Supplemental Data

Supplementary fig S1. **Functional characterization of recombinant HisS-tagged neurotrophin prodomains**.

(A) Coomassie staining of purified polypeptides of HisS-NGFpro (residues Glu^{1}- Arg^{102}) and HisS-BDNFpro (residues Ala^{1}-Arg^{110}) cloned into the pET-30 fXa/LIC vector (Novagen) that adds the two N-terminal poly-histidine and S-peptide tags (left panel). The presence of both tags is verified by Western blotting and detection by using a primary antibody against histidine followed by incubation with HRP-conjugated secondary antibody (middle panel) or with a directly HRP-conjugated version of S-protein (right panel).

(B) SPR analysis of the binding of concentration series (10, 20, 50, 100, 200, and 500 nM) of the recombinant pro-domains of NGF (upper panel) and BDNF (lower panel) to the immobilized extracellular domain of sortilin.

Supplementary fig S2. **Competitive binding of the NGF- and BDNF pro-domains to sortilin in SPR assay.**

A flow cell with immobilized sortilin was tested for three different ligand conditions as indicated. In one experiment, an initial injection of 100 nM (at 200sec) is continued by a similar second injection of more HisS-NGFpro at 700sec, whereas another experiment the second injection is changed to a mixture of 100 nM HisS-NGFpro together with 1 μM of HisS-BDNFpro. Both conditions led to a similar ligand association equal to 375 RU, thus suggesting that the presence of bound HisS-NGFpro preoccupy further association of the HisS-BDNFpro domain. As a positive control for the interaction with HisS-BDNF, a third experiment with an initial injection of buffer and subsequent test of 1 μM of HisS-BDNFpro at 700 sec shows that this amount of ligand on its own lead to an increase of 150 RU.

Supplementary fig S3. Single residues sortilin mutants.

(A) Silverstained non-reducing SDS-PAGE analysis of equal amounts of sortilin domain single residue mutants after purification by Ni^{2+}-NTA chromatography.

(B) Similar amount for each mutant protein was immobilized onto a biosensor chip and tested for binding to GST-NGFpro as compared to the wild-type (WT) sortilin domain. An identical concentration series of 10, 20, 30, 40, 50 nM of the GST-NGFpro was applied for each mutant, though showing very similar binding behavior. Affinities were estimated using the biaevaluation software, using the 1:1 Langmuir binding isotherm, and the kinetics are provided in table 2.

(C) CD analysis for sortilin proteins WT (black), R163A (blue), F165A (green), R166A (grey), F170A (cyan), K172A (yellow), and 4A (red) in the range of 200 to 260 nm expressed in ellipticity (millidegrees) with all spectra plotted together.

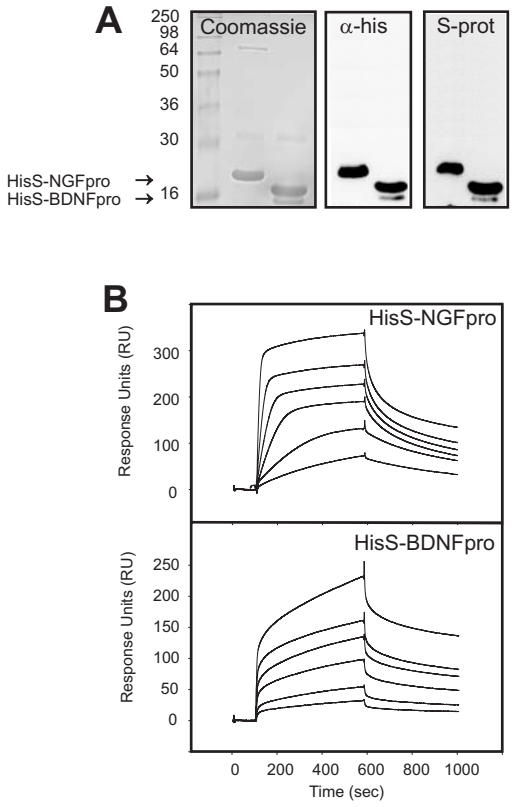

Figure S1
Andersen *et al*.

