Supplementary Table S1 - Oligonucleotides used in this study

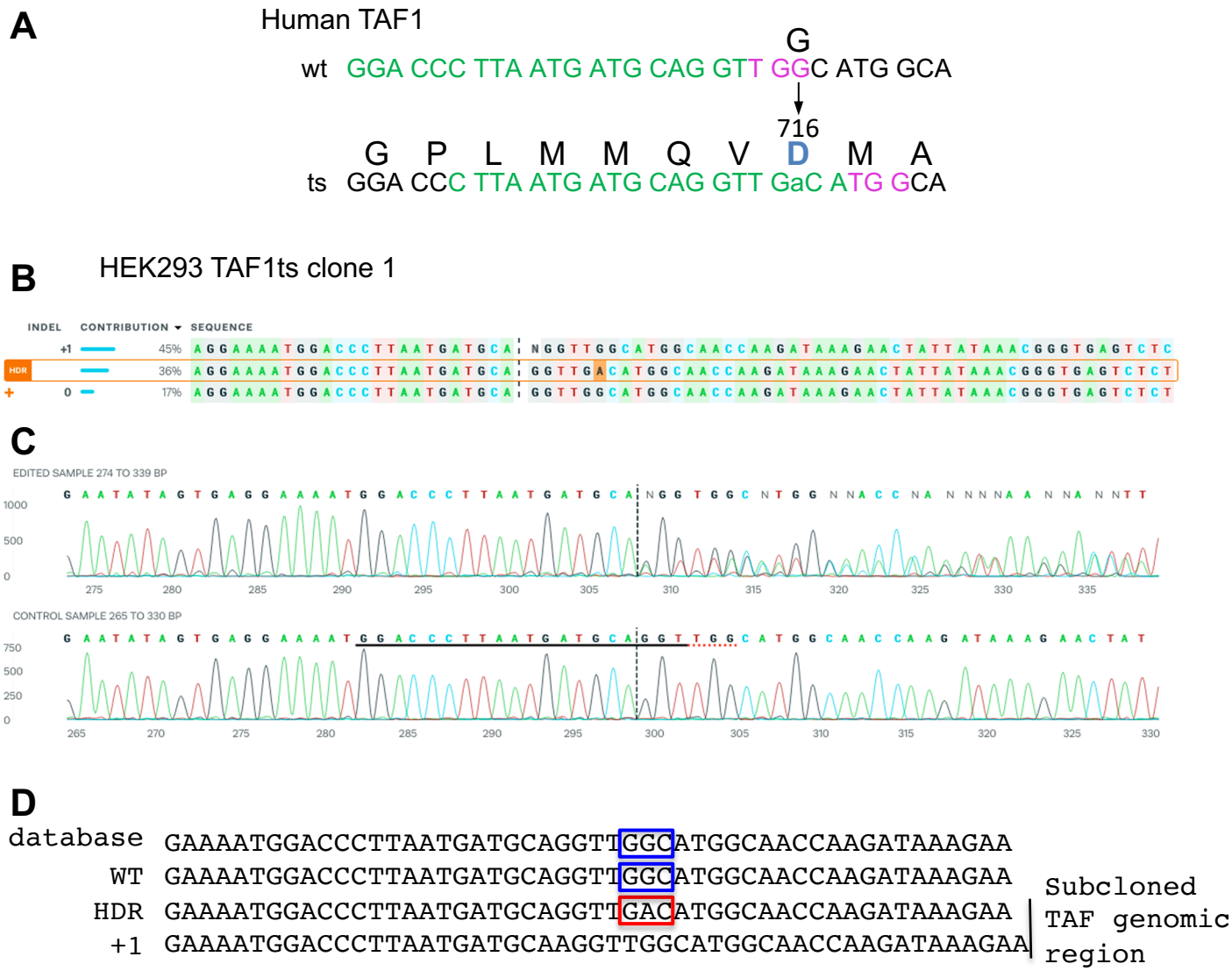
Guide for targeting human TAF1, aa716	
TAF1_g1_fw	caccGGACCCTTAATGATGCAGGT
TAF1_g1_re	aaacACCTGCATCATTAAGGGTCC
ssODN for creating ts mutation (G716D) in human TAF1	
TCTGAGCAGAGACTCACCCGTTTATAATAGTTCTTTATCTTGGTTGCCATGtCAAC CTGCATCATTAAGGGTCCATTTTCCTCACTATATTCTGCAAGAATAA	
Primers flanking the TAF1 guide site, to amplify 554bp fragment	
TAF1_hum_gen554_fw	gcagaacccatacatggatatggagg
TAF1_hum_gen554_re	tatggtatatgttcacagattaccag
Guide targeting the mutant human TAF1	
humTAF1_tsmut_g2_fw	caccgCTTAATGATGCAGGTTGaCA
humTAF1_tsmut_g2_re	aaacTGtCAACCTGCATCATTAAGc
ssODN for correcting ts mutation to make wt human TAF1	
CTGAGCAGAGACTCACCCGTTTATAATAGTTCTTTATCTTGGTTGCCATGCCAACC TGCATCATTAAGGGTCCATTTTCCTCACTATATTCTGCAAGAAT	
Primers flanking the TAF1L guide site, to amplify 879bp fragment	
TAF1L_gen879_fw	CCAAGCAACAGGGTCTTCGG
TAF1L_gen879_re	AATCAGACTTAAGCACCCACCAG
Guide for C-terminus of human PSMB6	
CR_B6_stop_g2_fw	caccgTAGAATCCCAGGATTCAGGC
CR_B6_stop_g2_re	aaacGCCTGAATCCTGGGATTCTAc
Another guide targeting C-terminus of human PSMB6 (used with HeLa ts)	
CR_B6_stop_g1_fw	caccgAATCCCAGGATTCAGGCGGG
CR_B6_stop_g1_re	aaacCCCGCCTGAATCCTGGGATTc
Primers to make human PSMB6-YFP donor template in pBluescript KS- with 1 kb homology arms	
SalI_b6_frg1_fw	ctcgag**gtcgac**cactattctgccatcctgcaggtcctacatcg
HindIII_b6_frg1_re	ggtggc**aagctt**ggcgggtggtaaagtggcaacggcgaatttggg
HindIII_ATG_Clover/YFP_fw	cccgcc**aagctt**gccaccatggtgagcaagggcgagg
BamHI_Clover/YFP_rev	gattca**ggatcc**agctcgagatctgagtccggacttgtacagctcg
BamHI_b6_Frg2_fw	cgagct**ggatcc**tgaatcctgggattctagtatgcaataagagatg
XbaI_b6_Frg2_re	ggccgc**tctaga**gcagtgagccaagaccaggctactgcactccagc
Guide for human ABL exon 2	
CRISPR_ABL_ex2_g3_fw	caccgAGATGCTACTGGCCGCTGAA
CRISPR_ABL_ex2_g3_re	aaacTTCAGCGGCCAGTAGCATCTc
Primers to make donor DNA for CMV-Clover-polyA cassette insertion into human ABL exon 2 using pBluescript KS- with 900-1000bp homology arms	
EcoRI_ABL_ex2_frg1_fw	ta**gaattc**gtctcgaggtgggggtgatcaccaaaaaaacagctctg
BamHI_ABL_ex2_frg1_re	gt**ggatcc**agggcttctggaagagaaagggg
pCDNAClov_BglII_fw	atcggg**agatct**cccgatcc
pCDNAClovPA_NotI_re	caacga**gcggccgc**tcagaagccatagagccc
NotI_ABL_ex2_frg2_fw	ta**gcggccgc**tcgttggaactccaaggaaaaccttc
SacI_ABL_ex2_frg2_re	ta**gagctc**gtctctggccaggactgctctcactctcacgcacc

Primers for checking integration into the genomic ABL locus	
CMV_integ_re	ccatatatgggctatgaactaatg
Up_AblEx2_frg1_fw (this primer is upstream of the left ABL exon 2 homology arm in the genomic sequence)	cgctgtcctagttggaagaacaggcagtgaag
Primers for amplifying 600bp region around PSMB6 stop codon	
psmb6_600gen_around_stop_fw	ACCAAGGAAGAGTGTCTGCAATTCACTGCC
psmb6_600gen_around_stop_rev	CAAGGACCTGGATATGGTGGGGGAAAGGAA
Guide for targeting aa35 of PSMB6	
Guide_B6_aa35_fw	caccgCACGGCCATGATAGTGGTCT
Guide_B6_aa35_re	aaacAGACCACTATCATGGCCGTGc
Primers to amplify 600 bp region surrounding T35A mutation site	
PSMB6_600bpgen_aa35_fw	GAGGTACCCCGAAGATGGTG
PSMB6_600bpgen_ aa35_re	TCTCATCCAAACTGAAGCGG
ssODN for creating T35A mutation in PSMB6	
TTTGTGTCTGCTGGAGAATGTAGACTTACTCCTTTTTCCCTTTTGCCAGGCCACTATCATGGCCGTGCAGTTTGACGGGGGCGTGGTTCTGGGGGCGGA	
Control guide non-targeting in human	
BFP_g2_fw	caccgCTGCACGCCGTGGGTCAGGG
BFP_g2_re	aaacCCCTGACCCACGGCGTGCAGc
Control ssODN	
ACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACATACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGAC	

Supplementary Table S2: Evaluation of CRISPR guides

Gene targeted	Guide	CHOP CHOP efficiency score	Off targets	Notes
ABL	AGATGCTACTGGCCGCTGAA	60.86	0.0.0.6	
PSMB6 stop g2	TAGAATCCCAGGATTCAGGC	64.52	0.0.0.26	Used in 293 ts
PSMB6 stop g1	AATCCCAGGATTCAGGCGGG	63.14	0.0.2.43	Used in HeLa ts
PSMB6 aa35	CACGGCCATGATAGTGGTCT	49.90	0.0.1.7	
TAF1 wt	GGACCCTTAATGATGCAGGT	64.49	1.0.0.11	
TAF1 ts	CTTAATGATGCAGGTTGaCA	49.72	1.0.1.17	wt seq used for evaluation
Non-targeting guide	CTGCACGCCGTGGGTCAGGG	No match (Synthego tool)		

sgRNAs used in this study were evaluated using the CHOPCHOP [38] or Synthego validation tools, as indicated. For the sgRNA guide targeting the ts mutation, the wild type sequence was used to evaluate the score and off target possibilities, as there is no natural target for this guide in the human genome. The off target possibilities are indicated as the number of off target sites found with 0, 1, 2, or 3 mismatches. Note that the TAF1 guides each have one “0-mismatch” off-target, due to the TAF1L gene.

