

Supplementary Information

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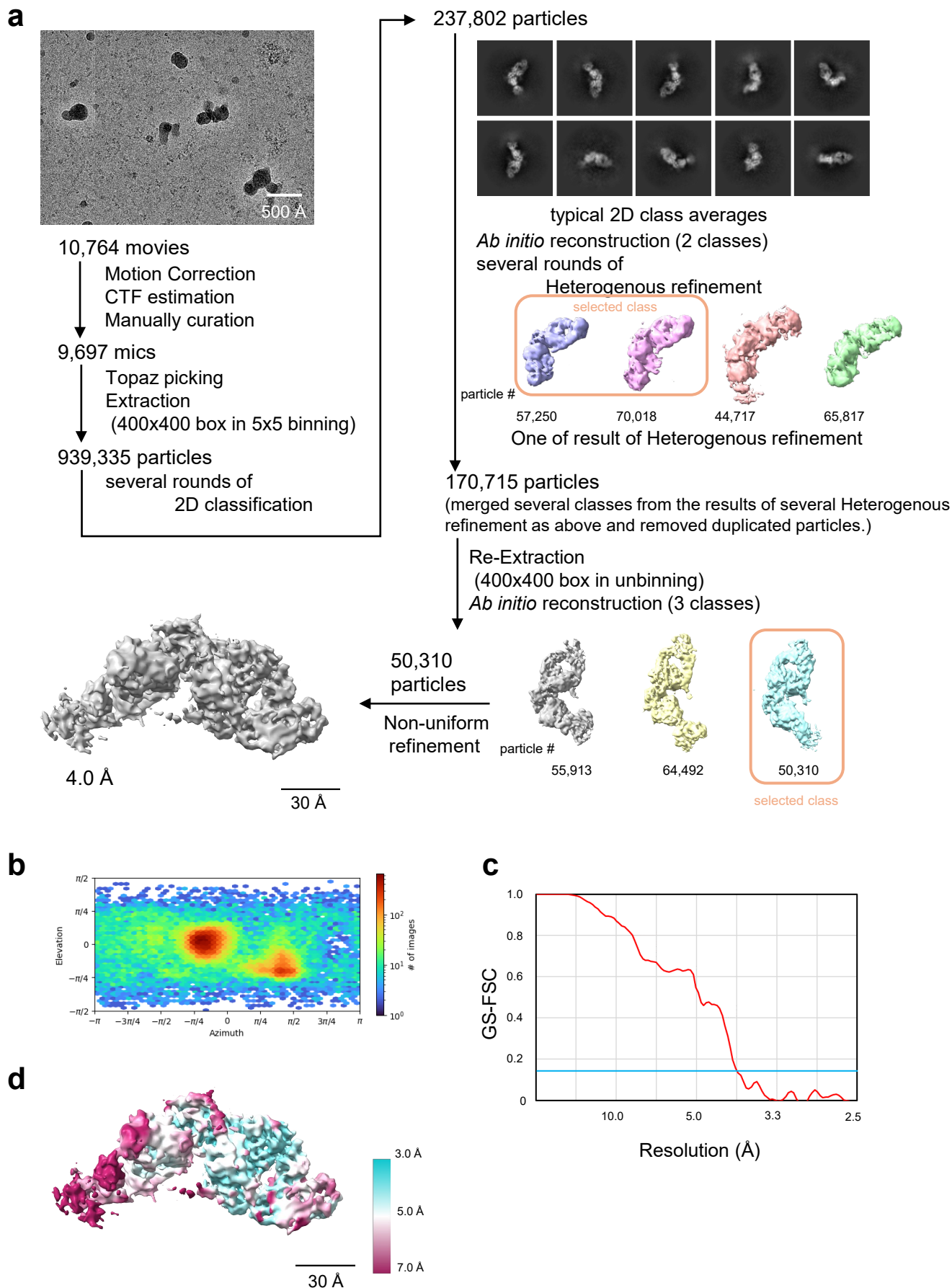
Affinity- and epitope-dependent pathogenicity of GM-CSF autoantibodies in autoimmune pulmonary alveolar proteinosis

