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MAPK-directed activation of the whitefly transcription factor *CREB* leads to P450-mediated imidacloprid resistance

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Abstract

The evolution of insect resistance to pesticides poses a continuing threat to agriculture and human health. While much is known about the proximate molecular and biochemical mechanisms that confer resistance far less is known about the regulation of the specific genes/gene families involved, particularly by *trans*-acting factors such as signal-regulated transcription factors. Here we resolve in fine detail the *trans*-regulation of *CYP6CM1*, a cytochrome P450 that confers resistance to neonicotinoid insecticides in the whitefly, *Bemisia tabaci*, by the mitogen-activated protein kinase (MAPK)-directed activation of the transcription factor *cAMP-response element binding protein* (*CREB*). Reporter gene assays were used to identify the putative promoter of *CYP6CM1*, but no consistent polymorphisms were observed in the promoter of a resistant strain of *B. tabaci* (IMR), that overexpresses this gene, compared to a susceptible strain (IMS). Investigation of potential *trans*-acting factors using *in vitro* and *in vivo* assays demonstrated that the bZIP transcription factor *CREB* directly regulates *CYP6CM1* expression by binding to a cAMP-response element (CRE)-like site in the promoter of this gene. *CREB* is overexpressed in the IMR strain and inhibitor, luciferase, and RNA interference assays revealed that a signaling pathway of MAPKs mediates the activation of *CREB*, and thus the increased expression of *CYP6CM1*, by phosphorylation-mediated signal transduction. Collectively, these results provide new mechanistic insights into the regulation of xenobiotic responses in insects, and implicate both the MAPK signaling pathway and a novel transcription factor in the development of pesticide resistance.

Key Words

Cytochrome P450, CREB, MAPK, Neonicotinoid insecticide, Insecticide resistance

Significance statement

Most studies of pesticide resistance have focused on the identification and functional analysis of resistance genes, but few studies have considered the signaling pathways involved in their regulation. In this work, we discovered that overexpression of a P450 that confers resistance to neonicotinoid insecticides in whitefly is *trans*-regulated by the transcription factor *CREB*. Further studies demonstrated that exposure to neonicotinoid insecticides activates a key pathway involved in the cellular response to extracellular signals, the MAPK signaling pathway, that activates *CREB* by phosphorylation. *CREB* then binds to a specific site on the promoter of *CYP6CM1* resulting in its increased expression. These findings reveal new mechanisms underlying the regulation of P450-mediated pesticide resistance, and also provide a potential target for pest control.

Introduction

Insect resistance to synthetic insecticides is an ongoing challenge to sustainable pest management while also an exceptional model system to study adaptive evolution. Work on this topic has provided a range of insights into the genomic and transcriptomic changes that occur in response to strong selective pressures. One such molecular alteration frequently implicated in resistant insect populations is increased production of metabolic enzymes that detoxify or sequester insecticides before they reach the target-site. A key enzyme system in this regard is insect cytochrome P450s (P450s), which play a central role in the metabolism of a wide range of natural and synthetic xenobiotics including insecticides (1, 2). The increased production of P450s in insecticide resistant insects has been shown to most commonly result from gene duplication/amplification, or from mutations in *cis*-acting promoter sequences (3–6). In contrast evidence for *trans*-acting factors regulating resistance-associated P450s comes from just a handful of studies (7-10).

Control of gene expression in *trans* is most commonly mediated by transcription factors, and regulation of genes involved in the metabolism of xenobiotics has been shown to involve several superfamilies, including basic leucine zipper (bZIP), basic-helix-loop-helix/Per-ARNT-Sim (bHLH-PAS), and nuclear receptors (10-12). Well characterized examples in mammals are the human steroid and xenobiotic receptor (SXR) and constitutive androstane receptor (CAR) which appear to function in a partially redundant manner in regulating a range of phase I and phase II detoxification enzymes (13). In insects, much less is known about the transcription factors that regulate the response to xenobiotics with much of our understanding stemming from work on *Drosophila melanogaster*. In this species the nuclear receptor *DHR96* and the bZIP factor *Cap n collar isoform C* (*CncC*) have been shown to play key roles in the regulation of xenobiotic responses, in part by modulating P450 transcription (14, 15). Much less is known about the role of transcription factors in the xenobiotic response of non-model insect species, however, recent work has also implicated *CncC*, and the bZIP factor small *muscle aponeurosis fibromatosis* (*maf*), in the overexpression of P450 genes involved in insecticide resistance in several crop pest species and disease vectors (7-9). Taken together these studies suggest members of the bZIP superfamily may play a central role in regulating the transcriptional response of insects to xenobiotics. A further member of this superfamily, *CREB* is involved in wide ranging processes including glucose homeostasis, growth factor-dependent cell survival, nervous system development, learning, memory, synaptic plasticity, and drug adaptive responses (16). In insects CREB has also been shown to play a role in diverse processes including long-term memory formation, waking and rest homeostasis, starvation resistance, age-dependent labor division and environment-induced behavioral plasticity (17-21). However, the role of *CREB* in regulating detoxification enzymes in the context of insect resistance to insecticides has never been explored.

Transcription factors such as *CREB* may be themselves regulated by activity-inducible kinases such as MAPKs, components of signaling cascades that respond to extracellular stimuli such as environmental stress. MAPKs have been extensively studied and a wealth of

information is available from a variety of vertebrates (22) and invertebrates, including *D. melanogaster*, *Caenorhabditis elegans*, and *Anopheles gambiae* (23–25). Three classical kinase subfamilies have been described: extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 kinases (22). To date, the role of the MAPK signaling pathway in the regulation of P450 genes associated with insecticide resistance is essentially unknown, however, a *MAP4K4* gene has recently been shown to alter the expression of genes involved in resistance to *Bacillus thuringiensis* Cry1Ac toxin in diamondback moth, *Plutella xylostella* (26).

The sweet potato whitefly, *Bemisia tabaci*, is a highly destructive pest of many protected and field crops worldwide that causes damage via direct feeding and as a vector of more than 100 plant viruses (27). Control of *B. tabaci* has largely relied on the use of synthetic insecticides with members of the neonicotinoid class, such as imidacloprid, particularly widely used. As a result, imidacloprid resistance has been widely reported in the two most economically important species of the *B. tabaci* complex from a range of geographical origins worldwide, and linked to the overexpression of a single P450 gene, *CYP6CM1* (28, 29). Subsequent functional analyses have demonstrated the capacity of the encoded enzyme to catalyze the hydroxylation of imidacloprid and other insecticides (30, 31). Despite the importance and global distribution of this mechanism the molecular basis for *CYP6CM1* overexpression in neonicotinoid resistant strains has never been elucidated. In the present study we investigated the factors that regulate the expression of *CYP6CM1* and lead to the development of resistance to imidacloprid in *B. tabaci*.

Results

Imidacloprid resistance in the IMR strain of *B. tabaci* is conferred by overexpression of *CYP6CM1* not mutation of the nicotinic acetylcholine receptor (nAChR).

A strain of *B. tabaci* MED collected in the field was split into two cultures and maintained under selection with 2.0 mM imidacloprid for more than 30 generations to form the IMR strain, or left unexposed to insecticides to form the IMS strain. At the time of this study the IMR strain exhibited 23-fold resistance to imidacloprid in comparison to the IMS strain (*SI Appendix*, Table S2). To examine if the resistance of the IMR strain was associated with mutation of the imidacloprid target site, the nicotinic acetylcholine receptor (nAChR), nine nAChR subunit genes (eight α subunits and one β subunit genes) present in the genome of *B. tabaci* were cloned and sequenced from the IMR and IMS strains. No non-synonymous mutations were observed in the sequences obtained that consistently distinguish the two strains (Dataset S4), thus target-site insensitivity plays no role in the resistance of the IMR strain. As detailed in the introduction, the primary mechanism of metabolic resistance to imidacloprid in *B. tabaci* worldwide is enhanced expression of the P450 *CYP6CM1*. Quantitative PCR (qPCR) was therefore used to assess the expression of *CYP6CM1* in the two strains, and revealed 10-fold greater expression of this P450 gene in the IMR strain than in the IMS strain (Fig. 1*A*). To confirm that this translated to altered expression of the encoded enzyme we used a rabbit

polyclonal antibody raised against a synthetic peptide of CYP6CM1 in western blot to show that the CYP6CM1 protein is expressed at higher levels in every developmental stage of the IMR strain than in the IMS strain (Fig. 1*B*, *SI Appendix*, S3*E*). To demonstrate the causal role of *CYP6CM1* in the imidacloprid resistance of the IMR strain, RNA interference (RNAi) was used to knockdown its expression and the effect of this on the lifespan of imidacloprid-treated adults of the IMR strain examined. After 48 h of feeding on a diet containing dsRNA specific for *CYP6CM1* the mRNA levels of this gene decreased by 49.7% (*SI Appendix*, Fig. S3*A*). Knockdown of *CYP6CM1* significantly decreased the lifespan of IMR adult whiteflies, relative to the control (fed *EGFP* dsRNA), when adults were treated with imidacloprid at 0.4 mM (median survival -23 h, $p=2.05 \times 10^{-147}$, Fig. 1*C*) or at 2.0 mM (median survival -9 h, $p=1.44 \times 10^{-52}$, *SI Appendix*, Fig. S6*A*, S8*A*, Dataset S1). In contrast, no significant shift was seen in the sensitivity of the IMS strain after *CYP6CM1* knockdown. Together these results demonstrate that reducing *CYP6CM1* mRNA level is sufficient to decrease the resistance of the IMR strain to imidacloprid.

CYP6CM1 promoter analysis. To identify the putative promoter of *CYP6CM1*, a series of constructs comprising eleven different regions upstream of the gene (-235 to +1, -458 to +1, -570 to +1, -702 to +1, -761 to +1, -812 to +1, -865 to +1, -920/ to +1, -939 to +1, -968 to +1, and -1196 to +1) were created, and the ability of each of these to drive expression of a reporter gene assessed using dual luciferase reporter assays. The construct containing the region -939 to +1 (pGL4.10-CYP6CM1$^{-939\ to\ +1}$) showed the most pronounced expression (14.3-fold higher than the reference reporter plasmid) of the reporter suggesting that the promoter is located in this region, with particularly important elements located between -920 and -939 bp (Fig. 2*A*).

***CYP6CM1* overexpression is not associated with polymorphisms in *cis*-acting promoter elements.** In the first study of *CYP6CM1* overexpression in *B. tabaci* SNPs were identified in a non-coding region of the gene that associate with imidacloprid resistance suggesting *CYP6CM1* may be regulated by *cis*-acting factors (28). To investigate if polymorphisms within the promoter of *CYP6CM1* are associated with its overexpression in the IMR strain the promoter region defined above, along with 6 kb upstream of this region, was PCR amplified and sequenced in 6 pools of DNA extracted from the IMR and IMS strains. While single nucleotide polymorphisms (SNPs) were observed at certain positions in the sequences obtained, no consistent polymorphisms were observed between sequences of the two strains (Dataset S2). This finding indicates that the increased expression of *CYP6CM1* in the IMR strain does not result from *cis*-acting elements in the promoter of this gene or in upstream enhancers within 6 kb.

The bZIP transcription factor *CREB* mediates *CYP6CM1* expression. Transcription factors that sense xenobiotic stress are known as xenobiotic sensors and we hypothesized that one or more of these factors may be involved in regulating *CYP6CM1*. Based on an extensive literature search we selected 16 transcription factors with potential roles in xenobiotic stress response from the recently sequenced genome of *B. tabaci* (detailed information on these

transcription factors is provided in Dataset S3). To explore if any of these regulate *CYP6CM1* expression, the 16 transcription factors were individually cloned into the pAC5.1b expression vector and co-transfected into S2 cells with pGL4.10-CYP6CM1S$^{-939\ to\ +1}$. The successful expression of these transcription factors in S2 cells was confirmed using QPCR (*SI Appendix*, Fig. S3*F*). In reporter gene assays a marked (5.98-fold) and significant ($p=1.49 \times 10^{-7}$) increase in expression driven by the *CYP6CM1* promoter was only observed in combination with the construct expressing the transcription factor *CREB* (Fig. 2*B*). In a follow-on experiment, *CREB* expression also significantly (p=0.022) increased reporter expression driven by the *CYP6CM1* promoter in HEK293T cells (*SI Appendix*, Fig. S3*B*).

The full-length gene that encodes *CREB* in *B. tabaci* contains an 846-bp ORF encoding 282 amino acid residues with high pairwise amino acid similarity to members of the *CREB* family from other organisms. The deduced amino acid sequence of the protein includes important conserved domains common to the bZIP family including a basic leucine zipper DNA-binding and dimerization domain (bZIP) and a central kinase-inducible domain (KID) which interacts with protein kinases (*SI Appendix*, Fig. S1). To investigate the importance of these domains in activating the promoter of *CYP6CM1* two mutated forms of *CREB*, CREB-DN1, which lacks the KID domain, and CREB-DN2, which lacks the bZIP domain, were created and co-transfected into S2 cells with pGL4.10-CYP6CM1$^{-939\ to\ +1}$. As shown in Fig. 2*C*, expression of either CREB-DN1 or CREB-DN2 significantly (DN1: $p=1.05 \times 10^{-4}$, DN2: $p=9.81 \times 10^{-7}$) reduced the activity of the *CYP6CM1* promoter compared to the control (pAC5.1b-CREB). Interestingly, in assays with CREB-DN1 reporter gene expression remained significantly ($p=9.43 \times 10^{-5}$) higher than that of the negative control (pAC5.1b empty vector) whereas expression driven by CREB-DN2 was no different to the negative control. Thus these findings reveal that both the KID and bZIP domains are important for *CYP6CM1* promoter activation *in vitro* with the latter essential.

CREB stimulates target gene expression at promoters that contain cAMP response elements (CREs) (32). These typically appear as either palindromic sequences (TGACGTCA) or half-site sequences (TGACG or CGTCA). Significantly, the promoter region of *CYP6CM1* contains a CRE half-site-like sequence TGATTG between positions –930 and -926bp, which we identified as containing elements of major effect on gene expression. When this site was disrupted a significant decrease was observed in reporter gene expression driven by the mutant *CYP6CM1* promoter suggesting this region is important for CREB binding (*SI Appendix*, Fig. S2). To investigate this further a DNA probe of the region -920 to -968bp encompassing this site was produced, and the CREB protein expressed in *E. coli* cells and purified. Purified CREB protein and the DNA probe were then used in electrophoretic mobility shift assays (EMSA) to examine protein-nucleic acid interactions. Dose-dependent binding of CREB protein to the CRE-like containing DNA probe was observed (Fig. 2*D*), providing strong evidence that *CREB* enhances *CYP6CM1* expression *in vitro* as a result of its binding to a CRE-like site in the upstream promoter of this gene. To determine whether *CREB* affects *CYP6CM1* gene expression *in vivo*, RNAi was used to knockdown the expression of *CREB* in

adult whiteflies, and the effect of this on expression of both *CREB* and *CYP6CM1* examined using qPCR. After 48 h of feeding on a diet containing dsRNA specific for *CREB*, the mRNA levels of this gene decreased by 40.2% (*SI Appendix*, Fig. S3*D*). Furthermore, a corresponding significant decrease (50.3%) in the transcript and protein levels of CYP6CM1 was observed (Fig. 2*E, SI Appendix*, Fig. S4*D*). Taken together, both *in vitro* and *in vivo* assays unequivocally demonstrate that *CREB* directly regulates *CYP6CM1* expression.

***CREB* is overexpressed in the IMR strain and RNAi knockdown of *CREB* reduces resistance to imidacloprid.** To explore the expression of *CREB* in the IMR and IMS strain, we used qPCR and a rabbit polyclonal antibody raised against a synthetic peptide of CREB protein in western blot to measure mRNA and protein levels respectively. *CREB* mRNA levels were 2.9-fold greater in the IMR strain than in the IMS strain (*SI Appendix*, Fig. S3*C*) with western blot confirming that the encoded protein is overexpressed in the IMR strain as a result (Fig. 2*F*). To further examine the role of *CREB* overexpression in imidacloprid resistance in the IMR strain, we used RNAi to knockdown the expression of *CREB* in adult whiteflies and examined the effect of this on the lifespan of IMR adults that were treated with imidacloprid. Knockdown of *CREB* significantly decreased the lifespan of whiteflies relative to the control (fed ds*EGFP*) when treated with imidacloprid at 0.4 mM (median survival -14 h, $p=2.37 \times 10^{-52}$, Fig. 2*G*) and at 2.0 mM (median survival -9 h, $p=9.28 \times 10^{-58}$, *SI Appendix*, Fig. S6*B*, S8*B*, Dataset S1). This finding confirmed that reducing *CREB* transcript levels (and thereby reducing *CYP6CM1* expression) is sufficient to decrease *B. tabaci* resistance to imidacloprid. Sequencing of the *CREB* promoter failed to identify consistent polymorphisms in the region 2.2kb upstream of the translation start site between the IMR and IMS strains (Dataset S2) suggesting overexpression of *CREB* results from *trans*-acting factors.

The ERK and p38 MAPK signaling pathways activate *CREB* transcription. CREB proteins are themselves regulated via phosphorylation by several kinases, including protein kinase A (PKA) (33) and MAPKs (34). To investigate whether PKA and MAPKs activate *CREB in vitro*, S2 cells were treated with a specific inhibitor of PKA (H89) (35) and with three inhibitors (PD98059, SB203580, and SP600125) (36) of three classical MAPK pathways (ERK, p38, and JNK respectively) and co-transfected with pGL4.10-CYP6CM1$^{-939 \text{ to } +1}$. As shown in Fig. S4*A*, reporter gene expression driven by the *CYP6CM1* promoter was much greater in cells treated with H89 than in untreated control cells, suggesting PKA suppresses the expression of *CYP6CM1* in this system. In contrast, inhibition of the ERK and p38 pathways significantly (ERK: $p=6.35 \times 10^{-6}$, p38: $p=4.73 \times 10^{-5}$) decreased reporter gene expression driven by CYP6CM1$^{-939 \text{ to } +1}$ suggesting that these signaling pathways may positively regulate *CYP6CM1* expression. Inhibition of the JNK pathway significantly ($p=4.52 \times 10^{-7}$) increased the expression of *CYP6CM1* (Fig. 3*A*) suggesting a negative regulatory role on *CYP6CM1* expression. To examine the effect of inhibition of the ERK and p38 pathways *in vivo*, PD98059 and SB203580 were fed to IMR adults in artificial diet and the effect of this on the lifespan of IMR adults that were treated with imidacloprid examined. As shown in Fig. S4*C* both ERK inhibition (median survival -19 h, $p=5.84 \times 10^{-91}$) and p38 inhibition (median survival

-16 h, $p=2.27 \times 10^{-45}$) significantly decreased the resistance of the IMR strain to imidacloprid. The efficacy of these inhibitors was further explored on tomato plants in combination with imidacloprid (0.4 mM) with 100 *B. tabaci* adults released onto each plant. The lifespan of adult *B. tabaci* was significantly decreased on plants exposed to 0.4 mM imidacloprid treatment + PD98059 or SB203580 in comparison to plants exposed to imidacloprid without inhibitors (PD98059 treatment: median survival -3.96 days, $p=8.65 \times 10^{-184}$, SB203580 treatment: median survival -26 h, $p=7.46 \times 10^{-91}$, *SI Appendix*, Fig. S4*E*).

To examine if inhibition of the ERK and p38 pathways increased sensitivity to imidacloprid as a result of their effects on *CYP6CM1* gene expression in *B. tabaci*, the mRNA levels of the *ERK* and *p38* MAPKs were knocked down using RNAi and the effect of this on the expression of *CYP6CM1* explored using qPCR. As shown in Fig. S4*D*, the mRNA transcript levels of *ERK* and *p38* decreased by 47.2 and 41.4%, respectively, after 48 h of feeding on a diet containing *ERK* and *p38* dsRNA (0.5 μg/μL), and knock down of the MAPKs *ERK* and p38 resulted in a significant decrease (65.7% and 61.9% respectively) in the expression of *CYP6CM1* in the IMR strain. Moreover, a corresponding significant decrease in protein levels of CYP6CM1 was observed (Fig. 3*B*). Furthermore, knock-down of *ERK* or *p38* significantly decreased the lifespan of IMR adults treated with 0.4 mM imidacloprid (*ERK* treatment: median survival -23 h, $p=1.04 \times 10^{-133}$; *p38* treatment: median survival -26 h, $p=2.05 \times 10^{-147}$, Fig. 3*C* and *E*, Dataset S1) and 2.0 mM imidacloprid (*ERK* treatment: median survival -9 h, $p=9.22 \times 10^{-31}$; *p38* treatment: median survival -12 h, $p=1.44 \times 10^{-52}$, *SI Appendix*, Fig. S6*C* and *D*, S8 *C* and *D*, Dataset S1) demonstrating that a reduction in *ERK* and *p38* expression is sufficient to decrease *B. tabaci* resistance to imidacloprid. No difference was observed in the expression of *ERK* in the IMR and IMS strains, however, the expression of *p38* was significantly higher in the IMR strain (*SI Appendix*, Fig. S4*B*). Both of the MAPKs are phosphorylated in order to activate down-stream proteins (analysis of phosphorylation sites is shown in *SI Appendix*, Fig. S5). To explore if differences were observed in the phosphorylation profile of ERK and p38 protein in the IMR and IMS strains, homo phosphorylation antibodies were used in western blots with the two strains. A significant increase in the level of protein phosphorylation of ERK was observed in the IMR strain compared to the IMS strain (Fig. 3*D*). Furthermore, when the IMR strain was treated with imidacloprid, the level of protein phosphorylation of ERK increased rapidly (within 0.5 h, Fig. 3*D*), suggesting that differential phosphorylation, rather than differential expression, of ERK plays a role in the resistance of the IMR strain. Similarly, a dramatic increase in the level of protein phosphorylation of p38 was observed in the IMR strain compared to the IMS strain, and when adult whiteflies were treated with imidacloprid, phosphorylation occurred much more rapidly and reached higher levels in the IMR strain (Fig. 3*D*), suggesting that both differential phosphorylation and expression of p38 plays a role in the resistance of the IMR strain.

To further explore the role of phosphorylation in resistance we investigated phosphorylation of *CREB* itself. In humans MAPKs activate *CREB* by phosphorylation of

Ser133 which promotes the recruitment of transcriptional coactivators (37). The equivalent site in *B. tabaci CREB* is Ser111 (RRP**S**YRK). We first demonstrated the importance of this site on function by mutating site 111 (Ser to Tyr) and cotransfecting S2 cells with the mutant construct and plasmid pGL4.10-CYP6CM1$^{-939\ to\ +1}$ and examining the effect on luciferase activity. Mutation of *CREB* at site 111 significantly (p=0.005) decreased reporter gene expression driven by the promoter of *CYP6CM1* (Fig. 4*A*) demonstrating its functional role. A rabbit polyclonal antibody for the phosphorylated form of *B. tabaci* CREB protein was then raised and used to determine the phosphorylation profile of this enzyme in the IMR and IMS strain by western blot. Consistent with the elevated phosphorylation of the upstream MAPKs we observed higher levels of the phosphorylated form of CREB in the IMR strain suggesting that resistance results from increased production of the activated (phosphorylated) form of this transcription factor (Fig. 4*B*).

Increased phosphorylation of CREB, ERK and p38 is also observed in imidacloprid resistant field strains of *B. tabaci*. To examine if the key findings on the regulation of resistance in the IMR strain extend to imidacloprid resistant field populations of *B. tabaci* MED we conducted further analysis on four field samples collected in 2018 (*SI Appendix*, Table S1). In insecticide bioassays the NK and YC strains exhibited strong resistance to imidacloprid, the LF strain showed modest resistance and the SY strain was susceptible to this compound (*SI Appendix*, Table S2). Western blot was used to estimate the total protein level of CYP6CM1, CREB, ERK, p38 and the phosphorylation level of CERB, ERK and p38 (Fig. 4D). The expression of CYP6CM1 protein was higher in the resistant NK and YC strains than the SY susceptible strain. In parallel, the level of both total CREB and total P38, and their phosphorylated forms, was greater in the two field resistance strains than the susceptible strain. While no difference was seen in the level of total ERK protein in the four field strains, the level of phosphorylated ERK was higher in the NK and YC strains than both of the other strains. RNAi knockdown of *CYP6CM1*, *CREB*, *ERK* and *p38* in the NK resistant strain (*CYP6CM1* treatment: median survival -21 h, p=3.96 × 10^{-289}; *CREB* treatment: median survival -6 h, p=7.63 × 10^{-67}, *ERK* treatment: median survival -6 h, p=3.41 × 10^{-54}; *p38* treatment: median survival -6 h, p=9.76 × 10^{-37}) and YC resistant strain (*CYP6CM1* treatment: median survival -18 h, p=1.70 × 10^{-291}; *CREB* treatment: median survival -15 h, p=6.06 × 10^{-279}, *ERK* treatment: median survival -18 h, p=5.65 × 10^{-249}; *p38* treatment: median survival -18 h, p=7.21 × 10^{-274}) significantly decreased the resistance levels to imidacloprid at 2.0 mM (*SI Appendix*, Fig. S7). In contrast, no significant shift was seen in the sensitivity of the SY strain after these genes were knocked down. Thus the increased expression of *CYP6CM1* in field strains of *B. tabaci* MED also appears to be mediated, at least in part, by CREB and phosphorylation-mediated signal transduction involving the MAPK signaling pathway.

Discussion

Herbivorous insects have evolved complex defense systems to protect themselves against xenobiotic stressors such as the secondary metabolites produced by their host plants,

however, how these toxins are sensed and this signal transduced into a physiological response is poorly understood. From an applied perspective such knowledge is important as some of these natural insect defense mechanisms have been co-opted during the evolution of insect resistance to synthetic insecticides. Our data demonstrate that *CYP6CM1*, a P450 recruited during the evolution of *B. tabaci* resistance to neonicotinoid insecticides, is regulated by *CREB* a transcription factor belonging to the bZIP superfamily. Members of this superfamily have been previously implicated in the regulation of P450 genes involved in resistance to plant allelochemicals and insecticides, however, CREB has never previously been implicated in this role. Indeed, only a single very recent study has previously linked *CREB* to the regulation of a P450, in this case human *CYP2J2*, which is highly expressed in the human brain, and is responsible for metabolizing endogenous polyunsaturated fatty acids (38, 39). More broadly in mammals, *CREB* has been shown to play a role in a remarkably diverse range of processes. Of particular relevance to this study is the role of *CREB* in the response to stressful stimuli including oxidative stress, ischemia, and excitotoxicity, with evidence from knockout mice revealing a role in neuroprotection and neuronal survival (32). Exactly how CREB mediates this activity is not fully understood. Here we demonstrate that CREB performs a neuroprotective function in *B. tabaci* by upregulating a key detoxification enzyme. This in turn increases the metabolic conversion of imidacloprid, an agonist of insect nAChRs in neuronal cells, into its less toxic 5-hydroxy form.

