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SUPPLEMENTARY INFORMATION

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Mutations in the SWI/SNF complex induce a targetable dependence on oxidative phosphorylation in lung cancer

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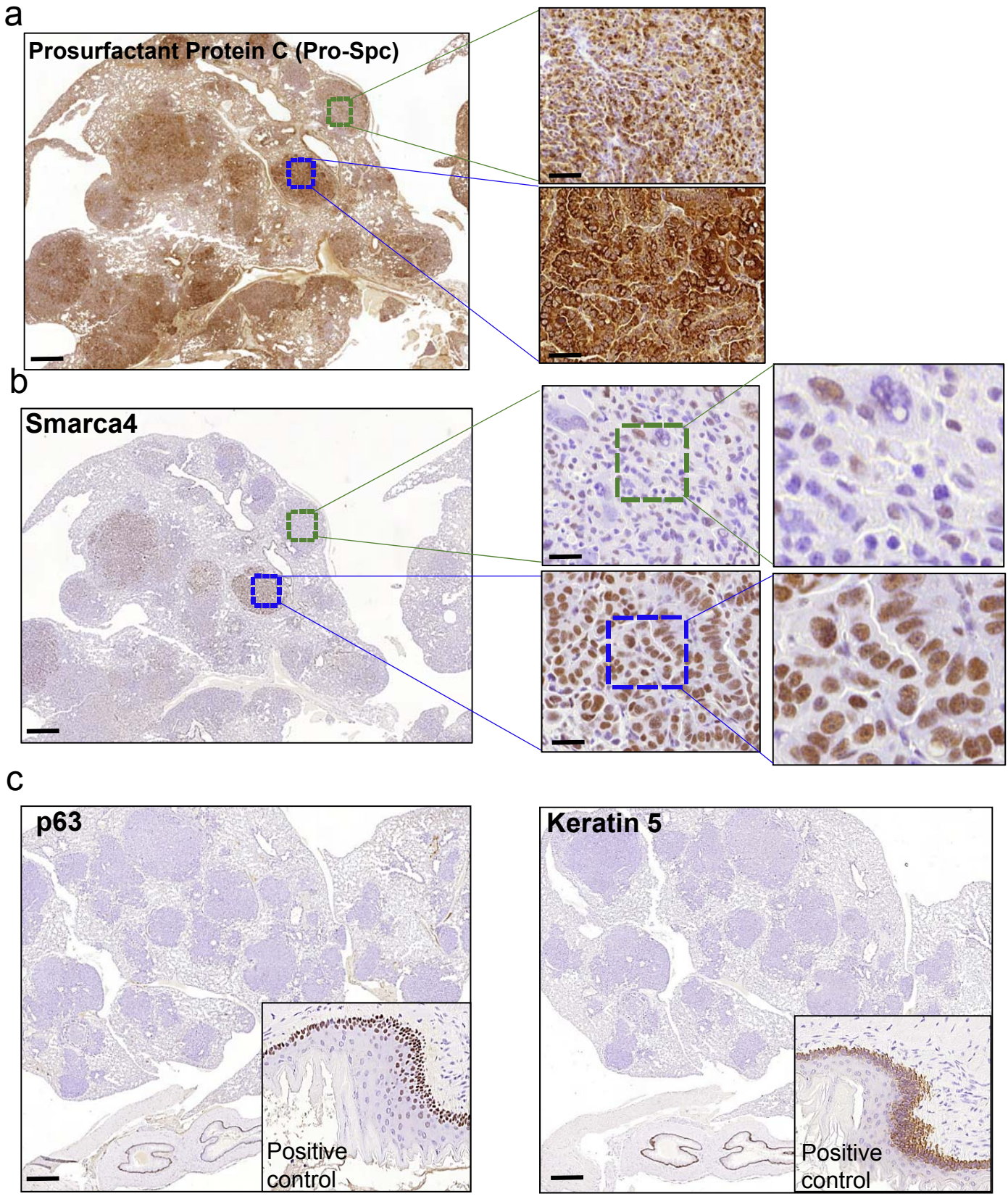
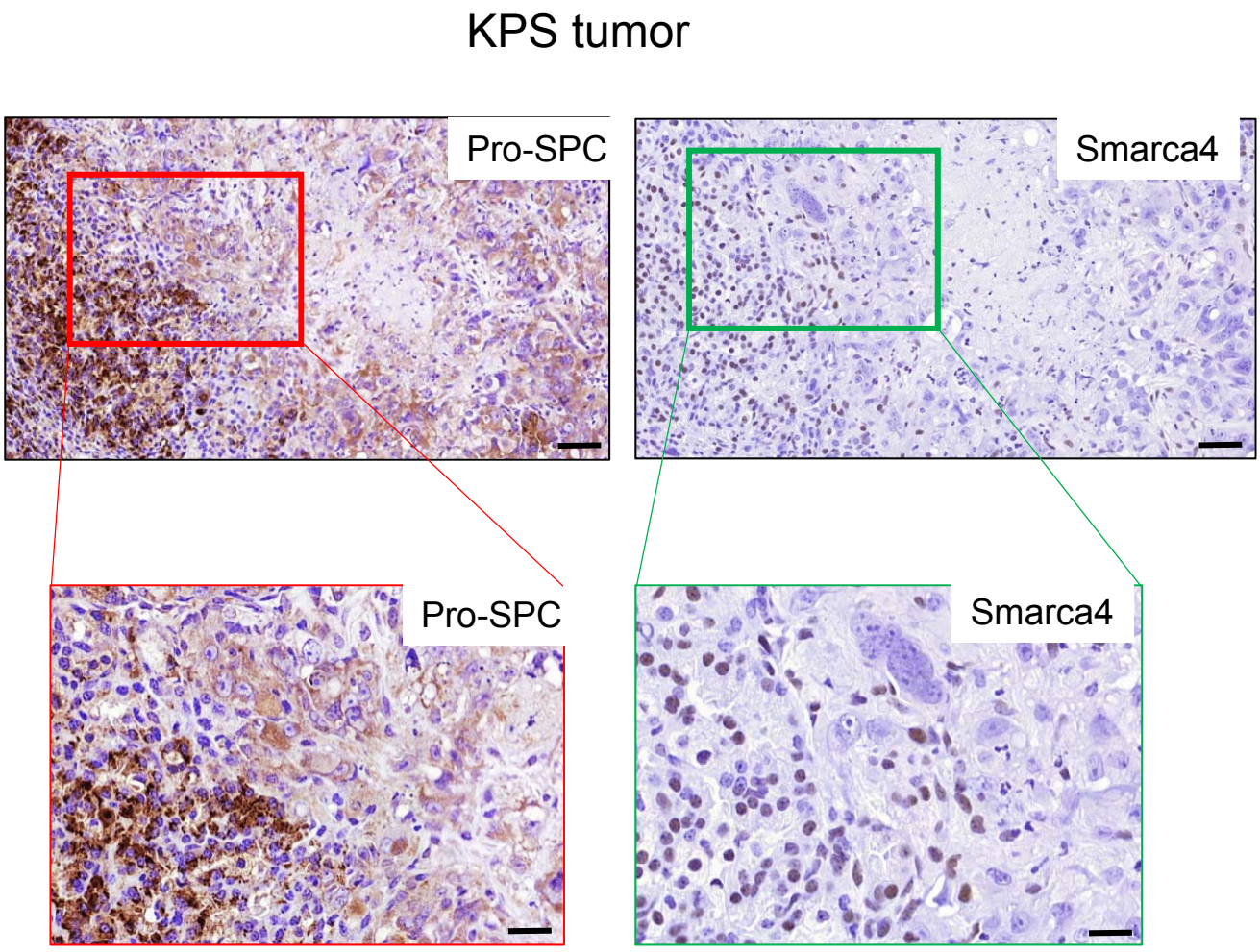


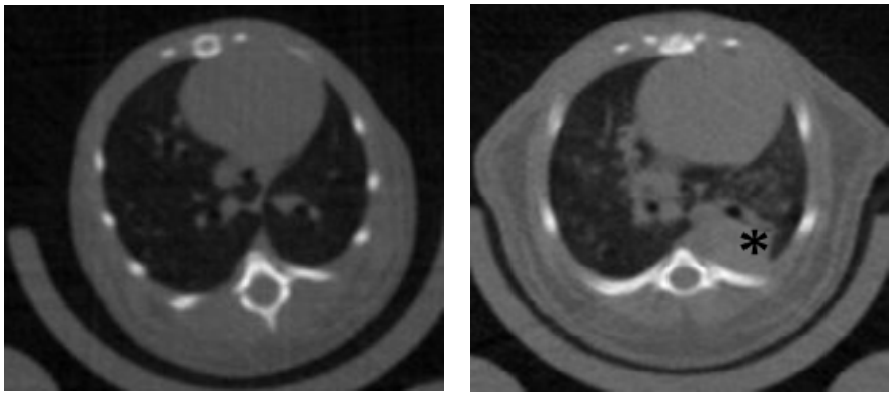
Supplementary Figure 1. Histopathological characterization of GEMM tumors indicates classic features of adenocarcinoma and heterogeneity (continued on next page).

