

Supplementary Information for

Nanobodies and chemical cross-links advance the structural and functional analysis of PI3K α

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This PDF file includes:

Figures S1 to S8
Tables S1 to S4

Other supplementary materials for this manuscript include the following:

Dataset S1 – Excel file

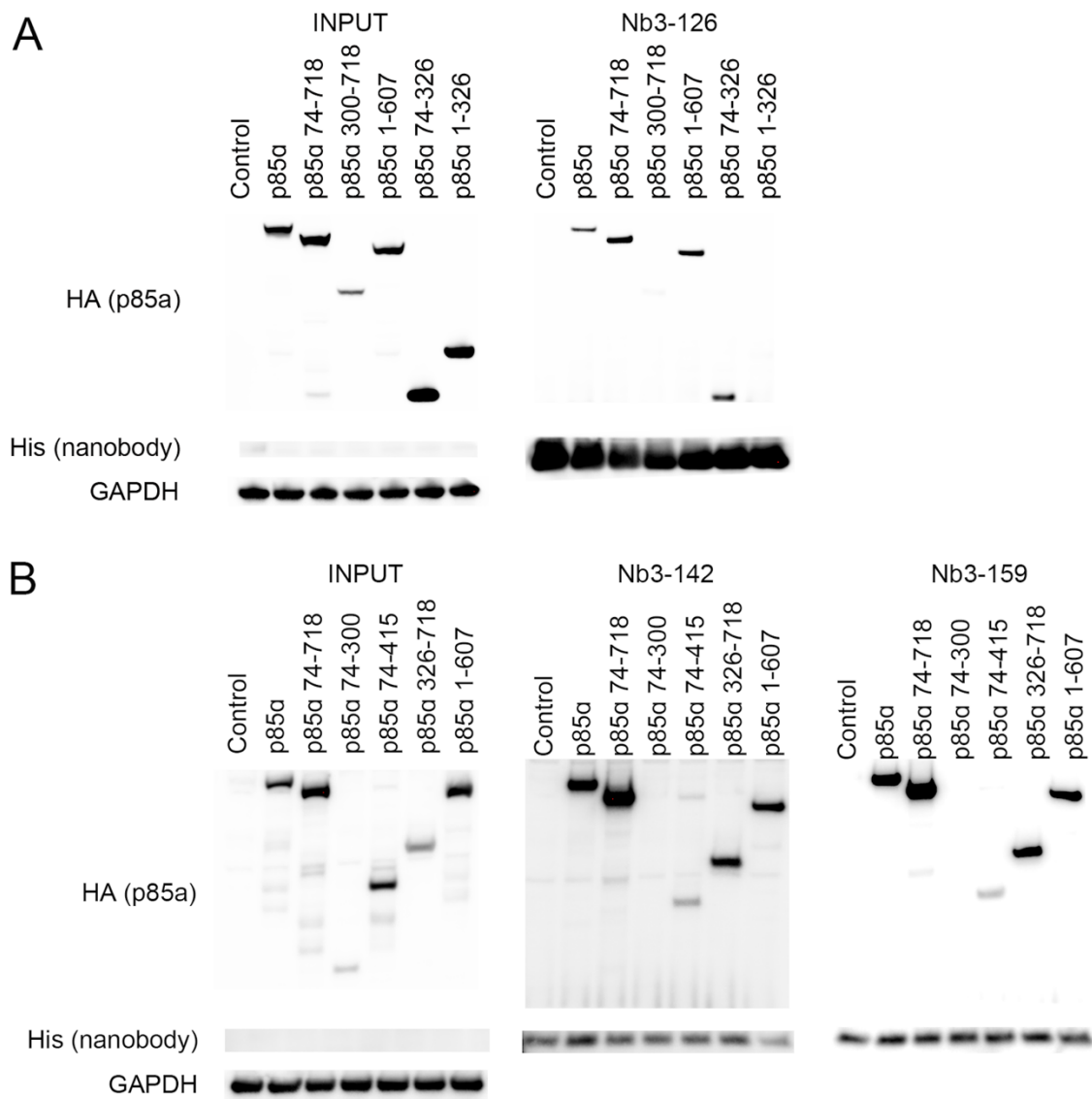


Fig. S1. Co-immunoprecipitation of p110 α with indicated N-terminally HA-tagged p85 α truncation constructs. (A) Nb3-126 specifically interacts with p85 α 74-326, the BH domain. (B) Nb3-142 and Nb3-159 specifically interact with the nSH2 domain in the 326-415 region.

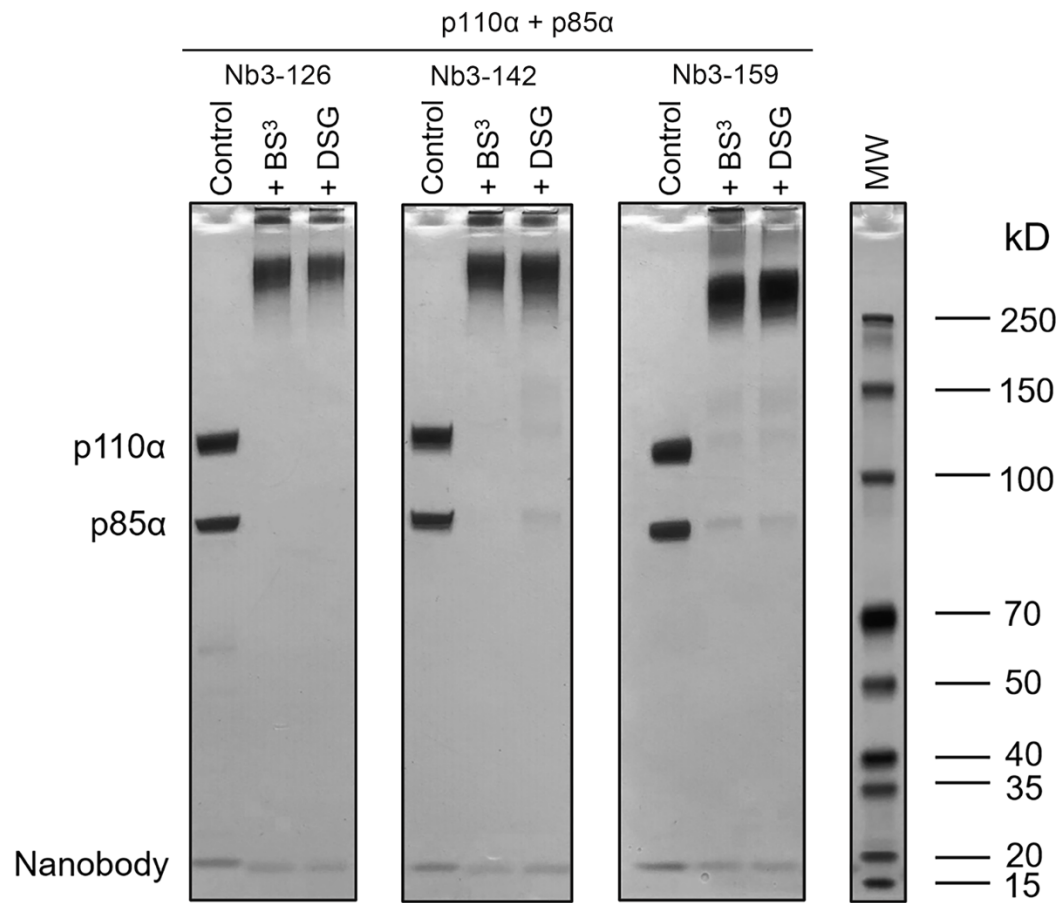


Fig. S2. Cross-linking products of PI3K α with Nb3-126, Nb3-142 and Nb3-159 were evaluated using SDS-PAGE.

