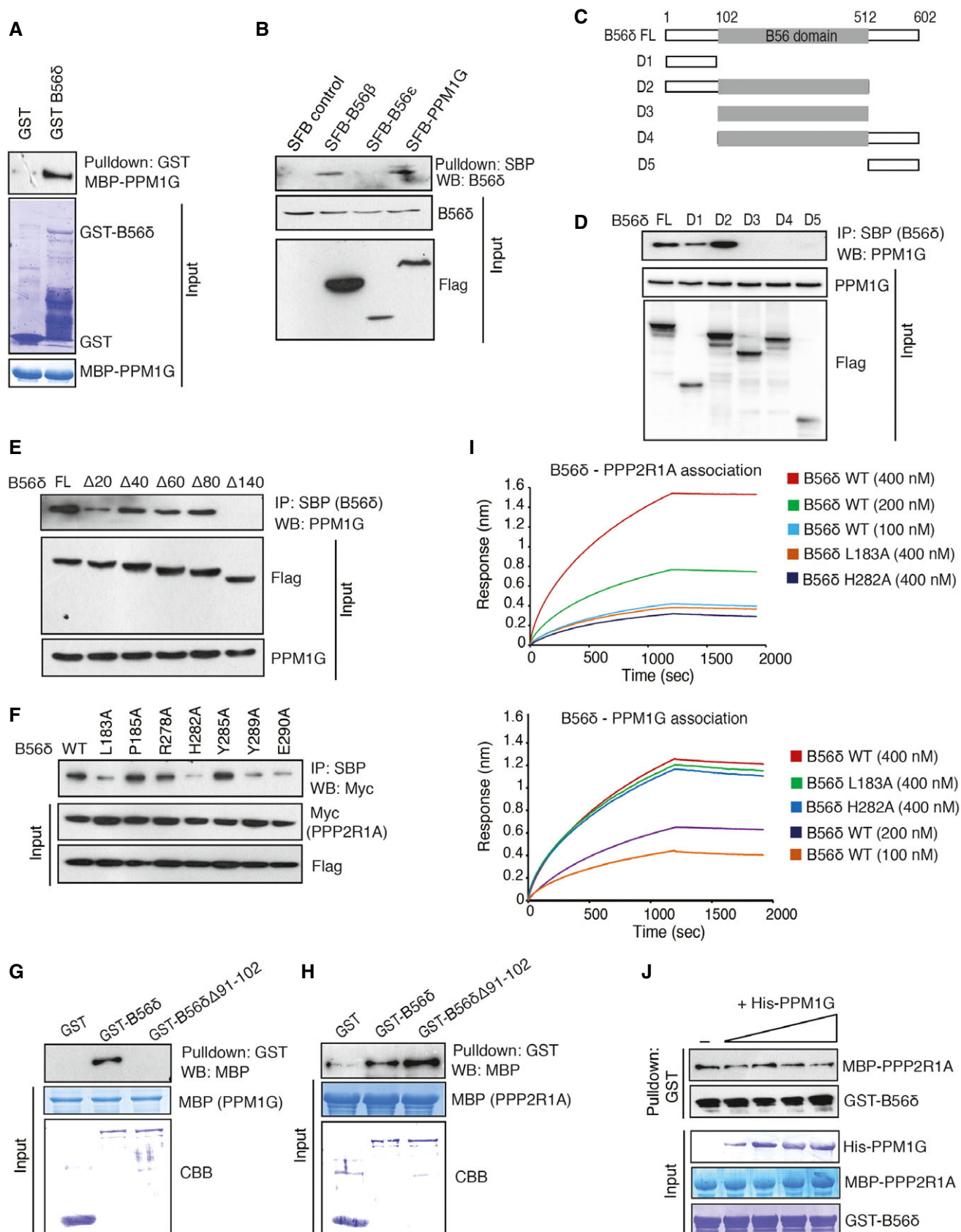


Expanded View Figures

Figure EV1. Characterization of PPM1G-B56 δ interaction.

