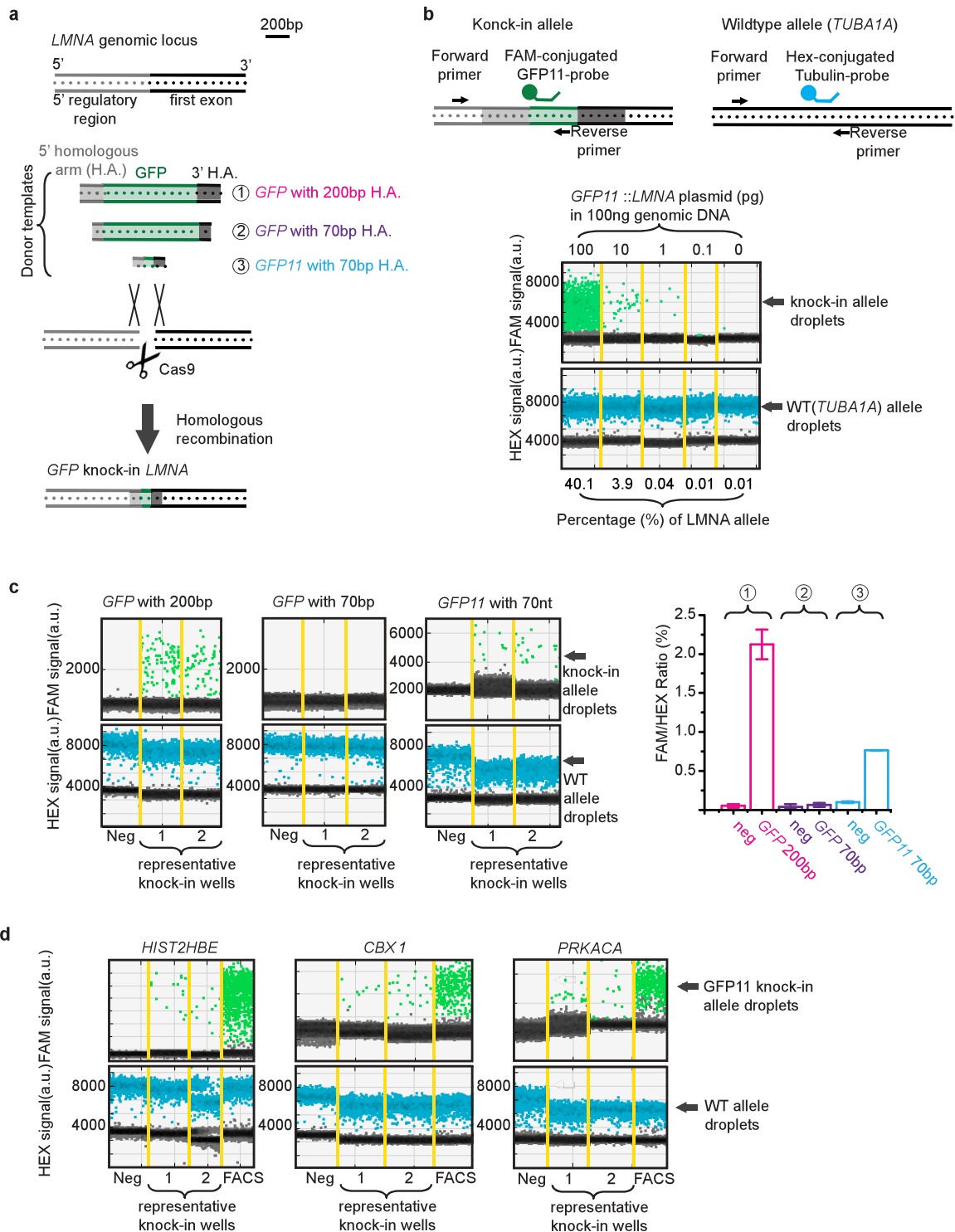


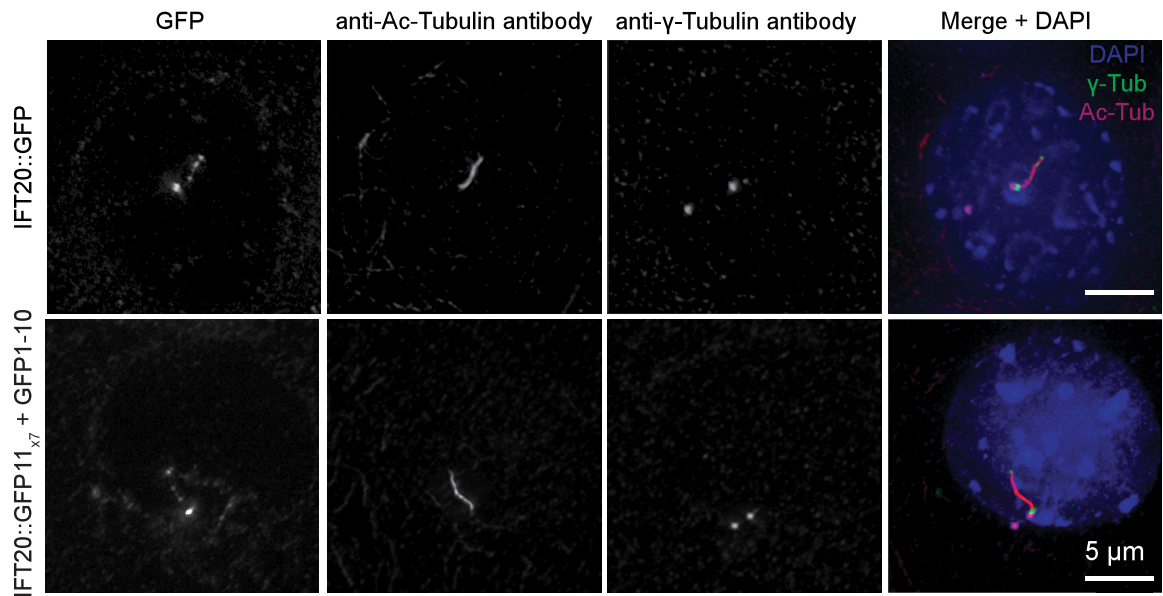
Supplementary Figure 1: Verification of GFP11 labeling, showing images of HeLa cells co-expressing GFP1-10 and GFP11:: β -actin, compared to controls with either sfGFP:: β -actin, GFP11:: β -actin alone or GFP1-10 alone. CD86::SNAP-tag + benzylguanine (BG) - Alexa Fluor 647, which labels the plasma membrane, was used as a transfection marker. Scale bars indicate 5 μ m.



Supplementary Figure 2: TaqManPCR-ddPCR combined assay for knock-in efficiency.

(a) Schematic for genetic knock-in via CRISPR/Cas9-mediated homologous recombination. Three different donor templates were used to target the same *LMNA*

genomic locus. In addition to GFP11 with 70 nt homology arms (ssDNA), we also tested full length GFP with 200 bp or 70 bp homology arms (double-strand DNA). **(b)** Schematic of TaqManPCR-ddPCR combined assay to quantify the percentage of knock-in alleles. **(c)** ddPCR with indicated amounts of *GFP11::LMNA* plasmid in 100 ng of genomic DNA. Percentages were calculated by taking concentration ratio of a knock-in-allele-specific FAM probe and a wild-type-allele-specific HEX probe. The result indicates that 70 bp homology arm is too short for efficient knock-in of full length GFP using a dsDNA donor. **(c)** ddPCR for *GFP*- or *GFP11*-knock-in alleles at a *LMNA* gene locus. As a negative control, non-targeting sgRNA was used. Quantified data are shown as a bar graph. **(d)** ddPCR for *GFP11*-knock-in alleles at indicated loci. Quantified data are shown in Figure 2a. After enriching GFP⁺ cells by fluorescence-activated cell sorting (FACS), GFP11-knock-in signal was significantly increased in each case. Dots show droplets containing indicated alleles. Yellow lines indicate the borders between samples.



Supplementary Figure 3. Images of mouse IMCD3 cells expressing either IFT20::GFP or IFT20::GFP11_{x7} + GFP1-10 (see Supplementary Movie 1).

