Title: Tacrolimus, but not voclosporin, inhibits insulin secretion from human islets at a clinical trough dose

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Abstract

Context. The incidence of new onset diabetes after transplant (NODAT) has increased over the past decade. It has been suggested that NODAT is caused by exposure to calcineurin inhibitors, particularly tacrolimus (TAC). Voclosporin (VCS), a next generation calcineurin inhibitor is reported to cause fewer incidences of NODAT.

Objective*.* Whilst calcineurin plays important roles in pancreatic β-cell survival, proliferation, and function, the effects of calcineurin inhibitors on human β-cells remain understudied. *In particular, we do not understand why some inhibitors have* more profound effects on the incidence of NODAT.

Design*.* Here, we compared the effects of TAC and VCS on the dynamics of insulin secretory function, the programmed cell death rate, and the transcriptomic profile of human islets. We studied two clinically relevant doses of TAC (10 ng/ml, 30 ng/ml) and VCS (20 ng/ml, 60 ng/ml), meant to approximate the clinical trough and peak concentrations.

Results**.** TAC, but not VCS, caused a significant impairment of 15 mM glucose-stimulated insulin secretion and 30 mM KCl-stimulated insulin secretion. This points to molecular defects in the distal stages of exocytosis after voltage-gated Ca^{2+} entry. No significant effects on islet cell survival or total islet insulin content were identified. RNA sequencing showed that TAC, and to a lesser extent VCS, decreased the expression of genes that regulate insulin exocytosis, trafficking, and processing, including *SYT16, SYT5*, *PITPNM1*, and *PAM*.

Conclusions*.* TAC directly inhibits insulin secretion, likely via transcriptional control of exocytosis machinery. VCS was found to be gentler on isolated human islets than TAC.

Introduction

New onset diabetes after transplant (NODAT) is a clinical problem that has increased in incidence over the past decade. It is widely believed that NODAT is caused by exposure to steroids or high dose calcineurin inhibitors, particularly tacrolimus, on cell types that are critical for the maintenance of glucose homeostasis (1). Tacrolimus (TAC; also known as FK506) is a mainstay for prevention of transplant rejection, but effective immunosuppression targeting a concentration range of 4-11 ng/mL still results in NODAT in > 20% of patients (2). There is an unmet clinical need for calcineurin inhibitors that do not cause diabetes in this already vulnerable patient population.

Voclosporin (VCS) is a next-generation calcineurin inhibitor that is structurally related to cyclosporine A (CsA) with the addition of a carbon molecule at amino acid-1 of (CsA). This modification results in enhanced binding of the VCS-cyclophilin complex to calcineurin and shifts metabolism, resulting in increased potency and a consistent pharmacokinetic-pharmacodynamic profile as compared to (CsA). VCS was previously evaluated in a Phase IIb, multi-center, open-label, concentration-controlled study in patients undergoing *de novo* renal transplantation, evaluating the efficacy and safety of three doses of VCS (0.4, 0.6, and 0.8 mg/kg BID) as compared to TAC (3). In that study, the low-dose VCS group had similar efficacy to tacrolimus in controlling acute rejection with a significantly decreased incidence of NODAT (1.6% vs. 16.4%, respectively, p=0.031) (3). In the AURA study for lupus nephritis, 1.1% of patients receiving VCS (23.7 mg) and 1.1% of patients in the placebo group reported diabetes (4), which can be compared to an approximate 25.7% of patients who developed diabetes while taking a standard dose of TAC for 1 year (5). Thus, a difference appears to exist between the clinical diabetes seen with TAC and VCS.

While the etiology of NODAT is not widely understood, calcineurin and NFAT are involved in a number of cellular processes outside of immunosuppression, including direct roles in normal pancreatic β-cell development and physiology (6-8). For example, Heit et al. reported that β-cell

selective knockout of calcineurin in mice reduced functional β-cell mass and caused diabetes that could be rescued by over-expressing active NFATc1 (7). The effects of acute calcineurin inhibition on human and mouse islet function was previously investigated in a study by our group comparing clinically relevant concentrations of TAC, CsA, and rapamycin. *In vitro* experiments showed a direct decrease in β-cell function after only 24 hours of exposure to TAC (6). Furthermore, we and others have shown that inhibition of the calcineurin pathway by TAC impaired human islet graft function *in vivo* (6,9). Thus, TAC has multiple pathological effects on pancreatic islets.

Here, we directly tested the hypothesis that VCS has lower human β-cell toxicity compared with TAC, and we conducted studies designed to identify the molecular mechanisms involved (Fig 1A). We found that human islets exposed for 48 hours to TAC exhibited significantly reduced glucose-stimulated insulin secretion, while VCS showed no statistically significant inhibition. RNA sequencing showed that TAC, and to a lesser extent VCS, decreased the expression of genes that regulate exocytosis. In support of the clinical observations, our data suggest that VCS is less toxic to human pancreatic β-cells at human-correlated doses. Thus, more profound effect of tacrolimus on NFAT when compared to VCS or CsA could provide an explanation of why the risk of NODAT is higher in patients taking TAC than VCS.

Methods

Human islet culture

High-quality research specific human islets from consenting donors were obtained from IsletCore Laboratory in Edmonton Alberta (10), and used under ethical approval from the UBC Clinical Research Ethics Board (H13-01865). Islets were shipped in CMRL media (Thermo Fisher Scientific) overnight from Edmonton to the University of British Columbia. Upon arrival, islets were immediately purified by handpicking under a stereomicroscope and suspended in RPMI 1640 medium (Thermo Fisher Scientific) supplemented with 5.5 mmol glucose, 10% FBS, and 100

units/ml penicillin/streptomycin. Groups of 100 islets were placed in non-treated 100-mm polystyrene petri dishes (Fisher Scientific), treated with vehicle DMSO, TAC (10 ng/ml, 30 ng/ml) or VCS (20 ng/ml, 60 ng/ml), followed by 48-hr incubation at 37°C and 5% CO_2 (Fig. 1A). Donor and isolation characteristics can be found in Figure 1B

Immunosuppression-related assays

The Calcineurin inhibitor assay and the T-cell proliferation assay are described in more detail elsewhere (11,12). Briefly, peripheral blood mononuclear cell (PBMC) lysates were used in the calcineurin assay after 25 min stimulation with PMA-A23187. After lysis with a calcineurin assay buffer (no detergents) and 3 freeze-thaw cycles, the soluble fraction was probed by Western blot with 1:200 Santa Cruz 13033 anti-NFAT2 antibody. Western blot analysis of NFAT accumulation after treatment with indicated doses of VCS and TAC was then conducted to identify the relative concentrations that elicited a similar response.

Dynamic analysis of insulin secretion from human islets

To define the dynamic effects of VCS and TAC on insulin secretion from human islets, we used human islet perifusion studies (6). We assessed the effects of TAC (10 ng/ml, 30 ng/ml) and VCS (20 ng/ml, 60 ng/ml) on the dynamics of insulin secretion in response to two stimuli that are diagnostic of changes in metabolism/signalling or exocytosis. Our standard approach (6,13-18) compared the response to 15 mM glucose stimulation and direct depolarization with 30 mM KCl. More specifically, 65 islets per column were perifused (0.4 ml/min) with 3 mM glucose KRB solution as described previously (6) for 60 min to equilibrate the islets to the KRB and flow rate, and then with the indicated condition (as described in corresponding figure legends). DMSO, VCS and TAC were present at the indicated concentrations during the entirety of the perifusion experiment. We repeated this experiment on islets from 17 donors to take into account the

significant variability between human islet preparations (10). Samples were stored at −20 °C and insulin secretion was quantified using human insulin radioimmunoassay kits (Cedarlane).

Cell death assays

Pancreatic islets from human cadaveric donors were dispersed and seeded into 384-well plates and cultured in similar manner as previous described (19-22). Cells were stained with 50 ng/mL Hoechst 33342 (Invitrogen) and 0.5 µg/mL propidium iodide (PI) (Sigma-Aldrich) in RPMI 1640 medium (Invitrogen) with 5 mM glucose (Sigma-Aldrich), 100 U/mL penicillin, 100 µg/mL streptomycin (Invitrogen), 10% vol/vol fetal bovine serum (FBS) (Invitrogen) with or without a cytokine cocktail (25 ng/mL TNF-α, 10 ng/mL IL-1β, and 10 ng/mL IFN-γ; R&D Systems). We have previously shown that neither of these chemicals affect islet cell viability on their own at these concentrations (22). After 2 hours of staining, cells were incubated with different concentrations of DMSO, tacrolimus or VCS (as described in figure 5C) and with a cytokine cocktail (25 ng/mL TNF-α, 10 ng/mL IL-1β, and 10 ng/mL IFN-γ; R&D Systems). Immediately after the addition of the test drugs, the cells were imaged at 37°C and 5% CO_2. Images were captured every second hour for 48 hours using a robotic microscope (ImageXpressMICRO XLS, Molecular Devices) and analyzed using MetaXpress multi-wave cell score Software (Molecular Devices). To adjust for the difference in basal cell death for each individual run, each timepoint was normalized to the first timepoint then further normalized to the cytokine control.

