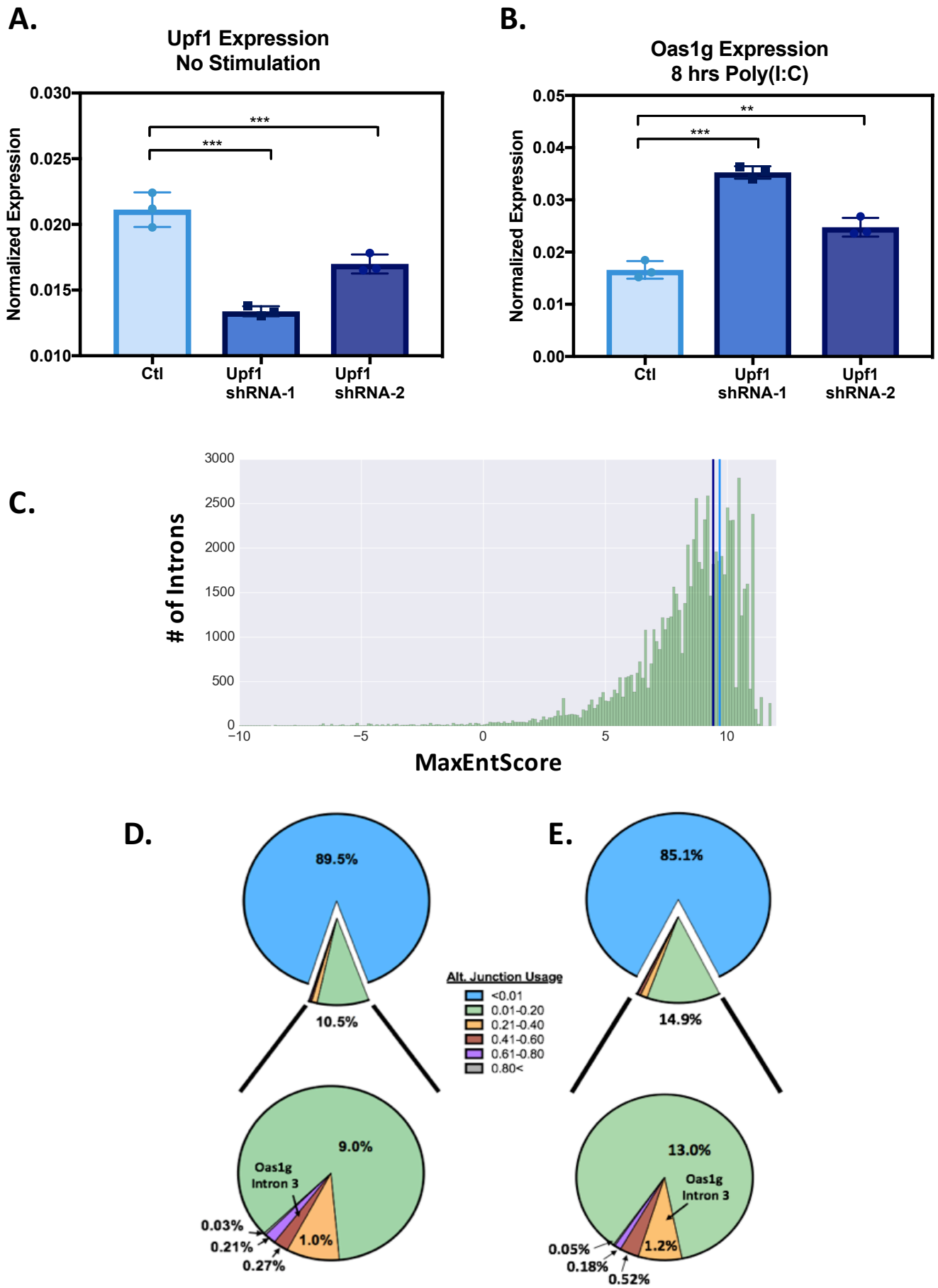


Figure S1. Related to Figure 1.

