Supplementary information

Materials and Methods

Zebrafish Wild type Tu line and transgenic line *tg(mylpfa:EGFP)* embryos were raised at 28.5℃ and staged according to description by Kimmel *et al* [1]. All animal experiments were approved by Institutional Animal Care and Use Committee (IACUC) of Peking University. The reference from IACUC of Peking University is LSC-ZhangB-1.

Csy4 expression and purification Full-length cDNA for *csy4* was amplified from pCsy4 (Plasmid #44252, addgene) and cloned into pET-28a vector. Then Csy4 protein was expressed in *E. coli* Rosetta (DE3) cells and induced with 1 mM IPTG at 18℃ overnight. Cells were lysed by sonication in lysis buffer [50 mM NaH_2PO4, 300 mM NaCl, 10 mM imidazole, 0.2 mg/mL lysozyme, 1 mM TCEP, protease inhibitor cocktail (Sigma), pH 7.4]. The cleared lysate was incubated with Ni-NTA affinity resin (QIAGEN). The bound protein was eluted with elution buffer (15.5 mM Na_2HPO_4, 4.5 mM NaH_2PO_4, 500 mM NaCl, 300 mM imidazole, 1 mM TCEP, 5% glycerol, pH7.4) and further dialyzed against storage buffer (100 mM HEPES pH7.5, 150 mM KCl, 5% glycerol, 1 mM TCEP). The concentration of soluble Csy4 protein was determined by Bradford Assay kit. 10% SDS-PAGE was used for the detection of the His-tagged Csy4.

Cas9, csy4 mRNA and gRNA synthesis *Cas9* mRNA was *in vitro* transcribed from an *Xba*I linearized z*Cas9* vector using the T3 mMESSAGE mMACHINE kit (Ambion) [2]. *Csy4*

mRNA was transcribed *in vitro* from an *Xba*I linearized pCS2^{+}-*csy4* vector using the Sp6 mMESSAGE mMACHINE kit (Ambion). The Csy4-gRNA template was produced by overlap PCR from pMD19-gRNA scaffold [3]. The gRNAs were transcribed *in vitro* using T7 MAXIscript Kit (Ambion) and incubated with Csy4 protein in reaction buffer (20 mM HEPES pH7.5, 100 mM KCl) at 25℃ for 20 minutes. The molar ratio of gRNA and Csy4 protein added is 1:10. Then the reaction was quenched by the addition of acid phenol-chloroform (Ambion) and purified by RNeasy FFPE kit (QIAGEN). We validated gRNA purity and sizes by 12% denaturing PAGE, and concluded that the column-purified gRNA could be directly used for injection. All primers used are listed in supplementary Table S1. Csy4 protein is now available at Shenzhen Shengjie Biotech Co. (shengjiebiotech@163.com).

Microinjection and genotyping One-cell stage zebrafish embryos were injected with 2 nL of a solution containing 200 ng/μL Cas9, 20 ng/μL gRNA. The mutagenesis efficiency for the *susd4* target sites was assessed by *Bts*CI (NEW ENGLAND Biolabs) digestion. In other cases, the amplified genome DNA of F_0 founders (6 embryos/sample) was assessed by T7 Endonuclease I Assay (NEW ENGLAND Biolabs) and cloned into pEASY-T1 simple (TRANS) for sequencing. Then the digested samples were resolved by electrophoresis through a 2% agarose gel. The band intensity was quantified using Quantity One software (BIO-RED).

Imaging Embryos were anesthetized with 0.03% Tricaine (Sigma-Aldrich) and mounted in

4% methylcellulose. All images were captured by a Zeiss Axio Imager Z1 microscope and processed by Adobe Photoshop CS5 software.

REFERENCE

1 Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev Dyn* 1995; **203**:253-310.

2 Liu D, Wang Z, Xiao A *et al.* Efficient Gene Targeting in Zebrafish Mediated by a Zebrafish-Codon-Optimized Cas9 and Evaluation of Off-Targeting Effect. *Journal of Genetics and Genomics* 2014; **41**:43-46.

3 Chang N, Sun C, Gao L *et al.* Genome editing with RNA-guided Cas9 nuclease in zebrafish embryos. *Cell research* 2013; **23**:465-472.