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Supplemental Information

PAR2-Mediated cAMP Generation Suppresses TRPV4-Dependent Ca^{2+} Signaling in Alveolar Macrophages to Resolve TLR4-Induced Inflammation

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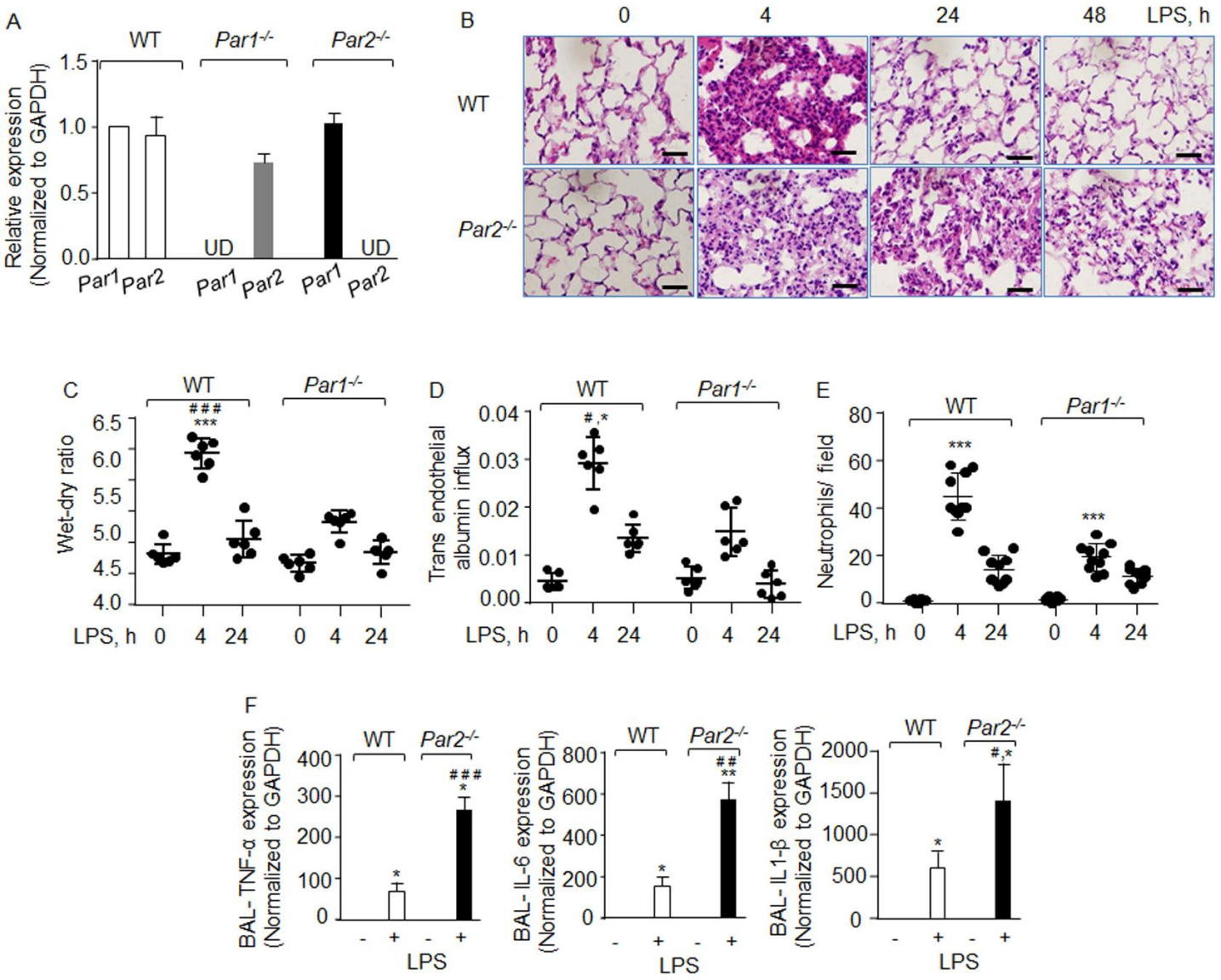

Figure S1. LPS failed to induce lung injury in PAR1 null mice. Related to Figure 1.

(A**)** Lungs from WT, *Par1*$^{-/-}$ and *Par2*$^{-/-}$ mice were harvested and the expression of *Par1* and *Par2* was determined using qPCR with GAPDH as an internal control. Data are represented as mean ± SD from three independent experiments.

