Supplementary Figure Legends

Supplementary Figure 1. Table S1: Viability of *E2f3*$^{-/-}$ mice in different strain backgrounds. Genotypic analysis of offspring obtained from intercrosses of *E2f3*$^{+/-}$ mice with the indicated strain backgrounds. Mice used for these crosses were back-bred at least five times into the indicated mouse strains. Note that viable *E2f3*$^{-/-}$ offspring were obtained when *E2f3*$^{+/-}$ mice with different strain backgrounds were used in the crosses. Fisher exact probability test was performed on each genetic group in comparison to the *wild type* group; highly significant results are indicated by an (^{b}).

Supplementary Figure 2. Statistical analysis for the MEF proliferation assays was performed on *E2f1*$^{-/-}$*E2f2*$^{-/-}$*E2f3a*$^{-/-}$ (four independent *E2f1*$^{-/-}$*E2f2*$^{-/-}$*E2f3a*$^{-/-}$ MEF lines were plated in duplicate; a total of four independent experiments) and *E2f1*$^{-/-}$*E2f2*$^{-/-}$*E2f3b*$^{-/-}$ (two independent *E2f1*$^{-/-}$*E2f2*$^{-/-}$*E2f3b*$^{-/-}$ MEF lines were plated in duplicate and the entire experiment was repeated twice; a total of four independent experiments) by using a GLM. Statistical analysis is described in detail in "Supplementary Methods".

Supplementary Figure 3. *E2f3a*$^{-/-}$ and *E2f3b*$^{-/-}$ mice are developmentally normal. Tissue sections of selected organs from 21 day-old mice with the indicated genotypes were processed for H&E staining. These organs included the testis, lung, liver and skin. Higher magnification images of the same tissues are presented in the bottom panels of each quadrant.

Supplementary Figure 4. Multiple organ defects in *E2f1*$^{-/-}$*E2f3a*$^{-/-}$ mice. **a**. Organ weights for different tissues in 21 day-old mice with the indicated genotypes; organ weight was normalized to total body weight. **b**. Quantification of Ki67 positive cells in the indicated tissues of 21 day-old mice is presented as the average ± SD percentage of cells that are Ki67 positive. At least 1000 cells per section were counted. Five sections per tissue in five different mice for each genotype were analyzed. (adre gl.), adrenal gland; (kidney c.), kidney cortex; (kidney m.), kidney medulla; (pan. exo.), pancreas exocrine tissue; (pan. endo.), pancreas endocrine tissues; (skin fol.), skin follicle; (skin epi.), skin epidermis. **c.** Graphical representation showing percentage of food absorption in *E2f1*$^{+/+}$*E2f3a*$^{+/+}$ and *E2f1*$^{-/-}$*E2f3a*$^{-/-}$ mice at one month of age. **d.** Serum triglycerides (TG), cholesterol (Ch) and leptin (Lep) levels in the blood of fasting 21 day-old mice (n=5) with the indicated genotypes. **e**. Serum growth hormone (GH) and Insulin-like growth factor 1 (IGF-1) levels in the blood of non-fasting 21 day-old mice (n=5) with the indicated genotypes. Student t-tests were performed for all the analyses comparing mutant and wild type groups and only indicated when $p < 0.05$.

Supplementary Figure 5. Impaired adrenal gland morphology and function in *E2f1*$^{-/-}$*E2f3a*$^{-/-}$ mice was rescued by *E2f3a*^{3bki} and *E2f3a*^{1ki}. H&E staining of the adrenal gland in 21 day-old mice with the indicated genotypes. High magnification images of the same tissues are presented in the bottom panels. (m) adrenal gland medulla; (cortex) adrenal gland cortex.

Supplementary Figure 6. Table S6: Primer sequences used for genotyping and RT-PCR quantification of gene expression as described in text and Materials and Methods.

References:

1. Jandacek, R. J., Heubi, J. E. & Tso, P. A novel, noninvasive method for the measurement of intestinal fat absorption. Gastroenterology 127, 139-44 (2004).
2. Wu, L. et al. The E2F1-3 transcription factors are essential for cellular proliferation. Nature 414, 457-62 (2001).