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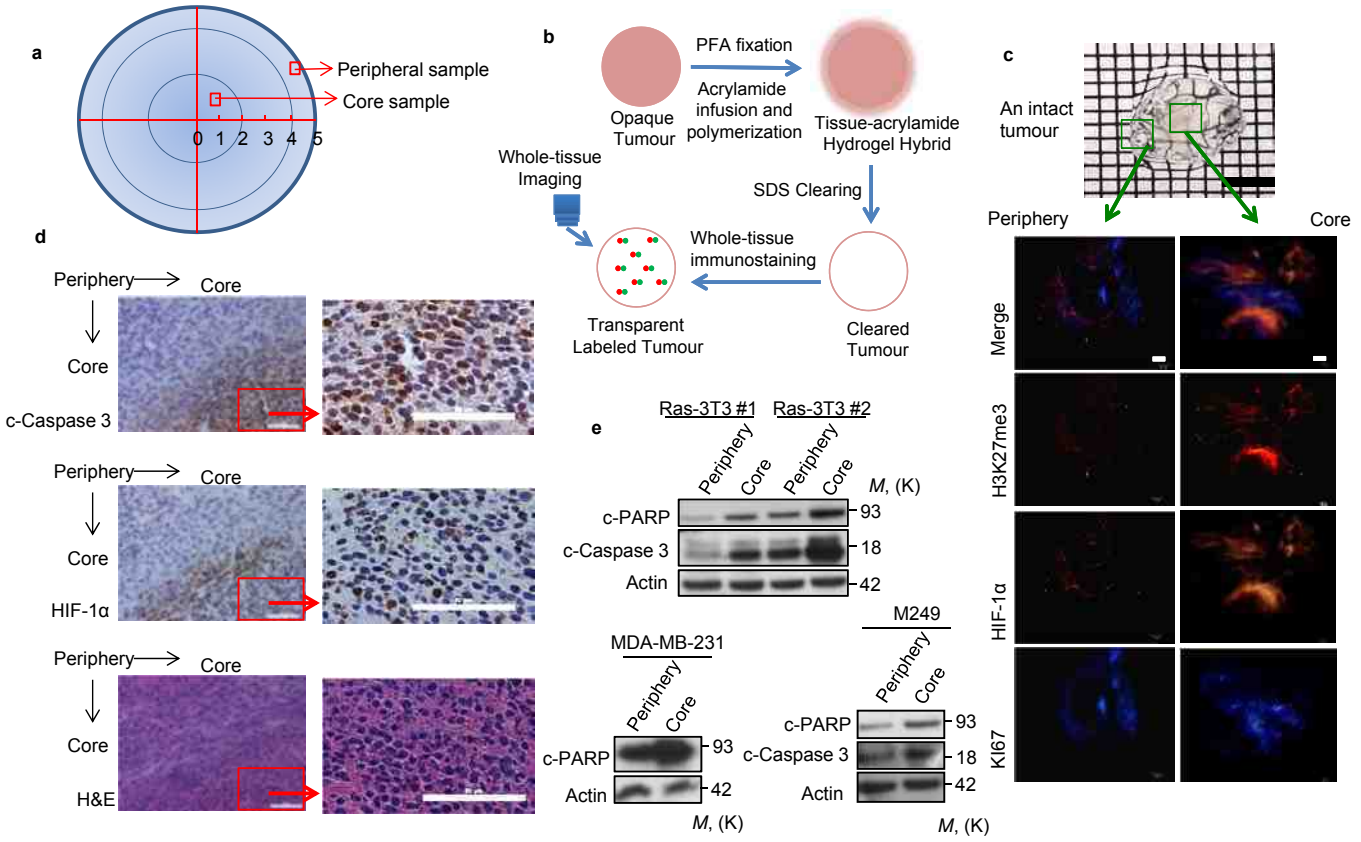
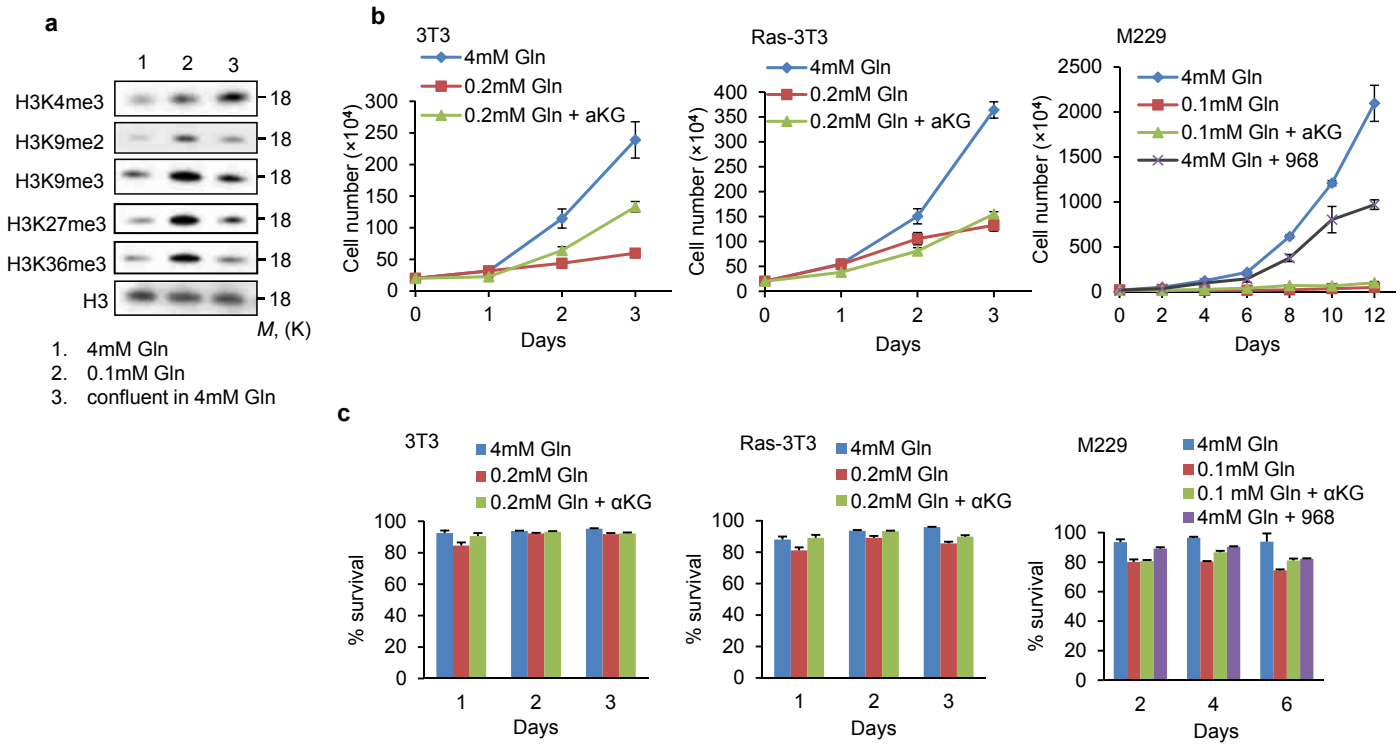
nature cell biology

Supplementary Figure 1. Tumour core regions display heterogeneity.



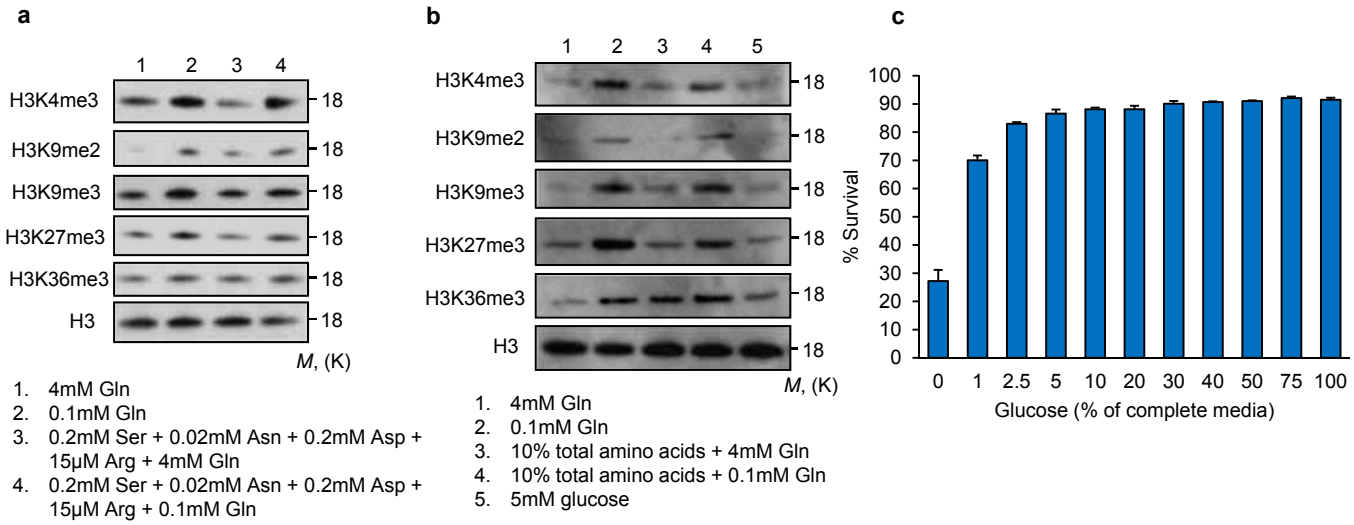
Supplementary Figure 1 Tumour core regions display heterogeneity. (a) Schematic of tumour dissection. One unit equals 20% of the radius. (b) Schematic of PACT (defined as Passive CLARITY) Technique procedure. (c) 3D imaging of M229 xenograft tumour depicting H3K27me3 (red), Hif1α (orange) and KI67 (blue) staining. Periphery and core of tumour are as indicated. Scale bar = 250 μm. (d) Immunohistochemistry staining in xenograft tumours. Whole tumours were sliced and stained with indicated antibodies (cleaved caspase 3, HIF-1α). H&E staining is also shown. Scale bar = 80 μm. (e) Tissues from periphery and core regions of xenograft tumours were lysed to collect whole cell lysate. Proteins were assessed by western blotting with specified antibodies. Unprocessed original scans of blots are shown in Supplementary Fig. 8.

Supplementary Figure 2. Histone methylation induced by low glutamine levels is not due to low proliferation rate of cells.