d



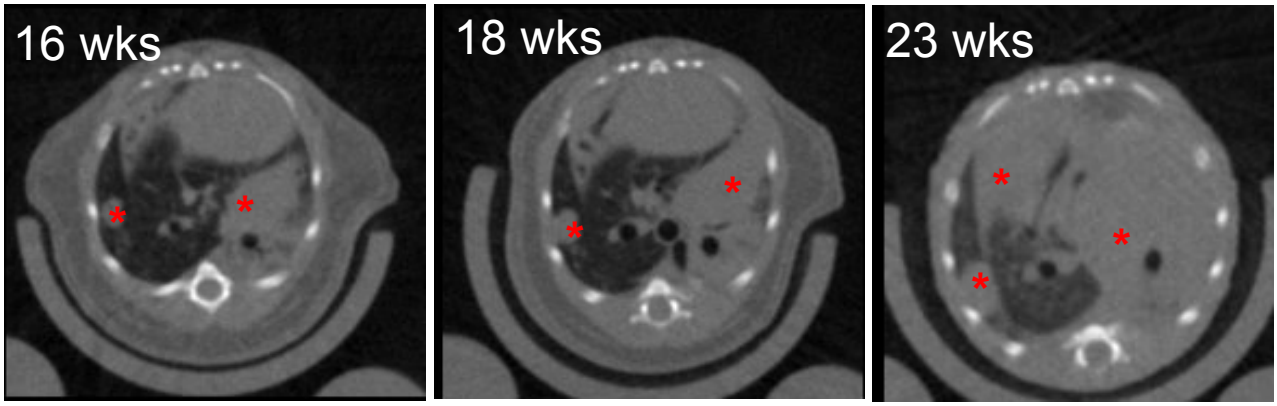
Supplementary Figure 1. Histopathological characterization of GEMM tumors indicates classic features of adenocarcinoma (continued from previous page).
a, Immunohistochemistry of a representative KPS GEMM tumor showing multifocal, pleomorphic tumors spread across most lobes of the lung. Formalin fixed paraffin embedded tissue was stained using antibody against pro-surfactant protein c (pro-SPC). Insets shows close up of two separate tumors showing strong (blue box) and modest (green box) staining for pro-SPC. Scale bar represents 500μM in primary image and 30μM in inset. Experiments repeated four times. **b**, Immunohistochemistry of a representative KPS tumor stained with Smarca4 antibody. Insets show close up of two independent tumors showing absence (blue box) and presence (green box) of nuclear Smarca4 indicating the highly heterogeneous nature of tumors partly caused by inefficient Cre mediated recombination of Smarca4 floxed allele. Scale bar represents 500μM in primary image and 30μM in inset. Experiments repeated four times. **c**, Immunohistochemistry of a KPS tumor stained with p63 antibody (left panel) and Keratin 5 antibody (right panel) shows no immunoreactivity. Inset shows positive signal on the same slide from adjacent esophageal squamous epithelial cells. Scale bar represents 500μM in primary image and 30μM in inset. Experiments repeated three times. **d**, Immunohistochemistry of a KPS tumor stained with Pro-surfactant protein c (pro-SPC) and Smarca4 antibody. Scale bar represents 60μM in primary image and 30μM in inset. Experiments repeated three times on independent tumors.

a



Smarca4$^{L/L}$ *Kras*$^{LSLG12D/+}$ *p53*$^{L/L}$ **(KP)**

b



Kras$^{LSLG12D/+}$, *p53*$^{L/L}$ *Smarca4*$^{L/L}$

(KPS)

Supplementary Figure 2. MicroCT imaging of GEMM lung tumors.
a, MicroCT imaging was done on mice 16 weeks after administration of AdenoCre virus. Images show thoracic scan of *Smarca4*$^{fl/fl}$ and *Kras*$^{LSL\text{-}G12D/+}$, *p53*$^{fl/fl}$ (referred to as KP) demonstrating presence of solid tumor (asterix) only in the lungs of KP mice. **b,** Serial microCT imaging was done on mice 16, 18 and 23 weeks after administration of AdenoCre virus. Images show thoracic scan of *Kras*$^{LSL\text{-}G12D/+}$, *p53*$^{fl/fl}$, *Smarca4*$^{fl/fl}$ (referred to as KPS) mice demonstrating presence of several rapidly growing solid tumors (red asterisks) in the lungs. Imaging was done on n=5 mice per cohort.

a

Pathway enrichment FDR *q-value*=0.0024

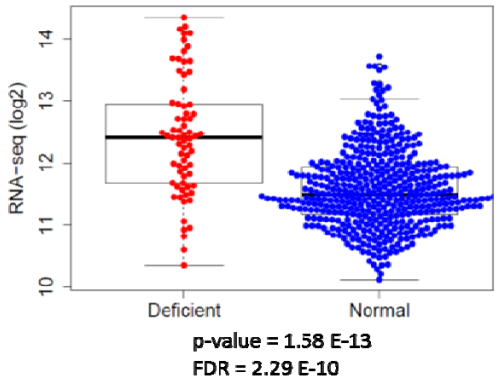
OXPHOS Genes Enriched in KPS tumors			
FDX1	ALAS1	COX7A2	MRPL15
PHYH	NDUFB3	HSPA9	COX6C
MGST3	ALDH6A1	ATP5G3	MTRR
POR	NDUFV2	NDUFS4	NDUFA9
MRPL11	MTRF1	ETFA	GOT2
PDP1	PRDX3	HCCS	VDAC3
TIMM10	MRPS22	ACADM	VDAC2
ATP5L	MRPS12	UQCRFS1	ATP6V1E1
NDUFB4	TIMM9	DLAT	SUCLA2
POLR2F	MRPL35	LDHA	TOMM70A
COX8A	SDHC	CYB5R3	MRPS11
COX5B	UQCRB	MTX2	IMMT
ECHS1	MRPL34	CYCS	NDUFA3
NDUFB8	SDHD	GPX4	TIMM50
NDUFA5	ATP6V1H	VDAC1	AFG3L2
UQCR10	ACAT1	MDH1	NDUFS3
BDH2	COX7C	NDUFA1	COX15
TIMM8B	COX11	IDH3A	UQCR11
NDUFA7	FXN	NDUFA8	NDUFV1
NDUFA6	TOMM22	COX7A2L	ATP5C1
NDUFC1	ATP6V1D	COX6A1	UQCRC2
ATP5G2	ACADSB	ATP5J	TIMM13
ATP1B1	ISCU	UQCRH	NQO2
NDUFS6	BAX	ATP5F1	LDHB
TIMM17A	ATP5E	ATP6V1G1	ATP5J2
NDUFB6	NDUFC2	NDUFB5	ACAA2
COX5A	MRPS30	UQCRQ	GRPEL1
NDUFA2	COX7B	ATP6V1F	
NDUFA4	NDUFS8	COX6B1	

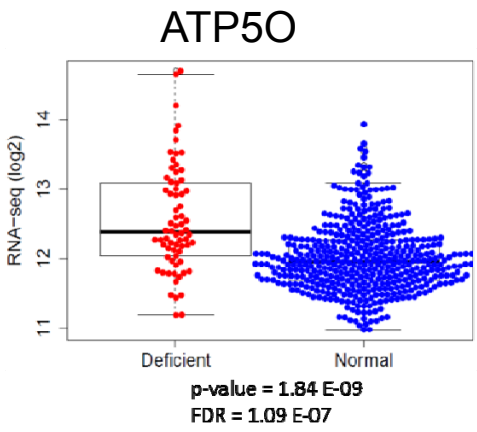
b

Pathway enrichment FDR *q-value*=0

OXPHOS Genes Enriched in SWI/SNF mutant human lung adenocarcinoma			
MRPS15	NDUFB4	LDHA	LRPPRC
COX7A2	VDAC2	COX15	FH
COX7A2L	PDHX	NDUFC2	NDUFA5
ATP5F1	COX5A	ATP5J2	NDUFA8
TIMM8B	ATP5C1	FDX1	DLD
ATP5L	IDH1	ACAA2	TCIRG1
UQCRH	COX7B	HCCS	NDUFB6
ATP5G3	ATP5G1	CYC1	ACADM
PRDX3	COX6A1	DLAT	COX11
MDH1	ATP6V1E1	NDUFV2	HSD17B10
MRPS22	VDAC3	MRPS30	UQCRQ
SDHD	NDUFAB1	SUCLA2	UQCRC1
TOMM22	COX5B	TIMM17A	ECHS1
ATP5J	HADHB	COX6B1	ATP5A1
NDUFB3	NDUFC1	NDUFA4	ATP6AP1
MRPL35	ETFA	GRPEL1	UQCRB
ATP5G2	ATP5H	UQCRC2	ISCA1
NDUFB5	SDHC	RHOT2	COX7C
SUCLG1	NDUFA9	NDUFA1	ATP5E
MGST3	ATP5I	COX17	TIMM10
MRPL15	ATP5B	DECR1	GLUD1
MRPS11	MRPS12	COX4I1	SLC25A11
SDHB	NDUFS3	OGDH	MAOB
NDUFB8	NDUFS1	POLR2F	CYB5R3
MRPL11	TOMM70A	TIMM50	MRPL34
ATP5O	CYCS	COX6C	COX8A
NDUFA6	UQCR10	NDUFA3	SLC25A5
NDUFB1	PHB2	NDUFS4	NDUFB2
UQCRFS1	PDHB	HTRA2	
TIMM9	MTX2	MTRF1	

