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Inhibition of SUV39H1 as a potent therapeutic target in multiple myeloma

Laura Alibert[1], Sara Ovejero[1,2], Julie Devin[1], Morgane Thomas[1], Alboukadel Kassambara[3], Matthieu Angles[1], Guilhem Requirand[2], Nicolas Robert[2], Laure Vincent[4], Guillaume Cartron[4,5], Anne Marie Martinez[1], Charles Herbaux[1,4,5], Giacomo Cavalli[1], Jérôme Moreaux[1,2,5,6,§], Caroline Bret[1,2,5§].

[1] Institut de Génétique Humaine, Univ Montpellier, CNRS, Montpellier, France;
[2] Department of Biological Hematology, CHU Montpellier, Montpellier, France;
[3] Helomnix, Montpellier, France;
[4] CHU Montpellier, Department of Clinical Hematology, Montpellier, France.
[5] UFR Médecine, Univ Montpellier, Montpellier, France.
[6] Institut Universitaire de France (IUF), Paris, France
[§] Equal contribution

Corresponding authors: caroline.bret@igh.cnrs.fr and jerome.moreaux@igh.cnrs.fr

Short title: Inhibition of SUV39H1 in MM

Data availability

For original data, please contact c-bret@chu-montpellier.fr and jerome.moreaux@igh.cnrs.fr. Gene expression data have been deposited in the ArrayExpress public database under accession numbers E-TABM-937, E-MTAB-372 and E-TABM-1088.

Conflict of Interest Disclosures

The authors declare no conflict of interest.

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Author's contributions

LA, SO and JD performed research and participated in the writing of the paper. MT, GR and NR participated in the research. GC, LV, and CH participated in clinical data analysis and participated in the writing of the paper. AK and MA participated in bio-informatic analyses. AMM and GC participated in the research and in the writing of the paper. JM and CB supervised the research and the writing of the paper.

Multiple Myeloma (MM) is a frequent hematological malignancy characterized by the accumulation of malignant plasma cells within the bone marrow. Genomic studies have shown substantial heterogeneity and genomic instability, a complex mutation profile, and branching patterns of clonal development(1). Despite significant improvements in patient outcomes over the past decade through the use of immunomodulatory drugs (IMiDs), proteasome inhibitors, and the introduction of immunotherapies, patients ultimately experience disease recurrence(1).

Recent studies have identified that alongside a diverse array of genetic mutations, epigenetic changes may play significant roles in the development of MM and mechanisms of drug resistance(2). These discoveries could shed light on new mechanisms fundamental to MM's origin and potentially reveal innovative approaches and targets for effective treatment.

Biallelic TP53 inactivation is the most important high-risk factor associated with poor survival in MM. We previously described that *TP53* bi-allelic events are associated with a specific H3K9me3 signature in MM cells(2). Three proteins primarily catalyze H3K9me3 including SUV39H1, SUV39H2 and SETDB1 histone methyltransferases (HMTs). SUV39H1, the histone methyltransferase that creates the H3K9me3 mark, is repressed by p53(3). Overexpression of SUV39H1 maintains high H3K9me3 at p53 target promoters, reduces p53 recruitment and dampens p53-dependant transcription and apoptosis, whereas SUV39H1 knockdown enhances p53 target gene expression(3). This suggests that *TP53* alterations could alter H3K9me3 levels on these promoters, thereby affecting the p53-mediated apoptotic response. Thus, we investigated if SUV39H1 and SUV39H2 HMTs play a role in MM pathophysiology.

Investigating *SUV39H1* and *SUV39H2* gene expression in normal B to plasma cell (PC) differentiation, purified MM cells from newly-diagnosed patients (n=97) and human myeloma cell lines (HMCLs) (n=33), we identified a significant higher expression of *SUV39H1* and *SUV39H2* in HMCLs compared to primary MM cells from patients and normal PCs (Figure 1A and Supplementary Figure S1A). We included a validation cohort with Affymetrix microarray data (Supplementary Figure S1B-C). We also identified a significant upregulation of *SUV39H1* in plasma cell leukemia (PCL) compared to MM patients (Supplementary Figure S1D). Of interest, high expression of *SUV39H1* and *SUV39H2* is associated with a poor outcome in newly diagnosed MM patients of the CoMMPASS cohort (Figure 1B). These data were validated in two other independent cohorts of newly diagnosed MM patients and in a cohort of MM patients at relapse treated by Daratumumab(4) (Supplementary Figure S1E-F). In COX analyses with high-risk cytogenetic abnormalities (HR CAs) (CoMMPASS cohort), high expression of *SUV39H1* or *SUV39H2* remained independent prognostic factors when compared to del17p or 1q gain (Supplementary Table S1). Only high *SUV39H1* expression is associated with a significant poor outcome in MM patients with del17p and in patients without del17p (Figure 1C). High *SUV39H1* expression was also associated with a prognostic value in patients with 1q gain and in patients without 1q gain CA (Figure 1D). In MM, accumulation of high-risk CAs worsens the prognosis of patients(5). We have assessed the effect of additional high risk CAs (del17p, 1q gain and t(4;14)) on the prognosis of patients with high *SUV39H1* expression in the CoMMPASS cohort. Of interest, the overall survival (OS) of patients with high *SUV39H1* expression significantly decreased when associated with other HR CAs ($P < 0.0001$) (Figure 1E). *SUV39H1* expression was significantly higher in patients with del13, del17p, del1p, 1q gain, t(4;14), t(11,14) and biallelic TP53/del17p alteration

cytogenetic abnormalities (Figure 2A and 2B). Furthermore, patients with DIS3, MAP3K1, MAX, SETD2, TP53 and TRRAP mutations are associated with higher *SUV39H1* expression (Figure 2C). Among the signature of genes specifically associated with H3K9me3 in TP53 double hit patients, 27% were enriched in promoter regions (Supplementary Figure S1J-K), Of interest, among these genes, we identified 5 potential tumor suppressor (*PHLDA3, TMEM74, COL27A1, ACKR3 and SPRY4*) genes that are specifically associated with H3K9me3 repressive mark in TP53 double hit patients in association with a poor outcome when under-expressed in MM cells and also significantly downregulated in TP53 double hit patients and cell lines compared to wild type TP53 status (Supplementary Figure S1L-M). Investigating the correlation between *SUV39H1* gene expression and protein expression by western blot, we identified a significant correlation ($P < 0.02$) in a panel of 11 HMCLs (Supplementary Figure S1N).

Thus, we decided to investigate the biological functions of SUV39H1 HMT in MM biology. To investigate the functional significance of SUV39H1 overexpression in MM cells, two HMCLs with a high SUV39H1 (XG7 and XG19) and LP1 with intermediate expression were transduced with a doxycycline-inducible lentivirus containing a SUV39H1 shRNA. We included one cell line without De17p and no TP53 mutation (XG7), a cell line without Del17p and with TP53 mutation (LP1) and a cell line with Del17p and TP53 mutation (XG19). No significant correlation between SUV39H1 expression and recurrent translocation was identified (Supplementary Figure S1I). Doxycycline treatment induced a 51–85% reduction of *SUV39H1* expression at RNA and protein levels ($P < 0.01$; Figure 2D and Supplementary Figure S2A) and a significant reduction of H3K9me2 and H3K9me3 levels ($P < 0.05$; Figures 2E and Supplementary Figure S2B). SUV39H1 depletion induced a progressive inhibition of

MM cell growth after doxycycline addition (Figure 2F, *P* = 0.03 and Supplementary Figure S2C). Furthermore, SUV39H1 depletion induced apoptosis in HMCL (Figure 2G, *P* = 0.01 and Supplementary Figure S2D). SUV39H1 depletion also affected the cell cycle distribution with an increase in the G2/M phase (Figure 2H, *P* < 0.05 and Supplementary Figure S2E). SUV39H1 depletion was also associated with the accumulation of DNA double-strand breaks, as evidenced the accumulation of 53BP1 foci and increased phosphorylation of the histone variant H2AX (Figures 3A-C). The results presented above indicate that the overexpression of SUV39H1 in MMCs could protect them from spontaneous DNA damage. Since chemotherapy also induces DNA breaks, we next investigated whether SUV39H1 depletion could potentiate melphalan toxicity in MM cells. We found that the direct depletion of SUV39H1 with shRNAs sensitized XG7 and XG19 MMCs to melphalan (Figure 3D and Supplementary Figures S2F). Furthermore, we identified a significant correlation between *SUV39H1* basal gens expression and response to melphalan in a panel of HMCLs (Supplementary Figure 2G).

Chaetocin has been shown to be an inhibitor of SUV39H HMTs(6). Chaetocin was also shown to affect the protein stability and expression of SUV39H1 at low concentration (7). With higher concentration, chaetocin was also reported to inhibit another HMT, G9a, which affects H3K9me2/3 levels and has been implicated in MM pathobiology(8). We tested the effects of chaetocin on MM cell growth using 11 different MM cell lines. We identified that chaetocin has a significant anti-myeloma effect at low doses, with an IC_{50} comprised between 4 and 17 nM in the 11 HMCLs tested (Figure 3E). A high *SUV39H1* expression was correlated with higher sensitivity to chaetocin (r = -0.55, *P* = 0.05). As reported with SUV39H1 depletion, chaetocin treatment inhibits H3K9 trimethylation in LP1 cells (Supplementary Figure S2H). Chaetocin treatment induced

a significant increase in apoptotic cell death, accompanied by a marked reduction of the cells in S phase (Supplementary Figure S2I and S2J). We therefore aimed to validate these results using primary MM cells from patients co-cultured with their bone marrow (BM) microenvironment and exogeneous IL-6 (2ng/ml). Primary BM samples were collected with the approval of the institutional board of Montpellier University Hospital (DC2008-417) after patients written informed consent. We identified a specific toxicity of chaetocin at nanomolar concentrations on primary MM cells from patients (n=5 MM patients, Figure 3F, $P < 0.05$) whereas no significant toxicity was observed on normal bone marrow cells in the co-culture (Figure 3G). SUV39H1 protein expression in HMCLs and primary MM cells from patients was validated by flow cytometry (Figure 3H). Altogether, our data suggest that targeting of SUV39H1 may represent a promising therapeutic approach in MM.