- A Glutathione Sepharose beads bound with bacterially expressed recombinant GST or GST-B56 δ proteins were incubated with bacterially purified recombinant MBP-PPM1G, and interaction of B56 δ with PPM1G was detected by immunoblotting with MBP antibody. Recombinant protein expression is shown by Coomassie staining.
- B Cells expressing SFB vector, SFB-B56 β , SFB-B56 ϵ , and SFB-PPM1G were lysed and pulled down with streptavidin beads. Interaction of B56 δ with these proteins was detected by immunoblotting with specific antibody.
- C Schematic representation of B56 δ full length (FL), and various deletion domains.
- D B56 δ FL and various deletion domains were expressed and pulled down using streptavidin beads. Interaction of PPM1G with these proteins was detected by immunoblotting using PPM1G antibody.
- E Interaction of PPM1G with SFB-tagged B56 δ full length (FL) and its N-terminal deletion mutants was detected using pull down with streptavidin beads and immunoblotting by PPM1G antibody.
- F Interaction of Myc-tagged PPP2R1A with SFB-tagged B56 δ wild type (WT) or its various point mutants was detected using pull down with streptavidin beads and immunoblotting by myc antibody.
- G Glutathione Sepharose beads bound with GST, GST-B56 δ , or GST-B56 δ Δ 91–102 proteins were incubated with bacterially purified recombinant MBP-PPM1G, and interaction of PPM1G was detected by immunoblotting with MBP antibody.
- H Bacterially purified recombinant GST, GST-B56 δ , and GST-B56 δ Δ 91–102 immobilized on glutathione Sepharose beads were incubated with bacterially purified recombinant MBP-PPP2R1A, and interaction of PPP2R1A was detected by immunoblotting with MBP antibody.
- I Binding affinities of wild type (WT), L183A, and H282A mutants of B56 δ with PPP2R1A and PPM1G were determined by bio-layer interferometry. 6xHis-tagged PPM1G and PPP2R1A were immobilized on Ni-NTA biosensors and incubated with the GST-B56 δ wild type (WT) or L183A and H282A mutants at indicated concentrations. Curves represent experimental trace obtained from the BLI experiments ($n = 3$ independent experiments).
- J GST-B56 δ bound on glutathione Sepharose beads were first incubated with MBP-PPP2R1A. The B56 δ -PPP2R1A complex was treated with increasing concentrations of His-PPM1G, and its effect on interaction of B56 δ -PPP2R1A interaction was assessed by immunoblotting with MBP antibody after pull down with GST beads.

Source data are available online for this figure.



