**Supplementary Materials**

*Selection of siRNA for MADL2 knockdown*

Three small interfering RNAs (siRNAs) were designed for the NM_001127325.2 isoform of *MAD2L2* that consists of 8 coding exons and encodes a 211 aa protein. The siRNA_MAD2L2_1 sequence is located at the border of the first (non-coding) and second exons of the transcript, siRNA_MAD2L2_2 and siRNA_MAD2L2_3 are both complementary to the last exon (Figure S1). These two siRNAs have deoxythymidine residues at the 3'-end of the strand, and siRNA_MAD2L2_1 has a double uracil residue. Transfection of siRNA was performed with the TurboFect™ Transfection Reagent (Thermo Fisher Scientific, USA). Cells were analyzed 24 h after transfection.

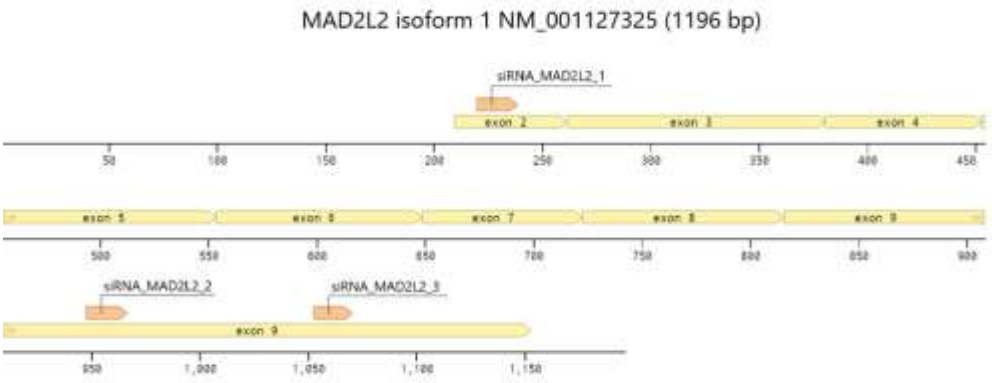


**Figure S1.** MAD2L2 and anti-*MAD2L2* siRNAs. First exon is non-coding and isn't shown.

Transfection efficacy (97.1%) was measured using a FAM-labeled siRNA (siRNA_flu) with a sequence that was non-specific for the human genome. Negative controls included untransfected cells, cells with transfection reagent only (Turbofect), and cells transfected with siRNA_flu. MAD2L2 expression in the cells transfected with siRNA_flu was set to 1 and othe samples were compared to it. Cells at all conditions showed the same normal survival 24h after transfection (data not shown). All 3 anti-MAD2L2 siRNAs demonstrated expression knockdown down to 0.3 (p=0.012), 0.09 (p=0.017), and 0.1 (p=0.017) compared to MAD2L2 expression in the cells transfected with siRNA_flu (Figure S2). siRNA_MAD2L2_2 demonstrated more than 10-fold knockdown and was selected for further experiments; it is called as siRNA_MAD2L2 in the main text.

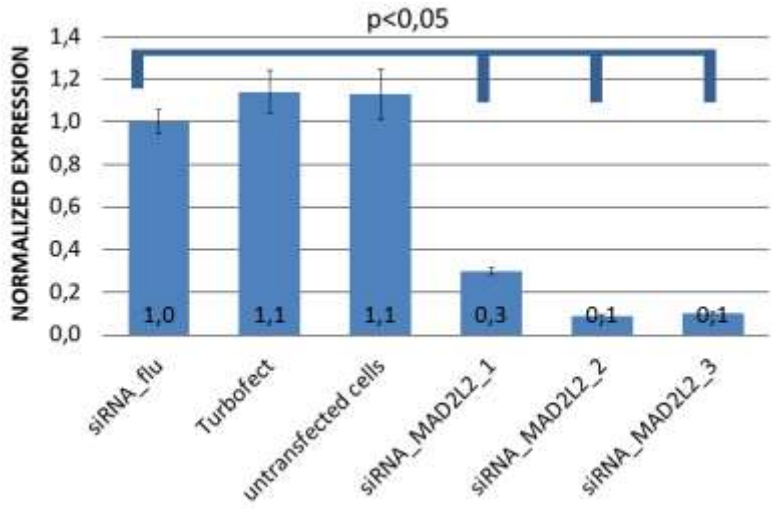


**Figure S2**. Expression of *MAD2L2* mRNA. Data is represented as mean and 95% CI, differences in expression levels were analyzed using the Mann-Whitney test. More details are in the text.

