Supporting Information

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SI Materials and Methods

Cloning of Plasmids Encoding Multiple Genes. The ^{552}RK-DD p110γ mutant as well as the tetra-alanine p101 mutants were done using the Quick-change strategy (Stratagene). For PI3Kγ-dependent GFP-Grp1 translocation experiments in mammalian cells, we used coexpression plasmids that allow for the simultaneous expression of all of the desired proteins in every transfected cell. Plasmids expressing myc-tagged human p110γ subunit, FLAG-mKateII (RFP) tagged porcine p101, and the myc-GFP-tagged $Grp1_{PH}$ domain were cloned into a single pcDNA3.1 vector using the Infusion enzyme (Clontech). Each of the genes was initially cloned individually into a pcDNA3.1 vector. Then, the p110γ gene with the promoter and terminator regions was introduced in the vector expressing the GFP-$Grp1_{PH}$ receptor gene. The latter vector had been digested with MluI restriction enzyme, and the PCR fragment encompassing the promoter-p110γ-terminator DNA possessed homologous sites with regions flanking the MluI restriction site. Primers were also designed to keep only the MluI restriction site on the 5′ end, so that other promoter-gene-terminator DNA sequences could be introduced into plasmids expressing multiple genes.

We used the MultiBac expression system to coexpress p110γ/p101 wild type or mutants that were used for lipid kinase assays in vitro. The two genes were assembled into a single plasmid using a "multiplication module" approach where a I-CeuI/BstXI fragment containing promoter, p101, and terminator sequences obtained by digestion of a p101/pAceBac1 plasmid was ligated into a I-CeuI–digested p110γ/pAceBac1 vector (1). Multigene plasmids coexpressing both genes were integrated into EMBacY baculoviral DNA via Tn7 transposition. EMBacY encodes YFP, which facilitates monitoring baculovirus infection of insect cells (1).

Phosphatidylinositol (3,4,5)-Trisphosphate Reporter Translocation Assay. HEK293T cells stably expressing the formyl-MET-LEU-PHE (fMLP) receptor were grown at 37 °C with 5% (vol/vol) CO_2 in DMEM complemented with 10% (vol/vol) heat-inactivated FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1 μg/mL puromycin. For confocal microscopy experiments, cells were plated in glass-bottom chambers (LabTek, two-well chambers) and transfected using Lipofectamine 2000 (Invitrogen) as described by the manufacturer. Each transfection was done with 300 ng of plasmids total per well in a six-well plate. After 24 h, transfected cells were starved in serum-free media for 14 h before imaging on a confocal microscope.

Confocal microscopy analysis was done on an Andor revolution XD system built around an inverted Nikon Ti microscope. For each transfection, movies were recorded by taking images every 10–15 s for at least 5 min after manual addition of 1 μM final fMLP peptide (Sigma; 10 μM stock in DMEM). Five to six fields were analyzed for every well. All cells in every field were analyzed, and the ratio of cells that showed fMLP-mediated GFP-$Grp1_{PH}$ translocation to cells unaffected was calculated. For a cell to be counted as positive, translocation had to be seen at anytime within 5 min after agonist addition. For each construct, 5–18 fields were analyzed, resulting in observation of at least 100 cells.

Akt Activation. HEK293E cells, grown in DMEM/10% (vol/vol) FBS, were transfected without or with HA-p101 (1.35 μg), myc-Akt (0.05 μg), and either myc-WT-p110γ (0.8 μg) or myc-DD-p110γ (0.75 μg plus 0.05 μg empty vector). Transfections were performed using Fugene at a Fugene (μL)/DNA (μg) ratio of 3:1. At 24 h posttransfection, cells were starved (1× DMEM + 100 U/mL pennicillin, 100 μg/mL streptomycin + 1 mM sodium pyruvate) overnight. The cells were then treated without or with 10 μM lysophosphatidic acid (LPA) for 5 min, lysed, and immunoprecipitated with anti-myc antibody. Anti-myc immunoprecipitates or whole-cell lysates were blotted with anti-myc, anti–pT308-Akt, or anti-HA antibodies. Results are shown as the ratio of pAkt over total Akt.

Protein Expression in Insect Cells and Purification. Expression of p110γ-His6 and of p110γ/His6-p101 was done in Sf9 insect cells. Cells (3 L) were infected with a single bacmid vector encoding either p110γ alone or p110γ/p101 present on the same plasmid, each under the control of an independent polyhedrin promoter, using a MultiBac system (1). After 55 h of infection, cells were harvested and lysed by sonication for 4 min and centrifuged for 20 min at 110,000 × *g*. The supernatant was filtered through a 0.45-μm Minisart filter unit (Sartorius Biotech) before loading onto a 5-mL HisTrap FF column (GE Healthcare). Protein was eluted with a 15- to 150-mM Imidazole gradient and subsequently purified on a 5-mL HiTrap Q-HP column (GE Healthcare). After elution with a 0–1 M NaCl gradient, the complex was concentrated using AMICON 50K centrifugal filters (Millipore) and loaded onto a 16/60 Superdex 200 gel filtration column (GE Healthcare) at 4 °C running with buffer containing 20 mM Hepes, pH 7.5, 100 mM NaCl, and 2 mM tris(2-chloroethyl) phosphate (TCEP). The proteins were concentrated to about 5 mg/mL, frozen in liquid nitrogen, and stored at −80 °C.

Purification of Gβγ and H-Ras Expressed in Insect Cells. Recombinant human $G\beta_1$ and bovine N-terminally hexahistidine-tagged wild-type $G\gamma_2$ and $G\gamma_2$(C68S) mutants were produced in Sf9 cells and purified following the procedure described previously (2). N-terminally hexahistidine-tagged human H-Ras was produced in Sf9 insect cells. After 55 h of infection with baculoviruses encoding H-Ras, the cells were collected by centrifugation at 1,000 × *g* for 5 min and washed with PBS. Isoprenylated H-Ras was isolated from the Sf9 cells using the Triton X-114 partition method as described previously (3). Subsequently, the detergent-enriched phase containing isoprenylated H-Ras was supplemented with 15 mM imidazole and incubated with Ni Sepharose 6 Fast Flow beads (GE Healthcare; 1 mL of beads/1.2 × 10^9 infected cells) for 1 h at 4 °C. The mixture was loaded onto a column cartridge and washed (6 column volumes) with a buffer containing 20 mM Hepes, pH 7.5, 200 mM NaCl, 5 mM $MgCl_2$, 200 μM GDP, 10 mM β-mercaptoethanol, 15 mM imidazole, and 0.5% (wt/vol) sodium cholate. Hexahistidine-tagged H-Ras was eluted with a buffer containing 20 mM Tris, pH 8, 20 mM NaCl, 5 mM $MgCl_2$, 10 mM β-mercaptoethanol, 200 mM imidazole, and 0.5% (wt/vol) sodium cholate. Eluted protein was loaded onto a 1-mL Resource 15Q HR 5/5 column (GE Healthcare) equilibrated with a buffer containing 20 mM Tris, pH 8, 5 mM $MgCl_2$, 2 mM DTT, and 0.5% (wt/vol) sodium cholate. Bound proteins were eluted and fractionated with a continuous gradient elution (0–600 mM NaCl). Peak fractions were pooled and concentrated with Amicon 10 concentrators (Millipore).

H-Ras was loaded with guanosine-5′-[(β,γ)-imido]triphosphate (GppNHp; Jena Bioscience) by incubation with a 30-M excess of the nucleotide in the presence of 5 mM $MgCl_2$ and 15 mM EDTA for 12 h at 4 °C. Thereafter, $MgCl_2$ was added to the mixture to a final concentration of 15 mM. GppNHp-bound H-Ras was separated from the free pool of GppNHp using a Superdex 200 10/300 GL gel filtration column (GE Healthcare).

Peak fractions were pooled and concentrated with Amicon 10 concentrators. The posttranslational processing and lipidation of the protein was verified by MS analysis. Proteins were stored at −80 °C.

