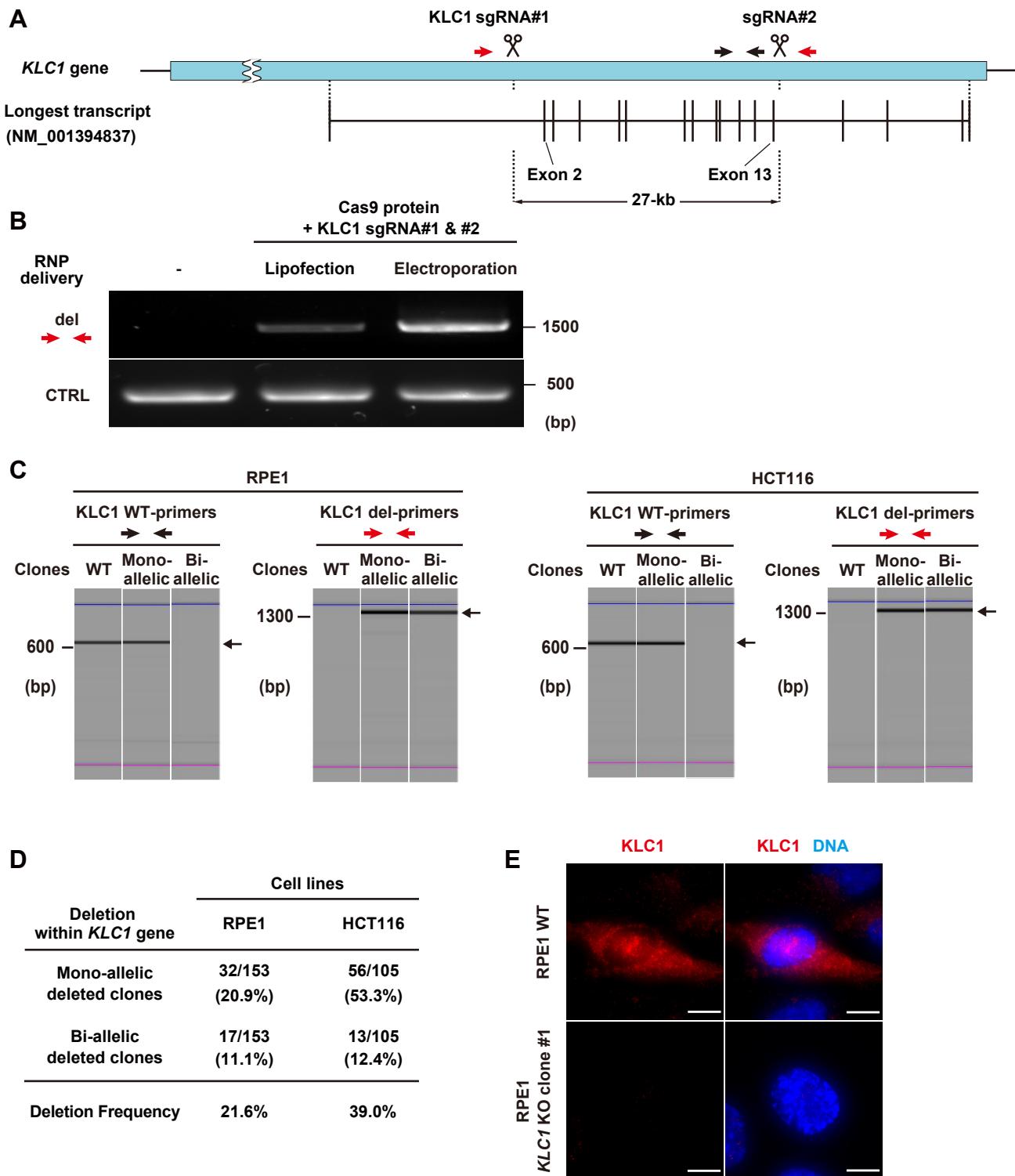


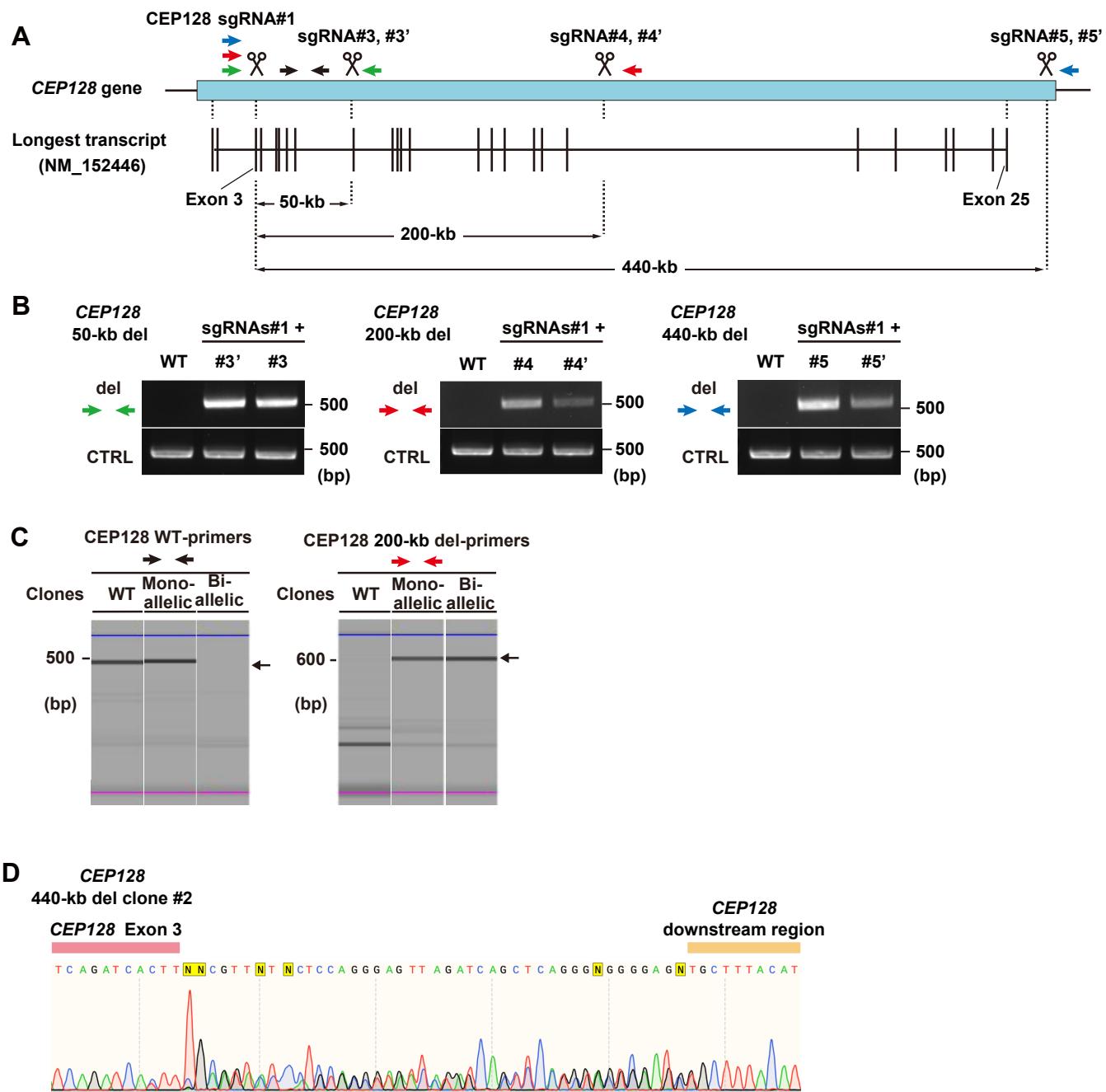
**Fig. S1. Validation of CEP128 deletion mutants.**

