

**Supplemental Figure 1. Acridine orange staining in V0D2 KO cells**

(A, B) Cells were stained with acridine orange and emissions at 520 and 620 nm were detected for the internal marker (520 nm) and endosomal acidification (620 nm) by flow cytometry. MFI of 520 nm or 620 nm were shown by histogram (A) and bar graph (B).

**A**

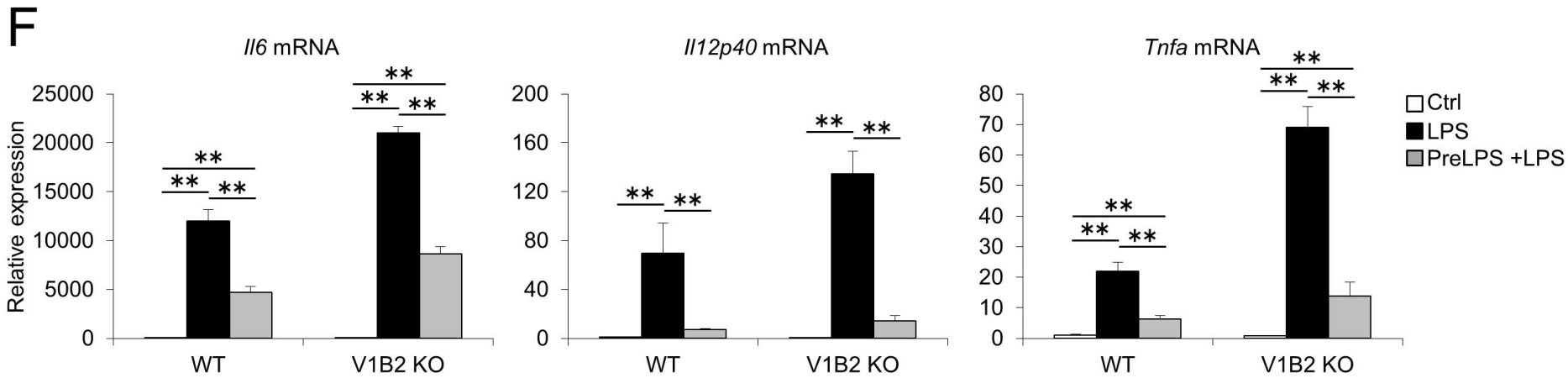
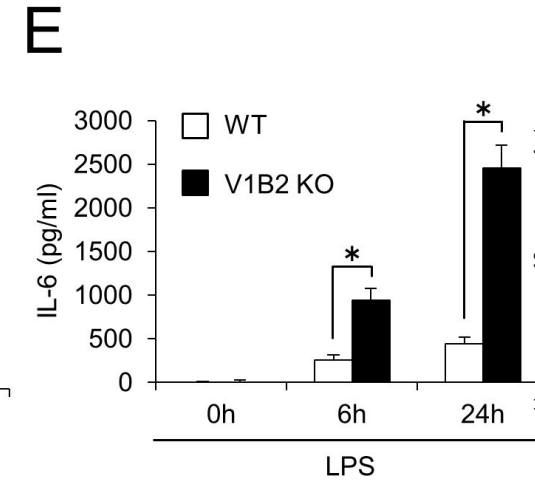
