SUPPLEMENTAL MATERIALS

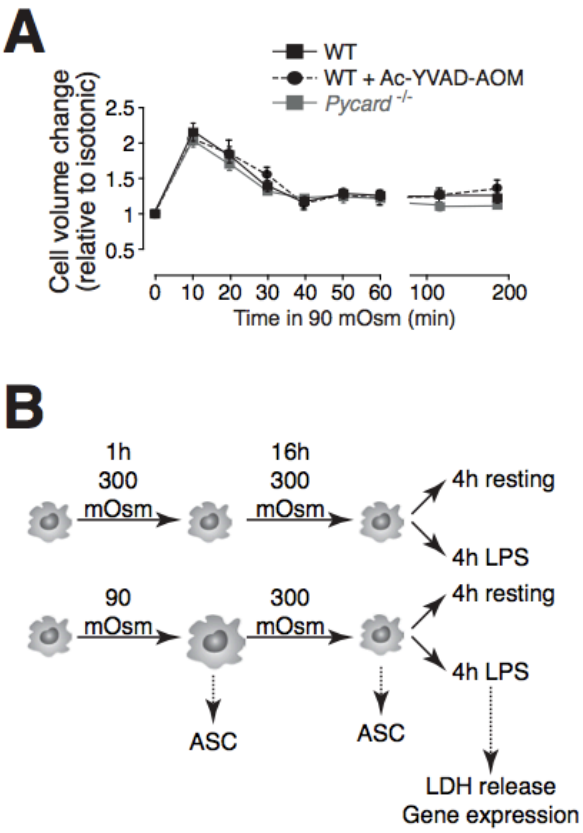

Figure S1. Cell swelling and RVD are independent on ASC or caspase-1 activity. (**A**) Relative cell area of immortalized bone marrow derived macrophages from wild type (WT) or ASC-deficient (*Pycard*$^{-/-}$) mice incubated in hypotonic solution in the presence or absence of caspase-1 inhibitor (Ac-YVAD-AOM). Average and s.e.m. of n = 50 cells/condition and representative of 3 independent experiments. (**B**) Experimental design to study long-term effects of ASC oligomerization.

