Supplementary information for

Precise CAG repeat contraction in a Huntington's Disease mouse model is enabled by gene editing with SpCas9-NG

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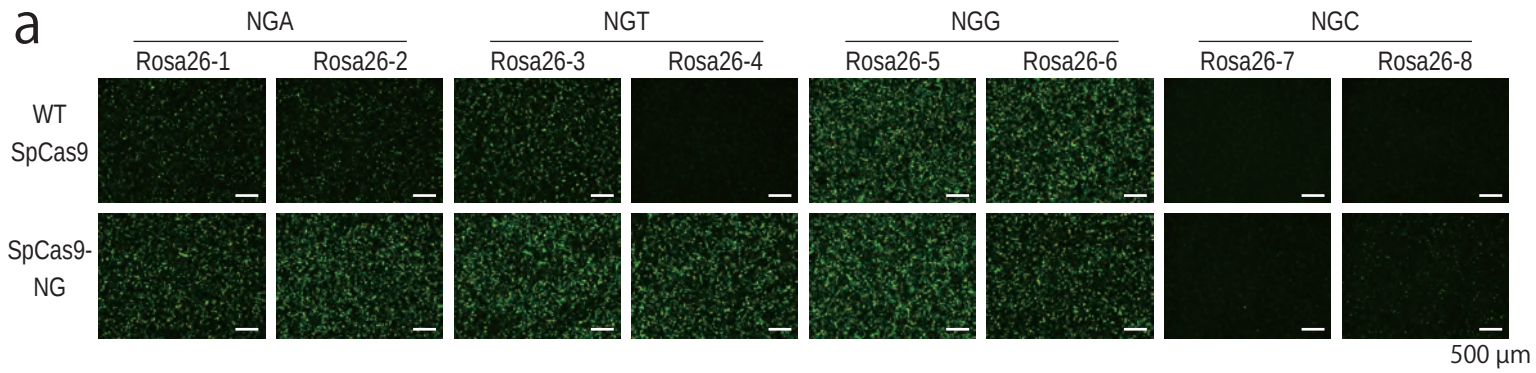
Supplementary Figure 1 to 10

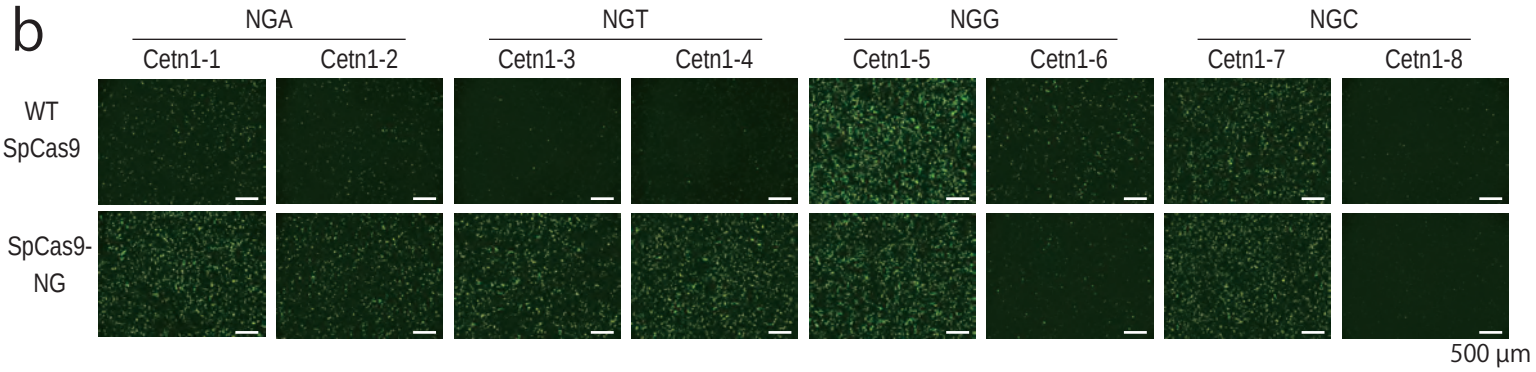
Other Supplementary materials for this manuscript include the following:

