Supporting Information

Dynamic changes in β-cell [Ca^{2+}] regulates NFAT activation, gene transcription and islet gap junction communication

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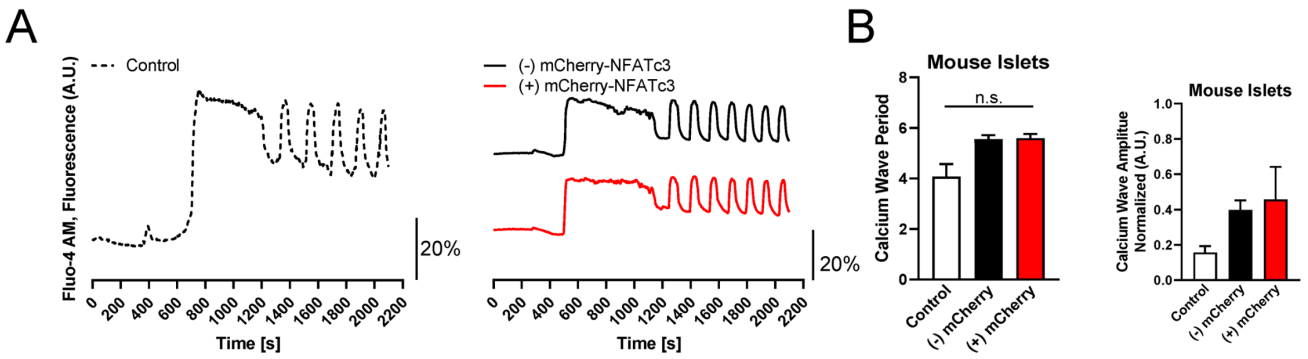
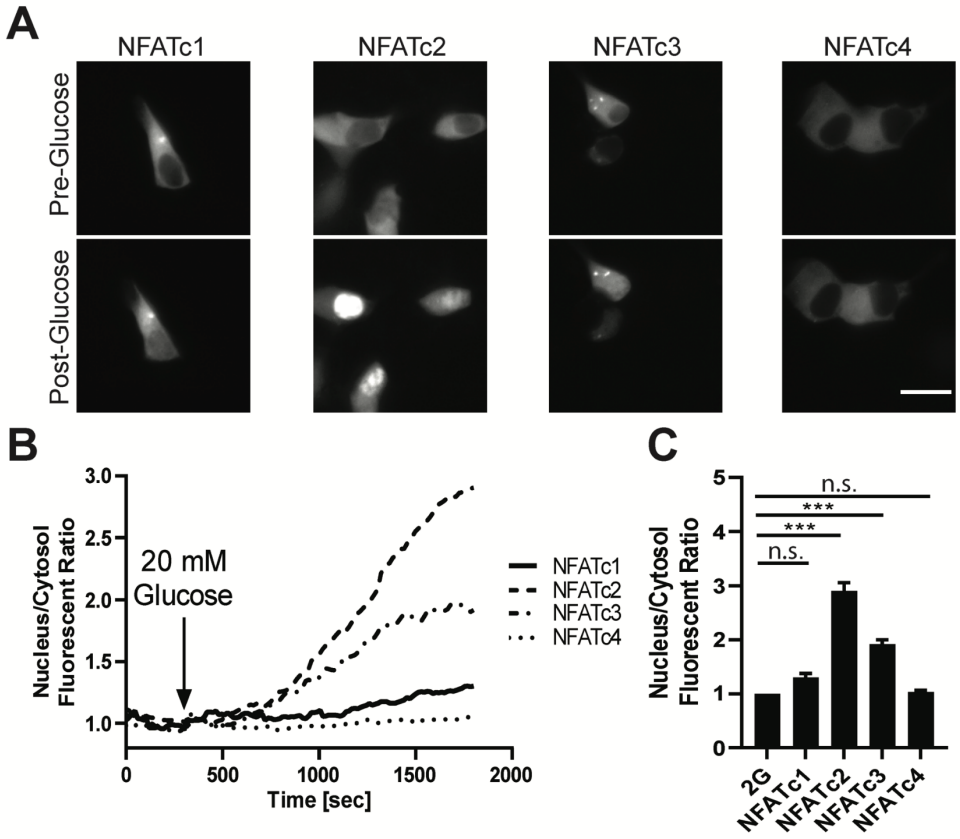
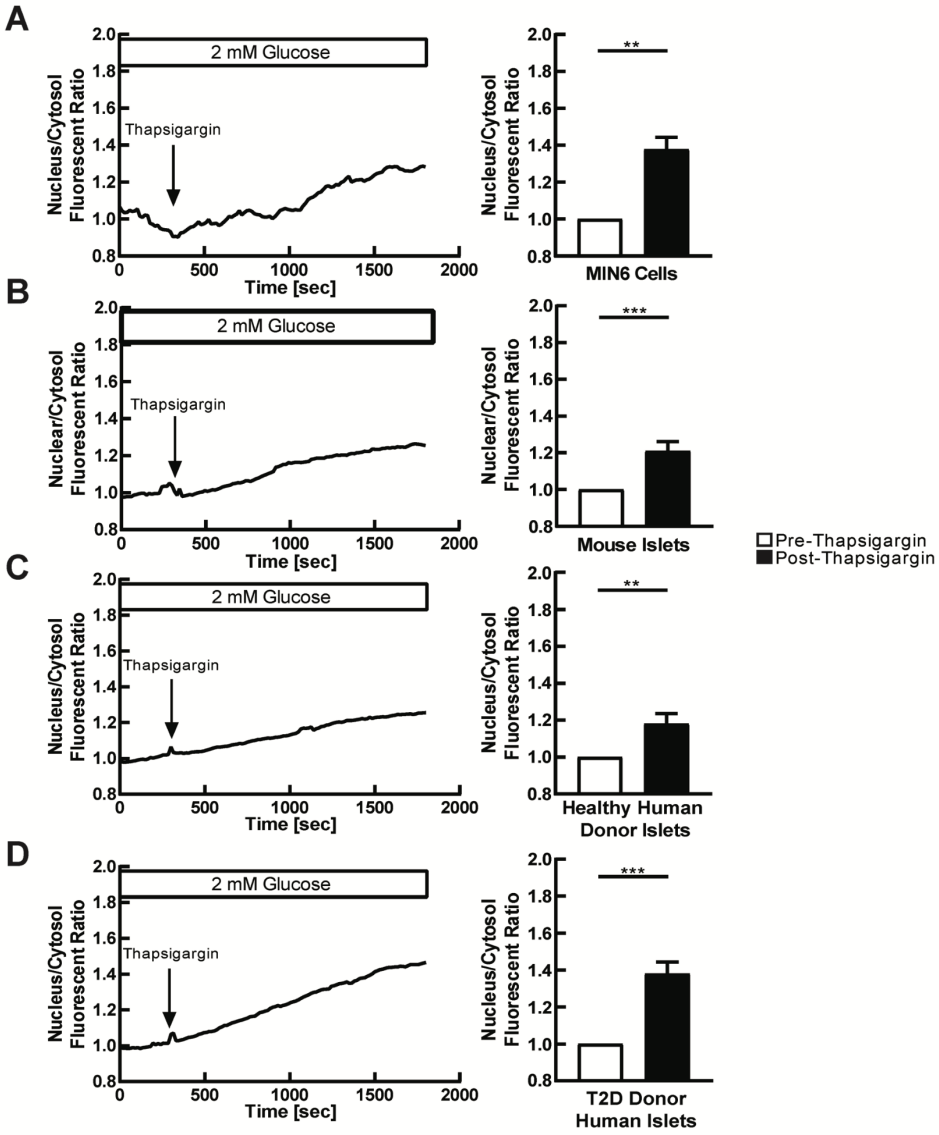