Lipid Kinase Assays. Lipid kinase assays to test for PI3Kγ activity were done by measuring radioactive phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) formation following a similar procedure as described previously (2). Briefly, lipid vesicles of the following composition were prepared: 5% brain-phosphatidylinositol 4,5-bisphosphate (PIP_2) (Sigma), 20% brain-phosphatidylserine (PS) (Sigma), 45% brain-phosphatidylethanolamine (PE) (Avanti), 15% 1,2-dioleoyl PC (DOPC) (Avanti), 10% cholesterol (Sigma), and 5% egg-sphingomyelin (Sigma). Percentages are weight percentages. Vesicles were used at a final concentration of 0.8 mg/mL. First, 2 μL of 0.5 mg/mL BSA solution was added to a well of a 384-well plate (Costar). Then 2 μL of a fivefold concentrated solution containing PI3Kγ constructs was added (at 600 nM for basal activity and 10 nM for Gβγ-stimulated activity in 20 mM Hepes, pH 7.5, 100 mM NaCl, and 2 mM TCEP). Subsequently, 2 μL of a solution containing 1.5 μM Gβγ (or buffer with matched concentration of CHAPS) was added. Substrate stock solutions containing lipids at 4 mg/mL were prepared, and 2 μL of this solution was added to the mixture. Reaction was started by adding 2 μL of a 400-μM ATP solution containing 0.2 μCi/mL of [γ^{32}P]ATP. Reactions were carried out in 20 mM Hepes (pH 7.5), 100 mM NaCl, 2 mM TCEP, 7 mM $MgCl_2$, 1 mM EGTA, and 50 μM CHAPS. The reaction was stopped after 60 min by transferring 5 μL of reaction mixture to 5 μL of a 20-mM EDTA quench solution. Lipid kinase activity was determined using a modified membrane capture radioactive assay measuring production of ^{32}P-labeled PIP_3 (4). A 3-μL aliquot of this mixture was then spotted onto a nitrocellulose membrane. The membrane was dried and washed six times with a 1-M NaCl/1% (vol/vol) phosphoric acid solution. The membrane was then air-dried before exposure to a phosphor screen (Molecular Dynamics) for 50 min. Intensity of the spots on the membrane was imaged using a Typhoon PhosphorImager (GE Healthcare) and quantified with the ImageQuant software (GE Healthcare).

Hydrogen–Deuterium Exchange Mass Spectrometry Measurements. Hydrogen–deuterium exchange mass spectrometry (HDX-MS) analyses of PI3Kγ were done following a similar protocol as described previously (2). Full-length p110γ-His6, EE-tagged-p101, and isoprenylated $G\beta_1$/His6-$G\gamma_2$ were used in all HDX-MS experiments. The same lipid vesicle composition as for lipid kinase assays was used in all experiments. To map interactions between p110γ and p101, the rate of exchange between p110γ alone and a p110γ/p101 heterodimer was compared. Protein stock solutions at 3 μM were prepared in 20 mM Hepes (pH 7.5), 100 mM NaCl, and 2 mM DTT. Exchange reactions were started by mixing 10 μL of protein stock in 40 μL of a 85% (vol/vol) D_2O solution containing 10 mM Hepes (pH 7.5) and 50 mM NaCl, reaching a final concentration of 69% D_2O. Deuterium exchange reactions were run for 3, 30, and 300 s of on-exchange at 23 °C before quenching the reaction. On-exchange was stopped with 20 μL of quench buffer containing 2.4% (vol/vol) formic acid and 3.2 M guanidine–HCl, which lowered the pH to 2.6. Samples were then immediately frozen in liquid nitrogen and stored at −80 °C for no longer than 7 d. Experiments mapping lipid-binding sites either on p110γ alone or on a p110γ/p101 heterodimer compared the rate of exchange for the enzymes in the absence or presence of lipid vesicles. Lipid vesicles at 5 mg/mL were diluted eightfold with the 98% D_2O solution described above, and the latter solution was used for deuterium incorporation experiments. Exchange reactions were started by the addition of 10 μL of protein stock to 40 μL of lipid-containing D_2O solution, reaching a final concentration of 69% D_2O. Deuterium exchange reactions ran for the same time points described above. Effects of Gβγ were analyzed on a p110γ/p101 heterodimer in the presence of membranes. To shift the equilibrium toward the PI3Kγ-lipidated Gβγ complex and minimize the concentration of free PI3Kγ, the Gβγ concentration (10 μM) was in excess of the PI3Kγ concentration (3 μM). Exchange reactions were performed with lipid-containing D_2O solution as described above. Every time point and state was a unique experiment, and every HDX-MS experiment was repeated twice.

Measurement of Deuterium Incorporation. Samples were rapidly thawed on ice and injected onto a UPLC system immersed in ice. The protein was run over an immobilized pepsin column (Applied Biosystems, Poroszyme, 2–3131-00) at 130 μL/min and collected over a particle vanguard pre-column (Waters) for 3 min. The trap was then eluted in line with an Acquity 1.7-μm particle, 100- × 1-mm C18 UPLC column (Waters) using a 5–36% gradient of buffer A (0.1% formic acid) and buffer B (100% acetonitrile) over 20 min and injected onto a LTQ Orbitrap XL (Thermo Scientific) to acquire mass spectra of peptides ranging from 350 to 1,500 *m/z*.

Protein Digestion and Peptide Identification. Mass analysis of the peptide centroids was performed as described previously, using the software HD-Examiner (Sierra Analytics) (5). Initial peptide identification was done by running tandem MS/MS experiments, using a 5–35% B gradient over 60 min with an LTQ Orbitrap XL (Thermo Scientific). Peptides were identified using a Mascot search in Thermo Proteome Discoverer software v. 1.2 (Thermo Scientific) based on fragmentation and peptide mass. The MS tolerance was set at 3 ppm with a MS/MS tolerance of 0.5 Da. All peptides with a Mascot score >15 were analyzed by the HD-Examiner software. Any ambiguous peptides were excluded from the analysis. The full list of peptides was then manually validated by searching a nondeuterated protein sample MS scan to test for correct *m/z* state and check for the presence of overlapping peptides. The HD-Examiner software was used to automate the initial analysis of deuterium incorporation, but every peptide was manually verified at every state and time to check for correct charge state, *m/z* range, presence of overlapping peptides, and proper retention time.

Mass Analysis of Peptide Centroids. Selected peptides were then manually examined for deuterium incorporation and accurate identification. Results are presented as relative levels of deuteration with no correction for back exchange because no fully deuterated protein sample could be obtained. However, a correction was applied to compensate for differences in the level of deuterium in the exchange buffer (78 or 69% in experiments with lipids). The real level of deuteration will be ∼25–35% higher than what is shown, based on tests performed with fully deuterated standard peptides. The average error was ≤0.2 Da for corrected data of two replicates. The deuterium incorporation was also plotted versus the on-exchange time.

Transformation Assays. NIH 3T3 cells grown in DMEM/10% (vol/vol) newborn calf serum were transfected with p110γ and p101 constructs. Two days after transfection, cells (2,500 cells/well) were plated in 1 mL of 0.3% top agar over 1 mL of 0.6% (wt/vol) bottom agar in a six-well dish. Cell colonies were counted 3 weeks later. Each figure is representative of two separate experiments.

Chemotaxis and Boyden-Chamber Assays. HEK293E cells (10% confluent in 60-cm dishes) were transfected with empty vector or constructs for p101 and wild-type or mutant p110γ in a pcDNA3.1 vector. After 24 h, the cells were trypsinized and seeded on 10-cm dishes to keep cell density low; after attachment (6 h) the cells

were incubated in serum-free DMEM containing 0.8% BSA overnight. The following day, the cells were dislodged without trypsin by pipetting, and cell clumps were disrupted using cell strainer tubes (BD Biosciences). The cells were plated onto transwell filters that had been pretreated for 2 h at 37 °C with 100 μL of 30 μg/mL collagen in 0.02 N acetic acid at a density of 65,000 cells per well in 200 μL serum-free DMEM/0.8% (wt/vol) BSA. Cells were exposed to 10 μM LPA in the lower chambers for 15 h and then fixed with 4% (wt/vol) paraformaldehyde for 15 min and washed with PBS for 5 min. After mechanical removal of cells remaining on the upper face of the filters, the filters were cut and mounted using DAPI-Fluoromount-G (Southern Biotech). Cells were imaged by fluorescence microscopy, and the average number of cells in 10 nonoverlapping fields from each filter was determined; two to three filters per condition were analyzed. To combine data from multiple experiments, the number of migrating cells under each condition was normalized to the maximal signal (LPA-stimulated cells expressing p101 and wild-type p110γ).

