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Supplemental material

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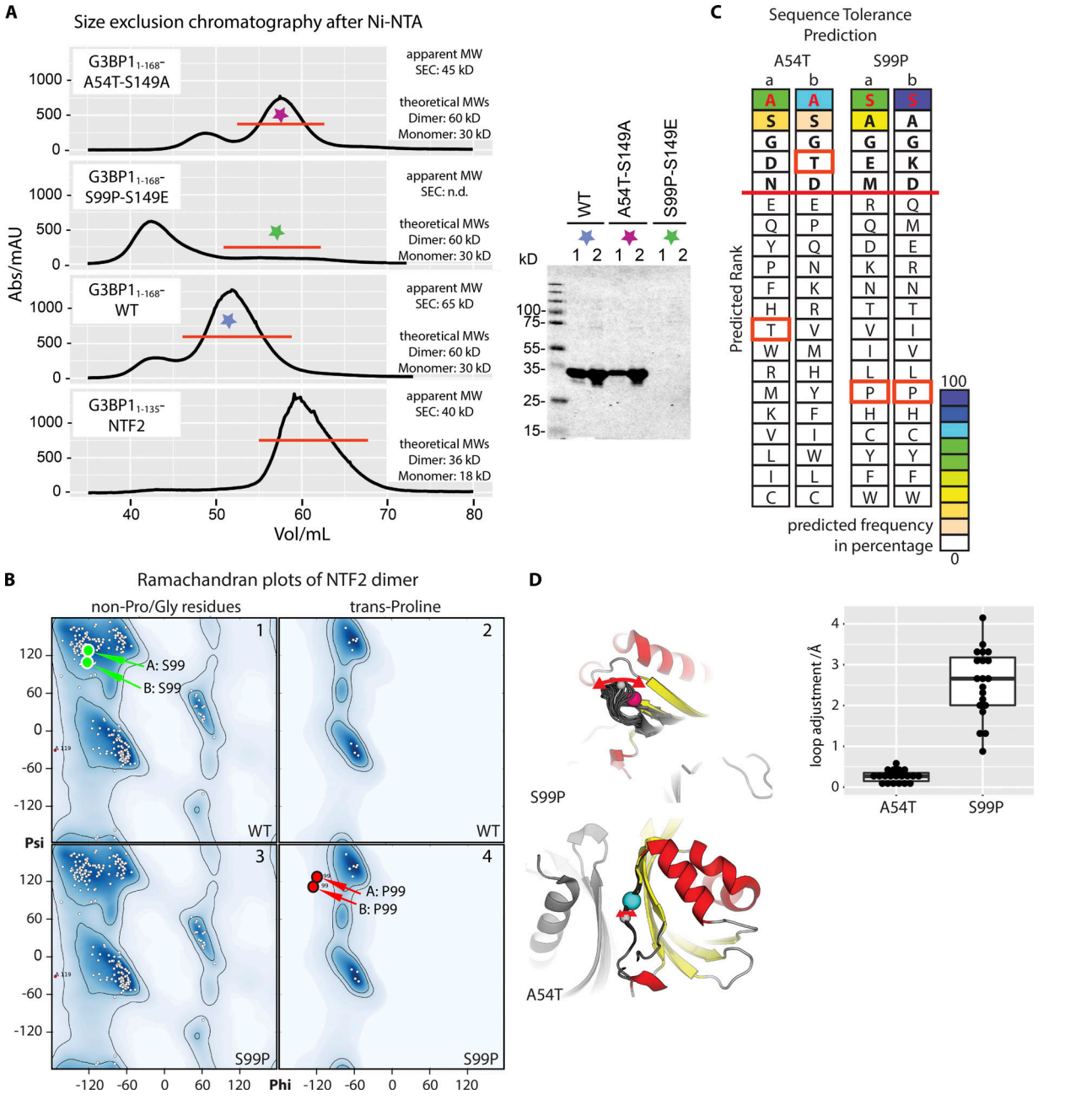

