File S1

Supplementary methods

TALEN contruction and expression

For the expression of TALENs in mammalian cells we used the expression vector pCAG-TALEN, that contains a CAG hybrid promoter region and a transcriptional unit comprising a sequence coding for the N-terminal amino acids 1 - 176 of TAL nuclease, located upstream of a pair of *Bsm*BI restriction sites, as described (17). This N-terminal regions includes an ATG start codon, a nuclear localisation sequence, a FLAG Tag sequence, a glycine rich linker sequence, a segment coding for 110 amino acids of the TAL protein AvrBs3 and the invariable N-terminal TAL repeat of the Hax3 TAL effector. Downstream of the central *Bsm*BI sites, the transcriptional unit contains 78 codons including an invariable C-terminal TAL repeat and 44 residues derived from the TAL protein AvrBs3, followed by the coding sequence of the FokI nuclease domain and a polyadenylation signal sequence. DNA segments coding for arrays of TAL repeats can be inserted into the *Bsm*BI sites of pCAG-TALEN in frame with the up- and downstream coding regions to enable the expression of predesigned TAL-Fok nuclease proteins. To derive TAL element DNA-binding domains we used the TAL effector motif (repeat) #11 of the Xanthomonas Hax3 protein (LTPEQVVAIAS**NI**GGKQALETVQRLLPVLCQAHG) to recognize A, the TAL effector motif #5 (LTPQQVVAIAS**HD**GGKQALETVQRLLPVLCQAHG) derived from the Hax3 protein to recognize C, and the TAL effector motif #4 (LTPQQVVAIAS**NG**GGKQALETVQRLLPVLCQAHG) from the *Xanthomonas* Hax4 protein to recognize T. To recognize a target G nucleotide we used the TAL effector motif #4 from the Hax4 protein with replacement of the amino acids 12 into N and 13 into N (LTPQQVVAIAS**NN**GGKQALETVQRLLPVLCQAHG). These elements were obtained by gene synthesis (Genscript, Piscataway, NJ, USA) and further amplified by PCR using primers that include BsaI sites outside of the coding region. For a 15 bp TALEN target sequence seven elements each are pooled in a pair of reactions together with *Bsa*I and T4 DNA ligase to create and ligate unique overhangs. Full length ligation products were recovered by gel extraction and inserted by seamless cloning (Gibson assembly, New England Biolabs) into pCAG-TALEN opened with *Bsm*BI. The integrity of all TALEN expression vectors was confirmed by DNA sequencing. The complete sequence of the vector encoded TALEN proteins is shown below [blue letters, nuclear localization sequence; red letters, FLAG tag; green letters, AvrBs3 N- and C-terminal sequences; black letters, TAL repeats (RVDs are underlined); orange letters, FokI nuclease domain; gray letters, linker sequences]:

TALEN-Fus15A

MGPKKKRKVAAADYKDDDDKPGGGGSGGGGVPASPAAQVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVA

VKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQSGLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPQQVVAIASH

DGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQ

QVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQA

HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQR

LLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQ

ALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIAS

HDGGRPALESIVAQLSRPDPALARSALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPEGSDRLNQLVKSELEEKKSELRHKLKYVPHEYIE

LIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW

KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

TALEN-Fus15B

MGPKKKRKVAAADYKDDDDKPGGGGSGGGGVPASPAAQVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVA

VKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQSGLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNI

GGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVV

AIASNIGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGL

TPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPV

LCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALET

VQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDG

GRPALESIVAQLSRPDPALARSALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPEGSDRLNQLVKSELEEKKSELRHKLKYVPHEYIELIEIA

RNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYP

SSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

TALEN-Rik2A

MGPKKKRKVAAADYKDDDDKPGGGGSGGGGVPASPAAQVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVA

VKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQSGLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPQQVVAIASN

NGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQ

QVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQA

HGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLL

PVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQA

LETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASH

DGGRPALESIVAQLSRPDPALARSALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPEGSDRLNQLVKSELEEKKSELRHKLKYVPHEYIELI

EIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKV

YPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

TALEN-Rik2B

MGPKKKRKVAAADYKDDDDKPGGGGSGGGGVPASPAAQVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVA

VKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQSGLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPQQVVAIASH

DGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQ

QVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQ

AHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQ

RLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQA

LETVQRLLPVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNK

GGRPALESIVAQLSRPDPALARSALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPEGSDRLNQLVKSELEEKKSELRHKLKYVPHEYIELIE

IARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVY

PSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF

TALEN RVD arrays:

TALEN	**RVD sequence (w/o last half repeat)**
TALEN-Fus15A Target sequence (5'-3')	**HD NG NG NG HD NI NN NN NN NN HD NN NI NN** ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ **C T T T C A G G G G C G A G**
TALEN-Fus15B Target sequence (5'-3')	**NI NI NG NI NG NN NN HD HD NG HD NG HD HD** ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ **A A T A T G G C C T C T C C**
TALEN-Rik2A Target sequence (5'-3')	**NN NG HD NN NI HD NG NI NG HD NG NN HD HD** ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ **G T C G A C T A T C T G C C**
TALEN-Rik2B Target sequence (5'-3')	**HD NG NN NG HD NG NG NN NN HD NI NI HD NI** ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ **C T G T C T T G G C A A C A**

Construction of reporter plasmids

Nuclease reporter plasmids were generated by the insertion of annealed sense and anti-sense oligonucleotides (Metabion, Martinsried, Germany), harboring the TALEN target sequences, (underlined) into the generic reporter plasmid pTAL-Rep, as described (17).

Fus target sense oligonucleotide:

5'-cggccaccatggtcgtctttcaggggcgagcacagacaggatcgcagggagaggccatattatg-3'

Fus target anti-sense oligonucleotide:

5'-cataatatggcctctccctgcgatcctgtctgtgctcgcccctgaaagacgaccatggtggc-3'

C9orf72 target sense oligonucleotide:

5'-cgtgtcgactatctgccccccaccatctcctgctgttgccaagacaga-3'

C9orf72 target anti-sense oligonucleotide:

5'-tctgtcttggcaacagcaggagatggtgggggcagatagtcgaca-3'

The integrity of all reporter plasmids was confirmed by DNA sequencing.

For the assessment of TALEN nuclease activities, expression vectors were cotransfected with the corresponding reporter plasmid into HEK 293 cells. Two days after transfection the cells were lysed and β-galactosidase was determined by chemiluminescense, as described (17).

RNA isolation and cDNA synthesis

Total RNA was isolated from tail tips using Trizol® (Life Technologies, Carlsbad, USA). Total RNA was reverse transcribed into cDNA using the Protoscript M-MuLV Taq-RT PCR kit (New England Biolabs) in presence of oligo $dT_{23}VN$, according to the manufacturer's protocol. Synthesized cDNA was diluted four times for further experiments.

PCR amplification, cloning and sequencing

Amplification of the diluted cDNA was performed by using Herculase II polymerase in 50 µl reactions with 30 cycles of {95 °C, 20 s; 51.5 °, 20 s; 72 °C, 30 s} using *Fus* specific primers (Table S3) to amplify 341 nucleotides spanning exon 14, exon 15 (which

encodes the NLS) and the 3'-UTR of the *Fus*, mRNA sequence. The resulting amplified product was subcloned using the StrataClone Blunt PCR Cloning Kit (Agilent, Waldbronn, Germany) and sequenced.

A hot start PCR protocol was employed for the amplification of genomic *Fus* gene fragments covering sequences 3.5kb upstream and 3.3 kb downstream of codon 513. PCR was carried out using Herculase II polymerase in 50 µl reactions with the 30 cycles of {95 °C, 20 s; 63 °, 20 s; 72 °C, 2 mins} using the gene specific primers (See Table S3: *Fus* 5' US and *Fus* 3' DS). PCR products were isolated by gel extraction (Qiagen, Hilden, Germany) and directly sequenced.

