

### *Supplementary material*

Supplementary Figure S1: Differential protein expression of EZH2 and MECP2 in Ts65Dn hippocampus compared with euploid. Western blots with proteins extracted from hippocampal tissue (n=4 for each group) of Ts65D (T) and euploid (E) mice, hybridized with either anti-EZH2 or anti-MECP2 antibodies. For quantitative analysis of Western blots, optical intensity of bands was evaluated with MultiGuage software. The optical intensity data for each Western blot band was divided by its respective GAPDH intensity to normalize for gel loading variation. (B) Relative intensity averaged from Ts65Dn and euploid control Western blot bands after normalization with GAPDH  $\pm$  SEM for EZH2 ( $p=0.07$ ) and MECP2 ( $p=0.06$ ).

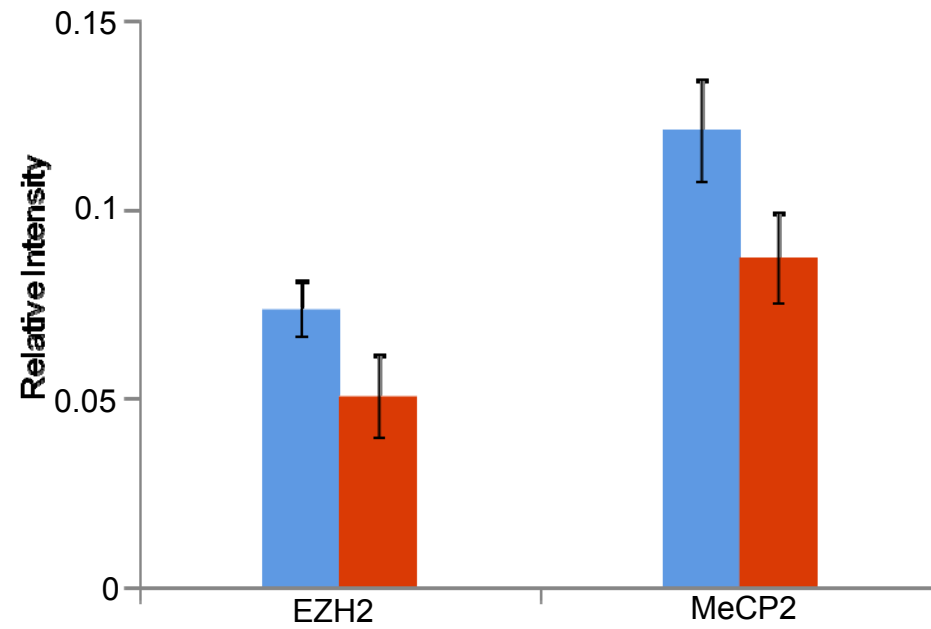
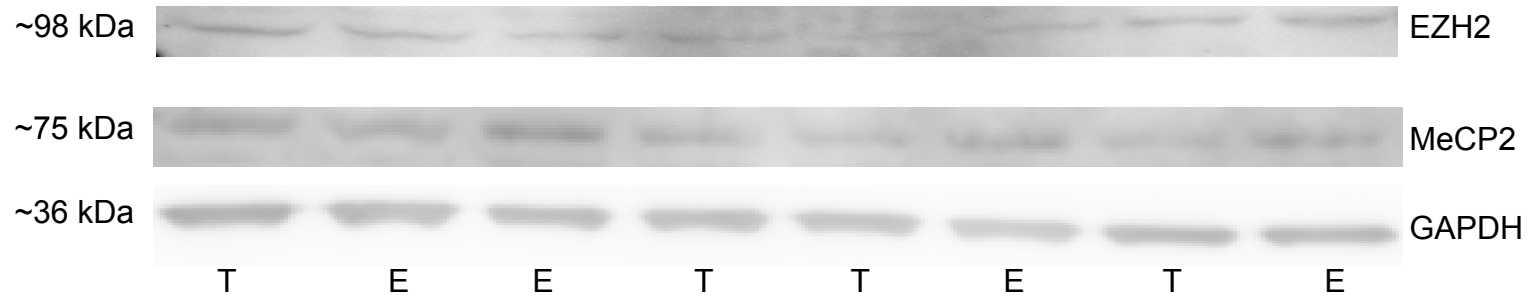
Supplementary Figure S2: Histopathological analysis of Ts65Dn and euploid mouse lungs (A) Representative example of euploid pulmonary vessel, wall measurement of 13 samples was  $\sim 42 \mu\text{m}$  in width, measured from the endothelium through the tunica intima, tunica media and the adventitia. Tissue surrounding the vessel is normal alveolar septae and within the lumen of the vessel are myriad erythrocyte, H&E 400x. (B) Representative example of Ts65Dn pulmonary vessel, wall measurement of all 10 samples was  $\sim 41.5 \mu\text{m}$  in width, measured as for euploid samples, H&E 400x. (C) Representative example of euploid pulmonary vessel, with the vessel wall composed of the endothelium overlying a small amount of fibrous connective tissue of the tunica intima, bounded by the smooth muscle of the tunica media (red) and merging with the fibrous outer layer of the adventitia (blue), Masson's Trichrome, 400x. (D) Representative example of Ts65Dn pulmonary vessel, with histological features identical to euploid samples, with no increased amounts of collagen deposition or hypertrophy of leiomyocytes noted, Masson's Trichrome, 400x.

