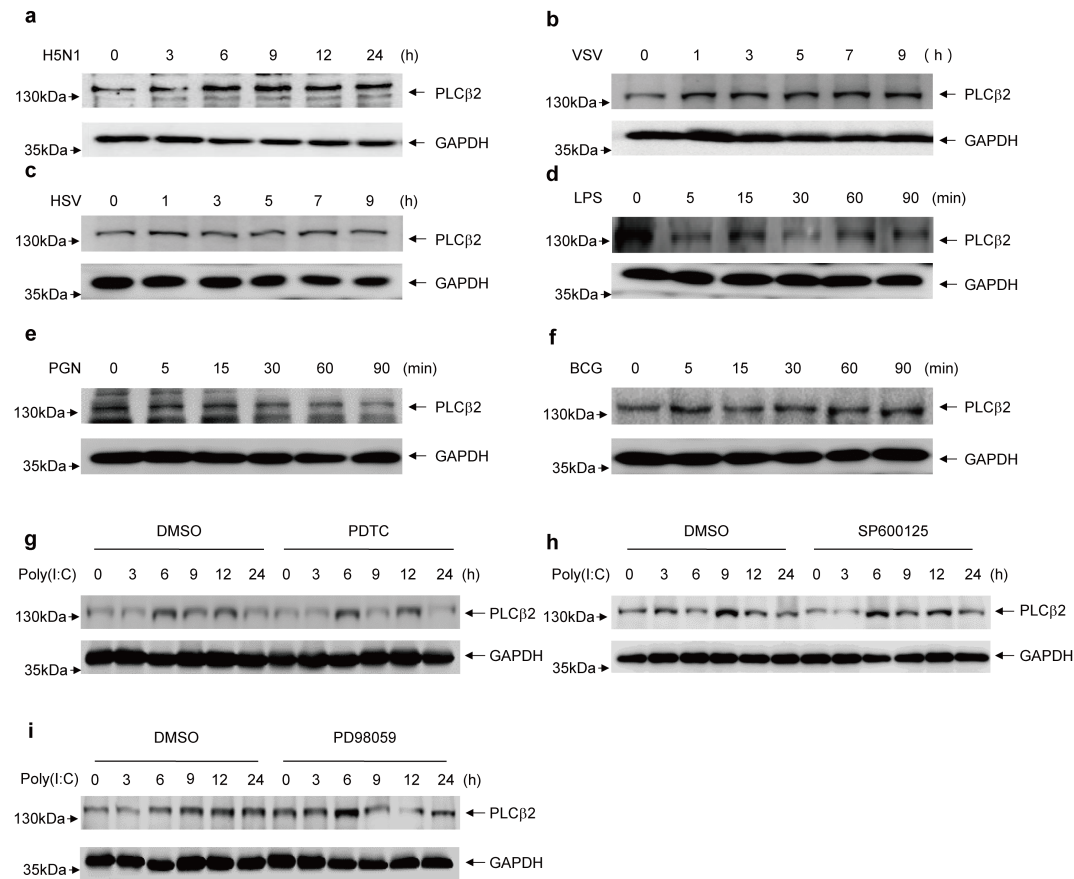


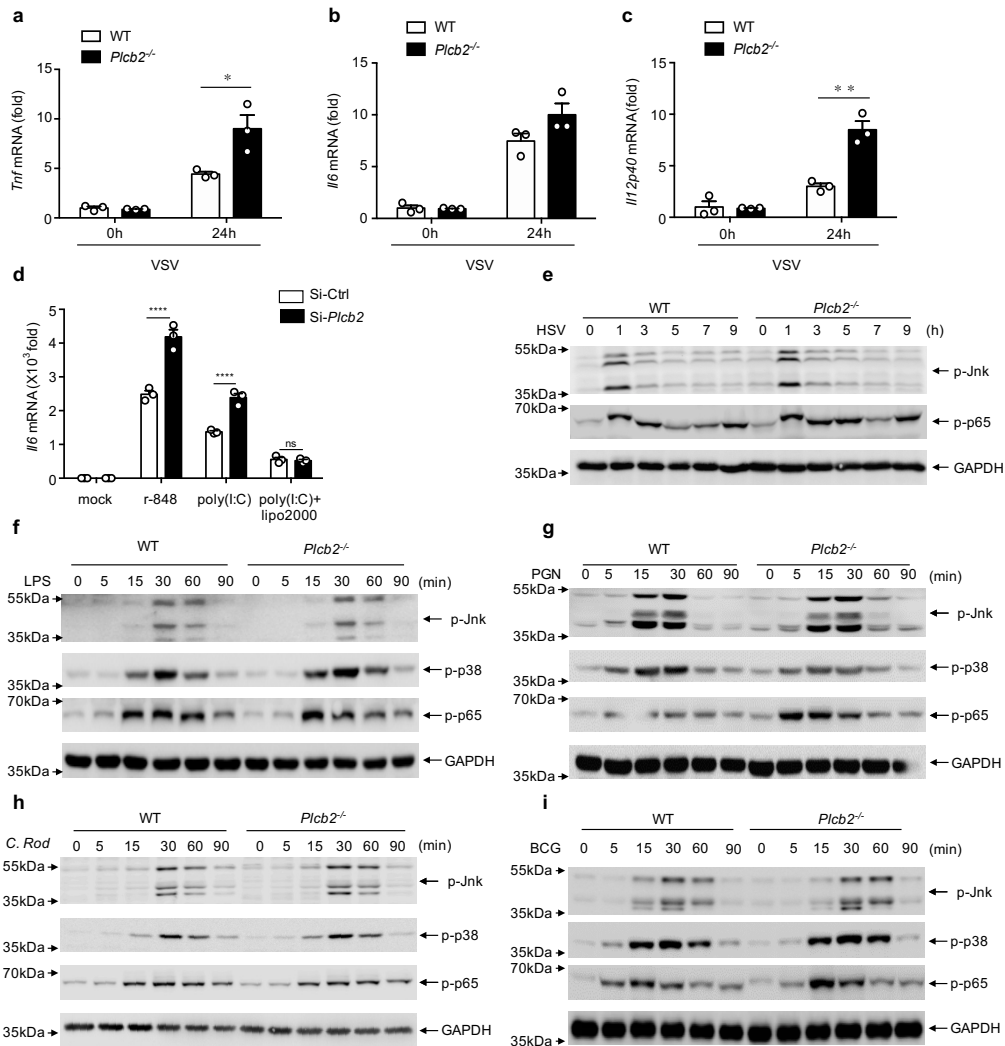
PLC β 2 Negatively Regulates the Inflammatory Response