Loss of SUV420H2 promotes EGFR inhibitor resistance in NSCLC through upregulation of MET via LINC01510

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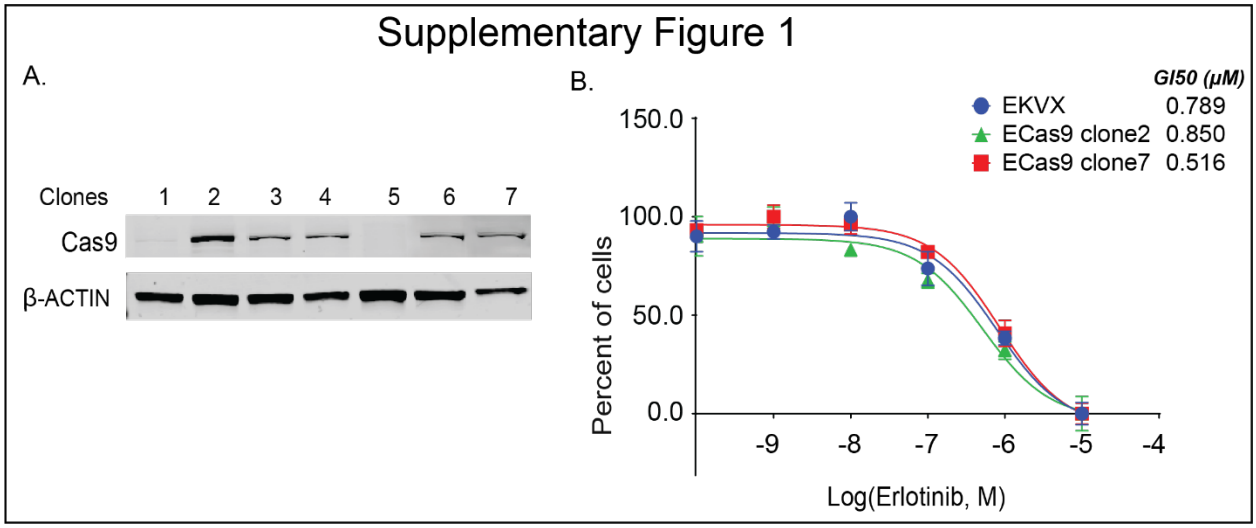