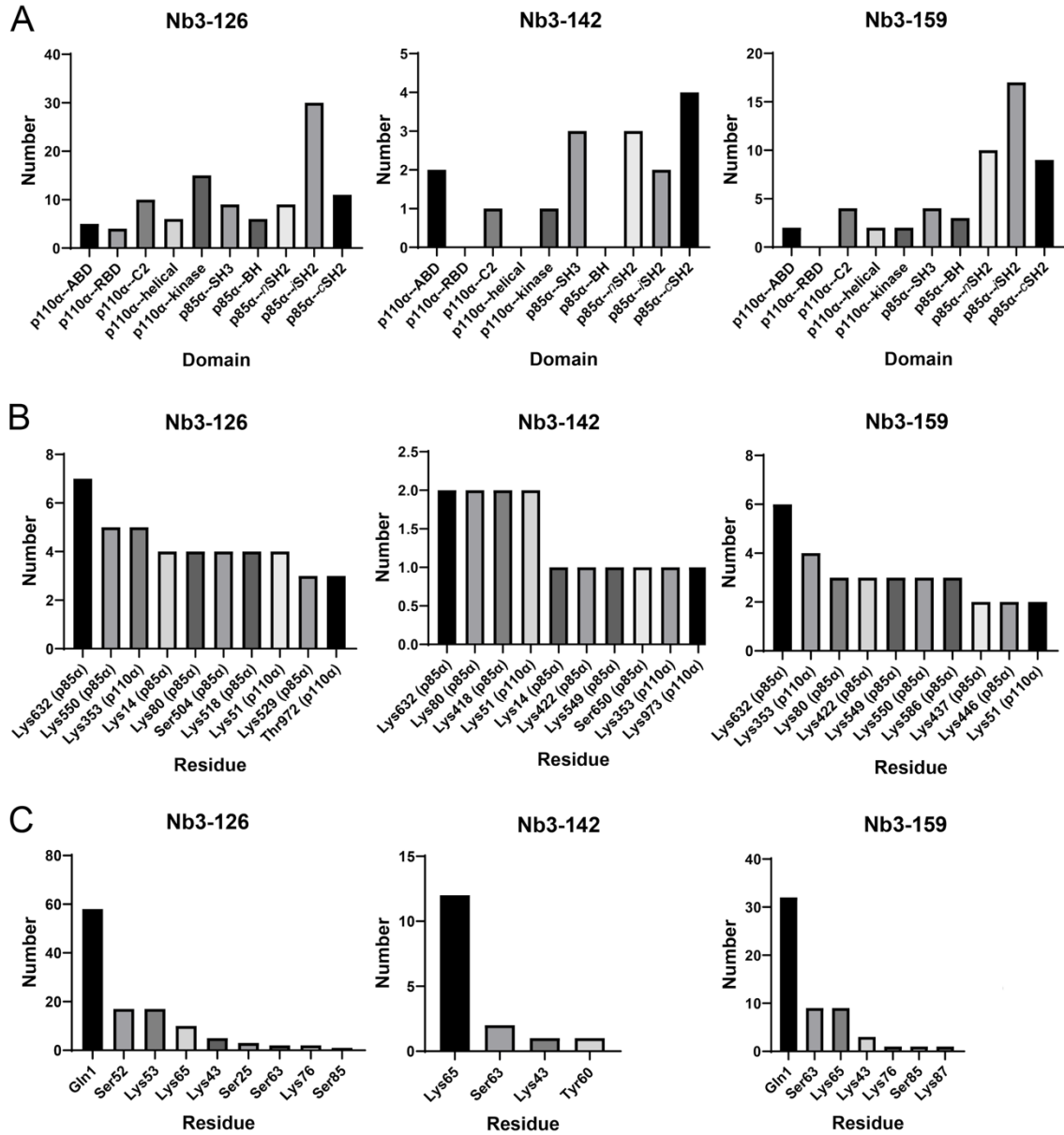


Fig. S3. Cross-links assigned between sites of PI3K α and the three nanobodies. (A) The number of identified cross-links between each domain of PI3K α and the nanobodies. (B) The top 10 residues in PI3K α having the largest number of cross-links with the nanobodies. (C) The number of identified cross-links between a specific residue of the nanobody and that of PI3K α .

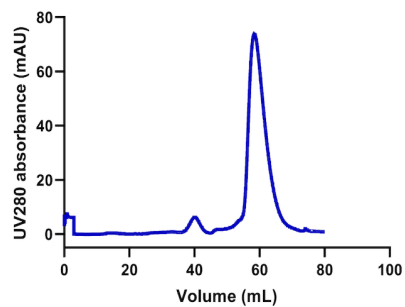
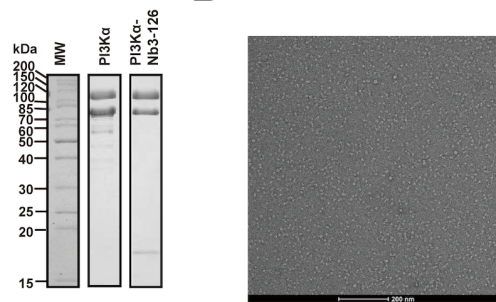
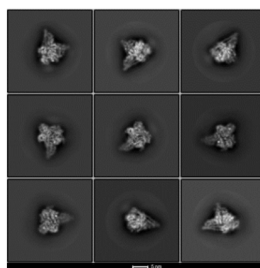
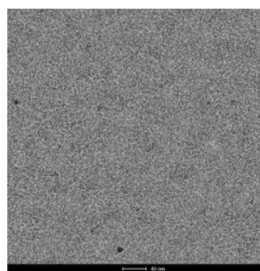
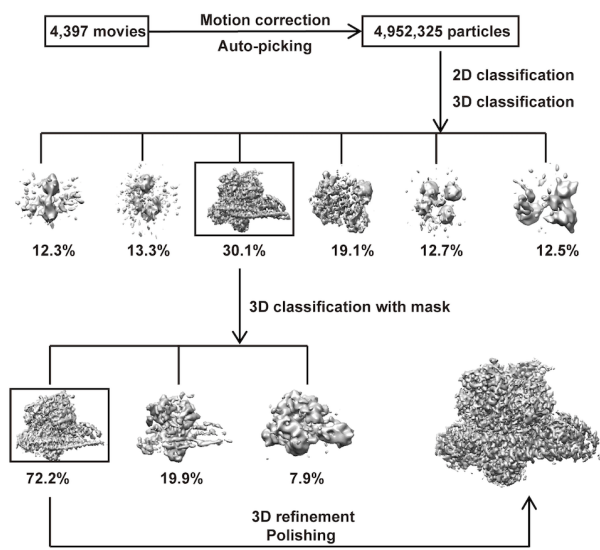
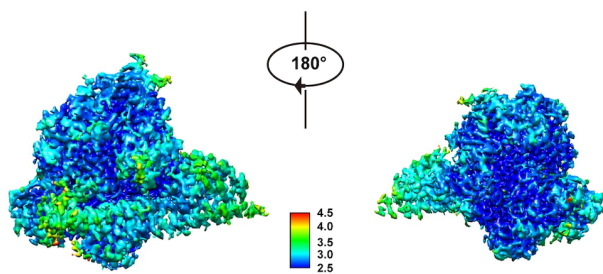
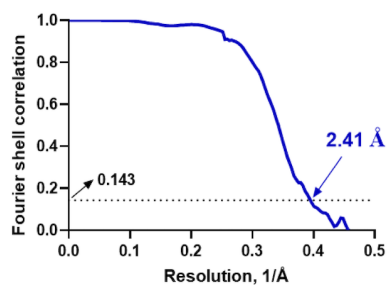
A**B****C****D****E****F**

Fig. S4. Characterization and cryo-EM analysis of the Nb3-126 complex. (A) Analytical size exclusion chromatography, SDS-PAGE/Coomassie blue stain of the purified Nb3-126 complex. (B) Representative negative staining image of the purified Nb3-126 complex. (C) Representative cryo-EM micrographs of the Nb3-126 complex. Representative 2D class averages showing distinct secondary structure features from different views. (D) Workflow of cryo-EM data processing for the Nb3-126 complex. (E) Cryo-EM maps are colored by local resolution (Å). (F) 'Gold-standard' FSC curves of the Nb3-126 complex, indicating the resolution is 2.41 Å at a FSC of 0.143.

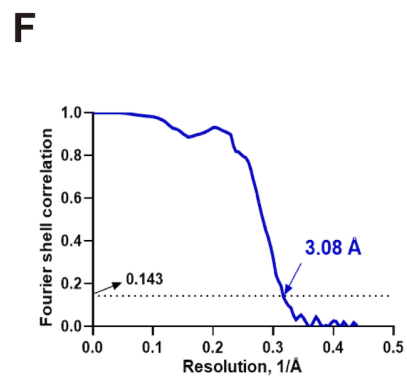
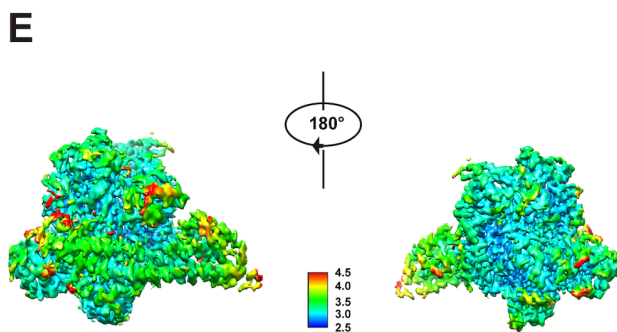
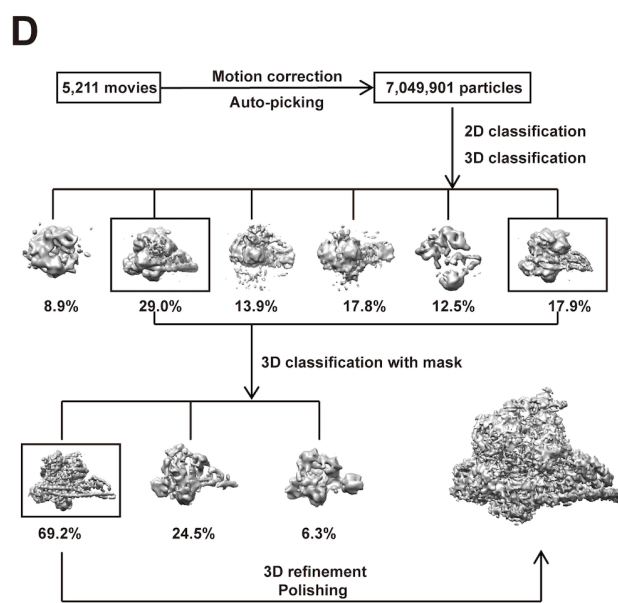
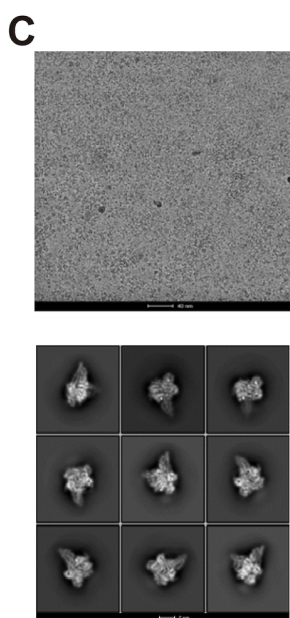
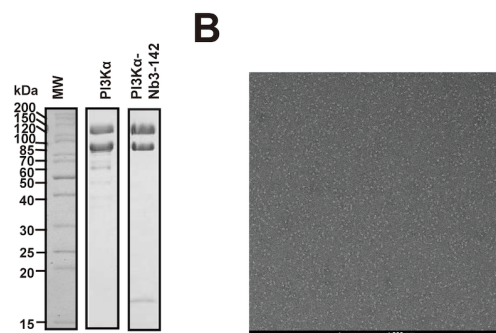
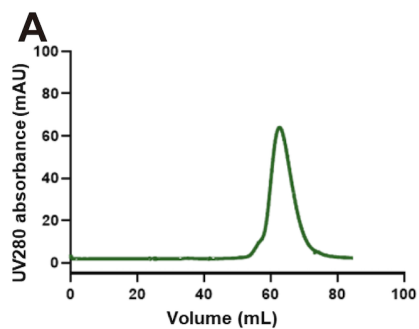


