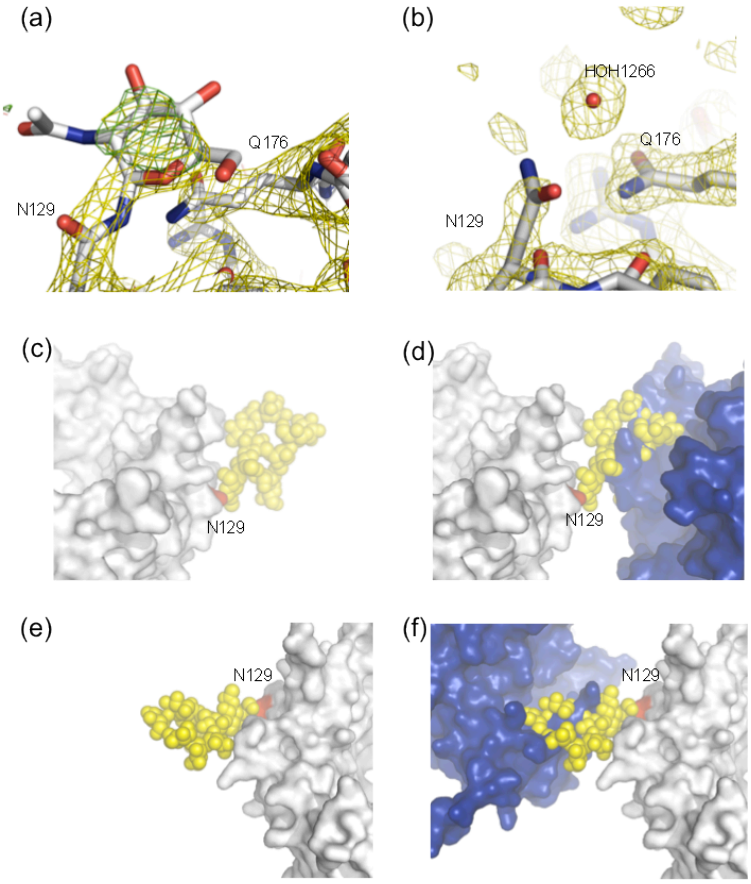
Supplementary Material

Peptide	Observed mass	Calculated mass	Comments
Asp^{123}-Arg^{134}(Asn^{129})	(1434.86) 1435.85 1437.83	1434.75	Asn^{129} is observed to be N-glycosylated, however a very minor fraction is observed unmodified (=mass in parenthesis)
Leu^{63}-Arg^{76} (Asn^{65})	(1679.83) 1680.81 1682.82	1679.82	Asn^{65} is observed to be N-glycosylated, however a very minor fraction is observed unmodified (=mass in parenthesis)
Asn^{423}-Lys^{438} (Asn^{423})	1873.85	1816.87 1873.87 (1 IAA)	Asn^{423} is non-glycosylated
Trp^{228}-Arg^{255} (Asn^{241})	2069.95 2071.95	2011.89 2068.89 (1 IAA)	Asn^{241} seems to be fully N-glycosylated
His^{360}-Arg^{378} (Asn^{373})	(2113.09) 2114.03 2116.04	2113.01	Asn^{373} is observed to be N-glycosylated, however a very minor fraction is observed unmodified (=mass in parenthesis)
Gln^{629}-Lys^{654} (Asn^{651})	(3213.33) 3214.52 3216.55	3042.33 3213.45 (3 IAA)	Asn^{651} is observed to be N-glycosylated, however a very minor fraction is observed unmodified (=mass in parenthesis)
Ser^{547}-Lys^{572} (Asn^{549})	NOT OBSERVED	3087.48	We can clearly see from all the structures that Asn^{549} is glycosylated. We have no explanation as to why it could not be identified by MS.

Supplementary table 1.

MS results for mapping of glycosylation sites of human sortilin. The data has been arranged according to size of the analyzed peptides. See “Materials and Methods” for further details.

