Supplementary materials for“Environmental and genetic activation of hypothalamic BDNF modulates T cell immunity to exert an anticancer phenotype”

**This file contains four supplemental figures.**



**Supplemental Figure 1**. **Mouse characteristics prior to tumor injection and at time of sacrifice.** Male, three week-old B6 mice were housed in EE or SE cages for six weeks, at which point blood was analyzed for ELISA analysis of serum leptin. (**A**) Body weight before intraperitoneal injection with 200 μg of CD8-depleting (CD8) or IgG-control (IgG) antibody. (**B**) Serum leptin in EE mice was reduced by 80%. (**C**)Representative flow cytometry of blood from CD8-depleted mouse demonstrated efficacy of CD8 depleting antibody. (**D**) Comparison of mononuclear cells per milligram of tumor. (**E**) Comparison of CTL frequency within the tumor mass. (**F**) Tumor infiltrating CTLs were compared for their surface expression of PD1 protein. (**G**) At sacrifice, total splenocyte numbers were reduced in EE mice compared to SE. (**H**) Comparison by flow cytometry of lymph node CD8 T cells that express memory phenotype markers CD44+CD122+. Data are represented as the mean ± SEM; n = 9-10 per group. \* *P* <0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001



**Supplemental Figure 2. Mouse physiology following EE.** (**A**)mRNA overexpression of BDNF in the hypothalamus of SE and EE miceafter 4-weeks (**B**) Mouse body weight was unchanged between SE and EE mice after 1-week, but EE mice weighed less after 4-weeks. (**C**) Total splenocytes. (**D**) The frequency of CTLs that express memory marker CD44+ was unchanged after short and long-term enrichment. Data are represented as the mean ± SEM; n = 5 per group. \*\* *P* < 0.01.



**Supplemental Figure 3. Overexpressing BDNF in the hypothalamus mimics EE’s effects on T cells**. (**A**) GFP fluorescence in the hypothalamus. Scale bar: 200 μm. ARC: arcuate nucleus, VMH: ventromedial hypothalamus, DMH: dorsomedial hypothalamus, 3V: third ventricle. (**B**) Comparison of BDNF mRNA overexpression in the hypothalamus. (**C**) Mouse body weight at sacrifice. (**D**) Total splenocyte numbers. (**E**) The T cell ratio of CD4/CD8 was reduced in BDNF spleens at 5-weeks and in (**F**) blood at 3-weeks. (**G**) The frequency of CTL cells expressing the memory-associated marker CD44, was non-significantly changed between groups. (**H**) T cell surface expression for Th1, Th2, and Th17 associated chemokines after 3-weeks. (**I**) Stimulation with CD3/CD28 beads revealed no change in INFγ, IL4 or IL17 secretion between BDNF or GFP injected mice after 3-weeks.



**Supplemental Figure 4. Knockdown BDNF in the hypothalamus blocks EE’s effects.** (**A**) mRNA expression of hypothalamic BDNF. (**B**) Mouse body weight at time of sacrifice. (**C**) Absolute splenocyte numbers. Data are represented as the mean ± SEM; n = 5-8 per group. \* *P* <0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001.