Supporting Information

Frost and Olson 10.1073/pnas.1118922109

SI Methods

Mice. To generate the *Let-7* transgenic mice, a genomic region on chromosome 13 comprising the genes for *Let-7a*, *Let-7d*, and *Let-7f* was amplified from C57BL/6 DNA using the following primers: forward, 5′-TTTGTCTCATGAACATCTTTTCC-3′; reverse, 5′-CCTGCTTCCCTCATTTGGTA-3′. The fragment was then subcloned into the pDONR221 gateway vector (Invitrogen) to create pENTR221-*Let-7a,d,f.* Concomitantly, attB sites for Gateway cloning were introduced into the Sall site of the CAG-Z-EGFP 1.0 vector (Invitrogen). Then the *Let-7a,d,f* DNA fragment from pENTR221-*Let-7a,d,f* was transferred by LR-Clonase (Invitrogen) recombination reaction into the pDEST-CAG-Z-EGFP vector to create pEXp-CAG-Z-EGFP-*Let-7a,d,f.* pEXP-CAG-Z-EGFP-*Let-7a,d,f* was linearized with Spe l and Swa l and used to generate transgenic mice by pronuclear injection. Founder mice were screened by lacZ staining of toe tips and PCR for the transgene construct. Transgene-positive offspring, *Let-7* Tg OFF mice, were then crossed to appropriate *Cre* lines to activate the transgene construct globally or in specific tissues. C57BL/6 mice, *CAG*-Cre, *MCK-Cre*, *Albumin-Cre*, *Nestin-Cre*, and *Fabp4-Cre* transgenic mice were purchased from Jackson Laboratory. Mice were housed in a SPF facility. The high-fat diet (HFD), containing 60 kcal% fat, was obtained from Research Diets (D12492).

Northern Blot Analysis. RNA was isolated using TRIzol (Invitrogen). For Northern blot analysis, 5 μg of total RNA was denatured for 5 min at 70 °C in a buffer containing 50% formamide and 10 mM EDTA. After electrophoresis on a 17% polyacrylamide gel, RNA was transferred onto a Hybond N membrane (Amersham) in 0.5× Tris/Borate/EDTA buffer at 80 V for 1 h. Hybridization was then done at 39 °C with ^{32}P-labeled StarFire oligonucleotide probes (Integrated DNA Technologies) directed against individual *Let-7* family members.

Histological and Immunohistological Examinations. For immunohistology, tissues were fixed with 4% paraformaldehyde (PFA) in PBS, embedded in paraffin wax, cut into 5-μm-thick sections, and then deparaffinized. The primary antibodies were anti-insulin (Dako) and anti-glucagon (Millipore) in 1:300 dilutions. Nuclei were counterstained with DAPI. For neutral lipid staining, liver sections were fixed overnight in 4% PFA, cryoprotected with increasing sucrose concentrations (10–18%), cryoembedded, stained with Oil Red O, and counterstained with hematoxylin.

Glucose and Insulin Measurements. Blood glucose was measured with an Accu-Check Compact Plus Blood Glucose Meter (Roche) using whole blood. For glucose tolerance tests (GTTs), mice were fasted for 7 h before receiving i.p. glucose injection (1.5 mg/g body weight for transgenic mice and 2.0 mg/g lean mass for antimiR-treated mice). Blood glucose levels were measured before injection and at 15, 30, 60, and 90 min after glucose stimulation. For insulin tolerance tests (ITTs), insulin (1.0 U/kg body weight) was injected i.p. in fed mice. Blood glucose levels were measured before injection and at 15, 30, 45, 60, and 90 min after insulin injection.

