

Supplementary Material:

Table 1. Sequence ID for B56 isoforms					
% Identity	B56 α	B56 β	B56 γ	B56 δ	B56 ϵ
B56 α	100	77.3	63.6	66.1	67.9
B56 β	77.3	100	63.1	63.1	66.3
B56 γ	63.6	63.1	100	69.4	71.7
B56 δ	66.1	63.1	69.4	100	77.5
B56 ϵ	67.9	66.3	71.7	77.5	100

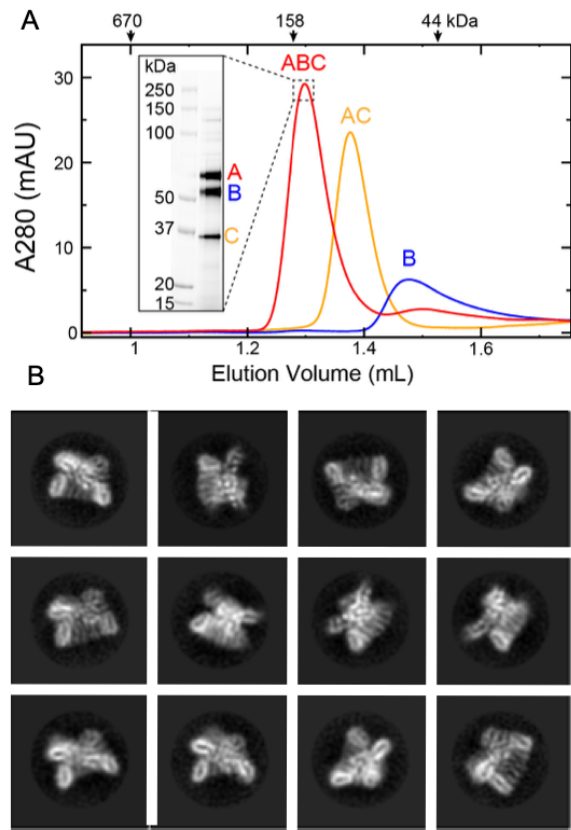


Fig 1. Assembly of PP2A heterotrimers. (A) B56 β PP2A complex was assembled using individually expressed and purified PP2A subunits. The heterotrimeric complex was purified using size-exclusion chromatography. All three subunits co-sediment as a complex as confirmed by SDS-PAGE. (B) B56 ϵ containing PP2A heterotrimeric complexes were imaged using cryo-EM. Processing of individual particles reveals high resolution features in the 2D class averages.

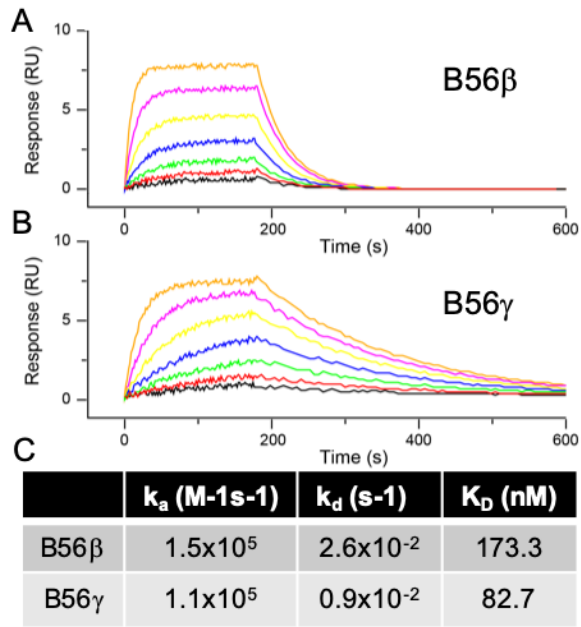


Fig 2. B56 β and B56 γ behave differently in vitro. SPR data demonstrate that purified (A) B56 β has a significantly reduced binding affinity and accelerated off-rate for the PP2A AC core complex as compared to (B) B56 γ . (C) Cumulative binding data for B56 β and B56 γ .

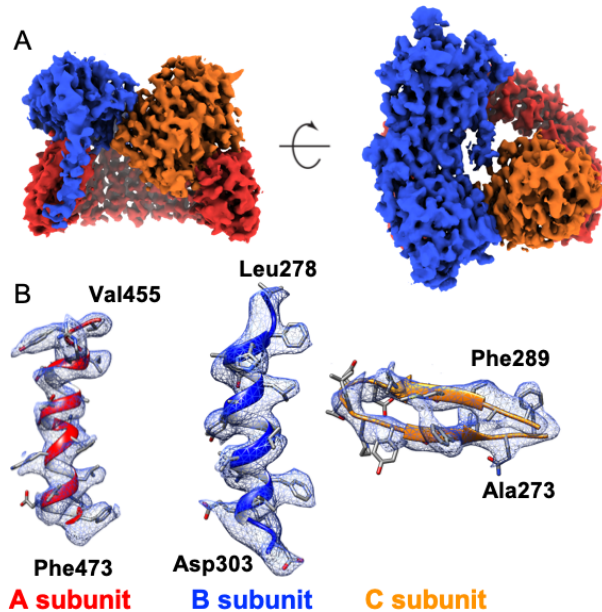


Fig 3. Cryo-EM reconstruction of the B56 ϵ containing PP2A heterotrimeric complex. (A) The 3D structure of the B56 γ -containing PP2A complex was solved to an average resolution of 3.8Å. The B56 ϵ subunit is colored blue, the catalytic C-subunit is orange, and the scaffold A-subunit is blue. Arrow indicates 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.

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