Supplemental Material

Alterations in *TCF7L2* expression define its role as a key regulator of glucose metabolism

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Supplementary Figure S1. Regulatory landscape of BACs RP11-57H14 and RP11-357H24.

(A) The location of enhancer-trapping human BACs RP11-57H14 and RP11-357H24 at the *TCF7L2* locus. Non-coding sequence conservation between human and chicken is displayed as red peaks (VISTA genome browser). The type 2 diabetes (T2D) associated interval is highlighted in red. A black asterisk marks the location of SNP rs7903146. (B) The endogenous expression of *Tcf7l2* is shown in the top row while two independent mouse lines harboring enhancer-trapped BAC RP11-57H14 are shown below. Images of whole embryos at e12.5 are shown in the first column with expression in the brain and olfactory tissues that overlaps with endogenous *Tcf7l2* activity. Images of brain (dorsal view) and olfactory epithelium (Olfactory) at e15.5 are shown in the adjacent columns (left to right). Regulatory activity consistent with endogenous *Tcf7l2* expression is present within the diencephalon of the brain and the olfactory epithelium in enhancer-trapped BAC RP11-57H14. (C) The endogenous expression of *Tcf7l2* is shown in the top row while two independent mouse lines harboring enhancer-trapped BAC RP11-357H24 are shown below. Images of the rib cage at e17.5 are show expression within the axial skeleton in enhancer-trapped BAC RP11-357H24.

Supplementary Figure S2. Postnatal expression profiles of BACs RP11-466I19 and RP11-139K1.

Postnatal expression patterns at 21 days of age (P21) in enhancer-trapped BACs RP11-466I19 (upper panel) and RP11-139K1 (lower panel). (A) Expression profile within the brain. Whole brains are shown in the first column from dorsal (top) and ventral (bottom) views. Sagittal sections of brain show pervasive expression in BAC RP11-466I19 with arrows pointing to expression within the thalamus (T) and hypothalamus (H) while BAC RP11-139K1 has strong expression exclusively in the thalamus (T). (B) Lower and higher magnifications of the forelimb (left to right). Expression can be seen in epiphyseal plates of the phalanges (P) and in metacarpals (M) in both BACs. (C) Lower and higher magnifications of the hindlimb (left to right). Expression can be seen in epiphyseal plates of the phalanges (P) and in metacarpals (M) in both BACs. (D) Images of the stomach. Stomach expression is localized to the pyloric stomach (P) in both BACs. Stomachs from stained wild-type (WT) non-transgenic littermates are shown in the lower right corners. (E) Lower and higher magnifications of the pancreas (left to right). Expression can be seen in the exocrine pancreas (pancreatic ducts and acinar cells, D). (F) Images of the upper gastrointestinal tract. Intestines show distinct foci of expression in the intestinal wall in both BACs. Intestines from stained wild-type (WT) non-transgenic littermates are shown in the lower right corners. A higher magnification of the internal gut wall at the right shows staining within the villi (V). (G) Images of the colon show strong expression. Stained wild-type (WT) colon from non-transgenic littermates are shown in the lower left corner.

Supplementary Figure S3. Histology of expression profiles in BACs RP11-466I19 and RP11-139K1.

(A) Brain expression from sagittal and coronal section (left and right respectively) of brains at e15.5 in BAC RP11-139K1 and RP11-466I19 (upper and lower panel respectively). Arrows point to strong staining in the epithalamus (developing thalamus, T), the diencephalon (developing hypothalamus, H), and the telencephalon (developing cortex, C). Rostral and caudal directions are marked by the letters R and C respectively. (B) Sagittal section of the stomach at e15.5 in BAC RP11-139K1. Arrow points to pyloric stomach (P) expression in columnar epithelial cells. (C) Coronal sections of the forelimbs in BACs RP11-139K1 (upper panel) and RP11-466I19 (lower panel) at e15.5. Images of digits, elbow and skeletal muscles are shown from left to right. Arrows point to bone expression in the epiphyseal plate of phalanges (P), metacarpals (M), and the epiphyseal plate of the radius (R) and ulna (U) in the first column. Expression in the humerus (H) and in skeletal muscles (SM) is highlighted by arrows in the remaining columns. (D) Sagittal section of the hindlimbs in BAC RP11-139K1 (upper panel) and RP11-466I19 (lower panel) at e15.5. In BAC RP11-139K1, arrows point to expression in metacarpals (M). A higher magnification of the hindlimb is shown at the right. In BAC RP11-466I19, light staining is present within the tibia (T) while skeletal muscle (SM) expression is present in the adjacent image. (E) Pancreatic staining in BAC RP11-139K1 at postnatal day 21 (P21). Lower to higher magnifications are shown from left to right. An arrow points to a pancreatic islet (I), which lacks staining. Pancreatic staining is visible in the exocrine pancreas.

