Expression of CD47 in the pancreatic β-cells of young people with recent-onset type 1 diabetes varies according to disease endotype

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Data Availability: Data supporting the conclusions reported in this paper are available from the authors on reasonable request.

Abstract

Aims: We are studying the dialogue between β-cells and the immune system in type 1 diabetes and have identified a cell surface receptor, Signal Regulatory Protein-alpha (SIRPα) as an important component in the regulation of β-cell survival. SIRPα interacts with another protein, CD47, to mediate signalling. In the present work, we have studied the expression and role of CD47 in human islet cells in type 1 diabetes.

Methods: Clonal EndoC-βH1 cells were employed for functional studies. Cells were exposed to pro-inflammatory cytokines and their viability monitored by flow cytometry after staining with propidium iodide. Targeted knockdown of CD47 or SIRPα was achieved with small interference RNA molecules and the expression of relevant proteins studied by Western blotting or immunocytochemistry. Human pancreas sections were selected from the Exeter Archival Diabetes Biobank and used to examine the expression of CD47 by immunofluorescence labelling. Image analysis was employed to quantify expression.

Results: CD47 is abundantly expressed in both α and β cells in human pancreas. In type 1 diabetes, the levels of CD47 are increased in α cells across all age groups whereas the expression in β-cells varies according to disease endotype. Knockdown of either CD47 or SIRPα in EndoC-βH1 cells resulted in a loss of viability.

Conclusions: We conclude that the CD47 plays a previously unrecognised role in the regulation of β-cell viability. This system is dysregulated in type 1 diabetes suggesting that it may be targeted therapeutically to slow disease progression.

Introduction

During the progression of type 1 diabetes, pancreatic β-cells are targeted by autoreactive immune cells which infiltrate the islets of Langerhans to mediate a selective cytotoxicity [1-5]. This process probably evolves at a different rate in each individual but it culminates, ultimately, in the demise of the β-cells and the loss of effective glucose homeostasis. Until recently, it had been widely held that such selective targeting is driven largely by defects in immune regulation but it is now increasingly accepted that this is an over-simplification and that β-cells engage in an active dialogue with the immune system as a means to influence the rate and progression of cytotoxicity [6]. This is achieved in a number of ways including by the mounting of a vigorous cellular response designed to repel the autoimmune attack. Thus, as insulitis develops, β-cells upregulate various cell surface molecules having the potential to impair the functional capacity of immune cells. Such responses include an elevation in the display of non-classical cell-surface HLA molecules (such as HLA-E and -F) which disrupt the responses of certain innate immune cells, including macrophages, dendritic cells and NK cells[7]. They also increase the expression of PDL-1 to actively suppress the activity of influent PD-1-bearing CD8+ T-cells [8]. In addition, β-cells retain a variety of additional mechanisms to resist autoimmune attack, including the expression of cytoprotective pathways deriving from receptor-mediated activation of the transcription factor, STAT6, by anti-inflammatory molecules such as interleukin (IL)-4 and IL-13. This leads to the induction of specific downstream effectors that antagonise pro-apoptotic signalling and thereby also promote cell viability in the face of active autoimmunity [9].

We have been studying the defensive responses of β-cells to targeted autoimmunity in type 1 diabetes and have discovered that a further component in their armoury involves the upregulation of Signal Regulatory Protein-alpha (SIRPα); a protein whose expression is controlled by the state of activation of STAT6 [9]. SIRPα is a transmembrane glycoprotein which transduces signals that sustain cell viability and we have shown that depletion of SIRPα from β-cells leads to a direct decline in their viability [9,10]. Importantly, SIRPα also serves as a receptor for a second surface protein, CD47, which acts as a ligand to promote signalling, implying that these two proteins may act in concert to regulate β-cell viability[11,12].

The interactions between SIRPα and CD47 have been studied most widely in the immune system, where they fulfil a very specific role. In particular, CD47 has become known as a “don’t eat me” signal since it can engage SIRPα present on the surface of macrophages, with the result that rates of phagocytosis are inhibited and the active engulfment of CD47-bearing cells minimised [10,13,14]. These mechanisms are subverted to advantage by certain tumour cells (including some pancreatic neuroendocrine tumours) which overexpress CD47 and thereby minimise their susceptibility to targeting and removal by cells of the innate immune system [15-17]. In addition, the mechanism may also be relevant to type 1 diabetes since enhanced signalling has been implicated in delaying the progression of insulitis in NOD mice where CD47 expression by islet cells is thought to exert a protective influence against the early macrophage infiltration seen in these animals [18-20]. A similar mechanism may also operate in humans early in the disease process although, overall, macrophages appear to play a much less significant role in human insulitis than in NOD mice. This implies that a fundamentally different mode of action may apply to the CD47/SIRPα system in human β-cells.

In the present study, we have addressed this possibility and show that CD47 is present on both α- and β-cells in human islets but that its expression levels are altered on these cells in type 1 diabetes. We also provide evidence that ligation of CD47 and SIRPα may be important for the normal regulation of β-cell viability suggesting that selective alterations in this pathway may represent an additional mechanism employed by β-cells to resist the cytotoxic effects of autoreactive immune cells during the progression of type 1 diabetes.

Methods

Immunostaining

Pancreas samples (Supplementary Table 1) were from the Exeter Archival Diabetes Biobank (http://foulis.vub.ac.be/) and used with full ethical approval[21]. For immunoperoxidase staining, pancreas sections were dewaxed, rehydrated and heated in citrate buffer (pH 7.4) for 20min in a microwave oven at full power. Sections were blocked in normal goat serum (5% vol/vol) before probing with primary and secondary antibodies (Supplementary Table 1). Counter staining was performed with haematoxylin prior to mounting sections with distryrene-xylene mountant. Where two or more antigens were stained on the same section, sequential staining was performed with primary antibodies and species-specific fluorescently labelled secondary antibodies.

Fluorescence images were captured using a Leica AF6000 microscope (Leica Microsystems, Milton Keynes, UK) and image quantification achieved using standardised microscope and camera settings after random selection of insulin containing islets (ICIs) from individuals with and without diabetes. Mean fluorescence intensity (MFI) was computed using an in-house MATLAB Script (version R2015b), VIOLA (www.mathworks.com/products/new_prodocts/release2015b.html) and values were obtained only from areas which were also immunopositive for either glucagon or insulin.

