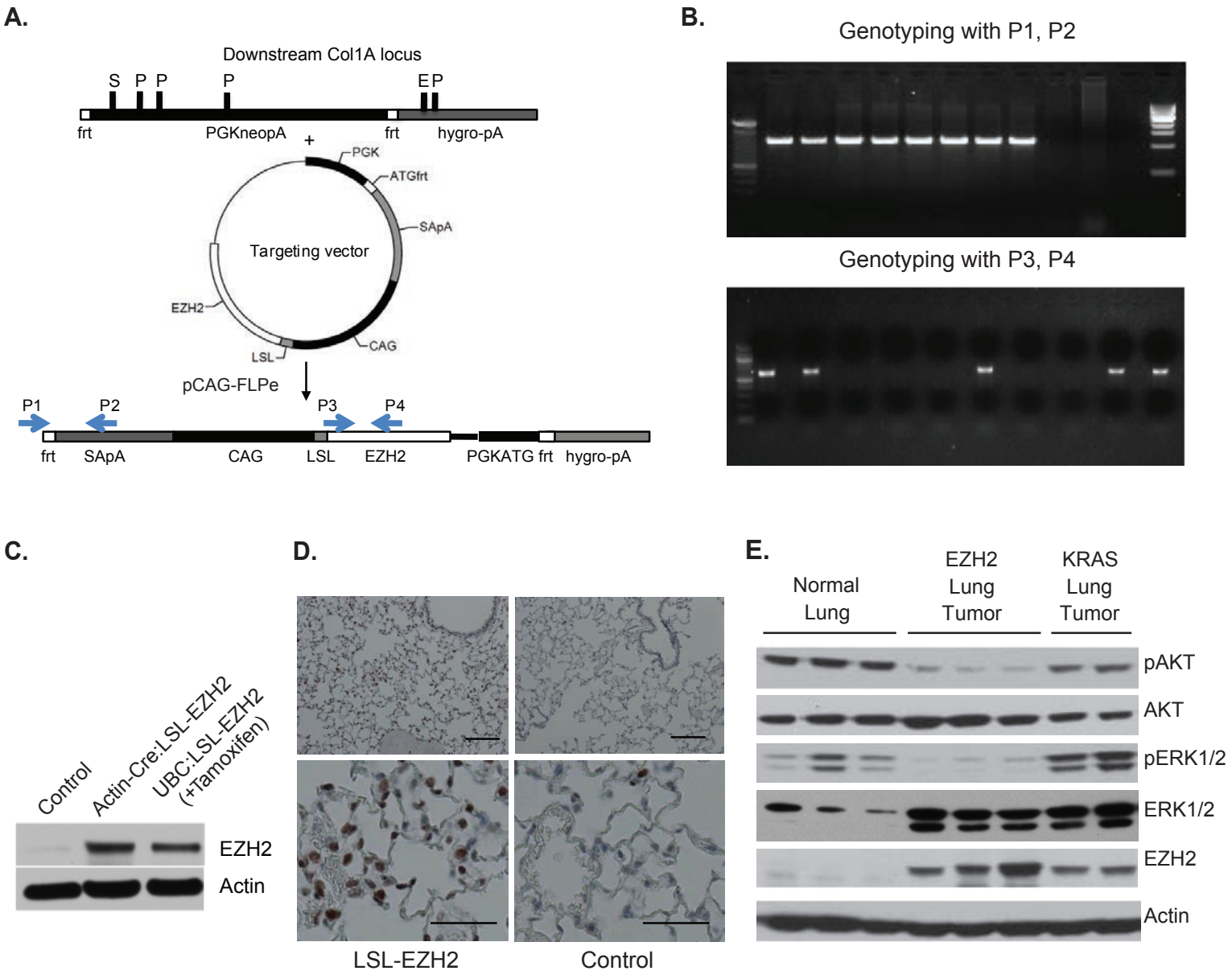


Supplementary Figure S1. Generation of LSL-EZH2 conditional transgenic mice.