RNA-sequencing and pathway analysis

Groups of 200 islets from 7 independent donors were treated for 48 hours with vehicle (DMSO), tacrolimus (30 ng/ml) or VCS (60 ng/ml) then immediately flash-frozen in liquid nitrogen and stored at -80°C. RNA isolation, library preparation, RNA sequencing and bioinformatics support were provided by the UBC Biomedical Research Centre Core Facility. Briefly, sample quality control was performed using the Agilent 2100 Bioanalyzer. Qualifying samples were then prepped

following the standard protocol for the NEBnext Ultra ii Stranded mRNA (New England Biolabs). Sequencing was performed on the Illumina NextSeq 500 with Paired End 42bp × 42bp reads. De-multiplexed read sequences were then aligned to the reference sequence using STAR (https://www.ncbi.nlm.nih.gov/pubmed/23104886) aligners. Assembly and differential expression were estimated using Cufflinks (http://cole-trapnell-lab.github.io/cufflinks/) through bioinformatics apps available on Illumina Sequence Hub. FKPM reads were filtered to exclude genes with a value <1 in the control islets (leaving 13,208 genes). We then sorted differentially expressed genes by uncorrected *p* value < 0.01 using the comparison of DMSO vs TAC or DMSO vs VCS to produce the heat map and a list of candidate genes.

Protein-protein interaction networks and pathway enrichment analyses were derived from these genes and generated by the visual analytic platform, NetworkAnalyst 3.0 (http://www.networkanalyst.ca/) (23). PPI network data are based on STRING interactome. Proteins that are linked to NFAT and calcineurin were selected and extracted as sub-networks using the “Extract module” function.

Statistics and data analysis

Data were analyzed using a one-way multiple comparisons ANOVA followed by a *post hoc* t-test using the Tukey HSD method, unless otherwise indicated. Data are expressed as means ± SEM, and $p < 0.05$ was considered significant. n values represent the number of unique islet donors studied.

Results

Effects of TAC and VCS on NFAT activity

Both TAC and VCS exert their immunosuppressive effects through inhibition of calcineurin phosphatase activity, preventing the dephosphorylation and activation of NFATc (24,25). Each of these two drugs is distinct with regard to its molecular mechanism of action. TAC interacts with

the immunophilin FKBP12 prior to binding calcineurin, whereas VCS interacts with cyclophilins. The *in vitro* activity of TAC, VCS (and the structurally related inhibitor CsA) were compared in a calcineurin inhibition assay and for their downstream ability to inhibit the division of activated T cells. The IC_{50} of TAC against calcineurin was found to be approximately 4-fold lower than VCS, in accordance with clinical dosing where the therapeutic range of TAC is 5-30 ng/mL and VCS target trough concentrations are around 20-60 ng/mL (Fig 2A). Surprisingly, the potency difference between TAC and VCS widened to 50-fold, in favor of TAC, when looking at inhibition of T-cell proliferation (Fig 2A), leading to additional experiments comparing the impact of each drug on their common pathway effector, NFAT. Maximal activation of NFAT was observed at 56 ng/mL of VCS and only 1.4 ng/mL of TAC, a potency difference of 40-fold (Fig. 2B,C,D). These data demonstrate that, at therapeutic concentrations, TAC has a far more profound impact on NFAT than VCS (Fig. 2E).

Effects of TAC or VCS on basal and glucose stimulated insulin secretion

Insulin is released from pancreatic islets, *in vivo* and *in vitro*, in a biphasic pattern that includes both a rapid 1st phase of pre-docked insulin granules and a 2nd sustained phase involving granules that are recruited to the plasma membrane (26). There is significant inter-individual variability in the rate of insulin secretion from human islets (10) and the effects of immunosuppressants (6), so we examined insulin secretion from high quality human islets isolated from 17 donors exclusively for research purposes. We employed the dynamic perifusion system with frequent sampling to evaluate basal insulin secretion at 3 mM glucose, both phases of insulin secretion during 15 mM glucose stimulation, and insulin secretion in response to direct depolarization with 30 mM KCl. TAC at 10 ng/ml (clinical trough) and 20 ng/ml (clinical peak) significantly inhibited total insulin secretion at 15 mM glucose (Fig. 3A,B), with a specific effect on the 2nd phase but not the 1st phase (Fig. 3C,D). The insulin secretory response to 30 mM KCl was also significantly inhibited by both concentrations of TAC (Fig. 3E). In contrast, VCS concentrations approximately

2 times higher than TAC had milder effects on glucose-stimulated insulin secretion. Specifically, the clinical trough concentration of 20 ng/ml VCS was ~50% less inhibitory to the total and 2nd phase of insulin secretion and did not result in statistically significant inhibition (Fig. 3A,B,D). No significant differences in basal insulin secretion were observed at 3 mM glucose for either drug at either of the concentrations tested (Fig. 3F). Similar results were observed when the perifusion data were normalized to basal insulin secretion (Fig. 3G,H). We were unable to detect significant differences in acid-ethanol extractable insulin protein content in human islets treated with either drug, at either concentration, for 48 hr (Fig. 4). These results indicate that the specific significant inhibitory effects of TAC on total and 2nd phase glucose-stimulated insulin secretion, as well as insulin secretion stimulated by direct depolarization, are likely to be direct effects on the exocytosis machinery rather than a result of reduced insulin production. Collectively, these data also demonstrate that VCS has milder effects on human islet secretory function when compared with TAC in this sensitive *in vitro* assay system.

Effects of TAC or VCS on human islet cell survival

We next assessed the effects of TAC and VCS on the survival of dispersed human islet cells using our long-term high-content imaging protocols that measure both apoptotic and non-apoptotic cell death over multiple days of culture in the presence of proinflammatory cytokines (19-22) (Fig. 5A,B). We studied both TAC and VCS over a 5 point-dose response curve. We observed no significant differences in human islet cell survival following either drug treatment (Fig. 5C). This is consistent with our data showing no changes in insulin content following 48 hrs of drug treatment, further pointing to the primacy of the direct effect of TAC on insulin secretion.

Effects of TAC or VCS on human islet gene expression

In order to provide clues as to the underlying molecular mechanisms by which TAC, but not VCS, significantly inhibits insulin secretion we performed RNAseq on human islets from 7 donors

(Fig 1B). As expected, there was significant donor to donor variation in gene expression (Fig. 6A). Notwithstanding, we were able to make several conclusions from these data (Fig. 6A,B). The most significantly differentially expressed gene was synaptotagmin 16 (*SYT16*), a member of the synaptotagmin family of exocytosis-regulating proteins that has not previously been directly implicated in insulin secretion. While we did not observe large statistical changes in specific genes, pathway analysis showed that there was a significant over-representation of nominally differentially expressed ($p < 0.001$) genes the Syntaxin-Binding 'Molecular Function' Gene Ontology Term, as well as many related Cellular Component terms (Fig. 6B). These observations further support the contention that TAC directly inhibits insulin granule trafficking and/exocytosis in human β-cells.

Finally, we sought to bioinformatically connect the differentially expressed genes to the NFAT/calcineurin signalling network known to mediate the proximal effects of these immunosuppressants. Based on protein-protein interaction sub-network analysis, we identified differentially expressed genes in both VSC and TAC treatments that are linked to NFAT/calcinerin signaling (Fig. 7A,B). FOS and PTGS2 (COX2) are associated with NFAT subunits and were downregulated by both treatments. ALB, upregulated by VSC, and MAPK4 and PPP1R1A, altered by TAC, are associated with calcineurin subunits (PPP3CA/B/C). These analyses show how VCS and TAC treatment affect the expression of specific genes via calcineurin signalling and lead us to our current working model (Fig. 7C).

Discussion

The effects of calcineurin inhibitors on human β-cells remain understudied. A deeper understanding of how immunosuppressive agents affect human islets is necessary to elucidate the pathogenesis of NODAT and to identify immunosuppressants with improved safety profiles. Thus, the objective of the present study was to determine the mechanism by which the next-

generation calcineurin inhibitor, VCS, has lower human β-cell toxicity compared with TAC. To examine this, we compared the effects of TAC and VCS on the dynamics of insulin secretory function, the programmed cell death, and the transcriptomic profile of isolated human islets. We found that TAC, but not VCS, caused a significant impairment of both 15 mM glucose and 30 mM KCl-stimulated insulin secretion at clinically relevant doses. We saw no effect on total insulin content or human islet cell survival with either drug. Gene pathway enrichment analysis of RNAseq data showed that TAC decreased the expression of genes that regulate exocytosis and genes that regulate transport from the ER to the Golgi apparatus. VCS treatment also induced these changes; however, it did so to a lesser extent. Thus, this greater inhibition could provide an explanation of why the risk of NODAT is higher in patients taking TAC than the next generation calcineurin inhibitor VCS. The equivalently immunosuppressive dose ranges of VCS and TAC we used have dramatically different potencies on NFAT, suggesting that NFAT-mediated gene expression mediates the observed effects on exocytosis regulating gene expression.