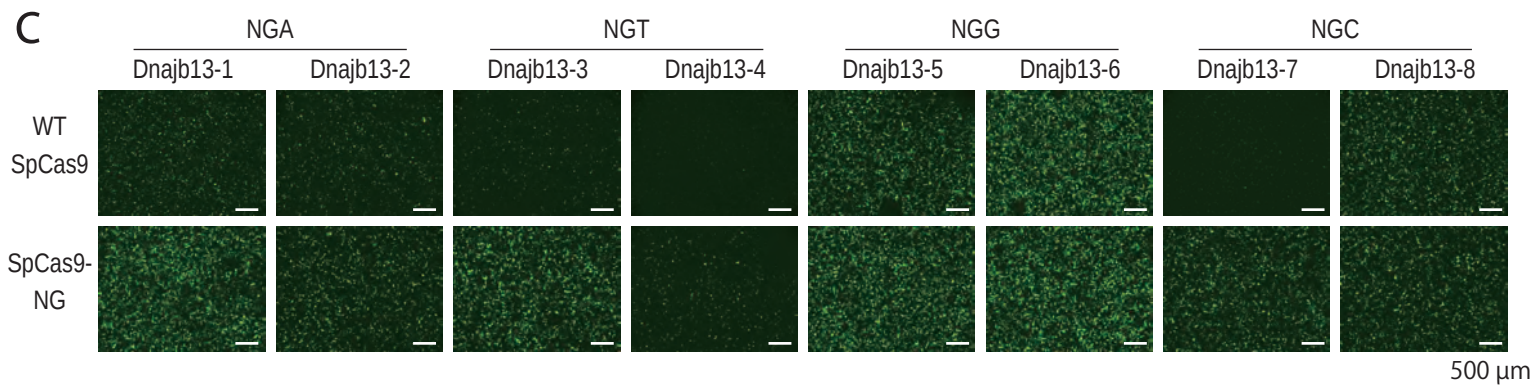
Supplementary Data 1 to 6

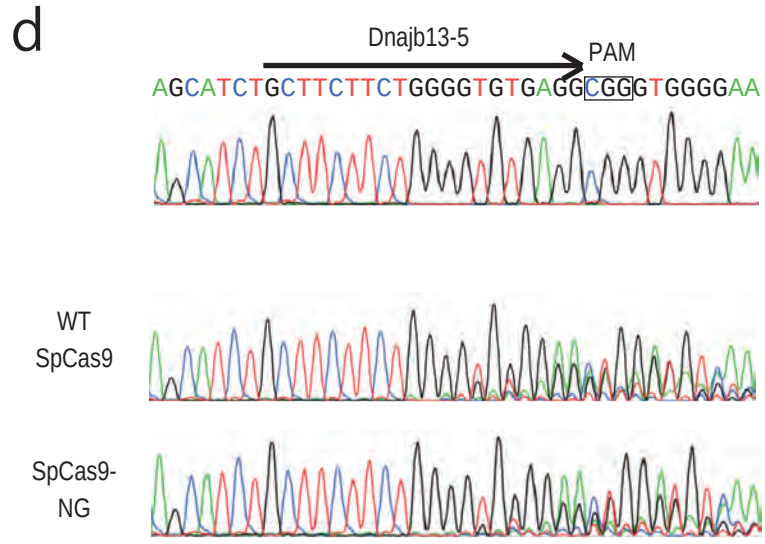
Supplementary Movie 1 and 2

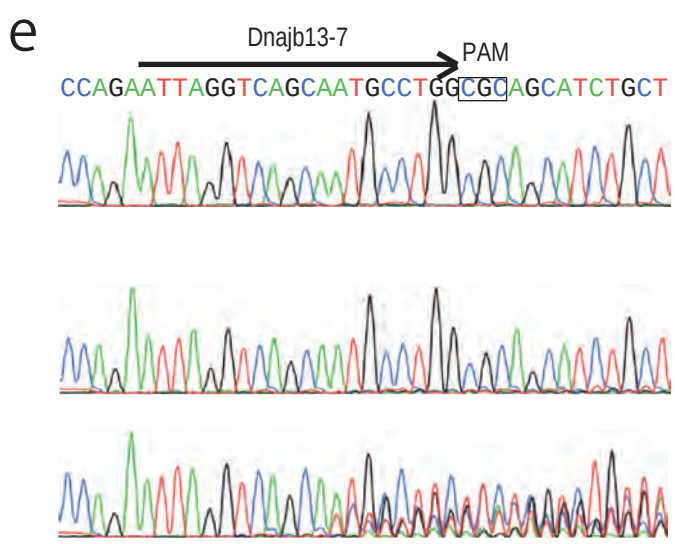
Supplementary Figure 1











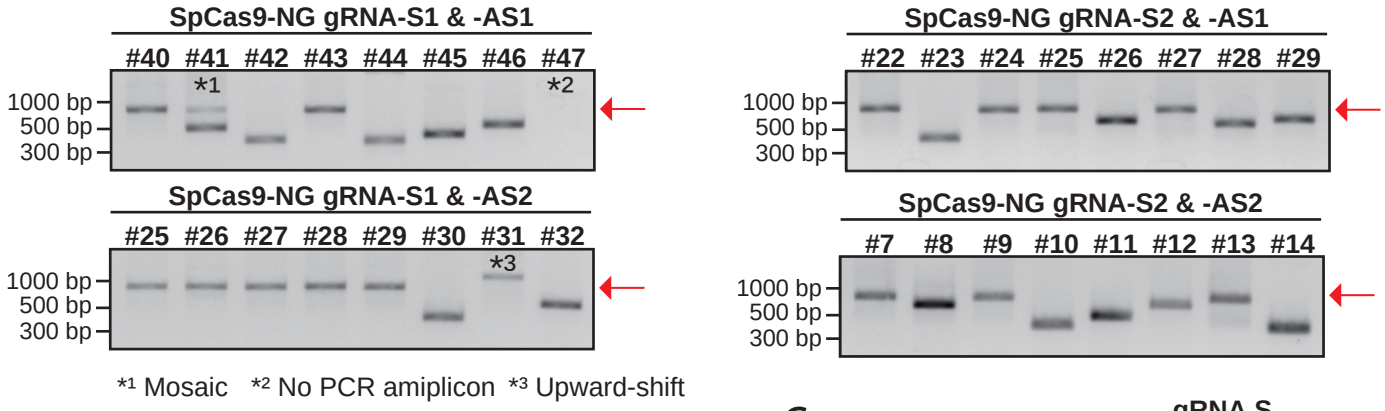
Supplementary Figure 1. Genome editing with SpCas9-NG, related to Fig. 1

a–c The EGFP fluorescence images were taken 36 hours after transfection in the SSA assay.

d, e Sequence of pooled ES cell genome samples. Black arrows and boxes show gRNA target sequence and PAM, respectively.

