Supplementary Information

m^{6}A mRNA Methylation Controls Functional Maturation in Neonatal Murine β Cells

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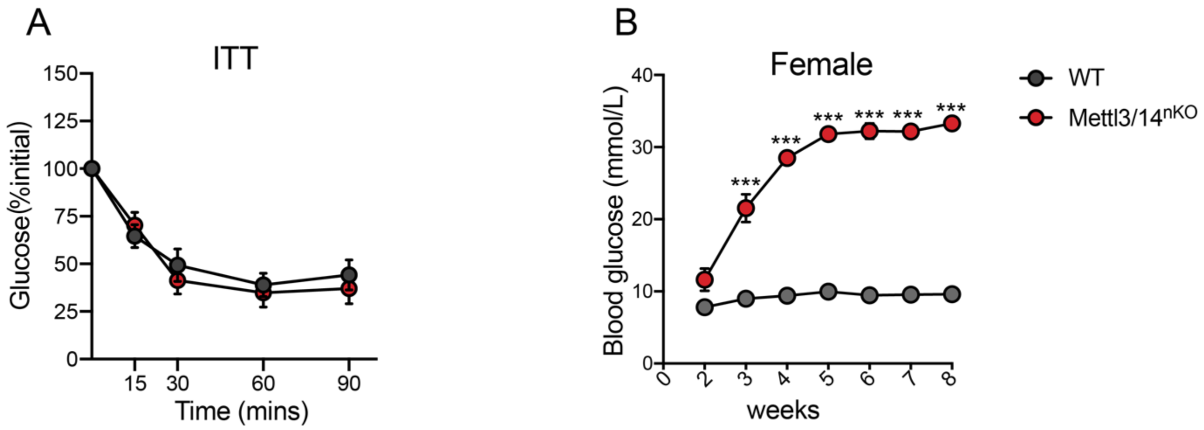
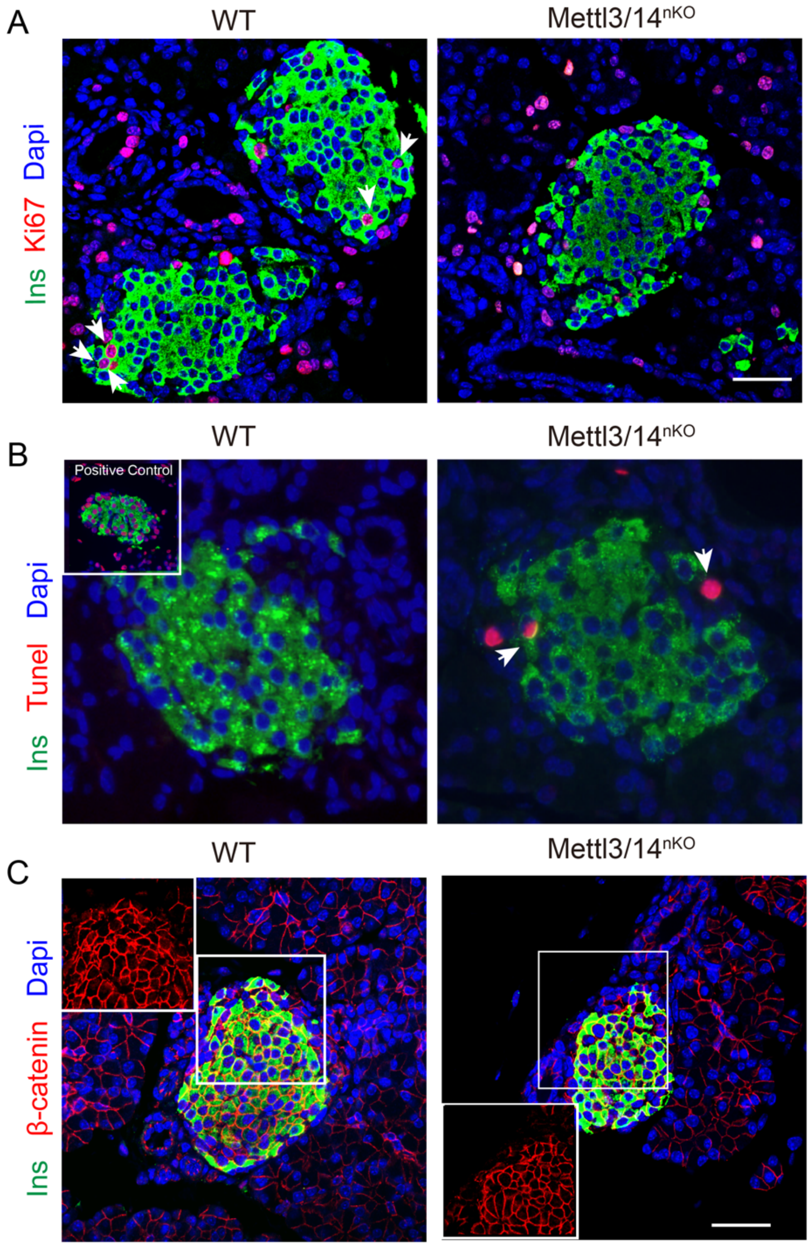


