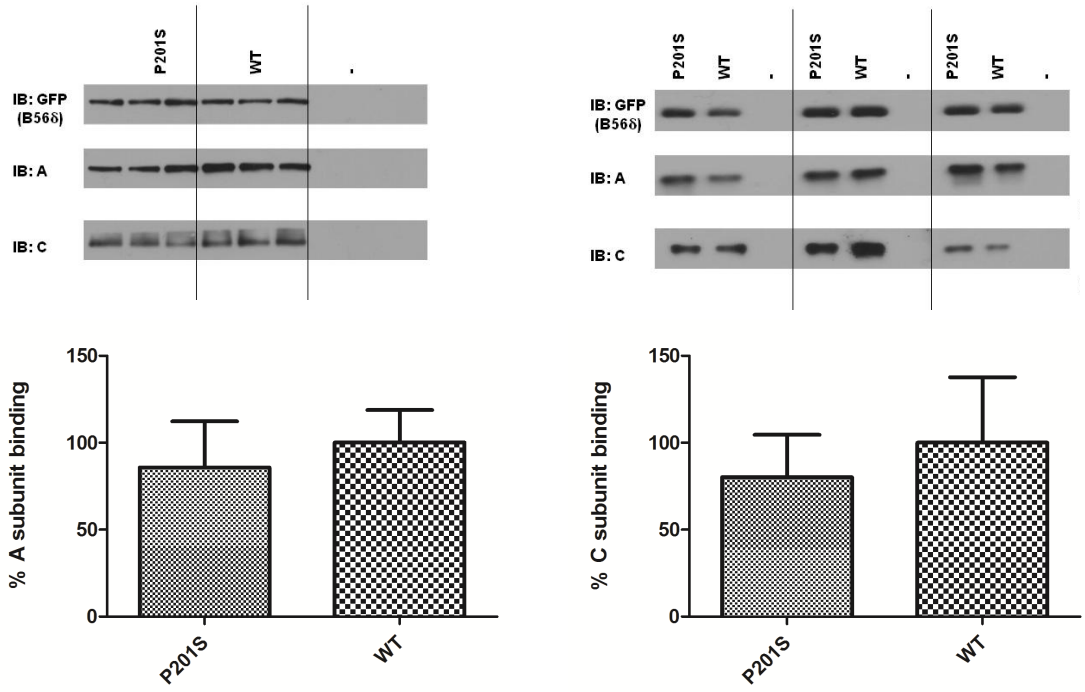
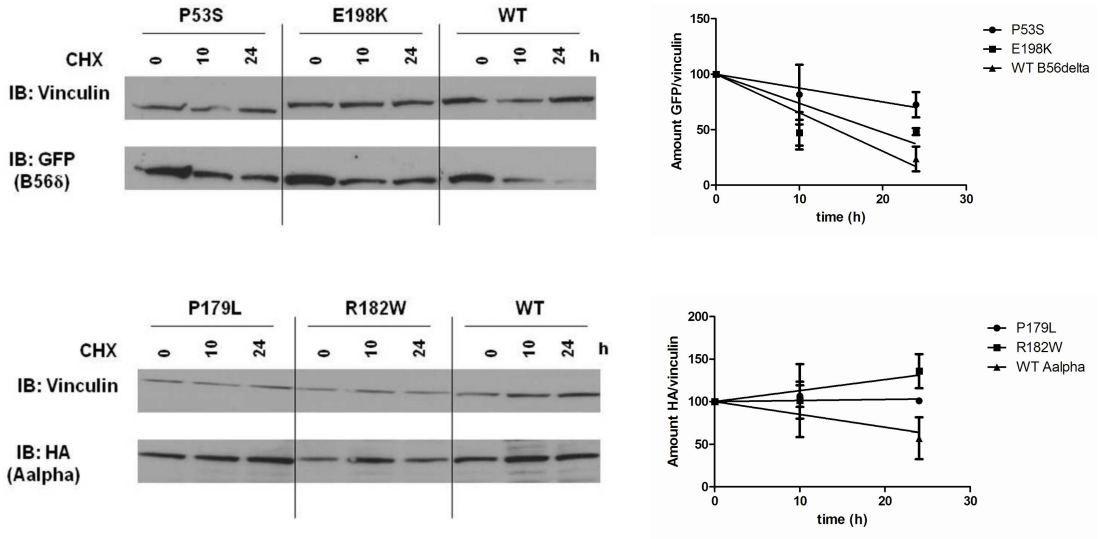
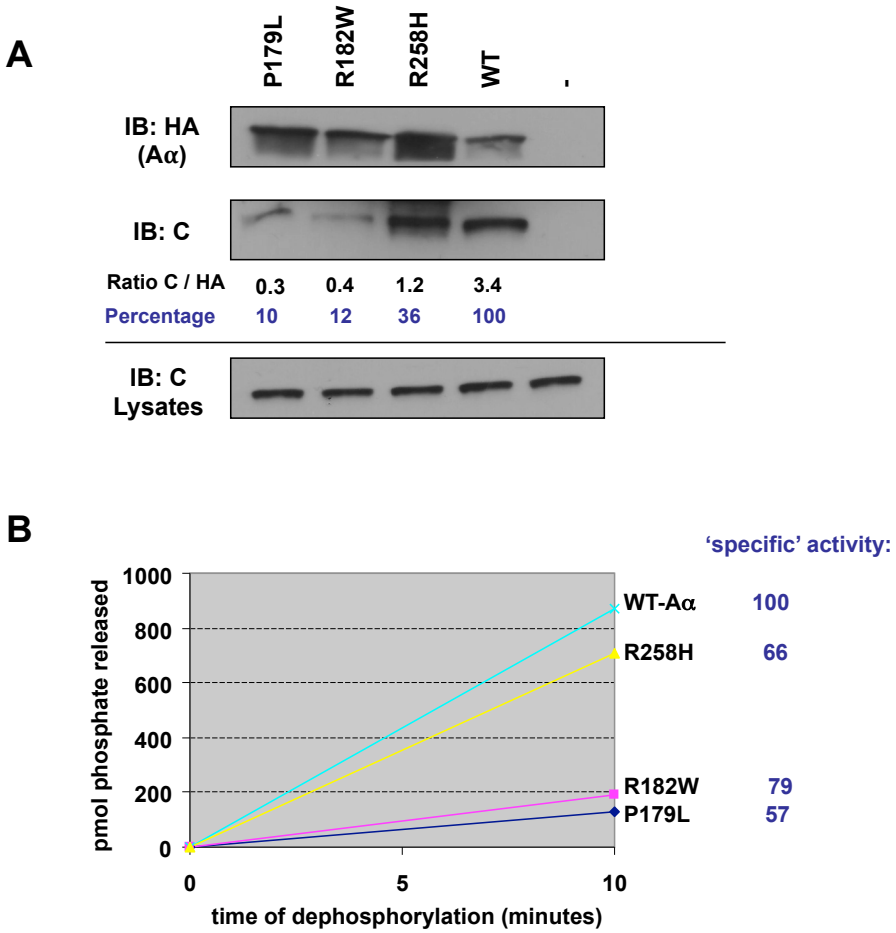
Supplementary Figure 1: Cellular binding assay of the B56δ P201S SNP found in the EVS database. EGFP-tagged wild-type B56δ, B56δ P201S, or EGFP alone (-) were ectopically expressed in HEK293 cells. Following EGFP-trapping, the presence of endogenous PP2A A and C subunits in the trapped complexes was examined by immunoblotting (IB). After quantification of the band intensities with Image J, the ratios between EGFP and C, and between EGFP and A signals were determined, and calculated relative to B56δ wild-type control. The graphs that show A or C binding abilities displays mean values of 6 independent experiments (all shown).



