Supplementary methods

Quantitative Real-time PCR:

Quantitative real-time PCR for stemness and differentiation genes was performed using primers purchased from Qiagen (QuantiTect primer assay). RNA was isolated from cells according to the manufacturer's instructions using Trizol (Invitrogen). The High Capacity cDNA Reverse Transcription Kit (Life Technologies) was used to prepare cDNA from 2 µg of RNA. Subsequently, real-time PCR was performed using the SYBR Green from Roche (Lightcycler 480 SYBR Green I Master) as per the manufacturer's instructions using a Light Cycler II (Roche). All data were normalized to the housekeeping gene 18S (18S QuantiTect Primer Assay; Qiagen).

RNA seq and analysis

RNA seq on octyl 2HG treated MIA-PACA2 and S2VP10 cells were done at the Oncogenomic Core of the Sylvester Comprehensive Cancer Center, University of Miami, FL

Raw paired-ended FASTQ data were assessed for quality with FastQC (ver.11.5, Andrews, S. 2010). Trimmomatic (ver.0.32) was then used to remove adapters, platform-specific sequences, and low-quality leading and trailing bases from reads (Bolger, et al., 2014). Then STAR (ver.2.5.0) was utilized to map reads to the reference genome GRCh38 (Dobin, et al., 2013). After that, the mapped data were assigned with genomic features with featureCounts ver.1.5.0 (Liao, et al., 2014). Fold changes of differential expression were estimated through DESeq2 (Love, et al., 2014). The differential pathway activation was performed through Ingenuity Pathway Analysis (IPA) with absolute log fold-change larger than 0.585 (e.g. relative change larger than 50%) and q-value less than 0.05.

(The MiaPaca_HypoxiavsMiaPaca_Normoxi and S2VP10_OLHGvs2VP10_ Normoxia differential genes were selected with same fold changes and q-value 0.01 due to very large amount of differential genes founded with the default setting (> 8000) while the differential genes in MiaPaca_LDH_MDH_siRNAvsMiaPaca_Hypoxia comparison were filtered with q-value 0.25 without the fold changes because too few differential genes were found with the default setting.)

Colony formation assay:

Human pancreatic cancer cells were cultured in normoxic or hypoxic environment for 48 hours. To see the effect of L-2HG on colony formation cells were cultured in the presence of octyl L-2HG at mentioned concentration for 48 hrs. After treatment cells were counted and plated at a density of 1000 cells per well. Colonies were counted after 4 days of plating. The results are presented as no. of colonies from 1000 cells per well.

Supplementary Figure Legends:

Supplementary Figure 1. Hypoxia suppressed expression of genes involved in differentiation in pancreatic cancer cell line MIA-PACA2 (A) and S2VP10 (B). Further, exposure to hypoxia increased CD133+ population in MIA-PACA2 cells (C).

Supplementary Figure 2. Hypoxia (A)and L-2HG (B) increased colony forming units in S2VP1o pancreatic cancer cells.

Supplementary Figure 3. ITRAQ analysis of samples under hypoxia compared to normoxia. String DB analysis of protein-protein interaction (A). Canonical pathways identified in L-2HG and hypoxia samples (B) GO analysis of biological processes (C) and parts of whole analysis of pathways altered in hypoxia (D).

Supplementary Figure 4. Methylation status of H3K4 (A), H3K9 (B) and H3K27 (C) under hypoxia (1% O2). Pathway analysis of identified genes following RNA seq after treating MIA-PACA2 (D) and S2VP10 (E) cells with octyl 2-HG.

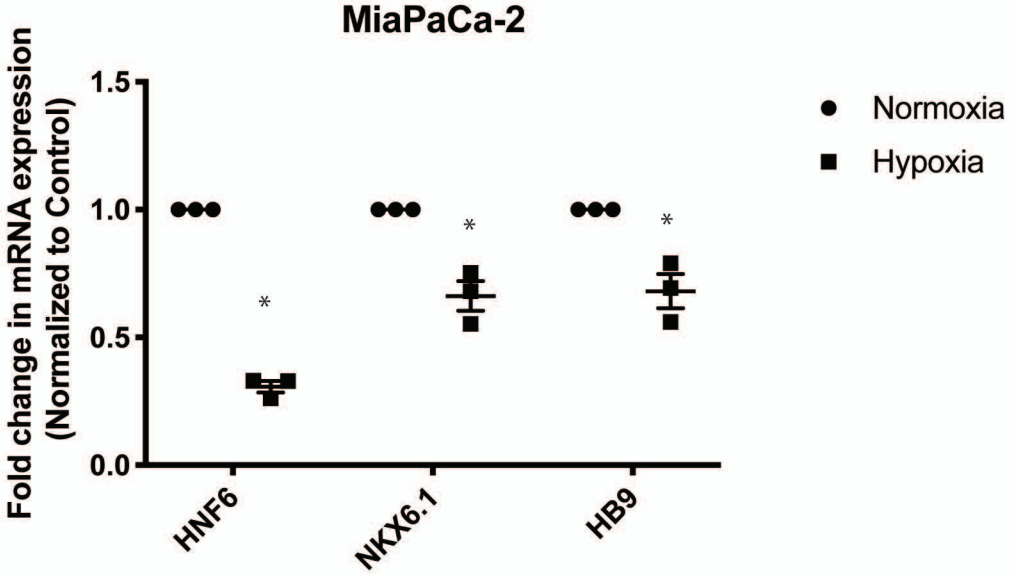
Supplementary Figure 5. PDX1 was not monomethylated during hypoxia indicating repression of gene expression (A) while CD133 not trimethylated during hypoxia indicating its activation (B). Methylation of H3K4 (C) and H3K27 (D) did not change in the promoter of CD133 and PDX1.