Supplementary Figure 2

a

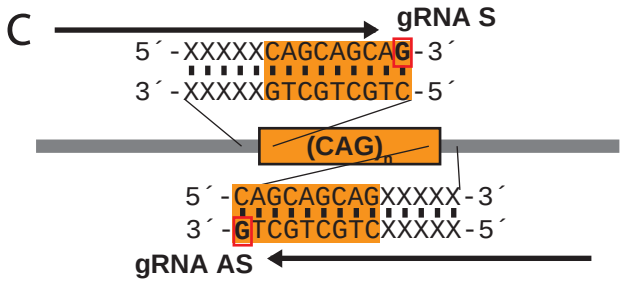


*1 Mosaic *2 No PCR amiplicon *3 Upward-shift

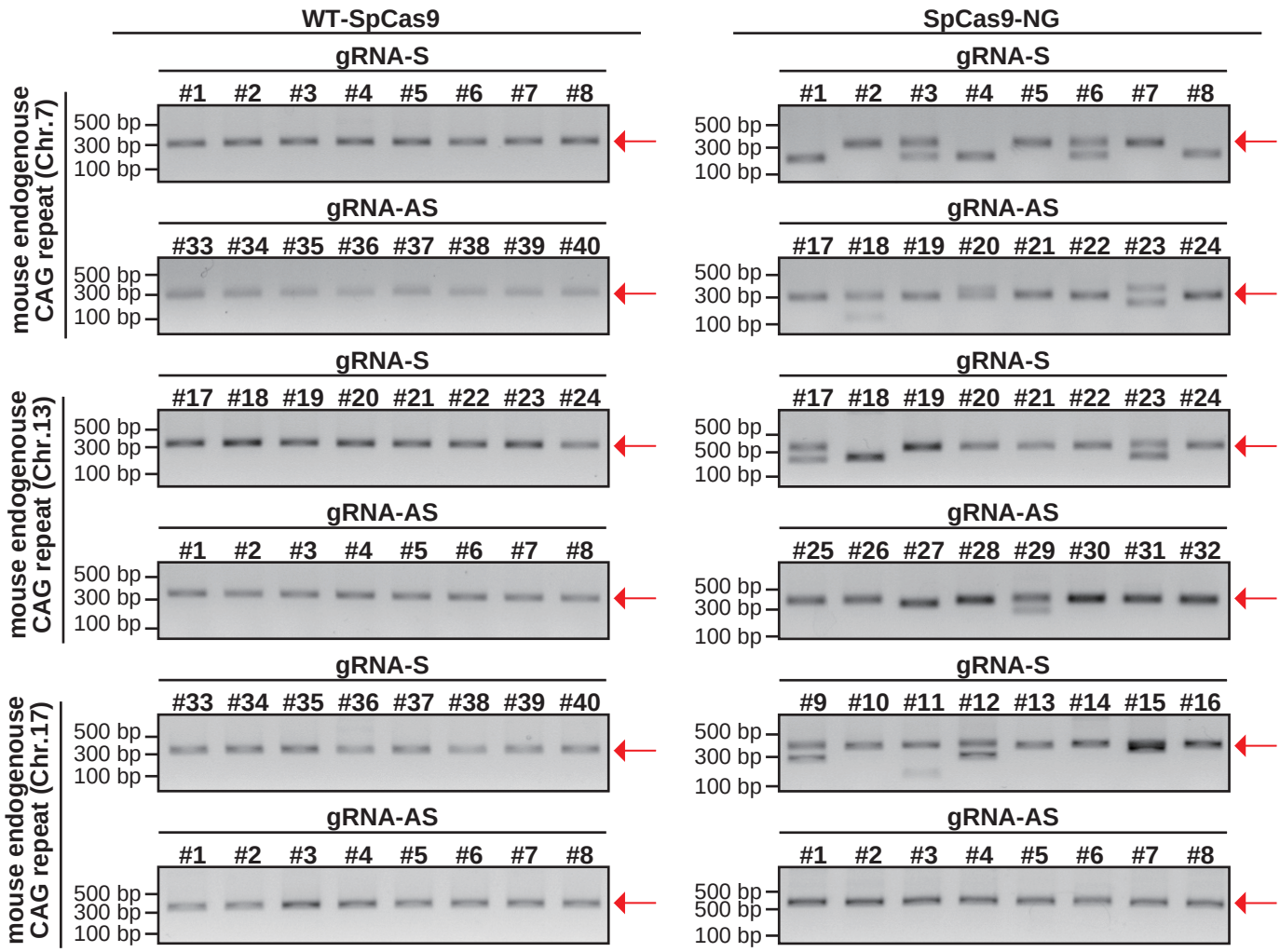
b

gRNA	Examined clones	contracted	in-frame
gRNA S1 & AS1	40	10	2
gRNA S1 & AS2	48	8	3
gRNA S2 & AS1	39	13	4
gRNA S2 & AS2	44	10	1

