SI materials and methods

Plasmid Generation

Original plasmids of the wild-type p110α, p110δ and p85α were a kind gift from the Williams laboratory at the LMB. Immunodeficiency single substitution APDS1 mutations in p110 or the APDS2 splice variant of p85 were generated using site-directed mutagenesis according to published protocols.

Protein Expression and Purification

All WT and p110δ mutant PI3K complexes were similarly expressed as previously described (12). To express PI3K complexes, an optimised ratio of p110δ:p85α baculovirus was used to co-infect *Spodoptera frugiperda* (Sf9) cells between 1-2×10^6 cells/mL. Co-infections were harvested between 40-72 hours and washed with ice-cold PBS before snap-freezing in liquid nitrogen. PI3K WT and APDS1 mutant proteins were purified in an identical method by lysing cells and performing nickel affinity, anion exchange, and size exclusion purifications. All steps in protein purification were carried out on ice, or in a 4°C cold room. Frozen Sf9 pellets were re-suspended in lysis buffer (20 mM Tris pH 8.0, 100 mM NaCl, 10 mM imidazole pH 8.0, 5% glycerol (v/v), 2 mM bME, protease inhibitor (Protease Inhibitor Cocktail Set III, Sigma)) and sonicated on ice for 1 minute (15s on, 15s off, level 4.0, Misonix sonicator 3000). Triton X-100 was added to the lysate at a concentration of 0.1% and centrifuged at 20,000 g for 45 minutes (Beckman Coulter Avanti J-25I, JA 25.50 rotor). The supernatant was then loaded onto a 5 mL HisTrap™ FF column (GE Healthcare) that had been equilibrated in NiNTA A buffer (20 mM Tris pH 8.0, 100 mM NaCl, 10 mM imidazole pH 8.0, 5% (v/v) glycerol, 2 mM bME). The column was washed with 20 mL of NiNTA buffer, 20 mL of 6% NiNTA B buffer (20 mM Tris pH 8.0, 100 mM NaCl, 200 mM imidazole pH 8.0, 5% (v/v) glycerol, 2 mM bME) before being eluted with 100% NiNTA B. The elution was loaded onto a 5 mL HiTrap™ Q HP column (GE Healthcare) equilibrated in Hep A buffer (20 mM Tris pH 8.0, 100 mM NaCl, 5% (v/v) glycerol, 2 mM bME). Protein was eluted using a gradient of Hep A buffer and Hep B buffer (20 mM Tris pH 8.0, 1 M NaCl, 5% (v/v) glycerol, 2 mM bME). Fractions were pooled and concentrated in a 50,000 MWCO Amicon concentrator (Millipore). Concentrated proteins were injected onto a Superdex™ 200 10/300 GL Increase size-exclusion column (GE Healthcare) equilibrated in Gel Filtration Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM tris(2-carboxyethyl)phosphine (TCEP)). Proteins were concentrated and frozen at a concentration between 0.5-10 mg/mL.

Purification of p110/p85α (Δ434-475) APDS2 Mutants

Complexes of p110 catalytic subunits with APDS2 mutants p85α (Δ434-475) were highly unstable, and expressed at a much lower level than WT or APDS1 mutants. The only way to generate pure protein (>90% by SDS page analysis) was

through the use of a N-terminal streptavidin tag. Protein was unstable to concentration, and for this reason all purifications and analyses were carried out within 10 hours. Both PI3K wild-type (p110/p85α) and PI3K APDS2 mutants (p110/p85α Δ434-475) containing a N-terminal streptavidin (strep) tag (Strep-tag® II) in the p110 subunit were expressed and lysed as previously described. All steps were performed at 4°C or on ice. The supernatant was loaded onto a 1 mL StrepTrap™ HP column (GE Healthcare) equilibrated in Hep A-Deletion buffer (20 mM Tris pH 8.0 RT, 100 mM NaCl, 10% (v/v) glycerol, 2 mM βME). The StrepTrap™ HP column was washed with 3 mL of Hep B-Deletion buffer (20 mM Tris pH 8.0 RT, 1 M NaCl, 10% (v/v) glycerol, 2 mM βME) followed by 3 mL of HEP A-Deletion buffer. To cleave the strep-tag, 1 mL of a TEV protease solution (~0.08 mg/mL) was loaded onto the column and incubated for 3 hours. Protein was eluted using 2 mL of Hep A-Deletion buffer. The entire StrepTrap™ HP elution was loaded onto a 1 mL HiTrap™ Q HP column (GE healthcare) equilibrated in Hep A-Deletion buffer. The HiTrap™ Q HP column was washed with 3 mL of Hep A-Deletion buffer to remove the TEV protease. PI3Ks were eluted using 2 mL of HEP Elution Buffer (20 mM Tris pH 8.0 RT, 350 mM NaCl, 10% (v/v) glycerol, 2 mM βME) into 100 μL fractions. The concentration of PI3K in each fraction was determined via NanoDrop (Thermo Scientific) and corrected using band intensities following Coomassie staining on SDS-PAGE. The fractions with the highest concentrations were pooled and used for subsequent experiments.

Lipid Vesicle Preparation

Two different types of lipid vesicles were made, one mimicking the composition of the plasma membrane (5% brain phosphatidylinositol 4,5- bisphosphate (PIP_2), 30% brain phosphatidylserine (PS), 50% brain phosphatidylethanolamine (PE), 15% brain phosphatidylcholine (PC)) and a highly negatively charged (5% C8:PIP_2, 95% brain PS) substrate, which is the optimal substrate for class IA PI3Ks. Vesicles were prepared by combining lipid components dissolved in organic solvent and evaporating the solvent under a stream of N_2 gas. The lipid film was desiccated under vacuum for 60 minutes. Lipids were resuspended at 1 mg/mL in lipid buffer (20 mM HEPES pH 7.5 (RT), 100 mM KCl, 0.5 mM EDTA) followed by sonication for 10 minutes. The vesicle solutions were subjected to three freeze-thaw cycles. Vesicles were extruded 11 times through a 100 nm filter using an Avanti mini-extruder (Avanti). Vesicles were snap-frozen in liquid nitrogen and stored at -80°C.

Lipid Kinase Assays

Lipid kinase assays monitoring hydrolysis of ATP were carried out using the Transcreener ADP^2 Fluorescence Intensity (FI) assay (Bellbrook labs). Lipid vesicles were used at a final concentration of 0.45 mg/ml, with ATP present at 100 μM. Protein solutions containing either pY (PDGFR residues 735–767, with pY740 and pY751, referred to afterwards as

pY; final concentration in assay 5 μM) or blank solution in 2X PI3K kinase buffer (100 mM HEPES pH 7.5, 200 mM NaCl, 6 mM $MgCl_2$, 2 mM EDTA, 0.06% CHAPS, 2 mM TCEP) was equilibrated briefly at 23°C. Kinase reactions were started by addition of 2 μL of protein solution to 2 μL of 2X substrate solution (0.9 mg/mL lipid vesicles, 200 μM ATP) in a 384-well black microplate (Corning). The reaction was allowed to proceed at 23°C for 60 minutes before the addition of 2X Stop and Detect buffer (1X Stop and Detect Buffer, 8 nM ADP Alexa594 Tracer, 93.7 μg/mL ADP[2] Antibody-IRDye QC-1). Antibody, tracer, and ADP were equilibrated for 60 minutes. Fluorescence intensity was measured using a Spectramax M5 plate reader with $\lambda_{excitation} = 590$ nm and $\lambda_{emission} = 620$ nm (20 nm bandwidth; Molecular Devices). Specific activity was calculated using an ATP/ADP standard curve according to the Transcreener ADP FI protocol. Fold activation shown in Fig. 1 was determined by normalising mutant specific activity values to the specific activity of the wild-type PI3K complex.

The potent PI3K inhibitor Idelalisib (SelleckChem) was used for IC50 measurements. Dilutions were generated from a 1 mM master stock of Idelalisib in 100% DMSO. Inhibitor was diluted to 100 μM in DMSO, and subsequent dilutions were all carried out in 1% final DMSO. Inhibitor dilution curves were carried out in triplicate and then mixed with substrate solution. All other lipid kinase assay steps were carried out according to the protocol described above. Values were imported into Prism (GraphPad Software, La Jolla, CA) for graphing and calculations of IC50 values. Statistical analysis for differences in lipid kinase activity shown in figures were carried out using a paired student t-test.

Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS)

HDX experiments were conducted in 50 μL reactions with a final concentration of 120 nM for APDS1 mutants and WT PI3Kδ. Three conditions were tested: PI3K alone, and PI3K in the presence of phosphopeptide (5 μM pY) with and without lipid vesicles (5% PIP_2, 30% PS, 50% PE, 15% PC present at 100 μg/mL). Deuterium exchange was initiated by the addition of 40 μL deuterated buffer (10 mM HEPES pH 7.5, 100 mM NaCl, 98% (v/v) D_2O). Exchange was carried out for three time points (3 s, 30 s, and 300 s at 23°C) and terminated by the addition of 20 μL ice-cold quench buffer (2 M guanidine-HCl, 3% formic acid). For APDS2 experiments, 70 μL reactions with a final concentration of 60 nM were used. Deuterium exchange was initiated by the addition of 50 μL deuterated buffer and exchange was carried out for two time points (3s, 300s at 23°C). The reaction was terminated by the addition of 25 μL quench buffer. All experiments were carried out in triplicate. Samples were immediately frozen in liquid nitrogen and stored at -80°C.

Protein samples were rapidly thawed and injected onto a UPLC system at 2°C. The protein was run over two immobilized pepsin columns (Applied Biosystems; porosyme, 2-3131-00) at 10°C and 2°C at 200 μL/min for 3 minutes, and peptides were collected onto a VanGuard precolumn trap (Waters). The trap was subsequently eluted in line with an Acquity 1.7 μm particle, 100 × 1 mm^2 C18 UPLC column (Waters), using a gradient of 5-36% B (buffer A 0.1% formic acid, buffer B

100% acetonitrile) over 16 minutes. Mass spectrometry experiments were performed on an Impact II TOF (Bruker) acquiring over a mass range from 150 to 2200 *m/z* using an electrospray ionization source operated at a temperature of 200°C and a spray voltage of 4.5 kV. Peptides were identified using data-dependent acquisition methods following tandem MS/MS experiments (0.5 s precursor scan from 150-2000 m/z; twelve 0.25 s fragment scans from 150-2000 m/z). MS/MS datasets were analyzed using PEAKS7 (PEAKS), and a false discovery rate was set at 1% using a database of purified proteins and known contaminants.

HD-Examiner Software (Sierra Analytics) was used to automatically calculate the level of deuterium incorporation into each peptide. All peptides were manually inspected for correct charge state and presence of overlapping peptides. Deuteration levels were calculated using the centroid of the experimental isotope clusters. Results for these proteins are presented as relative levels of deuterium incorporation and the only control for back exchange was the level of deuterium present in the buffer (78%-PI3K, 70%-Deletion). The real level of deuteration will be ~25–35% higher than shown, based on tests performed with fully deuterated standard peptides. The average error of all time points and conditions for each HDX project was less than 0.2 Da. Therefore, changes in any peptide at any time point greater than both 7% and 0.7 Da between conditions with a paired t-test value of $p<0.05$ was considered significant. The full deuterium incorporation for all experiments is shown in Fig. S4+S5+S6. Differences between conditions in APDS1 are shown in Fig. S7 and mapped onto a structural model in Fig. S8, and a representative example of HDX data processing is shown in Fig S2.

