Supplementary Materials for

Circularization of genes and chromosome by CRISPR in human cells

CRISPR-C: Circularization of genes and chromosome by CRISPR in human cells

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Fig. S1. TRE-ECC biosensor vector map and sequence.

Fig. S2. CAG-ECC biosensor vector map and sequence.

Fig. S3. Model of CAG-ECC biosensor induction by CRISPR-pairs. Mediated by DNA repair, the two simultaneous double-stranded breaks, introduced by transient cell-transfection of CRISPR-pairs Cr1 and Cr2, will lead to formation of [*EGFP*^{circle}] and Δ*EGFP* or to the inversion of *EGFP*.

Fig. S4. Testing the CAG-ECC biosensor vector in HEK293T cells. (A) Graphical map of vector with sites of CRISPR-pairs (Cr1+Cr2). (B) Outline of plasmid assay experiment. (C) Light (LM) and fluorescence microscopy, green (EGFP), red (mCherry). Images (40x) in triplicates of HEK293T cells transfected with CAG-ECC biosensor vector together with Cr1+Cr2. (D) Images of serial sets of control transfection experiments. Tet, tetracycline. Transfection of EGFP plasmid with Cr1+Cr2 served as a positive control for green cells and negative for red cells.

Fig. S5. CAG-ECC biosensor microscopy assay at higher magnification in HEK293T cells. (A) Graphical map of the vector showing CRISPR-pairs (Cr1+Cr2). (B) Outline of plasmid assay experiment. (C) Fluorescence microscopy (100x) in different channels; blue (DAPI), green (EGFP), red (mCherry), merged images. Cr1+Cr2, gRNAs; pUC19, negative control transfection, EGFP plasmid, positive EGFP control; white bar, 50 µm.

Fig. S6. Testing the TRE-ECC biosensor vector in HEK293T cells. (A) Graphical map of vector with sites of CRISPR-pairs (Cr1+Cr2). (B) Outline of plasmid assay experiment. (C) Light (LM) and fluorescence microscopy, green (EGFP), red (mCherry). Images (40x) in triplicates of HEK293T cells transfected with TRE-ECC biosensor vector together with Cr1+Cr2 -/+ tetracycline (Tet). (D) Images of control HEK293T cell transfections.

Fig. S7. TRE-ECC biosensor microscopy assay at higher magnification in HEK293T cells. (A) showing CRISPR-pairs (Cr1+Cr2). (B) Outline of experiment. (C) Fluorescence microscopy (100x) in different channels; blue (DAPI), green (EGFP), red (mCherry), merged images. Cr1+Cr2, gRNAs; pUC19, negative control transfection; Tet, tetracycline; white bar, 50 µm.

Fig. S8. Assessment of cell clones with integrated CAG-ECC biosensor. (A, upper part) Outline of stable genomic integration of pCAG-ECC in HEK293T cells. (B) Copy-number DNA evaluation by Southern blot with EGFP-probe after KpnI digestion. (A, lower part) Outline of experiment for assessment of integrated TRE-ECC by CRISPR gRNAs (Cr1+Cr2). (C) FACS gating images of the nine isolated clones and treatment with Cr1+Cr2. (D) Histograms of fluorescence cell percentages of the nine CAG-ECC clones after CRISPR-C and FACS analysis.

Fig. S9. Integrated TRE-ECC assay of fifteen cell clones. (A, upper part) Outline of stable genomic integration of TRE-ECC in HEK293T cells. (A, lower part) Outline of CRISPR gRNAs (Cr1+Cr2) assay for assessment of integrated TRE-ECC clones. (B-C) FACS gating images of isolated clones after treatment with Cr1+Cr2 in (B) absence or (C) presence of tetracycline (Tet).

Fig. S10. Correlation between percentage of GFP+ or mCherry+ median intensity with copy numbers of ECC biosensor. Correlation was carried for the co-colored cells (GFP+mCherry+), Pearson rank test.

Fig. S11. Validation of CAG-ECC genotypes after dual-CRISPR. (A) PCR and Sanger sequencing test of genotypes formed after dual-CRISPR gRNA (Cr1+Cr2) in CAG-ECC clone 2 and clone 4, confirming

EGFP^{inversion} and [*EGFP*^{circle}] formation. C, negative CRISPR control; NC, non-template control. (B) Southern blot, probed with EGFP on HEK293T purified and digested DNA from untreated (C, *KpnI*) and Cr1+Cr2 treated cells (clone 2). X=*XbaI*, H=*HindIII*.

Fig. S12. Anticipated outcomes after CRISPR-C gene editing. *, junctions sites after dual-CRISPR restriction with specific gRNAs (scissors) and subsequent repair of DNA breakage sites, mediated by non-homologous end-joining or microhomology mediated end-joining.

Fig. S13. EccDNA detection by three different purification methods in HEK293T cells. (A) Map of chromosome 1, 2-3 kb downstream of the *CRP* locus. gRNAs: Cr3, Cr4; diagnostic oligos: p5 to p8. (B) Experimental outline of DNA purification and enrichment of eccDNA by exonuclease (Exo) removal of linear DNA (+, 15U; ++ 90U). (C) Gel-images of corresponding PCR products across the junctions of [*dsCRP*$^{circle\ chr1:159,708-159,71Mb}$], along with control test for linear DNA (p31+p32) and plasmid DNA (pUC19), using templates before (-Exo) and after exonuclease treatment (+Exo/++Exo) at three different cell concentrations (10^5, 10^4, 10^2 cells). Blue rectangles 1 and 2 represent [*dsCRP*$^{circle\ 1q23.2:0.57\ kb}$] PCR product purified from genomic DNA preparation (G.DNA-prep) and cell lysate treated with exonuclease treatment (++Exo), respectively. (D) Chromatograms from Sanger sequencing of PCR products from the [*dsCRP*$^{circle\ 1q23.2:0.57\ kb}$], blue rectangles 1 and 2. C, negative CRISPR control (HEK293T cell lysate); NC, non-template control.

Fig. S14. Detection of eccDNA by three different purification methods in HMF cells. (A) Map of chromosome 1, 2-3 kb downstream of the *CRP* locus. gRNAs; Cr3, Cr4; diagnostic oligos; p5 to p8. (B) Experimental outline of DNA purification and enrichment of eccDNA by exonuclease (Exo) removal of linear DNA (+, 15U; ++ 90U). (C) Gel-images of corresponding PCR products across the junctions of [*dsCRP*$^{circle\ 1q23.2:0.57\ kb}$], along with control test for linear DNA (p31+p32) and plasmid DNA (pUC19), using templates before (-Exo) and after exonuclease treatment (+Exo/++Exo) at three different cell concentrations (from 10^5 down to 10^2 cells). (D) Chromatograms from Sanger sequencing of PCR products, blue boxes, from purification of [*dsCRP*$^{circle\ 1q23.2:0.57\ kb}$] from genomic DNA preparation (G.DNA-prep) and cell lysate. C, negative CRISPR control (HEK293T cell lysate); NC, non-template control.

Fig. S15. Validation of deletion after CRISPR-pairs at the *UPA* locus. (A) Map of chromosome 10 map at the *PLAU (UPA)* locus. gRNA, Cr5 and Cr6, diagnostic oligos; p9 to p12. (B) Experimental outline. (C) Gel-image of corresponding PCR products across the junctions of the Δ*UPA* and corresponding chromatogram from Sanger sequencing, red box.

Fig. S16. Deletion and inversion validation at the *TRIM28* locus after CRISPR-pairs. (A) Chromosome 19 map at the *TRIM28* locus. gRNA, Cr7 and Cr8, diagnostic oligos; p13 to p16. (B) Experimental outline. (C) PCR confirmation of the Δ*TRIM28* after Cr7+Cr8. (D) PCR and sequencing confirmation of the *TRIM28*^{inversion}. C, negative CRISPR control of HEK293T cell lysate.

Fig. S17. Indel distribution across the junction of [*TRIM28*^{circle}] in HEK293T.

Fig. S18. CAG-ECC time-course of [*EGFP*^{circle}] expression and retention in cell culture. (A) Outline of time-course experiment. (B) Left, percent fluorescence cells from 1 to 6 cell passages (1:3 split-ratio. (C) Corresponding FACS gating images from B. (D) Outward PCR analysis of [*EGFP*^{circles}] at passage 1 to 6. Cr1+Cr2 (1+2), CRISPR gRNAs; C, control – gRNA; ctrl, DNA template control. (E) Quantification of [EGFP^{circles}] by normalizing to *GAPDH* (solid line) using Image J. Theoretical 1/3 dilution curve was presented as dash line.

Fig. S19. Genotyping of Δ*EGFP*, [*EGFP*^{circle}], *EGFP*^{INV} of FACS-sorted ECC reporter cells after CRISPR-C. (A) Presentation of gating examples of FACS-sorted ECC reporter cells after CRISPR-C; P1-

P4 represent the different population of cells. (B) PCR genotyping. *GAPDH* was used as internal control. (C) Quantification of PCR genotyping results from (B) by Image J.

Fig. S20. Dot plots of fluorescent intensity (GFP), GFP+ cell counts in control and CRISPR-C treated ECC reporter cells over 6 passages. Control (CTRL) cells were transfected with a pUC19 control plasmid, and CRISPR-C treated cells were treated with Cr1+Cr2.

Fig. S21. Metaphase spreads and FISH of telomere and whole chromosome 18 painting. Karyotyping of chromosome 18 in wildtype (WT) HEK293T cells and after treatment with CRISPR-pairs (Cr1+Cr2), using telomere staining (Telo) and whole chromosome 18 painting (WCP18). Blue, DAPI; Red, telomere; Cyan, chromosome 18.

Supplementary Tables

Table S1

gRNA name	5'-3' (sense)	3'-5' (anti-sense)	hg38 region	CRISPR target
Cr1	CACCGAGAGCCCCAGAGACCGGCAC	AAACGTGCCGGTCTCTGGGGCTCTC	-	ECC-biosensor
Cr2	CACCGCTTGAACGCGTCCCGGCTTG	AAACCAAGCCGGGACGCGTTCAAGC	-	ECC-biosensor
Cr3	CACCGTGATAGCTCTAAAAGCACA	AAACTGTGCTTTTAGAGCTATCAC	1q23.2	*dsCRP*
Cr4	CACCGATAATTGCTTAATCACACA	AAACTGTGTGATTAAGCAATTATC	1q23.2	*dsCRP*
Cr5	CACCGTGGCCACACAAATGTGAGG	AAACCCTCACATTTGTGTGGCCAC	10q22.2	*uPA*
Cr6	CACCGACAAGTTGGGAAGGCTTCA	AAACTGAAGCCTTCCCAACTTGTC	10q22.2	*uPA*
Cr7	CACCGAGAGCGCCTGCGACCCGAG	AAACCTCGGGTCGCAGGCGCTCTC	19q13.43	*TRIM28*
Cr8	CACCGTGCTTCTCCAAAGACATCG	AAACCGATGTCTTTGGAGAAGCAC	19q13.43	*TRIM28*
Cr9	CACCGAGCTGGTCAGTAACTCCTCT	AAACAGAGGAGTTACTGACCAGCTC	1q23.1	chr1
Cr10	CACCGTGAGATCCTGGAGAAAAGC	AAACGCTTTTCTCCAGGATCTCAC	1q23.1	chr1
Cr11	CACCGTGGAAGACAAATTCCATTAA	AAACTTAATGGAATTTGTCTTCCAC	1q23.1	chr1
Cr12	CACCGAGTTCTCAGTCCACCATTAA	AAACTTAATGGTGGACTGAGAACTC	1q23.1	chr1
Cr13	CACCGTAAGGCTTTTTATTGCAGG	AAACCCTGCAATAAAAAGCCTTAC	1q23.1	chr1
Cr14	CACCGATTAGGAGGTCAGAAACTTG	AAACCAAGTTTCTGACCTCCTAATC	1q23.1	chr1
Cr15	CACCGAAGGTGACCTTCAAATTTGT	AAACACAAATTTGAAGGTCACCTTC	1q23.1	chr1
Cr16	CACCGTGGCATACAAGAAATAATGA	AAACTCATTATTTCTTGTATGCCAC	1q23.1	chr1
Cr17	CACCGATGGTCAGTGTCTCGTGGGC	AAACGCCCACGAGACACTGACCATc	18p11.22	Ring-chr1 (left arm)
Cr18	CACCGATTGGTCAGATGTCTGCCAA	AAACTTGGCAGACATCTGACCAATc	18p11.22	Ring-chr1 (left arm)
Cr19	CACCGTGCTGATTACTATGAGGTA	AAACTACCTCATAGTAATCAGCAC	18q21.2	Ring-chr1 (right arm)
Cr20	CACCGCTCAGGAAATCATCATTGC	AAACGCAATGATGATTTCCTGAGC	18q21.2	Ring-chr1 (right arm)

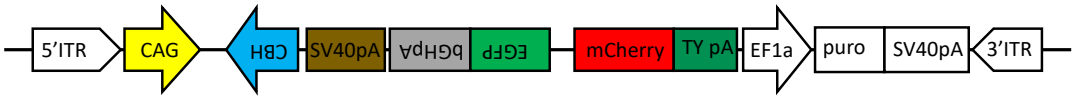
Table S2.