Fig. S5. Characterization and cryo-EM analysis of the Nb3-142 complex. (A) Analytical size exclusion chromatography, SDS-PAGE/Coomassie blue stain of the purified Nb3-142 complex. (B) Representative negative staining image of the purified Nb3-142 complex. (C) Representative cryo-EM micrographs of the Nb3-142 complex. Representative 2D class averages showing distinct secondary structure features from different views. (D) Workflow of cryo-EM data processing for the Nb3-142 complex. (E) Cryo-EM maps are colored by local resolution (Å). (F) 'Gold-standard' FSC curves of the Nb3-142 complex, indicating the resolution is 3.08 Å at a FSC of 0.143.

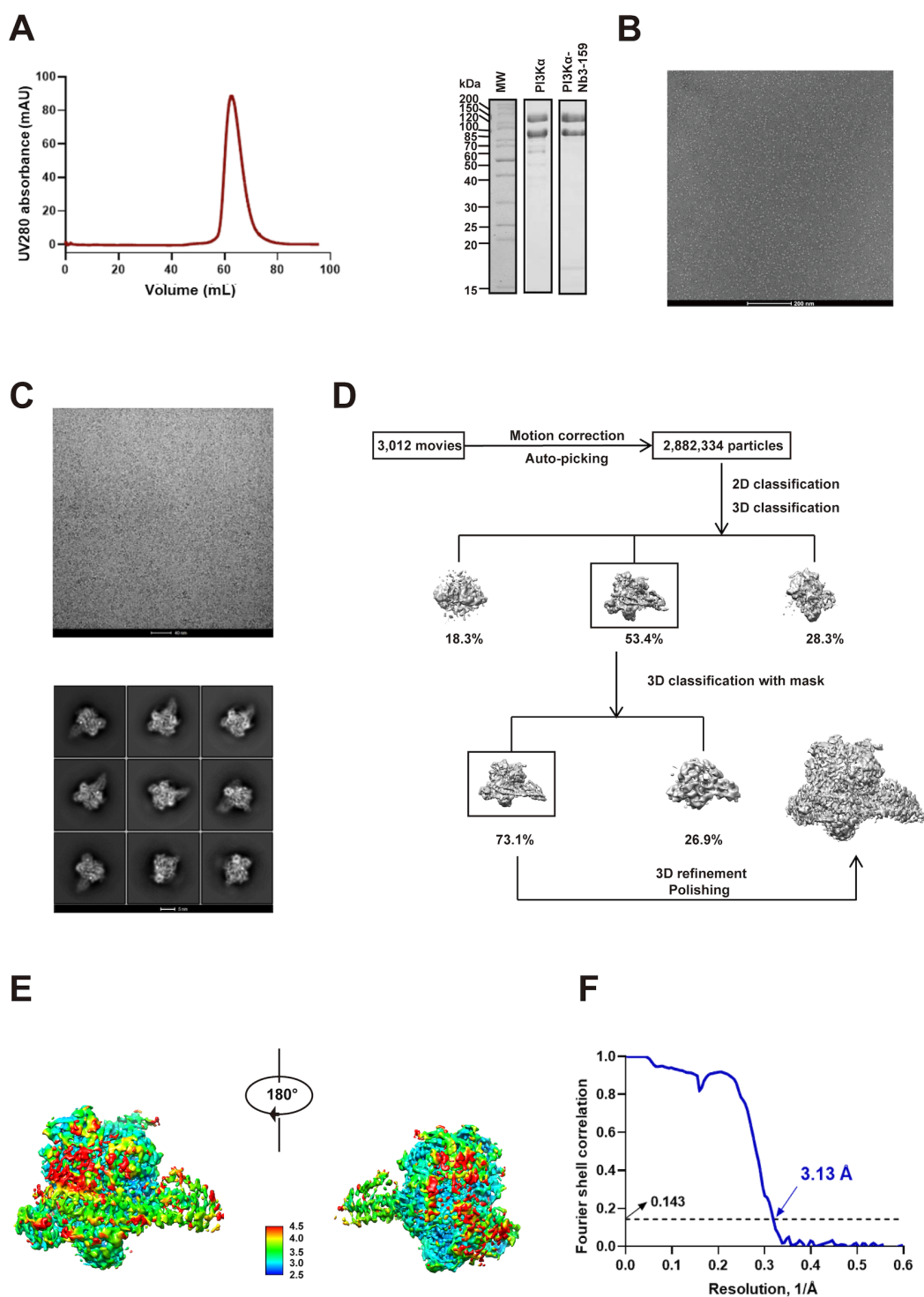
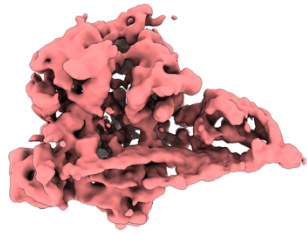


Fig. S6. Characterization and cryo-EM analysis of the Nb3-159 complex. (A) Analytical size exclusion chromatography, SDS-PAGE/Coomassie blue stain of the purified Nb3-159 complex. (B)

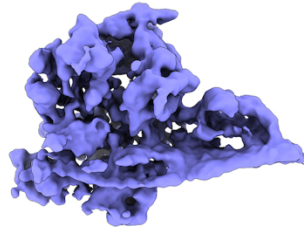
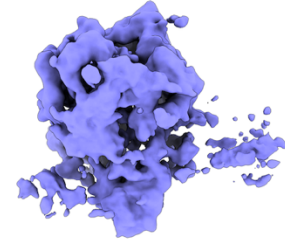
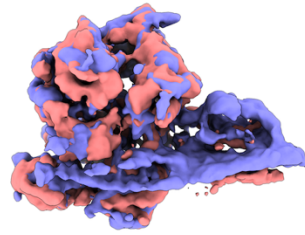
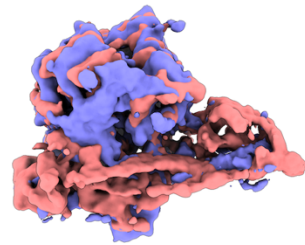
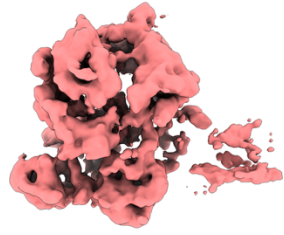
Representative negative staining image of the purified Nb3-159 complex. (C) Representative cryo-EM micrographs of the Nb3-159 complex. Representative 2D class averages showing distinct secondary structure features from different views. (D) Workflow of cryo-EM data processing for the Nb3-159 complex. (E) Cryo-EM maps are colored by local resolution (Å). (F) 'Gold-standard' FSC curves of the Nb3-159 complex, indicating the resolution is 3.13 Å at a FSC of 0.143.

