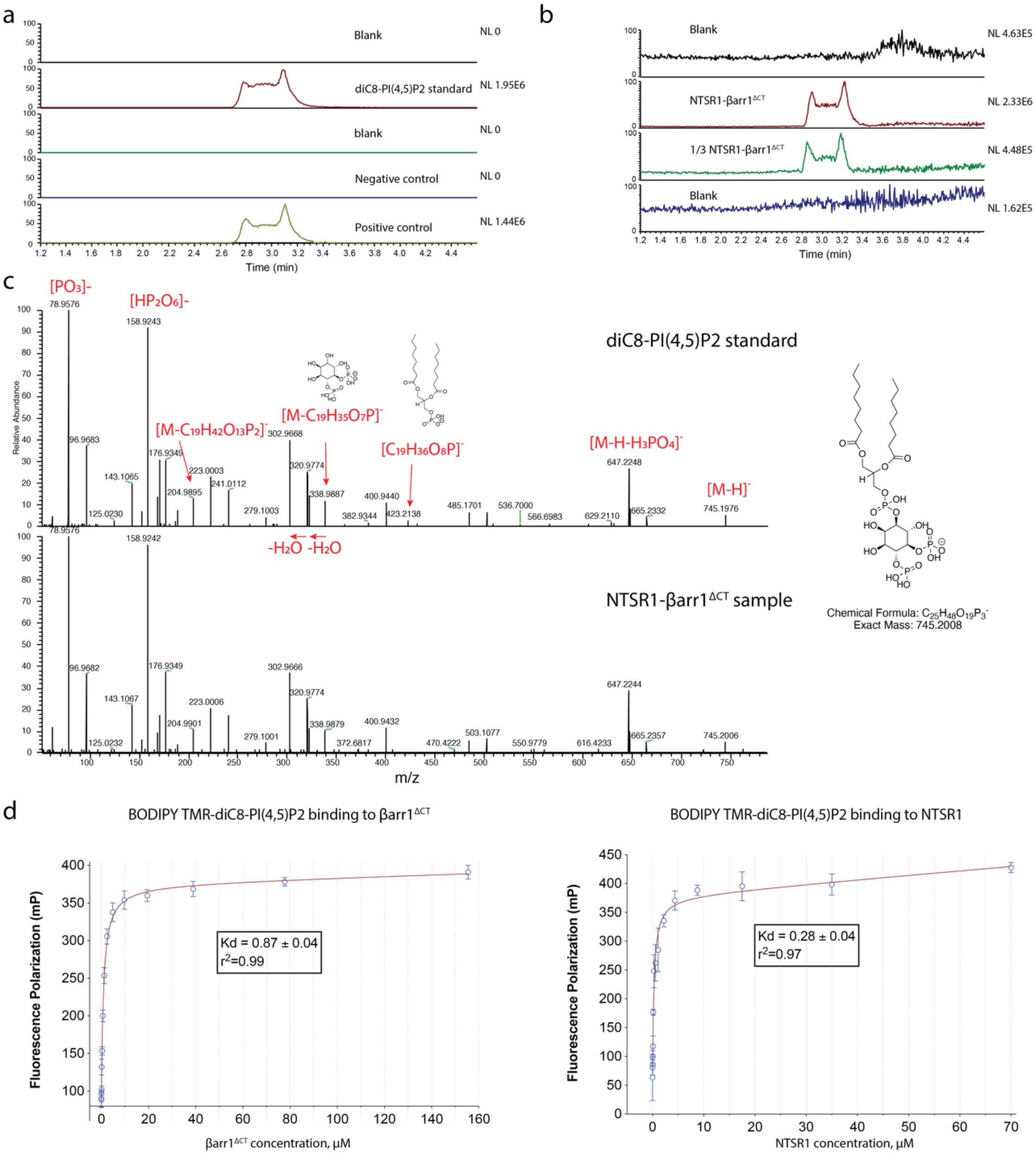
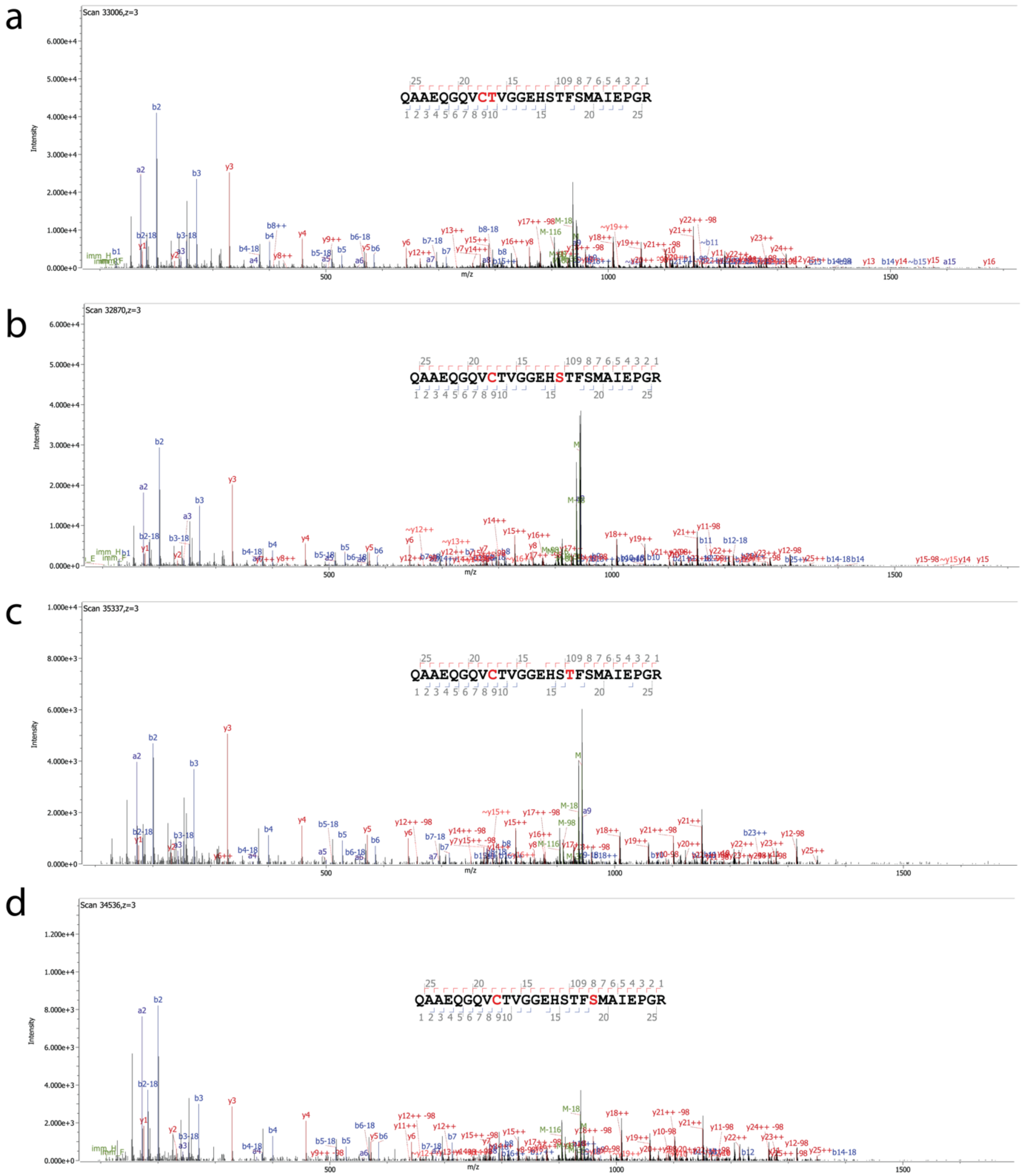


Supplementary Figure 1: NTSR1-βarr1$^{\Delta CT}$ complex samples contain diC8-PI(4,5)P2.

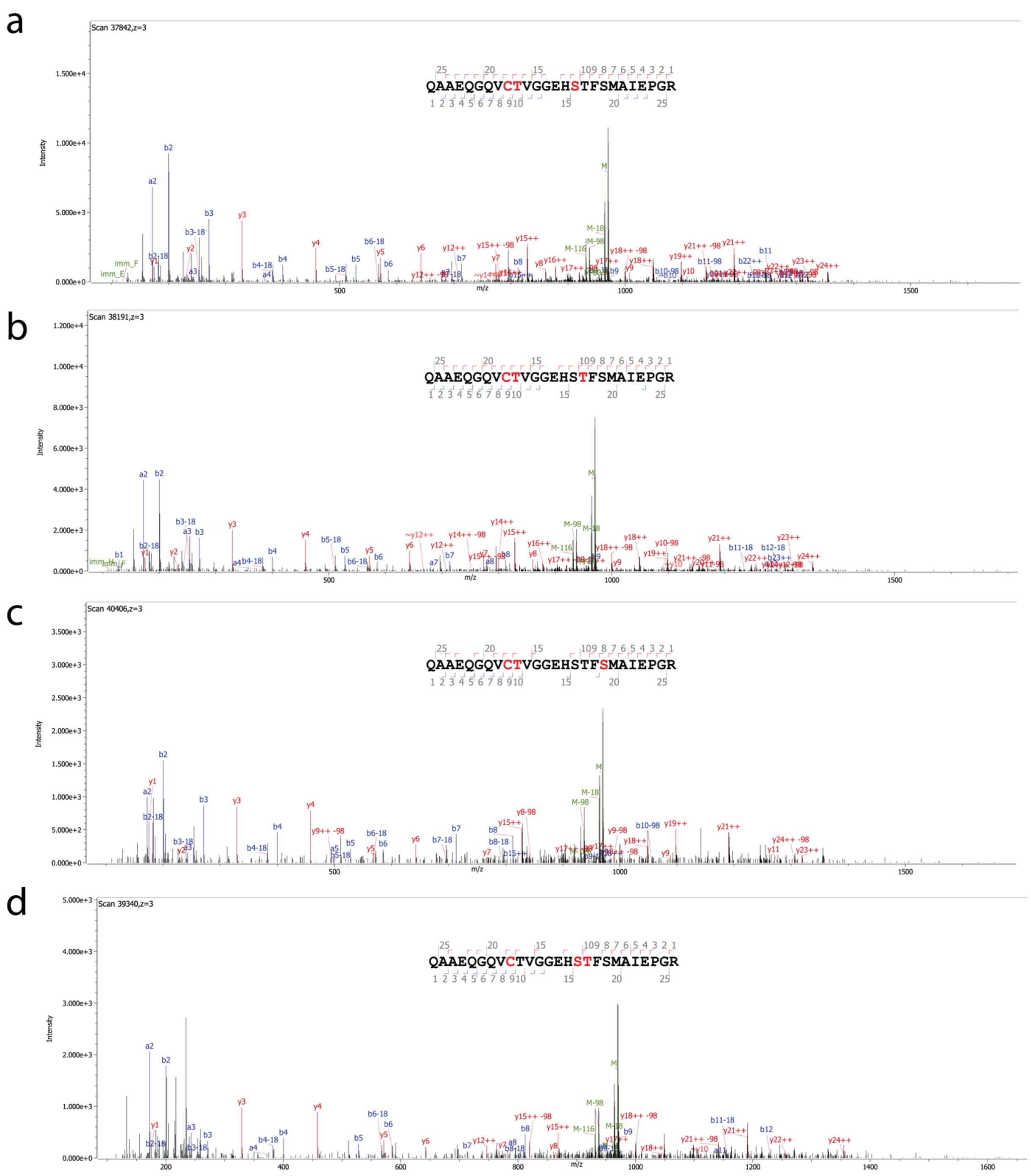
a) Extracted ion chromatogram (EIC) for diC8-PI(4,5)P2 745.2052 ± 5 ppm. Vertical axes are scaled individually for each run. Samples are listed top to bottom representing the order in which they were run. 1 µM diC8-PI(4,5)P2 was used for the standard run. Two samples were prepared where an equimolar mixture of NTSR1 and βarr1$^{\Delta CT}$ were combined in cryoEM SEC buffer to a final concentration of 1 µM each. To one sample (positive control) diC8-PI(4,5)P2 was added to 1 µM, and to the other sample buffer alone was added (negative control). These data show that intact diC8-PI(4,5)P2 can be measured in a protein-containing sample. Experiments were performed independently twice, with similar results. **b)** EICs for diC8-PI(4,5)P2

745.2052 ± 5 ppm show that a protein sample used for cryoEM, for which diC8-PI(4,5)P2 was not added during the complexing step, contains diC8-PI(4,5)P2. This experiment was performed independently once on a single cryoEM preparation. **c)** MS2 spectra of isolated 745.2069 m/z species, fragmented by HCD at 30 eV, observed in both diC8-PI(4,5)P2 standard and NTSR1-βarr1$^{\Delta CT}$ complex samples. Diagnostic fragment ions for diC8-PI(4,5)P2 are observed in both spectra and plausible chemical identities are annotated (only on top spectrum). Experiments were performed independently twice, with similar results. **d)** Fluorescence anisotropy measurements using BODIPY TMR-labeled PI(4,5)P2 show micromolar binding affinities for both βarr1$^{\Delta CT}$ (left panel) and NTSR1 (right panel). Values are shown as mean ± SD; n=3 technical replicates. Experiments were each performed independently once.



