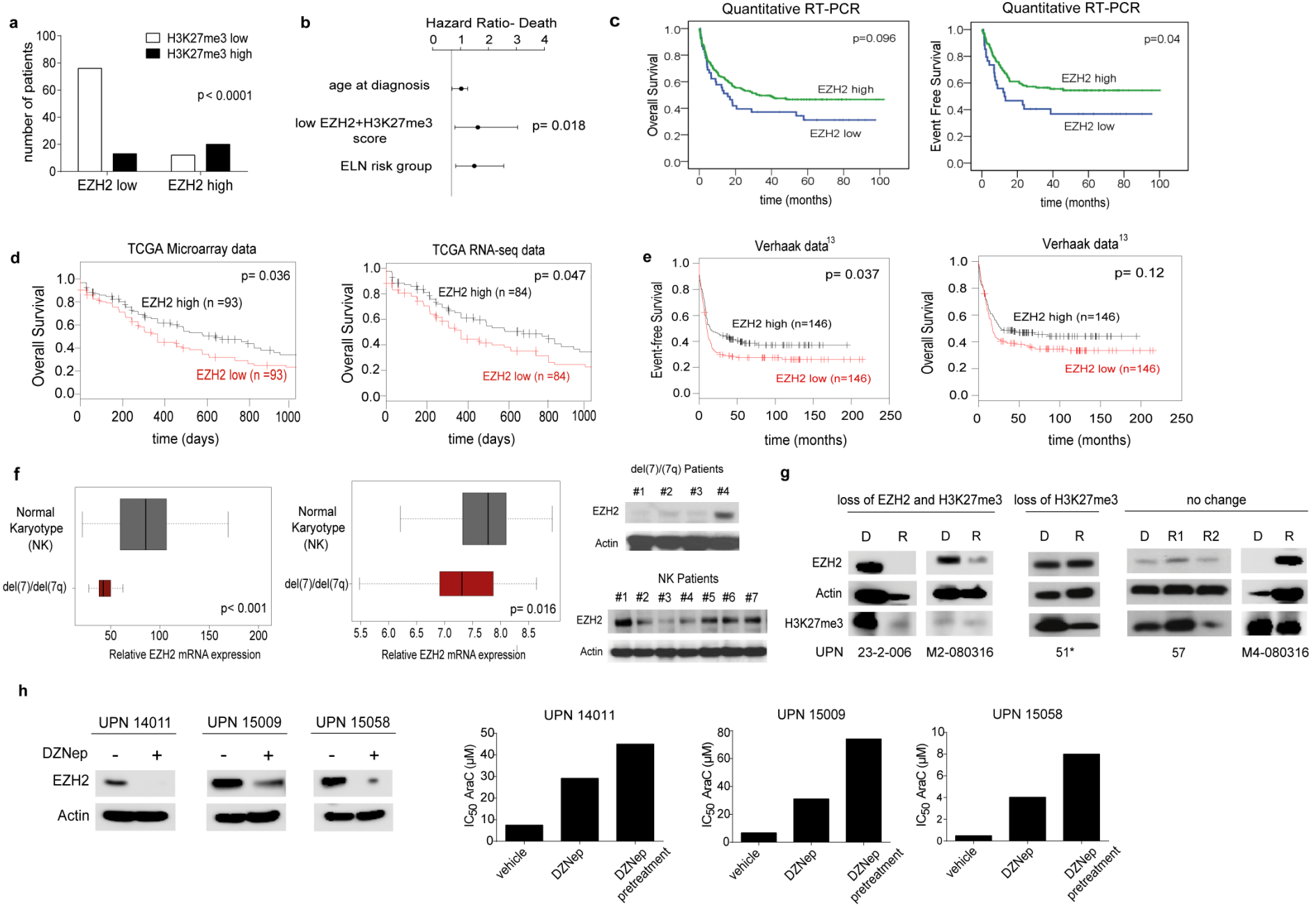
Supplementary Figure 1



Supplementary Figure 1, related to Figure 1. Reduced EZH2 levels correlate with poor prognosis and chemoresistance in AML patients

(a) Association between EZH2 protein expression and H3K27me3 levels of patients analyzed in Tissue Microarray experiments (Fisher`s exact test, $p< 0.0001$). Clinical data of the cohort are provided in Supplementary Table 1.

(b) A multivariate Cox-regression analysis for death risk of AML patients was performed including combined EZH2+H3K27me3 expression. Hazard ratios with 95% confidence intervals are indicated. A combined low EZH2+H3K27me3 protein level was independently associated with poor overall survival ($p= 0.018$).

(c) *EZH2* mRNA expression of 221 diagnosed primary AML patients was analyzed by Quantitative RT-PCR (qRT-PCR). Clinical data of the cohort are provided in Supplementary Table 2. *EZH2* mRNA expression was normalized to *GAPDH*. EZH2 high/low cutoff was set at the 40^{th} percentile. Kaplan-Meier Plots for overall survival (OS) and event free survival (EFS) are shown.

(d) Kaplan-Meier plots for overall survival (OS) of AML patients discriminated by high and low EZH2 mRNA expression from microarray (left) and RNA seq data (right) of the TCGA data set[1].

(e) Kaplan-Meier plots for overall (OS) and event-free survival (EFS) of AML patients discriminated by high and low EZH2 mRNA expression in the Verhaak microarray data set[2].

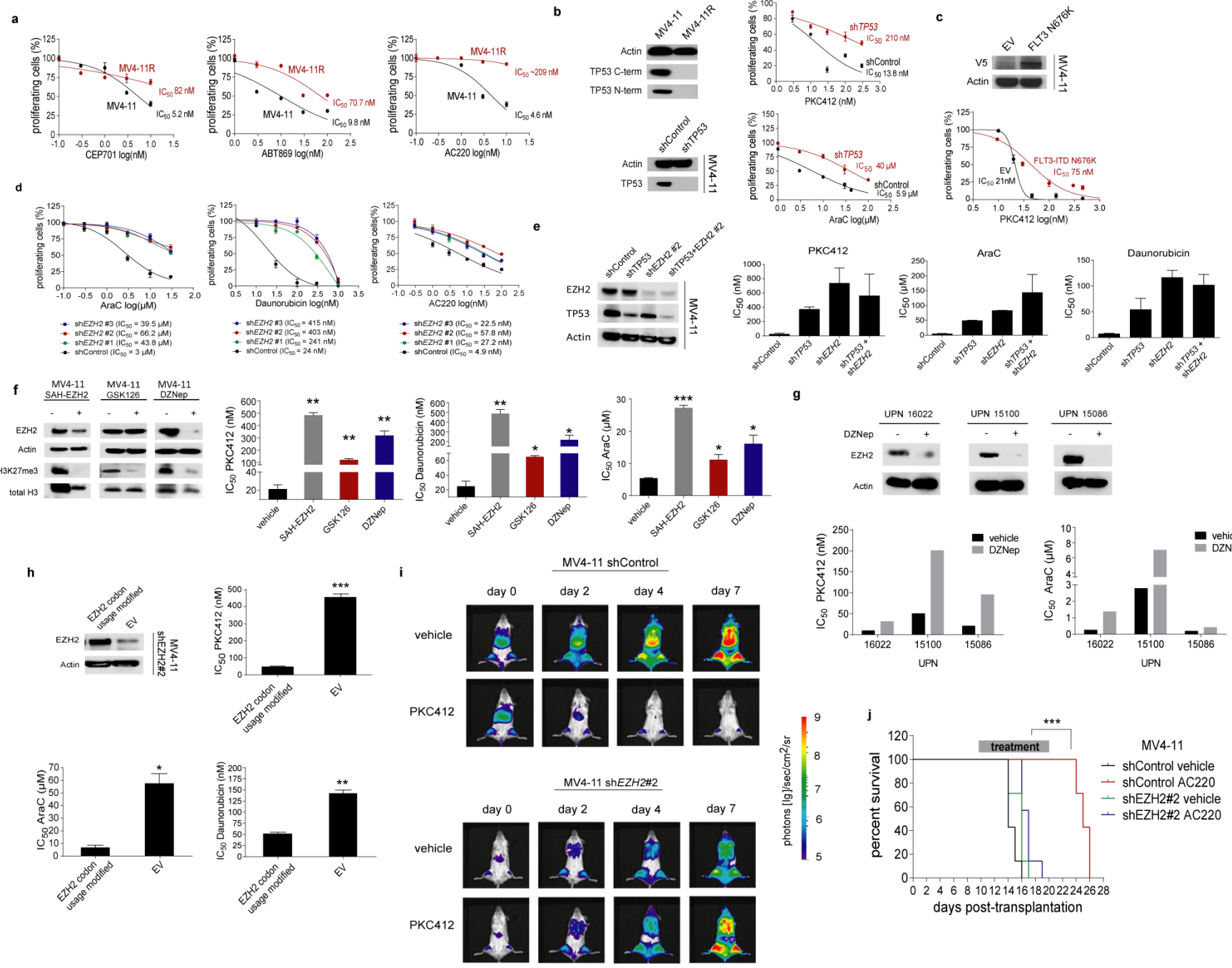
(f) *EZH2* mRNA expression analysis of AML patients with normal karyotype (NK) and patients with del(7)/del(7q) from Valk microarray data set[3] (left). Protein extracts were prepared from blasts of AML patients with normal karyotype (NK) and del7/del7q and analyzed for EZH2 expression (right).

(g) Frequency of EZH2 and H3K27me3 loss at diagnosis (D) and relapse (R). Protein extracts were prepared from matched patients blasts at diagnosis and relapse. Immunoblots were performed probing membranes with anti-EZH2, anti-beta Actin and anti-H3K27me3 antibodies. UPN= unique patient number. The asterisk indicates patients with ASXL1 mutations at relapse. See also Fig. 1c and Suppl. Table 3.

(h) Primary AML cells from patients with normal karyotype (NK) were exposed to vehicle or 1 µM of the methyltransferase inhibitor DZNep for 24 hours. EZH2 protein

levels were analyzed by Western Blot (left). AML blasts were either pretreated with DZNep for 24 hours and subsequently exposed to increasing concentrations of AraC or DZNep and AraC were simultaneously applied. IC_{50} values for AraC are given (right). Patients characteristics are given in Suppl. Table 4.

Supplementary Figure 2



Supplementary Figure 2, related to Figure 2. Effects of EZH2 knockdown on drug resistance *in vitro* and *in vivo*

(a) MV4-11 and MV4-11R cells were exposed to increasing concentrations of the TKIs CEP-701, ABT869 and AC220 for 72 hours. MTS assays were performed to determine the percentage of viable, proliferating cells. Means are shown for three independent experiments ± s.d.

(b) In MV4-11R cells TP53 mutations were identified (see Suppl. Table 5) leading to loss of TP53 protein as analyzed by Western Blot (top left). Sensitive MV4-11 cells were lentivirally transduced with a shRNA targeting *TP53* or shRNA scrambled control. Protein extracts were prepared and analyzed for the expression of TP53 (bottom left). MV4-11 shControl and sh*TP53* cells were treated with increasing concentrations of PKC412 (top right) and AraC (bottom right), respectively, for 72 hours. MTS assays were performed to determine the percentage of viable, proliferating cells. Means are shown for two independent experiments ± s.d.

(c) FLT3-ITD-N676K-V5 was lentivirally expressed in sensitive MV4-11. Protein extracts were analyzed for expression of V5 and Actin (top). MV4-11 EV and N676K overexpressing cells were treated with increasing concentrations of PKC412 for 72 hours. MTS assays were performed to determine the percentage of viable, proliferating cells (bottom). Means are shown for two independent experiments ± s.d.

(d) MV4-11 shControl and sh*EZH2* cells were exposed to increasing concentrations of AraC, daunorubicin or AC220, respectively, for 72 hours. MTS assays were performed to determine the percentage of viable, proliferating cells. Means are shown for three independent experiments ± s.d.

(e) Sensitive MV4-11 cells were lentivirally transduced with a shRNA targeting *TP53, EZH2* or shRNA scrambled control. Further, a TP53/EZH2 double knockdown was lentivirally introduced in sensitive MV4-11 cells. Protein extracts were prepared and analyzed for the expression of TP53 and EZH2 (left). MV4-11 shControl, sh*TP53*, sh*EZH2* and sh*TP53*+sh*EZH2* cells were treated with increasing concentrations of PKC412, AraC and daunorubicin, respectively, for 72 hours. MTS assays were performed to determine the percentage of viable, proliferating cells and IC_{50} values were calculated. Means are shown for three independent experiments ± s.d. (right).

(f) Sensitive MV4-11 cells were pretreated for 7 days with the peptide SAH-EZH2, for 4-7 days with GSK126 or for 24 hours with DZNep. Protein lysates were

analyzed for the expression of EZH2, H3K27me and total H3 (left). Vehicle- and inhibitor- pretreated cells were incubated with increasing concentrations of PKC412, AraC and daunorubicin, respectively, for 72 hours. MTS assays were performed to determine the percentage of viable, proliferating cells and IC_{50} values were calculated. Means are shown for three independent experiments ± s.d. (*** p= 0.001, ** p < 0.01, * p < 0.03) (right).

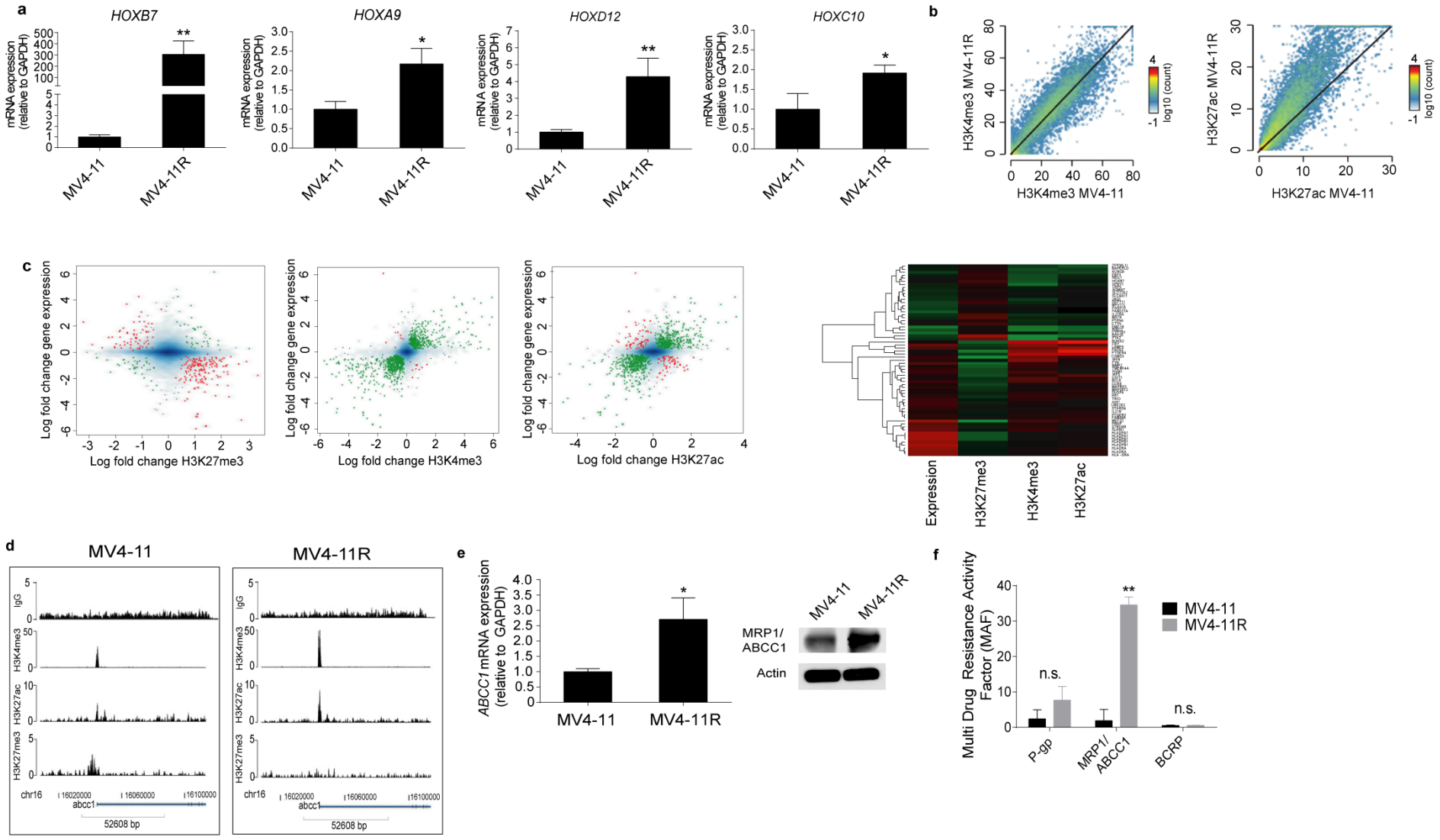
(g) Primary AML cells from patients with FLT3-ITD mutation were exposed to vehicle or 1 µM of the methyltransferase inhibitor DZNep for 24 hours. EZH2 protein levels were analyzed by Western Blot (top). Vehicle- and DZNep-pretreated AML blasts were exposed to increasing concentrations of PKC412 and AraC, respectively, for 48 hours. IC_{50} values are indicated (bottom). UPN= unique patient number.

(h) Codon-usage adapted EZH2 or Empty Vector (EV) control were lenitivirally overexpressed in MV4-11 sh*EZH2*#2 cells and protein lysates were analyzed for expression of EZH2 (top left). Cells were incubated with increasing concentrations of PKC412, AraC and daunorubicin, respectively, for 72 hours. MTS assays were performed to determine the percentage of viable, proliferating cells and IC_{50} values were calculated. Means are shown for three independent experiments ± s.d. (*** p= 0.0001, ** p < 0.02, * p < 0.04, top right and bottom).

(i) NSG mice engrafted with $5x10^6$ MV4-11 shControl-luc or MV4-11 sh*EZH2*#2-luc cells were treated with vehicle or PKC412 (75 mg/kg/d) orally once daily for 9 consecutive days starting at day 3 post-transplantation. Leukemic bone marrow infiltration was monitored by noninvasive luciferase imaging. Representative imaging pictures from each group (n=6 for PKC412 treatment group and n=4 for vehicle group) are shown. See also Figure 2c.

(j) NSG mice engrafted with MV4-11 control or EZH2 knockdown cells, respectively, were treated with vehicle or AC220 (10mg/kg orally) once daily for 10 days starting at day 9 after transplantation (n=7 per group). Kaplan-Meier analysis of animal survival is shown (*** p= 0.0003 for MV4-11 control plus AC220 vs. MV4-11 EZH2 knockdown plus AC220). Grey bar denotes treatment period.

Supplementary Figure 3



Supplementary Fig. 3, related to Figure 2. Bioinformatic analysis of MV4-11 and MV4-11R cells

(a) mRNA expression of representative differentially regulated *HOX* genes in MV4-11 and MV4-11R cells. Means are shown for three independent experiments ± s.d. (* $p< 0.05$, ** $p< 0.01$).

(b) Scatter plots indicate the global correlation of histone modifications H3K4m3 (left) and H3K27ac (right) in MV4-11 vs. MV4-11R cells as analyzed by ChIP-seq.

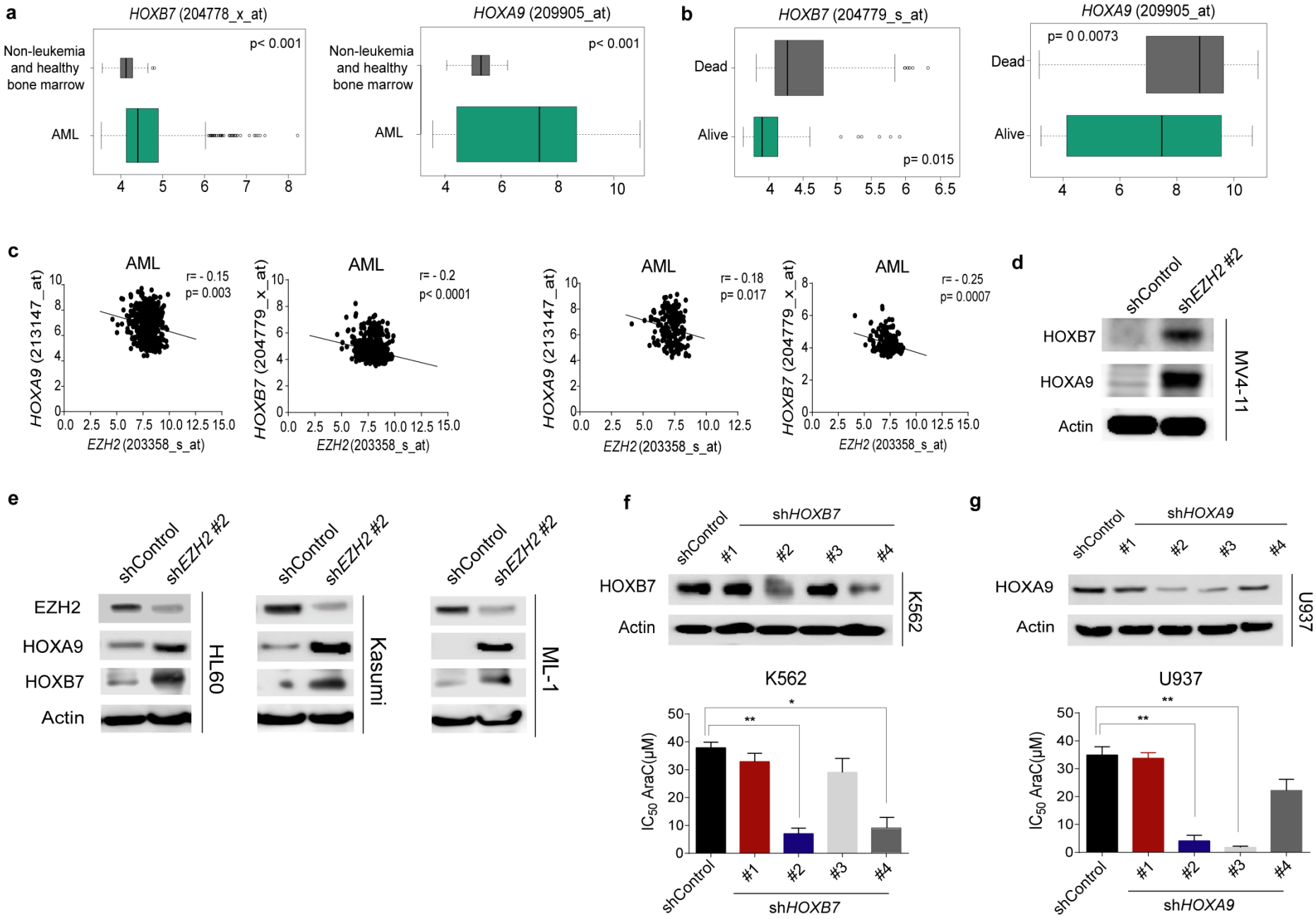
(c) Correlation between differences in mRNA expression and differences in histone modifications H3K27me3, H3K27ac and H3K4me3, respectively. Smoothed scatter plots indicate mRNA expression differences observed between resistant and sensitive cells on the y-axis versus the differences in histone modification observed at the respective gene locus on the x-axis. The point density is indicated by different color shades varying from light blue (low density) to dark blue (high density). Genes classified as differential in expression and histone modification are plotted as green points (same direction) or red points (inverse direction). Overall, the scatter plot between differences in mRNA expression and H3K27me3 levels revealed a negative correlation ($r= -0.09$), whereas positive correlations were observed for the active histone marks H3K27ac ($r= 0.29$) and H3K4me3 ($r= 0.33$) (left). Heatmap display of unsupervised hierarchical clustering of 58 genes differentially regulated in resistant vs. sensitive cells when mRNA expression was correlated with H3K27me3, H3K4me3 and H3K27ac ChIP-seq data using an integrative analysis (right) (see also Suppl. Table 6).

(d) Comparison of ChIP-seq peaks near the transcriptional start site (TSS) of *ABCC1* (coding for MRP1 protein). In resistant cells, loss of H3K27me at the *ABCC1* promoter is accompanied by a gain of H3K4me3 and H3K27ac marks.

(e) qRT-PCR analysis for *ABCC1* mRNA (left) and MRP1 protein expression (right) in MV4-11 and MV4-11R cells. Data represent three independent experiments.

(f) The Multidrug Resistance Activity Factor (MAF) for P-Glycoprotein (P-gp), MRP1 and BCRP was measured in MV4-11 and MV4-11R cells in the absence or presence of specific ABC-transporter inhibitors followed by FACS staining. Means ± s.d. are shown for three independent experiments (** $p< 0.005$).

Supplementary Figure 4



Supplementary Figure 4, related to Figure 2. *HOX* genes are upregulated by loss of EZH2 in drug resistant cells

(a) *HOXB7* and *HOXA9* mRNA expression in healthy bone marrow and AML patients analyzed using datasets published by Haferlach[4].

(b) *HOXB7* and *HOXA9* mRNA expression are significant survival indicators as analyzed using the Cancer Genome Atlas Research Network (TCGA) data[1] .

