SUPPLEMENTAL MATERIAL

Efficient and flexible tagging of endogenous genes by homology-independent intron targeting

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Detailed protocol for generating and isolating fluorescent cell clones by homology-independent intron targeting

Materials and Reagents

- HEK293 cells (ATCC CRL-1573)
  - Can also work with (but not limited to) HeLa cells, H9 hESCs, or HAP1 cells.
- Cell culture reagents (Thermo Fischer Scientific): DMEM, trypsin, Opti-MEM
- Transfection-grade PEI (Polysciences, cat. #24765)
- Plasmid encoding generic fluorescent tag donor
  - Any vector can work as long as it contains the sequences listed in Supplemental Tables S1 and S2.
  - Consider whether the tag will be spliced in-frame with the coding sequence; if not, use a donor with the appropriate frameshift mutations on either end.
- Plasmid encoding validated target sgRNA
  - The appropriate spacer sequences can be cloned into the lentiGuide-Puro plasmid (Addgene #52963). sgRNA sequences can be chosen using the sgRNA designer provided by the Broad institute (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design).
- Plasmid encoding sgRNA against donor
  - See Supplemental Table S3 for the utilized spacer sequence.
- Plasmid encoding Cas9
  - Can be used separately, such as on the lentiCas9-Blast plasmid (Addgene #53962), or included on the sgRNA plasmids (e.g. Addgene #52961).

Equipment

- Cell sorter (BD FACSAria Fusion or any other similar cell sorter)
- Flow cytometry tubes (12 × 75 mm)
- Cell Straining Kit (70 µm)
- Cell culture plates (12-well plate, 10 cm dish, 96-well plate)
- Cell culture incubator (37°C, 5% CO_2)

Procedure

Day 1: Plate cells

1) Plate HEK293 cells into at least 2 wells of a 12-well plate, such that they will be 60-80% confluent on the following day (160-200 × 10^3 cells/well).

Day 2: Transfect cells

2) To 100 µl Opti-MEM, add plasmids encoding the generic donor, the donor sgRNA, the target sgRNA, and Cas9 in a 5:1:1:1 molar ratio, respectively. A total amount of 1360 ng of plasmid DNA is mixed with 4.08 µl PEI (1 µg DNA : 3 µl PEI ratio).
3) An additional reaction with PEI reagent only is used as the “mock” transfection.
4) Vortex and incubate transfection mixtures for at least 15 minutes.
5) Add each mixture dropwise to a single well of a 12-well plate.

Day 3: Wash and replate cells

6) Trypsinize cells in 100 µl trypsin, resuspend in 500 µl DMEM, and transfer entire cell suspensions to a 10 cm dish containing 10 ml DMEM.

Day 7-8: Analyze and sort cells

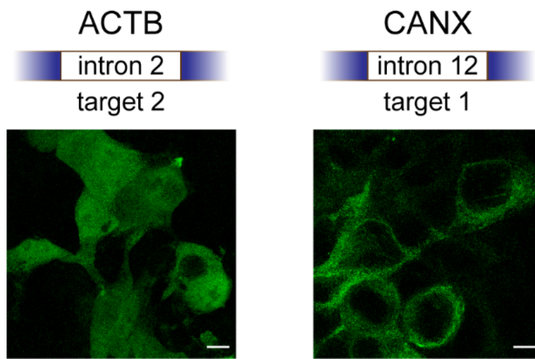
7) Prepare a 96-well plate filled with 100 µl DMEM per well. Store in cell culture incubator.

8) Trypsinize cells in 1 ml trypsin and resuspend in 2.5 ml DMEM.
9) Pass cell suspension through a cell strainer into a flow cytometry tube (this step is important to prevent clogs in the flow cytometer). Place tube immediately on ice.
10) Initialize cell sorter according to the manufacturer’s protocol. Set up gates to differentiate between the “mock”- and truly-transfected cells.
11) Sort single fluorescent cells directly into a 96-well plate. Place immediately in cell culture incubator to grow and expand.