To analyze the transcriptional programs regulated by SUV39H1 in MM cells, XG19 and XG7 transduced with a doxycycline-inducible lentivirus containing a SUV39H1 shRNA were treated with Doxycycline for 48h and gene expression profiles were analyzed using Affymetrix microarrays. LP1, XG19 and XG7 HMCLs were also treated with chaetocin and the transcriptomic profiles were analyzed. 215 genes were significantly upregulated in the treated cells and SUV39H1 depleted cells compared with control (fold change ≥2; false-discovery rate < 0.05; Supplementary Figure S2K). No significantly downregulated genes were identified. GSEA analysis revealed a significant enrichment in TP53 and RB1 target genes, polycomb PRC2 target genes, genes associated with DNA methylation, mature plasma cell genes, and genes involved in ferroptosis and inflammatory response. We previously reported the presence of H3K9me3 and H3K27me3 co-localization in gene promoters of tumor suppressor genes in MM cells(2). Our data underlined deregulation of genes related to

TP3 pathway and associated with epigenetic regulation reported to participate in the pathophysiology of the disease(2, 9-12).

SUV39H1 has a significant impact on cancer development and progression across multiple types of cancer. Specifically, the H3K9me3 modification catalyzed by SUV39H1 can suppress the tumor suppressor gene *p16INK4a*, which leads to unrestricted cell proliferation in both acute myeloid leukemia (AML) and lung cancer cells(13, 14). Cheatocin was reported to induce differentiation of acute myeloid leukemia cells and synergistic toxicities when combined with other epigenetic drugs(7). Recently, Lopez-Cobo S et al. demonstrated that SUV39H1 ablation enhances long-term CAR-T function in solid tumors(15) underlining potential common interest to target MM cells and improve immune based therapies.

Treatment outcomes of patients with MM have significantly improved. However, a subpopulation of MM patients with high-risk cytogenetic abnormalities still experiences early death due to disease progression. We believe our study may provide new therapeutic avenues in the development of targeted therapies based on molecular pathology that may help to improve the outcome of patients with high-risk MM.

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Figure Legends

Figure 1: SUV39H1 depletion induced cell growth inhibition and apoptosis associated with H3K9me3 repression.

(A) SUV39H1 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: **: $P < 0.01$; ****: $P < 0,0001$. **(B)** Kaplan-Meier showing the overall survival (OS) of patients from the CoMMpass cohort (n = 674) based on their SUV39H1 and SUV39H2 expression levels. High SUV39H1/2 expression was significantly associated with increased risk in MM patients. **(C, D)** Patients with or without del17p **(C)** or with without 1q gain **(D)** of the CoMPASS cohort (n = 674) were ranked according to SUV39H1 level and a maximum difference in OS was splitting patients into high or low SUV39H1 expression. High expression of SUV39H1 was significantly associated with high-risk in MM patients. **(E)** Kaplan-Meier showing the overall survival of newly diagnosed patients with MM (CoMMpass cohort) with high SUV39H1 expression according to the association with other high-risk CAs (HR CAs). HR CAs are defined by the presence of del(17p), t(4;14), and/or gain(1q). The green curve corresponds to patients with high SUV39H1 expression without other HR CAs; the orange curve to patients with 1 CA; and the red curve to patients with ≥2 other HR CAs. *P* value is determined by the log-rank test comparison.

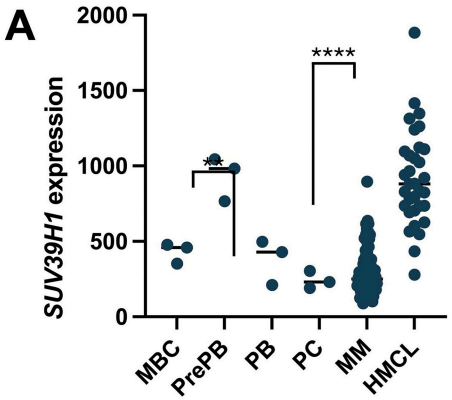
Figure 2: SUV39H1 expression is a prognostic factor in high-risk MM patients.

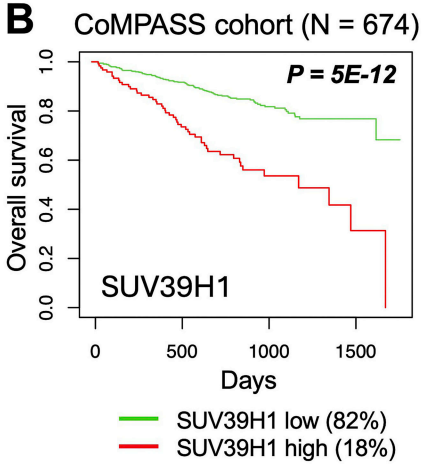
(A) SUV39H1 expression in patients from the CoMMpass cohort without CA (light blue) and patients with CA (dark blue). **(B)** SUV39H1 expression in patients from the CoMMpass cohort with the double hit (dark blue) or without (light blue) TP53mut/del17p. **(C)** SUV39H1 expression in patients from the CoMMpass cohort without the mutation (light blue) or with the mutation (dark blue) on the indicated gene. Statistical difference was tested using a Wilcoxon test. **(D-E)** XG7 doxycycline-inducible shSUV39H1 cells were treated or not with doxycycline (1 mg/ml) for 3 days. Cells were collected, and the indicated proteins were analyzed by western blot in whole-cell lysates. **(F)** The curves represent cumulative cell growth without induction (Control) and with induction of the anti-SUV39H1 shRNA following doxycycline 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* value was calculated using a paired Student's *t*-test. **(G-H)** XG7 doxycycline-inducible shSUV39H1 cells were treated or not with doxycycline (1 mg/ml) for 7 days. **(G)** Annexin V was detected by flow cytometry. **(H)** BrdU (10µg/ml) was added during the last 1.5h of treatment. Cells were fixed and processed to detect BrdU incorporation and total DNA (DAPI) by flow cytometry. Data are those of one experiment representative of 3 independent experiments. *P* values were calculated using a paired Student's *t*-test.

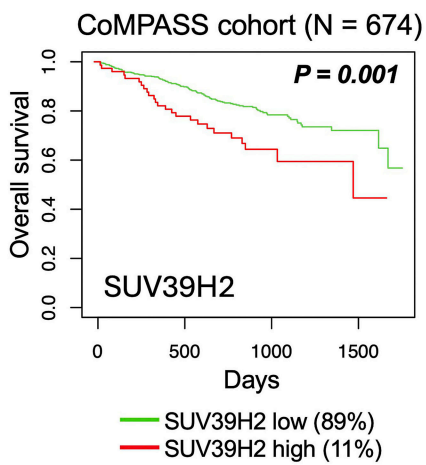
Figure 3: SUV39H1 depletion induced DNA damages.

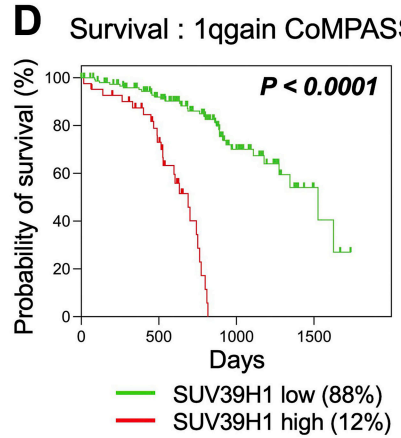
(A) Cells were treated with doxycycline (1 mg/ml) for 3 days, deposited onto slides by cytospin centrifugation, fixed with 4% PFA (10 min, RT) and IF was performed to detect 53BP1 foci. DNA was stained with DAPI. **(B)** Dot plots showing the distribution of 53BP1 foci per cell. Statistical analysis was performed using an ImageJ macro on at least 100 nuclei. P value was calculated using Mann–Whitney U test. Data are those of one experiment representative of 3 independent experiments. **(C)** Cells were treated as indicated for 3 days, collected and the indicated proteins were analyzed by western blot in whole-cell lysates. Data are those of one experiment representative of 3 independent experiments. **(D)** XG7 doxycycline-inducible shSUV39H1 cells were pretreated or not with doxycycline (1 mg/ml) for 2 days. Then, treated with increasing concentrations of melphalan (0.015 to 40 µM) for 4 days. IC50 was calculated after viability assessment by CellTiter-Glo (CTG) luminescent cell viability assay. The graph shows average +/- SD of 3 independent replicates. **(E)** HMCLs were cultured for 4 days with culture medium (control) and increasing concentrations of chaetocin (0.039 to 200 nM). IC50 was calculated after viability assessment by CTG luminescent cell viability assay. IC50 for each cell line was calculated using GraphPrism software. Data are mean values of three experiments determined on sextuplet culture wells. **(F, G)** Primary cells from bone marrow sample of 5 MM patients were treated with chaetocin at the indicated concentrations for 4 days. Myeloma cells (CD38+, CD138+) **(F)** and non-myeloma cells (CD38-, CD138-) **(G)** were analyzed by flow cytometry and expressed in % of control. Data shown are mean values of the results from samples of 5 MM patients. *Indicates a significant difference compared to control cells using a Wilcoxon test for pairs ($P \leq 0.05$). **(G)** SUV39H1 expression was assessed using flow cytometry in a panel of 8 HMCLs and at the surface of MM cells in bone marrow samples from 4 patients. Mean fluorescence intensity data confirmed SUV39H1 protein expression in primary MM cells from patients.