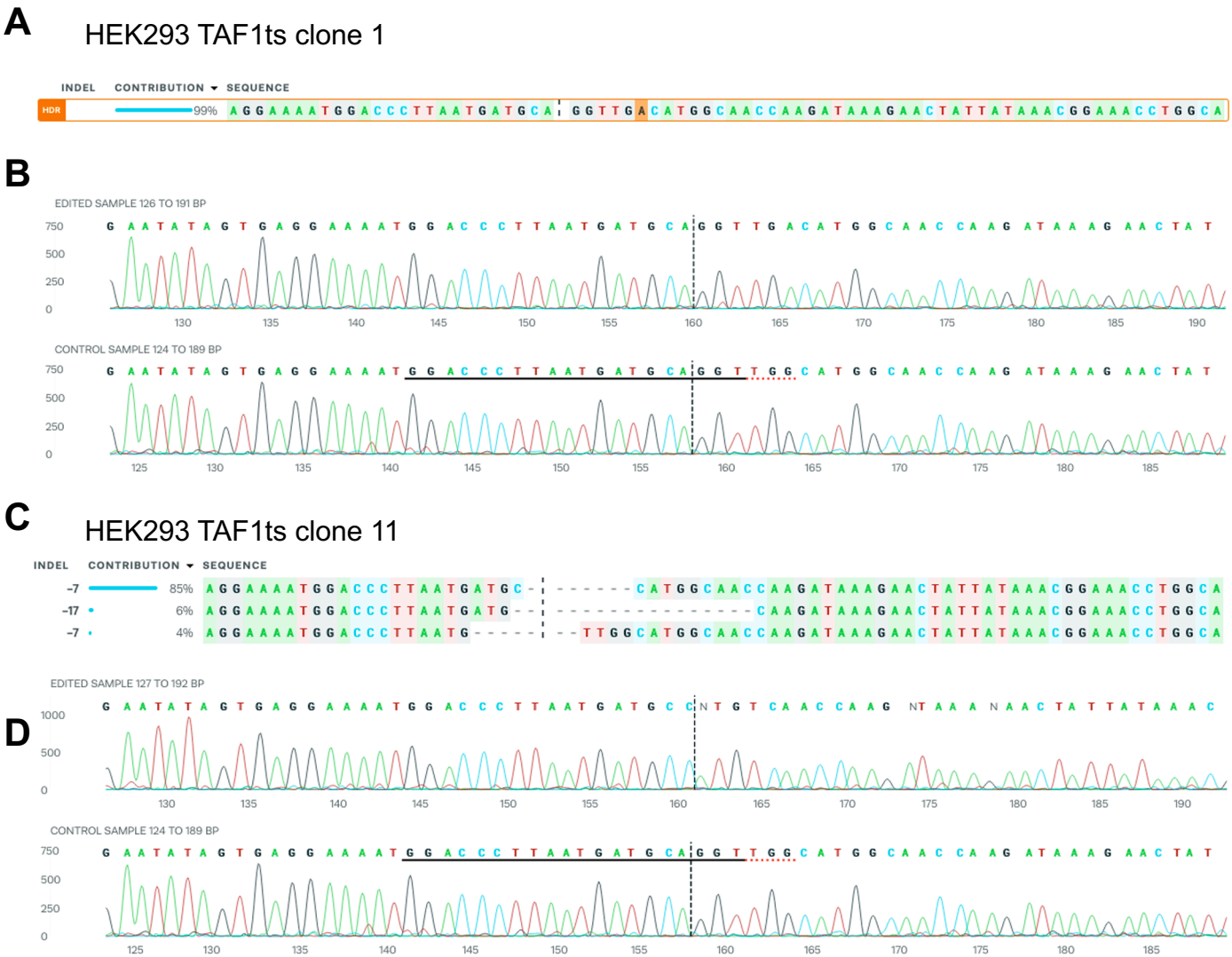


Supplementary Figure S1. Genotypic and phenotypic characterization of HEK293 TAF1 ts cells. (A) sgRNAs used to target human TAF1. The guide RNAs are shown in green, with the PAM sequences in pink. The guide used to create the TAF1 ts cells (G716D) is indicated on the upper wt sequence. The guide used to target the TAF1ts cells is indicated on the lower, ts sequence. **(B, C, D) Sequences of the TAF1 alleles in HEK293 TAF1ts clones 1.** The 554 bp fragment from the ts clone was amplified, sequenced, and the results were analyzed by using the Synthego ICE tool (Synthego Performance Analysis, ICE Analysis. 2019. v2. Synthego) **(B)**. The results show the planned G716D mutation (marked HDR), as well as +1 insertion. The insertion causes frame-shifting and early termination (10 amino acid addition, then stop codon). **(C)** Sanger sequencing results of the edited and control samples. The guide sequence is underlined, the PAM underlined with a dotted red line, and the location of the Cas9 cleavage indicated by the vertical dashed line. **(D)** In order to experimentally separate sequences of CRISPR TAFts clones, PCR on the respective genomic regions was done with the addition of BamHI and XhoI sites and cloned into pCDNA. Sequencing of 6 clones demonstrated HDR in 4 clones and +1 insertion in 2 clones, thus confirming the results of ICE analysis. The third allele of TAF1 in this clone had a large out-of-frame insertion of 174 bp downstream from the cut site, which was not well analyzed by ICE analysis of the 554bp fragment. Here (B), it was detected as a small contribution of wt sequence. Subcloning and sequencing of the 554bp and 728bp PCR products revealed the sequence of this allele, confirming that it will lead to a knocked-out gene product.

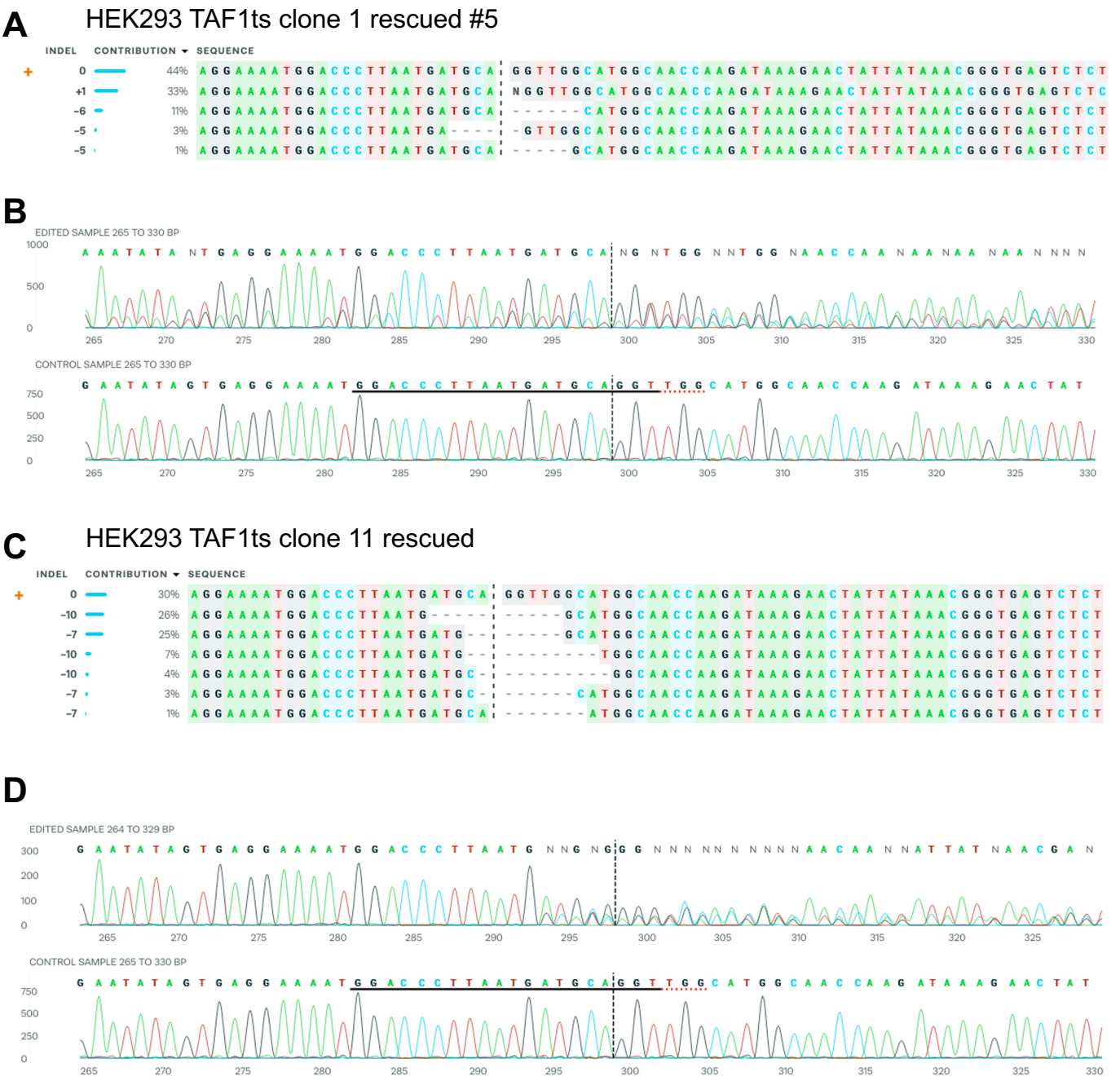
C

```
database GAAAATGGACCCTTAATGATGCAGGTTGGCATGGCAACCAAGATAAAGAA
      WT GAAAATGGACCCTTAATGATGCAGGTTGGCATGGCAACCAAGATAAAGAA
     HDR GAAAATGGACCCTTAATGATGCAGGTTGACATGGCAACCAAGATAAAGAA| subcloned
     -10 GAAAATGGACCCTTAATG:::::::::GCATGGCAACCAAGATAAAGAA| TAF genomic
      -7 GAAAATGGACCCTTAATGATG:::::::GCATGGCAACCAAGATAAAGAA| region
```