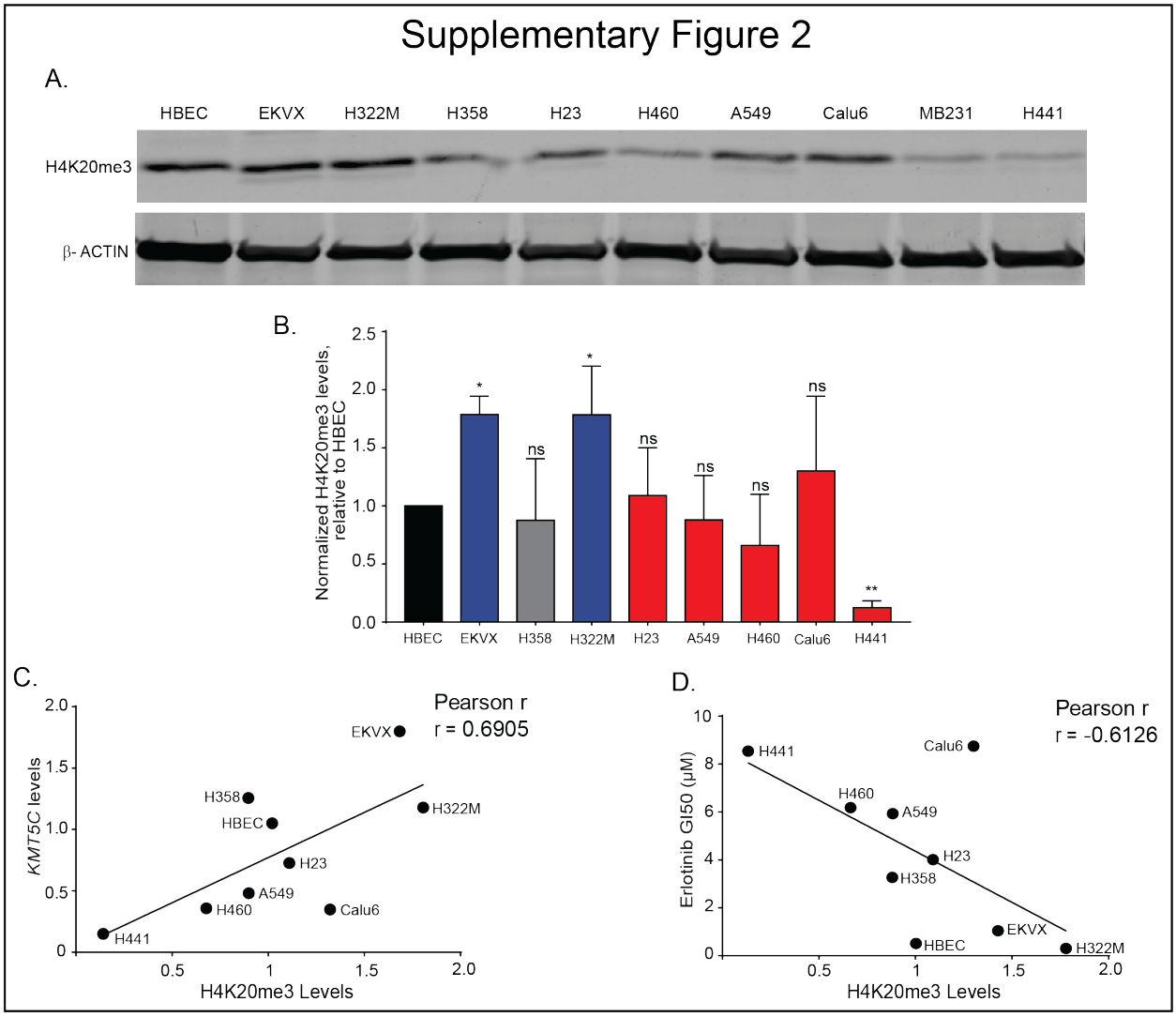
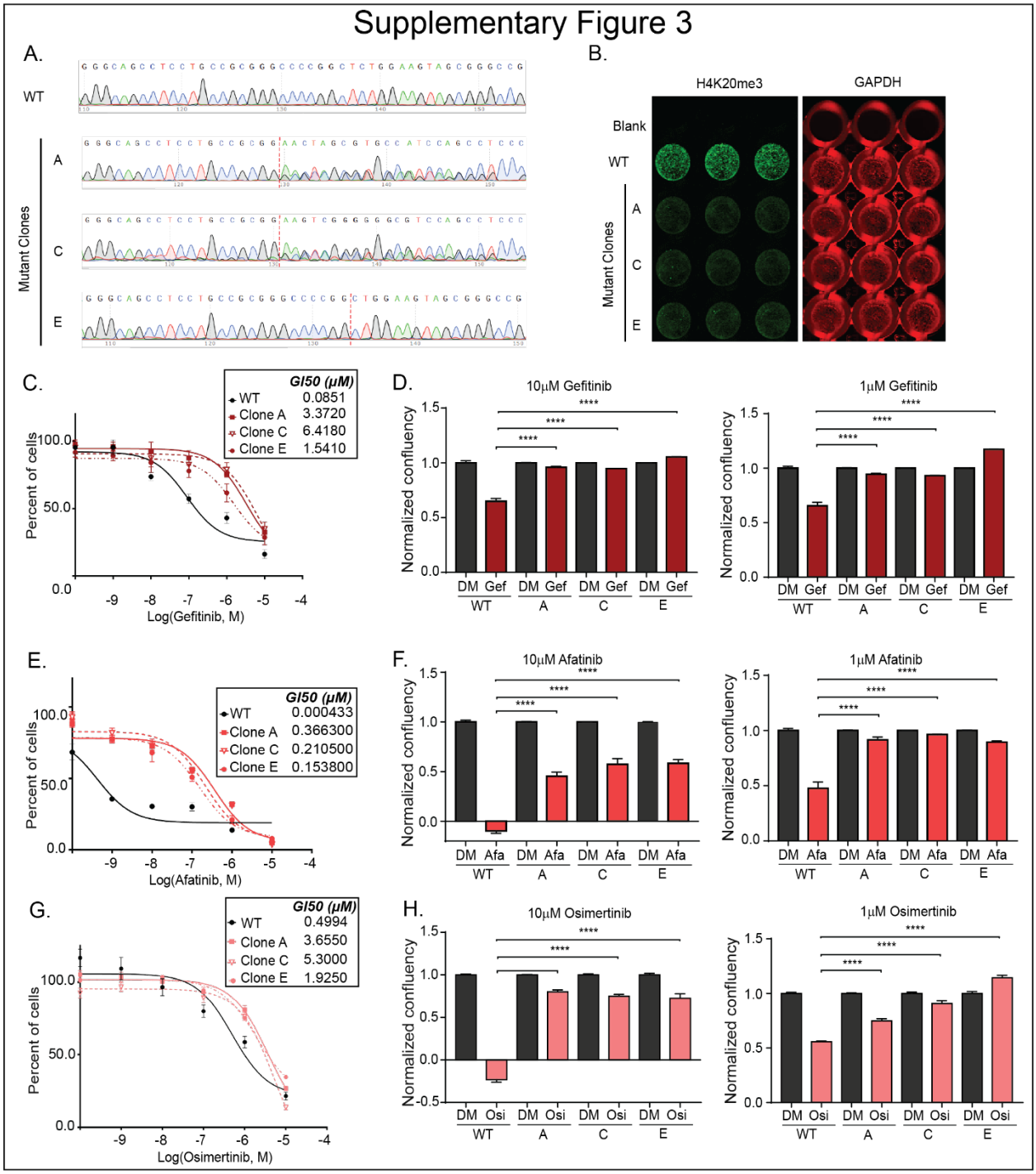
Supplemental Information:



Supplementary Figure 1: Characterization of Cas9 expressing EKVX clones. A) Western Blot analysis of Cas9 levels in EKVX clones stably expressing Cas9. β-ACTIN was used as a loading control. B) Parental EKVX cells, ECas9 clone 2, and ECas9 clone 7 were exposed to varying concentrations of erlotinib or the highest equivalent volume of dimethyl sulfoxide (DMSO, negative control) containing media for 72 hours. Erlotinib dose response was evaluated using the SRB assay. Post-normalization, the GI50 concentration of erlotinib was calculated from the respective dose curve for each cell line.

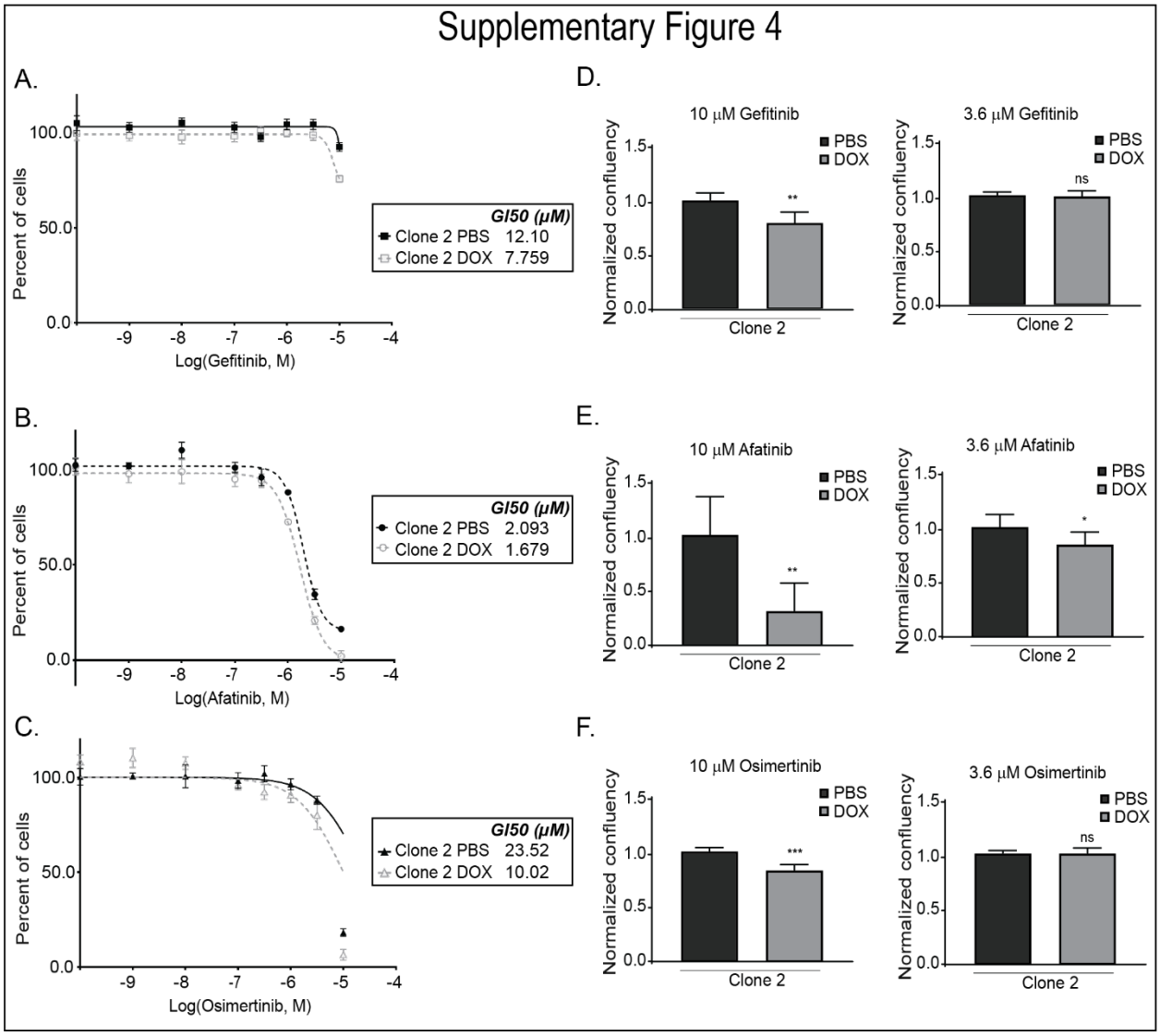


Supplementary Figure 2: Reduced H4K20me3 correlates with erlotinib resistance in NSCLC cells. A) Representative western blot of H4K20me3 in a panel of NSCLC cells. β-ACTIN was used as a loading control. MB231, a breast cancer cell line was included as a control cell line reported to have low levels of SUV420H2 (Shinchi et al., 2015). B) Protein quantification of H4K20me3 levels relative to β-ACTIN normalized to HBEC (n=4). One-way ANOVA followed by Dunnett's Multiple Comparison test was used to evaluate statistical significance of H4K20me3 relative to HBEC cells. C) Correlation analysis between quantified H4K20me3 levels from panel B and GI50 erlotinib values from Figure 2B. D) Correlation analysis between quantified H4K20me3 levels from panel B and *KMT5C* from Figure 2A. Evaluations in C and D were conducted using the Pearson correlation test.