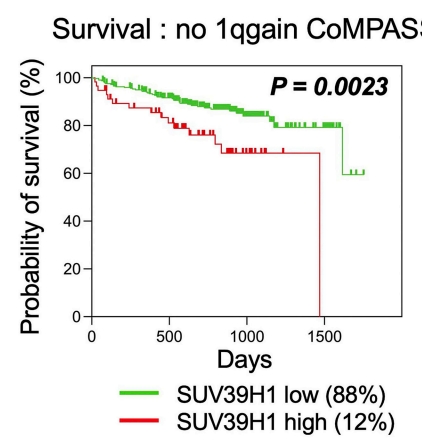
Figure 1

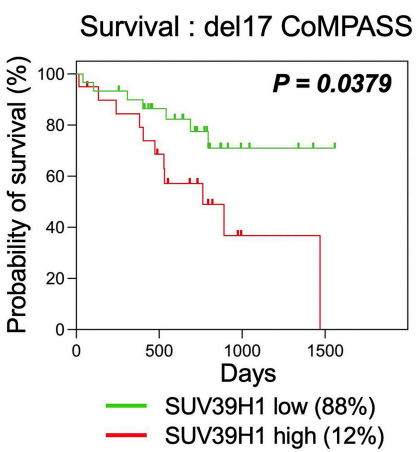

A
SUV39H1 expression
2000
1500
1000
500
0
****
MBC
PreP
PB
PC
MM
HMCL

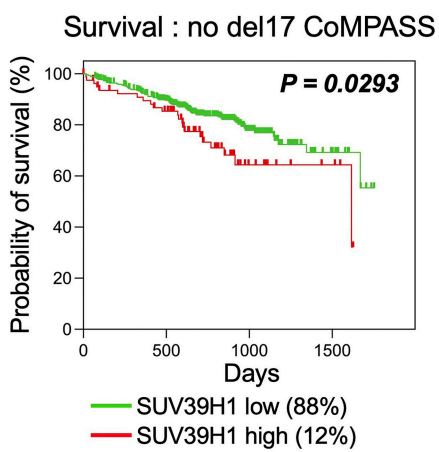

B
CoMPASS cohort (N = 674)
P = 5E-12
SUV39H1
Overall survival
Days
SUV39H1 low (82%)
SUV39H1 high (18%)


CoMPASS cohort (N = 674)
P = 0.001
SUV39H2
Overall survival
Days
SUV39H2 low (89%)
SUV39H2 high (11%)


D
Survival : 1qgain CoMPASS
P < 0.0001
Probability of survival (%)
Days
SUV39H1 low (88%)
SUV39H1 high (12%)


Survival : no 1qgain CoMPASS
P = 0.0023
Probability of survival (%)
Days
SUV39H1 low (88%)
SUV39H1 high (12%)


Survival : del17 CoMPASS
P = 0.0379
Probability of survival (%)
Days
SUV39H1 low (88%)
SUV39H1 high (12%)


Survival : no del17 CoMPASS
P = 0.0293
Probability of survival (%)
Days
SUV39H1 low (88%)
SUV39H1 high (12%)

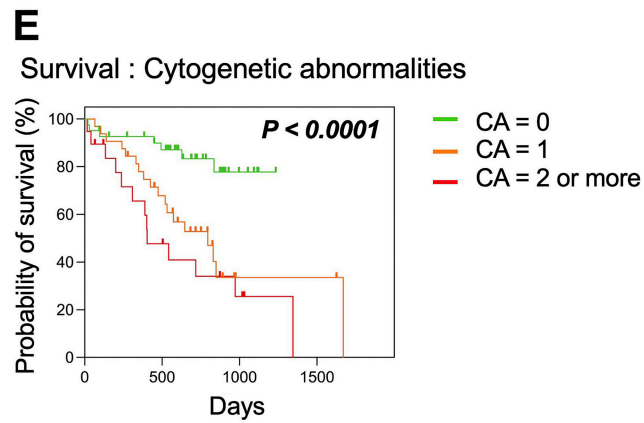
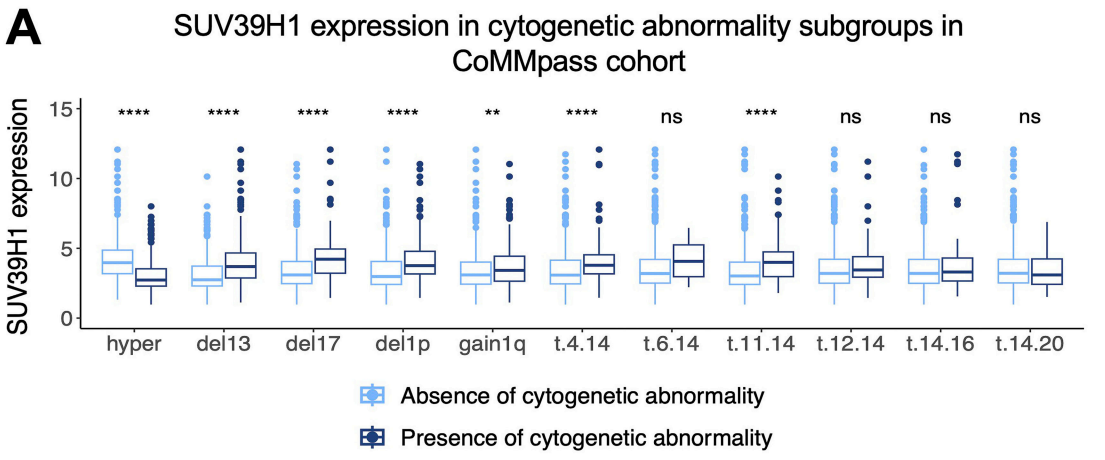
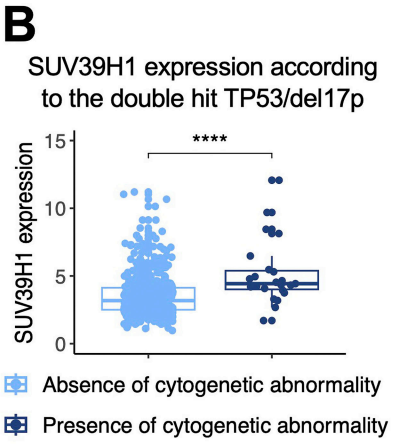
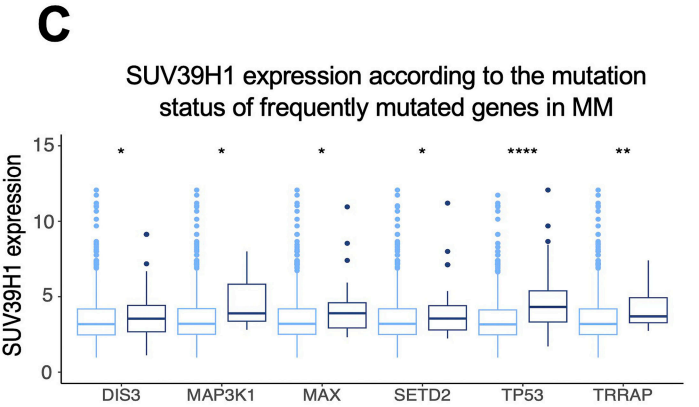
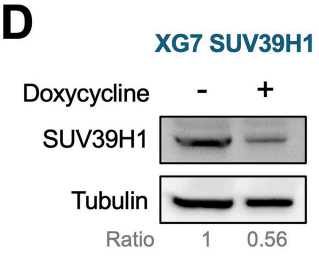

E
Survival : Cytogenetic abnormalities
P < 0.0001
Probability of survival (%)
Days
CA = 0
CA = 1
CA = 2 or more

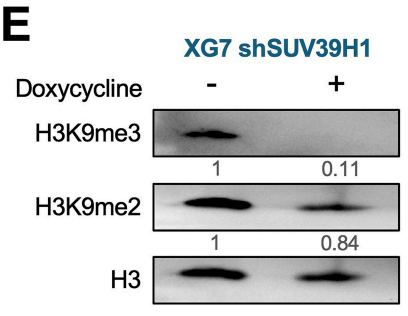
Figure 2

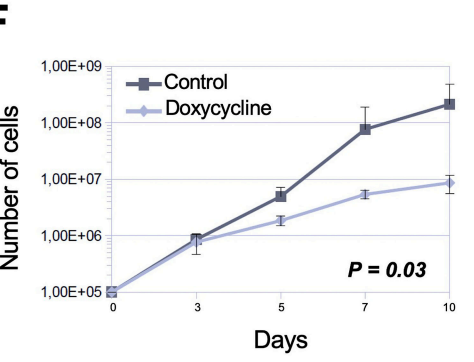

A
SUV39H1 expression in cytogenetic abnormality subgroups in CoMMpass cohort
SUV39H1 expression
hyper
del13
del17
del1p
gain1q
t.4.14
t.6.14
t.11.14
t.12.14
t.14.16
t.14.20
Absence of cytogenetic abnormality
Presence of cytogenetic abnormality

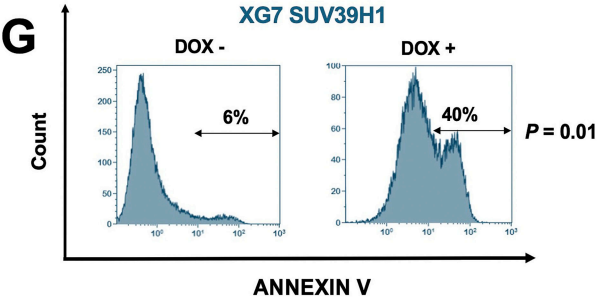

B
SUV39H1 expression according to the double hit TP53/del17p
SUV39H1 expression
Absence of cytogenetic abnormality
Presence of cytogenetic abnormality