Cell Culture and analysis

Mouse Alpha-TC cells were obtained from the ATCC and were cultured in DMEM supplemented with 1mM glucose (Sigma-Aldrich, UK), sodium pyruvate 100mM (Lonza, UK), 10% FBS (Gibco), 1mM HEPES, amino acids (diluted from a 50x stock; Merck, UK: #M5550) and 0.02% bovine serum albumin (BSA; fraction V), 100 U/ml penicillin, 100ug/ml streptomycin [22]. Cells were exposed to 20ng/ml of IL1β, IFNγ, TNFα, IL-6 (all from R&D Systems, Abingdon, UK) for 48h as reported[9].

Human EndoC-βH1 cells were provided by Prof R Scharfmann via Univercell-Biosolutions (Toulouse, France) and cultured as described previously[23]. Cells were maintained in DMEM (Gibco) containing 5.5mM glucose, 2% (w/v) BSA; fraction V, 10mM nicotinamide, 50µM β-mercaptoethanol, 100 U/ml penicillin, 100ug/ml streptomycin, 2mM L-glutamine, 2.7nM sodium selenite and 5.5ug/ml transferrin[23]. Cells were routinely tested for mycoplasma contamination and were negative.

Cell viability was estimated by flow cytometry after staining with propidium iodide (Merck) as described previously. These experiments were repeated at least three times on separate occasions[24].

Western blotting

Cellular proteins were extracted and used for western blotting as described previously[24]. Mouse anti-CD47 antibody (R&D Systems, Abingdon, UK) and anti-GAPDH (Proteintech, UK) were added in blocking solution at 4^0C overnight. Membranes were washed for 15min in Tris-buffered saline-Tween (TBST) and probed with alkaline phosphatase-conjugated secondary antibodies (all from Merck, Darmstadt, Germany) for 1h at room temperature. CDP-star Chemiluminescent substrate (Merck) was used for detection in a Licor Odyssey detection system (Licor, Cambridge, UK). Densitometry analysis were performed using image studio version 5.2 (https://www.licor.com/bio/products/software/image_studio/).

Interference RNA-mediated knockdown

Interference RNA methods were used to knockdown human CD47 and SIRPα. Target-specific sequences (CD47 ID: 145977, SIRPα ID: 109944) and scrambled control RNA were purchased from Thermofisher (Paisley, UK). Cells were transfected by combining siRNA with Optimem reagent (Thermofisher, Paisley, UK) and lipofectamine RNAi Max (Invitrogen, Boston, MA, USA) according to the manufacturers' instructions. Knockdown was confirmed by immunostaining and by qRT-PCR.

RT-qPCR

RNA for RT-qPCR was extracted using RNeasy mini kits (Qiagen, Hilden, Germany) and quantified by NanoDrop (Thermofisher, Paisley, UK). cDNA synthesis was achieved using the RT2 First Strand kit (Qiagen). Primers were sourced from Qiagen and reactions were performed on a QuantStudio Flex 12K instrument (Applied Biosystems, Boston, MA, USA). Gene expression was obtained by the comparative threshold cycle method ($2^{-\Delta\Delta C_t}$) after normalising with housekeeping genes, GAPDH and ACTB [25].

Statistical analysis

Statistical analysis was performed using Graphpad Prism and data are presented as mean values ± SEM. Statistical significance was evaluated using either a Mann-Whitney test or ANOVA (with Tukey's post hoc test) unless otherwise stated, with $p<0.05$ considered as statistically significant

Results

Expression of CD47 in human pancreas

Immunohistochemical staining of human pancreas sections from non-diabetic donors revealed a strong expression of CD47 in all islets, with much lower levels in the surrounding exocrine tissue (Fig. 1a). Co-staining with fluorescently labelled antisera confirmed that CD47 is present in both α- (co-stained with glucagon) and β- (co-stained with insulin) cells and analysis of the respective mean fluorescence intensity (MFI) values suggested that CD47 is present at higher levels in β-cells. (Fig. 1b,c).

Islet cell CD47 expression is altered in type 1 diabetes

Analysis of CD47 expression in pancreas sections from individuals with recent-onset type 1 diabetes revealed marked changes when compared with similarly aged controls. Initially, comparison of α-cell immunostaining was undertaken and this revealed that CD47 is increased significantly ($p<0.001$) in islet α-cells in type 1 diabetes (Fig. 2a,b). This response was observed across all age-groups studied. Since type 1 diabetes is characterised by islet inflammation, in vitro studies were then performed to investigate the possible molecular basis of this phenomenon. In the absence of a suitable human α-cell line, mouse alpha-TC1 cells were employed and were treated with a cocktail of pro-inflammatory cytokines (IL1β, IFNγ, TNFα, IL-6) for 48h to simulate the islet milieu in type 1 diabetes. Subsequent Western blotting analysis revealed that CD47 was markedly elevated in response to cytokine exposure in the α-cell line (Fig 2c). At least three separate protein bands were immunostained and all were apparently up-regulated under these conditions.

To study the presence of CD47 in human β-cells in diabetes, pancreas samples were sub-divided according to their immune cell profiles since these have been shown recently to define two endotypes, known respectively as type 1 diabetes endotypes 1 (T1DE1) and 2 (T1DE2). These segregate according to age at diagnosis and we therefore examined three pancreases from children diagnosed in the younger age group (under 13 years; T1DE1) and compared the staining patterns with those found in a further three pancreas specimens from the older group (>13y; T1DE2). Relevant similarly-aged controls were employed in both cases.

As expected, CD47 was expressed strongly in the β-cells of control subjects but important differences were noted when the residual β-cells were examined in those with type 1 diabetes (Fig. 3). These differences varied according to disease endotype. In the residual β-cells of subjects classified as T1DE1, CD47 was increased significantly by comparison with controls, whereas in those classified as T1DE2, the levels of CD47 were reduced in β-cells (Fig. 3a). To quantify these changes, seven insulin-containing

islets (ICIs) were selected at random in each pancreas section and these were imaged using standardised microscope and camera settings for all subjects. Analysis of the MFI confirmed a significant upregulation of CD47 in ICIs in T1DE1 when compared to age matched controls and a corresponding reduction in those with T1DE2 (Fig. 3b).

