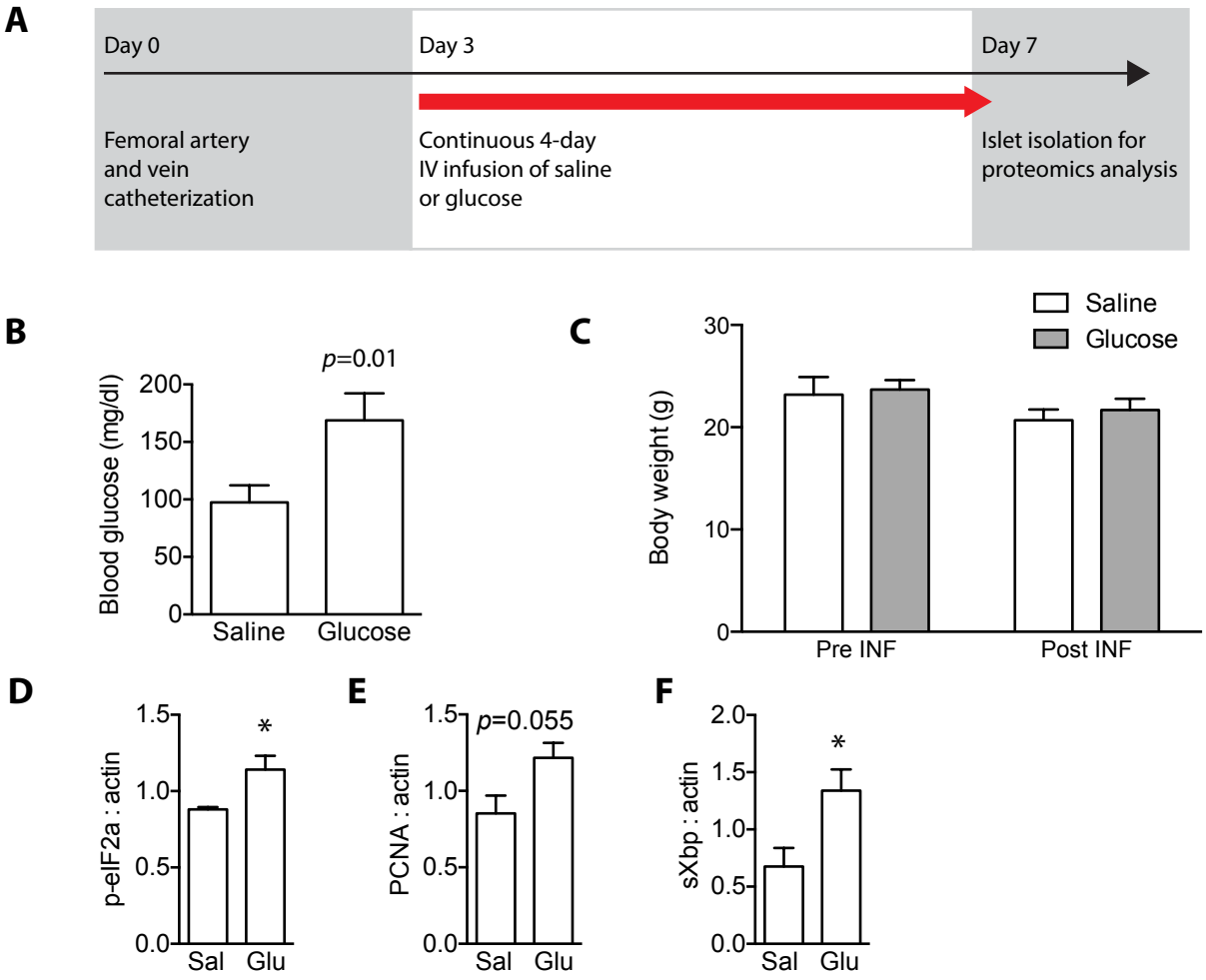
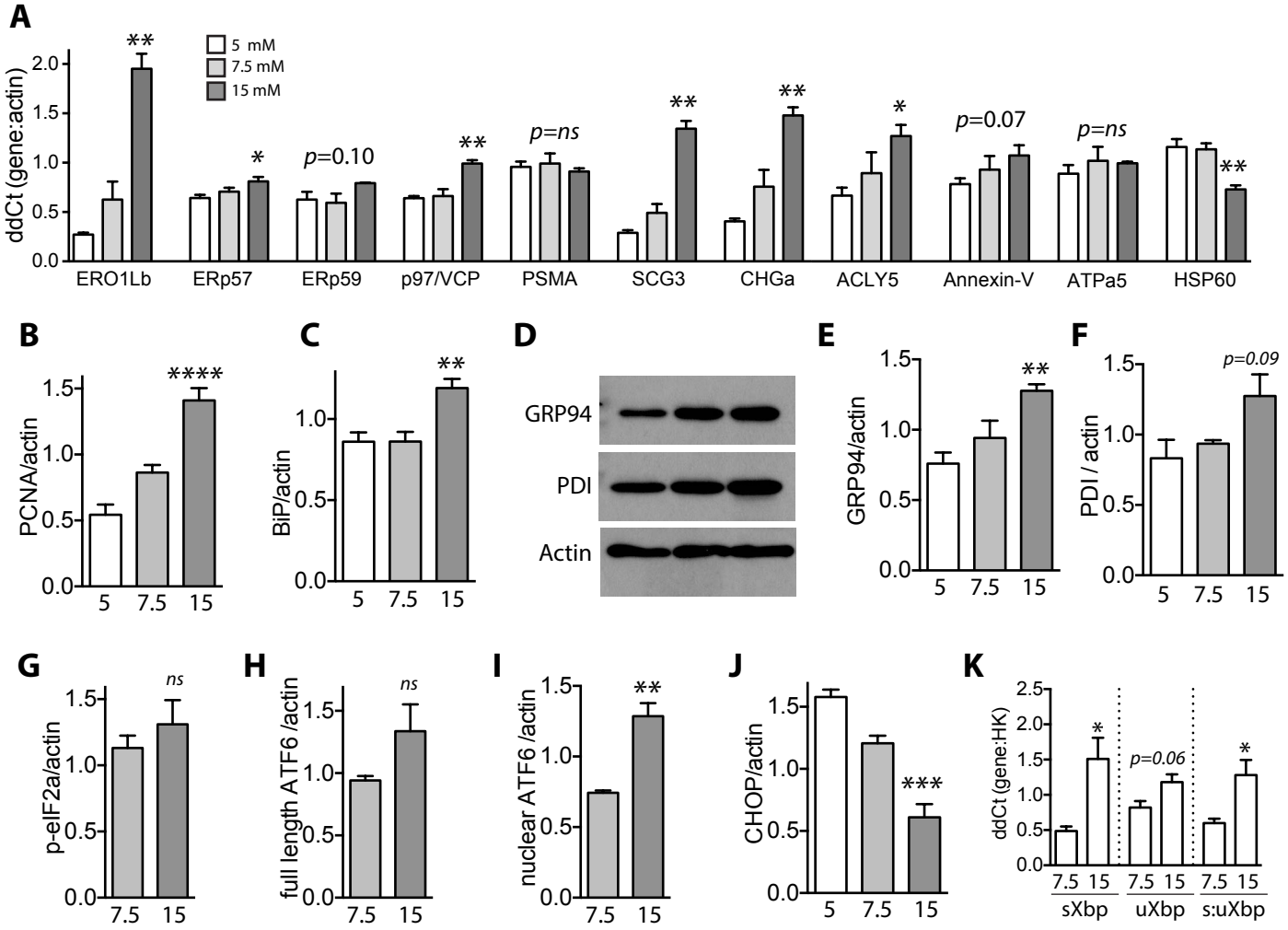
Supplemental Figure 1