(B) WT or PAR2-null lung sections were stained with hematoxylin and eosin to assess lung histology. Images show a representative trace from three individual experiments (Scale bars = 20μm).

(C-D) WT and *Par1*$^{-/-}$ mice were exposed to nebulized LPS (1 mg/ml) for 45 min. Thirty minutes before sacrificing the mice at indicated times, Evans blue–labelled albumin was injected retro-orbitally into each mouse. Lung vascular inflammatory injury was determined by measuring albumin influx and lung wet-dry ratio. *, $p < 0.05$ and *** $p < 0.001$ indicate significant as compared to unchallenged control group (0h). $^{\#}$, $p < 0.05$ and $^{\#\#\#}$ $p < 0.001$ indicate values that are significantly different from corresponding *Par1*$^{-/-}$ group. n=6 mice/group

(E) Neutrophil count was performed (per field) on hematoxylin and eosin stained WT and *Par1*$^{-/-}$ lung sections at indicated times. The plot shows individual values from three independent experiments with mean ± SD. ***, $p < 0.001$ indicates a significant increase in neutrophil count as compared to corresponding unchallenged control group.

(F) After 24 h following LPS-induced injury, bronchoalveolar lavage fluid was obtained from WT and *Par2*$^{-/-}$ mice. The macrophages were isolated from BAL fluid and RNA isolated. The expression of indicated cytokines was determined using qPCR. Data are represented as mean ± SD from experiments that were performed three times individually. *, $p < 0.05$ and **, $p < 0.01$ indicate values that were significantly different from mice receiving vehicle alone (0h). $^{\#}$, $p < 0.05$; ##, $p < 0.01$ and $^{\#\#\#}$, $p < 0.001$ indicate values that were significantly different from WT-mice post-24 h LPS challenge.

