Inventory of Supplemental Information

Supplemental Information

Mitochondrial retrograde signaling in mammals is mediated by the transcriptional cofactor GPS2 via direct mitochondria-to-nucleus translocation.

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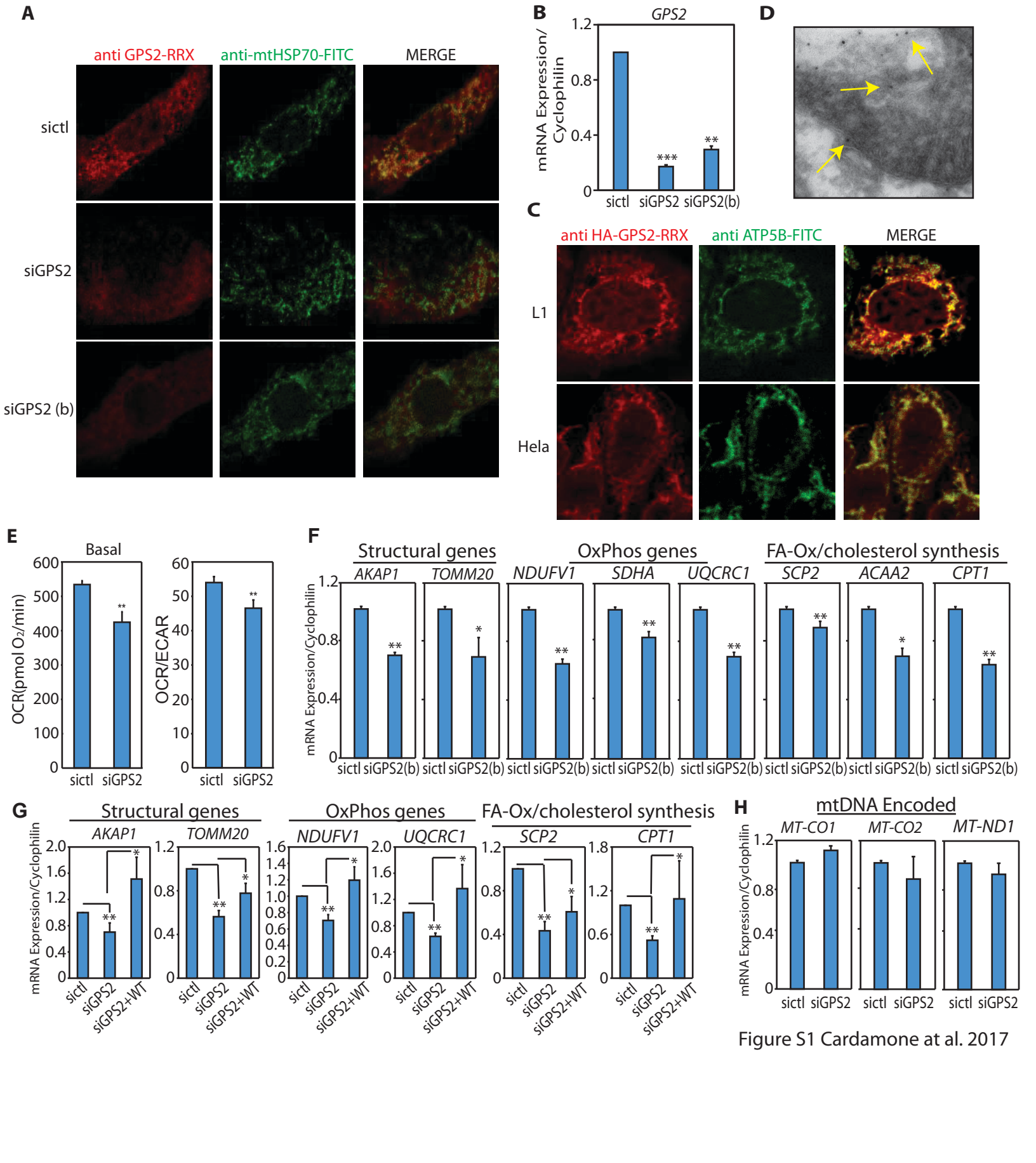


Figure S1 Cardamone at al. 2017

Supplemental Figure S1. Related to Figure 1.