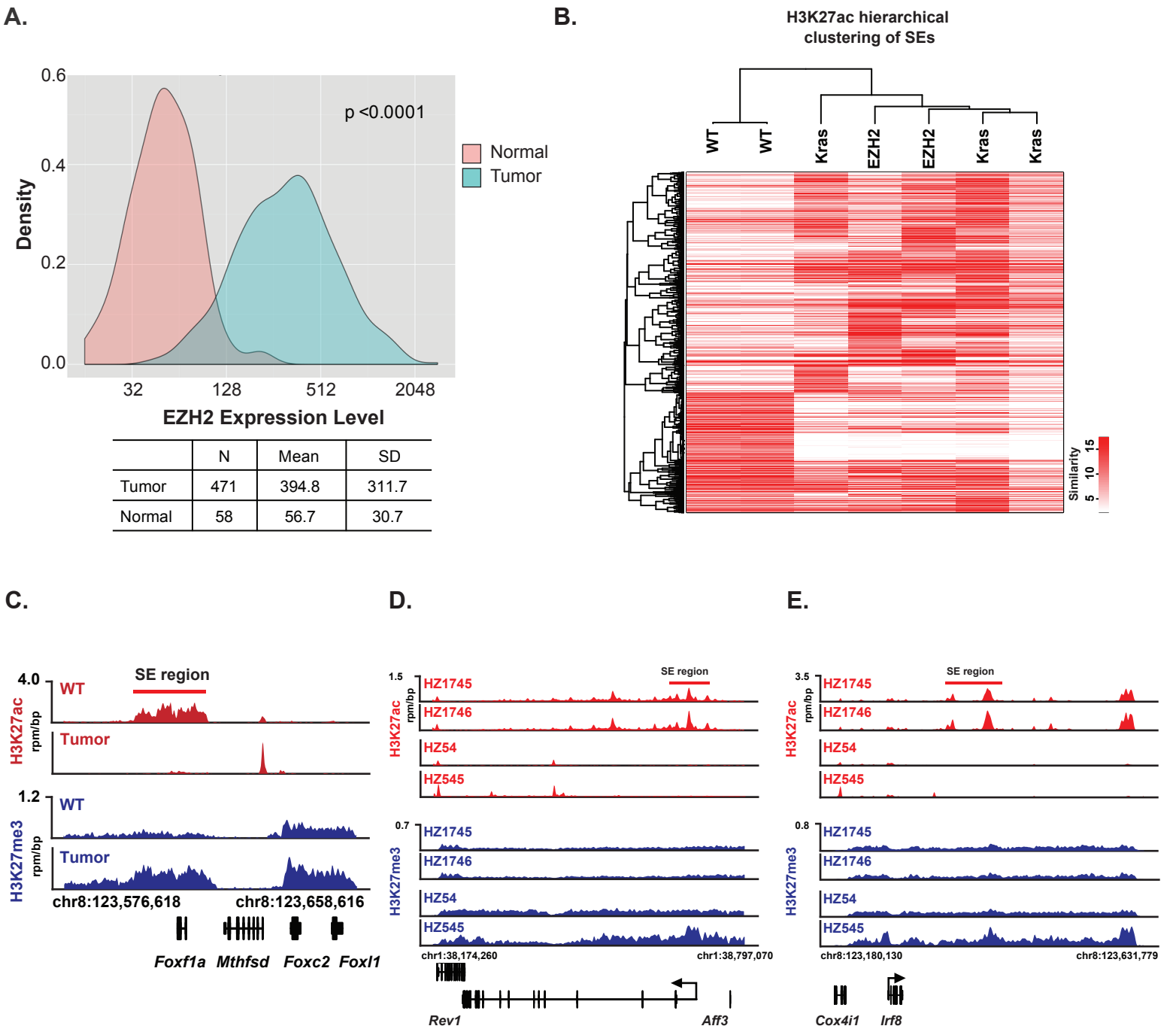
(A) Schematic for generating EZH2 transgenic mice by Frt-mediated homologous recombination. The targeting vector carrying EZH2 cDNA and pgkATGfrt was targeted to a modified site located ~500 bp downstream of the 3' untranslated region of the ColA1 locus by co-electroporation with FLPe transient expression vector. A LOX-STOP-LOX (LSL) cassette placed between the CAG promoter and EZH2 cDNA ensures the EZH2 transgene is only expressed in the presence of Cre-mediated excision of LSL cassette. E=EcoRI; P=PstI; S=SpeI

(B) Genotyping of mouse tail DNA with two different primer sets—P1 and P2, or P3 and P4. The target of the first primer set spans the integration site, ensuring a correct recombination.

(C) Lysates were prepared from lungs of wildtype (control), Actin-Cre:LSL-EZH2 and UBC:LSL-EZH2 mice. UBC:LSL-EZH2 mouse was treated with tamoxifen at 6 weeks of age and tissue was harvested two weeks later. Lysates were analyzed for EZH2 protein expression levels by Western blotting.