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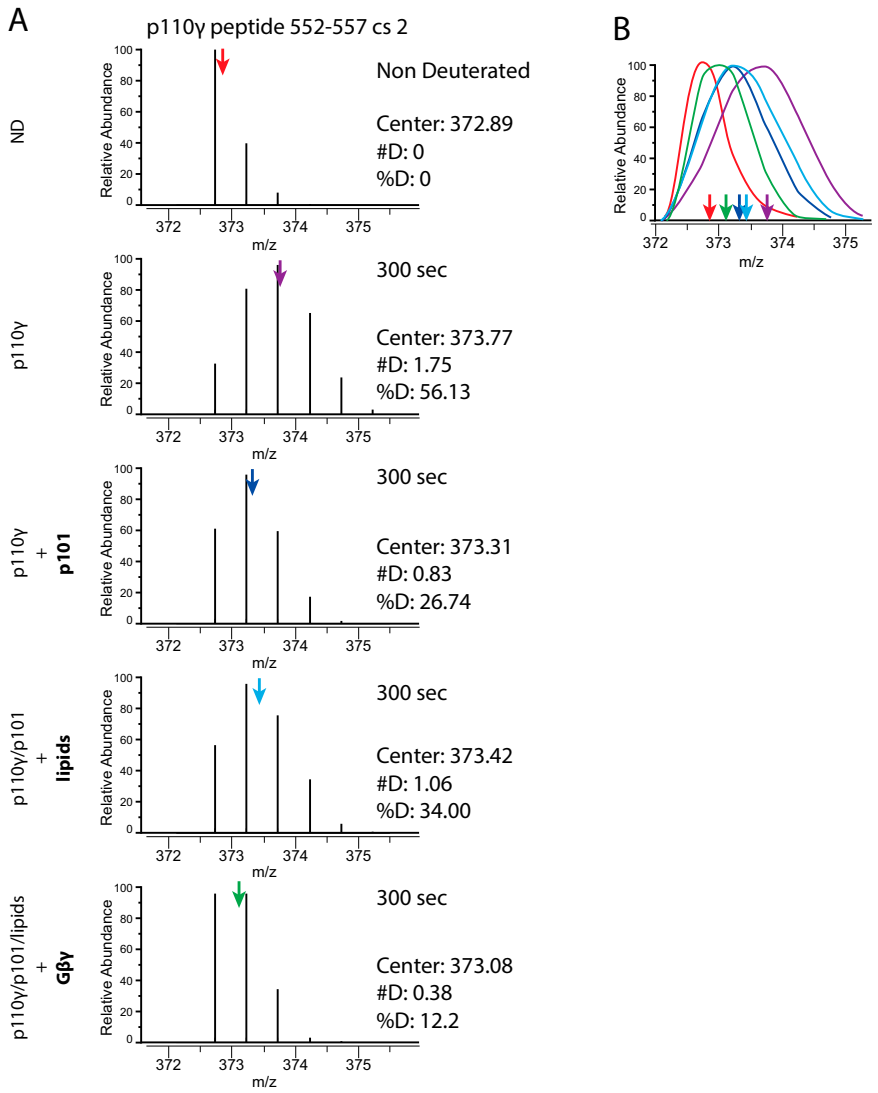


Fig. S1. HDX-MS raw data. (*A*) Raw data for a selected peptide in p110γ, showing deuterium incorporation as a measure of peptide mass centroid shift. All MS traces are after 300 s of deuterium incorporation, except for the nondeuterated sample. (*B*) Representation of isotopic envelope for the same peptides represented in *A* to better highlight the changes in mass centroid in every state.

p110γ peptide map (95% coverage)

p101 peptide map (74% coverage)

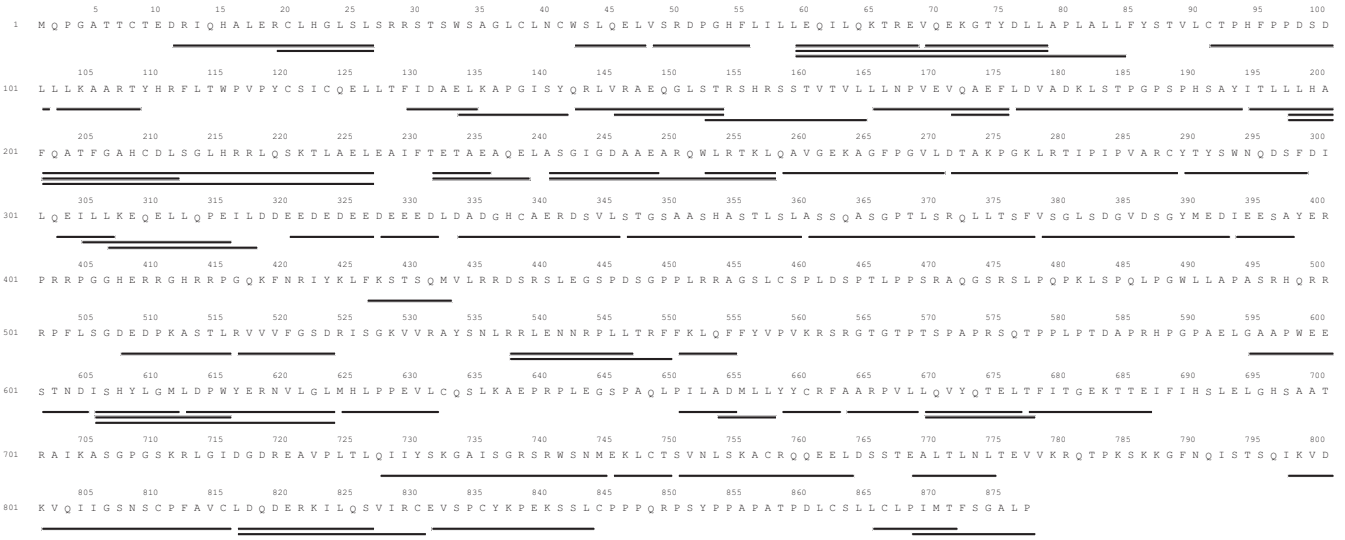


Fig. S2. Peptic peptides used in the HDX-MS analysis for p110γ and for p101. Protein coverage for p110γ and p101 is 95 and 74%, respectively.