Supplementary Figure 2: Stability of ID-associated B56δ and Aα mutants

HEK293 cells were transfected with EGFP-B56δ (wild-type), EGFP-B56δ-P53S or EGFP-B56δ-E198K mutants (upper panels), or with HA-Aα (wild-type), HA-Aα-R183W or HA-Aα-P179L mutants (lower panels), and incubated with 50 µM cycloheximide (CHX) for different time points (0h, 10h and 24h). After lysis, the protein extracts were analyzed by immunoblotting (IB) with anti-vinculin, anti-HA or anti-GFP antibodies. Band intensities were quantified using ImageJ software. Relative HA/vinculin or GFP-vinculin levels were calculated from three independent experiments and the mean values +/- standard deviation plotted in a graph defined by linear regression (and with relative PP2A subunit/vinculin levels at time point 0h designated as 100%).





Supplementary Figure 3: Reduced specific PP2A C activity in HA-(mutant)Aα-C complexes. In panel **A**, binding of C to the HA-tagged (mutant) A subunits is determined (as described in Figure 3A). In panel **B**, the pmole number of released phosphate from the K-R-pT-I-R-R phosphopeptide (350 µM in assay) was determined by Malachite Green assay for each of the HA-(mutant)Aα-C complexes. The assay was done at 30°C for 10 minutes. Specific C activities were obtained by dividing the absolute amount of pmoles released by the amount of C in the resp. samples, as determined by immunoblotting (IB) and following quantification by Image J. All specific activities were eventually recalculated relative to Aα wild-type control (which was set to 100%).

Supplementary Table 1: Overview of additional exomic alterations of potential interest.

Case	Gene	Genomic change	Prediction / evaluation
1	*MEP1B*	*De novo* missense variant	Polymorphism
2	-	No other plausible findings	-
3	*ABCB7D*	Inherited missense variant	Polymorphism
4	*COG1*	Compound heterozygous missense variants	The phenotype was incompatible with CDG type IIG
5	*OCRL*	Inherited missense variant	The phenotype was incompatible with Lowe syndrome
6	*?*	No information available	
7	*TMEM204* *SUV420H2*	*De novo* missense variants	No known disease association
8	*?*	Unknown – detected by targeted (MIP) assay	-
9	-	No other plausible findings	-
10	-	No other plausible findings	-
11	*ELMO2*	*De novo* missense variant	No known disease association
12	-	No other plausible findings	-
13	*PKHD1*	Compound heterozygous missense variants	The phenotype was incompatible with ARPKD
14	*LST1*	*De novo* missense variant	No known disease association
15	*TMEM67*	Heterozygous inherited splice mutation	Phenotype was partly reminiscent of a ciliopathy, but a second mutation was not detected.
16	-	No other plausible findings	-

Supplementary Table 2: Oligonucleotides used for site-directed mutagenesis

Primers B56δ mutations	
P53S Forward	5'-GTCTCAGCCATCGTCATCCAAC-3'
P53S Reverse	5'-GTTGGATGACGATGGCTGAGAC-3'
E198K Forward	5'-GACCCAGAGAAAGATGAGCCC-3'
E198K Reverse	5'-GGGCTCATCTTTCTCTGGGTC-3'
E200K Forward	5'-GACCCAGAGGAAGATAAGCCCACCCTGGAAGCTGC-3'
E200K Reverse	5'-GCAGCTTCCAGGGTGGGCTTATCTTCCTCTGGGTC-3'
P201R Forward	5'-GACCCAGAGGAAGATGAGCGCACCCTGGAAGCTGCTTGGCC-3'
P201R Reverse	5'-GGCCAAGCAGCTTCCAGGGTGCGCTCATCTTCCTCTGGGTC-3'
P201S Forward	5'-GACCCAGAGGAAGATGAGAGCACCCTGGAAGCTGCTTGGCC-3'
P201S Reverse	5'-GGCCAAGCAGCTTCCAGGGTGCTCTCATCTTCCTCTGGGTC-3'
W207R Forward	5'-GCCCACCCTGGAAGCTGCTAGGCCACATCTCCAGCTCG-3'
W207R Reverse	5'-CGAGCTGGAGATGTGGCCTAGCAGCTTCCAGGGTGGGC-3'
Primers Aα mutations	
P179L Forward	5'-GCTCAGATGACACCCGCATGGTGCGGCGGGC-3'
P179L Reverse	5'-GCCCGCCGCACCATGCGGGTGTCATCTGAGC-3'
R182W Forward	5'- ACCCCCATGGTGTGGCGGGCCGCA-3'
R182W Reverse	5'- TGCGGCCCGCCACACCATGGGGGT-3'
R258H Forward	5'-AAGACAAGTCCTGGCACGTCCGCTACATGGT-3'
R258H Reverse	5'-ACCATGTAGCGGACGTGCCAGGACTTGTCTT-3'