Supplementary Information

A Cell Cycle-dependent CRISPR-Cas9 Activation System Based on an Anti-CRISPR Protein Shows Improved Genome Editing Accuracy

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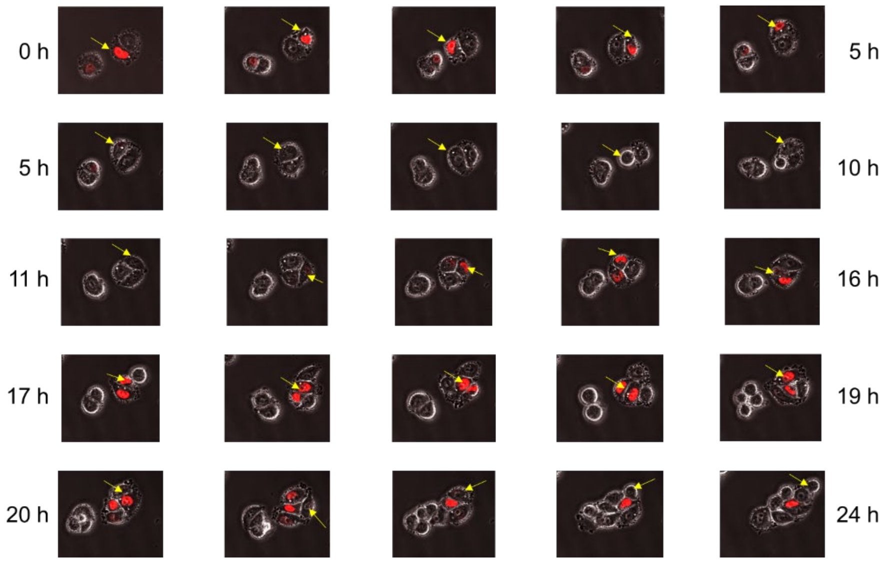
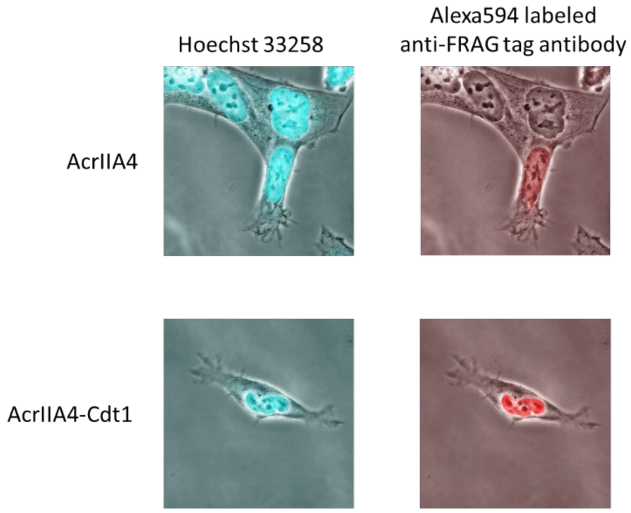
Supplementary Information: Figures

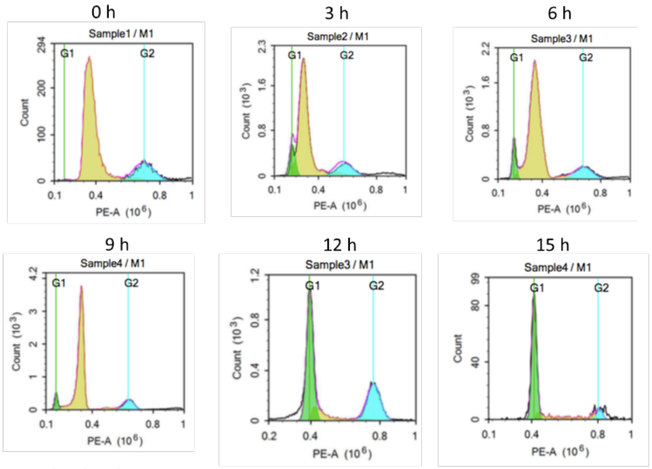


Supplementary Figure 1. Change of the expression level of mKO2-Cdt1 (Fucci) depending on time-lapse. Time indicate hours from the start of observation.