**A**, Genomic PCR to detect the 20-kb deletion in cells electroporated with Cas9 and the indicated sgRNAs. **B**, Western blotting to analyze the protein expression of CEP128 in the cell lysate of WT and a *CEP128* 20-kb deleted clone at 72 hr after transfection of the indicated siRNA. Asterisks show smaller fragments of CEP128 protein. **C**, Immunofluorescence imaging of CEP128 and centrin in the cells of WT, the 20-kb or 440-kb *CEP128*-deleted clones. Scale bar: 5  $\mu$ m (1  $\mu$ m for insets). **D**, Immunofluorescence imaging of Centriolin and  $\gamma$ -tubulin in the cells of WT, the 20-kb or 440-kb *CEP128*-deleted clone. Scale bar: 5  $\mu$ m (1  $\mu$ m for insets). **E**, Quantification of relative Centriolin intensity at the centrosome from (**D**), 50 cells for each sample. Data are represented as mean and *P* value was calculated by Mann–Whitney U test. \*\*\**P* < 0.001.



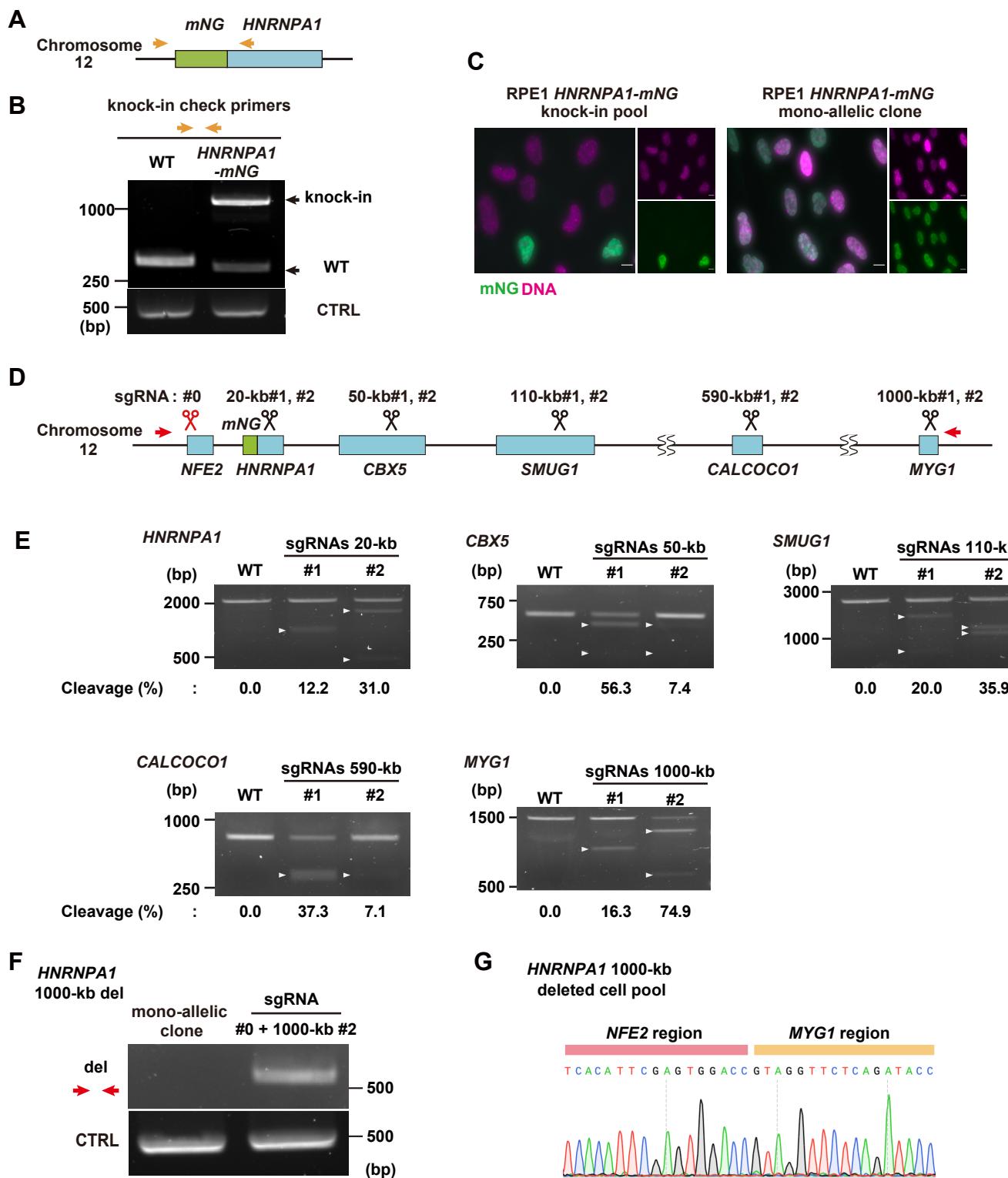
**Fig. S2. A large chromosomal deletion within the *KLC1* gene in human diploid cells by the optimized CRISPR-del pipeline.**

