

## Supplemental Figure Legends

**Figure S1.** *Interactions among genes with high 5hmC peaks in MetS-MSCs.* (a): Clusters of physical and functional protein interactions among mitochondria-related genes with high 5hmC peaks in MetS-MSCs (higher strength of interaction represented by multiple strings between the genes shown in different colors). (b): Expression (qPCR) of the candidate mitochondrial genes Hydroxyacyl-CoA Dehydrogenase Trifunctional Multienzyme Complex Subunit Alpha (*HADHA*) and Aldehyde Dehydrogenase 5 Family Member A1 (*ALDH5A1*) was higher in passage (p)-0 MetS-MSCs versus p-0 Lean-MSCs.

**Figure S2.** *Interactions among genes with low 5hmC peaks in MetS-MSCs.* (a): Clusters of physical and functional protein interactions among mitochondria-related genes with low 5hmC peaks in MetS-MSCs (higher strength of interaction represented by multiple strings between the genes shown in different colors). (b): Expression (qPCR) of mitochondrial genes NADH: Ubiquinone Oxidoreductase Subunit B6 (*NDUFB2*) and Cytochrome C Oxidase Assembly Factor Heme A Farnesyltransferase COX10 (*COX10*) was lower in p-0 MetS-MSCs versus p-0 Lean-MSCs. (\* $p < 0.05$  vs. Lean-MSCs).

**Figure S3.** *MetS alters the entire 5hmC profile of swine adipose tissue derived MSCs.* (a): Volcano plot showing 317,733 5-hydroxymethylcytosine (5hmC) peaks in genes, of which 7,022 were higher and 9,733 lower in MetS-MSCs compared to Lean-MSCs. The vertical axis (y-axis) corresponds to  $-\log_2$  (p-value) and the horizontal axis (x-axis) displays  $\log_2$  fold change (MetS-/Lean-MSCs). Higher ( $n=7,022$  peaks in 2,142 genes) and lower ( $n=9,733$  peaks in 2,604 genes) 5hmC peaks in MetS- versus Lean-MSCs are shown as red and blue dots, respectively, while non-significant peaks are shown as gray dots ( $p$ -value  $< 0.05$  and fold changes  $\geq 2$  are indicated by black dashed lines). (b): Gene set enrichment analysis (GSEA) of genes with high 5hmC peaks in MetS-MSCs versus Lean-MSCs (c): GSEA of genes with low 5hmC peaks in MetS-MSCs versus Lean-MSCs.

**Figure S4.** *Vitamin (Vit)-C reversed few high and low 5hmC peaks in MetS-MSCs.* (a): Volcano plot showing that among 172 high 5hmC peaks in mitochondria-related genes in MetS-MSCs compared to Lean-MSCs, 6 (corresponding to 6 genes) were reversed in MetS-MSCs treated with Vit-C (50 $\mu$ g/mL for 48hrs). The vertical axis (y-axis) corresponds to  $-\log_2$  (p-value) and the horizontal axis (x-axis) displays the  $\log_2$ -fold change (MetS-MSCs+Vit-C/MetS-MSCs). Peaks with lower ( $n=6$  peaks in 6 genes) 5hmC in MetS-MSCs+Vit-C versus MetS-MSCs are shown in blue dots, while non-significant or high peaks are shown as gray dots. (b): Volcano plot showing that among 416 low 5hmC peaks in mitochondria-related genes in MetS-MSCs compared to Lean-MSCs, 9 (corresponding to 9 genes) were reversed in MetS-MSCs treated with Vit-C (50 $\mu$ g/mL for 48hrs). The vertical axis (y-axis) corresponds to  $-\log_2$  (p-value) and the horizontal axis (x-axis) displays the  $\log_2$ -fold change (MetS-MSCs+Vit-C/MetS-MSCs). Peaks with higher ( $n=9$  peaks in 9 genes) 5hmC in MetS-MSCs+Vit-C versus MetS-MSCs are shown in red dots, while non-significant or high peaks are shown as gray dots.

**Figure S5.** *Vitamin (Vit)-C modulates 5hmC levels in mitochondria-related genes in Lean-MSCs.* (a): Volcano plot showing 23,944 5hmC peaks in mitochondria-related genes identified in Lean-MSCs treated with Vit-C (50 $\mu$ g/mL for 48hrs) versus Lean-MSCs untreated (Lean-MSCs+Vit-C/Lean-MSCs). The vertical axis (y-axis) corresponds to  $-\log_2$  (p-value) and the horizontal axis (x-axis) displays the  $\log_2$ -fold change (Lean-MSCs+Vit-C/Lean-MSCs). Peaks with higher ( $n=5$  peaks in 5 genes) and lower ( $n=23$  peaks in 21 genes) 5hmC in Lean-MSCs+Vit-C versus Lean-MSCs are shown as red and blue dots, respectively, while non-significant peaks are shown as gray dots ( $p$ -value  $< 0.05$  and fold changes  $\geq 2$  are indicated by black dashed lines) (b): Heat map representing 5 peaks in mitochondria-related genes with higher (left) and lower (right) 5hmC levels in Lean-MSCs+Vit-C versus Lean-MSCs ( $n=3$  each). (c): Venn diagram showing that only 4 of these genes also exhibited hyper or hypo- hydroxymethylated peaks in Vit-C-treated MetS-MSCs compared to untreated MetS-MSCs.

**Figure S6.** *Vitamin (Vit)-C modulates 5hmC levels in swine MetS-MSC genes.* (a): Volcano plot showing 301,647 5hmC peaks in MetS-MSCs treated with Vit-C (50 $\mu$ g/mL for 48hrs) versus MSCs untreated (MetS-MSCs+Vit-C/MetS-MSCs). The vertical axis (y-axis) corresponds to  $-\log_2$  (p-value) and the horizontal axis (x-axis) displays the  $\log_2$ -fold change (MetS-MSCs+Vit-C/MetS-MSCs). Peaks with higher ( $n=210$  peaks in 197 genes) and lower ( $n=210$  peaks in 182 genes) 5hmC in MetS-MSCs+Vit-C versus MetS-MSCs are shown as red and blue dots, respectively, while non-significant peaks are shown as gray dots ( $p$ -value  $< 0.05$  and fold changes  $\geq 2$  are indicated by black dashed lines) ( $n=3$  each) (b):

Pie chart showing that epigenetic changes in mitochondrial genes accounted for 10.6% of all epigenetic changes induced by Vit-C.

**Figure S7.** *Global 5mC levels are similar among the groups. (a):* Representative images of immunofluorescence (original magnification: X40) for 5mC (red) and its quantification in Lean- and MetS-MSCs untreated and treated with Vit-C (n=6 each).

**Figure S8.** *MetS increases expression of epigenetic enzymes in swine MSCs.* Gene expression (qPCR) of the epigenetic enzymes Ten-Eleven Translocation (TET) methylcytosine dioxygenases 1, 2, and 3 in MetS- compared to Lean-MSCs (n=6 each) (\*p<0.05 vs. Lean-MSCs).