PCR target	PCR test	5'- 3' Forward	5'- 3' Reverse	Expected size (bp)	hg38 region	Oligo ID
PLAU (uPA)	Deletion/wt	TACGAACAGGGTGAGGCGAGAG	TGCCAACGGACATTTTAAAGATGTC	258+654	10q22.2	p9 + p12
PLAU (uPA)	EccDNA	AGTGGAAGTGGTAAGGGGTGGC	ATTTGGGGACAGGGAGGGATGG	199	10q22.2	p10+p11
dsCRP_2kb	Deletion/wt	CTCCGTCTTGCACTAGGGGTAT	GGTTGTCGTACTTGCCTGGATT	639 +1232	1q23.2	p8+p5
dsCRP_2kb	EccDNA	TGCTTCTTTAAACGTGACCACCT	GGTCTTCCCCTTAGCCTACTTCT	530	1q23.2	p6+p7
dsCRP_2kb	Inversion	GGTTGTCGTACTTGCCTGGATT	GGTCTTCCCCTTAGCCTACTTCT	453	1q23.2	p5+p7
dsCRP_2kb	Inversion +/-SINE	CTCCGTCTTGCACTAGGGGTAT	TGCTTCTTTAAACGTGACCACCT	756+500	1q23.2	p8+p6
TRIM28	Deletion/wt	AGCCTCGGCCTCTGCCTCAGCC	GTTCGCATCCTGGGCGTCGGTG	171+661	19q13.43	p13+p16
TRIM28	EccDNA	TTGTAACAGTCTCCCACATCCCT	TCGTATGCAGGACCCAGAAAGTA	297	19q13.43	p14+p15
TRIM28	Inversion	TCGTATGCAGGACCCAGAAAGTA	GTTCGCATCCTGGGCGTCGGTG	340	19q13.43	p15+ p35
pUC19	Plasmid control	CTGCAGGTCGACTCTAGAGGAT	CCATTCAGGCTGCGCAACTG	195	-	p33+ p34
EGFP (ECC biosensor)	Deletion/wt	GTTATCCTCCTCGCCCTTGC	TCCGTACCACTTCCTACCCT	142+2059	-	p4+p0
EGFP (ECC biosensor)	EccDNA	AAGGGATGGTTGGTTGGTGG	GTGCTGCTTCATGTGGTCGG	361	-	p1+p3
EGFP (ECC biosensor)	Inversion	AACAGCTCCTCGCCCTTG	TCCGTACCACTTCCTACCCT	127	-	p2+p0
mCherry (ECC biosensor)	Inversion	AAGGGATGGTTGGTTGGTGG	GTTATCCTCCTCGCCCTTGC	156	-	p1+p4
dsCRP_106kb	EccDNA	TGCTTTATGTCACATTGCTTTGC	TGCTTCTTTAAACGTGACCACCT	666/756	1q23.1	P24+P19
dsCRP_106kb	Linear DNA	CACAAAAGGGCGTAGAGTTACC	GGTTGTCGTACTTGCCTGGATT	591/500	1q23.1	P23+P17
dsCRP_52kb	EccDNA	TCAAGGTCTTTTATACCCAAGTCA	TGCTTCTTTAAACGTGACCACCT	617	1q23.1	P21+P17
dsCRP_52kb	Linear DNA	AGACGCCTTCTGTCACATCC	GGTTGTCGTACTTGCCTGGATT	788	1q23.1	P22+P17
dsCRP_54kb	EccDNA	CATCTGGGTCTAGCGCATTT	TGCTTCTTTAAACGTGACCACCT	785	1q23.1	P21+P17
dsCRP_54kb	Linear DNA	GACATTCTTCCTCTACCTCCCC	GGTTGTCGTACTTGCCTGGATT	833	1q23.1	P22+P17
upCRP_101kb	EccDNA	GGTTGTCGTACTTGCCTGGATT	TTAGTCCATCTGTCCACTGAGC	724/707	1q23.1	P17+P25
upCRP_101kb	Linear DNA	TGCTTCTTTAAACGTGACCACCT	ATTAACTGTCCTTCCATGTGCC	615/635	1q23.1	P19+P26
upCRP_12kb	EccDNA	GGTTGTCGTACTTGCCTGGATT	TCAAACACTATGGGAATGAGGC	672/680	1q23.1	P19+P20
upCRP_12kb	Linear DNA	TGCTTCTTTAAACGTGACCACCT	GTGACCCCTTAATCAATCTCTCC	763/763	1q23.1	P17+P18
CRP_207kb	EccDNA	TGCTTTATGTCACATTGCTTTGC	TTAGTCCATCTGTCCACTGAGC	589/686	1q23.1	P24+P25
CRP_207kb	Linear DNA	CACAAAAGGGCGTAGAGTTACC	ATTAACTGTCCTTCCATGTGCC	573/479	1q23.1	P23+P26
Ring-chrom18	EccDNA	CATGGAAGTTTGGACAACAATGCTG	AGGATTACTCTGGTGACCTGGCATG	562/478/505/421	18p11.22-18q21.2	P28+P29
Ring-chrom18	Linear DNA	CTCCCAACAAACAGCCTGAGCGTCC	ATCAGCACAGCACCATCTTAGGTAC	541/641/597/681	18p11.22-18q21.2	P27+P30

wt=wildtype; up=upstream; ds=downstream

Fig. S1

pCAG-ECC biosensor vector



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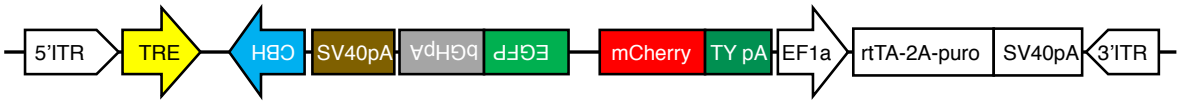
Cr1

Cr2

Fig. S2

pTRE-ECC biosensor vector



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TCGCTTTTTATAGGGCCGCCGCCGCCGCCGCCTCGCCATAAAAGGAAACTTTCGGAGCGCGCCGCTCTGATTGGCTGCCGCCGCACCTCTCCGCCTC
GCCCCGCCCCGCCCCTCGCCCCGCCCCGCCCCGCCTGGCGCGCGCCCCCCCCCCCCCCCCGCCCCCATCGCTGCACAAAATAATTAAAAAATAAATA
AATACAAAATTGGGGGTGGGGAGGGGGGGGAGATGGGGAGAGTGAAGCAGAACGTGGGGCTCACCTCGACCATGGTAATAGCGATGACTAATACGTA
GATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCACAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTT
GGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATTG
ACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGGGTACCTCTAGAGCCATTTGTCTGCAATAAAG
CAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCCCATAGAGCCCACCGCATCCCCAGCATGCCTGCTATTGTCTTCCCAATCCTCC
CCCTTGCTGTCCTGCCCCACCCCACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGACAGTGG
GAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGCTGATCGGCCGCTCTA
GAACTAGTGGATCCCCCGGGCTGCATCCAACCTCGAGTTACTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAG
GACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGCGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATG
GGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGT
CGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGAT
GCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCT
TCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGG
GCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTT
GTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATGGTGGCGGTGCCCGGGAGA
CGCCAACCGCAAGCCGGGACGCGTTCAAGCTTCGCCTGGAGACGATCCGCCGCCACCatggtgagcaagggcgaggaggataacatggccatcatca
aggagttcatgcgcttcaaggtgcacatggagggctccgtgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgagggcac
ccagaccgccaagctgaaggtgaccaagggtggccccctgcccttcgcctgggacatcctgtcccctcagttcatgtacggctccaaggcctacgtg
aagcaccccgccgacatccccgactacttgaagctgtccttccccgagggcttcaagtgggagcgcgtgatgaacttcgaggacggcggcgtggtga
ccgtgacccaggactcctccctgcaggacggcgagttcatctacaaggtgaagctgcgcggcaccaacttcccctccgacggccccgtaatgcagaa
gaagaccatgggctgggaggcctcctccgagcggatgtaccccgaggacggcgccctgaagggcgagatcaagcagaggctgaagctgaaggacggc
ggccactacgacgctgaggtcaagaccacctacaaggccaagaagcccgtgcagctgcccggcgcctacaacgtcaacatcaagttggacatcacct
cccacaacgaggactacaccatcgtggaacagtacgaacgcgccgagggccgccactccaccggcggcatggacgagctgtacaagtaaGTTTAAAC
GGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTGGGT
CGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTGGCACTCTGTCGATACCCCACCGAGACCCCATTGGGGCCAATACGCCCGCGTTTCTTCCTT
TTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGC…3′

Cr1

Cr2

Fig. S3

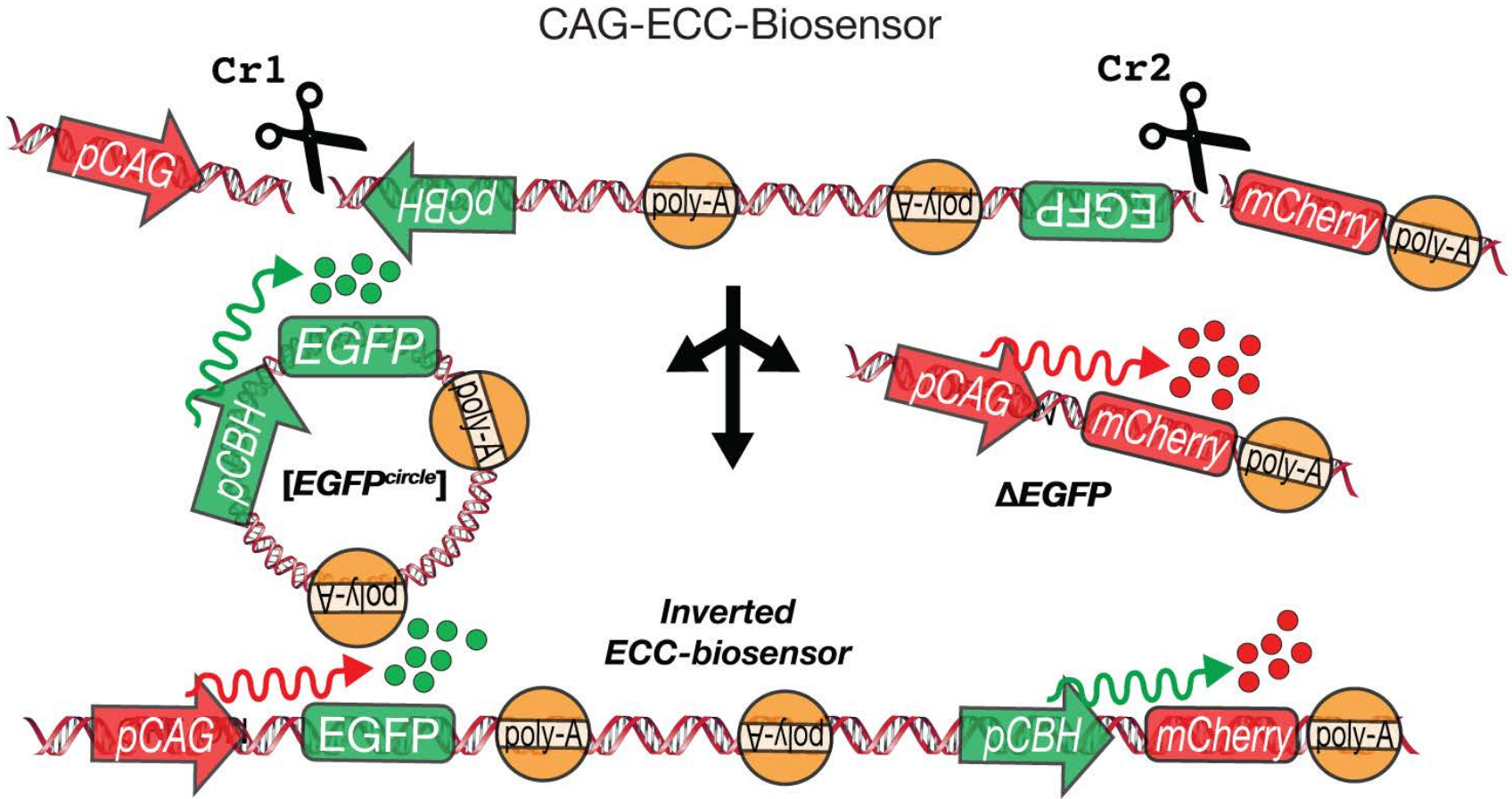

CAG-ECC-Biosensor
Cr1
Cr2
pCAG
pCBH
poly-A
EGFP
mCherry
[EGFPcircle]
ΔEGFP
Inverted
ECC-biosensor