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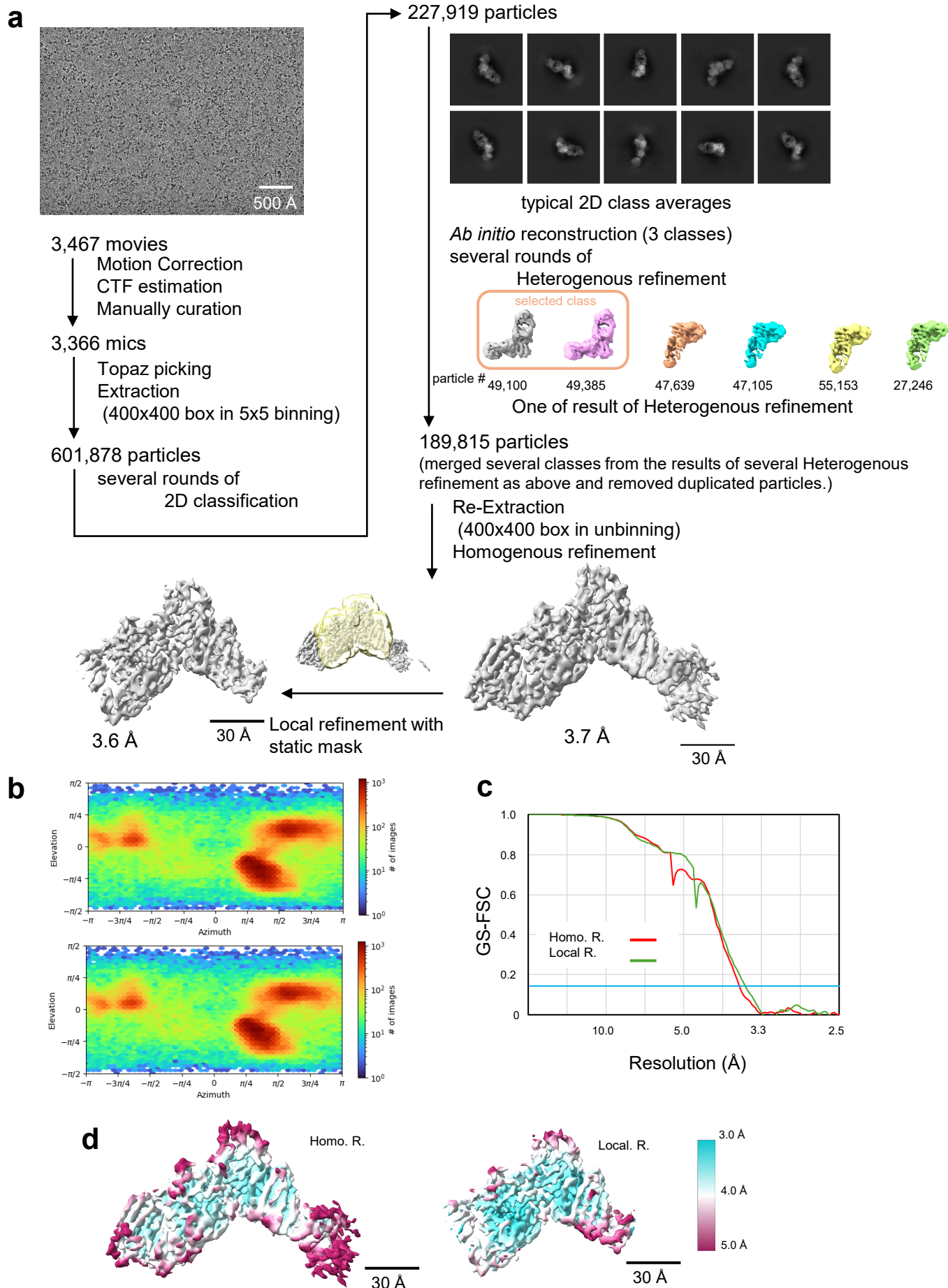
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This Supplementary Information file contains:

- 1. Supplementary Figures 1-6**
- 2. Supplementary Tables 1-2**

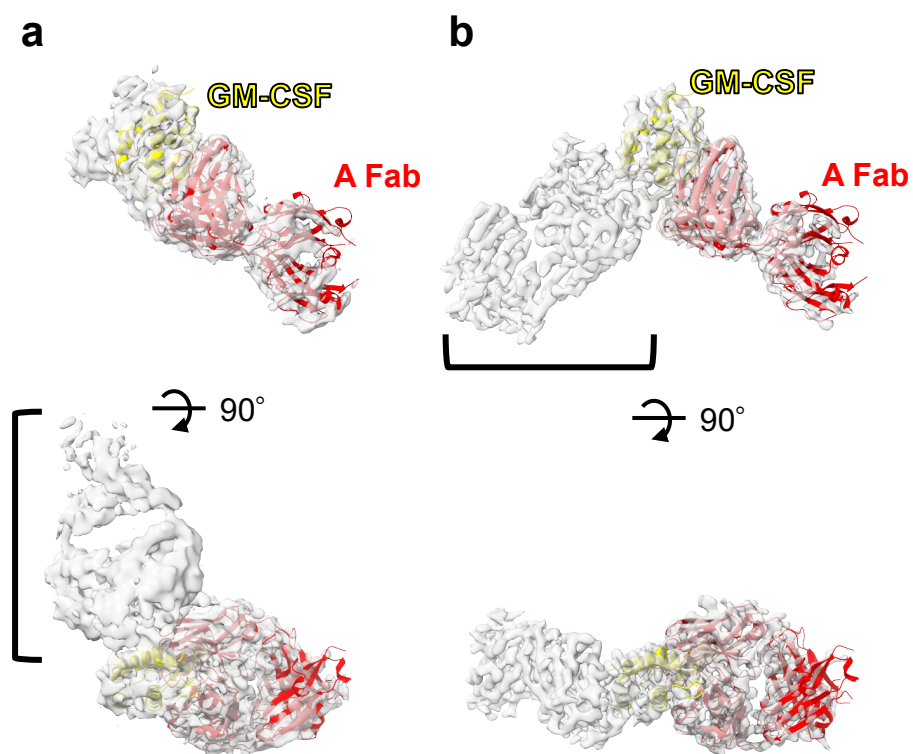


Supplementary Figure 1. Flow chart of single particle cryo-EM for the GM-CSF:A Fab:BD Fab complex.
a The image processing for the GM-CSF:A Fab:BD Fab complex are summarized. **b** Angular distribution of the particles used for final NU-refinement. **c** GS-FSC curve for the obtained map (red). The resolution criterion (GS-FSC=0.143) is indicated by blue line. **d** Local resolution of the obtained map.



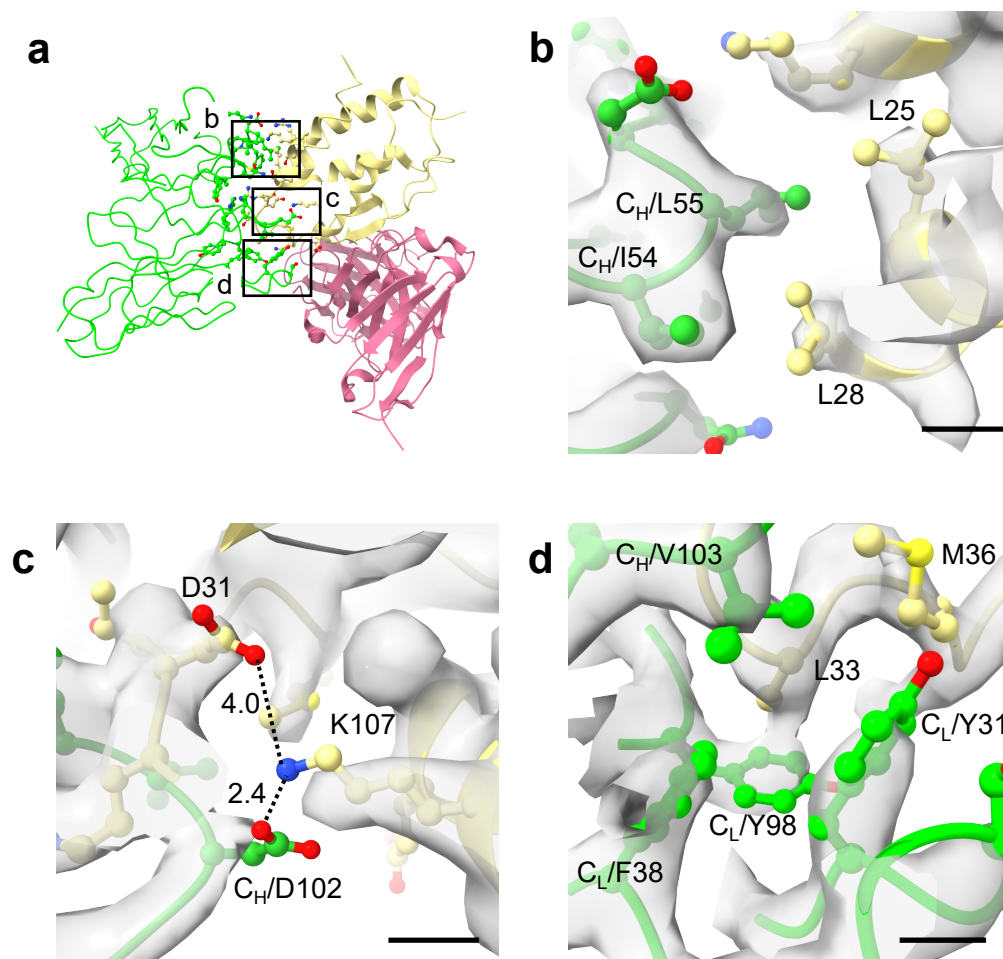
Supplementary Figure 2. Flow chart of single particle cryo-EM for the GM-CSF:A Fab:C Fab complex.

