Malinová et al., https://doi.org/10.1083/jcb.201701165

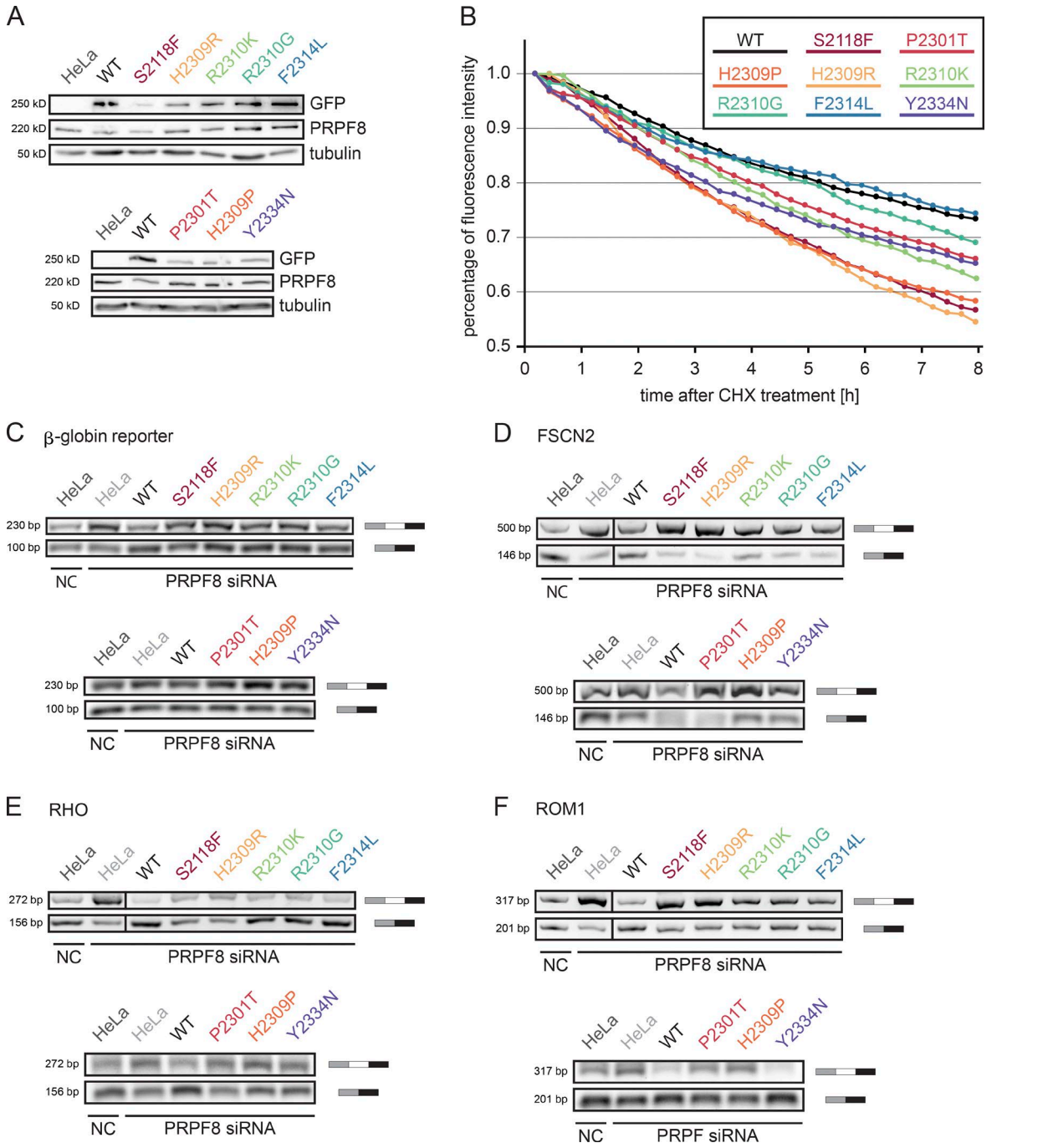

Figure S1. **Expression and stability of PRPF8 RP mutants, and their effect on splicing.** (A) Images represent WBs made with extracts of cells expressing the indicated PRPF8-LAP and showing the expression of endogenous PRPF8 (anti-PRPF8 antibody line) and LAP-tagged PRPF8 expressed from BACs (anti-GFP antibody line). Tubulin was used as a loading control. (B) Measurements of protein degradation rates. The amount of proteins was determined using time-lapse microscopy after inhibition of protein synthesis with cycloheximide (CHX). GFP fluorescence intensity was measured in 15-min intervals for 8 h. Each line represents a mean of three experiments (six for WT), and in each experiment, 8–10 cells were analyzed. (C–F) RT-PCR gels of the β-globin gene reporter (C) and the retina-specific reporter genes FSCN2 (D), RHO (E), and ROM1 (F) in cells knocked down for endogenous PRPF8 and expressing the indicated LAP-tagged PRPF8 variants. The upper bands represent unspliced variants, and the lower bands represent spliced variants. Black lines indicate that intervening lanes have been spliced out.

