

8 Supplementary Fig. 1- List of p110 α and p85 α constructs used.





- 10 Supplementary Fig. 2. SDS PAGE gel images of all p110α constructs used in this study. These
- 11 images (n=1) show the purity of each protein construct from a representative prep of each protein.
- 12 Uncropped gel images are presented in the source data.
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15 Supplementary Fig. 3 Comparison between kinase active (KA) and kinase dead (KD) p110α

A. Protein-Lipid FRET assay performed with kinase active and kinase dead p110α constructs under basal
 and pY activated states on liposomes containing 5% PIP₂, 10% Dansyl PS, 25 % PS, 65% PE. Experiments
 were carried out at saturating concentrations of PI3K concentrations ranging from 0.015 to 1µM, 1µM pY,
 and 16.65 µg/ml of lipid vesicles (technical replicates, error bars are S.D., n=3).

(B-D). The #D difference in deuterium incorporation for p110α and p85α in each experiment, with each point representing a single peptide, with error shown as the sum of SD across all time points (n=3 for each time point). comparing the following conditions: C. p110α/p85α WT kinase active vs kinase dead. D. WT kinase active in solution and membrane. E. WT kinase dead in solution and membrane. Peptides in p110α and p85α that showed significant differences in HDX upon binding to 100 nm extruded 5% PIP₂/PS/PE

25	vesicles are colored in red (greater than 0.4 Da and 5% difference at any timepoint, with a two tailed t-test
26	p<0.01).
27	E. %D graphs for a selection of peptides comparing WT kinase active vs Kinase dead under pY active state
28	and membrane bound state. (Mean is shown, with error bars representing S.D., n =3) Full HDX-MS data
29	available in source data.
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41 Supplementary Fig. 4 HDX-MS differences for experiments done with ΔABD

42 **A.** The #D difference in deuterium incorporation for p110 α between p110 α core and full-length p110 α /p85 α 43 complex, each point represents a single peptide with error shown as the sum of SD across all time points 44 (n=3 for each time point). Peptides in p110 α that showed significant differences in HDX are colored in red 45 (greater than 0.4 Da and 5% difference at any timepoint, with a two tailed t-test p<0.01).

46 **B.** %D graphs for a selection of peptides comparing p110α core and full-length p110α/p85α (Mean is shown,

- 47 with error bars representing S.D., n =3). Full HDX-MS data available in source data.
- 48 **C.** The sum #D difference in deuterium incorporation across all time points for p110α core upon binding 5%

49 PIP₂ membranes with error as SD (n=3 for each time point). Peptides in p110α that showed significant

50 differences in HDX are colored in red (greater than 0.4 Da and 5% difference at any timepoint, with a two

51 tailed t-test p<0.01).

52 **D.** %D graphs for a selection of peptides comparing for p110α core in the presence and absence of 5% 53 PIP₂ membranes (Mean is shown, with error bars representing S.D., n = 3). Note that the intrinsic exchange 54 rate of different regions explains some of the differences in H/D exchange seen upon membrane binding. 55 Regions with stable secondary structure in the absence of membrane are protected primarily at later time 56 point (this is due to membrane binding further stabilising the secondary structure, see 735-744). The C-57 terminus undergoes a putative disorder-order transition (1056-1068), and shows stabilisation at all time 58 points, with rapid exchange in the absence of membranes. Finally, regions with limited secondary structure 59 (343-354) show protection at only early timepoints of D₂O exchange.

60 **E.** Protein-Lipid FRET testing membrane recruitment of p110α on PIP₂/PS/PE and PM mimic membranes. 61 FRET assays were performed with saturating concentrations of PI3K (0.5 μ M) and 16.65 μ g of lipid (error 62 bars are S.D., n=3). Two tailed p-values represented by the symbols as follows: **<0.001; *<0.05; n.s.>0.05. 63 **F.** Protein-Lipid FRET testing membrane recruitment of p85α on PIP₂/PS/PE and PM mimic membranes. 64 FRET assays were performed with saturating concentrations of p85 (0.5 μ M) and 16.65 μ g of lipid (error 65 bars are S.D., n=3). Two tailed p-values represented by the symbols as follows: **<0.001; *<0.05; n.s.>0.05. 66 Source data for this figure are provided in the Source Data file.