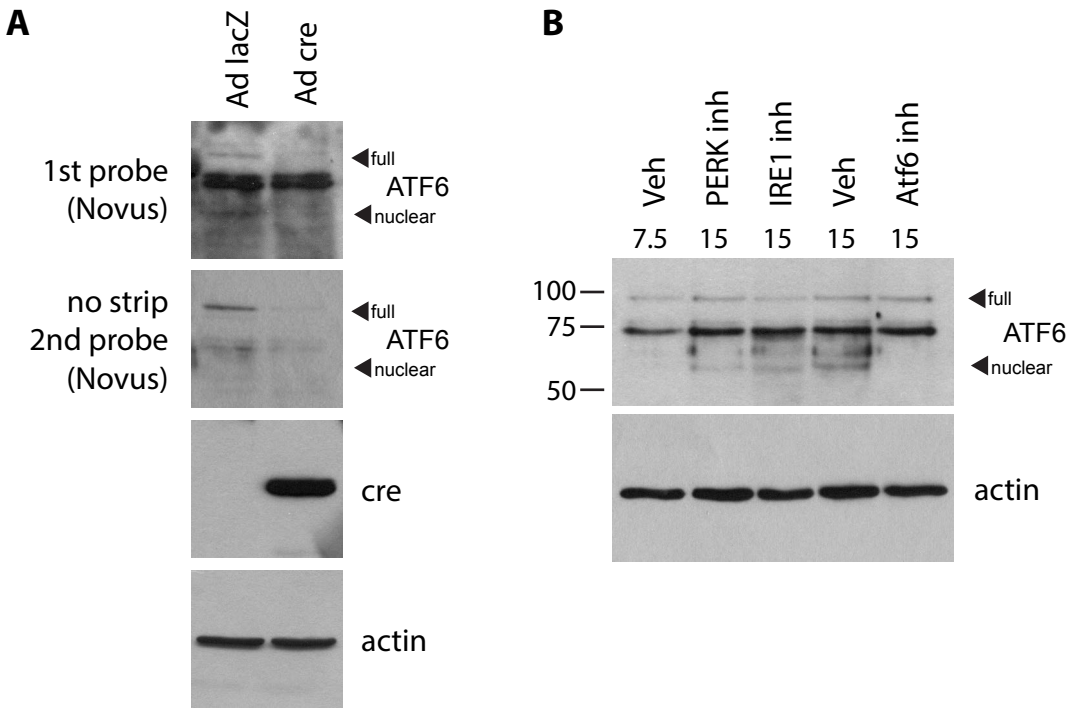
Supplemental Figure 1. (A) Schematic of experimental timeline for glucose infusion for proteomics analysis. Mice were catheterized in the femoral artery and vein on Day 0, allowed to recover for three days, and then received continuous intravenous infusion of saline (control) or 50% glucose through the femoral vein. After 4 days infusion mice were anesthetized and islets immediately isolated for proteomics screen. (B) Average blood glucose measured on blood obtained from the arterial catheter (unhandled mouse) over the 4-day infusion was modestly increased in mice receiving IV glucose. (C) Saline and glucose infused mice were of similar body weight before and after infusion. (D-F) Quantification of immunoblot performed on islets after 4-day infusion, corresponds to images shown in Figure 1F; n=4. Data are represented as mean +/- SEM. *p<0.05 by Student's T test.

Supplemental Figure 2



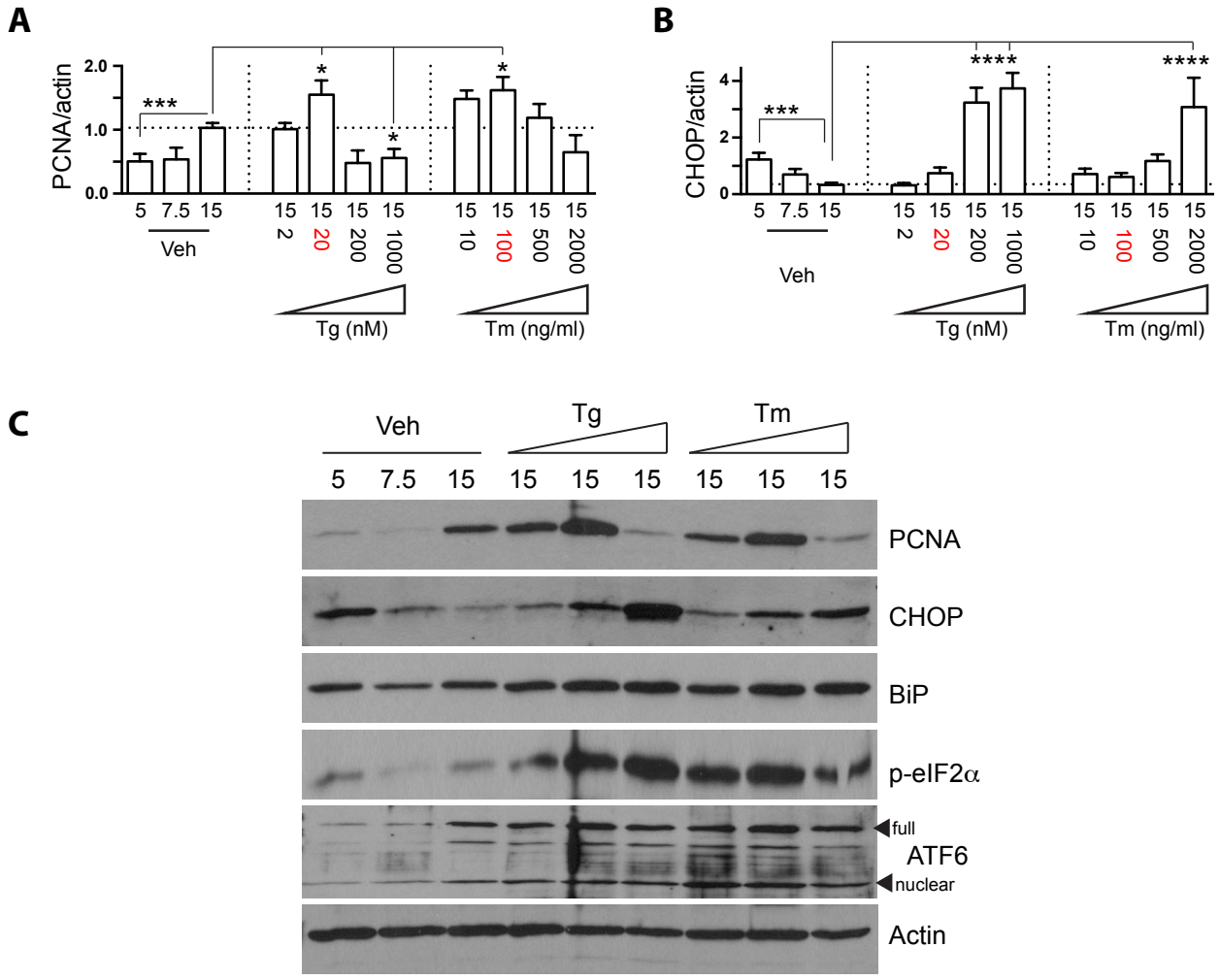
Supplemental Figure 2. Ex vivo islet glucose exposure models in vivo induction of proliferation, UPR, and targets induced in the proteomics screen (goes with Figure 2). (A) Mouse islet cells cultured in 5, 7.5 or 15 mM glucose for 24 hours were assessed for mRNA expression of the indicated genes corresponding to proteins altered in abundance on the proteomics screen (n=3; p values reflect comparison between 5mM and 15mM glucose. (B-C) quantification of immunoblots in Figure 2D (n=3). (C-E) Immunoblots on mouse islet cells cultured in 5, 7.5 or 15mM glucose for other UPR-associated proteins (n=3). (F-I) quantfication of immunoblots in Figure 2G and 2K (n=3). (J) Results of a qPCR assay for spliced and total Xbp. This is a different assay than the gel-based agarose assay shown in Figure 2F. Data are represented as mean +/- SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by Student's T test

