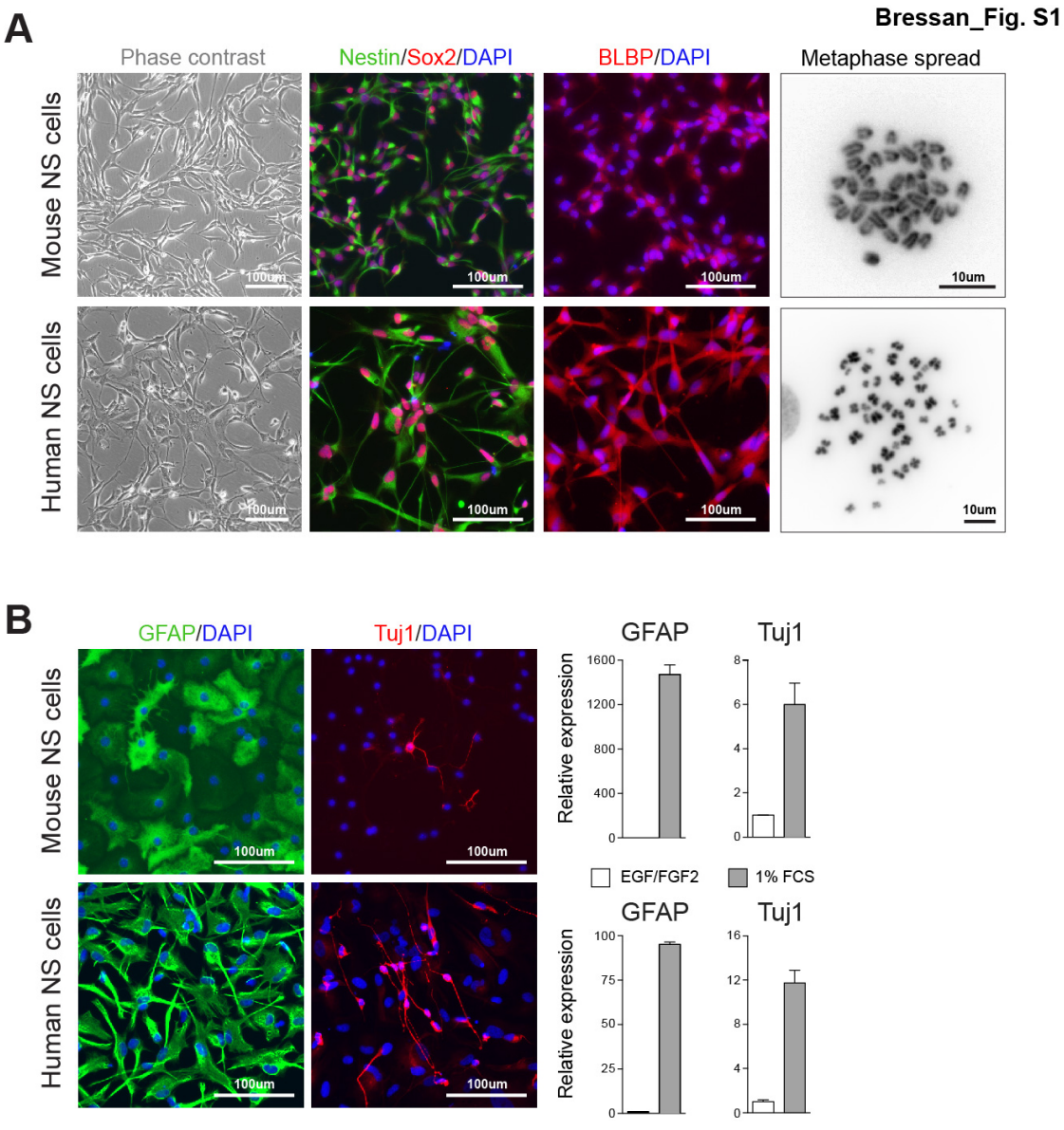

Figure S1 | Characterisation of parental mouse and human NS cell lines used in the study.

(A) Newly derived mouse and human NS cell lines (ANS4 and U3, respectively) displayed the characteristic NS cell morphology and uniformly expressed the defining NS markers Sox2 and Nestin as well as the radial glia marker BLBP. Analysis of metaphase spreads indicated normal chromosomal number after culture expansion

of both lines (modal chromosomal number = 40 in mouse cells and = 46 in human cells). Mouse and human lines were analyzed at passage 25 and 14, respectively.

(C) In differentiation conditions (see methods) astrocytes and neurons emerge, as shown by ICC and qPCR for the lineage markers GFAP (astrocyte) and TuJ-1 (neurons).

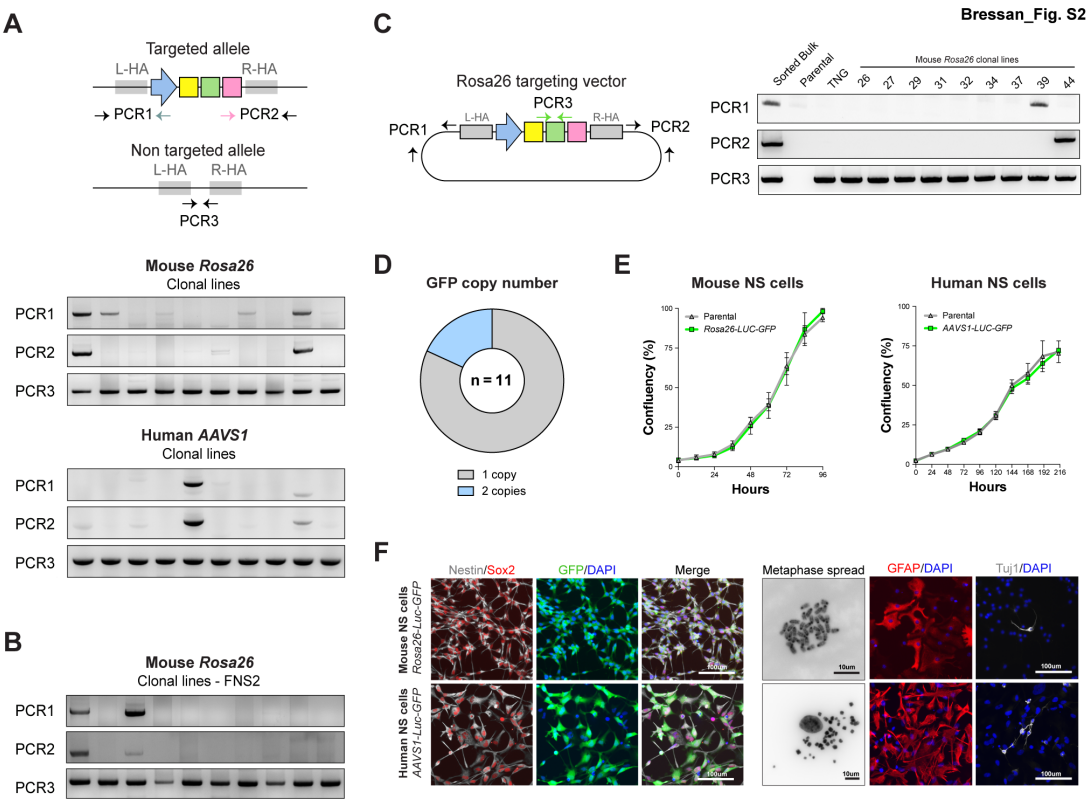

Figure S2 | Characterisation of mouse *Rosa26* and human *AAVS1* edited NS clonal lines.