**A**, Schematic representation of *KLC1* gene and the longest transcript variant annotated in genome databases. The target positions of sgRNAs and the expected length of a large deletion are shown. Black and red arrows indicate primers to detect WT and the deleted region of *KLC1* gene, respectively. **B**, Genomic PCR to detect the 27-kb deletion in RPE1 cells electroporated or lipofected with Cas9 protein and the indicated sgRNAs using the indicated primers. **C**, Genomic PCR for detection of WT and the deleted alleles of *KLC1* gene in RPE1 and HCT116 cells, analyzed by the automated microchip electrophoresis system. Each electrophoresis pattern was adjusted according to the upper (blue) and lower (pink) size makers. The arrows on the right side of electrophoresis images indicate the specific PCR product. **D**, Summary for the efficiency of mono- and bi-allelic deletions within *KLC1* gene in RPE1 and HCT116 cells. **E**, Immunofluorescence imaging of KLC1 in WT and bi-allelic deletion mutant RPE1 cells. Scale bar: 5 μm.



**Fig. S3. Large chromosomal deletions within the *CEP128* gene by the CRISPR-del pipeline.**

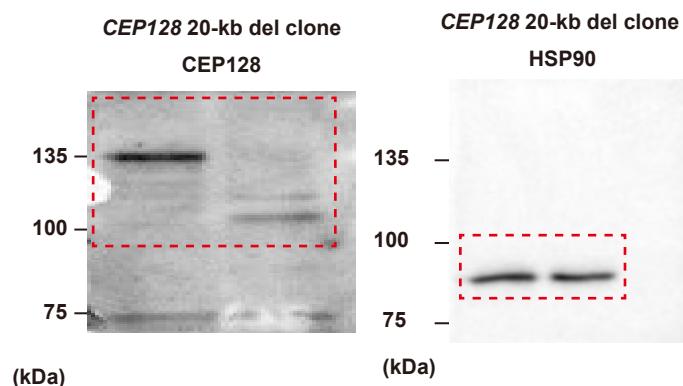
**A**, Schematic representation of *CEP128* gene and the longest transcript variant annotated in genome databases. The target positions of sgRNAs and the expected lengths of large deletions are shown. Green, red, blue and black arrows indicate primers to detect the 50-kb, 200-kb and 440-kb deleted and WT regions, respectively. **B**, For the validation of sgRNA combinations, chromosomal deletions with different lengths within the *CEP128* gene were detected by genomic PCR. *CEP128* sgRNA#3, #4 and #5, together with sgRNA#1, were used for further analyses in Fig. 2 and Fig. S2. **C**, Genomic PCR for detection of WT and the 200-kb deleted alleles of *CEP128* gene, analyzed by the automated microchip electrophoresis system. Each electrophoresis pattern was adjusted according to the upper (blue) and lower (pink) size makers. The arrows on the right side of electrophoresis images indicate the specific PCR product. **D**, Sequencing result of the *CEP128* deleted alleles in the 440-kb deleted clone #2.