**Figure S9.** *Vitamin (Vit)-C restored AMPK immunoreactivity in MetS-MSCs. (a):* Immunofluorescence staining for 5' AMP-activated protein kinase (AMPK) and its quantification in Lean- and MetS-MSCs untreated or treated with Vit-C (n=6 each) (\*p<0.05 vs. Lean-MSCs, †p<0.05 vs. Lean-MSCs+Vit-C, ‡p<0.05 vs. MetS-MSCs).

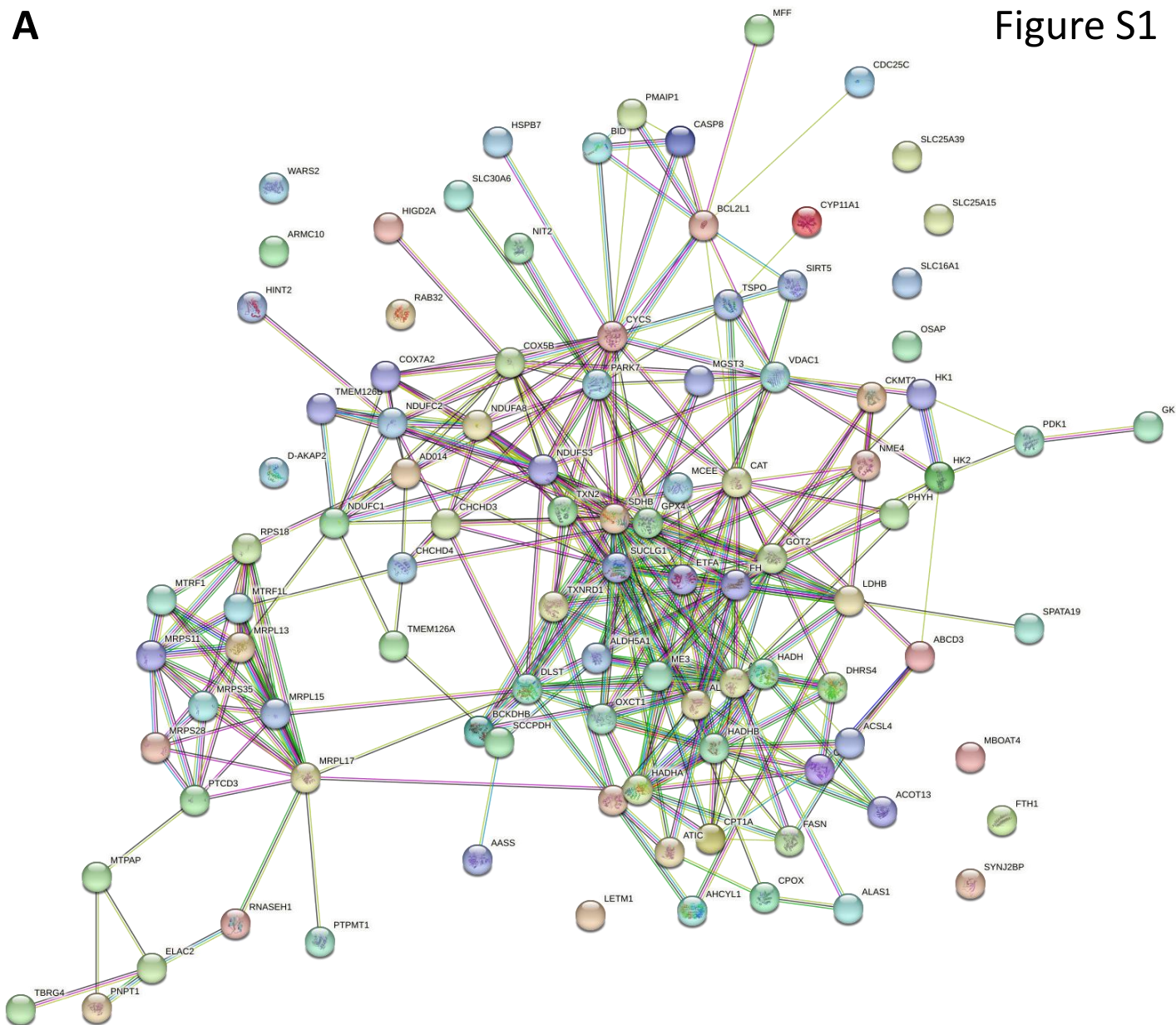
**Figure S10.** *Dimethyl alpha-ketoglutarate (DMαKG).* Expression (qPCR) of mitochondrial genes *HADHB*: Hydroxyacyl-CoA Dehydrogenase Trifunctional Multienzyme Complex Subunit Beta, *FASN*: Fatty Acid Synthase, *ETFB*: Electron Transfer Flavoprotein Subunit Beta, and *CYB5A*: Cytochrome B5 Type A in Lean-MSCs and MetS-MSCs untreated or treated with DMαKG. (\*p<0.05 vs. Lean-MSCs). \*p<0.05 vs. Lean-MSCs; †p<0.05 vs. Lean+MSCs+DMαKG; ‡p<0.05 vs. MetS-MSCs.

**Figure S11.** *DMαKG ameliorates oxidative stress and improves membrane potential in MetS-MSCs. (a):* Representative images of immunofluorescence (original magnification: X40) for the mitochondrial superoxide indicator MitoSOX (red) and the mitochondrial membrane potential marker tetramethylrhodamine ethylester (TMRE, green) of Lean- and MetS-MSCs, untreated and treated with DMαKG (n=6 each). Quantification of mitochondrial reactive oxygen species (b) and membrane potential (c) in all groups. \*p<0.05 vs. Lean-MSCs; †p<0.05 vs. Lean+MSCs+DMαKG; ‡p<0.05 vs. MetS-MSCs.



