

Inhibition of SUV39H1 as a potent therapeutic target in multiple myeloma

Authors

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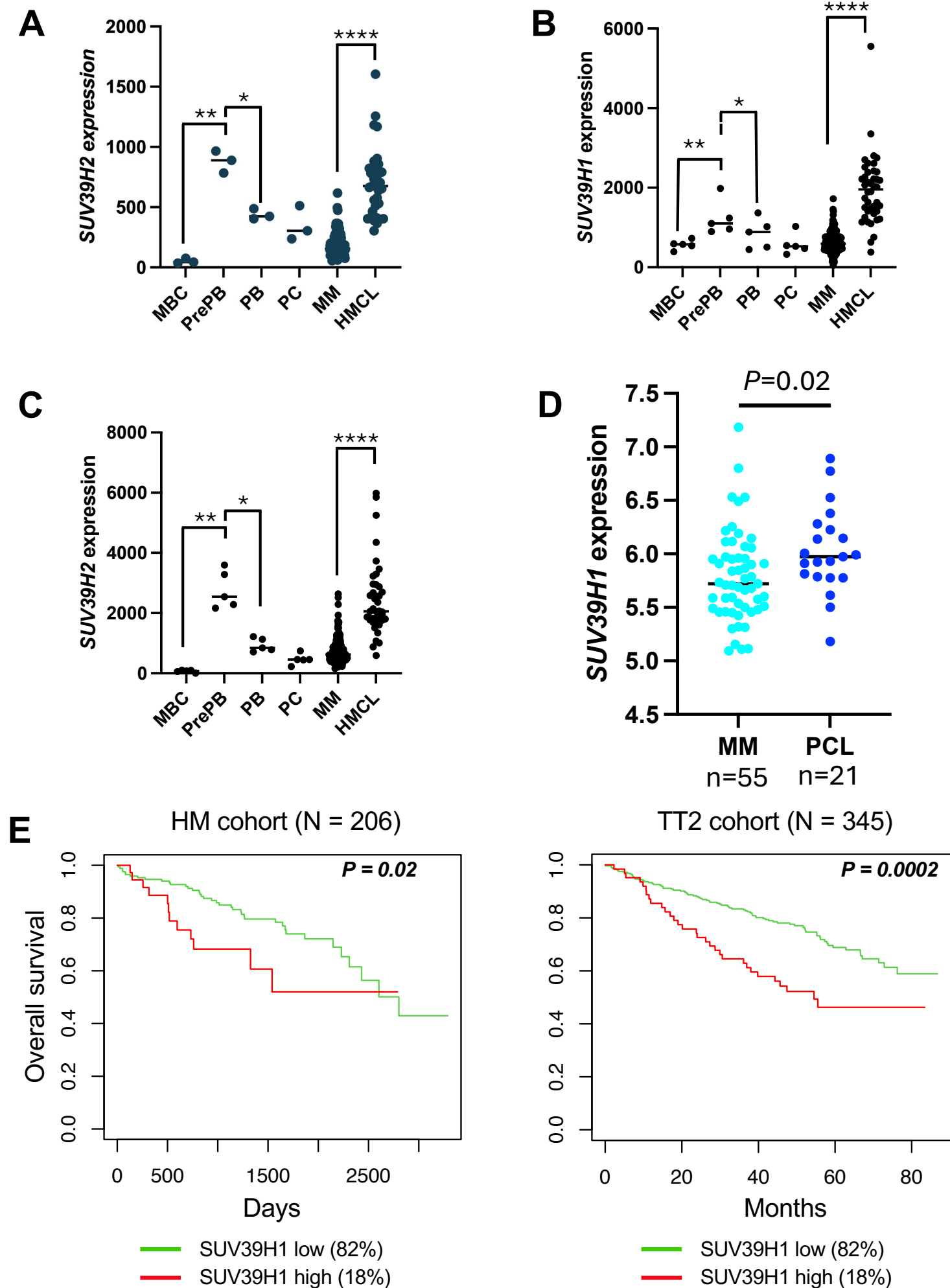
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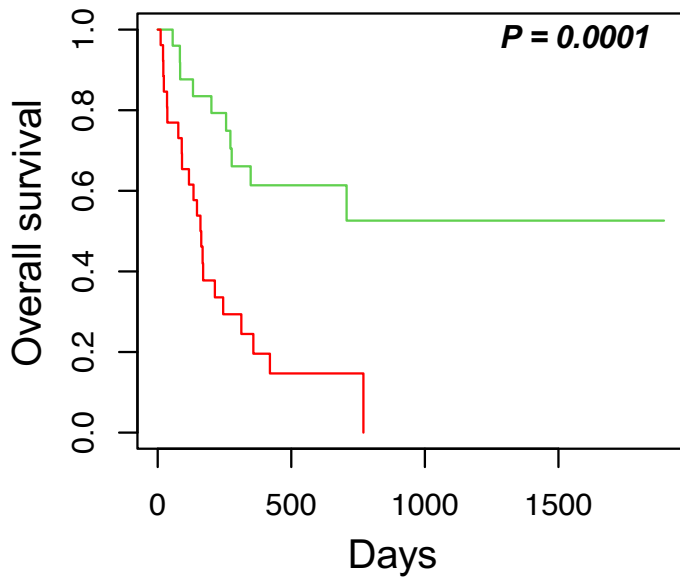
Supplementary Figure S1



Supplementary Figure S1

F

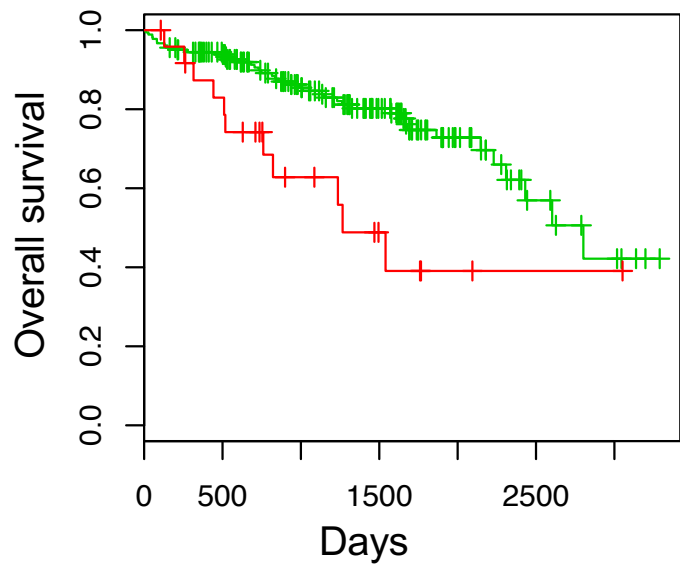
Dara cohort (N = 51)



SUV39H1 low (n=25)
SUV39H1 high (n=26)