(A) General PCR-based genotyping strategy for determining correct gene targeting at *Rosa26* and *AAVS1*, and representative genotyping results of clonal lines derived from the GFP sorted population. Biallelic targeting was not achieved, as PCR with primer set 3 was in all cases able to amplify the non-targeted allele.

(B) Targeting at *Rosa26* in the primary foetal mouse NS cell (FNS2).

(C) PCR-based strategy to detect vector backbone sequences in correctly targeted clones, and exemplar results of *Rosa26-Luc-GFP* mouse clonal lines. Mouse ES cell line TNG (Nanog-GFP-IRES-Puromycin) was used as control.

(D) Summary of GFP copy number analysis using quantitative PCR in *Rosa26-Luc-GFP* mouse clonal lines. 9/11 clones were shown to contain a single copy integrated in the genome.

(E) Growth curves of mouse and human clonal NS lines targeted with LUC-GFP cassette at *Rosa26* and *AAVS1* loci demonstrate similarly doubling time to unedited controls.

(F) Edited clonal lines displayed typical NS cell morphology, uniformly expressed the NS markers Nestin and Sox2 (left) and maintained diploid karyotype (modal chromosome number of 40 and 46 for mouse and human, respectively) as well as glial and neuronal differentiation potential (right).

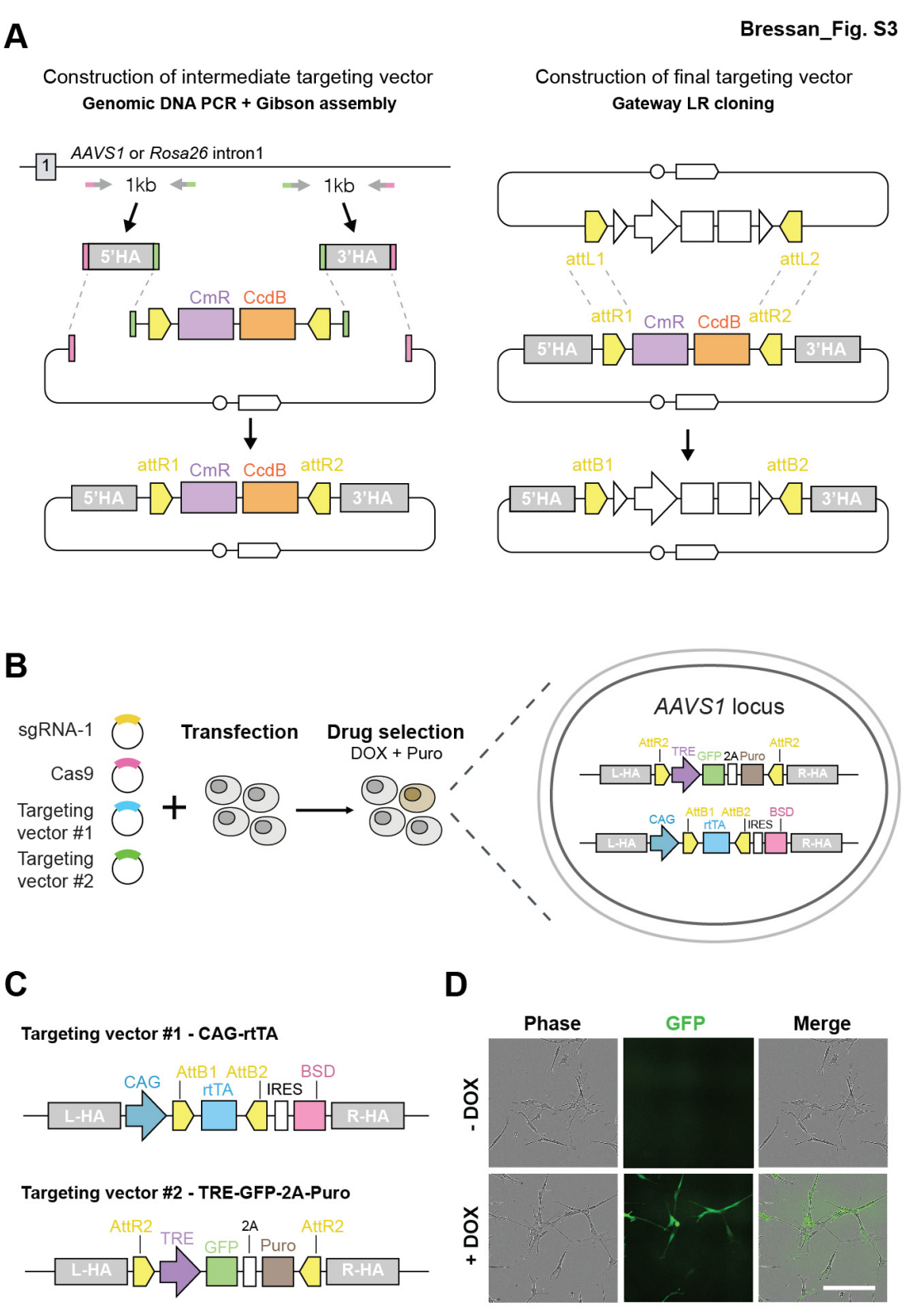

Figure S3 | General strategy for construction of Gateway compatible targeting vectors and generation of Dox-inducible GFP human NS cells by gene

targeting at *AAVS1*.

(A) Construction of intermediate targeting vectors (left panel) involved PCR amplification of homology arms, followed by Gibson assembly of a Gateway compatible vector containing the bacterial double-selection cassette CmR-CcdB. LR Gateway reaction (right panel) was then used to exchange the bacterial selection by a mammalian expression cassette of interest. Empty white boxes represent a generic expression cassette. For generation of targeting vectors for the knockout experiments, the bacterial Zeo-PheS double-selection cassette was used in the intermediate vectors and Ef1a-Puro cassette introduced by LR-Gateway into the final vector (see Material and Methods).

(B) Experimental strategy for biallelic knockin at *AAVS1* in human NS cells. Cells were transfected with two targeting vectors together with sgRNA1 and Cas9 expression plasmids. Cells targeted with both constructs were selected with puromycin in the presence of Doxycyclin (DOX).

(C) Schematic representation of the *AAVS1* targeting vectors containing the components of the Tet-On inducible expression system. rtTA was introduced in one intermediate vector through LR Gateway cloning, while a second vector containing the TRE driving expression of GFP-2A-Puro was generated through conventional cloning (see details in Materials and Methods).

(D) Live cell imaging of drug-selected cells in the presence and absence of DOX, confirming functionality of the Tet-on inducible system in human NS cells. Scale bar 100 um

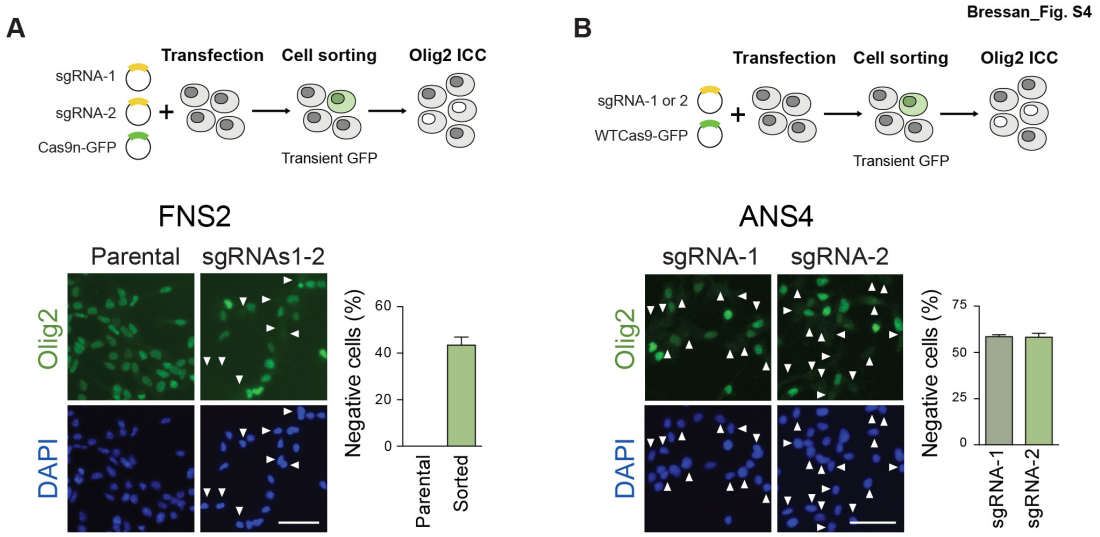

Figure S4 | Olig2 knockout in mouse NS cells using CRISPR/Cas9-induced NHEJ.

