antibodies

MDPI

Supplementary: A strategy to optimize the generation of stable chromobody cell lines for visualization and quantification of endogenous proteins in living cells

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Supplementary information

DNA oligo name	Sequence, 5′ - 3′
NB-ubi-for	ATA TAT CTG CAG GAG TCT GGG GGA GGC TTG GTG CA
NB-ubi-rev	ATA TAT TCC GGA GGA GAC GGT GAC CTG GGT CCC
β-actin-promoter-for	GGA ATT AAT ACT GCC TGG CCA CTC CAT G
β-actin-promoter-rev	TCC GCT AGC TCG GCA AAG GCG AGG C
β-actin-promoter-mutPstI-for	AGA GCT CCT TGT GCA GGA GCG
β-actin-promoter-mutPstI-rev	TGG AGG GCA TGG AGT GGC
AAVS1-CB-donor-fragment-2-for	TAG AGG CGG CAA TTG TTC A
AAVS1-CB-donor-fragment-2-rev	TGT TGT TAA CTT GTT TAT TGC AGC
Seq-AAVS1-CB-donor-1	TAT GGA AAA ACG CCA GCA AC
Seq-AAVS1-CB-donor-2	ATG TGG CTC TGG TTC TGG G
Seq-AAVS1-CB-donor-3	AGC GGC TCG GCT TCA C
Seq-AAVS1-CB-donor-4	CCT TAG ATG TTT TAC TAG CCA GAT
genPCR-AAVS1-int-for	TCG ACT TCC CTT CTT CCG ATG
genPCR-AAVS1-int-rev	CTC AGA TTC TGG GAG AGG GTA
EF1α-promoter (gBlock® gene fragment)	TTACCGCCATGCATTAGTTATTAATGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACAT CGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCATTTGAACCGGTGCCTAGAG AAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCG AGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAA CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCT TTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTCCAGTACGTGATTC TTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGG AGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGC GAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCC AAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGC GTCCCAGCGCACTTGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGA CGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGTCTCGCGCCGCCGTGTAT CGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAG ATGGCCGCTTCCCGGCCCTGCTCCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCG CTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGC TTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCAC ACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGA ATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAG TTTTTTTCTTCCATTTCAGGTGTCGTGAGCTAGCGCCACCATGCAGATCTTCG
AAVS1-CB-donor fragment 1 (gene synthesis, plasmid DNA)	CCTTTTGCTGGCCTTTTGCTCACATGTTGCTTTCTCTGACCAGCATTCTCTCCCCTGGGCC TGTGCCGCTTTCTGTCTCCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCCCAGATCCT TCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCTGCTG TGTTGCTGCCCAAGGATGCTCTTTCCGCAGCACTTCCTTCTCGGCGCTGCACCACGTGAT GTCCTCTGAGCGCATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACC CAACCCCATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCC ATCTCTCGTTTCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCT TCTTGTAGGCCTGCATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTG GCTTTAGCCACCTCTCCATCCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGAT TCGGGTCACCTCTCACTCCTTTCATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTC TGTGCAAGCTCTTCCAGCCCCCTGTCATGGCATCTTCCAGGGGTCCGAGAGCTCAGCTA GTCTTCTTCCTCCAACCCGGGCCCCTATGTCCACTTCAGGACAGCATGTTTGCTGCCTCC AGGCATCCTGTGTCCCCGAGCTGGGACCACCTTATATTCCCAGGGCCGGTTAATGTGGC TCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGGGGCAAGCTTCTGACCT CTTCTCTTCCTCCCACAGGGCCTCGAGAGATCTGGCAGCGGAGAGGGCAGAGGAAGTC TTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTAGGCTCGAGATGACAGAATA CAAACCCACGGTGCGGCTCGCGACTCGCGATGACGTGCCCCGAGCGGTGAGAACATTG GCAGCAGCGTTCGCAGACTATCCGGCTACGCGGCATACTGTCGATCCTGATCGACATAT TGAACGGGTCACCGAGTTGCAGGAGCTTTTCCTCACACGCGTTGGATTGGATATTGGCA AAGTATGGGTCGCGGACGACGGGGCAGCTGTCGCCGTGTGGACCACCCCCGAGTCAGT GGAGGCTGGAGCGGTATTCGCTGAGATCGGCCCTCGGATGGCAGAATTGAGCGGCTCC AGACTGGCGGCTCAACAGCAGATGGAAGGGCTCCTCGCCCCTCATAGACCTAAGGAAC CTGCTTGGTTCCTCGCAACCGTGGGCGTCTCACCAGACCATCAGGGGAAGGGCCTTGG GTCTGCGGTGGTCTTGCCGGGGGTCGAGGCaGCAGAGAGAGCTGGGGTACCCGCGTTTT TGGAAACAAGTGCGCCCCGAAACCTCCCGTTTTACGAACGGCTTGGCTTTACAGTCACA