Importantly, we show that, while CREB is overexpressed in resistant *B. tabaci* a marked increase is also observed in the activated form of this transcription factor as a result of phosphorylation of a key amino acid (Ser111) in the kinase-inducible domain. This finding suggests that additional upstream activators of CREB also play a role in CYP6CM1-mediated insecticide resistance. We confirmed this by demonstrating that two protein kinases, ERK and p38, that are focal points of two different MAPK signaling pathways, are involved in CYP6CM1-mediated resistance to imidacloprid in *B. tabaci*. The MAPK signaling pathway plays a key role in responses to both endogenous and xenobiotic substances (22); increased expression of cytochrome P450 2E1 induces heme oxygenase-1 through the ERK MAPK pathway (40), and p38 regulates the nuclear receptor CAR that activates the *CYP2B6* gene in human primary hepatocytes (41). Furthermore, the MAPK p38 pathway has been implicated in insect defense against pore-forming toxins such as Bt Cry toxins (42). We demonstrate that exposure of *B. tabaci* to imidacloprid results in the phosphorylation of p38 and ERK, and thus implicate the MAPK signaling pathway in resistance to synthetic insecticides for the first time. MAPKs function in multi-tiered signaling cascades (43), in which an activated MAP4K phosphorylates and activates a MAP3K, which, in turn, activates a downstream MAP2K, which actives a MAPK (ERK/p38/JNK) that can regulate transcription factors. A *MAP4K4* gene was identified in *P. xylostella*, that is constitutively transcriptionally-activated in resistant larvae and alters expression of midgut *ALP* and *ABCC* genes leading to resistance to Bt Cry1Ac toxin (26). Furthermore, a MAPK gene was found to be up-regulated in *Anopheles stephensi* after exposure to pyrethroid for 24 and 48 hours (44). In the case of whiteflies RNA-

seq analysis of a thiamethoxam resistant and susceptible strain of *B. tabaci* MEAM1 identified the MAPK signaling pathway as a significantly enriched biological pathway in the differentially expressed genes (45). Together with our results these findings suggest that the MAPK signaling pathway may play a previously unappreciated role as a *trans*-regulator of important insecticide resistance gene effectors in a range of insect species.

Exactly how do the *p38* and *ERK* MAPK pathways and *CREB* work together to increase the expression of *CYP6CM1*? We present a model for the regulation of *CYP6CM1* in the resistant IMR strain in Fig 4*C* and S9. In this model, the activation of the MAPK signaling pathway upon insecticide exposure via an unknown receptor triggers a signaling cascade resulting in the phosphorylation of the MAPKs *ERK* and *p38* by MAPK kinases (MAPKKs), these in turn phosphorylate CREB; once phosphorylated, *CREB* activates the expression of *CYP6CM1* by binding to a CRE-like site in the promoter of this gene; as shown previously (28), increased production of *CYP6CM1* results in imidacloprid resistance in *B. tabaci*. Overexpression of *CREB* plays an additional role in the increased production of CYP6CM1 protein. This model sets out a framework for future studies to identify potential genetic changes at key points in this pathway that result in the constitutive overexpression of *CYP6CM1* observed in imidacloprid resistant strains of *B. tabaci*.

The receptor that triggers the initiation of the signaling pathway illustrated in Fig 4C is unknown. However, several previous studies have demonstrated a role for G-protein-coupled receptors (GPCRs) in modulating the expression of P450 genes that lead to resistance (46-48). These previous findings and the fact that GPCRs can activate the conserved MAPK pathway (49, 50) makes these receptors candidate upstream activators of the MAPK signaling pathway associated with imidacloprid resistant *B. tabaci*.

In conclusion, we uncover the *trans*-regulatory pathway involved in the increased expression of a key resistance gene in *B. tabaci* and implicate both the MAPK signaling pathway and a novel transcription factor in the development of resistance to synthetic insecticides. Collectively, these results advance our understanding of the regulation of xenobiotic responses in insects, and illustrate the remarkable sophistication of the molecular pathways involved. Finally, from an applied perspective identification of the pivotal genes involved in the regulation of CYP6CM1 also highlight potential new targets for control interventions against a global crop pest. In this regard we provide preliminary demonstration of the potential use of inhibitors of these pathways to enhance the effectiveness of imidacloprid applications against *B. tabaci*.

Materials and Methods

Detailed information on insect strains and cell lines, extraction of DNA and RNA, qRT-PCR, promoter analysis, dual-luciferase reporter assay, RNAi experiments, lifespan measurement, Western blot and inhibitor assays, and statistical analysis are described in *SI Appendix, SI Materials and Methods*.

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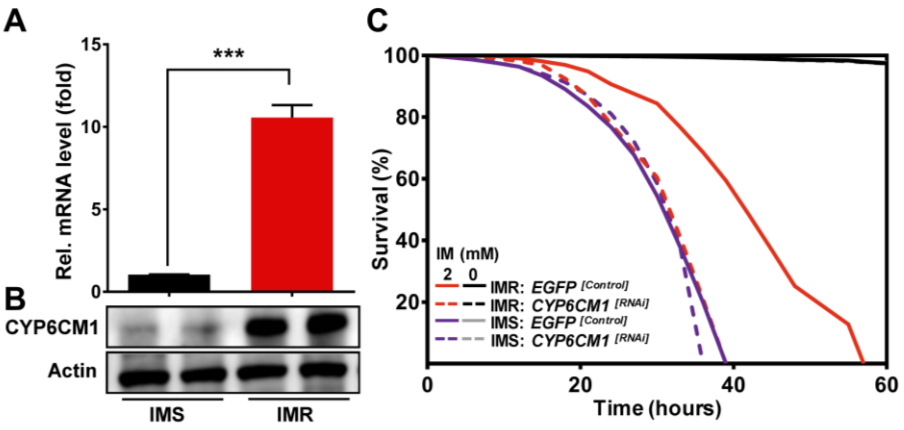

570

571 **Figure 1. *CYP6CM1* contributes to imidacloprid (IM) resistance in the *B. tabaci* strain**
572 **IMR.** (**A**) Relative expression of *CYP6CM1* mRNA (A) and protein (B) in the imidacloprid-
573 resistant strain IMR and the susceptible strain IMS as determined by qPCR and Western blot
574 respectively (qPCR: n = 3, mean ± SE; *** P < 0.001, two-tailed Student's t-test). Actin was
575 used as a loading control in Western blot. (**C**) Lifespan of adults of the IMR and IMS strains
576 exposed to 2.0 mM imidacloprid (2) or no imidacloprid (0) after RNAi knockdown of
577 *CYP6CM1*. Adults fed on ds*EGFP* were used as a negative control.

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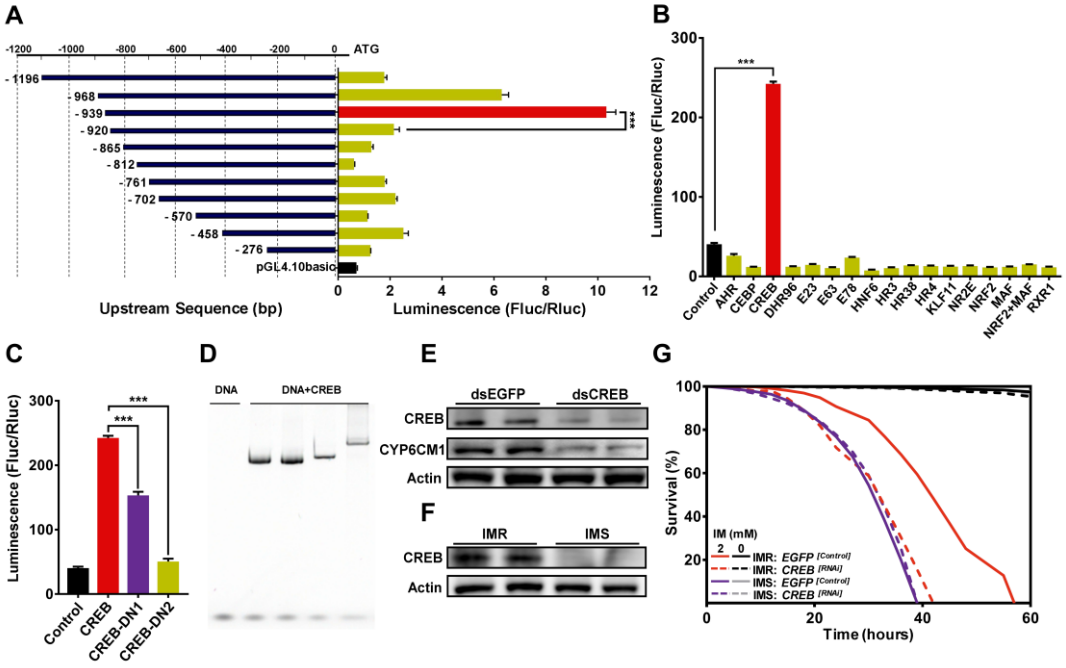

Figure 2. The transcription factor *CREB* regulates *CYP6CM1* expression. (**A**) Identification of the promoter of *CYP6CM1* using progressive deletion constructs in dual luciferase reporter assays. S2 cells were cotransfected with pGL4.10 reporter plasmids carrying the indicated promoter regions conjugated to firefly luciferase and a reference reporter plasmid (pGL4.73, containing the *hRluc* reporter gene and an SV40 early promoter). Fluc/Rluc represents the ratio of Firefly to Renilla luciferase activity (n = 3, mean ± SE; *** P < 0.001, ANOVA with Tukey's HSD post hoc test). (**B**) Influence of 16 different *B. tabaci* transcription factors on expression driven by the promoter of *CYP6CM1* in dual luciferase reporter assays. pGL4.10-CYP6CM1$^{-939\ to\ +1}$ and pGL4.73 were cotransfected into S2 cells with each of the 16 transcription factors in pAC5.1b. Empty pAC5.1b was used as a control (n = 3, mean ± SE; *** P < 0.001, ANOVA with Tukey's HSD post hoc test). (**C**) Identification of important functional domains in CREB using dual luciferase reporter assays. pGL4.10-CYP6CM1$^{-939\ to\ +1}$ and pGL4.73 were co-transfected into S2 cells with pAC5.1b-CREB (CREB), with modified *CREB* in which the KID domain (CREB-DN1) or the bZIP domain (CERB-DN2) was deleted, or with the pAC5.1b empty vector (control) (n = 3, mean ± SE; *** P < 0.001, two-tailed Student's t-test). (**D**) *CREB* binds to the upstream promoter sequences of *CYP6CM1*. EMSA results showing dose-dependent binding of CREB to a CRE-like containing DNA probe. (**E**) Expression of CREB and CYP6CM1 protein after RNAi knockdown of CREB in the IMR strain as assessed by Western blot. Actin was used as a loading control. (**F**) Expression of CREB protein in the IMR and IMS strain as assessed by Western blot. Actin was used as a loading control. (**G**) Lifespan of adults of the IMR and IMS strains exposed to 2.0 mM imidacloprid (2) or no imidacloprid (0) after feeding on *CREB* dsRNA for 48 h. Adults fed on ds*EGFP* were used as a negative control.

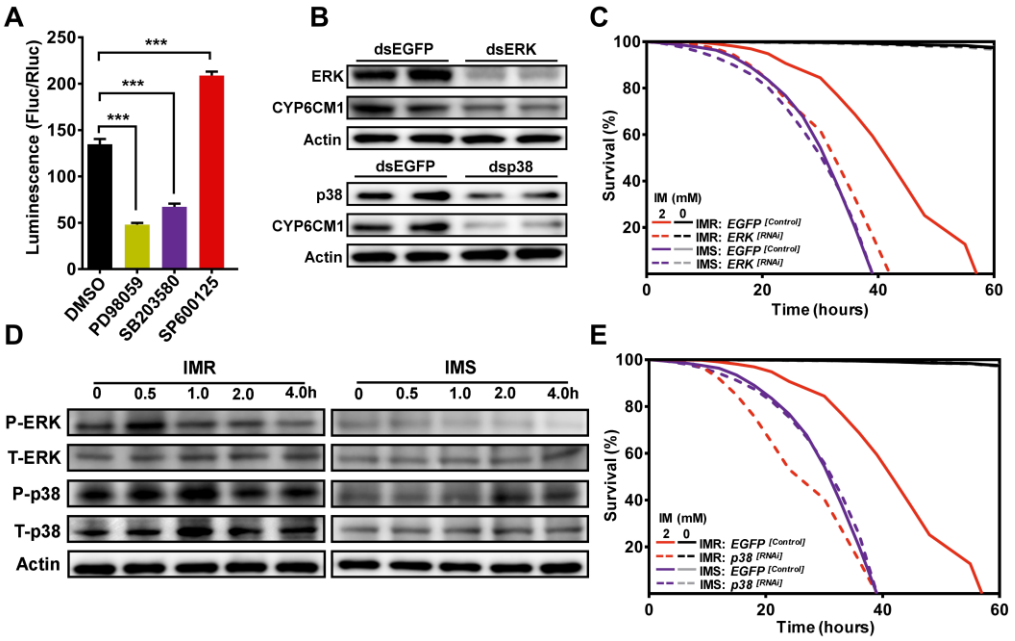

Figure 3. The MAPK signaling pathway regulates *CYP6CM1* expression and imidacloprid resistance. (**A**) Expression driven by the promoter of *CYP6CM1* in the presence of inhibitors of the ERK, p38 and JNK MAPK pathways. Dual luciferase reporter assays, in which pGL4.10-CYP6CM1$^{-939\ to\ +1}$ and pGL4.73 were cotransfected into S2 cells with either the ERK inhibitor PD98059 (75 μM), the p38 inhibitor SB203580 (53 μM), or the JNK inhibitor SP600125 (45 μM) are shown. Fluc/Rluc represents the ratio of Firefly to Renilla luciferase activity. (n = 3, mean ± SE; *** P < 0.001, ANOVA with Tukey's HSD post hoc test). (**B**) Expression of CYP6CM1 protein after knockdown of *ERK* or *p38* in the IMR strain as assessed by Western blot. Actin was used as a loading control. (**C**) Lifespan of adult whiteflies of the IMR and IMS strains after feeding on *ERK* dsRNA for 48 hours followed by exposure to 2.0 mM imidacloprid. Adults fed on ds*EGFP* were used as a negative control. (**D**) Levels of Total-ERK, Total-p38, and phosphorylated ERK and p38 in IMR and IMS adults assessed by Western blot at four time points after exposure to 2.0 mM imidacloprid. Actin was used as a loading control. (**E**) Lifespan of adult whiteflies of the IMR and IMS strains after feeding on *p38* dsRNA for 48 hours followed by exposure to 2.0 mM imidacloprid (2) or no imidacloprid (0). Adults fed on ds*EGFP* were used as a negative control.

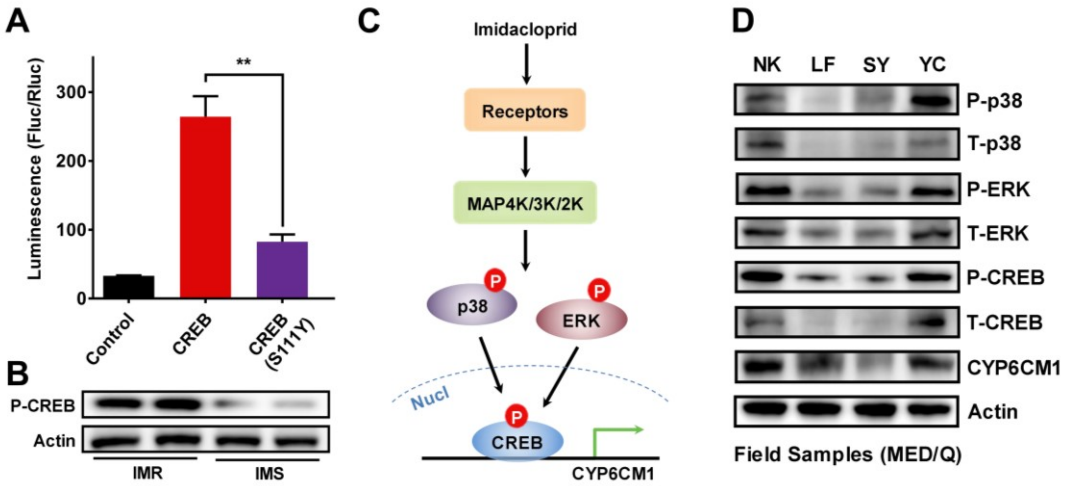

Figure 4. Model of the regulation of *CYP6CM1* in *B. tabaci*. (**A**) Expression driven by the promoter of *CYP6CM1* in the presence of wild type or mutated (Ser111Tyr) CREB. pGL4.10-CYP6CM1$^{-939\ to\ +1}$ and pGL4.73 plasmids were cotransfected into S2 cells with either CREB or mutated CREB (Ser111Tyr) or the pAC5.1b empty vector (control) and expression assessed using dual luciferase reporter assays (n = 3, mean ± SE; ** P < 0.01, two-tailed Student's t-test). (**B**) Phosphorylation levels of Ser111 in CREB in the IMR and IMS strain as assessed by Western blot. Actin was used as a loading control. (**C**) A model of the regulation of *CYP6CM1* in *B. tabaci*. In this model, exposure to imidacloprid is detected by an unknown receptor that actives mitogen-activated protein kinases of the MAPK pathway to phosphorylate ERK and p38. These in turn phosphorylate CREB, which then activates the expression of *CYP6CM1*. Activation and over-expression of CREB in the resistant strain increases the production of CYP6CM1 protein, which reduces the toxicity of imidacloprid. (**D**) Levels of Total-CYP6CM1, Total-CREB, Total-ERK, Total-p38, and phosphorylated CREB, ERK, and p38 in four field strains of *B. tabaci* MED. Actin was used as a loading control.

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Supplementary Information for

MAPK-directed activation of the whitefly transcription factor *CREB* leads to P450-mediated imidacloprid resistance

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

SI Results

Lifespan of no-imidacloprid controls

The mean mortality observed at the conclusion of the experiment in all of the no-imidacloprid controls run in RNAi experiments was 13% and did not exceed 17% in any strain (Dataset S1). No significant difference was observed between the survival curves of no-imidacloprid controls treated with control *EGFP* dsRNA and those treated with whitefly gene-specific dsRNA (Dataset S1). No significant difference was observed in the no-imidacloprid survial curves obtained from the IMS and IMR strains (Dataset S1). A significant difference was observed between the no-imidacloprid survial curves obtained from the IMS strain and the three field strains, NK, YC, SY, however in all cases the mortality at the conclusion of the experiment was very similar for all four strains (Dataset S1).

SI Materials and Methods

Insect Strains. A field strain of *B. tabaci* MED was collected in 2011 from Hangzhou in Zhejiang Province and split into two cultures one of which was treated with 2.0 mM imidacloprid for more than 30 generations by soil drench application to form the IMR strain, and the other left unexposed to insecticides to form the IMS strain. The IMR and IMS strains were maintained, and experiments were conducted, at 25 ^{o}C with a 14 h: 10 h light: dark cycle and 70% humidity on pepper (cv. Zhongjiao #4, *Capsicum annuum*) plants. Four field strains of *B. tabaci* MED were collected in 2018 from northern China (*SI Appendix*, Table S1). A total of approximately 2000 adults were collected per location, 500 of which were snap frozen in liquid nitrogen and stored at -80^{o}C for protein extraction and western bolt analysis, the remaining adults were used immediately in insecticide bioassays and RNAi experiments.

Extraction of DNA and RNA and qRT-PCR. Genomic DNA was extracted from a pool of 50 adult whiteflies of the IMR strain using the MiniBEST universal genomic DNA extraction kit (Takara Biotech) following the manufacturer's instructions. Total RNA was isolated from 50 whole adult whiteflies using standard TRIZOL (Invitrogen) protocols; the extracted RNA was converted to cDNA using oligo (dT) primer and Superscript II reverse transcriptase with gDNA Eraser (Taraka Biotech). qRT-PCR was performed using Power SYBR Green PCR Master Mix (Tiangen) in quadruplicate on the 7500 system (Applied Biosystems). A total of 150 adults (three biological replicates, n = 50) from different populations were used for qRT-PCR analysis. Primers were designed to amplify a 90-200 bp fragment. The total reaction volume of 20-µl comprised 1 µl of diluted cDNA, 10 µl of SYBR® Green Real-time PCR Master Mix (Tiangen) and 0.3 µl of each primer. Samples were run on an ABI 7500 real-time system using the following temperature cycling conditions: 15 min of activation at 95°C followed by 40 cycles of 30 s at 95°C and 40 s at 60°C. A 2-fold dilution series of cDNA was used to construct a relative standard curve, and the PCR efficiency was determined (see Dataset S5 for amplification efficiencies). PCR efficiency was used to convert quantification cycle (Ct-values) into processed data (relative quantities). The

fold-changes in the expression of whitefly genes of interest, normalized to three reference genes (*Actin*, *EF1α* and *RPL29*), were calculated using the $2^{-\triangle\triangle Ct}$ method (1).

Cell Culture. *Drosophila* S2 cells were cultured in Hyclone SFX-insect medium (Thermo Scientific) at 27 °C. Human HEK293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified incubator containing 5% CO_2. Approximately 5×10^{-5} cells per well were added to 24-well plates 6 h before transfection. Plasmids were transfected into the S2 and HEK293T cells using Lipofectamine 2000 (Invitrogen).

Promoter Analysis of *CYP6CM1*. The promoter sequence of *CYP6CM1* was analyzed by PCR and sequencing. Primers were designed based on the genomic sequence of *B. tabaci* (2) and used to characterize the ~6-kb putative promoter region immediately upstream of the translation start codon of *CYP6CM1* of the IMR strain (listed in Dataset S2). PCR products were recovered from agarose gels, PCR amplified using LA Taq (TaKaRa) and direct-sequenced. For reporter gene assays, a 1.2 kb promoter sequence was amplified from genomic DNA extracted from the IMR strain using a high-fidelity taq (TaKaRa). These were ligated into pGL4.10-Basic (Promega) and transformed into Trans1-T1 (Transgen). Plasmids were extracted with the EndoFree Mini Plasmid Kit (TIANGEN), sequenced, and adjusted to 100 ng/μL for use in dual luciferase assays.

Dual-luciferase Reporter and Dominant Negative Assays. The 5'-flanking and first intron regions of *CYP6CM1* were amplified from *B. tabaci* genomic DNA by PCR and were subcloned into the pGL4.10 reporter plasmids (Promega) carrying the indicated promoter regions conjugated to firefly luciferase. Reporter plasmids carrying deleted and mutated 5'-flanking regions of *CYP6CM1* were constructed from the $pGL4.10^{-1196}$ plasmids. The primers used for the construction of reporter plasmids are listed in Dataset S5. The coding sequence of *CREB* and 15 other transcription factors (3-22) were cloned into the pAC5.1b/V5/His expression vector (Invitrogen) for expression in S2 cells and into the pcDNA3.1V5/His vector for expression in 293T cells. q-PCR was used to estimate the expression of each transcription factor in cells. Two mutated forms of CREB (DN1, deletion of amino acids 178-282; and DN2, deletion of amino acids 1-206) were also cloned into the pAC5.1b/V5/His expression vector. pGL4.10-$CYP6CM1^{-939\ to\ +1}$ (200 ng) and a reference reporter pGL4.73 plasmid (100 ng, containing the hRluc reporter gene and an SV40 early promoter) were then cotransfected with pAC5.1b-transcription factors (600 ng) into S2 cells (24-well plates) and were kept at 27 °C. Luciferase activity was determined using the Dual-Luciferase Reporter Assay System and a GloMax 96 Microplate Luminometer (Promega) at 48 h post-transfection. Construct luciferase activity was normalized to Renilla luciferase activity.

RNAi Experiments. dsRNA was prepared using the T7 RiboMAX Expression RNAi system (Promega) following the manufacturer's instructions. *Enhanced green fluorescent protein* (*EGFP*) was used to generate control *EGFP* dsRNA. All primers used for producing dsRNA are listed in Dataset S4. dsRNAs were fed to adult whiteflies, within 2 days after emergence, using previously

described methodology (23). Briefly, dsRNA was fed to whitefly adults in a feeding chamber comprising a glass tube (20 mm in diameter × 50 mm long, open at both ends), which was covered at the top by one layer of Parafilm-membrane stretched as thinly as possible. A total of 0.2 mL of diet solution (5% yeast extract and 30% sucrose, wt/vol) was pipetted onto the outer surface of the stretched Parafilm. Insecticide or dsRNA (0.5 μg/μL) was dissolved in the diet solution. A second layer of Parafilm was stretched on top of the first membrane to form a feeding sachet. Fifty adult whiteflies (mixed sexes) were transferred into each tube, and the remaining opening was sealed with a black cotton plug and covered with a shade cloth. The tubes were placed in an environmental chamber (Panasonic MLR-352H, Gunma, Japan) at 25 °C and with a photoperiod of L14: D10 and 80% RH. The ends of the tubes with the Parafilm sachets were turned toward the light source, which was approximately 0.2 m away. Whiteflies were collected after 48 h of feeding and snap frozen in liquid nitrogen prior to molecular analyses.

Lifespan Measurements. The effect of the neonicotinoid insecticide imidacloprid (Sigma) on the lifespan of *B. tabaci* adults was determined in glass tubes as described by Yang et al (23). Two imidacloprid concentrations (0.4 and 2.0 mM) were dissolved in the diet solution, and introduced to adult whiteflies using the same satchet feeding system used for RNAi. An additional control comprised whiteflies fed on diet without imidacloprid.100–200 adult whiteflies were tested for each whitefly strain for each treatment/control with each glass tube containing 10 adult whiteflies. Mortality was recorded every 3 hours. The Kaplan-Meier method was used for survival curve analysis, and the log-rank (Mantel-Cox) test was used to determine the statistical significance of differences between survival curves using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA) and SPSS 19.0 (SPSS for Windows, Chicago, SPSS Inc.). Plant lifespan experiments used tomato plants (*Lycopersicon esculentum*, cv. Zhongza 9) treated with imidacloprid (both spray and soil drench) for 7 days. Inhibitors were then applied by spray application and 100 whiteflies placed on plants, with mortality recorded every day until all whiteflies had died.

Insecticide Bioassays. Leaf-dip bioassays were conducted with formulated imidacloprid (700 g kg-1 WG, Bayer Hangzhou Crop Science China Co. Ltd.) on adult whiteflies using the method described by Feng et al (24). Imidacloprid was dissolved and diluted with distilled water containing Triton X-100 (0.01%) to obtain serial concentrations of insecticide. Leaf discs (22 mm diameter) from cotton plants were dipped for 10 s in the insecticide solution or in distilled water (containing 0.01% triton X-100) as a control. After they were air dried, the leaf discs were placed with their adaxial surface downwards on agar (2 mL of 15 g/L) in a flat-bottomed glass tube (78 mm long). 15–30 adult whiteflies were added to each tube and kept in an incubator at 25°C with a 14:10 (L:D) photoperiod. Mortality was recorded after 48 h, and each combination of population and imidacloprid concentration was represented by four replicate tubes.

