Supplemental Information

AUTS2 controls neuronal lineage choice through a novel PRC1-independent complex and BMP inhibition

Zhuangzhuang Geng[1], Qiang Wang[1], Weili Miao[2], Trevor Wolf[3], Jessenia Chavez[1], Emily Giddings[3], Ryan Hobbs[4], David J. DeGraff[5,6], Yinsheng Wang[2], James Stafford[3], Zhonghua Gao[1,6,7,#]

1, Departments of Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey, PA 17033

2, Department of Chemistry, University of California at Riverside, Riverside, CA 92521

3, Department of Neurological Sciences, Larner College of Medicine, University of Vermont, Burlington, VT 05405

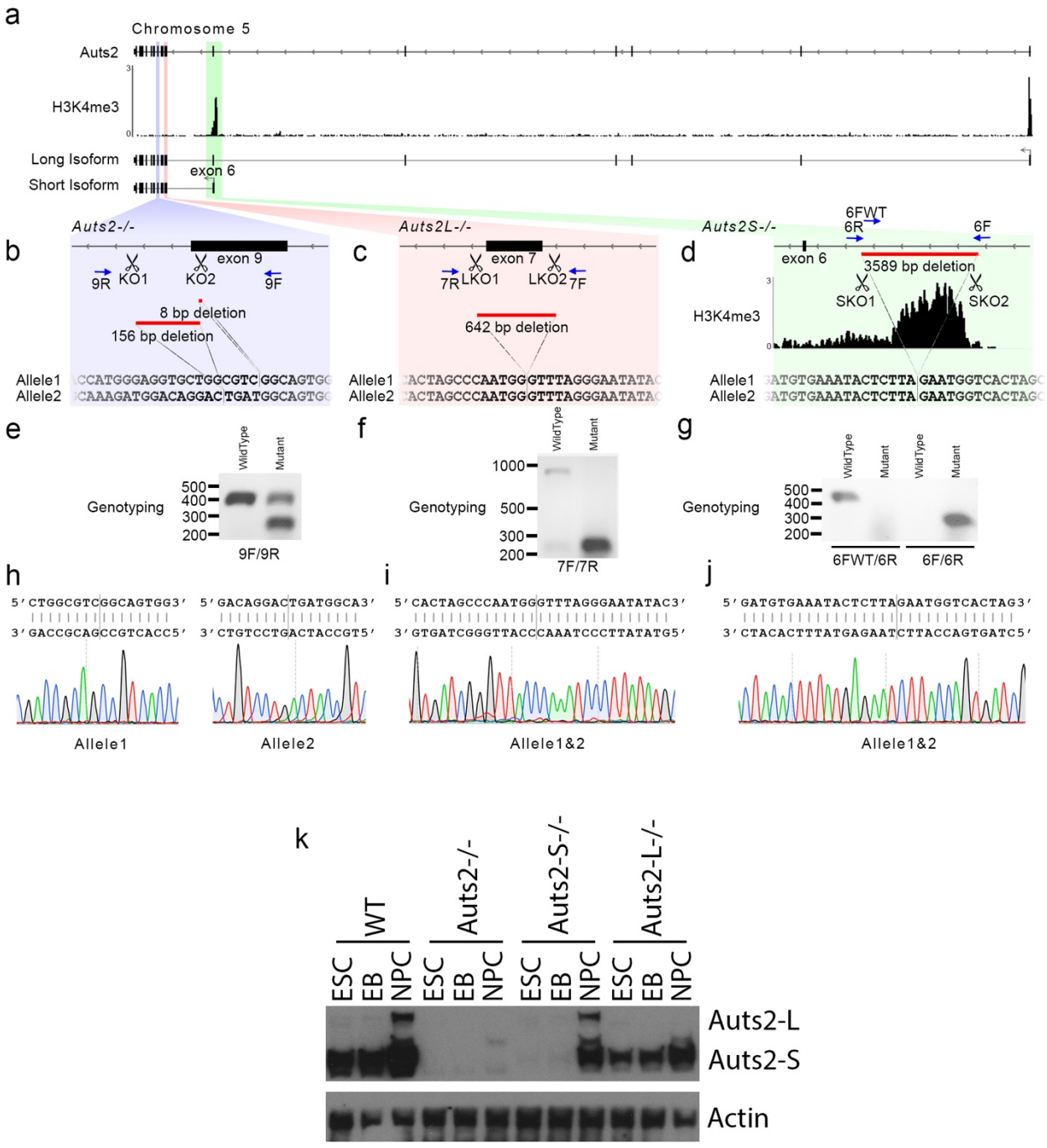
4, Department of Dermatology, Penn State College of Medicine, Hershey, PA 17033