Supplementary Table 1: Sequence of *GFP1-10* and *sfCherry1-10*

Sequence
<i>GFP1-10</i> ATGTCCAAAGGAGAAGAAGTGTGTTACCGGTGTTGTGCCAATTTTGGTTGAACTCGATGG TGATGTCAACGGACATAAGTTCTCAGTGAGAGGCGAAGGAGAAGGTGACGCCACCATTG GAAAATTGACTCTTAAATTCATCTGTAATACTGGTAAACTTCTGTACCATGGCCGACT CTCGTAACAACGCTTACGTACGGAGTTCAGTGCTTTTCGAGATACCCAGACCATATGAA AAGACATGACTTTTTTAAGTCCGGCTATGCCTGAAGGTTACGTGCAAGAAAGAACAATTT CGTTCAAAGATGATGGAAAATATAAACTAGAGCAGTTGTTAAATTTGAAGGAGATACT TTGGTTAACCGCATTGAACTGAAAGGAACAGATTTTAAAGAAGATGGTAATATTCTTGG ACACAAACTCGAATACAATTTTAATAGTCATAACGTATAACATCACTGCTGATAAGCAA AGAACGGAATTAAGCGAATTTTACAGTACGCCATAATGTAGAAGATGGCAGTGTTCAA CTTGCCGACCATTACCAACAAAACACCCCTATTGGAGACGGTCCGGTACTTCTTCCTGA TAATCACTACCTCTCAACACAAACAGTCCTGAGCAAAGATCCAAATGAAAA
<i>sfCherry1-10</i> ATGGAGGAGGACAACATGGCCATCATCAAGGAGTTCATGAGATTCAAGGTGCACATGGA GGGCAGCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCACCCCTACG AGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGG GACATCCTGAGCCCCAGTTCATGTACGGCAGCAAGGCCTACGTGAAGCACCCCGCCGA CATCCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCACCTGGGAGAGAGTGATGA ACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACAGCAGCCTGCAGGACGGCGAG TTCATCTACAAGGTGAAGCTGCTGGGCACCAACTTCCCAGCGACGGCCCCGTGATGCA GAAGAAGACCATGGGCTGGGAGGCCAGCACCGAGAGAATGTACCCCGAGGACGGCGCCC TGAAGGGCGAGATCAACCAGAGACTGAAGCTGAAGGACGGCGGCCACTACGACGCCGAG GTGAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTGGA CATCAAGCTGGACATCACCAGCCACAACGAGGAC