> pMC-mNG2$_{11}$
vector –
CCACACGAAATCGATTCGCTCTTCTAATCTCCTCTCTTCTCCTCTCTCCAG[_]GGTGGCTCT
GGAAGTTCAGGTGGAGGCTCGGGTGGCGGCAGTTCGACCGAGCTCAACTTCAAGGAGTG
GCAAAAGGCCTTTACCGATATGATGGGATCCGGAAGTGGCTCAAGCGGAGGAGGAAGTAG
TGGAAGTTCT[_]GTAAGTATTGGTTAAGAGCGAATCGATTTCGTGAGG
– *vector*

> pMC-mClover3
vector –
CCACACGAAATCGATTCGCTCTTCTAATCTCCTCTCTTCTCCTCTCTCCAG[_]GGTGGCTCT
GGAAGTTCAGGTGGAGGCTCGGGTGGCGGCAGTTCGGTGAGCAAGGGCGAGGAGCTGTT
CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCA
GCGTCCGCGGCGAGGGCGAGGGCGATGCCACCAACGGCAAGCTGACCCTGAAGTTCATC
TGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGG
CGTGGCCTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGC
CATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCTTTCAAGGACGACGGTACCTACAA
GACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGG
GCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACA
GCCACTACGTCTATATCACGGCCGACAAGCAGAAGAACTGCATCAAGGCTAACTTCAAGAT
CCGCCACAACGTTGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCC
CCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCCATCAGTCCAAG
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCC
GCCGGGATTACACATGGCATGGACGAGCTGTACAAGGGATCCGGAAGTGGCTCAAGCGG
AGGAGGAAGTAGTGGAAGTTCT[_]GTAAGTATTGGTTAAGAGCGAATCGATTTCGTGAGG
– *vector*

Supplemental Table S1. DNA sequences of the mNG2$_{11}$ and mClover3 donor regions in the pMC-mNG2$_{11}$ and pMC-mClover3 plasmids. The donor plasmid protospacer sequence is highlighted red. Linker regions are highlighted blue. The sequence of mNG2$_{11}$ or mClover3 is highlighted green. Splice sites are in black and directly adjacent to the sequences incorporated into the coding sequence. Blue brackets ("[_]") indicate locations were additional nucleotides should be added to change the frame of the tag.



Supplemental Figure S1. Additional intronic tagging locations for *ACTB* and *CANX*. Microscopy images of HEK293 cells stably expressing $mNG2_{1-10}$ tagged with $mNG2_{11}$ targeting the indicated genomic sites. Images are maximum projections of z-stacks and scale bars correspond to 10 µm.

> pMC-mNG2$_{11}$-BSD(-)
vector –
CCACACGAAATCGATTCGCTCTTCTAATCTCCTCTCTTCTCCTCTCTCCAG[_]GGTGGCTCT
GGAAGTTCAGGTGGAGGCTCGGGTGGCGGCAGTTCGACCGAGCTCAACTTCAAGGAGTG
GCAAAAGGCCTTTACCGATATGATGGGATCCGGAAGTGGCTCAAGCGGAGGAGGAAGTAG
TGGAAGTTCT[_]GTAAGTATTGGTGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGC
TATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCA
TTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTAC
AAATGTGGTATGGCTGATTATGATCCTCTGGAGATTTAGCCCTCCCACACATAACCAGAGG
GCAGCAATTCACGAATCCCAACTGCCGTCGGCTGTCCATCACTGTCCTTCACTATGGCTTT
GATCCCAGGATGCAGATCGAGAAGCACCTGTCGGCACCGTCCGCAGGGGCTCAAGATGC
CCCTGTTCTCATTTCCGATCGCGACGATACAAGTCAGGTTGCCAGCTGCCGCAGCAGCAG
CAGTGCCCAGCACCACGAGTTCTGCACAAGGTCCCCCAGTAAAATGATATACATTGACACC
AGTGAAGATGCGGCCGTCGCTAGAGAGAGCTGCGCTGGCGACGCTGTAGTCTTCAGAGAT
GGGGATGCTGTTGATTGTAGCCGTTGCTCTTTCAATGAGGGTGGATTCTTCTTGAGACAAA
GGCTTGGCCATGGTGGCGGATCCCGCGTCACGACACCTGTGTTCTGGCGGCAAACCCGT
TGCGAAAAAGAACGTTCACGGCGACTACTGCACTTATATACGGTTCTCCCCCACCCTCGGG
AAAAAGGCGGAGCCAGTACACGACATCACTTTCCCAGTTTACCCCGCGCCACCTTCTCTAG
GCACCGGTTCAATTGCCGACCCCTCCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGC
TCTGCCCACTGACGGGCACCGGAGCCTAAGAGCGAATCGATTTCGTGAGG
– *vector*