Supplementary Figure 2: Spectra used for assignment of the GRK5 phosphosites in NTSR1.

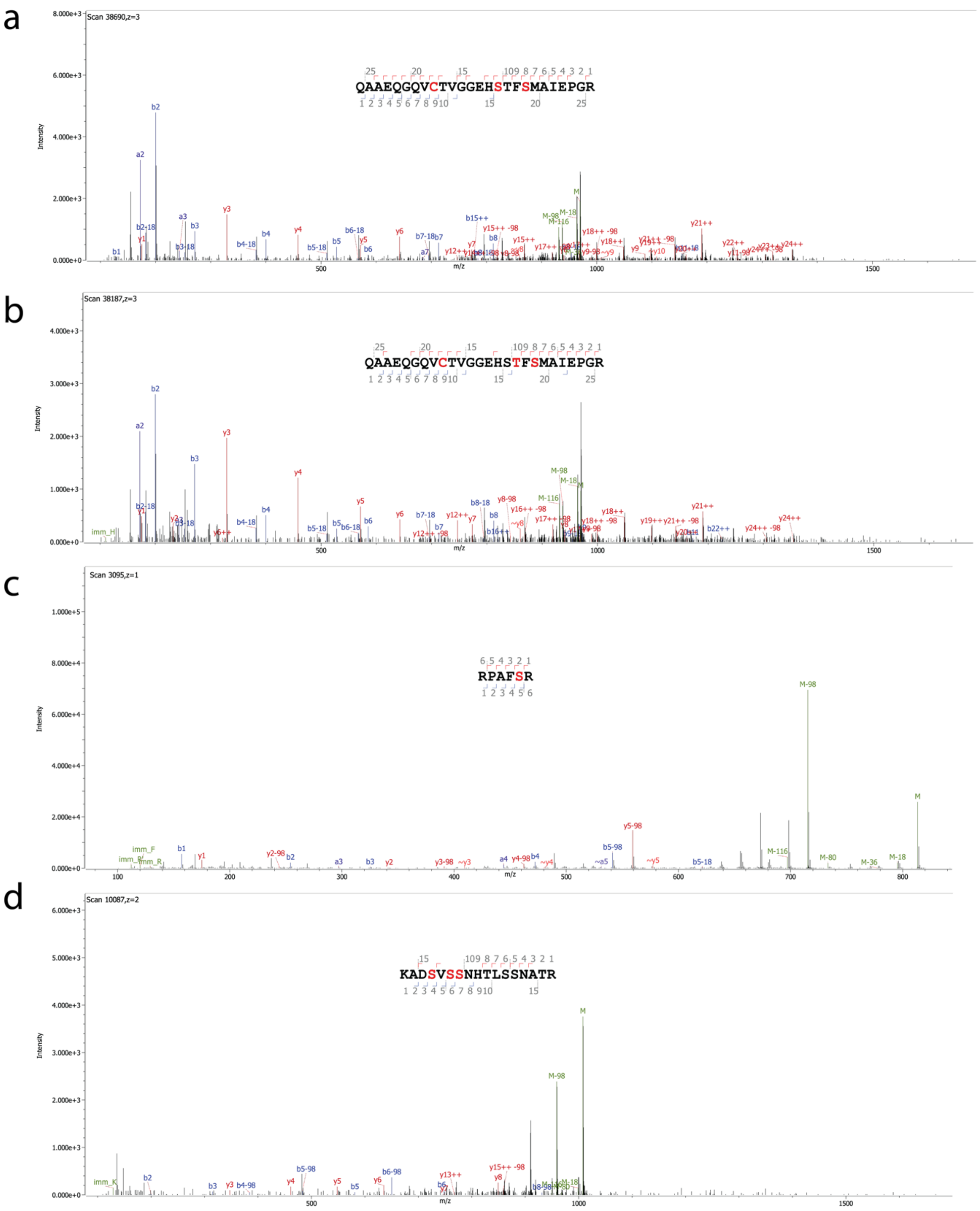
a) Representative MS2 [PASEF(943.0842)] of QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFSMAIEPGR bearing pT278. **b)** Representative MS2 [PASEF(943.0842)] of QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFSMAIEPGR bearing pS284. **c)** Representative MS2 [PASEF(943.0842)] of QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FSMAIEPGR bearing pT285. **d)** Representative MS2 [PASEF(943.0842)] of QAAEQGQVC(carbamidomethyl)TVGGEHSTFS(phospho)MAIEPGR bearing pS287. These are representative spectra from experiments that were performed independently twice, and analyzed by two or three technical replicates each time, all with similar results. PASEF stands for Parallel Accumulation-Serial Fragmentation and is the designation used for scans from the Bruker timsTOF Pro; eg. PASEF(x) denotes the MS2 scan recorded for species x. y ions are denoted by red markings, a and b ions by blue markings and other ions (including neutral loss) by green markings. The "~" denotes the fragment ion after loss of all labile modifications. The red letters in the peptide sequence denote modified residues. Numbering above and below are the numbering of the corresponding y and b/a ions. The charge of the isolated ion is shown in the top left corner.



Supplementary Figure 3: Spectra (continued) used for assignment of the GRK5 phosphosites in NTSR1.

a) Representative MS2 [PASEF(969.7328)] of QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHS(phospho)TFSMAIEPGR bearing pT278 and pS284. **b)** Representative MS2 [PASEF(969.7328)] of QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHST(phospho)FSMAIEPGR bearing pT278 and pS285. **c)** Representative

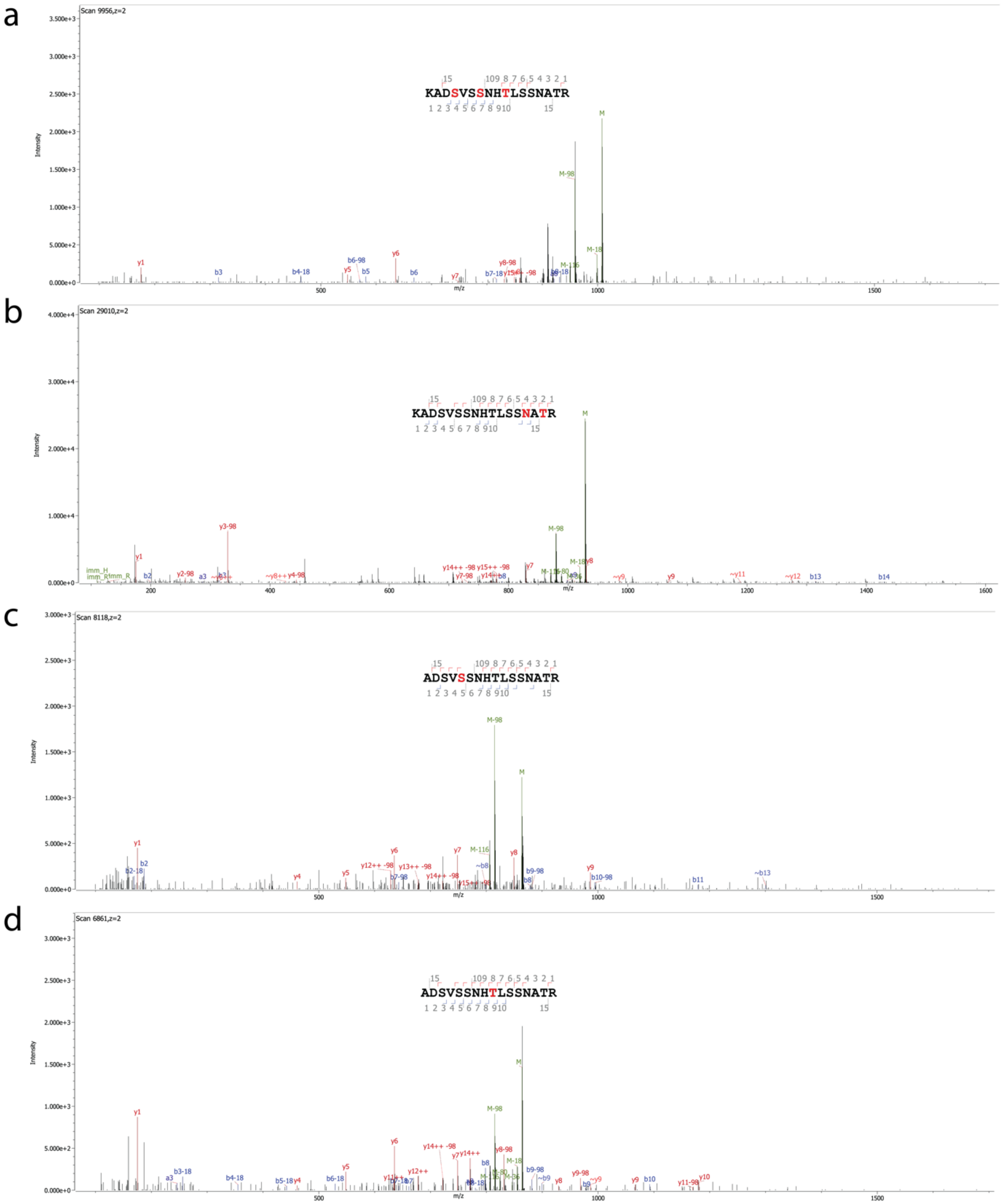
MS2 [PASEF(969.7328)] of QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFS(phospho)MAIEPGR bearing pT278 and pS287. **d)** Representative MS2 [PASEF(969.7328)] of QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)T(phospho)FSMAIEPGR bearing pS284 and pT285. These are representative spectra from experiments that were performed independently twice, and analyzed by two or three technical replicates each time, all with similar results. PASEF stands for Parallel Accumulation-Serial Fragmentation and is the designation used for scans from the Bruker timsTOF Pro; eg. PASEF(x) denotes the MS2 scan recorded for species x. y ions are denoted by red markings, a and b ions by blue markings and other ions (including neutral loss) by green markings. The "~" denotes the fragment ion after loss of all labile modifications. The red letters in the peptide sequence denote modified residues. Numbering above and below are the numbering of the corresponding y and b/a ions. The charge of the isolated ion is shown in the top left corner.



Supplementary Figure 4: Spectra (continued) used for assignment of the GRK5 phosphosites in NTSR1.

a) Representative MS2 [PASEF(969.7328)] of QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFS(phospho)MAIEPGR bearing pS284 and pS287. **b)** Representative MS2 [PASEF(969.7328)] of QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FS(phospho)MAIEPGR bearing pT285 and pS287. **c)** Representative

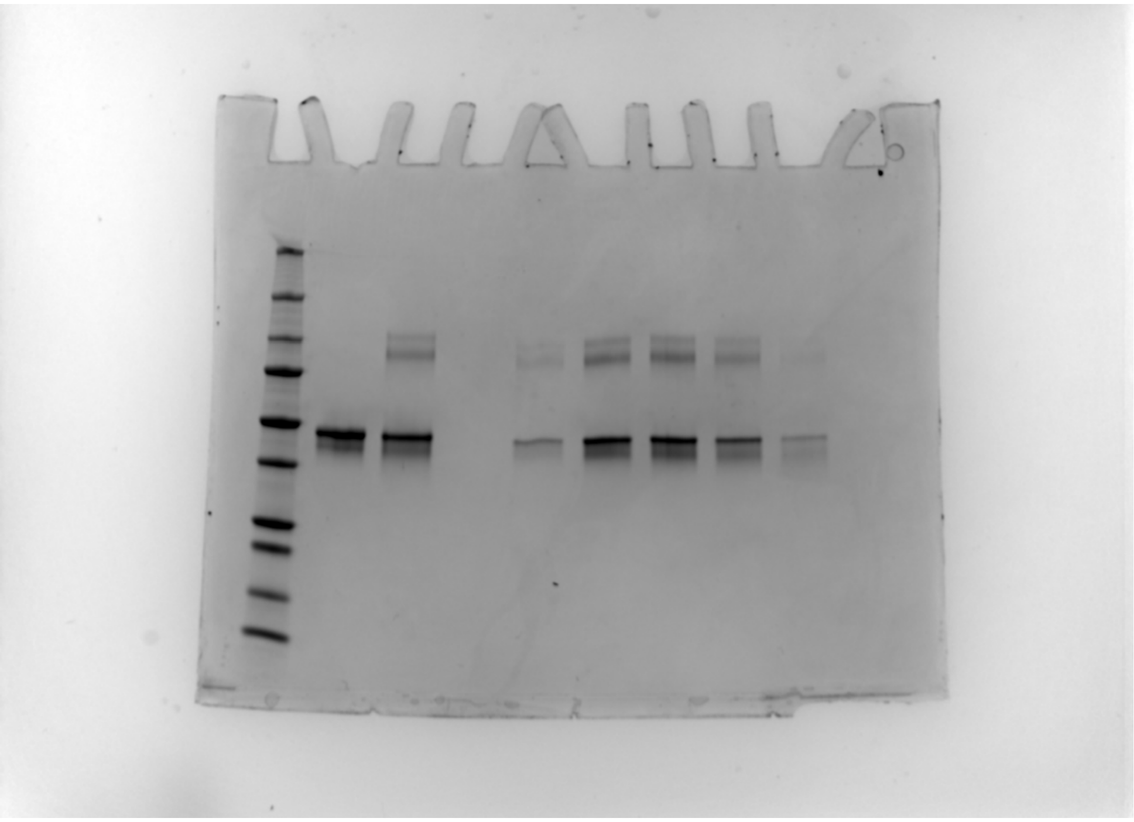
MS2 [PASEF(813.3849)] of RPAFS(phospho)R bearing pS396. **d)** Representative MS2 [PASEF(969.7328)] of KADS(Phospho)VS(Phospho)S(Phospho)NHTLSSNATR bearing pS401, pS403 and pS404. These are representative spectra from experiments that were performed independently twice, and analyzed by two or three technical replicates each time, all with similar results. PASEF stands for Parallel Accumulation-Serial Fragmentation and is the designation used for scans from the Bruker timsTOF Pro; eg. PASEF(x) denotes the MS2 scan recorded for species x. y ions are denoted by red markings, a and b ions by blue markings and other ions (including neutral loss) by green markings. The “~” denotes the fragment ion after loss of all labile modifications. The red letters in the peptide sequence denote modified residues. Numbering above and below are the numbering of the corresponding y and b/a ions. The charge of the isolated ion is shown in the top left corner.



