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Supplementary Materials for

The ER structural protein Rtn4A stabilizes and enhances signaling through the receptor tyrosine kinase ErbB3

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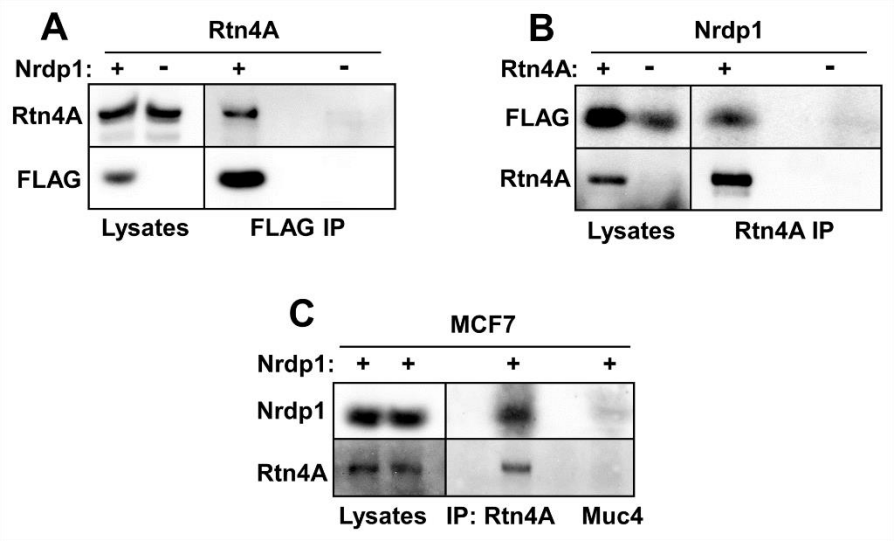

Fig. S1. Rtn4A physically interacts with Nrdp1.
There is no detectable endogenous Rtn4A or ERBB3 and only modest amounts of Nrdp1 in HEK293T human embryonic kidney cells (*13*). Because Nrdp1 is highly labile and undergoes very efficient autoubiquitination and degradation in many cell types (*3, 31, 69*), cells were treated overnight with 1.5 μM MG132 prior to immunoprecipitation to inhibit proteasomal degradation. **(A)** HEK293T cells were co-transfected with constructs encoding Rtn4A and either empty vector control (-) or FLAG-tagged Nrdp1 (+). Lysates from cells treated overnight with MG132 were immunoprecipitated with anti-FLAG, and lysates (left lanes) and precipitates (right lanes) were immunoblotted with antibodies recognizing FLAG and Rtn4A. **(B)** HEK293T cells were transfected constructs encoding Nrdp1 and either empty vector control (-) or FLAG-tagged Rtn4A (+). Lysates from cells treated overnight with MG132 were immunoprecipitated with anti-Rtn4A, and lysates and precipitates immunoblotted to detect Rtn4A and FLAG. **(C)** MCF7 cells were transfected with untagged Nrdp1 (+) and treated with MG132 overnight. Cell lysates were immunoprecipitated with anti-Rtn4A or an isotype-matched antibody to an unrelated protein (Muc4), and lysates and precipitates immunoblotted to detect Rtn4A and Nrdp1. Each panel represents at least three independent experiments.