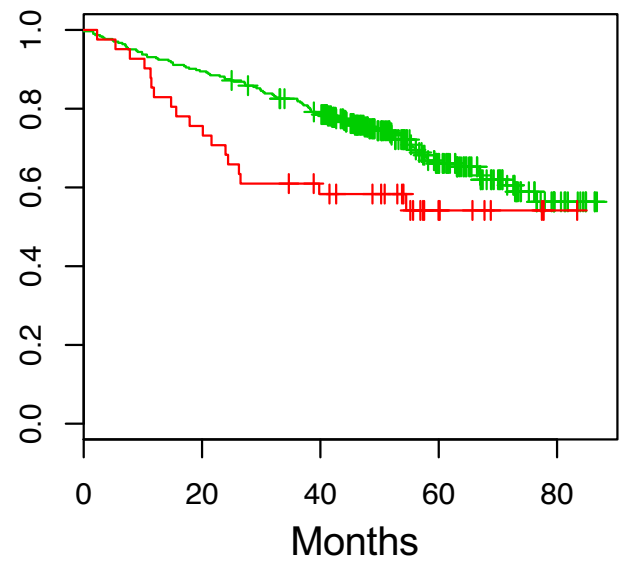
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HM cohort (N = 206)



SUV39H2 low (89%)
SUV39H2 high (11%)

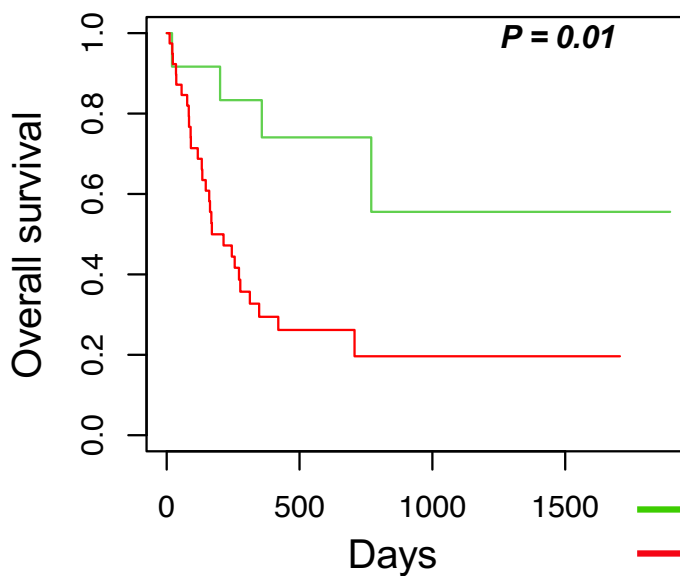
TT2 cohort (N = 345)



SUV39H2 low (89%)
SUV39H2 high (11%)

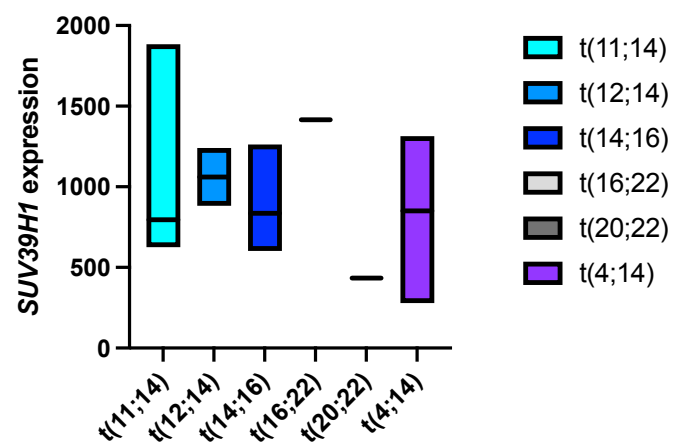
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Dara cohort (N = 51)



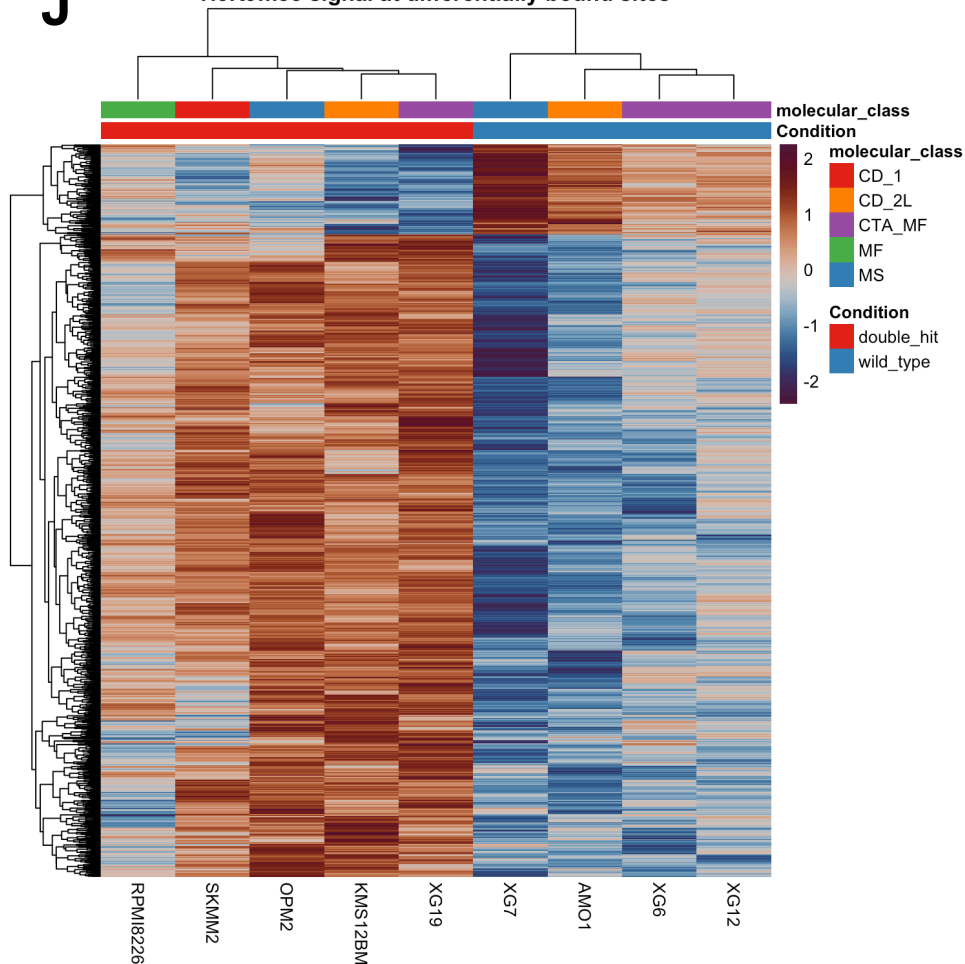
SUV39H2 low (n=12)
SUV39H2 high (n=39)

