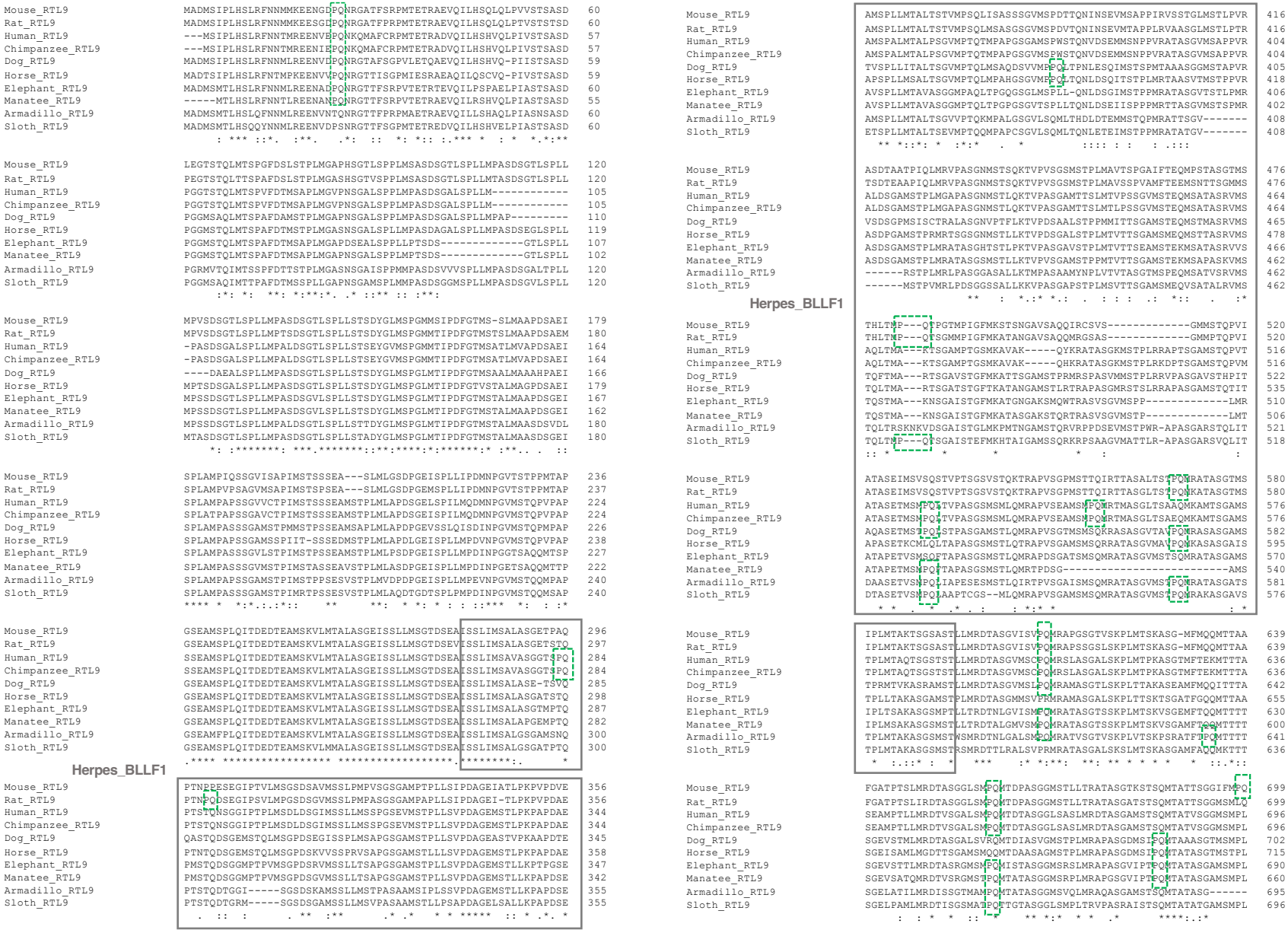

Mouse_RTL9
Rat_RTL9
Human_RTL9
Chimpanzee_RTL9
Dog_RTL9
Horse_RTL9
Elephant_RTL9
Manatee_RTL9
Armadillo_RTL9
Sloth_RTL9
Herpes_BLLF1

Fig. S1-1


PHA03247
ΔC
Maximal GAG-like region
PHA03247
Capsid domain
Minimal GAG-like region

Fig. S1-2

Fig. S1 Comparison of the RTL9 protein in the 10 representative eutherian species

Multiple alignment of the RTL9 protein sequences in 10 eutherian species: the mouse, rat, human, chimpanzee, dog, horse, elephant, manatee, armadillo and sloth. The identical amino acids in the 10 species are depicted with an asterisk (*) below the alignment. Three regions corresponding to the herpes BLLF1 super family, PHA03247 super family and capsid domain of sushi-ichi GAG (Fig. 1A) are shown as gray, orange and blue boxes, respectively. Maximal and minimal GAG-like regions are shown as red boxes and red dashed boxes, respectively. The end of RTL9ΔC, just before the maximal GAG-like region, is indicated by a red arrow with a short vertical line on its head. Details are provided in the Comparative Genome analysis in the Materials and Methods section.


