"Supplemental material"

MicroRNA-155 controls CD8$^+$ T cell responses by regulating interferon signaling

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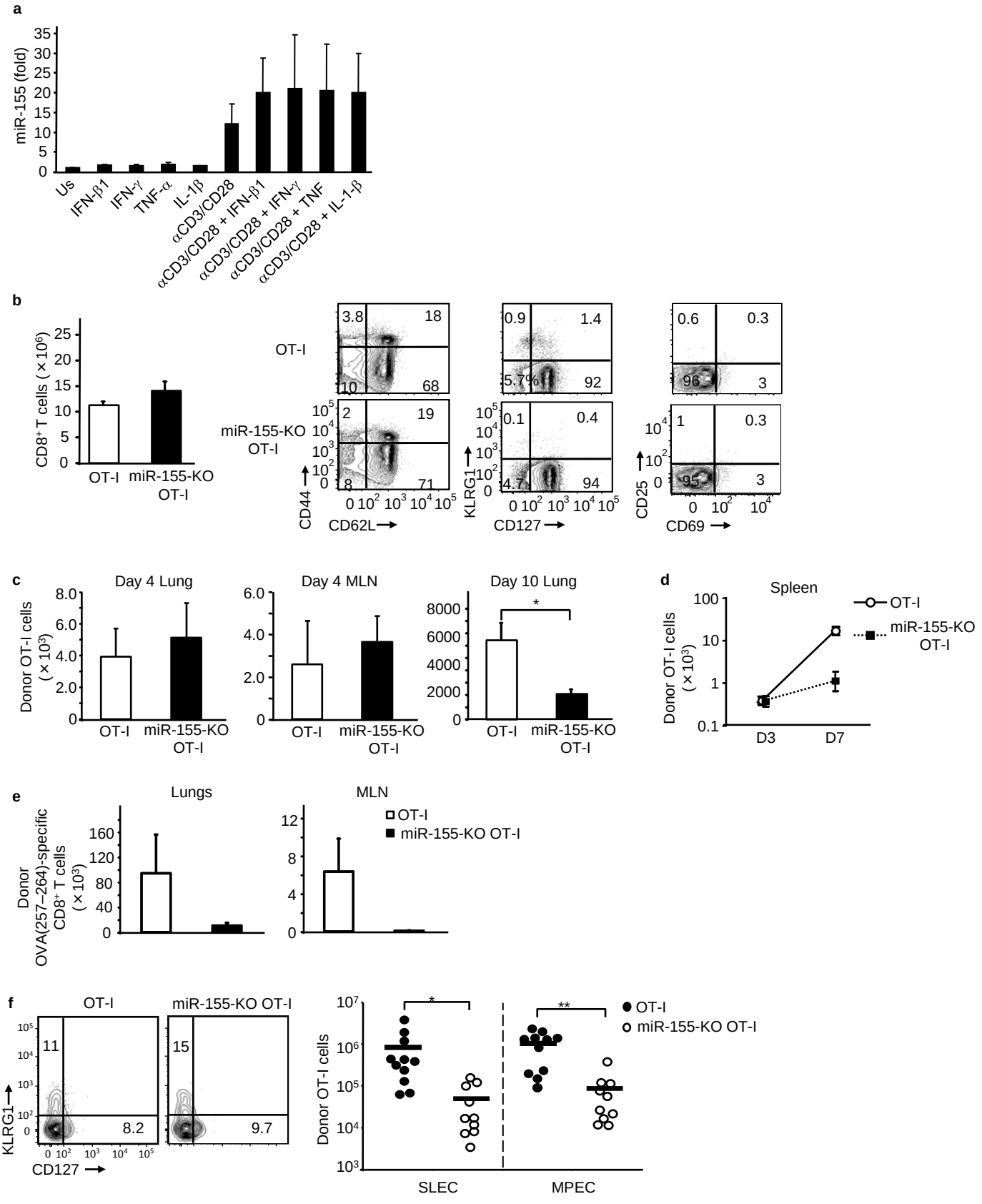
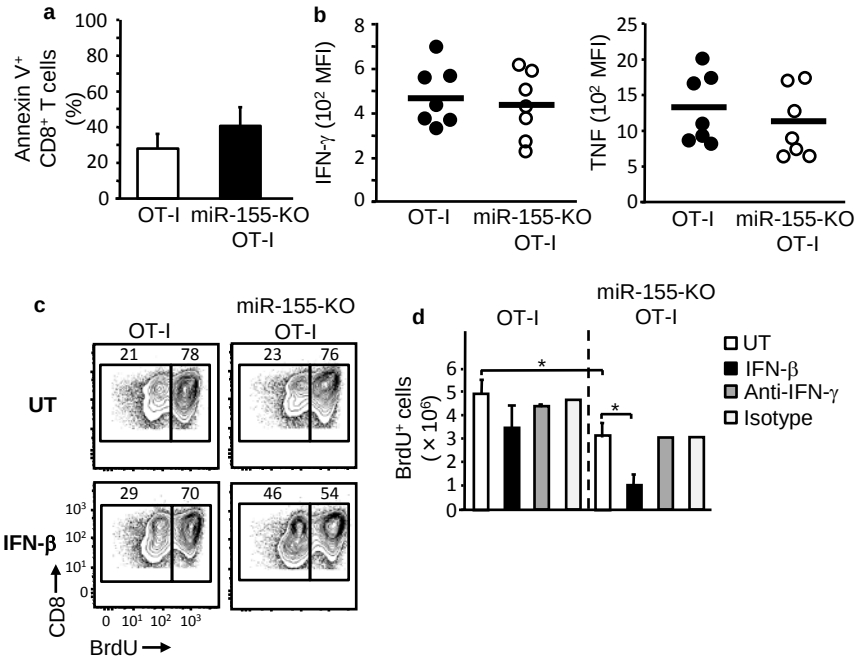
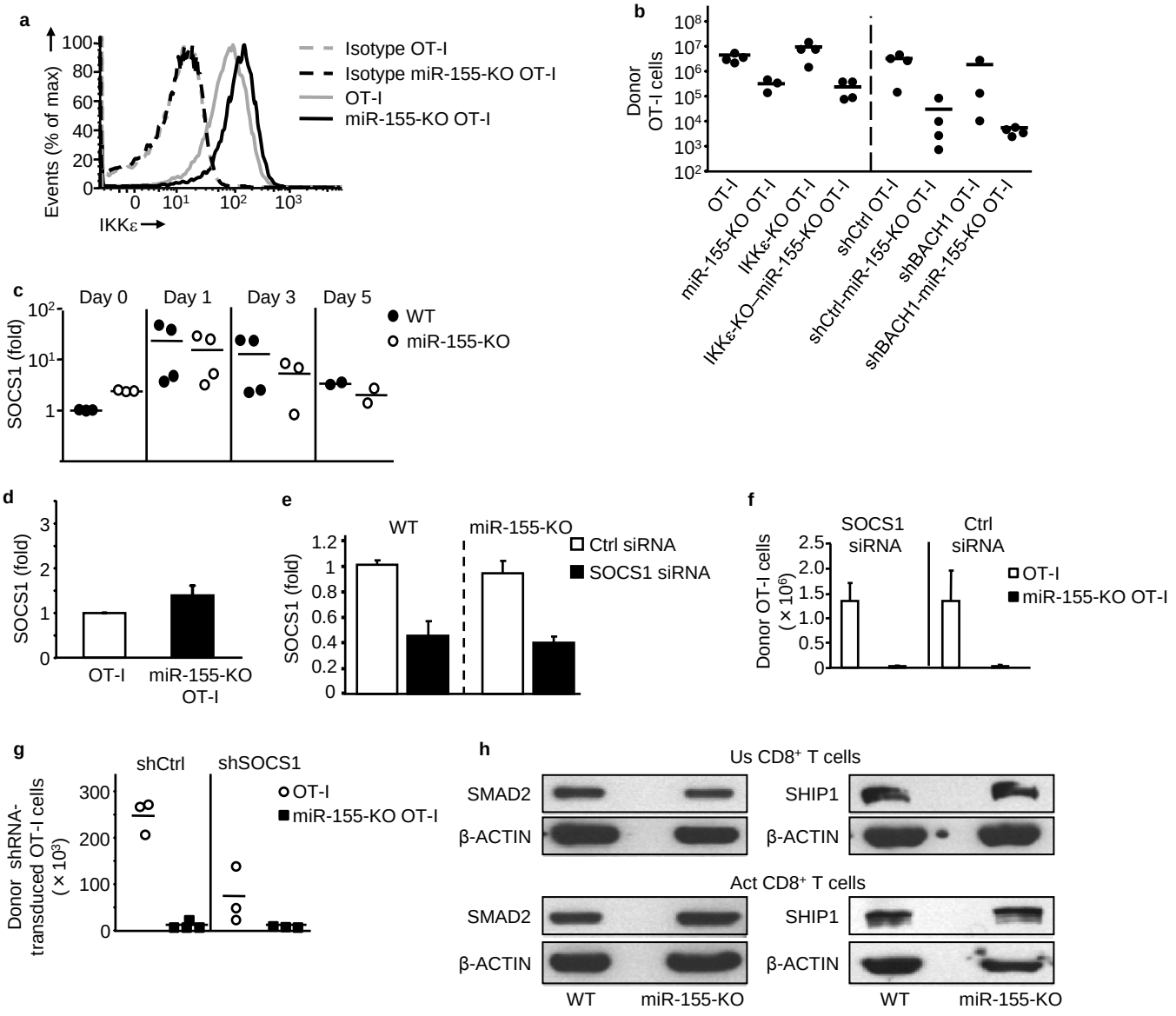

a
miR-155 (fold)
Us
IFN-β1
IFN-γ
TNF-α
IL-1β
αCD3/CD28
αCD3/CD28 + IFN-β1
αCD3/CD28 + IFN-γ
αCD3/CD28 + TNF
αCD3/CD28 + IL-1β
b
CD8+ T cells (×10⁶)
OT-I
miR-155-KO OT-I
CD44
CD62L
KLRG1
CD127
CD25
CD69
c
Day 4 Lung
Day 4 MLN
Day 10 Lung
Donor OT-I cells (×10³)
d
Spleen
Donor OT-I cells (×10³)
D3
D7
e
Lungs
MLN
Donor OVA(257–264)-specific CD8+ T cells (×10³)
f
SLEC
MPEC
Donor OT-I cells

Supplementary Figure 1. MiR-155 induction by cytokines, and phenotype and tissue distribution of miR-155-KO OT-I cells**. (a)** Treatment of unstimulated naive $CD8^+$ T cells with 10ng/ml of TNF, IFN-γ, IL-1-β or 1000U/ml IFN-β for 24h does not increase miR-155 levels. These cytokines only modestly increased further these levels in activated $CD8^+$ T cells. Pooled data from 5 experiments shown. **(b)** $CD8^+$ T cell numbers, naive/memory phenotype and activation status of splenic $CD8^+$ T cells in uninfected OT-I and miR-155-KO OT-I mice. Representative flow cytometric plots shown from 3 experiments performed. Numbers in plots indicate percent of $CD8^+$ T cells. **(c)** Day 4 lung and MLN miR-155-KO OT-I cell numbers after adoptive transfer into WSN-OVA infected animals. Day 10 lung miR-155-KO OT-I cells shown for comparison. In order to visualize cells early, 5×10^5 cells were transferred. Data from 2 experiments and n=6. *P<0.05 (Student's t-test). **(d)** Days 3 and 7 splenic miR-155-KO OT-I cell numbers after adoptive transfer into LM-OVA infected animals. In order to visualize cells early, 5×10^5 cells were transferred. Data from 2 experiments and n=5-6. **(e)** Day 60 memory miR-155-KO OT-I and wild-type OT-I in the MLN and lungs of mice that received adoptive transfers of wild type and miR-155-KO $CD8^+$ T cells and were infected with WSN-OVA influenza virus. Memory in spleens is shown in Figure 1. Bars show mean ± SEM and are from 2 independent experiments (n=5). **(f)** MiR-155 deficiency does not selectively affect SLEC or MPEC during the primary response. Both SLEC and MPEC numbers are reduced on day 10 of infection. Left: Representative flow cytometric plots of day 10 shown. Numbers in plots indicate percent of $CD8^+$ T cells. Right: Data from 3 experiments n=9-11. *P<0.05, **P<0.003 (Student's t-test).