Supplementary Figure 6: Estimation of lactate (A) lactate: pyruvate ratio (B) in the wild type tumor bearing mice showed a decrease after treatment with LDH inhibitor
while Pyruvate (C) alone did not show any change. In order to determine the efficacy of LDH inhibitor on LDH activity, orthotopic tumor bearing animals were sacrificed every 7 days after starting treatment with LDH inhibitor and intratumoral LDH activity was estimated (D). At end point, tumor L2HG levels did not show significant change (E). LDH inhibitor decreased

expression of stemness (F, H) and differentiation genes (G, I) in MIA-PACA2 and S2VP10 cells respectively.

Supplementary Figure 7. In CD8 KO animals, estimation of lactate (A) and lactate: pyruvate ratio (B) showed a decrease after treatment with LDH inhibitor. LDH activity was decreased in both WT and CD8KO animals (C).

A.



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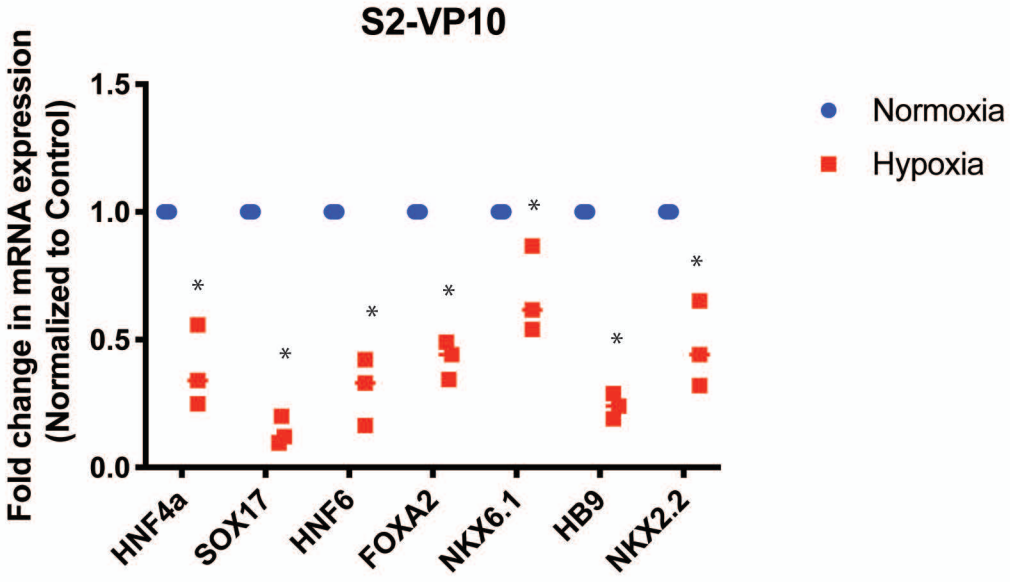

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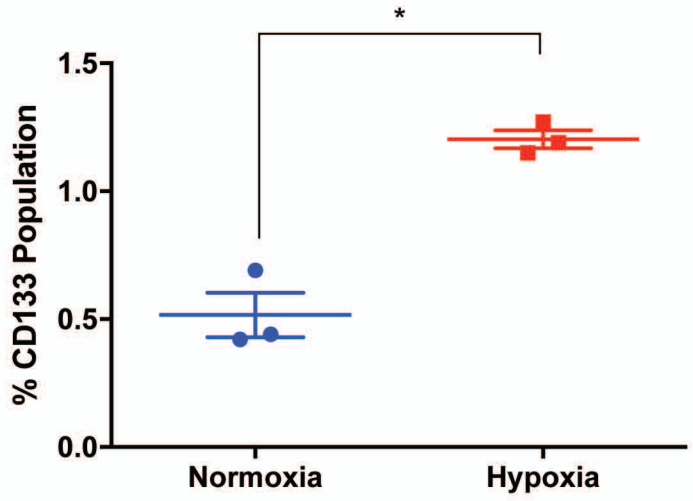