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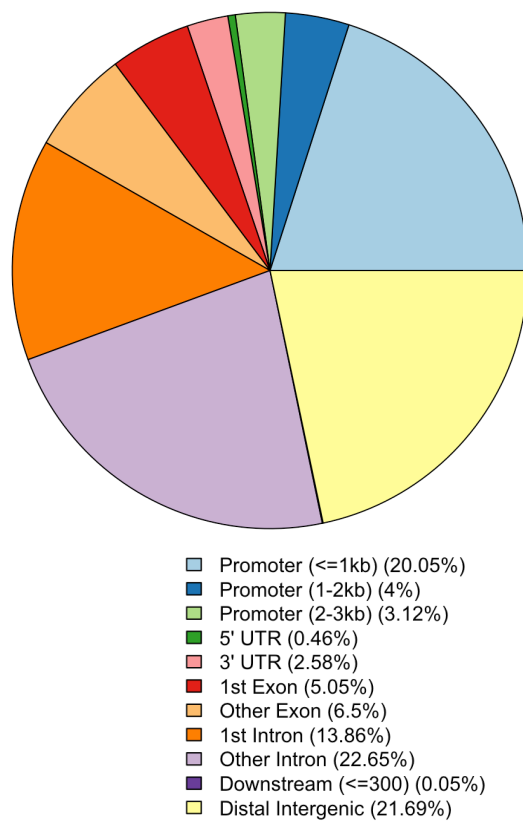


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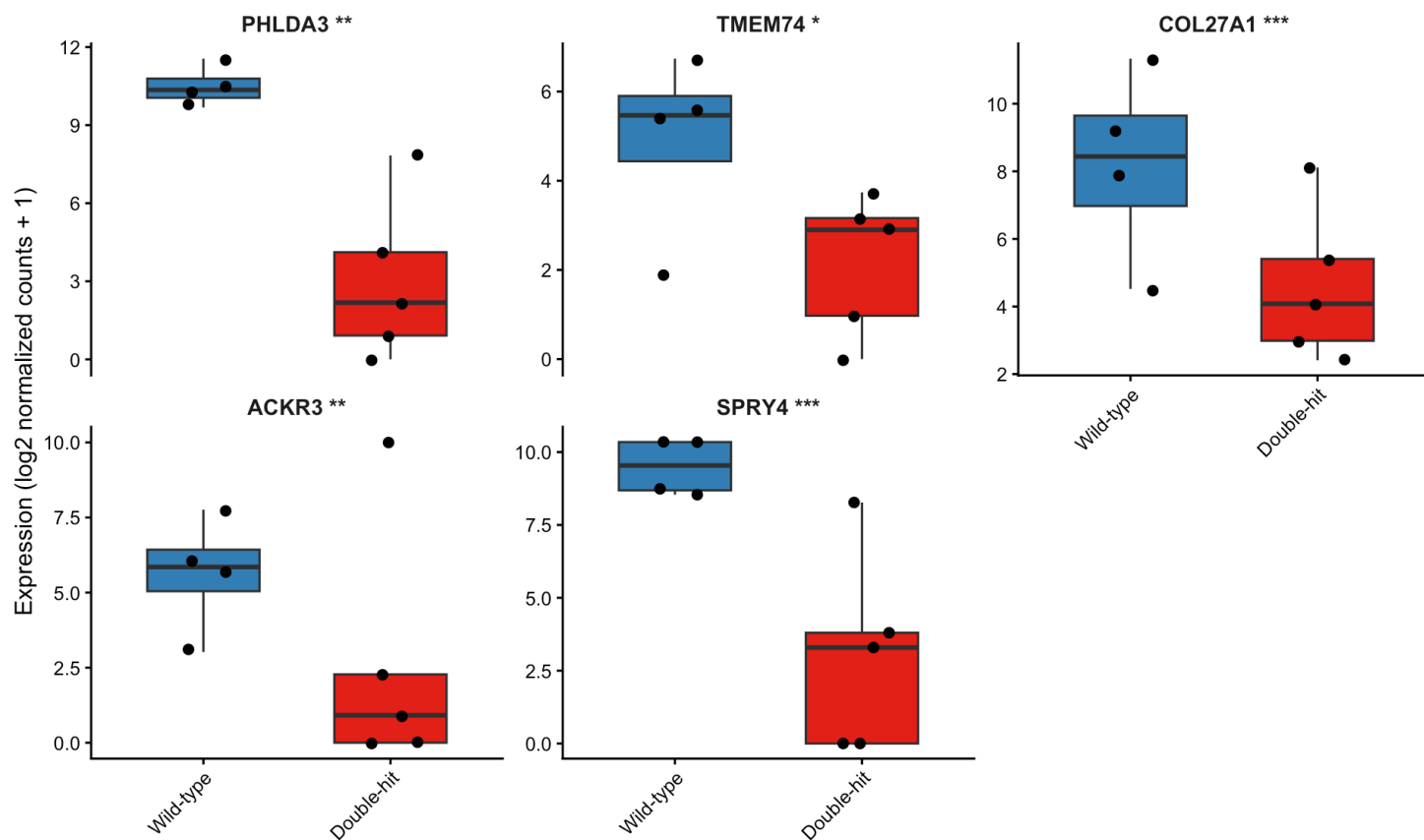
H3K9me3 signal at differentially bound sites



