FIGURE 1

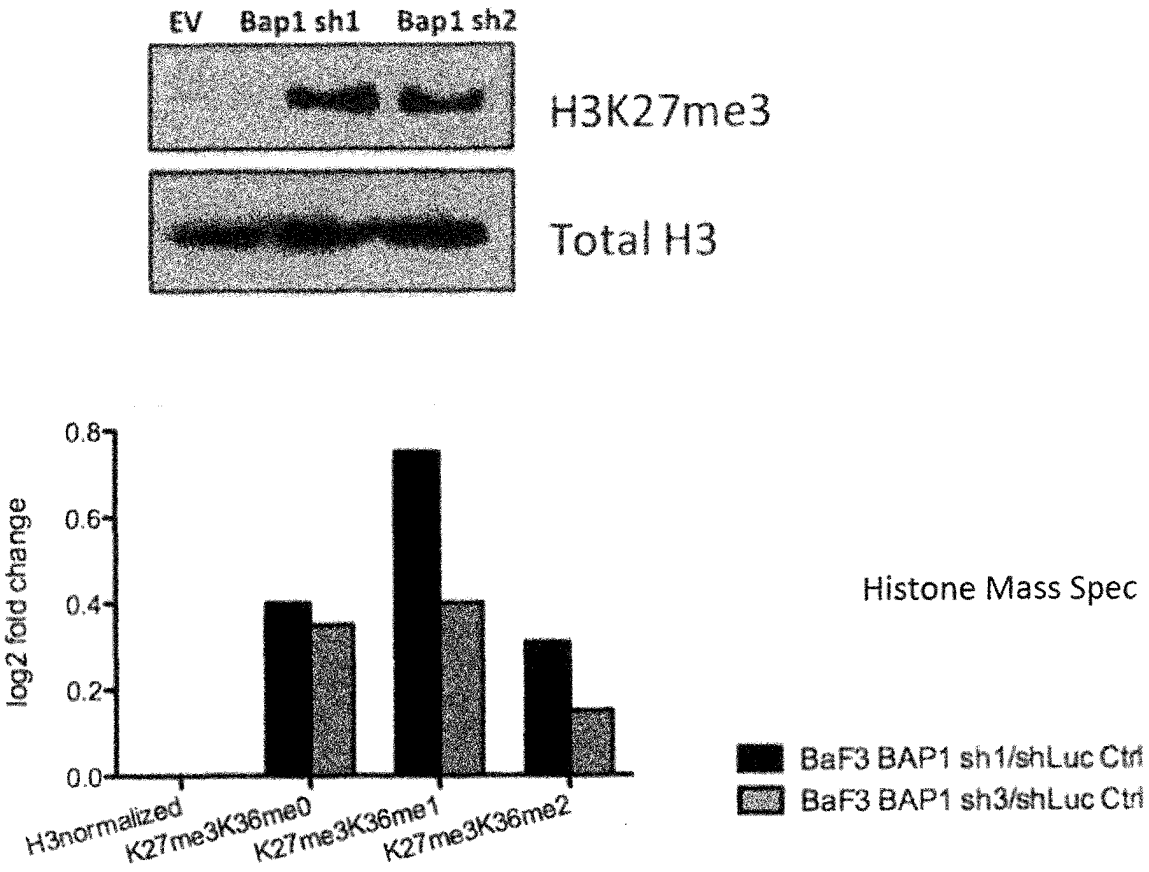

EV
Bap1 sh1
Bap1 sh2
H3K27me3
Total H3
log2 fold change
0.8
0.6
0.4
0.2
0.0
H3normalized
K27me3K36me0
K27me3K36me1
K27me3K36me2
Histone Mass Spec
BaF3 BAP1 sh1/shLuc Ctrl
BaF3 BAP1 sh3/shLuc Ctrl

FIGURE 2

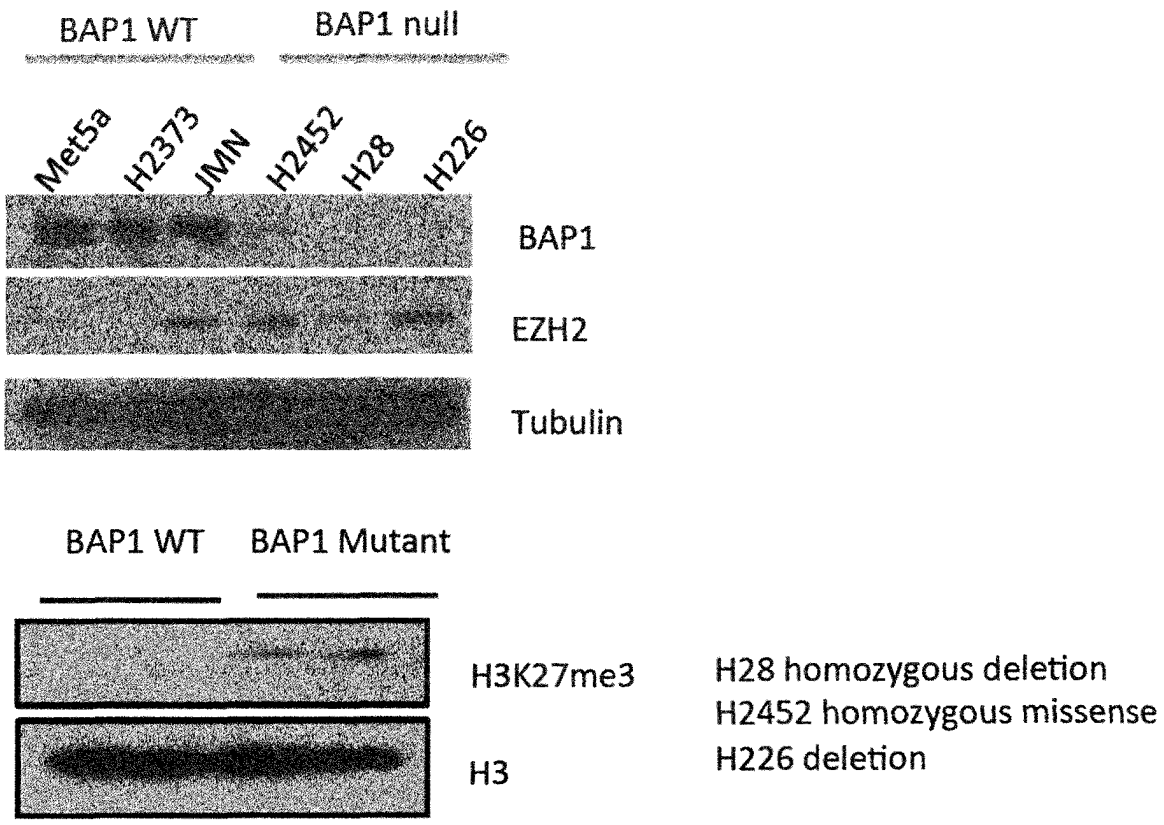

BAP1 WT
BAP1 null
Met5a
H2373
JMN
H2452
H28
H226
BAP1
EZH2
Tubulin
BAP1 WT
BAP1 Mutant
H3K27me3
H3
H28 homozygous deletion
H2452 homozygous missense
H226 deletion

FIGURE 3


293T
BAP1 cDNA
BAP1
SUZ12
EZH2
Tubulin
SET2
EV
Bap1 sh1
Bap1 sh2
BAP1
SUZ12
Tubulin

FIGURE 4

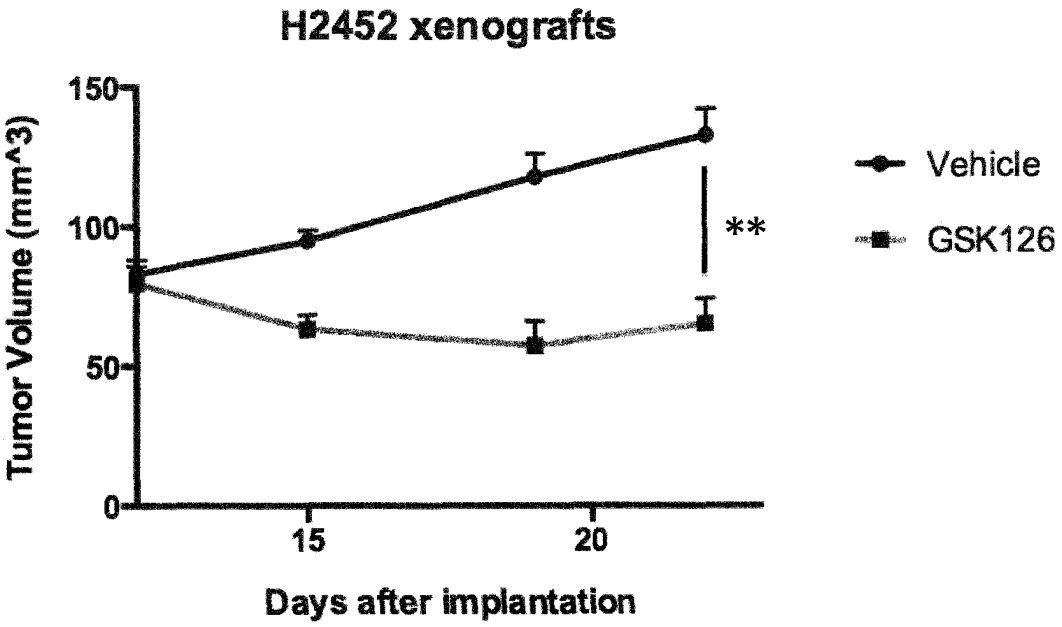

H2452 xenografts
Tumor Volume (mm^3)
150
100
50
0
15
20
Days after implantation
**
Vehicle
GSK126

