Figure S1, Related to Figure 1

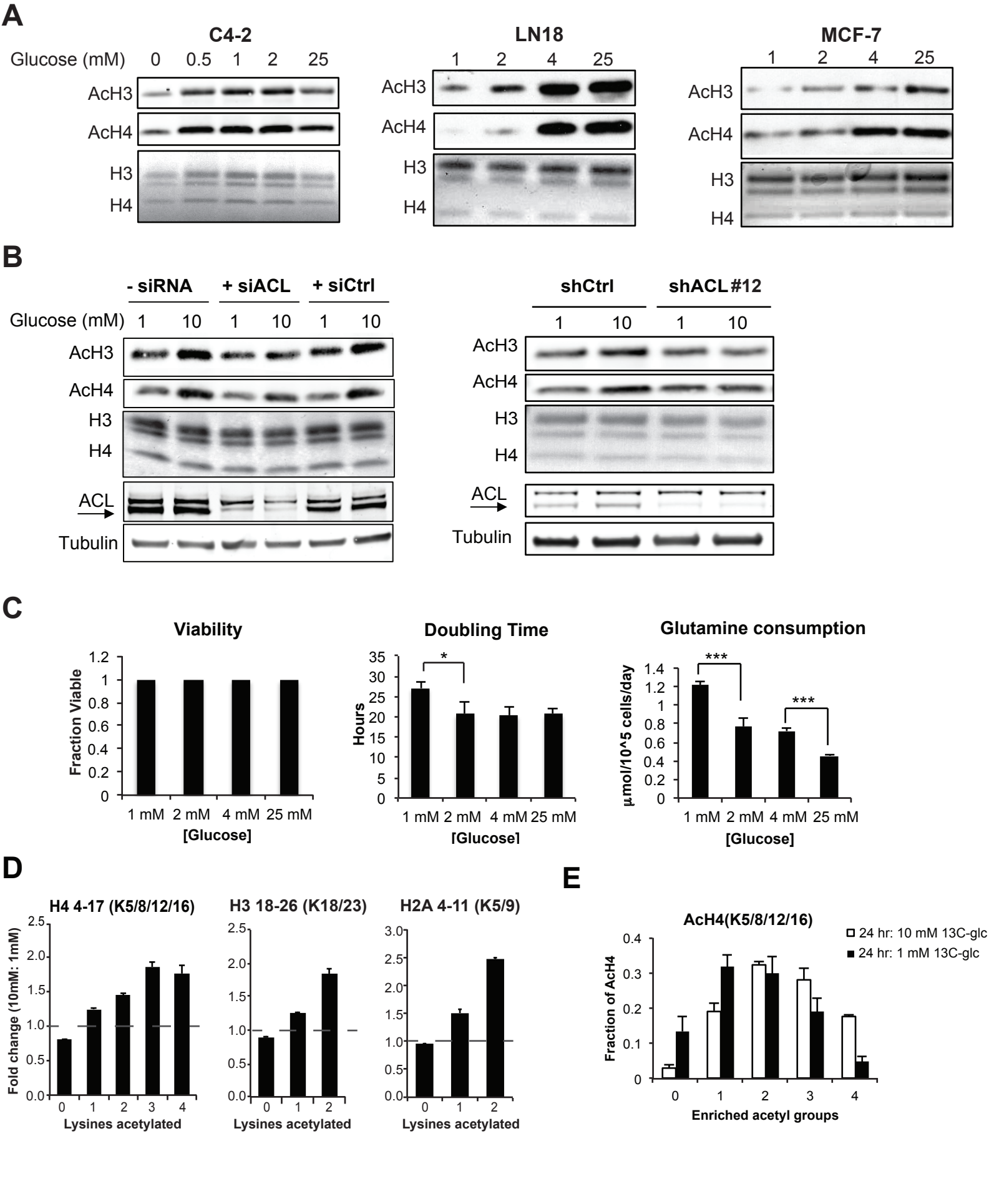
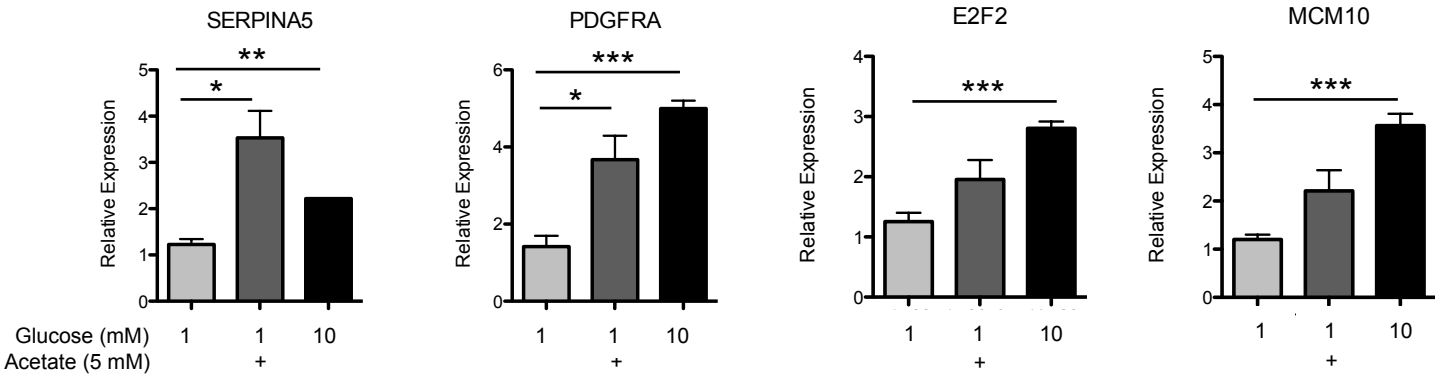


Figure S2, Related to Figure 1

A



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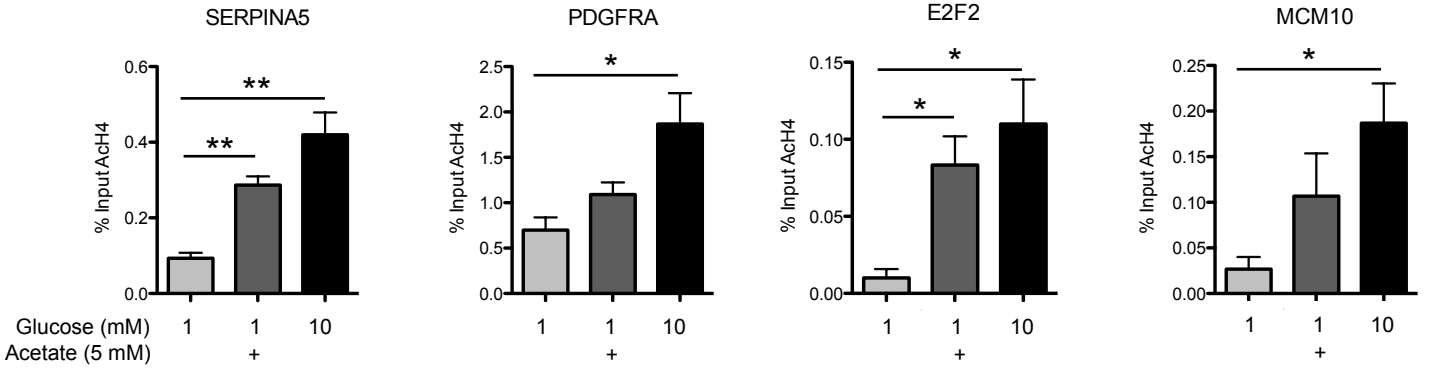


