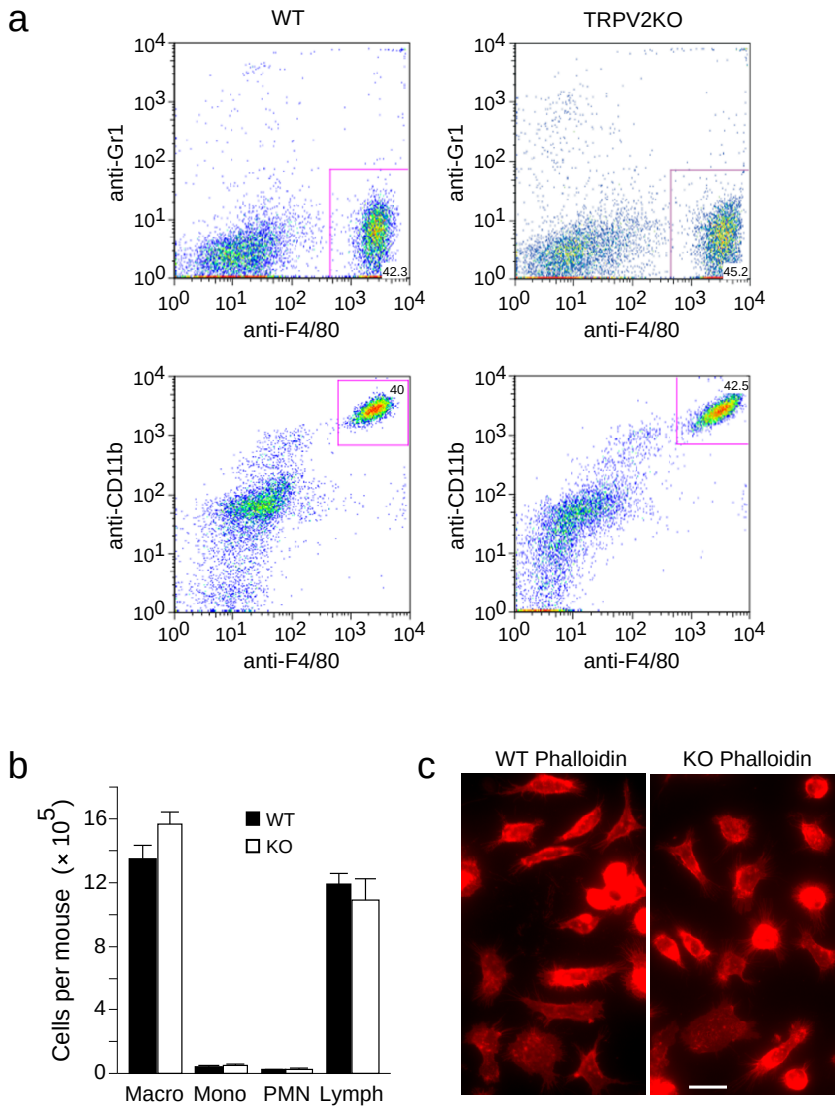
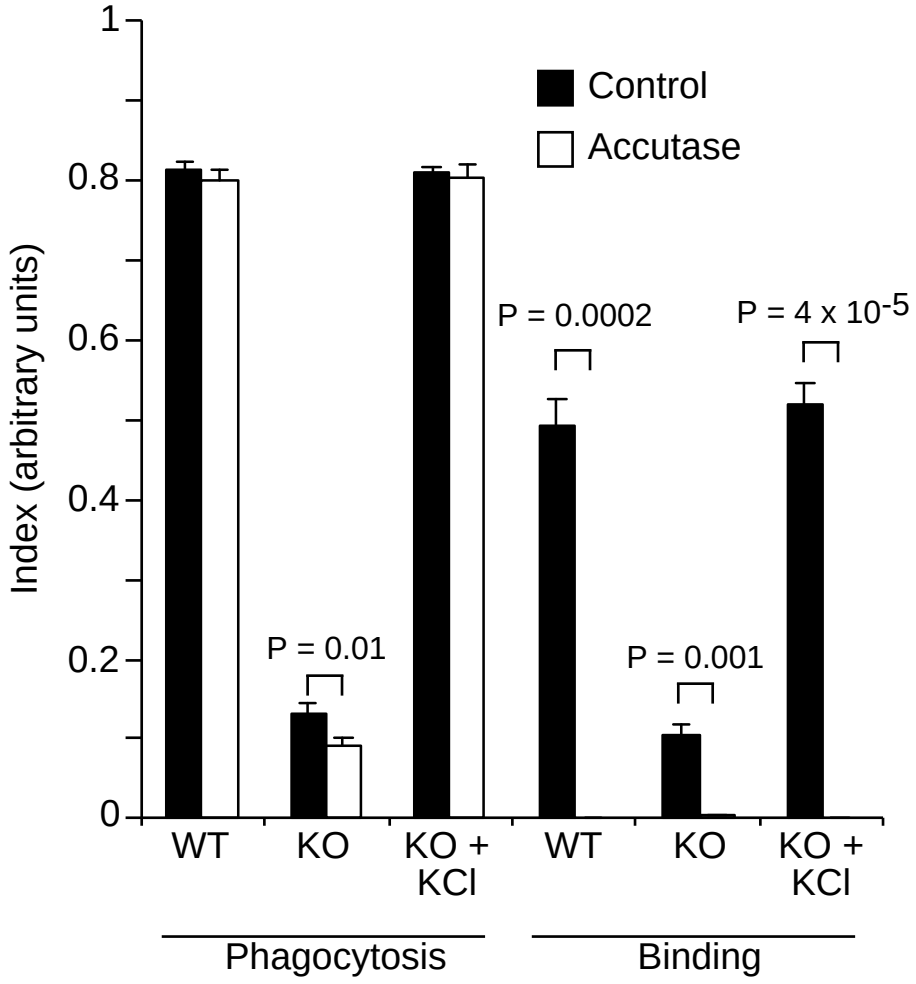
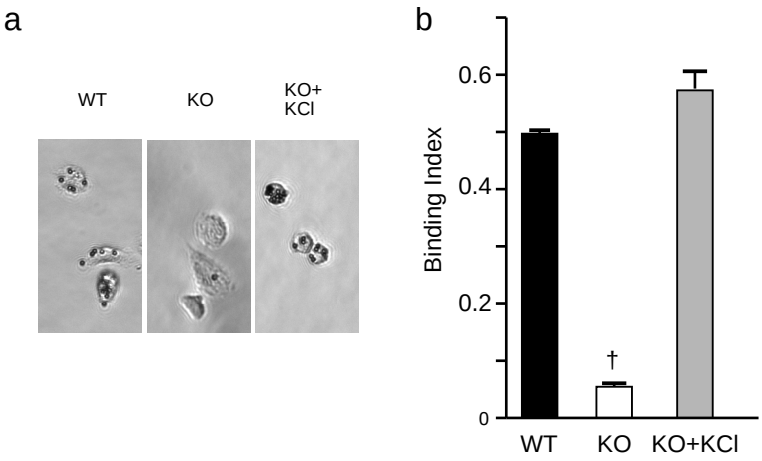