Supplementary Table 1: List of miRNA genes with fold change and statistical significance of differential expression. Values are reported for the three groups: (a) miRNA with significant changes in expression level between trisomy and euploid groups of mice ( $p < 0.05$ ), (b) overexpressed miRNAs in trisomy mice (Fold Change  $\geq 2$ ), and (c) overexpressed miRNAs in euploid mice (Fold Change  $\geq 2$ ). Undetermined fold change values refer to those situations when the miRNA expression could not be detected in one of the biological groups.

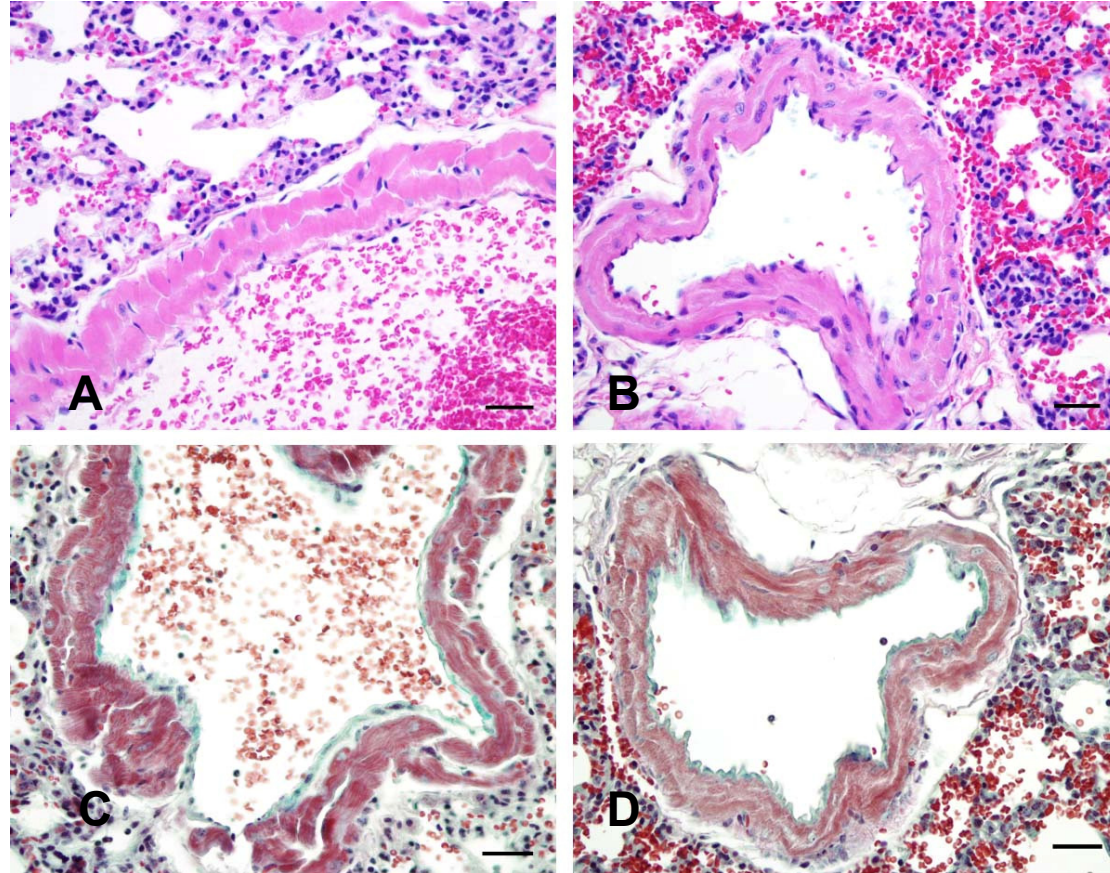
Supplementary Table 2: *In silico* gene targets of 12 differentially expressed miRNAs selected from TaqMan array and individual assay results. Targets were selected based on an in-house developed computer program that utilizes publicly available data from the following five resources: (i) [microrna.org](http://www.microrna.org/) [http://www.microrna.org/], (ii) [mirDB](http://mirdb.org/miRDB/) [http://mirdb.org/miRDB/], (iii) [RNA22](http://cbcsrv.watson.ibm.com/) [http://cbcsrv.watson.ibm.com/] and (iv) [TargetScan](http://www.targetscan.org/) [http://www.targetscan.org/] (v) [microT](http://diana.cslab.ece.ntua.gr/microT/) (http://diana.cslab.ece.ntua.gr/microT/). We selected gene targets for miRNA only when they were common predictions from all five resources mentioned here. A conservative search strategy was employed to reduce SNR (signal to noise ratio) by selecting a miTG score cutoff of 13 based on predictions by the microT server. It should be noted that some genes are targeted by more than one miRNAs. The genes involved in synaptic plasticity and neuroglia signaling pathway represented in Fig. 7 have been highlighted.

Supplementary Table 3: miRNAs expression in Ts65Dn lung tissue. Relative expression levels in 5 trisomy samples  $\pm$  SEM is shown with respect to 5 euploid controls. No detectable expression was observed for miR-802 in lung tissue of neither trisomy nor control mice.

Supplementary Table 4: MiRNA sequences used in individual miRNA assays and primers sequence used in real-time PCR experiments to quantify mRNAs expression.