Supplemental Figure 3



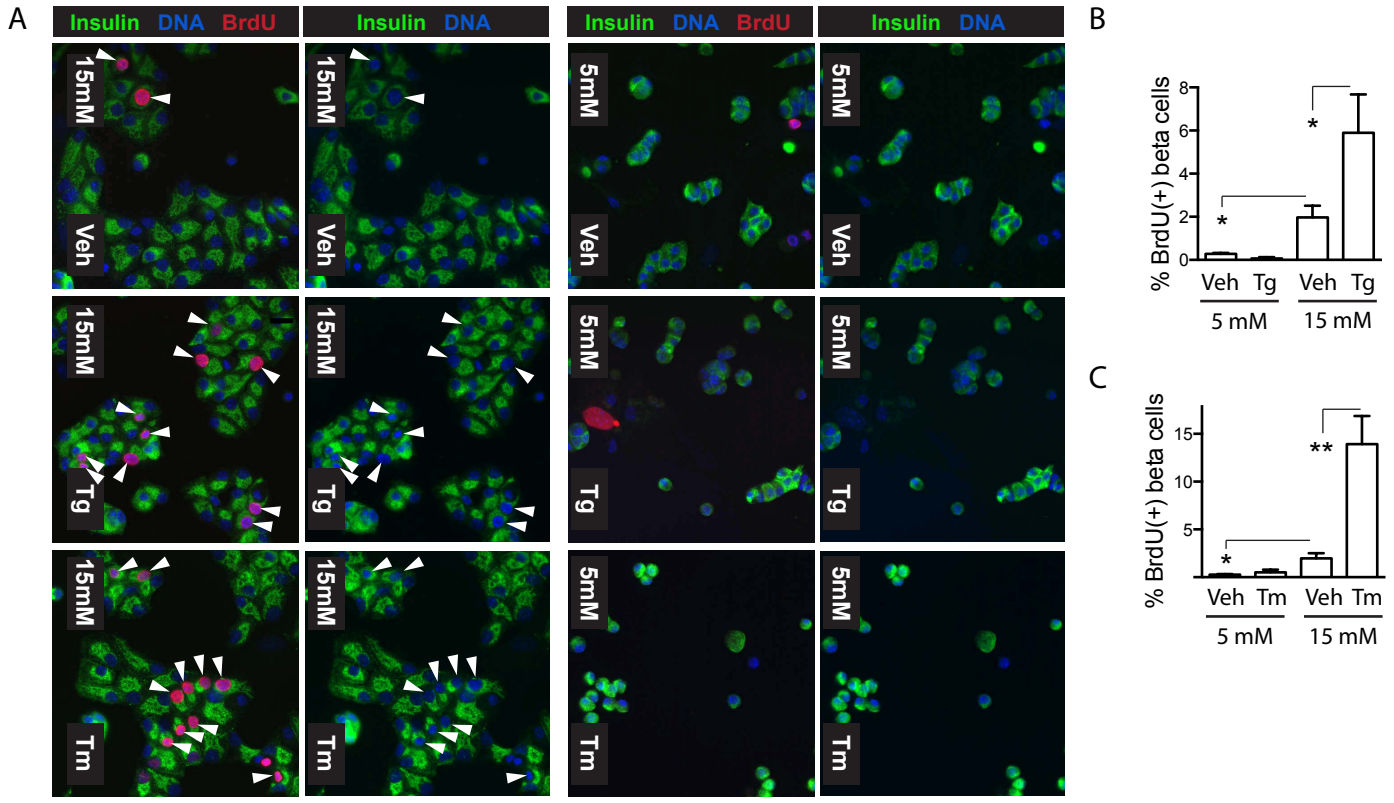
Supplemental Figure 3. Validation of ATF6 antibody. Most commercially available ATF6 antibodies did not detect bands of the correct size in mouse islets in our hands. One antibody, lot#AB121107A-02 from Novus (but not lot#AB060711A-05 from Novus), did detect bands of the correct size for full and nuclear ATF6 in dispersed mouse islet cultures. To test whether these bands were indeed ATF6, we tested (A) islets from ATF6-alpha flox/flox mice (generous gift from G. Hotamisligil) transduced with adeno-lacZ (control) or adeno-cre (for deletion). On first probe the bands are visible but faint, with extensive background; on second probing with the same antibody (with additional washing but without stripping) the bands of correct size are visible in the control and reduced in the Ad-cre treated islets. We also tested the antibody on islet cells treated with the ATF6 inhibitor (B); in this case, although PERK and IRE1 inhibitors did not reduce the nuclear/active ATF6 band, the ATF6 inhibitor did.

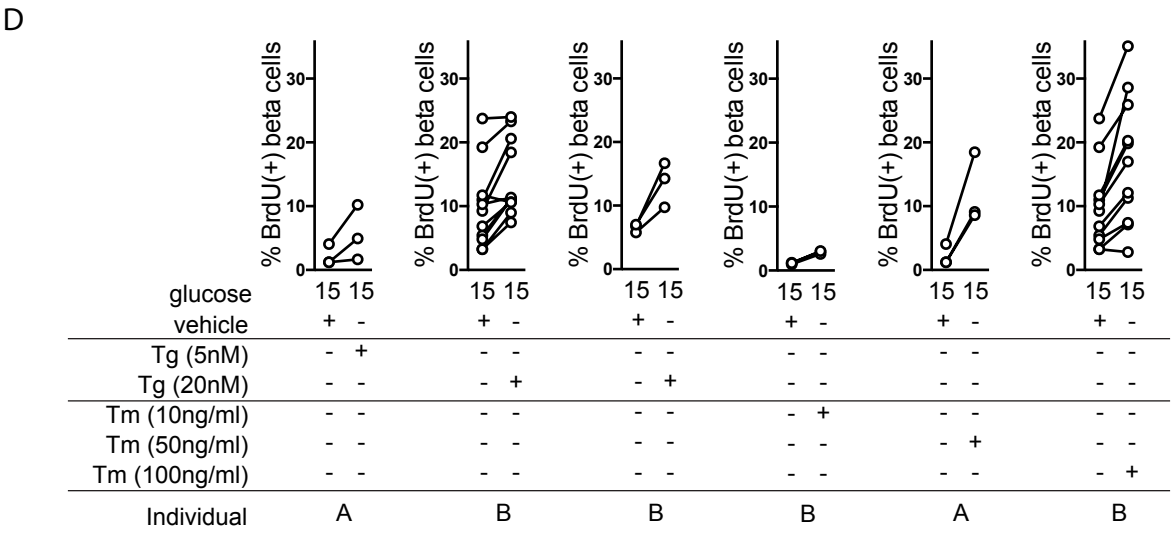
Supplemental Figure 4



Supplemental Figure 4. In vitro immunoblot quantification of proliferation and decompensated ER stress in mouse islet cells treated with Tg and Tm. (A-B) Quantification of the immunoblot experiments shown in Figure 4A. Mouse islet cells cultured in 5, 7.5 or 15 mM glucose, plus vehicle or a range of doses of thapsigargin (Tg) or tunicamycin (Tm). Cultures were assessed for abundance of PCNA (A) or CHOP (B); n=5-7. Data are represented as mean +/- SEM. *p<0.05, ***p<0.001, ****p<0.0001 by ANOVA. (C) A second example of the proliferative effect (increased PCNA) of low-dose Tg or Tm and the threshold effect, showing that at higher dose Tg or Tm PCNA is lost and CHOP induced. Doses used for Tg are 2, 20 and 500nM, and for Tm are 10, 100 and 1000 ng/ml.