Conserved domains on [lcl|NM_001040434.2]
View Concise Results
NM_001040434.2 Mus musculus retrotransposon Gag like 9 (Rtl9), mRNA
Graphical summary
Zoom to residue level
show extra options »
RF +1
Superfamilies
Herpes_BLLF1
PHA03247
Retrotrans
Search for similar domain architectures
Refine search
List of domain hits
Name Accession Description Interval E-value
Retrotrans_gag super family cl41948 Retrotransposon gag protein; Gag or Capsid-like proteins from LTR retrotransposons. There is a ... 3505-3780 7.31e-08
Retrotransposon gag protein; Gag or Capsid-like proteins from LTR retrotransposons. There is a central motif QGXXEXXXXXFXXLXXH that is common to Retroviridae gag-proteins, but is poorly conserved.
The actual alignment was detected with superfamily member pfam03732:
Pssm-ID: 455293 Cd Length: 97 Bit Score: 51.56 E-value: 7.31e-08
NM_001040434.2 1169 LVSLHL-GAAERW--SILQMEVGNPISSDN--KAFLRRsqgIYDSLSEIDILSAVLCHPKQGKKSVRQYATDFLLLAR-- 1241
Cdd:pfam03732 2 LAVHSLrGAALTWwkSLVARSIDAFDSWDEIkDAFLKR----FFPSIRKDLLRNELRSLRQGTESVREYVERFKRLARql 77
NM_001040434.2 1242 -HLSWSDAILRTRFLEGLSE 1260
Cdd:pfam03732 78 pHHGRDEEALISAFLRGLRP 97
PHA03247 super family cl33720 large tegument protein UL36; Provisional 2359-3423 1.61e-06
large tegument protein UL36; Provisional
The actual alignment was detected with superfamily member PHA03247:
Pssm-ID: 223021 [Multi-domain] Cd Length: 3151 Bit Score: 53.02 E-value: 1.61e-06
NM_001040434.2 787 LVRPPASGEIAPHSRTPVYGTISAPHMTTTASGVMTMSPMKTSVPVSESATLLRPTDSGVMSIPLTRTPASRAKSRPQMA 866
Cdd:PHA03247 2698 LADPPPPPPTPEPAPHALVSATPLPPGPAAARQASPALPAAPAPPAVPAGPATPGGPARPARPPTTAGPPAPAPPAAPAA 2777
NM_001040434.2 867 TAcgdmcplPVRAPATAGISPSPVRSPASStlstllrrPSDGAVTAELERVLGPAQFAAMTPGEMSKPLMRA--SAPGTT 944
Cdd:PHA03247 2778 GP-------PRRLTRPAVASLSESRESLPS---------PWDPADPPAAVLAPAAALPPAASPAGPLPPPTSAqpTAPPPP 2842
NM_001040434.2 945 TMPLMSPMTSGEMSMP---LMKTTPSGTMSTLQTKVMSSRATSLPQPrnaasgvianPPQRAPASGAGSTPLMRVSGSGM 1021
Cdd:PHA03247 2843 PGPPPPSLPLGGSVAPggdVRRRPPSRSPAAKPAAPARPPVRRLARP----------AVSRSTESFALPPDQPERPPQPQ 2912
NM_001040434.2 1022 MSTPLLGATASGGMSMPQMAPPTSGDMFSPLMRSPAPGIMSTPQtafGMTPTLNVKATDSGEASTSHTRFtapgskSTPH 1101
Cdd:PHA03247 2913 APPPPQPQPQPPPPPQPQPPPPPPRPQPPLAPTTDPAGAGEPS---GAVPQPWLGALVPGRVAVPRFRV-------PQPA 2983
NM_001040434.2 1102 MTSTAPEMKTPPPKEVPSFGMLTPALCYLLEQEAARGSS 1141
Cdd:PHA03247 2984 PSREAPASSTPPLTGHSLSRVSSWASSLALHEETDPPPVS 3023

Fig. S2-1

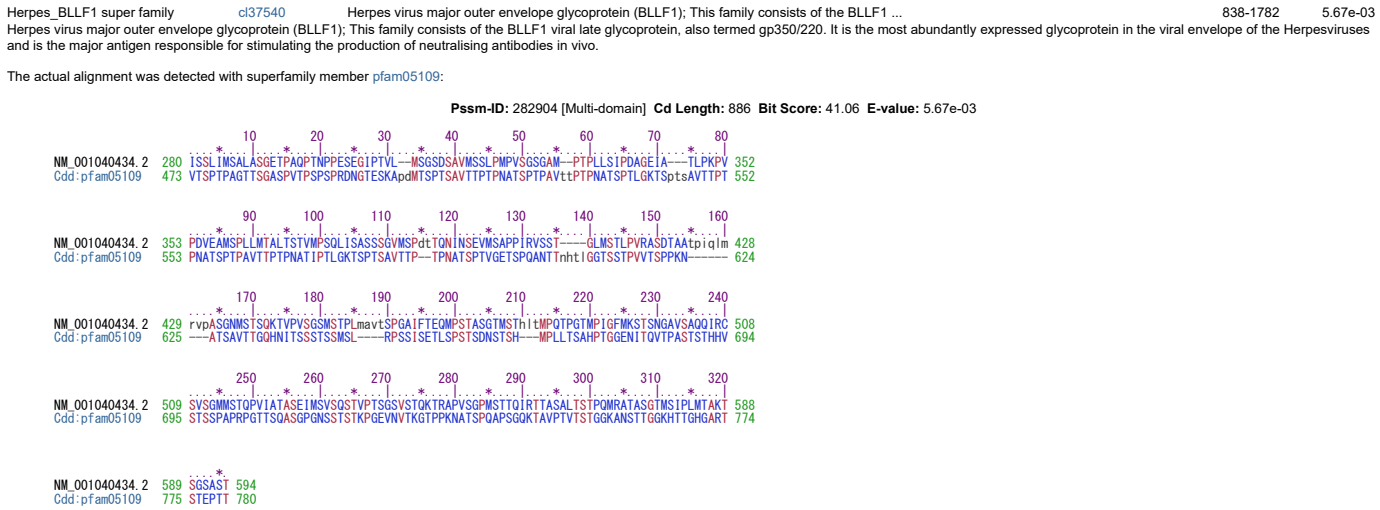


Fig. S2 The result of a conserved domain search using NCBI CDD

Three domains, the retrotransposon gag protein, large tegument protein UL36 and herpes virus major outer envelope glycoprotein (BLLF1), are listed in this order. The identical amino acids in the superfamily motifs and RTL9 are depicted in red.