5, Department of Pathology and Laboratory Medicine, Penn State College of Medicine, Hershey, PA 17033

6, Penn State Hershey Cancer Institute, Hershey, PA 17033

7, The Stem Cell and Regenerative Biology Program, Penn State College of Medicine, Hershey, PA 17033

#, correspondence author, zgao1@pennstatehealth.psu.edu


a
Chromosome 5
Auts2
H3K4me3
Long Isoform
Short Isoform
exon 6
b
Auts2-/-
exon 9
9R
KO1
KO2
9F
8 bp deletion
156 bp deletion
Allele1
Allele2
c
Auts2L-/-
exon 7
7R
LKO1
LKO2
7F
642 bp deletion
Allele1
Allele2
d
Auts2S-/-
6FWT
6R
6F
exon 6
3589 bp deletion
SKO1
SKO2
H3K4me3
Allele1
Allele2
e
Genotyping
WildType
Mutant
500
400
300
200
9F/9R
f
Genotyping
WildType
Mutant
1000
500
300
200
7F/7R
g
Genotyping
WildType
Mutant
WildType
Mutant
500
400
300
200
6FWT/6R
6F/6R
h
5′CTGGCGTCGGCAGTGG3′
3′GACCGCAGCCGTCACC5′
5′GACAGGACTGATGGCA3′
3′CTGTCCTGACTACCGT5′
Allele1
Allele2
i
5′CACTAGCCCAATGGGTTTAGGGAATATAC3′
3′GTGATCGGGTTACCCAAATCCCTTATATG5′
Allele1&2
j
5′GATGTGAAATACTCTTAGAATGGTCACTAG3′
3′CTACACTTTATGAGAATCTTACCAGTGATC5′
Allele1&2

k
WT
Auts2-/-
Auts2-S-/-
Auts2-L-/-
ESC
EB
NPC
Auts2-L
Auts2-S
Actin

Figure S1. Design, generation and validation of mouse ESC lines with Auts2 deletion. ***a***, Schematic representation of the genetic locus of mouse *Auts2*. H3K4me3 peaks, indicating the two transcription start sites, were obtained from previous ChIP-seq analysis [6]. Two major transcripts, one referred to as "long" and the other referred to as "short" isoforms are shown at the bottom. ***b-d***, Targeting strategy and final products of CRISPR/Cas9-mediated gene editing of *Auts2* locus. In each cell line, a pair of sgRNAs (labeled by scissors) were used and the deleted regions are indicated by red bars. Blue arrows indicate genotyping primers. Post-editing sequences are shown for both alleles. The two alleles in the *Auts2-/-* line were edited differently; one has an 8 bp frameshift deletion and the other a 156 bp deletion that spans the splicing junction (***b***). Identical editing events are present in both *Auts2L-/-* (***c***) and *Auts2S-/-* (***d***) lines. ***e-g***, Genotyping PCR results for edited lines. ***h-j***, Sanger sequencing results for edited lines. ***k***, Immunoblotting to confirm the disruption of specific isoforms of *Auts2* in edited lines.