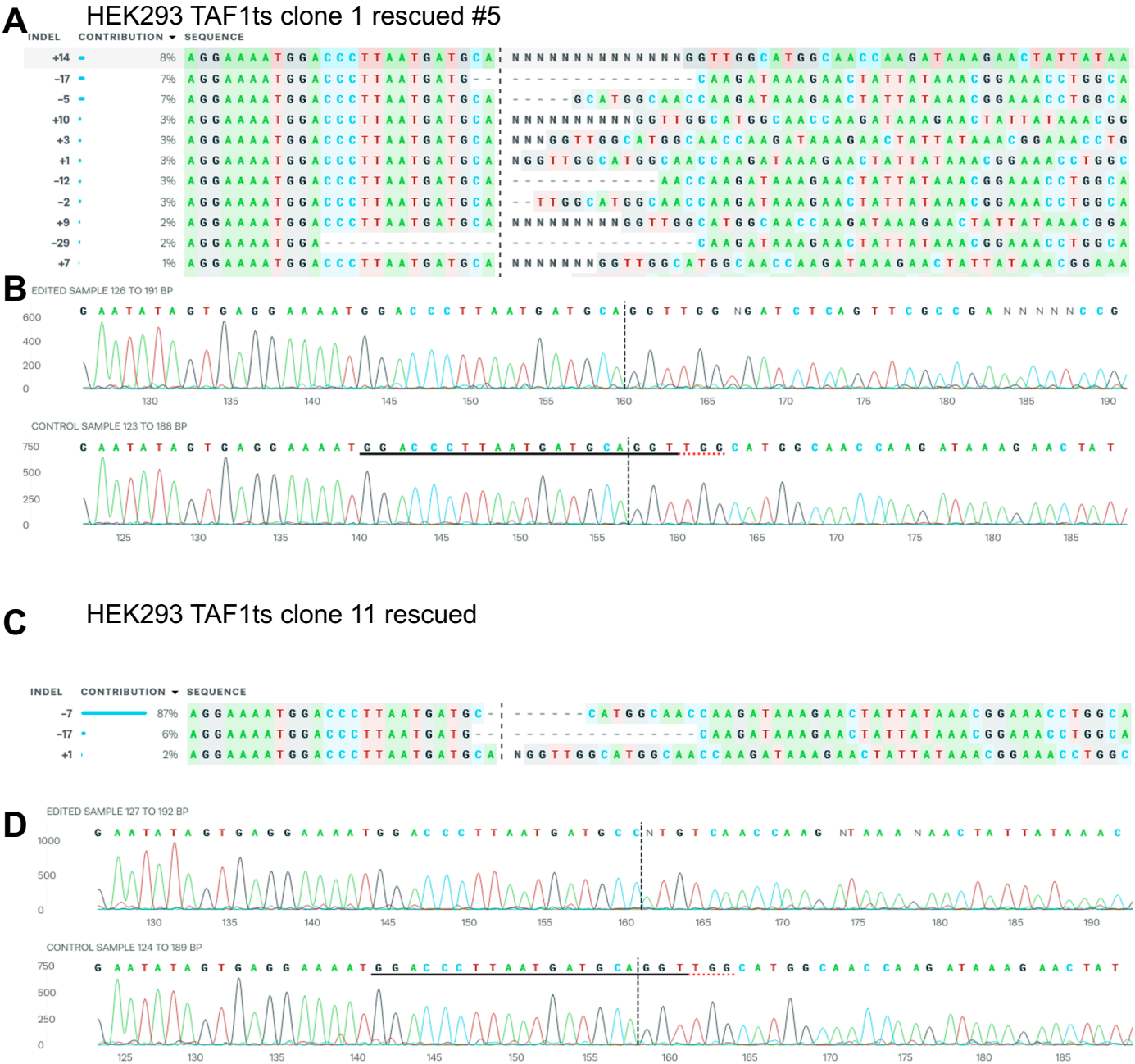
Supplementary Figure S2. TAF1 sequencing analysis of HEK293 TAF1 ts cells (clone11). The 554 bp fragment from the ts clone was amplified, sequenced, and the results were analyzed by using the Synthego ICE tool (Synthego Performance Analysis, ICE Analysis. 2019. v2. Synthego) **(A)**. The results show the planned G716D mutation (marked HDR), as well as -7 and -10 deletions. Another sequence suggestion is likely due to noisiness of the sequencing results. Both deletions cause frame-shifting and early termination (5-6 amino acid addition, then stop codon). **(B)** Sanger sequencing results of the edited and control samples. The guide sequence is underlined, the PAM underlined with a dotted red line, and the location of the Cas9 cleavage indicated by the vertical dashed line. **(C)** In order to experimentally separate sequences of CRISPR TAFts clones, PCR on the respective genomic regions was done with the addition of BamHI and XhoI sites and cloned into pCDNA. Sequencing of 11 clones demonstrated HDR in 6 clones, -10 deletion in 4 clones, and -7 deletion in 1 clone, thus confirming the results of ICE analysis



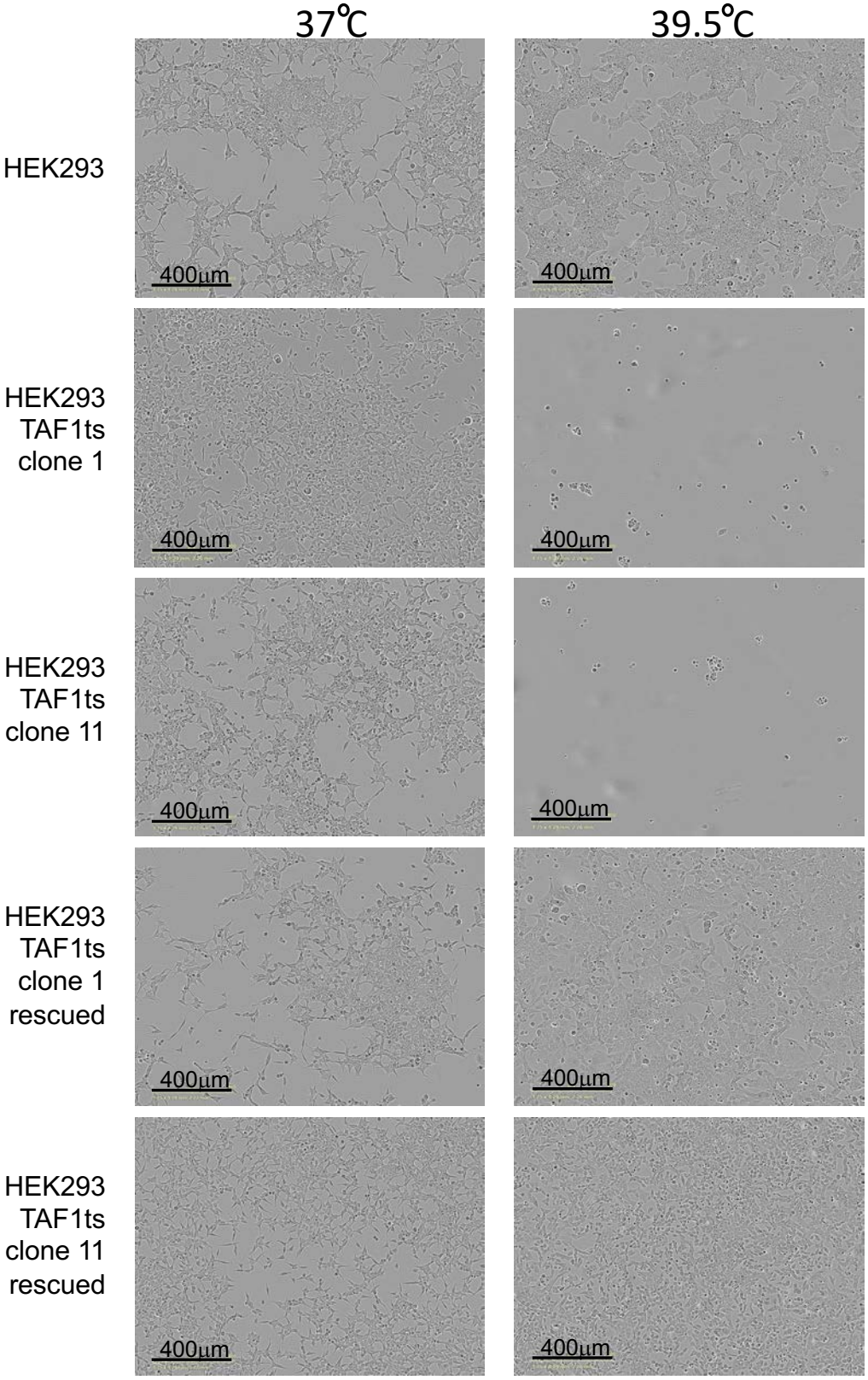
Supplementary Figure S3. TAF1L sequencing analysis of HEK293 TAF1 ts cells (clone1 and clone 11). An 879bp fragment from the ts clone was amplified, sequenced, and the results were analyzed by using the Synthego ICE tool (Synthego Performance Analysis, ICE Analysis. 2019. v2. Synthego) **(A)**. The results for HEK293 TAFts clone 1 show the TAF ts mutation. **(B)** Sanger sequencing results of the edited and control samples. The guide sequence is underlined, the PAM underlined with a dotted red line, and the location of the Cas9 cleavage indicated by the vertical dashed line. **(C)** The results for HEK293 TAFts clone 11 show -7 and -17 deletions. **(D)** Sanger sequencing results are presented, as in (B).