Supplementary Figure S4. Immunohistochemistry of pancreatic expression in BAC RP11-466I19.

(A) A stained whole pancreas from an enhancer-trapped BAC RP11-466I19 transgenic pup at birth (P0) is shown. Staining is present throughout the pancreas. (B) Immunohistochemistry of P0 pancreas shows staining within the endocrine pancreas. Endogenous insulin staining is shown in green while β-galactosidase (β-Gal.) staining is shown in red. At the right are merged images. (C) A second independent pancreatic islet showing the same pattern as in (B).

Supplementary Figure S5. Sequence of Tcf7l2 in zinc finger nuclease knockout mice.

The amino acid sequence of wild-type Tcf7l2 protein is shown in the top row while the amino acid sequences of Tcf7l2 protein in the three null lines (Line 1-3) are shown below. Blue highlights represent the wild-type Tcf7l2 amino acid sequence. Yellow highlights represent altered amino acids in null animals. Red stop marks the end of the amino acid sequence. Amino acid sequence numbers are shown in brackets.

Supplementary Figure S6. Phenotype of female *Tcf7l2*$^{+/-}$ mice.

(A) Growth curves for wild-type (gray, n = 22) and heterozygous null (blue, n = 27) mice from 1 to 6 weeks of age. (B) Body length in wild-type (+/+, n = 10) and heterozygous null (+/-, n = 10) mice at 6-7 weeks of age. (C) Lean and fat body mass in wild-type (+/+, n = 10) and heterozygous null (+/-, n = 10) mice. (D) Scatter plots of femur bone mineral density measurements in wild-type (+/+, n = 10) and heterozygous null (+/-, n = 10) mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