(A) Mitochondria localization of endogenous GPS2 by IHF staining in murine 3T3-L1 cells, with two different siRNA against GPS2 used to confirm signal specificity. Co-staining of endogenous mtHSP70 serves as a mitochondrial marker.

(B) siRNA efficiency measured by qPCR analysis in transiently transfected 3T3-L1 cells.

(C) Mitochondria localization of overexpressed HA-GPS2 by IHF staining in murine 3T3-L1 and human Hela cells. Co-staining of endogenous mtATP5B serves as a mitochondrial marker.

(D) Mitochondria labeling by Immunogold EM for GPS2 in Hela cells.

(E) Decreased basal respiration and bioenergetic profile in Hela cells upon GPS2 downregulation.

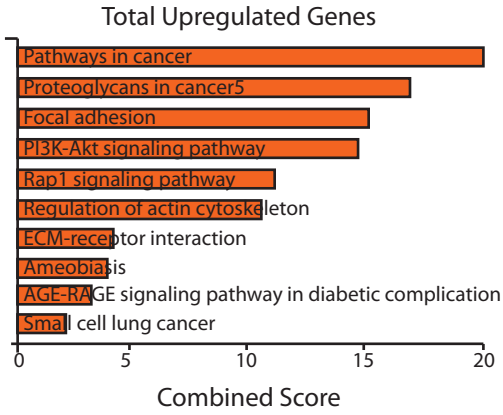
(**F)** RT-qPCR analysis of different classes of neMITO genes expression in 3T3-L1 cells transfected with siCTL or siGPS2(b).

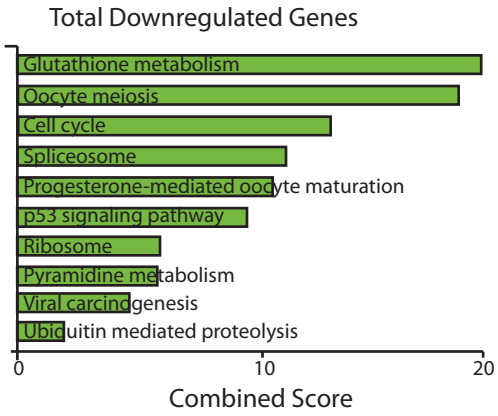
(G) Rescued expression of representative neMITO genes in GPS2-depleted 3T3-L1 cells by overexpressing HA-GPS2.

(H) RT-qPCR analysis of the expression of representative mitochondria-encoded genes in 3T3-L1 cells transfected with siCTL or siGPS2.

* indicate p-value<0.05; ** indicate p-value<0.01.

A





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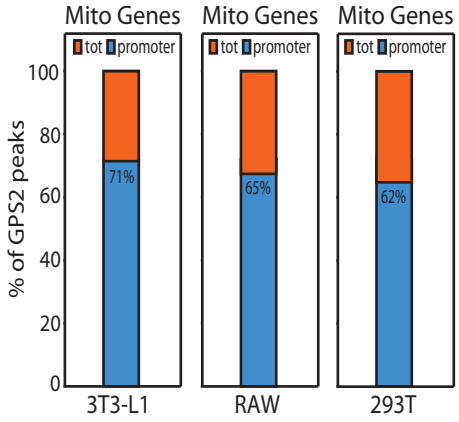


C

Cardinal Motifs enriched on GPS2 promoter peaks of Mitochondrial Genes

Motif	Name	P-value	%
	Sp1	1e-164	38.11%
	NFY	1e-97	24.64%
	ETS	1e-74	19.32%
	NRF1	1e-49	6.39%
	GFY	1e-35	4.84%
	YY1	1e-22	6.04%
	TATA-Box	1e-11	11.94%

Other Motifs enriched on GPS2 promoter peaks of Mitochondrial Genes

Motif	Name	P-value	%
	Klf5	1e-122	54.99%
	Klf4	1e-66	7.71%

Figure S2 Cardamone at al. 2017

Supplemental Figure S2. Related to Figure 2.

