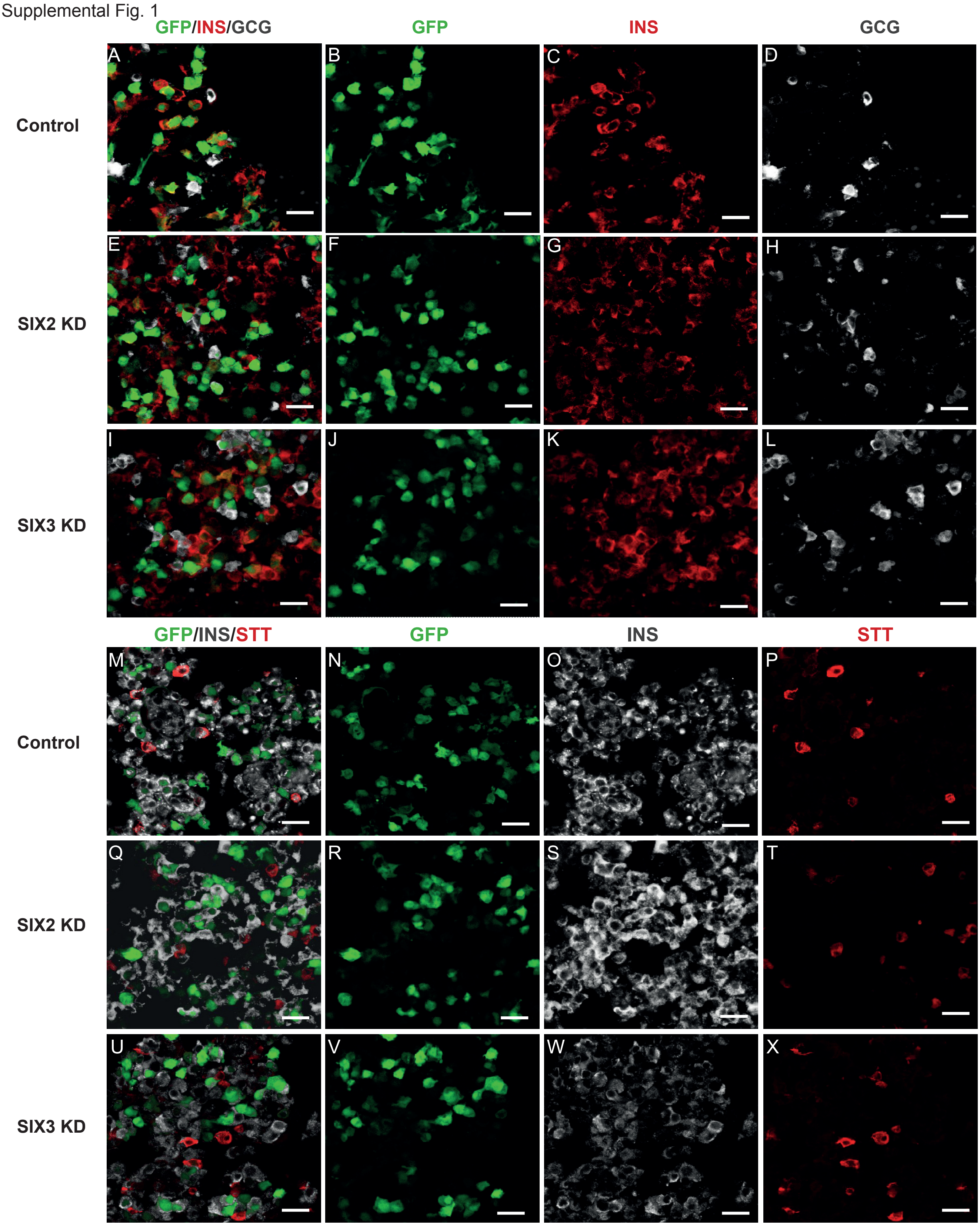
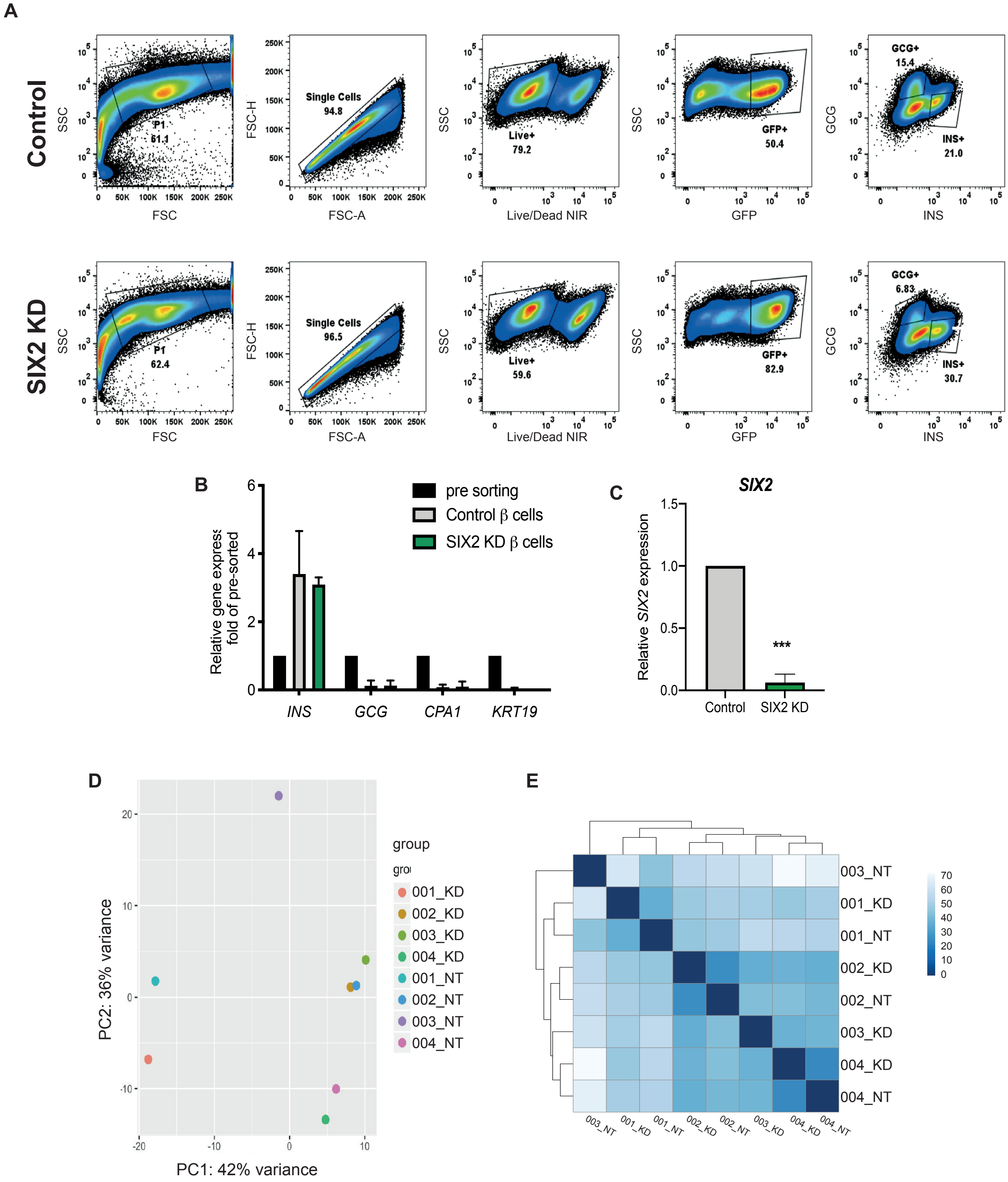
