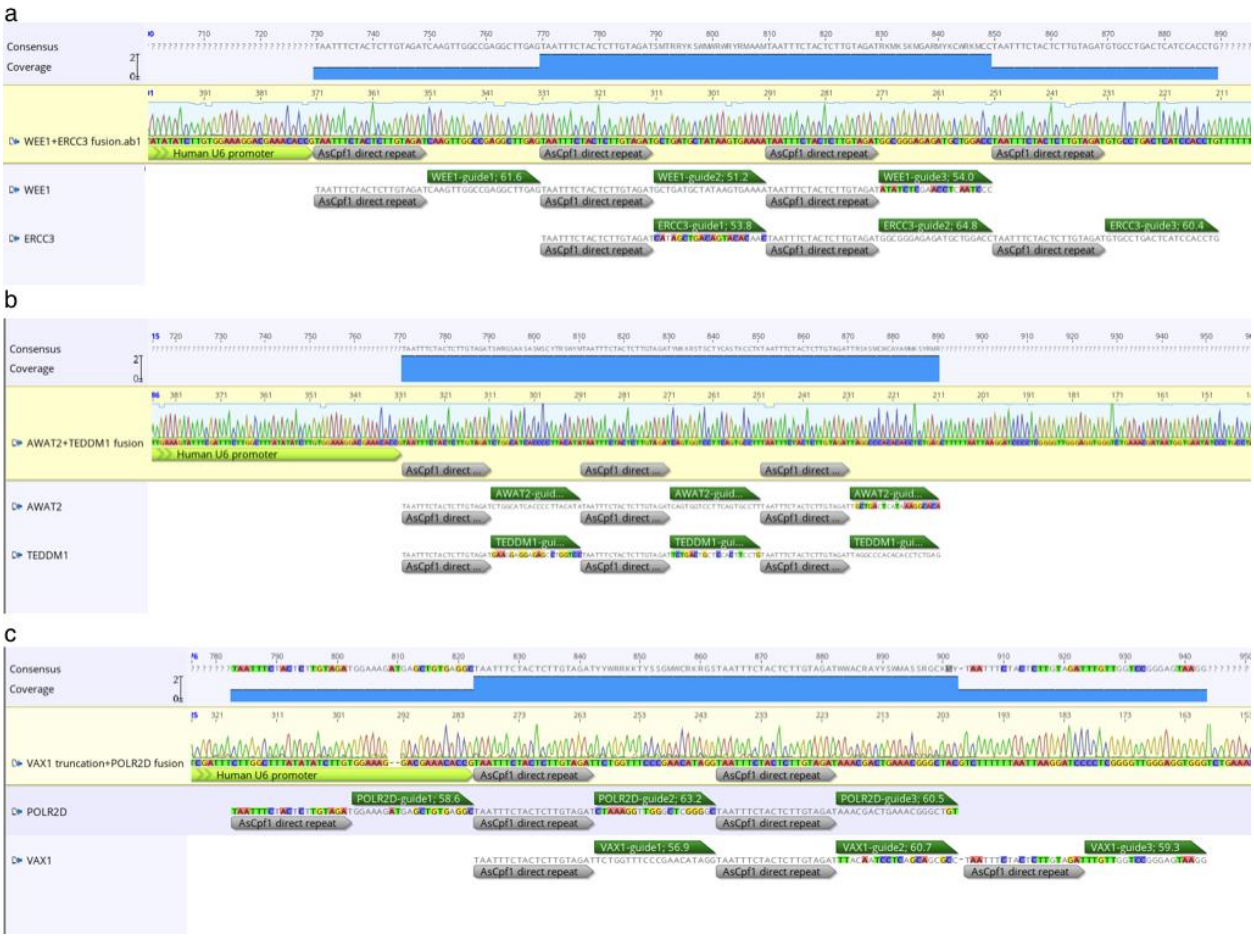
Supplementary Information

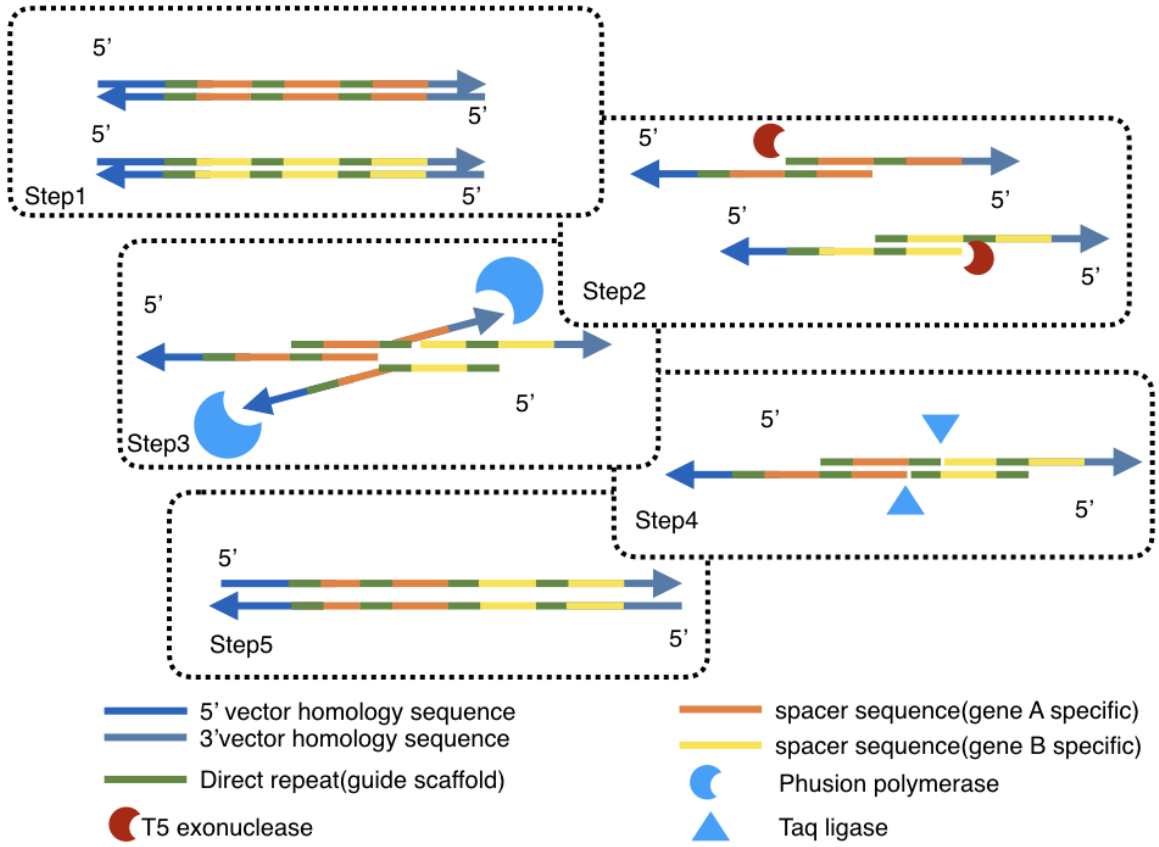
Pooled library screening with multiplexed Cpf1 library