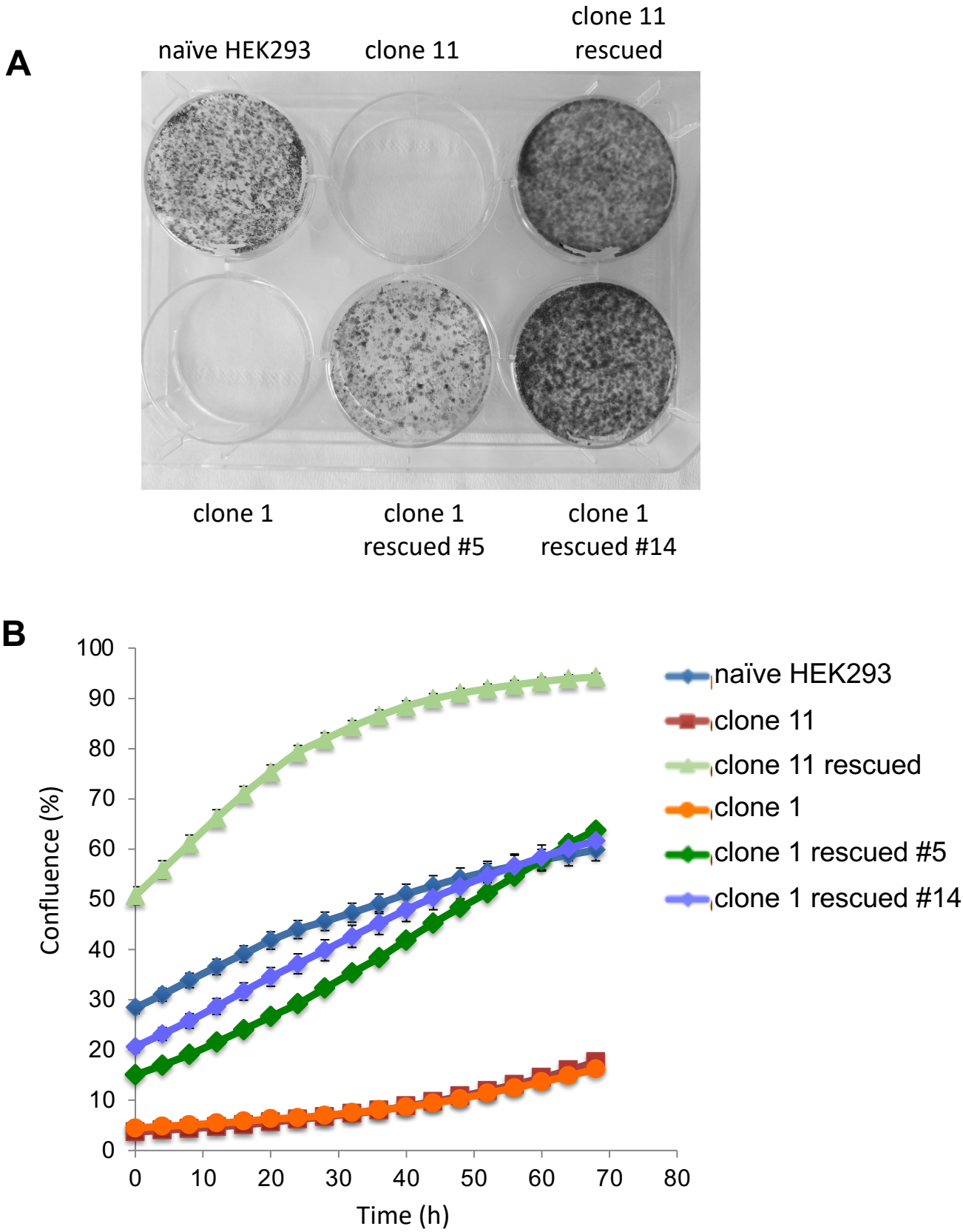
Supplementary Figure S4. TAF1 sequencing analysis of rescued clones of 293 TAF1 ts cells (clones 1 and 11). (A,B) HEK293 TAF1 ts cells (clone1 rescued #5). The 554 bp fragment from the corrected clone was amplified, sequenced, and the results were analyzed by using the Synthego ICE tool (Synthego Performance Analysis, ICE Analysis. 2019. v2. Synthego) **(A)**. The results show the complete correction of G716D mutation to the WT (marked 0), and a previously detected +1 insertion. The other sequence suggestions are likely due to noisiness of the sequencing results. **(B)** Sanger sequencing results of the edited and control samples. The guide sequence is underlined, the PAM underlined with a dotted red line, and the location of the Cas9 cleavage indicated by the vertical dashed line. **(C,D) HEK293 TAF1 ts cells (clone 11 rescued) (C)** The results from the ICE analysis show complete correction of G716D mutation back to the WT (marked 0), and the previously detected -7 and -10 deletions. The other sequence suggestions are likely due to noisiness of the sequencing results. **(D)** Sanger sequencing results of the edited and control samples.



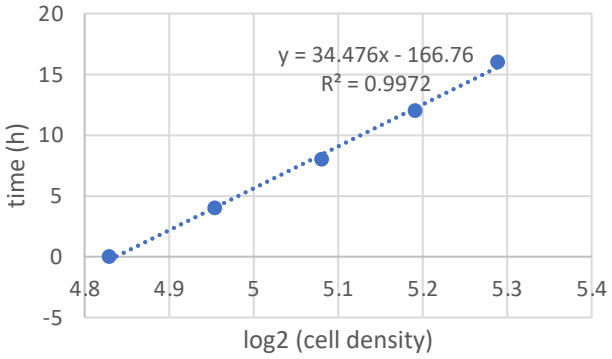
Supplementary Figure S5. TAF1L sequencing analysis of rescued clones of 293 TAF1 ts cells (clones 1 and 11). (A,B) HEK293 TAF1 ts cells (clone1 rescued #5). The 879 bp TAF1L fragment from the rescued clone was amplified, sequenced, and the results were analyzed by using the Synthego ICE tool (Synthego Performance Analysis, ICE Analysis. 2019. v2. Synthego) **(A)**. The results show a number of suggested sequences, likely due to the difficulty for the software to identify the large indel. **(B)** Sanger sequencing results of the edited and control samples. The guide sequence is underlined, the PAM underlined with a dotted red line, and the location of the Cas9 cleavage indicated by the vertical dashed line. **(C,D) HEK293 TAF1 ts cells (clone 11 rescued) (C)** The results from the ICE analysis show the previously detected -7 and -17 deletions. **(D)** Sanger sequencing results of the edited and control samples.



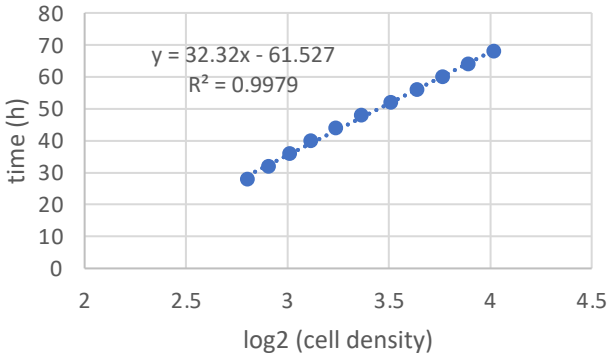
Supplementary Figure S6. Morphology of HEK293 naïve, TAF1ts, and TAF1ts rescued. HEK293 naïve, TAF1ts clones 1 and 11, and CRISPR-corrected clones of the ts cells were plated in 24 well plates at 14,000 cells/well. Cells were photographed by the Incucyte®SX1 Live-Cell Analysis System at 10x magnification. Pictures of cells growing exponentially at 37℃ 2-3 days post-plating are shown. Cells growing at 39.5 ℃ were photographed 5 days post-plating.