Fig. S4

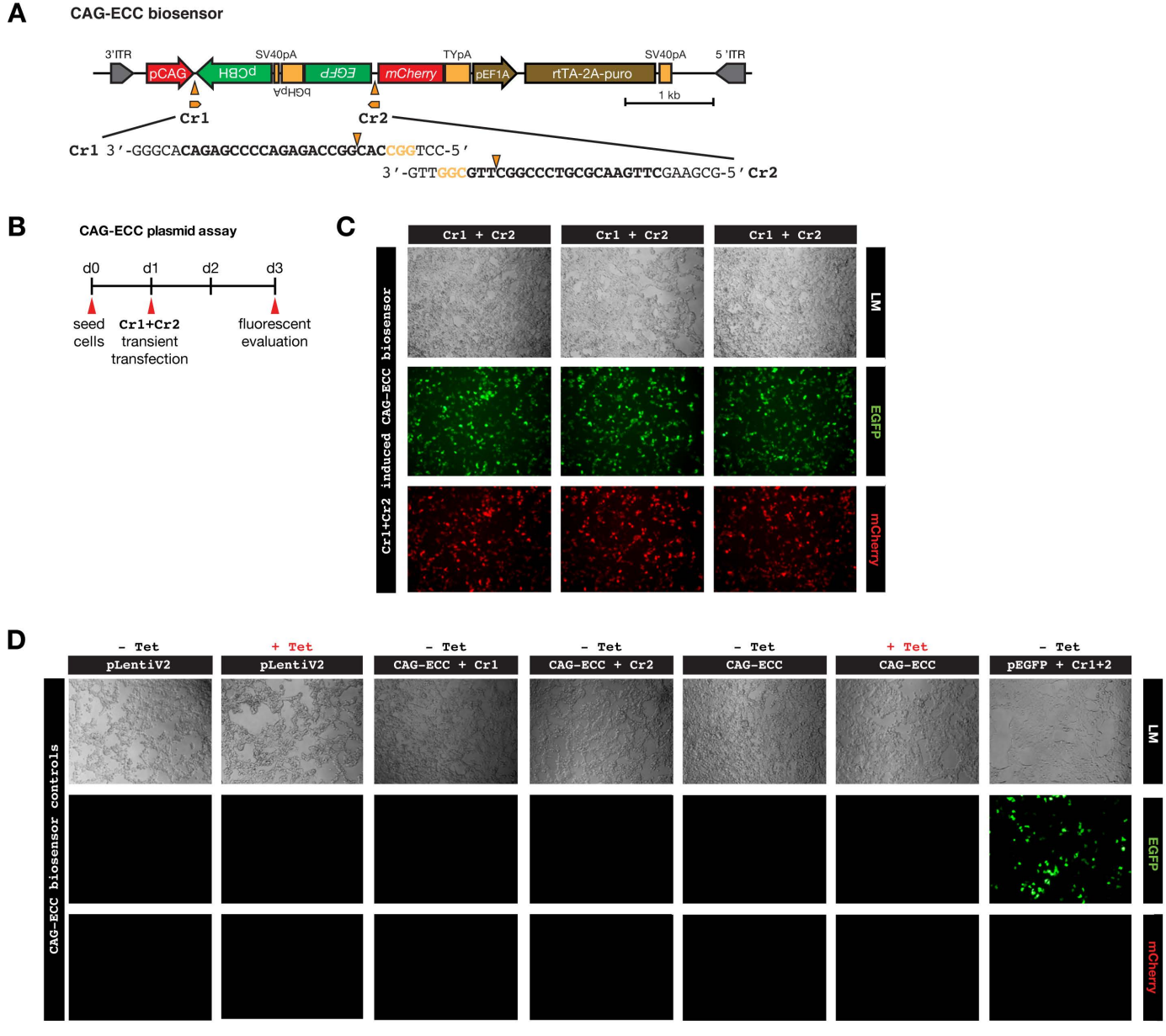

A
CAG-ECC biosensor
3'ITR
pCAG
SV40pA
pCBH
EGFP
bGHpA
TYpA
mCherry
pEF1A
rtTA-2A-puro
SV40pA
5 'ITR
1 kb
Cr1
Cr2
Cr1 3'-GGGCACAGAGCCCCAGAGACCGGCACCGGTCC-5'
3'-GTTGGCGTTCGGCCCTGCGCAAGTTCGAAGCG-5' Cr2
B
CAG-ECC plasmid assay
d0
d1
d2
d3
seed cells
Cr1+Cr2 transient transfection
fluorescent evaluation
C
Cr1 + Cr2
Cr1 + Cr2
Cr1 + Cr2
Cr1+Cr2 induced CAG-ECC biosensor
LM
EGFP
mCherry
D
- Tet
pLentiV2
+ Tet
pLentiV2
- Tet
CAG-ECC + Cr1
- Tet
CAG-ECC + Cr2
- Tet
CAG-ECC
+ Tet
CAG-ECC
- Tet
pEGFP + Cr1+2
CAG-ECC biosensor controls
LM
EGFP
mCherry

Fig. S5

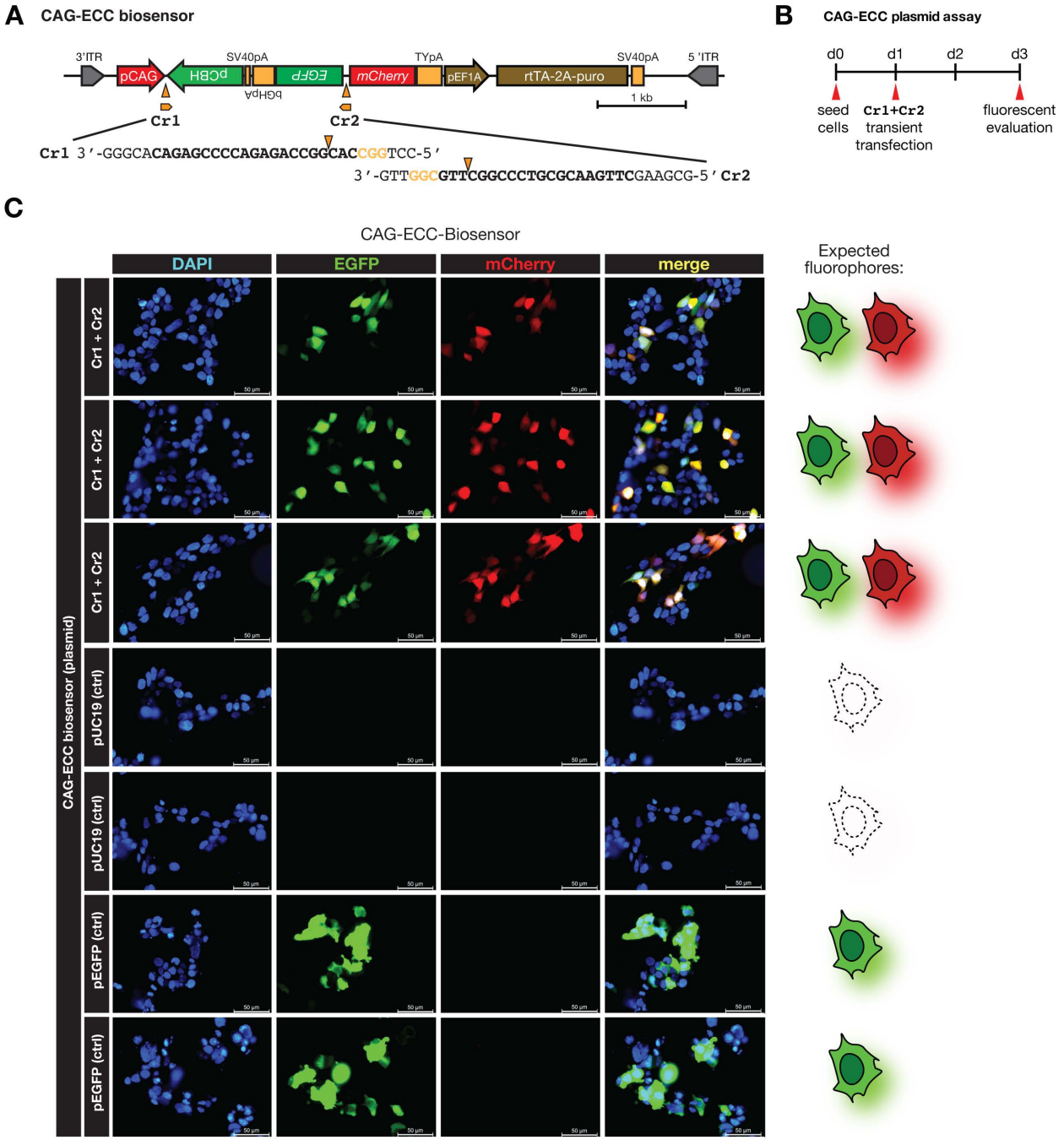

A
CAG-ECC biosensor
3'ITR
pCAG
SV40pA
pCBH
EGFP
bGHpA
mCherry
TYpA
pEF1A
rtTA-2A-puro
SV40pA
5 'ITR
1 kb
Cr1
Cr2
Cr1 3'-GGGCACAGAGCCCCAGAGACCGGCACCGGTCC-5'
3'-GTTGGCGTTCGGCCCTGCGCAAGTTCGAAGCG-5' Cr2
B
CAG-ECC plasmid assay
d0
d1
d2
d3
seed cells
Cr1+Cr2 transient transfection
fluorescent evaluation
C
CAG-ECC-Biosensor
DAPI
EGFP
mCherry
merge
Expected fluorophores:
CAG-ECC biosensor (plasmid)
Cr1 + Cr2
Cr1 + Cr2
Cr1 + Cr2
pUC19 (ctrl)
pUC19 (ctrl)
pEGFP (ctrl)
pEGFP (ctrl)
50 µm

Fig. S6

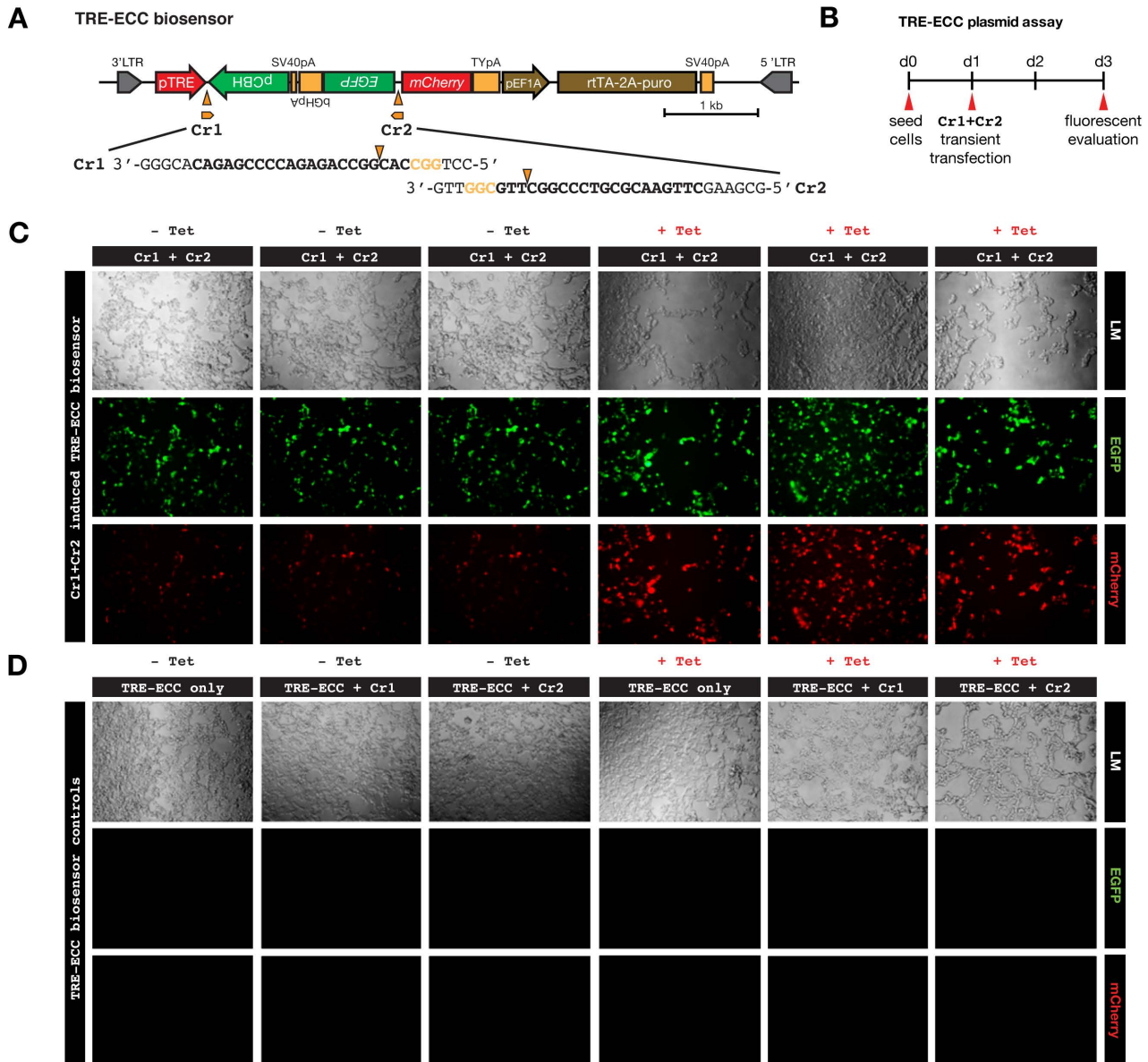

A
TRE-ECC biosensor
3'LTR
pTRE
pCBH
SV40pA
bGHpA
EGFP
mCherry
TYpA
pEF1A
rtTA-2A-puro
SV40pA
5 'LTR
1 kb
Cr1
Cr2
Cr1 3'-GGGCACAGAGCCCCAGAGACCGGCACCGGTCC-5'
3'-GTTGGCGTTCGGCCCTGCGCAAGTTCGAAGCG-5' Cr2
B
TRE-ECC plasmid assay
d0
d1
d2
d3
seed cells
Cr1+Cr2 transient transfection
fluorescent evaluation
C
- Tet
- Tet
- Tet
+ Tet
+ Tet
+ Tet
Cr1 + Cr2
Cr1 + Cr2
Cr1 + Cr2
Cr1 + Cr2
Cr1 + Cr2
Cr1 + Cr2
Cr1+Cr2 induced TRE-ECC biosensor
LM
EGFP
mCherry
D
- Tet
- Tet
- Tet
+ Tet
+ Tet
+ Tet
TRE-ECC only
TRE-ECC + Cr1
TRE-ECC + Cr2
TRE-ECC only
TRE-ECC + Cr1
TRE-ECC + Cr2
TRE-ECC biosensor controls
LM
EGFP
mCherry