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75 Supplementary Fig. 5 Source data for experiments comparing WT and different c-terminal mutants

76 **A.** The sum of the #D difference in deuterium incorporation across all time points for p110 α and p85 α in 77 between WT and the indicated p110a mutant or deletion, each point represents a single peptide with error 78 shown as the sum of SD across all time points (n=3 for each time point). Peptides in p110 α and p85 α that 79 showed significant differences in HDX are colored in red (greater than 0.4 Da and 5% difference at any 80 timepoint, with a two tailed t-test p<0.01). Peptide graphs for HDX differences between WT and mutants 81 showing changes in both p110 α and p85 α **B-C**. % D graphs for a selection of peptides showing significant 82 differences between and WT and mutants (Mean is shown, with error bars representing S.D., n =3). Full 83 HDX-MS data available in the source data.

D. Protein-Lipid FRET assay performed with different p110 α constructs under basal and pY activated states on PM mimic liposomes (5% PIP₂, 10% Dansyl PS, 15 % PS, 40% PE, 10% cholesterol, 15% PC and 5% SM) and 5% PIP₂, 10% Dansyl PS, 25 % PS, 65% PE liposomes. Experiments were carried out at saturating concentrations of PI3K (1µM) and 16.65 µg/ml of lipid vesicles (Mean is shown, with error bars representing S.D., n =3). Two tailed p-values represented by the symbols as follows: **<0.001; *<0.05; n.s.>0.05. Source data for this figure are provided in the Source Data file.

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98 A-C. HDX-MS comparing basal and pY activated states of (A) WT (B) H1047R and (C) the Δ C-terminus.

99 The differences with H1047R were mapped onto p110α/iSH2-nSh2 (PDB: 3HHM) and cSH2 (2Y3A). The

100 differences between WT and ΔCter were mapped onto p110α/iSH2-nSH2 (PDB: 4OVU) and nSH2

101 (2Y3A)

102 **D-F.** The sum of the #D difference in deuterium incorporation across all time points for p110 α and p85 α 103 upon pY binding for the indicated p110 α mutant or deletion, each point represents a single peptide with 104 error shown as the sum of SD across all time points (n=3 for each time point). Peptides in p110 α and p85 α that showed significant differences in HDX are colored in red (greater than 0.4 Da and 5% difference at any
timepoint, with a two tailed t-test p<0.01).

107	G. %D graphs for a selection of peptides with differences in exchange, with all HDX-MS data available in
108	the source data. Error bars are SD (n=3). Note that the intrinsic exchange rate of different regions explains
109	some of the differences in H/D exchange seen upon pY binding. Regions with stable secondary structure
110	in the absence of pY are protected primarily at later time points (this is due to pY binding further stabilising
111	the secondary structure, see 1014-1021). Regions with less stable secondary structure show changes
112	throughout the time course (see peptide 444-475). Source data for this figure are provided in the Source
113	Data file.
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126 Supplementary Fig. 7: Predicted molecular mechanism of activation for various PIK3CA

127 mutations. Mutations at the nSH2 interface/helical domain lead to disengagement of the nSH2 while the

128 mutations at the ABD/C2 promote disengagement of ABD-p85 from the catalytic core. Mutations at the

129 kinase domain promote membrane recruitment either by addition of positively charged residue at the

130 membrane binding interface (E726K) or alter the regulatory motif.

139 Supplementary Table 1a. - HDX-MS processing details

Experiment	Comparing WT PI3K and p110 α core. HDX Stats table for data shown in Figure 2 and Figure 3.			
Data set	WT	$p110\alpha$ core + Memb		
HDX reaction	%D ₂ O=69%	%D ₂ O=69%	%D ₂ O=69%	
details	pH _(read) =7.5	$pH_{\text{(read)}}=7.5$	$pH_{\text{(read)}}=7.5$	
	Temp=18°C	Temp=18°C	Temp=18°C	
HDX time course	3s, 30s, 300s	3s, 30s, 300s	3s, 30s, 300s	
(seconds)				
HDX controls	N/A	N/A	N/A	
Back-exchange	No correction, deuterium	No correction, deuterium	No correction, deuterium	
	levels are relative	levels are relative	levels are relative	
Replicates	3	3	3	
Protein p110a				
Number of peptides	125	125	125	
Sequence coverage	89.9%	89.9%	89.9%	
Average peptide	Length= 13.9	Length= 13.9	Length= 13.9	
/redundancy	Redundancy= 1.8	Redundancy= 1.8	Redundancy= 1.8	
Repeatability	Average StDev= 0.6%	Average StDev=0.4%	Average StDev=0.6%	