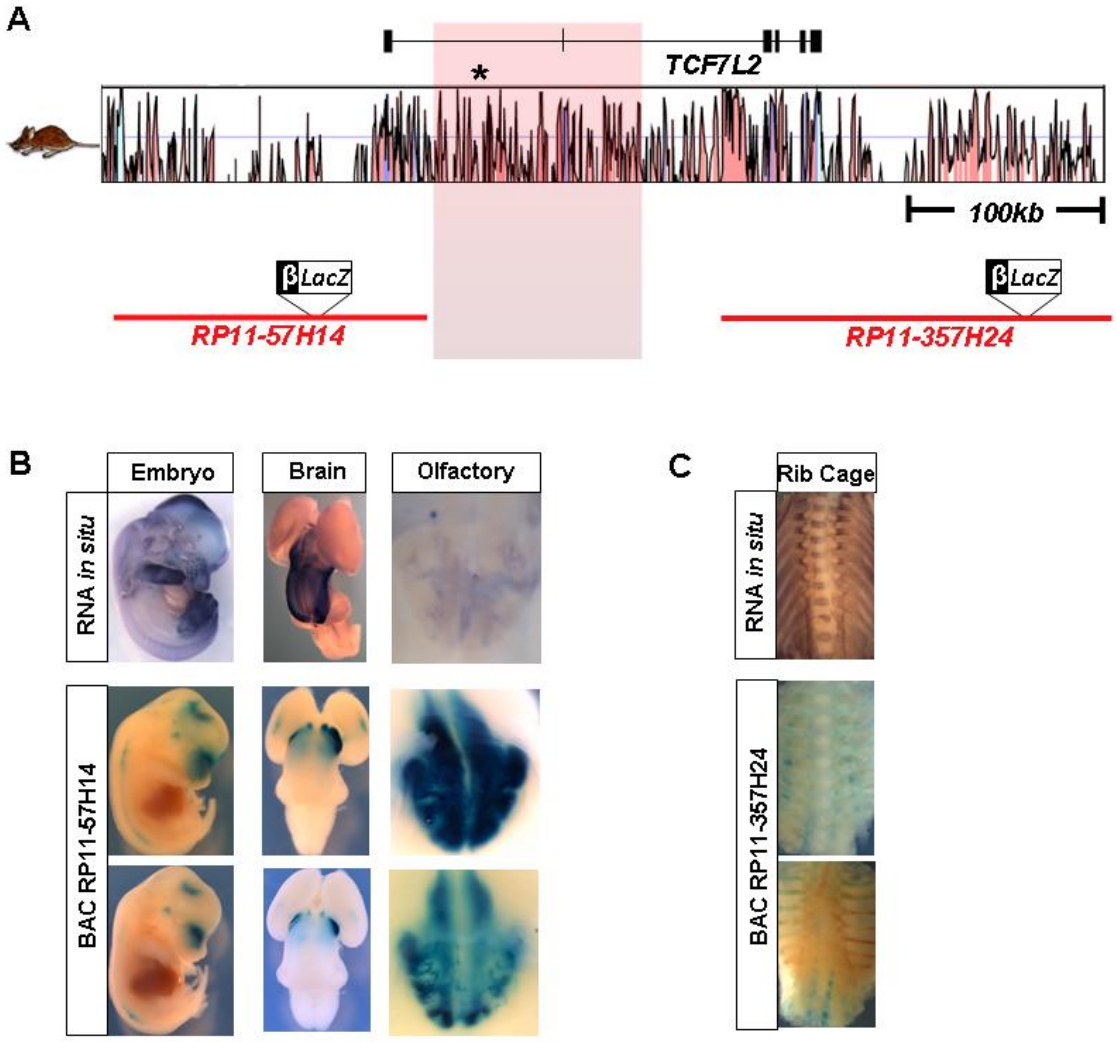
Supplementary Figure S7. Phenotype of male *Tcf7l2*$^{+/-}$ mice.

(A) Body length in wild-type (gray, n = 12) and heterozygous (blue, n = 13) mice at 6-7 weeks of age. (B) Scatter plots of femur bone mineral density measurements in wild-type (+/+, n = 12) and heterozygous (+/-, n = 13) mice. (C) Energy expenditure (2 day averages) in wild-type (+/+, n = 9) and heterozygous null (+/-, n = 10) mice. P values are for the averages during light and dark phases. (D) Drinking in wild-type (+/+, n = 8) and heterozygous null (+/-, n = 10) mice. (E) Activity (movement) in wild-type (+/+, n = 9) and heterozygous null (+/-, n = 10) mice during light and dark phases. (F) Percent weight gain after 10 weeks on a high fat diet (HFD) in wild-type (gray, n = 11) and heterozygous null (blue, n = 12) animals.