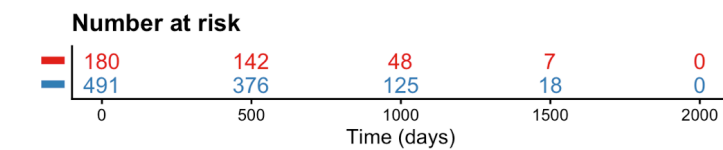
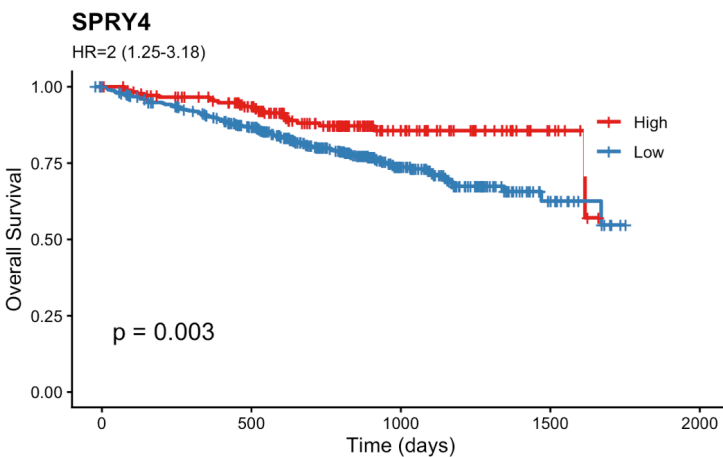
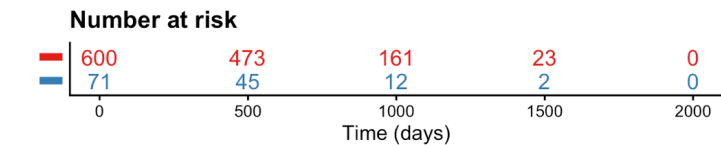
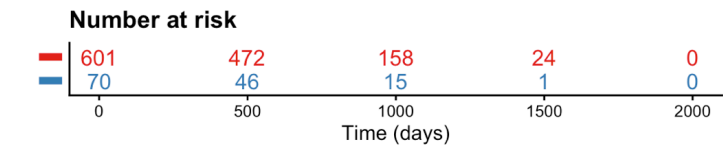
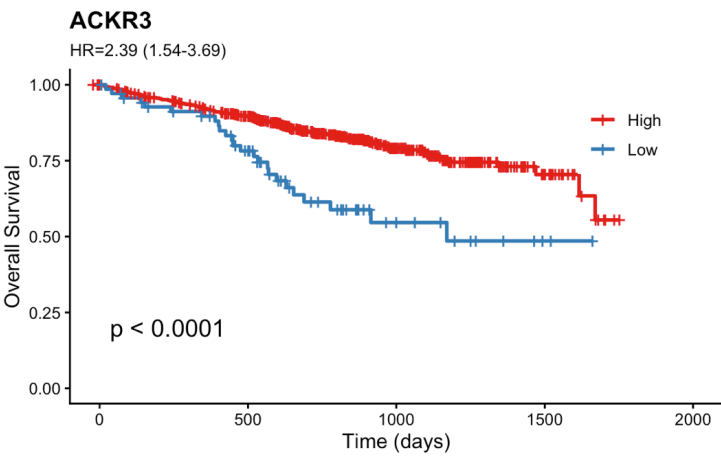
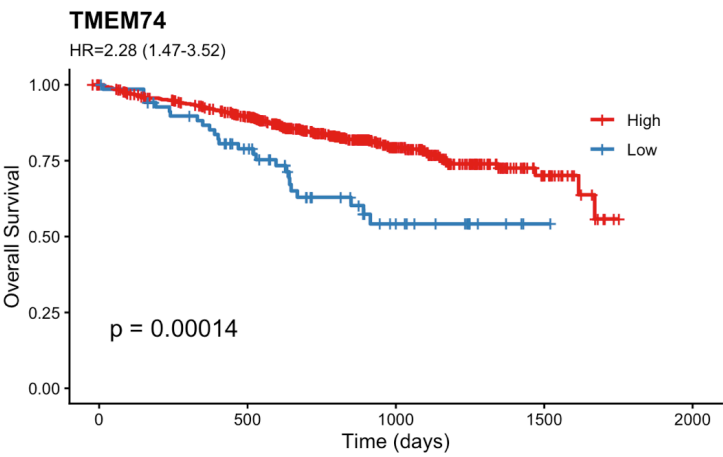
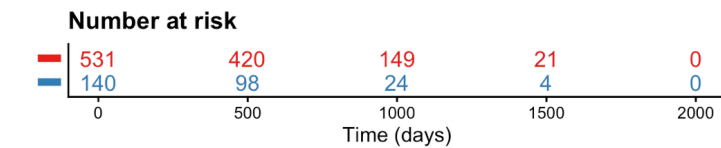
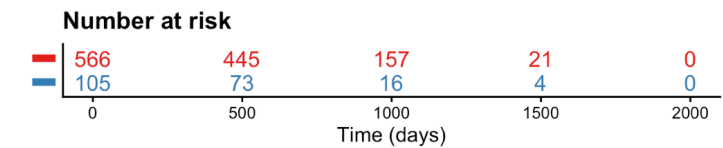
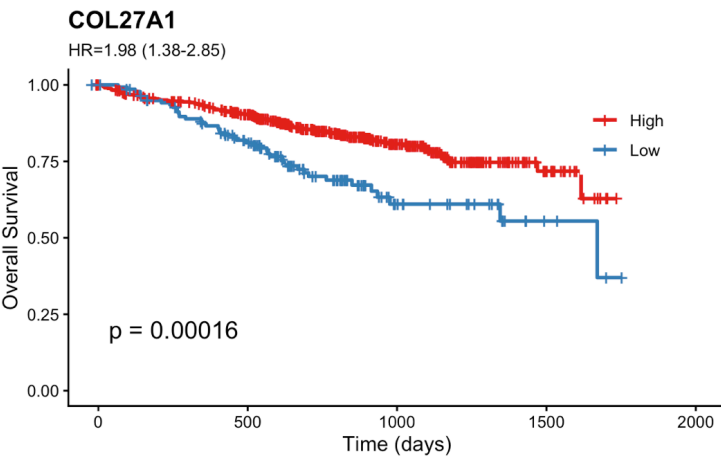