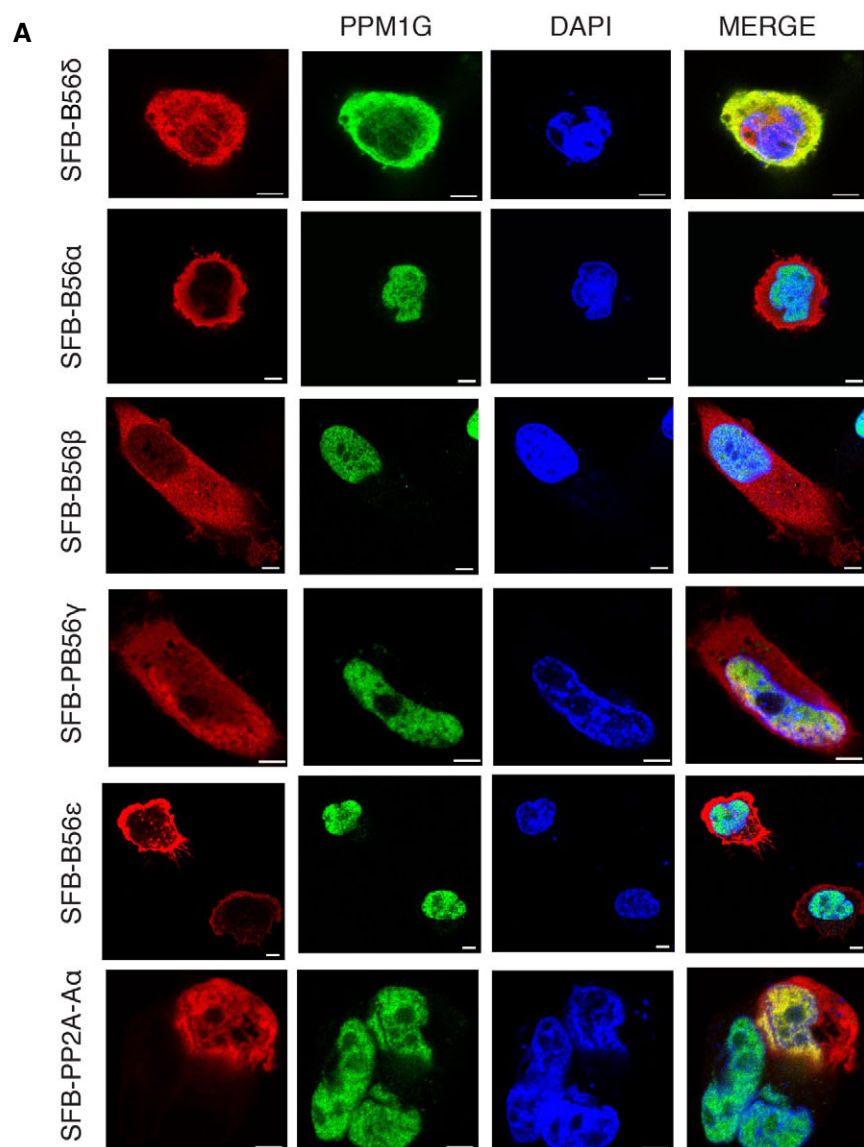


Figure EV2. Effect of B56 δ on PPM1G localization.

- A SFB-B56 α , SFB-B56 β , SFB-B56 γ , SFB-B56 ϵ , and SFB-PP2A-A α were expressed in MDA-MB-231 cells, and PPM1G localization was determined by confocal imaging after staining with PPM1G antibodies. Scale bar: 5 μ m.
- B Myc-B56 δ expressing MDA-MB-231 cells were treated with Leptomycin B (LMB) for 3 h, and then, cells were fixed and imaged using MYC and PPM1G specific antibodies. Scale bar: 5 μ m.

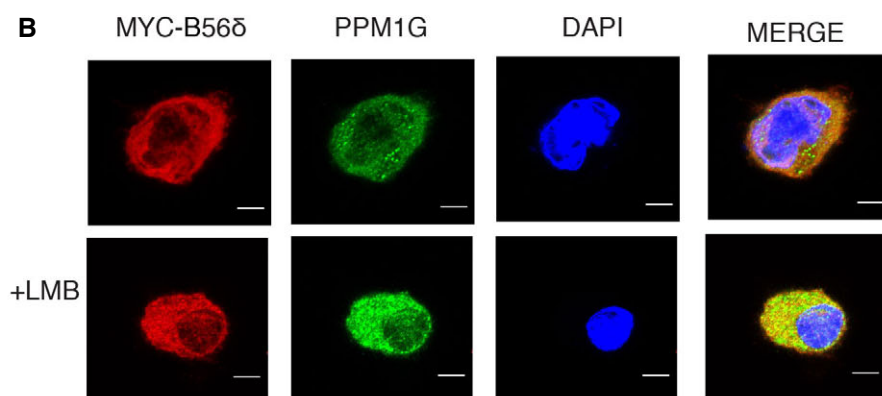


Figure EV3. Characterization of PPM1G- α -catenin interaction.

- A List of HCIPs (high confidence interacting proteins) found in purification of SFB-PPM1G using tandem affinity purification and mass spectrometric identification.
- B SFB- α -catenin and Myc-PPM1G were expressed in 293T cells. Interaction of PPM1G with α -catenin was detected by Western blotting with Myc antibody after immunoprecipitation with either IgG or Flag antibody.
- C Nuclear and cytoplasmic fractions were collected from cells expressing SFB- α -catenin. Interaction of α -catenin with PPM1G and β -catenin was detected by immunoprecipitation with Flag followed by blotting with PPM1G and β -catenin antibody, respectively.
- D Schematic representation of PPM1G full length (FL), along with its various deletion mutants (PD: phosphatase domain, AD: acidic domain).
- E 293T cells were co-transfected with the indicated SFB-tagged PPM1G constructs along with Myc-tagged α -catenin, and their interaction was determined by pull down using streptavidin beads followed by immunoblotting with Myc antibody.
- F SFB-tagged PPM1G wild type (WT) or PPM1G Δ NLS mutant were expressed in cells, and their localization was determined by immunostaining with Flag antibody. DAPI was used to counterstain nucleus.