to Virus Infection by

Inhibiting Phosphoinositide-mediated Activation of TAK1

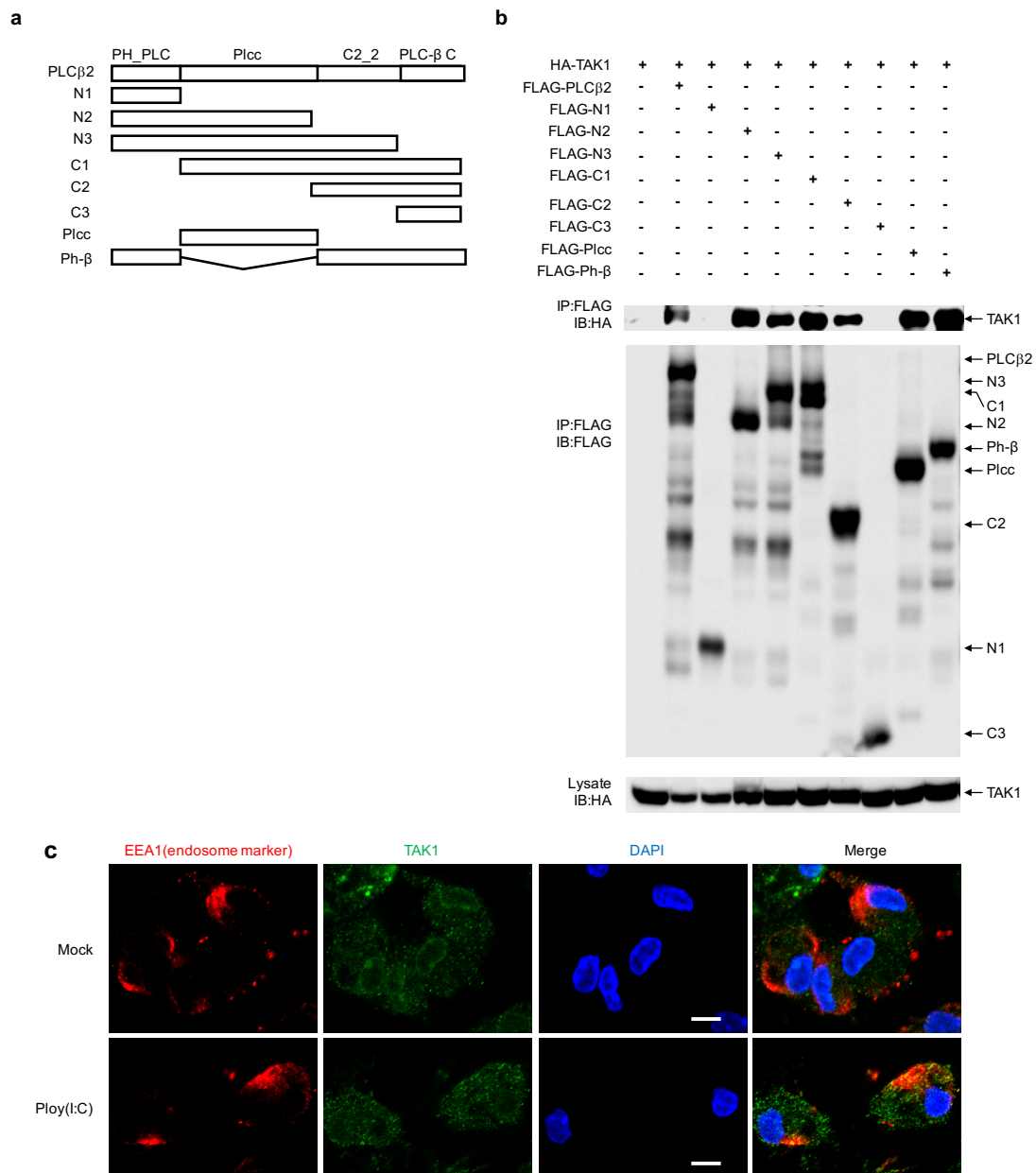
Wang et al.