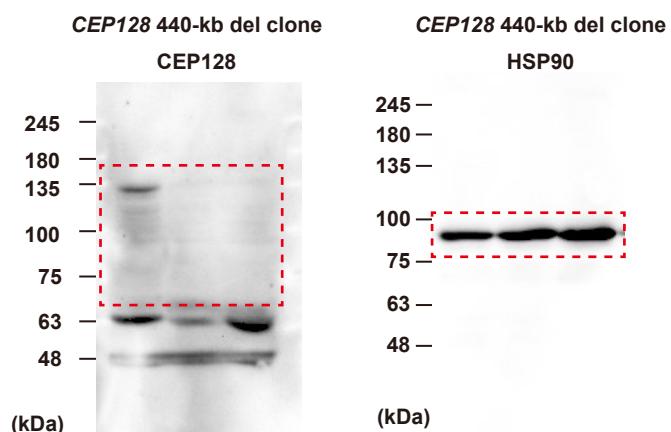
**Fig. S4. Large deletion of a chromosome region including *HNRNPA1* gene locus by the CRISPR-del.**

**A**, Schematic representation of the *HNRNPA1* gene locus in RPE1 *HNRNPA1-mNG* cells. Orange arrows indicate the primers to detect the knock-in allele. **B**, Genomic PCR to detect WT and the knock-in alleles of the *HNRNPA1* gene in the indicated cells. **C**, Fluorescence imaging of HNRNPA1-mNG in the indicated cells. Scale bar: 10 μm. **D**, Schematic representation of the chromosome region around the *HNRNPA1-mNG* locus in RPE1 *HNRNPA1-mNG* cells. The target positions of sgRNAs are shown. Red arrows indicate the primers to detect the 1000-kb chromosomal deletion. **E**, The T7E1 assay to detect genome editing at the indicated gene loci in RPE1 WT cells electroporated with Cas9 protein and the indicated sgRNAs. **F**, Genomic PCR to detect the 1000-kb deletion in WT cells and cells electroporated with Cas9 protein and the indicated sgRNA pair. **G**, Sequencing result of the deletion band shown in (F).

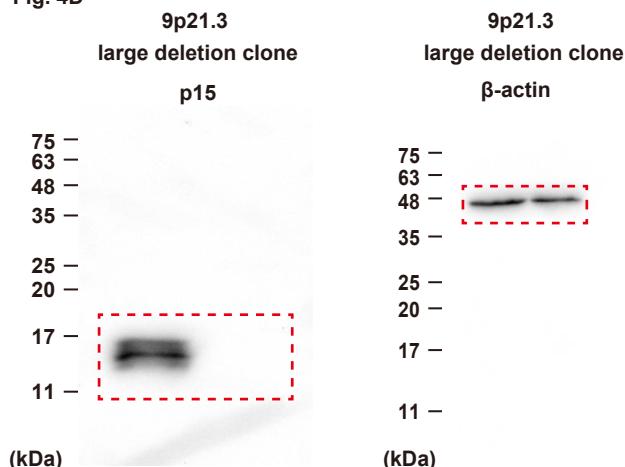
**Fig. 1E**



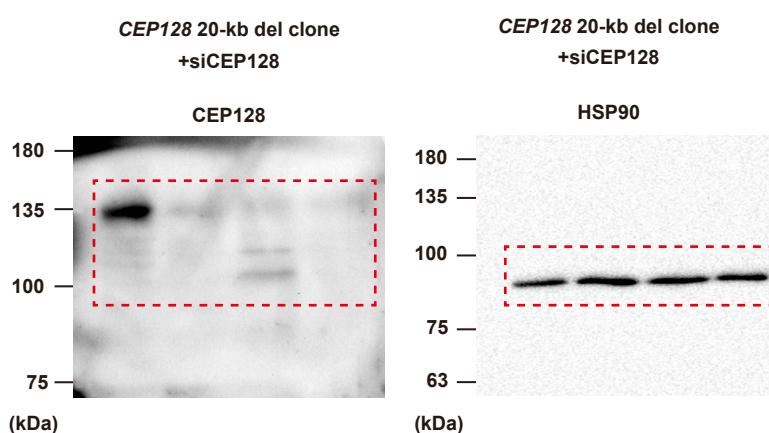
**Fig. 2F**



**Fig. 4D**



**Fig. S1B**



**Fig. S5. Blot transparency.**

Uncropped scans of western blots for Fig. 1E, 2F, 4D and S1B.

