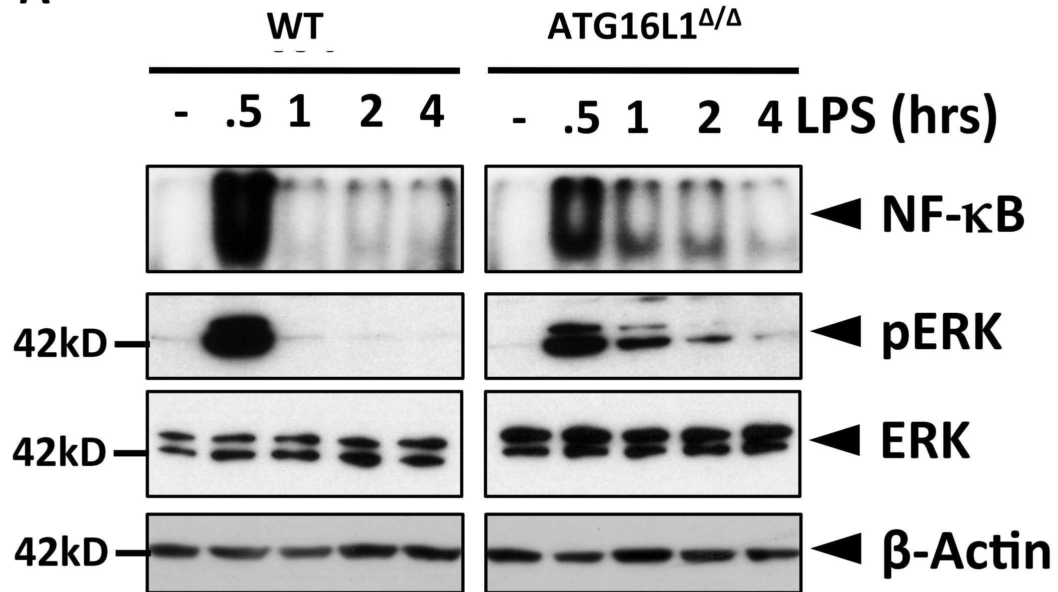
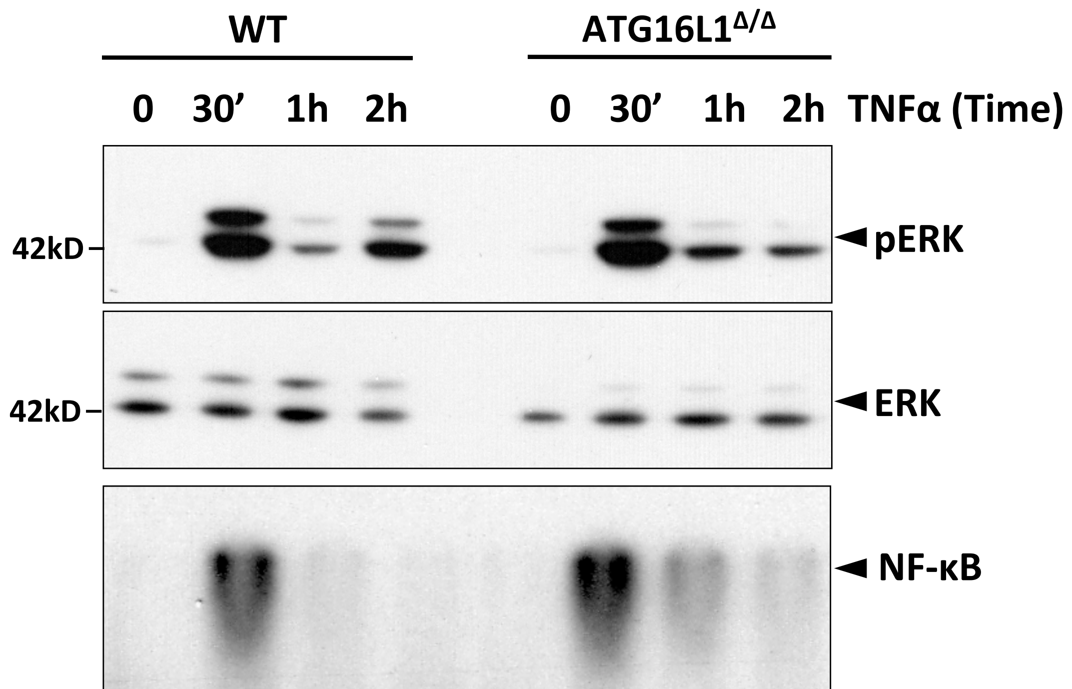
Supplemental Information

Supplemental Figure 1 ATG16L1 does not regulate LPS or TNF-a signaling. WT or ATG16L1-deficient MEF were stimulated with LPS (100 ng/ml) or TNF-a (1 ng/ml) as indicated and activation of pERK by IB and NF-kB by EMSA was measured.

