Generation of a C57Bl/6J mouse strain expressing the CD45.1 epitope to improve HSC engraftment and adoptive cell transfer experiments

Supplementary information

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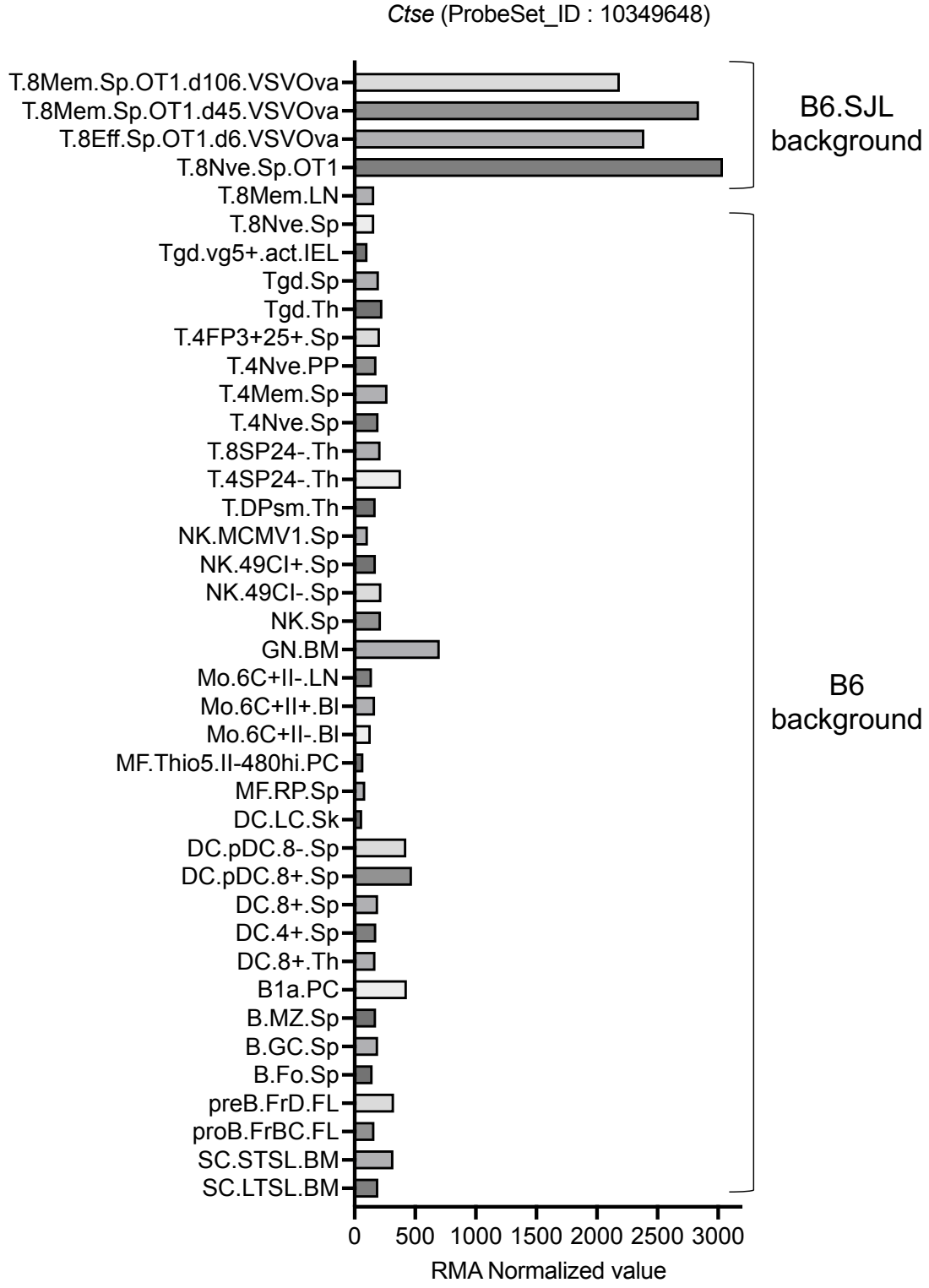
	Name	Sequence	PCR size
	tracrRNA	5'AAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC[U*U*U*]U3'	
CTSE	CTSE-guide RNA1	5*5*5*-CCCGUAGUUUCUUCCGAGUUUUAGAGCUAUGCUGUUUUG	
	CTSE- guide RNA2	7*5*6*-ACUCGAAUACACAUUACGUUUUAGAGCUAUGCUGUUUUG	
	CTSE_FWD	TTGCTGCTTCTGGTCTT	480 bp
	CTSE_REV	CCTCTTGAAGAAAGCTCT	
CD45 mutation A	mutA-guide RNA	5'[A*U*A*]CUUCAAUUUGUUUGGAGGUUUUAGAGCUAUGCUGUUUUG	
	mutA-ssODN	A*A*C*TTCTCTGCCCAGCATCGTACCTGGCTCACAGTGGAGTACATATGAAATATTGTCACTGTTGCATTTTCTGAAATCAAGGTTTTCTGTCTTCCACTCGAGGCAAATTGAAGTATTAGCCTTTTCTTTTGGTGTG*C*A*G	
	mutA_FWD	AAGAAGCATCAGACCTTGGGAG	540 bp
	mutA_REV	GAAAGCAACCTCCGGTGTGG	
CD45 mutation B	mutB-guide RNB	[G*C*A*]GACUCAGGUUUAGAUACGUUUUAGAGCUAUGCUGUUUUG	
	mutB-ssODN	T*T*T*CTGTTTTTAACAGTTCCAGAAACGCCTAAGCCTAGTTGTGGGGATCCAGCTGCAAGAAAAACGTTAGTCTCTTGGCCTGAGCCTGCATCCAAACCTGATCCTGCATCTAAACCCCATGGATATGTTTTATGCT*A*T*A	
