DNA demethylase ALKBH1 promotes adipogenic differentiation via regulation of HIF-1 signaling

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Supplementary Information

Page 2 Figure S1

Page 3 Figure S2, Figure S3

Page 4 Figure S4, Figure S5

Page 5 Table S1

Page 6-8 Uncropped images of blots

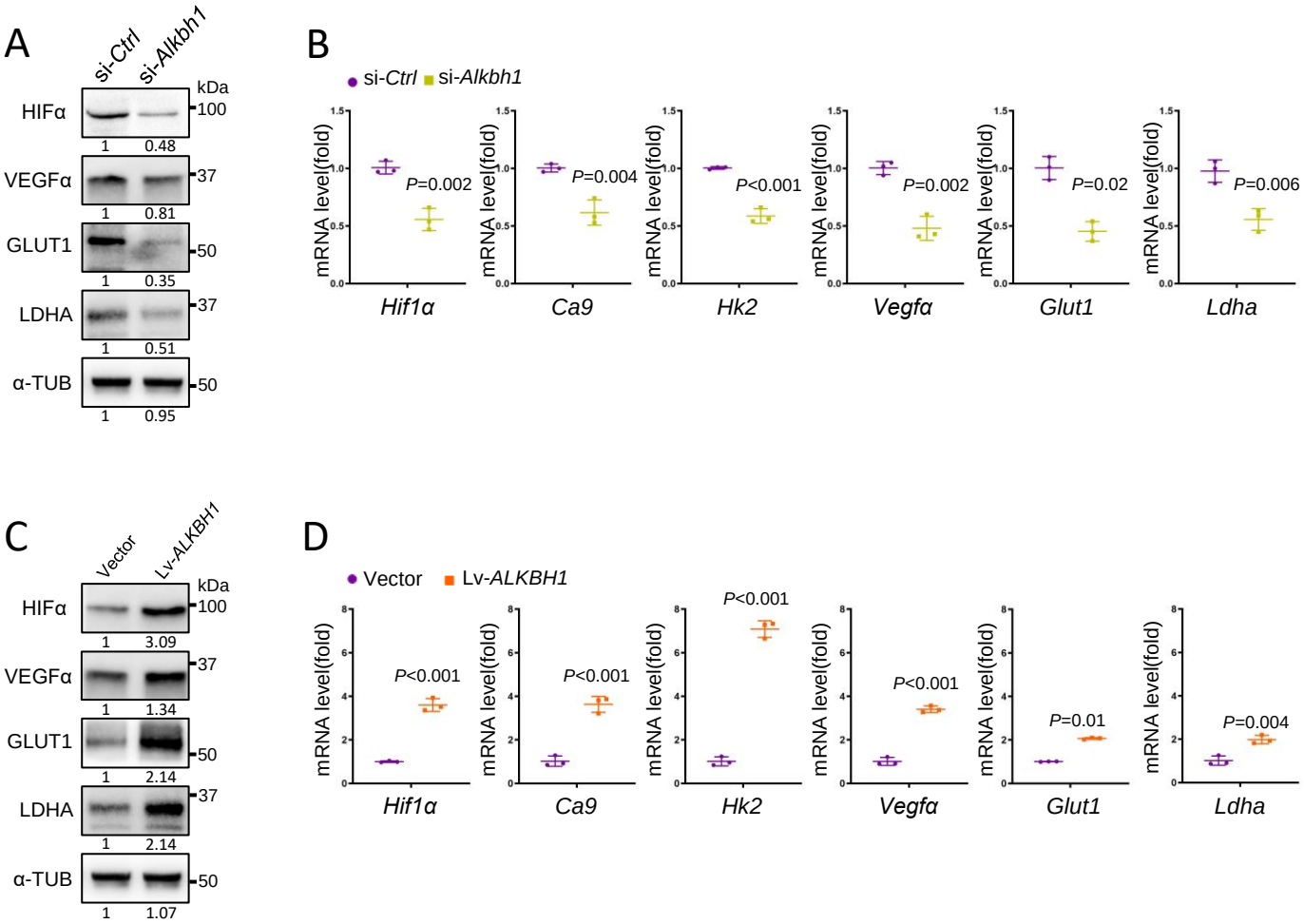


Figure S1. ALKBH1 activates HIF-1 pathway in 5% oxygen conditions. (A) Western blots of hypoxia-related proteins in *Alkbh1*-knocked 3T3-L1 cells in 5% oxygen conditions. **(B)** qRT-PCR analysis of *Hif-1α, Ca9, Hk2, Vegfα, Glut1* and *Ldha* in *Alkbh1*-deficient 3T3-L1 lines in 5% oxygen conditions (n=3). **(C)** Western blots and **(D)** qRT-PCR analysis confirm the activation of hypoxia axis in *ALKBH1*-overexpressed cells under 5% concentration of oxygen (n=3). The *P* values were calculated by two-tailed Student's *t* test. Scatter plots show individual data points±SD.