Figure S3, Related to Figure 3

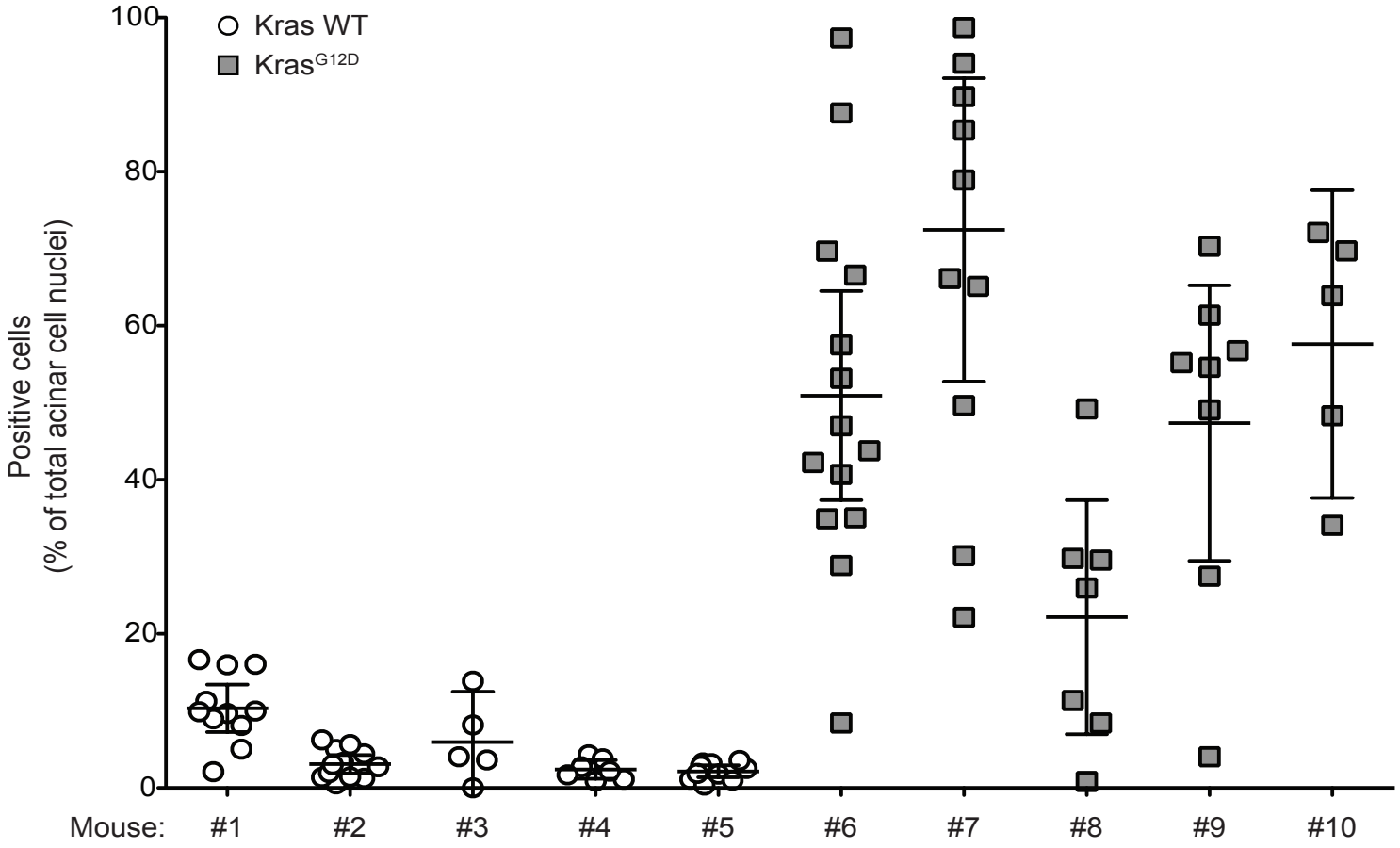


Figure S4,
Related to Figure 4

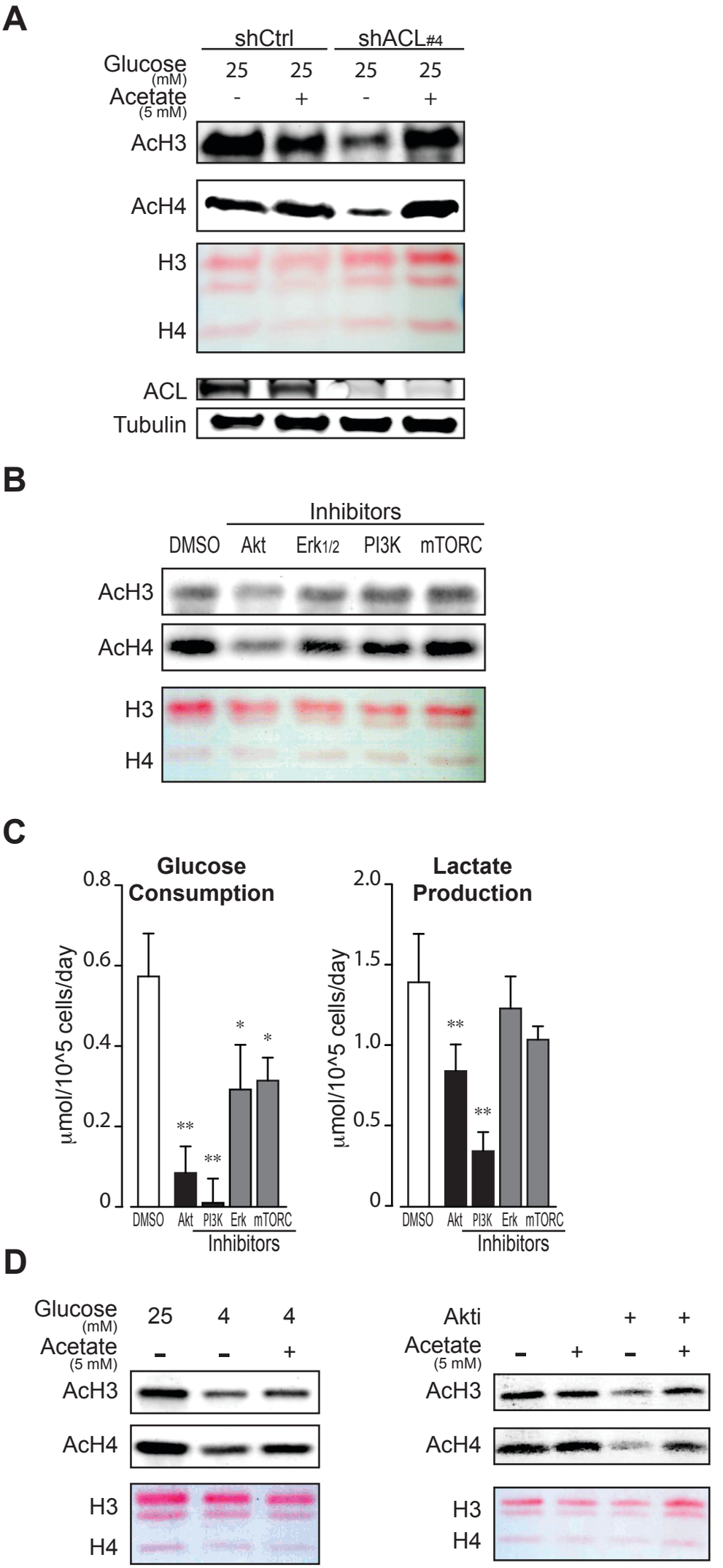