(A) Efficient biallelic knockout of Olig2 using transient plasmid delivery of Cas9n-2A-GFP and sgRNA pair in primary foetal forebrain mouse NS cells (FNS2). White arrows indicate Olig2-negative cells. Plot shows percentage of negative cells in relation to the total DAPI-stained nuclei. Scale bar: 50 μm.

(B) Delivery of wild type Cas9 and individual sgRNAs resulted in relatively higher Olig2 knockout efficiencies in the mouse NS cell line ANS4 following GFP-sorting. White arrows indicate Olig2-negative cells. Plot shows percentage of negative cells in relation to the total DAPI-stained nuclei. Scale bar: 50 μm.

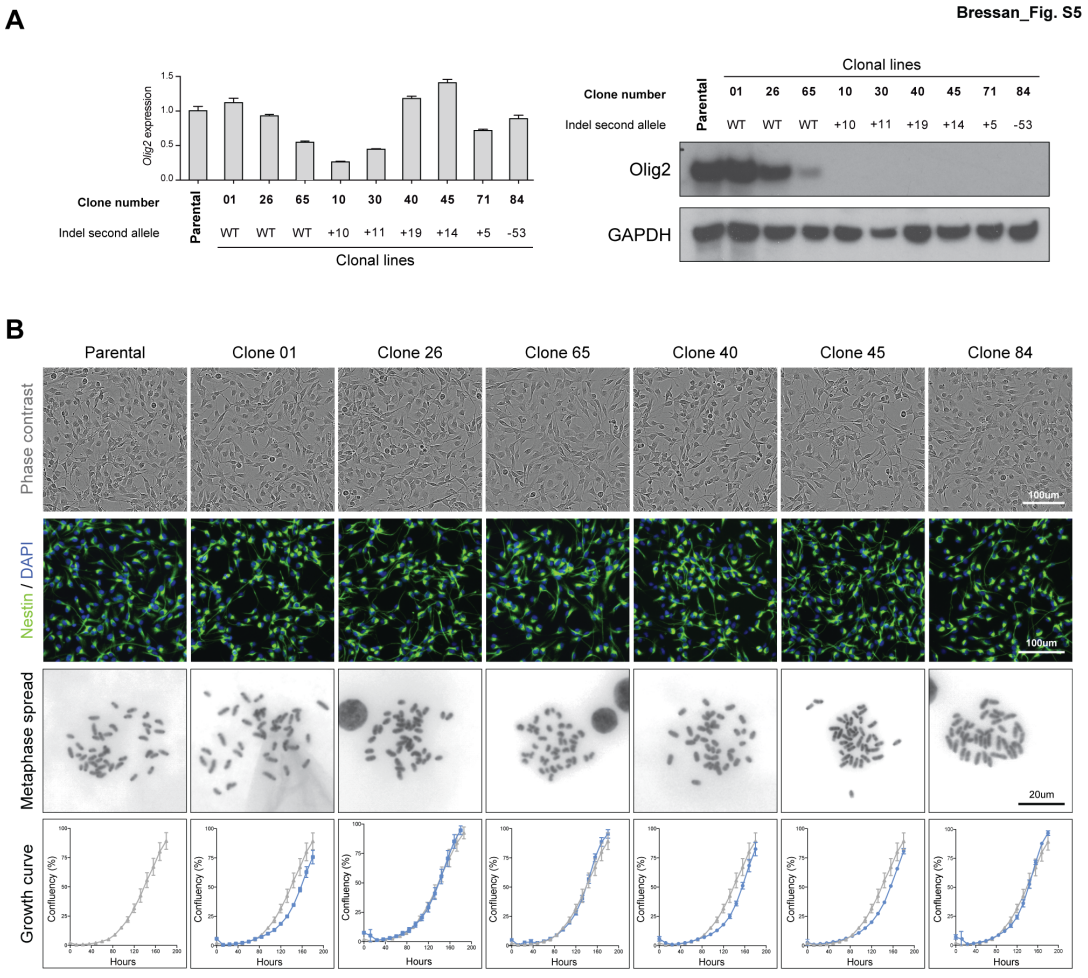

Figure S5 | Characterisation of Olig2 mutant mouse NS clonal lines generated via CRISPR/Cas9-assisted gene targeting

(A) qPCR and Western blotting confirmed complete ablation of Olig2 protein, but not mRNA levels, in clonal lines harboring frame shifting indels on the second allele. Parental cells and targeted clones with non-mutated second alleles were used for comparison.

(B) Olig2 mutant clonal lines maintained a normal karyotype as determined by metaphases spread (modal chromosomal number = 40; n = 20-30), typical NS morphology and expression of Nestin (middle lanes) and proliferated normally under optimal self-renewing conditions (bottom lane). Blue lines represent the confluence curves of the indicated clones. Growth of parental cells (grey line) is shown for comparison.

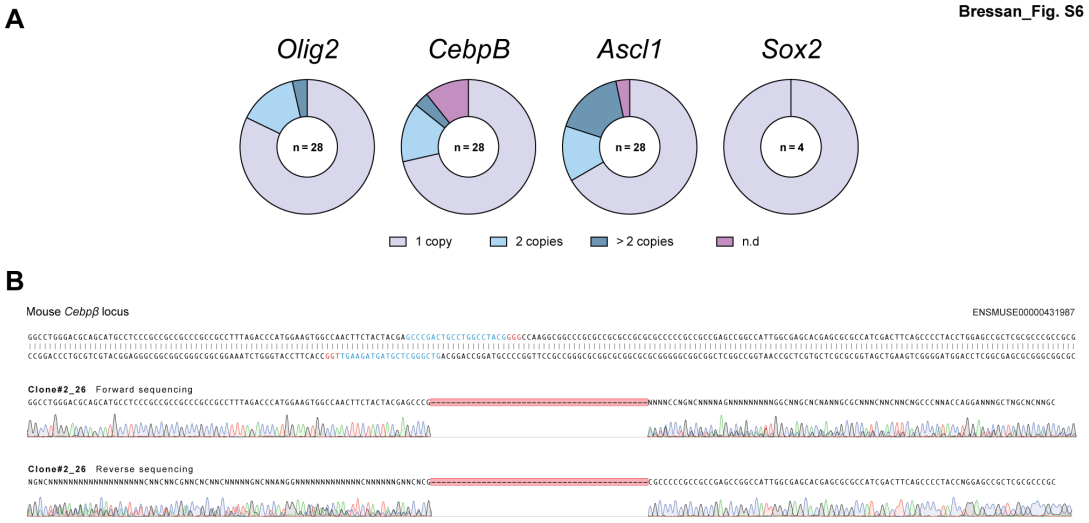

Figure S6 | Summary of copy number analysis of puromycin-resistance gene in mouse clonal lines.

