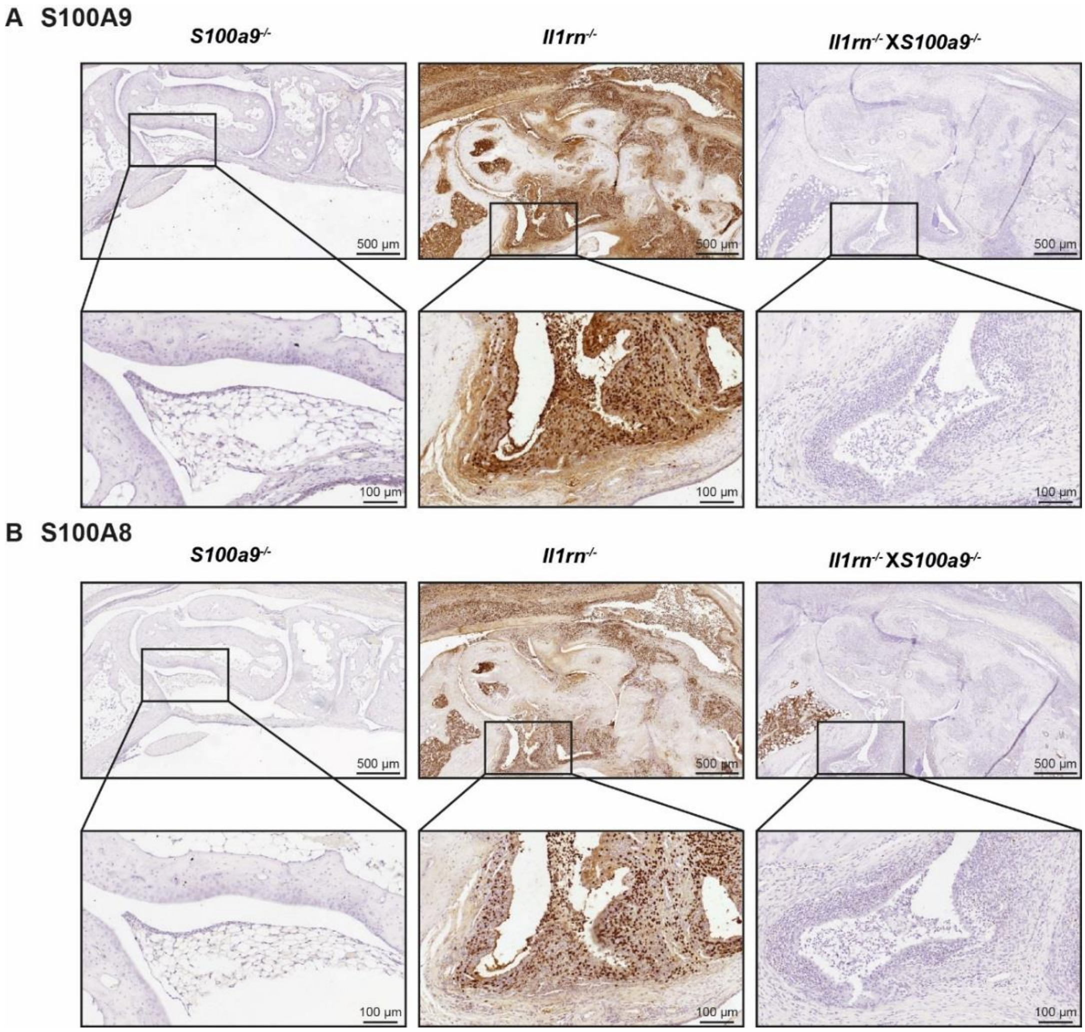
Additional File 5



S100a9$^{-/-}$XIl1rn$^{-/-}$ do not show rescued expression of S100A8 in inflammatory cells within the arthritic joints.

The expression of S100A8 and A9 in the ankle joint sections of 20-weeks-old *S100a9$^{-/-}$, Il1rn$^{-/-}$* and *Il1rn$^{-/-}$*X*S100a9$^{-/-}$* mice was determined with immunohistochemistry. Hereto, sections were digested with proteinase-free chondroitinase ABC (0.25 units/ml in 0.1 M Tris-HCl, pH 8.0; Sigma-Aldrich) for antigen retrieval followed by overnight incubation with rabbit anti-S100A8 or anti-S100A9 (*own facilities*). Sections were then incubated with biotinylated goat anti-rabbit IgG (Dako) as a secondary antibody followed by incubation with avidin-streptavidin-peroxidase (Elite-kit, Vector). Antibody binding was visualized using 3,3′-diaminobenzidine (DAB; Powervision DAB, Immunologic) and sections were counterstained with hematoxylin. Representative photomicrographs of S100A9 **(A)** and S100A8 **(B)** immunohistochemical staining are shown. S100A9 positive cells were absent in bone marrow and in synovium of *S100a9$^{-/-}$* and of *Il1rn$^{-/-}$*X*S100a9$^{-/-}$* mice, whereas numerous *S100A9* positive cells were visible in bone marrow (BM) and inflamed ankles of Il*1rn$^{-/-}$* mice. High numbers of S100A8 positive cells were present in the ankle joints of *Il1rn$^{-/-}$* mice. Note that expression of S100A8 protein is visible in BM of *Il1rn$^{-/-}$*X*S100a9$^{-/-}$* mice but not in the inflamed synovium suggesting that S100A8 expression is not rescued in the periphery under circumstances of high IL-1 signaling.