WT BMDMs were seeded on coverslips, left untreated (A), or stimulated with LPS for 40 minutes (B). After the indicated time of the treatment, cells were washed, fixed with 4% paraformaldehyde in PBS, pH 7.2, for 15 min, and permeabilized with 0.1% Triton X-100 for 10 min in PBS. RelA was labeled with rabbit anti RelA (p65) antibodies from Abcam (ab7970) and nuclei were stained with DAPI (Molecuar probes). Highly cross-adsorbed donkey anti- rabbit IgG antibodies, labeled with Alexa Fluor565, obtained from Life Technologies-Molecular Probes were used as secondary antibodies. Controls in which the primary or secondary antibodies were omitted were included in these runs. Immunofluorescence microscopy of optical sections was performed by using a confocal laser scanning microscope Leica TCS SP5 X (Leica MicroSystems). The fluorophores were excited with selected lines from a tunable white light laser (460–670 nm) or a diode laser (405 nm). Leica Application Suite Advanced Fluorescence software (LAS AF, version 2.7.3.9723, Leica MicroSystems) was used. *Scale bar*, 20 μm.

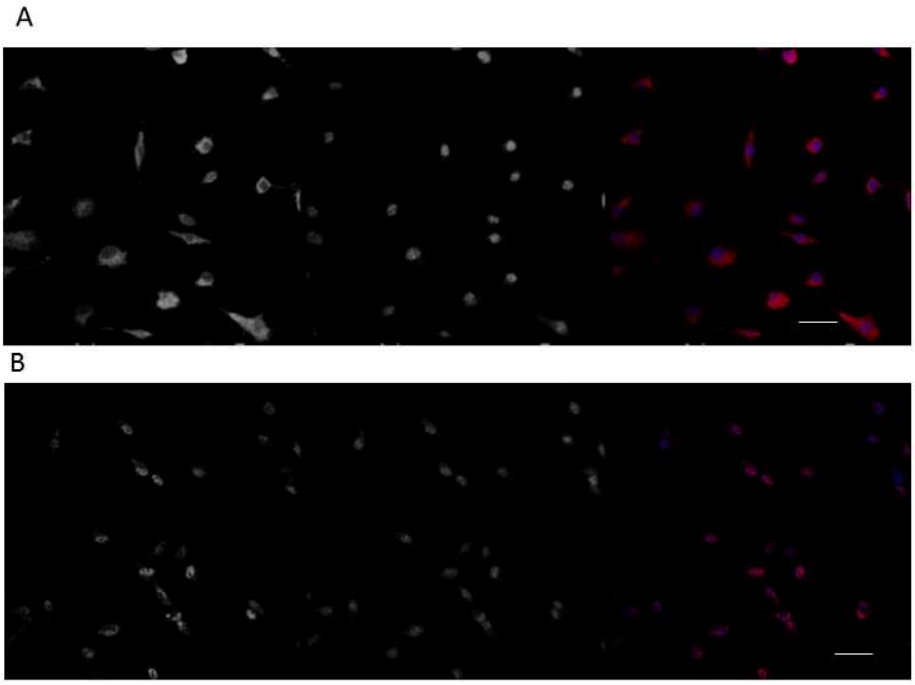

Figure S1 Translocation of Nuclear factor NF-kappa-B p65 subunit (RelA) (red) into the nucleus (blue) of LPS treated BMDMs.

Monolayer BMDMs at 80% confluence were first washed with ice-cold PBS, spleen cell lysates were prepared as described in Section 2. Protein concentration was determined with Bradford reagent (Bio-Rad). The lysates (20μg of protein) were subjected to electrophoresis on 15% SDS-polyacrylamide gels. Western blotting as described in section 2. Western blots were probed with stefin B antibodies Rabbit anti-stefin B (ab53725) from Abcam. Protein bands were visualized with ECL (Amersham Biosciences) according to the manufacturer's instructions. Lane 1 WT BMDM , lane 2 WT spleen, labe 3 KO BMDM lane 4 KO spleen.

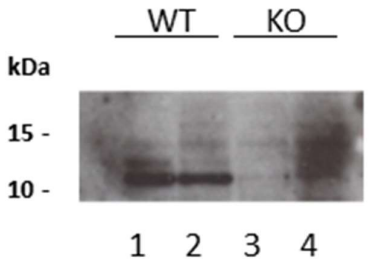

Figure 2. Lack of stefin B protein in BMDMs and spleens from stefin B deficient mice.