

## **Integrative analyses of translome and transcriptome reveal important translational controls in brown and white adipose regulated by microRNAs**

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## Supporting Information Legends

### Figure S1. Characterization of the adipocyte differentiation system.

(A) ORO staining picture of the representative samples during brown and white adipogenesis time course. (B) Key adipogenic marker (Fabp4 and Pparg) expression during the differentiation time course detected by realtime PCR. N=4. Primary adipocyte precursors were isolated as described in methods. ORO staining and realtime PCR were performed at the indicated time point after induction of differentiation.

### Figure S2. Correlation of ribosome profiling and/or RNA-seq between different samples

(A) Correlation of RNA-seq data between replicates.  
(B) Correlation of ribosome profiling data between replicates.  
(C-E) GSEA analysis of Oxidative phosphorylation across the ranked genes based on their RNA FCs during brown adipogenesis (C), white adipogenesis (D) and BAT vs. WAT (E).  
(F) Heatmap of the correlation efficiency for ribosome profiling data between different samples.  
(G) Heatmap of the correlation efficiency for RNA-seq data between different samples.

### Figure S3. Translational control contributes to gene expression change during adipogenesis.

(A) Heatmap of genes with  $\geq 4$  RPF FCs during both brown and white adipogenesis.  
(B) Correlation of RPF FCs during white adipogenesis and brown adipogenesis.  
(C) Correlation of RNA FCs during white adipogenesis and brown adipogenesis.  
(D) The relative contribution of RNA change and Eff change to the total translational change.  
(E,F,G) GSEA of ranked genes based on  $\log_2 \Delta FC_{\text{Eff}} = (\text{Eff}_{\text{BATD5}} / \text{Eff}_{\text{BATD0}}) / (\text{Eff}_{\text{WATD5}} / \text{Eff}_{\text{WATD0}})$  in brown and in white adipogenesis.

**Figure S4.** Comparison of proteomics between BAT vs WAT by ITRAQ.

(A,B) GSEA of genes ranked by their protein FCs between BAT and WAT.

(C) GSEA were performed using ranked genes based on their RPF-, RNA-, and protein FCs between BAT and WAT. Normalized enrichment scores (NES) of GSEA pathways significantly altered in all three analysis (FDR<0.25) are plotted in a heatmap.

(E) Correlation between protein FCs and RPF FCs in BAT vs. WAT.

(F) Correlation between protein FCs and RNA FCs in BAT vs. WAT.

**Figure S5.**

(A-C) Cumulative curves of miR-203's targets and non-targets were plotted based on their expression in BAT. (A) based on RPF, (B) base on RNA, (C) based on Eff. The KS test was used for P value.

(D) The FCs of targets vs non-targets were calculated in BAT and in WAT. The stack bar indicates the percentage of miRNAs whose targets are more repressed in BAT (black) and miRNAs whose targets are more repressed in WAT (grey).

The repressive extent of miRNA targets is not related to microRNA abundance.

(B, C) The distribution of (B) RPF and (C) RNA FCs between targets vs non-targets across miRNA abundance derived from small RNA-seq in BAT.

**Figure S6.** The whole gel of Ndufaf1 and Acad9.

## Supplemental datasets

Download from

<https://www.dropbox.com/sh/xp3x9ueklzzawqj/AACJibtDRYyOHpKNriVmUjQka?dl=0>

### *S01 Ribo+RNA+eff\_Protein\_processed 2*

Expression values in Ribo, RNA and Protein of each gene in all samples. It also includes the differential expression between different samples.

### *S02 Gene groups\_BATD5vsD0\_FC\_Ribo>4*

Genes with total translation FC >4 during brown adipogenesis are divided into 4 groups as shown in Figure 2G.

### *S03 Gene groups\_WATD5vsD0\_FC\_Ribo>4*

Genes with total translation FC >4 during white adipogenesis are divided into 4 groups as shown in Figure 2H.

### *S04 Gene groups\_BATvsWAT\_FC\_Ribo>2*

Genes with total translation FC >2 between BAT and WAT are divided into 4 groups as shown in Figure 3D.