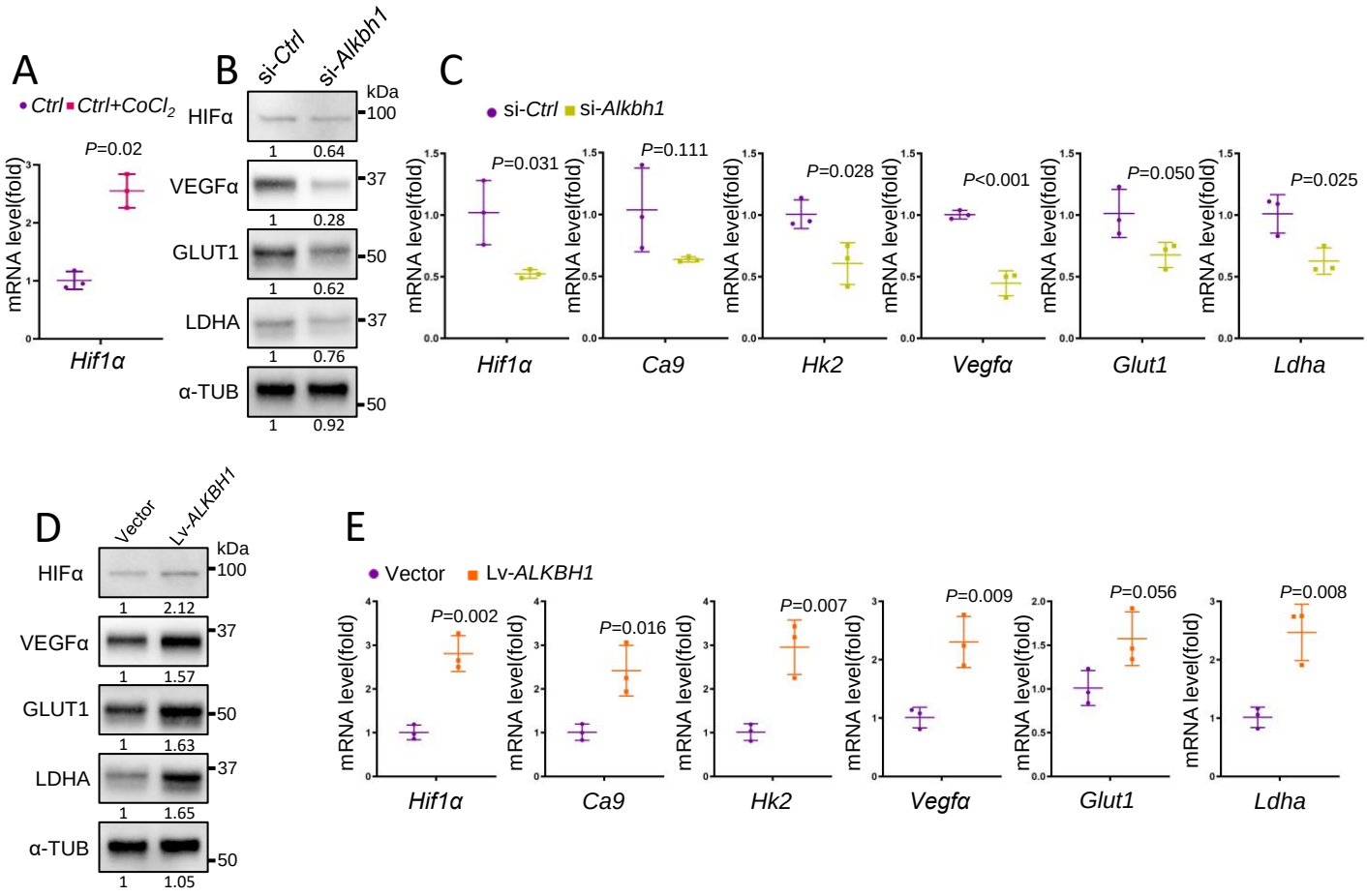


Figure S2. ALKBH1 activates HIF-1 pathway in hypoxia induced by $CoCl_2$. (A) qRT-PCR of *Hif-1α* at 24-hour treatment of $CoCl_2$. $CoCl_2$ activates HIF-1 pathway in 3T3-L1 cells (n=3). **(B)** Western blots of hypoxia-related proteins in *Alkbh1*-knocked 3T3-L1 cells in hypoxia induced by $CoCl_2$. **(C)** qRT-PCR analysis of *Hif-1α, Ca9, Hk2, Vegfα, Glut1* and *Ldha* in *Alkbh1*-deficient 3T3-L1 lines in hypoxia (n=3).). **(D)** Western blots and **(E)** qRT-PCR analysis confirm the overactivation of hypoxia axis in *ALKBH1*-overexpressed cells (n=3). The *P* values were calculated by two-tailed Student's *t* test. Scatter plots show individual data points±SD.