A Partial list of interacting partners

Unique peptides	Total peptides	Uniprot ID	Gene Symbol
37	67	Q04637	EIF4G1
34	78	Q93009	USP7
24	39	P12956	XRCC6
20	35	P13010	XRCC5
12	16	P60842	EIF4A1
12	12	P20585	MSH3
11	73	Q8IZQ5	SELENOH
10	10	Q9BW19	KIFC1
9	9	P43246	MSH2
8	9	P35221	CTNNA1
8	9	O96017	CHEK2
8	8	P52701	MSH6
7	10	P05412	JUN
7	7	O75449	KATNA1
7	7	P85037	FOXK1
6	97	Q96KK5	HIST1H2AH
5	6	Q9BVA0	KATNB1
5	5	P17275	JUNB
5	5	P11802	CDK4
5	5	Q01167	FOXK2
2	32	Q93079	HIST1H2BH
2	2	P62805	HIST1H4A

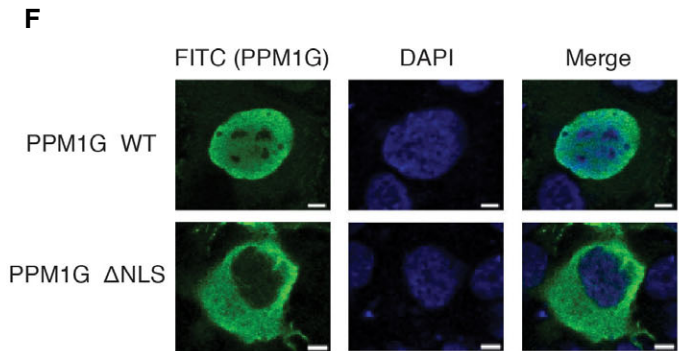
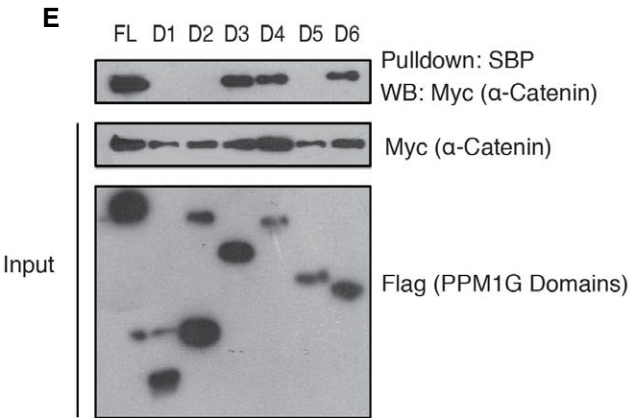
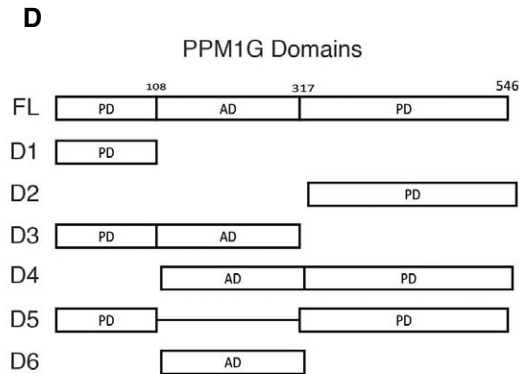
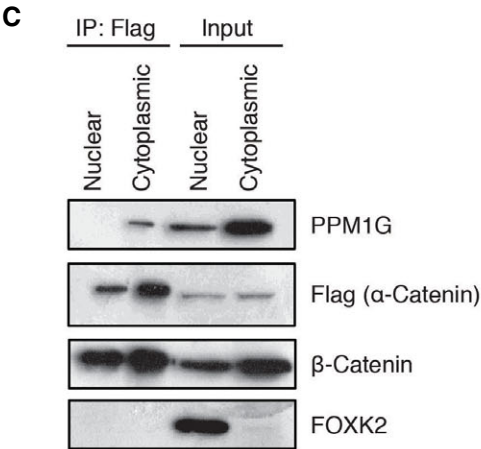
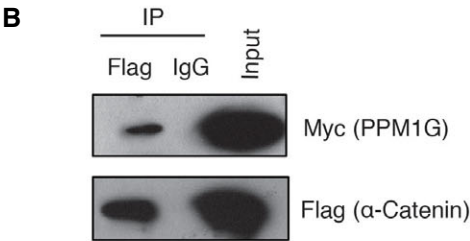