We also confirmed that *MAD2L2* influences its protein level. We stained the cells with anti-MAD2L2 antibodies and demonstrated that the number of MAD2L2 positive cells decreased 1.8-fold 24 h after transfection compared to control samples exposed to Turbofect only (S3 Fig).

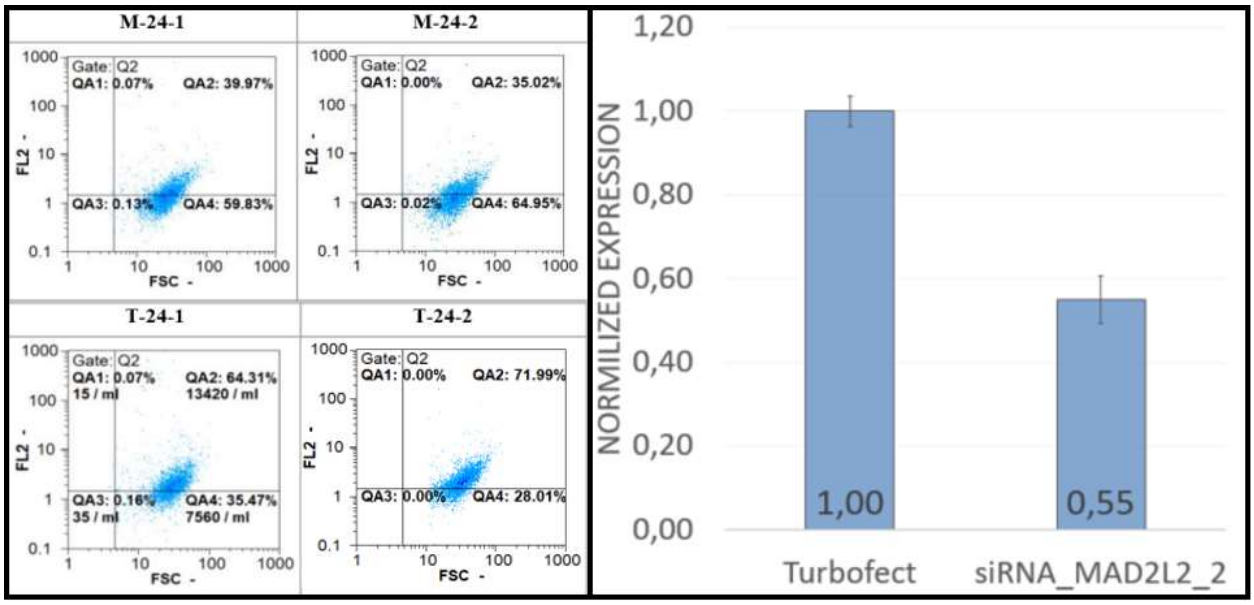


**Figure S3**. MAD2L2 protein knockdown with siRNA_MAD2L2. MAD2L2 was stained with primary (Recombinant AntiMad2L2/REV7 antibody [EPR13657], Abcam, UK) and secondary antibodies (Alexa Fluor 594 goat anti-rabbit IgG (H+L), Invitrogen, USA). A - Dot plot generated by the Flomax software; forward side scatter is along the X axis, fluorescent intensity, FL2, is along the y axis. M-24-1, M-24-2 – cells with knockdown of *MAD2L2,* 24 h after transfection, T-24-1, T-24-2 - control cells exposed to Turbofect only, 24 h after transfection. B – Proportion of MAD2L2-positive cells after *MAD2L2* knockdown. Mean and 95% CI. * $p < 0.05$.

*Verification of Cas9-SCAI expression*

Cas9-SCAI expression was confirmed by staining SCAI with primary (recombinant Anti-SCAI antibody [EPR4128] (ab124688), Abcam, UK) and secondary antibodies (Alexa Fluor 594 goat anti-rabbit IgG (H+L), Invitrogen, USA) 48 h after transfection with the Cas9-SCAI vector. Analysis of 62,959 cells transfected with Cas9-SCAI and 14,577 cells transfected with Cas9 only (control) confirmed that SCAI exhibits nuclear localization (S6 Fig) and its expression level is 12% higher in cells transfected with Cas9-SCAI (p=0.0001).

