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Supplemental Information

Oxidant Sensing by TRPM2 Inhibits Neutrophil Migration and Mitigates Inflammation

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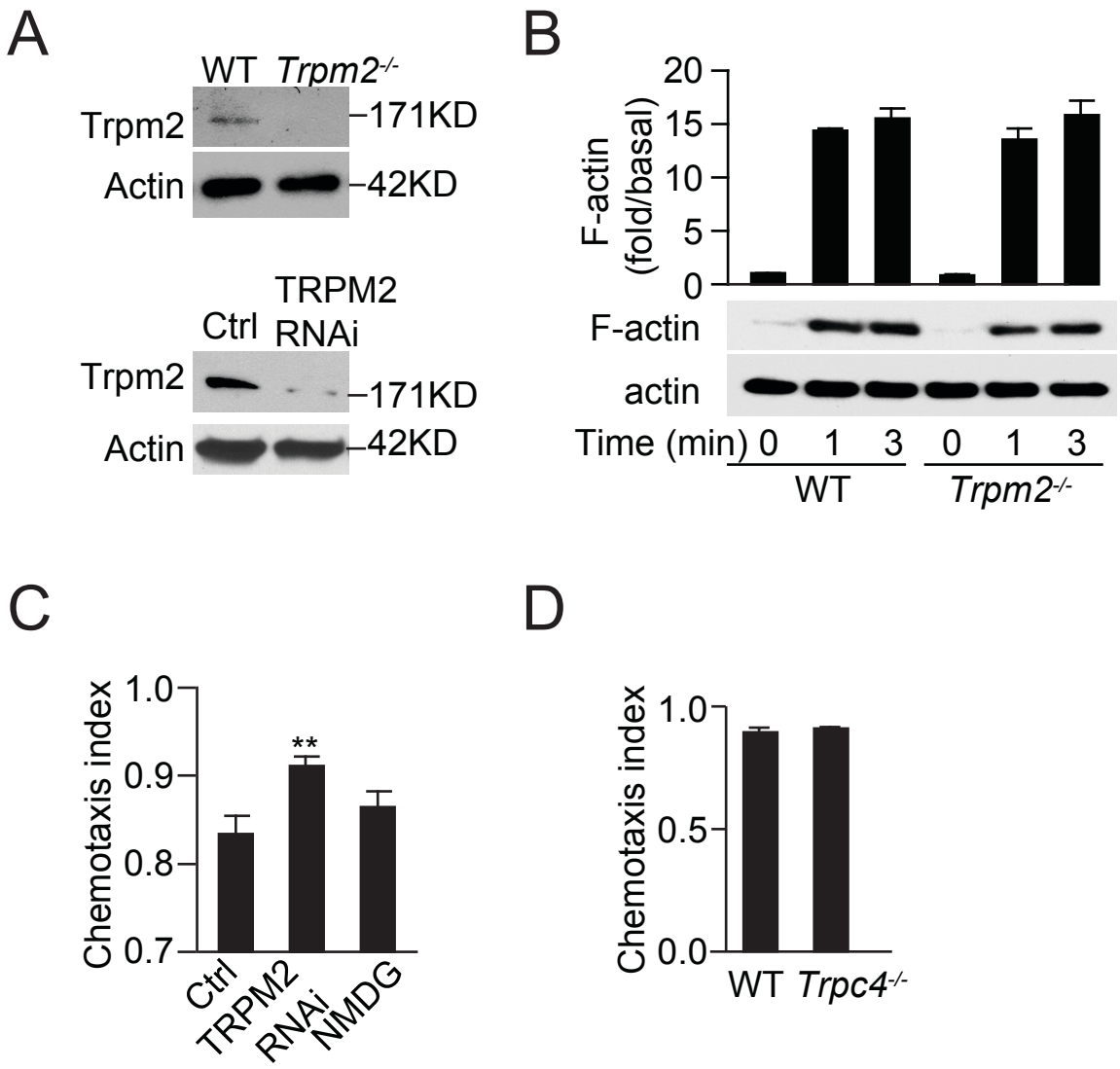

Figure S1, related to Figure 1-2. Knockout or knockdown effect of TRPM2.
(A) Immunoblots (IB) of TRPM2 in WT and *Trpm2*$^{-/-}$ neutrophils (top panels), or in ctrl and TRPM2 RNAi-treated HL60 cells (bottom panels). Actin is used as loading control. (B) Quantification (top) and blots (bottom) of filament actin (F-actin) or total actin in WT and *Trpm2*$^{-/-}$ neutrophils stimulated with fMLF at indicated times. (C) CI of ctrl, TRPM2 RNAi, or control HL60 cells treated with NMDG in a 100 nM fMLF gradient. **, $P < 0.01$ (Student's *t*-test). (D) CI of WT, *Trpc4*$^{-/-}$ mouse neutrophils in a 10 nM fMIFL gradient. Data are representative of (A, blot in B), or from (graph in B-D) three independent experiments (mean and s.e.m. in B-D).