	GCAGATGTTGAAGTACCGGAGGGACCAAGGACCTGGTGCATGACCCGCAAGCCGGGA GCTTGATCAAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTG CCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCC CACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATT CTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATA GCAGGCATGCTGGGGATGCGGTGGGCTCTATGGACTAGTATTAATTAAATCTAGAGGC GGCAATTGTTCACTCCTCAGGTGCAGGC
AAVS1-CB-donor fragment 2 (gBlock® gene fragment)	AAATCTAGAGGCGGCAATTGTTCACTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGT GGCTGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATCTTTTTCCCTCTGCCA AAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAA ATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATAT GGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACAT ATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGA AACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTT TTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTAGATGTTT TACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCGT CGACAGTACTAAGCTTTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCTAGTCTC CTGATATTGGGTCTAACCCCCACCTCCTGTTAGGCAGATTCCTTATCTGGTGACACACCC CCATTTCCTGGAGCCATCTCTCTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGAGC CAGAGAGCATCCTGGGAGGGAGAGCTTGGCAGGGGGTGGGAGGGAAGGGGGGGATG CGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCCAGAACCTGAGCTG CTCTGACGCGGCTGTCTGGTGCGTTTCACTGATCCTGGTGCTCCAGCTTCCTTACACTTC CCAAGAGGAGAAGCAGTTTGGAAAAACAAAATCAGAATAAGTTGGTCCTGAGTTCTA ACTTTGGCTCTTCACCTTTCTAGTCCCCAATTTATATTGTTCCTCCGTGCGTCAGTTTTAC CTGTGAGATAAGGCCAGTAGCCAGCCCCGTCCTGGCAGGGCTGTGGTGAGGAGGGGG GTGTCCGTGTGGAAAACTCCCTTTGTGAGAATGGTGCGTCCTAGGTGTTCACCAGGTCG TGGCCGCCTCTACTCCCTTTCTCTTTCTCCATCCTTCTTTCCTTAAAGAGTCCCCAGTGCT ATCTGGGACATATTCCTCCGCCCAGAGCAGGGTCCCGCTTCCCTAAGGCCCTGCTCTGG GCTTCTGGGTTTGAGTCCTTGGCAAGCCCAGGAGAGGCGCTCAGGCTTCCCTGTCCCCC TTCCTCGTCCACCATCTCATGCCCCTGGCTCTCCTGCCCCTTCCCTACAGGGGTTCCTGG CTCTGCTCTCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGG

Table S1: List of DNA oligonucleotides and synthesized gene fragments used in this study

	Expression construct
1.	pEGFP-N1 (Clontech)
2.	Ub-M-BC1-CB [1]
3.	Ub-R-BC1-CB [1]
4.	LMN-CB [2]
5.	Ub-M-LMN-CB (this study)
6.	Ub-R-LMN-CB (this study)
7.	(EF1α)-Ub-R-BC1-CB (this study)
8.	(β-actin)-Ub-R-BC1-CB (this study)
9.	mCherry [3]
10.	mCherry-CTNNB1 [1]
11.	Cas9_gRNA [4]
12.	AAVS1_Ub-R-BC1-CB (this study)
13.	Actin-CB [5]
14.	AAVS1_Ub-R-ACT-CB (this study)

Table S2: List of mammalian expression constructs used in this study

Supplementary Figures

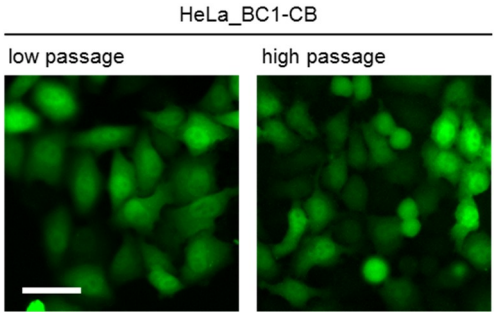

Figure S1 Stable HeLa_BC1-CB cell line displays heterogeneity in CB expression at high cell passages.

Illustration of HeLa cells stably expressing the BC1-CB driven by the CMV promoter at an early passage (passage number 4, left side) and at a high passage (passage number 20, right side). Scale bar: 50 μm.

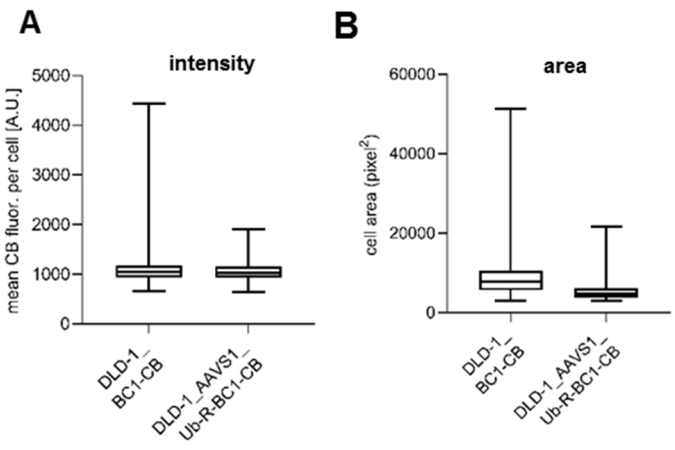

Figure S2 Population-wide analysis of BC1-CB signal in DLD-1_BC1-CB cells and DLD-1_AAVS1_Ub-R-BC1-CB.