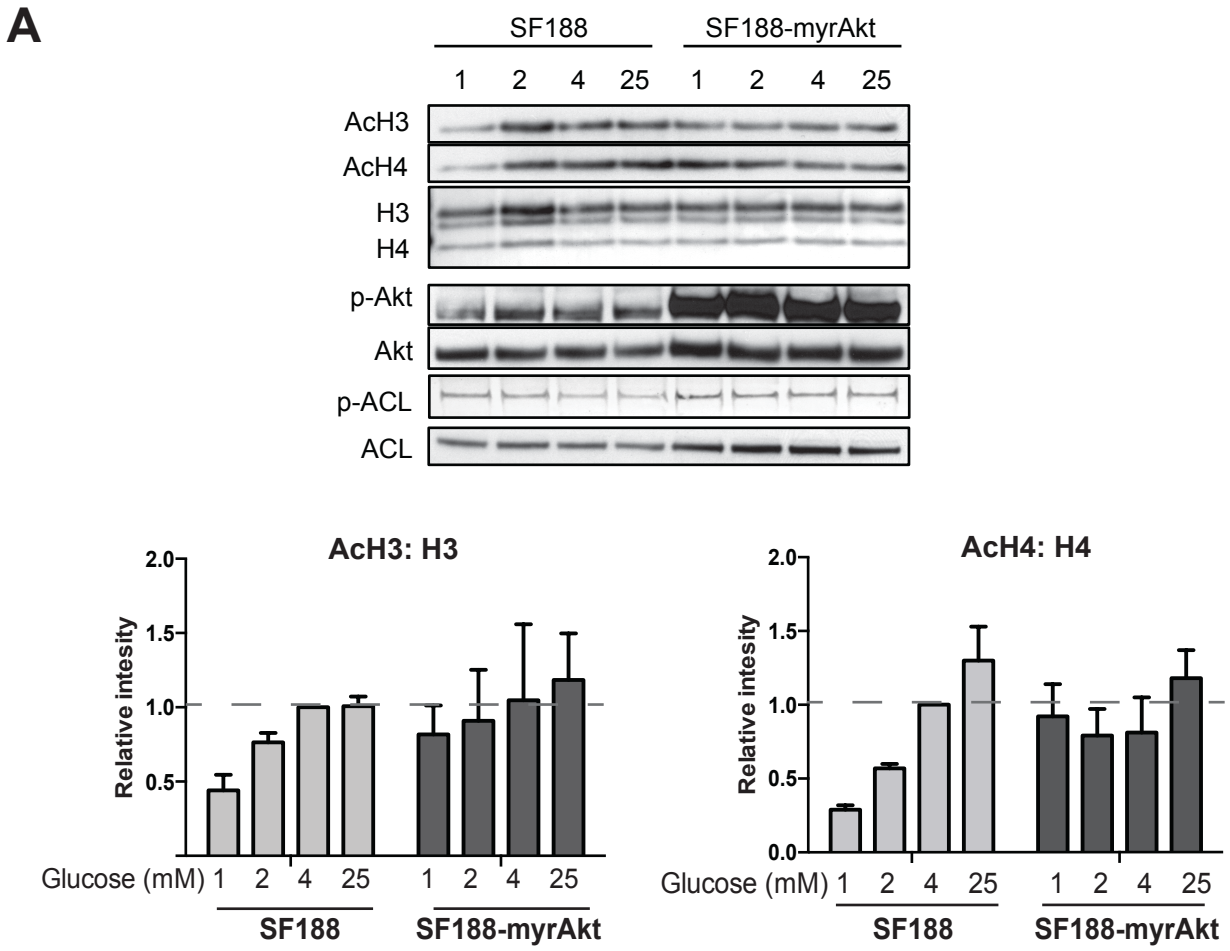


Figure S5, Related to Figure 5



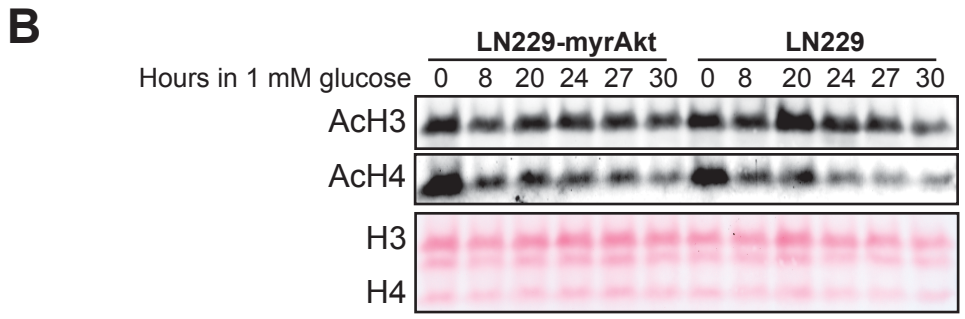
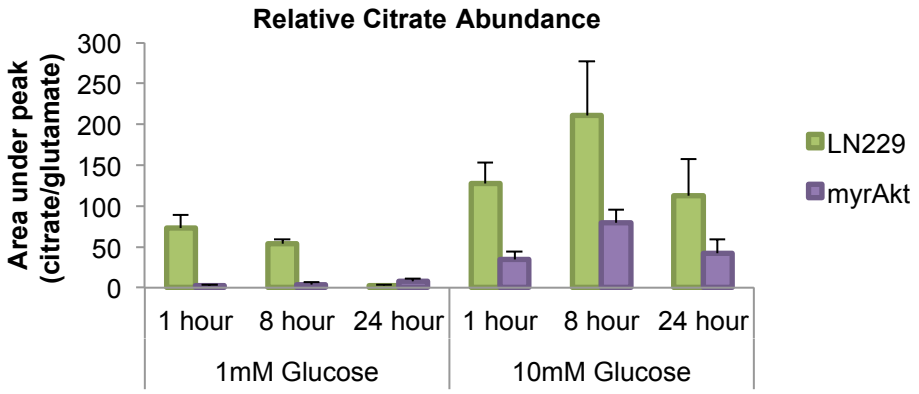