Liu et al



Supplementary Figure 1 Examples of amplicon rearrangement caused by Gibson assembly.

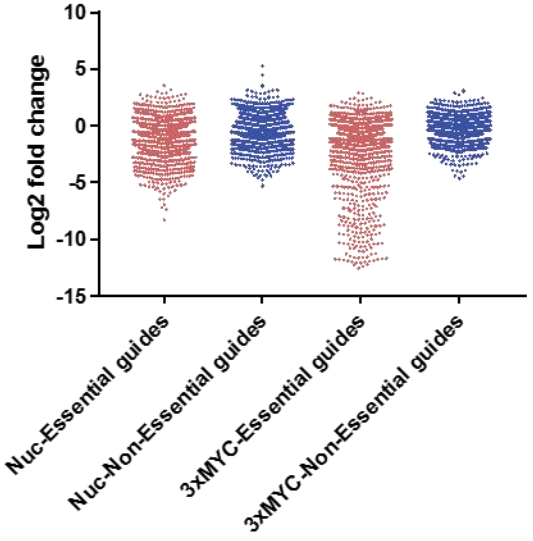
Sanger sequencing results of randomly picked colonies showing rearranged molecule generated from 2 different 3-guide array amplicon, resulting 4-guide hybrid **(a),** 3-guide hybrid **(b),** and truncated 2-guide hybrid**(c).**



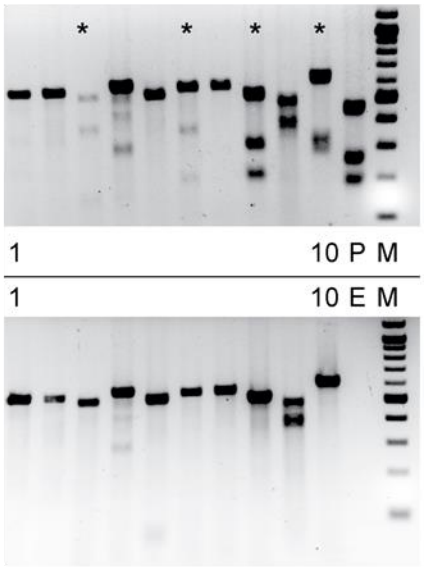
Supplementary Figure 2 Proposed amplicon rearrangement caused by Gibson assembly.

After PCR amplicon of oligo duplexes are added into Gibson assembly reaction (step 1), T5 exonuclease starts to erode the duplex from both strands’ 5’ terminals, exposing the direct repeat sequence of AsCpf1 guides as single strand ready for hybridization (step 2). Different guide arrays hybrid together because of homology in guides’ direct repeats, leaving a long imperfect tail. Phusion polymerase binds to the tails and use its 3’->5’ exonuclease activity to degrade the tails until it meets the perfect hybridization regions consisting of guide direct repeats (step3). Taq polymerase seals nicks between the hybrid chains (step 4) and the recombined molecule is generated and cloned into vector (step 5).