Fig. S7

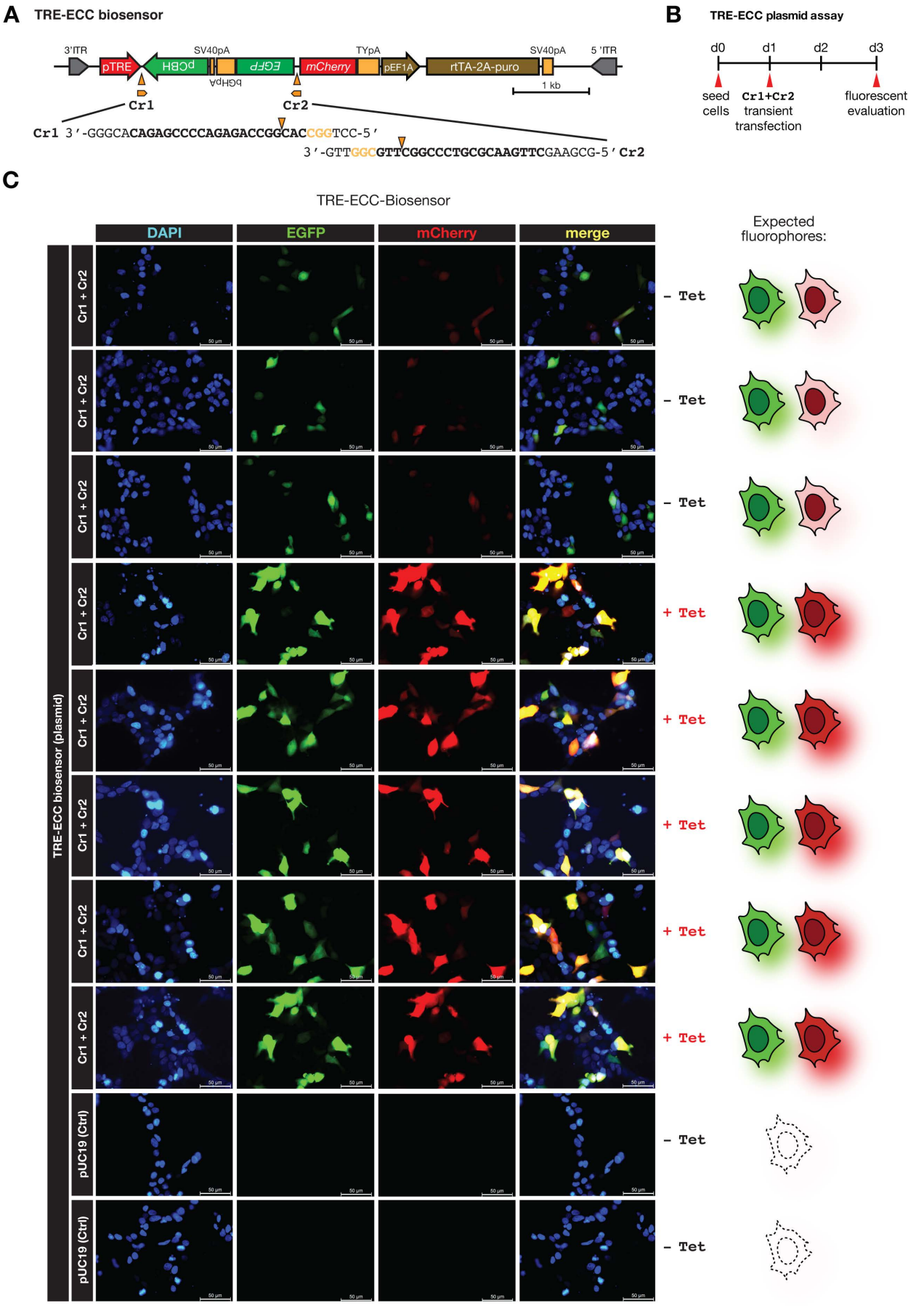
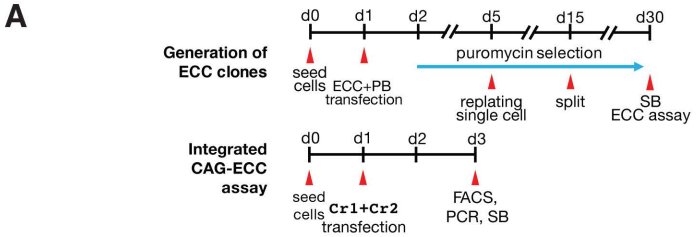
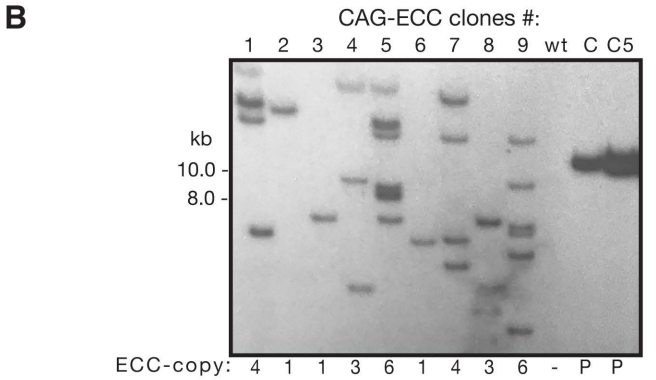
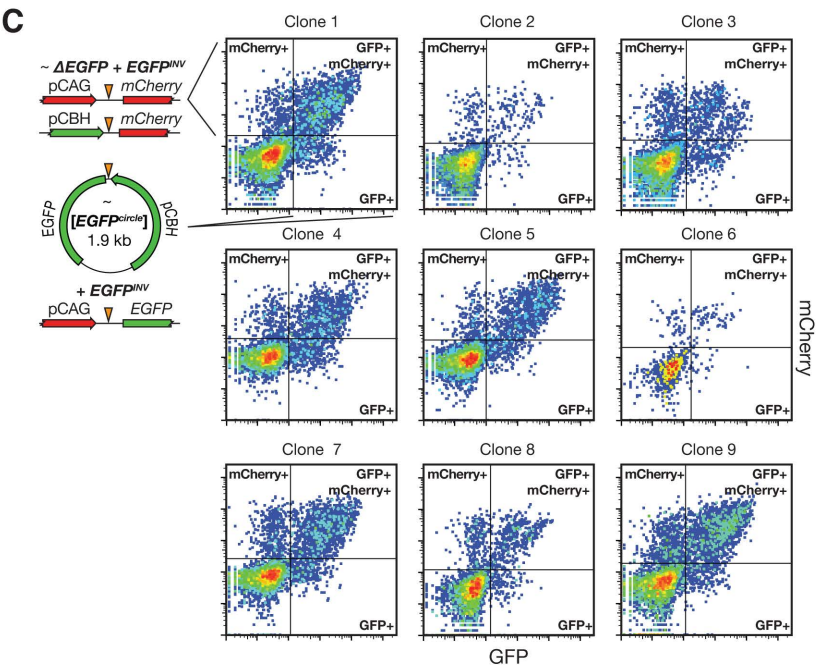

A
TRE-ECC biosensor
3'ITR
pTRE
pCBH
SV40pA
bGHpA
EGFP
mCherry
TYpA
pEF1A
rtTA-2A-puro
SV40pA
5 'ITR
1 kb
Cr1
Cr2
Cr1 3′-GGGCACAGAGCCCCAGAGACCGGCACCGGTCC-5′
3′-GTTGGCGTTCGGCCCTGCGCAAGTTCGAAGCG-5′ Cr2
B
TRE-ECC plasmid assay
d0
d1
d2
d3
seed cells
Cr1+Cr2 transient transfection
fluorescent evaluation
C
TRE-ECC-Biosensor
DAPI
EGFP
mCherry
merge
Expected fluorophores:
TRE-ECC biosensor (plasmid)
Cr1 + Cr2
pUC19 (Ctrl)
- Tet
+ Tet

Fig. S8


A
Generation of ECC clones
d0
d1
d2
d5
d15
d30
seed cells
ECC+PB transfection
puromycin selection
replating single cell
split
SB ECC assay
Integrated CAG-ECC assay
d0
d1
d2
d3
seed cells
Cr1+Cr2 transfection
FACS, PCR, SB


B
CAG-ECC clones #:
1 2 3 4 5 6 7 8 9 wt C C5
kb
10.0
8.0
ECC-copy: 4 1 1 3 6 1 4 3 6 - P P


C
~ ΔEGFP + EGFPINV
pCAG mCherry
pCBH mCherry
EGFP
[EGFPcircle]
1.9 kb
pCBH
+ EGFPINV
pCAG EGFP
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
Clone 7
Clone 8
Clone 9
mCherry+
GFP+
mCherry+
GFP+
mCherry
GFP

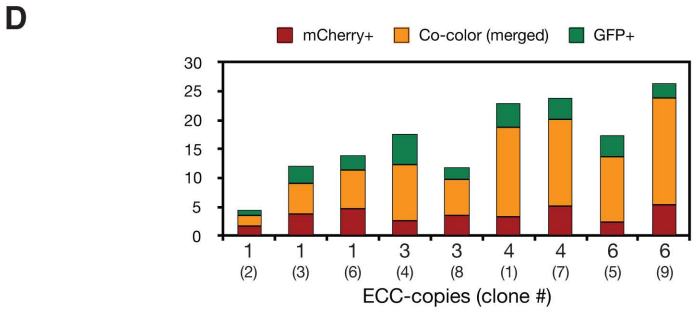

D
mCherry+
Co-color (merged)
GFP+
30
25
20
15
10
5
0
1 (2)
1 (3)
1 (6)
3 (4)
3 (8)
4 (1)
4 (7)
6 (5)
6 (9)
ECC-copies (clone #)