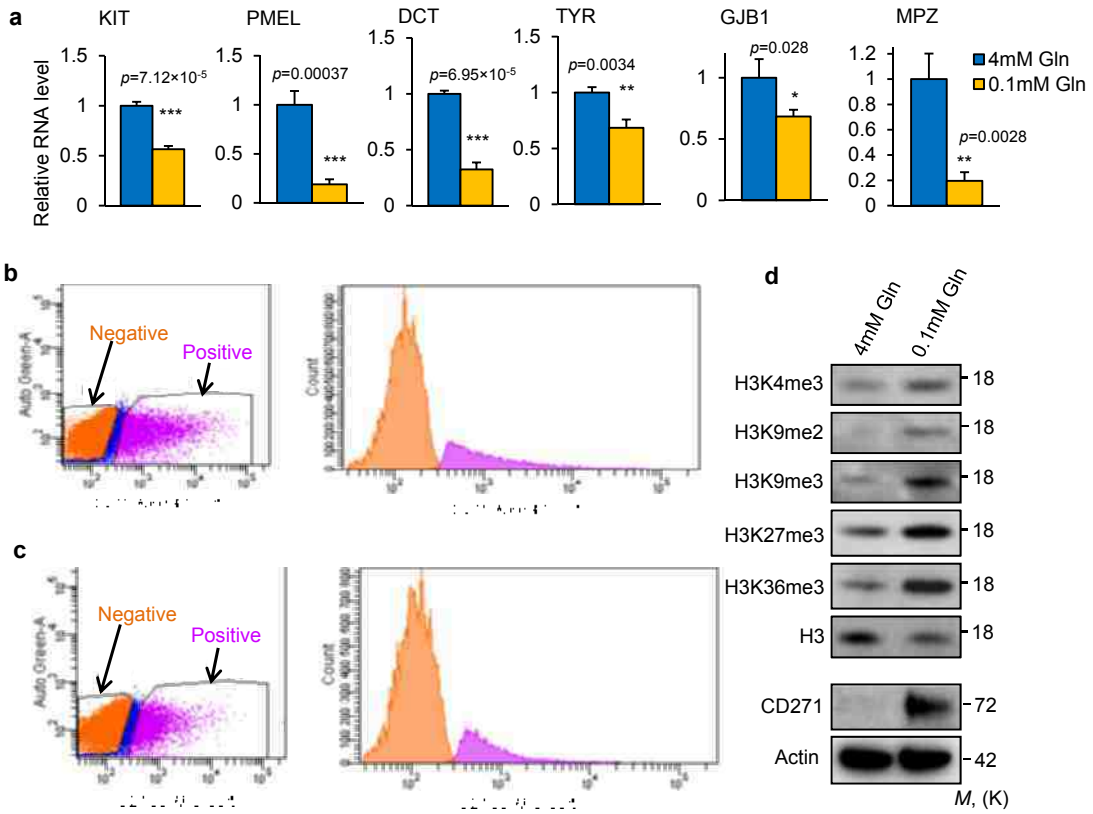
Supplementary Figure 2 Histone methylation in low glutamine is not due to low proliferation rate of cells. **(a)** For lane 1 and 2, 50% confluent M229 cells were cultured in complete (4mM glutamine) or 0.1mM glutamine medium for 4 days. For lane 3, 100% confluent M229 cells were cultured in complete medium for 4 days. Cells were lysed for histone extraction and histone lysine methylation levels were assessed by western blotting. Total histone H3 was used as loading control. Unprocessed original scans of blots are shown in Supplementary Fig. 8. **(b)** Cells were cultured under different conditions as indicated and cell number was determined by cell counting everyday (3T3 and Ras-3T3) or every other day (M229). Data represent mean ± S.D., n=3 independent experiments. **(c)** Cells were cultured under different conditions as indicated. After PI staining, cell survival was assessed by flow cytometry. Data represent mean ± S.D., n=3 independent experiments. Source data for **b** and **c** are shown in Supplementary Table 4.

Supplementary Figure 3. Low glutamine is the major driver of histone methylation.



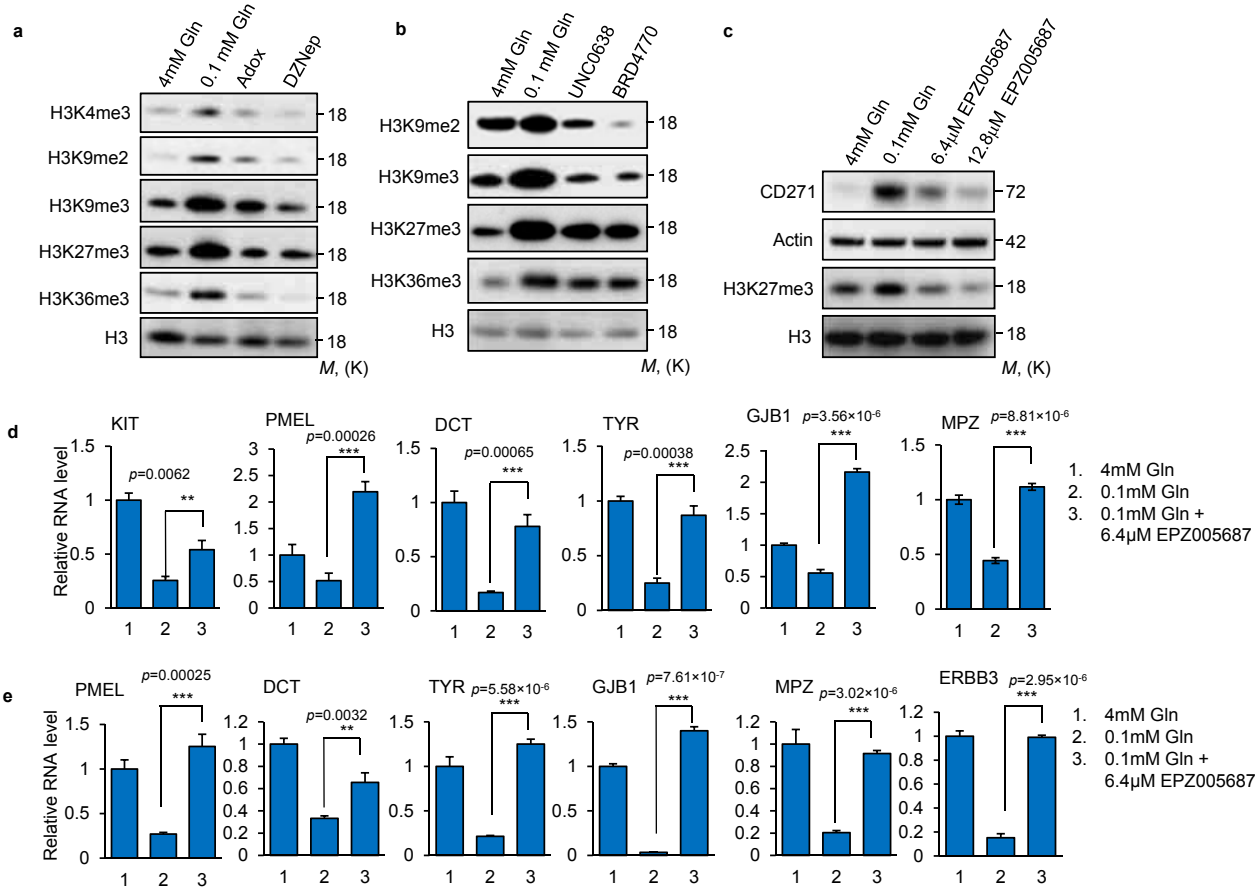
Supplementary Figure 3 Low glutamine is the major driver of histone methylation. **(a,b)** M229 cells were cultured under different conditions for 4 days as indicated. After that, cells were lysed for histone extraction and histone lysine methylation levels were assessed by western blotting. Total histone H3 was used as loading control. Unprocessed original scans of blots are shown in Supplementary Fig. 8. **(c)** M229 cell survival in low glucose medium. M229 cells were cultured in complete medium (25mM glucose) or medium with different glucose concentrations for 4 days. Medium was changed twice everyday. Cells were stained with PI and cell survival was measured by Flow cytometry. Data represent mean ± S.D., n=3 independent experiments.

Supplementary Figure 4. Low glutamine suppresses differentiation genes and induces CD271.



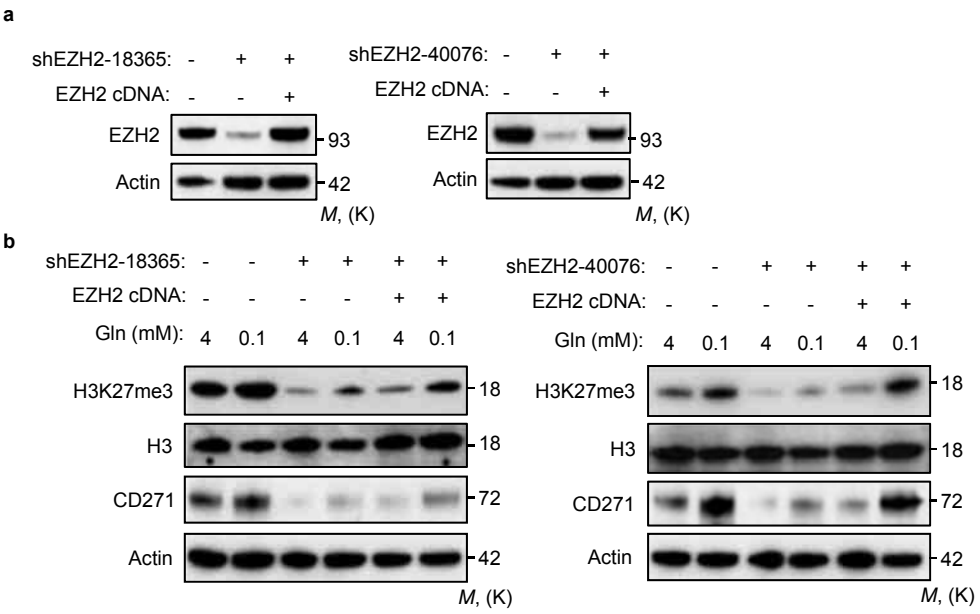
Supplementary Figure 4 Low glutamine induces suppression of differentiation genes, which can be reversed by EPZ005687. **(a)** M249 cells were cultured in complete or 0.1mM glutamine medium for 12 days, then RNA was extracted and gene expression was assessed by qPCR. Data represent mean ± S.D. of three independent RNA extracts. $*P<.05$, $**P<.01$, $***P<.001$ by unpaired Student's *t*-test. Source data can be found in supplementary Table 4. **(b)** M229 cells were stained with Alexa647 conjugated CD271 antibody. Cells were then sorted by flow cytometry and CD271- cells were collected. **(c)** The CD271- cells were cultured for 2 days and then stained with APC conjugated CD133 antibody. The stained cells were sorted again and CD271-/CD133- cells were collected. **(d)** CD271-/CD133- cells were cultured in complete (4mM Gln) or 0.1mM Gln medium for 4 days, then cells were harvested for histone extraction or whole cell lysate collection. Histone lysine methylation and protein levels were assessed by western blotting. Total histone H3 and Actin were used as loading control. Unprocessed original scans of blots are shown in Supplementary Fig. 8.

Supplementary Figure 5. Low glutamine-induced differential gene expression is reversed by H3K27me3 inhibitor.