	mutB_FWD	TTGGGTAGTCTTCAGATCAGTG	436 bp
	mutB_REV	GGACTCTAGTCCTGTCCACTATTT	
OT-mutA_1	OT-mutA_1_FWD	GGTGGGGTTACTTGCTGCTC	723 pb
	OT-mutA_1_REV	GCCAATGGGGTAGATGTGG	
OT-mutA_2	OT-mutA_2_FWD	AATACAGCGCCCTCAAACTGC	503 pb
	OT-mutA_2_REV	AGCCAAACAGATGTGCTTCCTAC	
OT-mutA_3	OT-mutA_3_FWD	GAGCACCCAGCCTACTCTAC	837pb
	OT-mutA_3_REV	AGCCTGGCCTTCATCCTAGC	
OT-mutA_4	OT-mutA_4_FWD	TTGCAAAATCTATGCCAAGGTTCC	791 pb
	OT-mutA_4_REV	ACACGTGACCCTCAACCCAG	
OT-mutA_5	OT-mutA_5_FWD	CATAAGACTGAGGCAGGTTGC	569 pb
	OT-mutA_5_REV	GGTTGGTCTGCCTGCTTGAC	
OT-mutA_6	OT-mutA_6_FWD	TAAGAGTGTTTACTGTGTGAGCG	920 pb
	OT-mutA_6_REV	CTTGGCATGCGTATAGCTGG	
OT-mutA_7	OT-mutA_7_FWD	TAACGCACAGTTTAAACGTCACC	704 pb
	OT-mutA_7_REV	ACTCTAGTGGGAGGGGAAAG	
OT-mutA_8	OT-mutA_8_FWD	CTTGCCCAGCTTGTGTGAAC	752 pb
	OT-mutA_8_REV	ACGTTCAGAGGCTCCCTTTTC	
OT-mutA_9	OT-mutA_9_FWD	AGCTCCCTTGTTTAGGGGTG	497pb
	OT-mutA_9_REV	TACACAGTCACAAAACCTTGCC	
OT-mutB_1	OT-mutB_1_FWD	CATGTTTGTGTGAGGTGCCAG	465 pb
	OT-muB_1_REV	ACACAGGAAAGTTTCAGCACCTC	
OT-mutB_2	OT-mutB_2_FWD	TACACATGCAGGCAGGTAGC	703 pb
	OT-muB_2_REV	ATAGGTGAATTCTAAGCAAGCCC	
Supplementary Table 1 : List of oligonucleotides			

