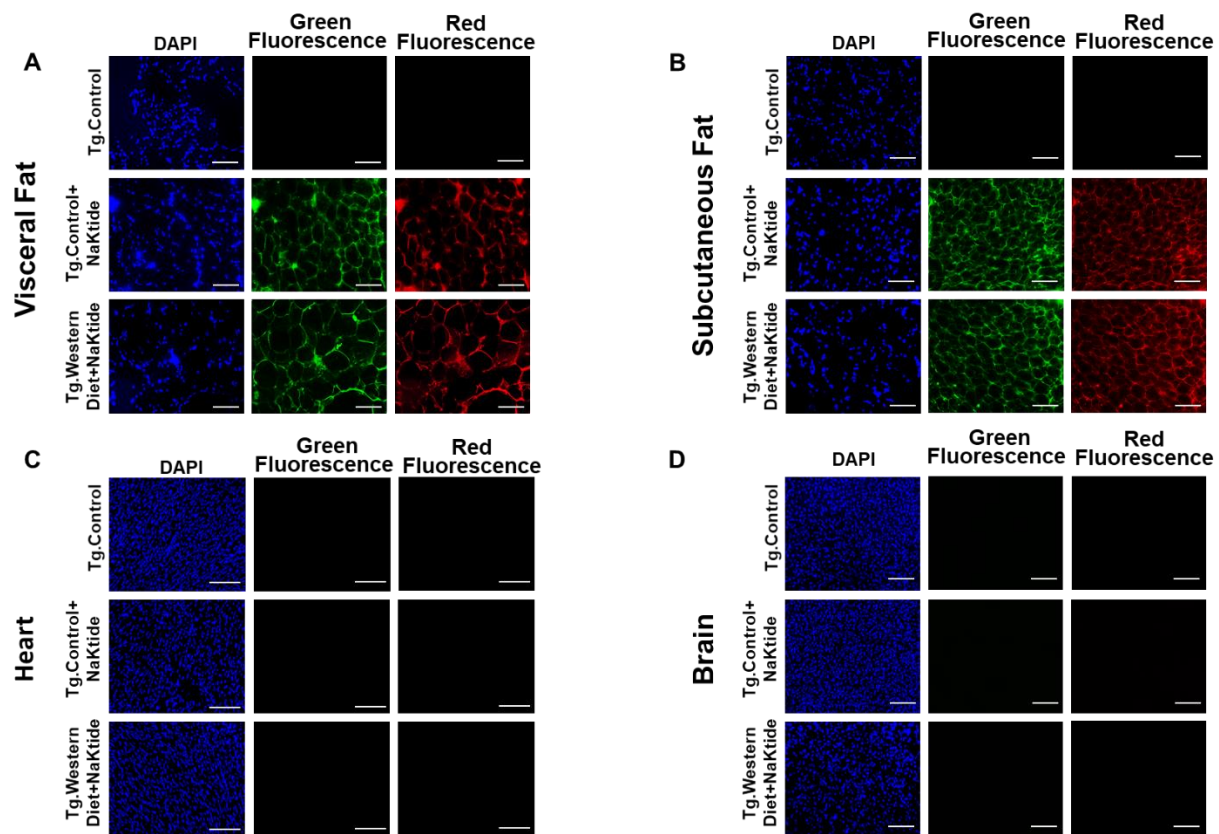


Supplemental information

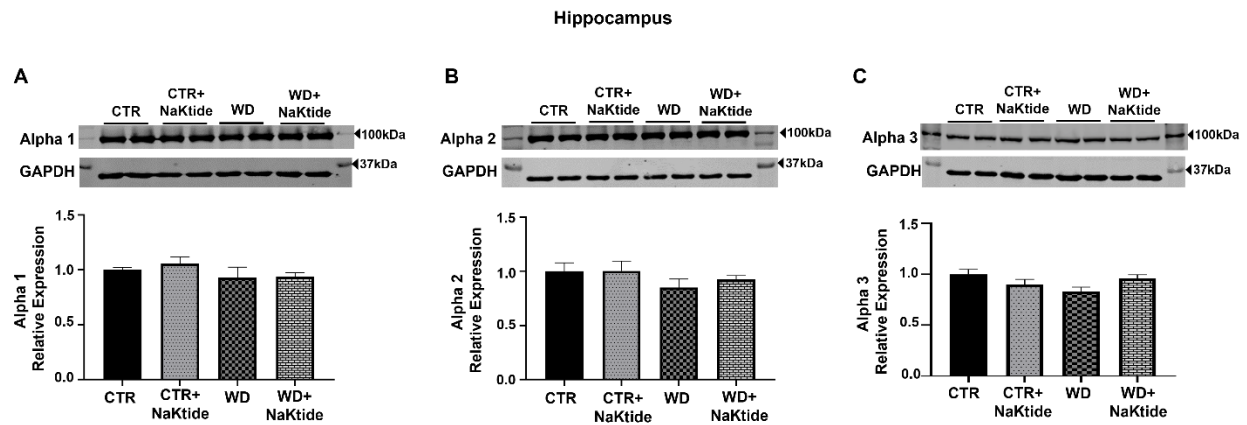
**Role of adipocyte Na,K-ATPase oxidant
amplification loop in cognitive
decline and neurodegeneration**

Komal Sodhi, Rebecca Pratt, Xiaoliang Wang, Hari Vishal Lakhani, Sneha S. Pillai, Mishghan Zehra, Jiayan Wang, Lawrence Grover, Brandon Henderson, James Denvir, Jiang Liu, Sandrine Pierre, Thomas Nelson, and Joseph I. Shapiro