Nb3-159

Component 2

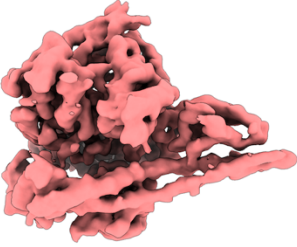


Component 1

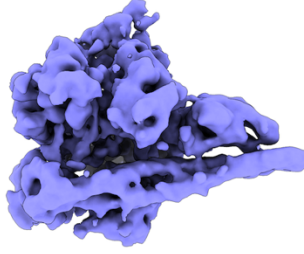
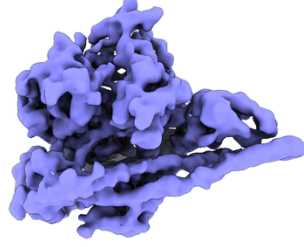
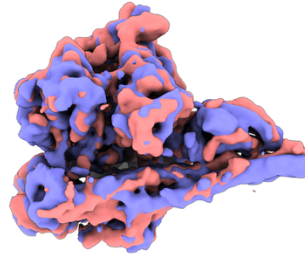
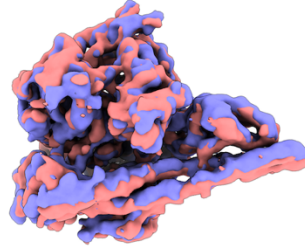
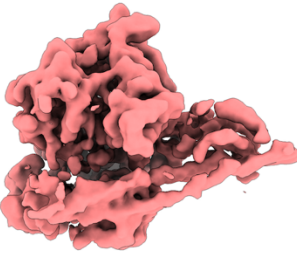


Nb3-142

Component 2

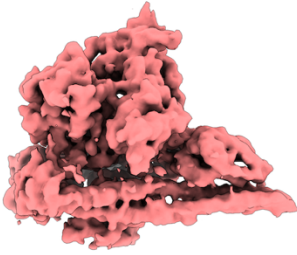


Component 1



Nb3-126

Component 2



Component 1

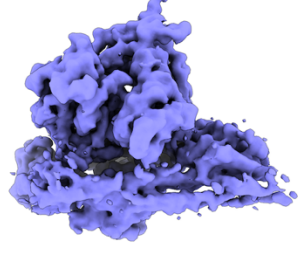
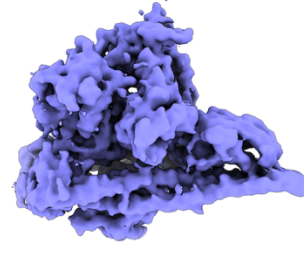
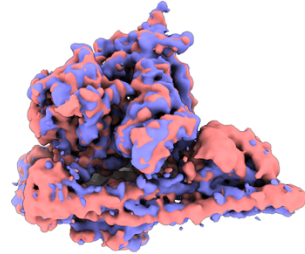
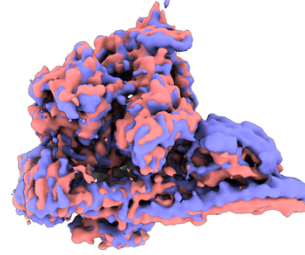
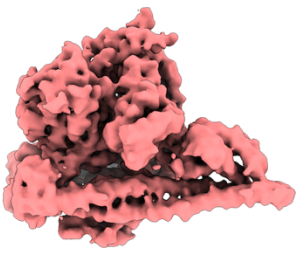


Fig. S7. 3D variability analysis of Nb3-126, Nb3-142, and Nb3-159. The indicated complexes are shown as positive (red) and negative (blue) variations with the components displayed. Nb3-126 and Nb3-142 both show two motions of the ABD and iSH2 corresponding to a side-to-side and up-and-down motion of the ABD-iSH2 region. However, Nb3-159 shows a radical deflection of the ABD-iSH2 domain in component 1 positive (red) and near complete disorder of the ABD and iSH2 domains in the component 2 negative (blue). Both of these motions are accompanied by major structural changes in the kinase domain including narrowing of the active site.

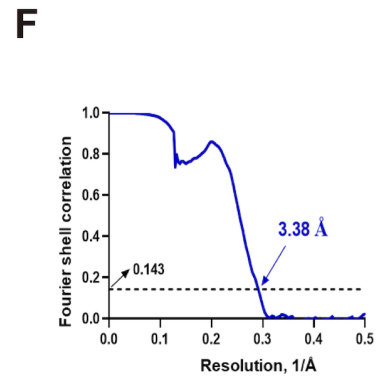
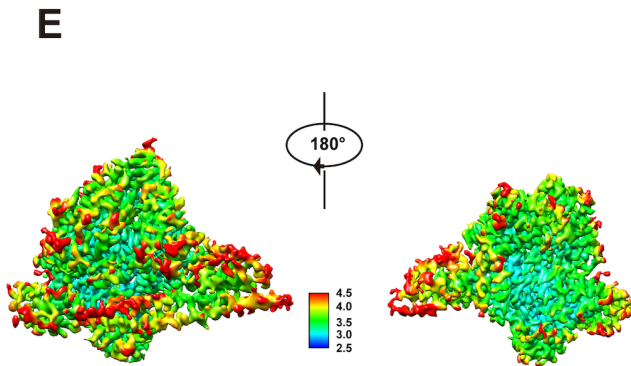
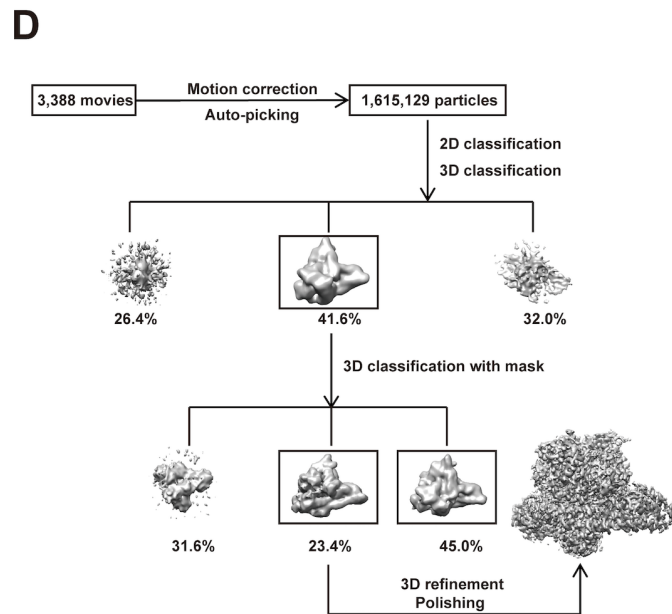
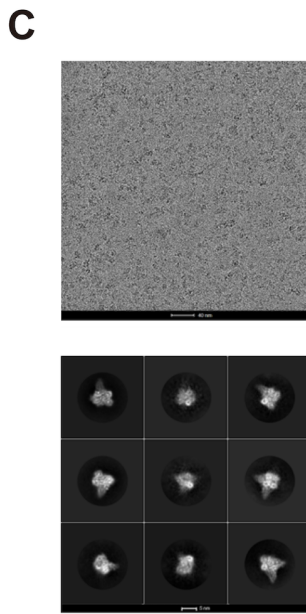
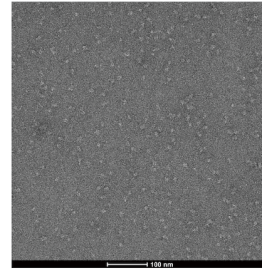
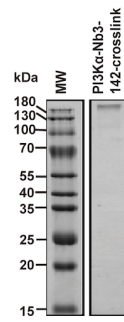
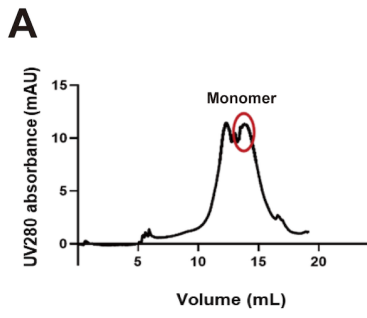


Fig. S8. Characterization and cryo-EM analysis of the DSG cross-linked Nb3-142 complex. (A) Analytical size exclusion chromatography, SDS-PAGE/Coomassie blue stain of the purified cross-linked Nb3-142 complex. (B) Representative negative staining image of the purified cross-linked Nb3-142 complex. (C) Representative cryo-EM micrographs of the cross-linked Nb3-142 complex. Representative 2D class averages showing distinct secondary structure features from different views. (D) Workflow of cryo-EM data processing for the cross-linked Nb3-142 complex. (E) Cryo-EM maps are colored by local resolution (Å). (F) 'Gold-standard' FSC curves of the cross-linked Nb3-142 complex, indicating the resolution is 3.38 Å at a FSC of 0.143.

Table S1. Inter-protein cross-links identified between PI3K α and Nb3-126.