p110γ peptides

Start	End	CS	#D	RT	GLOBAL HDX LEVELS p110γ 3	p110γ 30	p110γ 300	GLOBAL HDX LEVELS p110γ/ p101 3	p110γ/ p101 30	p110γ/ p101 300	GLOBAL HDX LEVELS p110γ/ p101 + Lipids 3	p110γ/ p101 + Lipids 30	p110γ/ p101 + Lipids 300	GLOBAL HDX LEVELS p110γ/ p101/ Lipids + Gβγ 3	p110γ/ p101/ Lipids + Gβγ 30	p110γ/ p101/ Lipids + Gβγ 300
4	12	2	6	12.82-13.21	84%	83%	84%	78%	83%	83%	80%	82%	84%	78%	83%	82%
36	41	1	3	14.63-15.10	1%	1%	3%	0%	1%	4%	0%	0%	3%	0%	0%	4%
42	58	4	13	11.96-12.43	22%	30%	35%	18%	29%	32%	20%	27%	34%	17%	26%	32%
59	72	3	12	9.62-9.93	1%	4%	7%	1%	5%	6%	1%	4%	6%	0%	3%	5%
59	74	3	14	10.45-10.58	2%	5%	6%	2%	5%	5%	2%	5%	6%	1%	4%	4%
59	78	4	18	12.65-13.08	0%	2%	5%	0%	2%	6%	0%	2%	4%	0%	1%	3%
60	75	3	14	12.00-12.43	1%	1%	3%	1%	2%	2%	1%	1%	2%	1%	1%	2%
60	78	4	17	12.39-12.56	0%	0%	2%	0%	0%	2%	-1%	0%	1%	-1%	0%	1%
71	78	2	6	12.26-12.69	0%	1%	2%	1%	0%	1%	0%	0%	1%	0%	0%	1%
77	84	1	6	9.88-10.10	27%	46%	57%	23%	43%	55%	20%	44%	55%	19%	41%	53%
83	87	1	3	12.69-12.86	48%	62%	68%	35%	61%	58%	33%	59%	61%	26%	51%	55%
87	99	3	10	14.20-14.63	2%	4%	5%	1%	3%	4%	2%	3%	4%	1%	3%	4%
100	106	1	5	9.84-10.23	23%	42%	47%	20%	40%	46%	20%	37%	46%	18%	34%	43%
107	113	2	5	12.99-13.29	16%	34%	50%	11%	28%	45%	12%	27%	45%	10%	28%	43%
107	119	2	11	15.01-15.19	6%	15%	26%	5%	12%	22%	4%	12%	22%	5%	12%	19%
114	121	1	6	12.69-12.95	17%	18%	17%	10%	18%	18%	11%	17%	18%	9%	16%	17%
122	137	3	13	11.05-11.48	2%	5%	10%	1%	4%	9%	1%	4%	9%	1%	3%	9%
125	137	4	10	10.45-10.83	2%	6%	9%	1%	5%	7%	2%	4%	7%	1%	3%	6%
138	145	2	4	6.61-7.11	47%	47%	53%	41%	45%	47%	42%	45%	50%	38%	42%	45%
138	150	2	9	10.40-10.79	21%	36%	47%	18%	35%	47%	19%	32%	48%	17%	31%	44%
150	157	2	6	13.08-13.33	1%	3%	14%	1%	2%	11%	1%	1%	10%	0%	1%	9%
158	173	2	14	14.11-14.41	20%	30%	40%	19%	30%	38%	20%	29%	38%	18%	28%	37%
174	187	3	11	11.31-11.44	1%	1%	6%	1%	1%	5%	0%	1%	4%	0%	1%	5%
176	182	2	5	8.44-8.88	3%	4%	11%	1%	2%	7%	1%	1%	7%	1%	1%	8%
176	195	3	16	10.92-11.22	5%	10%	19%	4%	9%	17%	5%	9%	15%	2%	8%	14%
186	195	3	7	9.62-10.01	10%	20%	33%	8%	20%	31%	8%	19%	29%	6%	17%	26%
196	211	3	11	16.00-16.48	24%	34%	44%	22%	33%	42%	23%	32%	43%	22%	32%	42%
212	221	2	8	14.58-15.06	14%	26%	44%	10%	26%	42%	11%	24%	43%	10%	23%	42%
222	232	3	9	8.05-8.44	23%	25%	27%	21%	24%	24%	21%	24%	25%	18%	21%	21%
233	245	2	9	13.81-14.11	8%	11%	20%	8%	11%	18%	8%	10%	18%	8%	10%	18%
270	281	3	10	15.14-15.57	1%	1%	3%	0%	1%	3%	1%	1%	3%	1%	1%	2%
282	291	2	7	11.91-12.13	6%	7%	7%	5%	6%	6%	5%	6%	6%	4%	5%	4%
292	307	4	14	12.65-12.99	2%	10%	18%	2%	10%	16%	2%	9%	17%	1%	8%	16%
308	315	1	3	12.21-12.69	8%	35%	47%	6%	34%	42%	6%	31%	41%	6%	30%	39%
316	335	3	17	16.52-16.65	43%	49%	56%	31%	43%	46%	32%	42%	49%	29%	42%	47%
328	339	2	10	12.73-12.90	52%	54%	53%	22%	37%	45%	23%	36%	47%	18%	34%	45%
336	350	2	13	11.14-11.48	26%	28%	30%	8%	14%	24%	8%	13%	24%	7%	12%	22%
340	350	3	9	10.71-11.01	20%	22%	23%	10%	15%	22%	10%	15%	22%	9%	12%	18%
355	381	5	23	14.45-14.71	11%	17%	23%	9%	16%	20%	9%	14%	19%	8%	13%	18%
370	379	2	7	13.42-13.76	44%	59%	69%	38%	56%	59%	39%	51%	59%	32%	47%	56%
382	394	2	11	12.86-13.25	8%	14%	18%	6%	13%	16%	5%	12%	16%	5%	12%	15%
395	406	2	8	9.06-9.41	7%	12%	18%	5%	12%	17%	4%	10%	16%	3%	8%	14%
414	428	3	12	14.54-14.75	5%	9%	19%	4%	6%	11%	4%	6%	11%	4%	5%	10%
417	428	3	9	11.91-12.17	2%	5%	13%	1%	2%	3%	1%	1%	3%	0%	1%	1%
442	462	4	18	7.07-7.42	18%	18%	19%	17%	19%	18%	18%	18%	19%	16%	17%	17%
467	484	5	16	14.32-14.71	6%	15%	20%	3%	11%	16%	3%	10%	16%	2%	9%	15%
498	515	3	15	9.80-10.06	12%	21%	29%	9%	17%	24%	9%	17%	27%	7%	14%	26%
515	519	1	3	15.40-15.40	0%	0%	0%	1%	0%	0%	0%	0%	0%	1%	0%	0%
520	551	5	24	12.60-12.82	41%	48%	53%	35%	42%	42%	39%	45%	46%	38%	46%	46%
530	551	4	16	11.61-11.78	52%	56%	55%	45%	47%	49%	49%	50%	54%	46%	48%	51%