Fig. S2-2

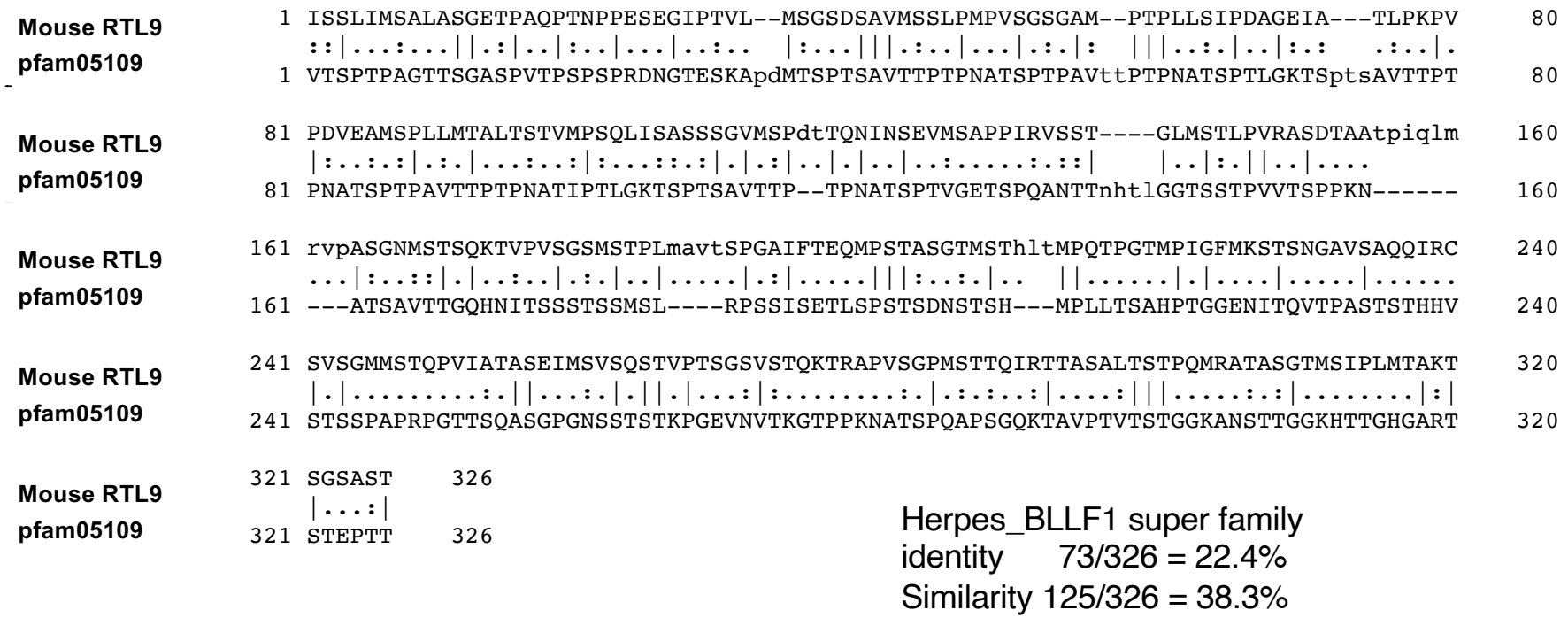


Fig. S3 Pairwise alignment and identity between pfam05109 (BLLF1) and mouse RTL9

The N-terminal part of mouse RTL9 (280 aa – 594 aa) possessing homology to herpes BLLF1 super family motif was re-analyzed using EMBOS Water program and EMBOSS needle program as described in Materials and Methods. One and two dots represent similarities of amino acids, such as weekly and strong similar, respectively.

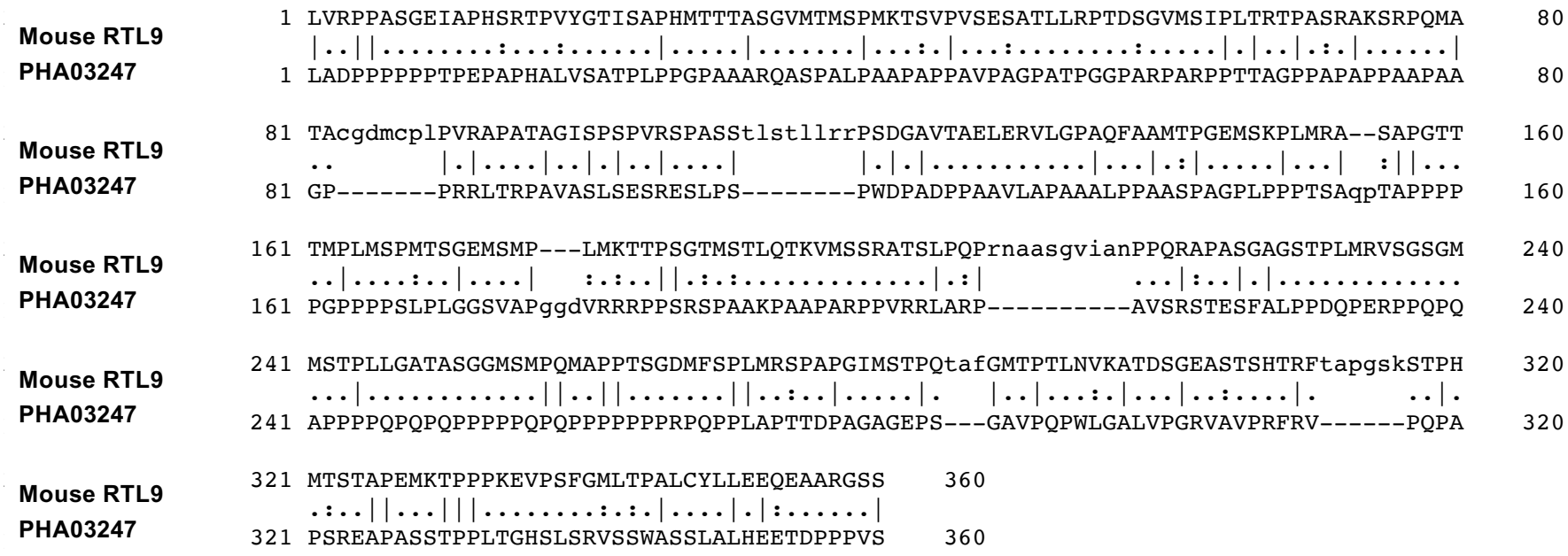
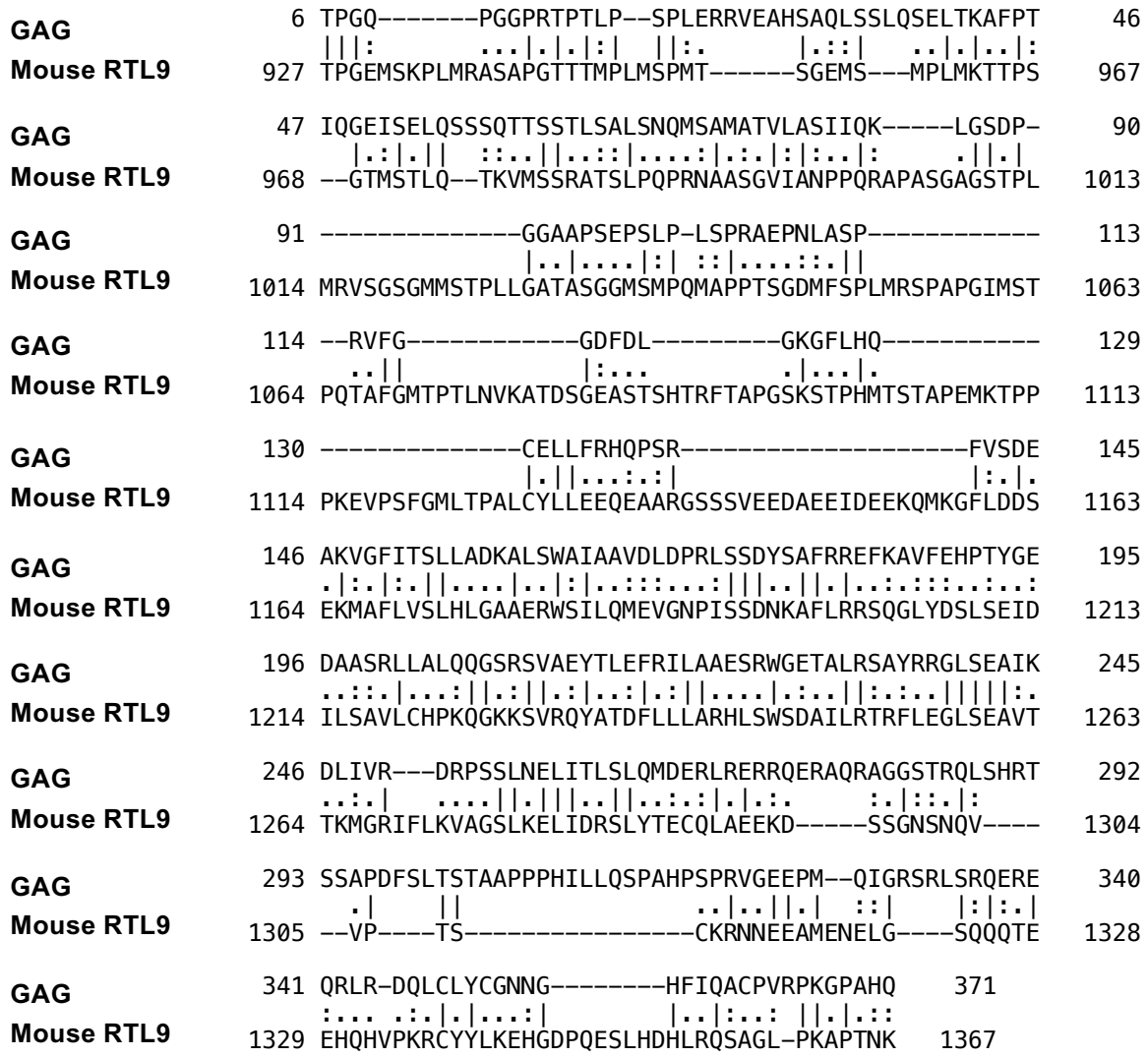