Figure EV3.

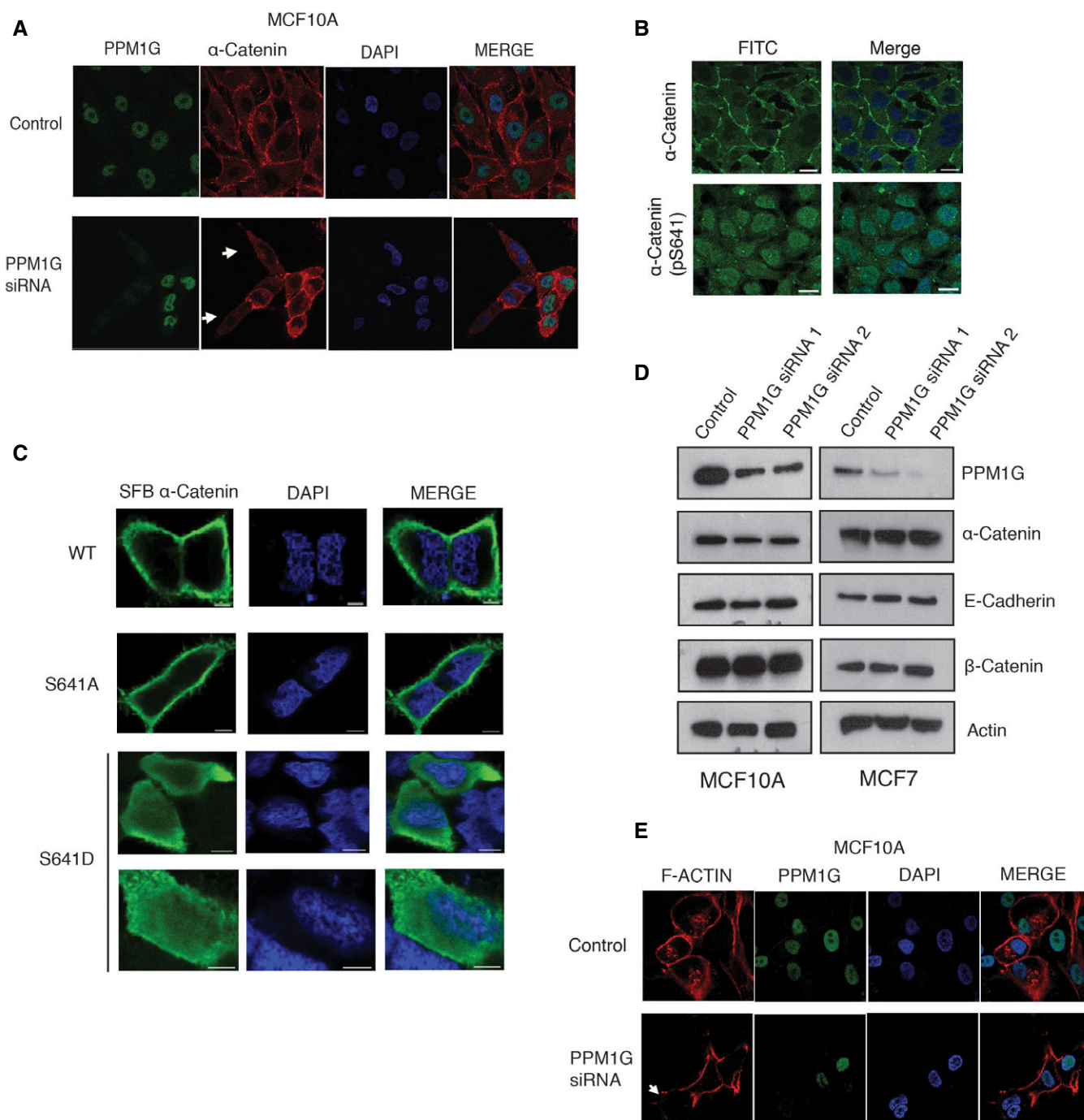


Figure EV4. Role of PPM1G-B56δ on α-catenin localization at adherens junctions.

