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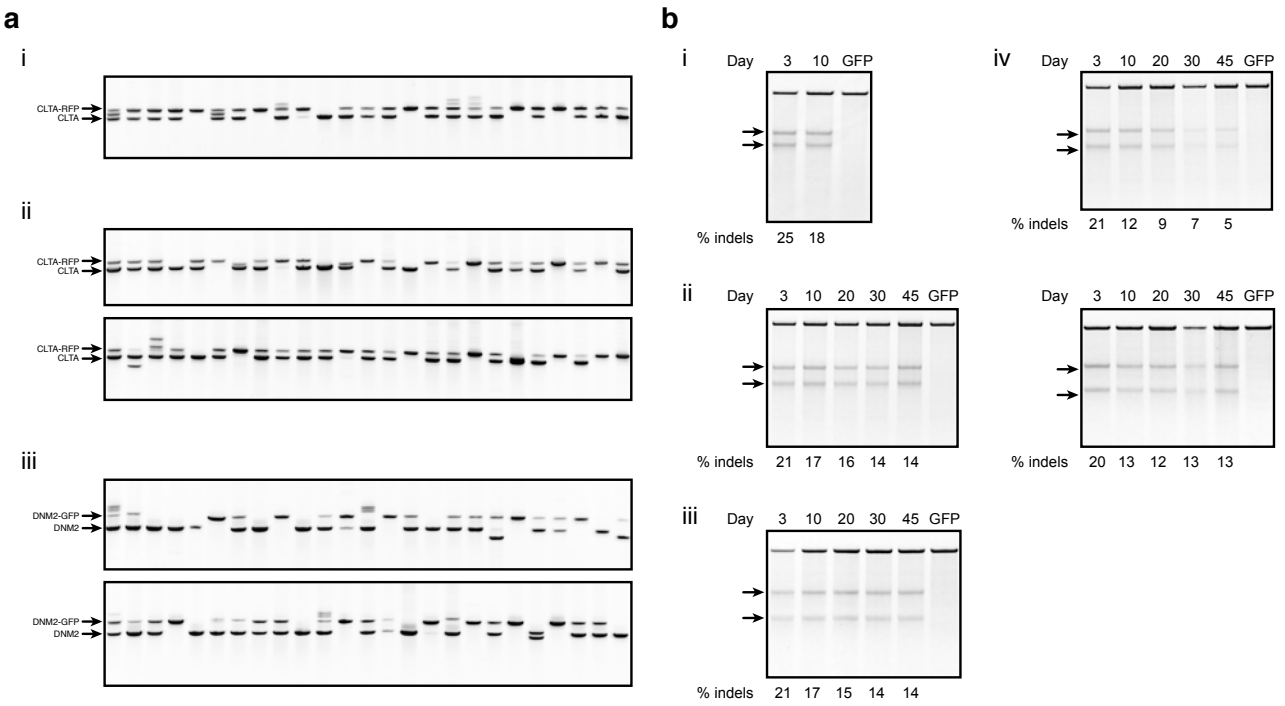

Figure S1 Genotypic analysis of genome-edited cell lines. (**a**) Representative editing efficiency using FACS enrichment. RFP-positive (i) BSC-1 or (ii and iii) SK-MEL-2 cells were enriched by FACS and individual clones were selected from colonies in 96-well plates. After ~4 weeks, clones were analyzed by out-out PCR for CLTA and DNM2 using the oligos listed in Supplementary Information, Table S4 as "TI." (i) 25 randomly chosen clones of the total 26, or (ii) 50 randomly chosen clones of the total 73 that were genotyped are shown. (iii) GFP-positive SK-MEL-2 clones were analyzed by out-out PCR for DNM2. (**b**) Stability of ZFN-modified BSC-1 and SK-MEL-2 cells. (i) BSC-1 cells were transfected with CLTA ZFNs, harvested at the indicated days post transfection, and processed for PCR and the Cel-1 cleavage assay using primers as described in Supplementary Information, Table S4. (ii to iv) SK-MEL-2 cells were transfected with (ii) CLTA ZFNs, (iii) DNM2 ZFNs, or (iv) both (top panel, CLTA; lower panel, DNM2), and processed similarly. GFP, ZFN transfection control. Arrows indicate the expected position of the Cel-1 cleavage products. % indels was calculated using the fraction of the cleaved signal. (**c and d**) Representative sequence genotyping for two genome-edited clones. (**c**) BSC-1 CLTA-RFP clone mkCLTA^{EN} was processed by out-out PCR (as in Fig. 1b) and the resulting amplicons were cloned into pCR2.1. 24 colonies were sequenced using CLTA-SQ-R (Supplementary Information, Table S4). Of the 23 clones that produced legible sequence, 17 perfectly matched WT CLTA near the ZFN cleavage site, and six matched the expected sequence of the RFP donor. "Stop" denotes the position of the endogenous or donor-provided stop codons. An asterisk (*) marks the location of SNPs in the ZFN-binding site of the donor. Red shading indicates the RFP coding sequence provided by the donor. (**d**) SK-MEL-2 DNM2-GFP clone hDNM2^{EN-1} was processed as in (c). 24 colonies were sequenced using DNM2-SQ-R (Supplementary Information, Table S4). Of the 22 clones that produced legible sequence, 19 perfectly matched WT DNM2 near the ZFN cleavage and donor insertion sites, and three matched the expected sequence of the GFP donor (note that the transgenic allele is a less efficient substrate both for cloning and for amplification). "Stop" denotes the position of the endogenous or donor-provided stop codons. Green shading indicates the GFP coding sequence provided by the donor.