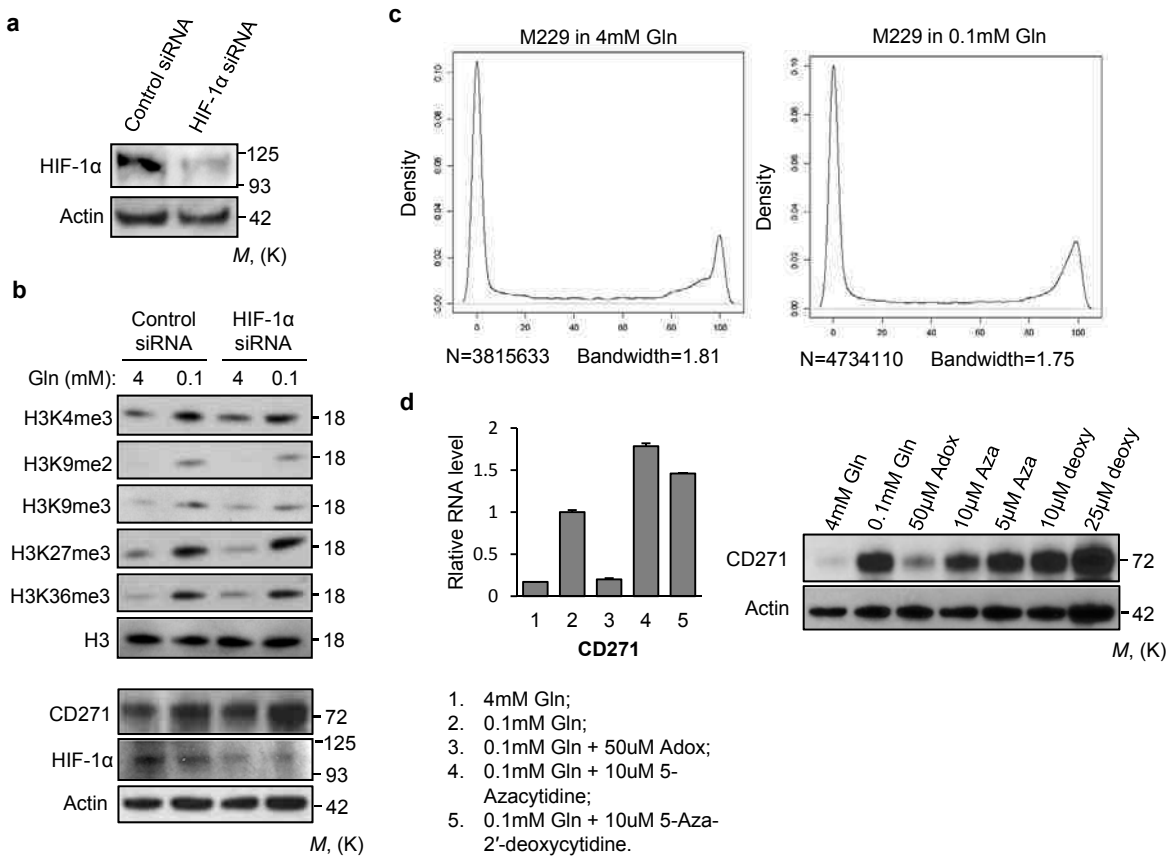
Supplementary Figure 5 Low glutamine-induced differential gene expression is reversed by H3K27me3 inhibitor. **(a,b)** M229 cells were cultured in complete medium or 0.1mM glutamine medium with or without global histone methylation inhibitors **(a)** or H3K9 specific methylation inhibitors **(b)** for 4 days; histones were extracted and protein levels were assessed by western blotting. Total histone H3 was used as loading control. Unprocessed original scans of blots are shown in Supplementary Fig. 8. **(c)** M229 cells were cultured in complete medium or 0.1mM glutamine medium with or without H3K27 methylation inhibitor EPZ005687 for 4 days, whole cell lysates were collected and protein levels were assessed by western blotting. Unprocessed original scans of blots are shown in Supplementary Fig. 8. **(d)** M229 and **(e)** M249 cells were cultured in complete medium or 0.1mM glutamine medium with or without H3K27 specific methylation inhibitor EPZ005687 for 4 days, then RNA was extracted and gene expression was assessed by qPCR. Data represent mean ± S.D. of three independent RNA extracts. $^{**}P<.01$, $^{***}P<.001$ by Student's *t*-test.

Supplementary Figure 6. Ectopic EZH2 expression rescues the EZH2 shRNA effects.



Supplementary Figure 6 Ectopic EZH2 expression rescues the EZH2 shRNA effects. **(a)** EZH2 was knocked down with two different shRNAs in M229 cells, then the cells were transiently transfected with EZH2 cDNA. Cells were harvested after 4 days and EZH2 protein was measured by western blotting. Actin was used as loading control. **(b)** EZH2 was knocked down with two different shRNAs in M229 cells, then the cells were transiently transfected with EZH2 cDNA in the medium with 4mM or 0.1mM Gln. Cells were harvested after 4 days, EZH2 protein and H3K27me3 were assayed by western blotting. Total H3 and Actin were used as loading control. Unprocessed original scans of blots are shown in Supplementary Fig. 8.

Supplementary Figure 7. HIF-1α or DNA methylation is not involved in low glutamine-induced CD271 expression.



Supplementary Figure 7 Neither HIF-1α nor DNA methylation is involved in low glutamine-induced epigenetic modification. **(a)** M229 cells were transfected with HIF-1α siRNA in a 6-well plate at day 1. At day 6, cells were lysed to collect whole cell lysate and HIF-1α level was assessed by western blotting. Actin was used as loading control. Unprocessed original scans of blots are shown in Supplementary Fig. 8. **(b)** M229 cells were transfected with HIF-1α siRNA in a 6-well plate at day 1. From day 2, cells were cultured in complete (4mM Gln) or 0.1mM Gln medium for 4 days. At day 6, cells were harvested for histone extraction or whole cell lysate collection. Histone lysine methylation and protein levels were assessed by western blotting. Total histone H3 and Actin were used as loading control. Unprocessed original scans of blots are shown in Supplementary Fig. 8. **(c)** M229 cells were cultured in complete or 0.1mM glutamine medium for 4 days, then DNA was extracted and used for whole genome DNA methylation sequencing. Methylation status of detected CpG sites (density) is shown. **(d)** M229 cells were cultured in complete medium, 0.1mM glutamine medium with or without histone methylation inhibitor Adox, DNA methylation inhibitors 5-Azacytidine and 5-Aza-2′-deoxycytidine. RNA and protein were harvested after 4 days for qPCR (left) and western blotting (right). PCR data represent mean ± S.D. of three independent experiments. Unprocessed original scans of blots are shown in Supplementary Fig. 8.

Figure 1d Ras-3T3

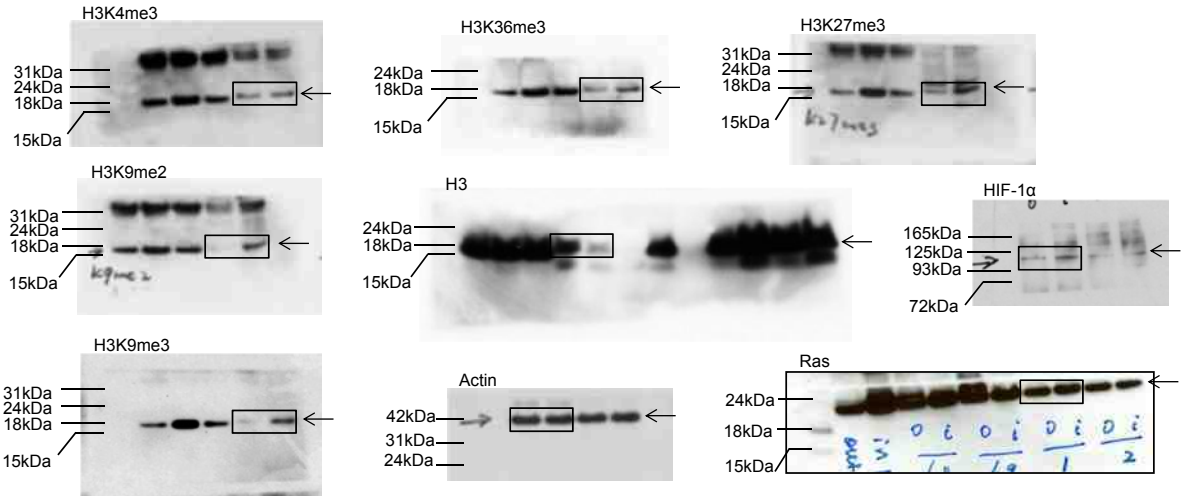
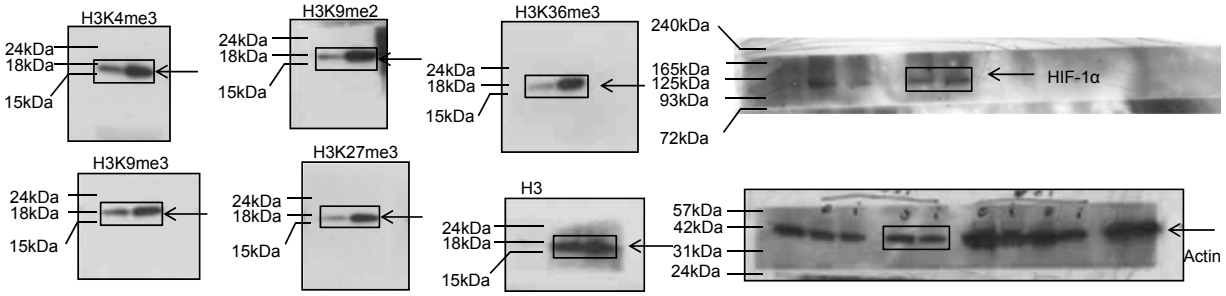
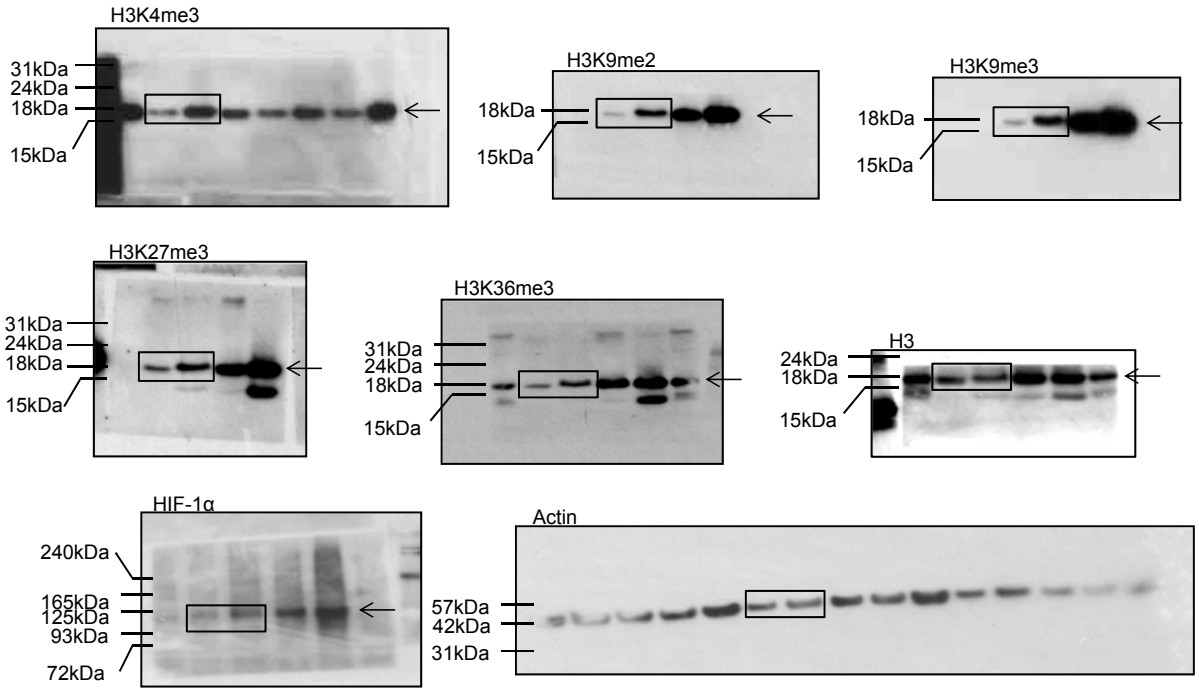


Figure 1d MDA-MB-231



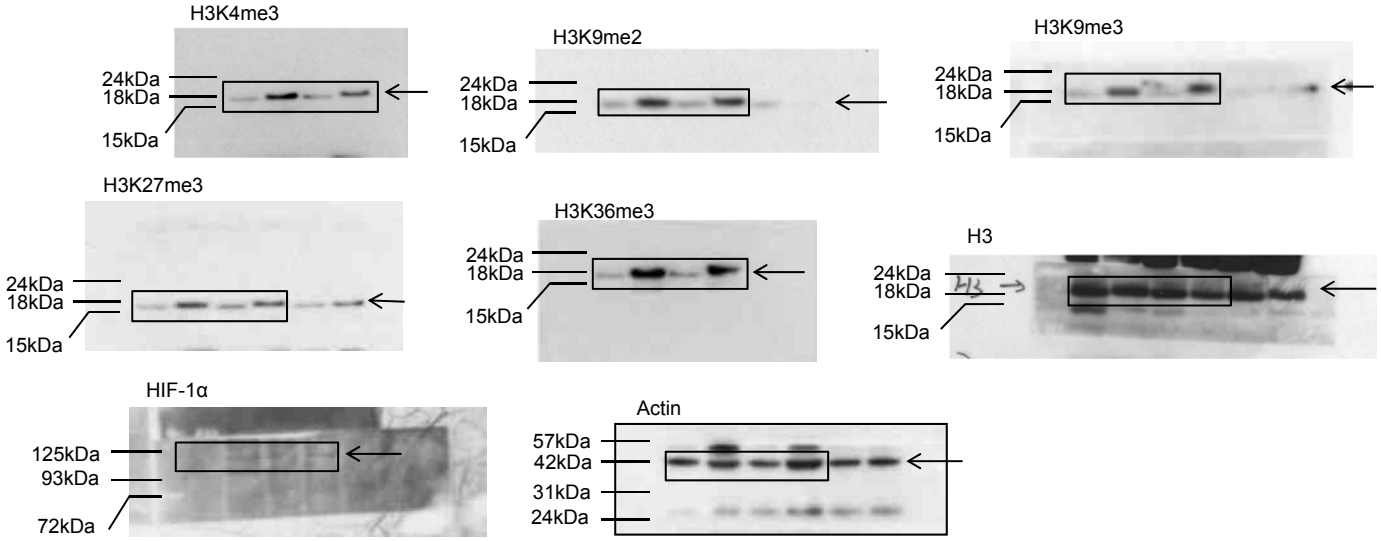
Supplementary Figure 8 Unprocessed blots of all figures. Black boxes indicate cropped portions that appear in the figures.