Supplemental Figure 1

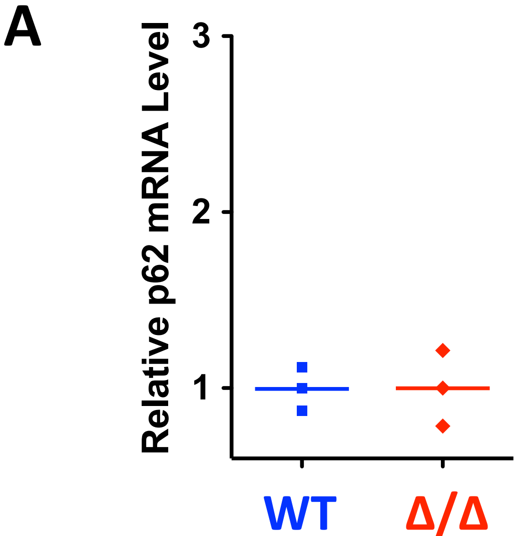




Supplemental Figure 2 ATG16L1 does not regulate p62 at the mRNA level. A. The levels of p62 mRNA in WT or ATG16L1-deficient MEF were measured by qPCR. **B.** GFP-p62 accumulates upon proteasome inhibition. WT or ATG16L1-deficient (Δ/Δ) MEF were transfected with GFP-p62 and treated with MG132 (10 μM) for 4 hrs and the levels of the indicated proteins were measured by immunoblotting.

