Kinetics of poly(ADP-ribosyl)ation, but not PARP1 itself, determines the cell fate in response to DNA damage *in vitro* and *in vivo*

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Supplementary Figure Legends

Figure S1: Generation of PARP1$^{D993A/D993A}$ mice

a. PARP1 gene targeting strategy, including the positions of the D993A point mutation, the neomycine resistance cassette (neo), the Thymidylate kinase (Tk) as well as the restriction enzymes (B: BspH1); X: XbaI, the probes (P6.4 or P7.6) and the expected fragments in kilobases (kB) for Southern Blotting before and after successful gene targeting. Black boxes indicate exons. **b** and **c**. Confirmation of PARP1^{D993A} gene targeting (**b**) and CRE-recombinase-mediated neo-removal (**c**) from the targeted mouse embryonic stem cells by Southern Blotting using the strategies shown in (**a**). **d**. Mendelian genotype distribution obtained from PARP1$^{Ki/+}$ intercrosses. **e**. Sequencing of cDNA isolated from the livers of PARP1$^{D993A/D993A}$ and PARP1$^{+/+}$ littermate mice. **f**. Semi-quantitative PCR of fragments within PARP1 and GAPDH using cDNA samples from (**e**). The PARP1 transcripts were quantified relative to GAPDH and are displayed as % of WT. N=3 mice per genotype.

Figure S2: PARP1^{D993A} mutation slows DNA damage-induced PAR formation in pMEFs

a. Representative images of the PAR-IF of pMEFs with the indicated PARP1 genotypes (wild type PARP1 (+/+); PARP1$^{D993A/D993A}$ (D993A) and PARP1 knockout (-/-)) treated with or without H_2O_2 (1 mM, 30 min). **b**. Quantification of nuclear PAR signals of pMEFs treated for the indicated durations with 1 mM H_2O_2. The data are the means ± SEM of at least two pMEFs littermate pairs in duplicates, expressed as percentage (%) of the maximal induction of +/+ littermates. Similar results were obtained in two independent experiments. **c** and **d**. Quantification of the nuclear PAR induction in pMEFs (see (**a**)) of the indicated PARP1 genotypes treated with 1260 µM MNU (**c**) or 125 nM CPT (**d**) for the indicated time. The data are expressed as the mean differences (Δ) versus DMSO-treated control cells at 0 min ± SEM of at least 1000 cells per condition. Asterisks (*), color-coded in (**b, c, d**), indicate the significance versus +/+. **: p <0.01; ***: p <0.001. n.s.: not significant, as determined by a 2-way ANOVA with a Tukey's post-test (**b**) and with a Bonferroni´s post-test (**c**, **d**).

Figure S3: H_2O_2 treatment induces replication-associated DNA damage signaling in pMEFs

a. Gating of sub-phases of the S-phase using EdU and DAPI signals. **b**. Quantification of the average sum of γH2AX signal intensities in the indicated sub-phases of the S-phase. **c**. Heat-map showing relative γH2AX signals in a cycle-dependent manner at the indicated time points. The data are derived from >1000 individual cells analyzed per condition. The error bars are the S.E.M.. Similar results were obtained in 5 independent experiments. * within the bars in B show the comparison to the previous time point. *: p <0.05; ***: p <0.001; n.s.: not significant, as determined by a 2-way ANOVA with a Sidak's post-test.

Figure S4: HiMAC analysis of pMEFs following CPT treatment

a. pMEFs with the indicated PARP1 genotype (wild type PARP1 (+/+); $PARP1^{D993A/D993A}$ (D993A) and PARP1 knockout (-/-)) were treated or not with 125 nM CPT for the indicated duration and then subjected to HiMAC analysis. Quantification of γH2AX signal intensity sum (upper panel) and the EdU intensities (lower panel) in S-phase pMEFs (EdU^{+}) normalized against the respective DMSO-treated controls. Color-coded asterisks (*) indicate the significance versus +/+ (WT), as determined by a 2-way ANOVA with a Bonferroni´s post-test. *: p <0.05; **: p <0.01. Data are the means ± S.E.M. of four replicates.