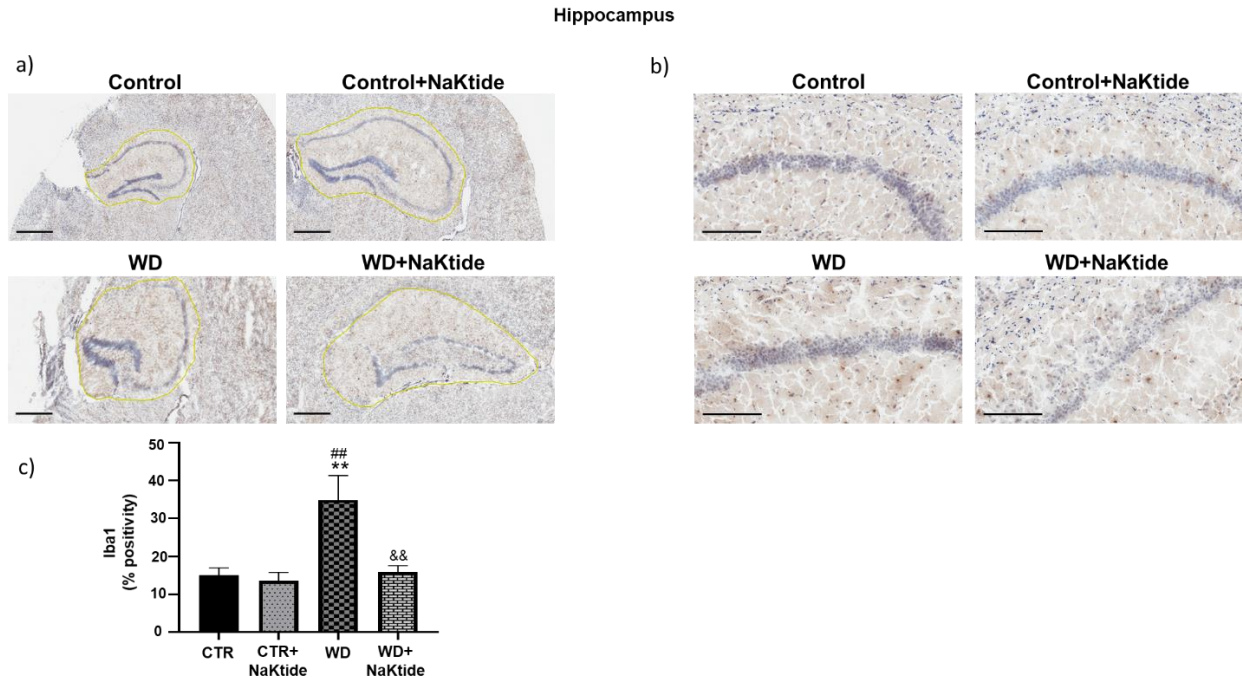
Supplemental Information:



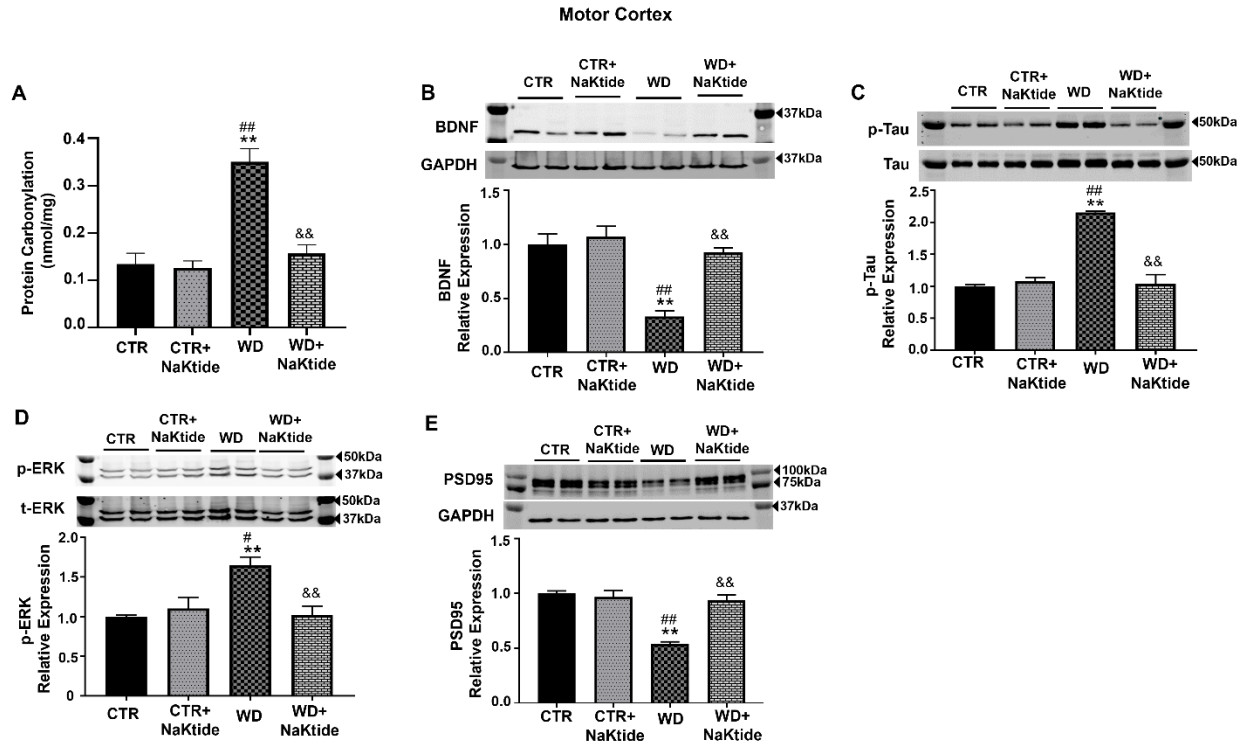
Supplemental Figure 1: Specificity of doxycycline induced NaKtide expression in visceral adipose tissue. Related to Figure 2. Immunofluorescence staining demonstrating readily detectable GFP (green) and NaKtide (red) expression in (A) visceral adipose sections and (B) subcutaneous adipose sections of Tet-On Tg mice fed with doxycycline added normal chow or WD. No fluorescence detected in either channel in Tet-Off Tg-control mice in visceral and subcutaneous adipose tissues. No fluorescence detected in GFP (green) and RFP (red) channel in Tet-Off Tg-control and in Tet-On Tg mice fed with doxycycline added normal chow or WD for (C) heart and (D) brain tissues. Representative images taken with 20X objective lens; scale represents 100 μ m. N=6/group.