(A) Quantitative PCR analysis using Taqman Copy number assay determining the number of genomic copies of puromycin-resistance cassette in mouse targeted clonal lines. Approximately seventy-five percent of clones showed a single copy insertion.

(B) Exemplar mixed sequencing trace of the non-targeted allele from a *CebpB*-targeted mosaic colony.

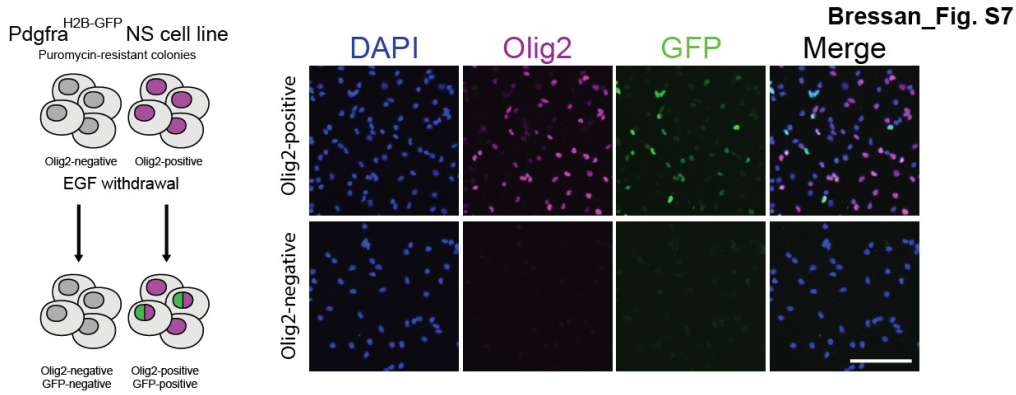

Figure S7 | Olig2 targeting in a mouse foetal PDGFRa $^{H2B\text{-}GFP}$ reporter NS cell line. Following transfection with Olig2 targeting vector and CRISPR sgRNAs, puromycin-resistant colonies were differentiated for 4 days in the absence of EGF. Olig2-negative colonies did not generate GFP-positive, oligodendrocyte precursor-like cells.

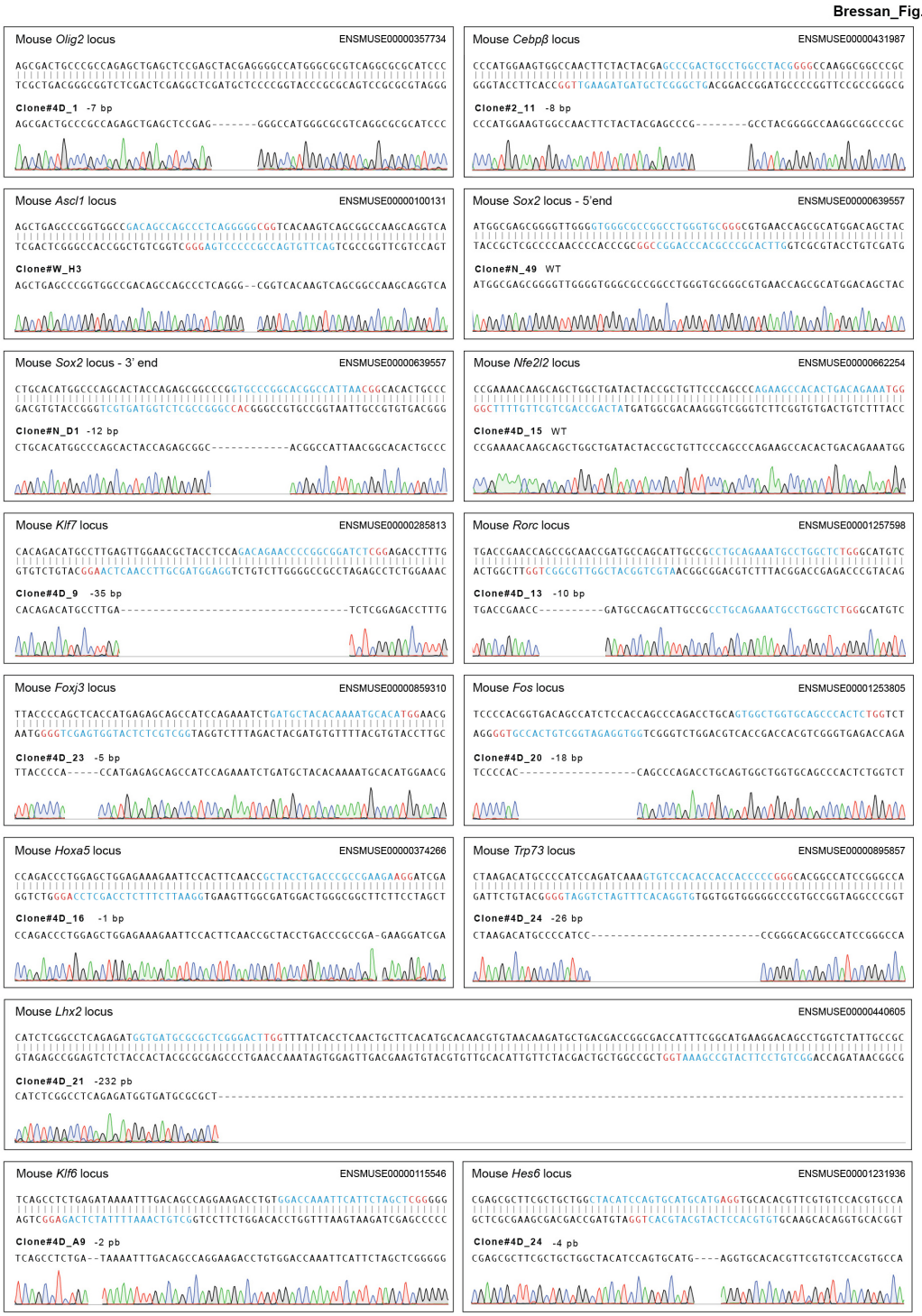

Figure S8 | Representative indels found in the second alleles of the 14 successfully targeted genes. Sanger sequencing traces identify the status of the genomic sequence around the target site of the sgRNA pairs used in the knockout experiments. Targeted gene exons can be identified by the Ensembl exon accession number shown at the top right corner of each box.