Supplementary Figure 1. Phenotype of *Mettl3/14^{nKO}* mice.

(A). Blood glucose levels of 4-week-old male WT and *Mettl3/14^{nKO}* mice after intraperitoneal injection of insulin (1 IU/kg body weight). (n=5-7)

(B). Random blood glucose of female *Mettl3/14^{nKO}* and WT mice were monitored weekly. (n=3)

Data were presented as mean ± SEM of independent experiment indicated as above, ***p<0.001. Student's t test.



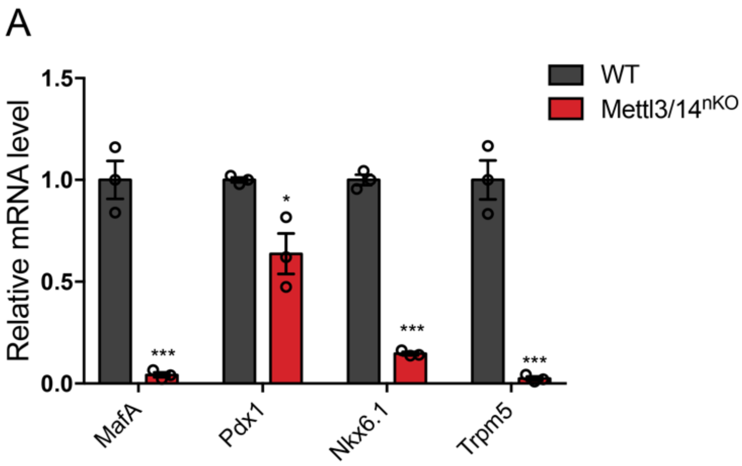
Supplementary Figure 2. Proliferation, apoptosis, cell size of β cells from P14 *Mettl3/14^nKO^* mice.

(A). Representative pancreatic sections immunostained for ki67(red) and insulin (green) in *Mettl3/14^nKO^* and WT pancreas at P14.