Fig. S9

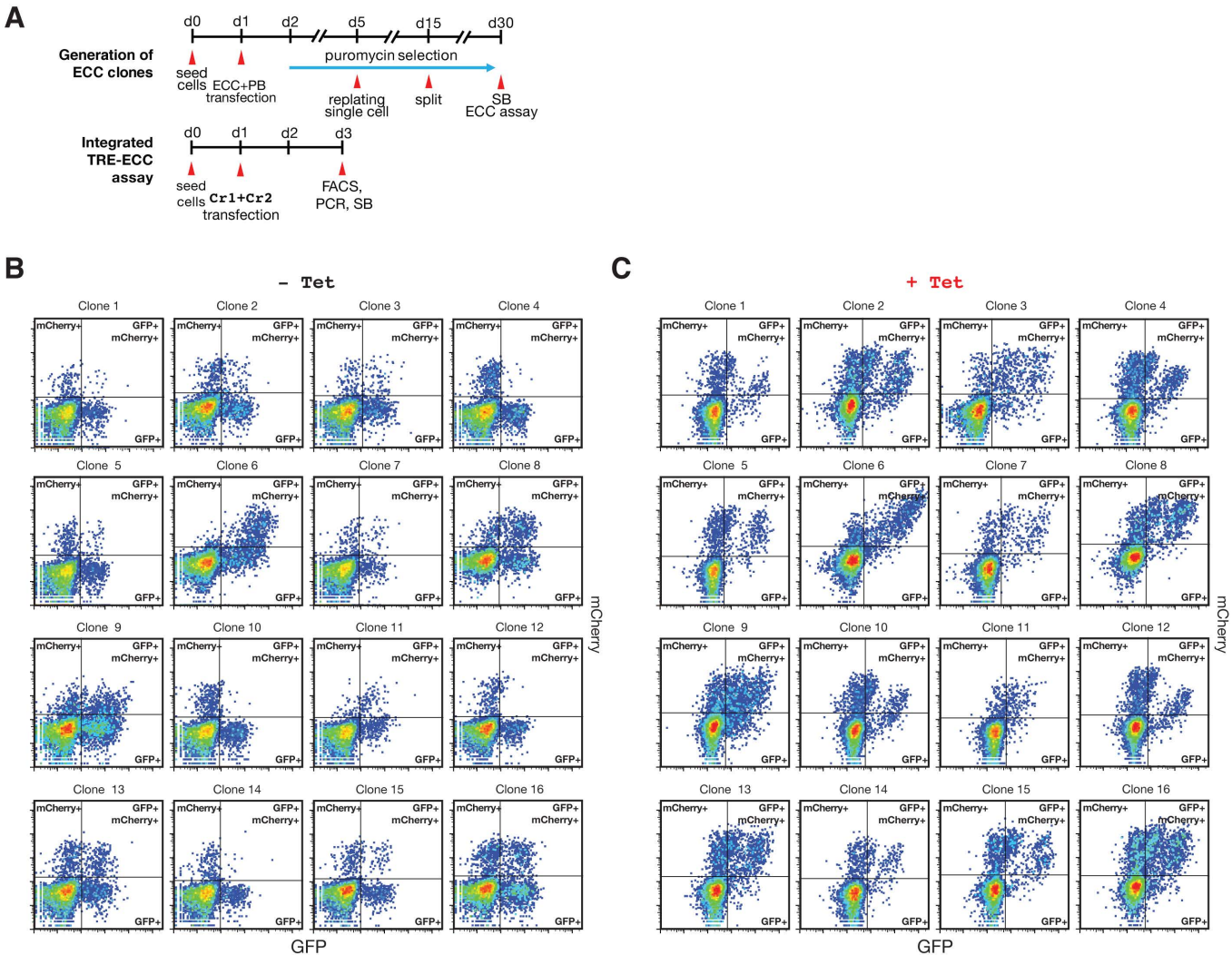

A
Generation of ECC clones
d0
d1
d2
d5
d15
d30
seed cells
ECC+PB transfection
puromycin selection
replating single cell
split
SB ECC assay
Integrated TRE-ECC assay
d0
d1
d2
d3
seed cells
Cr1+Cr2 transfection
FACS, PCR, SB
B
- Tet
C
+ Tet
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
Clone 7
Clone 8
Clone 9
Clone 10
Clone 11
Clone 12
Clone 13
Clone 14
Clone 15
Clone 16
mCherry+
GFP+
mCherry+
GFP+
mCherry
GFP

Fig. S10

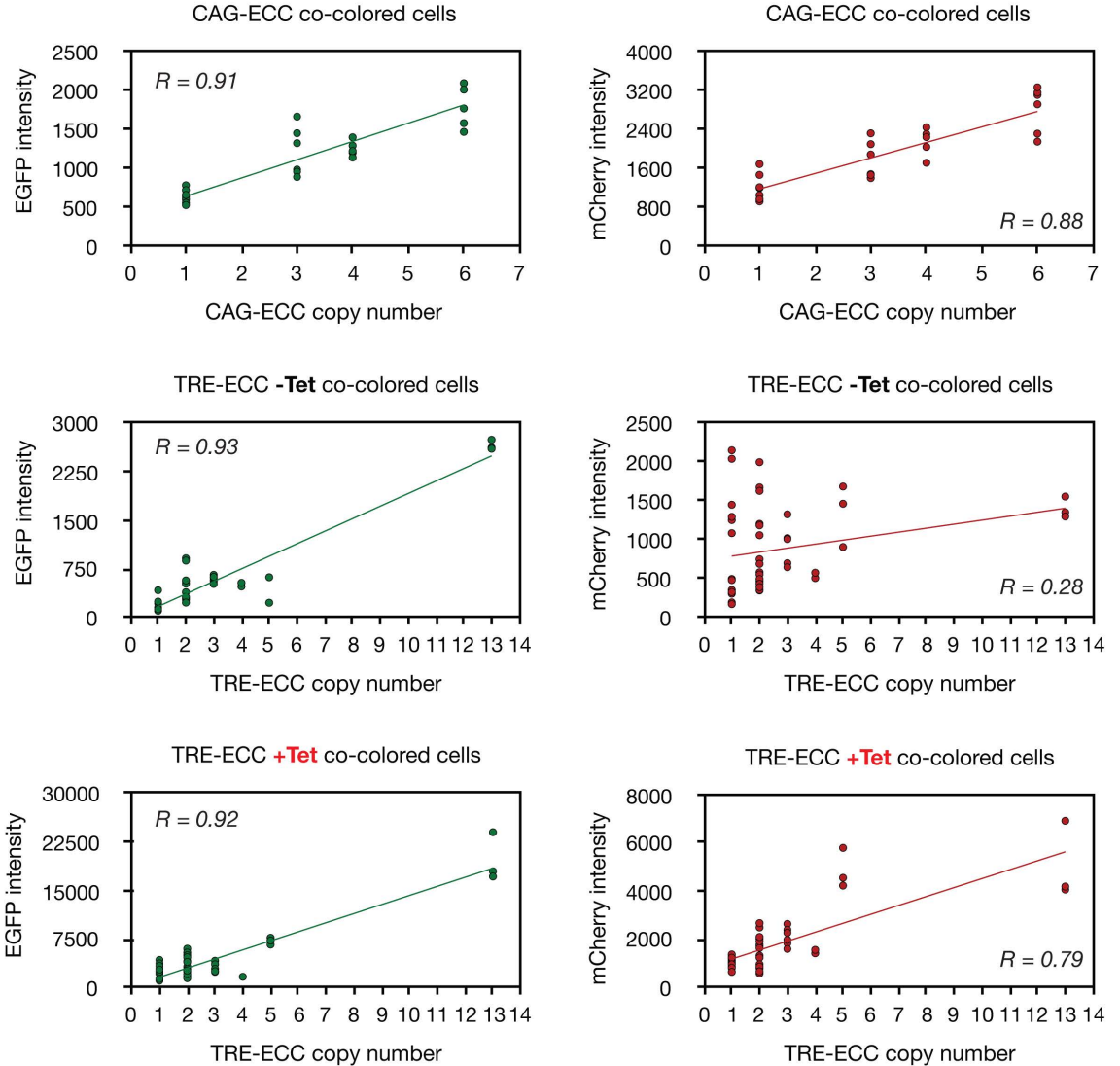

CAG-ECC co-colored cells
EGFP intensity
CAG-ECC copy number
R = 0.91
CAG-ECC co-colored cells
mCherry intensity
CAG-ECC copy number
R = 0.88
TRE-ECC -Tet co-colored cells
EGFP intensity
TRE-ECC copy number
R = 0.93
TRE-ECC -Tet co-colored cells
mCherry intensity
TRE-ECC copy number
R = 0.28
TRE-ECC +Tet co-colored cells
EGFP intensity
TRE-ECC copy number
R = 0.92
TRE-ECC +Tet co-colored cells
mCherry intensity
TRE-ECC copy number
R = 0.79

Fig. S11

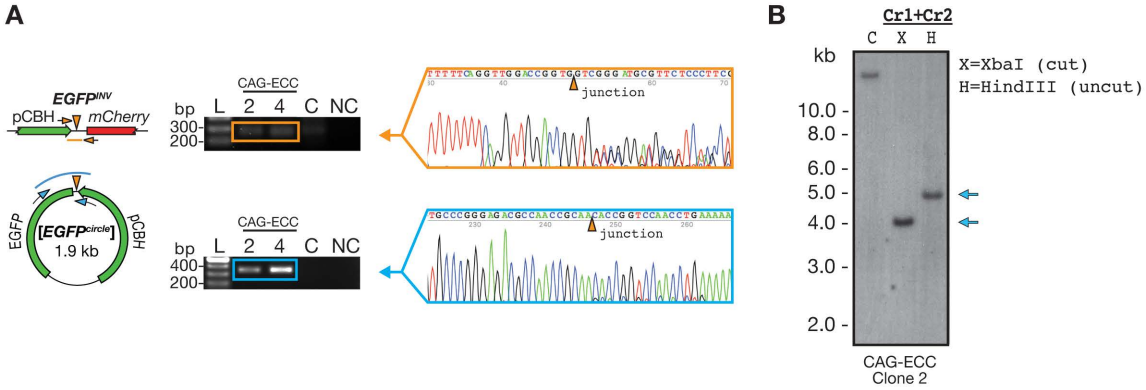

A
EGFP^INV
pCBH
mCherry
CAG-ECC
bp
L
2
4
C
NC
300
200
junction
EGFP
[EGFP^circle]
1.9 kb
pCBH
400
200
B
Cr1+Cr2
C
X
H
kb
10.0
8.0
6.0
5.0
4.0
3.0
2.0
X=XbaI (cut)
H=HindIII (uncut)
CAG-ECC
Clone 2

Fig. S12

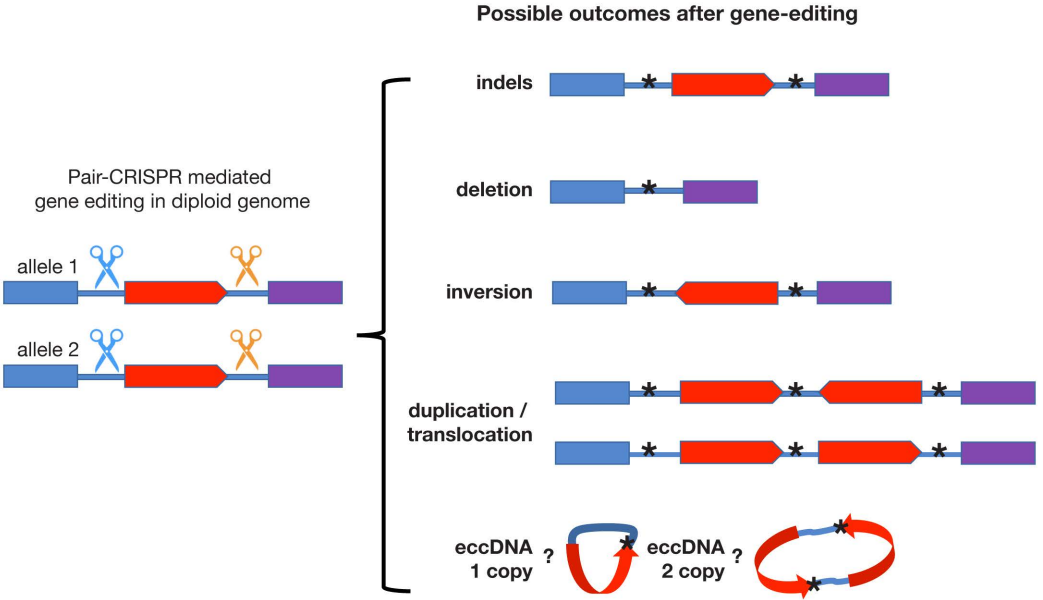

Possible outcomes after gene-editing
Pair-CRISPR mediated
gene editing in diploid genome
allele 1
allele 2
indels
deletion
inversion
duplication /
translocation
eccDNA
1 copy
?
eccDNA
2 copy
?