> pMC-mNG2$_{11}$-BSD(+)
vector –
CCACACGAAATCGATTCGCTCTTCTAATCTCCTCTCTTCTCCTCTCTCCAG[_]GGTGGCTCT
GGAAGTTCAGGTGGAGGCTCGGGTGGCGGCAGTTCGACCGAGCTCAACTTCAAGGAGTG
GCAAAAGGCCTTTACCGATATGATGGGATCCGGAAGTGGCTCAAGCGGAGGAGGAAGTAG
TGGAAGTTCT[_]GTAAGTATTGGTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCG
CCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAG
GTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGG
TGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTT
GCCGCCAGAACACAGGTGTCGTGACGCGGATCCGCCACCATGGCCAAGCCTTTGTCTCA
AGAAGAATCCACCCTCATTGAAAGAGCAACGGCTACAATCAACAGCATCCCCATCTCTGAA
GACTACAGCGTCGCCAGCGCAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGTGTCAAT
GTATATCATTTTACTGGGGGACCTTGTGCAGAACTCGTGGTGCTGGGCACTGCTGCTGCT
GCGGCAGCTGGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGAACAGGGGCATCTTG
AGCCCCTGCGGACGGTGCCGACAGGTGCTTCTCGATCTGCATCCTGGGATCAAAGCCATA
GTGAAGGACAGTGATGGACAGCCGACGGCAGTTGGGATTCGTGAATTGCTGCCCTCTGGT
TATGTGTGGGAGGGCTAAATCTCCAGAGGATCATAATCAGCCATACCACATTTGTAGAGGT
TTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAA
TTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAA
ATTTCACAAATAAAGCATTTTTTTCACTGCTAAGAGCGAATCGATTTCGTGAGG
– *vector*

> pMC-mClover3-BSD(-)
vector –
CCACACGAAATCGATTCGCTCTTCTAATCTCCTCTCTTCTCCTCTCTCCAG[_]GGTGGCTCT
GGAAGTTCAGGTGGAGGCTCGGGTGGCGGCAGTTCGGTGAGCAAGGGCGAGGAGCTGTT
CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCA

GCGTCCGCGGCGAGGGCGAGGGCGATGCCACCAACGGCAAGCTGACCCTGAAGTTCATC
TGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGG
CGTGGCCTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGC
CATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCTTTCAAGGACGACGGTACCTACAA
GACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGG
GCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACA
GCCACTACGTCTATATCACGGCCGACAAGCAGAAGAACTGCATCAAGGCTAACTTCAAGAT
CCGCCACAACGTTGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCC
CCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCCATCAGTCCAAG
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCC
GCCGGGATTACACATGGCATGGACGAGCTGTACAAGGGATCCGGAAGTGGCTCAAGCGG
AGGAGGAAGTAGTGGAAGTTCT[_]GTAAGTATTGGTGCAGTGAAAAAAATGCTTTATTTGTG
AAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACA
ACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAA
GTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCCTCTGGAGATTTAGCCCTCCCACA
CATAACCAGAGGGCAGCAATTCACGAATCCCAACTGCCGTCGGCTGTCCATCACTGTCCTT
CACTATGGCTTTGATCCCAGGATGCAGATCGAGAAGCACCTGTCGGCACCGTCCGCAGGG
GCTCAAGATGCCCCTGTTCTCATTTCCGATCGCGACGATACAAGTCAGGTTGCCAGCTGCC
GCAGCAGCAGCAGTGCCCAGCACCACGAGTTCTGCACAAGGTCCCCCAGTAAAATGATAT
ACATTGACACCAGTGAAGATGCGGCCGTCGCTAGAGAGAGCTGCGCTGGCGACGCTGTA
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TCTTGAGACAAAGGCTTGGCCATGGTGGCGGATCCCGCGTCACGACACCTGTGTTCTGGC
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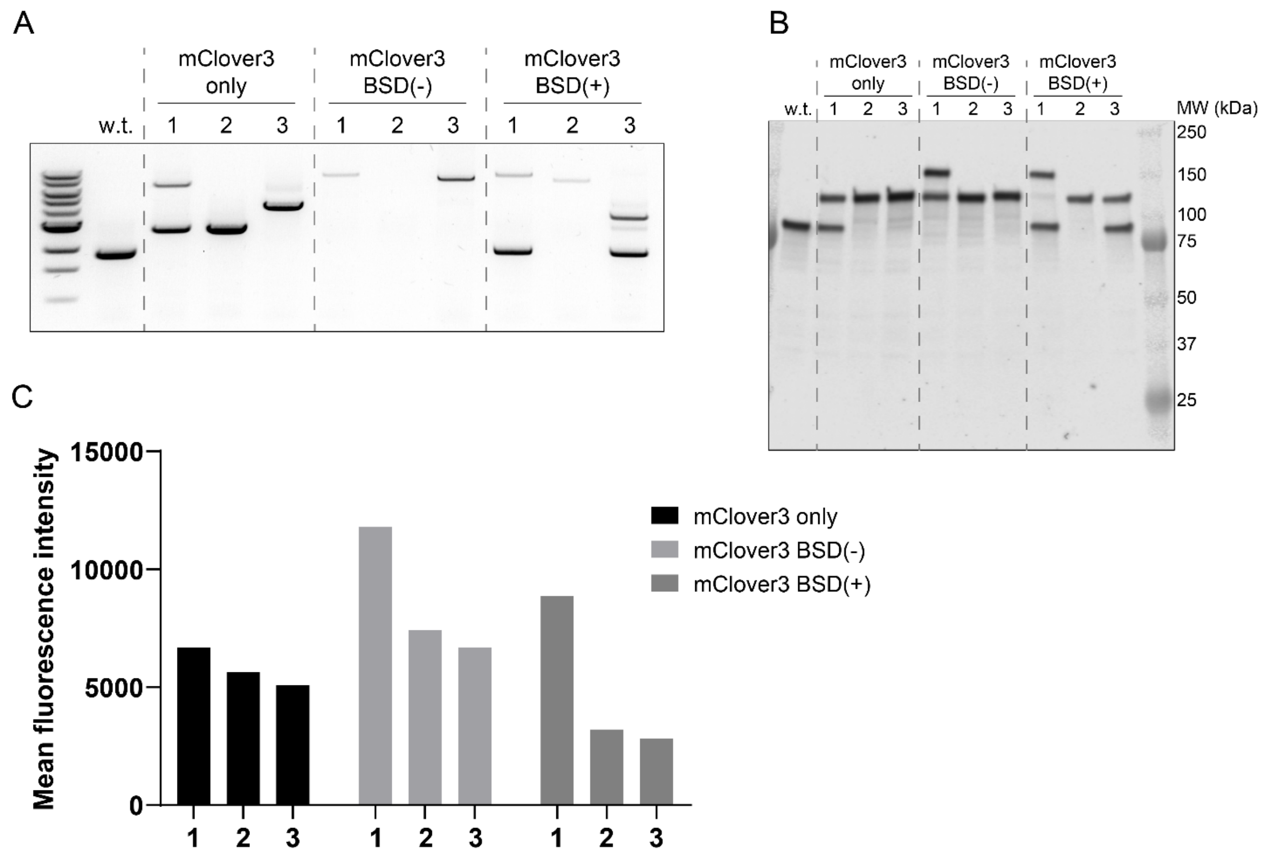
CCACCCTCGGGAAAAAGGCGGAGCCAGTACACGACATCACTTTCCCAGTTTACCCCGCGC
CACCTTCTCTAGGCACCGGTTCAATTGCCGACCCCTCCCCCCAACTTCTCGGGGACTGTG
GGCGATGTGCGCTCTGCCCACTGACGGGCACCGGAGCCTAAGAGCGAATCGATTTCGTG
AGG
– *vector*

