

Structural Insights into Selective and Dual Antagonism of EP2 and EP4 Prostaglandin Receptors

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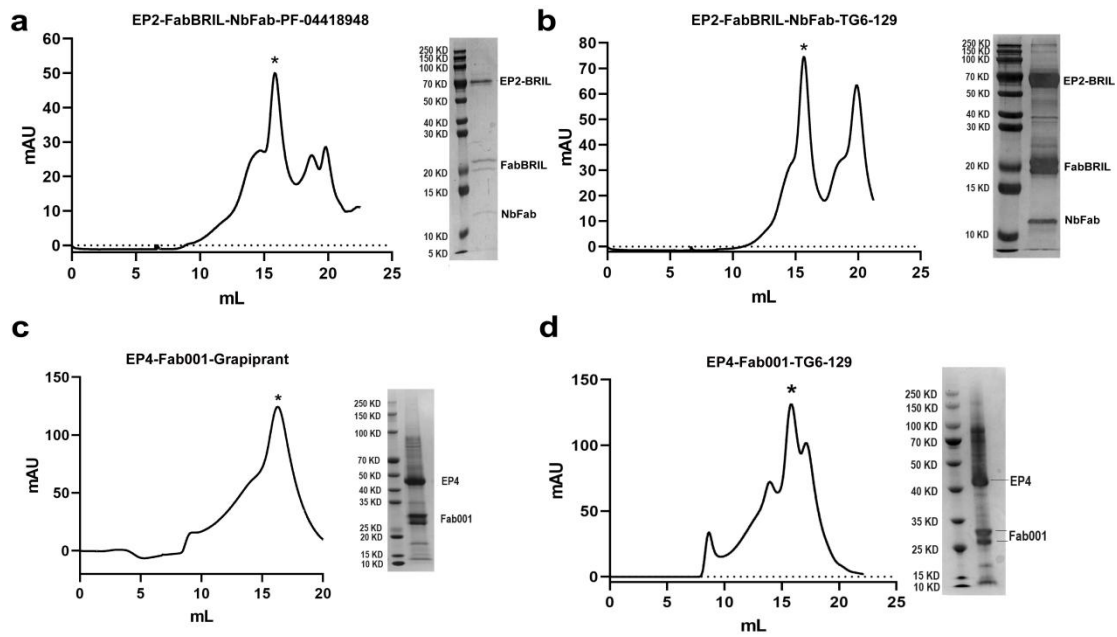
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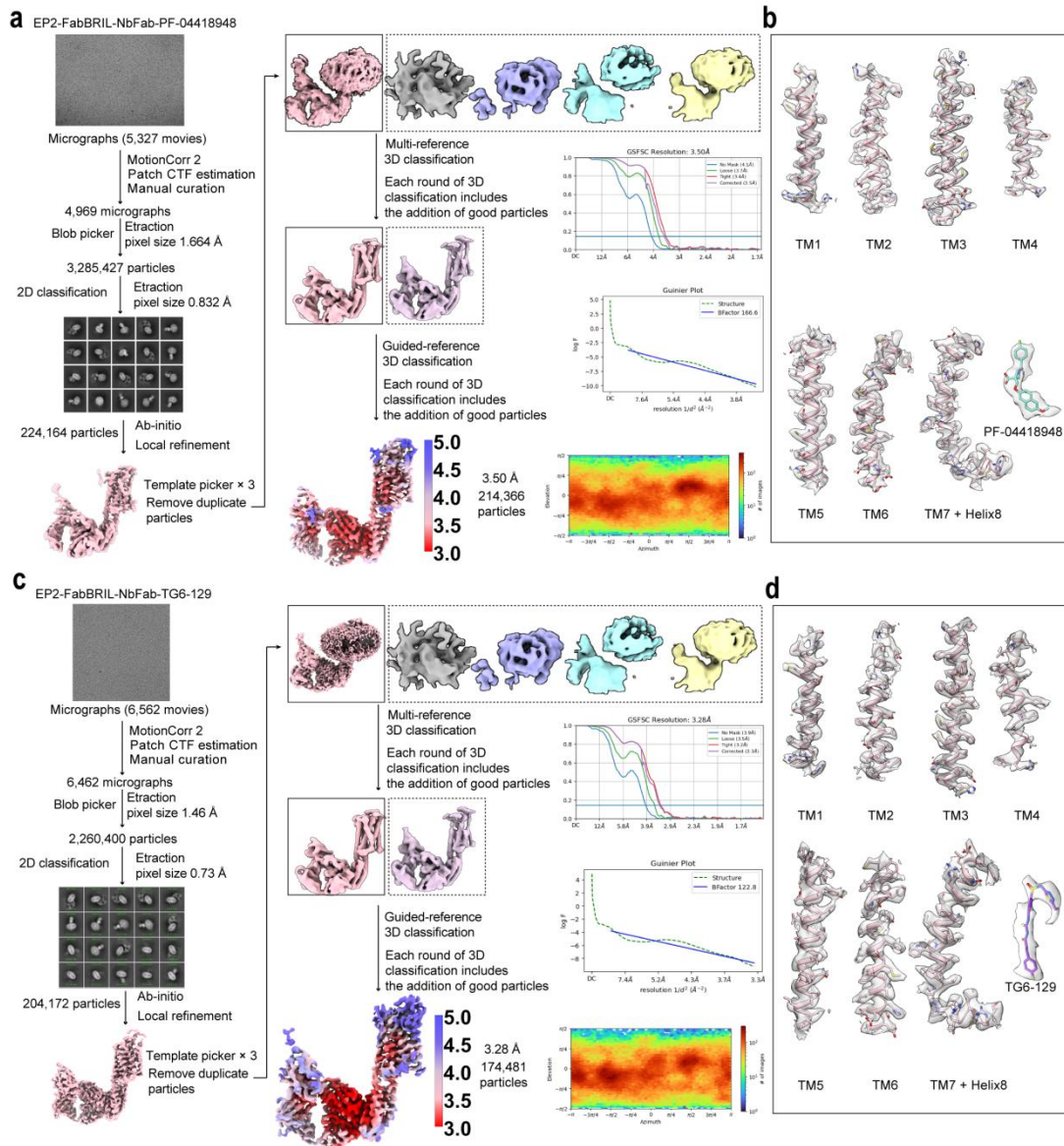
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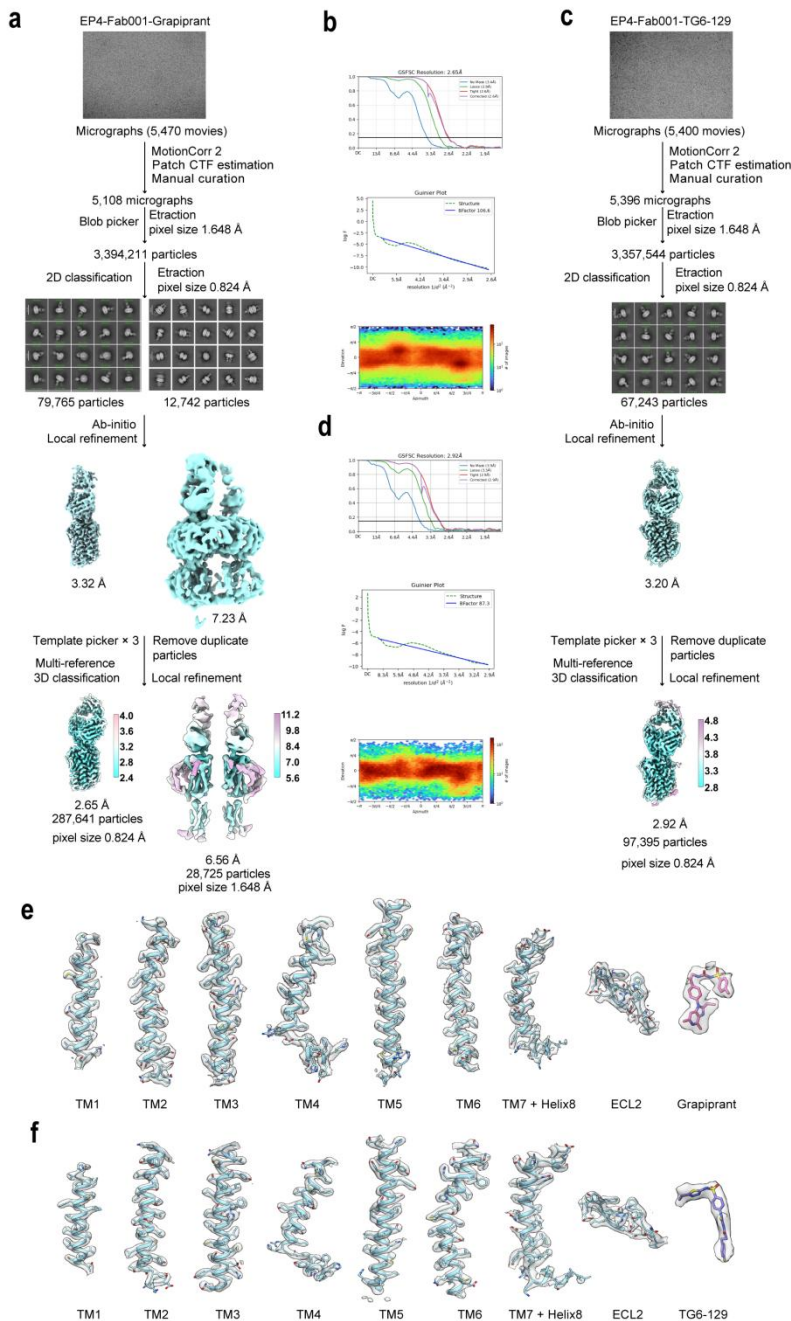
