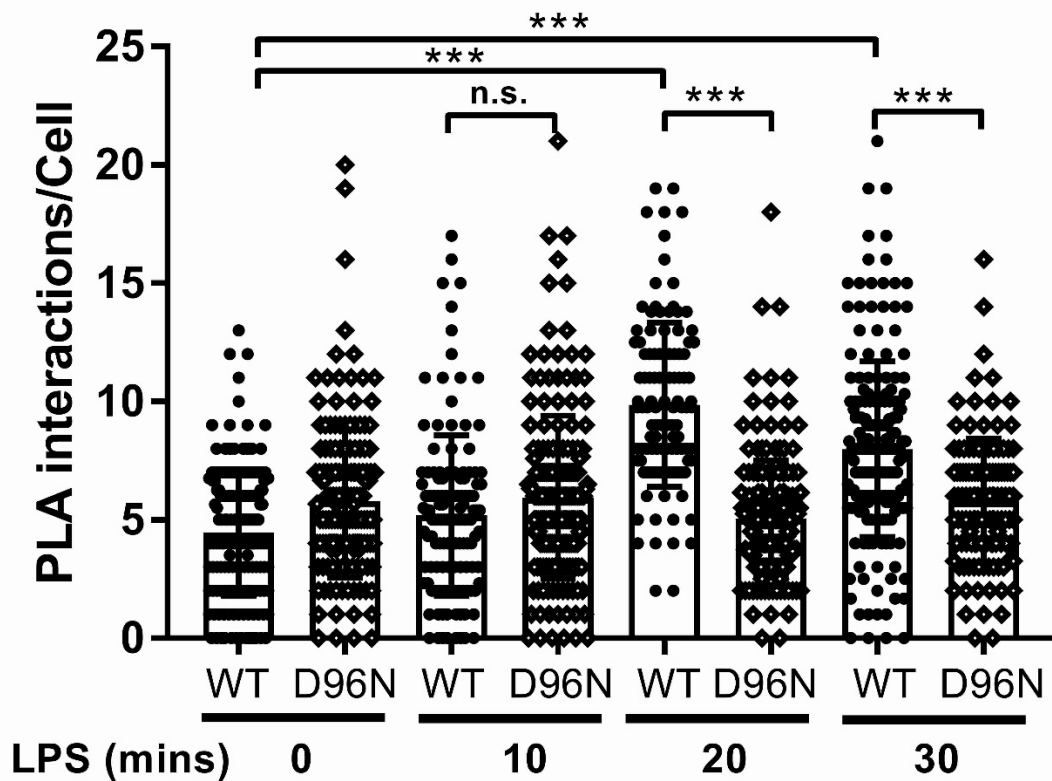


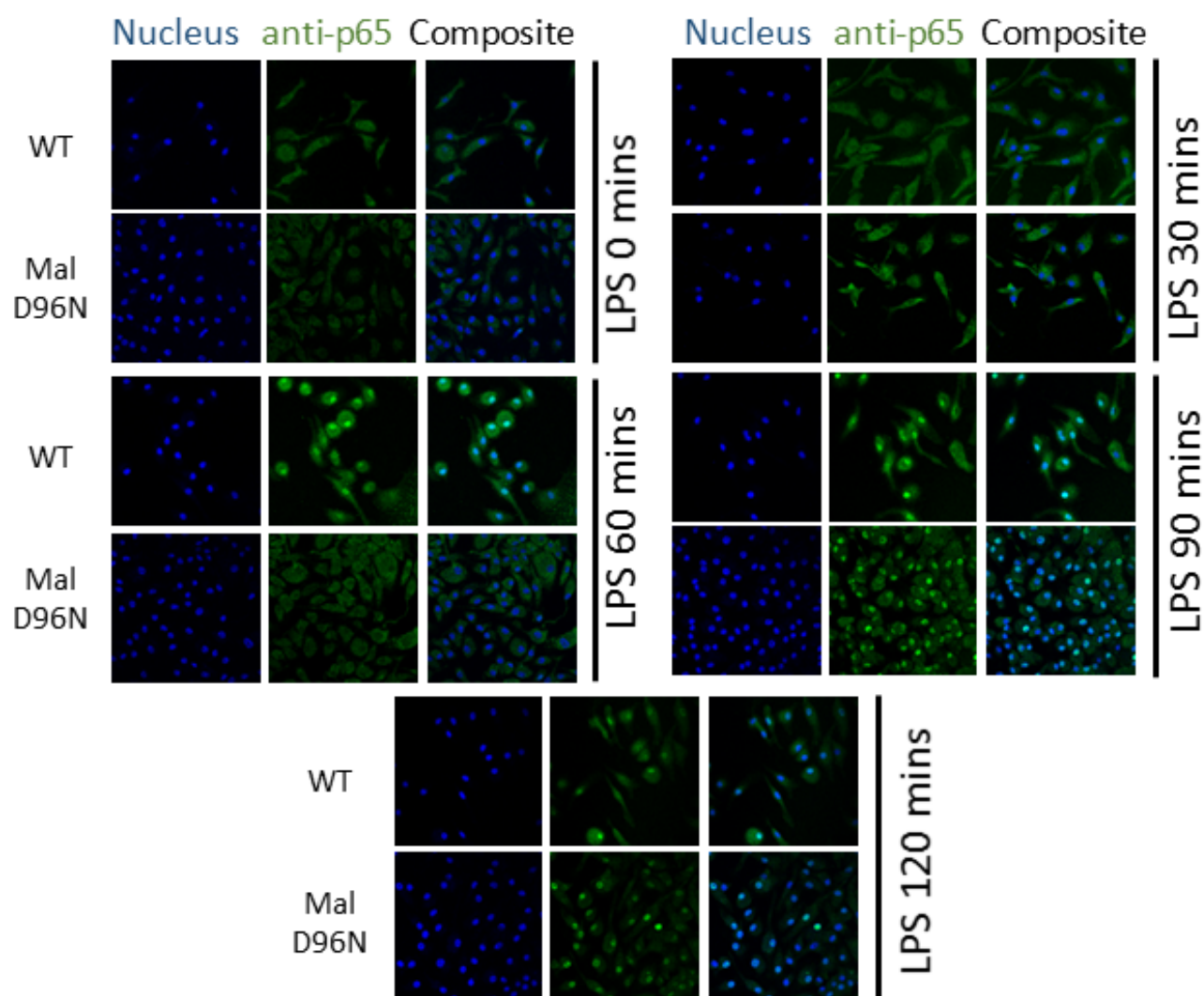
Supplemental Figure 1: Overt phenotype of MalD96N mice

WT mice and mice homozygous for the MalD96N display similar (A) body and (B) organ weights.

(c) Expression levels of Mal by real-time PCR; Mal D96N BMDMs exhibit commensurate levels of Mal expression as compared to wild type mice.



Supplemental Figure 2: Mal-D96N macrophages display attenuated interaction with MyD88 following TLR4 stimulation. Proximity Ligation assay; data points in each group represent the number of proximity sites observed per cell across the five fields of view chosen randomly for the data represented in Figure 2 (main text), approximately 150 cells per group. Data are presented as mean \pm SD from three independent experiments. One-way ANOVA *** $p < 0.001$; n.s., not significant.



Supplemental Figure 3. Mal D96N macrophages display delayed NF-κB nuclear translocation.

Bone marrow-derived macrophages (BMDMs) generated from WT and Mal D96N mice were plated at 1×10^4 cells/well, 24 h prior to stimulation with 100ng/ml of LPS for indicated times. Cells were treated with DAPI to identify nuclei for 10 mins prior to harvesting. Cells were washed with PBS (4°C), permeabilized and incubated with anti-p65 antibody (1:50), prior to incubation with Alexaflor-488 secondary antibody. Images are representative of 3 random fields of approximately >30cells/field.