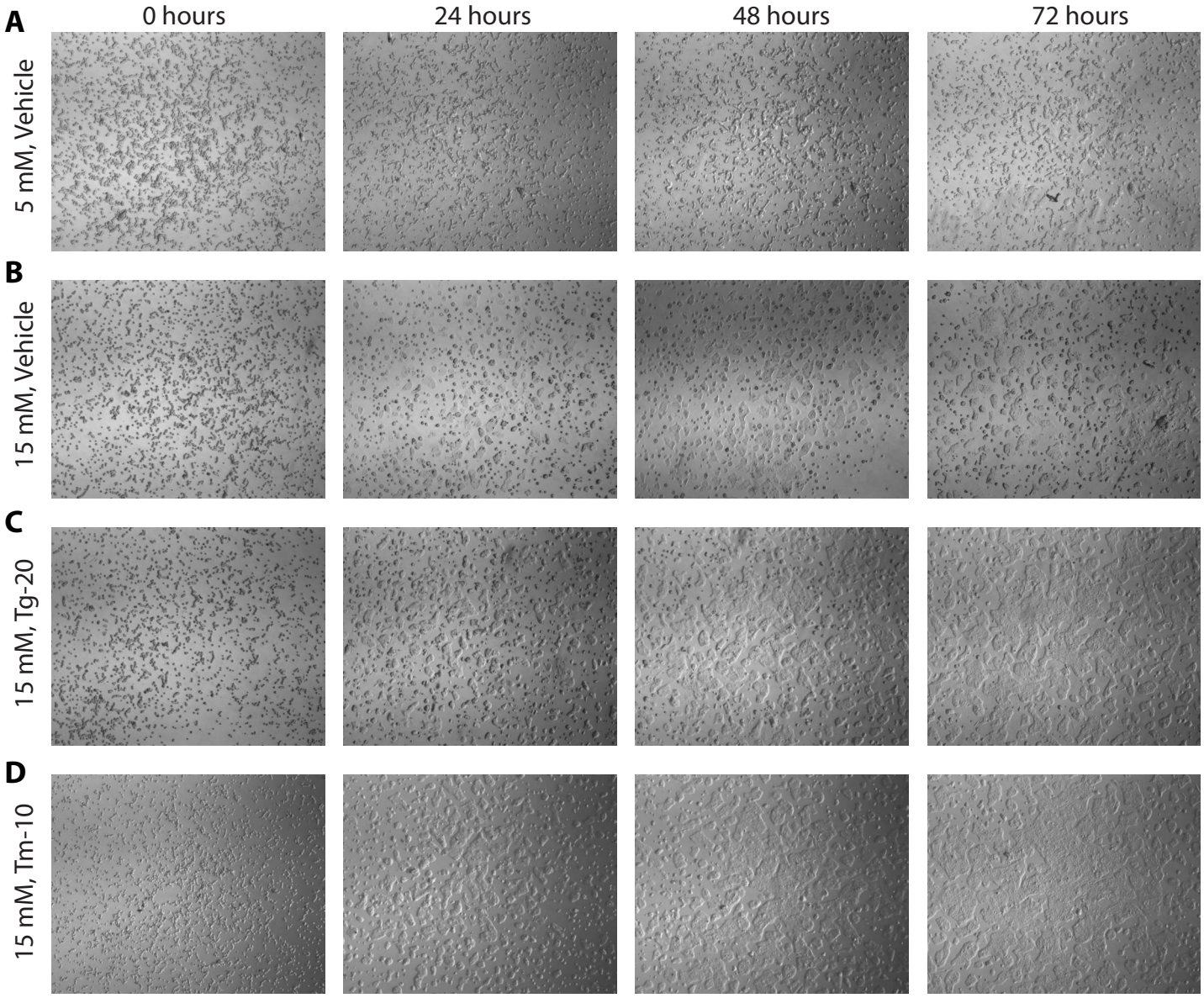
Supplemental Figure 5





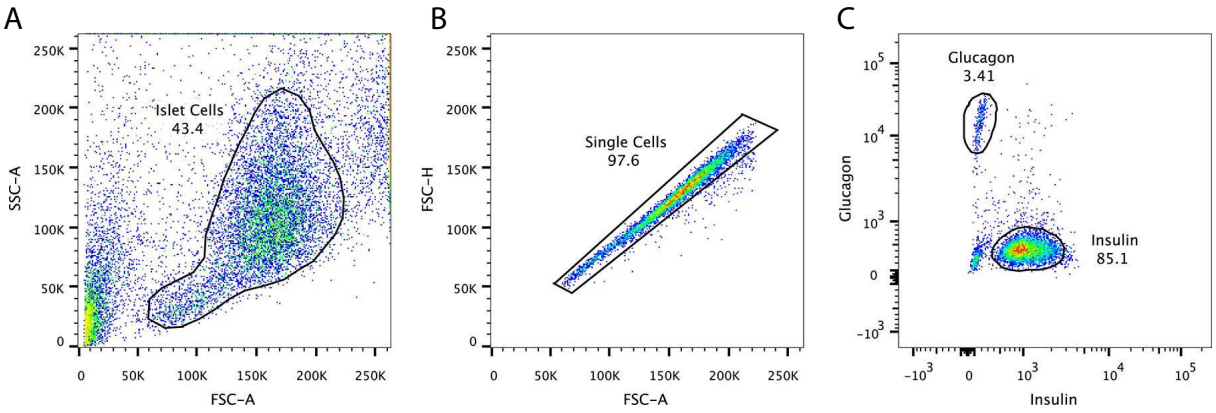
Supplemental Figure 5. Low dose thapsigargin (Tg) and tunicamycin (Tm) increase mouse beta cell proliferation (goes with Figure 4). Primary dispersed mouse islet cells were cultured in 5 or 15 mM glucose with vehicle or low-dose Tg (20 nM) or Tm (50 ng/ml) for 72 hours. BrdU was added for the final 24 hours of culture. Cultures were immunostained for insulin, BrdU and dapi, imaged, blinded and counted. (A) The images from Figure 4C are repeated here adjacent to panels showing insulin/dapi alone, to facilitate evaluation of the nuclear nature of the BrdU stain. (B-C) Quantification of BrdU counts from the first experiment exposing islet cell cultures to low dose thapsigargin (B) or tunicamycin (C). B-C was performed by a different individual in the lab from the experiment shown in Figure 4D-E (n=3-5). (D) Individual data points of all experiments, including the plots in Figure 4D-E, panels B-C above, and a third unpublished experiment, showing that proliferation increased when low dose Tg or Tm were added to 15mM glucose, independent of maximal proliferation in any given prep or of experimentalist. Data are represented as mean +/- SEM. *p<0.05, **p<0.01 by Student's T test. Images were acquired at 200X.

Supplemental Figure 6



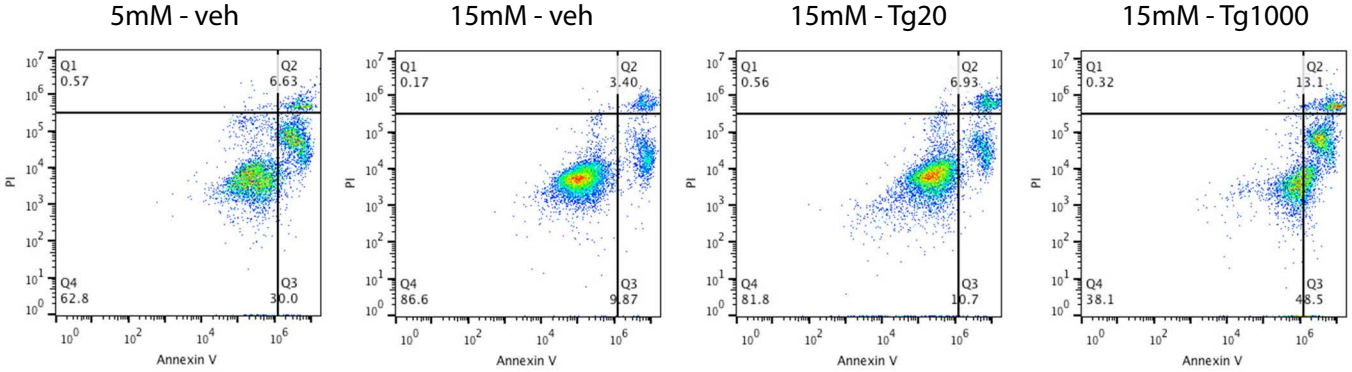
Supplemental Figure 6. Cell confluence increases in wells treated with low dose Tg and Tm. Wells containing dispersed mouse islet cells were imaged sequentially, at the same time each day, with brightfield microscopy. Time "0 hours" refers to one day after dispersion and plating, when the dispersed islet cultures were treated with the additives indicated. Tg-20 refers to 20 nM thapsigargin; Tm-10 refers to 10 ng/ml tunicamycin. The images in each row represent the same well imaged over time. Note the apparent increase in cell number at 24 and 48 hours, which is not captured by the BrdU exposure between 48-72 hours, or the pHH3 measurement, which reflects M-phase at the time of fixation. Between 0 and 24 hours, cells grown in 15mM glucose undergo spreading, which accounts for some of the increase in apparent confluence at 24 hours. Images acquired at 40X. These images are representative of n=3 independent biological replicates.