**Supplementary Figure S1**



**Supplementary Figure S2**

**Supplementary Table 1:** List of miRNA genes with fold change and statistical significance of differential expression.

Not detectable in Euploid samples(NDBEu)

Not detectable in Ts65Dn samples(NDBTs)

<b>Genes</b>	<b>p-value</b>	<b>Fold Change</b>	<b>Direction</b>
<b>(a) p &lt;= 0.05</b>			
mmu-miR-7b	0.002	1.68	↓
mmu-miR-210	0.006	2.01	↑
mmu-miR-181c	0.017	1.48	↓
mmu-miR-155	0.022	2.21	↑
mmu-miR-214	0.022	3.33	↑
mmu-miR-138	0.023	1.54	↑
mmu-miR-423-5p	0.024	>>10 (NDBEu)	↑
mmu-miR-369-5p	0.024	1.36	↓
mmu-miR-361	0.025	1.31	↓
mmu-miR-135b	0.041	1.70	↓
mmu-miR-223	0.049	2.40	↑
<b>(b) overexpressed in trisomy (Fold Change &gt;= 2)</b>			
mmu-miR-423-5p	0.024	>>10 (NDBEu)	↑
mmu-miR-296-3p	0.056	>>10 (NDBEu)	↑
rno-miR-343	0.169	5.33	↑
mmu-miR-743a	0.330	3.67	↑
mmu-miR-680	0.355	3.48	↑
mmu-miR-214	0.022	3.33	↑
rno-miR-598-5p	0.213	3.10	↑
mmu-miR-449b	0.385	3.07	↑
rno-miR-409-5p	0.262	2.62	↑
mmu-miR-504	0.301	2.43	↑
mmu-miR-223	0.049	2.40	↑
mmu-miR-490	0.479	2.39	↑
mmu-miR-467d	0.424	2.23	↑
mmu-miR-155	0.022	2.21	↑
mmu-miR-325	0.440	2.21	↑
mmu-miR-210	0.006	2.01	↑

**(c) overexpressed miRNAs in euploid mice (Fold Change  $\geq 2$ )**

mmu-miR-466h	0.191	>>10 (NDBTs)	↓
rno-miR-466b	0.158	>>10 (NDBTs)	↓
mmu-miR-450b-5p	0.228	>>10 (NDBTs)	↓
mmu-miR-590-5p	0.362	>>10 (NDBTs)	↓
mmu-miR-540-5p	0.262	4.73	↓
rno-miR-207	0.451	3.60	↓
mmu-miR-200b	0.166	3.59	↓
mmu-miR-582-3p	0.242	3.45	↓
mmu-miR-542-5p	0.206	3.10	↓
rno-miR-344-3p	0.538	3.03	↓
mmu-miR-182	0.302	2.99	↓
mmu-miR-200a	0.235	2.99	↓
mmu-miR-211	0.367	2.73	↓
mmu-miR-142-5p	0.095	2.35	↓
mmu-miR-682	0.117	2.29	↓
mmu-miR-380-3p	0.293	2.18	↓
mmu-miR-503	0.435	2.11	↓
mmu-miR-466h	0.191	>>10 (NDBTs)	↓

Gene Symbol	miRNA ID	Gene Function
1700049G17Rik	mmu-miR-181c	RIKEN cDNA 1700049G17 gene
2210418O10Rik	mmu-miR-181c	RIKEN cDNA 2210418O10 gene
2310067B10Rik	mmu-miR-181c	RIKEN cDNA 2310067B10 gene
2700078E11Rik	mmu-miR-155	RIKEN cDNA 2700078E11 gene
2810046L04Rik	mmu-miR-181c	RIKEN cDNA 2810046L04 gene
2810046L04Rik	mmu-miR-214	RIKEN cDNA 2810046L04 gene
4921524J17Rik	mmu-miR-181c	RIKEN cDNA 4921524J17 gene
4933426M11Rik	mmu-miR-214	RIKEN cDNA 4933426M11 gene
4933433P14Rik	mmu-miR-181c	RIKEN cDNA 4933433P14 gene
A930001N09Rik	mmu-miR-181c	RIKEN cDNA A930001N09 gene
Aak1	mmu-miR-361	AP2 associated kinase 1
Abl2	mmu-miR-223	v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)
Adamts1	mmu-miR-181c	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1
Adcy1	mmu-miR-181c	adenylate cyclase 1
AF064781	mmu-miR-181c	zinc fingerprotein 619
AI314180	mmu-miR-135b	expressed sequence AI314180
<b>Akt3</b>	<b>mmu-miR-181c</b>	<b>thymoma viral proto-oncogene 3</b>
Ank3	mmu-miR-135b	ankyrin 3, epithelial
Ankrd12	mmu-miR-7b	ankyrin repeat domain 12
Ankrd44	mmu-miR-138	ankyrin repeat domain 44
Ankrd50	mmu-miR-181c	ankyrin repeat domain 50
Antxr2	mmu-miR-181c	anthrax toxin receptor 2
Api5	mmu-miR-181c	apoptosis inhibitor 5
Arf6	mmu-miR-181c	ADP-ribosylation factor 6
Arhgef7	mmu-miR-181c	Rho guanine nucleotide exchange factor (GEF7)
Arid4a	mmu-miR-7b	AT rich interactive domain 4A (RBP1-like)
Arl5a	mmu-miR-181c	ADP-ribosylation factor-like 5A
Asxl1	mmu-miR-7b	additional sex combs like 1 (Drosophila)
Atf2	mmu-miR-181c	activating transcription factor 2
Atg14	mmu-miR-135b	VATG14 autophagy related 14 homolog (S. cerevisiae)
Atg5	mmu-miR-181c	autophagy-related 5 (yeast)
Atp1b1	mmu-miR-181c	ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, beta 1 polypeptide
Atp8a1	mmu-miR-135b	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1
Atxn1l	mmu-miR-135b	ataxin 1-like
Bach1	mmu-miR-135b	BTB and CNC homology 1
Bach2	mmu-miR-181c	BTB and CNC homology 2
Bai3	mmu-miR-181c	brain-specific angiogenesis inhibitor 3