(A) DE genes between WT and GPS2-KD 3T3-L1 cells by RNAseq. Analysis of the 10 most significant pathways associated with total upregulated (left) and downregulated (right) genes by EnrichR (KEGG Pathways).

(B) Percentage of GPS2 peaks on mitochondrial genes localized to promoter areas (+1kb/-200bp around TSS) in ChIPseq data sets from 3T3-L1 (GSE57779), 293T (GSE35197) and RAW (GSE66774) cells.

(C) Cardinal DNA-binding motifs and other TFs binding motifs enriched in the GPS2 peaks in 3T3-L1 cells as computed by HOMER Known Motifs enrichment analysis.

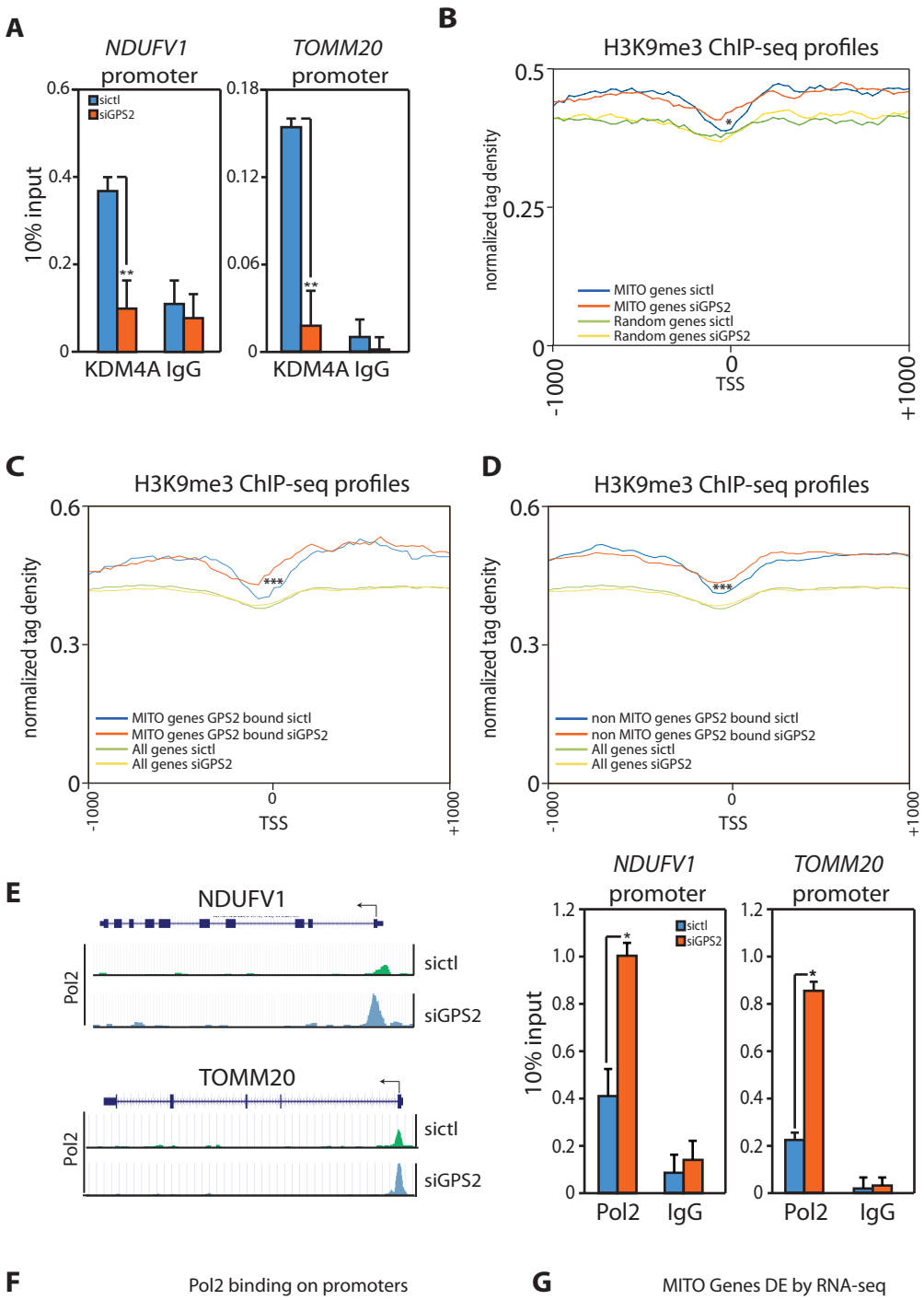


Figure S3 Cardamone at al. 2017

Supplemental Figure S3. Related to Figure 3.