**Table S1. List of sgRNAs used in this study.**

sgRNA/crRNA Target	Name	Sequence
CEP128 Exon3	CEP128 sgRNA#1	GACTCAATCGGTACAGACAG
CEP128 Intron8(20-kb)	CEP128 sgRNA#2	GATTTTTCGGATGTACCAA
CEP128 Intron8(50-kb)	CEP128 sgRNA#3	CCTGACAAGGAACCTTATTG
CEP128 Intron19(200kb)	CEP128 sgRNA#4	TCAAGAAGGGCACTTTACGT
CEP128 downstream region(440-kb)	CEP128 sgRNA#5	GATGTATAGATAAACACGGGG
KLC1 Intron1	KLC1 sgRNA#1	GAGGTGCTACTTGATAACAC
KLC1 Exon13	KLC1 sgRNA#2	CAACGTGGACGTGGTCAAGT
NFE2	HNRNPA1#0	TCACATTGAGTGGACCATC
HNRNPA1	HNRNPA1 20-kb#1	ACGCTTCAAGGAGGTGTAT
HNRNPA1	HNRNPA1 20-kb#2	GGATTGAGAGTGATCACTCA
CBX5	HNRNPA1 50-kb#1	ACCCAGGGAGCACAATACTT
CBX5	HNRNPA1 50-kb#2	TACCCAGGGAGCACAATACT
SMUG1	HNRNPA1 110-kb#1	GAAGTCTCTTATAACCCACGG
SMUG1	HNRNPA1 110-kb#2	TGGGAACCATCCAATCCCT
CALCOCO1	HNRNPA1 590-kb#1	GTGGGAATAGAATCGTCAC
CALCOCO1	HNRNPA1 590-kb#2	TGTGGGAATAGAATCGTCCA
MYG1	HNRNPA1 1000-kb#1	TGGCACCTTCACTGCAGC
MYG1	HNRNPA1 1000-kb#2	GGTATCTGAGAACCTACCTC
MTAP	9p21.3 sgRNA#1	AAGTAAGCAGTTCTCCACG
DMRTA1	9p21.3 sgRNA#2	TAGTGGATGTGGAGCCAAA
HNRNPA1	crRNA HNRNPA1	ATTAGGTAAGTAAGCACCTT