Link et al. Supplementary Figure 1



Link et al. Supplementary Fig. 2



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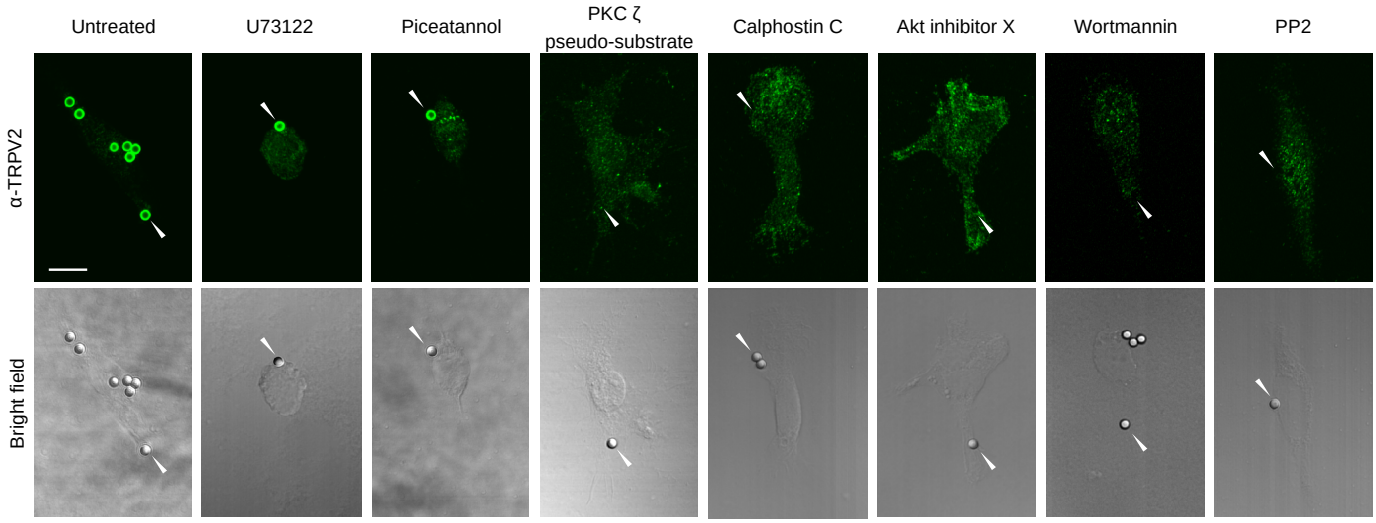
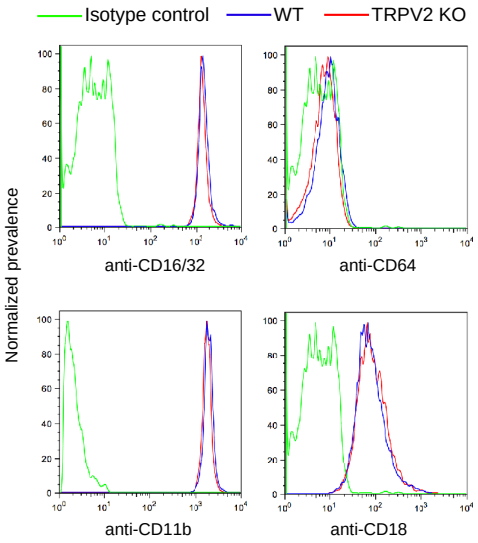
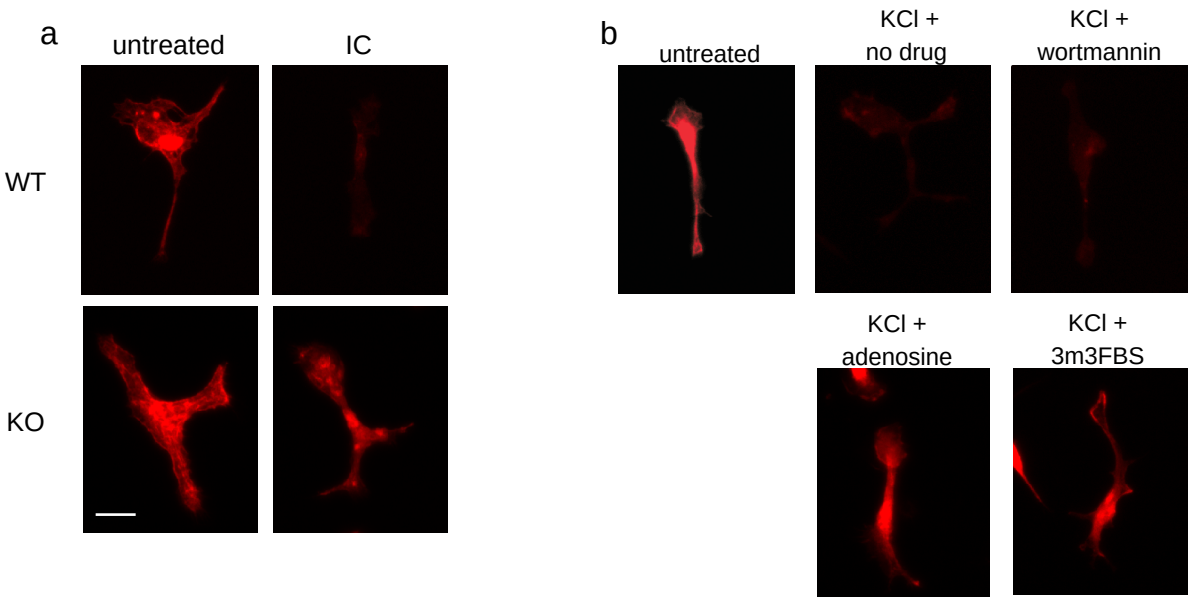