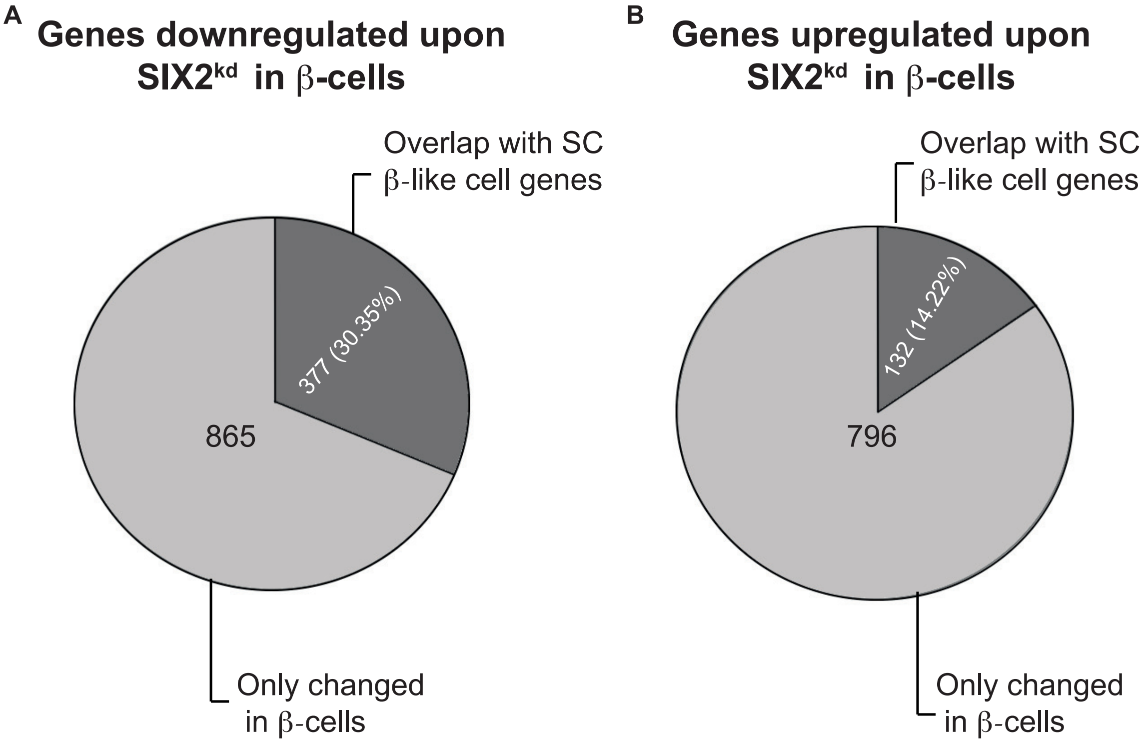
Supplemental Fig. 1



