

## Supplementary Information

for

### Structure of the Neurotensin Receptor 1 in complex with $\beta$ -arrestin 1

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## Content:

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Contains Supplementary Figure 1 that shows that NTSR1- $\beta$ arr1<sup>ACT</sup> complex samples contain diC8-PI(4,5)P2, Supplementary Figures 2 through 5 that show the spectra used for assignment of the GRK5 phosphosites in NTSR1 and Supplementary Figure 6 which shows the uncropped gel displayed in Extended Data Figure 3b. It also contains Supplementary Tables 1 through 7 which present information about crosslinks observed in the NTSR1- $\beta$ arr1<sup>ACT</sup> complex. There is one table for each band excised from the SDS-PAGE gel, two for global analysis (one for each protein) and one for mapping crosslinks. Lastly it contains Supplementary Tables 8 through 39 that enumerate the observed fragmentation ions that led to the phosphosite assignments presented in the text.

## List of Supplementary Items

**Supplementary Figure 1 – NTSR1- $\beta$ arr1<sup>ACT</sup> complex samples contain diC8-PI(4,5)P2**

**Supplementary Figure 2 – Spectra used for assignment of the GRK5 phosphosites in NTSR1**

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

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

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

**Supplementary Figure 6 – Uncropped gel from Extended Data Figure 3b**

**Supplementary Table 1 – NTSR1- $\beta$ arr1<sup>ACT</sup> complex sample, SDS-PAGE band 1**

**Supplementary Table 2 – NTSR1- $\beta$ arr1<sup>ACT</sup> complex sample, SDS-PAGE band 2**

**Supplementary Table 3 – NTSR1- $\beta$ arr1<sup>ACT</sup> complex sample, SDS-PAGE band 3**

**Supplementary Table 4 – NTSR1- $\beta$ arr1<sup>ACT</sup> complex sample, SDS-PAGE band 4**

**Supplementary Table 5 – Global analysis of modified residues in  $\beta$ arr1**

**Supplementary Table 6 – Global analysis of modified residues in NTSR1**

**Supplementary Table 7 – Mapped crosslinks in NTSR1- $\beta$ arr1<sup>ACT</sup>**

**Supplementary Table 8.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FSMAIEPGR bearing pT285.

**Supplementary Table 9.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FSMAIEPGR bearing pT285.

**Supplementary Table 10.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHSTFS(phospho)MAIEPGR bearing pS287.

**Supplementary Table 11.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHSTFS(phospho)MAIEPGR bearing pS287.

**Supplementary Table 12.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFSMAIEPGR bearing pS284.

**Supplementary Table 13.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFSMAIEPGR bearing pS284.

**Supplementary Table 14.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFSMAIEPGR bearing pT278.

**Supplementary Table 15.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFSMAIEPGR bearing pT278.

**Supplementary Table 16.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHS(phospho)TFSMAIEPGR doubly phosphorylated at positions T278 and S284.

**Supplementary Table 17.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHS(phospho)TFSMAIEPGR doubly phosphorylated at positions T278 and S284.

**Supplementary Table 18.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHST(phospho)FSMAIEPGR doubly phosphorylated at positions T278 and T285.

**Supplementary Table 19.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHST(phospho)FSMAIEPGR doubly phosphorylated at positions T278 and T285.

**Supplementary Table 20.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFS(phospho)MAIEPGR doubly phosphorylated at positions T278 and S287.

**Supplementary Table 21.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)T(phospho)VGGEHSTFS(phospho)MAIEPGR doubly phosphorylated at positions T278 and S287.

**Supplementary Table 22.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)T(phospho)FSMAIEPGR doubly phosphorylated at positions S284 and T285.

**Supplementary Table 23.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)T(phospho)FSMAIEPGR doubly phosphorylated at positions S284 and T285.

**Supplementary Table 24.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFS(phospho)MAIEPGR doubly phosphorylated at positions S284 and S287.

**Supplementary Table 25.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHS(phospho)TFS(phospho)MAIEPGR doubly phosphorylated at positions S284 and S287.

**Supplementary Table 26.** a and b ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FS(phospho)MAIEPGR doubly phosphorylated at positions T285 and S287.

**Supplementary Table 27.** y ions observed for peptide

QAAEQGQVC(carbamidomethyl)TVGGEHST(phospho)FS(phospho)MAIEPGR doubly phosphorylated at positions T285 and S287.

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

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

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

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

**Supplementary Table 32.** a and b ions observed for peptide

KADS(Phospho)VSS(Phospho)NHT(Phospho)LSSNATR phosphorylated at positions S401, S404, T407.

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

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

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

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

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

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

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