Figure S6, Related to Figure 5

A



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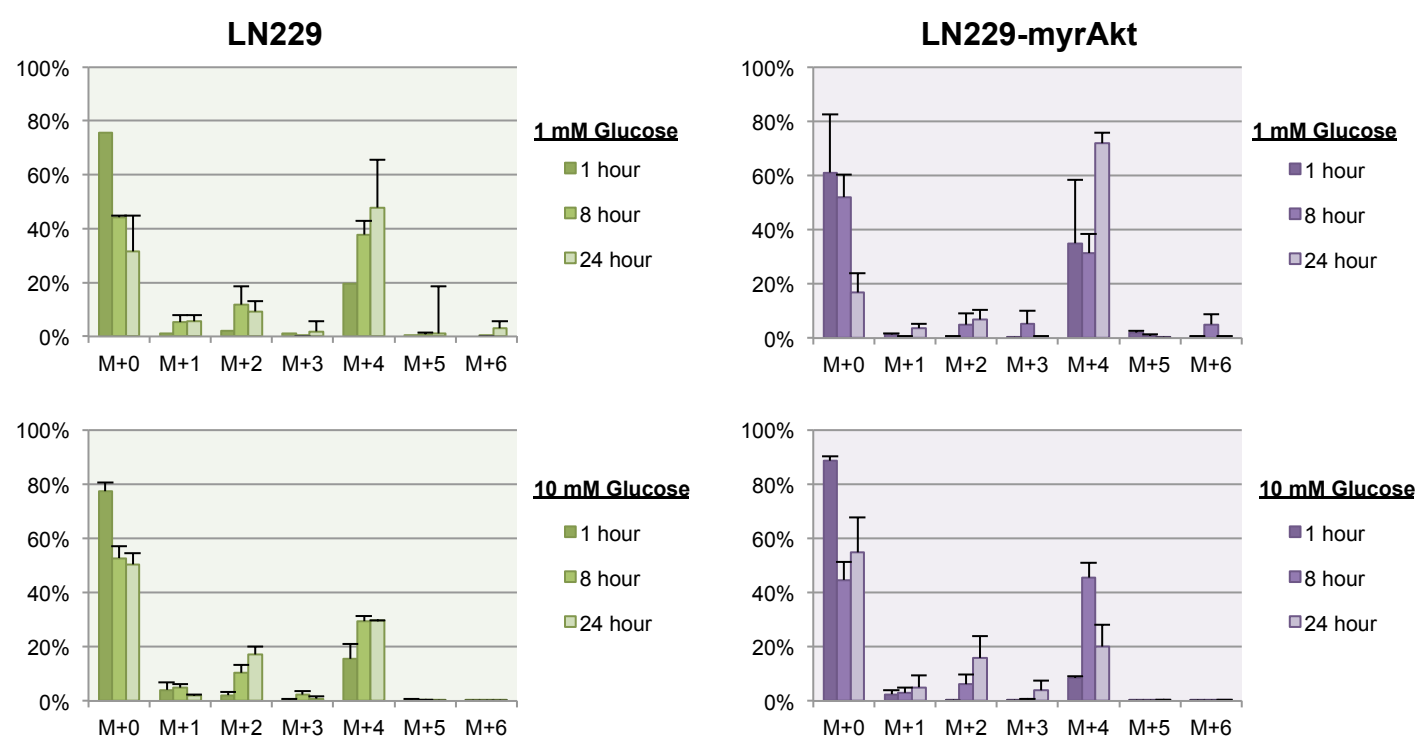
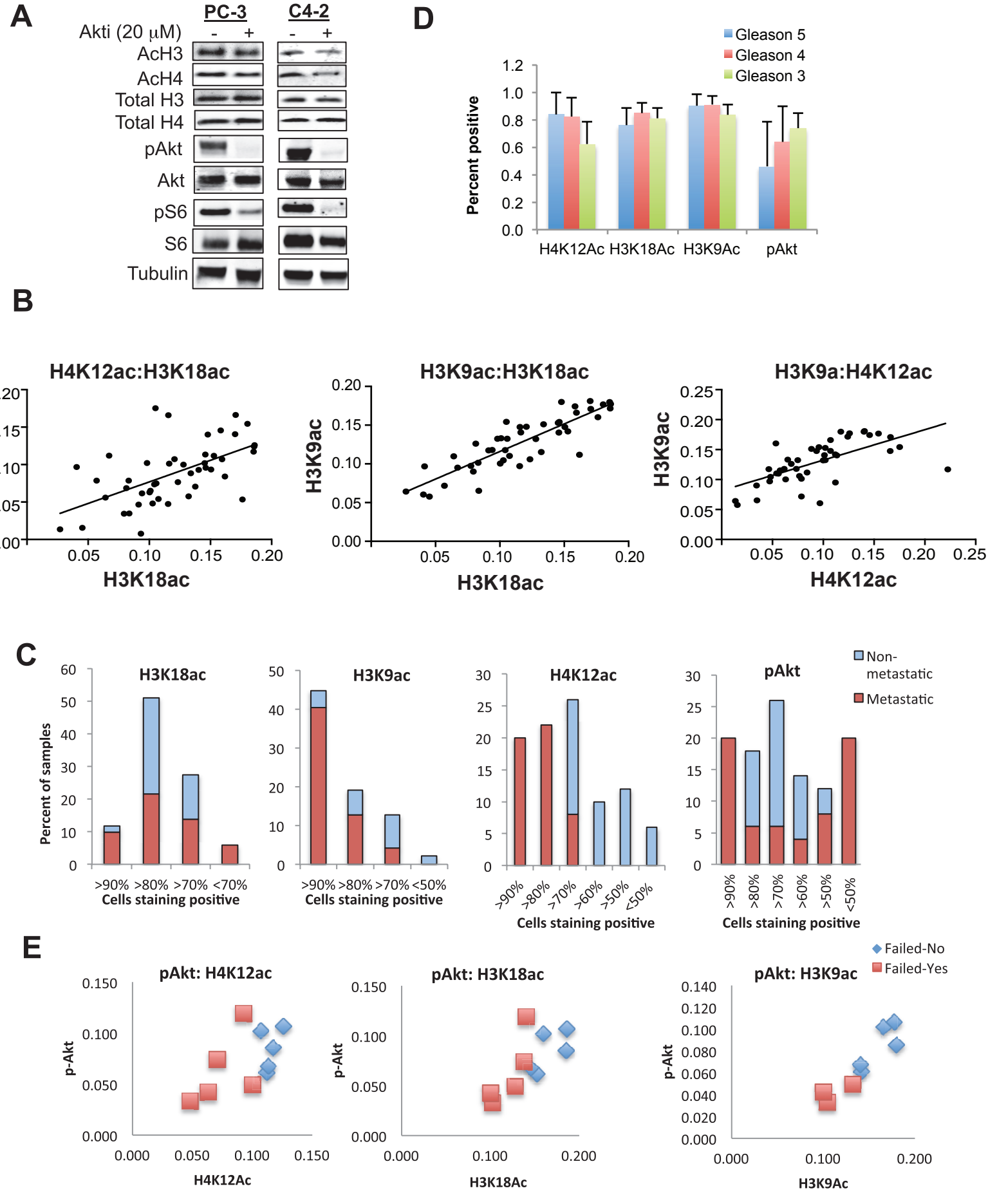


Figure S7, Related to Figure 7



Supplementary Figure Legends

Figure S1, Related to Figure 1. Glucose availability regulates histone acetylation in cancer cells. (A) Western blot analysis of histones acetylation in cancer cell lines cultured under indicated glucose conditions for 24 hours. Total acid-extracted histones were stained by Coomassie or Ponceau. (B) LN229 cells were transfected with Ctrl or ACL-targeting siRNA (left panel) or transduced with Ctrl or ACL-targeting shRNA (right panel). Cells were starved in 1 mM glucose, then treated with 1 mM or 10 mM glucose for 24 hours. Western blot analysis of acid extracted histones and other signaling components in whole cell lysates. (C) Viability, doubling time, and glutamine consumption were measured in LN229 cells after 24 hours of growth under indicated glucose concentrations, mean +/- SEM of triplicates (*, $p<0.05$; ***, $p<0.0005$). (D) Indicated histone tail peptides were analyzed by mass spectrometry. Fold change in acetylation between 10 mM: 1 mM glucose is graphed, mean +/- SEM of 10 mM sample triplicates over average 1 mM value. See Table S1 for complete data set. (E) MS analysis of acid extracted histone proteins from LN229 cells incubated with [U-$^{13}C_6$]-glucose for 24 hours. Enrichment of tetra-acetylated H4 after 24 hours in 1mM and 10mM [U-$^{13}C_6$]-glucose, mean +/- SD of triplicates. See Table S2 for complete data set.

Figure S2, Related to Figure 1: Histone acetylation at relevant loci is regulated by acetyl-CoA availability. (A) QPCR validation of select genes identified by RNA-Seq as glucose- and acetate-regulated, mean +/- SEM of triplicates (*, $p<0.05$; **, $p<0.005$; ***, $p<0.0005$) (B) AcH4 ChIP at promoter of each of genes in part (A) normalized to input, mean +/- SEM of triplicates (*, $p<0.05$; **, $p<0.005$).