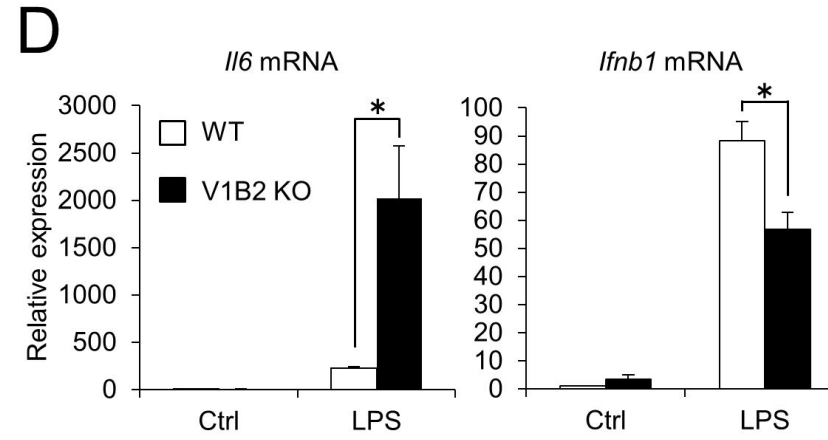
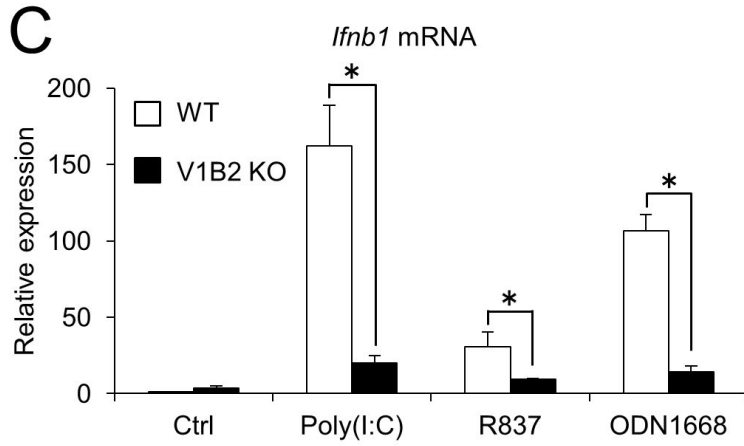
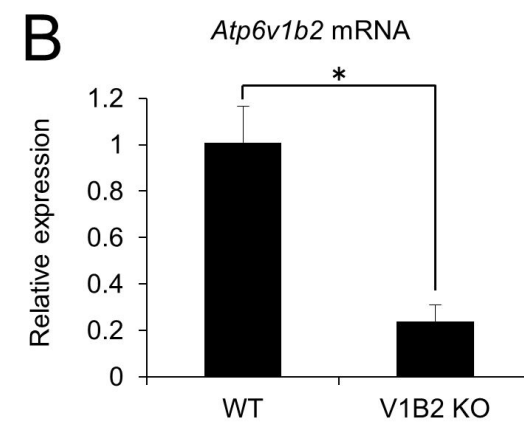
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V1B2-WT-Exon1      1  ATGGCGTTGCGAGCGATGCGGGGAATCGTGAACGGGGCCGCACCCGAACTGCCCGTGCCC 60
V1B2-KO-Exon1-chrom1 1  ATGGCGTTGCGAGCGATGCGGGGAATCGTGAACGGGGCCGCACCCGAACTGCCCGTGCCC 60
V1B2-KO-Exon1-chrom2 1  ATGGCGTTGCGAGCGATGCGGGGAATCGTGAACGGGGCCGCACCCGAACTGCCCGTGCCC 60