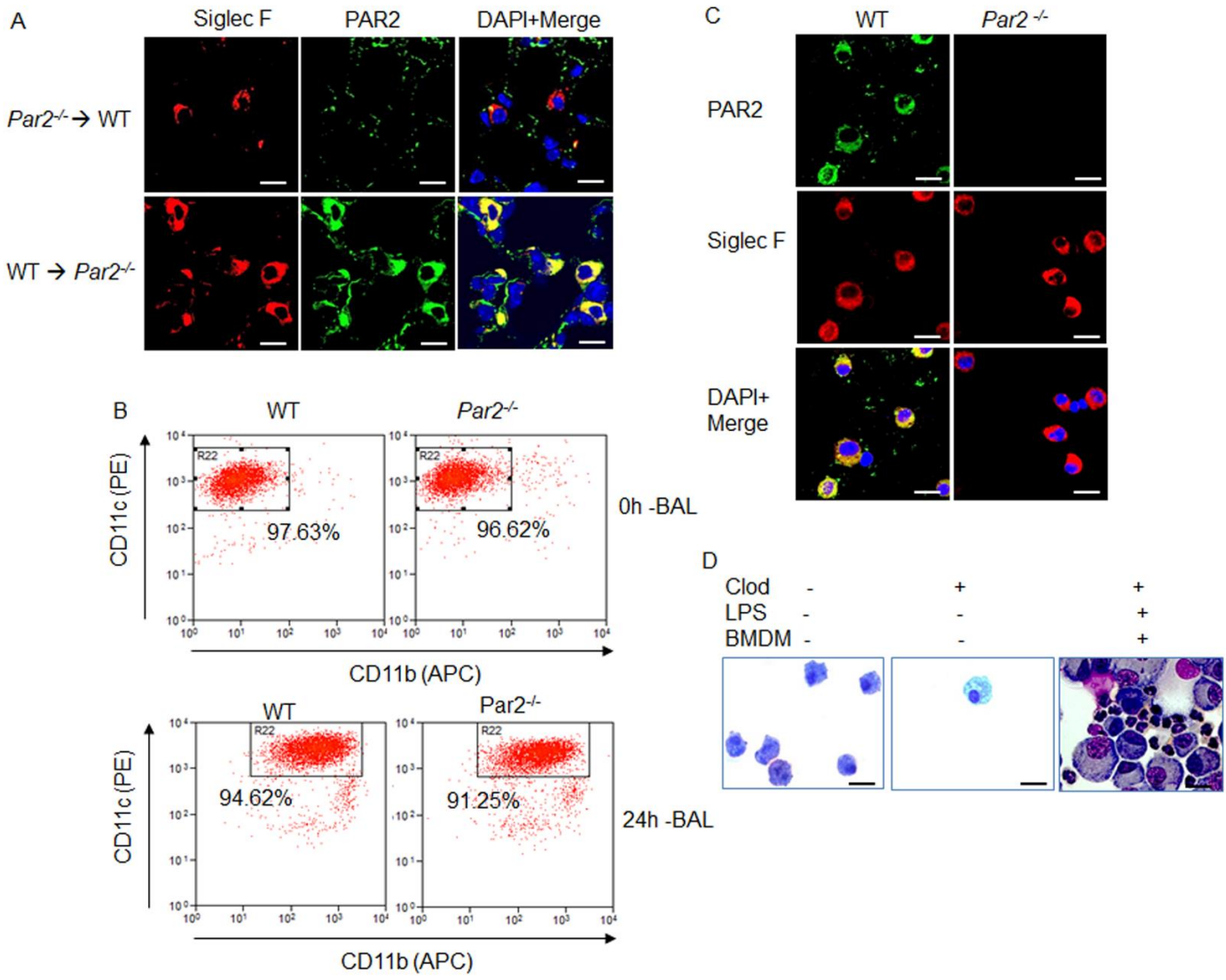

Figure S2. PAR2 in AM resolves lung injury. Related to Figure 2.

(A**)** Lung sections were stained with Siglec-F and PAR2 followed by appropriate secondary antibody treatment against PAR2. DAPI was used to stain the nuclei. The imaging was done under confocal microscope (Scale bar = 5µm). Representative images are shown from three independent experiments.

(B**)** The bronchoalveolar lavage from WT or *Par2*$^{-/-}$ mice was obtained, and cells were stained with CD11c-PE, CD11b-APC, PE-Cy7 CD45 and EF450 Ly6G cell markers. The cells were gated as CD45^{+} and Ly6G^{-}. Representative scatter plot is shown. The experiment was independently repeated three times.

(C**)** BAL cells from WT or PAR2 null lungs were stained with Siglec-F, anti-PAR2 antibody and appropriate secondary antibody to assess PAR2 positive AM. Representative images are shown from three independent experiments (Scale bar =10µm)

(D**)** H and E staining of BAL showing AM depletion following clodronate injection and repletion after adoptive transfer of BMDM in WT mice lungs. Representative images are shown from three independent experiments (Scale bar = 20µm).

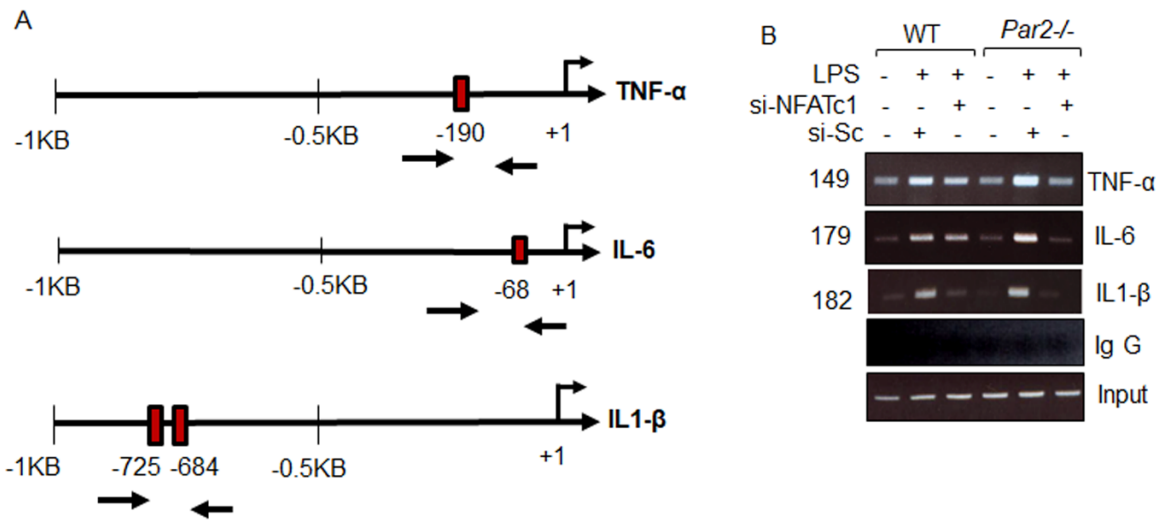

Figure S3: ChIP assay of NFκB binding to the cytokine promoters in BMDM. Related to Figure 4.
(A) Schematic diagram shows NFκB binding sites on the indicated cytokine promoters. Arrows indicate position of forward and reverse primers used in the assay.
(B) WT and *Par2*$^{-/-}$ BMDMs were transfected with si-NFATc1. After 48 h, the cells were stimulated with LPS for 4h and immunoprecipitated (IP) with IgG or antibody against NFκB and the resulting chromatin fragments were subjected to PCR amplification using primers spanning the IL-6, TNF-α and IL1-β consensus sequences. Gels represent ChIP assays of the TNF-α, IL-6, and IL1-β promoters.

