Appendix to “p53 status determines the epigenetic response to demethylating agents Azacitidine and Decitabine”. Hands et al.

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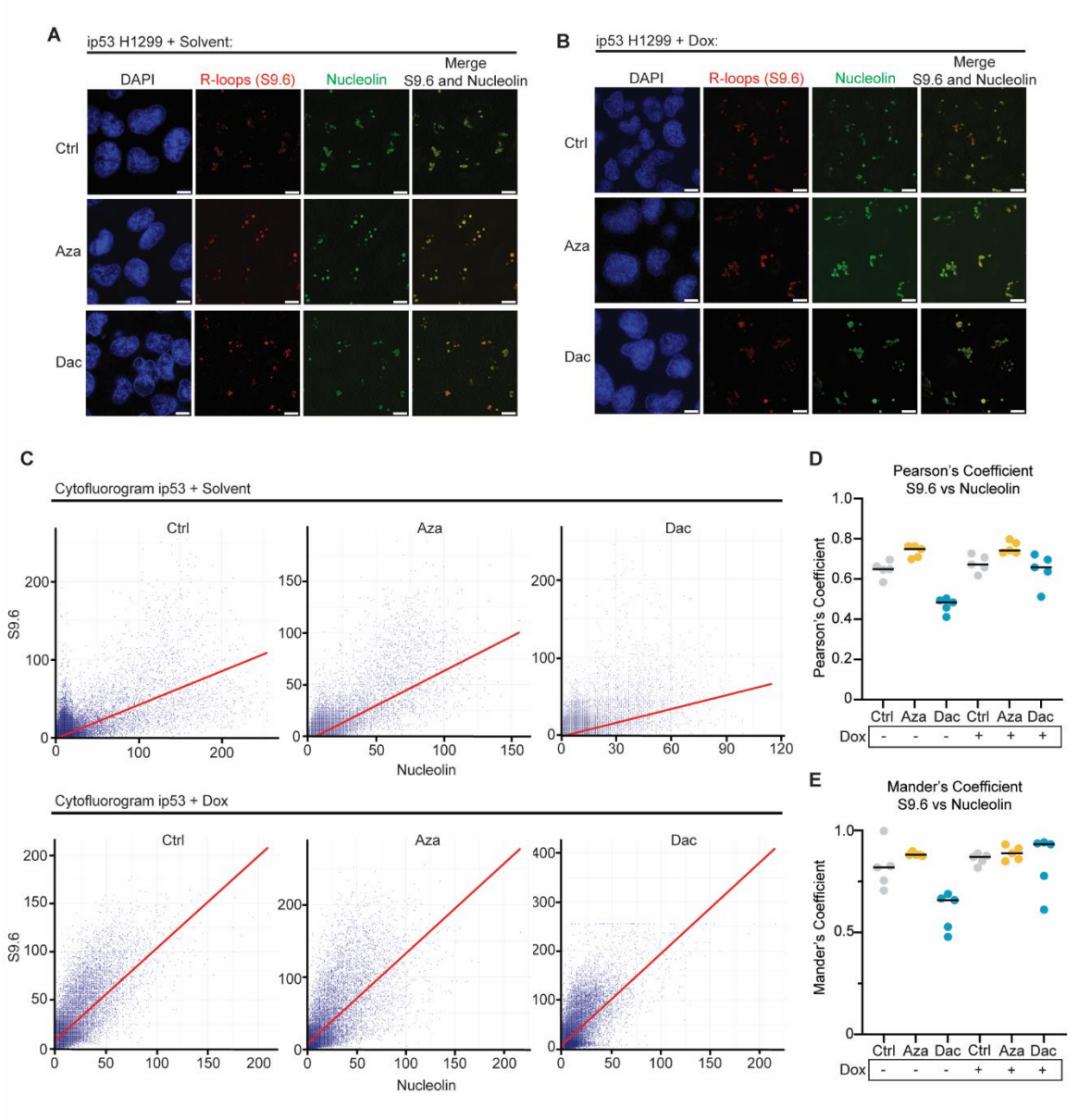
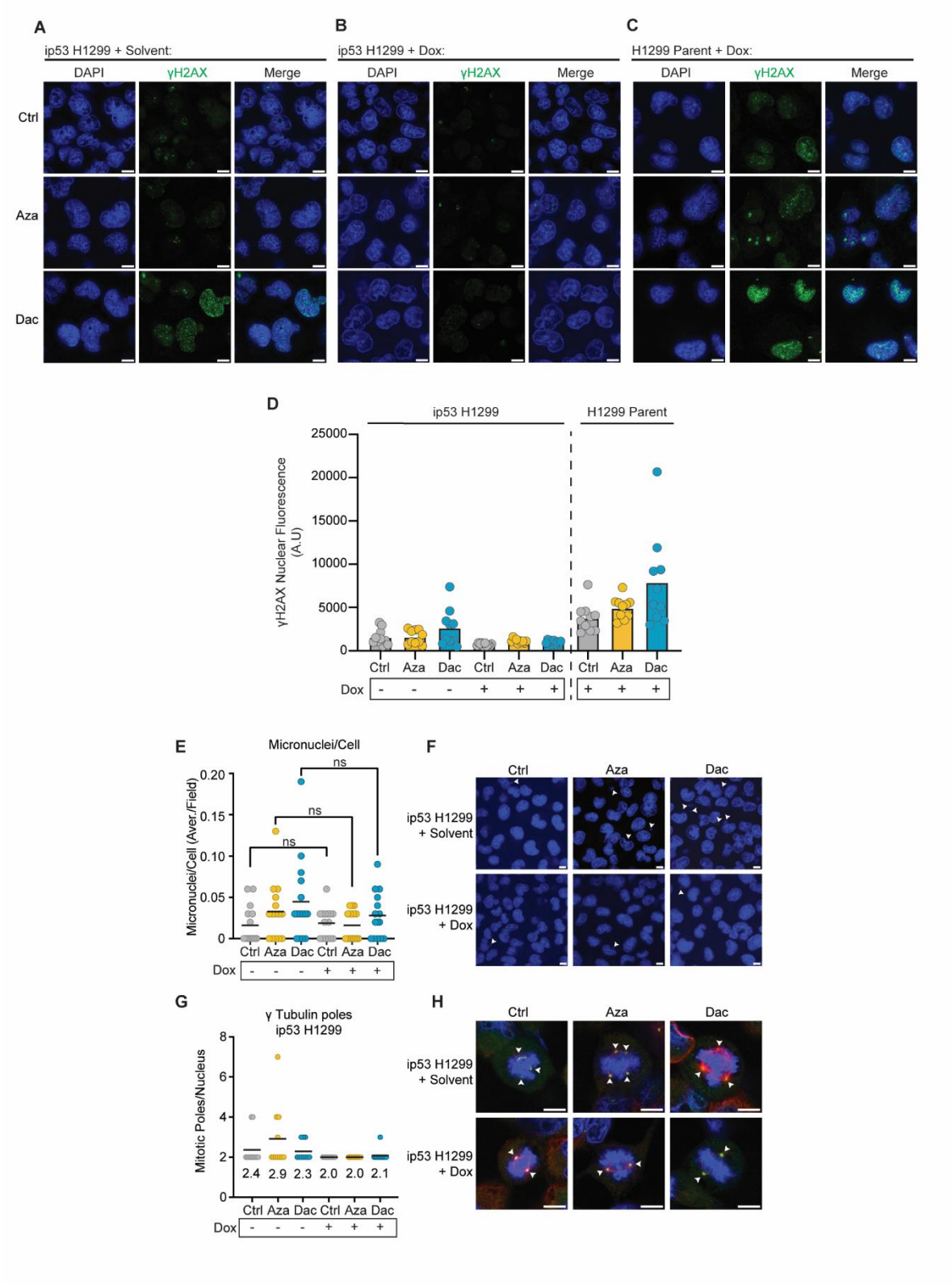