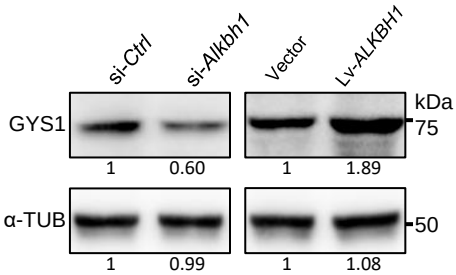


Figure S3. Positive relationship between ALKBH1 and GYS1 in hypoxia. Western blots confirms the relationship of ALKBH1 and GYS1 in 5% oxygen. GYS1 work as a target of ALKBH1.

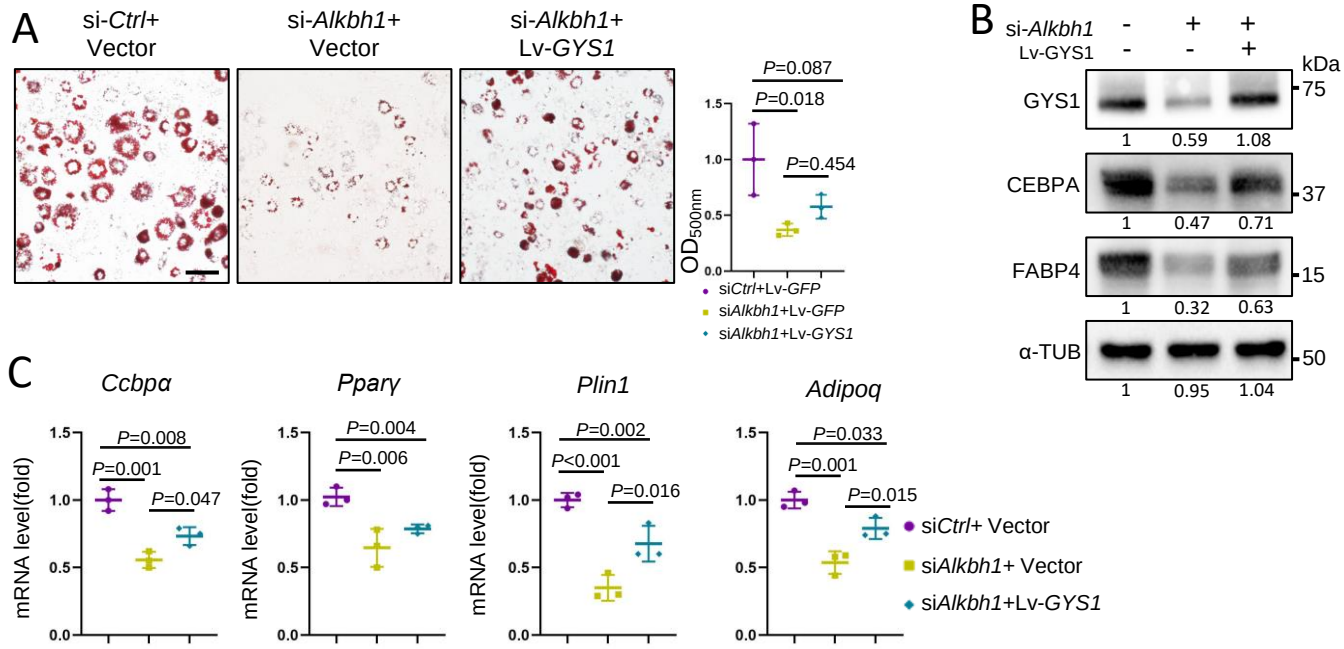


Figure S4. ALKBH1 demethylates DNA 6mA of GYS1. (A) Representative images of Oil red O staining in 3T3-L1 cells and quantitation analysis (n=3). Scale bar: 25μm. **(B)** Western blot analysisof HIF-1α , CEBPA and FABP4. Overexpression of GYS1 failed to fully rescued the decrease of adipogenic markers. **(C)** qRT-PCR shows the mRNA level of *Cebpα, Pparγ, Plin1* and *Adipoq* in si-*Ctrl*+Vector, si-*Alkbh1*+Vector and si-*Alkbh1*+Lv-*GYS1* group at 5 days of differentiation (n=3). The P values were calculated one-way ANOVA with Tukey's post hoc test. Scatter plots show individual data points±SD.