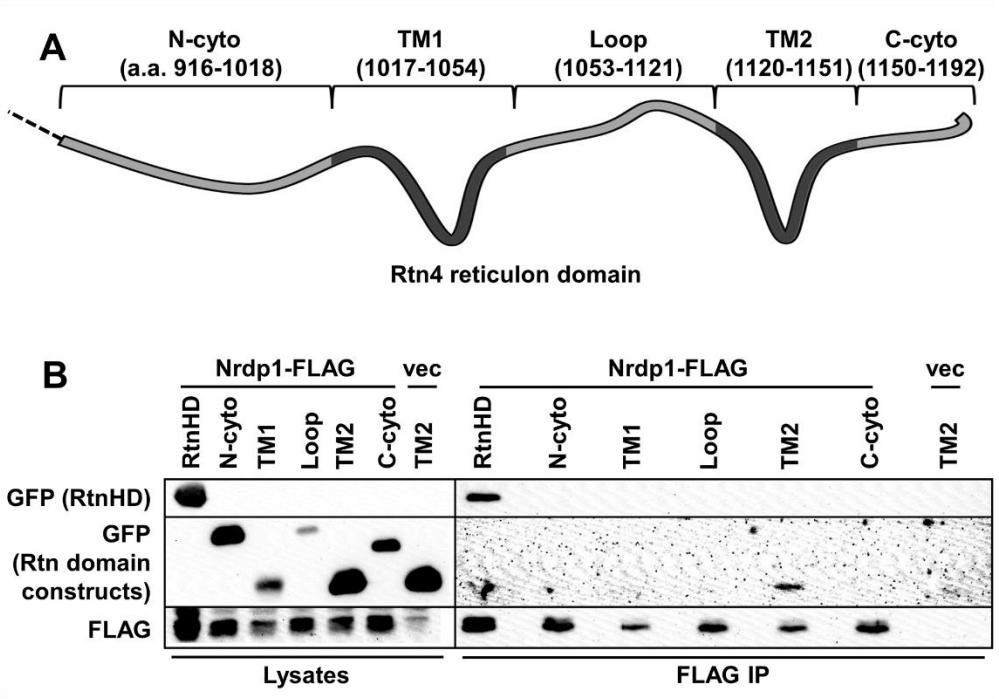

Fig. S2. Nrdp1 interacts with the second transmembrane domain of Rtn4A.
(A) A schematic of the reticulon domain of Rtn4 is illustrated, and the portions of the domain used in each reticulon domain construct is noted. All constructs are tagged with N-terminal GFP. **(B)** HEK293T cells were co-transfected with either vector control (vec) or FLAG-tagged Nrdp1 along with the constructs illustrated in panel A, as indicated, and treated overnight with 1.5 μM MG132 to allow Nrdp1 accumulation. Lysates (left lanes) were immunoprecipitated with anti-FLAG beads (right lanes), and lysates and precipitates were immunoblotted using antibodies that recognize FLAG and GFP. Panel B is representative of three independent experiments.