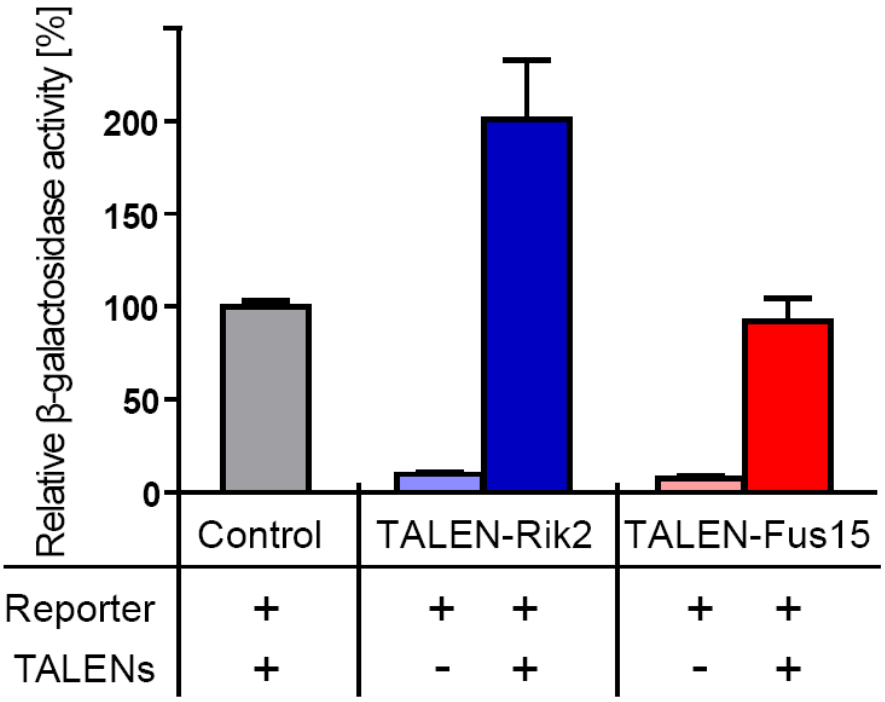

Figure S1 TALEN activity in HEK 293 cells.

Cotransfection of reporter plasmids with TALEN-Rik2 or TALEN-Fus-15 vectors into HEK293 cells leads to nuclease dependent gene repair and β-galactosidase expression, as compared to the transfection of the reporter alone. Values are expressed as nuclease stimulated increase of β-galactosidase activity as compared to background levels without nuclease vectors (-) and to a TALEN/reporter standard (Control). The error bars represents s.d. in three replicates.