Figure 1d M249



Supplementary Figure 8 continued

Figure 1e



Supplementary Figure 8 continued

Figure 2a Ras-3T3

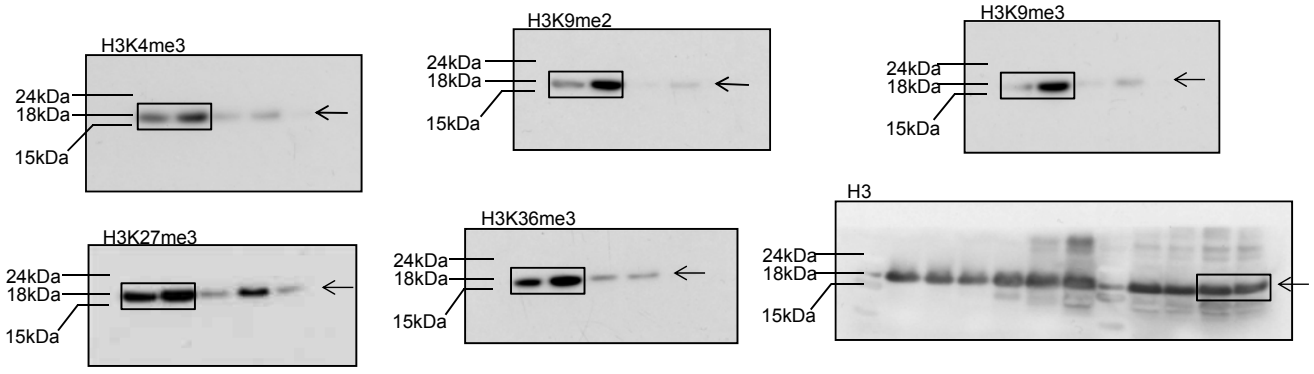
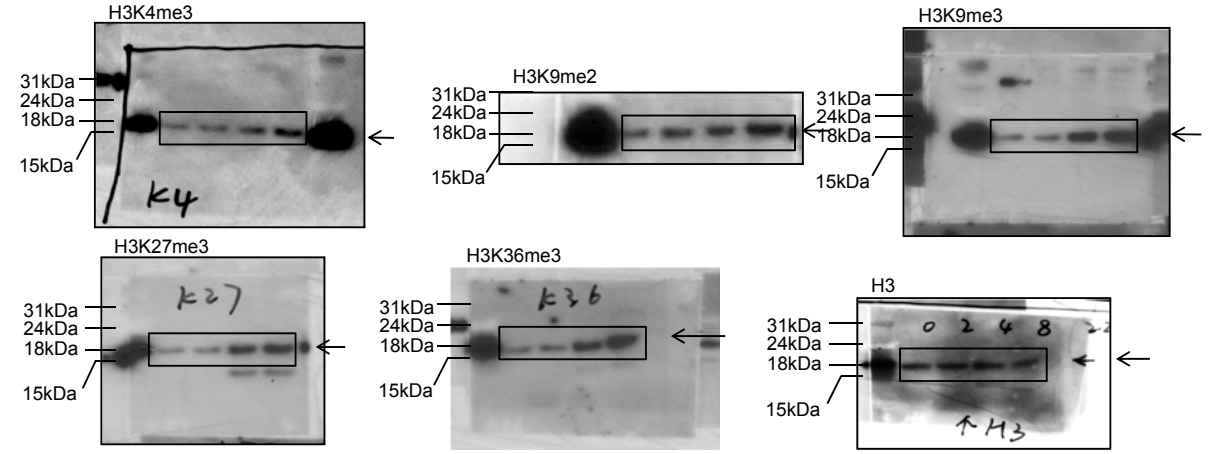


Figure 2a M229



Supplementary Figure 8 continued

Figure 2b

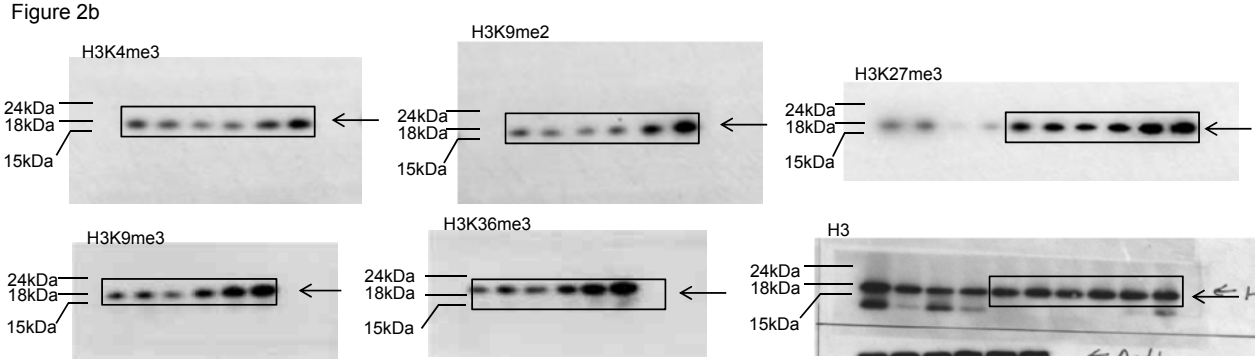
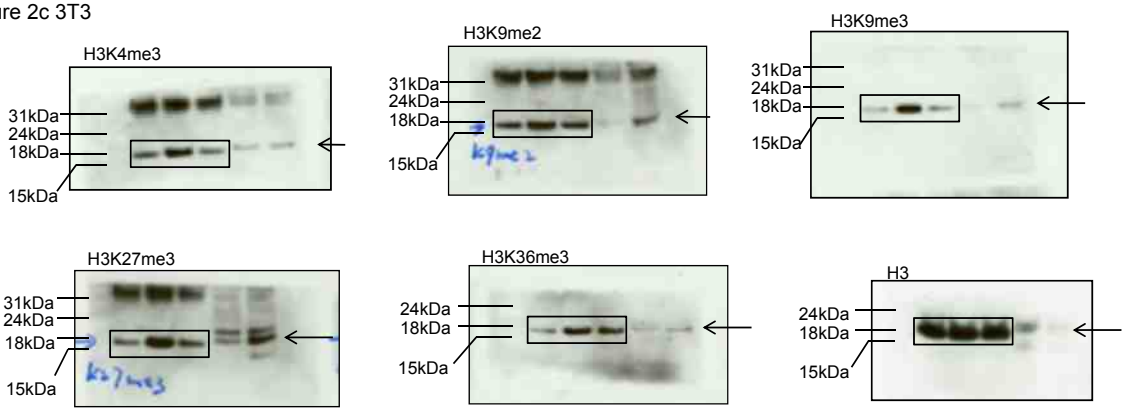


Figure 2c 3T3



Supplementary Figure 8 continued

Figure 2c Ras-3T3

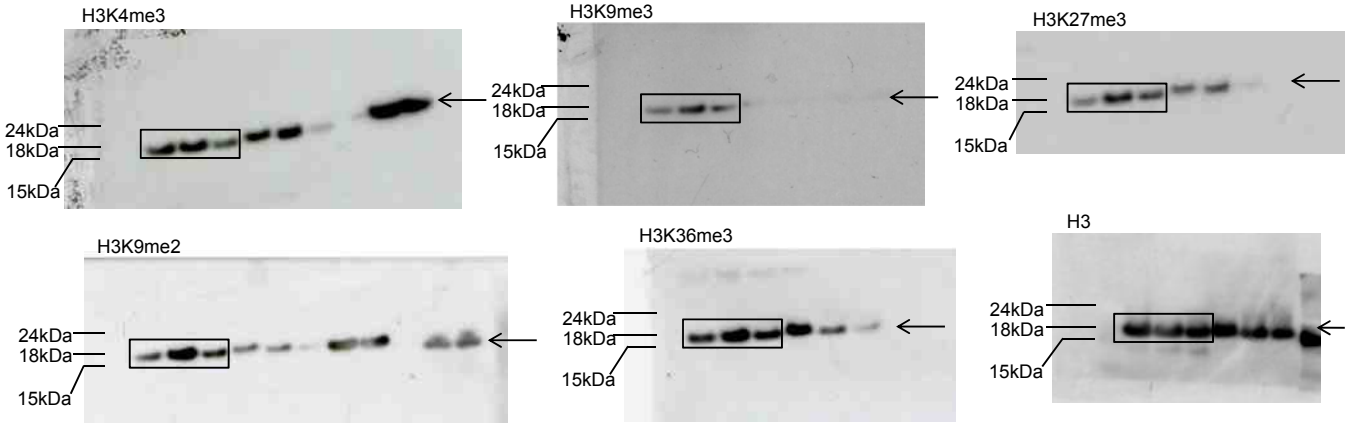
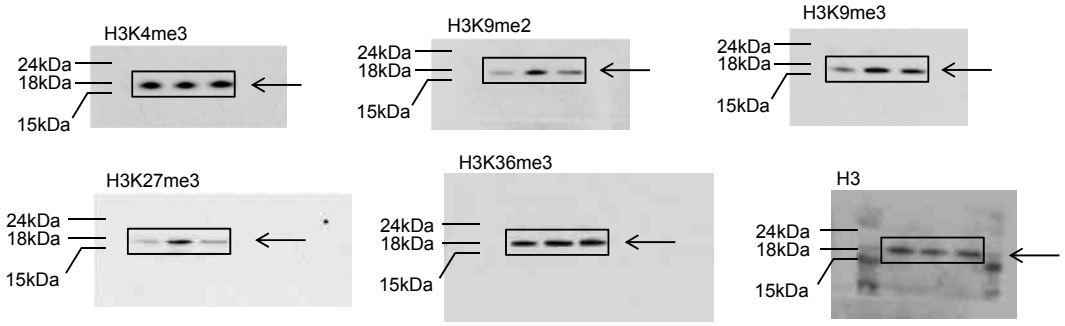


