Figure S1. Doksani and de Lange

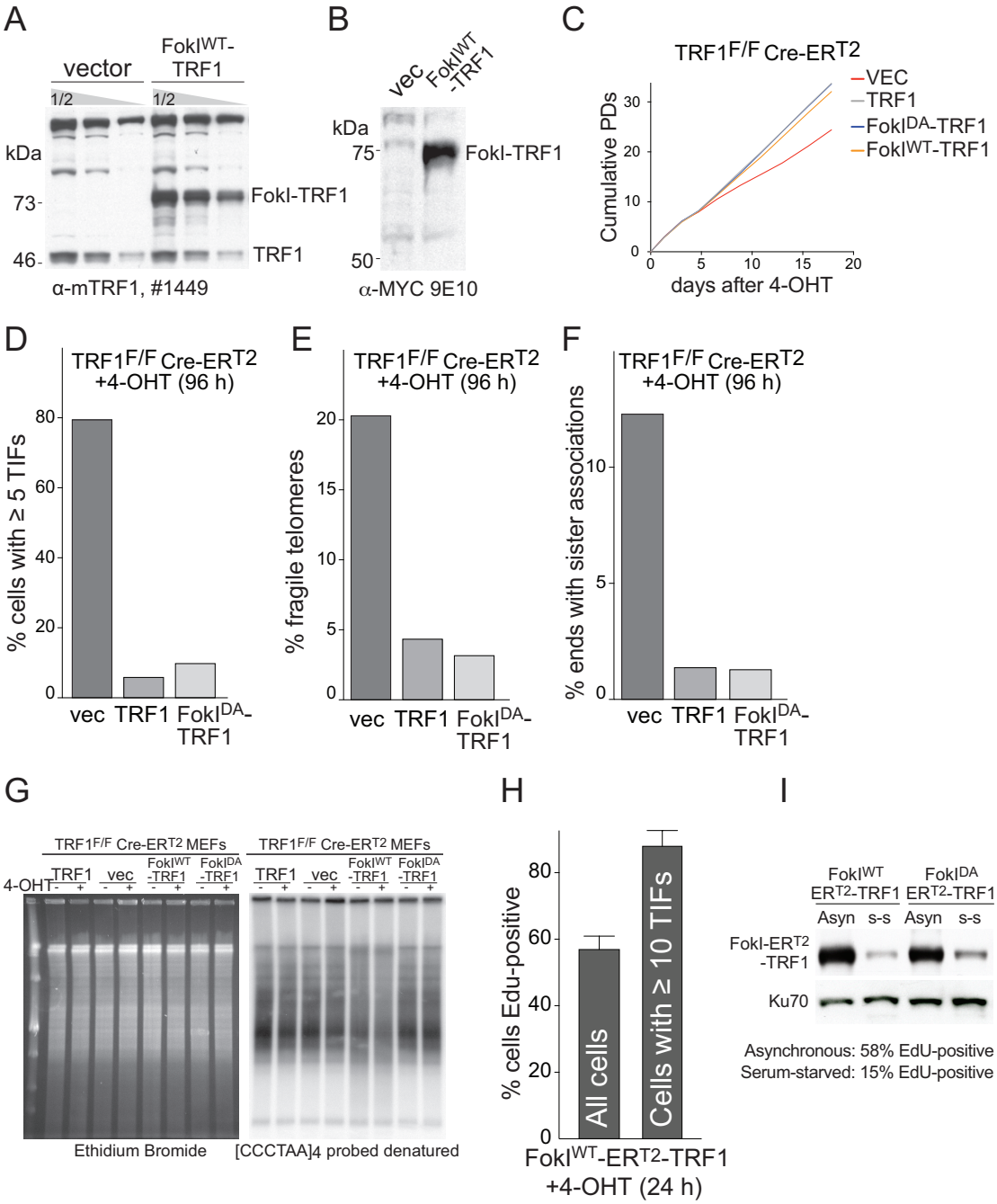

Figure S1. FokI-TRF1 expression: the effect on cell growth, complementation of endogenous TRF1 deletion and S-phase induction of TIFs , related to Figure 1

(A) Western blot control of $FokI^{WT}$-TRF1 overexpression. SV40LT-immortalized MEFs were infected with either $FokI^{WT}$-TRF1 or the empty vector. Two-fold serial dilutions were loaded to determine the level of $FokI^{WT}$-TRF1 compared to the endogenous TRF1.