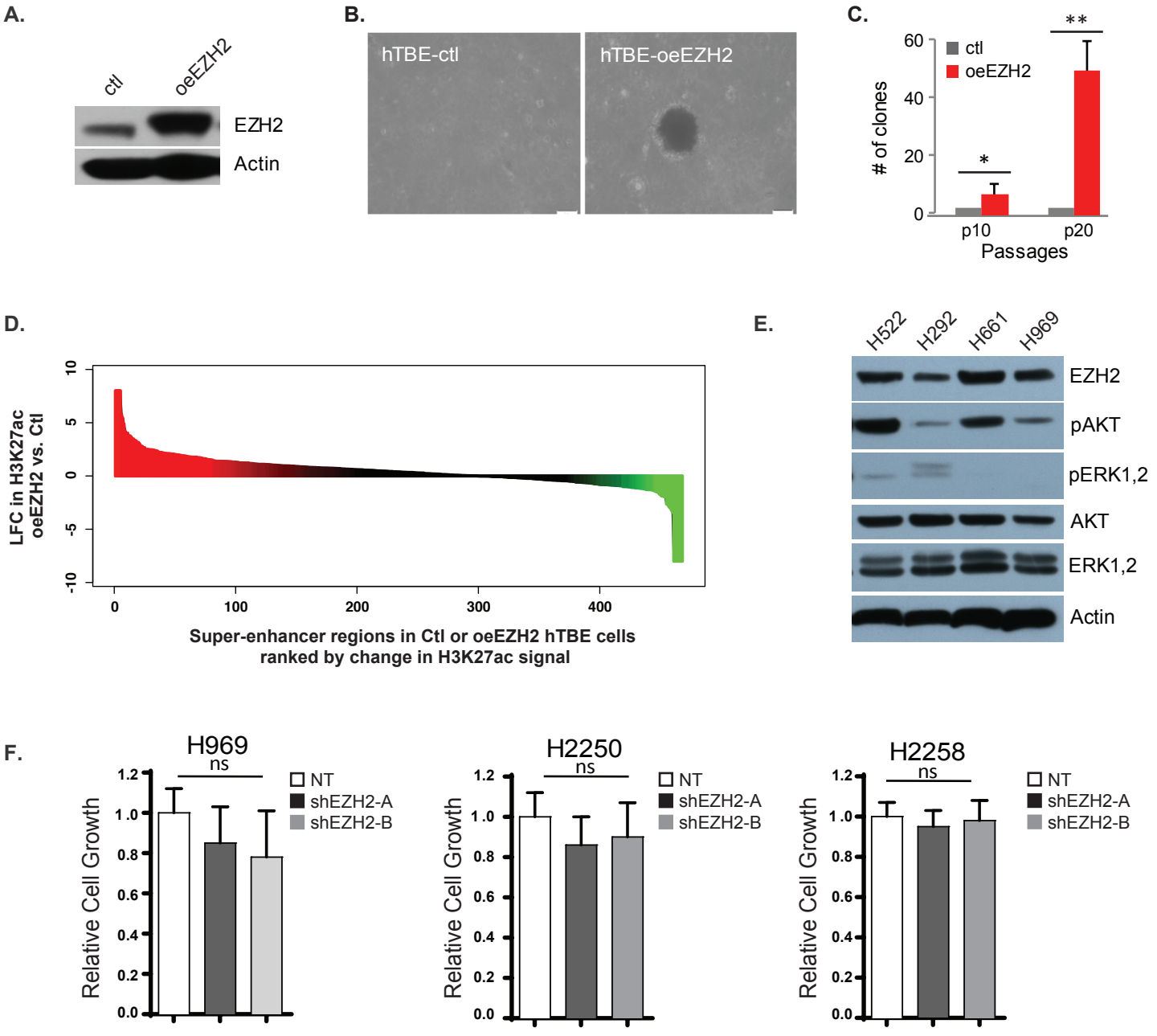
(D) Lungs from wildtype and Actin-Cre:LSL-EZH2 mice were sectioned and stained for EZH2 expression by immunohistochemistry. Scale bar represents 50 um.

(E) Western blots of EZH2, AKT, p-AKT, ERK1,2 and p-ERK1,2 expression in normal, EZH2-induced tumor, and KRAS-induced tumor lung tissues from mice.