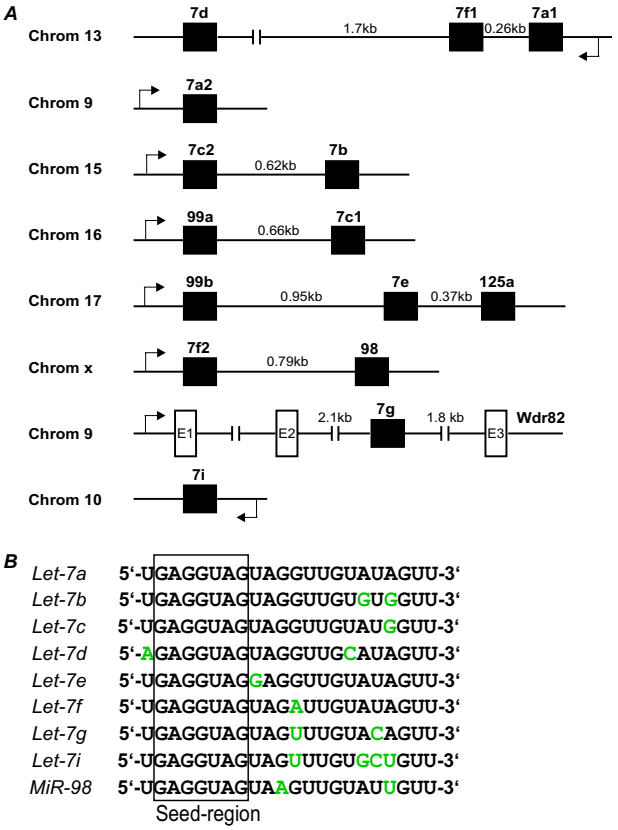

Fig. S1. Scheme of the genomic localization of the *Let-7* genes. *(A)* Eleven of the 12 genes of the *Let-7* family are located in intergenic regions. Only *Let-7g* is located in an intron of the *WD repeat domain 82 (Wdr82)* gene. Several *Let-7* family members are clustered with other *Let-7* genes or other microRNAs, including *miR-99a/b* and *miR-125a*. (*B*) Alignment of the nine *Let-7* family members. The seed region (nucleotides 2–8) is indicated by a black box.

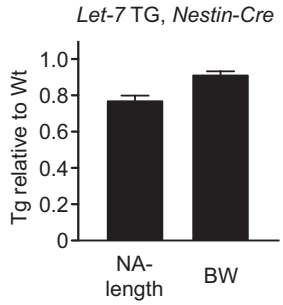

Fig. S2. Overexpression of *Let-7* in the CNS results in reduced body size. Activation of the *Let-7* transgene predominantly in the CNS by *Nestin-Cre* resulted in a similar reduction in body size [nose-to-anus (NA) length] as seen with global *Let-7* overexpression, but only an attenuated reduction in body weight (BW).

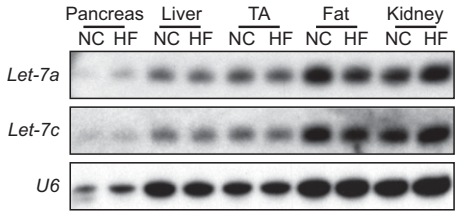

Fig. S3. *Let-7* expression in HFD-induced obesity. Total RNA was isolated from multiple organs of three male C57BL/6 mice that were fed an HFD (HF) for 10 wk starting at age 6 wk. Control animals were age- and sex-matched but received normal chow (NC). Expression of *Let-7a* and *Let-7c* was determined by Northern blot analysis. U6 was used as a loading control.

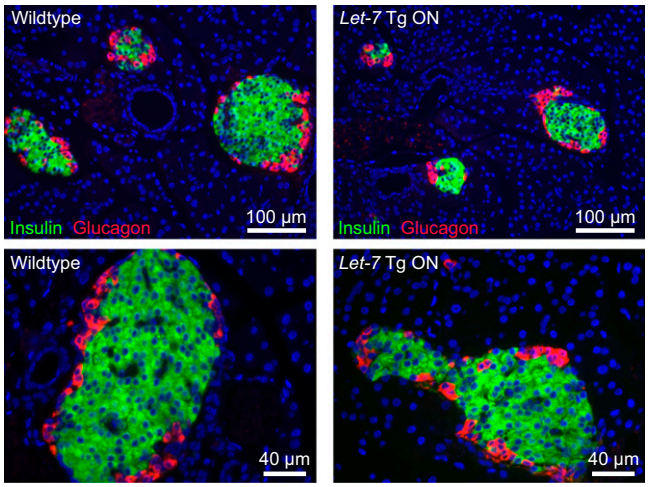

Fig. S4. *Let-7* overexpression does not change pancreatic islet structure. PFA-fixed sections of pancreata from 6-wk-old WT and *Let-7* Tg ON mice were deparaffinized and then used for immunofluorescence staining for insulin (green) and glucagon (red). Nuclei were counterstained with DAPI (blue).

