SUPPLEMENTARY DATA

Activation of Nrf2 is required for normal and ChREBPα-amplified glucose-stimulated β-cell proliferation.

Anil Kumar, Liora S. Katz, Anna M. Schulz, Misung Kim, Lee B. Honig, Lucy Li, Bennett Davenport, Dirk Homann, Adolfo Garcia-Ocaña, Mark A. Herman, Cole M. Haynes, Jerry E. Chipuk, and Donald K. Scott

Supplementary Table S1. Sequences of RT-PCR Primers.

Gene	Forward primer	Reverse Primer
PGC1α	ACAGACAGCTTTCTGGGTGG	AATCCGTCTTCATCCACCGG
PGC1β	ATGCCAGTGACTTCGACTCA	ATCCAGGGTCTTGGTAAGCG
Nrf1	GGCTGCAGTCAGGACTTTTC	TGAGACTGGTGGAGTTGCTG
Nrf2	CCTTCCTCTGCTGCCATTAG	GTTCAGTGAAATGCCGGAGT
Atf3	GCTGGAGTCAGTCACCATCA	ACACTTGGCAGCAGCAATTT
CHOP	GTCTCTGCCTTTCGCCTTTG	CTACCCTCAGTCCCCTCCTC
HO1	ACAGGGTGACAGAAGAGGCTAA	CTGTGAGGGACTCTGGTCTTTG
NQO1	CAGCGGCTCCATGTACT	GACCTGGAAGCCACAGAAG
ChREBPβ	TCTGCAGATCGCGCGGAG	CTTGTCCCGGCATAGCAA C
Tfam	CAAGTCAGCTGATGGGCTTAG	TCATCCTTAGCCTCCTGGAA
Polrmt	TCGGACTTTCTTCGAGTGCT	TCCGTCAGCATGATGAACAC
Tfb1m	GGAAAGCTGGCAGTCTAGCA	GAGTTTTCCAGGTGCTGCAT
Tfb2m	TTAAGGAAGAATGCGGATGG	ACACCTGCTGACCAAGGAAC
Polg	GCCCATATCAGGGAGCAGTA	GGACTTCTGACCTCGCTTTG
Gabpb1	CCGAGCCGGTGTAAGTAGAG	AAAGCTCCACCACCTCTTGA
Gabpb2	AGAAGTGCTCCTTCGAGCTG	TGGACATCAGCCCCATATTT
P62	CCACCTCTGCAGAAAGTTCC	CTGTGGATGGGTCCACTTCT
Ulk1	AAGACACTGTCAGGCTGTTCC	TCTTGACTCGGATGTTGCTG
Ulk2	GCAAAGGGATAATCCACAGG	GTGTCGCTGCCATTGTGTTA
D-loop (Mitochondrial DNA)	GGTTCTTACTTCAGGGCCATCA	GATTAGACCCGTTACCATCGAGAT
GAPDH (genomic DNA)	ACCATGCTTCACTGACATTCTGA	GGTCTGCCTCCCTGCTAACC

©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

SUPPLEMENTARY DATA

Supplementary Table S2. ChREBPα increases glycolytic and TCA intermediates.

	Ad.LacZ		Ad.ChREBPα	
	2mM glucose	20mM glucose	2mM glucose	20mM glucose
Glucose-6-phosphate	100	169.8 ± 10.4[a]	216.9 ± 9.5[b]	210 ± 26.6
Fructose 1,6-Bisphosphate	100	232.9 ± 39.6[a]	96.6 ± 23.4	544.2 ± 164.9[c]
α-glycerol-3-phosphate	100	228.8 ± 26.5[a]	226.7 ± 23.8[b]	1860 ± 310[c]
Pyruvate	100	283.6 ± 43.9[a]	355.8 ± 55.9[b]	1247 ± 276.9[c,d]
Lactate	100	129 ± 17.5	167.4 ± 11.8[b]	403.8 ± 18[c,d]
α-ketoglutarate	100	101 ±4 .44	84 ± 5.79	98 ± 11.7
Succinate	100	137 ± 5.7[a]	270 ± 85[b]	697 ± 108[c,d]
Fumarate	100	120.6 ± 15.4	97.8 ± 4.5	213.2 ± 42[c,d]
Malate	100	158 ± 39.6	120 ± 2.8[b]	504 ± 111[c,d]
Aspartic acid	100	85 ± 27	392.8 ± 116[b]	226 ± 72[d]
6-phospho-Dgluconate	100	443 ± 58[a]	217 ± 73[b]	2704 ± 812[c,d]
Ribose-5-Phosphate	100	138 ± 32.5	142 ± 19	359 ± 124[c,d]

