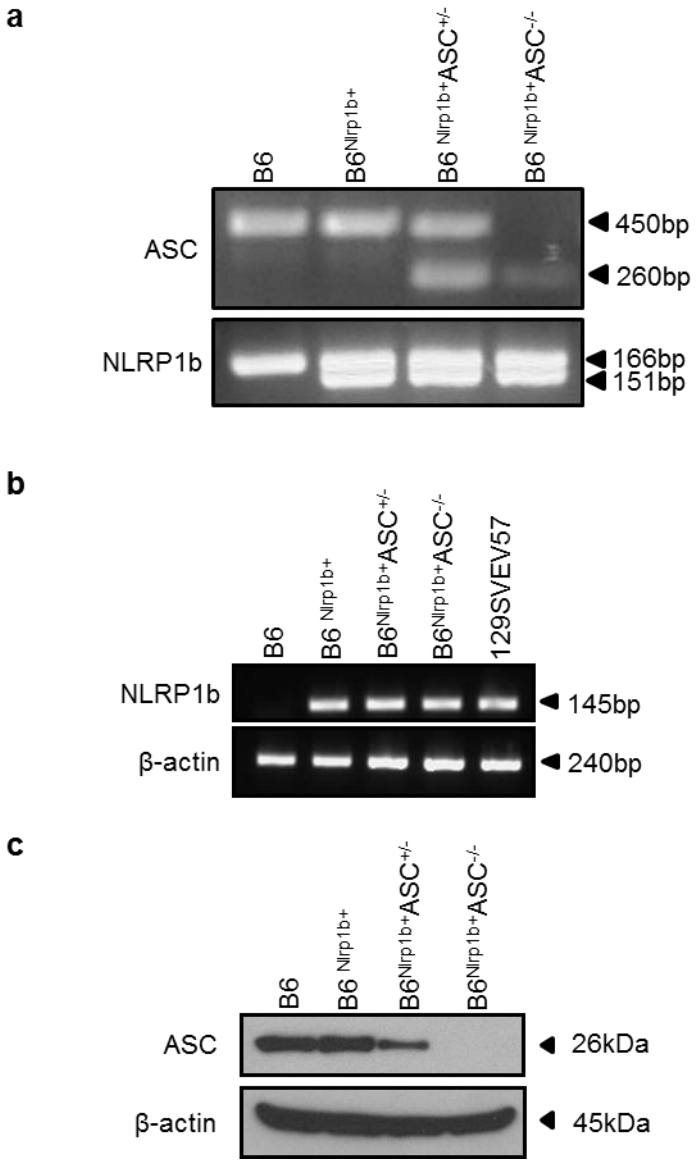
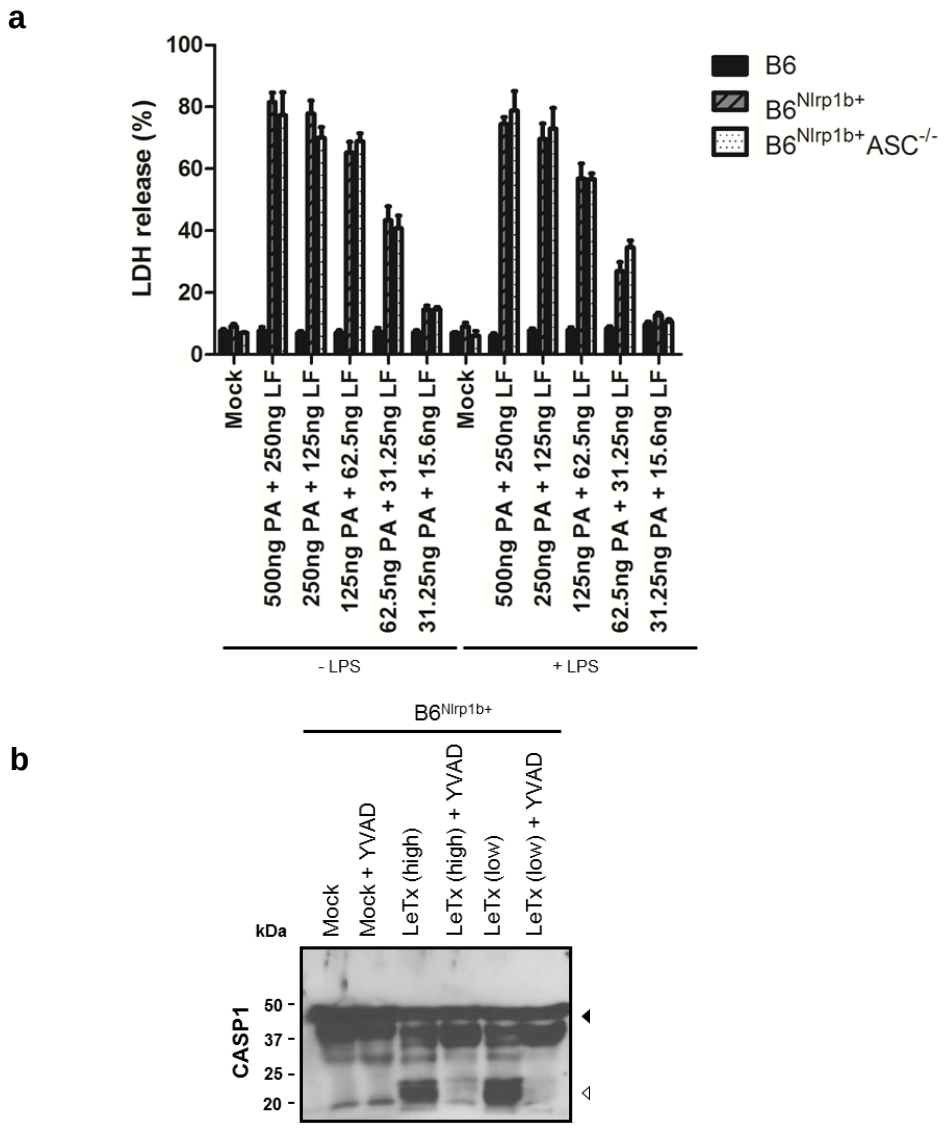
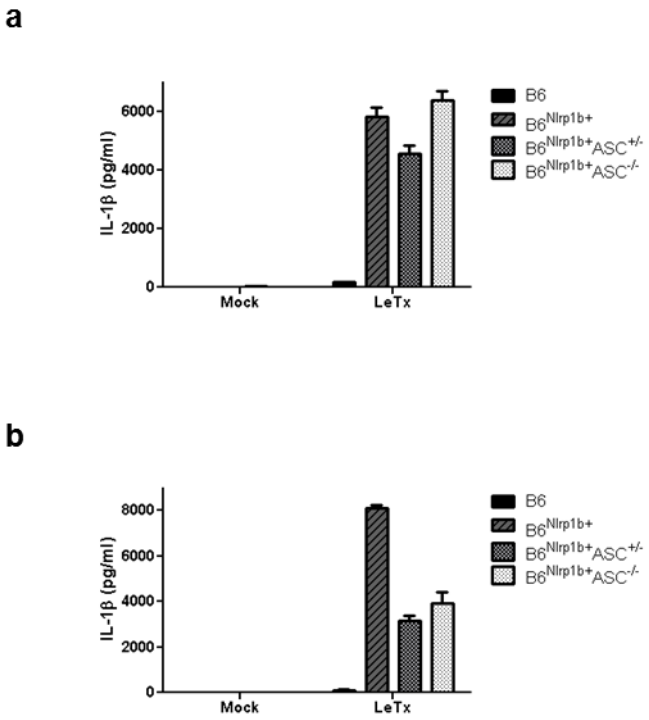


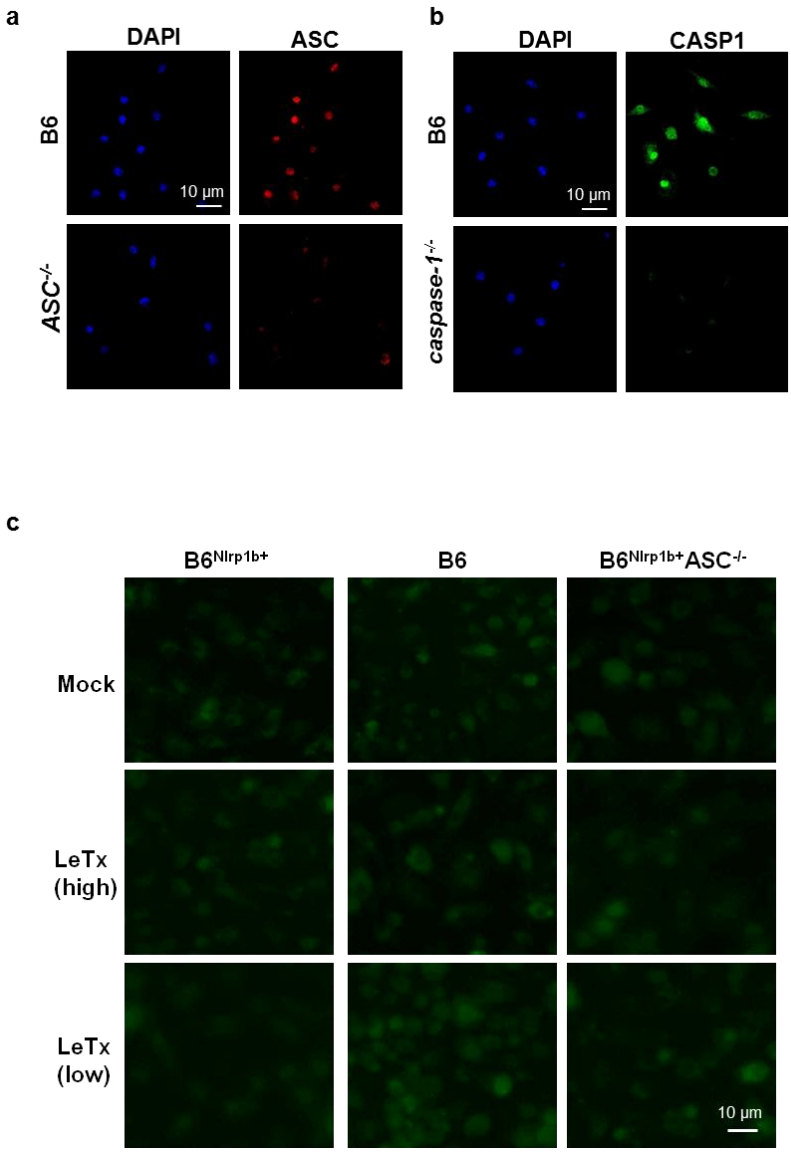
Supplementary Figure 1. Genotyping and expression analysis of ASC and NLRP1b in mice and macrophages. **a**) Genotyping for ASC and NLRP1b alleles on genomic DNA of B6, $B6^{Nlrp1b+}$, $B6^{Nlrp1b+}ASC^{+/-}$ and $B6^{Nlrp1b+}ASC^{-/-}$ mouse tails. **b**) RT-PCR analysis of functional NLRP1b expression on cDNA from B6, $B6^{Nlrp1b+}$, $B6^{Nlrp1b+}ASC^{+/-}$, $B6^{Nlrp1b+}ASC^{-/-}$ and 129SVEV57 BMDMs. **c**) B6, $B6^{Nlrp1b+}$, $B6^{Nlrp1b+}ASC^{+/-}$ and $B6^{Nlrp1b+}ASC^{-/-}$ BMDMs lysates were immunoblotted for the indicated proteins. Results are representative for 3 independent experiments.