**A**

Figure S1



# B

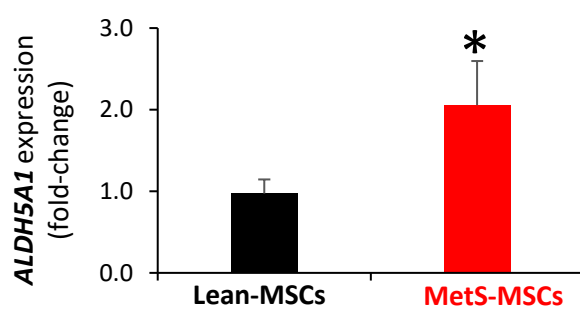
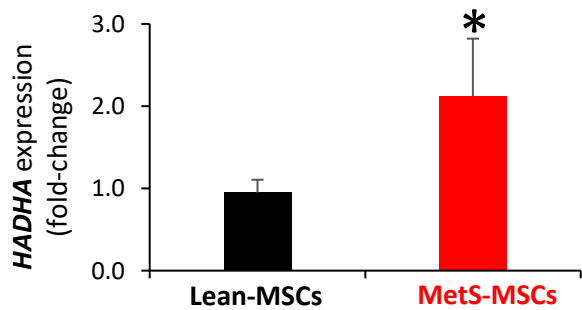
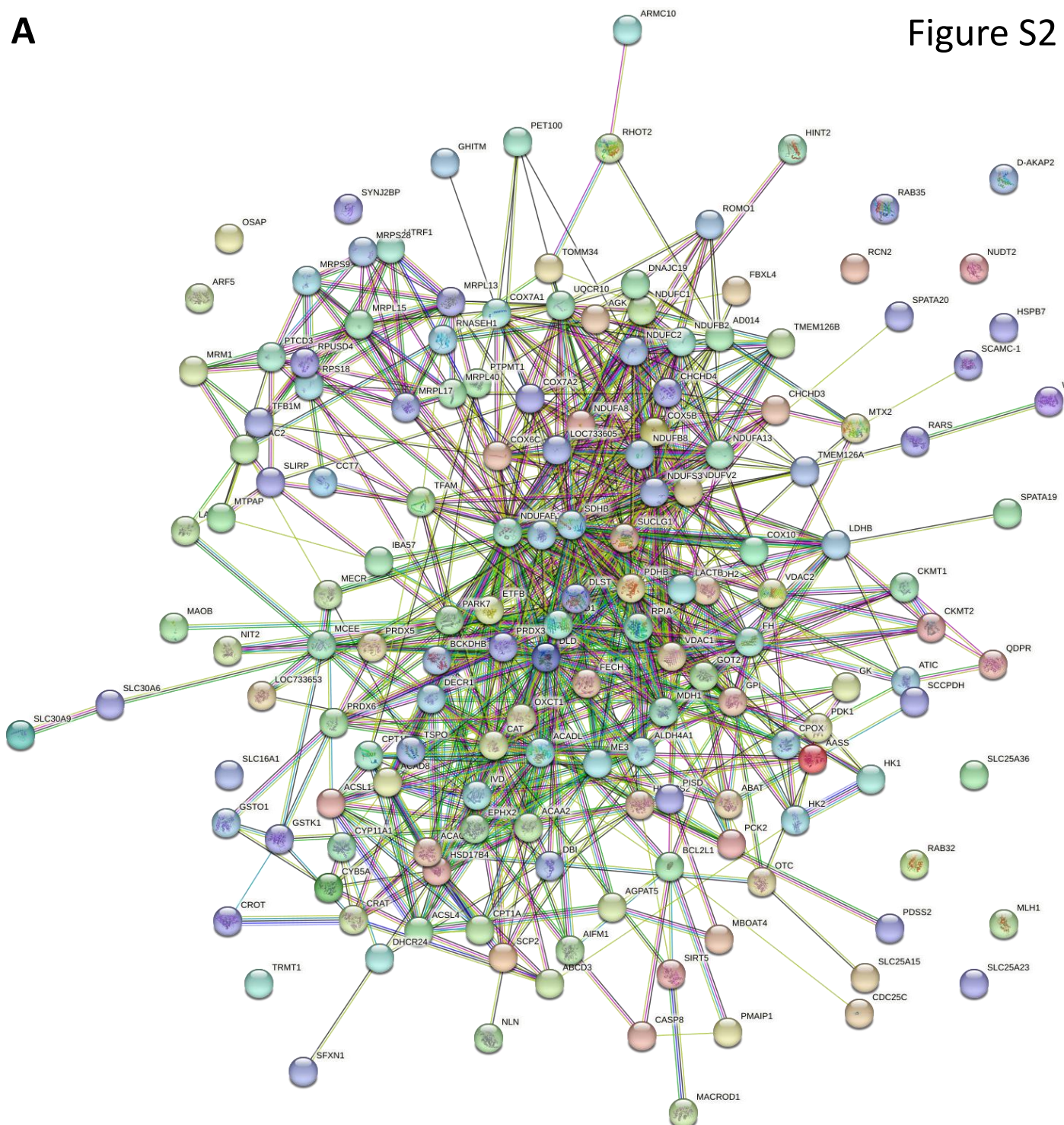


Figure S2



# B

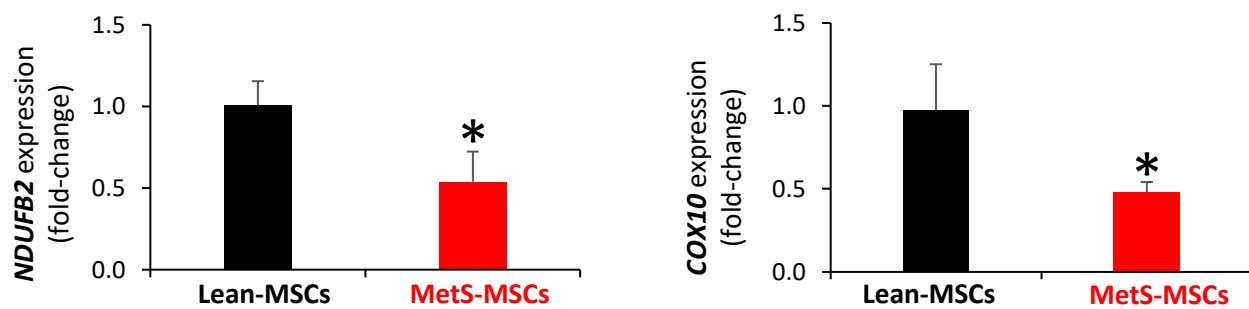
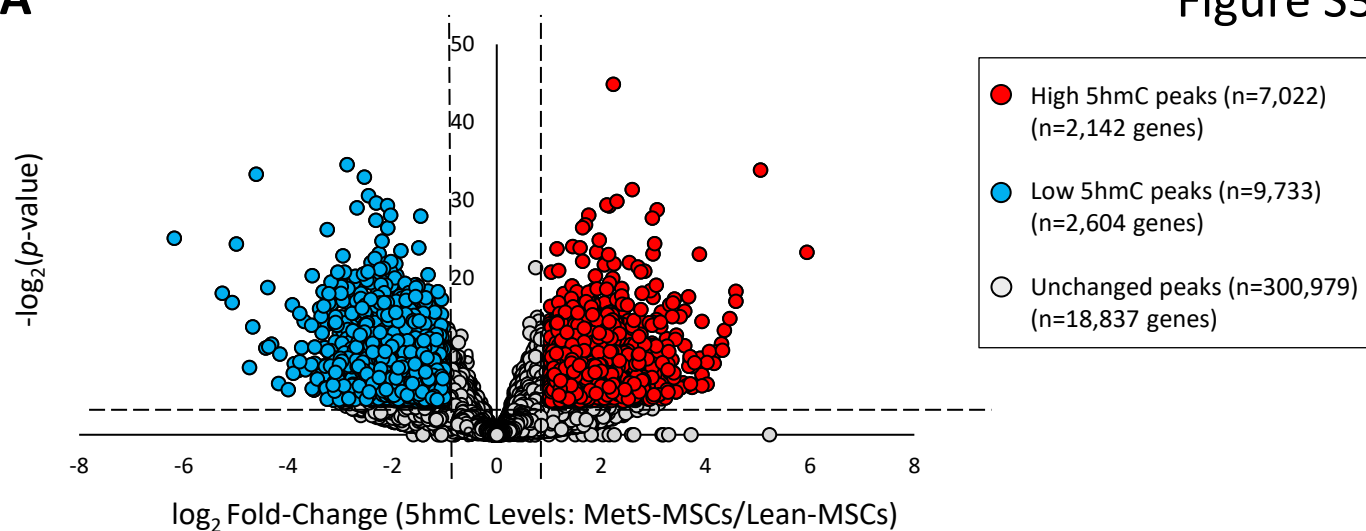
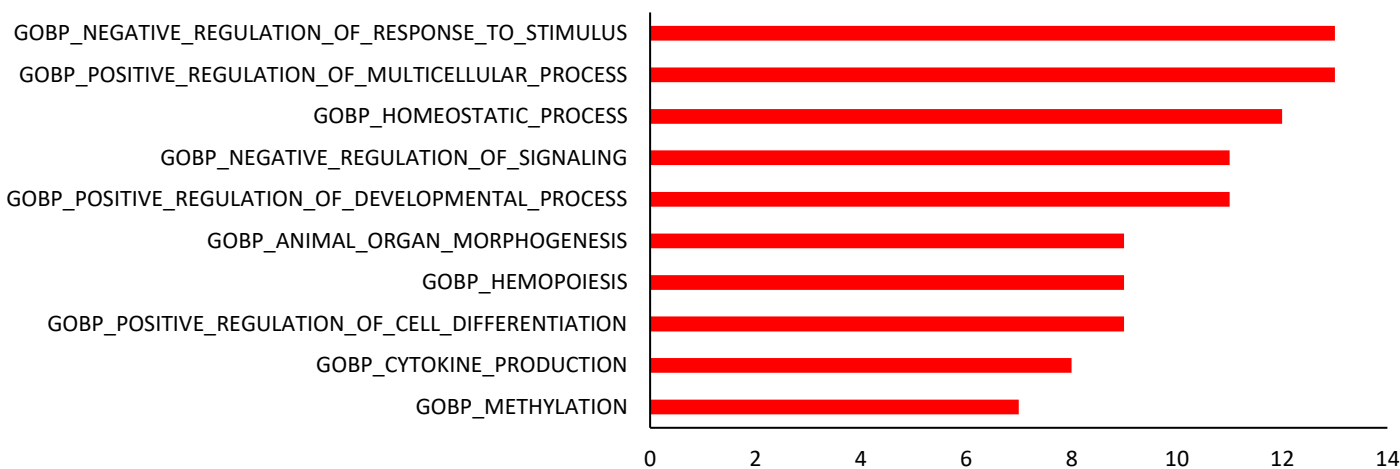