Western Blot. Total protein was extracted from 200 adult whiteflies per sample with the ProteinExt Mammalian Total Protein Extraction Kit (Transgen) following the manufacturer's

instructions. Rabbit polyclonal antibodies of CYP6CM1, total CREB, phosphorylated CREB, total ERK, and total p38 were raised against a synthetic peptide. The sequence of the peptide of CYP6CM1 was T-T-P-K-T-P-K-K-I-T-F-D-T-N (from tyrosine 486 to asparagine 500). The sequence of the peptide of total CREB was S-G-D-P-L-S-S-S-P-S-A-N-T-T (from serine 11 to tyrosine 25). The sequence of the peptide of phosphorylated CREB was D-I-L-T-R-R-P-pS-Y-R-K-I-L-N (from aspartic acid 103 to asparagine 117), in which Ser-111 was phosphorylated. Western blots were probed for phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (#4370), phosphor-p38 (Thr180/Tyr182) (#4631) (Cell Signaling Technologies). The sequence of the peptide of total ERK was L-E-Q-Y-Y-D-P-A-D-E-P-V-A-E (from serine 315 to tyrosine 328), and the sequence of the peptide of total p38 was D-P-E-D-E-P-T-S-P-P-Y-D-Q-S (from serine 309 to tyrosine 322), and β-actin antibody (#ab8227) (Abcam). The sequence of peptides of total CYP6CM1, CREB, ERK, and p38 were analyzed in the genome of *B tabaci* MED, they are present only in the target proteins. β-actin was used as a loading control in Western blot.

Inhibitor Assays. The following kinase inhibitors were purchased from Selleck: PD98059 (#S1102), SB203580 (#S1076), and SP600125 (#S1460). Dual luciferase reporter assays, in which pGL4.10-CYP6CM1$^{-939\ to\ +1}$ and pGL4.73 were co-transfected into S2 cells with either the ERK inhibitor PD98059 (75 μM), the p38 inhibitor SB203580 (53 μM), or the JNK inhibitor SP600125 (45 μM) were performed. Luciferase activity was determined using the Dual-Luciferase Reporter Assay System and a GloMax 96 Microplate Luminometer (Promega) at 48 h post-transfection. Inhibitors were dissolved in DMSO as stock solutions with DMSO alone added to the cell culture as a control. An ERK inhibitor (PD98059 at 1.8mM) or a p38 inhibitor (SB203580 at 1.3 mM) were fed to adult whiteflies, within 2 days after emergence, using the RNAi system as previously described (23). The efficacy of these inhibitors was further explored on tomato plants in combination with imidacloprid (0.4 mM) with 100 *B. tabaci* adults released onto each plant. The lifespan of adult *B. tabaci* was analyzed on plants exposed to 0.4 mM imidacloprid treatment + PD98059 or SB203580 in comparison to plants exposed to imidacloprid without inhibitors.

Electrophoretic Mobility Shift Assays. EMSA assays were performed using the Electrophoretic Mobility Shift Assay (EMSA) Kit (Invitrogen #E33075) according to the manufacturer's protocols. The assays were conducted using a synthetic DNA segment that contained putative *CREB* binding sites. The samples of CREB protein were expressed using the pEASY-Blunt E1 Expression Kit (Transgen) and purified with the CapturemTM His-tagged purification Kit (TaKaRa). The DNA amount was constant at 1.0 μg per reaction, and the protein/DNA ratios were 1:1, 1:2, 1:3, and 1:4. Samples were size-fractionated by gel electrophoresis using 6% TBE DNA retardation native gels at 150 V for 30 min. The DNA in the gel was stained using SYBR Green (provided in the same kit) and was visualized using the GE Typhoon LFA 9500 Imaging System (GE Healthcare LifeScience).

Statistical Analysis. The statistical significance of differences between samples was analyzed using Student's t-test and ANOVA with Tukey's HSD post hoc test (GraphPad 6.0, San Diego, CA). All quantitative data are reported as means ± SEMs from at least three independent experiments.

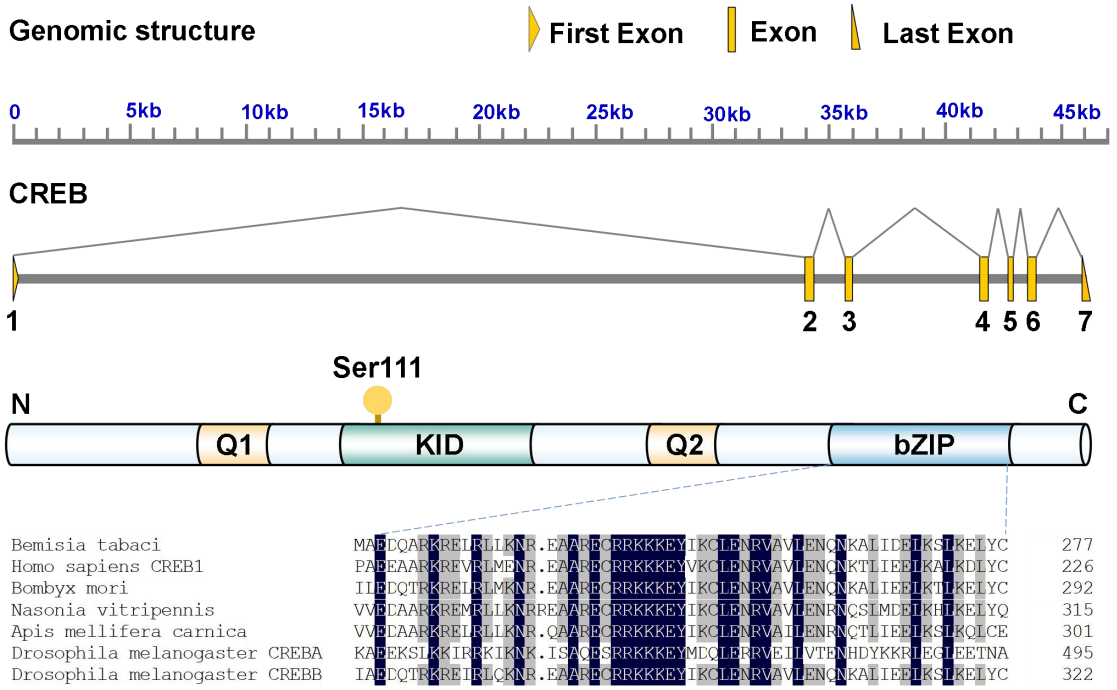

Fig. S1. Gene structure of *CREB* from the whitefly *B. tabaci*. The full-length gene that encodes *CREB* in *B. tabaci* contains an 846-bp ORF encoding 282 amino acid residues. Genomic structure analysis indicated that *CREB* contains 7 exons and 6 introns. *CREB* family members share similar modular organization: they all contain a carboxy-terminal basic Leu zipper (bZIP) DNA-binding and dimerization domain and an amino-terminal transactivation domain (TAD). The TAD contains a central kinase-inducible domain (KID) and two Gln-rich (Q1 and Q2) constitutive activation domains. A homo CREB1 Ser133-like site (RRP**S**YRK) is present in the *B. tabaci* KID domain (Ser 111). The deduced amino acid sequence includes important conserved domains common to other bZIP families, such as the leucine zipper and basic motif.

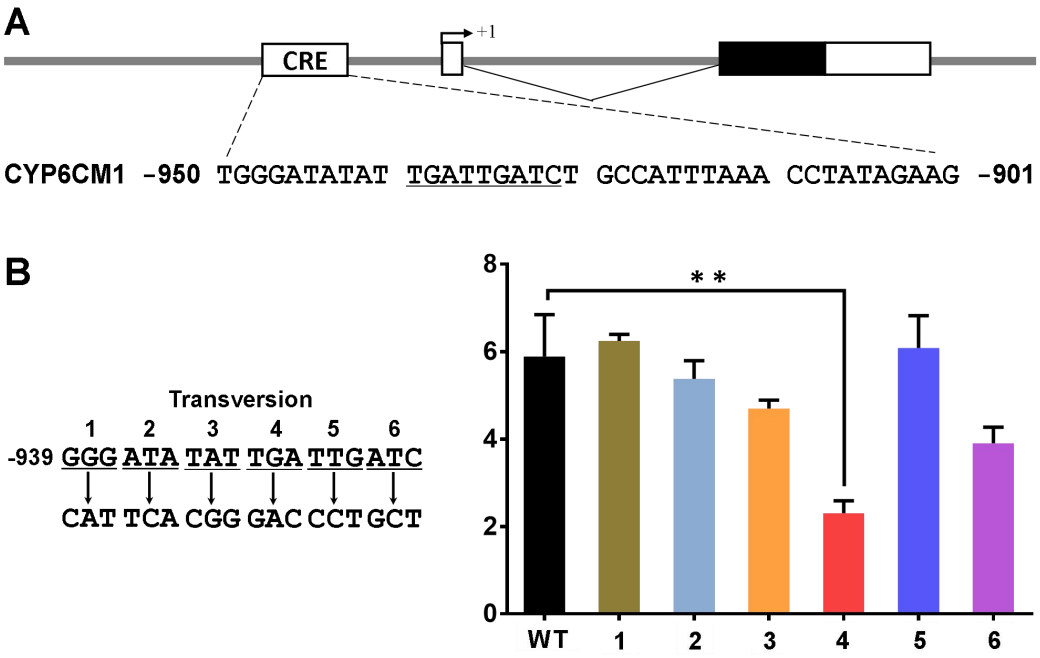

Fig. S2. The CRE-like site upstream of *CYP6CM1*. (**A**) Analysis of the CRE site in *CYP6CM1*. The upstream sequences of the *CYP6CM1* gene were analyzed in the MED/Q genomic sequences, and a half CRE-like site was identified at position –930 and -926bp. (**B**) The functionality of the CRE-like region was assayed with mutations causing a triplet transversion (each triplet has a transition) in the −939 to −920bp region of the upstream sequences of the *CYP6CM1* reporter (pGL4.10-*CYP6CM1*$^{-939\ to\ +1}$). Mutations of the core sequence of the CRE-like region (TGA) significantly decreased the expression of *CYP6CM1*. Values are means ± SEs (n=3). Data were analyzed using the Tukey-Kramer test (** $P < 0.01$).

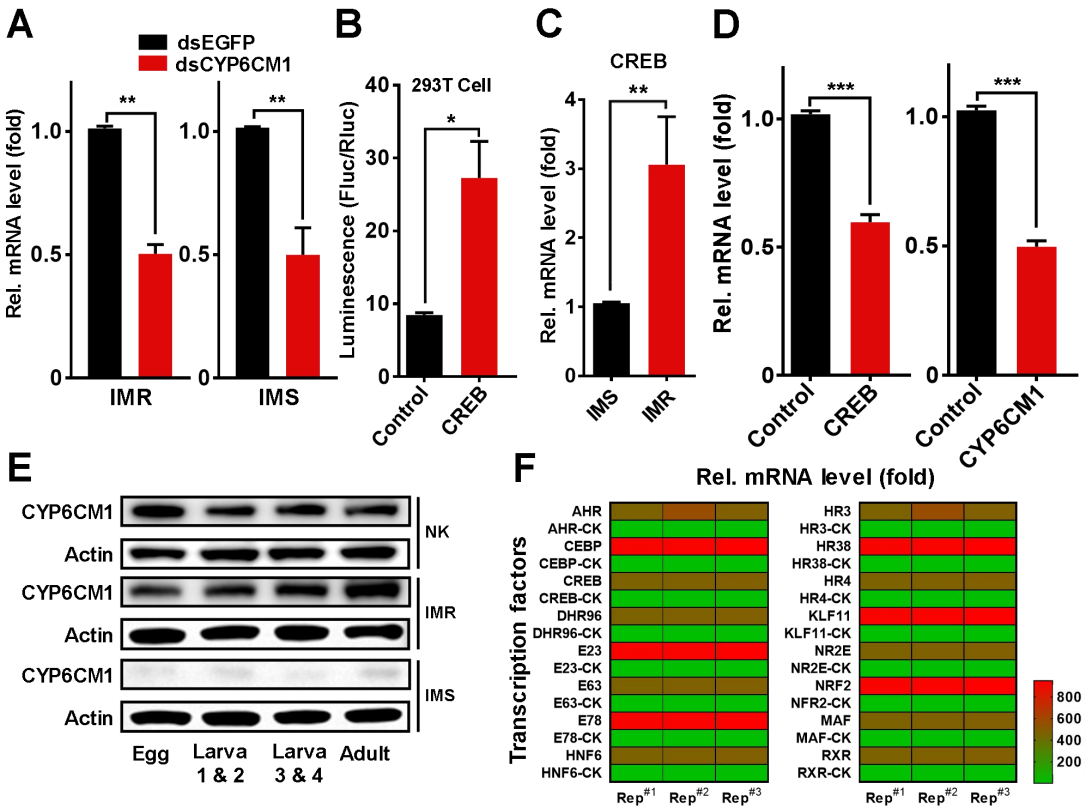

Fig. S3. (**A**) Efficiency of RNA knockdown of *CYP6CM1* as assessed by qRT-PCR. Relative mRNA abundance was measured in *B. tabaci* adults (IMR strain) that were fed ds*CYP6CM1*; adults that were fed ds*EGFP* served as the negative control. (**B**) Dual luciferase reporter assay, in which pGL4.10-*CYP6CM1*$^{-939\ \text{to}\ +1}$ and pGL4.73 plasmids were cotransfected into 293T cells with either the pcDNA3.1-CREB vector or the pcDNA3.1 empty vector (control). (**C**) Expression of *CREB* mRNA levels in the IMR and IMS strain as determined by qPCR. (**D**) The effect of feeding *CREB* dsRNA to *B. tabaci* for 48 h on the expression of *CREB* and *CYP6CM1*. mRNA levels were determined by qRT-PCR and are expressed as fold-change between control and treated whiteflies. Whiteflies fed on ds*EFGP* were used as a control. All values are means ± SEs (n=3). Data were analyzed using the Tukey-Kramer test (n = 3, mean ± SE; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two-tailed Student's t-test). (**E**) Levels of CYP6CM1 protein in different developmental stages of *B.tabaci* in the IMR and IMS strains, and a field resistance NK strain. Actin was used as a loading control. (**F**) The expression of 16 whitefly transcription factors after transfection into S2 cells in pAC5.1b as assessed by qPCR. Empty pAC5.1b was used as a control.

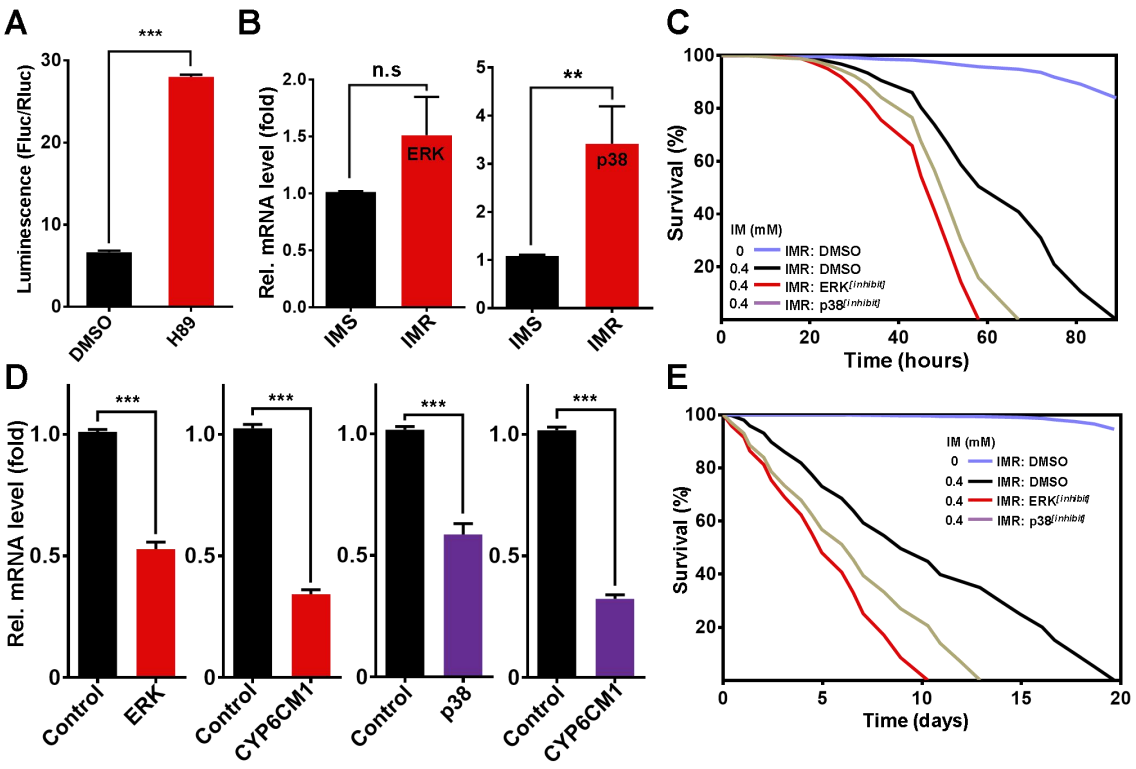

Fig. S4. (**A**) Dual luciferase reporter assays, in which pGL4.10-*CYP6CM1*$^{-939\ to\ +1}$ and pGL4.73 plasmids were cotransfected into S2 cells with either the PKA inhibitor H89 or DMSO alone as the control. Fluc/Rluc represents the ratio of firefly to Renilla luciferase activity. (**B**) Real-time qPCR analysis of the mRNA levels of *ERK* and *p38* in the imidacloprid resistant IMR strain and the susceptible strain IMS. mRNA levels are shown as fold-change between the susceptible and resistant strain. (**C**) The lifespan of adult whiteflies of the IMR strain after feeding on the inhibitors PD98059 (1.8 mM) and SB203580 (1.3 mM) for 24 hours on exposure to 0.4 mM imidacloprid. Adults fed on equivalent concentrations of DMSO were used as a negative control. (**D**) Expression of *CYP6CM1* mRNA after feeding adult whiteflies dsRNA of *ERK* (red) or *p38* (purple) for 48 h. For reference the expression of *ERK* and *p38* after dsRNA feeding is also shown. Adults fed on ds*EFGP* were used as a control. mRNA levels were determined by qRT-PCR. (**E**) Inhibition of *ERK* and *p38* enhances the control of *B. tabaci* (IMR adults) on tomato using imidacloprid. Lifespan of adult *B. tabaci* was significantly decreased when adults were exposed to 0.4 mM imidacloprid and to an *ERK* inhibitor (PD98059 at 1.8mM) or a *p38* inhibitor (SB203580 at 1.3 mM). For the control, adults were exposed to 0.4 mM imidacloprid without inhibitor. All values are means ± SEs (n=3). Data were analyzed using the Tukey-Kramer test (n = 3, mean ± SE; ** $P < 0.01$, *** $P < 0.001$, two-tailed Student's t-test).

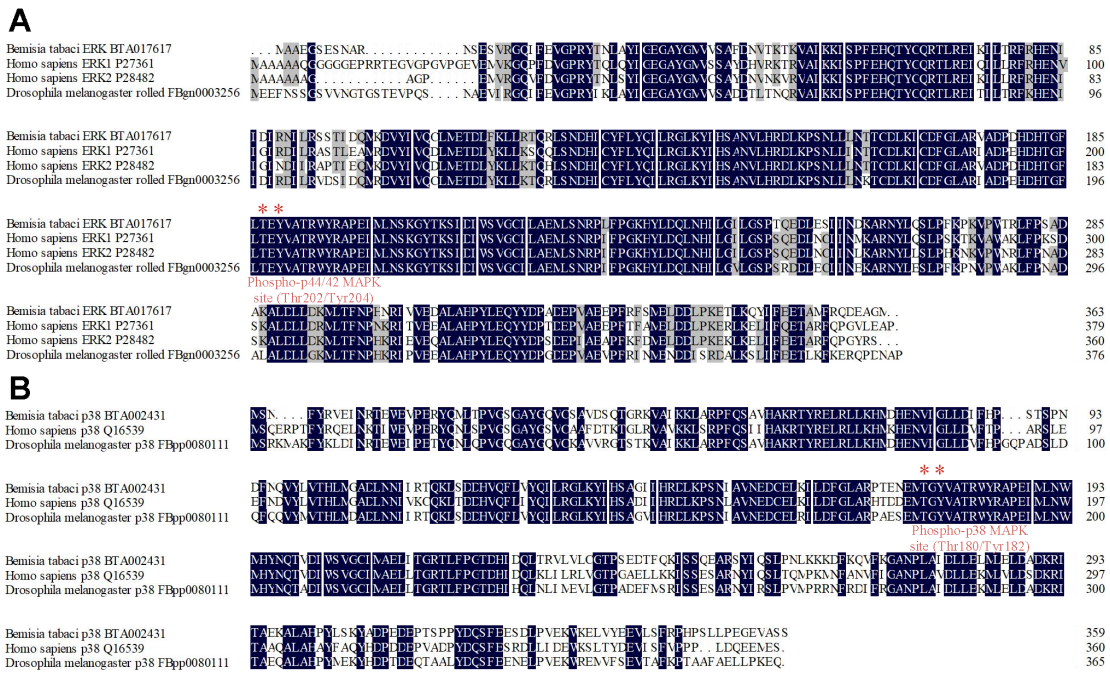

Fig. S5. Analysis of phosphorylation sites of the MAPKs *ERK* and *p38* in *B. tabaci* in comparison with the orthologs from *Homo sapiens* and *Drosophila melanogaster*. Asterisks indicate the phosphorylation sites of *Homo sapiens*.

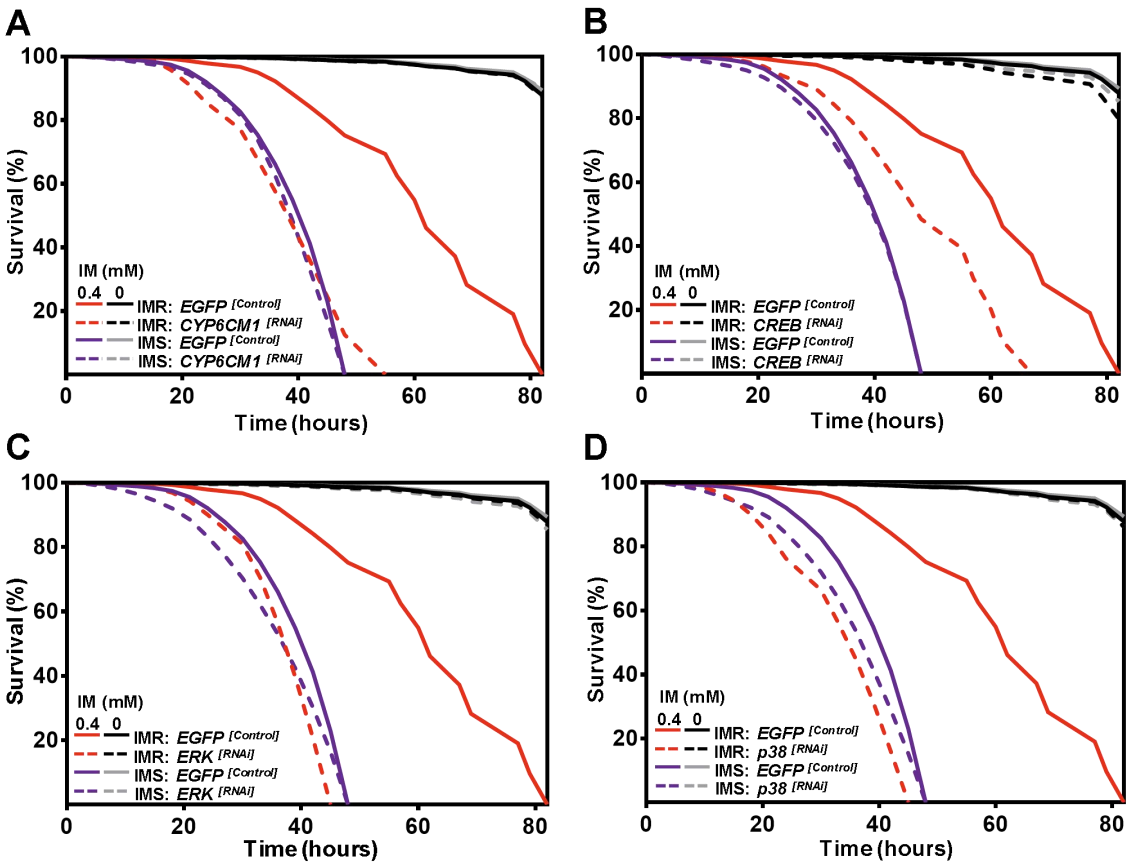

Fig. S6. Lifespan of IMR and IMS strains adults exposed to 0.4 mM imidacloprid (0.4) or no imidacloprid (0) after RNAi knockdown of *CYP6CM1* (**A**), *CREB* (**B**), *ERK* (**C**), and *p38* (**D**). Adults fed on ds*EFGP* were used as a negative control.

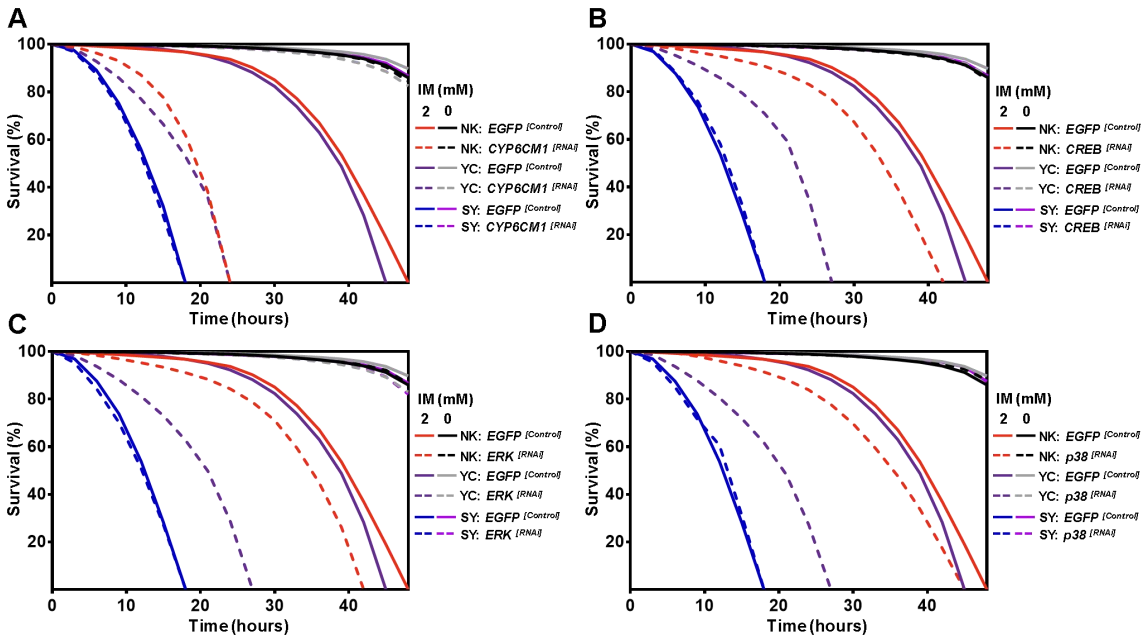

Fig. S7. Lifespan of three field resistance strains (NK, YC and SY) adults exposed to 2.0 mM imidacloprid (2) or no imidacloprid (0) after RNAi knockdown of *CYP6CM1* (**A**), *CREB* (**B**), *ERK* (**C**), and *p38* (**D**). Adults fed on ds*EFGP* were used as a negative control.