	N	Mean	SD
Tumor	471	394.8	311.7
Normal	58	56.7	30.7

Supplementary Figure S2. H3K27ac super enhancer analysis in EZH2 mouse lung tumors.
(A) Distribution of EZH2 expression in 471 human lung adenocarcinomas (blue) versus 58 normal lungs (red) from The Cancer Genome Atlas (TCGA). Mean EZH2 expression level is 394.8 in human lung adenocarcinomas and 56.7 in normal lungs.
(B) Heatmap showing hierarchical clustering of H3K27ac super enhancer (SE) regions in murine wildtype (WT) and tumor lung tissues (EZH2- or KRAS-driven). Relative H3K27ac levels are indicated by intensity of color.
(C-E) Gene tracks of ChIP-Seq signal in units of rpm/bp for H3K27ac and H3K27me3 at the (C) Foxf1a, (D) Aff3 or (E) Irf8 loci locus in either mouse WT or EZH2-overexpressing tumor lung tissues.



Supplementary Figure S3. Human NSCLC cells with low levels of EZH2 are not sensitive to disruption of EZH2.

(A) Western blot analysis of human tracheobronchial epithelial (hTBE) cells expressing control (ctl) or EZH2 (oeEZH2) constructs.

(B) Colony forming assay for hTBE cells over-expressing control (left) or EZH2 (right) constructs.

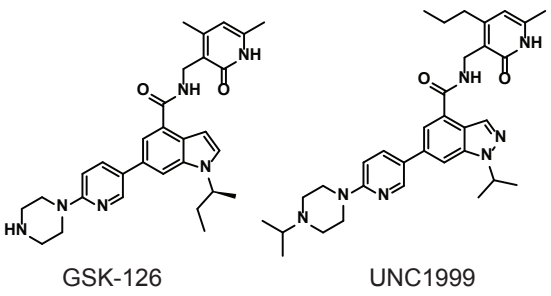
(C) hTBE cells expressing control (ctl) or EZH2 (oeEZH2) were cultured for 10 or 20 passages in vitro before seeding on soft agar to perform a colony formation assay. Error bars represent SEM, * $p<0.05$, ** $p<0.001$.

D) Waterfall plot showing rank-ordered change in H3K27ac signal at super enhancer-containing regions between hTBE cells over-expressing control or EZH2 constructs. Y-axis depicts the LFC in H3K27ac signal. Enhancers are ranked by LFC in signal with regions gaining the most H3K27ac in tumor at the left. Change in H3K27ac levels at super enhancers is colored by intensity of change from green to red.

(E) Western blot showing p-AKT, pERK1,2, total AKT and ERK1,2 in the NSCLC cell lines.

(F) Relative cell growth of H969, H2250 and H2258 cells expressing non-targeting control shRNA (NT) or two different shRNAs targeting EZH2 (shEZH2-A and shEZH2-B) was measured by MTS assay. Error bars represent SEM, n= 3.

A.

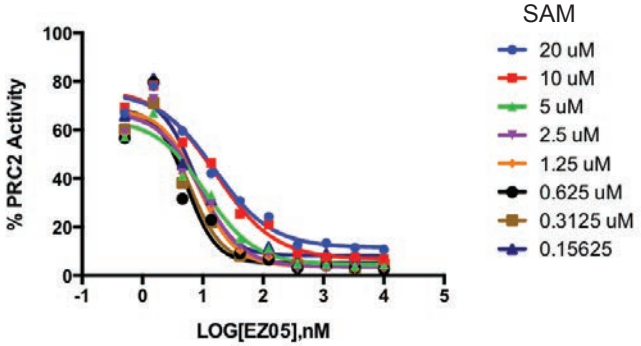


GSK-126 UNC1999

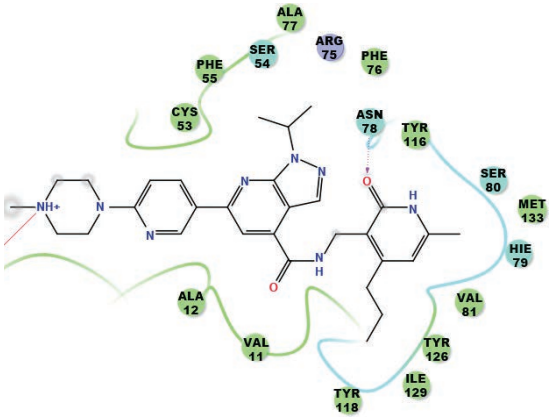
B.