c





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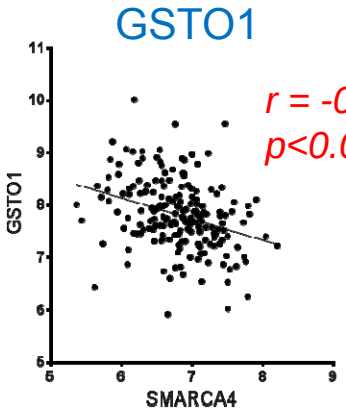
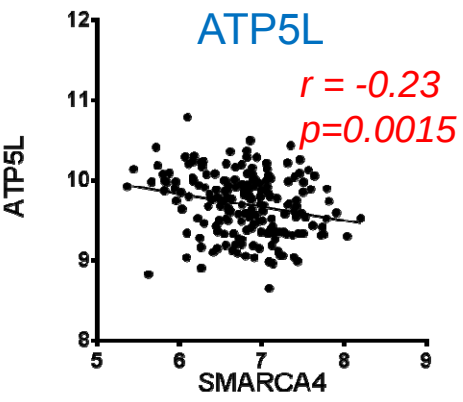
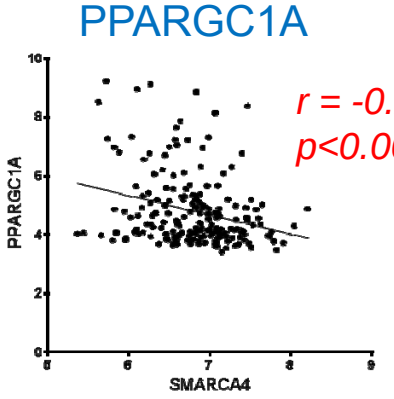
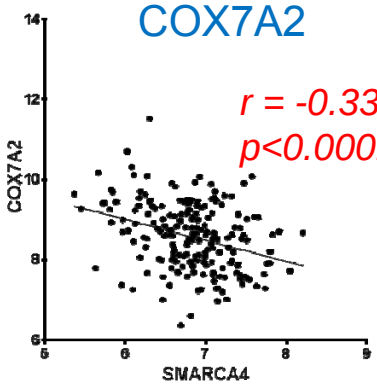
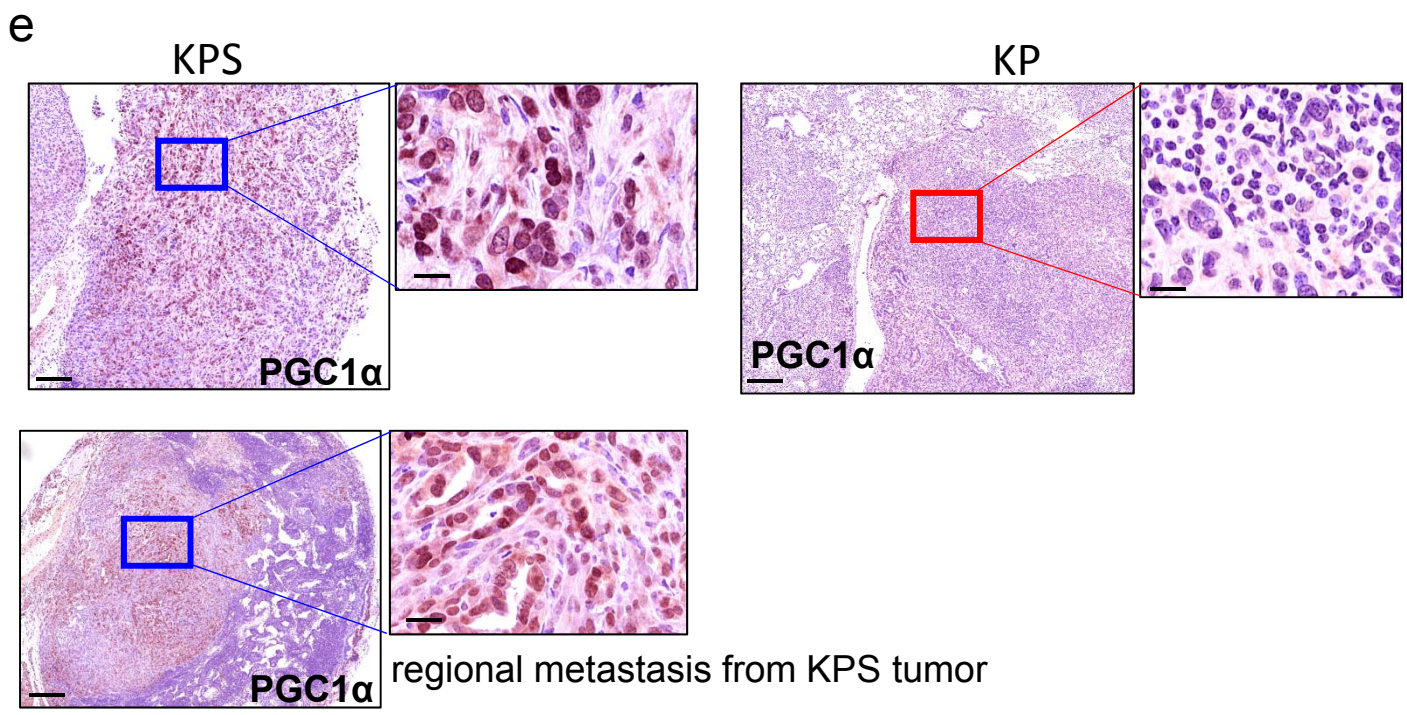


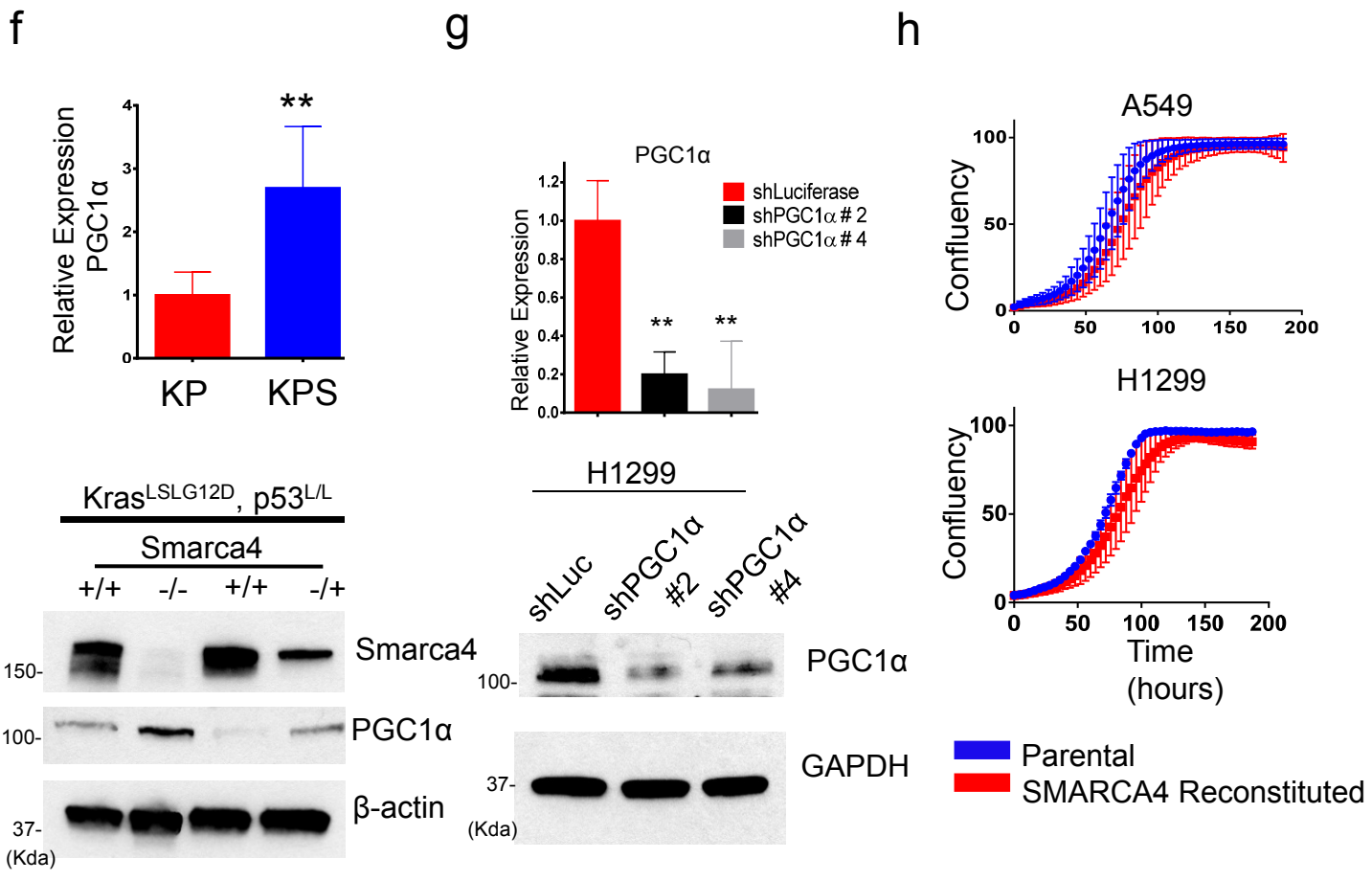






Supplementary Figure 3. OXPHOS genes are upregulated in *Smarca4* deficient tumors (continued on next page).