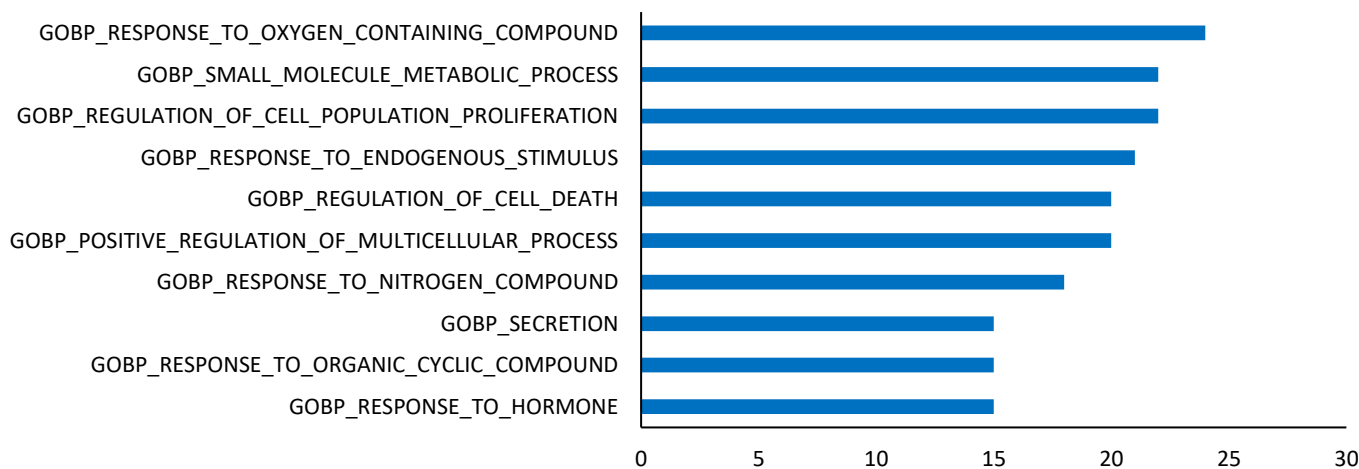
Figure S3

**A****B**

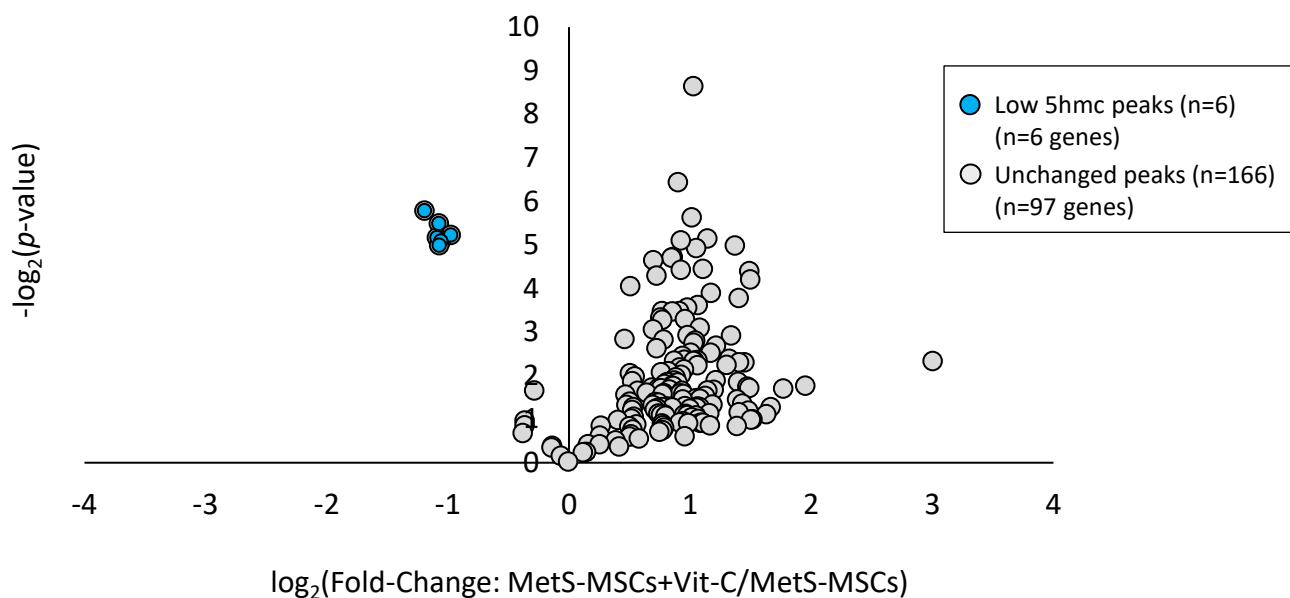
# Genes in Overlap (k)