V1B2-WT-Exon1      61  ACCGGTGGGCCGATGGCCGGAGCTCGGGAGCAGGCGCTGGCGGTGAGCCGGAAGTACTCTA 120
V1B2-KO-Exon1-chrom1 61  ACCGGTGGGCCGATGGCCGGAGCTCGGGAGC-----CTA 94
V1B2-KO-Exon1-chrom2 61  ACCGGTGGGCCGATGGCCGGAGCTCGGGAGC-----CTA 94

V1B2-WT-Exon1      121  TCCAGCCTCGTCTCA 136
V1B2-KO-Exon1-chrom1 95  TCCAGCCTCGTCTCA 110
V1B2-KO-Exon1-chrom2 95  TCCAGCCTCGTCTCA 110

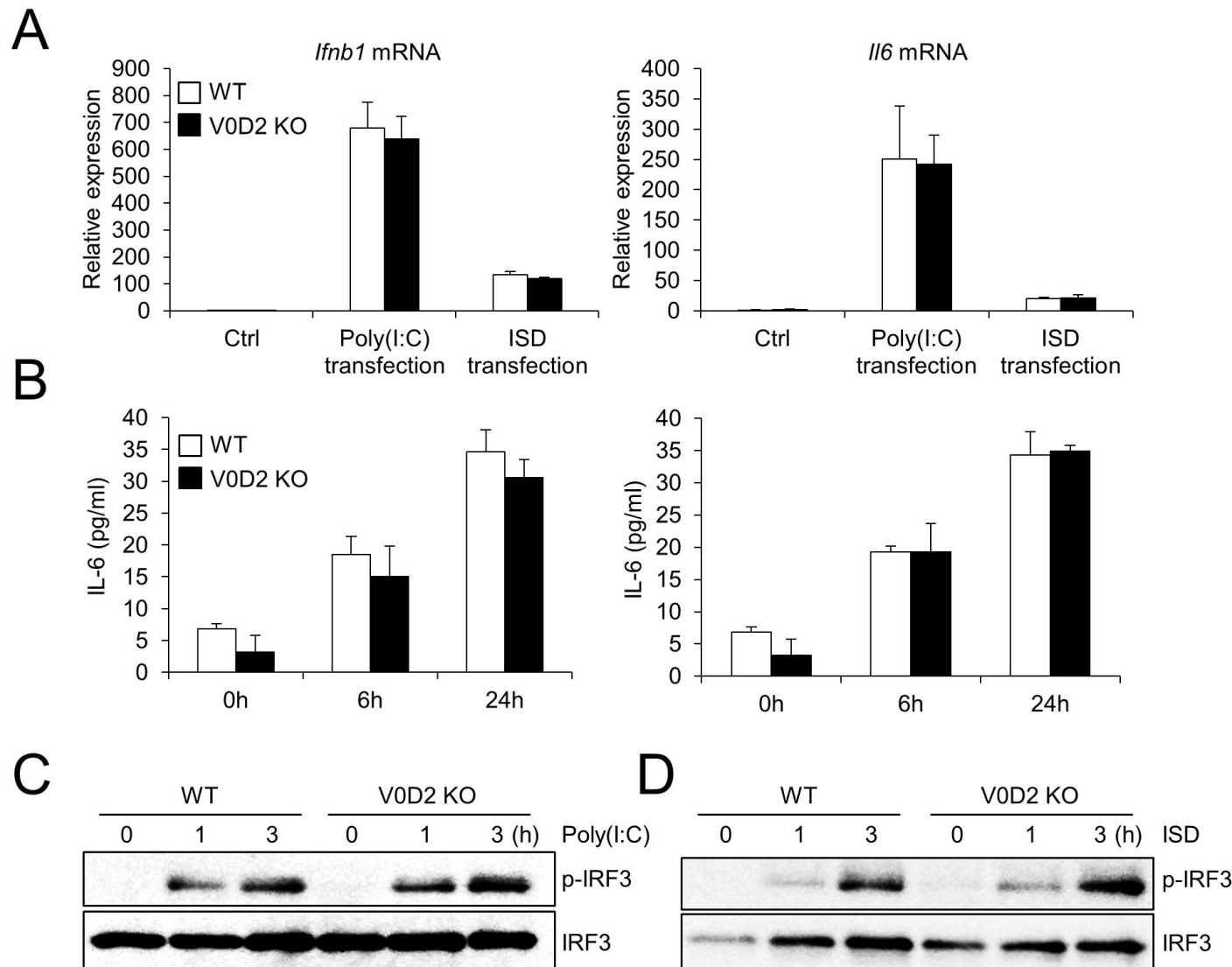
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### Supplemental Figure 2. Functional analysis of ATP6V1B2 KO cells