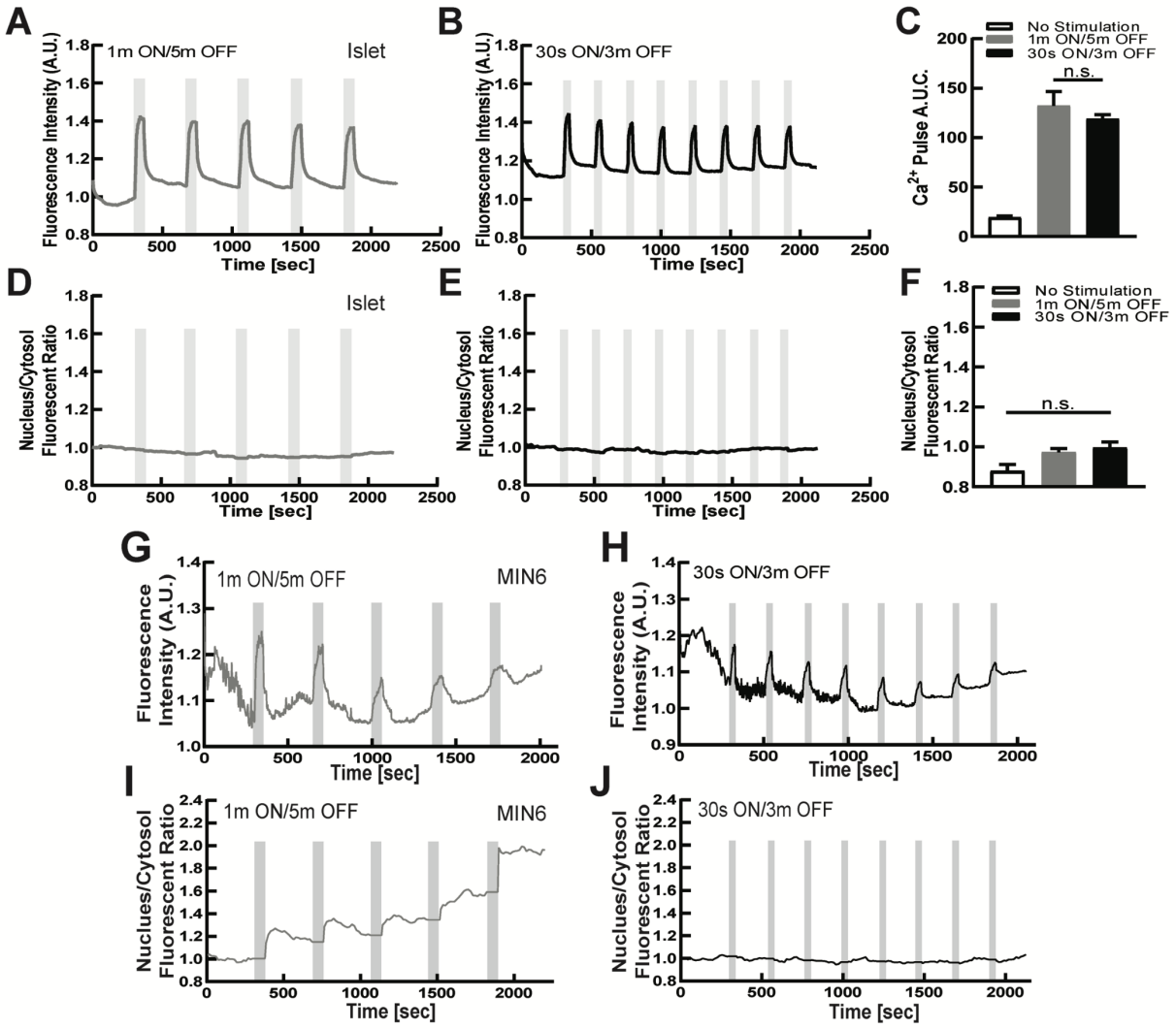
Supplementary Figure S1. Ca^{2+} response in mCherry-NFATc3 infected and non-infected cells. A) Ca^{2+} response following 11mM glucose treatment in mouse islets that were not infected (left) and infected with mCherry-NFATc3 (right) that showed either lack of expression (black) or expression (red). **B)** Quantification of Ca^{2+} response in terms of oscillation period (left) or oscillation amplitude (right) for non-infected mouse islets (empty) or infected mouse islets that showed either lack of expression (black) or expression (red). Data in B averaged over n=5 mice (20 islets).