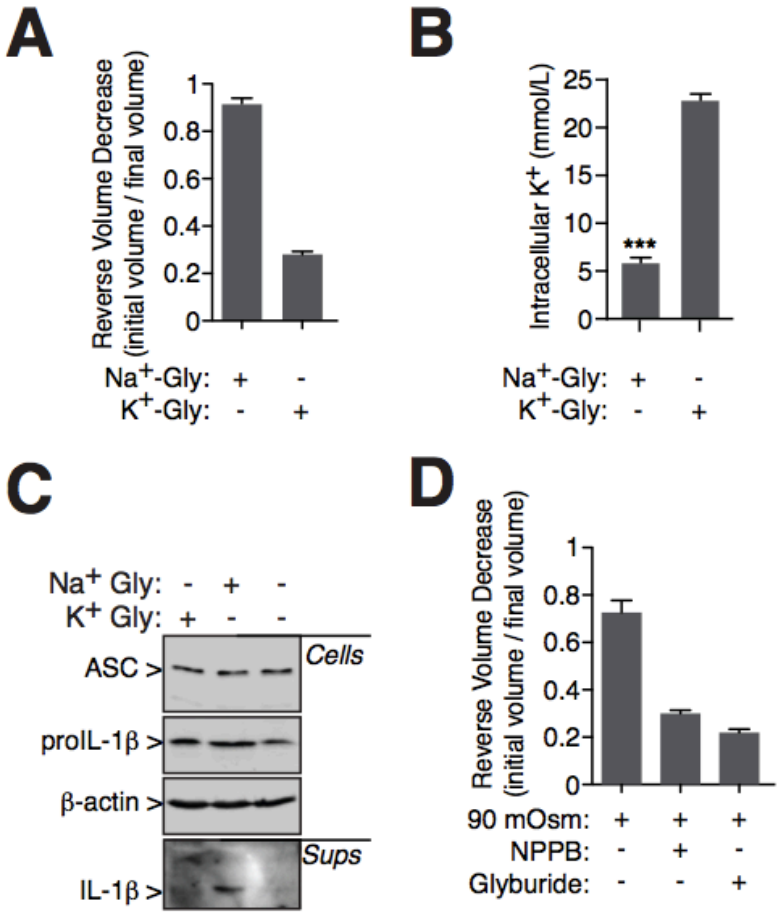

Figure S2. Regulatory volume decrease is blocked by high extracellular K^+, NPPB and glyburide. (**A**) Regulatory volume decrease measured as the ratio of the initial cellular volume and the final cellular volume after 3 h treatment with different hypotonic solutions of THP-1 cells primed with LPS and IFN-γ (100 ng/ml each, 16 h); hypotonic solutions were composed with: 120 mM glycerol with 140 mM of NaCl (Na^+-Gly) or 140 mM of KCl (K^+-Gly). (**B**) Relative intracellular K^+ concentration of THP-1 cells primed as in (A) but incubated for 1h in the presence of different solutions as indicated; data represent the average and s.e.m. of $n = 3$ independent experiments; ***$p < 0.001$. (**C**) Western blot analysis of the cleavage of pro-IL-1β to its active p17 form (IL-1β) and of ASC from THP-1 activated as in (A). *Cells*: cell lysates, *Sups*: supernatants; Western blot representative of 3 different experiments. (**D**) Regulatory volume decrease ratio of THP-1 cells primed with LPS and IFNγ (100 ng/ml each, 16 h) and incubated for 3 h with hypotonic solution in the presence or absence of NPPB (100 μM) or Glyburide (100 μM) as indicated. For (A) and (D), data represent average and s.e.m. of $n = 50$ cells/condition and representative of at least 3 independent experiments.

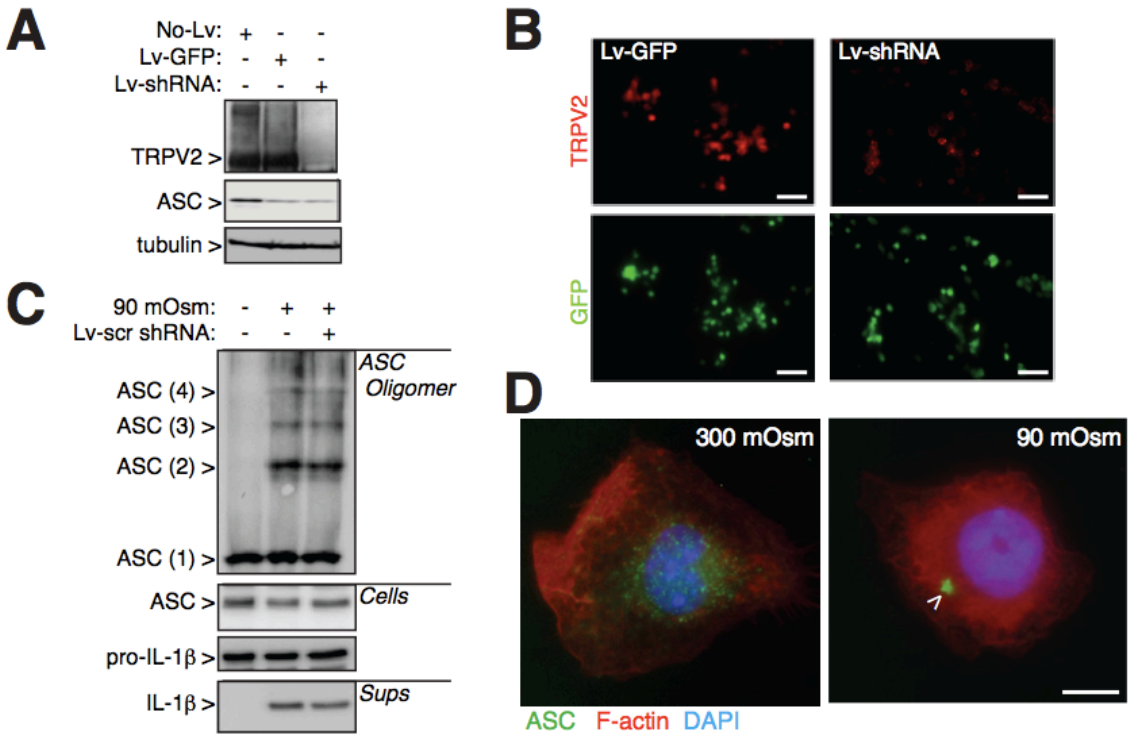

Figure S3. (A,B) Effective TRPV2 gene silencing in THP-1 cells. (**A**) Western blot for TRPV2, ASC and tubulin from THP-1 lysate of control non-infected cells (No-Lv), GFP-lentivirus infected cells (Lv-GFP) or cells infected with lentivirus coding for GFP and TRPV2 shRNA (Lv-shRNA). (**B**) TRPV2 immunostaining in THP-1 infected as in (A); scale bar 40 μm. (**C**) ASC and IL-1β Western blot from purified cross-linked ASC oligomer proteins (*ASC Oligomer*), total cell extracts (*Cells*) and cell supernatants (*Sups*) of THP-1 cells infected with lentivirus coding for scramble shRNA sequence primed with LPS and IFN-γ (100 ng/ml each, 16 h) and subsequently stimulated with 300 and 90 mOsm of extracellular osmolarity for 60 min. (**D**) Representative pictures of wild type murine peritoneal macrophages primed with LPS (1 μg/ml for 4 h) and stained for ASC (green), actin (Phalloidin-Texas red, red) and nuclei (DAPI, blue) after incubation for 40 min in isotonic or hypotonic solution. Scale bar 10 μm.