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Supplemental information

Immune regulator LGP2 targets Ubc13/UBE2N to mediate widespread interference with K63 polyubiquitination and NF-κB activation

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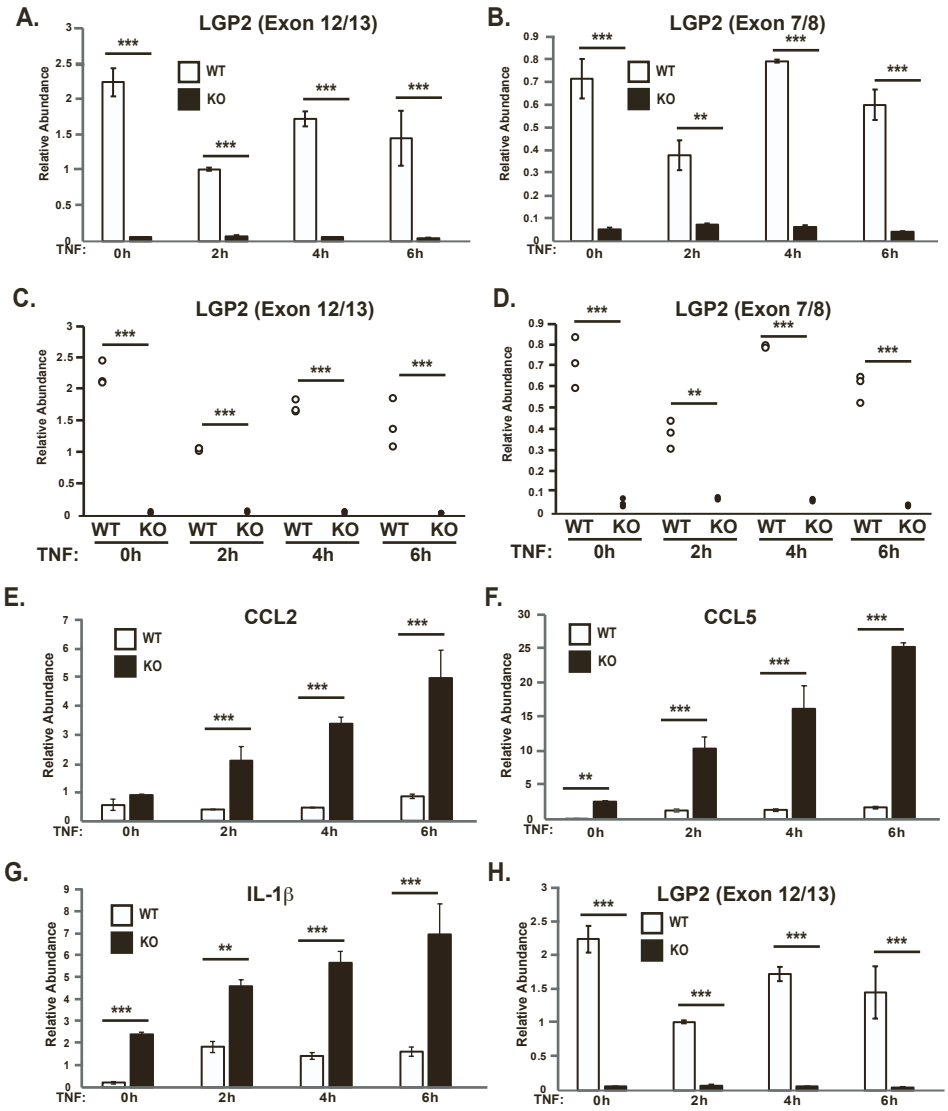

Figure S1: LGP2 Primer Data and Corresponding Bar Graphs Related to Figure 1
A-D) 2fTGH cell lines with (WT) and without (KO) LGP2 expression were stimulated for up to 6 hours with TNF-a prior to RNA isolation and RT-qPCR to measure mRNA levels of: A.) LGP2 (Exon 12/13), B.) LGP2 (Exon 7/8), and corresponding dot plots for C.) LGP2 (Exon 12/13), and D.) LGP2 (Exon 7/8). Bars represent average values ± standard deviation. *P<0.05, ** P < 0.005, *** P < 0.0005 by two-tailed Student's t test.
E-H) Representative bar graphs of RT-qPCR dot plots in Figure 1 D-G for E.) CCL2, F.) CCL5, G.) IL1-b, and H.) LGP2. Bars represent average values ± standard deviation. *P<0.05, ** P < 0.005, *** P < 0.0005 by two-tailed Student's t test.