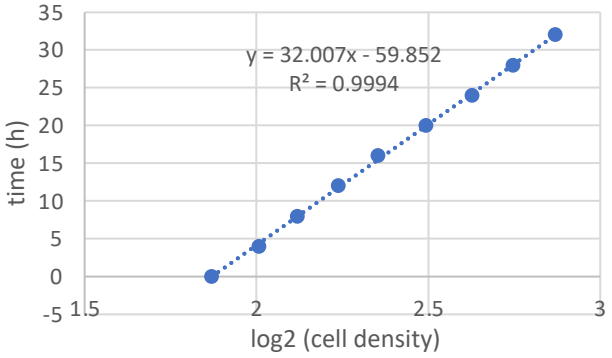
Supplementary Figure S7. Growth of HEK293 TAF1 ts and rescued clones at 39.5ºC and 37ºC. (A) Growth at 39.5ºC. HEK293 naïve, TAF1ts clones 1 and 11, and ts rescued clones were plated at 14,000 cells/well and incubated 7 days at 39.5ºC. Cells were stained with crystal violet. Two isolates of rescued clone 1 are shown (#5 and #14).**(B) Growth at 37ºC.** Cells were plated in quadruplicate in 24 well dishes at 14,000 cells/well and grown at 37ºC. Two days after plating, cells were photographed by the Incucyte®SX1 Live-Cell Analysis System at 10x magnification, 25 images per well, every 2h. Percent confluence was calculated by the Incucyte software, and was plotted vs. time.