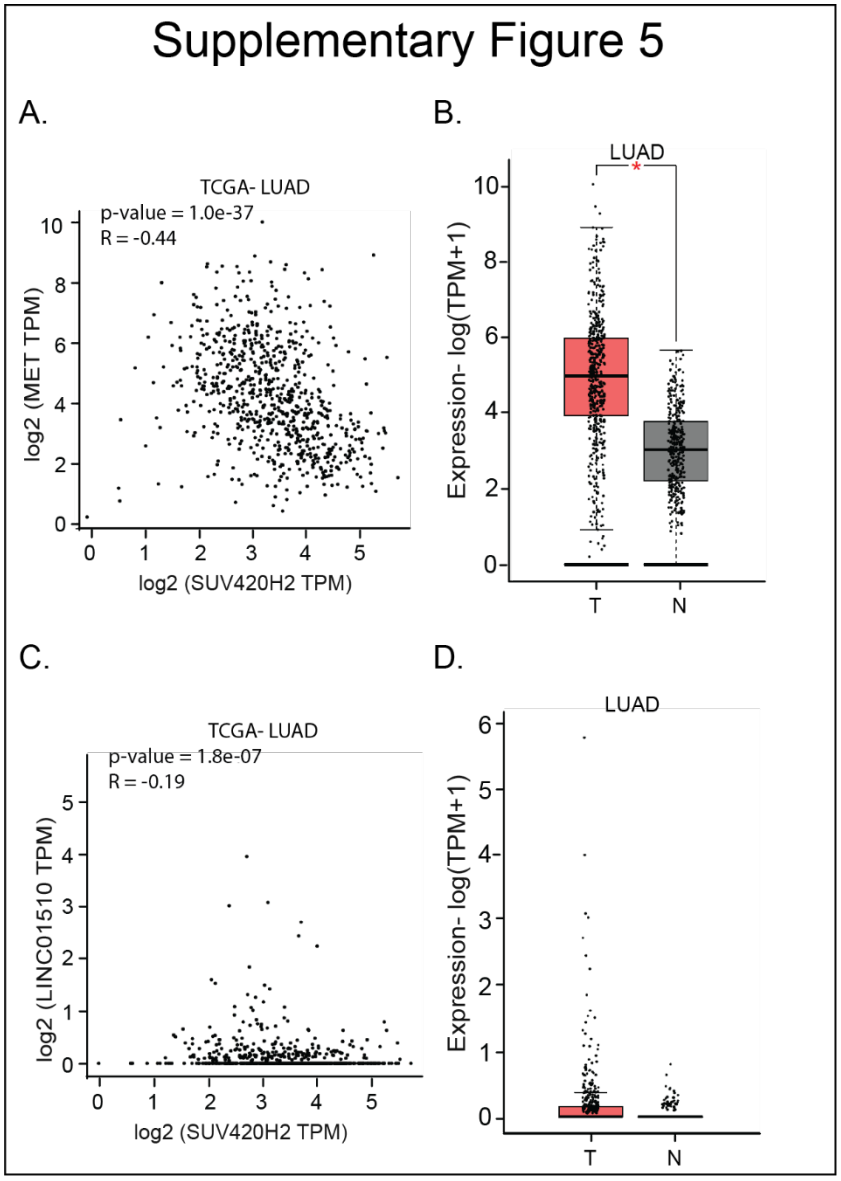


Supplementary Figure 3: SUV420H2 knock-out confers resistance to various EGFRi. A) Genomic DNA of WT cells or mutant clones A, C, E was isolated, the region targeted by CRISPR-Cas9 sgRNA targeting SUV420H2 was PCR amplified, purified and sequenced. Representative chromatograms of the wildtype SUV420H2 (WT) cells, and the specific mutations identified in mutant clones A, C, E. B) In-cell western of H4K20me3 levels in WT cells and mutant clones A, C, E. GAPDH serves as an endogenous control. C) Gefitinib, E) Afatinib, or G) Osimertinib dose response curves. Cells were exposed to the indicated concentration of drug or to the highest equivalent volume of vehicle control containing media for 72 hours. Following normalization, the