Figure S5: Correlation analysis of EdU incorporation and DNA damage markers after H_2O_2 in pMEFs

a.-c. Schematics of the gating strategy. Cells were treated with 100 µM H_2O_2 for 8 min before recovery in fresh medium for 4 or 9 hr. 45 min prior to HiMAC, cells were pulse-labelled with EdU for 45 min. **a.** (i). Gating of S-phase using EdU and DAPI signals. (ii). EdU signal intensities from individual cells were re-scaled from 0 (lowest) to 1 (highest) and then sorted ascendingly. Based on the signal intensities ("Global DNA synthesis"), different subsets of cells were defined as indicated "Stalling" "Replication restart" and "Replication progression". (iii) Plotting of the re-scaled EdU signal intensities versus the average of the corresponding DNA damage marker data of individual cells generates a smoothened curve (thick, central line). The upper and lower S.E.M. of these averages were used to plot the "Highest-" and "Lowest possible values", respectively. **b.** and **c.** Threshold setting. Re-scaled and ascending EdU signal intensities from

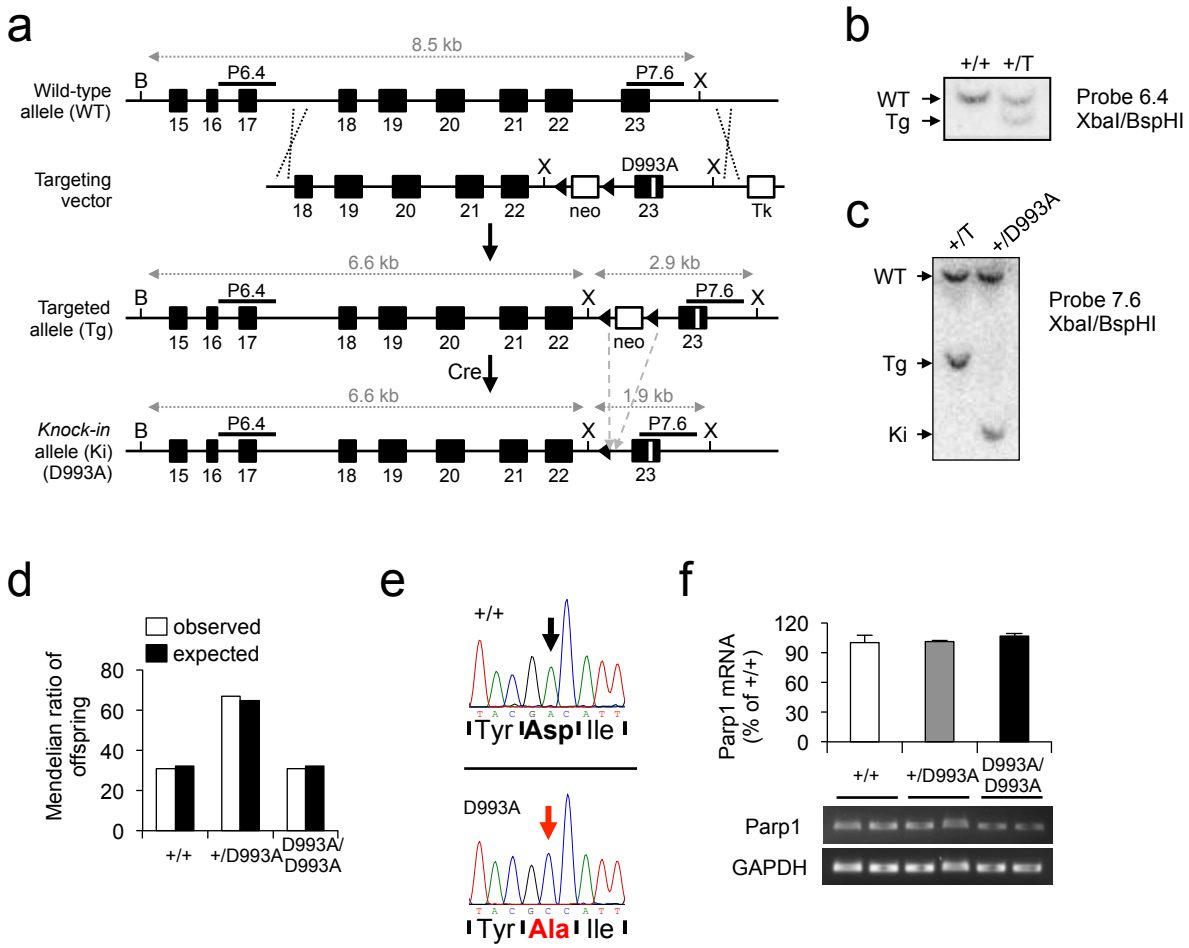
WT pMEFs in **a**(i) at 4 hr and 9 hr post/H_2O_2. The applied thresholds and the resulting subsets of cells are indicated. Note the similar slope of the curves where the thresholds were applied. **d**. The mean of the EdU signal intensities in the indicated sub-phases of the S-phase from untreated WT pMEFs. The signal intensities corresponding to the thresholds applied in **a** and **b** are indicated. **e**. The entire cell populations at both time points and the corresponding populations used to generate the panels in Fig. 4f. Correlation analysis of DNA synthesis (EdU) (X-axis) and γH2AX or 53BP1 signals (Y-axis) of pMEFs with the indicated color-coded PARP1 genotypes (wild type PARP1 (+/+); $PARP1^{D993A/D993A}$ (D993A) and PARP1 knockout (-/-)) at 4 hr (left) and 9 hr (right) post-H_2O_2. The single graphs are X/Y scatterplots (Re-scaled EdU versus the indicated DNA damage marker). All percentiles of the total EdU intensities of individual cells, i.e. the full range of the DNA replication rate within the population, are shown. Dark colored "smoothened curves" show the corresponding single-cell data over the indicated damage markers at the indicated time points after smoothening by moving averages with a period of 40 to facilitate trend identification. Light-colored curves flanking the dark-colored smoothened curves represent the S.E.M. from at least 500 cells per condition. The brackets indicate the respective region (percentiles) used in Fig. 4f.