Loss of CD47 is detrimental to β cell viability

The potential implications of a loss of CD47 (as observed in the β-cells among the T1DE2 cohort) was simulated by achieving the selective knockdown of CD47 in cultured human EndoC-βH1 cells using a small interference RNA approach. Transfection of the cells with siRNA targeted against transcripts encoding CD47 yielded a significant diminution at both the messenger RNA level (Fig. 4a) and when assessed at the protein level by immunocytochemistry (Fig. 4b,c). A parallel analysis demonstrated that knockdown of CD47 caused a significant loss of cell viability as judged by an increase in the proportion of PI positive cells (used as an index of cell death) when compared with cells transfected with scrambled control siRNA (Fig. 4d). In parallel experiments (Fig. 4a), SIRPα was knocked down independently and, in accord with our previous findings[9], this resulted in an equivalent loss of viability to that seen when CD47 levels were attenuated (Fig. 4d).

Finally, the effects of incubation with a cocktail of pro-inflammatory cytokines on the levels of CD47 in human EndoC-βH1 cells, was studied. In common with the response seen in the mouse alpha-TC cell line (Fig. 2c) this resulted in a marked increase in CD47 levels (Fig. 5).

Discussion

We present data implying that CD47 and its cognate ligand, SIRPα, may form part of a series of mechanisms deployed by pancreatic β-cells to resist the cytotoxic activity of immune cells in type 1 diabetes. CD47 and SIRPα have been widely understood to play important roles in immune regulation and CD47 is expressed on many cell types [14]. By contrast, the presence of SIRPα is more restricted but we have shown previously that it is present on pancreatic β-cells and is subject to regulation by anti-inflammatory cytokines acting via STAT6[9]. Although we have not proven conclusively that formal interactions between these molecules are required, our data provide strong evidence that CD47 and SIRPα have the propensity to interact within the confines of the islets of Langerhans to regulate β-cell viability. As such, we suggest that the physiological role of these proteins is likely to extend beyond their previously ascribed capacity to influence the phagocytic activity of macrophages and that, in the context of islet autoimmunity, their interactions may influence β-cell viability more directly.

Our studies confirm recent evidence that human islet cells express abundant levels of CD47[15,15,19] and we reveal that it is present on both α and β-cells. Analysis of the intensity of immunolabelling with an antiserum directed against CD47 implies that β-cells express higher levels of CD47 than α-cells in individuals without diabetes and this was true across all ages studied. However, in diabetes, a significant increase in α-cell CD47 occurs and this was seen both in children with the most aggressive form of type 1 diabetes (type 1 diabetes endotype 1 (T1DE1)) who are youngest in age at onset (typically <13y) and also in those with a less-aggressive autoimmune attack (T1DE2) who are older at diagnosis. Thus, the upregulation of CD47 on α-cells appears to be a characteristic (but previously unrecognised) feature of type 1 diabetes in humans. The factors that drive this response have not been fully defined but in vitro studies with mouse alpha-TC cells reveal that incubation with pro-inflammatory cytokines leads to a marked increase in CD47 expression. Thus, it is plausible that the pro-inflammatory milieu associated with islet inflammation is a contributory factor. It should also be noted that the antibody employed in these studies labelled at least three separate bands on the Western blots of alpha-TC1 cells consistent with the possibility that several of the known isoforms of CD47 may be present[26]. Further studies will be required to verify the precise isoform complement.

By analogy with the situation in other cell types (including some pancreatic neuroendocrine tumours) the increase in CD47 on islet α cells might be sensed by islet macrophages in a manner which then impairs their rate of phagocytosis[14,15]. However, this mechanism is unlikely to be of major significance in the development of type 1 diabetes since α-cell numbers are not depleted by macrophage engulfment in this disease**.** Rather, it seems more likely that the increase in α-cell CD47 occurs as an active response to minimise the rate of β-cell death and we suggest that this may involve the ligation of SIRPα. In support of this, we have demonstrated that knockdown of SIRPα in either rodent (INS-1E [9]) or human (EndoC-βH1) cells (Fig. 5) leads to a significant loss of viability, thereby suggesting that signalling via SIRPα helps to sustain β-cell viability. We now reveal that selective knockdown of CD47 is also associated with loss of viability in human β-cells and, taken together, these findings are consistent with the concept that interactions between CD47 and SIRPα may mediate anti-apoptotic signalling in these cells.

In drawing these conclusions, we emphasise that many of our functional data were derived from a culture system in which only a single cell type was present (i.e. β-cells were grown in the absence of α-cells and vice versa) and where cell contacts were largely minimised because the cells were maintained in a monolayer configuration. Hence, it is accepted that this system does not recapitulate the situation found within the 3D architecture of human islets in situ. Nevertheless, the data are informative since they point to the possibility that both "cis" (where the ligand and receptor exist on the same cell) and "trans" (where each component is expressed on a neighbouring cell) activation of SIRPα by CD47 could be relevant for the normal physiology of islet cells[27]. On this basis, the upregulation of CD47 seen on α-cells in type 1 diabetes may then represent a compensatory mechanism intended to sustain and promote β-cell viability in the face of declining SIRPα levels associated with loss of STAT6 [9]. Importantly, this does not preclude the possibility that "cis" activation between components present on the same individual β-cell within an islet might also be possible[10]. We note, too, that even if trans activation of CD47 and SIRPα on adjacent cells is the predominant cellular mechanism, then both heterotypic (alpha-beta cell) and homotypic (beta-beta cell) interactions could be involved in mediating this response. Leading on from these considerations, we accept that it has not been formally proven that the changes in cell viability reported here necessarily occur as a direct consequence of disrupted interactions between CD47 and SIRPα since we have studied each component separately. Nevertheless, they are fully consistent with this scenario.