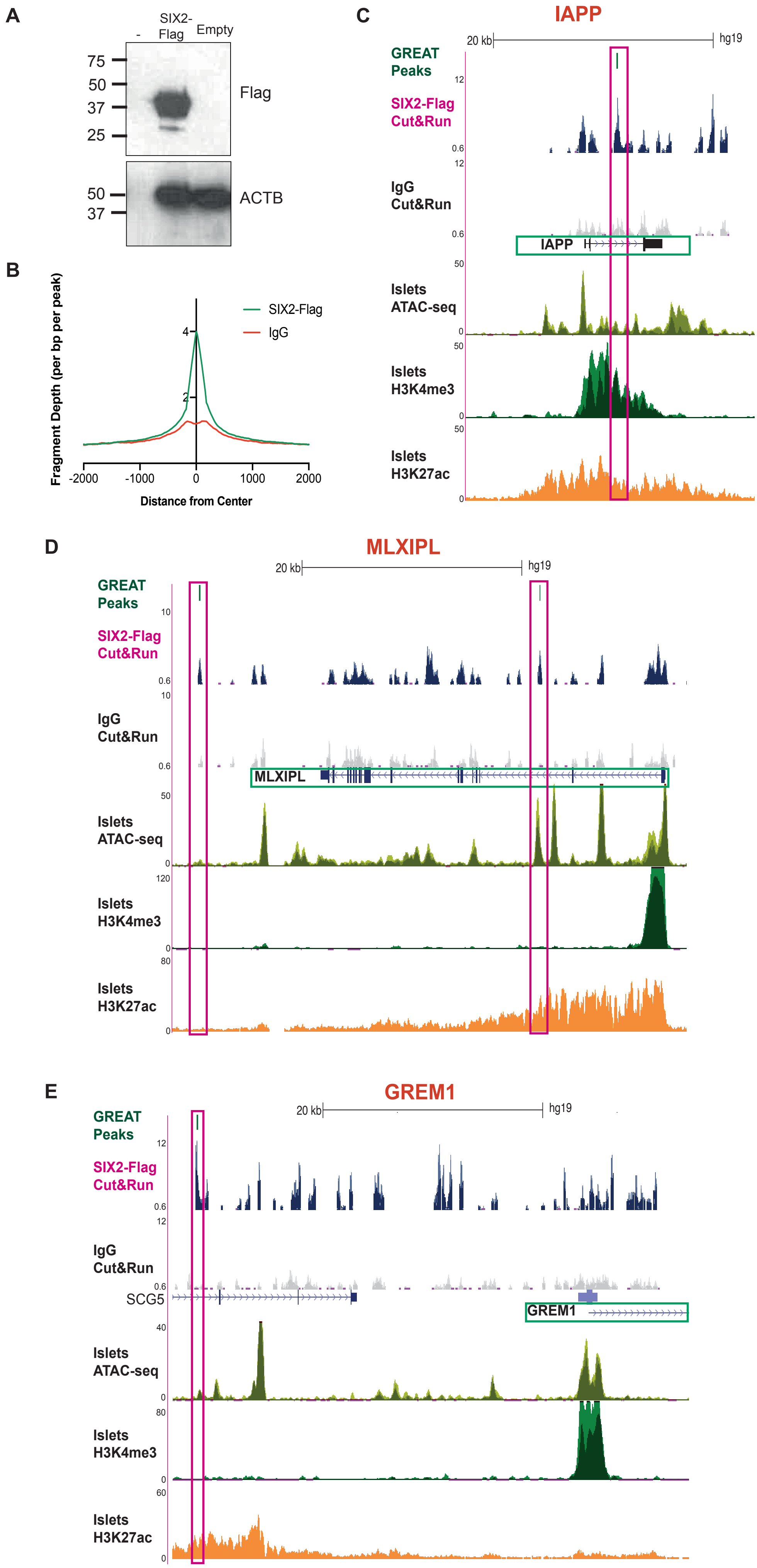
Supplemental Fig. 2



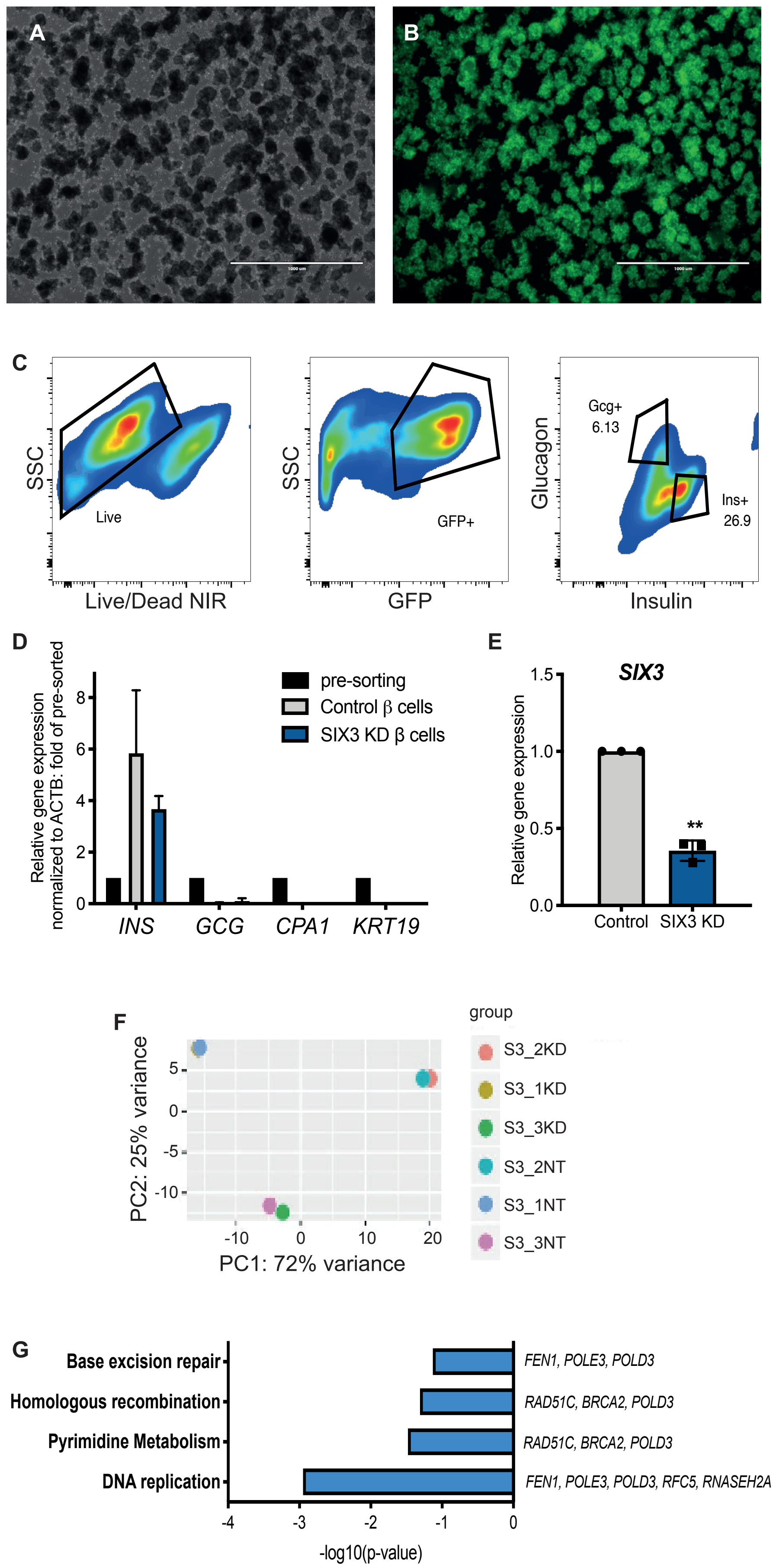
Supplemental Fig. 3



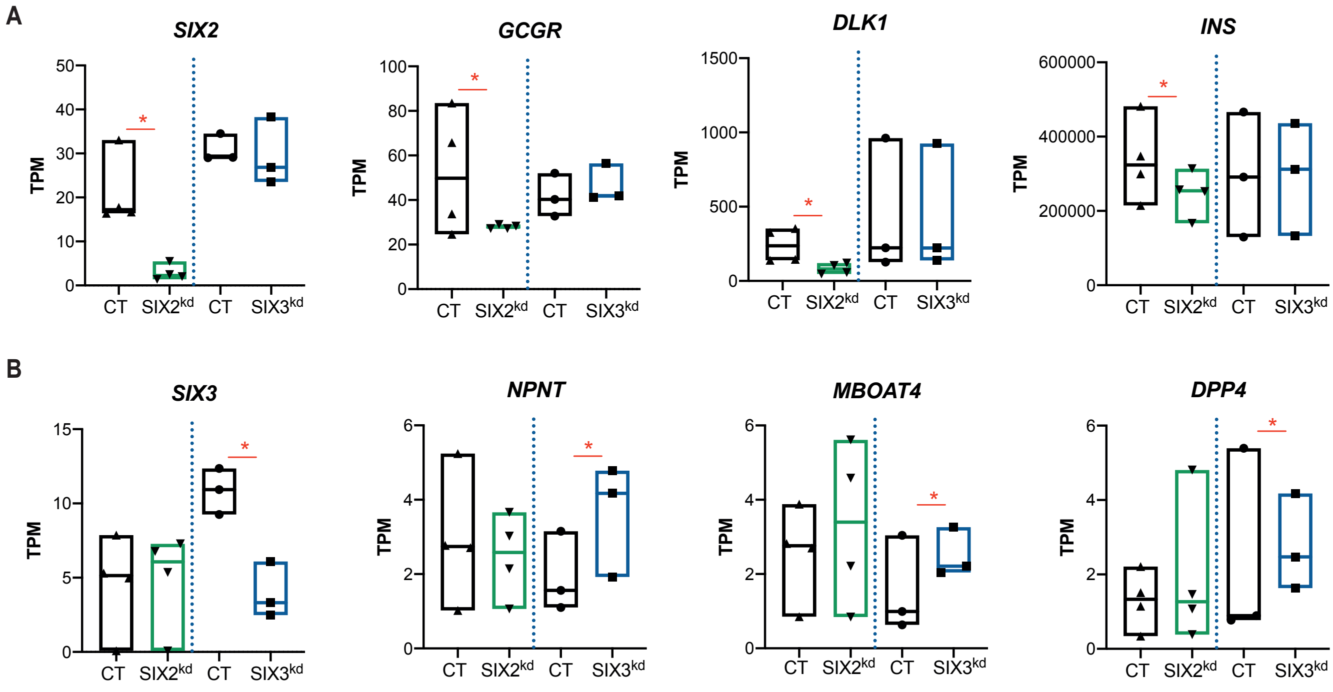
Supplemental Fig. 4