Supplemental Figures

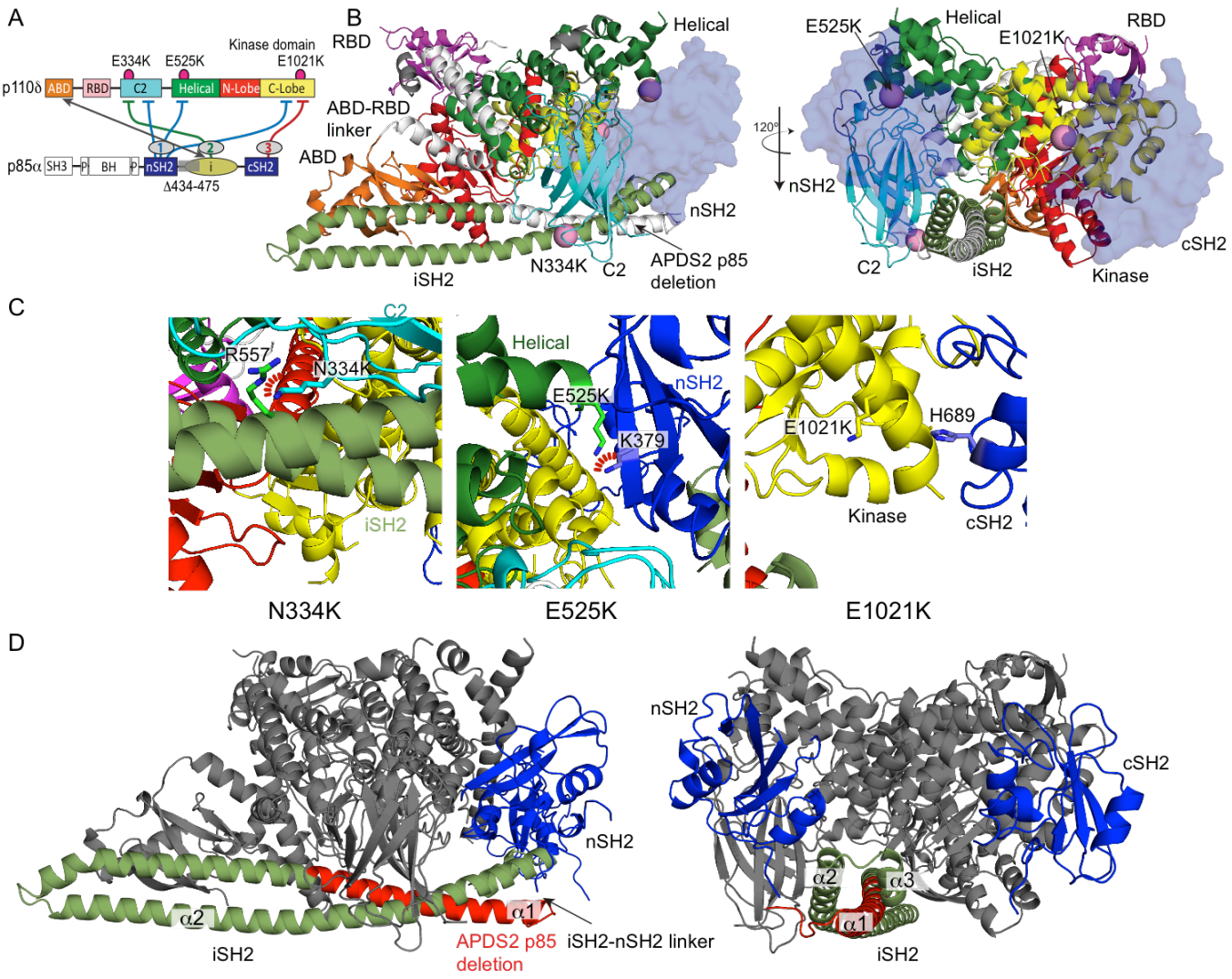


Fig. S1. Location of APDS1 and APDS2 mutations on a structural model of the p110δ/p85α nSH2-iSH2-cSH2 model. (**A**) Domain schematic of both p110δ/p85α with the binding interface between the iSH2-ABD highlighted, as well as inhibitory interfaces between the nSH2-C2/helical/kinase domains (**1**), iSH2-C2 domains (**2**), and cSH2-kinase domains indicated (**3**). (**B**) Structural model of the PI3Kδ complex based on the structure of the p110δ/p85α iSH2 structure (PDB:5DXU (37)), with the nSH2 modelled from the structure of p110α/p85α nSH2-iSH2 structure (PDB: 3HHM, (38)), and the cSH2 modelled from the structure of p110β/p85β iSH2-cSH2 (PDB:2Y3A (13)). The nSH2 and cSH2 are shown in a transparent surface representation, with APDS1 mutations studied shown as pink spheres. The APDS2 truncation in the N-terminus of the p85α iSH2 is coloured in white. (**C**) Highlight of the environment of APDS1 mutations. The N334K mutation is located at the C2/iSH2 domain interface, and there would be a predicted charge/charge repulsion between K334 and R557. The E525K mutation is located at the nSH2-helical domain interface, and there would be a predicted charge/charge repulsion between K525 and K379. The E1021K mutation is located in the C-terminus of the kinase domain, near the cSH2 interface in

the kinase domain. (**D**) The APDS2 p85α deletion mapped onto the structural model previously described in Fig. S1.B, with SH2 domains shown in cartoon representation. The p110δ subunit is coloured in grey, the iSH2 domain is coloured green with the three helices of the coiled coil labelled, the nSH2 and cSH2 are coloured in blue and the APDS2 p85α deletion is highlighted as red.

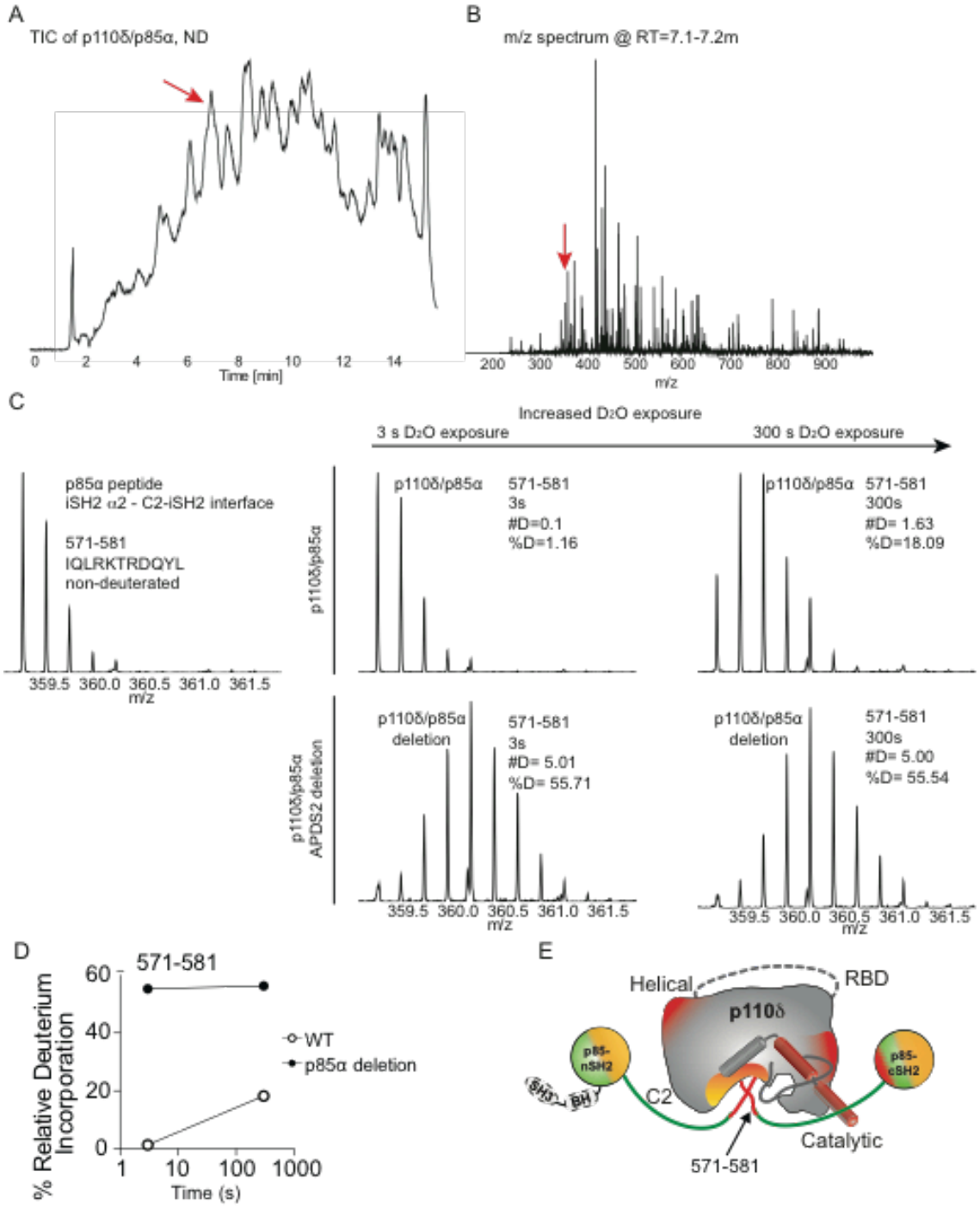


Fig. S2. HDX-MS methodology. (**A**) Representative UPLC total ion count (TIC) trace of a p110δ/p85α peptic digest. (**B**) MS spectra for the time window selected in the TIC in A. (**C**) Raw data for the peptide 571-581 in p85α showing the shift in mass centroid upon deuterium incorporation after 0 (nondeuterated, ND), 3 and 300 s exposure to D_2O buffer. The difference in HDX in this peptide is highlighted for the WT and p85 APDS2 deletion constructs. (**D**) HDX incorporation plots for this peptide in the WT and p85 APDS2 deletion constructs. (**E**) Cartoon model of PI3Kδ indicating the region with increased deuterium incorporation.