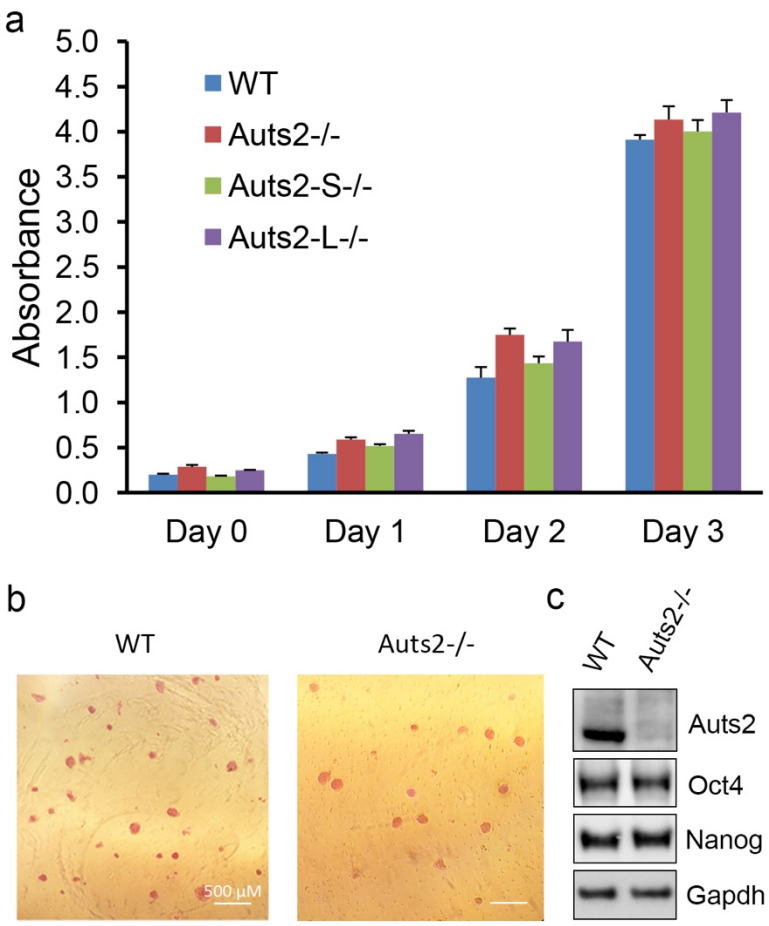


Figure S2. Loss of Auts2 does not affect ESC self-renewal and proliferation. ***a***, Alkaline phosphatase activity staining in WT and *Auts2*-/- ESCs. ***b***, Immunoblotting shows no obvious difference in the protein level of Oct4 and Nanog, two pluripotent markers, between WT and *Auts2*-/- ESCs. ***c***, MTT cell proliferation assay to measure the growth rate of ESCs at various days. All mean values and standard deviations were calculated from four independent measurements. No significant differences were found among ESCs of all genotypes.