542	551	2	7	11.27-11.57	71%	77%	76%	65%	73%	71%	65%	71%	71%	50%	58%	64%
551	557	2	5	10.19-10.32	3%	12%	50%	1%	7%	23%	0%	7%	27%	0%	1%	7%
552	557	2	4	7.46-8.05	10%	26%	60%	5%	16%	31%	3%	16%	37%	1%	6%	15%
558	573	2	12	14.20-14.45	24%	38%	59%	21%	33%	48%	20%	33%	53%	18%	34%	49%
558	578	3	17	17.65-18.04	14%	24%	49%	12%	21%	34%	12%	21%	40%	12%	22%	34%
558	592	3	29	14.71-15.06	8%	19%	45%	7%	15%	26%	7%	15%	36%	6%	13%	26%
574	578	2	3	17.30-17.74	2%	3%	22%	0%	1%	2%	0%	1%	10%	0%	0%	1%
579	592	4	10	10.01-10.23	4%	14%	40%	1%	6%	13%	2%	7%	28%	1%	4%	11%
593	601	1	7	12.73-12.90	3%	23%	71%	1%	7%	26%	1%	10%	56%	1%	9%	44%
593	611	3	17	16.13-16.44	3%	11%	65%	2%	6%	17%	2%	6%	38%	1%	5%	20%
602	607	1	4	8.00-8.44	1%	6%	60%	0%	0%	3%	0%	1%	23%	-1%	0%	2%
611	622	2	10	13.21-13.38	51%	57%	56%	47%	58%	55%	49%	57%	58%	46%	55%	57%
623	630	2	6	16.00-16.39	1%	12%	33%	1%	10%	16%	1%	9%	18%	0%	7%	18%
631	635	1	3	14.71-15.06	3%	9%	36%	0%	3%	20%	0%	6%	31%	0%	5%	36%
636	642	1	5	7.91-8.09	6%	8%	19%	4%	5%	8%	3%	5%	11%	3%	4%	9%
643	650	2	6	10.53-10.66	10%	13%	17%	7%	11%	13%	6%	11%	13%	6%	10%	11%
643	657	2	13	15.40-15.57	9%	15%	20%	7%	14%	16%	7%	13%	17%	7%	13%	19%
651	657	1	5	10.71-10.92	14%	41%	67%	12%	41%	65%	10%	37%	66%	9%	35%	64%
655	662	2	6	16.83-17.17	0%	1%	15%	0%	1%	13%	0%	1%	15%	-1%	1%	16%
663	677	3	12	13.46-13.89	2%	4%	6%	1%	4%	5%	1%	4%	5%	1%	3%	5%
678	694	5	15	10.10-10.40	1%	1%	1%	0%	1%	0%	0%	0%	0%	-1%	0%	-1%
681	697	5	15	14.41-14.71	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
698	713	5	14	11.35-11.52	3%	4%	12%	2%	4%	10%	2%	4%	10%	1%	4%	9%
718	729	1	10	14.63-14.80	0%	4%	9%	1%	4%	8%	-1%	3%	7%	0%	1%	7%
730	738	1	7	12.13-12.47	0%	-1%	0%	0%	0%	0%	0%	0%	0%	-1%	-1%	0%
742	747	1	4	12.09-12.34	3%	24%	51%	2%	24%	51%	1%	18%	48%	1%	17%	43%
748	767	3	18	15.06-15.49	73%	73%	74%	70%	73%	71%	69%	73%	74%	65%	71%	71%
762	782	3	18	14.15-14.41	46%	73%	86%	43%	73%	84%	39%	66%	86%	38%	66%	84%
783	796	3	10	14.15-14.37	1%	3%	10%	1%	3%	10%	1%	2%	9%	1%	2%	9%
797	815	4	16	14.37-14.84	5%	8%	14%	5%	9%	13%	5%	7%	12%	4%	7%	11%
816	842	4	24	15.88-16.22	14%	25%	36%	13%	24%	34%	12%	22%	34%	11%	23%	35%
824	842	4	17	14.63-14.97	3%	11%	21%	3%	11%	19%	2%	9%	19%	2%	9%	20%
843	848	1	4	17.91-18.30	0%	-1%	0%	1%	1%	0%	0%	-1%	1%	0%	-3%	-1%
849	862	2	12	17.30-17.48	2%	5%	13%	2%	5%	12%	2%	5%	12%	1%	5%	12%
863	878	2	13	16.18-16.65	1%	3%	11%	1%	3%	10%	1%	3%	10%	1%	3%	10%
879	901	3	21	13.55-13.89	35%	44%	51%	33%	43%	48%	33%	42%	52%	30%	43%	54%
888	910	4	21	14.54-14.67	26%	32%	36%	24%	31%	34%	25%	31%	37%	23%	30%	38%
902	910	2	7	14.15-14.28	0%	1%	8%	0%	1%	7%	1%	0%	7%	0%	0%	6%
902	924	5	20	13.33-13.46	4%	8%	13%	3%	9%	12%	5%	7%	13%	4%	6%	13%
925	934	2	8	13.03-13.12	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%
935	939	1	3	10.58-10.92	1%	1%	1%	0%	0%	0%	0%	1%	0%	0%	0%	0%
940	953	3	12	14.50-14.93	1%	1%	4%	0%	1%	3%	1%	2%	6%	0%	1%	6%
954	960	1	5	11.40-11.65	2%	8%	20%	1%	8%	18%	1%	6%	18%	1%	6%	18%
961	976	4	14	17.26-17.74	45%	49%	50%	42%	49%	47%	41%	48%	52%	41%	47%	52%
973	992	3	16	16.83-17.09	20%	23%	27%	19%	24%	25%	20%	25%	32%	19%	26%	36%
976	992	2	13	17.17-17.61	14%	17%	21%	12%	17%	19%	14%	19%	27%	12%	19%	32%
993	1014	4	19	13.21-13.68	2%	2%	3%	1%	3%	3%	2%	3%	6%	1%	3%	6%
1015	1027	2	10	11.27-11.52	0%	0%	3%	0%	0%	2%	0%	0%	2%	0%	0%	2%
1031	1035	1	4	17.56-17.87	0%	-1%	-1%	-1%	0%	0%	0%	-1%	-1%	-1%	-1%	-2%
1035	1050	2	13	14.93-15.40	27%	40%	56%	24%	39%	52%	21%	37%	52%	21%	36%	54%
1050	1071	5	20	13.51-13.81	10%	10%	13%	8%	10%	11%	9%	10%	11%	7%	8%	10%
1072	1084	3	11	15.27-15.53	9%	23%	43%	8%	23%	42%	15%	38%	49%	13%	39%	48%
1085	1090	2	4	17.78-17.91	7%	42%	58%	5%	41%	57%	5%	23%	57%	2%	20%	49%
1089	1108	3	18	6.70-6.84	18%	22%	24%	16%	22%	23%	14%	20%	23%	11%	17%	19%