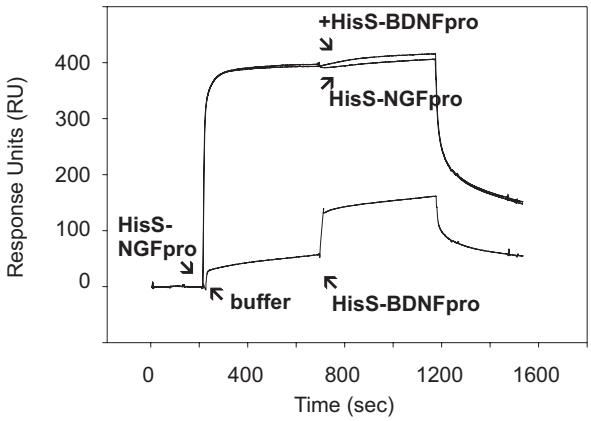

Figure S2
Andersen *et al*.

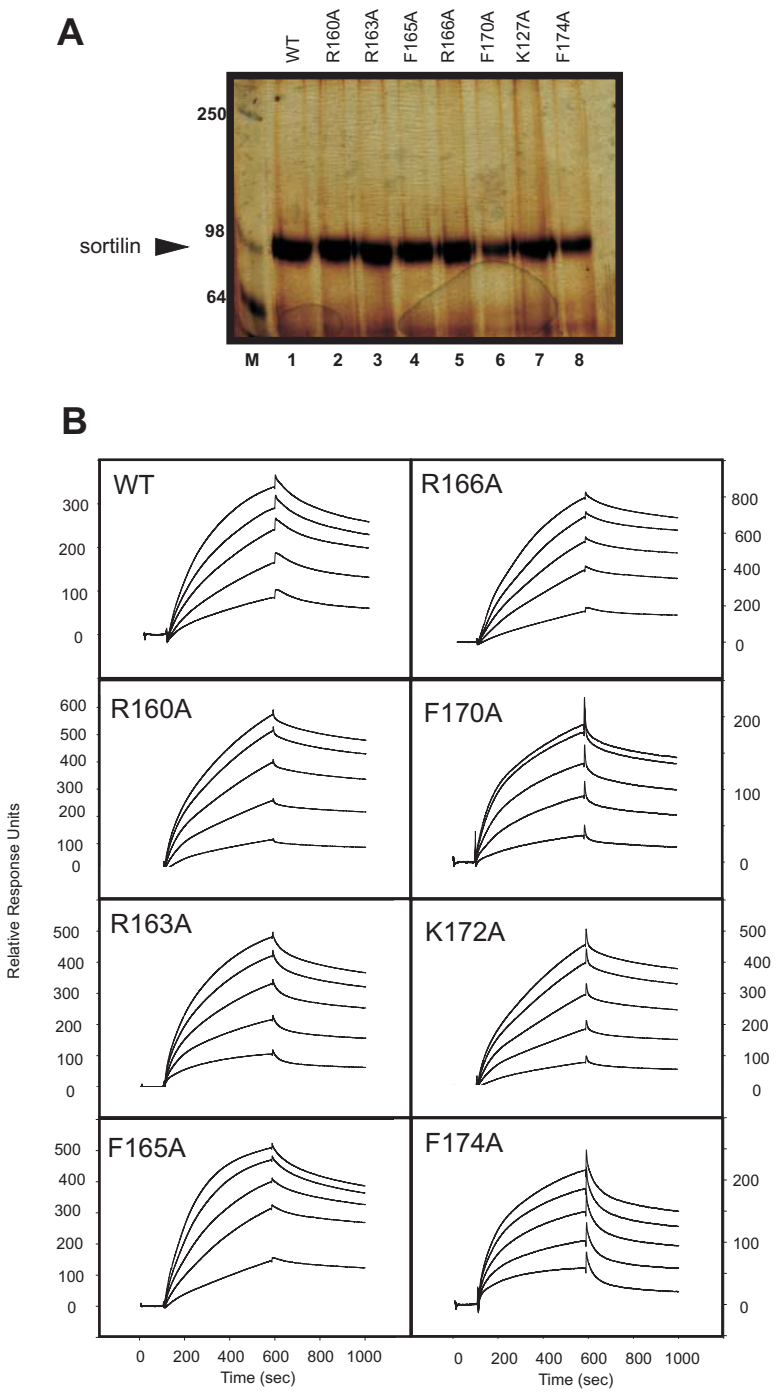

Figure S3
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