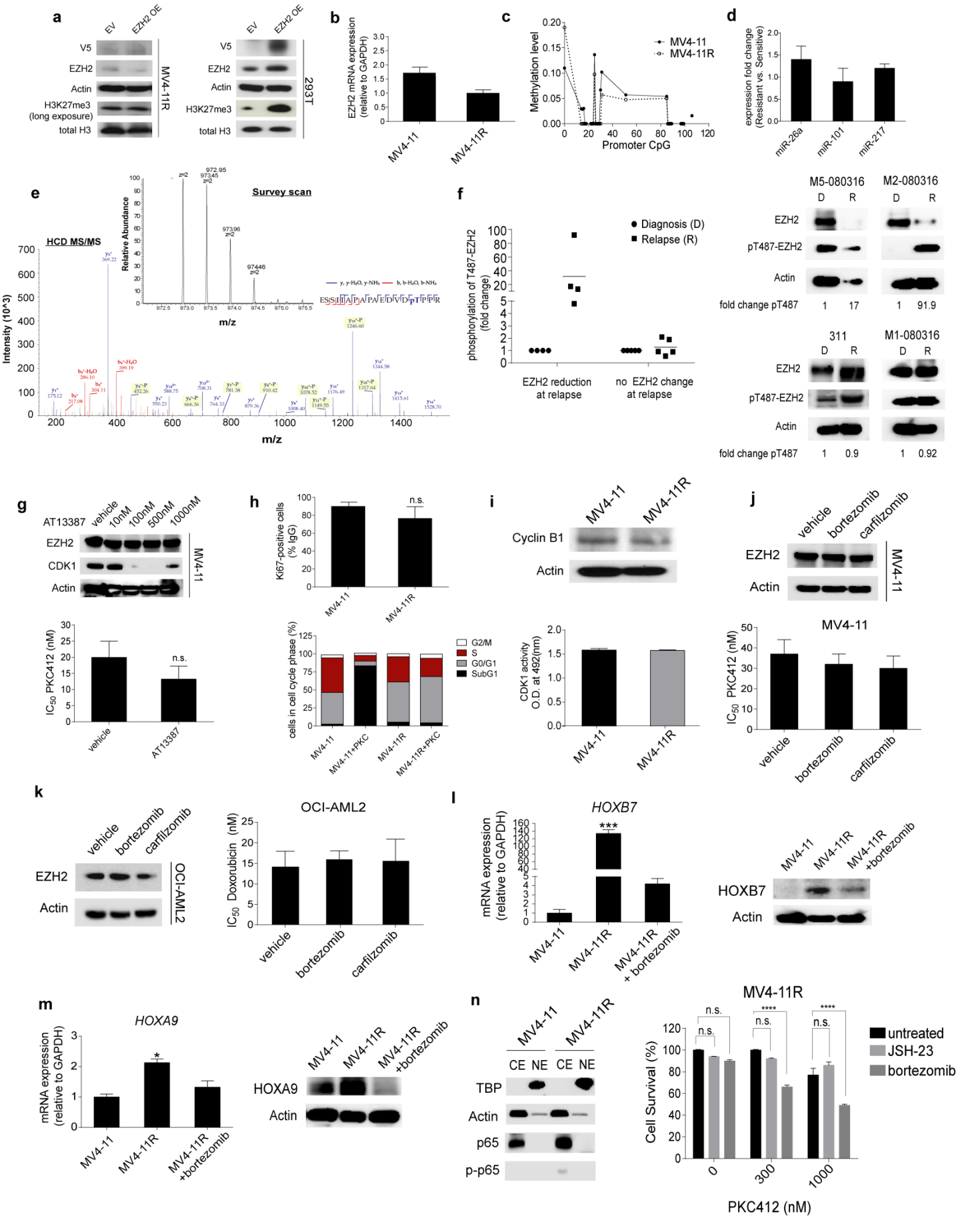
(c) Inverse correlation of *EZH2* and *HOXA9* or *HOXB7* mRNA expression, respectively, in AML patients (Haferlach data[4] (left), TCGA data[1] (right)).

(d) *HOXB7* and *HOXA9* protein expression was determined in MV4-11 control and MV4-11 s*hEZH2* #2 cells by western blotting. Blots are representative of two independent experiments.

(e) shRNA- mediated knockdown of EZH2 leads to upregulation of HOXB7 and HOXA9 proteins in MLL-fusion protein negative AML cell lines. Western Blot data are representative of two independent experiments per cell line.

(f) and **(g)** shRNA- mediated knockdown of HOXB7 and HOXA9 in MLL-fusion protein negative K562 cells (f) and U937 cells (g). K562 and U937 cell lines were chosen as they express high endogenous levels of HOXB7 and HOXA9, respectively. The efficiency of HOXB7 or HOXA9 knockdown versus scrambled control was analyzed by western blot. Data are representative of two independent experiments. (top). shControl and knockdown cells were exposed to increasing concentrations of AraC for 72 hours. MTS assays were performed to determine the percentage of viable, proliferating cells. Means of IC_{50} values from three independent experiments ± s.d. are shown (* p= 0.012, ** p< 0.007) (bottom).

Supplementary Figure 5



Supplementary Figure 5, related to Figure 3 and 4. EZH2 is post-translationally regulated in resistant cells

(a) Resistant MV4-11R (left) and 293T cells (right) were lentivirally transduced with pCDH vector expressing V5-tagged wild-type EZH2. Protein lysates were analyzed for expression of EZH2, H3K27me3 and V5. Data are representative of two independent experiments. EV (Empty Vector), OE (EZH2 overexpression vector).

(b) qRT-PCR analysis for *EZH2* mRNA in MV4-11 and MV4-11R cells. Means ± s.d. of three independent experiments are given.

(c) Analysis of *EZH2* Promoter CpG Methylation in MV4-11 and MV4-11R cells by Reduced Representation Bisulfite Sequencing (RRBS). For better visualization only CpGs with a coverage of >30 reads and methylation values of more than 0 are shown (n=19).

(d) qRT-PCR for microRNA -26a, -101 and -217 expression comparing MV4-11 and MV4-11R cells. Means ± s.d. of three independent experiments are shown. U6 snRNA expression was used for normalization.

(e) MS/MS spectrum of the tryptic peptide of EZH2 bearing phosphorylated T487. Protein bands containing immunoprecipitated EZH2 were cut off from SDS-PAGE gel and then subjected to in-gel digestion followed by phosphopeptide enrichment and LC-MS/MS analysis. Peptide bearing phosphorylated T487 (ESSIIAPAPAEDVDpTPPR), which was observed as a doubly charged ion with m/z of 972.95 as showed on the inset, was selected for fragmentation in the mass spectrometer. Matched b- and y- series of fragment ions are annotated with red and blue color codes, respectively. Fragment ions with neutral loss of phosphate group are highlighted as well.

(f) EZH2 pT487 phosphorylation at diagnosis (D) and relapse (R). Protein lysates of matched samples from diagnosis and relapse (presented in Figure 1c and Suppl. Figure 1g) were analyzed for pT487-EZH2, total EZH2 and Actin by immunoblotting. EZH2 and pT487-EZH2 levels were normalized to Actin. pT487-EZH2/EZH2 ratios were calculated for diagnosis and relapse samples and set to 1 in the diagnosis sample as a reference (left). Representative Western Blots of matched Diagnosis -Relapse samples are shown (right). Two diagnosis-relapse pairs could not be analyzed due to non-detectable EZH2 in one of the matched samples.

(g) Sensitive MV4-11 cells were treated with the HSP90 inhibitor AT13387 for 24 hours. Protein lysates were analyzed for expression of EZH2 and CDK1 by Western

Blot. Data are representative of two independent experiments (top). Sensitive cells were pretreated with AT13387 for 6 hours and proliferation of cells was analyzed in the presence of increasing concentrations of PKC412 after 48 hours by MTS assay. Means ± s.d. are shown for three independent experiments (p = 0.591, bottom).

(h) Proliferative Index of MV4-11 and MV4-11R cells. The percentage of Ki67-positive cells is shown. Means ± s.d. are given for three independent experiments (p= 0.07, top). Cell cycle phase distribution of sensitive and resistant MV4-11 cells with and without PKC412 for 72h as analyzed by BrDU staining and flow cytometry (bottom).

(i) Cyclin B1 protein levels were detected in protein lysates from sensitive and resistant cells. Data are representative of two independent experiments (top). CDK1 activity in sensitive and resistant cells was analyzed by use of MESACUP® Cdc2/Cdk1 Kinase Assay Kit. Bars represent means of OD_{492} values from three independent experiments ± s.d. (bottom).

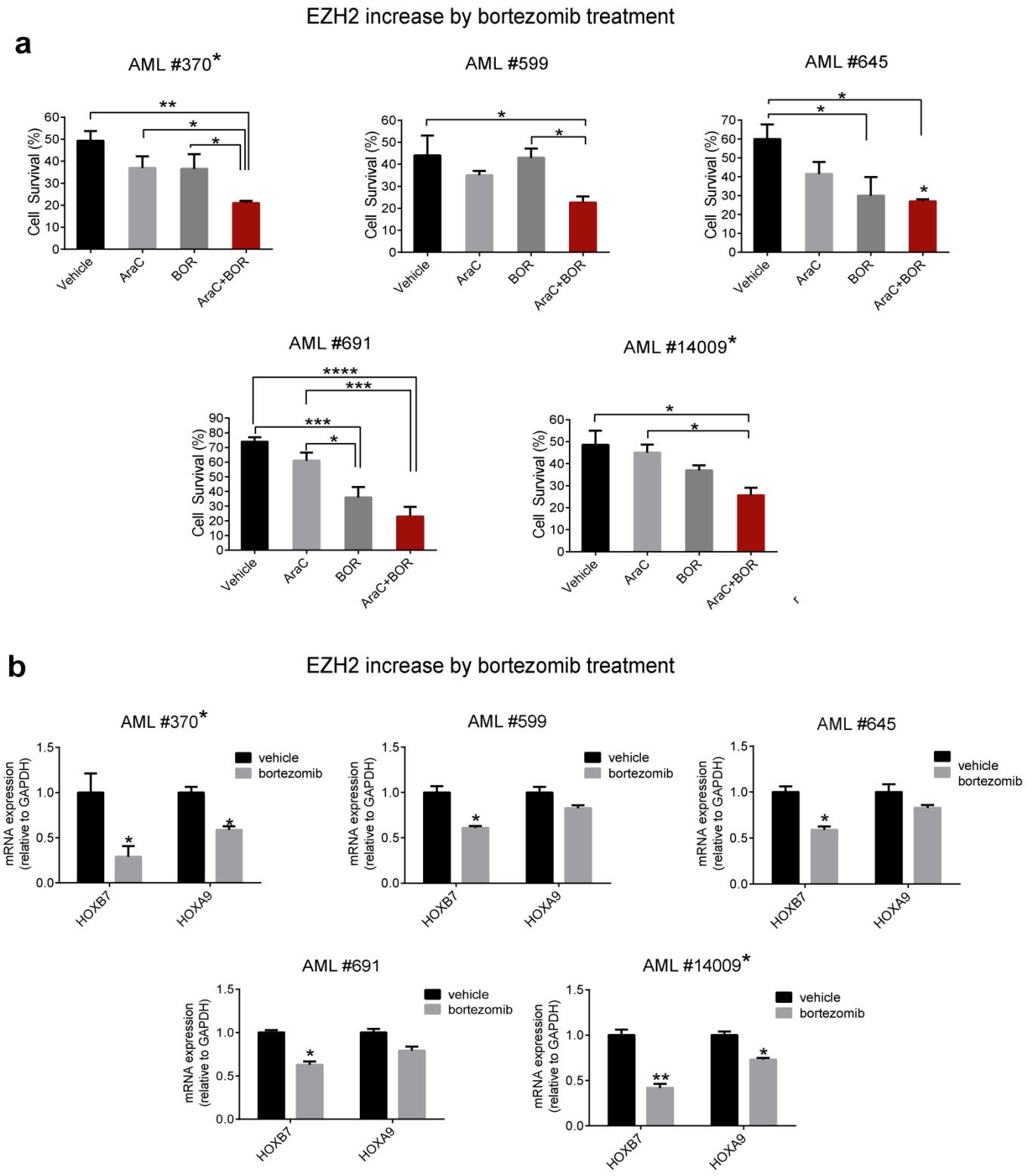
(j) and **(k)** Sensitive MV4-11 (j) or sensitive OCI-AML2 cells (k), respectively, were incubated for 24 hours with 10nM of the proteasome inhibitors bortezomib or carfilzomib. Protein lysates were analyzed for the expression of EZH2. Data are representative of two independent experiments (top and left, respectively). Sensitive cells were pretreated for 24 hours with 10nM of the proteasome inhibitors bortezomib or carfilzomib and proliferation of cells was analyzed in the presence of increasing concentrations of PKC412 or doxorubicin, respectively, after 72 hours by MTS assay. Means of IC_{50} values ± s.d. are shown for two independent experiments (bottom and right, respectively).

(l) and **(m)** *HOXB7* (l) and *HOXA9* (m) mRNA (left) and protein expression (right) in MV4-11, MV4-11R and MV4-11R cells treated with bortezomib. Means are shown for three independent experiments ± s.d. (* $p< 0.05$, *** $p< 0.003$).

(n) Cytoplasmic (CE) and nuclear extracts (NE) were prepared from MV4-11 and MV4-11R cells and analyzed for protein expression of total and phosphorylated p65. Expression of Actin was used as loading control for cytoplasmic extracts. Expression of TATA-Box Binding Protein (TBP) was used as loading control for nuclear extracts. Western Blot data are representative of two independent experiments (left). Resistant MV4-11R cells were pretreated with JSH-23, inhibiting translocation of p65 from the cytoplasm to the nucleus, or bortezomib for 6 hours and subsequently exposed to increasing concentrations of PKC412 for 72 hours. MTS assays were performed to

determine the percentage of viable, proliferating cells. Data were analyzed by two-way ANOVA followed by Sidak´s multiple comparison test. Means are shown for three independent experiments ± s.d. (**** $p < 0.0001$) (right).

Supplementary Figure 6

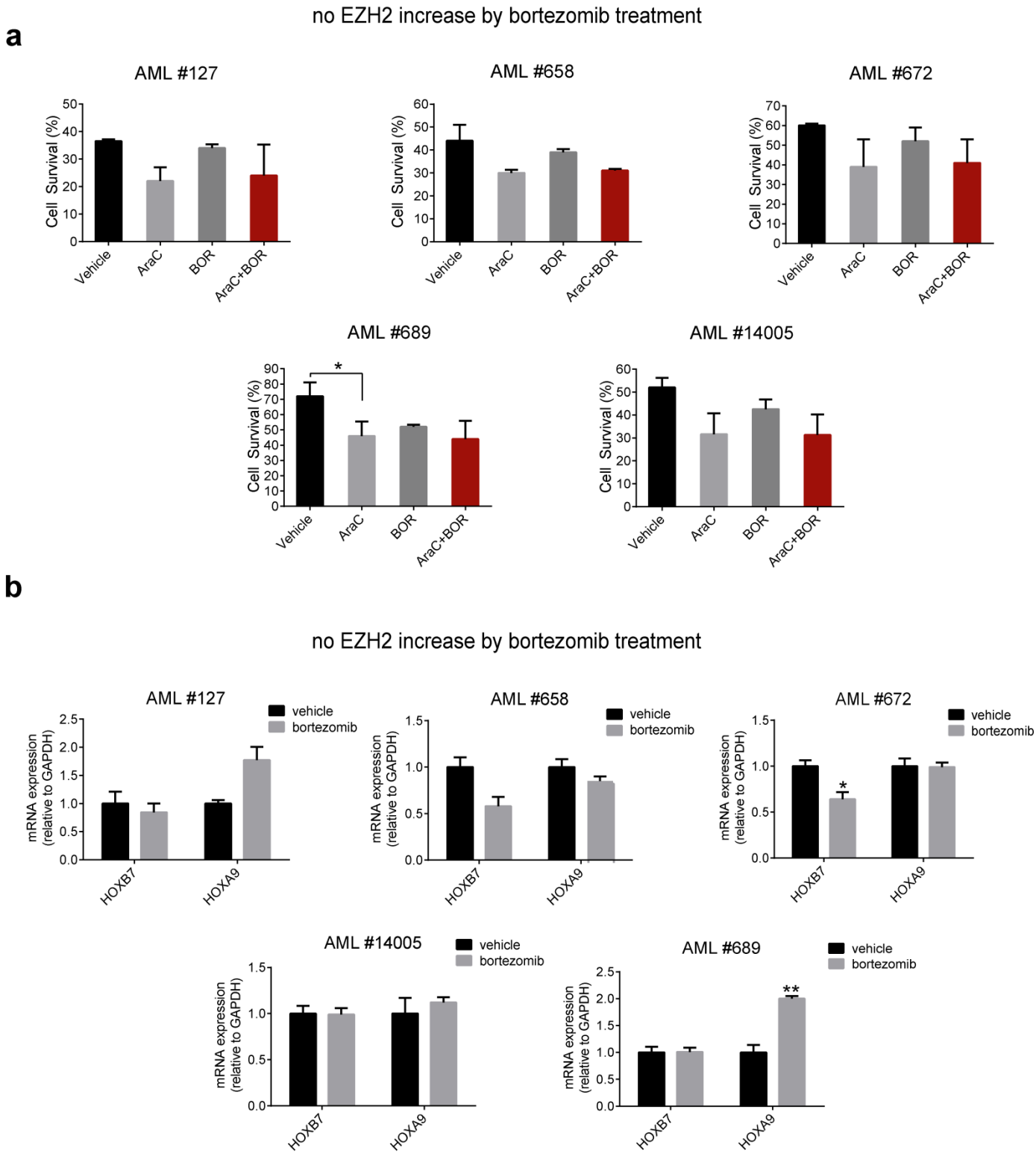


Supplementary Figure 6, related to Figure 4. Bortezomib treatment of patients blasts can increase EZH2 protein levels and improve treatment response

(a) Viability of patients blasts *ex vivo* in the presence of 3µM AraC, 10nM bortezomib (BOR) or the combination of both. Data were analyzed by one-way ANOVA followed by Tukey´s *post-hoc* test. Means ± s.d. of three technical replicates are shown (* $p< 0.02$, ** $p< 0.01$, *** $p< 0.001$, **** $p< 0.0001$). Relapse samples are marked by asterisks.

(b) *HOXB7* and *HOXA9* mRNA expression of patient samples treated with vehicle or with bortezomib. Expression of each vehicle-treated sample was set at 1 as a reference. Means ± s.d. of three technical replicates are shown (* $p< 0.02$, ** $p< 0.01$). Relapse samples are marked by asterisks.

Supplementary Figure 7



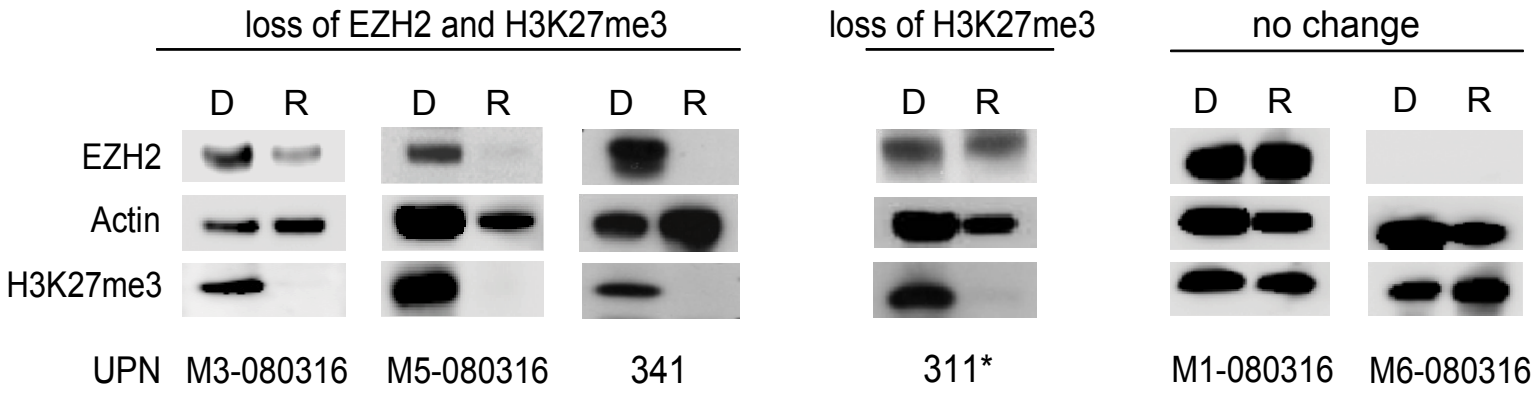
Supplementary Figure 7, related to Figure 4. Treatment response is not improved in patient samples without EZH2 increase upon bortezomib treatment

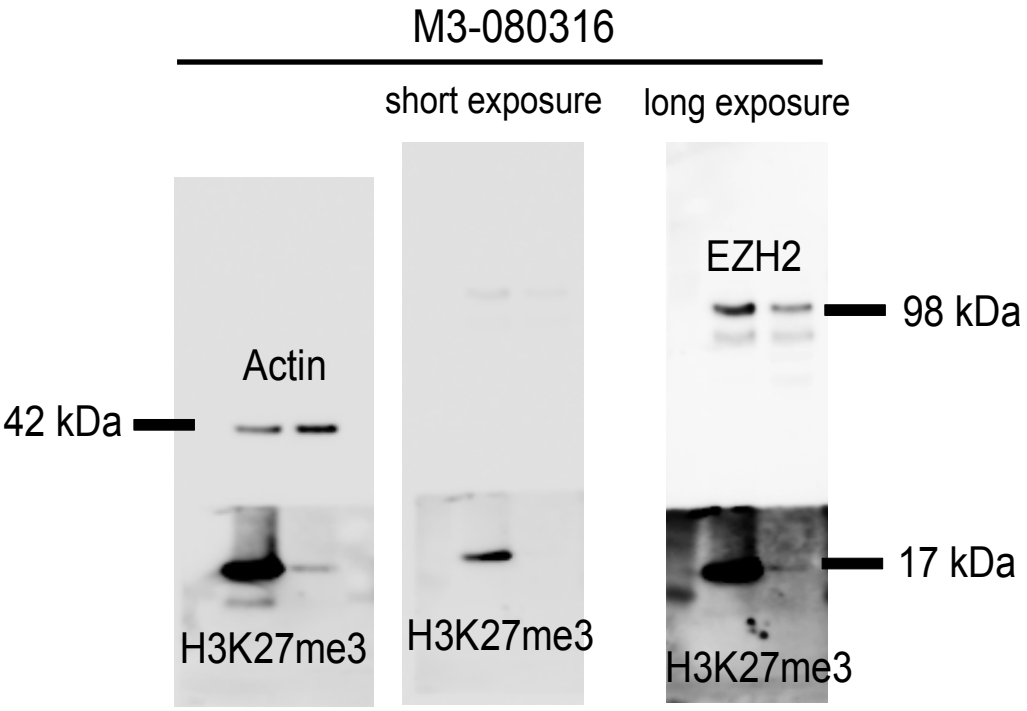
(a) Viability of patients blasts *ex vivo* in the presence of 3µM AraC, 10nM bortezomib (BOR) or the combination of both. Data were analyzed by one-way ANOVA followed by Tukey´s *post-hoc* test. Means ± s.d. of three technical replicates are shown (* $p < 0.02$).

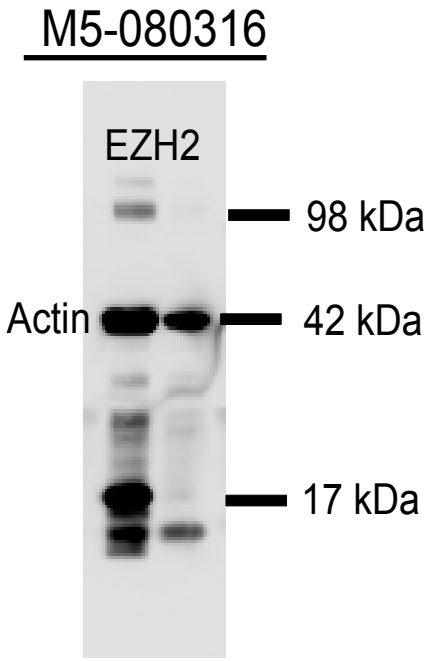
(b) *HOXB7* and *HOXA9* mRNA expression of patient samples treated with vehicle or with bortezomib. Expression of each vehicle-treated sample was set at 1 as a reference. Means ± s.d. of three technical replicates are shown.

