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Supporting Information

Cryo-electron microscopy reveals a single domain antibody with a unique binding epitope on fibroblast activation protein alpha

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| FAP to structurally aligned regions in DPPs. | |



- (A) Kinetic fits (red lines) for 1:1 model from FAP-MBP-I3 binding data (green lines). R² = 0.98 X² = 0.01
- (B) Steady state analysis of FAP-MBP-I3 binding data. R² = 0.998 R_{max} = 0.2403 \pm 0.004. K_d = 1.80 \pm 0.09 μ M



Figure S2. Cryo-EM data processing workflow. (A) Processing workflow used in cryoSPARC to obtain both reconstructions of one and two SUMO-I3 molecules bound to FAP. (B) Particle view distribution for one SUMO-I3 bound to FAP (C1 symmetry). (C) Particle view distribution for two SUMO-I3 bound to FAP (C2 symmetry).



Table S1. Cryo-EM data collection parameters and model refinement statistics.

| | FAP-(SUMO-I3)1 | FAP-(SUMO-I3) ₂ |
|---|----------------|----------------------------|
| Data collection and image | | |
| processing | | |
| Microscope | Titan Krios | Titan Krios |
| Voltage (kV) | 300 | 300 |
| Camera | K3 | K3 |
| Magnification | 105,000 x | 105,000 x |
| Electron exposure (e ⁻ /Å ²) | 65 | 65 |
| Exposure time (s) | 2.43 | 2.43 |
| Number of frames | 45 | 45 |
| Defocus range (µm) | -0.5 to 2.5 | -0.5 to 2.5 |
| Super resolution pixel size (Å) | 0.43 | 0.43 |
| Number of movies | 5,450 | 5,450 |
| Particles for final reconstruction (no.) | 272,711 | 238,246 |
| Symmetry imposed | C1 | C2 |
| Map resolution (Å) | 2.7 | 2.7 |
| Haif map FSC threshold 0.143 | | |
| EMDB accession code | | |
| Model refinement statistics | | |
| Model composition | | |
| Non-hydrogen atoms | 12,793 | 13,694 |
| Protein residues | 1,560 | 1,682 |
| Ligands | NAG x8 | NAG x8 |
| Cross correlation | | |
| Mask | 0.93 | 0.84 |
| Volume | 0.92 | 0.83 |
| RMSD | | |
| Bond length (Å) | 0.007 | 0.004 |
| Bond Angles (°) | 0.651 | 0.721 |
| Ramachandran | | |
| Favored (%) | 94.34 | 94.74 |
| Allowed (%) | 5.66 | 5.26 |
| Outlier (%) | 0.00 | 0.00 |
| Validation | | |
| MolProbity score | 1.60 | 2.02 |
| Clashscore | 3.71 | 7.41 |
| Rotamer outliers (%) | 1.17 | 2.13 |
| PDB accession code | | |

Figure S3. Map and model overlay. (A) Cartoon model of FAP-I3 overlaid with the final reconstruction map showing various orientations. (B) Map-to-model FSC for FAP-I3. (C) Cartoon model of FAP + 2 SUMO-I3 overlaid with the final reconstruction map showing different orientations. (D) Map-to-model FSC for FAP + 2 SUMO-I3.



Figure S4. Overall alignment of FAP with DPPs and comparison of I3 epitope region of FAP to structurally aligned regions in DPPs. PDB codes: FAP (1Z68), DPP4 (2ONC), DPP8 (6EOO), DPP9 (7A3F).