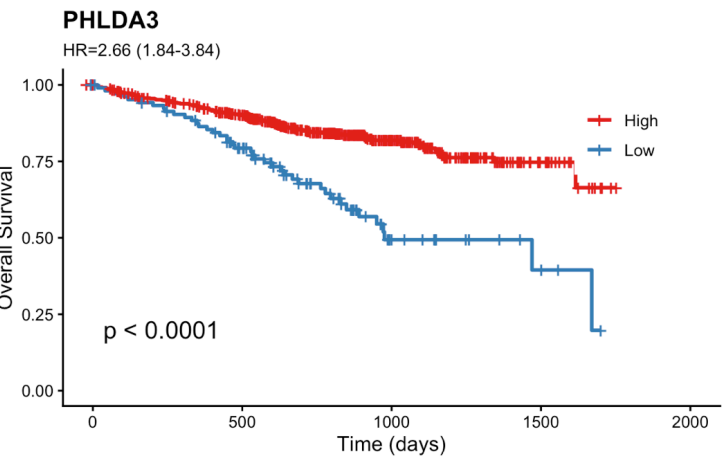
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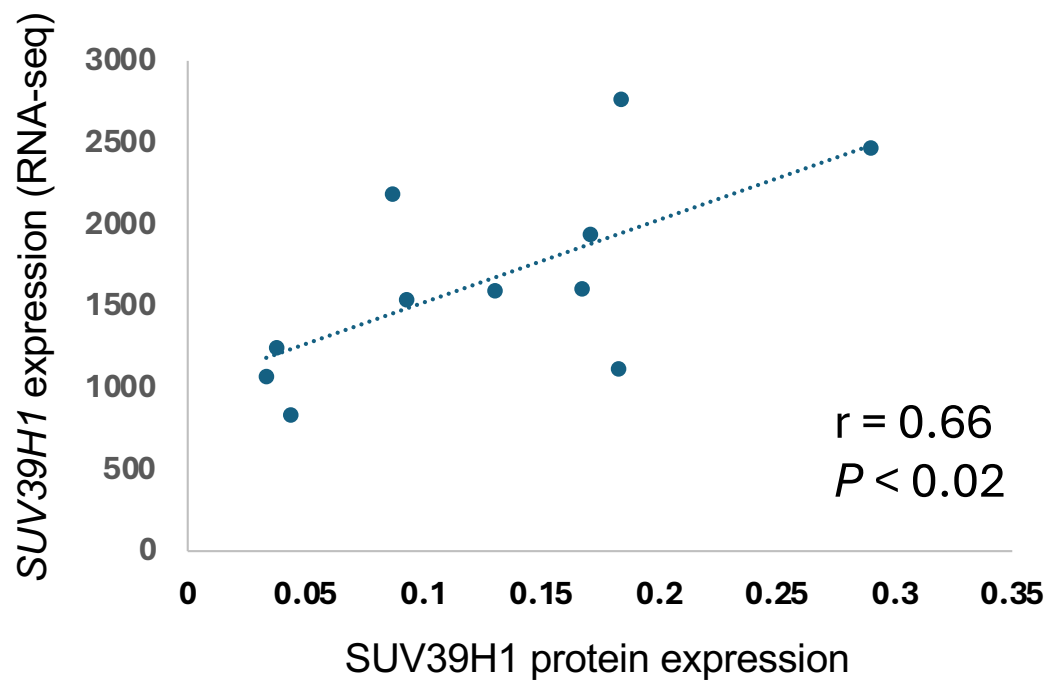
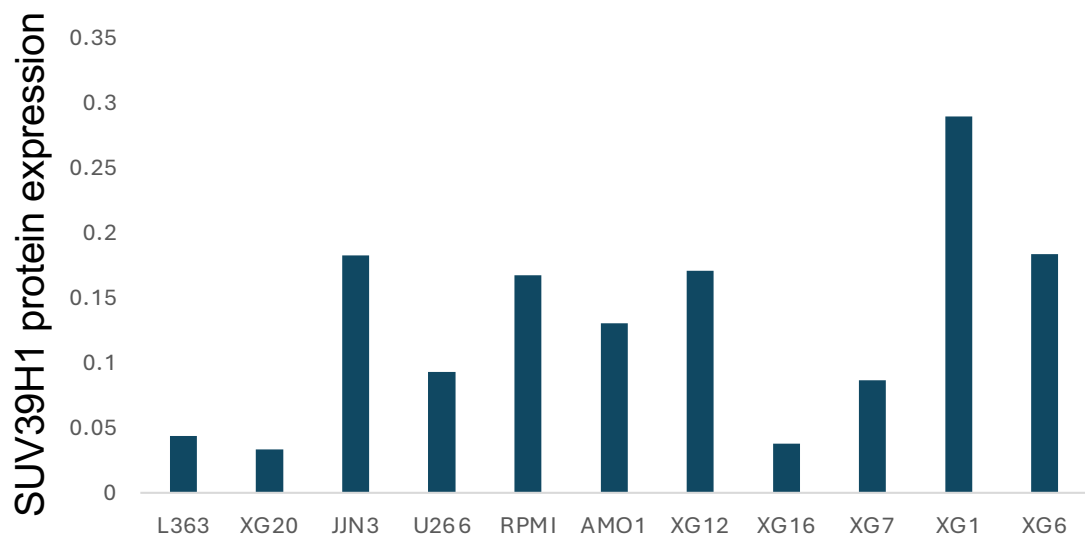
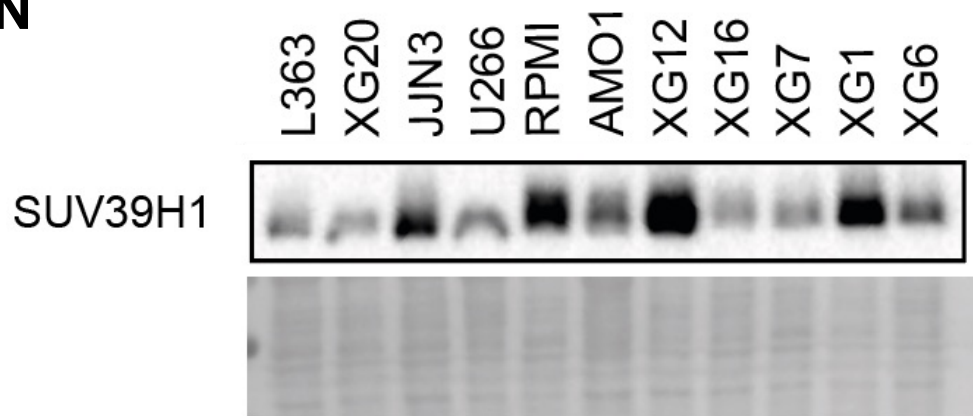
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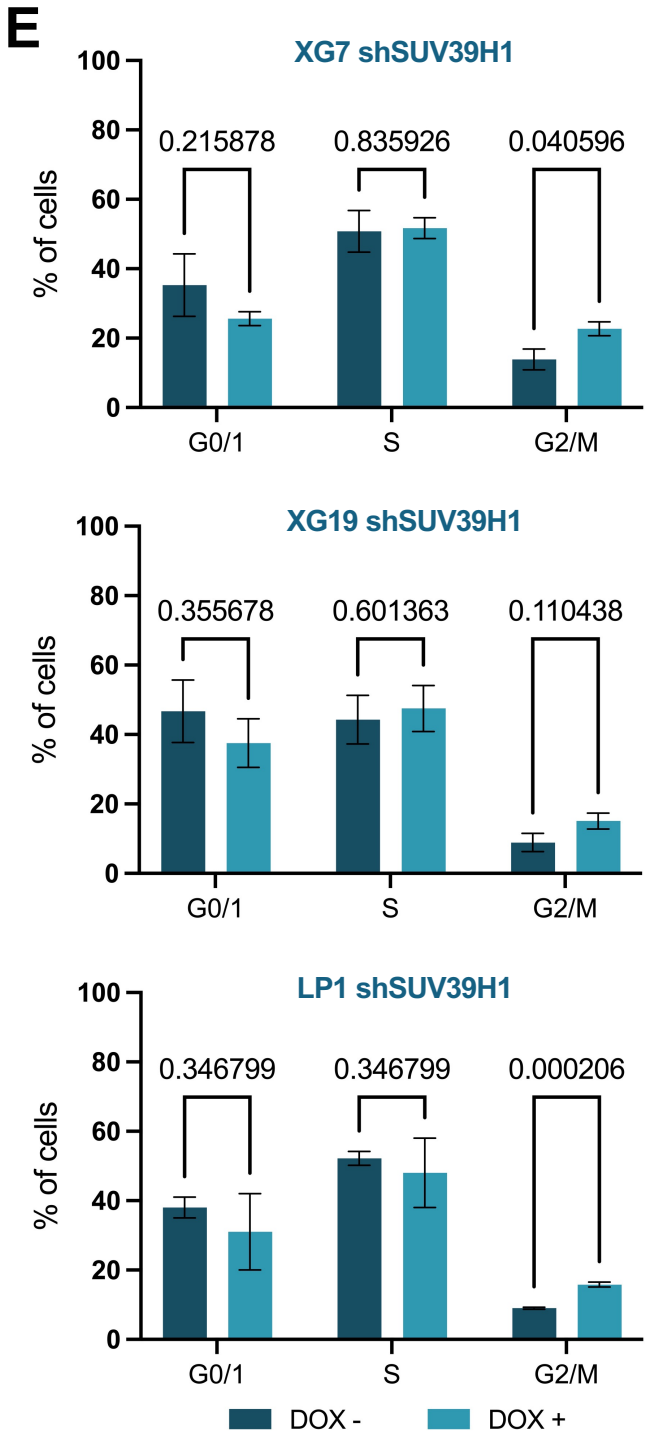
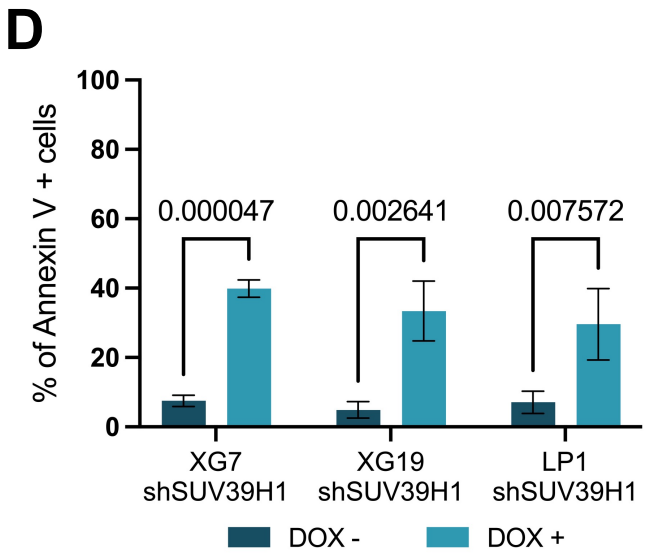