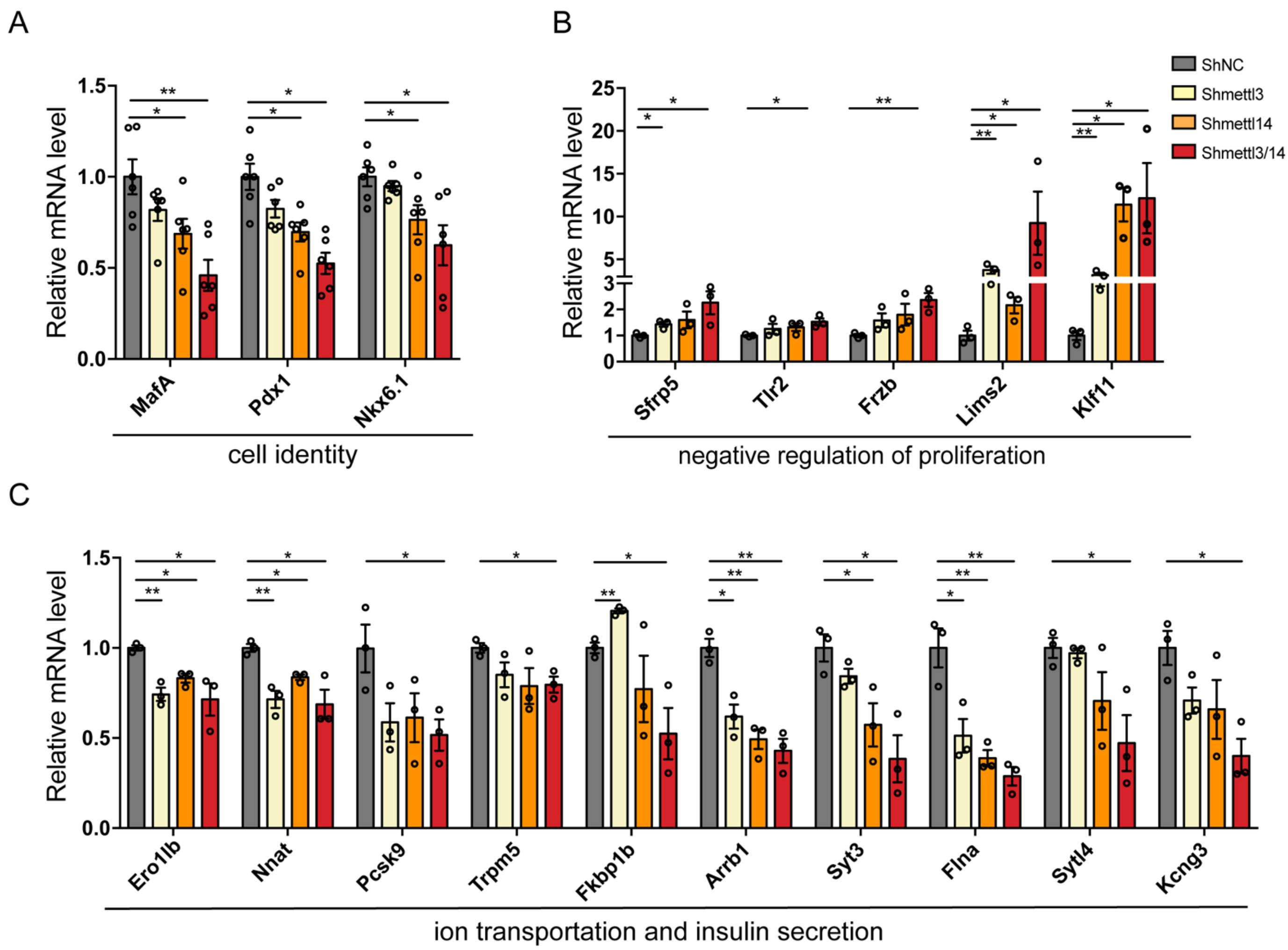
(B). Representative pancreatic sections immunostained for Tunel (red) and insulin (green) in *Mettl3/14^nKO^* and WT at P14. DNase I treatment was used as positive control.

(C). Representative pancreatic sections immunostained for β-catenin (red) and insulin (green) in *Mettl3/14^nKO^* and WT at P14.