Figure S6: The $PARP1^{D993A}$ mutation has no effect on proliferation of pMEFs

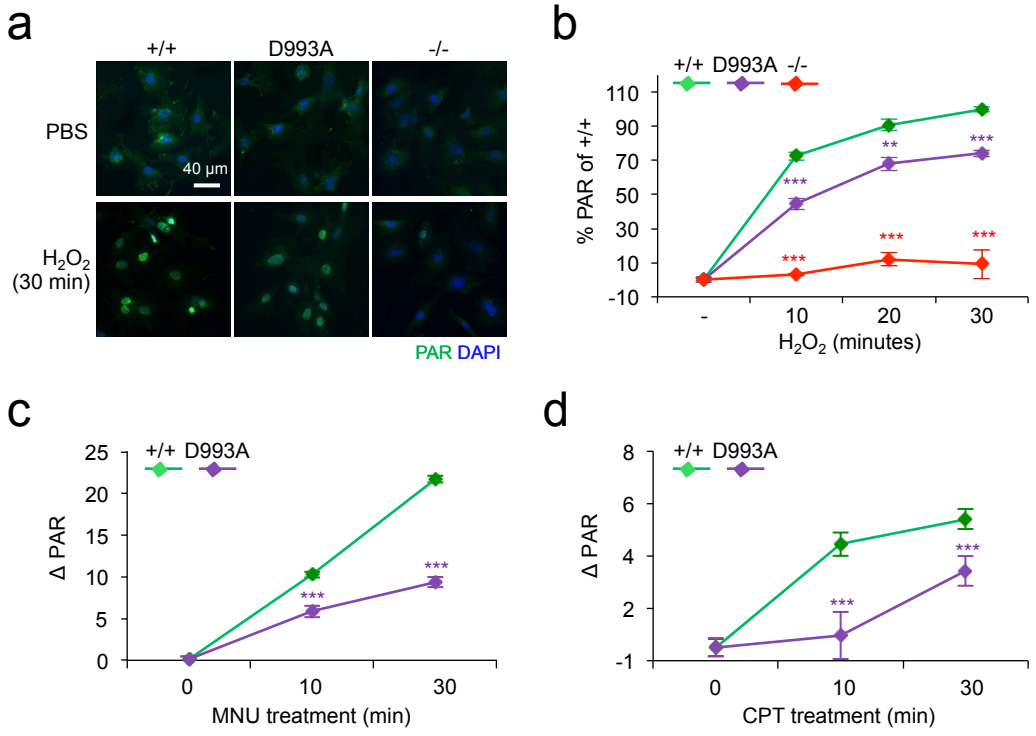
Proliferation of pMEFs of the indicated PARP1 genotypes (wild type PARP1 (+/+); $PARP1^{D993A/D993A}$ (D993A)) in culture according to a 3T3 protocol (n = 4). **a**. Cumulative cell number. **b**. The 3T3 proliferation curve replating 1.5 x 10^5 cells every three days. The data are the means ± S.E.M of two independent pMEF littermate pairs in duplicates.