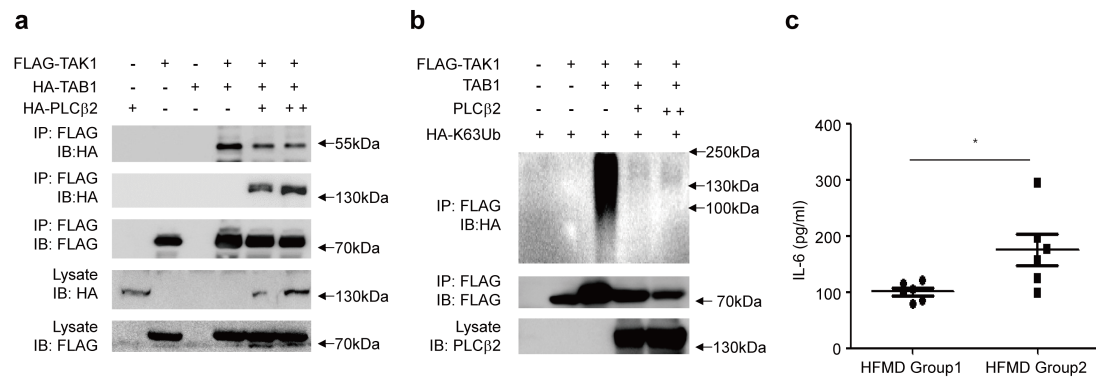
Supplementary Figure 1 RNA virus infection specifically induces PLCβ2 expression (a-f) IB of PLCβ2 in lysates of mouse peritoneal macrophages infected or stimulated with H5N1 (a), VSV (b), HSV (c), LPS (d), PGN (e) or BCG (f) for indicated times. (g-i) IB of PLCβ2 in mouse peritoneal macrophages pretreated for 1 h with the NF-κB inhibitor PDTC (g), JNK inhibitor SP600125 (h) or MEK inhibitor PD98059 (i) before poly(I:C) stimulation for the indicated times. Data are representative of three experiments with at least three independent biological replicates (mean and s.e.m. of n = 3 cultures in a-i).