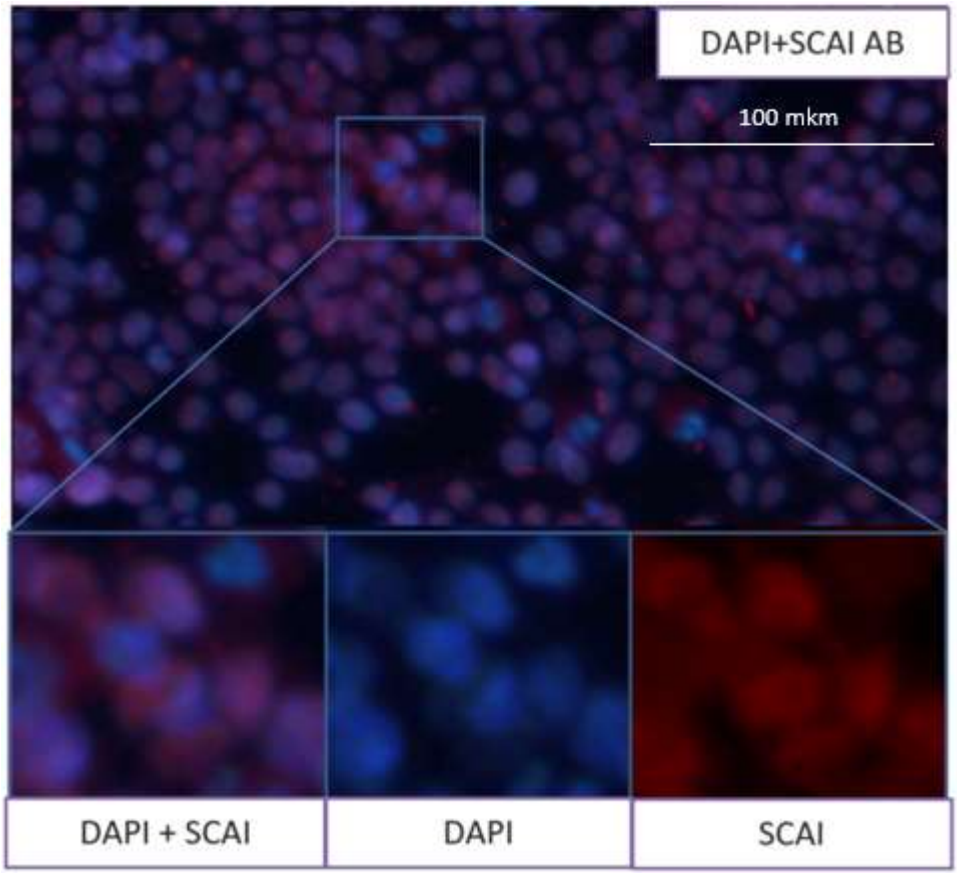


**Figure S4.** SCAI is localized in the nuclei of the transfected HEK293 cells stained with Anti-SCAI antibodies (SCAI AB) and DAPI.

*SCR7: choosing optimal time and concentration*

SCR7 which inhibits ligase IV was added to transfected cells at the time of transfection or 24 h before. Cells were transfected with the Cas9 to restore the *eGFP* gene by HDR. The number of GFP+ cells was 1.8 times higher (p=0.009) when exposed to SCR7 simultaneously with Cas9-sgRNA/ssODN transfection, while the administration of SCR7 24 h prior to transfection even reduced the number of successfully edited cells (by 2.4 times, p=0.009) (S4 Fig).