Figure S7: Cell cycle exit after MNNG in pMEFs

a and **b**. High content analysis of Ki67 positivity (**a**) and EdU incorporation (**b**) of pMEFs with the indicated genotypes (wild type PARP1 (+/+); $PARP1^{D993A/D993A}$ (D993A) and PARP1 knockout (-/-)) at 1, 2, or 3 days post-treatment (dp) for 30 min with 10 µM MNNG or DMSO (Co.). **c**. Western blotting of Cyclin A of the whole cell extracts of pMEFs with the indicated genotypes at 3dp after 10 µM MNNG treatment. β-Actin is a loading control. The data are the means ± S.E.M. derived from at least three independent experiments using two pMEFs littermate pairs and are expressed as % of total cells in (**a**) and % of controls in (**b**). Asterisks (*) indicate significant differences versus +/+ littermate. *: p <0.05; **: p <0.01; ***: p <0.001; n.s.: not significant, as determined by a 2-way ANOVA with a Sidak's post-test.



Suppl Fig. S1 - Schuhwerk *et al*.



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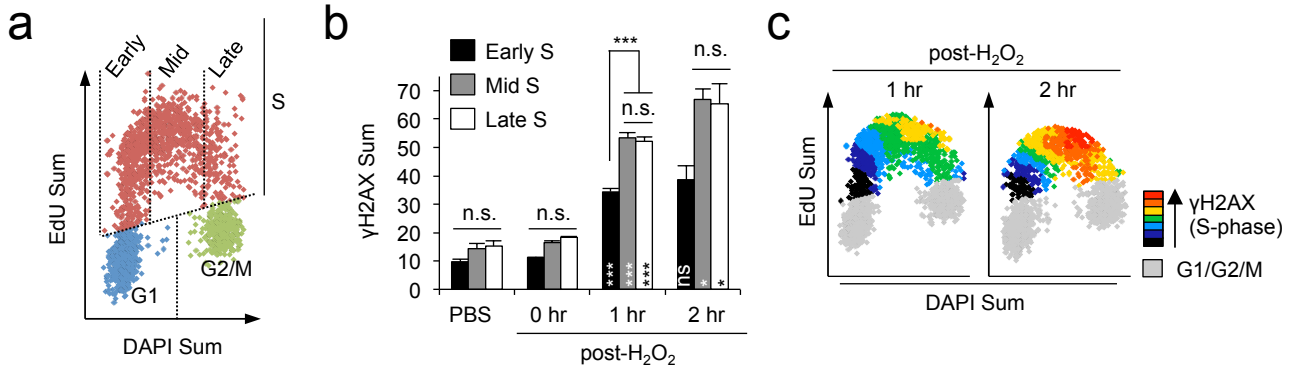
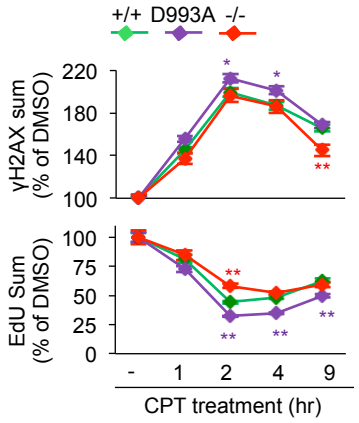


Suppl Fig. S3 - Schuhwerk *et al*.