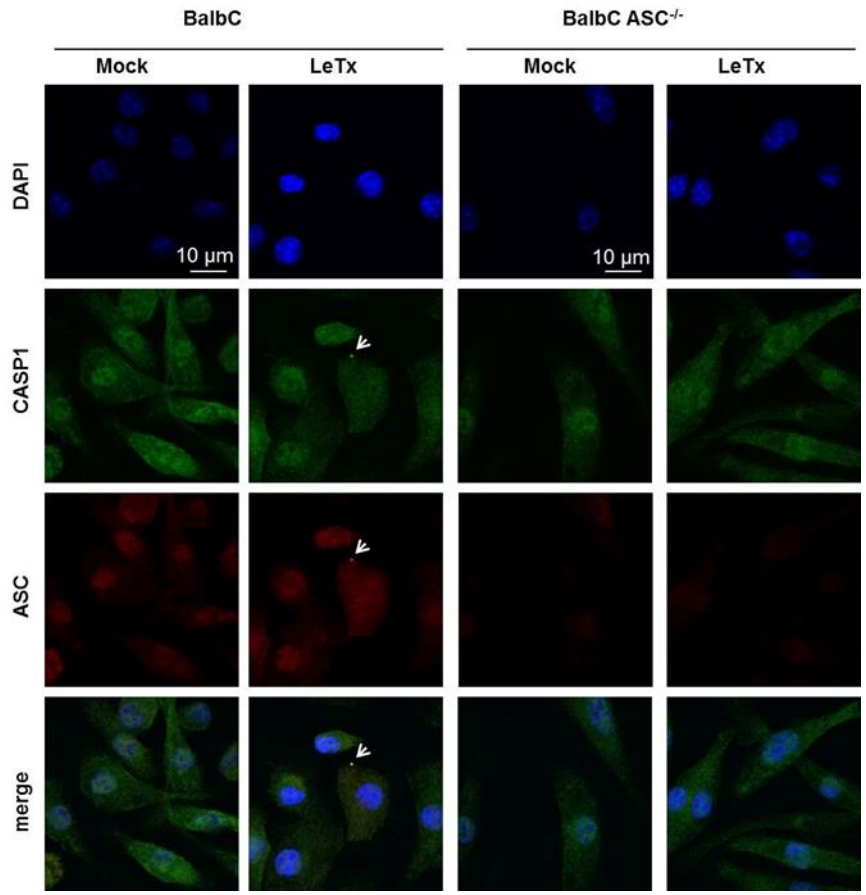
Supplementary Figure 2. Dose-dependent cell death induction in LeTx-treated macrophages. a) B6, B6$^{Nlrp1b+}$, B6$^{Nlrp1b+}$ and B6$^{Nlrp1b+}$ASC$^{-/-}$ BMDMs were left untreated or primed with 5 µg ml^{-1} LPS prior to incubation with decreasing concentrations of LeTx for 3 h. LDH levels were determined in culture supernatants. Data are shown as mean ± S.E.M. from two independent experiments, with each condition performed in triplicate in each experiment. **b)** LPS-primed B6$^{Nlrp1b+}$ BMDMs were pretreated with 50 µM ac-YVAD-cmk for 30 min and then left untreated (Mock), or exposed to 10 µg PA + 10 µg LF (High) or to 500 ng PA + 250 ng LF (Low) for another 3 h. Cell lysates were immunoblotted for caspase-1. Results are representative for 3 independent experiments.



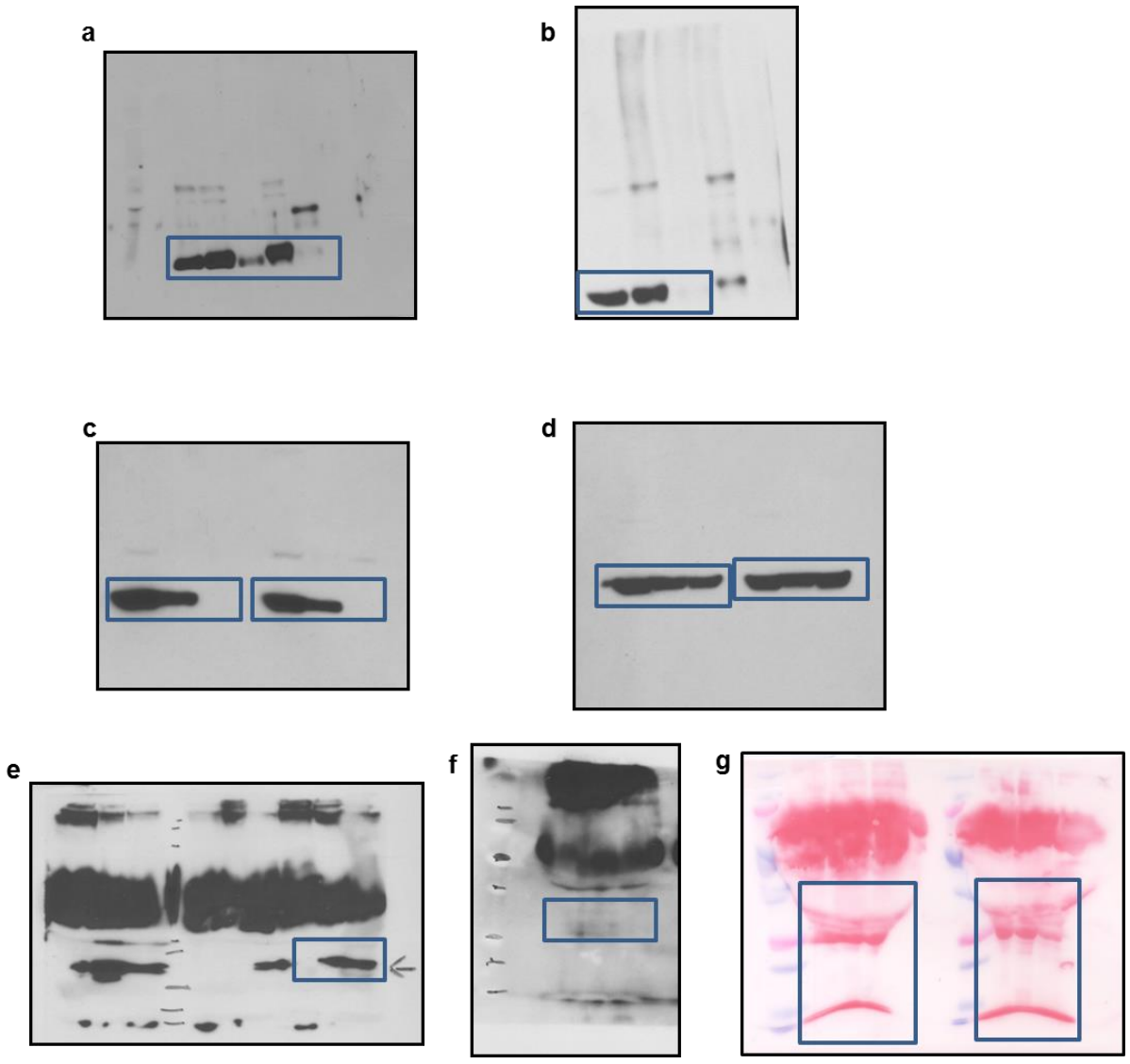
Supplementary Figure 3. LeTx-induced IL-1β secretion at 24h post-treatment. a-b) B6, $B6^{Nlrp1b+}$, $B6^{Nlrp1b+}ASC^{+/-}$ and $B6^{Nlrp1b+}ASC^{-/-}$ BMDMs were primed with LPS (5 μg ml^{-1} for 3 h) and then treated with LeTx (**a**, 10 μg PA + 10 μg LF; **b,** 500 ng PA + 250 ng LF) for 24h. Secreted IL-1β was determined by ELISA assay, and data are shown as mean ± S.D. from a single representative experiment out of 2, with each condition performed in triplicate.