Supplementary Table 2: Sequences of *GFP11* and *sfCherry11* repeat arrays

Name	Sequence
<i>GFP11</i> (10aa linker)	ATGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCCGGTATCACCGGGAGTGG TGGGTCCGGCGGGAAATTCATG
<i>GFP11</i> _{x3} (15aa linker)	ATGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATTACAGGTGGCGG CGGAAGTGGAGGTGGAGGCTCGGGTGGAGGCAGTTCGCGAGACCATATGGTTCTCCACG AATACGTA AACGCAGCAGGCATCACTGGCGGTGGCGGAAGTGGCGGAGGAGGTTTCGGGC GGAGGCAGTTCGCGTGACCATATGGTCCTCCATGAATATGTAAATGCAGCTGGCATTAC TGGAGGTGGCGGTAGTGGCGGAAAATTCATG
<i>GFP11</i> _{x7} (15aa linker)	ATGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATTACAGGTGGCTC TGGAAGTTCAGGTGGAGGCTCGGGTGGCGGCAGTTCGAGAGATCATATGGTTCTCCACG AATACGTTAACGCCGCAGGCATCACTGGCAGTGGTGGATCTGGCAGCGGGAGCGGCTCT GGAGGTAGCAGTCGCGACCATATGGTACTACATGAATATGTCAATGCAGCCGGAATAAC CGGATCCGGAAGTGGCTCAAGCGGAGGAGGAAGTAGTGAAGTTCCTCGGGATCACATGG TGCTGCATGAGTATGTGAACGCGGGCGGGTATAACTGGTTCGGGAGGCTCAGGTAGCGGC AGTTCAGGAGGAAGCGGGTCCCAGACCATATGGTGCTTCACGAATACGTAAACGCAGC TGGCATTACTGGGTGAGGAGGTTTCAGGAGGGTCTGGTTCCTGGATCAGGAGGTAGCAGGG ATCACATGGTACTCCATGAGTACGTGAACGCTGCTGGAATCACAGGCGGTAGCAGTGGT GGAAGTAGCGGCAGCGGCGGCAGTAGCTCACGGGACCATATGGTCTGCACGAATATGT CAATGCTGCCGGTATCACCGGGAGTGGTGGGTCCGGCGGGAAATTCATG
<i>GFP11</i> (5aa linker)	ATGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATTACAGGTGGCGG CAAATTC
<i>GFP11</i> _{x7} (5aa linker)	ATGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATTACAGGTGGCTC TGGAGGTAGAGATCATATGGTTCTCCACGAATACGTTAACGCCGCAGGCATCACTGGCG GTAGTGGAGGACGCGACCATATGGTACTACATGAATATGTCAATGCAGCCGGAATAACC GGAGGGTCCGGAGGCCGGGATCACATGGTGCTGCATGAGTATGTGAACGCGGCGGGTAT AACTGGTGGGTCCGGCGGACGAGACCATATGGTGCTTCACGAATACGTAAACGCAGCTG GCATTACTGGCGGATCAGGTGGCAGGATCACATGGTACTCCATGAGTACGTGAACGCT GCTGGAATCACAGGCGGTAGCGGCGGTCCGGACCATATGGTCTGCACGAATATGTCAA TGCTGCCGGTATCACCGGCGCAAATTCATG
<i>sfCherry11</i>	ATGTACACCATCGTGGAGCAGTACGAGAGAGCCGAGGGCAGACACAGCACCCGGCGGC
<i>sfCherry11</i> _{x4} (5aa linker)	ATGTACACAATCGTTGAACAGTACGAGAGGGCAGAGGGTAGGCATAGCACAGGTGGAGG GGGCAGTGGCGGCTACACCATCGTCGAGCAATATGAGAGAGCTGAAGGCCGCCACTCTA CAGGTGGAGGAGGTTCTGGTGGGTATACCATCGTTGAACAGTACGAACGCGCTGAGGGC AGGCACTCCACAGGAGGTGGTGGTAGTGGGGGTATACCATCGTCGAGCAGTACGAGCG CGCCGAGGGCAGGCACAGTACCGGTGGTGGAGGATCTGGCGGG

GFP11 sequence

sfCherry11 sequence

Linker sequence

Start codon

Supplementary Table 3: Sequence of *GFP1-10::VP64::NLS*

Sequence
<i>GFP1-10</i> <i>VP64</i> <i>NLS</i>
ATGTCCAAAGGAGAAGAAGTGTACCGGTGTTGTGCCAATTTTGGTTGAACTCGATGG TGATGTCAACGGACATAAGTTCTCAGTGAGAGGCGAAGGAGAAGGTGACGCCACCATTG GAAAATTGACTCTTAAATTCATCTGTACTACTGGTAAACTTCTGTACCATGGCCGACT CTCGTAACAACGCTTACGTACGGAGTTCAGTGCTTTTCGAGATACCCAGACCATATGAA AAGACATGACTTTTTTAAGTCGGCTATGCCTGAAGGTTACGTGCAAGAAAGAACAATTT CGTTCAAAGATGATGGAAAATATAAAACTAGAGCAGTTGTTAAATTTGAAGGAGATACT TTGGTTAACCGCATTGAACTGAAAGGAACAGATTTTAAAGAAGATGGTAATATTCTTGG ACACAAACTCGAATACAATTTAATAGTCATAACGTATACATCACTGCTGATAAGCAA AGAACGGAATTAAGCGAATTTACAGTACGCCATAATGTAGAAGATGGCAGTGTTCAA CTTGCCGACCATTACCAACAAAACACCCCTATTGGAGACGGTCCGGTACTTCTTCTGA TAATCACTACCTCTCAACACAAACAGTCCTGAGCAAAGATCCAAATGAAAAAGTTTAA ACGGTAGTGATGCTTTAGACGATTTTGACTTAGATATGCTTGGTTCAGACGCGTTAGAC GACTTCGACCTAGACATGTTAGGCTCAGATGCATTGGACGACTTCGATTTAGATATGTT GGGCTCCGATGCCCTAGATGACTTTGATCTAGATATGCTAGGTAGTactagtGGTGGCG GCGGAAGTCCCAAGAAGAAGCGCAAGGTGTAA