FIGURE 5A-L

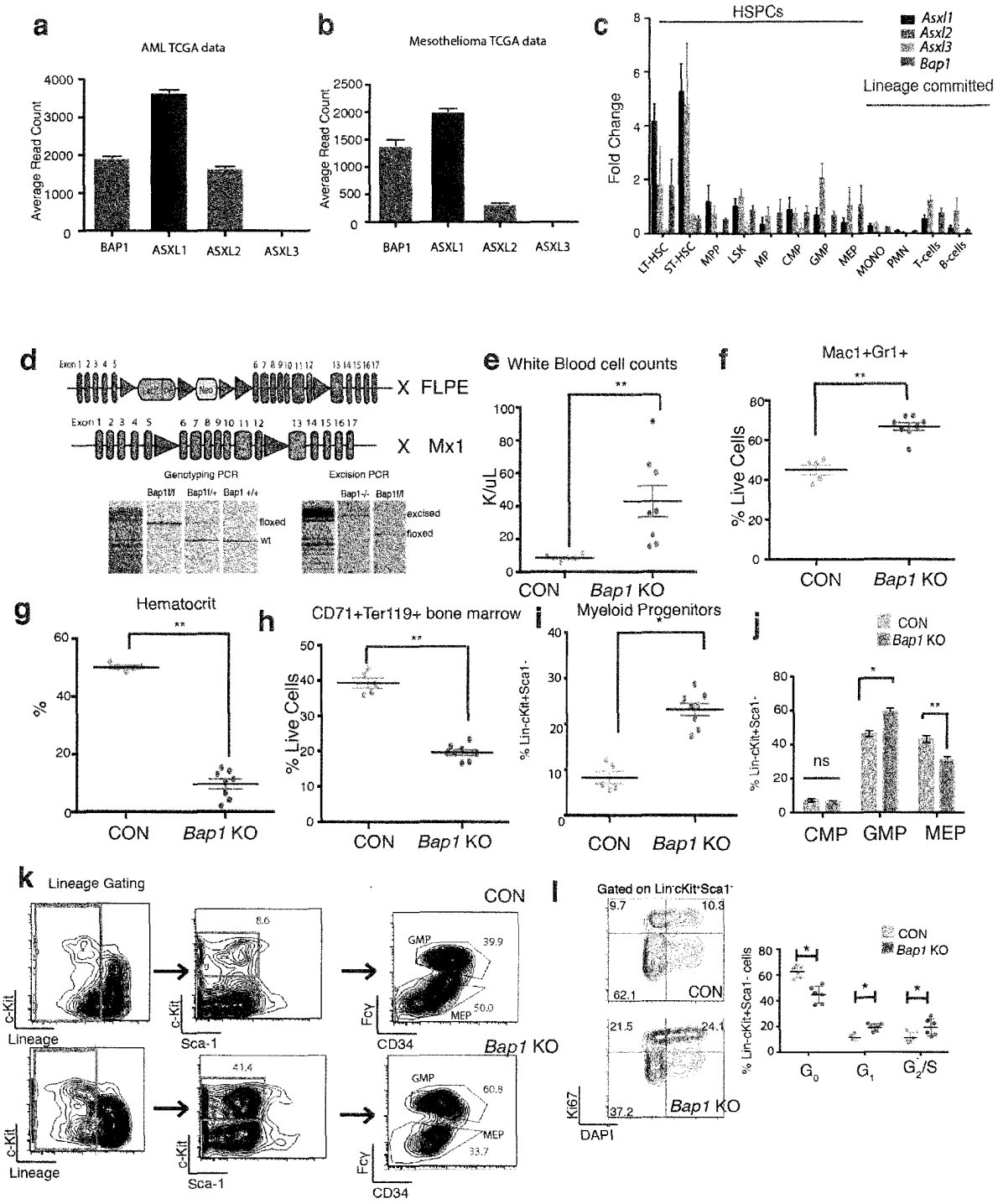

a
AML TCGA data
Average Read Count
BAP1
ASXL1
ASXL2
ASXL3
b
Mesothelioma TCGA data
c
HSPCs
Asxl1
Asxl2
Asxl3
Bap1
Lineage committed
Fold Change
LT-HSC
ST-HSC
MPP
LSK
MP
CMP
GMP
MEP
MONO
PMN
T-cells
B-cells
d
X FLPE
X Mx1
Genotyping PCR
Excision PCR
floxed
wt
excised
e White Blood cell counts
K/uL
CON
Bap1 KO
f
Mac1+Gr1+
% Live Cells
g
Hematocrit
%
h
CD71+Ter119+ bone marrow
i
Myeloid Progenitors
% Lin-cKit+Sca1-
j
CON
Bap1 KO
ns
CMP
GMP
MEP
k
Lineage Gating
c-Kit
Lineage
Sca-1
CD34
Fcγ
GMP
MEP
l
Gated on Lin-cKit+Sca1-
Ki67
DAPI
% Lin-cKit+Sca1- cells
G0
G1
G2/S

FIGURE 6A-L

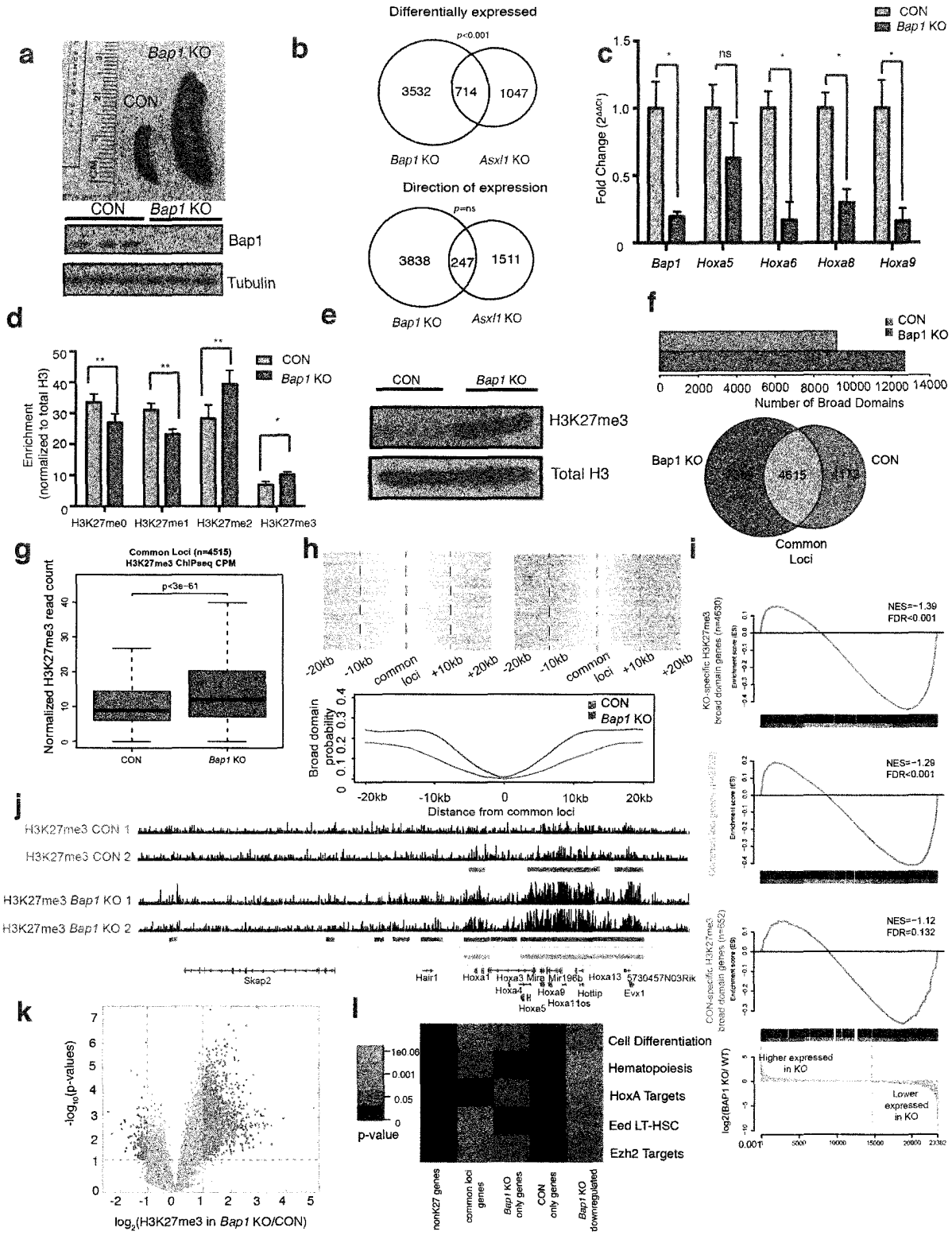

a
Bap1 KO
CON
CON
Bap1 KO
Bap1
Tubulin
b
Differentially expressed
p<0.001
3532
714
1047
Bap1 KO
Asxl1 KO
Direction of expression
p=ns
3838
247
1511
Bap1 KO
Asxl1 KO
c
CON
Bap1 KO
Fold Change (2^-ΔΔCt)
ns
Bap1
Hoxa5
Hoxa6
Hoxa8
Hoxa9
d
Enrichment (normalized to total H3)
CON
Bap1 KO
H3K27me0
H3K27me1
H3K27me2
H3K27me3
e
CON
Bap1 KO
H3K27me3
Total H3
f
CON
Bap1 KO
Number of Broad Domains
Bap1 KO
CON
4515
Common Loci
g
Common Loci (n=4515)
H3K27me3 ChIPseq CPM
p<3e-61
Normalized H3K27me3 read count
CON
Bap1 KO
h
-20kb
-10kb
common loci
+10kb
+20kb
Broad domain probability
CON
Bap1 KO
Distance from common loci
i
KO-specific H3K27me3 broad domain genes (n=4630)
NES=-1.39
FDR<0.001
NES=-1.29
FDR<0.001
NES=-1.12
FDR=0.132
Higher expressed in KO
Lower expressed in KO
log2(BAP1 KO/ WT)
j
H3K27me3 CON 1
H3K27me3 CON 2
H3K27me3 Bap1 KO 1
H3K27me3 Bap1 KO 2
Skap2
Hair1
Hoxa1
Hoxa3
Mirg
Mir196b
Hoxa13
5730457N03Rik
Hoxa4
Hoxa9
Hotlip
Evx1
Hoxa5
Hoxa11os
k
-log10(p-values)
log2(H3K27me3 in Bap1 KO/CON)
l
p-value
1e0.06
0.001
0.05
0
Cell Differentiation
Hematopoiesis
HoxA Targets
Eed LT-HSC
Ezh2 Targets
nonK27 genes
common loci genes
Bap1 KO only genes
CON only genes
Bap1 KO downregulated