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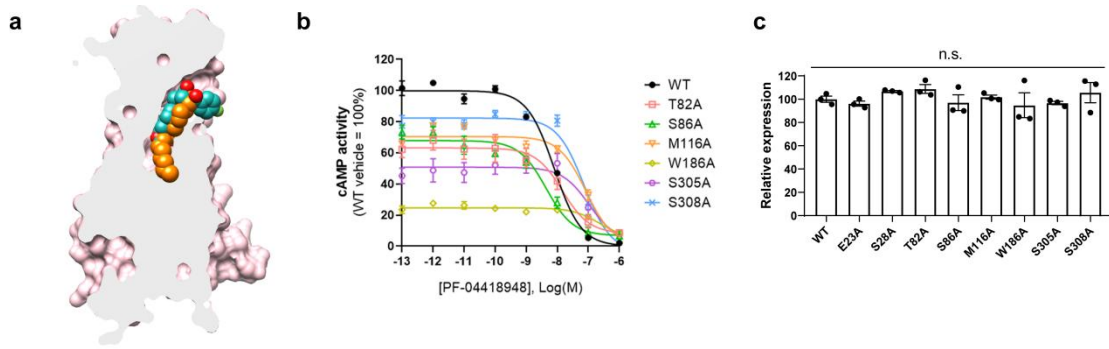
Supplementary Figure 1. Purification of EP2-FabBRIL-NbFab-antagonist complexes. **a-d**, Diagram of size-exclusion chromatography and SDS-PAGE analysis of EP2-FabBRIL-NbFab-PF-04418948, EP2-FabBRIL-NbFab-TG6-129, EP4-Fab001-grapiprant and EP4-Fab001-TG6-129 complex, respectively.