Supplementary Figure 2. Apoptosis, cytokine production and IFN sensitivity of miR-155-KO CD8$^+$ T cells. **(a)** *In vitro* apoptosis of miR-155-KO OT-I and OT-I cells stimulated with peptide loaded irradiated splenocytes. Day 4 cultures shown. Data from 5 experiments and *n*=5 per group. **(b)** MFI for IFN-γ and TNF intracellular stain after *ex vivo* peptide stimulation of miR-155-KO OT-I and OT-I cells. Day 10 post-infection shown. Data from 3 experiments and *n*=7. **(c)** and **(d)** miR-155-KO CD8$^+$ T cells are sensitive to Type I IFN-mediated inhibition. miR-155-KO and wild-type OT-I cells were stimulated with OVA(257–264)-pulsed irradiated splenocytes and cultured with media containing IL-7, IL-15 and IL-2 for 6 days. During the last 3 days cell were treated ± IFN-β or left untreated (UT). BrdU was added during the last 20h. **(c)** Representative flow cytometric plots showing reduced BrdU incorporation by miR-155-KO OT-I CD8$^+$ T cells in the presence of IFN-β. Numbers in plots indicate percent of CD8$^+$ T cells. **(d)** Bar graph depicting mean ± SEM reduction in number of live BrdU$^+$ miR-155-KO OT-I CD8$^+$ T cells with addition of IFN-β. Data from 3 independent experiments (*n*=3 per group). **P*< 0.008 (Student's t-test).


a
Events (% of max)
IKKε
Isotype OT-I
Isotype miR-155-KO OT-I
OT-I
miR-155-KO OT-I
b
Donor OT-I cells
OT-I
miR-155-KO OT-I
IKKε-KO OT-I
IKKε-KO–miR-155-KO OT-I
shCtrl OT-I
shCtrl-miR-155-KO OT-I
shBACH1 OT-I
shBACH1-miR-155-KO OT-I
c
SOCS1 (fold)
Day 0
Day 1
Day 3
Day 5
WT
miR-155-KO
d
SOCS1 (fold)
OT-I
miR-155-KO OT-I
e
SOCS1 (fold)
WT
miR-155-KO
Ctrl siRNA
SOCS1 siRNA
f
Donor OT-I cells (× 10⁶)
SOCS1 siRNA
Ctrl siRNA
OT-I
miR-155-KO OT-I
g
Donor shRNA-transduced OT-I cells (× 10³)
shCtrl
shSOCS1
OT-I
miR-155-KO OT-I
h
Us CD8⁺ T cells
SMAD2
β-ACTIN
SHIP1
β-ACTIN
Act CD8⁺ T cells
SMAD2
β-ACTIN
SHIP1
β-ACTIN
WT
miR-155-KO
WT
miR-155-KO

Supplementary Figure 3. IKKε, Bach1, SOCS-1, SHIP-1 and SMAD2 do not mediate the defect of miR-155-KO $CD8^+$ T cells. **(a)** Representative histogram showing increased IKKε protein expression in activated miR-155-KO $CD8^+$ T cells. Data representative of 4 independent experiments shown. **(b)** IKKε deficiency and Bach1 shRNA retroviral knockdown in miR-155-KO OT-I cells does not restore responses. Dot plots depict numbers of donor OVA(257–264)-specific $CD8^+$ T cells in the lungs day 10 post-infection. Recipient mice were