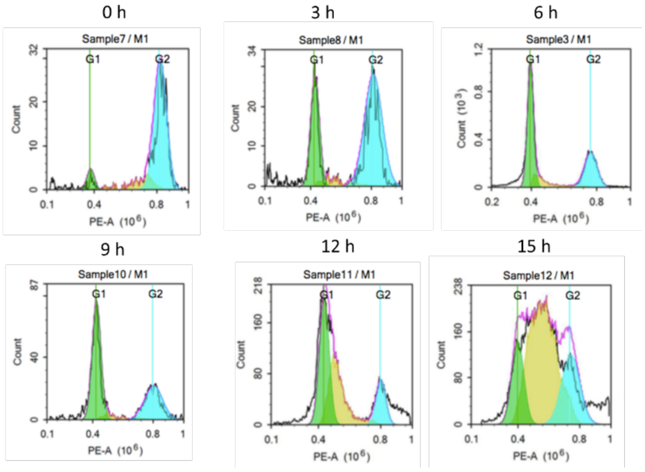


Supplementary Figure 2. Confirmation of expression and localization of AcrIIA4 and AcrIIA4-Cdt1in 293A cell. Immunostaining was performed 48 h after transfection. AcrIIA4 and AcrIIA4-Cdt1 were labeled by Alexa594 labeled anti-FLAG tag antibody. Nucleus was stained by Hoechst 33258.

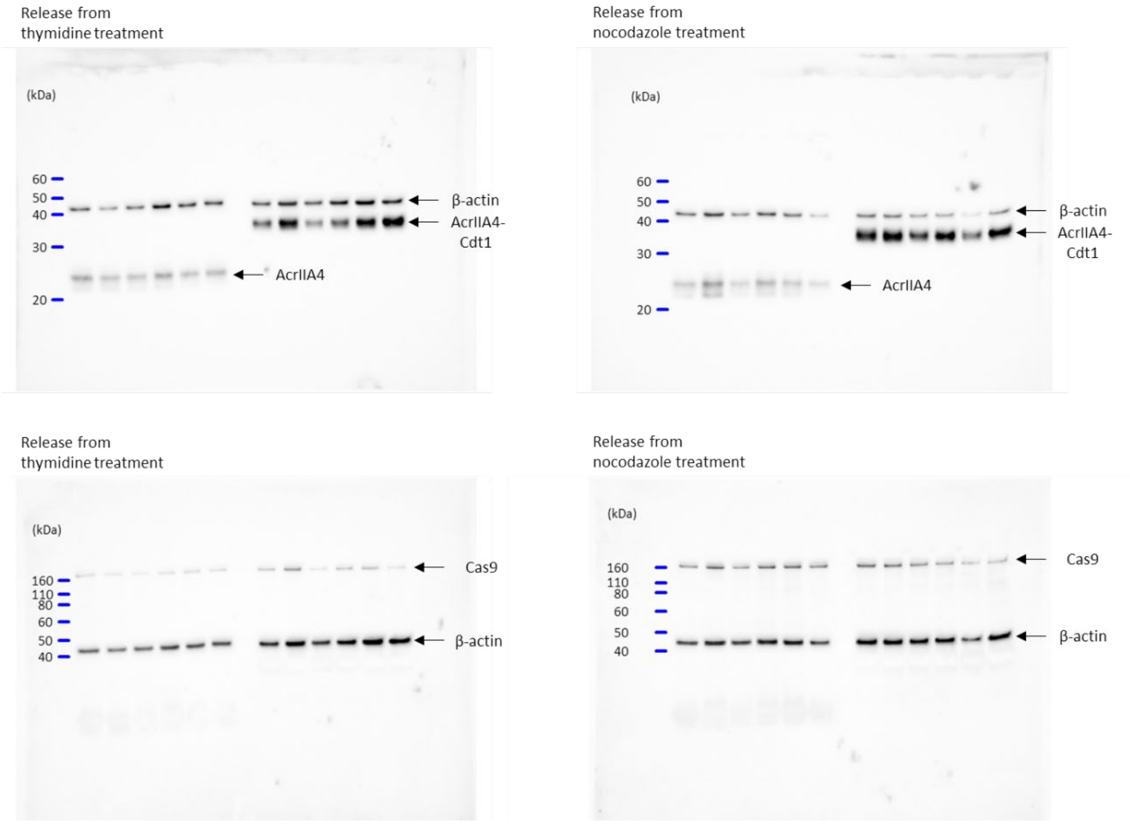
A Thymidine release



Nocodazole release



Supplementary Figure 3. FACS analysis for cell cycle. Cells were synchronized by double Thymidine block or Nocodazole. Cell cycle population was analyzed at each time after release from the drugs. Green range shows G_1 phase. Yellow range shows S phase. Blue range shows G_2 phase.