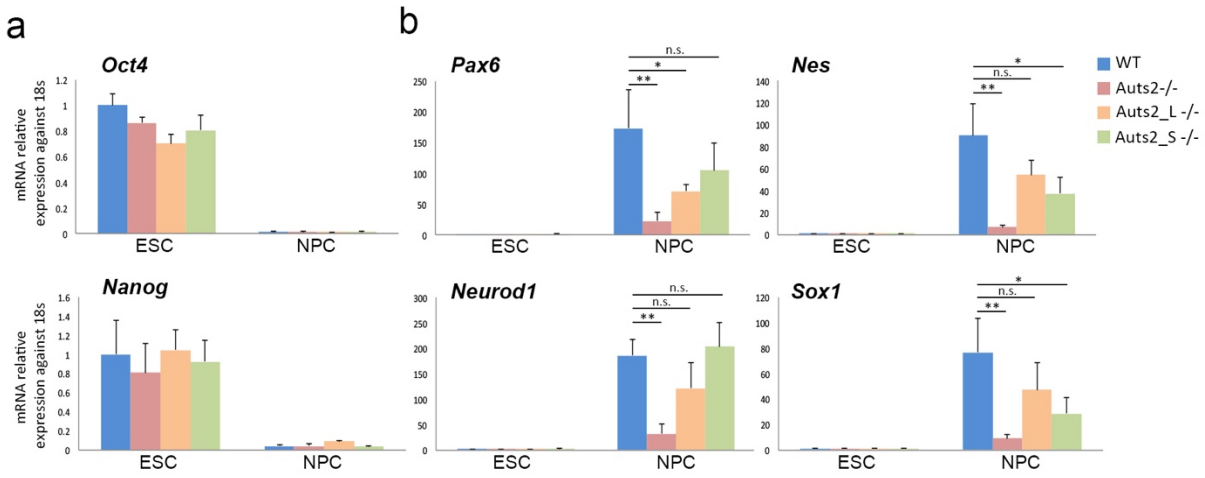


Figure S3. Expression of pluripotency and NPC markers in ESCs with Auts2 deletion. ***a***, Quantitative RT-PCR analysis of expression pluripotency markers (*Oct4* and *Nanog*) in WT, *Auts2*-/-, *Auts2L*-/- and *Auts2S*-/- ESCs and NPCs. ***b***, Quantitative RT-PCR analysis of NPC markers (*Pax6*, *Nes*, *Neurod1* and *Sox1*). Expression levels are normalized relative to those in WT ESCs. All mean values and standard deviations were calculated from three independent measurements. * P<0.05, ** P<0.01, n.s., not significant, by two-sided t-test.

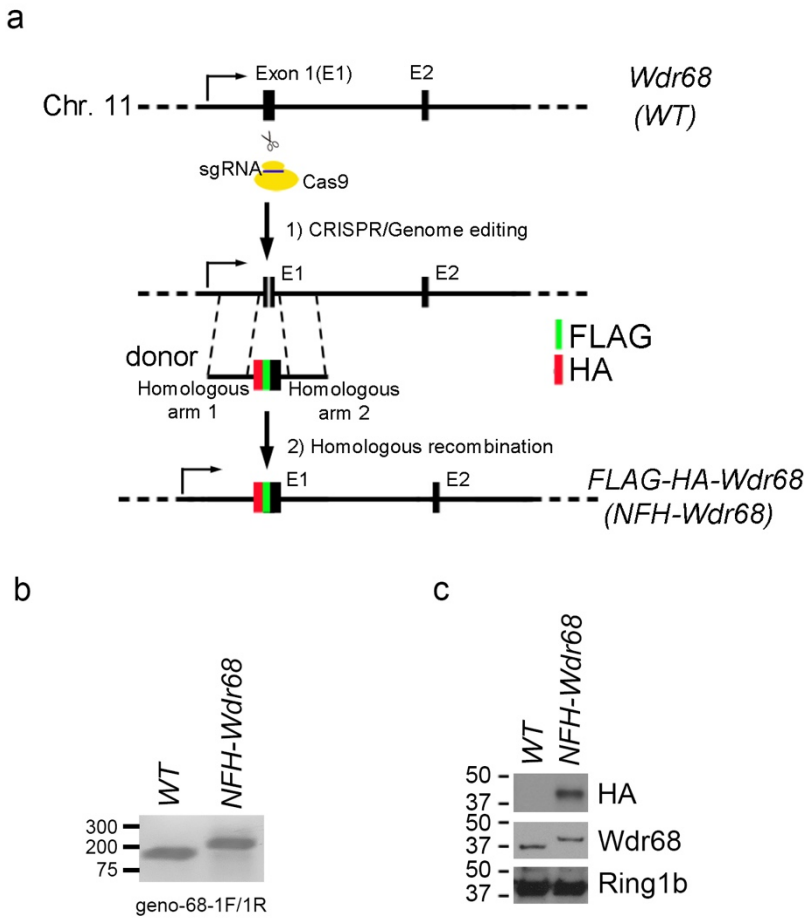


Figure S4. Generation of an ESC line that expresses FLAG-HA-Wdr68. ***a***, Schematic for the CRISPR/Cas9-mediated knock-in of FLAG-HA-Wdr68 in E14 cells. ***b***, Agarose gel picture shows the PCR products of genomic DNA isolated from WT and FLAG-HA-Wdr68 cells. ***c***, Immunoblotting detected the expression of FLAG-HA-Wdr68 in edited cells.