Supplemental Figure 7



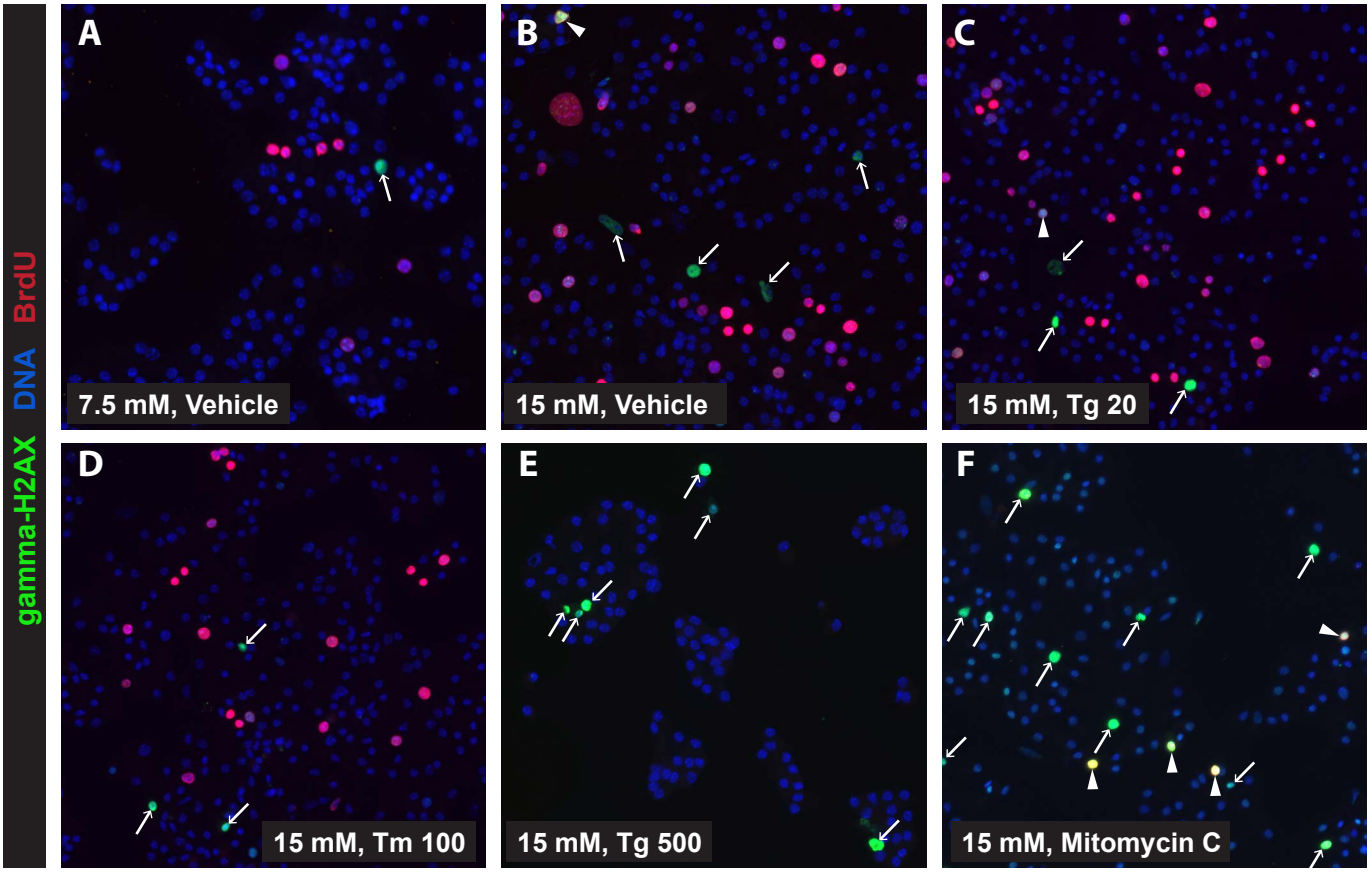
Supplemental Figure 7. Scatter plots showing procedure for identifying single alpha, beta and islet cells for the cell number counts per unit time experiments (Figure 4H-4I-4J). Dispersed mouse islet cells were cultured on plastic as described above. After lifting from the plate using trypsin, cells were immunostained for insulin and glucagon to identify beta and alpha cells, then counted by flow cytometry. Gating was performed to (A) capture healthy appearing islet cells, that (B) were not doublets, and (C) stained for either glucagon or insulin. Flow plots are representative of n=5-7 replicates.

Supplemental Figure 8



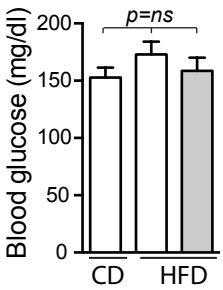
Supplemental Figure 8. Scatter plots for Annexin V / PI experiments. Dispersed mouse islet cells cultured with the indicated reagents were lifted using trypsin, stained for annexin V and PI, and analyzed by flow cytometry. The fraction of AnnexinV(+)/PI(+) cells was clearly increased by high dose thapsigargin (Tg; 1000 nM), as expected, but was not increased by low (proliferative) dose thapsigargin (20 nM). These are the raw data from one of n=4 experiments summarized in Figure 4L.