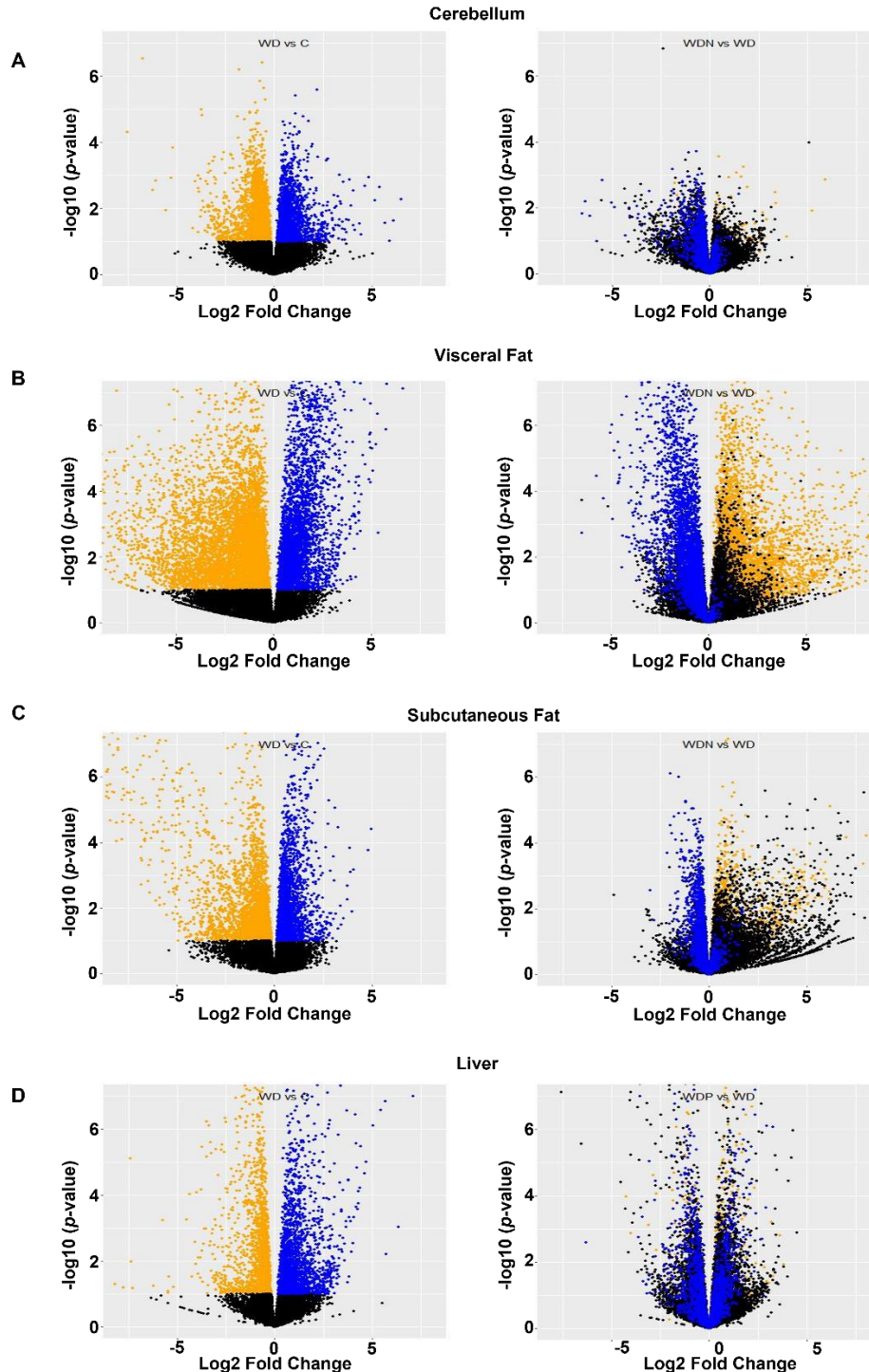
Supplemental Figure 2: Doxycycline induced adipocyte-specific NaKtide expression shows no effect on Na,K-ATPase isoforms in hippocampus of mice fed a WD. Related to Figure 3. Immunoblot analysis of (A) α -1 subunit, (B) α -2 subunit and (C) α -3 subunit with data shown as mean band density normalized to GAPDH. Results are expressed as means \pm SEM. N=4-6/group. Statistical analysis by one-way ANOVA, multiple comparison using Tukey's test. Data not significant.



