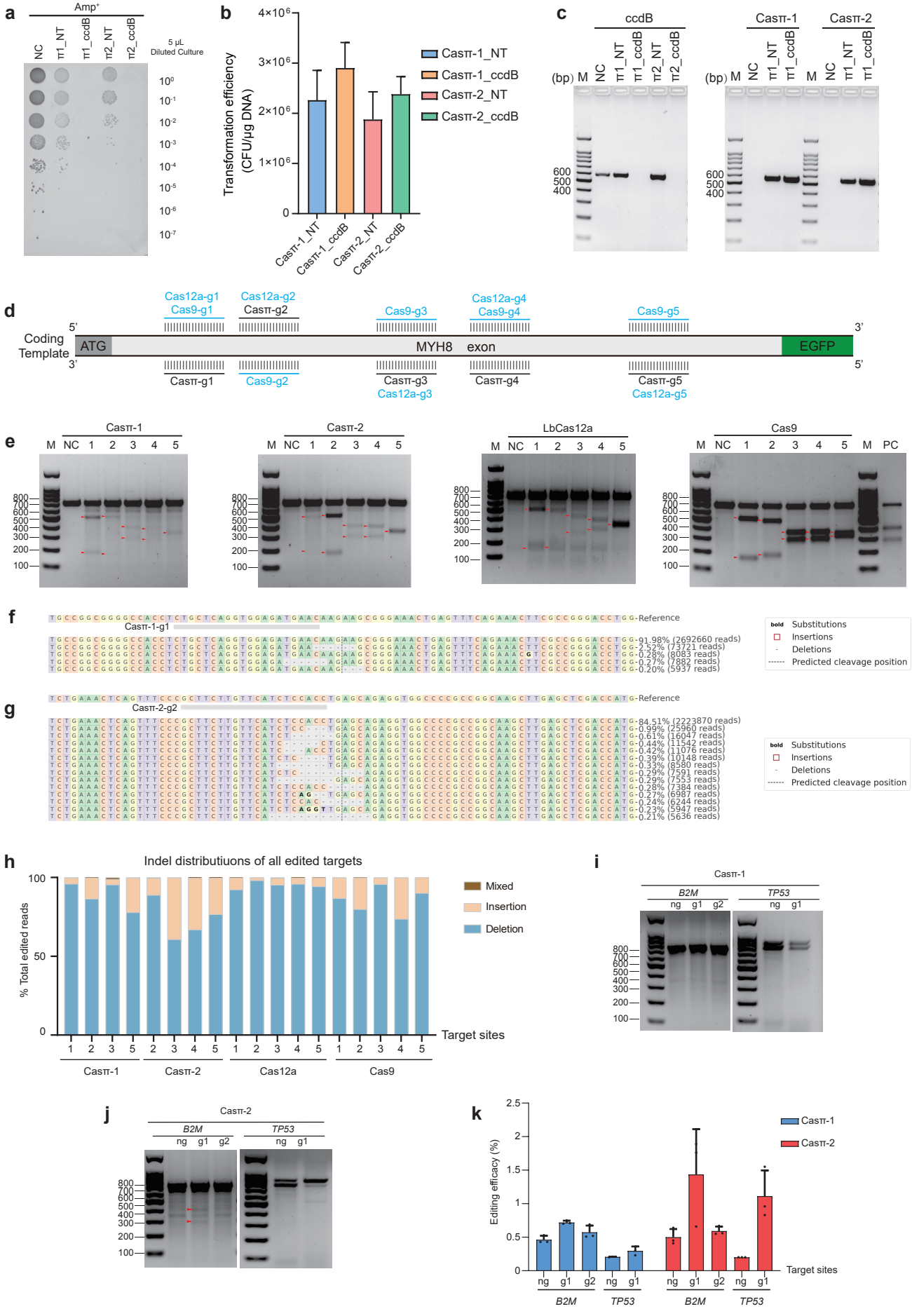
a
Amp+
NC
π1_NT
π1_ccdB
π2_NT
π2_ccdB
5 μL Diluted Culture
10^0
10^-1
10^-2
10^-3
10^-4
10^-5
10^-6
10^-7
b
Transformation efficiency (CFU/μg DNA)
4×10^6
3×10^6
2×10^6
1×10^6
0
Casπ-1_NT
Casπ-1_ccdB
Casπ-2_NT
Casπ-2_ccdB
c
ccdB
Casπ-1
Casπ-2
(bp) M NC π1_NT π1_ccdB π2_NT π2_ccdB
(bp) M NC π1_NT π1_ccdB M NC π1_NT π1_ccdB
600
500
400
d
Cas12a-g1
Cas9-g1
Cas12a-g2
Casπ-g2
Cas9-g3
Cas12a-g4
Cas9-g4
Cas9-g5
5'
3'
Coding Template
ATG
MYH8 exon
EGFP
Casπ-g1
Cas9-g2
Casπ-g3
Cas12a-g3
Casπ-g4
Casπ-g5
Cas12a-g5
e
Casπ-1
Casπ-2
LbCas12a
Cas9
M NC 1 2 3 4 5
M NC 1 2 3 4 5 M PC
800
700
600
500
400
300
200
100
f
Casπ-1-g1
Reference
91.98% (2692660 reads)
2.52% (73721 reads)
0.28% (8083 reads)
0.27% (7882 reads)
0.20% (5937 reads)
bold Substitutions
Insertions
Deletions
Predicted cleavage position
g
Casπ-2-g2
Reference
84.51% (2223870 reads)
0.99% (25960 reads)
0.61% (16047 reads)
0.44% (11542 reads)
0.42% (11076 reads)
0.39% (10148 reads)
0.33% (8580 reads)
0.29% (7591 reads)
0.29% (7553 reads)
0.28% (7384 reads)
0.27% (6987 reads)
0.24% (6244 reads)
0.23% (5947 reads)
0.21% (5636 reads)
bold Substitutions
Insertions
Deletions
Predicted cleavage position
h
Indel distributiuons of all edited targets
% Total edited reads
100
50
0
Mixed
Insertion
Deletion
1 2 3 5 2 3 4 5 1 2 3 4 5 1 2 3 4 5
Target sites
Casπ-1
Casπ-2
Cas12a
Cas9
i
Casπ-1
B2M
TP53
ng g1 g2
ng g1
800
700
600
500
400
300
200
100
j
Casπ-2
B2M
TP53
ng g1 g2
ng g1
800
700
600
500
400
300
200
100
k
Editing efficacy (%)
2.5
2
1.5
1
0.5
0
Casπ-1
Casπ-2
ng g1 g2 ng g1 ng g1 g2 ng g1
Target sites
B2M
TP53
B2M
TP53

