
Supplementary Information for “A comparative study of metformin and nicotinamide riboside in alleviating tissue aging in rats”

Supplemental Figures

Figure S1. Lifespan and tissue weights in MET- and NR-treated rats.

(A) Survival curves of O-CTRL ($n = 12$), O-MET ($n = 7$), and O-NR rats ($n = 6$) (Log-rank test).

(B) Organ weights of lung, liver, heart, brain, and kidney in Y-CTRL ($n = 5$), O-CTRL ($n = 5$), O-MET ($n = 7$), and O-NR ($n = 3$) groups (mean \pm SEM, unpaired Student's t -test).

(C) Relative organ weights of lung, liver, heart, brain, and kidney in Y-CTRL ($n = 5$), O-CTRL ($n = 5$), O-MET ($n = 7$), and O-NR ($n = 3$) groups (mean \pm SEM, unpaired Student's t -test).

Figure S2. Changes in the transcriptional profiles of different tissues during aging, MET, and NR treatment.

(A) Heatmaps showing the distribution of rescue DEGs (green), failure to rescue DEGs (orange), MET- or NR-specific DEGs (dark blue), and pro-aging DEGs (pink) in the nine tissues. Each column represents one gene. Orange, upregulated [$\log_2(\text{fold change}) > 1.5$, adjusted P -value < 0.05]; green, downregulated [$\log_2(\text{fold change}) < -1.5$, adjusted P -value < 0.05]; light grey, unchanged [$|\log_2(\text{fold change})| \leq 1.5$ or adjusted P -value ≥ 0.05].

(B) Rose charts showing the numbers of MET or NR pro-aging DEGs, and MET- or NR-specific rescue DEGs in the nine tissues (orange, upregulated; green, downregulated).

(C) Point plots showing the aging DEGs, MET DEGs, and NR DEGs shared by at least five tissues. The color keys from light grey to orange or green indicate $-\log_{10}(P\text{-value})$ from low to high.

(D) Representative GO terms and pathways enriched in aging DEGs shared by at least four tissues. The color keys from light grey to orange or green indicate $-\log_{10}(P\text{-value})$ from low to high.

(E) Gene set enrichment analysis plots showing a decreased interferon- α response in different tissues with MET or NR treatment.

(F) Heatmap showing the genes shared between rescue DEGs and aging-associated genes in the Aging Atlas database among nine tissues. The color key from green to orange indicates $\log_2(\text{fold change})$ from low to high.

Figure S3. Changes in the transcriptional regulatory networks and side-effects of different tissues upon MET and NR treatment.

(A) Rose charts showing the numbers of aging TFs, MET or NR TFs, and MET or NR rescue TFs, in the nine tissues (orange, upregulated; green, downregulated).

(B, C) Point plots showing the upregulated (orange) and downregulated (green) MET rescue TFs, NR rescue TFs, and common rescue TFs shared by at least two tissues upon MET or NR treatment.

(D) Pie charts showing the percentages of pro-aging DEGs upon MET or NR treatment in the nine tissues.

Figure S4. Changes in gene expression in response to MET and NR treatment.

(A) Heatmaps showing common rescue DEGs upon MET and NR treatment in the nine tissues. The color key from green to orange indicates z-score from low to high.

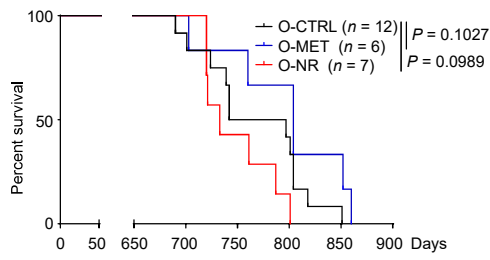
(B) Venn diagrams showing the numbers of common rescue DEGs upon both MET and NR treatment in the nine tissues.

(C) Representative GO terms and pathways enriched in common rescue DEGs shared by at least two tissues upon both MET and NR treatment. The color keys from light grey to orange or green indicate $-\log_{10}(P\text{-value})$ from low to high.

