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Supplemental Information

Passenger Mutations Confound Phenotypes of SARM1-Deficient Mice

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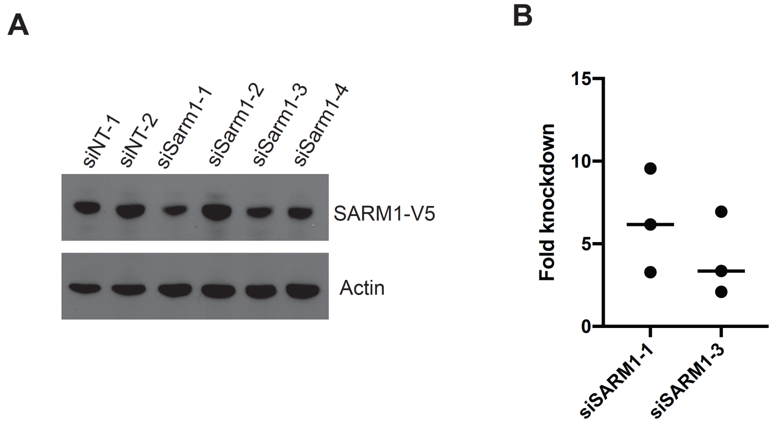
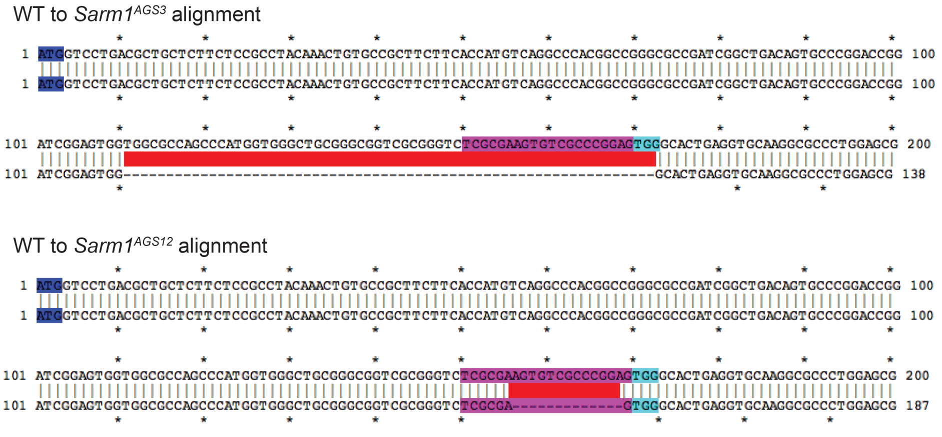
Figure S1. Knockdown experiments, Related to Figure 3.



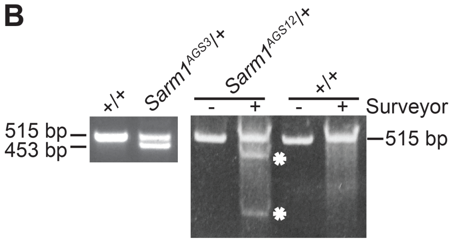
(A) Full western blot images for Figure 4C. (B) Fold knockdown of siRNAs compared to the average of siNT-1 and siNT-2 from 3 separate experiments.

Figure S2. *Sarm1* CRISPR alleles and absence of off-target CRISPR-mediated cleavage in *Sarm1*^{AGS3} mice, Related to Figure 4.