Supplemental Fig. 5



Supplemental Fig. 6



Supplemental Fig. 7

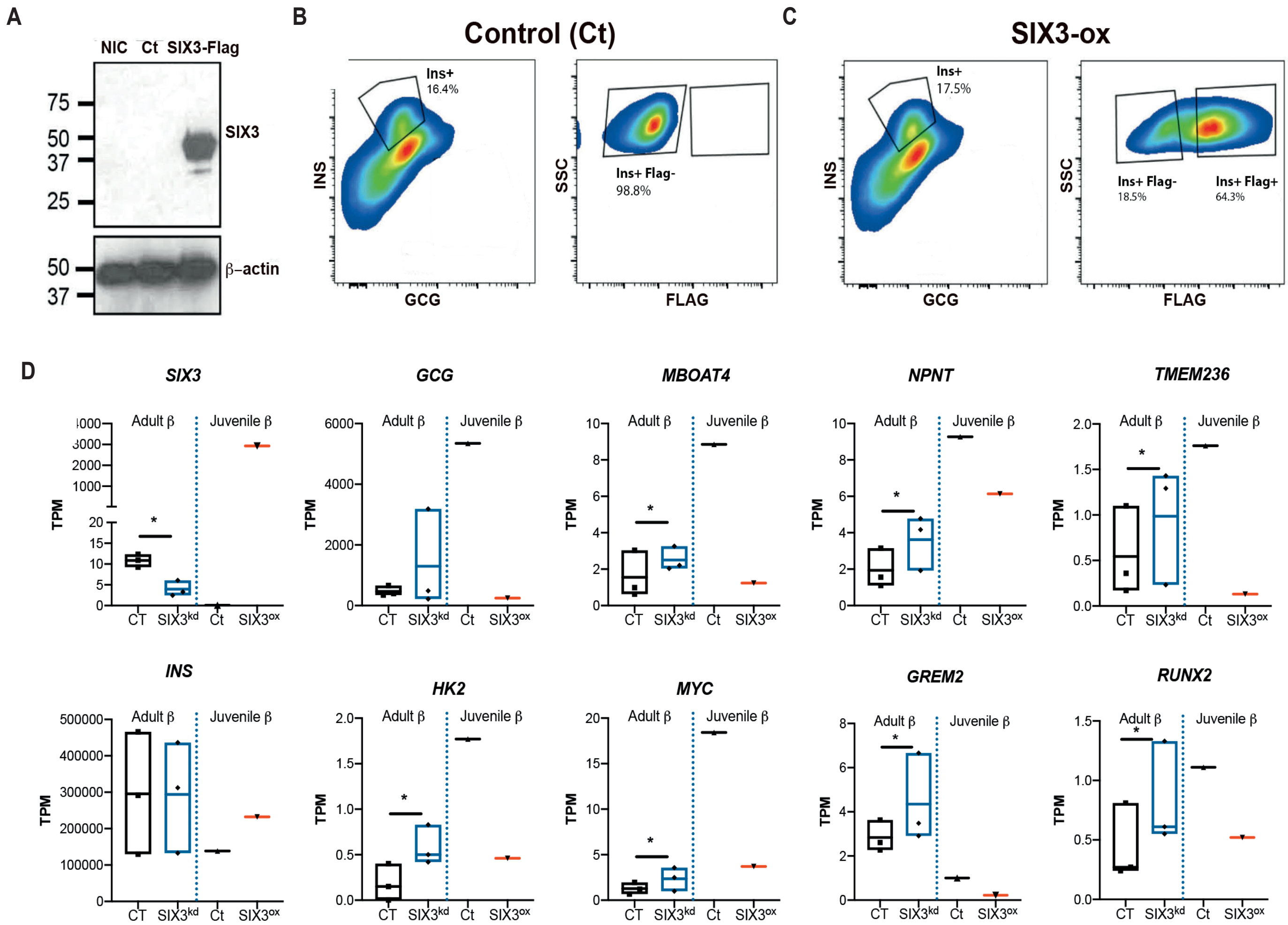


Table S1. Information of pancreatic islet donors used in this study.