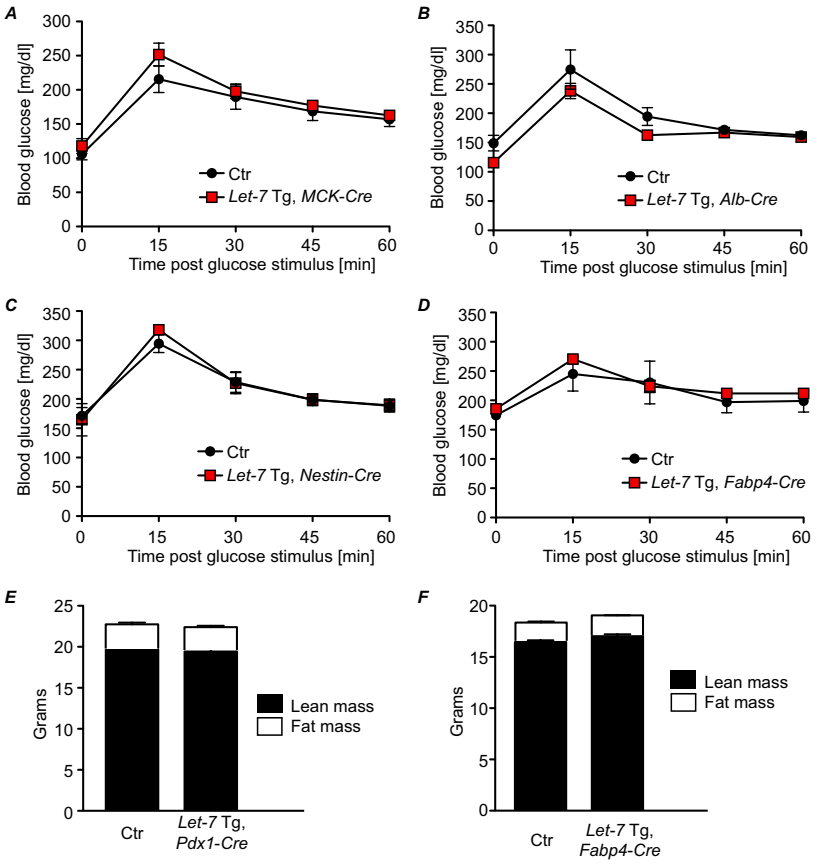

Fig. S5. Overexpression of *Let-7* in CNS, fat, muscle, or liver does not result in impaired glucose tolerance under baseline conditions. (*A*–*F*) To enrich *Let-7* expression in specific tissues, *Let-7* Tg OFF mice were crossed with transgenic mice expressing *MCK-Cre* (muscle), *Albumin-Cre* (liver), *Nestin-Cre* (CNS), and *Fabp4-Cre* (fat). To determine glucose tolerance, 6-wk-old double-transgenic mice were fasted for 7 h, after which glucose levels were measured before and at 15, 30, 45, and 60 min after glucose stimulation (1.5 mg/g body weight i.p.). WT, *Let-7* Tg OFF, or Cre^{+} single transgenic littermates served as controls (Ctr). n = 3–5 (*E* and *F*) Body composition was assessed at age 6 wk in control (Ctr) vs. *Let-7, Pdxl-Cre* and in Ctr vs. *Let-7, Fabp4-Cre* double-transgenic mice using an EchoMRI-100 body composition analyzer (Echo Medical Systems).

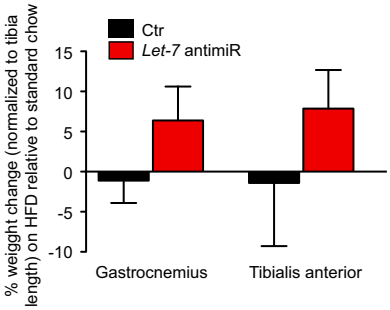

Fig. S6. *Let-7* antimiR treatment enhances muscle growth. Male C57BL/6 mice were fed for 10 wk with HFD and either treated once a week with *Let-7* antimiR (20 mg/kg body weight) or saline control. At the end of the study, gastrocnemius and tibialis anterior muscles were harvested and muscle weights were compared with mice on a standard diet. $n = 8$.

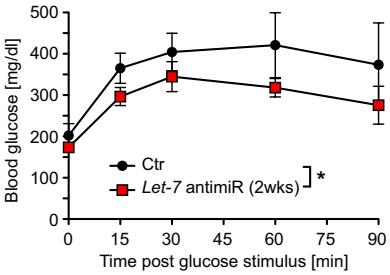

Fig. S7. The 2-wk treatment with *Let-7* antimiR is sufficient to improve impaired glucose tolerance. A GTT was performed at 2 wk after onset of *Let-7* antimiR administration in the treatment study. $*P < 0.05$ for Ctr vs. *Let-7* antimiR-treated mice by ANOVA.