**Supplementary Figure 1.**

**a**, Summary table of the number of mutations identified in *PPP2R1A* in the TCGA and MSK-IMPACT EMCA tumor cohorts, separated into groups defined by the EMCA histological subtype. **b**, Compiled stage distribution of tumors that harbor a *PPP2R1A* P179 site mutation revealed that at least 60% of patients have high stage, metastatic (stages III, IV) disease. Also shown are the stage distributions of wildtype uterine tumors with serous carcinoma (USC), endometrioid carcinoma (UEC), or carcinosarcoma (UCS) histology. **c**, Kaplan-Meier curves for disease free survival in P179-mutant tumors versus wildtype USC and UEC tumors (note: survival data for UCS was not available on cbioportal.com). **d**, Mutational burden and frequency of microsatellite instability (MSI)-positivity, and **e**, Copy number alteration data for P179*-*mutant tumors and wildtype USC, UEC, and UCS tumors. Data presented as mean ± SEM. **f**, Frequency of alteration in four patient cohorts (P179 mutant tumors; all USC tumors; all UEC tumors; all UCS tumors) of genes that are commonly altered in serous or endometrioid tumors [8]. In all instances, the alteration frequency in P179-mutant tumors trends with that seen in USC. All data was accessed through cbioportal.com under the MSK-IMPACT sequencing cohort, or the TCGA uterine and TCGA uterine CS studies for the PanCancer Atlas [64, 65].

**Supplementary Figure 2.**

**a**, Percentage of the patient population harboring a *PPP2R1A* site mutation, including hotspot sites P179, S256, R183, in three different tumor biobanks (TCGA and MSK-IMPACT data accessed through cBioportal.org) [64, 65]. **b**, Summary table of the Case CCC biobank tumor samples found to harbor a HEAT 5 or 7 ‘hotspot’ mutation following targeted Sanger sequencing of this region. **c**, Plots of the Sanger sequencing results for several tumors with P179 site mutations (arrow indicates residue 179). Sequencing results for UT89 is provided as an example of the wildtype sequence. Stable cell lines derived from the tumors UT89 and UT42 were used for experiments carried out in this report.

**Supplementary Figure 3.**

**a**, In a cell-free pull-down assay, GST-tagged B56α binds both wildtype Aα and Cα subunits. When wildtype (WT) Aα is replaced with P179R or P179L mutant isoforms, B56α displays markedly reduced binding with both Aα and Cα subunits. WT and mutant samples were run on the same gel; asterisks (\*) denote two lanes of samples that have been cropped. **b**, Across three replicates, B56α interaction with A- and C-subunits was significantly reduced with P179 mutant Aα isoforms relative to wildtype Aα (\*\*\* p<0.001).

**Supplementary Figure 4.**

**a-d**, Convergence plots of CV1 φ (**a**) and CV2 ψ (**b**), as calculated from Well-tempered metadynamics simulations, and diffusive behavior of CV1 (**c**) and CV2 (**d**) over the course of the apo WT-Aα (P179) simulation. **e-h**, Corresponding plots from the WT Aα/C complex simulation. **i-l**, Corresponding plots from the apo P179R-Aα (R179) simulation. **m-p**, Corresponding plots from the P179R Aα/C complex simulation. **q-t**, *Cis-trans* isomerisation of the ω bond as calculated in simulations for apo Aα P179 (**q**) and apo Aα R179 (mutant) (**s**), and for P179 (**r**) and R179 (**t**) in the Aα/C complex. The ω dihedral angle is defined as Cα-C-N-Cα preceding the residue at position 179 (0o for *cis* and ±180o for *trans* configuration).

**Supplementary Figure 5.**

**a**, Western blots of protein isolated from patient tumor tissue. Samples are part of the Case CCC biobank; tumor ID, stage / grade, and *PPP2R1A* status are indicated above images. Samples have been evaluated for abundance of PP2A subunits A, B55α, and C. Normalization std = isolated tumor protein that was prepped at double volumes and loaded equally in both gels. **b**, Calculated ratio of C-subunit or B55α-subunit relative to A-subunit for individual tumor samples. Quantifications for each protein were normalized to densitometry of stainfree images of total protein content per lane. **c**, Stainfree images of each membrane. Data presented as mean ± SEM. Statistical significance was determined by Student’s t-test. \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001, n.s. = not significant.