### *S05 FC during adipogenesis\_mitochondria, Lipids, translation genes*

Fold changes (at Ribo, RNA and Eff levels) for Mitochondria, Lipids, Translation GOs-related genes during brown and white adipogenesis, which were used to generate figure 2K-P.

### *S06 FC between BAT vs WAT\_mitochondria, Lipids, translation genes*

Fold changes (at Ribo, RNA and Eff levels) for Mitochondria, Lipids, Translation GOs-related genes between BAT and WAT, which were used to generate Figure 3 F-H

#### *S07 Protein\_ITRAQ*

Protein quantification data in ITRAQ analysis

#### *S08 Number of miRsites vs RPF (BATvsWAT)\_binned*

The distribution of the conserved miRNA binding sites on mRNAs which were binned into 12 groups according to the Ribo fold changes (FC) between BAT and WAT. Data were used for Figure 4J.

#### *S09 Number of miRsites vs RNA (BATvsWAT)\_binned*

The distribution of the conserved miRNA binding sites on mRNAs which were binned into 12 groups according to the RNA fold changes (FC) between BAT and WAT. Data were used for Figure 4K.

#### *S10 Number of miRsites vs Eff (BATvsWAT)\_binned*

The distribution of the conserved miRNA binding sites on mRNAs which were binned into 12 groups according to the Eff fold changes (FC) between BAT and WAT. Data were used for Figure 4L.

#### *S11 FC\_P\_miR-targets vs non-targets in BAT or WAT*

*For each microRNA, its target gene expression at RPF, RNA and Eff levels are compared with its non-target gene expression in BAT or WAT.*

*S12 Targets and non-targets\_BATvsWAT*

For each microRNA, its target gene expression ratio between BAT and WAT are calculated for non-target genes vs. target genes.