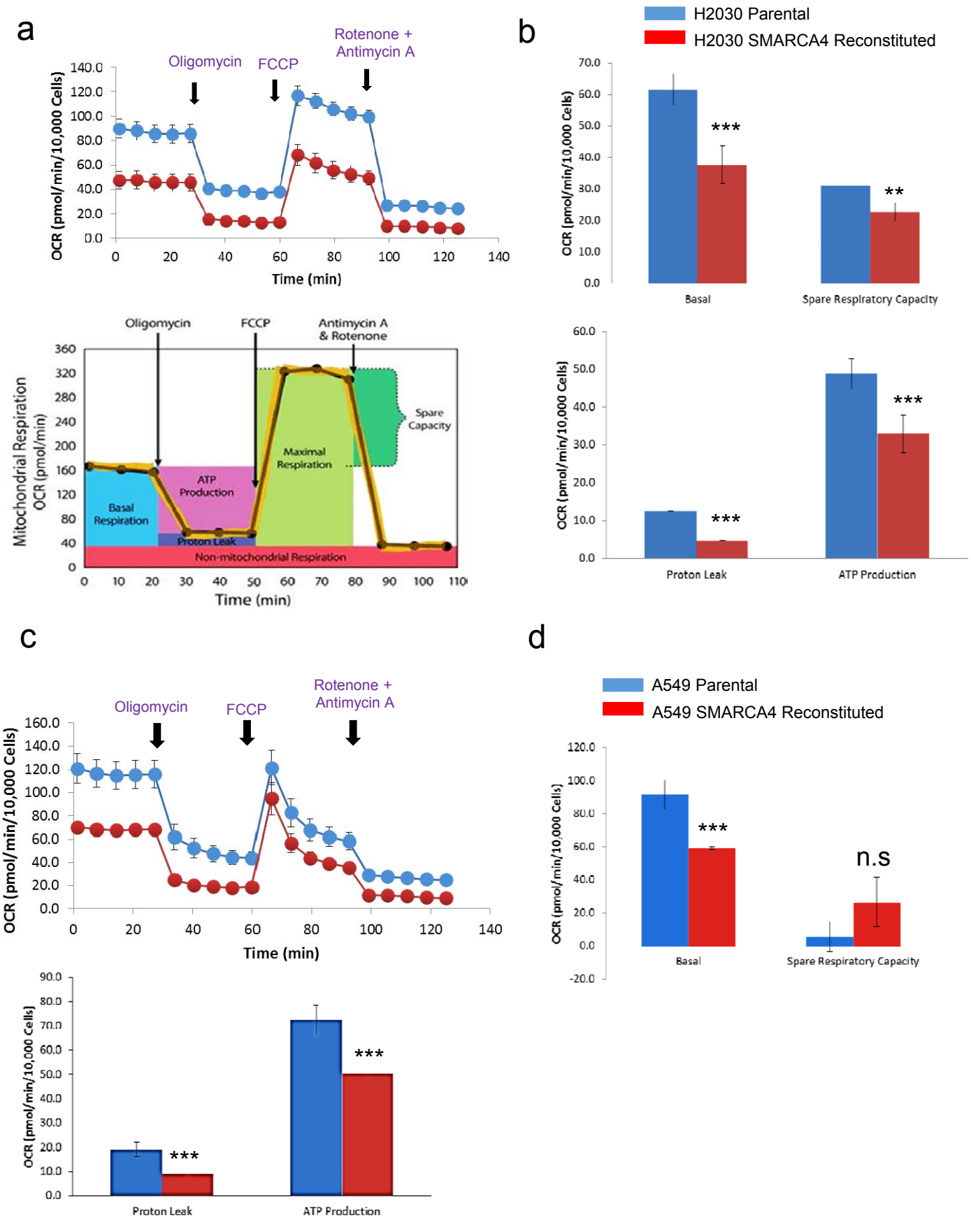




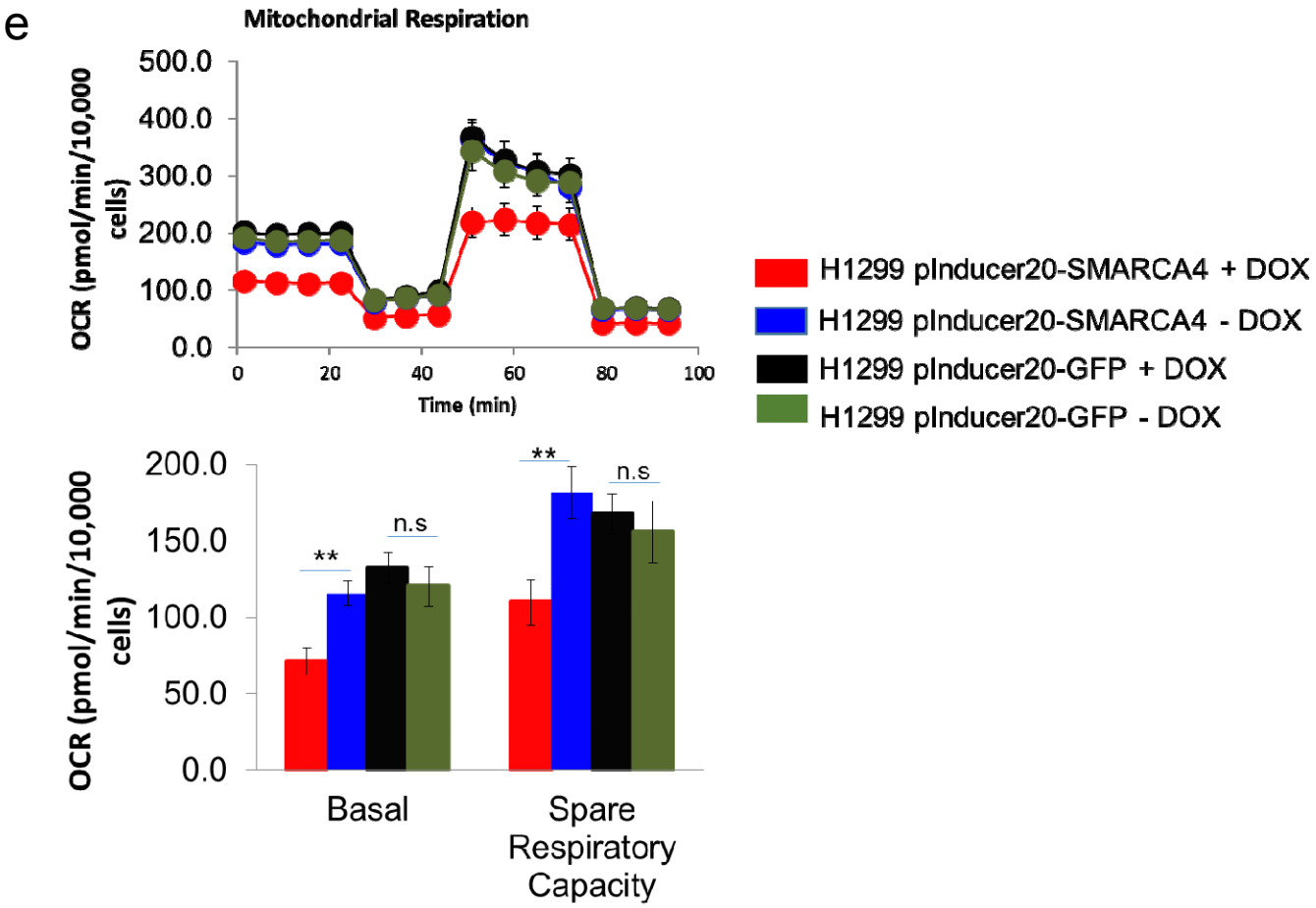
Supplementary Figure 3. OXPHOS genes are upregulated in *Smarca4* deficient tumors (continued on next page).

Supplementary Figure 3. OXPHOS genes are upregulated in *Smarca4* deficient tumors (continued from previous page).

a, GSEA generated list of OXPHOS genes identified as being enriched in KPS tumors compared to KP tumors (n=4 independent tumors, pathway enrichment FDR adjusted *p*-value =0.0024 and **b**, in SWI/SNF mutant (*SMARCA4* or *ARID1A* homozygous deleted or mutated) human TCGA lung adenocarcinoma tumors in comparison to SWI/SNF proficient tumors (pathway enrichment FDR adjusted p-value =0). WT, n= 445, *SWI/SNF* mutated n= 70 independent tumors **c**, log2 normalized RNA-Seq data show COX7A2 and ATP5O are elevated in SWI/SNF mutant human lung adenocarcinoma tumors. WT, n= 445, *SWI/SNF* mutated n= 70 independent tumors. Box-plots representation: from top to bottom: maximum value, 75th percentile, median, 25th percentile and minimum values. **d**, mRNA expression of *SMARCA4* and several OXPHOS genes were collected from the BATTLE trial (n=192). *SMARCA4* expression is plotted on x-axis and OXPHOS genes on y-axis. Pearson correlation coefficients show statistically significant negative correlation between *SMARCA4* and the indicated genes as shown by the p-values. **e**, Immunohistochemistry shows elevated PGC1α in KPS tumor (top left panel) including in a regional metastasis of a KPS tumor (lower panel) compared to a KP tumor (upper right panel). Scale bar represents 200μM in primary images and 30 μM in insets. Experiments repeated three times on independent tumors while the metastasis image is a single example. **f**, Top panel: mRNA expression of PGC1α in cell lines derived from KPS and KP tumors by qPCR (n=4 independent tumors). Bars represent mean+/- s.d and ** p-value=0.022 by two-sided Student's t-test. Bottom panel: Cropped immunoblot of PGC1α and SMARCA4 in lysates of cell lines derived from KPS and KP tumors demonstrating highest expression of PGC1α in cells with complete loss of SMARCA4 and lowest expression in SMARCA4 competent KP line. Experiment repeated twice and uncropped images available in Supplementary Fig.11-3. **g**, H1299 cells were stably transduced with control shRNA against luciferase and two independent PGC1α shRNAs. Top panel: qRT-PCR shows mRNA expression of PGC1α. (n=3 independent cell cultures). Bars represent mean+/- s.d and ** p=0.0005 for sh#2 and p=0.0016 for sh#4 by two-sided Student's t-test Bottom panel: Cropped western blot shows protein expression of PGC1α in control and PGC1α shRNA knockdown cells and uncropped images available in Supplementary Fig.11-3. Experiment repeated twice **h,** Incucyte measurement of cellular proliferation in A549 and H1299 parental or SMARCA4 re-expressing cell lines showing similar growth rate when grown under standard cell culture media (RPMI1640+Glutamine+10% FBS). Plots represent mean +/- s.d and n=3 independent cell cultures.



Supplementary Figure 4. Mitochondrial respiration is increased in SMARCA4 deficient cells (continued on next page).



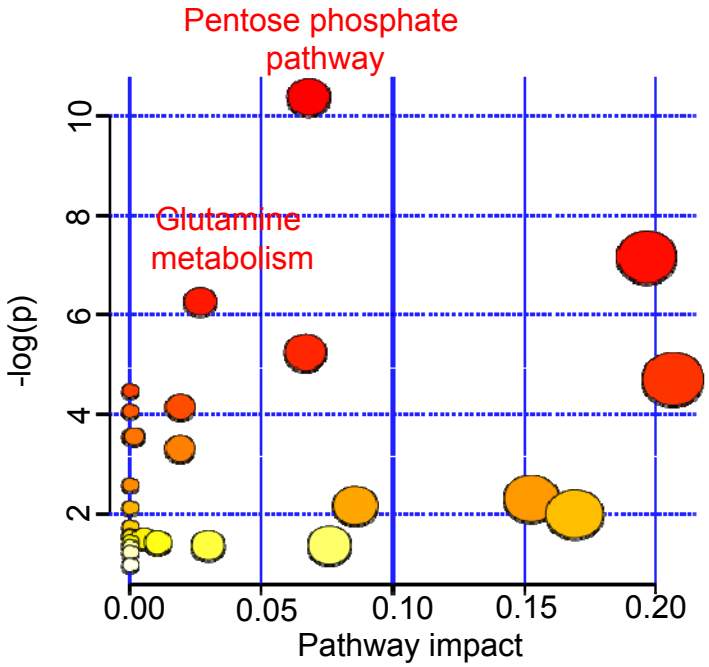
Supplementary Figure 4. Mitochondrial respiration is increased in *SMARCA4* deficient cell lines (continued from previous page).