FIGURE 7A-C

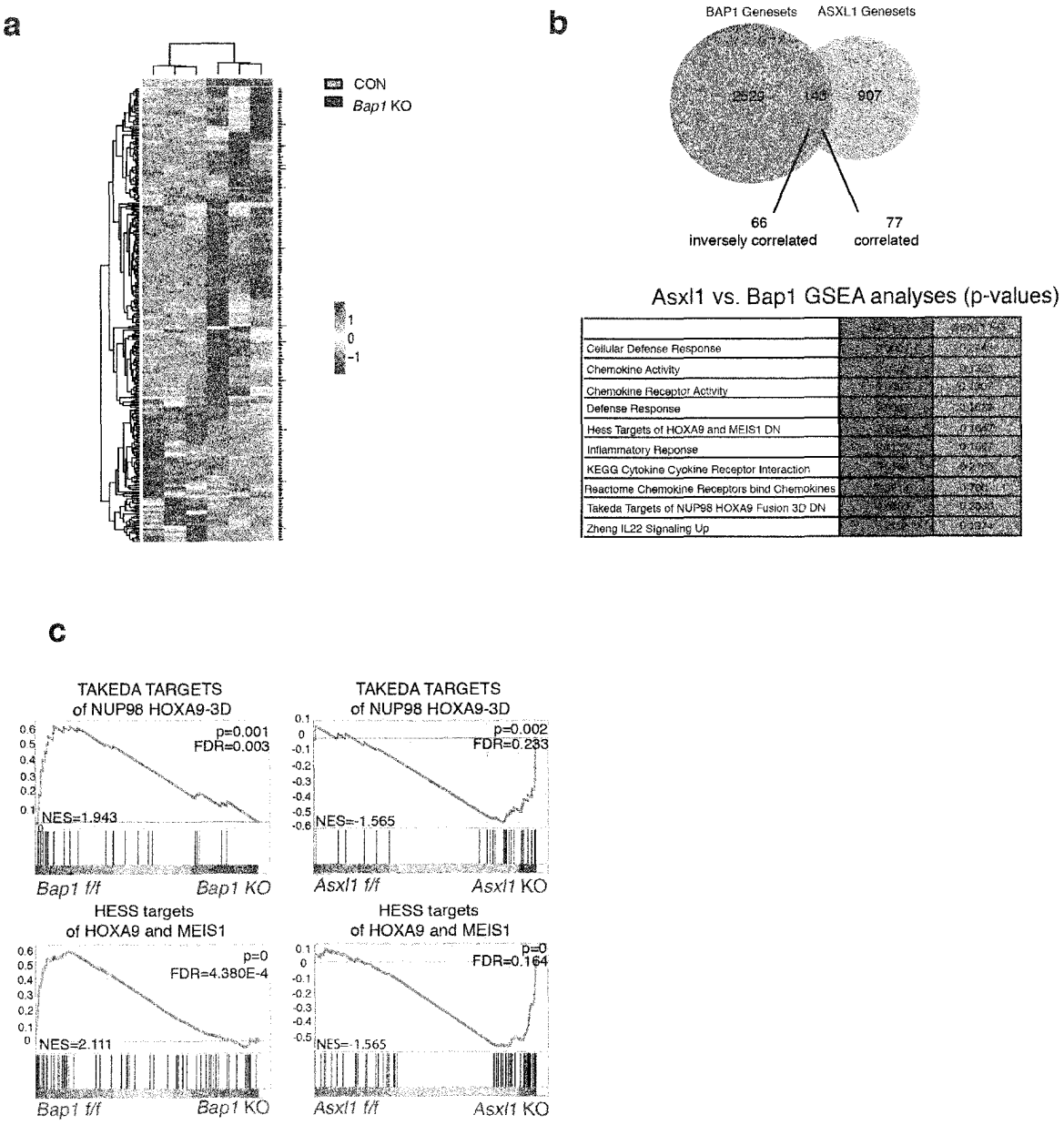

a
CON
Bap1 KO
1
0
-1
b
BAP1 Genesets
ASXL1 Genesets
907
66
inversely correlated
77
correlated
Asxl1 vs. Bap1 GSEA analyses (p-values)
Cellular Defense Response
Chemokine Activity
Chemokine Receptor Activity
Defense Response
Hess Targets of HOXA9 and MEIS1 DN
Inflammatory Reponse
KEGG Cytokine Cyokine Receptor Interaction
Reactome Chemokine Receptors bind Chemokines
Takeda Targets of NUP98 HOXA9 Fusion 3D DN
Zheng IL22 Signaling Up
c
TAKEDA TARGETS
of NUP98 HOXA9-3D
p=0.001
FDR=0.003
NES=1.943
Bap1 f/f
Bap1 KO
TAKEDA TARGETS
of NUP98 HOXA9-3D
p=0.002
FDR=0.233
NES=-1.565
Asxl1 f/f
Asxl1 KO
HESS targets
of HOXA9 and MEIS1
p=0
FDR=4.380E-4
NES=2.111
Bap1 f/f
Bap1 KO
HESS targets
of HOXA9 and MEIS1
p=0
FDR=0.164
NES=-1.565
Asxl1 f/f
Asxl1 KO

FIGURE 8A-C

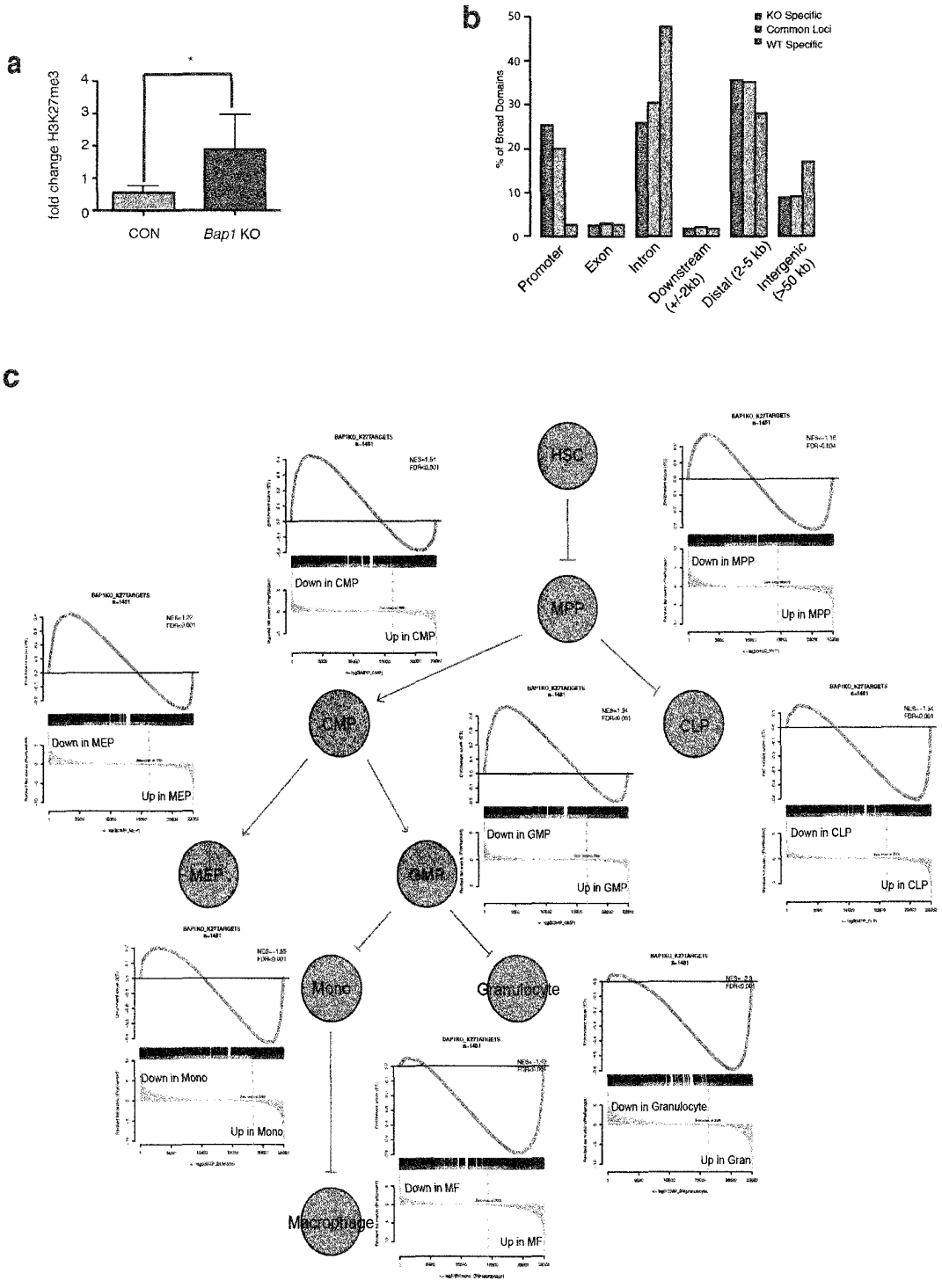

a
fold change H3K27me3
CON
Bap1 KO
b
% of Broad Domains
KO Specific
Common Loci
WT Specific
Promoter
Exon
Intron
Downstream (+/-2kb)
Distal (2-5 kb)
Intergenic (>50 kb)
c
HSC
MPP
CMP
CLP
MEP
GMP
Mono
Granulocyte
Macrophage
Down in CMP
Up in CMP
Down in MPP
Up in MPP
Down in MEP
Up in MEP
Down in GMP
Up in GMP
Down in CLP
Up in CLP
Down in Mono
Up in Mono
Down in Granulocyte
Up in Gran
Down in MF
Up in MF