Fig. S4 Pairwise alignment and identity between PHA03247 family and mouse RTL9 protein

The middle of mouse RTL9 (787 aa –1141 aa) possessing homology to the PHA03247 super family motif was re-analyzed using the EMBOSS Water program and EMBOSS needle program. One and two dots represent similarities of amino acids, such as weekly and strong similar, respectively.

Fig. S5 Pairwise alignment and identity between sushi-ichi GAG and mouse RTL9 (1)

A conserved domain search using NCBI CDD indicated that the C-terminal portion of mouse RTL9 (1169 aa –1260 aa) possesses homology to the Capsid motif of sushi-ichi retrotransposon GAG. However, the regions before and after the Capsid motif (1148 aa-1168 aa and 1261 aa-1367 aa) also exhibit homology to GAG (**Minimal GAG-like region**). The AAs in *Rtl9* exon 2 are shown in red. The AAs of the CCHC motif of GAG are shown in light blue. One and two dots represent similarities of amino acids, such as weekly and strong similar, respectively.



Sushi-ichi GAG
identity 108/490 = 21.8%
similarity 175/490 = 35.7%

Fig. S6 Pairwise alignment and identity between sushi-ichi GAG and mouse RTL9 (2)

Entire sushi-ichi GAG exhibits homology to mouse RTL9 (927 aa-1357 aa, **Maximal GAG-like region**).

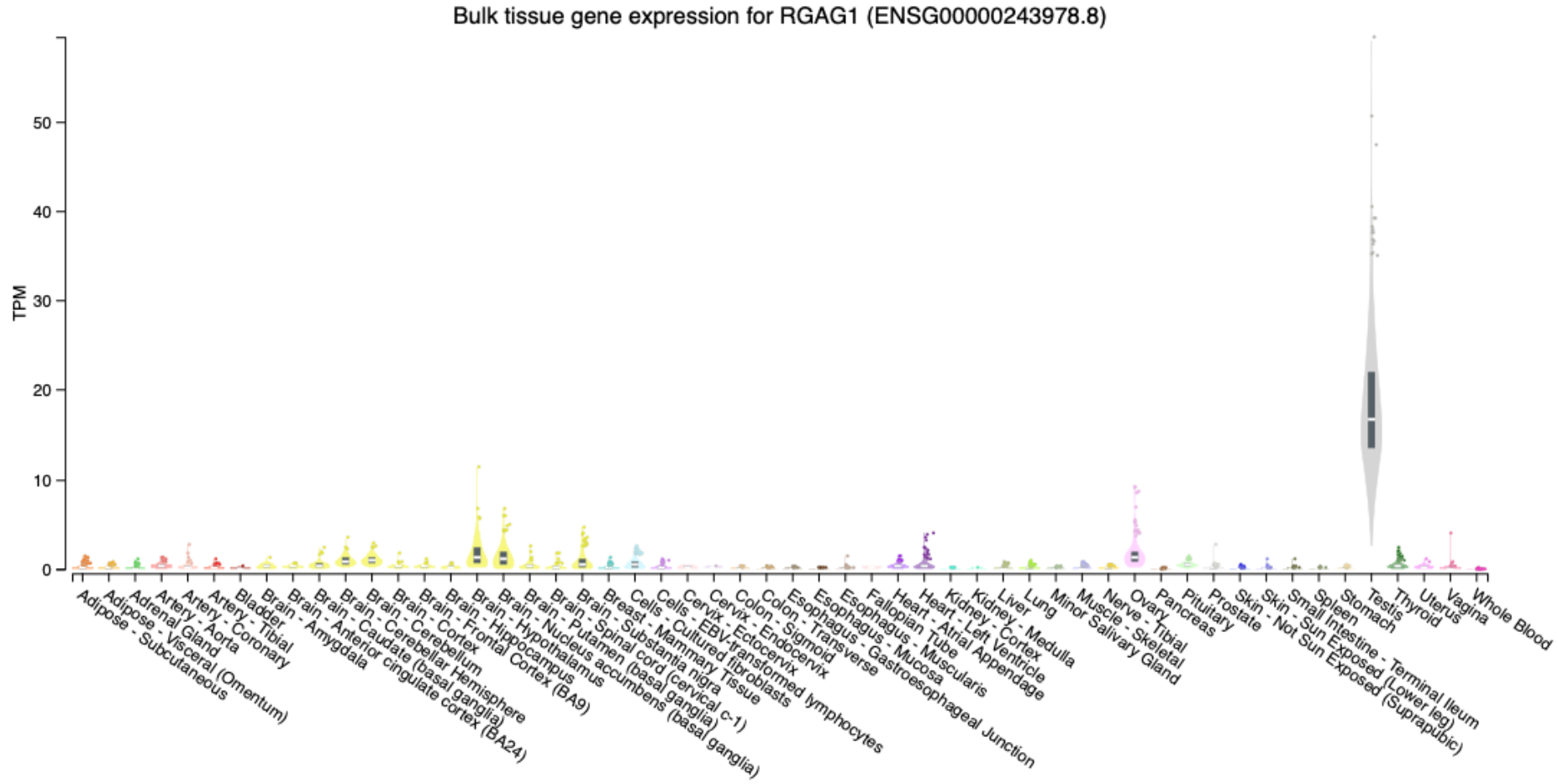


Fig. S7 *RTL9* (*RGAG1*) expression in human tissues and organs

See the original data at https://gtexportal.org/home/gene/RGAG1 (accessed on 2 June 2023)

