



Key role of caspase-1 in bacterial clearance during *S. aureus* infection of osteoblasts-like cells.

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CONTEXT & AIM

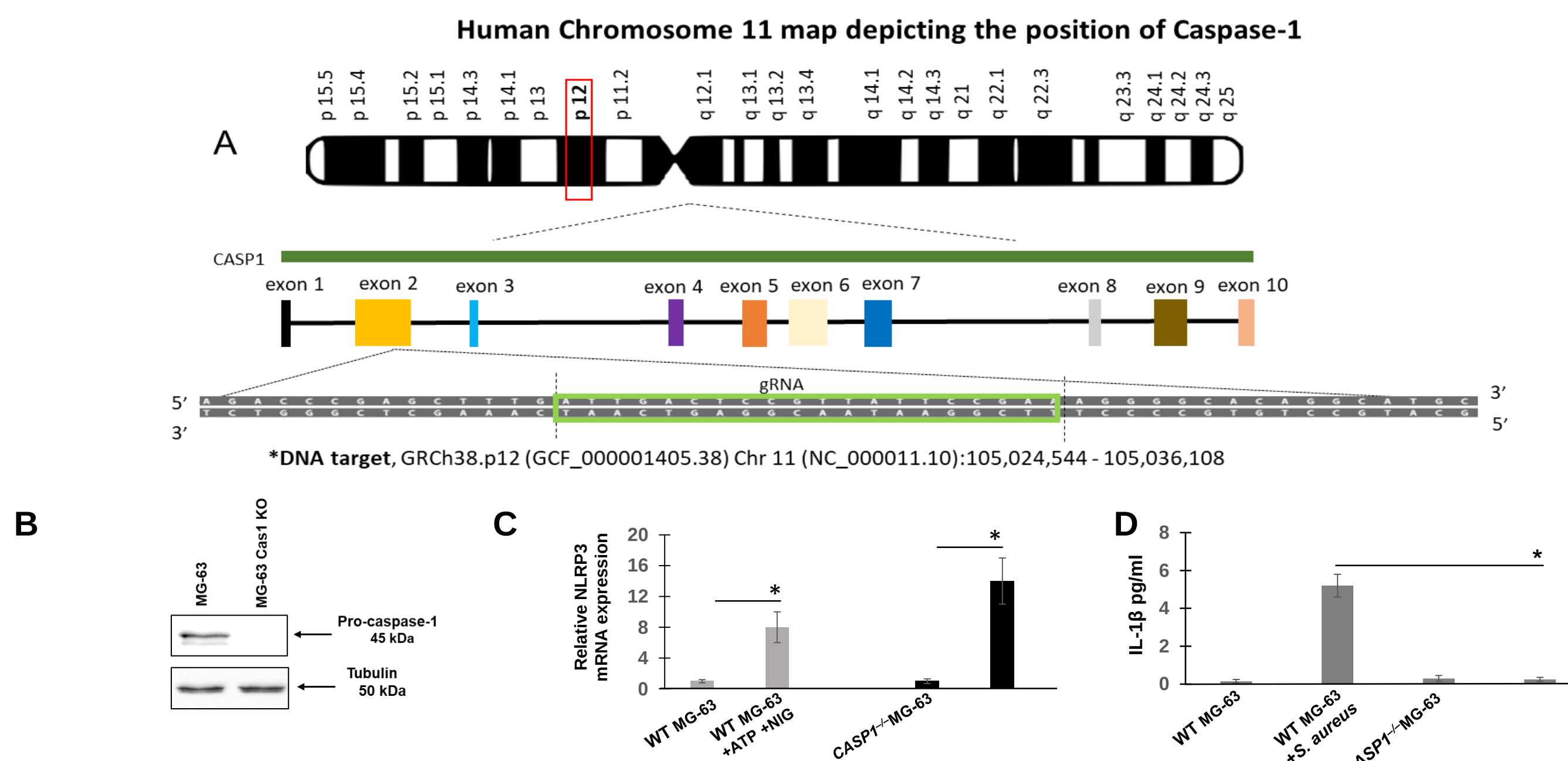
- The inflammasome is a multi-protein signaling platform that assembles after recognition of danger signals and/or pathogens. Once assembled, inflammasomes initiate signaling by activation of downstream proteases, most notably Caspase-1 and Caspase-11, which then proteolytically mature pro-IL-1 β , pro-IL-18, and pro-IL-33.
- Staphylococcus aureus* is a gram-positive bacterium that can cause several fatal infections and is also the predominant cause of bone infections worldwide. The quorum-sensing system in *S. aureus* known as the accessory gene regulator (Agr). Agr regulates the expression of many virulence factors including the expression of PSMs encoding genes.
- In this study, we investigated the involvement of inflammasomes in the model of persistent infection of human osteoblast-like cells with the help of the *CASP1*^{-/-} MG-63 cell line that was established using the CRISPR-Cas9 gene editing system.