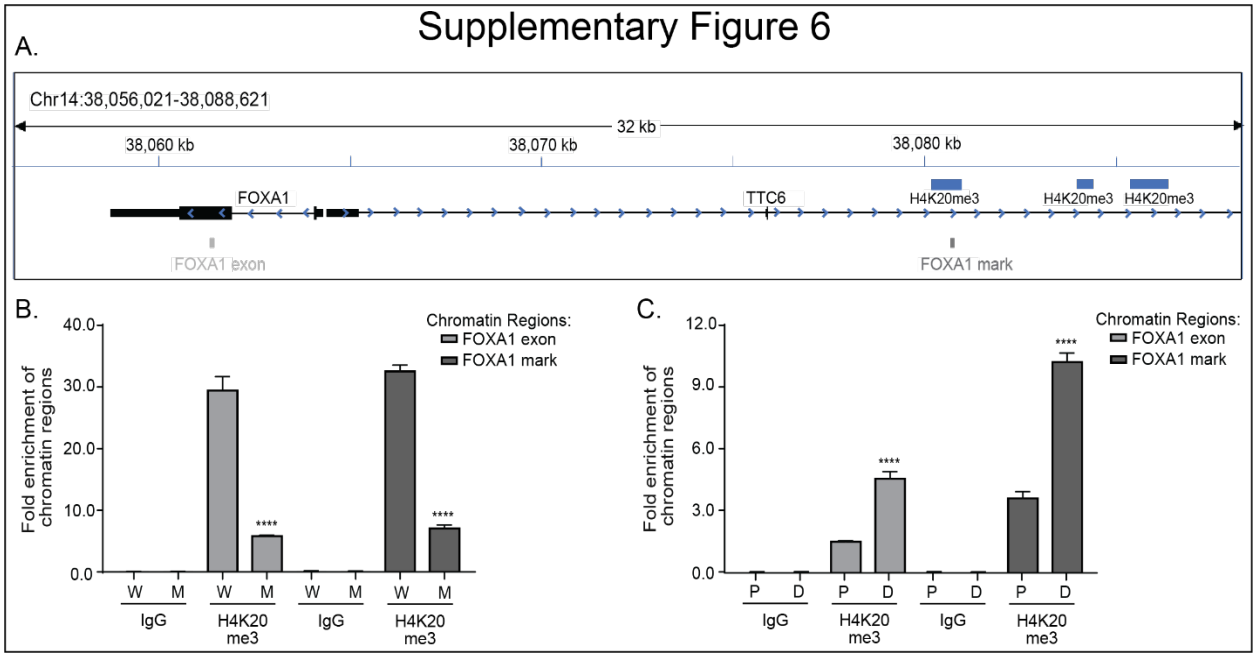
GI50 concentration of each inhibitor was calculated from the respective dose curve for each cell line. Proliferation of WT cells or mutant clones A, C, E was evaluated using the Incucyte. Cells were exposed to varying concentrations of D) Gefitinib (Gef) F) Afatinib (Afa) or H) Osimertinib (Osi) or the highest equivalent volume of DMSO (DM) containing media for 72 hours. Data relative to respective normalized DMSO control treatments is represented. One-way ANOVA followed by Dunnett's Multiple Comparison test was utilized to evaluate statistical significance of normalized confluency of clones A, C, E in the presence of 10 or 1μM of gefitinib, afatinib or osimertinib compared to WT cells.



Supplementary Figure 4: Ectopic expression of SUV420H2 partially sensitizes resistant cells to additional EGFRi. Dose response measured by SRB was evaluated after a two week exposure to PBS or DOX containing media for Calu6 or clones 1, 2 for A) gefitinib , B) afatininb, C) osimertinib. Erlotinib treatments were for 72 hours. Following normalization, the GI50 concentration of erlotinib was calculated from the respective dose curve for each cell line. Proliferation of clone 2 was evaluated using the Incucyte. Cells grown in PBS or DOX containing media for two weeks, were exposed to varying concentrations of D) gefitinib, E) afatinib, F) osimertinib, or the highest equivalent volume of DMSO containing media for 72 hours. Unpaired t-test was used to evaluate statistical significance of normalized confluency of DOX-cultured clone 2 cells in the presence of either 10 or 3.6 μM of gefitinib, afatinib, or osimertinib compared to respective normalized confluency of PBS-treated cells.