C
SUV39H1 expression according to the mutation status of frequently mutated genes in MM
SUV39H1 expression
DIS3
MAP3K1
MAX
SETD2
TP53
TRRAP


D
XG7 SUV39H1
Doxycycline - +
SUV39H1
Tubulin
Ratio 1 0.56


E
XG7 shSUV39H1
Doxycycline - +
H3K9me3
1 0.11
H3K9me2
1 0.84
H3


F
Control
Doxycycline
Number of cells
P = 0.03
Days


G
XG7 SUV39H1
DOX -
DOX +
6%
40%
P = 0.01
Count
ANNEXIN V

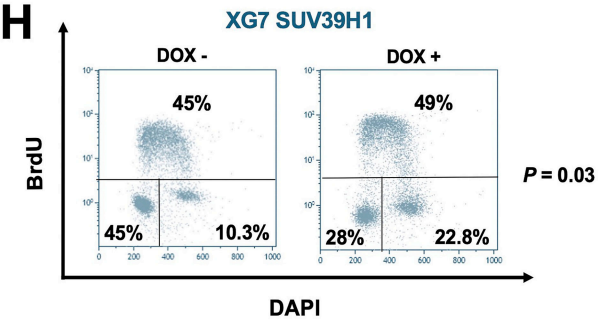
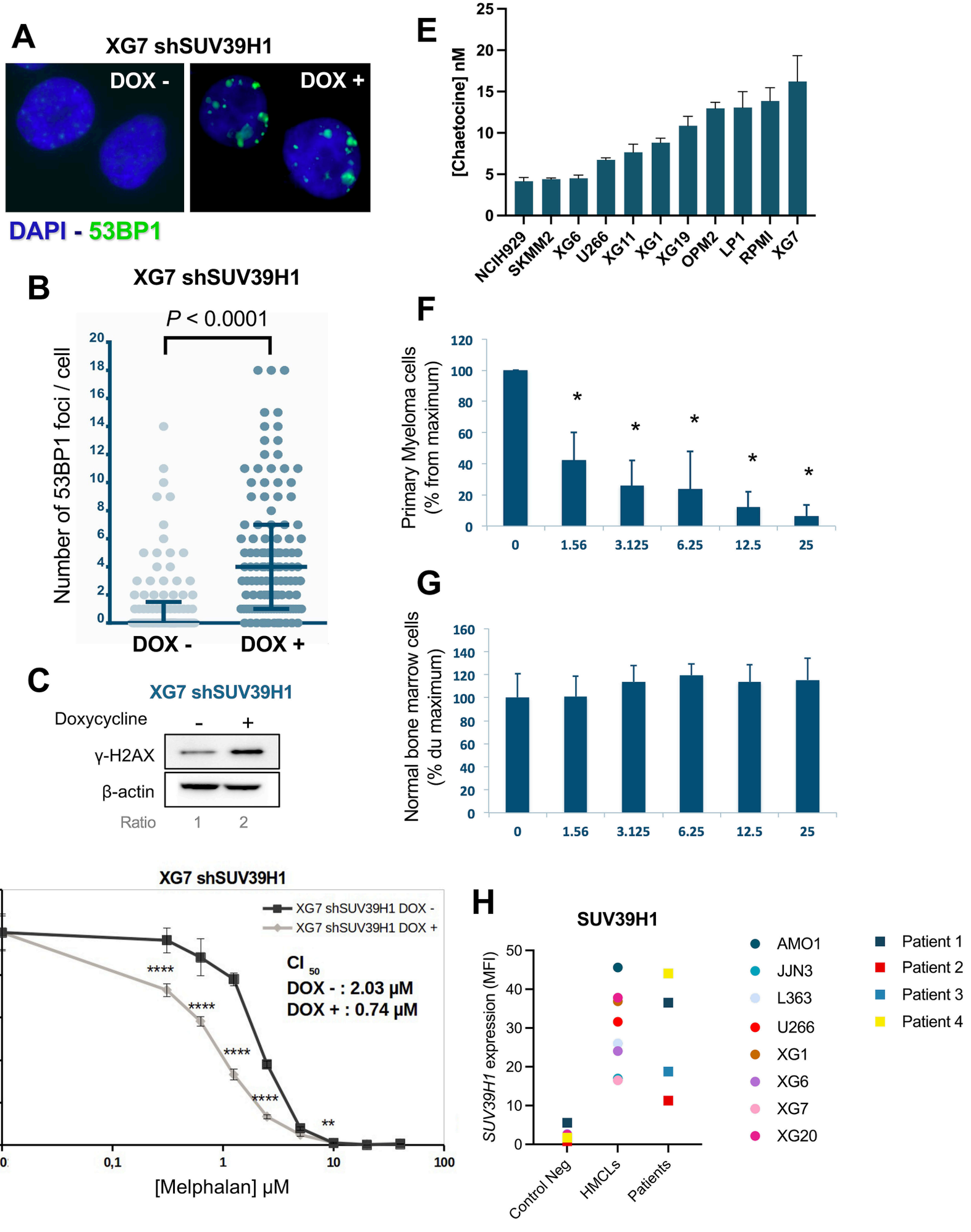
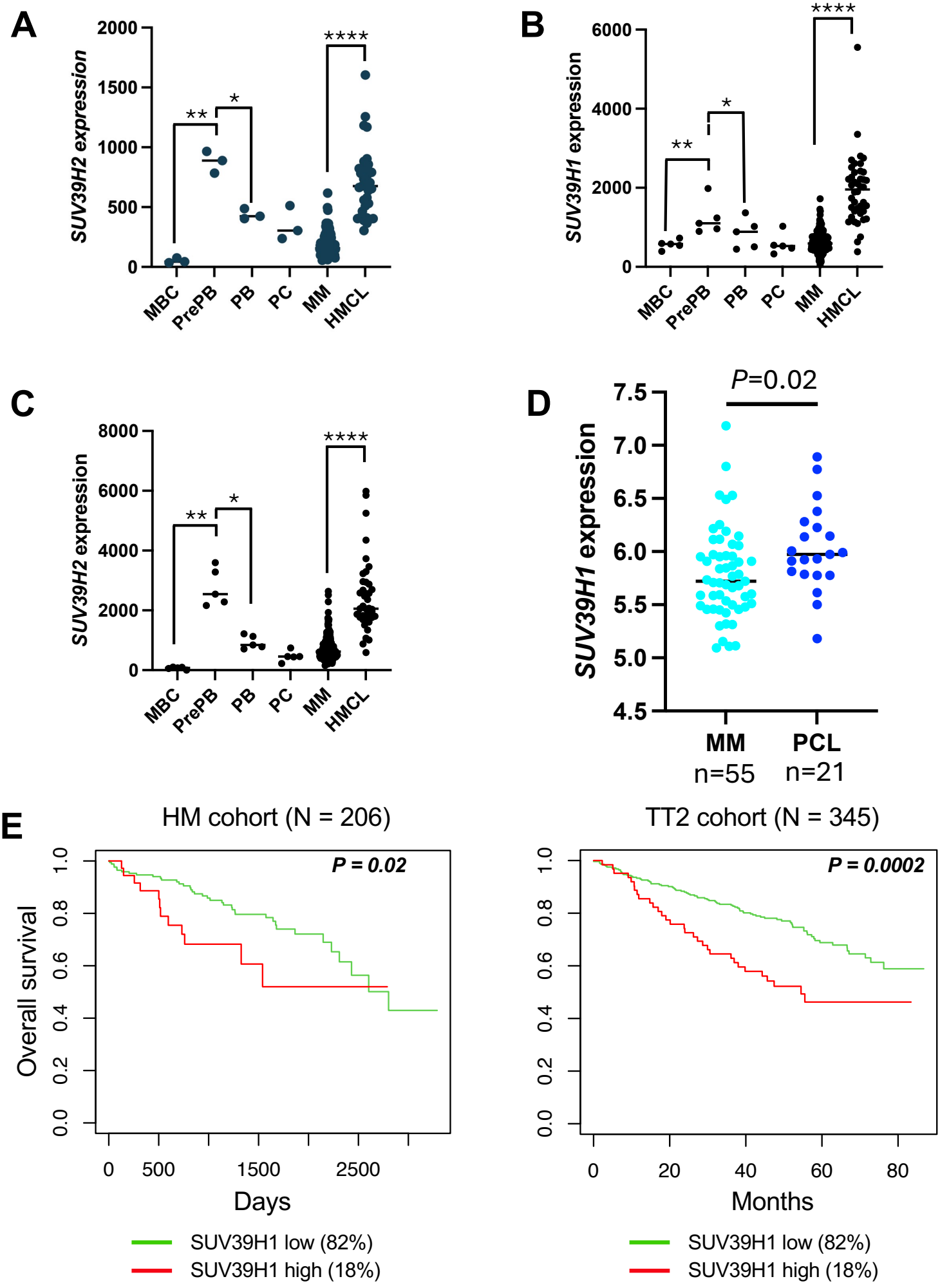