Donor prep source	Identification	Sex	Age	BMI	Usage
IIDP	SAMN08617638	M	49	34	SIX3-KD RT-qPCR +GSIS
Alberta University	R250	F	26	25.4	SIX3-KD RT-qPCR +GSIS
IIDP	AEJT193A		51	29	SIX3-KD RT-qPCR +GSIS
IIDP	AEJE412		55	30.1	SIX3-KD RT-qPCR +GSIS
Alberta University	R267	F	64	23.7	SIX3-KD RT-qPCR +GSIS
Alberta University	R268	F	48	29.2	SIX3-KD/SIX2-KD RT-qPCR +GSIS
IIDP	SAMN09370567	M	32	28.5	SIX3-KD/SIX2-KD RT-qPCR +GSIS
IIDP	SAMN09862214	M	31	31.8	SIX3-KD/SIX2-KD RT-qPCR +GSIS
Alberta University	R286	M	41	20.4	SIX3-KD/SIX2-KD RT-qPCR +GSIS
AFAO295	SAMN08611213	F	63	24.97	SIX3-KD/SIX2-KD RT-qPCR +GSIS
UCSF	rHIP-122	M	58	27.82	SIX3-KD/SIX2-KD RT-qPCR +GSIS
UCSF	rHIP-144	F	47	23.51	SIX3-KD/SIX2-KD GSIS
IIDP	SAMN16191825		25	31.3	SIX3-KD/SIX2-KD GSIS
IIDP	SAMN15942269	F	63	26.9	SIX3-KD/SIX2-KD GSIS Whole islet RT-qPCR -non-diabetic
IIDP	SAMN09867385	M	24	27.2	SIX3-KD RNA-seq
IIDP	SAMN10079665	M	25	31	SIX3-KD RNA-seq
Alberta University	R288	F	69	27.7	SIX3-KD RNA-seq
Alberta University	R318	M	54	20.5	SIX2-KD RNA-seq
UCSF	RHIP-133	F	26	25	SIX2-KD RNA-seq
Pittsburgh		M	24	31.8	SIX2-KD RNA-seq
Alberta University	R326	M	26	27	SIX2-KD RNA-seq
IIDP	SAMN13938639	F	50	39.2	RIP-SIX2-Flag CUT&RUN
Alberta University	R388	M	45	33.8	RIP-SIX2-Flag CUT&RUN
IIDP	SAMN16427178	F	42	31.2	RIP-SIX2-Flag CUT&RUN
CAOP	AHAX150	F	3	n.a	SIX3-overexpression, Beta cell RT-qPCR
IIAM	18 month old		18 month	n.a	SIX3-ox, Beta cell RT-qPCR and RNA-seq
Alberta University	R282	M	57	26.4	Whole islet RT-qPCR-non-diabetic
Alberta University	R283	M	22	22.5	Whole islet RT-qPCR -non-diabetic
Alberta University	R357	M	64	24.3	Whole islet RT-qPCR -non-diabetic
Alberta University	R348	F	43	16.4	Whole islet RT-qPCR -non-diabetic
IIDP	SAMN10977276	M	52	27.2	Whole islet RT-qPCR -non-diabetic
CAOP	AHHQ227	F	18	34.9	SIX3-KD/SIX2-KD GSIS; Whole islet RT-qPCR -non-diabetic
Alberta University	R347	M	57	27.9	Whole islet RT-qPCR -T2D-diabetic
NDRI	AGJU173	F	53		Whole islet RT-qPCR -T2D-diabetic
HPAP	AHBD-489	F	50	30.5	Whole islet RT-qPCR -T2D-diabetic
HPAP	AHGP286	M	40	37	Whole islet RT-qPCR -T2D-diabetic
HPAP-PANC-DB	HPAP-003	M	29	24.5	Non diabetic Beta, alpha cells RNA-seq, islet perifusion

HPAP-PANC-DB	HPAP-006	M	46	19.1	Non diabetic Beta, alpha cells RNA-seq, islet perifusion
HPAP-PANC-DB	HPAP-008	F	24	31.9	Non diabetic Beta, alpha cells RNA-seq, islet perifusion
HPAP-PANC-DB	HPAP-004	F	24	32.2	Non diabetic Islet perifusion
HPAP-PANC-DB	HPAP-014	F	43	30.9	Non diabetic Beta, alpha cells RNA-seq
HPAP-PANC-DB	HPAP-017	M	30	23.7	Non diabetic Beta, alpha cells RNA-seq, islet perifusion
HPAP-PANC-DB	HPAP-001	M	47	32.2	T2-Diabetic Beta, alpha cells RNA-seq
HPAP- PANC-DB	HPAP-007	F	65	42.6	T2-Diabetic Islet perifusion
HPAP- PANC-DB	HPAP-010	F	42	36.8	T2-Diabetic Beta, alpha cells RNA-seq, perifusion
HPAP-PANC-DB	HPAP-013	F	28	41.6	T2-Diabetic Beta, alpha cells RNA-seq, perifusion

Table S7. *SIX2*^{kd} in primary β-cells results in de-regulation of genes whose expression changes with age in β-cells (see methods for details).

***SIX2*^{kd} downregulated genes (*P*<0.05) enriched in adult β-cells (*P*<0.01)**				***SIX2*^{kd} upregulated genes (*P*<0.05) enriched in juvenile β-cells (*P*<0.01)**		
AACS	FAM107A	NOMO1	SORL1	ACAT2	PARD6B	POMZP3
ABLIM1	FAM3B	NPAS2	SPINT2	ARHGAP11B	PBX2	PTGFR
ACAT1	FAM47E	NRCAM	SPTBN4	ARIH2OS	PCGF6	ZBTB2
ADAP2	FBLN7	OLFM1	ST14	ARSK	PHF19	ZNF581
ADCYAP1	FBXO2	OMA1	STEAP2	ATG2A	PHLDA1	
ADSSL1	FBXO44	P2RX2	STEAP3	AURKA	PIM2	
AGT	FRRS1L	P4HA2	STUB1	BAK1	PIP5K1A	
AKR7A3	GALNT11	PAM	STXBP6	BAZ1A	PLAGL2	
ALDOA	GCGR	PAPSS2	SULT4A1	BTK	POLE4	
ANKH	GDA	PCDHA10	SUN1	C12orf4	PPM1D	
ANKRD24	GHDC	PCDHA6	SUSD4	C16orf72	PRR14	
ANKRD34C	GLT8D2	PCDHB5	SYNE2	C19orf54	PTGS2	
ANXA7	GOLGA8A	PCDHGA4	SYNPO	CACNB1	PYCR1	
APCDD1L	GOLGA8B	PCDHGA8	SYT16	CCL20	RAB42	
APH1A	GPRASP1	PCDHGB7	TBC1D8	CCL5	RACGAP1	
AQP3	GREM1	PEBP1	TGOLN2	CCNA2	RASGRP3	
ATP6V0E2	GRIA2	PFKP	THBD	CCNB2	RASSF3	
BNIP3	GRIA3	PIGZ	TMEM132D	CCNYL1	RET	
C1orf115	GRIPAP1	PLCB1	TMEM176B	CD3EAP	RFX7	
C1orf127	GRM1	PLOD2	TMEM246	CDC25A	RGMA	
C4orf3	GSN	PLXND1	TMEM255A	CDCA7L	RMI2	
C7orf50	HGD	PNMA2	TMEM59	CDKN2AIP	SIAH2	
CACNA1C	HHAT	POP4	TMEM74B	CDR2L	SLC16A4	
CACNA1D	HIBADH	PPIP5K1	TMOD1	CENPM	SLC38A2	
CAPN13	HIVEP3	PPL	TMX4	CITED2	SMC4	
CAPN2	HOOK2	PPP2R2C	TNS1	CLDN15	SNAI1	
CBX7	HOPX	PRDX1	TPD52	CLSPN	SOD2	
CCDC149	HPRT1	PRDX4	TSPAN13	CXorf38	SPNS1	
CCDC85C	HSF4	PRKAG2	TSPAN7	DAPP1	SRF	
CD5	HSPA12A	PRMT2	TUBA1A	DCAF12	SRSF3	
CD9	IAPP	PROCR	TXNDC5	DCUN1D3	STK17B	
CD99	IDS	PRSS23	TYW3	ESPL1	SUV39H2	
CDKN1A	IGFBP3	PRUNE2	UCHL1	EXOSC5	TCEANC2	
CDKN1C	IGIP	PSAP	UNC79	FAM189B	TMC6	
CHGB	IGSF21	PTPN3	VAT1L	FAM83D	TMEM170A	
CHRM3	IL17RB	PTPRN	VWA5A	FANCA	TMPO	
CLCN3	IL20RA	QPCT	VWDE	FOSL2	TNFRSF10C	
CLU	ITPR3	RAB17	WDR45B	GAS2L3	TONSL	
CMTM4	JAKMIP3	RAP1GAP2	WFS1	H2AFX	TOR4A	
CMTM8	KCND3	RASGRF1	WNT4	HIST1H2BK	TP53	
CNKSR2	KCNK1	RIC3	WSCD2	HIST1H4H	TPX2	
COG2	KCNK17	RIN2	WWC3	HIST2H2AA4	TRIAP1	
CPB1	KCNK3	RUFY3	XDH	HMGCR	TXNIP	
CPE	KIRREL3	RYR2	XKR4	IFFO2	USP42	
CPLX1	KLHL36	SCD5	ZBTB22	IFITM3	VNN3	
CPLX2	LAMA5	SCG5	ZFYVE28	IL1RN	WDR47	
CPNE4	LGI3	SCGB2A1	ZNF395	ISG20L2	WDR62	
CRYL1	LIMCH1	SCN1B	AK4	KBTBD2	XAF1	