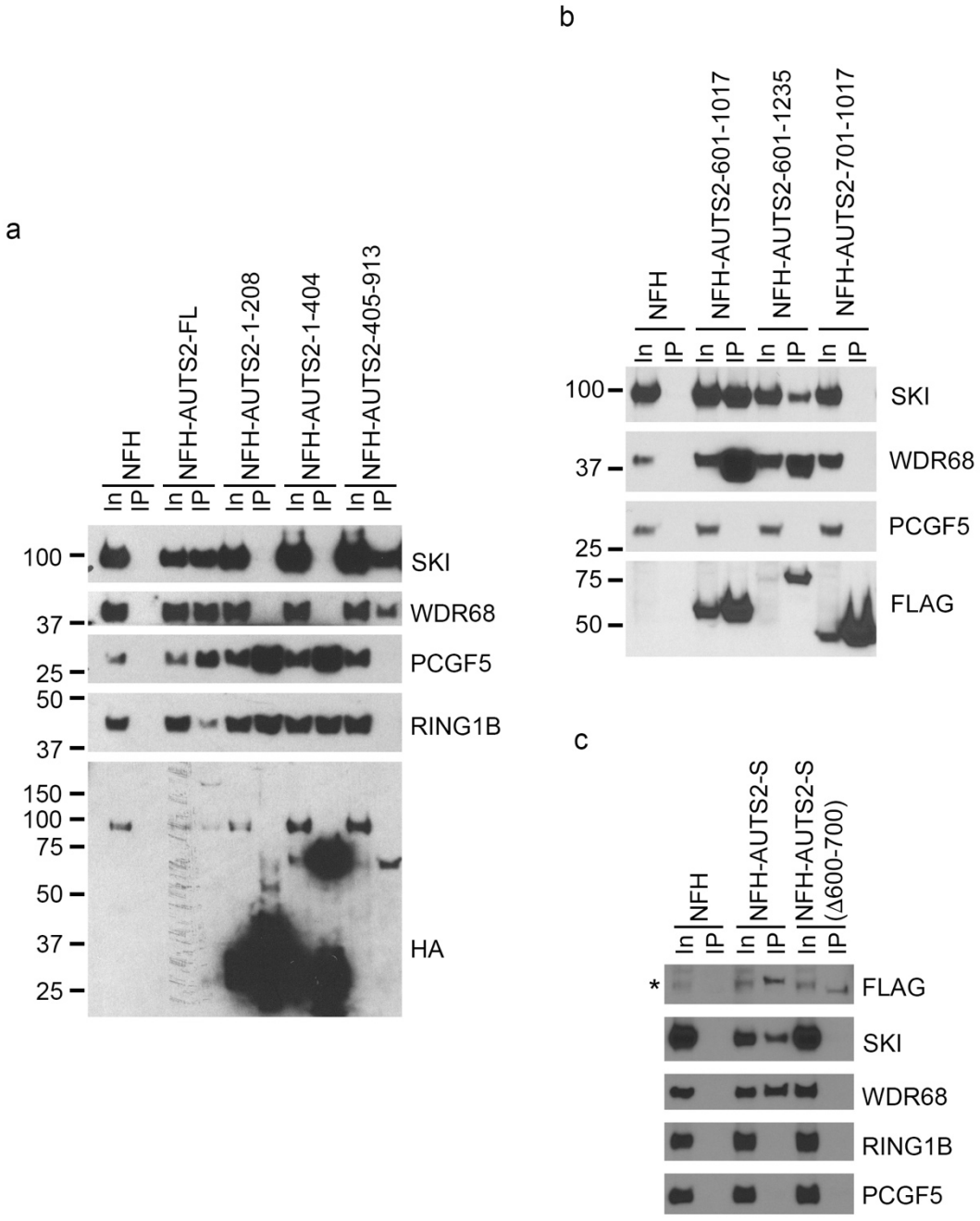


Figure S5. Domain mapping of AUTS2 for interaction with various partner proteins. ***a***, ***b*** and ***c***, HEK293T cells were plasmids expressing various lengths of FLAG and HA tagged AUTS2 as indicated. IP was performed using M2 beads. Bound proteins were resolved on SDS-PAGE and detected by Western blotting for the indicated antigens.