Previous studies have found that calcineurin inhibitors impair glucose-stimulated insulin secretion (7,27-35), including in human islets (6,9,36-42). Our study extends these previous findings by rigorously examining the kinetics of insulin release *in vitro* and by implicating a direct effect on insulin granule trafficking. TAC at 10 ng/ml (clinical trough) and 20 ng/ml (clinical peak) significantly inhibited total insulin secretion in response to 15 mM glucose and this inhibition was directly due to a specific effect on the 2nd phase of insulin release. Furthermore, KCl-stimulated insulin secretion was decreased to a similar degree, implicating secretory pathways distal to Ca^{2+} entry via voltage-gated Ca^{2+} channels. Together, these data suggest that the readily releasable pool of insulin granules is not robustly affected by TAC-mediated calcineurin inhibition; instead, TAC impairs pathways associated with insulin granule trafficking to the plasma membrane. While we did not directly look at exocytotic events in this study, the results of our RNAseq analysis are consistent with a TAC-mediated effect on insulin granule traffic and release. Our RNAseq data provide the impetus for follow-up studies on several genes, including *SYT16* which has not

previously been directly implicated in glucose-stimulated insulin secretion but that is known to be glucose responsive in human islets (43) and under circadian control in mouse islets (44). Network analysis demonstrated that calcineurin signalling was linked PTGS2 (a.k.a. Cox2), FOS, PPP1R1A, and MAPK4 in TAC-treated human islets. Collectively, these data further solidify the role of calcineurin signaling and NFAT-mediated gene transcription in human β-cell function and provide insight into the molecular mechanisms controlling 2nd phase insulin exocytosis.

Calcineurin activity has been ascribed a number of distinct functions in pancreatic β-cells (7-9,37,45-48). For example, cultured insulinoma cells treated with the calcineurin inhibitor cypermethrin exhibited decreased insulin exocytosis in response to both glucose and KCl stimulation (49). In contrast to this, some studies suggest that calcineurin has a negative role in insulin exocytosis. However, these were indirect findings obtained by examining the inhibition of β-cell exocytosis via neurotransmitter-mediated activation of calcineurin (50,51), as opposed to direct effects observed following inhibition of calcineurin. Nevertheless, our study now shows that VCS is gentler on human islets because it does not cause the same level of inhibition on insulin exocytosis compared to TAC.

In our previous work, we examined the different effects of immunosuppressive drugs (TAC, cyclosporine, and rapamycin) on induced endoplasmic reticulum (ER) stress and caspase-3-dependent apoptosis in human islets (6). In that study, we found that TAC impaired glucose-stimulated insulin release from human islets, but did not significantly elevate the protein levels of CHOP or cleaved caspase-3, indicating that it's effects were mostly on β-cell function rather than fate (6). In our current study we measured human islet cell survival over a longer period by using our high-content imaging protocol. We found that neither TAC nor VCS had significant deleterious effect on islet cell survival or insulin content over 48 hours, under the current testing conditions. Similarly, our post RNA-seq pathway analysis did not identify significant changes in genes involved in β-cell apoptosis and/or survival. While an increase in β-cell ER-stress and apoptosis

is a plausible pathophysiological mechanism for the diabetic effects of immunosuppressants in the post-transplant clinical setting, it is unlikely that this is the mechanism explaining why the risk of NODAT is higher in patients taking TAC when compared with VCS.

Limitations of our study include the following. Although we had the benefit of directly examining human islets, our *in vitro* experiments may be impacted by islet isolation and culture. Nevertheless, the fundamental *in vivo* mechanisms appear to be well conserved in high quality isolated islets such as those used in our study (10). Mechanistically, our analysis of insulin secretion in response to high glucose and high KCl, together with the transcriptomic profiling, points to direct effects on the distal processes of insulin exocytosis. Future studies could directly measure exocytosis with electrophysiological capacitance measurements. Our data point to differences in NFAT activation between VCS and TAC than can explain the distinct impacts on insulin secretion, but our work employed non-β-cells for the dose-finding experiment and ideally future studies will be repeated in human β-cells or human β-cell lines. Given the clinical importance of calcineurin inhibitor mediated β-cell dysfunction, we hope that our work stimulates more experimentation in this field.

In summary, we have demonstrated that TAC significantly inhibits the 2nd phase of glucose-stimulated insulin secretion from human islets. This decrease in secretion is not mediated by effects on islet cell health or insulin content and is most likely a direct consequence of decreased insulin granule exocytosis. VCS, a next generation calcineurin inhibitor, does not cause the same degree of inhibition of insulin secretion. Thus, this is a plausible physiological mechanism explaining the lower incidence of NODAT in patients taking VCS.

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Author Contributions:

JK designed, conducted and analyzed experiments, and co-wrote the manuscript.

LB conducted and analyzed experiments and edited the manuscript.

PO designed, conducted and analyzed experiments, and edited the manuscript.

HHC designed and analyzed experiments and edited the manuscript.

EP designed, conducted and analyzed experiments, and edited the manuscript.

DRU designed, conducted and analyzed experiments, and edited the manuscript.

JLC designed experiments and edited the manuscript.

RBH designed experiments and edited the manuscript.

JDJ designed and analyzed experiments and co-wrote the manuscript.

Data Availability:

All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

References

1. Gala-Lopez BL, Pepper AR, Pawlick RL, O'Gorman D, Kin T, Bruni A, Abualhassan N, Bral M, Bautista A, Manning Fox JE, Young LG, MacDonald PE, Shapiro AM. Antiaging Glycopeptide Protects Human Islets Against Tacrolimus-Related Injury and Facilitates Engraftment in Mice. *Diabetes*. 2016;65(2):451-462.
2. Porrini E, Moreno JM, Osuna A, Benitez R, Lampreabe I, Diaz JM, Silva I, Dominguez R, Gonzalez-Cotorruelo J, Bayes B, Lauzurica R, Ibernon M, Moreso F, Delgado P, Torres A. Prediabetes in patients receiving tacrolimus in the first year after kidney transplantation: a prospective and multicenter study. *Transplantation*. 2008;85(8):1133-1138.
3. Busque S, Cantarovich M, Mulgaonkar S, Gaston R, Gaber AO, Mayo PR, Ling S, Huizinga RB, Meier-Kriesche HU, Investigators P. The PROMISE study: a phase 2b multicenter study of voclosporin (ISA247) versus tacrolimus in de novo kidney transplantation. *Am J Transplant*. 2011;11(12):2675-2684.
4. Rovin BH, Solomons N, Pendergraft WF, 3rd, Dooley MA, Tumlin J, Romero-Diaz J, Lysenko L, Navarra SV, Huizinga RB, Group A-LS. A randomized, controlled double-blind study comparing the efficacy and safety of dose-ranging voclosporin with placebo in achieving remission in patients with active lupus nephritis. *Kidney Int*. 2019;95(1):219-231.
5. Torres A, Hernandez D, Moreso F, Seron D, Burgos MD, Pallardo LM, Kanter J, Diaz Corte C, Rodriguez M, Diaz JM, Silva I, Valdes F, Fernandez-Rivera C, Osuna A, Gracia Guindo MC, Gomez Alamillo C, Ruiz JC, Marrero Miranda D, Perez-Tamajon L, Rodriguez A, Gonzalez-Rinne A, Alvarez A, Perez-Carreno E, de la Vega Prieto MJ, Henriquez F, Gallego R, Salido E, Porrini E. Randomized Controlled Trial Assessing the Impact of Tacrolimus Versus Cyclosporine on the Incidence of Posttransplant Diabetes Mellitus. *Kidney Int Rep*. 2018;3(6):1304-1315.
6. Johnson JD, Ao Z, Ao P, Li H, Dai LJ, He Z, Tee M, Potter KJ, Klimek AM, Meloche RM, Thompson DM, Verchere CB, Warnock GL. Different effects of FK506, rapamycin, and

mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. *Cell Transplant*. 2009;18(8):833-845.

7. Heit JJ, Apelqvist AA, Gu X, Winslow MM, Neilson JR, Crabtree GR, Kim SK. Calcineurin/NFAT signalling regulates pancreatic beta-cell growth and function. *Nature*. 2006;443(7109):345-349.

8. Goodyer WR, Gu X, Liu Y, Bottino R, Crabtree GR, Kim SK. Neonatal beta cell development in mice and humans is regulated by calcineurin/NFAT. *Dev Cell*. 2012;23(1):21-34.

9. Dai C, Walker JT, Shostak A, Padgett A, Spears E, Wisniewski S, Poffenberger G, Aramandla R, Dean ED, Prasad N, Levy SE, Greiner DL, Shultz LD, Bottino R, Powers AC. Tacrolimus- and sirolimus-induced human beta cell dysfunction is reversible and preventable. *JCI Insight*. 2020;5(1).

10. Lyon J, Manning Fox JE, Spigelman AF, Kim R, Smith N, O'Gorman D, Kin T, Shapiro AM, Rajotte RV, MacDonald PE. Research-Focused Isolation of Human Islets From Donors With and Without Diabetes at the Alberta Diabetes Institute IsletCore. *Endocrinology*. 2016;157(2):560-569.

11. Batiuk TD, Pazderka F, Enns J, DeCastro L, Halloran PF. Cyclosporine inhibition of calcineurin activity in human leukocytes in vivo is rapidly reversible. *J Clin Invest*. 1995;96(3):1254-1260.

12. Kuo J, Bobardt M, Chatterji U, Mayo PR, Trepanier DJ, Foster RT, Gallay P, Ure DR. A Pan-Cyclophilin Inhibitor, CRV431, Decreases Fibrosis and Tumor Development in Chronic Liver Disease Models. *J Pharmacol Exp Ther*. 2019;371(2):231-241.

13. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, O'Dwyer S, Quiskamp N, Mojibian M, Albrecht T, Yang YH, Johnson JD, Kieffer TJ. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat Biotechnol*. 2014;32(11):1121-1133.

