Supplementary Figures:

Supplementary Figure 1. Inflammatory and microglial cell response in the hypothalamus of female WT- and Tg21-mice are not different on NCD or HFD-feeding. (A, B) Representative hypothalamic sections from age-matched (8 weeks) female Tg21- and WT-mice during NCD (A) or after three weeks of HFD-feeding (B) stained for nuclear marker DAPI, inflammatory marker TNFα, and microglial cells marker Iba1 in MBH. (C, D) Quantification of (A) and (B) (E) Gene expression of markers Ccl2, Il6, and Il1β in the hypothalamus of age-matched (8 weeks) female Tg21- and WT-mice on NCD or after 3 weeks of HFD determined by quantitative RT-PCR, normalized to WT control mice on NCD, and adjusted to Gapdh gene expression. Box and whisker plots denote bounds ranging from 25th to 75th percentile, line represents the median, whiskers ranging from minimum to maximum values, and includes outliers. Statistical significance is indicated by *** p<0.001, ** p<0.01, *p<0.05 (2-way ANOVA) (n=8-10/group). Scale bar 100μm; magnification 40X, 3V: third ventricle.

Supplementary Figure 2. Tg21- and WT-female mice show no difference in P2Y12+ microglial cells during NCD or HFD-feeding, and no detectable CD169+ cells in either condition (A) Representative hypothalamic sections from age-matched (8 weeks) female Tg21- and WT-mice during NCD (A) or after three weeks of HFD-feeding (B) stained for nuclear marker DAPI, CD169, andP2Y12 in MBH. (C) Quantification of (A) and (B) to measure CD169- and P2Y12-positive cells. Box and whisker plots denote bounds ranging from 25th to 75th percentile, line represents the median, whiskers ranging from minimum to maximum values, and includes outliers. Statistical significance was tested by 2-way ANOVA (n=6-8/group). Scale bar 100μm; magnification 40X, 3V: third ventricle. Each slide is representative of n=8-10/group.
Supplementary Figure 3. EpoR gene deletion via nestin-promoter driven cre-recombinase expression is specific in the brain and not in the spleen and does not affect hematocrit. (A, B) EpoR gene expression in the whole brain and spleen at age 4 weeks of nestin-cre;EpoR<sup>loxP/loxP</sup> and nestin-cre;EpoR<sup>WT/WT</sup> mice normalized to WT control mice, and adjusted to Gapdh gene expression. (C) Hematocrit levels in nestin-cre;EpoR<sup>loxP/loxP</sup> and nestin-cre;EpoR<sup>WT/WT</sup> mice at age 4 weeks. Box and whisker plots denote bounds ranging from 25<sup>th</sup> to 75<sup>th</sup> percentile, line represents the median, whiskers ranging from minimum to maximum values, and includes outliers. Statistical significance was tested by 2-way ANOVA (n=6-8/group).
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