> pMC-mClover3-BSD(+)
vector –
CCACACGAAATCGATTCGCTCTTCTAATCTCCTCTCTTCTCCTCTCTCCAG[_]GGTGGCTCT
GGAAGTTCAGGTGGAGGCTCGGGTGGCGGCAGTTCGGTGAGCAAGGGCGAGGAGCTGTT
CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCA
GCGTCCGCGGCGAGGGCGAGGGCGATGCCACCAACGGCAAGCTGACCCTGAAGTTCATC
TGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGG
CGTGGCCTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGC
CATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCTTTCAAGGACGACGGTACCTACAA
GACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGG
GCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACA
GCCACTACGTCTATATCACGGCCGACAAGCAGAAGAACTGCATCAAGGCTAACTTCAAGAT
CCGCCACAACGTTGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCC
CCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCCATCAGTCCAAG
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCC
GCCGGGATTACACATGGCATGGACGAGCTGTACAAGGGATCCGGAAGTGGCTCAAGCGG
AGGAGGAAGTAGTGGAAGTTCT[_]GTAAGTATTGGTGGCTCCGGTGCCCGTCAGTGGGCA
GAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGG
TGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCT
TTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTT
CGCAACGGGTTTGCCGCCAGAACACAGGTGTCGTGACGCGGGATCCGCCACCATGGCCA

AGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGCTACAATCAACAGCAT
CCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAGCGACGGCCGCATCTT
CACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGTGCAGAACTCGTGGTGCTGGGC
ACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGAAC
AGGGGCATCTTGAGCCCCTGCGGACGGTGCCGACAGGTGCTTCTCGATCTGCATCCTGG
GATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGCAGTTGGGATTCGTGAATT
GCTGCCCTCTGGTTATGTGTGGGAGGGCTAAATCTCCAGAGGATCATAATCAGCCATACCA
CATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACAT
AAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGC
AATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCTAAGAGCGAATCGATTTCGT
GAGG
– *vector*

Supplemental Table S2. DNA sequences of the $mNG2_{11}$- and mClover3- BSD(-) and BSD(+) donor regions. The donor plasmid protospacer sequence is highlighted red. Linker regions are highlighted blue. The sequences of $mNG2_{11}$ and mClover3 are highlighted green, and the blasticidin resistance gene (BSD), EF1a promoter, and SV40 poly(A) sequence are highlighted orange. Splice sites are in black and directly adjacent to the sequences incorporated into the coding sequence. Blue brackets (“[ ]”) indicate locations were additional nucleotides should be added to change the frame of the tag.



Supplemental Figure S2. Clonal HAP1 cells tagged with mClover3-only or mClover3-BSD(-/+) at *CANX* intron 14, sgRNA target 1. (**A**) PCR analysis of the targeted *CANX* locus. Wild-type locus is 1921 bp; tagging with mClover3-only or -BSD(-/+) adds multiples of 881 or 1734 bp, respectively (ladder is NEB 1kb N3232). (**B**) Uncropped Western blot of CANX from Figure 3E. Molecular weight (MW) of ladder is indicated. (**C**) Mean fluorescence intensities of mClover3-tagged HAP1 clones measured by flow cytometry.

Gene	Intron	Target	Spacer sequence
VIM	8	n/a	CACTAGACTACCTCAATATG
CBX1	3	1	TTGGAGTGATTATTCATCAA
CBX1	3	2	TTAGTCCTGAAATCTTAGGT
ACTB	2	1	CCCCCACCCCGGAAACCGGG
ACTB	2	2	CAAGGGCGCTTTCTCTGCAC
ACTB	2	3	AGCCTCCCGGTTTCCGGGGT
ACTB	3	1	GTGGGTGTAGGTACTAACAC
ACTB	3	2	TAGAACCTGCAGAGTTCCAA
ACTB	3	3	CCTACTTAATACACACTCCA
ACTB	5	1	GACAGCTCCCCACACACCAC
ACTB	5	2	CTGAGCTGACCTGGGCAGGT
ACTB	5	3	CTGCCCAGGTCAGCTCAGGC
CANX	10	1	TTGCAACTATAAAAAGACTG
CANX	10	2	AGATTGTCCAGACTCAGCTG
CANX	10	3	TTTATAATCTCTACAAAGAG
CANX	12	1	GGCACAATAAACGGCCACTG
CANX	12	2	AAAGCTGATTATTGCCCAAG
CANX	12	3	TAACTAAGATATGTTGCCTG
CANX	14	1	ATGAACCCATCTATGGACAA
CANX	14	2	GAGACCAGATTTAGACACAG
CANX	14	3	ATACTAAAAGTGCTAGAGGT
donor plasmid	n/a	n/a	AAGAGCGAATCGATTTCGTG

Supplemental Table S3. List of sgRNAs used.