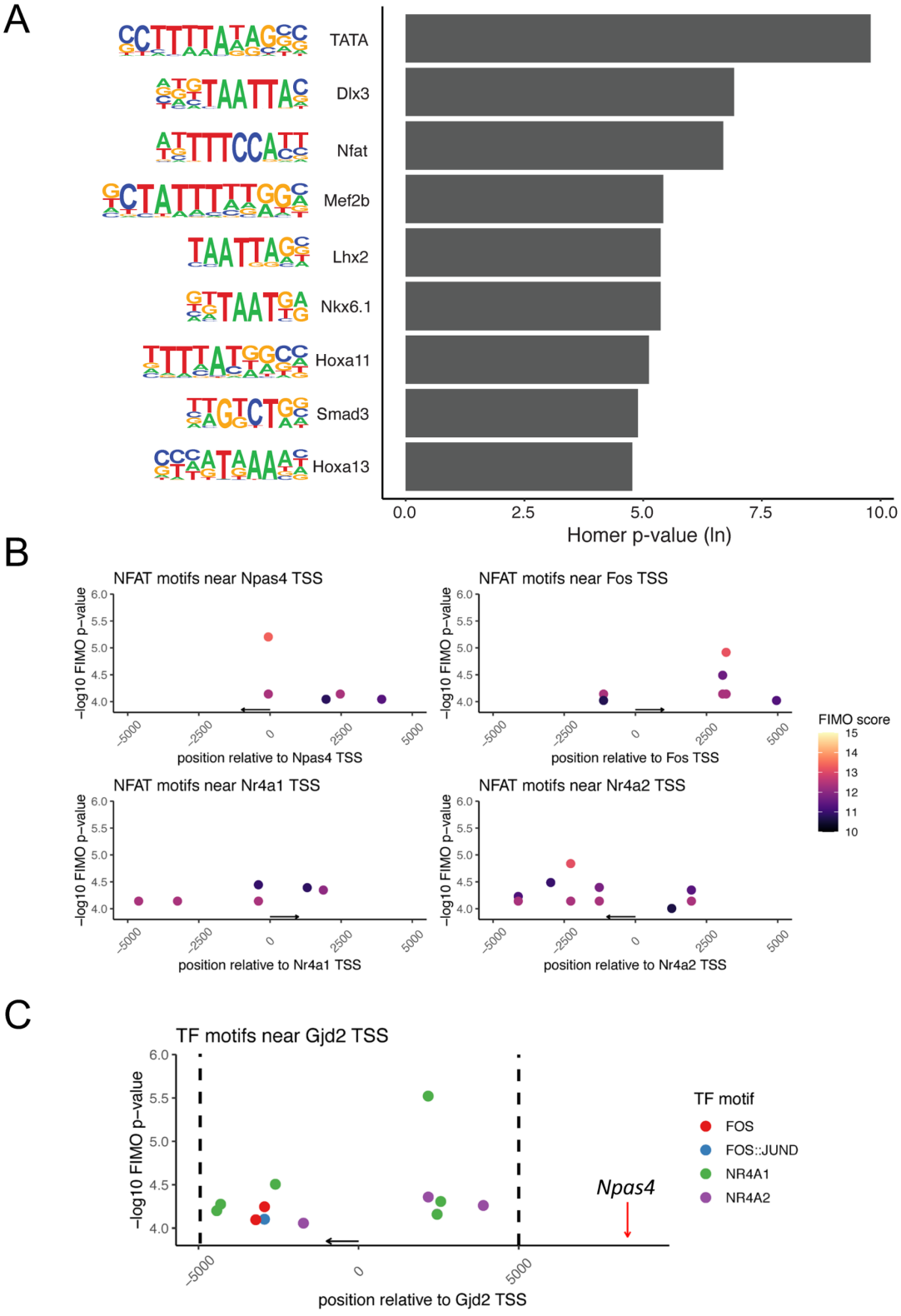
Supplementary Figure S2. Glucose dependent activation of NFATc1-c4 in MIN6 cells. A) Images of MIN6 cells transiently transfected with mCherry-NFATc1,c2,c3,c4 at low glucose and 30 minute after addition of high glucose. **B)** Time-course for the ratio of nuclear to cytoplasmic fluorescence of NFATc1-4. Traces represent average of all experiments performed in MIN6 cells. **C)** mean nuclear-cytoplasmic mCherry-NFATc1-c4 ratio 30minutes after treatment in MIN6 cells. Statistical analysis was done using ANOVA with Tukey HSD post hoc test. *** represent p=0.0005, respectively and n.s. means no significance (p>0.2). Data in C averaged over n=4 plates (6,35,15,27 cells).