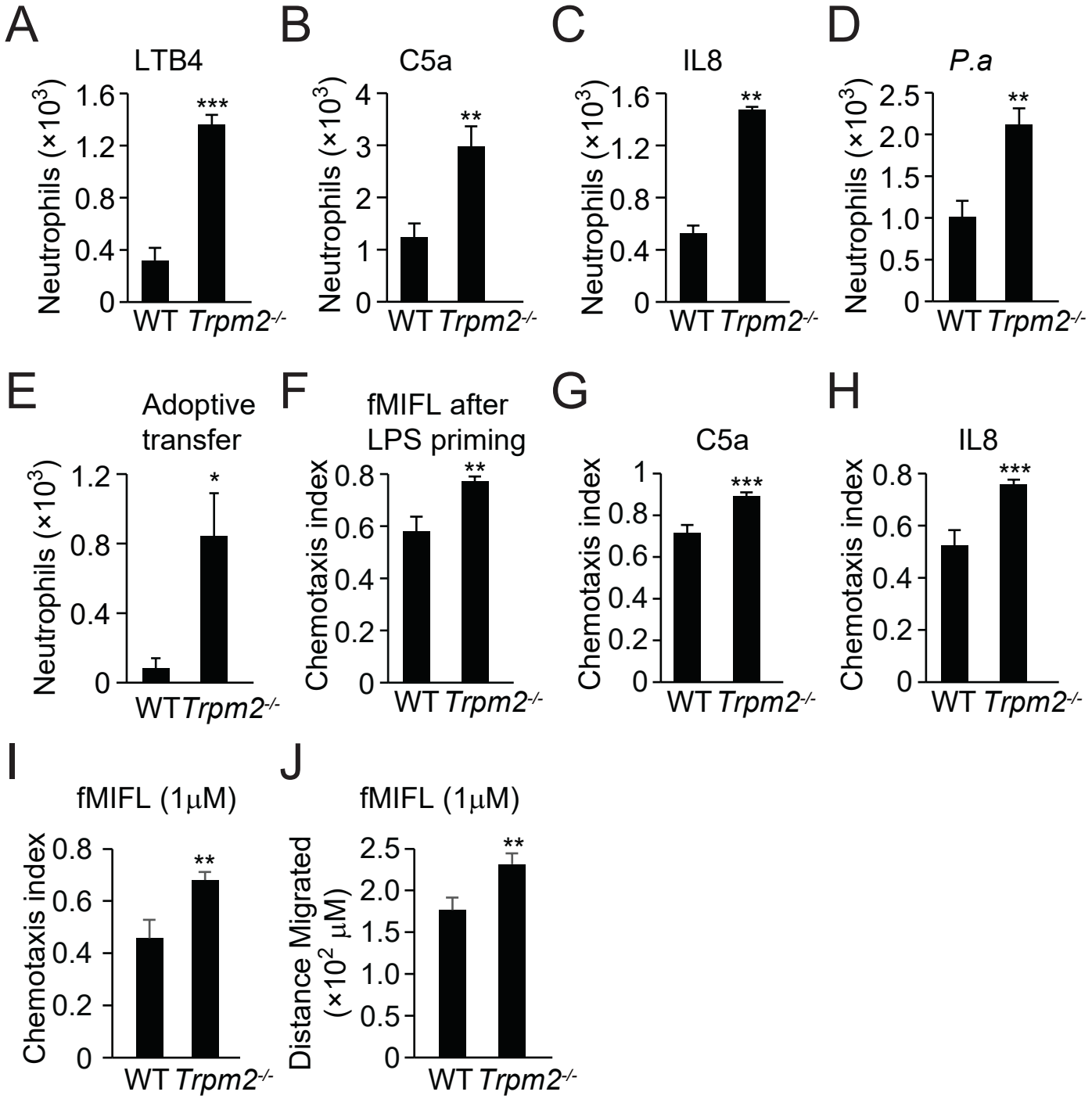

Figure S2, related to Figure 1-2. Deletion of TRPM2 enhances neutrophil transmigration and chemotaxis. (A-D) Neutrophils emigration into the lungs of WT and *Trpm2*$^{-/-}$ mice 4h after stimulation with LTB4 (A), C5a (B), IL8 (C) or *Pseudomonas aeruginosa* (D, *P.a*). (E) CFSE-labelled WT and *Trpm2*$^{-/-}$ neutrophils were i.v. injected into WT mice, neutrophil emigration into the lungs were assessed 4h after fMIFL stimulation. (F-I) CI of WT and *Trpm2*$^{-/-}$ neutrophils exposed to a gradient of fMIFL (F, after LPS priming), C5a (G), IL8 (H), or high dose fMIFL (1μM, I). (J) Distance of WT and *Trpm2*$^{-/-}$ neutrophils exposed to a gradient of 1μM fMIFL, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ (Student's *t*-test). Data are from three experiments (mean and s.e.m.).