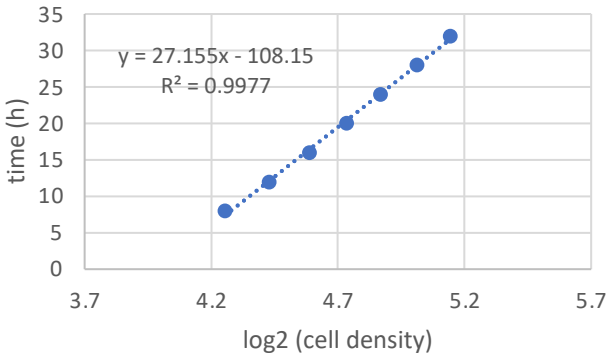
HEK293: 34.5 h



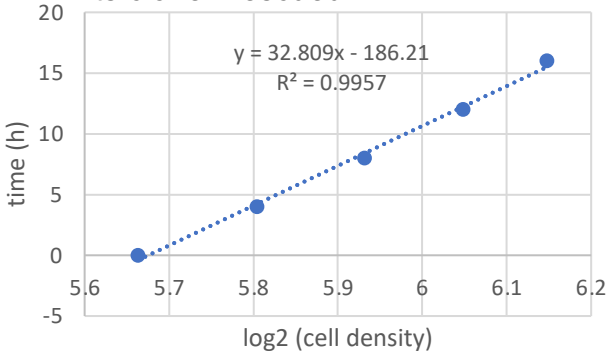
TAFts clone1: 32.3 h



TAFts clone11: 32.0 h

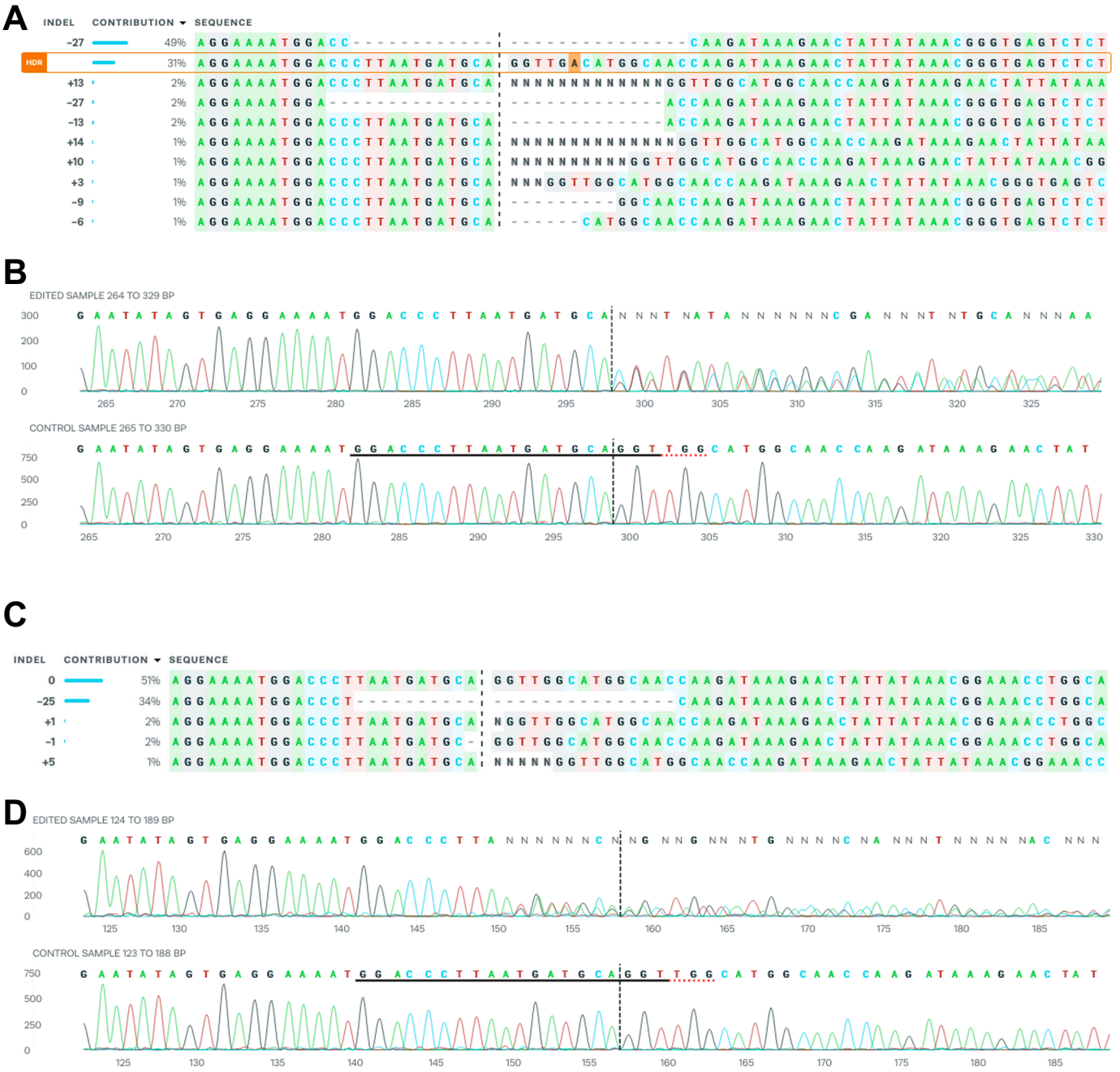


TAFts clone1 rescued: 27.2 h

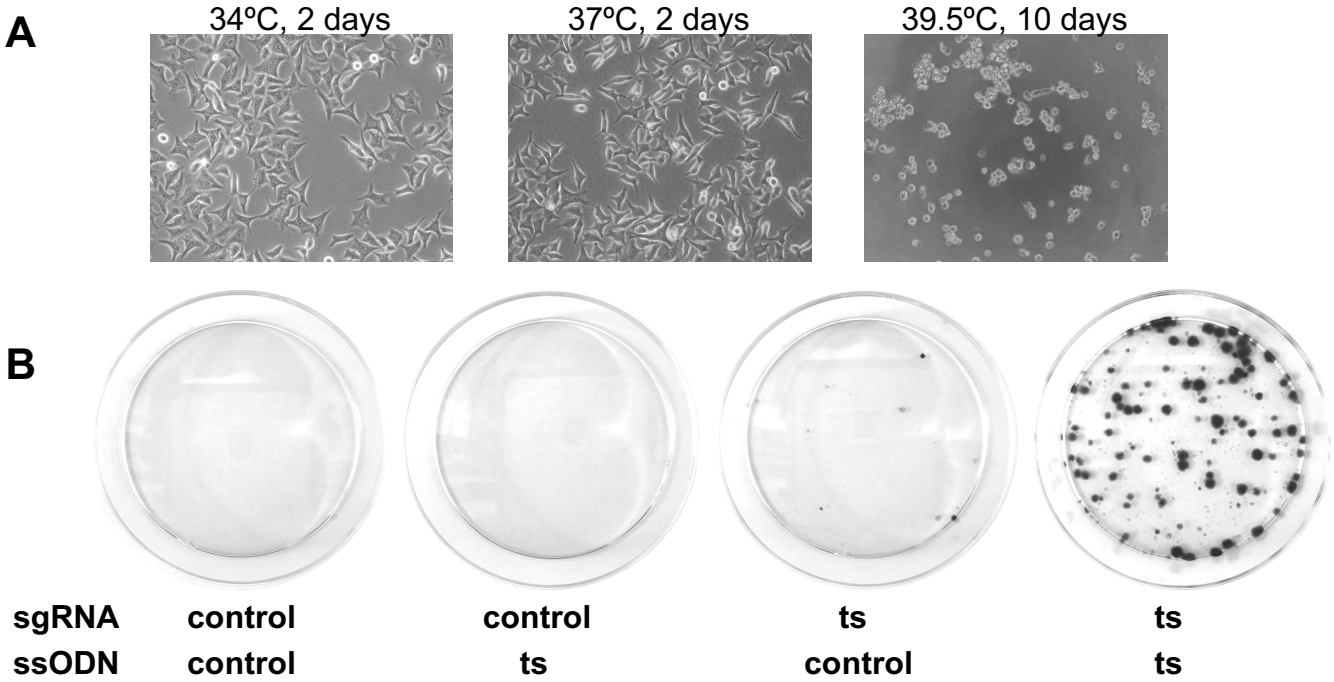


TAFts clone11 rescued: 32.8 h

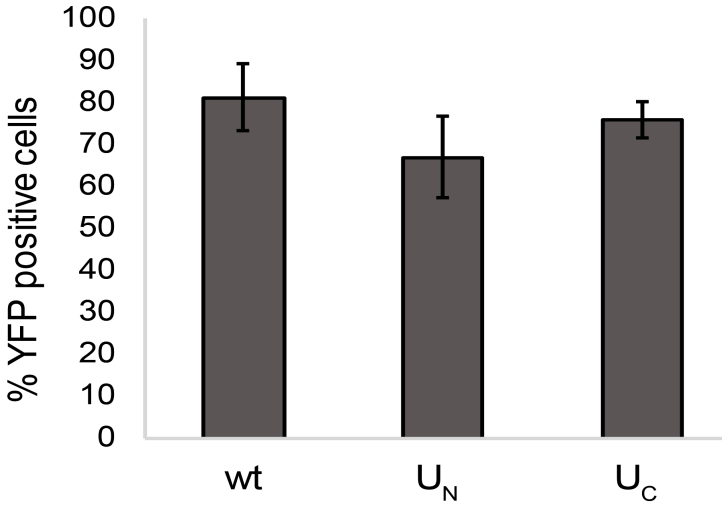
Supplementary Figure S8. Doubling time calculation of naïve, ts, and ts rescued cells. HEK293 naïve, TAF1ts clones 1 and 11, and CRISPR-rescued clones of the ts cells were plated in quadruplicate in 24 well plates at 14,000 cells/well. Cells were photographed by the Incucyte®SX1 Live-Cell Analysis System at 10x magnification, 25 images per well, every 2h. Percent confluence was calculated by the Incucyte analysis software. Log2 of the confluence was plotted vs time for cells in exponential growth, with the slope of the line being the doubling time of the cells. The calculated doubling time is indicated below each graph.



Supplementary Figure S9. TAF1 and TAF1L sequencing analysis of HeLa TAF1 G716D ts cells. (A) The 554 bp TAF1 fragment from the ts clone was amplified, sequenced, and the results were analyzed by using the Synthego ICE tool (Synthego Performance Analysis, ICE Analysis. 2019. v2. Synthego). The results show the planned G716D mutation (marked HDR) as well as large -27 deletion including the G716 position. The other sequence suggestions are likely due to noisiness of the sequencing results. **(B)** Sanger sequencing results of the edited and control samples. The guide sequence is underlined, the PAM underlined with a dotted red line, and the location of the Cas9 cleavage indicated by the vertical dashed line. **(C)** The 879 bp TAF1L fragment was analyzed as in (A). **(D)** Sanger sequencing results of the edited and control TAF1L samples, as in (B).



Supplementary Figure S10. Growth of HeLa TAF1 ts cells and rescue of the ts mutation. (A) HeLa TAF1 ts were grown at 34ºC, 37ºC and 39.5ºC, and photgraphed two or ten days post-plating, as indicated. **(B)** HeLa TAF1 ts cells were transfected with Cas9/sgRNA-encoding plasmids and ssODN. Two days post-transfection, cells were replated, and transferred the next day to 39.5 °C. 18 days later, colonies were stained with crystal violet.



Supplementary Figure S11. Co-editing of PSMB6-YFP in HEK293 TAF1 ts cells with MRN-recruiting Cas9 constructs. HEK293 TAF1 ts cells were transfected as described in Figure 4B, in quadruplicates, using either wt Cas9, or the MRN-recruiting Cas9 constructs U_N and U_C. Heat-selected colonies were analyzed for YFP expression, and the percent of YFP positive colonies out of total heat-resistant colonies is summarized. N=4, error bars represent SEM.

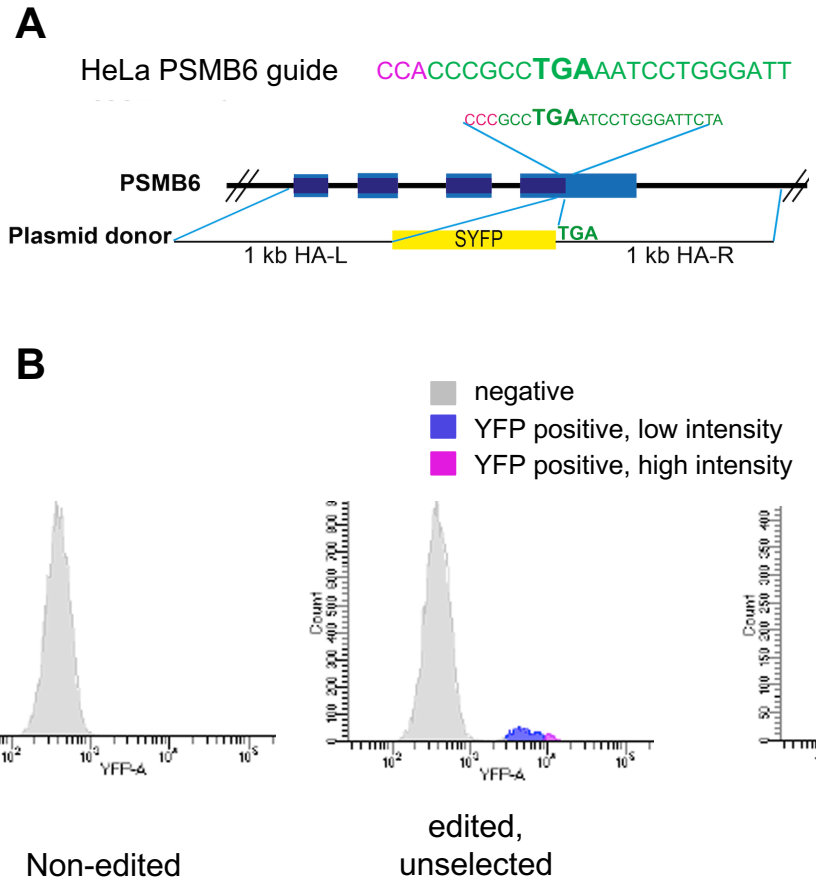
Unselected, co-edited pool : no editing observed