Compound	IC50 (nM) [95% CI]
JQEZ5	11.1 ± 2.9
JQEZ23	> 10,000
GSK-126	7.2 ± 2.5
UNC1999	6.4 ± 1.5

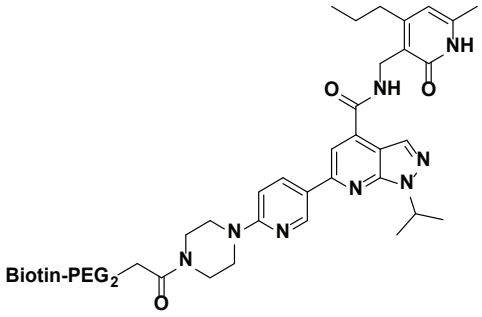
C.



D.



E.

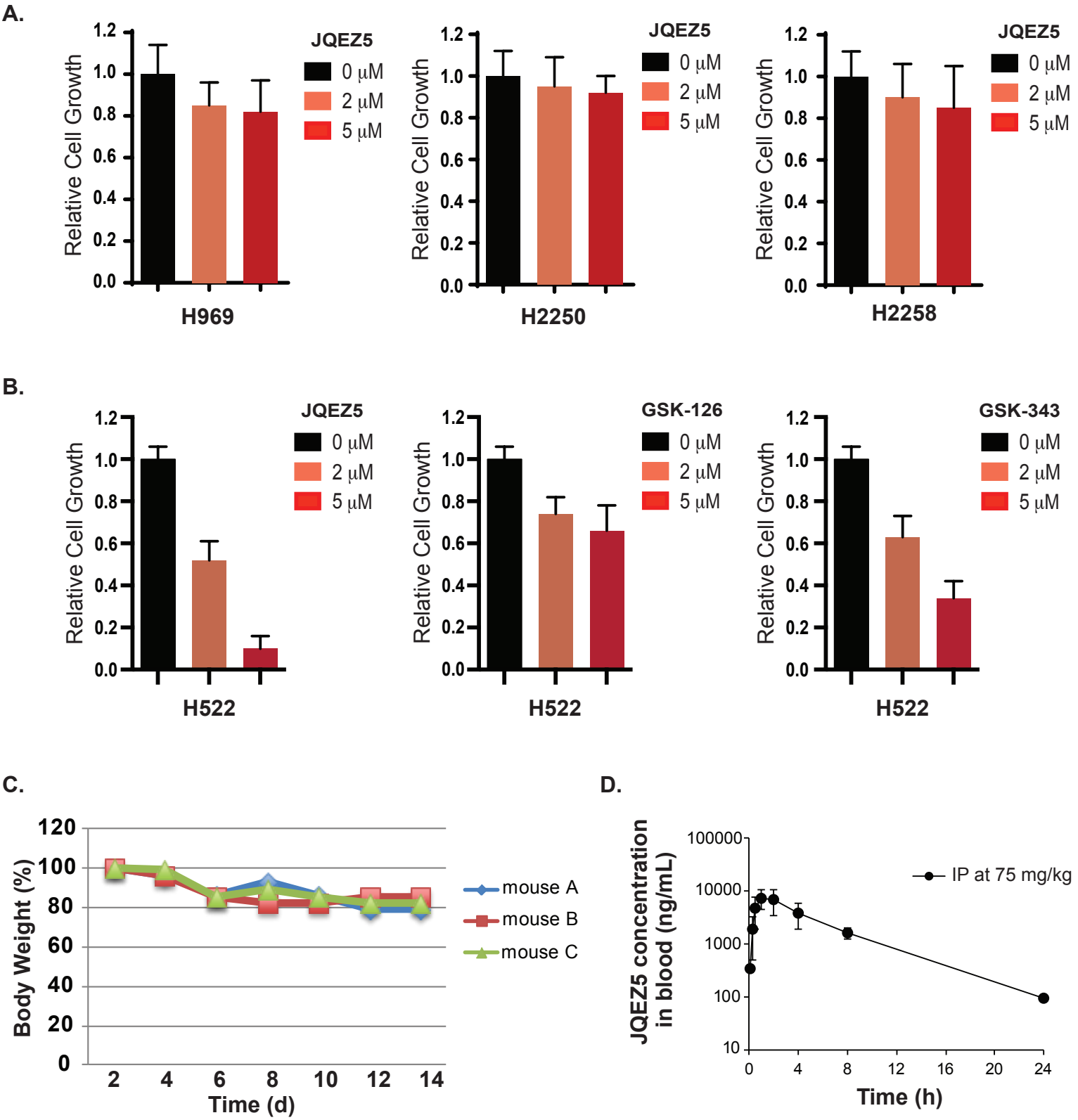


JQEZ6

F.