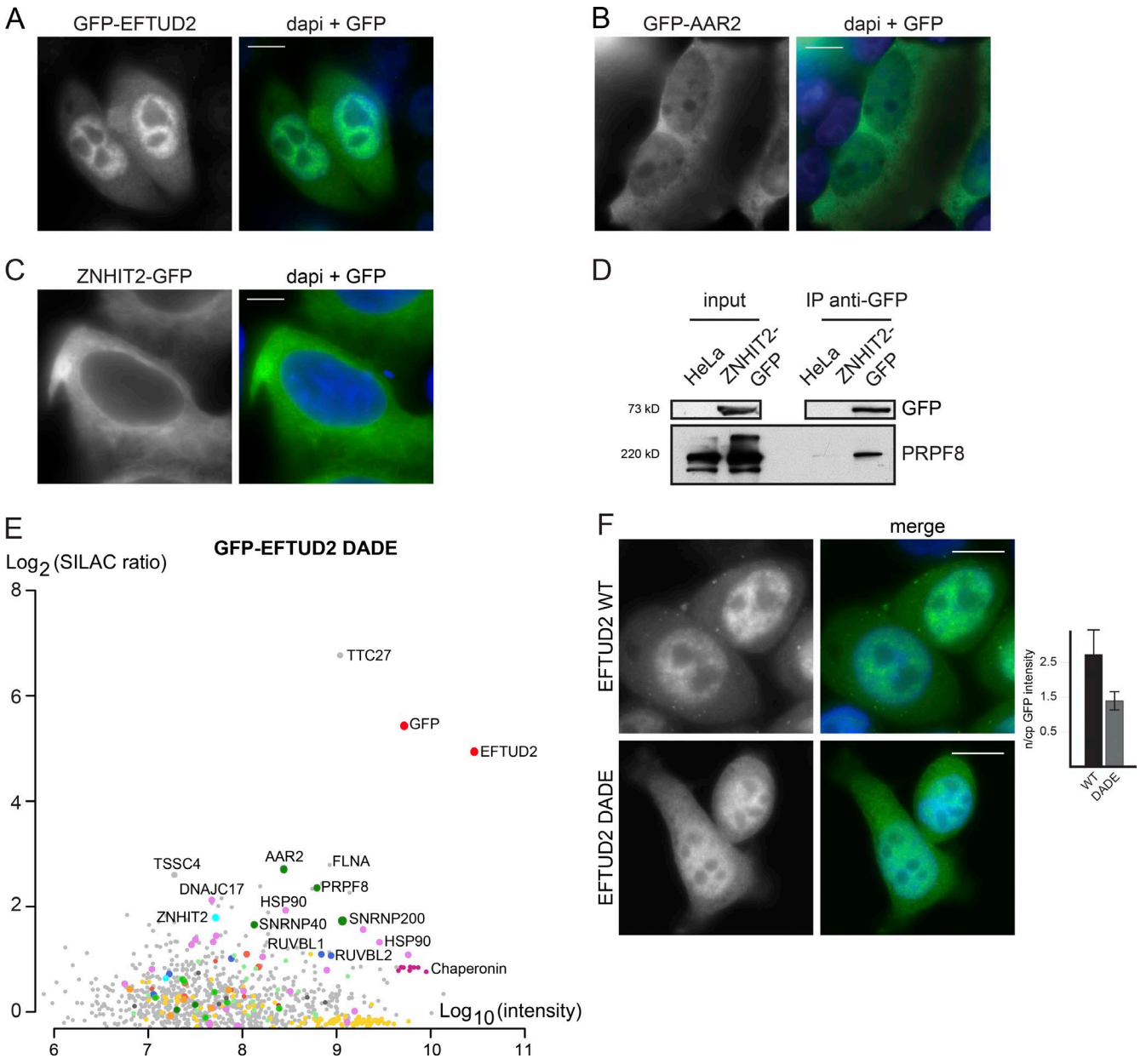

Figure S2. **Localization of ZNHIT2-GFP, GFP-EFTUD2 WT/DADE mutant, and GFP-AAR2, interaction of PRPF8 with ZNHIT2-GFP, and proteomic analysis of GFP-EFTUD2 DADE mutant.** (A–C) HeLa cells stably expressing the indicated fusion proteins. (D) Interaction between PRPF8 and GFP-ZNHIT2. WBs made with extracts of parental HeLa cells (HeLa) or HeLa cells transiently expressing GFP-ZNHIT2 (GFP-ZNHIT2; input), and immunoprecipitated with anti-GFP antibody (IP anti-GFP). Inputs contain 5% of the materials in the IP. Antibodies used to probe the WBs are indicated on the right. (E) Proteomic analyses of the EFTUD2 DADE mutant interactome. The graph displays SILAC ratios (y axis) as functions of signal abundance (x axis) measured by quantitative proteomic analysis of extracts from the HeLa cell–expressing GFP-EFTUD2 DADE mutant and immunoprecipitated with anti-GFP antibody. SILAC ratios are calculated from a control IP done with parental HeLa cells. Significance values are given in Table S1. (F) Microscopy images of HeLa cells stably expressing GFP-EFT UD2-WT or DADE mutant. Quantification of the GFP signals is shown on the left, expressed as ratios of green versus red (after background subtraction) intensities. Values are means calculated from >30 cells, and the error bars indicate SD. Green, GFP; blue, DAPI. Bars, 10 µm. n/cp, nucleocytoplasmic.

