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Inflammasomes involvement in *Staphylococcus aureus* infection of human osteoblast-like cells MG-63

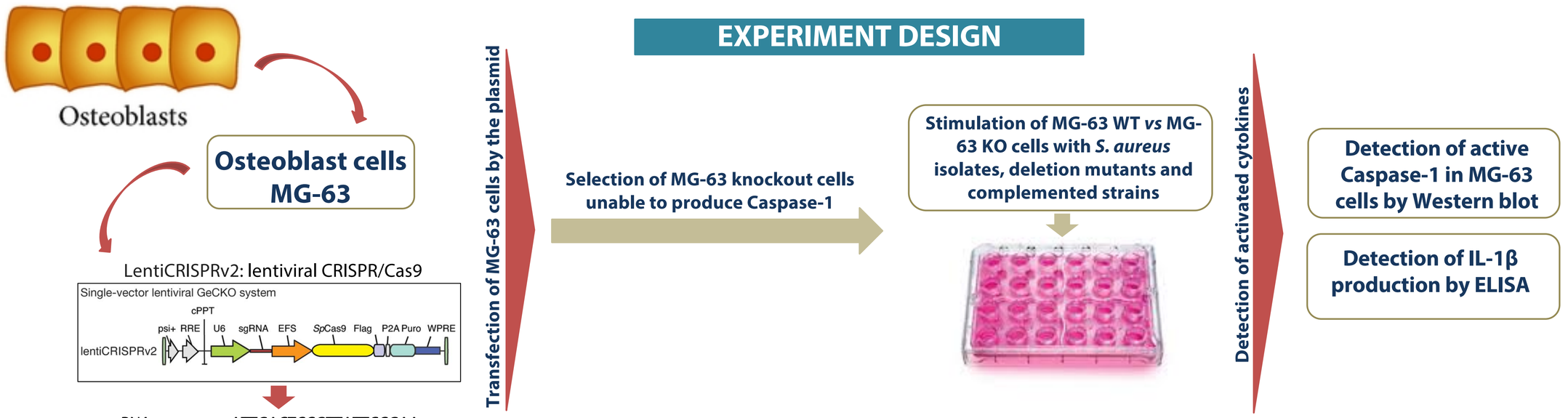
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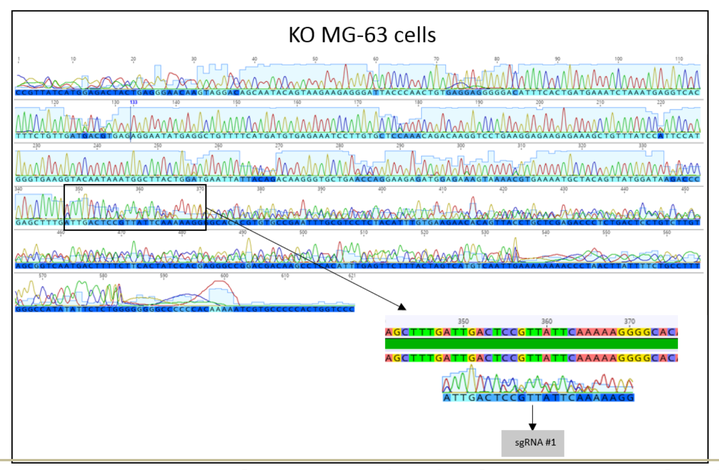
CONTEXT

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-1β, pro-IL-18, and pro-IL-33, and promote their secretion from the cell. *Staphylococcus aureus* is a gram-positive bacterium that can cause several fatal infections and is also the predominant cause of bone infections worldwide. In this study, we investigated the involvement of inflammasomes in the model of persistent infection of human osteoblast-like cells.