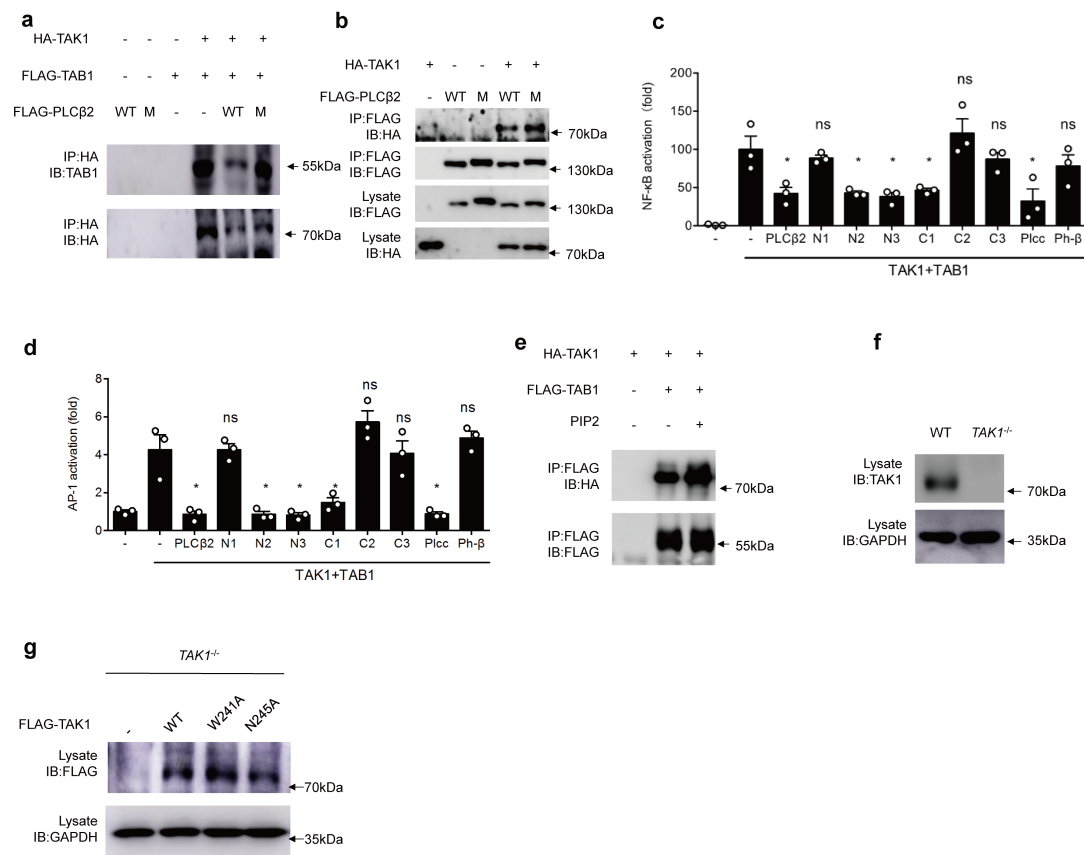
Supplementary Figure 2 PLCβ2 suppresses RNA virus- induced inflammation (a-c) Q-PCR analysis of relative *Tnf* (a), *Il6* (b) and *Il12p40* (c) mRNA in wild-type or *Plcb2*^{-/-} macrophages infected with VSV for the indicated times. (d) Q-PCR analysis of relative *Il6* in *Plcb2* knockdown macrophages stimulated with r-848 (TLR7 ligand) or poly(I:C) for 4 hours or transfected with poly(I:C) for 6 hours. (e-i) IB of lysates from wild-type or *Plcb2*^{-/-} macrophages infected or stimulated with HSV (e), LPS (f), PGN (g), C.Rod (h) or BCG (i) for the indicated times using phospho-antibodies. Data are representative of three experiments with at least three independent biological replicates (mean and s.e.m. of n=3 mice in a or n = 3 cultures in d-i).



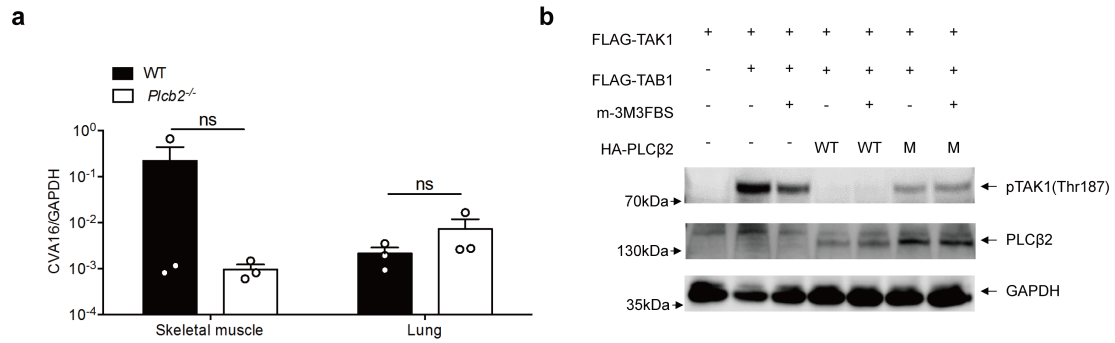
Supplementary Figure 3 PLCβ2 interacts with TAK1 (a) Deletion mutants of PLCβ2. (b) IP and IB analysis of lysates of HEK293T cell expressing PLCβ2 mutants and TAK1. (c) Confocal analysis of location of TAK1 in peritoneal macrophages with mouse anti-TAK1 antibody and EEA1 antibody (endosome marker). Scale bar, 5μm. Data are representative of three experiments with at least three independent biological replicates.



Supplementary Figure 4 PLC β 2 inhibits TAK1 activation (a) IB and IP of cell lysates from HEK293 cells expressing TAK1 and TAB1 in the presence or absence of PLC β 2. (b) IP and IB analysis of cell lysates from HEK293T cells expressing K63-Ub with TAK1 and TAB1 in the presence or absence of PLC β 2. (c) Enzyme-linked immune sorbent assay (ELISA) of IL-6 in serum from HFMD patients. Data are representative of three experiments with at least three independent biological replicates. * $p < 0.05$ by unpaired t -test (c). Data are representative of three experiments with at least three independent biological replicates.



Supplementary Figure 5 PLCβ2 inhibits TAK1 activation via PIP2 (a, b) IB of cell lysates from HEK293 cells transfected with various plasmids as indicated (c, d) Reporter assay of NF-κB (c) and AP-1 (d) activation in HEK293T cells expressing TAK1 and TAB1 in the presence or absence of PLCβ2 or PLCβ2 mutants. (e) IB and IP of cell lysates from HEK293 cells expressing HA-tagged TAK1 and FLAG-tagged TAB1 treated with PIP2 in a lipid carrier for 10 mins. (f) IB of TAK1 in wild-type (WT) or *TAK1*^{-/-} A549 cells with rabbit anti-TAK1 antibody. (g) IB and IP of cell lysates from *TAK1*^{-/-} A549 cells transfected with wild-type TAK1 or TAK1 mutants. **p* < 0.05 by unpaired *t*-test (c, d). Data are representative of three experiments with at least three independent biological replicates.