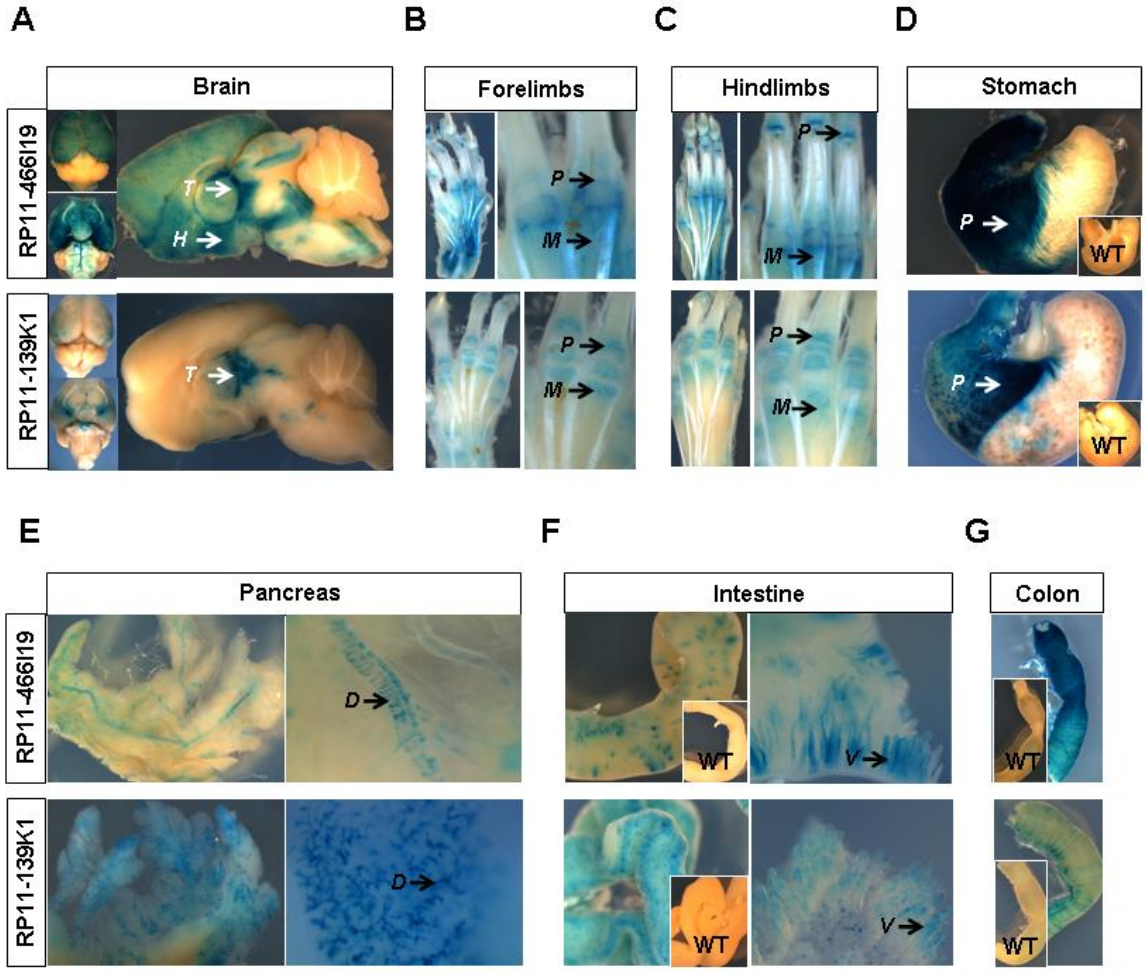
Supplementary Figure S8. Phenotype of*Tcf7l2* BAC transgenic mice.

(A) BAC copy number using eight F1 mice. (B) Growth curves of wild-type (gray, n = 26) and *Tcf7l2* BAC transgenic (orange, n = 15) mice from 1 to 6 weeks of age. (C) Body mass composition in wild-type (gray, n = 7) and *Tcf7l2* BAC transgenic (orange, n = 6) animals. (D) Scatter plots of femur bone mineral density measurements in wild-type (+/+, n = 7) and *Tcf7l2* BAC transgenic (BAC, n = 6) mice. (E) Energy expenditure (2 day averages) in wild-type (+/+, n = 7) and *Tcf7l2* BAC transgenic (BAC, n = 5) mice. P values are for the averages during light and dark phases. (F) Drinking in wild-type (+/+, n = 7) and *Tcf7l2* BAC transgenic (BAC, n = 5) mice. (G) Activity (movement) in wild-type (+/+, n = 7) and *Tcf7l2* BAC transgenic (BAC, n = 5) mice during light and dark phases. (H) Percent weight gain after 10 weeks on a high fat diet (HFD) between wild-type (gray, n = 13) and *Tcf7l2* BAC transgenic (orange, n = 10) animals.

Supplementary Figure S1. Regulatory landscape of BACs RP11-57H14 and RP11-357H24.



Supplementary Figure S2. Postnatal expression profiles of BACs RP11-466I19 and RP11-139K1.



