

Supplementary Material

Peripheral Insulin Regulates A Broad Network of Gene Expression in Brain and Hypothalamus

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Supplementary Figure Legends

Supplementary Figure 1: Physiological data of mice undergoing euglycemic clamps with

indicated doses of insulin infusion. A, Schematic of euglycemic clamps and brain tissue collection. Male C56BL/6J mice were infused with either saline, 4 and 12 mU/kg/min insulin for 3 h with glucose levels clamped at euglycemia (33). **B,** Blood glucose levels of mice at indicated time points during the clamps. $N = 6$. *, $P < 0.05$, saline vs 4 mU/kg/min insulin; #, $P < 0.05$, saline vs 12 mU/kg/min insulin. Repeated-measures 2-way ANOVA followed by Tukey's multiple comparisons. **C,** Glucose infusion rates of low and high insulin-infused groups at indicated time points during the clamps. $N = 6$. *, $P < 0.05$. Repeated-measures 2-way ANOVA followed by Sidak's multiple comparisons. **D,** Total glucose infused during the clamps. Student's t -test was performed between low- and high-insulin infused groups, $N = 6$. **E,** Plasma insulin levels of 4 mice randomly selected from each group following 3 h insulin clamps. $N = 4$. One-way ANOVA followed by Tukey's multiple comparison test. **, $P < 0.01$; ****, $P < 0.0001$. **F,** Serum insulin levels of male C56BL/6J mice at basal or 5 min after i.p. glucose injection (3g/kg). $N = 3$. Student's t -test. ***, $P < 0.001$. Data are shown as mean \pm s.e.m.

Supplementary Figure 2. Insulin triggers robust transcriptional regulation of both protein

coding and non-coding RNAs in the hypothalamus. A, Pie chart showing the percentages of significantly regulated protein-coding and non-coding RNAs by low-dose insulin in the hypothalamus ($P < 0.01$, $|FC| > 1.5$). Volcano plot showing the regulation of the coding and non-coding RNAs by low-dose insulin infusion. *Red* indicates significantly regulated non-coding RNAs ($P < 0.01$, $|FC| > 1.5$). **B,** Heatmap of top 50 non-coding RNAs regulated by low-dose insulin. **C,** Heatmap of top 50 protein-coding RNAs regulated by low-dose insulin.

Supplementary Figure 3. Regulation of pyruvate carboxylase, lactate dehydrogenases

and monocarboxylate transporters by insulin. Regulation of pyruvate carboxylase, lactate

dehydrogenases and monocarboxylate transporters by low dose insulin. Upregulation of genes is represented by fold change (Insulin/Saline). Suppression of genes is represented by – fold change (Saline/Insulin). Solid bars indicate $FDR < 0.1$; open dashed bars indicate $FDR \geq 0.1$.

Supplementary Figure 4. Expression of genes related to glycolysis, glutamate receptors

and GABA receptors in mouse model of insulin deficiency. **A**, Serum insulin levels of mice received citrate buffer alone as controls, mice injected with 150 mg/kg streptozotocin (STZ), or mice with subcutaneous insulin pellet treatment 14 days after initial injections. **B**, Blood glucose levels of mice control and STZ diabetic mice, followed by insulin pellet treatment or left untreated. **C**, Relative mRNA expression of genes involved in glucose uptake and glycolysis in the hypothalamus of control, STZ and STZ+Insulin groups. *Tbp* was used as internal control. $N = 6$. **D**, Relative mRNA expression of the subunits of ionotropic and metabotropic glutamate and GABA receptors in the hypothalamus of control, STZ and STZ+Insulin groups. *Tbp* was used as internal control. $N = 6$. One-way ANOVA followed by Tukey's multiple comparison test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Data are shown as mean \pm s.e.m.

Supplementary Figure 5. Transcriptional regulation of the genes involved in

neurotransmitter receptors, glucose uptake and glycolysis in primary cultured neurons

and astrocytes. **A**, Relative mRNA expression of the subunits of ionotropic and metabotropic glutamate and GABA receptors in primary neuron cultures stimulated with 100 nM insulin for 3 h. *Tbp* was used as internal control. $N = 6$. **B**, Relative mRNA expression of genes involved in glucose uptake and glycolysis in primary cultured neurons in response to 100 nM insulin stimulation for 3 h. *Tbp* was used as internal control. $N = 6$. **C**, Relative mRNA expression of genes involved in glucose uptake and glycolysis in primary cultured astrocytes in response to

100 nM insulin stimulation for 6 h. *Tbp* was used as internal control. $N = 4$. Student's *t*-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Data are shown as mean \pm s.e.m.

Supplementary Figure 6. Transcriptional regulation of the genes involved in long-chain fatty acyl-CoA and cholesterol biosynthesis in primary cultured neurons and astrocytes.

A and B, Relative mRNA expression of genes involved in long-chain fatty acyl-CoA biosynthesis in primary cultured neurons (**A**) and astrocytes (**B**) in response to 100 nM insulin stimulation for 3 h (neurons) or 6 h (astrocytes). **C and D**, Relative mRNA expression of genes involved in de novo cholesterol biosynthesis in primary cultured neurons (**C**) and astrocytes (**D**) in response to 100 nM insulin stimulation for 3 h (neurons) or 6 h (astrocytes). *Tbp* was used as internal control. $N = 6$ for neurons. $N = 4$ for astrocytes. Student's *t*-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Data are shown as mean \pm s.e.m.