(A) Genomic sequence of the *Atp6v1b2* exon 1 locus in V1B2 KO cells. (B) *Atp6v1b2* expression in WT and V1B2 KO RAW264.7 cells was measured by RT-qPCR. (C) WT and V1B2 KO RAW264.7 cells were stimulated with 50  $\mu$ g/ml poly(I:C), 5  $\mu$ g/ml R837, or 1  $\mu$ M ODN1668 for 6 h, and then *Ifnb1* expression was measured by RT-qPCR. (D) WT and V1B2 KO RAW264.7 cells were stimulated with 1  $\mu$ g/ml LPS for 6 h, and then *Il6* and *Ifnb1* expression was measured by RT-qPCR. (E) WT and V1B2 KO RAW264.7 cells were stimulated with 1  $\mu$ g/ml LPS for 6 or 24 h, and then IL-6 concentrations were measured by an ELISA. (F) WT and V1B2 KO RAW264.7 cells were stimulated with 10 ng/ml LPS for 18 h. The cells were washed to remove LPS and re-stimulated with 100 ng/ml LPS for 6 h. *Il6*, *Il12p40*, and *Tnfa* expression was measured by RT-qPCR. Data are representative of three independent experiments and mean values and standard errors are depicted. \* $P < 0.05$  (Student's *t*-test) and \*\* $P < 0.05$  (one-way ANOVA with Tukey test).

## Supplemental Figure 2

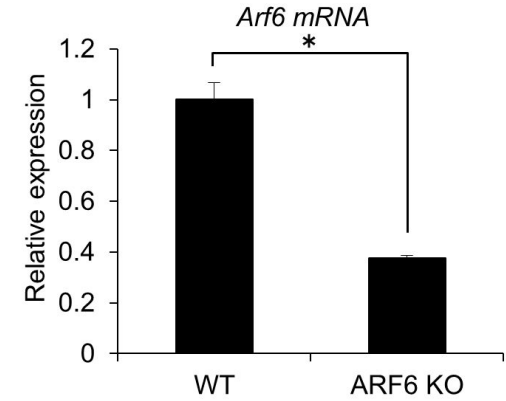


**Supplemental Figure 3. Dispensable role of ATP6V0D2 in cytosolic nucleic acid-mediated innate immune responses** (A, B) WT and V0D2 KO RAW264.7 cells were transfected with 1  $\mu$ g poly(I:C) or 1  $\mu$ g ISD for 6 h or 24 h, and then *Ifnb1* and *Il6* mRNA expression or production was measured by RT-qPCR (A) or ELISA (B). (C, D) WT and V0D2 KO RAW264.7 cells were stimulated by transfection of 1  $\mu$ g poly(I:C) (C) or 1  $\mu$ g ISD (D) for the indicated periods, and then lysates were immunoblotted with the indicated antibodies. Data are representative of three independent experiments and mean values and standard errors are depicted.