14. Yang YH, Szabat M, Bragagnini C, Kott K, Helgason CD, Hoffman BG, Johnson JD. Paracrine signalling loops in adult human and mouse pancreatic islets: netrins modulate beta cell apoptosis signalling via dependence receptors. *Diabetologia*. 2011;54(4):828-842.

15. Alejandro EU, Lim GE, Mehran AE, Hu X, Taghizadeh F, Pelipeychenko D, Baccarini M, Johnson JD. Pancreatic beta-cell Raf-1 is required for glucose tolerance, insulin secretion, and insulin 2 transcription. *Faseb J*. 2011;25(11):3884-3895.

16. Jeffrey KD, Alejandro EU, Luciani DS, Kalynyak TB, Hu X, Li H, Lin Y, Townsend RR, Polonsky KS, Johnson JD. Carboxypeptidase E mediates palmitate-induced beta-cell ER stress and apoptosis. *Proc Natl Acad Sci U S A*. 2008;105(24):8452-8457.

17. Dror V, Kalynyak TB, Bychkivska Y, Frey MH, Tee M, Jeffrey KD, Nguyen V, Luciani DS, Johnson JD. Glucose and endoplasmic reticulum calcium channels regulate HIF-1beta via presenilin in pancreatic beta-cells. *J Biol Chem*. 2008;283(15):9909-9916.

18. Luciani DS, Johnson JD. Acute effects of insulin on beta-cells from transplantable human islets. *Molecular and Cellular Endocrinology*. 2005;241(1-2):88-98.

19. Yang YH, Wills QF, Johnson JD. A live-cell, high-content imaging survey of 206 endogenous factors across five stress conditions reveals context-dependent survival effects in mouse primary beta cells. *Diabetologia*. 2015;58(6):1239-1249.

20. Yang YH, Vilin YY, Roberge M, Kurata HT, Johnson JD. Multiparameter Screening Reveals a Role for Na(+) Channels in Cytokine-Induced beta-Cell Death. *Mol Endocrinol*. 2014;28(3):406-417.

21. Yang YH, Manning Fox JE, Zhang KL, MacDonald PE, Johnson JD. Intraislet SLIT-ROBO signaling is required for beta-cell survival and potentiates insulin secretion. *Proc Natl Acad Sci U S A*. 2013;110(41):16480-16485.

22. Yang YH, Johnson JD. Multi-parameter single-cell kinetic analysis reveals multiple modes of cell death in primary pancreatic beta-cells. *J Cell Sci*. 2013;126(Pt 18):4286-4295.

23. Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. *Nucleic Acids Res*. 2019;47(W1):W234-W241.

24. Dumont FJ. FK506, an immunosuppressant targeting calcineurin function. *Curr Med Chem*. 2000;7(7):731-748.

25. Sieber M, Baumgrass R. Novel inhibitors of the calcineurin/NFATc hub - alternatives to CsA and FK506? *Cell Commun Signal*. 2009;7:25.

26. Braun M, Ramracheya R, Johnson PR, Rorsman P. Exocytotic properties of human pancreatic beta-cells. *Ann N Y Acad Sci*. 2009;1152:187-193.

27. Xu C, Niu YJ, Liu XJ, Teng YQ, Li CF, Wang HY, Yin JP, Wang LT, Shen ZY. Tacrolimus reversibly reduces insulin secretion, induces insulin resistance, and causes islet cell damage in rats. *Int J Clin Pharmacol Ther*. 2014;52(7):620-627.

28. Ozbay LA, Smidt K, Mortensen DM, Carstens J, Jorgensen KA, Rungby J. Cyclosporin and tacrolimus impair insulin secretion and transcriptional regulation in INS-1E beta-cells. *Br J Pharmacol*. 2011;162(1):136-146.

29. Radu RG, Fujimoto S, Mukai E, Takehiro M, Shimono D, Nabe K, Shimodahira M, Kominato R, Aramaki Y, Nishi Y, Funakoshi S, Yamada Y, Seino Y. Tacrolimus suppresses glucose-induced insulin release from pancreatic islets by reducing glucokinase activity. *Am J Physiol Endocrinol Metab*. 2005;288(2):E365-371.

30. Paty BW, Harmon JS, Marsh CL, Robertson RP. Inhibitory effects of immunosuppressive drugs on insulin secretion from HIT-T15 cells and Wistar rat islets. *Transplantation*. 2002;73(3):353-357.

31. Redmon JB, Olson LK, Armstrong MB, Greene MJ, Robertson RP. Effects of tacrolimus (FK506) on human insulin gene expression, insulin mRNA levels, and insulin secretion in HIT-T15 cells. *J Clin Invest*. 1996;98(12):2786-2793.

32. Ishizuka J, Gugliuzza KK, Wassmuth Z, Hsieh J, Sato K, Tsuchiya T, Townsend CM, Jr., Fish JC, Thompson JC. Effects of FK506 and cyclosporine on dynamic insulin secretion from isolated dog pancreatic islets. *Transplantation*. 1993;56(6):1486-1490.

33. Wakugami T, Islam MR, Higa S, Murakami K, Mimura G. Effect of FK506 on the development of diabetes in BB rats in comparison with that of cyclosporin. *Tohoku J Exp Med*. 1993;169(1):21-29.

34. Herold KC, Nagamatsu S, Buse JB, Kulsakdinun P, Steiner DF. Inhibition of glucose-stimulated insulin release from beta TC3 cells and rodent islets by an analog of FK506. *Transplantation*. 1993;55(1):186-192.

35. Carroll PB, Goncalves AA, Boschero AC, Tzakis AG, Starzl TE, Atwater I. Effect of the immunosuppressant FK 506 on insulin release from adult rat islets of Langerhans. *Transplant Proc*. 1991;23(1 Pt 1):337-339.

36. Kloster-Jensen K, Sahraoui A, Vethe NT, Korsgren O, Bergan S, Foss A, Scholz H. Treatment with Tacrolimus and Sirolimus Reveals No Additional Adverse Effects on Human Islets In Vitro Compared to Each Drug Alone but They Are Reduced by Adding Glucocorticoids. *J Diabetes Res*. 2016;2016:4196460.

37. Soleimanpour SA, Crutchlow MF, Ferrari AM, Raum JC, Groff DN, Rankin MM, Liu C, De Leon DD, Naji A, Kushner JA, Stoffers DA. Calcineurin signaling regulates human islet {beta}-cell survival. *J Biol Chem*. 2010;285(51):40050-40059.

38. Bugliani M, Masini M, Liechti R, Marselli L, Xenarios I, Boggi U, Filipponi F, Masiello P, Marchetti P. The direct effects of tacrolimus and cyclosporin A on isolated human islets: A functional, survival and gene expression study. *Islets*. 2009;1(2):106-110.

39. Polastri L, Galbiati F, Bertuzzi F, Fiorina P, Nano R, Gregori S, Aldrighetti L, Pozza G, Secchi A, Adorini L, Davalli AM. Secretory defects induced by immunosuppressive agents on human pancreatic beta-cells. *Acta Diabetol*. 2002;39(4):229-233.

40. Ricordi C, Zeng Y, Alejandro R, Tzakis A, Carroll P, Rilo HL, Venkataramanan R, Fung JJ, Bereiter D, Starzl TE. Effect of FK 506 on human pancreatic islets following renal subcapsular transplantation in diabetic nude mice. *Transplant Proc*. 1992;24(3):1042.

41. Ricordi C, Zeng YJ, Alejandro R, Tzakis A, Venkataramanan R, Fung J, Bereiter D, Mintz DH, Starzl TE. In vivo effect of FK506 on human pancreatic islets. *Transplantation*. 1991;52(3):519-522.

42. Tze WJ, Tai J, Cheung S. In vitro effects of FK-506 on human and rat islets. *Transplantation*. 1990;49(6):1172-1174.

43. Hall E, Dekker Nitert M, Volkov P, Malmgren S, Mulder H, Bacos K, Ling C. The effects of high glucose exposure on global gene expression and DNA methylation in human pancreatic islets. *Mol Cell Endocrinol*. 2018;472:57-67.

44. Perelis M, Marcheva B, Ramsey KM, Schipma MJ, Hutchison AL, Taguchi A, Peek CB, Hong H, Huang W, Omura C, Allred AL, Bradfield CA, Dinner AR, Barish GD, Bass J. Pancreatic beta cell enhancers regulate rhythmic transcription of genes controlling insulin secretion. *Science*. 2015;350(6261):aac4250.

45. Li X, Chan LWC, Li X, Liu C, Yang G, Gao J, Dai M, Wang Y, Xie Z, Liu J, Zhou F, Zheng T, Feng D, Guo S, Li H, Sun K, Yang S. Obesity-Induced Regulator of Calcineurin 1 Overexpression Leads to beta-Cell Failure Through Mitophagy Pathway Inhibition. *Antioxid Redox Signal*. 2020;32(7):413-428.

46. Zhao J, Zong W, Zhao Y, Gou D, Liang S, Shen J, Wu Y, Zheng X, Wu R, Wang X, Niu F, Wang A, Zhang Y, Xiong JW, Chen L, Liu Y. In vivo imaging of beta-cell function reveals glucose-mediated heterogeneity of beta-cell functional development. *Elife*. 2019;8.