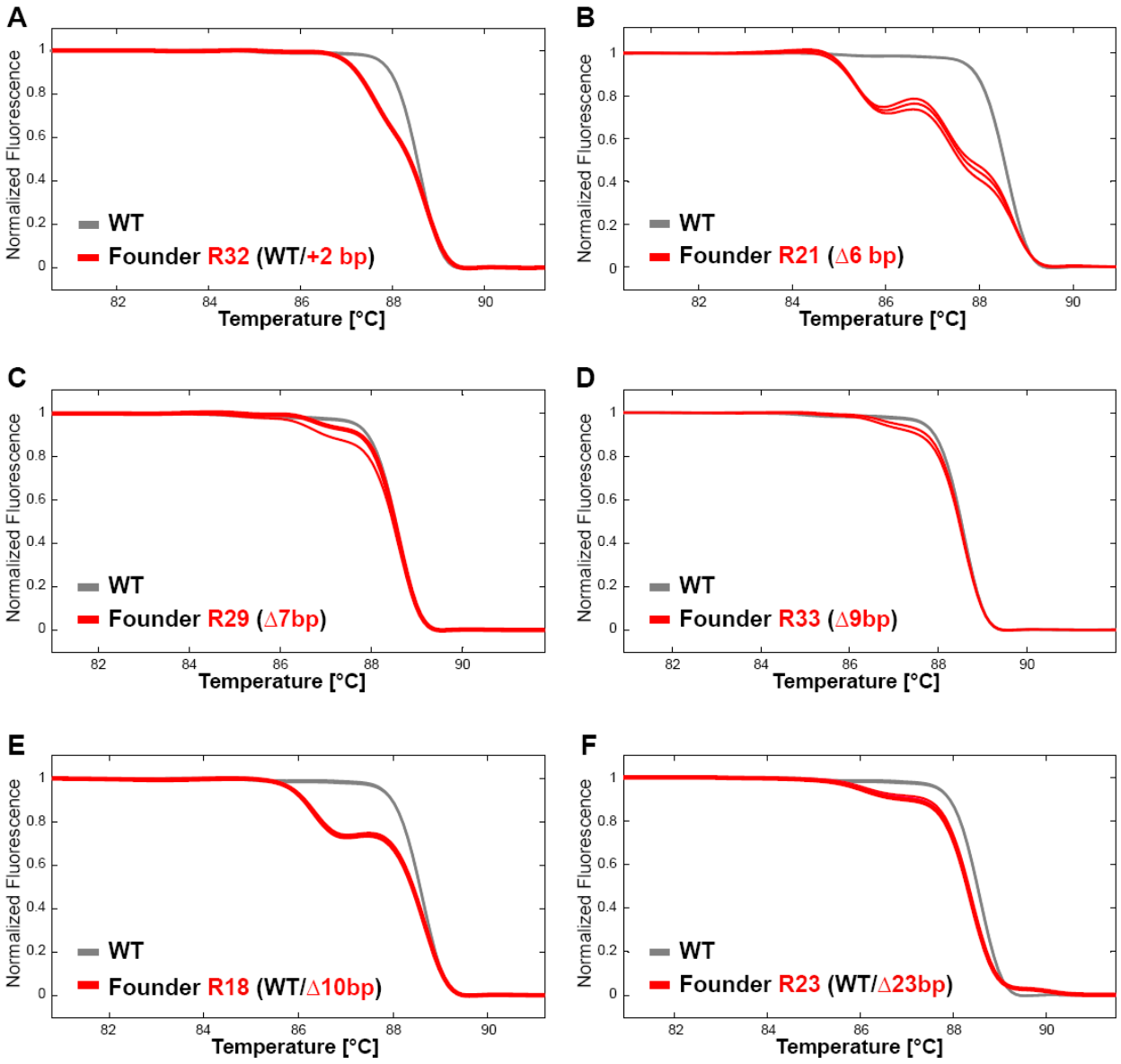

Figure S2 HRMA for the identification of *C9orf72* founder mutants.

Melting analysis of PCR products amplified in triplicate from tail DNA of founder R32 **(A)**, R21 **(B)**, R29 **(C)**, R33 **(D)**, R18 **(E)**, and R23 **(F)** (red curves, overlayed) or from a wildtype control mouse (grey curves, overlayed) demonstrated the presence of mutant allele(s). The genotype of mutant alleles is indicated in parentheses; see Figure 3 for the allele sequences.

