Supporting Information

Seijsing et al. 10.1073/pnas.1417717111

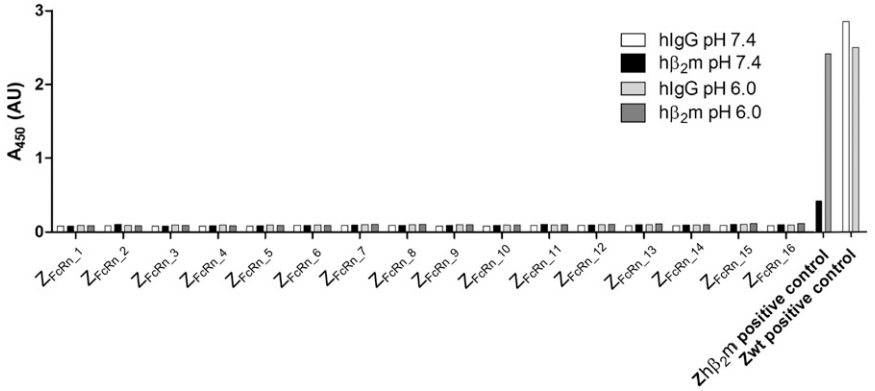

Fig. S1. Affibody specificity analysis by ELISA. An anti-ABD antibody was immobilized in the bottom of the wells and used to capture the affibody–ABD fusion proteins. Biotinylated hIgG or hβ_2m was added to the wells at pH 6.0 or 7.4, followed by addition of HRP-conjugated streptavidin and development with TMB substrate. The Zhβ_2m and Zwt positive controls were included to ascertain the function of the ELISA sandwich. The Zhβ_2m experiment used an affibody molecule binding to β_2m, taken from the initial screen after phage display selection. The Zwt experiment used the original affibody scaffold (binding IgG) fused to ABD. The *y*-axis corresponds to the A_{450} measured after development.

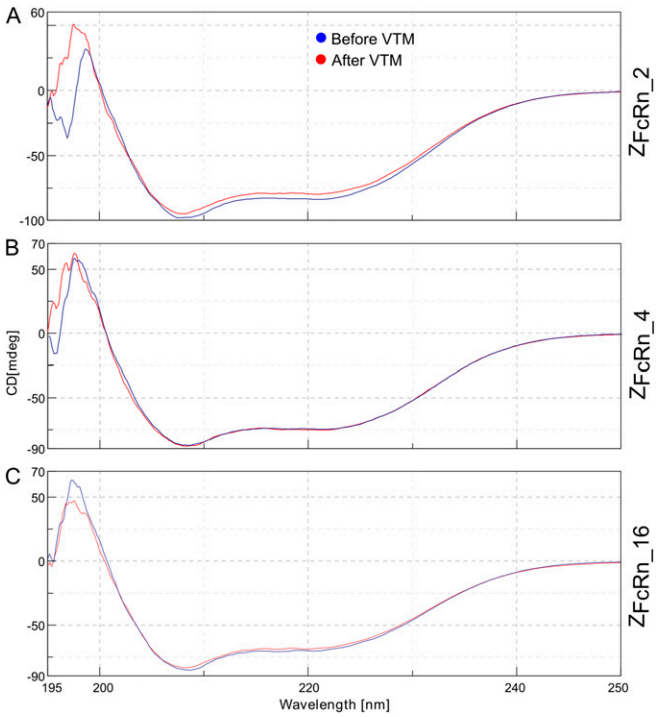

Fig. S2. Circular dichroism spectra from 250 to 195 nm, recorded before and after VTM for Z_{FcRn_2} (*A*), Z_{FcRn_4} (*B*), and Z_{FcRn_16} (*C*). VTM consisted of thermal unfolding of the proteins by heating to 90 °C.