H
XG7 SUV39H1
DOX -
DOX +
45%
49%
45%
10.3%
28%
22.8%
P = 0.03
BrdU
DAPI

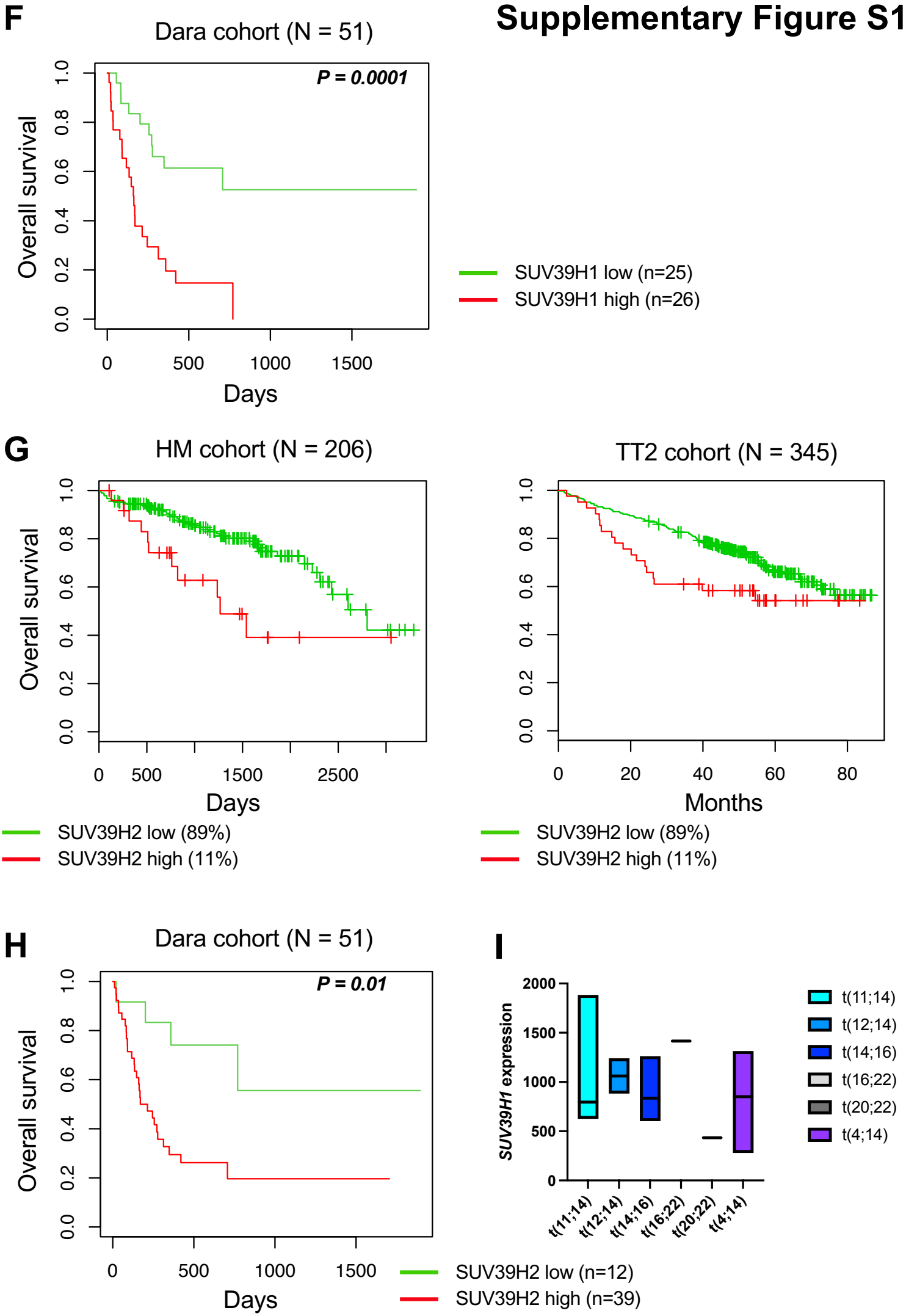
Figure 3

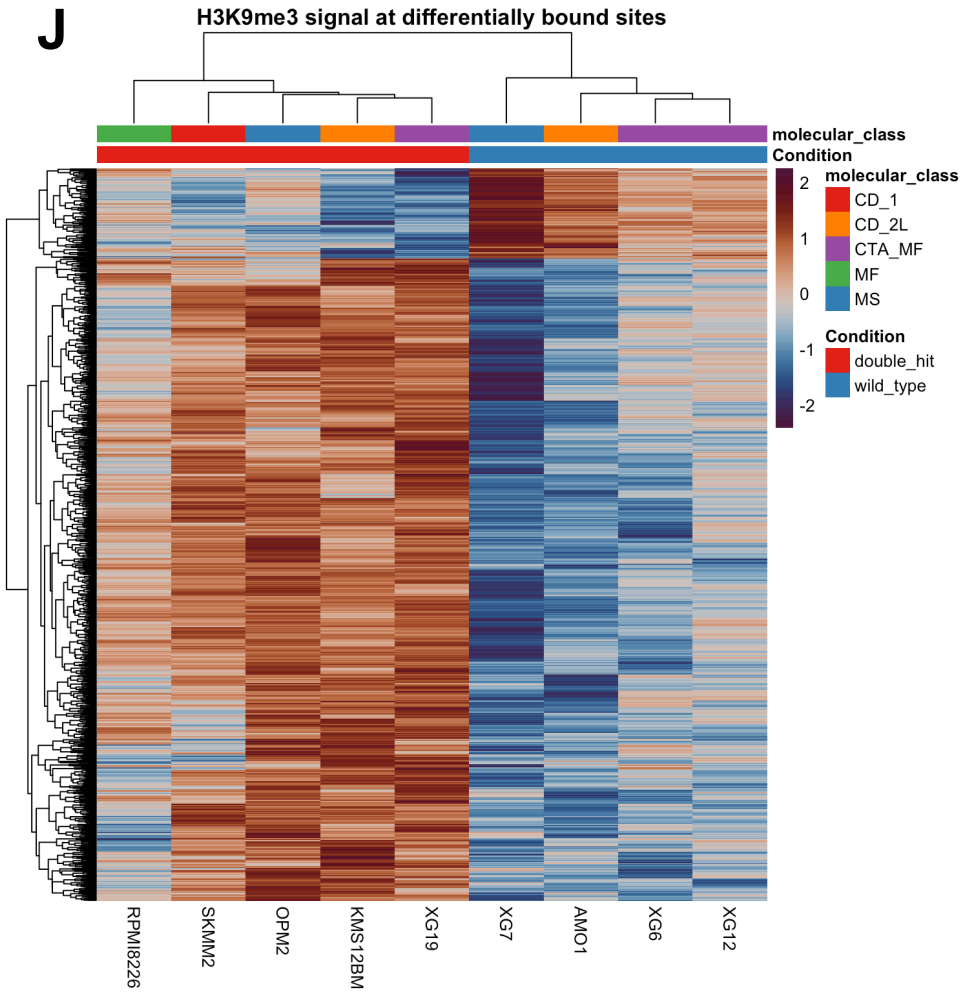

A
XG7 shSUV39H1
DOX -
DOX +
DAPI - 53BP1
B
XG7 shSUV39H1
P < 0.0001
Number of 53BP1 foci / cell
DOX -
DOX +
C
XG7 shSUV39H1
Doxycycline
-
+
γ-H2AX
β-actin
Ratio
1
2
D
XG7 shSUV39H1
XG7 shSUV39H1 DOX -
XG7 shSUV39H1 DOX +
CI 50
DOX - : 2.03 µM
DOX + : 0.74 µM
% of survival
[Melphalan] µM
E
[Chaetocine] nM
NCIH929
SKMM2
XG6
U266
XG11
XG1
XG19
OPM2
LP1
RPMI
XG7
F
Primary Myeloma cells
(% from maximum)
G
Normal bone marrow cells
(% du maximum)
H
SUV39H1
SUV39H1 expression (MFI)
Control Neg
HMCLs
Patients
AMO1
JJN3
L363
U266
XG1
XG6
XG7
XG20
Patient 1
Patient 2
Patient 3
Patient 4