Supplemental Figure 2

A



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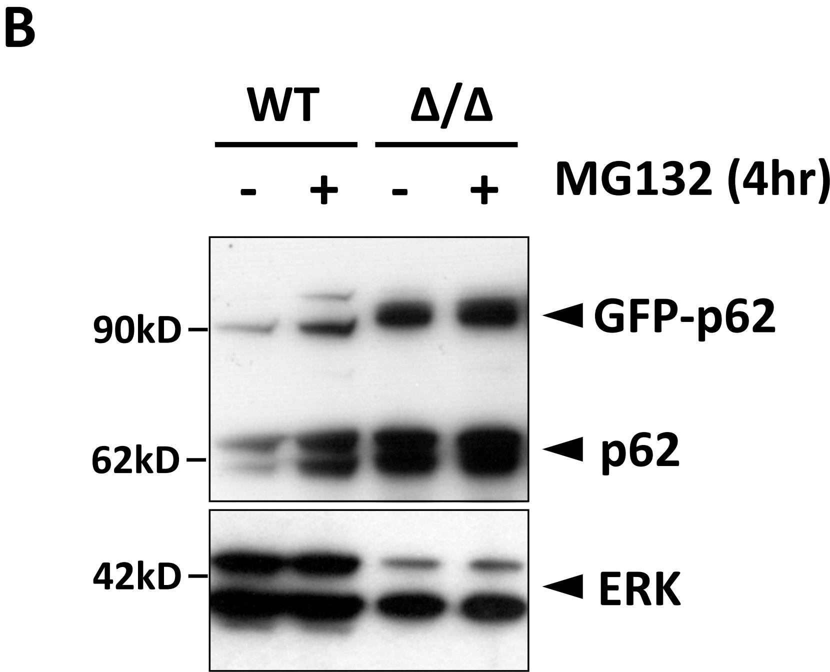
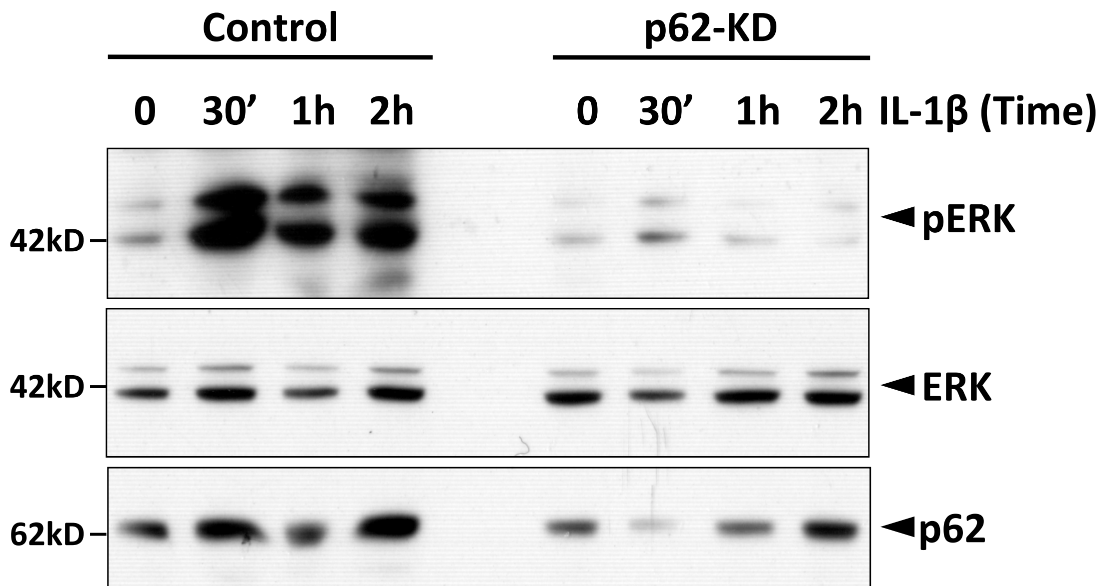
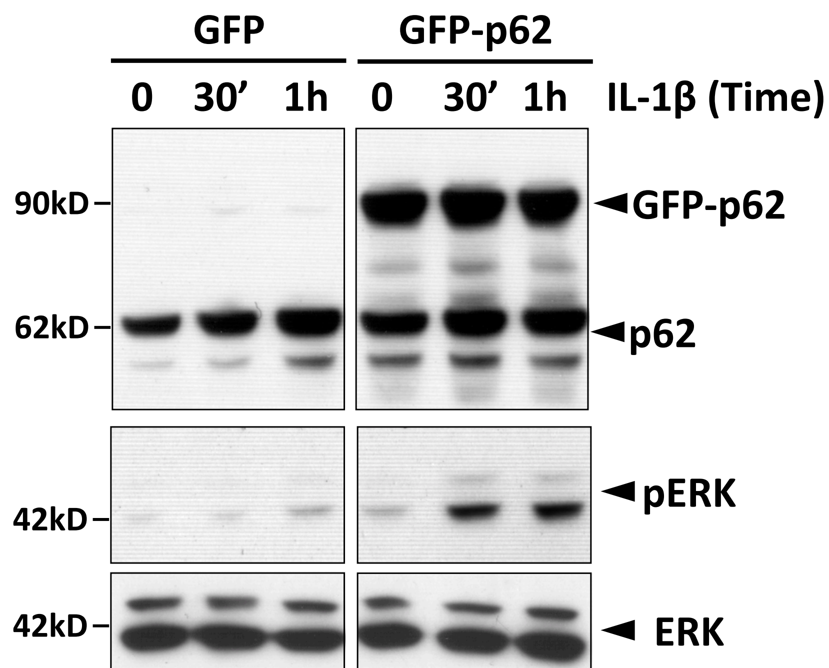


Supplemental Figure 3. P62 enhances IL-1β signaling in MEF. A. WT MEF were transfected with either control or p62 siRNA and stimulated with IL-1β as indicated. The levels of the indicated proteins after IL-1β stimulation (1 ng/ml) were determined by IB. **B.** WT MEF were transfected with either GFP or GFP-p62 expression vector and stimulated with IL-1β as indicated. The levels of the indicated proteins after IL-1β stimulation (1 ng/ml) were determined by IB.