(B) Western blot of the samples described in (A) analyzed with MYC antibody to detect $FokI^{WT}$-TRF1.

(C) Growth rate of cells expressing the indicated proteins. Experimental procedure as in Fig. 1B.

(D-F) $FokI^{DA}$-TRF1 complements the loss of the endogenous TRF1. The indicated constructs were introduced by retroviral infection in SV40LT-immortalized $TRF1^{F/F}$ RsCre-ER^{T2} MEFs and after selection, 4-OHT was added to induce Cre-mediated deletion of the endogenous TRF1 gene. Samples were taken 96 h after 4-OHT and analyzed for TIF-positive cells (D), fragile telomeres (E), and sister telomere associations (F).

(G) Ethidium Bromide staining showing equal loading and total telomeric signal for the PFGE gel shown in Fig. 1D.

(H) The TIF response occurs primarily in S-phase cells. The conditional $FokI^{WT}$-ER^{T2}-TRF1 construct was introduced by retroviral infection in SV40LT-immortalized MEFs. 4-OHT was added to induce $FokI^{WT}$-ER^{T2}-TRF1 and before harvesting, cells were exposed to a 30-60 min EdU pulse. Samples were harvested 24 h after 4-OHT and processed for EdU detection followed by IF-FISH for 53BP1 and telomeres. The percentage of EdU-positive cells in the whole cell population (all cells) and in TIF-positive cells is reported. Bars represent mean with SD from three independent experiments.

(I) Immunoblots for expression of FokI-TRF1 fusion proteins in cycling vs G0 (serum-starved) cells. Serum was withdrawn from fully confluent SV40LT-immortalized MEFs expressing FokI-ER^{T2}-TRF1 proteins as follows: day 0: 10% FBS, day 1: 5% FBS, day 2: 2% FBS, day 3 and day 4: 1% FBS. On day 5 cells were given a 60 min EdU pulse before harvest. Asynchronous cells were kept in 10% FBS throughout the experiment.