Release from thymidine treatment

Release from nocodazole treatment

Release from thymidine treatment

Release from nocodazole treatment

Supplementary Figure 4. Western blotting images for Fig. 3. Molecular sizes are shown based on the protein marker images. Cells were synchronized by double Thymidine block or Nocodazole.

H2B target site

TCAGACACAAGACTTCAACTCTTCACCTTATTTGCATAAGCGATTCTATATAAAAGCGCCTTGTCATACCCTGCTCAC
GCTGTTTTTCCTTTTCGTTGGCGCTTTATAGCTACACAGTGCTATGCCAGAGCCAGCGAAGTCTGCTCCCGCCCCGAA
AAAGGGCTCCAAGAAGGCGGTGAC

Sequence after HDR

TCAGACACAAGACTTCAACTCTTCACCTTATTTGCATAAGCGATTCTATATAAAAGCGCCTTGTCATACCCTGCTCAC
GCTGTTTTTCCTTTTGGATCCCTCGAGGCGGCCGCCGTTGGCGCTTTATAGCTACACAGTGCTATGCCAGAGCCAGCG
AAGTCTGCTCCCGCCCCGAAAAAGGGCTCCAAGAAGGCGGTGAC

AAVS1 target site

AGGAGGAGGCCTAAGGATGGGGCTTTTCTGTCACCAATCCTGTCCCTAGTGGCCCCACTGTGGGGTGGAGGGGACAGA
TAAAAGTACCCAGAACCAGAGCCACATTAACCGGCCCTGGGAATATAAGGTG

Sequence after HDR

AGGAGGAGGCCTAAGGATGGGGCTTTTCTGTCACCAATCCTGTCCCTAGTGGCCCCACTGTGGAAGCTTGGATCCGGA
GGGGACAGATAAAAGTACCCAGAACCAGAGCCACATTAACCGGCCCTGGGAATATAAGGTG

EMX1 target site

CAGGTGAAGGTGTGGTTCCAGAACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAG
CAGAAGAAGAAGGGCTCCCATCACATCAACCGGTGGCGCATTGCCACGAAGCAGGCCAATGGGGAGGACATCGATG

Sequence after HDR

CAGGTGAAGGTGTGGTTCCAGAACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAG
CAGAAGAAGCTTGGATCCAAGGGCTCCCATCACATCAACCGGTGGCGCATTGCCACGAAGCAGGCCAATGGGGAGGAC
ATCGATG

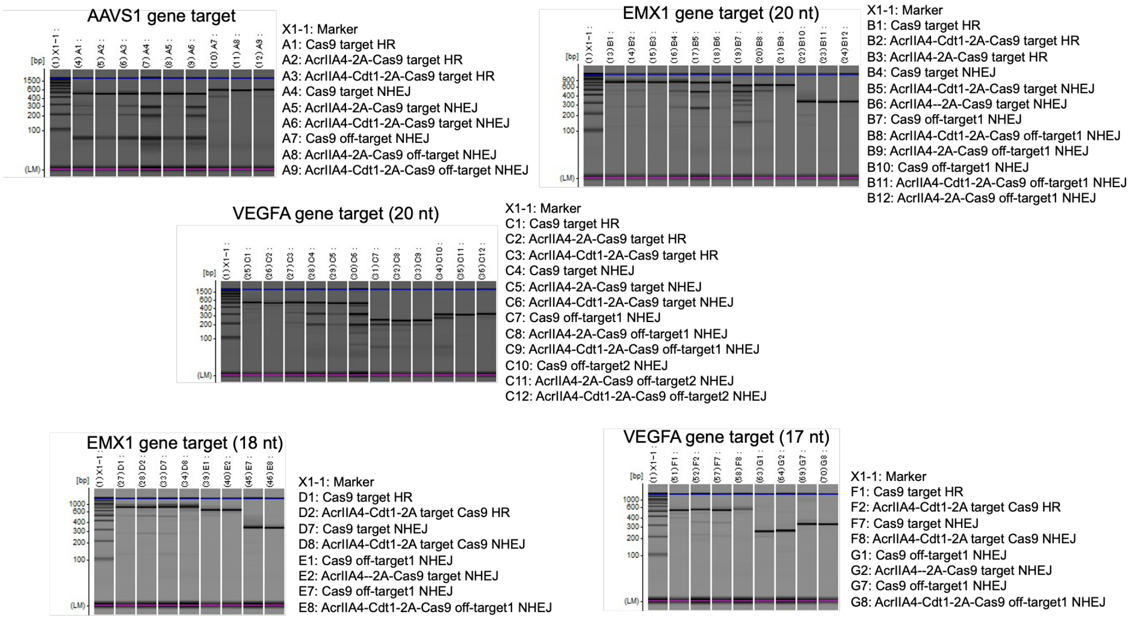
VEGFA target site

GAAGCTGGGTGAATGGAGCGAGCAGCGTCTTCGAGAGTGAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGTGTGCGT
GTGGGGTTGAGGGCGTTGGAGCGGGGAGAAGGCCAGGGGTCACTCCAGGATTCCAATAGATCTGTGTGTCCCTCTC

