

Figure S1. Experimental scheme of *in vitro* reconstitution of center part of cartwheel structure by HsSAS-6 fragment

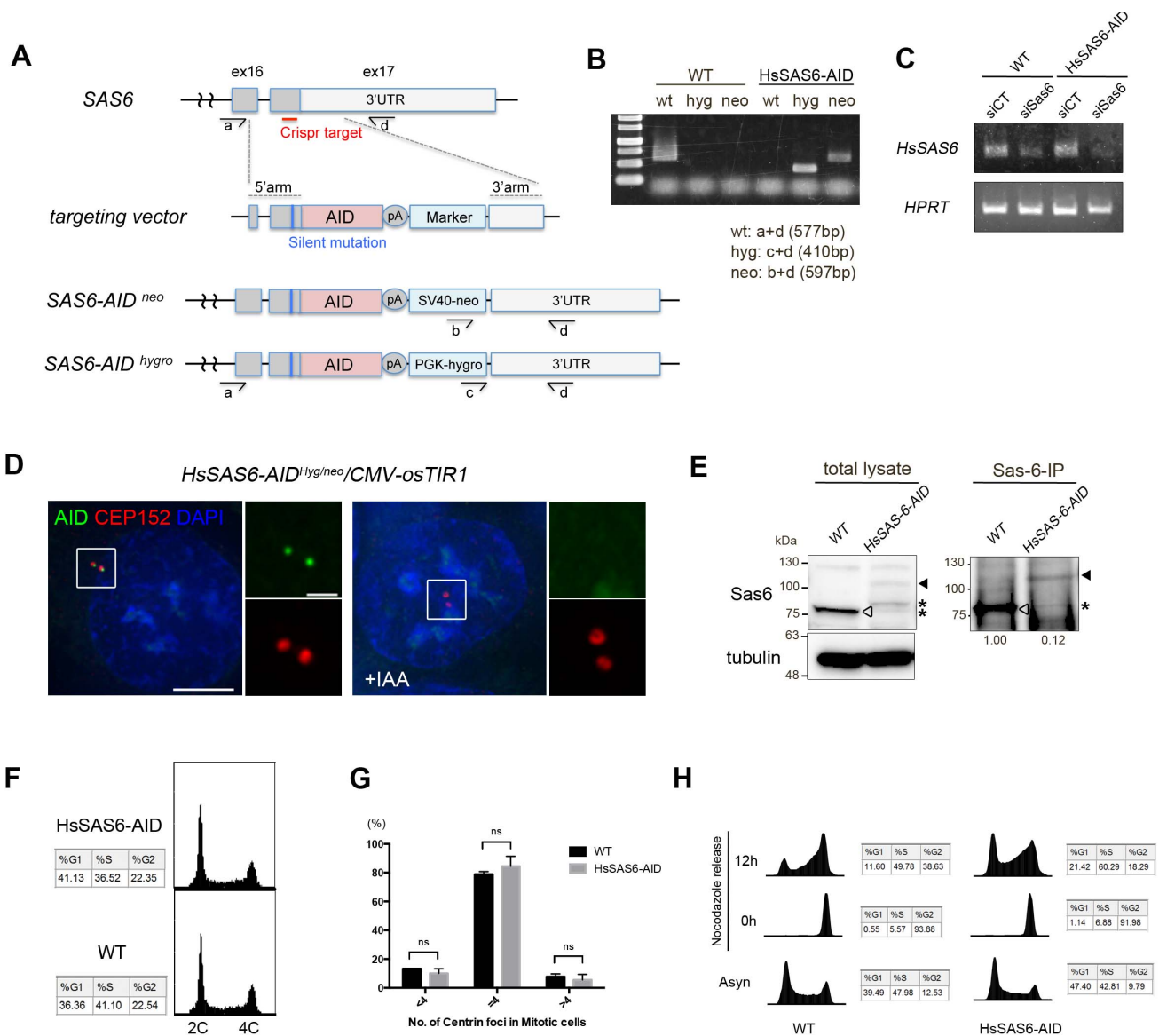


Figure S2. Generation of HsSAS-6-AID cell line

(A) Schematic representation of bi-allelic C-terminus AID-tagging on HsSAS-6 alleles by CRISPR/Cas9 system. Alphabets with arrows below the genes (A-D) indicate the positions of primers for genotyping used in B. (B) Bi-allelic AID knock-in was confirmed by genome PCR. Primer pairs used were indicated below. (C) Expression level of *HsSAS-6-AID* was semi-quantitatively tested by RT-PCR. *HsSAS-6* depletion by ORF-target *siHsSAS-6* reduced *HsSAS-6-AID* level, as is the case of *WT*. (D) The response to IAA in *HsSAS-6-AID* cell line was also confirmed by immunofluorescence with anti-AID antibody (related to Fig. 2A). (E) The amount of cytoplasmic HsSAS-6 protein was tested in *WT* and *HsSAS-6-AID* cells. Total lysates and IPs with anti-HsSAS-6 antibody were analyzed by western blotting using the indicated antibodies. White and black arrowheads point to endogenous HsSAS-6 and HsSAS-6-AID bands, respectively. The asterisk indicates a non-specific band. The blot and the signal intensities below shown are a representative from three independent experiments. (F)

HsSAS-6-AID cells show slightly more accumulation in G1 phase compared to WT cell by flow cytometry analysis. (G) Centriole duplication was normal in HsSAS-6-AID cell line. The number of centrin foci in mitotic cells was quantified. Values are mean percentages \pm s.e.m. from three independent experiments ($n \geq 50$ for each condition). The statistical significance between the data sets was determined by two-tailed unpaired *t*-test. ns, not significantly different ($p > 0.05$). (H) Cell cycle profile after nocodazole arrest release was tested in WT and HsSAS-6-AID cell lines. HsSAS-6-AID cells show a slight delay at 12hr after release compared to WT cells.