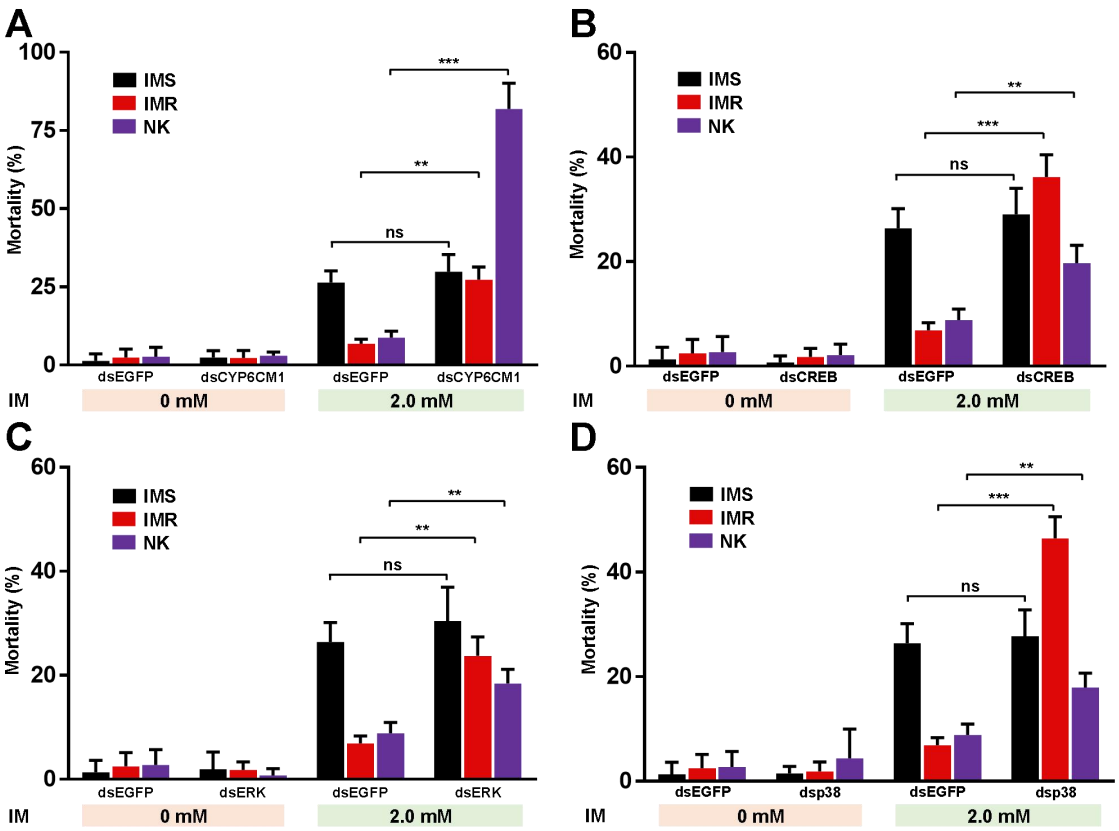

Fig. S8. Bioassays of one susceptible strain (IMS) and two resistance strains (IMR and NK) adults exposed to 2.0 mM imidacloprid (2.0) or no imidacloprid (0) for 24 hours after RNAi knockdown of *CYP6CM1* (**A**), *CREB* (**B**), *ERK* (**C**), and *p38* (**D**). Adults whitefly fed on ds*EFGP* were used as a negative control. (bioassays: n = 3, mean ± SE; ** $P < 0.05$,*** $P < 0.001$, two-tailed Student's t-test).

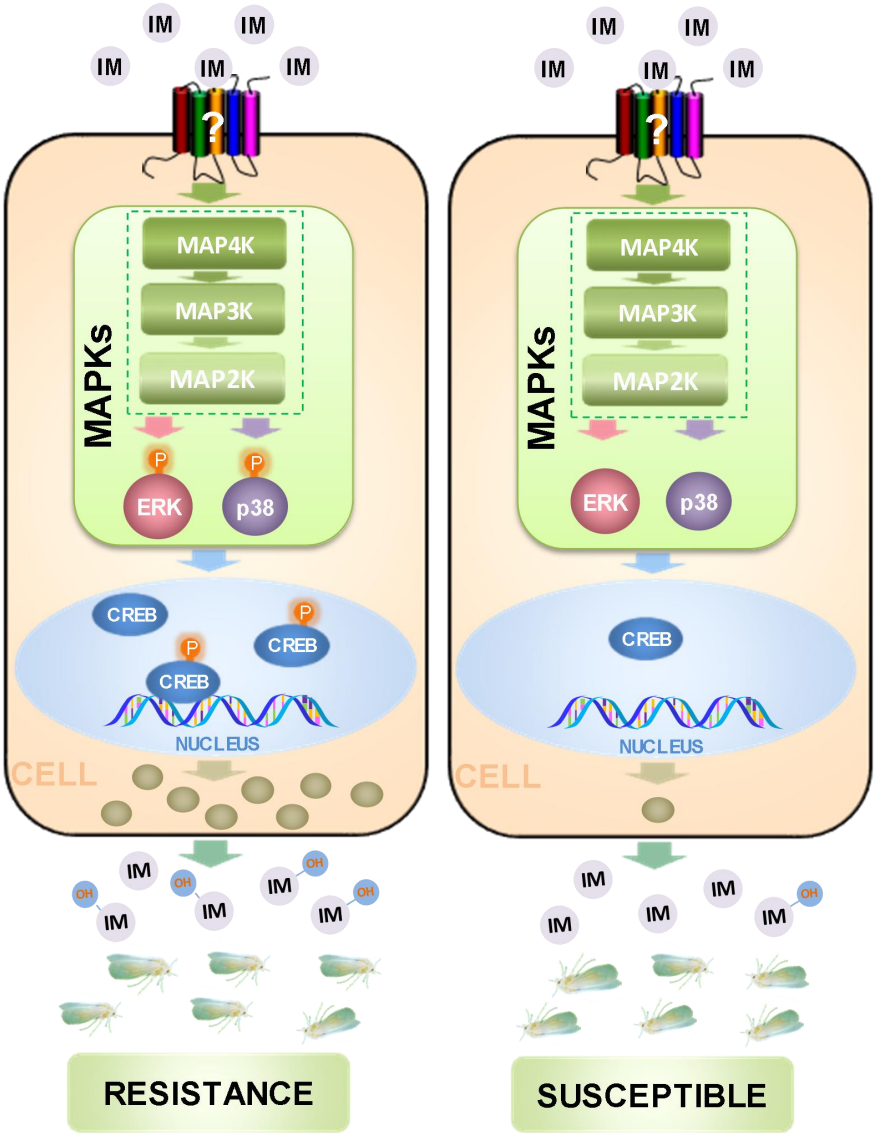

Fig. S9. A model of the regulation of *CYP6CM1* in *B. tabaci*. In this model, mitogen-activated protein kinases of the MAPK pathway phosphorylate ERK and p38. These in turn phosphorylate CREB, which then activates the expression of *CYP6CM1*. Activation and over-expression of *CREB* in the resistant strain increases the production of CYP6CM1 protein, which reduces the toxicity of imidacloprid.

Table S1. Origin of the *Bemisia tabaci* strains used in this study.

Population code	Population name	Sampling location	Sampling date	Host plant	*B. tabaci* composition
1	IMS/IMR	Hangzhou, Zhejiang (30°20′ N, 12°38′ E)	2011.10	Melon	MED/Q
2	NK	Nankou, Beijing (40°13′ N, 116°08′ E)	2018.09	Tomato	MED/Q
3	LF	Langfang, Hebei (39°36′ N, 116°35′ E)	2018.09	Pepper	MED/Q
4	YC	Yuncheng, Shanxi (35°26′ N, 110°58′ E)	2018.10	Cotton	MED/Q
5	SY	Shunyi, Beijin (40°11′ N, 116°43′ E)	2018.11	Tomato	MED/Q

Table S2: Log-dose probit-mortality data for *B. tabaci* strains in response to imidacloprid.

Strain	N[a]	Slope (±SE)	LC_{50} (mg L^{-1})	95% FL[b]	df[c]	χ^2	RR[d]
IMS	486	2.16 (± 0.18)	123	98-154	4	5.47	1
IMR	424	1.56 (± 0.19)	2790	638-3125	4	4.68	23
NK	639	1.72 (± 0.12)	1674	894-6869	3	7.81	14
YC	444	1.83 (± 0.24)	2146	1437-4731	4	5.36	17
LF	852	1.26 (± 0.10)	505	337-801	5	16.69	4
SY	353	1.17 (± 0.16)	35	7-71	3	3.86	0.3

[a]N = Number of *B.tabaci* used in each bioassay.

[b]df = Degrees of freedom.

[c]FL = Fiducial limit.

[d]RR (Resistance Ratio) = LC_{50} of the sample strains/LC_{50} of strain IMS.

Other Supplementary Material for this manuscript includes the following:

Dataset S1. Results and analysis of lifespan bioassays conducted on the IMR, IMS, SY, NK and YC strains.

Dataset S2. Upstream sequence analysis of *CYP6CM1* and *CREB* in the IMR and IMS strains.

Dataset S3. Information on the proteins chracterised in this study.

Dataset S4. Analysis of the sequences of eight nAChR α subunits and one β subunit in the IMR and IMS strains.

Dataset S5. Sequences of the oligonucleotide primers used in this study.

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A. Survival data for IMR, IMS, NK and YC strains: *CYP6CM1*$^{[RNAi]}$ (related to Fig. 1C; S.6A; S.7A)

Strain	Insecticide/RNAi Treatment	Sample size[a] (n Dead)	Tested time[b] (hours)	Median survival[c] (hours)	Percentage Decrease[d] (hours)	Chi square[e]	df[f]	p-value (log rank)[g] vs control
IMS Strain	**0.4 mM Imidacloprid**							
	Control (EGFP)	168 (168)	81	39				
	CYP6CM1 $^{[RNAi]}$	159 (159)	81	39	0	0.004205	1	0.94830
	2.0 mM Imidacloprid							
	Control (EGFP)	117 (117)	81	33				
	CYP6CM1 $^{[RNAi]}$	139 (139)	81	33	0	0.02655	1	0.87060
	0 mM Imidacloprid							
	Control (EGFP)	130 (16)	81	Undefined				
	CYP6CM1 $^{[RNAi]}$	111 (15)	81	Undefined	Undefined	1.955	1	0.162
IMR Strain	**0.4 mM Imidacloprid**							
	Control (EGFP)	156 (156)	81	62				
	CYP6CM1 $^{[RNAi]}$	114 (114)	81	39	37.1	1926	1	2.05×10^{-147}
	2.0 mM Imidacloprid							
	Control (EGFP)	189 (189)	81	42				
	CYP6CM1 $^{[RNAi]}$	153 (153)	81	33	21.4	878	1	1.44×10^{-52}
	0 mM Imidacloprid							
	Control (EGFP)	138 (17)	81	Undefined				
	CYP6CM1 $^{[RNAi]}$	125 (16)	81	Undefined	Undefined	0.9398	1	0.3323
NK Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	107 (107)	48	42				
	CYP6CM1 $^{[RNAi]}$	123 (123)	48	21	50	1321	1	3.96×10^{-289}
	0 mM Imidacloprid							
	Control (EGFP)	98 (12)	48	Undefined				
	CYP6CM1 $^{[RNAi]}$	108 (14)	48	Undefined	Undefined	0.8432	1	0.9269
YC Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	146 (146)	48	39				
	CYP6CM1 $^{[RNAi]}$	120 (120)	48	21	46.2	1713	1	1.70×10^{-291}
	0 mM Imidacloprid							
	Control (EGFP)	116 (16)	48	Undefined				
	CYP6CM1 $^{[RNAi]}$	101 (14)	48	Undefined	Undefined	0.253	1	0.615
SY Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	108 (108)	48	15				
	CYP6CM1 $^{[RNAi]}$	116 (116)	48	15	0	1.549	1	0.2133
	0 mM Imidacloprid							
	Control (EGFP)	106 (13)	48	Undefined				
	CYP6CM1 $^{[RNAi]}$	121 (15)	48	Undefined	Undefined	0.9041	1	0.34170

a	The total number of whiteflies tested for the treatment, with the number dead at the conclusion of the experiment shown in brackets
b	The total tested time for the treatment.
c	Median survial time in hours (time at which fractional survival equals 50%). Note this cannot be computed for no-imidacloprid controls as survival exceeded 50% at the conclusion of the experiment
d	The percentage decrease in median survival (hours) between the control and test group
e	Chi-square value reported by the logrank test
f	Degree of freedom
g	P value of logrank (Mantel-Cox) test of the survival curves of control versus treatment (null hypothesis is that treatment did not change survival)

B. Survival data for IMR, IMS, NK and YC strains: *CREB*$^{[RNAi]}$ (related to Fig. 2G; S.6B; S.7B)

Strain	Insecticide/RNAi Treatment	Sample size[a] (n Dead)	Tested time[b] (hours)	Median survival[c] (hours)	Percentage Decrease[d] (hours)	Chi square[e]	df[f]	p-value (log rank)[g] vs control
IMS Strain	**0.4 mM Imidacloprid**							
	Control (EGFP)	168 (168)	81	39				
	CREB $^{[RNAi]}$	150 (150)	81	42	-7.7	3.72	1	0.05370
	2.0 mM Imidacloprid							
	Control (EGFP)	117 (117)	81	33				
	CREB $^{[RNAi]}$	123 (123)	81	33	0	1.593	1	0.05200
	0 mM Imidacloprid							
	Control (EGFP)	130 (16)	81	Undefined				
	CREB $^{[RNAi]}$	122 (17)	81	Undefined	Undefined	0.833	1	0.3614
IMR Strain	**0.4 mM Imidacloprid**							
	Control (EGFP)	156 (156)	81	62				
	CREB $^{[RNAi]}$	144 (144)	81	48	22.6	835	1	2.37×10^{-52}
	2.0 mM Imidacloprid							
	Control (EGFP)	189 (189)	81	42				
	CREB $^{[RNAi]}$	220 (220)	81	33	21.4	1099	1	9.28×10^{-58}
	0 mM Imidacloprid							
	Control (EGFP)	128 (17)	81	Undefined				
	CREB $^{[RNAi]}$	112 (19)	81	Undefined	Undefined	0.1909	1	0.6621
NK Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	107 (107)	48	42				
	CREB $^{[RNAi]}$	110 (110)	48	36	14.3	298.3	1	7.63×10^{-67}
	0 mM Imidacloprid							
	Control (EGFP)	98 (12)	48	Undefined				
	CREB $^{[RNAi]}$	123 (16)	48	Undefined	Undefined	1.922	1	0.1657
YC Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	146 (146)	48	39				
	CREB $^{[RNAi]}$	99 (99)	48	24	38.5	1274	1	6.06×10^{-279}
	0 mM Imidacloprid							
	Control (EGFP)	116 (16)	48	Undefined				
	CREB $^{[RNAi]}$	103 (14)	48	Undefined	Undefined	0.3889	1	0.5329
SY Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	108 (108)	48	15				
	CREB $^{[RNAi]}$	97 (97)	48	15	0	1.996	1	0.1577
	0 mM Imidacloprid							
	Control (EGFP)	106 (13)	48	Undefined				
	CREB $^{[RNAi]}$	135 (17)	48	Undefined	Undefined	1.04	1	0.30780

a	The total number of whiteflies tested for the treatment, with the number dead at the conclusion of the experiment shown in brackets
b	The total tested time for the treatment.
c	Median survial time in hours (time at which fractional survival equals 50%). Note this cannot be computed for no-imidacloprid controls as survival exceeded 50% at the conclusion of the experir
d	The percentage decrease in median survival (hours) between the control and test group
e	Chi-square value reported by the logrank test
f	Degree of freedom
g	P value of logrank (Mantel-Cox) test of the survival curves of control versus treatment (null hypothesis is that treatment did not change survival)

C. Survival data for IMR, IMS, NK and YC strains: ERK $^{[RNAi]}$ (related to Fig. 2G; S.6C; S.7C)

Strain	Insecticide/RNAi Treatment	Sample size[a] (n Dead)	Tested time[b] (hours)	Median survival[c] (hours)	Percentage Decrease[d] (hours)	Chi square[e]	df[f]	p-value (log rank)[g] vs control
IMS Strain	**0.4 mM Imidacloprid**							
	Control (EGFP)	168 (168)	81	39				
	ERK [RNAi]	169 (169)	81	39	0	2.83	1	0.06170
	2.0 mM Imidacloprid							
	Control (EGFP)	117 (117)	81	33				
	ERK [RNAi]	142 (142)	81	33	0	2.65	1	0.07560
	0 mM Imidacloprid							
	Control (EGFP)	130 (16)	81	Undefined				
	ERK $^{[RNAi]}$	120 (15)	81	Undefined	Undefined	0.4805	1	0.4881
IMR Strain	**0.4 mM Imidacloprid**							
	Control (EGFP)	156 (156)	81	62				
	ERK $^{[RNAi]}$	126 (126)	81	39	37.1	1926	1	1.04×10^{-133}
	2.0 mM Imidacloprid							
	Control (EGFP)	189 (189)	81	42				
	ERK $^{[RNAi]}$	162 (162)	81	36	14.3	2677	1	9.22×10^{-31}
	0 mM Imidacloprid							
	Control (EGFP)	138 (17)	81	Undefined				
	ERK $^{[RNAi]}$	118 (15)	81	Undefined	Undefined	1.97	1	0.1605
NK Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	107 (107)	48	42				
	ERK $^{[RNAi]}$	139 (139)	48	36	14.3	240.3	1	3.41×10^{-54}
	0 mM Imidacloprid							
	Control (EGFP)	98 (12)	48	Undefined				
	ERK $^{[RNAi]}$	130 (19)	48	Undefined	Undefined	0.1756	1	0.6751
YC Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	147 (147)	48	39				
	ERK $^{[RNAi]}$	86 (86)	48	21	46.2	1463	1	5.65×10^{-249}
	0 mM Imidacloprid							
	Control (EGFP)	116 (16)	48	Undefined				
	ERK $^{[RNAi]}$	123 (17)	48	Undefined	Undefined	0.021	1	0.8848
SY Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	108 (108)	48	15				
	ERK $^{[RNAi]}$	116 (116)	48	15	0	1.243	1	0.123
	0 mM Imidacloprid							
	Control (EGFP)	106 (13)	48	Undefined				
	ERK $^{[RNAi]}$	121 (16)	48	Undefined	Undefined	0.2497	1	0.61730

a	The total number of whiteflies tested for the treatment, with the number dead at the conclusion of the experiment shown in brackets
b	The total tested time for the treatment.
c	Median survial time in hours (time at which fractional survival equals 50%). Note this cannot be computed for no-imidacloprid controls as survival exceeded 50% at the conclusion of the ex
d	The percentage decrease in median survival (hours) between the control and test group
e	Chi-square value reported by the logrank test
f	Degree of freedom
g	P value of logrank (Mantel-Cox) test of the survival curves of control versus treatment (null hypothesis is that treatment did not change survival)

D. Survival data for IMR, IMS, NK and YC strains: p38$^{[RNAi]}$ (related to Fig. 2G; S.6D; S.7D)

Strain	Insecticide/RNAi Treatment	Sample size[a] (n Dead)	Tested time[b] (hours)	Median survival[c] (hours)	Percentage Decrease[d] (hours)	Chi square[e]	df[f]	p-value (log rank)[g] vs control
IMS Strain	**0.4 mM Imidacloprid**							
	Control (EGFP)	168 (168)	81	39				
	p38 [RNAi]	168 (168)	81	39	0	2.28	1	0.05610
	2.0 mM Imidacloprid							
	Control (EGFP)	117 (117)	81	33				
	p38 [RNAi]	132 (132)	81	33	0	1.593	1	0.06160
	0 mM Imidacloprid							
	Control (EGFP)	130 (16)	81	Undefined				
	p38 [RNAi]	119 (15)	81	Undefined	Undefined	0.6104	1	0.4346
IMR Strain	**0.4 mM Imidacloprid**							
	Control (EGFP)	156 (156)	81	62				
	p38 [RNAi]	156 (156)	81	33	46.8	857	1	2.05×10^{-147}
	2.0 mM Imidacloprid							
	Control (EGFP)	189 (189)	81	42				
	p38 [RNAi]	147 (147)	81	33	21.4	1609	1	1.44×10^{-52}
	0 mM Imidacloprid							
	Control (EGFP)	138 (17)	81	Undefined				
	p38 [RNAi]	134 (16)	81	Undefined	Undefined	0.1387	1	0.7096
NK Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	107 (107)	48	42				
	p38 [RNAi]	139 (139)	48	36	14.3	160.3	1	9.76×10^{-37}
	0 mM Imidacloprid							
	Control (EGFP)	98 (12)	48	Undefined				
	p38 [RNAi]	106 (13)	48	Undefined	Undefined	0.0931	1	0.7603
YC Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	147 (147)	48	39				
	p38 [RNAi]	115 (115)	48	21	46.2	1648	1	7.21×10^{-274}
	0 mM Imidacloprid							
	Control (EGFP)	116 (16)	48	Undefined				
	p38 [RNAi]	125 (15)	48	Undefined	Undefined	0.5304	1	0.4665
SY Strain	**2.0 mM Imidacloprid**							
	Control (EGFP)	108 (108)	48	15				
	p38 [RNAi]	123 (123)	48	15	0	0.9062	1	0.3411
	0 mM Imidacloprid							
	Control (EGFP)	106 (13)	48	Undefined				
	p38 [RNAi]	98 (11)	48	Undefined	Undefined	0.0193	1	0.88950

a	The total number of whiteflies tested for the treatment, with the number dead at the conclusion of the experiment shown in brackets
b	The total tested time for the treatment.
c	Median survial time in hours (time at which fractional survival equals 50%). Note this cannot be computed for no-imidacloprid controls as survival exceeded 50% at the conclusion of the experim
d	The percentage decrease in median survival (hours) between the control and test group
e	Chi-square value reported by the logrank test
f	Degree of freedom
g	P value of logrank (Mantel-Cox) test of the survival curves of control versus treatment (null hypothesis is that treatment did not change survival)

E. Survival data for IMR: *ERK/p38 [inhibit]* on *B.tabaci* (related to Fig. S.4C)

Strain[a]	Insecticide/RNAi Treatment	Sample size[a] (n Dead)	Median survival[b] (hours)	Percentage Decrease[c] (hours)	Chi square[d]	df[e]	p-value (log rank)[f] vs control
IMR Strain	**0.4 mM Imidacloprid**						
	Control (DMSO)	136 (136)	67				
	ERK [PD98059]	144 (144)	48	28.4	836	1	5.84×10^{-91}
	0 mM Imidacloprid						
	Control (DMSO)	120 (7)	Undefined				
	ERK [PD98059]	112 (7)	Undefined	Undefined	1.714	1	0.1904
	0.4 mM Imidacloprid						
	Control (DMSO)	136 (136)	67				
	p38 [SB203580]	116 (116)	51	23.9	409	1	2.27×10^{-45}
	0 mM Imidacloprid						
	Control (DMSO)	120 (7)	Undefined				
	p38 [SB203580]	125 (8)	Undefined	Undefined	0.7982	1	0.3716

E. Survival data for IMR > *ERK/p38 [inhibit]* on plant (related to Figure S.4E)

Strains[a]	Insecticide/RNAi Treatment	Sample size[a] (n Dead)	Median survival[b] (days)	Percentage Decrease[c] (days)	Chi square[d]	df[e]	p-value (log rank)[f] vs control
IMR Strain	**2.0 mM Imidacloprid**						
	Control (DMSO)	100 (100)	8.92				
	ERK [PD98059]	100 (100)	4.96	44.4	849	1	8.65×10^{-184}
	0 mM Imidacloprid						
	Control (DMSO)	100 (5)	Undefined				
	ERK [PD98059]	100 (7)	Undefined	Undefined	0.4234	1	0.5152
	2.0 mM Imidacloprid						
	Control (DMSO)	100 (100)	8.92				
	p38 [SB203580]	100 (100)	6.54	26.7	562	1	7.46×10^{-91}
	0 mM Imidacloprid						
	Control (DMSO)	100 (5)	Undefined				
	p38 [SB203580]	100 (8)	Undefined	Undefined	3.023	1	0.0821

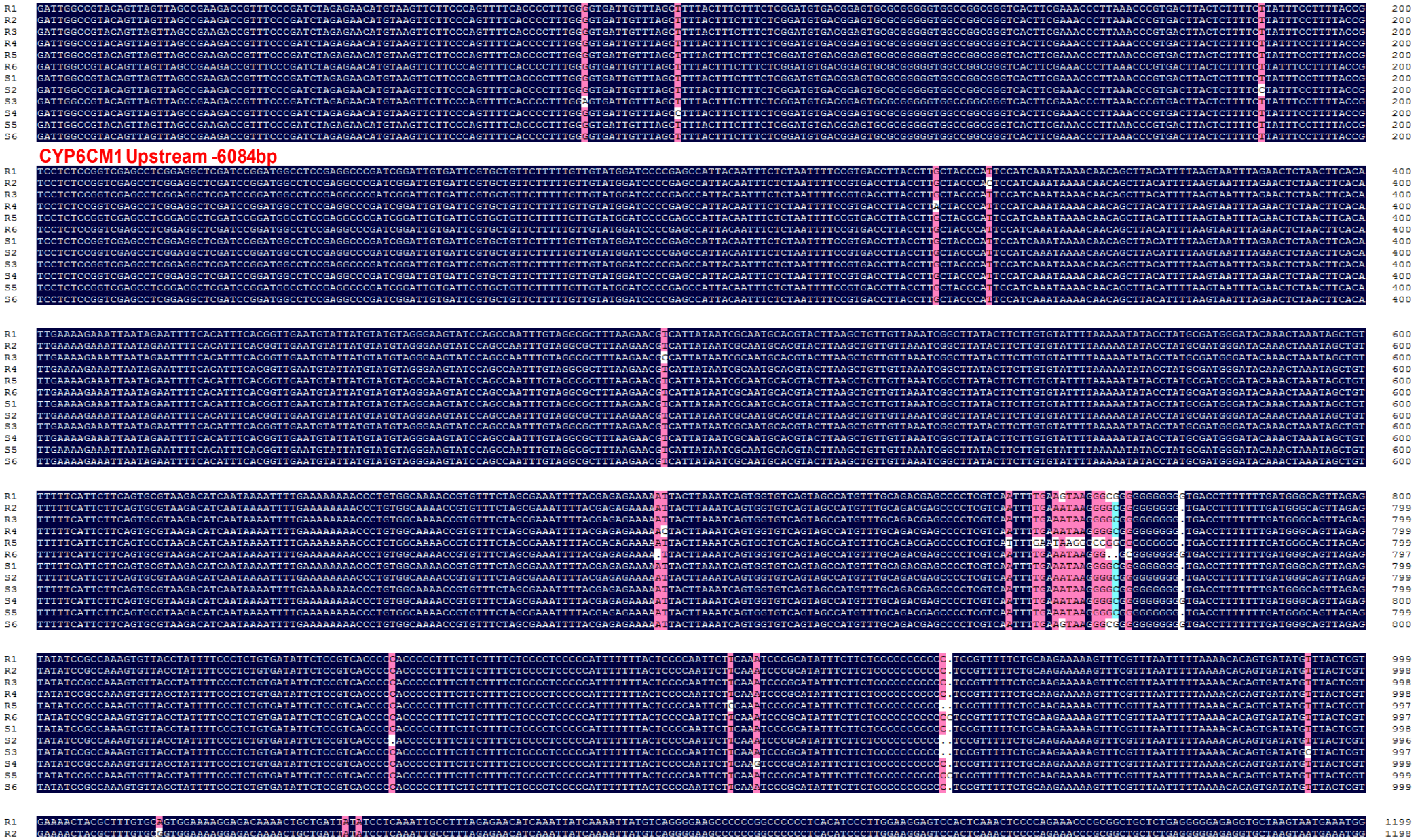
a	The total number of whiteflies tested for the treatment, with the number dead at the conclusion of the experiment shown in brackets
b	Median survial time in hours (time at which fractional survival equals 50%). Note this cannot be computed for no-imidacloprid controls as survival exceeded 50% at the conclusion of t
c	The percentage decrease in median survival (hours) between the control and test group
d	Chi-square value reported by the logrank test
e	Degree of freedom
f	P value of logrank (Mantel-Cox) test of the survival curves of control versus treatment (null hypothesis is that treatment did not change survival)