	Nb3-126 Cross-link pair	Linker	Peptide sequence	Best E- value^a	Best score^b
1	Nb3-126(1)- p110 α (51)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-HELFKEAR(5)	7.22E- 13	5.65E- 01
2	Nb3-126(1)- p110 α (100)	DSG	QVQLQESGGGLVQAGH SLR(1)- LFQPFLKVIEPVGNREE K(7)	8.32E- 05	1.22E- 02
3	Nb3-126(1)- p110 α (111)	BS ³	QVQLQESGGGLVQAGH SLR(1)- VIEPVGNREEKILNR(11)	2.36E- 06	8.73E- 02
4	Nb3-126(1)- p110 α (148)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- NILNVCKEAVDLR(7)	1.21E- 07	1.93E- 01
5	Nb3-126(1)- p110 α (173)	BS ³	QVQLQESGGGLVQAGH SLR(1)- AMYVYPPNVESSPELPK HIYNK(11)	3.08E- 05	1.18E- 01
6	Nb3-126(1)- p110 α (179)	BS ³	QVQLQESGGGLVQAGH SLR(1)- AMYVYPPNVESSPELPK HIYNK(17)	4.86E- 05	1.11E- 01
7	Nb3-126(1)- p110 α (260)	BS ³	QVQLQESGGGLVQAGH SLR(1)- VCGCDEYFLEKYPLSQY K(7)	1.84E- 04	3.27E- 05
8	Nb3-126(1)- p110 α (264)	DSG	QVQLQESGGGLVQAGH SLR(1)- VCGCDEYFLEKYPLSQY K(11)	6.89E- 10	3.26E- 04
9	Nb3-126(1)- p110 α (323)	DSG	QVQLQESGGGLVQAGH SLR(1)- ISTATPYMNGETSTKSL WVINSALR(13)	6.86E- 09	5.60E- 04
10	Nb3-126(1)- p110 α (324)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- ISTATPYMNGETSTKSL WVINSALR(14)	6.74E- 10	4.50E- 02
11	Nb3-126(1)- p110 α (325)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- ISTATPYMNGETSTKSL WVINSALR(15)	1.96E- 10	6.84E- 03

			QVQLQESGGGLVQAGH SLR(1)- RISTATPYMNGETSTKS LWVINSALR(16)		
12	Nb3-126(1)- p110α(337)	BS ³	QVQLQESGGGLVQAGH SLR(1)- IKILCATYVNVNIR(2)	4.95E- 06	3.89E- 01
13	Nb3-126(1)- p110α(353)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-DIDKIYVR(4)	5.01E- 04	1.08E- 01
14	Nb3-126(1)- p110α(413)	BS ³	QVQLQESGGGLVQAGH SLR(1)- KGAKEEHCP LAWGNINL FDYTDTLVSGK(1)	4.45E- 06	5.00E- 03
15	Nb3-126(1)- p110α(416)	BS ³	QVQLQESGGGLVQAGH SLR(1)- KGAKEEHCP LAWGNINL FDYTDTLVSGK(4)	1.55E- 11	8.05E- 04
16	Nb3-126(1)- p110α(507)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- EAGFSYSHAGLSNR(5)	1.76E- 08	3.50E- 01
17	Nb3-126(1)- p110α(509)	BS ³	QVQLQESGGGLVQAGH SLR(1)- EAGFSYSHAGLSNR(7)	6.18E- 05	1.57E- 05
18	Nb3-126(1)- p110α(528)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- DNELRENDKEQLK(9) QVQLQESGGGLVQAGH SLR(1)-ENDKEQLK(4)	2.88E- 06	3.69E- 02
19	Nb3-126(1)- p110α(656)	BS ³	QVQLQESGGGLVQAGH SLR(1)-KALTNQR(1)	5.31E- 06	5.60E- 02
20	Nb3-126(1)- p110α(700)	DSG	QVQLQESGGGLVQAGH SLR(1)- ACGMYLKHLNR(7)	3.90E- 07	6.36E- 01
21	Nb3-126(1)- p110α(729)	BS ³	QVQLQESGGGLVQAGH SLR(1)- KDETQKVQMK(6)	4.48E- 04	7.91E- 04
22	Nb3-126(1)- p110α(733)	BS ³	QVQLQESGGGLVQAGH SLR(1)- VQMKFLVEQMR(4)	3.02E- 09	1.52E- 02
23	Nb3-126(1)- p110α(972)	BS ³	QVQLQESGGGLVQAGH SLR(1)-GAQECTK(6)	2.73E- 05	4.39E- 02
24	Nb3-126(1)- p110α(985)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- FQEMCYKAYLAIR(6)	6.96E- 05	1.20E- 01

25	Nb3-126(1)-p110α(986)	BS ³	QVQLQESGGGLVQAGH SLR(1)- FQEMCYKAYLAIR(7)	1.80E-07	7.85E-03
26	Nb3-126(1)-p110α(1030)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- TLALDKTEQEALEYFMK (6)	7.13E-14	3.44E-02
27	Nb3-126(1)-p110α(1041)	BS ³	QVQLQESGGGLVQAGH SLR(1)- TLALDKTEQEALEYFMK QMNDAAHHGGWTTK(17)	2.00E-06	1.53E-01
28	Nb3-126(1)-p110α(1063)	BS ³	QVQLQESGGGLVQAGH SLR(1)- MDWIFHTIKQHALN(9)	9.79E-11	1.53E-03
29	Nb3-126(1)-p85α(14)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-ALYDYKK(6)	8.99E-08	3.91E-01
30	Nb3-126(1)-p85α(15)	BS ³	QVQLQESGGGLVQAGH SLR(1)- KEREEDIDLHLGDILTVN K(1)	2.46E-05	6.04E-02
31	Nb3-126(1)-p85α(80)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-KISPPTPK(1) QVQLQESGGGLVQAGH SLR(1)-KKISPPTPK(2)	1.00E-11	3.65E-01
32	Nb3-126(1)-p85α(140)	BS ³	QVQLQESGGGLVQAGH SLR(1)-LVEAIEKK(7)	1.56E-04	3.76E-01
33	Nb3-126(1)-p85α(141)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-KGLECSTLYR(1)	2.71E-11	4.74E-02
34	Nb3-126(1)-p85α(254)	DSG	QVQLQESGGGLVQAGH SLR(1)- LSQTSSKNLLNAR(6)	4.69E-04	6.73E-01
35	Nb3-126(1)-p85α(255)	BS ³	QVQLQESGGGLVQAGH SLR(1)- LSQTSSKNLLNAR(7)	1.62E-05	1.56E-03
36	Nb3-126(1)-p85α(361)	BS ³	QVQLQESGGGLVQAGH SLR(1)- DASTKMHGDYTLTLR(4)	1.94E-14	6.96E-01
37	Nb3-126(1)-p85α(388)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- DGKYGFSDPLTFSSVVE LINHYR(3)	3.24E-15	3.60E-02
38	Nb3-126(1)-p85α(389)	BS ³	QVQLQESGGGLVQAGH SLR(1)- YGFSDPLTFSSVELIN HYR(1)	2.21E-11	2.27E-03

39	Nb3-126(1)-p85α(392)	BS ³	QVQLQESGGGLVQAGH SLR(1)- YGFSDPLTFSSVVELIN HYR(4)	4.63E-12	2.34E-03
40	Nb3-126(1)-p85α(398)	BS ³	QVQLQESGGGLVQAGH SLR(1)- YGFSDPLTFSSVVELIN HYR(10)	1.03E-04	4.41E-03
41	Nb3-126(1)-p85α(491)	DSG	QVQLQESGGGLVQAGH SLR(1)- TAIEAFNETIKIFEEQCQ TQER(11)	7.29E-22	2.18E-02
42	Nb3-126(1)-p85α(652)	BS ³	QVQLQESGGGLVQAGH SLR(1)- ESSKQGCYACSVVVDG EVK(4)	3.68E-08	5.03E-05
43	Nb3-126(1)-p85α(673)	BS ³	QVQLQESGGGLVQAGH SLR(1)- HCVINKTATGYGFAEPY NLYSSLK(6)	1.71E-09	5.05E-05
44	Nb3-126(1)-p85α(674)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- HCVINKTATGYGFAEPY NLYSSLK(7)	1.74E-19	1.40E-03
45	Nb3-126(1)-p85α(418)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- NESLAQYNPKLDVK(10)	7.28E-08	6.73E-01
46	Nb3-126(1)-p85α(422)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- LDVKLLYPVSK(4)	9.54E-12	5.07E-01
47	Nb3-126(1)-p85α(437)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- YQQDQVVKEDNIEAVG K(8) QVQLQESGGGLVQAGH SLR(1)- YQQDQVVKEDNIEAVG KK(8)	2.11E-11	6.77E-03
48	Nb3-126(1)-p85α(446)	BS ³	QVQLQESGGGLVQAGH SLR(1)- YQQDQVVKEDNIEAVG KK(17) QVQLQESGGGLVQAGH SLR(1)-EDNIEAVGKK(9)	1.73E-06	2.11E-01

49	Nb3-126(1)-p85α(447)	BS ³	QVQLQESGGGLVQAGH SLR(1)- KLHEYNTQFQEK(1)	1.45E-15	9.73E-03
50	Nb3-126(1)-p85α(458)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- LHEYNTQFQEKSR(11) QVQLQESGGGLVQAGH SLR(1)- KLHEYNTQFQEKSR(12)	6.08E-11	2.84E-01
51	Nb3-126(1)-p85α(504)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-YSKEYIEK(2)	1.54E-09	3.91E-01
52	Nb3-126(1)-p85α(529)	BS ³	QVQLQESGGGLVQAGH SLR(1)-IMHNYDKLK(7)	4.76E-08	2.00E-02
53	Nb3-126(1)-p85α(549)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-RLEEDLKK(7)	2.58E-08	4.66E-01
54	Nb3-126(1)-p85α(550)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-KQAAEYR(1)	9.35E-13	1.92E-01
55	Nb3-126(1)-p85α(566)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- MNSIKPDLIQLR(5)	5.48E-08	6.02E-01
56	Nb3-126(1)-p85α(584)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- DQYLMWLTQKGV(8) QVQLQESGGGLVQAGH SLR(1)- TRDQYLMWLTQKGV(10)	5.70E-07	1.86E-01
57	Nb3-126(1)-p85α(586)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)- DQYLMWLTQKGV(10) QVQLQESGGGLVQAGH SLR(1)- TRDQYLMWLTQKGV(12)	1.35E-17	6.51E-01
58	Nb3-126(1)-p85α(632)	BS ³ , DSG	QVQLQESGGGLVQAGH SLR(1)-NKAENLLR(2)	7.47E-11	6.34E-01
59	Nb3-126(25)-p110α(972)	DSG	LSCAASGR(6)- GAQECTKTR(6)	1.58E-08	3.28E-01
60	Nb3-126(25)-p85α(518)	DSG	LSCAASGR(6)- EGNEKEIQR(5)	3.84E-08	5.80E-01
61	Nb3-126(25)-p85α(632)	BS ³ , DSG	LSCAASGR(6)- NKAENLLR(2)	8.78E-10	1.04E-01
62	Nb3-126(43)-p85α(14)	DSG	RAPGKER(5)- ALYDYKK(6) APGKER(4)- ALYDYKK(6)	2.73E-14	7.98E-01