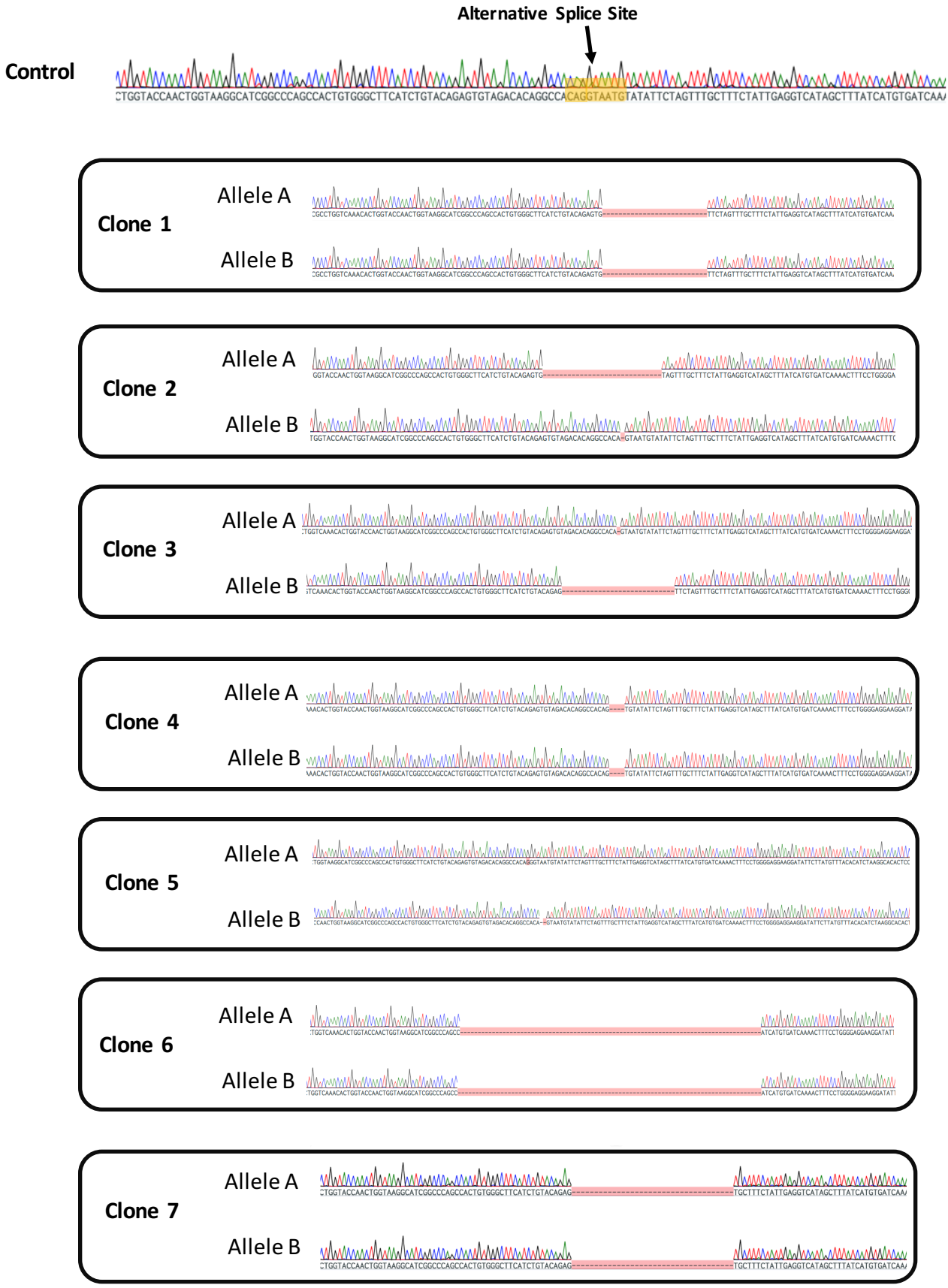
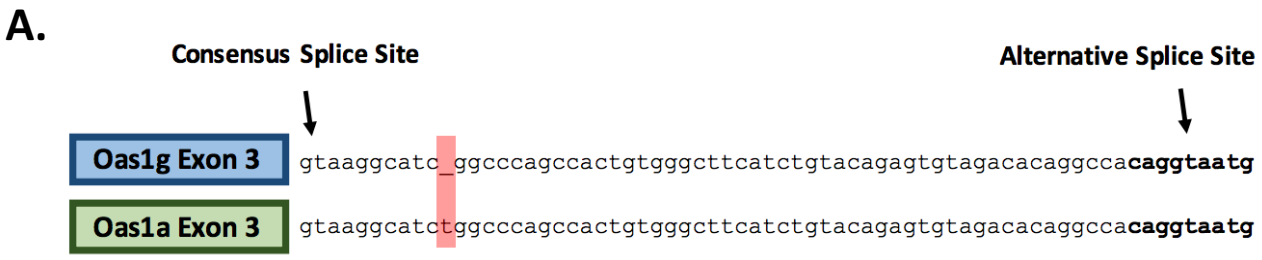
