Supporting Information

Reduction of oligomer size modulates the competition between cluster formation and phase separation of the tumor suppressor SPOP

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4 SI figures

SI Figures

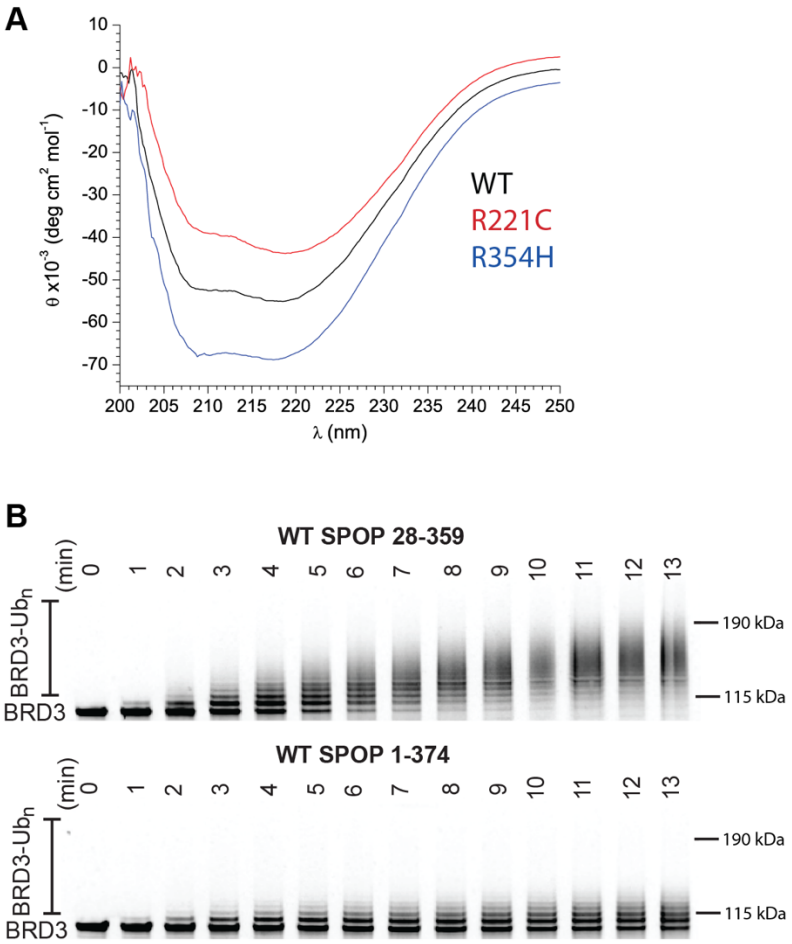

Figure S1: Biophysical and biochemical characterization of SPOP.

(A) CD spectra of WT and mutant SPOP. Slightly different concentrations between 1 and 2 μM protein were used to enable comparison of the offset spectra. **(B)** In vitro ubiquitination assay monitoring polyubiquitination of BRD3 by WT SPOP$^{28\text{-}359}$ vs full-length SPOP.

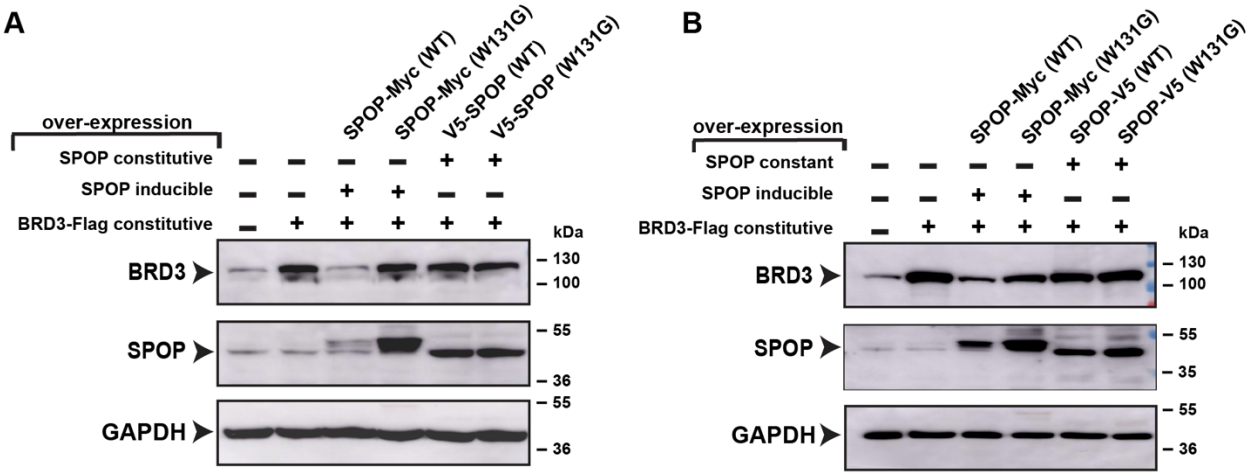

Figure S2. SPOP expression induced close to endogenous levels mediates BRD3 turnover.

