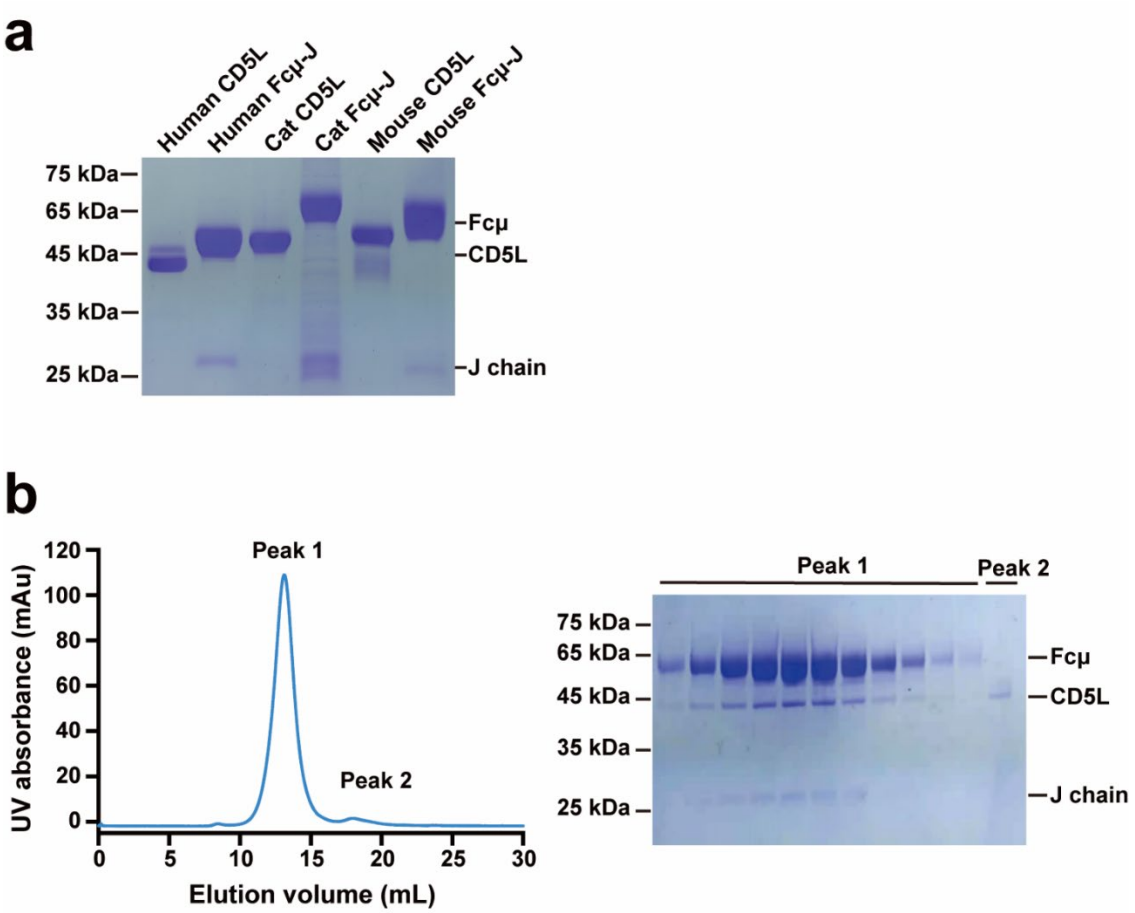
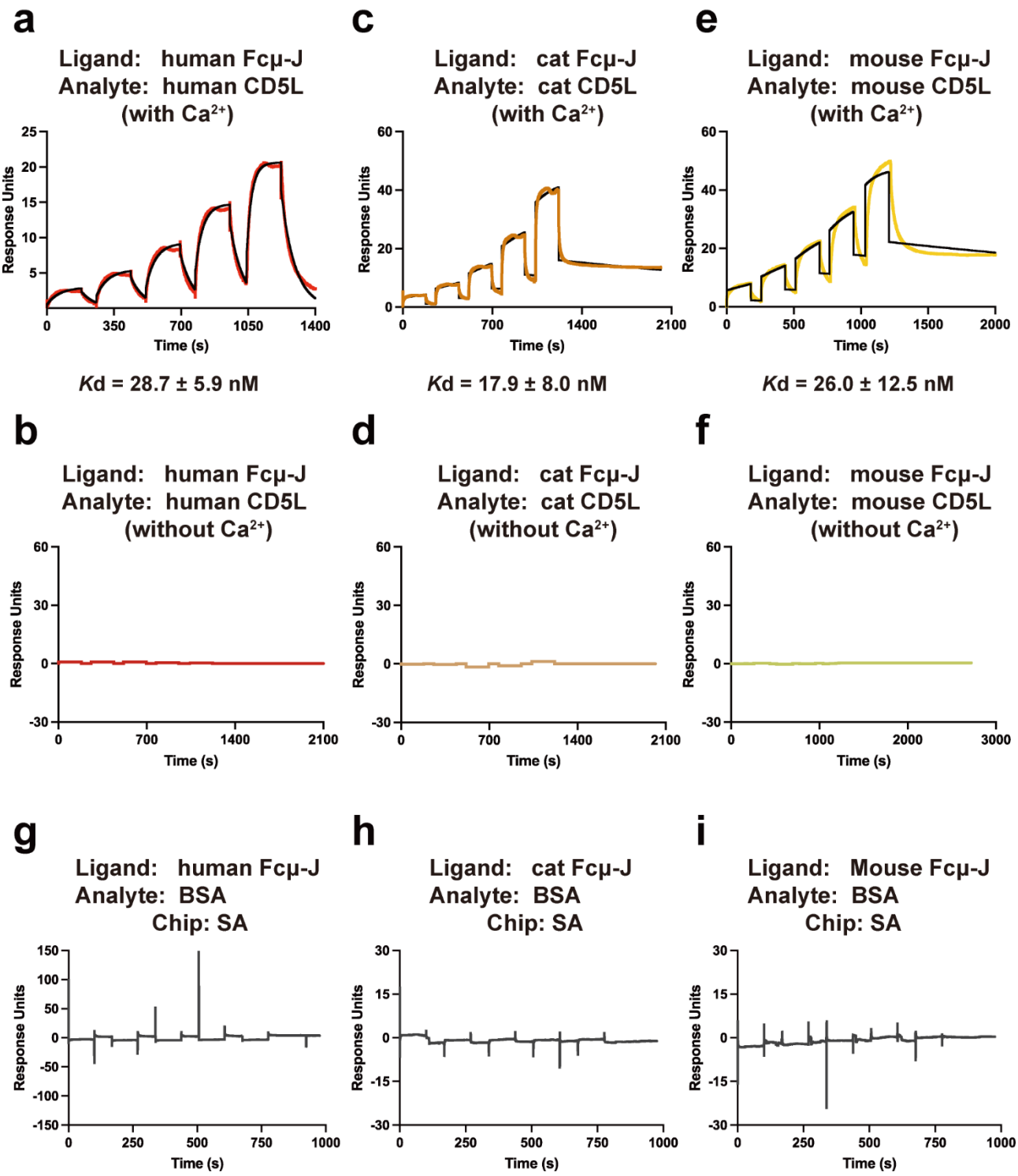