Generation of *CASP1*^{-/-} MG-63 cells using the CRISPR-Cas9 gene editing system

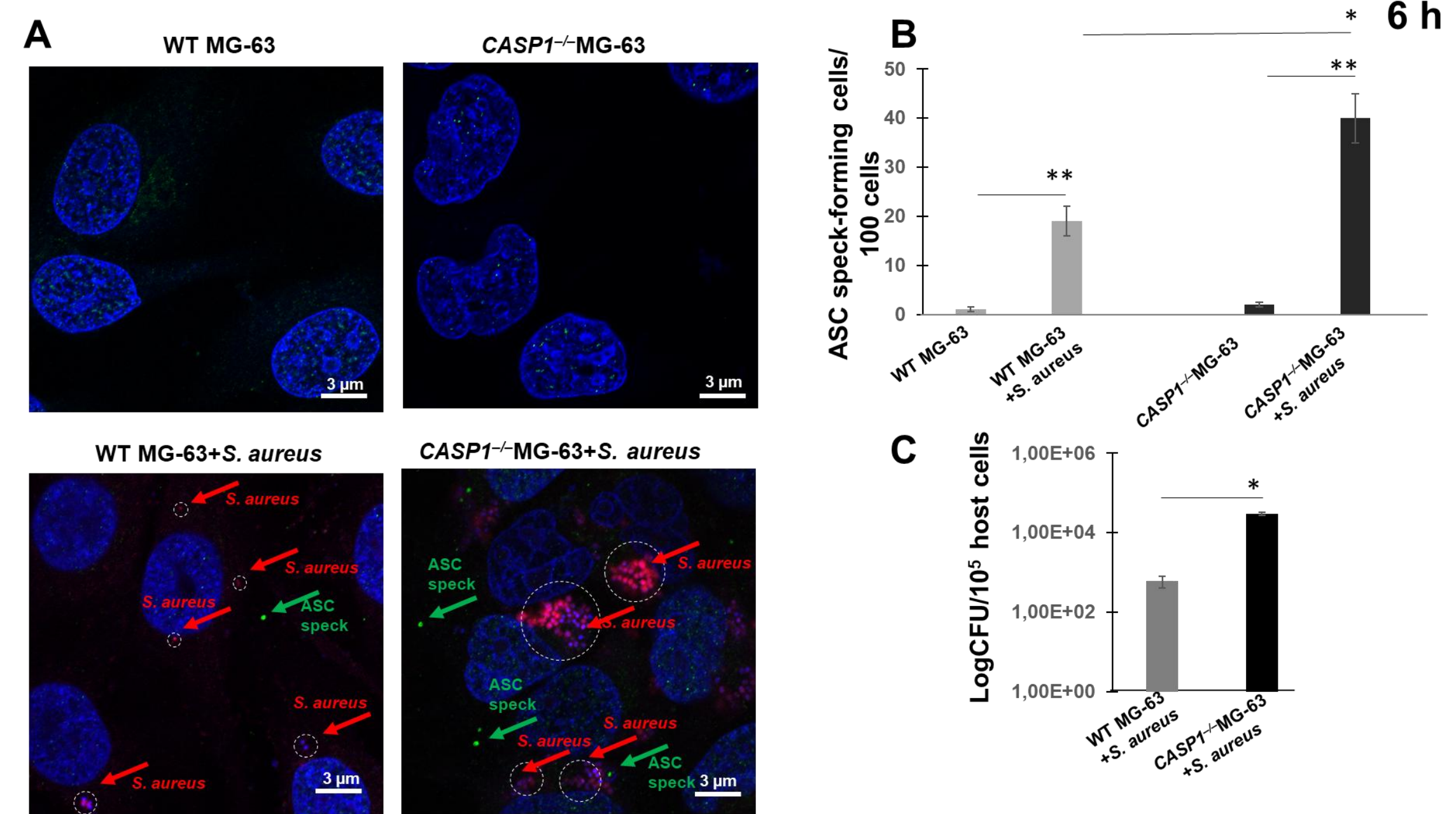
RESULTS

Higher number of ASC specks in *CASP1*^{-/-} MG-63 cells compared to WT MG-63 cells