CTAGE6	LPIN3	SCN8A	APBA1	KCNJ2	ZNF264
CTSB	LRP2BP	SEC11C	CRYZ	KDM2B	ZNF490
DAP	LYRM9	SEMA5A	DLK1	KDM6A	ZNF527
DDR1	MAN1C1	SETD7	EFR3B	KIFC1	ZNF570
DGCR2	MAPT	SGSM1	FA2H	KLF16	ZNF574
DHRS2	MBP	SH3YL1	GPX2	KLF3	ZNF669
DIRAS3	MCOLN3	SHROOM1	GREM2	KLF4	ZNF829
DLGAP4	MDH1B	SIAE	HIGD1A	KLHL15	ZNF835
DMKN	ME3	SIX2	HIST1H1E	KPNA2	ZNF850
DOCK9	METRN	SLC16A12	KIF26B	LEAP2	ZSWIM8
DPH6	MICAL3	SLC25A27	KREMEN1	LSMEM1	ARL4A
DPP7	MICU2	SLC35D3	LPCAT1	MCL1	BBC3
DYNLT3	MLXIPL	SLC35F3	MALL	MID1IP1	BRCA2
DZIP1L	MT1E	SLCO5A1	NFIC	MSMO1	C1QTNF9
ECHDC2	MT1F	SMAD9	SDK2	MTF1	C2CD2L
EHBP1	MT1X	SMARCD3	SLC7A4	MTG2	CCDC116
EHF	MUC13	SMIM5	SLC9A3R2	NEB	CCDC168
ENO2	MYH14	SMOC1	TMEM132B	NETO2	CD300LB
ENPP5	MYO1D	SMPDL3A	TMEM184B	NLRC4	CDC45
ENTPD3	MYO5C	SNAP25	TSKU	NR4A2	HSPBAP1
ENTPD6	NBEAL1	SNCB	TTC7B	NRAS	ITGA2B
EPB41L4B	NDRG1	SNRNP35	UNC5B	NUSAP1	LAT
EPCAM	NEIL2	SNTA1	WSCD1	OLFML3	LRRN3
EPHX2	NFASC	SNX29			

Table S8. Genes showing DE upon *SIX2*^{kd} and *SIX3*^{kd} in the adult β-cell (P<0.05)

A1CF	DDX55	ITM2C	RADIL	VWA1	ZNF829	SF3A2	SLIT1
ACCS	EIF5	KCNA5	RBM15	WDR81	RRM2B	RNF38	SLC44A2
ACOT7	FEN1	KHSRP	RSAD1	ZIK1	GLT8D2	GPRASP1	TSKU
ACSL6	FFAR4	KLHL29	SH2B3	ZNF547	GDF15	CAPN13	FBLN1
ANKRD40	FST	LRP1	SLC9A3R2	ZNF57	FXYD6	PPIP5K1	PLXDC2
ARFGEF3	GAREM1	LRRC75A	SMIM32	ZNF77	SYT7	ZFYVE28	NEB
ARL14	GDNF	MAST1	SPEG	ZSWIM4	RGS11	ZBTB22	CCL20
ATP2A3	GHRL	MAX	SRA1	TPX2	SCD5	SHROOM1	
B4GALNT3	GPR142	MPV17L2	STOM	GAS2L3	RAP1GAP	NCKAP5	
BRCA2	GRIN2C	MYO1A	SVOP	CDC37L1	HILPDA	PFKP	
BRINP1	HGH1	OLIG1	TAF5	ZNF556	OBSL1	KCNMB2	
BST2	HIPK2	OVGP1	THOC5	ZNF296	SNCB	GREM2	
C4B	HIST2H2AA3	PCDHA7	TLE2	CDR2L	RAP1GAP2	KCND3	
CACNA1H	HS6ST3	PHF7	TMEM132A	PSAT1	ITPR3	COL6A1	
CAMK2N1	IDI1	PLCH2	TMEM237	CD3EAP	TRPM3	PTPRS	
CD300A	IFIT2	POLE3	TREX1	PROCR	NDRG1	HAP1	
COL27A1	INSYN1	PTK7	TSPOAP1	CRELD2	CCDC85C	HGD	
CXCL5	ISG20	RAD21	URGCP.MRPS24	SUPV3L1	TNS1	PBX2	

Table S9. *SIX3*^{kd} in primary β-cells results in upregulation of genes enriched in juvenile β-cells (see methods for details).