Baz2b	mmu-miR-181c	bromodomain adjacent to zinc finger domain, 2B
Bcl6b	mmu-miR-181c	B-cell CLL/lymphoma 6, member B
Bcor1	mmu-miR-361	BCL6 co-repressor-like 1
Birc6	mmu-miR-181c	baculoviral IAP repeat-containing 6
Brdt	mmu-miR-214	bromodomain, testis-specific
Brpf3	mmu-miR-223	bromodomain and PHD finger containing, 3
Brwd1	mmu-miR-181c	bromodomain and WD repeat domain containing 1
Bsn	mmu-miR-135b	bassoon
Btbd3	mmu-miR-181c	BTB (POZ) domain containing 3
C77370	mmu-miR-181c	expressed sequence C77370
Cacna1e	mmu-miR-135b	calcium channel, voltage-dependent, R type, alpha 1E subunit
Cacnb4	mmu-miR-155	calcium channel, voltage-dependent, beta 4 subunit
Calb1	mmu-miR-181c	calbindin 1
Camk1g	mmu-miR-135b	calcium/calmodulin-dependent protein kinase I gamma
Camk2d	mmu-miR-361	calcium/calmodulin-dependent protein kinase II, delta
Cblb	mmu-miR-181c	Casitas B-lineage lymphoma b
Cds2	mmu-miR-181c	CDP-diacylglycerol synthase (phosphatidate cytidyltransferase) 2
Cecr2	mmu-miR-181c	cat eye syndrome chromosome region, candidate 2 homolog (human)
Cenpb	mmu-miR-135b	centromere protein B
Cggbp1	mmu-miR-181c	CGG triplet repeat binding protein 1
Chd3	mmu-miR-181c	B-cell CLL/lymphoma 6, member B
Chd7	mmu-miR-181c	chromodomain helicase DNA binding protein 7
Chrn2	mmu-miR-138	cholinergic receptor, nicotinic, beta polypeptide 2 (neuronal)
Clasp1	mmu-miR-181c	CLIP associating protein 1
Clock	mmu-miR-181c	circadian locomotor output cycles kaput
Cntnap1	mmu-miR-135b	contactin associated protein-like 1
Cops2	mmu-miR-181c	COP9 (constitutive photomorphogenic) homolog, subunit 2 (Arabidopsis thaliana)
Cpeb4	mmu-miR-181c	cytoplasmic polyadenylation element binding protein 4
Cplx1	mmu-miR-135b	complexin 1
Cplx2	mmu-miR-135b	complexin 2
Cramp1l	mmu-miR-135b	Crm, cramped-like (Drosophila)
Cttnbp2	mmu-miR-135b	cortactin binding protein 2
Cxcl12	mmu-miR-214	chemokine (C-X-C motif) ligand 12
Cyp1b1	mmu-miR-181c	cytochrome P450, family 1, subfamily b, polypeptide 1



D430041D05Rik	mmu-miR-181c	RIKEN cDNA D430041D05 gene
Ddx19b	mmu-miR-214	DEAD (Asp-Glu-Ala-Asp) box polypeptide 19b
Ddx3x	mmu-miR-181c	expressed sequence C77371
Dio2	mmu-miR-181c	deiodinase, iodothyronine, type II
Dip2c	mmu-miR-181c	DIP2 disco-interacting protein 2 homolog C (Drosophila)
Dirc2	mmu-miR-135b	disrupted in renal carcinoma 2 (human)
Dmxl2	mmu-miR-181c	Dmx-like 2
Dnajc13	mmu-miR-181c	DnaJ (Hsp40) homolog, subfamily C, member 13
Dock4	mmu-miR-181c	dedicator of cytokinesis 4
Dpp10	mmu-miR-361	dipeptidylpeptidase 10
Dtna	mmu-miR-135b	dystrobrevin alpha
E2f7	mmu-miR-181c	E2F transcription factor 7
Eif4a2	mmu-miR-181c	eukaryotic translation initiation factor 4A2
Elavl2	mmu-miR-181c	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)
Elavl4	mmu-miR-181c	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D)
En2	mmu-miR-181c	engrailed 2
Entpd7	mmu-miR-135b	ectonucleoside triphosphate diphosphohydrolase 7
Epha4	mmu-miR-181c	Eph receptor A4
Esrra	mmu-miR-135b	estrogen related receptor, alpha
Evi5	mmu-miR-135b	ecotropic viral integration site 5
F730047E07Rik	mmu-miR-361	RIKEN cDNA F730047E07 gene
Fastkd2	mmu-miR-181c	FAST kinase domains 2
Fbn2	mmu-miR-181c	fibrillin 2
Fbxl3	mmu-miR-181c	F-box and leucine-rich repeat protein 3
Fbxo11	mmu-miR-181c	F-box protein 11
Fgf11	mmu-miR-135b	fibroblast growth factor 11
Fign	mmu-miR-181c	fidgetin
Fndc3a	mmu-miR-181c	fibronectin type III domain containing 3A
Fndc3b	mmu-miR-181c	fibronectin type III domain containing 3B
Fos	mmu-miR-181c	FBJ osteosarcoma oncogene
Foxn3	mmu-miR-135b	forkhead box N3
Foxp1	mmu-miR-181c	forkhead box P1
Frmpd4	mmu-miR-135b	FERM and PDZ domain containing 4
Gabrb2	mmu-miR-135b	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2
Glrb	mmu-miR-135b	glycine receptor, beta subunit
Golga4	mmu-miR-361	golgi autoantigen, golgin subfamily a, 4
Gpr17	mmu-miR-138	G protein-coupled receptor 17
Gpr21	mmu-miR-135b	G protein-coupled receptor 21
Gpr22	mmu-miR-181c	G protein-coupled receptor 22
Grik2	mmu-miR-181c	glutamate receptor, ionotropic, kainate 2 (beta 2)

