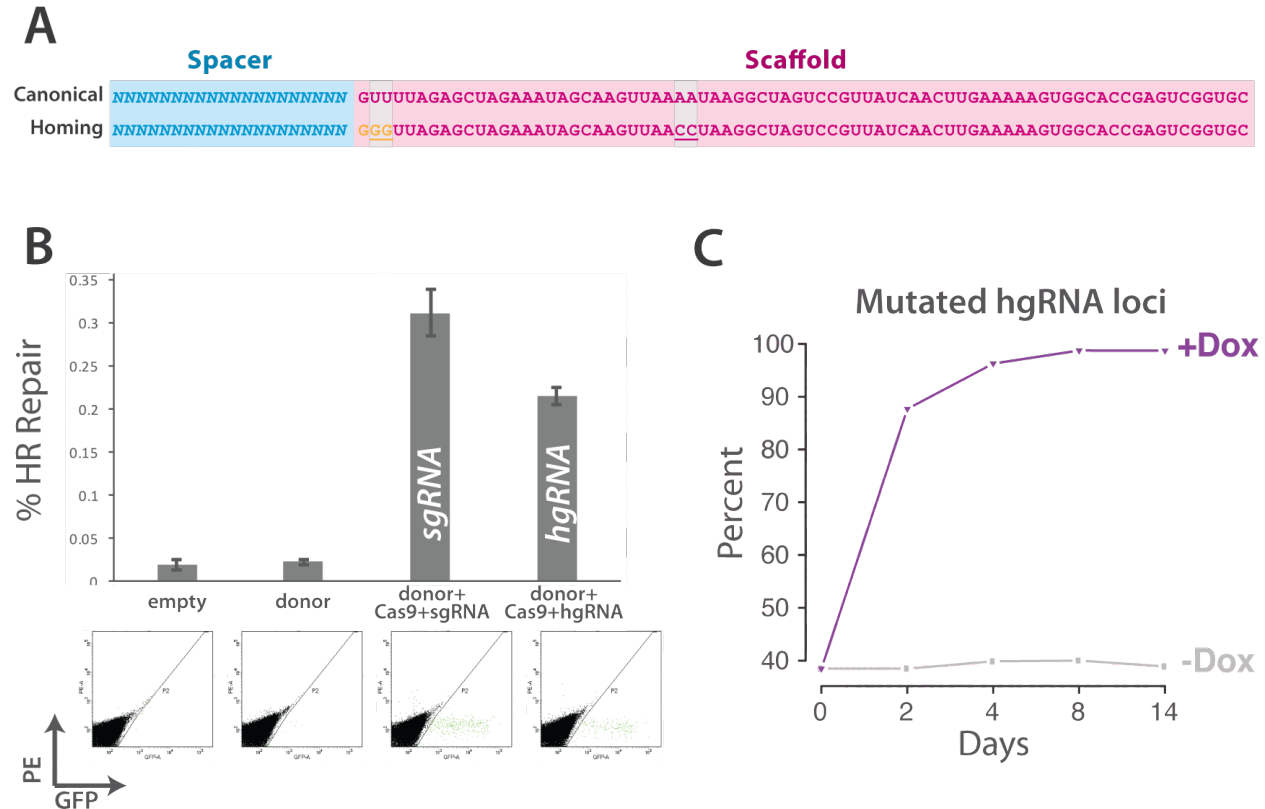


Supplementary Figures



Supplementary Figure 1. (A) Primary sequences of standard and homing gRNAs are shown and the positions that were mutated to create a hgRNA are underlined. Orange bases mark the position of PAM in the hgRNA. Grey boxes mark the positions where hgRNA is mutated compared to the wild type gRNA. **(B)** Results of a HR-based assay to evaluate to functionality of hgRNAs. A genomically integrated GFP coding sequence is disrupted by the insertion of a stop codon and a 68-bp genomic fragment from the AAVS1 locus. Restoration of the GFP sequence by HR with an appropriate donor sequence results in GFP+ cells that can be quantified by FACS. AAVS1 locus contains a site known as T1 which matches the spacer sequence of hgRNA-A21. Bar graph on top depicts HR efficiencies induced by wild type and homing T1 guide RNAs, as measured by FACS. Representative FACS plots of the targeted cells are depicted below. Data are shown as means \pm SEM (N = 4). **(C)** Sequencing results showing above-background accumulation of mutations in hgRNA locus upon Cas9 expression. Cas9 expression is induced in cells with a lentivirally integrated hgRNA-A21. DNA samples harvested before (t=0 days) and at various points after induction are characterized by high-throughput sequencing to quantify mutations in the DNA locus

that encodes hgRNA-A21. Any mismatch, deletion, or insertions compared to the original locus is considered a mutation. The high initial fraction of mutants is due to sequencing error (materials and methods) as the steady level of mutations in the non-induced sample suggests there is no significant Cas9 expression leakage in the course of our experimental times.

Supplementary Sequences

>hgRNA-A21

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC **GGTCCCCTCCACCCACAGTGGGGTTAGAGC**
TAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCT **TTTT**

U6 Promoter

Predicted TSS

Spacer

gRNA Scaffold

U6 Terminator

>sgRNA-A21

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC **GGTCCCCTCCACCCACAGTGGTTTTAGAGC**
TAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCT **TTTT**

U6 Promoter

Predicted TSS

Spacer

gRNA Scaffold

U6 Terminator

>A21-Target

AAGAGGATGGTGCAGCAACCAAG **AACA** **GAAAGTATTCGATTTCTTGGCTTTATATATCTTGTGGAAAG**
GACGAAACACCG **GTCCCCTCCACCCACAGT** **GGG** **TTAGAGCTAGAAATAGCAAGTTAAGGTAAG** **GTGAG**
AGGCACCTGTCAGATTGA

Truncated Promoter

Spacer

Truncated Scaffold with **PAM**

Primer-binding Sites

>hgRNA-B21

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC **GGTCGGAGGGAGGTGGGTTAGGGGTTAGAGC**
TAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCT **TTTT**

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-C21

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTACACCCTCAAGCAGTGTGGGGTTAGAGC
TAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTT
TTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-D21

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTCACGCTACGGCCAAGGTGGGGTTAGAGC
TAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTT
TTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-E21

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTCCAGAGATACTGACGTGGGGTTAGAGC
TAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTT
TTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-F21

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA

GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTGTGTAGCGATGGCTACAGGGGTAGAGC
TAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-G21

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTATGCGGATGCAATCTCCGGGGTTAGAGC
TAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-A'26

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTAGACGCACCTCCACCCACAGTGGGGTT
AGAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-A'51

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTAGACGCGGTCACACTGATGCAGCTAGTA
TGCACTCCACCCACAGTGGGGTTAGAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAAC
TTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-A'76

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC^GGTAGACGCTGTGACAGAGCCAACACGCAGT
CTCGGTCACACTGATGCAGCTAGTAT^{TGCACCTCCACCCACAGTG}GGGTTAGAGCTAGAAATAGCAAGTT
AACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT

U6 Promoter

Predicted TSS

Spacer

gRNA Scaffold

U6 Terminator

>hgRNA-A'101

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTA
GTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT
TTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC^GGTAGACGGCTAGATGAAGAGCAAGCGCATG
GACTGTGACAGAGCCAACACGCAGTCTCGGTCACACTGATGCAGCTAGTAT^{TGCACCTCCACCCACAGTG}
GGGTTAGAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT
CGGTGCTTTTTTTT

U6 Promoter

Predicted TSS

Spacer

gRNA Scaffold

U6 Terminator