A



B

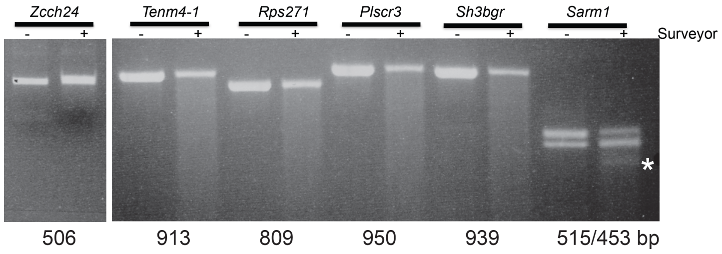


C

Guide: TCGCGAAGTGTCGCCCGGAGTGG

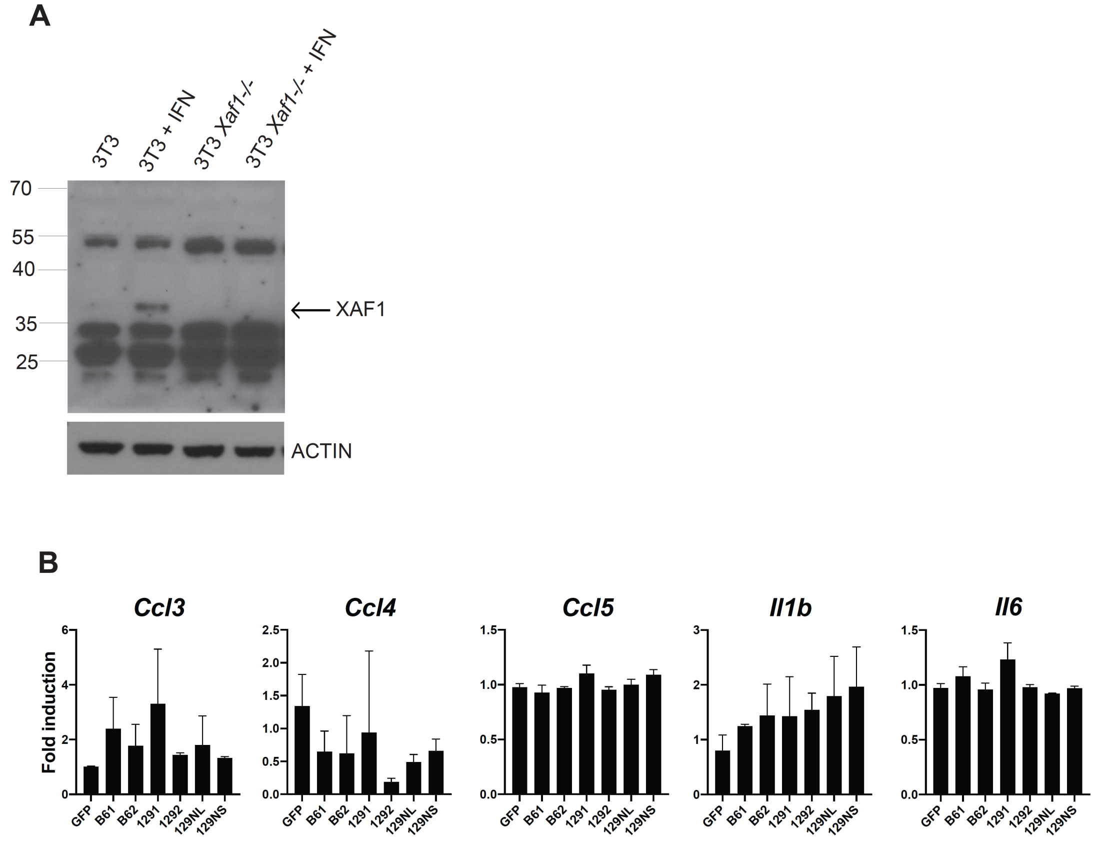
Quality score: 97

Sequence	Score	Mismatches	Gene
TCGGCAAGGGCCGCCCGGAGCAG	0.5	4MMs [4:5:9:11]	Zcchc24
TGGAGGAGTGTGGCCCGGAGTAG	0.4	4MMs [2:4:6:12]	Tenm4
CCGCGCCGTGTCGCCCGGACAAG	0.3	4MMs [1:6:7:20]	Rps271
TCCCGCAGTGACGCCCGGGGGAG	0.2	4MMs [3:6:11:19]	Sh3bgr
TCGCGAAGTGTGGGCCGCATCAG	0	4MMs [12:14:18:20]	Plscr3



(A) Sequence alignment of WT and Sarm1^{AGS3}, and WT and Sarm1^{AGS12} alleles. The start codon is indicated in blue, the guide sequence in pink, and the PAM sequence in cyan. (B) WT or Sarm1^{AGS3}/+ genomic DNA was amplified by PCR and run on an agarose gel (left). WT or Sarm1^{AGS12}/+ genomic DNA was amplified by PCR, digested with Surveyor Nuclease, and run on a TBE gel (right). (C) Genes with off-target cleavage sites in exonic regions were amplified by PCR of genomic DNA from a cross of the Sarm1^{AGS3} founder mouse to WT (Sarm1^{AGS3}/+), digested with Surveyor nuclease, and run on agarose gels. Sizes of PCR products are listed below the gel. The Sarm1 locus served as a positive control for Surveyor cleavage. Note both the Sarm1^{AGS3} and the Sarm1^{AGS12} alleles resulted from the same mosaic founder mouse.