c

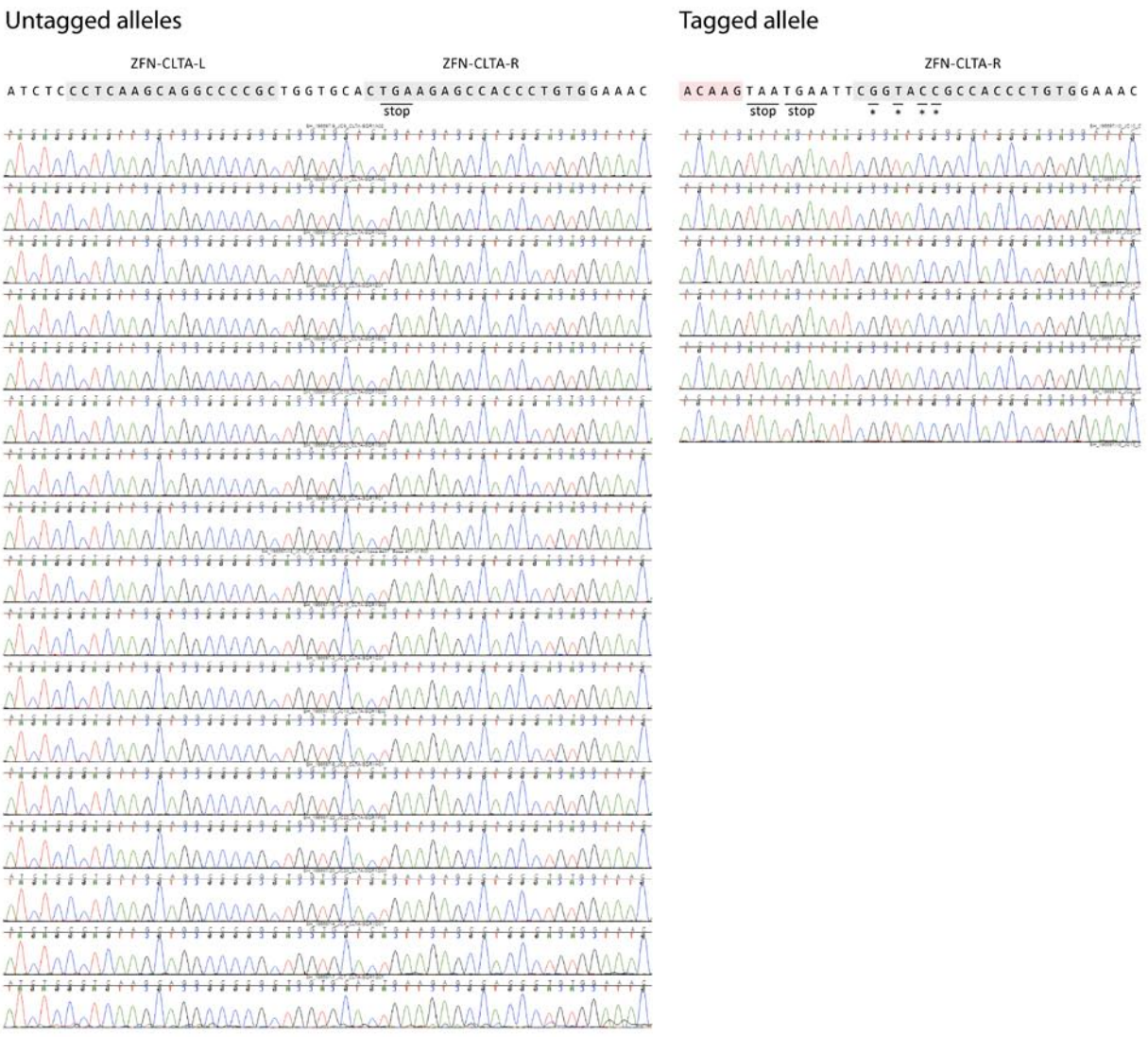

Figure S1 continued

d

Figure S1 continued

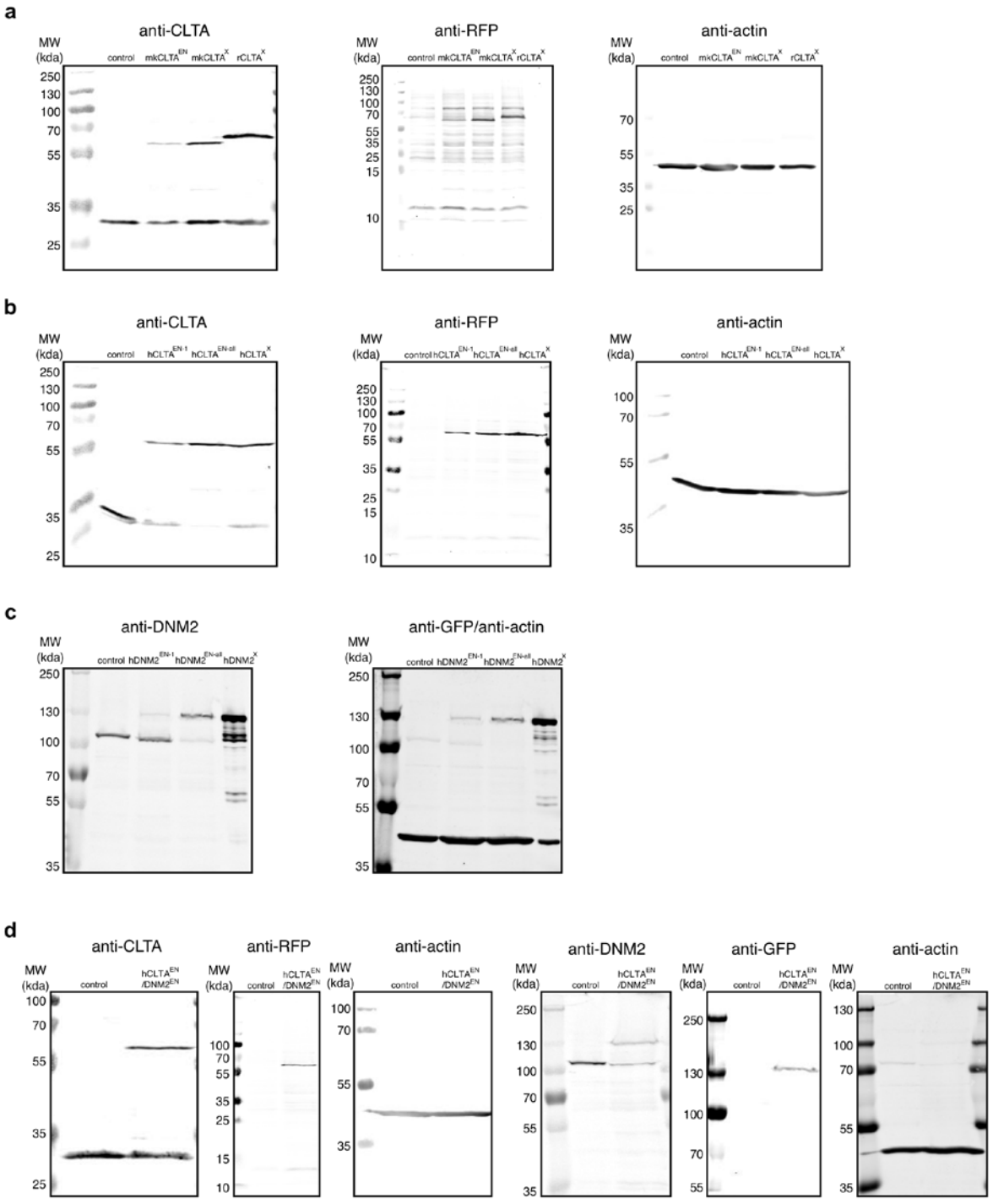

Figure S2 Western immunoblot analysis of genome-edited cell lines. Lysates of (**a**) BSC-1 or (**b to d**) SK-MEL-2 cells were immunoblotted against CLTA, DNM2, RFP, GFP, or actin. Control, parental cell line. (a) BSC-1 cell lines: mkCLTA^{EN}, single-allele CLTA-RFP tagged genome-edited line; mkCLTA^{X}, stable CLTA-RFP overexpression line; rCLTA^{X}, stable GFP-CLTA (rat brain-derived) overexpression line. (b) SK-MEL-2 CLTA-RFP cell lines: hCLTA^{EN-1}, single-allele CLTA-RFP tagged genome-edited line; hCLTA^{EN-all}, all-allele tagged genome-edited line; stable CLTA-RFP overexpression line. (c) SK-MEL-2 DNM2-GFP cell lines: hDNM2^{EN-1}, single-allele tagged genome-edited line; hDNM2^{EN-all}, all-allele tagged genome-edited line; hDNM2^{X}, stable overexpression DNM2-EGFP cell line. (d) SK-MEL-2 hCLTA^{EN}/hDNM2^{EN}, single-allele tagged CLTA-RFP and all-allele tagged DNM2-GFP genome-edited line. Note RFP antibody cross-reactivity with GFP.