**Table S2. List of primers used in this study.**

Primer for PCR-assembled DNA templates	Name	Sequence
sgRNA amplification primers	Universal Design Primer_Fw	TTCTAAATACGACTCATATAAG
	Universal Design Primer_Rv	AAAAGGCCGACTCGGTG
crRNA_1, crRNA	crRNA_tracrRNA	GTTTAAAGCTGAAATAGCAAGTTAAATTAGCTGGCCGTTACACTGAAAGTGCCAGCAGTCGGTCTT
CEP128 sgRNA#1	sgRNA_CEP128_#1_Fw	TCTTAATACGACTCATAGCTAACATCGTCAGCGACAG
CEP128 sgRNA#2	sgRNA_CEP128_#1_Rv	TCTAGCTCTAAACCTGGTAGCTCGCGATTGAGTC
CEP128 sgRNA#3	sgRNA_CEP128_#2_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
CEP128 sgRNA#3	sgRNA_CEP128_#2_Rv	TCTAGCTCTAAACCTGGTAGCTCGCGATTGAGTC
CEP128 sgRNA#3	sgRNA_CEP128_#3_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
CEP128 sgRNA#4	sgRNA_CEP128_#3_Rv	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
CEP128 sgRNA#4	sgRNA_CEP128_#4_Fw	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
CEP128 sgRNA#5	sgRNA_CEP128_#5_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
KLC1 sgRNA#1	sgRNA_KLC1_#1_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
KLC1 sgRNA#2	sgRNA_KLC1_#1_Rv	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
KLC1 sgRNA#2	sgRNA_KLC1_#2_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1#0	sgRNA_#0_Fw	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 20-kb#1	sgRNA_de120-kb_#1_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 20-kb#1	sgRNA_de120-kb_#1_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 20-kb#2	sgRNA_de120-kb_#2_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 20-kb#2	sgRNA_de120-kb_#2_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 50-kb#1	sgRNA_de150-kb_#1_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 50-kb#1	sgRNA_de150-kb_#1_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 50-kb#2	sgRNA_de150-kb_#2_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 50-kb#2	sgRNA_de150-kb_#2_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 110-kb#1	sgRNA_de1110-kb_#1_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 110-kb#1	sgRNA_de1110-kb_#1_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 110-kb#2	sgRNA_de1110-kb_#2_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 110-kb#2	sgRNA_de1110-kb_#2_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 590-kb#1	sgRNA_de590-kb_#1_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 590-kb#1	sgRNA_de590-kb_#1_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 590-kb#2	sgRNA_de590-kb_#2_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 590-kb#2	sgRNA_de590-kb_#2_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 1000-kb#1	sgRNA_de11000-kb_#1_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 1000-kb#1	sgRNA_de11000-kb_#1_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1 1000-kb#2	sgRNA_de11000-kb_#2_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1 1000-kb#2	sgRNA_de11000-kb_#2_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
9p21.3 sgRNA#1	sgRNA_9p21.3_#1_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
9p21.3 sgRNA#1	sgRNA_9p21.3_#1_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
9p21.3 sgRNA#2	sgRNA_9p21.3_#2_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
9p21.3 sgRNA#2	sgRNA_9p21.3_#2_Rv	TCTAGCTCTAAACACGTTAAGTGCCTTCCTG
HNRNPA1	HNRNPA1_cRNA_Fw	TCTTAATACGACTCATAGCTAACCTGGTAGCTCGCGATTGAGTC