63	Nb3-126(43)- p85α(80)	DSG	RAPGKER(5)- KISPPTPK(1)	1.23E- 08	7.81E- 01
64	Nb3-126(43)- p85α(549)	DSG	RAPGKER(5)- RLEEDLKK(7) RAPGKER(5)- LEEDLKK(6)	8.23E- 10	6.66E- 01
65	Nb3-126(43)- p85α(550)	DSG	RAPGKER(5)- KQAAEYR(1) APGKER(4)- KQAAEYR(1)	5.57E- 05	7.88E- 01
66	Nb3-126(43)- p85α(632)	DSG	RAPGKER(5)- NKAENLLR(2) APGKER(4)- NKAENLLR(2)	3.01E- 08	7.43E- 01
67	Nb3-126(52)- p110α(51)	BS ³	EFVAGISKGENYR(7)- HELFKEAR(5)	4.17E- 13	4.43E- 01
68	Nb3-126(52)- p110α(353)	BS ³ , DSG	EFVAGISKGENYR(7)- DIDKIYVR(4)	3.57E- 05	4.25E- 01
69	Nb3-126(52)- p110α(416)	BS ³	EFVAGISKGENYR(7)- KGAKEEHCP LAWGNINL FDYTDTLVSGK(4)	1.47E- 07	1.37E- 02
70	Nb3-126(52)- p110α(507)	BS ³	EFVAGISKGENYR(7)- EAGFSYSHAGLSNR(5)	6.76E- 07	2.80E- 01
71	Nb3-126(52)- p110α(528)	DSG	EFVAGISKGENYR(7)- DNELRENDKEQLK(9)	7.33E- 04	5.94E- 01
72	Nb3-126(52)- p110α(985)	BS ³	EFVAGISKGENYR(7)- FQEMCYKAYLAIR(6)	4.44E- 04	9.24E- 02
73	Nb3-126(52)- p110α(986)	BS ³ , DSG	EFVAGISKGENYR(7)- FQEMCYKAYLAIR(7)	1.38E- 09	6.57E- 01
74	Nb3-126(52)- p110α(1061)	BS ³	EFVAGISKGENYR(7)- MDWIFHTIKQHALN(7)	5.73E- 05	1.15E- 02
75	Nb3-126(52)- p110α(1063)	BS ³	EFVAGISKGENYR(7)- MDWIFHTIKQHALN(9)	1.48E- 05	3.32E- 01
76	Nb3-126(52)- p85α(80)	BS ³	EFVAGISKGENYR(7)- KISPPTPK(1) EFVAGISKGENYR(7)- KKISPPTPK(2)	1.96E- 05	2.49E- 01
77	Nb3-126(52)- p85α(287)	DSG	EFVAGISKGENYR(7)- FSAASSDNTENLIKVIEIL ISTEWNER(14)	6.62E- 04	5.08E- 01
78	Nb3-126(52)- p85α(504)	BS ³ , DSG	EFVAGISKGENYR(7)- YSKEYIEK(2)	1.25E- 07	2.06E- 01
79	Nb3-126(52)- p85α(505)	BS ³	EFVAGISKGENYR(7)- YSKEYIEK(3)	1.33E- 04	1.92E- 03
80	Nb3-126(52)- p85α(518)	BS ³	EFVAGISKGENYR(7)- EGNEKEIQR(5)	3.21E- 04	3.14E- 01

81	Nb3-126(52)- p85α(529)	BS ³ , DSG	EFVAGISKGENYR(7)- IMHNYDKLK(7)	6.47E- 07	5.56E- 01
82	Nb3-126(52)- p85α(550)	DSG	EFVAGISKGENYR(7)- KQAAEYR(1)	8.58E- 18	4.84E- 01
83	Nb3-126(52)- p85α(632)	BS ³ , DSG	EFVAGISKGENYR(7)- NKAENLLR(2)	9.18E- 06	3.33E- 01
84	Nb3-126(53)- p110α(51)	BS ³	EFVAGISKGENYR(8)- HELKFKEAR(5)	8.80E- 13	1.54E- 01
85	Nb3-126(53)- p110α(353)	BS ³ , DSG	EFVAGISKGENYR(8)- DIDKIYVR(4)	2.20E- 07	8.15E- 01
86	Nb3-126(53)- p110α(416)	BS ³	EFVAGISKGENYR(8)- KGAKEEHCP LAWGNINL FDYTDTLVSGK(4)	7.50E- 05	4.11E- 01
87	Nb3-126(53)- p110α(972)	BS ³	EFVAGISKGENYR(8)- GAQECTKTR(6)	9.70E- 04	4.48E- 01
88	Nb3-126(53)- p110α(985)	BS ³ , DSG	EFVAGISKGENYR(8)- FQEMCYKAYLAIR(6)	5.60E- 11	1.65E- 01
89	Nb3-126(53)- p110α(986)	BS ³ , DSG	EFVAGISKGENYR(8)- FQEMCYKAYLAIR(7)	1.01E- 12	2.62E- 04
90	Nb3-126(53)- p85α(14)	BS ³ , DSG	EFVAGISKGENYR(8)- ALYDYKK(6)	2.26E- 06	2.02E- 01
91	Nb3-126(53)- p85α(80)	BS ³	EFVAGISKGENYR(8)- KISPPTPK(1)	7.07E- 05	2.17E- 01
92	Nb3-126(53)- p85α(381)	BS ³	EFVAGISKGENYR(8)- LIKIFHR(3)	1.35E- 06	5.50E- 01
93	Nb3-126(53)- p85α(447)	BS ³	EFVAGISKGENYR(8)- KLHEYNTQFQEK(1)	6.65E- 04	4.92E- 01
94	Nb3-126(53)- p85α(503)	DSG	EFVAGISKGENYR(8)- YSKEYIEK(1)	5.09E- 04	4.60E- 02
95	Nb3-126(53)- p85α(504)	DSG	EFVAGISKGENYR(8)- YSKEYIEK(2)	9.01E- 08	6.62E- 03
96	Nb3-126(53)- p85α(505)	BS ³	EFVAGISKGENYR(8)- YSKEYIEK(3)	1.33E- 06	7.98E- 03
97	Nb3-126(53)- p85α(518)	BS ³ , DSG	EFVAGISKGENYR(8)- EGNEKEIQR(5)	1.04E- 04	6.21E- 01
98	Nb3-126(53)- p85α(529)	BS ³ , DSG	EFVAGISKGENYR(8)- IMHNYDKLK(7)	9.61E- 07	1.40E- 01
99	Nb3-126(53)- p85α(550)	DSG	EFVAGISKGENYR(8)- KQAAEYR(1)	3.22E- 10	7.37E- 01
100	Nb3-126(53)- p85α(632)	BS ³ , DSG	EFVAGISKGENYR(8)- NKAENLLR(2)	1.79E- 10	8.21E- 02
101	Nb3-126(63)- p110α(51)	DSG	SYADSVKGR(5)- HELKFKEAR(5)	5.75E- 08	4.95E- 01
102	Nb3-126(63)- p85α(437)	DSG	SYADSVKGR(5)- YQQDQVVKEDNIEAVG K(8)	1.84E- 05	4.86E- 01

103	Nb3-126(65)- p110α(353)	BS ³ , DSG	SYADSVKGR(7)- DIDKIYVR(4)	8.95E- 12	8.04E- 01
104	Nb3-126(65)- p110α(973)	DSG	SYADSVKGR(7)- GAQECTKTR(7)	1.60E- 15	2.98E- 01
105	Nb3-126(65)- p85α(14)	DSG	SYADSVKGR(7)- ALYDYKK(6)	2.10E- 11	6.97E- 01
106	Nb3-126(65)- p85α(254)	DSG	SYADSVKGR(7)- LSQTSSKNLLNAR(6)	1.94E- 04	2.27E- 01
107	Nb3-126(65)- p85α(446)	DSG	SYADSVKGR(7)- EDNIEAVGKK(9)	1.03E- 08	2.96E- 01
108	Nb3-126(65)- p85α(504)	BS ³ , DSG	SYADSVKGR(7)- YSKEYIEK(2)	3.73E- 05	3.79E- 01
109	Nb3-126(65)- p85α(518)	DSG	SYADSVKGR(7)- EGNEKEIQR(5)	1.75E- 13	2.98E- 01
110	Nb3-126(65)- p85α(550)	DSG	SYADSVKGR(7)- KQAAEYR(1)	3.95E- 04	4.72E- 01
111	Nb3-126(65)- p85α(632)	BS ³ , DSG	SYADSVKGR(7)- NKAENLLR(2)	3.77E- 14	6.22E- 01
112	Nb3-126(65)- p85α(650)	DSG	SYADSVKGR(7)- ESSKQGCYACSVVVDG EVK(2)	2.34E- 04	1.59E- 02
113	Nb3-126(76)- p110α(353)	DSG	DNAKSTVYLQMNSLKP DDAAVYYCAAK(4)- DIDKIYVR(4)	5.95E- 04	8.12E- 01
114	Nb3-126(76)- p85α(388)	DSG	DNAKSTVYLQMNSLKP DDAAVYYCAAK(4)- DGKYGFSDPLTFSSVVE LINHYR(3)	2.19E- 08	3.17E- 05
115	Nb3-126(85)- p85α(632)	DSG	STVYLQMNSLKPDDAA VYYCAAK(9)- NKAENLLR(2)	1.76E- 06	5.23E- 01