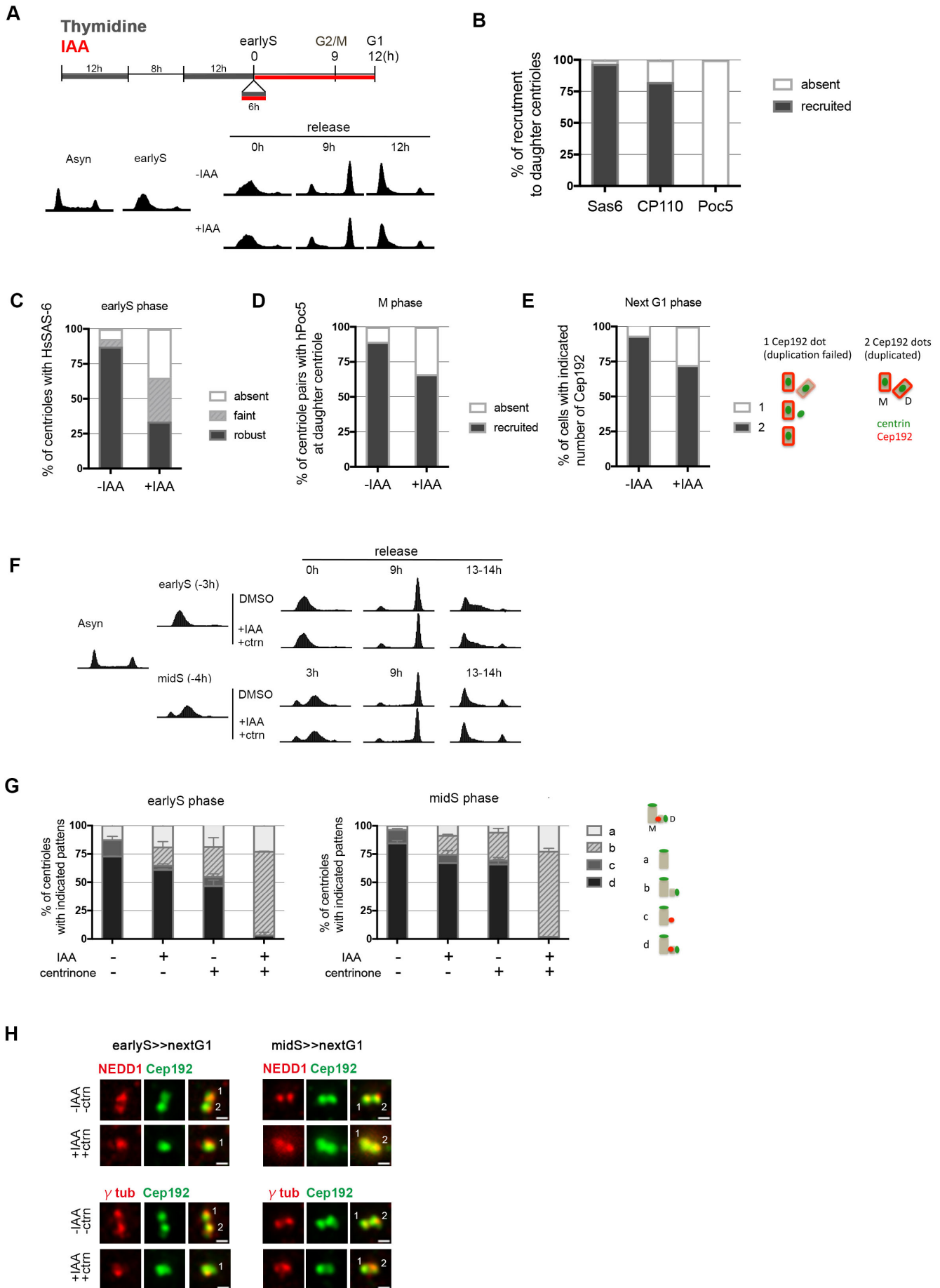


Figure S3. The effect of IAA on HsSAS-6-AID cell line

(A) Experimental scheme of HsSAS-6 removal in the S-phase arrested cells. Cells were synchronized at G1/S border (early S phase) by double thymidine block. For HsSAS-6-AID removal, cells were treated with 0.5 mM IAA for 6 h, and released into fresh medium with IAA until next G1. Cell cycle progression was monitored by flow cytometry (below). (B) Recruitment of HsSAS-6, CP110 and Poc5 at early S phase was tested. Values are mean percentages from two independent experiments (total n = 100, n = 34 and n = 50, respectively). (C) The number of centrosomes with HsSAS-6 after IAA treatment was quantified according to the indicated category. (D) The number of centriole pairs without or with hPoc5 at daughter centrioles at M phase was quantified. (E) The number of centrioles with the indicated number of Cep192 in the next G1 phase was quantified according to the patterns shown right. Values are mean percentages from two independent experiments (total n ≥ 100; C-E). (F) FACS profiles related to Fig. 4C-H are shown. After IAA and/or Centrinone treatment at early S phase or middle S phase, cells are released and proceed to next G1 phase within 13 to 14 h. (G) The number of centrioles with the indicated patterns after the drug treatment in Fig. 4D was quantified. Values are mean percentages from two independent experiments (total n ≥ 100). Left panel, in the early S phase; right panel, in the middle S phase. (H) Representative patterns of NEDD1 and γ -tubulin at next G1 phase after HsSAS-6 removal in the early S or middle S phase. Numbers in the panels indicate the number of mature centrioles.