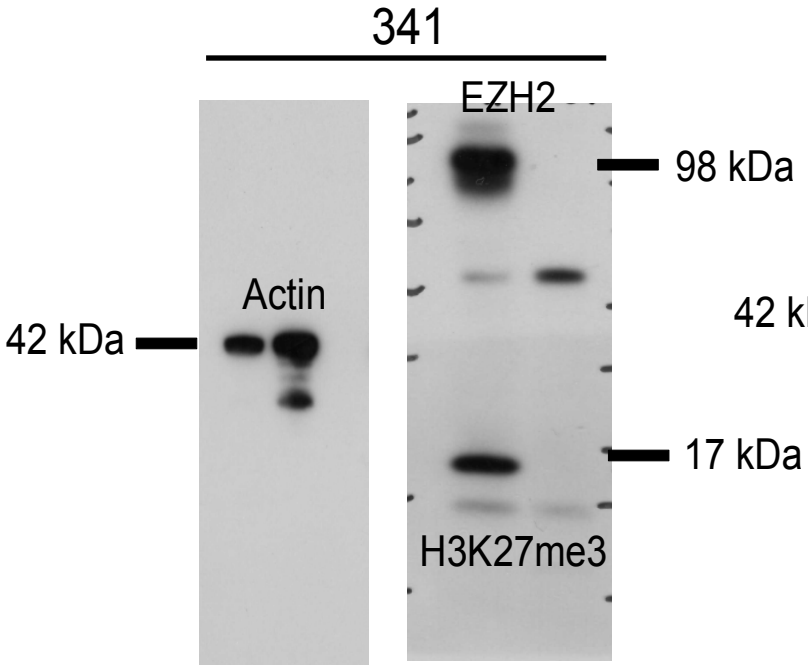
Supplementary Figure 8

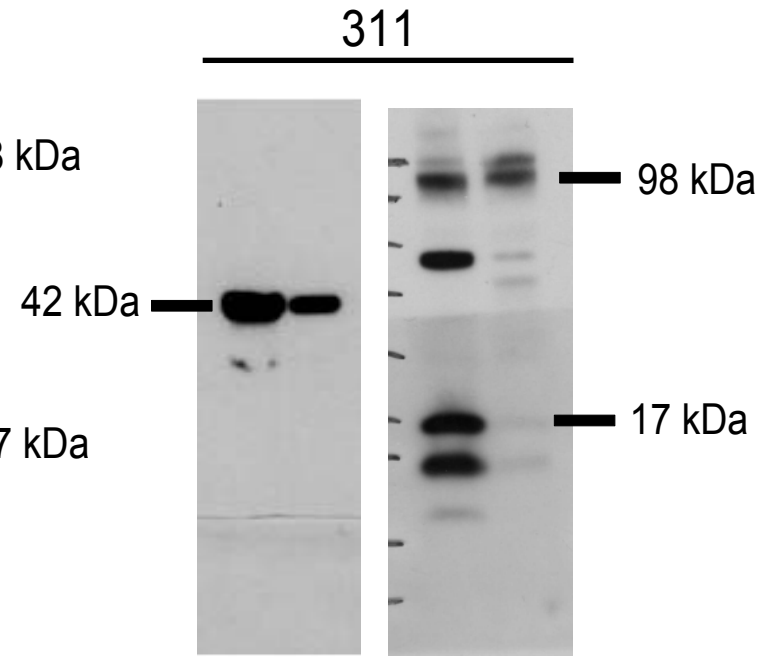
WBs for Figure 1c



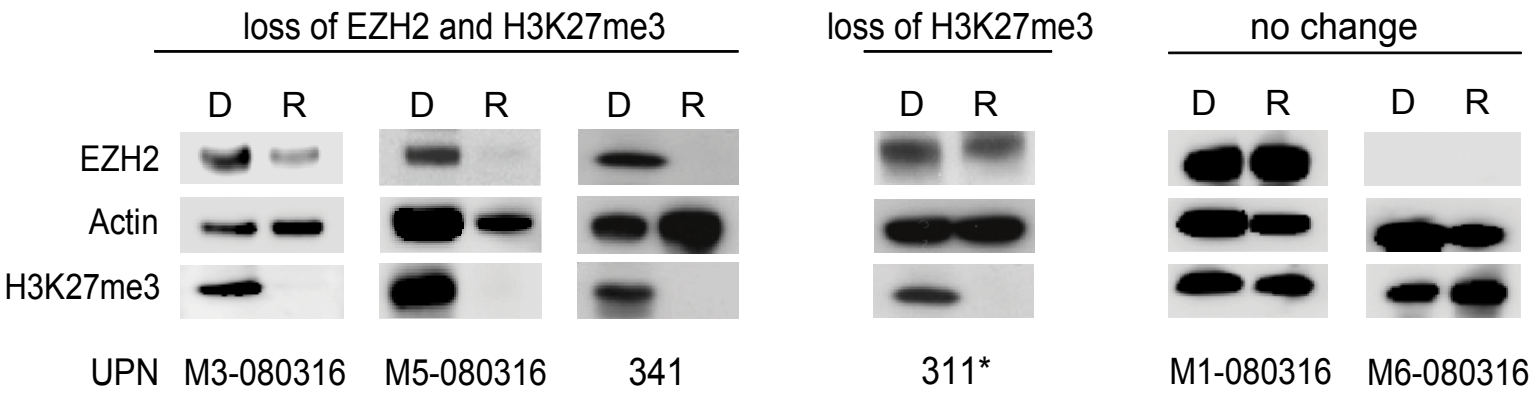


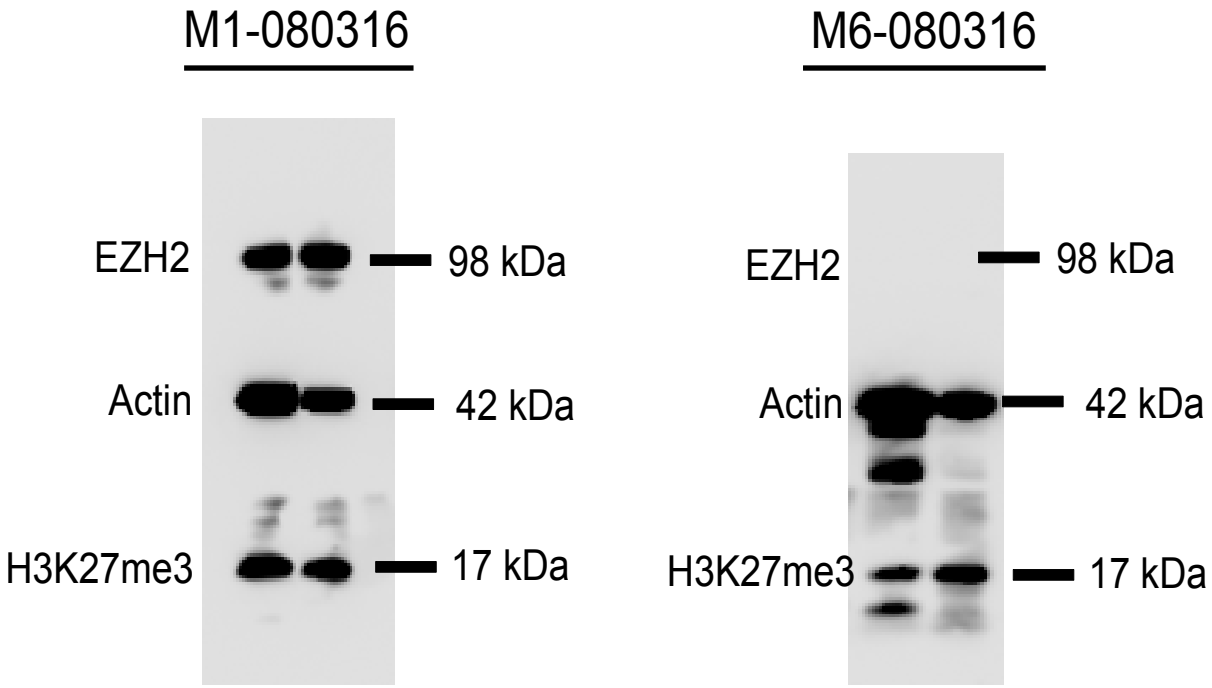




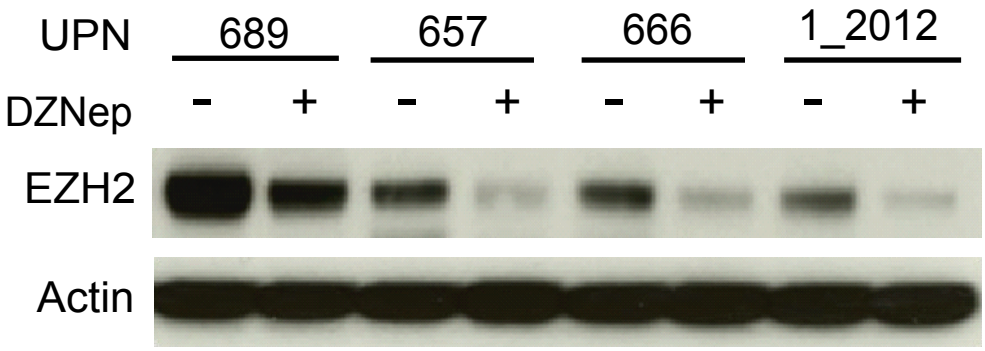


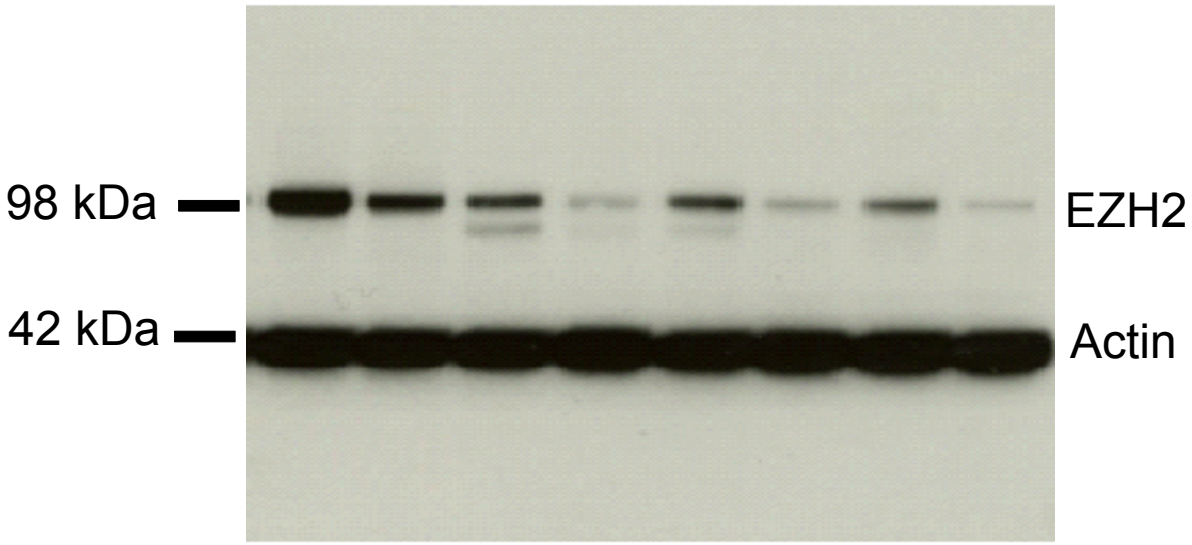
WBs for Figure 1c



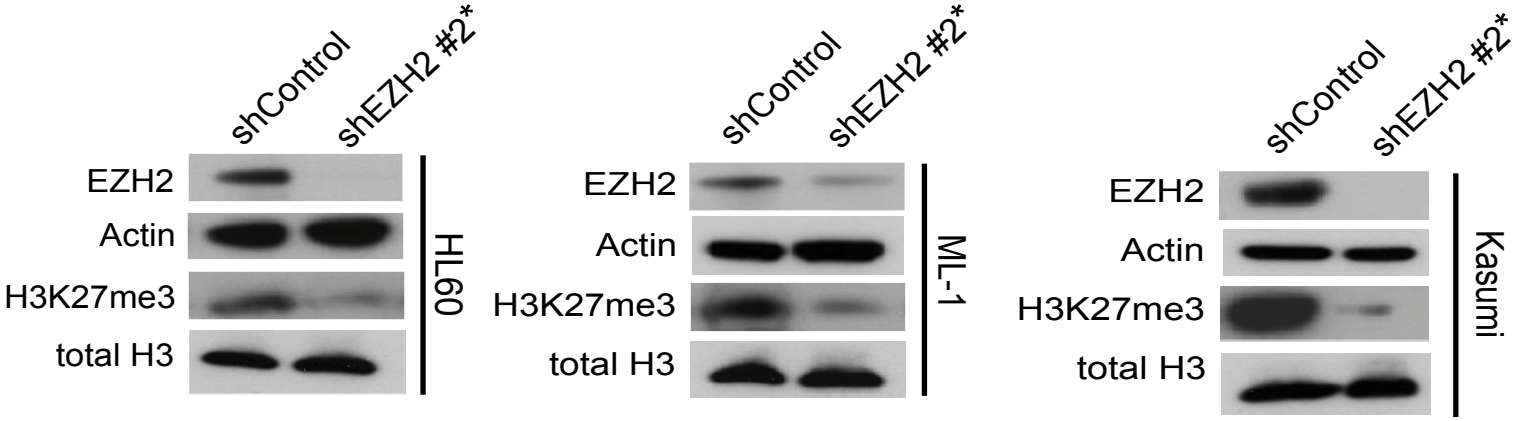


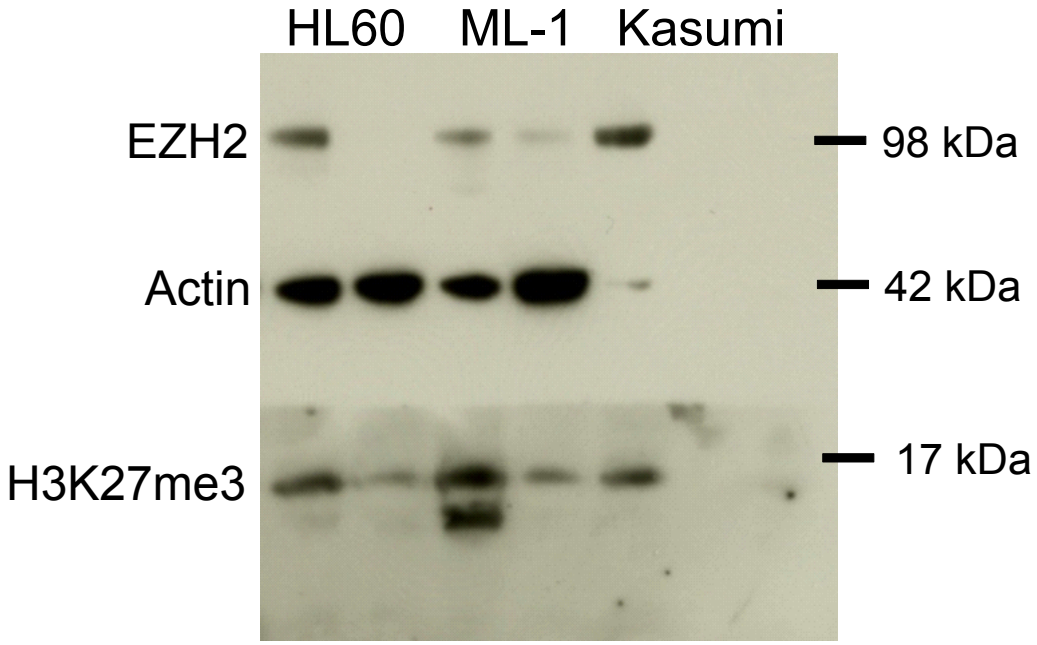
WBs for Figure 1d



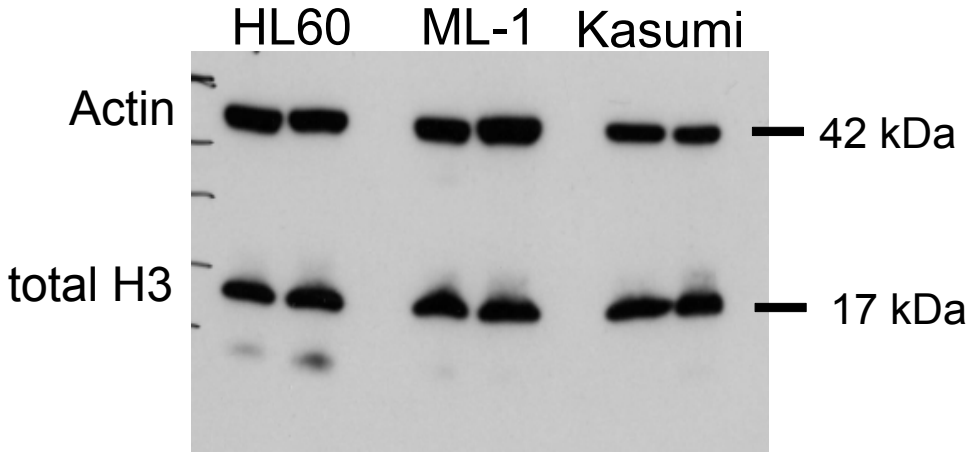


WBs for Figure 1e

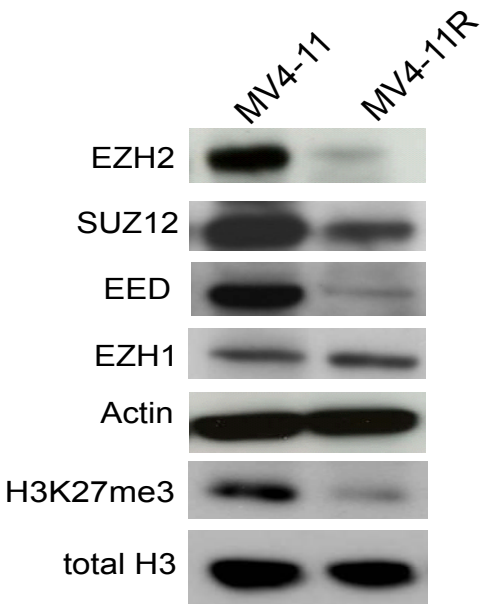


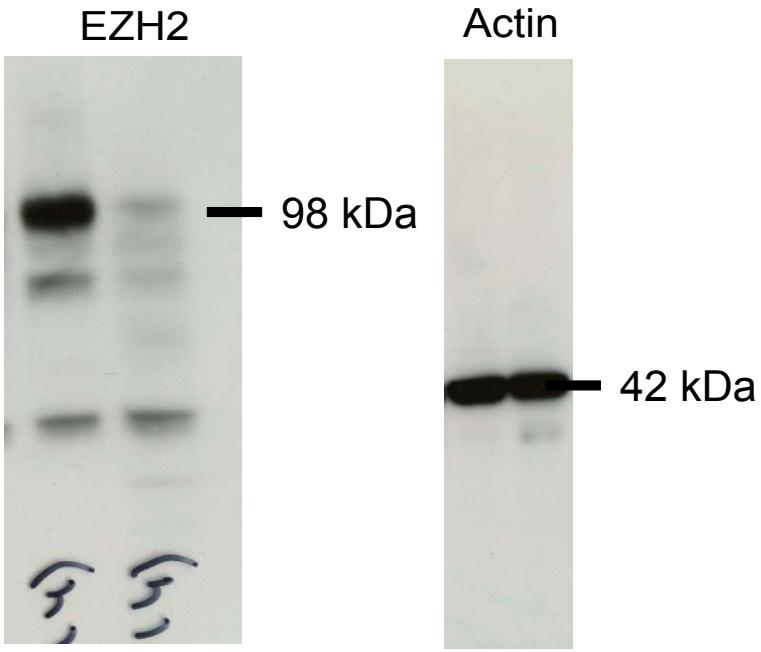


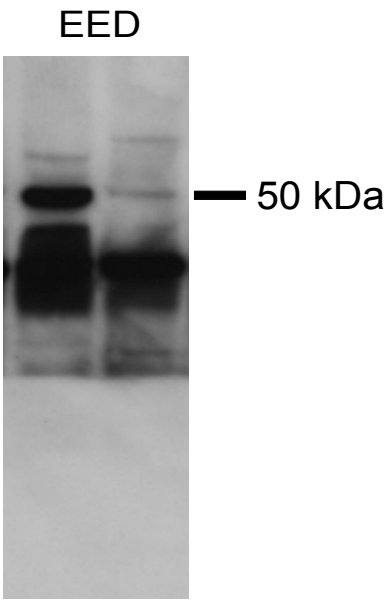
Repetition Actin Blot (+total H3)