Appendix Figure S1: (**A-B**) Representative confocal images of ip53 H1299 using S9.6 to stain for R-loops (red), Nucleolin to stain for the Nucleoli (green), and DAPI as a counter stain for DNA (blue). A merge between the red and green channels is shown. Cells treated with Dox express p53, whilst cells treated with solvent do not. Scale bars are 10µm. (**C-E**) S9.6 and Nucleolin localisation analysis. For all analysis n = 2 biological repeats, ≥ 5 fields per replicate. (**C**) Cytofluorograms of Nucleolin staining vs S9.6 staining, with linear regression line (red), in ip53 H1299 cells. (**D**) Dot plot of Pearson's Coefficient scores from S9.6 and Nucleolin staining in ip53 H1299 cells. Each dot represents the score for one image containing ≥ 30 cells, a black line marks the median. (**E**) Dot plot of Mander's Coefficient scores from S9.6 and Nucleolin staining in ip53 H1299 cells. Each dot represents the score for one image containing ≥ 20 cells, a black line marks the median.



Appendix Figure S2: (**A-D**) γH2AX Analysis. (**A-B**) Representative Confocal images of γH2AX staining in ip53 H1299 cells, treated with (**A**) solvent control or (**B**) with Doxycycline. DAPI was used as a counter stain for DNA. Scale bars are 10μm. (**C**) Representative Confocal images of γH2AX staining in H1299 Parent cells, treated with

Doxycycline. DAPI was used as a counter stain for DNA. Scale bars are 10μm. (**D**) Bar graph of γH2AX Nuclear fluorescence intensity in ip53 H1299 cells and H1299 Parent cells. n = 2 biological repeats, five fields analysed per repeat. (**E-F**) Micronuclei analysis. The DAPI channel of the S9.6 and Nucleolin stained cells (see Appendix Figure S1A-B), and the DAPI channel of the γH2AX stained cells were used for Micronuclei analysis. An Ordinary one-way ANOVA with Šídák's multiple comparisons test was performed. (**E**) Dot plot of micronuclei quantification (count per cell) in ip53 H1299 cells treated with solvent or Doxycycline. Black line denotes the mean. n = 3 biological repeats with a minimum of 5 fields analysed per replicate. (**F**) Representative confocal images of micronuclei in ip53 H1299 cells. White arrows indicate the location of micronuclei, scale bars are 10μm. (**G-H**) γ-tubulin poles analysis**.** (**G**) Dot plot displaying quantification of mitotic poles in ip53 H1299 cells (all dividing cells in a field were analysed and the average γ-tubulin poles per nuclei was plotted as one data point). Black line indicates the mean. n = 2 biological repeats with a minimum of 10 fields analysed per repeat. (**H**) Representative confocal images of α-tubulin (red) and γ-tubulin (green) staining in ip53 H1299 cells. Arrows point out the γ-tubulin poles. Scale bars are 10μm. DAPI (blue) is used to counterstain DNA.