a The image processing for the GM-CSF:A Fab:C Fab complex are summarized. **b** Angular distribution of the particles used for final Homogenous refinement and Local refinement, respectively. **c** GS-FSC curves for the obtained maps. The resolution criterion (GS-FSC=0.143) is indicated by blue line. **d** Local resolution of the obtained maps.



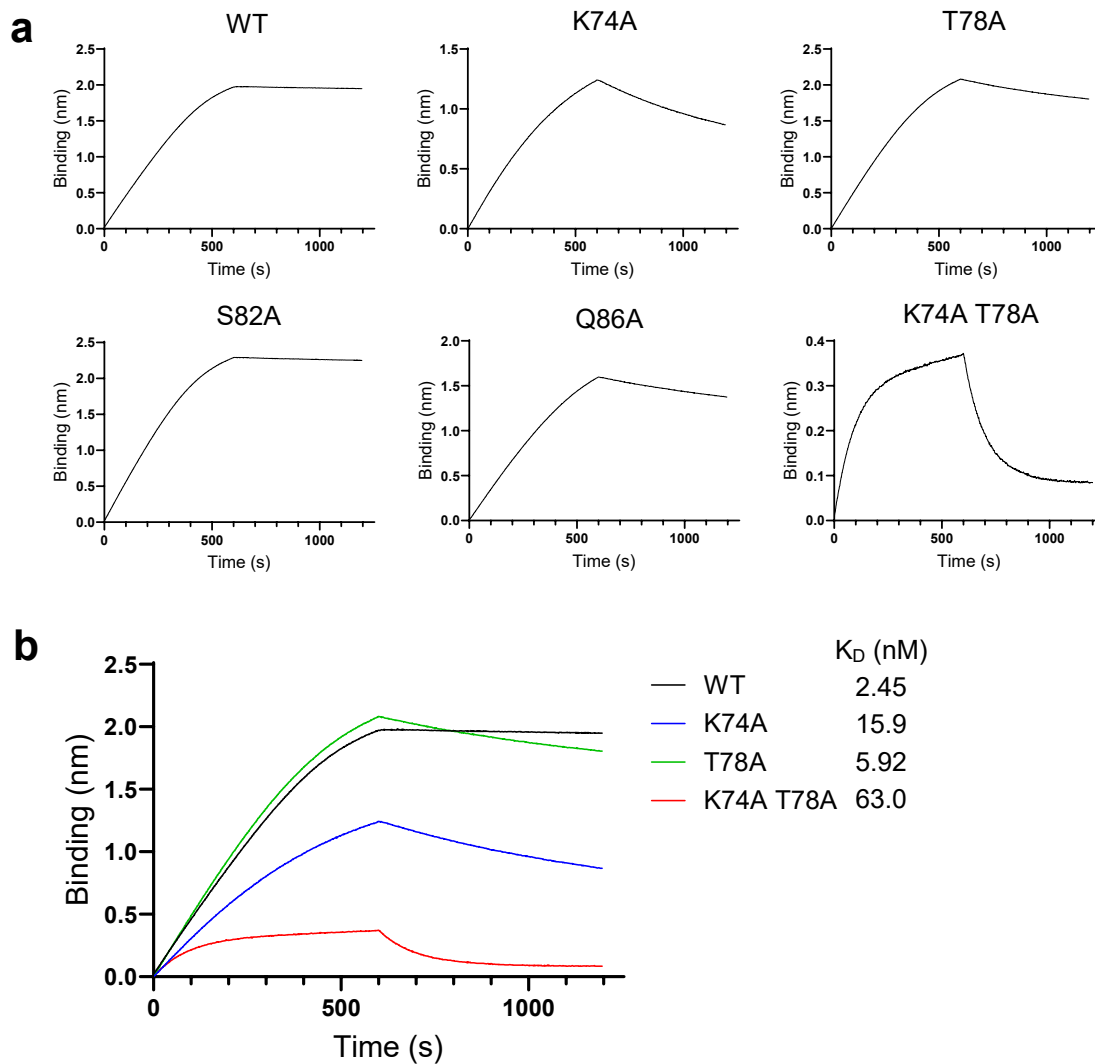
Supplementary Figure 3. The rigid body fitted models for the obtained maps.