Supplementary Figure 6 PLCB2 PLCβ2-deficient mice are more susceptible to CVA16 infection (a) Fourteen-day-old wild-type or *Plcb2*^{-/-} mice were infected intraperitoneally with 50 μl of CVA16 (1.5×10^4 PFU/mouse). The viral loads in the skeletal muscle and lung tissues of infected mice were measured by qRT-PCR on days 3. (b) IB and IP of cell lysates from HEK293 cells treated with PLC activator m-3M3FBS for 1h before transfecting plasmid encoding TAK1 and TAB1 in the presence or absence of PLCβ2. Data are representative of three experiments with at least three independent biological replicates. Data are representative of three experiments with at least three independent biological replicates (mean and s.e.m. of n = 3 mice in a).

Figure 1d

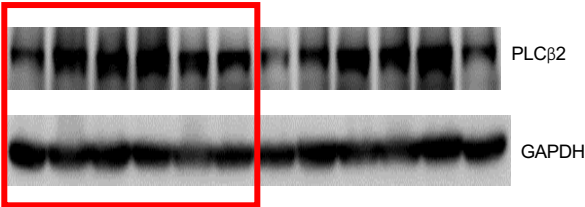


Figure 2f

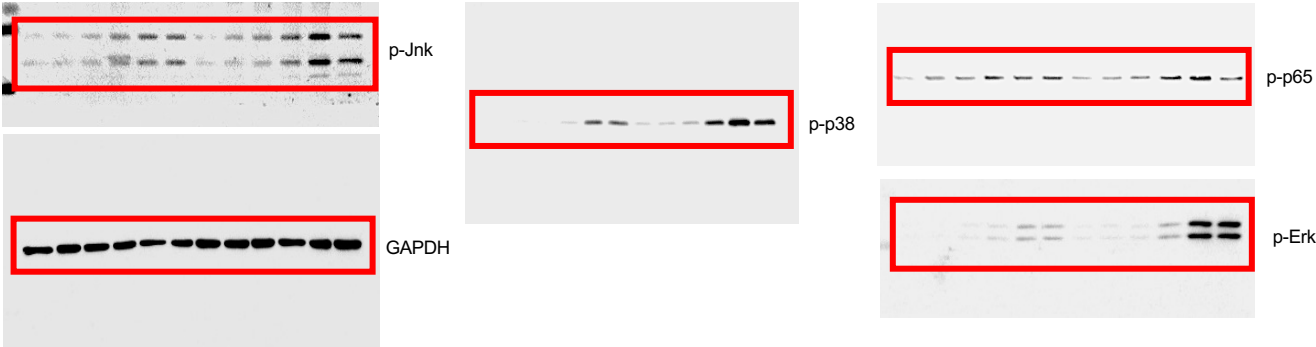


Figure 3a