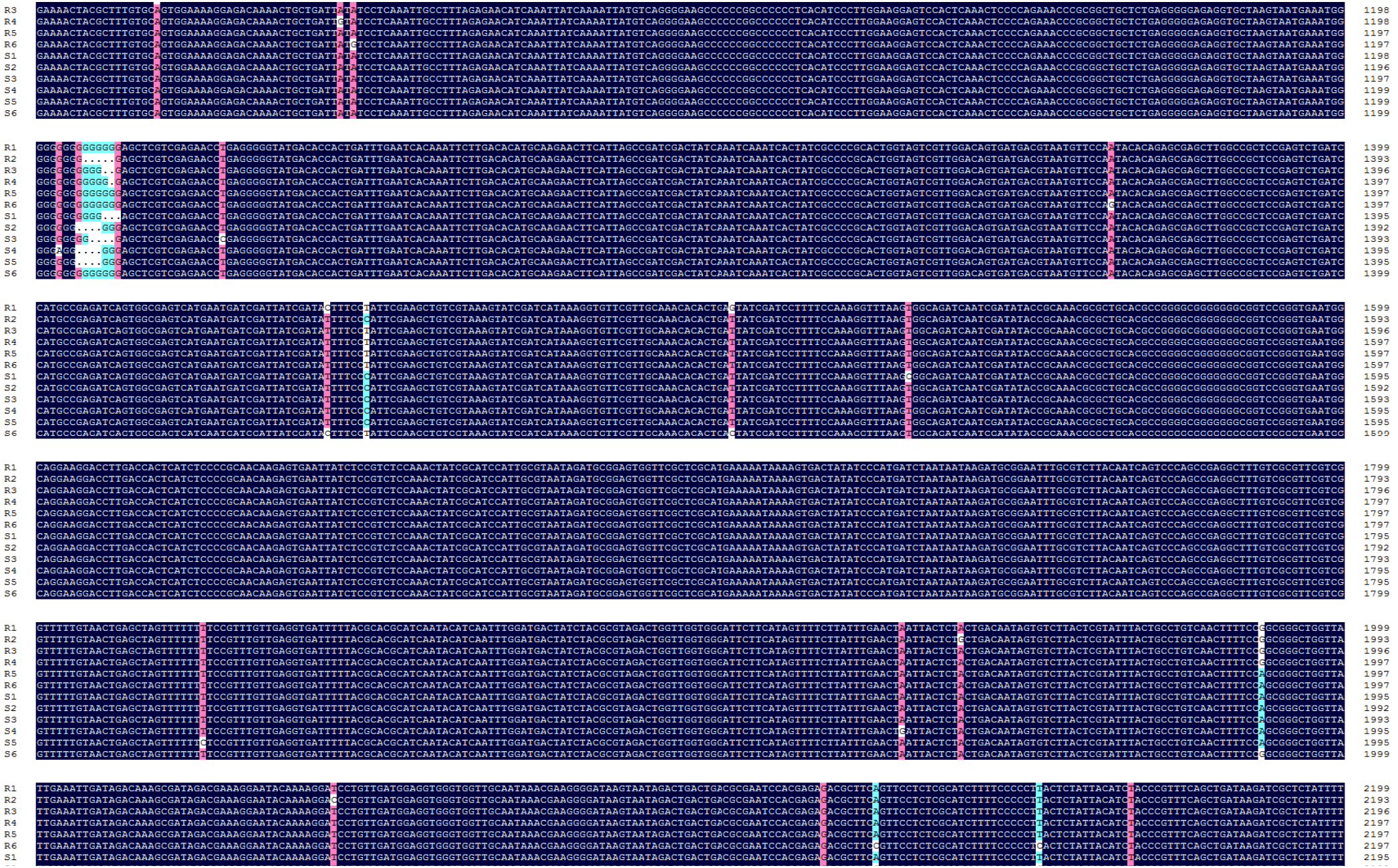
F. Survival data for no-imidacloprid controls (comparison of data dervied from the IMR, IMS, YC, NK and SY strains).

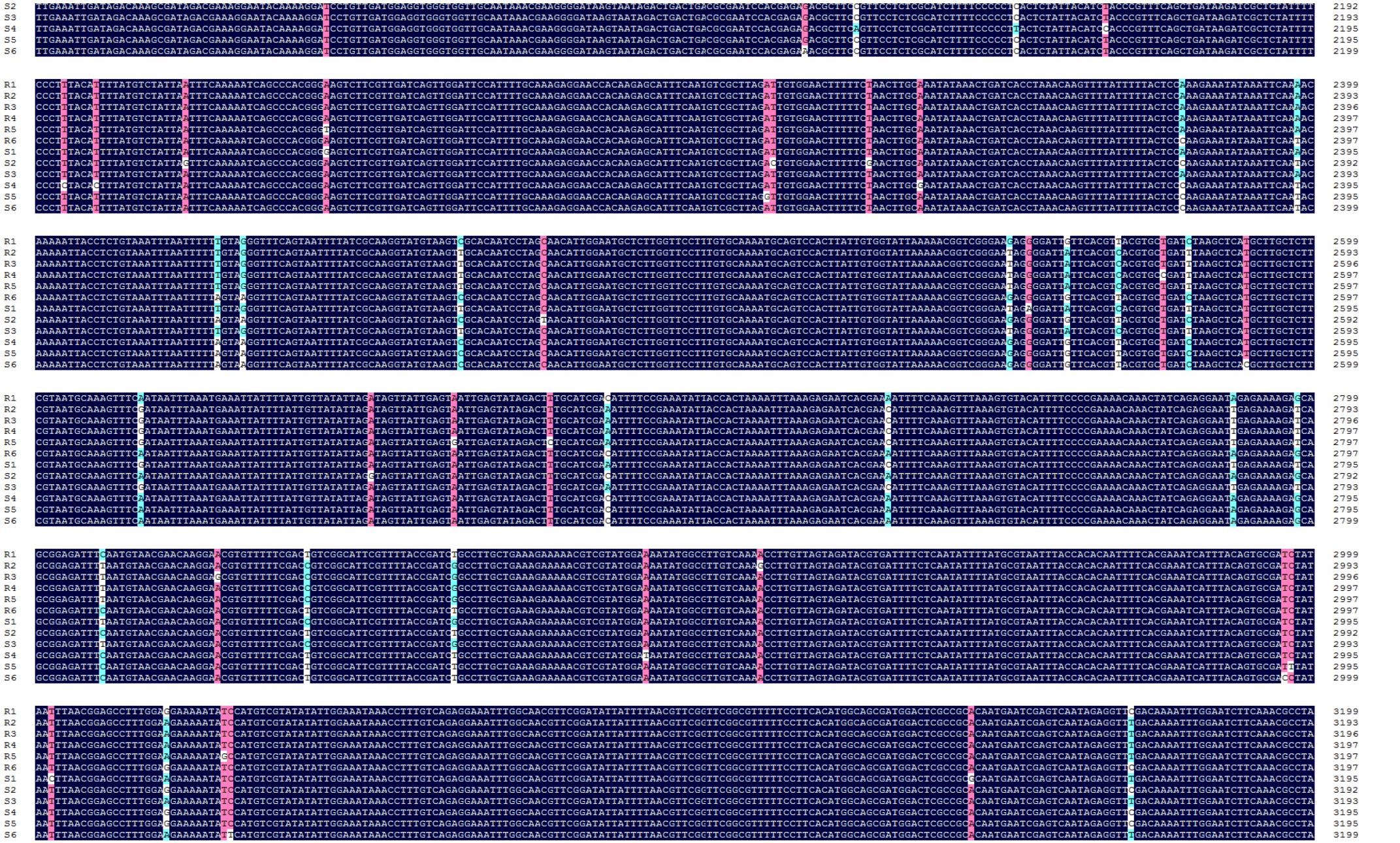
Strain	RNAi Treatment	Sample size[a] (n Dead)	Tested time[b] (hours)	Median survival[c] (hours)	Percentage Decrease[d] (hours)	Chi square[e]	df[f]	p-value (log rank)[g] vs IMS control
IMS	Control (EGFP)	130 (16)	81	Undefined				
IMR	Control (EGFP)	138 (17)	81	Undefined	Undefined	1.234	1	0.2666
NK	Control (EGFP)	98 (12)	48	Undefined	Undefined	53.2	1	1.03×10^{-28}
YC	Control (EGFP)	116 (16)	48	Undefined	Undefined	145.9	1	1.38×10^{-33}
SY	Control (EGFP)	106 (13)	48	Undefined	Undefined	156.4	1	7.04×10^{-36}
IMS	*CYP6CM1* [RNAi]	111 (15)	81	Undefined				
IMR	*CYP6CM1* [RNAi]	125 (16)	81	Undefined	Undefined	2.281	1	0.131
NK	*CYP6CM1* [RNAi]	108 (14)	48	Undefined	Undefined	103.4	1	2.74×10^{-24}
YC	*CYP6CM1* [RNAi]	101 (14)	48	Undefined	Undefined	125.8	1	3.36×10^{-29}
SY	*CYP6CM1* [RNAi]	121 (15)	48	Undefined	Undefined	125.4	1	1.09×10^{-24}
IMS	*CREB* [RNAi]	122 (17)	81	Undefined				
IMR	*CREB* [RNAi]	112 (19)	81	Undefined	Undefined	0.0573	1	0.8108
NK	*CREB* [RNAi]	123 (16)	48	Undefined	Undefined	168.4	1	1.64×10^{-38}
YC	*CREB* [RNAi]	103 (14)	48	Undefined	Undefined	137.1	1	1.23×10^{-31}
SY	*CREB* [RNAi]	135 (17)	48	Undefined	Undefined	122	1	2.29×10^{-28}
IMS	*ERK* [RNAi]	120 (15)	81	Undefined				
IMR	*ERK* [RNAi]	118 (15)	81	Undefined	Undefined	0.1374	1	0.7109
NK	*ERK* [RNAi]	130 (19)	48	Undefined	Undefined	150.5	1	1.33×10^{-34}
YC	*ERK* [RNAi]	123 (17)	48	Undefined	Undefined	137.4	1	9.71×10^{-32}
SY	*ERK* [RNAi]	121 (16)	48	Undefined	Undefined	130.3	1	3.44×10^{-30}
IMS	*p38* [RNAi]	119 (15)	81	Undefined				
IMR	*p38* [RNAi]	134 (16)	81	Undefined	Undefined	2.275	1	0.1315
NK	*p38* [RNAi]	106 (13)	48	Undefined	Undefined	96.94	1	7.12×10^{-23}
YC	*p38* [RNAi]	125 (15)	48	Undefined	Undefined	98.99	1	2.53×10^{-23}
SY	*p38* [RNAi]	98 (11)	48	Undefined	Undefined	114.5	1	1.01×10^{-26}

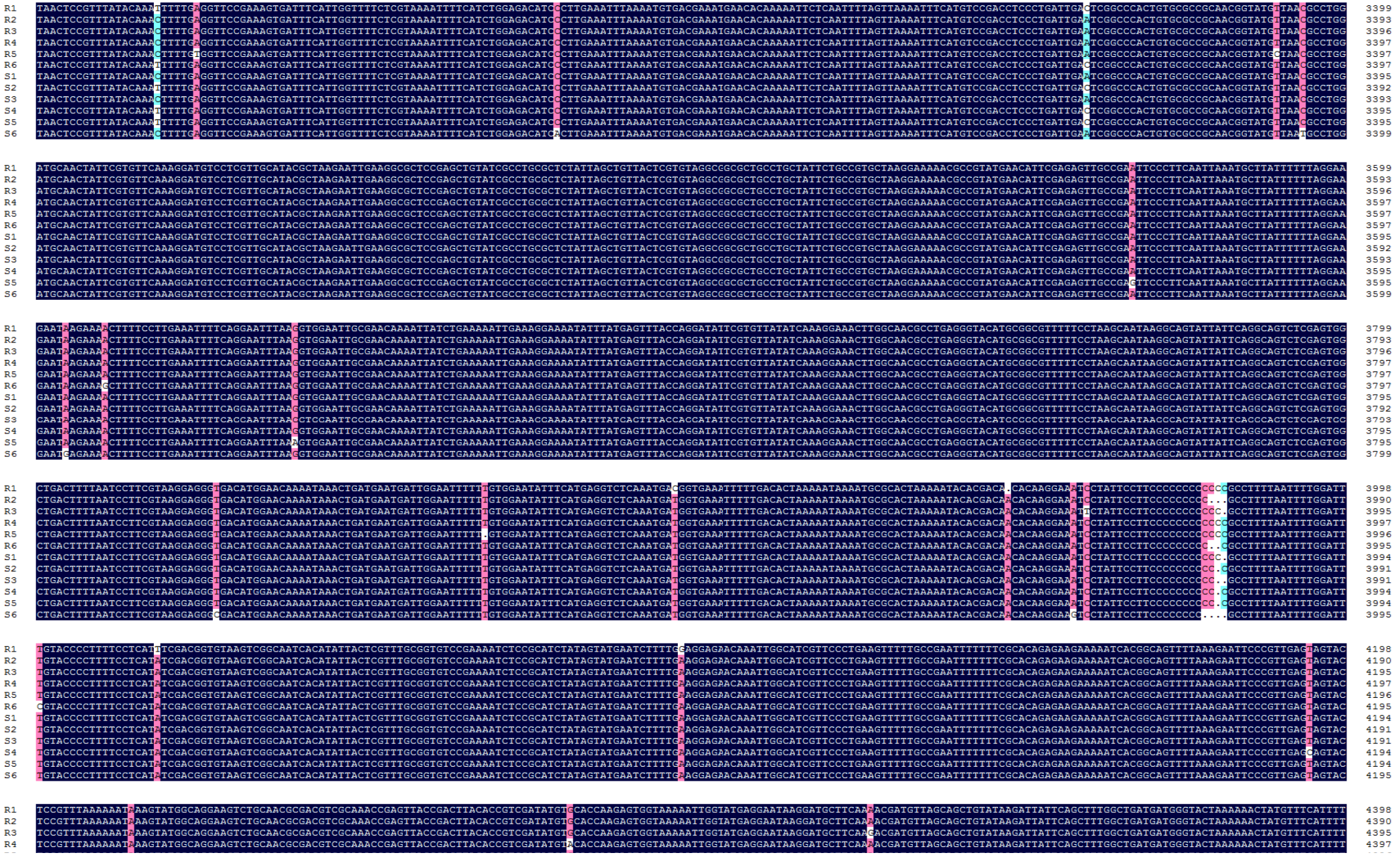
a	The total number of whiteflies tested for the treatment, with the number dead at the conclusion of the experiment shown in brackets
b	The total tested time for the treatment.
c	Median survial time in hours (time at which fractional survival equals 50%). Note this cannot be computed for no-imidacloprid controls as survival exceeded 50% at the conclusion of the ex
d	The percentage decrease in median survival (hours) between the control and test group
e	Chi-square value reported by the logrank test
f	Degree of freedom
g	P value of logrank (Mantel-Cox) test of the survival curves of each strain compared to the IMS strain (null hypothesis is there is no significant difference in survival)

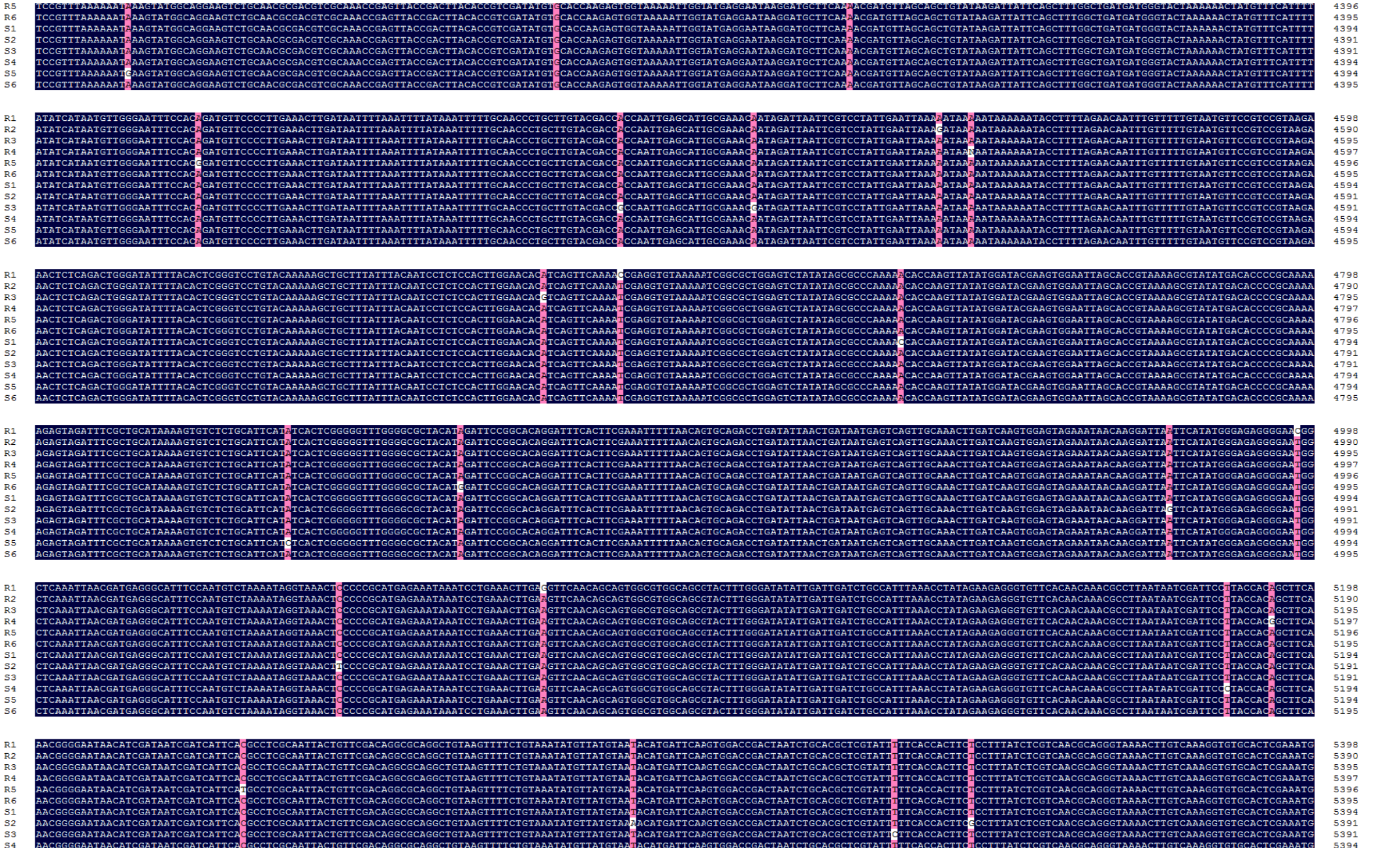
Analysis the upstream seuquences of CYP6CM1