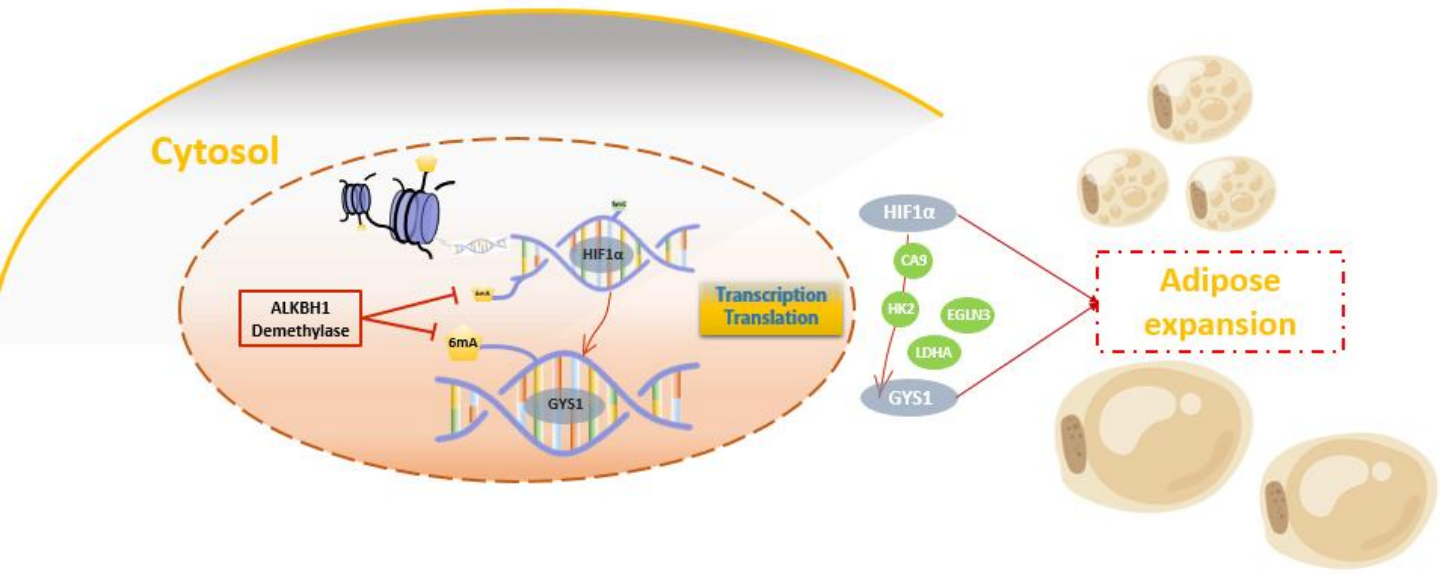


Figure S5. ALKBH1 enhances adipogenesis through activating HIF-1 axis. Intranuclear ALKBH1 demethylates the 6mA of *HIF1α* and *GYS1*, leading the active transcription of these two genes. Numerous transcriptions result in the excessive accumulation of HIF1α and GYS1 proteins. The activation of HIF-1 pathway promotes the adipogenic differentiation of mesenchymal stem cells.

siRNA sequences	
siRNA-sense	GGUCUAAAGAAGUGACUAATT
siRNA-antisense	UUAGUCACUUCUUUAGACCTT