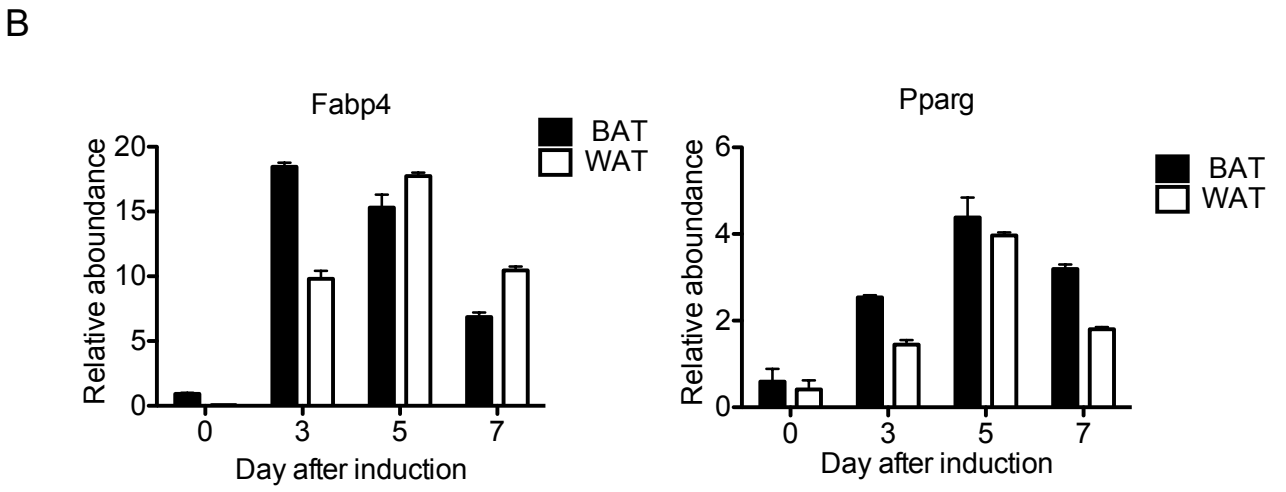
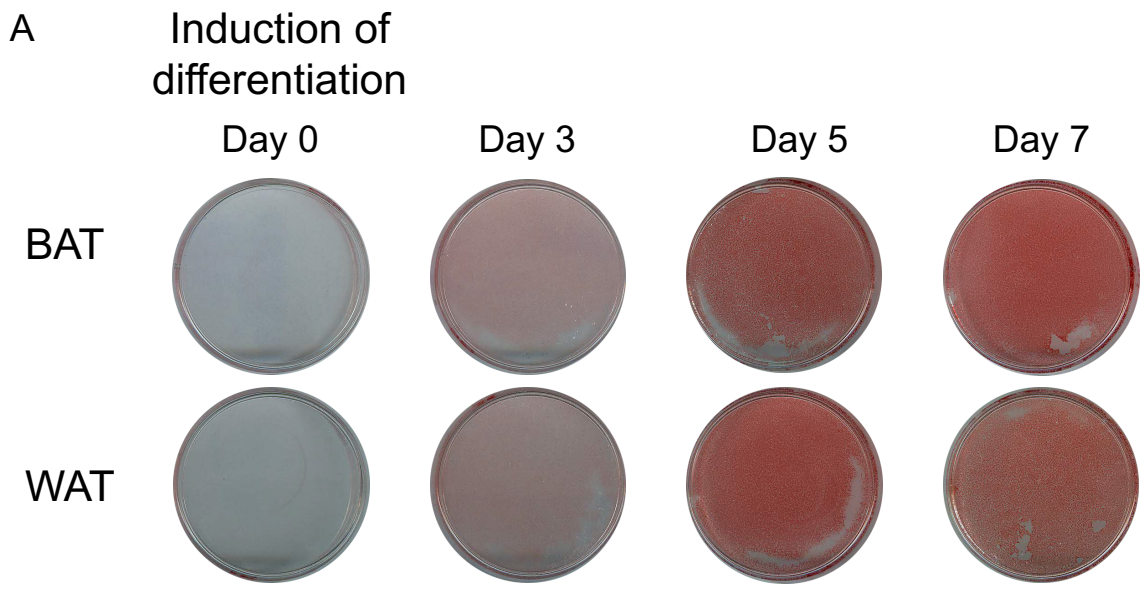