Gse1	mmu-miR-181c	genetic suppressor element 1
Gsk3b	mmu-miR-135b	glycogen synthase kinase 3 beta
Hcn2	mmu-miR-135b	hyperpolarization-activated, cyclic nucleotide-gated K+ 2
Hic2	mmu-miR-181c	hypermethylated in cancer 2
Hif1an	mmu-miR-135b	hypoxia-inducible factor 1, alpha subunit inhibitor
Hnf4a	mmu-miR-135b	hepatic nuclear factor 4, alpha
Hoxa11	mmu-miR-181c	homeobox A11
Il1a	mmu-miR-181c	interleukin 1 alpha
Insl3	mmu-miR-181c	genetic suppressor element 2
Ippk	mmu-miR-181c	inositol 1,3,4,5,6-pentakisphosphate 2-kinase
Kcna4	mmu-miR-181c	potassium voltage-gated channel, shaker-related subfamily, member 4
Kcnab3	mmu-miR-135b	potassium voltage-gated channel, shaker-related subfamily, beta member 3
Kcnc1	mmu-miR-214	potassium voltage gated channel, Shaw-related subfamily, member 1
Kcnd1	mmu-miR-135b	potassium voltage-gated channel, Shal-related family, member 1
Kcnh7	mmu-miR-181c	potassium voltage-gated channel, subfamily H (eag-related), member 7
Kcnma1	mmu-miR-181c	potassium large conductance calcium-activated channel, subfamily M, alpha member 1
Kif16b	mmu-miR-7b	kinesin family member 16B
Kif3b	mmu-miR-135b	kinesin family member 3B
Klf6	mmu-miR-181c	Kruppel-like factor 6
Klhl1	mmu-miR-181c	kelch-like 1 (Drosophila)
Kpna1	mmu-miR-181c	karyopherin (importin) alpha 1
Lats2	mmu-miR-135b	large tumor suppressor 2
Lin28b	mmu-miR-181c	lin-28 homolog B (C. elegans)
Lmtk2	mmu-miR-214	lemur tyrosine kinase 2
Lnp	mmu-miR-214	limb and neural patterns
Lonrf1	mmu-miR-135b	LON peptidase N-terminal domain and ring finger 1
Lrrn1	mmu-miR-181c	leucine rich repeat protein 1, neuronal
Macf1	mmu-miR-138	microtubule-actin crosslinking factor 1
Macf1	mmu-miR-214	microtubule-actin crosslinking factor 1
Macf1	mmu-miR-223	microtubule-actin crosslinking factor 1
Man1a	mmu-miR-135b	mannosidase 1, alpha
Mapk1	mmu-miR-181c	mitogen-activated protein kinase 1
Mapre1	mmu-miR-214	microtubule-associated protein, RP/EB family, member 1
Mapre2	mmu-miR-135b	microtubule-associated protein, RP/EB family, member 2