$p < 0.05$ by student T test

a – Ad.LacZ 2mM glucose versus Ad.LacZ 20mM glucose

b - Ad.LacZ 2mM glucose versus Ad.ChREBPα 2mM glucose

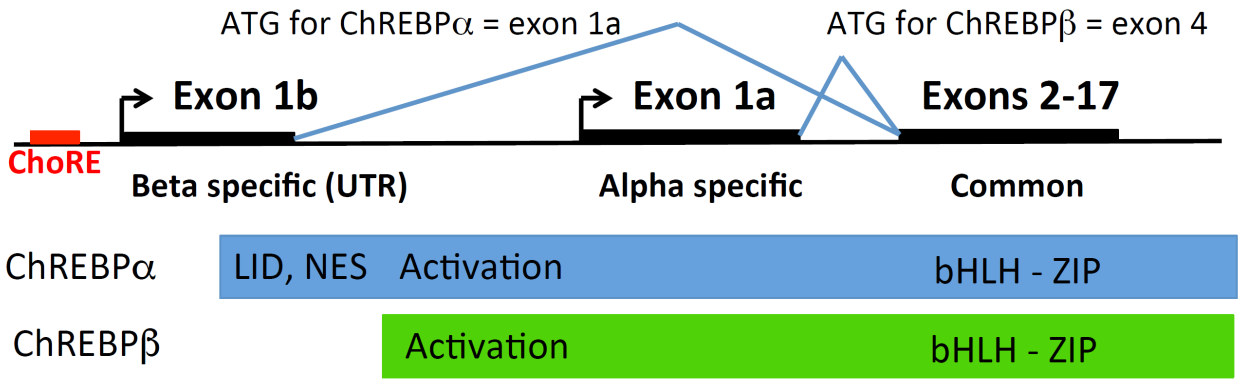
c- Ad.ChREBPα 2mM glucose versus Ad.ChREBPα 20mM glucose

d- Ad.LacZ 20mM glucose versus Ad.ChREBPα 20mM glucose

©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

SUPPLEMENTARY DATA

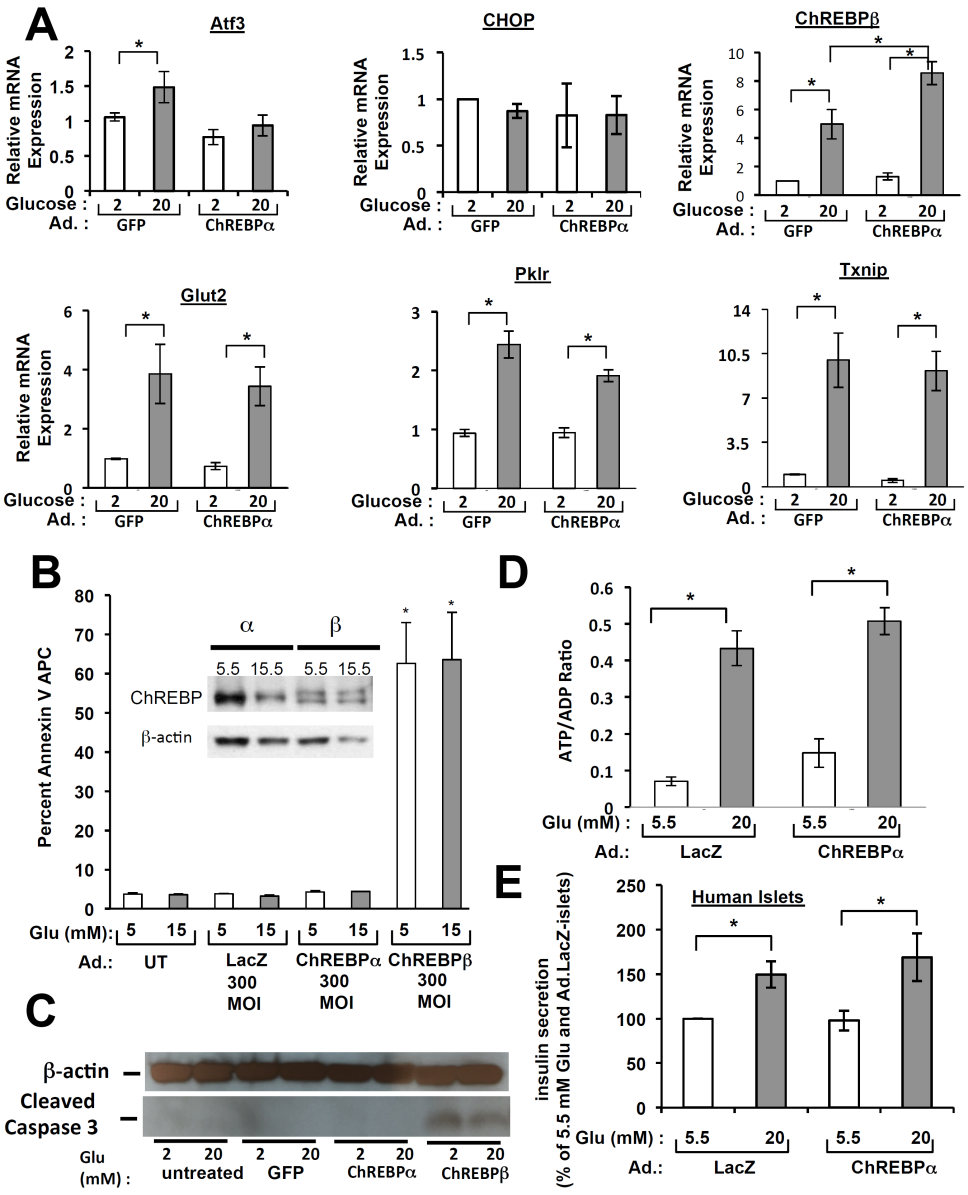
Supplementary Figure S1. ChREBP exists as 2 major isoforms. ChREBPα is the "classical" full-length, glucose-regulated form. ChREBPβ is an alternatively spliced isoform that begins translating at an alternative ATG and therefore lacks the low glucose inhibitory domain (LID, also containing nuclear export signals, NES), and is unrestrained by low glucose, constitutively nuclear and more potent than its longer counterpart. ChREBPβ is induced by a carbohydrate response element (ChoRE) located near the start site of an alternative promoter for ChREBPβ (exon 1b). Thus the induction of the beta isoform leads to an autoregulated feed-forward loop, with the newly synthesized ChREBPβ binding to the carbohydrate response element on its own promoter (and other ChREBP target genes), leading to still more production of ChREBPβ. UTR, untranslated region. bHLH-ZIP, basic, helix loop helix, zipper domain.