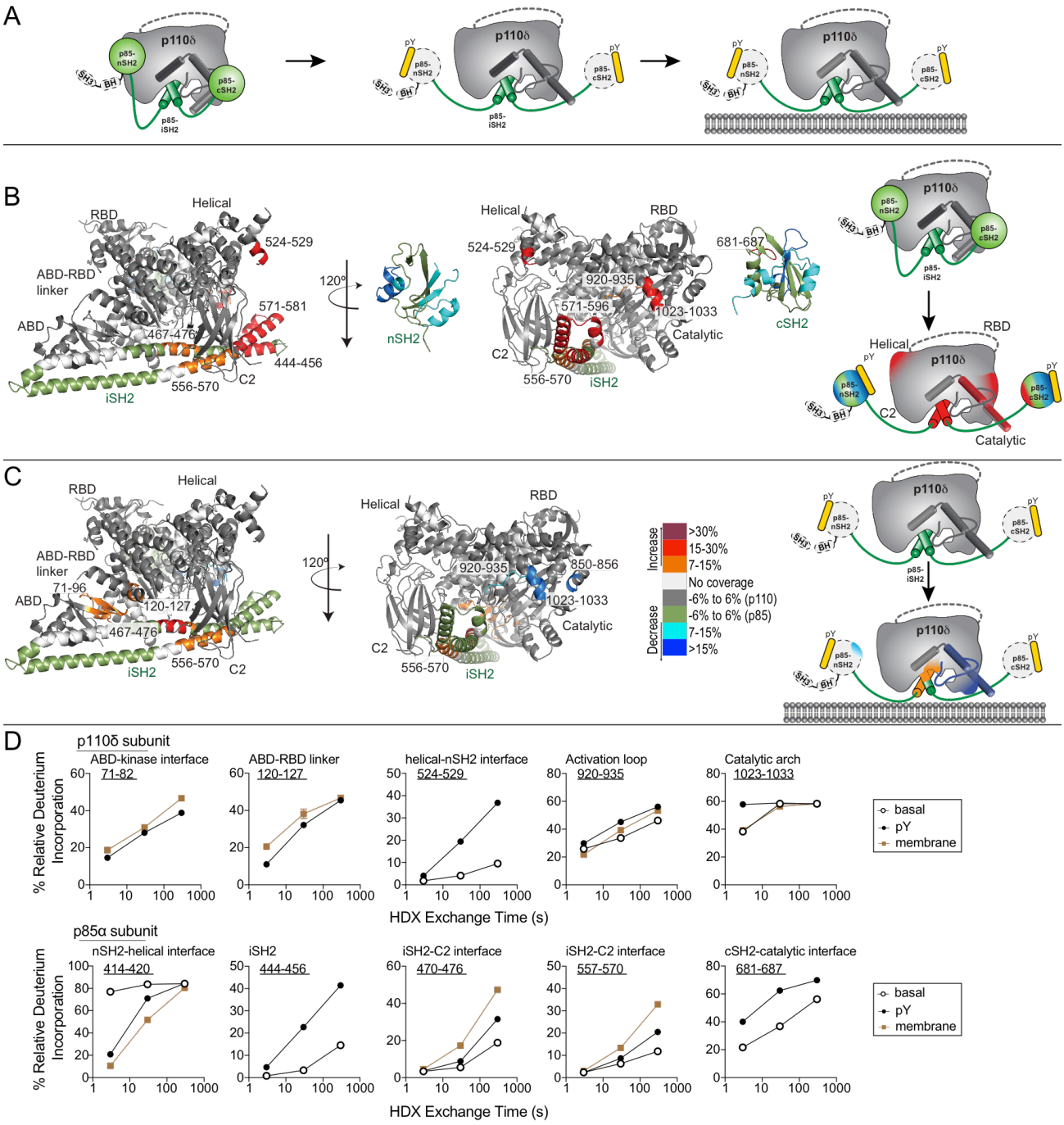
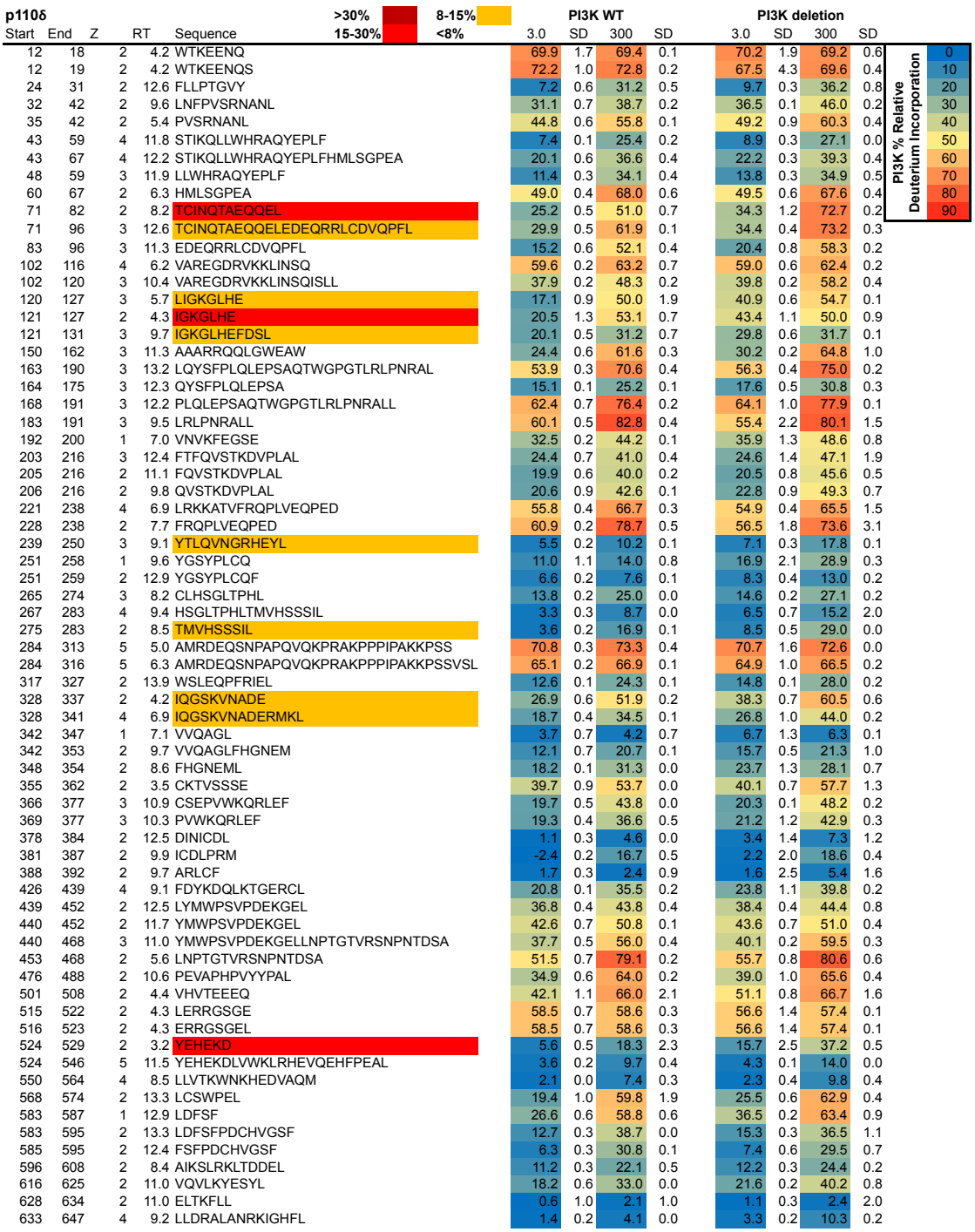


Fig. S3. Activation cycle of PI3Kδ and HDX-MS data for WT PI3Kδ in presence of pY and membranes. (**A**) The catalytic cycle of PI3Kδ is shown in cartoon representation, with the basal cytosolic inhibited form shown on the left, the pY activated form in the centre, and the pY activated form on membranes on the right. (**B**) Peptides in p110δ and the iSH2 of p85α that showed differences in HDX both greater than 0.7 Da and 7% between the basal and pY-activated WT or (**C**) between the pY-activated WT with and without membranes, are highlighted on both the structure of p110δ /iSH2-p85α (PDB: 5DXU) and on a schematic as in panel (**A**). Upon pY binding to the nSH2 and cSH2 of p85α, there were increases in exchange seen in the helical domain interface with the nSH2, the kinase domain interface with the cSH2, as well as the

activation loop. Upon membrane binding, the catalytic domain showed increases in exchange in the ABD domain at the interface with the kinase domain, the ABD-RBD linker, as well as regions of both the C2 and iSH2 domains at the C2/iSH2 interface. Decreases in exchange were observed in the putative membrane-binding surface in the activation loop and kα11 helix of the kinase domain. (**D**) Time course of deuterium incorporation for a selection of peptides in both p110δ and p85α that showed differences in HDX either upon pY or membrane addition (or both).