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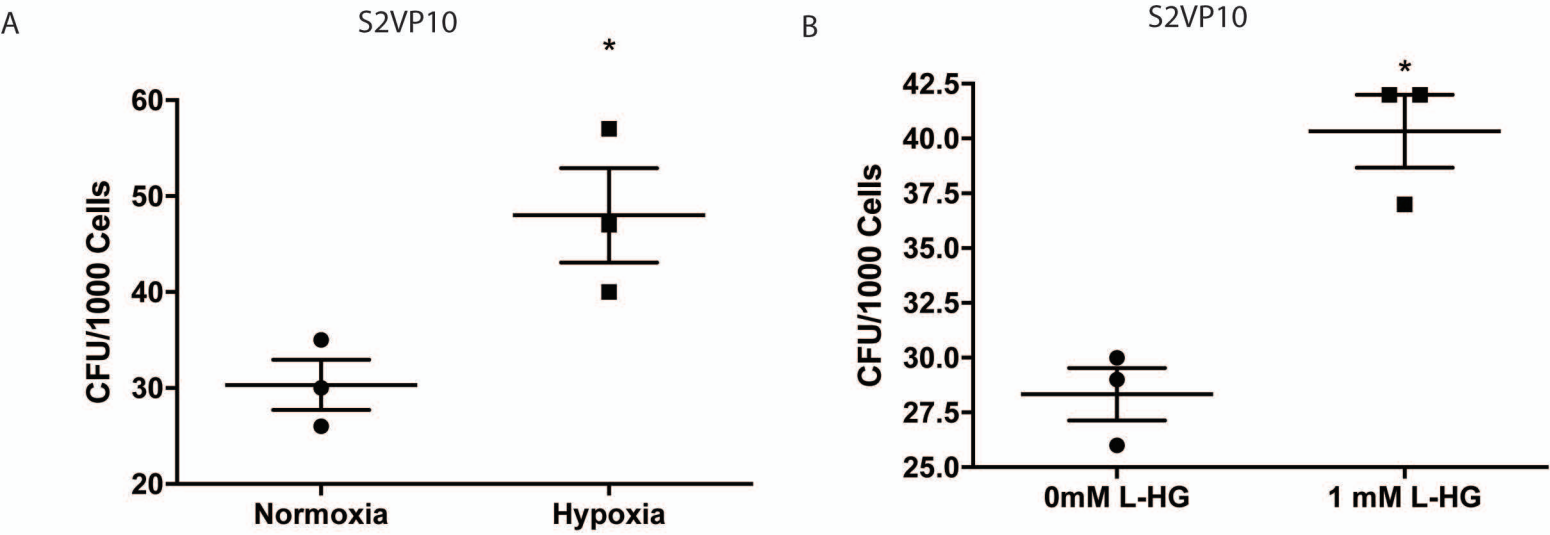
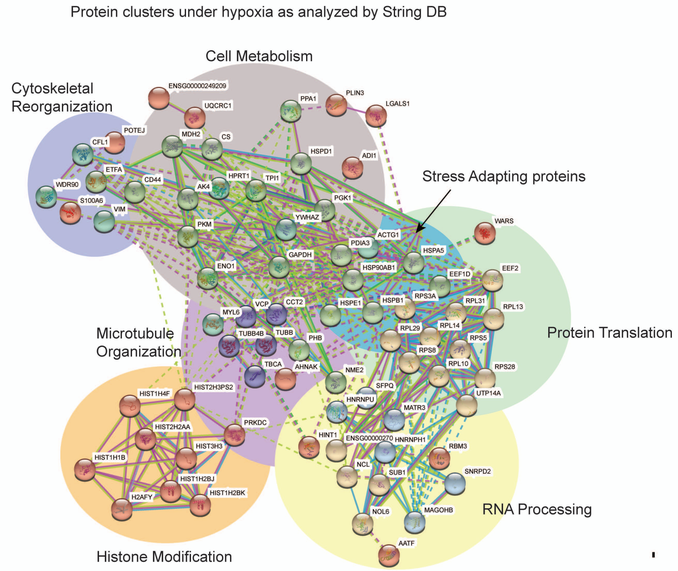


Supplementary Figure 2. Hypoxia (A) and L-2HG (B) increased colony forming units in S2VP10 pancreatic cancer cells

A.



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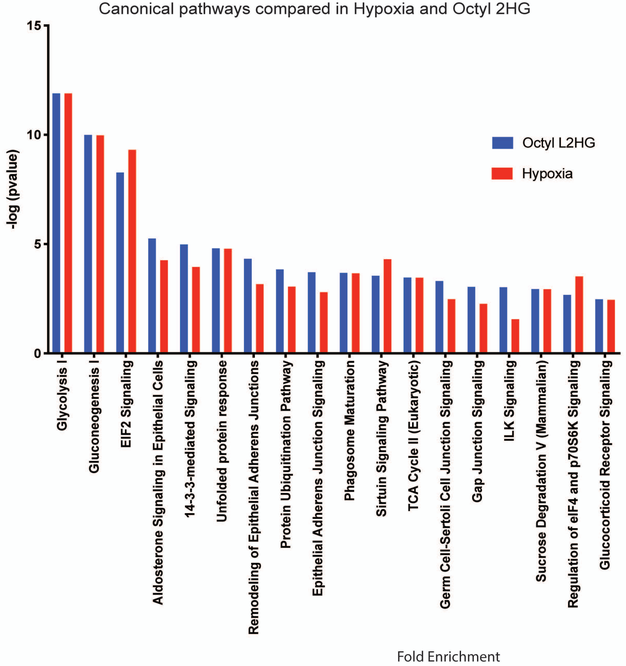

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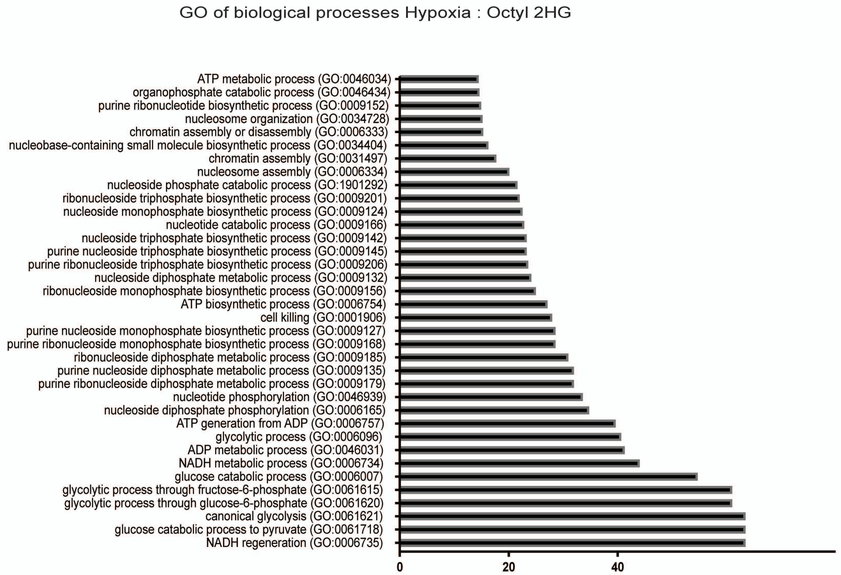