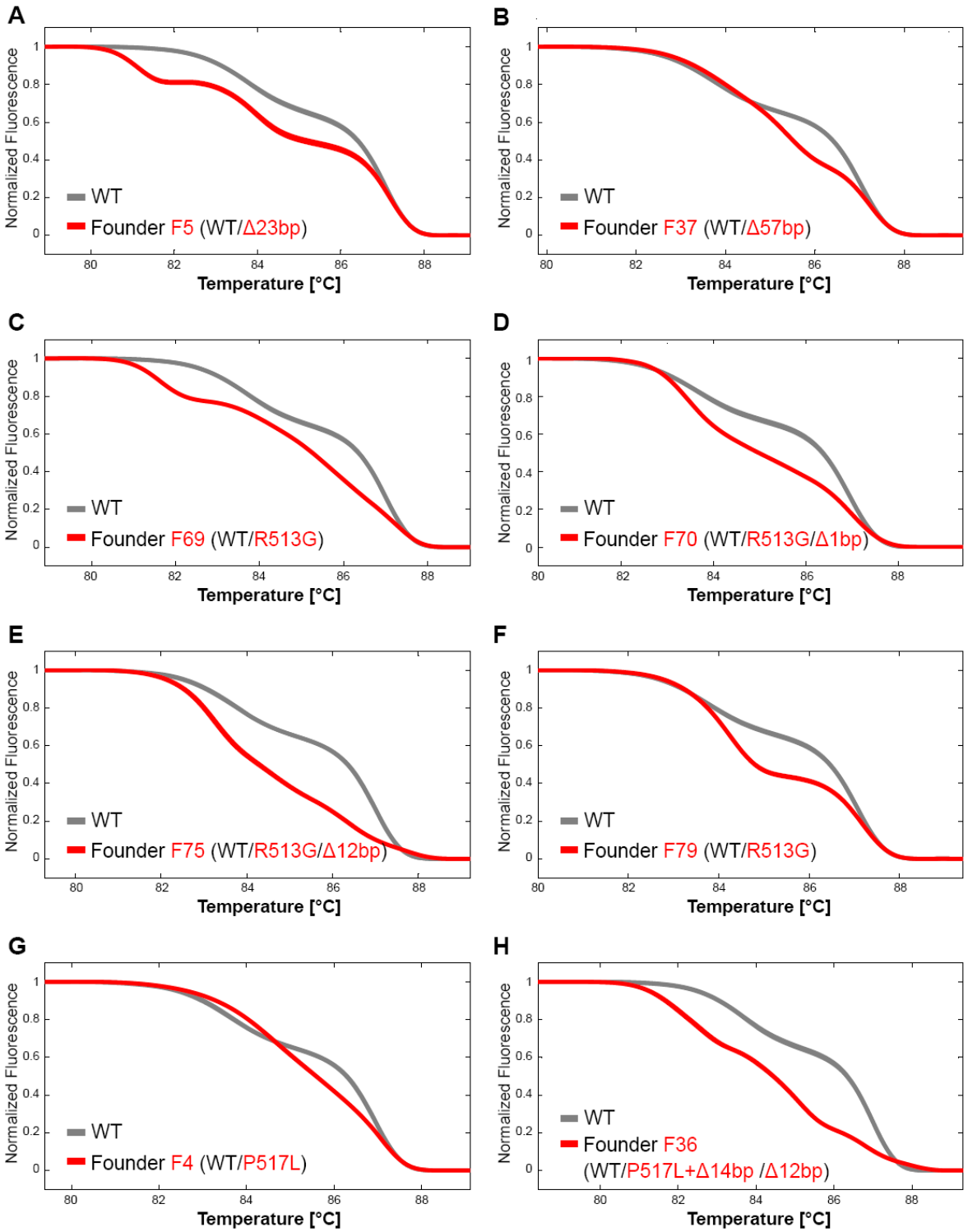

Figure S3 HRMA for the identification of *Fus* founder mutants.
Melting analysis analysis of PCR products amplified in triplicate from tail DNA of founder F5 **(A)**, F37 **(B)**, F69 **(C)**, F70 **(D)**, F75 **(E)**, and F79 **(F)** using TALEN-Fus15 and ODN^{R513G} and of founder F4 **(G)**, and F36 **(H)** using TALEN-Fus15 and ODN^{P517L} (red curves, overlayed), or from a wildtype control mouse (grey curves, overlayed). The genotype of mutant alleles is indicated in parentheses; see Figure 4 for the allele sequences.