**C**

# Genes in Overlap (k)



## High 5hmC peaks in MetS-MSCs versus Lean-MSCs



## Low 5hmC peaks in MetS-MSCs versus Lean-MSCs

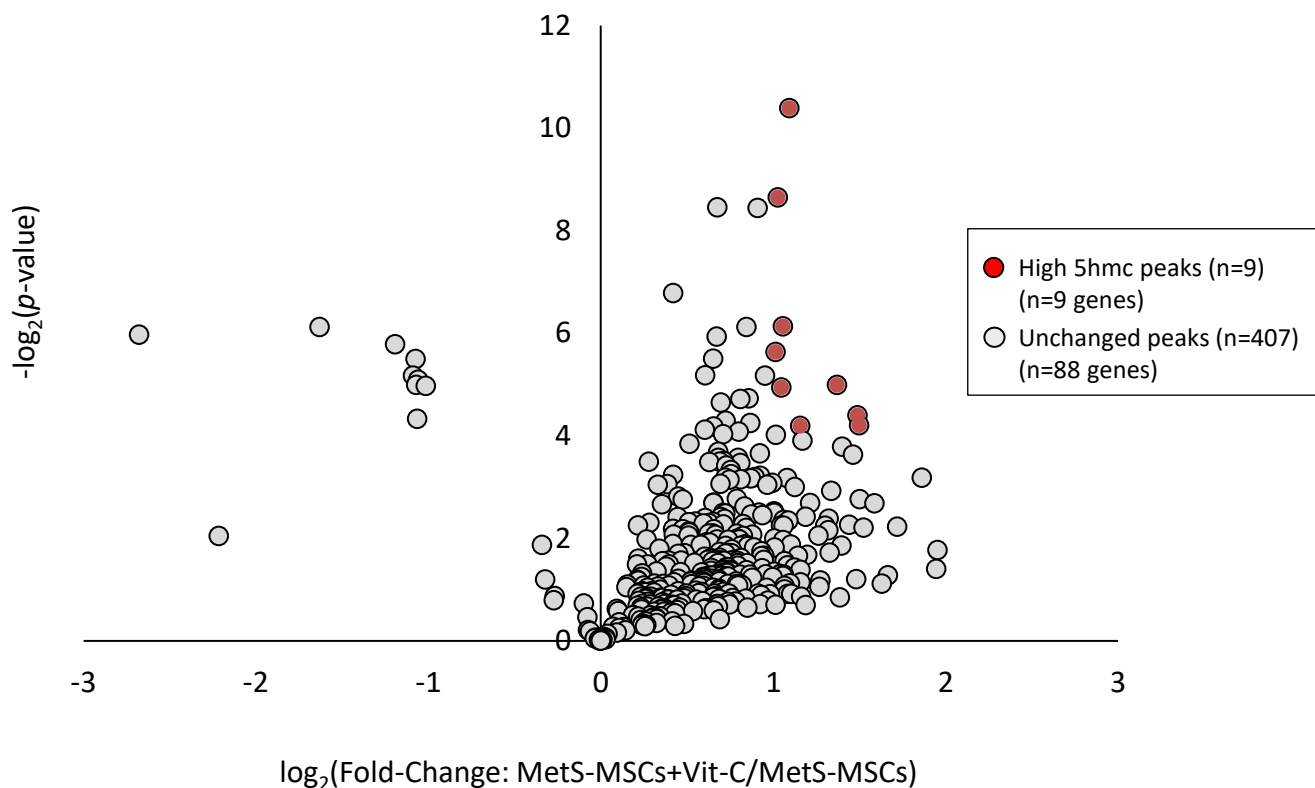


Figure S5

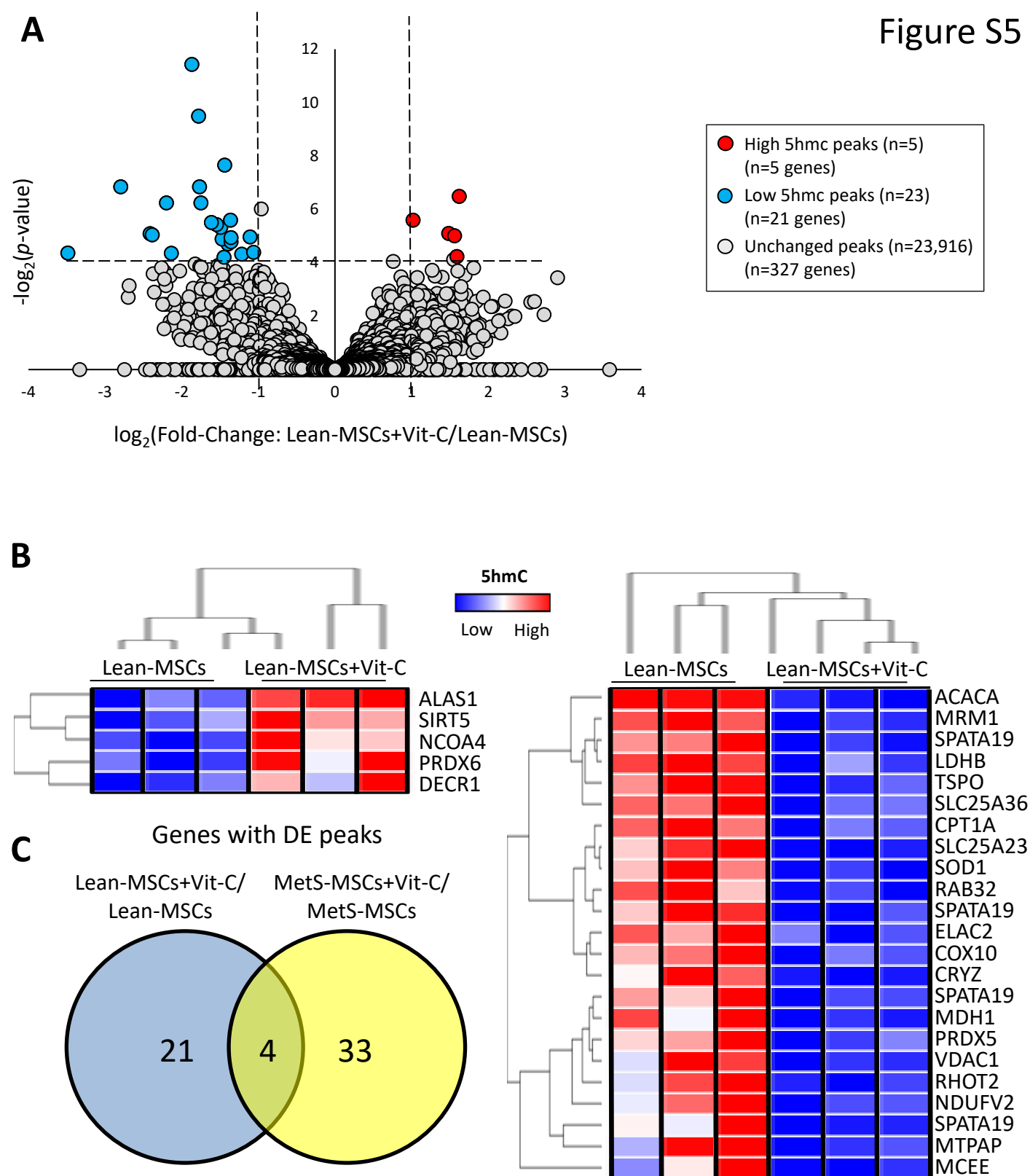




Figure S6