Supplementary Figure 1. Purification of the CD5L, Fcμ–J and Fcμ–J–CD5L.

a. SDS–PAGE analyses of the purified CD5L and Fcμ–J form human, mouse and cat.

b. Size-exclusion chromatography of the Fcμ–J–CD5L complex on a Superose 6 Increase column and SDS–PAGE analyses.



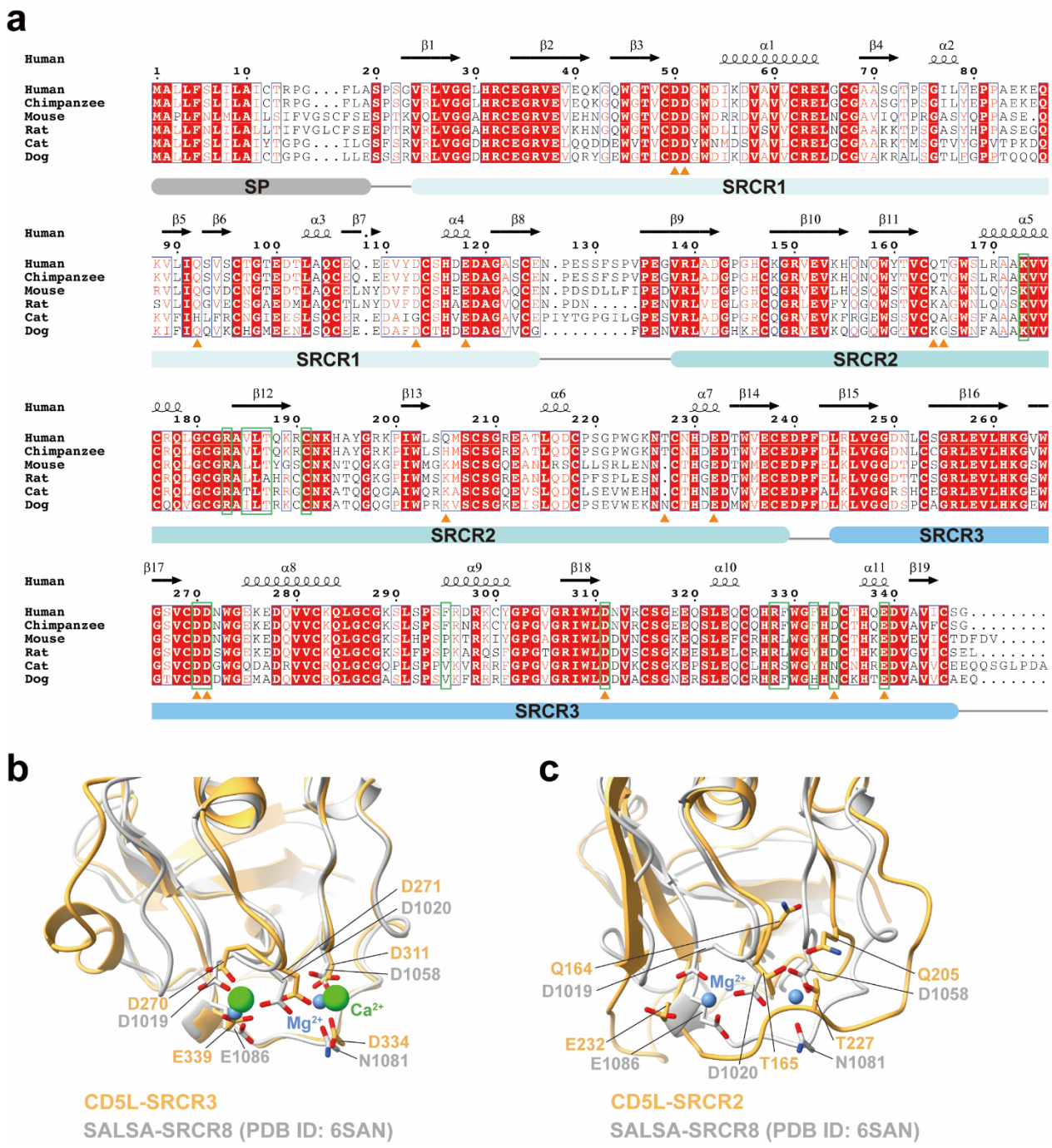
Supplementary Figure 2. SPR analyses of the Fcμ–J–CD5L interactions.