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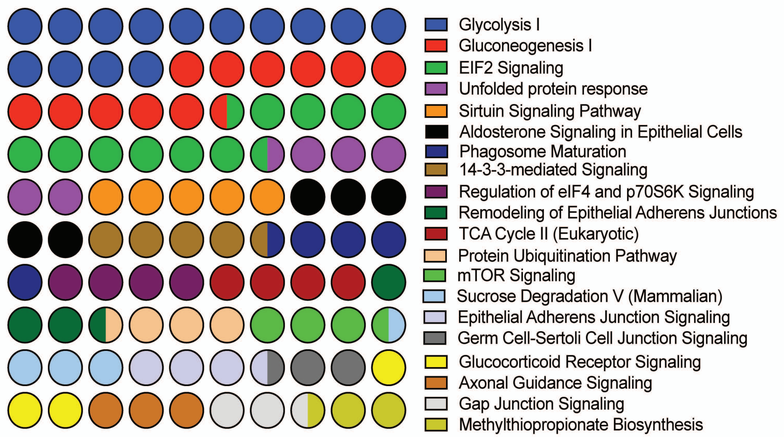

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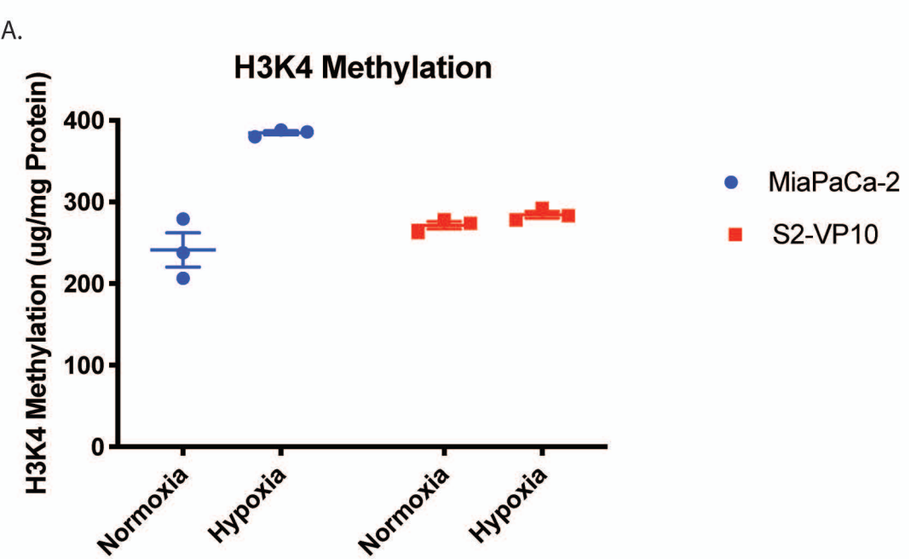