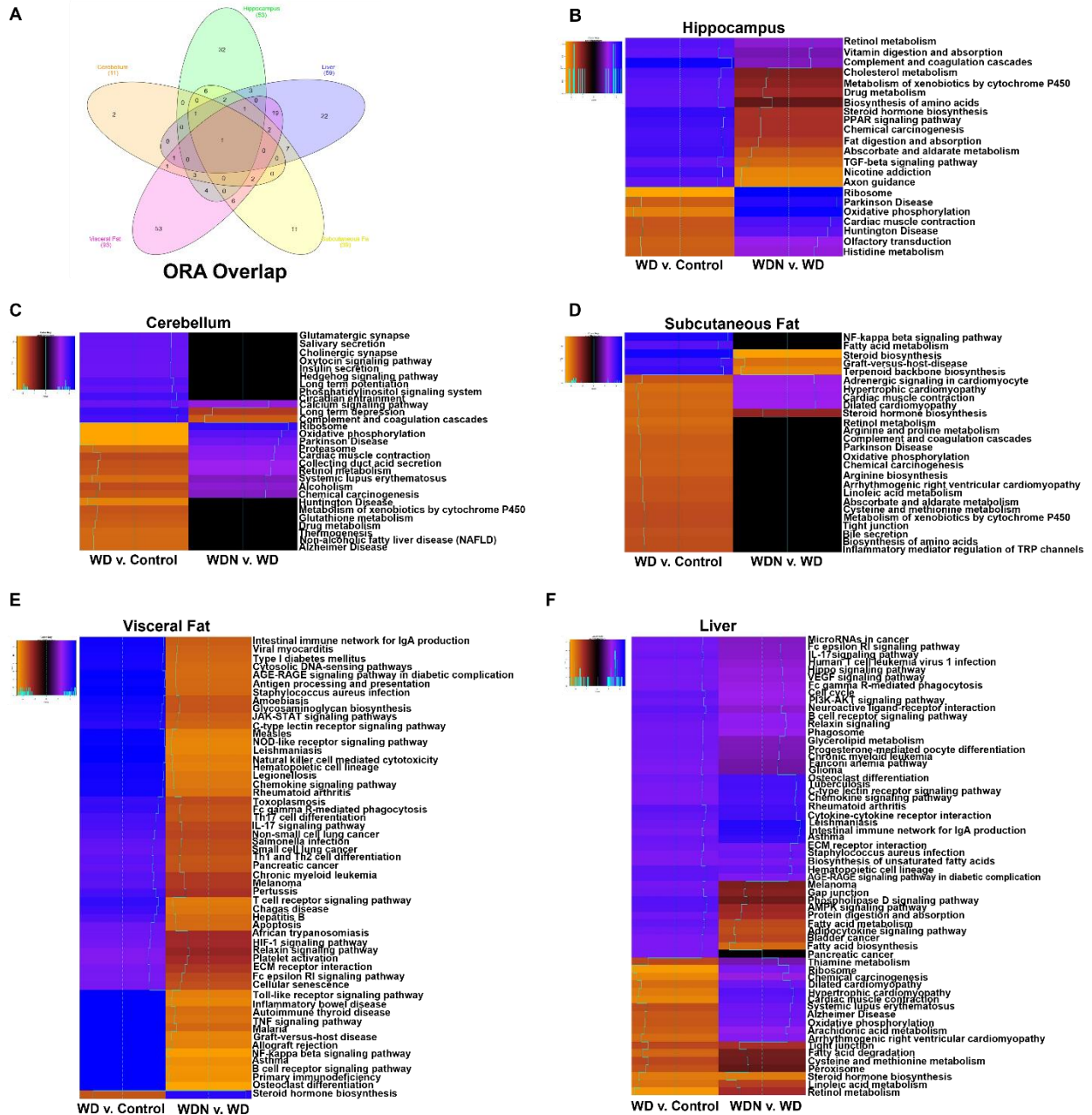
Supplemental Figure 3: Immunohistochemical staining for microglial activation in hippocampus of mice fed a WD. Related to Figure 3. Representative images of Iba1 staining in hippocampus section showing Iba1-positive cells. Yellow lines indicate the hippocampal area used to quantify the Iba1 positive staining. (a) Representative images in the left panel with scale bar = 600µm. (b) Representative images on the right panel with scale bar = 200µm, showing CA1 area of the hippocampus from images in the left panel (a). Percentage of Iba1 positive cells were quantified (c). Results are expressed as means \pm SEM. N=4-8/group. Statistical analysis by one-way ANOVA, multiple comparison using Tukey's test, where * $p < 0.05$ vs. CTR, ** $p < 0.01$ vs. CTR, # $p < 0.05$ vs. CTR+NaKtide, ## $p < 0.01$ vs. CTR+NaKtide, & $p < 0.05$ vs. WD, && $p < 0.01$ vs. WD.



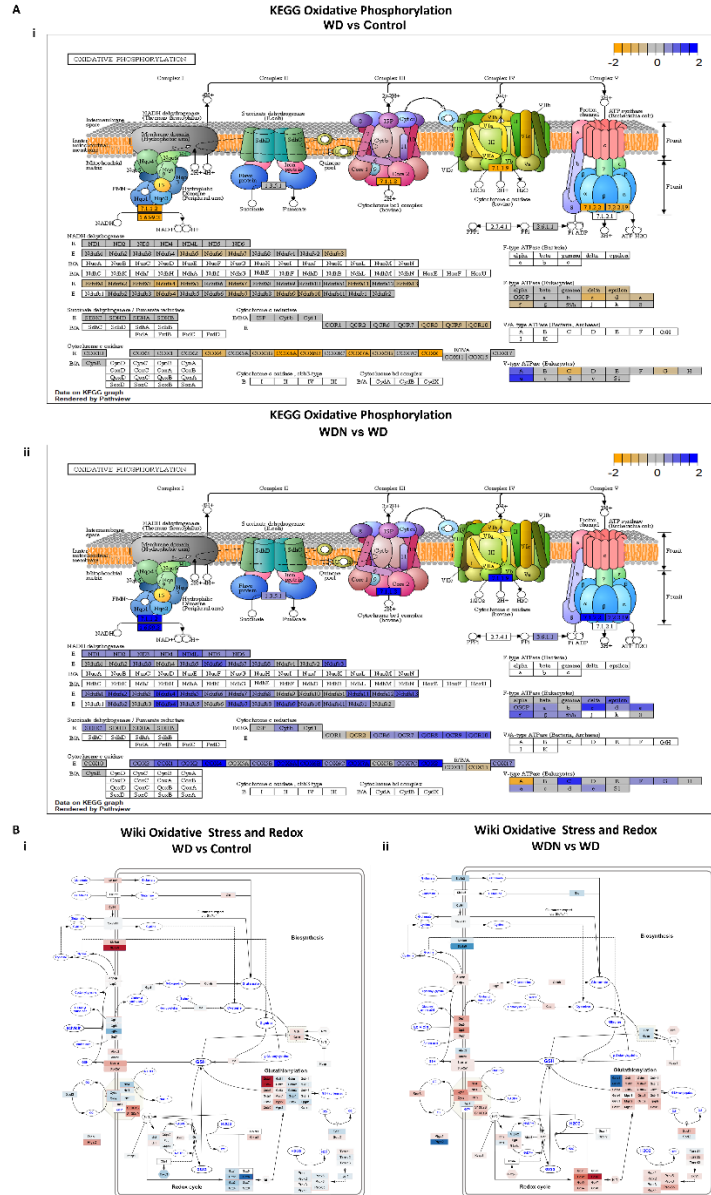
Supplemental Figure 4: Doxycycline induced NaKtide expression improves markers of cognitive function in the motor cortex of mice fed a WD. Related to Figure 3. (A) Protein carbonylation assay in motor cortex homogenates. Immunoblot