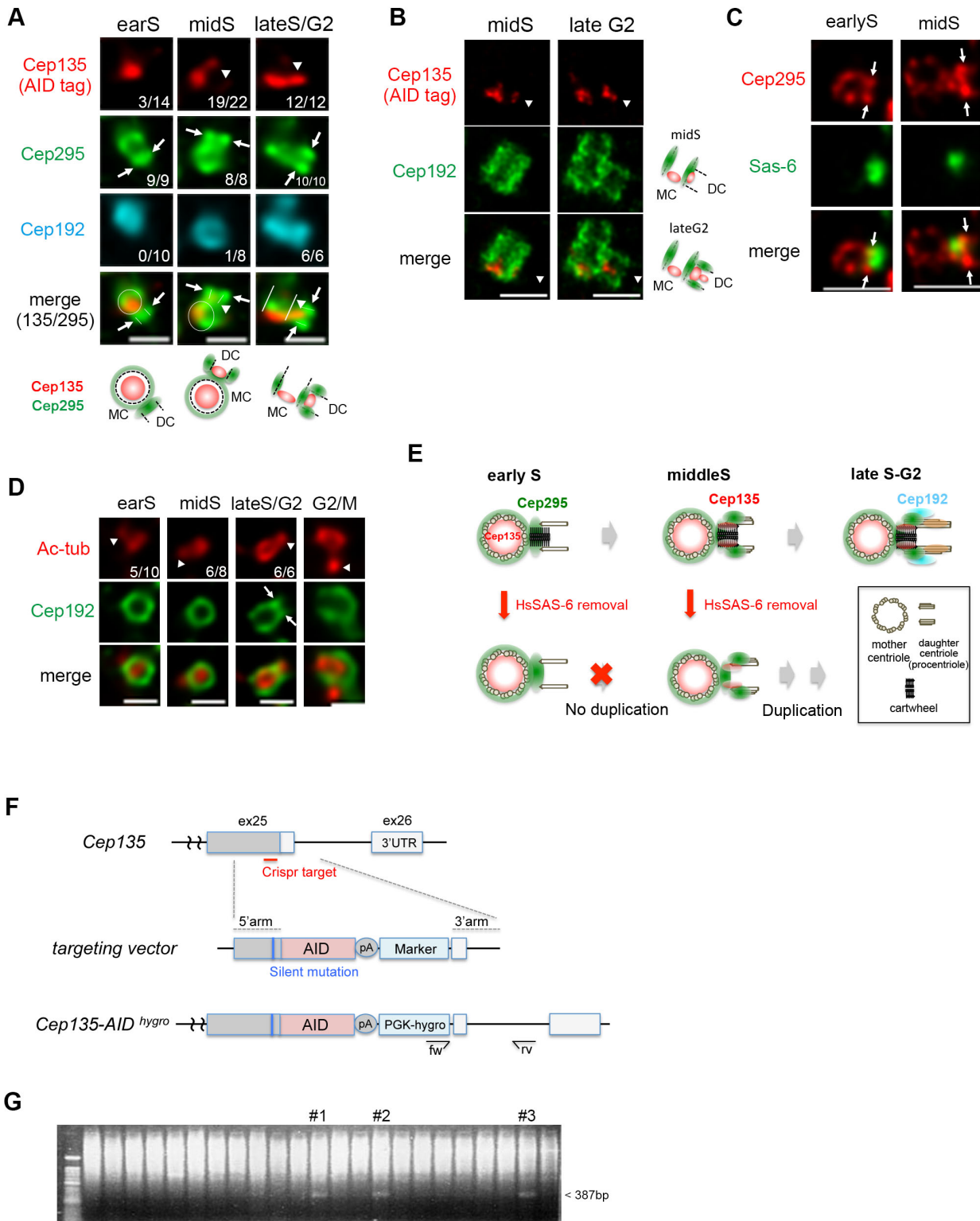


Figure S4. Ordered loading of centriolar proteins through earlyS phase to G2 phase in a growing procentriole.