Supplementary Figure 4. Control stainings in fluorescence and confocal microscopy. a) Untreated B6 and $ASC^{-/-}$ BMDMs were fixed and stained for ASC and mounted in ProLong Gold Antifade reagent containing DAPI. **b**) Untreated B6 and caspase-$1^{-/-}$ BMDMs were fixed, stained for caspase-1, and mounted in ProLong Gold Antifade reagent containing DAPI. Micrographs were acquired by confocal microscopy at 60x magnification. **c**) Analysis of autofluorescence by B6, $B6^{Nlrp1b+}$ and $B6^{Nlrp1b+}ASC^{-/-}$ BMDMs that were treated with LeTx (high, 10 µg PA + 10µg LF; low, 500 ng PA + 250 ng LF) for 30 min, and then incubated with vehicle control (DMSO) for 45 min. Fluorescence micrographs were taken at 20x magnification. Results are representative for 3 independent experiments.



Supplementary Figure 5. ASC is critical for LeTx-induced speck formation in BALB/c background. BALB/c and BALB/c ASC$^{-/-}$ BMDMs were grown on coverslips and treated with LeTx (10 µg PA and LF, respectively) for 60 min after which cells were fixed in 4 % paraformaldehyde and immunostained for ASC (red) and caspase-1 (green). DAPI (blue) was included to localize nuclei. Arrows indicate specks. Confocal micrographs were acquired at 60x magnification. Results are representative for 3 independent experiments.



Supplementary Figure 6. Uncropped scans of blots shown in figures 6 and 7. a-b) shown are the blot scans used to generate a part of the panel of Fig. 6j and 6k, respectively. **c-g**) shown are the blot scans for the panels of Fig. 7a and 7b. The cropped areas used in the figures are marked by boxes.