Supplemental Data

mTORC1 to AMPK Switching Underlies β-Cell Metabolic Plasticity During Maturation and Diabetes

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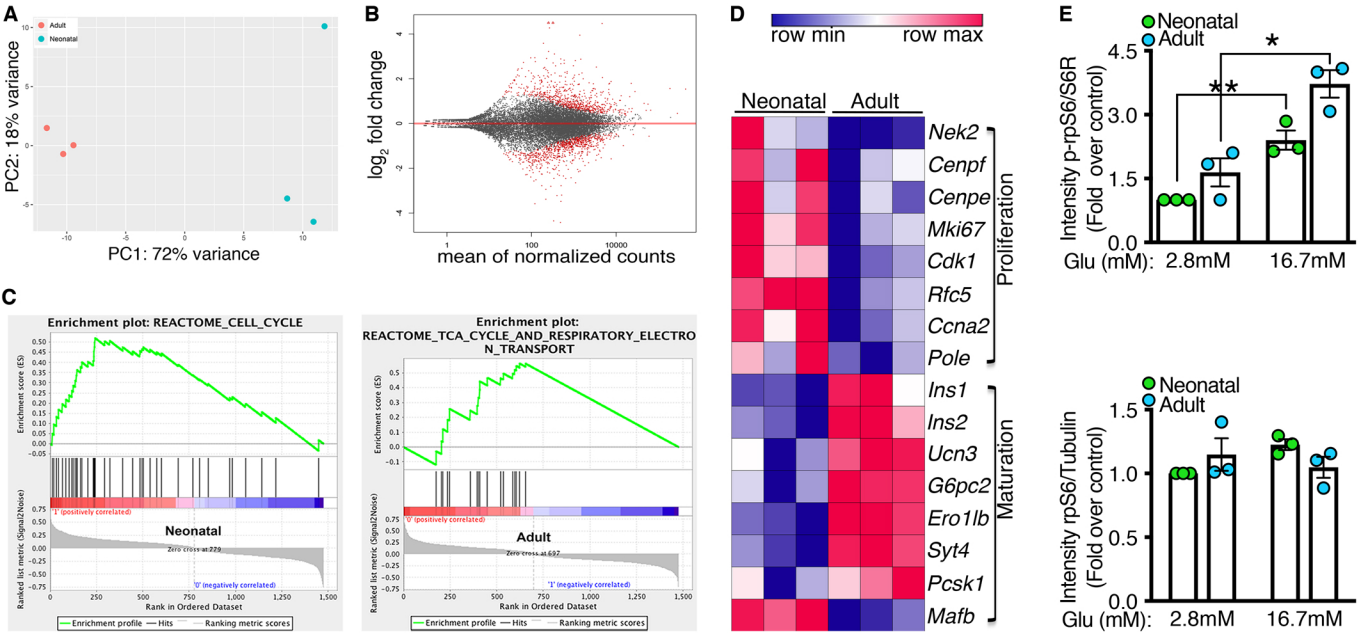
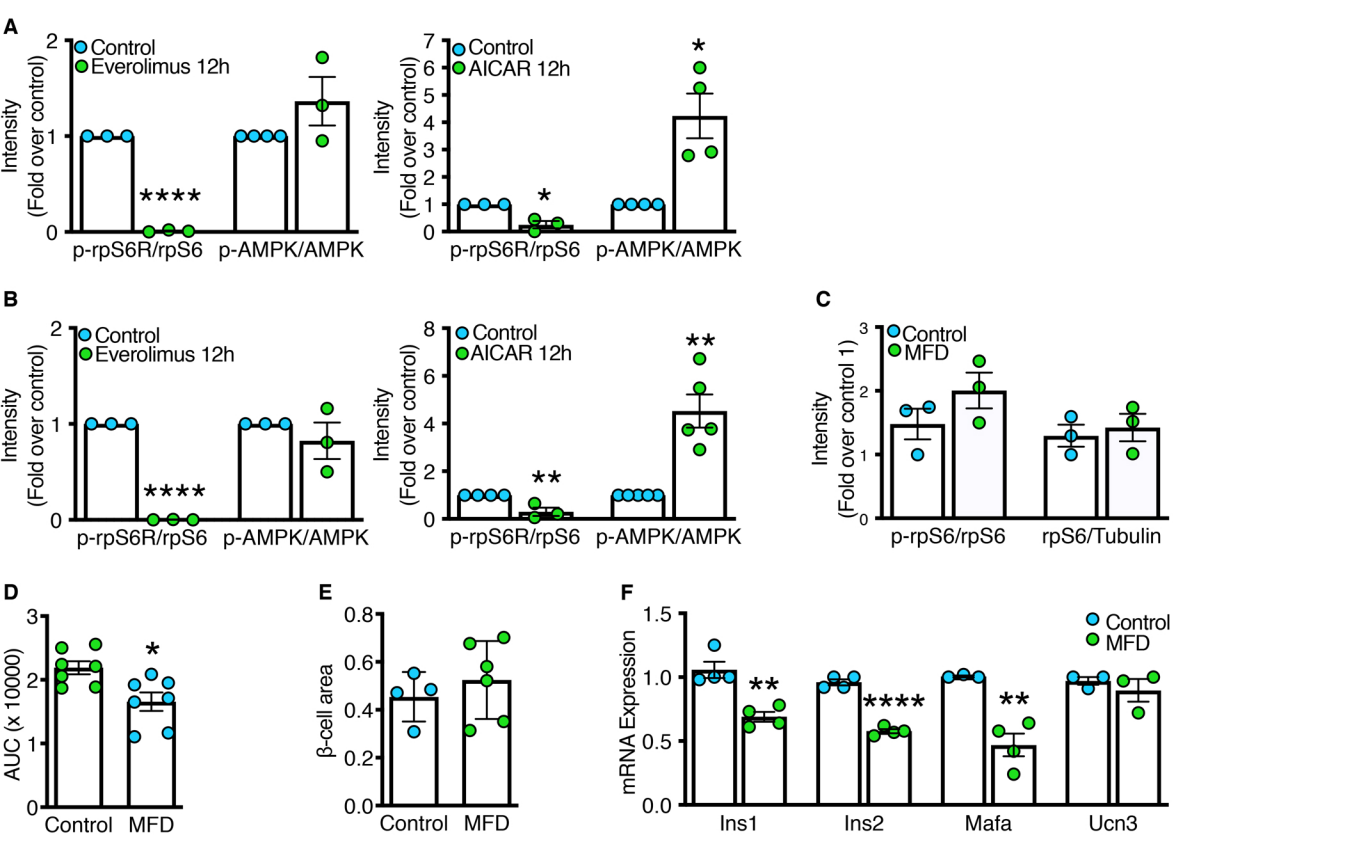
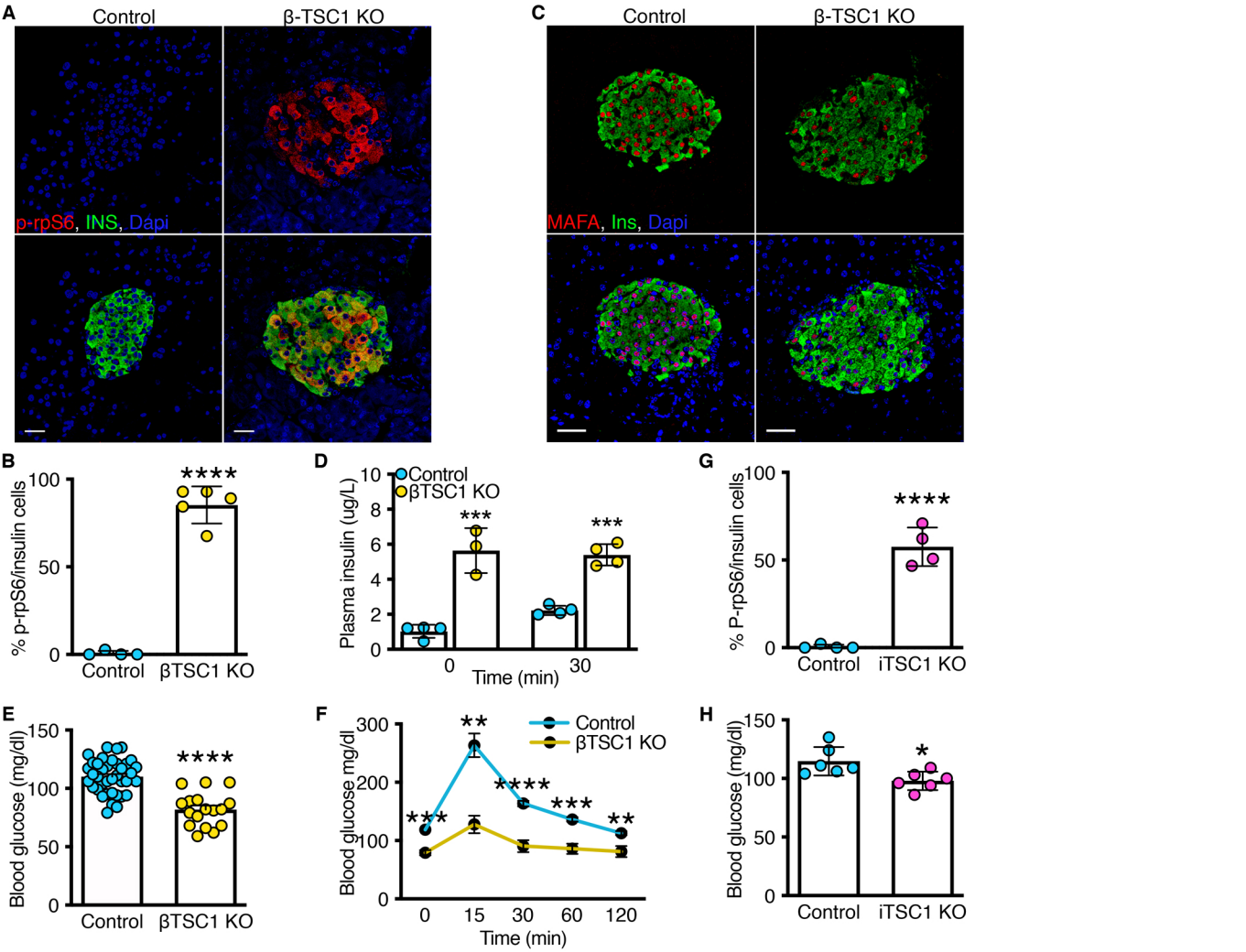