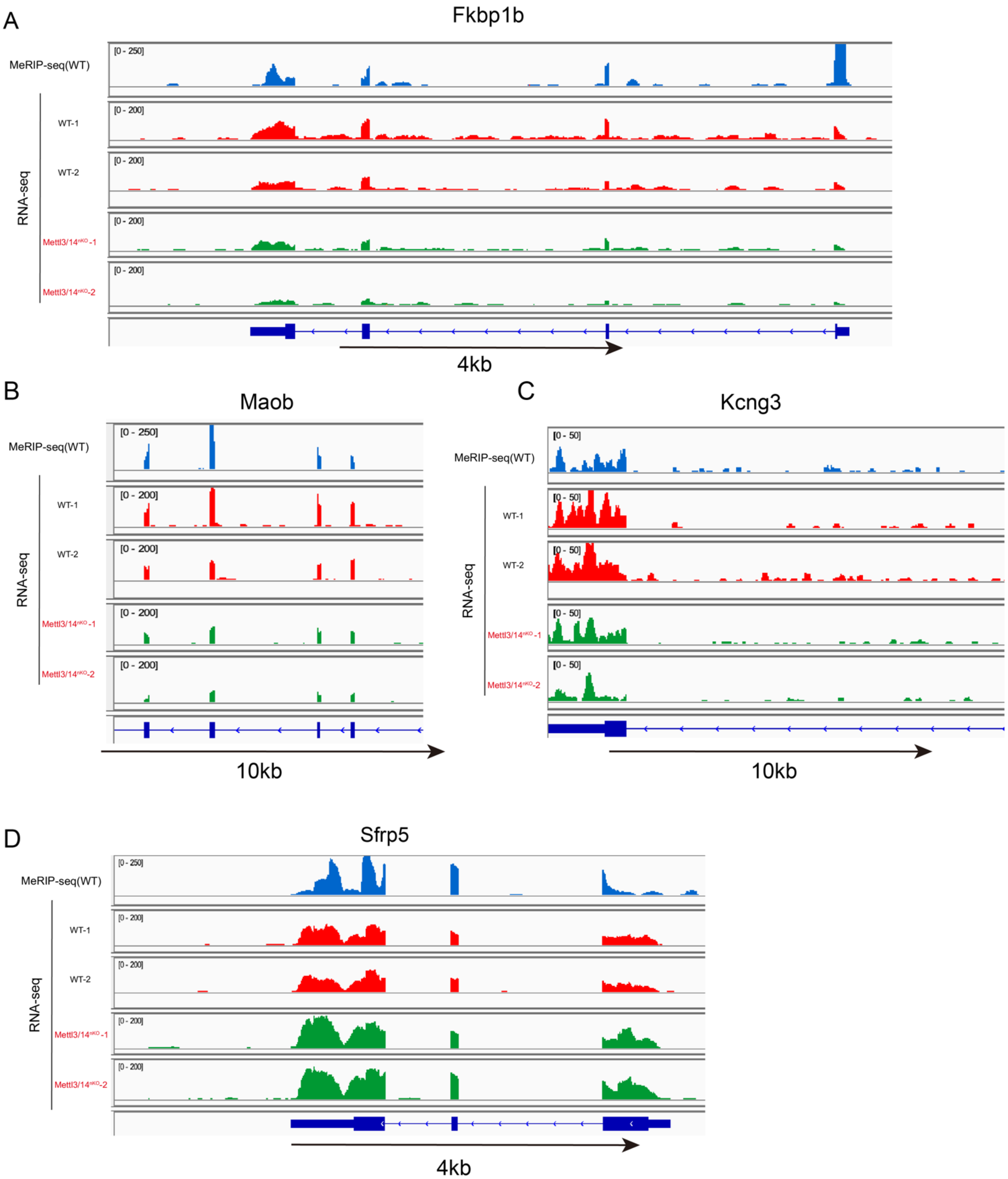
Nuclei were counterstained with Dapi (blue). Scale bars, 20μm.



Supplementary Figure 3. Mettl3/14 regulates critical β cell genes in 8-week *Mettl3/14^{nKO}* islets.
(A). RT-PCR analysis of critical β cell genes in isolated form 8-week *Mettl3/14^{nKO}* and WT islets. (n=3) **p*<0.05, ****p*<0.001. Student's t test.