©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

SUPPLEMENTARY DATA

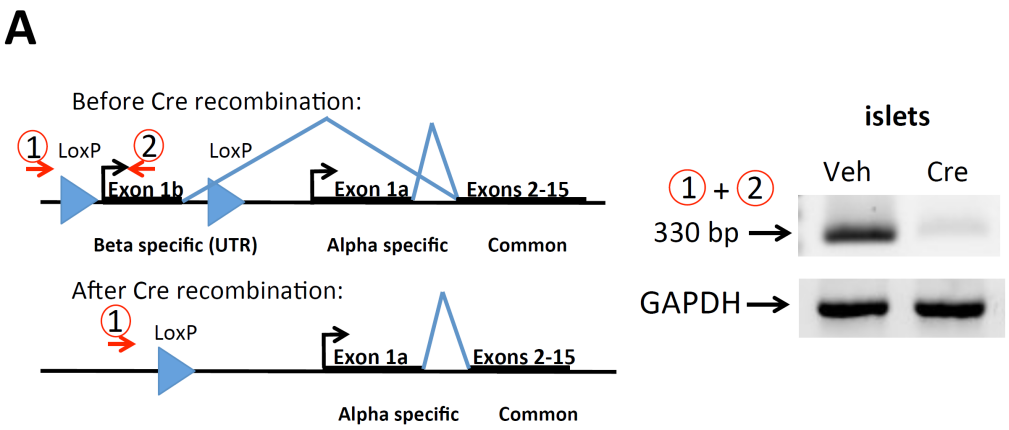
Supplementary Figure S2. Overexpression ChREBPα does not induce ER stress or apoptosis, and only modestly increases ChREBPβ without increasing other ChREBP target genes. A. INS1 cells were transduced with control adenovirus (LacZ), or adenovirus expressing ChREBPα, and were cultured in 2 or 20 mM glucose for 6 h. Total RNA was isolated and processed for RT-PCR using primers specific for the indicated genes. Results are fold change after normalizing to β-actin mRNA using the ΔΔCT method. B. INS1 cells were transduced with control adenovirus (LacZ), or adenovirus expressing ChREBPα or Cherry-tagged ChREBPβ and cultured for 48 h in 2 mM or 20 mM glucose. Inset shows immunoblot shows similar expression and loading using antibodies against C-terminus of ChREBP and β-actin. Cells were fixed and stained with Annexin V APC and the percent Annexin positive cells were determined by flow cytometric analysis. C. Immunoblot using antibodies against cleaved caspase 3 and β-actin as a loading control. D. ATP/ADP ratios were determined. E. Glucose stimulated insulin secretion. Human islets were treated with Ad.ChREBPα or Ad.LacZ. Insulin secretion and content were measured after 30 min in 5.5 or 20 mM glucose and the results were expressed relative to the Ad.LacZ-treated islets that were stimulated with 5.5 mM glucose. Data shown are the mean ± SE, n=4 *p < 0.05.

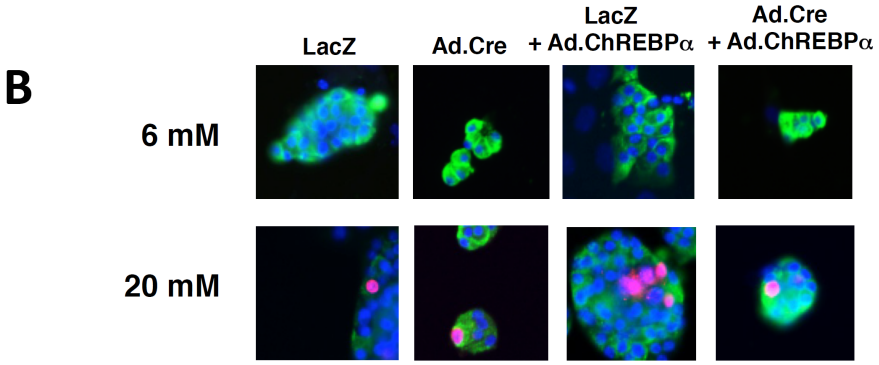


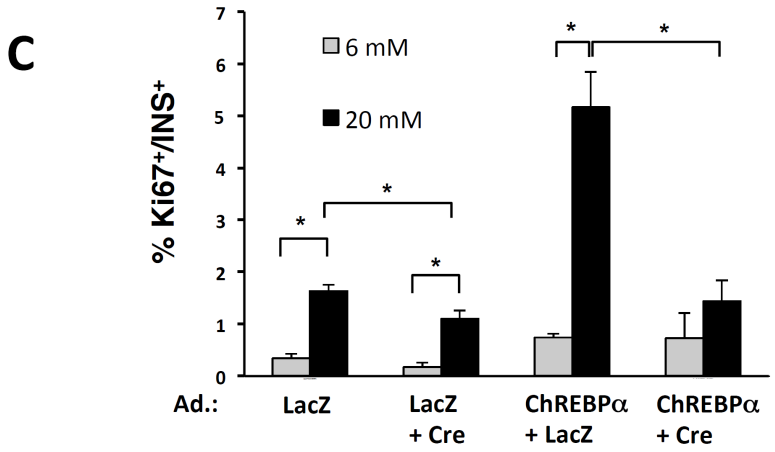
©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