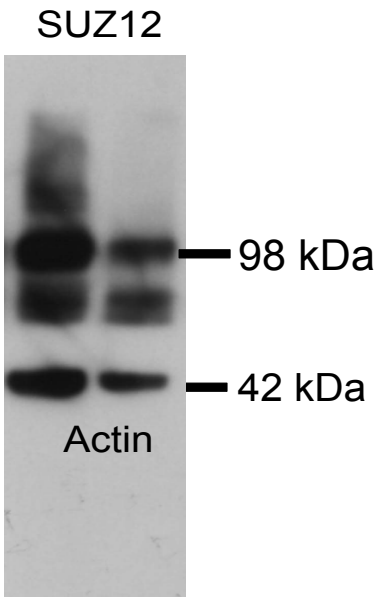


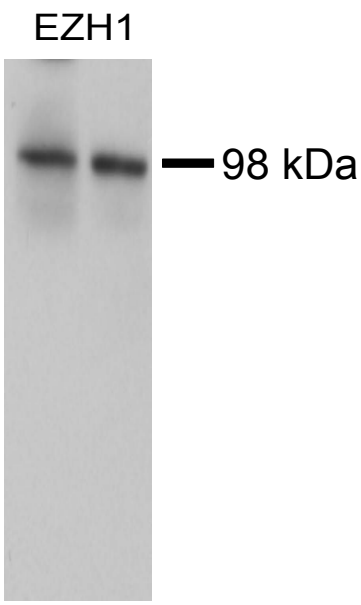
WBs for Figure 2a

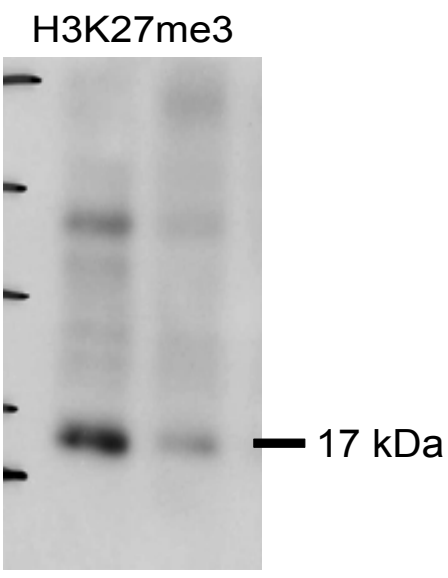


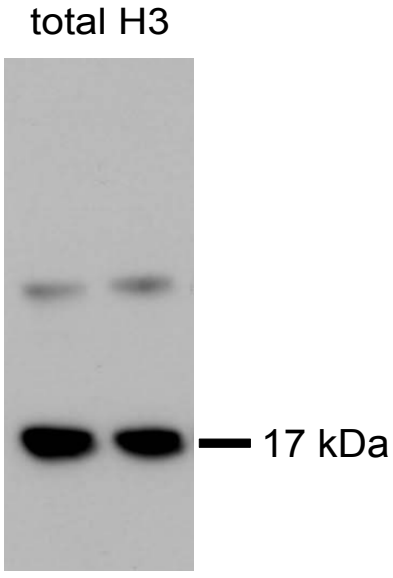




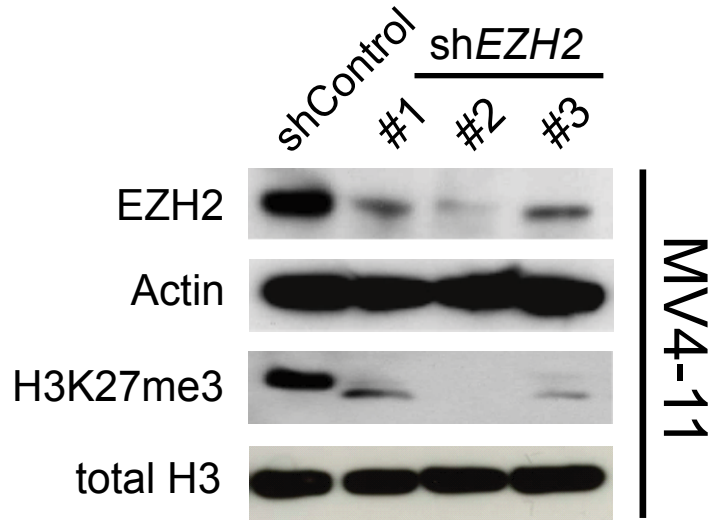


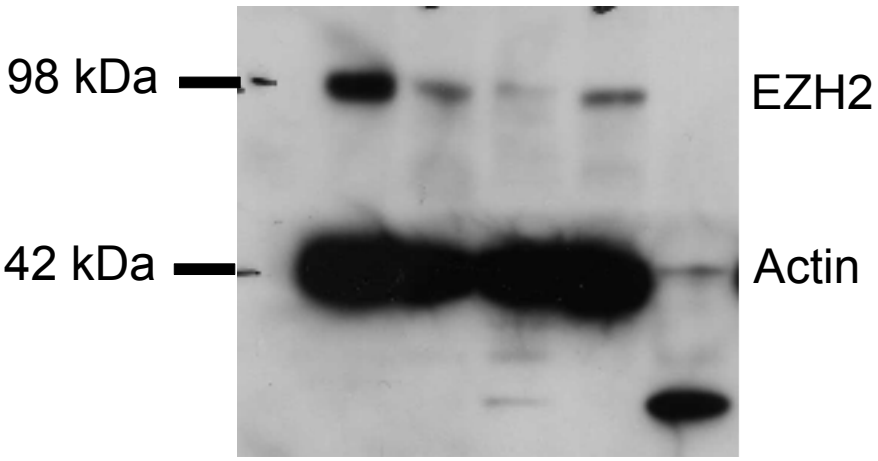


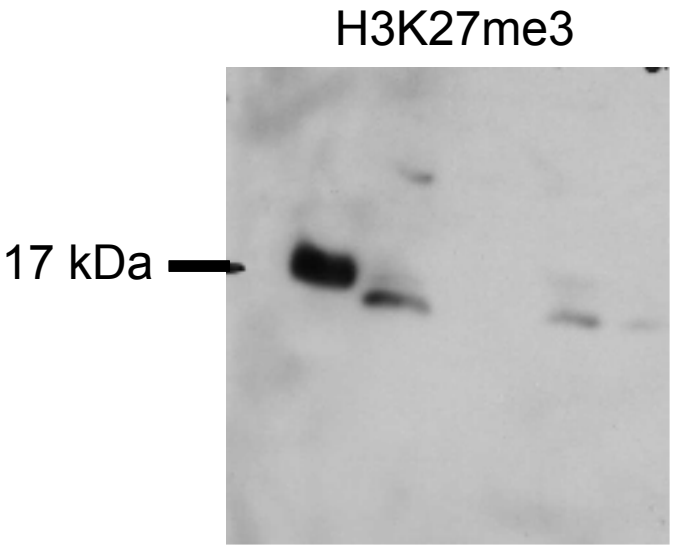


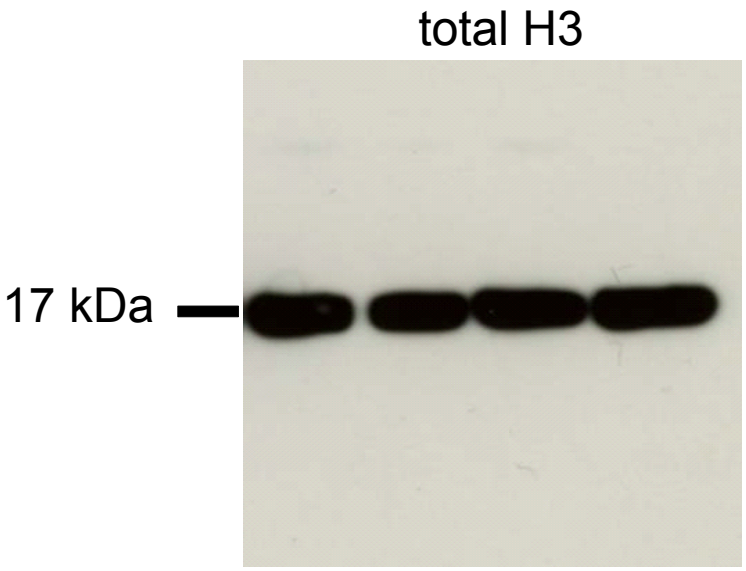


WBs for Figure 2b

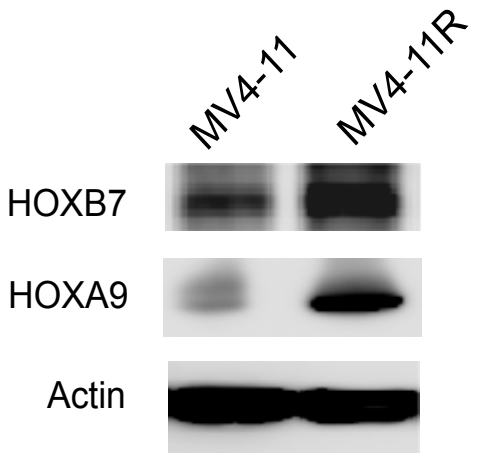


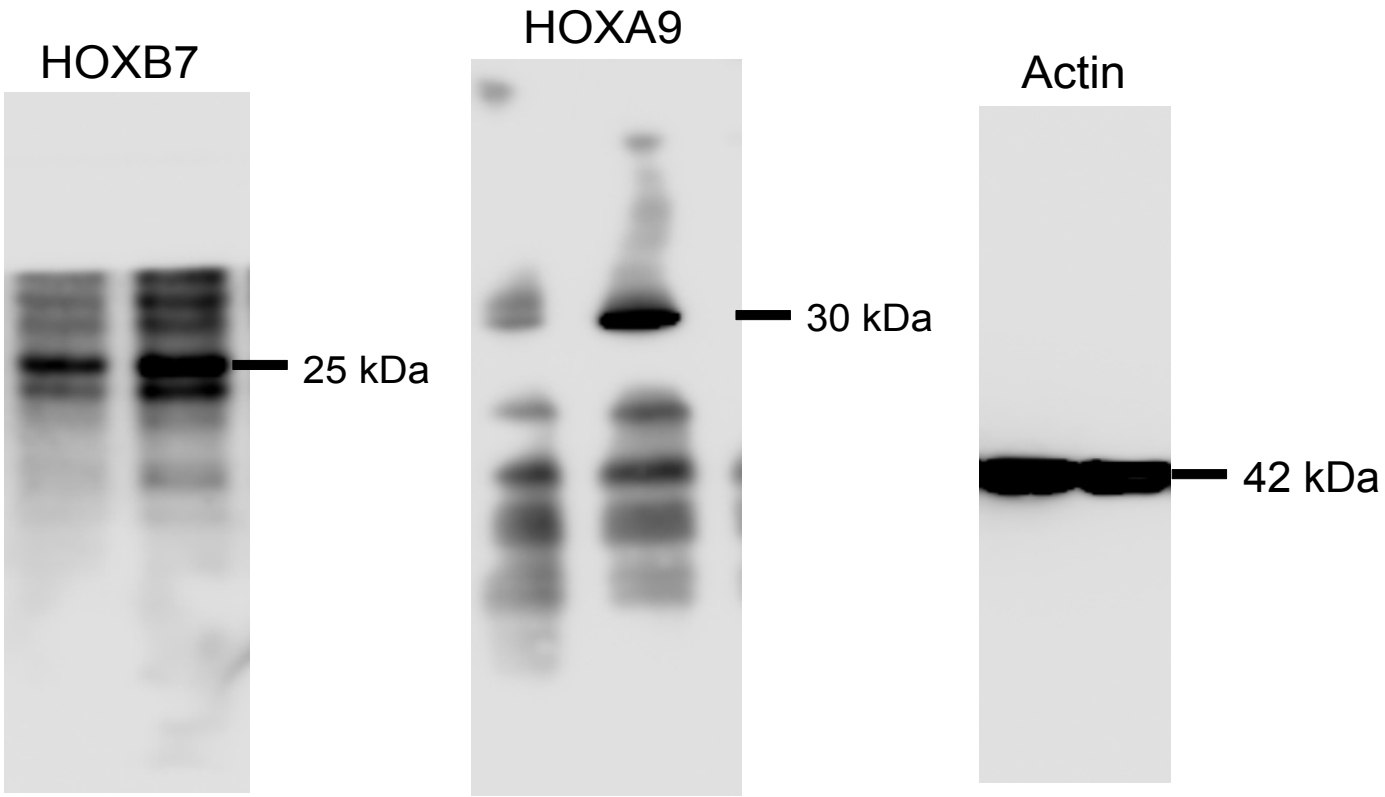




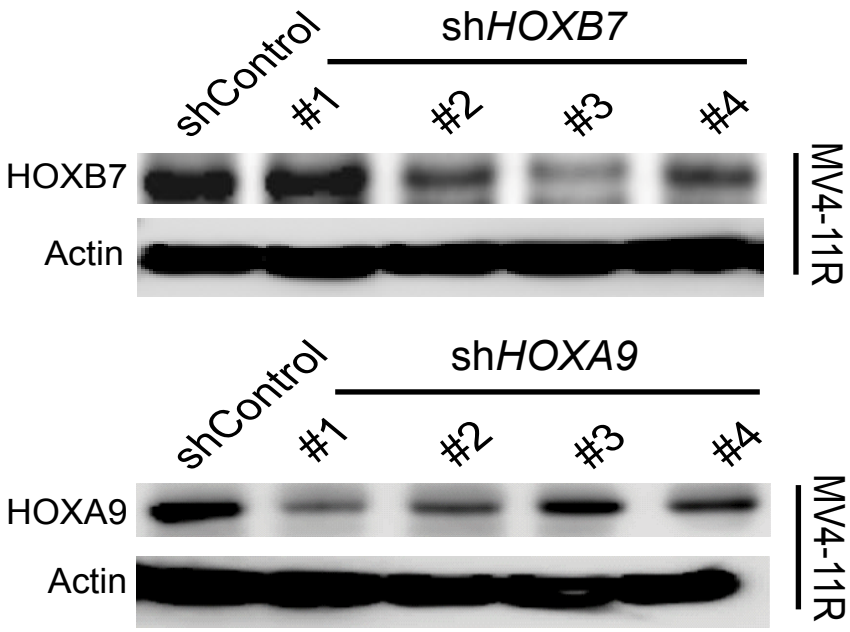


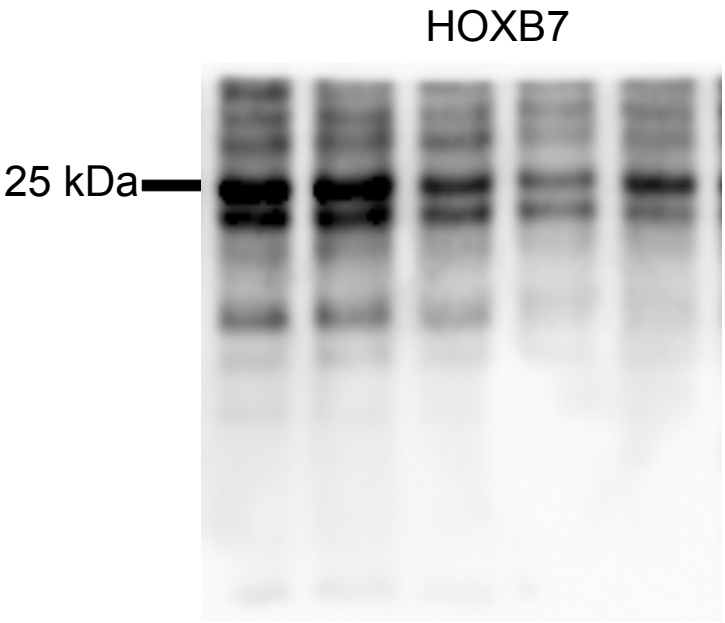
WBs for Figure 2g

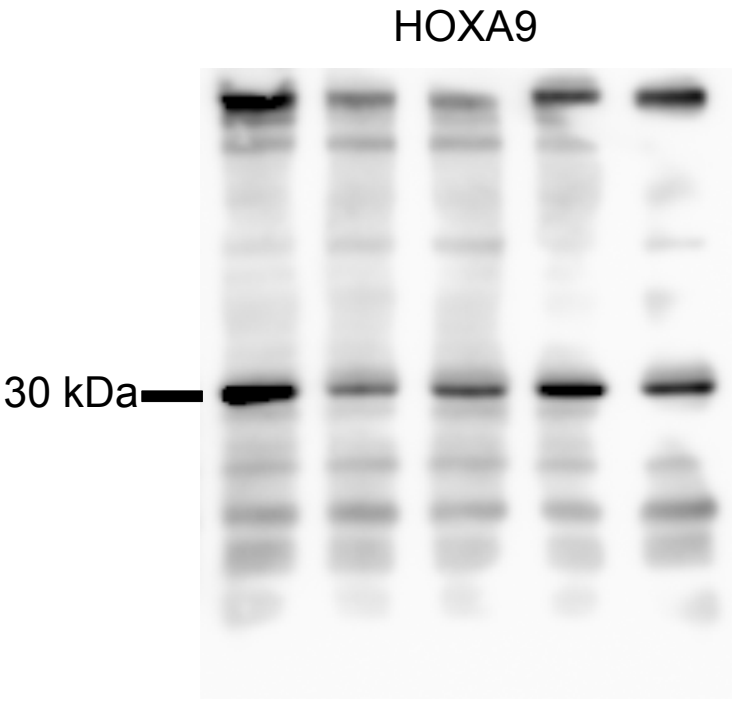


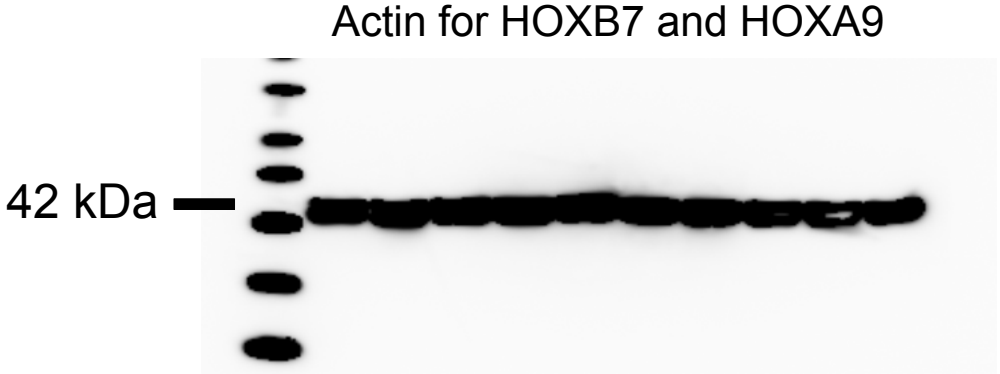


WBs for Figure 2h

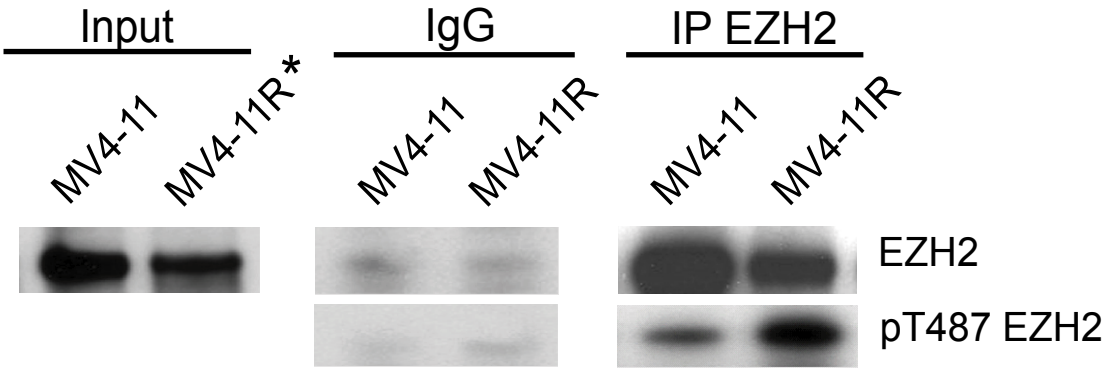


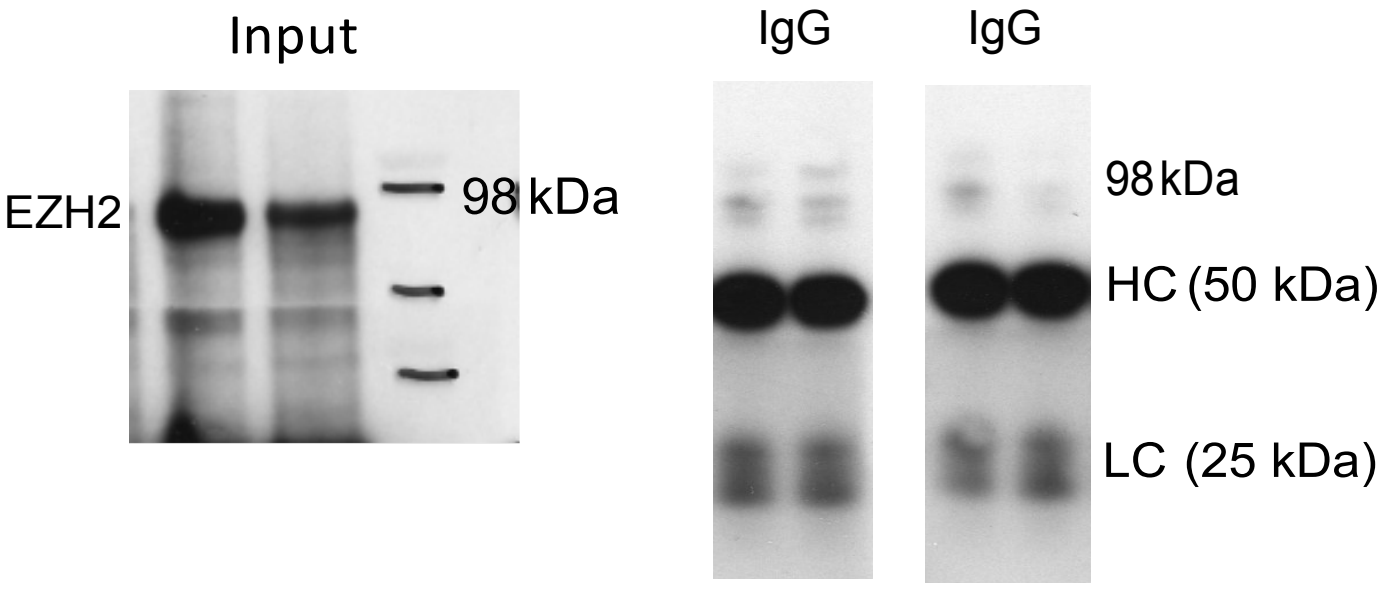


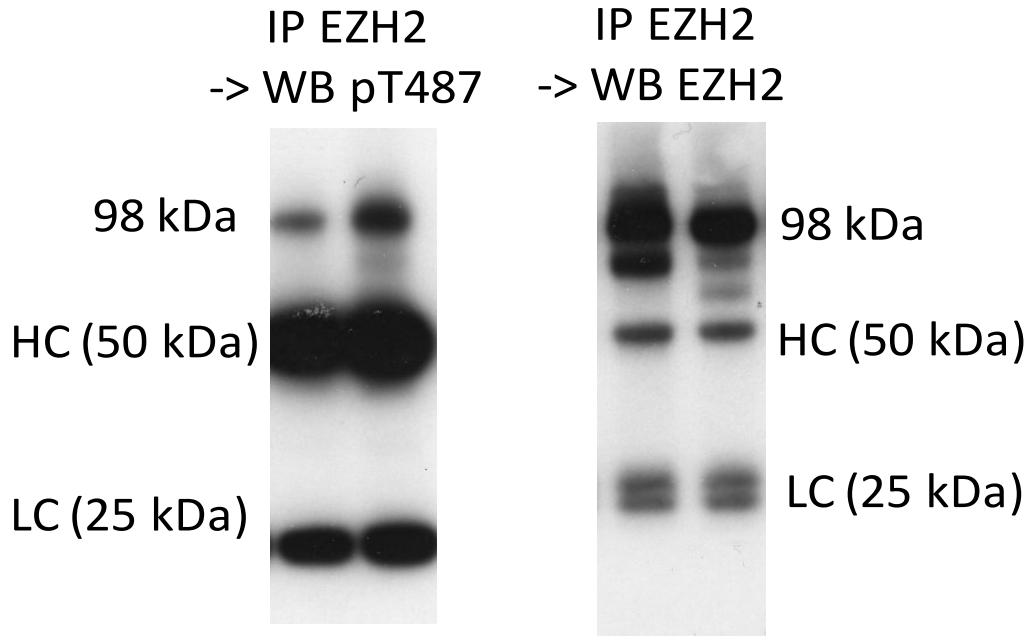




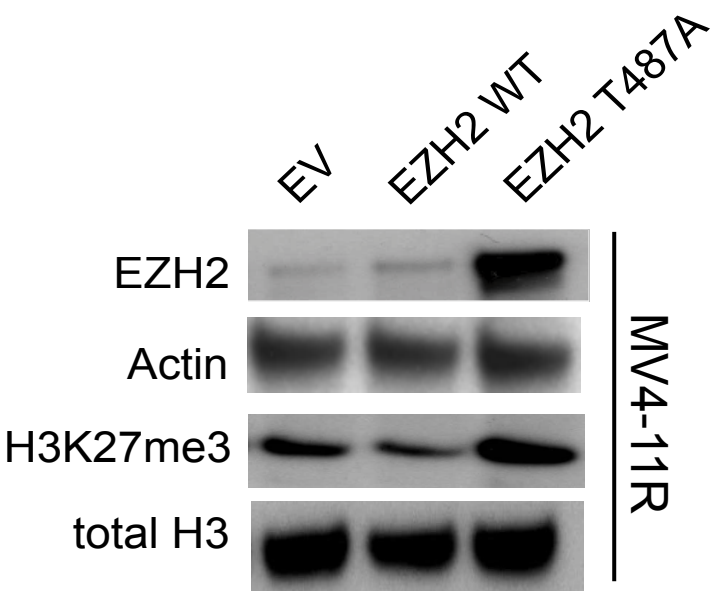
WBs for Figure 3a