HNRNPA1	HNRNPA1_cRNA_Rv	AAGTGCCTTACTTACCTAATCTACCAAGATGTAAGAATTC
dsDNA repair template	Name	Sequence
HNRNPA1	HNRNPA1_dsDNA repair template_Fw	CACTTGAAACTTTAAAAGAAAAATTGTAATTTCAGGTGCTATGGCGGTGAGTCAG
HNRNPA1	HNRNPA1_dsDNA repair template_Rv	TTGGAGCCGGTCAAGGTGCG
Primer for deletion check	Name	Sequence
CEP128 del-primers_20-kb	del-primer_CEP128_20-kb_Fw	ATATGGCTTAAATGGGACCGCT
CEP128 del-primers_20-kb	del-primer_CEP128_20-kb_Rv	GGGAGAAATTACATGGGAGAGTCGA
CEP128 del-primers_50-kb	del-primer_CEP128_50-kb_Fw	TGGCGAGAATCATCCAGCGAACT
CEP128 del-primers_50-kb	del-primer_CEP128_50-kb_Rv	ATTATCCATGAGCATGACCGCTGC
CEP128 del-primers_200-kb	del-primer_CEP128_200-kb_Fw	TGGCGAGAATCATCCAGCGAA
CEP128 del-primers_200-kb	del-primer_CEP128_200-kb_Rv	GGGAGATCAAAGCAAGCAGCAGT
CEP128 del-primers_440-kb	del-primer_CEP128_440-kb_Fw	TGGCGAGAATCATCCAGCGAACT
CEP128 del-primers_440-kb	del-primer_CEP128_440-kb_Rv	TGTTGGCATTCATCACGTC
CEP128 WT-primers_20-kb	WT-primer_CEP128_20_Fw	TTTCTCCATGAGTGGTC
CEP128 WT-primers_20-kb	WT-primer_CEP128_20_Rv	TGGCGACTGGTCAGGTAAC
CEP128 WT-primers_50-440-kb	WT-primer_CEP128_50-440_Fw	CCACGTGACAAAGAACCCCA
CEP128 WT-primers_50-440-kb	WT-primer_CEP128_50-440_Rv	TGAGATGCCCTCGTCAT
KLC1 WT-primers	WT-primer_KLC1_Fw	TGCGGCAATCTGCTTATTGCT
KLC1 WT-primers	WT-primer_KLC1_Rv	GTCCCATGCGTCCGGTTAG
KLC1 del-primers	del-primer_KLC2_Fw	CTGGCTTTCAGTGAAGTC
KLC1 del-primers	del-primer_KLC2_Rv	AGCATGCCAGATACGACAGCA
NEK2 WT-primers	WT-primer_NEK2_Fw	TGGGTTTTTACATCTGTGTGTGA
NEK2 WT-primers	WT-primer_NEK2_Rv	ACTCCATCAACTAAAGACCAAC
HNRNPA1 del-primers_1000-kb	del-primer_HNRNPA1_1000-kb_Fw	CCCTAAAGCTCAACTGCT
HNRNPA1 del-primers_1000-kb	del-primer_HNRNPA1_1000-kb_Rv	ACTGTCACAATCTGCACCA
MTAP_outside WT-primers	WT-primer_MTAP_outside_Fw	AGTGTAGCGCACTGGGAG
MTAP_outside WT-primers	WT-primer_MTAP_outside_Rv	TTCAGACCTTCGGTTC
MTAP WT-primers	WT-primer_MTAP_Fw	GGAGCTTTCCTCGTCA
MTAP WT-primers	WT-primer_MTAP_Rv	AGTTTACAGTCGTTGG
CDKN2A WT-primers	WT-primer_CDKN2A_Fw	AGAATTCCTCCGTCGTA
CDKN2A WT-primers	WT-primer_CDKN2A_Rv	CGTTCTCTCGCGGATA
CDKN2B WT-primers	WT-primer_CDKN2B_Fw	CACTGAGGAGATTCGGC
CDKN2B WT-primers	WT-primer_CDKN2B_Rv	GACATCCACAGGACCAT
DMRT1A1 WT-primers	WT-primer_DMRT1A1_Fw	AGACTTACTGGCAACAG
DMRT1A1 WT-primers	WT-primer_DMRT1A1_Rv	GCAATATCAAACTGGCG
DMRT1A1_outside WT-primers	WT-primer_DMRT1A1_outsideFw	CCGATCAAACATGGGAC
DMRT1A1_outside WT-primers	WT-primer_DMRT1A1_outsideRv	ACACCCGAAATCCTAAAGCAA
9p21.3 del-primers	9p21.3_del-primerFw	AGAGGGTACGCTTCAAATGA
9p21.3 del-primers	9p21.3_del-primerRv	AAAACACGTGCTGCGCTA
Primer for knock-in check	Name	Sequence
HNRNPA1 Knock-in check primers	Ki-primer_HNRNPA1_Fw	AAAGATGTCGTGAGCTACTGCTGG
HNRNPA1 Knock-in check primers	Ki-primer_HNRNPA1_Rv	ACTATGTTGCACTGCTCAGCTA
Primer for surveyor assay	Name	Sequence
Surveyor 20-kb	Surveyor-20kb-Fw	CGCTTGCAGATTCTCTAGCAC
Surveyor 20-kb	Surveyor-20kb-Rv	CTGGCTTAATGCCGCTCAT
Surveyor 50-kb	Surveyor-50kb-Fw	TCTTTTTGTCGCAATGTCCTCT
Surveyor 50-kb	Surveyor-50kb-Rv	GAGAAGCGCAAGGGAGTGTGTT
Surveyor 110-kb	Surveyor-110kb-Fw	TGGGCTTTCATGAGGTGATG
Surveyor 110-kb	Surveyor-110kb-Rv	GCATGCCCTTCAGAAGATT
Surveyor 590-kb	Surveyor-590kb-Fw	CCCACATGGAAAGGGAGTGTG
Surveyor 590-kb	Surveyor-590kb-Rv	TGTGGAGAGATGCGAGGAGA
Surveyor 100-kb	Surveyor-100kb-Fw	TAGTCGGTCGCGCTGCTCTA
Surveyor 100-kb	Surveyor-100kb-Rv	ACTGTCACCAACTGCCACAA