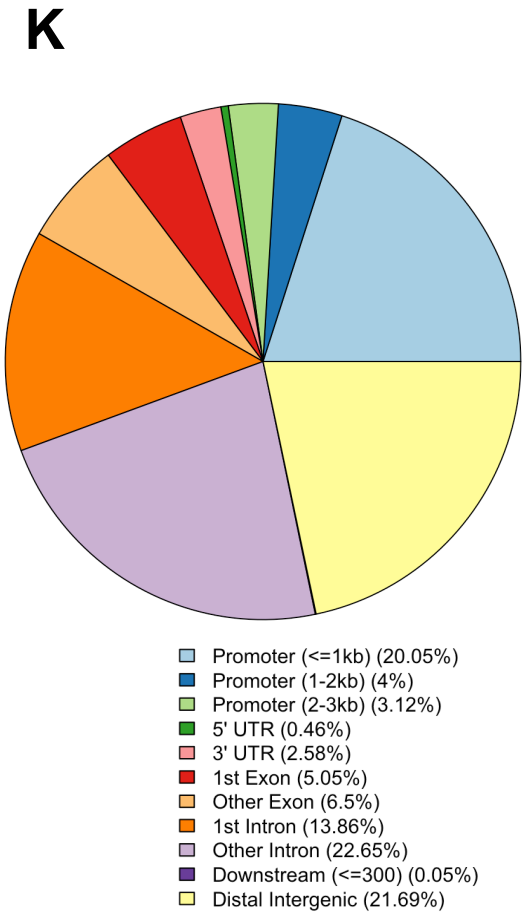
Supplementary Figure S1

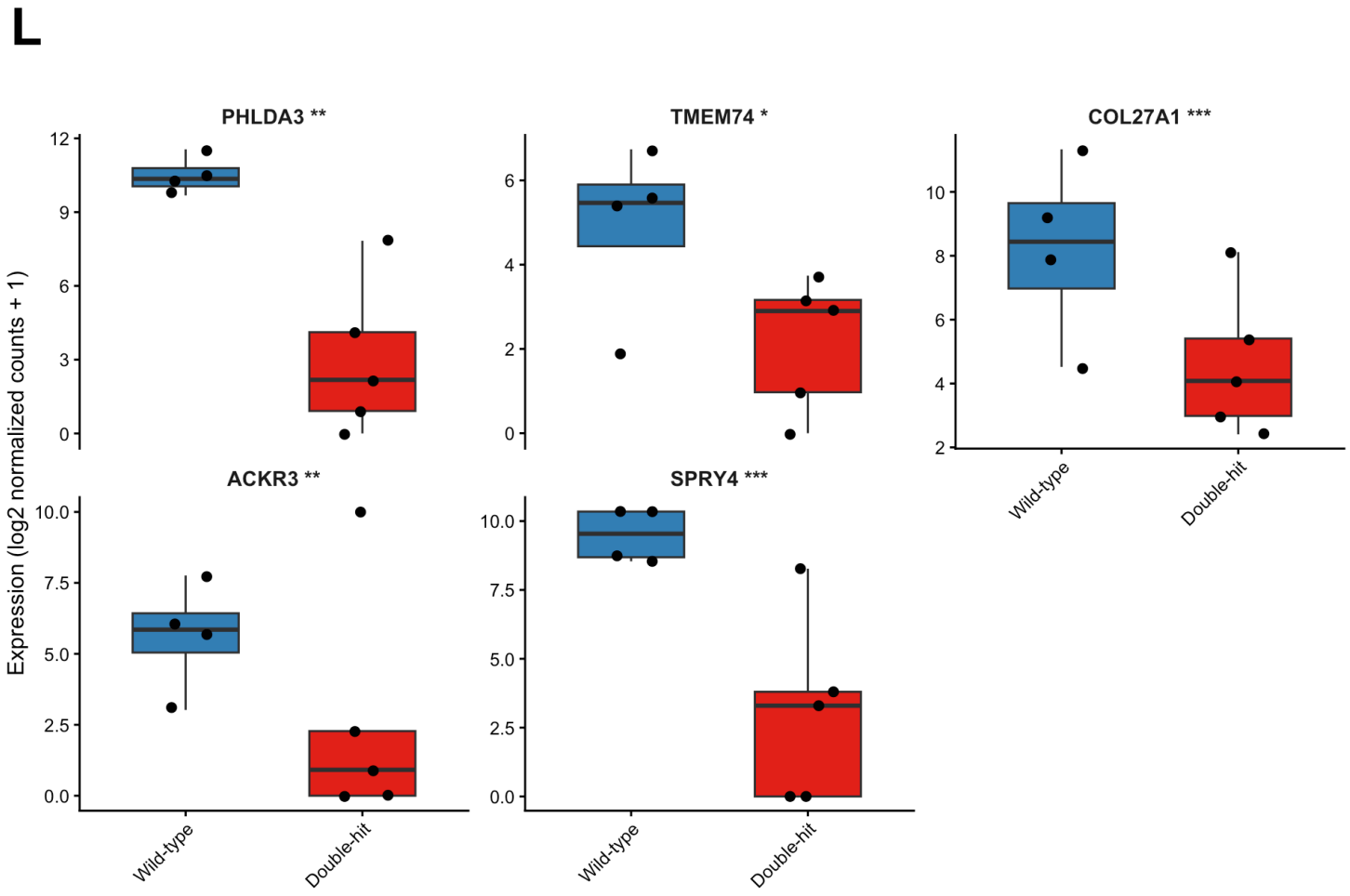


Supplementary Figure S1

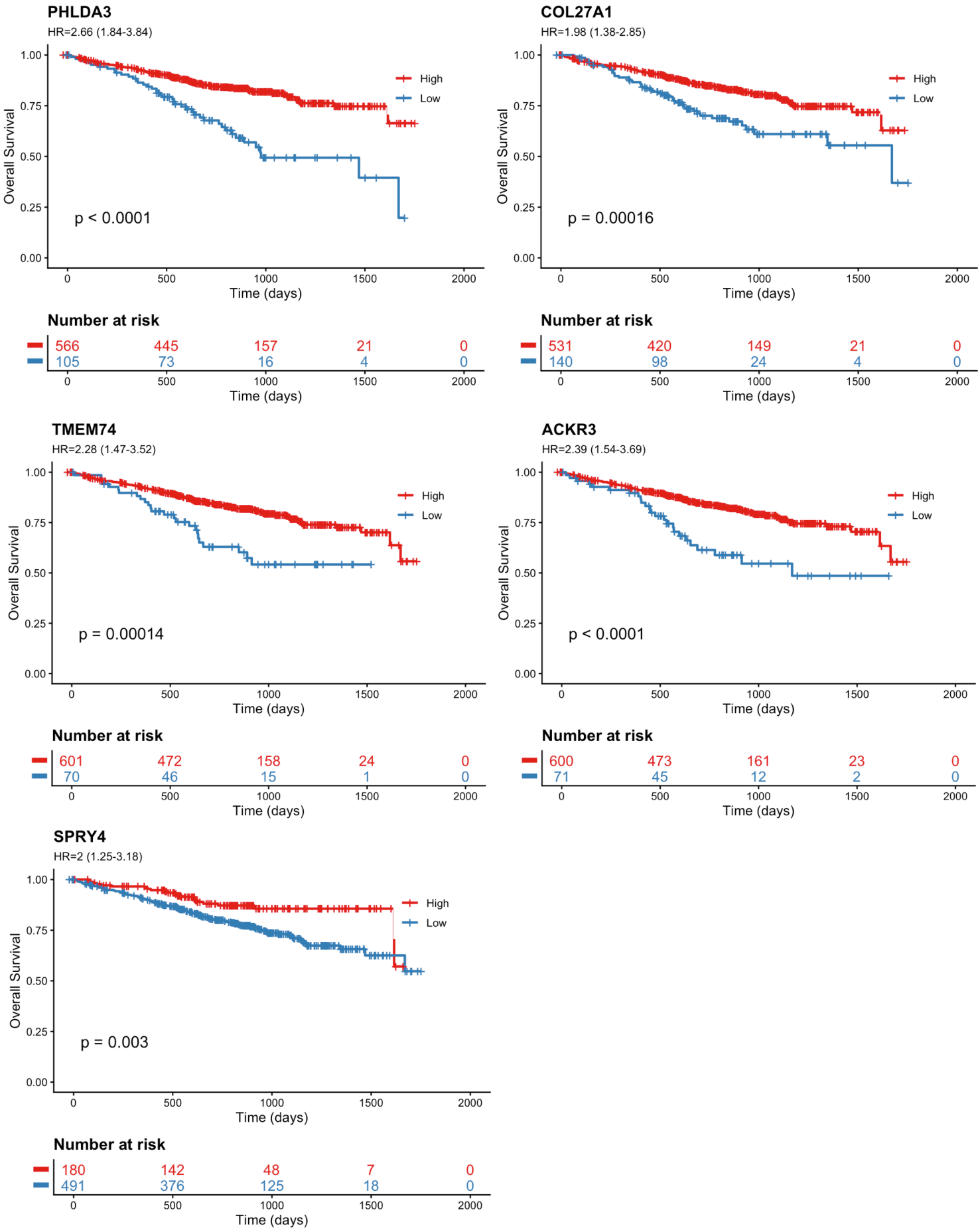



J
H3K9me3 signal at differentially bound sites
molecular_class
Condition
molecular_class
CD_1
CD_2L
CTA_MF
MF
MS
Condition
double_hit
wild_type
2
1
0
-1
-2
RPMI8226
SKMM2
OPM2
KMS12BM
XG19
XG7
AMO1
XG6
XG12

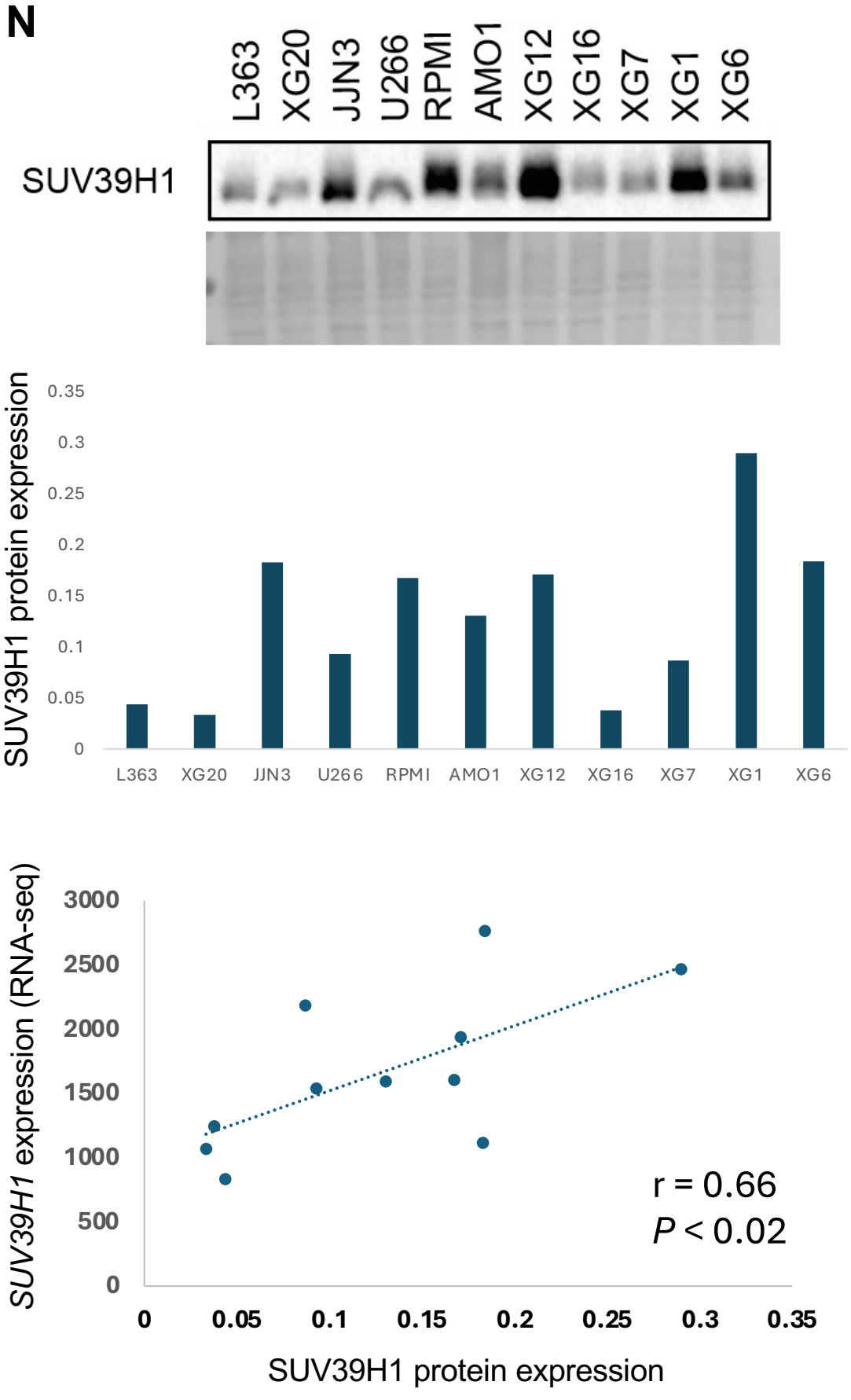

K
Promoter (<=1kb) (20.05%)
Promoter (1-2kb) (4%)
Promoter (2-3kb) (3.12%)
5' UTR (0.46%)
3' UTR (2.58%)
1st Exon (5.05%)
Other Exon (6.5%)
1st Intron (13.86%)
Other Intron (22.65%)
Downstream (<=300) (0.05%)
Distal Intergenic (21.69%)


