

Cell line	Target	Type of transfection	Gene editing (indel)	Phenotype rate
HeLa	<i>PLK1</i>	Solid-phase rec. Cas9 + gRNA	24.3%	4.75% \pm 0.47%
	<i>PLK1</i>	All-in-one cDNA	17.6%	8.33% \pm 1.54%
	<i>PLK1</i>	Lentiviral particles	19.7%	9.26% \pm 0.94%
	<i>PLK1</i>	Liquid-phase rec. Cas9 + gRNA	12.2%	1.84% \pm 0.07%
	<i>KIF11</i>	Solid-phase rec. Cas9 + gRNA	21.7%	8.85% \pm 1.33%
	<i>KIF11</i>	All-in-one cDNA	18.1%	11.23% \pm 1.65%
	<i>KIF11</i>	Lentiviral particles	14.2%	5.32% \pm 0.40%
	<i>KIF11</i>	Liquid-phase rec. Cas9 + gRNA	7.8%	2.63% \pm 0.44%
	<i>INCENP</i>	Solid-phase rec. Cas9 + gRNA	15.3%	5.13% \pm 0.31%
	<i>INCENP</i>	All-in-one cDNA	14.7%	3.85% \pm 0.52%
	<i>INCENP</i>	Lentiviral particles	12.6%	4.77% \pm 0.76%
	<i>INCENP</i>	Liquid-phase rec. Cas9 + gRNA	4.5%	1.32% \pm 0.36%
HEK293T	<i>PLK1</i>	Solid-phase rec. Cas9 + gRNA	25.1%	10.42% \pm 0.74%
	<i>PLK1</i>	All-in-one cDNA	18.8%	14.58% \pm 1.49%
	<i>PLK1</i>	Liquid-phase rec. Cas9 + gRNA	17.1%	6.22% \pm 0.82%
	<i>KIF11</i>	Solid-phase rec. Cas9 + gRNA	25.6%	9.01% \pm 0.44%
	<i>KIF11</i>	All-in-one cDNA	18.7%	7.66% \pm 1.30%
	<i>KIF11</i>	Liquid-phase rec. Cas9 + gRNA	23.3%	9.92% \pm 0.13%
	<i>INCENP</i>	Solid-phase rec. Cas9 + gRNA	15.0%	2.79% \pm 0.22%
	<i>INCENP</i>	All-in-one cDNA	15.3%	4.78% \pm 0.68%
	<i>INCENP</i>	Liquid-phase rec. Cas9 + gRNA	15.0%	1.48% \pm 0.30%

Supplemental Table S4 Gene editing efficiencies as produced indels and phenotype incidences. The transfections were performed in 384-well plates (solid-phase reverse transfection of rec. Cas9 + gRNA as well as all-in-one cDNA) and in 96-well plates (liquid-phase transfection of rec. Cas9 + gRNA). For the HeLa cell line, the indel and phenotype incidences that were produced after lentiviral transduction of the CRISPR all-in-one constructs are also shown. The transductions were performed in 384-well plates. Cells were fixed or lysed 48 hours post transfection or transduction, respectively, followed by PCR target amplification and genomic cleavage detection assay. Phenotype analysis and quantification were performed as described in Methods.