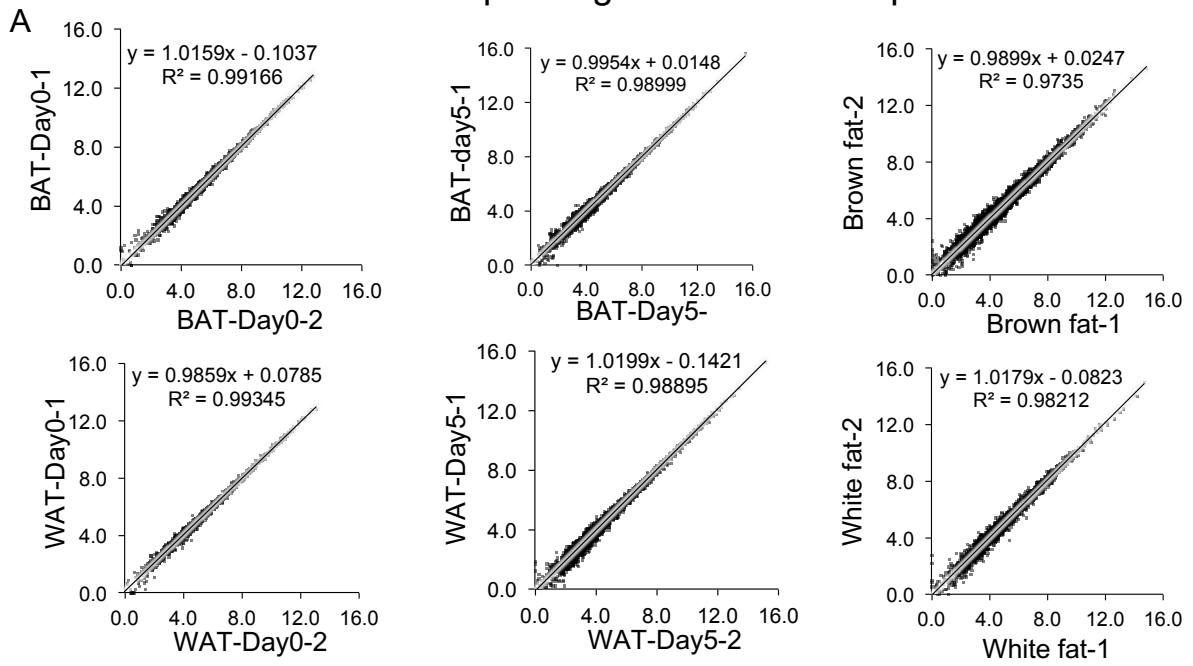