c



d



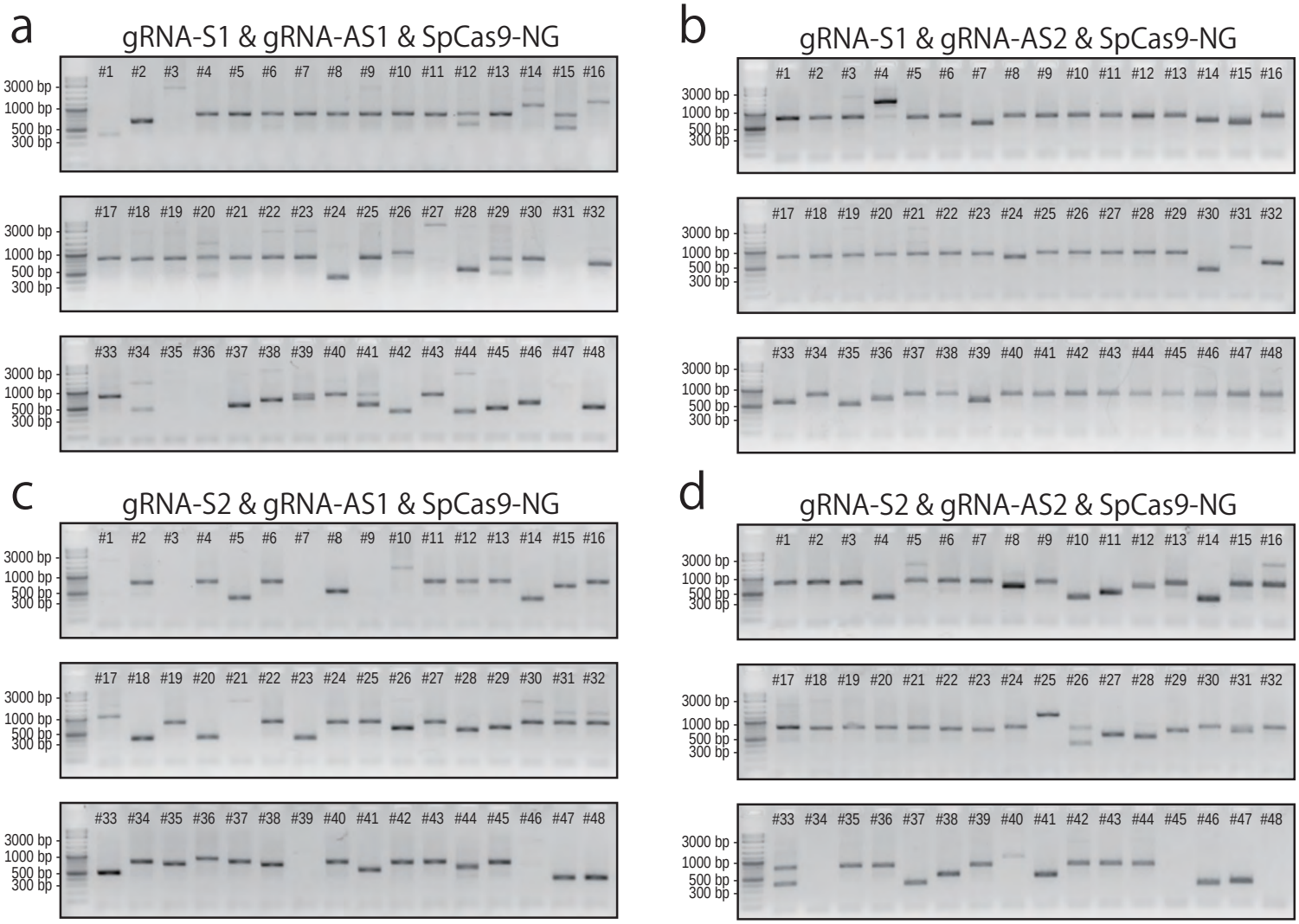
e

Target	gRNA	WT-SpCas9 Clones	WT-SpCas9 Contracted	SpCas9-NG Clones	SpCas9-NG Contracted
CAG repeat (Chr.7)	gRNA S	48	0	43	23
	gRNA AS	48	0	45	2
CAG repeat (Chr.7)	gRNA S	47	0	48	12
	gRNA AS	48	0	43	4
CAG repeat (Chr.7)	gRNA S	48	1	47	18
	gRNA AS	48	0	46	3

Supplementary Figure 2. Two-hit method for CAG repeat contraction and targeting of mouse endogenous CAG repeats, related to Fig. 2

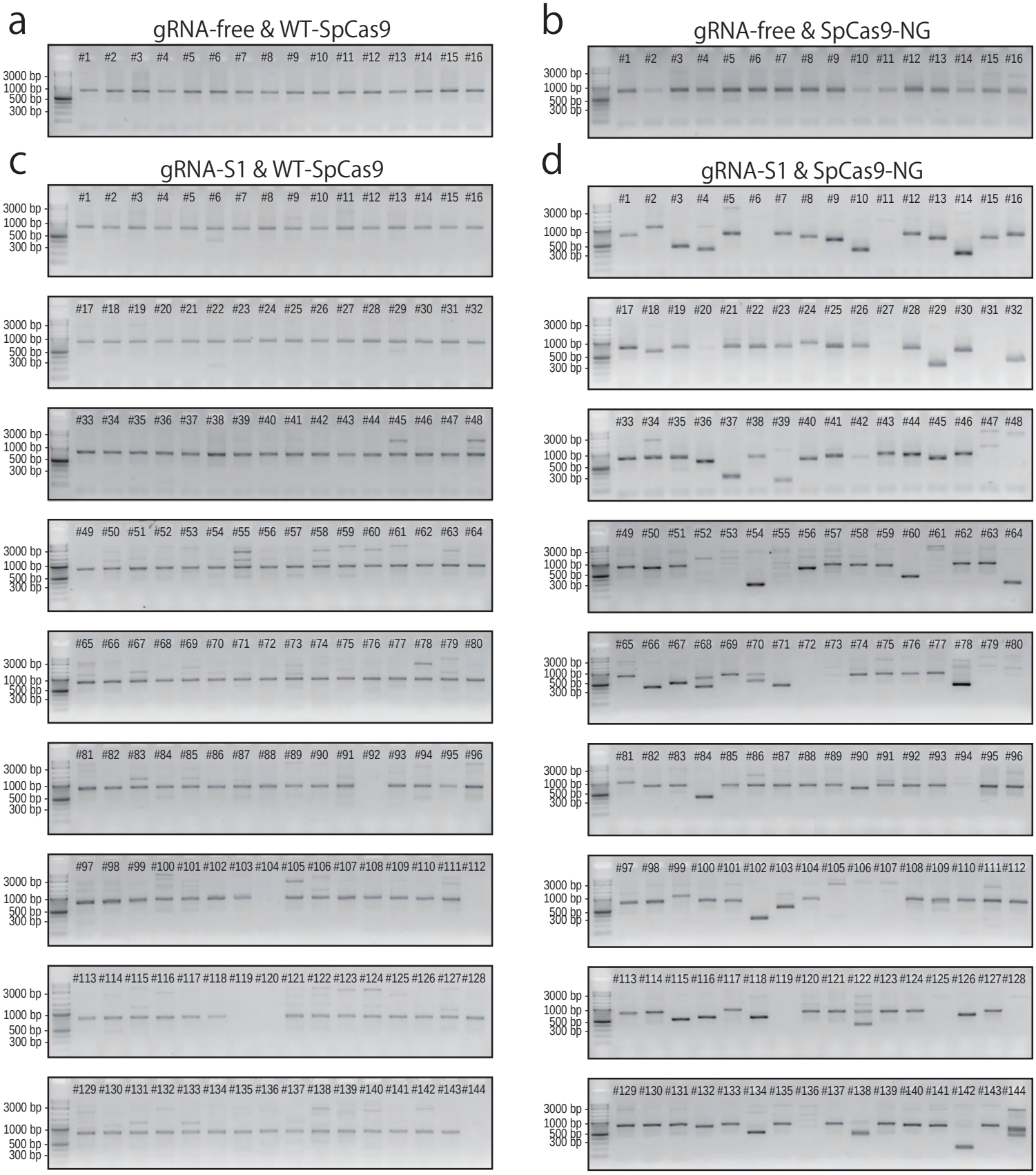
a PCR-based screen for successfully contracted clones. The red arrows indicate the original size of the PCR amplicon. **b** Summary of PCR-based screening in 2A and direct sequencing. **c** Design of gRNAs for targeting mouse endogenous CAG repeats. The orange background color shows the area of the repeat tract. Bold characters with a red box in the DNA sequence indicate the second G in NGN-PAMs. **d** PCR-based confirmation of CAG repeat contraction. The red arrow indicates the original size of the PCR amplicon. **e** Summary of PCR-based confirmation of repeat contraction in 2C.