SUPPLEMENTARY DATA

Supplementary Figure S3. ChREBPβ is required for the augmented glucose stimulated beta cell proliferation mediated by ChREBPα. A. Diagram of floxed ChREBPβ mice indicating LoxP sites flanking exon 1b of the ChREBP (Mlxipl) locus before and after Cre recombination. The PCR products on the right show effective removal of exon 1b after Cre recombination using an adenovirus on isolated islets. B. Isolated and dispersed islets from floxed ChREBPβ mice were treated for 48 h with the indicated adenoviruses (all groups at a total of 300 MOI). The cells were cultured in 6 or 20 mM glucose for 20 h and then fixed and immunostained for insulin or Ki67. C. Quantification of insulin positive and Ki67 positive cells from B. Data are the mean +/- SE, n=5, *, $p < 0.05$.



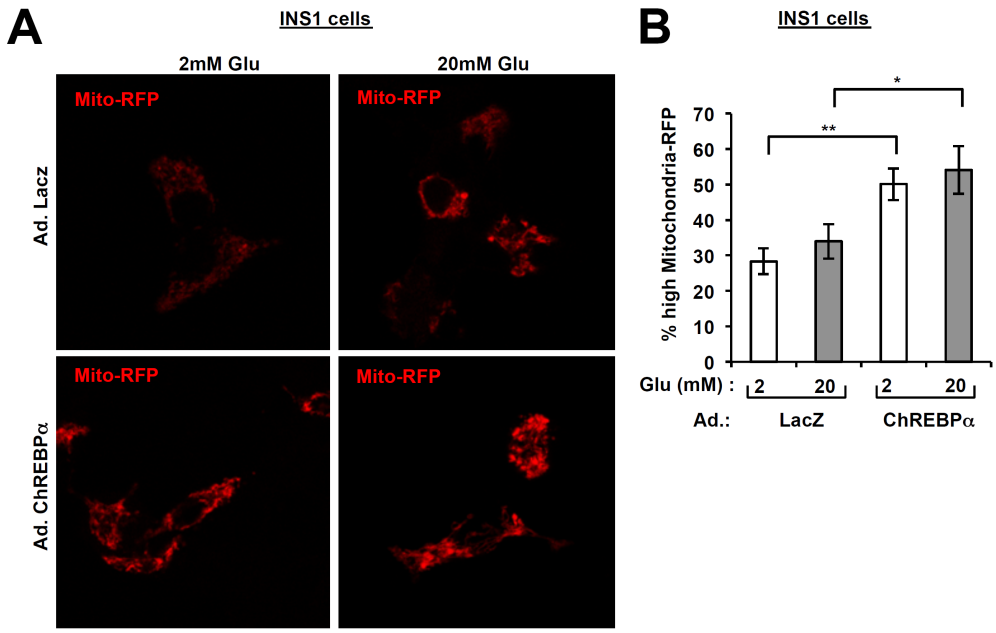




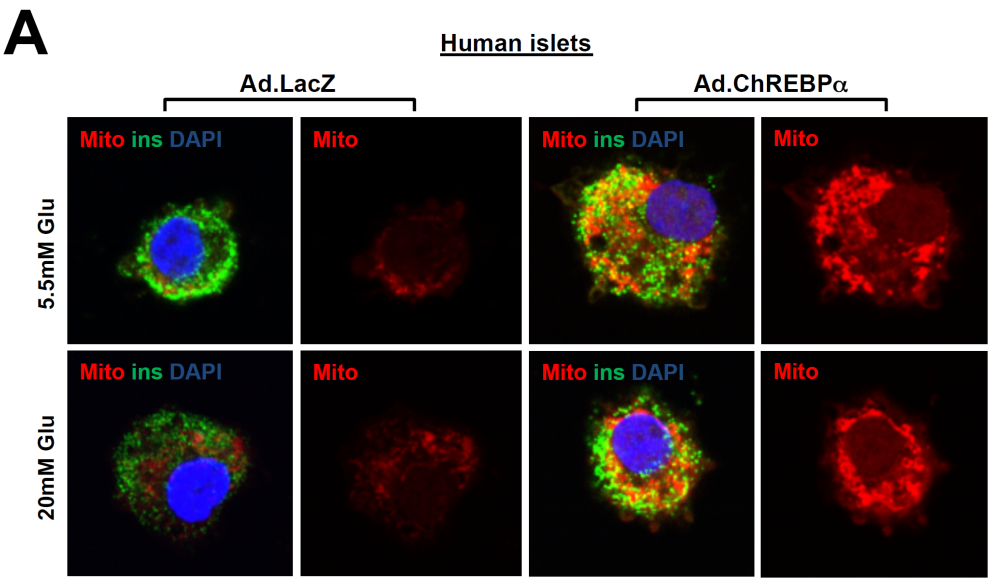
©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

SUPPLEMENTARY DATA

Supplementary Figure S4. ChREBPα increases mitochondrial labeling. A. INS1 cells were cotransduced with baculovirus expressing mitochondria-targeted RFP (Mito-RFP) with either Ad.LacZ or Ad.ChREBPα and treated for 6 h with either 2 mM or 20 mM glucose. A. Cells were imaged by indirect immunofluorescence using a Zeiss Axioplan2 microscope. B. Cells were suspended by trypsinization and mitochondrial content was determined by flow cytometric analysis. Results are the percentage of highly fluorescing RFP positive cells. Mean ± SE, n=3, *p < 0.05, **p < 0.01.



