

FIGURE S2

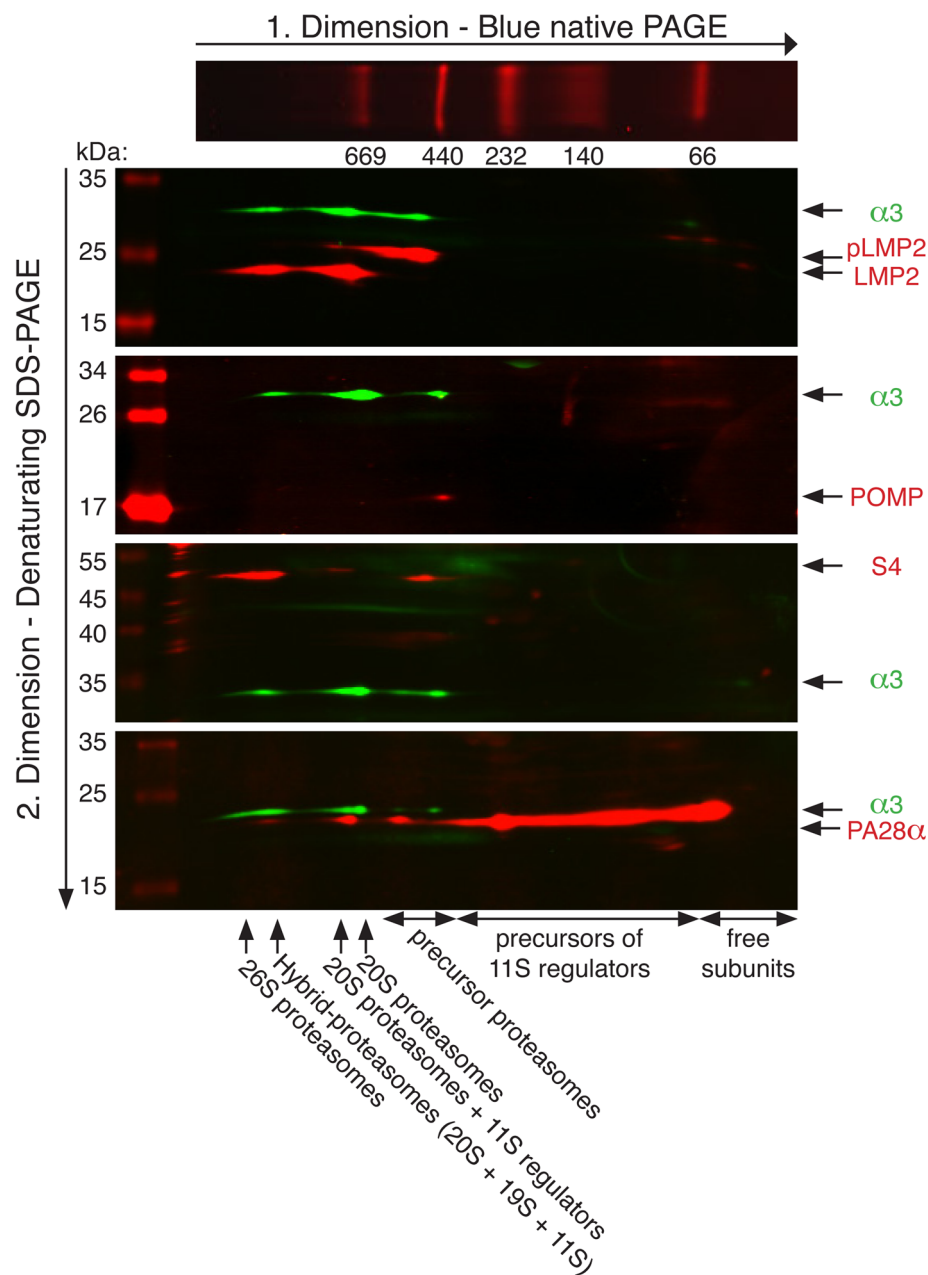


Figure S2: Identification of mature proteasome and precursor complexes by 2D two-colour fluorescent immunoblot analysis

Organ-lysates of spleens of *Imp7*^{-/-} mice, which were infected i.v. with 5×10^3 cfu of *L. monocytogenes* 4 days before were analysed by 2D two-colour fluorescent immunoblot analysis. First, protein complexes were separated by Blue Native-PAGE and in the second dimension subjected to SDS-PAGE, which was followed by two-colour fluorescent immunoblot analysis. Each membrane was stained for $\alpha 3$ (green signal) and as it is present in all early to mature complexes in proteasome assembly, $\alpha 3$ serves as a marker for the presence and positions of 13-15S precursor proteasomes, 20S proteasomes and 20S proteasomes + 11S and 19S regulators complexes. Staining for known components identified the various complexes: 13-15S precursor proteasomes were identified by the presence of unprocessed LMP2 (pLMP2) and POMP, 19S regulators by presence of ATPase subunit S4 and 11S regulators by staining for PA28 α (red signals). Arrows indicate the positions of the various identified proteasome complexes. Organs of three to four mice per group were pooled for the analysis.