

Appendix for

Alternative splicing broadens antiviral diversity at the human *OAS2* locus

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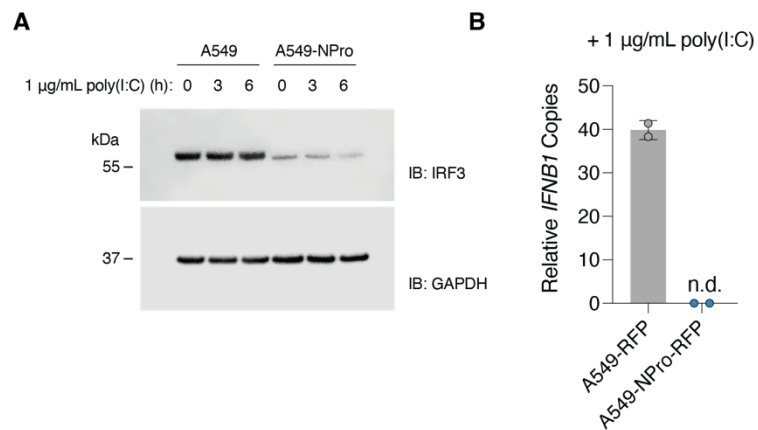
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Appendix Figure S1 – BVDV NPro degrades IRF3 and inhibits IFN signalling.

(A) A549-RFP and A549-NPro-RFP were transfected with 1 $\mu\text{g/mL}$ poly(I:C), and IRF3 levels were monitored at multiple timepoints, by Western blotting.

(B) A549-RFP and A549-NPro-RFP were transfected with 1 $\mu\text{g/mL}$ poly(I:C) and IFNB1 transcript levels were measured at 6 h, by RT-qPCR. n.d., not detected.