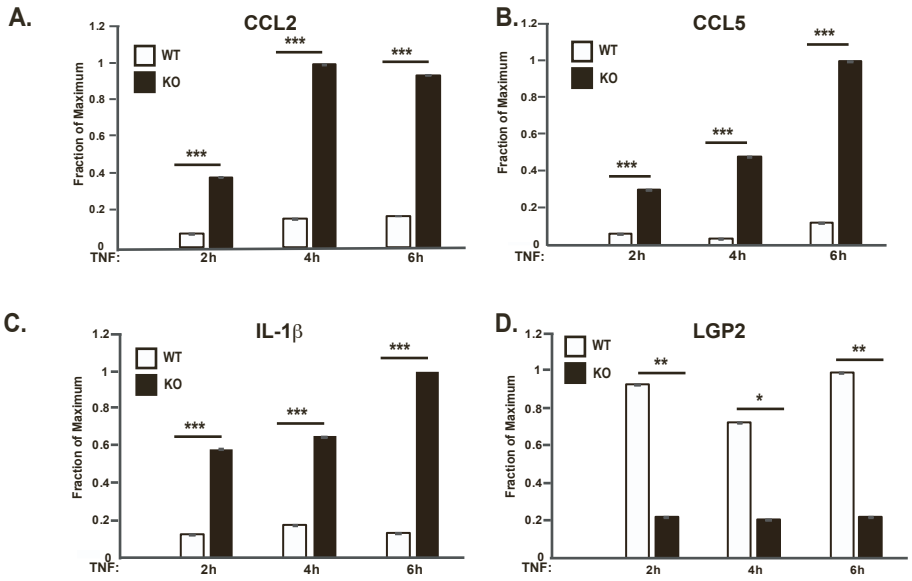

Figure S2: Additional Data Related to Figure 1
A-D) 2fTGH cell lines with (WT) and without (KO) LGP2 expression were stimulated for up to 6 hours with TNF-a prior to RNA isolation and RT-qPCR to measure mRNA levels of: A.) CCL2, B.) CCL5, C.) IL1-b, and D.) LGP2. Bars represent average values (n=3) ± standard deviation. *P<0.05, ** P < 0.005, *** P < 0.0005 by two-tailed Student's t test.

