

Cell surface receptor SLAMF5 enhances DC-autophagy via IRF8

Dóra Bencze, Zsófia Agod, Árpád Lányi

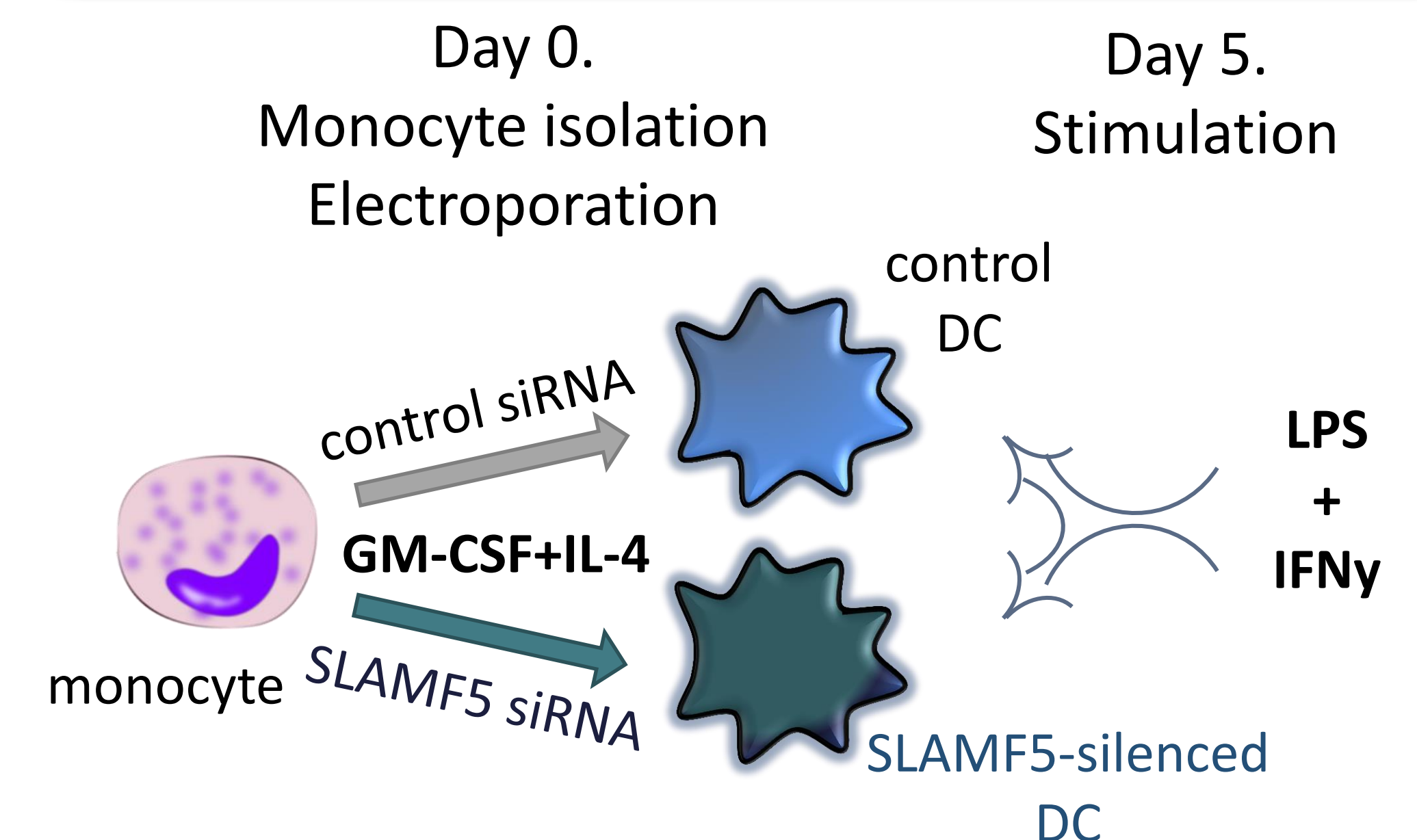
Department of Immunology, University of Debrecen,
Debrecen, Hungary