Selected, co-edited pool #1: 6% HDR

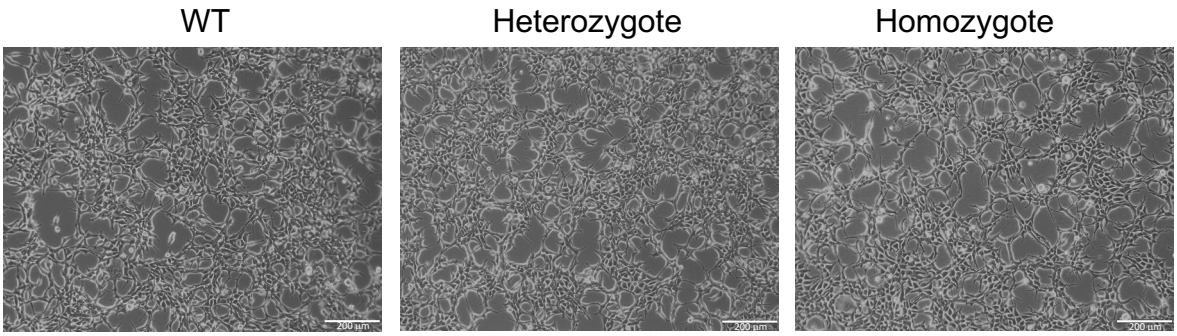
Selected, co-edited pool #1: 5% HDR

Selected, co-edited pool #1: 6% HDR

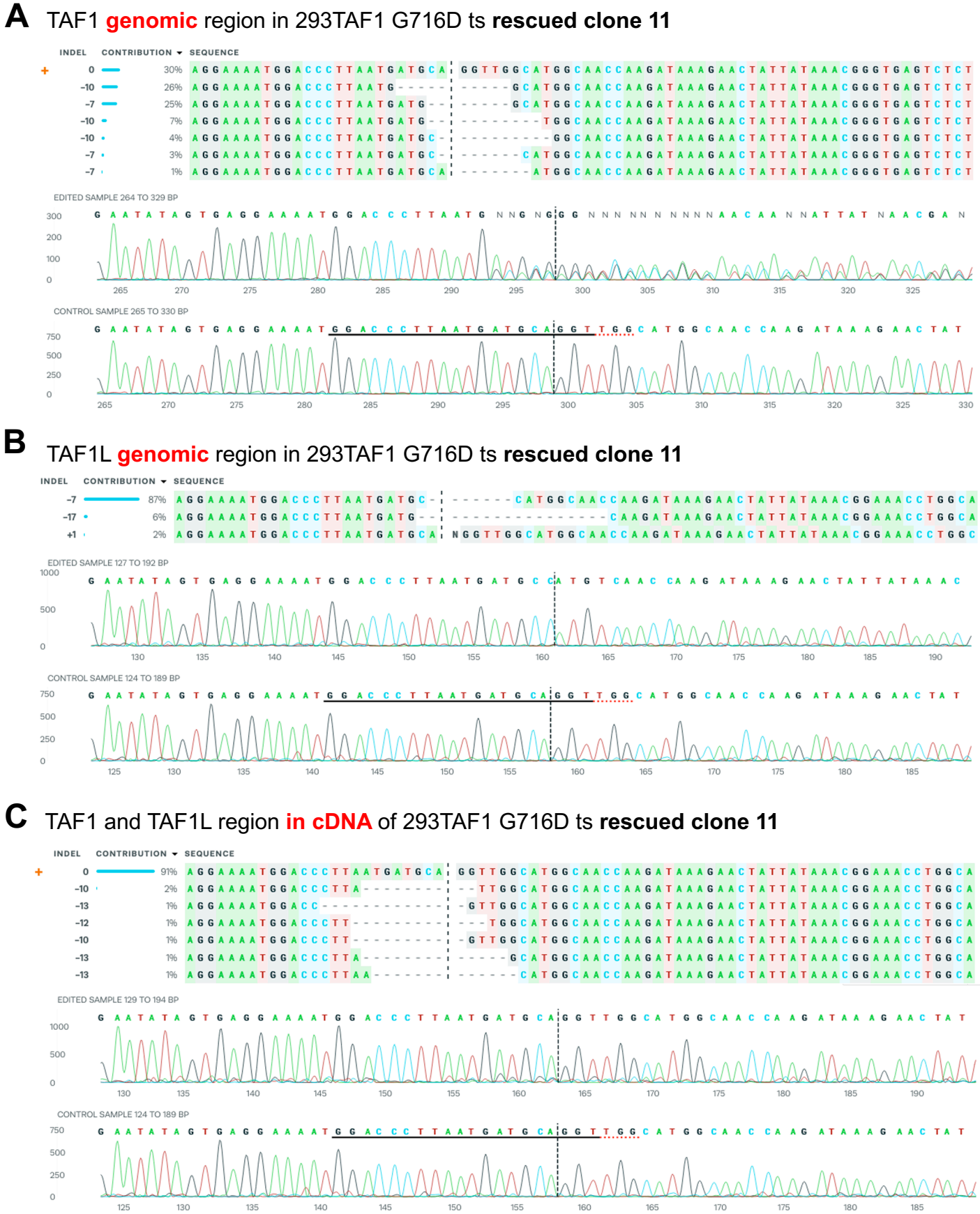
Supplementary Figure S12. Synthego ICE analysis of PSMB6-Flag edited cells. HEK293 TAF1 ts cells were transfected as described in Figure 4D. The 554 bp fragment from pools of unselected selected was amplified, sequenced, and the results were analyzed by using the Synthego ICE tool. Results from three independent pools of selected cells and one unselected are shown. The orange + indicates wt sequence, +1 indicates a 1nt insertion, HDR shows the inserted Flag tag.



Supplementary Figure S13.PSMB6-YFP editing of HeLa cells. (A) Guide RNA used for editing HeLa TAF1ts. The original guide and the schematic for the PSMB6-YFP donor plasmid are shown below. (**B) FACS of HeLa ts co-edited for PSMB6-YFP.** HeLa ts cells co-edited for PSMB6-YFP as described in Figure 5 were analyzed by FACS with 30,000 cells per point in triplicates. FACS histogram of YFP positive cells showing two separate populations of low and high intensity.

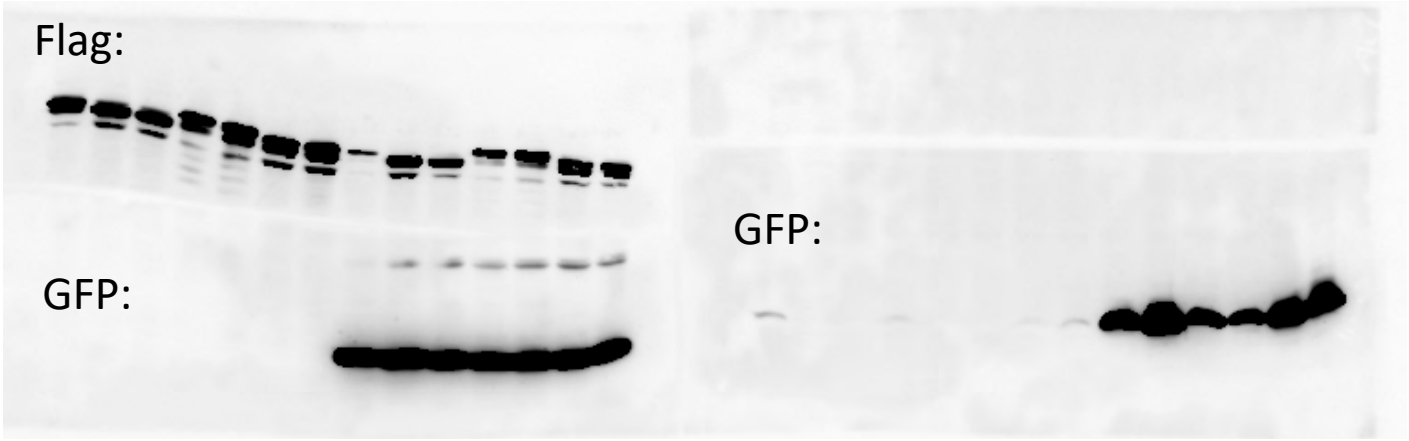


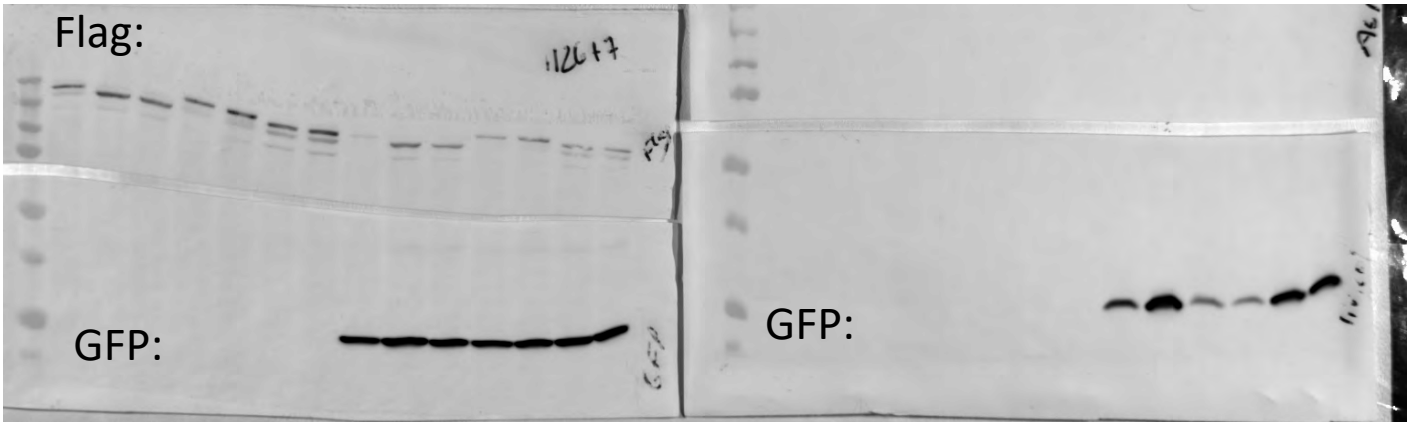
Supplementary Figure S14. Cells with PSMB6 T35A have normal morphology.

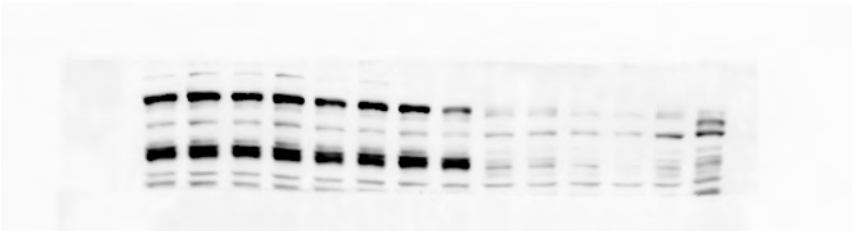


Supplementary Figure S15. Only wt TAF1 is expressed in rescued ts cells. A rescued clone of HEK293 TAF1ts clone 11 was analyzed by sequencing the genomic loci of TAF1 (A) and TAF1L (B), as also shown in Sup. Figures S4,5. Sequences were analyzed using Synthego ICE analysis tool. This sequencing shows the rescued TAF1 ts allele restored to wt, as well as the alleles with -7 and -10 deletions. The TAF1L locus has -7 and -17 deletions. Sequencing of the cDNA (C) showed that only the wt allele was detected, meaning that the deleted alleles were absent from the mRNA, likely due to nonsense-mediated decay.

