

Supporting Information

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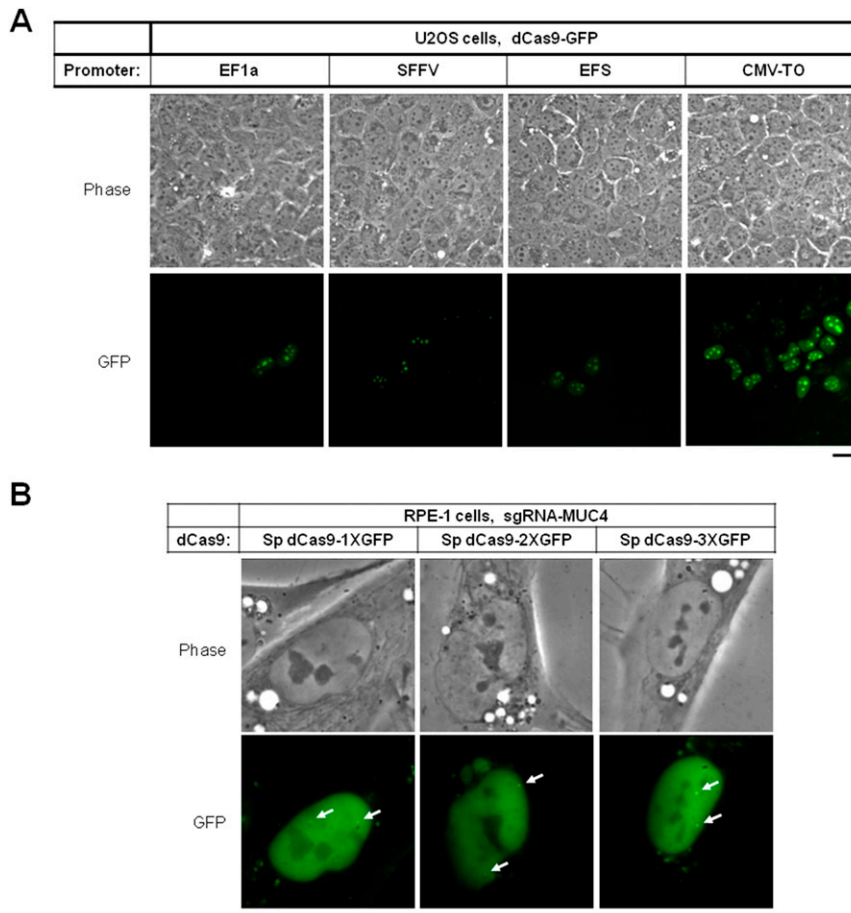


Fig. S1. Optimization of GFP-tagged Sp Cas9 expression. (A) Promoter optimization for CRISPR labeling using Sp Cas9-GFP. U2OS cells were transfected with 150 ng of dCas9-GFP expression plasmids under the control of a series of promoters: EF1 α (Left), SFFV (Middle Left), EFS (Middle Right), and CMV-TO (Right). Less than 5% of cells were GFP-positive when using the EF1 α , SFFV, and EFS promoters; however, the percentage of GFP-positive cells increased to 50% with use of the CMV-TO promoter. (Scale bar: 20 μ m.) (B) Increased labeling by fusing 3XGFP to Sp Cas9. RPE-1 cells were cotransfected with 250 ng of sgRNA plasmid targeting to the MUC4 gene locus and 50 ng of plasmids encoding Sp dCas9-1XGFP, Sp dCas9-2XGFP, or Sp dCas9-3XGFP. The fluorescent images in the bottom row were obtained within the same range of linear sensitivity and scaled to the same threshold. The brightness of foci (arrows) gradually increased when Sp dCas9 was tagged with 1XGFP, 2XGFP, or 3XGFP. (Scale bar: 5 μ m.)