Supplementary Figure 5: LINC01510 correlates poorly with LUAD prognosis. Correlation analysis between A) *MET* and *KMT5C* and C) *LINC01510* and *KMT5C* transcript levels in TCGA-LUAD dataset, evaluated using GEPIA.GEPIA analysis for B) *MET* and D) *LINC01510* transcript levels in normal (N, n = 347) and tumor samples (T, n = 483) form LUAD data obtained from TCGA and GTEx databases. The majority of the samples in the normal subgroup had undetectable levels of *LINC01510*. TPM= Transcripts per million



Supplementary Figure 6: H4K20me3 is enriched at the FOXA1 locus in an SUV420H2 dependent manner. A) ChIP-qPCR primers designed to evaluate enrichment of H4K20me3 at the FOXA1 exonic region (FOXA1 exon), and at the predicted H4K20me3 modification upstream of the FOXA1 promoter region (FOXA1 mark). ChIP was performed using either IgG or H4K20me3 primary antibodies on chromatin isolated from B) WT or SUV420H2 mutant clone C or C) inducible SUV420H2 cells (in the presence of DOX or PBS). qPCR using the immunoprecipitated chromatin was conducted using primers shown in A (Table 3). Data are represented as fold enrichment of the chromatin region pulled-down by the H4K20me3 primary antibody relative to IgG and was evaluated for significance using one-way ANOVA. W = WT cells, M = SUV420H2 mutant clone C cells, P = Calu6 clones grown in PBS containing media, D = Calu6 clones grown in DOX containing media.

Supplementary Table 1: Candidate genes identified from the CRISPR-Cas9 knock out screen. Thirty-five significant hits identified by MAGeCK-VISPR analysis and β-score, p-value, and false discovery rate (FDR)

Target	β-score	p-value	FDR
SUV420H2	97	8.30E-05	0.07
ADSS	91	0.00021	0.07
OPA3	89	0.00028	0.07
LEPREL4	88	0.00032	0.07
GAREM	86	0.00049	0.07
ISG15	83	0.00065	0.07
PROM2	83	0.00065	0.07
hsa-mir-602	77	0.00082	0.07
CCDC130	81	0.00088	0.07
PCSK2	80	0.00091	0.07
FAM120AOS	79	0.001	0.07
CCL23	79	0.0011	0.07
TNFSF12	76	0.0028	0.07
hsa-mir-27b	74	0.0081	0.11
SMN2	25	0.012	0.16
OR6V1	74	0.012	0.16
SYBU	72	0.012	0.17
CASP8	73	0.012	0.17
LDLRAP1	71	0.013	0.17
PFDN2	70	0.013	0.17
CPA3	68	0.013	0.17
PP2D1	68	0.013	0.17
TMEM234	68	0.013	0.17
TMEM147	67	0.013	0.17
hsa-mir-5699	62	0.016	0.21
hsa-mir-512-1	50	0.016	0.21

MLL2	22	0.016	0.21
hsa-mir-648	43	0.016	0.21
AGAP9	22	0.016	0.21
hsa-mir-4669	43	0.016	0.21
RPL41	38	0.016	0.21
hsa-mir-3183	37	0.016	0.21
hsa-mir-1268a	34	0.017	0.22
hsa-mir-147b	34	0.017	0.22
hsa-mir-148a	27	0.018	0.24

Supplementary Table 2: Primer sequences used to conduct the CRISPR-Cas9 knock-out screen. Multiple PCR2 primers were used, each with an independent barcode that allows for sorting of sample-specific sgRNAs post sequencing.

PCR	Sample	Primer name	Primer direction	Primer sequence
PCR 1	All samples	1st PCR primer	Forward	TCTTTCCCTACACGACGCTCTTCCGATC TNNNNAATGGACTATCATATGCTTACC GTAACTTGAAAGTATTTCG
		1st PCR primer	Reverse	GTGACTGGAGTTCAGACGTGTGCTCTTC CGATCTNNNNGCACCGACTCGGTGCCA CTTTTTCAAGTTGATAACGGACTAGCC
PCR2	EKVX-Baseline 1	UDA5050	Forward	AATGATACGGCGACCACCGAGATCTAC ACTGACAATGTCACACTCTTTCCCTACA CGAC
		UDA7143	Reverse	CAAGCAGAAGACGGCATACGAGATAG AAGCCAATGTGACTGGAGTTCAGACGT G
	EKVX-Replicate 1	UDA5051	Forward	AATGATACGGCGACCACCGAGATCTAC ACCGACCTAACGACACTCTTTCCCTACA CGAC
		UDA7142	Reverse	CAAGCAGAAGACGGCATACGAGATGAC TCACTAAGTGACTGGAGTTCAGACGTG
	EKVX-Baseline 2	UDA5052	Forward	AATGATACGGCGACCACCGAGATCTAC ACTAGTTCGGTAACACTCTTTCCCTACA CGAC
		UDA7141	Reverse	CAAGCAGAAGACGGCATACGAGATAGT CTGTCGGGTGACTGGAGTTCAGACGTG
	EKVX-Replicate 2	UDA5053	Forward	AATGATACGGCGACCACCGAGATCTAC ACGCCGCACTCTACACTCTTTCCCTACA CGAC