Figure 2c M229



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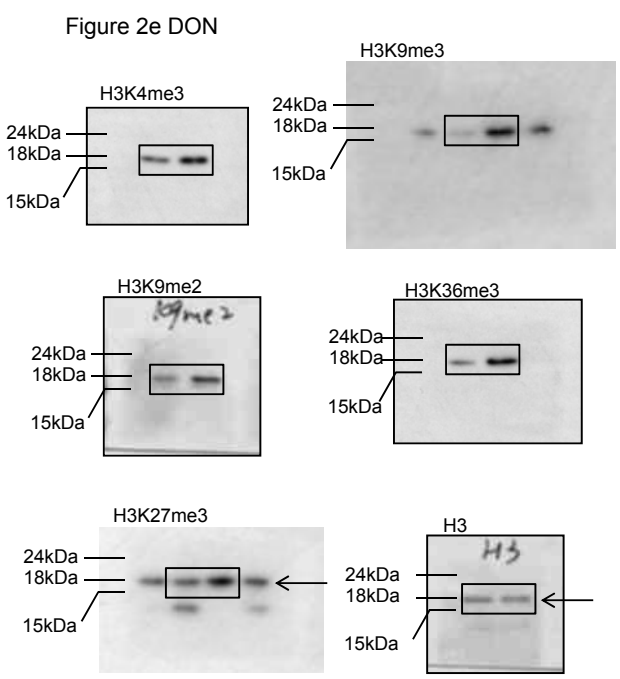
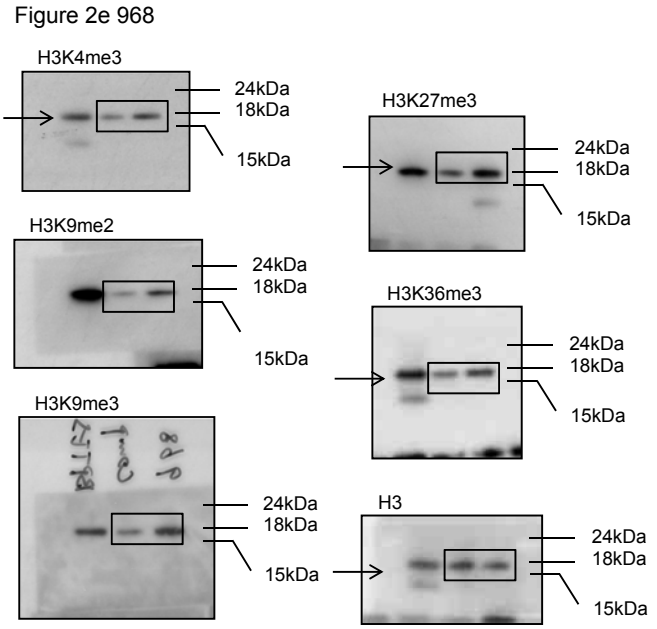




Supplementary Figure 8 continued

Figure 3a

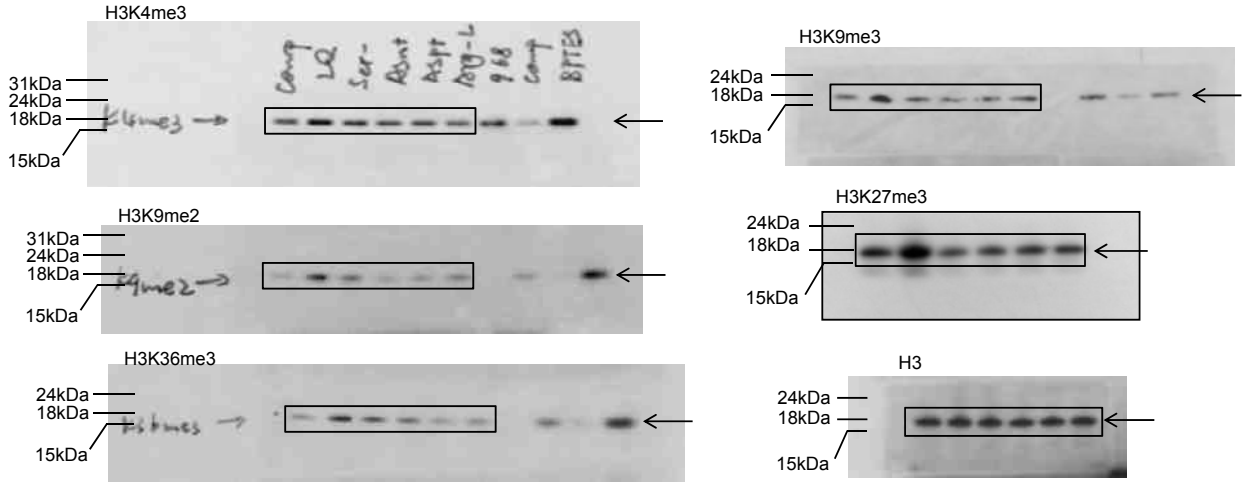
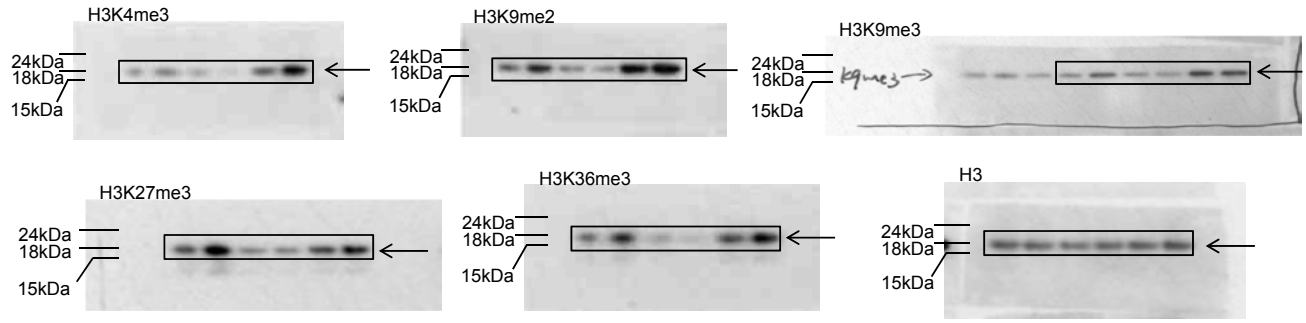


Figure 3b



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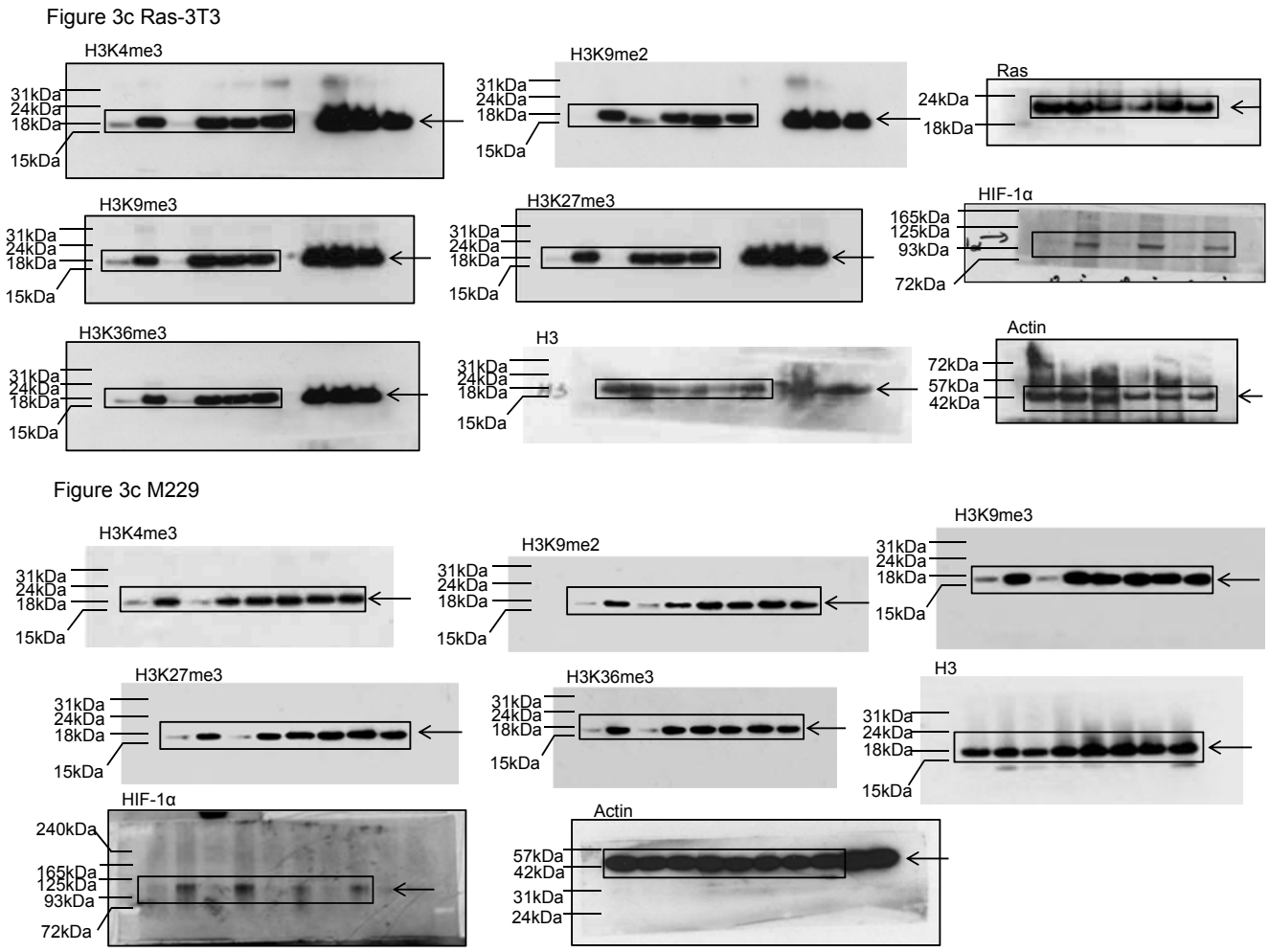
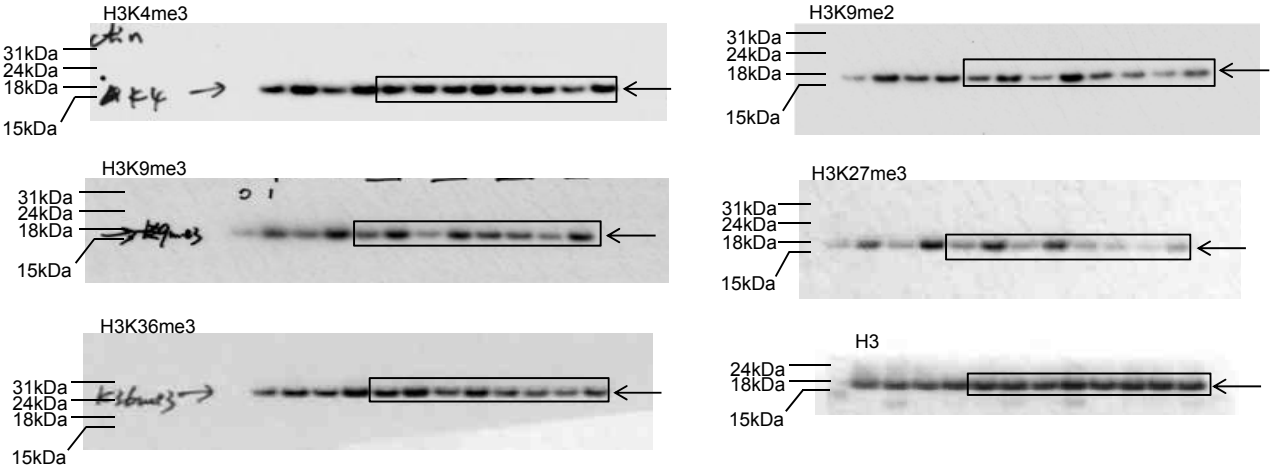