0% 10% 20% 30% 40% 50% 60% 70%

Fig. S3. Global HDX in p110γ for the following states: p110γ alone, p110γ/p101 complex, p110γ/p101 with lipid vesicles, and p110γ/p101 with prenylated Gβγ-containing lipid vesicles. The HDX for each peptide is shown at 3, 30, and 300 s. The beginning and ending residues for each peptide are illustrated along with the charge state (CS), number of amide deuterons (#D), and retention time (RT).

p110γ peptides

Start	End	CS	#D	RT	GLOBAL HDX LEVELS p110γ 3	30	300	GLOBAL HDX LEVELS p110γ + Lipids 3	30	300
4	12	1	6	11.08-11.39	82%	84%	82%	81%	84%	82%
36	41	1	3	12.81-12.99	0%	0%	8%	1%	1%	5%
42	58	4	13	10.36-10.63	26%	33%	41%	23%	30%	36%
60	75	3	14	10.41-10.68	1%	2%	5%	1%	2%	4%
71	78	2	5	10.59-10.77	0%	0%	4%	0%	0%	1%
77	86	2	8	7.70-8.08	45%	56%	65%	36%	53%	63%
79	86	1	6	7.75-7.89	58%	73%	80%	50%	71%	81%
87	99	3	10	12.86-12.99	3%	5%	7%	3%	5%	6%
100	106	1	5	8.08-8.35	25%	42%	51%	23%	40%	49%
107	113	2	5	11.26-11.52	19%	36%	52%	18%	37%	50%
107	121	2	13	13.65-13.83	13%	17%	32%	13%	19%	31%
114	121	1	6	10.81-11.04	18%	19%	23%	18%	20%	22%
122	137	3	13	9.46-9.78	3%	8%	16%	2%	6%	14%
125	137	4	10	8.73-8.97	3%	7%	12%	3%	6%	10%
138	150	3	9	8.64-8.88	23%	38%	49%	24%	40%	48%
150	157	2	6	11.30-11.57	1%	2%	15%	1%	3%	14%
158	173	2	14	12.59-12.90	21%	30%	40%	22%	31%	40%
174	195	4	18	10.19-10.36	5%	9%	18%	5%	7%	14%
176	182	2	5	6.89-7.13	1%	2%	11%	2%	3%	8%
186	195	3	7	7.75-8.03	10%	19%	35%	10%	20%	30%
196	211	3	11	14.41-14.85	25%	34%	45%	24%	33%	43%
212	221	2	8	12.90-13.08	17%	29%	51%	14%	28%	48%
222	232	3	9	6.65-6.84	25%	24%	28%	24%	25%	26%
233	245	2	9	12.14-12.41	9%	11%	22%	9%	12%	20%
249	269	4	18	9.24-9.51	43%	47%	49%	42%	48%	49%
270	281	3	10	13.74-14.05	1%	1%	4%	1%	2%	4%
282	291	2	7	9.96-10.32	7%	6%	8%	7%	7%	7%
282	307	5	23	13.79-14.14	3%	11%	16%	3%	10%	16%
292	307	4	14	10.86-11.13	3%	12%	19%	3%	13%	19%
308	315	1	3	10.23-10.45	10%	36%	50%	10%	35%	44%
316	335	3	17	15.17-15.44	42%	49%	56%	43%	48%	57%
316	339	3	21	14.68-14.85	40%	44%	51%	39%	44%	50%
316	350	5	32	13.56-13.92	28%	32%	36%	24%	28%	34%
328	339	2	10	10.90-11.21	52%	52%	57%	52%	53%	53%
336	350	2	13	9.51-9.78	30%	28%	31%	31%	31%	31%
355	381	5	23	13.12-13.43	12%	17%	24%	10%	15%	22%
382	394	2	11	11.21-11.48	8%	14%	19%	7%	16%	17%
395	406	2	8	7.22-7.41	9%	13%	20%	7%	11%	17%
408	413	1	4	17.64-17.81	20%	36%	49%	9%	29%	44%
414	428	3	12	13.17-13.34	6%	8%	21%	6%	13%	28%
417	428	3	9	10.19-10.41	1%	4%	14%	2%	10%	23%
442	462	3	18	9.42-9.60	29%	36%	36%	27%	34%	32%
467	484	5	16	13.12-13.39	5%	14%	20%	4%	14%	23%
498	513	2	13	7.03-7.27	14%	22%	34%	15%	29%	55%
515	519	1	3	13.61-13.83	1%	1%	0%	1%	1%	1%
520	551	4	24	10.81-11.13	41%	48%	53%	41%	50%	53%
530	551	4	16	9.91-10.19	51%	53%	55%	50%	54%	55%
542	551	2	7	9.55-9.87	71%	76%	76%	70%	76%	75%
551	557	2	5	8.31-8.59	3%	14%	61%	6%	54%	68%
552	557	2	4	6.31-6.60	8%	24%	67%	12%	69%	75%
558	573	2	12	12.68-12.95	24%	39%	66%	29%	62%	68%
558	578	3	17	16.57-16.84	14%	24%	55%	18%	55%	63%
558	592	4	29	13.48-13.74	9%	19%	53%	10%	56%	61%
574	578	2	3	16.02-16.38	2%	5%	35%	8%	40%	47%
579	592	4	10	8.03-8.40	3%	14%	44%	8%	41%	46%
593	601	2	7	10.95-11.17	4%	23%	75%	11%	73%	76%
593	611	3	17	14.81-15.30	4%	12%	69%	6%	75%	79%
602	607	1	4	6.70-6.89	0%	6%	69%	5%	74%	80%
611	622	2	10	11.61-11.92	54%	58%	60%	53%	59%	59%
623	630	1	6	14.36-14.54	2%	14%	61%	5%	51%	85%
631	635	1	3	12.99-13.39	3%	10%	46%	7%	38%	52%
636	642	2	5	6.55-6.79	6%	7%	26%	8%	26%	63%
643	650	2	6	8.59-8.83	10%	12%	20%	11%	18%	44%
651	657	1	5	8.83-9.15	15%	42%	69%	14%	43%	69%
655	662	2	6	15.44-15.66	0%	1%	18%	0%	2%	21%
663	677	3	12	11.88-12.19	2%	4%	6%	2%	5%	6%
678	694	4	15	8.45-8.64	0%	0%	0%	0%	0%	-1%
681	697	5	15	12.95-13.08	0%	0%	0%	0%	0%	0%
698	713	5	14	9.64-9.87	2%	4%	13%	3%	6%	15%
700	713	4	12	7.51-7.84	2%	4%	13%	2%	5%	13%
718	729	2	10	13.08-13.30	1%	4%	10%	1%	5%	11%
730	738	2	7	10.27-10.68	1%	0%	1%	0%	0%	0%
742	747	2	4	10.27-10.54	4%	25%	57%	3%	22%	54%
748	754	2	5	9.10-9.33	81%	83%	82%	82%	82%	83%
748	767	3	18	13.56-13.79	71%	73%	75%	70%	72%	74%
762	782	3	18	12.77-12.99	45%	72%	84%	42%	70%	86%
783	796	2	10	12.37-12.81	2%	3%	13%	2%	3%	11%
797	815	4	16	12.81-13.26	7%	10%	17%	6%	8%	14%
816	823	2	5	8.88-9.15	15%	41%	70%	13%	45%	68%
824	842	4	17	13.26-13.48	3%	11%	22%	3%	12%	21%
844	848	1	0	14.85-15.03	0%	0%	0%	0%	0%	-1%
849	862	2	12	16.07-16.34	2%	3%	13%	1%	5%	13%
863	878	2	13	14.68-15.03	1%	3%	12%	2%	4%	11%
879	901	2	21	12.10-12.28	34%	44%	52%	34%	45%	55%
888	901	2	12	7.70-8.03	49%	52%	59%	49%	54%	62%
902	910	3	7	12.46-12.68	0%	1%	9%	1%	2%	8%
902	924	4	20	11.66-11.92	5%	8%	15%	5%	8%	15%
925	934	2	8	11.30-11.61	0%	0%	0%	0%	0%	0%
935	939	1	3	8.59-9.06	0%	1%	0%	0%	0%	0%
940	953	3	12	13.04-13.34	1%	1%	5%	1%	3%	7%
954	960	1	5	9.55-9.91	2%	9%	22%	2%	9%	21%
961	972	2	10	15.39-15.89	31%	37%	41%	29%	38%	42%
961	975	2	13	15.26-15.30	38%	43%	46%	37%	45%	49%
962	992	5	27	16.34-16.57	30%	33%	37%	29%	36%	43%
973	992	4	16	15.57-15.89	21%	23%	29%	21%	28%	38%
976	992	3	13	16.07-16.52	15%	18%	23%	15%	23%	35%
993	1014	4	19	11.70-12.06	2%	3%	5%	2%	5%	9%
1015	1026	2	10	8.03-8.35	1%	1%	4%	1%	1%	5%
1015	1027	4	11	9.69-9.87	1%	0%	4%	1%	1%	1%
1031	1035	1	3	16.16-16.38	0%	-1%	-1%	0%	0%	0%
1035	1050	2	13	13.30-13.56	28%	39%	59%	26%	44%	59%
1043	1050	1	6	9.37-9.64	14%	21%	51%	13%	25%	53%
1050	1071	4	20	12.01-12.32	10%	11%	14%	10%	12%	14%
1072	1084	3	11	13.74-14.10	10%	25%	46%	18%	47%	55%
1085	1090	2	4	16.57-16.88	9%	41%	63%	6%	24%	51%
1089	1108	5	18	5.83-6.03	19%	22%	24%	15%	20%	22%