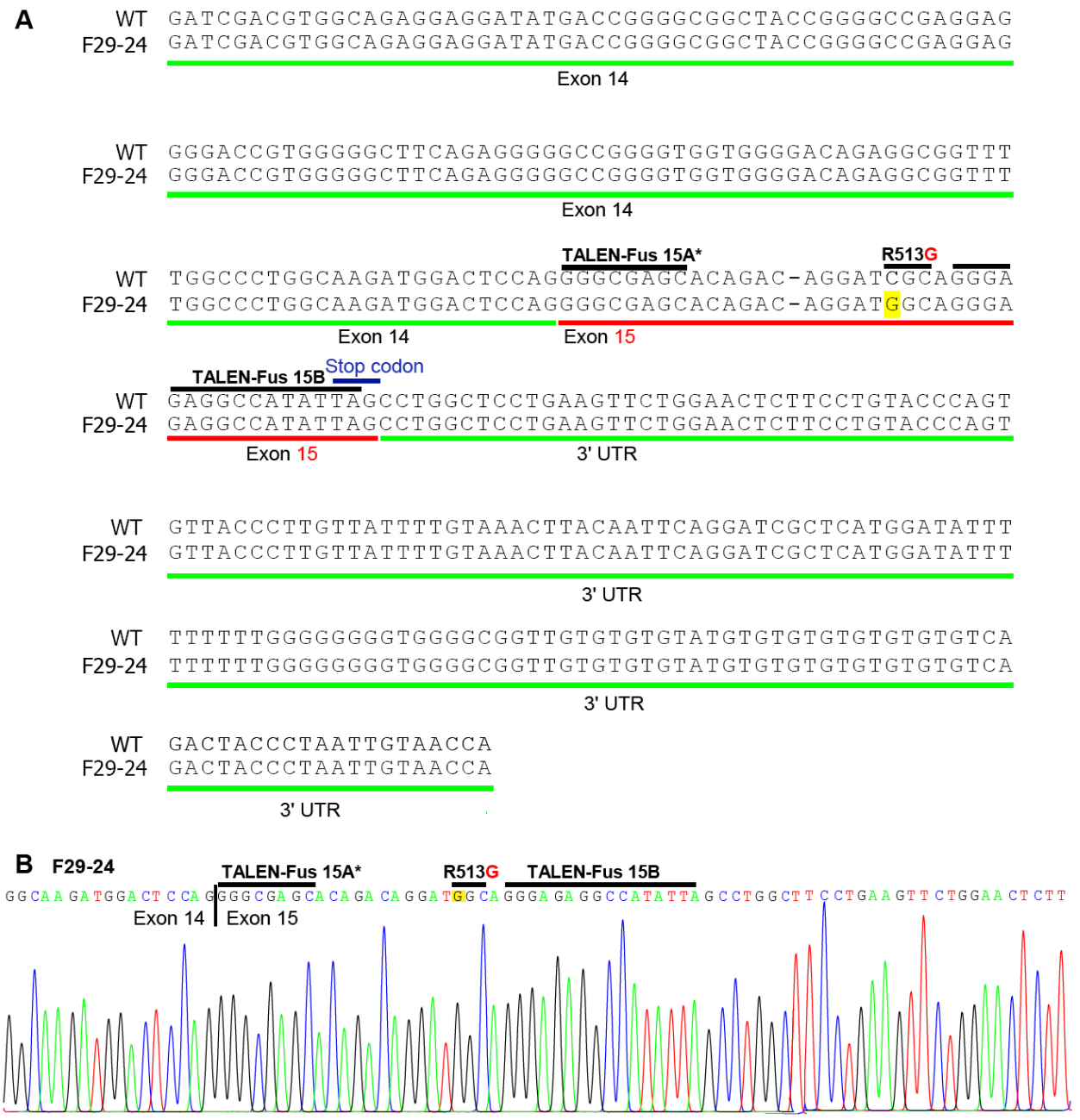

Figure S4 Sequence analysis of *Fus*^{R513G} cDNA.
(A) Sequence comparison of the cloned PCR product representing 341 bp of the *Fus* cDNA sequence, including exon 14, exon 15 and 3´UTR sequences, derived from the mutant pup F29-24 in comparison to wildtype cDNA sequence. **(B)** Chromatogram showing the sequencing peaks of the cloned PCR product covering the *Fus* codon 513 (nucleotide replacement highlighted in yellow). The positions of exon 14, exon 15, exon boundary, stop codon, 3' UTR, TALEN binding sites and of the R513G replacement are indicated.