a, A representative trace of OCR values (mean +/-s.d) of mitochondrial stress test using XF-96 analyzer showing H2030 parental (blue) and H2030 cell line reconstituted with SMARCA4 (red) from which **b**, basal respiration, spare respiratory capacity, proton leak and ATP production were computed. A two-sided Student's t-test computed. *** indicate p-values <0.0001 and ** indicates p=0.003 Graphs indicate mean and error bars denote s.d from 8 wells (from 2 experiments). Lower panel adapted from http://www.agilent.com/cs/library/usermanuals/public/XF_Cell_Mito_Stress_Test_Kit_User_Guide.pdf **c,** A representative trace of OCR values (mean +/-s.d) of mitochondrial stress test using XF-96 analyzer showing A549parental (blue) and A549 cell line reconstituted with SMARCA4 (red) from which **d**, basal respiration, spare respiratory capacity, proton leak and ATP production were computed. A two-sided Student's t-test computed. *** indicate p-values <0.0001 and for the n.s (not significant) test p=0.27. Graphs indicate mean and error bars denote s.d from 8 wells (from 2 experiments). **e,** A representative trace (mean +/-s.d) of mitochondrial stress test using XF-96 analyzer showing H1299 cells stably transduced with control pInducer20-GFP or pInducer20-SMARCA4 plasmids treated with doxycycline (0.5µg/ml) or not (Top Panel). This data was used to compute basal and spare respiratory capacity showing no significant change in control cells were treated with doxycycline whereas SMARCA4 reconstituted cells have lower mitochondrial respiration. A two-sided Student's t-test computed. ** indicate p-values <0.0001. Graphs indicate mean and error bars denote s.d from 8 wells (from 2 experiments).

a

Metabolite	p-value	Fold change in Smarca4 null cells
3-phospho-serine	0.0118	5.5
thiamine-phosphate	0.0372	3.6
glucose-6-phosphate	0.0311	3.0
D-erythrose-4-phosphate	0.0398	2.5
fructose-6-phosphate	0.0411	2.3
UMP	0.0495	2.0
Phenylpropiolic acid	0.0453	1.9
glutamine	0.0488	1.9
2-deoxyglucose-6-phosphate	0.0079	1.8
hexose-phosphate	0.0051	1.8
1,3-diphopshateglycerate	0.0155	1.8
a-ketoglutarate	0.0380	1.8

b



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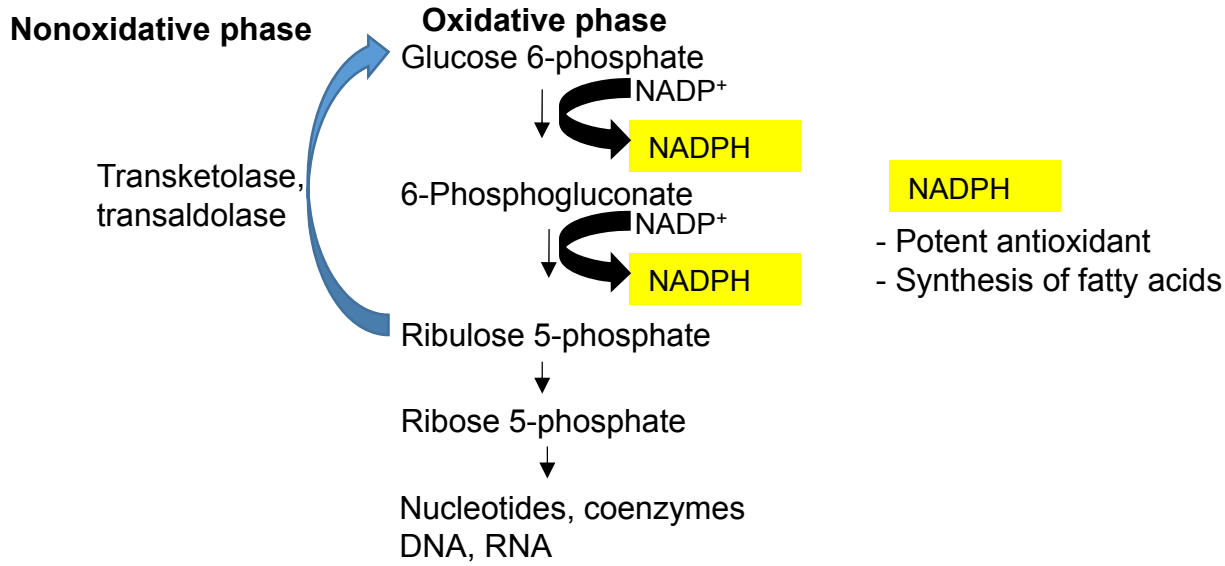
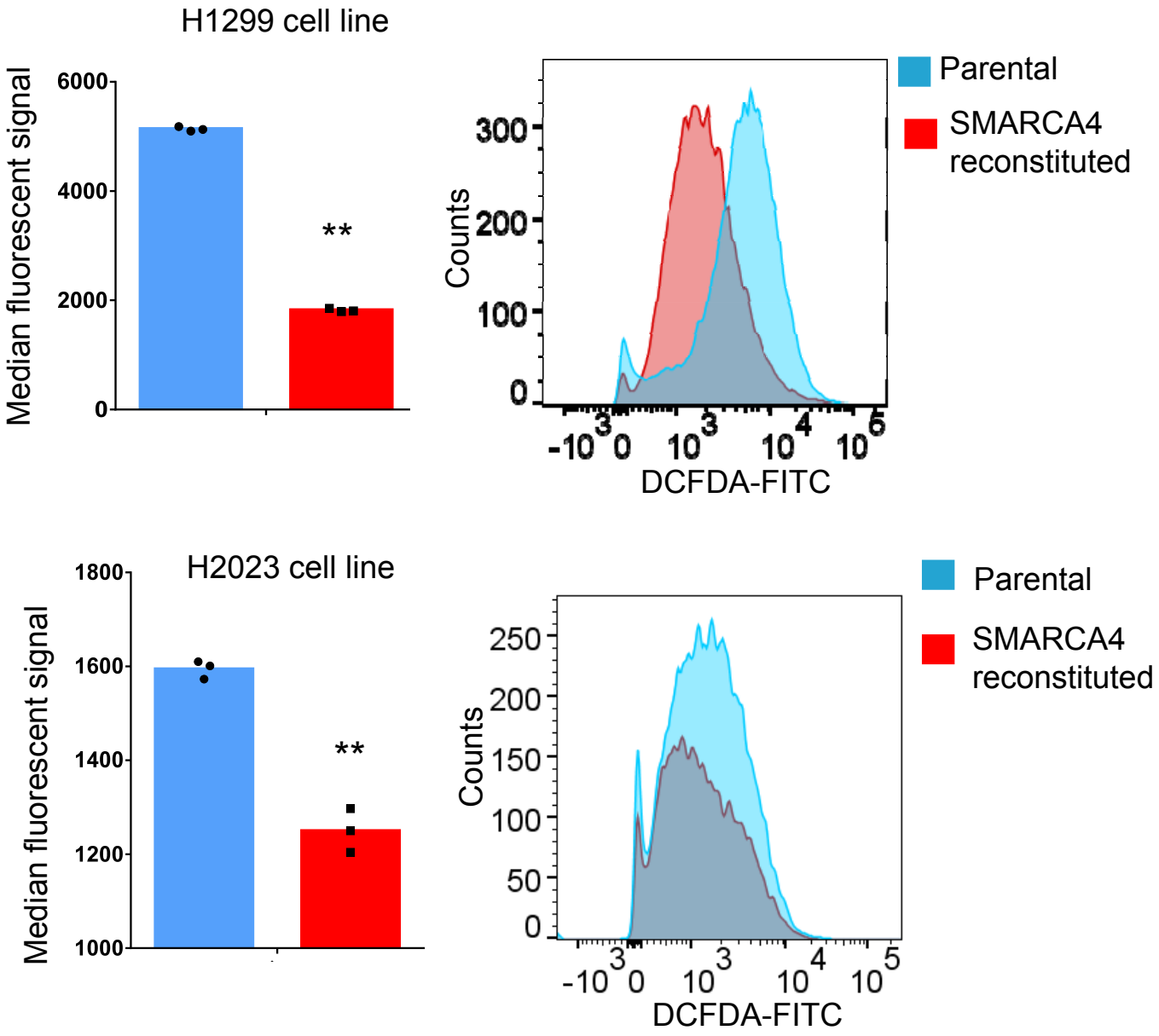


Supplementary Figure 5. Metabolomics analysis in SMARCA4 deficient cells (continued on next page).

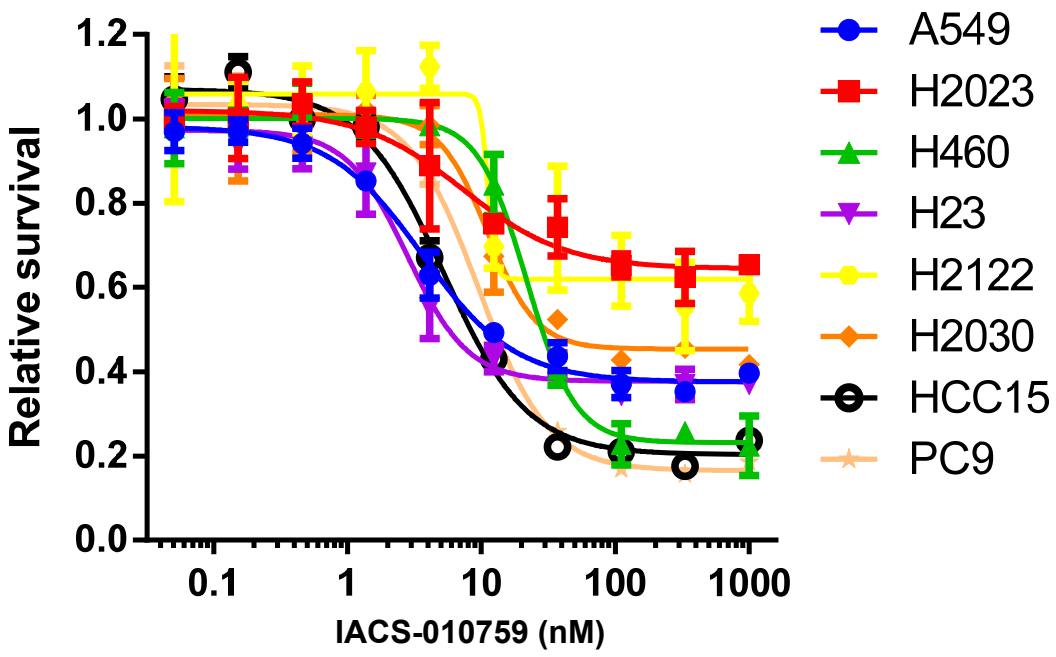
Supplementary Figure 5 Unlabeled polar metabolomic analysis of *SMARCA4* deficient and proficient cells (continued from previous page).