transferred with either miR-155-KO OT-I, IKKε-KO OT-I, miR-155-KO IKKε-KO OT-I or wild-type OT-I cells, and then infected one day later with WSN-OVA. For Bach1 knockdowns, miR-155-KO OT-I or control OT-I cells were transduced with retroviruses expressing Bach1 shRNA or control shRNA and 48h later intravenously transferred into recipient mice which were then infected with WSN-OVA. **(c)** SOCS-1 mRNA levels are not increased in activated purified miR-155-KO compared to wild-type $CD8^+$ T cells. RT-PCR data from 2 experiments shown. **(d)** Activated miR-155-KO OT-I cells do not express significantly more SOCS1 mRNA than wild-type OT-I. miR-155-KO and wild-type OT-I cells were stimulated with OVA(257–264)-pulsed irradiated splenocytes for 4 days and SOCS1 measured by RT-PCR. **(e)** SOCS-1 siRNA reduces SOCS-1 mRNA levels by ~60% in purified OT-I and miR-155-KO OT-I cells after 72h. Non-targeting control siRNA had no effect on SOCS-1 levels. **(f)** SOCS-1 siRNA transfection of miR-155-KO OT-I cells does not restore their *in vivo* expansion. Numbers of donor OVA(257–264)-specific $CD8^+$ T cells in lungs shown. Cells were transfected for 72h and then adoptively transfer into congenic hosts that were infected with WSN-OVA influenza virus. Data are from 2 independent experiments and n=6 mice per group. **(g)** Transduction of miR-155-KO OT-I cells with retroviruses expressing SOCS1 shRNA failed to restore their expansion. Cells were retrovirally transduced for 48h, adoptively transferred and infected as described above (n=3-4 mice per group). **(h)** Protein levels of SHIP1 and SMAD2 by immunoblotting in unstimulated (Us) and activated (Act) miR-155-KO $CD8^+$ T cells did not differ from wild-types. $CD8^+$ T cells were activated with anti-CD3 and anti-CD28 antibodies. Blots are representative of 2 independent experiments (n=2 per group).

Supplementary Table 1: Differentially expressed genes between *in vitro* activated wild-type and miR-155-KO $CD8^+$ T cells, as identified by SAM analysis (delta=1.6, FDR=0.082).

Downregulated				**Upregulated**			
>2.00 - fold							
Hist1h2ab				*Fbxw10*	*Rtp4*	*Trim16*	*Klhdc1*
Hist1h2bb				*Slfn5*	*Rundc3b*	*Xaf1*	*Tas2r143*
				Dhrs3			
1.99–1.50 - fold							
Slc16a3	*Hist1h4h*			*Ifit3*	*Gstt1*	*Art2a*	*OTTMUSG00000005523*
Gm5452	*Hist1h4i*			*Gm5970*	*Sgms1*	*Trib2*	*Lyst*
Tk1-1	*Fanca*			*Gm12258*	*Zbp1*	*Slfn9*	*Irf7*
Mrpl39	*Dsc1*			*Nr1d2*	*Ect2l-11*	*5830416P10Rik*	*Ppp3cc-1*