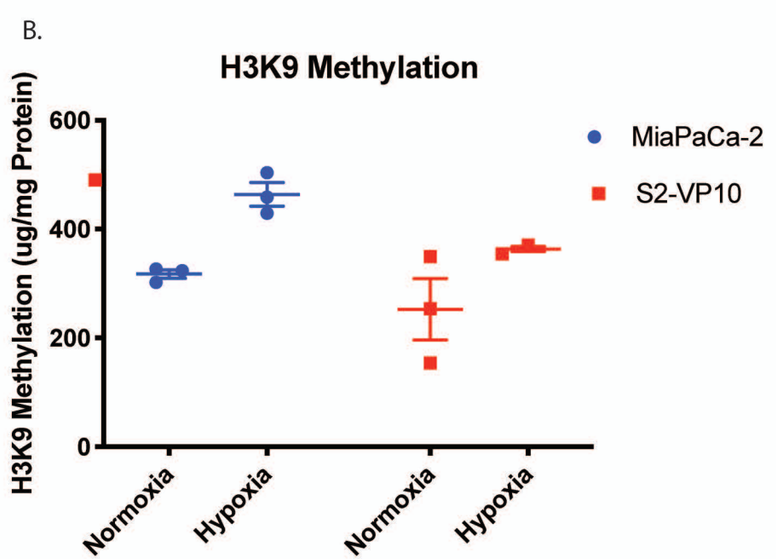

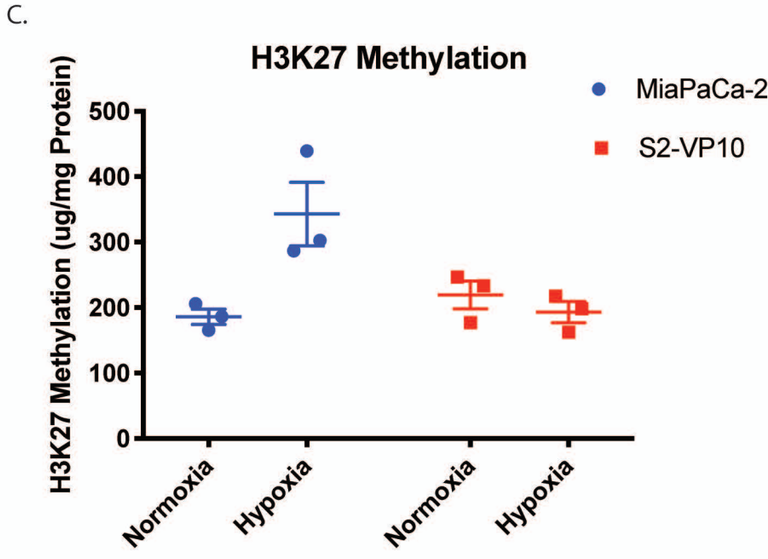

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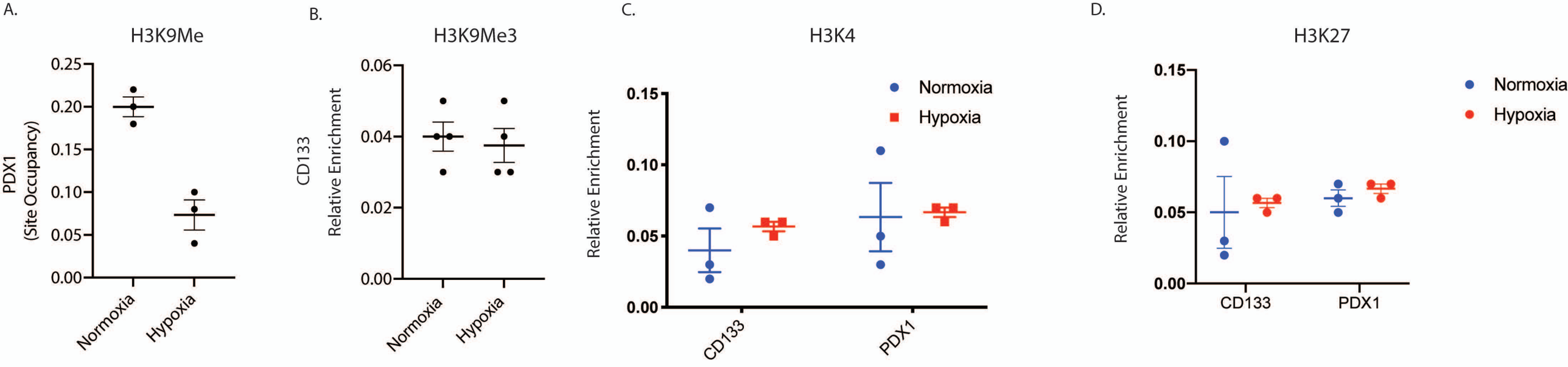