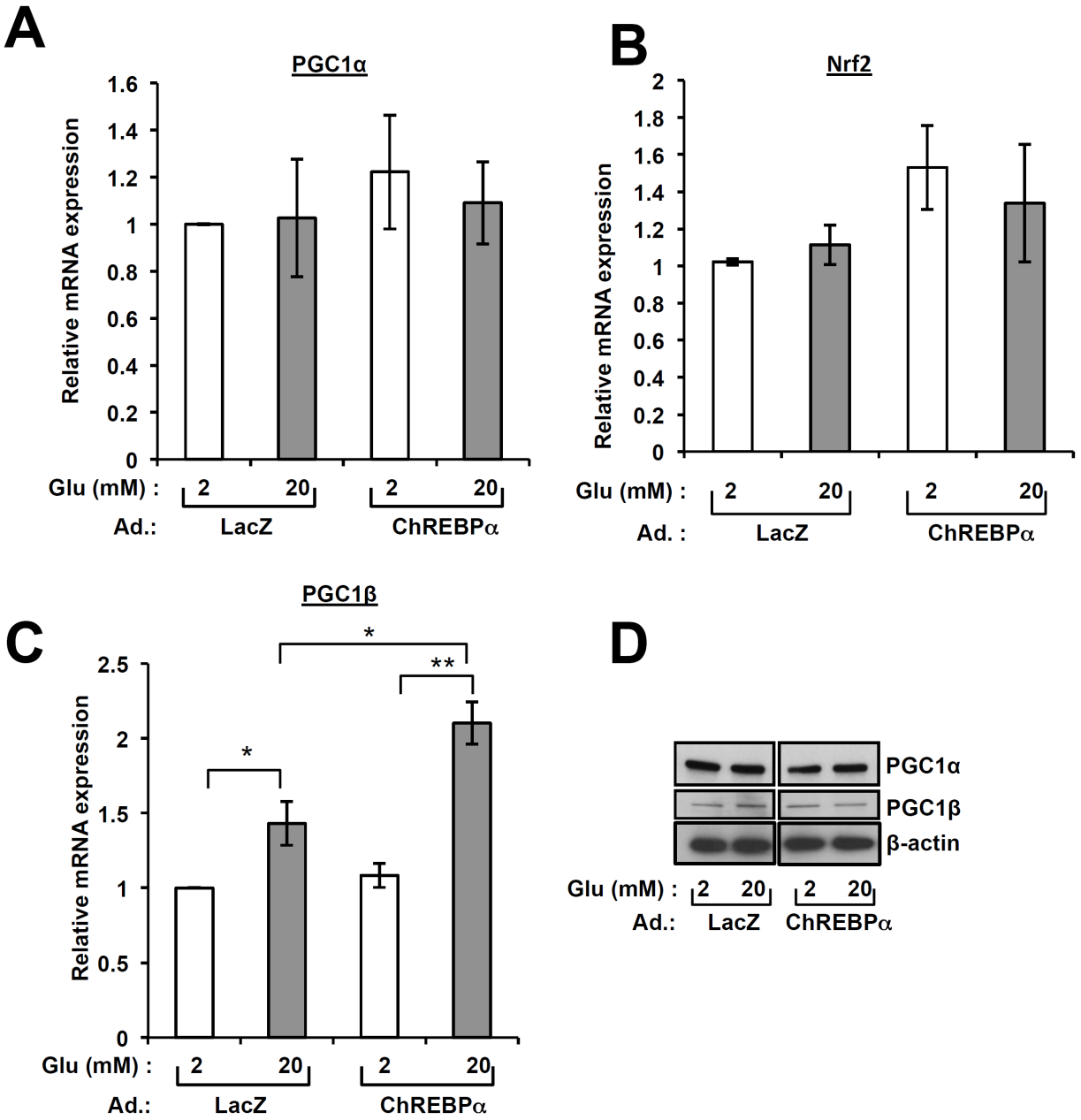
Supplementary Figure S5. ChREBPα increases mitochondrial labeling in human β-cells. A. Dispersed human islets were treated as indicated, and stained with MitoTracker Red CMX-Rosamine and an anti-insulin antibody.