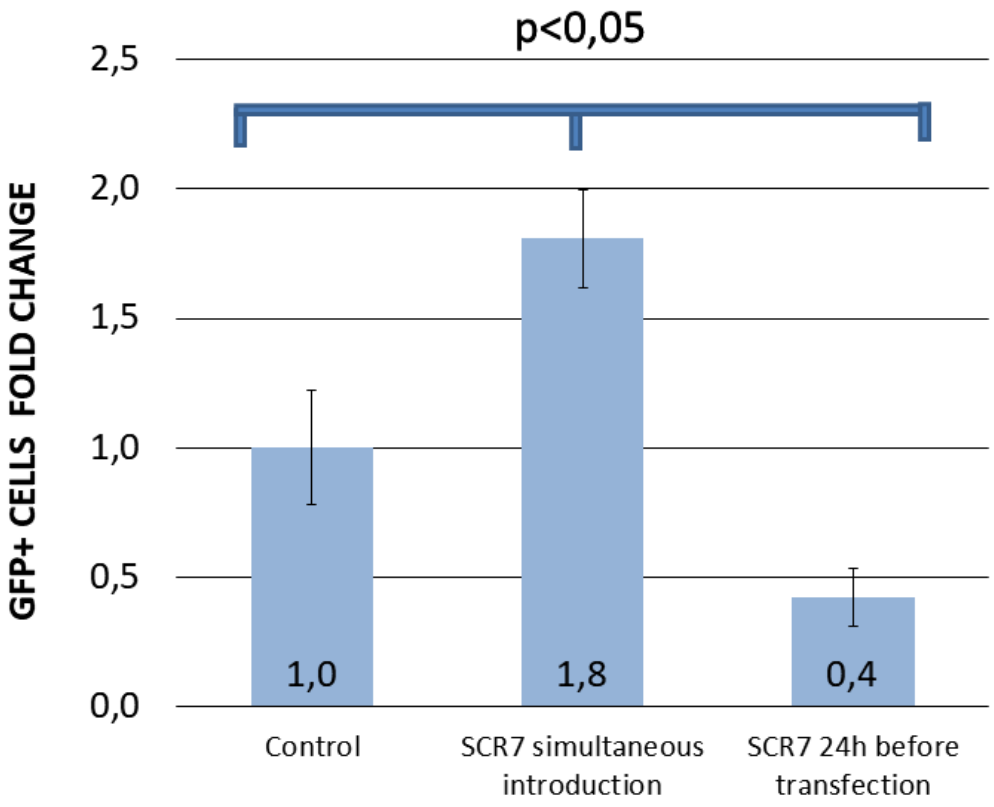


**Figure S5.** Influence of SCR7 added at different time points on the number of GFP+ cells after genome editing by Cas9-sgRNA/ssODN. Mean and 95% CI.

Next we added SCR7 at 4 different concentrations simultaneously with transfection. All of them were efficient in increasing the HDR efficiency (S5 Fig), there were no differences between different concentrations ($p > 0.4$). There was also no difference in cell survival (data not shown); the concentration of 1 µM was chosen as the most frequently used by other authors.

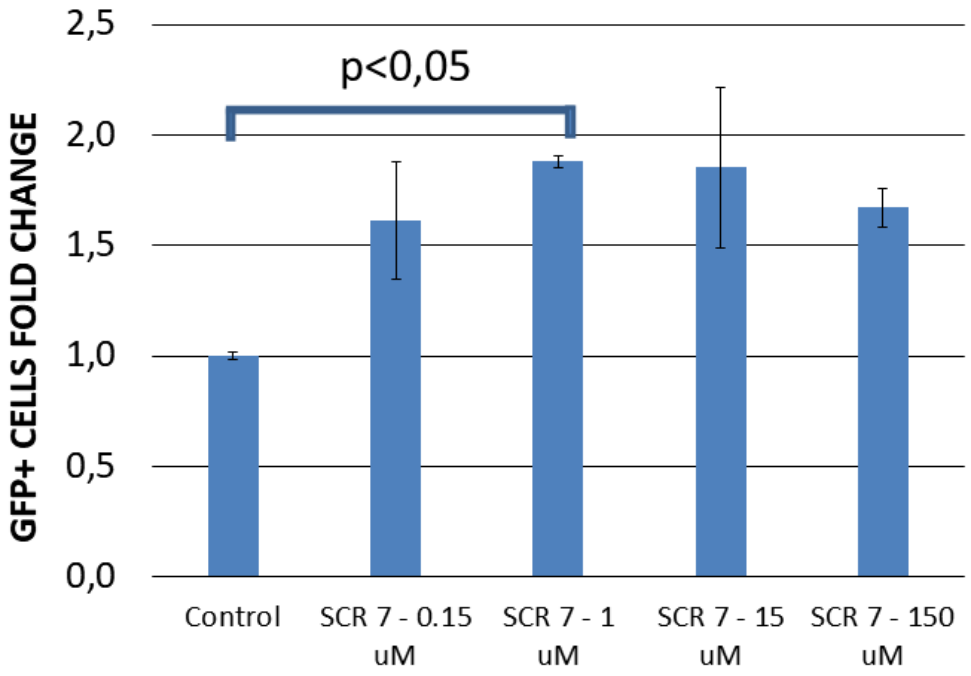


**Figure S6.** Influence of SCR7 added at different concentrations on the number of GFP+ cells after genome editing by Cas9-sgRNA/ssODN. Mean and 95% CI.

***Supplementary Methods***

*Microscopy*

Microscopy was performed using an Axio vert. A1 fluorescence microscope (Carl Zeiss, Germany) and Zen2.3 software.