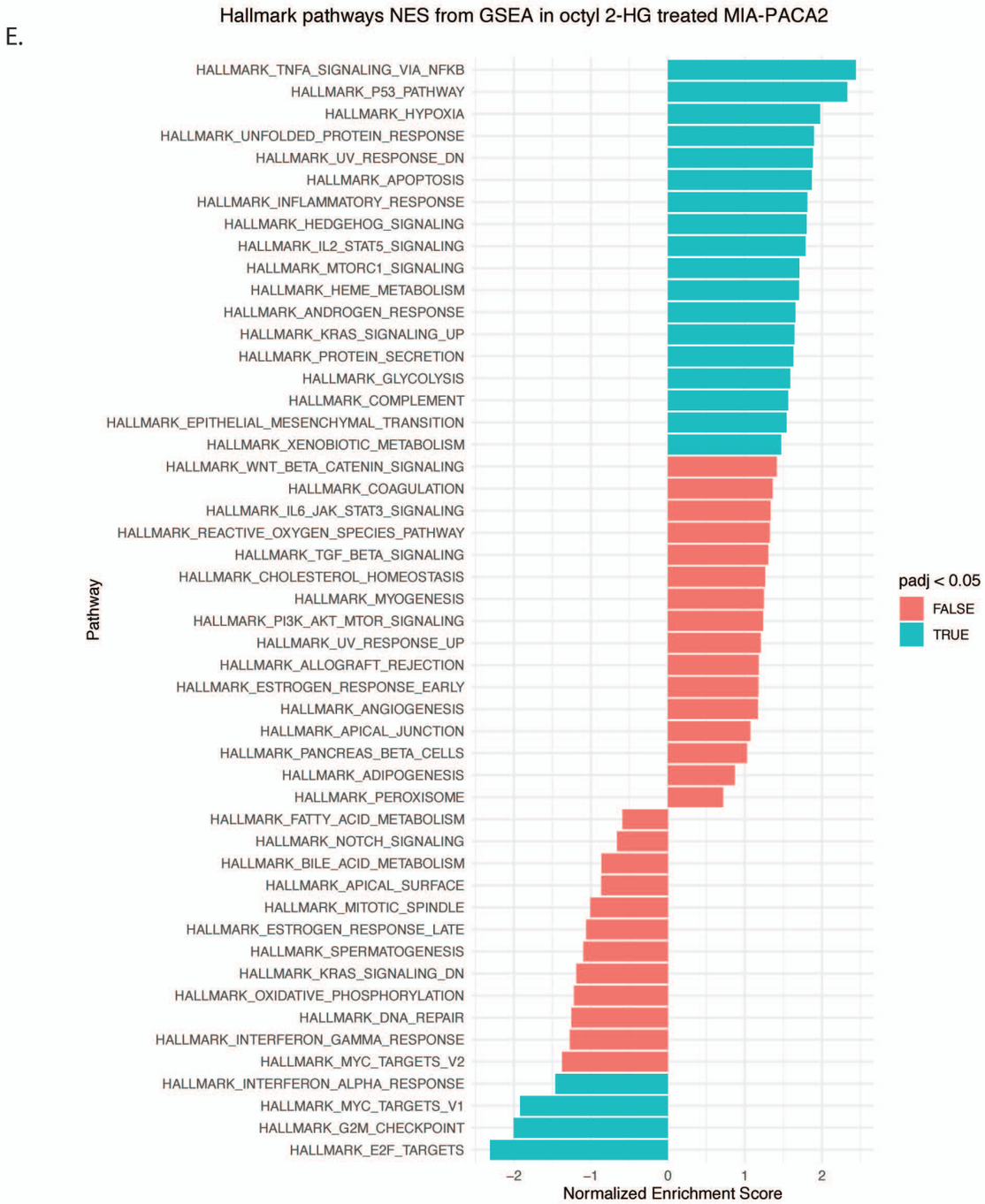

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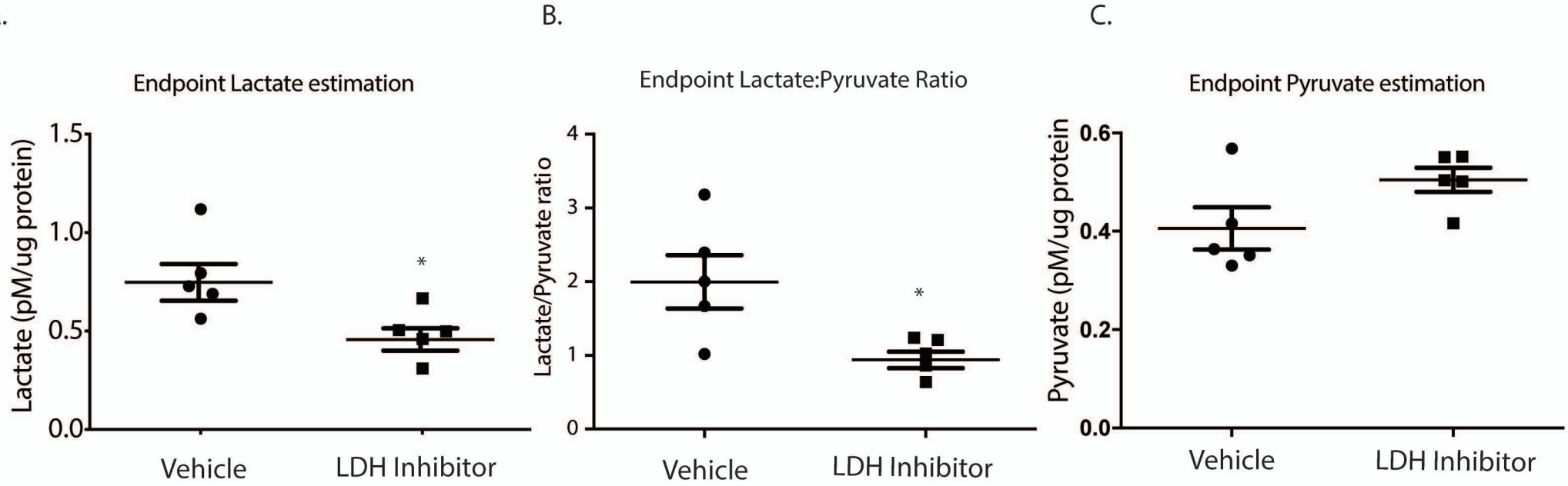