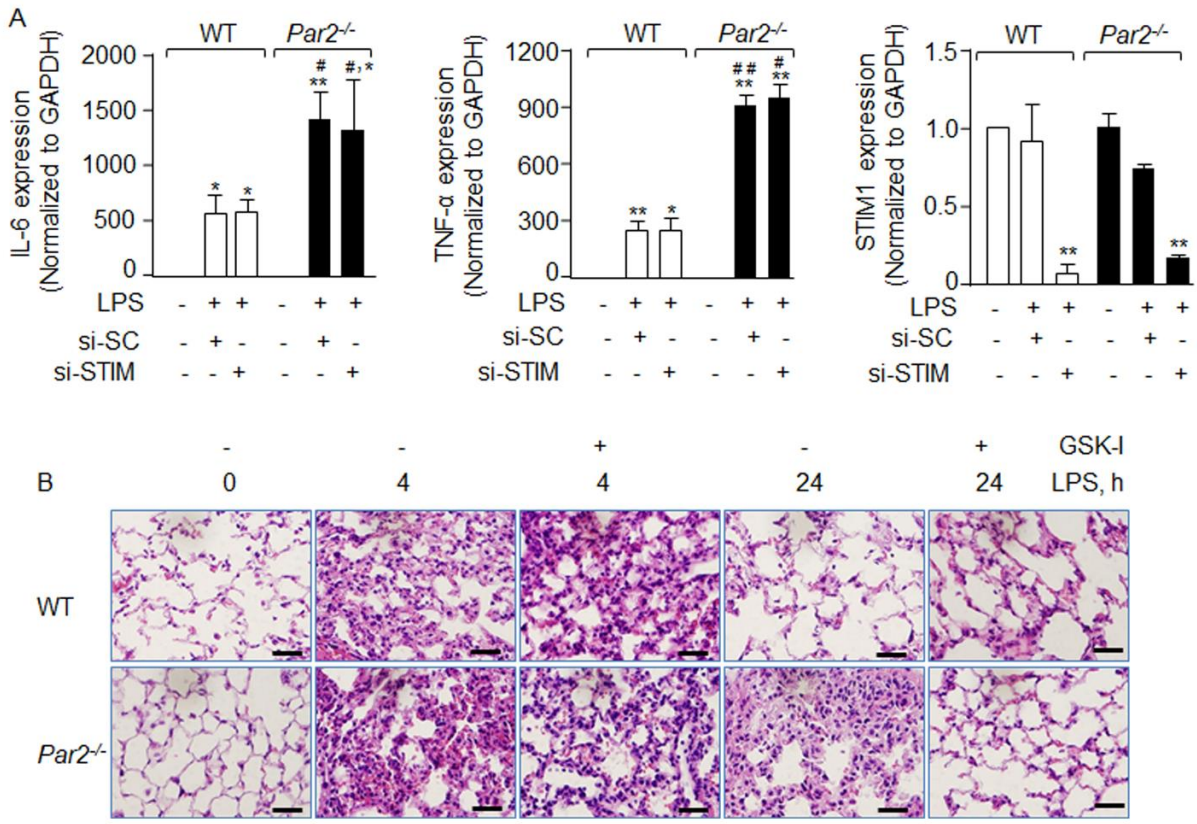

Figure S4. Thrombin activates TRPV4 activity. Related to Figure 6 & 7.

(A) Related to Figure 6. BMDM transfected with control or STIM1 siRNA were stimulated with 1 μg/ml of LPS for 4 h and expression of STIM1, IL-6 and TNF-α was determined by qPCR. The data are represented as mean ± SD from three independent experiments. *, $p < 0.05$ and **, $p < 0.01$ indicate significance from control BMDM. #, $p < 0.05$ and ##, $p < 0.01$ indicate significance from WT-BMDM post-LPS challenge.

(B) Related to Figure 7. WT or PAR2-null lungs receiving vector or GSK-1 post LPS challenge were stained with hematoxylin and eosin to assess lung histology. Images show a representative trace from three individual experiments (Scale bar =20μm).