47. Alejandro EU, Kalynyak TB, Taghizadeh F, Gwiazda KS, Rawstron EK, Jacob KJ, Johnson JD. Acute insulin signaling in pancreatic beta-cells is mediated by multiple Raf-1 dependent pathways. *Endocrinology*. 2010;151(2):502-512.

48. Bernal-Mizrachi E, Cras-Meneur C, Ye BR, Johnson JD, Permutt MA. Transgenic overexpression of active calcineurin in beta-cells results in decreased beta-cell mass and hyperglycemia. *PLoS One*. 2010;5(8):e11969.

49. Wang R, McGrath BC, Kopp RF, Roe MW, Tang X, Chen G, Cavener DR. Insulin secretion and Ca2+ dynamics in beta-cells are regulated by PERK (EIF2AK3) in concert with calcineurin. *J Biol Chem*. 2013;288(47):33824-33836.

50. Renstrom E, Ding WG, Bokvist K, Rorsman P. Neurotransmitter-induced inhibition of exocytosis in insulin-secreting beta cells by activation of calcineurin. *Neuron*. 1996;17(3):513-522.

51. Braun M, Wendt A, Buschard K, Salehi A, Sewing S, Gromada J, Rorsman P. GABAB receptor activation inhibits exocytosis in rat pancreatic beta-cells by G-protein-dependent activation of calcineurin. *J Physiol*. 2004;559(Pt 2):397-409.

Figure Legends

Figure 1| Experimental design, and human islet donor and isolation characteristics (A) Experimental design for study of VCS and TAC effects on high quality primary human islets isolated from cadaveric donors. Details of each isolation are available from IsletCore.ca (the example image shown from the R297 isolation). **(B)** High-quality, research-specific human islets from consenting donors were obtained from IsletCore Laboratory in Edmonton, Alberta. Islet donors and isolation characteristics are listed here. Stimulation index is the average of 3 technical replicates of the 16.7 mM to 2.8 mM glucose response as provided by the IsletCore Laboratory quality control database.

Figure 2| *Differences between TAC, VCS and CsA on* calcineurin activity, lymphocyte proliferation assay, and NFAT accumulation. (A-D) Sister aliquots of PBMC were treated in the presence of drug or vehicle control for analysis of lymphocyte cell division (Div), calcineurin activity (CN), and cytosolic NFAT. **(A)** Lymphocyte division was determined by CFSE analysis following 3-day stimulation with CD3 antibody. Calcineurin activity was determined by radioactive 32P-RII peptide assay following 30-min stimulation with PMA+A23187. **(B)** Cytosolic NFAT relative to CypA (loading control protein) was determined by Western blotting and densitometric quantitation following 30-min stimulation with PMA+A23187. **(C)** K562 cells stably transfected with an NFAT-luciferase reporter were stimulated for 22 hr with PMA + A23187 in the presence of drug or vehicle control. Cells were separated into separate aliquots for measurement of calcineurin activity and luciferase activity for NFAT promoter activation. **(D)** Percent inhibition was determined by normalizing values to vehicle control (100% activity) and to 2 µg/ml VCS (100% inhibition). These observations were replicated 5 times. **(E)** Calculated IC50 values in PBMC and K562-NFAT-Luc. n = 8-12 experiments for PBMC and 4-7 experiments for K562-NFAT-Luc.

Figure 3| Basal, glucose-stimulated and KCl-stimulated insulin secretion from human islets treated with peak and trough concentrations of VCS and TAC. (A) Averaged traces of dynamic insulin secretion measurements in the context of 3 mM glucose, 15 mM glucose, or 30 mM KCl (as indicated). **(B)** Total area under the curve (AUC) of the 15 mM glucose response. (**C, D, E)** AUCs of 1st phase and 2nd phase 15 mM glucose responses, as well as the KCl response. **(F)** Baseline insulin secretion. **(G, H)** Insulin secretion normalized to baseline, including AUC. n=17

Figure 4| Insulin content from isolated human islets treated with VCS and TAC. Insulin protein measured by radioimmunoassay from 20 islets treated for 48 hours with VCS or TAC (concentrations as indicate) after acid-ethanol extraction of insulin.

Figure 5| Islet cell survival from dispersed human islets treated with VCS and TAC. (A,B) Cell death was assessed every 30 minutes over 48 hours using Propidium Iodide (PI) and Hoechst staining, with a significant increase seen in the presence of a β-cell toxic cytokine cocktail. **(C)** Shown is the maximum cell death normalized measurement (see methods) for each condition TAC and VCS dose, normalized to the DMSO control cultures. Results shown are quantified from 5 cultures per drug condition, over two separate runs.

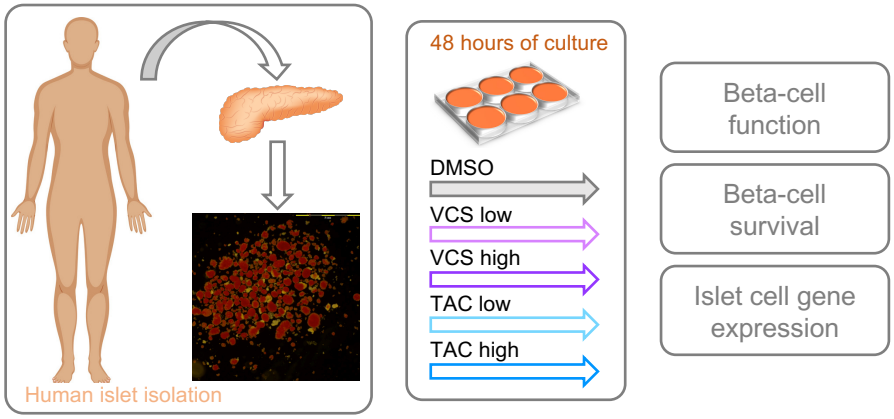
Figure 6| RNA sequencing analysis of human islets treated with TAC and VCS at peak doses. (A) mRNAs that were nominally differentially expressed ($p < 0.01$, uncorrected) are shown in blocks organized by primary difference (1 increased in TAC vs control, 2 increased in VCS vs control, 3 decreased TAC vs control, 2 decreased in VCS vs control). Raw CPM values from each biological replicate are shown in the heatmap table. p values < 0.001 are shown in grey for Control vs Tac and in purple for Control vs VCS. **(B)** Significantly enriched gene categories (Top, GO Molecular Function; Bottom, GO Cellular Component) according to Gene Ontology.

Figure 7| Protein-protein interaction networks in human islets treated with TAC and VCS at peak doses. Protein-protein interaction networks highlight connections between calcineurin/NFAT pathway and deferentially expression genes in **(A)** VCS or **(B)** TAC treatment. Up or down regulated genes are shown in red or green, respectively. **(C)** Working model of differential regulation of insulin exocytosis by VCS and TAC. In our model, TAC has more potent inhibitory effects on NFAT, and therefore results in the stronger suppression of key genes.

Figure 1

Overall study design and human islet donor characteristics

A

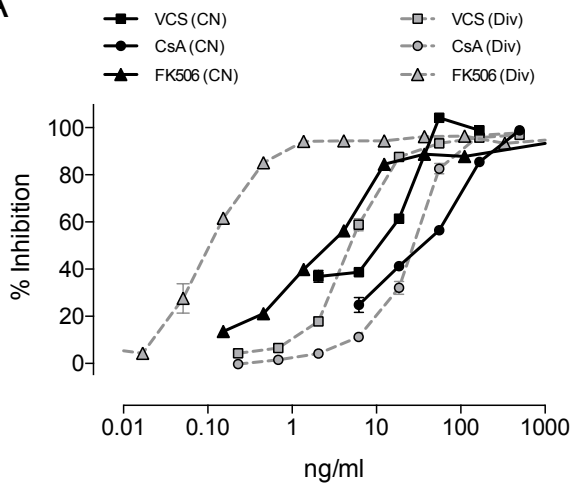


B

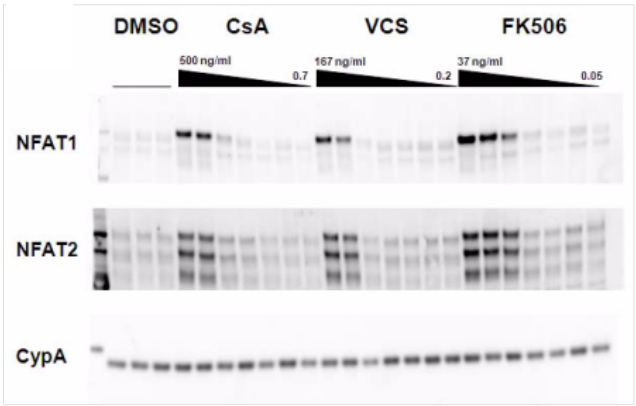
Donor ID	Age (years)	Sex	HbA1c (%)	BMI	Islet Purity	Stimulation Index	Experiments
R297	69	Male	No data	27.2	90%	5.05	Perifusion
R300	30	Female	No data	25.3	75%	2.05	Perifusion
R301	18	Male	5.0	19.0	75%	9.21	Perifusion
R302	62	Female	6.2	32.3	40%	3.57	RNAseq
R303	56	Female	No data	24.1	85%	2.64	Perifusion
R305	60	Male	5.6	21.0	80%	1.29	Perifusion, RNAseq
R306	22	Female	5.3	21.1	80%	19.13	Perifusion
R308	20	Male	5.5	19.8	80%	5.90	Perifusion
R309	47	Female	5.5	27.4	80%	9.08	Perifusion, RNAseq
R310	25	Male	5.4	26.4	90%	3.81	Perifusion, RNAseq
R314	31	Female	5.0	30.3	80%	6.53	Perifusion, RNAseq
R316	52	Male	5.7	26.0	50%	6.93	Perifusion, RNAseq
R317	54	Male	5.1	26.4	90%	10.78	RNAseq
R318	54	Male	5.0	20.5	90%	2.76	Perifusion
R319	68	Male	5.0	27.8	90%	5.04	Perifusion
R322	44	Female	4.9	23.2	90%	3.09	Perifusion
R325	50	Male	No data	30.3	75%	2.86	Perifusion
R326	26	Male	5.5	27.0	90%	5.34	Perifusion
R327	57	Male	5.8	95%	95%	3.59	Perifusion
R335	25	Male	4.9	24.6	80%	11.92	Cell death
R338	30	Male	5.3	25.5	90%	6.73	Cell death

