Supplementary information

Supplementary materials and methods

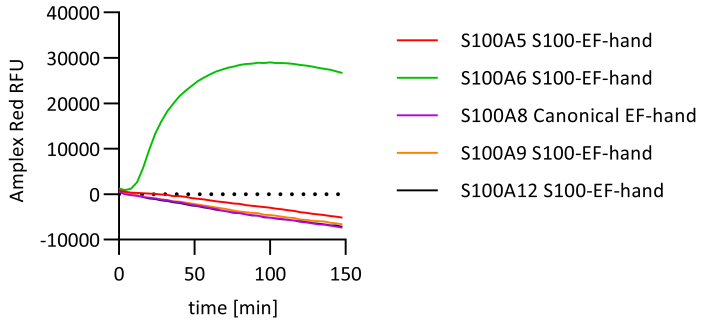
Cell-based binding assay:

Recombinant S100 proteins, anti-SIRL-1, and anti-LAIR-1 antibodies were dissolved in PBS to a final concentration of 10 µg/ml. Collagen I was dissolved in PBS with 2 mM acetic acid to a final concentration of 25 µg/ml. 150 µl of the solutions were incubated in wells of a MaxiSorp 96-well plate at 4°C overnight to allow immobilization. The next day, wells were washed three times with PBS and incubated with 1% (w/v) BSA solution in PBS for 60 min at RT, and the plate was again washed three times with PBS. K562 is a human lymphoblast cell line, which we transduced with full-length SIRL-1 analogous to the LAIR-1-overexpressing K562 cells, described previously [1]. K562 cells (wt, LAIR-1-overexpressing, and SIRL-1-overexpressing) were concentrated to $5x10^6$ cells/ml and incubated with the fluorescent dye calcein dissolved in PBS for 30 min at 37°C in a cell culture incubator. Cells were then washed three times with RPMI 1640 + 1% fetal bovine serum (FBS), and $1.5x10^5$ cells in 100 µl RPMI 1640 + 1% FBS were added to the microtiter plate and incubated for 6 h at 37°C in a cell culture incubator to allow adherence to the plate-coated proteins. After incubation, cells were washed 15 times with RPMI 1640 + 1% FBS, and calcein fluorescence was recorded (Ex/Em = 485/527 nm) in a plate reader before every wash step to measure the decrease in calcein fluorescence intensity as a consequence of cell detachment. Results are shown in supplementary figure 2.

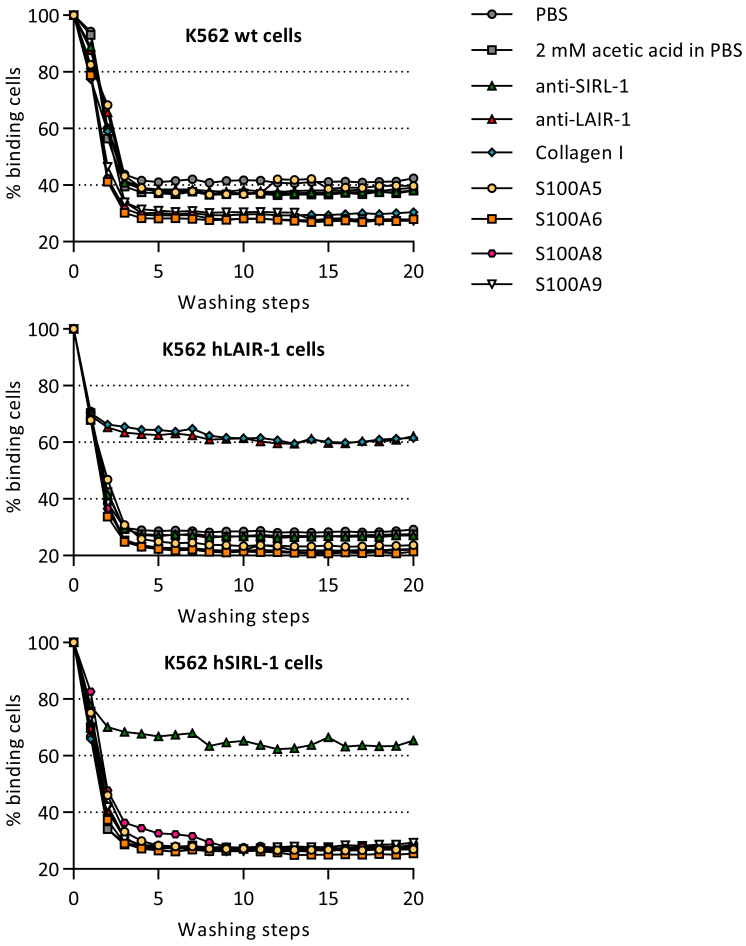
ELISA-based binding assay:

Recombinant S100 proteins were dissolved in PBS to a final concentration of 10 µg/ml. Anti-SIRL-1 (clone 1A5, own production) and anti-LAIR-1 (clone 8A8, own production) antibodies and bovine serum albumin (BSA) were dissolved in PBS to a final concentration of 5 µg/ml. 100 µl of the solutions were incubated in wells of a MaxiSorp 96-well plate at 4°C overnight to allow immobilization. The next day, wells were washed three times with PBS and incubated with 3% (w/v) BSA solution in PBS for 60 min at RT. The plate was then washed five times with PBS. Fusion proteins containing ectodomains of SIRL-1 or LAIR-1 and the Fc dimerization tag, respectively SIRL-1-Fc or LAIR-1-Fc, were added to the wells to a concentration of 10 µg/ml in PBS + 1% BSA and incubated for 2 h at RT. Wells were washed five times with PBS. Anti-human-IgG-HPR Ab was added to 0.2 µg/ml in PBS + 1% BSA and incubated for 1 h at 4°C. Wells were washed five times with PBS, and ELISA was developed with TMB substrate and stopped with 1 M H_2SO_4. Absorbance was measured at 450 nm. Results are shown in supplementary figure 3.

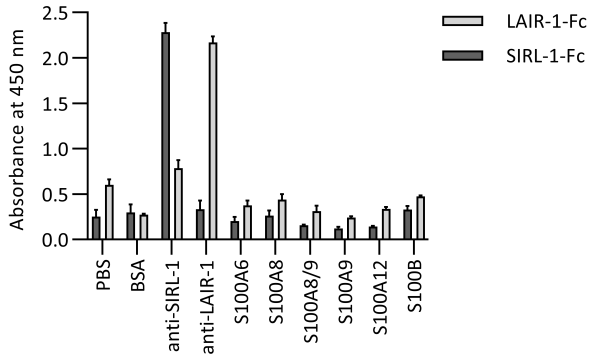
Supplementary figures



Supplementary figure 1: EF-hand motifs of selected S100 proteins were tested for induction of ROS in human neutrophils. The S100-specific EF-hand of S100 protein A6 was the only one that induced ROS. Representative traces of one experiment.



Supplementary figure 2: SIRL-1 overexpressing and control K562 cells were incubated with plate-coated S100 proteins as well as control antibodies and ligands. LAIR-1 overexpressing cells bound plate-coated collagen I and anti-LAIR-1 antibody. SIRL-1 overexpressing cells bound to plate-coated anti-SIRL-1 antibody, but no binding was observed to S100 proteins A5, A6, A8, and A9. A representative experiment is shown.



Supplementary figure 3: Recombinant SIRL-1-Fc fusion protein selectively bound to anti-SIRL-1, but not to plate-coated S100 proteins A6, A8, A8/9 heterodimer, A9, A12, or B. Recombinant LAIR-1-Fc fusion protein selectively bound to anti-LAIR-1, and was used as a control. Mean and SEM of one representative experiment are shown.

Supplementary references

1 **Lebbink, R. J., de Ruiter, T., Adelmeijer, J., Brenkman, A. B., van Helvoort, J. M., Koch, M., Farndale, R. W. et al.,** Collagens are functional, high affinity ligands for the inhibitory immune receptor LAIR-1. *J Exp Med* 2006. **203**: 1419-1425.