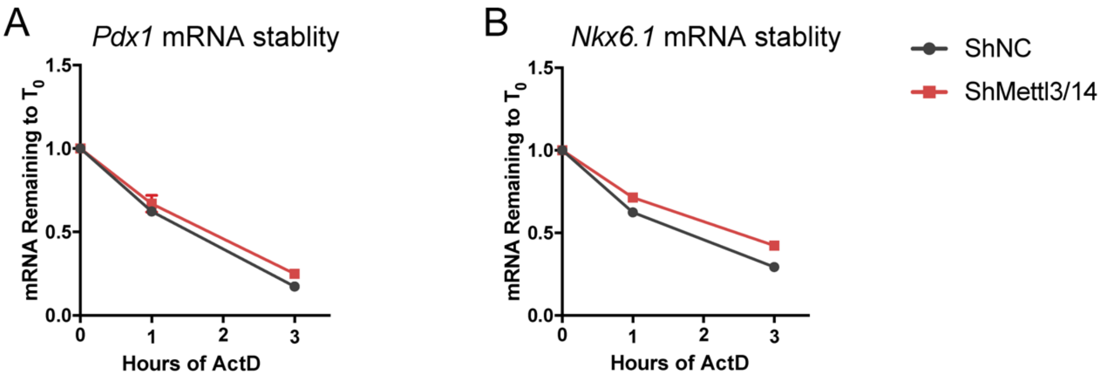


Supplementary Figure 4. Mettl3/14 regulated critical β cell genes in MIN6 cells. (A-C) MIN6 cells were treated with ShMettl3, ShMettl14, ShMettl3/14 or control lentiviruses for 48h, then RT-PCR analysis were performed to examine the expression levels of critical β cell transcription factors (A) and genes involved in proliferation inhibition (B), genes involved in ion transportation and insulin secretion (C) (n=3-6).

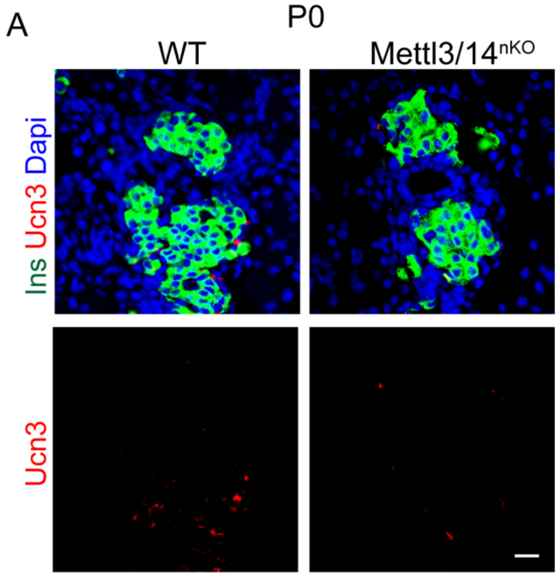


Supplementary Figure 5. Differentially expressed genes with m^{6}A modifications in P14 *Mettl3/14^{nKO}* islets.

(A-D). Integrative Genomics Viewer (IGV) tracks showed RNA-seq reads distribution in Fkbp1b(A), Maob(B), Kcng3(C) and Sfrp5 (D) mRNA of P14 WT and *Mettl3/14^{nKO}* islets (lower panels), and MeRIP-seq reads distribution in mRNA (upper panels).



Supplementary Figure 6. Mettl3/14 does not affect *Pdx1, Nkx6.1* mRNA stability in MIN6 cells. (A, B) The relative amount of *Pdx1*(A) and *Nkx6.1* (B) mRNA in MIN6 cells after 0，1，3h ActD treatment with or without shMettl3/14 were determined. (n=3)



Supplementary Figure 7. Ucn3 was not detected in P0 *Mettl3/14^nKO^* and WT islets. (A) Representative pancreatic sections from P0 WT and *Mettl3/14^nKO^* were co-immunostained for insulin (Ins, green) and Ucn3 (red). Nuclei were counterstained with Dapi (blue). Scale bar: 20μm.

Supplementary Table 1. Clinical Characteristics of ND and T2D individuals.

ND group							
Case ID	Gender (F/M)	Age (years)	BMI (kg/m²)	FBG (mmol/L)	Course of T2D (years)	Antidiabetic therapy	
639059	F	51	24.34	5.59	N/A	/	pancreatic serous microcystadenoma
621219	M	64	23.03	4.58	N/A	/	pancreatic serous microcystadenoma
644731	F	50	18.51	4.97	N/A	/	pancreatic mucinous cystadenoma
640422	M	58	24.62	5.12	N/A	/	pancreatic serous microcystadenoma
648986	F	55	18.51	5.54	N/A	/	pancreatic mucinous cystadenoma
Mean		55.6	21.8	5.16			
SEM		2.5	1.37	0.19			

