Supplementary information, Data S1 Materials and Methods

Animals

Golden Syrian hamsters were purchased from Vital River Laboratories (Beijing, China). The animals were maintained on a 12-hour light/12-hour dark cycle at 24°C and were provided water ad libitum and fed a standard laboratory chow diet. The Principles of Laboratory Animal Care (NIH publication no.85Y23, revised 1996) was followed, and the experimental protocol was approved by the Animal Care Committee, Peking University Health Science Center (LA2010-059).

Collection and culturing of hamster zygotes

Superovulation was induced in 8- to 12-week-old female golden Syrian hamsters by intraperitoneal injection of 20 IU of pregnant mare serum gonadotropin (Ningbo Sansheng Pharmaceutical Co., Ltd., Ningbo, China) on the 2nd day in a 4 day estrous cycle when the post-estrous discharge was visible, followed by intraperitoneal injection of 20 IU of human chorionic gonadotropin hormone (hCG,) 72 hours later, and then the females were mated with sexually mature males.

Next, 18-20 hours after hCG injection, the females were killed by an overdose of anesthesia, and then the oviducts were excised and placed into 37°C warmed M2 medium (Sigma-Aldrich，St. Louis, MO, USA). Under a dissecting microscope, the swollen ampullary region of the oviducts was ruptured using fine forceps, and the cumulus-oocyte complexes were extruded. Then, the zygotes were freed from the cumulus cells by 1 minute treatment with 0.1% hyaluronidase (Sigma-Aldrich) in M2 medium.

For in vitro culturing, the zygotes were transferred into culture drops consisting of 70 μl

HECM-10 medium (NaCl 113.8 mM, KCl 3 mM, NaHCO3 25 mM, sodium lactate 4.5 mM, $CaCl_2$ 1 mM, MgCl2 2 mM, glutamate 0.01 mM, glutamine 0.2 mM, glycine 0.01 mM, histidine 0.01 mM, lysine 0.01 mM, proline 0.01 mM, serine 0.01 mM, asparagine 0.01 mM, aspartate 0.01 mM, cysteine 0.01 mM, taurine 0.5 mM, pantothenate 0.003 mM, PVA 0.1 mg/ml, Sigma-Aldrich) [1], covered with mineral oil (Sigma-Aldrich) and incubated at 37.5°C in a 10% CO2 incubator for 2-4 hours. It was critical that the culture drops in the dish were equilibrated in the incubator for a minimum of 2 hours before use.

Microinjection of eGFP lentiviral vector and embryo transfer

The lentiviral suspension was purchased from Neuron Biotech Co., Ltd (Shanghai, China). The titer of the lentiviral stock was 2×10^9 titer units (TU)/ml. A schematic representation of pLOV-UbiC-EGFP within the lentiviral vector is shown in Figure 1B.

The pronuclear-stage embryos from the zygotes incubated in the culture drops were transferred into M2 medium covered with mineral oil. The embryo was held with a holding pipette, and the tip of the injection pipette was pushed through the zona pellucida. The viral solution (50-100 pl) was injected into the perivitelline space (between the zona pellucida and the cytoplasmic membrane) of the embryo.

Lentiviral vector-infected embryos were cultured in HECM-10 medium for 1-2 hours and were then transferred into the oviducts of the pseudopregnant female hamsters (15-20 per oviduct) through the fimbriae. The pseudopregnancy was induced by mating the females with vasectomized males.

PCR and Southern blot analysis of proviral DNA

To detect the eGFP gene, GFP-F forward (5'- AAATTGTCCGCTAAATTCTGG -3') and

GFP-R reverse primers (5'-TCACCTTGATGCCGTTCTT-3') were used to yield a 591-bp product. After the initial denaturation step at 94°C for 5 min, 35 cycles of amplification were performed consisting of 45 s of denaturation at 94°C, 45 s of annealing at 57°C, and 45 s of elongation at 72°C. Then, the samples were incubated at 72°C for 10 min to ensure complete strand extension. pLOV-UbiC-EGFP plasmid DNA was used as a positive control.

For Southern blot detection, 30 micrograms (for F0 hamsters) or 10 micrograms (for F1 hamsters) of genomic DNA was digested with *Eco*RI overnight at 37°C. DNA fragments were fractionated by 1% agarose gel electrophoresis and transferred to a nylon membrane. The transgene was detected using a 591-bp DIG-labeled probe (DIG High Prime Lab/Det Kit II; Roche Diagnostics, Mannheim, Germany) against the eGFP sequence (Figure 1D).

Fluorescence imaging

Fluorescence images of the live hamsters and organs were obtained using a Maestro in vivo spectrum imaging system (Cambridge Research and Instrumentation, Woburn, MA). The excitation filter was set as 435-480 nm, and the emission filter was a 490 nm long-pass filter.

FACS analysis of peritoneal macrophages and bone marrow cells

Resident peritoneal macrophages were isolated by injecting 50-60 ml of sterile PBS (Ca^{2+}- and Mg^{2+}-free) into the peritoneal cavity followed by gentle massaging of the abdomen. Abdominal skin was cut, and the resulting peritoneal fluid was collected. Red blood cells (RBCs) were lysed by ammonium chloride-potassium (ACK) buffer. The remaining cells were cultured in RPMI-1640 medium (10% fetal calf serum, 1% penicillin-streptomycin) and were allowed to adhere for 4 hours. Non-adherent cells were washed off with PBS, and new

culture medium was added. Adherent cells were digested with 0.25% trypsin. Bone marrow cells (BMCs) were extracted from the femur and tibia of the hamsters. RBCs were lysed, and the BMCs were centrifuged and resuspended in PBS.

After washing with PBS, the percentage of GFP-positive cells was counted using a FACSCalibur flow cytometer (Becton Dickinson). Data acquisition was performed with FlowJo software.

References

1. Lee ST, Kim TM, Cho MY, et al. Development of a hamster superovulation program and adverse effects of gonadotropins on microfilament formation during oocyte development. Fertil Steril 2005; 83 Suppl 1: 1264-74.