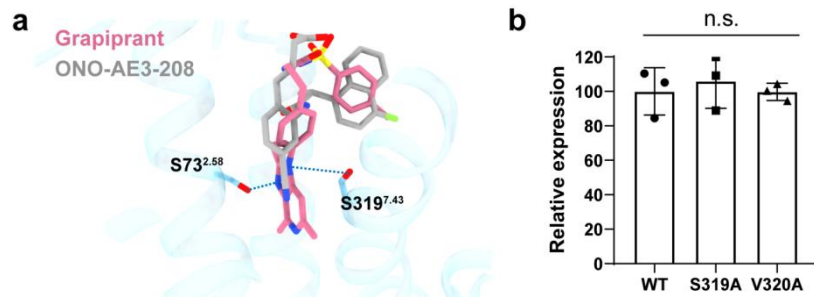
Supplementary Figure 2. Cryo-EM data processing and Representative cryo-EM density maps of EP2-FabBRIL-NbFab-antagonist complexes. **a**, Computational sorting of cryo-EM particle images, the “Gold-standard” FSC curve of EP2-FabBRIL-NbFab-PF-04418948 complex. **b**, Cryo-EM density maps of the seven transmembrane (TM) helices of PF-04418948 bound EP2. **c**, Computational sorting of cryo-EM particle images, the “Gold-standard” FSC curve of EP2-FabBRIL-NbFab-TG6-129 complex. **d**, Cryo-EM density maps of the seven TM helices of TG6-129 bound EP2. The cryo-EM sample preparation and data collection were performed once.