Mast2	mmu-miR-138	microtubule associated serine/threonine kinase 2
Mbnl2	mmu-miR-181c	muscleblind-like 2
Mecp2	mmu-miR-138	methyl CpG binding protein 2
Mecp2	mmu-miR-155	methyl CpG binding protein 2
Mecp2	mmu-miR-361	methyl CpG binding protein 2
Med13	mmu-miR-135b	mediator complex subunit 13
Mesdc2	mmu-miR-214	mesoderm development candidate 2
Mier3	mmu-miR-181c	mesoderm induction early response 1, family member 3
Mll1	mmu-miR-181c	myeloid/lymphoid or mixed-lineage leukemia 1
MLxip	mmu-miR-138	MLX interacting protein
MLxip	mmu-miR-214	MLX interacting protein
Mmp16	mmu-miR-135b	matrix metalloproteinase 16
Msi2	mmu-miR-181c	Musashi homolog 2 (Drosophila)
Mtap2	mmu-miR-361	microtubule-associated protein 2
Mtf2	mmu-miR-181c	metal response element binding transcription factor 2
Mtif3	mmu-miR-138	mitochondrial translational initiation factor 3
Mtrr	mmu-miR-361	5-methyltetrahydrofolate-homocysteine methyltransferase reductase
Mtus1	mmu-miR-135b	mitochondrial tumor suppressor 1
Mybl1	mmu-miR-181c	myeloblastosis oncogene-like 1
Myh10	mmu-miR-181c	myosin, heavy polypeptide 10, non-muscle
Myh10	mmu-miR-223	myosin, heavy polypeptide 10, non-muscle
Myo18a	mmu-miR-214	myosin XVIIIa
Ncoa2	mmu-miR-181c	nuclear receptor coactivator 2
Neto2	mmu-miR-135b	neuropilin (NRP) and tolloid (TLL)-like 2
Nf1	mmu-miR-135b	neurofibromatosis 1
Nf1	mmu-miR-214	neurofibromatosis 1
Nfic	mmu-miR-135b	mannosidase 1, alpha
Nova1	mmu-miR-181c	neuro-oncological ventral antigen 1
Nptn	mmu-miR-181c	neuroplastin
Nr1d2	mmu-miR-181c	nuclear receptor subfamily 1, group D, member 2
Nr3c1	mmu-miR-181c	nuclear receptor subfamily 3, group C, member 1
Nr3c2	mmu-miR-135b	nuclear receptor subfamily 3, group C, member 2
Nr4a3	mmu-miR-181c	nuclear receptor subfamily 4, group A, member 3
Ntrk2	mmu-miR-135b	neurotrophic tyrosine kinase, receptor, type 2
Nup153	mmu-miR-135b	nucleoporin 153
Oxtr	mmu-miR-138	oxytocin receptor
Pak7	mmu-miR-215	microtubule-associated protein, RP/EB family, member 2

Palm2	mmu-miR-181c	nuclear receptor subfamily 4, group A, member 4
Pank3	mmu-miR-361	pantothenate kinase 3
Papd5	mmu-miR-181c	PAP associated domain containing 5
Pcdh15	mmu-miR-138	protocadherin 15
Pcdha11	mmu-miR-181c	protocadherin alpha 11
Pcnp	mmu-miR-181c	PEST proteolytic signal containing nuclear protein
Pcyt1b	mmu-miR-135b	phosphate cytidyltransferase 1, choline, beta isoform
Pdcl	mmu-miR-135b	phosducin-like
Pde4b	mmu-miR-361	phosphodiesterase 4B, cAMP specific
Pdgfra	mmu-miR-181c	platelet derived growth factor receptor, alpha polypeptide
Pdpk1	mmu-miR-214	3-phosphoinositide dependent protein kinase 1
Pds5b	mmu-miR-223	PDS5, regulator of cohesion maintenance, homolog B ( <i>S. cerevisiae</i> )
Peli2	mmu-miR-135b	pellino 2
Phactr1	mmu-miR-181c	phosphatase and actin regulator 1
Phf15	mmu-miR-181c	PHD finger protein 15
Phf15	mmu-miR-214	PHD finger protein 15
Phox2b	mmu-miR-181c	paired-like homeobox 2b
Pik3cd	mmu-miR-7b	phosphatidylinositol 3-kinase catalytic delta polypeptide
Plch2	mmu-miR-138	phospholipase C, eta 2
Plxna2	mmu-miR-361	plexin A2
Ppp2r5c	mmu-miR-135b	protein phosphatase 2, regulatory subunit B (B56), gamma isoform
Prkcd	mmu-miR-181c	protein kinase C, delta
Psme4	mmu-miR-135b	proteasome (prosome, macropain) activator subunit 4
Pten	mmu-miR-181c	phosphatase and tensin homolog
Rab14	mmu-miR-214	RAB14, member RAS oncogene family
Rab3gap1	mmu-miR-361	RAB3 GTPase activating protein subunit 1
Rab8b	mmu-miR-223	RAB8B, member RAS oncogene family
Rac1	mmu-miR-361	RAS-related C3 botulinum substrate 1
Rad23b	mmu-miR-181c	RAD23b homolog ( <i>S. cerevisiae</i> )
Rad51l3	mmu-miR-181c	RAD51-like 3 ( <i>S. cerevisiae</i> )
Rap1gds1	mmu-miR-135b	RAP1, GTP-GDP dissociation stimulator 1
Rapgef6	mmu-miR-135b	Rap guanine nucleotide exchange factor (GEF) 6
Rassf8	mmu-miR-139	oxytocin receptor
Rbak	mmu-miR-135b	RB-associated KRAB repressor
Rbbp7	mmu-miR-181c	retinoblastoma binding protein 7
Rbfox2	mmu-miR-135b	RNA binding protein, fox-1 homolog ( <i>C. elegans</i> ) 2