FIGURE 9A-C

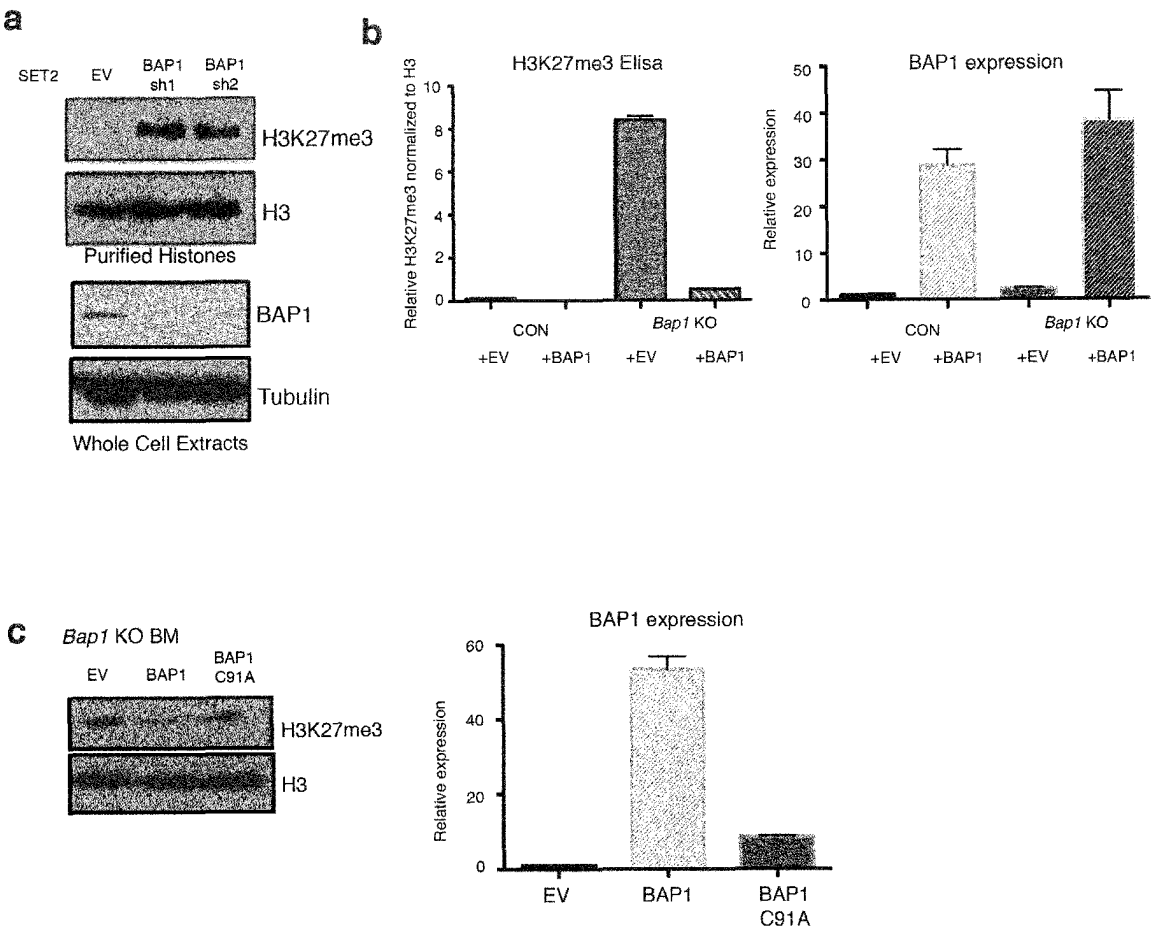

a
SET2
EV
BAP1 sh1
BAP1 sh2
H3K27me3
H3
Purified Histones
BAP1
Tubulin
Whole Cell Extracts
b
H3K27me3 Elisa
Relative H3K27me3 normalized to H3
CON
Bap1 KO
+EV
+BAP1
+EV
+BAP1
BAP1 expression
Relative expression
CON
Bap1 KO
+EV
+BAP1
+EV
+BAP1
c
Bap1 KO BM
EV
BAP1
BAP1 C91A
H3K27me3
H3
BAP1 expression
Relative expression
EV
BAP1
BAP1 C91A

FIGURE 10A-D

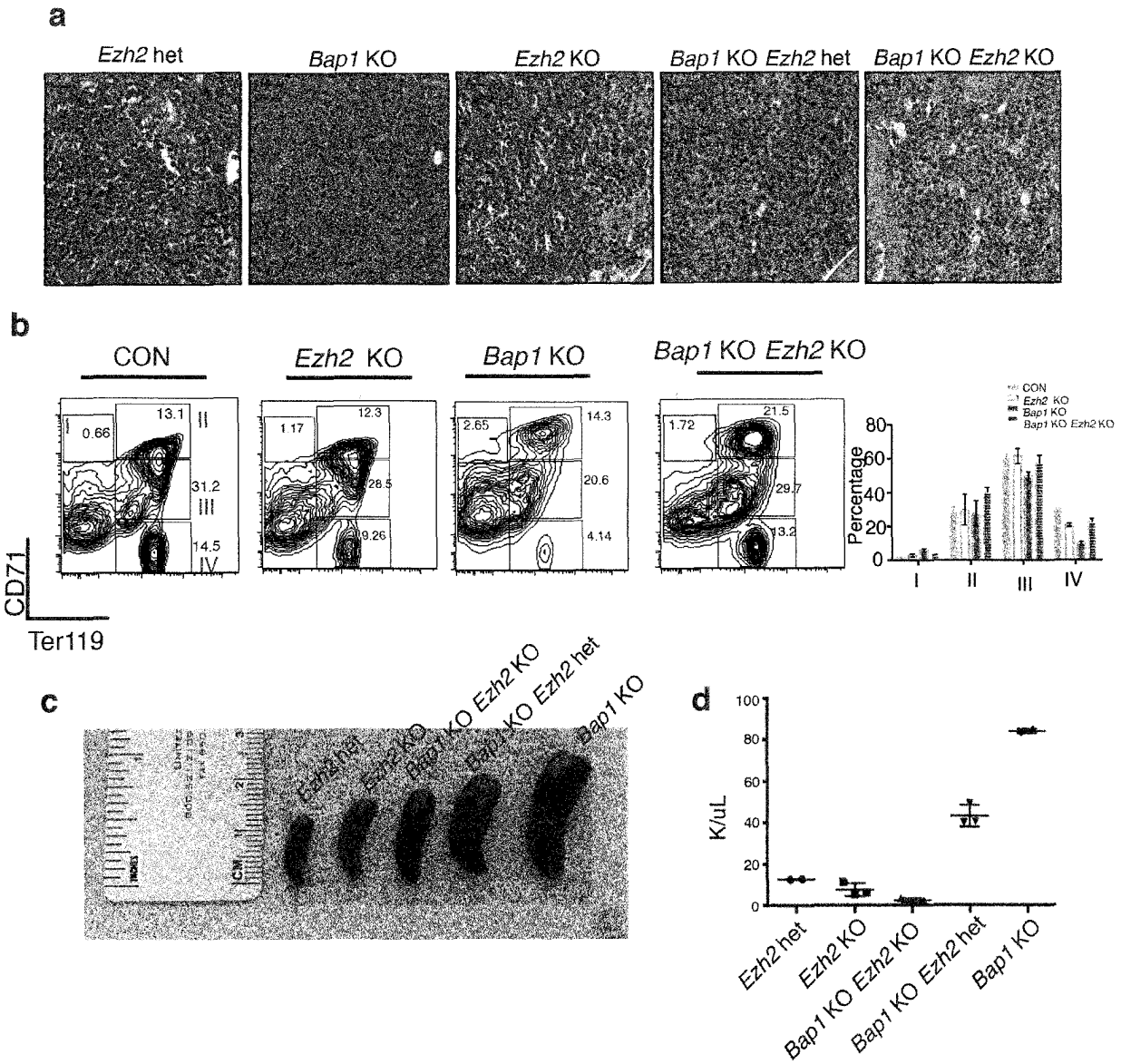

a
Ezh2 het
Bap1 KO
Ezh2 KO
Bap1 KO Ezh2 het
Bap1 KO Ezh2 KO
b
CON
Ezh2 KO
Bap1 KO
Bap1 KO Ezh2 KO
CD71
Ter119
0.66
13.1
31.2
14.5
1.17
12.3
28.5
9.26
2.65
14.3
20.6
4.14
1.72
21.5
29.7
13.2
Percentage
I
II
III
IV
c
d
K/uL
Ezh2 het
Ezh2 KO
Bap1 KO Ezh2 KO
Bap1 KO Ezh2 het
Bap1 KO