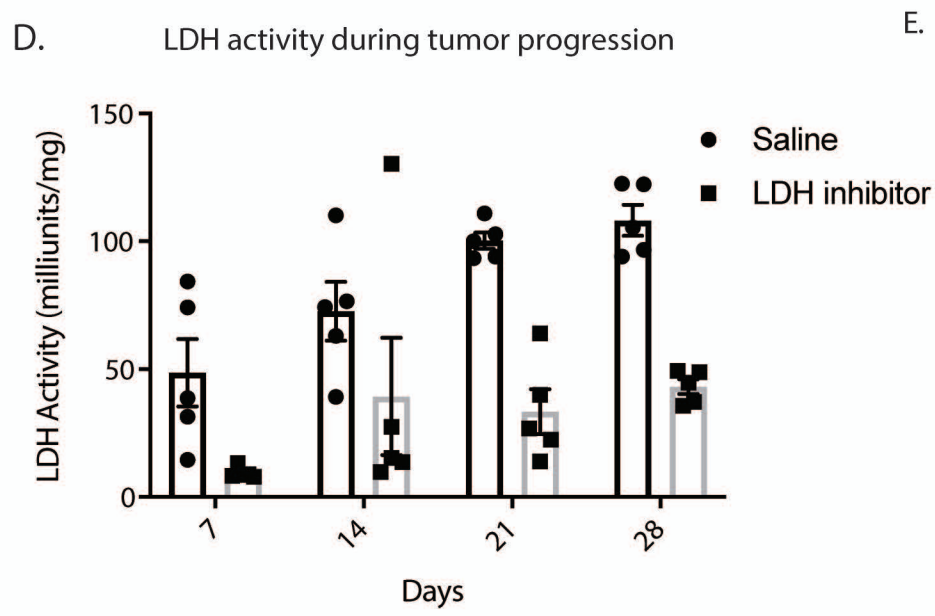

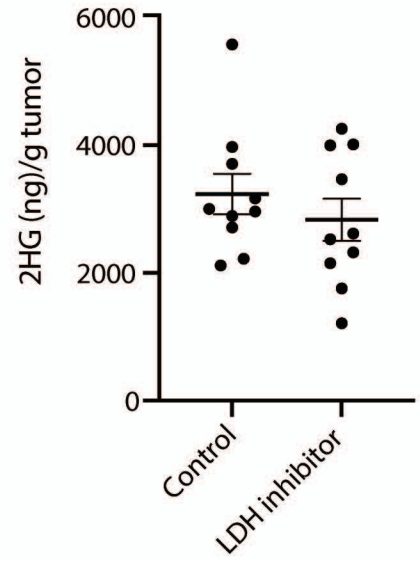

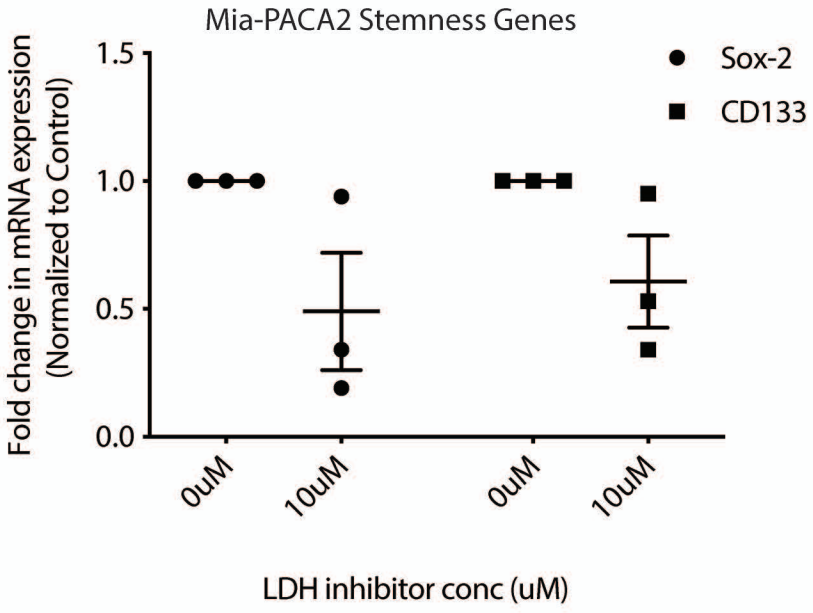

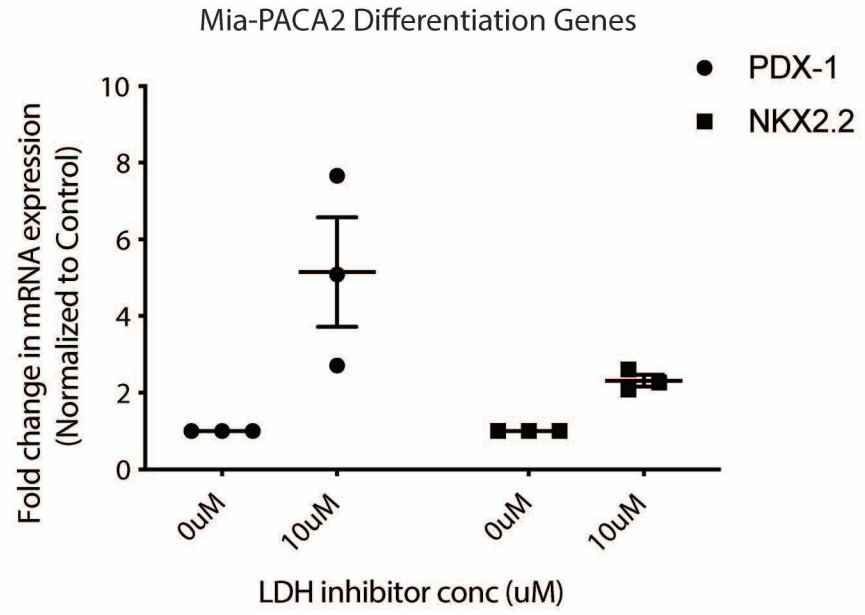

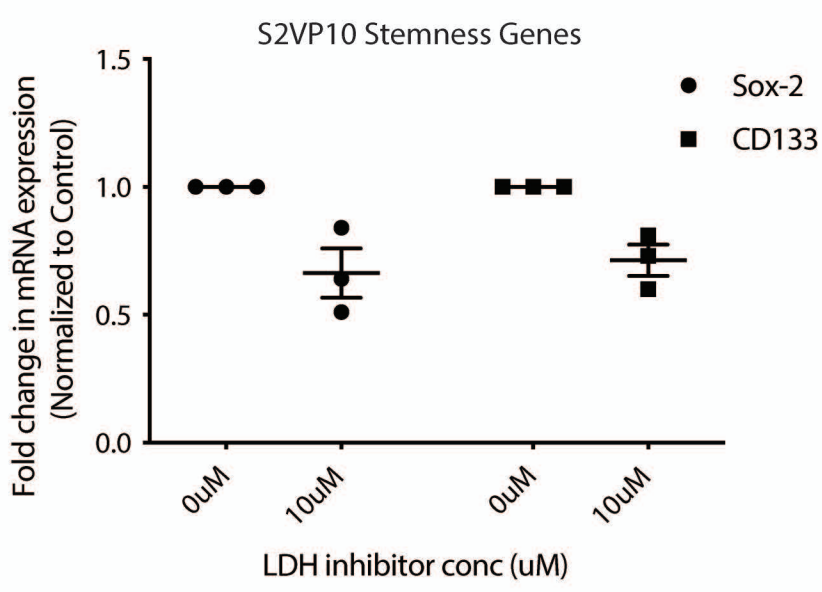

