

FIGURE S5

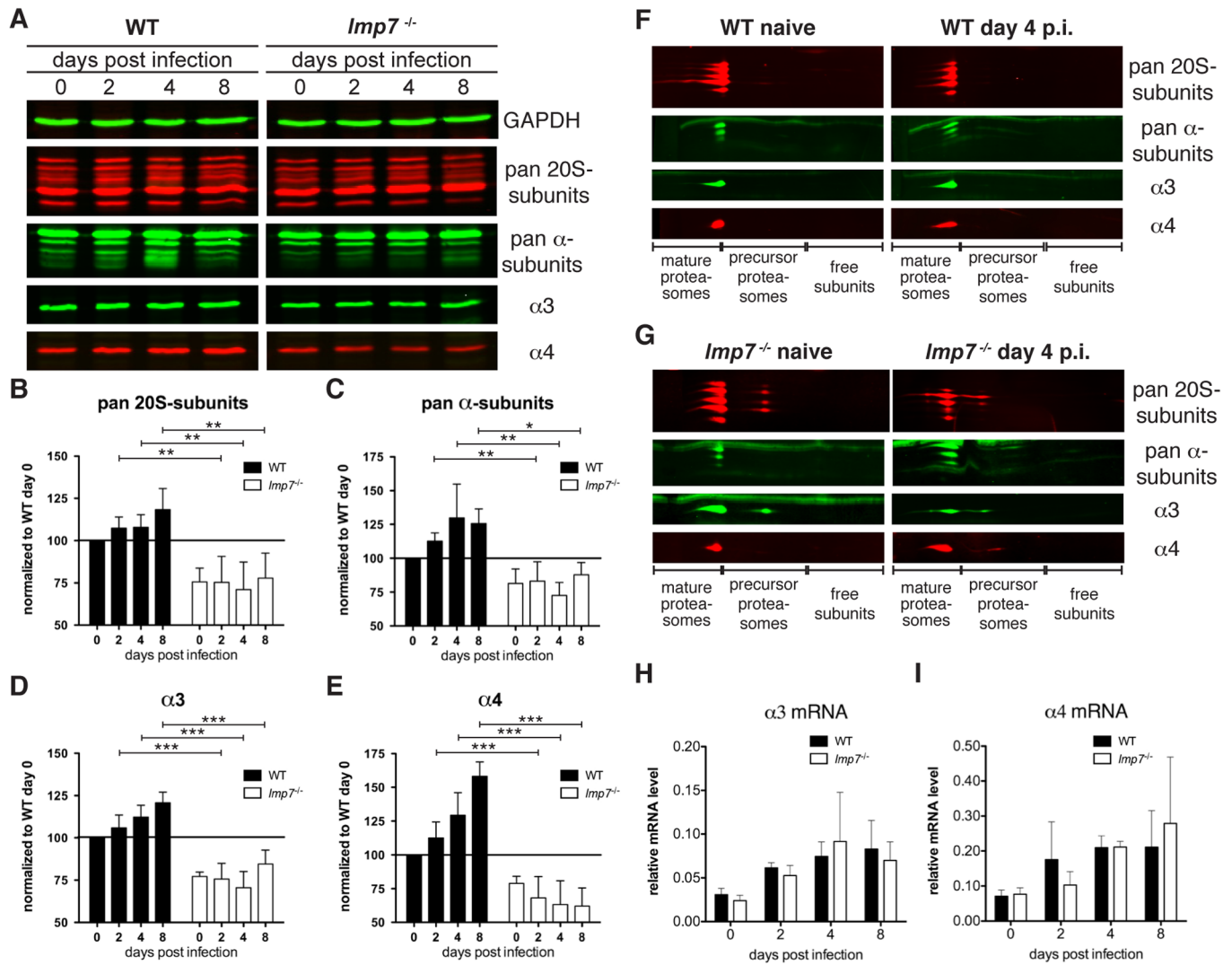


Figure S5: Relative Quantification of mature proteasomes in WT and *Imp7^{-/-}* mice during the course of listeria infection

Both groups of mice were infected i.v. with 5×10^3 cfu of listeria and spleens of three to four mice per group were pooled for two-colour fluorescent immunoblot analysis. Naïve mice (day 0) were used as controls in both groups of mice. Each membrane was stained against GAPDH as loading control. Further, membranes were stained with antibodies recognizing pan-20S-subunits, pan- α -subunits, $\alpha 3$ and $\alpha 4$ as indicated. Representative blots of three independent experiments are shown (A). Following densitometric analysis the relative protein abundance of pan-20S subunits (B), pan- α subunits (C), $\alpha 3$ (D) and $\alpha 4$ (E) was expressed as band intensities normalized to WT day 0, which was calculated as follows: (band intensity of proteasome subunit X at day Y / band intensity of GAPDH at day Y) / (band intensity of proteasome subunit X in WT day 0 / band intensity of GAPDH in WT day 0) \times 100. The given results are means \pm standard deviation of three independent infection experiments and each sample was analysed in duplicates during immunoblot analysis (B-E). To analyse in which proteasome complexes the analysed structural subunits are integrated, 2D Two-colour fluorescent immunoblot analysis was performed on spleen lysates of naïve and infected WT (F) and *Imp7^{-/-}* mice (G). Brackets mark the areas in which the different proteasome fractions are found. qPCR analysis on total splenic cDNA was performed to assess the mRNA expression of $\alpha 3$ (H) and $\alpha 4$ (I) during the course of listeria infection in WT and *Imp7^{-/-}* mice. The relative mRNA expression of the proteasome subunits was calculated by normalization to the house keeping gene RPS9 using the $\Delta\Delta CT$ method. Each value represents mean \pm standard deviation of three individual mice.