Rbm16	mmu-miR-361	RNA binding motif protein 16
Rbm26	mmu-miR-181c	RNA binding motif protein 26
Rex2	mmu-miR-181c	reduced expression 2
Rgl1	mmu-miR-135b	ral guanine nucleotide dissociation stimulator,-like 1
Rgl2	mmu-miR-362	RNA binding motif protein 17
Rlf	mmu-miR-181c	rearranged L-myc fusion sequence
Rnf157	mmu-miR-135b	ring finger protein 157
Rnf34	mmu-miR-181c	ring finger protein 34
Rock2	mmu-miR-135b	Rho-associated coiled-coil containing protein kinase 2
Rragb	mmu-miR-361	Ras-related GTP binding B
Rsad1	mmu-miR-181c	radical S-adenosyl methionine domain containing 1
Rsb1	mmu-miR-7b	rosbin, round spermatid basic protein 1
Rufy2	mmu-miR-135b	RUN and FYVE domain-containing 2
Rufy2	mmu-miR-155	RUN and FYVE domain-containing 2
Sbno1	mmu-miR-181c	sno, strawberry notch homolog 1 (Drosophila)
Schip1	mmu-miR-181c	schwannomin interacting protein 1
Scn2b	mmu-miR-135b	sodium channel, voltage-gated, type II, beta
Sdk1	mmu-miR-361	sidekick homolog 1 (chicken)
Sel1l	mmu-miR-181c	sel-1 suppressor of lin-12-like (C. elegans)
Sema6d	mmu-miR-181c	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D
Setd7	mmu-miR-135b	SET domain containing (lysine methyltransferase) 7
Sfrs18	mmu-miR-181c	serine/arginine-rich splicing factor 18
Sgpp1	mmu-miR-181c	sphingosine-1-phosphate phosphatase 1
Sirt1	mmu-miR-181c	sirtuin 1 (silent mating type information regulation 2, homolog) 1 (S. cerevisiae)
Slc24a2	mmu-miR-135b	solute carrier family 24 (sodium/potassium/calcium exchanger), member 2
Slc35a5	mmu-miR-361	solute carrier family 35, member A5
Slc6a2	mmu-miR-181c	PAP associated domain containing 6
Slc6a5	mmu-miR-135b	solute carrier family 6 (neurotransmitter transporter, glycine), member 5
Slc6a5	mmu-miR-361	solute carrier family 6 (neurotransmitter transporter, glycine), member 5
Slc8a1	mmu-miR-135b	solute carrier family 8 (sodium/calcium exchanger), member 1
Slc8a1	mmu-miR-181c	solute carrier family 8 (sodium/calcium exchanger), member 1
Slc8a1	mmu-miR-214	solute carrier family 8 (sodium/calcium exchanger), member 1
Slc8a1	mmu-miR-223	solute carrier family 8 (sodium/calcium exchanger), member 1

Smad5	mmu-miR-135b	MAD homolog 5 (Drosophila)
Smad7	mmu-miR-181c	MAD homolog 7 (Drosophila)
Sos1	mmu-miR-181c	son of sevenless homolog 1 (Drosophila)
Sox6	mmu-miR-181c	SRY-box containing gene 6
Sp3	mmu-miR-135b	trans-acting transcription factor 3
Spin1	mmu-miR-181c	spindlin 1
Spire1	mmu-miR-181c	spire homolog 1 (Drosophila)
Srrm2	mmu-miR-138	serine/arginine repetitive matrix 2
Ss18l1	mmu-miR-181c	synovial sarcoma translocation gene on chromosome 18-like 1
Ssx2ip	mmu-miR-181c	synovial sarcoma, X breakpoint 2 interacting protein
St8sia3	mmu-miR-181c	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3
Stk32a	mmu-miR-210	serine/threonine kinase 32A
Stt3b	mmu-miR-181c	STT3, subunit of the oligosaccharyltransferase complex, homolog B ( <i>S. cerevisiae</i> )
Stx8	mmu-miR-214	syntaxin 8
Stxbp6	mmu-miR-7b	syntaxin binding protein 6 (amisyn)
Suv420h2	mmu-miR-135b	suppressor of variegation 4-20 homolog 2 (Drosophila)
Syncrip	mmu-miR-181c	synaptotagmin binding, cytoplasmic RNA interacting protein
Syncrip	mmu-miR-223	synaptotagmin binding, cytoplasmic RNA interacting protein
Syncrip	mmu-miR-361	synaptotagmin binding, cytoplasmic RNA interacting protein
Tacc1	mmu-miR-214	transforming, acidic coiled-coil containing protein 1
Tardbp	mmu-miR-181c	TAR DNA binding protein
Tcerg1	mmu-miR-181c	transcription elongation regulator 1 (CA150)
Tcf7l2	mmu-miR-135b	transcription factor 7-like 2, T-cell specific, HMG-box
Tcf7l2	mmu-miR-181c	transcription factor 7-like 2, T-cell specific, HMG-box
Tgfbr1	mmu-miR-181c	transforming growth factor, beta receptor I
Tgm2	mmu-miR-138	transglutaminase 2, C polypeptide
Tmem87b	mmu-miR-181c	transmembrane protein 87B
Tmod2	mmu-miR-138	tropomodulin 2
Tnfrsf11b	mmu-miR-181c	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)
Tnpo1	mmu-miR-135b	transportin 1
Tnpo1	mmu-miR-181c	transportin 1
Tnrc6b	mmu-miR-181c	trinucleotide repeat containing 6b
Tomm70a	mmu-miR-135b	translocase of outer mitochondrial membrane 70 homolog A (yeast)
Topors	mmu-miR-135b	topoisomerase I binding, arginine/serine-rich