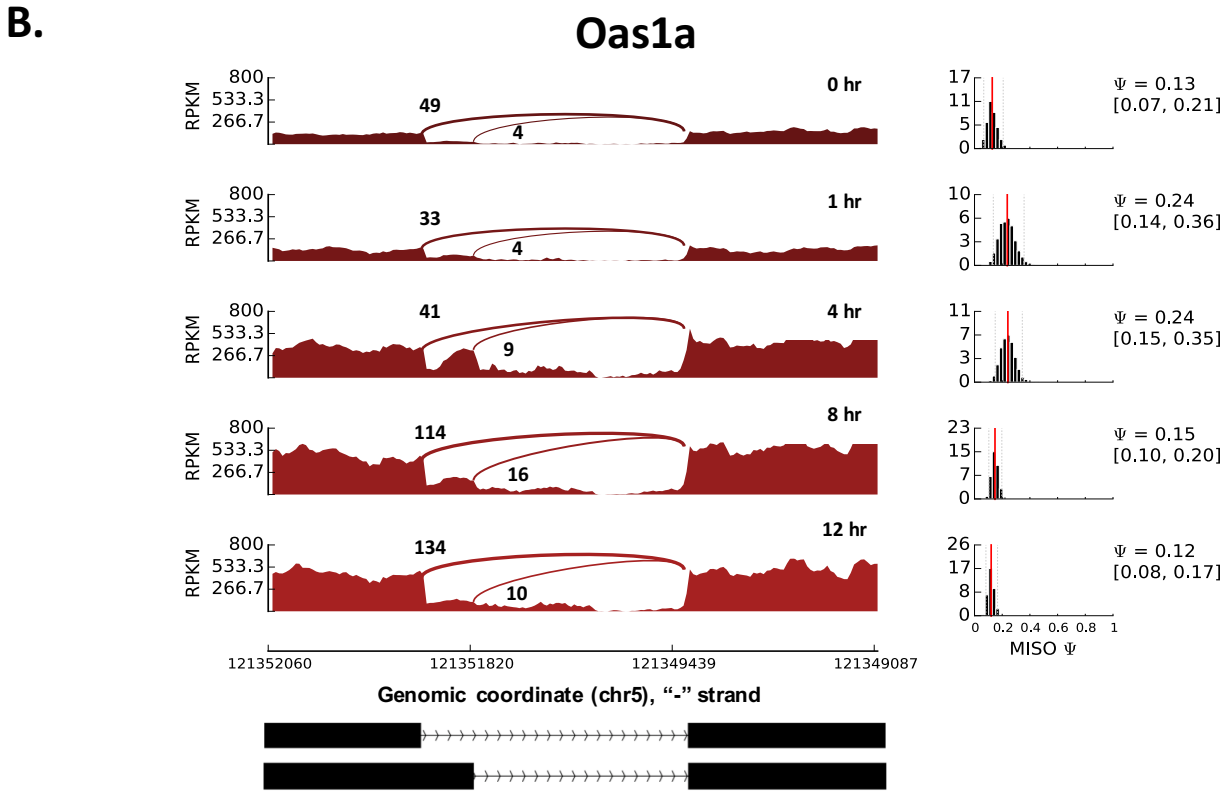
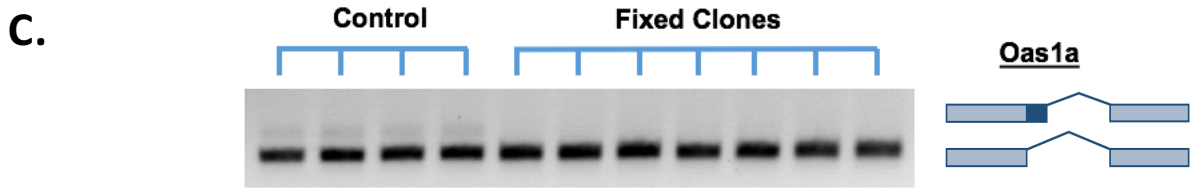


