Supplementary Material:

Table 1. Sequence ID for B56 isoforms

% Identity	B56α	B56β	B56γ	B56δ	B56ε
B56α	100	77.3	63.6	66.1	67.9
B56β	77.3	100	63.1	63.1	66.3
B56γ	63.6	63.1	100	69.4	71.7
B56δ	66.1	63.1	69.4	100	77.5
B56ε	67.9	66.3	71.7	77.5	100

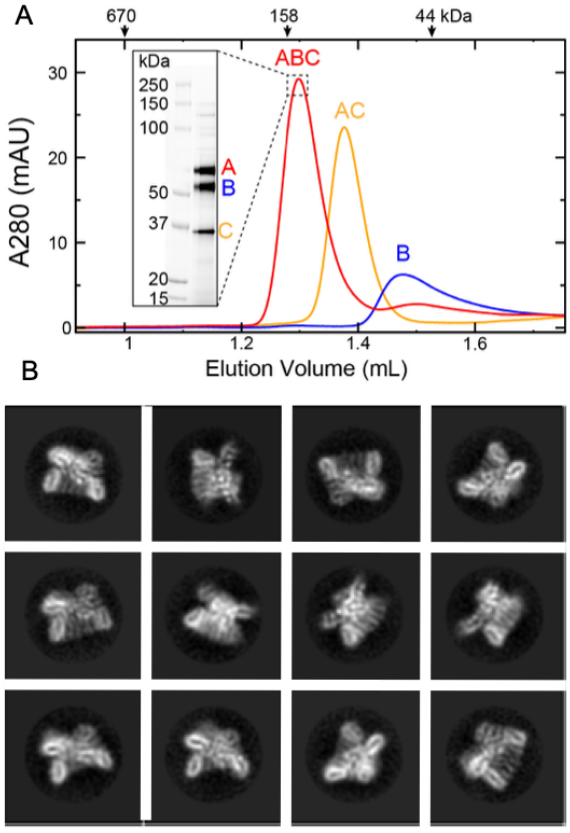
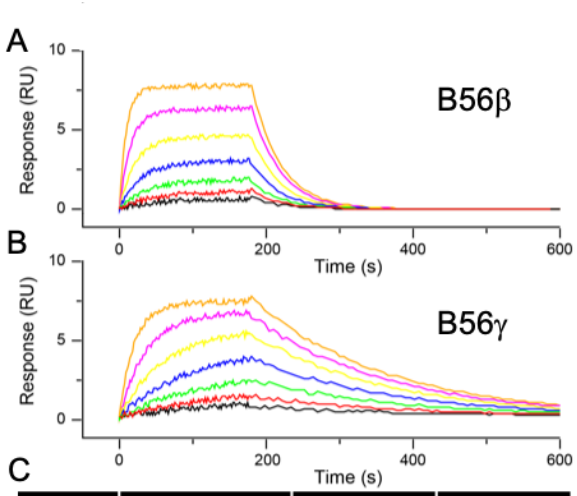


Fig 1. Assembly of PP2A heterotrimers. (A) B56β PP2A complex was assembled using individually expressed and purified PP2A subunits. The heterotrimeric complex was purified using size-exclusion chromatography. All three subunits co-sediment as a complex as confirmed by SDS-PAGE. **(B)** PP2A heterotrimeric complexes were imaged using cryo-EM. Processing of individual particles reveals high resolution features in the 2D class averages.



	k_a (M-1s-1)	k_d (s-1)	K_D (nM)
B56β	$1.5x10^5$	$2.6x10^{-2}$	173.3
B56γ	$1.1x10^5$	$0.9x10^{-2}$	82.7

Fig 2. B56β and B56γ behave differently in vitro. SPR data demonstrate that purified **(A)** B56β has a significantly reduced binding affinity and accelerated off-rate for the PP2A AC core complex as compared to **(B)** B56γ. **(C)** Cumulative binding data for B56α and B56γ.

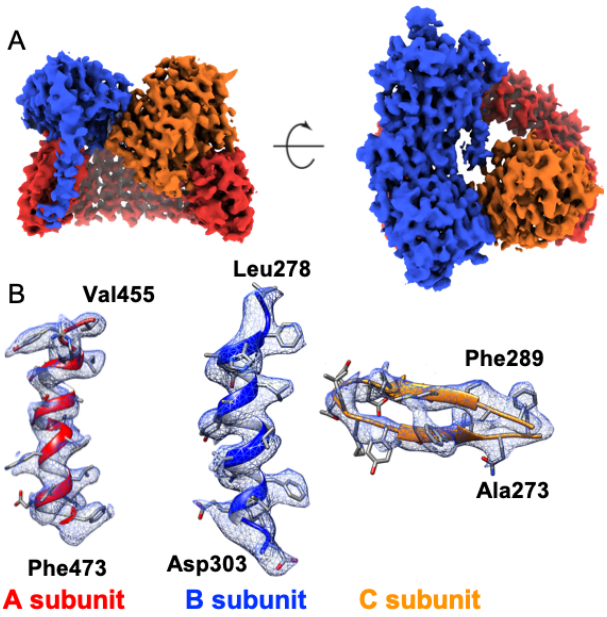


Fig 3. Cryo-EM reconstruction of the B56γ containing PP2A heterotrimeric complex. (A) The 3D structure of the B56γ-containing PP2A complex was solved to an average resolution of 3.8Å. The B56γ subunit is colored blue, the catalytic C-subunit is orange, and the scaffold A-subunit is blue. Arrow indicates a 90o rotation of the structure. **(B)** Models of different regions fit into the cryo-EM density reveals secondary structure, along with several amino acid side-chains.