Fig. S4 Casπ mediated gene manipulation in prokaryotic and eukaryotic cells.

(**a**) Bacteria survival result on LB-Amp$^+$ agar plates. (NT, plasmid with Casπ and non-target sgRNA; ccdB, plasmid with Casπ and sgRNA targeting *ccdB* gene).

(**b**) Transformation efficiency of Casπ plasmids validated on LB-Strep$^+$ agar plates (n=3 each, mean ± SD).

(**c**) Left panel, PCR detection of ccdB plasmids in edited cells. The two PCR primers respectively locates at the cleavage-site upstream and downstream. Right panel, PCR detection of Casπ plasmids in edited cells.

(**d**) Distribution of targeting sites across *MYH8* exon for Casπ, Cas12a and Cas9 effectors.

(**e**) T7E1 cleavage on the re-annealed target amplified from edited genome of Casπ-1, Casπ-2, Cas12a and Cas9. Cleavage products were indicated by red arrows. PC indicates the positive control offered in the manufacture kit for T7E1 assay.

(**f**) Summary of indels generated by Casπ-1 on the MYH8-target site1. Only indels with frequencies ≥ 0.2% of the total reads are shown.

(**g**) Summary of indels generated by Casπ-2 on the MYH8-target site2. Only indels with frequencies ≥ 0.2% of the total reads are shown.

(**h**) INDEL distributions of all five targets by Cas effectors analyzed by NGS of the MYH8 target (Mixed means insertion and deletion, only targets with editing efficacies ≥ 1% are shown).

(**i**) T7E1 cleavage on the re-annealed target amplified from edited genome of Casπ-1 on *B2M* and *TP53* (ng means non-target sgRNA; n=3 each, mean ± SD).

(**j**) T7E1 cleavage on the re-annealed target amplified from edited genome of Casπ-2 on *B2M* and *TP53*. Cleavage products were indicated by red arrows (n=3 each, mean ± SD).

(**k**) Editing efficacies determined by NGS for 3 more targets mediated by Casπ-1 and Casπ-2 (n=3 each, mean ± SD).