**Supplementary Figure 6.**

**a**, Representative images of colony formation by UT89 cells expressing EGFP, WT-Aα, or P179R-Aα (n=3). **b**, Growth of UT89 EGFP, WT-Aα, or P179R-Aα tumors that formed following sub-cutaneous cell injection in nude mice (n=10). Volume measurements presented as group mean ± SEM. Statistical significance was determined by Student’s t-test. \* p ≤ 0.05. **c**, Summary table of the number of animals per experimental group and distribution across days of intra-uterine UT42 cell injection. Compiled images of the isolated gynecologic tracts show the uterine corpus gross appearance and tumor presence; image borders are color-coded to indicate the day of injection. # - denotes animals judged to have no macroscopic / gross tumor presence. **d**, Summary table of the number of animals per group presenting with primary uterine tumor formation and metastasis at 8 weeks post-injection of tumor cells expressing EGFP or WT-Aα (n=7). Tumor cell presence in uterine tumor foci and suspected metastatic nodules was confirmed through microscopic inspection of H&E stained slides. **e**, H&E stained section of representative metastatic nodules (left, middle) and a liver parenchymal metastasis (left, right) from an EGFP animal. Images are 0.36x (scale bar = 1 mm), 4x (scale bar = 100 μm), and 10x (scale bar = 100 μm). **f**, Wound closure was assessed via scratch wound assay and revealed no significant reduction in migratory behavior for cells expressing WT-Aα relative to those expressing EGFP (n=3).

**Supplementary Figure 7.**

**a**, Sensitivity to SMAP-061 was evaluated for four EMCA cell lines: the *PPP2R1A* P179R+ mutant cell line UT42, and three *PPP2R1A* wildtype cell lines (UT89, UT150, UT185). All four lines are primary cell lines derived from patient tumors. Dose-Response curves were constructed following 72 hrs of treatment with SMAP-061 across a range of doses. % growth represents the fold change in cell count from t = 0 hr to t = 72 hr, and all values were normalized to that of DMSO (n=3). Nonlinear fit curves were generated using GraphPad Prism to determine the IC50 dose and 95% confidence interval (CI) for each cell line. **b**, Representative images of colony formation by UT42 and UT89 during a two-week treatment with SMAP-061 at the indicated doses (n=3). **c**, Western blotting was performed on protein isolates from UT42 PDX tumors treated with vehicle (DMA) or SMAP-1154 (tumors correspond to the *in vivo* tumor growth data presented in **Figure 6g-j**) to evaluate for dephosphorylation of the PP2A substrates Akt, GSK3β, and c-Myc. DMA tumors #3 and #4 were loaded on both gels. **d**, Quantification of western blotting band intensities presented as mean ± SEM. The phosphorylated protein signal was normalized to that of the total protein (P:T ratio), followed by normalization to the P:T ratio of DMA#3 from the same gel. Note: SMAP 50 mg/kg tumor #3 was excluded from the graphs.

**Additional Methods Information**

***Generation of stable cell lines*:** The Gateway V5-tagged lentiviral expression vector pLX304-*PPP2R1A* was obtained from the DNASU Plasmid Repository (HsCD00444402), deposited by the ORFeome Collaboration. Site-directed mutagenesis was performed using the QuickChange Lightning Kit (Agilent, 210513) to generate the pLX304-*PPP2R1A*-P179R, -S256F, and -R183W missense mutation plasmids. Gateway cloning was performed to insert EGFP into the pLX304 vector. All construct sequences were verified by Sanger sequencing.