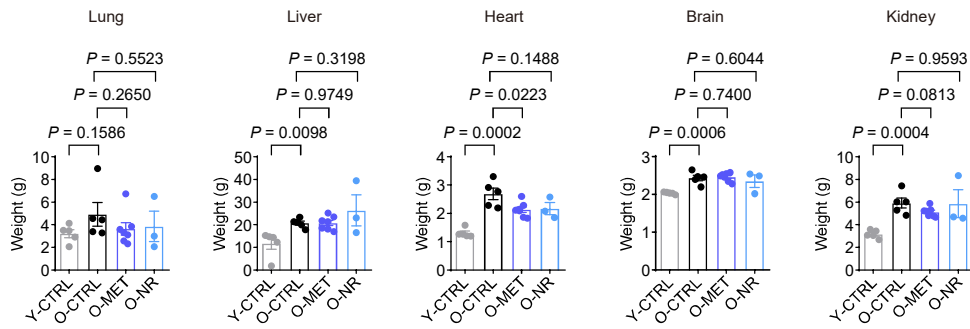
(D) Heatmap showing the common rescue DEGs shared by at least three tissues upon both MET and NR treatment. The color key from green to orange indicates $\log_2(\text{fold change})$ from low to high.

Figure S1

A



B



C

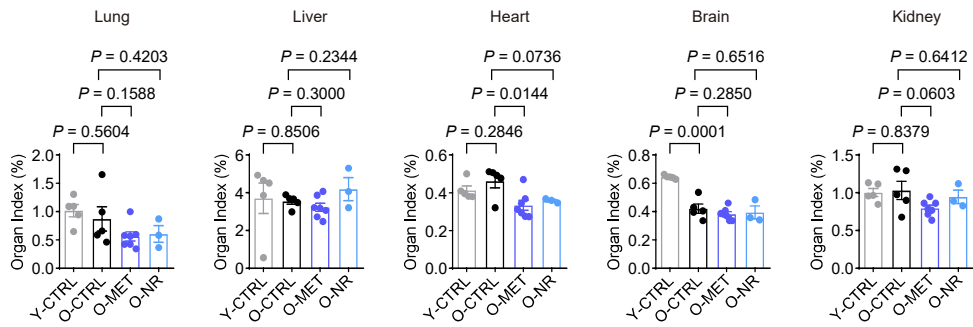
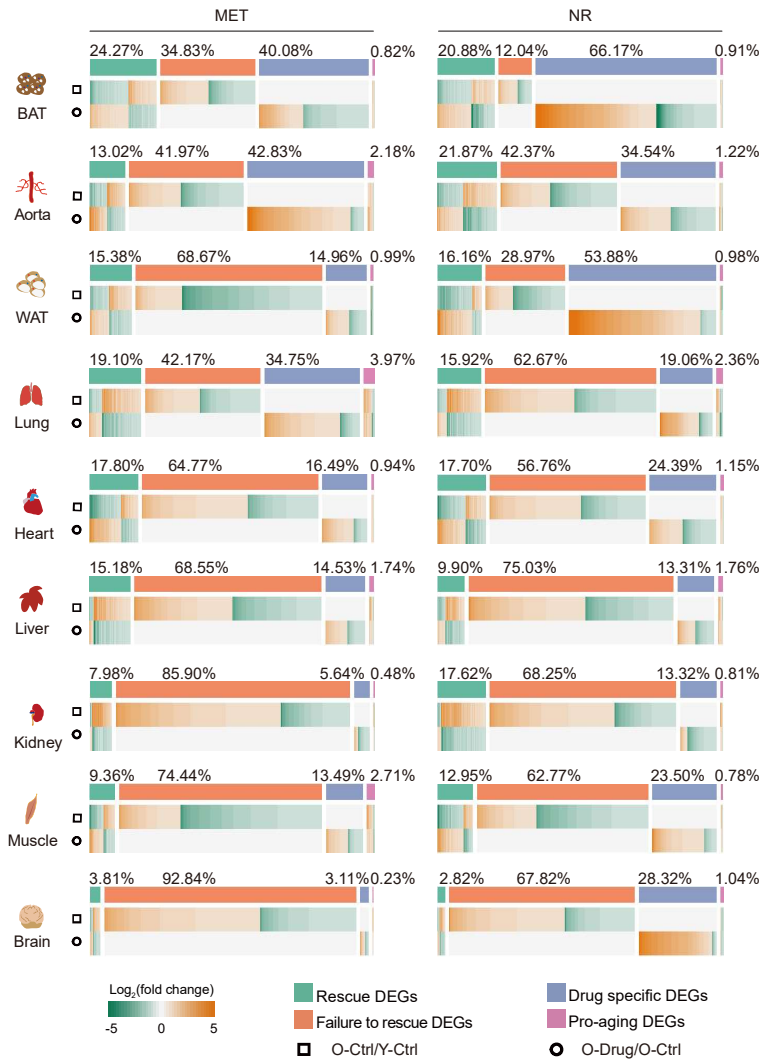
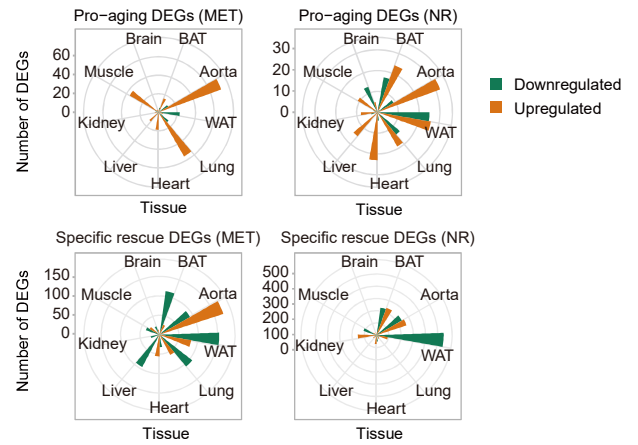