Fluorescence images of DLD-1_BC1-CB cells and DLD-1_AAVS1_Ub-R-BC1-CB (as illustrated in **Figure 6C**) were subjected to quantitative image analysis. Using a customized image segmentation algorithm (see Material and Methods), the mean fluorescence intensity of the CB (**A**) and the cell area (**B**) were determined for each individual cell imaged. Number of analyzed cells: DLD-1_BC1: n=2478; DLD1_AAVS1_Ub-R-BC1-CB: n=2090.

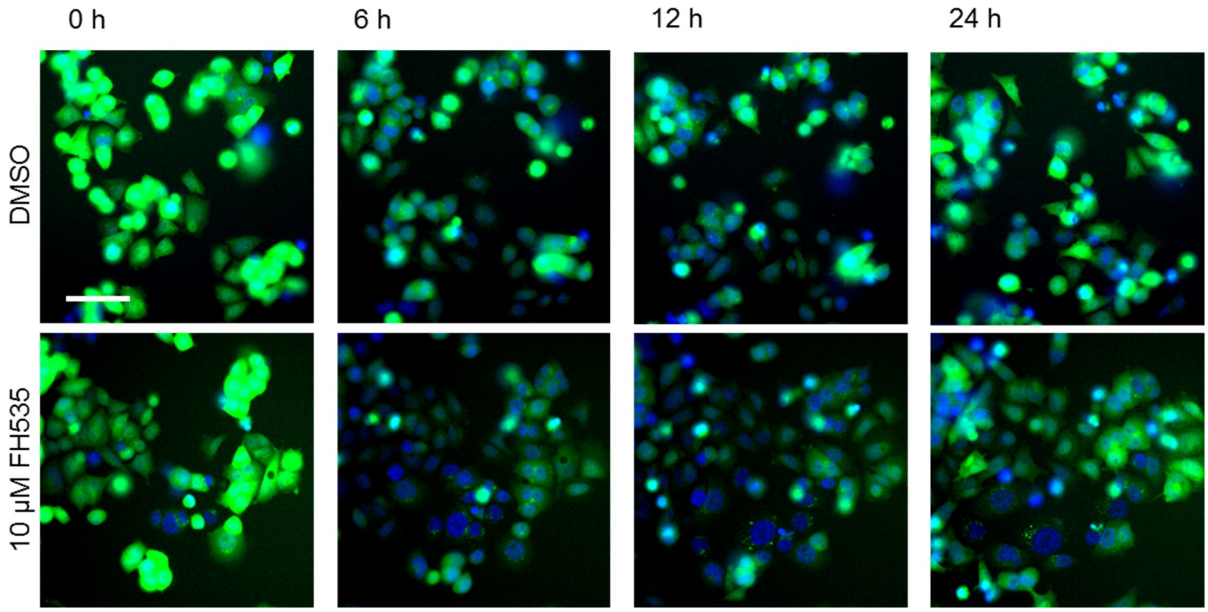

Figure S3 Fluorescence images of DLD-1_AAVS1_Ub-R-BC1-CB cells upon compound treatment.

DLD-1_AAVS1_Ub-R-BC1-CB were either treated with 10 µM FH535 or 0.01 % DMSO as control and were continuously imaged every 2 h for up to 24 h. Representative fluorescence images are shown after 0, 6, 12 and 24 h, scale bar: 50 µM.

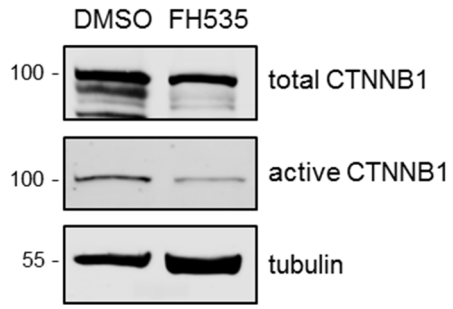

Figure S4: FH535 reduces the amount of active CTNNB1 in DLD-1_AAVS1_Ub-R-BC1-CB cells.

DLD-1_AAVS1_Ub-R-BC1-CB were either treated with 10 μM FH535 or 0.01 % DMSO as control for 24 h. Cells were lysed using lysis buffer comprising 0.5% NP-40 and equal protein amounts of the soluble fraction were subjected to SDS-PAGE followed by immunoblot analysis using antibodies specific for total CTNNB1, active CTNNB1 and tubulin as loading control.

References

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