Figure S2. Doksani and de Lange

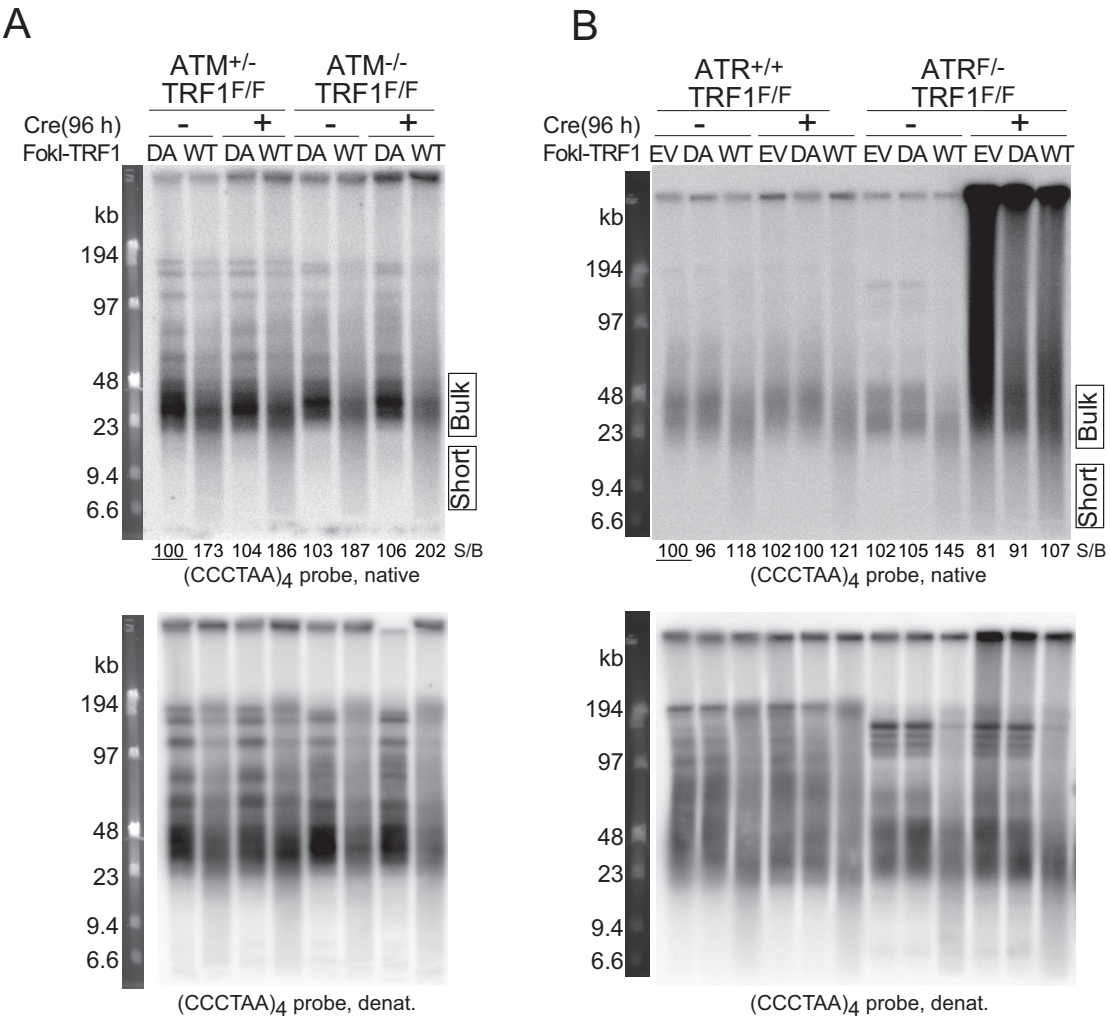

Figure S2. FokI-mediated cutting of telomeric DNA in ATM- and ATR-deficient cells, related to Figure 2.

(A) PFGE analysis of telomeric DNA. FokI-TRF1 fusions were introduced in the indicated cell lines by retroviral infection. After selection, cells were infected with H&R Cre retrovirus to induce deletion of the endogenous TRF1 and samples were analyzed 96 h later. The PFGE procedure is described in Fig. 1D. The values below the gel are reported relative to underlined sample, which is set to 100. Top: native hybridization. Bottom: denatured.

(B) PFGE analysis of telomeric DNA as in (A) but with cells lacking ATR after Cre treatment.