Supplementary Table 4: sgRNA sequences

sgRNA target	DNA sequence
<i>HIST2H2BE</i>	GTCCCGGCAGGGACTCACT TGG
<i>CBX1</i>	GAAAGCTGGCGGGCACTAT GGG
<i>LMNA</i>	GCCATGGAGACCCCGTCCCAG CGG
<i>PRKACA</i>	GGCCGCCGCCGCCGCGAT GGG
<i>ERN1</i> (non-targeting sgRNA)	GACTCCAGACGCCCTCTGAGCG AGG
<i>CxCR4</i>	GCAGACGCGAGGAAGGAGGGCGC
<i>CxCR4</i> (negative control)	GAACGACTAGTTAGGCGTGTA

Guide sequences of 20-25 bases upstream of a PAM sequence (**bold**) were selected.

Supplementary Table 5: Oligo-nucleotide donor DNA sequences

sgRNA target	DNA sequence
<i>HIST2H2BE</i> (200 nt)	GCCCCGGCGAGCTGGCCAAGCACGCCGTGTCCGAGGGCACCAAGGCGGTACCAAGTACA CCAGCTCCAAGGGTGGCGGCCCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCT GGGATTACATGAGTCCCTGCCGGGACCTGGCGCTCGCTCGCTCGAGTCGCCGGCTGCTT GACTCCAAAGGCTCTTTTTTCAGAG
<i>CBX1</i> (200 nt)	CTAATGCCCTTTTTATTTTATTTTATCATTTTAGCAGCGTCACCCTTTACACCAGAAAGC TGGCGGGCACTATGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATT ACAGGTGGCGGCCGGGAAAAACAACAAGAAGAAAGTGGAGGAGGTGCTAGAAGAGGA GGAAGAGGAATATGTGGTGGAAA
<i>LMNA</i> (200 nt)	TCCTTCGACCCGAGCCCCGCGCCCTTTCCGGGACCCCTGCCCGCGGGCAGCGCTGCCA ACCTGCCGGCCATGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATT ACAGGTGGCGGCCGAGACCCCGTCCCAGCGGCGCGCCACCCGCAGCGGGGCGCAGGCCAG CTCCACTCCGCTGTGCCCCACCC
<i>PRKACA</i> (200 nt)	GGGGCCGCCGGCCGCGAGCCAGCACCCGCCGCGCCGCGAGCTCCGGGACCGGCCCGGCCG CCGCCCGCGCATGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCTGGGATT ACAGGTGGCGGCCGGCAACGCCGCCGCCCAAGAAGGGCAGCGAGCAGGAGAGCGGTGA GTGCCCGGGCTGTGACCCCGATC

GFP11x1 sequence

Linker sequence

Regulatory region sequence

Coding region sequence

Start or stop codon

Supplementary Table 6: TaqMan probes and primers for ddPCR

	Forward primer	GFP11-specific FAM-probe	GFP11-specific Reverse primer
<i>HIST2H2BE</i> <i>::GFP11</i>	CAAGCGCTCCACCAT CACAT	TGAAGGACCATGTGGTCACG GC	CCCAGCAGCATTACATA CTCA
<i>GFP11::CBX1</i>	TCCCTGGTTTTCTTA CCATCCA	ATGCGTGACCACATGGTCCT TCAT	CCTGTAATCCCAGCAGCA TTTA
<i>GFP11::LMNA</i>	GGACCTATTAGAGCC TTTGCC	ATGCGTGACCACATGGTCCT TCAT	CCTGTAATCCCAGCAGCA TTTA
<i>GFP11:: PRKACA</i>	CAGCGGCAGAGATCT TGGG	ATGCGTGACCACATGGTCCT TCAT	CCTGTAATCCCAGCAGCA TTTA

	Forward primer	EGFP-specific FAM-probe	EGFP-specific Reverse primer
<i>EGFP::LMNA</i>	GGACCTATTAGAGCC TTTGCC	ATGGTGAGCAAGGGCGAGGA	TCCAGCTCGACCAGGATG

	Forward primer	Wild-type allele specific HEX-probe	Reverse primer
Wild-type allele (<i>TUBA1A</i>)	GCGTGAGTGCATCTC CAT	CTCTACTGCCTGGAACACGG CATC	ACTTGGCATCTGGCCATC