**Supplementary Materials and Methods**

**Cell cultures**

HEK293 and HEK293T cells (human transformed embryonic kidney cell lines) and Huh7 cells (a human hepatocarcinoma cell line) were cultured in Dulbecco’s Modified Eagle’s Medium (WAKO; Tokyo, Japan) supplemented with 10% fetal bovine serum, streptomycin (100 μg/mL; NacalaiTesque; Kyoto, Japan) and penicillin (100 U/mL; NacalaiTesque). H1299 cells (a human non-small cell lung carcinoma cell line) were cultured in RPMI 1640 (Sigma-Aldrich; St. Louis, MO) supplemented with 10% fetal bovine serum, streptomycin (100 μg/mL) and penicillin (100 U/mL). HeLa cells (a human cervical cancer cell line) were cultured in Dulbecco’s Modified Eagle’s Medium low glucose (Sigma-Aldrich) supplemented with 10% fetal bovine serum, streptomycin (100 μg/mL) and penicillin (100 U/mL).

**Ad vectors**

Ad vectors were prepared by improved in vitro ligation method[1,2]. Briefly, generated pHM-based plasmids were digested with I-CeuI and PI-SceI and cloned into pAdE4-122aT[3]. Ad vector plasmids linearized by digestion with PacI were transfected and subsequently amplified in HEK293 cells. Ad vectors were purified by two rounds of cesium-chloride-gradient ultracentrifugation. The virus particle (VP) titers were determined by spectrophotometric method[4]. The infectious units (IFU) were determined using an Adeno-X Rapid Titer Kit (Clontech Laboratories; Mountain view, CA).

**Plasmids**

DNA fragments of the U6 promoter and cognate crRNA sequences for AsCas12a and LbCas12a were isolated from BPK3079 (Addgene #78741; Cambridge, MA) and BPK3082 (Addgene #78742) and cloned into pHM5[2] generating pHM-U6-asgRNA and pHM-U6-lbgRNA, respectively. DNA fragments encompassing a hybrid chicken beta-actin (CBh) promoter together with AsCas12a or LbCas12a coding regions were isolated from previously generated pHM-CBh-AsCpf1 and pHM-CBh-LbCpf1[5] and cloned upstream of the U6 promoter in pHM-U6-asgRNA and pHM-U6-lbgRNA, and subsequently double-stranded oligonucleotides for gRNAs for the Adeno-associated virus integration site 1 (*AAVS1*) region were cloned at BsmBI sites generating pHM-CBh-AsCas12a-AAVS1 and pHM-Cbh-LbCas12a-AAVS1, respectively. A synthetic liver-specific promoter composed of apolipoprotein E enhancer, the hepatocyte control region, and human α1-antitrypsin (AHA) promoter region isolated from pBS-ApoEHCR-hAATp-FIX-Int-bpA16[6] and AsCas12a and LbCas12a coding regions from pcDNA3.1-hAsCpf1 (Addgene #69982) and pcDNA3.1-hLbCpf1 (Addgene #69988) were cloned upstream of U6 promoter in pHM-U6-asgRNA and pHM-U6-lbgRNA, and subsequently double-stranded oligonucleotides for gRNAs for *AAVS1* region were cloned at BsmB sites generating pHM-AHA-AsCas12a-AAVS1 and pHM-AHA-LbCas12a-AAVS1, respectively. The fragment containing the tetracycline-responsive promoter (TRE) and the 3G rtTA expression cassette from pTetone (Clontech Laboratories; Mountain view CA) was cloned upstream of U6 promoter in pHM-U6-asgRNA-BbsI and pHM-U6-lbgRNA-BbsI, where BsmBI site was converted to BbsI, and AsCas12a and LbCas12a coding regions were cloned downstream of TRE. Double-stranded oligonucleotides for gRNA for *AAVS1* region were cloned at the BbsI site generating pHM-teton-AsCas12a-AAVS1 and pHM-teton-LbCas12a-AAVS1, respectively. pHM-CBh-GFP was generated by replacing hSpCas9 of pHM-CBh-hCas9[5] with the GFP coding region isolated from pHM-CA-GFP[7]. The newly constructed pHM-based plasmids as well as previously constructed pHM-CBh-AsCpf1 and pHM-CBh-LbCpf1[5] were cloned into pAdE4-122aT[3]. shRNA sequences for AsCas12a and LbCas12a were designed using siDIRECT[8] (Supplementary table S1) and double-stranded oligonucleotides encoding shRNAs were cloned into pLKO.1 puro[9] (Sigma-Aldrich) digested with AgeI and EcoRI sites, generating pLV-shAsCas12a and pLV-shLbCas12a.

**gRNA selection**

Candidate target sequences for *AAVS1* region were selected using CHOPCHOP v2[10]. The double-stranded oligonucleotides were cloned into BsmBI sites in BPK3079 and BPK3082. Four hundred ng of each plasmid was co-transfected with 600 ng of pHM-CBh-hAsCas12a or pHM-CBh-hLbCas12a[5] into HEK293 cells. Genomic DNAs were prepared 48 hours later and subjected to T7 endonuclease I (T7E1) assay.