- A MCF10A cells were transfected with either control siRNA or PPM1G siRNA, and the localization of α-catenin was determined by using immunofluorescence with a specific antibody. Arrows indicate α-catenin staining in knock down cells.
- B Localization of α-catenin and pS641 α-catenin was detected by immunofluorescence after staining the cells with specific antibodies. Scale bars, 20 μm.
- C SFB-tagged α-catenin, S641A, and S641D mutants were expressed, and their localization was detected by staining with Flag antibody. Scale bars, 5 μm.
- D Either control or two different PPM1G siRNAs were transfected into MCF10A and MCF7 breast epithelial cells. Levels of indicated proteins were determined by Western blotting with specific antibodies.
- E F-Actin in control and PPM1G-depleted cells was stained using rhodamine-labeled phalloidin, and the actin cytoskeleton was visualized by using confocal microscope. Arrows indicate F-actin staining in knock down cells.

Figure EV5. Effect of PPM1G-B56 δ on cell migration.

- A Transwell migration assays were performed with MCF10A cells expressing either control siRNA or two different PPM1G siRNAs. Quantification data for migrated cells per field (derived from average from 3 different fields) from three independent experiments are shown. Error bars indicate standard deviation ($n = 3$), *** $P < 0.001$ (one-way ANOVA, post hoc test: Bonferroni's multiple comparison test).
- B Various SFB-tagged acidic domain mutants of PPM1G derived from COSMIC database were expressed in 293T cells, and their interaction with α -catenin was detected by immunoblotting with α -catenin antibody after pull down using streptavidin beads.
- C Phosphatase activity of bacterially purified recombinant PPM1G wild type (WT) or various point mutants was measured by using pNPP as a substrate. Amount of released phosphate from pNPP was assayed colorimetrically. Error bars indicate standard deviation ($n = 3$ independent experiments), *** $P < 0.001$ (one-way ANOVA, post hoc test: Bonferroni's multiple comparison test).
- D, E Various SFB-tagged acidic domain mutants of PPM1G were expressed in MDA-MB-231 cells. Representative images from transwell migration assay (D) are shown, and (E) quantified data for migrated cells per field from three independent experiments were plotted. Error bars indicate standard deviation ($n = 3$), *** $P < 0.001$ (one-way ANOVA, post hoc test: Bonferroni's multiple comparison test).
- F Data from the migration assays performed with MCF10A cells expressing either control shRNA or two different B56 δ shRNAs were plotted. Error bars indicate standard deviation ($n = 3$ independent experiments), *** $P < 0.001$ (one-way ANOVA, post hoc test: Bonferroni's multiple comparison test).
- G MDA-MB-231 cells expressing either control shRNA, PPM1G shRNA, or B56 δ shRNA were plated in equal numbers, and rate of cell proliferation for the indicated days after plating, derived by cell counting, was plotted. Error bars indicate standard deviation ($n = 3$ independent experiments); n.s (one-way ANOVA, post hoc test: Bonferroni's multiple comparison test).
- H SFB-B56 δ was expressed in control shRNA and PPM1G shRNA-transduced MCF7 cells, and expression of different proteins was determined by immunoblotting with the indicated antibodies.
- I SFB-PPM1G was expressed in control shRNA and B56 δ shRNA-transduced MCF7 cells, and expression of different proteins was determined by immunoblotting with the indicated antibodies.
- J Control shRNA and B56 δ shRNA-transduced MCF7 cells were transfected with MYC-PPM1G and MYC-PPM1G Δ NLS, and expression of different proteins was determined by immunoblotting with the indicated antibodies.
- K SFB-tagged α -catenin S641A and S641D mutants were expressed in PPM1G shRNA and B56 δ shRNA-transduced MCF7 cells, and expression of different proteins was determined by immunoblotting with the indicated antibodies.