p110δ					>30%	8-15%	PI3K WT				PI3K deletion			
Start	End	Z	RT	Sequence	15-30%	<8%	3.0	SD	300	SD	3.0	SD	300	SD
12	18	2	4.2	WTKEENQ			69.9	1.7	69.4	0.1	70.2	1.9	69.2	0.6
12	19	2	4.2	WTKEENQS			72.2	1.0	72.8	0.2	67.5	4.3	69.6	0.4
24	31	2	12.6	FLLPTGVY			7.2	0.6	31.2	0.5	9.7	0.3	36.2	0.8
32	42	2	9.6	LNFPVSRNANL			31.1	0.7	38.7	0.2	36.5	0.1	46.0	0.2
35	42	2	5.4	PVSRNANL			44.8	0.6	55.8	0.1	49.2	0.9	60.3	0.4
43	59	4	11.8	STIKQLLWHRAQYEPLF			7.4	0.1	25.4	0.2	8.9	0.3	27.1	0.0
43	67	4	12.2	STIKQLLWHRAQYEPLFHMLSGPEA			20.1	0.6	36.6	0.4	22.2	0.3	39.3	0.4
48	59	3	11.9	LLWHRAQYEPLF			11.4	0.3	34.1	0.4	13.8	0.3	34.9	0.5
60	67	2	6.3	HMLSGPEA			49.0	0.4	68.0	0.6	49.5	0.6	67.6	0.4
71	82	2	8.2	TCINQTAEQQEL			25.2	0.5	51.0	0.7	34.3	1.2	72.7	0.2
71	96	3	12.6	TCINQTAEQQELEDEQRRLCDVQPFL			29.9	0.5	61.9	0.1	34.4	0.4	73.2	0.3
83	96	3	11.3	EDEQRRLCDVQPFL			15.2	0.6	52.1	0.4	20.4	0.8	58.3	0.2
102	116	4	6.2	VAREGDRVKKLINSQ			59.6	0.2	63.2	0.7	59.0	0.6	62.4	0.2
102	120	3	10.4	VAREGDRVKKLINSQISLL			37.9	0.2	48.3	0.2	39.8	0.2	58.2	0.4
120	127	3	5.7	LIGKGLHE			17.1	0.9	50.0	1.9	40.9	0.6	54.7	0.1
121	127	2	4.3	IGKGLHE			20.5	1.3	53.1	0.7	43.4	1.1	50.0	0.9
121	131	3	9.7	IGKGLHEFDSL			20.1	0.5	31.2	0.7	29.8	0.6	31.7	0.1
150	162	3	11.3	AAARRQQLGWEAW			24.4	0.6	61.6	0.3	30.2	0.2	64.8	1.0
163	190	3	13.2	LQYSFPLQLEPSAQTWGPGTLRLPNRAL			53.9	0.3	70.6	0.4	56.3	0.4	75.0	0.2
164	175	3	12.3	QYSFPLQLEPSA			15.1	0.1	25.2	0.1	17.6	0.5	30.8	0.3
168	191	3	12.2	PLQLEPSAQTWGPGTLRLPNRALL			62.4	0.7	76.4	0.2	64.1	1.0	77.9	0.1
183	191	3	9.5	LRLPNRALL			60.1	0.5	82.8	0.4	55.4	2.2	80.1	1.5
192	200	1	7.0	VNVKFEGSE			32.5	0.2	44.2	0.1	35.9	1.3	48.6	0.8
203	216	3	12.4	FTFQVSTKDVPLAL			24.4	0.7	41.0	0.4	24.6	1.4	47.1	1.9
205	216	2	11.1	FQVSTKDVPLAL			19.9	0.6	40.0	0.2	20.5	0.8	45.6	0.5
206	216	2	9.8	QVSTKDVPLAL			20.6	0.9	42.6	0.1	22.8	0.9	49.3	0.7
221	238	4	6.9	LRKKATVFRQPLVEQPED			55.8	0.4	66.7	0.3	54.9	0.4	65.5	1.5
228	238	2	7.7	FRQPLVEQPED			60.9	0.2	78.7	0.5	56.5	1.8	73.6	3.1
239	250	3	9.1	YTLQVNGRHEYL			5.5	0.2	10.2	0.1	7.1	0.3	17.8	0.1
251	258	1	9.6	YGSYPLCQ			11.0	1.1	14.0	0.8	16.9	2.1	28.9	0.3
251	259	2	12.9	YGSYPLCQF			6.6	0.2	7.6	0.1	8.3	0.4	13.0	0.2
265	274	3	8.2	CLHSGLTPHL			13.8	0.2	25.0	0.0	14.6	0.2	27.1	0.2
267	283	4	9.4	HSGLTPHLTMVHSSSIL			3.3	0.3	8.7	0.0	6.5	0.7	15.2	2.0
275	283	2	8.5	TMVHSSSIL			3.6	0.2	16.9	0.1	8.5	0.5	29.0	0.0
284	313	5	5.0	AMRDEQSNPAPQVQKPRAKPPPIPAKKPSS			70.8	0.3	73.3	0.4	70.7	1.6	72.6	0.0
284	316	5	6.3	AMRDEQSNPAPQVQKPRAKPPPIPAKKPSSVSL			65.1	0.2	66.9	0.1	64.9	1.0	66.5	0.2
317	327	2	13.9	WSLEQPFRIEL			12.6	0.1	24.3	0.1	14.8	0.1	28.0	0.2
328	337	2	4.2	IQGSKVNADE			26.9	0.6	51.9	0.2	38.3	0.7	60.5	0.6
328	341	4	6.9	IQGSKVNADERMKL			18.7	0.4	34.5	0.1	26.8	1.0	44.0	0.2
342	347	1	7.1	VVQAGL			3.7	0.7	4.2	0.7	6.7	1.3	6.3	0.1
342	353	2	9.7	VVQAGLFHGNEM			12.1	0.7	20.7	0.1	15.7	0.5	21.3	1.0
348	354	2	8.6	FHGNEML			18.2	0.1	31.3	0.0	23.7	1.3	28.1	0.7
355	362	2	3.5	CKTVSSSE			39.7	0.9	53.7	0.0	40.1	0.7	57.7	1.3
366	377	3	10.9	CSEPVWKQRLEF			19.7	0.5	43.8	0.0	20.3	0.1	48.2	0.2
369	377	3	10.3	PVWKQRLEF			19.3	0.4	36.6	0.5	21.2	1.2	42.9	0.3
378	384	2	12.5	DINICDL			1.1	0.3	4.6	0.0	3.4	1.4	7.3	1.2
381	387	2	9.9	ICDLPRM			-2.4	0.2	16.7	0.5	2.2	2.0	18.6	0.4
388	392	2	9.7	ARLCF			1.7	0.3	2.4	0.9	1.6	2.5	5.4	1.6
426	439	4	9.1	FDYKDQLKTGERCL			20.8	0.1	35.5	0.2	23.8	1.1	39.8	0.2
439	452	2	12.5	LYMWPSVPDEKGEL			36.8	0.4	43.8	0.4	38.4	0.4	44.4	0.8
440	452	2	11.7	YMWPSVPDEKGEL			42.6	0.7	50.8	0.1	43.6	0.7	51.0	0.4
440	468	3	11.0	YMWPSVPDEKGELLNPTGTVRSNPNTDSA			37.7	0.5	56.0	0.4	40.1	0.2	59.5	0.3
453	468	2	5.6	LNPTGTVRSNPNTDSA			51.5	0.7	79.1	0.2	55.7	0.8	80.6	0.6
476	488	2	10.6	PEVAPHPVYYPAL			34.9	0.6	64.0	0.2	39.0	1.0	65.6	0.4
501	508	2	4.4	VHVTEEEQ			42.1	1.1	66.0	2.1	51.1	0.8	66.7	1.6
515	522	2	4.3	LERRGSGE			58.5	0.7	58.6	0.3	56.6	1.4	57.4	0.1
516	523	2	4.3	ERRGSGEL			58.5	0.7	58.6	0.3	56.6	1.4	57.4	0.1
524	529	2	3.2	YEHEKD			5.6	0.5	18.3	2.3	15.7	2.5	37.2	0.5
524	546	5	11.5	YEHEKDLVWKLRHEVQEHFPEAL			3.6	0.2	9.7	0.4	4.3	0.1	14.0	0.0
550	564	4	8.5	LLVTKWNKHEDVAQM			2.1	0.0	7.4	0.3	2.3	0.4	9.8	0.4
568	574	2	13.3	LCSWPEL			19.4	1.0	59.8	1.9	25.5	0.6	62.9	0.4
583	587	1	12.9	LDFSF			26.6	0.6	58.8	0.6	36.5	0.2	63.4	0.9
583	595	2	13.3	LDFSFPDCHVGSF			12.7	0.3	38.7	0.0	15.3	0.3	36.5	1.1
585	595	2	12.4	FSFPDCHVGSF			6.3	0.3	30.8	0.1	7.4	0.6	29.5	0.7
596	608	2	8.4	AIKSLRKLTDDEL			11.2	0.3	22.1	0.5	12.2	0.3	24.4	0.2
616	625	2	11.0	VQVLKYESYL			18.2	0.6	33.0	0.0	21.6	0.2	40.2	0.8
628	634	2	11.0	ELTKFLL			0.6	1.0	2.1	1.0	1.1	0.3	2.4	2.0
633	647	4	9.2	LLDRALANRKIGHFL			1.4	0.2	4.1	0.0	3.3	0.2	10.3	0.2

PI3K % Relative Deuterium Incorporation: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90

Fig. S4. All HDX peptide data for experiments examining conformational changes in APDS2 mutation (labelled as p85 deletion) for both p110δ and p85α. The charge state (Z), residue start, residue end number, retention time (RT) and sequence are displayed for every peptide. The two time points are labelled for the conditions tested. The relative level of HDX is coloured according to the amount of deuterium incorporated, on a blue to red continuum. Peptides with differences in exchange have the sequence colour according to the legend. The data listed are the average of three independent experiments, with SD shown next to all HDX values.

p110δ						PI3K WT				PI3K deletion			
Start	End	Z	RT	Sequence	3.0	SD	300	SD	3.0	SD	300	SD
634	647	4	8.0	LDRALANRKIGHFL	2.6	0.1	7.5	0.3	5.1	1.0	17.3	0.2
635	646	4	6.4	DRALANRKIGHF	3.7	0.4	9.8	0.3	6.8	0.8	21.0	0.1
636	646	4	5.6	RALANRKIGHF	3.8	0.8	10.3	0.9	6.6	1.8	21.8	2.5
647	651	2	13.0	LFWHL	0.3	0.4	1.1	0.6	2.9	0.6	9.6	0.2
648	655	3	10.1	FWHLRSEM	1.2	0.5	1.5	0.3	4.7	0.3	7.7	0.9
662	668	2	13.3	LRFGLIL	1.3	0.3	1.8	0.1	3.6	0.2	10.5	0.2
683	689	2	5.9	MKQGEAL	1.9	0.8	4.5	0.2	11.0	1.2	23.9	4.9
698	713	5	5.0	FVKLSSQKTPKPQTKE	39.2	0.7	58.1	0.6	42.9	1.1	60.1	0.3
698	715	4	6.9	FVKLSSQKTPKPQTKELM	28.6	0.6	46.8	0.0	32.3	0.3	50.5	1.0
726	740	2	10.6	EALSHLQSPLDPSTL	7.6	0.5	27.0	0.5	12.6	0.8	35.6	0.3
726	741	2	12.1	EALSHLQSPLDPSTLL	5.9	0.3	24.1	0.5	11.7	1.8	33.9	0.6
728	740	2	10.2	LSHLQSPLDPSTL	8.4	0.5	31.3	0.2	15.7	0.0	42.1	0.4
746	751	1	9.0	VEQCTF	4.9	0.4	37.1	0.1	9.2	1.7	47.0	2.3
752	760	3	10.3	MDSKMKPLW	22.1	0.5	34.7	0.3	24.3	0.3	39.4	0.4
752	762	3	12.3	MDSKMKPLWIM	13.3	0.4	22.6	0.0	16.4	0.1	28.6	0.2
753	760	3	10.2	DSKMKPLW	23.3	0.5	38.4	0.8	32.8	0.9	46.3	0.2
753	762	3	12.4	DSKMKPLWIM	12.8	0.4	23.3	0.3	15.3	0.3	32.0	0.8
767	775	1	3.7	EAGSGGSVG	45.0	1.1	56.5	0.2	45.5	2.0	56.2	1.5
776	784	2	9.4	IIFKNGDDL	19.5	0.4	28.7	0.2	21.6	0.3	33.3	0.6
801	807	2	7.2	WKQEGLD	4.5	0.4	27.0	0.7	9.6	0.6	33.9	0.1
808	826	3	10.7	LRMTPYGCLPTGDRTGLIE	5.2	0.4	13.3	0.2	8.4	0.1	22.2	0.0
809	824	3	9.7	RMTPYGCLPTGDRTGL	5.5	0.6	16.4	0.2	11.6	2.3	30.4	1.5
827	836	2	6.5	VVLRSDTIAN	23.7	0.2	41.6	0.0	27.8	0.5	55.2	1.6
830	836	2	3.8	RSDTIAN	0.6	0.2	15.8	0.5	9.5	1.4	44.4	1.1
850	856	2	9.4	FNKDALL	57.5	5.7	69.7	0.5	61.7	1.8	73.4	1.7
851	856	2	7.6	NKDALL	46.4	0.5	65.4	0.1	46.5	1.4	67.7	0.8
856	868	3	9.8	LNWLKSKNPGEAL	35.1	0.3	49.4	0.0	37.7	0.4	51.3	0.1
857	868	3	9.0	NWLKSKNPGEAL	38.5	0.3	49.5	0.4	40.3	0.1	51.7	0.2
869	874	2	4.5	DRAIEE	2.3	0.4	6.5	0.2	7.0	0.8	14.8	1.4
875	881	1	9.6	FTLSCAG	-1.7	0.3	-1.3	0.1	1.9	0.6	3.0	0.6
882	886	1	6.4	YCVAT	-0.6	0.0	-0.1	0.3	1.9	0.8	2.6	0.3
887	898	3	7.9	YVLGIGDRHSDN	6.5	0.4	15.9	0.1	13.2	2.6	24.8	0.2
887	900	2	10.0	YVLGIGDRHSDNIM	3.0	0.2	9.2	0.2	7.4	0.2	16.3	0.1
901	907	2	5.8	IRESGQL	6.1	0.2	26.8	0.3	11.8	0.3	33.3	0.4
908	912	2	11.5	FHIDF	1.9	0.4	17.8	1.6	6.5	3.2	32.5	0.6
908	915	3	11.8	FHIDFGHF	13.1	0.9	28.7	0.6	18.9	0.2	34.1	0.2
908	919	3	13.8	FHIDFGHFLGNF	25.5	0.7	43.5	0.1	33.8	0.3	44.9	0.8
909	919	3	12.8	HIDFGHFLGNF	29.5	1.0	47.9	0.3	36.9	1.2	47.1	0.6
920	934	4	10.2	KTKFGINRERVPFIL	30.7	0.6	46.9	0.1	35.0	0.8	53.4	0.7
920	935	4	9.8	KTKFGINRERVPFILT	31.9	0.5	53.9	0.3	40.4	0.3	60.5	0.1
939	958	5	6.3	VHVIQQGKTNNSEKFERFRG	20.0	0.4	30.5	0.2	24.3	0.4	33.6	0.4
964	973	3	8.5	YTILRRHGLL	8.2	0.0	17.1	0.4	9.0	0.5	20.2	1.3
964	973	4	8.5	YTILRRHGLL	9.8	0.8	17.8	0.4	8.6	1.0	19.8	2.0
964	974	4	10.6	YTILRRHGLLF	6.9	0.3	15.7	0.3	7.5	0.2	18.9	0.6
978	989	2	12.5	FALMRAAGLPEL	22.1	0.5	59.8	0.2	32.4	1.9	62.4	0.2
982	989	2	9.5	RAAGLPEL	32.8	1.0	80.3	0.6	48.7	1.3	82.1	0.3
1010	1019	3	8.0	EALKHFRVKF	0.5	0.1	10.8	0.2	2.9	0.3	21.5	1.4
1024	1033	4	8.5	RESWKTKVNW	50.8	0.7	61.4	0.7	61.0	1.6	61.5	0.4
1024	1044	5	8.2	RESWKTKVNWLAHNVSKDNRQ	48.7	1.0	54.4	1.2	52.9	0.9	53.2	0.5
1034	1044	3	3.3	LAHNVSKDNRQ	55.2	1.2	55.4	0.0	53.3	0.8	54.6	0.6

Fig. S4 All HDX peptide data for experiments examining conformational changes in APDS2 mutation (labelled as PI3K deletion) for both p110δ and p85α (cont).