FIGURE 11A-K

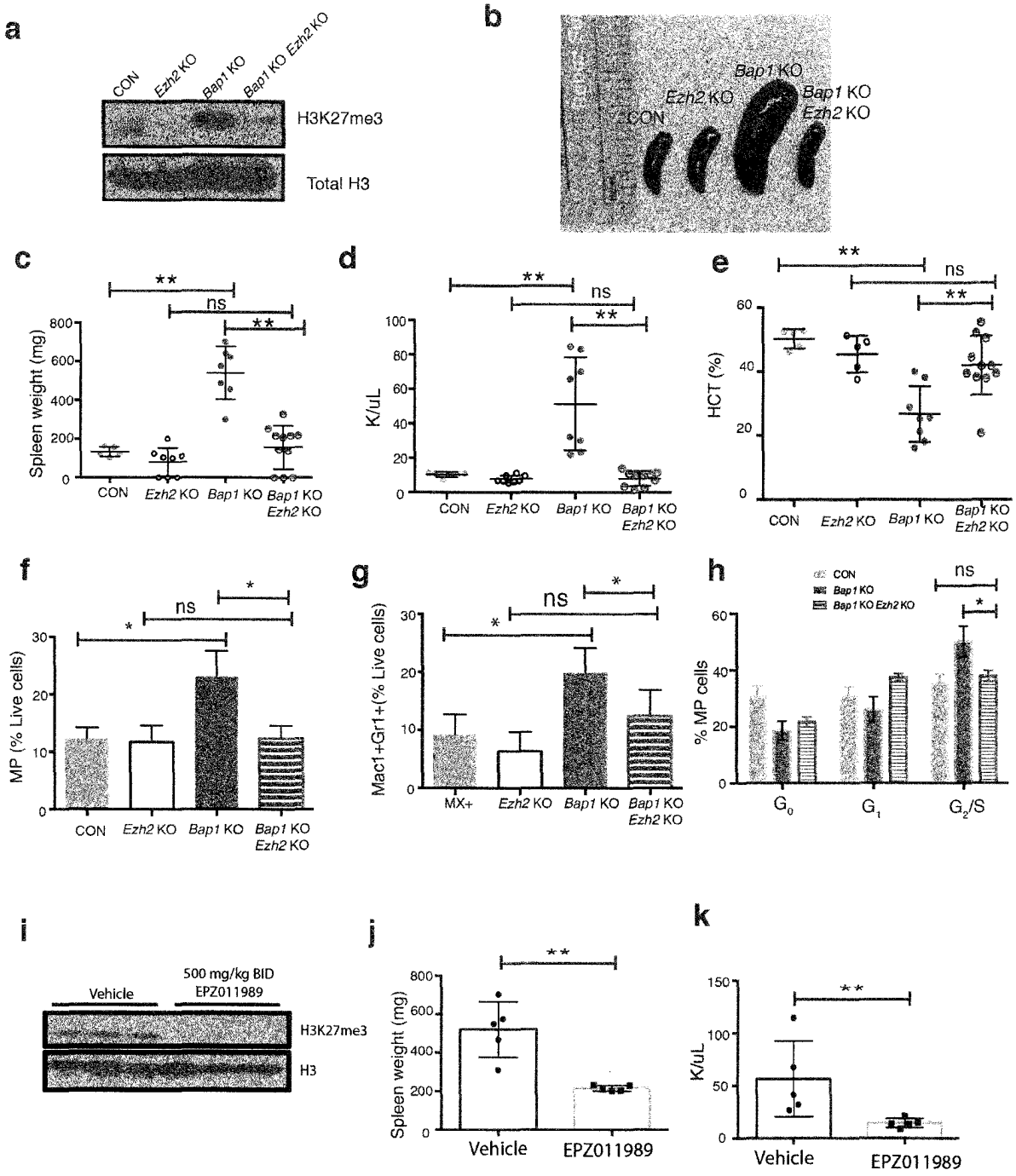
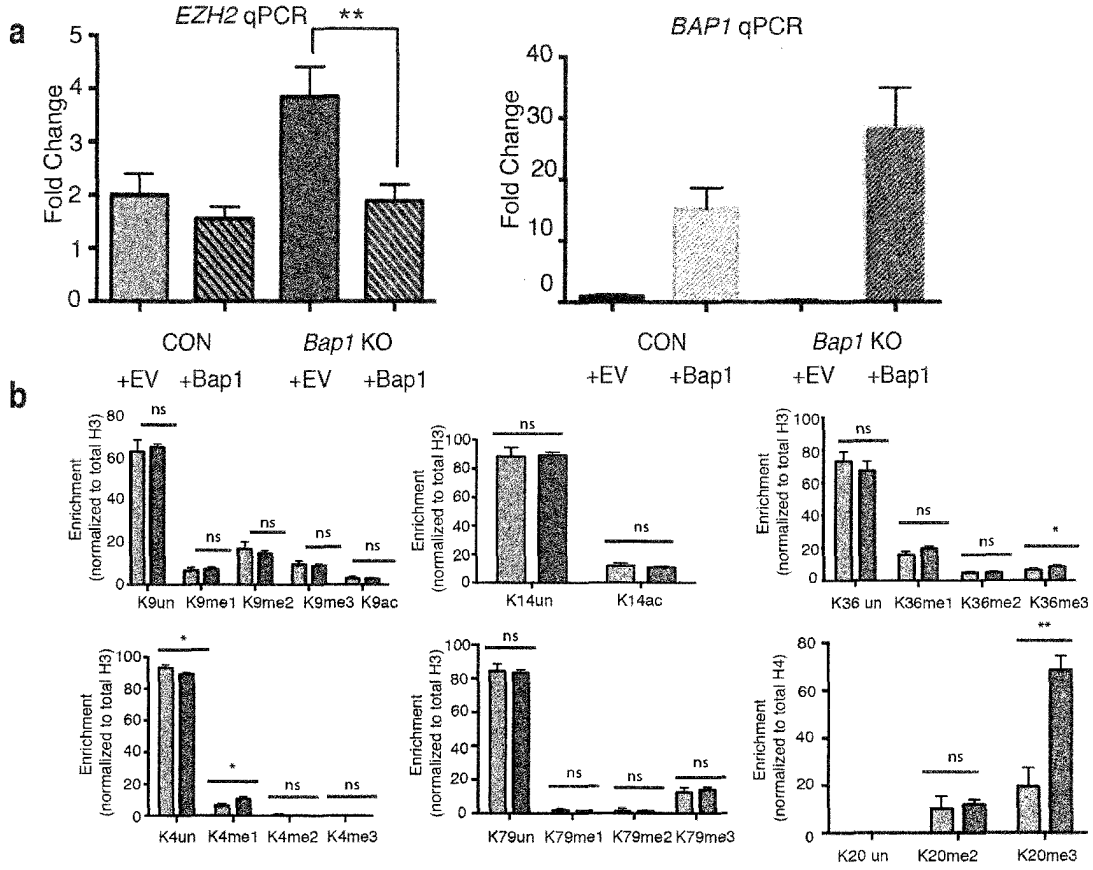

a
CON
Ezh2 KO
Bap1 KO
Bap1 KO Ezh2 KO
H3K27me3
Total H3
b
Bap1 KO
Ezh2 KO
Bap1 KO Ezh2 KO
CON
c
Spleen weight (mg)
CON
Ezh2 KO
Bap1 KO
Bap1 KO Ezh2 KO
d
K/uL
e
HCT (%)
f
MP (% Live cells)
g
Mac1+Gr1+(% Live cells)
MX+
h
% MP cells
G0
G1
G2/S
i
Vehicle
500 mg/kg BID EPZ011989
H3K27me3
H3
j
Spleen weight (mg)
Vehicle
EPZ011989
k
K/uL
Vehicle
EPZ011989

FIGURE 12A-C


a
EZH2 qPCR
**
Fold Change
CON
Bap1 KO
+EV
+Bap1
BAP1 qPCR
b
Enrichment (normalized to total H3)
K9un K9me1 K9me2 K9me3 K9ac
K14un K14ac
K36 un K36me1 K36me2 K36me3
K4un K4me1 K4me2 K4me3
K79un K79me1 K79me2 K79me3
Enrichment (normalized to total H4)
K20 un K20me2 K20me3
ns

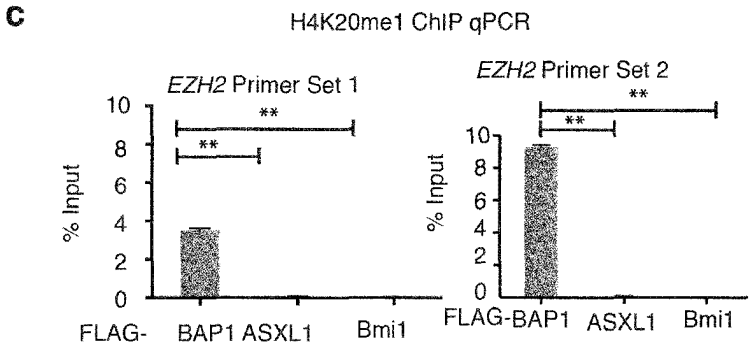

c
H4K20me1 ChIP qPCR
EZH2 Primer Set 1
EZH2 Primer Set 2
% Input
FLAG-
BAP1
ASXL1
Bmi1