SCAI expression was analyzed using a Leica TCS SP8 Confocal Laser Scanning Microscope (Leica Microsystems, Germany). Images were processed and analyzed using the open-source software CellProfiler version 3.0.0. Cell nuclei and cytoplasm were identified, after which the percentage of positive cells and fluorescence intensity in each cell were measured.

*Immunostaining*

Harvested cells were washed with the Dulbecco's Phosphate-Buffered Saline (DPBS), then transferred into 1.7 ml tubes and fixed with 4% formaldehyde, permeabilized with 0.25% Triton X100 in PBS and blocked with 1% BSA in PBST. After that, the cells were incubated for 30 min at room temperature with primary antibodies (Recombinant Anti-Mad2L2/REV7 antibody [EPR13657], Abcam, UK, 1:70; or Recombinant Anti-SCAI antibody [EPR4128] (ab124688), 1:160). Then the cells were washed with DPBS and incubated for 30 min with secondary antibodies (Alexa Fluor594 goat anti-rabbit IgG(H+L), Invitrogen, USA; 1:150). After washing with DBPS, the cells were analyzed by flow cytometry.

**Table S1.** Oligonucleotides used in the study

| **Types of oligos** | **Description** | **Sequence** |
|---|---|---|
| **Primers** | for sequencing of *eGFP* locus | F 5’-ACGTAAACGGCCACAAGTTCA-3’<br>R 5'-CTGCCGTCCTCGATGTTGT-3' |
| | for cloning of *eGFPmut* cds in lentiviral vector | F 5’-GCCTGCTAGCGAGGGCAGAGGAAGTCTTCTAA-3’<br>R 5'-CTTTGTCGACTCACTTGTACAGCTCGTCCATGC CGAGAGTGA-3’ |
| | for cloning of *TurboRFP* cds in lentiviral vector | F 5’-GTTTGGATCCGCCACCATGGTGGGTGAGGATAG CGT-3’<br>R 5'-GTTTGCTAGCGCCTCTGTGCCCCAGTTTGCTAG GGAGGT-3’ |
| | for RT-PCR of *MAD2L2* gene | F 5’-CACTGCGTCAAGCCACTCCT-3’<br>R 5’-CAGCGAGTCTGAGCTGATGG-3’ |
| | for reference house-keeping gene *TFRC* | F 5’-TCCTTGCATATTCTGGAATCCC-3’<br>R 5’-ATCACGAACTGACCAGCG-3’ |
| | for reference house-keeping gene *TBP* | F 5’-CGGAGAGTTCTGGGATTGTAC-3’<br>R 5’-GTGGTTCGTGGCTCTCTTATC-3’ |
| | for reference house-keeping gene *B2M* | F 5-’CTGCCGTGTGAACCATGTGA-3’<br>R 5’-CAATCCAAATGCGGCATCTTC-3’ |
| | For RT-PCR of *SCAI* gene | F 5’-CAGAGGTTCTGGTGATAGCAGTC-3’<br>R 5’-CTGCCACTGCTTCTGTCCAT-3’ |
| **Linker sequence** | For fusion of *Cas9* and *SCAI* | NT: 5’-TCCGGCTCCGAGACCCCCGGCACCTCCGA GTCCGCCACCCCCGAGTCC-3’<br>AA: SGSETPGTSESATPES |
| **gRNA** | guide RNA complementary a part of *eGFP* coding sequence with mutation p.337delG | 5’-TGTCGCCCTCGAACTTCACT-3’<br>3-’ACAGCGGGAGCTTGAAGTGA-5’ |
| **ssODN** | single-stranded oligodeoxynucleotide for | 5’-<br>TTCTTTAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTG AAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAA GGGCATCGACTTCAAGGAGGACGGCAACATCCTCGGG-3’ |

| | | |
|---|---|---|
| | homology directed repair | |
| **siRNAs** | siRNA_MAD2L2_1 | 5’-CCUCGACUUCUCAGCCUCCUU-3’<br>3’-UUGGAGCUGAAGAGUCGGAGG-5’ |
| | siRNA_MAD2L2_2 | 5’GUGCUCUUAUCGCCUCUGUdTdT3’<br>3’dTdTCACGAGAAUAGCGGAGACA5’ |
| | siRNA_MAD2L2_3 | 5’-GGAUGACCACGCUCACACGdTdT-3’<br>3’-dTdTCCUACUGGUGCGAGUGUGC-5 |
| | siRNA_flu | 5’FAM –AGGUCGAACUACGGGUCAAdTdC-3’<br>3’-dGdAUCCAGCUUGAUGCCCAGUU-FAM5’ |