REAGENT or RESOURCE	SOURCE	IDENTIFIER
FITC anti-mouse CD45 (clone 30F11)	ThermoFisher Scientific	Cat#11-0451-85 (AB_465051)
FITC anti-mouse KLRG1 (clone 2F1)	ThermoFisher Scientific	Cat#11-5893-82 (AB_1311265)
FITC anti-mouse IFNg (clone XMG1.2)	BD Biosciences	Cat#562019 (AB_395375)
PerCP-Cy5.5 anti-mouse CD4 (clone RM4-5)	BD Biosciences	Cat#550954 (AB_393977)
PE anti-mouse CD3e (clone 145-2C11)	ThermoFisher Scientific	Cat#12-0031-82 (AB_465496)
PE anti-mouse CD45.2 (clone 104)	BD Biosciences	Cat# 560695 (AB_1727493)
PECy7 anti-mouse CD11b (clone M1/70)	BD Biosciences	Cat#561098 (AB_394491)
PECy7 anti-mouse TNF (clone MP6-XT22)	BD Biosciences	Cat#554420 (AB_398553)
APC anti-mouse IL-2 (clone JES6-5H4)	Biolegend	Cat#503809 (AB_315303)
APC anti-mouse CD45R/B220 (clone RA3-6B2)	BD Biosciences	Cat#553092 (AB_398531)
APC anti-mouse NK1.1 (clone PK136)	ThermoFisher Scientific	Cat#17-5941-82 (AB_469479)
APC B8R dextramer (TSYKFESV)	Immudex	
Alexa Fluor 700 anti-mouse CD45 (clone 30-F11)	Biolegend	Cat#103128 (AB_493714)
Alexa Fluor 700 anti-mouse CD8a (clone 53-6.7)	ThermoFisher Scientific	Cat#56-0081-82 (AB_494005)
APC/Cyanine7 anti-mouse CD45R/B220 (clone RA3-6B2)	Biolegend	Cat#103224 (AB_313006)
APC-eFluor 780 anti-mouse NK1.1 (clone PK136)	ThermoFisher Scientific	Cat#47-5941-82 (AB_2735070)
Brilliant Violet 421 anti-mouse CD3e (clone 145-2C11)	Biolegend	Cat#100336 (AB_10898314)
Brilliant Violet 510 anti-mouse CD62L (clone MEL-14)	Biolegend	Cat#104441 (AB_2561537)
Brilliant Violet 605 anti-mouse CD44 (clone IM7)	Biolegend	Cat#103047 (AB_2562451)
Brilliant Violet 605 CD45R/B220 (clone RA3-6B2)	Biolegend	Cat#103243 (AB_11203907)
Brilliant Violet 650 anti-mouse NK1.1 (clone PK136)	BD Biosciences	Cat#564143 (AB_2738617)
Brilliant Violet 711 anti-mouse CD11b (clone M1/70)	Biolegend	Cat#101242 (AB_11218791)
Brilliant Violet 711 anti-mouse NKG2D (clone CX5)	BD Biosciences	Cat#563694 (AB_2722498)
BUV395 anti-mouse CD8a (clone 53-6.7)	BD Biosciences	Cat#563786 (AB_2732919)
BUV496 anti-mouse CD4 (clone GK1.5)	BD Biosciences	Cat#612952 (AB_2813886)
BUV737 anti-mouse CD45.1 (clone A20)	BD Biosciences	Cat#564574 (AB_2738850)
Biotin anti-mouse CTSE (Polyclonal goat IgG)	R&D	Cat#BAF1130
Fixable Viability Dye eFluor 780	ThermoFisher Scientific	Cat#65-0865-14
Zombie UV Fixable Viability Kit	Biolegend	Cat#423108
Supplementary Table 2 : List of antibodies		