a, Relative fold-change (upregulation) of unlabeled polar metabolites significantly increased in *SMARCA4* deficient lung cancer cell lines (H1299, A549, H2023 and H2030) compared to SMARCA4 reconstituted cells (out of 296 metabolites quantified by mass spectrometry). n=4 independent cell line pairs and two-sided Student's t-test performed to derive p-values. **b**, Inference of metabolic pathway enrichment using Metaboanalyst 3.0. All metabolites and their fold change values were entered in the online software (http://www.metaboanalyst.ca/) which calculated enrichment values for pathways that either generate or use these metabolites. Result shows enrichment of pentose-phosphate pathway and glutamine metabolism in *SMARCA4* deficient cells. **c**, Schematic representation of the oxidative phase of pentose-phosphate pathway critical in generating reducing potential NADPH.



Supplementary Figure 6. Elevated reactive oxygen species (ROS) in SMARCA4 mutants. ROS levels were measured by labeling H1299 (Top Panel) and H2023 (Bottom Panel) parental and SMARCA4 reconstituted cells with the general oxidative stress indicator CM-H_2DCFDA and FACS quantification. Median fluorescence intensity from live cells of three biological replicates plotted using FlowJo. A representative histogram of fluorescent intensity and count plotted using FlowJo. Bar graphs represent median+/- s.d and ** denotes p=0.0003 for H2023 and p<0.0001 for H1299 by two-tailed Student's t-test.

a



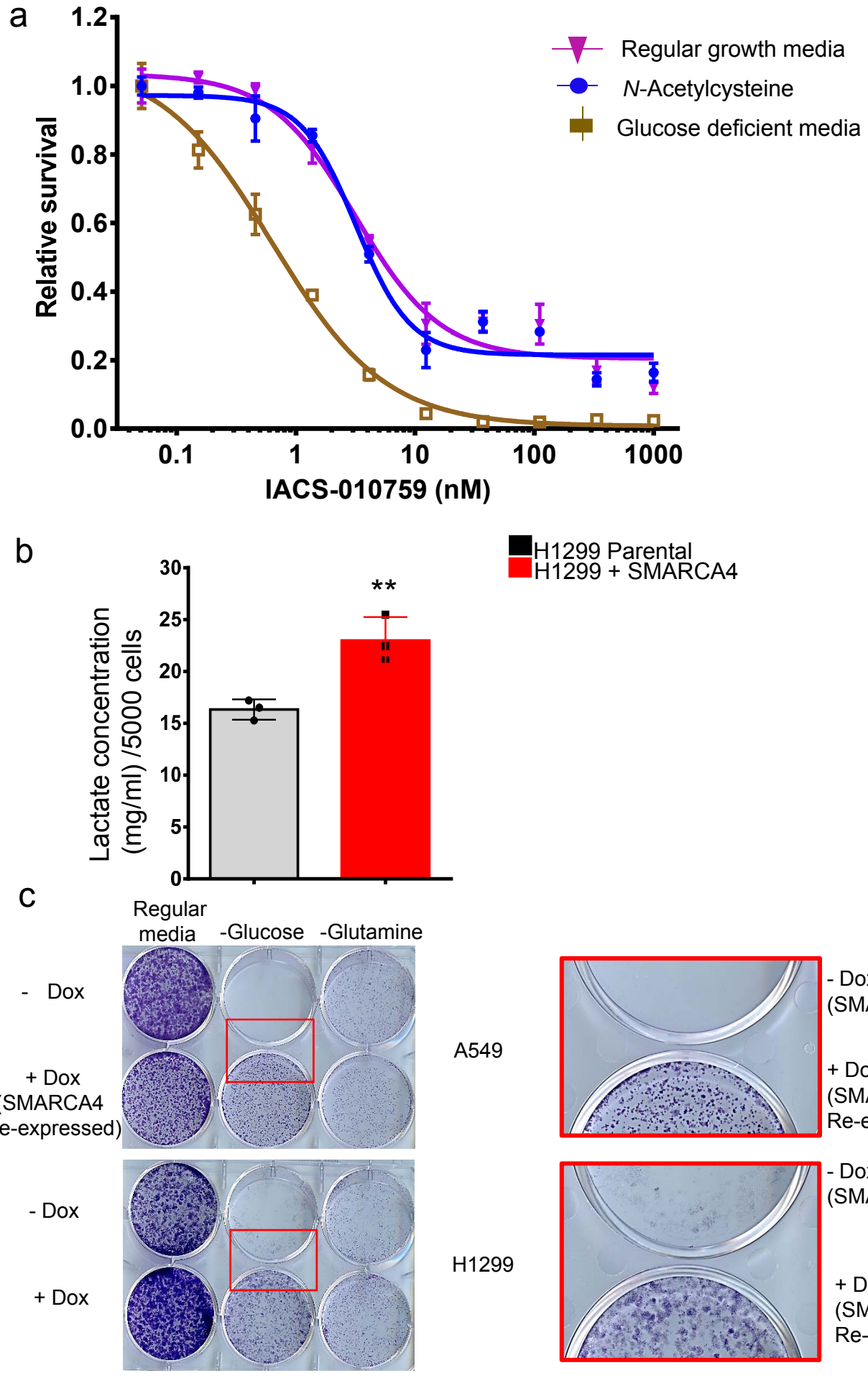
b

Cell line	GI50 (nM)	SWI/SNF mutations
A427	4	SMARCA4 hom.del
Hop62	4	n.d
H23	6	SMARCA4 K1566N* (homozy.)
HCC15	8	SMARCA4 *p* .M272*fs* *31 (homozy.)
A549	11	SMARCA4 Q729C*fs* *4 (homozy.)
PC9	13	n.d
H1650	13	SMARCA4 p.N1164Y (homozy.)
H3122	17	ARID1b p.L1072R, p.L1090R
H460	28	ARID1A *in frame* I2135_L2136del (homozy.)
H2030	30	SMARCA4 hom.del
H2009	49	SMARCC2 p. I901M (het.), SMARCD3 p. D161H (het.)
H2023	>1000	SMARCA4 S828P*fs* *3 (homozy.)
H2122	>1000	n.d
H1975	>1000	n.d
HCC827	>1000	n.d
H3255	>1000	n.d
HCC2279	>1000	n.d
H2228	>1000	n.d
H838	>1000	ARID1A P1876S*fs* *22 (het.)
H358	>1000	n.d

Supplementary Figure 7. Sensitivity of SWI/SNF mutant cells to inhibition of OXPHOS by IACS-010759 (continued on next page).

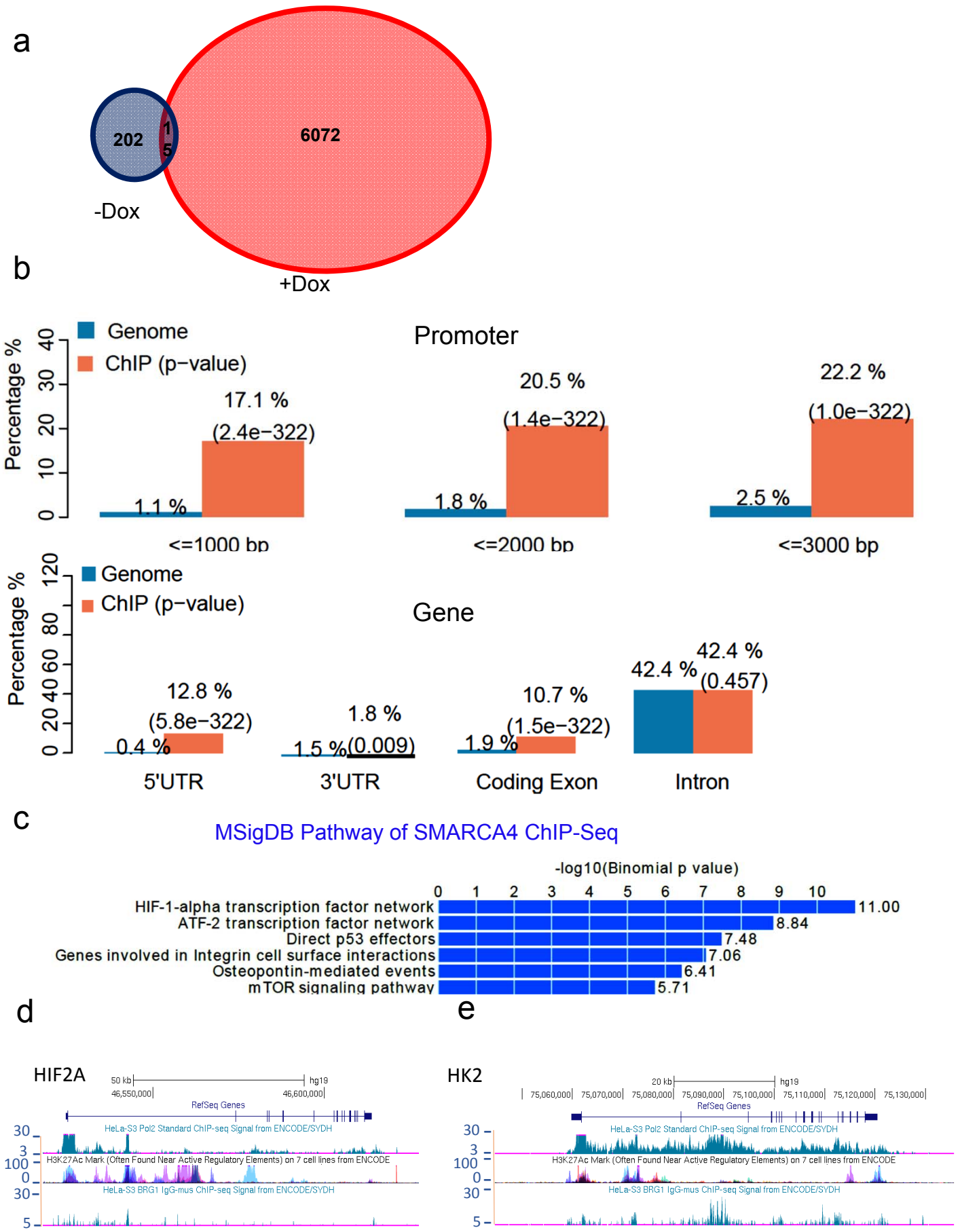
Supplementary Figure 7. Sensitivity of SWI/SNF mutant cells to inhibition of OXPHOS by IACS-010759 (continued from previous page).