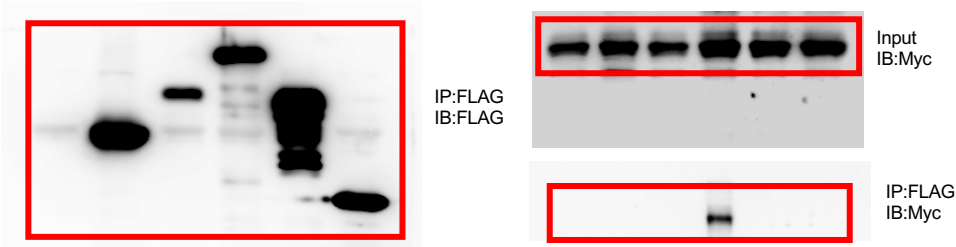


Figure 3c



Figure 3d

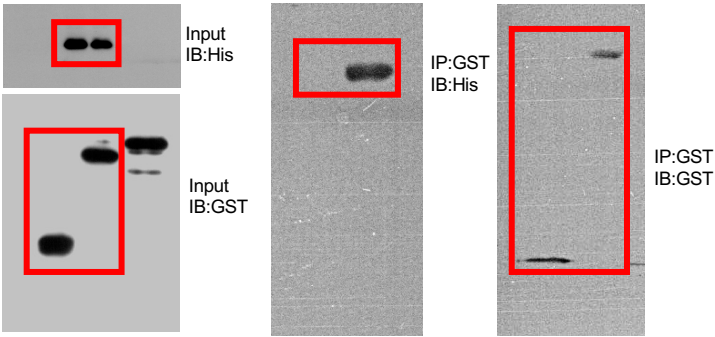


Figure 3e

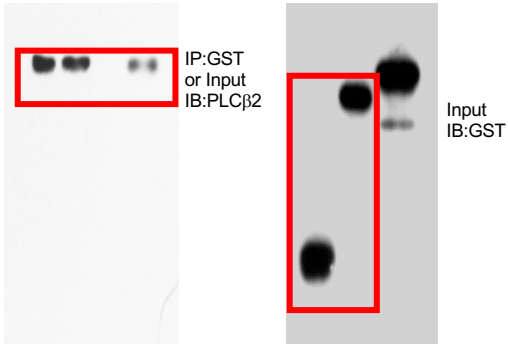


Figure 3h

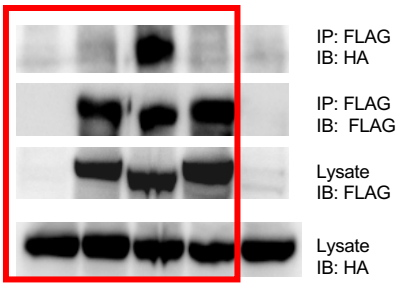


Figure 4d

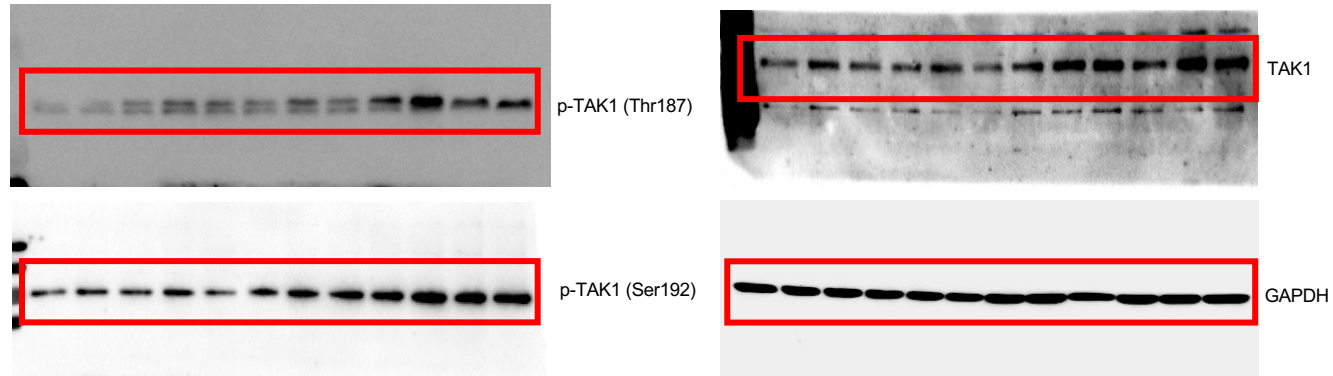


Figure 5c

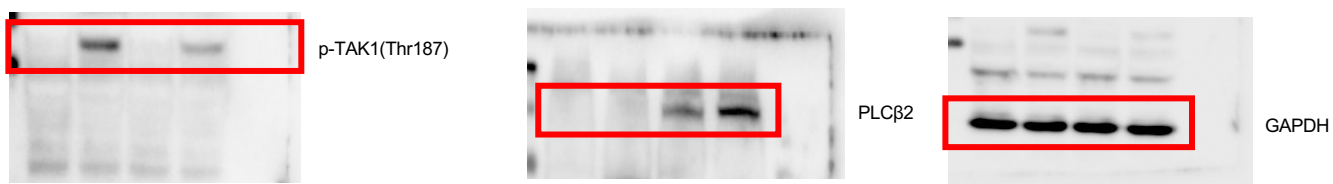


Figure 5d

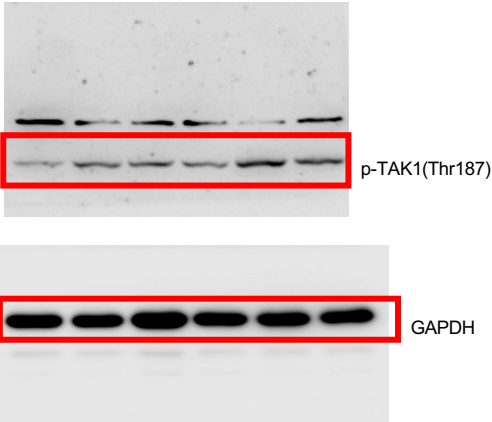


Figure 5e

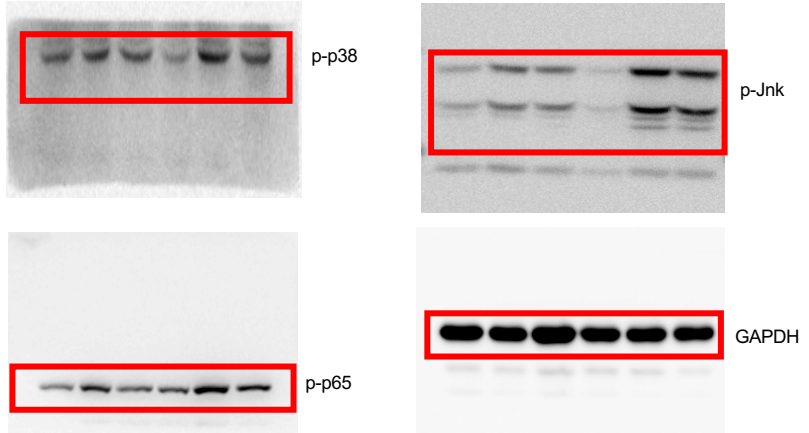


Figure 5f