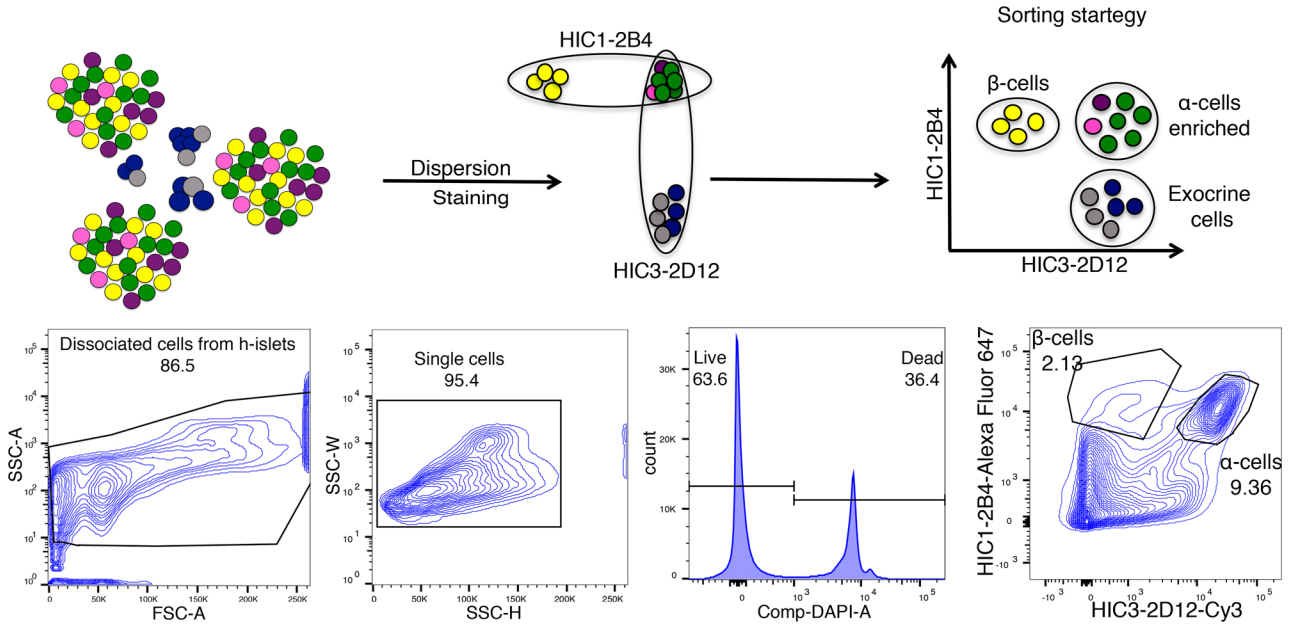
Supplemental Figure 1. A. Principal components analysis (PCA) of RNAseq libraries from P6 and P45 pancreatic β-cells. **B.** Plot of normalized mean expression counts vs. log2-fold change for P6 vs. P45 β-cells. **C.** GSEA of RNAseq data, showing enrichment by reactome cell cycle pathway genes in neonatal β-cells, and instead, enrichment by reactome TCA cycle and respiratory electron transport chain genes in adult β-cells ($p<0.05$). **D.** Heatmap, showing β-cell transcriptional profiles including relative upregulation of genes related to proliferation at P6 and maturation at P45 ($p<0.05$). **E.** Quantification of data in Figure 1E, with p-rpS6 normalized to total rpS6 (top) and total rpS6 normalized to tubulin (bottom). **p = 0.006 *p = 0.0109 (unpaired t-test corrected for multiple comparison using Holm-Sidak method).