Supplementary Figure 8 continued

Figure 3e



Supplementary Figure 8 continued

Figure 4g

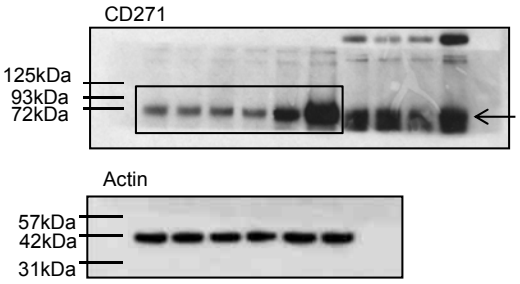


Figure 4i

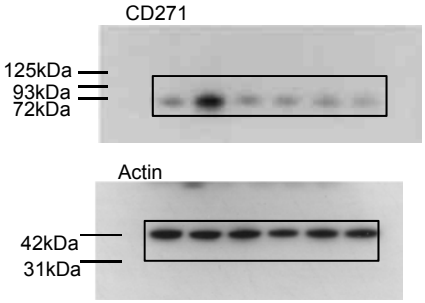


Figure 4h

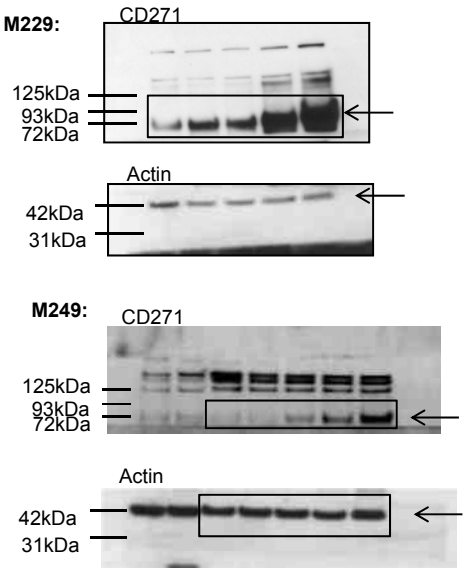
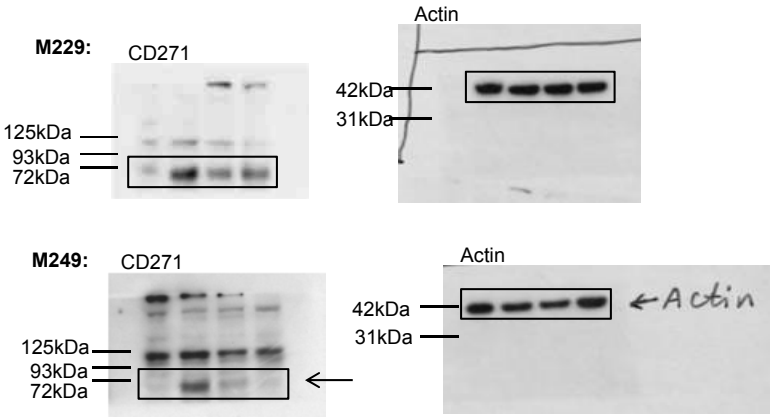


Figure 4j



Supplementary Figure 8 continued

Figure 4k left panels

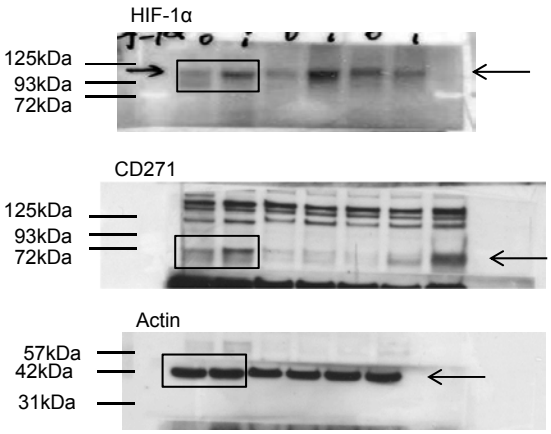


Figure 4k right panels

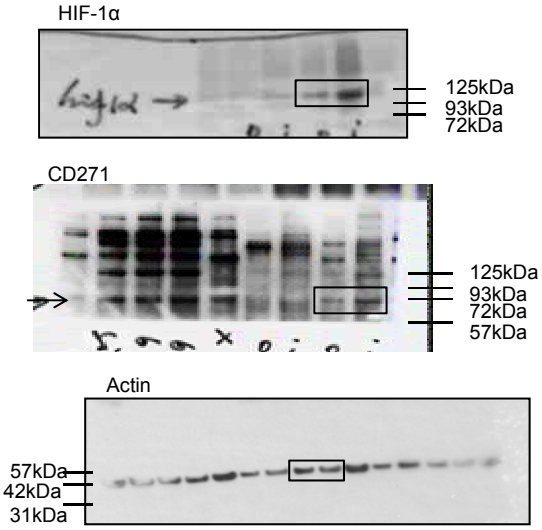
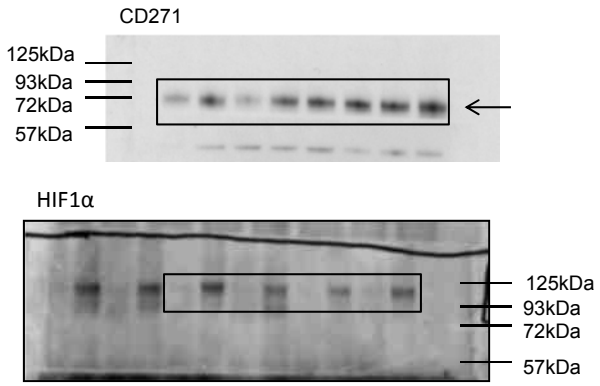
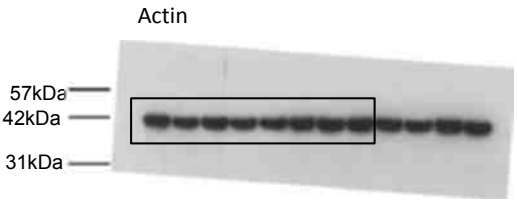


Figure 4l





Supplementary Figure 8 continued

Figure 6a

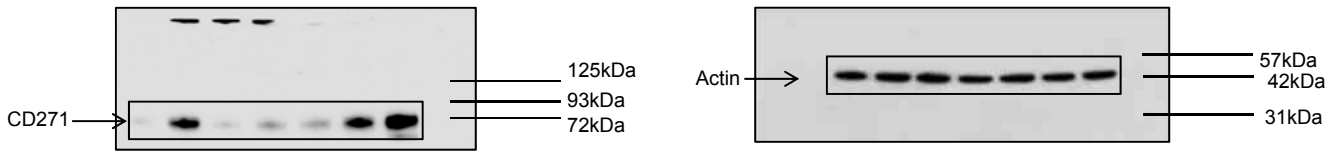


Figure 6c

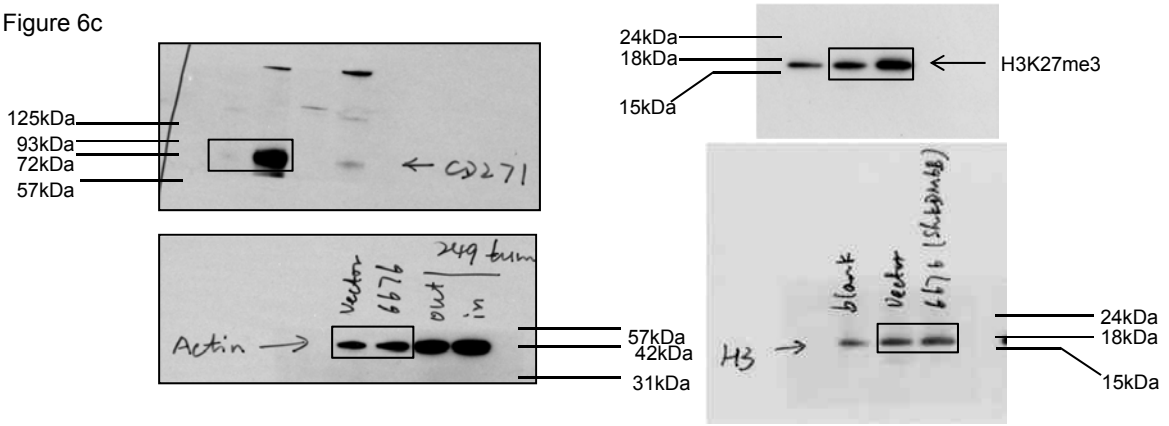


Figure 6d

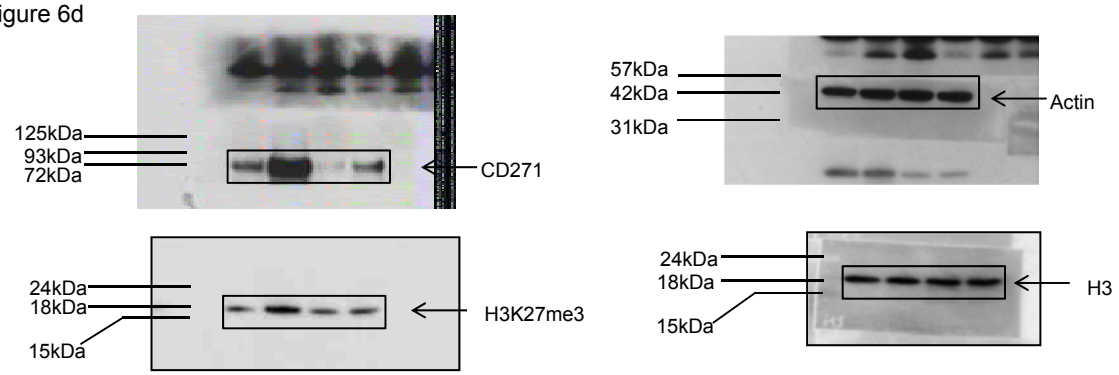


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Figure 7e

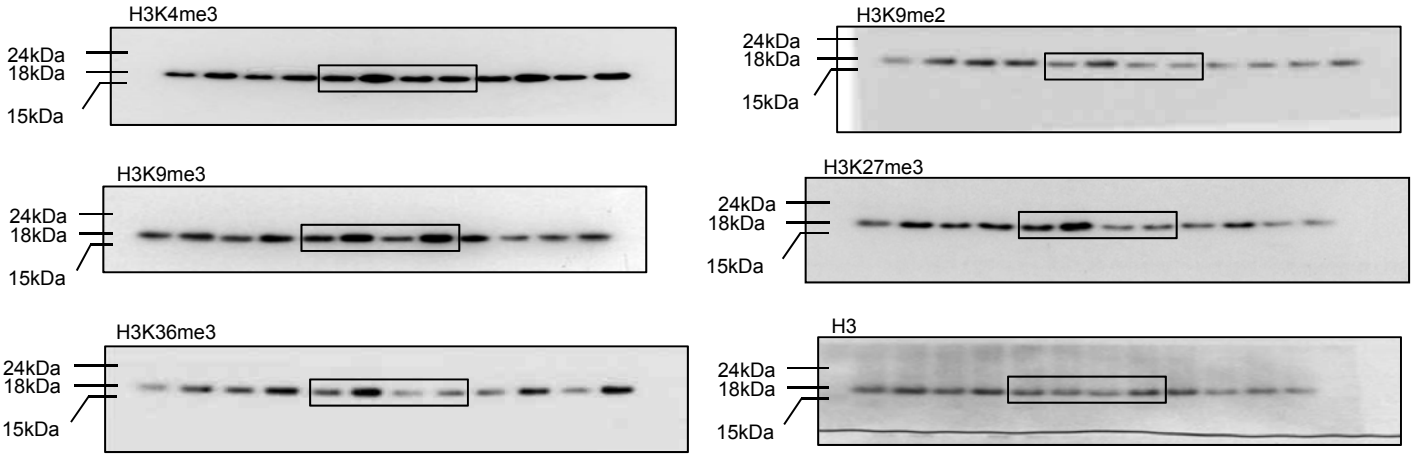
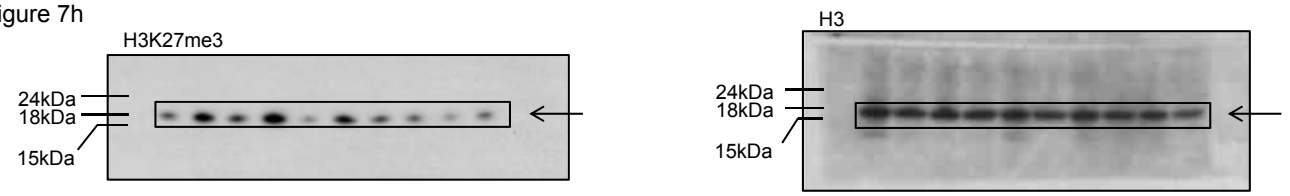
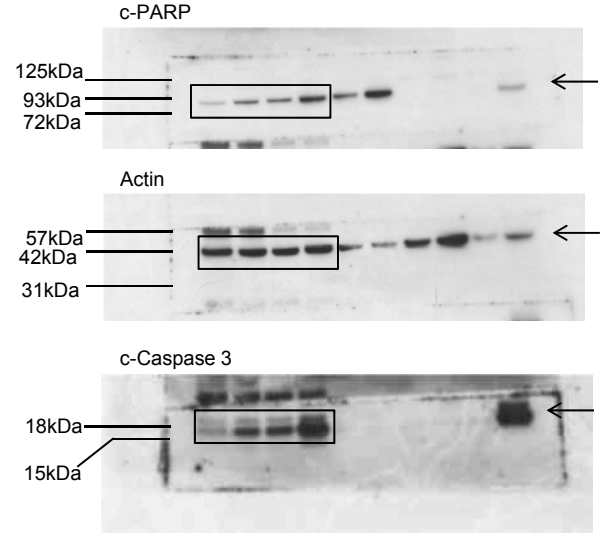