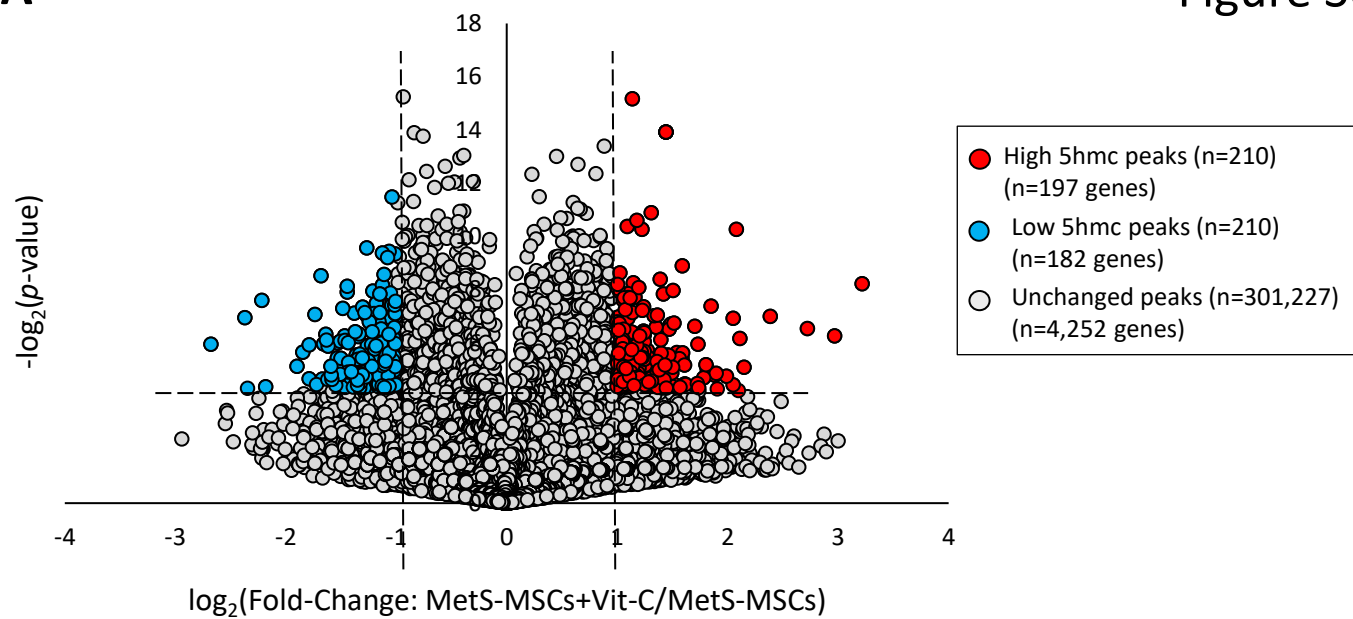
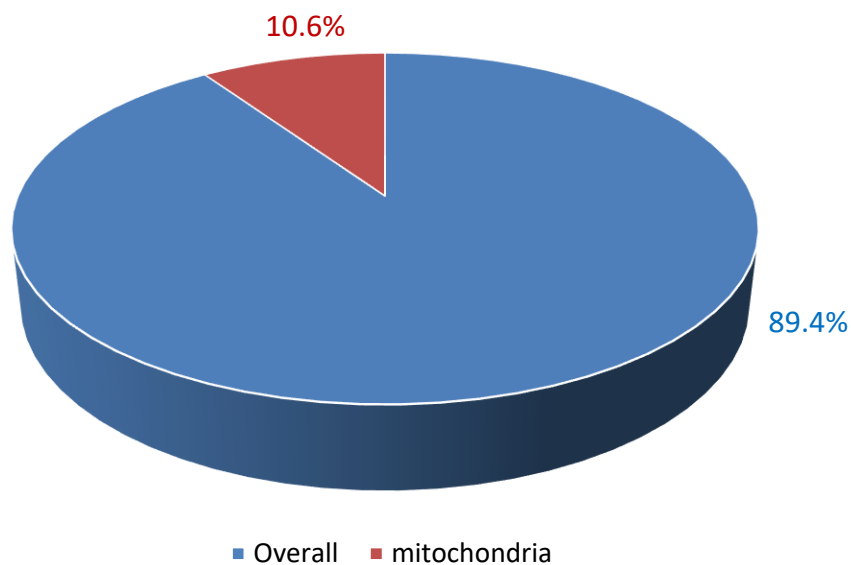
**A****B**

Figure S7

Lean-MSCs

Lean-MSCs+Vit-C

MetS-MSCs

MetS-MSCs+Vit-C

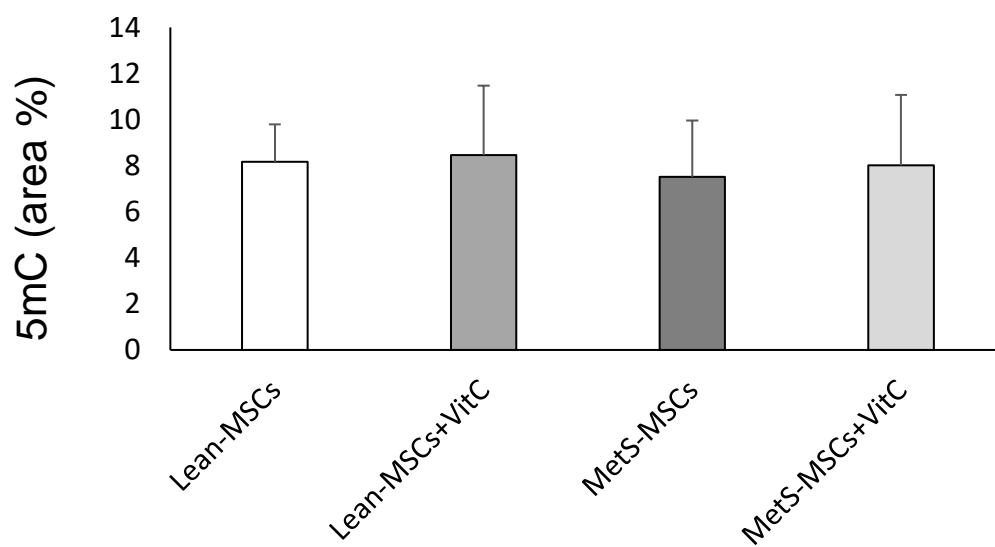
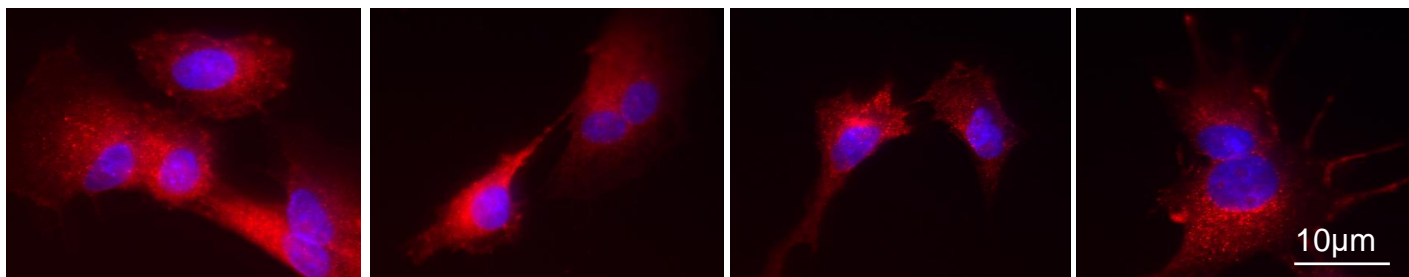