a-b. Fcμ–J was immobilized on a CM5 chip via amine coupling, and twofold serial dilutions of human CD5L (50 nM to 3.125 nM) were flowed over the chip for SPR measurement. No significant interaction between human CD5L and Fcμ–J is detected in the presence of EDTA.

c–d. Twofold serial dilutions of cat CD5L (200 nM to 12.5 nM) binds to immobilized cat Fcμ-J on CM5 chip, and no binding is detected in presence of EDTA.

e-f. Twofold serial dilutions of mouse CD5L (200 nM to 12.5 nM) binds to immobilized mouse Fcμ-J on CM5 chip, and no binding is detected in presence of EDTA.

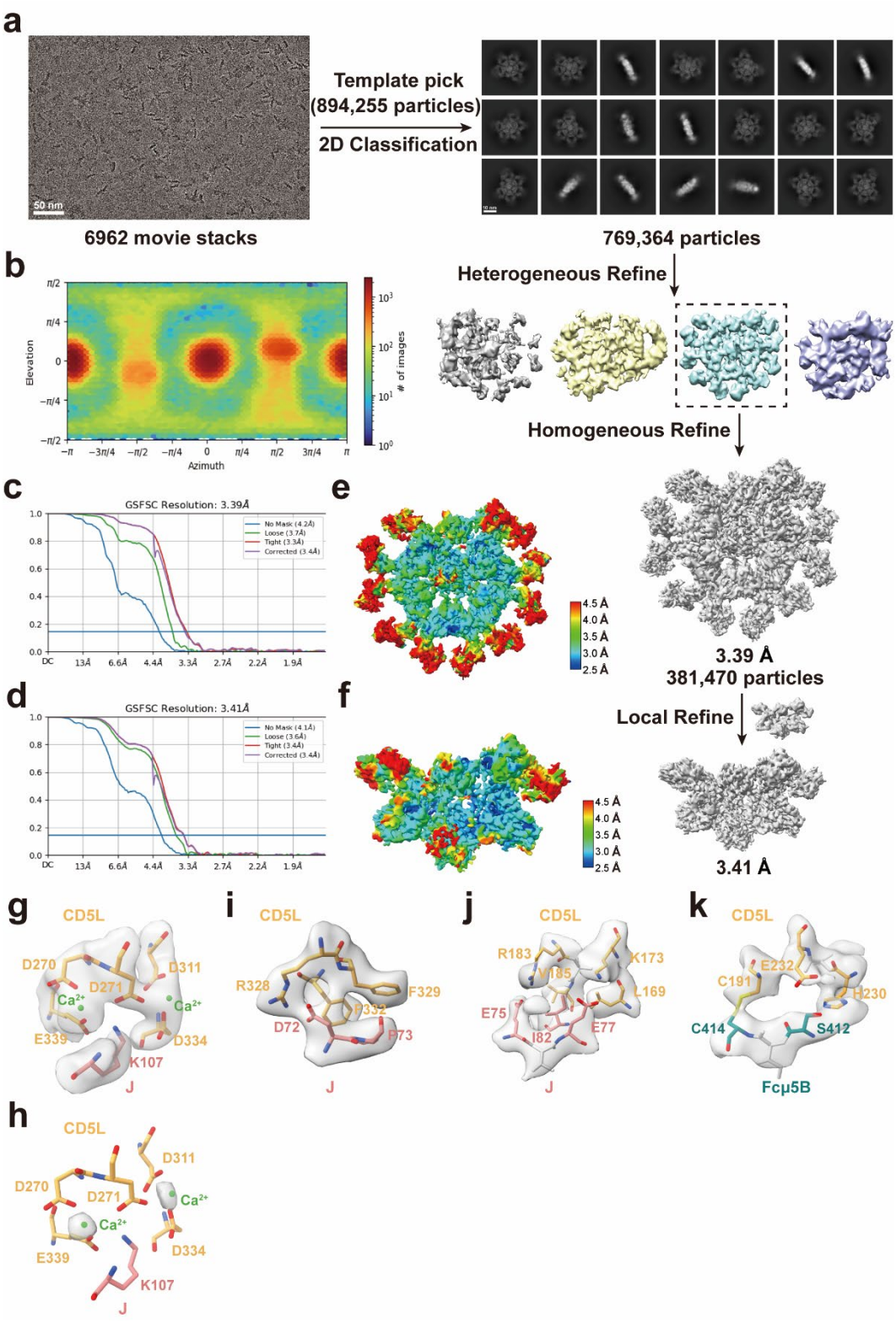
g-i. No significant interaction between BSA and Fcμ–J is detected.



Supplementary Figure 3. Sequence and structural analyses of CD5L.

a. Sequence alignment of CD5L from human and other animals. Residues in human CD5L that are involved in binding to Fcμ–J are highlighted in green boxes. The signal peptide (SP) is depicted in grey. Three SRCR domains are shown in light cyan, light blue and blue, respectively. Putative Ca^{2+}-binding sites in each SRCR domain are denoted by orange triangles.

b-c. Structural comparison of CD5L-SRCR3 (b) and SRCR2 (c) with SALSA-SRCR8 (PDB ID: 6SAN). SALSA-SRCR8 is displayed in grey. Ca^{2+} ions (green) in CD5L and Mg^{2+} ions (blue) in SALSA are represented as spheres.