Fig. S13

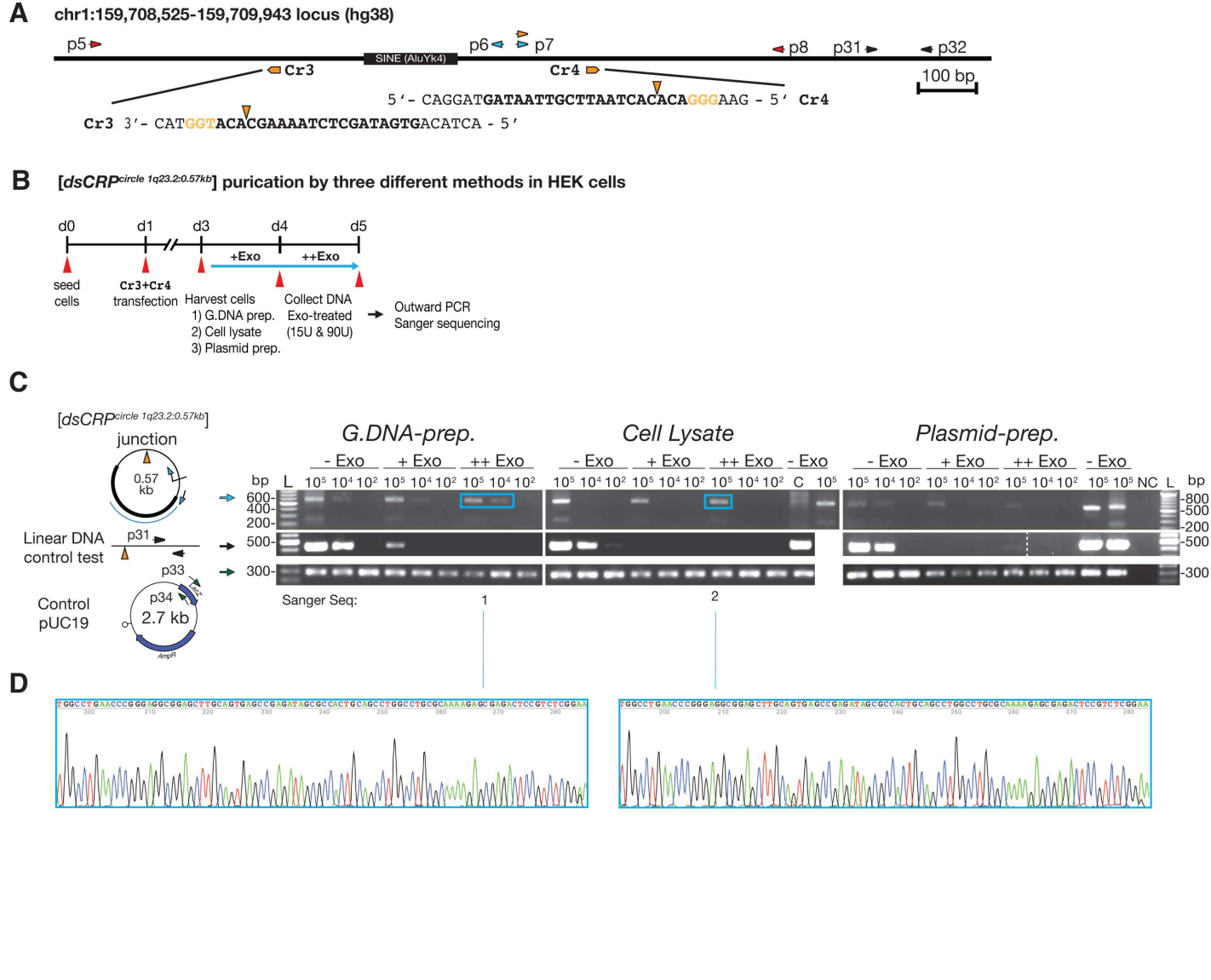

A
chr1:159,708,525-159,709,943 locus (hg38)
p5
p6
p7
p8
p31
p32
SINE (AluYk4)
Cr3
Cr4
100 bp
5′- CAGGATGATAATTGCTTAATCACACAGGGAAG - 5′ Cr4
Cr3 3′- CATGGTACACGAAAATCTCGATAGTGACATCA - 5′
B
[dsCRPcircle 1q23.2:0.57kb] purication by three different methods in HEK cells
d0
d1
d3
d4
d5
+Exo
++Exo
seed cells
Cr3+Cr4 transfection
Harvest cells
1) G.DNA prep.
2) Cell lysate
3) Plasmid prep.
Collect DNA
Exo-treated
(15U & 90U)
Outward PCR
Sanger sequencing
C
[dsCRPcircle 1q23.2:0.57kb]
junction
0.57 kb
Linear DNA control test
p31
p33
p34
2.7 kb
Control pUC19
AmpR
ORI-2
G.DNA-prep.
Cell Lysate
Plasmid-prep.
- Exo
+ Exo
++ Exo
bp
L
600-
400-
200-
500-
300-
800-
500-
200-
500-
300-
Sanger Seq:
1
2
D

Fig. S14

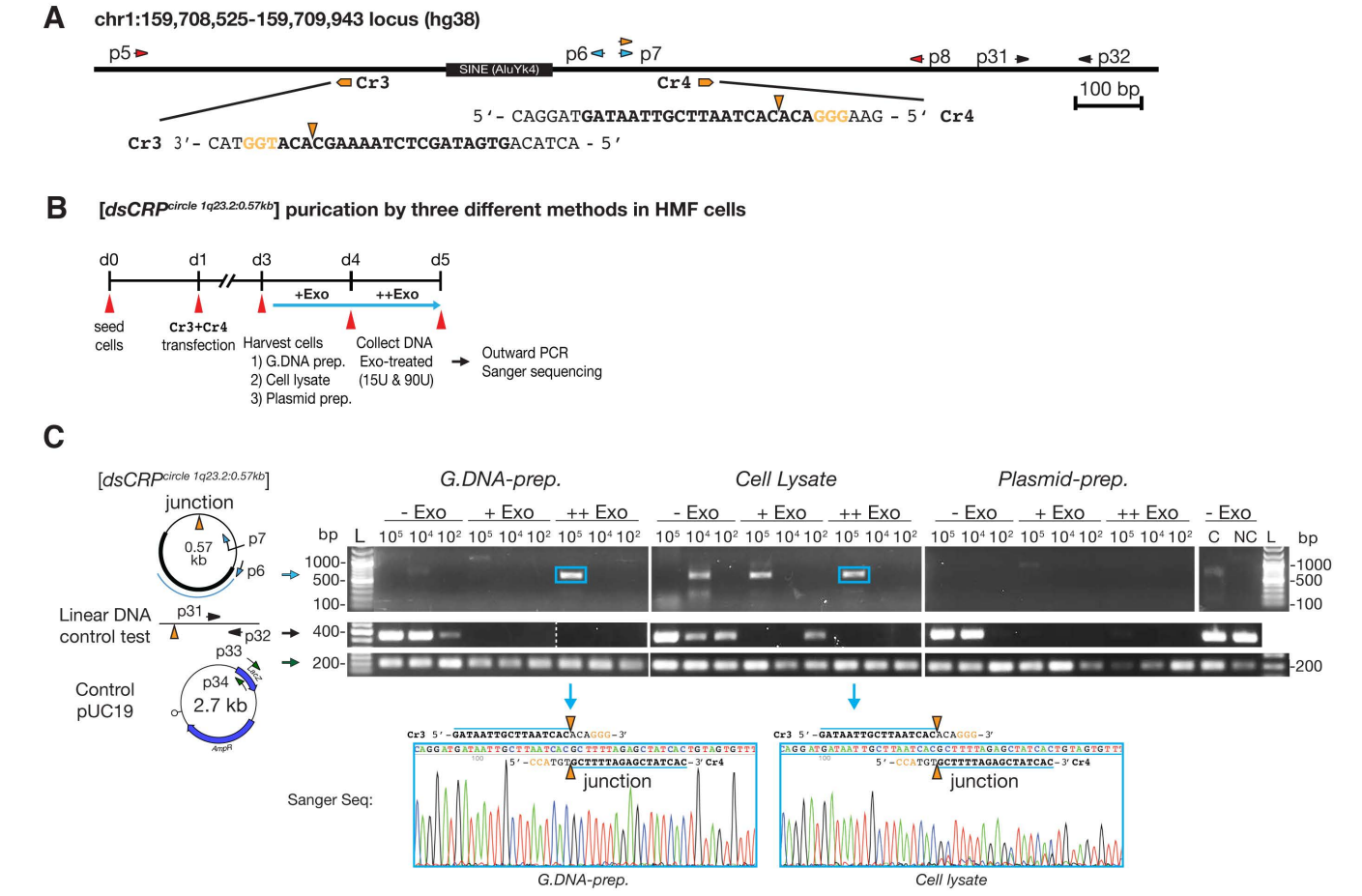

A
chr1:159,708,525-159,709,943 locus (hg38)
p5
p6
p7
p8
p31
p32
SINE (AluYk4)
Cr3
Cr4
100 bp
5′- CAGGATGATAATTGCTTAATCACACAGGGAAG - 5′ Cr4
Cr3 3′- CATGGTACACGAAAATCTCGATAGTGACATCA - 5′
B
[dsCRPcircle 1q23.2:0.57kb] purication by three different methods in HMF cells
d0
d1
d3
d4
d5
+Exo
++Exo
seed cells
Cr3+Cr4 transfection
Harvest cells
1) G.DNA prep.
2) Cell lysate
3) Plasmid prep.
Collect DNA
Exo-treated
(15U & 90U)
Outward PCR
Sanger sequencing
C
[dsCRPcircle 1q23.2:0.57kb]
junction
0.57 kb
p7
p6
Linear DNA control test
p31
p32
p33
p34
2.7 kb
Control pUC19
AmpR
ORI2
G.DNA-prep.
Cell Lysate
Plasmid-prep.
- Exo
+ Exo
++ Exo
bp
L
C
NC
1000
500
400
200
100
Sanger Seq:
Cr3 5′-GATAATTGCTTAATCACACAGGG-3′
5′-CCATGTGCTTTTAGAGCTATCAC-3′ Cr4
junction
G.DNA-prep.
Cell lysate

Fig. S15

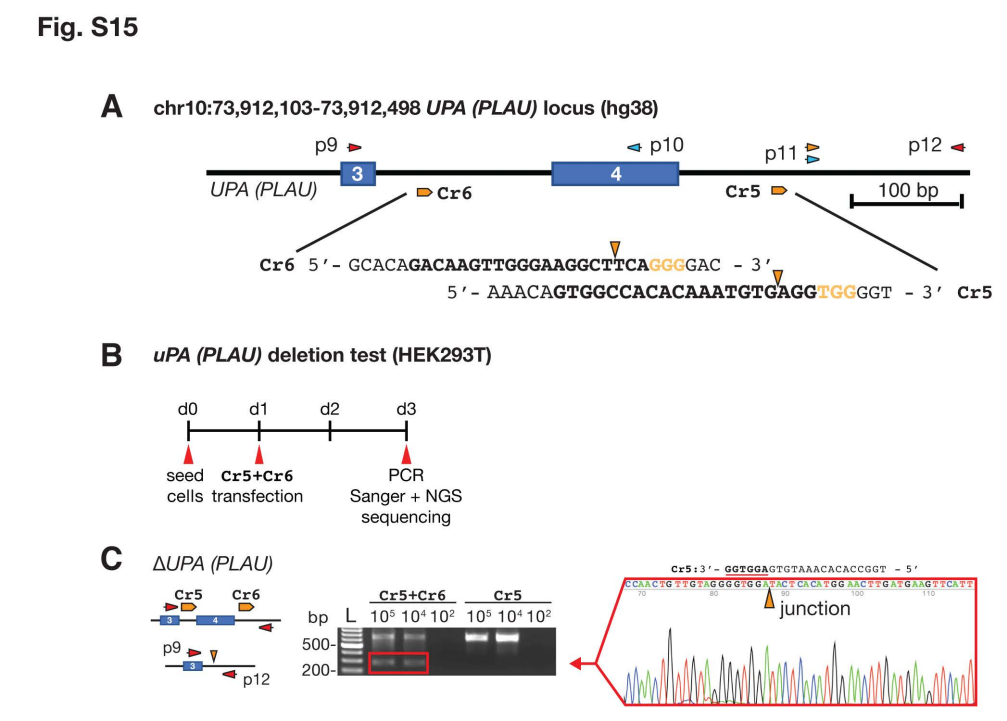

A
chr10:73,912,103-73,912,498 UPA (PLAU) locus (hg38)
p9
p10
p11
p12
3
4
UPA (PLAU)
Cr6
Cr5
100 bp
Cr6 5′- GCACAGACAAGTTGGGAAGGCTTCAGGGGAC - 3′
5′- AAACAGTGGCCACACAAATGTGAGGTGGGGT - 3′ Cr5
B
uPA (PLAU) deletion test (HEK293T)
d0
d1
d2
d3
seed cells
Cr5+Cr6 transfection
PCR Sanger + NGS sequencing
C
ΔUPA (PLAU)
Cr5
Cr6
p9
p12
bp
L
Cr5+Cr6
Cr5
10^5 10^4 10^2
10^5 10^4 10^2
500-
200-
Cr5:3′- GGTGGAGTGTAAACACACCGGT - 5′
junction