Supplemental Figure 2. A. Quantification of the immunoblots in Figure 2A for p-rpS6 and p-AMPK performed on mouse islets treated for 12 hours with 40μM everolimus (left) or 1mM AICAR (right) normalized to total rpS6 or total AMPK, respectively (n=3 per group). * p= 0.0104, ****p < 0.0001 (unpaired t-test corrected for multiple comparison using Holm-Sidak method). **B.** Quantification of the immunoblots in Figure 2B for p-rpS6 and p-AMPK performed on human islets treated for 12 hours with 40μM everolimus (left) or 1mM AICAR (right) normalized to total rpS6 or total AMPK, respectively (n=3 per group). **p= 0.0051 ** p= 0.0018, and ****p < 0.0001 (unpaired t-test corrected for multiple comparison using Holm-Sidak method). **C.** Quantification of data in Figure 2D, showing p-rpS6 normalized to total rpS6 and total rpS6 normalized to tubulin. **D.** Figure 2E corresponding area under the curve n=7. *p= 0.011 (two-tailed unpaired t-test). **E.** Calculated β-cell area in islets from control mice and MFD mice (n=5-6). p=0.4720 (two-tailed unpaired t-test). **F.** qPCR, showing relative mRNA levels of β-cell maturation markers in control and MFD mice n=3-4. **p= 0.008, ****p<0.0001 (unpaired t-test corrected for multiple comparison using Holm-Sidak method).



Supplemental Figure 3. **A.** Immunostaining for insulin (green), p-rpS6 (red), and DAPI (blue) in representative pancreatic sections from control and β-TSC1KO mice (scale bar: 50 μm). **B.** Percentage of β-cells positive for both p-rpS6 and insulin in adult β-TSC1KO and control mice (n=4-5). ****p<0.0001 (two-tailed unpaired t-test). **C.** Immunostaining for insulin (green), MafA (red) and DAPI (blue) in representative pancreatic sections from adult β-TSC1KO and control mice (scale bar: 50 μm). **D.** Plasma insulin levels after glucose challenge in β-TSC1KO and control mice (n=5), ***p=0.0009 and ***p=0.00015 (unpaired t-test corrected for multiple comparison using Holm-Sidak method). **E.** Fasting blood glucose levels in β-TSC1KO mice and littermate controls (n=17-38) ****p<0.0001 (two-tailed unpaired t-test). **F.** IPGTTs for β-TSC1 KO mice and littermate controls (n=4-11). **p<0.01, ***p<0.0001, and ****p<0.0001 (unpaired t-test corrected for multiple comparison using Holm-Sidak method). **G.** Percentage of cells positive for both p-rpS6 and insulin in islets from iTSC1KO and control mice (n=4). ****p<0.0001 (two-tailed unpaired t-test). **H.** Fasting blood glucose levels in iTSC1KO mice and littermate controls (n=6). **p=0.0173 (two-tailed unpaired t-test).



Supplemental Figure 4. Gating strategy for sorting β-cells from human islets.