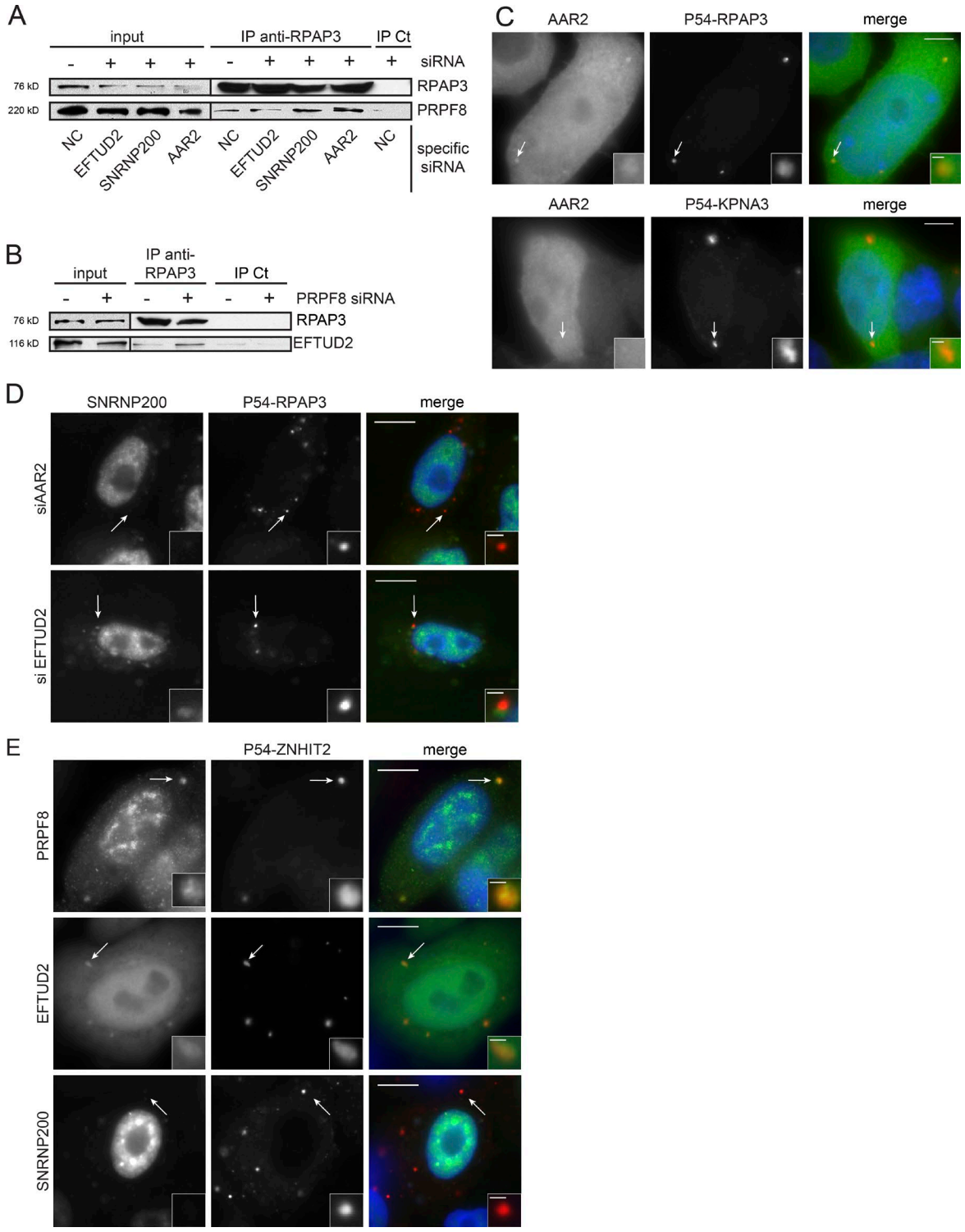

Figure S3. **Interaction of RPAP3 and ZNHIT2 with PRPF8, EFTUD2, AAR2, and SNRNP200.** (A) Interaction between RPAP3 and PRPF8 in the presence and absence of other U5 proteins and AAR2. Images represent WBs made with extracts of HeLa cells treated with the indicated siRNAs and immunoprecipitated with anti-RPAP3 antibody (IP anti-RPAP3) or IgG as control (Ct; IP Ct). Inputs contain 5% of the materials in the IP. Antibodies used to probe the WBs are indicated on the right. (B) Interaction between RPAP3 and EFTUD2 in the presence and absence of PRPF8. Legend is as in A. Black lines indicate that intervening lanes have been spliced out. (C) Analysis of the interaction between AAR2 and RPAP3 with a cytoplasmic corecruitment assay. Top rows are microscopy images of HeLa cells stably expressing a p54-DsRed2-RPAP3 fusion and transiently transfected with a plasmid expressing GFP-AAR2. Bottom rows are images of HeLa cells expressing p54-DsRED2-KPNA3 instead of p54-DsRed2-RPAP3 as a control. (D) Analysis of the interaction between SNRNP200 and RPAP3 with a cytoplasmic corecruitment assay. Microscopy images of HeLa cells stably expressing a p54-DsRed2-RPAP3 fusion and transiently transfected with both a plasmid expressing GFP-SNRNP200 and siRNAs against AAR2 (top) or EFTUD2 (bottom). Legend is as in C. (E) Analysis of the interaction between ZNHIT2 and PRPF8, EFTUD2, and SNRNP200 with a cytoplasmic corecruitment assay. Microscopy images of HeLa cells transiently expressing a p54-DsRed2-ZNHIT2 fusion and a plasmid expressing GFP-SNRNP200 (bottom). PRPF8 and EFTUD2 were detected by indirect IF (top and middle, respectively). Legend is as in C. Insets are magnifications of the dots indicated by arrows. Bars: (main images) 10 µm; (insets) 1 µm.