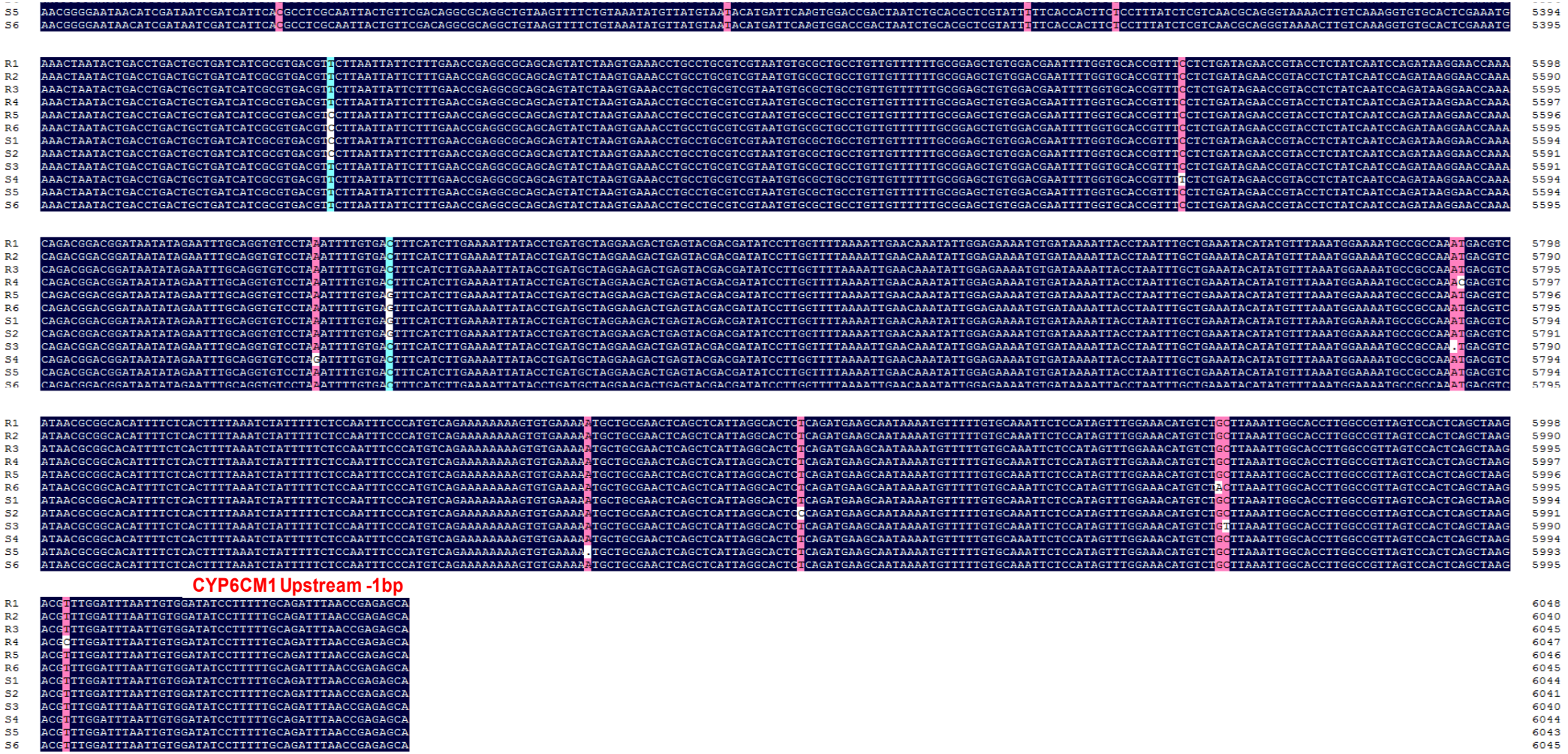




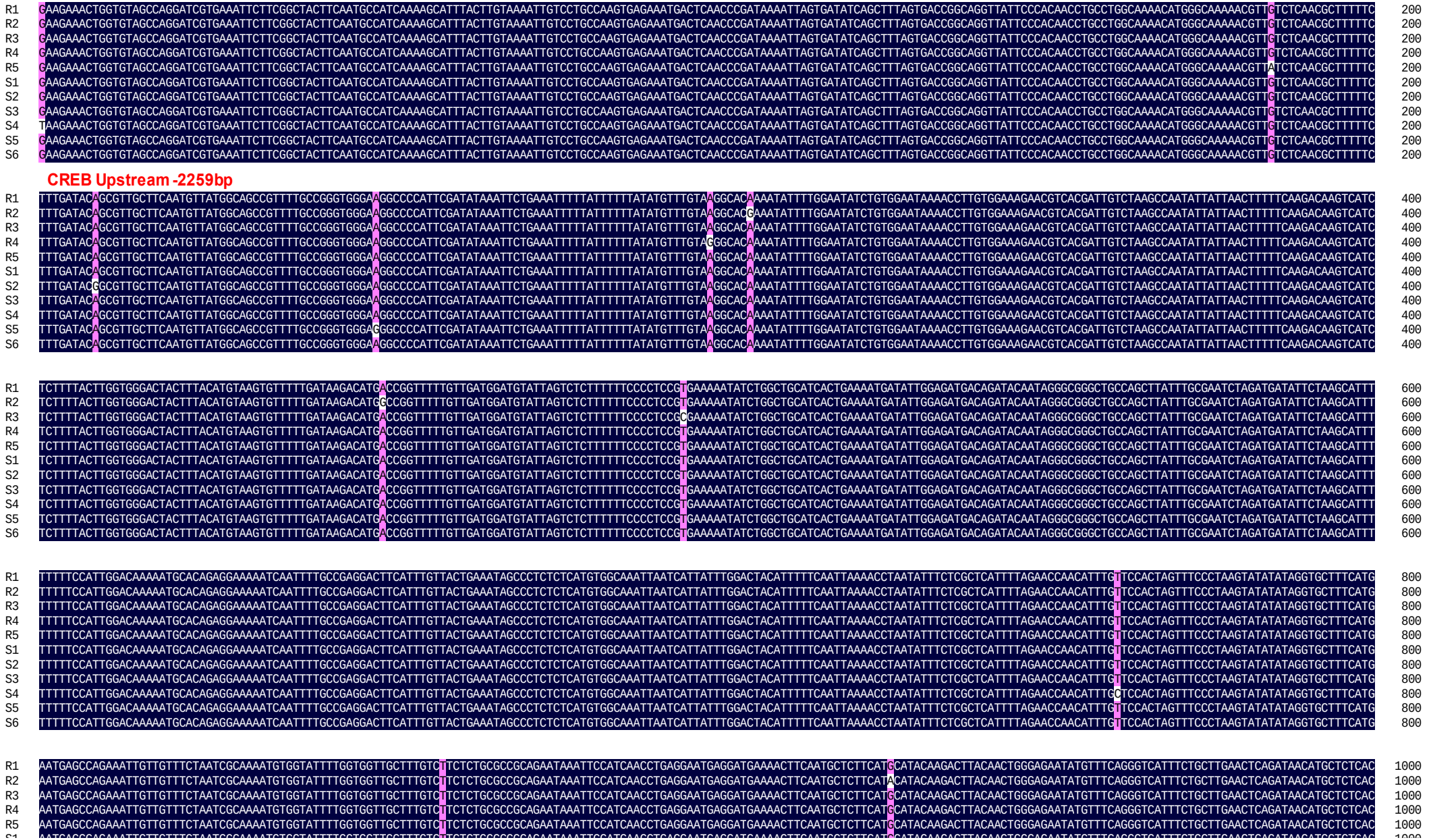


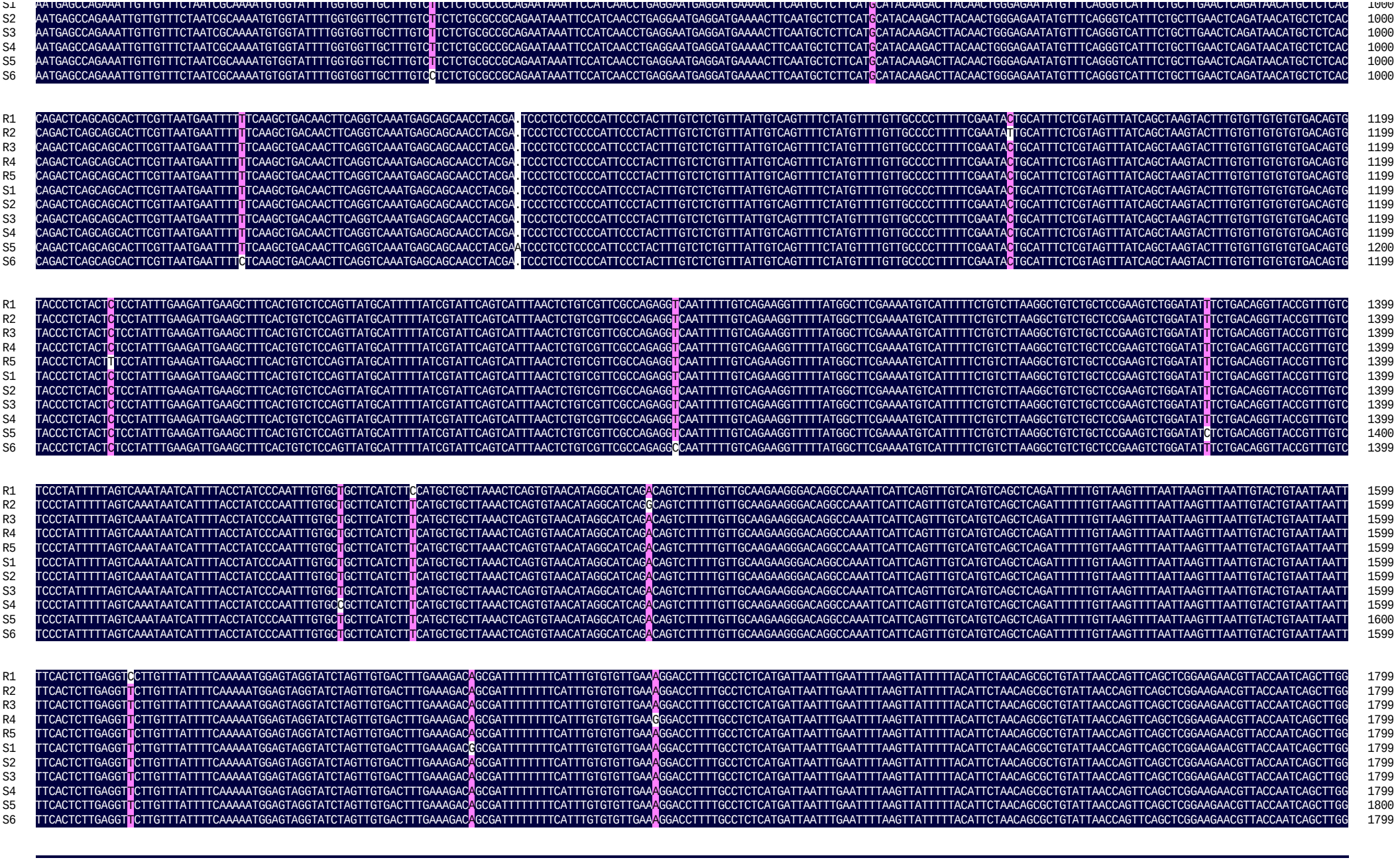


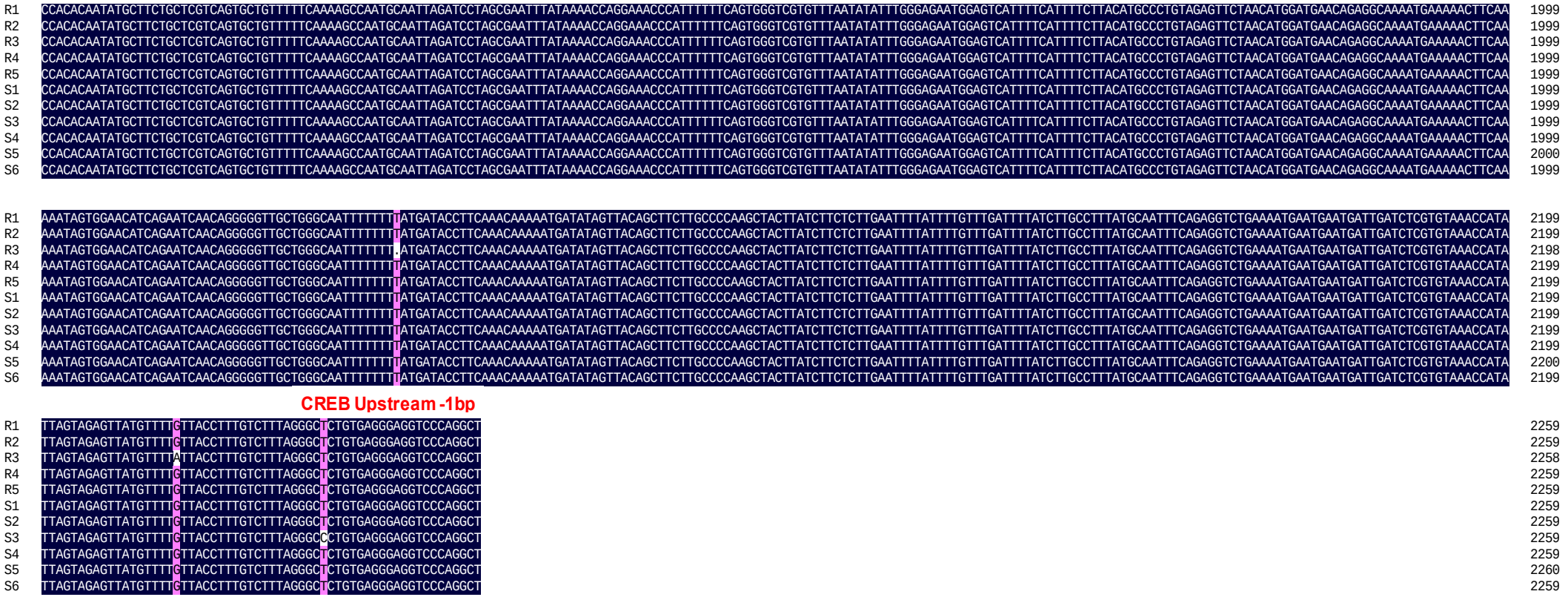



CYP6CM1 Upstream -1bp

Analysis the upstream seuquences of CREB



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R2 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 1999
R3 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 1999
R4 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 1999
R5 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 1999
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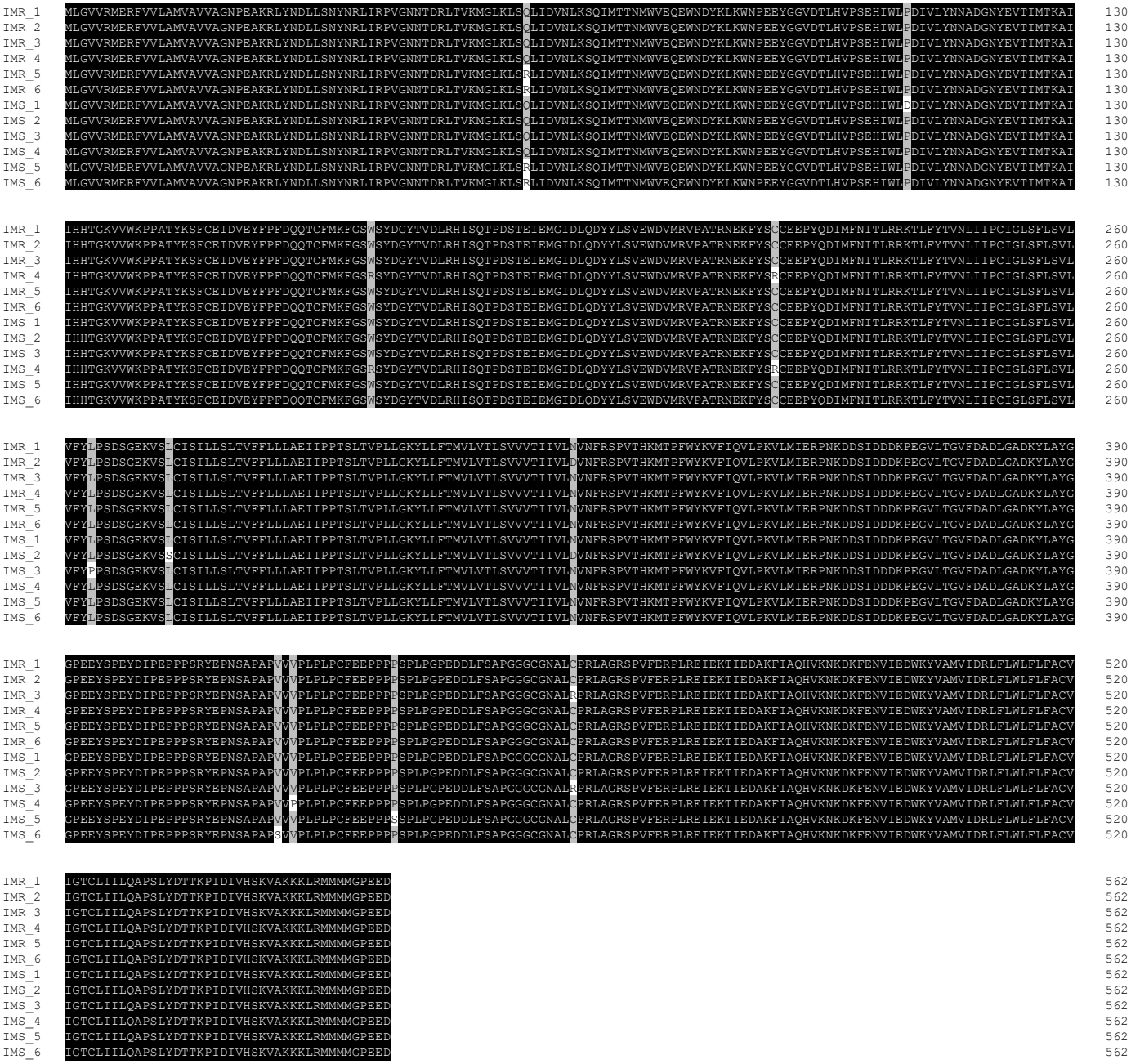
S2 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 1999
S3 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 1999
S4 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 1999
S5 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 2000
S6 CCACACAATATGCTTCTGCTCGTCAGTGCTGTTTTTCAAAAGCCAATGCAATTAGATCCTAGCGAATTTATAAAACCAGGAAACCCATTTTTTCAGTGGGTCGTGTTTAATATATTTGGGAGAATGGAGTCATTTTCATTTTCTTACATGCCCTGTAGAGTTCTAACATGGATGAACAGAGGCAAAATGAAAAACTTCAA 1999
R1 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
R2 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
R3 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTT.ATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2198
R4 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
R5 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
S1 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
S2 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
S3 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
S4 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
S5 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2200
S6 AAATAGTGGAACATCAGAATCAACAGGGGGTTGCTGGGCAATTTTTTTTTATGATACCTTCAAACAAAAATGATATAGTTACAGCTTCTTGCCCCAAGCTACTTATCTTCTCTTGAATTTTATTTTGTTTGATTTTATCTTGCCTTTATGCAATTTCAGAGGTCTGAAAATGAATGAATGATTGATCTCGTGTAAACCATA 2199
CREB Upstream -1bp
R1 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2259
R2 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2259
R3 TTAGTAGAGTTATGTTTTATTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2258
R4 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2259
R5 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2259
S1 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2259
S2 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2259
S3 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCCCTGTGAGGGAGGTCCCAGGCT 2259
S4 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2259
S5 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2260
S6 TTAGTAGAGTTATGTTTTGTTACCTTTGTCTTTAGGGCTCTGTGAGGGAGGTCCCAGGCT 2259

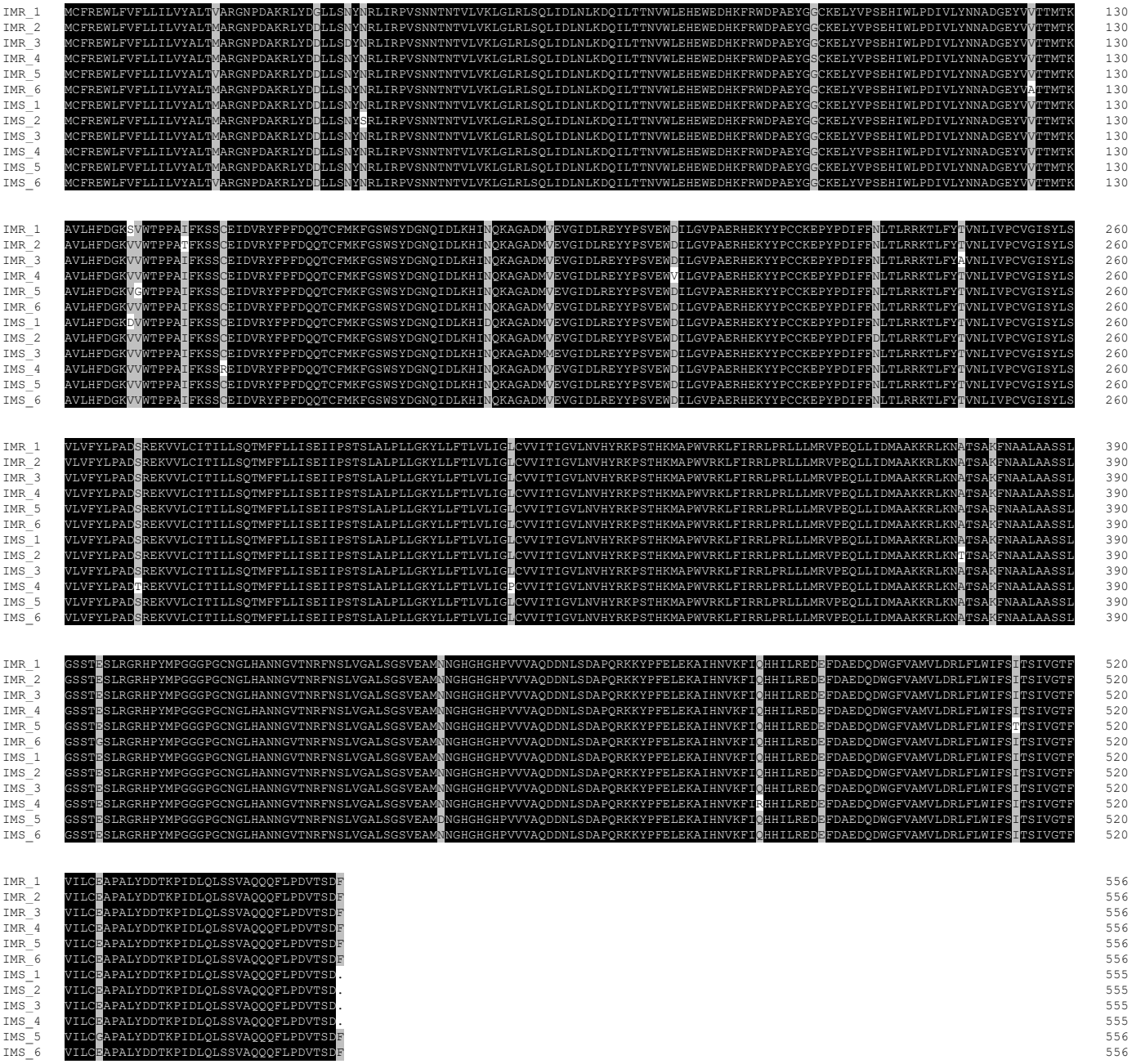
Name	MEAM1 Genome ID	Sequences	Length (Aa)	MW (kDa)	pI	References	
CREB	Bta05712	MDGMVEENGTGSGDPLSSSPSANTTNVATSVQSVIQANQQSVIQTATGNIQPAVLTKGNVILVSKPNSVIQTTQGSLQTLQVVVEANSDD SLSAEDDSTRKRRDILTRRPSYRKILNDLGGAEIAGCKVESSNSDCDSNLDSELSSHSLPTHYPTVIPAGSLQLCSQGEGAQGIQSITMTNAS SGGTIVQYAGQDGQFFVPGEIVVTQGSTLPGVPLMAEDQARKRELRLLKNREAARECRRKKKEYIKCLENRVAVLENQNKALIDELKSL KELYCQQKTD-	282	30.29	5.4	1. Doi J, et al. (2002) Salt-inducible kinase represses cAMP-dependent protein kinase-mediated activation of human cholesterol side chain cleavage cytochrome P450 promoter through the CREB basic leucine zipper domain. J Biol Chem 277(18):15629–15637. 2. Bani MH, Tohkin M, Ushio F, Fukuhara M (1998) Evidence for involvement of cAMP-dependent pathway in the phenobarbital-induced expression of a novel hamster cytochrome P450, CYP3A31. Arch Biochem Biophys 356(2):100–106.	2
AhR	Bta13125	MSALAHSVLGINPSNAVQKRHASSLGSDEEDPSGSKYGRMEESNDLQDKERFASRENHCEIERRRRNKMTAYITELSDMVPTCSALARKP DKLTILRMAVAHMKALRGTGNTSDGTYKPSFLTDQELKHLILEAADGFLFVVSCDTGHIIYVSDSISPVLNHSQSDWFGSCLYDHVHPDD VEKVREQLSTQEPQSTGRILDLKSGTVKKEGHQSAMRVCMGSRRGFICRIKVGNLGPDAMASGHLNRLKQRNSLGPSKDGHNYAVIHCT GFIKNWPPSGQFDNPLAGVQMDRGVEEESKHFCLVAIGRLQLTSTPNASDLSGGNSNAEFISRHSTDGKFSFVDQRVINILGYTPPELLGK SCFDFFHPEDVSHMKESFDQVLKLKGQVMSVIYRFRVRNRNDYVWLRTSAFAFLNPYTDDIEYIVSTNTLAKSLQSPGNSETSEQPYQPS VDYSLQQRYPSHLILNPQQHIHTSSSQQQQQQQRSNSSQMYESYENSQQQNSPSIGYNNSPTPQSSNPASVLNRISKSSPTPAQTAWTLR	653	72.39	6.45	1. Hakkola J, Juvonen R, Rahnasto-Rilla M, Raunio H, A Lang M (2013) Function and regulation of the Cyp2a5/CYP2A6 genes in response to toxic insults in the liver. Curr Drug Metab 14(1): 137–150. 2. Gerbal-Chaloin S, et al. (2006) Role of CYP3A4 in the regulation of the aryl hydrocarbon receptor by omeprazole sulphide. Cell Signal 18(5):740–750.	2
DHR96	Bta02014	MDLKHDTAYMRGGPGPGDGIKSESRVHSPCFGAPEISVGRPLSVVASNGLFSGLGSSKTLRSDDWLSTAHSPNSHSHSGHNHNPTSQTSV PLTPSPGPPANQFTVISSNGYSSPMSSGSYDPYSPTNGRIGKEELSPANSLNGYSVDSCDASRKKKGGTGRQQEELCLVCGDRASGYHYN ALTCEGCKGFFRRSITKNAVYQCKYGNNCEIDMYMRRKCQECRLKKCLSVGMRPECVVPEFQCAVKRKEKKAQKDKDKPNSTTSCSP DGIKQEIDPQRLDTDSQLLSVNGVKPITPEQEELIHRLVYFQNEYEHPSPEDIKRIVNAAPEEENVAEERFRHITEITILTVQLIVEFSKRLPGF DKLIREDQIALLKACSSEVMMFRMARRYDAETDSILFATNQPYTRESYTVAGMGDTVEDLLRFCRHMCAMKVDNAEYALLTAIVIFSERP SLSEGWKVEKIQEIYIEALKAYVENRRKPYATTIFAKLLSVLTELRTLGNMNSETCFSLKLKNRKVPSFLEEIWDVVS-	530	59.29	7.47	1. King-Jones K, Horner MA, Lam G, Thummel CS (2006) The DHR96 nuclear receptor regulates xenobiotic responses in Drosophila. Cell Metab 4(1):37–48. 2. Lin GH, Kozaki T, Scott JG (2011) Hormone receptor - like in 96 and Broad - Complex modulate phenobarbital induced transcription of cytochrome P450 CYP6D1 in Drosophila S2 cells. Insect Mol Biol 20(1): 87–95.	2
HNF6	Bta12954	MVSTPSPPKIIQQTIQTAPPSAATTTISIKVNQSNNCSIGMGSGSGSGSDLEEINTKELAQRISAELKRYSIPQAIFAQRVLCRSQGTLSDLLR NPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAAAQIPQRSSEVFEHMNTGTNAFASAACKRKEDPQIMSEHTPATKKPRLVFTD LQRRTLQAIFKETKRPSKEMQVTIARQLGLEPTTVGNFFMNARRRSMDKWKDEEPAKAGVGVGVGMGALGMGALSDDQPSPNNLGGQ HHDL-	273	30.18	9.96	1. Li J, et al. (2015) Sex-dependent regulation of hepatic CYP3A by growth hormone: roles of HNF6, C/EBPα, and RXRα. Biochem Pharmacol 93(1):92–103.	1
Nrf2	Bta00634	MTSALPQPDRSVLLHNATLAPPVGDLNSSCPYPTIGANSNFGSAVATSMNLTNSSEPIGNENSGASFKMEPSSHDMMYYQNSSSEINQTD GFLSSILNDEDLQLMDMAMNEEMYSMRMLEGAVGGNNSMMGCHTTGERAGTDSDSAVSSMGSERVPSLSSDNEWMETNSDSGHNIAD HYNSDYRKYRCYDYQYSTRSHTASENSTSSRLPVAPQKKYHMYGKRYLQEQASGAGPDLSAATPEEAAIAPLKYEPSTSTAMPVPSMLP AHDMKAQQESKYSCSIEFACNNLLNSRIGDHVNHNHTYHLPPENIGTMQRPYLKDKQKCRRNDEHQQQMTRDEKRARALNIPISVMDII NLPMDEFNERLSKYDLSESQLSLIRDIRRRGKNKVAAQNCRKRKLDQIICLADEVQQMRDRKNRAEKERHYLITERHRIKELFNQLYRHIF QNLRDPDGNPYSSYEYSLQMAADGSIVLMPRDNGTISPLVDPSEYSHLRGRHKDPPHKQ-	505	57.1	6.1	1. Jin M, Kumar A, Kumar S (2012) Ethanol-mediated regulation of cytochrome P450 2A6 expression in monocytes: role of oxidative stress-mediated PKC/MEK/Nrf2 pathway. PloS One 7(4):e35505. 2. Satarug S, Marks GC, Lang MA, Moore MR (2004) Acute cadmium chloride administration induces hepatic and renal CYP2A5 mRNA, protein and activity in the mouse: involvement of transcription factor NRF2. Toxicol Lett 148(3):199–210.	2
Maf-S	Bta07674	MNRKYERKISSLPPLSPNPLLDISDDELVSISVRDLNRQLKLRGLTKEEIVKMKQRRRTLKNRGYAASCRIKRIEQKDELETEKSQELRDLE MMEKDNSLIKEEIEQIVIKFEALKKFALMRKVPIPLDLESLKSGCL-	138	16.2	9.97		
RXR1	Bta11398	MEGSERGLSLENNISLNSVGPQSPLDMKPDTASLLGGQFSPGGGPNSPNSFGMAHSSLIVGSAGGGSKNNSITSPLPPNHPLSGSKHLCSI CGDRASGKHYGVYSCEGCKGFFKRTVRKDLSYACREEKNCLIDKRQRNRCQYCRYKKCLSMGMKREAVQEERQRTKENQNEVESTSS NHSDLAIDRILEAERRVECNLDQNTNSLVKAAEKQMIQLVEWAKHIPHFTSLPLEDQVCLLRAGWNELLIASFSHRSIDARDGIVLTTGLTI HRNSAHQAGMGNIFDRVLTELVTKMKDMKMDKTELGCLRTIILFNPDTKGLKSVQEVGLLREKVFTILEEYCRTTHPEEPGRFAKLLLRLP SLRSIGLKCSEYLFFYKLIGDSQVDVFLVEMLENSDRTL-	400	44.69	8.46	1. Evans RM, Mangelsdorf DJ (2014) Nuclear receptors, RXR, and the big bang. Cell 157(1):255–266. 2. Burk O (2005) Antimalarial artemisinin drugs induce cytochrome P450 and MDR1 expression by activation of xenosensors pregnane X receptor and constitutive androstane receptor. Mol Pharmacol 67(6):1954–1965.	2
NR2E	Bta00533	MEGLSETQCKVCGDKASGKHYGVASCDGCRGFFKRSIRRNLEYVCKENGKCIVDVTRRNQCQACRFSKCLLVNMKKDAVQHERTSRQ NSHHHHHRFHRSRELCSPIFPSAFIYRYPHPFPYSVAAIMSKSELHQTTPASRPLGAFNNYLALHDCQYISVSKDCQQLLKEDEVSSSEEVQ HITPQDVTKSSPISPKKERQDDVSVDMPNEGIYESAAKLLFLAVKWARSIPSFIQLPSHDQSLLLEDSWSELFALSAAQWSFALDQGFLVIN SAAPEYRHSALEETVRKLKGIVNRILTMRLDHTEFTCLKALVLFKSDVAGLSEPSYIELLQDQTQVMLREYISVTSKVRFGKLLLLLPVLAS LEKRNLEELFFRKTIGNVSISSLLTDMLRQ-	393	44.73	8.6	1. Sluder AE, Maina CV (2001) Nuclear receptors in nematodes: themes and variations. Trends Genet 17(4):206–213.	1
CEBP	Bta03941	MDSPQMYDTPAQTAATNGTNGTGNGSLTAQEIVNMKNHQKNINLHQLKRYNDLADLNTPEISLDLQNLIDDSQFNEGLFTEILNQGNSR QLQISKTHVQQQFQTSRGPQSTTALHYLPQPVHYEAATPGTGGHIKEEPPETPEFRNVVPSANPAYSVVNGTAPQFTPAGVNHLNLKHHH HHSHRKSSKSVDKNTDEYKRRRERNNIAVRKSREKAKIRSRETEEKVKILVKENERLQKRIELLTEELNVLRSLFTNVGVLPEHLHRELNK HFDTYPHHGVQ-	281	32.01	8.94	1. Tinel M, et al. (2003) Downregulation of cytochromes P450 in growth-stimulated rat hepatocytes: role of c-Myc induction and impaired C/EBP binding to DNA. J Hepatol 39(2):171–178.	1
KLF11	Bta10336	MVLTIDTSEGALMMTDCSRSRYLKGMDLCTRNYPRCFDLPQSSPLSLTPQPSDSEGDEMDVDYGKKLAQPIMSATPPHSPTSCEAKVTSV PVSVIMRAHKDGSCSPEPSSPEPERHSPLNCTYAPSSASGGGPDFSPRSSPGLFKPVPNHLEINPLKSIRYKMNTGKEEVFINSKDTNRDRA DGESEEDRSSGPKTLPIAPRPFLPLSPAQTVILTGGTLIPLDPNRRPILFAPPAENSPFVFVAPPAAPAPLGAPLASRRERGAQNLDPRRRVFE CHYENCGKNYFKSSHLKAHMRTHTGEKPFVCQWEGCERRFSRSDELSRHKRTHTGEKKFGCEVCARRFMRSDHLAKHVKRHTKDQLLI SAQRNLVQSRPAPPANPPHPHHHAAAVAPTPEGPLRLGFVIPVKNFLTQMI-	415	45.86	9.18	1. Zhang H, et al. (2013) Mouse KLF11 regulates hepatic lipid metabolism. J Hepatol 58(4):763–770. 2. Zheng Y, et al. (2014) Epigenetic regulation of uterine biology by transcription factor KLF11 via posttranslational histone deacetylation of cytochrome p450 metabolic enzymes. Endocrinology 155(11):4507–4520.	2
E23	Bta04225	MVKPGELLAVMGPSGCGKTTLLNCLAGRIKLDSGCIRLNRERLNKRWKRRICYVLQQDIFFPDLTLRQTLEYTALLRLPDSFSYVQKMQY VDHIIDVLDLRNCQDTIIGDYIKRGLSGGEKKRANIACELLTNPALMLLDEPMSGLDSHAAFNLMALLKRYAEKEGKTIVVTAHQPSSQMF HMFDKLLLLCNGQTAYFGETHKVVDFFNNVGLTLMPHYNPADFIMEQIKGSEEVKEKILAASQEARLSSDYPVELRPNYFSNSICDKYLS NYHTNSLHSIGHLGGEFHRDWSHHFTSPPPPPSSDLCVAISPPHTNENQGHVYTTIAVKEEENKTLWLDDQSHASSSVSSEDDYGWQWPT CFWTQFKVLSRRNFQEARPRMLSTLNWVQTIGLGLMAGLLWFQLPRSEEFLHDIQGWMFFSMTYWMLFAHFGALSSFPSEREVINKERQ [illegible]	640	16.12	4.94		
E63	Bta13636	MADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAF RVFDKDGNGFISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVTMMTSK-	150	16.81	4.09		
E78	Ssa11354	METVTTPTPQSPQPSSPKSCTFISVKQEPNNCEVESPDSAAPKNNEQHYVAADSNSSTPTKSFVPCKVCGDKASGYHYGVTSCEGCKGF FRRSIQKQIEYRCLRDGKCLVIRLNRNRCQYCRFKKCLAVGMSRDSVRYGRVPKRSRERGEGSDVRVTTSDSSSDQSCDIDNKHLAIYDV ILTVSQAYQSNCDYTEEQTKTLTRKPLVSHPSPVCSSPEVASSTADSNSKQRIWLWQQFATHMTPSVQRVVEFAKRVPGFCDLSQDDQLI LIKVGFFELWLGHVSRLTSECSLTFSDGTYVTRQQMELMYDSEFVYSIFHFANSFNALALNDTELGLFSAVVLLTSDRPGVTDLKAIKQHQ DRLLDALKLQVGRNHANEPQVLSNVLLKLPELRNLGAKHSAHLDWFRLNWDKLRLPPLFAEIFDIPKCEEDLQ-	434	48.89	7.82	1. Luan JB, Ghanim M, Liu SS, Czosnek H (2013) Silencing the ecdysone synthesis and signaling pathway genes disrupts nymphal development in the whitefly. Insect Biochem Mol Biol 43(8):740–746. 2. Brown MR, Sieglaff DH, Rees HH (2009) Gonadal ecdysteroidogenesis in Arthropoda: occurrence and regulation. Annu Rev Entomol 54:105–125. 3. Fahrbach SE, Smagghe G, Velarde RA (2012) Insect nuclear receptors. Annu Rev Entomol 57:83–106. 4. Nakagawa Y, Henrich VC (2009) Arthropod nuclear receptors and their role in molting. FEBS J 276(21):6128–6157.	4
HR3	Bta04882	MSRDAVKFGRMSKKQREKVEDEVRFHRAQLSRAQTETAPDSSVFDQAQTPSSSDQLHQYNGGYPYSTSDIGNYTNSPYGYSTPVGAHA LQYEINGVDSFVDSTTAYDPRPTSLDSLTPDNGIMGNVVTTDPAQISELLSKTIADAHARTCLYTLEHIHDMFRKQQDISKLIYYKGLSHEE LWLECAQKLTTVIQQIIEFAKMVPGFMKLSQDDQIVLLKAGSFELAVIRMSRYYDLSQNCVLYADTLLPQDAFYTQDTLEMKLVNLAFE VARSIAELKLTETELALYSAVVLLSHDRPGLKGLAEISRLGQAVSKALRLELDRNHGPIKGDVTVYDALLAKVPSLRELSMLHMEALAKFK RSAPHLEFPALHKELFSVDS-	381	42.59	5.66		
HR4	Bta02331	MEDLHLIKSGTSVDIKKTDGKVHSAIVSGVNFDTKSVTVEWFEKGETKGKEIEVETHLALNGHLAAPNNINNFNVPNNKVPKYSTNKATPA PVVSNKGSYRSTKLQSRPTNIITNGHSETRGTENLPSTPTTPVVPSRETFISSHIPGAQNVRGSVTSSLANATTINVQQRQQQKQQQQQQ QELQQQDSQGGKNRRSHVVKEVEKLKKNREERRQRQAELREEKEALINMDPGQQNWEFLGMIRDYRSQINFRPLRDAEPVENHQISVC VRKRPLNKKELARKEVDVITVPTKDQIVVHEPKNKVDLTKYLENQLFRFDYAFDDSCDNDLVYKFTAKPLVETIFEGGMATCFAYGQTG SGKTHTMGGDFQGKTQDCKKGIYAMAAKDVFKWLRSPKYKPLNLIVSASFFEIYSGKVFDLLADKAKLRVLEDGKQQVQIVGLTERIVE [illegible]	754	84.85	8.81		
HR38	Bta11834	MLAWKGCVYLESDKVSFAQLEELLPLHLQSESQFASKTLSPSPDLISVGTDLEPLPLPELPELKVCVSVSPRSQSLDSLDSQLLKPDLLPSCVP CPSDEEPPFESESRQELCERVLRGSDPSLHESCSDLTVSQLSPQPILPDRDDSEAGHPAGTGLPQASSSMLLLQTQTSFGSSSFAELLSAPYT CGDSAACQHYGVRTCEGCKGFFKRTVQKGSKYVCLAEKCCPVDKRRRNRCQFCRFQKCLQVGMVKEVVRTDSLKGRRGRLPSKPKS PQESPPSPPVSLITALVRGHLDTSPDLSSLDYSQYREPSPLEPVITEAEKIMQFYTLLTTSIDVIKLFVEKIPGFSDLCPEDQELLFQSACLELFV LRLAYRTRATDTKLVFCNGVVLEKQQCVRSFGDWLFAILDFCQSMHAMEIDLSAFACLCALTIITERHGLKEPHKVEQLQSRISSALQDHV [illegible]	519	57.89	5.41		19

