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Supplementary information

Generation of CRISPR–Cas9-mediated genetic knockout human intestinal tissue–derived enteroid lines by lentivirus transduction and single-cell cloning

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Supplementary Methods

For the two vector system, which is not detailed in the Procedure but has been used for generating a *FUT2-KO* (16), separate Cas9-expressing and sgRNA-expressing lentivral vectors are used. A Cas9-expressing lentiviral vector (lentiCas9-BLAST, Addgene #52962) and sgRNA expression vector (lentiGuide-puro, Addgene #52963) were obtained from Addgene. The sgRNA sequence for the human *FUT2* gene (5'-CCAGCCAGCTCAGGGGGATG-3') was cloned into the lentiGuide-puro vector. Lentiviruses were produced by cotransfecting HEK-293FT cells with a combination of a lentivirus plasmid (lentiCas9-BLAST or lentiGuide puro) and three other plasmids including a packaging plasmid (pMDLg/ pRRE [Addgene #12251], an envelope plasmid pMD2.G [Addgene #12259], and a pRSV-Rev plasmid [Addgene #12253]) at a ratio of 3.5:2:1:1, respectively, using polyethylenimine HCl Max molecular weight (MW) 40,000 (Polysciences). The culture supernatant was harvested 60 to 72 h post-transfection, filtered through a 0.45-μm filter, and concentrated by using a LentiX-concentrator (TaKaRa-Clontech #631232), and then suspended in WRNE medium for HIE transduction. The HIEs were initially transduced by lentiCas9-BLAST, selected by Blasticidin S (5 μg/mL) to obtain Cas9-expressing HIEs. Those HIEs were then re-transduced by lentiGuide-Puro-FUT2 and selected by puromycin (2 μg/mL) to obtain cells expressing both Cas9 and *FUT2* sgRNA simultaneously for single cell cloning. The HIEs transduced with the two separate vectors, the integrated Cas9 cassette and the *FUT2* sgRNA cassette, are now resistant to both Blasticidin S and puromycin.