Figure S3, Related to Figure 3: KrasG12D expression increases histone H4 acetylation in pancreatic acinar cells *in vivo*

Pancreata from either wild type (Kras WT) or oncogenic Kras (KrasG12D)-expressing mice were harvested at 6 weeks of age (n=5, each group). Acinar cell nuclei positive for acetylated histone H4 (AcH4) were quantified by immunohistochemistry. The scientist who performed the quantitation was blinded to the genotypes of the mice. Percentages of AcH4-positive acinar cell nuclei were calculated in different, non-overlapping microscopic fields and plotted (each dot refers to a different microscopic fields). Each column indicates a different animal. Bars denote means and interquartile ranges.

Figure S4, Related to Figure 4. Akt regulates histone acetylation in PanIN and PDA cells.

(A) PanIN-derived primary cells were transduced with control (shCtrl) or ACL-targeting (shACL#4) short hairpin RNA. Cells were cultivated under indicated glucose concentrations, +/- 5 mM acetate for 24 hours. Histones were acid-extracted and analyzed by Western Blot. Ponceau staining is shown as loading control for histones. (B) Mouse PDA-lesion derived cells from KPCY mice were cultured and either mock-treated (DMSO) or treated with the indicated inhibitors for 24 hours. Acetylation of acid-extracted histones was assessed by Western Blot. (C) Glucose consumption and Lactate production were measured in PDA-derived primary mouse cells treated as in part A, mean +/- SD of triplicates (**p≤0.005; *,p≤0.05). (D) PDA-derived primary cells were cultured in high (25 mM) or low (4 mM) glucose conditions, +/- Akt inhibitor, +/- 5 mM acetate for 24 hours and histone acetylation levels were assessed by Western Blot. Ponceau staining for total histones is shown as loading control.

Figure S5, Related to Figure 5. Akt promotes histone acetylation under glucose-limited conditions. (A) Western blot analysis of proteins and histones from SF188 cells with or without myrAkt. Quantitation represents 3 independent experiments, mean +/- SEM, with SF188 4mM samples set to 1. (B) LN229 and LN229-myrAkt cells were incubated in 1 mM glucose for indicated times. Histones were extracted and analyzed by Western blot.

Figure S6, Related to Figure 5. Activated Akt-ACLY pathway does not stimulate glutamine reductive carboxylation.

A) Comparison of citrate levels in LN229 cells and LN229-myrAkt cells treated with 2mM [$^{13}C_5{}^{15}N_2$]-glutamine and 1mM or 10mM glucose for the indicated times. The graph depicts total citrate normalized to total glutamate. The total of each metabolite was calculated as the summation of isotopologues generated for the metabolite. (B) Comparison of citrate isotopologues in LN229 and LN229-myrAkt cells treated with 2 mM [$^{13}C_5{}^{15}N_2$]-glutamine and 1 mM or 10 mM glucose for the indicated times. The graph indicates the percent of carbons contributed by glutamine for each isotopologue. M+4 citrate is generated through glutamine oxidative metabolism while M+5 citrate is generated by reductive carboxylation.

Figure S7, Related to Figure 7. Histone acetylation marks correlate with one another in human prostate tumors. A) Prostate cancer cells C4-2 and PC-3 were cultured in 10mM glucose in RPMI medium and were either mock-treated (EtOH) or treated with Akt inhibitor for 24 hours. Whole cell lysates were analyzed by Western blot. B) Levels of histone acetylation marks (H3K18ac, H4K12ac and H3K9ac) strongly correlated with one another in tumors (H3K18ac and H4K12ac: r=0.58, $p \leq 0.0001$, H3K18ac and H3K9ac: r=0.866, $p \leq 0.0001$; H3K9ac and H4K12ac: r=0.6412, $p \leq 0.0001$).

C) Percentage distribution of positive nuclei for select histone marks (H3K18ac, H4K12ac and H3K9ac) between metastatic and non-metastatic tumors. D) Distribution of percent positivity for select histone marks (H3K18ac, H4K12ac and H3K9ac) in tumors based on Gleason score, mean +/ SD. E) Relationship between pAkt and histone acetylation marks in the 10 patients with biochemical failure data shown in Fig 7C.

		10 mM glucose						1 mM glucose					
		Replicate 1		Replicate 2		Replicate 3		Replicate 1		Replicate 2		Replicate 3	
		Peak Area	Enrichment	Peak Area	Enrichment	Peak Area	Enrichment	Peak Area	Enrichment	Peak Area	Enrichment	Peak Area	Enrichment
H3K9K14 acetylation and methylation Peptide: KSTGGKAPR	0 ac, 0 me1	1.55E+09	18.76%	1.40E+09	18.96%	1.52E+09	19.47%	4.75E+08	10.53%	2.40E+09	22.27%	1.85E+09	20.20%
	0 ac, 1 me1	8.73E+08	10.56%	9.86E+08	13.39%	9.05E+08	11.58%	4.29E+08	9.50%	1.42E+09	13.15%	9.90E+08	10.83%
	0 ac, 1 me2	2.10E+09	25.37%	1.69E+09	23.00%	1.83E+09	23.41%	1.26E+09	27.92%	2.66E+09	24.67%	2.38E+09	26.09%
	0 ac, 1 me3	1.30E+09	15.77%	1.09E+09	14.77%	1.18E+09	15.10%	8.02E+08	17.77%	1.75E+09	16.26%	1.56E+09	17.11%
	1 ac, 0 me1	5.10E+08	6.18%	4.53E+08	6.15%	4.90E+08	6.27%	2.25E+08	4.99%	5.84E+08	5.41%	4.80E+08	5.26%
	1 ac, 1 me1	4.84E+08	5.86%	4.56E+08	6.19%	5.08E+08	6.50%	5.96E+08	13.19%	5.51E+08	5.11%	4.26E+08	4.67%
	1 ac, 1 me2	9.91E+08	11.99%	8.80E+08	11.96%	9.49E+08	12.14%	5.01E+08	11.09%	9.61E+08	8.91%	9.89E+08	10.82%
	1 ac, 1 me3	4.18E+08	5.05%	3.79E+08	5.15%	4.02E+08	5.14%	2.09E+08	4.63%	4.19E+08	3.88%	4.27E+08	4.67%
	2 ac, 0 me1	3.80E+07	0.46%	3.19E+07	0.43%	3.16E+07	0.40%	1.77E+07	0.39%	3.78E+07	0.35%	3.13E+07	0.34%
H3K18K23 acetylation and methylation Peptide: KQLATKAAR	0 ac, 0 me1	5.32E+10	66.99%	4.88E+10	66.77%	5.43E+10	66.47%	2.07E+10	80.14%	6.62E+10	70.64%	6.67E+10	71.01%
	0 ac, 1 me1	6.25E+08	0.79%	5.55E+08	0.76%	6.36E+08	0.78%	3.38E+08	1.31%	6.71E+08	0.72%	8.01E+08	0.85%
	0 ac, 2 me1	6.68E+07	0.08%	4.34E+07	0.06%	7.21E+07	0.09%	9.15E+07	0.35%	2.22E+07	0.02%	3.95E+07	0.04%
	1 ac, 0 me1	2.36E+10	29.72%	2.16E+10	29.63%	2.45E+10	30.01%	4.70E+09	18.20%	2.45E+10	26.18%	2.47E+10	26.27%
	2 ac, 0 me1	1.92E+09	2.41%	2.03E+09	2.78%	2.16E+09	2.65%	0.00E+00	0.00%	2.29E+09	2.44%	1.72E+09	1.83%
AcH4 4-17 acetylation Peptide: GKGGKGLGKGGAKR	0 ac	3.72E+10	50.33%	3.29E+10	50.99%	3.75E+10	50.69%	1.11E+10	72.66%	4.75E+10	56.38%	4.87E+10	57.72%
	1 ac	2.90E+10	39.21%	2.41E+10	37.32%	2.85E+10	38.51%	3.42E+09	22.48%	2.85E+10	33.85%	2.99E+10	35.41%
	2 ac	5.83E+09	7.89%	5.65E+09	8.74%	6.02E+09	8.14%	6.14E+08	4.04%	6.42E+09	7.62%	4.60E+09	5.44%
	3 ac	1.71E+09	2.31%	1.68E+09	2.60%	1.76E+09	2.38%	1.04E+08	0.68%	1.64E+09	1.94%	1.08E+09	1.28%
	4 ac	1.96E+08	0.27%	2.20E+08	0.34%	2.03E+08	0.27%	2.08E+07	0.14%	1.77E+08	0.21%	1.26E+08	0.15%
H2A 4-11 acetylation Peptide: GKQGGKAR	0 ac	2.43E+09	86.50%	2.26E+09	86.61%	2.14E+09	84.63%	1.48E+09	90.30%	3.35E+09	91.74%	2.80E+09	90.34%
	1 ac	3.63E+08	12.89%	3.32E+08	12.75%	3.72E+08	14.72%	1.55E+08	9.47%	2.93E+08	8.02%	2.89E+08	9.34%
	2 ac	1.73E+07	0.62%	1.66E+07	0.64%	1.66E+07	0.66%	3.80E+06	0.23%	8.76E+06	0.24%	9.71E+06	0.31%