Figure S3. Specificity of XAF1 antibody and XAF1 overexpression, Related to Figure 6.



(A) 3T3 and 3T3 *Xaf1-/-* cells were mock treated, or treated with 1000 U universal type I IFN for 24 hrs. before western blotting for XAF1. (B) Neuro2A cells were transfected with the indicated XAF1 isoforms and cytokine/chemokine induction was determined by qPCR at 24 hrs. Data is normalized to 18S and expressed as fold induction relative to GFP control.

Table S1. *Sarm1* Alleles, Related to Figure 4.

Allele	Deletion (bp)	Nucleotide position	Protein length (aa)
WT	-	-	764
Sarm1^{AGS3}	Δ62	+111 to +172	38
Sarm1^{AGS3}	Δ13	+156 to +168	74

Table S2. SNP analysis of $\mathit{Sarm1}^{AD}$ and $\mathit{Sarm1}^{MSD}$ mice, Related to Figure 5.

SNP#	Chromosome	NCBI Assembly bp	B6J	$\mathit{Sarm1}^{-/-}$	$\mathit{Sarm1}^{-/-MSD}$
11	01-11	75525463	V V		F F
21	01-21	144953207	F F		V V
115	05-15	104143745	V V		V F
180	08-14	88318808	F F		V V
205	10-01	7775347	F F		V F
207	10-03	20325683	F F		V F
209	10-05	38965551	V V		V F
211	10-07	48685723	F F		V F
213	10-09	67238174	F F	V F	V V
225	11-01	9552730	F F		V F
229	11-05	37252460	V V		F F
230	11-06	45819381	F F		V V
231	11-07	54267162	V V		F F
233	11-09	65095597	V V		F F
235	11-11	75647055	F F	V V	V V
248	12-06	34055903	F F		V F
260	12-18	114730692	F F		V F
275	13-15	102573089	F F		V F
277	13-17	115537300	F F		V F
290	14-12	86216087	V V		V F
292	14-14	101001947	V V		V F
297	15-01	10575512	F F		V F
299	15-03	22519205	F F		V F
301	15-05	35126500	F F		V V
329	17-03	23972821	F F		V F
331	17-05	33380773	F F		V F
349	18-09	58557879	F F		V F
356	19-02	17770192	F F		V F
362	19-08	59241076	V V		V F

F=FAM probe
V=VIC probe

Table S3. *Xaf1* Transcripts, Related to Figure 6.

Transcript	Size (bp)	Size (aa)	Size (kDa)	Accension
B6 transcript 1	822	273	31	ENSMUST00000146233.7
129 transcript 1	813	270	30	MGP_129S1SvImJ_T0030476.1
B6 transcript 2	537	178	20	ENSMUST00000140842.8
129 transcript 2	528	175	20	MN366017
129 novel long	600	199	23	MN366018
129 novel short	315	104	12	MN366019

Table S4. Differentially expressed transcripts between WT and *Sarm1*^{AGS3}, Related to figure 6.

Gene symbol	Gene name	Log2 Fold Change
Mock infected		
Atp5k	ATP synthase, subunit E (complex V)	0.702
Ndufa3	NADH: ubiquinone oxioreductase, subunit A3 (complex I)	0.671
Ndufb3	NADH: ubiquinone oxioreductase, subunit B3 (complex I)	0.591
Uqcrh	Cytochrome b-c1, subunit 6 (complex III)	0.365
Rpl38	Ribosomal protein L38	0.947
Rps29	Ribosomal protein S29	0.891
Rpl36a	Ribosomal protein L36a	0.633
Rps21	Ribosomal protein S21	0.624
Rpl37	Ribosomal protein L37	0.583
Rpl23	Ribosomal protein L23	0.551
Rpl5	Ribosomal protein L5	0.339
Rpl9	Ribosomal protein L9	0.316
Aatk	Apoptosis-associated tyrosine kinase	-0.286
Metrn	Meteorin, glial cell differentiation regulator	-0.351
Nbl1	Neuroblastoma, suppression of tumorigenicity	-0.494
Gabra6	GABA A receptor, subunit alpha 6	-1.27
WNV-KUN infected		
Rps29	Ribosomal protein S29	0.804
Ifi27k2a	Interferon-alpha inducible protein 27 like 2A	0.746
Tfrc	Transferrin receptor	-0.71
Malat1	Metastasis associated lung adenocarcinoma transcript 1	-0.722
Tug1	Taurine up-regulated (lnRNA)	-0.735
Srxn1	Sulfiredoxin 1	-0.779
Adcyap1	Adenylate cyclase activating peptide 1	-0.865
Fndc9	Fibronecting type III domain containing 9	-1.13
Prl	Prolactin	-1.38

Table S5. Primer sequences, Related to STAR methods.