Supplementary Figure S3. ER Ca^{2+} release activates NFATc3. A) Mean time-course for the ratio of nuclear to cytoplasmic fluorescence of MIN6 cells transiently transfected by mCherry-NFATc3, at 2 mM glucose and thapsigargin after addition of thapsigargin (left); together with mean nuclear-cytoplasmic mCherry-NFATc3 ratio before and 30min after thapsigargin addition (right). **B)** As in A for primary mouse islets infected with mCherry-NFATc3. **C)** As in A for human islets from healthy donors, infected with mCherry-NFATc3. **D)** As in A for human islets from donors with T2D, infected with mCherry-NFATc3. Statistical analysis was done using ANOVA with Tukey HSD post hoc test. *, **, and *** represent p=0.05, p=0.005, p=0.0005, respectively. Data in A-C averaged over n=8 plates (18 cells), n=5 mice (10 islets), n=8 healthy donors (31 islets) and n=8 donors with T2D (n=28 islets).

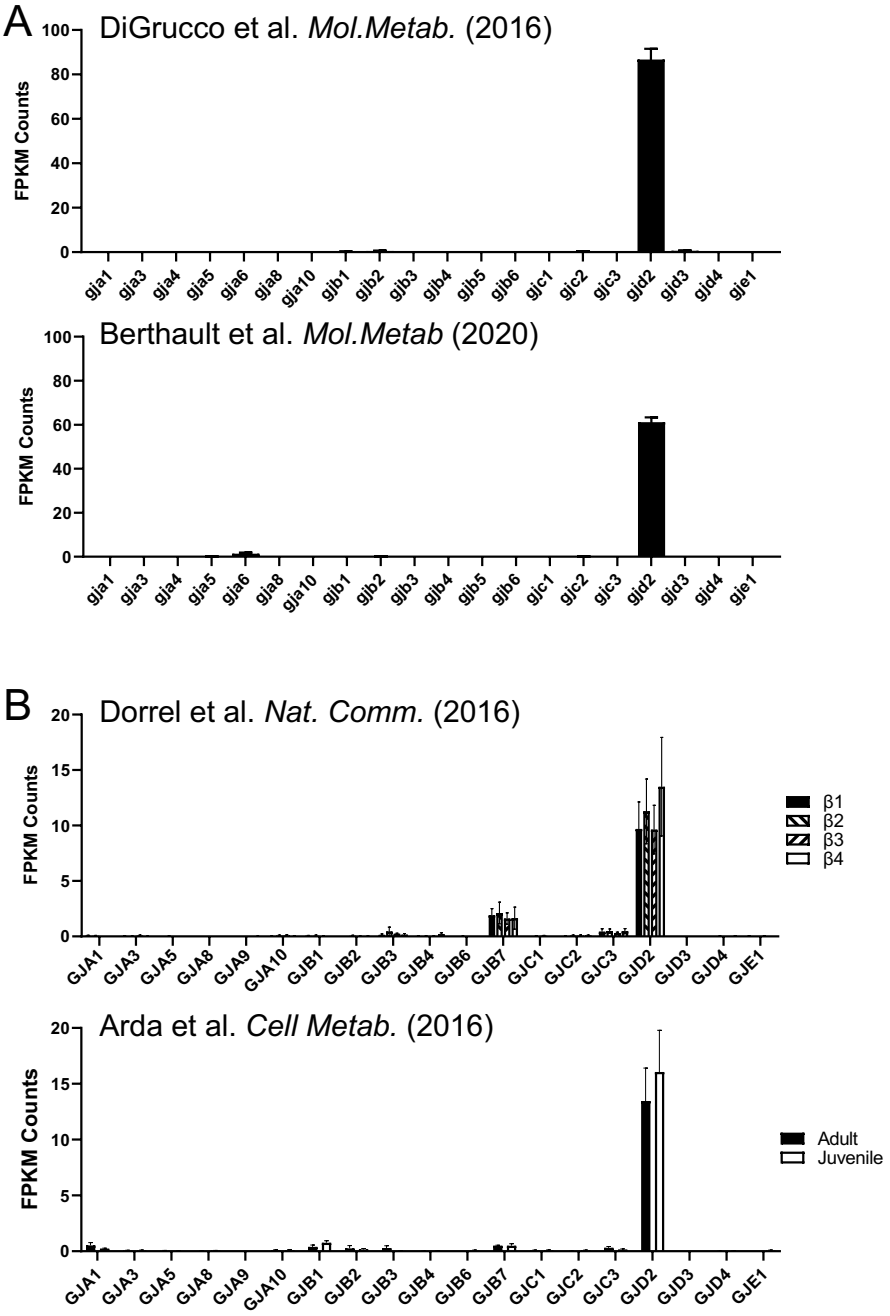


Supplementary Figure S4. Dynamic Ca^{2+} changes via optogenetics activates NFATc3. A) mean Ca^{2+} changes in mouse islets expressing ChR2 in the beta-cell, at 2mM glucose following 1m ON/5m OFF optical stimulation protocol. Grey indicates duration of optical stimulation. **B)** as in A for 30s ON/3m OFF optical stimulation protocol. **C)** Area under the curve (AUC) for each stimulation pulse protocol, as well as time course lacking stimulation. **D)** Mean time-course of mCherry-NFATc3 nucleus/cytosol fluorescent ratio in ChR2-expressing islets following the 1m ON/5m OFF optical stimulation pulse protocol. **E)** As in D for the 30s ON/3m OFF protocol. **F)** Mean nuclear-cytoplasmic mCherry-NFATc3 ratio 30minutes after optical stimulation. **G)** mean Ca^{2+} changes in MIN6 cells transiently transfected with ChR2, at 2mM glucose following 1m ON/5m OFF optical stimulation protocol. Grey indicates duration of optical stimulation. **H)** as in G for 30s ON/3m OFF optical stimulation protocol. **I)** Mean time-course of mCherry-NFATc3 nucleus/cytosol fluorescent ratio in MIN6 cells, following the 1m ON/5m OFF optical stimulation pulse protocol. **J)** As in I for the 30s ON/3m OFF protocol. Statistical analysis was done using ANOVA with Tukey HSD post hoc test. Data in C,F= averaged over n=4 mice (15, 14 islets).