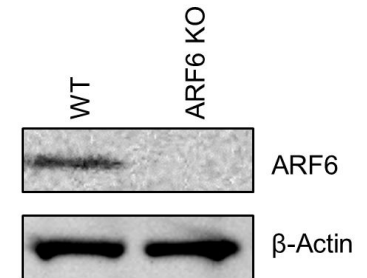
**A**

ARF6-WT-Exon2	1	ATGGGGAAGGTGCTATCCAAGATCTTCGGGAACAAGGAAATGCGGATCCTCATGCTGGGC	60
ARF6-KO-Exon2-chromo1	1	ATGGGGAAGGTGCTATCCAAGATCTTCGGGAACAAGGAAATGCGGATCCTCAT-----GC	55
ARF6-KO-Exon2-chromo2	1	ATGGGGAAGGTGCTATCCAAGATCTTCGGGAACAAGGAAATGCGGATCCTCATGCT--GC	58
ARF6-WT-Exon2	61	CTGGACGCAGCCGGCAAGACAACGATCCTGTACAAGTTGAAGCTGGGCAATCGGTGACC	120
ARF6-KO-Exon2-chromo1	56	CTGGACGCAGCCGGCAAGACAACGATCCTGTACAAGTTGAAGCTGGGCAATCGGTGACC	115
ARF6-KO-Exon2-chromo2	59	CTGGACGCAGCCGGCAAGACAACGATCCTGTACAAGTTGAAGCTGGGCAATCGGTGACC	118
ARF6-WT-Exon2	121	ACCATCCCCACGGTGGGCTTCAACGTGGAGACGGTGACTTACAAAAACGTCAAGTTCAAC	180
ARF6-KO-Exon2-chromo1	116	ACCATCCCCACGGTGGGCTTCAACGTGGAGACGGTGACTTACAAAAACGTCAAGTTCAAC	175
ARF6-KO-Exon2-chromo2	119	ACCATCCCCACGGTGGGCTTCAACGTGGAGACGGTGACTTACAAAAACGTCAAGTTCAAC	178
ARF6-WT-Exon2	181	GTGTGGGATGTGGGCGGCCAGGACAAGATCCGGCCGCTCTGGCGGCATTACTACACCGGG	240
ARF6-KO-Exon2-chromo1	176	GTGTGGGATGTGGGCGGCCAGGACAAGATCCGGCCGCTCTGGCGGCATTACTACACCGGG	235
ARF6-KO-Exon2-chromo2	179	GTGTGGGATGTGGGCGGCCAGGACAAGATCCGGCCGCTCTGGCGGCATTACTACACCGGG	238
ARF6-WT-Exon2	241	ACCCAGGGTCTGATCTTCGTGGTAGACTGCGCCGACCGCGACCGCATCGACGAGGCCCGC	300
ARF6-KO-Exon2-chromo1	236	ACCCAGGGTCTGATCTTCGTGGTAGACTGCGCCGACCGCGACCGCATCGACGAGGCCCGC	295
ARF6-KO-Exon2-chromo2	239	ACCCAGGGTCTGATCTTCGTGGTAGACTGCGCCGACCGCGACCGCATCGACGAGGCCCGC	298
ARF6-WT-Exon2	301	CAGGAGCTGCACCGCATTATCAATGACCGGGAGATGAGGGACGCCATCATCCTCATCTTC	360
ARF6-KO-Exon2-chromo1	296	CAGGAGCTGCACCGCATTATCAATGACCGGGAGATGAGGGACGCCATCATCCTCATCTTC	355
ARF6-KO-Exon2-chromo2	299	CAGGAGCTGCACCGCATTATCAATGACCGGGAGATGAGGGACGCCATCATCCTCATCTTC	358
ARF6-WT-Exon2	361	GCCAACAAGCAGGACCTGCCCGATGCCATGAAACCCCATGAGATCCAGGAGAAACTGGGC	420
ARF6-KO-Exon2-chromo1	356	GCCAACAAGCAGGACCTGCCCGATGCCATGAAACCCCATGAGATCCAGGAGAAACTGGGC	415
ARF6-KO-Exon2-chromo2	359	GCCAACAAGCAGGACCTGCCCGATGCCATGAAACCCCATGAGATCCAGGAGAAACTGGGC	418
ARF6-WT-Exon2	421	CTGACCCGGATTCGGGACAGGAAC TGGTATGTGCAGCCCTCCTGTGCCACCTCCGGGGAC	480
ARF6-KO-Exon2-chromo1	416	CTGACCCGGATTCGGGACAGGAAC TGGTATGTGCAGCCCTCCTGTGCCACCTCCGGGGAC	475
ARF6-KO-Exon2-chromo2	419	CTGACCCGGATTCGGGACAGGAAC TGGTATGTGCAGCCCTCCTGTGCCACCTCCGGGGAC	478
ARF6-WT-Exon2	481	GGACTCTATGAGGGGCTCACATGGTTAACCTCTAACTACAAATCCTAA	528
ARF6-KO-Exon2-chromo1	476	GGACTCTATGAGGGGCTCACATGGTTAACCTCTAACTACAAATCCTAA	523
ARF6-KO-Exon2-chromo2	479	GGACTCTATGAGGGGCTCACATGGTTAACCTCTAACTACAAATCCTAA	526

**B**



**C**



**Supplemental Figure 4. Generation of ARF6 KO in RAW264.7 cells**

(A) Genomic sequence of the *Arf6* exon 2 locus in ARF6 KO cells. (B, C) *Arf6* expression was measured by RT-qPCR (B) and immunoblotting (C). Data are representative of three independent experiments and mean values and standard errors are depicted. \*P < 0.05 (Student's *t*-test).