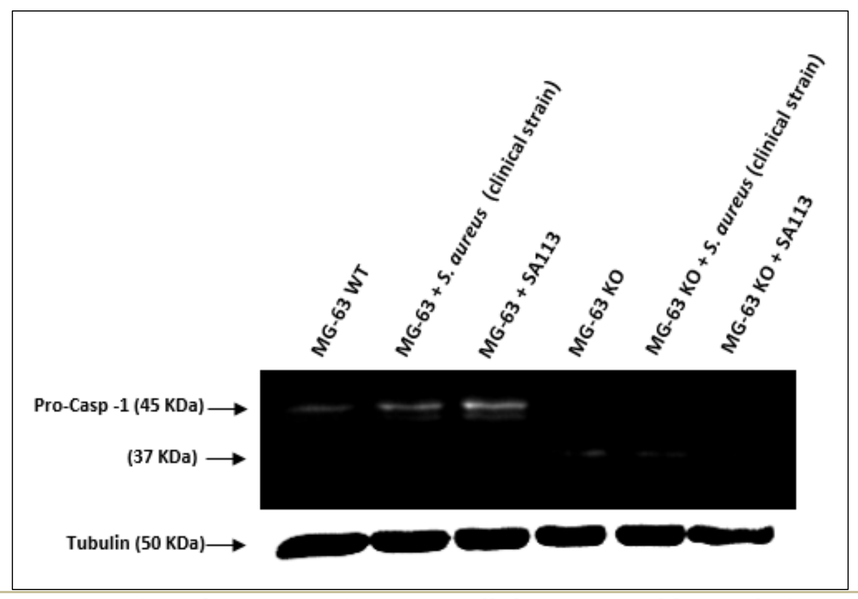
EXPERIMENT DESIGN



Results

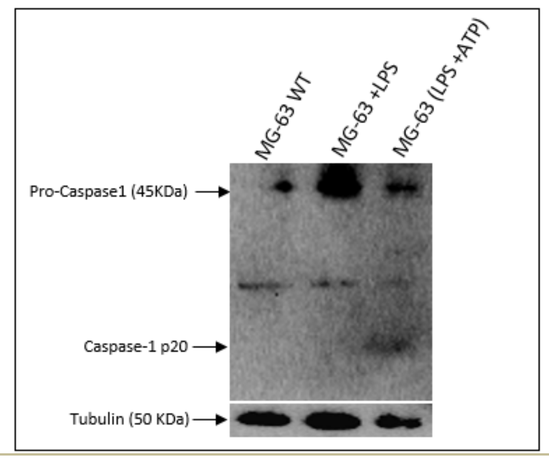


1 **KO Casp1 MG-63 shows a region in the sequence that is superimposed on the target sequence by sgRNA.**

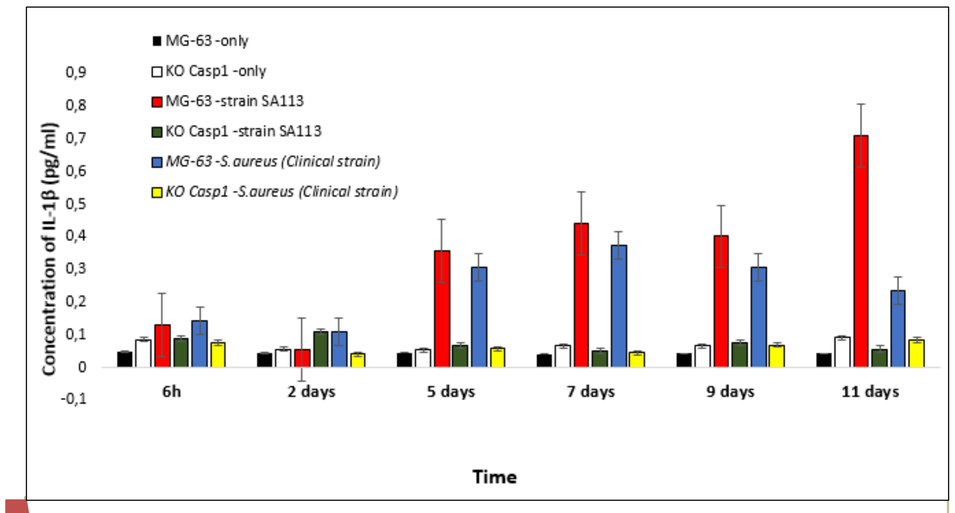


2 **Detection of Caspase-1 in MG-63 cells by Western blot analysis. The band corresponding to Pro-caspase 1 (45 KDa) was detected in MG-63 WT cells in contrast to MG-63 KO Caspase-1 cells, where 45 KDa band was absent. The results indicate that Pro-caspase-1 is not translated in MG-63 KO cells. There is a faint 37 KDa band in MG63 KO Casp1 cells that may be related to non-specific signal.**

Results



3 **Caspase-1 activation in the wild-type osteoblast-like MG-63 cells. MG-63 WT cells were primed for 3h with LPS (1µg/ml) and were stimulated or not with ATP (5mM). Extracts cells together with cell culture supernatants were immunoblotted with anti-Caspase-1 antibody. *Arrows* denote Pro-Caspase-1 and active Caspase-1 p20 subunit.**



4 **Detection of IL-1β in supernatants of MG-63 cells by ELISA. Exposure of MG-63 WT cells to *S. aureus* isolates resulted in increase of IL- 1β in their supernatants in contrast to supernatants of MG-63 KO cells. Thus, Il-1β release is likely associated to inflammasome assemblage in *S. aureus*-infected cells. Consequently Il-1β detection in supernatants of MG-63 WT *vs* MG-63 KO cells is employed to investigate inflammasome involvement during *S. aureus* infection.**

CONCLUSION

Non-phagocytic osteoblast-like MG-63 cells form inflammasomes in the response to *S. aureus* infection, however the time of inflammasomes formation was different compared to the professional phagocytes. Some virulence factors induce the inflammasomes assemblage.

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