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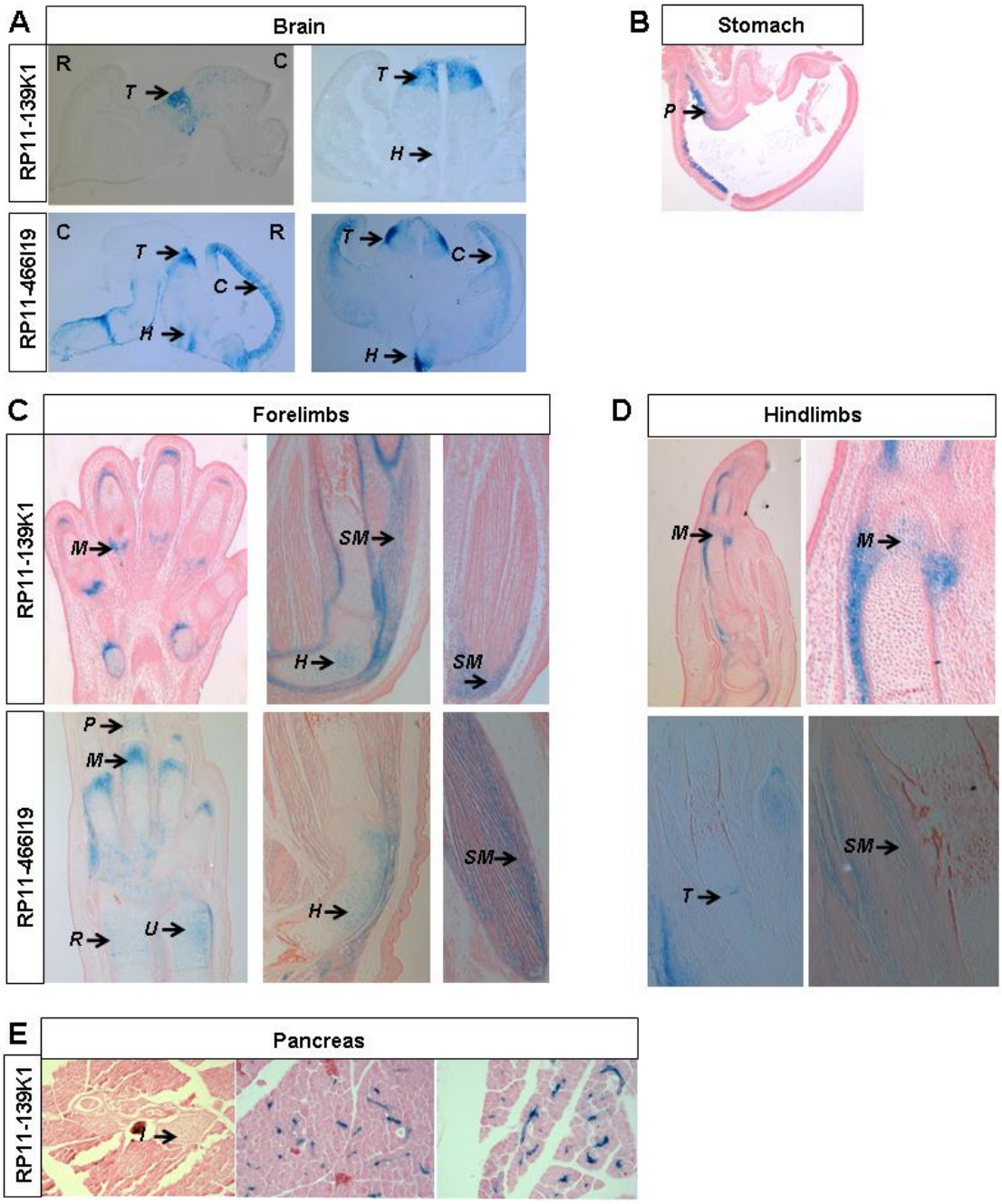
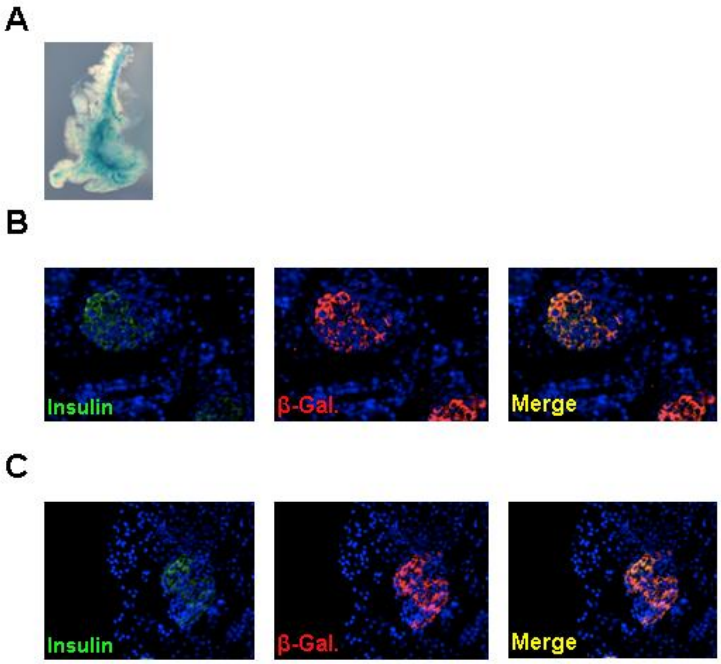