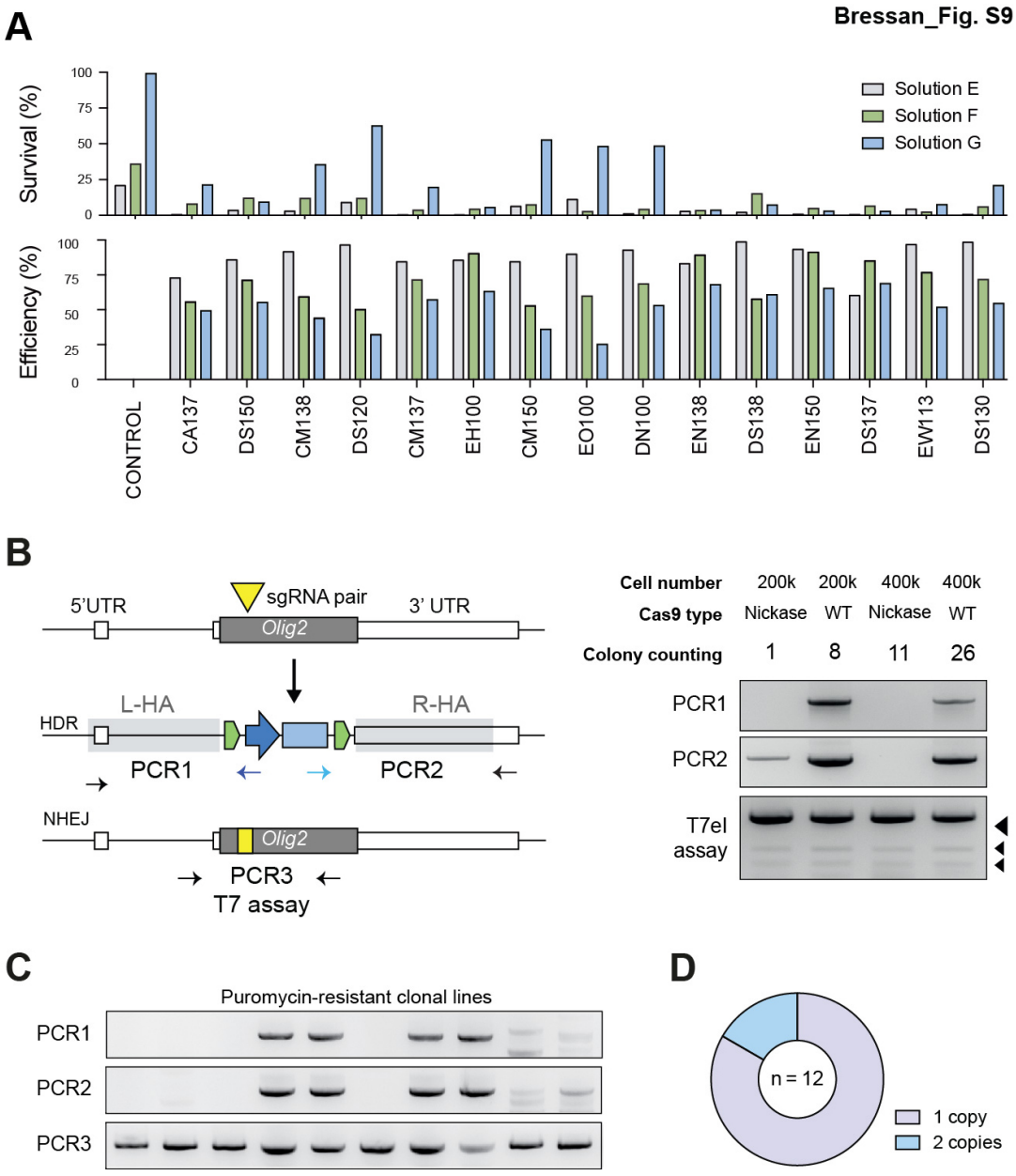

Figure S9 | Optimization of the 4D Amaxa nucleofection system for gene knockout via CRISPR/Cas9 assisted gene targeting.

(A) Optimisation of transfection buffer and nucleofection program using a GFP expression plasmid. Solution G and program DN100 produced the maximal results in terms of cell survival and transfection efficiency.

(B) Olig2 gene targeting in mouse NS cells using the optimized transfection protocol.

Left - Targeting and genotyping strategy used (same as in Fig.3). Right - puromycin-resistant colony counts and PCR genotyping results after transfection of different cell amounts and Cas9 type.

(C) Exemplar genotyping results of puromycin-resistant colonies following Amaxa 4D transfection using WTCas9 and 400k cells.

(D) qPRC copy number analysis of puromycin-resistant gene in *Olig2* correctly targeted clonal lines obtained with Amaxa 4D transfection system.

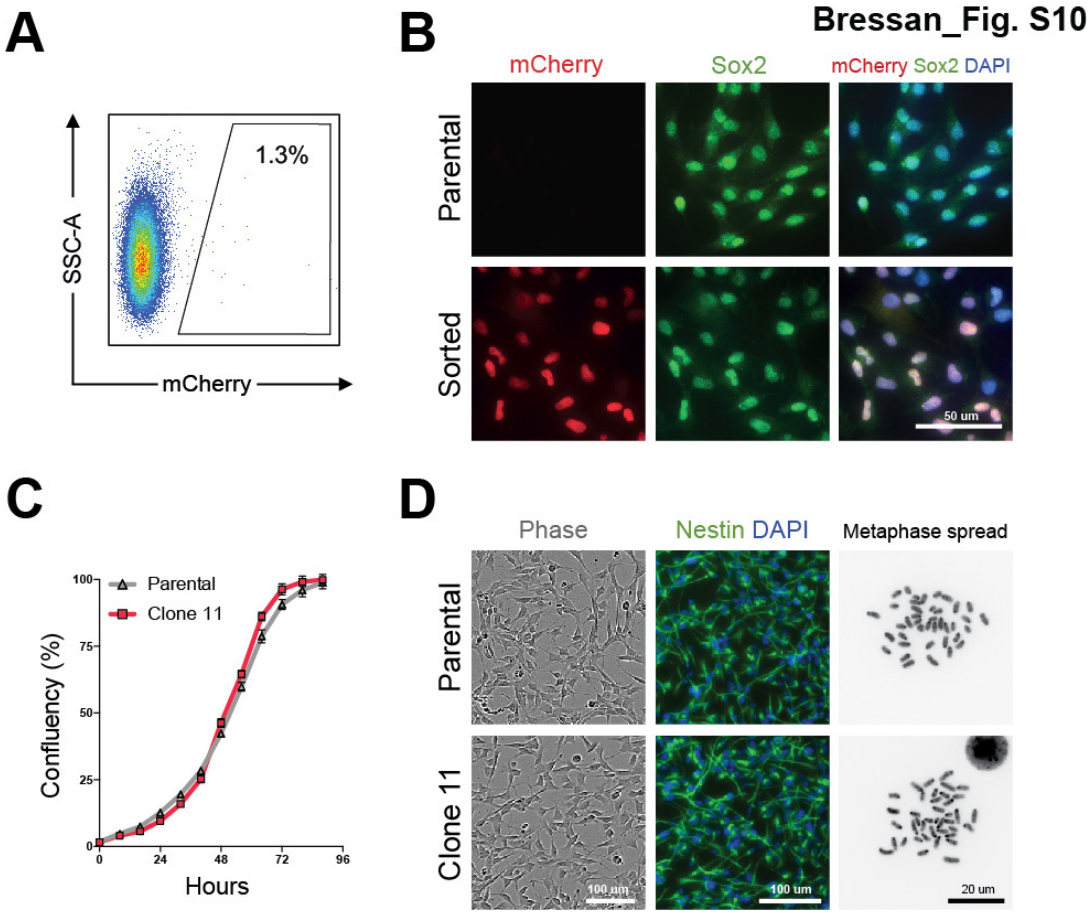

Figure S10| Characterization of mouse Sox2-mCherry reporter NS cell line.

(A) FACS plot indicating the percentage of mCherry positive cells 10 days post-transfection with a promoterless Sox2-mCherry targeting vector. Parental, non-transfected cells were used to set the gates. SSC, side scatter.

(B) ICC analysis confirmed co-localization of mCherry and Sox2 staining in the sorted but not in the parental cells. DAPI counterstaining was used to highlight nuclear localization of Sox2-mCherry staining.

(C) Growth curves of parental ANS4 (grey line) and homozygously targeted Sox-mCherry clonal line (red).

(D) Sox2-mCherry clonal line maintained normal NS morphology, uniform expression of nestin and diploid karyotype (modal chromosomal number = 40).