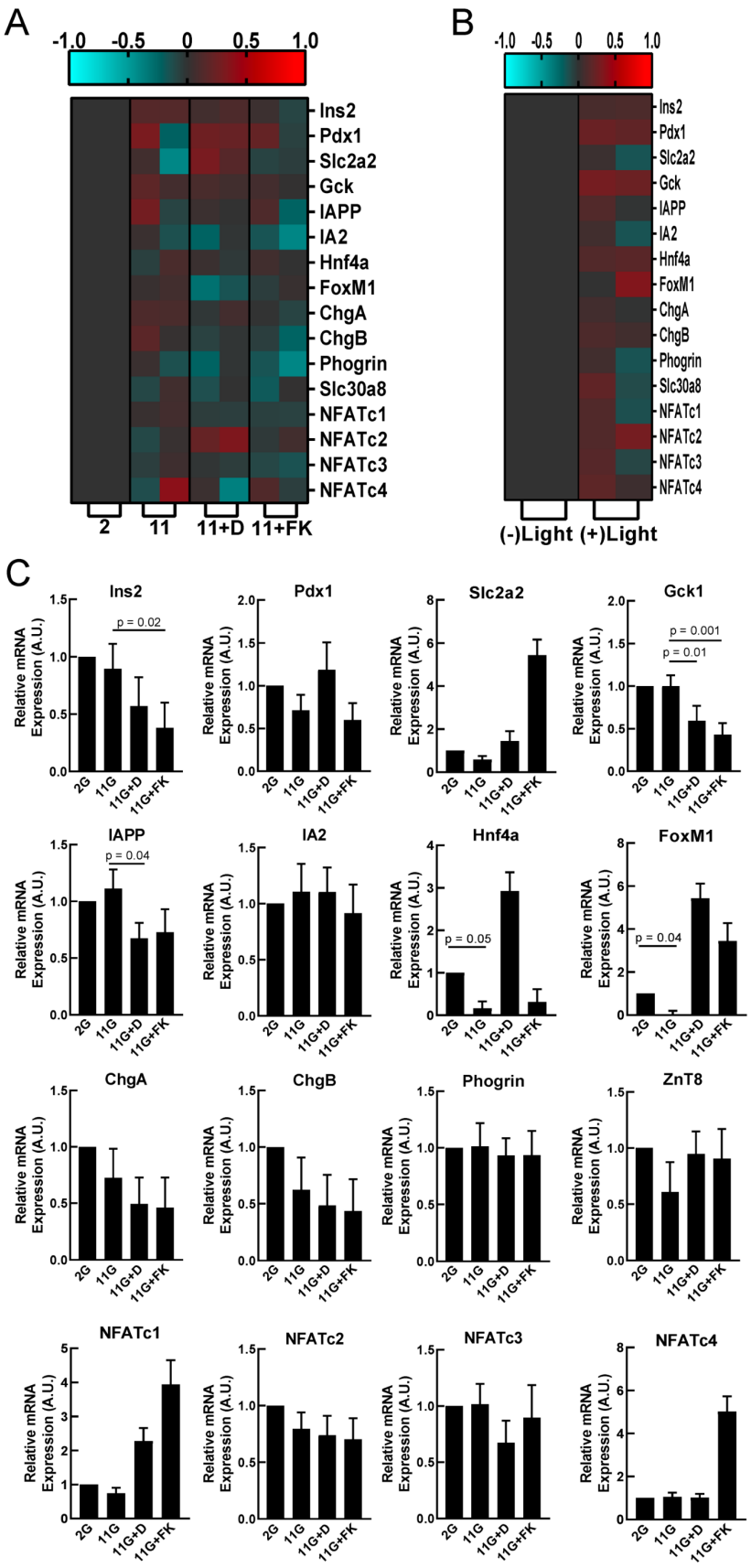


Supplementary Figure S5. Activity-dependent transcription factor binding sites. A) Enrichment of binding sites within 300bp of the transcription start site (TSS) for those genes that show expression changes upon glucose and glucose plus diazoxide (see Figure 3A), as identified by Homer analysis. **B)** Identification of NFAT binding sites within the subset of transcription factor genes identified to be regulated by beta cell excitability and [Ca^{2+}] elevation (see Figure 3B). Arrow indicates transcribed region of indicated gene. Analysis restricted to 5000bp of the TSS. **C)** Identification of those transcription

factor binding sites identified to be regulated by beta cell excitability and [Ca^{2+}] elevation (see Figure 3B) within 5000bp of the *Gjd2* TSS (vertical dashed lines). Known Npas4 binding site indicated by red arrow (no Npas4 sites identified within 5000bp of TSS).