Figure S1. **The S99P mutation causes the aberrant behavior of the presumed S149E variant. (A)** Size exclusion and SDS-PAGE analysis of heterologously produced G3BP1$_{1-168}$ WT, S149A (unintentional A54T), and S149E (unintentional S99P) constructs. The size-exclusion chromatography profile of the NTF2-like G3BP1 domain (sequence-derived molecular weight [MW] is 18 kD) used for crystallization is shown for comparison. WT G3BP1$_{1-168}$ (30 kD) yielded a major peak at a retention volume of ∼52 ml, corresponding to an apparent molecular weight of 65 kD, in agreement with the theoretical size of the dimer. The S149A (A54T) mutant eluted at ∼58 ml corresponding to an apparent molecular weight of 45 kD, in between the monomer and dimer. For the S149E (S99P) construct, a major peak at the void volume of the column was obtained, indicating aggregated protein. SDS-PAGE analysis showed protein bands at the expected molecular weight of ∼30 kD for the combined major peak fractions of G3BP1-WT and S149A (A54T). We did not detect any S149E (S99P) protein in the indicated fractions that were chosen based on the purification of the WT and S149A (A54T) protein. The major "void" peak was not analyzed in SDS-PAGE. Numbers 1 and 2 indicate before and after concentrating the combined fractions using ultracentrifugation. **(B)** In silico substitution of Ser-99 to Pro in monomers A and B shifts the formerly favored Phi/Psi angles of Ser (top 1, green arrows) into disallowed regions in the proline Ramachandran plot (bottom 4, red arrows). **(C)** The ranked table of residues with their respective predicted frequencies illustrates that both WT residues at positions 54 and 99 are favored in the two chains of the NTF2 dimer (a and b). The red line indicates a cutoff of the top five amino acid choices at each position. While threonine (red boxes) appears among the five highest ranked residues in one chain of the dimer, proline at position 99 (red boxes) is listed at the lower end of the ranked table of both chains. **(D)** "Backrub"-modeled structures of S99P mutants reveal a 2.5-Å perturbation of the Ser-99–associated loop. The models of A54T are almost identical to the WT structure with perturbations in the range of 0.2 Å. Cartoons were drawn and color-coded as in Fig. 1 B. The perturbation of the loops was measured as point-to-point distances of the highlighted WT Cα-atom (gray sphere) to the respective atom in the mutant structures.