Interestingly, despite the similarity in α-cell responses between the two endotypes of type 1 diabetes, we observed an important difference in CD47 expression between the β-cells of people with T1DE2 compared to those with T1DE1. Thus, unlike the situation in T1DE1, where β-cell CD47 levels were increased (possibly driven by the stronger pro-inflammatory response in the islets of these individuals[5]), the expression of CD47 was decreased on the majority of β-cells in subjects with T1DE2. As indicated earlier, this may be important mechanistically in terms of the maintenance of cell viability, but it is also of significance from the point of view of the definition of disease endotypes. This is because, as with immune cell profiles and differences in proinsulin processing[5,28], these new data point to additional underlying aetiological differences in the responses of islet cells to autoimmunity, according to age at diagnosis.

Our finding that CD47 is depleted in the β-cells of young people with T1DE2 at, or soon after, disease onset but is increased in those with T1DE1, might be taken to imply that, in T1DE2, the β-cells are more susceptible to the influence of pro-apoptotic stimuli. At first sight, this does not seem to accord with evidence that individuals defined as T1DE2 retain greater numbers of β-cells at diagnosis than those with T1DE1[28]. However, because in T1DE1 most β-cells have been destroyed by the time of disease onset (<10% of the original β-cell complement is retained at diagnosis compared with equivalently aged controls) it might also be argued that the few residual β-cells found in those with

T1DE1 represent cells which are endowed with specific features (such as elevated CD47) rendering them most resistant to the autoimmune attack. Resolution of this apparent conundrum will require further study and may be facilitated by access to pancreas samples from autoantibody-positive individuals who have not yet succumbed to clinical disease.

Overall, the current work reveals a previously unheralded aspect of the pathophysiology of the endocrine pancreas in type 1 diabetes. It suggests that the CD47/SIRPα axis displays important changes that may influence the rate of β-cell loss and, given that this axis is the target of newly developed agents for cancer therapy[16,17], its potential for therapeutic intervention in type 1 diabetes also merits consideration. Indeed, it may also be important to consider whether systemically-delivered agents which disrupt cellular interactions involving CD47 could lead to impairment of β-cell viability as has been seen with drugs targeting a different immune checkpoint pathway, that involving PDL-1 and PD-1[29]. To our knowledge, there are currently no reports of such adverse events in patients receiving anti-CD47 therapy but this should be kept under review.

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Author contributions: KAL performed experiments and analysed data. MAR and NGM designed the study and analysed data. KAL and NGM wrote the manuscript and all authors edited and agreed the final version. NGM is guarantor of the work and had full access to all data generated.

Conflicts of interest: The authors have no conflicts to declare.

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Figure legends.

Figure 1: **CD47 is expressed in human islets**. a) Representative image of a section of human pancreas from a control subject immunostained for CD47. b) Co-immunofluorescence staining of CD47 (green), insulin (cyan), glucagon (red) and nuclei (dapi, blue) in control human pancreas. c) Quantification of the MFI for immunostaining of CD47 in islet α- and β-cells. Data are mean values ± SEM plotted in parallel with values for each individual islet. Data were collected from 6 separate pancreases with 7 islets examined on each pancreas section. Differences between groups were analysed with a Mann-Whitney test ***p<0.001

Figure 2. **α-cell expression of CD47 is dysregulated in type 1 diabetes**. a,b) Representative images of immunofluorescence staining of sections of human pancreas for CD47 (green), insulin (cyan) and glucagon (red) from three individuals with type 1 diabetes diagnosed either <7y (1y, 3y & 4y respectively; T1DE 1) or three older individuals (19y, 25y & 42y respectively; T1DE2) with representative similarly aged controls (shown in Supplementary Table 2). Images from both insulin containing islets (ICI) and insulin deficient islets (IDI) are shown. MFI values were recorded from 7 islets per donor and data are presented as mean values ± SEM. c) Alpha-TC cells were either exposed to vehicle (control) or a cocktail of pro-inflammatory cytokines cytokines (IL1β, IFNγ, TNFα and IL-6) for 48h then extracted for monitoring of CD47 expression by Western blotting (upper panel) and densitometry (lower panel) from three experimental replicates, each performed in duplicate. Differences between groups were analysed either by Mann Whitney test or by one way ANOVA with Tukey's post hoc test (for multiple group comparison) *p<0.05; **p<01

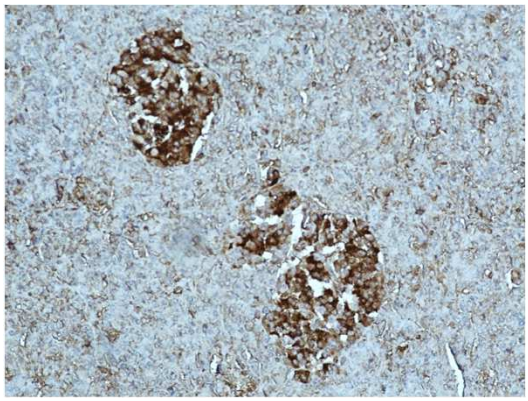
Figure 3. **CD47 expression is altered differentially in the β-cells of subjects with different endotypes of type 1 diabetes**. a,b) Representative immunofluorescence images showing immunostaining of CD47 (green), insulin (cyan), glucagon (red) and Dapi nuclei stain (blue) in islets from subjects classified as T1DE1 **(**three donors aged 1y, 3y & 4y) or T1DE2 (three donors aged 10y, 19y or 25y). Control donors spanned a similar range of ages (Supplementary table 2). Both insulin-containing and insulin-deficient islets are shown. MFI values were recorded and data are presented as mean values ± SEM (3 donors, 7 islets/donor). Differences between groups were analysed by Mann-Whitney test or one way ANOVA with Tukey's post hoc test **p<0.01 ***p<0.001

Figure 4. **Loss of either CD47 or SIRPa is detrimental to β cell survival**. a) siRNA was employed to knockdown either CD47 or SIRPα in EndoC-βH1 cells. The extent of knockdown was monitored by qRT-PCR (n=6). (b,c) Immunocytochemical staining of Endo-βH1 cells revealed a loss of CD47 after targeted knockdown when compared with cells incubated with scrambled control siRNA (n=9). Measurement of MFI values confirmed the extent of knockdown (panel c) (n=9)**.** d) The loss of viability was measured in cells exposed to either vehicle (control; left bar) or siRNA targeting CD47 (centre bar) or SIRPα (right bar) (n=9). Data are represented as mean values ± SEM from three experiments. Differences between groups were analysed by Mann-Whitney test **p<0.01, ***p<0.001