SUMMARY

Signaling lymphocyte activation molecule family (SLAMF) receptors are essential regulators of innate and adaptive immune responses. The function of **SLAMF5/CD84**, a family member with almost ubiquitous expression within the hematopoietic lineage is poorly defined. In light of the recently established link between autophagy, a degradative pathway, which plays a critical role in orchestration of the immune response and SLAMF receptors, we decided to examine the role of SLAMF5 in dendritic cell (DC) autophagy and in the cytokine production of DCs responding to inflammatory signals.

We performed RNA-interference targeting SLAMF5, IRF8 or SLAMF5 in combination with TRIM21 on freshly isolated monocytes then differentiated them into DCs. In DCs lacking SLAMF5 we found decreased autophagic flux both in the presence and absence of inflammatory stimuli. LPS/IFN γ treatment of SLAMF5-deficient DCs significantly increased production of IL-1 β and IL-23, while IL-12 secretion was diminished. Strikingly, we detected greatly reduced protein levels of the autophagy regulator **IRF8**, which could be reversed in the presence of the proteasome inhibitor MG132. Furthermore, silencing of **TRIM21**, an E3 ubiquitin ligase completely restored the level of IRF8 in SLAMF5 knock down cells. In addition, DCs made IRF8-deficient by RNA interference showed similar characteristics to those lacking SLAMF5. Taken together, data presented in this work indicate that SLAMF5 enhances autophagy and controls pro-inflammatory cytokine production of human DCs, presumably by inhibiting TRIM21 dependent proteasomal degradation of the autophagy master regulator transcription factor IRF8.

Figure 1a. Generation and activation of SLAMF5-silenced moDCs.



To study the role of SLAMF5-IRF8 interaction, we transfected freshly isolated human monocytes with SLAMF5-specific or with gene specific scrambled small interfering RNA (siRNA) as control, then differentiated them into DCs in the presence of GM-CSF and IL-4. On day 5 cells were stimulated with LPS and IFN γ or left untreated.

Figure 1b. SLAMF5-specific siRNA efficiently inhibited endogenous SLAMF5 expression.