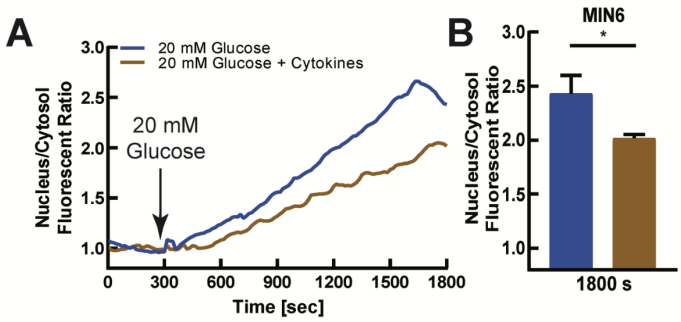


Supplementary Figure S6. Expression of gap junction genes in the moue and human β-cells. A) Expression of mouse gap junction genes in sorted mouse β-cells from published bulk RNAseq datasets. Top: β-cells sorted by fluorescent protein markers [DiGrucco et al. *Molecular Metabolism* (2016) doi:10.1016/j.molmet.2016.04.007]; Bottom: β-cells sorted by cell surface markers [Berthault et al. *Molecular Metabolism* (2020) doi:10.1016/j.molmet.2020.101060]. **B)** As in A for all human gap junction genes in sorted human β-cells. Top: β-cells sorted by cell surface markers, across β1-β4 subtypes [Dorrel et al. *Nature Communication* (2016) doi:10.1038/ncomms11756]. Bottom: β-cells sorted by surface markers, across juvenile and adult donors [Arda et al. *Cell Metabolism* (2016) doi: 10.1016/j.cmet.2016.04.002].

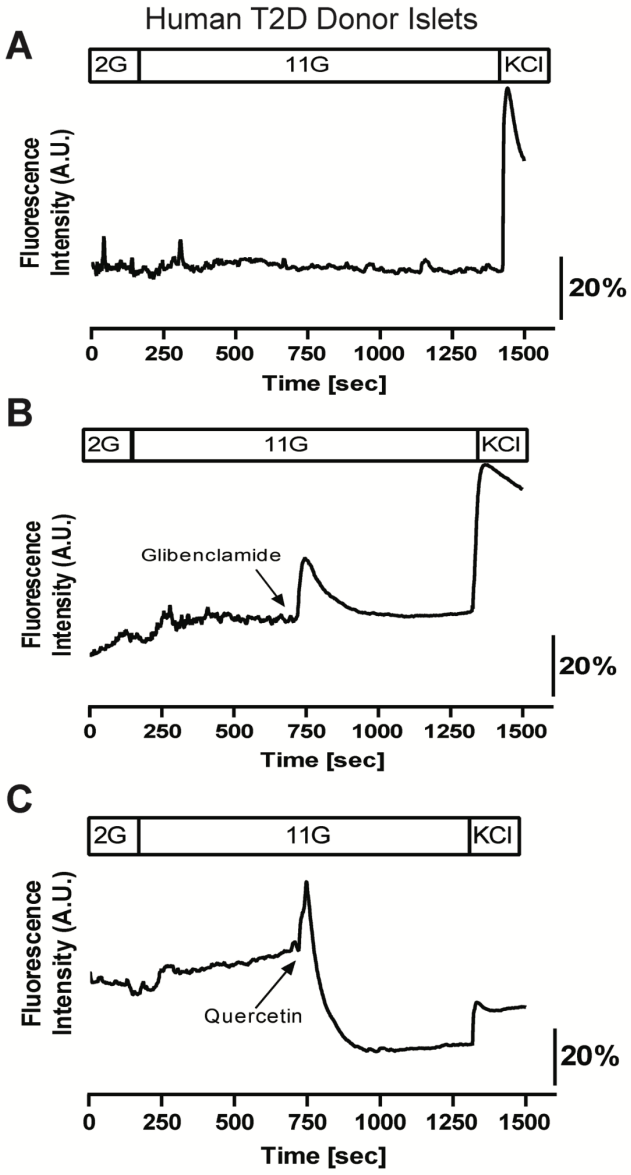


Supplementary Figure S7. Acute gene transcription changes by RNAseq and qPCR following elevated electrical activity A). Heat map depicting genes previously identified to regulate beta-cell

proliferation and insulin secretion, following glucose stimulation, application of K_{ATP} opener diazoxide upon glucose stimulation upon application of CaN inhibitor FK506 upon glucose stimulation. **B)** As in A for CR2-expressing islets in the absence and presence of stimuli applied in A. Displayed is the subset of genes that shows the highest expression changes across an optical stimulation protocol. **C)** qPCR measurements of gene expression for those genes in A. Statistical analysis for C was done using ANOVA with Tukey HSD post hoc test. Data in C averaged over n=5 experiments.



Supplementary Figure S8. Diminished NFATc3 activation in MIN6 cells treated with proinflammatory cytokes. Mean NFATc3 nuclear translocation over time, as measured by the ratio of nuclear to cytoplasmic fluorescence of mCherry-NFATc3, in untreated (blue) or cytokine-treated (brown) MIN6 cells, following elevated glucose. Cytokine treatment contains 0.005 μg/mL IL1-β, 0.01 μg/mL TNF-α, and 0.1 μg/mL IFN-γ for 2 h in DMEM or RPMI-1640 media. **B)** mean nuclear-cytoplasmic mCherry-NFATc3 ratio 30minutes after treatment in MIN6 cells, as in A. Statistical analysis was done using ANOVA with Tukey HSD post hoc test. * represent p=0.05, Data in B averaged over n=5 plates (17 cells).