analysis for (B) BDNF, (C) pTau, (D) pERK and (E) PSD95. Results are expressed as means \pm SEM. N=4-8/group. Statistical analysis by one-way ANOVA, multiple comparison using Tukey's test, where * $p < 0.05$ vs. CTR, ** $p < 0.01$ vs. CTR, # $p < 0.05$ vs. CTR+NaKtide, ## $p < 0.01$ vs. CTR+NaKtide, & $p < 0.05$ vs. WD, && $p < 0.01$ vs. WD.



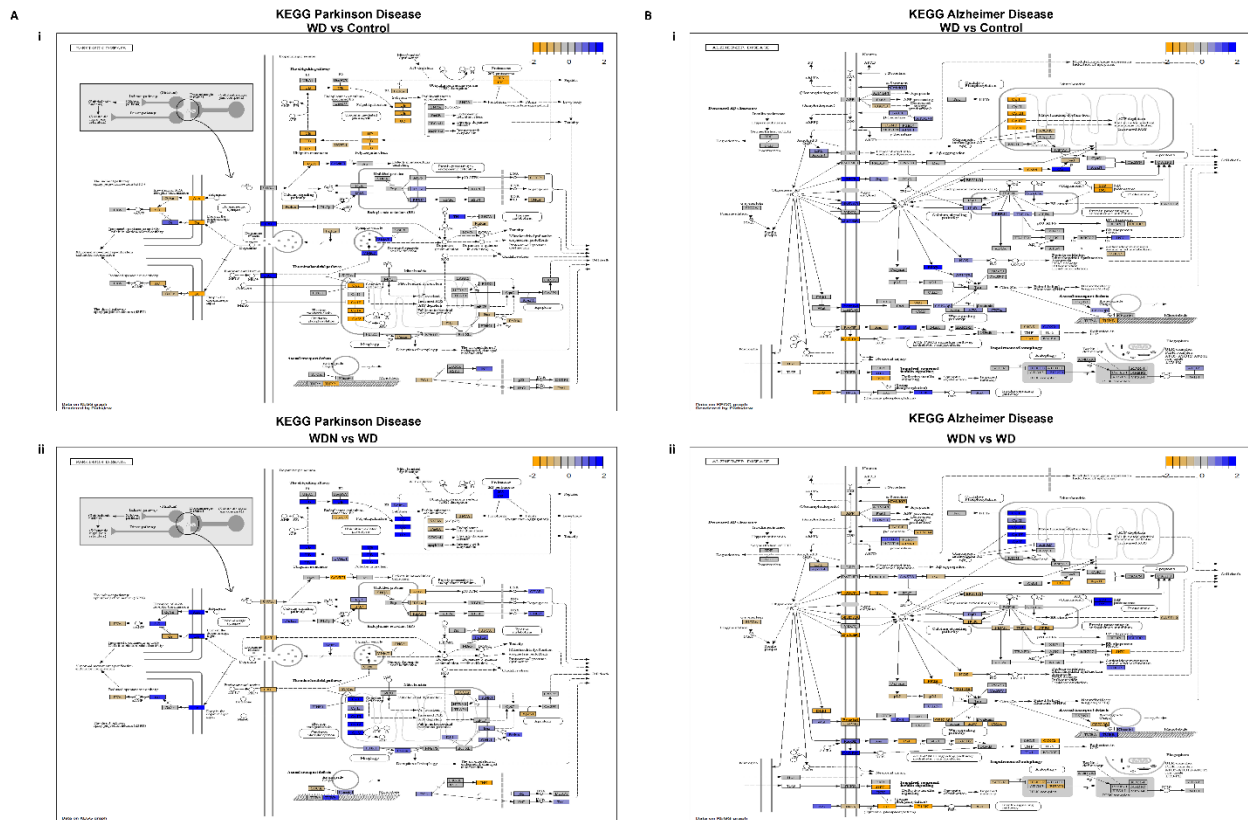
Supplemental Figure 5: Gene expression profiles of Tg mouse (A) cerebellum, (B) visceral fat, (C) subcutaneous fat and (D) liver tissues in response to (i) WD compared with control or (ii) WD+NaKtide expression compared to WD. Related to Figure 4. Data shown with genes down- or up-regulated by WD colored orange or blue, respectively ($p < 0.10$). We note that as was the case in hippocampus (Figure 4), doxycycline induced NaKtide expression appeared to antagonize the changes in gene expression induced by WD.



Supplemental Figure 6: Enriched biological pathways among tissues in Tg mice fed a WD. Related to Figure 5. (A) Venn diagram from pathway enrichment analysis performed using ORA, depicting the overlap of all enriched pathways among cerebellum, hippocampus, liver, subcutaneous