Sequence after HDR

GAAGCTGGGTGAATGGAGCGAGCAGCGTCTTCGAGAGTGAGGACGTGTGTGTCTGTGTGGGTGAGTGAGTGTGTAAGC
TTGGATCCTGTGGGGTTGAGGGCGTTGGAGCGGGGAGAAGGCCAGGGGTCACTCCAGGATTCCAATAGATCTGTGTGT
CCCTCTC

Supplementary Figure 5. Sequences around target sites and change of the sequence by HDR. Yellow highlights show seed sequences for SpyCas9 recognition and underlines indicate PAM sequences. Red characters are inserted sequences by HDR.



X1-1: Marker
A1: Cas9 target HR
A2: AcrIIA4-2A-Cas9 target HR
A3: AcrIIA4-Cdt1-2A-Cas9 target HR
A4: Cas9 target NHEJ
A5: AcrIIA4-2A-Cas9 target NHEJ
A6: AcrIIA4-Cdt1-2A-Cas9 target NHEJ
A7: Cas9 off-target NHEJ
A8: AcrIIA4-2A-Cas9 off-target NHEJ
A9: AcrIIA4-Cdt1-2A-Cas9 off-target NHEJ

X1-1: Marker
B1: Cas9 target HR
B2: AcrIIA4-Cdt1-2A-Cas9 target HR
B3: AcrIIA4-2A-Cas9 target HR
B4: Cas9 target NHEJ
B5: AcrIIA4-Cdt1-2A-Cas9 target NHEJ
B6: AcrIIA4--2A-Cas9 target NHEJ
B7: Cas9 off-target1 NHEJ
B8: AcrIIA4-Cdt1-2A-Cas9 off-target1 NHEJ
B9: AcrIIA4-2A-Cas9 off-target1 NHEJ
B10: Cas9 off-target1 NHEJ
B11: AcrIIA4-Cdt1-2A-Cas9 off-target1 NHEJ
B12: AcrIIA4-2A-Cas9 off-target1 NHEJ

X1-1: Marker
C1: Cas9 target HR
C2: AcrIIA4-2A-Cas9 target HR
C3: AcrIIA4-Cdt1-2A-Cas9 target HR
C4: Cas9 target NHEJ
C5: AcrIIA4-2A-Cas9 target NHEJ
C6: AcrIIA4-Cdt1-2A-Cas9 target NHEJ
C7: Cas9 off-target1 NHEJ
C8: AcrIIA4-2A-Cas9 off-target1 NHEJ
C9: AcrIIA4-Cdt1-2A-Cas9 off-target1 NHEJ
C10: Cas9 off-target2 NHEJ
C11: AcrIIA4-2A-Cas9 off-target2 NHEJ
C12: AcrIIA4-Cdt1-2A-Cas9 off-target2 NHEJ

X1-1: Marker
D1: Cas9 target HR
D2: AcrIIA4-Cdt1-2A target Cas9 HR
D7: Cas9 target NHEJ
D8: AcrIIA4-Cdt1-2A target Cas9 NHEJ
E1: Cas9 off-target1 NHEJ
E2: AcrIIA4--2A-Cas9 target NHEJ
E7: Cas9 off-target1 NHEJ
E8: AcrIIA4-Cdt1-2A-Cas9 off-target1 NHEJ

X1-1: Marker
F1: Cas9 target HR
F2: AcrIIA4-Cdt1-2A target Cas9 HR
F7: Cas9 target NHEJ
F8: AcrIIA4-Cdt1-2A target Cas9 NHEJ
G1: Cas9 off-target1 NHEJ
G2: AcrIIA4--2A-Cas9 target NHEJ
G7: Cas9 off-target1 NHEJ
G8: AcrIIA4-Cdt1-2A-Cas9 off-target1 NHEJ

Supplementary Figure 6. Images of each result in Figs. 6 and 7. These images were made from the peak intensities by MultiNA viewer software.