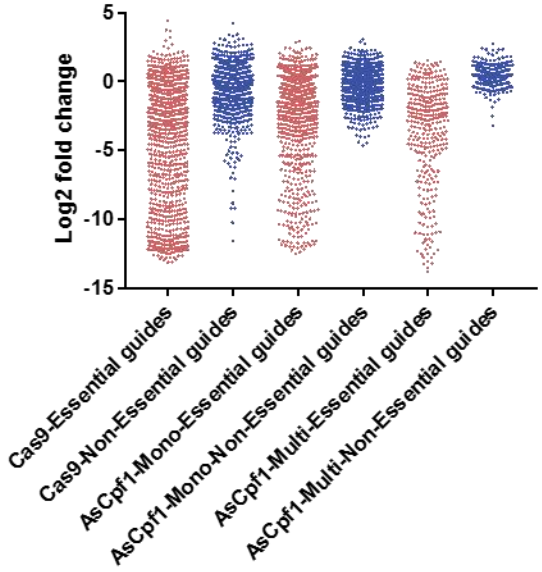
a



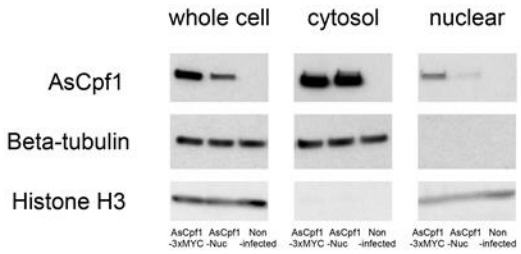
b



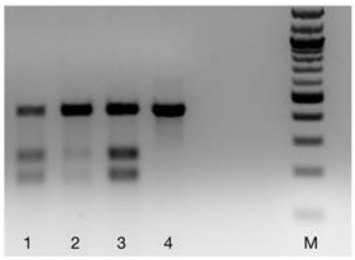
c



d



e

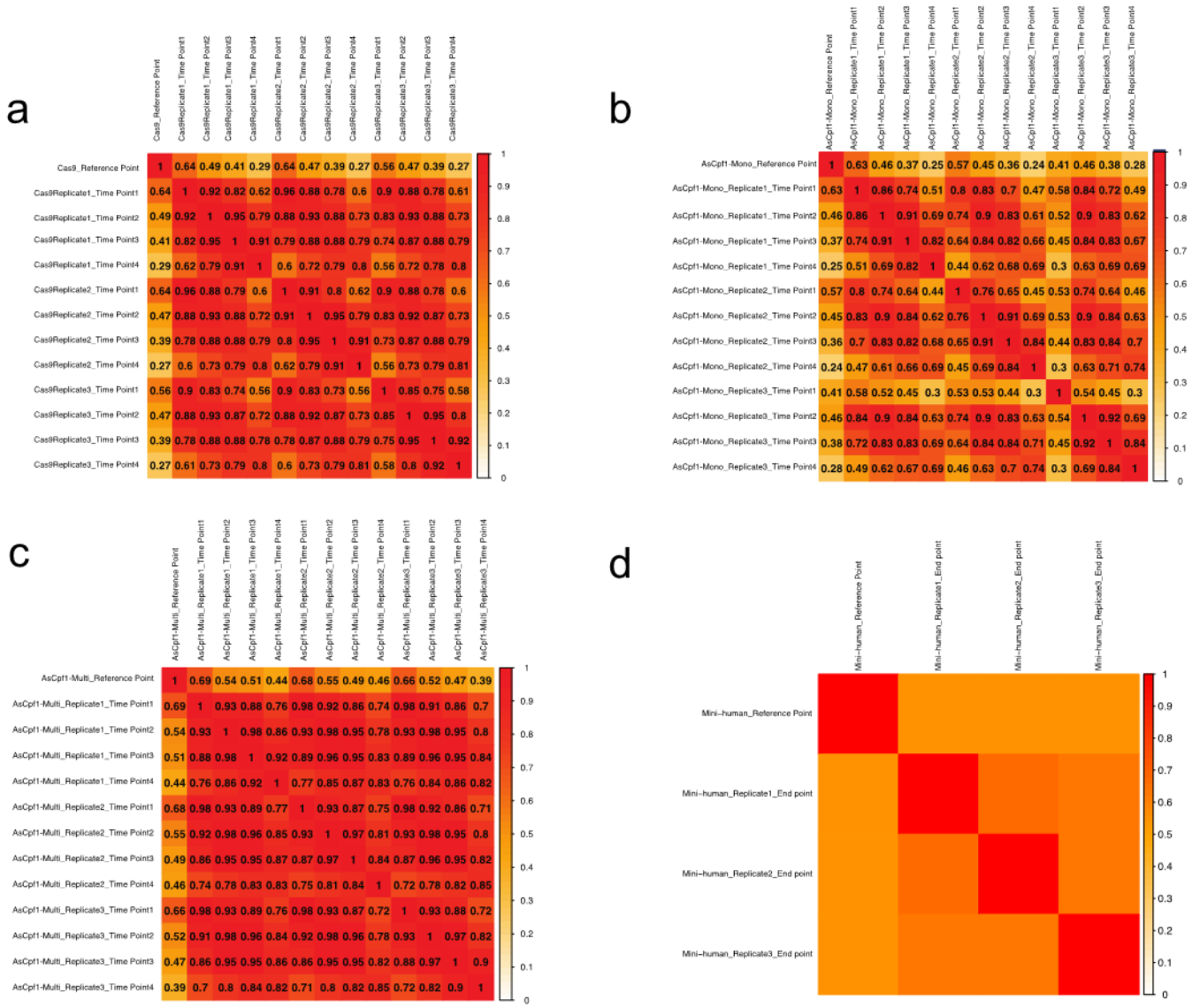


Editing efficacy 48% 9% 36% 0%

Lane	1	2	3	4	M
AsCpf1 variant	AsCpf1-3 xMYC	AsCpf1-Nuc	AsCpf1-SV40Nuc	No AsCpf1	Marker
N-NLS	3xMYC	-	2xSV40	N.A	N.A
C-NLS	-	1xNuc	1xNuc	N.A	N.A

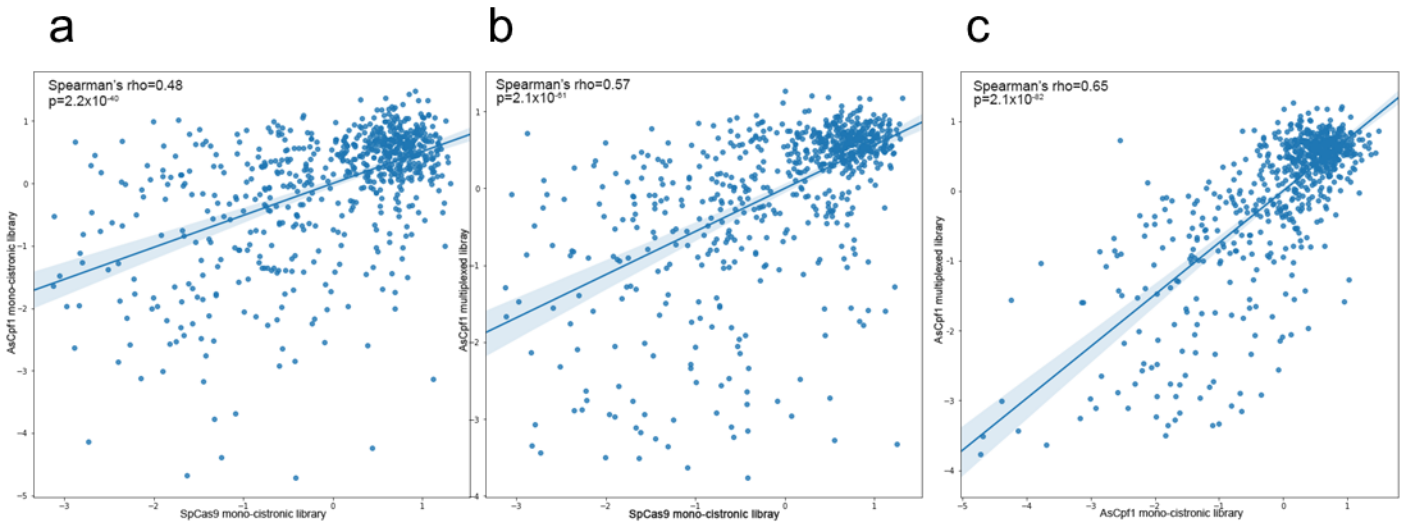
Supplementary Figure 3 AsCpf1 optimization for functional genomics