^aE-value means normalized refined_scores for filtered peptide spectrum matches (PSMs) by FDR, the smaller the more confident. Best E-value is the smallest E-value of all spectra of the cross-linked peptide. ^bScore, which is short for SVM score in pLink 2, is the prime measure for FDR estimation. Best score is the smallest value of all spectra of the cross-linked peptide.

Table S2. Inter-protein cross-links identified between PI3K α and Nb3-142.

Nb3-142					
	Cross-link pair	Linker	Peptide sequence	Best E-value^a	Best score^b
1	Nb3-142(43)-p85 α (632)	DSG	QTPGKER(5)-NKAENLLR(2)	6.58E-05	6.97E-01
2	Nb3-142(60)-p85 α (80)	DSG	YADSVKGR(1)-KISPPTPK(1)	8.59E-04	4.86E-01
3	Nb3-142(63)-p110 α (51)	BS ³	YADSVKGR(4)-HELFKEAR(5)	1.65E-06	8.14E-01
4	Nb3-142(63)-p85 α (418)	DSG	YADSVKGR(4)-NESLAQYNPKLDVK(10)	1.94E-06	5.34E-01
5	Nb3-142(65)-p110 α (51)	DSG	YADSVKGR(6)-HELFKEAR(5)	6.13E-09	4.58E-01
6	Nb3-142(65)-p110 α (353)	BS ³ , DSG	YADSVKGR(6)-DIDKIYVR(4)	5.75E-13	4.27E-01
7	Nb3-142(65)-p110 α (973)	DSG	YADSVKGR(6)-GAQECTKTR(7)	7.79E-15	8.02E-02
8	Nb3-142(65)-p85 α (14)	BS ³ , DSG	YADSVKGR(6)-ALYDYKK(6)	5.33E-11	5.17E-01
9	Nb3-142(65)-p85 α (80)	BS ³ , DSG	YADSVKGR(6)-KISPPTPK(1)	2.94E-07	8.16E-01
10	Nb3-142(65)-p85 α (418)	DSG	YADSVKGR(6)-NESLAQYNPKLDVK(10)	1.98E-07	4.21E-01
11	Nb3-142(65)-p85 α (422)	DSG	YADSVKGR(6)-LDVKLLYPVSK(4)	8.29E-04	2.10E-01
12	Nb3-142(65)-p85 α (549)	DSG	YADSVKGR(6)-LEEDLKK(6)	1.62E-07	6.66E-01
13	Nb3-142(65)-p85 α (550)	DSG	YADSVKGR(6)-KQAAEYR(1)	5.37E-08	3.54E-01
14	Nb3-142(65)-p85 α (632)	BS ³ , DSG	YADSVKGR(6)-NKAENLLR(2)	1.97E-15	6.94E-01
15	Nb3-142(65)-p85 α (650)	DSG	YADSVKGR(6)-ESSKQGCVVVDGEVK(2)	3.96E-04	9.66E-02
16	Nb3-142(65)-p85 α (651)	DSG	YADSVKGR(6)-ESSKQGCVVVDGEVK(3)	6.11E-04	1.86E-01

^aE-value means normalized refined_scores for filtered peptide spectrum matches (PSMs) by FDR, the smaller the more confident. Best E-value is the smallest E-value of all spectra of the cross-linked peptide. ^bScore, which is short for SVM score in pLink 2, is the prime measure for FDR estimation. Best score is the smallest value of all spectra of the cross-linked peptide.

Table S3. Inter-protein cross-links identified between PI3K α and Nb3-159.

Nb3-159					
	Cross-link pair	Linker	Peptide sequence	Best E-value^a	Best score^b
1	Nb3-159(1)-p110 α (51)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)-HELKFEAR(5)	1.75E-12	5.29E-01
2	Nb3-159(1)-p110 α (353)	BS ³ ,	QVQLQESGGGLVQTGGS LR(1)-DIDKIYVR(4)	2.40E-04	5.56E-02
3	Nb3-159(1)-p110 α (507)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)- EAGFSYSHAGLSNR(5)	1.17E-07	1.78E-02
4	Nb3-159(1)-p110 α (528)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)- DNELRENDKEQLK(9)	1.63E-05	2.78E-02
5	Nb3-159(1)-p110 α (532)	DSG	QVQLQESGGGLVQTGGS LR(1)- ENDKEQLKAISTR(8)	1.33E-05	5.67E-01
6	Nb3-159(1)-p110 α (973)	BS ³	QVQLQESGGGLVQTGGS LR(1)-GAQECTKTR(7)	5.24E-04	3.26E-04
7	Nb3-159(1)-p110 α (1063)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)- MDWIFHTIKQHALN(9)	9.30E-11	8.14E-01
8	Nb3-159(1)-p85 α (14)	BS ³	QVQLQESGGGLVQTGGS LR(1)-ALYDYKK(6)	2.41E-05	2.25E-02
9	Nb3-159(1)-p85 α (80)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)-KISPPTPK(1) QVQLQESGGGLVQTGGS LR(1)-KKISPPTPK(2)	9.89E-11	4.84E-01
10	Nb3-159(1)-p85 α (253)	BS ³	QVQLQESGGGLVQTGGS LR(1)- LSQTSSKNLLNAR(5)	9.35E-04	1.09E-03
11	Nb3-159(1)-p85 α (254)	BS ³	QVQLQESGGGLVQTGGS LR(1)- LSQTSSKNLLNAR(6)	5.78E-05	1.85E-02
12	Nb3-159(1)-p85 α (255)	BS ³	QVQLQESGGGLVQTGGS LR(1)- LSQTSSKNLLNAR(7)	2.32E-04	1.27E-01
13	Nb3-159(1)-p85 α (361)	DSG	QVQLQESGGGLVQTGGS LR(1)- DASTKMHGDYTLTLR(4)	1.06E-04	2.65E-02
14	Nb3-159(1)-p85 α (381)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)-LIKIFHR(3)	4.94E-09	6.65E-01
15	Nb3-159(1)-p85 α (389)	BS ³	QVQLQESGGGLVQTGGS LR(1)- YGFSDPLTFSSVVELINHY R(1)	9.37E-12	5.51E-13

16	Nb3-159(1)-p85α(392)	BS ³	QVQLQESGGGLVQTGGS LR(1)- YGFSDPLTFSSVVELINHY R(4)	5.63E-04	1.14E-04
17	Nb3-159(1)-p85α(398)	BS ³	QVQLQESGGGLVQTGGS LR(1)- YGFSDPLTFSSVVELINHY R(10)	7.53E-05	7.82E-02
18	Nb3-159(1)-p85α(399)	BS ³	QVQLQESGGGLVQTGGS LR(1)- YGFSDPLTFSSVVELINHY R(11)	5.87E-09	7.18E-02
19	Nb3-159(1)-p85α(418)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)- NESLAQYNPKLDVK(10)	1.96E-09	1.28E-04
20	Nb3-159(1)-p85α(422)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)-LDVKLLYPVSK(4)	6.27E-09	9.73E-02
21	Nb3-159(1)-p85α(437)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)- YQQDQVVKEDNIEAVGK(8) QVQLQESGGGLVQTGGS LR(1)- YQQDQVVKEDNIEAVGKK(8)	2.30E-13	5.17E-04
22	Nb3-159(1)-p85α(446)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)- YQQDQVVKEDNIEAVGKK(17) QVQLQESGGGLVQTGGS LR(1)-EDNIEAVGKK(9)	1.93E-08	1.57E-02
23	Nb3-159(1)-p85α(447)	DSG	QVQLQESGGGLVQTGGS LR(1)-KLHEYNTQFQEK(1)	9.61E-06	2.85E-02
24	Nb3-159(1)-p85α(458)	DSG	QVQLQESGGGLVQTGGS LR(1)- LHEYNTQFQEKSR(11) QVQLQESGGGLVQTGGS LR(1)- KLHEYNTQFQEKSR(12)	2.20E-11	4.31E-01
25	Nb3-159(1)-p85α(504)	DSG	QVQLQESGGGLVQTGGS LR(1)-YSKEYIEK(2)	4.40E-07	6.69E-01
26	Nb3-159(1)-p85α(529)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)-IMHNYDKLK(7)	1.08E-05	7.16E-01
27	Nb3-159(1)-p85α(549)	DSG	QVQLQESGGGLVQTGGS LR(1)-LEEDLKK(6)	1.34E-08	6.41E-01