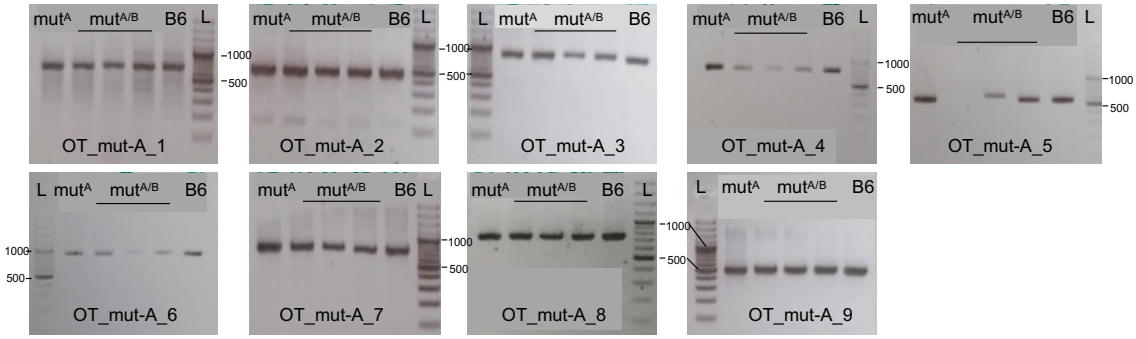
Supp Figure 1 : Ctse mRNA expression levels in various immune cell types from Immgen database

The Immunological Genome Project (ImmGen) provides an atlas of mRNA signatures across the immune system. The figure represents the *Ctse* mRNA levels from various immune cells type isolated from a mouse with a B6 or a B6.SJL background.

a.

Name	5' - 3'	PAM	Location	Distance from target
ON_mut-A	A T A C T T C A A T T T G T T T G G A G	T G G	chr1:138112261-138112283:-	-
OT_mut-A_1	T . . . . . T	A . A	chr1:120728775-120728797:+	17,383 Mbp
OT_mut-A_2	T . . T . . . . T . . .	. . A	chr1:23550699-23550721:-	114,562 Mbp
OT_mut-A_3	. . G . . . . A . . .	A . .	chr19:33622154-33622176:-	-
OT_mut-A_4	. . G . . . . . G	A . .	chr19:33784368-33784390:-	-
OT_mut-A_5	. . . . . A . . . . . C	A A .	chr16:82993647-82993669:+	-
OT_mut-A_6	. . T T . . T	. . .	chr12:96182417-96182439:-	-
OT_mut-A_7	. G . . A G	. . .	chr6:34638922-34638944:+	-
OT_mut-A_8	G . C T	G A .	chr8:35713243-35713265:-	-
OT_mut-A_9	. . T . . . T T	A . A	chr18:45580414-45580436:+	-

b.

