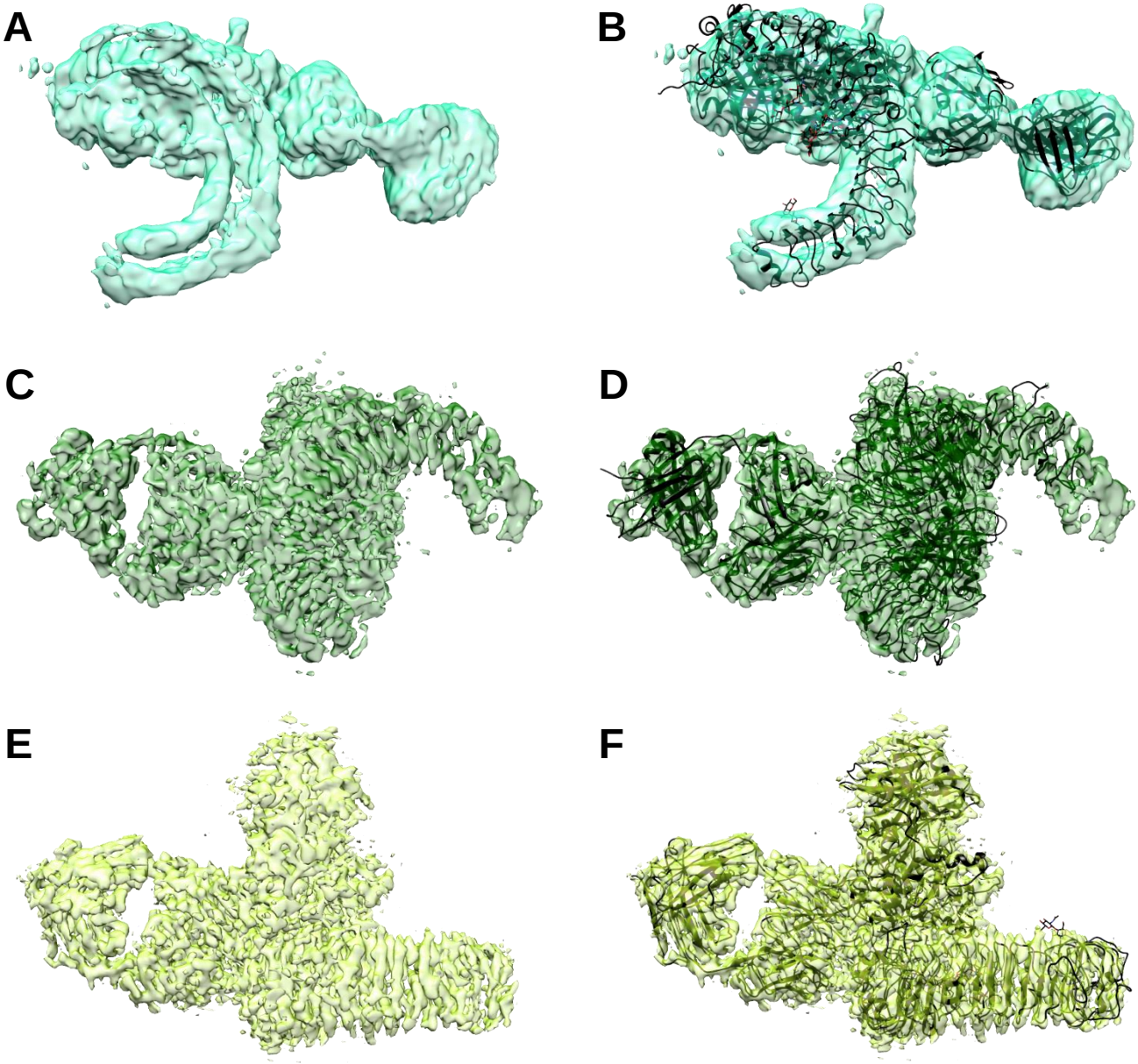
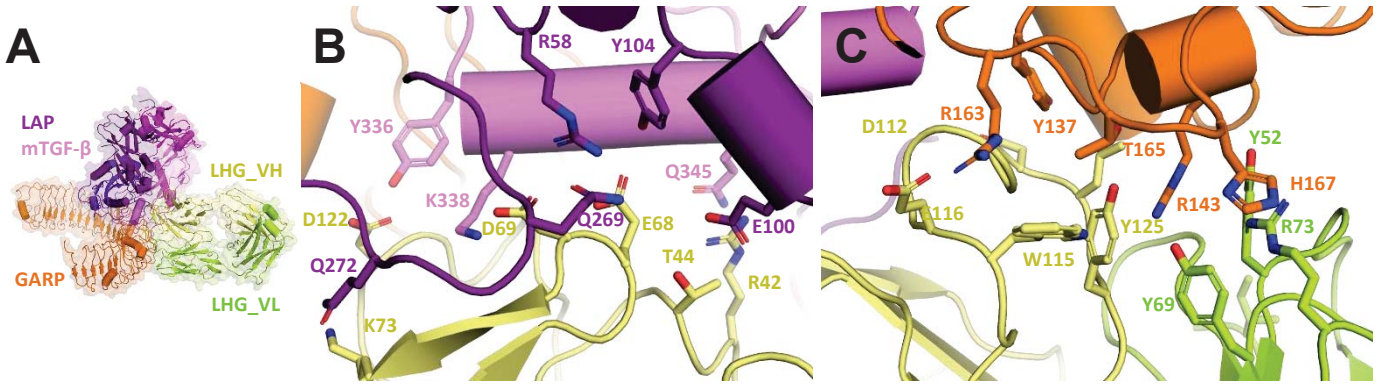
Supplementary Figure 1