(A) Decrease in JMJD2A/KDM4A recruitment to *Ndufv1* and *Tomm20* gene promoters by ChIP in GPS2-KD 3T3-L1 cells.

(B) Tag density plot showing the increase in H3K9me3 binding on mitochondrial genes in GPS2-KD 3T3-L1 cells compared to randomly selected groups of comparable size (2086 genes). P-values were computed using the Wilcoxon test based on the difference in normalized counts +/-200bp around the TSS.

(C) (D) Tag density profile showing the increase in H3K9me3 binding on 962 GPS2 promoters of mitochondrial genes **(C)** or 4915 GPS2 bound promoters of non-mitochondrial genes **(D)** in GPS2-KD 3T3-L1 cells as determined by GPS2-ChIPseq in 3T3-L1 cells. P-values were computed using the Wilcoxon test based on the difference in normalized counts +/-200bp around the TSS.

(E) Representative ChIPseq tracks for POL2 binding to *Ndufv1* and *Tomm20* gene promoters on the UCSC browser (left). Validation of POL2 accumulation on target promoters in GPS2-KD 3T3-L1 cells by hand-ChIP (right).

(F) Boxplots showing a significant increase in POL2 binding specific to both the promoters of mitochondrial genes and the promoters of GPS2-bound genes in GPS2-KD 3T3-L1 cells. P-values were computed using the Wilcoxon test based on the difference in normalized counts +/-200bp around the TSS. In green are represented the GPS2-bound mitochondrial gene promoters (pr mito Gb), in blue the non-mitochondrial GPS2-bound gene promoters (other pr Gb), in red all the mitochondrial gene promoters (all mito).

(G) Boxplot showing decreased transcription by GROseq for both down- and up-regulated mitochondrial genes as defined by RNAseq in 3T3-L1 transfected with siCTL or siGPS2 (p-value<2.2e-16 for both groups).

* indicate p-value<0.05; ** indicate p-value<0.01, *** indicate p-value<0.001.