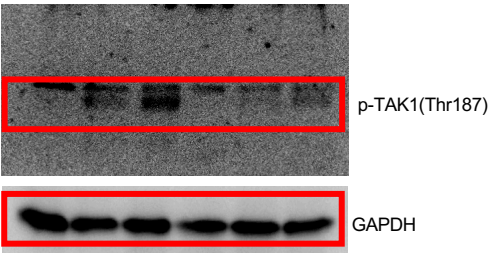


Figure 5g

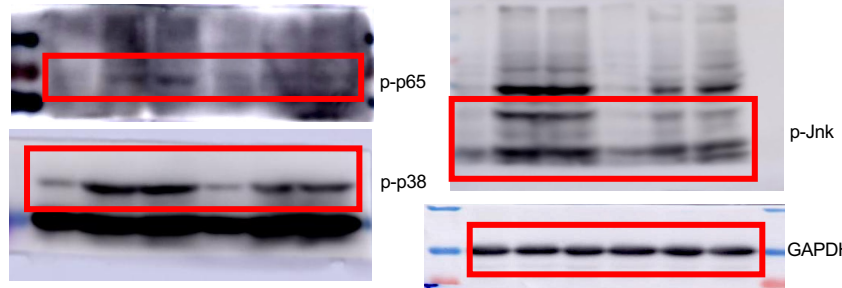


Figure 5j

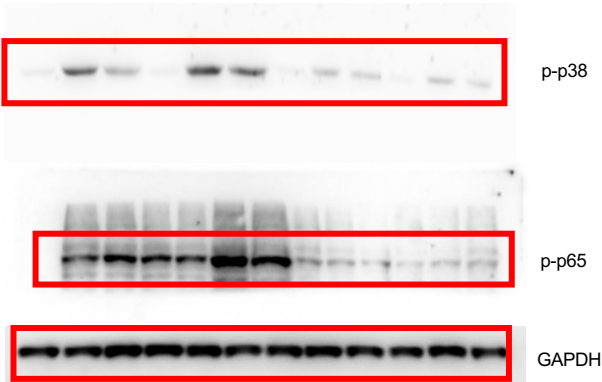


Figure 5m

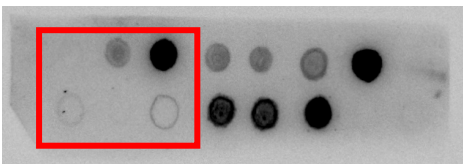


Figure 5n



Figure 5o

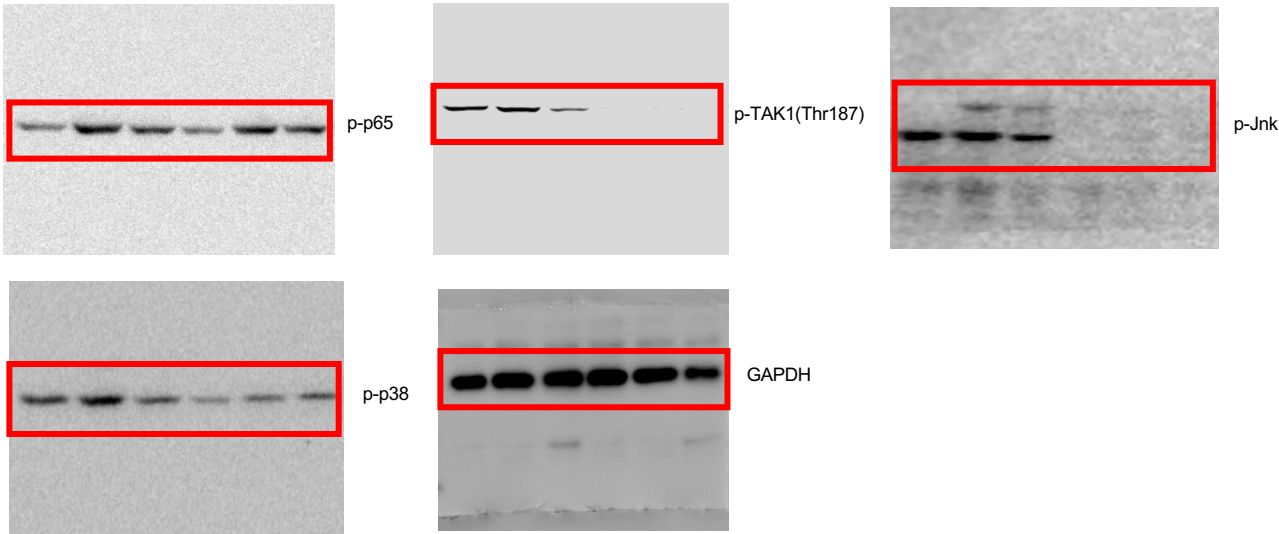


Figure 7a

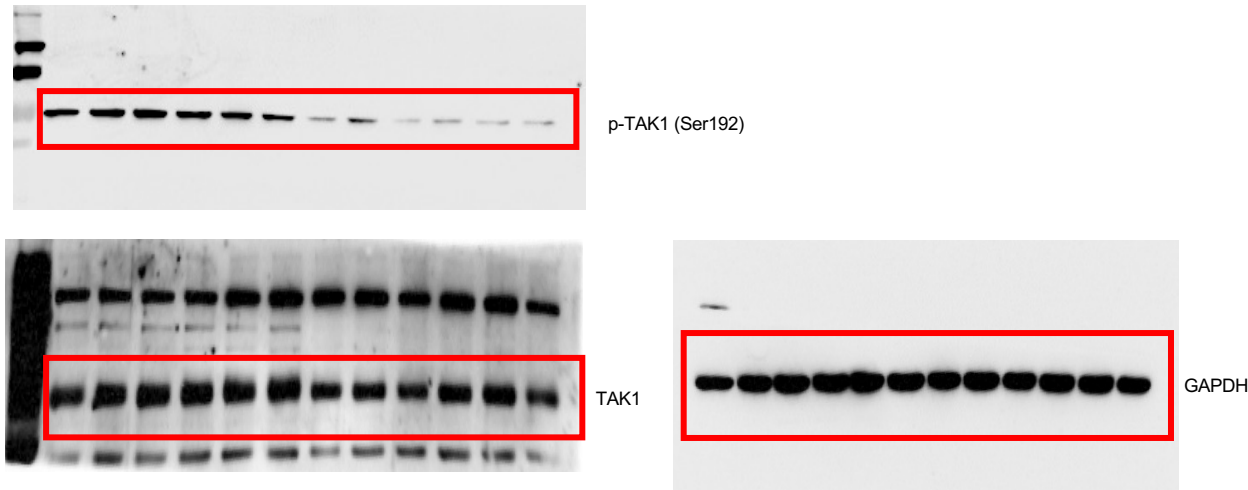
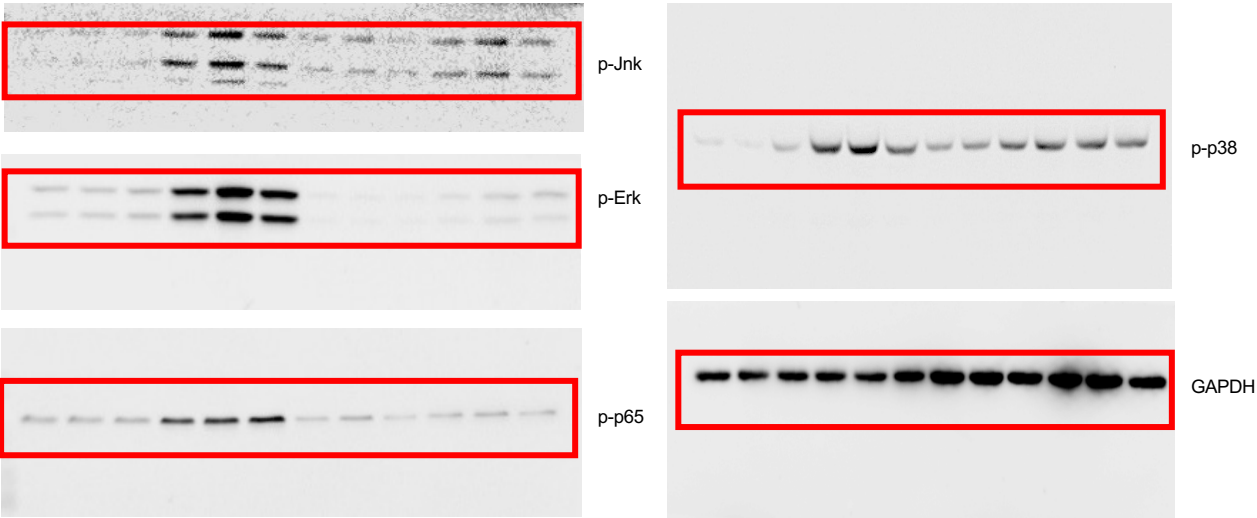


Figure 7b



Supplementary Figure 7 Original immunoblots shown in the main Figures