Figure S2. Related to Figure 2.







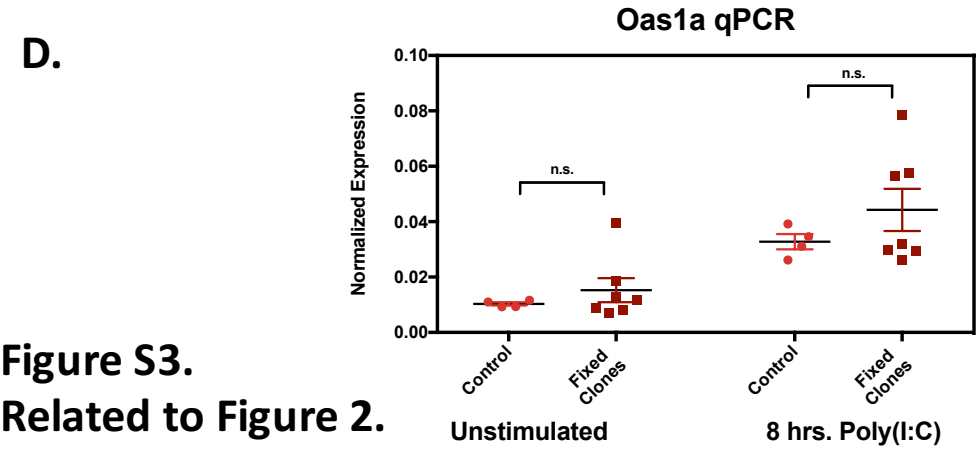


Figure S3.
Related to Figure 2.

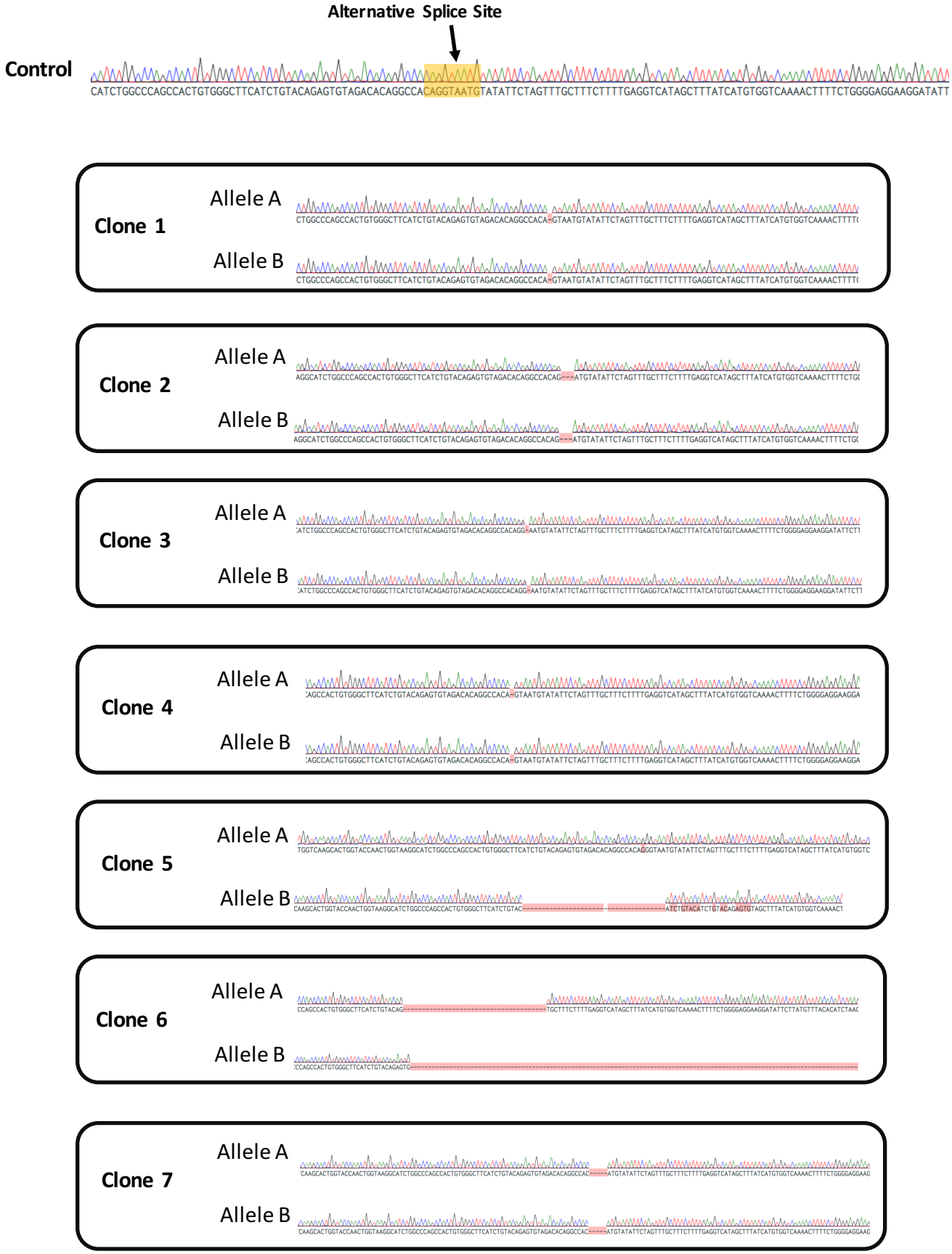


Figure S4. Related to Figure 2.