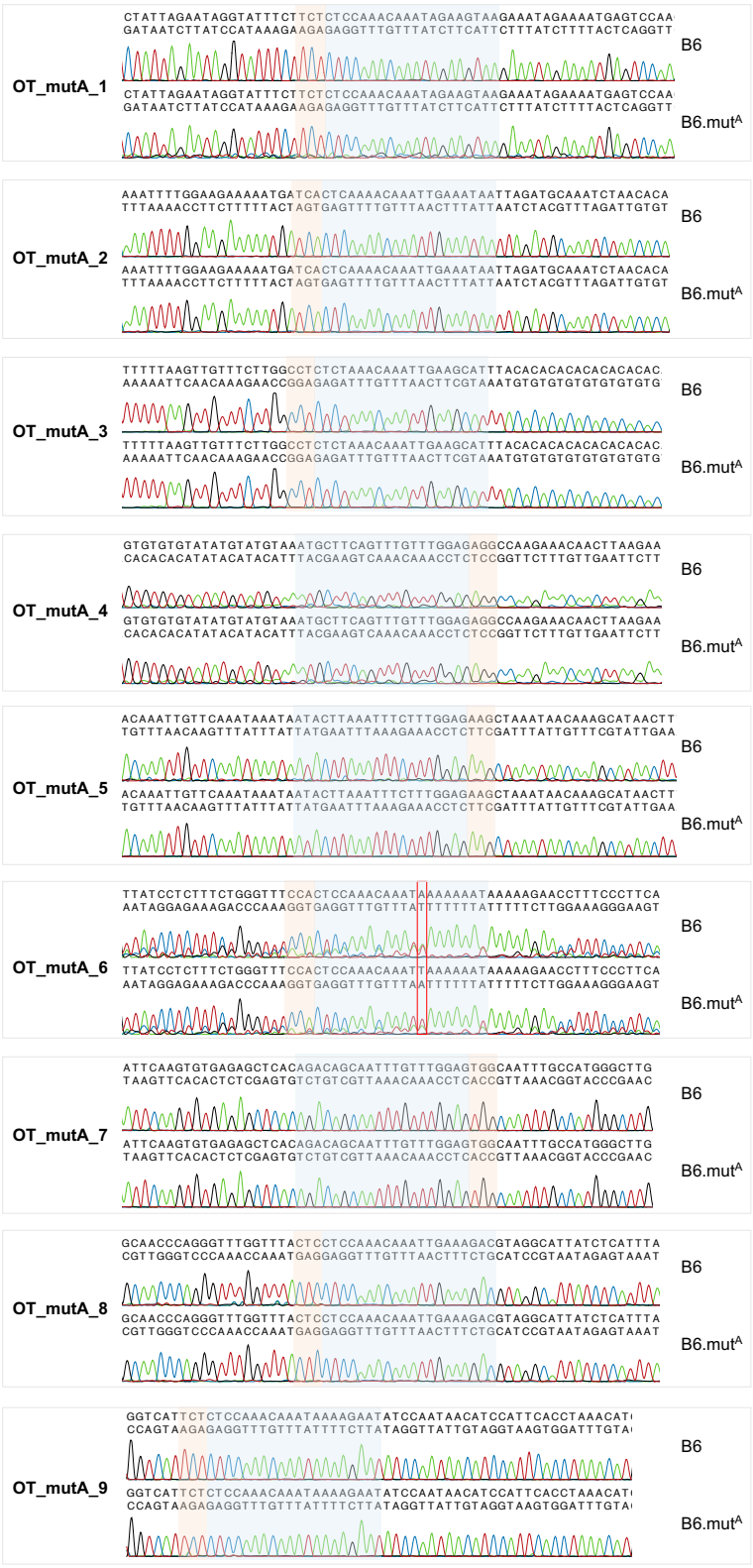


c.