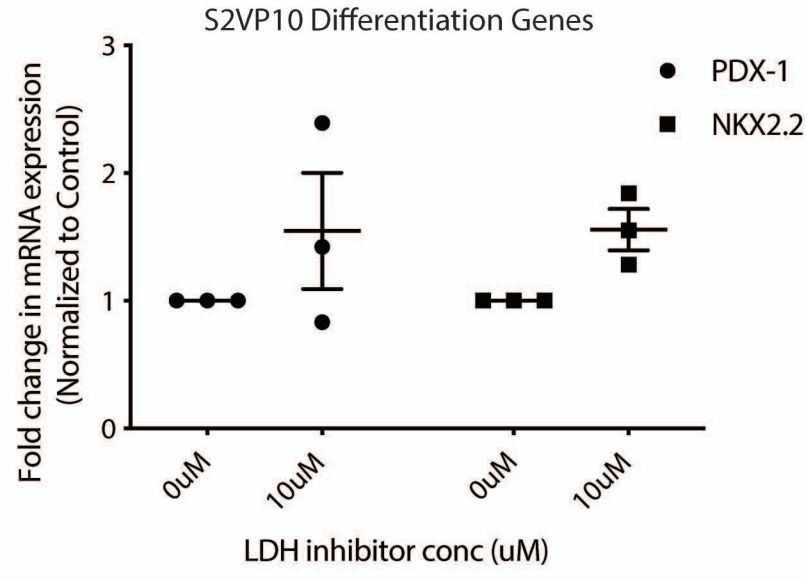

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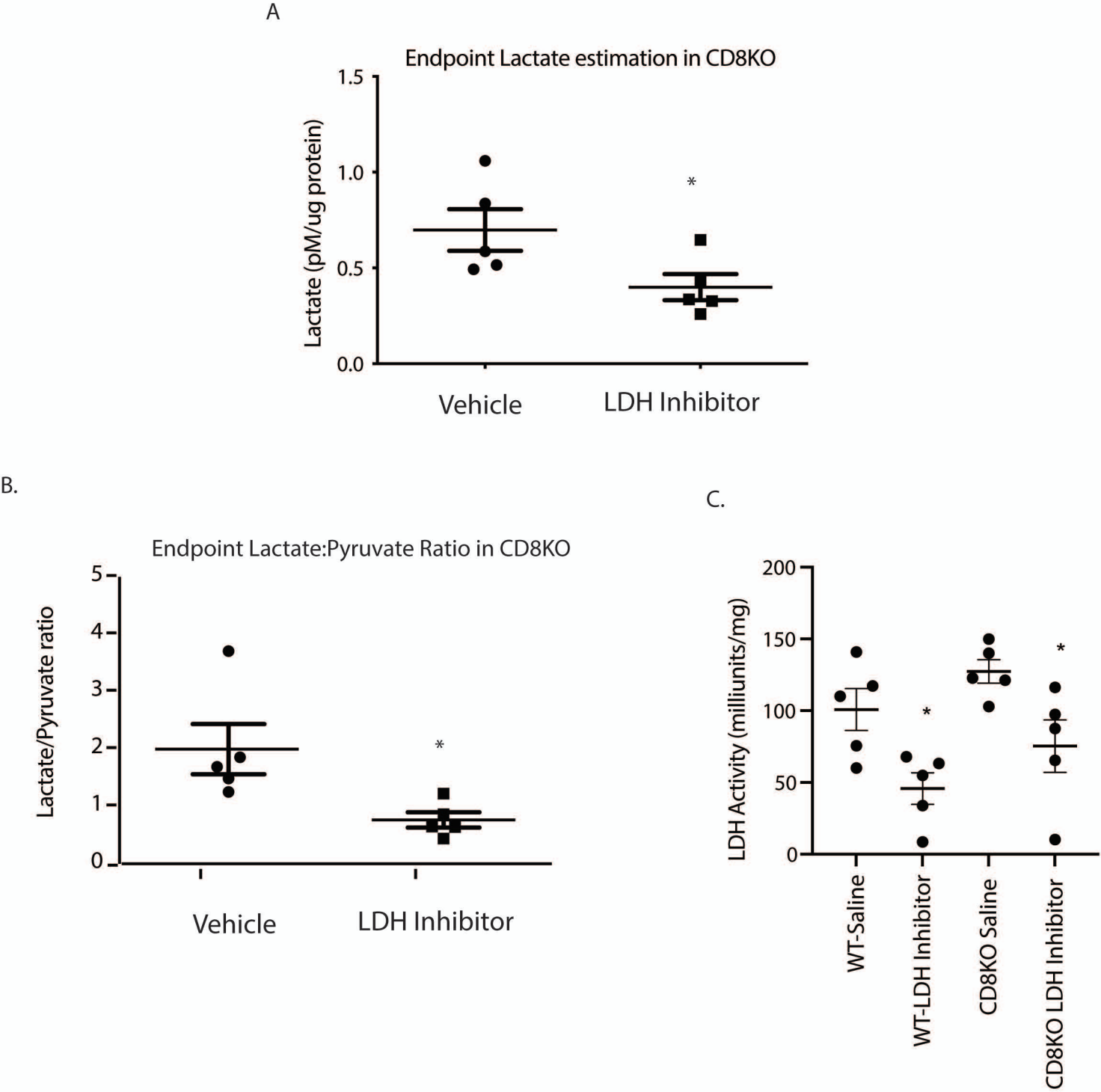

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