

% <i>orb2^R/orb2^R</i> exp./obs.	% <i>orb2^R/TM3 Ser</i> exp./obs.	% <i>TM3 Ser/TM3 Ser</i> exp./obs.	χ^2 p-value
33,33/21,00	66,67/79,00	Lethal balancer combination	< 0,0001(n=1500)

Fig. S1. Mendelian inheritance of *orb2^R* allele. Observed and expected offspring ratios and chi-square analysis of offspring with different genotypes from *orb2^R/TM3 Ser* intercross.

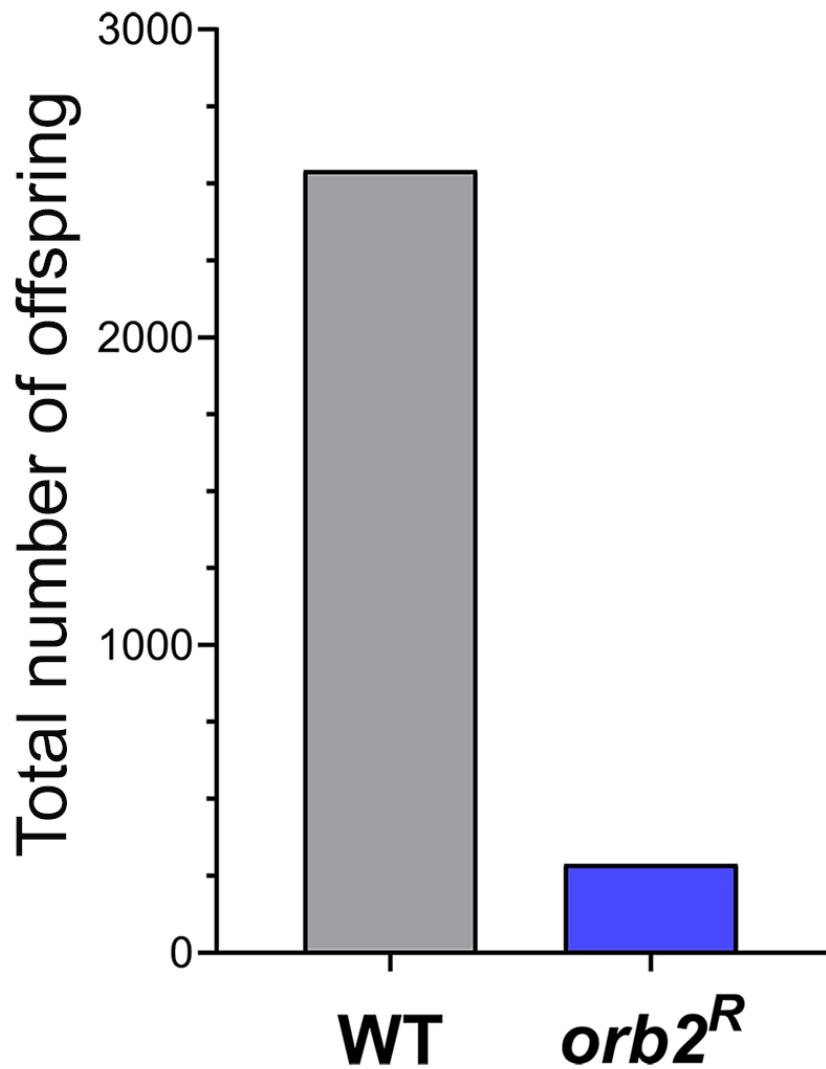


Fig. S2. Overall fertility assay. 35 WT or *orb2^R* males were mated with 70 WT females, and the total number of offspring reaching adulthood was counted. The overall fertility of *orb2^R* males is decreased tenfold.

Supplementary Materials and Methods

Sequences for Cas9 cut

Upstream cut, 5'- CTTCGTAATAGACCGTATTAT↓GTAAGG;

Downstream cut, 5'- CTTCGTGGAGTACTGCTGATA↓TCTTGG.

Primers used in qPCR

orb2 total mRNA, 5'-TAACACCAGCGAAAGGGGAC and 5'- CAGATGTGCGACGAGTGC;

orb2 primary transcript, 5'-GCTGTTGGTGCTGATGGA and 5'-AGCCTCTTCATCTGTTGTC;

GAPDH mRNA, 5'-CTACCTGTTCAAGTTCGATTGAC and 5'-AGTGGACTCCACGATGTATTG;

Soti mRNA, 5'-CCACCACGTCGGCGGAAGAA and 5'-TCCTCGCGAACGTGACGCAT;

Bruce mRNA, 5'-AGCGCAAGGATAAATCAGCTCGC and 5'-TCAGCGTAAGGCTGCGCCAA;

Unc mRNA, 5'-CTCGGCCTGAGAACACCAAA and 5'-GTTTGCGGGCATTGTAGG;

Yuri mRNA, 5'-TCTCCTGATCGGTGCACTGCC and 5'- GCAACTGGCAAGTGTGCATGTC;

Orb mRNA, 5'-GTCGAATGTTCGTTACCCTCT and 5'-GCAGCTGACGGTGTCTGCT.