L
PHLDA3 **
TMEM74 *
COL27A1 ***
ACKR3 **
SPRY4 ***
Expression (log2 normalized counts + 1)
Wild-type
Double-hit

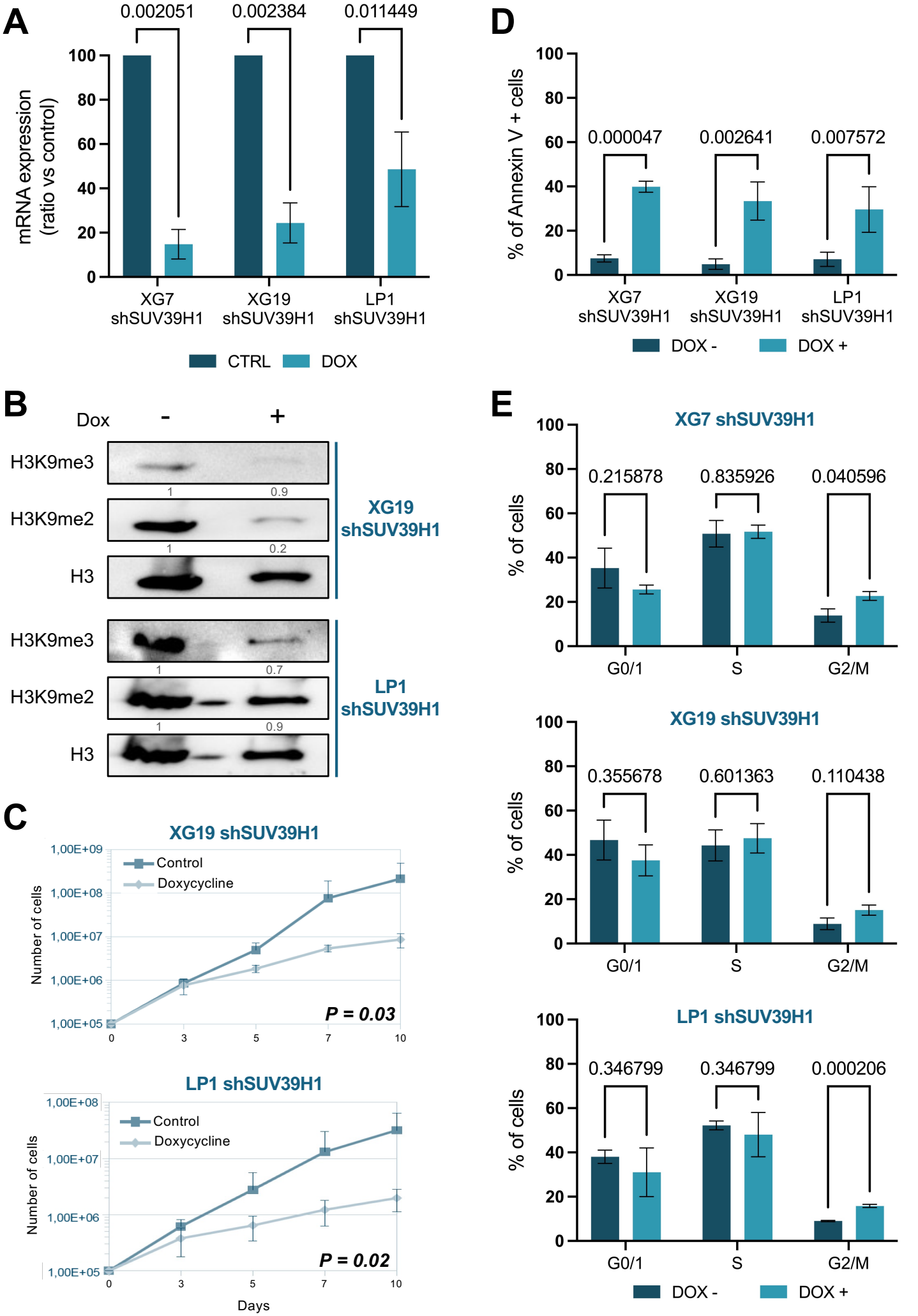
M


PHLDA3
HR=2.66 (1.84-3.84)
p < 0.0001
Overall Survival
Time (days)
High
Low
Number at risk
566 445 157 21 0
105 73 16 4 0
COL27A1
HR=1.98 (1.38-2.85)
p = 0.00016
531 420 149 21 0
140 98 24 4 0
TMEM74
HR=2.28 (1.47-3.52)
p = 0.00014
601 472 158 24 0
70 46 15 1 0
ACKR3
HR=2.39 (1.54-3.69)
p < 0.0001
600 473 161 23 0
71 45 12 2 0
SPRY4
HR=2 (1.25-3.18)
p = 0.003
180 142 48 7 0
491 376 125 18 0

N

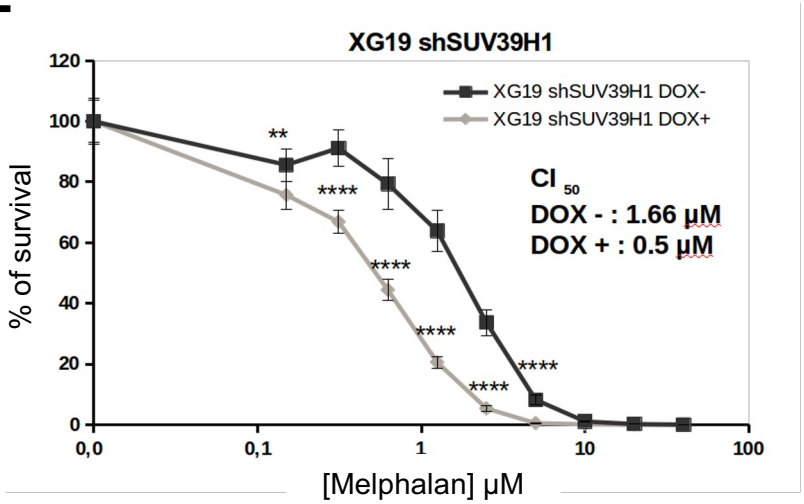

L363
XG20
JJN3
U266
RPMI
AMO1
XG12
XG16
XG7
XG1
XG6
SUV39H1
SUV39H1 protein expression
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
L363
XG20
JJN3
U266
RPMI
AMO1
XG12
XG16
XG7
XG1
XG6
SUV39H1 expression (RNA-seq)
3000
2500
2000
1500
1000
500
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
SUV39H1 protein expression
r = 0.66
P < 0.02