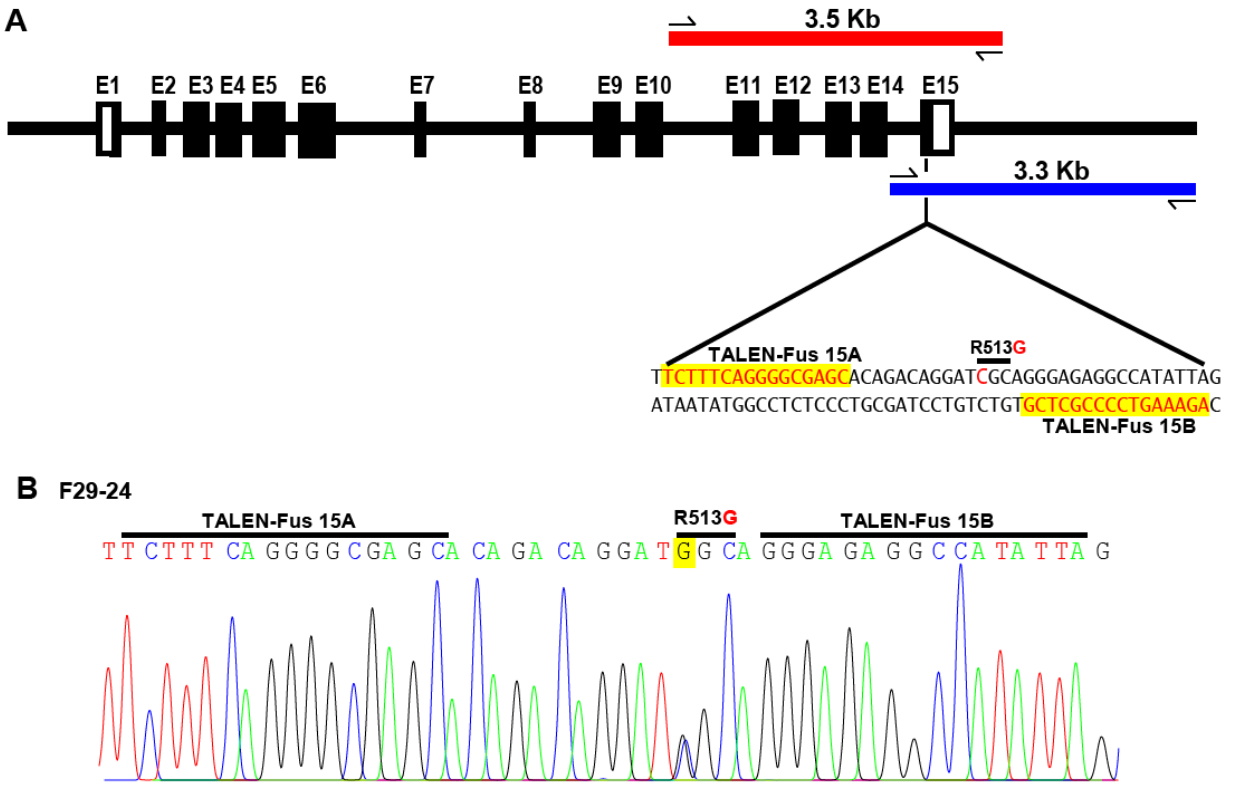

Figure S5 Sequence analysis of the *Fus*^{R513G} locus.
(A) Schematic drawing of the mouse *Fus* gene, showing the PCR amplified segments of 3.5 kb and 3.3 kb PCR product, which both overlap codon 513. Amplification was performed using tail DNA of the heterozygous (*Fus*^{R513G}) pup F29-24 such that equal amounts of PCR products are derived from the *Fus* wildtype and the *Fus*^{R513G} alleles are expected. **(B)** Chromatogram covering the sequence around codon 513, showing a mixed peak at the position of the targeted *Fus*^{R513G} nucleotide replacement.

Figure S6 Nucleotide polymorphisms in TALEN-Fus15 off-target sites 1 and 3.
Sequencing of HRMA PCR products revealed single nucleotide polymorphisms between wildtype C57BL/6 controls and mutant founder animals in the TALEN-Fus15 off-site 1 (**A**) and off-site 3 (**B**). These polymorphisms were identified as strain polymorphisms, resulting from the mixed genetic backgrounds (C57BL/6, DBA/2, and FVB/N) of founders. Predicted TALEN off-target binding sites and HRMA primer binding sites are indicated.

Table S1 Germline transmission of TALEN-induced mutations

Microinjection experiment	Founder	Pups	Mutant pups
TALEN-Rik2	R23	15	7
TALEN-Fus15 + ODN^{R513G}	F5	10	1
	F18	23	13
	F29	15	8
	F37	17	8
	F70	3	2
	F75	7	4
TALEN-Fus15 + ODN^{P517L}	F36	12	8

Table S2 Potential off-target sites of TALEN-Rik2 and TALEN-Fus15.