Lentivirus was produced through co-transfection of 10 μg plasmid with the second-generation packaging constructs pMD2.G (Addgene, 12259) and psPAX2 (Addgene, 12260) into HEK293T cells using X-tremeGENE HP DNA transfection reagent (Sigma). HEK293T cells were plated 24 hours in advance in Pen-Strep free DMEM. Lentivirus-containing media was collected at 24 and 48 hours, filtered through a 0.45 μm filter, supplemented with 8 μg/ml polybrene (Santa Cruz), and applied to target cells. Target cells were cultured for an additional 48 hours before beginning selection with 16 μg/ml Blasticidin (Invivogen).

***Immunoblotting*:** To obtain whole cell protein extracts, adherent cells were detached from plates with 0.25% Trypsin (ThermoFisher, SH30042.02), washed with ice cold PBS, and lysed with cold RIPA buffer (ThermoFisher, P189901). Tumor protein extracts were obtained through mechanical homogenization of tissue in T-PER buffer (ThermoFisher, 78510). Lysis buffers were prepared with protease and phosphatase inhibitor tablets (Roche, 05892791 and 04906837). Protein concentration was quantified using the Pierce BCA protein assay kit (ThermoFisher) and equal protein quantities were loaded into polyacrylamide TGX Stain-free gels (Bio-Rad) for separation by SDS-PAGE. Proteins were transferred to 0.2 μM nitrocellulose membranes using the Bio-Rad Trans-blot Turbo transfer system. Membranes were blocked for 1 hour at room temperature with 5% non-fat milk powder in TBS-T and incubated with primary antibodies diluted in 5% milk overnight at 4°C. Membranes were then washed with TBS-T and incubated with species-specific HRP-conjugated secondary antibody for 1 hour at room temperature. For the immunoblotting of tumor samples grown in mice, a conformation-specific anti-mouse secondary (Abcam, ab131368) was used when primary antibodies were from a mouse host. The ECL chemiluminescent detection system (Amersham) was utilized in conjunction with the Bio-Rad ChemiDoc imaging platform. Densitometry quantification was performed using Bio-Rad Image Lab software. Quantified densitometries were normalized to corresponding values for loading controls (Vinculin, GAPDH), unless otherwise noted.

Table listing the primary antibodies that were used for immunoblotting:

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***Real-time PCR*:** RNA was isolated using the High Pure RNA Isolation kit (Roche) and the concentration of eluted RNA was quantified using a NanoDrop. 1 μg of RNA was converted to cDNA using the iScript cDNA Synthesis kit (BioRad). cDNA was diluted 1:10 with sterile H2O and real-time PCR was performed in triplicate using SYBR-Green reagent according to manufacturer instructions.

Primer sequences utilized for RT-PCR:

PPP2CA: F1 5’-AGGAGCTGGTTACACCTTTG-3’

PPP2CA: R1 5’-GCACCAGTTATATCCCTCCATC-3’

PPP2CA: F2 5’-CCTCTGCGAGAAGGCTAAAG-3’

PPP2CA: R2 5’-GCCCATGCACATCTCCACAG-3’

PPP2R2A: F1 5’-CCACCTTTATCTCCTGTTGC-3’

PPP2R2A: R1 5’-TTTCTCAGGTGAAAGGAGCAG-3’

PPP2R2A: F2 5’-CCACCTTTATCTCCTGTTGCT-3’

PPP2R2A: R2 5’-GGTGTTTTTCTCAGGTGAAAGG-3’

PPP2R5A: F1 5’-CTTGTTGTTCATTTGAAGTGGC-3’

PPP2R5A: R1 5’-AGGGCTCGTCGCAGTTTC-3’

PPP2R5A: F2 5’-CACTGCTGCAACTTCTGACA-3’

PPP2R5A: R2 5’-AGGGCTCGTCGCAGTTT-3’

***Proteasome inhibition and half-life studies*:** Table providing details of the statistical analysis of data from the cycloheximide experiments (**Figure 4f-g**). Raw data had been Ln-transformed and submitted to linear regression analysis using GraphPad Prism. First, analysis of covariance (ANCOVA) was performed to determine if the regression lines were different, which revealed that at least one slope was significantly different between the groups (EGFP, WT, P179R). Next, an ANOVA with Tukey’s post-hoc t-tests was performed on the slope and standard error values provided by the Prism regression in order to identify between which specific pairs there was a significant difference.