Supplemental Figure 9



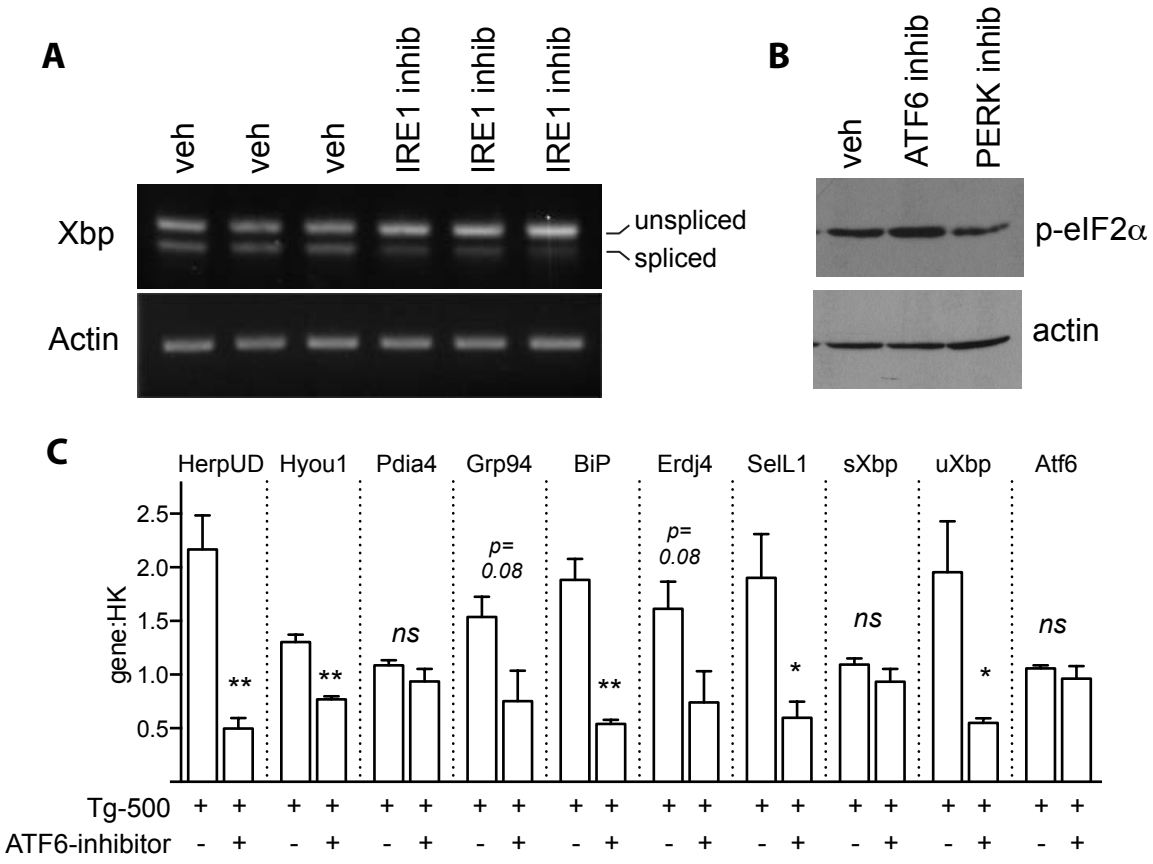
Supplemental Figure 9. Co-immunostaining for BrdU (proliferation) and gamma-H2AX (DNA damage) suggests that UPR-induced BrdU incorporation is not due to DNA damage. Dispersed mouse islet cells cultured in 7.5mM or 15mM glucose, plus additives including low dose 20nM thapsigargin (Tg20), high dose 500nM thapsigargin (Tg500), low dose 100ng/ml tunicamycin (Tm100), or mitomycin C to directly induce DNA damage, were cultured for 72 hours, with BrdU included during the final 24 hours of culture. Although rare cells positive for gamma-H2AX were evident under all conditions, cells with BrdU incorporation in low/high glucose or with low dose Tg or Tm do not show gamma H2AX staining. Directly inducing DNA damage by treatment with mitomycin C, conversely, induces widespread gamma H2AX staining, without many BrdU positive cells, suggesting that BrdU does not routinely label beta cells with DNA damage. Arrows: gamma-H2AX labeled cells. Arrowheads: cells labeled with both gamma-H2AX and BrdU.

Supplemental Figure 10



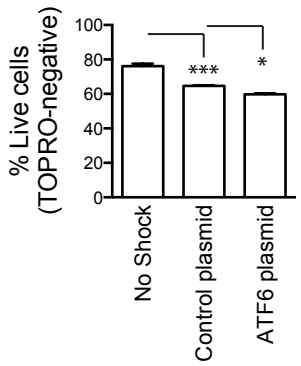
Supplemental Figure 10. Blood glucose in high fat diet (HFD) fed mice injected with TUDCA. Blood glucose levels at the end of the 7-day HFD exposure were not significantly different whether mice were injected with saline or with TUDCA. Neither group was significantly different from mice fed control diet (CD). n=5-8. Data are represented as mean ± SEM.

Supplemental Figure 11



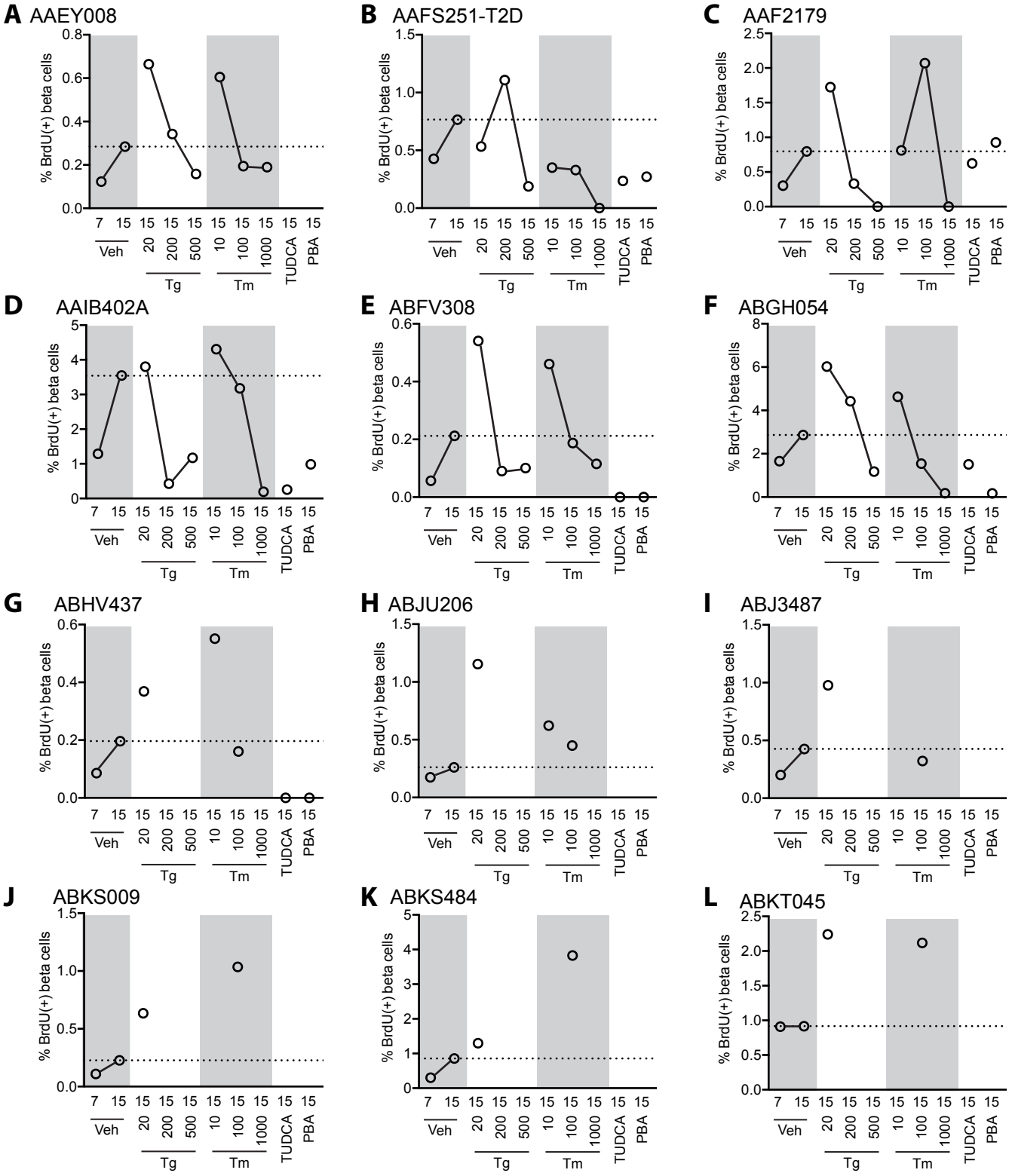
Supplemental Figure 11. IRE1, PERK and ATF6 inhibitors block activation of their expected targets. Mouse islets were dispersed and cultured on plastic for 48 (A, C) or 72 (B) hours, then harvested for RNA (A, C) or protein (B) analysis. For all panels, high dose thapsigargin (Tg) 500nM was added to activate ER stress. (A) IRE1 inhibitor prevented splicing of Xbp (n=3). (B) PERK inhibitor reduced phosphorylation of eIF2a. (C) ATF6 inhibitor reduced expression of known ATF6 transcriptional targets. As expected, the ATF6 inhibitor did not alter abundance of sXbp or ATF6. Data are represented as mean +/- SEM. *p<0.05, **p<0.01 by Student's t-test.