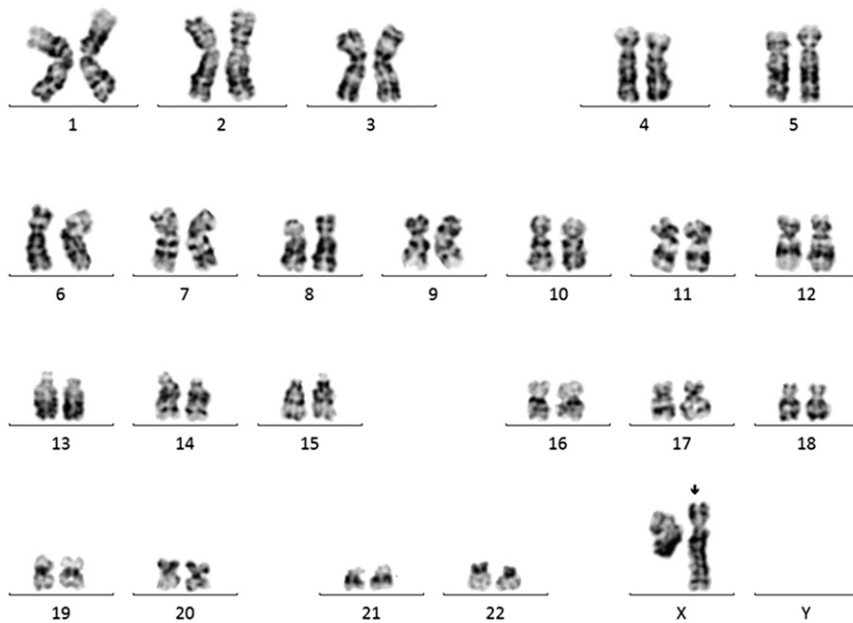


Fig. 55. Karyotype of RPE-1 cells. Chromosome analysis of RPE-1 cells (American Type Culture Collection CRL-4000) was performed. Twenty metaphase cells were analyzed, and all showed a near-diploid karyotype as shown, with a 2N chromosome number of 46. As shown in the image, one copy of the X carries an additional element at Xq28 (arrow).

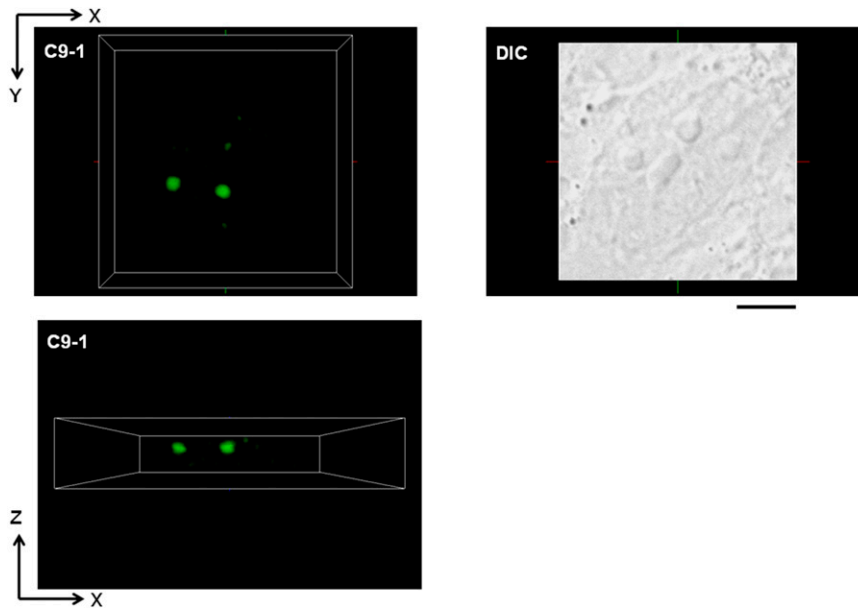


Fig. 56. Three-dimensional view of the chromosome 9 pericentromeric locus C9-1. U2OS cells were transfected with 150 ng of a plasmid encoding Sp dCas9-3XGFP and 750 ng of a plasmid encoding a SpsgRNA targeting to C9-1. Serial optical sections were then captured by confocal microscopy. The 3D images were reconstructed with Metamorph software. A representative example is shown in *x-y* (Upper Left) and *x-z* (Lower Left) projections. (Upper Right) The corresponding differential interference contrast (DIC) image. (Scale bar: 5 μm .)