a. Depletion of essential and non-essential gene targeting guide in AsCpf1-nuc variant and AsCpf1-3xMYC based screen. Pink: the mean value of log2 transformed fold change of essential gene targeting guides at the endpoint, n=1026. Purple: the mean value of log2 transformed fold change of non-essential gene targeting guides at the endpoint, n=1035. **b.** T7E1 assay of 10 different loci transfected with AsCpf1-nuc and corresponding guides. Active guides are indicated by "*". Upper panel: cells transfected with AsCpf1-nuc and guide. Lower panel: cells transfected with guides only. Lane1 to lane 10: T7E1 assay products targeting loci (from left to right) RESP18, NEUROD6, NAMPT, PRAMEF2, SLC22A9, COX7B2, TFAP2D, GUCY2F, ADAM30, and FNDC9. P: T7E1 assay product of DNMT1 edited allele as a positive control for T7 endonuclease activity. E: Empty lane. M: NEB 100bp DNA ladder. **c.** Depletion of essential and non-essential gene targeting guide in AsCpf1-3xMYC and SpCas9 based screen. Pink dot: the mean value of log2 transformed fold change of essential gene targeting guides at the endpoint, n=1026 for SpCas9 and AsCpf1 based mono-cistronic library, n=342 for AsCpf1 based multiplexed library. Purple dot: the mean value of log2 transformed fold change of non-essential gene targeting guides at the endpoint, n=1035 for SpCas9 and AsCpf1 based mono-cistronic library, n=345 for AsCpf1 based multiplexed library. **d.** Western blot of AsCpf1 variant. K-562 cells were infected with AsCpf1-Nuc (the commonly used variant) and AsCpf1-3xMYC (Kozak sequence and nucleus localization signal optimized variant) virus at the same multiplicity of infection **e.** T7E1 assay results of AsCpf1 variants on locus DNMT1. HEK293-T cells were transfected with different AsCpf1 variants and guide targeting DNMT1 locus (lane 1-3) in complexed with lipofectamine 3000. 3xMYC (3x tandem MYC NLS), 2x SV40 (2x tandem SV40 NLS), 1xNuc(1x Nucleoplasmin NLS), N.A.(Not applicable). Lane 4: negative control with guide transfection only. Lane M: NEB 100bp DNA ladder. Source data are provided as a Source Data file.



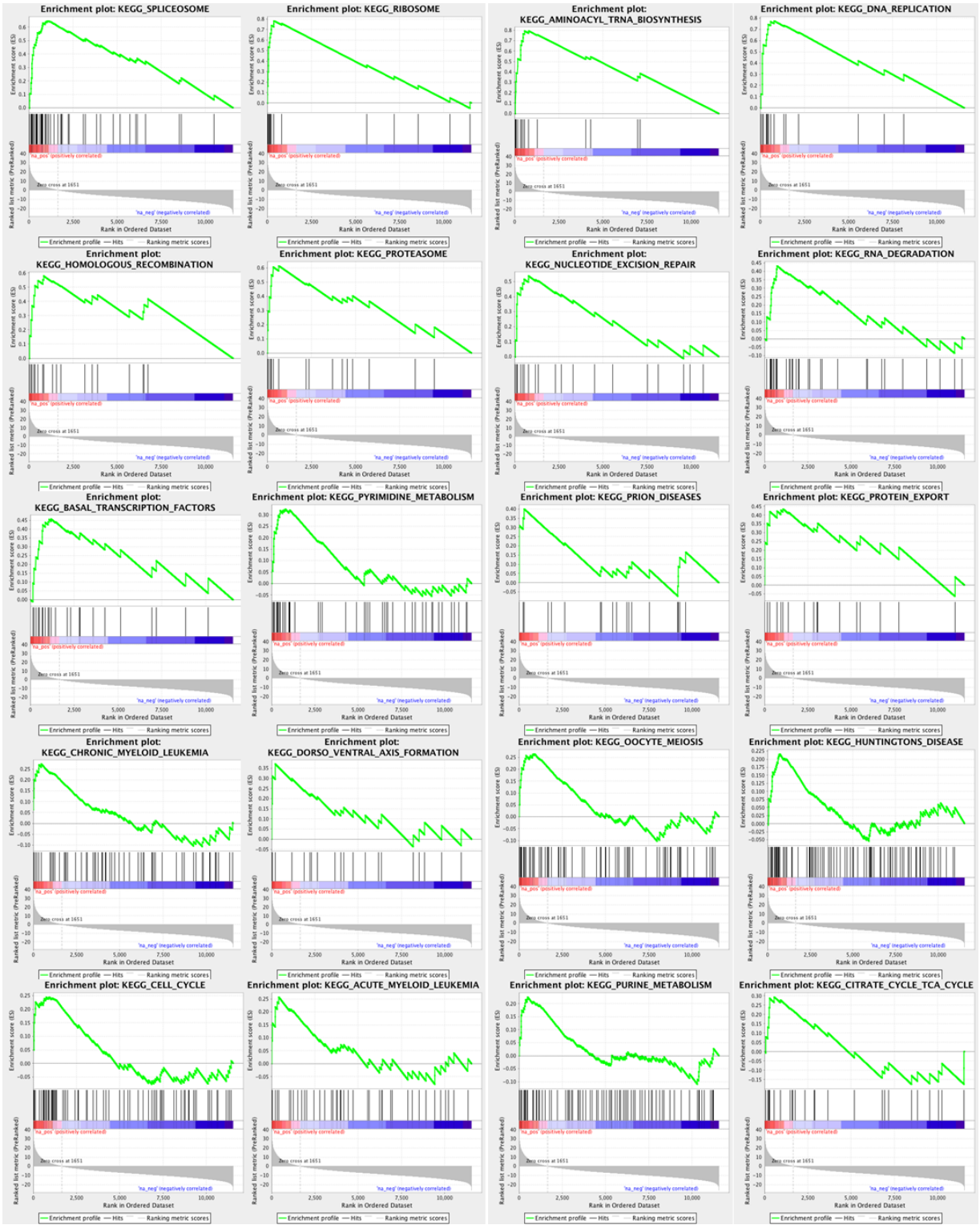
Supplementary Figure 4 Correlation of replicates in the pooled screenings