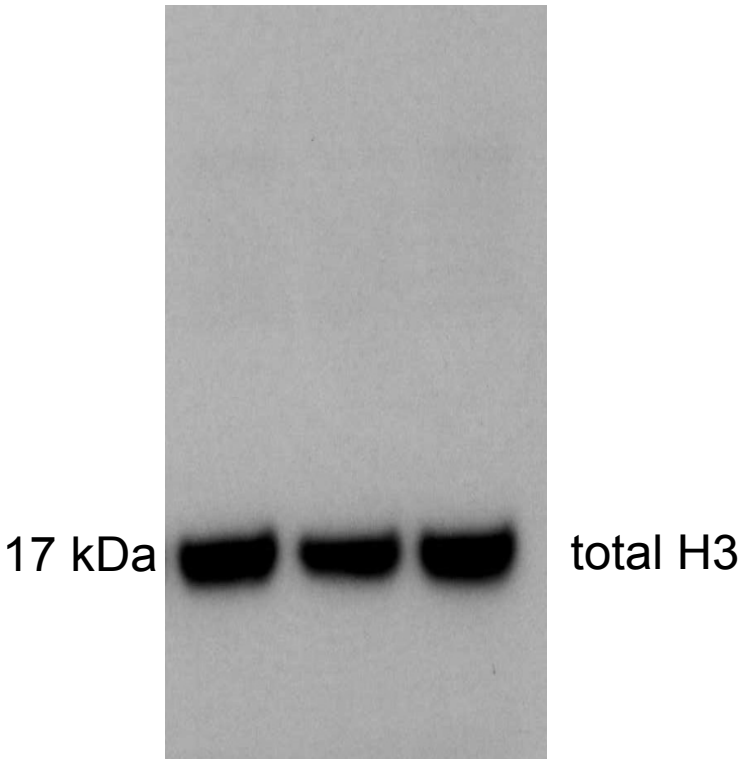


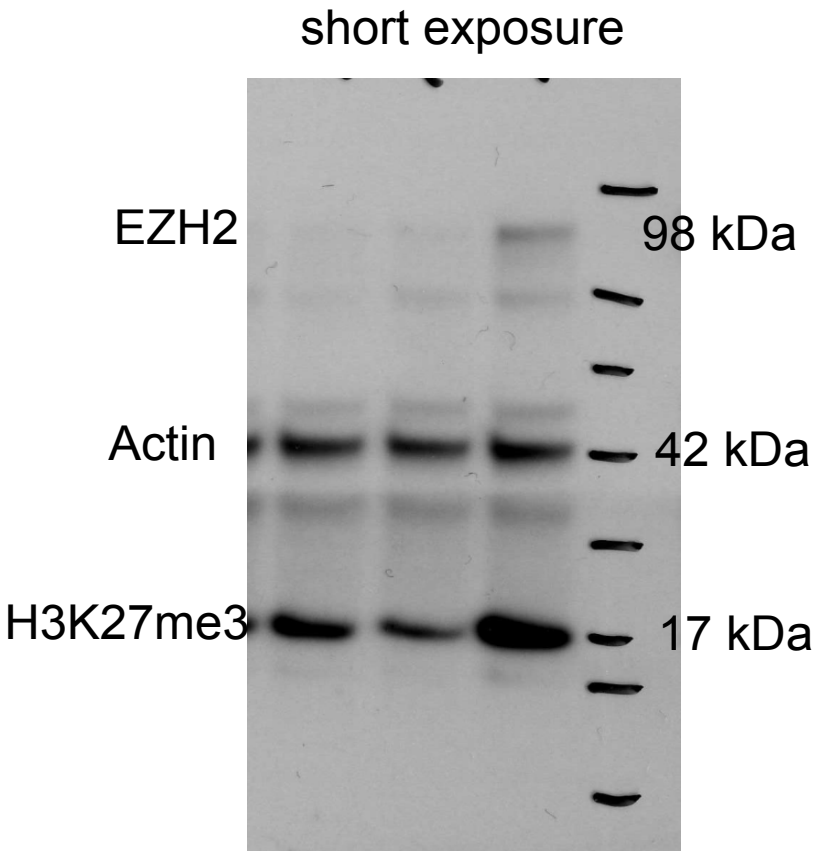


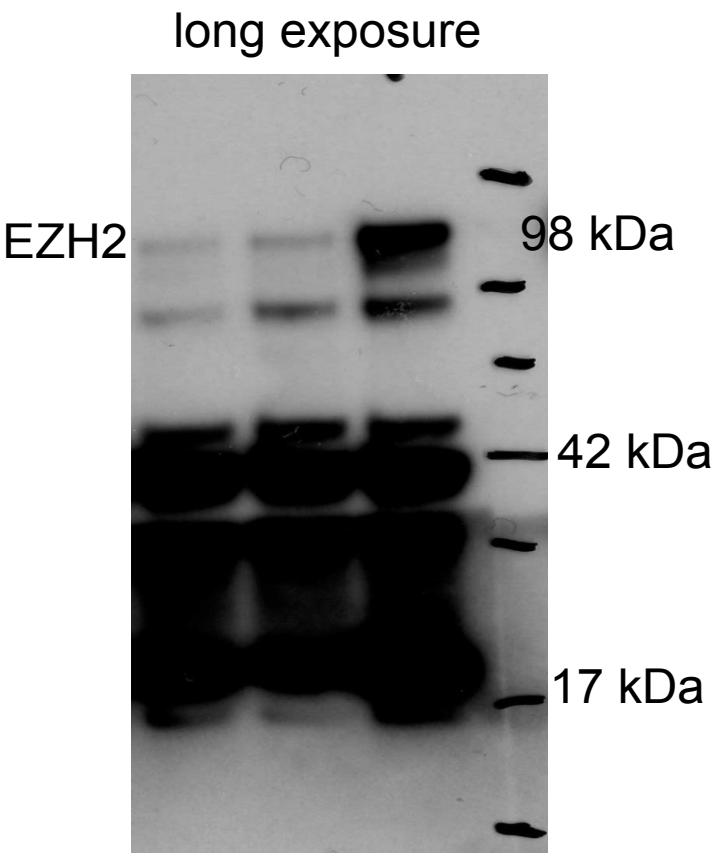


WBs for Figure 3b

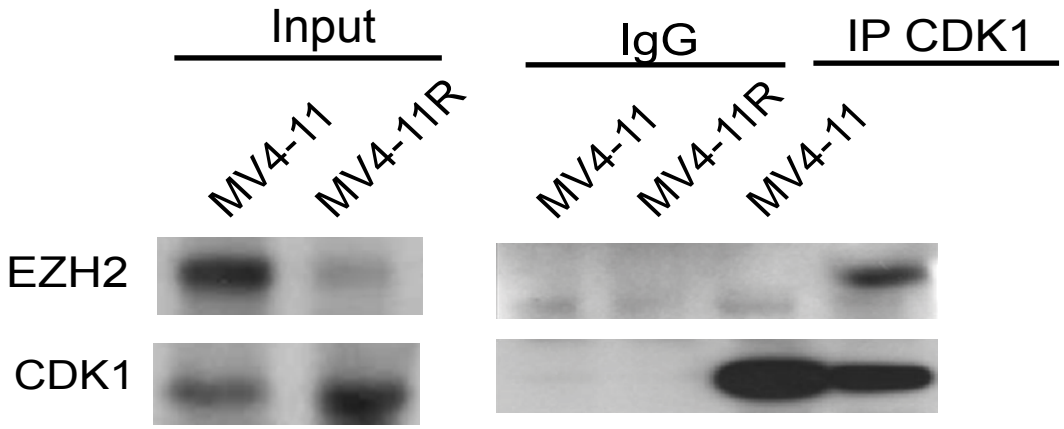


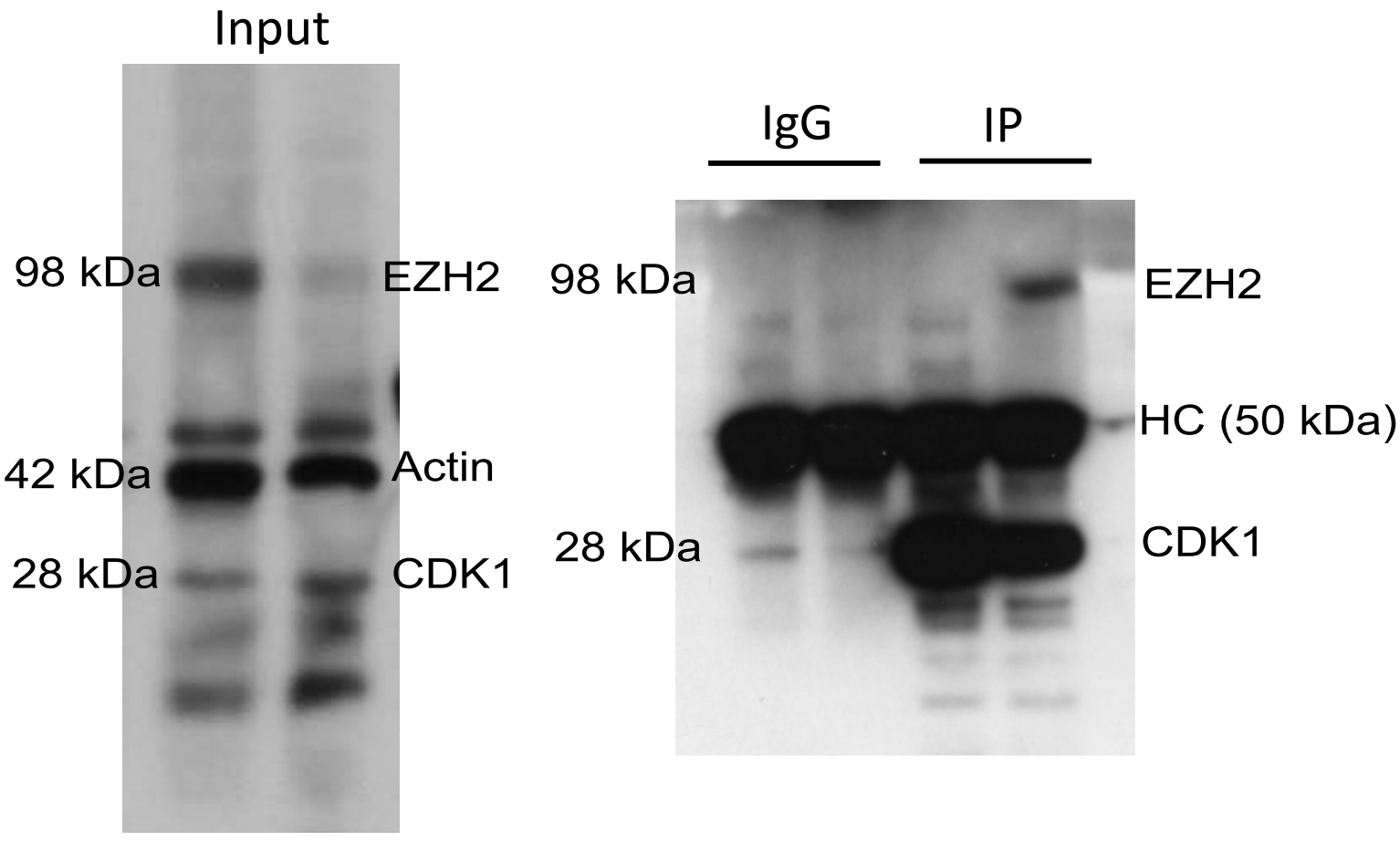




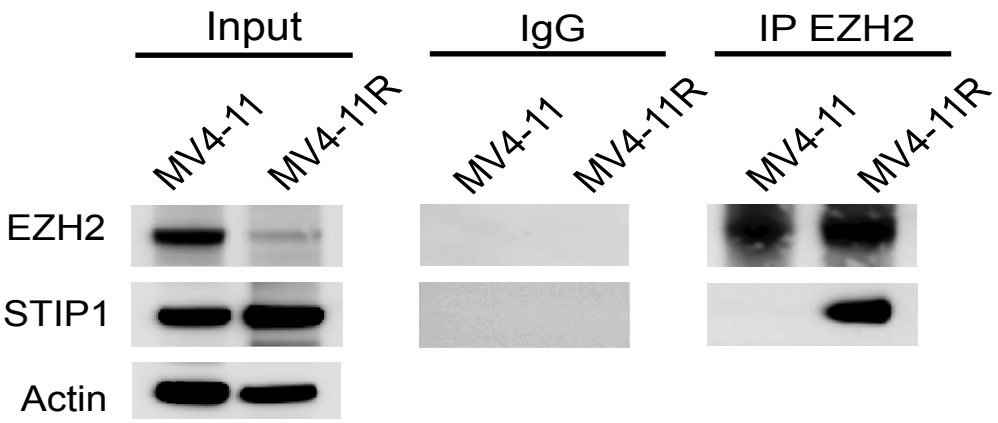


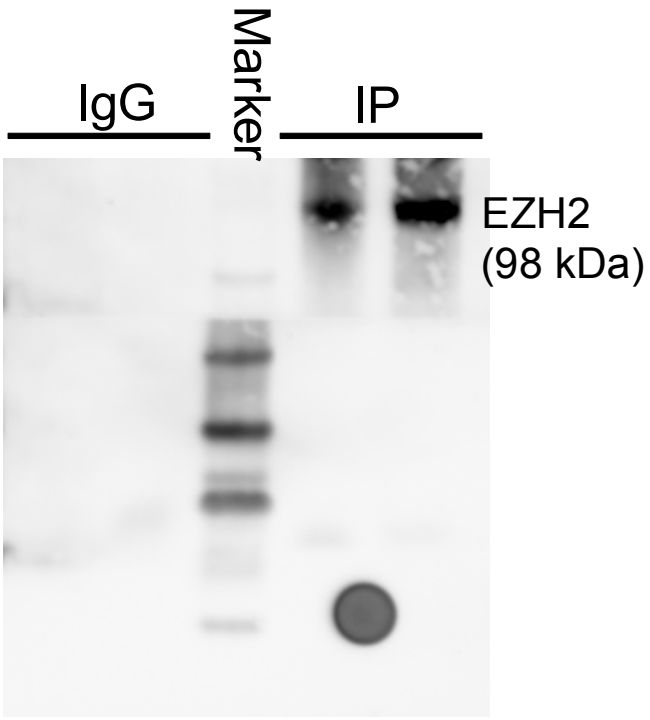
WBs for Figure 3c

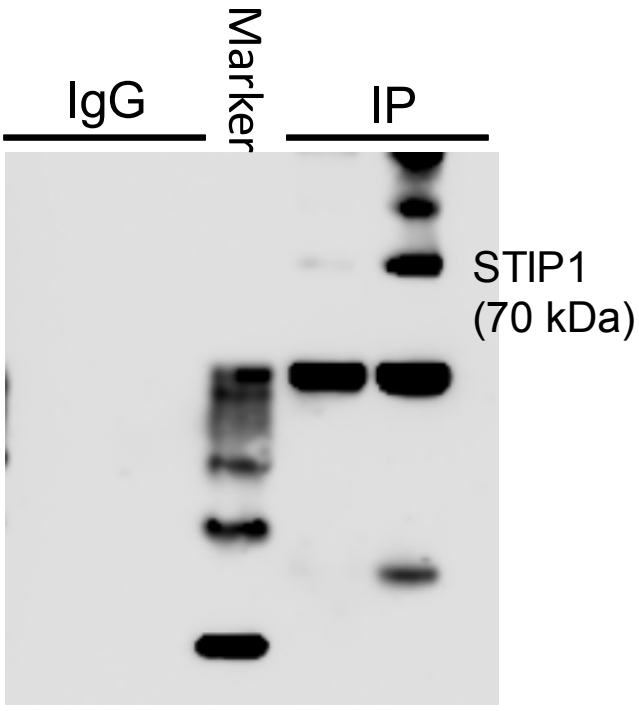




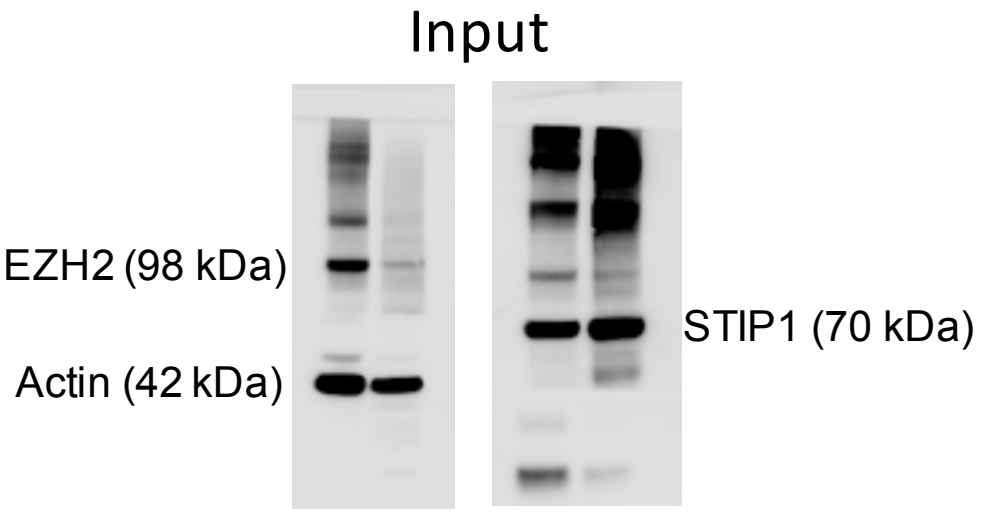
WBs for Figure 3d



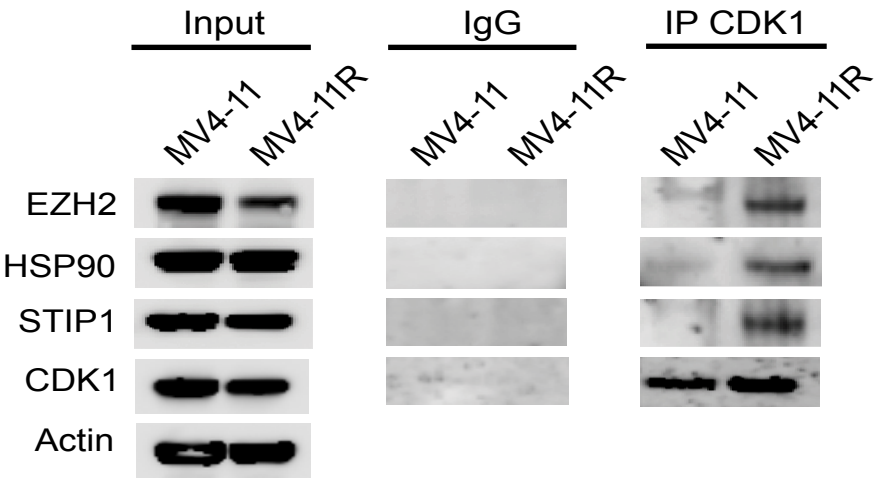


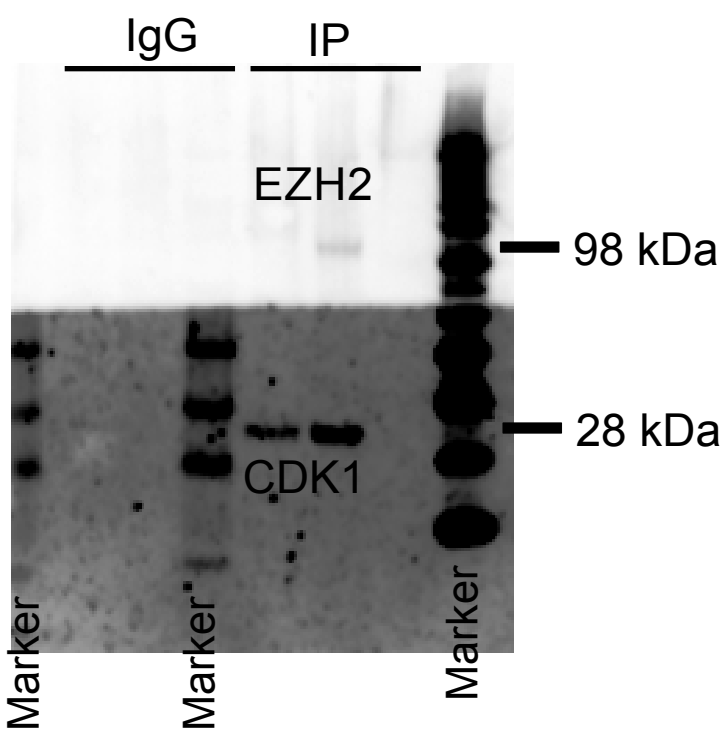


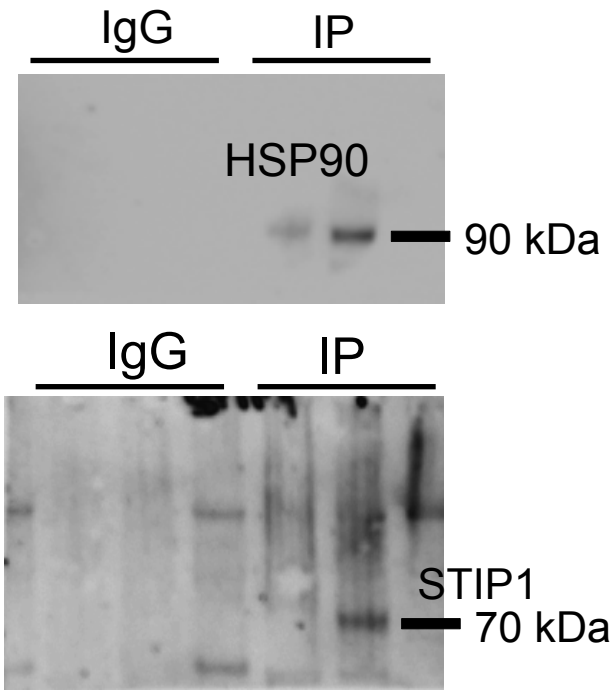
Note: These Co-IPs were performed with antibody crosslinked to beads.



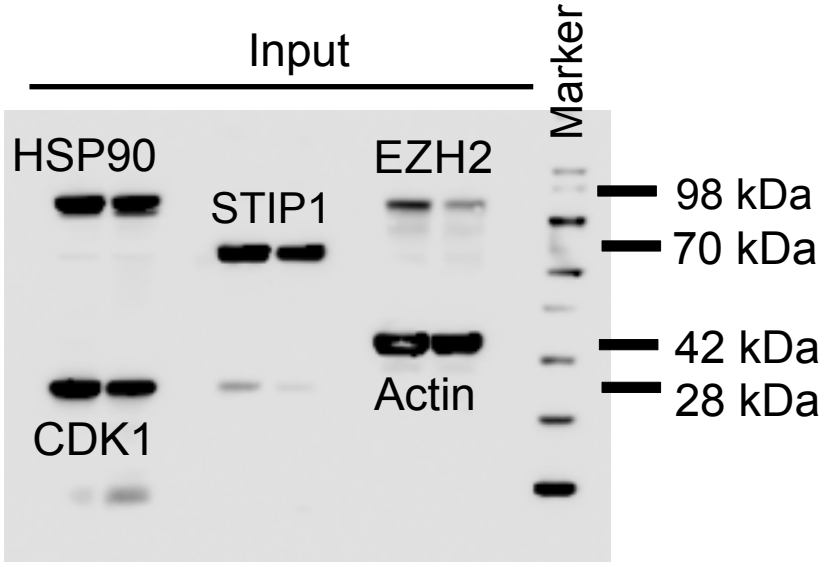
WBs for Figure 3e



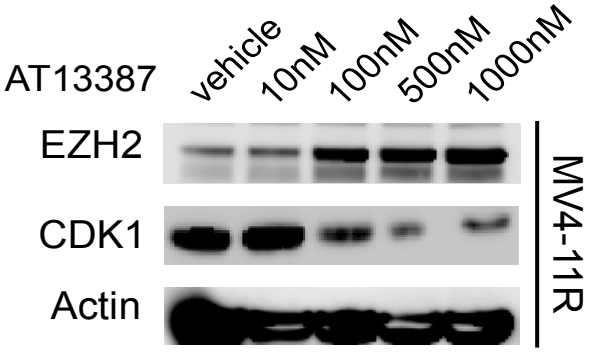


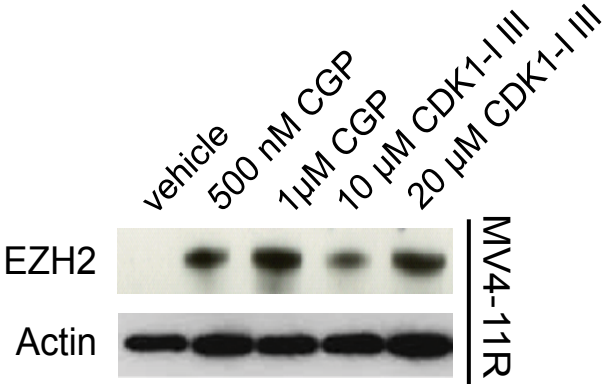


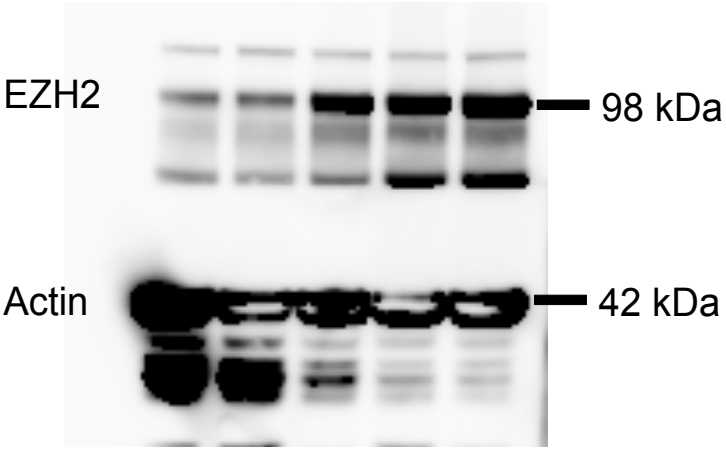
Note: These Co-IPs were performed with antibody crosslinked to beads.