0% 10% 20% 30% 40% 50% 60% 70%

Fig. S4. Global HDX for p110γ in the absence and presence of lipid vesicles Peptides are illustrated in Fig. S3.

p101 peptides

Start	End	CS	#D	RT	GLOBAL HDX LEVELS p110γ/ p101 3	30	300	GLOBAL HDX LEVELS p110γ/ p101 + Lipids 3	30	300	GLOBAL HDX LEVELS p110γ/ p101/ Lipids + Gβγ 3	30	300
11	26	4	14	16.08-16.56	18%	26%	34%	18%	25%	34%	18%	26%	34%
19	26	2	6	12.81-13.28	34%	40%	40%	34%	40%	41%	30%	38%	39%
42	47	1	4	17.42-17.60	1%	5%	31%	0%	3%	24%	1%	2%	28%
48	55	2	5	9.74-10.18	7%	20%	25%	5%	18%	25%	4%	15%	22%
59	68	3	8	10.78-11.00	1%	1%	3%	1%	1%	3%	0%	0%	2%
59	78	3	18	14.14-14.40	5%	16%	28%	4%	13%	27%	4%	13%	27%
59	84	3	23	17.90-18.29	4%	13%	25%	3%	11%	24%	3%	11%	24%
69	78	2	8	14.57-14.96	7%	29%	46%	7%	24%	45%	6%	24%	48%
91	101	2	6	13.71-13.97	11%	24%	32%	12%	21%	32%	8%	19%	32%
102	108	2	5	7.81-7.99	1%	1%	3%	0%	1%	2%	1%	0%	1%
129	134	1	4	15.35-15.61	1%	2%	2%	1%	2%	2%	0%	2%	2%
133	141	1	6	12.90-13.02	0%	0%	0%	0%	0%	0%	-1%	-2%	0%
142	153	2	10	10.13-10.35	11%	17%	18%	10%	15%	17%	10%	16%	19%
145	153	2	7	9.35-9.66	15%	23%	24%	13%	20%	22%	12%	18%	21%
152	164	2	11	10.18-10.35	1%	1%	1%	1%	2%	1%	1%	1%	1%
165	175	1	8	16.47-16.95	29%	66%	80%	24%	58%	77%	23%	59%	80%
171	175	1	3	13.07-13.45	35%	53%	71%	33%	53%	65%	31%	51%	69%
176	193	2	13	12.21-12.55	13%	23%	38%	13%	21%	34%	13%	23%	39%
194	226	4	31	16.77-17.03	6%	9%	11%	6%	8%	11%	6%	8%	11%
197	211	2	13	15.65-15.91	6%	11%	12%	6%	10%	12%	5%	9%	11%
197	226	5	28	14.79-14.96	7%	12%	14%	7%	11%	13%	7%	10%	13%
231	235	1	3	6.02-6.45	0%	1%	0%	1%	0%	0%	0%	0%	0%
231	238	1	6	7.59-8.03	0%	0%	0%	0%	0%	0%	0%	0%	-1%
240	248	1	7	9.79-10.18	27%	43%	61%	25%	40%	56%	23%	38%	55%
240	257	4	16	14.75-15.13	10%	16%	27%	9%	15%	25%	9%	15%	26%
252	257	2	4	12.51-12.77	1%	0%	1%	1%	0%	1%	1%	0%	0%
258	270	2	10	14.53-14.88	12%	33%	45%	11%	28%	43%	10%	28%	42%
271	288	4	13	11.82-12.16	24%	44%	50%	23%	42%	53%	21%	41%	50%
289	298	1	8	16.60-17.08	44%	55%	58%	45%	52%	59%	42%	53%	60%
302	306	1	3	15.65-16.04	0%	3%	35%	0%	2%	32%	0%	3%	39%
304	315	1	9	15.65-15.95	44%	73%	71%	37%	71%	73%	39%	71%	73%
306	317	2	9	15.69-15.95	43%	73%	71%	36%	70%	74%	39%	71%	72%
320	326	1	5	7.81-8.03	19%	25%	23%	18%	24%	26%	17%	23%	21%
327	331	1	3	6.78-7.10	20%	29%	25%	19%	27%	28%	17%	22%	27%
333	345	2	11	11.47-11.95	33%	37%	36%	35%	36%	37%	33%	35%	35%
346	359	2	12	12.81-13.11	46%	50%	49%	50%	49%	50%	46%	49%	49%
360	377	2	15	16.25-16.30	60%	66%	62%	63%	63%	64%	61%	65%	63%
378	392	1	13	13.76-14.23	44%	50%	47%	48%	48%	50%	45%	49%	46%
393	397	1	3	8.17-8.65	64%	74%	69%	65%	72%	71%	58%	59%	60%
426	432	2	5	9.66-9.96	54%	60%	60%	56%	58%	62%	55%	58%	60%
507	515	2	6	10.26-10.57	31%	38%	39%	30%	37%	40%	28%	37%	38%
516	523	2	6	12.64-13.02	13%	16%	14%	14%	15%	16%	12%	14%	15%
537	546	3	7	11.00-11.21	31%	52%	53%	28%	50%	53%	27%	49%	51%
537	549	3	10	12.42-12.68	18%	46%	47%	15%	40%	48%	17%	43%	46%
550	554	1	3	15.74-16.13	3%	23%	28%	2%	18%	29%	2%	18%	27%
594	604	1	8	13.15-13.58	53%	58%	57%	57%	57%	58%	54%	57%	57%
605	611	2	5	13.58-14.06	0%	2%	11%	0%	1%	9%	0%	0%	5%
605	615	2	8	17.90-18.16	0%	1%	8%	0%	1%	6%	0%	1%	3%
605	623	2	16	17.94-18.16	1%	2%	7%	1%	1%	6%	1%	1%	5%
612	623	2	9	17.64-17.90	1%	1%	6%	1%	1%	5%	0%	1%	5%
624	631	2	4	14.62-15.00	23%	56%	71%	20%	48%	69%	19%	48%	71%
650	654	1	3	11.99-12.42	0%	0%	13%	1%	1%	8%	1%	2%	10%
653	657	1	2	15.91-16.30	0%	0%	0%	0%	0%	0%	0%	0%	0%
658	662	2	4	14.23-14.62	0%	0%	1%	1%	1%	1%	-1%	-1%	0%
663	668	1	3	11.04-11.47	1%	14%	29%	1%	11%	30%	0%	12%	28%
669	676	1	7	14.66-15.09	0%	1%	1%	0%	0%	1%	0%	1%	1%
669	677	1	7	13.76-14.01	0%	1%	1%	0%	0%	2%	0%	-1%	0%
677	686	1	8	11.21-11.65	14%	28%	45%	13%	25%	42%	11%	23%	40%
727	744	4	16	12.42-12.64	13%	26%	29%	12%	24%	30%	10%	21%	25%
745	749	1	3	8.96-9.26	7%	46%	53%	3%	30%	54%	0%	13%	29%
750	763	3	12	11.95-12.38	45%	52%	52%	45%	51%	54%	41%	50%	52%
768	774	1	5	16.13-16.38	12%	31%	34%	7%	28%	34%	3%	14%	28%
797	815	3	16	15.44-15.91	4%	7%	6%	4%	7%	7%	4%	7%	7%
816	826	3	9	11.30-11.56	11%	40%	55%	8%	34%	55%	3%	21%	43%
816	830	4	13	15.05-15.48	9%	30%	39%	6%	26%	39%	2%	16%	31%
831	843	2	9	11.17-11.34	40%	53%	57%	42%	51%	58%	39%	49%	55%
844	861	3	9	13.07-13.24	68%	74%	71%	70%	71%	71%	69%	71%	71%
865	871	1	4	16.99-17.29	4%	2%	3%	5%	1%	5%	5%	5%	8%
868	877	1	7	17.03-17.25	4%	17%	32%	4%	14%	31%	3%	12%	29%

0% 10% 20% 30% 40% 50% 60% 70%

Fig. S5. Global HDX in p101 for the p110γ/p101 complex in solution in the presence of lipid vesicles and in the presence of Gβγ-containing lipid vesicles. Peptides are illustrated in Fig. S3.

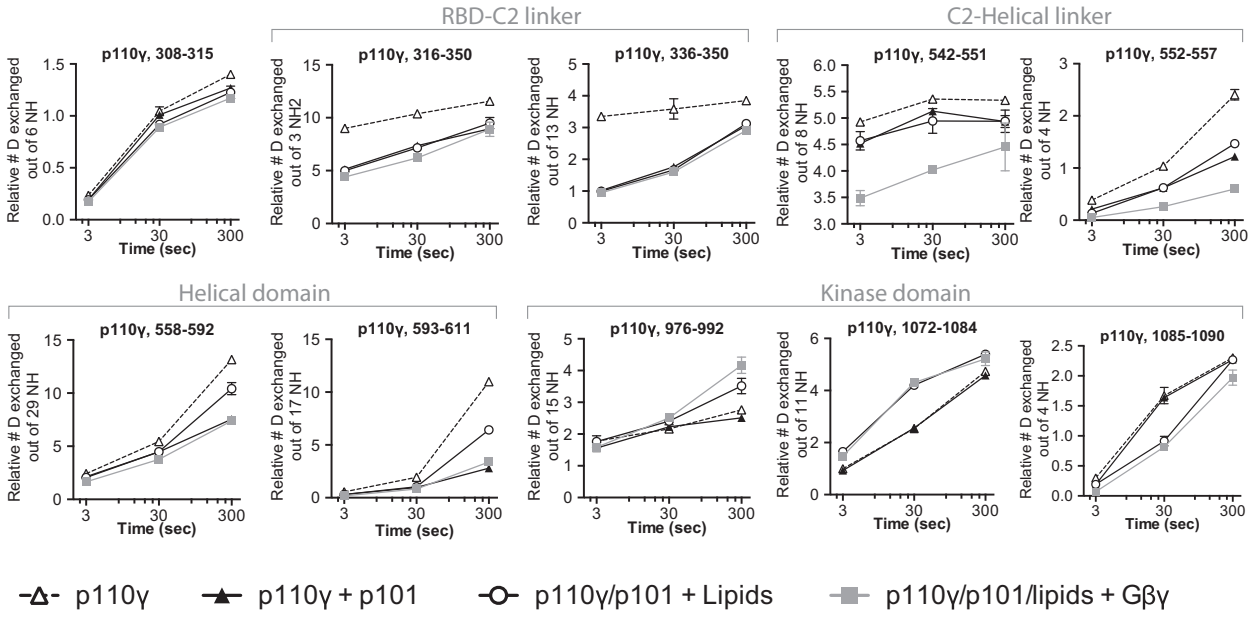


Fig. S6. HDX incorporation plots for selected peptides in p110γ for all regions showing changes in HDX rates between two states.

