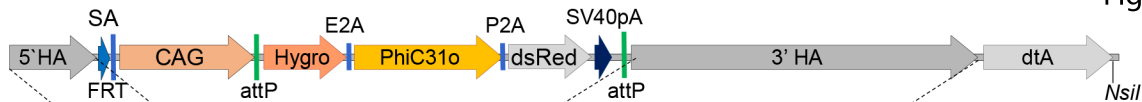
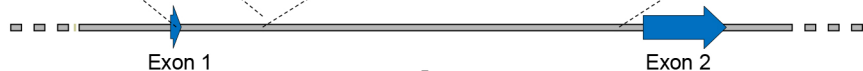


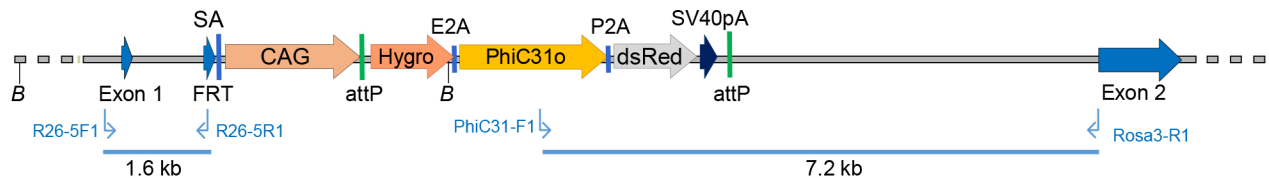
A



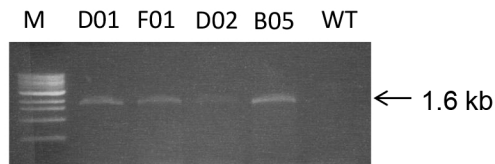
B



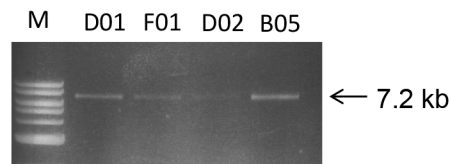
C



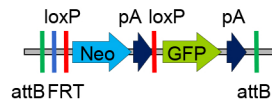
D



E



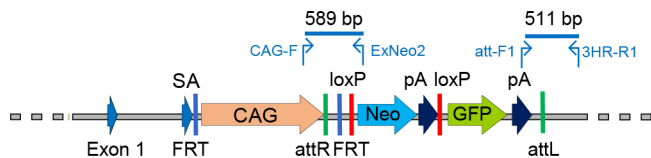
A



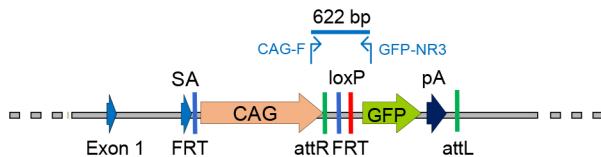
B



C



D



E

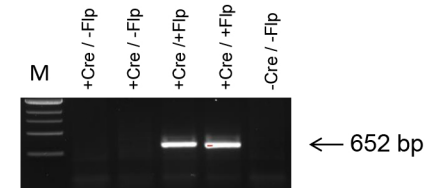
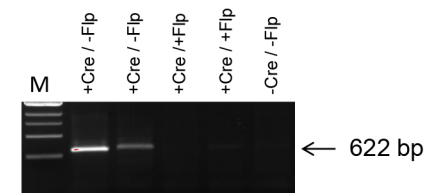
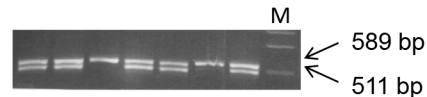
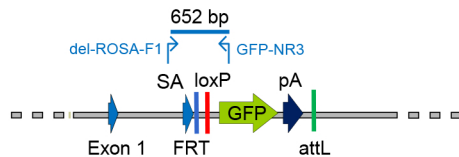
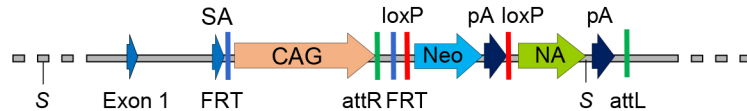
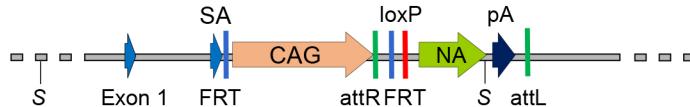


