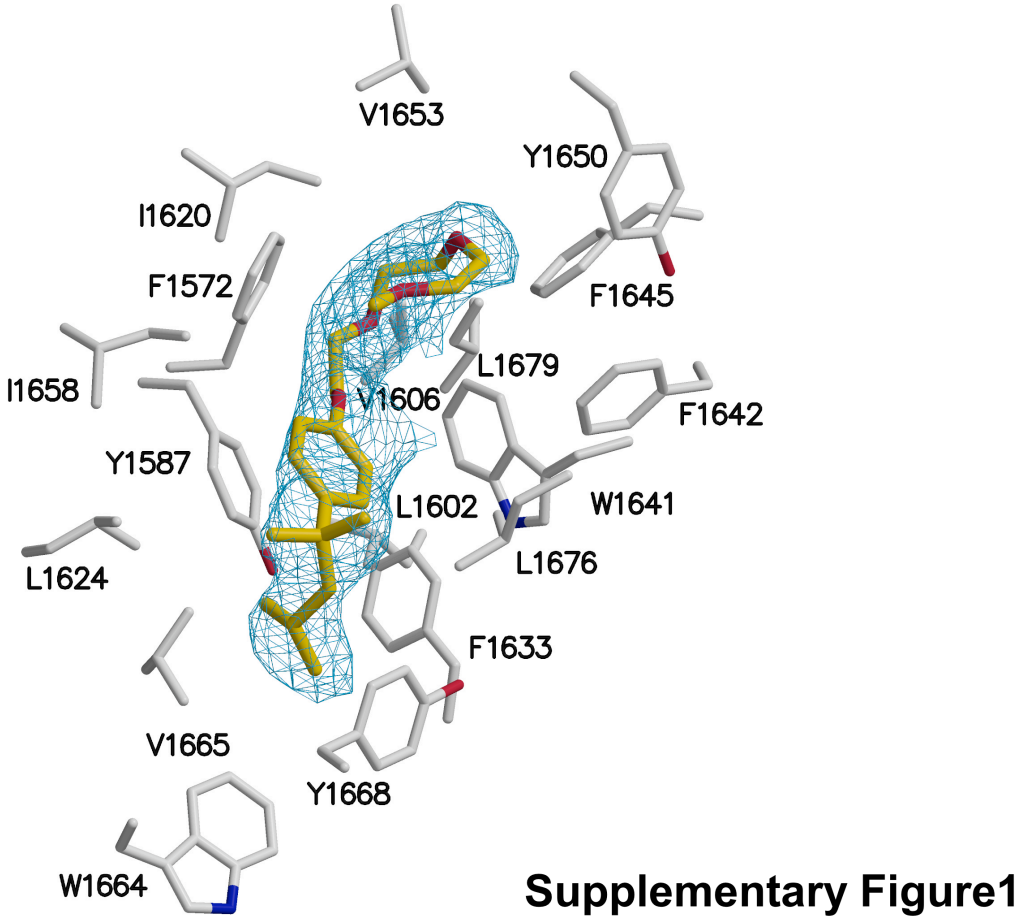
Supplementary Material 1:

Properties of the lipid binding cavity.

The lipid binding cavity in our structure is lined by hydrophobic residues and has a volume of 1,300 Å^3, spanning 17 Å to 21 Å on its longest side similar like in other Sec14p-like proteins (Min et al., 2003; Sha et al., 1998), but with no splitting of the lipid binding volume that has been observed in Supernatant Protein Factor (Stocker and Baumann, 2003; Stocker et al., 2002). The detergent molecule adopts a bent conformation (Supplementary Fig. 1), similarly as seen in α-Tocopherol Transfer Protein (Meier et al., 2003; Min et al., 2003) and presumably occupies the position of a functional ligand.



Supplementary Figure 1. Protein-ligand interaction for the bound Triton X-100. The detergent ligand is bound by extensive Van der Waals contacts through hydrophobic residues lining the Sec14p-like domain of neurofibromin. The polar tail of the detergent assumes a self-stabilizing bent conformation. A segment of 2Fo-Fc electron density (contoured at 1σ) is shown.

Supplementary Material 2:

For heavy atom derivatization the crystals were transferred for 8 hours to a stabilizing solution of mother liquor containing 1 mM $HgCl_2$ or K_2PtCl_4. Datasets from native and heavy atom treated crystals were collected under cryogenic conditions (100K) at the European Synchrotron Radiation Facility (ESRF, Beamline ID14-2). Data were processed using MOSFLM (v.6.2.2) (Collaborative Computational Project, 1994) or XDS (Kabsch, 1993) (Supplementary Table 1).