Supplementary Figure S16: Raw images of blots

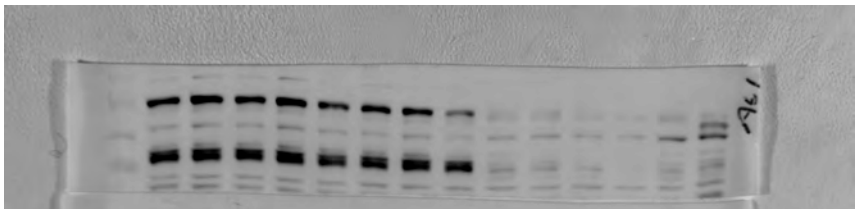


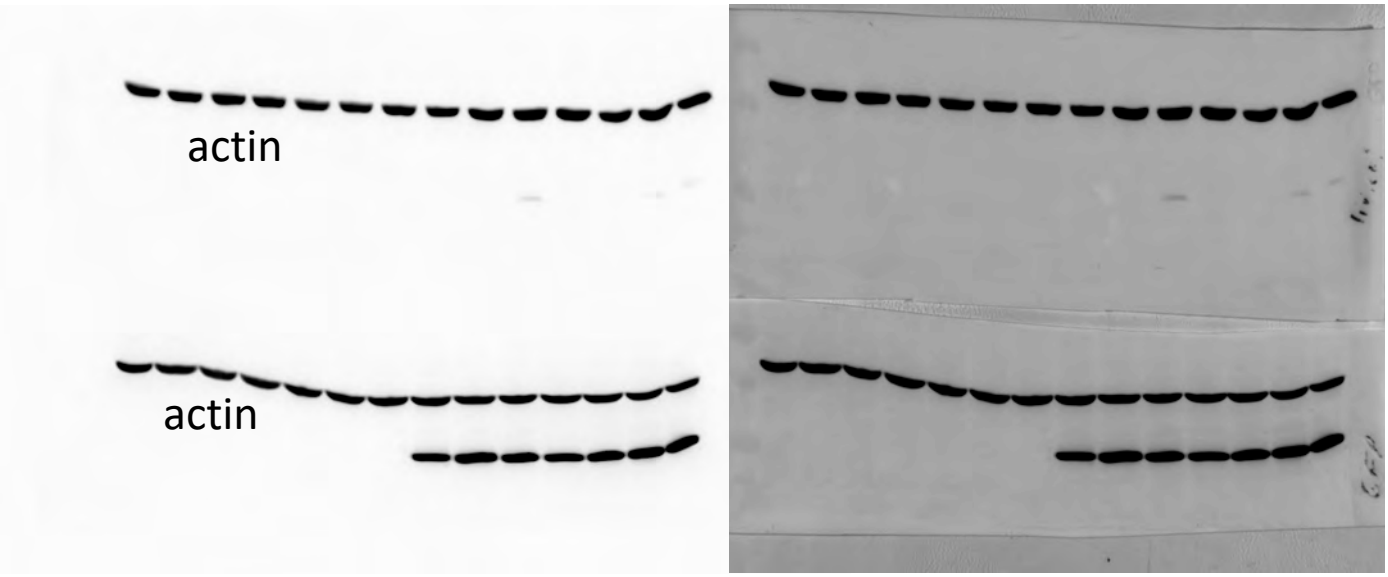




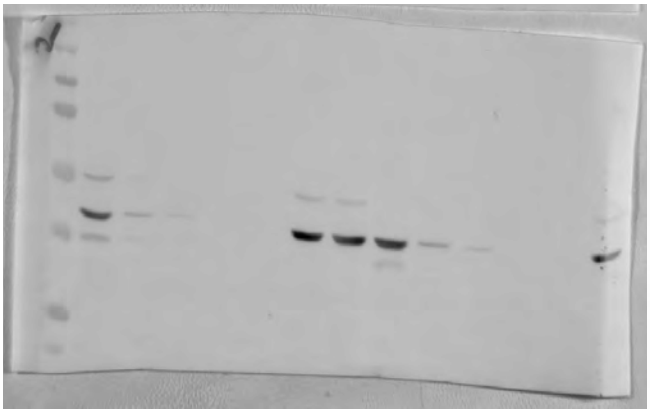
Abl

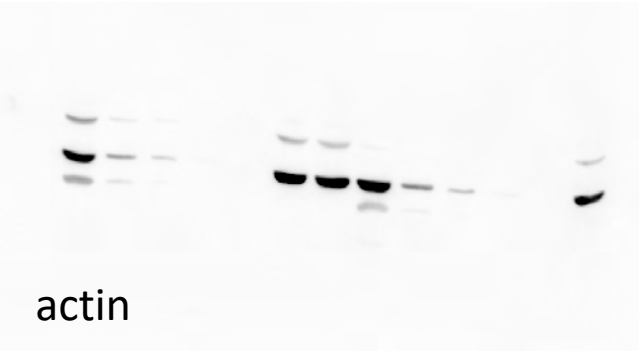
Blots used in Figure 3.

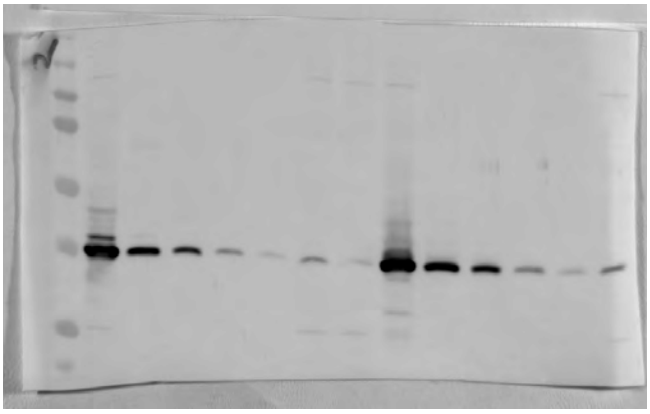


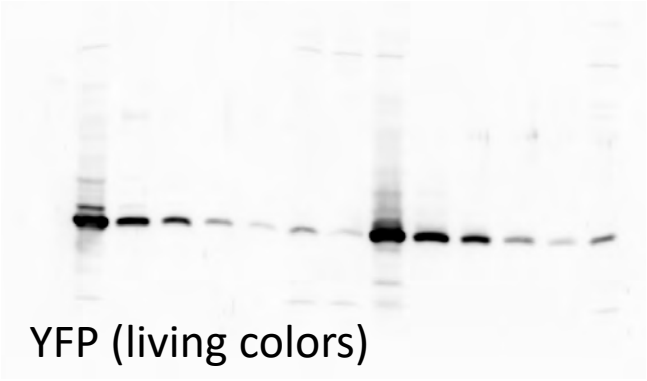


Blots used in Figure 4









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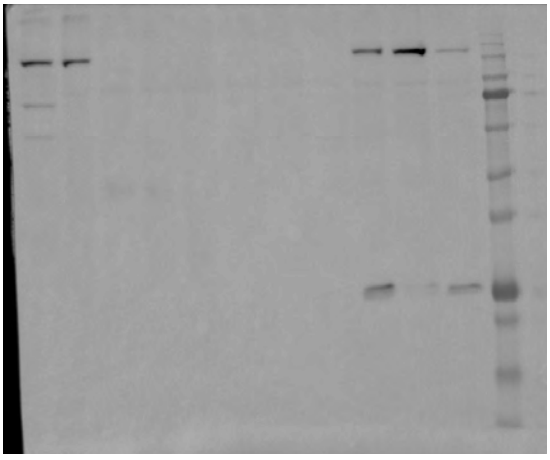

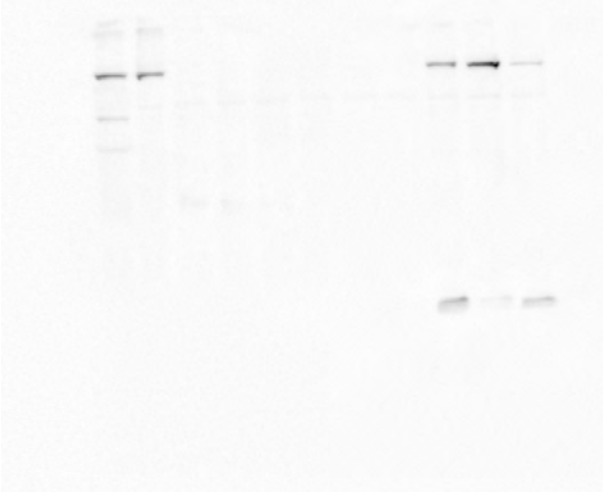

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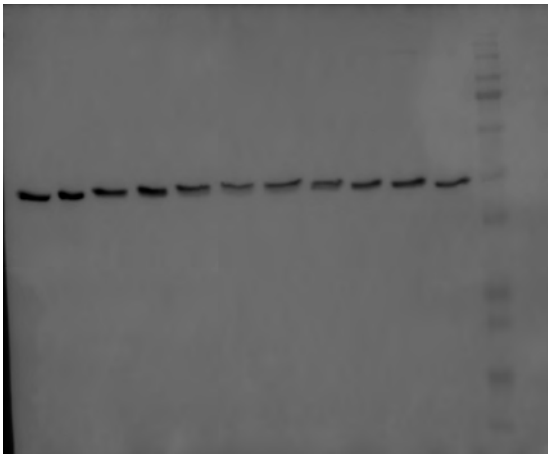

Blots used in Figure 5.

Living colors (YFP)

Actin