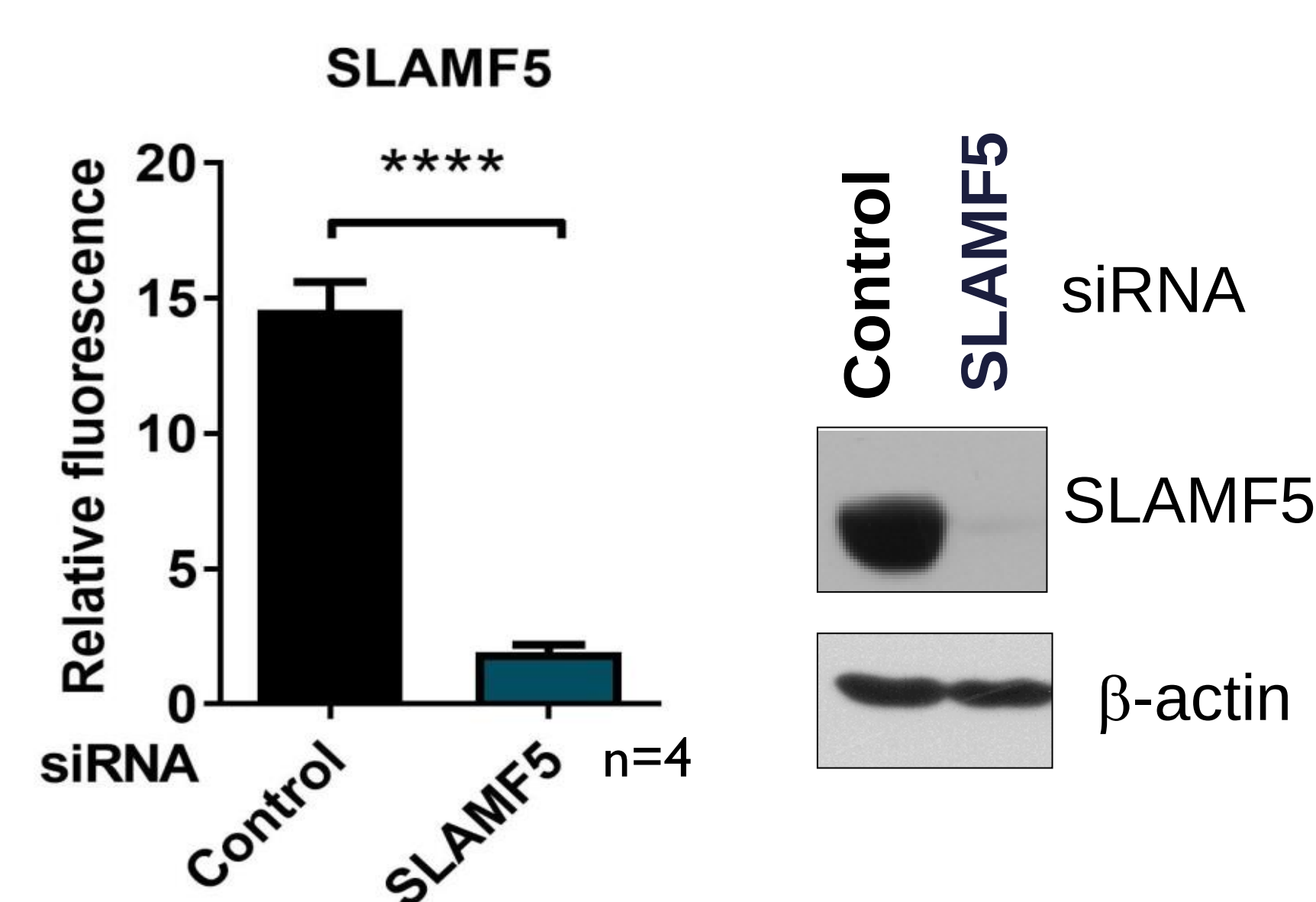


Figure 2. LPS/IFN γ -induced conversion of LC3-I to LC3-II is decreased in SLAMF5 knock down cells.