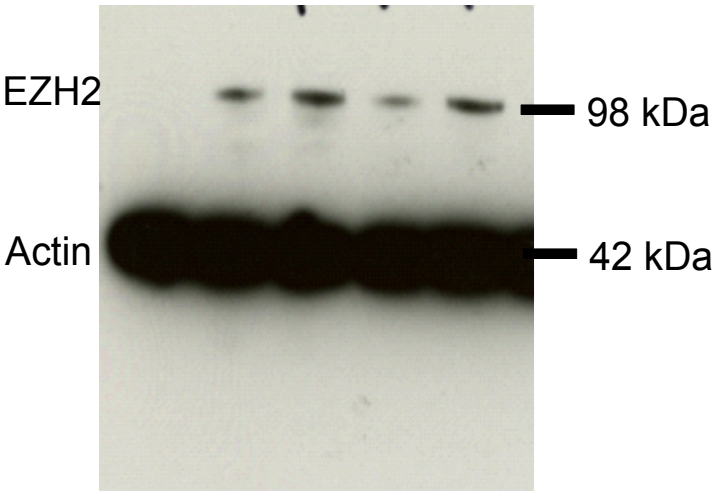


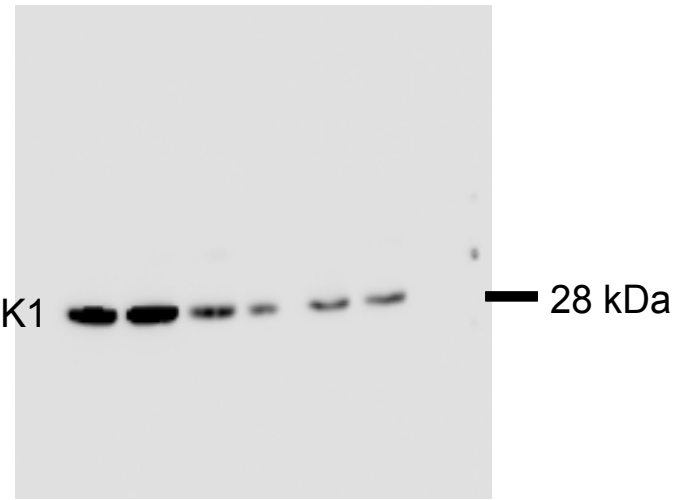
WBs for Figure 3f



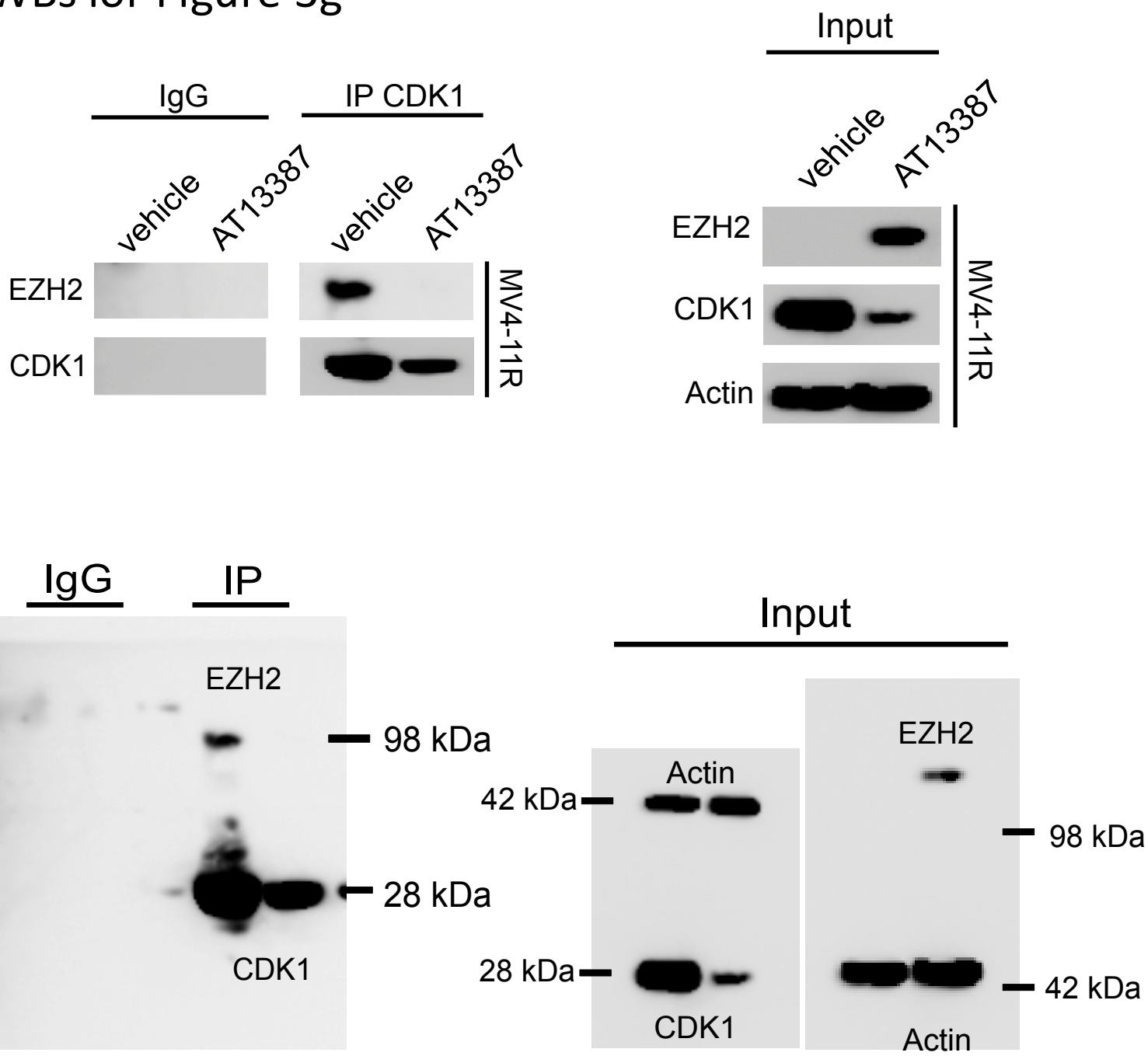




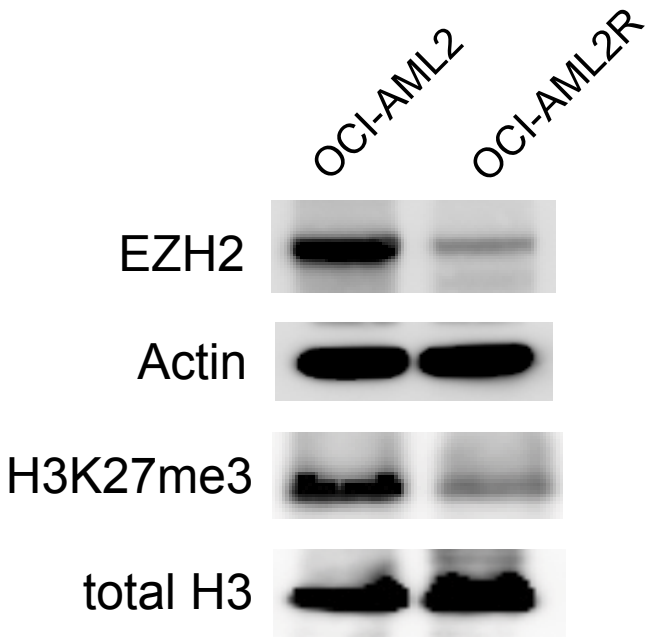


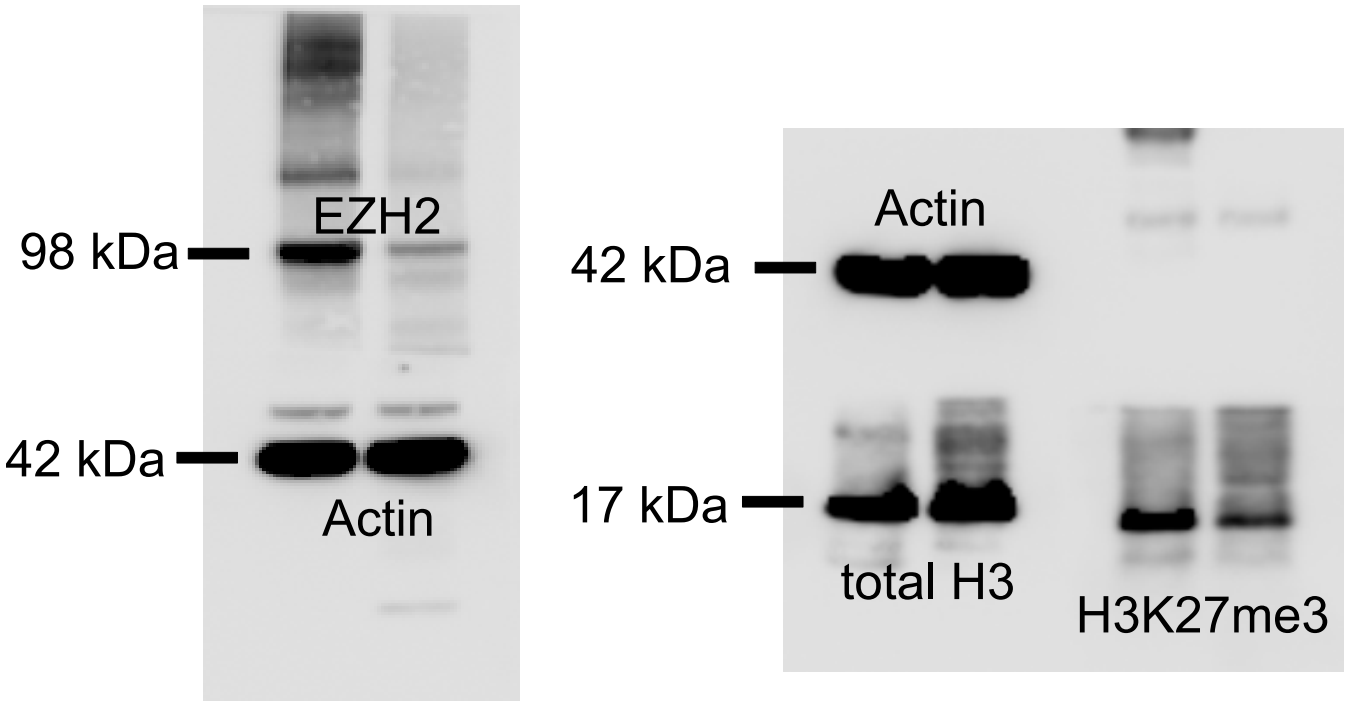


WBs for Figure 3g

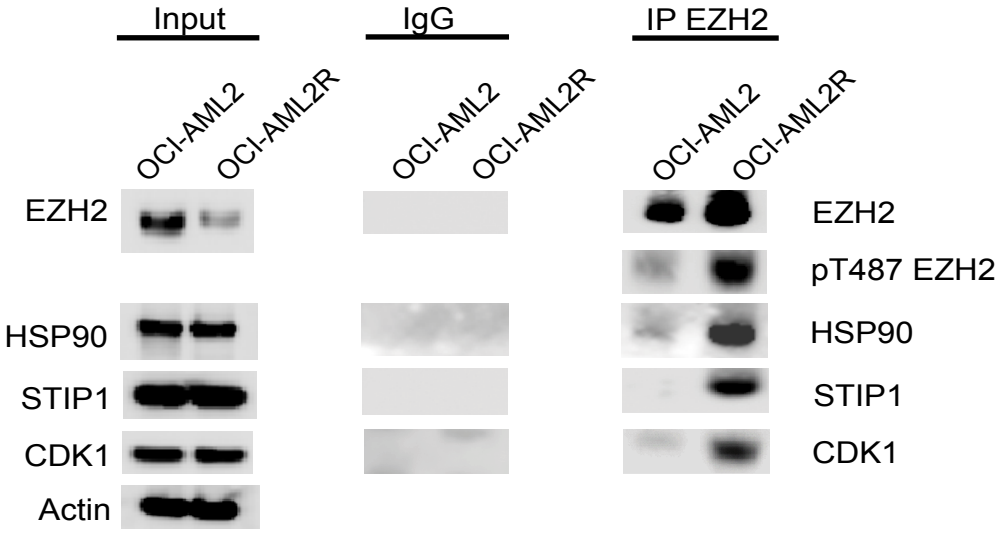


WBs for Figure 3h

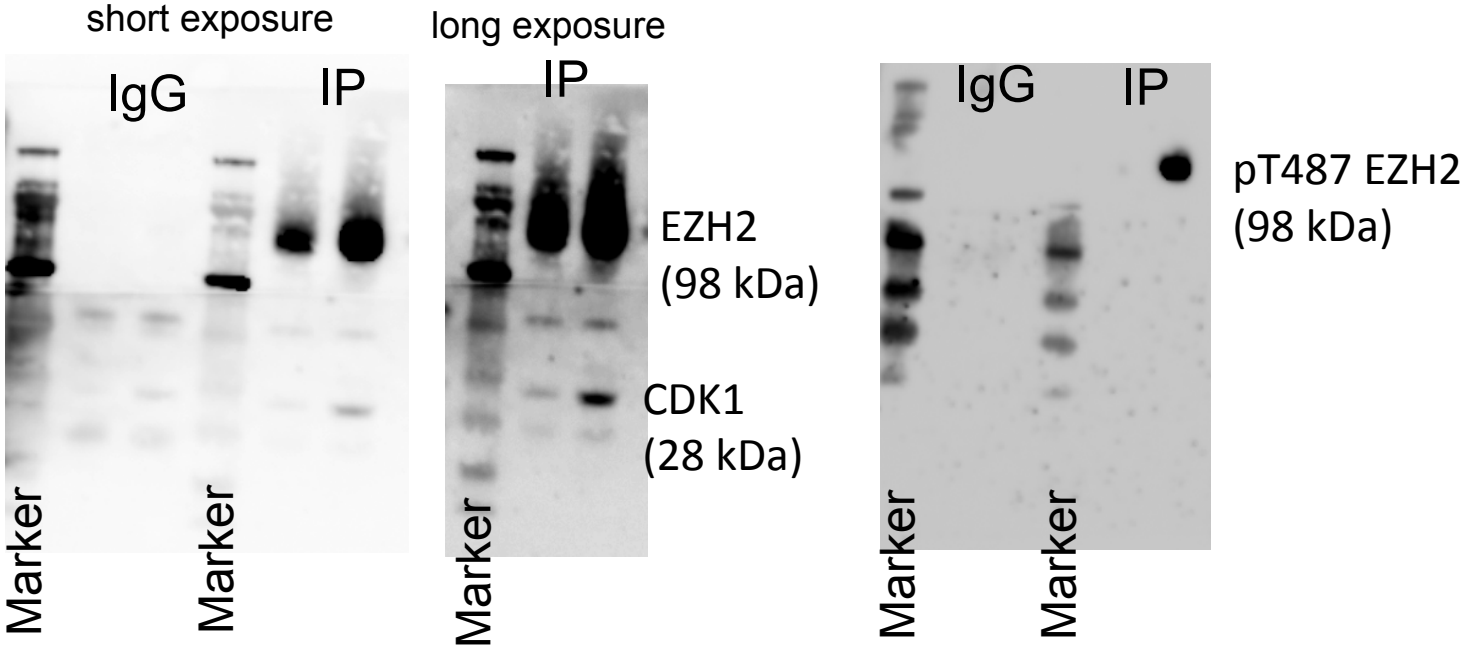


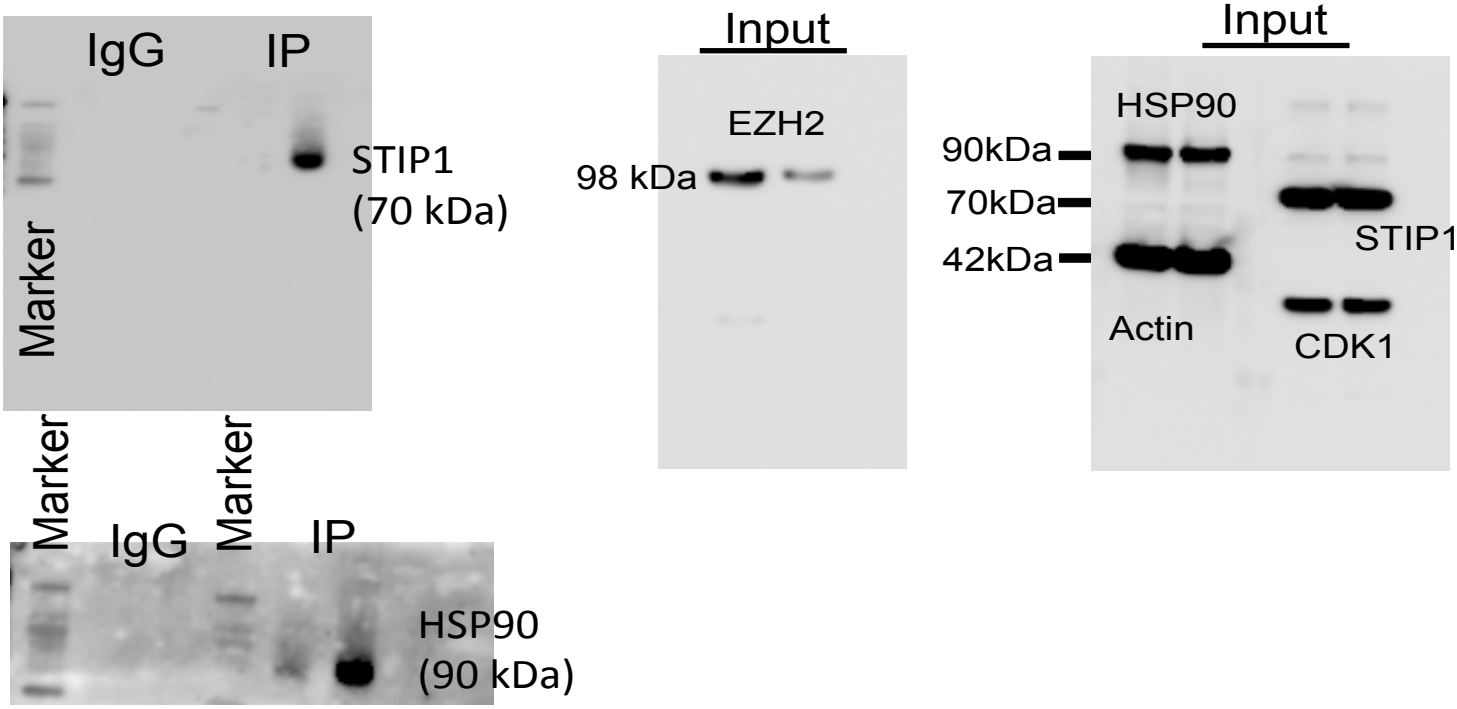


WBs for Figure 3i

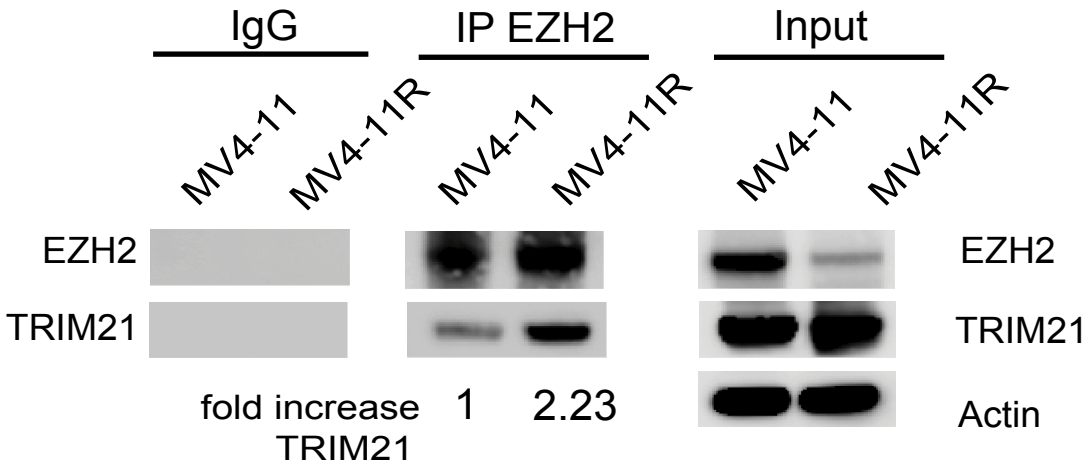


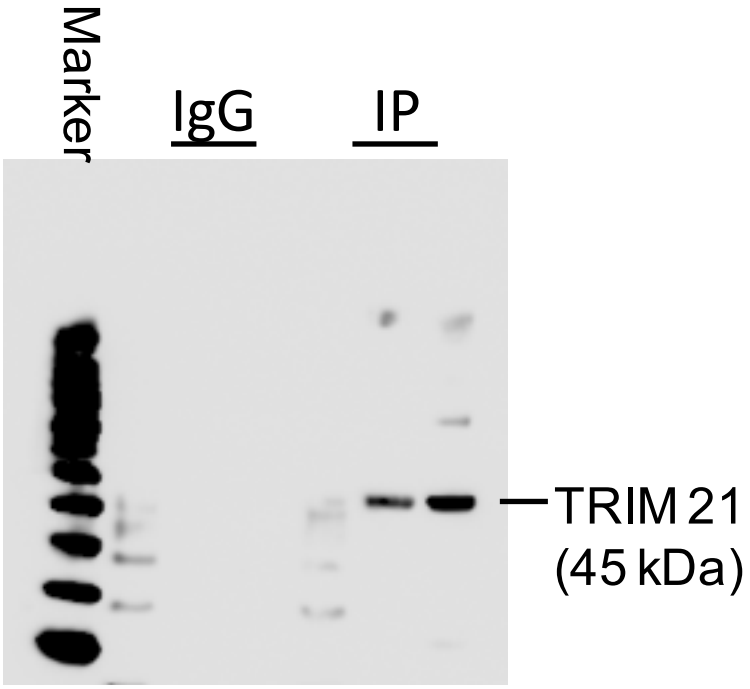
Note: These Co-IPs were performed with antibody crosslinked to beads.