Supplementary Figure 5: Spectra (continued) used for assignment of the GRK5 phosphosites in NTSR1.

a) Representative MS2 [PASEF(1008.4042)] of KADS(Phospho)VSS(Phospho)NHT(Phospho)LSSNATR bearing pS401, pS404, pT407. **b)** Representative MS2 [PASEF(928.9199)] of KADSVSSNHTLSSNAT(Phospho)R bearing pS416. **c)** Representative MS2 [PASEF(863.8839)] of ADSVS(Phospho)SNHTLSSNATR bearing pS403. **d)** Representative MS2 [PASEF(863.8853)] of

ADSVSSNHT(Phospho)LSSNATR bearing pT407. These are representative spectra from experiments that were performed independently twice, and analyzed by two or three technical replicates each time, all with similar results. PASEF stands for Parallel Accumulation-Serial Fragmentation and is the designation used for scans from the Bruker timsTOF Pro; eg. PASEF(x) denotes the MS2 scan recorded for species x. y ions are denoted by red markings, a and b ions by blue markings and other ions (including neutral loss) by green markings. The "~" denotes the fragment ion after loss of all labile modifications. The red letters in the peptide sequence denote modified residues. Numbering above and below are the numbering of the corresponding y and b/a ions. The charge of the isolated ion is shown in the top left corner.



Supplementary Figure 6: Uncropped gel from Extended Data Figure 3b.

Full scan of SDS-PAGE gel shown in Extended Data Figure 3b. Lanes from left to right are: molecular weight markers, pre-crosslinked NTSR1-βarr1$^{\Delta CT}$ complex, post-UV illumination (crosslinked) NTSR1-βarr1$^{\Delta CT}$ complex, empty lane, then five fractions from the SEC run post-UV illumination beginning with the earliest elution volume.

βarr1$^{\Delta CT}$								NTSR1						
Uniprot numbering	Construct numbering	Obs'd	%mod	Dead-end	xlink (self)	xlink (other)		Uniprot numbering	Construct numbering	Obs'd	%mod	Dead-end	xlink (self)	xlink (other)
N/A	14	16	12.5	0	1	1		N/A	3	0	ND	0	0	0
Lys4	28	34	26.4705882	3	3	3		Lys63	86	16	0	0	0	0
Lys10	34	9	22.2222222	0	1	1		Lys91	114	0	ND	0	0	0
Lys11	35	3	0	0	0	0		Lys92	115	0	ND	0	0	0
Lys17	41	34	100	11	22	6		Lys175	198	23	52.173913	3	6	3
Lys24	48	38	60.5263158	1	19	3		Lys177	200	17	76.4705882	5	4	4
Lys49	73	0	ND	0	0	0		Lys186	209	4	100	0	1	3
Lys77	101	38	65.7894737	13	12	0		Lys187	210	1	100	1	0	0
Lys94	118	92	13.0434783	0	12	0		Lys234	257	12	0	0	0	0
Lys95	119	15	0	0	0	0		Lys262	285	0	ND	0	0	0
Lys106	130	2	100	2	0	0		Lys391	414	80	25	16	2	2
Lys107	131	3	0	0	0	0		Lys398	421	7	0	0	0	0
Lys138	162	3	0	0	0	0								
Lys147	171	7	0	0	0	0								
Lys157	181	30	83.3333333	5	16	4								
Lys160	184	18	72.2222222	1	12	0								
Lys170	194	12	0	0	0	0								
Lys195	219	34	52.9411765	10	4	4								
Lys205	229	19	0	0	0	0								
Lys226	250	9	44.4444444	0	2	2								
Lys229	253	3	66.6666667	0	1	1								
Lys230	254	1	0	0	0	0								
Lys232	256	5	100	3	2	2								
Lys250	274	7	0	0	0	0								
Lys270	294	22		0	0	0								
Lys284	308	0	ND	0	0	0								
Lys292	316	9	33.3333333	1	2	0								
Lys294	318	6	83.3333333	3	2	0								
Lys322	346	5	0	0	0	0								
Lys324	348	0	ND	0	0	0								
Lys326	350	2	100	2	0	0								
Lys355	379	2	0	0	0	0								
Lys357	381	4	50	2	0	0								

Supplementary Table 1: NTSR1-βarr1$^{\Delta CT}$ complex sample, SDS-PAGE band 1.

Obs'd denotes the number of times that residue is covered within the MS data. %mod denotes the fraction of times that residue is modified out of the total number of times it is observed. Dead-end denotes the number of times the residue is modified by a dead-end crosslinker. Xlink (self) denotes the number of times that the peptide bearing this residue is modified by a crosslinker which intramolecularly crosslinks the peptide. Xlink (other) denotes the number of times that the peptide bearing this residue is crosslinked to another peptide. N/A for Uniprot number is used when the reactive residue is not part

of the natural protein sequence. ND (not determined) is used when there are no observations of the given residue. Construct numbering denotes the residue number in the context of the construct used in this study.

βarr1$^{\Delta CT}$								NTSR1						
Uniprot numbering	Construct numbering	Obs’d	%mod	Dead-end	xlink (self)	xlink (other)		Uniprot numbering	Construct numbering	Obs’d	%mod	Dead-end	xlink (self)	xlink (other)
N/A	14	44	45.4545455	13	6	1		N/A	3	0	ND			
Lys4	28	114	15.7894737	8	5	5		Lys63	86	14	0	0	0	0
Lys10	34	2	100	0		2		Lys91	114	0	ND			
Lys11	35	17	11.7647059	0		2		Lys92	115	0	ND			
Lys17	41	58	100	25		33		Lys175	198	13	30.7692308	3	1	0
Lys24	48	72	45.8333333	1	32	0		Lys177	200	6	50	2	1	0
Lys49	73	15	0	0	0	0		Lys186	209	0	ND			
Lys77	101	99	51.5151515	24	27	0		Lys187	210	0	ND			
Lys94	118	192	16.1458333	4	27	0		Lys234	257	9	0			
Lys95	119	81	11.1111111	4	5	0		Lys262	285	0	ND			
Lys106	130	4	100	2	1	1		Lys391	414	44	29.5454545	13		
Lys107	131	26	11.5384615	1	1	1		Lys398	421	0	ND			
Lys138	162	53	0	0	0	0								
Lys147	171	35	11.4285714	1	3	0								
Lys157	181	84	54.7619048	15	31	0								
Lys160	184	46	67.3913043	0	31	0								
Lys170	194	23	17.3913043		2	2								
Lys195	219	17	0	0	0	0								
Lys205	229	16	0	0	0	0								
Lys226	250	14	14.2857143	2	0	0								
Lys229	253	2	0	0	0	0								
Lys230	254	5	20	0	1	0								
Lys232	256	11	81.8181818	8	1	0								
Lys250	274	30	3.33333333	1	0	0								
Lys270	294	97	2.06185567	2	0	0								
Lys284	308	7	ND	0	0	0								
Lys292	316	20	70	3	11	0								
Lys294	318	22	68.1818182	3	11	1								
Lys322	346	21	0	1	1	1								
Lys324	348	2	50	1	0	0								
Lys326	350	7	71.4285714	5	0	0								
Lys355	379	20	0	1	2	0								
Lys357	381	19	15.7894737	1	2	0								

Supplementary Table 2: NTSR1-βarr1$^{\Delta CT}$ complex sample, SDS-PAGE band 2.

Obs’d denotes the number of times that residue is covered within the MS data. %mod denotes the fraction of times that residue is modified out of the total number of times it is observed. Dead-end denotes the number of times the residue is modified by a dead-end crosslinker. Xlink (self) denotes the number of times that the peptide bearing this residue is modified by a crosslinker which intramolecularly crosslinks the peptide. Xlink (other) denotes the number of times that the peptide bearing this residue is crosslinked to another peptide. N/A for Uniprot number is used when the reactive residue is not part

of the natural protein sequence. ND (not determined) is used when there are no observations of the given residue. Construct numbering denotes the residue number in the context of the construct used in this study.

βarr1$^{\Delta CT}$								NTSR1						
Uniprot numbering	Construct numbering	Obs’d	%mod	Dead-end	xlink (self)	xlink (other)		Uniprot numbering	Construct numbering	Obs’d	%mod	Dead-end	xlink (self)	xlink (other)
N/A	14	18	33.3333333	4	1	1		N/A	3	0	ND	0	0	0
Lys4	28	51	29.4117647	11	2	2		Lys63	86	16	6.25	1	0	0
Lys10	34	1	100	0	1	0		Lys91	114	2	100	0	0	2
Lys11	35	3	0	0	0	0		Lys92	115	2	0	0	0	0
Lys17	41	38	100	19	0	19		Lys175	198	17	11.7647059	1	1	0
Lys24	48	47	40.4255319	0	0	19		Lys177	200	7	100	3	2	2
Lys49	73	29	0	1	0	0		Lys186	209	0	ND	0	0	0
Lys77	101	69	53.6231884	17	19	1		Lys187	210	0	ND	0	0	0
Lys94	118	139	15.1079137	0	19	2		Lys234	257	8	0	0	0	0
Lys95	119	41	9.75609756	0	3	1		Lys262	285	0	ND	0	0	0
Lys106	130	3	100	3	0	0		Lys391	414	60	25	15	0	0
Lys107	131	22	4.54545455	1	0	0		Lys398	421	3	66.6666667	0	1	1
Lys138	162	46	0	0	0	0								
Lys147	171	17	17.6470588	1	2	0								
Lys157	181	60	60	13	23	0								
Lys160	184	40	60	1	23	0								
Lys170	194	11	0	0	0	0								
Lys195	219	19	0	0	0	0								
Lys205	229	18	0	0	0	0								
Lys226	250	9	0	0	0	0								
Lys229	253	0	0	0	0	0								
Lys230	254	1	0	0	0	0								
Lys232	256	5	0	0	0	0								
Lys250	274	23	17.3913043	2	2	0								
Lys270	294	53	3.77358491	0	2	0								
Lys284	308	1	ND	0	0	0								
Lys292	316	15	73.3333333	3	7	1								
Lys294	318	15	66.6666667	4	6	0								
Lys322	346	19	0	1	1	1								
Lys324	348	1	0	0	0	0								
Lys326	350	5	60	3	0	0								
Lys355	379	18	5.55555556	1	0	0								
Lys357	381	13	15.3846154	2	0	0								

Supplementary Table 3: NTSR1-βarr1$^{\Delta CT}$ complex sample, SDS-PAGE band 3.

Obs’d denotes the number of times that residue is covered within the MS data. %mod denotes the fraction of times that residue is modified out of the total number of times it is observed. Dead-end denotes the number of times the residue is modified by a dead-end crosslinker. Xlink (self) denotes the number of times that the peptide bearing this residue is modified by a crosslinker which intramolecularly crosslinks the peptide. Xlink (other) denotes the number of times that the peptide bearing this residue is crosslinked to another peptide. N/A for Uniprot number is used when the reactive residue is not part

of the natural protein sequence. ND (not determined) is used when there are no observations of the given residue. Construct numbering denotes the residue number in the context of the construct used in this study.