156 Supplementary Table 1b- HDX-MS processing details

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $								
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Experiment	Comparing basal PI3K WT vs mutants (H1047R, M1043L and Delta C) and in the presence of pY with H1047R and Delta C. HDX Stats table for data shown in Figure 5A, Figure 5C, Figure 5D and Figure S7						
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Data set	WT	WT+pY	H1047R	H1047R + pY	M1043L	Delta C	Delta C + pY
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	HDX	%D ₂ O=75%	%D ₂ O=75%	%D ₂ O=75%	%D ₂ O=75%	%D ₂ O=75%	%D ₂ O=75%	%D ₂ O=75%
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	reaction	$pH_{(read)}=7.5$	$pH_{(read)}=7.5$	$pH_{(read)}=7.5$	$pH_{(read)}=7.5$	$pH_{\text{(read)}}=7.5$	$pH_{(read)}=7.5$	$pH_{(read)}=7.5$
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	details	Temp=18°C	Temp=18°C	Temp=18°C	Temp=18°C	Temp=18°C	Temp=18°C	Temp=18°C
$ \begin{array}{cccc} course \\ (seconds) & 18^{\circ}\mathrm{C} & 18^{\circ}\mathrm{C} & 18^{\circ}\mathrm{C} & 3s at 0 {}^{\circ}\mathrm{C} & 3s at 0 {}^{\circ}\mathrm{C}$	HDX time	3s, 30s, 300s,	3s, 30s, 300s,	3s, 30s, 300s,	3s, 30s, 300s,	3s, 30s, 300s,	3s, 30s, 300s,	3s, 30s, 300s,
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	course	3000s at	3000s at	3000s at	3000s at	3000s at	3000s at	3000s at
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	(seconds)	18℃	18℃	18℃	18℃	18℃	18℃	18℃
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		3s at 0 °C	3s at 0 °C	3s at 0 °C	3s at 0 °C	3s at 0 °C	3s at 0 °C	3s at 0 °C
$ \begin{array}{ c c c c c } \hline controls & & & & & & & & & & $	HDX	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Back- exchangeNo correction deuterium levels are relativeNo correction relativeNo correction deuterium levels are relativeNo correction relativeNo correction relativeNo correction relativeNo correction relativeNo correction relative	controls							
exchangedeuterium levels are relativedeuterium levels are relativedeuterium relativedeuterium levels are relativedeuterium relativedeuterium levels are relativedeuterium relativedeuterium relativedeuterium relativedeuterium relativedeuterium relativedeuterium relativedeuterium relativedeuterium relative	Back-	No correction	No correction	No correction	No correction	No correction	No correction	No correction
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	exchange	deuterium	deuterium	deuterium	deuterium	deuterium	deuterium	deuterium
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$		levels are	levels are	levels are	levels are	levels are	levels are	levels are
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$		relative	relative	relative	relative	relative	relative	relative
$\begin{array}{ c c c c c c c c } \hline \begin{tabular}{ c c c c c c c } \hline \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Replicates	3	3	3	3	3	3	3 (2 for 3s 0
$\begin{array}{c c c c c c c c c c c c c c c c c c c $								°C at sample)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Significant	>5%	>5%	>5%	>5%	>5%	>5%	>5%
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	differences	and >0.4 Da	and >0.4 Da	and >0.4 Da	and >0.4 Da	and >0.4 Da	and >0.4 Da	and >0.4 Da
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	in HDX	and unpaired	and unpaired	and unpaired	and unpaired	and unpaired	and unpaired	and unpaired
Protein Instruction <		t-test ≤0.01	t-test ≤0.01	t-test ≤0.01	t-test ≤0.01	t-test ≤0.01	t-test ≤0.01	t-test ≤0.01
Number peptides 171 171 170 170 169 164 164 Sequence coverage 95.8% 13.7 Redundancy= Redundancy= Redundancy= 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.1	Protein		I			I	I	1
peptides95.8%95.8%95.8%95.8%95.8%95.8%Sequence coverage95.8%95.8%95.8%95.8%95.8%95.8%Average peptideLength= 13.7 Redundancy=Length= 13.7 Redundancy=Length= 13.7 Redundancy=Length= 13.7 Redundancy=Length= 13.7 	Number	171	171	170	170	169	164	164
Sequence coverage 95.8%	peptides							
coverageImage<	Sequence	95.8%	95.8%	95.8%	95.8%	95.8%	95.8%	95.8%
Average peptideLength= 13.7 Redundancy=Length= 13.7 Redundancy=Length= 13.7 	coverage							
peptideRedundancy= <td>Average</td> <td>Length= 13.7</td> <td>Length= 13.7</td> <td>Length= 13.7</td> <td>Length= 13.7</td> <td>Length=13.7</td> <td>Length= 13.7</td> <td>Length= 13.7</td>	Average	Length= 13.7	Length= 13.7	Length= 13.7	Length= 13.7	Length=13.7	Length= 13.7	Length= 13.7
/redundancy2.12.12.12.12.12.12.1RepeatabilityAverageAverageAverageAverageAverageAverageAverageStDev=0.5%StDev=0.5%StDev=0.6%StDev=0.5%StDev=0.5%StDev=0.5%StDev=0.5%ProteinI17I17I17I17I17I17	peptide	Redundancy=	Redundancy=	Redundancy=	Redundancy=	Redundancy=	Redundancy=	Redundancy=
RepeatabilityAverage<	/redundancy	2.1	2.1	2.1	2.1	2.1	2.1	2.1
StDev=0.5% StDev=0.5% StDev=0.6% StDev=0.5% StDev=0.5% StDev=0.5% Protein	Repeatability	Average	Average	Average	Average	Average	Average	Average
Protein Image: Second sec	D	StDev=0.5%	StDev=0.5%	StDev=0.6%	StDev=0.5%	StDev=0.5%	StDev=0.5%	StDev=0.5%
Number 117<	Protein	115	115	115	115	115	115	115
peptides	Number	117	117	117	117	117	117	117
	peptides		0.0.0.1			0.0.0.1		
Sequence 90.3% 90.3% 90.3% 90.3% 90.3% 90.3% 90.3%	Sequence	90.3%	90.3%	90.3%	90.3%	90.3%	90.3%	90.3%
coverage	coverage	1 1 1 ()	L 1 160	1 1 1 ()	1 1 1 ()	L 1 160	L 1 160	I 1 160
Average Length= 16.3	Average	Length= 16.3	Length= 16.3	Length= 16.3	Length= 16.3	Length= 16.3	Length= 16.3	Length= 16.3
redundancy= Re	peptide	κ equindancy =	Redundancy=	Redundancy=	Redundancy=	Redundancy=	Redundancy=	κ edundancy=
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	/redundancy	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Kepeataoliniy Average Average Average Average Average Average StDev=0.5% StDev=0.6% StDev=0.6% StDev=0.6% StDev=0.6% StDev=0.6% StDev=0.6% StDev=0.6%	Repeatability	Average StDev-0.5%	Average StDev-0.6%	Average StDev-0.6%	Average StDev-0.5%	Average StDev-0.6%	Average StDev-0.6%	Average StDev-0.6%