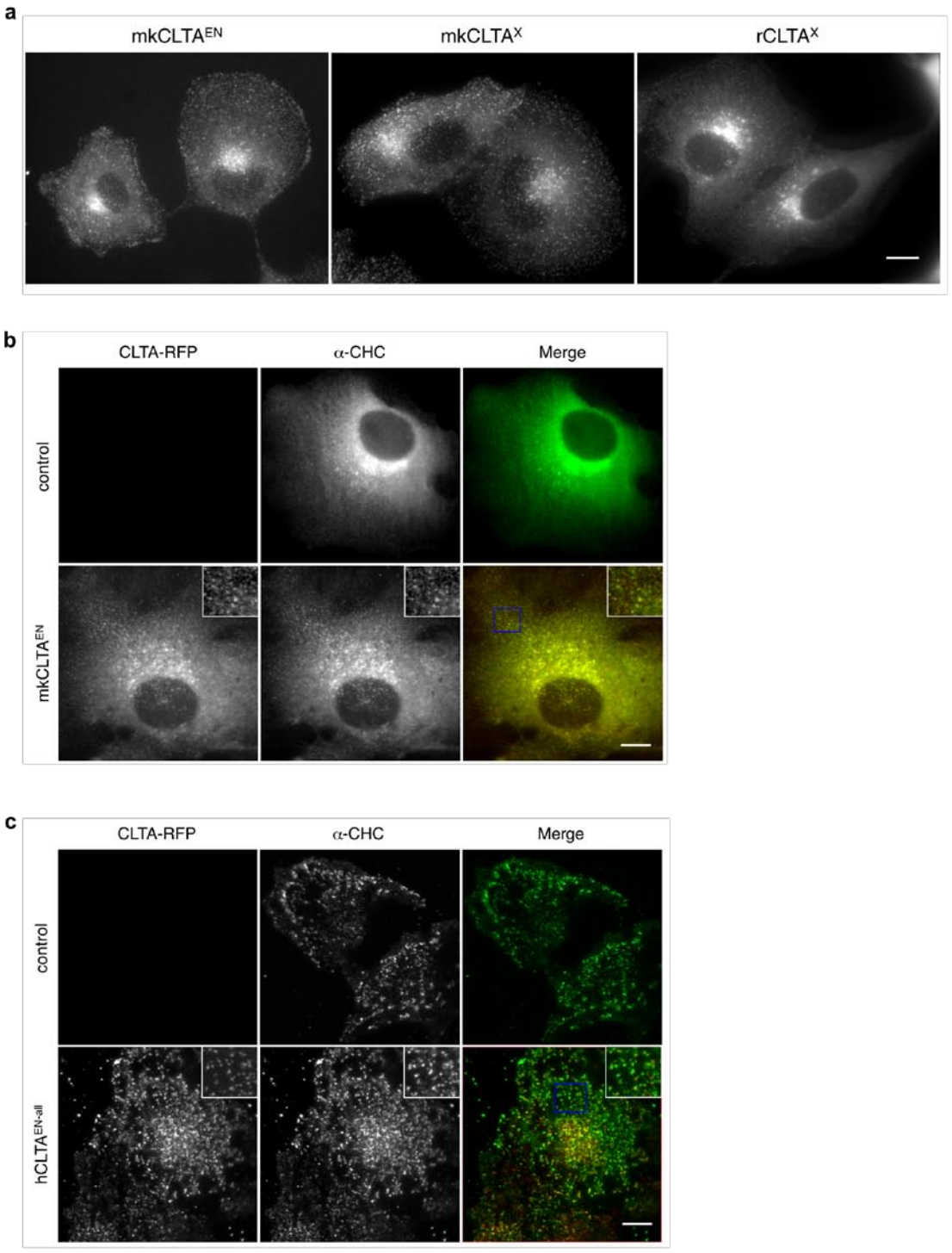

Figure S3 Fluorescence microscopy analysis of genome-edited cell lines. (**a**) Epifluorescence images of BSC-1 cell lines. mkCLTA^{EN}, single-allele tagged CLTA-RFP genome-edited cell line; mkCLTA^{X}, stable CLTA-RFP (monkey-derived) overexpression cell line; rCLTA^{X}, stable GFP-CLTA (rat brain-derived) overexpression cell line. Scale bar, 10 μm. (**b and c**) Immunostaining analysis of genome edited cell lines. Fixed (**b**) BSC-1 mkCLTA^{EN} and (**c**) SK-MEL-2 hCLTA$^{EN\text{-}all}$ cell lines were stained for clathrin heavy chain (CHC) and visualized by epi-fluorescence and TIRF microscopy, respectively. Scale bar, 10 μm.