Supplementary Figure 4. Cryo-EM 3D reconstruction of the Fcμ–J–CD5L complex.

a. Flowchart of cryo-EM data processing.

b. Angular particle distribution heat map.

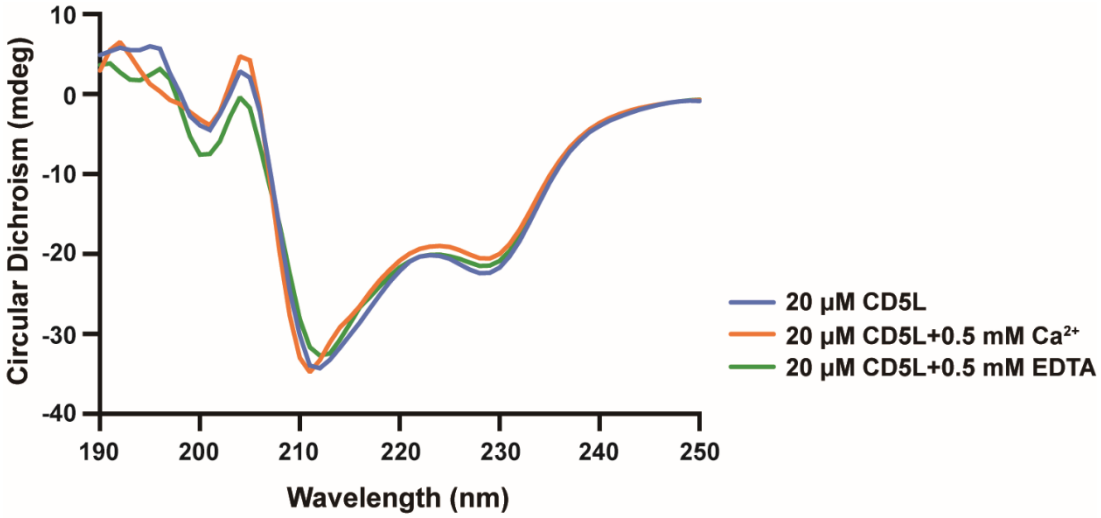
c-d. Gold-standard Fourier shell correlation (GSFSC) curves of the global and local Fcμ–J–CD5L complex, respectively.

e-f. Resolution estimations for the final maps of the global and local Fcμ–J–CD5L complex, respectively.

g-h. EM density of the DDE/D motif and two Ca^{2+} ions in CD5L-SRCR3. The EM density contour level for the DDE/D motif (g) is 0.18, while that for the two calcium ions (h) is 0.45.

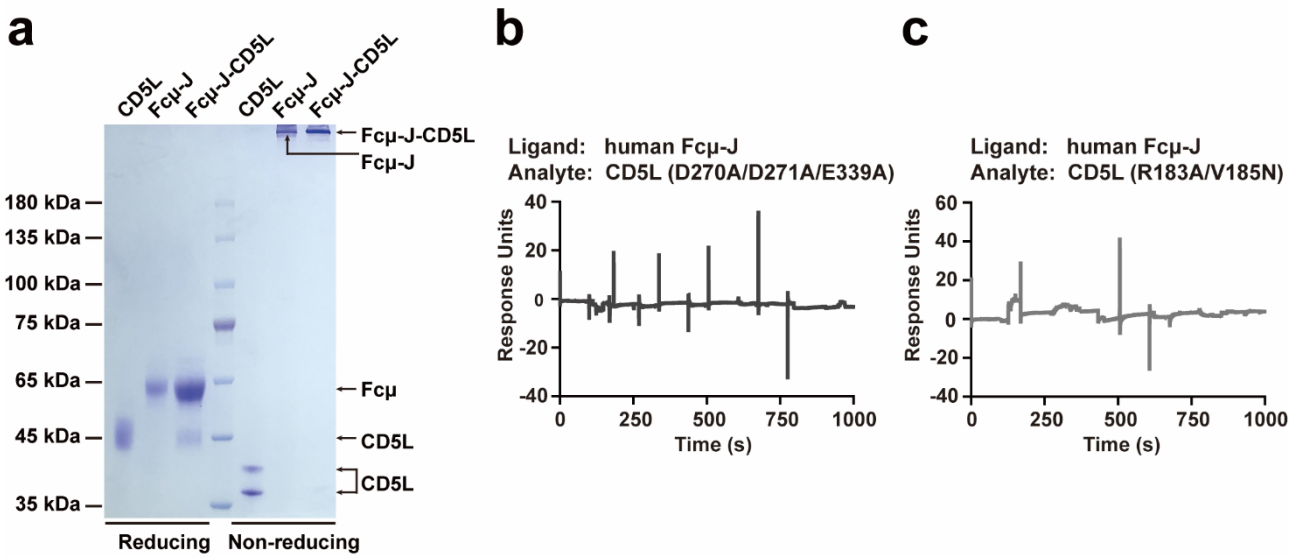
i-j. EM densities of the interface between the SRCR2–SRCR3 junction of CD5L and the β5–β6 hairpin of the J chain. The EM density contour levels for (i) and (j) are both 0.13.

k. EM density of the interface between CD5L-SRCR2 and Fcμ5B. The EM density contour level is 0.13.



