Supporting Materials for Kratz and de Lange

Supporting Figures S1-S3

Figure S1. Kratz and de Lange

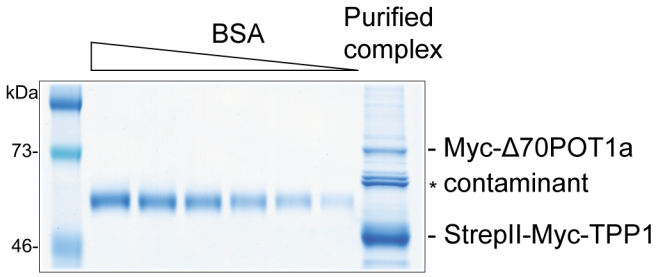
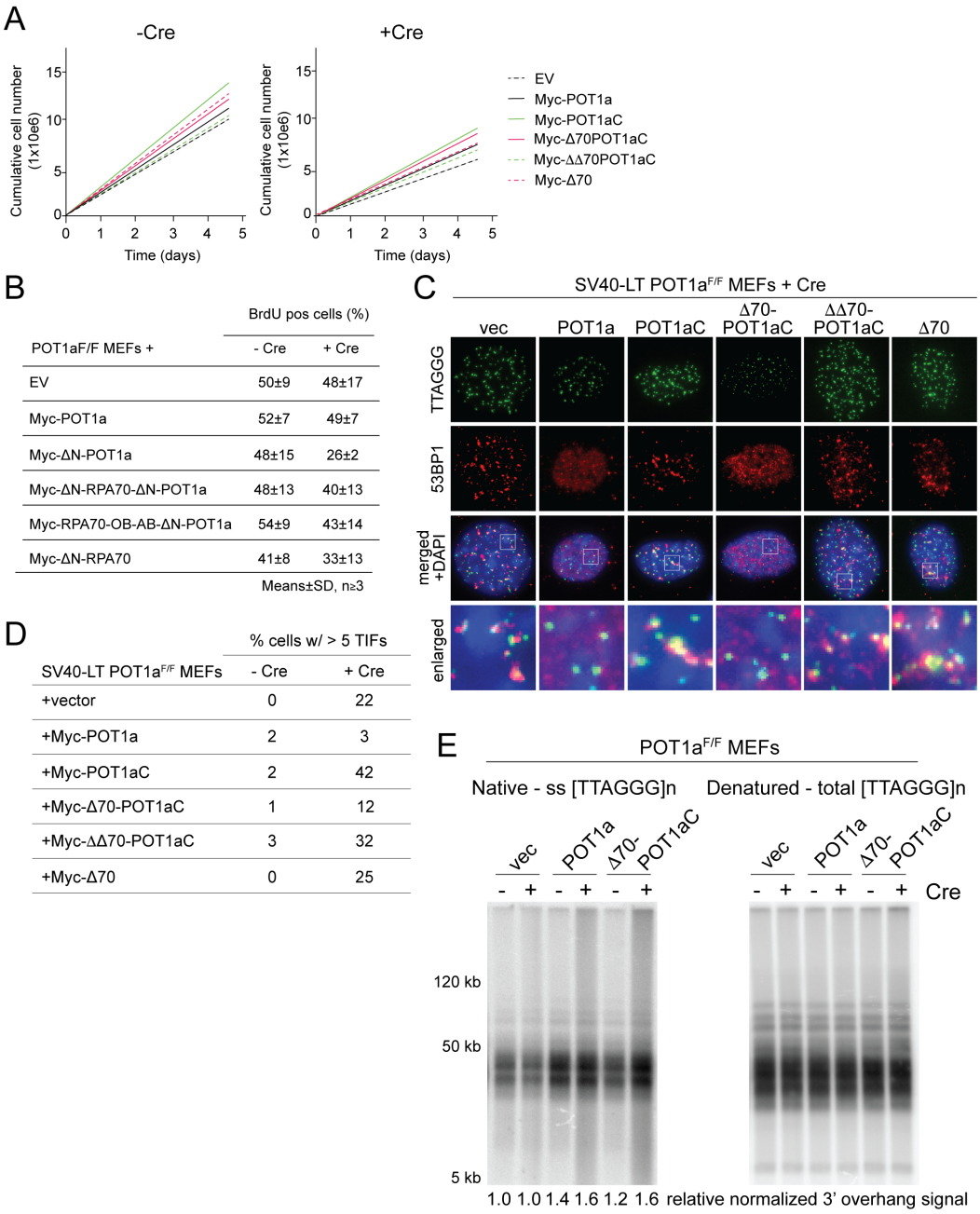