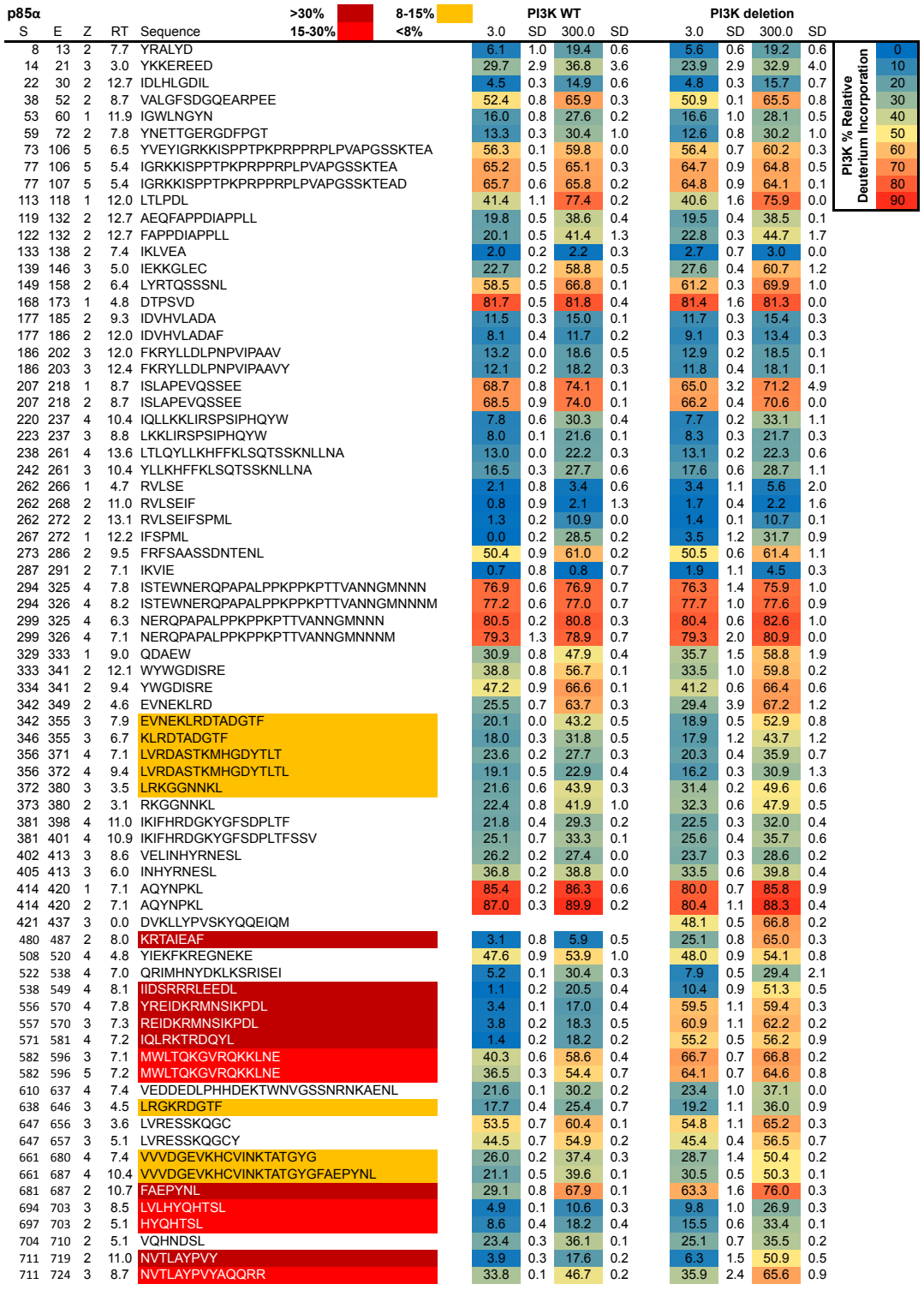


Fig. S4 All HDX peptide data for experiments examining conformational changes in APDS2 mutation (labelled as p85 deletion) for both p110δ and p85α (cont).

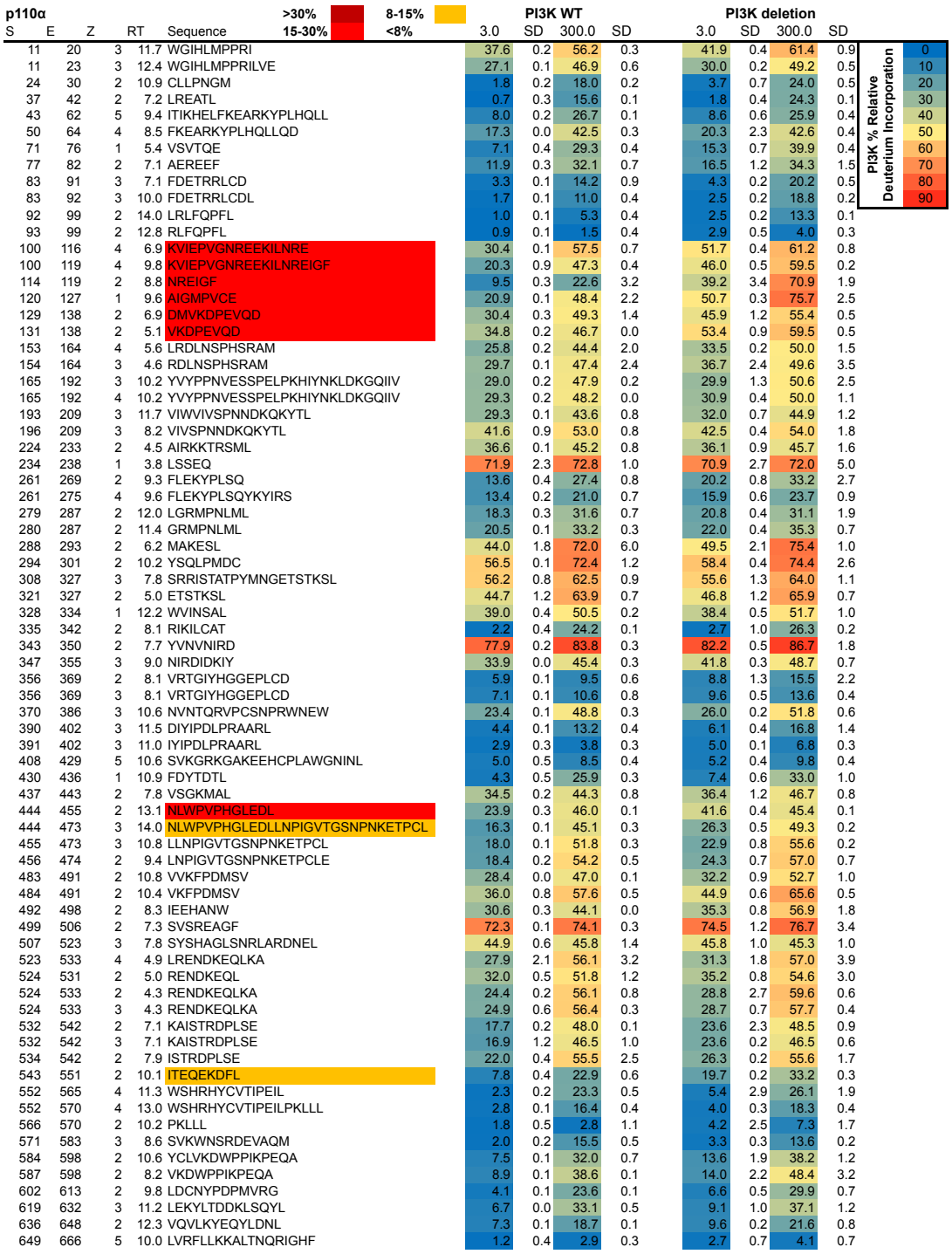
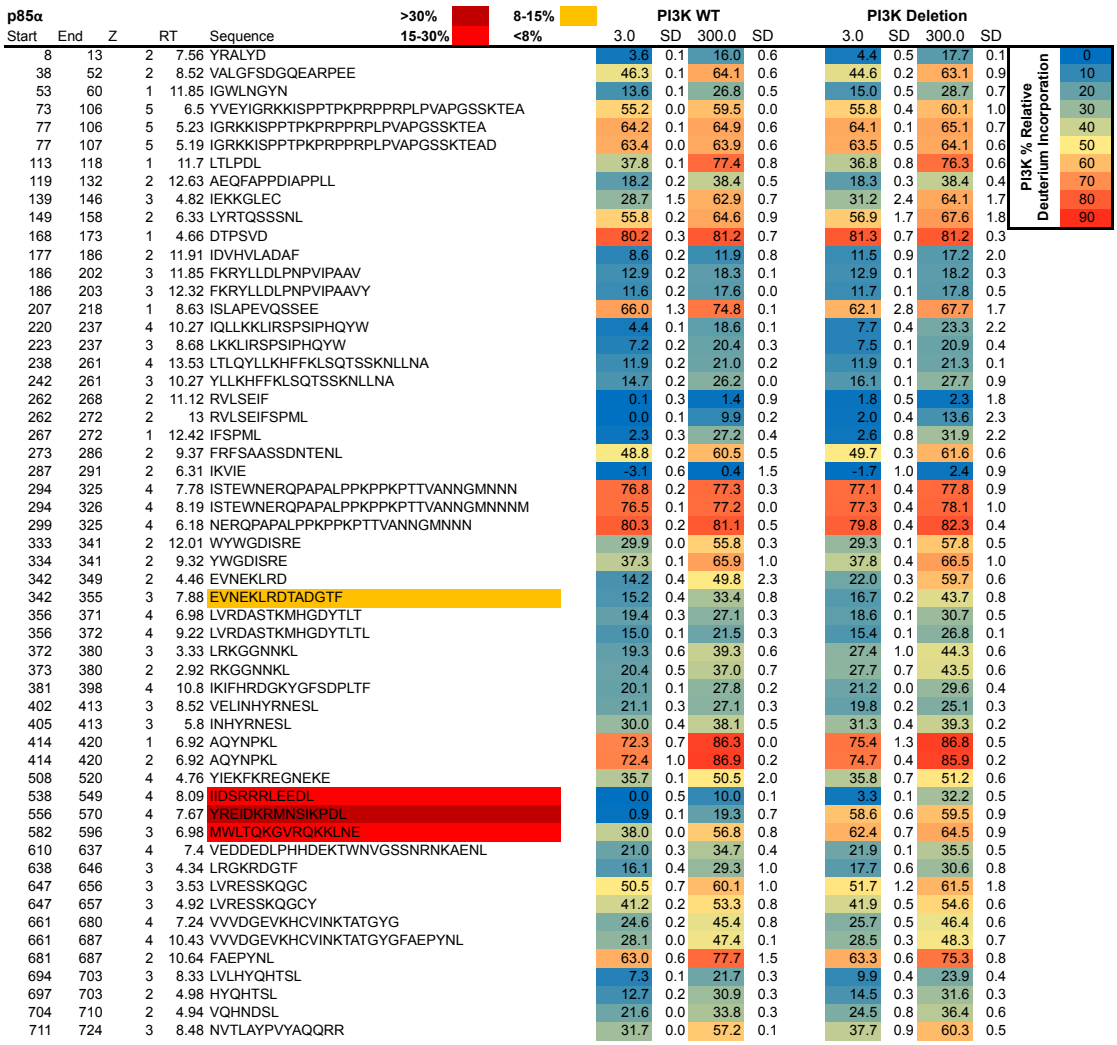