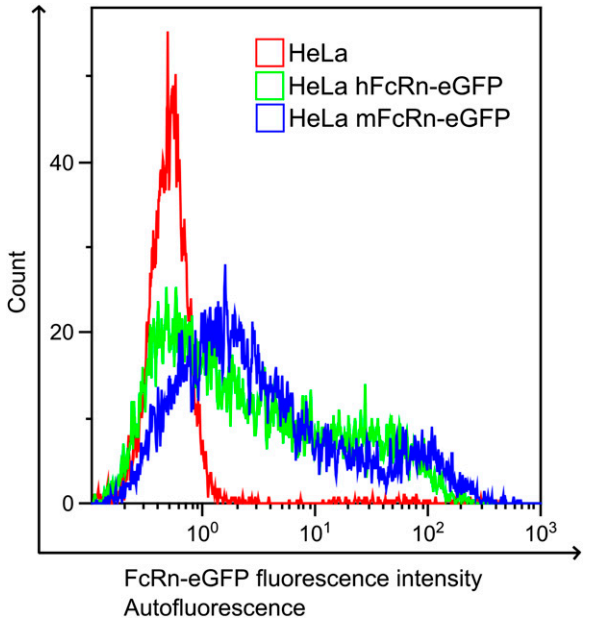

Fig. S3. Flow cytometry analysis of HeLa cells. The *x*-axis corresponds to an FcRn-eGFP/autofluorescence signal recorded in FL-1, and the *y*-axis corresponds to cell count. HeLa cells (red), HeLa cells expressing hFcRn-eGFP (green), and HeLa cells expressing mFcRn-eGFP (blue) were analyzed.

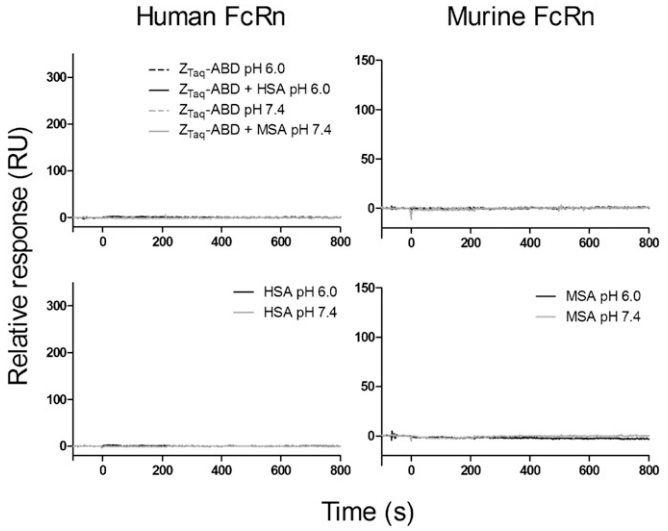

Fig. S4. Surface plasmon resonance analysis of the interaction between the control protein Z_{Taq}-ABD or SA and $FcRn_{ECD}$. Z_{Taq}-ABD was injected with or without previous incubation for 4 h with SA at pH 6.0 or 7.4. HSA or MSA was used, depending on the origin of $FcRn_{ECD}$. The binding of HSA and MSA to human or murine $FcRn_{ECD}$ was also analyzed at pH 6.0 and 7.4.

Table S1. Phage display selection parameters

Parameter	Selection track 1	2	3	4
Selection round 1				
Blocking	HSA	HSA	—	—
Target concentration, nM	100	100	100	100
Washes*	2	2	2	2
Elution, pH	8.0	2.2	8.0	2.2
Selection round 2				
Blocking	HSA	HSA	—	—
Target concentration, nM	50	50	50	50
Washes*	5	5	5	5
Elution, pH	8.0	2.2	8.0	2.2
Selection round 3				
Blocking	HSA	HSA	—	—
Target concentration, nM	25	25	25	25
Washes*	8	8	8	8
Elution, pH	8.0	2.2	8.0	2.2
Selection round 4				
Blocking	HSA	HSA	—	—
Target concentration, nM	10	10	10	10
Washes*	12	12	12	12
Elution, pH	8.0	2.2	8.0	2.2

*Number of washes of 2 min each.

Table S2. Protein sequences

Library*	VDAKYAKEXXXAXXEIXXLPNLTXXQXXAFIXKLXDDPSQSSELLSEAKKLNDSQAPK
Z_{FcRn_2}†	--------QDA-AH--RW-----FD-RV---H--A----------
Z_{FcRn_16}†	--------WMR-AH--RW-----FD-RV---H--E----------
Z_{FcRn_4}†	--------FES-AH--RW-----YD-RV---H--S----------

*Amino acid sequence of the library. X denotes varied positions in the library.
†"-" indicates identity with the library sequence.