©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

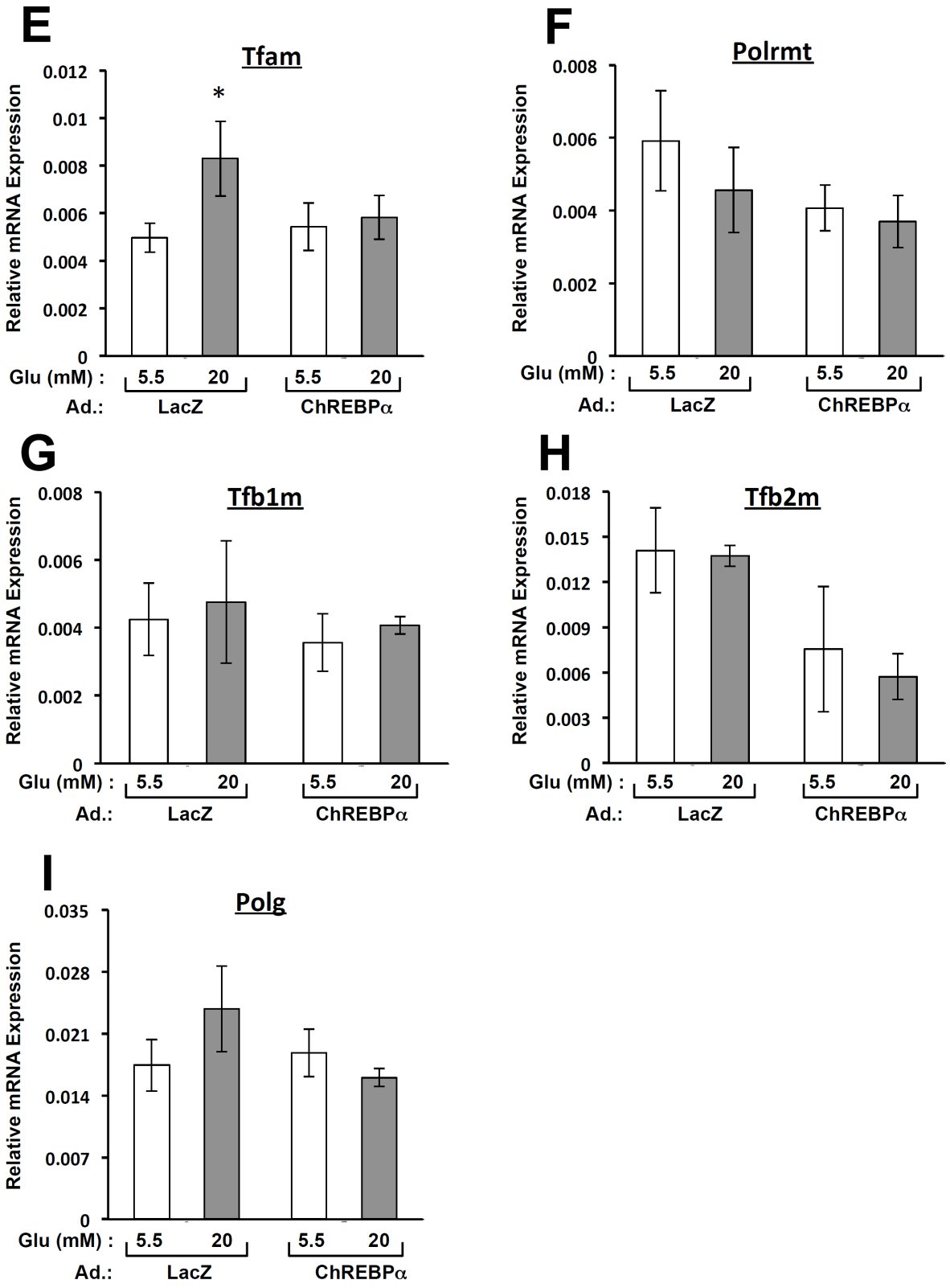
SUPPLEMENTARY DATA

Supplementary Figure S6. Effects of ChREBPα on mitochondrial biogenic genes. INS1 cells were transduced with Ad.ChREBPα or Ad. LacZ and treated with 2 or 20 mM glucose. Total RNA was isolated and processed for RT-PCR (A-C and E-I) using primers for the indicated genes, or lysates were processed for immunoblotting (D) using antibodies against PGC1α, PGC1β, or β-actin. The RT-PCR results are fold change after normalizing to β-actin mRNA using the ΔΔCT method. Mean ± SE, n=3-5, *p < 0.05, **p < 0.01.