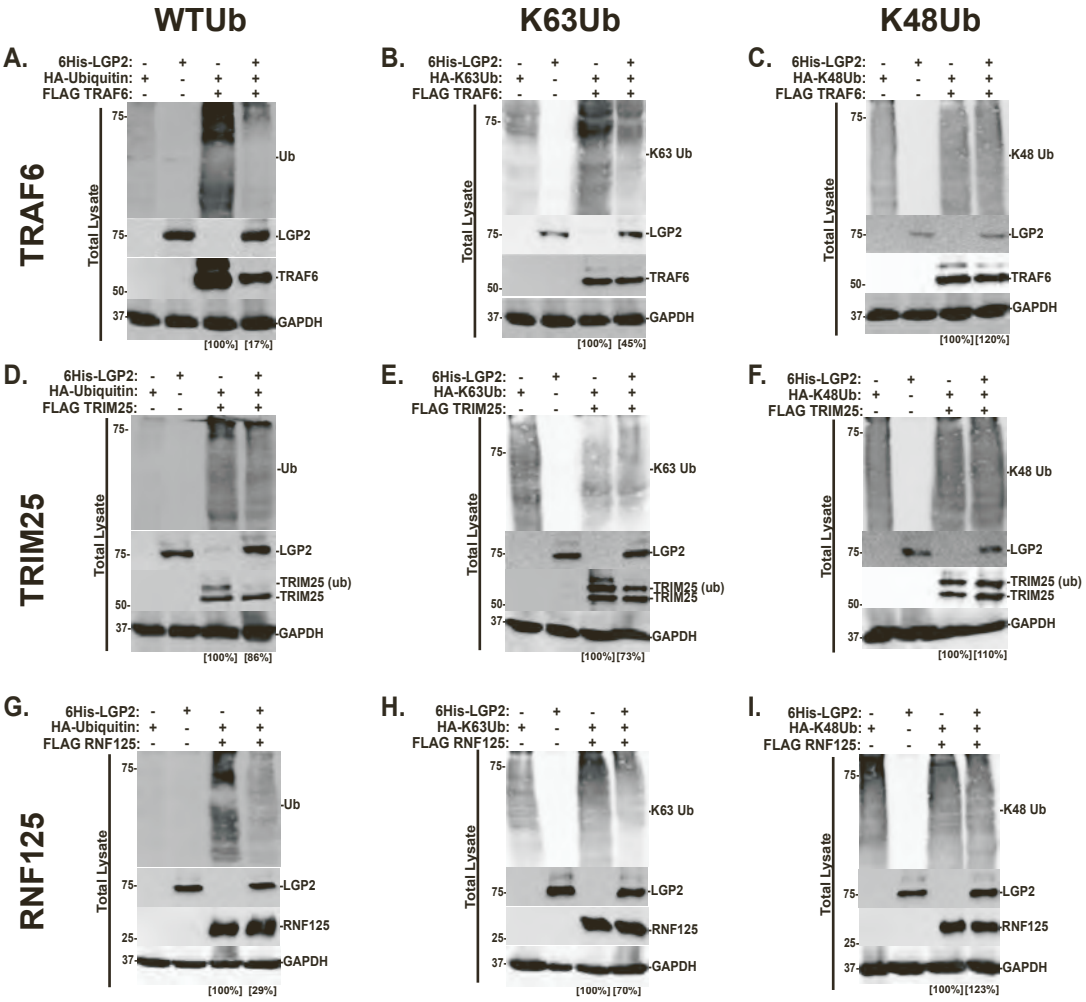

Figure S3: Loading Controls Related to Figure 2
A.-C.) Loading controls were subjected to immunoblot for HA to detect ubiquitins, FLAG to detect TRAF6, LGP2, or GAPDH specific antisera for Figures 2A.-C. Quantification for specific lanes is found in [ ] below.
D.-F.) Loading controls were subjected to immunoblot for HA to detect ubiquitins, FLAG to detect TRIM25, LGP2, or GAPDH specific antisera for Figures 2A.-C. Quantification for specific lanes is found in [ ] below.
G.-I.) Loading controls were subjected to immunoblot for HA to detect ubiquitins, FLAG to detect RNF125, LGP2, or GAPDH specific antisera for Figures 2D.-F. Quantification for specific lanes is found in [ ] below.