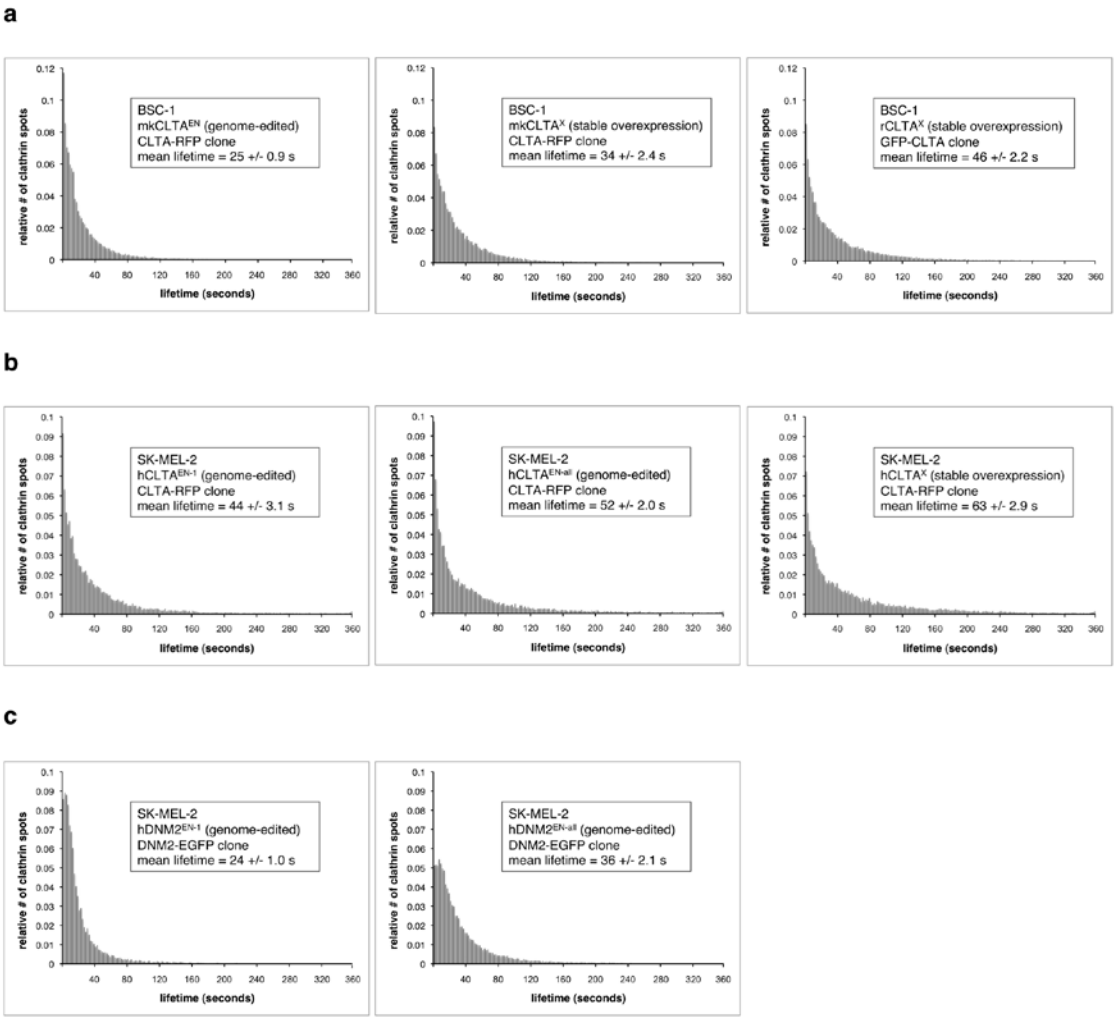

Figure S4 Endocytic protein lifetime distribution of genome-edited cell lines. Lifetime distribution for endocytic proteins was determined by the elapsed time between the appearance and disappearance of fluorescent structures present in the time series (6 min) from TIRF microscopy time-lapse videos of (**a**) BSC-1, (**b**) SK-MEL-2 CLTA-RFP and (**c**) SK-MEL-2 DNM2-GFP cell lines. Exposure time: 900 ms; Acquisition: 2 s/frame.

Supplementary Movie Legends

Movie S1 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative BSC-1 cell stably overexpressing rat brain-derived CLTA-GFP (rCLTA^{x}). Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 3.3 MB)

Movie S2 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative BSC-1 single-allele tagged genome-edited cell expressing monkey-derived CLTA-RFP (mkCLTA^{EN}). Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 3.3 MB)

Movie S3 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative BSC-1 cell stably overexpressing monkey-derived CLTA-RFP (mkCLTA^{x}). Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 3.3 MB)

Movie S4 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative SK-MEL-2 single-allele tagged genome-edited cell expressing human-derived CLTA-RFP (hCLTA^{EN-1}). Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 3.3 MB)

Movie S5 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative SK-MEL-2 cell stably overexpressing human-derived CLTA-RFP (hCLTA^{x}). Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 3.3 MB)

Movie S6 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative SK-MEL-2 all-allele tagged genome-edited cell expressing human-derived CLTA-RFP (hCLTA^{EN-all}). Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 3.3 MB)

Movie S7 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative SK-MEL-2 single-allele tagged genome-edited cell (hDNM2^{EN-1}) expressing human-derived DNM2-GFP. Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 3.3 MB)

Movie S8 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative SK-MEL-2 all-allele tagged genome-edited cell (hDNM2^{EN-all}) expressing human-derived DNM2-GFP. Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 3.3 MB)