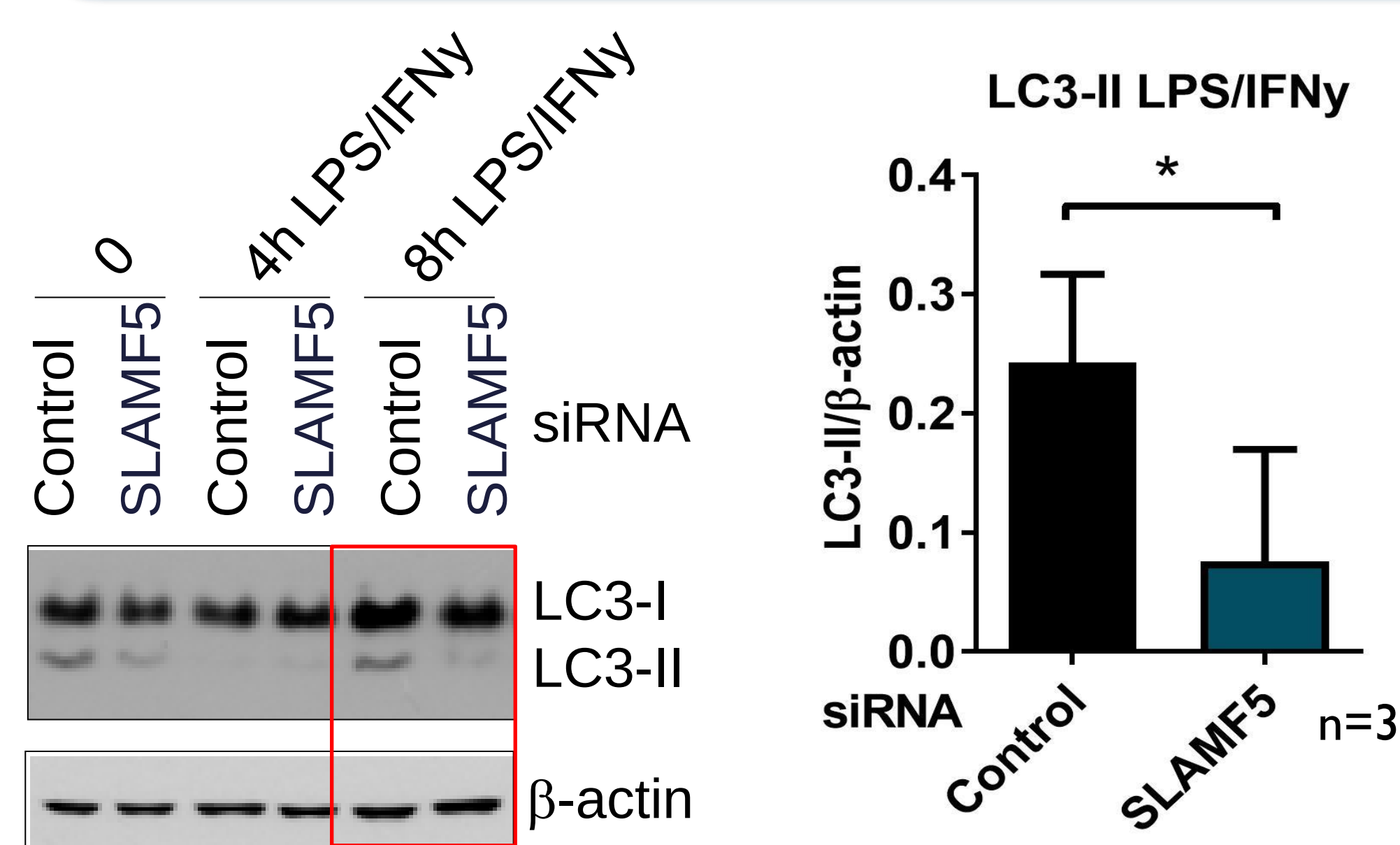


Figure 3. SLAMF5 enhances autophagy via IRF8.