166 Supplementary Table 1c- HDX-MS processing details

Experiment	Comparing Kinase Active PI3K and G1049R PI3K. HDX Stats table for data shown			
	in Figure 5B and Figure 5F			
Data set	WT	G1049R		
HDX reaction details	%D ₂ O=76%	%D ₂ O=76%		
	$pH_{\text{(read)}}=7.5$	$pH_{\text{(read)}}=7.5$		
	Temp=18°C	Temp=18°C		
HDX time course (seconds)	3s, 30s, 300s, 3000s	3s, 30s, 300s, 3000s		
HDX controls	N/A	N/A		
Back-exchange	No correction, deuterium levels are	No correction, deuterium levels are		
	relative	relative		
Replicates	3	3		
Significant differences	>5% and >0.4 Da and unpaired t-test	>5% and >0.4 Da and unpaired t-test		
in HDX	≤0.01	≤0.01		
Protein	p1	10a		
Number of peptides	169	169		
Sequence coverage	95.2%	95.2%		
Average peptide	Length=13.7	Length=13.7		
/redundancy	Redundancy=2.1	Redundancy=2.1		
Repeatability	Average StDev=0.6%	Average StDev=0.5%		
Protein	p	85		
Number of peptides	114	114		
Sequence coverage	89%	89%		
Average peptide	Length=16.4	Length=16.4		
/redundancy	Redundancy=2.5	Redundancy=2.5		
Repeatability	Average StDev=0.6%	Average StDev=0.5%		