Fig. S5. All HDX peptide data for experiments examining conformational changes in APDS2 mutation (labelled as p85 deletion) for both p110α and p85α. The charge state (Z), residue start, residue end number, retention time (RT) and sequence are displayed for every peptide. The two time points are labelled, and the relative level of HDX is coloured according to the amount of deuterium incorporated, on a blue to red continuum. Peptides with differences in exchange have the sequence coloured according to the legend. The data listed are the average of three independent experiments, with SD shown next to all HDX values.

p110α						WT				deletion			
S	E	Z	RT	Sequence	3.0	SD	300.0	SD	3.0	SD	300.0	SD
653	666	3	7.4	LLKKALTNQRIGHF	1.6	0.6	3.9	0.1	3.3	0.4	6.2	0.3
667	671	2	13.7	FFWHL	1.8	0.1	3.1	0.7	3.0	0.3	6.4	0.1
672	687	4	9.2	KSEMHNKTVSQRFGLL	6.7	0.3	24.6	0.6	10.1	0.8	27.6	0.6
691	697	2	7.1	YCRACGM	28.2	0.1	34.3	0.0	31.3	0.1	41.3	1.1
698	709	3	6.8	YLKHLNRQVEAM	2.4	0.3	5.0	0.1	4.2	0.4	13.3	1.2
713	734	5	10.7	INLTDILKQEKKDETQKVQMKF	47.3	0.1	60.0	0.7	49.4	0.2	65.9	3.3
720	734	5	6.7	KQEKKDETQKVQMKF	48.7	0.9	51.3	0.8	49.0	1.0	51.0	0.8
735	744	3	8.7	LVEQMRRPDF	18.8	0.5	68.7	0.7	35.6	1.5	72.3	2.1
745	764	3	14.1	MDALQGFLSPLNPAHQLGNL	2.0	0.1	11.7	1.0	5.0	1.6	14.2	1.7
745	766	3	14.0	MDALQGFLSPLNPAHQLGNLRL	7.3	0.2	16.0	0.0	8.9	0.8	16.8	0.9
746	764	2	13.7	DALQGFLSPLNPAHQLGNL	2.8	0.1	12.1	0.3	4.3	0.5	14.4	0.2
746	766	3	13.7	DALQGFLSPLNPAHQLGNLRL	8.1	0.1	16.5	0.3	8.8	0.1	18.4	0.5
751	764	2	11.7	FLSPLNPAHQLGNL	4.4	0.0	16.7	0.4	7.3	0.2	22.3	1.3
751	766	3	12.0	FLSPLNPAHQLGNLRL	11.3	0.1	20.9	0.3	12.8	0.3	24.2	1.2
769	781	4	10.4	CRIMSSAKRPLWL	17.4	0.2	32.4	0.4	20.1	0.6	33.7	2.4
782	789	1	11.2	NWENPDIM	33.7	0.2	61.1	0.3	36.1	0.9	64.0	0.2
799	807	2	9.6	IIFKNGDDL	19.8	0.2	29.9	0.5	24.2	1.9	35.1	1.6
799	811	3	9.5	IIFKNGDDLRQDM	11.7	0.1	30.5	2.5	14.6	0.6	28.4	1.3
815	821	2	9.6	QIIRIME	-1.4	0.0	-0.5	0.3	2.4	0.3	6.0	0.0
816	821	2	9.2	IIRIME	-0.5	0.3	-0.6	0.3	2.8	2.4	4.8	1.7
822	830	1	10.7	NIWQNQGLD	16.1	0.1	36.7	1.2	22.5	1.7	42.1	2.9
822	831	2	12.7	NIWQNQGLDL	11.8	0.1	31.3	0.6	14.1	0.2	32.3	0.6
831	839	2	12.5	LRMLPYGCL	3.7	0.1	17.1	0.5	6.0	0.0	21.0	0.5
832	839	2	11.5	RMLPYGCL	4.9	0.4	21.3	0.1	7.1	0.8	26.4	1.4
850	858	3	5.9	VVRNSHTIM	14.6	0.7	26.1	0.4	17.8	0.6	39.1	1.1
859	872	3	10.1	QIQCKGGLKGALQF	62.3	0.1	69.0	0.0	59.5	1.9	68.9	1.4
873	879	2	3.7	NSHTLHQ	11.1	0.5	37.3	0.4	12.9	2.5	35.1	1.3
880	893	4	7.8	WLKDKNKGEIYDAA	24.1	0.4	36.2	0.9	24.2	0.2	39.9	1.1
904	908	1	6.7	YCVAT	-2.2	0.1	-0.8	0.6	-0.7	0.9	5.9	1.5
909	920	3	9.5	FILGIGDRHNSN	7.4	0.3	16.9	0.8	10.5	0.5	22.9	1.4
909	921	3	10.4	FILGIGDRHNSNI	4.5	0.1	12.5	0.7	6.1	0.9	15.0	0.4
922	929	2	6.7	MVKDDGQL	2.8	0.7	10.5	1.0	4.5	0.3	12.9	0.9
930	934	2	11.7	FHIDF	4.3	0.4	24.3	1.3	7.7	0.9	29.5	3.2
930	937	3	12.0	FHIDFGHF	7.2	0.3	28.4	0.7	13.2	0.7	28.9	0.8
961	976	4	7.0	LIVISKGAQECTKTRE	29.4	0.8	44.0	0.4	28.6	2.2	48.9	1.1
962	970	2	5.7	IVISKGAQE	22.8	0.3	35.0	1.5	23.4	0.5	37.1	0.4
962	976	3	5.7	IVISKGAQECTKTRE	32.2	0.4	48.1	0.4	31.6	0.8	45.5	0.6
984	989	2	9.1	CYKAYL	-5.1	1.2	-3.3	1.5	-2.3	1.9	4.3	2.9
985	989	2	8.7	YKAYL	-1.8	0.2	-0.2	1.0	0.6	2.2	5.5	2.5
990	997	2	5.1	AIRQHANL	11.1	0.3	36.4	1.8	12.8	0.6	36.7	0.4
1002	1006	1	13.3	FSMML	2.9	0.5	24.3	0.2	6.9	0.5	36.8	1.6
1006	1013	1	10.9	LGSGMPEL	26.4	0.4	51.0	0.6	38.9	0.5	64.0	1.4
1006	1015	2	9.0	LGSGMPELQS	22.9	0.4	57.4	2.5	15.5	0.5	59.1	2.6
1039	1055	4	8.0	FMKQMNDAHHGGWTTKM	22.5	0.5	27.2	0.6	23.3	1.1	29.2	0.8
1039	1055	5	7.9	FMKQMNDAHHGGWTTKM	21.9	0.2	26.3	0.3	23.0	1.3	27.3	0.8
1040	1055	4	7.1	MKQMNDAHHGGWTTKM	23.9	0.2	28.3	0.4	25.6	0.3	31.6	1.0
1060	1068	2	4.0	HTIKQHALN	55.2	0.2	57.4	1.6	53.2	1.0	57.2	1.3
1060	1068	3	4.0	HTIKQHALN	57.1	0.8	58.5	0.9	54.0	0.1	58.0	1.6

Fig. S5 All HDX peptide data for experiments examining conformational changes in APDS2 mutation (labelled as p85 deletion) for both p110α and p85α. (cont).