βarr1$^{\Delta CT}$								NTSR1						
Uniprot numbering	Construct numbering	Obs’d	%mod	Dead-end	xlink (self)	xlink (other)		Uniprot numbering	Construct numbering	Obs’d	%mod	Dead-end	xlink (self)	xlink (other)
N/A	14	15	0	0	0	0		N/A	3	0	ND	0	0	0
Lys4	28	29	17.2413793	5	0	0		Lys63	86	11	0	0	0	0
Lys10	34	0	ND	0	0	0		Lys91	114	3	100	0	0	3
Lys11	35	2	0	0	0	0		Lys92	115	3	0	0	0	0
Lys17	41	22	100	9	13	0		Lys175	198	7	0	0	0	0
Lys24	48	29	44.8275862	0	13	0		Lys177	200	2	100	2	0	0
Lys49	73	10	0	0	0	0		Lys186	209	0	ND	0	0	0
Lys77	101	45	64.4444444	13	16	0		Lys187	210	0	ND	0	0	0
Lys94	118	103	15.5339806	0	16	0		Lys234	257	5	0	0	0	0
Lys95	119	23	0	0	0	0		Lys262	285	0	ND	0	0	0
Lys106	130	2	50	1	0	0		Lys391	414	36	25	9	0	0
Lys107	131	14	0	0	0	0		Lys398	421	1	ND	0	0	0
Lys138	162	35	0	0	0	0								
Lys147	171	9	0	0	0	0								
Lys157	181	27	66.6666667	9	9	0								
Lys160	184	16	56.25	0	9	0								
Lys170	194	12	0	0	0	0								
Lys195	219	16	0	0	0	0								
Lys205	229	15	0	0	0	0								
Lys226	250	5	0	0	0	0								
Lys229	253	0	0	0	0	0								
Lys230	254	0	0	0	0	0								
Lys232	256	3	100	3	0	0								
Lys250	274	11	18.1818182	0	1	1								
Lys270	294	21	0	0	0	0								
Lys284	308	0	ND	0	0	0								
Lys292	316	9	66.6666667	0	5	1								
Lys294	318	7	85.7142857	2	0	4								
Lys322	346	13	0	0	0	0								
Lys324	348	1	0	0	0	0								
Lys326	350	3	66.6666667	2	0	0								
Lys355	379	9	0	0	0	0								
Lys357	381	9	22.2222222	2	0	0								

Supplementary Table 4: NTSR1-βarr1$^{\Delta CT}$ complex sample, SDS-PAGE band 4.

Obs’d denotes the number of times that residue is covered within the MS data. %mod denotes the fraction of times that residue is modified out of the total number of times it is observed. Dead-end denotes the number of times the residue is modified by a dead-end crosslinker. Xlink (self) denotes the number of times that the peptide bearing this residue is modified by a crosslinker which intramolecularly crosslinks the peptide. Xlink (other) denotes the number of times that the peptide bearing this residue is crosslinked to another peptide. N/A for Uniprot number is used when the reactive residue is not part

of the natural protein sequence. ND (not determined) is used when there are no observations of the given residue. Construct numbering denotes the residue number in the context of the construct used in this study.

Uniprot numbering	Construct numbering	Total Obs'd	Total Dead-end	Total xlink (self)	Total xlink (other)	Major xlink	%mod	%dead-end	%xlink (self+other)
N/A	14	93	17	9	1		29	18	11
Lys4	28	228	27	10	10		21	12	9
Lys10	34	12	0	2	1		25	0	25
Lys11	35	25	0	0	2		8	0	8
Lys17	41	152	64	35	23	intrastrand	80	42	38
Lys24	48	186	2	64	22	intrastrand	47	1	46
Lys49	73	54	1	0	0		2	2	0
Lys77	101	251	67	74	1	intrastrand	57	27	30
Lys94	118	526	4	74	2	intrastrand	15	1	14
Lys95	119	160	4	8	1	intrastrand	8	3	6
Lys106	130	11	8	1	1	mostly dead-end	91	73	18
Lys107	131	65	2	1	1		6	3	3
Lys138	162	137	0	0	0		0	0	0
Lys147	171	68	2	5	0		10	3	7
Lys157	181	201	42	79	4	intrastrand	62	21	41
Lys160	184	120	2	75	0	intrastrand	64	2	63
Lys170	194	58	0	2	2		7	0	7
Lys195	219	86	10	4	4		21	12	9
Lys205	229	68	0	0	0		0	0	0
Lys226	250	37	2	2	2		16	5	11
Lys229	253	5	0	1	1	None, failed to validate	0	0	0
Lys230	254	7	0	1	0		14	0	14
Lys232	256	24	14	3	2	mostly dead end	79	58	21
Lys250	274	71	3	3	1		10	4	6
Lys270	294	193	2	2	0		2	1	1
Lys284	308	8	0	0	0		0	0	0
Lys292	316	53	7	25	2	intrastrand	64	13	51
Lys294	318	50	12	19	5	intrastrand	72	24	48
Lys322	346	58	2	2	2		10	3	7
Lys324	348	4	1	0	0		25	25	0
Lys326	350	17	12	0	0	all dead-end	71	71	0
Lys355	379	49	2	2	0		8	4	4
Lys357	381	45	7	2	0		20	16	4

Supplementary Table 5: Global analysis of modified residues in βarr1.

Total Obs'd denotes the number of total times that residue is covered in the MS data, for all four gel bands (sum of **Supplementary Tables 1-4**). Total dead-end denotes the total number of times that residue is modified by a dead-end crosslinker across all four gel bands. Total xlink (self) denotes the number of times that the peptide bearing this residue is modified by a crosslinker which intramolecularly crosslinks the peptide, across all four gel bands. Total xlink (other) denotes the number of times that the peptide bearing this residue is crosslinked to another peptide, across all four gel bands. Major xlink denotes the most common type of modification observed for that residue, if there is one that clearly dominates. Intrastrand means that crosslinking within a given peptide is the most commonly seen modification. Failed to validate means that the spectrum that led to this assignment was of insufficient quality (by manual inspection) to make an assignment. %mod denotes the fraction of times that residue is modified out of the total number of times it is observed. %dead-end denotes the fraction of times this residue is modified by a dead-end crosslinker, out of the total. %xlink (self+other) denotes the fraction of that this residue forms a crosslink, either intramolecularly or intermolecularly. N/A for Uniprot number is used when the reactive residue is not part of the natural protein sequence. Construct numbering denotes the residue number in the context of the construct used in this study. Lys 14, 28 and 381 (construct numbering) are not resolved in the model and are not represented in **Extended Data Figure 4**.

Uniprot numbering	Construct numbering	Total Obs’d	Total Dead-end	Total xlink (self)	Total xlink (other)	%mod	%dead-end	%xlink (self+other)
N/A	3	0	0	0	0	0	0	0
Lys63	86	57	1	0	0	2	2%	0%
Lys91	114	5	0	0	5	100	0%	100%
Lys92	115	5	0	0	0	0	0%	0%
Lys175	198	60	7	8	3	30	12%	18%
Lys177	200	32	12	7	6	78	38%	41%
Lys186	209	4	0	1	3	100	0%	100%
Lys187	210	1	1	0	0	100	100%	0%
Lys234	257	34	0	0	0	0	0%	0%
Lys262	285	0	0	0	0	0	0%	0%
Lys391	414	220	53	2	2	26	24%	2%
Lys398	421	11	0	1	1	18	0%	18%

Supplementary Table 6: Global analysis of modified residues in NTSR1.

Total Obs’d denotes the number of total times that residue is covered in the MS data, for all four gel bands (sum of **Supplementary Tables 1-4**). Total dead-end denotes the total number of times that residue is modified by a dead-end crosslinker across all four gel bands. Total xlink (self) denotes the number of times that the peptide bearing this residue is modified by a crosslinker which intramolecularly crosslinks the peptide, across all four gel bands. Total xlink (other) denotes the number of times that the peptide bearing this residue is crosslinked to another peptide, across all four gel bands. %mod denotes the fraction of times that residue is modified out of the total number of times it is observed. %dead-end denotes the fraction of times this residue is modified by a dead-end crosslinker, out of the total. %xlink (self+other) denotes the fraction of that this residue forms a crosslink, either intramolecularly or intermolecularly. N/A for Uniprot number is used when the reactive residue is not part of the natural protein sequence. Construct numbering denotes the residue number in the context of the construct used in this study. Lys 3, 414 and 421 (construct numbering) are not resolved in the model and are not represented in **Extended Data Figure 4**.

Gel band	Peptide	Protein Source	Xlink protein source	Xlink partner	Start AA peptide	End AA peptide	Xlink Partner Peptide Xlink Pos In Protein	Location within model	Approx. distance	comment
1	K.AFVAENLEEk.I	arrestin	arrestin	K.IHK.R	172	181	184	top of N lobe	9 Å	
1	K.AsPnGkLTVYLGK.R	arrestin	arrestin	K.AFVAENLEEK.I	36	48	181	top of N lobe	8-15 Å	
1	K.AsPnGkLTVYLGK.R	arrestin	arrestin	K.IHK.R	36	48	184	top of N lobe	9 Å	
2	K.ErR.V	arrestin	arrestin	K.ALGVDYEVK.A	74	76	171	top of N lobe	14 Å	
2	K.DLFVANVQSFPPAPEDkKPLTR.L	arrestin	arrestin	R.LQER.L	102	123	127	back helix of N lobe	5-10 Å	
1	K.IkISVR.Q	arrestin	arrestin	K.VPVAMEEADDTVAPSSTFSK.V	255	260	294	C lobe adjacent strands	11.2 Å	
3	R.EILGIIVSYk.V	arrestin	arrestin	K.VK.V	337	346	348	within C lobe top	< 10 Å	
1, 2, 3	R.DAcTYATALNVASLSVEr.Y	NTSR1	NTSR1	R.KK.S	172	189	114	TM4-TM5	20 Å	
3	R.KrPAFsr.K	NTSR1	NTSR1	self	415	420	n.a.	receptor Cterm	< 10 Å	
2	R.KrPAFSr.K	NTSR1	NTSR1	self	415	420	n.a.	receptor Cterm	< 10 Å	
2	R.YLAIcHPFkAk.T	NTSR1	NTSR1	self	190	200	n.a.	ICL2	< 10 Å	
1	R.YLAIcHPFkAk.T	NTSR1	NTSR1	self	190	200	n.a.	ICL2 to itself	< 10 Å	
1	K.TLmsrSR.T	NTSR1	NTSR1	R.TK.K	201	207	209	ICL2 to adjacent ICL2 peptide	< 10 Å	low confidence in base peptide due to triply mod
1	R.sRTk.K	NTSR1	NTSR1	K.TLMSRSR.T	206	209	205	ICL2 to adjacent ICL2 peptide	< 10 Å	
1	R.Rk.R	NTSR1	arrestin	G.PGGGLNDIFEAQKIEWHEGGSGGSGDK.G	413	414	14	Cterm of receptor to Nterm of arrestin	ND, not resolved	
1	K.rPAFsR.K	NTSR1	arrestin	K.RNSVR.L	415	420	185	Cterm of receptor to Nterm of arrestin	ND, not resolved	
4	R.kK.S	NTSR1	arrestin	R.QYADIVLFNTAQYK.V	114	115	274	ICL to C-lobe strand	10 Å	
3	R.kK.S	NTSR1	arrestin	R.QYADIVLFNTAQYKVPVAMEEADDTVAPSSTFSK.V	114	115	274	ICL to C-lobe strand	10 Å	
3	R.kK.S	NTSR1	arrestin	R.QYADIVLFNTAQYK.V	114	115	274	ICL to C-lobe strand	10 Å	

Supplementary Table 7: Mapped crosslinks in NTSR1-βarr1$^{\Delta CT}$.