Supplemental Table 1: Oligonucleotides

OLIGONUCLEOTIDE	SOURCE	IDENTIFIER
EMCV Forward qPCR Primer (5' - CCTCTTAATTCGACGCTTGAA- 3')	Pérez et. al., 2009	N/A
EMCV Reverse qPCR Primer (5' - GGCAAGCATAGTGATCGAAG- 3')	Pérez et. al., 2009	N/A
Rpl32 Forward qPCR Primer (5' - GCCTCTGGTGAAGCCCAAG - 3')	Ruiz-Villalba et. al., 2017	N/A
Rpl32 Reverse qPCR Primer (5' – TTGTTGCTCCCATAACCGATGT - 3')	Ruiz-Villalba et. al., 2017	N/A
Oas1g TaqMan	Thermo Fisher Scientific	Mm01730198_m1
Rpl32 TaqMan	Thermo Fisher Scientific	Mm02528467_g1
Oas1a TaqMan	Thermo Fisher Scientific	Mm00836412_m1

Supplemental Figure Legends

Figure S1. The Alternative 5' Splice Site Mediating the AS Event is of Similar Strength to the Consensus 5' Splice Site. (A) RT-qPCR analysis of *Upf1* mRNA levels in unstimulated macrophages expressing a scrambled shRNA (light blue) or one of two *Upf1* targeted shRNAs (darker blue). **(B)** RT-qPCR analysis of *Oas1g* mRNA levels in poly(I:C) stimulated (8 hrs.) macrophages expressing a scrambled shRNA (light blue) or one of two *Upf1* targeted shRNAs (darker blue). **(C)** Histogram representing the 5' splice site strength (MaxEntScore) of introns of expressed in BMDMs. The bin with which the consensus splice site falls is shown by the light blue line. The bin with which the alternative splice site falls is shown by the dark blue line. **(D)** Pie chart representing alternative junction usage for all expressed junctions upon 4 hrs. of poly(I:C) stimulation. The slice including the alternatively spliced third junction of *Oas1g* is labelled (Alt. Junction Usage 0.41-0.60). **(E)** Same as (D) but for 12 hrs. of poly(I:C) stimulation. The slice including the alternatively spliced third junction of *Oas1g* is labelled (Alt. Junction Usage 0.21-0.40). Data is representative of two independent experiments **(A, B)** from three biological replicates (error bars indicate SEM). * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$ using a Student's t test. Results are presented relative to those of *Rpl32*.

Figure S2. *Oas1g* Macrophage Cell Line Genotyping. Sanger sequencing gDNA from a control sample (very top) and the *Oas1g* SS KO clones. Sequencing is centered around the *Oas1g* alternative splice site. Sequencing is oriented such that the negative strand runs left to right.

Figure 3. *Oas1a* has a Similar Frequently Utilized AS-NMD Event. (A) Schematic depiction showing the homology between *Oas1a* and *Oas1g* at the alternatively spliced third junction. **(B)** (left) Sashimi plots centered at the third junction of *Oas1g* from BMDMs stimulated with poly(I:C) for 0, 1, 4, 8, and 12 hrs. Sequenced RNA was derived from the total nuclear fraction. The y-axis represents Reads Per Kilobase of transcript, per Million mapped reads (RPKM). Genomic coordinates represent the mm9 genome assembly. (right) Posterior distributions of the Ψ value for each individual time point. The mean Ψ is depicted by the red line. Mean and 95% confidence intervals are labelled to the right of the posterior distribution. **(C)** RT-PCR upon stimulation with poly(I:C) confirming alternative splice site usage in control populations and forced productive splicing in fixed clones. **(D)** RT-qPCR analysis of *Oas1g* mRNA levels in unstimulated and

stimulated (8 hrs poly(I:C)) macrophages. Control samples are represented in light blue, SS KO clones are represented in dark blue. Data is representative of two independent experiments **(D-F)** and is shown as mean (error bars indicate SEM). * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$ using a Student's t test. Results are presented relative to those of *Rpl32* **(D)**

Figure S4. *Oas1a* Macrophage Cell Line Genotyping. Sanger sequencing gDNA from a control sample (very top) and the *Oas1a* SS KO clones. Sequencing is centered around the *Oas1a* alternative splice site. Sequencing is oriented such that the negative strand runs left to right.

Supplemental Table 1: Oligonucleotides