Appendix Table S1 - List of oligonucleotides used in this study

Purpose	Target	Sequence
Mutagenesis	OAS2 D481A forward	CCTCAACGAAAGTGTGAGCTTTGCAGTGCTTCCTGCCTTTAA TGCACTGGG
Mutagenesis	OAS2 D481A reverse	AAAGCTGACACTTTCGTTGAGGACTTTGG
Mutagenesis	OAS2 CAFAKA forward	GGAATGGTTATCCTCTCCCGCCGCCGCGATGGGACTGGAA ACCCAATACCACC
Mutagenesis	OAS2 CAFAKA reverse	GGGAGAGGATAACCATTCCTTTGCTTC
Mutagenesis	OAS2 R529E/R533E forward	CTGTTTCACAGTCCTGCAGGAAACTTCATTGAATCCCGGCC CACCAAACATAAAGG
Mutagenesis	OAS2 R529E/R533E reverse	CTGCAGGACTGTGAAACAGGTAGAAAAC
Mutagenesis	OAS2 p71 727 forward	GCCTCAGACAGTGGTTCAAAG
Mutagenesis	OAS2 p71 727 reverse	CCGCCCTCGAGGAATTCGGCCAGAGAGGCCCTTAAAGCGAT TGCCAGATGATCTCCAGAAGTTCTTTTAGAATTATTATTCAG
Mutagenesis	OAS2 p71 Δ 18 forward	GCCTCAGACAGTGGTTCAAAG
Mutagenesis	OAS2 p71 Δ 18 reverse	CCGCCCTCGAGGAATTCGGCCAGAGAGGCCCTACTCATTGA CAATAGGATGGATCCTAGC
Mutagenesis	OAS2 core forward	GCCTCAGACAGTGGTTCAAAG
Mutagenesis	OAS2 core reverse	CCGCCCTCGAGGAATTCGGCCAGAGAGGCCCTACGGCACT TTCCAAGGTGGTATTGGGTTTC
Mutagenesis	OAS2 p71 VKVI forward	CTGAATAATAATTCTAAAAGAACTTCGTAAAAGTCATCTAGG GCCTCTCTGGCCGAATTCC
Mutagenesis	OAS2 p71 VKVI reverse	GAAGTTTCTTTTAGAATTATTATTCAGGATTCTATGGCTTCTG
Mutagenesis	OAS2 p69 V684A forward	GCCTCAGACAGTGGTTCAAAG
Mutagenesis	OAS2 p69 V684A reverse	CCGCCCTCGAGGAATTCGGCCAGAGAGGCCCTTAGATGACTT TTGCCGGCACTTTCCAAGG
Mutagenesis	OAS2 p69 K685A forward	GCCTCAGACAGTGGTTCAAAG
Mutagenesis	OAS2 p69 K685A reverse	CCGCCCTCGAGGAATTCGGCCAGAGAGGCCCTTAGATGACTG CTACCGGCACCTTTCCAAGG
Mutagenesis	OAS2 p69 V686A forward	GCCTCAGACAGTGGTTCAAAG
Mutagenesis	OAS2 p69 V686A reverse	CCGCCCTCGAGGAATTCGGCCAGAGAGGCCCTTAGATTGCTT TTACCGGCACCTTTCCAAGG
Mutagenesis	OAS2 p69 I687A forward	GCCTCAGACAGTGGTTCAAAG
Mutagenesis	OAS2 p69 I687A reverse	CCGCCCTCGAGGAATTCGGCCAGAGAGGCCCTTATGCGACTT TTACCGGCACCTTTCCAAGG
OAS2 KO Generation	sgOAS2_1	GTCTTAAGAGGCAACTCCGA
OAS2 KO Generation	sgOAS2_2	GGACGGAAAACAGTCTTAAG
OAS2 KO Generation	sgOAS2_3	GCTTACTCAGAGCGTTGAAGG
RNase L KO Generation	sgRNaseL_1	GCCGAGTTGCTGTGCAACG
RNase L KO Generation	sgRNaseL_2	TTATCCTCGCAGCGATTGCG
RNase L KO Generation	sgRNaseL_3	TATAGGACGCTTCGGAATGT
STAT1 KO Generation	sgSTAT1_1	GAGGTCATGAAAACGGATGG
RT-qPCR	HCoV-OC43 ORF1a forward	ATGTGGTGTAAGCAGGAAC
RT-qPCR	HCoV-OC43 ORF1a reverse	GCAAGAACAGTCCACGGTATA
RT-qPCR	HCoV-OC43 ORF1a Probe	TACTGGTCTGGACGCTGTTATGC
RT-qPCR	HCoV-OC43 N forward	CTACTTCGCGCACATCCAG
RT-qPCR	HCoV-OC43 N reverse	GTCAGGTGTTACACCAGAGG
RT-qPCR	HCoV-OC43 N Probe	AGCCTCTAGTGCAGGATCGCGTAG