“Gel band” denotes the source of the crosslink that is being evaluated, bands 1 through 4 correspond to the bands on the SDS-PAGE gel shown in Extended Data Figure 3b, and are numbered from highest to lowest apparent molecular weight. “Peptide” denotes the “parent” species while “xlink partner” denotes the second peptide in the crosslink pair. “Protein source” denotes the origin of the parent “peptide”. “Xlink protein source” is the source of the crosslink partner. Crosslinks are sorted so that arrestin-arrestin intramolecular peptides are together, as is true for NTSR1-NTSR1 crosslinks and NTSR1-arrestin intermolecular crosslinks. The convention used for the peptides is that N and C terminal cleavage sites are denoted by a “.” The observed peptide is the species between the two periods. Lower case letters denote modified residues (by any PTM). “Start/end AA peptide” denotes the construct number of the first and last residue in the parent peptide, respectively. “xlink partner peptide xlink pos in protein” denotes the starting amino acid (construct numbering) of the crosslinking partner peptide. “Location within model” denotes the region of the model where these residues are. “Approx distance” denotes an approximate distance measured (in pymol) between residues in the crosslinked peptide. For unresolved regions of the model this is designated “ND, not resolved”. Since only one end of the crosslinker is specific it is difficult to map the exact position of the crosslink, these numbers are meant only to provide a sense of distance (within range of crosslinker, at the limit of the crosslinker length, beyond the length of the crosslinker (12.5 Å spacer arm)). One crosslink, for which there is a comment, is ambiguous due to modest spectral quality and a heavily modified parent peptide, but is listed since a crosslink in this region is physically consistent with other observed crosslinks in this region.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659	129.0687	0.0028	111.0553			65.0366		
A	2	172.1081	172.1102	0.0022	200.103	200.1064	0.0035	182.0924	182.0957	0.0033	100.5551		
A	3	243.1452	243.1413	-0.0039	271.1401	271.1437	0.0037	253.1295	253.1325	0.003	136.0737		
E	4	372.1878	372.1884	0.0007	400.1827	400.1866	0.004	382.1721	382.1747	0.0026	200.595		
Q	5	500.2463	500.2441	-0.0022	528.2412	528.2464	0.0052	510.2307	510.2371	0.0064	264.6243		
G	6	557.2678	557.2758	0.008	585.2627	585.2729	0.0102	567.2521	567.2583	0.0061	293.135		
Q	7	685.3264			713.3213	713.3294	0.0081	695.3107	695.3195	0.0088	357.1643		
V	8	784.3948	784.3877	-0.0071	812.3897	812.4036	0.0139	794.3791	794.3821	0.003	406.6985	406.6976	-0.0009
C	9	944.4254	944.4309	0.0054	972.4203	972.4362	0.0159	954.4098	954.4284	0.0186	486.7138		
T	10	1045.4731			1073.468			1055.4575			537.2376		
V	11	1144.5415			1172.5364			1154.5259	1154.5492	0.0233	586.7719		
G	12	1201.563			1229.5579	1229.5432	-0.0147	1211.5473	1211.553	0.0057	615.2826		
G	13	1258.5844			1286.5794			1268.5688			643.7933		
E	14	1387.627			1415.6219	1415.6408	0.0188	1397.6114			708.3146	708.309	-0.0056
H	15	1524.6859			1552.6809			1534.6703			776.8441	776.845	0.001
S	16	1611.718			1639.7129			1621.7023			820.3601		
T	17	1792.732			1820.7269			1802.7163			910.8671		
F	18	1939.8004			1967.7953			1949.7847			984.4013		
S	19	2026.8324			2054.8273			2036.8168			1027.9173		
M	20	2157.8729			2185.8678			2167.8572			1093.4375		
A	21	2228.91			2256.9049			2238.8944			1128.9561		
I	22	2341.9941			2369.989			2351.9784			1185.4981		
E	23	2471.0367			2499.0316			2481.021			1250.0194		
P	24	2568.0894			2596.0843			2578.0738			1298.5458		
G	25	2625.1109			2653.1058			2635.0952			1327.0565		
R	26												

Supplementary Table 8: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FSMAIEPGR bearing pT285.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2699.1589			1350.0831	1350.0946	0.0115	25
A	2628.1218			1314.5645			24
E	2557.0847			1279.046	1279.0515	0.0056	23
Q	2428.0421			1214.5247	1214.5414	0.0167	22
G	2299.9835			1150.4954	1150.5117	0.0163	21
Q	2242.962			1121.9847	1121.9983	0.0136	20
V	2114.9035			1057.9554	1057.9696	0.0143	19
C	2015.835			1008.4212	1008.4342	0.013	18
T	1855.8044			928.4058	928.4193	0.0135	17
V	1754.7567			877.882	877.8923	0.0103	16
G	1655.6883	1655.7509	0.0626	828.3478	828.3548	0.007	15
G	1598.6669			799.8371	799.8505	0.0134	14
E	1541.6454			771.3263			13
H	1412.6028	1412.6108	0.008	706.805	706.8129	0.0078	12
S	1275.5439	1275.5639	0.02	638.2756			11
T	1188.5119	1188.5155	0.0036	594.7596			10
F	1007.4979			504.2526			9
S	860.4294	860.4354	0.006	430.7184			8
M	773.3974	773.4094	0.012	387.2023			7
A	642.3569	642.3625	0.0055	321.6821			6
I	571.3198	571.3264	0.0066	286.1635			5
E	458.2358	458.2413	0.0055	229.6215			4
P	329.1932	329.1942	0.001	165.1002			3
G	232.1404	232.138	-0.0024	116.5738			2
R	175.119	175.1222	0.0032	88.0631			1

Supplementary Table 9: y ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FSMAIEPGR bearing pT285.

"Seq." denotes the residue in the peptide, "#" denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. "a/b/y calc." denotes the calculated m/z for the corresponding a/b/y ion. "a/b/y obs." denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. "a/b/y delta" denotes the deviation (in m/z) between the calculated and observed species. "b/y++" denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the "Seq" column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659			111.0553			65.0366		
A	2	172.1081	172.1105	0.0025	200.103	200.1059	0.003	182.0924	182.0974	0.005	100.5551		
A	3	243.1452	243.1446	-0.0006	271.1401	271.1426	0.0025	253.1295	253.1325	0.003	136.0737		
E	4	372.1878	372.1912	0.0034	400.1827	400.1877	0.0051	382.1721	382.176	0.0039	200.595		
Q	5	500.2463			528.2412	528.2457	0.0045	510.2307	510.2397	0.009	264.6243		
G	6	557.2678	557.2798	0.012	585.2627	585.2735	0.0108	567.2521	567.2583	0.0061	293.135		
Q	7	685.3264	685.3277	0.0013	713.3213	713.3337	0.0124	695.3107	695.3175	0.0067	357.1643		
V	8	784.3948	784.4091	0.0143	812.3897	812.3973	0.0076	794.3791	794.3874	0.0083	406.6985		
C	9	944.4254			972.4203	972.4405	0.0202	954.4098	954.4337	0.0239	486.7138		
T	10	1045.4731			1073.468			1055.4575			537.2376		
V	11	1144.5415			1172.5364	1172.547	0.0105	1154.5259	1154.5385	0.0127	586.7719		
G	12	1201.563			1229.5579	1229.5464	-0.0115	1211.5473			615.2826		
G	13	1258.5844			1286.5794			1268.5688			643.7933		
E	14	1387.627			1415.6219			1397.6114	1397.6381	0.0268	708.3146		
H	15	1524.6859			1552.6809			1534.6703			776.8441		
S	16	1611.718			1639.7129			1621.7023			820.3601	820.3647	0.0047
T	17	1712.7656			1740.7606			1722.75			870.8839	870.8879	0.004
F	18	1859.8341			1887.829			1869.8184			944.4181	944.4263	0.0081
S	19	2026.8324			2054.8273			2036.8168			1027.9173		
M	20	2157.8729			2185.8678			2167.8572			1093.4375		
A	21	2228.91			2256.9049			2238.8944			1128.9561		
I	22	2341.9941			2369.989			2351.9784			1185.4981		
E	23	2471.0367			2499.0316			2481.021			1250.0194		
P	24	2568.0894			2596.0843			2578.0738			1298.5458		
G	25	2625.1109			2653.1058			2635.0952			1327.0565		
R	26												

Supplementary Table 10: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHSTFS(phospho)MAIEPGR bearing pS287.

"Seq." denotes the residue in the peptide, "#" denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. "a/b/y calc." denotes the calculated m/z for the corresponding a/b/y ion. "a/b/y obs." denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. "a/b/y delta" denotes the deviation (in m/z) between the calculated and observed species. "b/y++" denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the "Seq" column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2699.1589			1350.0831	1350.1065	0.0234	25
A	2628.1218			1314.5645			24
E	2557.0847			1279.046	1279.062	0.016	23
Q	2428.0421			1214.5247	1214.5423	0.0177	22
G	2299.9835			1150.4954	1150.5075	0.0121	21
Q	2242.962			1121.9847	1121.9941	0.0095	20
V	2114.9035			1057.9554	1057.9626	0.0072	19
C	2015.835			1008.4212	1008.4415	0.0203	18
T	1855.8044			928.4058	928.4195	0.0137	17
V	1754.7567			877.882	877.897	0.015	16
G	1655.6883			828.3478	828.354	0.0062	15
G	1598.6669			799.8371	799.8468	0.0098	14
E	1541.6454			771.3263			13
H	1412.6028			706.805	706.8133	0.0082	12
S	1275.5439	1275.5682	0.0243	638.2756	638.2773	0.0017	11
T	1188.5119	1188.5232	0.0113	594.7596			10
F	1087.4642	1087.4831	0.0189	544.2357			9
S	940.3958	940.4126	0.0168	470.7015			8
M	773.3974	773.4095	0.0121	387.2023			7
A	642.3569	642.3616	0.0047	321.6821			6
I	571.3198	571.3247	0.0049	286.1635			5
E	458.2358	458.2416	0.0058	229.6215			4
P	329.1932	329.1917	-0.0015	165.1002			3
G	232.1404	232.1426	0.0022	116.5738			2
R	175.119	175.1222	0.0032	88.0631			1

Supplementary Table 11: y ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHSTFS(phospho)MAIEPGR bearing pS287.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659	129.0694	0.0036	111.0553			65.0366		
A	2	172.1081	172.1108	0.0028	200.103	200.1057	0.0027	182.0924	182.0947	0.0023	100.5551		
A	3	243.1452	243.1472	0.0021	271.1401	271.1428	0.0027	253.1295	253.1309	0.0014	136.0737		
E	4	372.1878	372.1882	0.0005	400.1827	400.1873	0.0047	382.1721	382.1768	0.0047	200.595		
Q	5	500.2463	500.2471	0.0008	528.2412	528.2468	0.0056	510.2307	510.2414	0.0107	264.6243		
G	6	557.2678	557.2691	0.0013	585.2627	585.2696	0.0069	567.2521	567.2607	0.0086	293.135		
Q	7	685.3264	685.3212	-0.0051	713.3213	713.3301	0.0088	695.3107	695.321	0.0103	357.1643		
V	8	784.3948	784.3911	-0.0037	812.3897	812.3975	0.0078	794.3791	794.3925	0.0134	406.6985		
C	9	944.4254	944.4192	-0.0063	972.4203	972.4306	0.0103	954.4098	954.4297	0.0199	486.7138		
T	10	1045.4731	1045.4732	0.0001	1073.468	1073.4934	0.0254	1055.4575	1055.4763	0.0188	537.2376		
V	11	1144.5415			1172.5364	1172.5511	0.0146	1154.5259	1154.5437	0.0178	586.7719		
G	12	1201.563			1229.5579	1229.5855	0.0276	1211.5473	1211.5694	0.0221	615.2826		
G	13	1258.5844			1286.5794			1268.5688			643.7933		
E	14	1387.627			1415.6219	1415.6204	-0.0016	1397.6114	1397.6153	0.0039	708.3146	708.3142	-0.0004
H	15	1524.6859			1552.6809			1534.6703			776.8441	776.8544	0.0103
S	16	1691.6843			1719.6792			1701.6686			860.3432		
T	17	1792.732			1820.7269			1802.7163			910.8671		
F	18	1939.8004			1967.7953			1949.7847			984.4013	984.4192	0.0179
S	19	2026.8324			2054.8273			2036.8168			1027.9173	1027.9332	0.0159
M	20	2157.8729			2185.8678			2167.8572			1093.4375		
A	21	2228.91			2256.9049			2238.8944			1128.9561	1128.9863	0.0302
I	22	2341.9941			2369.989			2351.9784			1185.4981	1185.5153	0.0171
E	23	2471.0367			2499.0316			2481.021			1250.0194	1250.0368	0.0174
P	24	2568.0894			2596.0843			2578.0738			1298.5458		
G	25	2625.1109			2653.1058			2635.0952			1327.0565	1327.0556	-0.001
R	26												

Supplementary Table 12: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFSMAIEPGR bearing pS284.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2699.1589			1350.0831	1350.1016	0.0185	25
A	2628.1218			1314.5645			24
E	2557.0847			1279.046	1279.0608	0.0148	23
Q	2428.0421			1214.5247	1214.5383	0.0136	22
G	2299.9835			1150.4954	1150.509	0.0136	21
Q	2242.962			1121.9847	1121.9976	0.013	20
V	2114.9035			1057.9554	1057.967	0.0116	19
C	2015.835			1008.4212	1008.4326	0.0115	18
T	1855.8044			928.4058	928.4169	0.011	17
V	1754.7567			877.882	877.8917	0.0097	16
G	1655.6883	1655.7166	0.0283	828.3478	828.3579	0.0101	15
G	1598.6669	1598.674	0.0072	799.8371	799.8482	0.0111	14
E	1541.6454			771.3263	771.3345	0.0082	13
H	1412.6028			706.805	706.8126	0.0075	12
S	1275.5439	1275.5604	0.0165	638.2756			11
T	1108.5455	1108.5589	0.0134	554.7764			10
F	1007.4979			504.2526			9
S	860.4294	860.4392	0.0097	430.7184	430.7191	0.0008	8
M	773.3974	773.4073	0.0099	387.2023	387.202	-0.0003	7
A	642.3569	642.3663	0.0093	321.6821			6
I	571.3198	571.3272	0.0074	286.1635			5
E	458.2358	458.2408	0.0051	229.6215			4
P	329.1932	329.1925	-0.0007	165.1002			3
G	232.1404	232.1396	-0.0009	116.5738			2
R	175.119	175.1205	0.0015	88.0631			1