Figure S8

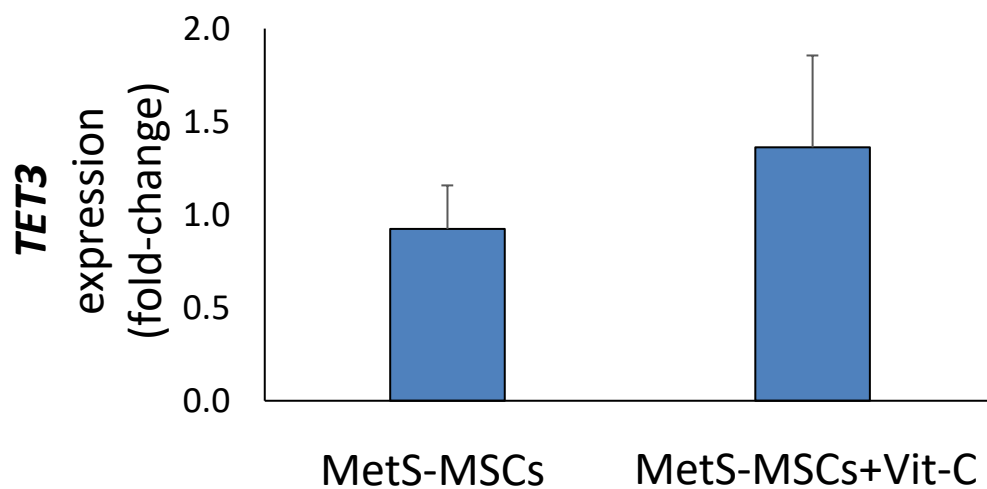
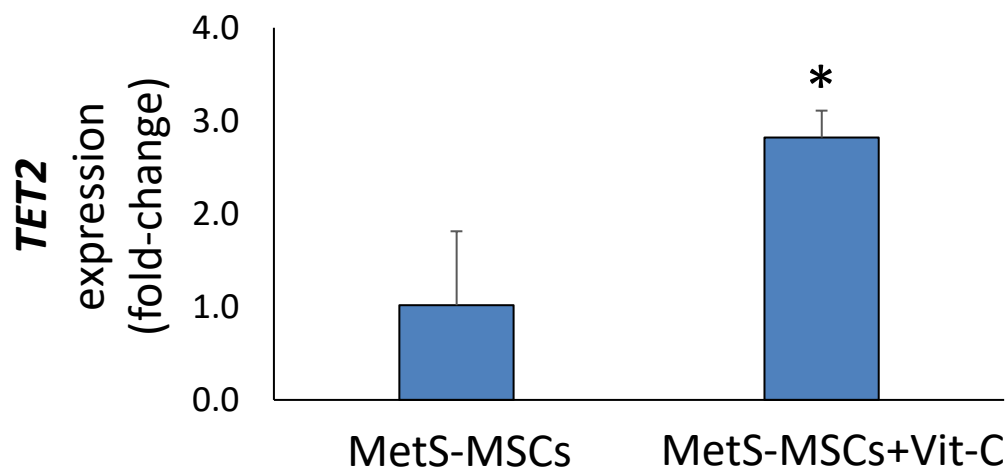
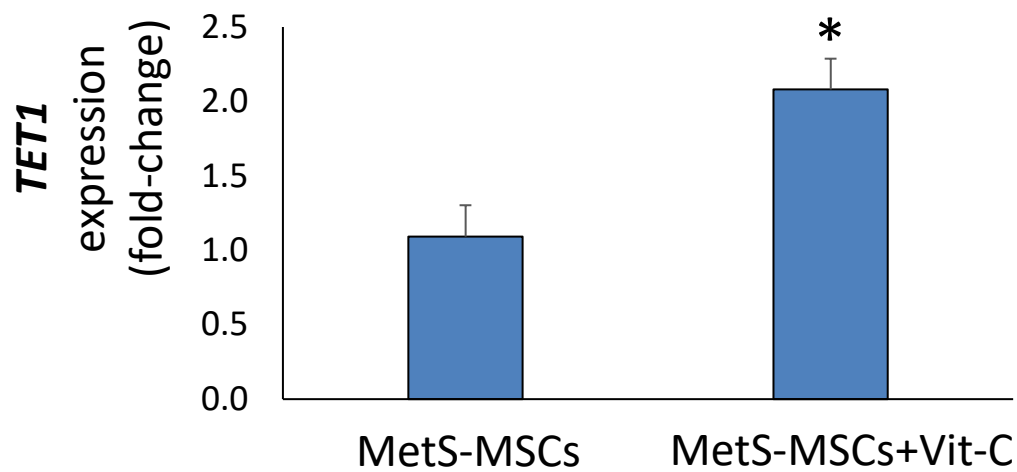