Supplementary Table 1

Name	Sequence (5' to 3')
CMV Forward	CGCAAATGGGCGGTAGGCGTG
BamHI_NLS-AcrIIA4_Fw	ctcctggatccgccaccatggGTGGCCCGAAGAAGAAACGGAAGGTAGGTGGCggcaatataaatgacctcataag
Acr-REsite_XbaI_Rv	GGAGGATCTAGATTACTCGAGCGGCCGCCAGTGT
pEBMultiHyg long Fw	ccgggggatccactagttctagagcGCCACCATGGGTGGCCCG
2A-Cas9	gacttcctctgccctcCTCGAGCGGCCGCCAGTG
NLS-Acr long Fw	GcggccgctcgaggagggcagaggaagtctgctaacatgcggtgacgtcgaggagaatcctggcccaGACAAGAAGTACTCCATTGGGC
pEBMulti-Hyg long Rv	atggctgattatgatctagagtcgcTCACACCTTCCTCTTCTTCTTGG
NLS-Acr-Cdt1 long Fw	CcaggacaccatcgagggcagaggaagtctgctaacatgcggtgacgtcgaggagaatcctggcccaGACAAGAAGTACTCCATTGGGC
pEBCas9 Fw	ccgggggatccactagttctagagcGCCACCATGGACAAGAAGTAC
pEBCas9 Rv	atggctgattatgatctagagtcgcTCACACCTTCCTCTTCTTCTTGG
gRNA_H2B_Fw	TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCgctataaagcgccaacgaaa
gRNA_H2B_Rv	GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTTTCGTTGGCGCTTTATAGC
gRNA_AAVS1_Fw	TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTGGCCCCACTGTGGGGTGG
gRNA_AAVS1_Rv	GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCCACCCCACAGTGGGGCCAC
gRNA_EMX1_Fw	TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGAGTCCGAGCAGAAGAAGAA
gRNA_EMX1_Rv	GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTTCTTCTTCTGCTCGGACTC
gRNA_VEGFA_Fw	TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTGAGTGAGTGTGTGCGTG
gRNA_VEGFA_Rv	GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCACGCACACACTCACTCACC
ssODN_AAVS1	TATATTCCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCGGATCCAAGCTTCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCC
ssODN_EMX1	TCCTCCCCATTGGCCTGCTTCGTGGCAATGCGCCACCGGTTGATGTGATGGGAGCCCTTGGATCCAAGCTTCTTCTGCTCGGACTCAGGCCCTTCCTCCTCCAGCTTCTGCCGTTTGTACTTTGTCCTC
ssODN_VEGFA	TATTGGAATCCTGGAGTGACCCCTGGCCTTCTCCCCGCTCCAACGCCCTCAACCCCACAGGATCCAAGCTTACACACTCACTCACCCACACAGACACACACGTCCTCACTCTCGAAGACGCTGCTCGCT
T7E1 H2B T Fw	GGCCCTAGAAGAGCGAGTCT

T7E1 H2B T Rv	GCGCTCGAAAATGTCGTTCACA
T7E1 AAVS1 T Fw	TTCGGGTCACCTCTCACTCC
T7E1 AAVS1 OT Fw	GCATTAGGGCTTGGTCATGT
T7E1 AAVS1 T Rv	GGCTCCATCGTAAGCAAACC
T7E1 AAVS1 OT Rv	TCCTCCCATGCTATGTCTCC
T7E1 EMX1 T Fw	GGAGCAGCTGGTCAGAGGGG
T7E1 EMX1 OT1 Fw	TCTCTCCTTCAACTCATGACCAGCT
T7E1 EMX1 OT2 Fw	TGAAATCTCACCTGGGCGAGA
T7E1 EMX1 T Rv	GGGAAGGGGGACACTGGGGA
T7E1 EMX1 OT1 Rv	ATCTGCACATGTATGTACAGGAGTCAT
T7E1 EMX1 OT2 Rv	TGCAGTCTGCCTTTTTGGGG
T7E1 VEGFA T Fw	TCCAGATGGCACATTGTCAG
T7E1 VEGFA OT1 Fw	GAGGGGGAAGTCACCGACAA
T7E1 VEGFA OT2 Fw	TCCTTTGAGGTTCATCCCCC
T7E1 VEGFA T Rv	AGGGAGCAGGAAAGTGAGGT
T7E1 VEGFA OT1 Rv	TACCCGGGCCGTCTGTTAGA
T7E1 VEGFA OT2 Rv	CCAATCCAGGATGATTCCGC