Appendix Table S2 - Exact P-values for main and expanded view figures

Figure	Test	Group Compared	P-value	Summary
2D	Welch's t-test	RFP vs. p71 24 h	0.9329	ns
		RFP vs. p69 24 h	0.3427	ns
		RFP vs. p71 48 h	0.8118	ns
		RFP vs. p69 48 h	0.0066	**
		RFP vs. p71 72 h	0.5057	ns
		RFP vs. p69 72 h	<0.0001	****
2E	One-way ANOVA with Dunnett's test	RFP vs. p71	0.6743	ns
		RFP vs. p69	<0.0001	****
2F	One-way ANOVA with Dunnett's test	RFP vs. p71	0.9494	ns
		RFP vs. p69	0.0193	*
2G	One-way ANOVA with Dunnett's test	RFP vs. p71	0.9631	ns
		RFP vs. p69	0.0060	**
2J	Welch's t-test	RFP vs. p71 24 h	0.1543	ns
		RFP vs. p69 24 h	0.0352	*
		p71 vs. p69 24 h	0.5696	ns
		RFP vs. p71 48 h	0.2028	ns
		RFP vs. p69 48 h	0.0364	*
		p71 vs. p69 48 h	0.0137	*
		RFP vs. p71 72 h	0.1099	ns
		RFP vs. p69 72 h	0.0035	**
2L	Welch's t-test	p71 vs. p69 48 h	0.0081	ns
		Cas9/sgOAS1_1 vs Cas9 24 h	0.4617	ns
		Cas9/sgOAS1_1 vs Cas9 48 h	0.5701	ns
		Cas9/sgOAS1_1 vs Cas9 72 h	0.04474	*
2N	Welch's t-test	Cas9/sgOAS1_1 vs Cas9 24 h	0.189	ns
		Cas9/sgOAS1_1 vs Cas9 48 h	0.0924	ns
		Cas9/sgOAS1_1 vs Cas9 72 h	0.0508	ns
3C	One-way ANOVA with Dunnett's test	Untreated vs. p71	<0.0001	****
		Untreated vs. p69	<0.0001	****
3E	One-way ANOVA with Dunnett's test	RFP vs. p71 ^{WT}	0.9162	ns
		RFP vs. p71 ^{G2A}	0.9902	ns
		RFP vs. p69 ^{WT}	<0.0001	****
		RFP vs. p69 ^{G2A}	>0.9999	ns
EV3C	One-way ANOVA with Dunnett's test	Untreated vs. p71	0.0008	***
		Untreated vs. p69	0.1045	ns
4F	One-way ANOVA with Dunnett's test	RFP vs. p69 ^{WT}	0.0096	**
		RFP vs. p69 ^{H529L/H533L}	0.5055	ns
4G	One-way ANOVA with Dunnett's test	RFP vs. p69 ^{WT}	0.0086	**
		RFP vs. p69 ^{H529L/H533L}	0.6653	ns
5B	One-way ANOVA with Dunnett's test	RFP vs. p69 ^{WT}	<0.0001	****
		RFP vs. p69 ^{U481A}	<0.0001	****
		RFP vs. p69 ^{CAI-AKA}	<0.0001	****
5C	One-way ANOVA with Dunnett's test	RFP vs. p69 ^{WT}	<0.0001	****
		RFP vs. p69 ^{U481A}	<0.0001	****
		RFP vs. p69 ^{CAI-AKA}	<0.0001	****
6D	One-way ANOVA with Tukey's test	Cas9 + RFP vs. Cas9 + p71	>0.9999	ns
		Cas9 + RFP vs. Cas9 + p69	0.0129	*
		Cas9 + RFP vs. Cas9/sgSTAT1 + RFP	0.9991	ns
		Cas9/RFP vs. Cas9/sgSTAT1 + p71	>0.9999	ns
		Cas9 + RFP vs. Cas9/sgSTAT1 + p69	0.0045	**
		Cas9 + p71 vs. Cas9 + p69	0.0128	*
		Cas9 + p71 vs. Cas9/sgSTAT1 + RFP	0.9991	ns
		Cas9 + p71 vs. Cas9/sgSTAT1 + p71	>0.9999	ns
		Cas9 + p71 vs. Cas9/sgSTAT1 + p69	0.0045	**
		Cas9 + p69 vs. Cas9/sgSTAT1 + RFP	0.0051	**
		Cas9 + p69 vs. Cas9/sgSTAT1 + p71	0.0210	*
		Cas9 + p69 vs. Cas9/sgSTAT1 + p69	0.9984	ns
		Cas9/sgSTAT1 + RFP vs. Cas9/sgSTAT1 + p71	0.9930	ns
		Cas9/sgSTAT1 + RFP vs. Cas9/sgSTAT1 + p69	0.0017	**
		Cas9/sgSTAT1 + p71 vs. Cas9/sgSTAT1 + p69	0.0075	**