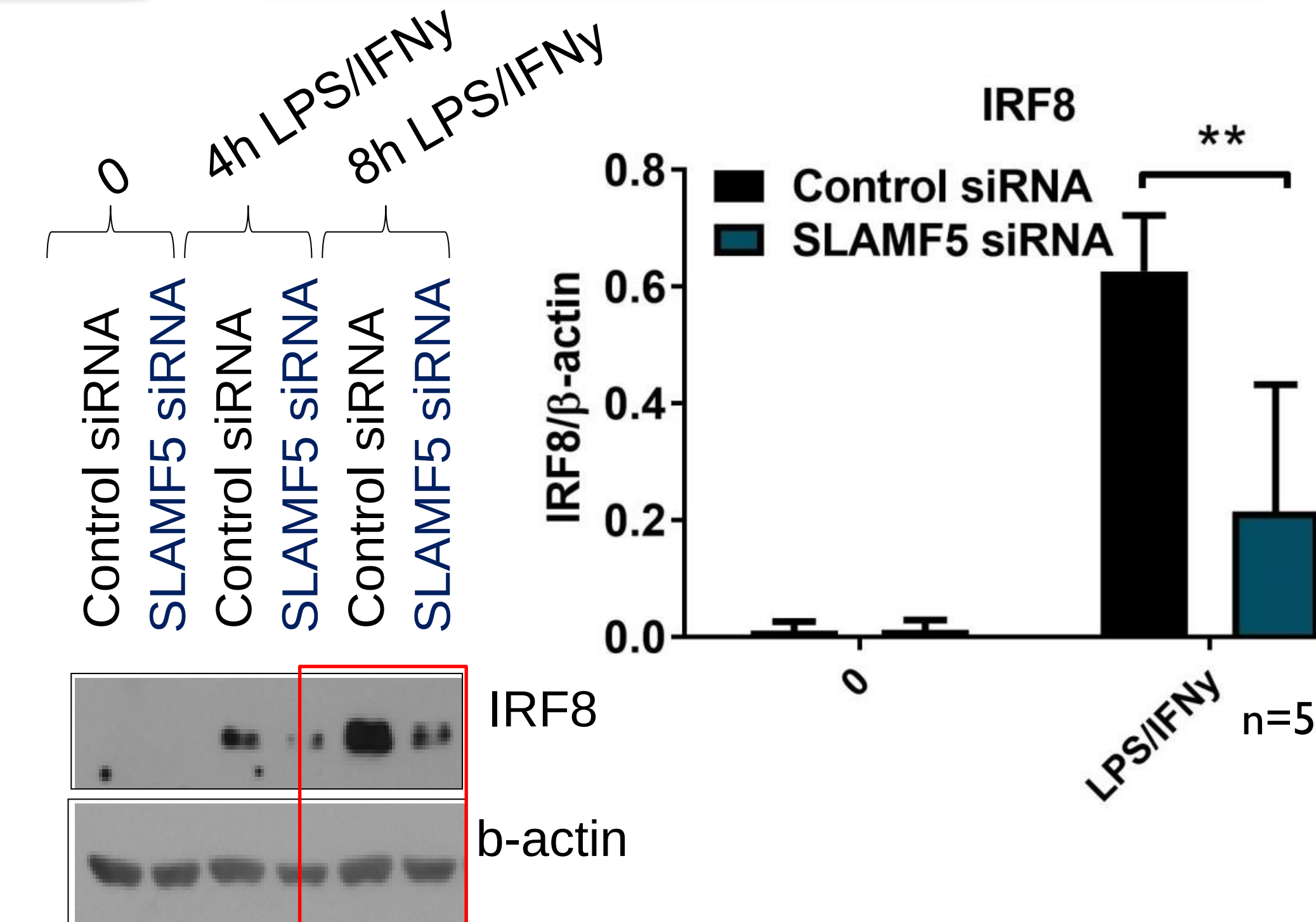


Figure 4. SLAMF5 prevents the proteosomal degradation of IRF8.