Table S1, Related to Figure 1. Cells in triplicate wells were cultured in 1 mM or 10 mM glucose for 24 hours. Histones were extracted and modifications analyzed by mass spectrometry as described in Experimental Procedures.

	10 mM ^{13}C-Glucose-2 hrs	10 mM ^{13}C-Glucose-24 hrs	1 mM ^{13}C-Glucose-2 hrs	1 mM ^{13}C-Glucose-24 hrs	Ratio 10 mM: 1 mM at 24 hours
H2A (1 ac enriched)	0.1666	0.5269	0.213	0.340	1.5
H3K18ac/K23ac	0.220	0.518	0.211	0.310	1.7
H3K18acK23ac (1 ac enriched)	0.236	0.315	0.181	0.215	1.5
H3K18acK23ac (2 ac enriched)	0.037	0.203	0.033	0.086	2.4
H3K9ac/K14ac (1 ac enriched)	0.219	0.546	0.210	0.341	1.6
H3K9me1K14ac	0.207	0.514	0.179	0.321	1.6
H3K9me2K14ac	0.201	0.515	0.184	0.319	1.6
H3K9me3K14ac	0.194	0.509	0.166	0.314	1.6
H4K16ac	0.148	0.520	0.143	0.359	1.4
H4 4-17 (2 ac; 1 enriched)	0.308	0.461	0.294	0.418	1.1
H4 4-17 (2 ac; 2 enriched)	0.062	0.330	0.061	0.186	1.8
H4 4-17 (3 ac; 1 enriched)	0.369	0.301	.0352	0.376	0.8
H4 4-17 (3 ac; 2 enriched)	0.123	0.371	0.118	0.247	1.5
H4 4-17 (3 ac; 3 enriched)	0.015	0.225	0.032	0.109	2.1
H4 4-17 (4 ac; 1 enriched)	0.415	0.193	0.376	0.321	0.6
H4 4-17 (4 ac; 2 enriched)	0.201	0.323	0.205	0.302	1.1
H4 4-17 (4 ac; 3 enriched)	0.062	0.0280	0.054	0.193	1.5
H4 4-17 (4 ac; 4 enriched)	0.006	0.175	0.009	0.051	3.4

Table S2, Related to Figure 1. **Regulation of histone lysine acetylation by glucose.**

LN229 glioblastoma cells were cultured overnight in 1 mM glucose. Triplicate plates were then washed and cultured in either 1 mM or 10 mM [U-$^{13}C_6$]-glucose for an additional 2 or 24 hours. Histones were extracted and fractional enrichment analyzed by mass spectrometry as described in Experimental Procedures.

Table S3, Related to Figure 1. Genes regulated by glucose and acetate. List of genes included in the heat map in Figure 1E and their cluster identification.

Supplemental Experimental Procedures

Antibodies and Inhibitors

Inhibitors used were as follows: Akt inhibitor VIII (Calbiochem, 20 μM), LY294002 (Cayman Chemical, 50 μM), PD325901 (LC Laboratories, 1 μM), rapamycin (50 nM). Antibodies used for Western blots include: AcH3, AcH4, H3, H4 (from Millipore), pAkt-Ser473, Akt, pACLY-Ser455, pERK, ERK, pS6, S6 (from Cell Signaling), tubulin (Sigma), ACLY (previously described (Wellen et al., 2009)). Antibodies used for IHC on murine tissues as follows: AcH4 (1:2000; clone 06-759-MN, Millipore), AcH3 (1:500; clone 06-599, Millipore), H3K4me1 (1:2000; #ab8895, Abcam), Ki67 (1:100; #ab16667 AbCam) pACL (1:100; #SAB4504020, Sigma), and pAkt (1:100; clone 736E11, Cell Signaling).

Cell Culture Conditions

Glioblastoma cell lines were cultured in RPMI (LN229 and LN18) or DMEM (SF188) supplemented with 10% FBS and penicillin/streptomycin mix and L-glutamine. C4-2 and PC-3 prostate cancer cells were cultured in RPMI + 5% FBS. Murine primary PanIN and PDA cells were previously described (Rhim et al., 2012). Cells were cultured in DMEM containing 50 mg/mL Glucose, 6 mg/mL Glutamine, supplemented with Soybean Trypsin Inhibitor (0.1 mg/mL; Sigma), ITS+ Premix (0.5% v/v; BD Biosciences), Bovine Pituitary Extract (25 mg/mL; Sigma), Nicotinamide (1.22 mg/mL; Sigma) and Penicillin/Streptomycin mix. To examine the effects of histone acetylation, cells were cultured in their respective medias with dialyzed FBS and described glucose concentrations for 24 hours before harvesting.

Protein lysate preparation for Western blot

Total protein was collected from adherent cells at 80% confluence on a 6-well dish. Cells were washed once with 1X PBS and directly lifting adherent cells off 6-well dishes in RIPA (1% NP-40, 0.5% Deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM Tris-HCL pH 8.0, freshly added 1X protease inhibitors). The lysates were sonicated at 30% duty cycle and an output control setting of 3-4. The sonicated samples were pelleted for 5 minutes and 16,000 rcf and the supernatant was collected and quantified by BSA (Thermo Scientific). Samples were prepared for western blot analysis through Invitrogen's NuPAGE and BOLT gel systems. Blot quantitation was performed using ImageJ software.