Supplementary Table 13: y ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFSMAIEPGR bearing pS284.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659	129.0696	0.0038	111.0553			65.0366		
A	2	172.1081	172.1105	0.0024	200.103	200.1057	0.0027	182.0924	182.0951	0.0027	100.5551		
A	3	243.1452	243.1466	0.0014	271.1401	271.1422	0.0022	253.1295	253.1319	0.0024	136.0737		
E	4	372.1878	372.1913	0.0036	400.1827	400.1874	0.0047	382.1721	382.1755	0.0034	200.595		
Q	5	500.2463	500.2491	0.0028	528.2412	528.246	0.0048	510.2307	510.2378	0.0071	264.6243		
G	6	557.2678	557.2544	-0.0134	585.2627	585.2706	0.0079	567.2521	567.2618	0.0097	293.135		
Q	7	685.3264	685.3291	0.0027	713.3213	713.3324	0.0111	695.3107	695.3218	0.0111	357.1643		
V	8	784.3948	784.4032	0.0085	812.3897	812.3973	0.0076	794.3791	794.3873	0.0082	406.6985	406.703	0.0045
C	9	944.4254	944.4221	-0.0033	972.4203	972.4325	0.0122	954.4098	954.4179	0.0081	486.7138		
T	10	1125.4394			1153.4344			1135.4238			577.2208		
V	11	1224.5078	1224.5126	0.0048	1252.5028	1252.5305	0.0278	1234.4922	1234.5188	0.0266	626.755		
G	12	1281.5293			1309.5242			1291.5137	1291.5404	0.0267	655.2657		
G	13	1338.5508			1366.5457	1366.5832	0.0376	1348.5351			683.7765		
E	14	1467.5934			1495.5883	1495.5952	0.0069	1477.5777			748.2978		
H	15	1604.6523	1604.6323	-0.0199	1632.6472			1614.6366			816.8272	816.8383	0.0111
S	16	1691.6843			1719.6792			1701.6686			860.3432		
T	17	1792.732			1820.7269			1802.7163			910.8671		
F	18	1939.8004			1967.7953			1949.7847			984.4013	984.4054	0.0041
S	19	2026.8324			2054.8273			2036.8168			1027.9173		
M	20	2157.8729			2185.8678			2167.8572			1093.4375		
A	21	2228.91			2256.9049			2238.8944			1128.9561	1128.9741	0.018
I	22	2341.9941			2369.989			2351.9784			1185.4981	1185.5272	0.0291
E	23	2471.0367			2499.0316			2481.021			1250.0194	1250.0205	0.0011
P	24	2568.0894			2596.0843			2578.0738			1298.5458		
G	25	2625.1109			2653.1058			2635.0952			1327.0565		
R	26												

Supplementary Table 14: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFSMAIEPGR bearing pT278.

"Seq." denotes the residue in the peptide, "#" denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. "a/b/y calc." denotes the calculated m/z for the corresponding a/b/y ion. "a/b/y obs." denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. "a/b/y delta" denotes the deviation (in m/z) between the calculated and observed species. "b/y++" denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the "Seq" column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2699.1589			1350.0831	1350.0893	0.0062	25
A	2628.1218			1314.5645	1314.5801	0.0156	24
E	2557.0847			1279.046	1279.0615	0.0156	23
Q	2428.0421			1214.5247	1214.5426	0.0179	22
G	2299.9835			1150.4954	1150.5105	0.0151	21
Q	2242.962			1121.9847	1121.9954	0.0108	20
V	2114.9035			1057.9554	1057.969	0.0137	19
C	2015.835			1008.4212	1008.4375	0.0163	18
T	1855.8044			928.4058	928.4168	0.011	17
V	1674.7904	1674.8072	0.0168	837.8988	837.9109	0.0121	16
G	1575.722	1575.7409	0.019	788.3646	788.3754	0.0108	15
G	1518.7005	1518.718	0.0175	759.8539	759.8611	0.0072	14
E	1461.6791	1461.6954	0.0164	731.3432	731.3539	0.0107	13
H	1332.6365	1332.6523	0.0158	666.8219	666.8305	0.0086	12
S	1195.5776	1195.5883	0.0107	598.2924			11
T	1108.5455	1108.5584	0.0129	554.7764			10
F	1007.4979			504.2526	504.2631	0.0105	9
S	860.4294	860.4396	0.0102	430.7184	430.7191	0.0008	8
M	773.3974	773.4062	0.0088	387.2023			7
A	642.3569	642.3635	0.0066	321.6821			6
I	571.3198	571.3271	0.0073	286.1635			5
E	458.2358	458.2412	0.0055	229.6215			4
P	329.1932	329.1924	-0.0008	165.1002			3
G	232.1404	232.1409	0.0005	116.5738			2
R	175.119	175.1196	0.0006	88.0631			1

Supplementary Table 15: y ions observed for peptide QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFSMAIEPGR bearing pT278.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659			111.0553			65.0366		
A	2	172.1081	172.1109	0.0029	200.103	200.1057	0.0027	182.0924	182.0945	0.0021	100.5551		
A	3	243.1452	243.1422	-0.0029	271.1401	271.1439	0.0038	253.1295	253.13	0.0005	136.0737		
E	4	372.1878	372.1886	0.0008	400.1827	400.1858	0.0031	382.1721	382.179	0.0069	200.595		
Q	5	500.2463			528.2412	528.2462	0.005	510.2307	510.2366	0.0059	264.6243		
G	6	557.2678			585.2627	585.2724	0.0097	567.2521	567.2588	0.0067	293.135		
Q	7	685.3264	685.3116	-0.0147	713.3213	713.33	0.0087	695.3107	695.3172	0.0065	357.1643		
V	8	784.3948			812.3897	812.4023	0.0126	794.3791			406.6985		
C	9	944.4254			972.4203	972.4204	0.0001	954.4098	954.422	0.0123	486.7138		
T	10	1125.4394			1153.4344			1135.4238			577.2208		
V	11	1224.5078			1252.5028	1252.5396	0.0369	1234.4922	1234.5047	0.0125	626.755		
G	12	1281.5293	1281.5031	-0.0262	1309.5242	1309.5281	0.0038	1291.5137	1291.5183	0.0046	655.2657		
G	13	1338.5508			1366.5457			1348.5351			683.7765		
E	14	1467.5934			1495.5883			1477.5777			748.2978		
H	15	1604.6523			1632.6472			1614.6366			816.8272	816.8347	0.0075
S	16	1771.6506			1799.6455			1781.635			900.3264		
T	17	1872.6983			1900.6932			1882.6827			950.8502		
F	18	2019.7667			2047.7616			2029.7511			1024.3845		
S	19	2106.7987			2134.7937			2116.7831			1067.9005		
M	20	2237.8392			2265.8341			2247.8236			1133.4207		
A	21	2308.8763			2336.8713			2318.8607			1168.9393	1168.9504	0.0111
I	22	2421.9604			2449.9553			2431.9447			1225.4813	1225.5015	0.0202
E	23	2551.003			2578.9979			2560.9873			1290.0026	1290.0082	0.0056
P	24	2648.0557			2676.0507			2658.0401			1338.529		
G	25	2705.0772			2733.0721			2715.0616			1367.0397		
R	26												

Supplementary Table 16: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHS(phospho)TFSMAIEPGR doubly phosphorylated at positions T278 and S284.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2779.1252			1390.0662			25
A	2708.0881			1354.5477	1354.5583	0.0106	24
E	2637.051			1319.0291	1319.0391	0.0099	23
Q	2508.0084			1254.5078	1254.524	0.0162	22
G	2379.9498			1190.4786	1190.4948	0.0163	21
Q	2322.9284			1161.9678	1161.9849	0.0171	20
V	2194.8698			1097.9385	1097.9546	0.0161	19
C	2095.8014			1048.4043	1048.4191	0.0148	18
T	1935.7707			968.389	968.4005	0.0115	17
V	1754.7567			877.882	877.8923	0.0103	16
G	1655.6883			828.3478	828.3573	0.0095	15
G	1598.6669			799.8371	799.849	0.0119	14
E	1541.6454			771.3263			13
H	1412.6028			706.805	706.8179	0.0129	12
S	1275.5439	1275.5557	0.0119	638.2756			11
T	1108.5455	1108.5495	0.004	554.7764			10
F	1007.4979	1007.5095	0.0116	504.2526			9
S	860.4294	860.4367	0.0073	430.7184			8
M	773.3974	773.4089	0.0115	387.2023			7
A	642.3569	642.3643	0.0074	321.6821			6
I	571.3198	571.3279	0.0081	286.1635			5
E	458.2358	458.2395	0.0038	229.6215			4
P	329.1932	329.1935	0.0004	165.1002			3
G	232.1404	232.1451	0.0047	116.5738			2
R	175.119	175.1207	0.0018	88.0631			1

Supplementary Table 17: y ions observed for peptide QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHS(phospho)TFSMAIEPGR doubly phosphorylated at positions T278 and S284.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659	129.0702	0.0044	111.0553			65.0366		
A	2	172.1081	172.1102	0.0021	200.103	200.1056	0.0027	182.0924	182.0941	0.0017	100.5551		
A	3	243.1452	243.1417	-0.0035	271.1401	271.1419	0.0019	253.1295	253.1323	0.0028	136.0737		
E	4	372.1878	372.1915	0.0038	400.1827	400.1874	0.0047	382.1721	382.174	0.0019	200.595		
Q	5	500.2463			528.2412	528.2496	0.0083	510.2307	510.2387	0.008	264.6243		
G	6	557.2678			585.2627	585.2669	0.0042	567.2521	567.2588	0.0067	293.135		
Q	7	685.3264	685.302	-0.0243	713.3213	713.3193	-0.002	695.3107			357.1643		
V	8	784.3948	784.4024	0.0076	812.3897	812.3997	0.01	794.3791			406.6985		
C	9	944.4254			972.4203	972.4428	0.0225	954.4098	954.4238	0.0141	486.7138		
T	10	1125.4394			1153.4344			1135.4238			577.2208		
V	11	1224.5078			1252.5028			1234.4922	1234.5038	0.0116	626.755		
G	12	1281.5293	1281.5416	0.0123	1309.5242			1291.5137	1291.4994	-0.0142	655.2657		
G	13	1338.5508			1366.5457			1348.5351			683.7765		
E	14	1467.5934			1495.5883			1477.5777			748.2978		
H	15	1604.6523			1632.6472			1614.6366			816.8272		
S	16	1691.6843			1719.6792			1701.6686			860.3432		
T	17	1872.6983			1900.6932			1882.6827			950.8502		
F	18	2019.7667			2047.7616			2029.7511			1024.3845		
S	19	2106.7987			2134.7937			2116.7831			1067.9005		
M	20	2237.8392			2265.8341			2247.8236			1133.4207		
A	21	2308.8763			2336.8713			2318.8607			1168.9393		
I	22	2421.9604			2449.9553			2431.9447			1225.4813		
E	23	2551.003			2578.9979			2560.9873			1290.0026	1290.0201	0.0175
P	24	2648.0557			2676.0507			2658.0401			1338.529		
G	25	2705.0772			2733.0721			2715.0616			1367.0397		
R	26												

Supplementary Table 18: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHST(phospho)FSMAIEPGR doubly phosphorylated at positions T278 and T285.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2779.1252			1390.0662			25
A	2708.0881			1354.5477	1354.5727	0.025	24
E	2637.051			1319.0291	1319.0592	0.03	23
Q	2508.0084			1254.5078	1254.5228	0.015	22
G	2379.9498			1190.4786	1190.4882	0.0097	21
Q	2322.9284			1161.9678	1161.9762	0.0083	20
V	2194.8698			1097.9385	1097.9495	0.0109	19
C	2095.8014			1048.4043	1048.4097	0.0054	18
T	1935.7707			968.389	968.4029	0.0139	17
V	1754.7567			877.882	877.8934	0.0114	16
G	1655.6883			828.3478	828.3576	0.0098	15
G	1598.6669			799.8371	799.8455	0.0085	14
E	1541.6454			771.3263			13
H	1412.6028			706.805	706.8187	0.0136	12
S	1275.5439	1275.5034	-0.0404	638.2756			11
T	1188.5119			594.7596			10
F	1007.4979	1007.4996	0.0018	504.2526			9
S	860.4294	860.4403	0.0109	430.7184			8
M	773.3974	773.4003	0.0029	387.2023			7
A	642.3569	642.3621	0.0051	321.6821			6
I	571.3198	571.3249	0.0051	286.1635			5
E	458.2358	458.2397	0.004	229.6215			4
P	329.1932	329.1963	0.0031	165.1002			3
G	232.1404	232.147	0.0066	116.5738			2
R	175.119	175.1192	0.0003	88.0631			1