	Forward primer	Reverse primer
Sarm1^{AGS3} genotyping	TCTCCGCCTACAAACTGTGC	GGATACCGTCTCCAACCACC
Sarm1^{AGS12} genotyping	GTCCTGACGCTGCTCTTCT	TGTGACAGCCTGTTTTGCTC
18S qRT-PCR	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Ccl1 qRT-PCR	CCCCTGAAGTTTATCCAGTGTTACAG	GTTGAGGCGCAGCTTTCTCTAC
Ccl2 qRT-PCR	TTGACCCGTAAATCTGAAGCTAAT	TCACAGTCCGAGTCACACTAGTTCAC
Ccl3 qRT-PCR	TGCCCTTGCTGTTCTTCTCT	GTGGAATCTTCCGGCTGTAG
Ccl4 qRT-PCR	AAGCTGCCGGGAGGTGTAAG	TGTCTGCCCTCTCTCTCCTCTTG
Ccl5 qRT-PCR	TGCCCACGTCAAGGAGTATTTC	TCCTAGCTCATCTCCAAATAGTTGATG
Ccl7 qRT-PCR	GGATCTCTGCCACGCTTCTG	TCCTTCTGTAGCTCTTGAGATTCCTC
Cxcl2 qRT-PCR	GTCCCTCAACGGAAGAACCAA	ACTCTCAGACAGCGAGGCACAT
Cxcl3 qRT-PCR	CTGGGATTCACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
Cxcl9 qRT-PCR	ATTGTGTCTCAGAGATGGTGCTAATG	TGAAATCCCATGGTCTCGAAAG
Cxcl10 qRT-PCR	TTCACCATGTGCCATGCC	GAACTGACGAGCCTGAGCTAGG
Cxcl11 qRT-PCR	AAAATGGCAGAGATCGAGAAAGC	CAGGCACCTTTGTCGTTTATGAG
Il1b qRT-PCR	TGTCTTGGCCGAGGACTAAGG	TGGGCTGGACTGTTTCTAATGC
Il6 qRT-PCR	TGAGATCTACTCGGCAAACCTAGTG	CTTCGTAGAGAACAACATAAGTCAGATACC
Il10 qRT-PCR	GGGTTGCCAAGCCTTATCG	TCTCACCCAGGGAATTCAAATG
Il12b qRT-PCR	CCTAAGTTCATCATGACACCTTTGC	CCAAGTGGAATGCTAGAATATCTATGC
Tnf qRT-PCR	AGAAACACAAGATGCTGGGACAGT	CCTTTGCAGAACTCAGGAATGG
Ifng qRT-PCR	TGCTGATGGGAGGAGATGTCTAC	TTTCTTTCAGGGACAGCCTGTTAC
Ifnb1 qRT-PCR	CACAGCCCTCTCCATCAACTA	CATTTCCGAATGTTCGTCCT
Sarm1 qRT-PCR	TCGCAATTTTGTCCTGGTG	AGCTTAAAGCAGTCACAATCTCC
Zcch24 Surveyor	GCTGTCTGCCATCGACACGA	CTGCTTACTAGGAGCAGGGCT
Tenm4-1 Surveyor	ATAAGCCTGGGGCCTAGTGA	TACTGCAGCGGTTACCAAGG
Rps271 Surveyor	TATTGTTCCGTGTGTCCCCC	GAACCCCTTTGTCGTTTGGC
Plscr3 Surveyor	GCCCGTCAGCTAGGATTAGG	TTGCTTCAGGAGGGAACGTC
Sh3bgr Surveyor	AGCTGGTGGAAGGAGAAAGC	AGGAGCATGAAACACTCCCC
Ccl5 SNP PCR	GCCAGGTGATGTAGCAGACA	CCACAACTGGCCTTTTCAGT
Xaf1 genotyping	GAACCCACACAGGGGTACAG	TCAGGCGGAGGACAGGAATA
Xaf1 cloning	ATGGAGGCTGACTTCCAAGTGT	TCACCAGCCCCACTGGAGTTT (B6) TCACTGGAGTTTCTTTTGGTGAG (129)