Experiment	Comparing Kinase Active PI3K and Frameshift. HDX Stats table for data shown		
	in Figure 5E		
Data set	WT	Frameshift	
HDX reaction details	%D ₂ O=81%	%D ₂ O=81%	
	$pH_{(read)}=7.5$	$pH_{(read)}=7.5$	
	Temp=18°C	Temp=18°C	
HDX time course (seconds)	3s, 30s, 300s, 3000s	3s, 30s, 300s, 3000s	
HDX controls	N/A	N/A	
Back-exchange	No correction, deuterium levels are	No correction, deuterium levels are	
	relative	relative	
Replicates	3 (2 for 3s sample)	3	
Significant differences in	>5% and >0.4 Da and unpaired t-test	>5% and >0.4 Da and unpaired t-test	
HDX	≤0.01	≤0.01	
Protein	p110a		
Number of peptides	141	139	
Sequence coverage	84.7%	84.7%	
Average peptide	Length= 13.7	Length= 13.7	
/redundancy	Redundancy= 1.6	Redundancy= 1.6	
Repeatability	Average StDev=0.9%	Average StDev=0.7%	
Protein	p:	85	
Number of peptides	180	180	
Sequence coverage	79%	79%	
Average peptide	Length= 16	Length= 16	
/redundancy	Redundancy= 2.6	Redundancy= 2.6	
Repeatability	Average StDev=0.9%	Average StDev=0.6%	

168 Supplementary Table 1d-HDX-MS processing details

Experiment	Comparing Kinase Active PI3K and Kinase Dead (KD) PI3K. HDX Stats table for data shown in Supplemental Figure 3			
Data set	WT	WT+memb	KD	KD + Memb
HDX reaction	%D ₂ O=72%	%D ₂ O=72%	%D ₂ O=72%	%D ₂ O=72%
details	$pH_{\text{(read)}}=7.5$	$pH_{\text{(read)}}=7.5$	$pH_{\text{(read)}}=7.5$	$pH_{\text{(read)}}=7.5$
	Temp=18°C	Temp=18°C	Temp=18°C	Temp=18°C
HDX time	3s, 300s	3s, 300s	3s, 300s	3s, 300s
course				
(seconds)				
HDX controls	N/A	N/A	N/A	N/A
Back-exchange	No correction,	No correction,	No correction,	No correction,
	deuterium levels are	deuterium levels are	deuterium levels are	deuterium levels are
	relative	relative	relative	relative
Replicates	3	3	3	3
Significant	>5% and >0.4 Da	>5% and >0.4 Da	>5% and >0.4 Da	>5% and >0.4 Da
differences in	and unpaired t-test	and unpaired t-test	and unpaired t-test	and unpaired t-test
HDX	≤0.01	≤0.01	≤0.01	≤0.01
Protein	p110a			
Number of	133	133	133	133
peptides				
Sequence	84.9%	84.9%	84.9%	84.9%
coverage				
Average	Length=13.1	Length=13.1	Length=13.1	Length=13.1
peptide	Redundancy=1.5	Redundancy=1.5	Redundancy=1.5	Redundancy=1.5
/redundancy				
Repeatability	Average StDev=0.7	Average StDev=0.7	Average	Average
			StDev=1.1%	StDev=1.3%
Protein	p85			
Number of	81	81	81	81
peptides				
Sequence	71.4%	71.4%	71.4%	71.4%
coverage				
Average	Length=15.1	Length=15.1	Length=15.1	Length=15.1
peptide	Redundancy=1.6	Redundancy=1.6	Redundancy=1.6	Redundancy=1.6
/redundancy				
Repeatability	Average StDev=0.7	Average	Average	Average StDev=1%
		StDev=0.6%	StDev=1.4%	