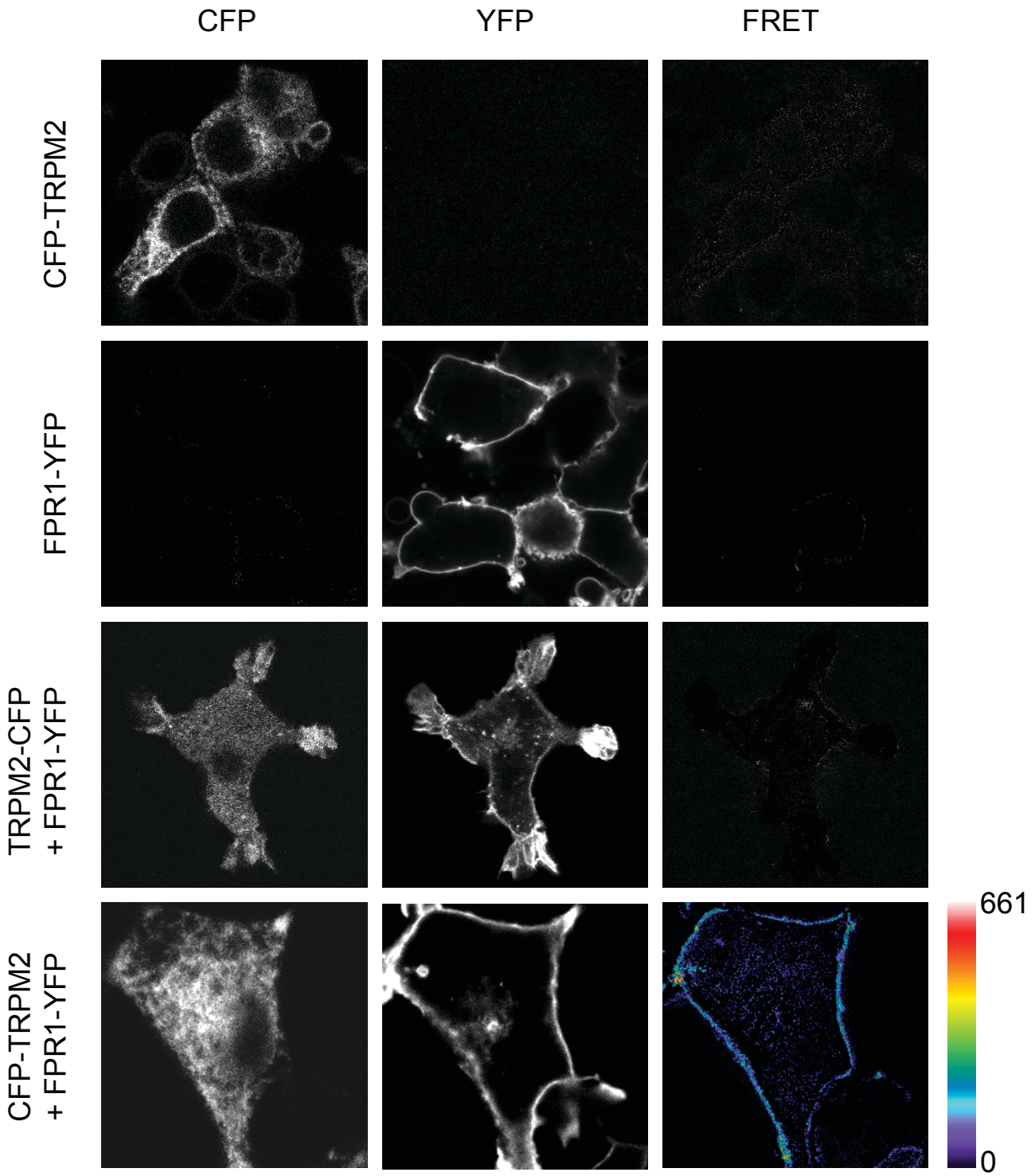

Figure S3, related to Figure 4. FRET imaging controls. HEK-293T cells were transfected with either CFP-TRPM2 (N-terminal) only, FPR1-YFP only, TRPM2-CFP (C-terminal) + FPR1-YFP, or CFP-TRPM2 (N-terminal) + FPR1-YFP and imaged in CFP, YFP, and FRET channels. The scale in net FRET images was color coded with a range from 0 - 661 pixel intensity units. Images are 90 μm^2 as shown. *N* = 3, each using >30 cells percondition, one representative experiment is shown.