Figure S2

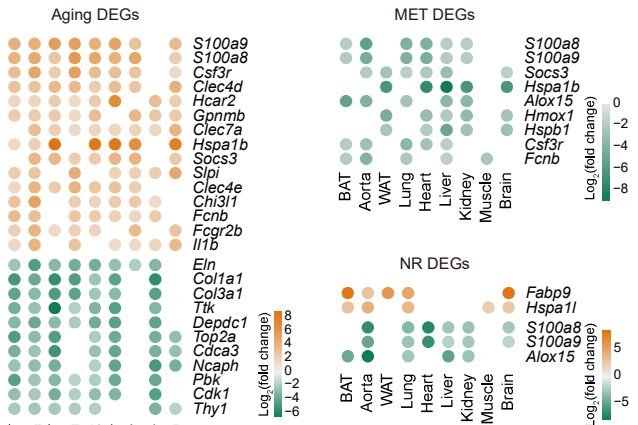
A



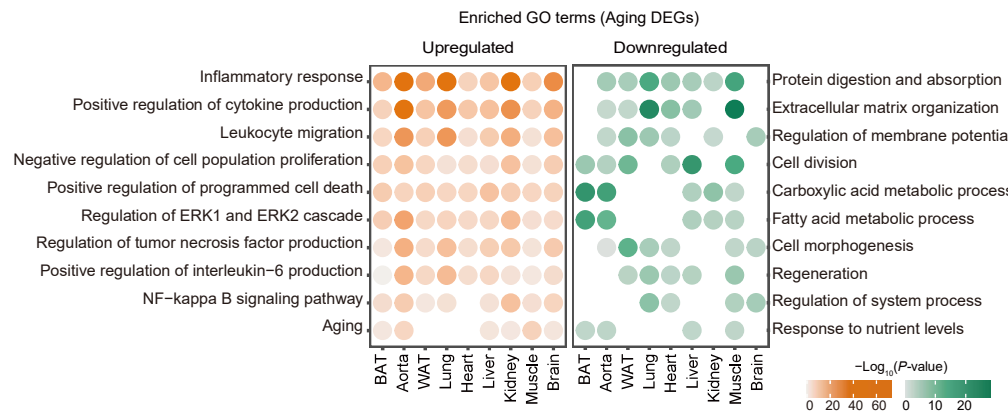
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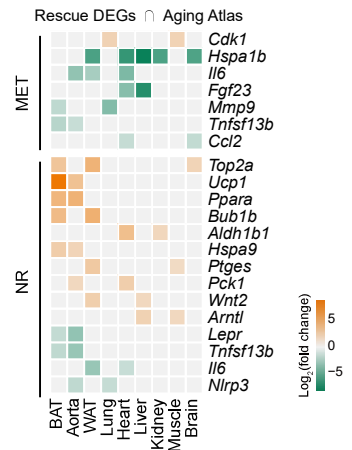
C