Supplementary Figure 3



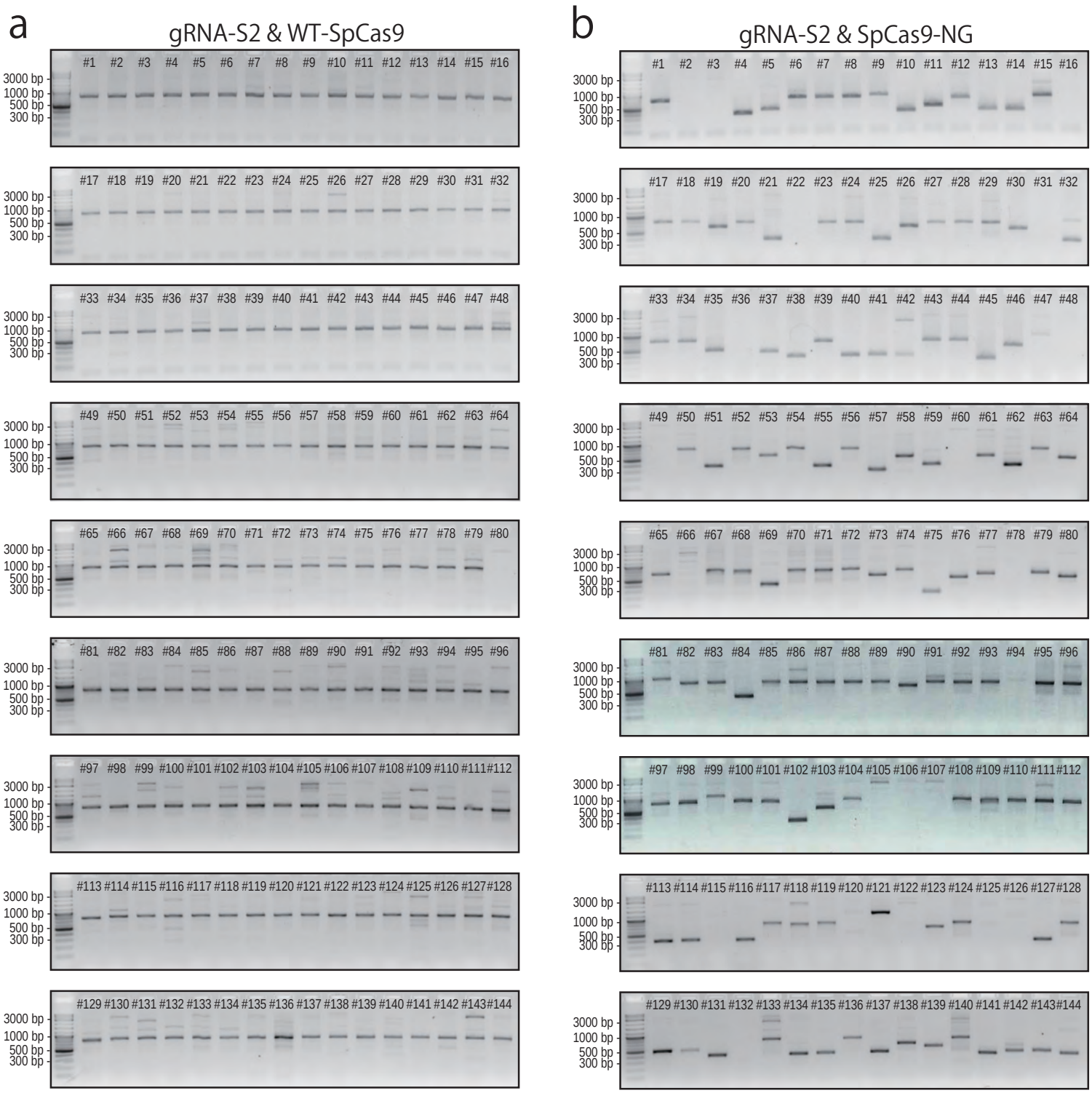
Supplementary Figure 3. Whole gel images of PCR based screeninig of ES cell clones, related to Supplementary Figure 2a.

Supplementary Figure 4



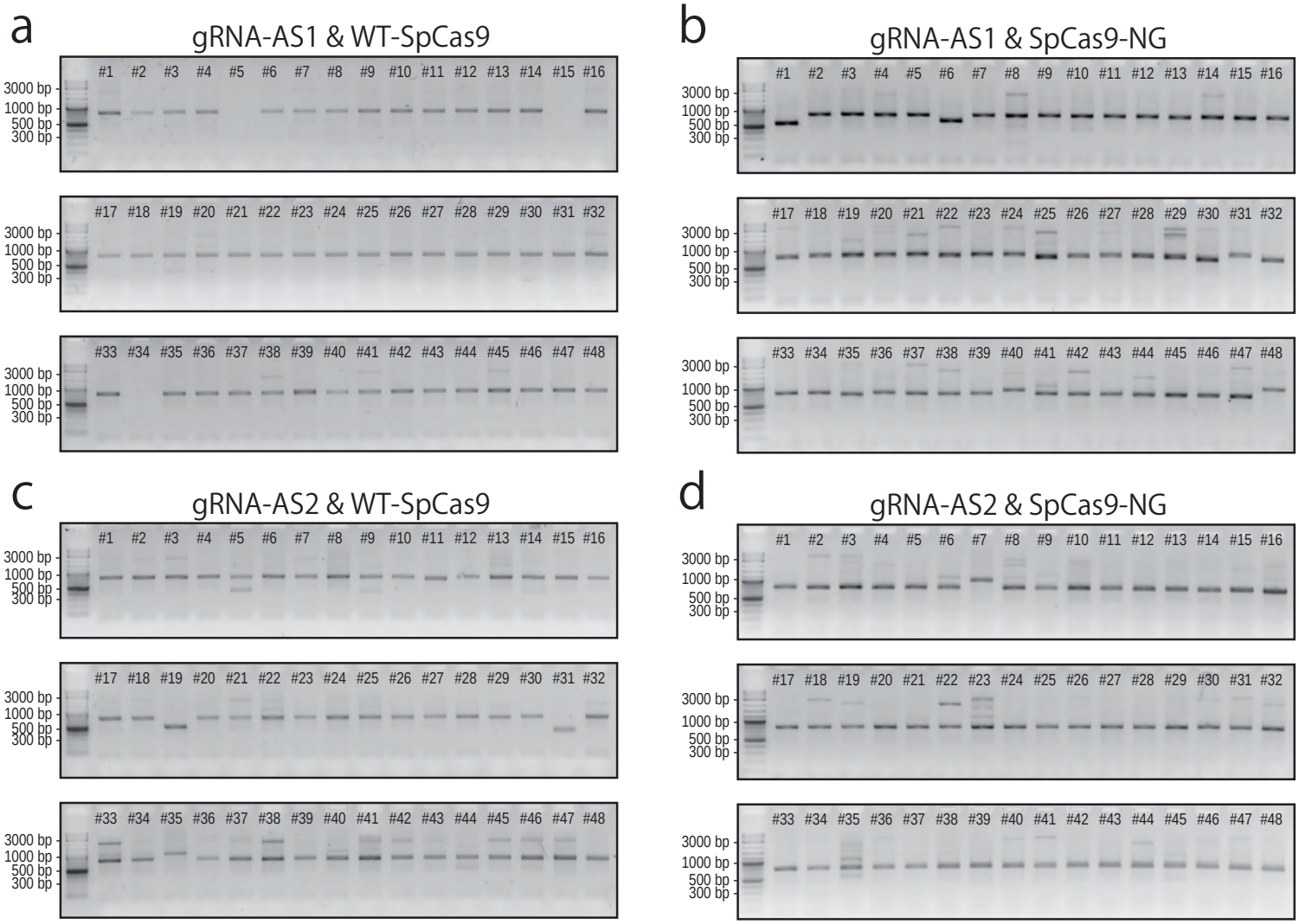
Supplementary Figure 4. Whole gel images of PCR based screeninig of ES cell clones, related to Figure 2c.