T2D group							
Case ID	Gender (F/M)	Age (years)	BMI (kg/m²)	FBG (mmol/L)	Course of T2D (years)	Antidiabetic therapy	pathological diagnosis
651833	F	49	26.22	5.79	5	Insulin+Acarbose	pancreatic mucinous cystadenoma
551593	F	58	19.92	6.82	17	Insulin+ Metformin+Pioglitazone	intraductal papillary mucinous neoplasm
605223	F	69	18.83	5.0	10	Metformin	pancreatic serous microcystadenoma
598838	F	46	24.24	5.80	9	Insulin+Metformin+Voglibose	pancreatic serous microcystadenoma
491670	M	71	26.04	5.46	10	Glimepiride	pancreatic serous oligocystic adenoma.
Mean		58.6	23.05	5.77	10.2		
SEM		5.1	1.55	0.30	1.9		

All clinical characteristics of human subjects are summarized.

Supplementary Table 2. RT-PCR primer sequences of target genes.

GENE	SPECIES	FORWARD	REVERSE
Mafa	Mouse	GCTTCAGCAAGGAGGAGGTCAT	TCTCGCTCTCCAGAATGTGCCG
Pdx1	Mouse	TTCCCGAATGGAACCGAGCCTG	TTTTCCTCGGGTTCCGCTGTGT
Nkx6.1	Mouse	TCTGGACAGCAAATCTTCGCCC	ACTTGGTCCTGCGGTTCTGGAA
Maob	Mouse	TACTTGGGGACCGAGTGAAGCT	CCAAAGCAGGTGGAATGGCACT
Trpm5	Mouse	GGTGTTCACACTTCGGCTCATC	CCACAAGCCATACGCTCAGGAA
Sfrp5	Mouse	GAGATGCTGCACTGCCACAAGT	TGCTCCATCTCACACTGGGCAC
Tlr2	Mouse	ACAGCAAGGTCTTCCTGGTTCC	GCTCCCTTACAGGCTGAGTTCT
Frzb	Mouse	CTGCCTCTGTCCTCCACTTACT	CTTACCAAGCCGATCCTTCCAC
Lims2	Mouse	CCTTGTCACGACAAGATGGGCA	CAGGAATGGCTTCTCACACTTGG
Klf11	Mouse	GCTCATCTTCGCACTCACACAG	TCTTCTCTCCCGTGTGAGTCCT
Ero1lb	Mouse	CTGGATGACTGTGAGCAGGCTA	TCACAAAAGTGGTCCTGCGAATC
Nnat	Mouse	GTGGTGGAGGAAGAGGGTTAAG	CACATTTTGGGGAGGGCTTTCG
Pcsk9	Mouse	ATGGCACCAGACAGAGGAAGAC	CACGCTGTTGAAGTCGGTGATG
Fkbp1b	Mouse	GGCAAACAGGAAGTCATCAAAGG	GGTAGCTCCATAGGCCACATCA
Arrb1	Mouse	CTTCTGTGCTGAGAACCTGGAG	GGAACTGTCTGGTAGTCTCAGC
Syt3	Mouse	TCCCAGCAAAGGACTCCAATGG	GGCACCGAGAACTGAAACGTCT
Flna	Mouse	CAGCAAGCTACAGGTGGAACCT	TCAGTGGTTGCCTCTCGGAAGA
Sytl14	Mouse	GTGGCATCTTGGGAGACAGAAG	ACTTCACCAGGTGGTCAATGTCC
Kcng3	Mouse	TCTGGATGACCGGAGCAGGTAC	GGTCTCTTGACAAACTCACACTTG
Mettl3	Mouse	CAGTGCTACAGGATGACGGCTT	CCGTCCTAATGATGCGCTGCAG
Mettl14	Mouse	AGAGTGCGGATAGCATTGGTGC	CTCCTTCATCCAGACACTTCCG
Gapdh	Mouse	CATGTTCCAGTATGACTCCACTC	GGCCTCACCCCATTTGATGT