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***Clonogenicity assay*:** Colony formation was assessed through seeding of 250 (UT89) or 500 (UT42) cells per well of a 6-well plate. Plates were incubated for 8-10 days, with media changes every 2-4 days (for colony formations with SMAP-061 treatment, media is changed every 2 days). At end, plates were triple-washed with cold PBS, fixed for 30 minutes with 10% acetic acid and 10% methanol in diH2O on a rotating shaker, and then stained with 1% crystal violet dissolved in methanol. Colonies were counted using ImageJ software.

***Protein expression, purification, crystallization and structure determination*:** Table elaborating the crystallographic data collection and refinement statistics as applies to the determination of a *PPP2R1A* P179R mutant crystal structure (**Figure 2d-e**). AU, asymmetric unit; Rmsd, root-mean-square deviation. Values in parentheses indicate the corresponding statistics in the highest-resolution shell. CC1/2 is the correlation coefficient between symmetry-related intensities taken from random halves of the dataset.

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***Molecular dynamics simulations*:** To sample conformations separated by high energy barriers, which may not have been visited using unbiased simulations, Well-tempered metadynamics (WTMetaD) of the PP2A wildtype and mutant structures was performed at 300 K using ACEMD software and the PLUMED plugin using an integration step of 4 fs [33, 34]. To study the influence of the mutations on the structure, we chose the dihedral angles of the WT/mutated residues to be the collective variables (CV). The choice of the CVs (CV1 = φ phi and CV2 = ψ psi) was based on the observation that the slowest motions in a protein are a function of their backbone flexibility [35]. Therefore, the differences in the structural effects resulting from the changes between wildtype and mutant should be pronounced in the dihedral angles. A total of 450 ns (P179 Aα), 550 ns (P179 Aα/C complex), 400 ns (R179 Aα), and 400 ns (R179 Aα/C complex) in the NVT ensemble were needed to reach full convergence of the free energy.

The free energy surface of the WTMetaD simulation as a function of the two CVs is readily obtained by integrating the deposited energy bias along the trajectory. The error on the minima and barriers of the free energy surface was estimated from the largest variation observed in the mono-dimensional projections along the collective variables during the last 100 ns of the simulation. It amounts to 0.5 kcal/mol. The sampling convergence was checked by comparing the reconstructed free energy surfaces at different time intervals during the last 50 ns of the simulations. Production simulations were initiated from the final snapshot of the corresponding equilibration simulations. The bias factor was determined by the kT value in the corresponding sampling temperature and the initial Gaussian height to 0.5 kcal/mol, deposited every 1 ps. The Gaussian width was set to 0.3 for the two CVs, respectively. The free energy surface of the metadynamics simulation as a function of the two CVs is readily obtained by integrating the deposited energy bias along the trajectory. The structures corresponding to the minima were selected from the metadynamics trajectories, based on the values of collective variables CV1 and CV2. To retrieve representative structures of the minima, the conformations corresponding to each minimum on the landscape were clustered based on Cα RMSD. The representative conformations of the most highly populated clusters are depicted.

The structures corresponding to the minima were selected from the WTMetaD trajectories, based on the values of collective variables CV1 and CV2. The RMSF was calculated from the unbiased simulations of 1000 ns of the wildtype and mutant, using the g\_rmsf tool of the GROMACS analysis toolkit [36]. To remove the effect of slow, large-scale conformational transitions, the RMSF was calculated on the Cα atoms in overlapping windows of 100 ns each and averaged afterwards. The first 200 ns of the simulation were considered equilibration time and omitted from the calculation. The dihedral angles for the wildtype and mutant protein systems were performed using GROMACS analysis toolkit [36]. All unbiased and biased simulations were analyzed using functionalities available on GROMACS 5.1.2 analysis toolkit. The first 150 ns of each simulation were considered equilibration time and omitted from the calculation. The charges were assigned on the proteins using PDB2PQR functionality [37]. The binding energy between the two subunits was calculated using the APBS program using a default protocol [38]. All structural figures were generated using ICM-Pro software [27].