D



F



E

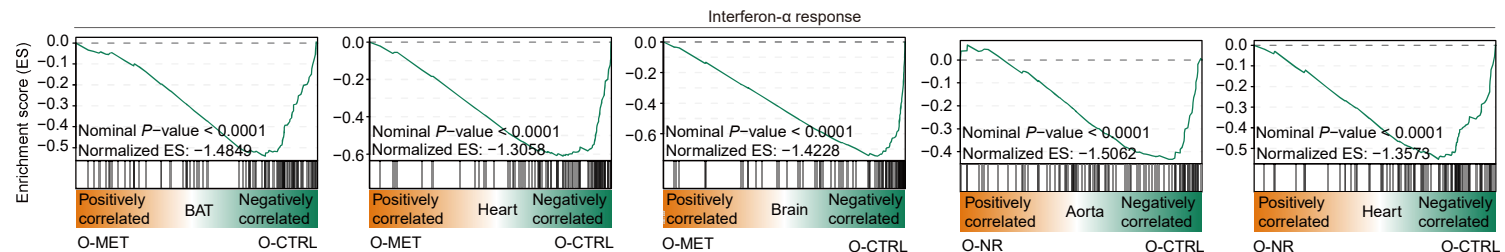
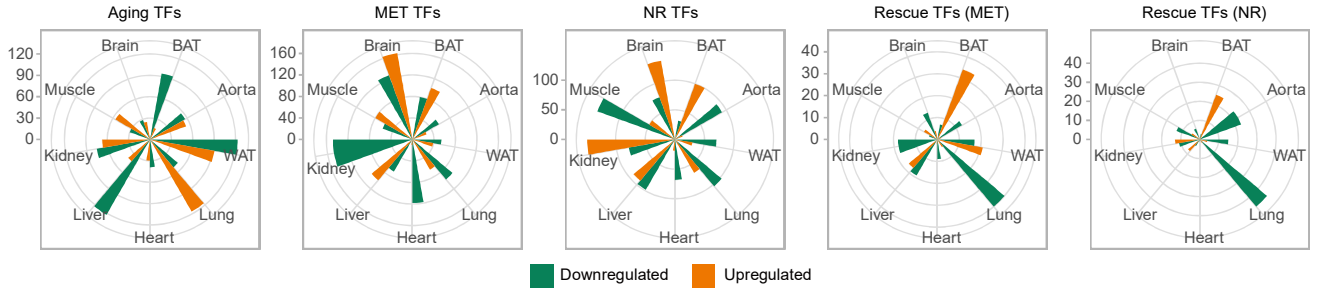
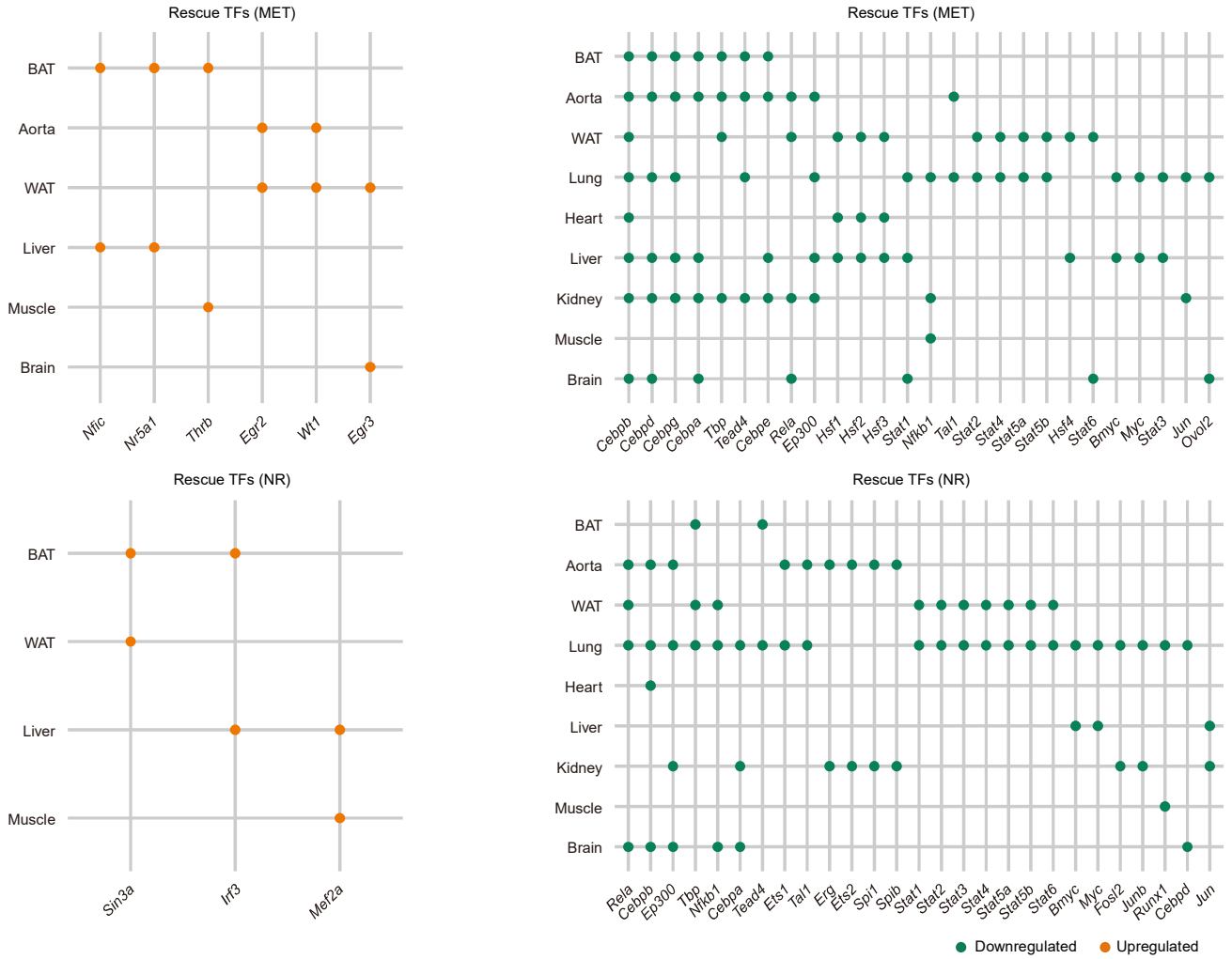