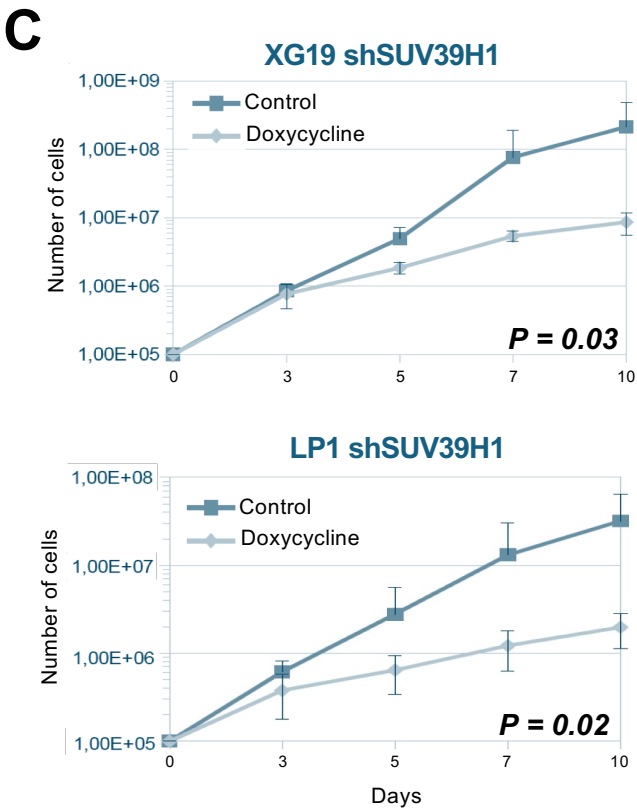
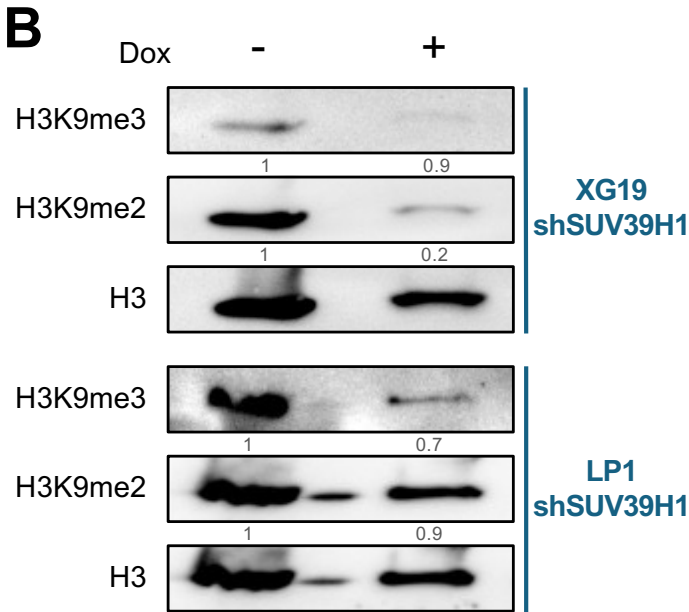
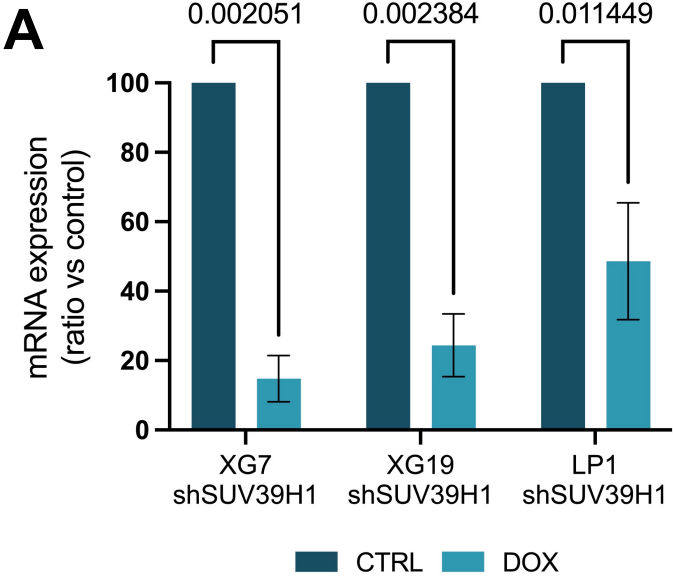
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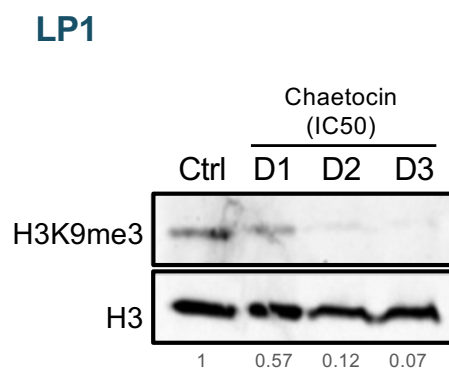
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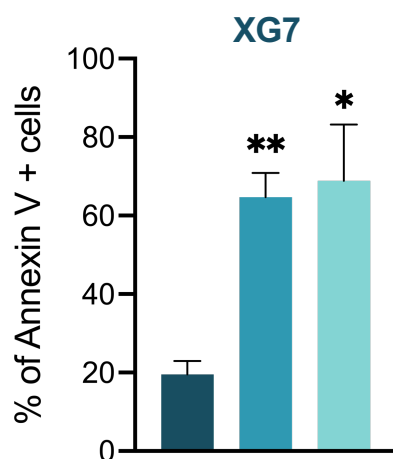
Supplementary Figure S2