7A	Welch's t-test	RFP vs. p71 8h	0.6210 ns
		RFP vs. p69 8 h	0.5567 ns
		RFP vs. p71 24 h	0.0132 *
		RFP vs. p69 24 h	0.1501 ns
		RFP vs. p71 48 h	0.0631 ns
		RFP vs. p71 48 h	0.5168 ns
7B	Welch's t-test	RFP vs. p71 8 h	0.2937 ns
		RFP vs. p69 8 h	0.3544 ns
		p71 vs. p69 8 h	0.8631 ns
		RFP vs. p71 24 h	0.0302 *
		RFP vs. p69 24 h	0.061 ns
		p71 vs. p69 24 h	0.1443 ns
		RFP vs. p71 48 h	0.0165 *
		RFP vs. p69 48 h	0.01 **
7D	Welch's t-test	p71 vs. p69 48 h	0.4419 ns
		Cas9/sgOAS1_1 vs Cas9 8 h	0.3173 ns
		Cas9/sgOAS1_1 vs Cas9 24 h	0.3255 ns
		Cas9/sgOAS1_1 vs Cas9 48 h	0.8079 ns
7F	Welch's t-test	Cas9/sgOAS1_1 vs Cas9 8 h	0.4141 ns
		Cas9/sgOAS1_1 vs Cas9 24 h	0.1542 ns
		Cas9/sgOAS1_1 vs Cas9 48 h	0.0911 ns
7G	One-way ANOVA with Dunnett's test	RFP vs. p71	0.0030 **
		RFP vs. p71 ^{G2A}	0.9370 ns
		RFP vs. p69	0.7989 ns
		RFP vs. p69 ^{G2A}	0.9963 ns
7I	One-way ANOVA with Dunnett's test	RFP vs. p71	0.0164 *
		RFP vs. p71 ^{D481A}	0.9621 ns
		RFP vs. p71 ^{H529E/H533E}	0.8377 ns
7K	One-way ANOVA with Tukey's test	Cas9 + RFP vs. Cas9 + p71	<0.0001 ****
		Cas9 + RFP vs. Cas9/sgRNaseL + RFP	0.0138 *
		Cas9 + RFP vs. Cas9/sgRNaseL + p71	0.0109 *
		Cas9 + p71 vs. Cas9/sgRNaseL + RFP	<0.0001 ****
		Cas9 + p71 vs. Cas9/sgRNaseL + p71	<0.0001 ****
		Cas9/sgRNaseL + RFP vs. Cas9/sgRNaseL + p71	0.9979 ns
7N	One-way ANOVA with Dunnett's test	NPro ^{RFP} vs. NPro ^{p71}	0.0026 **
		NPro ^{H1+P} vs. NPro ^{p69}	0.5758 ns
8H	One-way ANOVA with Dunnett's test	RFP vs. p71 ⁷²⁷	0.7651 ns
		RFP vs. p71	0.8156 ns
		RFP vs. p71 ^{Δ18}	0.8733 ns
		RFP vs. p69	0.0004 ***
		RFP vs. OAS2 ^{core}	>0.9999 ns
8I	One-way ANOVA with Dunnett's test	RFP vs. p71 ⁷²⁷	0.227 ns
		RFP vs. p71	0.0003 ***
		RFP vs. p71 ^{Δ18}	0.0281 *
		RFP vs. p69	0.3617 ns
		RFP vs. OAS2 ^{core}	0.4091 ns
8L	One-way ANOVA with Dunnett's test	RFP vs. p71	0.9998 ns
		RFP vs. p69	0.0429 *
		RFP vs. p69 ^{V684A}	0.0307 *
		RFP vs. p69 ^{K685A}	0.2151 ns
		RFP vs. p69 ^{V686A}	>0.9999 ns
		RFP vs. p69 ^{I687A}	0.9979 ns
8O	One-way ANOVA with Dunnett's test	RFP vs. p71	0.9615 ns
		RFP vs. p71 ^{VKVI}	0.9945 ns
		RFP vs. p69	0.0527 ns
EV5F	One-way ANOVA with Dunnett's test	RFP/RFP vs. p71/RFP	0.3476 ns
		RFP/RFP vs. p69/RFP	0.0005 ***
		RFP/RFP vs. p71/p69	0.3807 ns
EV5G	One-way ANOVA with Dunnett's test	RFP/RFP vs. p71/RFP	<0.0001 ****
		RFP/RFP vs. p69/RFP	0.7944 ns
		RFP/RFP vs. p71/p69	<0.0001 ****