Figure S3. Doksani and de Lange

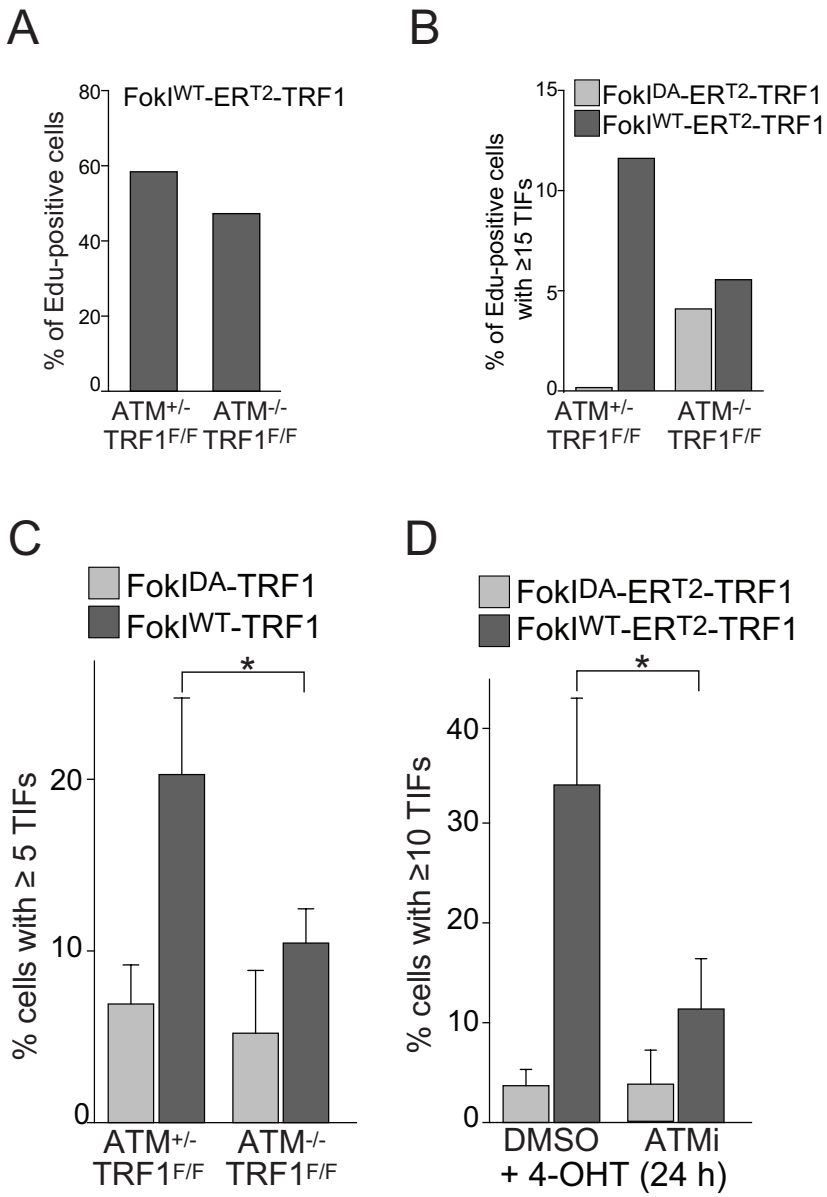

Figure S3. Control experiments for the ATM-dependent signaling at FokI-induced DSBs, related to Figure 2.

(A) Quantification of the EdU-positive fraction of the indicated cell lines. FokI^{WT}-ER^{T2}-TRF1 constructs were introduced in the indicated cell lines by retroviral infection. After selection, cells were incubated with EdU for 1 h before harvesting.

(B) Quantification of the TIF-response including only the EdU positive cells in the analysis. The conditional FokI-ER^{T2}-TRF1 constructs were introduced by retroviral infection in the indicated cell lines. After selection, 4-OHT was added to induce FokI-ER^{T2}-TRF1 and cells were exposed to an EdU pulse 1 h before harvesting (24 h after 4-OHT). Samples were processed for EdU detection followed by IF-FISH for 53BP1 and telomeres. The graph shows the quantification of the TIF response occurring only in EdU-positive cells. 53BP1-telomere co-localization counting was performed with the automated Fiji/ImageJ macro.

(C) Quantification of the TIF-positive cells in the presence of the endogenous TRF1. Same experimental procedure as the one in Fig. 2A, without the Cre-mediated deletion of TRF1. Bars represent mean with SD from 3 independent experiments (~100 cells each). P value from two tailed, paired t test.

(D) Effect of ATM inhibition on TIF response. The conditional FokI-ER^{T2}-TRF1 construct was introduced into SV40LT-immortalized MEFs by retroviral infection. After selection, the ATM inhibitor KU55933 (5 μM) was added for 24 h, followed by 4-OHT and fresh KU55933 for another 24 h. Bars represent means with SD from three experiments. P value from unpaired two-tailed t test.