p85α					>30%	8-15%	PI3K WT				PI3K Deletion			
Start	End	Z	RT	Sequence	15-30%	<8%	3.0	SD	300.0	SD	3.0	SD	300.0	SD
8	13	2	7.56	YRALYD			3.6	0.1	16.0	0.6	4.4	0.5	17.7	0.1
38	52	2	8.52	VALGFSDGQEARPEE			46.3	0.1	64.1	0.6	44.6	0.2	63.1	0.9
53	60	1	11.85	IGWLNGYN			13.6	0.1	26.8	0.5	15.0	0.5	28.7	0.7
73	106	5	6.5	YVEYIGRKKISPPTPKPRPPRPLPVAPGSSKTEA			55.2	0.0	59.5	0.0	55.8	0.4	60.1	1.0
77	106	5	5.23	IGRKKISPPTPKPRPPRPLPVAPGSSKTEA			64.2	0.1	64.9	0.6	64.1	0.1	65.1	0.7
77	107	5	5.19	IGRKKISPPTPKPRPPRPLPVAPGSSKTEAD			63.4	0.0	63.9	0.6	63.5	0.5	64.1	0.6
113	118	1	11.7	LTLPDL			37.8	0.1	77.4	0.8	36.8	0.8	76.3	0.6
119	132	2	12.63	AEQFAPPDIAPPLL			18.2	0.2	38.4	0.5	18.3	0.3	38.4	0.4
139	146	3	4.82	IEKKGLEC			28.7	1.5	62.9	0.7	31.2	2.4	64.1	1.7
149	158	2	6.33	LYRTQSSSNL			55.8	0.2	64.6	0.9	56.9	1.7	67.6	1.8
168	173	1	4.66	DTPSVD			80.2	0.3	81.2	0.7	81.3	0.7	81.2	0.3
177	186	2	11.91	IDVHVLADAF			8.6	0.2	11.9	0.8	11.5	0.9	17.2	2.0
186	202	3	11.85	FKRYLLDLPNPVIPAAV			12.9	0.2	18.3	0.1	12.9	0.1	18.2	0.3
186	203	3	12.32	FKRYLLDLPNPVIPAAVY			11.6	0.2	17.6	0.0	11.7	0.1	17.8	0.5
207	218	1	8.63	ISLAPEVQSSEE			66.0	1.3	74.8	0.1	62.1	2.8	67.7	1.7
220	237	4	10.27	IQLLKKLIRSPSIPHQYW			4.4	0.1	18.6	0.1	7.7	0.4	23.3	2.2
223	237	3	8.68	LKKLIRSPSIPHQYW			7.2	0.2	20.4	0.3	7.5	0.1	20.9	0.4
238	261	4	13.53	LTLQYLLKHFFKLSQTSSKNLLNA			11.9	0.2	21.0	0.2	11.9	0.1	21.3	0.1
242	261	3	10.27	YLLKHFFKLSQTSSKNLLNA			14.7	0.2	26.2	0.0	16.1	0.1	27.7	0.9
262	268	2	11.12	RVLSEIF			0.1	0.3	1.4	0.9	1.8	0.5	2.3	1.8
262	272	2	13	RVLSEIFSPML			0.0	0.1	9.9	0.2	2.0	0.4	13.6	2.3
267	272	1	12.42	IFSPML			2.3	0.3	27.2	0.4	2.6	0.8	31.9	2.2
273	286	2	9.37	FRFSAASSDNTENL			48.8	0.2	60.5	0.5	49.7	0.3	61.6	0.6
287	291	2	6.31	IKVIE			-3.1	0.6	0.4	1.5	-1.7	1.0	2.4	0.9
294	325	4	7.78	ISTEWNERQPAPALPPKPPKPTTVANNGMNNN			76.8	0.2	77.3	0.3	77.1	0.4	77.8	0.9
294	326	4	8.19	ISTEWNERQPAPALPPKPPKPTTVANNGMNNNM			76.5	0.1	77.2	0.0	77.3	0.4	78.1	1.0
299	325	4	6.18	NERQPAPALPPKPPKPTTVANNGMNNN			80.3	0.2	81.1	0.5	79.8	0.4	82.3	0.4
333	341	2	12.01	WYWGDISRE			29.9	0.0	55.8	0.3	29.3	0.1	57.8	0.5
334	341	2	9.32	YWGDISRE			37.3	0.1	65.9	1.0	37.8	0.4	66.5	1.0
342	349	2	4.46	EVNEKLRD			14.2	0.4	49.8	2.3	22.0	0.3	59.7	0.6
342	355	3	7.88	EVNEKLRDTADGTF			15.2	0.4	33.4	0.8	16.7	0.2	43.7	0.8
356	371	4	6.98	LVRDASTKMHGDYTLT			19.4	0.3	27.1	0.3	18.6	0.1	30.7	0.5
356	372	4	9.22	LVRDASTKMHGDYTLTL			15.0	0.1	21.5	0.3	15.4	0.1	26.8	0.1
372	380	3	3.33	LRKGGNNKL			19.3	0.6	39.3	0.6	27.4	1.0	44.3	0.6
373	380	2	2.92	RKGGNNKL			20.4	0.5	37.0	0.7	27.7	0.7	43.5	0.6
381	398	4	10.8	IKIFHRDGKYGFSDPLTF			20.1	0.1	27.8	0.2	21.2	0.0	29.6	0.4
402	413	3	8.52	VELINHYRNESL			21.1	0.3	27.1	0.3	19.8	0.2	25.1	0.3
405	413	3	5.8	INHYRNESL			30.0	0.4	38.1	0.5	31.3	0.4	39.3	0.2
414	420	1	6.92	AQYNPKL			72.3	0.7	86.3	0.0	75.4	1.3	86.8	0.5
414	420	2	6.92	AQYNPKL			72.4	1.0	86.9	0.2	74.7	0.4	85.9	0.2
508	520	4	4.76	YIEKFKREGNEKE			35.7	0.1	50.5	2.0	35.8	0.7	51.2	0.6
538	549	4	8.09	IIDSRRRLEEDL			0.0	0.5	10.0	0.1	3.3	0.1	32.2	0.5
556	570	4	7.67	YREIDKRMNSIKPDL			0.9	0.1	19.3	0.7	58.6	0.6	59.5	0.9
582	596	3	6.98	MWLTQKGVRQKKLNE			38.0	0.0	56.8	0.8	62.4	0.7	64.5	0.9
610	637	4	7.4	VEDDEDLPHHDEKTWNVGSSNRNKAENL			21.0	0.3	34.7	0.4	21.9	0.1	35.5	0.5
638	646	3	4.34	LRGKRDGTF			16.1	0.4	29.3	1.0	17.7	0.6	30.6	0.8
647	656	3	3.53	LVRESSKQGC			50.5	0.7	60.1	1.0	51.7	1.2	61.5	1.8
647	657	3	4.92	LVRESSKQGCY			41.2	0.2	53.3	0.8	41.9	0.5	54.6	0.6
661	680	4	7.24	VVVDGEVKHCVINKTATGYG			24.6	0.2	45.4	0.8	25.7	0.5	46.4	0.6
661	687	4	10.43	VVVDGEVKHCVINKTATGYGFAEPYNL			28.1	0.0	47.4	0.1	28.5	0.3	48.3	0.7
681	687	2	10.64	FAEPYNL			63.0	0.6	77.7	1.5	63.3	0.6	75.3	0.8
694	703	3	8.33	LVLHYQHTSL			7.3	0.1	21.7	0.3	9.9	0.4	23.9	0.4
697	703	2	4.98	HYQHTSL			12.7	0.2	30.9	0.3	14.5	0.3	31.6	0.3
704	710	2	4.94	VQHNDSL			21.6	0.0	33.8	0.3	24.5	0.8	36.4	0.6
711	724	3	8.48	NVTLAYPVYAQQRR			31.7	0.0	57.2	0.1	37.7	0.9	60.3	0.5

PI3K % Relative Deuterium Incorporation: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90

Fig. S5 All HDX peptide data for experiments examining conformational changes in APDS2 mutation (labelled as p85 deletion) for both p110α and p85α. (cont).

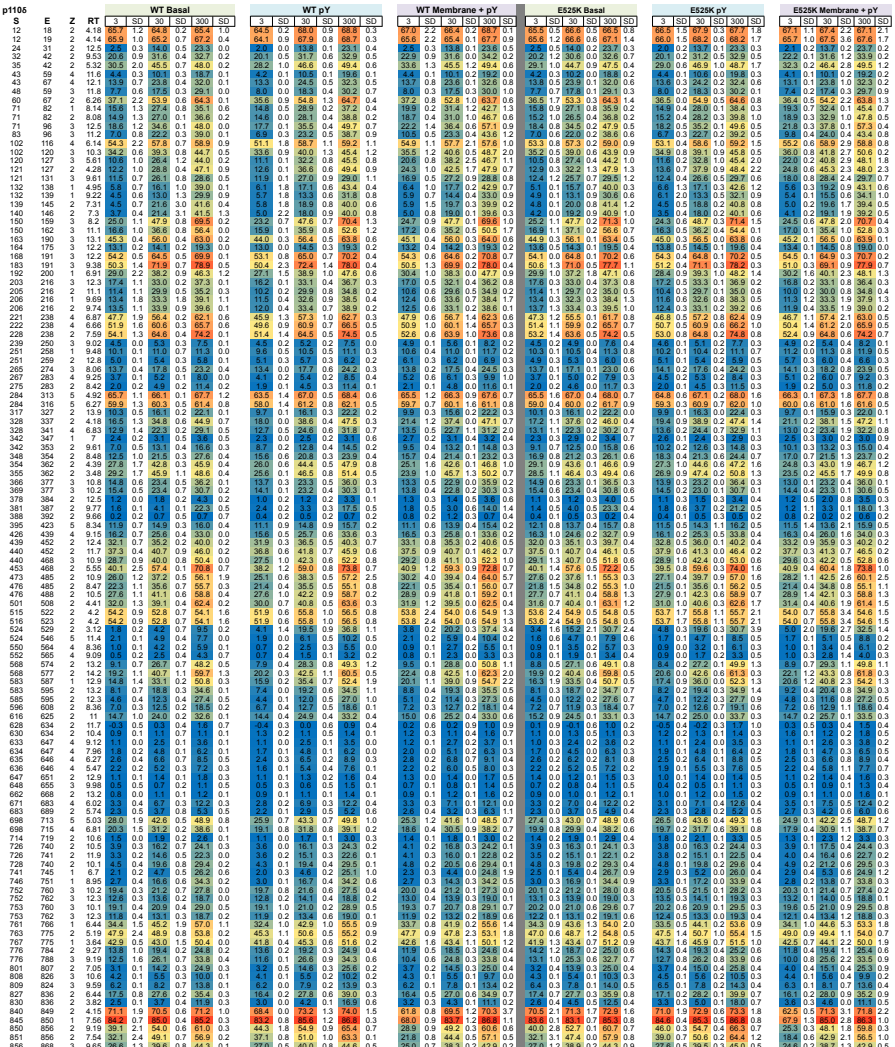


Fig. S6 All HDX peptide data for experiments examining conformational changes in APDS1 mutations under basal, pY-activated, and membrane-bound states for both p110δ and p85α.

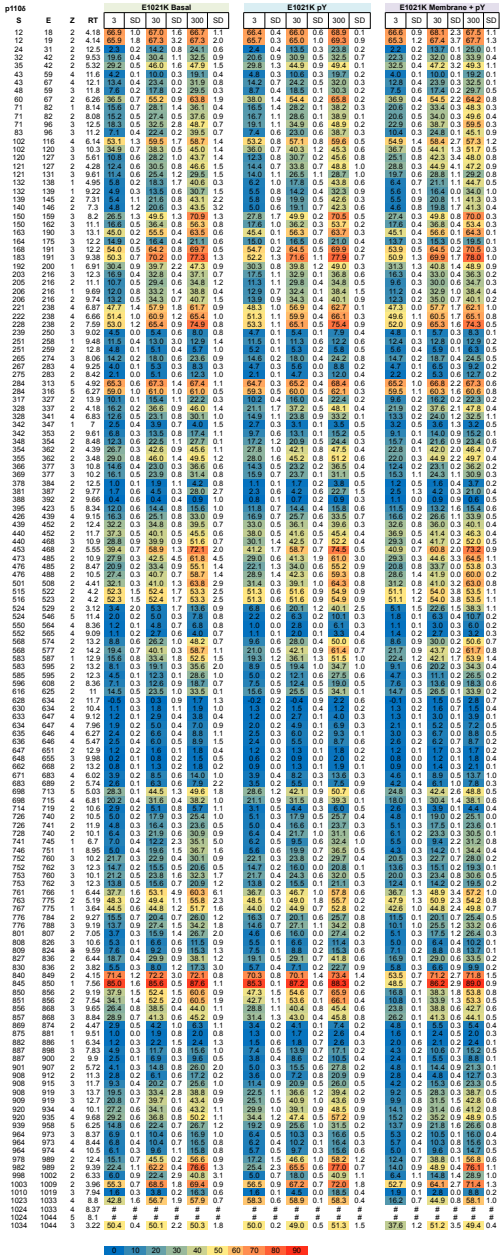


Fig. S6 All HDX peptide data for experiments examining conformational changes in APDS1 mutations under basal, pY-activated, and membrane-bound states for both p110δ and p85α.