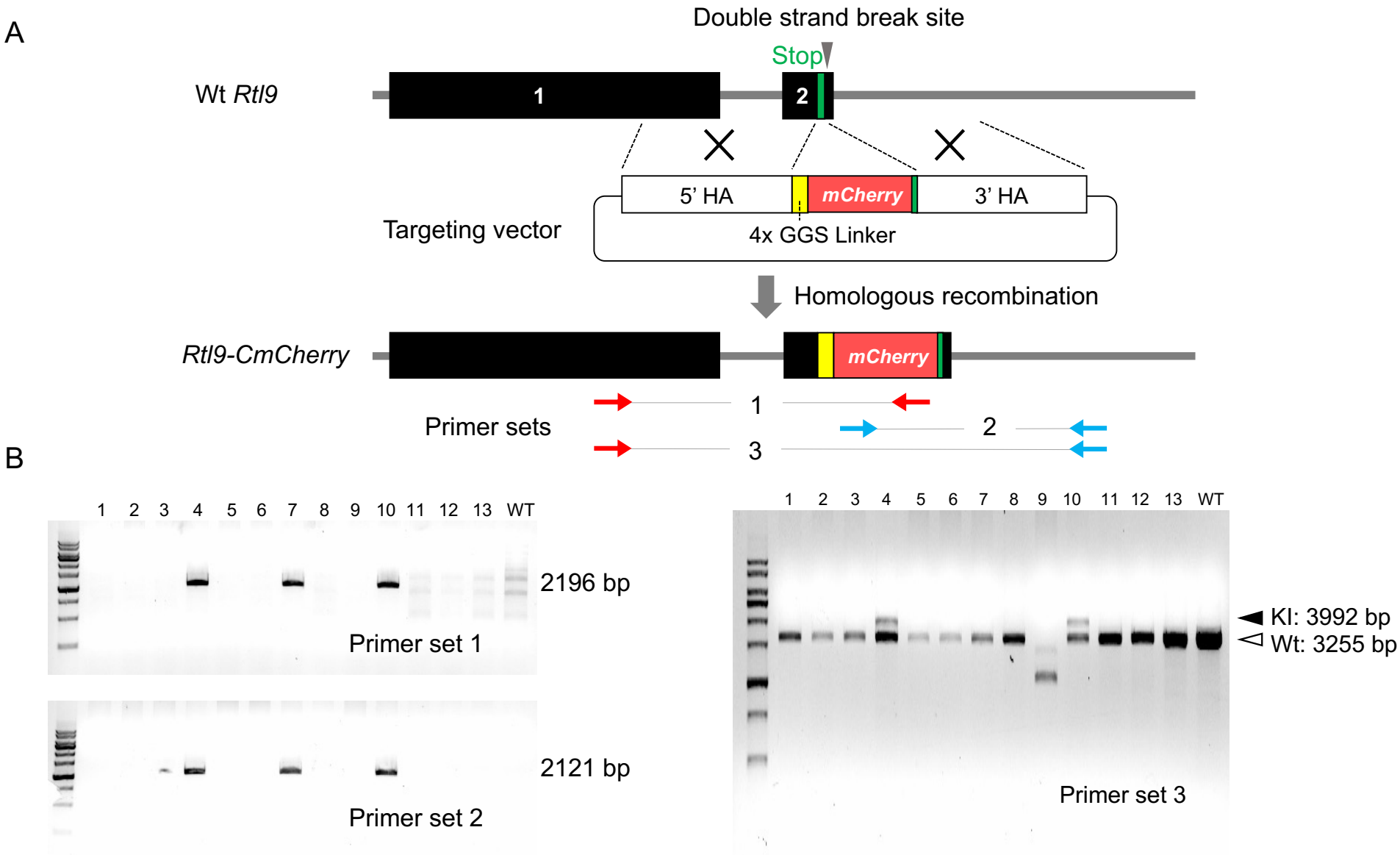


Fig. S8 Making of *Rtl9-mCherry* KI mouse

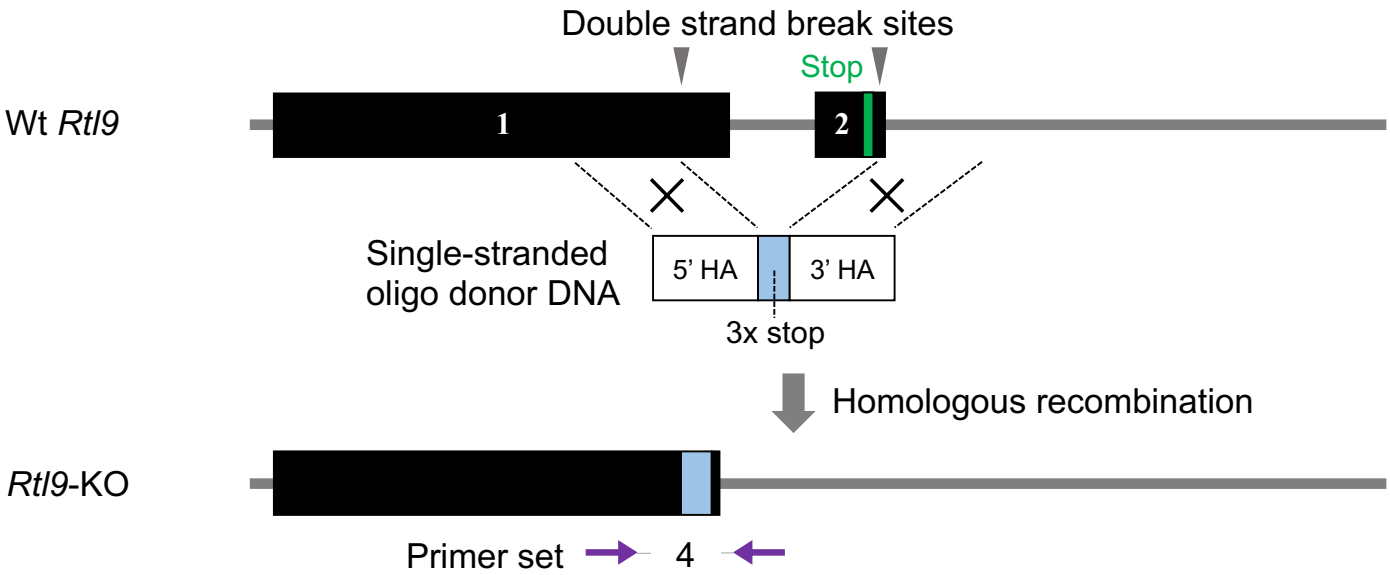
Fig. S8 Generation of the *Rtl9CmC* KI mouse

A) Schematic representation of the generation of the *Rtl9CmC* KI mouse. *Rtl9* exon 1 and 2 are numbered and shown in black. The stop codon is shown in green. The genomic locus near the stop codon in exon2 of *Rtl9* was cut out using the CRISPR/Cas system and homologous recombination was induced between the Wt *Rtl9* locus and a targeting vector containing 5' and 3' homology arms (5' HA and 3' HA), a 4x GGS linker and the mCherry coding sequence. The reconstructed *Rtl9CmC* has mCherry at the C-terminus. The position of the PCR primer sets for genotyping is indicated (red and light blue arrows). Cross symbols means homologous recombination. **B)** The results of genotyping 13 founder (F0) mice. Genomic PCR was performed using the primer sets 1 to 3 shown in A. The number (#) of F0 mice is indicated above the gel images. Wild type (WT) mouse genome was used as a control. Based on the genotyping PCR data, the offspring of F0 mouse #4 and #10 were used for further experiments.