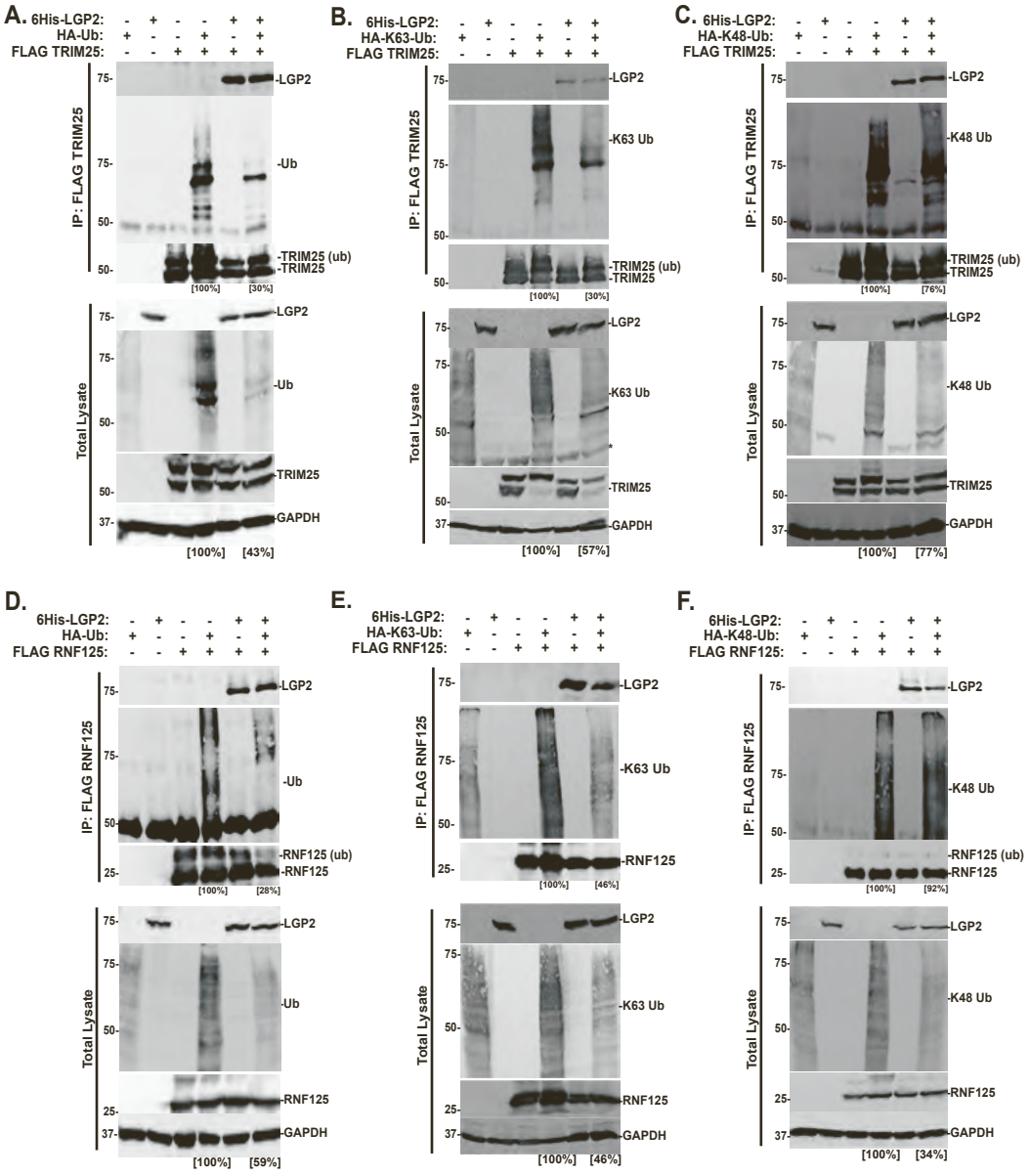

Figure S4: Additional Data Related to Figure 2
HEK293T cells were transfected with expression vectors for FLAG-tagged TRIM25 (A.-C.) or RNF125 (D.-F.), along with HA-tagged ubiquitin with all lysines (A. and D.), K63-only ubiquitin (B. and E.), or K48-only ubiquitin (C. and F.), and 6His-tagged LGP2. Lysates were subjected to FLAG M2 immunoaffinity purification and immunoblotting with HA to detect ubiquitins, FLAG to detect TRIM25 and RNF125, LGP2, or GAPDH specific antisera. Representative experiment of n=3 replicates. Quantification of specific lanes is shown in [ ] below.