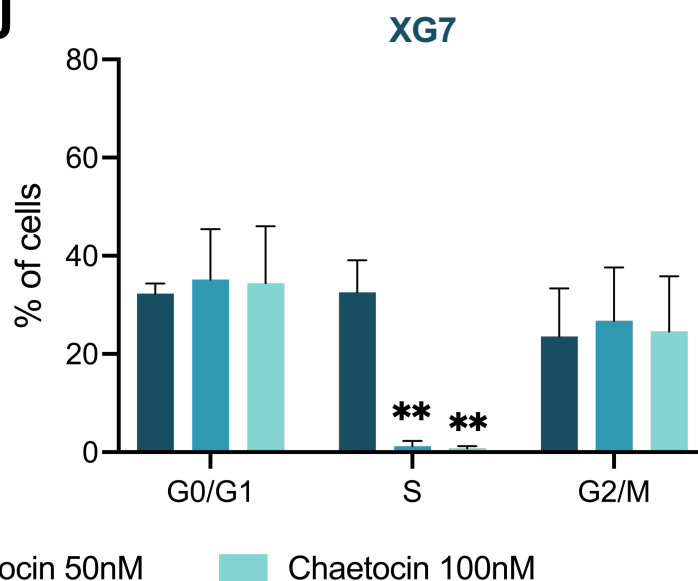
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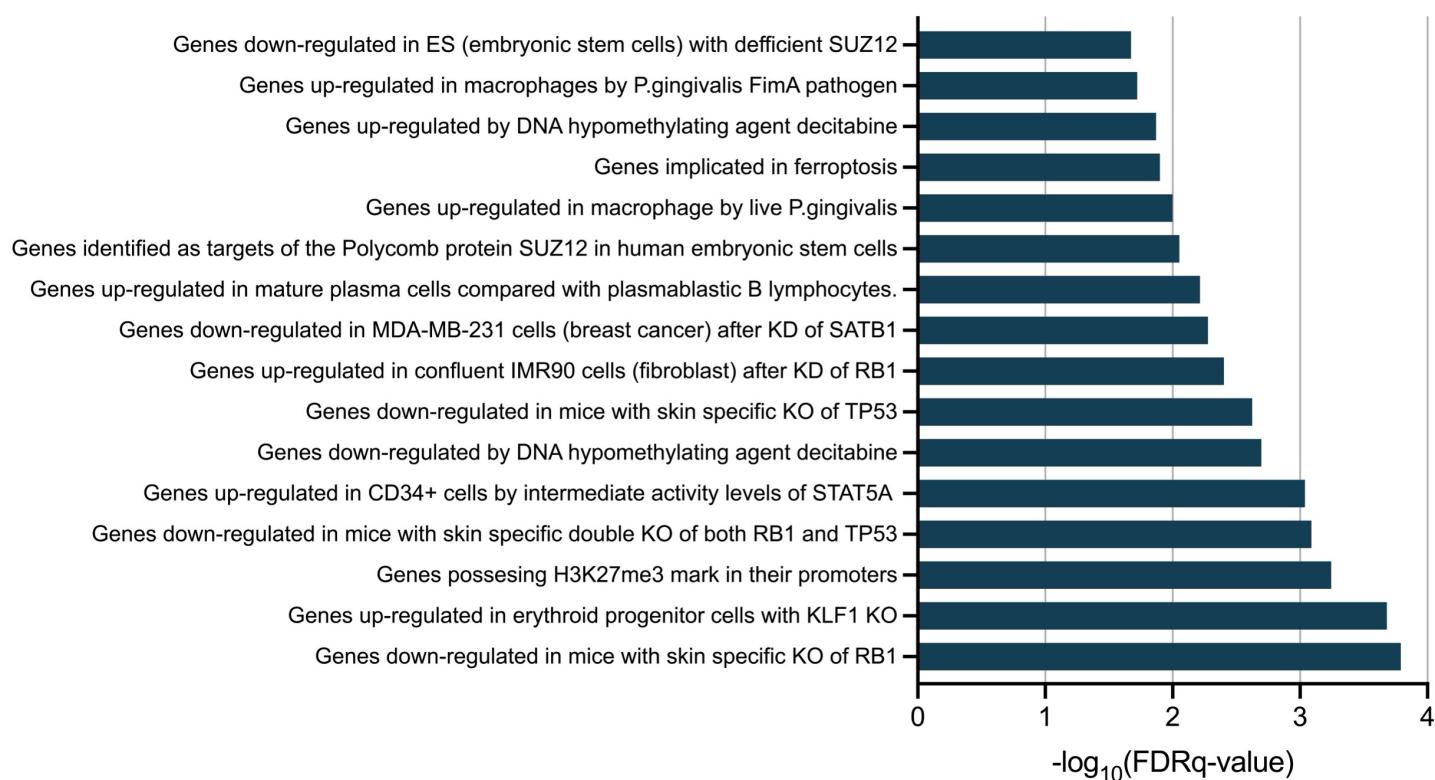