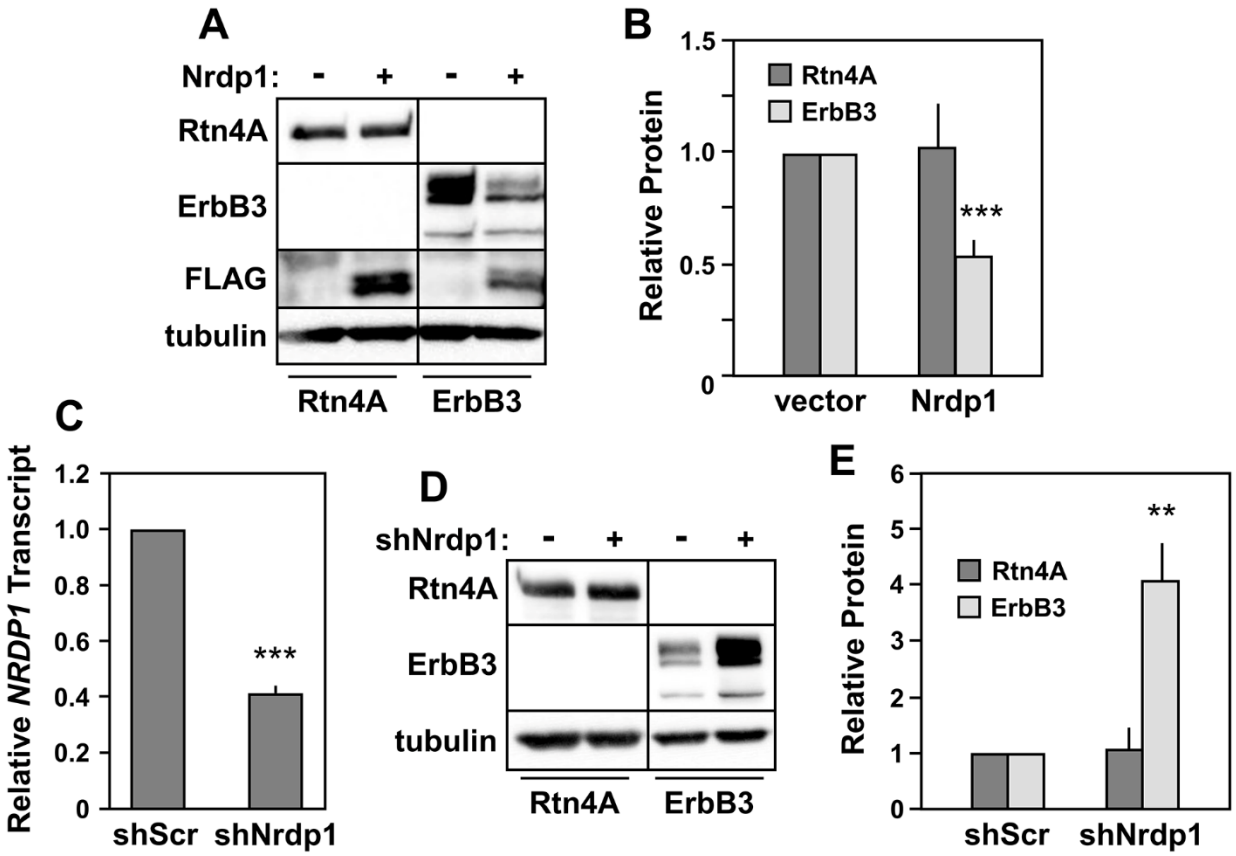

Fig. S3. Nrdp1 does not destabilize Rtn4A.
(A) HEK293T cells were co-transfected with constructs encoding either Rtn4A or ErbB3 along with vector control (-) or FLAG-tagged Nrdp1 (+), as indicated. Lysates were immunoblotted with antibodies recognizing Rtn4A, ErbB3, FLAG, and tubulin. **(B)** Six independent experiments such as that depicted in panel (A) were quantified, and the relative abundance of Rtn4A and ErbB3 protein was plotted. **(C)** HEK293T cells were transfected with either scrambled control shRNA (shScr) or Nrdp1-directed shRNA, and endogenous *Nrdp1* transcripts were measured in three independent experiments by qPCR. **(D)** Cells were co-transfected with either Rtn4A or ErbB3 along with scrambled control (-) or Nrdp1-directed shRNA (+), and lysates were immunoblotted to detect Rtn4A, ErbB3, and tubulin. **(E)** Blotted ErbB3 and Rtn4A bands from nine independent experiments similar to that depicted in (D) were quantified and plotted. **, $P < 5x10^{-3}$; ***, $P < 5x10^{-5}$ by Student's t-test.