Pearson correlation coefficient matrices of all samples for SpCas9 based mono-cistronic benchmark library screening **(a)**, AsCpf1 based mono-cistronic benchmark library screening **(b).** AsCpf1 based multiplexed benchmark library screening **(c).** Mini-human genome-wide screening **(d)**. Raw reads counts were used for the analysis. Source data are provided as a Source Data file.



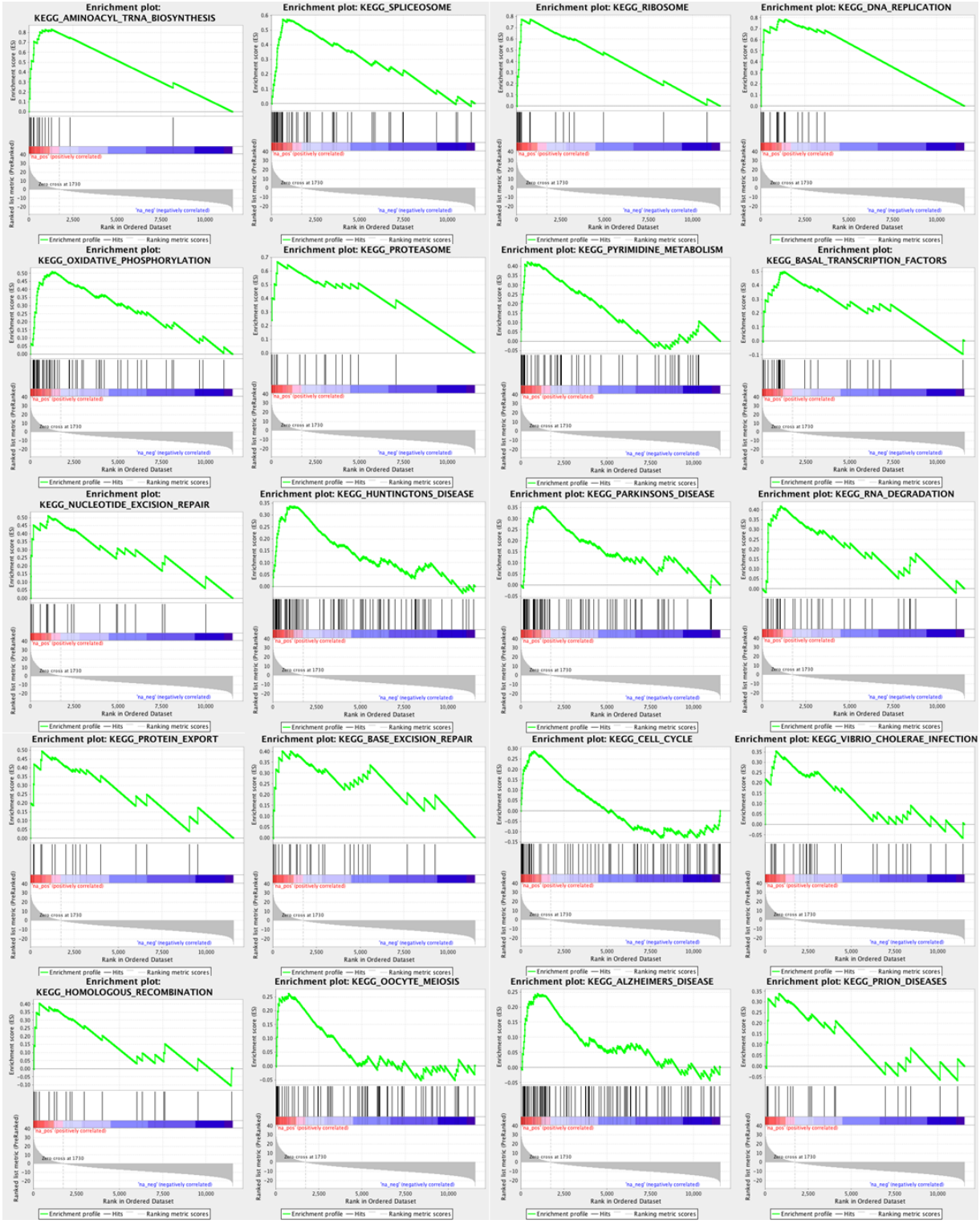
Supplementary Figure 5 Correlation of hit's ranks between different CRISPR libraries

Rank's correlations between benchmark library AsCpf1-Mono vs Cas9 **(a)**, AsCpf1-Multi vs Cas9 **(b)**, AsCpf1-Multi vs AsCpf1-Mono **(c).** Z scores of core-essential genes from each library were used. Line of best fit is plotted and the shaded area is presented as SD. Source data are provided as a Source Data file.