Figure S1

# Correlation of RNA profiling data between replicates



# Correlation of ribosome profiling data between replicates

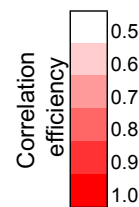
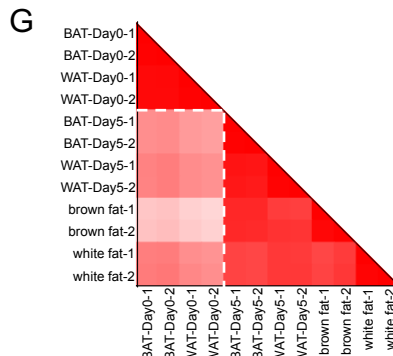
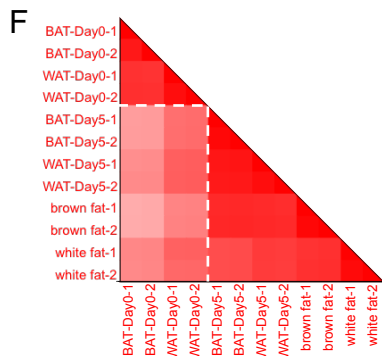
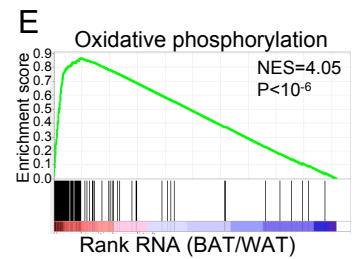
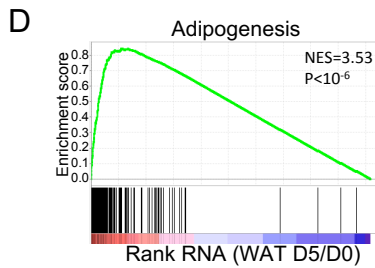
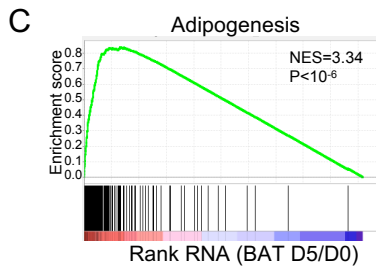
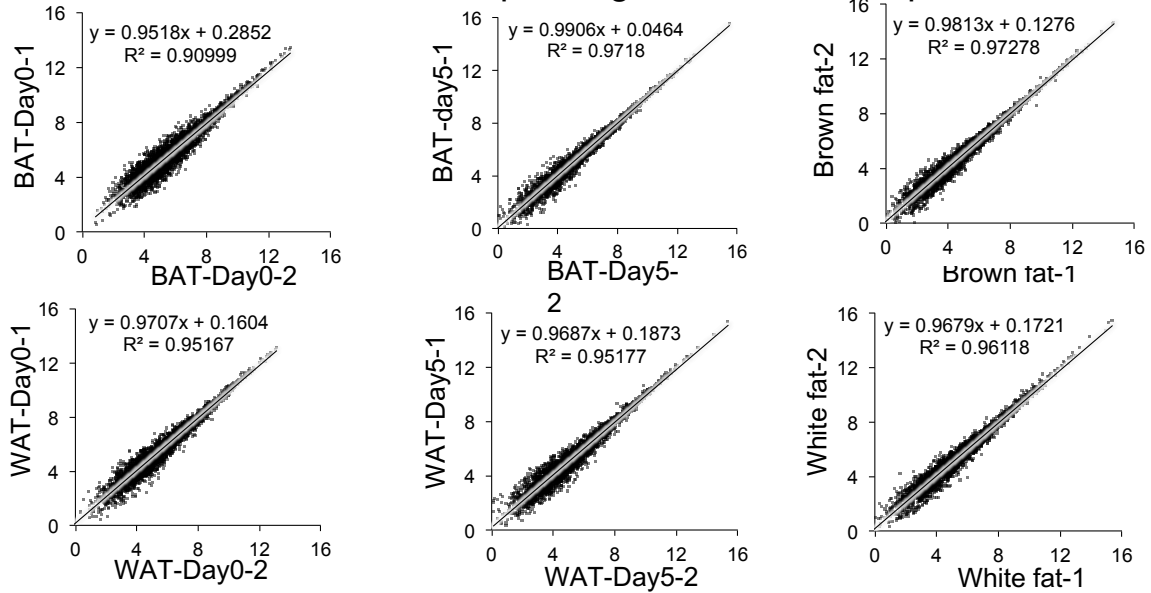