J



K

GSEA : Up-regulated genes in SUV39H1 depleted cells



Supplementary Figure S1:

(A) *SUV39H2* expression was quantified using RNA-Sequencing in memory B cells (MBC), pre plasmablasts (PrePB), plasmablasts (PB), and plasma cells (PC) (n = 3), primary multiple myeloma (MM) samples (n = 97), and human myeloma cell lines (HMCLs) (n = 33). *P* values were calculated using a wilcoxon test: **: p-value < 0.01; ****: p-value < 0,0001. **(B)** *SUV39H1* expression was investigated using Affymetrix U133P microarrays in memory B cells (MBC), pre plasmablasts (PrePB), plasmablasts (PB), and plasma cells (PC) (n = 5), primary multiple myeloma (MM) samples (n = 206), and human myeloma cell lines (HMCLs) (n = 42)(ArrayExpress public database under accession numbers E-TABM-937 and E-TABM-1088). *P* values were calculated using a paired or heteroscedastic Student's *t*-test: **: p-value < 0.01; ****: p-value < 0,0001. **(C)** *SUV39H2* expression was analyzed using Affymetrix U133P microarrays in memory B cells (MBC), pre plasmablasts (PrePB), plasmablasts (PB), and plasma cells (PC) (n = 5), primary multiple myeloma (MM) samples (n = 206), and human myeloma cell lines (HMCLs) (n = 42)(ArrayExpress public database under accession numbers E-TABM-937 and E-TABM-1088). *P* values were calculated using a wilcoxon test: **: p-value < 0.01; ****: p-value < 0,0001. **(D)** The *SUV39H1* expression was investigated in purified plasma cells from patients with MM (n=55) and plasma cell leukemia (PCL; n=21) using the Affymetrix publicly available data (Gene Expression Omnibus GSE39925). **(E-F)** Patients of the HM cohort (n = 206) (Array Express public database (E-MTAB-372), TT2 cohort (n = 345) (GSE24080) and Dara cohort (n = 51) were ranked according to *SUV39H1* level and the patients were split into high or low *SUV39H1* expression. High expression of *SUV39H1* was significantly associated with high-risk in MM patients. **(G-H)** Patients of the HM cohort (n = 206), TT2 cohort (n = 345) and Dara cohort (n = 51) were ranked according to *SUV39H2* level and the patients were split into high or low *SUV39H2* expression. High expression of *SUV39H2* was significantly associated with high-risk in MM patients. **(I)** *SUV39H1* expression was investigated using RNA-Sequencing in human myeloma cell lines (HMCLs) (n = 33) according to the different recurrent translocations. No significant differences were identified. **(J)** H3K9me3 signal heatmap at differentially bound sites. Row-scaled binding affinity (z-score) shows significant separation between TP53 double-hit HMCLs (red) and wild-type TP53 (blue) HMCLs with a specific H3K9me3 signature in TP3 double-hit MM cells. **(K)** Genomic features distribution of differentially bound regions (DBR). DBRs are enriched in promoter regions (27%) and intronic sequences (36%) with distal intergenic regions comprising 22% of sites. **(L)** Genes expression of PHLDA3, TMEM74 COL27A1, ACKR3 and SPRY4 in HMCLs with TP53 double-hits (red) and wild type TP53 (blue). Boxplots represent DESeq2-normalized log2 counts. Asterisks indicate DESeq2 *P* values. *SUV39H1* protein expression was investigated using western blot in a panel of 11 HMCLs. **(M)** Kaplan-Meier plots of potential tumor suppressive genes associated with specific H3K9me3 repressive marks (promoter) and underexpressed in TP53 double hit versus wild-type MMRF CoMMpass patients and cell lines. **(N)** *SUV39H1* protein expression was quantified and the correlation with *SUV39H1* gene expression (RNA-sequencing) was determined using the Pearson correlation test.

Supplementary Figure S2:

(A) The shRNAs to SUV39H1 (Invitrogen, Carlsbad, USA) were cloned in the pLenti4-EZ-miR plasmid (Invitrogen). This plasmid contains the shRNA sequence and also the *GFP* gene under the control of Tet operators. Total mRNA was extracted from XG7, XG19 and LP1 cells treated as indicated for 3 days, subjected to retro transcription and the level of SUV39H1 gene was quantified by qPCR. Graph show the average $\pm$ SD of 3 independent replicates. *P* values were calculated using Wilcoxon test for pairs. **(B)** Cells were treated or not with doxycycline (1 μ g/ml) for 3 days, collected and the indicated proteins were analyzed by western blot in whole-cell lysates. **(C)** The curves represent cumulative cell growth without induction (Control) and with induction of the anti-SUV39H1 shRNA following doxycycline (1 μ g/ml) treatment (Doxycycline). Cell growth was assessed over 10 days by trypan blue counting. The curves show the means, and the error bars indicate the standard deviations from three independent experiments. *P* values were calculated using a paired Student's *t*-test. **(D)** Cells were treated or not with doxycycline for 7 days. Annexin V + cells were detected by flow cytometry and quantified. Result shows the average $\pm$ SD of 3 independent replicates. *P* values were calculated using Wilcoxon test for pairs. **(E)** Cells were treated or not with doxycycline for 7 days. BrdU (10 μ g/ml) was added during the last 1.5h of treatment. Cells were fixed and processed to detect BrdU incorporation and total DNA by flow cytometry. The graph show average $\pm$ SD of 3 independent replicates. *P* values were calculated using Wilcoxon test for pairs. **(F)** XG19 doxycycline-inducible shSUV39H1 cells were pretreated or not with doxycycline (1 μ g/ml) for 2 days. Then, treated with increasing concentrations of melphalan (0,015 to 40 μ M) for 4 days. IC50 was calculated after viability assessment by CTG luminescent cell viability assay. The graph represents average $\pm$ SD of 3 independent experiments. *P* values were calculated using Wilcoxon test for pairs. **(G)** Correlation between *SUV39H1* expression and response to melphalan in 17 HMCLs. Linear regression analysis of *SUV39H1* expression (RNA-seq) and IC50 of melphalan in 17 HMCLs. *R* represent the Pearson correlation coefficient (Pearson correlation test). **(H)** LP1 cells were treated with or without chaetocin at the IC50 (14 nM) for 3 days. Cells were collected at day 1, 2 and 3, and the indicated proteins were analyzed by western blot in whole-cell lysates. **(I, J)** XG7 cells were treated or not with chaetocin (50 and 100 nM) for 24h. **(I)** Annexin V was detected by flow cytometry. **(J)** BrdU (10 μ g/ml) was added during the last 1.5h of treatment. Cells were fixed and processed to detect BrdU incorporation and total DNA by flow cytometry. The results represent the average $\pm$ SD of 3 independent replicates. *P* values were calculated using Wilcoxon test for pairs. **(K)** XG19 and XG7 transduced with a doxycycline-inducible lentivirus containing a SUV39H1 shRNA were treated with doxycycline (1 mg/ml) for 2 days and gene expression profiles were analyzed using Affymetrix U133 plus 2.0 microarrays. LP1, XG19 and XG7 HMCLs were also treated with chaetocin for 2 days. Total RNA was extracted. RNA sequencing was performed and GSEA was applied to find upregulated pathways. FDR: false discovery rate.

Supplementary table S1: Cox univariate and multivariate analyses to model overall survival in the CoMMpass cohort (n = 674 patients with MM) relative to the *SUV39H1*, *SUV39H2* expression and cytogenetic abnormalities. HR: Hazard Ratio.

A		
Univariate COX Analyses		
Prognostic variable	Proportional HR	P-value
SUV39H1 expression	1.638	0.0005
SUV39H2 expression	1.992	0.001
del17p	2.123	0.003
1q gain	2.211	<0.0001

B		
Multivariate COX analysis (cytogenetic abnormalities)		
Prognostic variable	Proportional HR	P-value
SUV39H1 expression	2.895	<0.0001
del17p	1.82	0.02
SUV39H1 expression	2.467	<0.0001
1q gain	2.08	<0.0001

C		
Multivariate COX analysis (cytogenetic abnormalities)		
Prognostic variable	Proportional HR	P-value
SUV39H2 expression	2.86	<0.0001
del17p	1.839	0.009
SUV39H2 expression	1.858	0.004
1q gain	2.227	<0.0001