Fig. S16

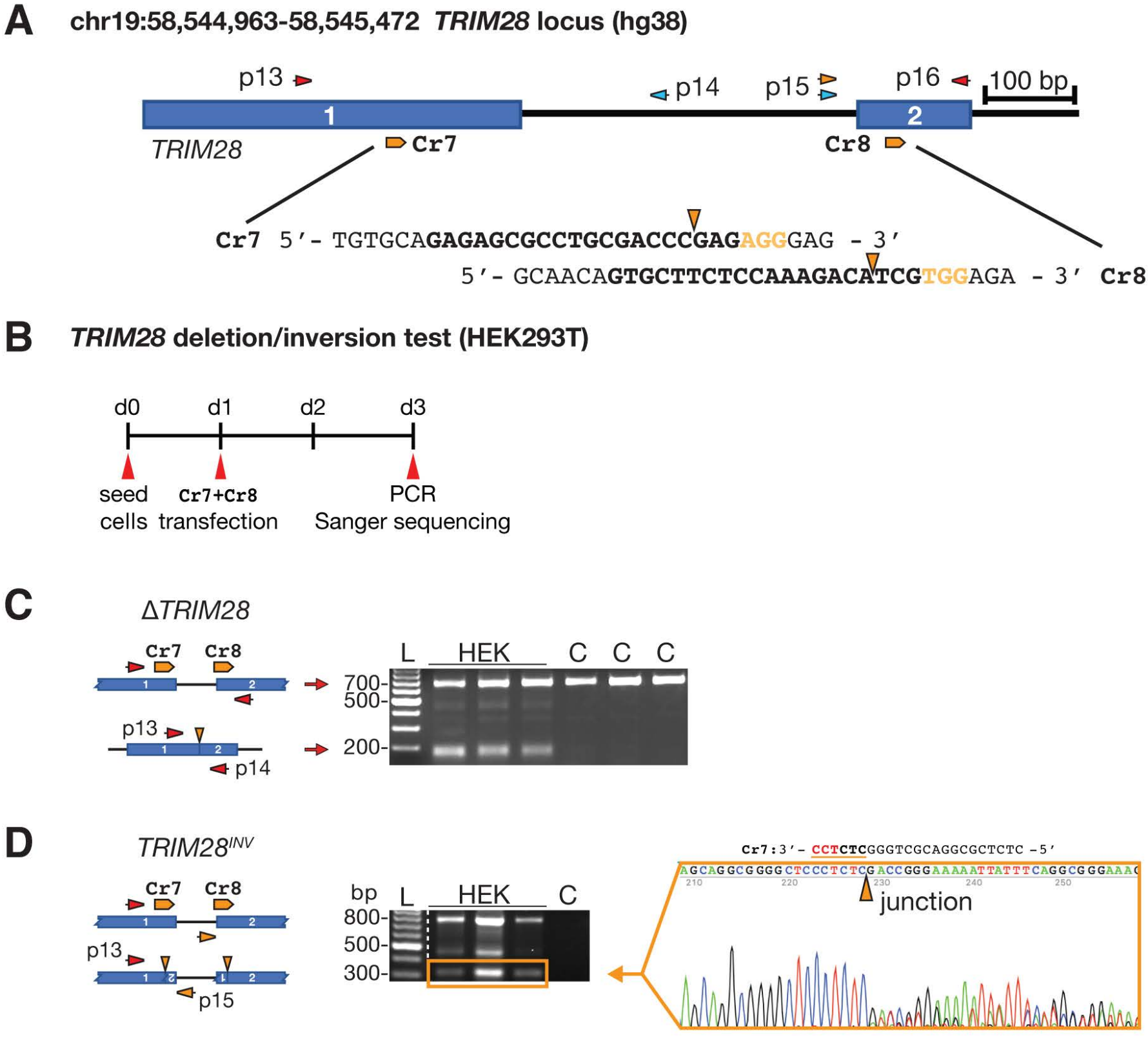
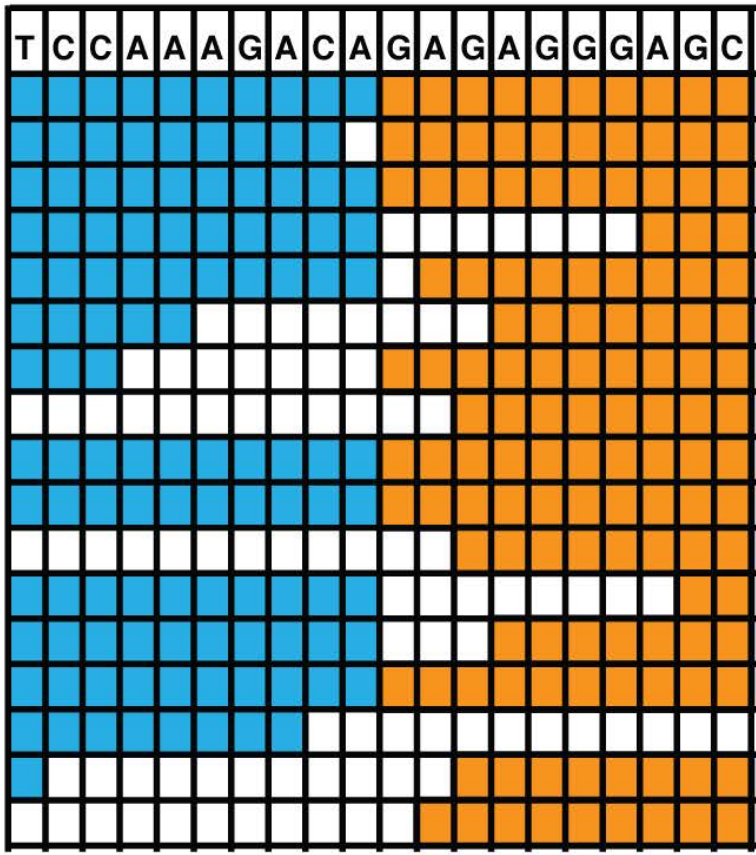

A
chr19:58,544,963-58,545,472 TRIM28 locus (hg38)
p13
p14
p15
p16
100 bp
1
2
TRIM28
Cr7
Cr8
Cr7 5′- TGTGCAGAGAGCGCCTGCGACCCGAGAGGGAG - 3′
5′- GCAACAGTGCTTCTCCAAAGACATCGTGGAGA - 3′ Cr8
B
TRIM28 deletion/inversion test (HEK293T)
d0
d1
d2
d3
seed cells
Cr7+Cr8 transfection
PCR
Sanger sequencing
C
ΔTRIM28
Cr7
Cr8
p13
p14
L
HEK
C
C
C
700-
500-
200-
D
TRIM28INV
Cr7
Cr8
p13
p15
bp
L
HEK
C
800-
500-
300-
Cr7: 3′- CCTCTCGGGTCGCAGGCGCTCTC - 5′
AGCAGGCGGGGCTCCCTCTCGACCGGGAAAAATTATTTCAGGCGGGAAAC
210
220
230
240
250
junction

Fig. S17

Indels of [*TRIM28*$^{circle\ exon\ 1\text{-}2}$] in HEK cells



T	C	C	A	A	A	G	A	C	A	G	A	G	A	G	G	G	A	G	C	Reads	%	INDEL	Indel Seq. 5'-
																				24,925	43.27	INS	C
																				7,579	13.16	DEL	A
																				3,911	6.79	BEJ*	-
																				2,901	5.04	DEL	GAGAGGG
																				2,625	4.56	DEL	G
																				2,265	3.93	DEL	AGACAGAG
																				2,182	3.79	DEL&INS	AAAGACA & C
																				2,101	3.65	DEL	24bp
																				1,879	3.26	INS	CC
																				1,690	2.93	INS	TC
																				1,186	2.06	DEL	12 bp
																				750	1.30	DEL	GAGAGGGA
																				540	0.94	DEL	GAG
																				241	0.42	INS	46 bp
																				186	0.32	DEL	12 bp
																				171	0.30	DEL	11bp
																				!""	0.23	DEL	11bp

Fig. S18

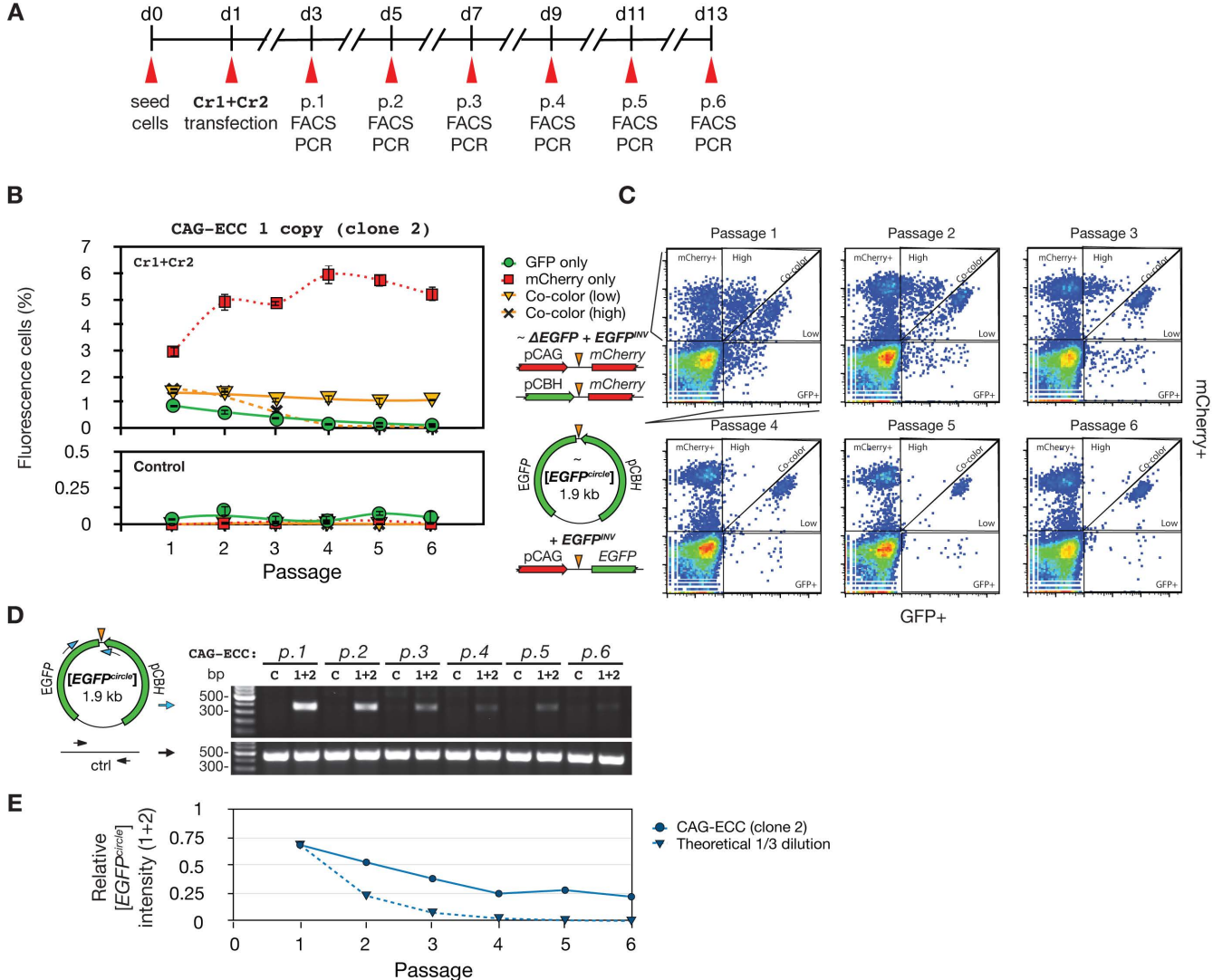

A
d0
d1
d3
d5
d7
d9
d11
d13
seed cells
Cr1+Cr2 transfection
p.1 FACS PCR
p.2 FACS PCR
p.3 FACS PCR
p.4 FACS PCR
p.5 FACS PCR
p.6 FACS PCR
B
CAG-ECC 1 copy (clone 2)
Cr1+Cr2
Control
Fluorescence cells (%)
Passage
GFP only
mCherry only
Co-color (low)
Co-color (high)
~ ΔEGFP + EGFPINV
pCAG
mCherry
pCBH
mCherry
EGFP
[EGFPcircle]
1.9 kb
pCBH
+ EGFPINV
pCAG
EGFP
C
Passage 1
Passage 2
Passage 3
Passage 4
Passage 5
Passage 6
mCherry+
High
Low
Co-color
GFP+
mCherry+
GFP+
D
CAG-ECC:
p.1
p.2
p.3
p.4
p.5
p.6
bp
C
1+2
500-
300-
EGFP
[EGFPcircle]
1.9 kb
pCBH
ctrl
E
Relative [EGFPcircle] intensity (1+2)
Passage
CAG-ECC (clone 2)
Theoretical 1/3 dilution