Supplementary Table 19: y ions observed for peptide QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHST(phospho)FSMAIEPGR doubly phosphorylated at positions T278 and T285.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659			111.0553			65.0366		
A	2	172.1081	172.1114	0.0034	200.103	200.1055	0.0025	182.0924	182.0949	0.0025	100.5551		
A	3	243.1452	243.1477	0.0025	271.1401	271.1437	0.0037	253.1295	253.1303	0.0008	136.0737		
E	4	372.1878			400.1827	400.1873	0.0046	382.1721	382.1825	0.0104	200.595		
Q	5	500.2463	500.2662	0.0199	528.2412	528.2461	0.0048	510.2307	510.2164	-0.0143	264.6243		
G	6	557.2678			585.2627	585.2714	0.0087	567.2521	567.2699	0.0178	293.135		
Q	7	685.3264			713.3213	713.3242	0.0029	695.3107	695.3139	0.0031	357.1643		
V	8	784.3948			812.3897	812.3953	0.0056	794.3791	794.3929	0.0138	406.6985		
C	9	944.4254			972.4203	972.4295	0.0092	954.4098	954.4274	0.0176	486.7138		
T	10	1125.4394			1153.4344			1135.4238			577.2208		
V	11	1224.5078			1252.5028			1234.4922			626.755		
G	12	1281.5293			1309.5242			1291.5137			655.2657		
G	13	1338.5508			1366.5457			1348.5351			683.7765		
E	14	1467.5934			1495.5883			1477.5777			748.2978		
H	15	1604.6523			1632.6472			1614.6366			816.8272	816.839	0.0118
S	16	1691.6843			1719.6792			1701.6686			860.3432		
T	17	1792.732			1820.7269			1802.7163			910.8671		
F	18	1939.8004			1967.7953			1949.7847			984.4013	984.4201	0.0189
S	19	2106.7987			2134.7937			2116.7831			1067.9005		
M	20	2237.8392			2265.8341			2247.8236			1133.4207		
A	21	2308.8763			2336.8713			2318.8607			1168.9393		
I	22	2421.9604			2449.9553			2431.9447			1225.4813		
E	23	2551.003			2578.9979			2560.9873			1290.0026		
P	24	2648.0557			2676.0507			2658.0401			1338.529		
G	25	2705.0772			2733.0721			2715.0616			1367.0397		
R	26												

Supplementary Table 20: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFS(phospho)MAIEPGR doubly phosphorylated at positions T278 and S287.

"Seq." denotes the residue in the peptide, "#" denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. "a/b/y calc." denotes the calculated m/z for the corresponding a/b/y ion. "a/b/y obs." denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. "a/b/y delta" denotes the deviation (in m/z) between the calculated and observed species. "b/y++" denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the "Seq" column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2779.1252			1390.0662			25
A	2708.0881			1354.5477			24
E	2637.051			1319.0291	1319.0292	0.0001	23
Q	2508.0084			1254.5078			22
G	2379.9498			1190.4786	1190.4911	0.0126	21
Q	2322.9284			1161.9678			20
V	2194.8698			1097.9385	1097.9421	0.0036	19
C	2095.8014			1048.4043	1048.4091	0.0047	18
T	1935.7707			968.389	968.4089	0.0198	17
V	1754.7567			877.882			16
G	1655.6883			828.3478	828.3606	0.0128	15
G	1598.6669			799.8371			14
E	1541.6454			771.3263			13
H	1412.6028			706.805			12
S	1275.5439	1275.5716	0.0277	638.2756			11
T	1188.5119			594.7596			10
F	1087.4642	1087.4858	0.0216	544.2357			9
S	940.3958	940.3987	0.003	470.7015			8
M	773.3974	773.3971	-0.0003	387.2023			7
A	642.3569	642.3645	0.0076	321.6821			6
I	571.3198	571.3329	0.0131	286.1635			5
E	458.2358	458.2412	0.0055	229.6215			4
P	329.1932	329.1924	-0.0008	165.1002			3
G	232.1404	232.136	-0.0044	116.5738			2
R	175.119	175.1207	0.0017	88.0631			1

Supplementary Table 21: y ions observed for peptide QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFS(phospho)MAIEPGR doubly phosphorylated at positions T278 and S287.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659			111.0553			65.0366		
A	2	172.1081	172.1119	0.0038	200.103	200.1067	0.0037	182.0924	182.0939	0.0015	100.5551		
A	3	243.1452	243.138	-0.0072	271.1401	271.1443	0.0043	253.1295	253.1322	0.0027	136.0737		
E	4	372.1878	372.1974	0.0097	400.1827	400.1843	0.0016	382.1721	382.1757	0.0036	200.595		
Q	5	500.2463			528.2412	528.2466	0.0054	510.2307			264.6243		
G	6	557.2678			585.2627	585.2737	0.011	567.2521	567.2649	0.0128	293.135		
Q	7	685.3264			713.3213	713.3257	0.0044	695.3107			357.1643		
V	8	784.3948	784.3636	-0.0312	812.3897	812.3923	0.0026	794.3791	794.3883	0.0092	406.6985		
C	9	944.4254	944.4099	-0.0156	972.4203	972.4509	0.0306	954.4098	954.422	0.0122	486.7138		
T	10	1045.4731			1073.468			1055.4575			537.2376		
V	11	1144.5415	1144.5302	-0.0113	1172.5364			1154.5259	1154.5135	-0.0124	586.7719		
G	12	1201.563			1229.5579	1229.5873	0.0294	1211.5473			615.2826		
G	13	1258.5844			1286.5794			1268.5688			643.7933		
E	14	1387.627			1415.6219			1397.6114			708.3146		
H	15	1524.6859			1552.6809			1534.6703			776.8441		
S	16	1691.6843			1719.6792			1701.6686			860.3432		
T	17	1872.6983			1900.6932			1882.6827			950.8502		
F	18	2019.7667			2047.7616			2029.7511			1024.3845		
S	19	2106.7987			2134.7937			2116.7831			1067.9005		
M	20	2237.8392			2265.8341			2247.8236			1133.4207		
A	21	2308.8763			2336.8713			2318.8607			1168.9393		
I	22	2421.9604			2449.9553			2431.9447			1225.4813		
E	23	2551.003			2578.9979			2560.9873			1290.0026		
P	24	2648.0557			2676.0507			2658.0401			1338.529		
G	25	2705.0772			2733.0721			2715.0616			1367.0397		
R	26												

Supplementary Table 22: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)T(phospho)FSMAIEPGR doubly phosphorylated at positions S284 and T285.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2779.1252			1390.0662			25
A	2708.0881			1354.5477	1354.5805	0.0328	24
E	2637.051			1319.0291			23
Q	2508.0084			1254.5078	1254.5352	0.0274	22
G	2379.9498			1190.4786	1190.4955	0.017	21
Q	2322.9284			1161.9678			20
V	2194.8698			1097.9385			19
C	2095.8014			1048.4043	1048.4329	0.0286	18
T	1935.7707			968.389	968.4002	0.0112	17
V	1834.7231			917.8652			16
G	1735.6546			868.331	868.3379	0.0069	15
G	1678.6332			839.8202			14
E	1621.6117			811.3095			13
H	1492.5691			746.7882	746.7955	0.0073	12
S	1355.5102			678.2587			11
T	1188.5119			594.7596			10
F	1007.4979			504.2526			9
S	860.4294	860.4333	0.0039	430.7184			8
M	773.3974	773.3897	-0.0077	387.2023			7
A	642.3569	642.3648	0.0079	321.6821			6
I	571.3198	571.3242	0.0044	286.1635			5
E	458.2358	458.2394	0.0036	229.6215			4
P	329.1932	329.1958	0.0026	165.1002			3
G	232.1404			116.5738			2
R	175.119	175.1206	0.0017	88.0631			1

Supplementary Table 23: y ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)T(phospho)FSMAIEPGR doubly phosphorylated at positions S284 and T285.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659	129.0657	-0.0002	111.0553			65.0366		
A	2	172.1081	172.1107	0.0027	200.103	200.1061	0.0031	182.0924	182.0952	0.0028	100.5551		
A	3	243.1452	243.1444	-0.0008	271.1401	271.1424	0.0023	253.1295	253.132	0.0025	136.0737		
E	4	372.1878			400.1827	400.1854	0.0027	382.1721	382.179	0.0069	200.595		
Q	5	500.2463			528.2412	528.2495	0.0083	510.2307	510.2407	0.01	264.6243		
G	6	557.2678			585.2627	585.2649	0.0022	567.2521	567.2658	0.0137	293.135		
Q	7	685.3264	685.3281	0.0017	713.3213	713.3295	0.0082	695.3107	695.3177	0.0069	357.1643		
V	8	784.3948	784.3993	0.0045	812.3897	812.3951	0.0054	794.3791	794.3836	0.0045	406.6985		
C	9	944.4254			972.4203			954.4098	954.4183	0.0085	486.7138		
T	10	1045.4731			1073.468			1055.4575			537.2376		
V	11	1144.5415			1172.5364			1154.5259	1154.4877	-0.0382	586.7719		
G	12	1201.563			1229.5579			1211.5473			615.2826		
G	13	1258.5844			1286.5794			1268.5688			643.7933		
E	14	1387.627			1415.6219			1397.6114			708.3146		
H	15	1524.6859			1552.6809			1534.6703			776.8441	776.8582	0.0141
S	16	1691.6843			1719.6792			1701.6686			860.3432		
T	17	1792.732			1820.7269			1802.7163			910.8671		
F	18	1939.8004			1967.7953			1949.7847			984.4013		
S	19	2106.7987			2134.7937			2116.7831			1067.9005		
M	20	2237.8392			2265.8341			2247.8236			1133.4207		
A	21	2308.8763			2336.8713			2318.8607			1168.9393		
I	22	2421.9604			2449.9553			2431.9447			1225.4813		
E	23	2551.003			2578.9979			2560.9873			1290.0026		
P	24	2648.0557			2676.0507			2658.0401			1338.529		
G	25	2705.0772			2733.0721			2715.0616			1367.0397		
R	26												

Supplementary Table 24: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFS(phospho)MAIEPGR doubly phosphorylated at positions S284 and S287.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2779.1252			1390.0662			25
A	2708.0881			1354.5477	1354.5592	0.0115	24
E	2637.051			1319.0291	1319.0398	0.0107	23
Q	2508.0084			1254.5078	1254.5241	0.0162	22
G	2379.9498			1190.4786	1190.4929	0.0144	21
Q	2322.9284			1161.9678	1161.9606	-0.0072	20
V	2194.8698			1097.9385	1097.9554	0.0168	19
C	2095.8014			1048.4043	1048.4169	0.0126	18
T	1935.7707			968.389	968.3962	0.0072	17
V	1834.7231			917.8652			16
G	1735.6546			868.331	868.3417	0.0107	15
G	1678.6332			839.8202			14
E	1621.6117			811.3095			13
H	1492.5691			746.7882	746.7923	0.0041	12
S	1355.5102			678.2587			11
T	1188.5119			594.7596			10
F	1087.4642	1087.4767	0.0125	544.2357			9
S	940.3958	940.4063	0.0105	470.7015			8
M	773.3974	773.4024	0.005	387.2023			7
A	642.3569	642.3655	0.0085	321.6821			6
I	571.3198	571.3263	0.0065	286.1635			5
E	458.2358	458.2404	0.0046	229.6215			4
P	329.1932	329.1931	-0.0001	165.1002			3
G	232.1404			116.5738			2
R	175.119	175.1181	-0.0008	88.0631			1

Supplementary Table 25: y ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFS(phospho)MAIEPGR doubly phosphorylated at positions S284 and S287.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta	b++ calc.	b++ obs.	b++ delta
Q	1	101.0709			129.0659			111.0553			65.0366		
A	2	172.1081	172.1105	0.0024	200.103	200.1057	0.0027	182.0924	182.0963	0.0039	100.5551		
A	3	243.1452	243.1437	-0.0015	271.1401	271.1407	0.0006	253.1295	253.1339	0.0044	136.0737		
E	4	372.1878			400.1827	400.1927	0.01	382.1721	382.1799	0.0078	200.595		
Q	5	500.2463			528.2412	528.2459	0.0046	510.2307	510.2421	0.0114	264.6243		
G	6	557.2678			585.2627	585.2587	-0.004	567.2521	567.2554	0.0033	293.135		
Q	7	685.3264			713.3213	713.3326	0.0113	695.3107	695.3184	0.0077	357.1643		
V	8	784.3948			812.3897	812.4002	0.0105	794.3791	794.3884	0.0093	406.6985		
C	9	944.4254			972.4203	972.4449	0.0245	954.4098	954.4325	0.0227	486.7138		
T	10	1045.4731			1073.468			1055.4575			537.2376		
V	11	1144.5415			1172.5364	1172.5553	0.0189	1154.5259			586.7719		
G	12	1201.563			1229.5579			1211.5473			615.2826		
G	13	1258.5844			1286.5794			1268.5688			643.7933		
E	14	1387.627			1415.6219			1397.6114			708.3146		
H	15	1524.6859			1552.6809			1534.6703			776.8441		
S	16	1611.718			1639.7129			1621.7023			820.3601	820.3514	-0.0087
T	17	1792.732			1820.7269			1802.7163			910.8671		
F	18	1939.8004			1967.7953			1949.7847			984.4013		
S	19	2106.7987			2134.7937			2116.7831			1067.9005		
M	20	2237.8392			2265.8341			2247.8236			1133.4207		
A	21	2308.8763			2336.8713			2318.8607			1168.9393		
I	22	2421.9604			2449.9553			2431.9447			1225.4813	1225.4528	-0.0285
E	23	2551.003			2578.9979			2560.9873			1290.0026		
P	24	2648.0557			2676.0507			2658.0401			1338.529		
G	25	2705.0772			2733.0721			2715.0616			1367.0397		
R	26												