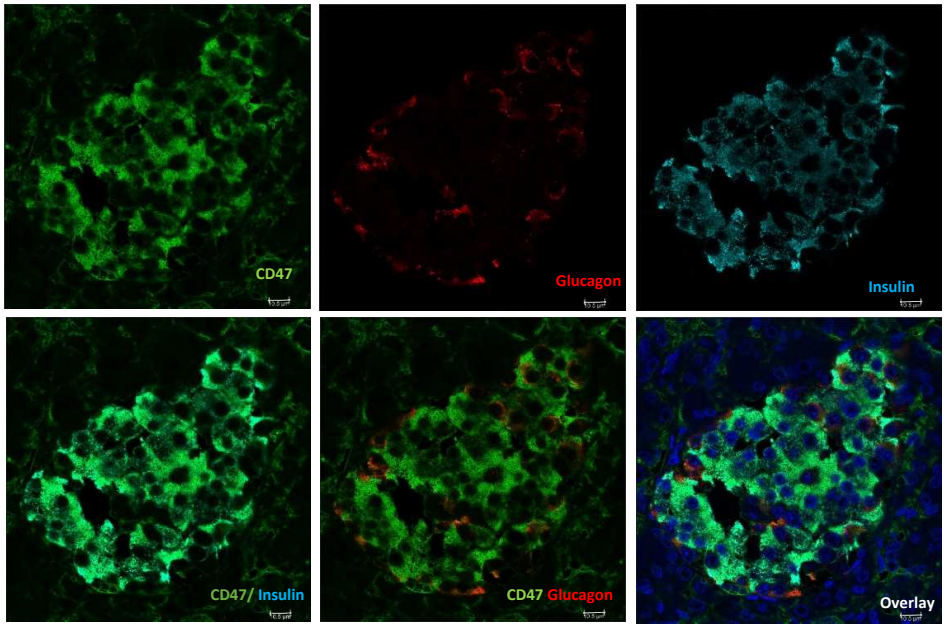
Figure 5. **Pro-inflammatory cytokines promote CD47 expression in EndoC-βH1 cells.** a) EndoC-βH1 cells were exposed to either vehicle (control) or to a cocktail of pro-inflammatory cytokines (IL1β, IFNγ, TNFα and IL-6) for 48h. Cells were then fixed and immunostained to show the presence of CD47 (green) and nuclei (dapi; blue). b) The expression of CD47 was quantified by measuring the MFI and data are shown ass mean values ± SEM from three experiments (n=9). Differences between groups were analysed by Mann-Whitney test ***p<0.001.

Figure 1

a.



b.


CD47
Glucagon
Insulin
CD47/ Insulin
CD47 Glucagon
Overlay

c.

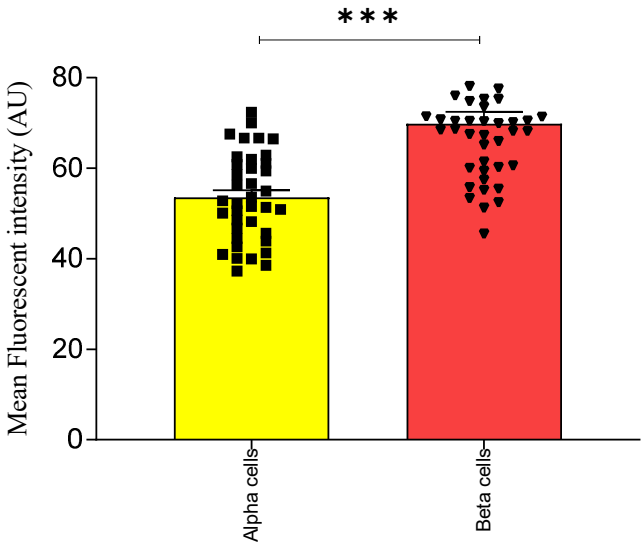
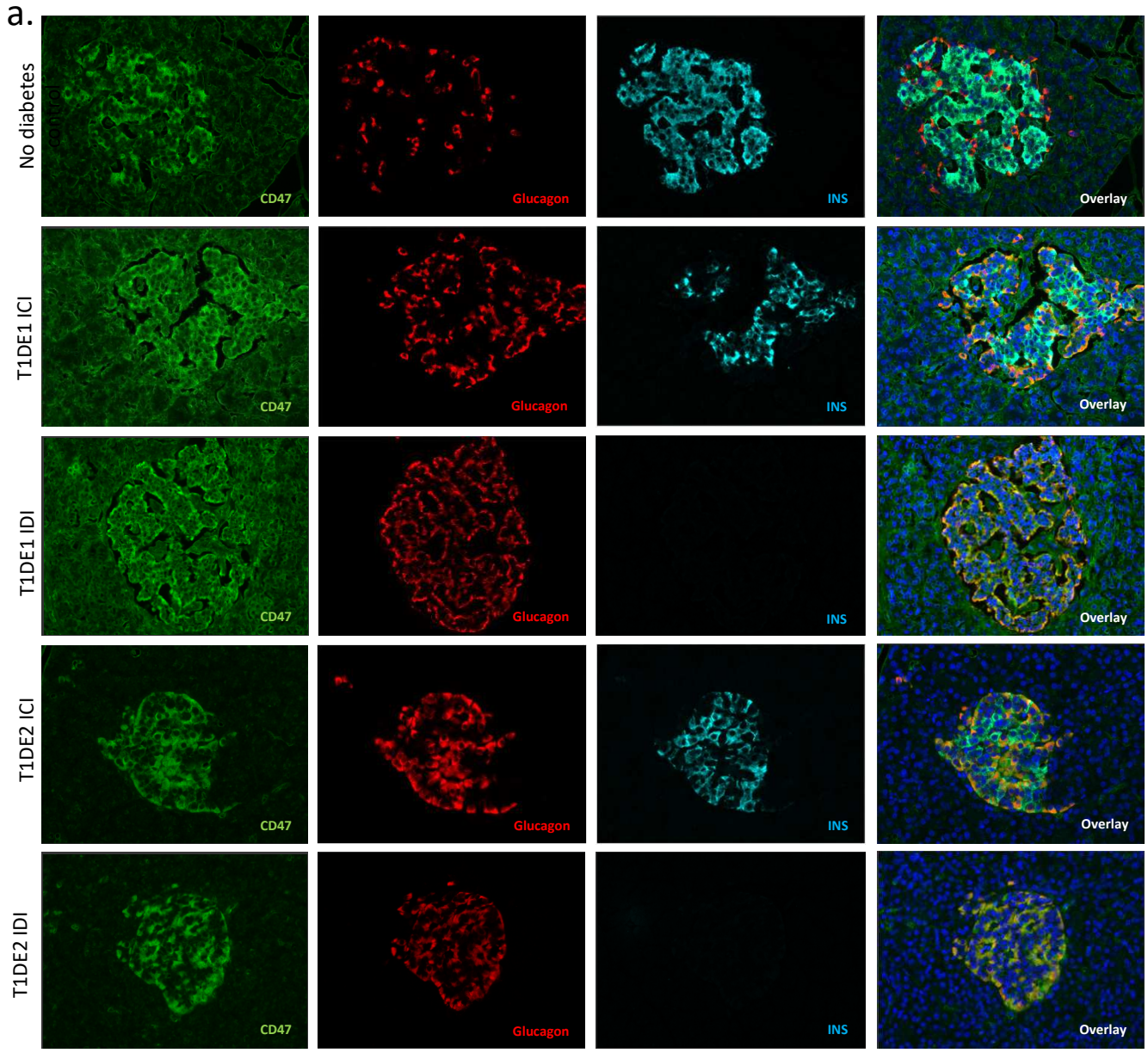
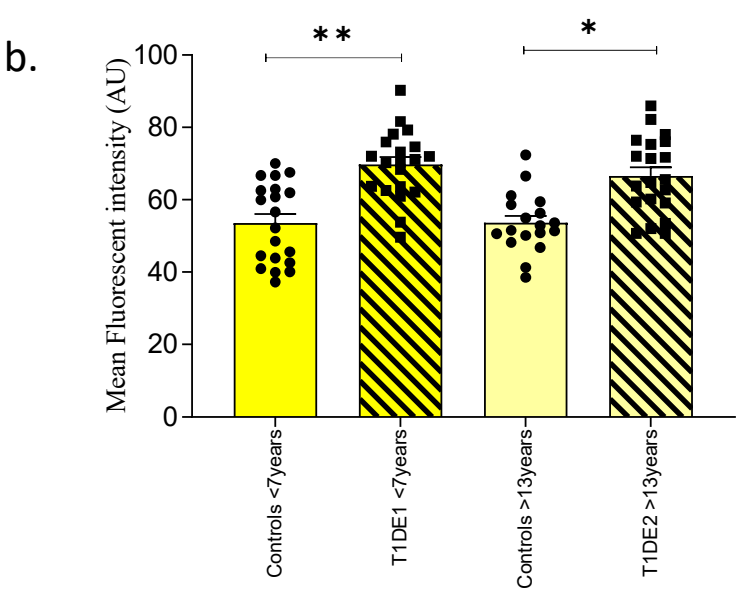