©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

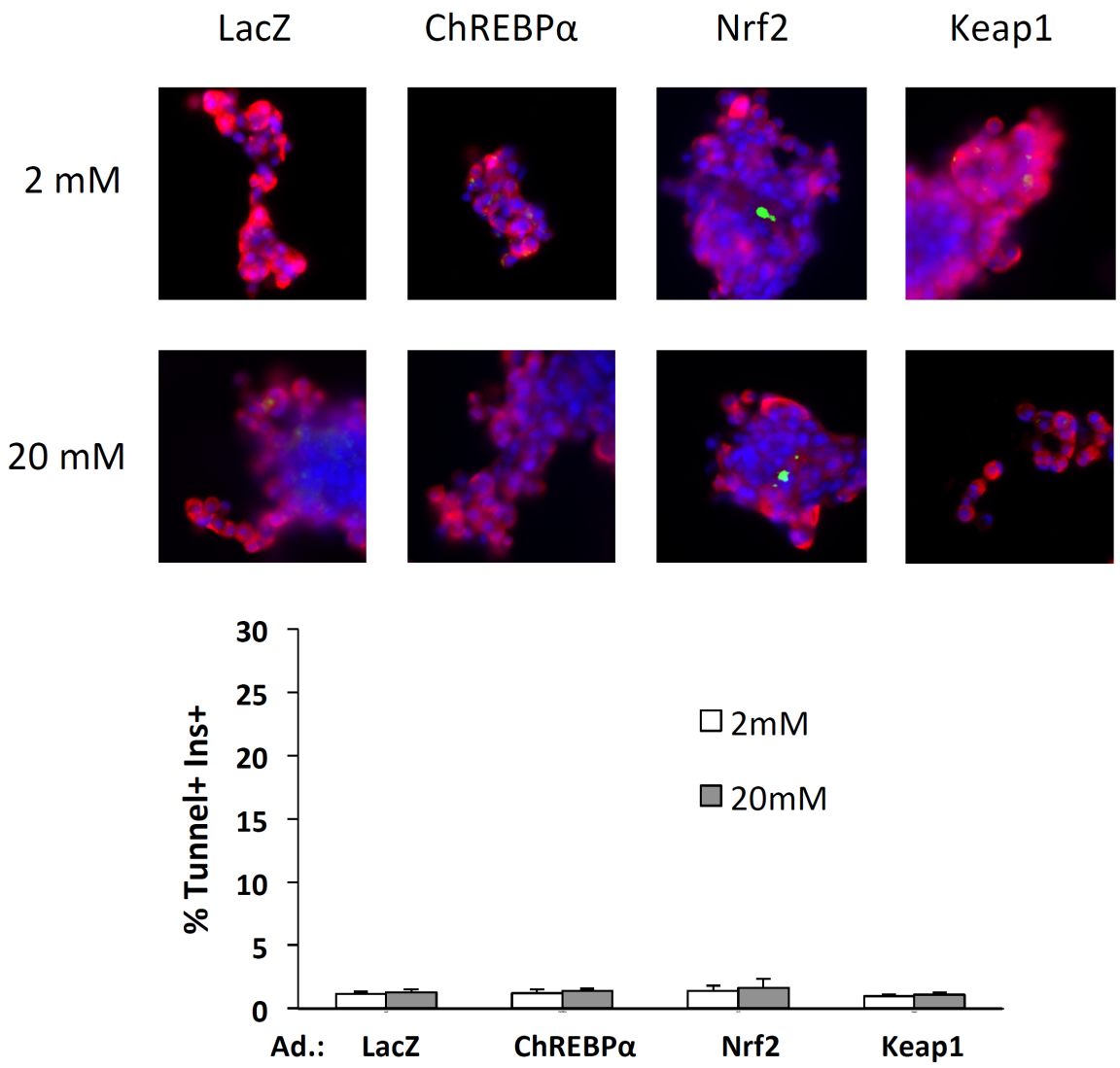
SUPPLEMENTARY DATA



©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

SUPPLEMENTARY DATA

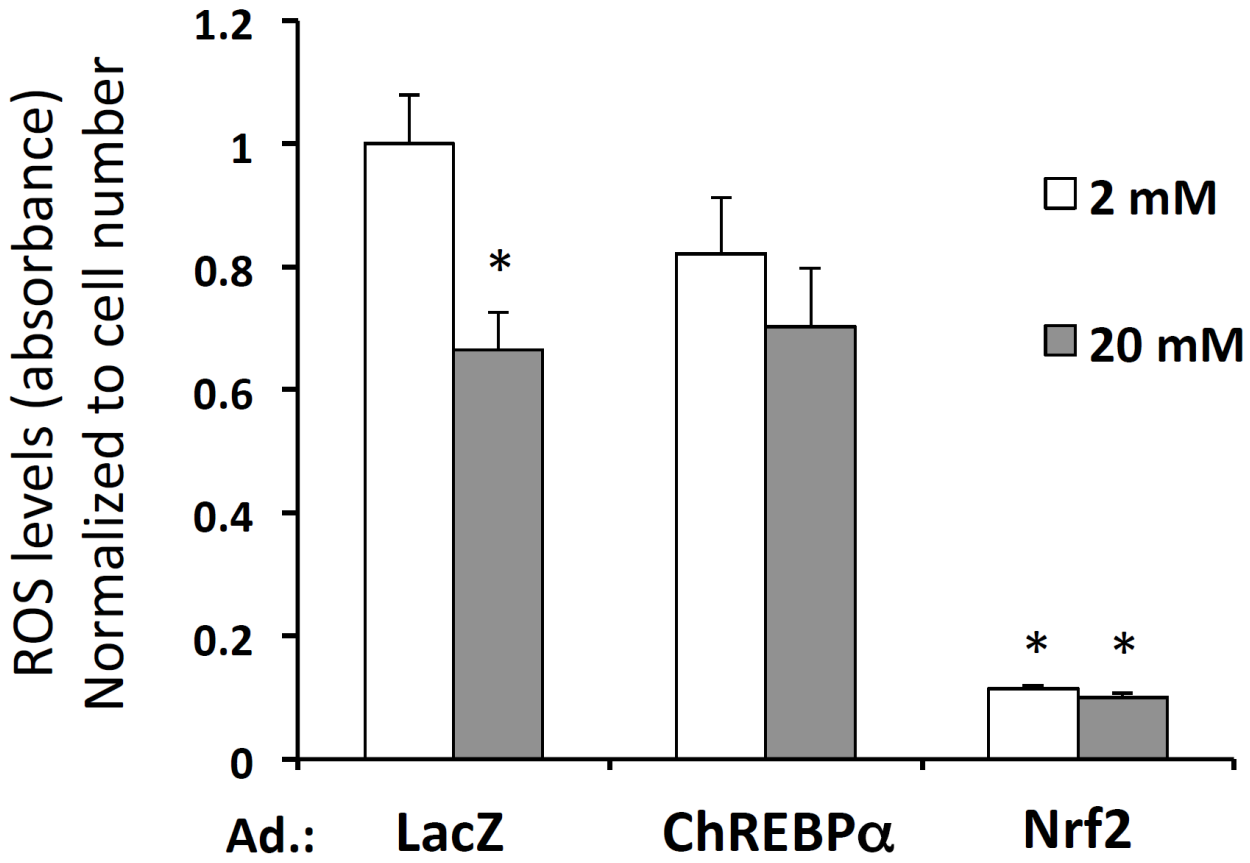
Supplementary Figure S7. Overexpression of ChREBPα, Nrf2 or Keap1 does not increase apoptosis in human beta cells. Human islets were dispersed and transduced with the indicated adenoviruses for 48 hours and then subjected to TUNEL assay. Results shown are the mean +/- SE, n=3.