T-REx cells were transfected with plasmids for BRD3-Flag and SPOP-Myc (inducible expression) or V5-SPOP (constitutive expression). Twenty-four hours post-transfection, cells were lysed, and the resulting lysates were immunoblotted using antibodies for BRD3, SPOP, and GAPDH (loading control). The expected difference in BRD3 turnover for cells expressing $SPOP^{WT}$ and $SPOP^{W131G}$ is more pronounced with the induced-expression SPOP constructs. The figure shows 2 replicates.

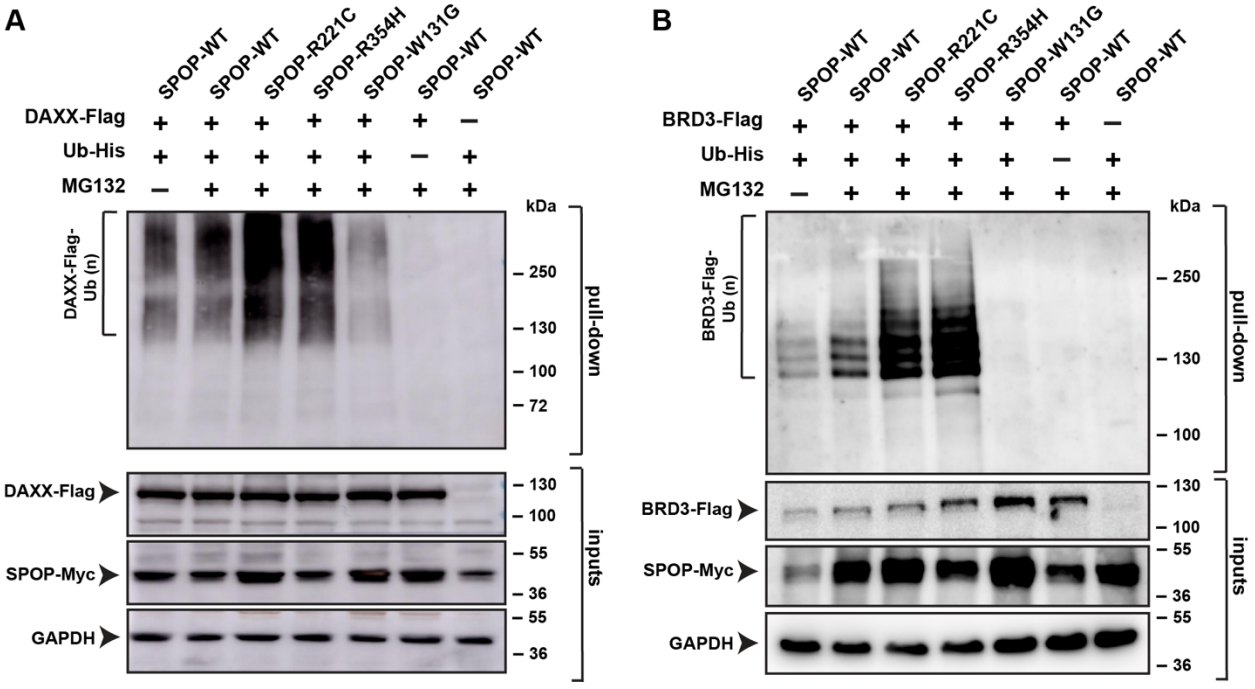

Figure S3. Replicates of in-cell ubiquitination assays.

(A) Immunoblot from replicate in-cell ubiquitination assay as in Fig. 4A. **(B)** Immunoblot from replicate in-cell ubiquitination assay as in Fig. 4C.