Supplementary Figure S4. Immunohistochemistry of pancreatic expression in BAC RP11-466I19.



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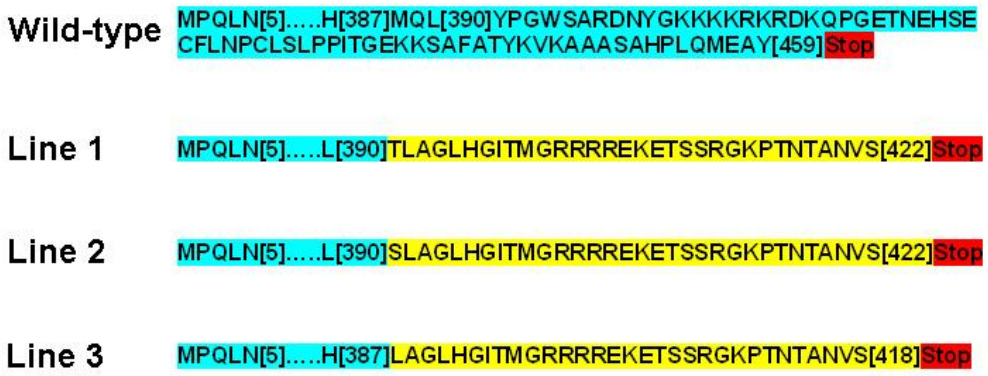
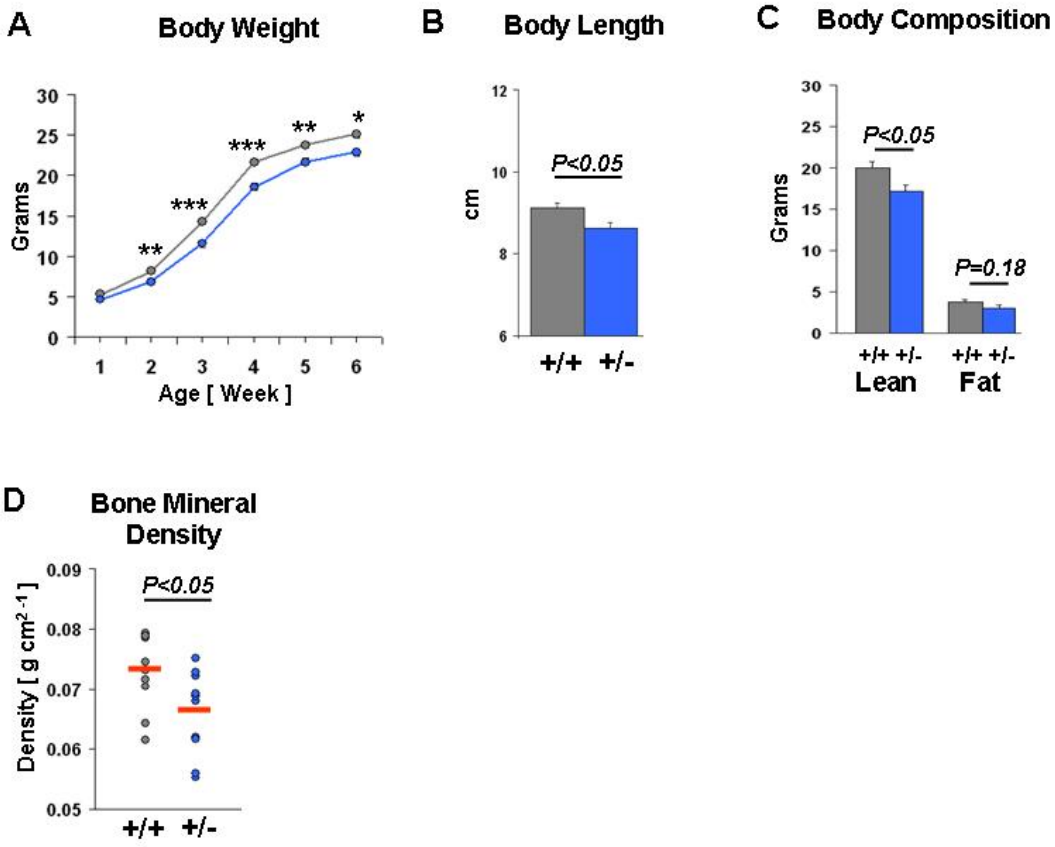