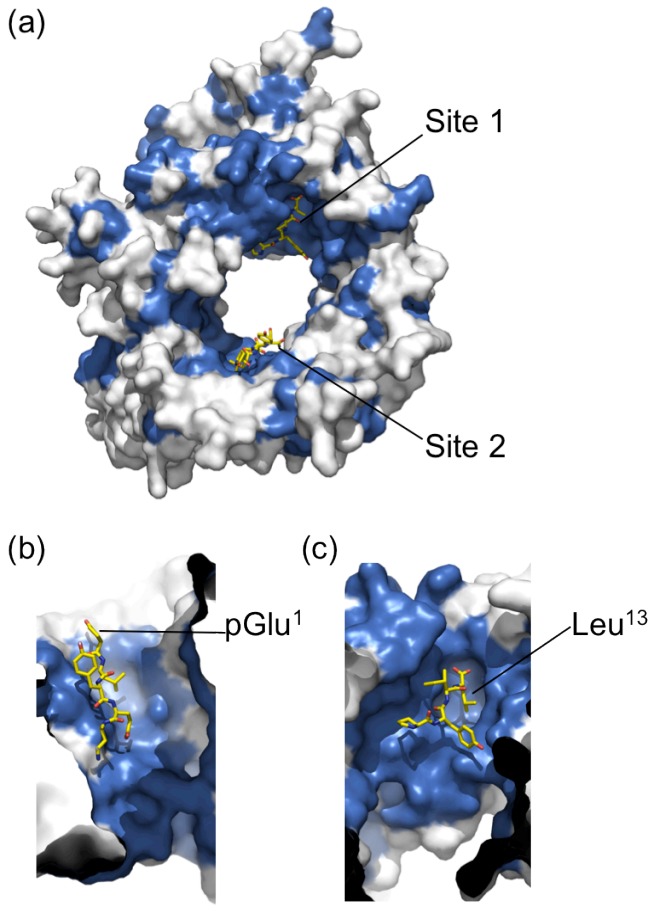


Supplementary figure 1.

The Asn129 glycosylation site. (**a)** The Asn129-linked glycosylation of the sSort:NT_{High} structure and its local surroundings are shown in sticks and coloured by atom type; C is grey, N is blue and O is red. The 2Fo-Fc electron density map contoured at 1σ is shown as a yellow wire mesh. The SA-omit map is shown in green wire mesh contoured at 3σ. (**b)** Asn129 and its surroundings in sSort:NT_{Low} are shown as in (a). Asn129 is apparently not glycosylated. Instead a water molecule is found within hydrogen bonding distance of the Asn129 side chain. **c)** sSort:NT_{Low} shown with an *in silico* modelled glycosylation at Asn129. sSortilin is shown in grey surface representation with Asn129 labelled and highlighted in red. The glycosylation is shown in a yellow space fill representation. **d)** Same as in c, but with a symmetry related molecule shown in blue surface representation. The modeled oligo-mannose glycosylation makes severe clashes with the 10CC-b domain of the symmetry related molecule. Note that 10CC is much farther away in the less compact crystal form (see fig. 1). **e-f)** Same as (c-d) but rotated 180° around a vertical axis.

Supplementary figure 2.

Multiple alignment of the Vps10 domains of human sortilin (without the signalpeptide and propeptide; residues 45-716), SorLA (without the signal- and propeptide; residues 82-761), SorCS1 (without the signal- and propeptide; residues 111-801), SorCS2 (without the signalpeptide; residues 51-784) and SorCS3 (without the signal- and propeptide; residues 134-825). Colored by conservation (threshold 30%) using the Zappo color scheme. The cysteines and glycosylation sites of sortilin as well as two key residues of binding site 1 (S283, R292) are labeled. The hydrophobic loop 97-108 and the hydrophobic tip of loop 557-563 are marked by red bars.



Supplementary figure 3.

Conservation of binding sites 1 and 2 mapped on the sSort:NT_{High} structure. The protein is shown in surface representation and is colored grey, except fully conserved residues, which are blue. Neurotensin is shown as sticks color coded according to residue type: carbon – yellow , oxygen – red , and nitrogen – blue. The calculation of conservation was done as described previously (Quistgaard et al. 2009), i.e. it is based on the multiple alignment of human sSortilin and seven vertebrate homologues, which is also represented in Fig. 2c. **a)** Cross-section showing that binding site 1 and a large area surrounding it is conserved. **b and c)** View from inside the tunnel of the conservation of binding sites 2 and 1 respectively.