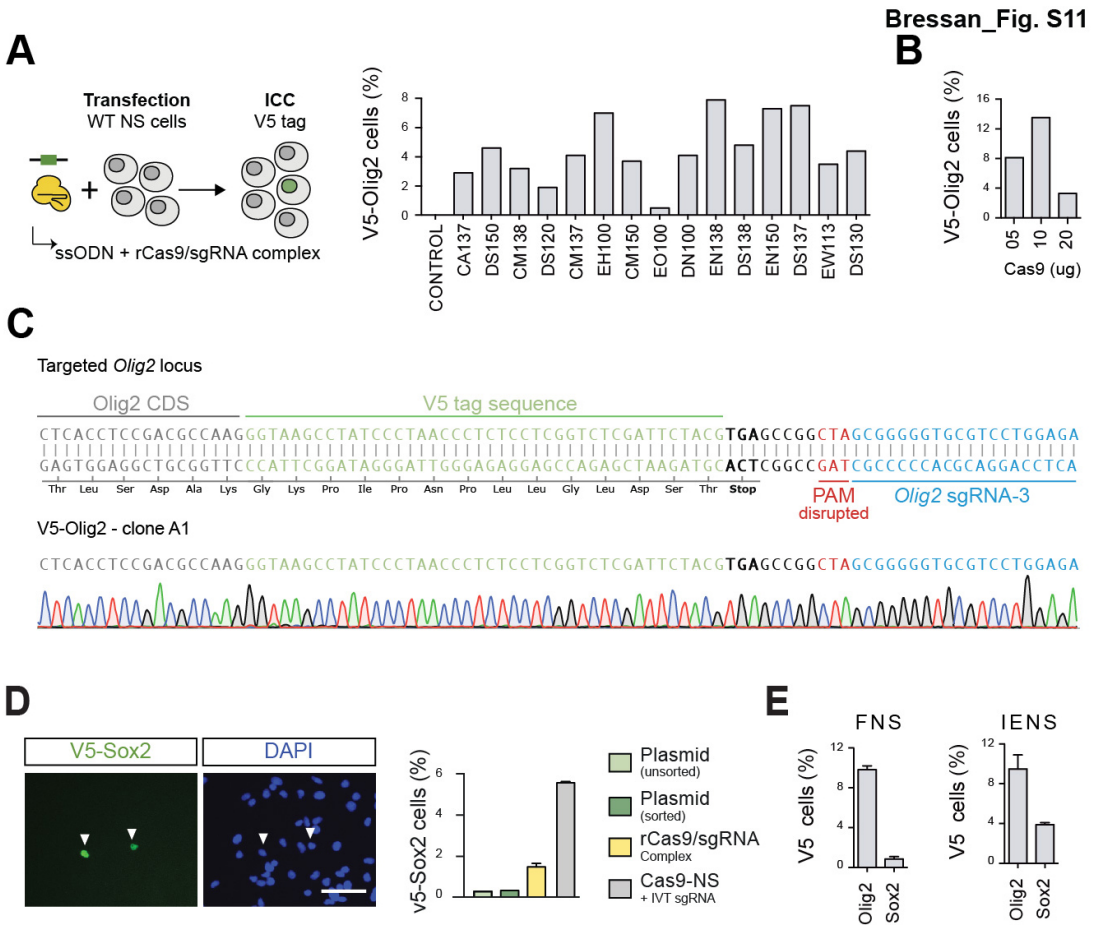

Figure S11 | Epitope tagging in mouse NS cells using single-strand DNA oligonucleotides as donor templates.

(A) Optimization of rCas9/IVT sgRNA delivery for Olig2 tagging using the Amaxa 4D system. 15 transfection programs were tested in SG cell line buffer and knock-in efficiencies quantified by V5 ICC. Program EN-138 shows maximum efficiency and was used for rCas9/IVT sgRNA delivery in all experiments.

(B) Varying amounts of rCas9 were compared for transfection and Olig2 V5 knock-in efficiency.

(C) Sanger sequencing confirms correct, in frame insertion of the V5 tag into Olig2 C'terminus in homozygously tagged clonal line. Different features are highlighted in the sequence shown; Olig2 coding region (grey), V5-tag sequence (green), stop

condon (bold black), PAM (red) and sgRNA sequence (blue). Note that PAM sequence was disrupted to avoid re-cutting after the HR event.

(D) Efficiencies of V5 tagging of Sox2 in mouse NS cells using the different delivery methods. Tagged cells were identified by V5 staining (indicated by the white arrows).

(E) Efficiencies of V5 tagging for Olig2 and Sox2 using rCas9/sgRNA delivery in two independent mouse NS cell lines. FNS – primary foetal forebrain NS cell line; IENS – tumour initiating mouse NS cell line.

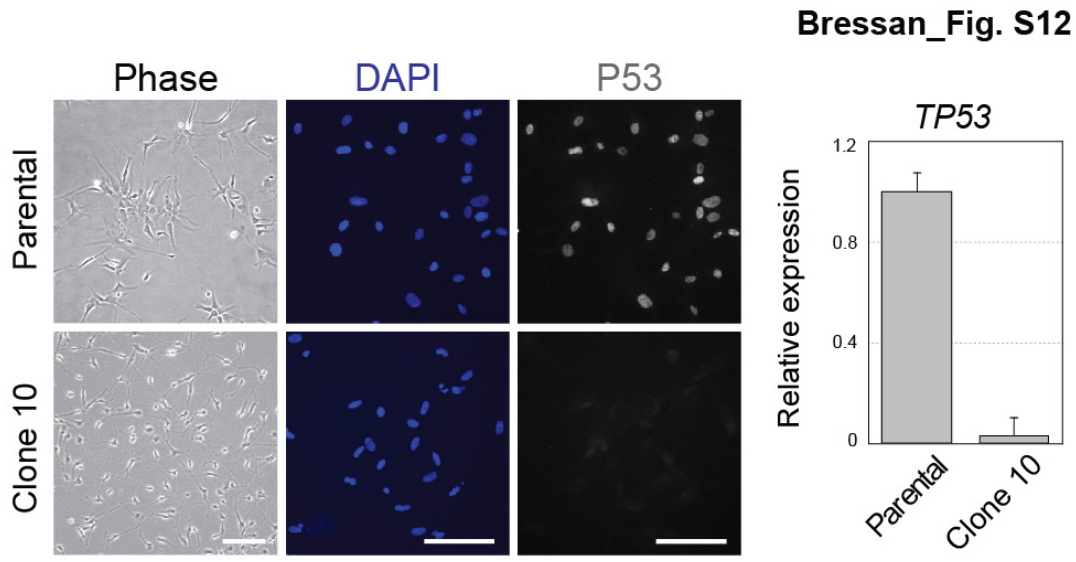

Figure S12 | P53 expression in *TP53*-deleted human NS cell clonal line. Phase contrast images and P53 staining of parental human NS cells and representative *TP53* deleted clonal line. Plot shows levels of *TP53* mRNA levels assayed by qPCR. Scale bar: 100um

Table S1. List of sgRNAs used in this study.

