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Ash2L enables P53–dependent apoptosis by favoring stable transcription pre–initiation complex formation on its pro-apoptotic target promoters

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1: Induction of P53 establishes the H3K4me3 histone mark on its target promoters. a, **b**, **c**, **d**, **e**, **f**, ChIP analysis showing P53 occupancy (**a, b, c**) and H3K4me3 enrichment (**d, e, f**) on P53 target promoters in EJ–P53 cells, 24 h after tetracycline removal from the culture medium (**a, d**) or in HCT116 cells treated with Nutlin3a for 16 h (**b, e**) or HCT116 cells treated with 20 μM of Etoposide for 24 h (**c**, **f**). The target sequences were detected by quantitative real-time PCR analysis of eluted DNA. Relative promoter occupancy over the % input is shown in the form of a bar diagram.

Supplementary Fig. 2: SUV39H1 silencing does not induce H3K4me3 mark enrichment or its reader Ash2L occupancy on P53 pro–apoptotic target

promoters. a, **b**, ChIP analysis showing H3K4me3 enrichment (**a**) or Ash2L occupancy (**b**) on P53 pro–apoptotic target promoters in HCT116 WT cells stably transduced with inducible sh-SUV39H1 and cultured in the presence of doxycycline for the indicated time points. The target sequences were detected by quantitative real-time PCR analysis of eluted DNA. Relative promoter occupancy over the % input is shown in the form of a bar diagram.

Supplementary Fig. 3: Induction of P53 does not alter the cellular levels of H3K4me3 reader levels. a, **b**, Real-time quantitative PCR of EJ-P53 cells induced for p53 for the indicated time points (**a**) or B5/589 cells treated with MI–219 (**b**). mRNA expression levels are normalized to relative levels of 18S RNA. **c**, **d**, Western blot analysis of EJ-P53 cells induced for p53 for the indicated time points (**c**) or B5/589 cells treated with MI–219 (**d**). Tubulin blot was used as a lysate control.

Supplementary Fig. 4: Transcriptional Induction of P53 increases H3K4me3 reader's occupancy on P53 target promoters. ChIP analysis showing Wdr5 (**a**), RbBP5 (**b**) and Ash2L (**c**) occupancy on P53 target promoters in EJ–P53 cells, 24 h after tetracycline removal from the culture medium. The target sequences were detected by quantitative real-time PCR analysis of eluted DNA. Relative promoter occupancy over the % input is shown in the form of a bar diagram.

Supplementary Fig. 5: DNA damage stabilized P53 increases H3K4me3 reader's occupancy on P53 target promoters. ChIP analysis showing Wdr5 (**a**), RbBP5 (**b**)

and Ash2L (**c**) occupancy on P53 target promoters in HCT116 cells treated with 20 μM of etoposide for 24 h. The target sequences were detected by quantitative real-time PCR analysis of eluted DNA. Relative promoter occupancy over the % input is shown in the form of a bar diagram.

Supplementary Fig. 6: Ash2L overexpression enhances P53–dependent apoptosis by enhancing P53–dependent transcription. a, Propidium iodide (PI) staining of B5/589 cells overexpressing Ash2L and treated with increasing doses of Paclitaxol. The % of cells undergoing apoptosis (less than 2N content of DNA) is shown in the form of a line diagram. **b**, Real-time quantitative PCR of HCT116 cells overexpressing Ash2L and treated with Nutlin3a for 16 h. mRNA expression levels are normalized to relative levels of 18S RNA.

Supplementary Fig. 7: Ash2L overexpression enhances P53 occupancy and H3K4me3 enrichment on its pro-apoptotic target promoters, in response to P53 induction. a, **b**, ChIP analysis showing P53 occupancy (**a**) or H3K4me3 enrichment (**b**) on P53 pro-apoptotic target promoters in HCT116 control or Ash2L overexpressing cells and treated with Nutlin3a for 16 h. The target sequences were detected by quantitative real-time PCR analysis of eluted DNA. Relative promoter occupancy over the % input is shown in the form of bar diagram.

Supplementary Fig. 8: Ash2L silencing decreases P53–dependent transcription and apoptosis. a, **b**, Real-time quantitative PCR (**a**) and Western blot (**b**) analysis of

HCT116 cells stably transduced with inducible sh–Ash2L (Sequence 1) and cultured in the presence of doxycycline for 48 h followed by either treating cells with Nutlin3a for 16 h (**a**) or increasing doses of etoposide for 48 h (**b**). mRNA expression levels are normalized to relative levels of 18S RNA and tubulin blot was used as a lysate control in the western blot experiments. **c**, Propidium iodide (PI) staining in B5/589 cells stably transduced with inducible sh–Ash2L. The cells were cultured in the presence of doxycycline for 48 h followed by treatment with increasing doses of Paclitaxol for another 48 h.

Supplementary Fig. 9: Ash2L silencing does not alter Wdr5 occupancy on P53 pro-apoptotic target promoters in response to P53 induction. ChIP analysis showing Wdr5 occupancy on P53 pro-apoptotic target promoters in HCT116 cells stably transduced with either inducible sh-GFP or inducible sh–Ash2L (2 sequences) and cultured in the presence of doxycycline for 48 h followed by treating cells with Nutlin3a for 16 h. The target sequences were detected by quantitative real-time PCR analysis of eluted DNA. Relative promoter occupancy over the % input is shown in the form of bar diagram.

Supplementary Fig. 10: Ash2L silencing alters Ser5–CTD occupancy on P53 target promoters in response to P53 induction. a, **b**, ChIP analysis showing RNAP II (**a**) and Ser5–CTD (**b**) occupancy on P53 pro–apoptotic target promoters in HCT116 cells stably transduced with either inducible sh-GFP or inducible sh–Ash2L (2 sequences) and cultured in the presence of doxycycline for 48 h followed by treating

cells with Nutlin3a for 16 h. The target sequences were detected by quantitative real-time PCR analysis of eluted DNA. Relative promoter occupancy over the % input is shown in the form of bar diagram.

Supplementary Fig. 11: Ash2L silencing does not alter cellular levels of GTFs in response to P53 induction. Western blot analysis of B5/589 cells stably transduced with inducible sh–Ash2L and cultured in the presence of doxycycline for 48 h followed by treating cells with MI–219 for 16 h. Tubulin blot was used as a lysate control.

Supplementary Fig. 12: Ash2L silencing alters TFIIF and TFIIB occupancy on P53 target promoters in response to P53 induction. a, **b**, ChIP analysis showing TFIIF (**a**) and TFIIB (**b**) occupancy on P53 pro–apoptotic target promoters in HCT116 cells stably transduced with either inducible sh-GFP or inducible sh–Ash2L (2 sequences) and cultured in the presence of doxycycline for 48 h followed by treating cells with Nutlin3a for 16 h. The target sequences were detected by quantitative real-time PCR analysis of eluted DNA. Relative promoter occupancy over the % input is shown in the form of bar diagram.

Supplementary Table 1: Shows the list of Primer Sequences used in this manuscript.