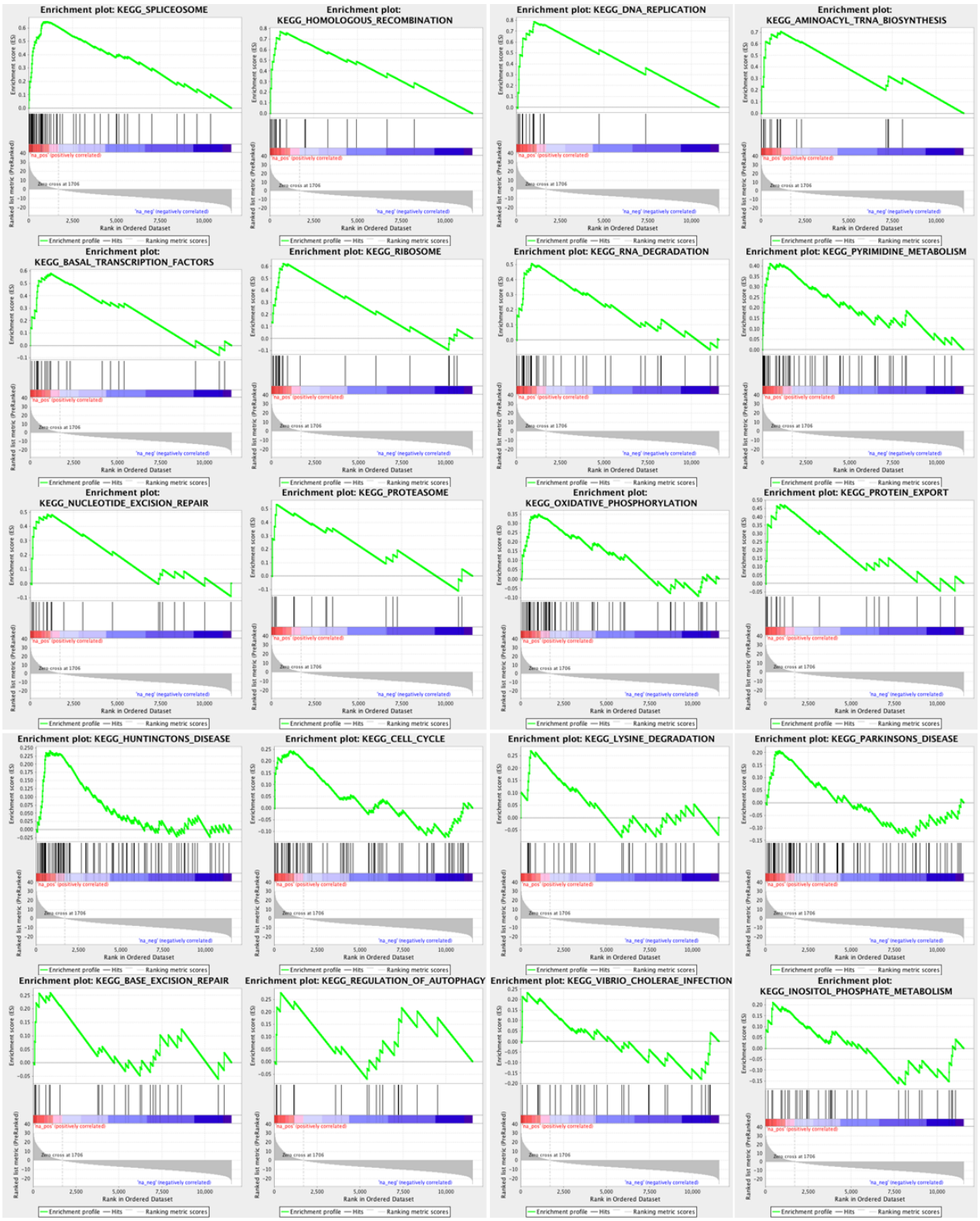
Supplementary Figure 6 Top 20 depleted pathways with Wang library

The gene level Bayesian factors (BF) generated from Wang library screen were used in the KEGG gene set enrichment analysis (GSEA). The enrichment plots of top 20 depleted pathways were listed.



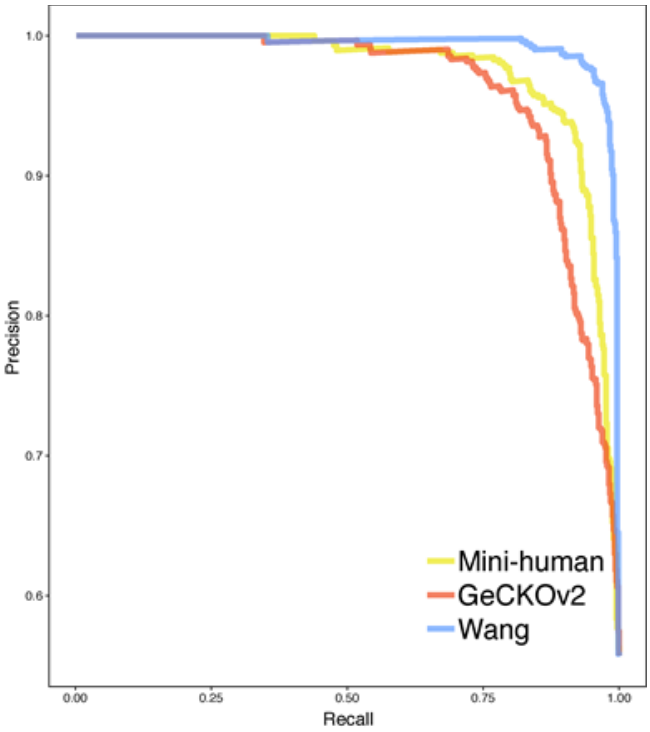
Supplementary Figure 7 Top 20 depleted pathways with GeCKO V2 library

The gene level Bayesian factors (BF) generated from GeCKOv2 library screen were used in the KEGG gene set enrichment analysis (GSEA). The enrichment plots of top 20 depleted pathways were listed.