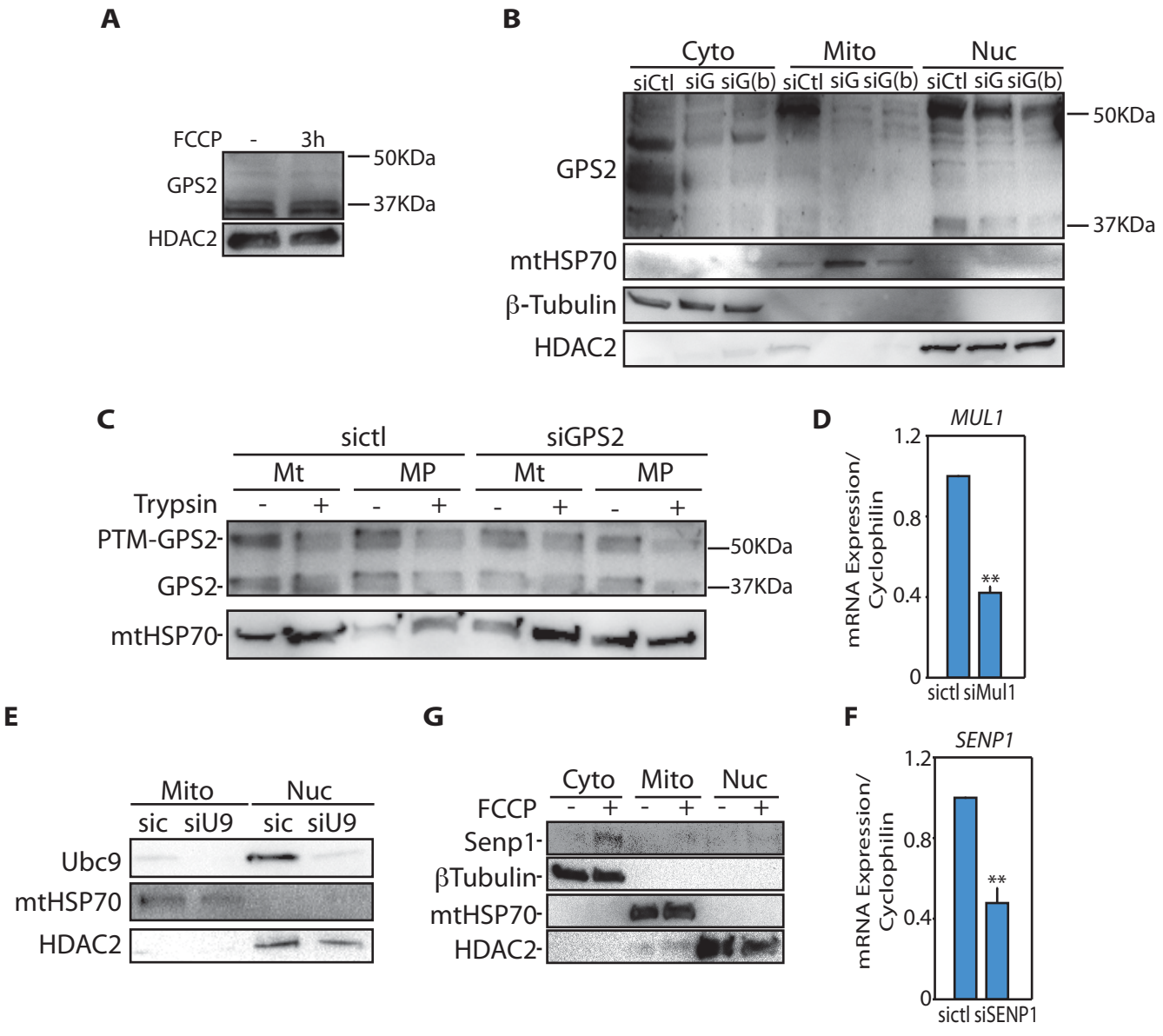


Figure S4 Cardamone at al. 2017

Supplemental Figure S4. Related to Figure 4.

(A) Total GPS2 protein levels not changing upon FCCP treatment as shown by WB of whole cell extracts.

(B) Two different siRNA against GPS2 -siG and siG(b)- were used to show by western blotting of fractionated extracts the specificity of GPS2 bands.

(C) Trypsin protection assay showing localization of GPS2 and post translational modified GPS2 (PTM-GPS2) in the mitochondria matrix and outer membrane respectively. Mt stands for isolated mitochondria, MP for mitoplasts.

(D) MUL1 siRNA efficiency by qPCR analysis.

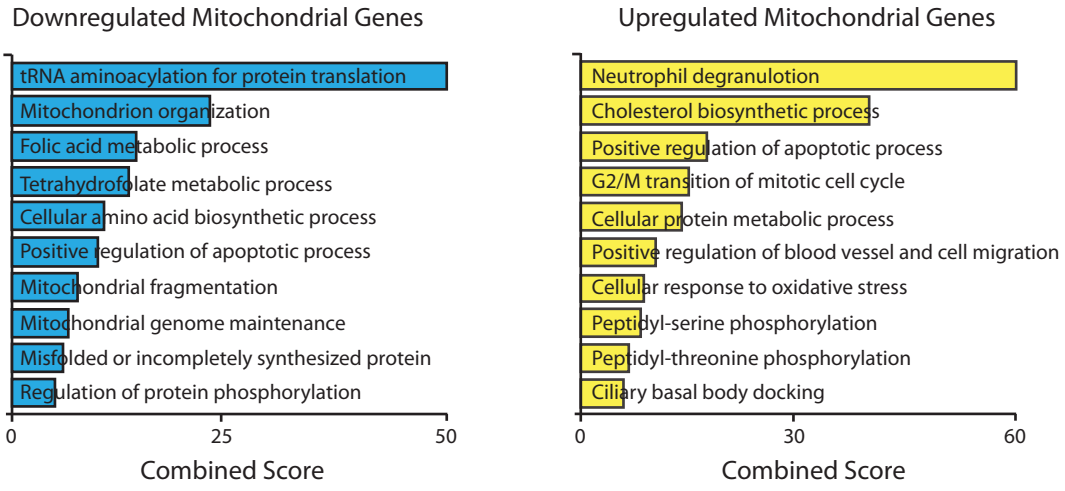
(E) Ubc9 siRNA efficiency by Western Blot analysis.