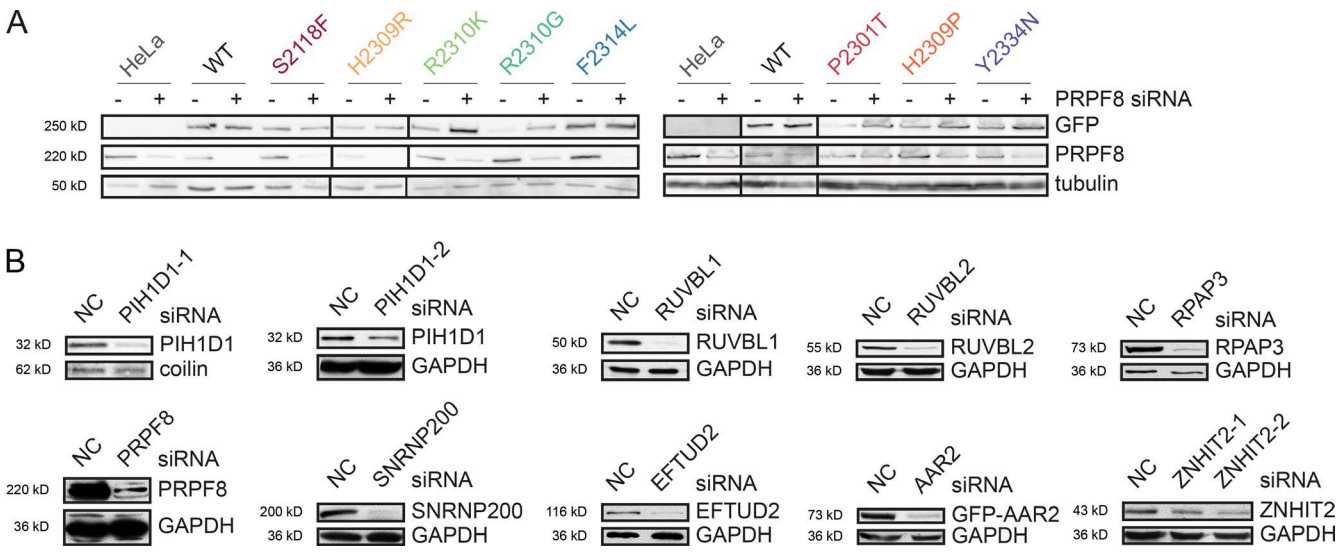

Figure S4. **KD efficiency of siRNAs.** (A) KD of endogenous PRPF8. WBs were made from extracts of cells expressing the PRPF8-LAP variants indicated at the top and treated with the siRNA targeting endogenous PRPF8 ("+") or an NC siRNA ("−"). Antibodies used to probe the WBs are indicated at the right. The PRPF8 bands correspond to the endogenous protein. Tubulin was used as the loading control. Black lines indicate that intervening lanes have been spliced out. (B) KD efficiency of individual siRNAs used in this work was measured by WBs made with extracts of cells treated with an NC siRNA and an siRNA targeting the indicated protein. Coilin or GAPDH were used as loading controls.

Table S1 is a separate Excel file showing a hit list of the proteomic experiments.