Supporting Information

Targeted disruption of *Rab10* causes early embryonic lethality

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Materials and methods

Construction of *Rab10* heterozygous mice

A 5.0 kb fragment located upstream of exon3 and another 5.0 kb fragment located downstream of exon5 were amplified via PCR using genomic DNA as a template. A neomycin resistance cassette was used to replace exons 3-5 of *Rab10* (Fig. 1A). The HSV-TK expression cassette was employed as a negative selection maker against random integration. The targeting vector was linearized and electroporated into the W4 embryonic stem (ES) cell line (129S6/SvEvTac-derived). After screening via PCR and Southern blot analysis, correctly targeted ES clones were injected into C57BL/6 blastocysts for chimera generation (Fig. 1B and 1C). Male chimeric offspring mice were bred with C57BL/6 females to generate F1 *Rab10* heterozygous ($Rab10^{+/-}$) mutants which were verified by genotyping (Fig. 1D). All of the experiments were performed according to guiding principles for the care and use of laboratory animals.

ES cells screening

The PCR primers used for screening the ES cells were as follows: primer 1, 5'-AGCAGGAAATGTTGGTGA-3', primer 2, 5'-TTGGCTGGACGTAAACTC-3', the product length was 5.8 kb; primer 3, 5'-CGCATTGTCTGAGTAGGT-3', primer 4, 5'-AGTCAAATCCAGGTTAGG-3' , the product length was 4.0 kb. The amplification protocol of the PCR reaction was performed as follows: 1 cycle of 95 ℃ for 5 min; 40 cycles of 95 ℃ for 30 s, 60 ℃ for 45 s and 68 ℃ for 6 min.

For the Southern blots, homologous recombination was verified by external restriction digests. At the 5’ terminus, genomic DNA was digested with NheI and hybridized with a 5’ probe. The probe hybridized to a 16.7 kb fragment derived from the wild-type allele or an 8.0 kb fragment from the allele with a correctly targeted replacement. Similarly, genomic DNA digested with ApaLI and hybridized with a 3’ probe resulted in either a 18.7 kb fragment from the wild-type allele or a 12.8 kb fragment from the properly disrupted gene. The locations of the 5’ probe and 3’ probe are shown in Fig.1A.

Genotyping

The pups and embryos generated by natural mating of *Rab10* heterozygote mice were identified via PCR using genomic DNA as a template. Tail snips or embryos were lysed in alkaline lysis buffer (25 mM NaOH, 0.2 mM EDTA, pH=12) at 95 ℃ for 20 min. Then, the same volume of neutralizing solution (40 mM Tris-HCl, pH=5) was added. The primer WG3R, 5’-GTTGGTGATGTCATACACTAGCATG-3’, primer WG5F, 5’-GCCGCTATGAAGTGTGGAGCTTATA-3’ and primer ES-1R3, 5’-AAGGGTTATTGAATATGATCGGA-3’ were used for the PCR reaction. The protocol was as follows: 1 cycle of 95 ℃ for 5 min, 35 cycles of 95 ℃ for 30 s, 58 ℃ for 30 s, and 72 ℃ for 30 s. The length of the PCR products in the wild-type allele using the primers WG5F and WG3R and in the targeted allele using the primers WG5F and ES-1R3 are 366 bp and 194 bp, respectively.

Collection of embryos and Western blot

Rab10 heterozygous male and female mice during oestrum were put together at 17:00 on the first day. The next morning, conception was identified in female mice carrying vaginal plug and the age of the embryos was defined as 0.5 days postcoitum (E0.5). Embryos of different ages were dissected from the deciduas, and the Reichert's membrane was removed using fine forceps in DMEM (Invitrogen) buffered with 25 mM HEPES (pH7.4) containing 10% FBS.

For Western blot, the embryos were washed in PBS (Gibco, 14190) twice, and then treated with lysis buffer (Beyotime, P0013) containing 1% protease inhibitor cocktail on ice for 20 min. The samples were boiled with SDS-PAGE protein loading buffer (Beyotime, P0015) for 5-10 min at 98 ℃and the supernatants were collected after centrifuging at 12000 rpm for 15 min at 4 ℃. Mouse monoclonal antibody anti-Rab10 was from Abcam.

Immunofluorescence assay

Histological sections were fixed in 4% PFA for 30 min at room temperature (RT) then washed three times for ten minutes. The sections were then dipped into PBS buffer containing 5% goat serum and 0.2% Triton X-100 for 30 min to 1 hour at RT. The samples were incubated with antibodies overnight at 4 ℃, washed with PBS four times, and then stained with fluorescently labeled secondary antibodies. Images of Z-stacks were acquired using microscope (FV1200, Olympus, Japan). Excitation of the

fluorophores was performed using 405, 488 and 561 nm diode lasers. Colocalization masks were analyzed using ImageJ software (National Institutes of Health).

Electron microscopy

The embryos were fixed in 2.5% glutaraldehyde in PBS with a pH of 7.4 at RT for 1 h and then post-fixed with 1% osmium tetroxide in 0.1 mol/L sodium cacodylate for 40 min. After dehydration and infiltration, the samples were embedded in Embed 812 and sliced into 65 nm sections using a Leica ultramicrotome EM UC6 (Leica, Germany). The sections were observed under a Tecnai 20 transmission electron microscope (FEI Company, The Netherlands) operating at 120 kV at a magnification of 40000 × after contrasting with uranyl acetate and lead citrate.

Statistical analysis

Student's *t*-test was used for statistical analysis using SigmaStat. A Significant difference is indicated by asterisks (*$p<0.05$, **$p<0.01$, ***$p<0.001$).