		UDA7140	Reverse	CAAGCAGAAGACGGCATACGAGATGTATTCTCTAGTGACTGGAGTTCAGACGTG
	EKVX-Baseline 3	UDA5054	Forward	AATGATACGGCGACCACCGAGATCTACACATTATGTCTCACACTCTTTCCCTACACGAC
		UDA7139	Reverse	CAAGCAGAAGACGGCATACGAGATACGCCTCTCGGTGACTGGAGTTCAGACGTG
	EKVX-Replicate 3	UDA5055	Forward	AATGATACGGCGACCACCGAGATCTACACAGAACCGAGTACACTCTTTCCCTACACGAC
		UDA7138	Reverse	CAAGCAGAAGACGGCATACGAGATTAACCGCCGAGTGACTGGAGTTCAGACGTG

Supplementary Table 3: Primers utilized in the study. Designed and purchased from Integrated DNA Technologies.

Primer use		**Primer direction**	**Primer sequence**
pLV-sgSUV420H2		Forward	CACCGCGGCCCGCTACTTCCAGAGC
		Reverse	AAACGCTCTGGAAGTAGCGGGCCGC
pLVX-Tetone-SUV420H2		Forward	TCGTAAAGAATTCACCATGGGGCCCGACAGAGT GACAGCA
		Reverse	GAGATCTGGATCCTCAGTACAGCTCTTCACCGCC GAC
pLVX-Tetone-SUV420H2-puro		Forward	CCGCTACGCGTTCAGAAGAACT
		Reverse	AGCGGCGTACGATGATTGAACA
KMT5C genomic locus amplification		Forward	GAGCAGATGGGAGGTGCGGCGACAGT
		Reverse	GAGCTCAGAAGAAAGGAGACAGAT
KMT5C genomic locus sequencing		Forward	CCTCTCCTTAGCCTGGTCCT
		Reverse	CAAGGGCTAGGAAGTCAGGG
KMT5C quantification		Forward	TCGGTTTCCGCACCCATAAG
		Reverse	CGGAGGTAGCGATAGACGTG
ChIP-QPCR	FOXA1 mark	Forward	AAGGAGAGGTGCGTTGTTTG
		Reverse	CATTCTCCCACGAAAGGCAG
	FOXA1 exon	Forward	AAGACTCCAGCCTCCTCAAC
		Reverse	CGGGTGGTTGAAGGAGTAGT
	Linc01510 mark	Forward	GCTTCTTGTCCCTCCCAGAT
		Reverse	GCAGAAGTGAGAGGAAGGGT
	Up 1	Forward	CACACTGGAGTTCTTGCCAC
		Reverse	TATGCACTCCTTCACTGGGG
	Up 2	Forward	GCAGTCCAGCTAAGCAATCC
		Reverse	GACATCTTGGGAAGGGGACA
	Up 3	Forward	CCTCTTCACATCCCACAGGT

		Reverse	CTCTGCTGGCTTGATCATTG
	MET	Forward	GATCAAGGAAATGGGGCGTT
		Reverse	GGGACTAGGGCCTATTGTCA
	Down 1	Forward	CCCTGCCTCTCATCAACTGA
		Reverse	GTTGAGCCACTAAACCACCC
	Down 2	Forward	TGCCTGGTCTCCTGTTAACA
		Reverse	ATCTGTCTTCTCCCTGTGCC
	Down 3	Forward	AGTCCAAGATCAAGGCACCA
		Reverse	AGGCCTTTCTTGTACCCCTT