Figure 7h

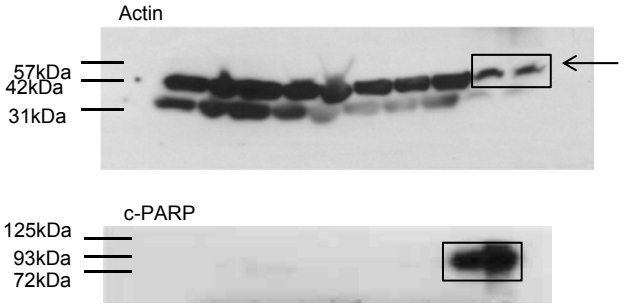


Supplementary Figure 8 continued

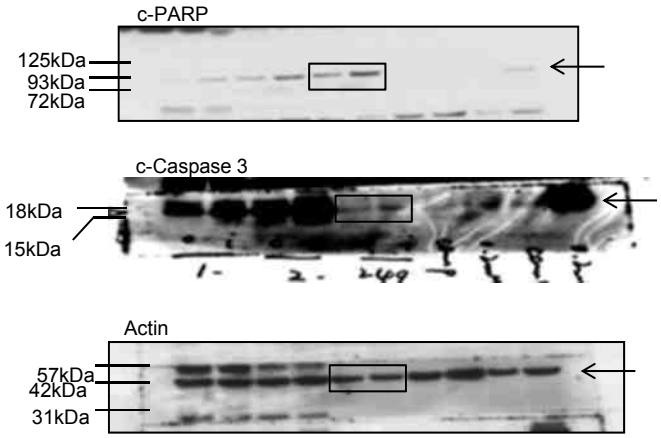
Supplementary Figure 1e Ras-3T3



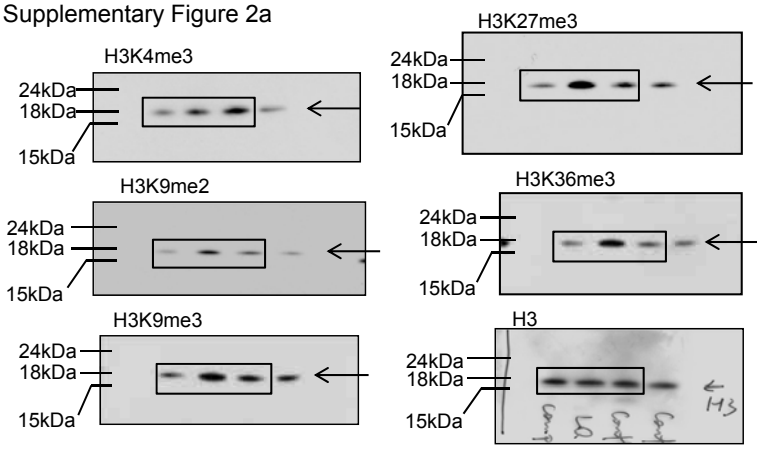
Supplementary Figure 1e MDA-MB-231



Supplementary Figure 1e M249



Supplementary Figure 2a



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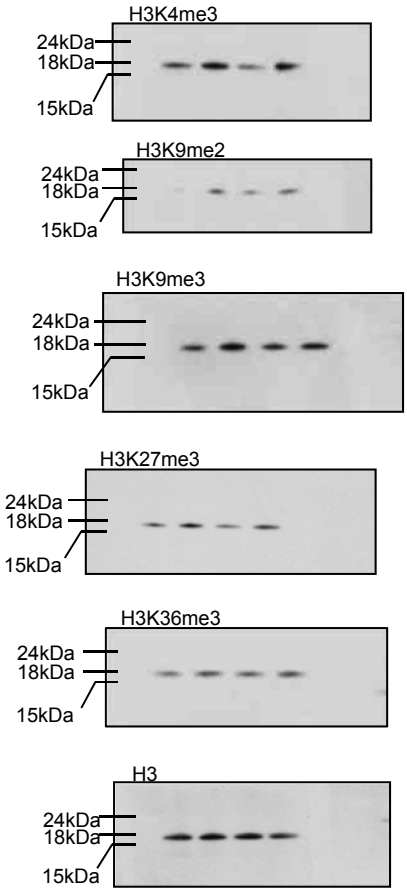
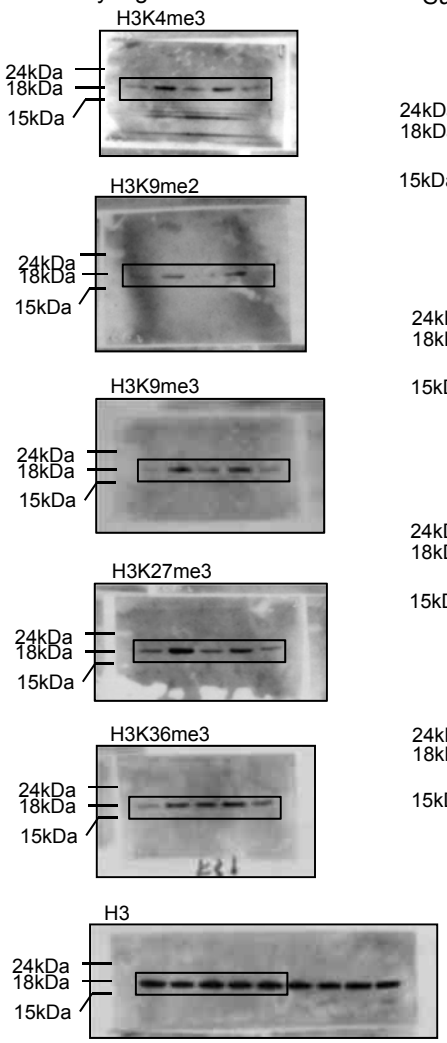
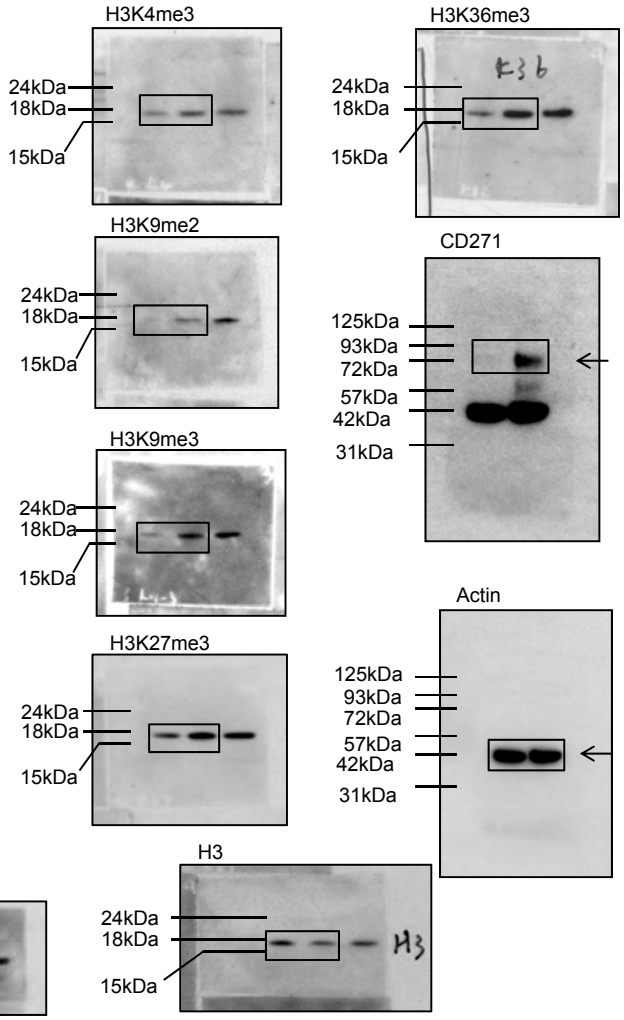
Supplementary Figure 3a



Supplementary Figure 3b



Supplementary Figure 4d



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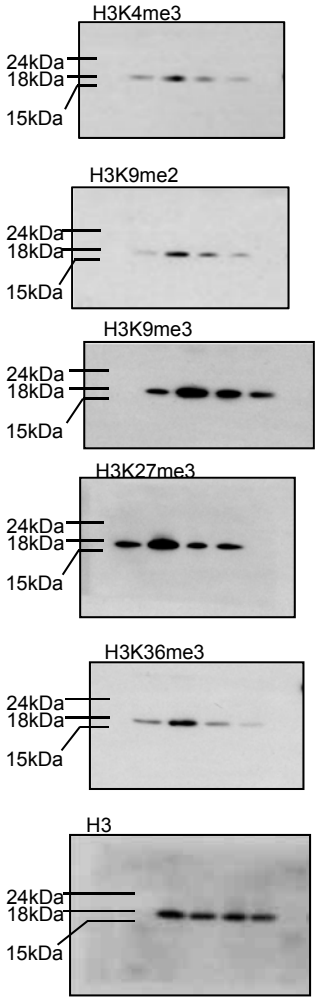
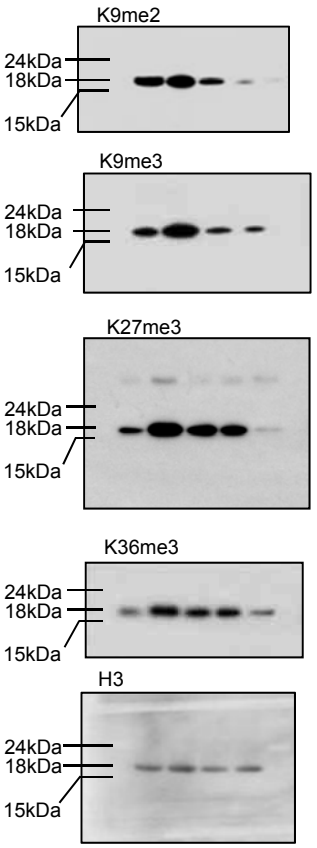
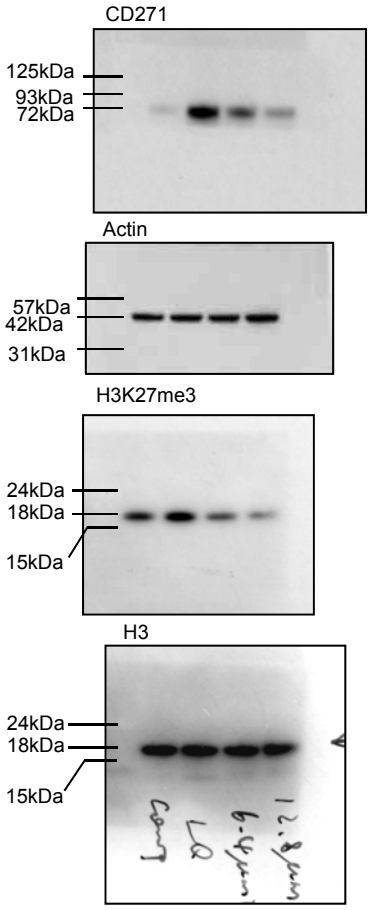