CYP6CM1	Bta07221	PILVVRDPELARIVLVKSFSSFSGRLKSPDTTLDPLSNHLFTLNGEKWRQVRHKTATAFSTAKLKNMFHSLKDCAREMDAYMERAIGDKG DVEFDALKVMSNYTLEVIGACAMGIKCDSIHDEETEFKRFSRDFFRFDARRMIFTLLDLLHPKLPVLLKWKAVRPEVENFFREAIKEAASL KESEAAARTDFLQILIDFQKSEKASKTDAGNDTELVFTDNIIGGVIGSFFSAGYEPTAAALTFCLYELARNPQVQAKLHEEILAVKEKLGDD IEYETLKEFKYANQVIDETLRLYPASGILVRTCTEPFKLPDSDVVIEKGTKVFVSSYGLQTDPRYFPEPEKFDPERFSEENKEKILPGTYLPFG	521	59.74	7.59
p38	Bta12848	MSNFYRVEINRTEWEVPERYQMLTPVGSGAYGQVCSAVDSQTGRKVAIKKLARPFQSAVHAKRTYRELRLLKHMDHENVIGLLDIFHPS TSPNDFNQVYLVTHLMGADLNNIIRTQKLSDDHVQFLVYQILRGLKYIHSAGIIHRDLKPSNIAVNEDCELKILDFGLARPTENEMTGYVA TRWYRAPEIMLNWMHYNQTVDIWSVGCIMAELITGRTLFPGTDHIDQLTRVLVLCGTPSEDTFQKISSQEARSYIQSLPNLKKKDFKQVF KGANPLAIDLLELMLELDADKRITAEKALAHPYLSKYADPEDEPTSPPYDQSFEESDLPVEKWKELVYEEVLSFRPHPSLLPEGEVASS-	359	41.07	5.68
ERK	Bta01850	MAAEGSETNARNSESVRGQIFEVGPRYTNLAYIGEGAYGMVVSAFDNVTKTKVAIKKISPFEHQTYCQRTLREIKILTRFRHENIIDIRNILR SSTIDQMKDVYIVQCLMETDLFKLLRTQRLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLARVADPDHDHTG FLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCILAEMLSNRPLFPGKHYLDQLNHILGILGSPTQEDLESIINDKARNYLQSLPFKPKVP WTRLFPSADAKALDLLDKMLTFNPHNRIVVEDALAHPYLEQYYDPADEPVAEEPFRFSMELDDLPKETLKQYIFEETAMFRQDEAGM-	363	41.91	5.91
nAChRs-α1	Bta11027	KWNPEEYGGVDTLHVPSEHIWLPDIVLYNNADGNYEVTIMTKAIIHHTGKVVWKPPATYKSFCEIDVEYFPFDQQTCFMKFGSWSYDGY TVDLRHISQTPDSTEIEMGIDLQDYYLSVEWDVMRVPATRNEKFYSCCEEPYQDIMFNITLRRKTLFYTVNLIIPCIGLSFLSVLVFYLPSDS GEKVSLCISILLSLTVFFLLLAEIIPPTSLTVPLLGKYLLFTMVLVTLSVVVTIIVLNVNFRSPVTHKMTPFWYKVFIQVLPKVLMIERPNKDD SIDDDKPEGVLTGVFDADLGADKYLAYGGPEEYSPEYDIPEPPPSRYEPNSAPAPVVVPLPLPCFEEPPPPSPLPGPEDDLFSAPGGGCGNAL	562	63.97	4.74
nAChRs-α2	Bta11032	WDPAEYGGCKELYVPSEHIWLPDIVLYNNADGEYVVTTMTKAVLHFDGKSVWTPPAIFKSSCEIDVRYFPFDQQTCFMKFGSWSYDGN QIDLKHINQKAGADMVEVGIDLREYYPSVEWDILGVPAERHEKYYPCCKEPYPDIFFNLTLRRKTLFYTVNLIVPCVGISYLSVLVFYLPAD SREKVVLCITILLSQTMFFLLISEIIPSTSLALPLLGKYLLFTLVLIGLCVVITIGVLNVHYRKPSTHKMAPWVRKLFIRRLPRLLLMRVPEQLLI DMAAKKRLKNATSAKFNAALAASSLGSSTESLRGRHPYMPGGGPGCNGLHANNGVTNRFNSLVGALSGSVEAMNNGHGHGHPVVVA	556	63.21	6.94
nAChRs-α3	Bta14972	YGGVEMLHVPSDHIWRPDIVLYNNADGNFEVTLATKATLNYTGRVEWKPPAIYKSSCEIDVEYFPFDEQTCVMKFGSWTYDGFQVDLRH IDEVFGSNVVNIGVDLSEFYTSVEWDILEVPAVRNEKFYTCCDEPYLDITFNITMRRKTLFYTVNLIIPCMGISFLTVLVFYLPSDSGEKVSLS ISILLSLTVFFLLLAEIIPPTSLVVPLLGKFVLFTMILDTFSICVTVVVLNVHFRSPQTHTMAPWVKRVFIHILPKLLVMRRPHYPVDKRGLAA SCQRIMVRTCNGNVELRDSSLFPHHAAPPALPSELLNTPAQYVFQHSSRDTEPTIIGSSCEIHGAPPALSQLFPDTLGENGCGGVTPEPSPNE	569	64.48	6.16
nAChRs-α4	Bta05486	GYKLRWDPNEYGGVEMLYVPSEHIWLPDIVLYNTADGNYEVTLMTKATLNYTGDVLWRPPAIYKSSCEMNVLYSPFDEQSCLMKFGSW TYNGFQIDLKHMEQVYGSNLVNVGVDLSEFHLSVEWDILEVPARRNEEYYPCCTEPYSDITFNITMRRKTLFYTVNLIIPCVGITFLTVLVF YLPSDSGEKVSLCSSILVSLTVFFLLLAEIIPPTSLAVPLLGKYLLFTMTLVTLSICVTVSVLNVHFRSPSTHKMSPWVKTVFLHFMPRLLMM RRPPYSPNDLYLENLSDASYVNDGDFRDSFSDGCPMEHKCSPKENRNMYKSNDCNHFHHGAQAPDLENVIPRHLSDDLLAALEGVRFIA	534	61.56	5.21
nAChRs-α5	Bta11534	LWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAVINTNVIVKHDGEVTWLSHGIYRSSCDIDVEYFPFDIQSCAMKWASWTYDGY MLDLVKQTEEGDVSNYQANGEFDLIGFDSIKNVITYSCCEEPYPDITYFIRLRRRPMFYVFNLILPCILINCIALLVFYVPSESGEKVTLGISA LLSMTVFLMTIRETLPPTEKTPLISMYYGVSICLVTLASGLSVVTLNIYHRGVRGTKVPGIIKATMLGKLARCLFLQFEPRKKNSQEARRCG PKMQINYTKPDRWTTMNDEYRYNSPKFIMRHSAQPAETTATSTGSRSFETQFMRMLQRVNVTVEQNELRLLDQERREQTELEWKQVAL	484	55.86	6.83
nAChRs-α6	Bta05486	SSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNDGTCLYIPPGIFKSTCKIDITWFPFDDQKCDMKFGSWTYDGFQLD LQLKSEEGGDLSDFIPNGEWYLLGMPGKRNVLYYQCCPEPYVDITFQIQIRRRTLYYFFNLIVPCVLISSMALLGFTLPPDSGEKLTLGVTIL LSLTVFLNLVAESMPTTSDAVPLIGTYFNCIMFTVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISIS HRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDETAMCSIAQREMQHILRELRFITGRLRKKDEEAEVIS	462	53.14	6.32
nAChRs-α7	Bta02663	SEFGNVRDLRIPPHRLWKPDILMYNSADEGFDGTYPTNVVVRSNGSCSYIPPGIFKSTCKIDITWFPFDDQRCEMKFGSWTYDGFQLDLQ LQDEGGGDISSFITNGEWDLLGVPGKKNVIYYNCCPEPYIDITFVIIIRRRTLYYFFNLIVPCVLIASMAVLGFTLPPDSGEKLSLSVTILLSLT VFLNMVAETMPATSDAVPLLGTYFNCIMFMVASSVVSTILILNYHHRNADTHEMSPWIKLLFLKWFPCILRMSRPEPETPVSGSGESARKL QLRELELKERSSKSLLANVLDIDDDFRHAPIPPPAPHGYLPRHGVGVGGHPHEDGHTGTLLHATSASCLGPHRELALILKELRVITEKIRKEE	504	56.88	5.71
nAChRs-α8	Bta05486	DYKLRWDPNEYGGVEMLYVPSEHIWLPDIVLYNNADGNYEVTLMTKATLNYTGDVLWRPPAIYKSSCEMNVLYFPFDEQSCLMKFGS WTYNGFQIDLKHMEQVYGSNLVNVGVDLSEFHLSVEWDILEVPARRNEEYYPCCTEPYSDITFNITMRRKTLFYTVNLIIPCVGITFLTVLV FYLPSDSGEKVSLCSSILVSLTVFFLLLAEIIPPTSLAVPLLGKYLLFTMTLVTLSICVTVCVLNVHFRSPSTHKMSPWVKTVFLHFMPRLLM MRRPPYSPNDLYLENLSDASYVNDGDFRDSFSDGCPMEHKGSPKENRNMYKSNDCNHFHHGAQAPDLENVIPRHLSDDLLAALEGVRFI	534	61.66	5.11
nAChRs-β1	Bta03349	DETDYGGIGVLRLPPDKVWKPDIVLFNNADGNYEVRYKSNVLIYPHGEVLWIPPAIYQSSCTIDVTYFPFDQQTCIMKFGSWTFNGDQVS LALYNNKNFVDLSDYWKSGTWDIIEVPAYLNTYPGVPTETDITFYIIIRRKTLFYTVNLILPTVLISFLCVLVFYLPAEAGEKVTLGISILLSLV VFLLLVSKILPPTSLVLPLIAKYLLFTFIMNTVSILVTVIIINWNFRGPRTHRMPVWIRSVFLYYLPIILLMKRPKKTRLRWMMEMPSMTNVS SVPPHPHYGSPTELPKNLAQASSKSKTEVMELSDFHHPNCKINRKASVERRESESSDSLLLSPEASKATEAVEFIVEHLRNEDQYIQIREDW	508	58.85	7.83
MEAM1 Genome Website		http://www.whiteflygenomics.org/cgi-bin/bta/blast.cgi			
MW & pI Prediction Website		https://www.bioinformatics.org/sms2/index.html			

Analysis the sequences of nAChR-α1 in IMR and IMS strain



Analysis the sequences of nAChR-α2 in IMR and IMS strain



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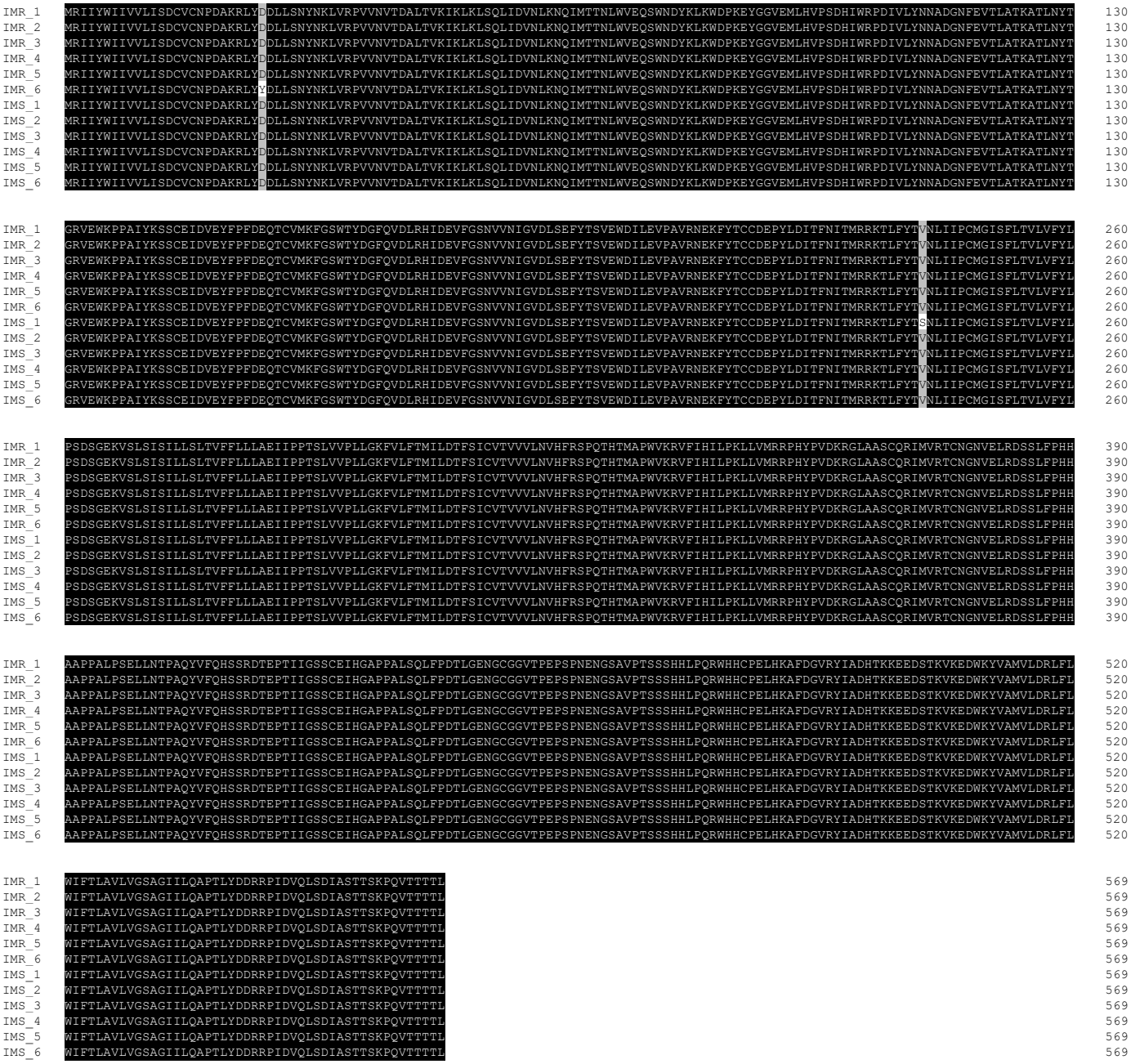
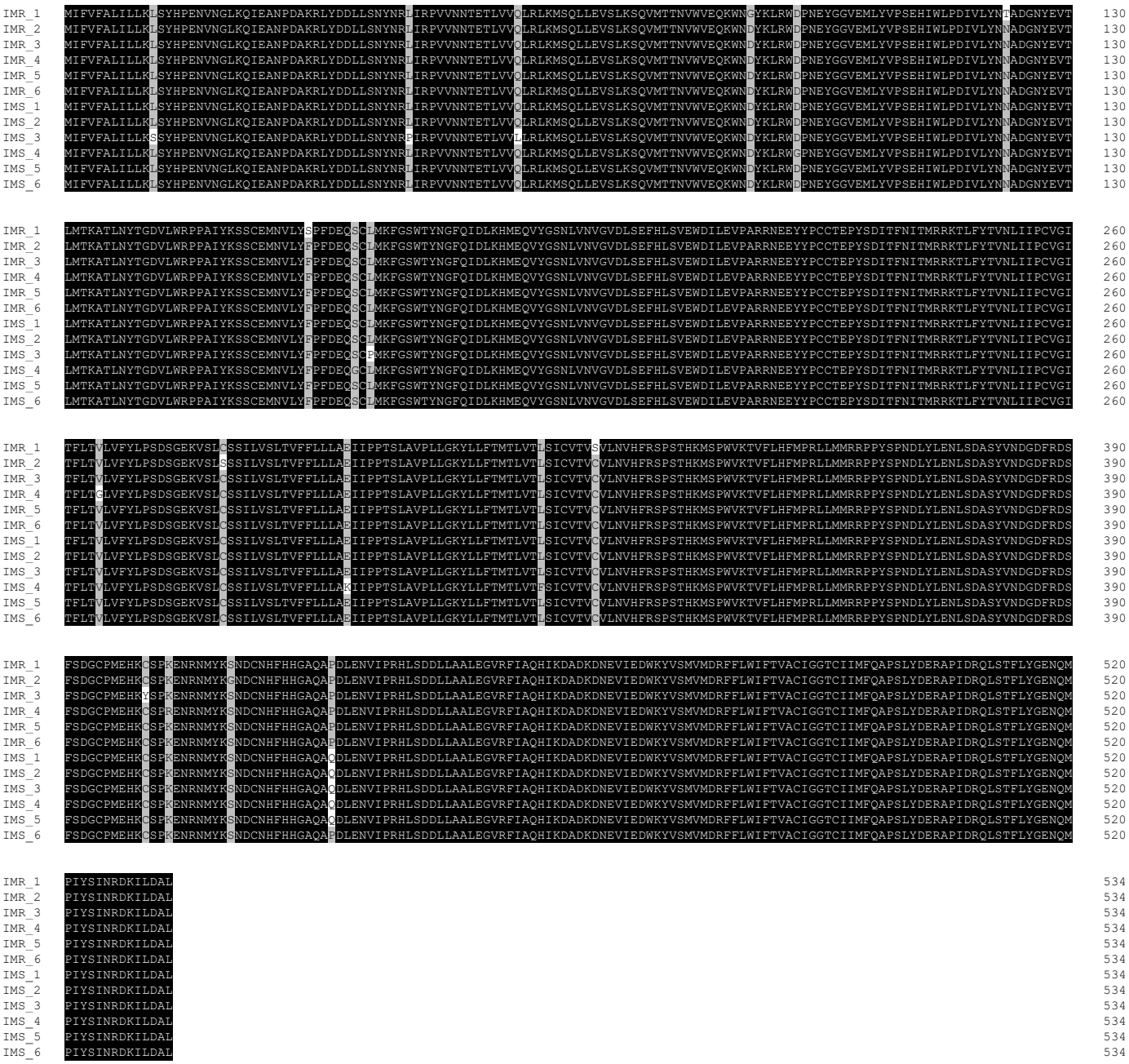


Analysis the sequences of nAChR-α4 in IMR and IMS strain



Analysis the sequences of nAChR-α5 in IMR and IMS strain

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IMR_2  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMR_3  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMR_4  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMR_5  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMR_6  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMS_1  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMS_2  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMS_3  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMS_4  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMS_5  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130
IMS_6  MRLLSDNRIFALLCVHSLLSFVSVICDGVEYALMRTLMENYDSSIRPAKNSTQSLKVEFGVSLHHIIDVDEKNQILTTNCWITQVWTDHHLLWNISDFDEITVLRIPYHKVWRPDIILYNNADSQYNSAV  130

IMR_1  INTNVIVKHDGEVTWLSHGIYRSSCDIDVEYFPFDIQSCAMKWASWTYDGYMLDLVKQTEEGDVSNYQANGEFDLIGFDSIKNVITYSCCEEPYPDITYFIRLRRRPMFYVFNLILPCILINCIALLVFY  260
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IMR_4  INTNVIVKHDGEVTWLSHGIYRSSCDIDVEYFPFDIQSCAMKWASWTYDGYMLDLVKQTEEGDVSNYQANGEFDLIGFDSIKNVITYSCCEEPYPDITYFIRLRRRPMFYVFNLILPCILINCIALLVFY  260
IMR_5  INTNVIVKHDGEVTWLSHGIYRSSCDIDVEYFPFDIQSCAMKWASWTYDGYMLDLVKQTEEGDVSNYQANGEFDLIGFDSIKNVITYSCCEEPYPDITYFIRLRRRPMFYVFNLILPCILINCIALLVFY  260
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IMS_2  INTNVIVKHDGEVTWLSHGIYRSSCDIDVEYFPFDIQSCAMKWASWTYDGYMLDLVKQTEEGDVSNYQANGEFDLIGFDSIKNVITYSCCEEPYPDITYFIRLRRRPMFYVFNLILPCILINCIALLVFY  260
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IMS_4  INTNVIVKHDGESTWLSHGIYRSSCDIDVEYFPFDIQSCAMKWASWTYDGYMLDLVKQTEEGDVSNYQANGEFDLIGFDSIKNVITYSCCEEPYPDITYFIRLRRRPMFYVFNLILPCILINCIALLVFY  260
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IMR_1  VPSESGEKVTLGISALLSMTVFLMTIRETLPPTEKTPLISMYYGVSICLVTLASGLSVVTLNIYHRGVRGTKVPGIIKATMLGKLARCLFLQFEPRKKNSQEARRCGPKMQINYTKPDRWTTMNDEYRYN  390
IMR_2  VPSESGEKVTLGISALLSMTVFLMTIRETLPPTEKTPLISMYYGVSICLVTLASGLSVVTLNIYHRGVRGTKVPGIIKATMLGKLARCLFLQFEPRKKNSQEARRCGPKMQINYTKPDRWTTMNDEYRYN  390
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IMS_1  VPSESGEKVTLGISALLSMTVFLMTIRETLPPTEKTPLISMYYGVSICLVTLASGLSVVTLNIYHRGVRGTKVPGIIKATMLGKLARCLFLQFEPRKKNSQEARRCGPKMQINYTKPDRWTTMNDEYRYN  390
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IMR_2  SPKFIMRHSAQPAETTATSTGSRSFETQFMRMLQRVNVTVEQNELRLLDQERREQTELEWKQVALVMDRLLLWIFIILTAISTTVILRGSPYGP  484
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IMR_4  SPKFIMRHSAQPAETTATSTGSRSFETQFMRMLQRVNVTVEQNELRLLDQERREQTELEWKQVALVMDRLLLWIFIILTAISTTVILRGSPYGP  484
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Analysis the sequences of nAChR-α6 in IMR and IMS strain