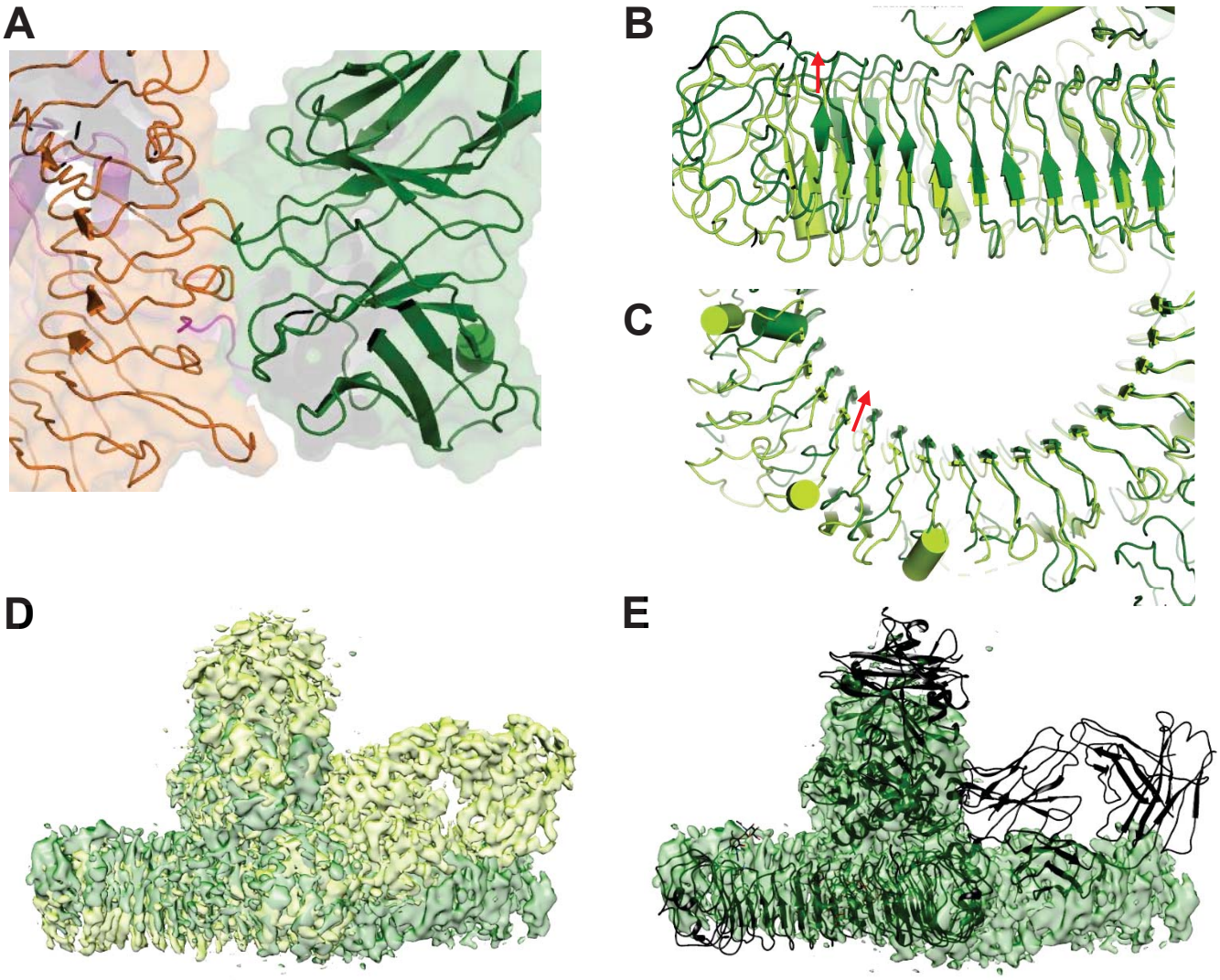
Cryo-EM maps and fitted models. Cryo-EM maps (**A, C, E**) and models (cartoon representation) fitted in the corresponding maps (**B, D, F**) of the GARP/L-TGF-β in complex with **A, B** Fab 28G11 (cyan) at 7.5 Å resolution, **C, D** LHG-10 (lemon green) at 2.7 Å resolution, and **E, F** 12G2B4 (dark green) at 3.4 Å resolution. All Fabs offer good model to map correlation considering the corresponding resolution.