***SIX3*^{kd} upregulated genes (*P*<0.05) enriched in juvenile β-cells (*P*<0.01)**						
ADD3	CELF3	ELF4	INSR	NFIB	PTPRS	VNN1
ANO6	CNN2	EVI2B	IQGAP2	NPAS4	SERPING1	WIZ
ANTXR2	CNOT3	FAM126A	KCNMB2	PBX2	SH3GL1	ZNF341
ANXA1	DMD	FBLN1	KCTD12	PCGF2	SLC1A5	C4B
ATG9A	DMTN	FOXP4	LYZ	PDK2	SLC44A2	CD302
BCL6	DOCK8	FSTL1	MVB12B	PHF12	THBS1	GHRL
BCL7A	DPP4	HILPDA	MYC	PLXDC2	TJP2	INPPL1
CCL20	DUSP2	HK2	NCKAP5	PLXNA1	TLE3	RNF182
CCNG2	EBF4	IER5L	NDST2	PPDPF	TMEM236	TNRC18
CD248	EHD2	INSM1	NEB	PTPN12	TSKU	VIT

Table S12. List of genes DE by *SIX2*^{kd} both in adult human β-cells (this study) and SC–β-like cells (Velazco-Cruz et al 2020). DESeq2 was used to calculate DE genes with log2FC (*P*<0.05)

Genes downregulated post *SIX2*^{kd} in human β-cells and SC–β-like cells					Genes upregulated post *SIX2*^{kd} in human β-cells and SC–β-like cells	
A1CF	DEPP1	ISYNA1	PCDHGA8	SLC35F3	AOC3	PLAGL2
ABCA2	DEPTOR	ITM2C	PCDHGB7	SLC4A3	ARL4A	POLE3
ABCC3	DHRS2	JAKMIP3	PCP4	SLC4A8	ARRDC4	POLR1E
ABCC4	DIRAS3	KCNA5	PCSK1N	SLC7A4	ATP8B2	POLR3D
ABHD14A	DLK1	KCND3	PCSK6	SLC9A3R2	B3GALNT2	PRG4
ABHD14B	DMKN	KCNIP2	PDE9A	SLCO5A1	BAZ1A	PRMT3
ABLIM1	DNM1	KCNJ6	PFKP	SMARCD3	BST2	PROB1
ACADVL	DOCK9	KCNK1	PFN2	SMIM32	C16orf72	PRR14
ACCS	DPP6	KCNK16	PHLDB2	SMIM6	C19orf48	PRRC1
ACOXL	DSCAML1	KCNK17	PIGZ	SMKR1	C21orf91	PRSS8
ACSL6	DZIP1L	KCNMA1	PIPOX	SMOC1	CALU	PRX
ACTN1	ECEL1	KCNMB2	PLA2G6	SNAP25	CCDC134	PTCH2
ADA2	EDN3	KCNQ2	PLCB1	SNCB	CCDC17	PTGFR
ADAMTS2	ELF3	KIAA1211L	PLCE1	SNTA1	CCNB1IP1	PTGS2
ADCY1	EMB	KIF12	PLD3	SORL1	CCSAP	PYCR1
ADCYAP1	ENO2	KIF5C	PLEKHB1	SPATA20	CD274	RALA
ADSSL1	ENTPD1	KIRREL3	PLEKHG6	SPON2	CDC25A	RASSF1
AK4	EPB41L1	KLHL29	PLXND1	SPTBN4	CDCA7L	RCC1
ALDOA	EPB41L4B	LGI3	PNMA2	SRGAP3	CEBPB	RCL1
ANGPTL4	EPHX2	LIMCH1	PNPLA4	SSTR2	CEP85	RGMA
ANKRD24	F12	LINGO2	POMGNT1	ST8SIA3	CFAP77	RIPK2
ANKRD34C	FAM131C	LMO1	PPL	STEAP2	CHKA	SAR1A
ANKRD37	FAM228B	LPIN3	PPP1R36	STEAP3	CITED2	SESN2
ANKRD6	FAM229B	LPP	PPP2R2C	STXBP1	CLDND2	SHMT1
APBA1	FBXO2	LRFN2	PPY	SULF2	CMYA5	SLC22A1
APPL2	FBXO44	LRP2BP	PRAG1	SULT4A1	COA7	SLC30A1
ARFGEF3	FFAR1	MAGI1	PRKACA	SUSD4	CREB3	SLC38A2
ARSD	FFAR4	MALL	PRKAG2	SYNGR1	CYTH3	SNX8
ASRGL1	FGF14	MAN1C1	PRPH	SYNPO	DCLRE1B	SRF
ATP2B4	FGFR1	MAP1LC3A	PRPS2	SYT16	DDX20	STX6
ATP6V0E2	FGGY	MAPK10	PRSS22	SYT17	DDX55	SUV39H2
B3GNT3	FRRS1L	MAPRE3	PSD	SYT7	DNAH11	TAF1D
BAALC	FSTL4	MDH1B	PSD4	TAGLN3	DUSP14	TAPT1
BABAM2	FXYD3	ME3	PTGDR2	TCERG1L	EIF5	TEAD2
BACE2	FXYD6	MEIS3	PTK2B	TCTN1	ESAM	TGIF2
BAIAP3	FYB2	METRN	PTPRN	TENM1	ESPL1	THAP9
BDKRB2	GABARAPL2	MGAT4C	PTPRS	THBD	FAM160A1	THUMPD2
BLOC1S6	GABRA2	MISP	PTPRT	TIAM1	FLCN	TMEM136

BRINP1	GALNT10	MLXIPL	PXK	TM4SF4	FOSL2	TMEM167B
BTN3A3	GAMT	MPP2	QDPR	TMEM125	FST	TMEM39A
C1orf127	GCG	MRAP2	QPCT	TMEM132A	HIST1H2BK	TMPO
C22orf42	GCK	MROH7	RAB26	TMEM132D	HIST1H4J	TOR4A
C9orf16	GDA	MS4A8	RAB31	TMEM158	HIST3H2A	TP53
CACNA1C	GDPD5	MSRB2	RAB3B	TMEM163	HMOX1	VPS37B
CAMK1D	GJB1	MTA3	RAB4B	TMEM255A	HRK	WDR90
CAMK2B	GLT8D2	MYH10	RAB9B	TMEM42	ICK	X9.Mar
CAMK2N1	GNB5	MYL7	RABL3	TMEM61	IFFO2	ZBTB24
CAPN2	GPD1	MYO1D	RASA4	TMEM74B	ISG20L2	ZBTB5
CASR	GPR148	MYO5B	RASGRF1	TMOD1	KBTBD8	ZHX1
CBX7	GPRASP1	MYT1L	RBP4	TMPRSS6	KIF24	ZIK1
CCDC159	GRIA2	NBEAL1	RDX	TMX4	KIF27	ZNF267
CCKBR	GRIN3B	NCAM1	RGS11	TNS2	KLF3	ZNF474
CCND2	GRM1	NCKAP5	RHOU	TP53I11	KLHL21	ZNF551
CCNI	GSN	NDRG1	RIMBP2	TPM4	KMT5A	ZNF57
CDH22	GSTM2	NECAB2	RTN1	TRIM2	LRRC66	ZNF581
CDKN1A	HAP1	NFAM1	RTN4RL1	TRMT9B	LYAR	ZNF749
CELF4	HEPACAM2	NFASC	RYR2	TSPAN1	LYG1	ZNF813
CERK	HOPX	NLRP1	S100B	TSPAN7	MCM2	ZNF841
CHD3	HSD3B7	NPAS2	SARDH	TSPOAP1	METTL1	ZNF850
CHDH	HSF4	NPDC1	SCG5	TTLL6	MGME1	
CHGB	HSPA12A	NPW	SCN1B	TUB	MMP14	
CLDN4	HSPD1	NRBP2	SDK2	UNC5A	MMS22L	
CLU	IAPP	NRXN2	SFTPD	UNC5B	MPV17L2	
COL6A1	IDNK	NSG1	SHISA4	VAT1L	MRAS	
COL9A2	IDS	OLFM1	SHISA6	VTN	MRE11	
CPE	IGFBP3	OMA1	SHROOM2	VWA5A	MSANTD2	
CPLX1	IGFBP7	PACRG	SIGIRR	WNT4	NETO2	
CPLX2	IGSF21	PACSIN1	SIGLEC15	WSCD2	NFKBIB	
CREG2	IL11RA	PALM2	SIL1	X1.Mar	NRAS	
CST3	IL17RB	PAX6	SIX2	XKR4	NUS1	
CTSB	IL1R1	PCBP4	SLC12A8	ZDHHC22	ORC6	
CYBA	IL20RA	PCDHA10	SLC16A12	ZFYVE28	PAIP2B	
DACH2	IMPDH1	PCDHB10	SLC18A2	ZNF860	PEX5	
DCTN1	INS	PCDHB13	SLC22A17			
DCX	INSYN1	PCDHB14	SLC25A27			
DDC	IRX3	PCDHB6	SLC35D3			