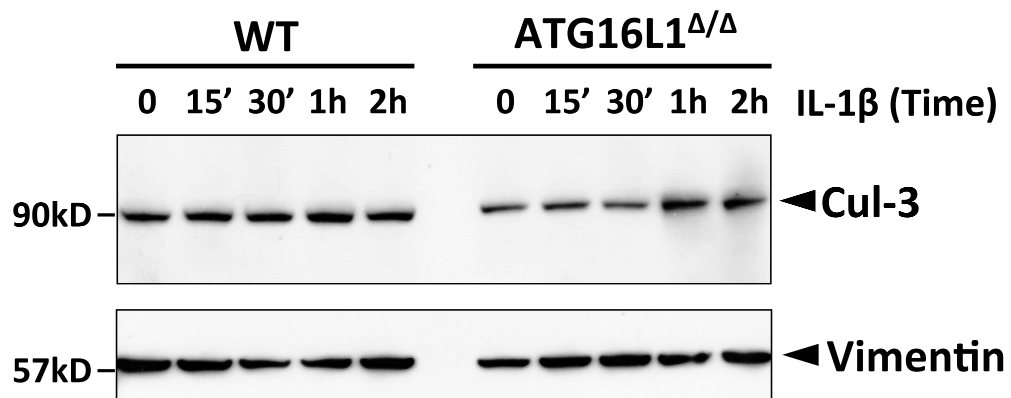
Supplemental Figure 3

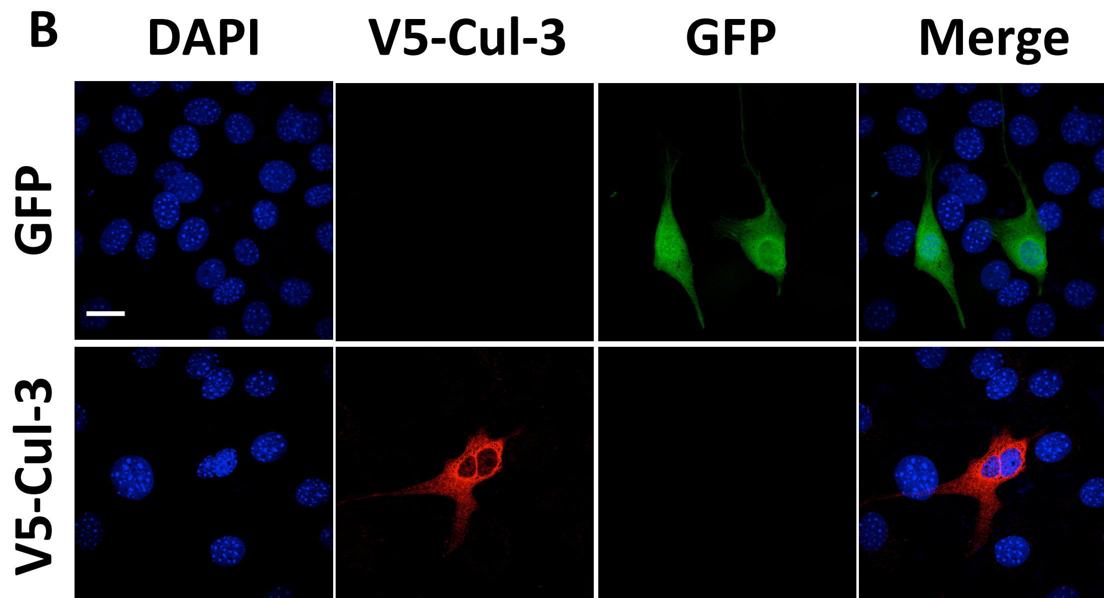




Supplemental Figure 4. ATG16L1 or IL-1β does not regulate expression of Cul-3 in MEF. A. WT ATG16L1-deficient MEF were stimulated with IL-1β (1 ng/ml) as indicated and the levels of the indicated proteins were measured by IB. **B.** WT MEF were transfected with either GFP-p62 of V5-Cul-3 and expression of these proteins was confirmed by immunofluorescence.

Supplemental Figure 4





Supplemental Figure 5. Regulation of p62 by Cul-3 is independent of Nrf2. A. Nrf2 knockdown does not significantly alter p62 degradation by proteasome. WT MEF were transfected with either control or Nrf2 siRNA (Santa Cruz Biotechnology) and treated with MG132 (10μM) as indicated. The levels of the indicated proteins were measured by immunoblotting. Nrf2 protein was not detectible under steady state conditions in MEF but accumulated upon proteasome inhibition. **B.** Nrf2 does not significantly influence p62 degradation by Cul-3. WT MEF were transfected with control, Cul-3, or Cul-3 plus Nrf2 siRNA and treated with MG132 (10μM) as indicated. The levels of the indicated proteins were measured by immunoblotting.

Supplemental Figure 5