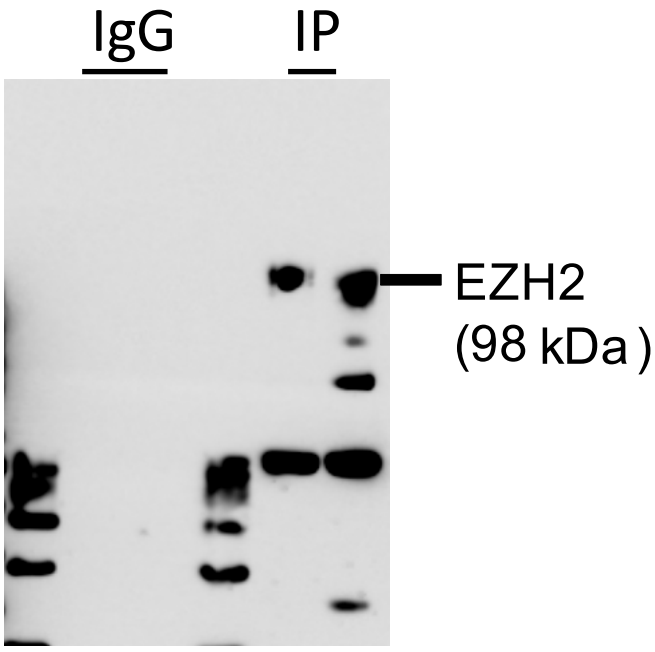


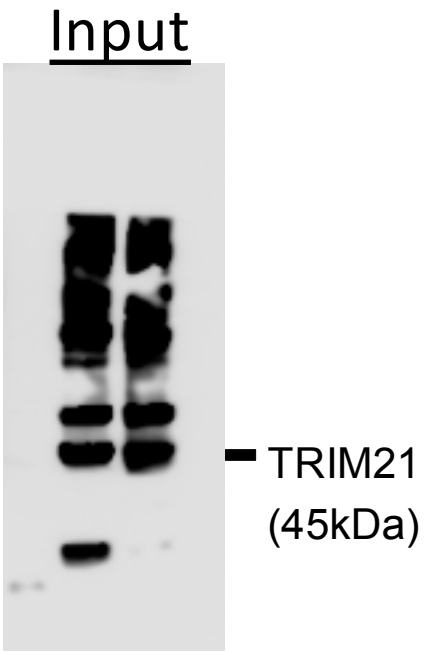


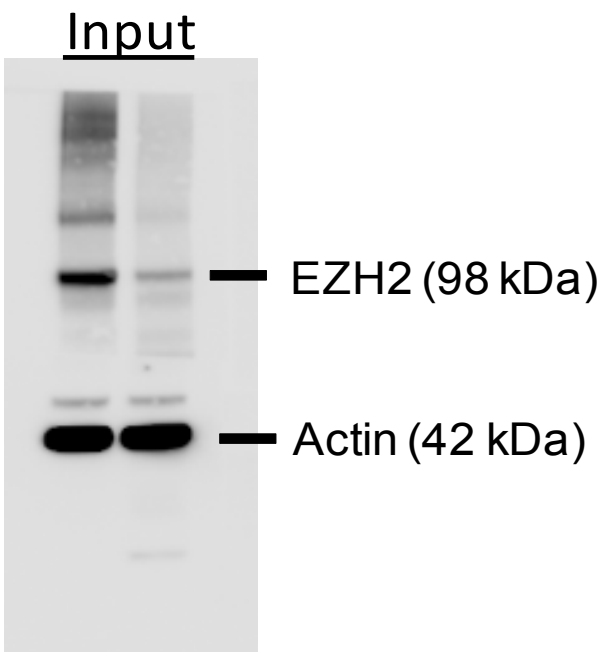
WBs for Figure 4a



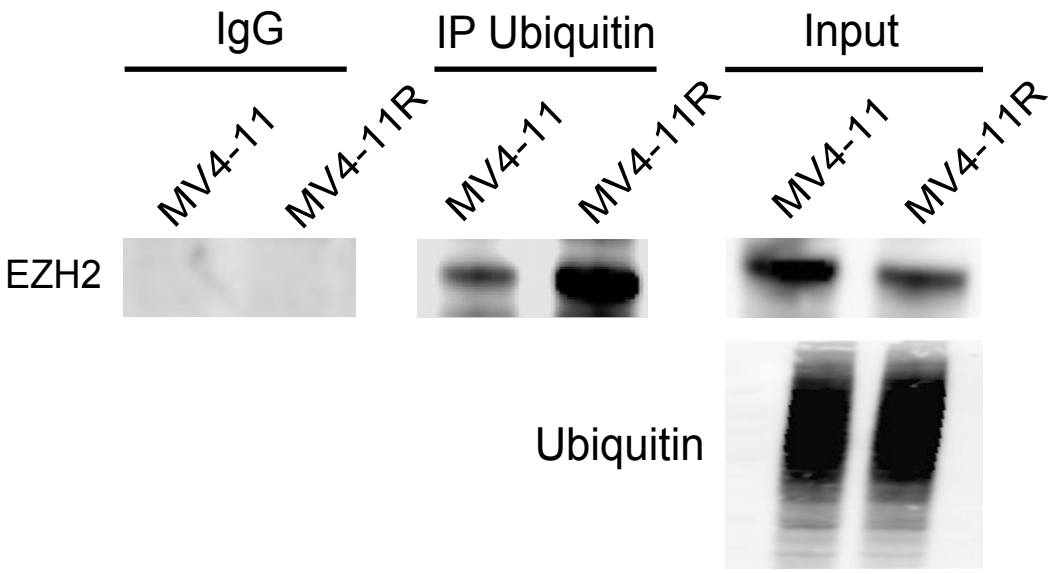


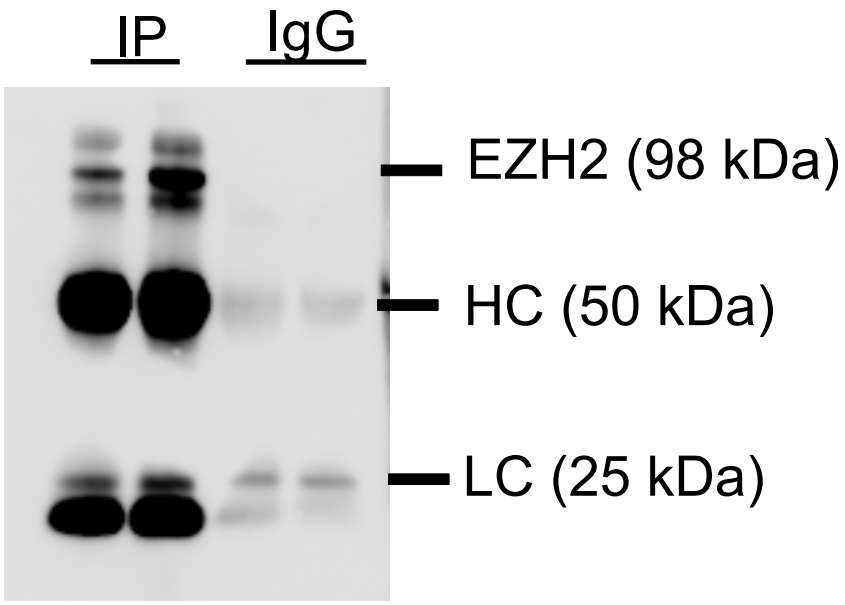


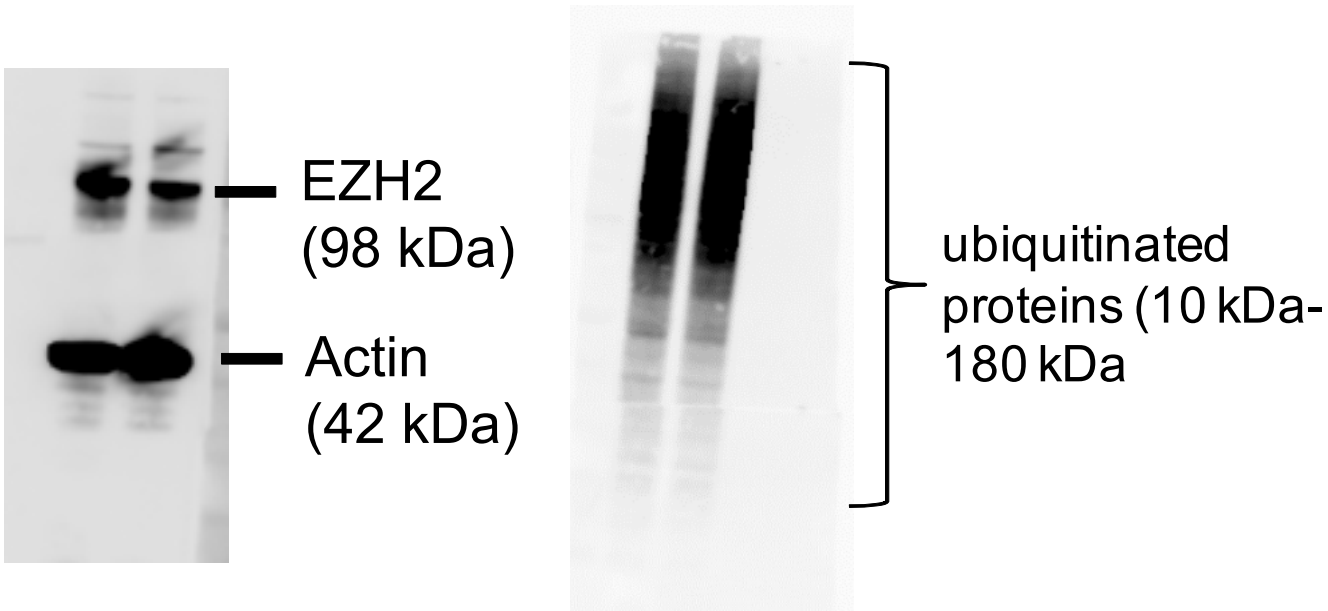




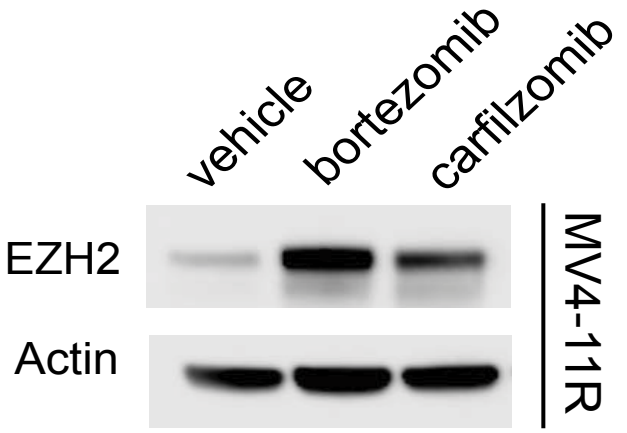
WBs for Figure 4b

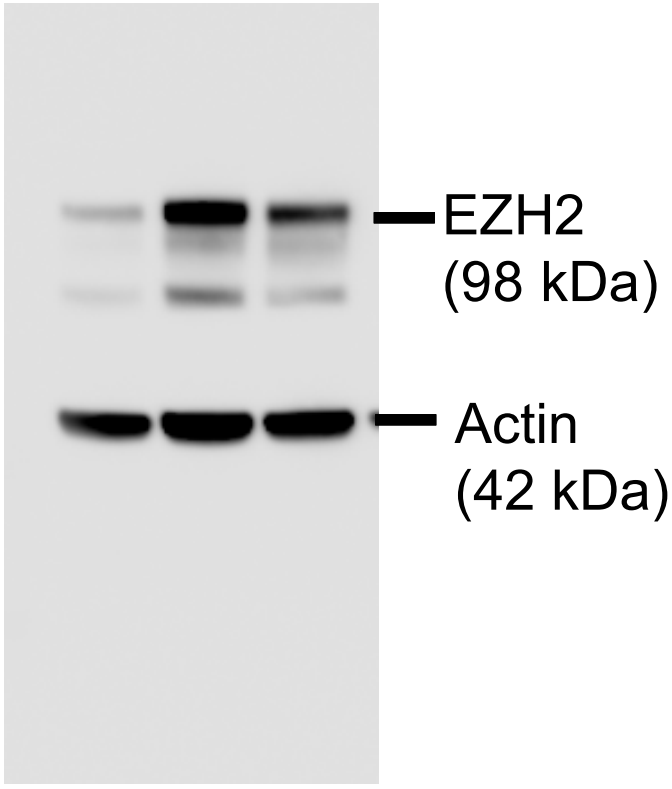




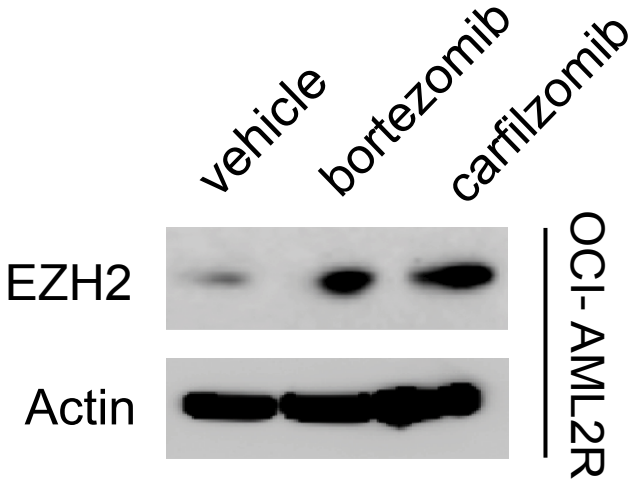


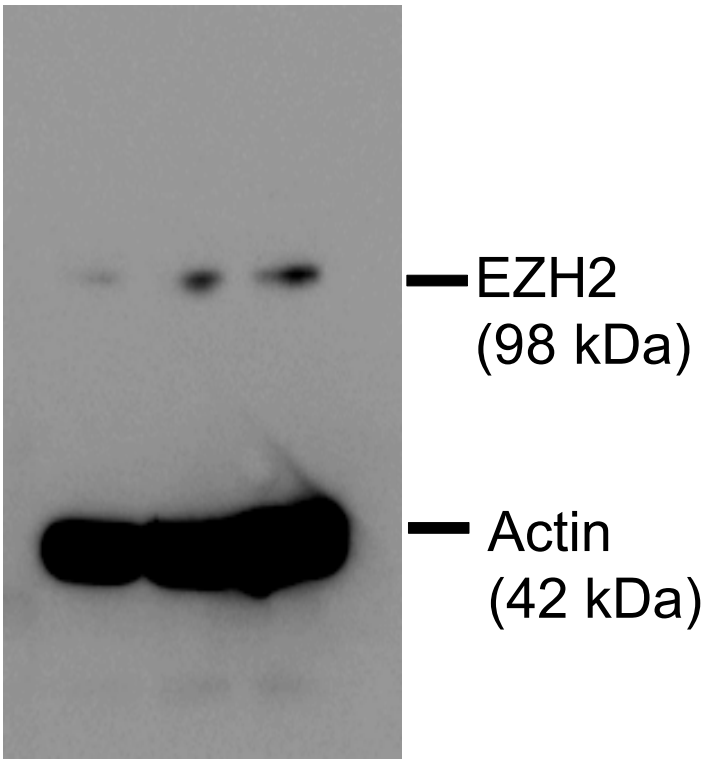
WBs for Figure 4d



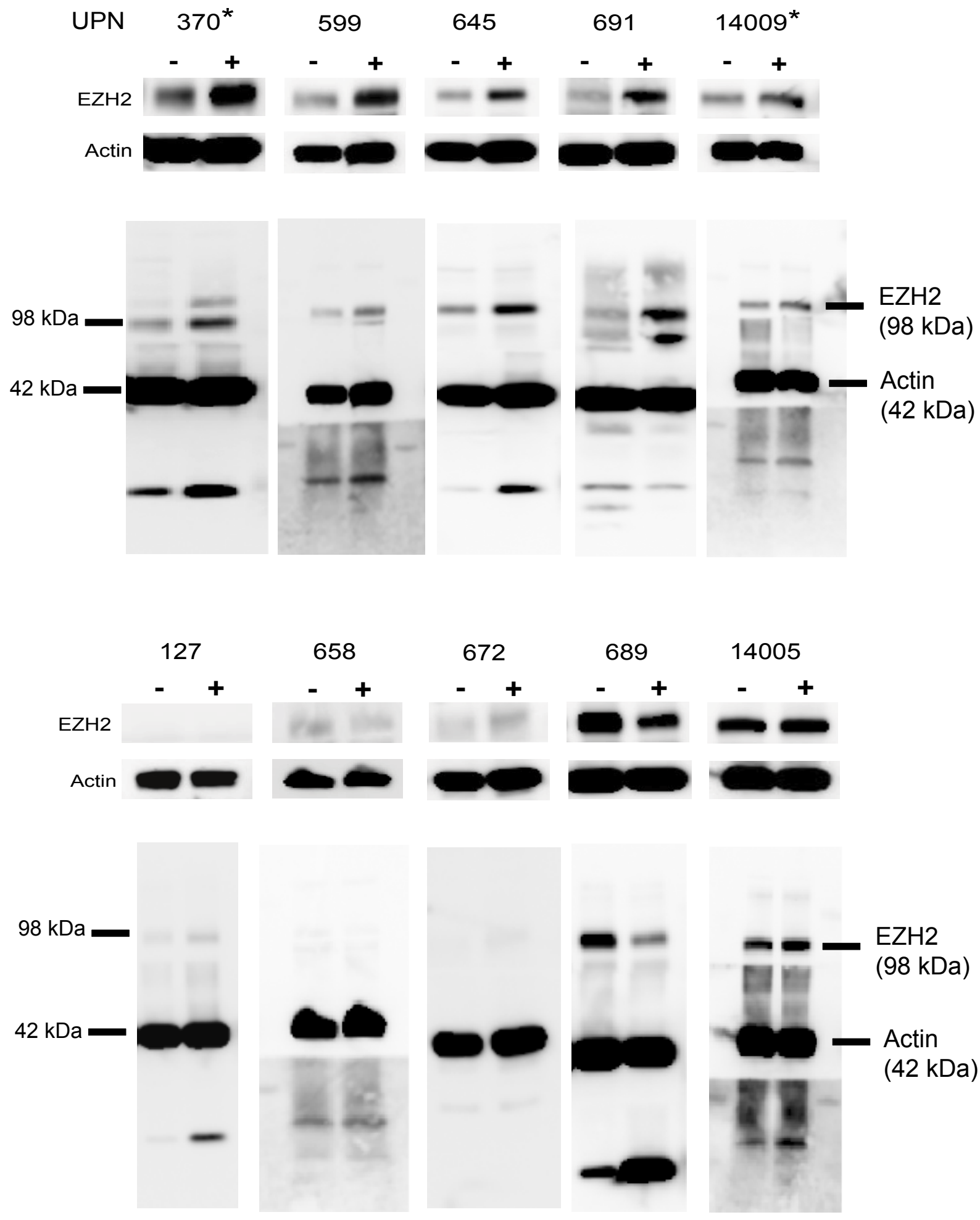


WBs for Figure 4e

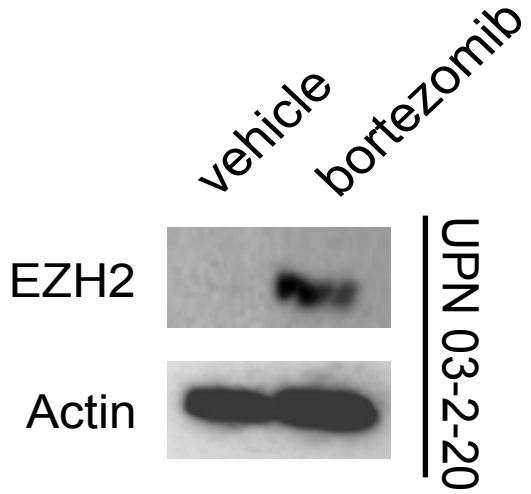


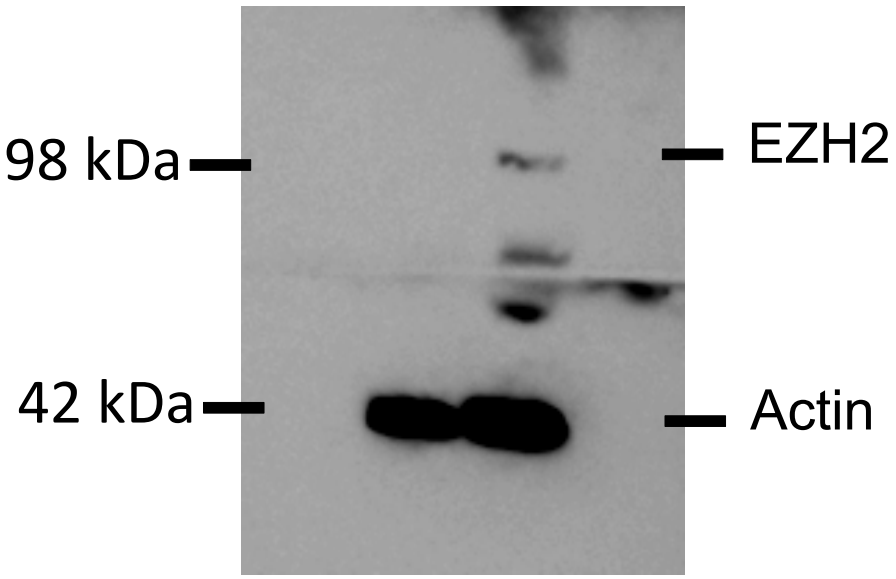


WBs for Figure 4f

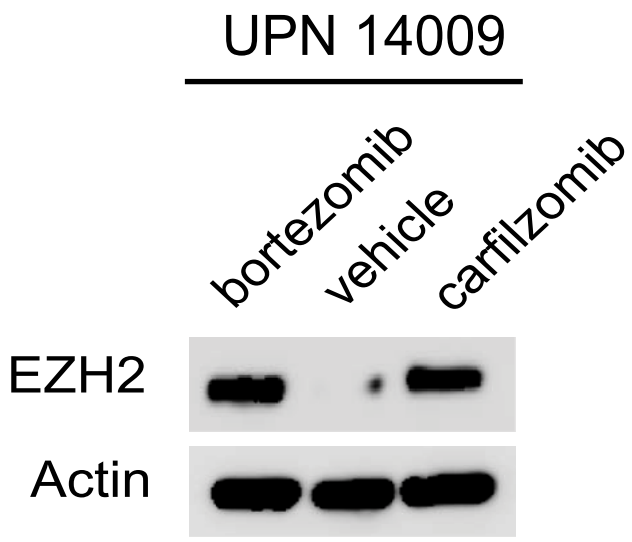


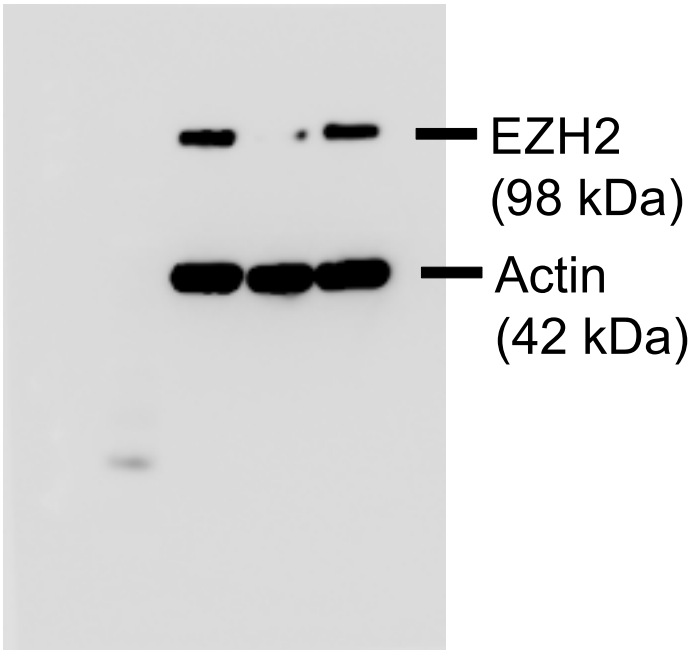
WBs for Figure 4i



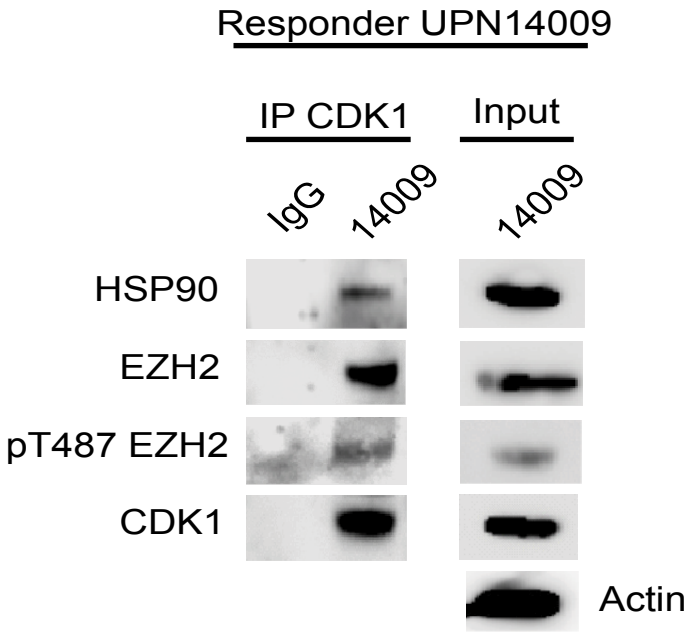


WBs for Figure 5a

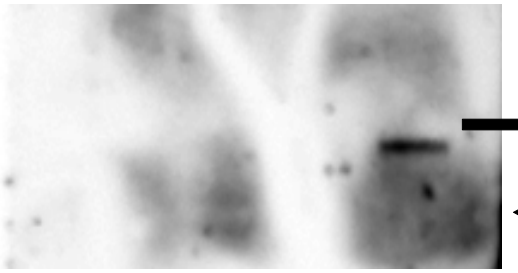


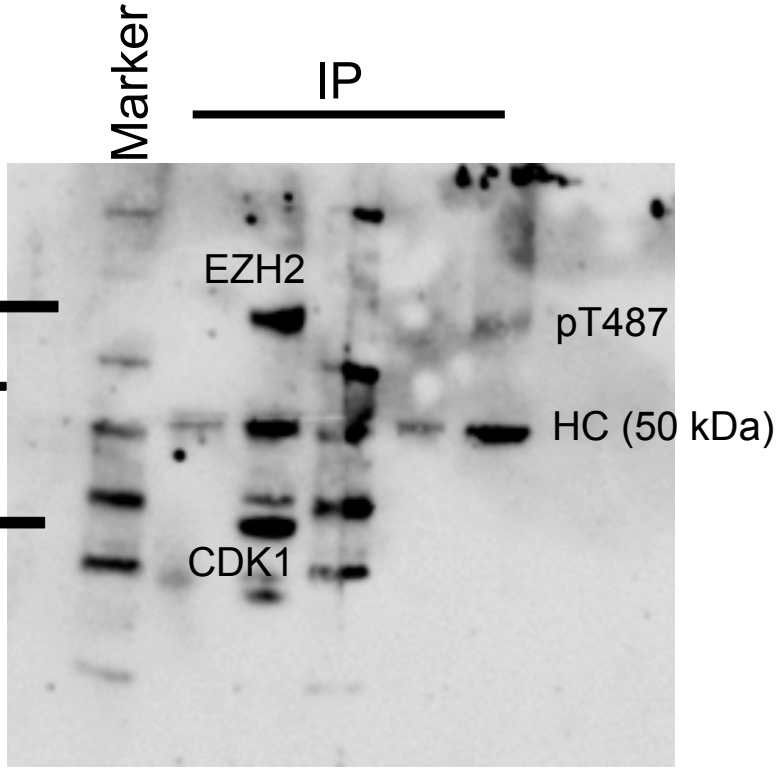


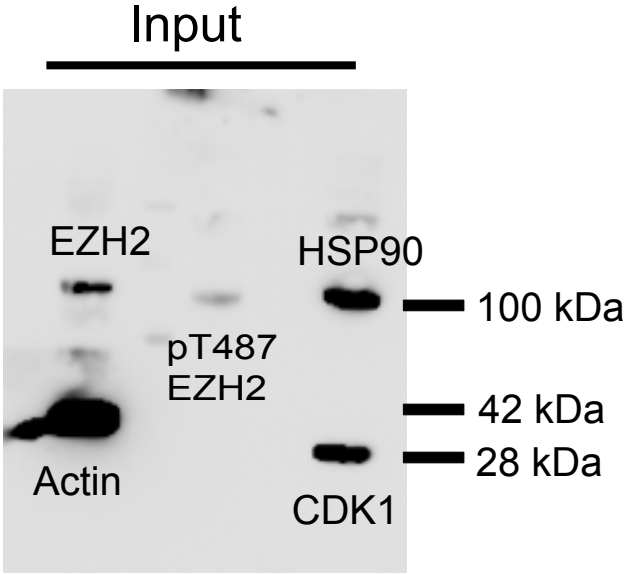
WBs for Figure 5b



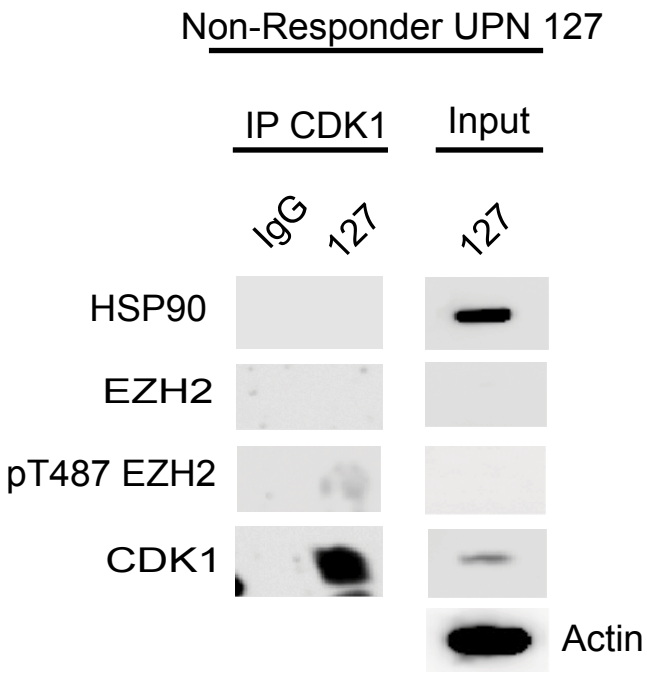
upper part of membran was reincubated with HSP90 antibody