Figure S9

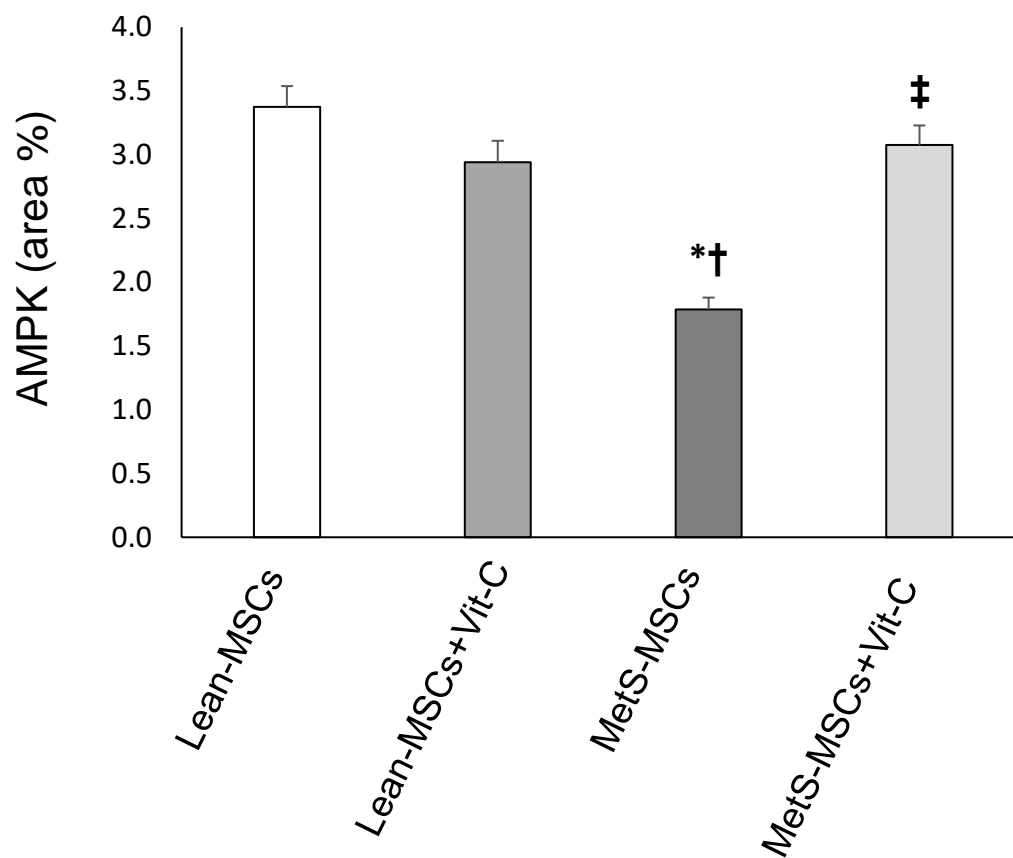
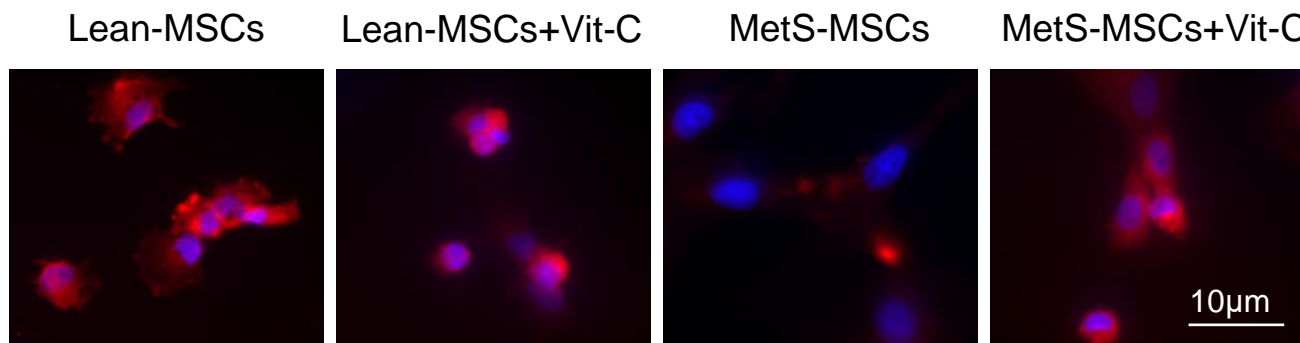


Figure S10

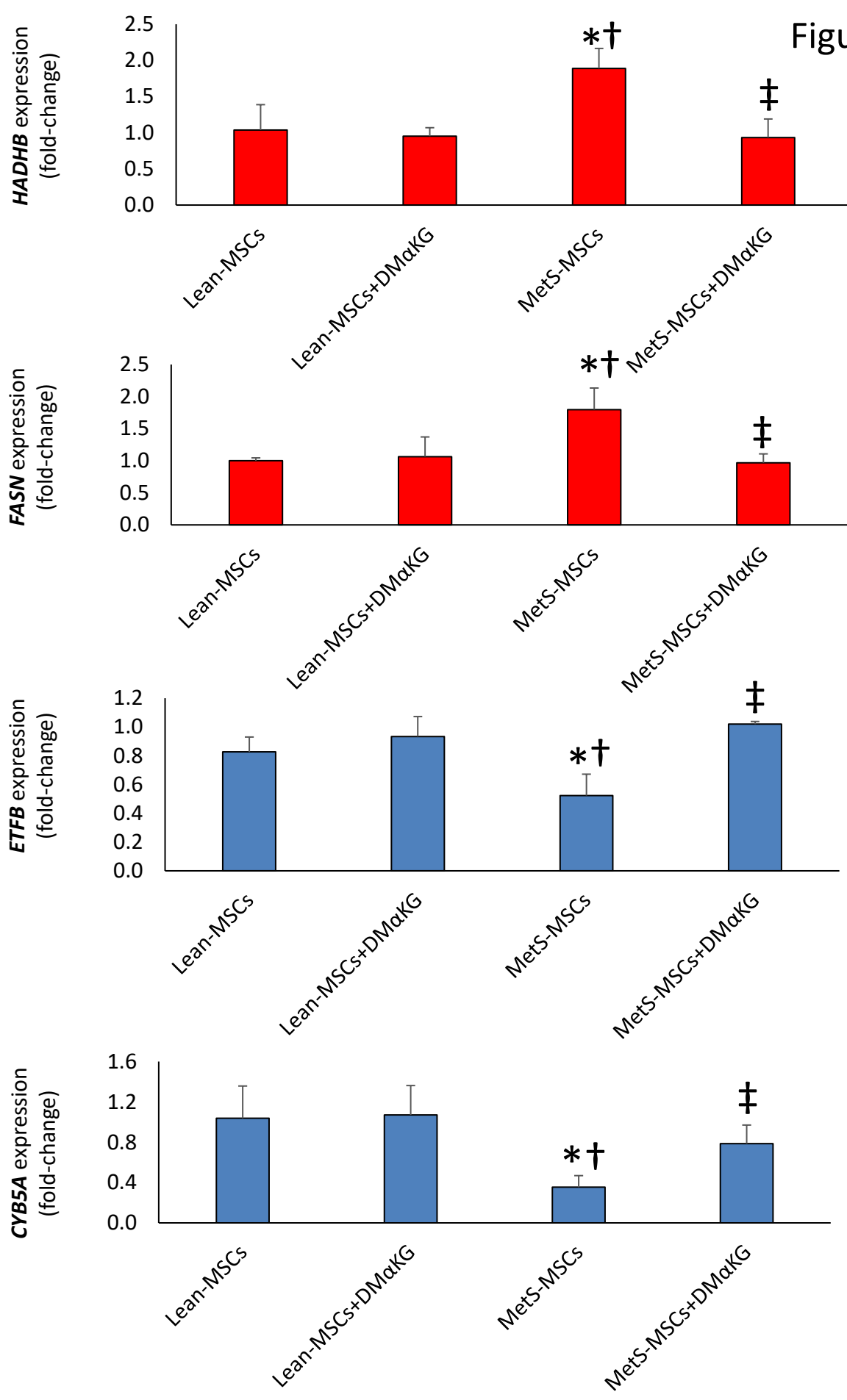


Figure S11