Figure S2



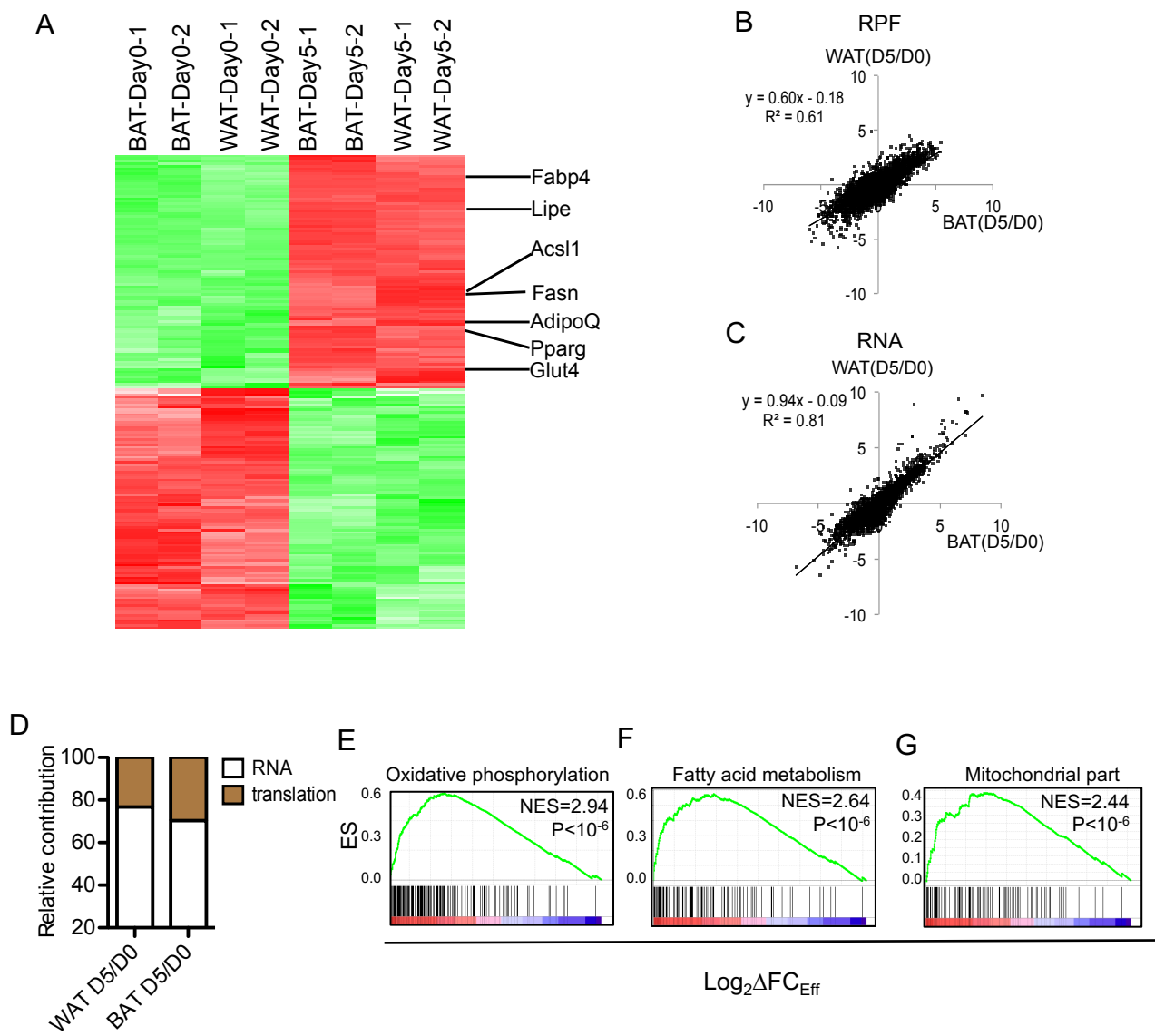


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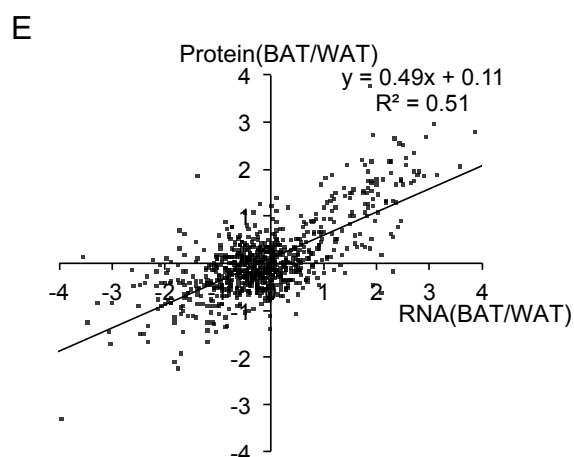