Figure S4. Doksani and de Lange

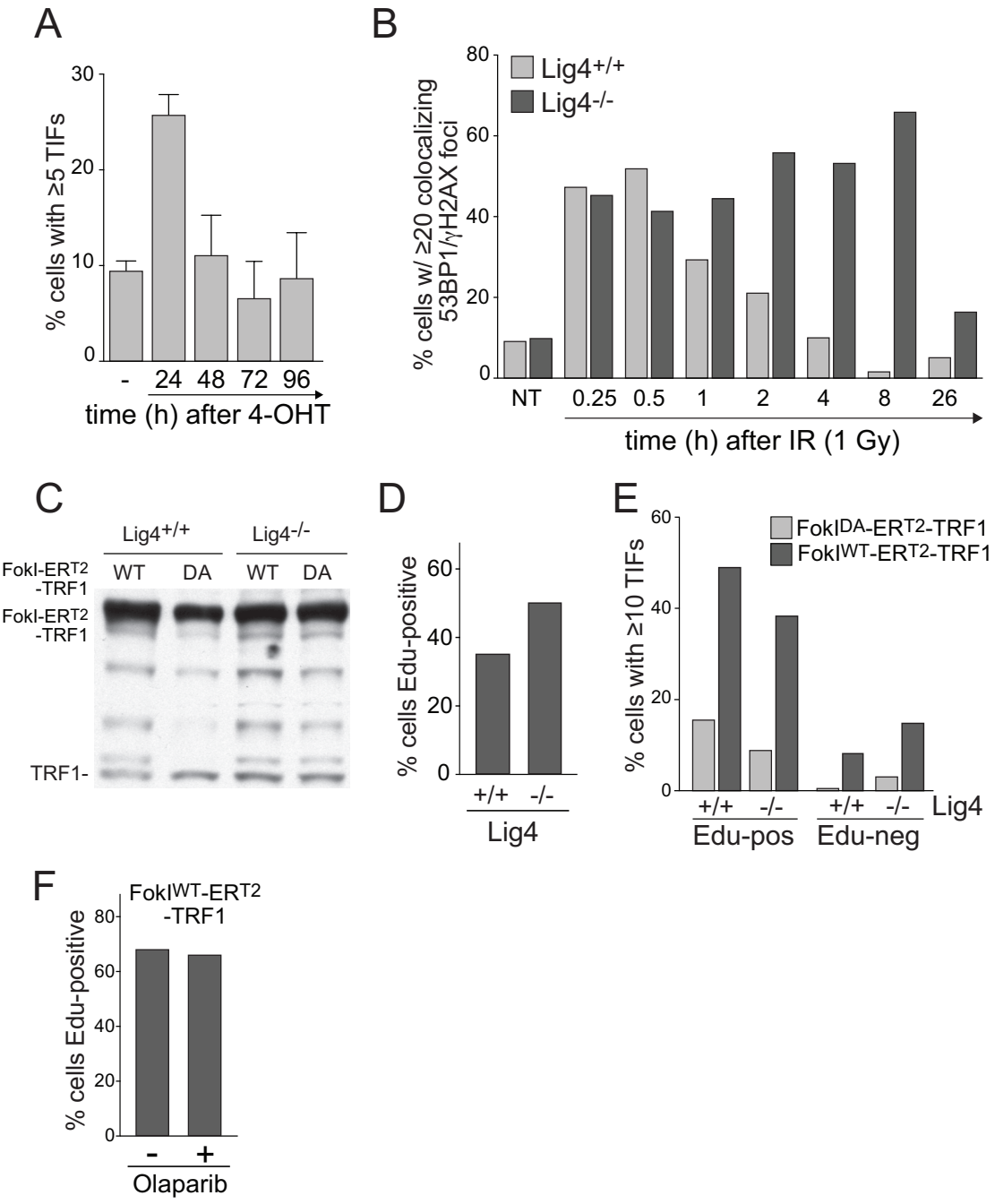

Figure S4. Control experiments for DSB repair at telomere-internal DSBs, related to Figure 4.
(A) Time course of TIFs disappearance after transient FokI^{WT}-ER^{T2}-TRF1 induction. SV40LT-immortalized MEFs were infected with the conditional FokI-ER^{T2}-TRF1 construct. After selection, 4-OHT was added for 2 h and cells washed with PBS and fresh media without 4-OHT was added. TIFs were quantified in samples were collected at the indicated time points. Bars represent mean with SD from three independent experiments.
(B) Kinetics of clearance of IR-induced foci in Lig4-proficient and –deficient cells. SV40LT-immortalized TRF2$^{F/+}$Lig4$^{+/+}$ and TRF2$^{F/F}$Lig4$^{-/-}$ MEFs were exposed to 1 Gy IR, fixed at the indicated time points, processed for IF for 53BP1 and gH2AX. 53BP1 and gH2AX foci were scored using the automated Fiji/ImageJ macro.
(C) Western blot for FokI-ER^{T2}-TRF1 expression for the experiment in Fig. 4A.
(D) Quantification of EdU-positive cells in Lig4$^{+/+}$ and Lig4$^{-/-}$ MEFs. Cells were incubated with EdU for 1 h before harvesting.
(E) Quantification of the TIF-response in EdU-positive or EdU-negative cells. The conditional FokI-ER^{T2}-TRF1 constructs were introduced by retroviral infection in the indicated cell lines. After selection, 4-OHT was added for 24 h with an EdU pulse during the last h. Samples processed for EdU detection followed by IF-FISH for 53BP1 and telomeres. The graph shows the quantification of the TIF response occurring in EdU-positive and -negative cells. 53BP1-telomere co-localizations were scored using the automated Fiji/ImageJ macro.
(F) Quantification of the effect of Olaparib treatment (5 mM, 24 h) on the frequency of EdU-positive cells. FokI^{WT}-ER^{T2}-TRF1 was introduced in SV40LT-immortalized MEFs. After selection, 4-OHT was added for 24 h with an EdU pulse during the last h and processed for EdU detection