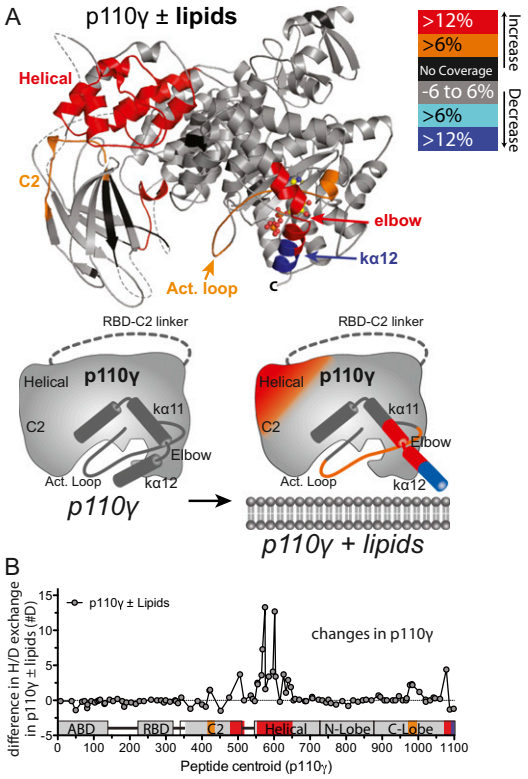
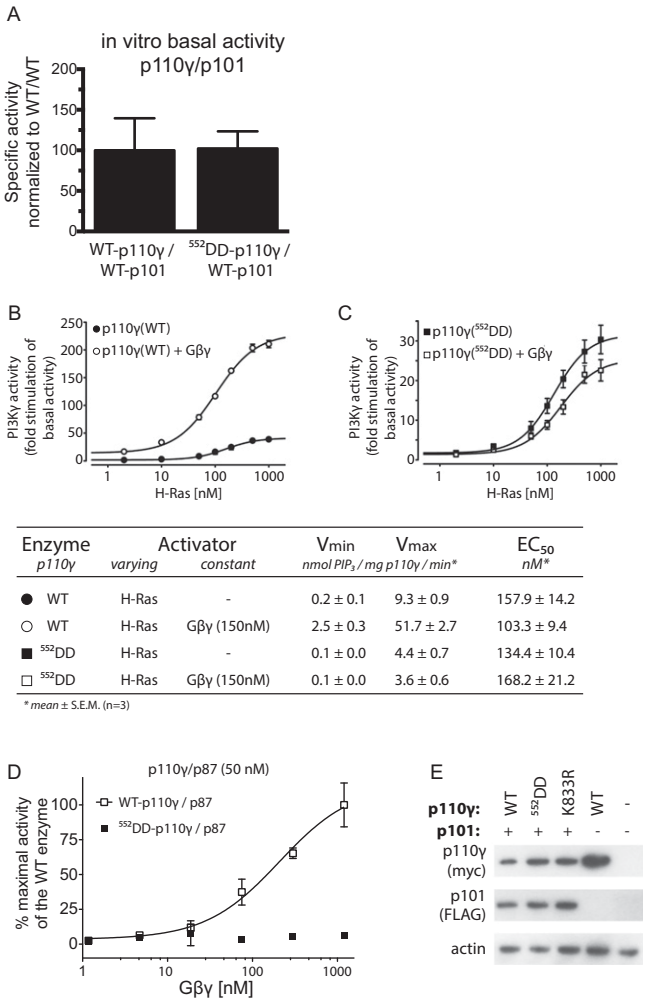


Fig. S7. Influence of lipid membranes on the isolated p110γ subunit. (*A*) Mapping of the changes in HDX rate induced by p110γ binding lipid membranes. Peptides with significant changes are colored on the ribbon diagram of the p110γ structure (Protein Data Bank ID 1E8X) according to the color scheme shown (red and orange indicate increased exposure on binding, and cyan and blue represent decreased exposure). (*Lower*) Schematic drawing illustrating the two states that were compared in the HDX-MS analysis. (*B*) A map of changes in exposure of p110γ on membrane binding as a function of residue number.



Enzyme *p110γ*	Activator *varying*	*constant*	Vmin *nmol PIP_3 / mg p110γ / min**	Vmax	EC_{50} *nM**
● WT	H-Ras	-	0.2 ± 0.1	9.3 ± 0.9	157.9 ± 14.2
○ WT	H-Ras	Gβγ (150nM)	2.5 ± 0.3	51.7 ± 2.7	103.3 ± 9.4
■ 552DD	H-Ras	-	0.1 ± 0.0	4.4 ± 0.7	134.4 ± 10.4
□ 552DD	H-Ras	Gβγ (150nM)	0.1 ± 0.0	3.6 ± 0.6	168.2 ± 21.2

** mean* ± S.E.M. (n=3)

Fig. S8. Effect of 552DD-p110γ mutation on activity. (*A*) In vitro basal lipid kinase activity of WT-p110γ/p101 and 552DD-p110γ/p101 complexes. HEK293T cells were cotransfected with WT or mutant myc-p110γ and HA-p101 for 3 d and then lysed and immunoprecipitated with anti-HA antibody, followed by a lipid kinase assay. The specific activity was calculated by dividing the kinase activity by the level of myc-p110γ in the HA immunopreciptates, as quantified by Western blotting and LICOR. To combine experiments, the specific activity within each experiment was normalized to the specific activity of WT p110γ /WT p101. The bars represent the means of the measurements, and the error bars represent the SDs of three replicates. (*B*) In vitro activity as a function of Ras (H-Ras-GppNHp) concentration of the wild-type p110γ in absence and presence of Gβγ heterodimers (150 nM). (*C*) In vitro activity as a function of Ras (H-Ras-GppNHp) concentration of the ^{552}DD-p110γ mutant in absence and presence of Gβγ heterodimers (150 nM). V_{min}, V_{max}, and EC_{50} derived from the data presented in *B* and *C* are shown below the graphs. (*D*) In vitro activity as a function of Gβγ concentration of the wild-type p110γ and ^{552}DD-p110γ in complexes with the p87 regulatory subunit. (*E*) Western blot analysis of the expression of wild-type and mutant myc-p110γ/ FLAG-mKateII-p101 complexes used for confocal microscopy analysis.

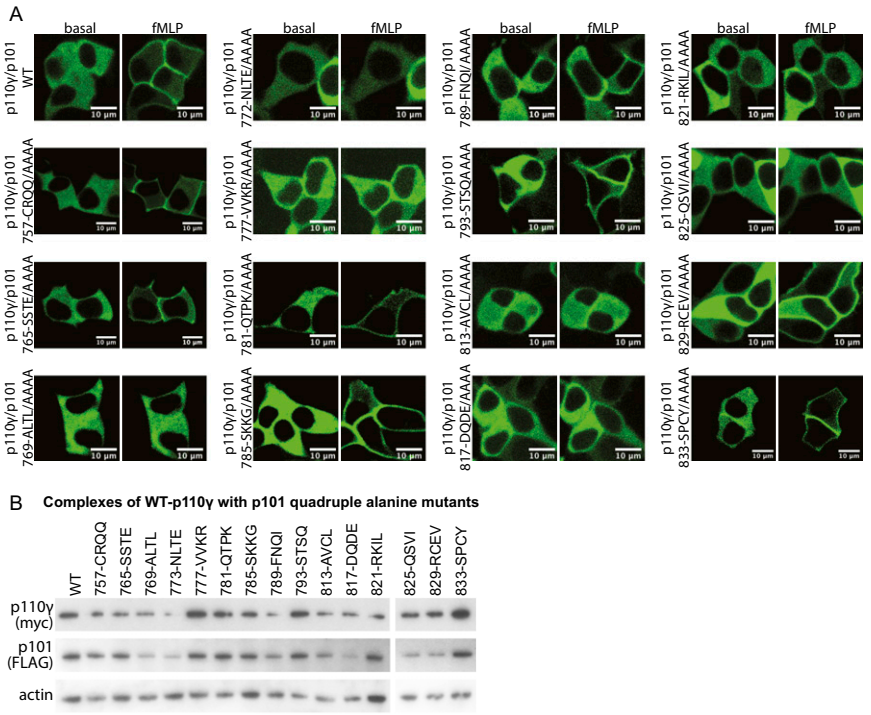
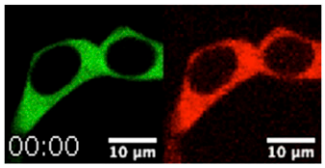
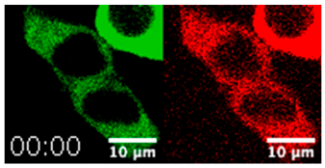


Fig. S9. Effects of p101 mutations on PI3K activity in vitro and in cells. (*A*) PIP_3 production in HEK293T cells for all of the p110γ/p101 mutant complexes. Translocation of the GFP-$Grp1_{PH}$ domain to the plasma membrane upon fMLP stimulation indicates p110γ/p101 activity. (*B*) Expression of FLAG-mKateII-p101 mutants and myc-p110γ in HEK293T cells as determined by Western blotting.



Movie S1. Activation of WT-p110γ/p101 in cells upon fMLP stimulation. HEK293T cells expressing the fMLP receptor, GFP-$Grp1_{PH}$, and WT-p110γ/p101 are stimulated by the addition of 1 μM final fMLP (white square mark). Localization of GFP-$Grp1_{PH}$ (green) and of FLAG-mKateII-p101 (red) every 15 s is shown.

Movie S1



Movie S2. Activation of ^{552}DD-p110γ/p101 in cells by fMLP. Localization of GFP-$Grp1_{PH}$ (green) and of FLAG-mKateII-p101 (red) every 10 s is shown using the same setup described in Movie S1 legend, this time expressing a mutated ^{552}DD-p110γ/p101 enzyme.

Movie S2