Acid extraction of histones

Adherent cells were cultured to 80% confluency in 6 well plates and nuclei were harvested by gentle lifting in cold NIB buffer (15mM Tris-HCL pH7.5, 60mM KCl, 15mM NaCl, 5mM $MgCl_2$, 1mM $CaCl_2$, 250mM sucrose, freshly added: 1mM DTT, 1X protease inhibitors, 10mM sodium butyrate, 0.1% NP-40). Nuclei were pelleted at 600 rcf for 5 min at 4 °C and washed twice using NIB buffer without NP-40. The pellets were immediately resuspended in 0.4N H_2SO_4 and rotated for at least 30 minutes at 4 °C in. After centrifugation at 11,000 rcf for 10 min at 4 °C, histones were precipitated from the supernatant by addition of 20% tricholoracetic acid (TCA) for at least 1 hour, followed by centrifugation at 16,000 rcf for 10 min at 4 °C. The pellet (or white film) was washed once with acetone containing 0.1% HCl, and finally with 100% acetone. Histone proteins were dried at room temperature and resuspended in water.

RNA interference

ACL-targeting siRNA pools were obtained from Dharmacon and transfected with RNAimax reagent, according to manufacturer's instruction. shRNA for stable silencing of murine ACL [previously described (Hatzivassiliou et al., 2005)] were subcloned into pLKO.1 vector and lentivirus generated from 293T cells. shRNA for silencing human ACL in pGIPZ vector were obtained from Open Biosystems (Clone ID: V2LHS_94212 used in figure). Cells were transduced with lentiviral shACL or shCtrl and either used immediately in experiments (LN229) or stable lines generated by puromycin selection (PanIN cells).

Chromatin Immunoprecipitation

Cells were grown to 80% confluency on 10 cm^2 dishes on day of harvest and were fixed on the dish with 1% formaldehyde for 10 minutes at room temperature. The reactions were quenched with 0.125 M glycine. The cells were then washed twice with 1X PBS and scraped in cell lysis buffer (10 mM Tris-HCl pH8.1, 10 mM NaCl, 1.5 mM $MgCl_2$, 0.5% NP-40), supplemented with protease inhibitors (Roche). The cell lysate was then incubated for 15 minutes on ice and crude nuclear extracts were collected by centrifugation at 600 rcf for 5 min at 4 °C. The pellet was resuspended in 0.5 mL of nuclear lysis buffer (50 mM Tris-HCl pH 8.1, 5 mM EDTA, 1% SDS) supplemented with protease inhibitors. The chromatin was fragmented with a Diagenode Bioruptor 300 (60 cycles of 30 s on followed by 30 s off, at 4 °C). To remove insoluble components, the samples were centrifuged at 13,000 rcf for 15 min at 4 °C and the supernatant were recovered. The supernatant were diluted 1:1 with dilution buffer (16.7 mM Tris-HCl pH 8.1, 1.1% Triton X-100, 0.01% SDS, 167 mM NaCl, 1.2 mM EDTA) supplemented with protease inhibitors and quantified by BCA. For each sample, 15 uL of protein G magnetic beads (Millipore 16-662) was added to 100 ug of protein in 500 uL of dilution

buffer and incubated with 2 uL of AcH4 antibody (Millipore 06-866) overnight at 4 °C. The next day, samples were washed with each of the following buffers, once, in the order of: low salt (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris HCl pH 8.1, 150 mM NaCl), high salt (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris HCl pH 8.1, 500 mM NaCl), LiCl (1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH 8.1, 250 mM LiCl), and TE (10mM Tris-HCl pH 8.1, 1 mM EDTA). ChIP DNA was eluted off the beads by incubating beads in 125 uL elution buffer (10 mM Tris-HCl pH 8.1, 1 mM EDTA, 1% SDS, 150 mM NaCl), supplemented with DTT to a final concentration of 5 mM, for 10 minutes at 65 °C. The supernatant was removed and ChIP DNA was eluted a second time in the same fashion. The combined supernatant was then incubated overnight at 65 °C to reverse crosslinks and proteinase K treated for 1 hour the next morning. Samples were purified using Macherey-Nagel DNA purification kit, with NTB binding buffer (for purification of DNA in high SDS).

Quantitative PCR

ChIP samples were diluted 1:20 and used as template in the Power Sybr Master Mix (ABI 4367659) and DNA was amplified using the ViiA-7 Real-Time PCR system. All primers were designed for the human gene at -1 kb upstream of the TSS. **SERPINA5** - Forward: GGATATCCAACAGCCACATAATTG; Reverse: GTTCAGCAAACACTGTCCATC. **PDGFRA** - Forward: GGGAGAAGGATGAAGGATGAC; Reverse: GATGCTCCAGGAACCAGAC. **E2F2** - Forward: GGAGAATCACTTTAACCCAGGA; Reverse: GTCTGAGGCAAGGTCTCTTT. **MCM10** - Forward: CAGCTCTCAGAAATGGTTGTATTC; Reverse: AACAGTCCTGATGCCATCTAC.

RNA was isolated from triplicate wells under each condition using TRIzol (Invitrogen) and cDNA synthesized using high-capacity RNA-to-cDNA master mix (Applied Biosystems), as per the kit instructions. cDNA was diluted 1:10 and used as template in the Power Sybr Master Mix (ABI 4367659) and DNA was amplified using the ViiA-7 Real-Time PCR system. Fold change in expression was calculated using ΔΔCt, with 18S as an endogenous control. All primers were designed at exons of the human gene. **SERPINA5** – Forward: TGGTCCCACACTTATCAGCA; Reverse: GTCCCAATGTCACACAGCAC. **PDGFRA** – Forward: GAAGAAGAGAGCTCCGATGTG; Reverse: TAGCAAGTGTACAACCCTGTG. **E2F2** – Forward: CGGAACAAACCAGTGGGATAA; Reverse: AGAAGGCTTCCACACAGATG. **MCM10** – Forward: TTTACCTCCTGAGCGAGTCA; Reverse: AGCACGTTGGTGATGTCATAG. **18S** – Forward: AAATCAGTTATGGTTCCTTTGGTC; Reverse: GCTCTAGAATTACCACAGTTATCCAA.

Acetyl-CoA and CoASH measurements

Cells were grown to 80% confluency on 10 cm^2 dish on day of harvest and were gently lifted, centrifuged at 500 rcf for 5 min and resuspended in 750 µL ice cold 10% TCA for extraction as described previously (Basu and Blair, 2012; Basu et al., 2011). The SILEC standard was added (250 µL) and samples were pulse sonicated with a probe tip sonicator on ice 30 times for 0.5 s. Samples were spun down for 10 min at 16,000 rcf at 4°C. SPE columns were activated with 1 mL methanol and equilibrated with 1 mL of water. The supernatant was transferred to the columns, filtrated, and then washed with 1 mL water. The acyl-CoAs were then eluted by washing the columns twice with 500 µL methanol containing 25 mM ammonium acetate into glass tubes. After evaporation to

dryness under nitrogen gas, the eluates were re-dissolved in 50 µL of 5 % 5-sulfosalicylic acid (w/v) and transferred to HPLC vials ready for LC-MS analysis.

Eight point standard curves were made using analytical grade commercial standards and the batch of SILEC standards used for analysis of cell samples. Standards were treated and extracted identically to the analyzed samples as previously described (Basu and Blair, 2012). Regression analyses of the standard curve and calculations of absolute concentrations were performed with Excel. All data points were interpolated from the standard curve.

Samples were kept at 4°C in a Leap CTC autosampler (CTC Analytics, Switzerland) with 10 µL injections used for LC-MS analysis. Chromatographic separation was performed using reversed phase Waters XBridge C18 column (2.1 x 150 mm, pore size 3 µm) on an Agilent 1100 HPLC system using a three solvent system: (A) 5 mM ammonium acetate in water, (B) 5 mM ammonium acetate in 95/5 acetonitrile/water (v/v), and (C) 80/20/0.1 (v/v/v) acetonitrile/water/formic acid, with a constant flow rate of 0.2 mL/min. Gradient elution was performed as follows: 2% B (isocratic) for 1.5 min, 2% to 20% (linear gradient) over 3.5 min, 20% to 100% B (linear gradient) B over 0.5 min, 100% B (isocratic) for 8 min, 100% C for 5 min, before equilibration at initial conditions for 5 min.