a, CellTitre-Glo based cell viability assays 3 days after administration of DMSO control or the indicated 8 doses of IACS-010759 across a cohort of lung cancer cell lines, experiment repeated twice. Plots show mean and s.d. of three technical replicates **b**, GI50 values for IACS-010759 across 20 lung cancer cell lines determined by a 3-day cell CellTitre-Glo viability assay. Values and cell lines in “red” font represent cell lines harboring mutations in either ARID1A or SMARCA4, hence referred to as “SWI/SNF mutants”.



Supplementary Figure 8 SMARCA4 deficient cells are hypersensitive to glucose deprivation (continued on next page).

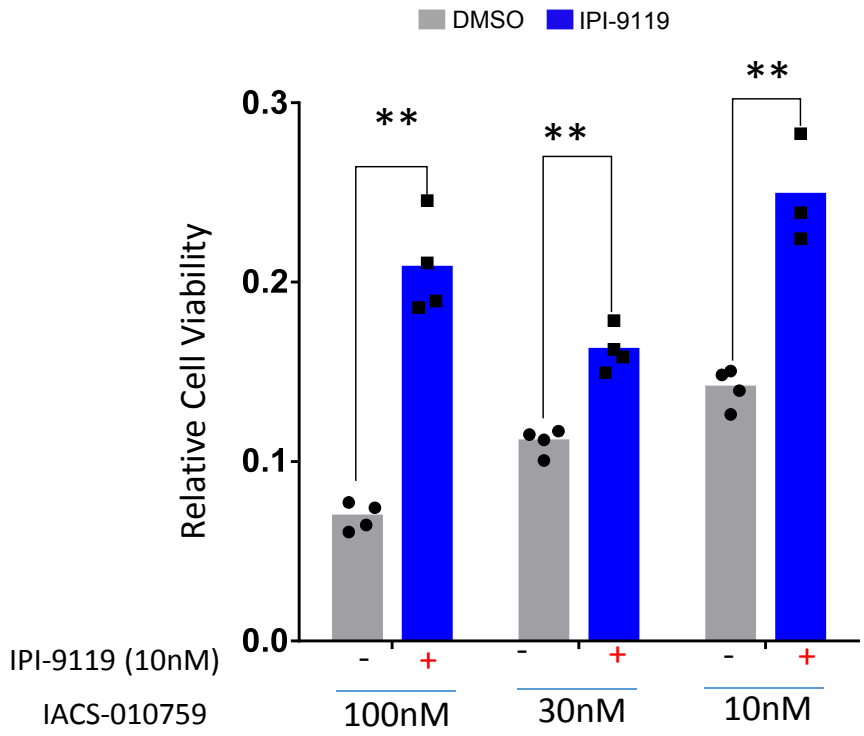
Supplementary Figure 8. *SMARCA4* deficient cells are hypersensitive to glucose deprivation (continued from previous page).
a, CellTitre-Glo based 72 hour cell viability assay of H1299 cells treated with indicated concentrations of IACS-010759 and that were grown in regular media, regular media supplemented with N-acetylcysteine (2mM) or in glucose deficient RPMI-1640 media. While N-acetylcysteine has no effect on the growth inhibitory activity of IACS-010759, glucose co-depletion resulted in dramatic loss of viability. n=3 independent cell cultures, values represent mean+/- s.d **b**, Extracellular lactate was measured in the spent media of H1299 cells that were stably transduced with an inducible SMARCA4 vector. Media was collected after inducing expression of SMARCA4 by administration of doxycycline or left un-induced (parental) as control. Graphs show mean+/- s.d of n=3 independent cell cultures and ** p=0.0094 by two-sided Student's t-test. **c,** Cell viability assay of A549 and H1299 cells stably transduced with an inducible SMARCA4 vector was conducted after inducing expression of SMARCA4 by administration of doxycycline or left un-induced as control. Cells were grown in regular media (RPMI-1640+2mM glutamine, 10% FBS), or RPMI-1640+glutamine 2mM media that lacked glucose (-Glucose), or RPMI-1640 with no glutamine supplementation (-Glutamine). Media was removed four days later, and cells were fixed and stained with crystal violet. Right panel shows a close up of red boxed area. Experiment repeated three times.



Supplementary Figure 9. Characterization of genomic localization of SMARCA4 using ChIP-Seq (continued on next page)

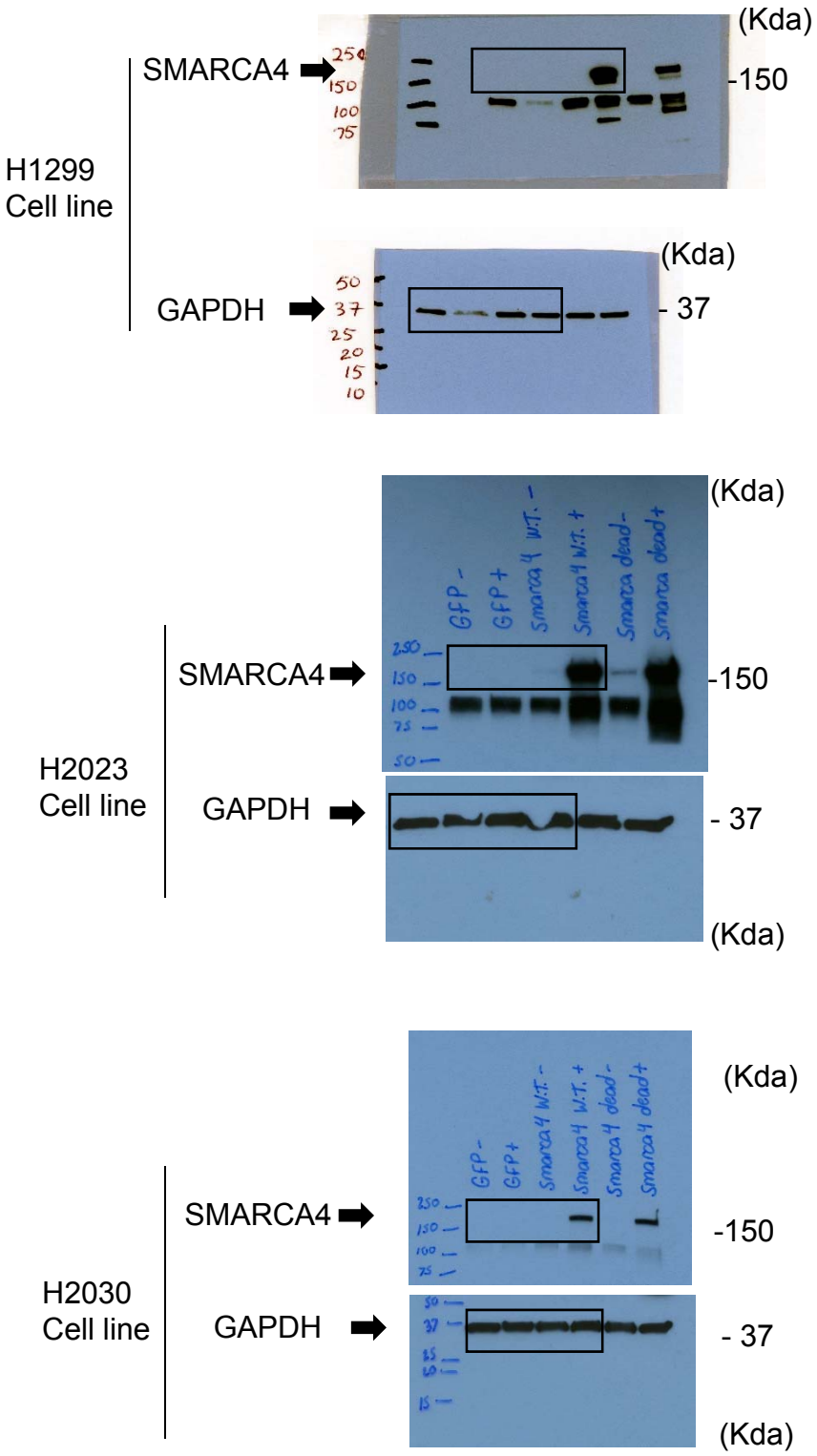
Supplementary Figure 9. Characterization of genomic localization of SMARCA4 using ChIP-Seq (continued from previous page).

a, Venn diagram showing number of peaks detected (MACS) in ChIP-seq performed using SMARCA4 antibody in H1299 cells stably expressing doxycycline inducible SMARCA4 vector and that were treated (+Dox) or not (-Dox) with doxycycline (0.5μg/ml) for 5 days to induce SMARCA4 expression. Only under +Dox condition do we see substantial number of peaks. **b,** Frequency of SMARCA4 ChIP-Seq peaks in defined regions of the genome compared to the normal distribution of such defined regions across the genome. CEAS (cis-regulatory element annotation system) was used. Defined regions include promoter (1000, 2000 or 3000bp from transcription start site), 3' and 5' UTRs, exons and introns and intergenic regions. SMARCA4 peaks are enriched in promoters and 5' UTR but are not enriched in introns. n=6072 genomic peak regions were used to perform enrichment statistics by single sided binomial test. **c,** ENCODE SMARCA4 ChIP-Seq data were used to identify genomic regions occupied by SMARCA4. GREAT analysis tool used to generate pathway enrichment in SMARCA4 ChIP-Seq peaks. n=1356 genomic peak regions were used to perform binomial statistical test by the online tool GREAT and generate p-value. **d**, Representative UCSC genome browser views of SMARCA4 ChIP-Seq peak from HeLa cells on the HIF2A genomic locus and **e**, hexokinase 2 genomic locus. ENCODE SMARCA4 ChIP-seq was done three times using isogenic replicates.