Figure S3

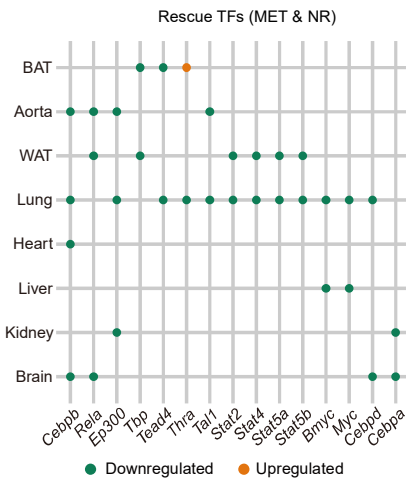
A



B



C



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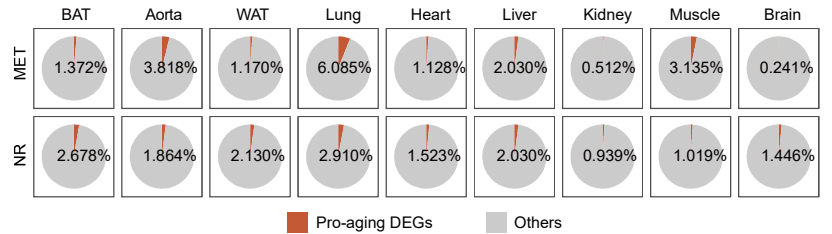
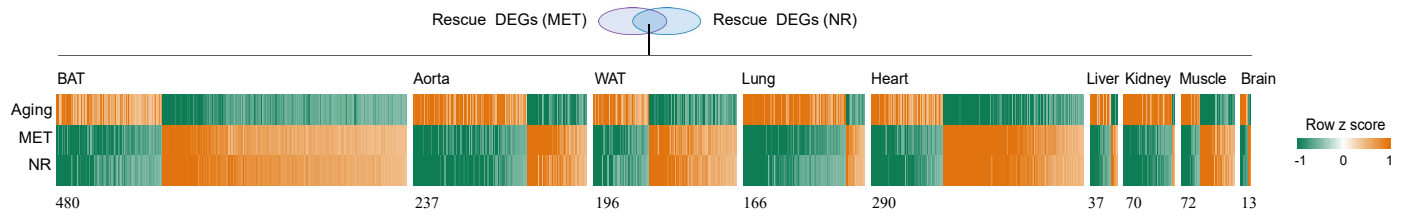
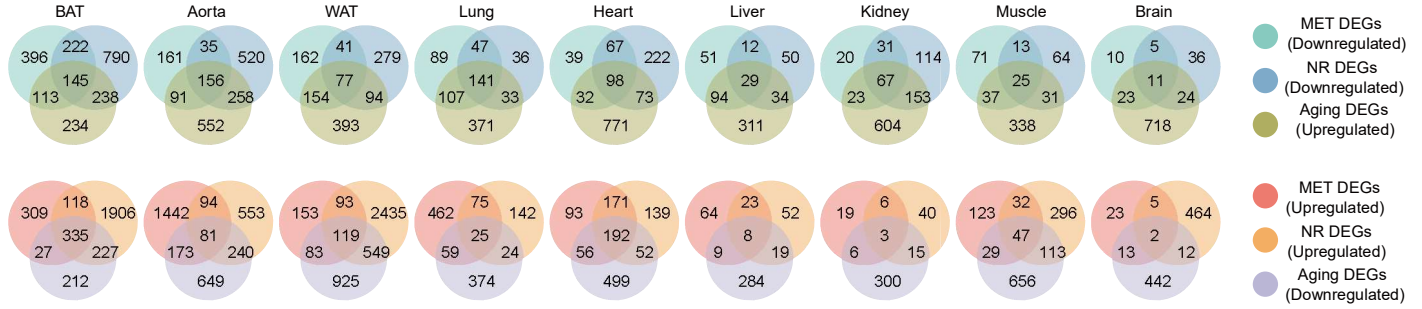


Figure S4

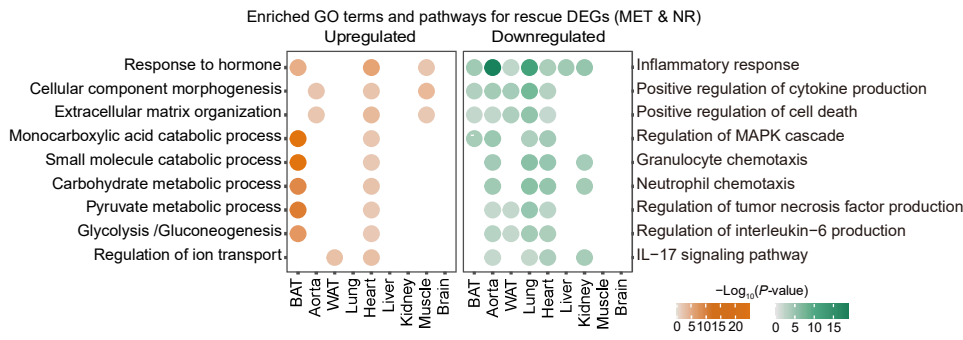
A



B



C



D