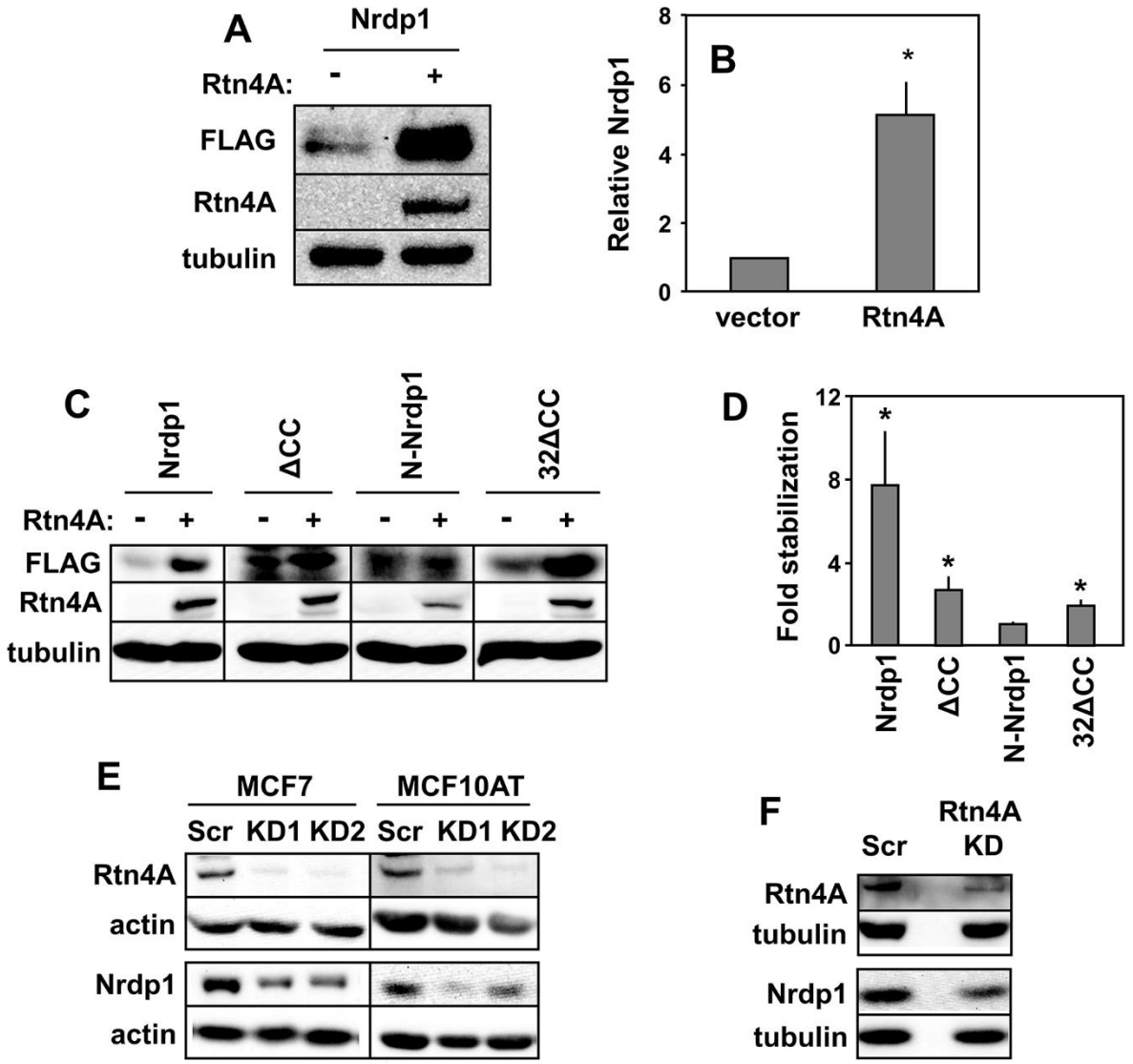

Fig. S4. Rtn4A increases Nrdp1 protein abundance.
When cells are not treated with MG132, the abundance of Nrdp1 is reduced, but forms of Nrdp1 that bind to Rtn4A are stabilized. **(A)** HEK293T cells were co-transfected with FLAG-tagged Nrdp1 and either vector control or Rtn4A, and lysates were immunoblotted with the antibodies recognizing FLAG, Rtn4A, and tubulin. **(B)** Six independent experiments similar to that depicted in panel (A) were quantified. **(C)** HEK293T cells were co-transfected with each of the Nrdp1 deletion constructs along with Rtn4A (+) or vector control (-). Lysates were immunoblotted to detect FLAG, Rtn4A, and tubulin. **(D)** Four independent experiments such as that illustrated in panel (C) were quantified, and the fold stabilization by Rtn4A was plotted for each Nrdp1 construct. **(E)** MCF7 (left lanes) and MCF10AT (right lanes) cells were treated with either scrambled control siRNA (Scr) or one of two different Rtn4A targeted siRNAs (KD1 and KD2). Lysates were blotted for Rtn4A, Nrdp1, and actin. Representative of five independent

experiments. **(F)** C2C12 myoblasts were treated with scrambled control siRNA (Scr) or Rtn4A-targeted siRNA (Rtn4A KD) then differentiated for three days. Lysates were blotted for Rtn4A, Nrdp1, and tubulin. Representative of six independent experiments. *, $P < 0.05$ by Student's t-test.