The atomic model of the GM-CSF:A Fab complex (PDBID: 6BFQ) into the density maps for the GM-CSF:A Fab:BD Fab complex (**a**) and the GM-CSF:A Fab:C Fab complex (**b**), respectively. Brackets indicate the additional densities corresponding to another Fab (BD or C Fabs). GM-CSF and A Fab are colored yellow and red, respectively.



Supplementary Figure 4. Specific interactions between human GM-CSF and C Fab.

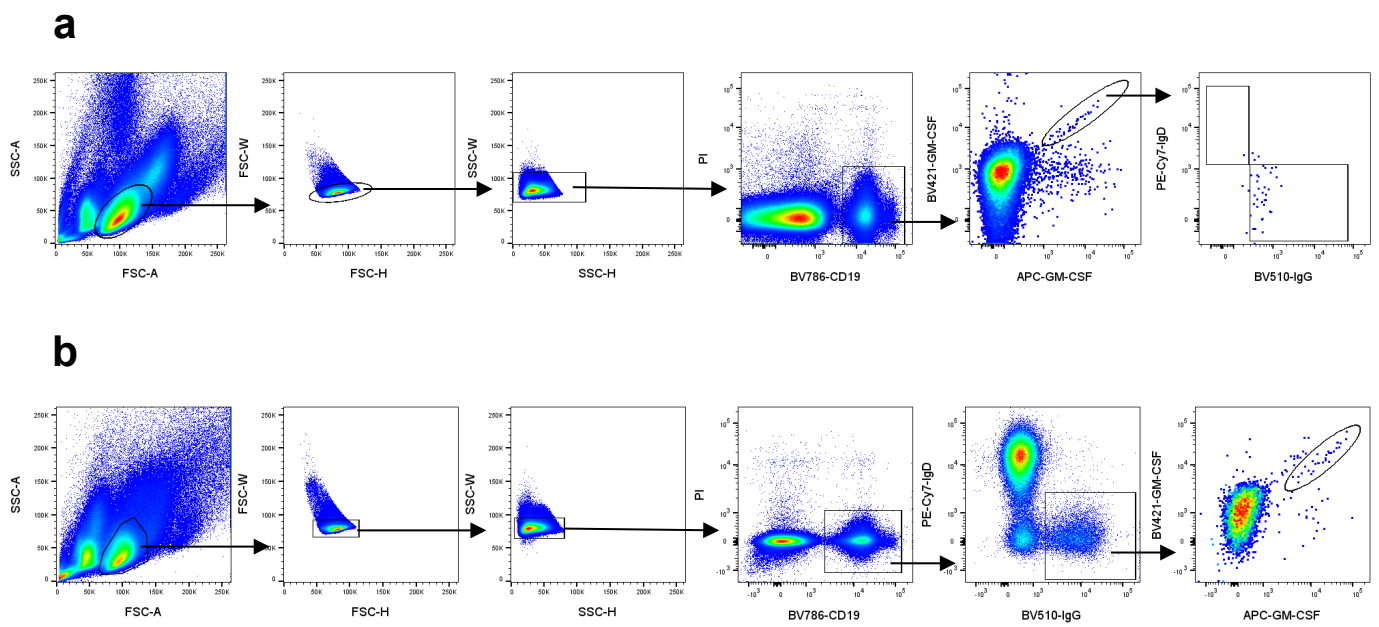
a Atomic model of the GM-CSF:A Fab:C Fab complex built in this study. GM-CSF, A Fab, and C Fab are colored yellow, magenta, and green, respectively. Rectangles indicate the regions enlarged in **b-d**. **b-d** show the interactions between human GM-CSF and C Fab. **b** and **d** hydrophobic interactions, **c** electrostatic interactions. Possible hydrogen bonds are indicated by dotted lines. The numerical values are distances (Å). C_H and C_L indicate heavy and light chain of C Fab, respectively. Scale bars, 3 Å.