Link et al. Supplementary Fig. 4



Link et al. Supplementary Figure 5



Link et al. Supplementary Figure 6

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Flow cytometric analysis of peritoneal macrophages. (**a**) Scatter plots from flow cytometric examination of resident peritoneal cells from a representative wild-type and TRPV2KO mouse pair. Percent macrophages are indicated in gated regions. b. Quantification of cell types in peritoneal lavage samples from each genotype. Mean ± SEM; wild-type, $n = 10$; TRPV2KO, $n = 9$. Macrophages were defined as F4/80 and CD11b high/Gr1, CD3, and B220 negative. Lymphocytes were defined as CD3 or B220 positive. Neutrophils were defined as Gr1 high/ CD11b medium. c. Actin staining of wild-type and TRPV2KO peritoneal macrophages with rhodamine-phalloidin. Scale bar, 20 μm.

Supplementary Figure 2: Discrimination between internalized versus noninternalized particles during phagocytosis and binding**.** Following phagocytosis (5 min) or binding (5 min) in the presence of 10 μM cytochalasin D of IgG-coated latex beads, binding or phagocytosis index was quantified in wild-type, TRPV2KO, or KCl-treated TRPV2KO macrophages before (black bars) and after (open bars) incubation with Accutase (100%, Sigma, 15 min, 37°C) to remove noninternalized particles. Mean ± SEM, $n = 3$ wells per condition per genotype. Note that Accutase treatment removes nearly all particles incubated under binding conditions, but spares most particles incubated under phagocytosis conditions in wild-type mice or in TRPV2KO cells treated with KCl. There is some reduction in particles associated with TRPV2KO cells even under phagocytosis conditions, suggesting that not all phagosomes had closed.

Supplementary Figure 3: Defective phagocytosis in TRPV2 deficient BMM and rescue by KCl. a. Representative photomicrographs of wild-type, TRPV2KO, and KCl-treated TRPV2KO BMMs following 5 min phagocytosis of IgG-coated latex beads (2 μm, left). Wild-type and TRPV2KO photos show cells exposed to beads under control conditions. KO + KCl, TRPV2KO cells were exposed to beads with KCl (50 mM) added to the medium. b. Corresponding phagocytic indices. Mean ± SEM, $n = 3$ mice per genotype, each assayed in duplicate. † $P < 10^{-4}$.

Supplementary Figure 4: Pharmacological inhibition of TRPV2 phagosomal recruitment. Wild-type macrophages are shown after 5 min phagocytosis of IgG-coated beads (arrowheads), without drugs or in the presence of PLC inhibitor U73122 (10 μM), Syk kinase inhibitor piceatannol (20 μM), PKC ζ pseudosubstrate (40 μM), gernal PKC inhibitor calphostin C (500 nM), Akt inhibitor X (10 μM), PI3 kinase inhibitor wortmannin (100 nM), or Src kinase inhibitor PP2 (1 μM). Top, TRPV2 immunofluorescence. Bottom, brightfield images. Scale bar, 8 μm.

Supplementary Figure 5: Phagocyte receptor expression levels are similar between wildtype and TRPV2 deficient macrophages. Representative histograms from flow cytometric data of wild-type macrophages (F4/80 positive, CD11b positive, CD3 negative, B220 negative) in acutely isolated peritoneal lavage. Histograms compare wild-type (blue traces) and TRPV2KO (red traces) macrophages and isotype controls (green traces), with respect to intensity of surface binding by antibodies against Fcγ

receptors (anti-CD16/32, anti-CD64) (6) and complement C3 receptor (anti-CD11b, anti-CD18) (7).

Supplementary Figure 6: IC-induced actin depolymerization**.** a. Representative photomicrographs of wild-type and TRPV2KO BMMs, stained with rhodamine-phalloidin, which are untreated or following a 2 min stimulation with ICs at 37˚C. b. Representative photomicrographs of TRPV2KO BMMs, stained with rhodamine-phalloidin, which are untreated, treated with KCl (50 mM), KCl + wortmannin (100 nM, PI(3)K inhibitor), KCl + adenosine (300 μM, PI4 kinase inhibitor), or KCl + 3m3FBS (10 μM, PLC activator). Scale bar, 10 μm.