(F) Western Blot showing increased SENP1 levels in 3T3-L1 cells treated with FCCP.

(G) SENP1 siRNA efficiency by qPCR analysis.

* indicate p-value<0.05; ** indicate p-value<0.01.

A



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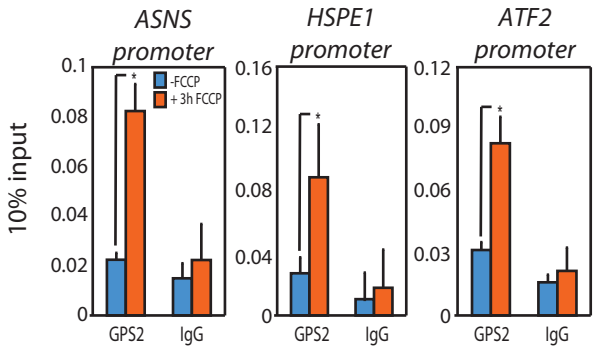

Figure S5 Cardamone at al. 2017

Supplemental Figure S5. Related to Figure 5.

(A) Analysis of 10 most enriched pathways among mitochondrial genes upregulated or downregulated in response to FCCP depolarization as identified by GROseq (EnrichR, KEGG Pathways).

(B) GPS2 ChIP-qPCR analysis in 3T3-L1 cells for genes representative of the pathways indicated in Figure 5C.

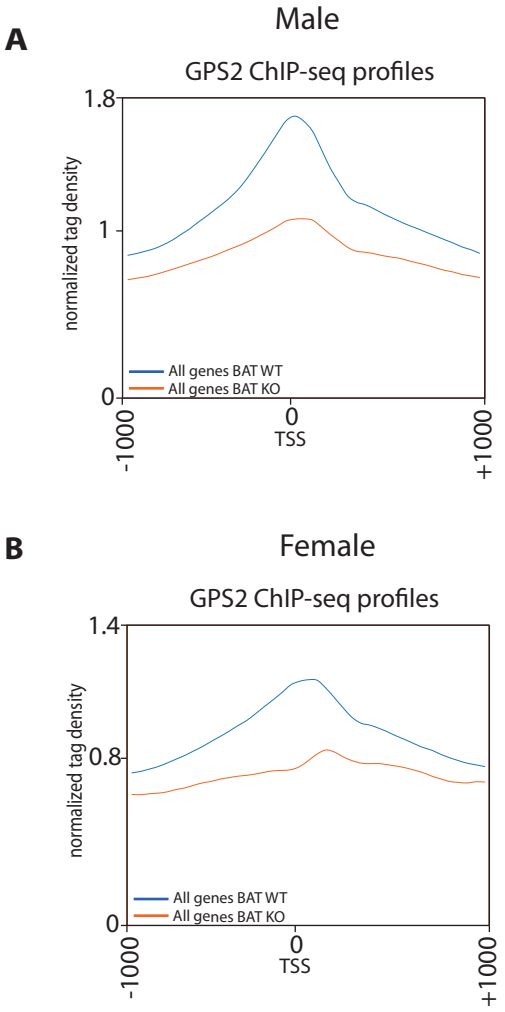

Figure S6 Cardamone at al. 2017

Supplemental Figure S6. Related to Figure 7.

(A) (B) Tag density profiles showing GPS2 binding on all gene promoters in Male **(A)** and Female **(B)** KO mice.