Nuf2	*Hist1h2bh*			*Arhgap26*	*Rragd*	*Slco3a1*	*Tspan14*
	Kif15			*Acpp*	*H2-T24*	*N4bp1*	*Gm6907*
				Fam26f	*9330175E14Rik*	*Oasl2*	
				D730003I15Rik	*Gbp3*		
1.49–1.20 - fold							
Kif11	*Pebp1-2*	*2210021J22Rik*	*Mcm5*	*Gvin1-2*	*Itga6*	*Lmbrd1*	*Ifit2*
Ppic	*Zmiz1*	*Mrps23*	*Pla2g15*	*Tbc1d23*	*Zfp420*	*Eif1-1*	*Uvrag*
Ak3l1-1	*Wdr90*	*Itgb3bp-1*	*Snrpe*	*Cirbp*	*2810021J22Rik*	*Brwd1*	*Nsun4*
Fam84a	*Snrpd2-2*	*Unc13a*	*Gm8944-1*	*Impg2*	*Ccpg1*	*Zpbp2*	*Cd151-1*
Prelid2	*Dtl*	*Tmsb10*	*Gm8944*	*Sgk3*	*6430601O08Rik*	*Sdcbp*	*1700034F02Rik*
Acsl6	*Pgk1-1*	*Ndufaf2*	*Gjb3*	*Icosl*	*Tgtp-1*	*Myo9a-8*	*Poli*
Cd160	*Pgk1-2*	*Snrpd2-4*	*Eif3i*	*Fam46c*	*B430306N03Rik*	*Gbp5*	*Hectd3*
Slc2a3	*Pgk1*	*Hn1l-2*	*Alkbh7*	*Ifngr1*	*Tgtp*	*Trappc2*	*Dnajc7*
Fam111a	*Eif4a3-2*	*Snrpd2*	*Slc2a1*	*Trat1*	*Arid4a*	*Kidins220*	*Irf1*
Ankrd37	*Calm3*	*Serf1-1*	*Hprt1*	*Dennd4c*	*Ifngr2*	*AI606181*	*Arl15*
Gm4924	*Tbl3*	*Acaca-1*	*1700001E04Rik*	*1700109H08Rik*	*Parp10*	*0610040B10Rik*	*Rab33b*
Hnrpll	*3110082I17Rik*	*E2f1*	*Nucb1*	*Stat2*	*D14Ertd668e*	*Cript*	*Bat5*
Tk1	*Cox4i1*	*1700065D16Rik*	*4930422G04Rik*	*Nqo1*	*Fam55c*	*Dtx3l*	*Mobkl3*
Pfkl	*Ankle1*	*Syce2*	*Angptl2*	*Ube2d1*	*1700102P08Rik*	*Mppe1*	*Kdm2a-1*
H2afx	*Gm5050*	*Cenpm*	*Erbb3*	*A530023O14Rik*	*Rps6ka5*	*Wdr37*	*Zfp263*
Ccdc50	*C1qbp*	*Cad*	*Cotl1*	*Il7r*	*Zbtb16*	*Prdm2*	*Picalm*
Anxa6	*Tomm7*	*Mtif3*	*Rgs1*	*Sft2d2*	*Serf2*	*Csnk1g2*	*9530048O09Rik*
E2f3-1	*Lsm3*	*Slc9a5*	*Ndufa8*	*9530009G21Rik*	*A530017D24Rik*	*Gm5039-2*	*Ubl3*
Ngfrap1	*Dnmt3a*	*D830030K20Rik-5*	*Mcm7*	*5930434B04Rik-1*	*AU042671-2*	*Gpr21*	*Map3k7ip2*
Fam162a	*Nup155*	*D830030K20Rik-2*	*Rnf26-1*	*Inpp4b*	*L1cam*	*Parp9*	*BC030336*
Mogs	*Prmt1*	*RP23-38E20.1-2*	*Med9*	*Jarid2*	*Ctns*	*Mef2a*	*Kbtbd3*
Prim1	*Pole*	*Pgam1-1*	*Ubash3a*	*Ncoa3*	*Sla2*	*Gm9964*	*Gm10374*
Smyd2	*Tmsb10-1*	*Pgam1*	*Agpat6*	*4930453N24Rik*	*Tgfbr1*	*Vps13c*	*Dapp1*
Ncapd2	*Dapl1*	*Nup85*	*Tubg2*	*Rb1cc1-1*	*Sike1*	*Eef2k*	*2010111I01Rik*
Gm12260	*5330426P16Rik*	*Gm8824*	*Atp5e*	*Msi2*	*Ahr*	*Gclc*	*Ccdc130*
Snrpd2-3	*Shmt1*	*Snrpd2-1*	*Chaf1b*	*Tnfaip3*	*Nfe2l2*	*Tspo*	*Rusc1*
Prmt1	*Pgm2*	*Gdf11*	*Tmsb10-1*	*Gimap9*	*D130062J21Rik*	*A230046K03Rik*	*Cdk3*
Pole	*Nrip3*	*Tctex1d2*	*Dapl1*	*Arhgef12*	*Kbtbd2*	*Sgcb*	*Mlycd*
	ENSMUSG00000079376	*9530058B02Rik*		*Tmx4*	*Vps54*	*Kras*	*Gpr146*
				Smad5	*Prickle3*	*Zfp512*	*Plk3*
				Smcr8	*Inpp5d*	*Klf7*	
				Nin	*Aff4*	*Pde4b*	