Supplementary Table 26: a and b ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FS(phospho)MAIEPGR doubly phosphorylated at positions T285 and S287.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
Q							26
A	2779.1252			1390.0662			25
A	2708.0881			1354.5477	1354.5619	0.0142	24
E	2637.051			1319.0291			23
Q	2508.0084			1254.5078			22
G	2379.9498			1190.4786	1190.4959	0.0173	21
Q	2322.9284			1161.9678	1161.9631	-0.0048	20
V	2194.8698			1097.9385	1097.9552	0.0167	19
C	2095.8014			1048.4043	1048.4196	0.0153	18
T	1935.7707			968.389	968.3969	0.0078	17
V	1834.7231			917.8652			16
G	1735.6546			868.331			15
G	1678.6332			839.8202			14
E	1621.6117			811.3095			13
H	1492.5691			746.7882	746.7946	0.0064	12
S	1355.5102			678.2587			11
T	1268.4782			634.7427			10
F	1087.4642			544.2357			9
S	940.3958	940.4109	0.0151	470.7015			8
M	773.3974	773.4088	0.0114	387.2023			7
A	642.3569	642.3699	0.013	321.6821	321.682	-0.0001	6
I	571.3198	571.3275	0.0077	286.1635			5
E	458.2358	458.2412	0.0054	229.6215			4
P	329.1932	329.1928	-0.0004	165.1002			3
G	232.1404	232.1403	-0.0001	116.5738			2
R	175.119	175.119	0.0001	88.0631			1

Supplementary Table 27: y ions observed for peptide QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FS(phospho)MAIEPGR doubly phosphorylated at positions T285 and S287.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta
R	1	129.1135			157.1084	157.1108	0.0024	139.0978		
P	2	226.1662			254.1611	254.1626	0.0015	236.1506		
A	3	297.2033	297.205	0.0017	325.1983	325.2027	0.0044	307.1877		
F	4	444.2718	444.2782	0.0064	472.2667	472.2687	0.002	454.2561		
S	5	611.2701			639.265			621.2545	621.2602	0.0057
R	6									

Supplementary Table 28: a and b ions observed for peptide RPAFS(Phospho)R phosphorylated at position S396.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.		#
R				6
P	657.2756			5
A	560.2228			4
F	489.1857			3
S	342.1173	342.1222	0.0049	2
R	175.119	175.1213	0.0023	1

Supplementary Table 29: y ions observed for peptide RPAFS(Phospho)R phosphorylated at position S396.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	b calc.	b obs.	b delta
K	1	129.1022		
A	2	200.1393	200.1416	0.0023
D	3	315.1663	315.1695	0.0032
S	4	482.1646		
V	5	581.2331	581.2353	0.0022
S	6	748.2314	748.2365	0.0051
S	7	915.2298		
N	8	1029.2727		
H	9	1166.3316		
T	10	1267.3793		
L	11	1380.4634		
S	12	1467.4954		
S	13	1554.5274		
N	14	1668.5703		
A	15	1739.6075		
T	16	1840.6551		
R	17			

Supplementary Table 30: a and b ions observed for peptide KADS(Phospho)VS(Phospho)S(Phospho) NHTLSSNATR phosphorylated at positions S401, S403, S404.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
K							17
A	1886.6719			943.8396			16
D	1815.6347			908.321			15
S	1700.6078			850.8075			14
V	1533.6094			767.3084	767.2994	-0.009	13
S	1434.541			717.7742			12
S	1267.5427			634.275			11
N	1100.5443			550.7758			10
H	986.5014			493.7543			9
T	849.4425	849.4508	0.0083	425.2249			8
L	748.3948	748.4012	0.0064	374.701			7
S	635.3107	635.3228	0.0121	318.159			6
S	548.2787	548.2821	0.0034	274.643			5
N	461.2467	461.2558	0.0091	231.127			4
A	347.2037	347.1949	-0.0088	174.1055			3
T	276.1666			138.587			2
R	175.119			88.0631			1

Supplementary Table 31: y ions observed for peptide KADS(Phospho)VS(Phospho)S(Phospho)NHTLSSNATR phosphorylated at positions S401, S403, S404.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta
K	1	101.1073			129.1022			111.0917		
A	2	172.1444			200.1393			182.1288		
D	3	287.1714			315.1663	315.1667	0.0004	297.1557		
S	4	454.1697			482.1646			464.1541	464.1435	-0.0106
V	5	553.2381			581.2331	581.2408	0.0077	563.2225		
S	6	640.2702			668.2651	668.2877	0.0226	650.2545		
S	7	807.2685			835.2634			817.2529	817.271	0.0182
N	8	921.3115	921.2792	-0.0323	949.3064			931.2958	931.3236	0.0277
H	9	1058.3704			1086.3653			1068.3547		
T	10	1239.3844			1267.3793			1249.3687		
L	11	1352.4684			1380.4634			1362.4528		
S	12	1439.5005			1467.4954			1449.4848		
S	13	1526.5325			1554.5274			1536.5169		
N	14	1640.5754			1668.5703			1650.5598		
A	15	1711.6125			1739.6075			1721.5969		
T	16	1812.6602			1840.6551			1822.6446		
R	17									

Supplementary Table 32: a and b ions observed for peptide KADS(Phospho)VSS(Phospho)NHT(Phospho)LSSNATR phosphorylated at positions S401, S404, T407.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	#
K				1
A	1886.6719			2
D	1815.6347			3
S	1700.6078			4
V	1533.6094			5
S	1434.541			6
S	1347.509			7
N	1180.5106			8
H	1066.4677			9
T	929.4088			10
L	748.3948	748.3926	-0.0022	11
S	635.3107	635.3201	0.0094	12
S	548.2787	548.2857	0.007	13
N	461.2467			14
A	347.2037			15
T	276.1666			16
R	175.119	175.1204	0.0015	17

Supplementary Table 33: y ions observed for peptide KADS(Phospho)VSS(Phospho)NHT(Phospho)LSSNATR phosphorylated at positions S401, S404, T407.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta
K	1	101.1073			129.1022		
A	2	172.1444			200.1393	200.1415	0.0022
D	3	287.1714	287.1777	0.0063	315.1663	315.1661	-0.0002
S	4	374.2034			402.1983		
V	5	473.2718			501.2667		
S	6	560.3038			588.2988		
S	7	647.3359			675.3308		
N	8	761.3788			789.3737	789.3704	-0.0033
H	9	898.4377	898.4133	-0.0244	926.4326		
T	10	999.4854			1027.4803		
L	11	1112.5695			1140.5644		
S	12	1199.6015			1227.5964		
S	13	1286.6335			1314.6284	1314.6677	0.0392
N	14	1401.6605			1429.6554	1429.6553	0
A	15	1472.6976			1500.6925		
T	16	1653.7116			1681.7065		
R	17						

Supplementary Table 34: a and b ions observed for peptide KADSVSSNHTLSSN(deamidated)AT(Phospho)R phosphorylated at position T416.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
K							1
A	1727.7232			864.3652			2
D	1656.6861			828.8467			3
S	1541.6592			771.3332	771.3499	0.0166	4
V	1454.6271			727.8172			5
S	1355.5587			678.283			6
S	1268.5267			634.767			7
N	1181.4947			591.251			8
H	1067.4517	1067.4101	-0.0416	534.2295			9
T	930.3928	930.4199	0.0271	465.7			10
L	829.3451	829.3625	0.0173	415.1762			11
S	716.2611			358.6342			12
S	629.229			315.1182			13
N	542.197			271.6021			14
A	427.1701			214.0887			15
T	356.133			178.5701			16
R	175.119	175.1233	0.0043	88.0631			17

Supplementary Table 35: y ions observed for peptide KADSVSSNHTLSSN(deamidated)AT(Phospho)R phosphorylated at position T416.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta
A	1	72.0444			54.0338		
D	2	187.0713	187.0725	0.0012	169.0608	169.0618	0.0011
S	3	274.1034			256.0928		
V	4	373.1718			355.1612		
S	5	540.1701			522.1596		
S	6	627.2022			609.1916		
N	7	741.2451			723.2345		
H	8	878.304	878.2988	-0.0052	860.2934		
T	9	979.3517			961.3411		
L	10	1092.4357			1074.4252		
S	11	1179.4678	1179.471	0.0032	1161.4572		
S	12	1266.4998			1248.4892		
N	13	1380.5427			1362.5322		
A	14	1451.5798			1433.5693		
T	15	1552.6275			1534.617		
R	16						

Supplementary Table 36: a and b ions observed for peptide KADSVS(Phospho)SNHTLSSNATR phosphorylated at position T403.

"Seq." denotes the residue in the peptide, "#" denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. "a/b/y calc." denotes the calculated m/z for the corresponding a/b/y ion. "a/b/y obs." denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. "a/b/y delta" denotes the deviation (in m/z) between the calculated and observed species. "b/y++" denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the "Seq" column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	#
A				16
D	1655.7021			15
S	1540.6751			14
V	1453.6431			13
S	1354.5747			12
S	1187.5763			11
N	1100.5443			10
H	986.5014	986.5099	0.0085	9
T	849.4425	849.4485	0.006	8
L	748.3948	748.4065	0.0117	7
S	635.3107	635.3124	0.0017	6
S	548.2787	548.2701	-0.0086	5
N	461.2467	461.2631	0.0164	4
A	347.2037			3
T	276.1666			2
R	175.119	175.1221	0.0031	1

Supplementary Table 37: y ions observed for peptide KADSVS(Phospho)SNHTLSSNATR phosphorylated at position T403.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	#	a calc.	a obs.	a delta	b calc.	b obs.	b delta	b-18 calc.	b-18 obs.	b-18 delta
A	1	44.0495			72.0444			54.0338		
D	2	159.0764			187.0713			169.0608		
S	3	246.1084	246.1119	0.0035	274.1034			256.0928	256.0952	0.0024
V	4	345.1769			373.1718			355.1612	355.1633	0.0021
S	5	432.2089			460.2038			442.1932	442.2038	0.0106
S	6	519.2409			547.2358			529.2253	529.2241	-0.0012
N	7	633.2838			661.2788	661.2918	0.0131	643.2682	643.269	0.0008
H	8	770.3428	770.3538	0.011	798.3377	798.3461	0.0085	780.3271	780.3277	0.0006
T	9	951.3568			979.3517	979.3701	0.0185	961.3411		
L	10	1064.4408			1092.4357	1092.4633	0.0276	1074.4252		
S	11	1151.4729			1179.4678			1161.4572		
S	12	1238.5049			1266.4998			1248.4892		
N	13	1352.5478			1380.5427			1362.5322		
A	14	1423.5849			1451.5798			1433.5693		
T	15	1524.6326			1552.6275			1534.617		
R	16									

Supplementary Table 38: a and b ions observed for peptide ADSVSSNHT(Phospho)LSSNATR phosphorylated at position T407.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.

Seq.	y calc.	y obs.	y delta	y++ calc.	y++ obs.	y++ delta	#
A							16
D	1655.7021			828.3547			15
S	1540.6751			770.8412	770.8494	0.0082	14
V	1453.6431			727.3252			13
S	1354.5747			677.791	677.8058	0.0148	12
S	1267.5427			634.275	634.2796	0.0046	11
N	1180.5106	1180.5476	0.037	590.759			10
H	1066.4677	1066.4948	0.027	533.7375			9
T	929.4088	929.4319	0.0231	465.208			8
L	748.3948	748.4001	0.0053	374.701			7
S	635.3107	635.3173	0.0066	318.159			6
S	548.2787	548.2837	0.005	274.643			5
N	461.2467	461.2489	0.0022	231.127			4
A	347.2037			174.1055			3
T	276.1666			138.587			2
R	175.119	175.1185	-0.0004	88.0631			1

Supplementary Table 39: y ions observed for peptide ADSVSSNHT(Phospho)LSSNATR phosphorylated at position T407.

“Seq.” denotes the residue in the peptide, “#” denotes the number from the beginning of counting fragment ions of that type. A letter shown in red denotes an assigned phosphosite. “a/b/y calc.” denotes the calculated m/z for the corresponding a/b/y ion. “a/b/y obs.” denotes the observed m/z for the ion, if observed. If a species is not observed the corresponding obs. cell will be empty. “a/b/y delta” denotes the deviation (in m/z) between the calculated and observed species. “b/y++” denotes double charged fragment ions, if not specified fragment ions are single charged. b-18 denotes an ion which has lost a water molecule. For clarity modifications are not explicitly listed in the “Seq” column, but explicitly stated in the table title.