a



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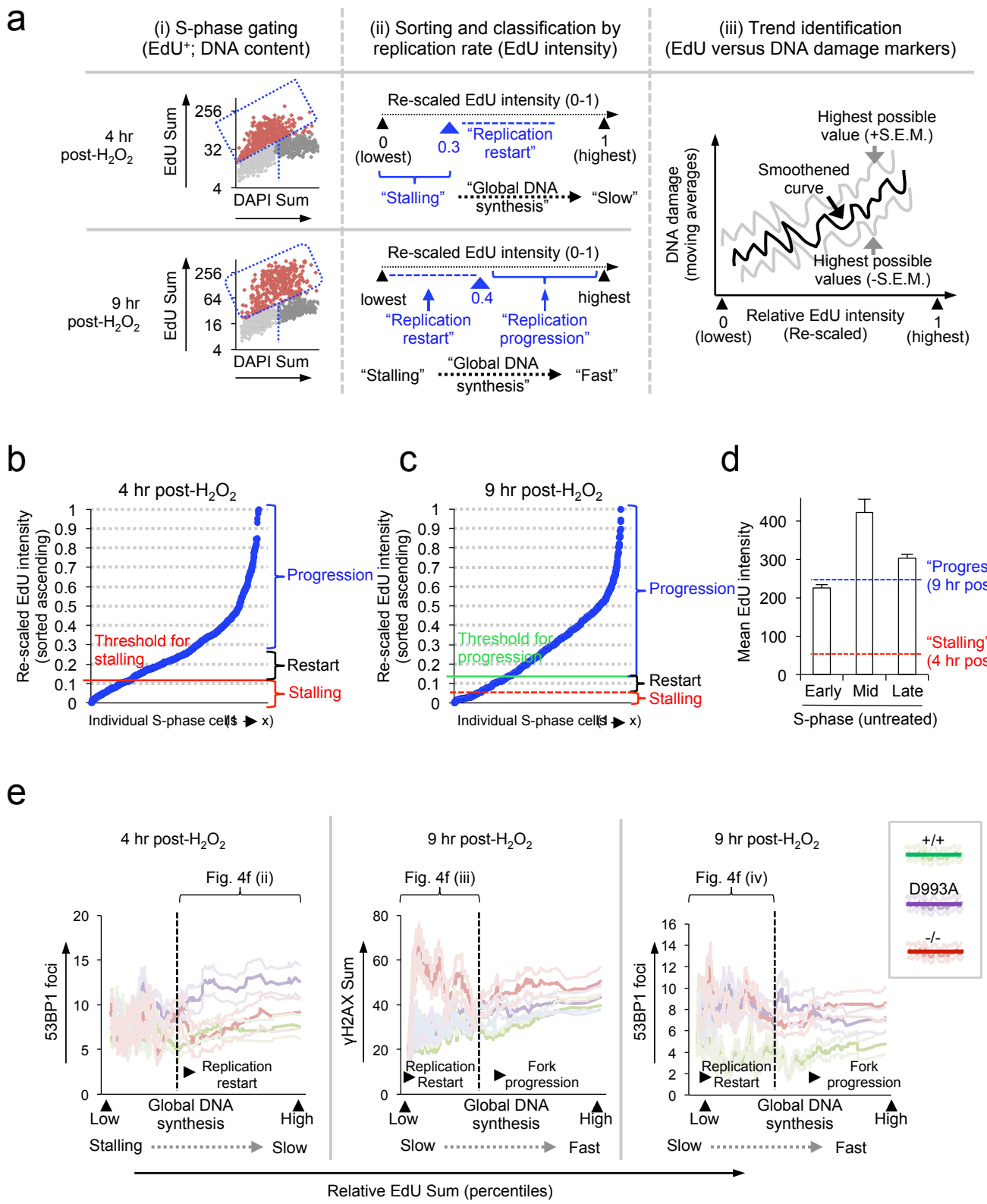

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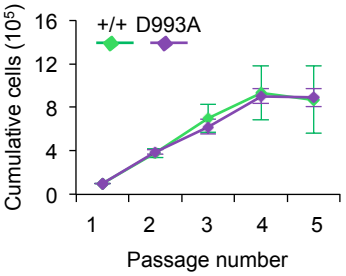
a



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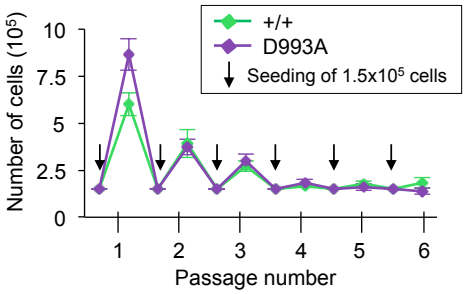

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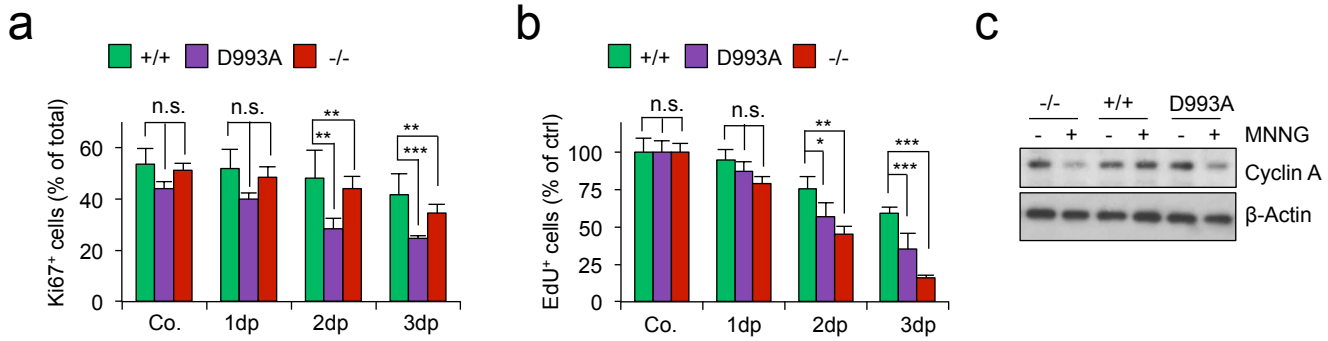

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