MOI 50:1

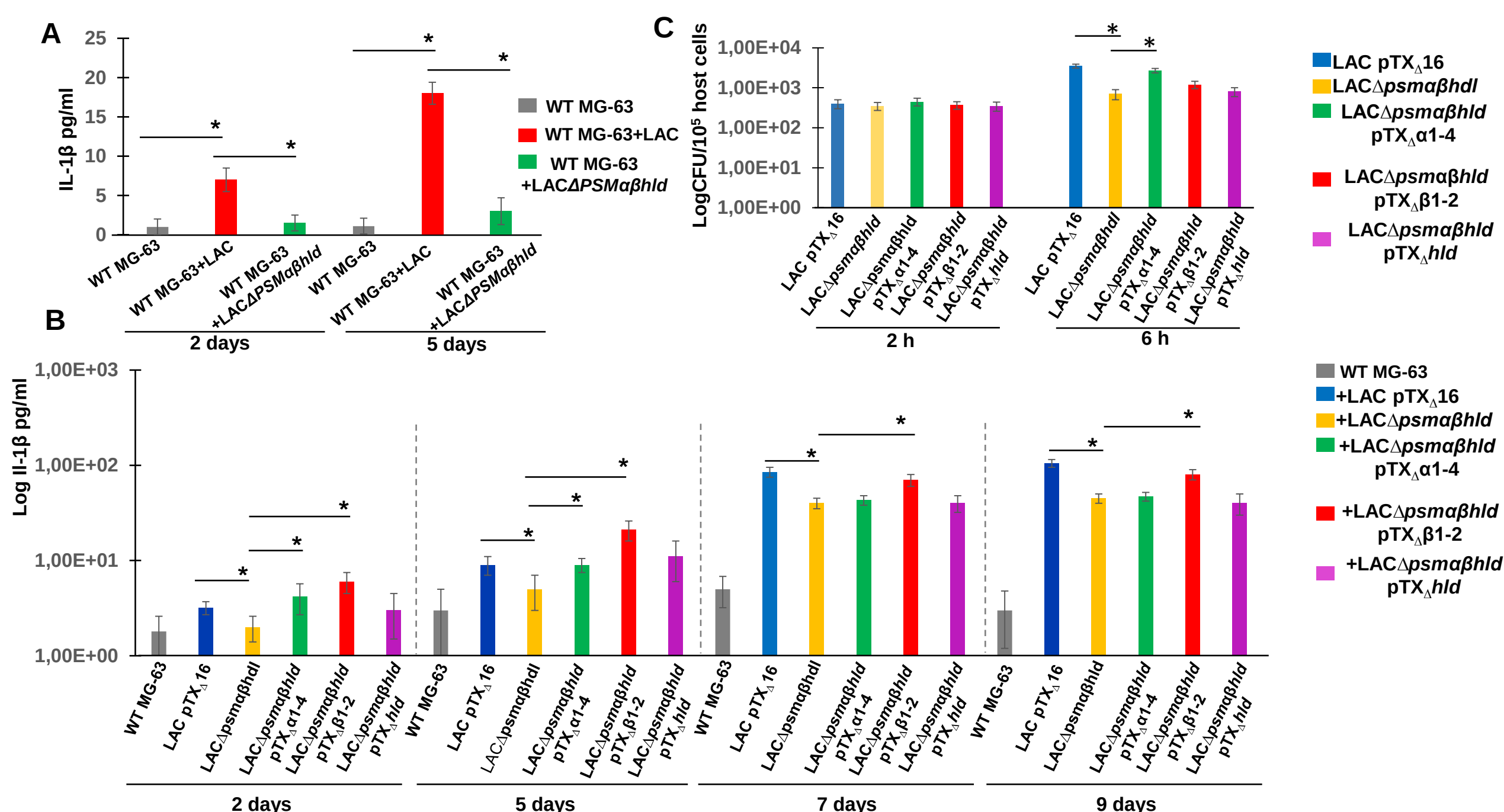


- Generation of *CASP1*^{-/-} MG-63 cells targeting exon 2 of the *CASP1* gene
- Detection of 45-kDa pro-caspase-1 in WT MG-63 vs *CASP1*^{-/-} MG-63 cells by Western blot analysis using anti-caspase-1 antibody.
- Analysis of mRNA levels of NLRP3 in WT MG-63, *CASP1*^{-/-} MG-63 cells by RT-qPCR.
- The level of IL-1 β in cell supernatants was determined by ELISA.