Figure 2

A



B



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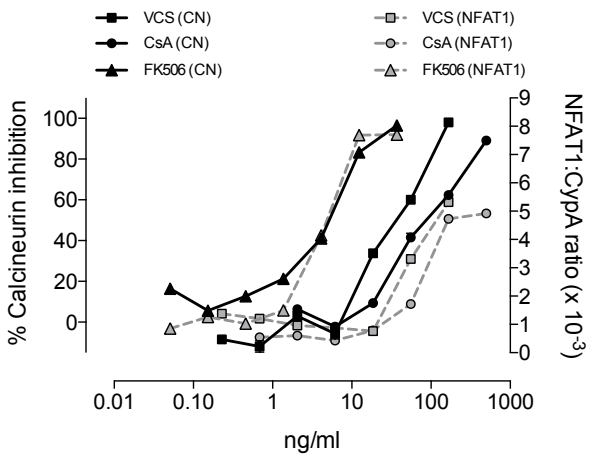
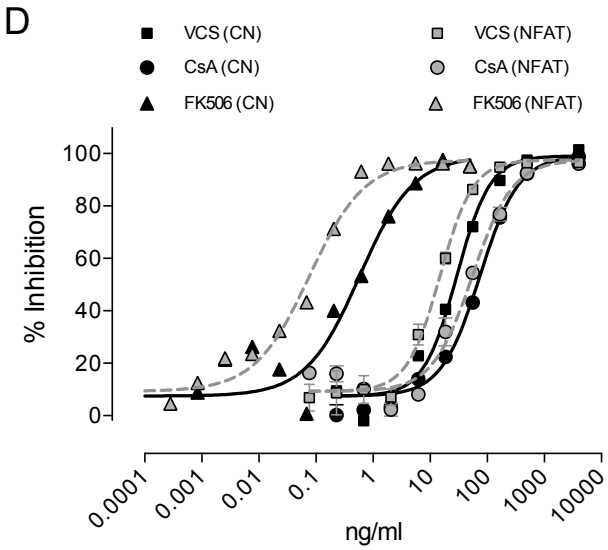


D



E

	PBMC Calcineurin Activity		PBMC Cell Division		K562-NFAT-Luc Calcineurin Activity		K562-NFAT-Luc NFAT Activity	
	IC50 (±SE) ng/ml	Relative Potency	IC50 (±SE) ng/ml	Relative Potency	IC50 (±SE) ng/ml	Relative Potency	IC50 (±SE) ng/ml	Relative Potency
CsA	83.7 (±18.7)	1	50.5 (±7.8)	1	201.6 (±52.7)	1	53.4 (±10.2)	1
VCS	26.3 (±4.1)	3.2	13.8 (±2.1)	3.7	65.2 (±15.9)	3.1	11.0 (±2.4)	4.9
FK506	2.6 (±0.7)	32	0.3 (±0.2)	168	1.1 (±0.3)	183	0.054 (±0.02)	989

Figure 3

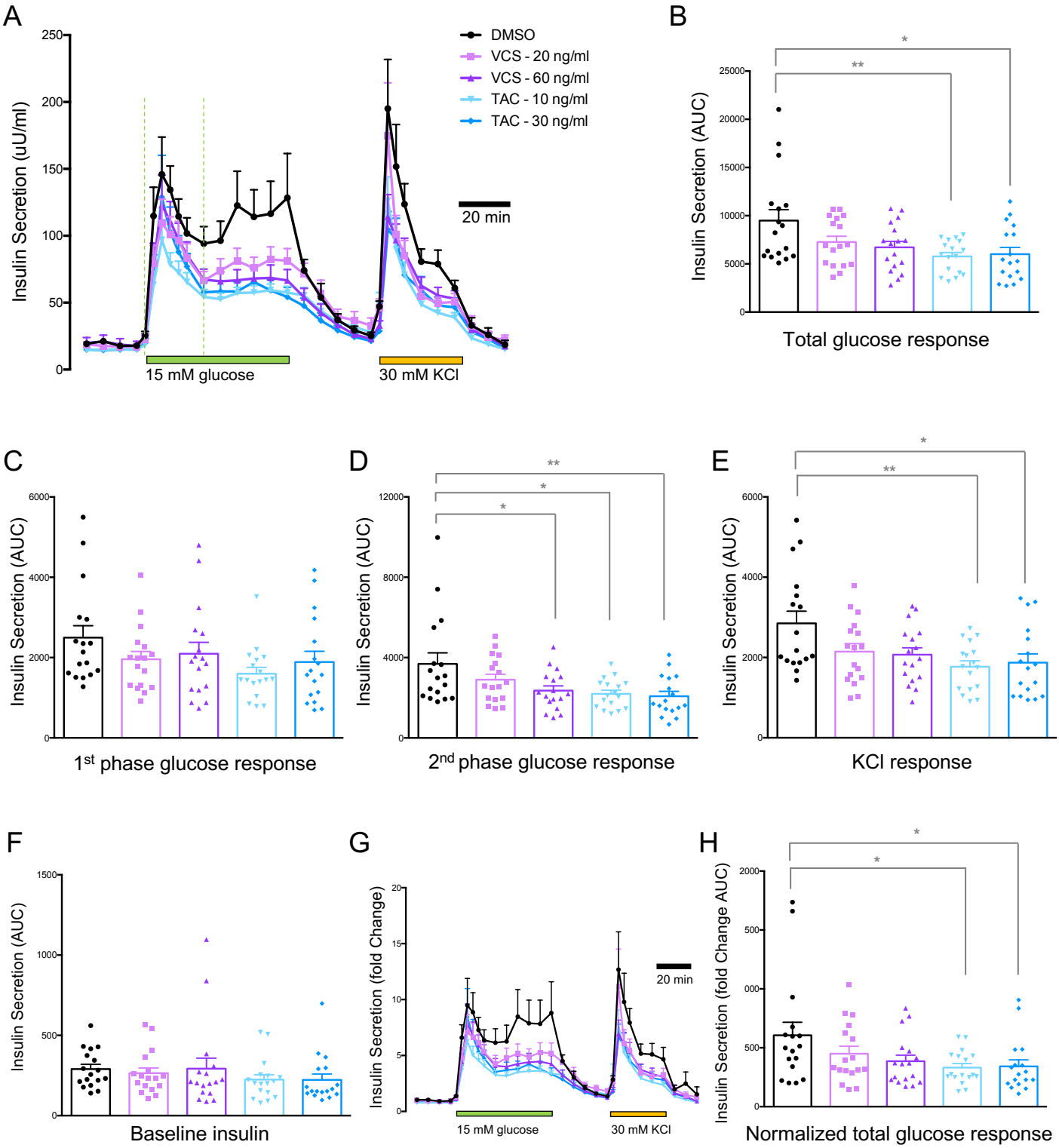

A
Insulin Secretion (uU/ml)
250
200
150
100
50
0
DMSO
VCS - 20 ng/ml
VCS - 60 ng/ml
TAC - 10 ng/ml
TAC - 30 ng/ml
20 min
15 mM glucose
30 mM KCl
B
Insulin Secretion (AUC)
25000
20000
15000
10000
5000
0
*
**
Total glucose response
C
Insulin Secretion (AUC)
6000
4000
2000
0
1st phase glucose response
D
Insulin Secretion (AUC)
12000
8000
4000
0
**
*
*
2nd phase glucose response
E
Insulin Secretion (AUC)
6000
4000
2000
0
*
**
KCl response
F
Insulin Secretion (AUC)
1500
1000
500
0
Baseline insulin
G
Insulin Secretion (fold Change)
20
15
10
5
0
20 min
15 mM glucose
30 mM KCl
H
Insulin Secretion (fold Change AUC)
2000
500
000
500
0
*
*
Normalized total glucose response