Methyltransferase:	JQEZ5 IC50 (M)	SAH IC50 (M)
DOT1	8.32E-06	1.41E-07
EZH1	1.30E-06	2.15E-05
EZH2	1.72E-07	1.52E-05
G9a	> 1.0E-05	6.67E-06
GLP	> 1.0E-05	3.19E-07
MLL1	> 1.0E-05	3.28E-06
MLL2	> 1.0E-05	1.79E-05
MLL3	> 1.0E-05	4.27E-05
MLL4	> 1.0E-05	1.20E-05
NSD2	> 1.0E-05	7.49E-06
PRMT1	> 1.0E-05	7.23E-07
PRMT3	> 1.0E-05	1.77E-06
PRMT4	> 1.0E-05	2.16E-07
PRMT5	> 1.0E-05	1.91E-07
PRMT6	> 1.0E-05	2.52E-07
SET1b	> 1.0E-05	6.69E-06
SET7/9	> 1.0E-05	1.21E-04
SET8	> 1.0E-05	1.40E-04
SETMAR	> 1.0E-05	5.61E-07
SMYD2	> 1.0E-05	8.24E-07
SUV39H1	> 1.0E-05	1.06E-04
SUV39H2	> 1.0E-05	2.11E-05

Supplementary Figure S4. Development and characterization of JQEZ5.
(A) Chemical structure of reported EZH2 inhibitors, GSK-126 and UNC1999.
(B) IC50 values of JQEZ5, the negative control compound, JQEZ23, and literature reported EZH2 inhibitors as determined in a radiometric Scintillation Proximity Assay (SPA) used to measure PRC2 activity.
(C) Structure of biotinylated JQEZ5 derivative, JQEZ6.
(D) JQEZ5 inhibitory activity against PRC2 was measured with increasing concentrations of S-adenosyl methionine (SAM) by SPA assay.
(E) Ligand interaction diagram (LID) depicts how JQEZ5 interacts with EZH2 residues.
(F) JQEZ5 activity was assayed against a panel of 22 methyltransferases. IC50 values for JQEZ5 and the control compound, S-adenosyl-homocysteine (SAH), are listed. The selectivity profiling with a methyltransferase panel showed that JQEZ5 selectively binds EZH2 in a panel of 22 methyltransferase assays.



Supplementary Figure S5. Cellular and in vivo characterization of JQEZ5.
(A) H969, H2250 and H2258 human lung cancer cells were incubated with increasing concentrations of JQEZ5 and relative cell growth was assessed by MTS assay. Error bars represent SD, n=3.
(B) H522 human lung cancer cells were incubated with increasing concentrations of JQEZ5, GSK-126 or GSK-343 and relative cell growth was assessed by crystal violet assay. Error bars represent SD, n=3.
(C) Male mice were treated with JQEZ5, 75 mg/kg IP daily, for 14 days and body weight was measured every other day, n=3.
(D) Mean JQEZ5 concentration in whole blood was measured over time following 75 mg/kg IP in male CD1 mice, n=3.

Supplementary Table S1. Summary of LSL-EZH2 mouse models.

Mouse Model LSL-EZH2	Lung Adenocarcinoma	Histiocytic Sarcoma (Liver)	Lymphoma	Mice with Tumors/ Total Mice
Actin-Cre	8	2	2	11/16[a]
UBC-Cre	4	2	1	5/10[b]
Adeno-Cre	5	1	0	5/12[a]

[a]One mouse had both lung adenocarcinoma and histiocytic sarcoma in the liver.
[b]Two mice had both lung adenocarcinoma and histiocytic sarcoma in the liver.

Fisher's exact test, $P>0.1$ for all comparisons.

Supplementary Table S2. Summary of lung adenocarcinoma in LSL-EZH2 mouse models.

Mouse Model LSL-EZH2	Lung Adenocarcinoma	Penetrance (n)	Avg Latency (wks±SEM)	Range (wks)
Actin-Cre	8	50% (16)	69.9±5.6	51.4-105.4
UBC-Cre	4	40% (10)	84.8±10.1	70.7-114.9
Adeno-Cre	5	42% (12)	88.2±14.6	53.0-122.9