Figure S3

A



B



C

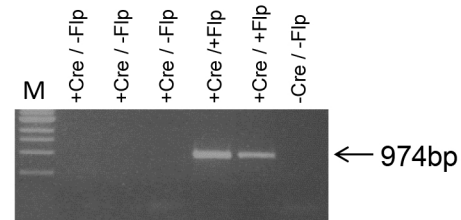
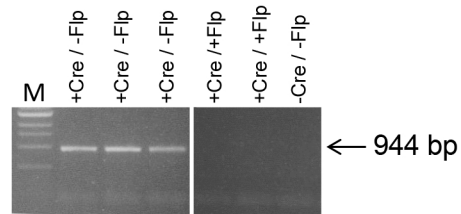
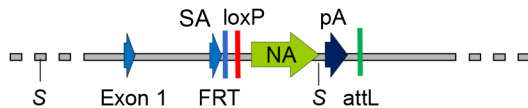
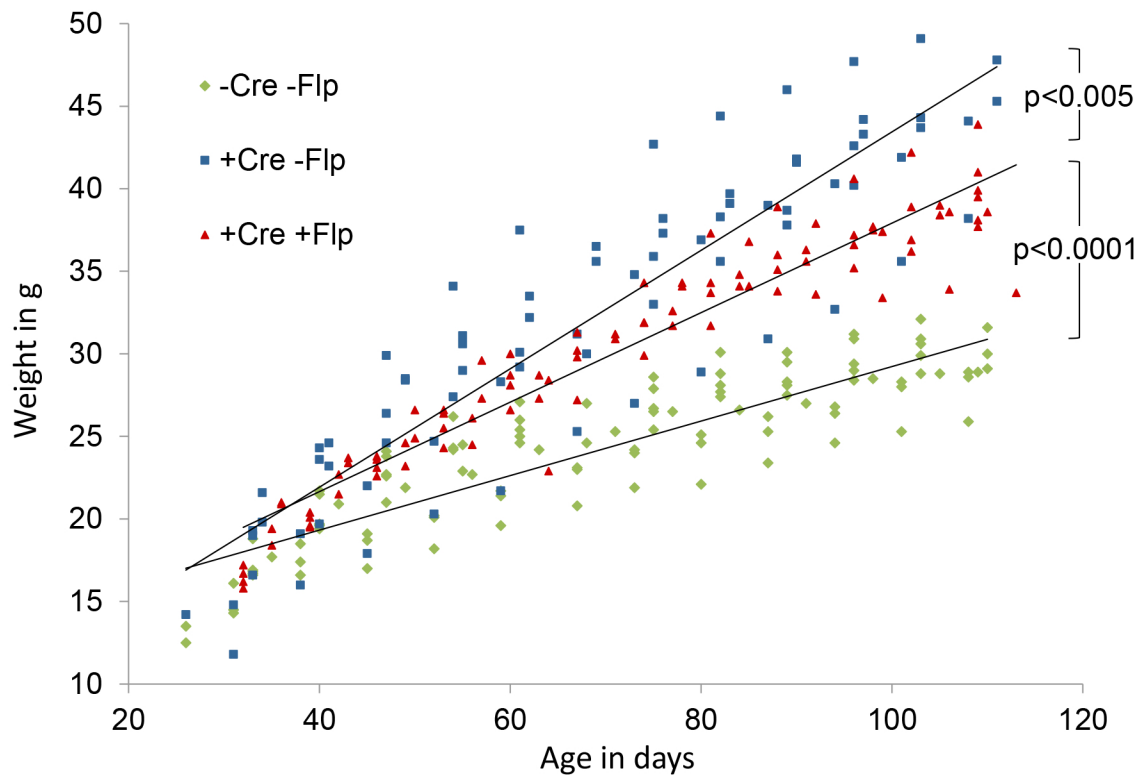


Figure S4



Supplementary Figure Legends

Figure S1 Generation of the RS-PhiC ES cell line (A) Targeting vector used to introduce the cassette exchange machinery into the *ROSA26* locus. HA, homology arms; SA, splice acceptor; E2A and P2A self-cleaving peptides. (B) Endogenous *ROSA26* locus. (C) Targeted *ROSA26* locus showing the positions of the primers used for long-range PCR analysis to detect the targeting event. (D) PCR confirmation of 5' targeting using primers R26-5F1 and R26-5R1 and (E) PCR confirmation of 3' targeting using primers PhiC31-F1 and Rosa3-R1.

Figure S2 Generation of the GFP promoter-switch allele (A) Cassette exchange vector, CB93-GFP. (B) *ROSA26* locus targeted with the cassette exchange machinery. (C) Left panel shows the cassette exchanged *ROSA26* locus, showing the integration of the GFP transgene and the excision of the Hygro-PhiC31-dsRed cassette, with the primers used for PCR screening of the integration events over the 5' attR and the 3' attL sites. Right hand panel shows the results of a typical screen showing the integration of the transgene array by cassette exchange (amplicons for 5' attR and 3' attL are present) or simple integration (only amplicon for 5' attR site is present). (D) Cre recombinase dependent deletion of the Neo cassette activating the expression of GFP. The allele conformation with primers for the screening of the Cre deletion event are shown in the left panel and the right panel shows some example genotyping. (E) Flp recombinase dependent deletion of the CAG promoter, linking the expression of GFP to the endogenous *ROSA26* promoter via the splice acceptor (SA). The allele conformation with primers for the screening of the Flp deletion event are shown in the left panel and the right panel shows some example genotyping.

Figure S3 Generation of the *Nonagouti* promoter-switch allele (A) The cassette exchanged *ROSA26* locus, showing the integration of the *Nonagouti* (NA) transgene. (B) Cre recombinase dependent deletion of the Neo cassette activating the expression of *Nonagouti*. The allele conformation with primers for the screening of the Cre deletion event are shown in the left panel and the right panel shows some example genotyping. (C) Flp recombinase dependent deletion of the CAG promoter, linking the expression of *Nonagouti* to the endogenous *ROSA26* promoter via the splice acceptor (SA). The allele conformation with primers for the screening of the Flp deletion event are shown in the left panel and the right panel shows some example genotyping.

Figure S4 Growth curves for male *Nonagouti* overexpression mice before (green diamonds; n=9) and after (blue squares; n=7) ubiquitous Cre recombinase activation and following the Flp recombinase promoter-switch (red triangles; n=8). p values were calculated with the Student's t-test using weights at 110 days of age.