









Supplemental Figure 1. Bone development in WT and  $S6KI^{-/-}$  embryos at E17.0. Lateral view of WT and  $S6KI^{-/-}$  mouse skeleton at E17.0 analyzed for ossification, using alcian blue and alizarine red. Arrow indicate areas of diverging ossification stages.

Supplemental Figure 2. Placenta from embryos at E13.5 and E19.5. (A-F) Cross section in placentas from embryos at E13.5: Chorionic plate (cp), labyrinthine trophoblast (lt), labyrinthine (lb), spongiotrophoblast (sp) and giant cell (gl) layers. In *S6K1*<sup>-/-</sup> placentas, note the reduced size of the spongiotrophoblast laye: (A-C) Histology by H&E staining; (D-F) Blood vessels by anti-PECAM-1 immunostaining. (G-J) Cross-sections of placenta at E19.5: (G-H) Histology by H&E staining; (I-J) Spongiotrophoblast-specific, Tpbp detection by ISH. Arrows indicate the improperly positioned Tpbp-expressing cells in the labyrinthine region of the *S6K1*<sup>-/-</sup> placenta. Scale bars: 500μm.

Supplemental Figure 3. Detection of Tg(*RIP-S6K1*)rip<sup>s6k</sup> transgene expression in embryonic pancreata. Pancreas sections from embryos at E16.5 analyzed for S6K1 (green) and insulin (red) expression by immunostaining. Nuclei were visualized with DAPI (blue). Scale bars: 25μm.

Supplemental Figure 4. Body weight of  $S6K1^{-1}$  Tg(RIP-S6K1)rip<sup>S6K1</sup> embryos at E16.5. Results from weight measurement of n=9-10 embryos per genotype. Values are mean  $\pm$  SEM. \*P < 0.05 vs. other genotypes, ANOVA.

**Supplemental Figure 5. Analysis of hypothalamic expression of Tg**(*RIP-S6K1*) *rip*<sup>s6k1</sup> **transgene** (**A**) Genotyping for *rip*<sup>s6</sup> Tg(*RIP-S6K1*) <sup>k1</sup> transgene iin mouse brain DNA. (**B**) Tg(*RIP-S6K1*) *S6K1* transgenic expression in hypothalamic RNA, determined by RT-PCR analysis. (**C**) Analysis of S6K1 expression in hypothalamus protein extracts by western blot with indicated antibodies. n=3-6 per genotype.