Source data are available online for this figure.

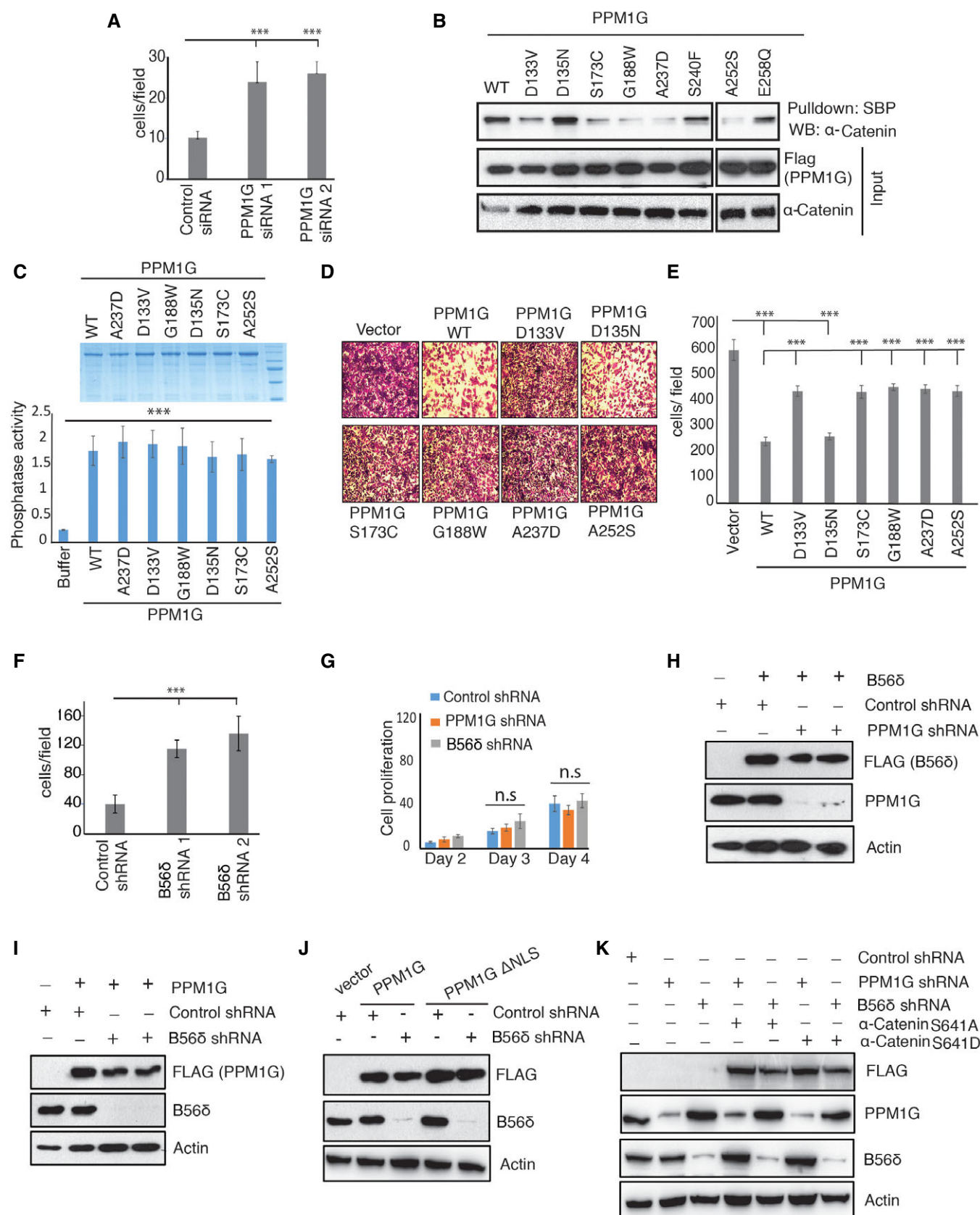


Figure EV5.