Supplementary Figure 5



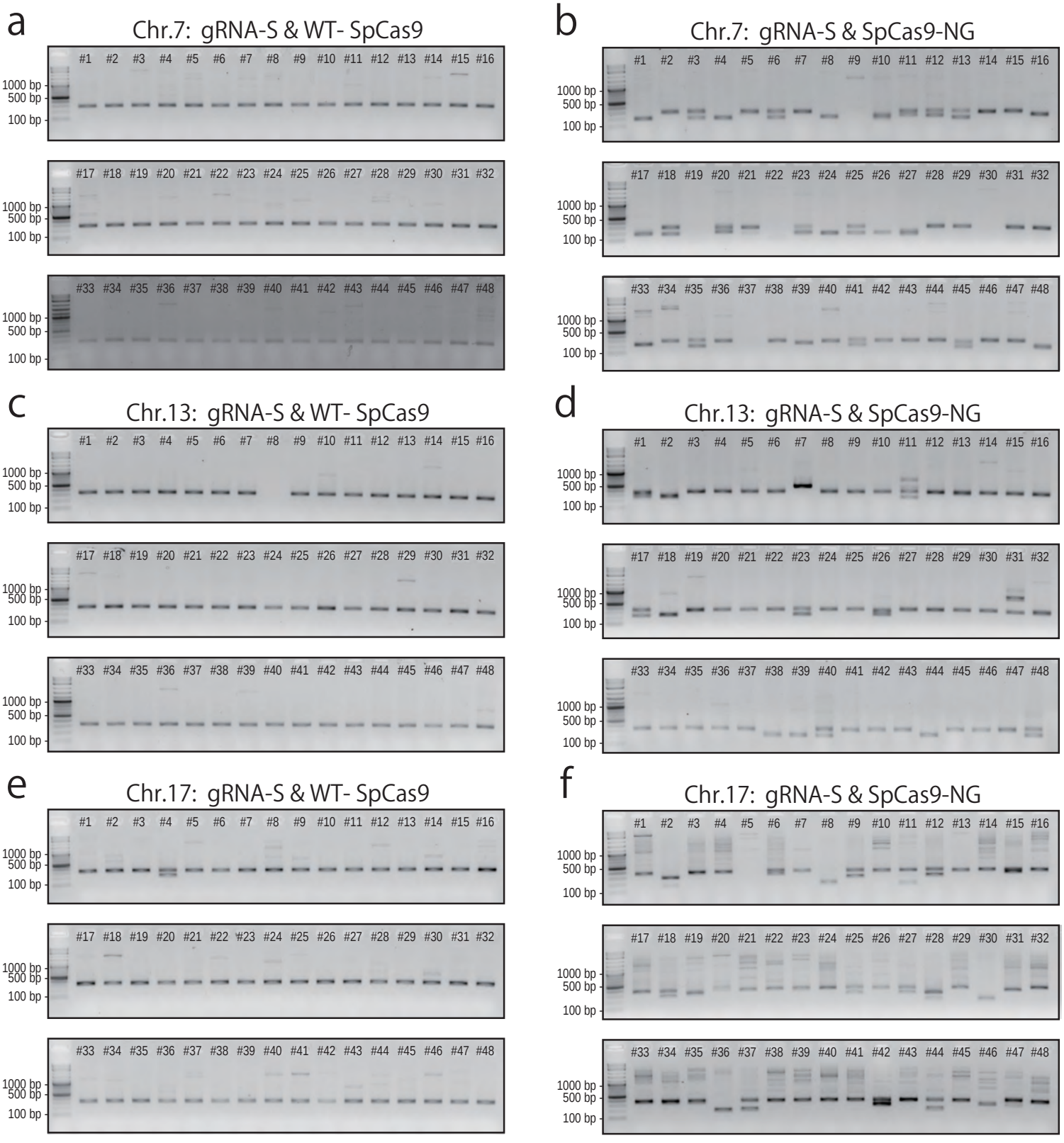
Supplementary Figure 5. Whole gel images of PCR based screeninig of ES cell clones, related to Figure 2c.