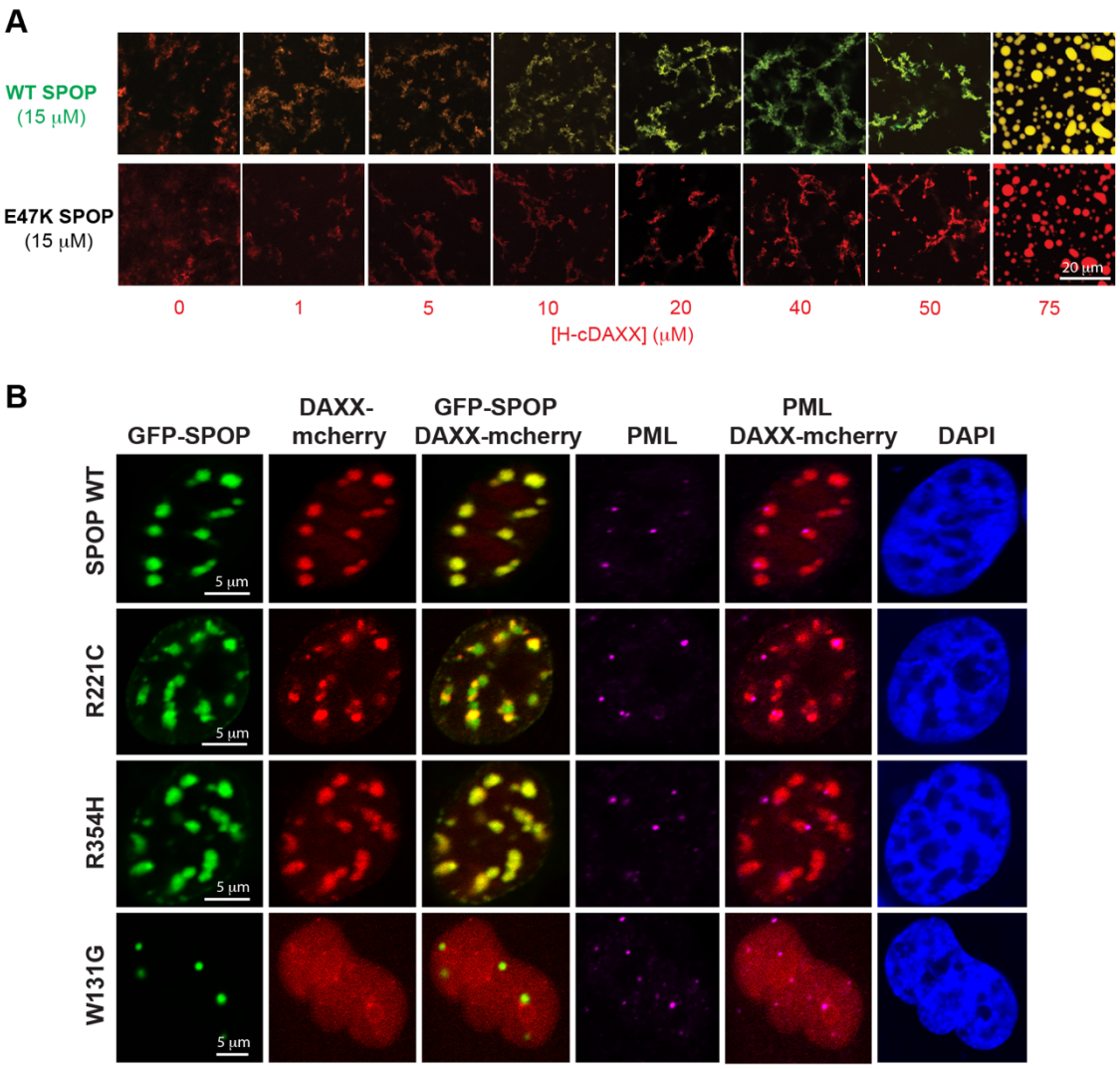

Figure S4. Phase separation and cellular localization of mutant SPOP.

(A) Confocal fluorescence microscopy images of $SPOP^{WT}$ (green) or $SPOP^{E47K}$ (unlabeled) as a function of cDAXX (red) concentration. All samples contain 10% w/v ficoll 70, and/or 500 nM Rhodamine-cDAXX. The samples with $SPOP^{WT}$ contain an additional 500 nM ORG-$SPOP^{WT}$. **(B)** Representative fluorescence confocal images of HeLa cells expressing GFP-SPOP and DAXX-mCherry constructs. Cells were transfected with the indicated plasmids. Twenty-four hours post-transfection, cells were fixed and immuno-stained using an antibody against PML bodies (magenta). DAPI (blue) marks nuclear DNA.