Supplemental Figure Legends

Figure S1. Pseudoislets exhibit re-aggregation of the principal islet cell-types after lentiviral transduction
(A-L) Immunostaining of INS (red) and GCG (white) paired with GFP (green) in human pseudoislets five days after transduction with (A-D) Control, (E-H) $SIX2^{kd}$ and (I-L) $SIX3^{kd}$ lentiviruses. (M-X) Immunostaining of INS (white) and STT (red) paired with GFP (green) in human pseudoislets five days after transduction with (M-P) Control, (Q-T) $SIX2^{kd}$ and (U-X) $SIX3^{kd}$ lentiviruses. Scale bars, 20 µm.

Figure S2. β-cells $SIX2^{kd}$ RNAseq
(A) *SIX2* mRNA expression in sorted β-cells of control (grey bar) and $SIX2^{kd}$ (green bar) (n=4 independent donors). (B) Insulin, Glucagon CPA-1 and KRT19 mRNA expression in pre-sorted (black bars), GFP+ Insulin+ control (grey bars) and $SIX2^{kd}$ (green bars) pseudoislet cells, data normalized to human beta actin. (C) Detailed FACS scheme used to sort GFP+ β–cells of Control and $SIX2^{kd}$ pseudoislets: single, live, GFP^+ cells were used for intracellular staining with INS and GCG antibodies, and INS^+ cells were used for RNA extraction and RNA-Seq library building: see methods. (D) PCA plot showing variance due to knockdown and donor conditions (n=4 independent donors). (E) Heatmap of the sample-to-sample distances of all RNA-Seq samples used in this experiment. The scale bar indicates the euclidean distance. The data is presented as mean, error bars represent the standard error. Two-tailed t tests were used to generate *P* values. *** $P<0.0001$

Figure S3. $SIX2^{kd}$ in β-cells results in a distinct set of differentially expressed genes compared to SC β-like cells: (A) $SIX2^{kd}$ downregulated genes (B) $SIX2^{kd}$ upregulated genes. For both datasets, DE genes were calculated using DEseq2, $P<0.05$ (see methods).

Figure S4. SIX2-Flag CUT&RUN in adult human islets. (A) Western blot using anti-Flag antibody on protein lysates from either SIX2-Flag or control (Empty construct) human islets. (B) Histogram plotting averaged reads showing enrichment of read densities in the peak centers for the SIX2-Flag libraries. (C-E) Tracks showing SIX2-Flag genomic regions associated to (D) IAPP, (E) MLXIPL, (F) GREM1. Accessible chromatin regions in human islets are shown by ATAC-seq, H3K27ac and H3K4me3 ChiP-seq tracks of whole human islets. SIX2 CUT&RUN (SIX2 C&R) peaks are shown in pink boxes, and regulated genes highlighted in green boxes.

Figure S5. β-cells $SIX3^{kd}$ RNAseq: (A,B) Human pseudoislets five days after transduction with $SIX3^{kd}$ lentiviruses: (A) bright field; (B) blue light (488 nm), scale bars = 1000 µm. (C) Detailed FACS scheme used to sort $SIX3^{kd}$ GFP^+ β–cells: live, GFP^+, INS^+ cells were sorted for RNA extraction and RNA-Seq library building. (D) *SIX3* mRNA expression in sorted β-cells of control (grey bar) and $SIX3^{kd}$ (blue bar) (n=3). (E) Insulin, Glucagon CPA-1 and KRT19 mRNA expression in pre-sorted (black bars), GFP+ Insulin+ control (grey bars) and $SIX3^{kd}$ (blue bars) pseudoislet cells (n=3 independent donors), data normalized to human beta actin. (F) PCA plot showing variance due to knockdown and donor conditions. (n=3). (G) KEGG pathways enriched in genes downregulated in β-cells post-$SIX3^{kd}$. The data is presented as mean, error bars represent the standard error. Two-tailed t tests were used to generate *P* values. ** $P<0.05$

Figure S6. SIX2 and SIX3 targets are distinct: Boxplots displaying normalized TPM counts of (A) selected S*IX2*^{kd} targets in the adult β-cell; the expression of which is not affected by *SIX3*^{kd}, control (grey bars), S*IX2*^{kd} (green bars), S*IX3*^{kd} (blue bars). (B) selected S*IX3*^{kd} targets in the adult β-cell; the expression of which is not affected by *SIX2*^{kd}, control (grey bars), S*IX2*^{kd} (green bars), S*IX3*^{kd} (blue bars). Box plots show the mean. *, $P<0.05$

Figure S7. *SIX3* overexpression in juvenile β-cells: (A) Western blot using anti-SIX3 antibody on protein lysates from either non infected control (NIC), infected Control (Ct, Empty construct) or SIX3-Flag overexpressed human islets. (B-C) FACS scheme used to sort Flag+ SIX3-ox juvenile β–cells, using intracellular staining with INS and GCG antibodies and intranuclear staining with Flag antibody, for the Empty Control (Ct) and *SIX3*-Flag (SIX3-ox) overexpressed human islets. (D) Boxplots displaying normalized TPM counts of *SIX3*, *INS* and selected S*IX3* targets in the adult β-cell; the expression of which is inversely affected by SIX3-ox in juvenile β-cells (n=1), control (black bars), S*IX3*^{kd} (blue bars), S*IX3*^{Ox} (red bars). Box plots show the mean. *, $P<0.05$