Supplementary Figure 6



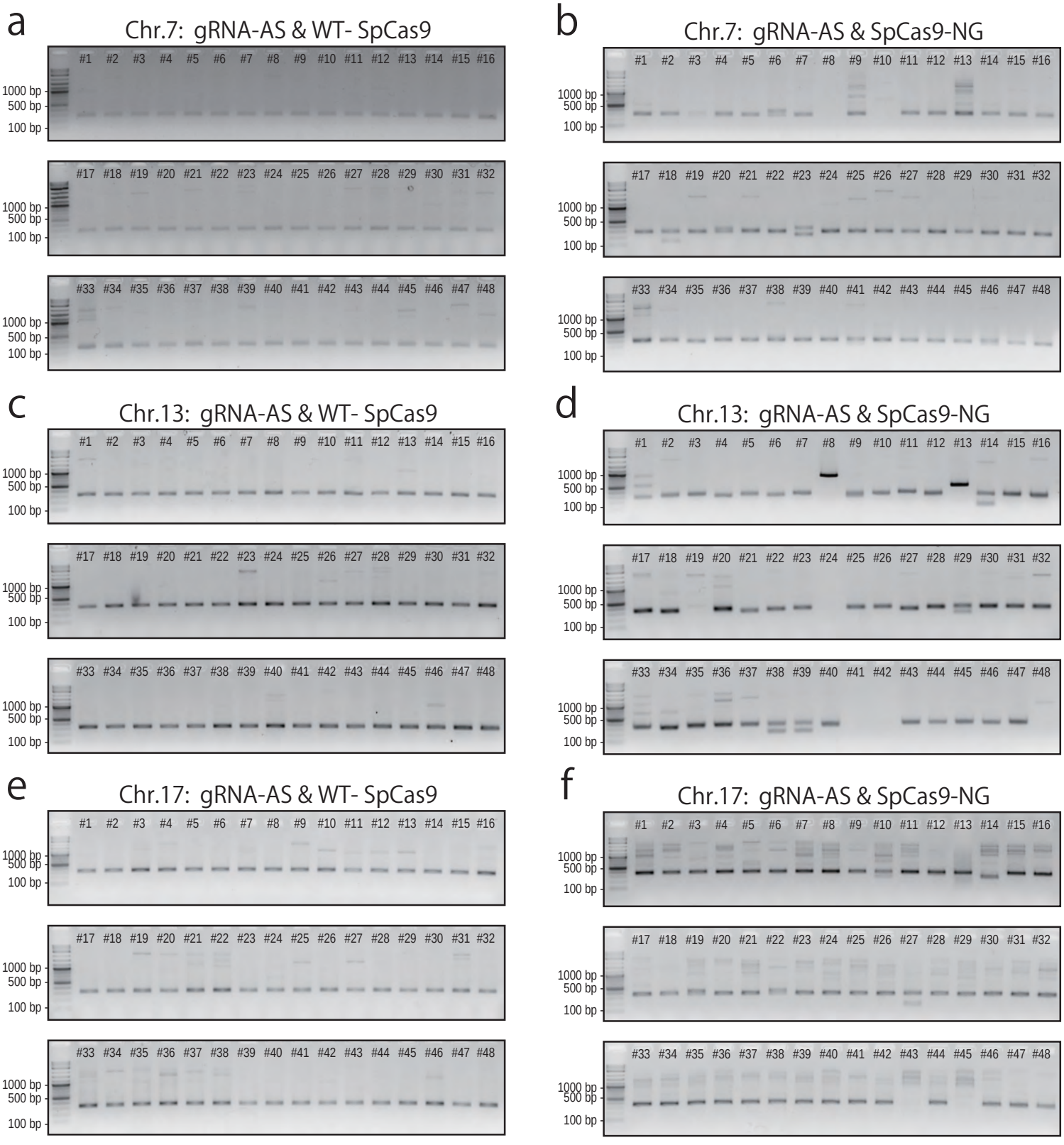
Supplementary Figure 6. Whole gel images of PCR based screeninig of ES cell clones, related to Figure 2c.

Supplementary Figure 7



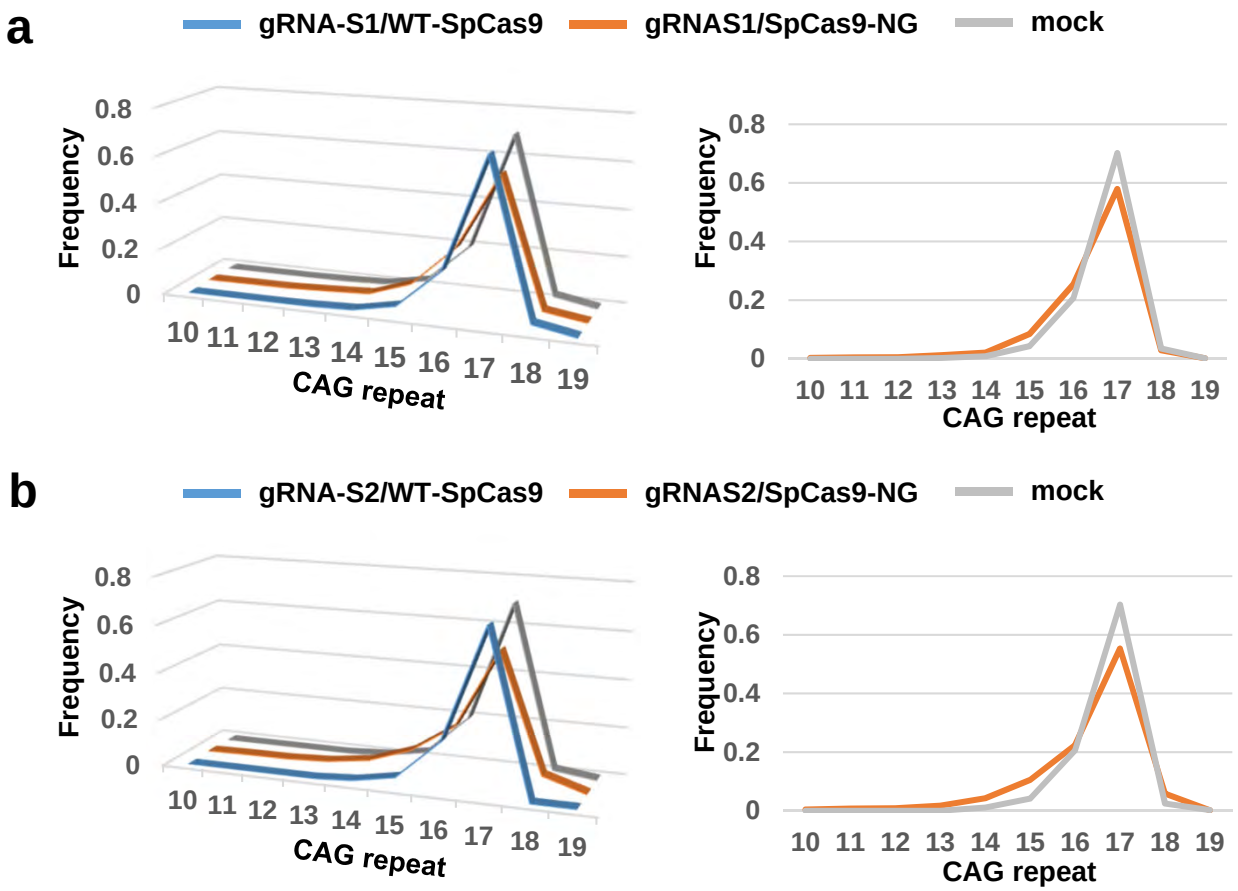
Supplementary Figure 7. Whole gel images of PCR based screeninig of ES cell clones, related to Supplementary Figure 2d.