OT_mutA_1	TTGGACTCATTTTCTATTTCTTACTTCTATTTGTTTGGAGAGAAGAAATACCTATTCTAATAG	B6
	TTGGACTCATTTTCTATTTCTTACTTCTATTTGTTTGGAGAGAAGAAATACCTATTCTAATAG	B6.mut^{A}
OT_mutA_2	TGTGTTAGATTTGCATCTAATTATTTCAATTTGTTTTGAGTGATCATTTTTCTTCCAAAATTT	B6
	TGTGTTAGATTTGCATCTAATTATTTCAATTTGTTTTGAGTGATCATTTTTCTTCCAAAATTT	B6.mut^{A}
OT_mutA_3	GTGTGTGTGTGTGTGTGTAAATGCTTCAATTTGTTTAGAGAGGCCAAGAAACAACTTAAAAA	B6
	GTGTGTGTGTGTGTGTGTAAATGCTTCAATTTGTTTAGAGAGGCCAAGAAACAACTTAAAAA	B6.mut^{A}
OT_mutA_4	GTGTGTGTATATGTATGTAAATGCTTCAGTTTGTTTGGAGAGGCCAAGAAACAACTTAAGAA	B6
	GTGTGTGTATATGTATGTAAATGCTTCAGTTTGTTTGGAGAGGCCAAGAAACAACTTAAGAA	B6.mut^{A}
OT_mutA_5	ACAAATTGTTCAAATAAATAATACTTAAATTTCTTTGGAGAAGCTAAATAACAAAGCATAACTT	B6
	ACAAATTGTTCAAATAAATAATACTTAAATTTCTTTGGAGAAGCTAAATAACAAAGCATAACTT	B6.mut^{A}
OT_mutA_6	TGAAGGGAAAGGTTCTTTTTATTTTTTTATTTGTTTGGAGTGGAAACCCAGAAAGAGGATAA	B6
	TGAAGGGAAAGGTTCTTTTTATTTTTTAATTTGTTTGGAGTGGAAACCCAGAAAGAGGATAA	B6.mut^{A}
OT_mutA_7	ATTCAAGTGTGAGAGCTCACAGACAGCAATTTGTTTGGAGTGGCAATTTGCCATGGGCTTG	B6
	ATTCAAGTGTGAGAGCTCACAGACAGCAATTTGTTTGGAGTGGCAATTTGCCATGGGCTTG	B6.mut^{A}
OT_mutA_8	TAAATGAGATAATGCCTACGTCTTTCAATTTGTTTGGAGGAGTAAACCAAACCCTGGGTTGC	B6
	TAAATGAGATAATGCCTACGTCTTTCAATTTGTTTGGAGGAGTAAACCAAACCCTGGGTTGC	B6.mut^{A}
OT_mutA_9	ATGTTTAGGTGAATGGATGTTATTGGATATTCTTTTATTTGTTTGGAGAGAATGACC	B6
	ATGTTTAGGTGAATGGATGTTATTGGATATTCTTTTATTTGTTTGGAGAGAATGACC	B6.mut^{A}

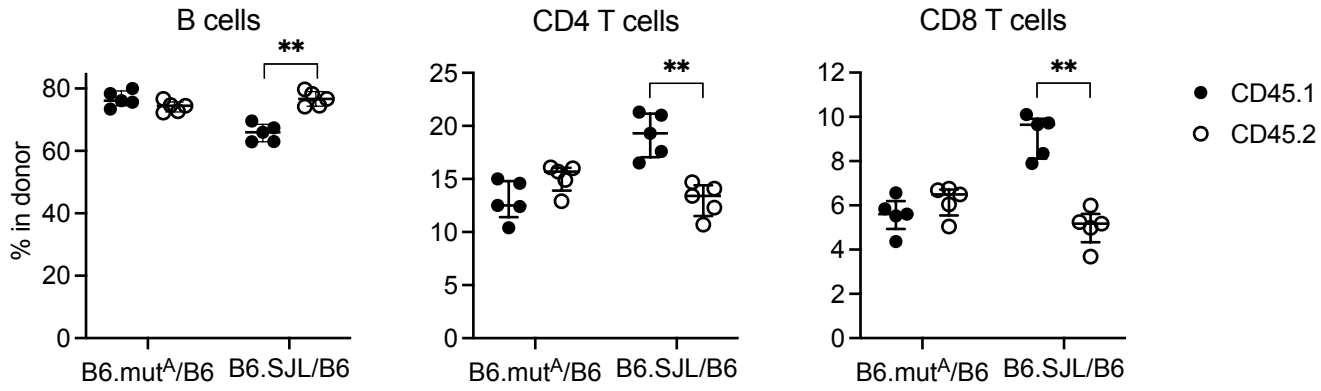
Supp Figure 2 : Analysis of potential off-target sites for mutation A insertion