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IMR-2   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFDITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNDGTCLY   140
IMR-3   MIRADSYFVLVVLMTQFGDSMQGRHERRLLDKLLDSYNVLERPVANESAALLVRFGITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNNGTCLY   140
IMR-4   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFDITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNDGTCLY   140
IMR-5   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFGITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPSRVWKPDILMYNSADEKFDGTFHTNVLVRNDGTCLY   140
IMR-6   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFGITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNNGTCLY   140
IMS-1   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFGITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNNGTCLY   140
IMS-2   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFGITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWDSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNNGTCLY   140
IMS-4   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFGITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNNGTCLY   140
IMS-5   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFGITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNNGTCLY   140
IMS-6   MIRADSYFVLVVLMTQFGDSIQGRHERRLLDKLLDSYNVLERPVANESAALLVRFGITLQQIIDVDEKNQILTTNAWLNLEWVDYSLGWNSSDYGGVKDLRITPNRVWKPDILMYNSADEKFDGTFHTNVLVRNDGTCLY   140

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IMR-2   IPPGIFKSTCKIDITWFPFDDQKCDMKFGSWTYDGFQLDLQLKSEEGGDLSDFIPNGEWYLLGMPGKRNVLYYQCCPEPYVDITFQIQIRRRTLYYFFNLIVPCVLISSMALLGFTLPPDSGEKLTLGVTILLSLTVFLN   280
IMR-3   IPPGIFKSTCKIDITWFPFDDQKCDMKFGSWTYDGFQLDLQLKSEEGGDLSDFIPNGEWYLLGMPGKRNVLYYQCCPEPYVDITFQIQIRRRTLYYFFNLIVPCVLISSMALLGFTLPPDSGEKLTLGVTILLSLTVFLN   280
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IMS-4   IPPGIFKSTCKIDITWFPFDDQKCDMKFGSWTYDGFQLDLQLKSEEGGDLSDFIPNGEWYLLGMPGKRNVLYYQCCPEPYVDITFQIQIRRRTLYYFFNLIVPCVLISSMALLGFTLPPDSGEKLTLGVTILLSLTVFLN   280
IMS-5   IPPGIFKSTCKIDITWFPFDDQKCDMKFGSWTYDGFQLDLQLKSEEGGDLSDFIPNGEWYLLGMPGKRNVLYYQCCPEPYVDITFQIQIRRRTLYYFFNLIVPCVLISSMALLGFTLPPDSGEKLTLGVTILLSLTVFLN   280
IMS-6   IPPGIFKSTCKIDVTWFPFDDQKCDMKFGSWTYDGFQLDLQLKSEEGGDLSDFIPNGEWYLLGMPGKRNVLYYQCCPEPYVDITFQIQIRRRTLYYFFNLIVPCVLISSMALLGFTLPPDSGEKLTLGVTILLSLTVFLD   280

IMR-1   LVAESMPTTSDAVPLIGTYFNCIMFTVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMR-2   LVAETLPQVSDAIPLLGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMR-3   LVAETLPQVSDAIPLLGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMR-4   LVAETLPQVSDAIPLLGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMR-5   LVAETLPQVSDAIPLLGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMR-6   LVAETLPQVSDAIPLLGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMS-1   LVAETLPQVSDAIPLLGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDGLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMS-2   LVAESMPTTSDAVPLIGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMS-4   LVAETLPQVSDAIPLLGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420
IMS-5   LVAESMPTTSDAVPLIGTYFNCIMFMVASSVVLTVLVLNYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNDSTNTTCHQRQGTTVSQSVDET   420
IMS-6   LVAESMPTTSDAVPLIGTYFNCIMFMVASSVVLTVLVLSYHHRTPENHKMSGFVQKLFLEYLPWVLRMGRPGKKITLKTISISHRMRELEIRERSSRSLLANILDLDDDLQRFRANVNNSTNTTCHQRQGITVSQSVDET   420

IMR-1   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMR-2   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMR-3   AMCSIAQREMQHVLRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMR-4   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMR-5   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMR-6   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMS-1   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMS-2   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMS-4   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMS-5   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
IMS-6   AMCSIAQREMQHILRELRFITGRLRKKDEEAEVISDWKFAAM   462
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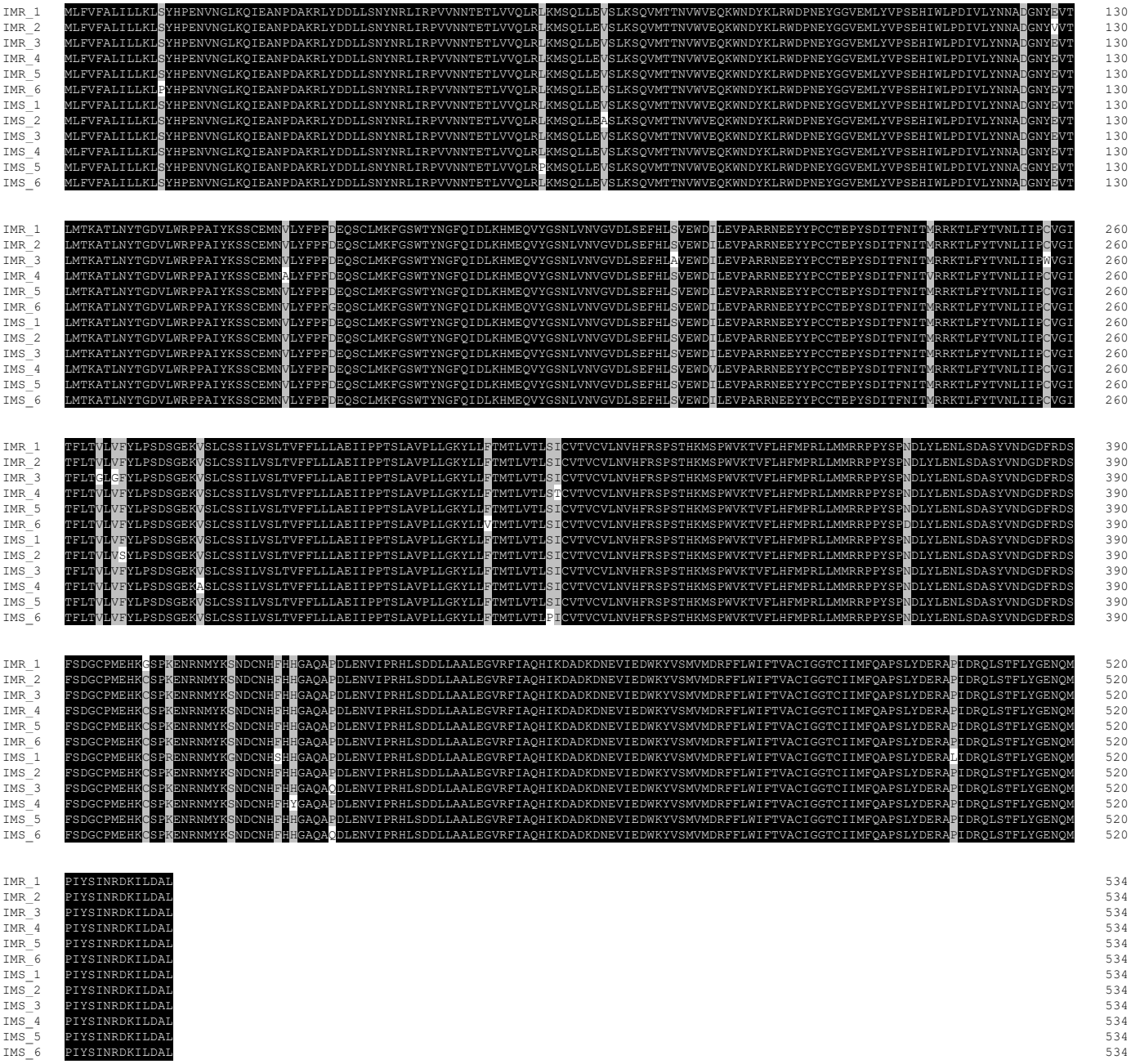
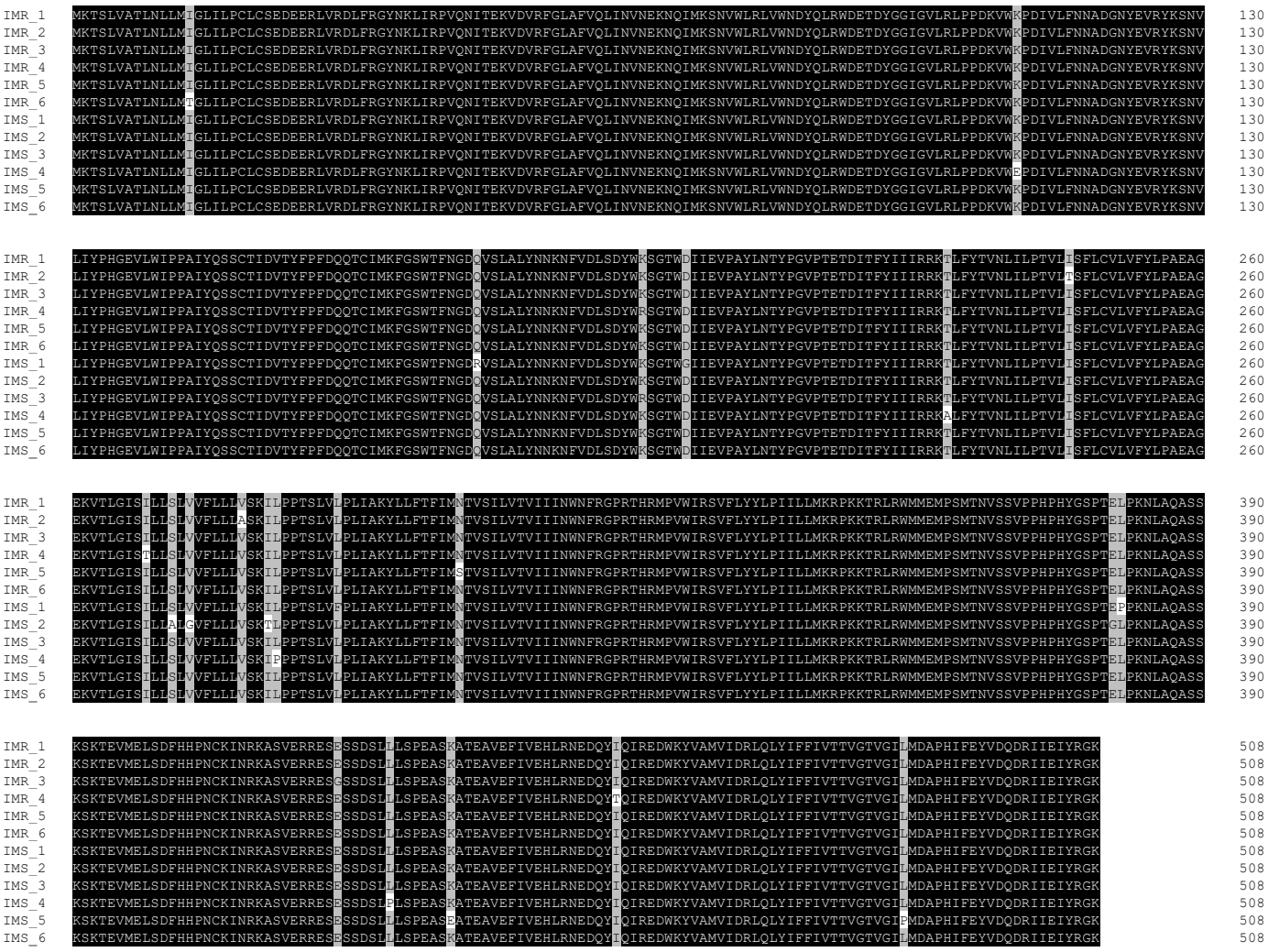
Analysis the sequences of nAChR-α7 in IMR and IMS strain

Analysis the sequences of nAChR-α8 in IMR and IMS strain



Analysis the sequences of nAChR-β1 in IMR and IMS strain



Dataset S4. Primers used in this study.

Gene Name	Abbreviation	Genome ID (MEAM1)	Direction	Primer (5'-3')	Annealing temperature (°C)	Product (bp)	Usage	Amplification efficiency (%)
CYP6CM1	CYP6CM1	Bta07221	Left	CACTCTTTTGGATTTACTGCACCC	62	110	qRT-PCR	99
			Right	GTGAAGCTGCCTCTTTAATGGC	61			
			Left	TAATACGACTCACTATAGGG ACTTTTTCAGGGAGGCCATT	58	378	RNAi	
			Right	TAATACGACTCACTATAGGG GTCGCAGCGTCTCATCAATA	58			
			CM1 upstream (-2146 to +1)	CGACAACACAAGGAAATCCTATTCC	63	2146	CYP6CM1 up sequences analysis	
				TGCTCTCGGTTAAATCTGCAAAAAG	64			
			CM1 upstream (-2026 to -4190)	GGTGGGATTCTTCATAGTTTTCTTATTTG	64	2165		
				CGTCGATATGAGGAAAAGGGGTAC	63			
			CM1 upstream (-4033 to -6084)	GATTGGCCGTACAGTTAGTTAGCC	61	2052		
				CCATCAACAGGATCCTTTTGTATTC	61			
cAMP-response element binding protein	CREB	Bta05712	Left	ATGGACGGGATGGTGGAGG	62	849	Gene clone	
			Right	TCAATCTGTTTTTTGCTGGCAATAGAGC	63			
			Left	ACTCAAGGCAGTCTCCAAACCC	62	154	qRT-PCR	99
			Right	TTTCTGCTCCGCCTAAATCGTT	63			
			Left	TAATACGACTCACTATAGGG AAGCGGTGACCCTTTATCCT	58	365	RNAi	
			Right	TAATACGACTCACTATAGGG TTGACGACTCCACTTTGCAG	58			
			Left	ATGACAAATGCATCATCAGGAGG	61	312	CREB-DN1	
			Right	ATCTGTTTTTTGCTGGCAATAGAGC	63			
			Left	ATGGACGGGATGGTGGAGG	63	618	CREB-DN2	
			Right	CACCACAATTTCACCTGGTACG	60			
			CREB upstream (-2259 to +1)	GAAGAAACTGGTGTAGCCAGGATC	61	2259	CREB up sequences analysis	
				AGCCTGGGACCTCCCTCACAG	65			
p38	p38	Bta12848	Left	ATGTCTAATTTTTACAGGGTTGAAATC	60	1080	Gene clone	
			Right	AGAGGAAGCAACTTCACCTTCC	59			
			Left	GAACGCCGTCGGAGGATACTT	63	115	qRT-PCR	103
			Right	TTGGCTCCTTTGAACACTTGC	61			
			Left	TAATACGACTCACTATAGGG AAGGGTTTTGGTGCTTTGTG	58	371	RNAi	
			Right	TAATACGACTCACTATAGGG CTGGCAGAAGAGAAGGATGG	58			
Extracellular signal-regulated kinase	ERK	Bta01850	Left	ATGGCGGCCGAGGGCTCG	65	1092	Gene clone	
			Right	CATGCCTGCCTCATCTTGCC	63			
			Left	AGATTATTTCCTTCAGCCGATGC	62	100	qRT-PCR	98
			Right	GGGCAAGGGCATCTTCAACTAC	63			
			Left	TAATACGACTCACTATAGGG AGGTGCTTATGGAATGGTCG	58	389	RNAi	
			Right	TAATACGACTCACTATAGGG GGTCACACGTTGTGTTGAGG	58			
pGL4.10-CYP6CM1$^{-276\ to\ +1}$	CM4.1F-276	—	Left	AATGGAAAATGCCGCCAAATG	64	276	Promoter analysis	
pGL4.10-CYP6CM1$^{-458\ to\ +1}$	CM4.1F-458		Left	GAACCAAACAGACGGACGGATAA	63	458		
pGL4.10-CYP6CM1$^{-570\ to\ +1}$	CM4.1F-570		Left	GAAACCTGCCTGCGTCGTAAT	62	570		
pGL4.10-CYP6CM1$^{-702\ to\ +1}$	CM4.1F-702		Left	CTTTATCTCGTCAACGCAGGGTA	61	702		
pGL4.10-CYP6CM1$^{-761\ to\ +1}$	CM4.1F-761		Left	GTAATACATGATTCAAGTGGACCGACT	62	761		
pGL4.10-CYP6CM1$^{-812\ to\ +1}$	CM4.1F-812		Left	GCAATTACTGTTCGACAGGCG	61	812		
pGL4.10-CYP6CM1$^{-865\ to\ +1}$	CM4.1F-865		Left	CTTACCACAGCTTCAAACGGGG	63	865		
pGL4.10-CYP6CM1$^{-920\ to\ +1}$	CM4.1F-920		Left	GCCATTTAAACCTATAGAAGAGGGTG	63	920		
pGL4.10-CYP6CM1$^{-939\ to\ +1}$	CM4.1F-939		Left	TATTGATTGATCTGCCATTTAAACCTAT	62	939		
pGL4.10-CYP6CM1$^{-968\ to\ +1}$	CM4.1F-968		Left	TCAACAGCAGTGGCGTGGC	63	968		
pGL4.10-CYP6CM1$^{-1196\ to\ +1}$	CM4.1F-1196		Left	GGGGCGCTACATAGATTCCG	62	1196		
pGL4.10-CYP6CM1^{+1}	CM4.1R-1		Right	TGCTCTCGGTTAAATCTGCAAAAAG	64			
Aryl hydrocarbon receptor	AHR	Bta13125	Left	ATGTCTGCATTAGCTCATTCTGTCCTT	64	1965	Gene clone	
			Right	CTCAAAGGGCCCATTGAACATAT	62			
			Left	GCGGAACTGTGAAGAAAGAAGGT	61	183	qRT-PCR	96
			Right	CAGCGTAGTTATGACCGTCCTTT	60			
CCAAT/enhancer-binding protein	CEBP	Bta03941	Left	ATGGACTCGCCGCAGATGTAC	62	846	Gene clone	
			Right	CTGAACACCGTGGTGAGGATAG	59			
			Left	CAAACACCACCATCACCACAGT	60	184	qRT-PCR	99
			Right	CAAGCGTTCGTTTTCTTTCACT	59			
Drosophila melanogaster nuclear hormone receptor HR96	DHR96	Bta02014	Left	ATGGACTTGAAACATGATACAGCG	61	1593	Gene clone	
			Right	TGAAACAACATCCCAAATCTCC	59			
			Left	GGCGAATAGTTTGAACGGGTA	59	137	qRT-PCR	95
			Right	TGAGGGCGTTGTAGTGGTAGC	60			

Ecdysone-Induced Protein 23	E23	Bta04225	Left	ATGGTCAAGCCTGGCGAACT	62	1956	Gene clone	
			Right	TTTGGGTGTTCGAAAATAACG	58			
			Left	CCAGGGATGGATGTTTTTCTCTA	60	101	qRT-PCR	98
			Right	GCTCTTTGTTTATGACCTCCCGT	61			
Ecdysone-Induced Protein 63	E63	Bta13636	Left	ATGGCTGATCAGCTGACGG	59	450	Gene clone	
			Right	CTTTGATGTCATCATTGTCACAAAC	59			
			Left	TGGAGATGGAACAATCACGACA	62	179	qRT-PCR	99
			Right	CGCTATCTGTGTCTTTCATTTTCCT	61			
Ecdysone-Induced Protein 78	E78	Ssa11354	Left	ATGGAGACCGTCACGACACC	60	1302	Gene clone	
			Right	CTGGAGATCCTCTTCACACTTAGG	59			
			Left	CATCAAACAGCATCAAGATAGGC	59	155	qRT-PCR	99
			Right	TAAACCAGTCTAAATGAGCGGAA	59			
Hepatocyte nuclear factor 6	HNF6	Bta12954	Left	ATGGTGTCCACGCCGTCG	63	822	Gene clone	
			Right	AAGTCGTGGTGTTGGCCGC	64			
			Left	ATGGACAAGTGGAAGGACGAGG	62	101	qRT-PCR	96
			Right	GATGGCTGGTCGTCGGAGA	61			
Hormone receptor 3	HR3	Bta04882	Left	ATGTCCAGAGATGCGGTGAAATTCG	63	1362	Gene clone	
			Right	GCTATCAACCGAGAACAACTCTTT	59			
			Left	TGGCTGTGATAAGGATGTCGC	60	195	qRT-PCR	99
			Right	CCGCAGAATACAGGGCAAGTT	61			
Hormone receptor 4	HR4	Bta02331	Left	ATGGAGGATTTACATCTCATCAAAT	60	2268	Gene clone	
			Right	ATTATAAAATCGGTGCGGAGTG	59			
			Left	TGTTCCGTTTTGATTACGCATTT	61	192	qRT-PCR	96
			Right	CTTTTTTACAATCTTGCGTTTTACCT	60			
Hormone receptor-like in 38	HR38	Bta11834	Left	ATGATTCTCGCGTGGCGGGG	62	2271	Gene clone	
			Right	AAATGGTAAACTAGCAACAAACATGT	59			
			Left	AGTTTTGGTGACTGGCTCTTCG	61	120	qRT-PCR	97
			Right	TCCGTGACGCTCTGTGATGATA	61			
Kruppel-like factor 11	KLF11	Bta10336	Left	ATGGTGCTCACTATTGATACTAGCG	60	1248	Gene clone	
			Right	AATCATTTGAGTGAGGAAGTTCTTC	58			
			Left	CGTTACCTGAAAGGAATGGACC	60	139	qRT-PCR	103
			Right	GCTTCTTTCCATAGTCAACATCCAT	61			
Muscle aponeurosis fibromatosis-S	Maf-S	Bta07674	Left	ATGAACAGGAAATATGAACGAAAAATT	61	417	Gene clone	
			Right	TAAGCATCCACTTTTTAAGGACTCC	60			
			Left	CGAAATGATGGAAAAAGATAACAGTC	61	107	qRT-PCR	99
			Right	GAATAGGCACTTTCCTCATCAGC	60			
Nuclear receptors 2E	NR2E	Bta00533	Left	ATGGAGGGTCTGTCGGAGACG	63	1182	Gene clone	
			Right	TTGCCGGAGCATGTCTGTG	60			
			Left	GAAAGAGGATGAAGTAAGCAGTTCAG	60	108	qRT-PCR	102
			Right	GAAACATCGTCCTGTCGCTCTT	60			
Nuclear factor E2-related factor-2	NRF2	Bta00634	Left	ATGACCTCCGCCCTCCCG	65	1518	Gene clone	
			Right	ATGATCCAGACTCCCCAGTTCC	62			
			Left	AACGGAACCATTTCTCCTCTTG	61	76	qRT-PCR	96
			Right	TGTGCGGAGGGTCCTTGTG	62			
Rretinoid X Receptor	RXR1	Bta11398	Left	ATGGAAGGCAGTGAAAGAGGAC	60	1203	Gene clone	
			Right	CAGTGTGCGATCGGAATTTTC	61			
			Left	ATCTCCTCTTCCACCAAACCATC	61	163	qRT-PCR	97
			Right	TTCACGGCAAGCATAAGACAAGT	61			
pAc5.1b	—	—	Left	GACACAAAGCCGCTCCATCAG	63	252	Vector linearization	
			Right	CCTTAGAAGGCACAGTCGAGGC	63			
pGL4.10	—	—	Left	TAGCAAAATAGGCTGTCCCCAG	61	283	Vector linearization	
			Right	CGTCGGTAAAGGCGATGGTG	63			
60S ribosomal protein L29	RPL29	Bta02466	Left	TCGGAAAATTACCGTGAG	60	144	qRT-PCR	101
			Right	GAACTTGTGATCTACTCCTCTCGTG	60			
elongation factor 1 alpha	EF-1a	Bta04011	Left	TAGCCTTGTGCCAATTTCCG	60	110	qRT-PCR	103
			Right	CCTTCAGCATTACCGTCC	60			
β-actin	Actin	Bta08780	Left	TCTTCCAGCCATCCTTCTTG	60	174	qRT-PCR	96
			Right	CGGTGATTTCCTTCTGCATT	60			

nAChRs-α1	α1	Bta11027	Left	ATGCTGGGCGTGGTTAGAATG	62	1686	Gene clone	
			Right	ATCCTCCTCGGGGCCCAT	63			
nAChRs-α2	α2	Bta11032	Left	ATGTGCTTCCGGGAGTGGCT	64	1671	Gene clone	
			Right	AAAATCGGAGGTGACGTCGG	62			
nAChRs-α3	α3	Bta14972	Left	ATGAGGATAATTTACTGGATAATCGTTG	61	1707	Gene clone	
			Right	TAGAGTCGTGGTAGTAACTTGCGGC	64			
nAChRs-α4	α4	Bta05486	Left	ATGATCTTCGTTTTTGCTTTAATCCT	61	1602	Gene clone	
			Right	AAGTGCATCTAAAATCTTGTCTCGG	61			
nAChRs-α5	α5	Bta11534	Left	ATGCGCCTGTTGAGTGACAATC	62	1452	Gene clone	
			Right	CGGGCCGTATGGGGATCC	65			
nAChRs-α6	α6	Bta05486	Left	ATGATTCGGGCCGACTCCTATT	63	1386	Gene clone	
			Right	TCACGCCTGGCTGTGGATACTT	64			
nAChRs-α7	α7	Bta02663	Left	ATGGTGCGGATCGGTGTTGT	63	1515	Gene clone	
			Right	GTTGACGATAATATGCGGGGC	62			
nAChRs-α8	α8	Bta05486	Left	ATGCTCTTCGTTTTTGCTTTAATCC	62	1602	Gene clone	
			Right	AAGTGCATCTAAAATTTTGTCTCGG	61			
nAChRs-β1	β1	Bta03349	Left	ATGAAAACGTCGCTAGTGGCTACT	61	1524	Gene clone	
			Right	CTTTCCACGGTAGATCTCAATTATTC	60			
MEAM1 Genome Website	http://www.whiteflygenomics.org/cgi-bin/bta/blast.cgi							