Supplementary Figure 5. Circular dichroism (CD) spectroscopy analyses of CD5L.

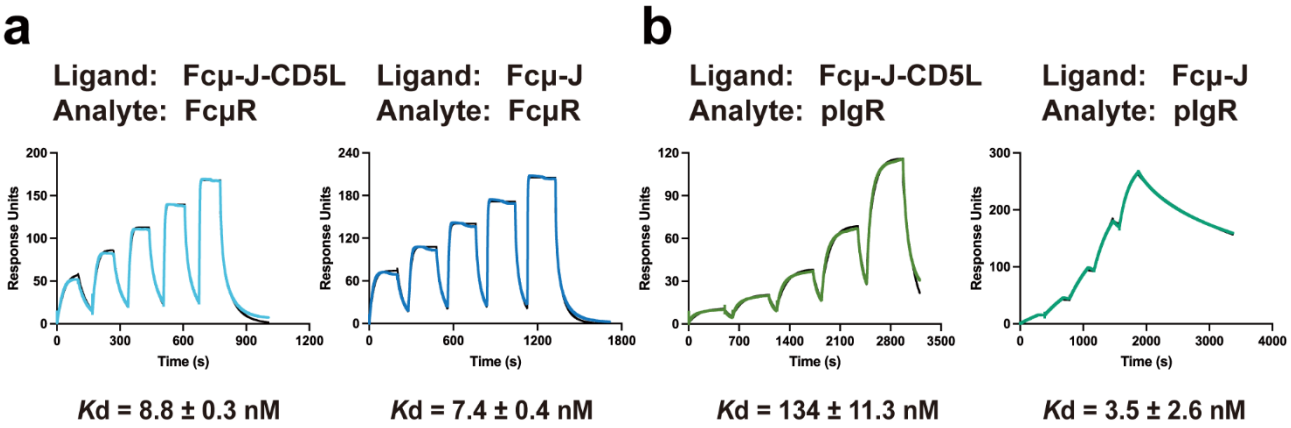
The CD spectroscopy curves for CD5L are measured in three conditions: alone (blue), with Ca^{2+} (orange), and with EDTA (green).



Supplementary Figure 6. Non-reducing SDS-PAGE and SPR analyses.

a. Reducing and non-reducing SDS-PAGE analyses of the CD5L, Fcμ–J and Fcμ–J–CD5L samples.

b-c. SPR analyses of the interactions between immobilized human Fcμ–J and two CD5L mutants. Serial dilutions of CD5L mutants (from 200 nM to 12.5 nM) were passed through immobilized Fcμ–J on the SA chip in the presence of Ca^{2+}.

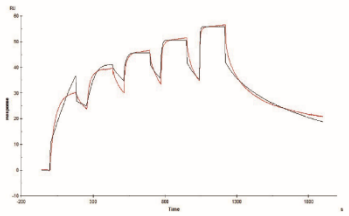


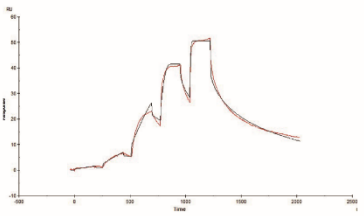
Supplementary Figure 7. SPR analyses of FcμR-D1 or pIgR-SC binding to immobilized Fcμ–J or Fcμ–J–CD5L.

a. SPR analyses of the interactions between immobilized Fcμ–J or Fcμ–J–CD5L and FcμR-D1. Fcμ–J–CD5L and Fcμ–J were immobilized on the CM5 chip via amine coupling. The binding studies were conducted by passing twofold serial dilutions of purified FcμR-D1 (from 100 nM to 6.25 nM) over the immobilized Fcμ–J–CD5L (left panel) or Fcμ–J (right panel).

b. SPR analyses of the interactions between immobilized Fcμ–J or Fcμ–J–CD5L and pIgR-SC. The binding studies were conducted by passing twofold serial dilutions of pIgR-SC (from 100 nM to 6.25 nM) over the immobilized Fcμ–J–CD5L (left panel) or Fcμ–J (right panel).

Chip: CM5
Ligand: Human Fcμ–J
Analyte: Human CD5L (with Ca^{2+})

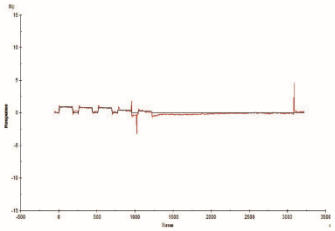




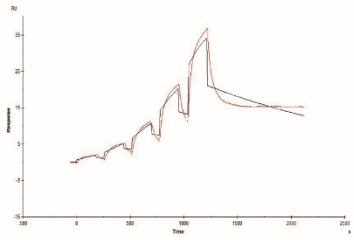
*K*d = 2.2 ×10^{-8} M
Chi^2 = 3.4

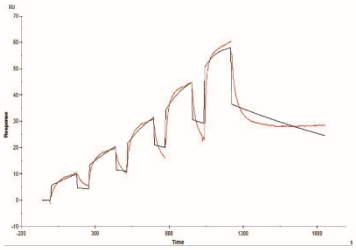
*K*d = 3.4 ×10^{-8} M
Chi^2 = 1.2

Chip: CM5
Ligand: Human Fcμ–J
Analyte: Human CD5L (without Ca^{2+})



Chip: CM5
Ligand: Mouse Fcμ–J
Analyte: Mouse CD5L (with Ca^{2+})