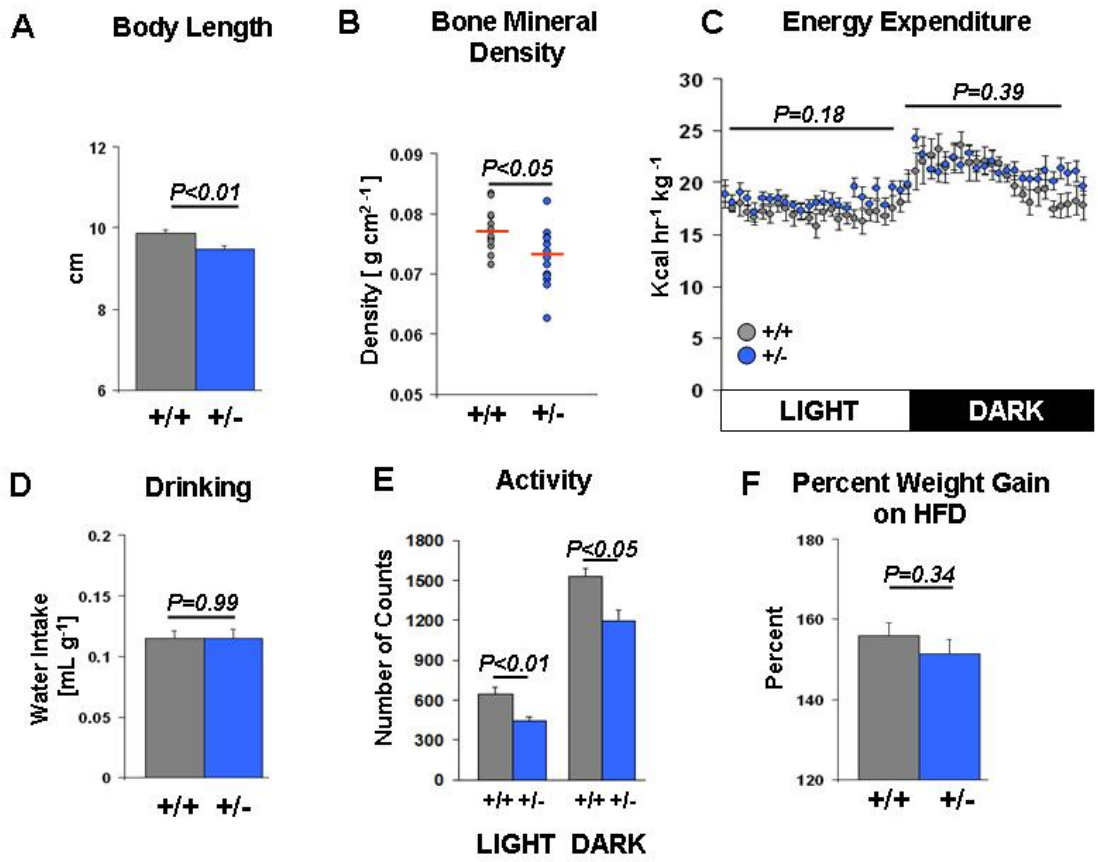


Supplementary Figure S6. Phenotype of female *Tcf7l2*$^{+/-}$ mice.



Supplementary Figure S7. Phenotype of male *Tcf7l2*$^{+/-}$ mice.



Supplementary Figure S8. Phenotype of *Tcf7l2* BAC transgenic mice.