Supplemental table 1

Log2 fold changes and associated p-value for genes presented in the heatmap shown in supplemental Fig 1D

Gene name	Log2 fold change	p-value
Nek2	-1.339034306	0.00941881
Cenpf	-0.977890623	0.03495696
Cenpe	-1.212366861	0.02397859
Mki67	-1.192655003	2.26E-005
Cdk1	-1.433178469	0.00792847
Rfc5	-1.528949667	0.00258173
Ccna2	-1.528532817	0.00103548
Pol2	-1.289185581	0.02712927
Ins1	1.566120644	2.68E-012
Ins2	1.540292745	5.16E-014
Ucn3	1.257778699	3.58E-006
G6pc2	1.425826996	5.60E-012
Ero1lb	1.19744314	1.04E-016
Syt4	1.81888941	3.14E-016
Pcsk1	0.455240727	0.04116302
Mafb	-1.753123494	1.19E-016

Supplemental table 2

Log2 fold changes and associated p-value for genes presented in the heatmap shown in Figure 1B

Gene name	Log2 fold change	p-value
Igf2	-1.669744396	0.00535097
Irs1	-1.009097768	0.0049805
Igf1r	-1.009466088	3.26E-005
Igf2bp1	-3.584108315	3.16E-013
Igf2bp2	-2.420619212	1.32E-016
Igf2bp3	-4.351045752	1.71E-027
Eif4g1	-0.496406801	0.00311579
Eif3b	-0.518685897	0.0341385
Eif4h	-0.614698641	0.00934488
Eif4a3	-0.701346773	0.01070156
Eif4a1	-0.867112366	0.01129465
Rps6ka2	-0.466920254	0.0290266
Foxo1	-0.709903032	0.01876581
Prkaa2	1.951447958	5.23E-005
Pten	0.58453832	0.00173429

Supplemental table 3

Log2 fold changes and associated p-value for genes presented in the heatmap shown in Figure 3E

Gene name	Log2 fold change	p-value
Ins1	-3.738244171	8.72E-37
Ins2	-5.099994098	6.81E-54
Ucn3	-2.693629409	1.66E-14
G6pc2	-3.299783607	1.94E-28
Ero1lb	-2.875381609	2.53E-21
Syt4	-3.447526233	7.78E-15
Pcsk1	-2.47241789	4.60E-08
Mafa	-2.5436652	1.14E-05

Supplemental table 4

Log2 fold changes and associated p-value for genes presented in the heatmap shown in Figure 4A

Gene name	Log2 fold change	p-value
mt-Co1	0.901261886	1.66E-005
mt-Co2	1.257060123	0.00234427
mt-Co3	0.70901422	0.01370815
mt-Cytb	1.133039722	2.56E-006
mt-Nd1	0.896537131	0.01299598
mt-Nd2	0.749238012	0.03711852
mt-Nd4	1.150902649	0.00212917
mt-Nd5	0.848395287	0.00056296
mt-Atp6	0.980945583	0.0206238
Atp5e	1.478860056	0.00308137
Atp6ap1	0.622265079	0.01749909
Cox5b	0.809685423	0.01566565
Cox6a2	2.706722493	4.16E-008
Cox7b	1.048435595	0.00300421
Cyb5a	0.865927615	0.00806819
Ndufa3	1.294458592	0.02502589
Uqcrh	1.231889143	0.00128698
Uqcrq	1.226963434	1.30E-005

Supplemental table 5

Log2 fold changes and associated p-value for genes presented in the heatmap shown in Figure 4E

Gene name	Log2 fold change	p-value
mt-Co1	-0.868310802	1.18E-11
mt-Co2	-0.911171573	1.18E-12
mt-Co3	-0.880341761	1.29E-25
mt-Nd1	-0.726717508	6.48E-08
mt-Nd2	-0.937632202	3.18E-12
mt-Nd4	-0.95314497	1.21E-13
mt-Nd5	-1.010724448	2.41E-16
mt-Atp6	-0.90594167	5.55E-22
Atp8	-0.844959725	2.59E-14
Atp2a2	-0.473449972	0.00017864
Atp6ap2	-0.611224628	3.96E-10
Atp6v1e1	-0.45885391	5.04E-09
Atp6v1d	-0.486891211	2.99E-24
Cox7a2l	-0.506881364	0.0003437
Cox6a2	-0.722191874	0.0003903
Ppargc1a	-0.523333512	0.039
Ppargc1b	-0.397202902	0.06413312

Supplemental table 6

Log2 fold changes and associated p-value for genes presented in the heatmap shown in Figure 5A