Supplementary Figure S9. [Ca^{2+}] response in islets from donors with T2D. A) [Ca^{2+}] elevations, as indicated by Fluo4 fluorescence, in human islets obtained from donors with T2D. Treatments include sequential stimulation for 2 minutes at 2 mM glucose, 20 minutes at 11 mM glucose, and 3 minutes with 20 mM KCl. **B)** As in A, but 10 minutes post adding 11 mM glucose, glibenclamide was added acutely. **C)** as in A but 10 minutes post adding 11 mM glucose, quercetin was added acutely. Data representative of islets 3 donors with T2D (11 islets).

Gene	Primer (5' – 3')	T_M (°C)	Predicted Amplicon Length (bp)	NCBI Reference Sequence
Insulin-2 (Ins2)	**F:** CACCAGCCCTAAGTGATCCG **R:** TTGTGCCACTTGTGGGTCCT	60 62	260	NM_001185083.2
Pancreatic and Duodenal Homeobox 1 (Pdx1)	**F:** CAGTGGGCAGGAGGTGCTTA **R:** GGTCCGTATTGGAACGCTCA	62 60	777	NM_008814.3
Glut2	**F:** ATAGCAAGCACACTACGGCT **R**: TGGGGTTGGAATCACAGCAT	60 60	397	X78722.1
Glucokinase (Gck)	**F:** TCAGCAGCTGTACGAAAAGATCA **R:** CTAGTGGACTGGGAGCATTTGT	60 60	614	NM_010292.5
Chromogranin-A (ChgA)	**F:** AGCCAGACTACAGACCCACT **R:** TCATCACCTTGGTGTCCCCT	60 60	174	NM_007693.2
Chromogranin-B (ChgB)	**F:** CCGAGCTCCTCCTACACTCA **R:** AATGCAGCGAGTCACCATTT	60 58	147	NM_007694.4
Islet Amyloid Peptide (IAPP)	**F:** CGGACCACTGAAAGGGATCTT **R:** CGTGTTGCACTTCCGTTTGT	60 60	149	NM_010491.2
Islet Antigen-2 (IA2)	**F:** TTGTCCGTTCTAAGGACCAGTT **R:** CAGATGCTCACACAGGCAGAA	59 60	148	NM_008985.2
Hepatocyte Nuclear Factor 4 alpha (Hnf4a)	**F:** CTTTGATCCAGATGCCAAGGG **R:** TACTCCCAGGTGCTCTTCTGA	60 60	606	NM_008261.3
Phogrin	**F:** AAGTGGAATCAGGCCGAAGG **R:** GTAGTGTGAGTTGTGGCGGA	60 60	163	NM_011215.2
Forkhead Box M1 (FoxM1)	**F:** CTGTGAGGGTCAAAGCTTGC **R:** TCTGATGTTTCACTCGGGGC	59 60	140	NM_008021.4
ZnT8	**F:** ACTGGACAGCGCATCAAACA **R:** GGCTTCTGTCGAAGTTCTCTGT	60 60	145	NM_172816.3
Connexin-36 (Gjd2)	**F:** TTTGCCCTCATTCGGGTGAT **R:** AAGCTGGCATGCTATCCTGG	60 60	409	AF226992.1
Connexin-43 (Gja1)	**F:** TTGACTTCAGCCTCCAAG **R:** AATGAACAGCACCGACAGC	59 60	172	Article Ref
NFATc1	**F:** TGCAAGCCGAATTCTCTGGT **R:** TAAATGTGCAACACGCCACG	60 60	251	NM_172390.2
NFATc2	**F:** TTTGAATCCGAACGAAGAAGAGC **R:** CAGAAACTTCTGCGGCCCTA	60 60	181	NM_012340.0
NFATc3	**F:** AGGTGCATCGAATCACTGGG **R:** CAAGGACAATGTGAGCCCCT	60 60	498	NM_173165.2
NFATc4	**F:** GTAAACGTCTGACCTGGGGC **R:** TCCAGTTCTTCCCCCAATCC	60 60	260	NM_001136022.2
Hypoxanthine Guanine Phosphoribosyl Transferase (HPRT1) (Reference)	**F:** TGGATACAGGCCAGACTTTGTT **R:** GGGAAAATACAGCCAACACTGC	60 60	626	NM_013556.2

Supplementary Table S1. qPCR primers. Primers used to test specific genes involved in cell function, insulin granule formation, and NFATc1-NFATc4. Table shows primer name, sequence from 5'-3', melting temperature, and predicted amplicon.