a, The potential off-target (OT) sites for mut^{A}-guide RNA were determined using CRISPOR software. Weaker NAG or NGA PAM are also taken into account even if corresponding OT are less likely to happen. Potential OT loci were PCR-amplified and sequenced using genomic DNA from founder B6.mut^{A} F1 mouse and compared to B6 control. **b**, Corresponding PCR gels are shown. O'GeneRuler 100bp Plus DNA Ladder was used as a ladder (L). **c**, Sequence alignments of potential OT regions for B6.mut^{A} and B6 mice. Sequence targeted by the guide RNA is in blue, PAM sequences are in orange (NGG, NGA or NAG) and nucleotide differences between B6 and B6.mut^{A} are in red.



Supp Figure 3 :Sequencing trace datas of potential off-target sites

Chromatograms of sequenced PCR products for each potential mut^{A} OT sites and associated double stranded sequences are represented for B6.mut^{A} and B6 mice. Sequence targeted by the guide RNA is highlighted in blue and PAM sequence in orange. Nucleotide differences are highlighted with a red square.

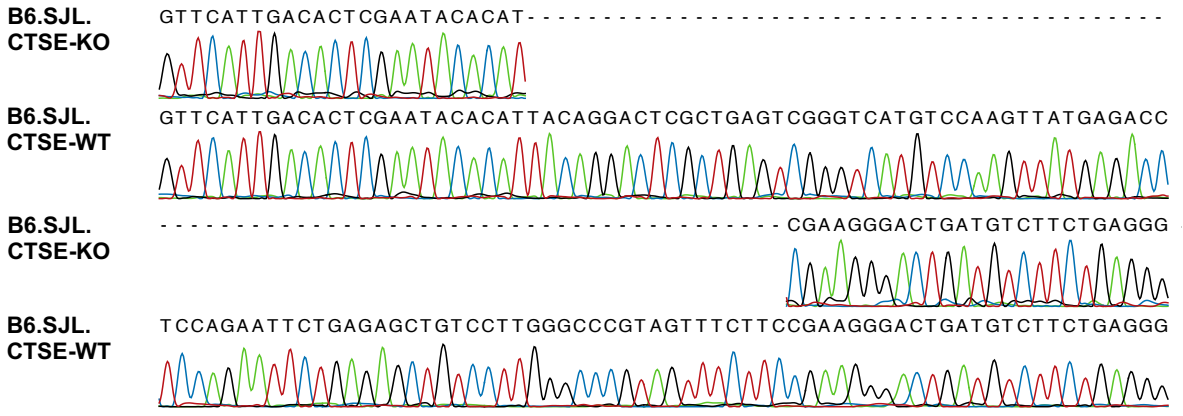


Supp Figure 4 : B6.mut^A mice have similar bone marrow reconstitution potential than B6 mice.

Irradiated B6/B6.SJL heterozygous mice received a 1:1 mixture of B6.mut^A or B6.SJL and B6 bone marrow cells. The immune cell composition of CD45.1 and CD45.2 donor compartment was determined 17 weeks after transfer in the spleen by flow cytometry. Results are expressed as the median ± range (n = 5 mice/group, one of two representative experiment). The statistical significance of differences was determined by Mann-Whitney test (*p<0.05, **p<0,01).

a

b



Supp Figure 5 : Generation of B6.SJL.CTSE-KO mice

B6.SJL.CTSE-KO mouse was generated by CRIPSR editing. An 83 bp deletion was made in exon 3 of *Ctse* gene in B6.SJL mouse using two guide RNAs simultaneously. **a**, The targeted *Ctse* gene sequence is represented with guide RNA sequences highlighted in red, PAM sequences in blue and primer sequences in grey. **b**, DNA Sanger sequencing of the targeted *Ctse* gene region for both B6.SJL.CTSE-KO and B6.SJL.CTSE-WT mice (sequences and trace data, antisens strand).