Table S1 Plasmids

	Plasmid Name	Source	Vector	Enzyme sites	Tags
1	pINTO-NFH-AUTS2-L	Gao et al., 2014	pINTO-NFH	KpnI/EcoRI	N-FLAG-HA
2	pINTO-NFH-AUTS2-S	This study	pINTO-NFH		N-FLAG-HA
3	pINTO-NFH-AUTS2-8-208	This study	pINTO-NFH	KpnI/XhoI	N-FLAG-HA
4	pINTO-NFH-AUTS2-8-404	This study	pINTO-NFH	KpnI/XhoI	N-FLAG-HA
5	pINTO-NFH-AUTS2-405-913	This study	pINTO-NFH	KpnI/XhoI	N-FLAG-HA
6	pINTO-NFH-AUTS2-601-1017	This study	pINTO-NFH		N-FLAG-HA
7	pINTO-NFH-AUTS2-601-1235	This study	pINTO-NFH		N-FLAG-HA
8	pINTO-NFH-AUTS2-701-1017	This study	pINTO-NFH		N-FLAG-HA
9	pINTO-NFH-AUTS2-701-1235	This study	pINTO-NFH		N-FLAG-HA
10	HA-SKI	Addgene, #10910	pCI-Neo	XhoI/HpaI	N-HA
11	HA-DDB1	Addgene, #19909	pCDNA3-HA2		N-HA
12	HA-ub	Iwahara et al., 2012			HA
13	FLAG-SMAD1	Addgene, #11735	pCMV5B	ClaI/BamHI	N-FLAG
14	DN-CUL4B	Addgene, #15822	pCDNA3.1(+)	BamHI/ApaI	C-FLAG
15	pLV-EF1a-IRES-Blast	Addgene, #85133	pLV-EF1a-IRES-Blast	BamHI/EcoRI	
16	pLV-hAUTS2-S	This study	pLV-EF1a-IRES-Blast	BamHI/EcoRI	
17	pLV-hAUTS2-Δ	This study	pLV-EF1a-IRES-Blast	BamHI/EcoRI	

Table S2 Antibodies used in this study

Antibody	Suppliers	Catalog No.	Lot No.	Applications
AUTS2	Gao et al., 2014			WB, IF
AUTS2	Sigma	HPA000390		WB
WDR68	Sigma	HPA022948	A76005	WB
SKI	Santa Cruz	sc33693		WB
SMAD1	Cell Signaling	6944	lot: 5	WB
SMAD2	Cell Signaling	5339	lot: 4	WB
SMAD4	Cell Signaling	38454	lot: 1	WB
SMAD5	Cell Signaling	12534	lot: 2	WB
pSMAD2	Cell Signaling	3108	lot: 8	WB
pSMAD1/5/9	Cell Signaling	13820	lot: 3	WB
RING1B	BETHYL	A302-869A	A302-869A-1	WB
PCGF3/5	Abcam	ab201510		WB
PCGF5	ABCAM	ab201511		WB
DDB1	Santa Cruz	sc376860	A2318	WB
NANOG	Invitrogen	PA1-41577		WB
OCT4	Santa Cruz	sc365509	L1817	WB
PAX6	DSHB	Pax6-s		WB
NESTIN	BD	611658		WB, IF
HA	Covance	MMS-101P		WB
HA	Abcam	ab9110	GR98618-3	WB
HA beads	Sigma	A2095		IP
FLAG	Sigma	F3165		WB
FLAG beads	Sigma	A2220		IP
β-TUBULIN	Abcam	ab6046		WB
GAPDH	Thermo Fisher Scientific	MA5-15738	GA1R	WB
ACTIN	Abcam	ab8277		WB
H3	Abcam	ab1791		WB