Gene	Intron	Target	Orientation	Complementary sequence
ACTB	2	1	upstream	CACCAGGTAGGGGAGCTG
ACTB	2	1	downstream	AGGGTGAGGATGCCTCTCTT
ACTB	2	2	upstream	CACCAGGTAGGGGAGCTG
ACTB	2	2	downstream	AGGGTGAGGATGCCTCTCTT
ACTB	2	3	upstream	CACCAGGTAGGGGAGCTG
ACTB	2	3	downstream	AGGGTGAGGATGCCTCTCTT
ACTB	3	1	upstream	TTGCTTTTTCCCAGATGAGC
ACTB	3	1	downstream	GAACACGGCTAAGTGTGCTG
ACTB	3	2	upstream	GCCCTTTCTCACTGGTTCTCT
ACTB	3	2	downstream	GCTTTACACCAGCCTCATGG
ACTB	3	3	upstream	TTGCTTTTTCCCAGATGAGC
ACTB	3	3	downstream	GAACACGGCTAAGTGTGCTG
ACTB	5	1	upstream	CCCAGCACAATGAAGATCAA
ACTB	5	1	downstream	ACATCTGCTGGAAGGTGGAC
ACTB	5	2	upstream	GACATCCGCAAAGACCTGTA
ACTB	5	2	downstream	GTGAGGACCCTGGATGTGAC
ACTB	5	3	upstream	GACATCCGCAAAGACCTGTA
ACTB	5	3	downstream	GTGAGGACCCTGGATGTGAC
CANX	10	1	upstream	TACCCTGCTCTTGGGTGCTA
CANX	10	1	downstream	AGGCCTAAAGCCTCACAACC
CANX	10	2	upstream	TACCCTGCTCTTGGGTGCTA
CANX	10	2	downstream	AGGCCTAAAGCCTCACAACC
CANX	10	3	upstream	TTAGGCCTCATGCAAAAATG
CANX	10	3	downstream	GCCAAGATCCTGCTGAAATG
CANX	12	1	upstream	ACCAAGCCATGTTTGGTGTT
CANX	12	1	downstream	CAGCAGGCAAAGCTGATTATT
CANX	12	2	upstream	CCAGATGGGAGCAGGATTTA
CANX	12	2	downstream	GAAGGTGAAGGCAGAATGGA
CANX	12	3	upstream	TAGCCCTTCCTGTGTTCCTG
CANX	12	3	downstream	ACAATAAACGGCCACTGAGG
CANX	14	1	upstream	TTGCCTCTCCTCACTGTGC
CANX	14	1	downstream	ACTGCTCATTGCCTGTTTCC
CANX	14	2	upstream	AGGGTGACAGGAGAGGAACA
CANX	14	2	downstream	GGAAGGCAGAGTTGTAGCTGA
CANX	14	3	upstream	GGAAACAGGCAATGAGCAGT
CANX	14	3	downstream	CACTTACATCCCCATGGAAAA
mNG2$_{11}$	n/a	n/a	forward	CTCCTCTCTTCTCCTCTCTCCA
mNG2$_{11}$	n/a	n/a	reverse	AACCAATACTTACAGAACTTCCA
CANX	14	1	upstream (1921 bp)	TGGCACTGTCAGTCAAGAGG
CANX	14	1	downstream (1921 bp)	CGTGGCTTTCTGTTTCTTGG

Supplemental Table S4. List of DNA primers used. “Orientation” refers to position of the primer binding site relative to the sgRNA target site. “Upstream” primers were mixed with mNG2$_{11}$ “reverse” for PCR amplification, and “downstream” primers were mixed with mNG2$_{11}$ “forward”.