Gene	sgRNA ID	Guide sequence (5' to 3')	PAM	Strand	Species
Olig2	Olig2-1	GCCCATGGCCCCTCGTAGCT	CGG	-	*M. musculus*
Olig2	Olig2-2	GCTGAGCTCCGAGCTACGAG	GGG	+	*M. musculus*
Olig2	Olig2-3	TCTCCAGGACGCACCCCCGC	TGG	-	*M. musculus*
CebpB	CebpB-1	GTCGGGCTCGTAGTAGAAGT	TGG	-	*M. musculus*
CebpB	CebpB-2	GCCCGACTGCCTGGCCTACG	GGG	+	*M. musculus*
Ascl1	Ascl1-1	GACTTGTGACCGCCCCCTGA	GGG	-	*M. musculus*
Ascl1	Ascl1-2	GACAGCCAGCCCTCAGGGGG	CGG	+	*M. musculus*
Sox2	Sox2-1	GTTCACGCCCGCACCCAGGC	CGG	-	*M. musculus*
Sox2	Sox2-2	GTGGGCGCCGGCCTGGGTGC	GGG	+	*M. musculus*
Sox2	Sox2-3	GGGGCCGCTCTGGTAGTGCT	GGG	-	*M. musculus*
Sox2	Sox2-4	GTGCCCGGCACGGCCATTAA	CGG	+	*M. musculus*
Sox2	Sox2-5	CAGCCCTCACATGTGCGACA	GGG	-	*M. musculus*
Sox2	Sox2-6	GAAAGAGATACAAGGGAATT	GGG	+	*M. musculus*
Sox2	Sox2-7	CCAGCCCTCACATGTGCGAC	AGG	-	*M. musculus*
Hes6	Hes6-1	GGTGCACCTCATGCATGCAC	TGG	-	*M. musculus*
Hes6	Hes6-2	GTACATCCAGTGCATGCATG	AGG	+	*M. musculus*
Nfe2l2	Nfe2l2-1	GTCAGCCAGCTGCTTGTTTT	CGG	-	*M. musculus*
Nfe2l2	Nfe2l2-2	GGAAGCCACACTGACAGAAA	TGG	+	*M. musculus*
Klf6	Klf6-1	GCTGTCAAATTTTATCTCAG	AGG	-	*M. musculus*
Klf6	Klf6-2	GGACCAAATTCATTCTAGCT	CGG	+	*M. musculus*
Klf7	Klf7-1	GGAGGTAGCGTTCCAACTCA	AGG	-	*M. musculus*
Klf7	Klf7-2	GACAGAACCCCGGCGGATCT	CGG	+	*M. musculus*
Rorc	Rorc-1	GTGCTGGCATCGGTTGCGGC	TGG	-	*M. musculus*
Rorc	Rorc-2	GCTGCAGAAGTGCCTGGCTC	TGG	+	*M. musculus*
Foxj3	Foxj3-1	GGCTGCTCTCATGGTGAGCT	GGG	-	*M. musculus*
Foxj3	Foxj3-2	GATGCTACACAAAATGCACA	TGG	+	*M. musculus*
Fos	Fos-1	GGTGGAGATGGCTGTCACCG	TGG	-	*M. musculus*
Fos	Fos-2	GTGGCTGGTGCAGCCCACTC	TGG	+	*M. musculus*
Hoxa5	Hoxa5-1	GGAATTCTTTCTCCAGCTCC	AGG	-	*M. musculus*
Hoxa5	Hoxa5-2	GCTACCTGACCCGCCGAAGA	AGG	+	*M. musculus*
Lhx2	Lhx2-1	GGCTGTCCTTCATGCCGAAA	TGG	-	*M. musculus*
Lhx2	Lhx2-2	GGTGATGCGCGCTCGGGACT	TGG	+	*M. musculus*
Trp73	Trp73-1	GTGGACACTTTGATCTGGAT	GGG	-	*M. musculus*
Trp73	Trp73-2	GTGTCCACACCACCACCCCC	GGG	+	*M. musculus*
Rosa26	Rosa26-1	CGCCCATCTTCTAGAAAGAC	TGG	-	*M. musculus*
Rosa26	Rosa26-2	AGTCTTTCTAGAAGATGGGC	GGG	+	*M. musculus*
AAVS1	AAVS1-1	GTCACCAATCCTGTCCCTAG	TGG	-	*H. sapiens*
AAVS1	AAVS1-2	GGGGCCACTAGGGACAGGAT	TGG	+	*H. sapiens*
H3F3A	H3F3A-1	GATCACCCCCTCCCCAAATC	TGG	-	*H. sapiens*
H3F3A	H3F3A-2	GGCAGGAAAAGTTGTATGTT	TGG	+	*H. sapiens*
TP53	TP53-1	GATGGCCATGGCGCGGACGC	GGG	-	*H. sapiens*
TP53	TP53-2	GAGCGCTGCTCAGATAGCGA	TGG	+	*H. sapiens*

Table S2. Primer sequences

Primers used for HDR-based knockout experiments - related to Figure 3 and Table 1