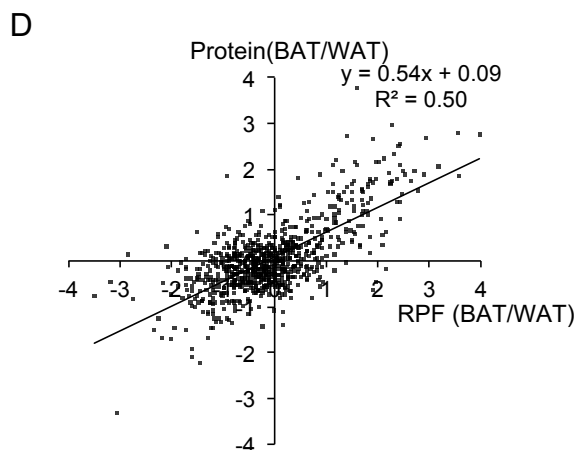
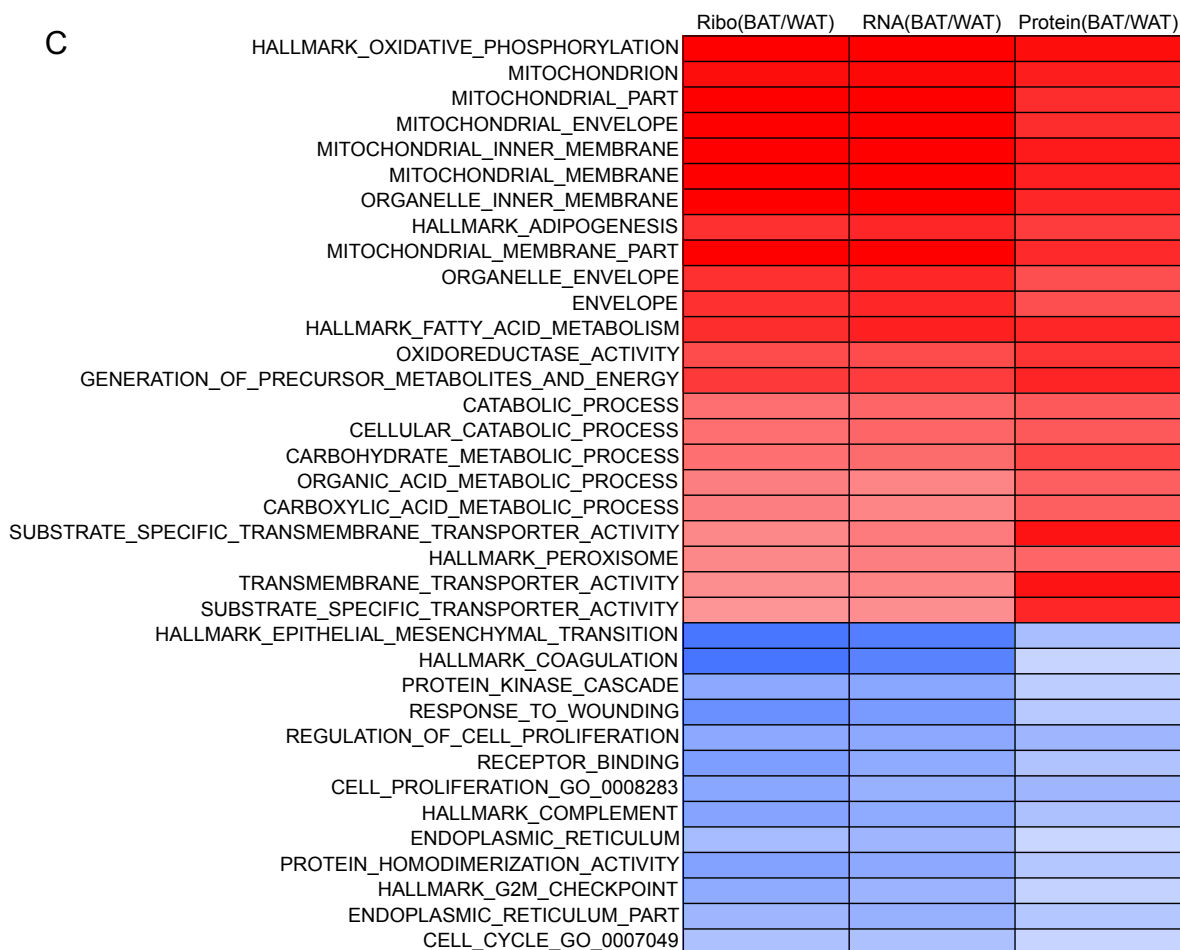
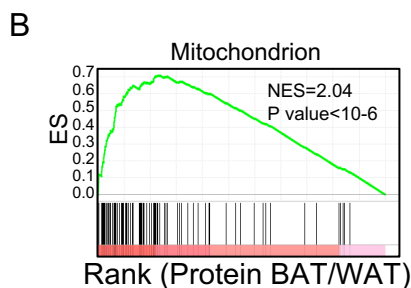
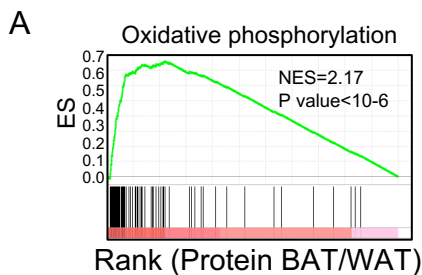