Table S1. dCas9-FPs

Promoter	dCas9 fusion protein	NLS
EF1 α	NLS-Sp dCas9-NLS-sfGFP	2X
SSFV	NLS-Sp dCas9-NLS-sfGFP	2X
EFS	NLS-Sp dCas9-NLS-sfGFP	2X
CMV-TetO	NLS-Sp dCas9-NLS-sfGFP	2X
CMV-TetO	NLS-Sp dCas9-NLS-2XsfGFP	2X
CMV-TetO	NLS-Sp dCas9-NLS-3XsfGFP	2X
CMV-TetO	NLS-Sp dCas9-NLS-3XmCherry	2X
CMV-TetO	NLS-Nm dCas9-NLS-3XsfGFP	2X
CMV-TetO	NLS-Nm dCas9-NLS-3XmCherry	2X
CMV-TetO	NLS-St1 dCas9-NLS-3XsfGFP	2X
CMV-TetO	NLS-St1 dCas9-2XNLS-3XsfGFP	3X
CMV-TetO	NLS-St1 dCas9-3XNLS-3XsfGFP	4X
CMV-TetO	NLS-St1 dCas9-3XNLS-3XTagBFP2	4X
CMV-TetO	NLS-St1 dCas9-3XNLS-3XsfGFP-NLS	5X
CMV-TetO	NLS-St1 dCas9-3XNLS-3XsfGFP-2XNLS	6X

Table S2. sgRNA vectors

Vector name	sgRNA expression vector cassette
pLH-Sp sgRNA2	U6 promoter-BbsI-CcdB-BbsI-Sp sgRNA2
pLH-Nm sgRNAm3	U6 promoter-BbsI-CcdB-BbsI-Nm sgRNAm3
pLH-Nm sgRNA1.1	U6 promoter-BbsI-CcdB-BbsI-Nm sgRNA1.1
pLH-St1 sgRNAm1	U6 promoter-BbsI-CcdB-BbsI-St1 sgRNAm1
pLH-St1 sgRNAm7	U6 promoter-BbsI-CcdB-BbsI-St1 sgRNAm7
pLH-St1 sgRNA1.1	U6 promoter-BbsI-CcdB-BbsI-St1 sgRNA1.1
pLH-St1 sgRNA2.1	U6 promoter-BbsI-CcdB-BbsI-St1 sgRNA2.1
pLH-St1 sgRNA3.1	U6 promoter-BbsI-CcdB-BbsI-St1 sgRNA3.1

Table S3. Single-guide RNA sequences

dCas9	Target	Guide RNA sequence	PAM
<i>S. pyogenes</i>	MUC4	GTGGCGTGACCTGTGGATGCTG	GG
<i>S. pyogenes</i>	Telo-TA	GGTTAGGGTTAGGGTTAGGG	TA
<i>S. pyogenes</i>	Telo-GT	AGGGTTAGGGTTAGGGTTAG	GT
<i>S. pyogenes</i>	Telo-AG	GTTAGGGTTAGGGTTAGGGT	AG
<i>S. pyogenes</i>	Sp-Telo	TTAGGGTTAGGGTTAGGGTT	GG
<i>S. pyogenes</i>	Telo-17	GGGTTAGGGTTAGGGTT	GG
<i>S. pyogenes</i>	Telo-13	TAGGGTTAGGGTT	GG
<i>S. pyogenes</i>	Telo-10	GTTAGGGTT	GG
<i>S. pyogenes</i>	Telo-06	AGGGTT	GG
<i>S. pyogenes</i>	C9-1	TGGAATGGAATGGAATGGAA	GG
<i>S. pyogenes</i>	C9-2	TGTCTGTGAGGAAGCTCCCC	GG
<i>S. pyogenes</i>	C13-1	TAAGCATGGACCATTCTTC	GG
<i>S. pyogenes</i>	C13-2	GGGCCAGGACCTCTAAAA	GG
		CCGGGGAAGTGCTGAGTC	GG
		TGGTGGGTGTAGACACGG	GG
<i>N. meningitidis</i>	Telo-AGGG	GGTTAGGGTTAGGGTTAGGGTTAG	AGGG
<i>N. meningitidis</i>	Telo-GGGT	GTTAGGGTTAGGGTTAGGGTTAGG	GGGT
<i>N. meningitidis</i>	Nm-Telo	TTAGGGTTAGGGTTAGGGTTAGGG	GGTT
<i>N. meningitidis</i>	Telo-GTTA	TAGGGTTAGGGTTAGGGTTAGGGT	GTTA
<i>N. meningitidis</i>	C13-1	CTCCATCCTGAAGGAATGGTCCAT	GCTT
<i>S. thermophilus</i>	St1-Telo	GGTTAGGGTTAGGGTTAGGG	AGGG
<i>S. thermophilus</i>	C9-1	ATGGAATGGAATGGAATGGA	GGAA