Movie S9 This movie shows a TIRF microscopy time-lapse video (6 min) of a representative SK-MEL-2 single-allele CLTA-RFP and all-allele DNM2-GFP tagged genome-edited cell (hCLTA^{EN}/DNM2^{EN}). Exposure time= 900 ms; Acquisition= 2 s / frame. (AVI, 6.7 MB)

Table S1 Summary of genome-editing statistics. Top panel: The number of genotyped clones is given for each target in each cell type, along with the number (percentage) of correctly tagged clones having at least one (but less than all) allele tagged, or all alleles tagged. Clones that had smaller, larger, or unexpected combinations of out-out PCR products are classified as "Imprecise or Ambiguous". Note that in all cases, cells were enriched by FACS for the cognate fluorescent marker prior to limiting dilution and genotyping. Lower panel: Summary of genotypes for genome-edited clones. Each clone was processed by out-out PCR (as in Figs. 1b, 2a, 3b, and 4a), and the number of topo clones representing tagged or untagged alleles is given. The genotype of these alleles is shown in parenthesis. An asterisk (*) denotes alleles containing the indicated short insertion/deletion (number in parentheses) at the predicted cleavage site for DNM2 ZFN pair, which is located 43 bp downstream of the DNM2 stop codon in the 3' UTR.

Target	Cell line	Clones genotyped	Correctly targeted clones		Imprecise or Ambiguous	Untagged
			1 or 2 copies tagged	All copies tagged		
CLTA	BSC-1	26	12 (46.2)	8 (30.8)	5 (19.2)	1 (3.8)
CLTA	SK-MEL-2	73	34 (46.6)	22 (30.1)	7 (9.6)	10 (13.7)
DNM2	SK-MEL-2	73	25 (34.2)	13 (17.8)	12 (16.4)	23 (31.5)

Designation	Clone	Cell line	Tagged Gene(s)	Topo clones sequenced	# of reads (allele)	
					untagged	tagged
mkCLTA^{EN}	26	BSC-1	CLTA	23	17 (WT)	6 (WT)
hCLTA^{EN-1}	96	SK-MEL-2	CLTA	15	11 (WT)	4 (WT)
hCLTA^{EN-all}	66	SK-MEL-2	CLTA	19	0	19 (WT)
hDNM2^{EN-1}	20	SK-MEL-2	DNM2	23	20 (WT)	3 (WT)
hDNM2^{EN-all}	95	SK-MEL-2	DNM2	24	0	31 (Δ13)*
hCLTA^{EN}/DNM2^{EN}	13	SK-MEL-2	CLTA	21	16 (WT)	5 (WT)
			DNM2	24	0	24 (+3)*

Table S2 ZFN target sites and designed zinc finger helix sequences (top panel). Bases in lower-case are skipped from a DNA recognition perspective by the zinc finger proteins (ZFPs). The amino acid sequences of the ZFPs are in the bottom panel, with the recognition alpha-helices underlined.

