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Chapter 3 FUCCI imaging of POT1a/b DKO cells treated with Cdh1 shRNA

Movie S7, related to Fig. S5A

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Chapter 1 Imaging of sorted diploid cells after re-expression of POT1a

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SUPPLEMENTARY MATERIALS

LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Figure 1, related to Fig. 1. Polyploidy due to persistent DNA damage signaling

(A) Immunoblotting showing POT1a/b deletion and Chk1 and Chk2 phosphorylation in POT1a$^{F/-}$POT1b$^{F/F}$ MEFs after introduction of Cre.

(B) Supernumerary centrosomes in POT1a/b DKO cells. Centrosomes were detected by immunofluorescence for pericentrin in POT1a/b knockout cells and controls (no Cre). The percentage of cells containing more than 2 centrosomes was scored on >50 cells for each group.

(C) Contribution of ATR to the induction of polyploidy. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated with ATR shRNA or vector control and polyploidy was measured by FACS after Cre expression (4 days after), zeocin treatment (48 hours after) or after no treatment. Quantification of the percentage of polyploid cells in 3 independent experiments and SDs are shown.

(D) Contribution of ATM to the induction of polyploidy in POT1a/b DKO cells and in zeocin-treated cells. POT1a$^{F/-}$POT1b$^{F/F}$ATM$^{-/-}$ and POT1a$^{F/-}$POT1b$^{F/F}$ATM$^{+/-}$ MEFs were treated with Cre, zeocin, or left untreated and the percentage of polyploidy was measured by FACS. Quantification of the percentage of polyploid cells in 4 independent experiments with SD is shown.

(E) No effect of the ATR shRNA on the S phase index. POT1a$^{F/-}$POT1b$^{F/F}$ATM$^{-/-}$ and POT1a$^{F/-}$POT1b$^{F/F}$ATM$^{+/-}$ were treated as in Fig. 1F. Cells were pulsed with BrdU for one hour and the percentage of BrdU positive cells was measured. Average values add SD are shown.

(F,G) UCN01 decreases polyploidization in POT1a/b DKO cells. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated with Cre or vector and after 2 days were synchronized in G1/S by double thymidine block and released. Cells were pulsed for 1 hour (t=0h to t=1h) with BrdU in order to mark a cell population and washed. After 2 hours from release (t=2h) UCN01 (2 μM) or vehicle was added and FACS was performed at the indicated time points (F). Tetraploid BrdU positive cells were gated. (G) Bargraphs showing the average percentage of tetraploid BrdU-positive cells after treatment with 2 or 5 μM UCN01.

(H) FACS analysis of TRF2$^{F/-}$Lig4$^{-/-}$ SV40 MEFs treated with Cre showing a moderate level of polyploidization after 4 days.

(I) Metaphase spread from TRF2$^{F/-}$Lig4$^{-/-}$p53$^{-/-}$ MEFs treated with Cre showing diplochromosomes. DAPI, red; Telomeric FISH, green. FISH-IF on metaphase spreads was performed as previously described (Celli and de Lange, 2005).

(J-L) Induction of polyploidy in mouse cells using various treatments to induce persistent DNA damage. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were continuously treated with the indicated drugs or with UV (20J/m^2 every 2 hours for two periods of 12 h with 12 h in between) and the percentage of polyploid cells was measured by FACS at the indicated time points. Alternatively, MEFs expressing TopBP1-ER were treated or not with OHT for 72 hours. Representative experiments (J) and quantification in two or more independent experiments (L) are shown. Immunoblotting showing phosphorylation of Chk2 after drug treatment at the indicated time points is shown in (K).

(M, N) Persistent DNA damage induces polyploidy in human cells. Human BJ fibroblasts expressing HPV E6 and E7 proteins were treated with the indicated drugs and polyploidy was measured by FACS at the indicated time points. Representative experiment (M) and quantification in 3 independent experiments (N) are shown.

(O) Absence of p53 is sufficient to allow polyploidization in MEFs. $p53^{-/-}$ MEFs were treated with zeocin and polyploidy was measured by FACS at the indicated time points. Average values from 2 independent experiments with standard error of the mean (SEM) are shown.

Supplementary Figure 2, related to Fig. 2. Impaired activity of Cdk1/CyclinB and APC/Cdc20

(A) Low Cdk1/CyclinB activity in POT1a/b DKO and zeocin treated cells. $POT1a^{F/-}$ $POT1b^{F/F}$ MEFs were treated with Cre or with continuous zeocin and Cdk1/CyclinB activity was measured at the indicated time points. Phopho-histone-H1, Coomassie staining of histone H1, immunoblotting showing Cdk1 and quantification of kinase activity are shown.

(B) Role of Chk1/Chk2 in stabilization of mitotic APC targets. $POT1a^{F/-}POT1b^{F/F}$ MEFs were treated as in Fig. S1F and immunoblotting for the indicated proteins at the indicated time points is shown.