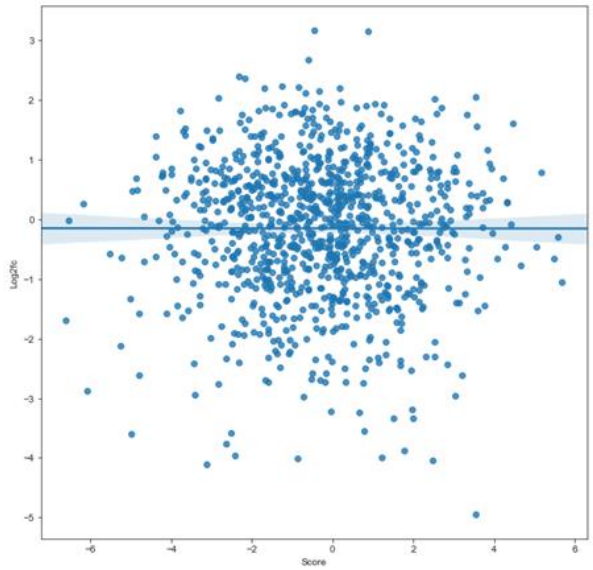
Supplementary Figure 8 Top 20 depleted pathways with Mini-human library

The gene level Bayesian factors (BF) generated from Mini-human library screen were used in the KEGG gene set enrichment analysis (GSEA). The enrichment plots of top 20 depleted pathways were listed.



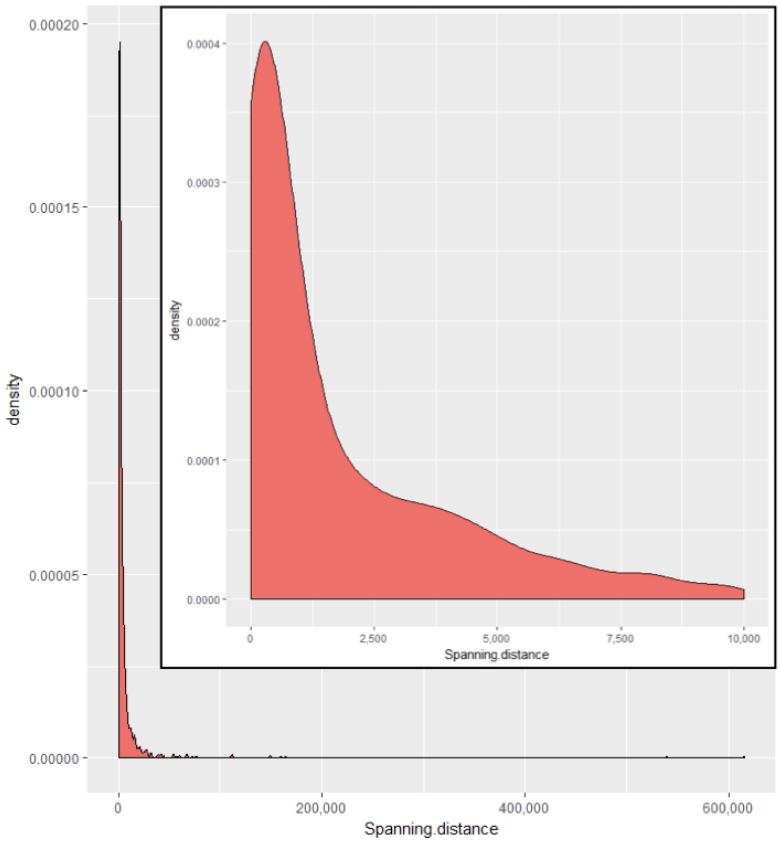
Supplementary Figure 9 The precision-recall curve plot of different CRISPR library screen

Blue *curve*: down-sampled Wang library (4 guides each gene). Red *curve*: GeCKO V2 library. Yellow *curve*: Mini-human library. Source data are provided as a Source Data file.



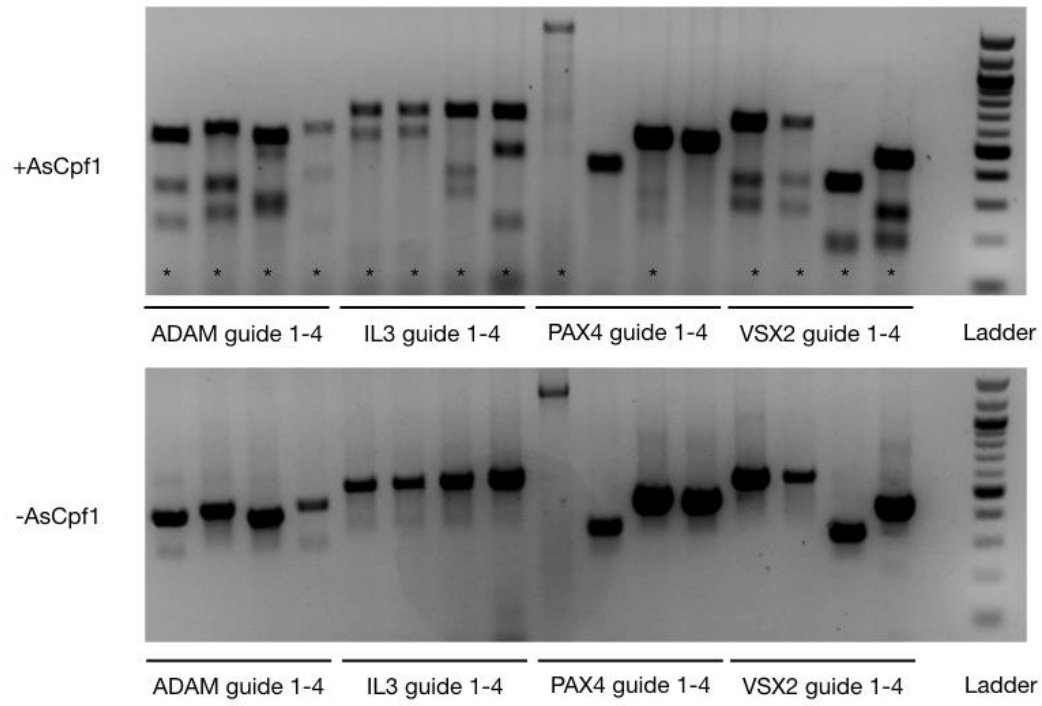
Supplementary Figure 10 Activity scores and depletion correlation of non-essential gene targeting guides.