PCR primer sets for genotyping of KI and KO mice

Mutant	Primer set name	Direction	Sequence
Rtl9-CmCherry	Primer set 1	F	GGAATGATGTCCACGCCACTA
		R	CTTCAGCTTCAGCCTCTGCT
	primer set 2	F	CCTGTCCCCTCAGTTCATGT
		R	CCTAGACTATTGGACCAGAGG
	Primer set 3	F	GGAATGATGTCCACGCCACTA
		R	CCTAGACTATTGGACCAGAGG
Rtl9-KO	Primer set 4	F	GAGAACACCAGCTTCTAGAGC
		R	GGGAGTTCAGAACCTCATACAC

A



B

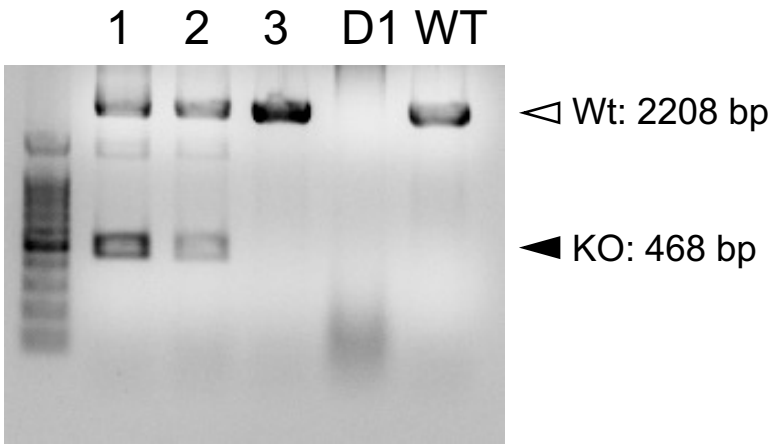


Fig. S9-1 Making of *Rtl9 KO* mouse

C

Fig. S9-2 Making of *Rtl9 KO* mouse

Fig. S9 Making of *Rtl9* KO mouse

A) Schematic representation of the generation of the *Rtl9* KO mouse. *Rtl9* exons 1 and 2 are numbered and shown in black. The stop codon is shown in green. The genomic locus encoding alanine 921 (A921) and downstream of the *Rtl9* stop codon was cut using the CRISPR/Cas system, and homologous recombination was induced between the Wt *Rtl9* locus and single-stranded oligo donor DNA containing a sequence of 3 stop codons (3x stop) and 5' and 3' homology arms (5' HA and 3' HA). In the reconstructed *Rtl9*-KO allele, the genomic region from glutamine 922 to the stop codon of *Rtl9* was deleted and replaced by a 3x stop. The position of the PCR primer set used for genotyping is indicated as purple arrows. **B)** Results of genotyping of 3 surviving and 1 dead (D1) F0 mice. Genomic PCR was performed with the primer set 4 shown in A. The number (#) of F0 mice, including D1, is indicated above the gel image. Wild type (WT) mouse genome was used as a control. **C)** Illustrated results of Sanger sequencing using the founder (F0) mice #1 and 2 shown in B. In F0 mouse #1 (top), a 3x stop was inserted at the expected genomic position just after A921 of *Rtl9*. In #2 (bottom), 12 extra bases (highlighted in red) were unexpectedly inserted just before a stop codon (highlighted in grey). Based on the Sanger sequencing data, the offspring of F0 mouse #1 were used for further experiments. The PCR primer sets for genotyping the KO mice are presented in the Fig. S6 legend.

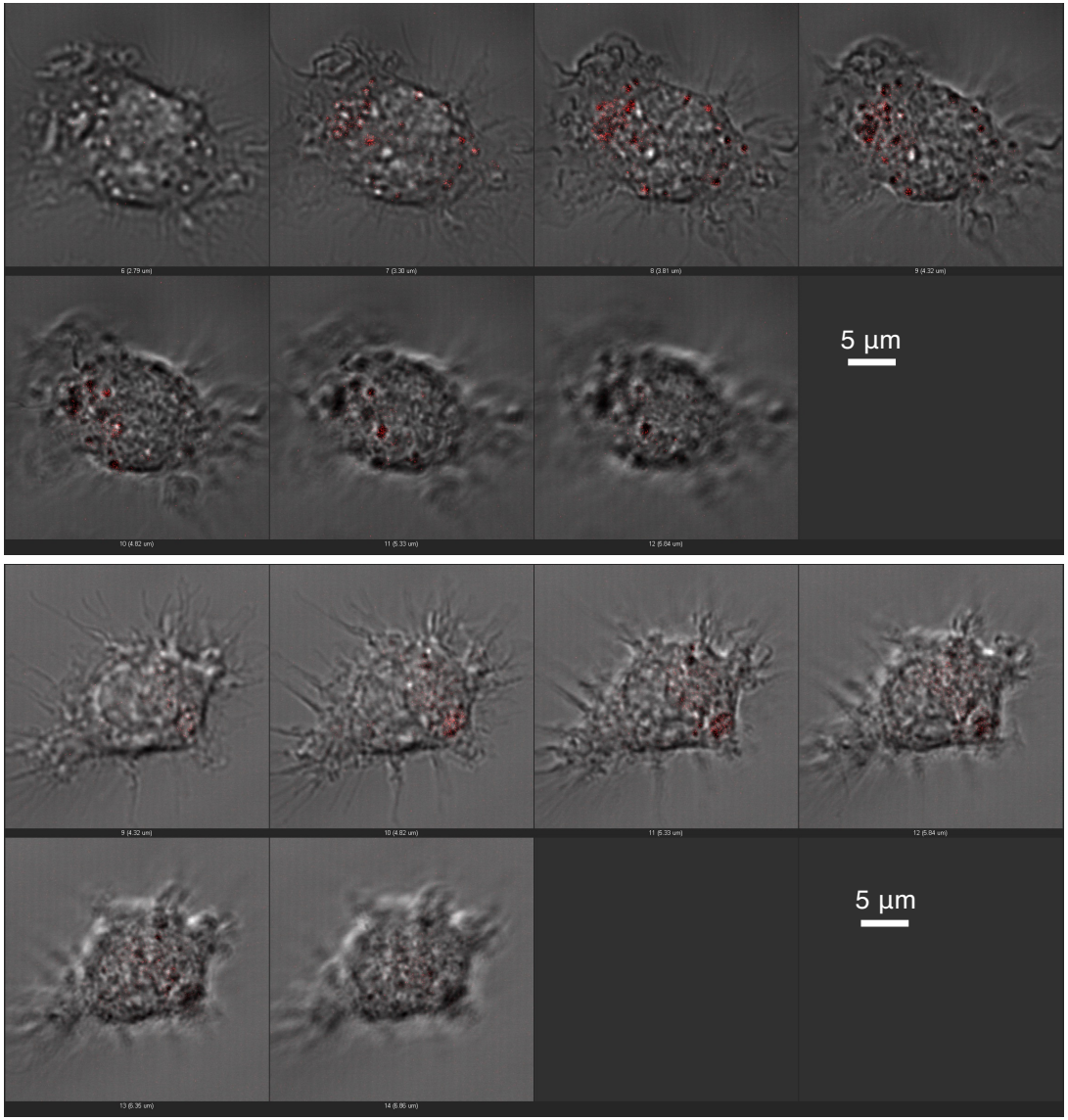


Fig. S10 mCherry signals in isolated microglia isolated from *Rtl9CmC* KI brain

Microglia were isolated from P0 neonatal brain and cultured for 10 days. The microglial cells were collected from the cultured Petri dishes (mixed glia cultures) by tapping (Lian et al., 2016; Irie et al., 2022).

