

Supplementary Figure 1

Homogenous Time Resolved Fluorescence (HTRF) assay of 384 bacterial crude extracts containing single clones from a DARPIn library obtained after the fourth round of selection against the extracellular domain of recombinant VCAM-1. The values are represented in arbitrary fluorescence intensity units.

Supplementary Figure 2

Screening for high affinity binders to VCAM-1 in a cell-based assay. 77 candidate binders were examined at a 1:200 dilution. A cut-off of 25% VCAM-1 positive cells was applied (red line). The red bar represents the signal from a monoclonal anti-VCAM-1 antibody used as positive control.

Supplementary Figure 3

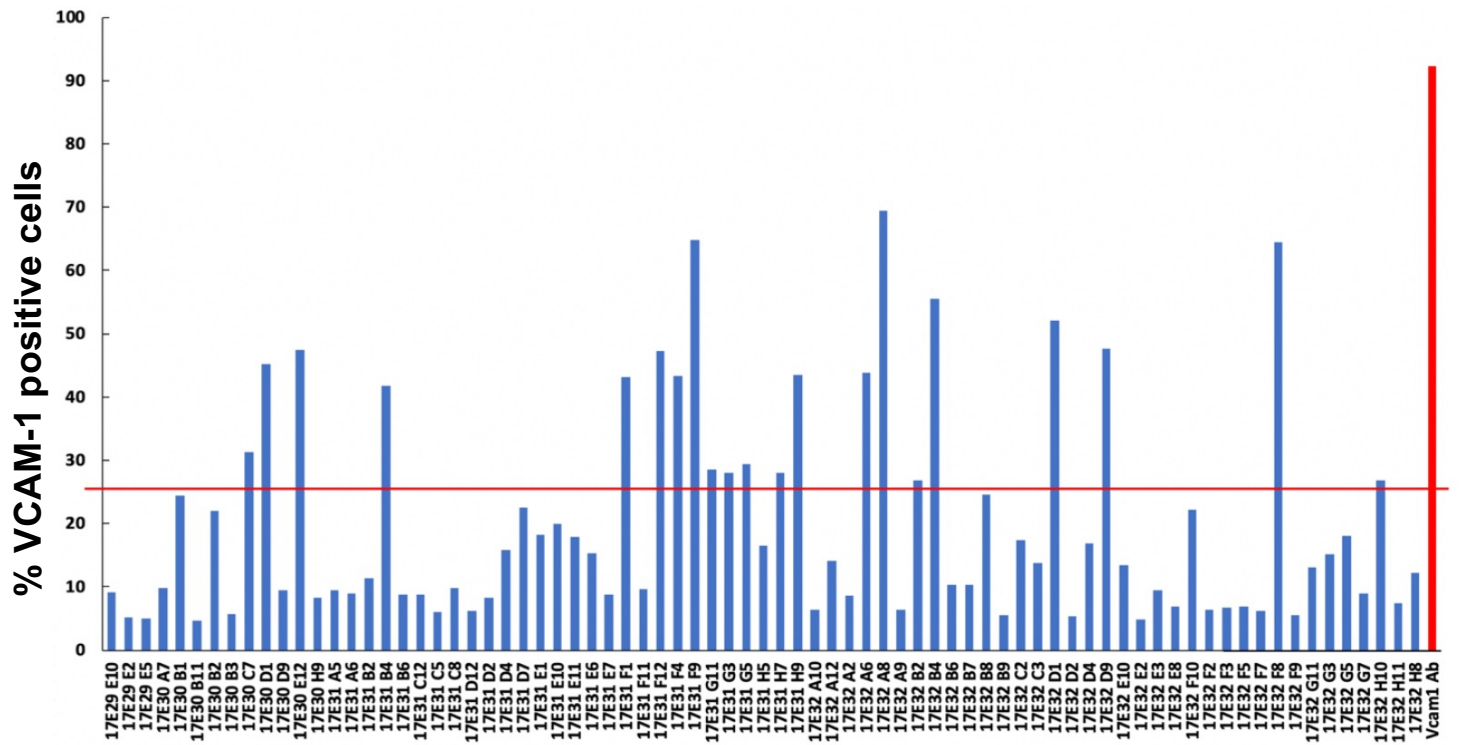
(A) SDS PAGE analysis of 5 purified DARPIn binders and an isotype control DARPIn (E3-5).

(B) SEC analysis of 5 purified DARPIns. All DARPIns except 1730_C7 were monomeric and did not show aggregation. M designates molecular mass standard.

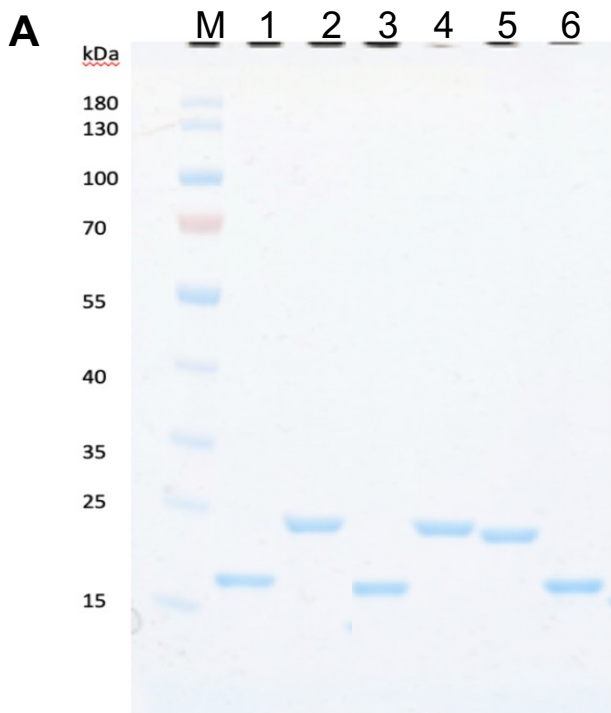
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



1 - 1730_E12 (N2C) 4 - 1732_F8 (N3C)
2 - 1731_F9 (N3C) 5 - E3_5 (N3C)
3 - 1732_D1 (N2C) 6 - 1730_C7 (N2C)