FIGURE 13A-L

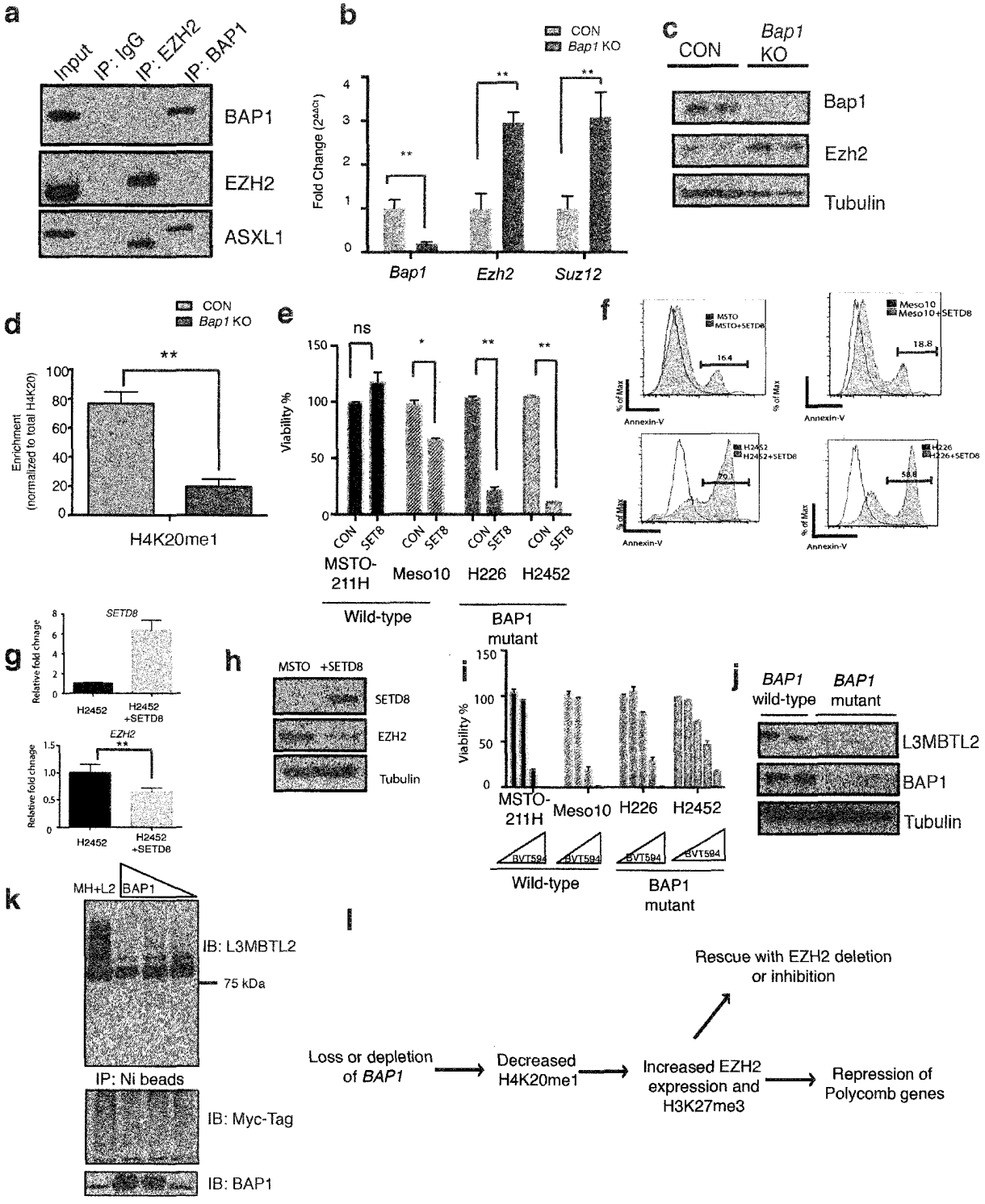
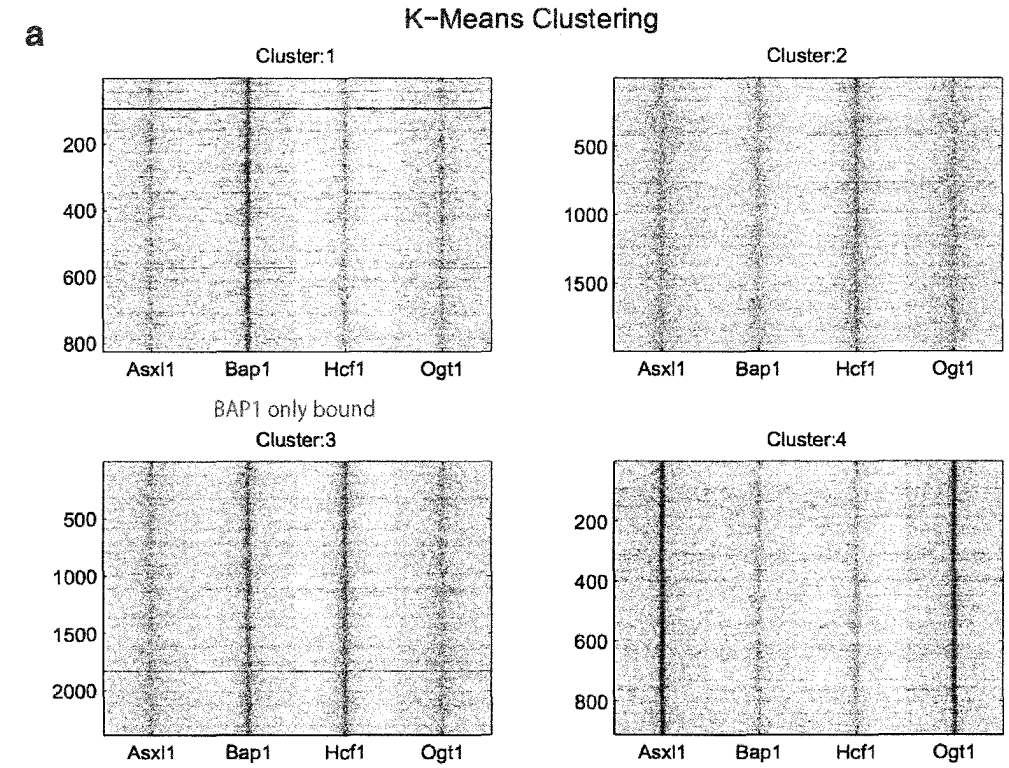

a
Input
IP: IgG
IP: EZH2
IP: BAP1
BAP1
EZH2
ASXL1
b
CON
Bap1 KO
Fold Change (2^ΔΔCt)
Bap1
Ezh2
Suz12
c
CON
Bap1 KO
Bap1
Ezh2
Tubulin
d
CON
Bap1 KO
Enrichment (normalized to total H4K20)
H4K20me1
e
ns
Viability %
CON
SETD8
MSTO-211H
Meso10
H226
H2452
Wild-type
BAP1 mutant
f
MSTO
MSTO+SETD8
Meso10
Meso10+SETD8
H2452
H2452+SETD8
H226
H226+SETD6
Annexin-V
% of Max
g
SETD8
Relative fold change
H2452
H2452 +SETD8
EZH2
h
MSTO +SETD8
SETD8
EZH2
Tubulin
i
Viability %
MSTO-211H
Meso10
H226
H2452
BVT594
Wild-type
BAP1 mutant
j
BAP1 wild-type
BAP1 mutant
L3MBTL2
BAP1
Tubulin
k
MH+L2
BAP1
IB: L3MBTL2
75 kDa
IP: Ni beads
IB: Myc-Tag
IB: BAP1
l
Loss or depletion of BAP1
Decreased H4K20me1
Increased EZH2 expression and H3K27me3
Repression of Polycomb genes
Rescue with EZH2 deletion or inhibition

FIGURE 14A-B



b

Cluster 1:

Motif Logo	Motif	p-value	% of targets	% of background
	USF2/E-box	1e-19	18.6	8.5

FIGURE 15A-I

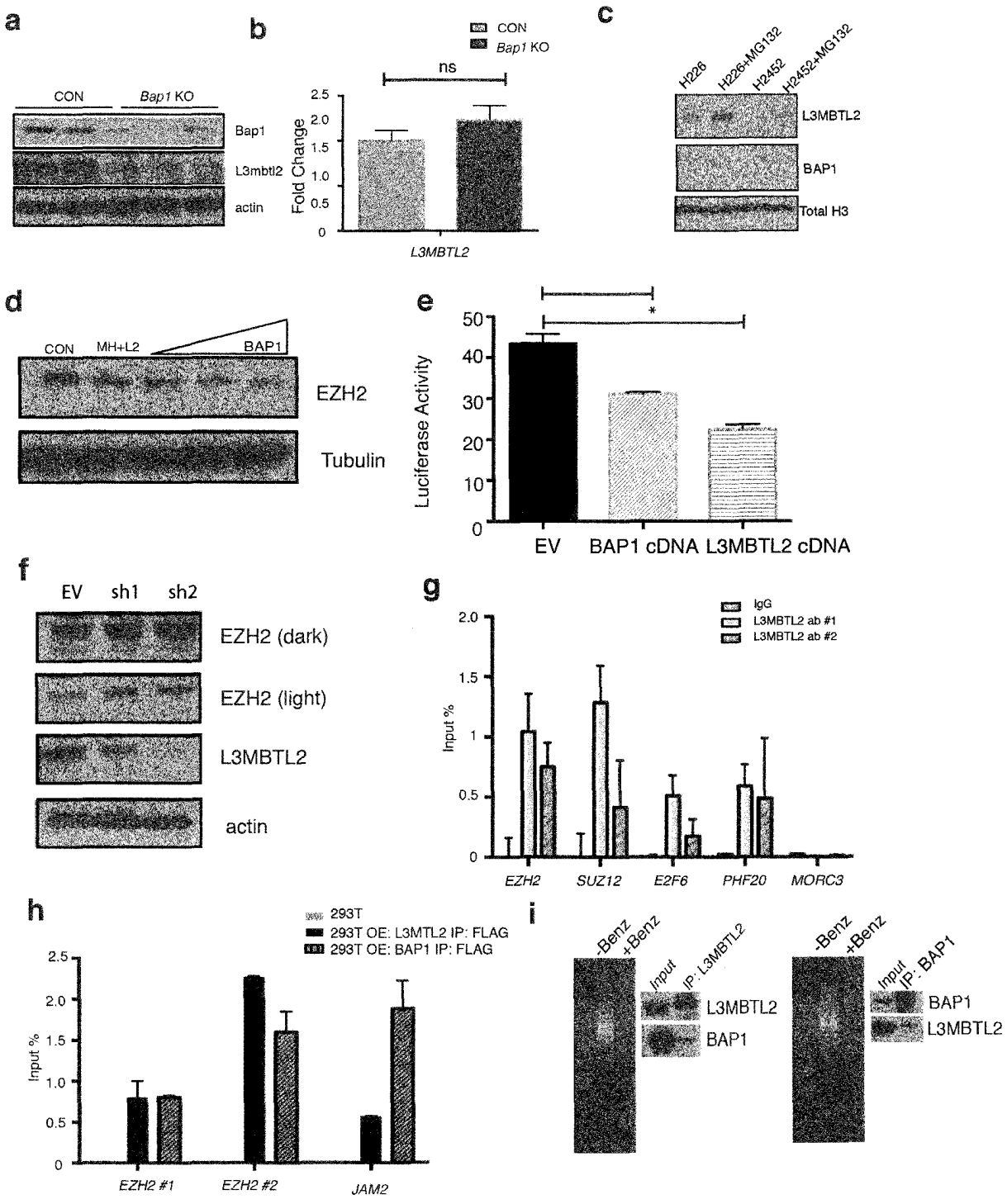

a
CON
Bap1 KO
Bap1
L3mbtl2
actin
b
CON
Bap1 KO
ns
Fold Change
L3MBTL2
c
H226
H226+MG132
H2452
H2452+MG132
L3MBTL2
BAP1
Total H3
d
CON
MH+L2
BAP1
EZH2
Tubulin
e
Luciferase Activity
EV
BAP1 cDNA
L3MBTL2 cDNA
f
EV
sh1
sh2
EZH2 (dark)
EZH2 (light)
L3MBTL2
actin
g
IgG
L3MBTL2 ab #1
L3MBTL2 ab #2
Input %
EZH2
SUZ12
E2F6
PHF20
MORC3
h
293T
293T OE: L3MTL2 IP: FLAG
293T OE: BAP1 IP: FLAG
Input %
EZH2 #1
EZH2 #2
JAM2
i
-Benz
+Benz
Input
IP: L3MBTL2
L3MBTL2
BAP1
IP: BAP1