(A) Loading of Cep295, Cep135 and Cep192 to procentrioles at the indicated stages was tested by immunofluorescence staining. Cep295 and Cep192 were detected by anti-Cep295 and anti-Cep192 antibodies, respectively. Cep135 was detected by anti-AID antibody against endogenously-tagged Cep135-AID (cell line information in F,G). Note that we also detected

similar patterns with anti-Cep135 antibody. The images were obtained by Leica TCS SP8 Hyvolution system. White arrowheads and white arrows indicate the signals of Cep135 and Cep295 at procentrioles, respectively. The numbers below represent the numbers of Cep135, Cep295 or Cep192 loading to procentrioles /total counts. Schematic illustrations are shown below. All scale bars in Figure S4, 500 nm. (B) Representative STED images of Cep135 loading to procentrioles in the middle S and late G2 phase. Schematic illustrations are shown on the right. (C) Representative STED images of Cep295 pattern at early and middle S phase. HsSAS-6 was used for a procentriole marker, and white arrows indicate procentriole sites. Notably Cep295 signals at procentriole sites seem to be increasing through early S to middle S phase. All scale bars in this figure, 500 nm. (D) Acetylation of tubulin in daughter centrioles was tested at the indicated stages. White arrowheads indicate procentriole assembly sites. Note that tubulin acetylation seems to occur earlier than other centriole maturation events, such as tubulin polyglutamylation and PCM recruitment. (E) Speculative illustration of the phenotypes by HsSAS-6 removal in the middle of procentriole formation. After Cep295 and Cep135 loading to the daughter centrioles, the cartwheel may be dispensable for further elongation and maturation. (F) Schematic representation of C-terminus AID-tagging on Cep135 allele by CRISPR/Cas9 system. The arrows below the gene (fw and rv) indicate the positions of primer pair for genotyping used in G. (G) Cep135-AID knock-in was screened by genomic PCR. Clone #1 was used in this study.

Table S1. Oligos used for the generation of HsSAS-6-AID and Cep135-AID cell lines.

Bold letters in guideRNAs show the sequence in the vector. Colored characters in 3' arm indicates the CRISPR/Cas9 target site. Note that PAM sequence is substituted, not to be targeted (Blue, guide RNA target site; green, part of PAM; red, substituted in PAM). BamHI site was inserted at the stop codon for the generation of targeting vector.

Gene	Oligo name	Sequence (5'-3')
HsSAS-6	gRNA fw	CACCGAATAGGCTGAAGACGCAGAG
	gRNA rv	AAACTCTGCGTCTTCAGCCTATTC
	3' arm	ttcctttacgcgactcagccagaacctatttagtaattcaggaagcagggatt tctggatctttgtactttatagttgaattaaattaatgtatgaagacttaataac cagtaacctttttgtcctcattgcagaccatcagagagatggcacttttagga gcattacatacatcttccaaccacagcgctaccctctgcttccagcctat ttccctgggacgtaccaaacagtggatccctctagtgtcatgtttacttttatt ggtatcttagaaactcaggtgcttaaaaaatgtcaacacaaaccagatcctc aaatagcaagttcaaatttacttgagctgttaaagactggatactttaagta gctttatggcagttataataggaacaaattgtacttggggattggtaaagat tgtgagtaaatgacatttttggttgtgaacatggcgtttatagaataata
Cep135	gRNA fw	CACCGGACCATGTCGACGCATCTCT
	gRNA rv	AAACAGAGATGCGTTCGACATGGTCC
	3' arm	aaaattgtacaaagacataggatttaaatttaataagtaactaagtaattattac aaattagctaataactcttatattgtttgccacttttgattttccctgggcttgaat atatctctctgtttgatcttactaacagagaacgagcaatcaagagatgctg cgacatggcttgctacaccacccttagtccactctgaggtctccttcacatt ctcctgaacatagaaatgtggatccctatcagaaaggtatgtatgtaacacc aaggacaggcaaaactaatctgtggtgtaaaaattcagaaagtagtttctgc atcaggaaaggggagattgcctggaaggcgtcaggagaacttctggagt aatggaaaagtctatacttggtgatagtagttatgtgggtatatacaattgtaa aattcataaaactgaccatttacagtgtatgctttttttttaagagatggg