***
Mean Fluorescent intensity (AU)
80
60
40
20
0
Alpha cells
Beta cells

Figure 2


a.
No diabetes control
T1DE1 ICI
T1DE1 IDI
T1DE2 ICI
T1DE2 IDI
CD47
Glucagon
INS
Overlay


b.
Mean Fluorescent intensity (AU)
100
80
60
40
20
0
**
*
Controls <7years
T1DE1 <7years
Controls >13years
T1DE2 >13years

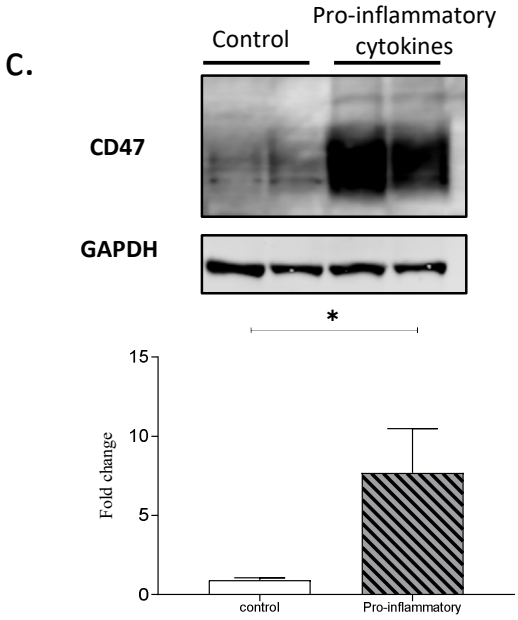
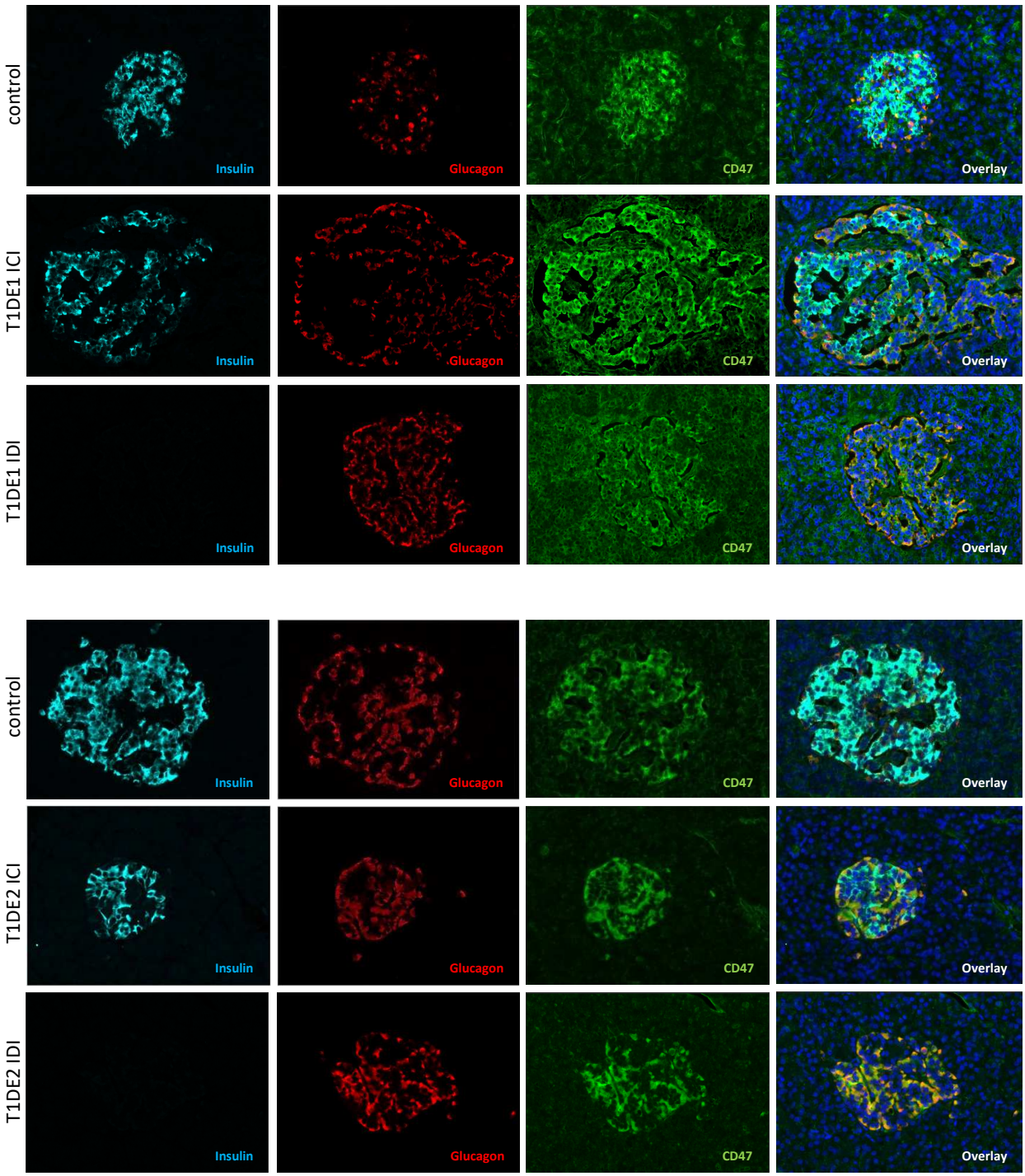