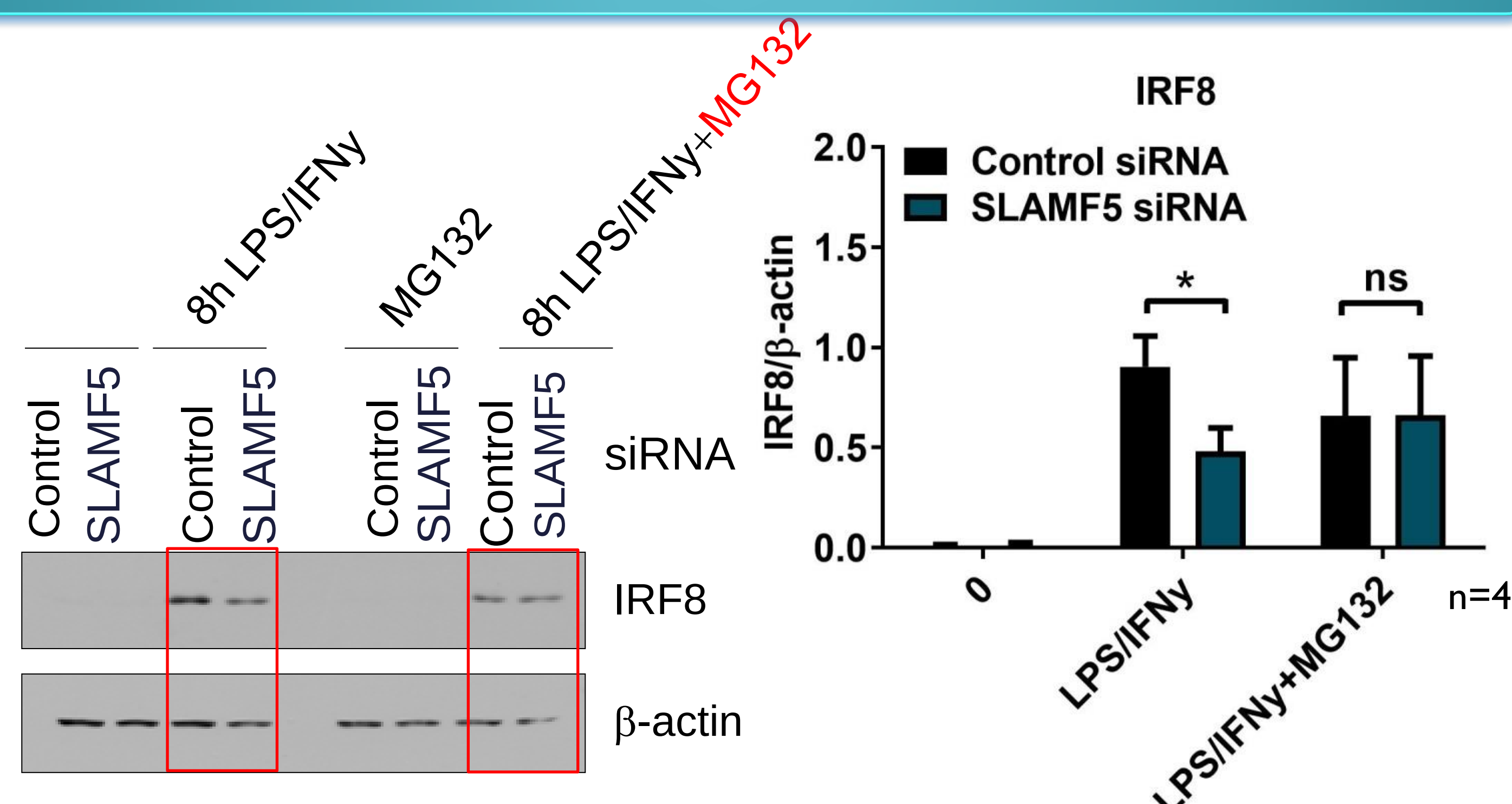


Figure 5. Degradation of IRF8 is dependent on TRIM21.