Supplementary Figure 3, related to Fig. 3. Polyploidization during live cell imaging

FACS profiles of cells used in the live-cell imaging shown in Fig. 3A at the beginning (t = 0 h) and at the end (t = 48 h) of the imaging session. Percentage of polyploid cells is shown.

Supplementary Figure 4, related to Fig. 4. Fluctuations of geminin, Cdt1 and other proteins in endoreduplicating cells

(A) $POT1a^{F/-}POT1b^{F/F}$ MEFs expressing FUCCI proteins were continuously treated with zeocin and imaged as in Fig. 4B (Movie S3). Selected time points are shown. Arrows

with the same orientation highlight the same cell over time. Two representative cells showing geminin and Cdt1 oscillation without mitotic events are indicated.

(B) Geminin degradation in the absence of mitosis in BJ cells treated with zeocin. BJ cells expressing SV40-LT were transduced with FUCCI lentiviral vectors and imaged in the absence (control) or presence of zeocin for 96 hours (Movie S4). The percentage of cells showing at least one event of geminin degradation in the absence of mitosis during the imaging was analyzed by scoring at least 100 cells for each condition. Similar data obtained using BJ cells expressing HPV-E6/E7 are shown.

(C) FUCCI imaging of synchronized cells. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs expressing FUCCI vectors were synchronized in G1 by mitotic shake-off and were imaged for 72 hours in the presence or absence of zeocin, Imaging was started 10 hours after the mitotic cells were plated. Selected time points (every 6 hours) are shown from a representative experiment. The corresponding movies are Movie S5.

(D) Analysis of the indicated cell cycle regulated proteins over time in zeocin treated cells. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were synchronized in G1/S by double thymidine block and released in the presence of zeocin. Immunoblotting for the indicated proteins at the indicated time points is shown.

(E) Analysis of the indicated cell cycle regulated proteins in POT1a/b DKO cells. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated with Cre, synchronized in G1/S by double thymidine block and released. Immunoblotting for the indicated proteins are shown.

(F) FACS analysis of cells treated as in (D). FACS analysis at the indicated time points is shown and the percentage of polyploid cells is indicated.

(G) POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated as in (E). FACS analysis at the indicated time points is shown and the percentage of polyploid cells is shown.

Supplementary Figure 5, related to Fig. 5. Involvement of Cdh1 and APC1 in endoreduplication

(A) Cells were treated with Cdh1 shRNA or the vector control and imaged as in Fig. 5D in the presence of zeocin (Movie S7). Selected time points (every 4 hours) are shown. Arrows with the same orientation highlight the same cell during time.

(B) Table showing the effect of Cdh1 knockdown on mitosis-independent geminin degradation in zeocin-treated cells. Cells were treated and imaged as in (A). At least 100 cells were followed throughout the movie and the percentage of cells showing at least one event of geminin degradation without mitosis was determined. Average values obtained from 3 independent experiments with SD are shown.

(C) Prolonged G2 phase after Cdh1 knockdown in zeocin-treated cells. Cells were treated and imaged as in (A) and the indicated number of cells were followed throughout the imaging session to determine the length of G1 (red), entry into S phase (yellow), and S/G2 (green). Average values (h) are shown.

(D-F) Diminished polyploidy after knockdown of APC1. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated shRNA to knockdown APC (immunoblot in (E)) and polyploidy was measured after POT1a/b deletion with Cre, after continuous zeocin treatment, or in untreated cells. FACS profiles from a representative experiment (D) and quantification of the percentage of polyploid cells in 3 independent experiments (F) are shown.

SUPPLEMENTARY MOVIES

Movie S1, related to Fig. 3

Chapter 1 Phase contrast imaging of control cells

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were infected with vector control an imaged (phase contrast, objective 10X) every 15 min for 48 h. Arrows highlight one dividing cell and its daughters. Selected frames from this movie are shown in Fig. 3A.

Chapter 2 Phase contrast imaging of POT1a/b DKO cells

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated with Cre and were imaged as in Chapter 1 (imaging started 72 h after introduction of Cre). Arrows highlight 3 representative cells that do not perform mitosis during the imaging session. Selected frames from this movie are shown in Fig. 3A.

Chapter 3 Phase contrast imaging of zeocin-treated cells

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were imaged as in Chapter 1 in the presence of zeocin (added two hours before starting imaging session and maintained in culture medium during the imaging session). Arrows highlight 3 representative cells not dividing during the session. Selected frames from this movie are shown in Fig. 3A.

Movie S2, related to Fig. 3B

Chapter 1 Imaging of control cells expressing CyclinE-eGFP

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were transduced with CyclinE-eGFP expressing vector and then treated with vector control. Cells were imaged (phase contrast and GFP channel, 20X objective) every 15 min. Arrows highlight representative cells showing raise in CyclinE-eGFP followed by mitosis.

Chapter 2 Imaging of POT1a/b DKO cells expressing CyclinE-eGFP

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were transduced with CyclinE-eGFP expressing vectors and then treated with Cre. After 72 h, cells were imaged as in Chapter 1. Arrows highlight representative cells showing CyclinE rising multiple times without an intervening mitosis.