Figure 4

A

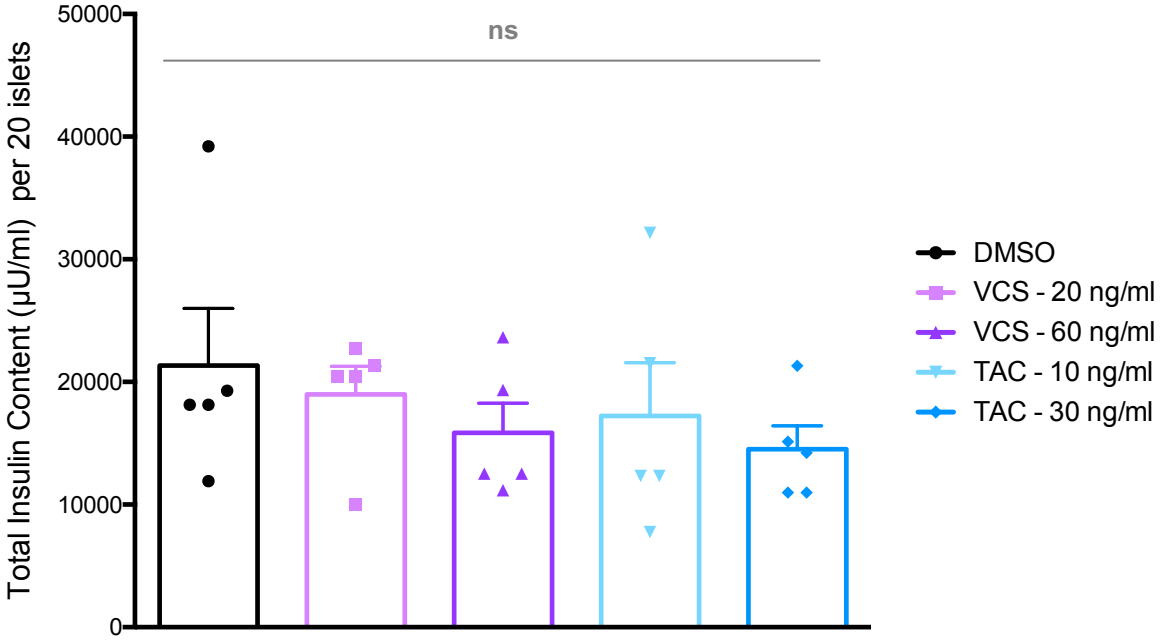

Total Insulin Content (µU/ml) per 20 islets
50000
40000
30000
20000
10000
0
ns
DMSO
VCS - 20 ng/ml
VCS - 60 ng/ml
TAC - 10 ng/ml
TAC - 30 ng/ml

Figure 5

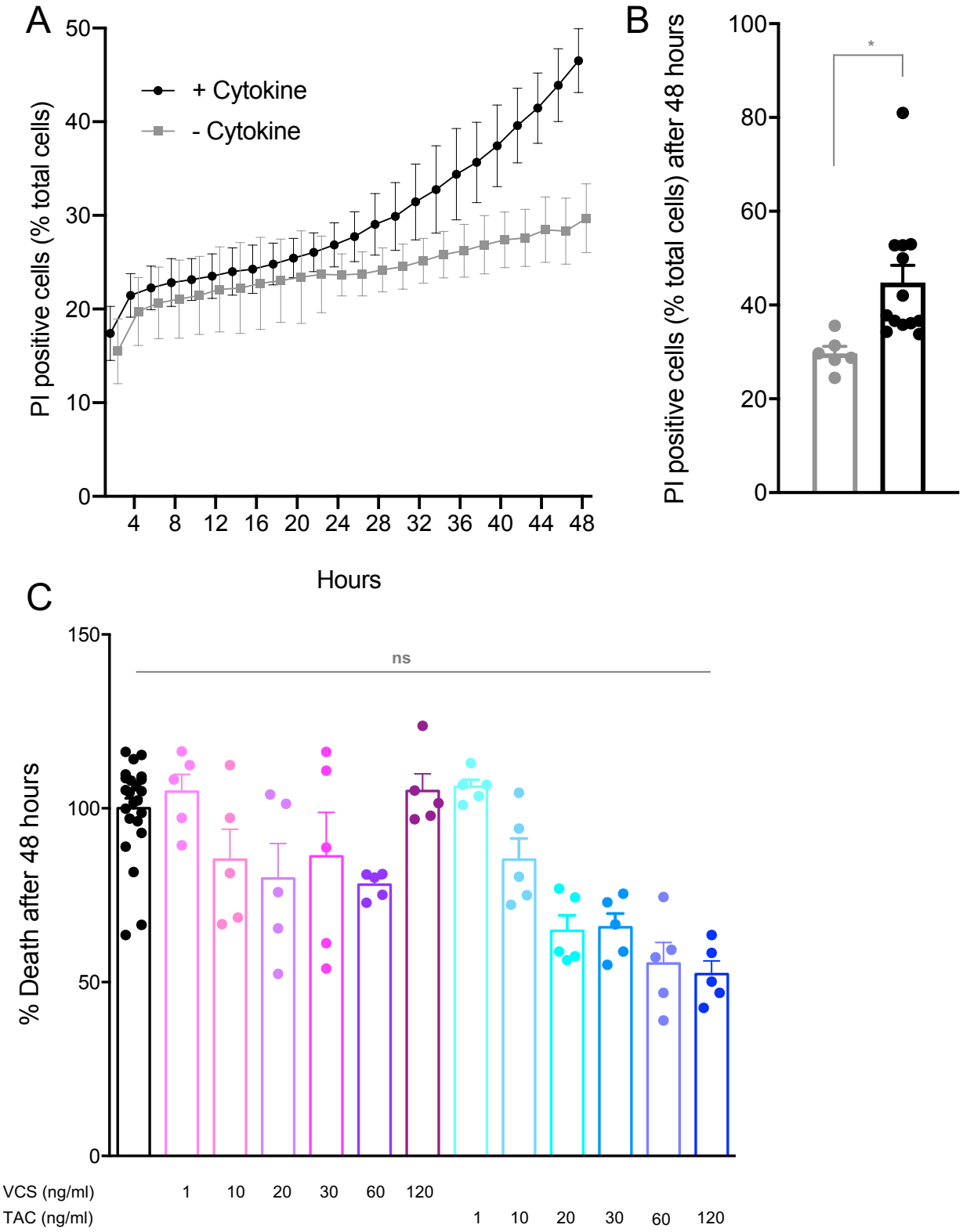

A
PI positive cells (% total cells)
+ Cytokine
- Cytokine
Hours
B
PI positive cells (% total cells) after 48 hours
*
C
% Death after 48 hours
ns
VCS (ng/ml)
TAC (ng/ml)

