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Supplemental information

A novel NF2 splicing mutant causes neurofibromatosis type 2 via liquid-liquid phase separation with large tumor suppressor and Hippo pathway

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Supplemental Items

Figure S1. Sequence alignment of NF2 protein in 15 organisms, related to Figure 1D-1F. The red box shows the deleted section of the NF2 protein. The green box indicates the five amino acid deletion in p.257-261.

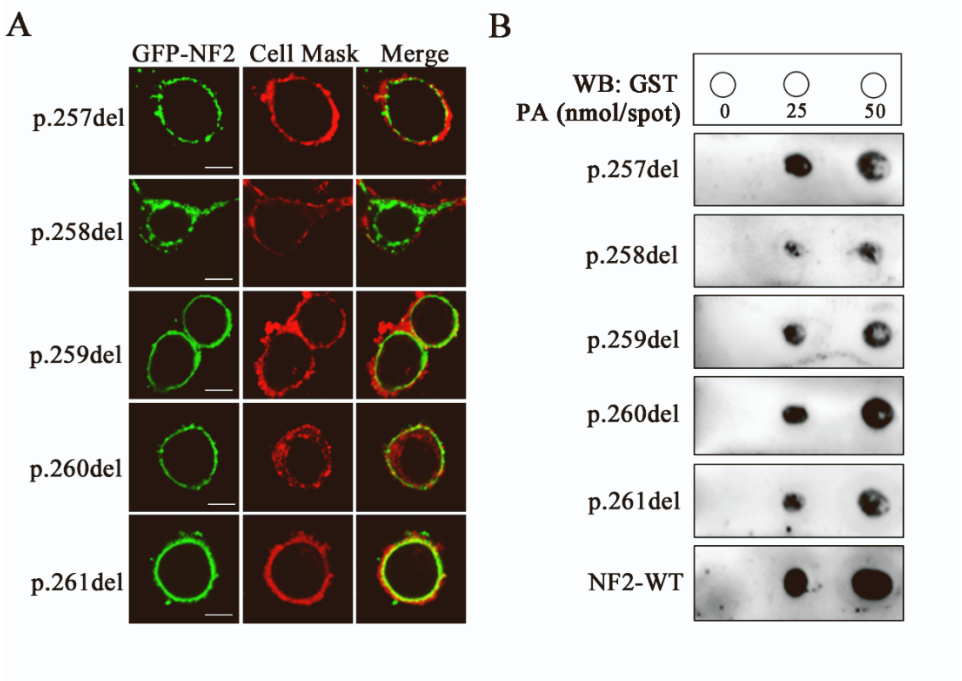

Figure S2. Effect of single amino acid residue deletion of NF2 at p.257-261 (Pro-Trp-Asn-Glu-Ile, respectively) on subcellular localization, related to Figure 2A and 2B. (A) HEK293T cells transfected with pEGFP-*NF2*-mut (p.257del, p.258del, p.259del, p.260del, p.261del) were labeled with CellMask™ plasma membrane stains. Fixed cells were immunostained as indicated. Scale Bar: 10μm. (B) Single amino acid residue deletion NF2 variant interact with PA. The purified NF2-mut (p.257del, p.258del, p.259del, p.260del, p.261del) proteins (0.5μg/mL) were subjected to the lipid dot-blot assay.

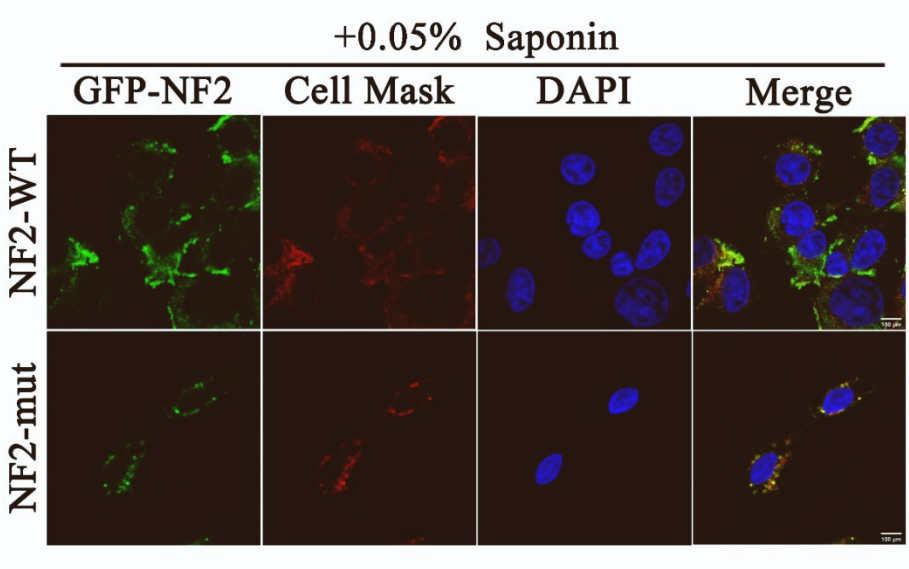

Figure S3. Effect of saponin treatment on NF2 puncta, related to Figure 2D. HEK293T HEK293T cells transfected with pEGFP-*NF2*-WT or pEGFP-*NF2*-mut were permeabilized with 0.05% saponin and analyzed by confocal microscopy.

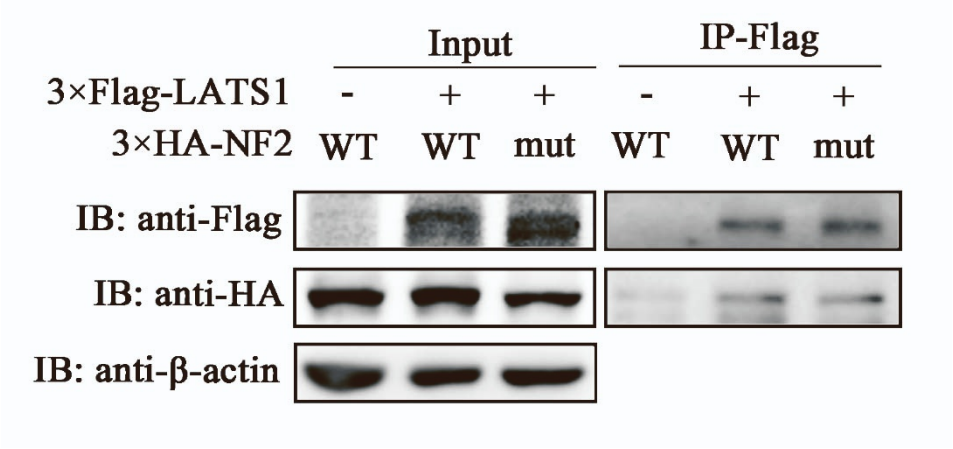

Figure S4. Co-IP assay on the interaction of LATS with NF2, related to Figure 3A. HEK293T cells transfected with p3×Flag-*LATS1* and p3×HA-*NF2* were immunoprecipitated with anti-Flag and immunoblotted as indicated.

Table S1. MD simulation systems in this study, related to Figure 1G-1M.

system	residues included	Initial size of the simulation box	total atoms	simulation time (ns)
wild-FERM	20-313	8.5×8.5×8.5 nm^3	61066	200
mutant-FERM	20-256, 262-313	8.5×8.5×8.5 nm^3	61026	200
Wild-Merlin	20-380, 459-595	9.3×9.3×9.3 nm^3	81037	200
Mutant-Merlin	20-256, 262-380, 459-595	9.3×9.3×9.3 nm^3	80953	200