

Supplementary Fig. 1. Histology of *Lin28a* KO mice. (A) Some *Lin28a* KO mice have a cardiac defect. H&E images of *Lin28a* +/- and -/- P0.5 newborn hearts are shown. IVS interventricular septum, LA left atrium, LV left ventricle, RA right atrium, RV right ventricle, VSD ventricular septal defect. (B) Representative images of different organs/tissues in *Lin28a* +/- vs. -/- mice. Scale bars 200 μm (A), 100 μm (B). **Related to Fig. 1.**

Supplementary Fig. 2. 7% of *Lin28a* *-/-* mice can survive to adulthood on a mixed background.

(A) Representative images of *Lin28a* *+/-* and *-/-* mice at different ages. (B) Representative images of *Lin28a* *+/-* and *-/-* mice after DEXA scan. **Related to Fig.1.**

Supplementary Fig. 3. Characterization of *Lin28b* KO and *Lin28a* *+/-;b* *-/-* mice. (A-B) *Lin28b* KO embryos and placentae show normal growth. N=9. (A) Weights of E18.5 embryos and placentae in *Lin28b* *+/-* vs. *-/-* mice. (B) Ratio of placenta/embryo weights in *Lin28b* *+/-* vs. *-/-* mice. (C) Organ weights normalized to total body weight in adult *Lin28b* *+/-* vs. *-/-* males. N=3. Epididymal fat pads were used to measure fat weights. (D) Weights of embryos and placentae of *Lin28a* *+/+;b* *-/-* vs. *Lin28a* *+/-;b* *-/-* embryos at E13.5. N=5-9. (E) Postnatal growth curve of *Lin28a* *+/+* vs. *+/-* males. N=9. * $p < 0.05$. Error bars represent SEM. **Related to Fig. 2.**

Supplementary Fig. 4. PCR genotyping confirms deletion of *Lin28a* (A-C) and *Lin28b* (D-F) alleles in various tissues of *Lin28a* *fl/-; UBC-CreER* (+) (A-C) and *Lin28b* *fl/-; UBC-CreER* (+) (D-F) mice. Tamoxifen was injected at E15.5 (A and D), P7-9 (B and E), or 6 weeks (C and F).

Related to Fig. 4.

Supplementary Fig. 5. Fetal *Lin28a/b*, not neonatal or adult *Lin28a/b*, regulates postnatal growth. (A-C) Postnatal growth curves of *Lin28a* *fl/-; UBC-CreER* (+) vs. *Lin28a* *fl/-* mice. Body lengths after tamoxifen (TAM) injections at (A) E15.5, (B) P7-9 and (C) 6 weeks. N=3-12. (D-F) Postnatal growth curves of *Lin28b* *fl/-; UBC-CreER* (+) vs. *Lin28b* *fl/-* mice. Body lengths after

TAM injections at (D) E15.5, (E) P7-9 and (F) 6 weeks. N=5-13. Arrows indicate ages at TAM injection. * $p < 0.05$, ** $p < 0.01$. Error bars represent SEM. **Related to Fig. 4.**

Supplementary Fig. 6. No expression of Lin28a/b in fat. Representative Lin28a (A) and Lin28b (B) immunostaining of wild-type fat tissues. Scale bars 100 μm . **Related to Fig. 5.**

Supplementary Fig. 7. Overexpression of *let-7* in skeletal muscle is sufficient to cause growth retardation. (A) *Let-7* levels in adult *Lin28a* +/- and KO mice. N=4. (B) *Let-7* levels in adult *Lin28b* +/- and KO mice. N=3. (C-D) Postnatal growth curves of *LSL-iLet-7s; Myf5-Cre(+)* females given DOX from conception. N=4-13. (C) Body weights. (D) Body lengths. (E) Postnatal growth curve of *Lin28a fl/fl; Myf5-Cre* mice. Body weights. N=4-13. (F) Representative images of skeletal muscles in *Lin28b fl/fl* vs. *Lin28b fl/fl; Myf5-Cre(+)* mice. * $p < 0.05$, ** $p < 0.01$. Error bars represent SEM. **Related to Fig. 6.**

Supplementary Fig. 8. Characterization of *Lin28a* KO and *Lin28b* KO muscles. (A-B) Representative type II myosin immunostaining of gastrocnemius in *Lin28a* +/- vs. *Lin28a* KO (A) and *Lin28b fl/fl* vs. *Lin28b fl/fl; Myf5-Cre(+)* (B) mice. (C-D) Mitochondrial DNA content analyzed by quantitative PCR in gastrocnemius (gast) and quadriceps femoris (quad) of *Lin28a* +/- vs. *Lin28a* KO (C) and *Lin28b fl/fl* vs. *Lin28b fl/fl; Myf5-Cre(+)* (D) mice. *Cox2* DNA was used for mtDNA and normalized to *Rsp18* for relative amount calculation. N=3-4. (E-F) *Nrf-1*, *Nrf-2*, *Pgc-1a*, *Pgc-1b*, *Ppara*, *Tfam*, *Tfb1m*, and *Tfb2m* mRNA analyzed by quantitative RT-PCR in

gastrocnemius of *Lin28a* +/- vs. *Lin28a* KO (E) and *Lin28b* fl/fl vs. *Lin28b* fl/fl; *Myf5-Cre*(+) (F) mice. N=3-4. Error bars represent SEM. Scale bars 100 μ m. **Related to Fig. 6.**

Supplementary Fig. 9. Hepatocyte- and pancreatic beta cell-specific *Lin28b* KO mice. (A-B) Postnatal growth curves of *Albumin-Cre; Lin28b* fl/fl males. N=7-9. (A) Body weights. (B) Body lengths. (C-D) Postnatal growth curves of *Ins2-Cre; Lin28b* fl/fl males. N=8-10. (C) Body weights. (D) Body lengths. Error bars represent SEM. **Related to Fig. 6.**

Supplementary Fig. 10. Targeted creation of a conditional *Lin28b* allele. (A) Upper: Genomic map of the *Lin28b* locus shows exons, restriction sites. Middle: *Lin28b* conditional targeting construct. Exon 2 is flanked by loxP sites. PGK-Neo cassette is flanked by a frt site. Lower: Targeted allele following a homologous recombination. Hi=HindIII, Xb=XbaI. (B) Southern blot showing ESC clones with the floxed allele. * denotes a non-specific band. (C) Western blot confirms no *Lin28b* proteins in *Lin28b* -/- ESC lysate. (D) PCR genotyping showing wild type (wt), floxed and deleted (null) alleles. **Related to Experimental Procedures.**

Supplementary Table. Numbers of *Lin28a* +/+; *b* -/- vs. *Lin28a* +/-; *b* -/- vs. *Lin28a* -/-; *b* -/- embryos collected at different ages. Numbers in parentheses show non-viable embryos, as defined by degenerative tissues or arrested heart. P-values were calculated by Fisher's exact probability test. **Related to Fig. 3E.**