Fig. S19

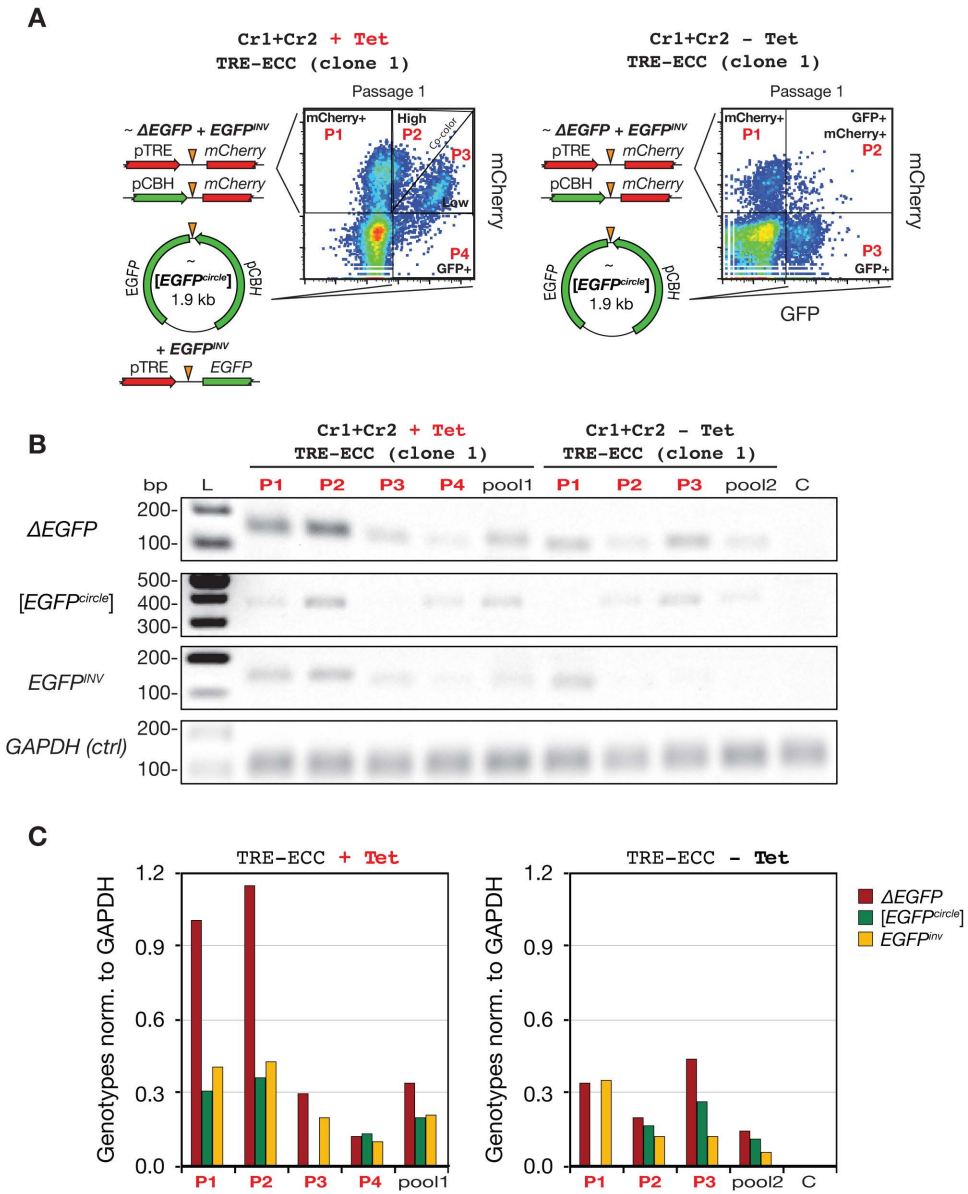
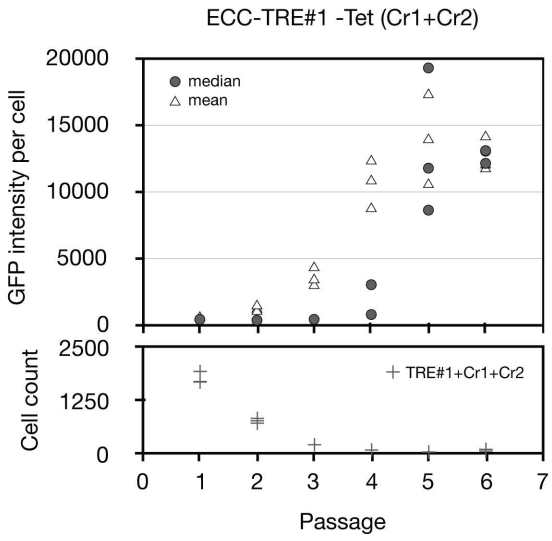
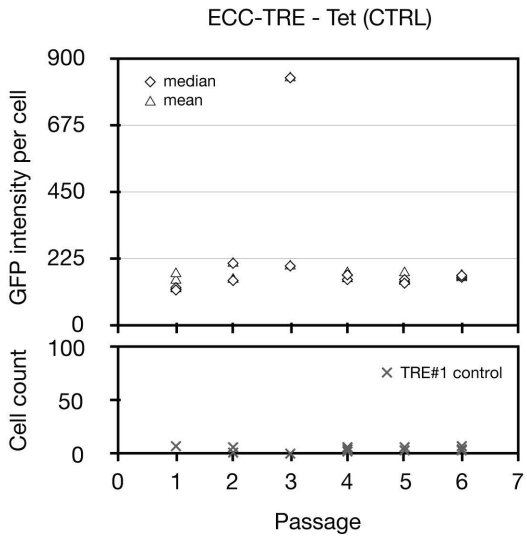
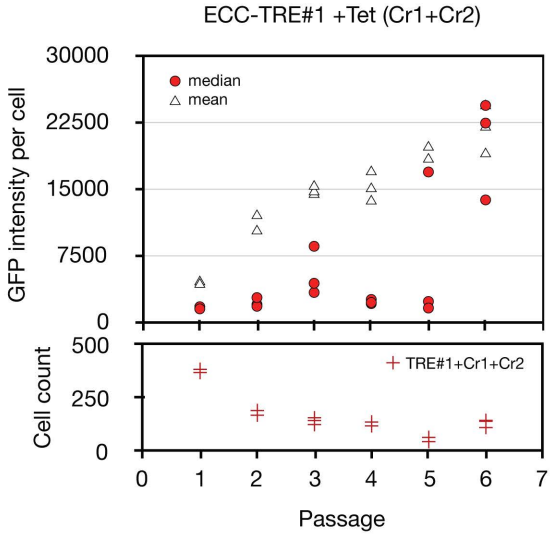
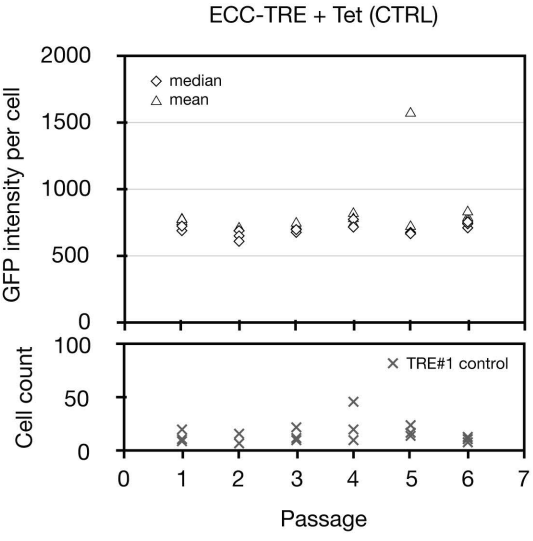

A
Cr1+Cr2 + Tet
TRE-ECC (clone 1)
Passage 1
~ ΔEGFP + EGFPINV
pTRE mCherry
pCBH mCherry
mCherry+
High
P1
P2
P3
P4
GFP+
Low
Cp-color
mCherry
EGFP
[EGFPcircle]
1.9 kb
pCBH
+ EGFPINV
pTRE EGFP
Cr1+Cr2 - Tet
TRE-ECC (clone 1)
Passage 1
~ ΔEGFP + EGFPINV
pTRE mCherry
pCBH mCherry
mCherry+
GFP+
mCherry+
P1
P2
P3
GFP+
mCherry
EGFP
[EGFPcircle]
1.9 kb
pCBH
GFP
B
Cr1+Cr2 + Tet
TRE-ECC (clone 1)
Cr1+Cr2 - Tet
TRE-ECC (clone 1)
bp
L
P1
P2
P3
P4
pool1
P1
P2
P3
pool2
C
ΔEGFP
200-
100-
[EGFPcircle]
500-
400-
300-
EGFPINV
200-
100-
GAPDH (ctrl)
200-
100-
C
TRE-ECC + Tet
TRE-ECC - Tet
Genotypes norm. to GAPDH
1.2
0.9
0.6
0.3
0.0
P1
P2
P3
P4
pool1
pool2
C
ΔEGFP
[EGFPcircle]
EGFPinv

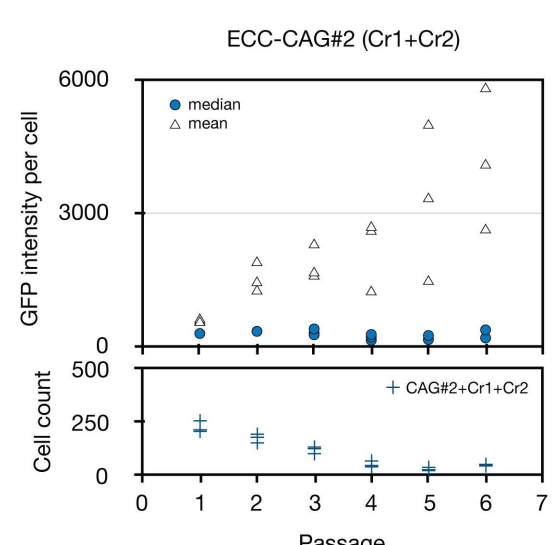
Fig. S20


ECC-TRE#1 -Tet (Cr1+Cr2)
GFP intensity per cell
median
mean
Cell count
TRE#1+Cr1+Cr2
Passage


ECC-TRE - Tet (CTRL)
GFP intensity per cell
median
mean
Cell count
TRE#1 control
Passage


ECC-TRE#1 +Tet (Cr1+Cr2)
GFP intensity per cell
median
mean
Cell count
TRE#1+Cr1+Cr2
Passage


ECC-TRE + Tet (CTRL)
GFP intensity per cell
median
mean
Cell count
TRE#1 control
Passage


ECC-CAG#2 (Cr1+Cr2)
GFP intensity per cell
median
mean
Cell count
CAG#2+Cr1+Cr2
Passage

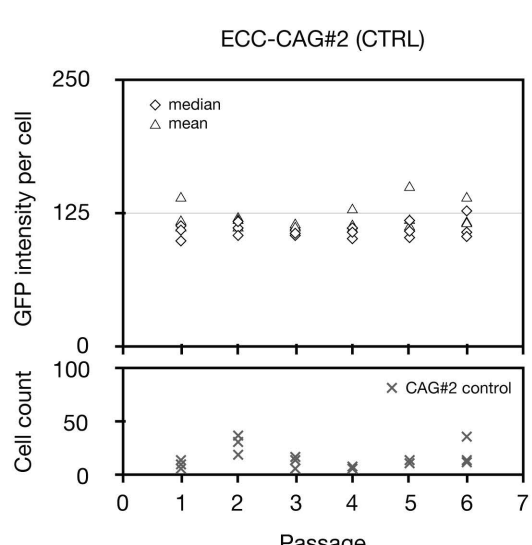
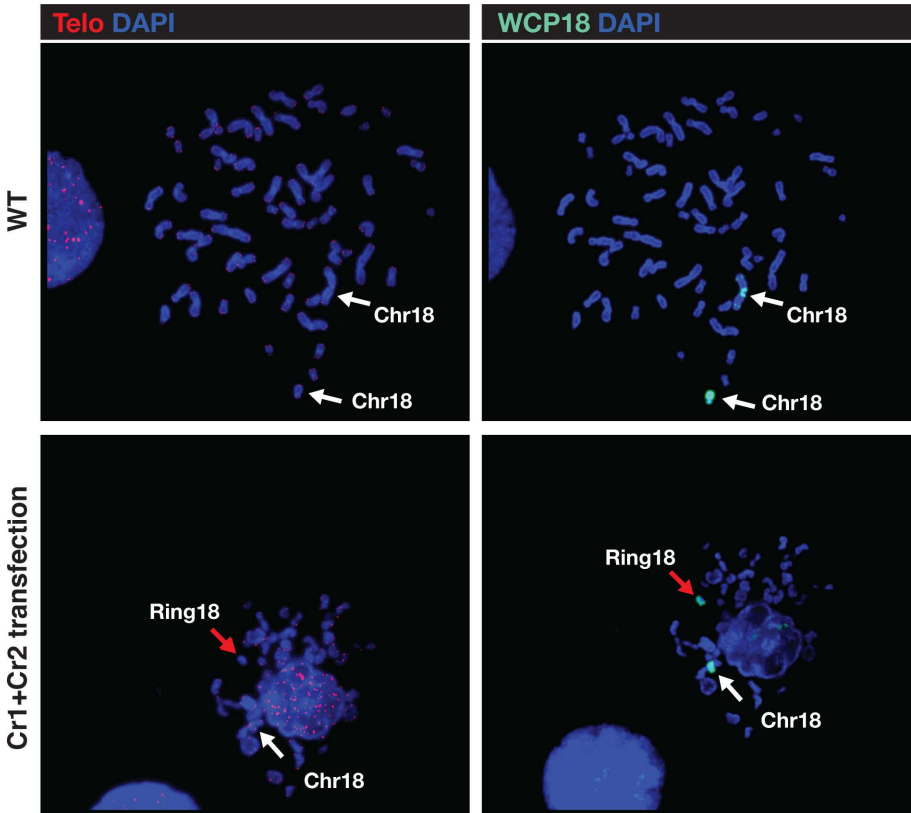

ECC-CAG#2 (CTRL)
GFP intensity per cell
median
mean
Cell count
CAG#2 control
Passage

Fig. S21


Telo DAPI
WCP18 DAPI
WT
Chr18
Chr18
Chr18
Chr18
Cr1+Cr2 transfection
Ring18
Chr18
Ring18
Chr18