Table S3 List of primers for qPCR

For RT-PCR

Name	Sequence (F/R)
mOct4	5'-AGATCACTCACATCGCCAATCA-3' / 5'-CGCCGGTTACAGAACCATACTC-3'
mSox9	5'-AGCGAACGCACATCAAGA-3' / 5'-CTGTAGTGAGGAAGGTTGAAGG-3'
mNanog	5'-AGGCTTTGGAGACAGTGAGGTG-3' / 5'-TGGGTAAGGGTGTTCAAGCACT-3'
mNes	5'-AGTGCCCAGTTCTAGTGGTGTCC-3' / 5'-CCTCTAAAATAGAGTGGTGAGGGTTG-3'
mTbx2	5'-ATGTACATCCACCCGGACAG-3' / 5'-GACAGCGATGAAGTCGGTCT-3'
mDpysl2	5'-CAGAATGGTGATTCCCGGAGG-3' / 5'-CAGCCAATAGGCTCGTCCC-3'
mSnai1	5'-CCGATGAGGACAGTGGCAAA-3' / 5'-CCCAGGCTGAGGTACTCCTT-3'
mNeurod1	5'-CGAGTCATGAGTGCCCAGCTTA-3' / 5'-CCGGGAATAGTGAAACTGACGTG-3'
mId2	5'-CTACTCCAAGCTCAAGGAACTG-3' / 5'-GATCTGCAGGTCCAAGATGTAA-3'
mPax6	5'-CTTGGGAAATCCGAGACAGA-3' / 5'-CTAGCCAGGTTGCGAAGAAC-3'
m18SrRNA	5'-GCAATTATTCCCCATGAACG-3' / 5'-GGCCTCACTAAACCATCCAA-3'
mTubb2a	5'-GGAGGTGATAAGCGATGAGCATG-3' / 5'-GGCTCCAGGTCCACTAGGATG-3'
mHoxa6	5'-GTGACCCTACTGCCATCTTAC-3' / 5'-GAATAATCACCGCAGGACTCT-3'
mWwc2	5'-TCATCTGGGAGCAGTCTAGGG-3' / 5'-TGATAGTCTGTGTCCATCTGGTC-3'
mHand1	5'-TCGCTACACTTCCTACCTAGAG-3' / 5'-GAAGGGAAAGGAAGGGAAAGAT-3'
mTubb3	5'-TGAGGCCTCCTCTCACAAGT-3' / 5'-GGCCTGAATAGGTGTCCAAA-3'
mGata6	5'-TGCAGGATTGCATCATGACAGA-3' / 5'-TGACCTCAGATCAGCCACGTT-3'
mVglut1	5'-TGCTACCTCACAGGAGAATGGA-3' / 5'-GCGCACCTTCTTGCACAAAT-3'
mGap43	5'-TGTGCCTGCTGCTGTCACTGAT-3' / 5'-AGGTTTGGCTTCGTCTACAGCG-3'
mGata4	5'-TTCCTCTCCCAGGAACATCAAA-3' / 5-GCTGCACAACTGGGCTCTACTT-3'
mSox1	5'-GGCCGAGTGGAAGGTCAT-3' / 5'-ACTTGTAATCCGGGTGTTCCT-3'
mNkx2-5	5'-GATGGGAAAGCTCCCACTATG-3' / 5'-GACACCAGGCTACGTCAATAAA-3'
mT(Brachyury)	5'-TGCACATTACACACCACTGACG-3' / 5'-AGAACCAGAAGACGAGGACGT-3'
mMap2	5'-AAAGGCCCGCGTAGATCAC-3' / 5'-GGGATTCGAGCAGGTTGATG-3'

For geno typing

Name	Sequence
mWdr68KI-1F	5'-CAGCCCGCTGCCTCTCTGG-3'
mWdr68KI-1R	5'-TTCCACGAAGCTGCCCAGCG-3'
mAuts2KO-7F	5'-TTTTATAGATTTTTCTTTATCCACATGT-3'
mAuts2KO-7R	5'-GGTGTGTGCCATCATTCCTGGG-3'
mAuts2KO-9F	5'- GGAACTGAACACGCGTTTCCTG-3'
mAuts2KO-9R	5'-CACAGTGCCAGCAATGGCCATC-3'
mAuts2KO-6F	5'-GCTGGTGTGAATGTATACACAAGT-3'
mAuts2KO-6R	5'-TGGCACCAAAGGTGTTACTATTCC-3'
mAuts2KO-6FWT	5'-CCTAATCCAGCTCTGGCTCCCA-3'
mSKI-F	5'-AAAGATGTGCGCCCAGTGCGCA-3'
mSKI-R	5'-CAGGATGCCCATGACTTTGAGGA-3'