Supplemental Figure 12



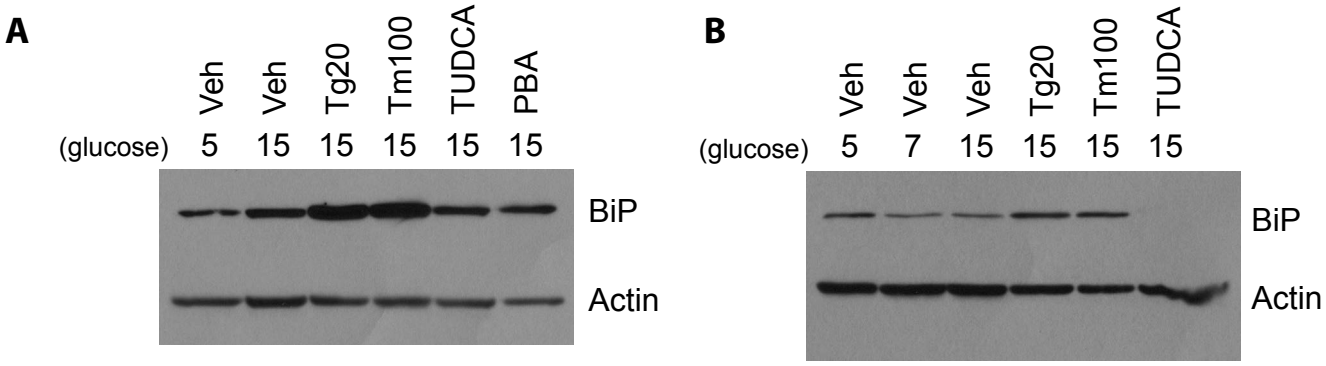
Supplemental Figure 12. Viability of mouse islet cells after electroporation. Mouse islets were dispersed using trypsin, and then either held as control ("No Shock"), electroporated with control plasmid or electroporated with ATF6 plasmid, then immediately treated with TOPRO dye to identify dead cells. The most significant loss of viability occurred after trypsinization, with moderate additional loss of viability after electroporation. n=3, quantified by flow cytometry. Data are represented as mean +/- SEM. *p<0.05, ***p<0.001 by Student's T test.

Supplemental Figure 13



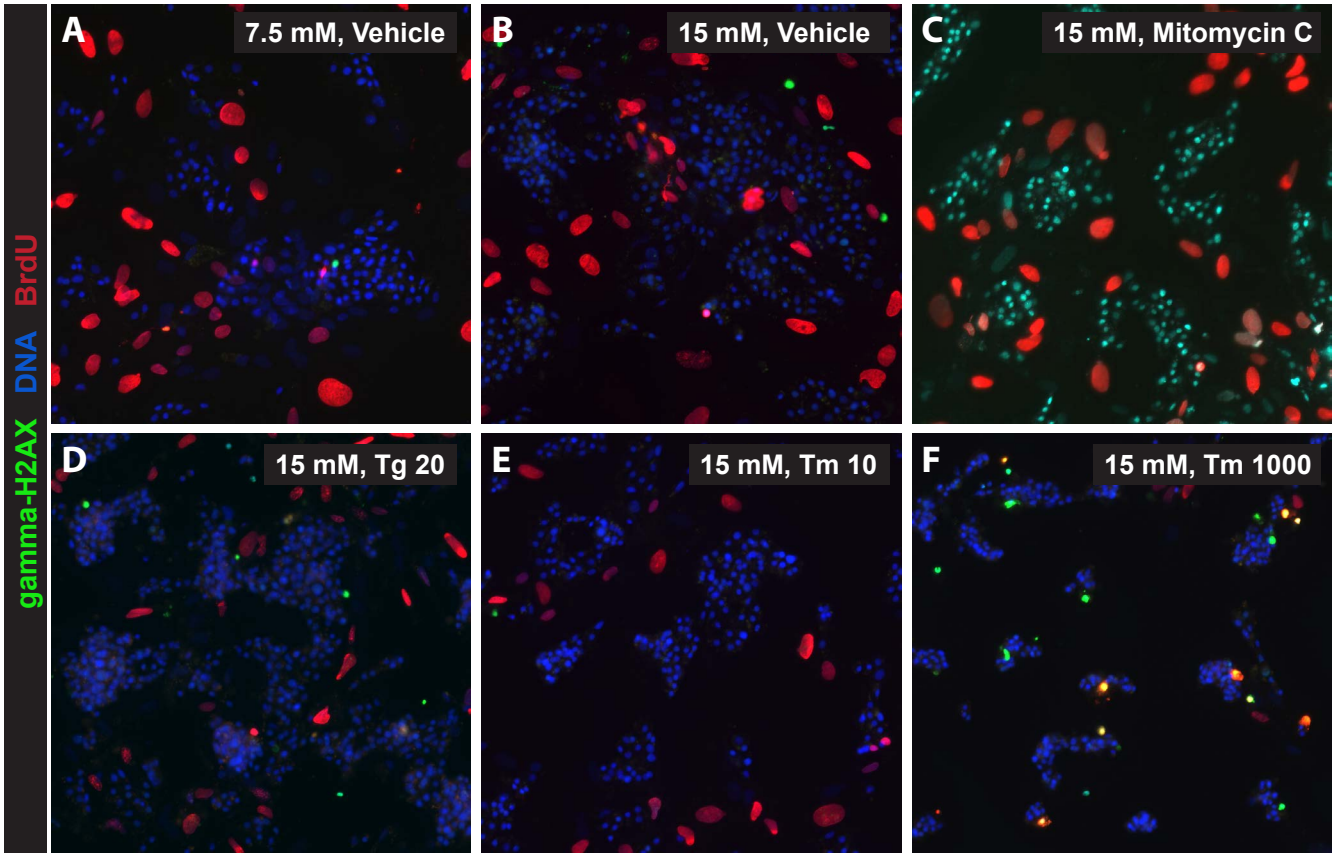
Supplemental Figure 13. **Raw, non-normalized data for human islet experiments.** Variability in human beta cell proliferation among donors necessitated normalizing the data to observe effects of thapsigargin (Tg) and tunicamycin (Tm) on proliferation (Figure 9). The raw, non-normalized data are shown here for all preps treated with Tg (20, 200 or 500 nM), Tm (10, 100 or 1000 ng/ml), and chaperones TUDCA and PBA. Note that in nearly all preps, low dose Tg and/or Tm increased proliferation over 15mM glucose baseline, and, when tested, TUDCA and PBA reduced proliferation below 15mM glucose baseline. Later preps (G-L) were not treated with high dose Tg or Tm, or chaperones (H-L). Note the variable Y-axis range, and the lack of proliferative effect of low dose Tg or Tm in the single prep from a donor with T2D (B).

Supplemental Figure 14



Supplemental Figure 14. Low dose Thapsigargin (Tg) and tunicamycin (Tm) increase ER stress in human islets, and TUDCA and PBA reduce ER stress in human islets. (A-B) Dispersed human islet cells from two different donors were cultured with thapsigargin (Tg; 20nM), tunicamycin (Tm; 100 ng/ml), TUDCA (100 ng/ml) or PBA (250 nM) for 96 hours before lysing cells for western blot. Tg and Tm increased BiP abundance, and TUDCA and PBA reduced BiP abundance, relative to 15mM glucose vehicle control.

Supplemental Figure 15



Supplemental Figure 15. Immunostaining human islet cells for gamma-H2AX shows that DNA damage relatively infrequent in these cultures. Under control conditions (7.5 and 15mM glucose with vehicle) or proliferative low dose Tg (20 nM) and Tm (10 ng/ml), only rare cells stain for gamma-H2AX, a marker of DNA damage. gamma-H2AX-positive cells do not co-localize with BrdU stain in conditions of widespread DNA damage caused by mitomycin C treatment, suggesting the BrdU incorporation is not a general artifact of DNA damage, although some co-localization was seen with high-dose Tm (1000 ng/ml). Images are representative of n=3 independent experiments.