Gene	ZFN Binding Sequence (underlined)	ZFN	Finger 1	Finger 2	Finger 3	Finger 4	Finger 5	Finger 6
CLTA	CCTCAAgCAGGCCCCGCTGgtgcactGAAGAGcCACCCTGTG	CLTA-R	RSDSLSV	HNDSRKN	DQSNLRA	RSANLAR	QSGNLAR	-
	GGAGTTcGTCCGGGGCGACcacgtgaCTTCTCgGTGGGACAC	CLTA-L	RSDHLSA	SYWSRTV	RSDALSV	DSSHRTR	RSDHLSE	NSRNRKT
DNM2	GGGGGCCTCaCGCACCCgcggcGCAGGAGCTTCAGTG	DNM2-R	RSDSLLR	QSADRTK	QSSDLRR	QSGHLQR	QSGDLTR	-
	CCCCCGGAGtGCGTGGgcgccgCGTCCTCGAAGTCAC	DNM2-L	DRSTLRQ	DRSDLSR	RSDNLTR	RSDDLTR	TSGHLSR	-

CLTA-R:

VPAAMAERPFQCRICMRNFS**RSDSLSV**HIRTHTGEKPFACDICGRKFA**HNDSRKN**HTKIHTGEKPFQCRICMRKFA**DQSNLRA**HTKIHTHPRAPIPKPFQCRICMRNFS**RSANLAR**HIRTHTGEKPFACDICGRKFA**QSGNLAR**HTKIHLRGS

CLTA-L:

VPAAMAERPFQCRICMRNFS**RSDHLSA**HIRTHTGEKPFACDICGRKFA**SYWSRTV**HTKIHTHPRAPIPKPFQCRICMRNFS**RSDALSV**HIRTHTGEKPFACDICGRKFA**DSSHRTR**HTKIHTGSQKPFQCRICMRNFS**RSDHLSE**HIRTHTGEKPFACDICGRKFA**NSRNRKT**HTKIHLRGS

DNM2-R:

VPAAMAERPFQCRICMRNFS**RSDSLLR**HIRTHTGEKPFACDICGRKFA**QSADRTK**HTKIHTGSQKPFQCRICMRKFA**QSSDLRR**HTKIHTGEKPFQCRICMRNFS**QSGHLQR**HIRTHTGEKPFACDICGRKFA**QSGDLTR**HTKIHLRGS

DNM2-L:

VPAAMAERPFQCRICMRKFA**DRSTLRQ**HTKIHTGEKPFQCRICMRNFS**DRSDLSR**HIRTHTGEKPFACDICGRKFA**RSDNLTR**HTKIHTHPRAPIPKPFQCRICMRNFS**RSDDLTR**HIRTHTGEKPFACDICGRKFA**TSGHLSR**HTKIHLRGS

Table S3 Lifetime analysis of endocytic proteins in various cell lines.

BSC-1

Designation	Description	Lifetime (seconds) mean	25th percentile	median	75th percentile	% > 358	SD	SEM	n = # tracks; k = # cells
rCLTA^{X}	GFP-CLTA stable overexpression (rat brain)	46.2	10	26	60	0.74	8.68	2.24	n=32,366 k=15
mkCLTA^{EN}	Clone 26 - genome-edited CLTA-RFP (african green monkey kidney)	25.4	6	14	32	0.03	2.81	0.85	n=50,250 k=11
mkCLTA^{X}	Clone OE 89 - stable overexpression CLTA-RFP (african green monkey kidney)	34.5	8	22	46	0.18	5.30	2.37	n=30,734 k=5