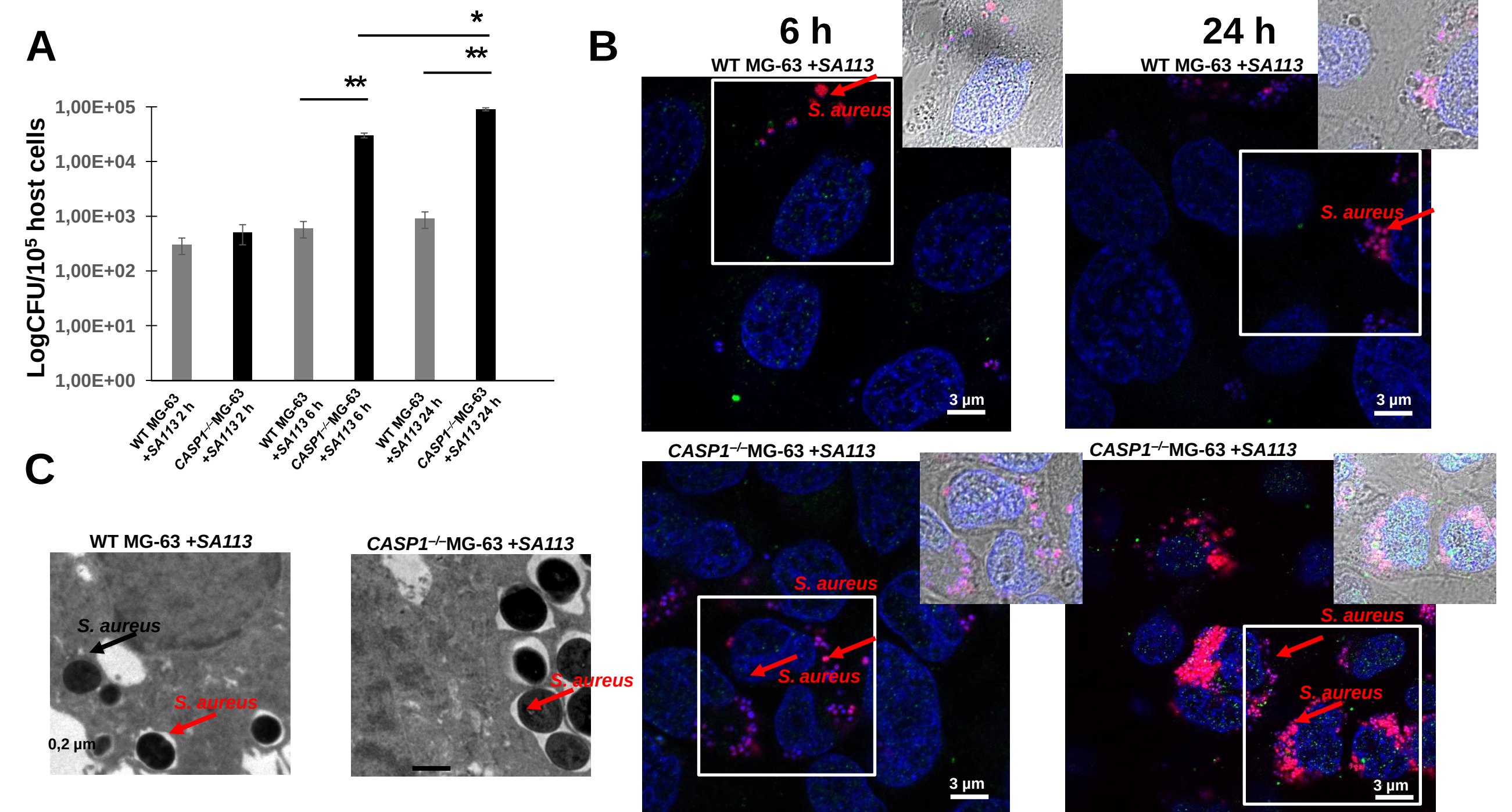
- WT MG-63 or *CASP1*^{-/-} MG-63 cells were exposed to *S. aureus* SA113 (red fluorescence, red arrows). Six hours post-infection cells were immunostained with anti-PYCARD antibody.
- Quantification of the amount of ASC-speck forming cells/100 cells.
- Cell lysates were plated on BHI agar, and CFU were determined. CFU values of cells exposed to *S. aureus* were normalized to 10⁵ host cells.