Structure solution of the wild-type protein crystals was performed using the CNS suite (Brunger et al., 1998). MIRAS phases were calculated in the 15-2.8 Å resolution range and improved by density modification algorithms as implemented in CNS, resulting in an electron density map, which was readily interpretable. The model was built using the program O (Jones and Kjeldgaard, 1997) and refined with CNS and REFMAC (Collaborative Computational Project, 1994). Figures were generated with the program POVSCRIPT (Fenn et al, 2003a).

Supplementary Material 3:

Purification of Glutathione-S-Transferase (GST) fused NF1-PH. An NF1-PH construct including part of the linker region was cloned into pGEX4T (AP biotech) vector, providing an N- terminal GST fusion. Expression was carried out in BL21(DE3)-Codon-plus-RIL (Novagen) cells, as described (Bonneau et al., 2004).

Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM Tris-HCl pH 8.5, 150 mM NaCl, 2 mM Di-Thio-Threitol) and lysed with an Emulsiflex (Avestin). The lysate was applied to a glutathione-Sepharose 4B column (Amersham

Supplementary Table 1: Summary of the Crystallographic Analysis

	Native	$HgCl_2$	K_2PtCl_4
Data Collection			
Soaking conditions	-	1 mM, 8 h	1 mM, 9 h
X-ray Source	ESRF ID14-2	ESRF ID14-2	ESRF ID14-2
Wavelength (Å)	0.933	0.933	0.933
Space Group	$P4_12_12$	$P4_12_12$	$P4_12_12$
Unit cell (Å, º)	a=b=113.5, c=125.6	a=b=113.4, c=127	a=b=112.9, c=125.6
Resolution (Å)	2.3	2.9	2.8
Highest shell (Å)	2.41-2.3	3.06-2.9	2.95-2.8
#Observations	527,844 (68,781)	115,156 (17,011)	195,431 (28,562)
Unique reflections	45,084 (5,784)	18,296 (2,624)	20,674 (2,951)
I/σ	5 (1.8)	6.3 (2.8)	5.8 (2)
R_{sym} (%)[1]	8.6 (34)	7 (28)	8.6 (29.6)
Completeness (%)	96 (100)	99.6 (100)	99.5 (100)
MIRAS Analysis			
Resolution range (Å)	15-2.8	15-2.8	15-3
Riso (%)[2]	-	21.2	15.4
# sites	-	6	2
Phasing power: MIR/anomalous[3]	-	0.97/1.2	0.55/0.47
Figure of merit of final map[3]	0.52	-	-
Refinement			
Resolution range (Å)	20-2.3	-	-
#reflections	34,716	-	-
R_{work}/R_{free} (%)[3]	22.5/27.2	-	-
#Atoms:		-	-
Protein	4,142	-	-
Solvent	113	-	-
Triton X-100	24	-	-
PP_i	9	-	-

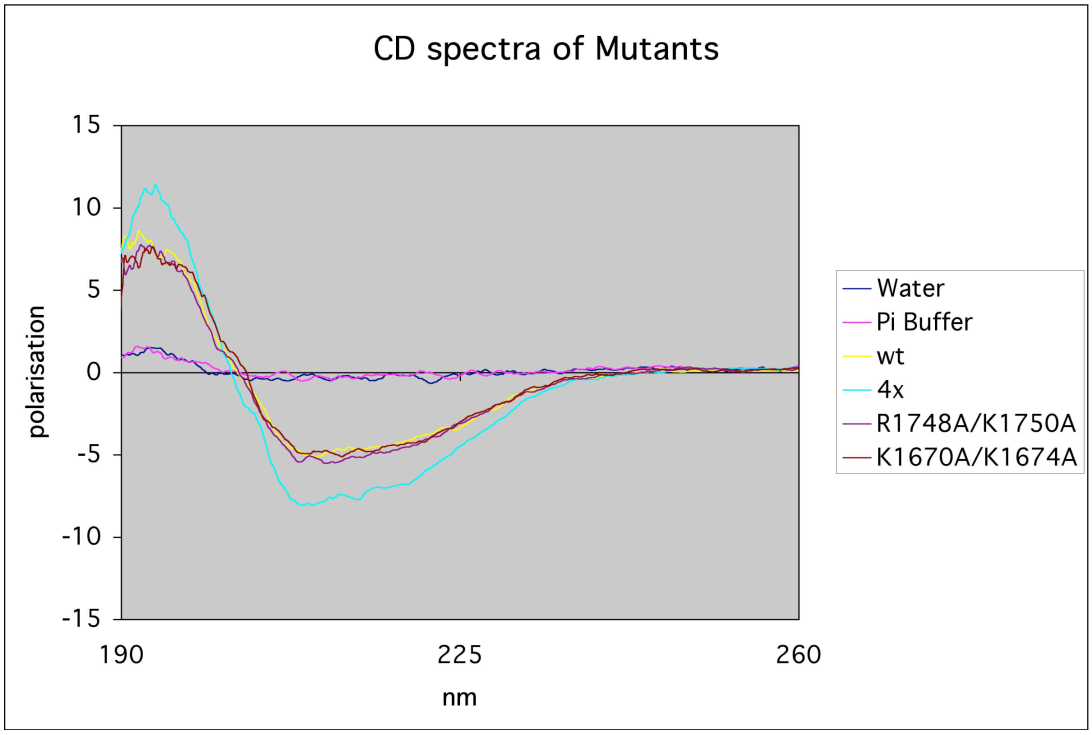
[1], as defined in MOSFLM (Collaborative Computational Project, 1994) or XDS (Kabsch, 1993)
[2], as defined in SCALA (Collaborative Computational Project, 1994),
[3], as defined in CNS (Brunger et al., 1998).