SK-MEL-2

Designation	Description	Lifetime (seconds) mean	25th percentile	median	75th percentile	% > 358	SD	SEM	n = # tracks; k = # cells
hCLTA^{EN-1}	Clone 96 - genome-edited CLTA-RFP (human skin)	43.8	8	24	54	1.45	8.77	3.10	n=14,868 k=8
hCLTA^{EN-all}	Clone 66 - genome-edited CLTA-RFP (human skin)	52.1	8	24	68	3.81	7.27	2.02	n=19,525 k=13
hCLTA^{X}	Clone OE 20 - stable overexpression CLTA-RFP (human skin)	62.9	12	36	86	3.86	9.54	2.88	n=19,190 k=11
hDNM2^{EN-1}	Clone 20 - genome-edited DNM2-EGFP (human skin)	23.8	6	14	28	0.06	3.29	1.04	n=14,726 k=10
hDNM2^{EN-all}	Clone 95 - genome-edited DNM2-EGFP (human skin)	36.3	10	22	46	0.14	7.60	2.11	n=29,681 k=13
hCLTA^{EN}/DNM2^{EN}	Clone 13 - genome-edited CLTA-RFP (human skin)	44.5	8	26	64	0.75	2.42	0.99	n=6,799 k=6
	Clone 13 - genome-edited DNM2-EGFP (human skin)	31.5	10	22	40	0.03	7.28	3.26	n=3,944 k=5

Table S4 Sequences of oligos used in the PCR, Cel-1, and sequencing reactions.

Oligo	Sequence
BSC-1 CLTA Cel-1 F	GCAGCAGAAGAAGCCTTTGT
BSC-1 CLTA Cel-1 R	TTACTCCTCCCCTTCCTCTC
SK-MEL-2 CLTA Cel-1 F	GCAGCAGAAGAAGCCTTTGT
SK-MEL-2 CLTA Cel-1 R	TTCCTCCTCTCCCTCCTCTC
SK-MEL-2 DNM2 Cel-1 F	CCCTCCCCACCTGTCTTTAT
SK-MEL-2 DNM2 Cel-1 R	GAGACTCCATCCCCCAAAGT
BSC-1 CLTA TI F	AAAACTGGCTTTGGGTCCTAGC
BSC-1 CLTA TI F	CATCACCTAAAACGAGCCAGGT
SK-MEL-2 CLTA TI F	ATTCTGGGCTGCACCTTATCAA
SK-MEL-2 CLTA TI R	CATCACCTAAAACGAGCCAGGT
SK-MEL-2 DNM2 TI F	GCTCAACATCATCGGTGACATC
SK-MEL-2 DNM2 TI R	GATTTCCTGGCCCCTCTACTGT
BSC-1 CLTA-HA-F	GTCCTTTCCGGCTGTAGCTCC
BSC-1 CLTA-HA-R	CATGTGCCTGGTAAATACTGCATTGG
SK-MEL-2 CLTA-HA-F	GGTGGGCTGACCTTGAT
SK-MEL-2 CLTA-HA-R	TCTATAACCTGTGCTATCCGAG
BSC-1 CLTA-QC-F	GCCCCGCTGGTGCACGGTACCGCCACCCTGTGGAAAC
BSC-1 CLTA-QC-R	GTTTCCACAGGGTGGCGGTACCGTGCACCAGCGGGGC
SK-MEL-2 CLTA-QC-F	CAAGCAGGCCCCGCTGGTGCACGGTACCGCCACCCTGTGGAAACACTACATCTGC
SK-MEL-2 CLTA-QC-R	GCAGATGTAGTGTTTCCACAGGGTGGCGGTACCGTGCACCAGCGGGGCCTGCTTG
BSC-1 RFP-Kpn-F	GACGTCGAGGGTACCAGCGGCGGAAGCATGGTGTCTAAGGGCGAAGAGCTG
BSC-1 RFP-Kpn-R	GACGTCGAGGGTACCGTGCACCAGCGGGGCCTGCTTGAGGG
SK-MEL-2 RFP-Kpn-F	GGTACCAGCGGCGGAAGCATGGTGTCTAAGGGCGAAGAGC
SK-MEL-2 RFP-Kpn-R	GGTACCGAATTCATTACTTGTACAGCTCGTCCATGC
DNM2-HA-F	TGTTTGCCAACAGTGACCTC
DNM2-HA-R	GTGCAGGGGGTCAGAGAATA
DNM2-QC-F	CCAGCCGAACCATCCCTGCTCGACGGTACCTCGAGGGGGGCGTGCTCT
DNM2-QC-R	AGAGCACGCCCCCCTCGAGGTACCGTCGAGCAGGGATGGTTCGGCTGG
GFP-Kpn-F	GGTACCAGCGGCGGAAGCATGGTGAGCAAGGGCGAG
GFP-Kpn-R	GGTACCGAATTCATTACTTGTACAGCTCGTCCATGC
CLTA-SQ R	ATGCCAGGGAGAACACAGT
DNM2-SQ R	GCCAGCGTTAAGGAAGAGG