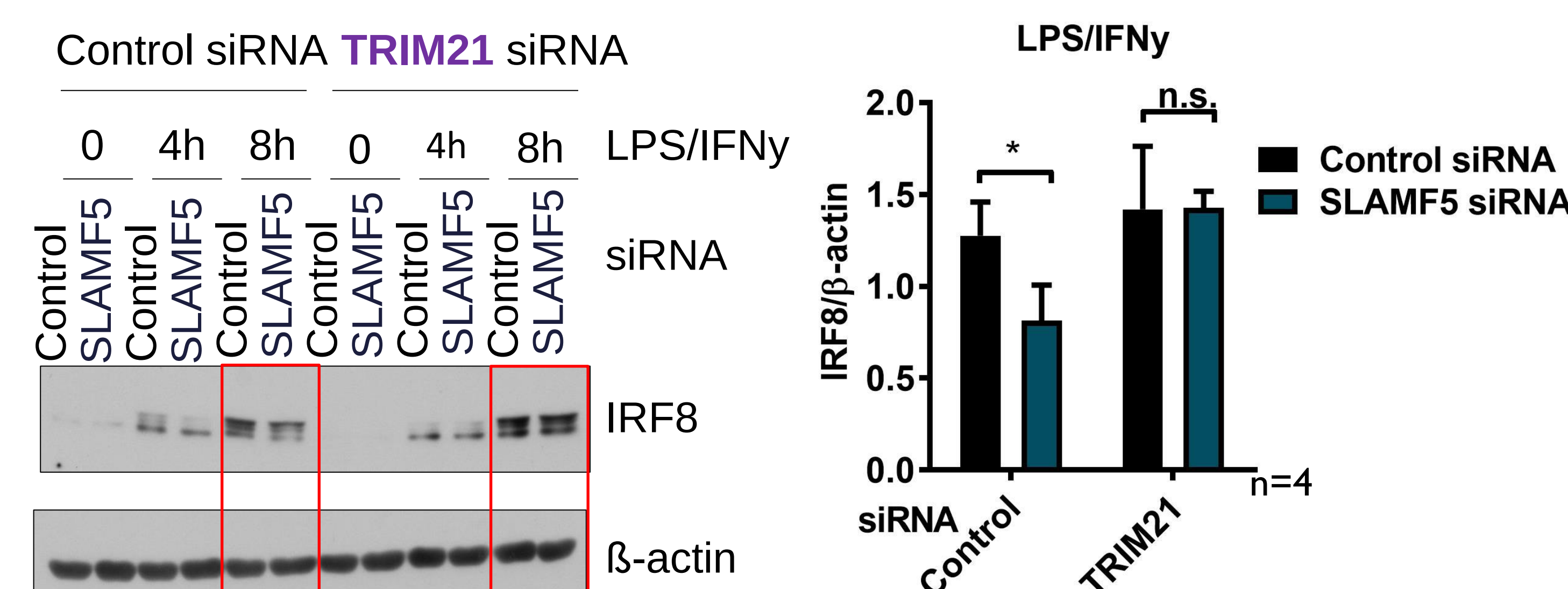


Figure 7. Overlapping changes in the cytokine secretion of SLAMF5- and IRF8- silenced moDCs.

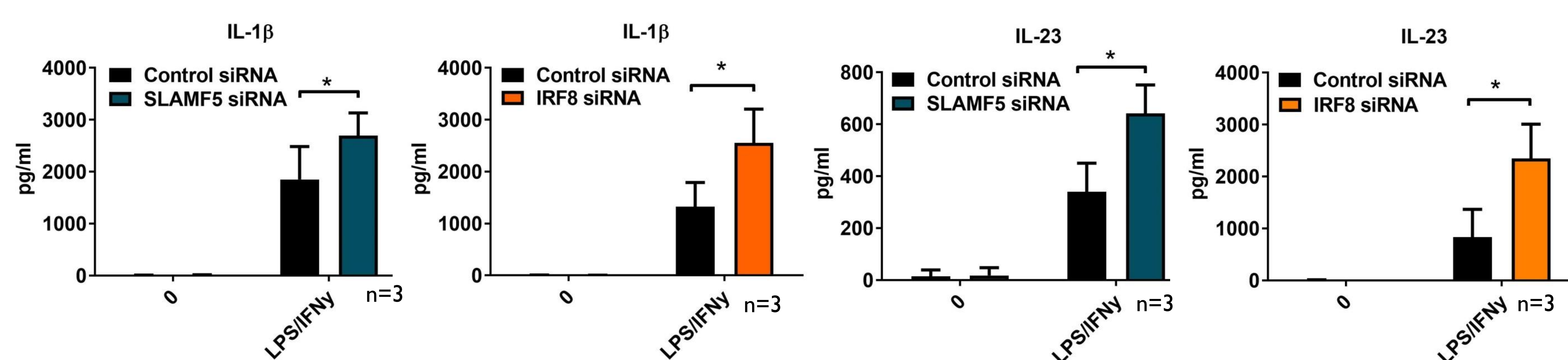


Figure 8. The SLAMF5-IRF8-TRIM21 pathway.