S. aureus phenol-soluble modulins stimulate IL-1 β release from infected osteoblast-like MG-63 cells



- WT MG-63 cells were exposed to wild type LAC (USA300) and its isogenic mutant LAC Δ *psma* θ *hld*. The level of IL-1 β in cell supernatants was determined by ELISA.
- MG-63 cells were exposed to USA300 LAC (pTX Δ 16), which carries the control plasmid, the deletion mutant LAC Δ *psma* θ *hld* (pTX Δ 16) and the complemented strains.
- WT MG-63 or *CASP1*^{-/-} MG-63 cells were exposed to USA300 LAC, the deletion mutant LAC Δ *psma* θ *hld* (pTX Δ 16) and the complemented strains. Then, cell lysates were plated on BHI agar, and CFU were determined. CFU values were normalized to 10⁵ host cells.

Involvement of caspase-1 in bacterial clearance



- WT MG-63 or *CASP1*^{-/-} MG-63 cells were exposed to a fluorescent *S. aureus* SA113 strains. CFU values were normalized to 10⁵ host cells.
- WT MG-63 or *CASP1*^{-/-} MG-63 cells exposed to *S. aureus* SA113. Six hours and 24 hr post-infection cells were stained with anti-PYCARD antibody (green staining). Nuclear DNA was labeled with DAPI (blue staining). The number of viable bacteria recovered from infected cells was significantly higher in *CASP1*^{-/-} MG-63 than in WT MG-63 cells.
- Transmission electron micrographs of WT MG-63 or *CASP1*^{-/-} MG-63 cells infected with SA113 strain.

CONCLUSION

- Osteoblast-like cells induce an immune response against *S. aureus* through inflammasomes activation and processing of IL-1 β .
- The active caspase-1 restrict intracellular replication of *S. aureus* in non-professional phagocytes in addition to professional phagocytes, suggesting the pivotal role of caspase-1 in *S. aureus* clearance independently from the type of cells.
- Inflammasomes related IL-1 β production by infected osteoblast-like cells appears to be particularly dependent on PSM β among PSMs.