c.
Control
Pro-inflammatory cytokines
CD47
GAPDH
*
Fold change
15
10
5
0
control
Pro-inflammatory

Figure 3

a.


control
T1DE1 ICI
T1DE1 IDI
control
T1DE2 ICI
T1DE2 IDI
Insulin
Glucagon
CD47
Overlay

b.

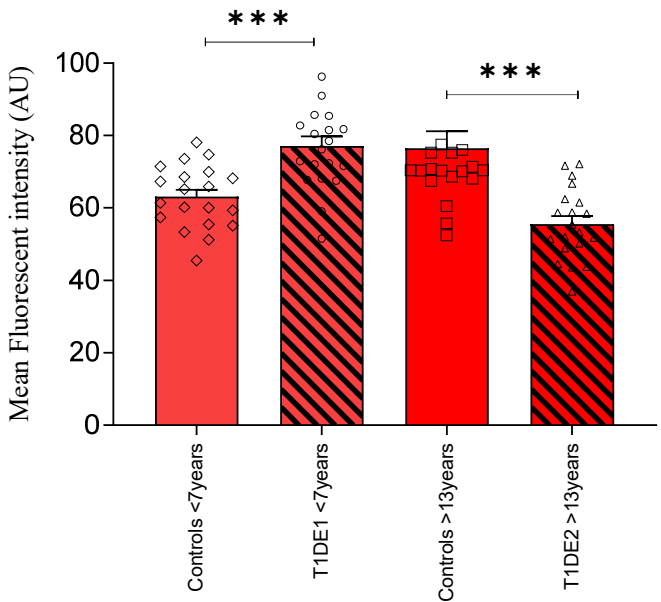
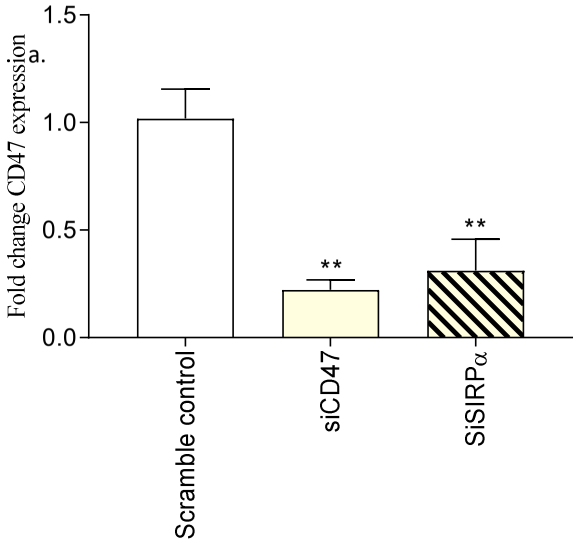
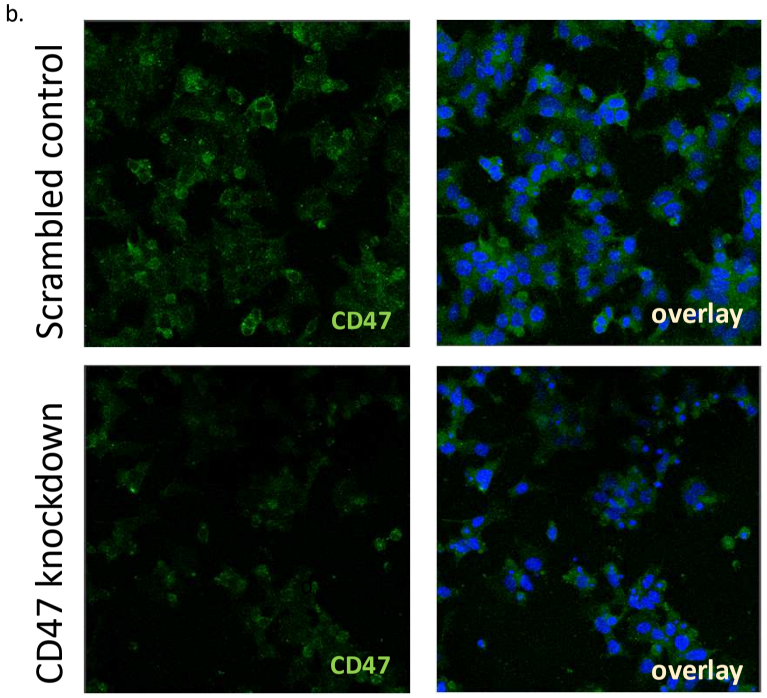
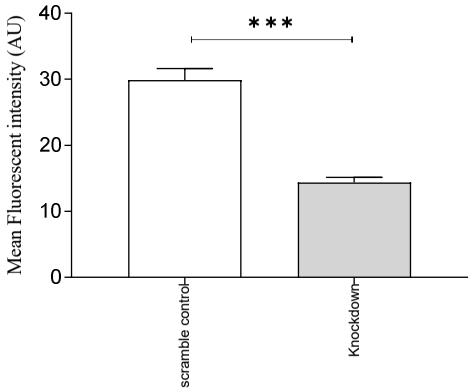