FIGURE 16A-H

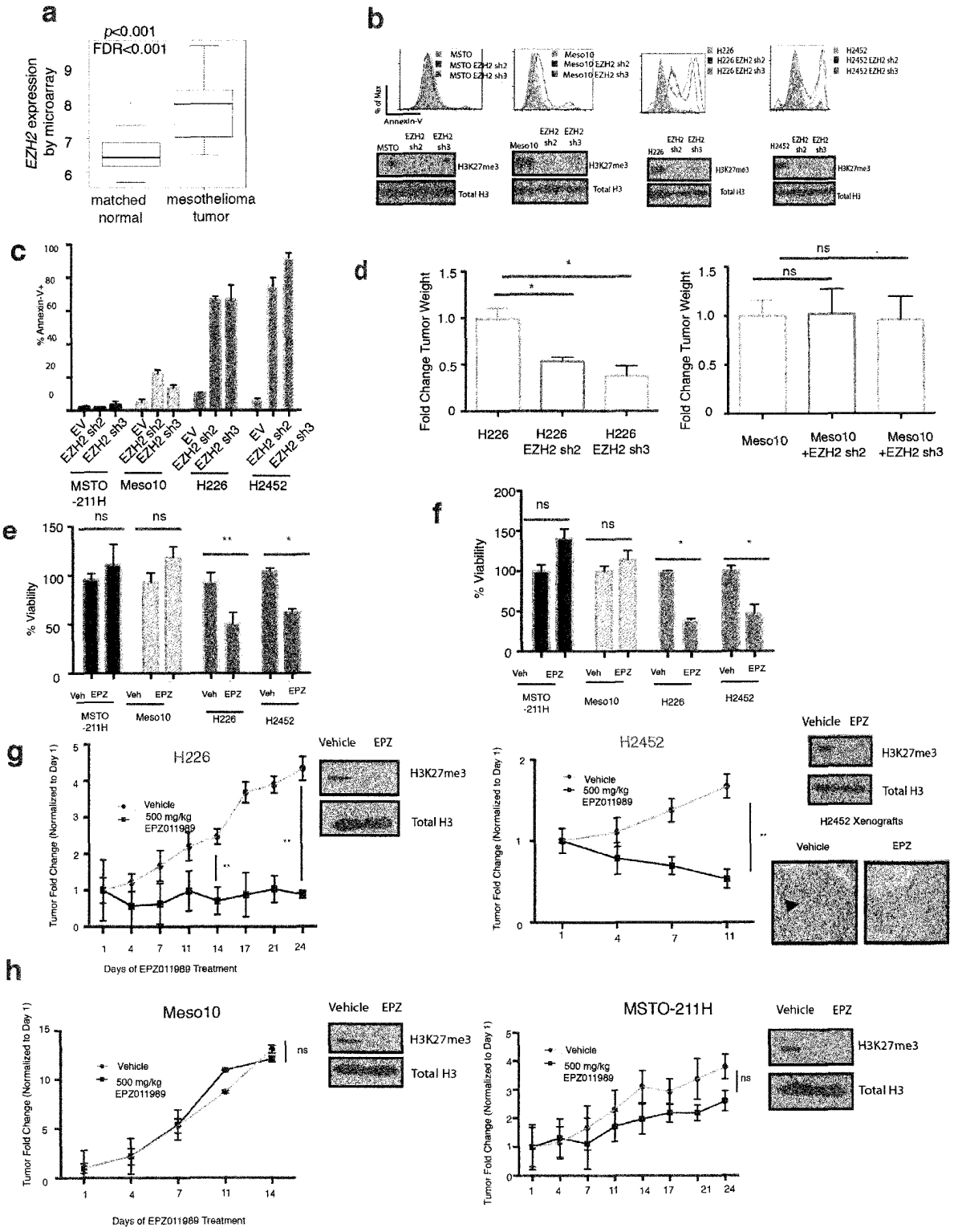

a
p<0.001
FDR<0.001
EZH2 expression by microarray
matched normal
mesothelioma tumor
b
MSTO
MSTO EZH2 sh2
MSTO EZH2 sh3
Meso10
Meso10 EZH2 sh2
Meso10 EZH2 sh3
H226
H226 EZH2 sh2
H226 EZH2 sh3
H2452
H2452 EZH2 sh2
H2452 EZH2 sh3
% of Max
Annexin-V
EZH2 sh2
EZH2 sh3
H3K27me3
Total H3
c
% Annexin-V+
EV
EZH2 sh2
EZH2 sh3
MSTO -211H
Meso10
H226
H2452
d
Fold Change Tumor Weight
H226
H226 EZH2 sh2
H226 EZH2 sh3
Meso10
Meso10 +EZH2 sh2
Meso10 +EZH2 sh3
ns
e
% Viability
Veh
EPZ
MSTO -211H
Meso10
H226
H2452
f
% Viability
g
H226
Vehicle
500 mg/kg EPZ011989
Tumor Fold Change (Normalized to Day 1)
Days of EPZ011989 Treatment
Vehicle EPZ
H3K27me3
Total H3
H2452
H2452 Xenografts
h
Meso10
MSTO-211H

FIGURE 17

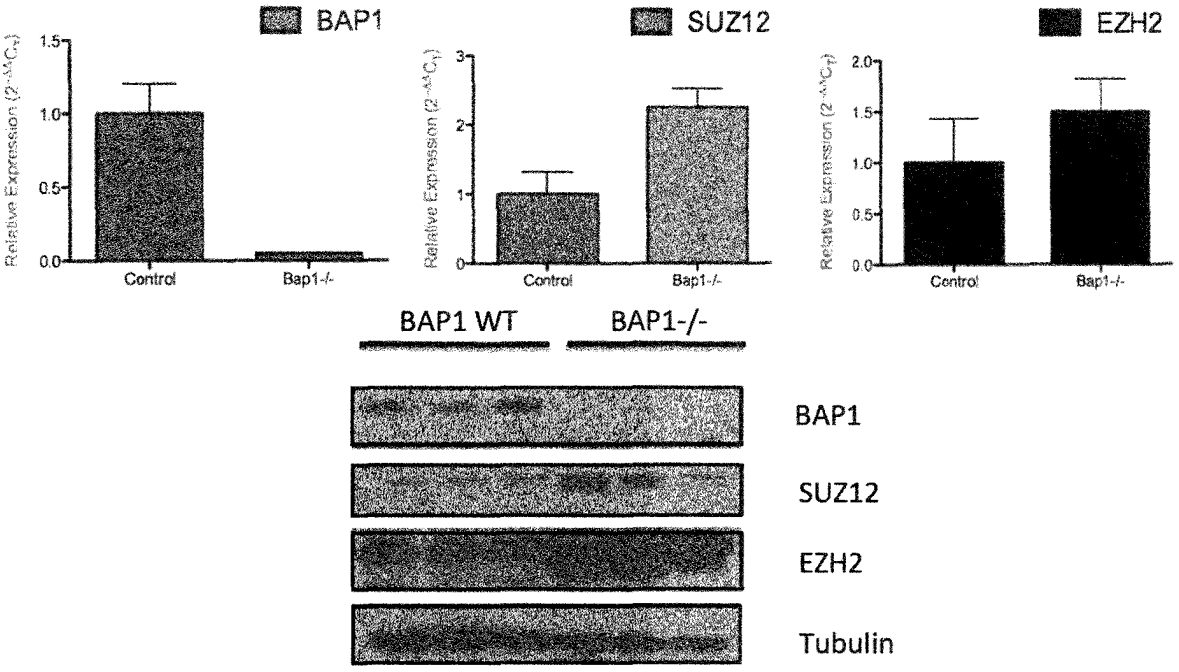

BAP1
SUZ12
EZH2
Relative Expression (2^-ΔΔCT)
1.5
1.0
0.5
0.0
3
2
1
0
2.0
1.5
1.0
0.5
0.0
Control
Bap1-/-
BAP1 WT
BAP1-/-
BAP1
SUZ12
EZH2
Tubulin

FIGURE 18

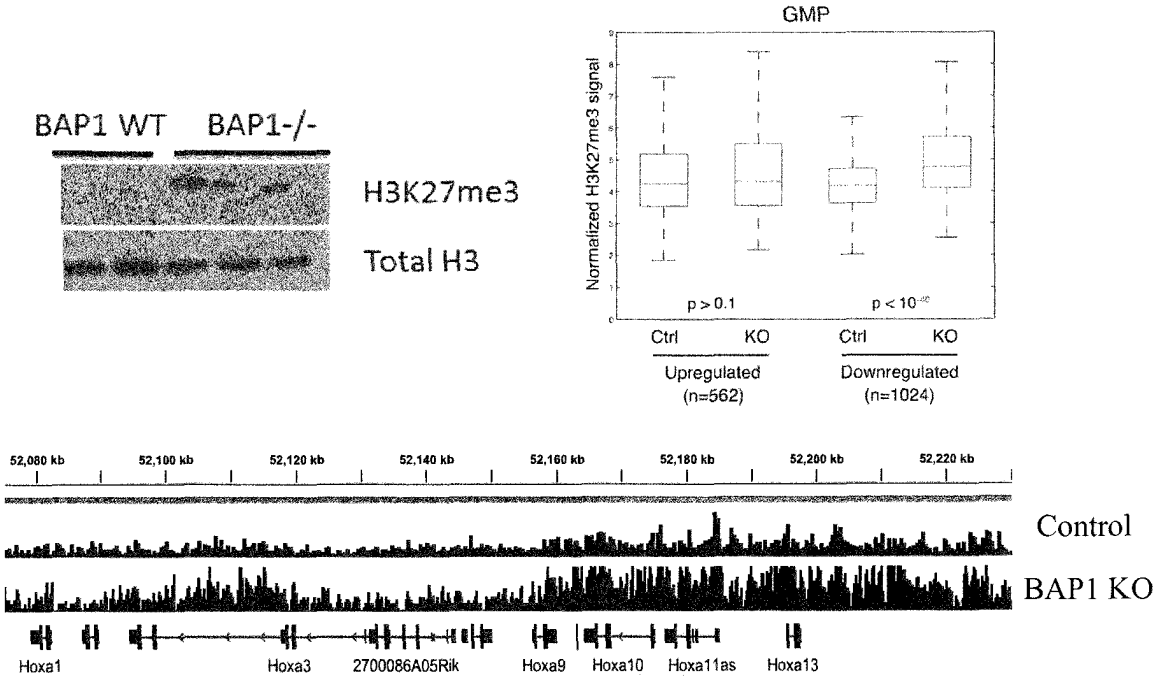

BAP1 WT
BAP1-/-
H3K27me3
Total H3
GMP
Normalized H3K27me3 signal
p > 0.1
p < 10
Ctrl
KO
Ctrl
KO
Upregulated
(n=562)
Downregulated
(n=1024)
52,080 kb
52,100 kb
52,120 kb
52,140 kb
52,160 kb
52,180 kb
52,200 kb
52,220 kb
Control
BAP1 KO
Hoxa1
Hoxa3
2700086A05Rik
Hoxa9
Hoxa10
Hoxa11as
Hoxa13