		Primers for cloning 5'HA		Primers for cloning 3'HA		Primers for screening 5' targeting		Primers for screening 3' targeting		Primers for screening NHEJ allele	
Locus	**Species**	Forward	Reverse	Forward	Reverse	Forward	Reverse	Forward	Reverse	Forward	Reverse
Olig2	*M. musculus*	CCTCCAGGAAAAGCACATTG	CCCAGGGATGATCTAAGCTCT	TGCAAGGCAGGTGTTTTCAGT	CTGCCAGTGATCACCCAAC	AATTAGCCGGGTGACATCAG	GCGATCTCTGGGTTCTACGTTAGTG	TCTATAGTCGCAGTAGGCGG	CAGCTGGGAGGAAAGAAACA	CTCTCCTCAAGTTCGGAAGG	TGGCGATCTTGGAGAGCTT
CebpB	*M. musculus*	GTTAGTGAAGCCCCAACGTC	CCCAACTCTTGGGAAACAGA	TGAGAACCTTTTCCGTTTCG	TGAGAGCCACAAGCTATCCA	GCTCACTCTCTGCCTTCCTG	AAGGCGCATAACGATACCAC	TCTATAGTCGCAGTAGGCGG	ACCCTGTGACAGTGGGAGAC	CCTTATAAACCTCCCGCTCG	GAGGTCGGAGAGGAAGTCG
Ascl1	*M. musculus*	ACACTTCCCCACACCACTGT	ATTTGGTTGAACCCCCTTTC	CCCTCTTAGCCCAGAGGAAC	GAAAGGCTGTCCGAGAACTG	AATGCCCTCCTTTCCCATAG	GCGATCTCTGGGTTCTACGTTAGTG	CATGTCTGGATCCGGGGGTACCGCGTCGAG	A	AGAGCTCTGGCAAGATGGAG	CTTGCTCATCTTCTTTGTTGGC
Sox2 (sgRNAs 1-2)	*M. musculus*	GCGACAACAAGAGAAACAAAACTA	CTCTTCTTTCTCCCAGCCCTA	CTCCATTATGCACAGTTTGAGA	AATGTGATGAGAGCGCACAA	CATCAGGACTTTCTTCCTTCTCTC	AAGGCGCATAACGATACCAC	CATGTCTGGATCCGGGGGTACCGCGTCGAG	CCCTTCCCAGTACCTTACCC	GCTTCCCCCCAACTATTCTC	TGATCTCCGAGTTGTGCATC
Sox2 (sgRNAs 3-4)	*M. musculus*	GCGACAACAAGAGAAACAAAACTA	CTCTTCTTTCTCCCAGCCCTA	CTCCATTATGCACAGTTTGAGA	AATGTGATGAGAGCGCACAA	CATCAGGACTTTCTTCCTTCTCTC	AAGGCGCATAACGATACCAC	CATGTCTGGATCCGGGGGTACCGCGTCGAG	CCCTTCCCAGTACCTTACCC	GCTTCCCCCCAACTATTCTC	TGATCTCCGAGTTGTGCATC
Hes6	*M. musculus*	AATGGATGGGTGAAGAGGTGGG	GACACAGTAGCGTGTCCCGTTC	CTCTGTGTTCCCCCACTTTCC	ATTATTTCATGCACCCTGCATC	CTCGTACTTTCCCCGGACTT	AAGGCGCATAACGATACCAC	TCTATAGTCGCAGTAGGCGG	TTTGGACCCCAACTTATGGA	GCCACGATAGTCACTGCTCA	CCGGTTTAGTTCAGCCTCTG
Nfe2l2	*M. musculus*	CCCACATTCCCAAACAAGATG	CCAAACTTGCTCCATGTCCTG	TTATAGCCATGTGTGGCAGTC	AGGGCCTTTGTTTTTATTCCC	GGGAGGTTATTGCCTAGCAC	GCGATCTCTGGGTTCTACGTTAGTG	TCTATAGTCGCAGTAGGCGG	CCCAGCACCCAACTTTACA	GATTTGGGGGTAGGGAATTG	CCGTCCAGGAGTTCAGAGAG
Klf6	*M. musculus*	GAGGCCGGGACTTAGGATGT	TTCACCCAACAGGACAGAAAT	CATCGTGCTATGTTGGGTTGC	TTCTCCTGGGAAAAAGAGCAC	GTCTTCGCCATTTCTTTGGA	GCGATCTCTGGGTTCTACGTTAGTG	CATGTCTGGATCCGGGGGTACCGCGTCGAG	CCATGACCCCACACTTCTTT	TGACCCACAATACCTGACACA	AATTTGGTCGTGGGTGAAAG
Klf7	*M. musculus*	ACAGACACCACATGACTTGC	AAATCCATAGAACCCCAACAT	TTCCTCTTTTGGGTGTTCCCTT	GATAGTGCAGGCCTTTAAGTCC	TGAAGCCCAAAGCTGATTTT	GCGATCTCTGGGTTCTACGTTAGTG	TCTATAGTCGCAGTAGGCGG	GCAACTTCCTTGGTGACACA	AGTCATTCCATGTGGCCTTG	GAGCTCTTCTCGCAGATGGT
Rorc	*M. musculus*	CAGTCGTGTCCTGTGCTCATA	CAGGGACCTCCAATTCATGTGC	TCAAGGCTCTTCAGCCTCTA	ACAGCTGAAGAAGGTTCAGGAA	CCAGTGCACATGAATTGGAG	AAGGCGCATAACGATACCAC	TCTATAGTCGCAGTAGGCGG	TCCCAGCTGGAGGAGTCTT	GGGGACTTTGGTGGATGTAG	GGAGCAGAGCCTGGAGTAGA
Foxj3	*M. musculus*	GGCTGATTTTTGCTATGCTT	GAAGAGAGAGGGGAGAAAGGG	ATATGGAGACCGAAGGACAAC	GCCTCACACTCACAGAGACAT	CACAGCTGGAAACTGTTGGA	AAGGCGCATAACGATACCAC	TCTATAGTCGCAGTAGGCGG	TGCTGAAATGCAAACTCCAG	AAGCTTTGCCTCATTTTGGA	CAACCACTGCCAGCTTCTCT
Fos	*M. musculus*	GCCCAGTGACGTAGGAAGTC	TTCCTGTATGCACCTCATCGGA	TTGACAGTTGGACCCAAGACA	TAAGTAGTGCAGCCCGGAGT	GGACCATCTCCGAAATCCTA	AAGGCGCATAACGATACCAC	TCTATAGTCGCAGTAGGCGG	TTGCCTTCTCTGACTGCTCA	GACACTAGGGCCACTTGCTC	GCAGTCAGTAGCGTCCCTTC
Hoxa5	*M. musculus*	TACAATGGCATGGATCTCAGC	TTAAAGTTCCAGCCGAGCTA	TGTAGACCCCAAACCCTCTG	TTCAACTCCCAGAGCTCCACGA	TAATGGAACTGCGAGGGAAA	AAGGCGCATAACGATACCAC	CATGTCTGGATCCGGGGGTACCGCGTCGAG	CCCGCCTCAATTCAGTCTT	CCAGAAAGGAAGGGAGAAGG	GTACTTTGGCCGCTCAGATG
Lhx2	*M. musculus*	AGCATAGCTGTAGGCTGTCC	CCTGCCTGAAATAGTTTTCC	TAGTTCATTGGTGATCCAGG	GGCTAGTGGGTGAGCTCTAA	AGCTTGGGCCTGGTAGATG	AAGGCGCATAACGATACCAC	TCTATAGTCGCAGTAGGCGG	CCCTCTTTTTGCTCAGTCACA	CAGTGACAGCAACAGCACCT	CTGGACTCTTGCGCTTTCTC
Trp73	*M. musculus*	GAGAAACCCTGTCTCGAAAA	GACCGTTGCATAAGGATGACA	AGCAAGGGTGTAGTCTGAGG	ACCGATCATCTCTGTCTTCCC	TTCATTGCATTTGCCTATGG	AAGGCGCATAACGATACCAC	TCTATAGTCGCAGTAGGCGG	CCTGTCCCACTGACATTCAA	ATCTGAAGCATGCCTGAAGC	TTCCTGTGAGGGGAGAGTTG
Rosa26	*M. musculus*	CCGCGGCAGGCCCTCCGA	TGCAACTCCAGTCTTTCTAG	AGATGGGCGGGAGTCTTCTG	CCTGACCTAAGGTCTTGTGA	AGAGCCTCGGCTAGGTAG	ATTGACGTCAATGGGCGG	AGCTTGGCGTAATCATGGTC	CCTGAAGAAGCTTGGCAAA	TAAGGGAGCTGCAGTGGA	AGGCGGATCACAAGCAATA
AAVS1	*H. sapiens*	TTACCATCCCTCCCTCGACT	CCGAGCTGGGACCACCTTAT	TCCTTAGGCCTCCTCCTTCC	TAGCACTGAAACCCTCAGTC	CCACTCTGTGCTGACCACTC	GGGCGTACTTGGCATATGAT	TATCCGCTCACAATTCCACA	TGACCAACCATCCCTGTTTT	CTTGCTTTCTTTGCCTGGAC	AGGATCCTCTCTGGCTCCAT
TP53	*H. sapiens*	CCCCTAGCAGAGACCTGTGGGAA	GTTGCTTTATCTGTTCACTT	TCTGATTCCTCACTGATTGCT	ATCCTGAGTAGTGGTAATCTAC	GTGAGCGGTGAGACAGTTGTTCTTCCAG	GCGATCTCTGGGTTCTACGTTAGTG	CATGTCTGGATCCGGGGGTACCGCGTCGAG	GTTCTGAAGTTAGTTAGCTACA	ATCACACCACTGTGCTCCAG	GGGAGGTCAAATAAGCAGCA
H3F3A	*H. sapiens*	GATAGTTTCCCTGTTCCCTCTG	AAGGCACAAACCACTGGAAG	ATCCCTAACCCTCTCCTCGGT	TAAAATGGTGGGTGGGAAGA	n/a	n/a	ATCCCTAACCCTCTCCTCGGT	TCTTCCCACCCACCATTTTA	n/a	n/a

CAG-LUC-2A-GFP-IRES-BSD specific primers

EF1a-PuroR specific primers

V5 tag specific primers

Gene specific primers

Primers used for epitope tagging experiments - related to Figure 5

V5 tag forward	ATCCCTAACCCTCTCCTCGGT"
Olig2 C' forward	TGTCCTCGGTCGGATCCAT
Olig2 C' reverse	TTCAAATGCTCCCATTGCTT

V5 tag specific primers