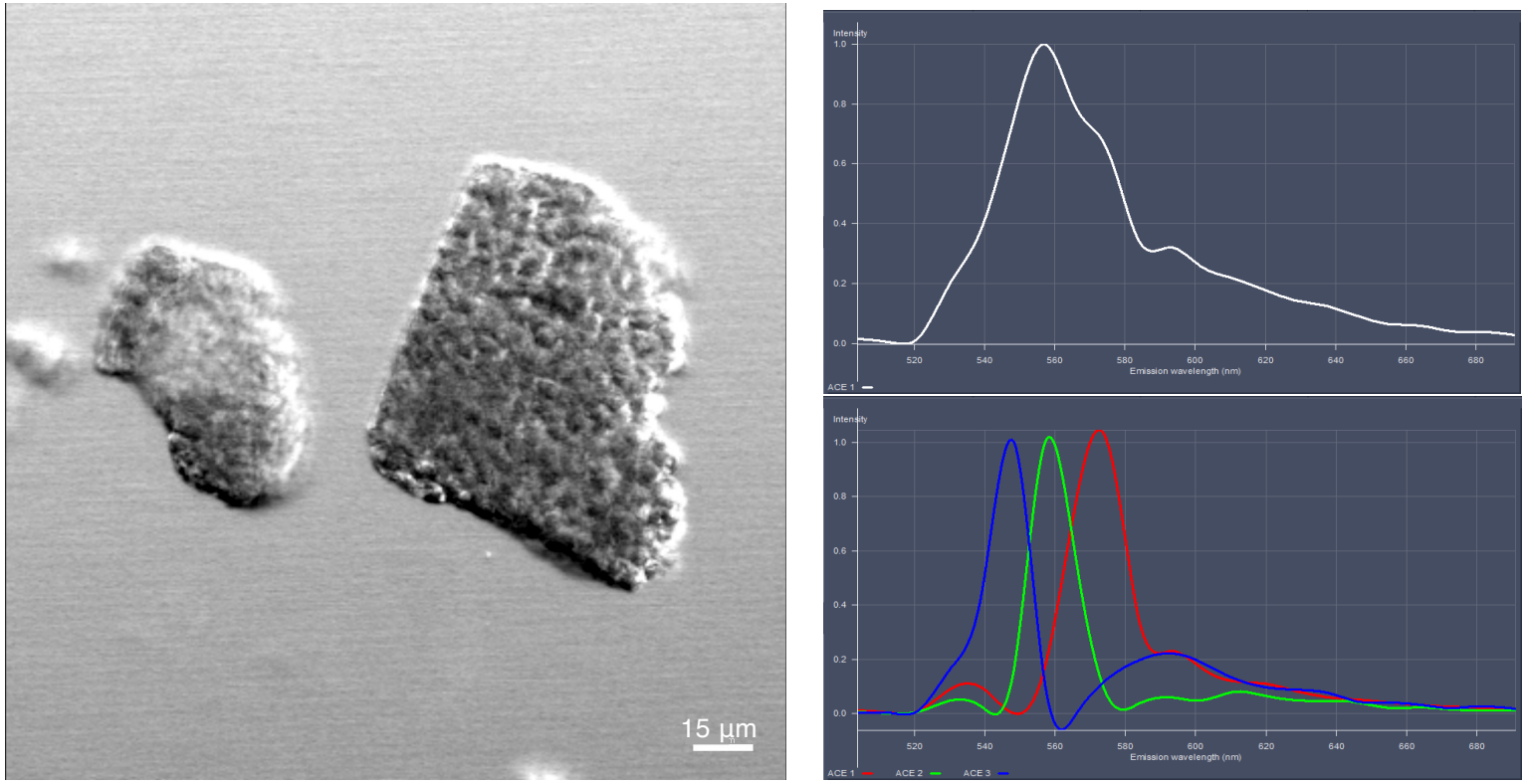


Fig. S11 The autofluorescence spectrum of Zymosan

Left: Transmission image. Right: Autofluorescence spectrum. Single (top) and/or the strongest signal (Maximum peak emission fluorescence wavelength: 570 nm) among 3 representative signals were used to detect zymosan.