Table S1. mouse siRNA sequences used in this study

Figure 1B

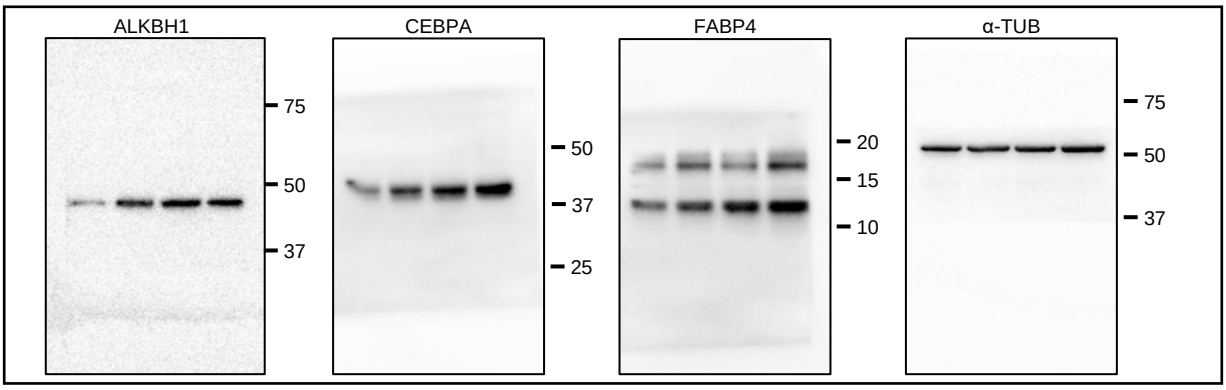


Figure 1D

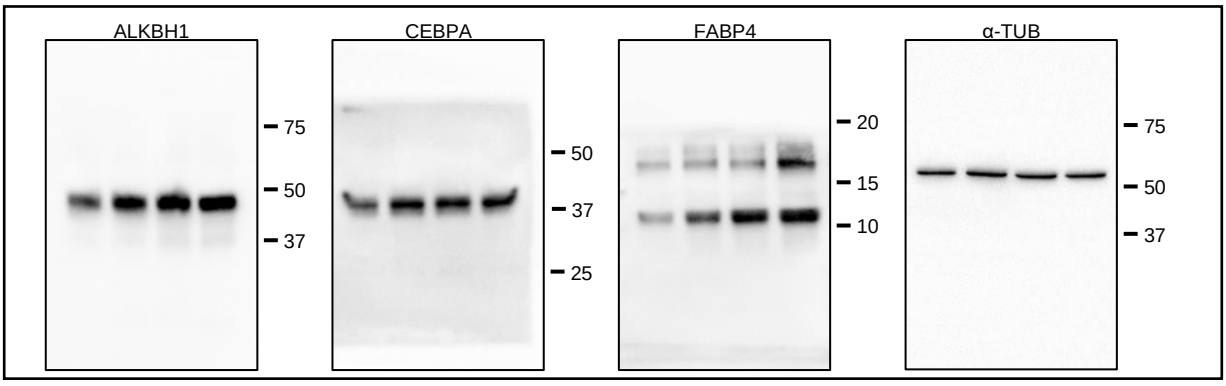


Figure 2A

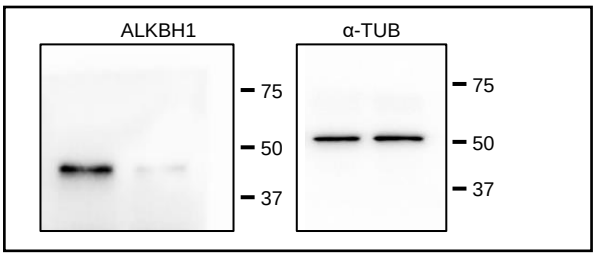


Figure 2E

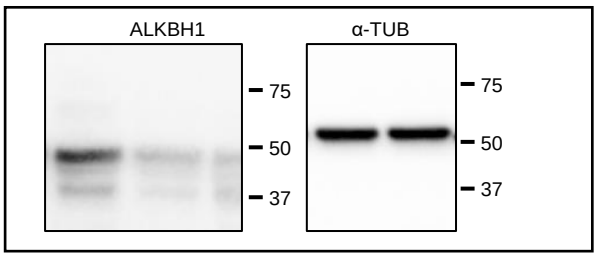


Figure 3A

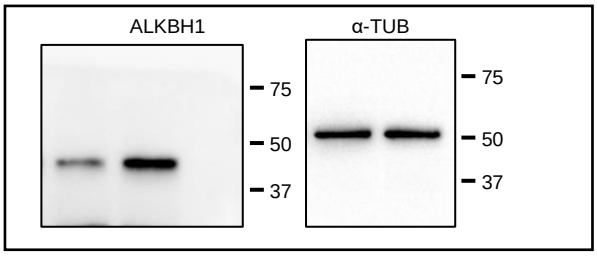
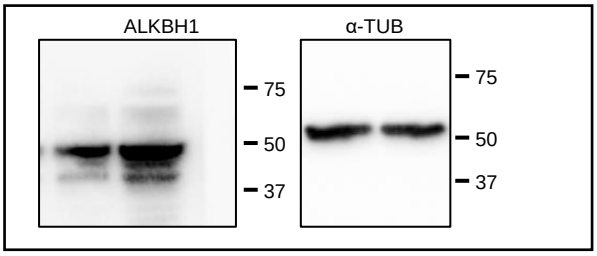