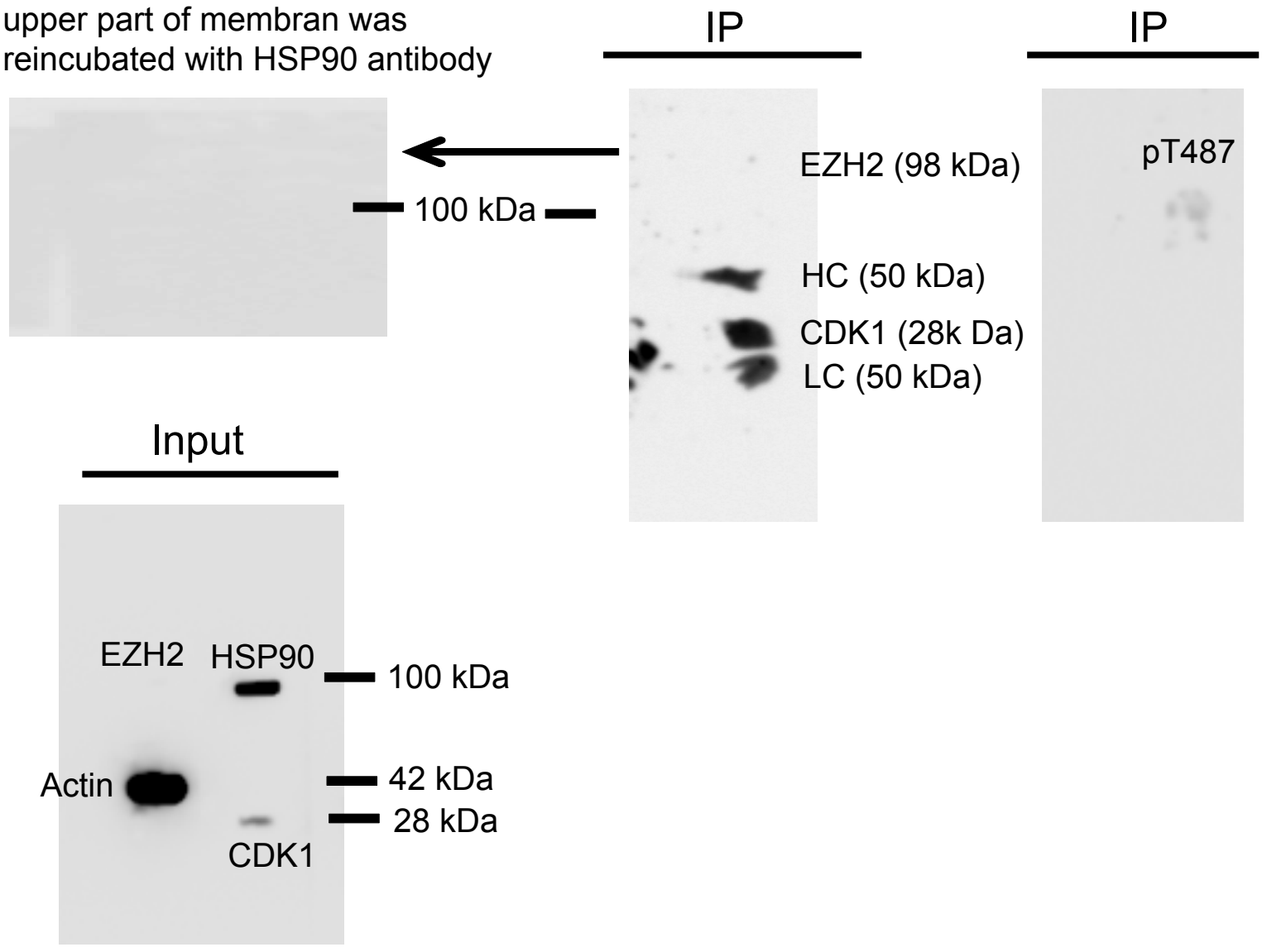




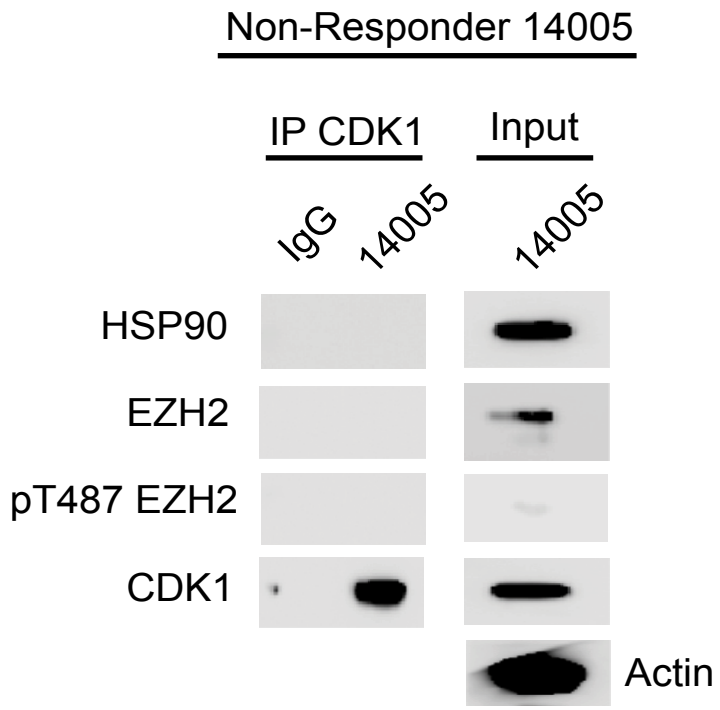


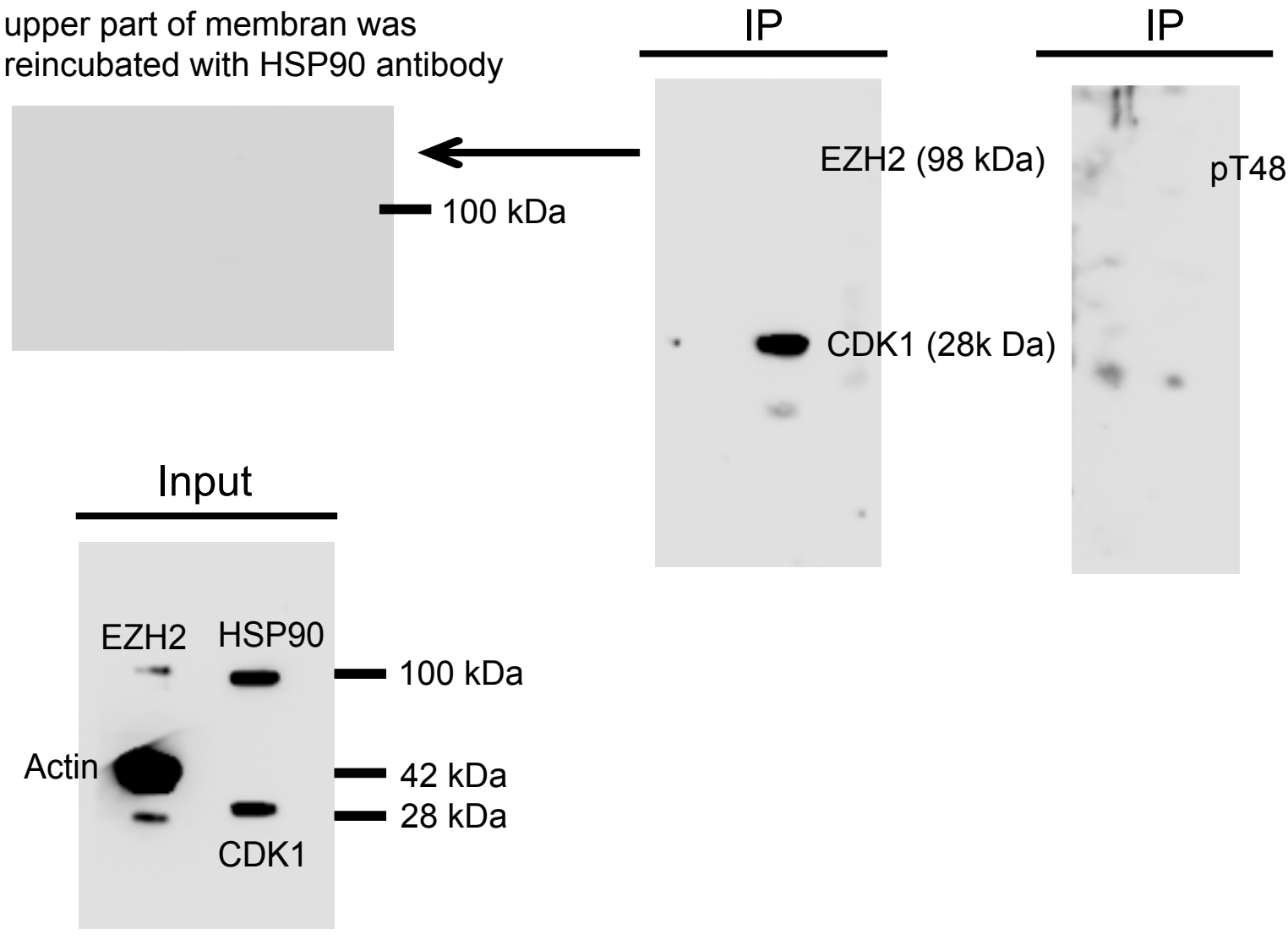
WBs for Figure 5b



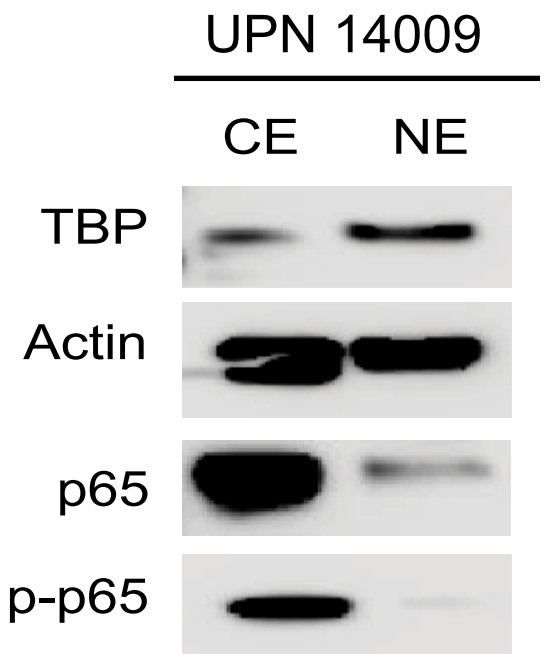


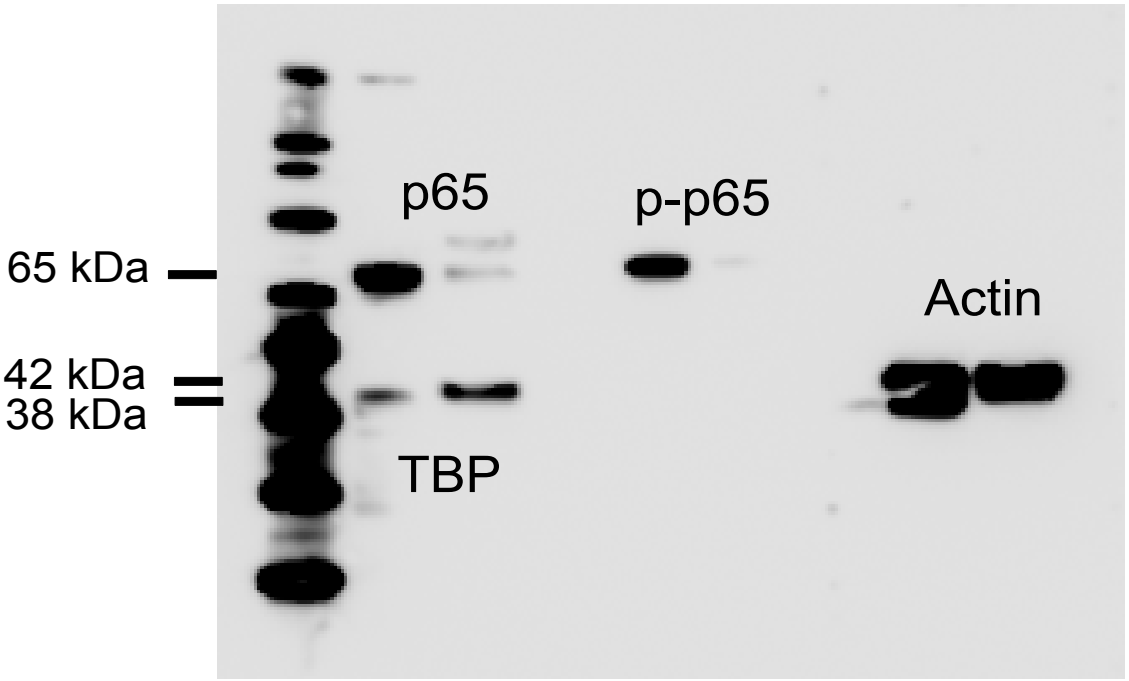
WBs for Figure 5b



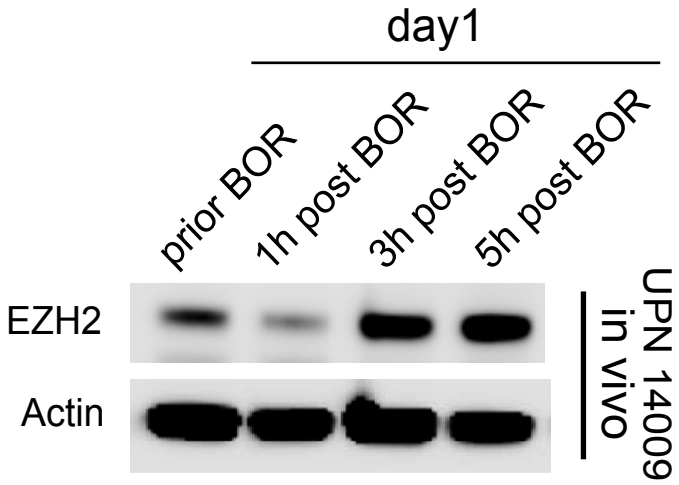


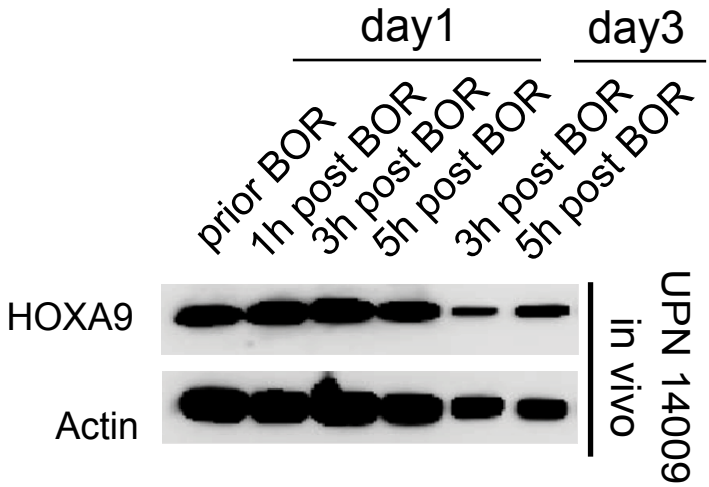
WBs for Figure 5c

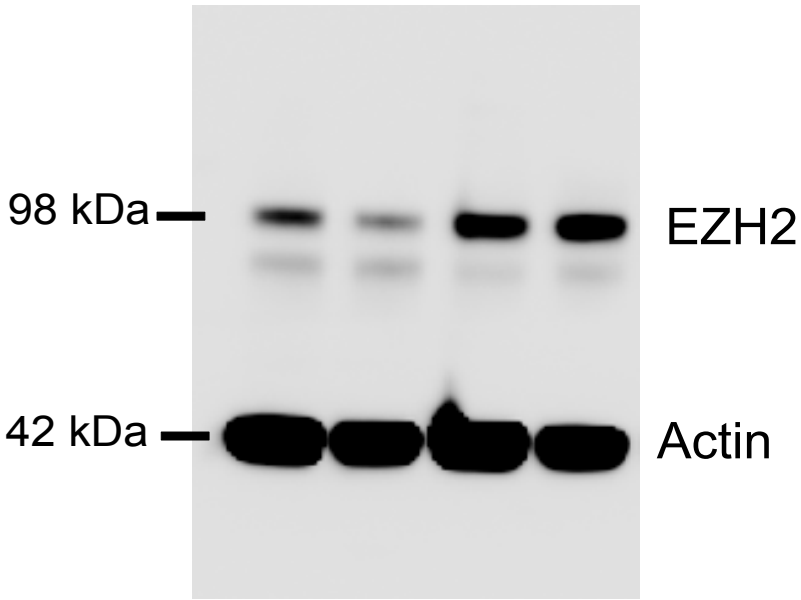


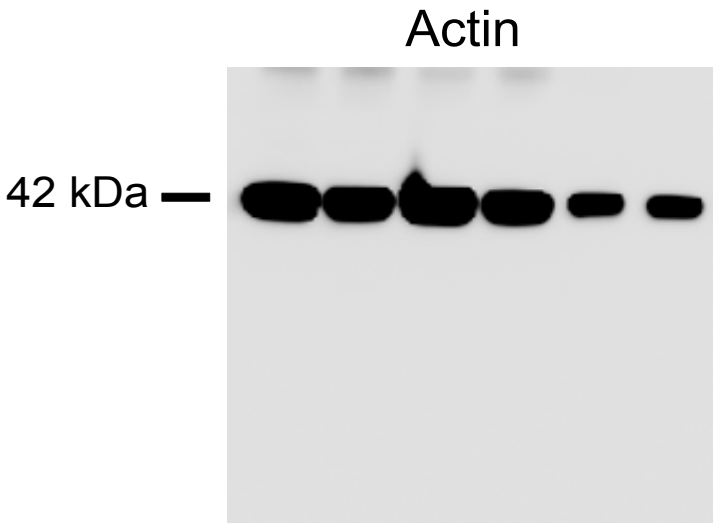


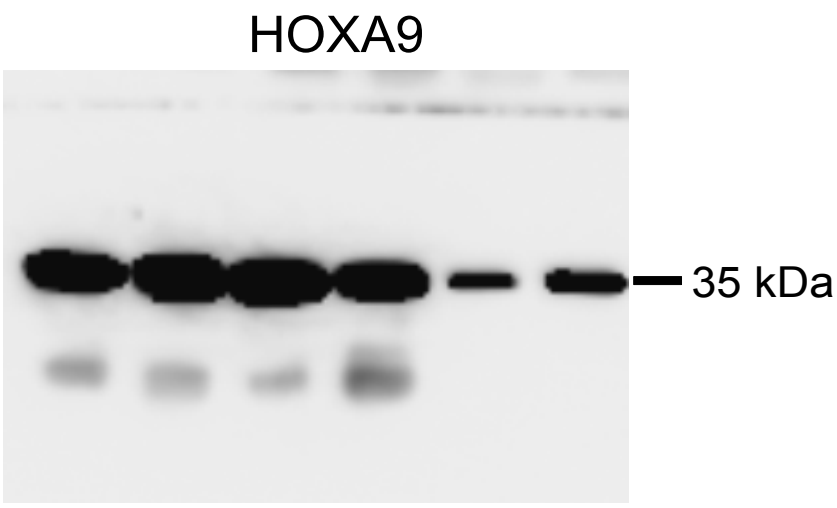
WBs for Figure 5d



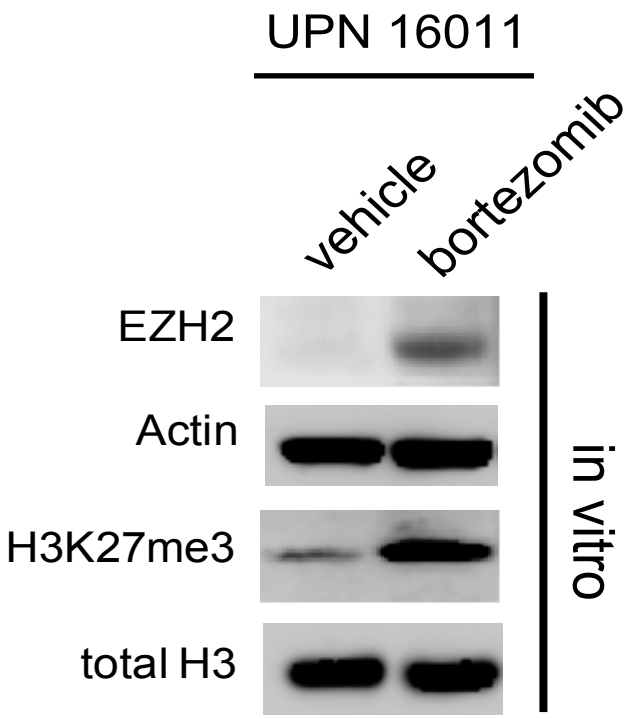


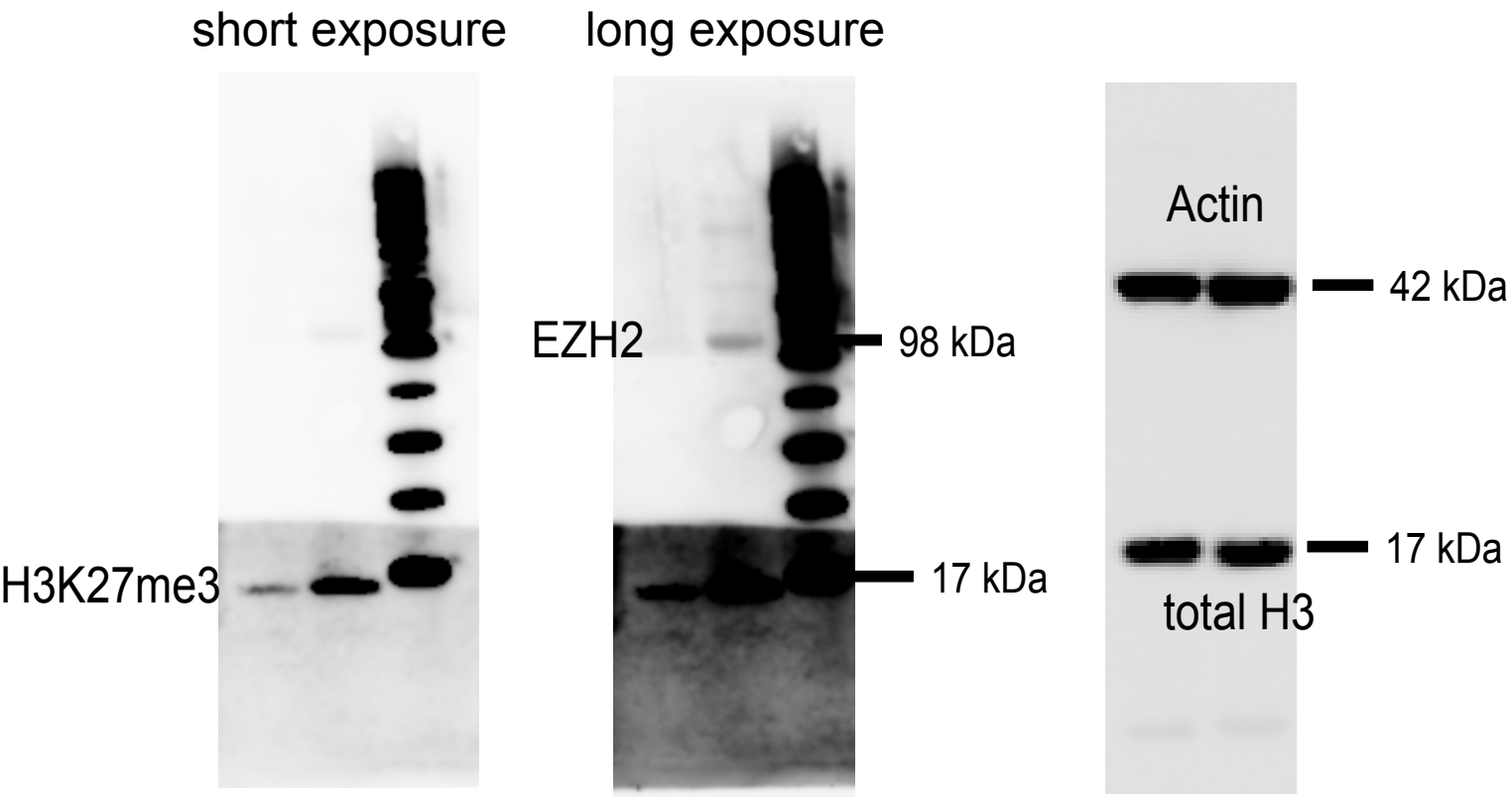






WBs for Figure 5g





Supplementary Figure 8, related to Figures 1-5. Uncropped western blots

All uncropped western blots for cropped versions in Figures 1-5 are provided in the order of their appearance in the article. Molecular weight standards are indicated.

References

1. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N. Engl. J. Med.* **368**, 2059-2074 (2013).

2. Verhaak, R. G. *et al*. Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling. *Haematologica* **94**, 131-134 (2009).

3. Valk, P. J. *et al*. Prognostically useful gene-expression profiles in acute myeloid leukemia. *N. Engl. J. Med.* **350**, 1617-1628 (2004).

4. Haferlach, T. *et al*. Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. *J. Clin. Oncol.* **28**, 2529-2537 (2010).

5. Huang da, W., Sherman, B. T. & Lempicki, R. A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **4**, 44-57 (2009).

Supplementary Table 1, related to Figure 1. Data of patients analyzed by Tissue Microarrays (TMA)

Number	124
Sex	Female: 51 (41.1 %) Male: 73 (58.9 %)
Median age at diagnosis	60.27 years (21-80)
Median WBC at diagnosis (per µl)	34,100 (800-409,670)
Median % of leukemic blasts **Bone Marrow (BM)** **Peripheral Blood (PB)**	 80 % (20-99) 45 % (0-98)
Cytogenetic Risk (ELN) Low Intermediate High unknown	 11 (8.9 %) 78 (62.9 %) 30 (24.2 %) 5 (4 %)
FAB 0 1 2 3 4 5 6 7 unknown	 5 (4 %) 15 (12.1 %) 16 (12.9 %) 5 (4 %) 32 (25.8 %) 43 (34.7 %) 4 (3.2 %) 1 (0.8 %) 3 (2.4 %)
FLT3 status WT Mutant unknown	 69 (55.6 %) 19 (15.3 %) 36 (29.0 %)

Supplementary Table 2, related to Supplementary Figure 1. Data of patients analyzed by Quantitative RT-PCR

Number	221
Sex	Female: 119 (53.8%) Male: 102 (46.2%)
Median age at diagnosis	52 years (18-60)
Median WBC at diagnosis (per µl)	21,295 (190-266,000)
Median % of leukemic blasts Bone Marrow (BM)	63 % (20-98)
FAB	
0	6 (2.7 %)
1	52 (23.5 %)
2	66 (29.9 %)
3	0
4	33 (14.9 %)
5	38 (17.2 %)
6	4 (1.8 %)
7	0
unknown	22 (10 %)
FLT3 status	
WT	163 (73.8 %)
Mutant	55 (24.9 %)
unknown	3 (1.4 %)

Supplementary Table 5, related to Figure 2. Mutations identified by Whole-Exome-Sequencing in MV4-11R vs. MV4-11 cells

Chromosome	Position	Gene Region	Gene Symbol	Protein Variant	Translation Impact
1	11710779	Exonic	FBXO2	45_46insA	in-frame
2	180725568	Intronic; micro-RNA	ZNF385B, miR1258		stop gain
4	71510354	Exonic	ENAM	G1071X	missense
8	113253982	Exonic	CSMD3		missense
9	140322570	Exonic	NOXA1	R116W	missense
10	105344322	Exonic	NEURL	R227W	missense
11	1258387	Exonic	MUC5B	R1097H	missense
11	113078076	Exonic	NCAM1	P241Q	missense
12	112888202	Exonic	PTPN11	T73I	missense
13	28602340	Exonic	FLT3	N676K	missense
13	49795190	Exonic	MLNR	Q241fs	frameshift
15	52258624	Exonic	LEO	D46Y	missense
15	91312369	Exonic	BLM	A772T	missense
16	19566996	Exonic	C16orf62	V71G	missense
17	7578377	Exonic	TP53	S146f*	frameshift
17	7578380	Exonic	TP53	S24_D25delinsY; S183_D184delinsY; S51_D52delinsY S144_D145delinsY	non-frameshift
19	56133473	Exonic	ZNF784	R206C	missense
20	62195710	Exonic	HELZ2	A920T; A1489T	missense
X	53224166	Exonic	KDM5C	D1062fs; D1129fs	frame-shift
Y	21154466	Exonic	CD24	S44T	missense

Supplementary Table 7, related to Figure 2. GO-term enrichment analysis[5] of target genes differentially regulated between MV4-11R and MV4-11 cells (TOP20 terms)

Term	p-value	% involved genes/total genes
PIRSF001991:class II histocompatibility antigen	0.005	50
GO:0004954 prostanoid receptor activity	0.006	7
GO:0002828~regulation of T-helper 2 type immune response	0.006	7
GO:0032395~MHC class II receptor activity	0.008	5.2
membrane	0.013	3.5
IPR001245:Tyrosine protein kinase	0.017	3.5
SM00219:TyrKc	0.022	3.5
GO:0030099~myeloid cell differentiation	0.025	7
GO:0032555~purine ribonucleotide binding	0.025	7
GO:0004715~non-membrane spanning protein tyrosine kinase activity	0.025	3.5
GO:0002829~negative regulation of T-helper 2 type immune response	0.026	3.5
GO:0035023~regulation of Rho protein signal transduction	0.028	19.3
phosphotransferase	0.028	3.5
GO:0001883~purine nucleoside binding	0.031	7
GO:0005524~ATP binding	0.031	3.5
GO:0030097~hemopoiesis	0.041	21
GO:0002684~positive regulation of immune system process	0.041	21
GO:0021700~developmental maturation	0.043	19
GO:0030554~adenyl nucleotide binding	0.044	5.2
GO:0001882~nucleoside binding	0.048	7

Supplementary Table 16, related to Figure 5. Chemotherapy dosage for patient UPN 14009

Stage	Treatment
Diagnosis	Cytarabine (100 mg/m² d1-7) + Daunorubicin (60 mg/m² d3-5) Reinduction: Mitoxantrone (7 mg/m²) , Cytarabine (2 x 1 g/m²) and Fludarabine (2 x 15 mg/m²) (Mito-FLAG) 1st Consolidation: High-dose Cytarabine (2 x 3 g/m² d1, 3, 5) 2nd Consolidation: High-dose Cytarabine (2 x 3 g/m² d1, 3, 5)
1st Relapse	Mitoxantrone (7 mg/m²) , Cytarabine (2 x 1 g/m²) and Fludarabine (2 x 15 mg/m²) (Mito-FLAG) with absence of remission followed by mini-ICE regimen (Mitoxantrone (7 mg/m²), Etoposide (100 mg/m²), Cytarabine (100 mg/m²) myeloablative conditioning regimen for 1st allogeneic hematopoietic stem cell transplantation (alloSCT)
2nd Relapse	Hydroxycarbamide (d1-3) + Cytarabine (200 mg/m^2, d3-5) myeloablative conditioning regimen for 2nd alloSCT
3rd Relapse	Bortezomib (1.3 mg/m^2 s.c. d1 and 5) + Cytarabine (2x 3g/m^2, d1, 3, 5)