Function category	Accession number	Protein name	Symbol	Number of peptides	Protein score	Protein score confidence interval (%)	Direction of change
Translation	gi|33859482	Eukaryotic translation elongation factor 2	EEF2	29	368	100	shift
Protein folding	gi|109730421	ERO1-like beta	ERO1LB	18	234	100	down
	gi|112293264	Protein disulfide isomerase associated 3	PDIA3	31	652	100	down
	gi|31981679	Heat shock 60kDa protein 1 (chaperonin)	HSPD1	23	241	100	down
	gi|6678413	Triosephosphate isomerase 1	TPI1	9	100	99.999	up
	gi|549060	T-complex protein 1 subunit eta (TCP-1-eta) (CCT-eta)	CCT7	20	324	100	down
	gi|129729	Protein disulfide-isomerase precursor (PDI)	PDI	17	207	100	up
	gi|2506545	78 kDa glucose-regulated protein precursor (GRP78, BIP)	HSPA5	23	215	100	up
Protein degradation	gi|1698572	Proteasome activator PA28 beta subunit	PSME2	9	109	100	up
	gi|6755198	Proteasome (prosome, macropain) subunit, alpha type 6	PSMA6	13	179	100	up
Secretory pathway	gi|1171774	N-ethylmaleimide sensitive fusion protein, isoform CRA_a	NSF	34	304	100	up
	gi|19353445	Secretogranin III	SCG3	12	71	99.13	up
	gi|6680932	Chromogranin A	CHGA	8	94	99.995	up
Metabolism	gi|29293809	ATP citrate lyase	ACLY	32	237	100	up
	gi|6680748	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	ATP5A1	14	113	100	up
Nuclear transport	gi|12846283	Ras-related nuclear protein	RAN	11	119	100	up
Misc	gi|6753060	Annexin A5	ANXA5	21	443	100	up

Supplemental Table 1. Proteomics screen results. 8 mice underwent catheterization and four-day intravenous infusion with saline (n=4) or glucose (n=4). Immediately after infusion pancreatic islets were isolated, hand-picked, and frozen. Protein lysates within each infusion group were combined, labeled with Cy3 (saline) or Cy5 (glucose), mixed, and separated by 2D electrophoresis. Protein spots with different visual intensity between the fluorophores, implying regulation by in vivo hyperglycemia, were excised from the gel, trypsinized, and identified by mass spectrometry. Of 24 spots picked, 21 were positively identified with a high degree of confidence, 5 of which were duplicates (charge shift) of the same proteins, leaving 17 unique proteins, listed above.

Supplemental Table 2: Human islet characteristics

Islet characteristics				*Donor characteristics*					
Unos ID	*Post Culture Viability*	*Post Culture Purity*	*% beta cells*	*Gender*	*Age*	*BMI*	*Diabetes*	*Ethnicity*	*Cause of Death*
AAEY008	80	90	89	F	55	39.9	No	White	Stroke
AAFS251	95	95	64	M	49	23.9	Yes	Asian	Stroke
AAF2179	93	83	41	M	65	37.4	No	White	Stroke
AAIB402A	95	90	69	M	30	37	No	Black	Anoxia
ABFV308	97	85	93	F	45	27.4	No	White	Stroke
ABGH054	95	85	73	M	25	33.8	No	Hispanic	Head Trauma
ABHV437	95	90	79	F	61	31	No	Black	Stroke
ABJU206	98	80	69	F	52	31.4	No	White	Stroke
ABJ3487	95	85	79	M	28	32.8	No	White	Stroke
ABKS009	94	90	60	M	15	23	No	White	Head trauma
ABKS484	98	70	79	M	63	38.6	No	White	Anoxia
ABKT045	95	70	86	M	35	32	No	Hispanic	Stroke
ACCK183	93	80	84	F	39	22.8	No	White	Stroke

Supplemental Table 2: Characteristics of human islet preparations. All preps were received from the IIDP. Post culture viability and purity, gender, age, BMI, diabetes history, ethnicity, and cause of death are per IIDP report. The % beta cells refers to the percent of cells adherent in coverslip cultures that stained for insulin divided by the number of dapi-stained nuclei.

Supplemental Table 3: Experimental use of each human islet preparation

Unos ID	*7.5 vs.15 mM gluc (Figure 9B)*	*Thapsigargin (Tg) (Figure 9C)*	*Tunicamycin (Tm) (Figure 9D)*	*TUDCA, PBA (Figure 9E)*	*Atf6 inhibitor (Figure 9F)*	*Ad-Atf6 (Figure 9G)*	*gH2AX (Suppl. Figure 13)*
AAEY008	x	x	x				
AAFS251	x	x	x	x			
AAF2179	x	x	x	x			
AAIB402A	x	x	x	x	x		
ABFV308	x	x	x	x			
ABGH054	x	x	x	x			
ABHV437	x	x	x		x		
ABJU206	x	x	x		x	x	x
ABJ3487	x	x	x		x	x	x
ABKS009	x	x	x		x		
ABKS484	x	x	x		x	x	
ABKT045	x	x	x		x	x	
ACCK183							x

Supplemental Table 3: Description of which human islet preparations were used for which experiment.

Primer Name	Forward	Reverse
ERO1LB	ACCCTGAGCTTCCTCTCAAGT	AAAGGACATGGTCGTTTCAGATT
PDIERp57	CGCCTCCGATGTGTTGGAA	CAGTGCAATCCACCTTTGCTAA
PDIERp59	GCCGCAAAACTGAAGGCAG	GGTAGCCACGGACACCATAC
TPI1	CCAGGAAGTTCTTCGTTGGGG	CAAAGTCGATGTAAGCGGTGG
PSMA	CCTTTCCTCTGACAGCAACA	CTATCTTCTCTCCCGAGGGC
P97/VCP	CGACCCAATCGGTTAATTGTTGA	AGCTTCCCGTCTTTTCTTTCC
SCG3	CCCCACAGGGAAGACGACT	TGGAGCTGGGAATAGCTTACTT
CHGa	ATCCTCTCTATCCTGCGACAC	GGGCTCTGGTTCTCAAACACT
BiP	AGGACAAGAAGGAGGATGTGGG	ACCGAAGGGTCATTCCAAGTG
sXBP	CAGCACTCAGACTATGTGCA	GTCCATGGGAAGATGTTCTGG
uXBP	GTCCATGGGAAGATGTTCTGG	CTGAGTCCGAATCAGGTGCAG
CHOP	CACATCCCAAAGCCCTCG	CTCAGTCCCCTCCTCAGC
Hyou1	TAGCCAGGTGTTCTCGAAGC	GACTAAGGAGGCTGGGATGC
GRP94	TGATGAAGTCGACGTGGATG	TCCTGTTCACTTCAGCTTGG
PDIA4	ATCGCCAAGATGGATGCTAC	CTTGGTCCTGCTCCTCTTTG
Erdj4	TGAATTTGCAGAGGTTTCACTG	CAAACTCAGCCCGACACATA
Sec24D	TGTAGGCTTTGCACCTGTTG	TCAAACCCTTCGCTGACATT
SSR3	ACCAGAACCAGGAACAGAGTG	CCGGAAGGAGAAAGACGAA
CalR	CTGCATAGGCCTCATCATTG	AGTTTTGCTGTACTGGGCCT
Ki67	CTGCCTGCGAAGAGAGCATC	AGCTCCACTTCGCCTTTTGG
PCNA	ACCTGCAGAGCATGGACTCG	GCAGCGGTATGTGTCGAAGC
ATF6 alpha	GACTCACCCATCCGAGTTGTG	CTCCCAGTCTTCATCTGGTCC
XBP-for agarose gel	GAACCAGGAGTTAAGAACACG	AGGCAACAGTGTCAGAGTCC
Actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA
Tubulin	ACAGTGTTCGGTCTGGCG	CTTGCTGATGAGCAGTGTGC

Supplemental Table 4. Primer sequences.