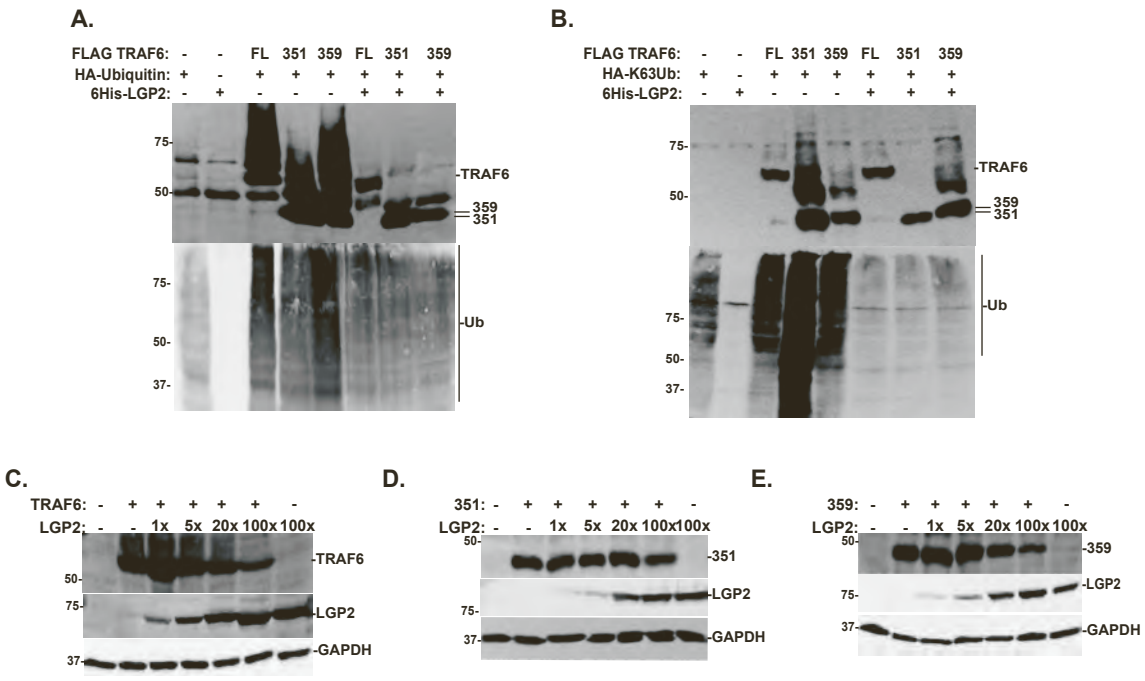

Figure S5: Loading Controls Related to Figure 3
A). Immunoblot loading control for HA-tagged ubiquitin and FLAG TRAF6 (6), 351, and 359 for Figure 3C. Lysates were subjected to immunoblot with anti-HA for ubiquitin.
B) Immunoblot loading control for HA-tagged K63-only ubiquitin (K63ub) and FLAG TRAF6 (6), 351, and 359 for Figure 3D. Lysates were subjected to immunoblot with anti-HA for ubiquitin and anti-FLAG for TRAF6, 351, and 359.
C-E.) Immunoblot loading controls for reporter gene assays in Figures 3D-F. Lysates were subjected to immunoblot with anti-FLAG to detect TRAF6 (Figure 3D), 351 (Figure 3E), or 359 (Figure 3F), LGP2, or GAPDH specific antisera.

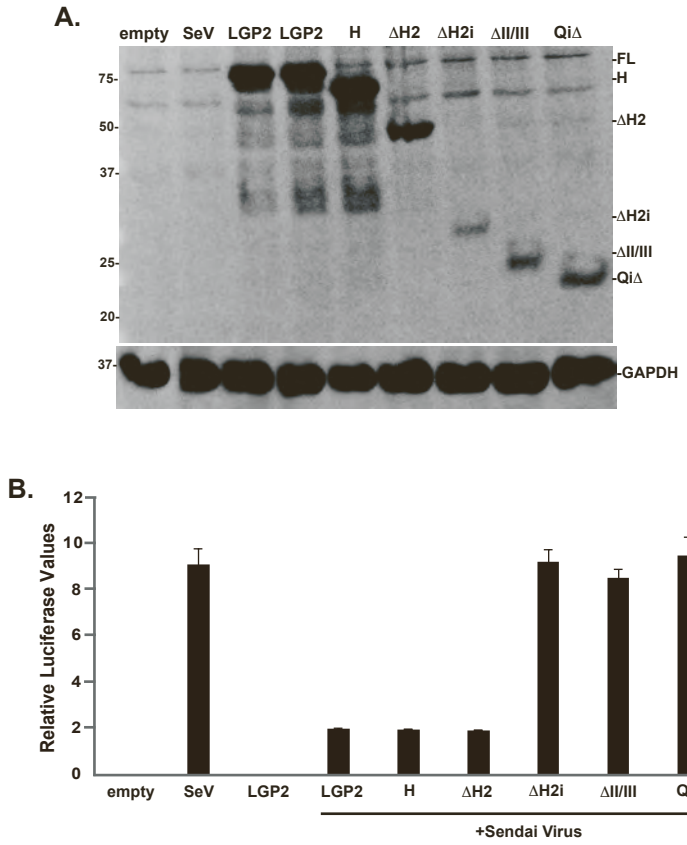

Figure S6: Additional Data Related to Figure 5
A) Immunoblot loading controls for reporter gene assay in Figure 5D. Lysates were subjected to immunoblot with anti-FLAG to detect LGP2 (FL), LGP2-H (H), ΔH2, ΔH2i, ΔII/III, and QiΔ. or GAPDH specific antisera

B) Representative bar graph of luciferase assay dot plots in Figure 5D.