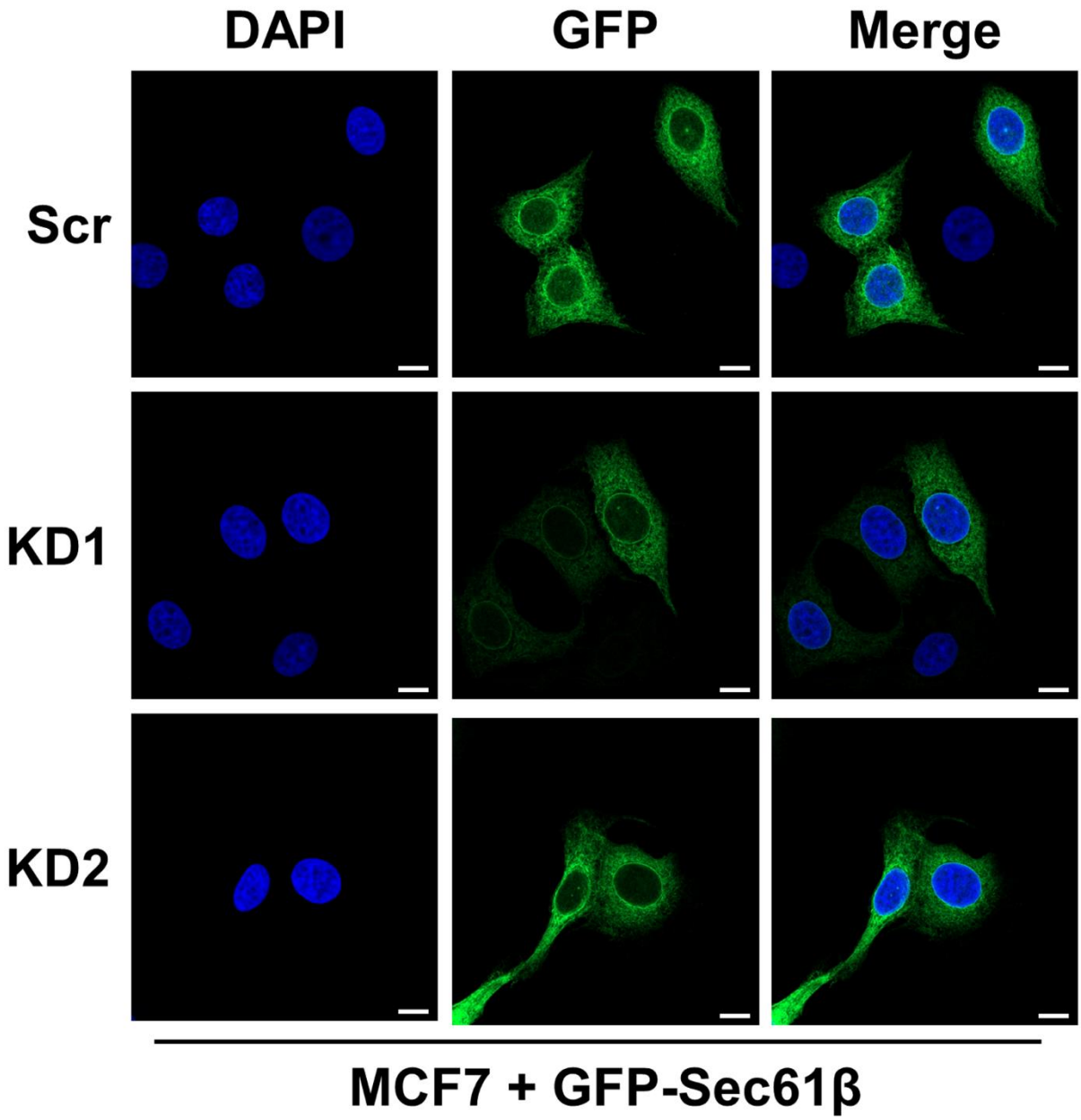

Fig. S5. Rtn4A knockdown does not alter ER morphology.
MCF7 cells were transfected with GFP-Sec61β to label the endoplasmic reticulum (ER) and then treated with scrambled control (Scr) or Rtn4A-targeted siRNA (KD1 or KD2) for five days. Cells were fixed, stained with DAPI, and imaged. Representative of three independent experiments. Scale bar: 10 μm.