Samples were analyzed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) in positive electrospray ionization (ESI) mode and data was analyzed using Analyst software as described previously (Basu and Blair, 2011, 2012). The mass spectrometer operating conditions were as follows: ion spray voltage (5.0 kV), compressed air as curtain gas (15 psi) and nitrogen as nebulizing gas (8 psi), heater (15 psi), and collision-induced dissociation (CID) gas (5 psi). The ESI

probe temperature was 450°C, the declustering potential was 105 V, the entrance potential was 10 V, the collision energy was 45 eV, and the collision exit potential was 15 V. CoA thioesters were monitored using the transitions described previously (Basu and Blair, 2012).

Citrate and glutamate measurements

Cells grown to 80% confluency on 10 cm^2 plates were cultured with 2mM [$^{13}C_5{}^{15}N_2$]-glutamine from Cambridge isotopes (Cambridge, MA), and indicated concentrations of glucose in 10% dialyzed FBS for the indicated time points. Cells were gently lifted by scraping and centrifuged at 500 rcf for 5 min. Following aspiration, cell pellets were resuspended in 1 mL of ice cold 90:10 Methanol/water. Samples were pulse sonicated on ice at a rate of 1 pulse per s for 60 s. Sonicated samples were then centrifuged at 16,000 rcf for 10 min. Supernatants were transferred to glass tubes and dried under nitrogen. Dried cell samples were derivatized by the addition of 50 µL acetonitrile and 50 µL of pentafluorobenzyl bromide in acetonitrile (20% by volume). The samples were capped and allowed to incubate at room temperature for 24 hours. Derivatized samples were evaporated to dryness under nitrogen and resuspended in 100 µL of 95:5 hexanes/ethanol for LC-MS analysis.

LC separation was performed on a Waters 2690 HPLC coupled with a Chiralpak ADH column (5 µm particle, 4.6 mm x 250 mm) equipped with a post-column split for methanol infusion (750 µL/min). Mobile phase A was hexanes and mobile phase B was 50/50 methanol/isopropanol. The LC flow rate was 1 mL/minute with the following gradient, 1% B to 3 minutes, 60% B at 25 minutes and held until 30 minutes, 1% B at 32 minutes, and 1% B at 40 minutes.

A Thermo TSQ Quantum equipped with an APCI source operating in negative SRM mode was used for LC-ECAPCI-MS/MS analysis as previously described (Singh et al., 2000). The discharge current was 25 μA, the vaporizer and capillary temperature were 300°C, sheath and auxiliary gases were 30 and 5(arbitrary untis), respectively. The following transitions were used to detect the M0-M6 isotopes of citrate in order of increasing mass; 551 → 111, 552 → 112, 553 → 113, 554 → 114, 555 → 115, 556 → 116, 557 → 116, and M0-M7 isotopes of glutamate in order of increasing mass; 308 → 224, 309 → 225, 310 → 226, 311 → 227, 312 → 228, 313 → 229, 314 → 229.

RNA-Seq data analysis and clustering

All the RNA-seq data were processed using RUM pipeline in the Functional Genomics Core at the University of Pennsylvania (Grant et al., 2011). Differential analysis also has been done via RUM and EdgeR package (Robinson et al., 2010). Genes differentially regulated by 1 mM glucose and 10 mM glucose using an FDR cut off of 0.05 were classified as glucose regulated genes. Genes differentially regulated by 1 mM glucose and 1 mM glucose + acetate using an FDR cut off of 0.05 were classified as acetate regulated genes. Glucose and acetate regulated genes were used for generation of the Venn diagram and heatmap. We did hierarchical clustering analysis using log2-transformed gene expression values from all conditions and replicates for previously selected differential genes. In general, R package, "fastcluster" (Mullner, 2013), was used both for genes (rows) and samples (columns), but with different linkage criteria, Ward's criterion for genes and complete-linkage criterion for samples. As a distance measure, 1 – (correlation coefficient) was used in common. Clustering heatmap was drawn using z-score that is scaled across samples for each gene. Functional annotation was performed using the DAVID Gene Functional Annotation Tool (Huang da et al., 2007).

Detailed protocol for immunohistochemistry and automated scoring for human gliomas

Immunostaining was performed using the Discovery XT processor (Ventana Medical Systems). Tissue sections were blocked for 30 min in 10% normal goat serum in 2% BSA in PBS. Sections were incubated for 5 hours with the anti-pAkt (Novocastro, NCL-Akt-Phos, 1:300) or anti-ACH4 (clone 06-759-MN, Millipore, concentration 1:500) antibodies. Tissue sections were then incubated for 60 min with biotinylated goat anti-rabbit IgG (Vector labs, PK6101) at 1:200 dilution. Blocker D, Streptavidin- HRP and DAB detection kit (Ventana Medical Systems) were used according to the manufacturer instructions.

For automated scoring, each slide was scanned using an Aperio Scanscope Scanner (Aperio Vista, CA) and viewed through Aperio ImageScope software program. An individual blinded to the experimental design captured JPEG images from each core (circular area of 315 cm^2 corresponding to the entire core) at 10X magnification on the Aperio ImageScope viewing program. Quantification of immunostaining on each JPEG was conducted using an automated analysis program with Matlab's image processing toolbox based on previously described methodology (Venneti et al., 2013). The algorithm used color segmentation with RGB color differentiation, K-Means Clustering and background-foreground separation with Otsu's thresholding. To arrive at a score the number of extracted pixels were multiplied by their average intensity for each core (represented as pixel units). The final score for a given case and marker was calculated by averaging the score of two cores (for each case) for a given marker.

Detailed protocol for immunohistochemistry and scoring of human prostate tumors

Antigen retrieval was performed with Target Retrieval Solution (Dako North America, Carpinteria, CA, USA; #S1699) by boiling the slides for 20 min followed by cooling at room temperature for 20 min. The sections were then incubated with Protein Block Serum Free solution (Dako North America; #X0909) at room temperature for 1 hour. Incubations with primary antibodies were performed at the following dilutions: C-myc (Epitomics, Inc., Burlingame, California; #1472-1) at 1:100, p-Akt (Cell Signaling Technology; Beverly, MA; #4060) at 1:25, H3K9Ac (Abcam, Cambridge, MA; #ab10812) at 1:500, H3K18Ac (Abcam; #ab61233) at 1:500. H4K12Ac (Abcam; # ab61238) at 1:2000. The slides were incubated with the antibodies for 1 hour at room temperature in a humidified chamber. The slides were then washed in phosphate buffered saline and incubated with secondary antibody using the Dako Envision + Detection System according to the manufacturer's instructions (Dako North America). The staining was developed with 3,3 0-diaminobenzidine (DAB). The immunohistochemistry of the TMA were analyzed by Nuance/Vectra/inForm software (PerkinElmer Inc, Waltham, MA). The TMA were imaged with the Vectra mutli-spectral microscope system (PerkinElmer Inc, Waltham, MA). The tumor and normal tissue were segmented by trainable algorithms of inForm software. All the segmentations were reviewed and the unsatisfactory spots were scored manually. Then the nuclei were segmented, and the nuclear optical density of the DAB stain was scored.

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