Movie S3, related to Fig. 4 and S4A

Chapter 1 FUCCI imaging of control cells

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were treated with vector control and imaged (phase contrast, GFP and rhodamine channels) every 15 min for 72 h (10X objective). Arrows highlight a representative cell. Selected frames from this movie are shown in Fig. 4A.

Chapter 2 FUCCI imaging of POT1a/b DKO cells

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were treated with Cre and imaged as in Chapter 1 (imaging started 72 h after introduction of Cre). Arrows highlight representative cells showing geminin and Cdt1 oscillation in the absence of mitotic events. Selected frames from this movie are shown in Fig. 4A.

Chapter 3 FUCCI imaging of zeocin-treated cells

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were imaged as in Chapter 1 in the presence of zeocin (added 2 hours before the session and maintained on the cells). Arrows highlight representative cells showing geminin and Cdt1 oscillation in the absence of mitotic events. Selected frames from this movie are shown in Fig. S4A.

Movie S4, related to Fig. S4B

Chapter 1 FUCCI imaging of control BJ-SV40 cells

BJ human fibroblasts expressing SV40-LT transduced with FUCCI vectors were imaged (phase contrast, GFP and rhodamine channels) every 15 min for 96 h (10X objective). Arrows highlight a representative cell dividing during time.

Chapter 2 FUCCI imaging of BJ-SV40 cells treated with zeocin

BJ human fibroblasts expressing SV40-LT transduced with FUCCI vectors were imaged as in Chapter 1 in the presence of zeocin. Arrows highlight a representative cells showing geminin and Cdt1 fluctuation in the absence of mitotic events.

Movie S5, related to Fig. S4C

Chapter 1 FUCCI imaging of synchronized control cells

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were synchronized in G1 by mitotic shake-off. Mitotic cells were plated and 10 h later, cells were imaged as in Movie S3. Selected frames from this movie are shown in Fig. S4C.

Chapter 2 FUCCI imaging of synchronized cells treated with zeocin POT1a$^{F/-}$ POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were synchronized in G1 by mitotic shake-off. Mitotic cells were imaged as in Chapter 1 in the presence of zeocin (added 2 hours before the imaging session and maintained throughout). Selected frames from this movie are shown in Fig. S4C.

Movie S6, related to Fig. 5D-F

Chapter 1 FUCCI imaging of cells treated with Cdh1 shRNA

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were treated with Cdh1 shRNA and imaged (phase contrast, GFP and rhodamine channels, 10X objective) every 15 min over a period of 60 h. Arrow shows a representative cell dividing during the imaging session.

Chapter 2 FUCCI imaging of POT1a/b DKO cells treated with vector control for Cdh1 shRNA

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were treated with Cre and the day after with empty vector (pLK0.1) used for the Cdh1 shRNA. Cells were imaged (at

72 h after introduction of Cre) as in Chapter 1. Arrows highlight representative cells. Selected time points from this movie are shown in Fig. 5D.

Chapter 3 FUCCI imaging of POT1a/b DKO cells treated with Cdh1 shRNA

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were treated with Cre and the day after with Cdh1 shRNA. At 72 h after introduction of Cre, cells were imaged as in Chapter 1. Arrows highlight representative cells showing prolonged arrest in G2 without geminin degradation. Selected time points from this movie are shown in Fig. 5D.

Movie S7, related to Fig. S5A

Chapter 1 FUCCI imaging of zeocin-treated cells infected with the Cdh1 shRNA vector control

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were treated with the empty vector (pLK0.1) used for Cdh1 shRNA. Cells were imaged as in Movie S6 in the presence of zeocin. Zeocin was added 2 h before the session and maintained on the cells. Arrows highlight representative cells over time. Selected time points from this movie are shown in Fig. S5A.

Chapter 2 FUCCI imaging of zeocin-treated cells infected with the Cdh1 shRNA

POT1a$^{F/-}$POT1b$^{F/F}$ MEFs transduced with FUCCI vectors were infected with Cdh1 shRNA and imaged in the presence of zeocin as in Chapter 1. Arrows highlight representative cells over time showing prolonged arrest in G2 without geminin degradation. Selected time points from this movie are shown in Fig. S5A.

Movie S8, related to Fig. 6D

Chapter 1 Imaging of sorted diploid cells after re-expression of POT1a

H2B-GFP expressing cell line #19 was treated as described in Fig. 6A and after sorting diploid cells were imaged in the absence of doxycycline (phase contrast and GFP

channel) every 15 min for 48 hours. Arrows highlight a representative cell dividing multiple times. Selected time points from this movie are shown in Fig. 6D.

Chapter 2 Imaging of sorted tetraploid cells after re-expression of POT1a

H2B-GFP expressing cell line #19 was treated as described in Fig. 6A and after sorting tetraploid cells were imaged as in Chapter 1. Arrows highlight representative cells dividing during time. Cells showing evidence of multipolar spindles are marked with three red arrows. Selected time points from this movie are shown in Fig. 6D.