Supplementary Figure 8



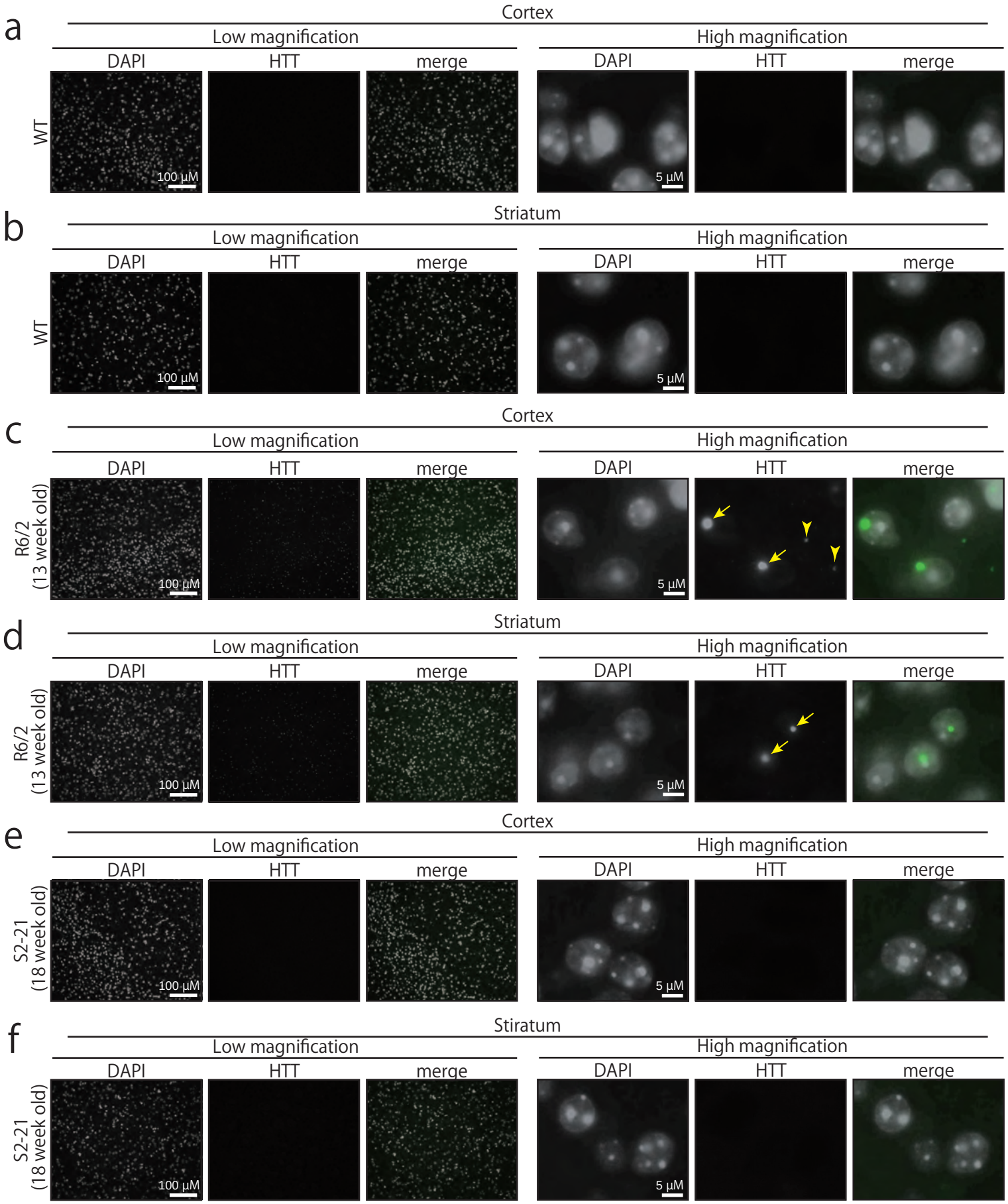
Supplementary Figure 8. Whole gel images of PCR based screeninig of ES cell clones, related to Supplementary Figure 2d.

Supplementary Figure 9



Supplementary Figure 9. Contraction of HTT CAG repeats in HEK293T cells. **a–b** Histogram of the number of HTT CAG repeat sequences in HEK293T cells transfected with gRNA-S1 (a) and -S2 (b) expression vectors in Figure 3. The frequency of X repeats was calculated by the following formula: [(Reads with X repeats)/ $\sum_{X=10}^{19}$(Reads with X repeats)].

Supplementary Figure 10



Supplementary Figure 10. HTT aggregation detection by Immunofluorescence, related to Figure 6i–n. **a–f** Immunofluorescent staining with anti-human HTT antibody. White arrows and arrowheads indicate HTT aggregates inside and outside the nucleus, respectively.sequence and PAM, respectively.