Figure 3D



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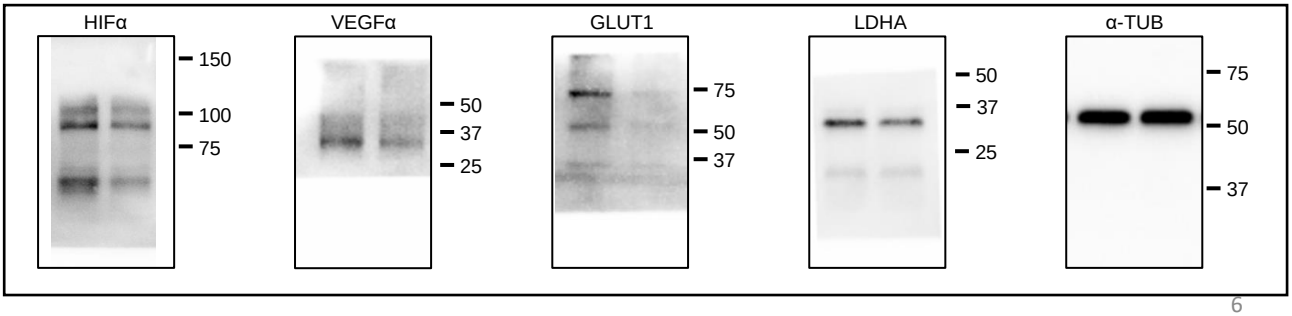