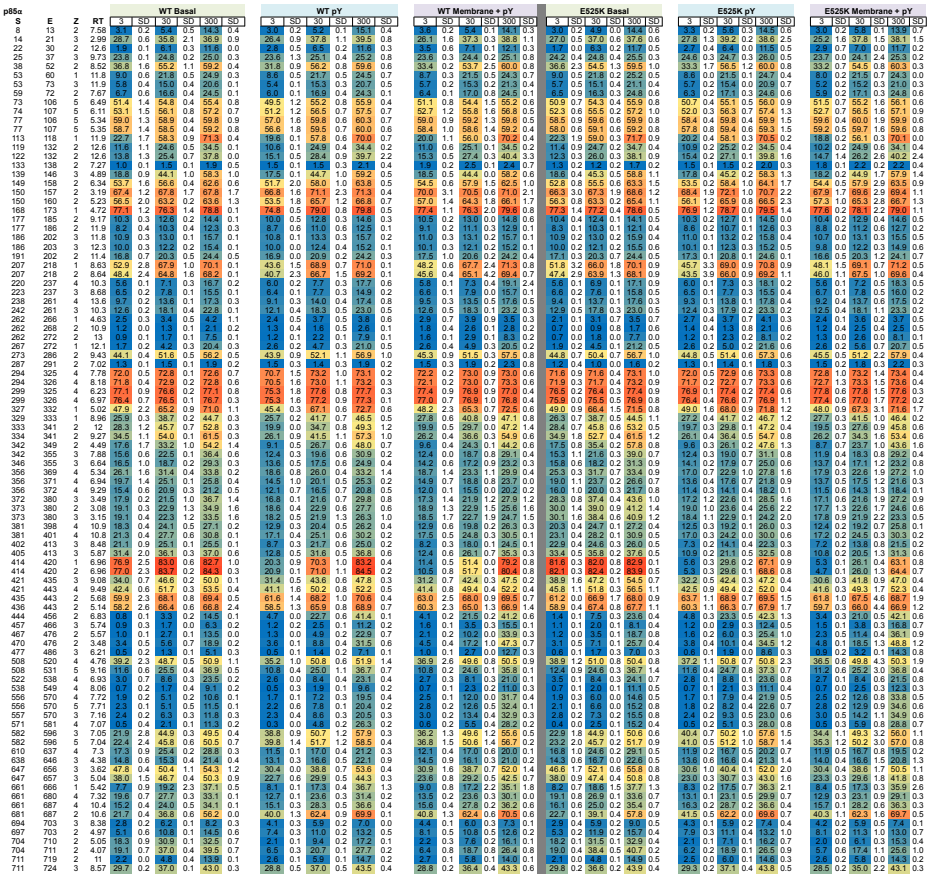


Fig. S6 All HDX peptide data for experiments examining conformational changes in APDS1 mutations under basal, pY-activated, and membrane-bound states for both p110δ and p85α.

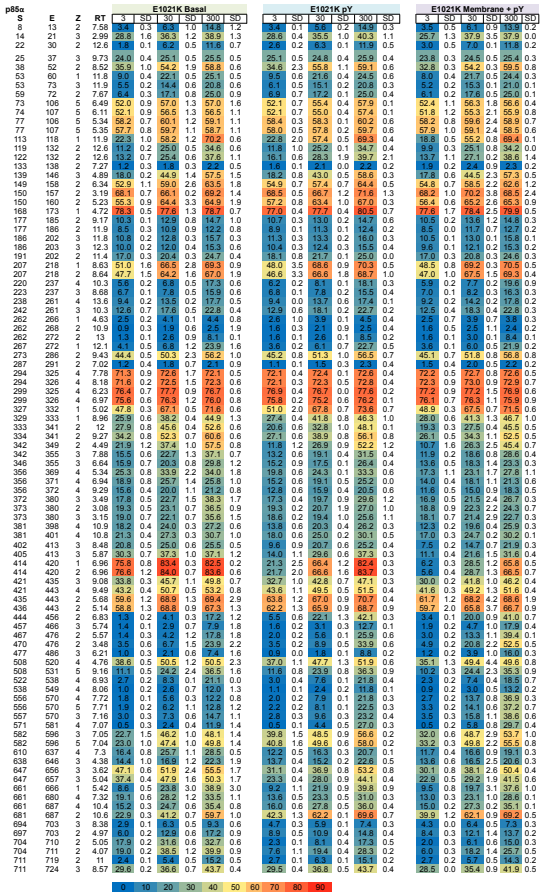


Fig. S6. All HDX peptide data for experiments examining conformational changes in APDS1 mutations under basal, pY-activated (pY), and membrane-bound (PMpY) states for both p110δ and p85α. The charge state (Z), residue start (S), residue end number (E), and retention time (RT) are displayed for every peptide. The three time points are labelled for the conditions tested. The relative level of HDX is coloured according to the amount of deuterium incorporated, on a blue to red continuum. The data listed are the average of three independent experiments, with SD shown next to all HDX values.

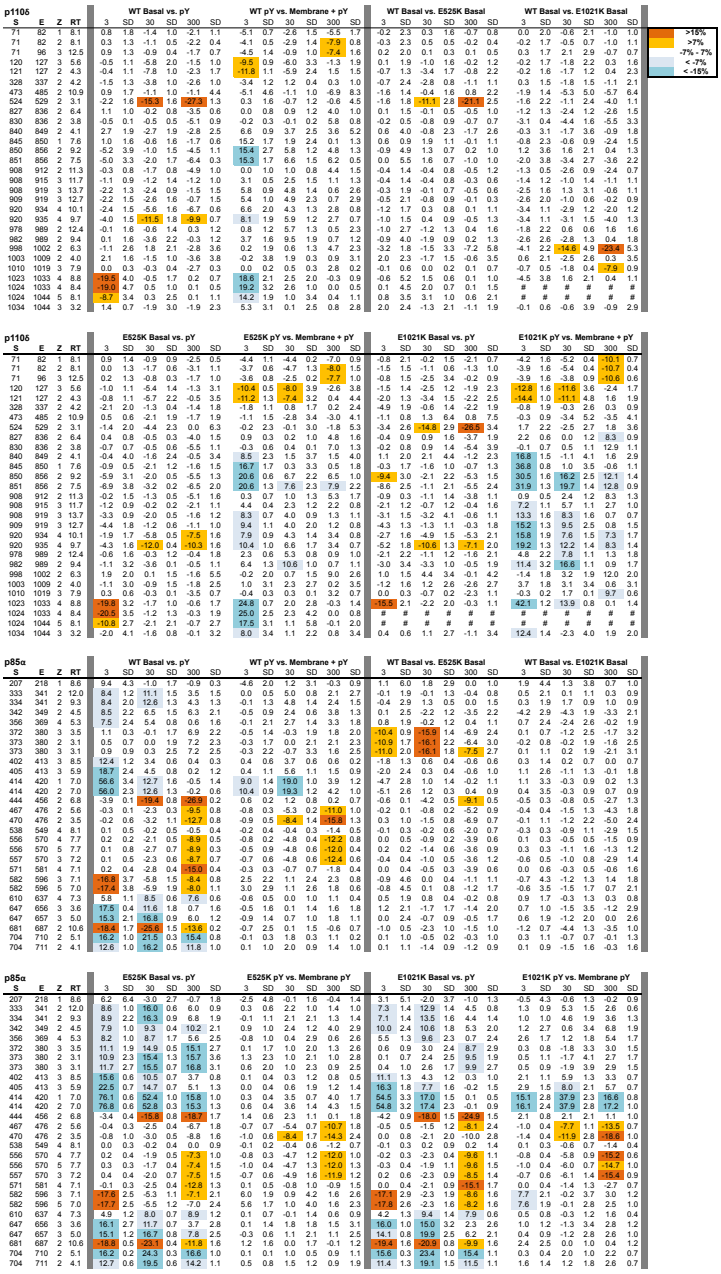


Fig. S7. HDX differences in APDS1 mutations and under different activation states (pY-bound, and membrane-bound). Differences in exchange at all time points for a selected set of peptides (some overlapping peptides have been removed), but all data can be found in Fig. S6) that undergo decreases (positive values) / increases (negative values) in exchange between conditions (WT and APDS1 mutations, pY-activated, membrane-bound) are shown. The specific conditions being compared are labelled above the selected columns. Increases/decreases greater than 0.7 Da and 7% are coloured according to the legend.

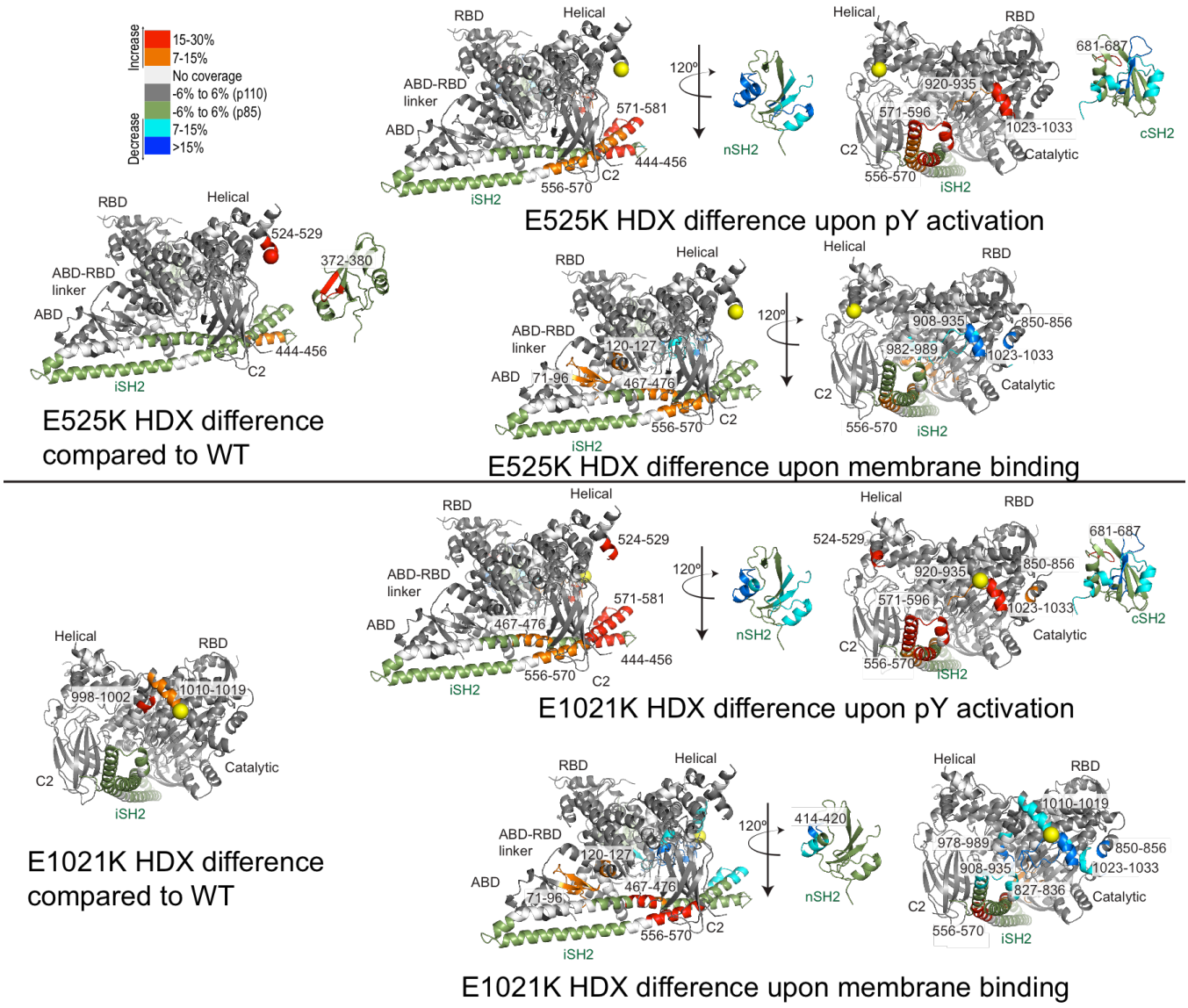


Fig. S8 HDX differences in APDS1 mutations and under different activation states (pY-bound, and membrane-bound). Peptides in p110δ and the iSH2 of p85α that showed differences in HDX both greater than 0.7 Da and 7% between the basal and pY-activated WT or between the pY-activated WT with and without membranes are highlighted on both the structure of p110δ/iSH2-p85α (PDB: 5DXU) according to the legend. Mutations are represented on the structure as yellow spheres.