Figure S1. Isolation of Δ70-POT1aC

TPP1 and Δ70-POT1aC were co-expressed in HEK293T cells and isolated using the StrepII-tag of TPP1. The Coomassie gel shows a BSA standard to determine the protein concentration of Δ70-POT1aC to calculate the relative K_ds in Figure 1G.

Figure S2. Kratz and de Lange



B

POT1aF/F MEFs +	BrdU pos cells (%) - Cre	+ Cre
EV	50±9	48±17
Myc-POT1a	52±7	49±7
Myc-ΔN-POT1a	48±15	26±2
Myc-ΔN-RPA70-ΔN-POT1a	48±13	40±13
Myc-RPA70-OB-AB-ΔN-POT1a	54±9	43±14
Myc-ΔN-RPA70	41±8	33±13

Means±SD, n≥3

D

SV40-LT POT1a$^{F/F}$ MEFs	% cells w/ > 5 TIFs - Cre	+ Cre
+vector	0	22
+Myc-POT1a	2	3
+Myc-POT1aC	2	42
+Myc-Δ70-POT1aC	1	12
+Myc-ΔΔ70-POT1aC	3	32
+Myc-Δ70	0	25

Figure S2. Characterization of RPA-POT1aC expressing cell lines

(A) Proliferation rate of the different cell lines used. (B) S phase indices of POT1a$^{F/F}$ MEFs with and without Cre treatment (96 h) expressing the indicated proteins. Average of 3 independent experiments and SDs. (C) Representative IF images of the DNA damage response at telomeres of Cre-treated (96 h) POT1a$^{F/F}$ MEFs expressing the indicated proteins. 53BP1 foci that co-localize with telomeres (TIFs) were detected with α-53BP1 antibody (red) combined with FISH for telomeric DNA (green). (D) Quantification of the TIF response (53BP1 foci at telomeres) in POT1a$^{F/F}$ MEFs expressing the indicated proteins with and without Cre treatment (96 h) as

shown in (C). (E) Quantitative analysis of the ss TTAGGG repeats at telomeres of $POT1a^{F/F}$ MEFs with and without Cre treatment expressing the indicated proteins (96 h). Numbers below the native gel (left) represent the relative 3' overhang signal normalized to the total TTAGGG repeat signal in the same lane.