Line of best fit is plotted and the shaded area is presented as SD. *(*Spearman's rho=-0.01, two-side p=0.65, Spearman's rank-order Correlation*).* Source data are provided as a Source Data file



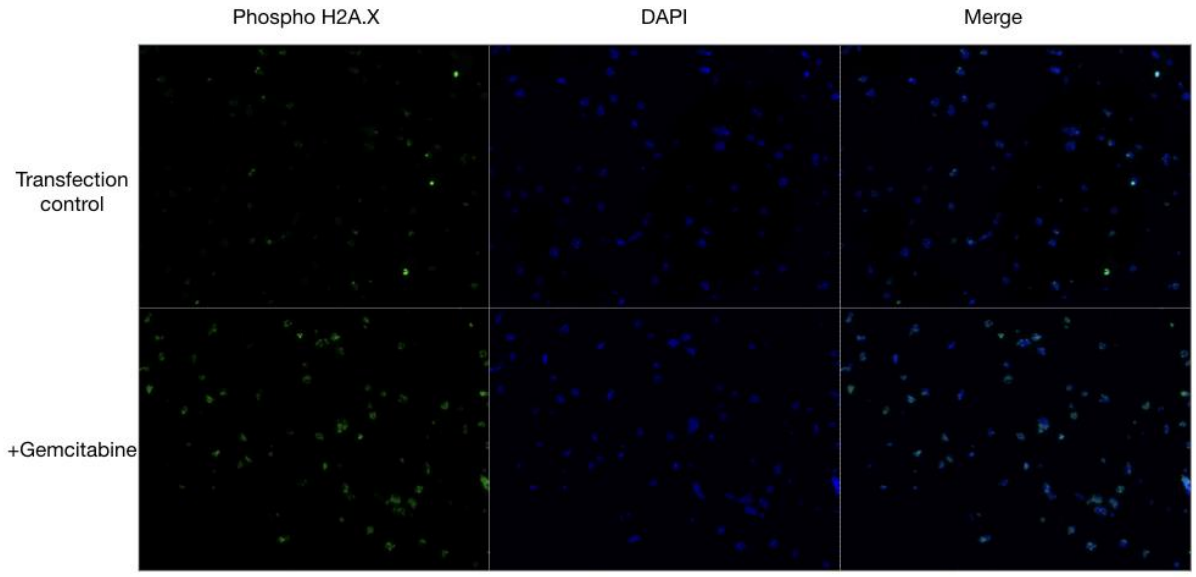
Supplementary Figure 11 Maximum distance of double strand breaks in AsCpf1 multiplexed library.

The maximum distance of double strand breaks was defined as the maximum distance of chromosomal coordinate between any two guides targeting the same gene. The upper enlarged right corner showed the distribution of the maximum distance shorter than 10 kilobases. Source data are provided as a Source Data file



Supplementary Figure 12 T7E1 assay results of AsCpf1 on non-essential gene loci.

HEK293-T cells were transfected with AsCpf1-3xMYC and guides in complexed with lipofectamine 3000. Active guides are indicated by "*". Upper panel: cells transfected with AsCpf1-3xMYC and guide. Lower panel: cells transfected with guides only. T7E1 assay products targeting loci (from left to right): ADAM, IL3, PAX4, VSX2. For each locus, 4 guides were selected from Mini-human library. Ladder: NEB 100bp DNA ladder. Source data are provided as a Source Data file.



Supplementary Figure 13 Immunofluorescence microscopy validation of p-H2A.X antibody.

HEK293-T cells were transfected with AsCpf1-3xMYC and empty guide vector in complexed with Lipofectamine 3000 (Transfection control) or treated with 1-hour 100nM gemcitabine incubation (+Gemcitabine) and stained for H2A.X phosphorylation and nucleus. Source data are provided as a Source Data file.

	Restriction digestion (rearranged/total colony)	Gibson assembly (rearranged/total colony)
NEB Stable (Q5 polymerase)	0/8	2/10
DH10B (Q5 polymerase)	Not tested	8/11
NEB Stable (KOD polymerase)	0/9	3/9
DH10B (KOD polymerase)	0/10	Not tested

Supplementary. Table.1 Rate of plasmid rearrangement using different library cloning techniques

Oligo pools were PCR amplified with either KOD polymerase or NEB Q5 polymerase and then cloned into vector by either restrictive digestion or Gibson assembly. Ligation products were transformed into either

NEB stable strain or DH10B strain. Transformants were then subjected to sanger sequencing and mapped back to reference to detect whether vector recombination was present.

Sequence of LentiUniversal-Puro vector:

>LentiUniversal-Puro
CCCGGGTGCAAAGATGGATAAAGTTTTAAACAGAGAGGAATCTTTGCAGCTAATGGACCTTCTA
GGTCTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACA
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Sequence of AsCpf1-3xMYC vector:

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Highlight:AsCpf1-3xMYC sequence