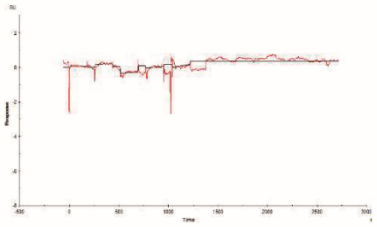




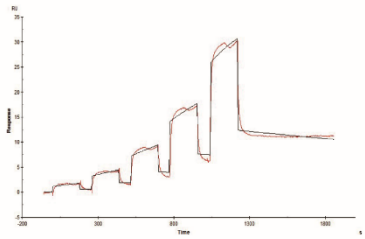
*K*d = 1.3 ×10^{-8} M
Chi^2 = 4.6

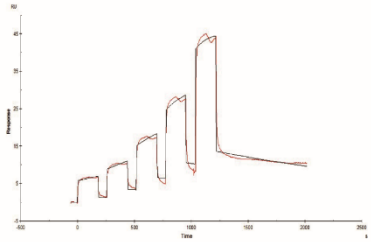
*K*d = 2.7 ×10^{-8} M
Chi^2 = 8.8

Chip: CM5
Ligand: Mouse Fcμ–J
Analyte: Mouse CD5L (without Ca^{2+})



Chip: CM5
Ligand: Cat Fcμ–J
Analyte: Cat CD5L (with Ca^{2+})

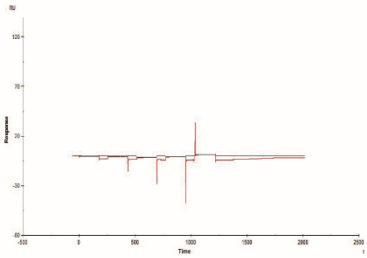




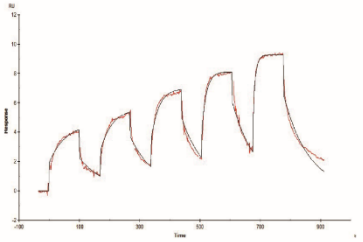
*K*d = 1.6 ×10^{-8} M
Chi^2 = 1.3

*K*d = 2.7 ×10^{-8} M
Chi^2 = 1.8

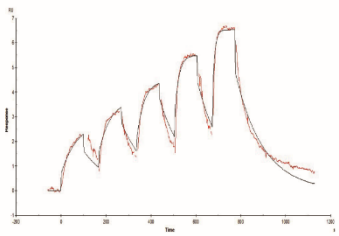
Chip: CM5
Ligand: Cat Fcμ–J
Analyte: Cat CD5L (without Ca^{2+})



Chip: SA
Ligand: Human Fcμ–J
Analyte: Human CD5L (with Ca^{2+})

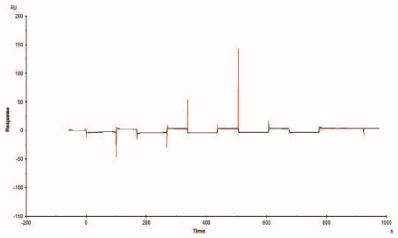


*K*d = 2.2 ×10^{-8} M
Chi^2 = 0.1

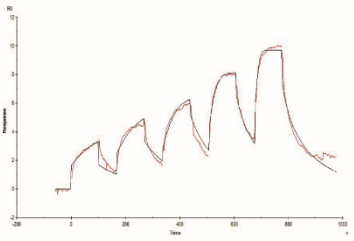


*K*d = 1.8 ×10^{-8} M
Chi^2 = 0.1

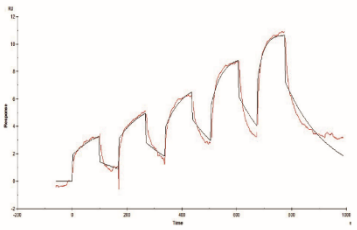
Chip: SA
Ligand: Human Fcμ–J
Analyte: BSA (with Ca^{2+})



Chip: SA
Ligand: Mouse Fcμ–J
Analyte: Mouse CD5L (with Ca^{2+})

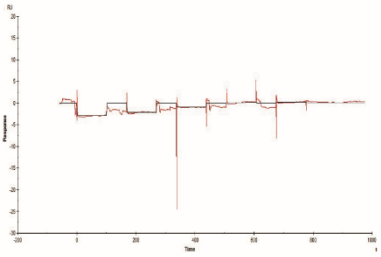


*K*d = 2.8 ×10^{-8} M
Chi^2 = 0.2

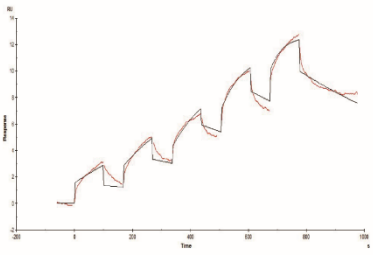


*K*d = 3.2 ×10^{-8} M
Chi^2 = 0.3

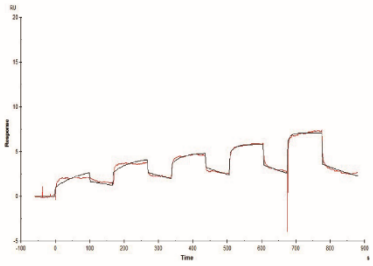
Chip: SA
Ligand: Mouse Fcμ–J
Analyte: BSA (with Ca^{2+})



Chip: SA
Ligand: Cat Fcμ–J
Analyte: Cat CD5L (with Ca^{2+})