			QVQLQESGGGLVQTGGS LR(1)-RLEEDLKK(7)		
28	Nb3-159(1)- p85α(550)	BS ³	QVQLQESGGGLVQTGGS LR(1)-KQAAEYR(1)	3.63E- 12	2.43E- 03
29	Nb3-159(1)- p85α(566)	DSG	QVQLQESGGGLVQTGGS LR(1)-MNSIKPDLIQLR(5)	1.30E- 08	1.45E- 02
30	Nb3-159(1)- p85α(586)	DSG	QVQLQESGGGLVQTGGS LR(1)- DQYLMWLTQKGVR(10) QVQLQESGGGLVQTGGS LR(1)- TRDQYLMWLTQKGVR(12)	2.34E- 23	2.71E- 01
31	Nb3-159(1)- p85α(632)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)-NKAENLLR(2)	2.11E- 09	2.52E- 01
32	Nb3-159(1)- p85α(674)	BS ³ , DSG	QVQLQESGGGLVQTGGS LR(1)- HCVINKTATGYGFAEPYN LYSSLK(7)	3.45E- 13	5.09E- 03
33	Nb3-159(43)- p110α(353)	BS ³ , DSG	QAPGKER(5)- DIDKIYVR(4)	3.86E- 06	8.14E- 01
34	Nb3-159(43)- p85α(550)	DSG	QAPGKER(5)- KQAAEYR(1)	6.99E- 08	2.45E- 01
35	Nb3-159(43)- p85α(632)	BS ³ , DSG	QAPGKER(5)- NKAENLLR(2)	7.75E- 06	8.07E- 01
36	Nb3-159(63)- P110α(51)	BS ³ , DSG	GSNTSYADSVKGR(9)- HELKFEAR(5)	4.58E- 06	4.21E- 01
37	Nb3-159(63)- p110α(353)	DSG	GSNTSYADSVKGR(9)- DIDKIYVR(4)	3.11E- 07	3.52E- 01
38	Nb3-159(63)- p85α(80)	BS ³ , DSG	GSNTSYADSVKGR(9)- KISPPTPK(1) GSNTSYADSVKGR(9)- KKISPPTPK(2)	3.42E- 08	8.15E- 01
39	Nb3-159(63)- p85α(422)	DSG	GSNTSYADSVKGR(9)- LDVKLLYPVSK(4)	5.15E- 10	4.07E- 01
40	Nb3-159(63)- p85α(437)	DSG	GSNTSYADSVKGR(9)- YQQDQVVKEDNIEAVGKK (8)	7.40E- 05	4.92E- 01
41	Nb3-159(63)- p85α(458)	DSG	GSNTSYADSVKGR(9)- LHEYNTQFQEKS(11)	3.18E- 05	6.74E- 01
42	Nb3-159(63)- p85α(549)	DSG	GSNTSYADSVKGR(9)- LEEDLKK(6)	7.39E- 14	2.71E- 01
43	Nb3-159(63)- p85α(586)	DSG	GSNTSYADSVKGR(9)- DQYLMWLTQKGVR(10)	5.61E- 11	1.08E- 01

44	Nb3-159(63)- p85α(673)	DSG	GSNTSYADSVKGR(9)- HCVINKTATGYGFAEPYN LYSSLK(6)	7.16E- 07	1.68E- 01
45	Nb3-159(65)- p85α(80)	BS ³ , DSG	GSNTSYADSVKGR(11)- KISPPTPK(1) GSNTSYADSVKGR(11)- KKISPPTPK(2)	2.27E- 12	8.02E- 01
46	Nb3-159(65)- p110α(353)	BS ³ , DSG	GSNTSYADSVKGR(11)- DIDKIYVR(4)	3.56E- 14	5.92E- 01
47	Nb3-159(65)- p85α(422)	DSG	GSNTSYADSVKGR(11)- LDVKLLYPVSK(4)	4.58E- 08	1.71E- 03
48	Nb3-159(65)- p85α(446)	DSG	GSNTSYADSVKGR(11)- EDNIEAVGKK(9)	6.72E- 06	6.42E- 01
49	Nb3-159(65)- p85α(549)	DSG	GSNTSYADSVKGR(11)- LEEDLKK(6) GSNTSYADSVKGR(11)- RLEEDLKK(7)	4.12E- 13	6.88E- 01
50	Nb3-159(65)- p85α(550)	BS ³ , DSG	GSNTSYADSVKGR(11)- KQAAEYR(1)	5.41E- 14	1.98E- 01
51	Nb3-159(65)- p85α(586)	DSG	GSNTSYADSVKGR(11)- DQYLMWLTQKGVR(10)	4.97E- 07	5.79E- 01
52	Nb3-159(65)- p85α(632)	BS ³ , DSG	GSNTSYADSVKGR(11)- NKAENLLR(2)	1.24E- 17	7.87E- 01
53	Nb3-159(65)- p85α(650)	DSG	GSNTSYADSVKGR(11)- ESSKQGCYACSVVVDGE VK(2)	5.38E- 09	7.37E- 01
54	Nb3-159(76)- p85α(632)	BS ³ , DSG	DNTKNTVALQMNSLKPED TAVYYCAADFGR(4)- NKAENLLR(2)	3.22E- 05	7.85E- 01
55	Nb3-159(85)- p85α(632)	DSG	NTVALQMNSLKPEDTAVY YCAADFGR(9)- NKAENLLR(2)	8.40E- 07	5.48E- 01
56	Nb3-159(87)- p85α(632)	BS ³	NTVALQMNSLKPEDTAVY YCAADFGR(11)- NKAENLLR(2)	1.48E- 06	1.19E- 04

^aE-value means normalized refined_scores for filtered peptide spectrum matches (PSMs) by FDR, the smaller the more confident. Best E-value is the smallest E-value of all spectra of the cross-linked peptide. ^bScore, which is short for SVM score in pLink 2, is the prime measure for FDR estimation. Best score is the smallest value of all spectra of the cross-linked peptide.

Table S4. Cryo-EM data acquisition and image processing.

Data collection				
Microscope	Titan Krios			
Voltage (kV)	300			
Corrected magnification	46,685			
Pixel size (Å)	1.071			
Detector	Gatan K3 Summit			
Defocus range (μm)	-1.5 to -2.5			
Defocus mean (μm)	-2.0			
Total electron exposure (e-Å ⁻² s ⁻¹)	70			
Exposure rate (e-Å ⁻² frame ⁻¹)	25			
Data processing	PI3Kα-Nb3-126	PI3Kα-Nb3-142	PI3Kα-Nb3-159	PI3Kα-Nb3-142 (cross-linked)
Useable image movies	4,397	5,211	3,012	3,388
Particles	4,952,325	7,049,901	2,882,334	1,615,129
Final particles	506,412	739,753	240,599	217,230
Map sharpening B factor (Å ²)	-81.3	-163.2	-144.3	-130.3
Resolution (Å)	2.41	3.08	3.13	3.38
Unmasked (0.143 FSC)	2.4	3.2	2.8	3.6
Masked (0.143 FSC)	2.4	3.1	2.8	3.4
Local resolution range (Å)	2.1-9.5	2.0-10.2	2.0-10.7	2.0-11.4
Model	PI3Kα-Nb3-126	PI3Kα-Nb3-142	PI3Kα-Nb3-159	PI3Kα-Nb3-142 (cross-linked)
EMDB accession code	EMD-27327	EMD-27334	EMD-27330	EMD-27336
PDB accession code	8DCP	8DD4	8DCX	8DD8
Model composition				
Chain	3	3	3	3
Non-hydrogen atom	10,620	10,425	9,646	10,727
Protein residue	1,284	1,255	1,169	1,300
Refinement				
Software	phenix.real_space_refine			
Resolution (Å)	2.3	3.1	3.1	3.5
CC (mask)	0.82	0.74	0.66	0.76

CC (peak)	0.70	0.70	0.51	0.74
CC (volume)	0.79	0.74	0.62	0.75
Average B factor (Å²)				
Protein	46.44	52.81	51.24	63.46
Ligand	19.91	33.94	---	---
R.M.S. deviation				
Bond length (Å) (# > 4σ)	0.003 (0)	0.003 (0)	0.002 (0)	0.003 (0)
Bond angle (°) (# > 4σ)	0.641 (0)	0.679 (1)	0.559 (1)	0.619 (1)
Validation				
Molprobrity score	2.26	1.68	1.75	1.94
Clashscore, all atoms	9.43	10.00	9.76	11.24
Rotamer outlier (%)	3.64	0.00	0.00	0.00
Cβ outlier (%)	0.00	0.00	0.00	0.00
CaBLAM outlier (%)	2.70	1.63	2.24	3.33
Ramachandran plot (%)				
Outlier	0.00	0.16	0.09	0.31
Allowed	4.80	2.74	3.50	5.21
Favored	95.2	97.10	96.41	94.84

Dataset S1. CXMS derived restraints and docking analysis for nanobody PI3K α complexes.