Biosciences) and extensively washed with lysis buffer. The protein was eluted with lysis buffer containing 20 mM reduced glutathione and stored in aliquots at -70°C.

Site Directed Mutagenesis and Purification of Mutants. Site directed mutagenesis was performed using the Quickchange kit (Stratagene) following manufacturer instructions. Expression was carried out similarly as described (Bonneau et al., 2004). After cell lysis without Triton X-100 the lysate was cleared by centrifugation and applied to a 1 ml HisTrap Nickel affinity column (AP biotech). The eluate was dialyzed against 20 mM Tris-HCl pH 8, 150 mM NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol for 48 hours in the presence Tobacco Etch Virus (TEV) protease for His tag cleavage. The protein was then concentrated to 1 ml (using 10 kDa cutoff Vivaspin concentrators) and applied to a size exclusion chromatography column (Superdex 200 HiLoad 16/60, AP biotech) equilibrated with dialysis buffer. Fractions containing the protein were pooled and concentrated to 10 mg/ml. The quality of mutant proteins was confirmed using mass spectroscopy, analytical gel filtration, Circular Dichroism (CD, see Supplementary Figure 2) or Nuclear Magnetic Resonance (NMR) spectroscopy. The double deletion ΔI1658/ΔY1659 could not be produced as a soluble protein.

Antibodies. Anti-NF1-Sec-PH antibodies were raised in rabbits with the RiBi adjuvant system (Corixa) according to standard protocols using the recombinant protein as antigen. Purification was performed with two affinity chromatography steps: an initial matrix (CNBr sepharose, AP biotech) was chemically linked to GST for clearing the serum of GST binders; a second matrix was linked to GST-NF1-Sec-PH for antibody binding. Antibodies were eluted with 200 mM glycine, 150 mM NaCl, pH 2.3 followed by immediate neutralization using 2 M Tris HCl, pH 9. The quality of the antibodies was assessed by western blotting using standard protocols

Suppl. Figure 2



Supplementary Figure 2: Circular dichroism (CD) analysis of wildtype and mutated NF1-Sec-PH showing proper folding for all proteins used in membrane overlay experiments. Spectra were recorded in the far UV range (260-190 nm) using a JASCO-710 temperature-controlled spectropolarimeter. All experiments were performed at RT with a protein concentration of 0.5 to 2 mg/ml in 5 mM PBS with pH corresponding to the one used during membrane overlay experiments (pH 6). The buffer baseline is also shown.

(Sambrook and Russell, 2001). GST directed antibodies (coupled to horse radish peroxidase, HRP, AP biotech) were applied according to manufacturer's instructions.

Supplementary Material 4

The strips were blocked for 1 hour in Buffer A: Phosphate Buffered Saline (PBS) pH 6 + 0.1% Tween 20® + 3% fatty acid free Bovine Serum Albumine (BSA, Sigma) and

then incubated at R/T or 4C with 1 µg/ml protein (10 µg) in Buffer A for 3 hours. A good signal was observed only when using PBS, with a pH value adjusted to 6. Washes were performed in Buffer B (as Buffer A but without BSA).

After 10 washing steps of 5 minutes each, the strips were incubated for 1 hour in buffer A with 1:10,000 Rabbit Anti-NF1-Sec-PH antibodies followed by washing steps (2x rinsing, 1x 1 hour, 3x 10 minutes in buffer B). Finally, the strips were incubated for 30 minutes with 1:5,000 goat HRP-coupled anti-rabbit antibodies (sigma) in buffer A and again washed (3x rinsing, 1x 1 hour, 4x 5 minutes in buffer B). Protein binding was detected by Enhanced Chemo-Luminescence (ECL), using the ECLplus kit (Amersham) following manufacturer's instructions. The assay was typically done at room temperature, but in some cases incubation at 4˚C considerably reduced the background. For the GST-fused NF1-PH construct detection was performed with HRP-coupled anti-GST antibody (Amersham).

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