Supplementary Figure 5. Binding kinetics of BD Fab to alanine substituted GM-CSF mutants.

a Binding kinetics of BD Fab (Pt9-2-28) to GM-CSF mutants were measured by BLI. Biotin-GM-CSF mutants were loaded onto streptavidin biosensors and incubated with BD Fab wildtype (WT) and mutants. Representative of two independent experiments.

b Overlaid sensorgram of WT, K74A, T78A, and K74A T78A mutants. Calculated affinity (K_D) is shown on the right.



Supplementary Figure 6. Gating strategy for flow cytometry.

a Representative gating strategy for the analysis and sorting of GM-CSF-specific B cells (**Fig. 2b**). After exclusion of doublets, lymphocytes were gated on live CD19⁺ cells. **b** Representative gating strategy for the analysis of GM-CSF-specific IgG⁺ B cells (**Fig. 4b** and **4f**). After exclusion of doublets, lymphocytes were gated on live CD19⁺IgG⁺IgD⁻ cells.

Supplementary Table 1. Antibodies and reagents used in flow cytometry.

Antibody and reagent	Dilution	Catalogue number
FITC anti-human CD38	1:50	#11-0388-42 Thermo Fisher Scientific
PE anti-human IgL	1:20	#12-9990-42 Thermo Fisher Scientific
APC Streptavidin	1:250 (biotinylated GM-CSF prelabelled with fluorophore-conjugated streptavidin)	#SA1005 Thermo Fisher Scientific
PE-Cy7 anti-human IgD	1:200	#348209 BioLegend
APC-Cy7 anti-human CD27	1:100	#356424 BioLegend
BV785 anti-human CD19	1:200	#302240 BioLegend
BV421 Streptavidin	1:50 (biotinylated GM-CSF prelabelled with fluorophore-conjugated streptavidin)	#405225 BioLegend
BV510 anti-human IgG	1:10	#563247 BD
Propidium iodide	1:10000	#537060 Sigma-Aldrich
anti-human Fc Receptor binding inhibitor	1:200	#14-9161-73 Thermo Fisher Scientific

Supplementary Table 2. Cryo-EM data collection and processing statistics.

GM-CSF:A Fab:BD Fab (EMDB-61568)		GM-CSF:A Fab:C Fab (EMDB-61569) (PDB 9JKR)
Data collection and processing		
Magnification		105,000
Voltage (kV)	300	
Electron Exposure (e-/Å ²)		65
Defocus range (µm)	-0.8 to -1.8	
Pixel size (Å/pix)		0.675
Symmetry imposed	C1	
Collected movies (#)	10,764	3,467
Initial particles (#)	939,335	601,878
Final particles (#)	50,310	189,815
Map resolution (Å)	4.0	3.7
FSC threshold		0.143
Refinement		
Initial model used (PDB)	-	6BFQ
Model resolution (Å)	-	3.7
FSC threshold		0.5
Map sharpening B factor (Å ²)	-	104.2
Model composition		
Non-hydrogen atoms	-	4,399
Protein residues	-	566
R.M.S. deviations		
Bond length (Å)	-	0.005
Bond angles (°)	-	0.770
Validation		
MolProbity score	-	2.09
Clashscore	-	12.09
Poor rotamer (%)	-	0.00
Ramachandran plot		
Favored (%)	-	91.73
Allowed (%)	-	8.27
Disallowed (%)	-	0