Figure 4G

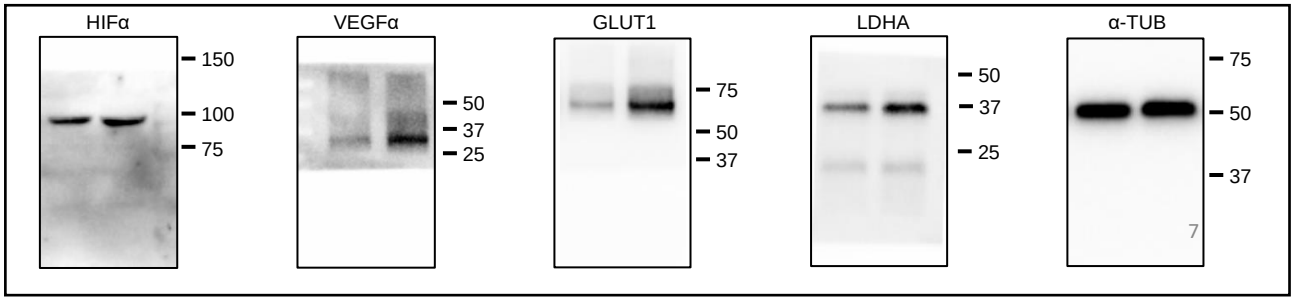

Figure 5A

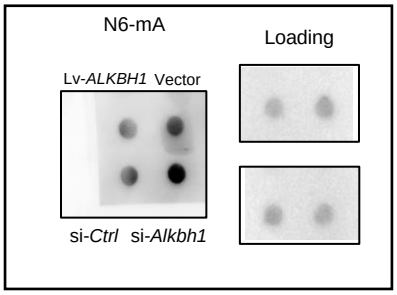

Figure 5E

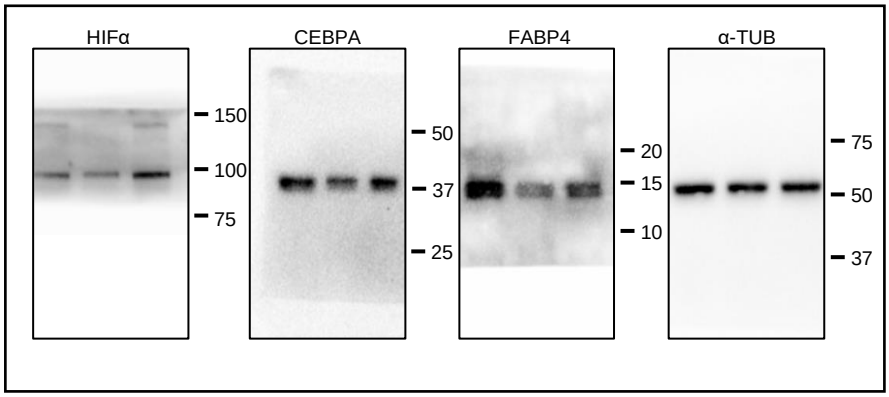

Figure 6C

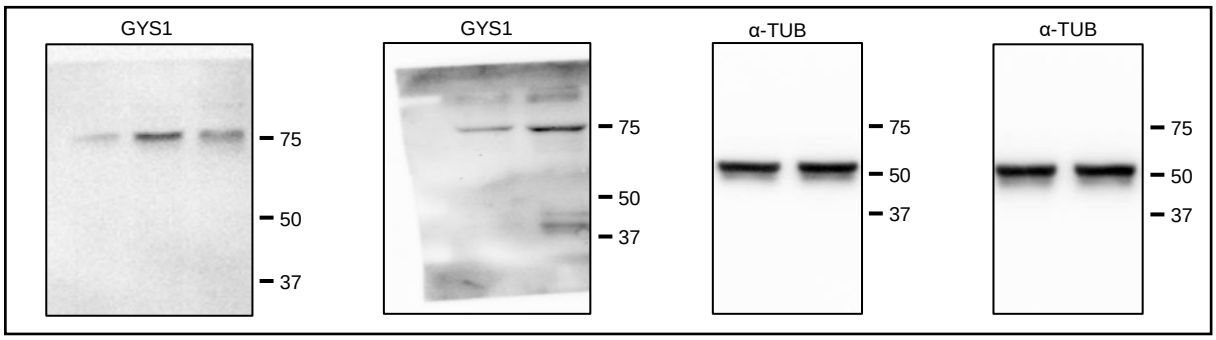

Figure 6E

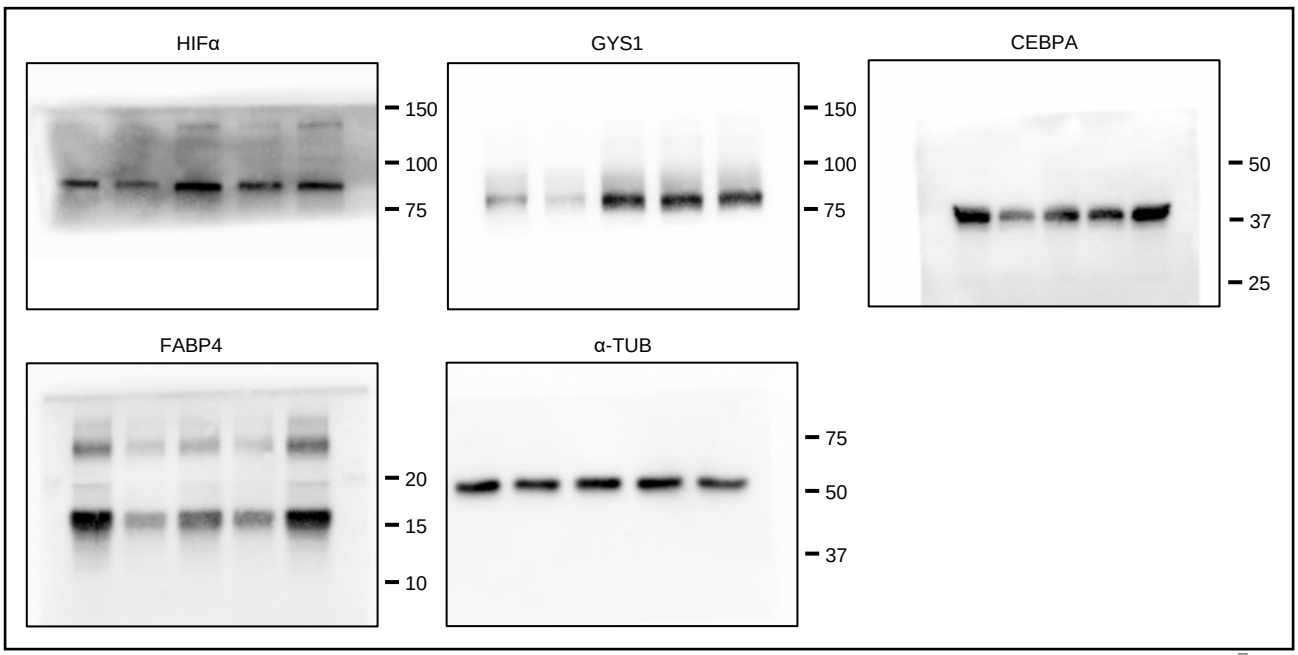

Figure S1A