Gene name	Log2fold change	p value
mTor	0.780377302	0.01287027
Akt1	0.705019398	0.00125506
Igf1r	0.970627039	0.00736922
igf2r	1.438017002	1.76E-09
Rps6ka1	1.20304205	0.00902307
Rps6ka2	0.618403979	0.04289504
Rps6ka4	0.919014983	0.00025866
Pdk1	1.094459248	0.00210561
Eif4a3	0.938303261	0.00085781
Eif4ebp1	1.63063821	3.61E-07
Eif4g1	0.508975641	0.02180279
Eif4h	0.474795353	0.04136719
Eif4b	1.188249422	4.29E-07
Eif4a3	0.938303261	0.00085781
Pik3r5	2.298347767	1.41E-07
Ulk1	1.155388941	3.27E-06
Ambra1	0.7668819	0.00379013
Rptor	1.162070397	8.44E-06
Prkaa1	-0.887260688	0.00629938
Ppp2ca	-1.251862877	8.82E-07
Fkbp1a	-0.665205399	0.0046429
Fkbp1b	-2.189758461	2.40E-21
Mapk1	-0.759869661	0.00323474
Ins2	-0.864623497	0.00080844
Ucn3	-1.093252337	6.26E-06
G6pc2	-0.877497222	0.00241343
Ero1lb	-1.817987497	5.89E-22
Pcsk1n	-1.257182893	2.31E-08

Supplemental table 7: qPCR primer sequences

	Forword primer	Reverse primer
PGC1a	GAATCAAGCCAGTACAGACACCG	CATCCCTCTTGAGCCTTTCGTG
PGC1b	CAGCCTCAGTTCCAGAAGTCAG	CACCGAAGTGAGGTGCTTATGC
Ins1	AGGACCCACAAGTGGAACAAC	GTGCAGCACTGATCCACAATG
Ins2	GCTCTCTACCTGGTGTGTGGG	CTCCACCCAGCTCCAGTTGTG
Ucn3	AAGCCTCTCCCACAAGTTCTA	GAGGTGCGTTTGGTTGTCATC
Glut2	GTTGGAAGAGGAAGTCAGGGCA	ATCACGGAGACCTTCTGCTCAG
Mafa	TCAGCAAGGAGGTCATCCGA	TGGCTCTGGAGCTGGCACTTCT

Supplemental table 8: list of disallowed genes.

Pullen et al.2010		
HBA	BLOC1S1	PFKL
NDRG2	C1qBP	PFKP
HBB	CAT	H6PD
SLC16A1	CD302	PGD
ABCA8A	COX5A	RPE
MYL9	CRLZ1	ACLY
LY6A	CXCL12	ACSS2
CAR2	FCGRT	HSD17b7
TST	HIGD1A	GDPD1
LY6C	IGFBP4	MVK
FXYD1	ISLR	SOAT1
SULT1A1	ITIH5	
GAS6	LDHA	
GDA	LMO4	
MGST3	MAF	
CXCL12	MCT1	
MGLL	MGST1	
GSTA4	MYLK	
LDHA	NOLA2	
PDGFRA	OAT	
TNS1	OGN	
OAT	PARP3	
FGF1	PCOLCE	
FAM59A	PDGFRA	
CD302	PRL24	
NFIB	PRL36	
DDAH1	SELENBP1	
ASPA	SMAD3	
CCL21	TGM2	
GAS1	UQCRB	
ACOT7	ZFP36L1	
NDRG4	ZYX	
YAP1	DNMT3a	
GALM	HK1	
GUCY1A3	HK2	
PLEC1	LDHA	
AK3	ALDOB	
ZDHHC9	TPI1	
IGFBP4	PGM1	
ZFP622	PGM2	
ARHGDIB	BPGM	

Thorrez et al. 2011
ZFP622
ARHGDIB
BLOC1S1
C1qBP
CAT
CD302
COX5A
CRLZ1
CXCL12
FCGRT
HIGD1A
IGFBP4
ISLR
ITIH5
LDHA
LMO4
MAF
MCT1
MGST1
MYLK
NOLA2
OAT
OGN
PARP3
PCOLCE
PDGFRA
PRL24
PRL36
SELENBP1
SMAD3
TGM2
UQCRB
ZFP36L1
ZYX

Dhawan et al. 2015
DNMT3a
HK1
HK2
LDHA
ALDOB
TPI1
PGM1
PGM2
BPGM
PFKL
PFKP
H^PD
PGD
RPE
ACLY
ACSS2
HSD17b7
GDPD1
MVK
SOAT1