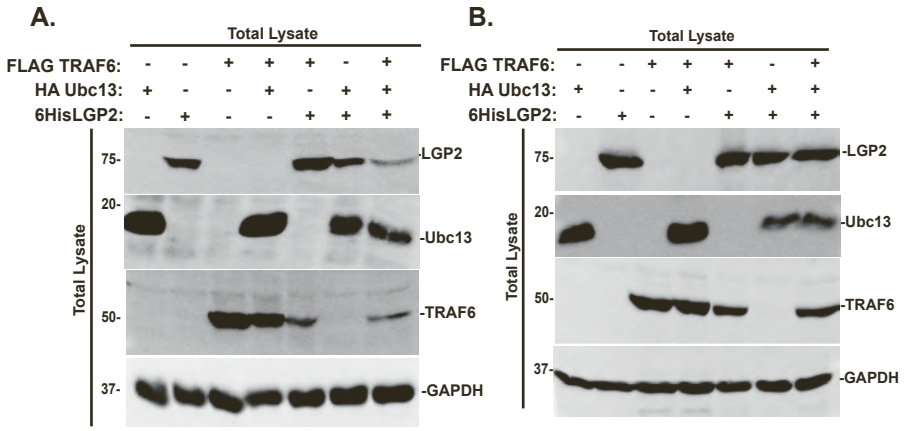

Figure S7: Loading Controls Related to Figure 6
A.) Immunoblot loading controls for Figure 6E. Immunoblot of lysates was carried out with antiserum for LGP2, FLAG (for TRAF6), or HA (for Ubc13/UBE2N).
B.) Immunoblot loading controls for Figure 6F. Immunoblot of lysates was carried out with antiserum for LGP2, FLAG (for TRAF6), or HA (for Ubc13/UBE2N).

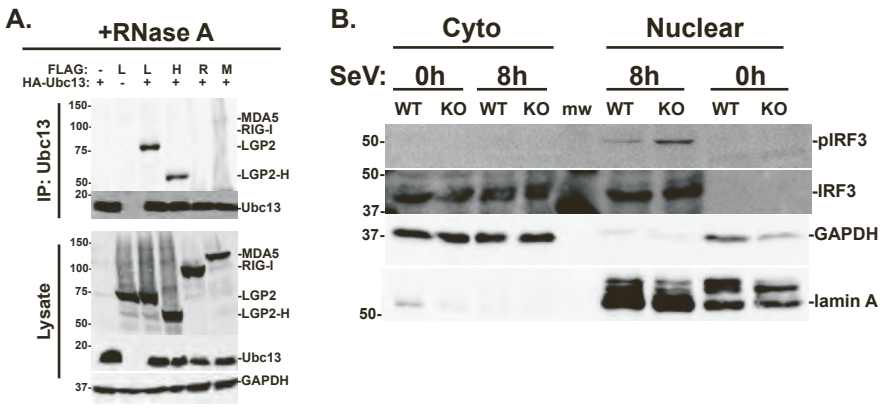

Figure S8: Analysis of Interaction and the Antiviral Response Related to Figure 4
A) HEK293T cells were transfected with expression vectors for FLAG-tagged LGP2 (L), LGP2-H (H), RIG-I (R), or MDA5 (M) with (+) or without (-) HA-tagged Ubc13/UBE2N. Lysates were subjected to HA immunoaffinity purification and detection of co-precipitation was carried out with anti-FLAG for LGP2, LGP2-H, RIG-I, or MDA5, anti-HA for Ubc13/UBE2N, or anti-GAPDH loading control.
B) Cytoplasmic (Cyto) and nuclear fractions of samples infected with SeV for 8h.