**Real-Time RT-PCR analysis**

Cells were seeded at 2.5x105 cells/well in 24-well plates and transfected with 400 ng of pHM-CBh-AsCpf1 or pHM-CBh-LbCpf1[5]. Total RNA was isolated from the cells 48 hours after transfection using ISOGEN (Nippon Gene; Tokyo, Japan). cDNA was synthesized using SuperScript II reverse transcriptase (Invitrogen Life Technologies) and a random primer. qPCR was performed with the primers listed in Table S1. The values were normalized by GAPDH expression.

**Western blot analysis**

Cells were seeded at 3.5x104 cells/well in 24-well plates and transduced with Ad vectors. Total cell lysates were prepared 48 hours later using RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific; Waltham, MA) containing a protease inhibitor cocktail (Sigma-Aldrich). Western blot analysis was performed as previously described[11]. Briefly, cell lysates were electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gels (PAGE), and transferred to polyvinyliden difluoride (PVDF) membranes (Millipore; Darmstadt, Germany). The HA-tagged Cas12a proteins were detected using mouse Anti-HA.11 Epitope Tag Antibody (BioLegend; San Diego, CA). Mouse anti-β-actin (Sigma-Aldrich) was used for loading controls.

**Refferences**

[1] H. Mizuguchi, M.A. Kay, Efficient construction of a recombinant adenovirus vector by an improved in vitro ligation method., Hum. Gene Ther. 9 (1998) 2577–83. doi:10.1089/hum.1998.9.17-2577.

[2] H. Mizuguchi, M.A. Kay, A simple method for constructing E1- and E1/E4-deleted recombinant adenoviral vectors., Hum. Gene Ther. 10 (1999) 2013–7. doi:10.1089/10430349950017374.

[3] K. Shimizu, F. Sakurai, K. Tomita, Y. Nagamoto, S.-I. Nakamura, K. Katayama, M. Tachibana, K. Kawabata, H. Mizuguchi, Suppression of leaky expression of adenovirus genes by insertion of microRNA-targeted sequences in the replication-incompetent adenovirus vector genome., Mol. Ther. Methods Clin. Dev. 1 (2014) 14035. doi:10.1038/mtm.2014.35.

[4] J.V.J. Maizel, D.O. White, M.D. Scharff, The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A, and 12., Virology. 36 (1968) 115–125.

[5] T. Tsukamoto, E. Sakai, S. Iizuka, M. Taracena-Gandara, F. Sakurai, H. Mizuguchi, Generation of the Adenovirus Vector-Mediated CRISPR/Cpf1 System and the Application for Primary Human Hepatocytes Prepared from Humanized Mice with Chimeric Liver., Biol. Pharm. Bull. 41 (2018) 1089–1095. doi:10.1248/bpb.b18-00222.

[6] C.H. Miao, K. Ohashi, G.A. Patijn, L. Meuse, X. Ye, A.R. Thompson, M.A. Kay, Inclusion of the hepatic locus control region, an intron, and untranslated region increases and stabilizes hepatic factor IX gene expression in vivo but not in vitro., Mol. Ther. 1 (2000) 522–532. doi:10.1006/mthe.2000.0075.

[7] E. Mukai, S. Fujimoto, F. Sakurai, K. Kawabata, M. Yamashita, N. Inagaki, H. Mizuguchi, Efficient gene transfer into murine pancreatic islets using adenovirus vectors., J. Control. Release. 119 (2007) 136–141. doi:10.1016/j.jconrel.2007.01.012.

[8] Y. Naito, T. Yamada, K. Ui-Tei, S. Morishita, K. Saigo, siDirect: highly effective, target-specific siRNA design software for mammalian RNA interference, Nucleic Acids Res. 32 (2004) W124–W129. doi:10.1093/nar/gkh442.

[9] S.A. Stewart, D.M. Dykxhoorn, D. Palliser, H. Mizuno, E.Y. Yu, D.S. An, D.M. Sabatini, I.S.Y. Chen, W.C. Hahn, P.A. Sharp, R.A. Weinberg, C.D. Novina, Lentivirus-delivered stable gene silencing by RNAi in primary cells., RNA. 9 (2003) 493–501.

[10] K. Labun, T.G. Montague, J.A. Gagnon, S.B. Thyme, E. Valen, CHOPCHOP v2: a web tool for the next generation of CRISPR genome engineering., Nucleic Acids Res. 44 (2016) W272-6. doi:10.1093/nar/gkw398.

[11] M. Machitani, F. Sakurai, K. Wakabayashi, K. Nakatani, K. Takayama, M. Tachibana, H. Mizuguchi, Inhibition of CRISPR/Cas9-Mediated Genome Engineering by a Type I Interferon-Induced Reduction in Guide RNA Expression., Biol. Pharm. Bull. 40 (2017) 272–277. doi:10.1248/bpb.b16-00700.

**Supplementary Figure Legend**

**Figure S1 Cell viability analysis of various cell lines transduced with the Ad vector inducibly expressing AsCas12a.**

Huh-7, H1299 and HeLa cells were transduced with Ad-teton-AsCas12a at the indicated MOIs and cultured in the presence or absence of DOX (1μg/ml) The cell viability was determined 48 hours transduction. Mock-transduced cells without DOX were set to 100%. Results were presented as mean±SD (n=3); \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001, N.S., not significant compared to with DOX (-). Statistical significance was determined using two-way ANOVA analysis followed by Bonferroni’s Multiple comparison Test.