Figure S4

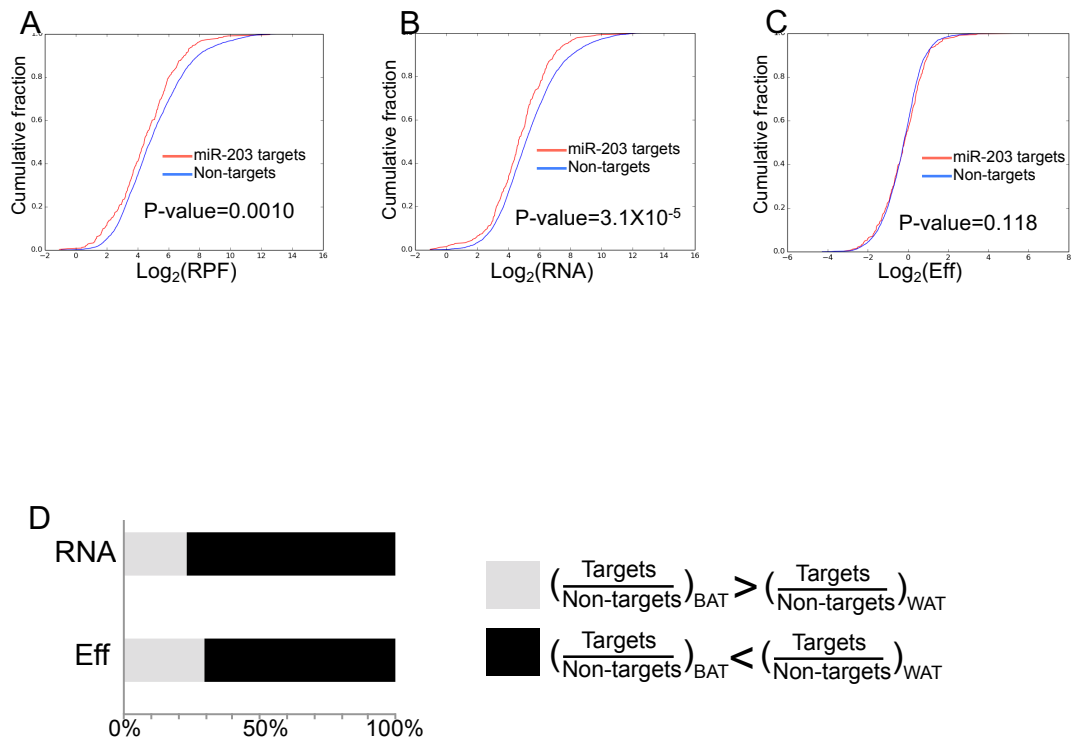


Figure S5

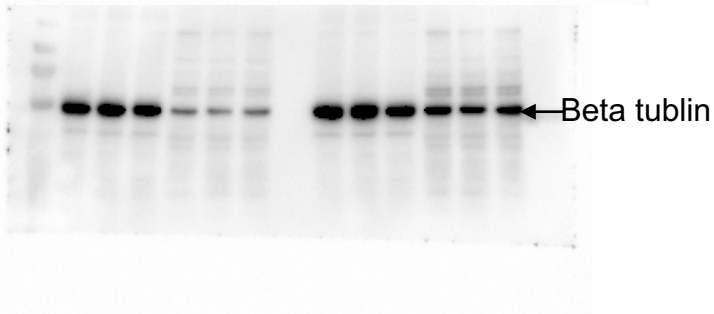
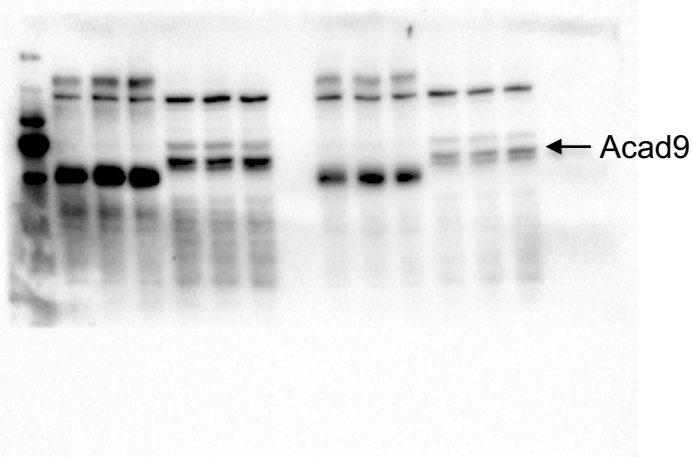
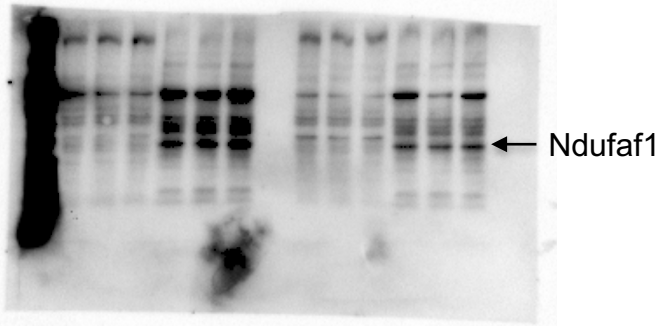


Figure S6