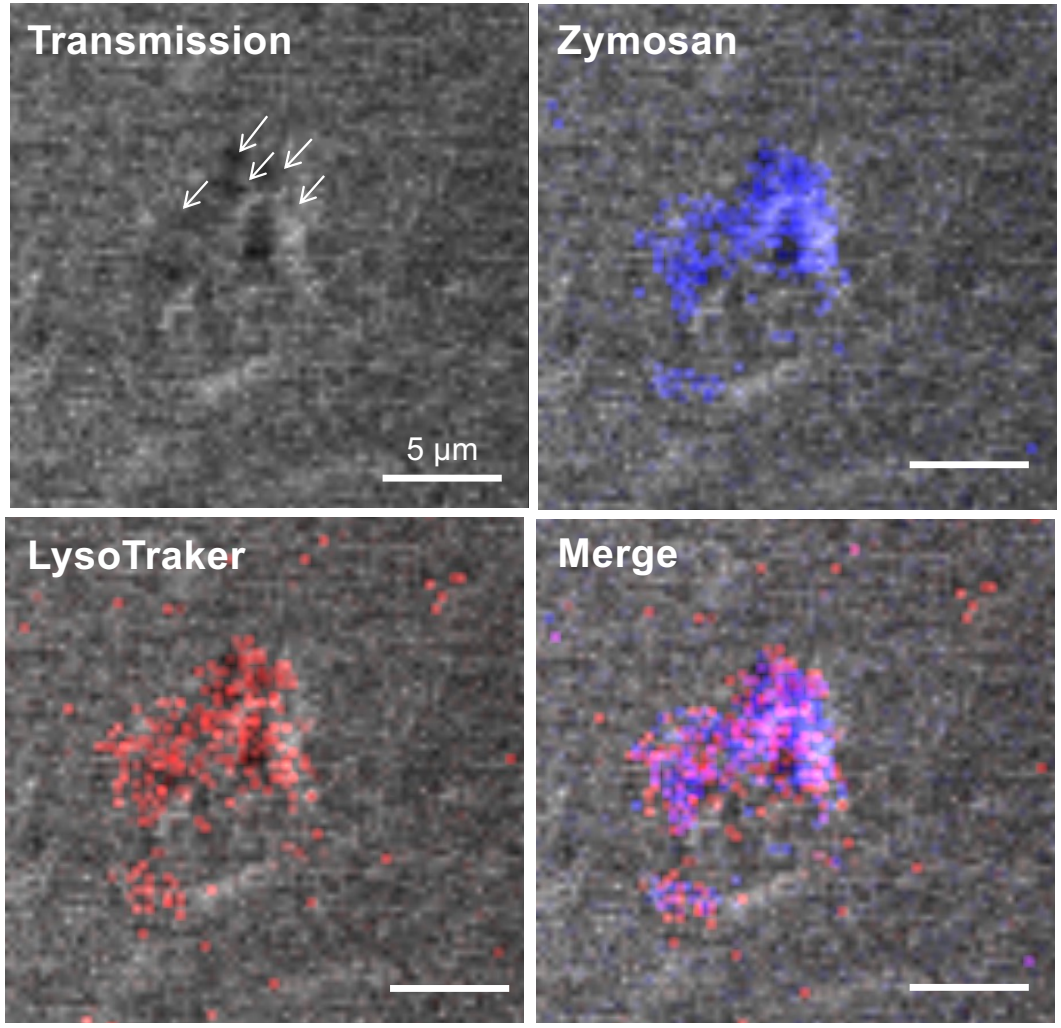


Fig. S12 Zymosan is visible in the transmission image

Zymosan incorporated in lysosomes in the *Rtl9* KO brain. Top, left: A transmission image. Arrows indicate zymosan aggregates. Top, right: zymosan autofluorescence (light blue). Bottom, left: The LysoTracker signal (red). Bottom, right: A merged image. Zymosan is distinguishable by its shape in the transmission image(s) in addition to its specific fluorescence signal. The same samples as in Fig. 3E were analyzed.

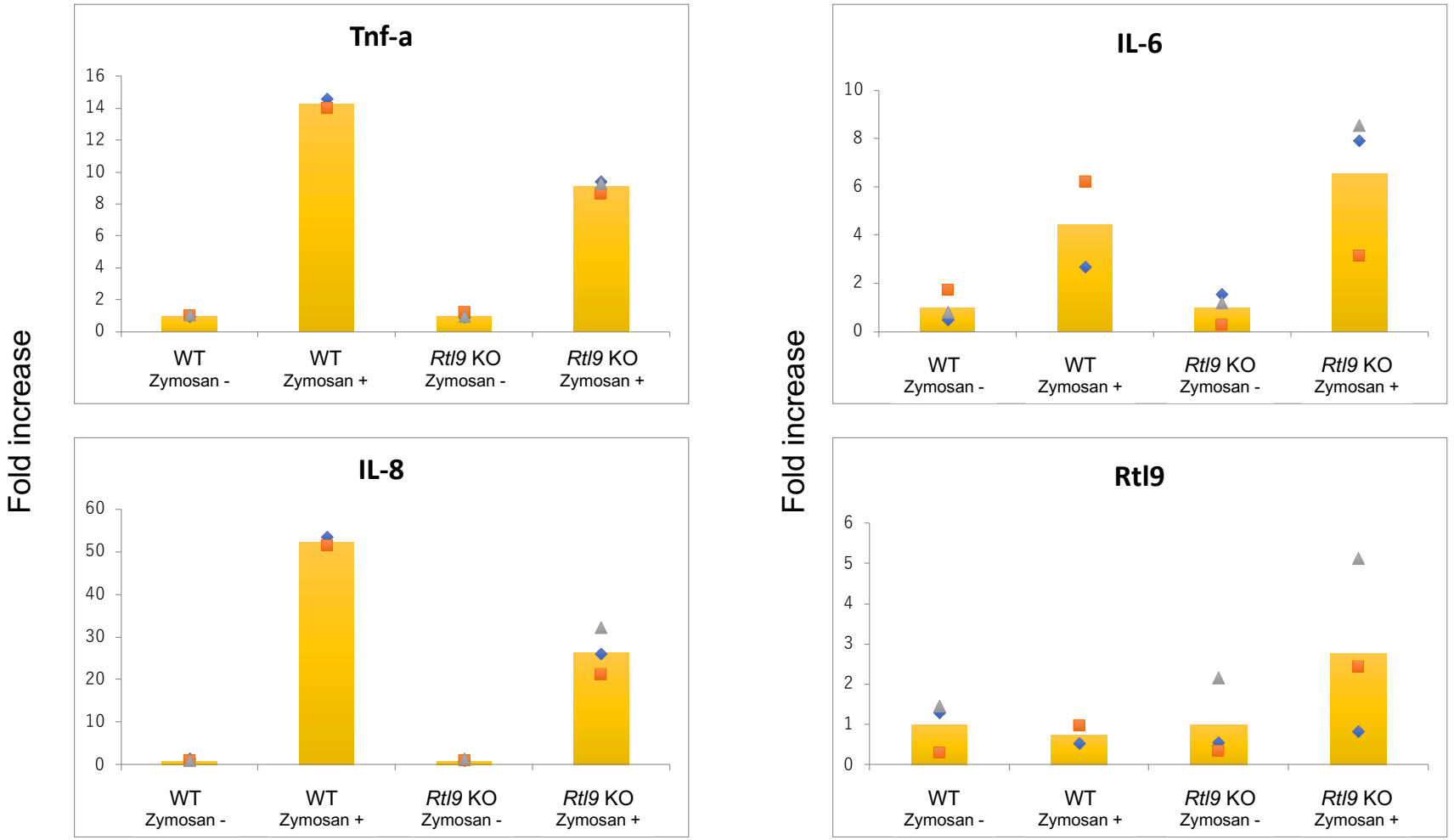


Fig. S13 Zymsan induces *Tnfa*, *Il6* and *Il8* expression in both WT and *Rtl9* KO microglia

Fig. S13 Zymosan induces *Tnfa*, *Il6* and *Il8* expression in both WT and *Rtl9* KO microglia

Tnfa, Il6, Il8 and *Rtl9* mRNA were analyzed at 0 min (no administration) and 30 min after zymosan administration to WT and *Rtl9* KO microglia (n=3 each, shown in different colors and shapes). The expression levels are averaged with non-administration samples as 1. *Rtl6* primers were designed for the 3'-UTR, and were therefore also present in the *Rtl9* KO microglia. It should be noted that the *Rtl9* mRNA itself increases upon zymosan administration in *Rtl9* KO microglia, presumably due to feedback regulation of the RTL9 protein.

The PCR primers used were as follows:

Tnfa, 5′-GACAAGGCTGCCCCGACTACG-3′ (forward) and 5′-CTTGGGGCAGGGGCTCTTGAC-3′ (reverse); *Il6*, 5′-AGTTGCCTTCTTGGGACTGA-3′(forward) and 5′-CCTCCGACTTGTGAAGTGGT-3′ (reverse); *Il8*: 5′-CTCTCAAGGGCGGTCAAAAAGTT-3′ (forward) and 5′-TCAGACAGCGAGGCACATCAGGTA-3′(reverse); *Rtl9*: 5'-TCACCTACATGCCTGTGACC-3' (forward) and 5'-CAACAACACCACATTGTTACGG-3' (reverse).