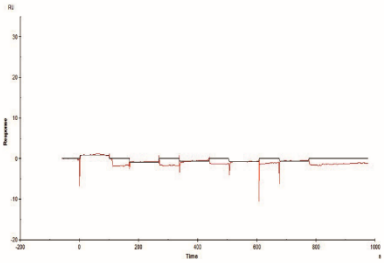


*K*d = 1.2 ×10^{-8} M
Chi^2 = 0.2

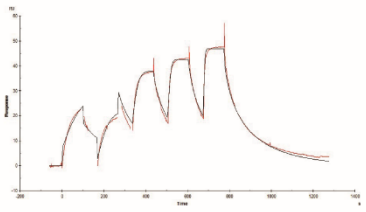


*K*d = 7.4 ×10^{-9} M
Chi^2 = 0.2

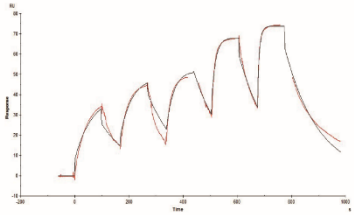
Chip: SA
Ligand: Cat Fcμ–J
Analyte: BSA (with Ca^{2+})



Chip: SA
Ligand: Human Fcμ–J
Analyte: Human CD5L C191S

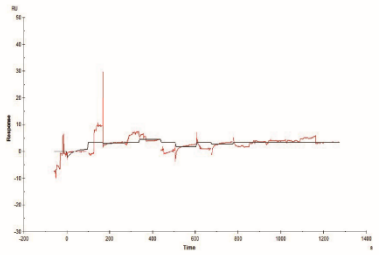


*K*d = 1.1 ×10^{-8} M
Chi^2 = 1.6

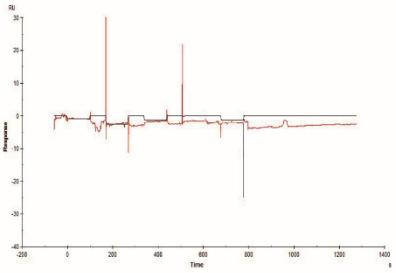


*K*d = 1.4 ×10^{-8} M
Chi^2 = 6.7

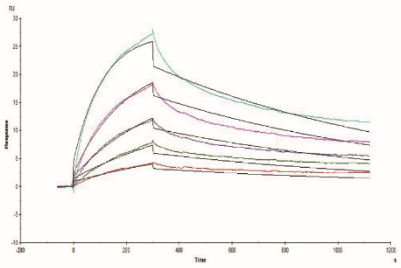
Chip: SA
Ligand: Human Fcμ–J
Analyte: Human CD5L (R183A/V185N)



Chip: SA
Ligand: Human Fcμ–J
Analyte: Human CD5L (D270A/D271A/E339A)