©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

SUPPLEMENTARY DATA

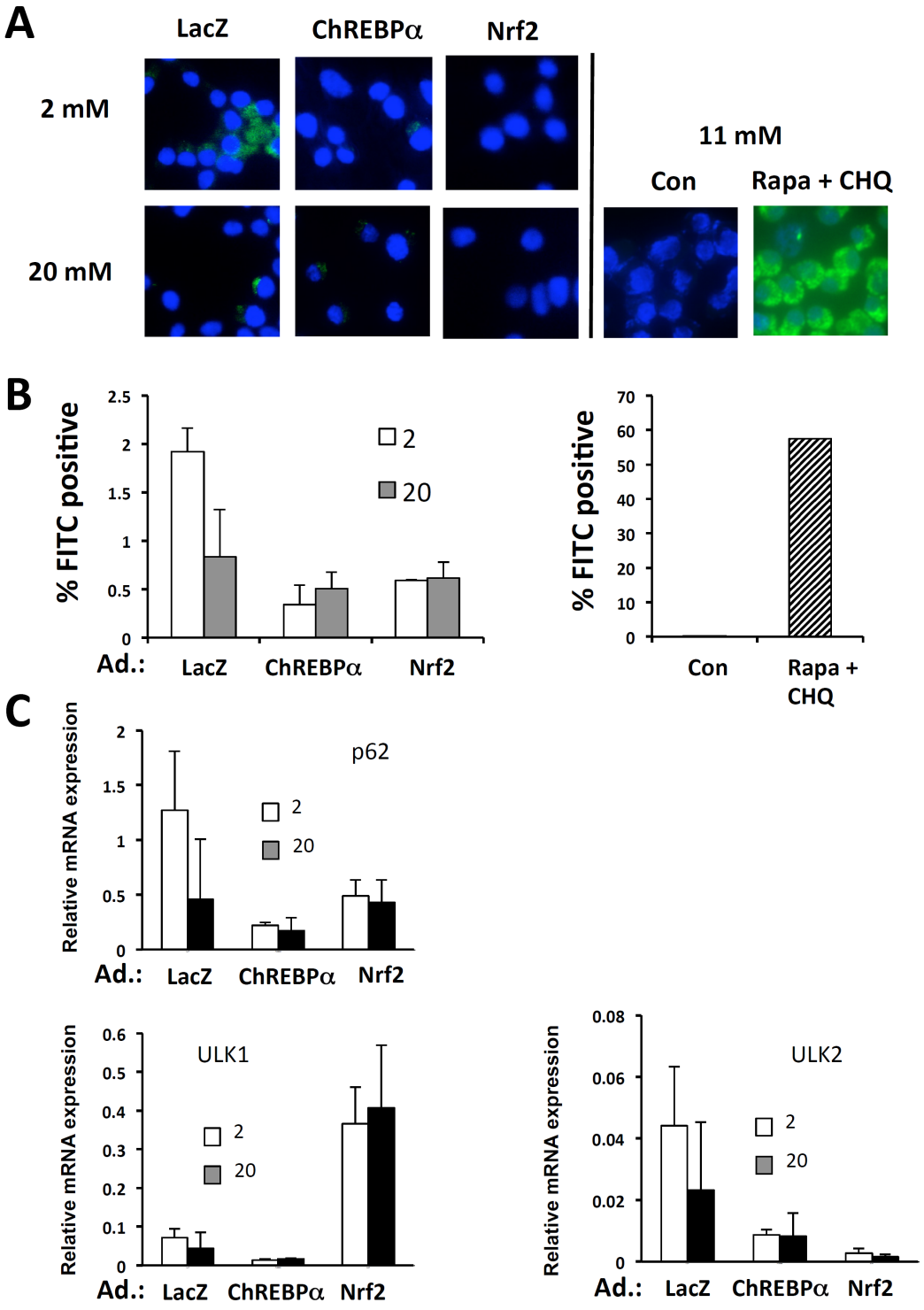
Supplementary Figure S8. Glucose and Nrf2 treatment decreases ROS production. INS1 cells were transduced with the indicated adenoviruses for 30 h, followed by culture in either 2 or 20 mM glucose for 18 h. ROS generation was measured and normalized to cell number. Data are mean +/- SE, n=3; *, p < 0.05 (vs. 2 mM glucose, LacZ control).



©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1

SUPPLEMENTARY DATA

Supplementary Figure S9. Overexpression of either ChREBPα or Nrf2 does not increase autophagy. INS1 cells were transduced with the indicated adenoviruses for 30 h, followed by culture in either 2 or 20 mM glucose for 18 h. A. The presence of autophagosomes was stained with CYTO-ID dye and quantified in B by flow cytometry. c. RT-PCR was performed and results are the means of relative expression after normalizing to β-actin mRNA using the ΔΔCT method +/- SE. *, $p < 0.05$



©2018 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0943/-/DC1