Supplementary Figure S2

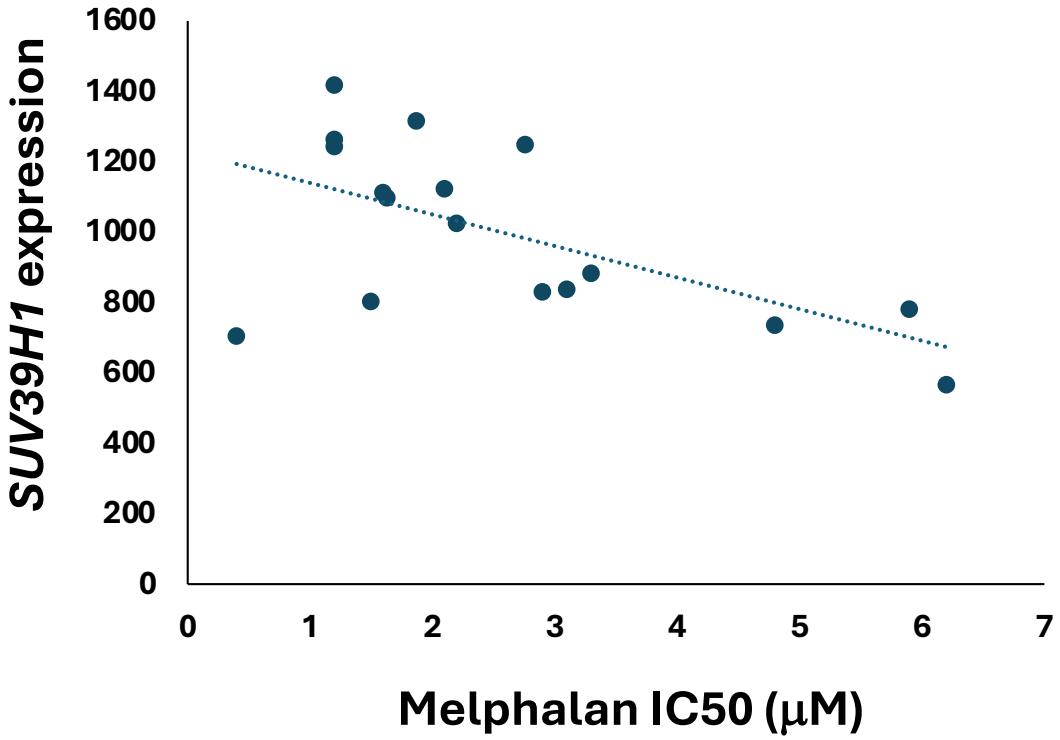


Supplementary Figure S2

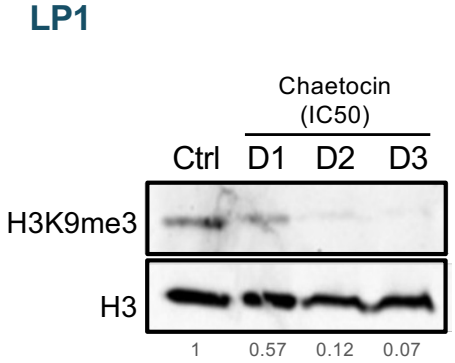
F

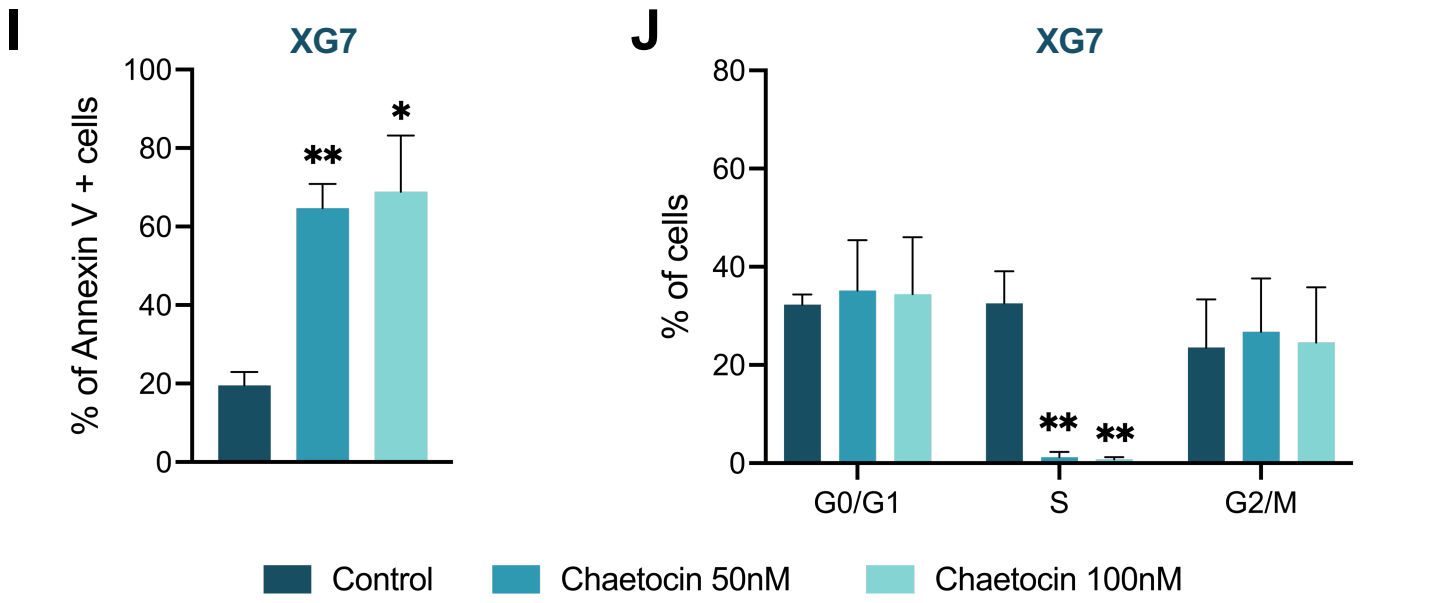


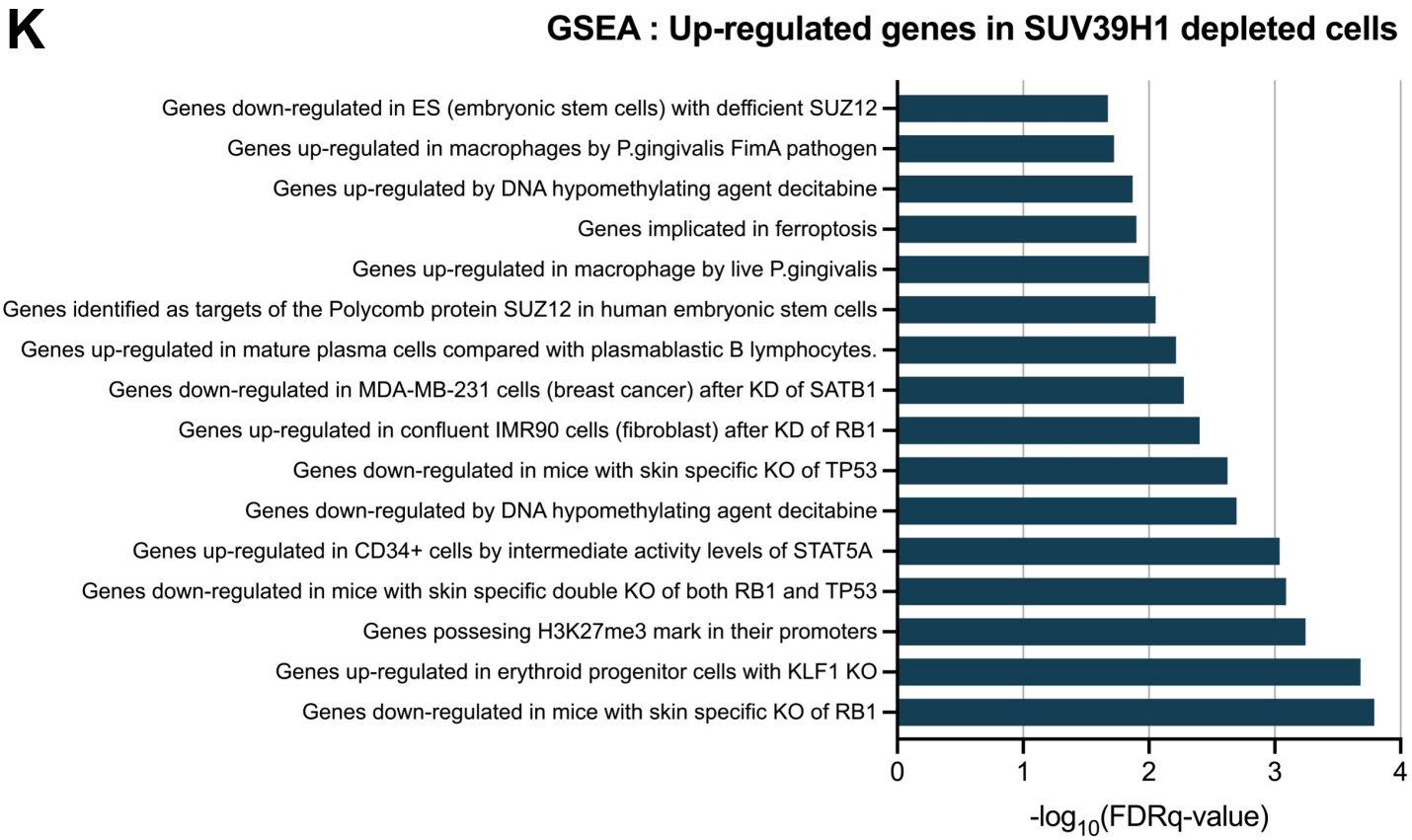
G



H




I
XG7
% of Annexin V + cells
100
80
60
40
20
0
**
*
J
XG7
% of cells
80
60
40
20
0
**
**
G0/G1
S
G2/M
Control
Chaetocin 50nM
Chaetocin 100nM


K
GSEA : Up-regulated genes in SUV39H1 depleted cells
Genes down-regulated in ES (embryonic stem cells) with defficient SUZ12
Genes up-regulated in macrophages by P.gingivalis FimA pathogen
Genes up-regulated by DNA hypomethylating agent decitabine
Genes implicated in ferroptosis
Genes up-regulated in macrophage by live P.gingivalis
Genes identified as targets of the Polycomb protein SUZ12 in human embryonic stem cells
Genes up-regulated in mature plasma cells compared with plasmablastic B lymphocytes.
Genes down-regulated in MDA-MB-231 cells (breast cancer) after KD of SATB1
Genes up-regulated in confluent IMR90 cells (fibroblast) after KD of RB1
Genes down-regulated in mice with skin specific KO of TP53
Genes down-regulated by DNA hypomethylating agent decitabine
Genes up-regulated in CD34+ cells by intermediate activity levels of STAT5A
Genes down-regulated in mice with skin specific double KO of both RB1 and TP53
Genes possesing H3K27me3 mark in their promoters
Genes up-regulated in erythroid progenitor cells with KLF1 KO
Genes down-regulated in mice with skin specific KO of RB1
0
1
2
3
4
-log10(FDRq-value)

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 +/- SD of 3 independent replicates. P values were calculated using Wilcoxon test for pairs. **(B)** Cells were treated or not with doxycycline (1 μg/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 μg/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 +/- 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 (10μg/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 +/- 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 μg/ml) for 2 days. Then, treated with increasing concentrations of melphalan (0,015 to 40μM) for 4 days. IC50 was calculated after viability assessment by CTG luminescent cell viability assay. The graph represents average +/- 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 μg/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 +/- 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