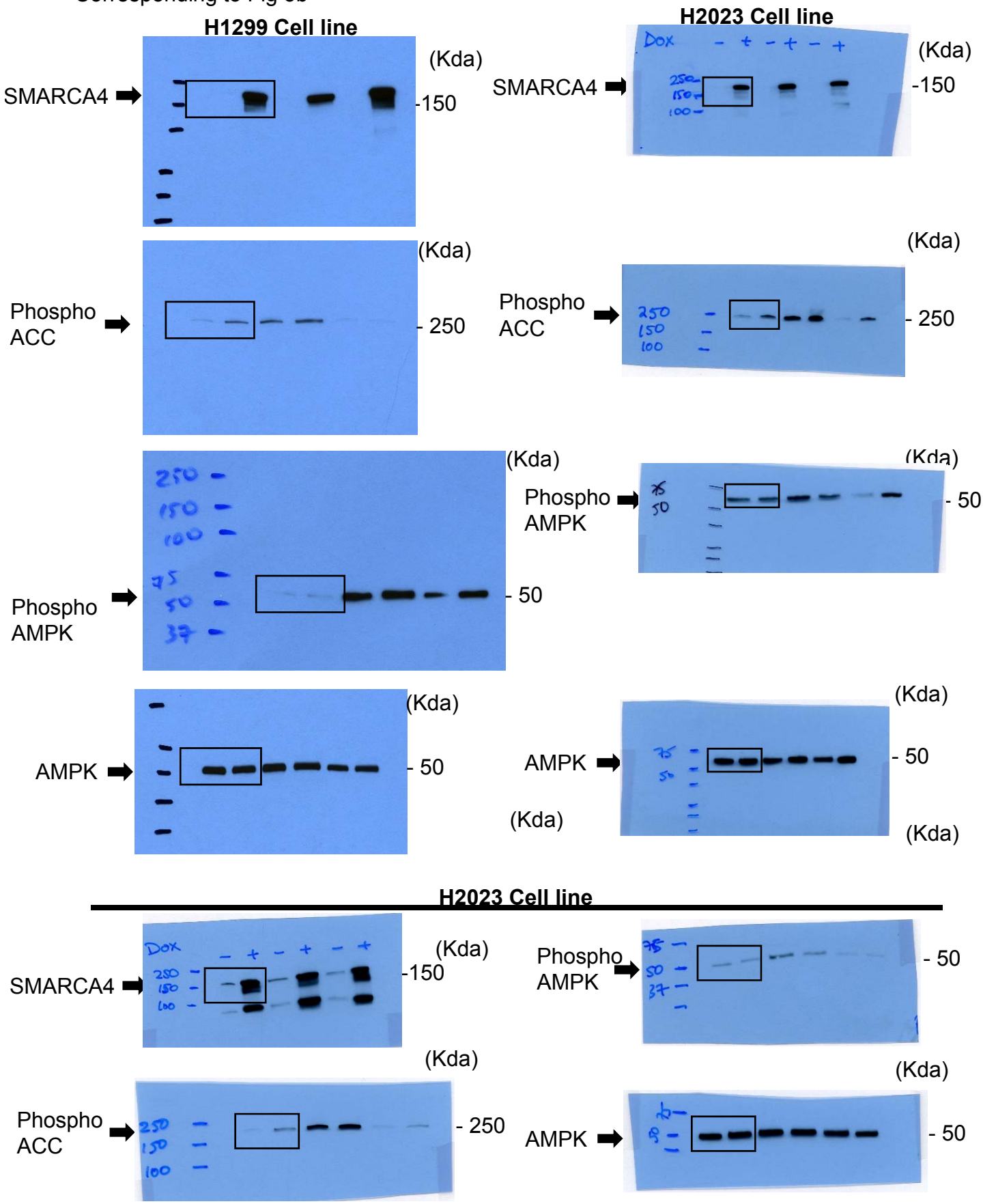
Supplementary Figure 10. Fatty acid synthesis inhibition partially rescues cell growth inhibition by IACS-010759.
Celltitre-Glo based viability assay of H1299 parental and SMARCA4 reconstituted cells treated with different doses of IACS-010759 (100, 30 or 10nM) alone or in combination with the fatty acid synthesis inhibitor IPI-9119 (10nM). Results were compared to DMSO control treated cells (set at 100%) and show partial rescue of growth inhibition at each dose by IPI-9119. Graph represents mean +/- s.d, of n=3 independent cell culture grown and treated cells, Two-sided Student's t-test used to derive p values. ** denotes p=0.0011, p=0.0004 and p<0.0001 for 10, 30 and 100nM IACS-010759 treated cells respectively.

Corresponding to Fig 2d



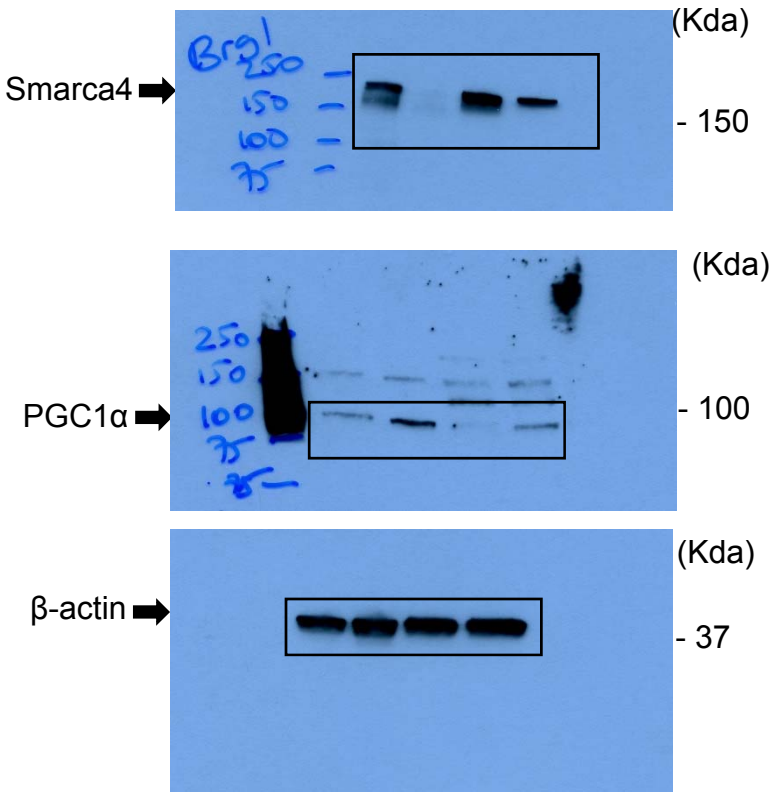
Supplementary Figure 11-1. Uncropped western blots for Fig. 2d
Each membrane was cut into two halves according to molecular weight. Top half was incubated with SMARCA4 antibody and bottom half was incubated with GAPDH antibody. Black boxes indicate the position of the specific bands and where blots were cropped.

Corresponding to Fig 5b

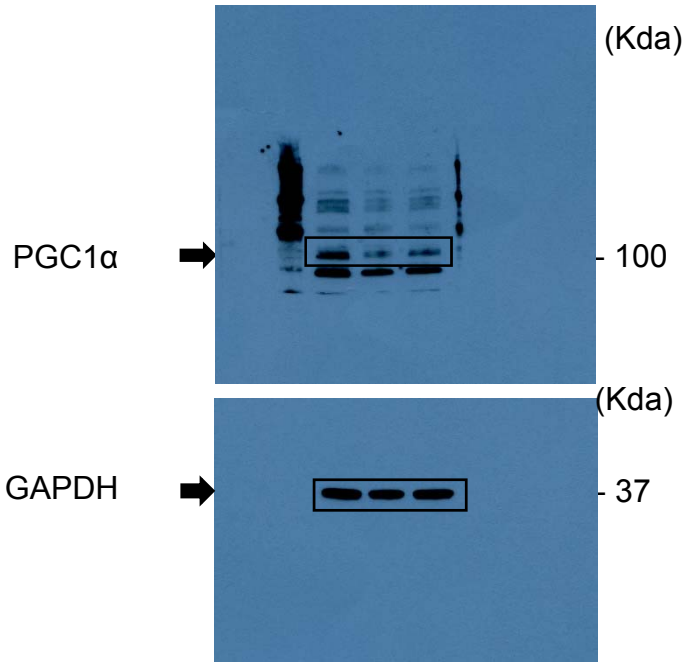


Supplementary Figure 11-2. Uncropped western blots for Fig. 5b
Each membrane was cut into several parts according to molecular weight and incubated with the indicated specific antibodies. Black boxes indicate the position of the specific bands and where blots were cropped.

Corresponding to Supplementary Figure 3f



Corresponding to Supplementary Figure 3g



Supplementary Figure 11-3. Uncropped western blots for Supplementary Fig. 3f-g
Each membrane was cut into several parts according to molecular weight and incubated with the indicated specific antibodies. Black boxes indicate the position of the specific bands and where blots were cropped.

Supplementary Table 1. Gene expression data for SMARCA4 and select OXPHOS genes from the BATTLE-2 lung cancer trial dataset

Affymetrix Human Gene 1.0 ST Array based mRNA expression profiling was done on n=192 independent human lung cancers according to manufacturer's guidelines. Data was background corrected by RMA, quantile normalized and log2 transformed. Expression values for SMARCA4, GSTO1, ATP5L, PPARGC1A and COX7A2 are provided.