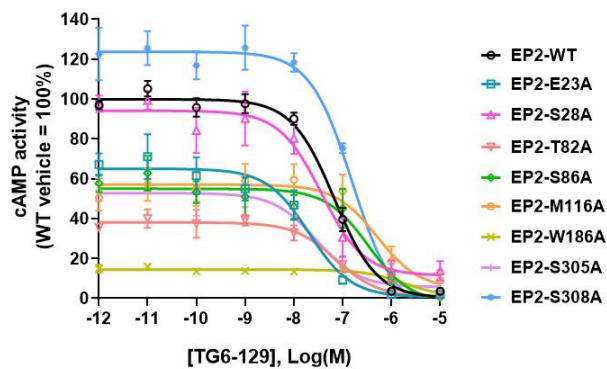
Supplementary Figure 3. Cryo-EM data processing and Representative cryo-EM density maps of EP4-Fab001-antagonist complexes. **a**, Computational sorting of cryo-EM particle images of EP4-Fab001-grapiprant complex. **b**, The “Gold-standard” FSC curve of EP4-Fab-grapiprant complex. **c**, Computational sorting of cryo-EM particle images of EP4-Fab001-TG6-129 complex. **d**, The “Gold-standard” FSC curve of EP4-Fab001-TG6-129 complex. **e**, Cryo-EM density maps of the seven transmembrane helices of grapiprant bound EP4. **f**, Cryo-EM density maps of the seven transmembrane helices of grapiprant bound EP4. The cryo-EM sample preparation and data collection were performed once.



Supplementary Figure 4. Additional data for inhibition of EP2. **a**, Comparison of binding pocket of PF-04418948 (light sea green) and PGE2 (orange) in inactive EP2. **b**, cAMP inhibition assay of key mutants in EP2 that bind to PF-04418948. Data are presented as mean \pm SEM; n=3 independent samples. **c**, Cell surface expression level of WT and mutant EP2 receptors. Data are presented as mean \pm SEM; n=3 independent samples, significance was determined with two-side unpaired t test; $P > 0.05$ was considered statistically not significant (n.s.).



Supplementary Figure 5. Additional data for selective inhibition of EP4. **a**, Comparison of binding pocket of grapiprant (pale violet red) and ONO-AE3-208 (gray) in inactive EP2. **b**, Cell surface expression level of WT and mutant EP4 receptors. Data are presented as mean \pm SEM; $n=3$ independent samples, significance was determined with two-side unpaired t test; $P>0.05$ was considered statistically no significant (n.s.).



Supplementary Figure 6. cAMP inhibition assay of key mutants in EP2 that bind to TG6-129. Data are presented as mean \pm SEM; n=3 independent samples.

Supplementary information, Table S1

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

	EP2-PF-04418948	EP2-TG6-129	EP4-Grapiprant	EP4-TG6-129
	PDB: 9JRO	PDB: 9JRT	PDB: 9JQZ	PDB: 9JQY
	EMDB: 61762	EMDB: 61763	EMDB: 61744	EMDB: 61743
Data collection and processing				
Magnification	105,000	105,000	105,000	105,000
Voltage (kV)	300	300	300	300
Electron exposure (e-/Å ²)	50	50	50	50
Defocus range (μm)	-1.2~-2.8	-1.2~-2.8	-1.2~-2.8	-1.2~-2.8
Pixel size (Å)	0.832	0.73	0.824	0.824
Symmetry imposed	C1	C1	C1	C1
Initial particle images (no.)	3,285,427	2,260,400	3,394,211	3,357,544
Final particle images (no.)	214,366	174,481	287,641	97,395
Map resolution (Å)	3.50	3.28	2.65	2.92
FSC threshold			0.143	
Map sharpening B factor (Å ²)	-166.6	-122.8	-106.6	-87.3
Refinement				
Initial mode used	From AlphaFold2	EP2-PF-04418948	From AlphaFold2	EP4-Grapiprant
Model resolution (Å)	3.43	3.26	2.68	2.97
FSC threshold			0.143	
Model-Map CC (mask)	0.68	0.68	0.55	0.56
Model composition				
Non-hydrogen atoms	6955	7061	5398	5393
Protein residues	896	909	662	682
B factors (Å ²)				
Protein	61.60	61.71	36.39	36.40
Ligand	20.00	30.00	30.00	30.00
R.m.s. deviations				
Bond lengths	0.002	0.002	0.002	0.002
Bond angles	0.505	0.537	0.528	0.525
Validation				
MolProbity score	1.73	1.78	1.96	1.87
Clash score	6.53	6.64	6.34	5.51
Rotamer outliers (%)	3.27	3.41	4.31	4.81
Ramachandran plot				
Favored (%)	98.17	97.98	97.30	97.75
Allowed (%)	1.83	2.02	2.70	2.25
Disallowed (%)	0.00	0.00	0.00	0.00