Figure S3. Kratz and de Lange

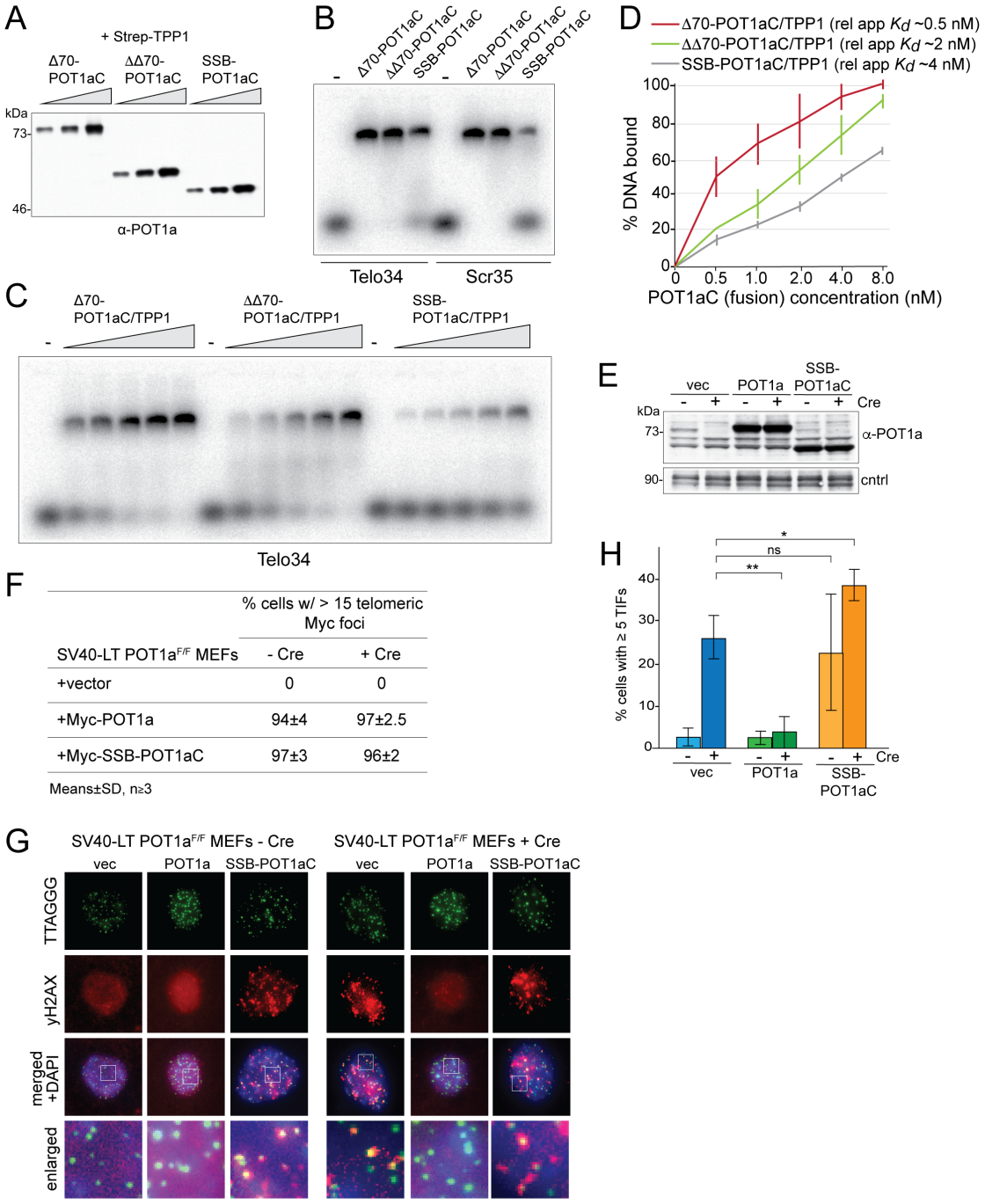


Figure S3. Shelterin-tethered SSB does not repress ATR

Immunoblot to determine the relative protein concentration of Strep-tagged TPP1 in complex with either the indicated RPA-fusion proteins or SSB-POT1C isolated from transfected 293T cells. EMSA showing binding of TPP1/Δ70-POT1aC, TPP1/ΔΔ70-POT1aC, and TPP1/SSB-POT1aC to telomeric and non-telomeric single-stranded DNAs. (C) Representative EMSA to

determine the apparent relative K_d of TPP1/Δ70-POT1aC, TPP1/ΔΔ70-POT1aC, and TPP1/SSB-POT1aC for Telo34. (D) Quantification of the affinity of TPP1/Δ70-POT1aC, TPP1/ΔΔ70-POT1aC and TPP1/SSB-POT1aC as shown in (C) from 3 independent experiments. Error bars represent SDs. (E) Immunoblot for POT1a in SV40-LT immortalized $POT1a^{F/F}$ MEFs expressing the indicated proteins before and after Cre treatment (96 h). The endogenous POT1a is lost upon Cre treatment and the introduced proteins are overexpressed compared to endogenous POT1a. (F) Quantification of the telomeric localization of the indicated proteins in $POT1a^{F/F}$ MEFs with and without Cre treatment (96 h). Averages of 3 independent experiments and SDs. (G) Representative IF images of the DNA damage response at telomeres of Cre-treated (96 h) $POT1a^{F/F}$ cells expressing the indicated proteins. γH2AX foci that co-localize with telomeres (TIFs) were detected with an α-γH2AX antibody (red) combined with FISH for telomeric DNA (green). (H) Quantification of the TIF response as shown in (G) in the indicated $POT1a^{F/F}$ cells with and without Cre treatment. Data represent averages from 3 independent experiments. P values as in Figure 2.