Supplementary Figure 2



Detailed representation of the GARP/L-TGF-β-LHG-10 epitope and paratope. A Schematic overview scheme of the GARP/L-TGF-β-LHG-10 complex. **B** The binding epitope of LHG-10 (yellow and lemon green) on LAP (dark purple) consists of residues 269-273, 58, 100, 104 and residues 336-338, 345 on mTGF-β (light purple). **C** GARP (orange) residues 137, 140-143, 161-163, 165 and 167 interact with the light (lemon green) and heavy (yellow) chain of the variable regions of Fab LHG-10.

Supplementary Figure 3



Conformational rearrangements upon binding of Fab 12G2B4. A 12G2B4 (dark green) binds exactly at the point where the horseshoe fold of GARP (orange) is completed by a β-strand addition of LAP (magenta). Here, the β -sheet at the N-terminus of LAP intercalates into the β -sandwich of GARP. **B** Upon binding of 12G2B4 a conformational rearrangement of GARP is induced. In comparison to the LHG-10 bound structure (lemon green), the C-terminal portion of 12G2B4 bound GARP (dark green) moves up towards L-TGF-β and **C** In addition, the curvature of the horseshoe folds increases in this region, resulting in a slight dislocation compared to the LHG structure. **D** These conformal changes are also clearly visible in the EM density of the structure, as shown by the superposition of the maps of LHG-10 (lemon green) and 12G2B4 (dark green)**. D** Overlay of 12G2B4 cryo-EM map and LHG-10 model (black), aligned on GARP N-terminal portion and L-TGF-β.

Supplemental Table 1: Data collection, processing and refinement statistics for GARP-L-TGF-β cryo -EM structures in complex with different Fabs.

Experimental data			
Protein	GARP - L-TGF-β	GARP - L-TGF-β	GARP - L-TGF-β
Fab	LHG-10	28G11	12G2B4
PDB /EMBD ID	EMD-16460 8C7H	EMD-16456	EMD-16459
Data Collection and Processing			
Microscope	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios
Voltage (kV)	300	300	300
Camera	Gatan K2 Summit	Gatan K3	Gatan K3
Exposure time (s)	8	8	8
Total Dose ($e^-/Å^2$)	40	40	40
Defocus range (μm)	0.8-2.8	0.8-2.8	0.8-2.8
Pixel size (Å) (calibrated)	1.08	0.83	0.83
Symmetry imposed	C1	C1	C1
Number of micrographs	2842	3354	8289
Initial particle number	2,837,425	3,639,216	2,602,167
Final particle number	418,886	488,857	613,675
Map resolution (Å)	2.7	4.3	3.4
FSC threshold	0.143	0.143	0.143
Refinement			
Model composition			
Protein (residues)	9172		
Ligands	BMA:3; NAG:6		
RMSD bond length (Å)	0.003		
RMSD bond angles (°)	0.661		
Rotamer outliers (%)	0.42		
ADP (B-factor) (min/max/mean)			
Protein	3.37/159.21/68.62		
Ligand	16.10/94.14/53.72		
Ramachandran plot			
Favoured (%)	95.61		
Allowed (%)	4.39		
Outliers (%)	0.00		
All-atom clashscore	8.20		
MolProbity score	1.75		