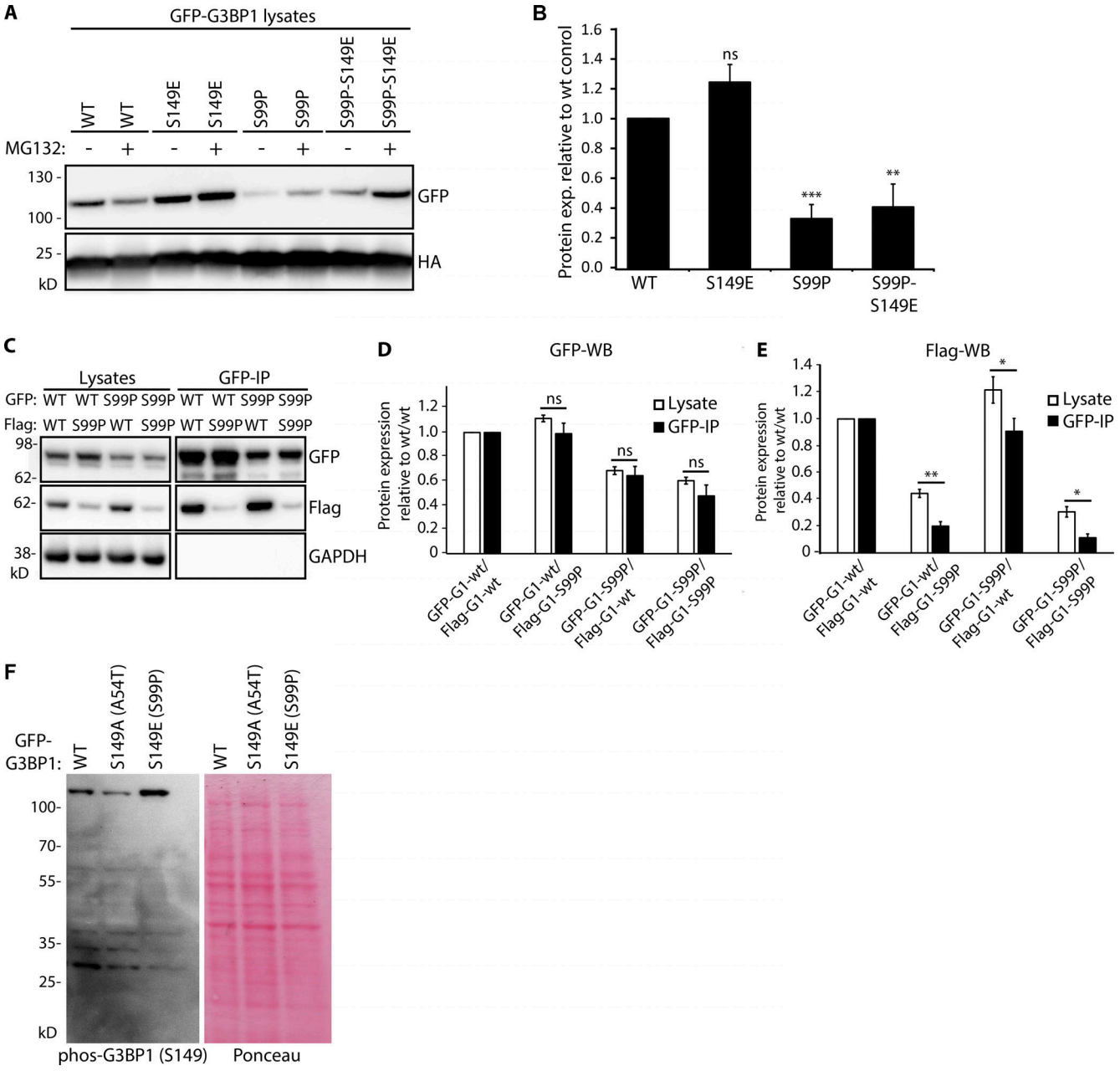

Figure S2. **S99P is less efficiently expressed and impaired in interacting with G3BP-WT and with itself. (A)** Transient coexpression of GFP-G3BP1 mutants with an HA-tagged reporter construct, untreated or treated with MG132 for 6 h, and blotted as indicated. **(B)** Coexpressed GFP-G3BP1 variants with an HA-tagged reporter, quantified by Western blot/densitometry for GFP and HA. GFP/HA expression was normalized relative to the WT, and plotted (mean ± SEM, *n* = 3). **(C)** Coexpression of indicated GFP-G3BP1 and Flag-G3BP1 constructs in ΔΔG3BP1/2 U2OS cells, immunoprecipitated with GFP-TRAP, and blotted as indicated. **(D)** GFP quantified from lysates and GFP IP Western blots using densitometry. Relative values to WT/WT cooverexpression were plotted. Mean ± SEM, *n* = 3. **(E)** Flag signal was quantified as in D (mean ± SEM, *n* = 3). **(F)** ΔG3BP1 U2OS cells stably expressing GFP-G3BP1-WT, S149A (A54T), and S149E (S99P) lysed and separated by SDS-PAGE, membrane-stained with Ponceau (right panel), and then blotted for phos-G3BP1-S149 (left panel; G8046; Sigma-Aldrich).

Table S1. **Primer sequences**

Mutation	Plasmid	Forward primer	Reverse primer
A54T	pEGFP-C1-G3BP1-WT	5′Phos-CCAGCAGATACTGTCTACGGACAGAAAG-3′	5′Phos-CTTTCCATTTGAATCCAATCCCCCATGGA-3′
S99P	pEGFP-C1-G3BP1-WT	5′Phos-GGGGCTTCTCCCTAACAACAACCAGG-3′	5′Phos-ATCACCTGGACTACCACACCATCATTTAGC-3′
S99P	pcDNA-Flag-G3BP1-WT	5′Phos-GGGGCTTCTCCCTAACAACAACCAGG-3′	5′Phos-ATCACCTGGACTACCACACCATCATTTAGC-3′
S149A	pEGFP-C1-G3BP1-A54T-S149A	5′Phos-CCAGCAGATGCAGTCTACGGACAGAAAG-3′	5′Phos-CTTTCCATTTGAATCCAATCCCCCATGGA-3′
S149E	pEGFP-C1-G3BP1-S99P-S149E	5′Phos-GGGGCTTCTCTCTAACAACAACCAGG-3′	5′Phos-ATCACCTGGACTACCACACCATCATTTAGC-3′

Table S2. **List of antibodies used in this study**

Antigen	Species	Catalog number (clone)	Source
Actin	Mouse	Sc-8432	Santa Cruz Biotechnology
Caprin1	Rabbit	15112-1-AP	Proteintech Group
eIF4G1	Rabbit	Sc-11373	Santa Cruz Biotechnology
eIF3b	Goat	Sc-16377 (N-20)	Santa Cruz Biotechnology
Flag	Mouse	F3165	Sigma-Aldrich
GAPDH	Mouse	Sc-47724	Santa Cruz Biotechnology
G3BP1 Epitope	Mouse	Sc-81940 (TT-Y) Recombinant hG3BP1	Santa Cruz Biotechnology
G3BP1 Epitope	Mouse	Sc-365338 (H-10) aa 158–251 hG3BP1	Santa Cruz Biotechnology
G3BP1 Epitope	Mouse	611126 aa 210–323 hG3BP1	BD Transduction Laboratories
phos-G3BP1-S149	Rabbit	G8046	Sigma-Aldrich
GFP	Rabbit	ab290	Abcam
GFP	Chicken	G160	ABM
HA	Mouse	MMS-101R	Covance
Sequestosome-1	Mouse	Sc-28359	Santa Cruz Biotechnology
TIA1	Goat	sc-1751	Santa Cruz Biotechnology
USP10	Rabbit	A300-900A	Bethyl Laboratories
USP10	Rabbit	A300-901A1	Bethyl Laboratories
Ubiquitin	Mouse	Sc-271289	Santa Cruz Biotechnology