Supplementary Figure 5b

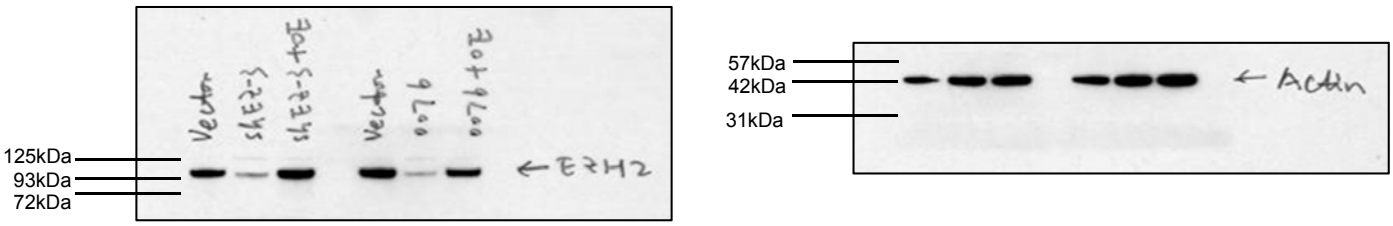


Supplementary Figure 5c

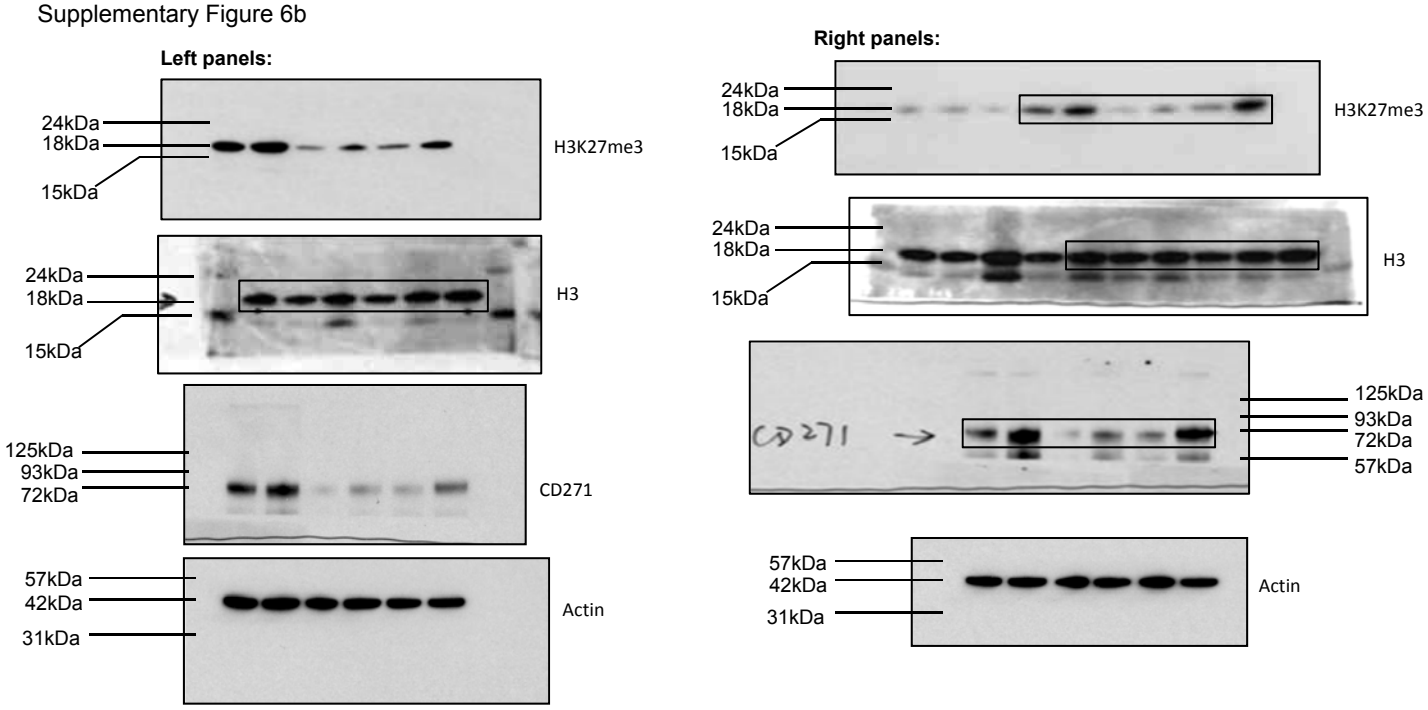


Supplementary Figure 8 continued

Supplementary Figure 6a

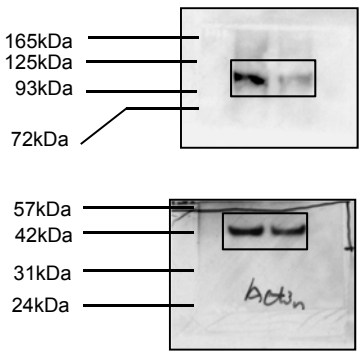


Supplementary Figure 6b

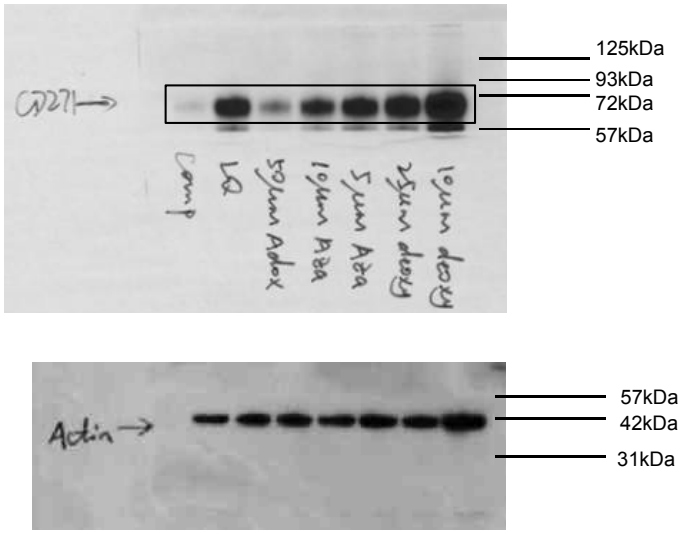


Supplementary Figure 8 continued

Supplementary Figure 7a

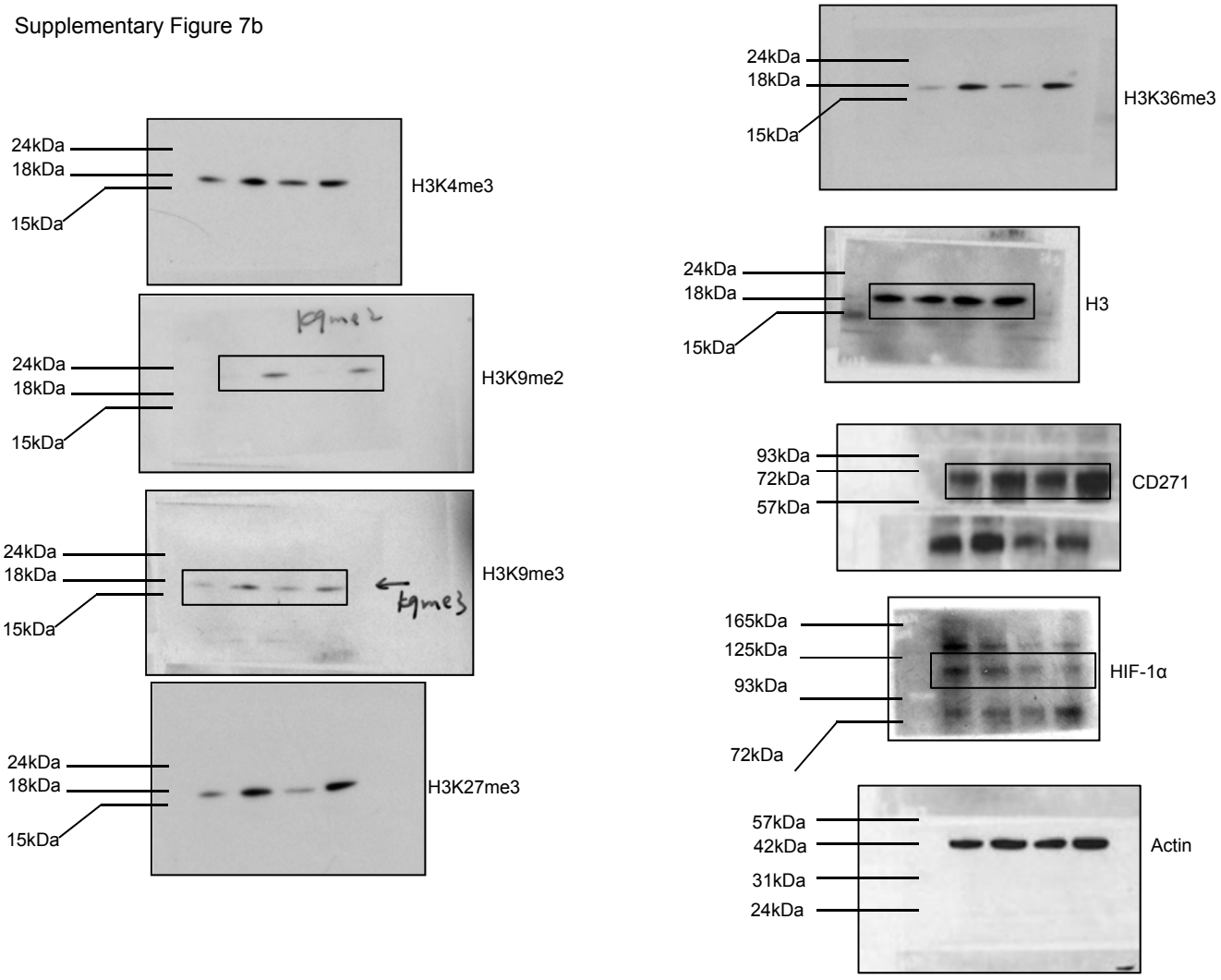


Supplementary Figure 7d



Supplementary Figure 8 continued

Supplementary Figure 7b



Supplementary Figure 8 continued

Supplementary Table Legends

Supplementary Table 1 Amino acid concentrations in tumour periphery and core regions. M229 xenograft tumours were harvested and separated into periphery and core samples (n=8 tumours). Metabolites were extracted from each sample and used for LC-MS analysis. Core/periphery ratio of each amino acid was shown. p value was calculated by Student's *t*-test.

Supplementary Table 2 Sequences of primers used in the study.

Supplementary Table 3 Information on antibodies used in the study.

Supplementary Table 4 Statistic source data.