Trim66	mmu-miR-214	tripartite motif-containing 66
Trim71	mmu-miR-181c	tripartite motif-containing 71
Trpc2	mmu-miR-214	transient receptor potential cation channel, subfamily C, member 2
Tsc22d2	mmu-miR-181c	TSC22 domain family, member 2
Tshz3	mmu-miR-155	teashirt zinc finger family member 3
Ttc14	mmu-miR-135b	tetratricopeptide repeat domain 14
Ttn	mmu-miR-138	titin
Ttpa	mmu-miR-181c	tocopherol (alpha) transfer protein
Ubox5	mmu-miR-135b	U box domain containing 5
Ubr5	mmu-miR-361	ubiquitin protein ligase E3 component n-recognin 5
Ulk2	mmu-miR-223	Unc-51 like kinase 2 (C. elegans)
Usp14	mmu-miR-181c	ubiquitin specific peptidase 14
Usp20	mmu-miR-214	ubiquitin specific peptidase 20
Usp49	mmu-miR-139	serine/arginine repetitive matrix 3
Vapb	mmu-miR-215	ubiquitin specific peptidase 21
Vcan	mmu-miR-135b	versican
Vcan	mmu-miR-181c	versican
Vegfa	mmu-miR-361	vascular endothelial growth factor A
Vps37c	mmu-miR-135b	vacuolar protein sorting 37C (yeast)
Wasl	mmu-miR-181c	Wiskott-Aldrich syndrome-like (human)
Wdr37	mmu-miR-181c	WD repeat domain 37
Wdr47	mmu-miR-7b	WD repeat domain 47
Wipf2	mmu-miR-7b	WAS/WASL interacting protein family, member 2
Wnk1	mmu-miR-181c	Wiskott-Aldrich syndrome-like (human)
Yipf6	mmu-miR-181c	Yip1 domain family, member 6
Ythdc2	mmu-miR-181c	YTH domain containing 2
Ythdf2	mmu-miR-181c	YTH domain family 2
Ythdf3	mmu-miR-181c	YTH domain family 3
Ywhag	mmu-miR-181c	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide
Zfp120	mmu-miR-181c	zinc finger protein 120
Zfp14	mmu-miR-181c	zinc finger protein 14
Zfp235	mmu-miR-135b	zinc finger protein 235
Zfp238	mmu-miR-361	zinc finger protein 238
Zfp286	mmu-miR-181c	zinc finger protein 286
Zfp335	mmu-miR-214	zinc finger protein 335
Zfp36l2	mmu-miR-181c	zinc finger protein 36, C3H type-like 2
Zfp518a	mmu-miR-135b	zinc finger protein 518A
Zfp618	mmu-miR-181c	zinc fingerprotein 618
Zfp820	mmu-miR-181c	zinc finger protein 820
Zfp945	mmu-miR-181c	zinc finger protein 945
Zfp97	mmu-miR-181c	zinc finger protein 97
Zhx3	mmu-miR-214	zinc fingers and homeoboxes 3

Zmat3	mmu-miR-361	zinc finger matrin type 3
Zxdb	mmu-miR-181c	zinc finger, X-linked, duplicated B



**Supplementary Table 3:** miRNAs expression in Ts65Dn Lung. Relative expression levels in 5 trisomy samples  $\pm$  SEM is shown with respect to 5 euploid controls. No expression was observed for miR-802 in Lung tissue of either trisomy or control mice.

<b>miRNA</b>	<b>Relative expression in lung tissue Ts65Dn/Euploid (N=5)</b>
miR-802	no detectable expression
miR-155	1.84 $\pm$ 0.28 (p=0.2)
miR-223	1.66 $\pm$ 0.16 (p=0.2)
miR-204	1.42 $\pm$ 0.14 (p=0.1)
miR-214	1.19 $\pm$ 0.06 (p=0.2)

**Supplementary Table 4**

<b>Name of miRNA/mRNA</b>	<b>Sequence</b>
miR-802	UCAGUAACAAAGAUUCAUCCUU (assay ID: 002029)
miR-155	UUAAUGCUAAUUGUGAUAGGGGU (assay ID:002571)
miR-223	UGUCAGUUUGUCAAAUACCCCA (assay ID: 002295)
miR-214	ACAGCAGGCACAGACAGGCAGU (assay ID: 002306)
miR-204	UUCCCUUUGUCAUCCUAUGCCU (assay ID: 000508)
U6	GTGCTCGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCT GCGCAAGGATGACACGCAAATTCGTGAAGCGTTCATATTTT (assay ID: 001973)
Ship1 Forward	CGGTTTCATCTTCCACAGCCAAC[FAM]G (Cat.#:10336022 161262 A02)
Ship1 Reverse	GCTTCCACCTTTCCAGATCC (Cat.#: 10336022 161229 D02)
Mecp2 Forward	CGCTTAGGAGGCTCACTGGAAAG[FC]G (Cat.#: 20439213 159899 A07)
Mecp2 Reverse	GGCGACCATAGGCTGAGTCTT (Cat.#: 20439213 159691 B02)
Ezh2 Forward	CGCTGTAAACCAAGAGTGAAGCAG[FC]G (Cat.#: 20454511 159848 A12)
Ezh2 Reverse	ACTCCCTAGTCCC GCGCAAT (Cat.#: 20454511 824E06)
Creb1 Forward	CAATCTTCAAGCCCAGCCACAGA[FT]TG (Cat.#: 20439213 159899 A08)
Creb1 Reverse	GGAGCAGATGATGTTGCATGAG (Cat.#: 20439213 159691 B03)