FIGURE 19

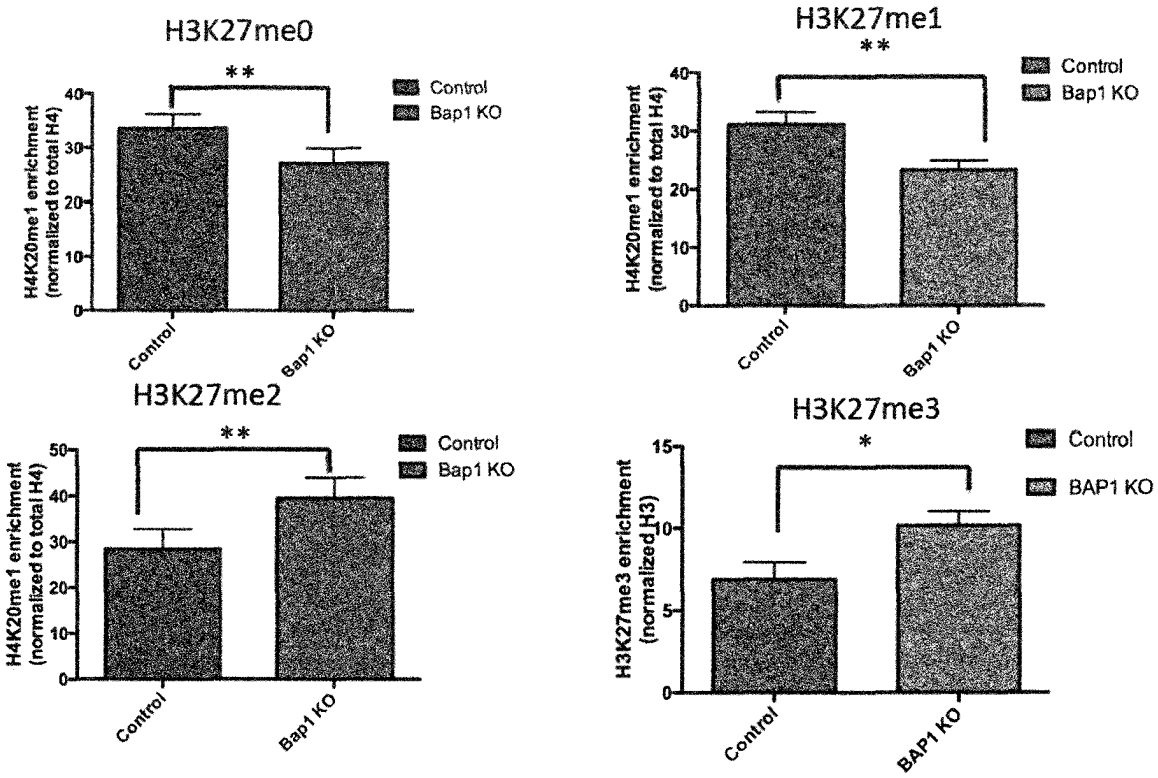

H3K27me0
**
Control
Bap1 KO
H4K20me1 enrichment (normalized to total H4)
0
10
20
30
40
Control
Bap1 KO
H3K27me1
**
Control
Bap1 KO
H4K20me1 enrichment (normalized to total H4)
0
10
20
30
40
Control
Bap1 KO
H3K27me2
**
Control
Bap1 KO
H4K20me1 enrichment (normalized to total H4)
0
10
20
30
40
50
Control
Bap1 KO
H3K27me3
*
Control
BAP1 KO
H3K27me3 enrichment (normalized H3)
0
5
10
15
Control
BAP1 KO

FIGURE 20A-D

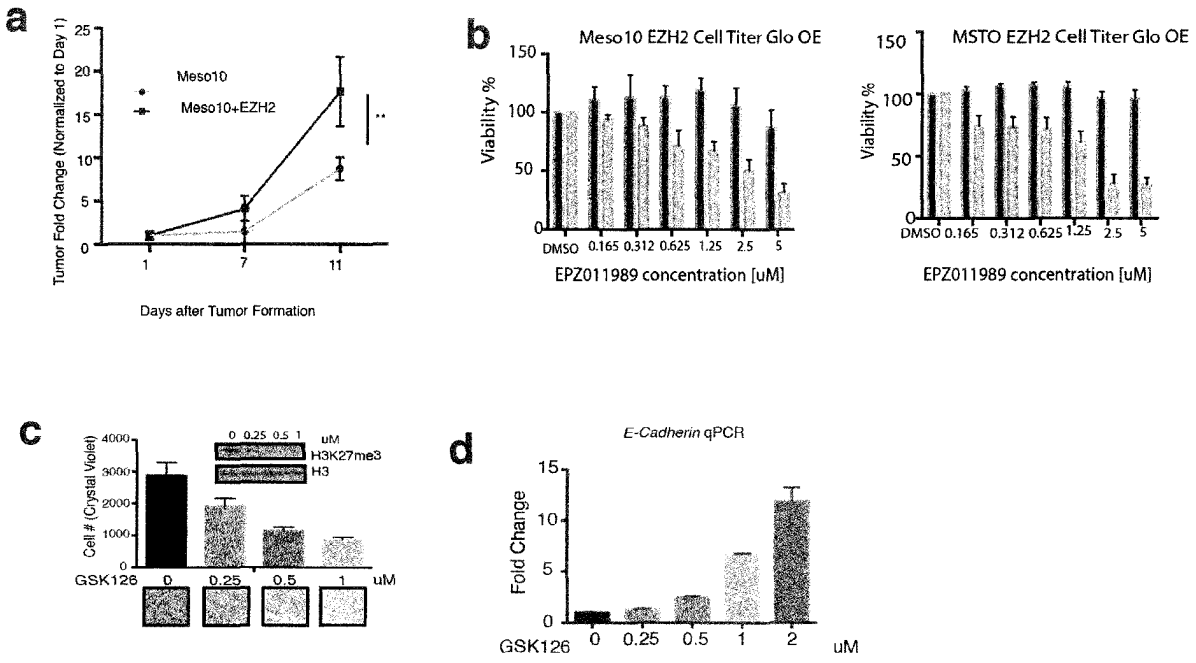
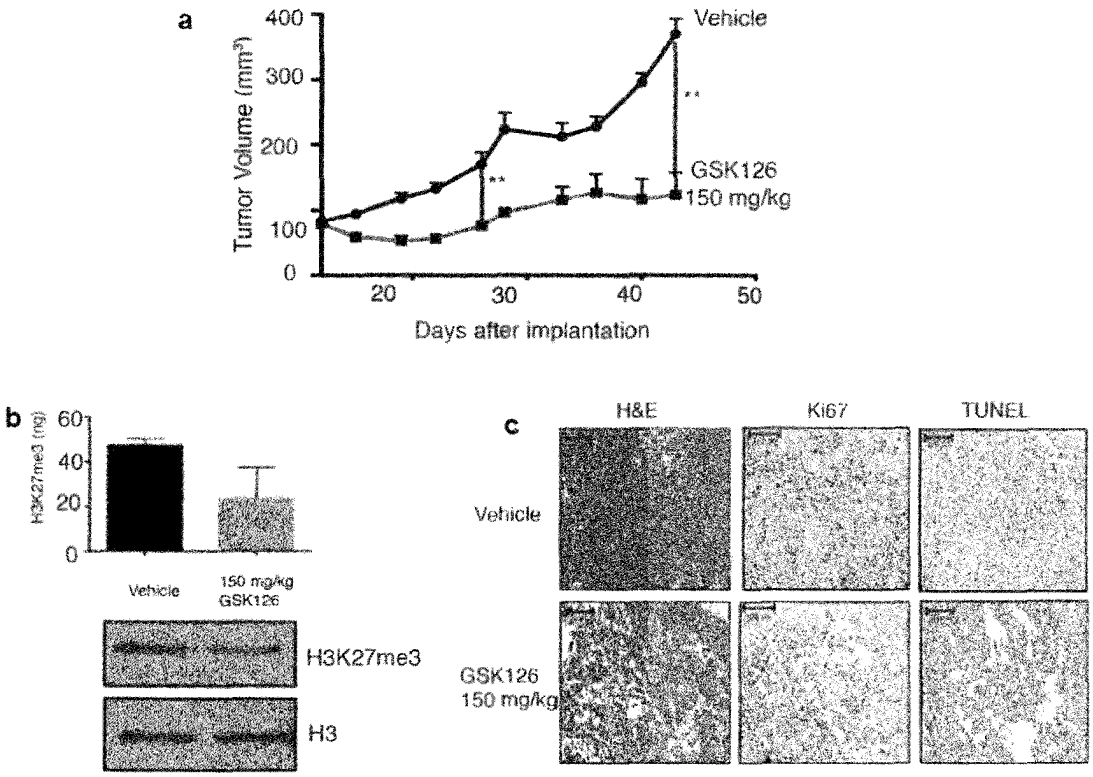

a
Tumor Fold Change (Normalized to Day 1)
Meso10
Meso10+EZH2
**
Days after Tumor Formation
b
Meso10 EZH2 Cell Titer Glo OE
Viability %
DMSO 0.165 0.312 0.625 1.25 2.5 5
EPZ011989 concentration [uM]
MSTO EZH2 Cell Titer Glo OE
Viability %
DMSO 0.165 0.312 0.625 1.25 2.5 5
EPZ011989 concentration [uM]
c
Cell # (Crystal Violet)
0 0.25 0.5 1 uM
H3K27me3
H3
GSK126 0 0.25 0.5 1 uM
d
E-Cadherin qPCR
Fold Change
GSK126 0 0.25 0.5 1 2 uM

FIGURE 21A-C


a
Tumor Volume (mm³)
400
300
200
100
0
20
30
40
50
Days after implantation
Vehicle
GSK126
150 mg/kg
**
**
b
H3K27me3 (ng)
60
40
20
0
Vehicle
150 mg/kg GSK126
H3K27me3
H3
c
H&E
Ki67
TUNEL
Vehicle
GSK126
150 mg/kg