Collection of embryos and preparation of fetal liver cell suspension
Timed pregnancy of Cited2$^{+/-}$ females was determined as 0.5 d.p.c if vaginal plug was found after overnight mating. Fetal livers were dissected at embryonic stages of 13.5 d.p.c, 14.5 d.p.c and 15.5 d.p.c. Fetal liver genotyping was performed by Southern blotting of genomic DNA with a probe described previously.[8] Individual fetal liver was washed twice in IMDM supplemented with 10% fetal bovine serum (FBS) (Hyclone). To prepare single cell suspension, fetal liver was minced and resuspended through a 1-mL sterile tip until no clumps were visible. The cell suspension was then filtered through a 100-μm cell strainer (BD Biosciences). The cell suspension was centrifuged at 300g (Allegra 6R, Beckman Coulter) for 5 min and the cell pellet was resuspended in 1 mL IMDM supplemented with 2% FBS (Stem Cell Technologies). Cell number was counted using a hemocytometer (Sigma) after diluting cells in 3% acetic acid.

Peripheral blood analysis
Peripheral blood samples were collected from tails after transplantation. Blood samples were lysed for 10 min at room temperature in buffer consisting of 155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA (pH8.0) and washed by HBSS with 2% FBS prior to staining.

Microarray analysis
Total RNA was extracted from fetal liver at 14.5d.p.c from Cited2$^{-/-}$ embryos and Cited2$^{+/+}$ littermate controls using TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA was further purified using RNeasy Micro Kit (Qiagen, Inc., Valencia, CA). Purified total RNA was delivered to the Gene Expression Array Core Facility at Case Western Reserve University (http://www.geacf.net). Computational analysis on microarray raw data was performed mainly with Data Mining Tool (DMT v. 3.0, Affymetrix) and Affymetrix Genechip Operating Software (v 1.3, Affymetrix). Probes that received an Affymetrix change call of 'increased,' 'decreased,' 'marginally increased' and 'marginally decreased' were retained for further analysis. The microarray data generated from each Cited2$^{-/-}$ fetal liver (n=3) sample was compared to that generated from Cited2$^{+/+}$ littermate fetal liver (n=3) to result in a total of 9 comparisons. Further consideration was given to the probe sets, which were present in more than 3 out of 9 comparisons.

Real-time PCR
2 μg total RNA was reverse transcribed into cDNA using SuperScript First-Strand Synthesis System for RT-PCR kit (Invitrogen, Carlsbad, CA). cDNA template was diluted 20 times and 2 μL diluted cDNA template was used for each real-time PCR reaction using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). Triplicate reactions from each sample were set up for detecting the expression of each gene. The real-time PCR was run on MyiQ (Bio-Rad Laboratories, Hercules, CA). Ct value was recorded to perform data analysis. The primers used for the study are listed in Table 1.