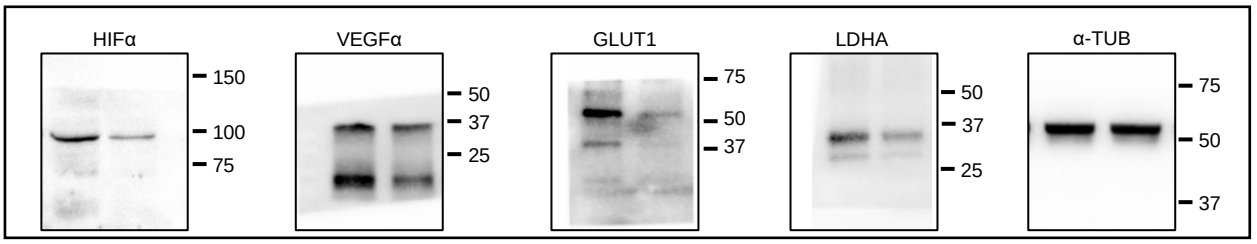

Figure S1C

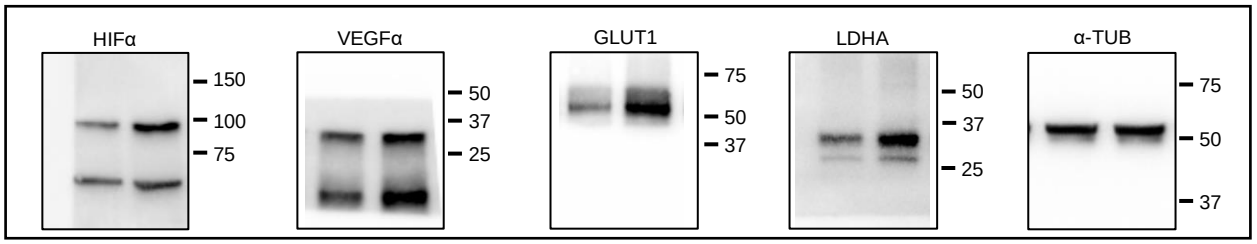

Figure S2B

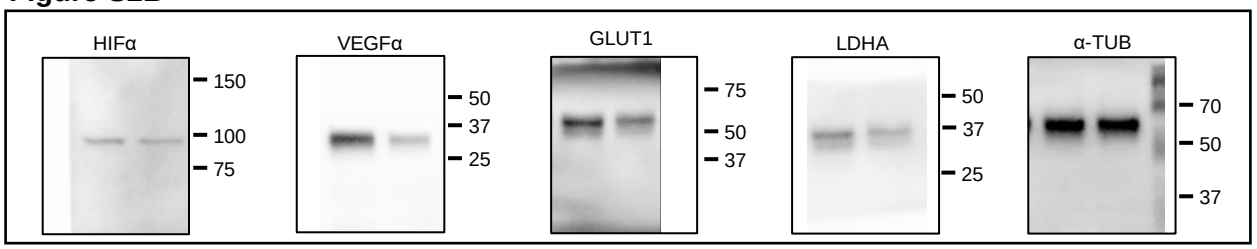

Figure S2D

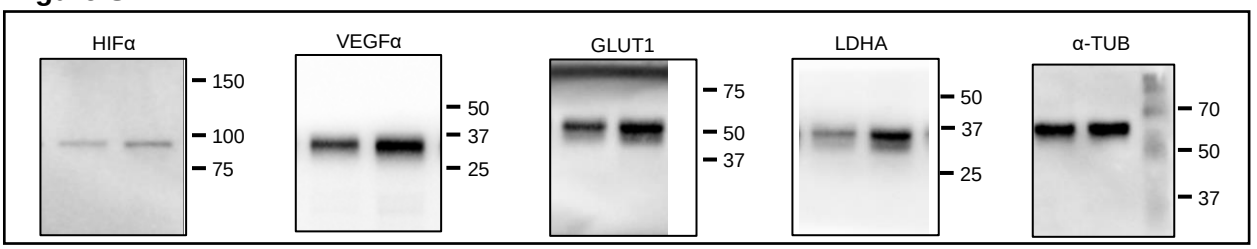

Figure S3A

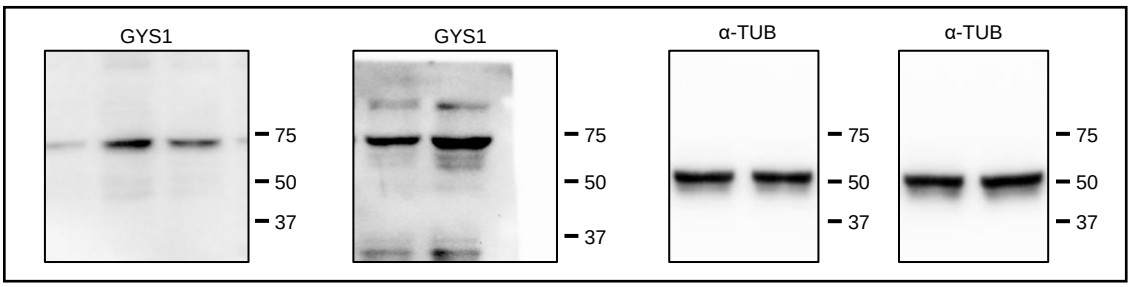

Figure S4B