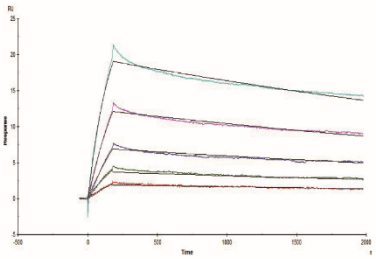


Chip: CM5
Ligand: FcμR
Analyte: Fcμ–J–CD5L



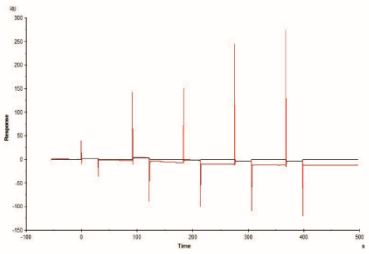
*K*d = 4.7 ×10^{-10} M
Chi^2 = 0.7

Chip: CM5
Ligand: FcμR
Analyte: Fcμ–J

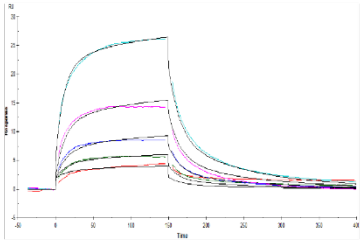


*K*d = 1.2 ×10^{-10} M
Chi^2 = 0.1

Chip: CM5
Ligand: FcμR
Analyte: BSA

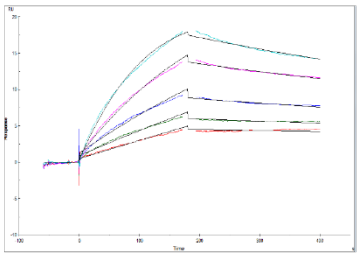


Chip: SA
Ligand: pIgR
Analyte: Fcμ–J–CD5L
Two-state reaction model



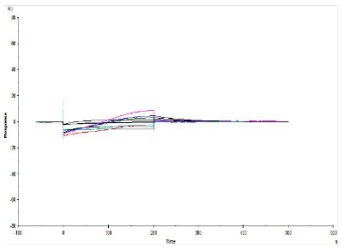
*K*d = 4.1 ×10^{-7} M
Chi² = 0.4

Chip: SA
Ligand: pIgR
Analyte: Fcμ–J

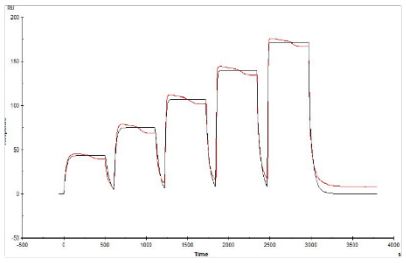


*K*d = 6.4 ×10^{-9} M
Chi² = 0.06

Chip: SA
Ligand: pIgR
Analyte: BSA

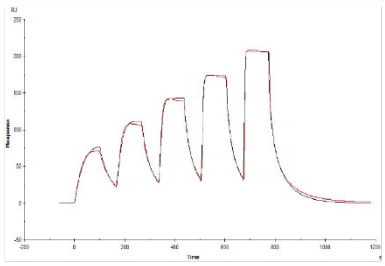


Chip: CM5
Ligand: Fcμ–J–CD5L
Analyte: FcμR



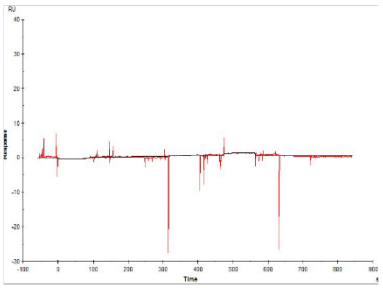
*K*d = 8.6 × 10^{-9} M
Chi² = 11.1

Chip: CM5
Ligand: Fcμ–J
Analyte: FcμR

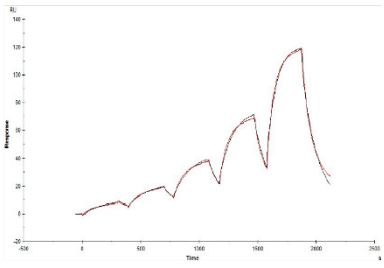


*K*d = 7.7 × 10^{-9} M
Chi² = 10.4

Chip: CM5
Ligand: Fcμ–J
Analyte: BSA

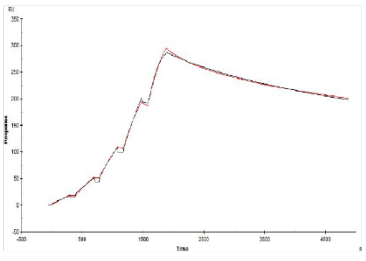


Chip: CM5
Ligand: Fcμ–J–CD5L
Analyte: pIgR



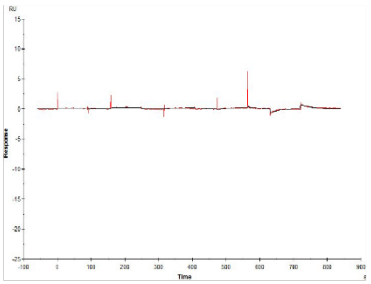
*K*d = 1.3 × 10^{-7} M
Chi² = 3.0

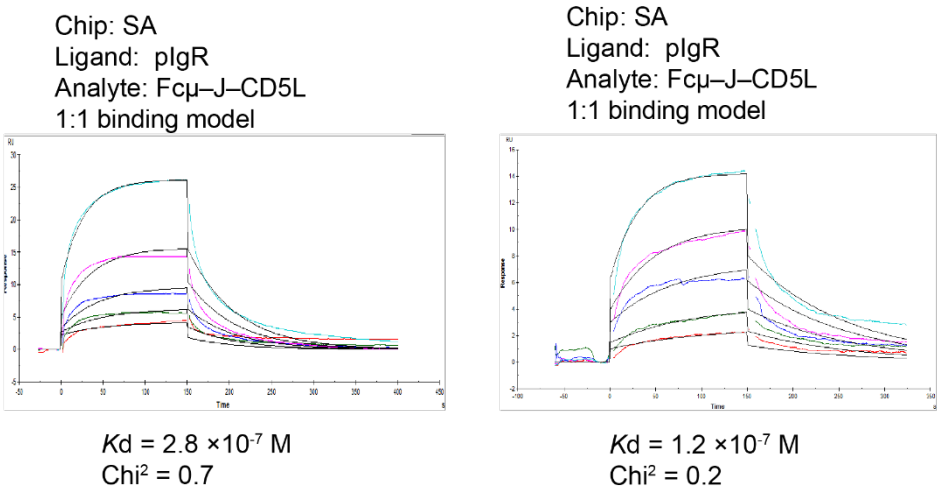
Chip: CM5
Ligand: Fcμ–J
Analyte: pIgR



*K*d = 1.6 × 10^{-9} M
Chi² = 7.9

Chip: CM5
Ligand: Fcμ–J–CD5L
Analyte: BSA





Supplementary Figure 8. Repeats of SPR assays.

Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics.

	IgM-Fc/J/AIM overall PDB ID: 8WYR EMDB: EMD-37936	IgM-Fc/J/AIM local PDB ID: 8WYS EMDB: EMD-37937
Data collection and processing		
Magnification	105,000	
Voltage (kV)	300	
Electron exposure (e–/Å^2)	59.5	
Defocus range (μm)	–1.0 to –1.5	
Pixel size (Å)	0.83	
Symmetry imposed	C1	
Initial particle images (no.)	894,255	
Final particle images (no.)	381,470	
Map resolution (Å) FSC threshold: 0.143	3.39	3.41
Refinement		
Initial model used (PDB code)	6KXS, 6LX3	6KXS, 6LX3
Model resolution (Å) FSC threshold: 0.143	3.39	3.41
Map sharpening *B* factor (Å^2)	113.4	107.6
Model composition		
Non-hydrogen atoms	2,0612	6,340
Protein residues	2,629	805
Ligands	14	6
B factors (Å^2)		
Protein	131.45	77.30
Ligand	89.66	66.45
R.m.s. deviations		
Bond lengths (Å)	0.007	0.016
Bond angles (°)	1.011	1.133
Validation		
MolProbity score	1.88	1.80
Clashscore	7.67	6.34
Poor rotamers (%)	0.51	0.70
Ramachandran plot		
Favored (%)	92.74	92.96
Allowed (%)	7.26	7.04
Disallowed (%)	0.00	0.00