and visceral adipose tissue. Heatmaps of differentially regulated pathways, enriched by GSEA, with and without NaKtide expression shown in (B) hippocampus (HC), (C) cerebellum (Cereb), (D) visceral fat (Vfat), (E) subcutaneous fat (Sfat) and (F) liver tissue.



Supplemental Figure 7: Gene expression in representative KEGG Pathways shown as heatmaps. Related to Figure 5. (Ai) KEGG Pathway for oxidative phosphorylation in WD compared with control. Gene expression in dataset shown as heatmaps with blue indicating up- and orange indicating down-regulation, respectively. (Aii) KEGG pathway for oxidative phosphorylation in WD + NaKtide compared with WD. Gene expression in dataset shown as heatmaps with blue indicating up- and orange indicating down-regulation, respectively. (Bi) Wiki Oxidative Stress and Redox pathway in WD compared with control. Gene expression in dataset shown as heatmaps with blue indicating up- and orange indicating down-regulation, respectively. (Bii) Wiki Oxidative Stress and Redox pathway in WD + NaKtide compared with WD. Gene expression in dataset shown as heatmaps with blue indicating up- and orange indicating down-regulation, respectively. All GSEA plots produced with fgsea package. All KEGG pathway maps produced with Pathview package. Wikipathway map produced using Cytoscape program utilizing Wikipathway software.



Supplemental Figure 8: Gene expression in representative KEGG Pathways shown as heatmaps. Related to Figure 5. (Ai) KEGG Pathway for Parkinson Disease in WD compared with control. Gene expression in dataset shown as heatmaps with blue indicating up- and orange indicating down-regulation, respectively. (Aii) KEGG pathway for Parkinson Disease in WD + NaKtide compared with WD. Gene expression in dataset shown as heatmaps with blue indicating up- and orange indicating down-regulation, respectively. (Bi) KEGG Pathway for Alzheimer Disease in WD compared with control. Gene expression in dataset shown as heatmaps with blue indicating up- and orange indicating down-regulation, respectively. (Bii) KEGG Pathway for Alzheimer Disease in WD + NaKtide compared with WD. Gene expression in dataset shown as heatmaps with blue indicating up- and orange indicating down-regulation, respectively. All GSEA plots produced with fgsea package. All KEGG pathway maps produced with Pathview package.