Figure 6

A

	Control							Voclosporin							Tacrolimus							Control vs Tac *p* value	Control vs VCS *p* value
FBXO28	7	7	7	8	7	7	7	8	7	8	9	7	7	8	8	9	8	8	9	8	8	0.00050	
TCP11L1	5	4	4	4	5	4	4	5	4	5	4	4	5	5	5	5	5	5	5	5	5		
EGR4	1	1	2	2	2	1	2	2	2	3	3	3	2	2	2	2	2	2	2	3	3		
CSNK2A2	17	18	16	17	16	16	17	20	16	17	18	19	19	19	17	19	18	18	18	18	20		
NFIC	20	18	21	21	22	26	23	25	24	23	22	26	26	30	26	32	25	23	28	30	26		
KLHL18	4	4	5	5	4	4	5	4	5	5	6	5	4	5	5	4	5	5	6	6	5		
ACVR2B	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	2	1	2	1	1		
RPS6KA5	2	1	2	1	1	1	2	2	2	2	2	1	1	2	2	2	2	2	3	2	2		
NAP1L1	108	116	104	102	111	111	103	113	118	109	132	120	136	112	122	123	116	153	123	117	141		
STX6	6	6	6	6	5	5	5	7	7	6	6	5	6	6	7	6	6	6	6	6	6		
MCM7	9	12	11	12	11	11	15	11	13	13	17	14	15	15	14	12	14	13	17	16	16		
DERA	31	33	28	28	26	32	29	35	34	30	40	26	37	31	33	33	30	33	34	35	37		
TAF5	1	2	1	1	1	1	1	1	2	2	2	2	1	1	2	1	2	2	2	2	2		
PTPRS	7	8	6	7	7	9	6	9	10	7	5	11	10	9	11	11	9	10	7	9	11		
TNRC6C	1	1	1	1	1	1	1	1	1	2	1	2	1	2	2	2	2	1	2	2	1		
WASF2	26	30	29	30	26	27	28	28	29	33	30	28	29	32	31	29	33	29	31	32	29		
ZNF596	1	2	2	2	2	1	2	2	2	2	2	3	2	2	1	2	2	2	2	2	2		
PCGF6	2	3	2	3	3	2	3	3	3	4	4	3	3	3	3	2	2	2	3	2	3		
SYT16	3	2	4	4	3	3	3	1	1	1	1	1	1	2	1	1	1	1	1	1	1	0.00006	0.00023
SCARB2	128	121	115	133	126	127	127	117	108	109	104	119	107	118	106	116	106	116	109	117	107	0.00031	
PITPNM1	33	26	24	35	33	33	31	24	22	18	25	24	22	27	21	25	17	22	21	29	19		
TBC1D30	4	4	4	4	4	6	4	3	2	2	2	3	3	3	2	2	2	2	2	2	2	0.00012	
FAM131A	16	15	15	17	18	19	12	13	11	10	10	13	14	14	11	12	10	14	10	12	13		
PCK1	6	7	4	15	7	5	14	2	3	1	3	2	1	3	3	2	1	2	2	1	1		
CCDC184	7	8	7	8	8	11	5	4	5	3	3	5	7	5	4	4	3	5	2	5	3	0.00022	
SYT5	36	30	37	42	50	69	36	18	19	12	13	26	37	24	14	16	11	18	12	27	19	0.00038	
PPP5C	35	32	35	32	34	37	37	31	31	32	31	31	34	31	32	31	30	33	28	30	33		
KIAA0319	7	6	8	7	7	9	5	5	4	4	2	6	6	6	4	5	4	6	3	4	4	0.00055	
MFSD1	41	38	40	38	42	39	40	39	38	34	34	39	36	35	33	39	37	37	33	30	35		
MLIP	7	8	12	7	9	11	6	6	5	7	3	7	6	7	3	3	4	4	2	5	5	0.0002	
NPTN	149	137	131	126	142	144	130	138	123	119	100	131	122	115	125	138	115	123	98	113	113		
ABCB8	9	9	8	8	9	9	8	7	9	8	9	7	8	7	7	7	7	7	8	6	7	0.00051	
SLC7A2	140	129	133	145	128	175	106	118	99	113	72	109	117	123	94	108	97	104	68	101	107		
ANXA7	165	148	155	174	170	166	161	150	144	136	149	140	160	161	137	144	131	163	132	143	142		
SGSM3	29	30	29	27	31	29	26	26	27	27	28	26	27	23	26	25	26	27	23	24	25		
CREM	18	20	19	26	22	17	27	15	17	15	22	14	15	16	16	13	13	16	19	16	13		
DAP	173	162	166	197	190	206	180	149	152	145	180	156	176	162	136	137	138	156	163	129	167		
ASB4	2	2	2	2	3	2	2	2	2	1	1	2	1	2	1	1	1	1	1	1	1	0.0001	
PAM	449	284	349	322	385	363	279	356	213	214	148	305	284	266	187	339	211	272	150	239	248		
LINC00936	3	2	3	2	3	4	3	2	1	1	2	2	3	2	1	1	1	2	1	1	2	0.00047	
PDK4	40	82	97	91	111	154	62	25	40	36	36	71	96	41	28	16	29	33	20	36	45	0.00097	
COPA	68	67	62	68	68	68	64	60	62	61	69	54	62	63	67	55	59	58	59	63	61		
TCTN3	21	21	24	22	24	22	19	19	19	19	18	22	22	19	18	19	21	21	17	18	17		
LGI2	1	2	2	3	1	3	1	0	1	1	0	1	1	1	1	0	1	1	0	0	1		
SMOC1	32	39	54	26	35	103	38	16	19	18	13	19	47	13	11	9	13	8	8	8	23		
TMED3	198	143	187	158	187	175	168	160	123	133	153	182	160	128	110	146	124	171	135	131	141		
B9D1	30	30	30	29	40	34	34	26	28	19	33	29	30	24	22	23	19	28	22	24	23	0.00022	
AAED1	17	14	15	19	17	23	15	14	13	11	15	13	18	14	12	12	11	14	12	12	14		
NUP210	9	7	10	8	9	10	9	9	7	9	6	9	7	8	7	9	8	8	7	6	6		
MRPL17	22	21	21	21	21	20	20	20	20	18	19	20	22	18	17	18	18	21	17	20	19		
BBS5	5	6	6	7	7	5	4	5	5	5	4	5	5	7	5	4	5	5	4	5	4		
TMED10	140	115	128	131	141	145	137	121	107	117	140	131	135	120	94	114	113	126	118	125	112		
MPV17	55	57	55	64	60	65	60	47	52	49	64	42	67	58	47	41	47	50	49	53	53	0.00042	
ARF1	342	332	358	345	348	369	369	317	333	333	380	314	385	327	322	303	323	333	318	335	337		
TASP1	9	8	8	8	7	8	9	7	7	8	7	6	6	7	6	7	7	6	7	8	8		
SKIV2L	13	12	13	14	14	14	13	12	11	13	12	12	12	13	13	13	11	13	11	12	13		
THNSL1	6	6	6	6	7	5	5	5	5	5	5	5	5	5	5	6	5	6	3	6	5		
CEP85L	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		

B

Functional enrichments in your network

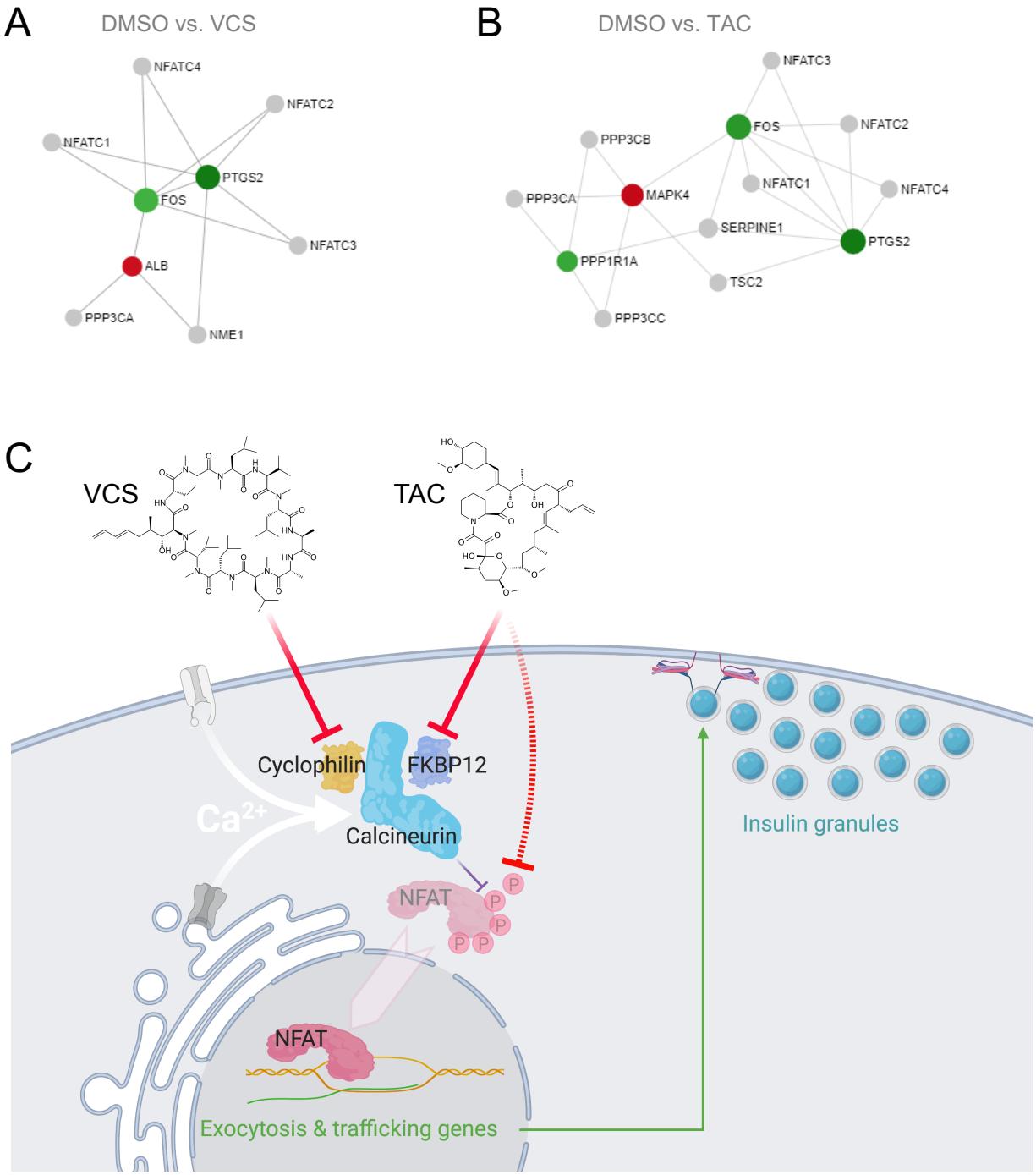
Molecular Function (GO)

GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0019905	syntaxin binding	4 of 83	0.0281

Cellular Component (GO)

GO-term	*description*	*count in gene set*	*false discovery rate*
GO:0030137	COPI-coated vesicle	3 of 22	0.0146
GO:0030662	coated vesicle membrane	5 of 161	0.0153
GO:0030135	coated vesicle	6 of 255	0.0153
GO:0030659	cytoplasmic vesicle membrane	9 of 724	0.0173
GO:0030133	transport vesicle	6 of 356	0.0295
GO:0030126	COPI vesicle coat	2 of 13	0.0346
GO:0098588	bounding membrane of organelle	14 of 1950	0.0391
GO:0030285	integral component of synaptic vesicle membrane	2 of 15	0.0391
GO:0030136	clathrin-coated vesicle	4 of 172	0.0442
GO:0098791	Golgi subcompartment	8 of 801	0.0476
GO:0030672	synaptic vesicle membrane	3 of 83	0.0476
GO:0030658	transport vesicle membrane	4 of 181	0.0476

Figure 7


A
DMSO vs. VCS
NFATC4
NFATC2
NFATC1
PTGS2
FOS
NFATC3
ALB
PPP3CA
NME1
B
DMSO vs. TAC
NFATC3
FOS
NFATC2
PPP3CB
NFATC1
NFATC4
PPP3CA
MAPK4
SERPINE1
PTGS2
PPP1R1A
TSC2
PPP3CC
C
VCS
TAC
Cyclophilin
FKBP12
Calcineurin
Ca2+
NFAT
P
NFAT
Insulin granules
Exocytosis & trafficking genes