***
***
Mean Fluorescent intensity (AU)
100
80
60
40
20
0
Controls <7years
T1DE1 <7years
Controls >13years
T1DE2 >13years

Figure 4


a.
Fold change CD47 expression
1.5
1.0
0.5
0.0
**
**
Scramble control
siCD47
SiSIRPα


b.
Scrambled control
CD47 knockdown
CD47
overlay
CD47
overlay


c.
Mean Fluorescent intensity (AU)
40
30
20
10
0
***
scramble control
Knockdown

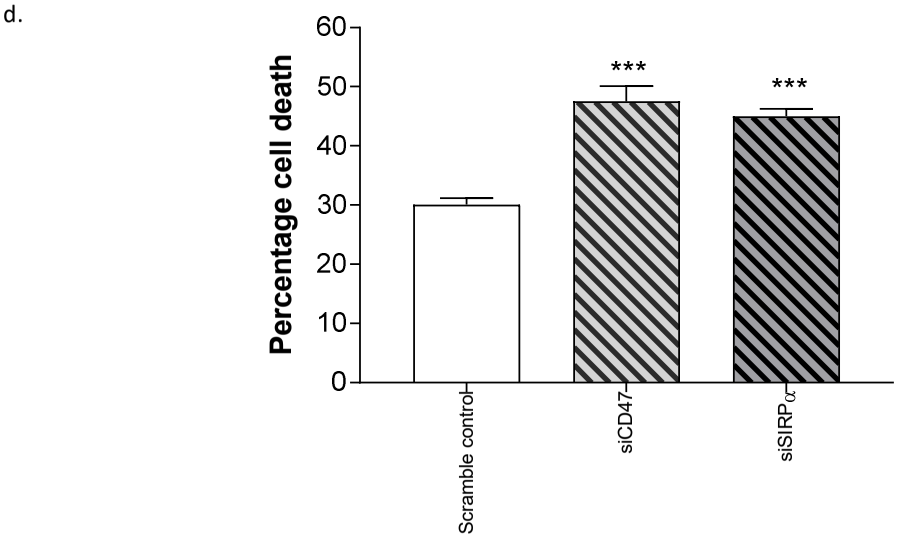
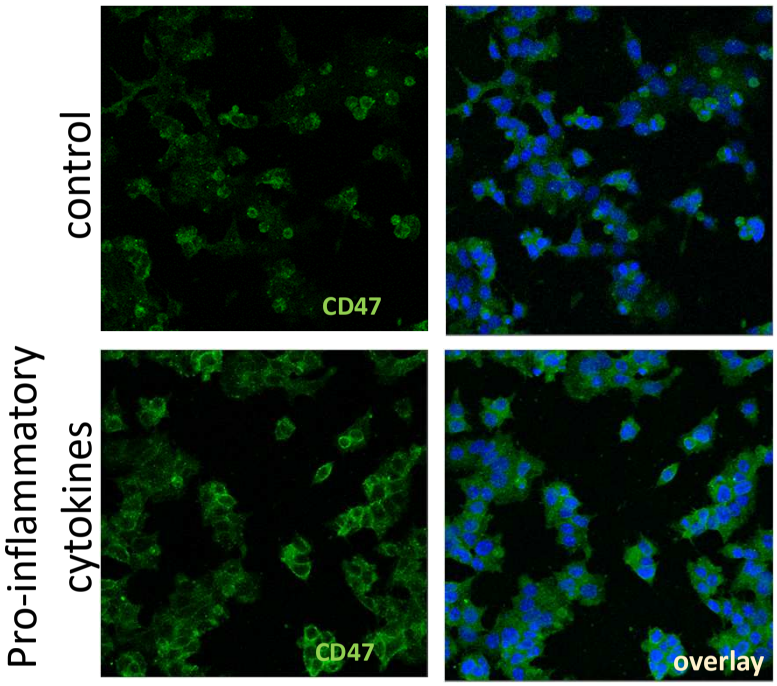
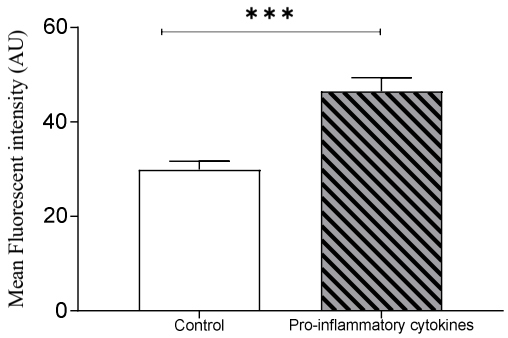

d.
Percentage cell death
60
50
40
30
20
10
0
***
***
Scramble control
siCD47
siSIRPα

Figure 5


control
Pro-inflammatory cytokines
CD47
CD47
overlay


***
Mean Fluorescent intensity (AU)
60
40
20
0
Control
Pro-inflammatory cytokines