Locus	Chromosomal position	Target sequence A (5′-3′)	Spacer length	Target sequence B (5′-3′)	Dimer type
C9orf72	***Chr4:35218811-35218854***	**T CTGTCTTGGCAACAG**	**14**	**T GTCGACTATCTGCCC**	A-B
Csmd1	*Chr8:16813743-16813785*	**T CTAACTTGACAACAA**	**13**	**T CTATCTAATTAACAA**	A-A
Intergenic	*Chr8:34491313-34491356*	**T CTGTCTTCCCACCAG**	**14**	**T ATCAACTACATAACC**	A-B
Gfra1	*Chr19:58314874-58314920*	**T CTGTGTTCACAAAAG**	**17**	**T CTGTCCTGACCACAG**	A-A
Intergenic	*Chr9:112015226-112015268*	**T CTGCCTTGACAACCT**	**13**	**T TTCAACTATGTGCCC**	A-B
Intergenic	*Chr19:4408724-4408766*	**T CAGCCTTGGCAACAT**	**13**	**T CTCTCTTCCCAAAAG**	A-A
Fus	***Chr7:127981783-127981827***	**T CTTTCAGGGGCGAGC**	**15**	**T AATATGGCCTCTCCC**	A-B
Intergenic	*Chr4:149785023-149785065*	**T CATTCAAGCCGAAGC**	**13**	**T AATATGACATCTCCC**	A-B
Zdhhc5	*Chr2:84705308-84705354*	**T CTATCAAAGGCAACC**	**17**	**T AAGATGACCACTCCA**	A-B
Sec23b	*Chr2:144575630-144575674*	**T CTTTACAGAACTAAC**	**15**	**T AAAATGACCTCCCCC**	A-B
AC122296.2-201	*Chr6:67120279-67120328*	**T CTTCCACAACCAAGC**	**20**	**T CTTTCCGGCCCAAGC**	A-A
Intergenic	*ChrX:35868551-35868598*	**T CTTACAAAGCCAAAC**	**18**	**T CATATAACTTCTCCA**	A-B

TALEN-Rik2 and TALEN-Fus15 binding sequences are shown in bold letters. Nucleotides of off-target sites matching the target are shown in black, mismatches in off-target sites are shown in red. A-B: heterodimeric target sequence, A-A: homodimeric target sequence.

Table S3 Oligonucleotides used in this study

Amplicon Name	Forward Primer (5'-3')	Reverse Primer (5'-3')
Fus	CTATGGAGATGATCGACGTG	TGGTTACAATTAGGGTAGTCTG
Fus HRMA	CATGGGTAGGGTAGTTCAGTAACACGT	ACACTGGGTACAGGAAGAGTTCCAG
Fus OS1	CCAACCACCCATGGCTTCTTATTA	GCACTGGGGTAGCATACACACATC
Fus OS2	GCTTTCCTTGTTTTAGCACTCTGC	CTGCAGCCACTCCCTAAACTTCTT
Fus OS3	CTCCCTTCCCTCTGTCTGTCTCTG	TTTCTGGGTTACCTGGGGTCAGTA
Fus OS4	CCCAAGCAGCTGGACTAAGGATCT	ACACCTGGTGGTAGTTCATTGCTT
Fus OS5	GTGAGTTCAAGTCCAGCCCAGTCT	GCTTTGTGGCAGGCACTTTTATTC
Fus R513G	TGGGTAGGGTAGTTCAGTAAC	ACAAGGGTAACACTGGGTAC
Fus 5' US	GTCATCAAGCACCTTTACCTG	TGGTTACAATTAGGGTAGTCTG
Fus 3' DS	TGGGTAGGGTAGTTCAGTAAC	ACTCTTGTCTAGCAGTGATTCTC
Rik	TTAGCTGAAATGGTTTGGAGAC	CATCACTGAGAAGCACTTGG
Rik HRMA	CGTTCGGATAATGTGAGACCTG	CAAAGGTAGCCGCCAACAAG
Rik OS1	TGACTGACAGGCTTCACAGAGAGC	CTACTGGGGACTGATGACTACCTG
Rik OS2	CACAGGGACTTTGTGTGCACTCTT	AAACCAGAGCATGGCTTTGAAAAA
Rik OS3	GAAGATAAACAGGAGCCGCATGAA	TGACTCACTAAGGGCCATTCCTTC
Rik OS4	TACAGGCTTCACTCTGTGGGGTTT	CCAGATCTGACTCAGGTAATGTGA
Rik OS5	AGTGGCCCCACACAGAAGAGTAAG	AGGAATGTGTGGGCAAATCTTGTT
Sequencing Primer		
Fus 5´RI 3	—	GCACACCCTTCAAGTCTCTG
Fus 5´RI 2	GCGGGAGTGGGTGGGTTTTTG	—
Fus 3´RI 5	GACAATTTTCCCGAAATGGGC	—