
Supplementary information

Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease

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Supplementary Information

Transposon-associated TnpB is an RNA-programmable DNA nuclease

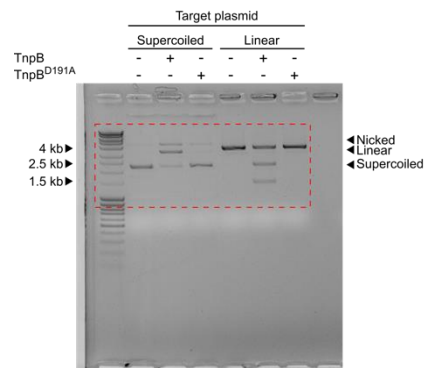
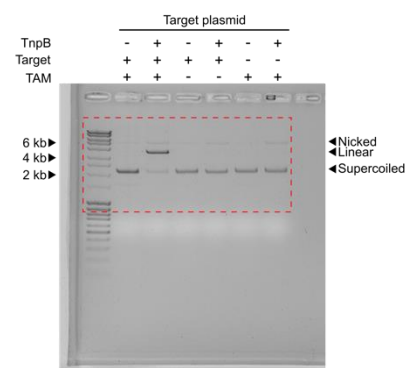
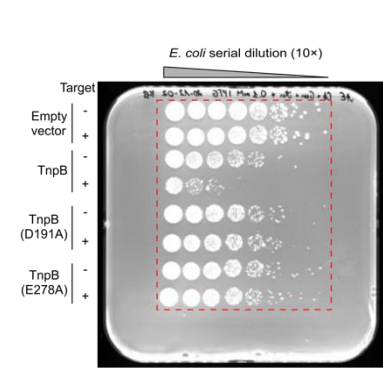
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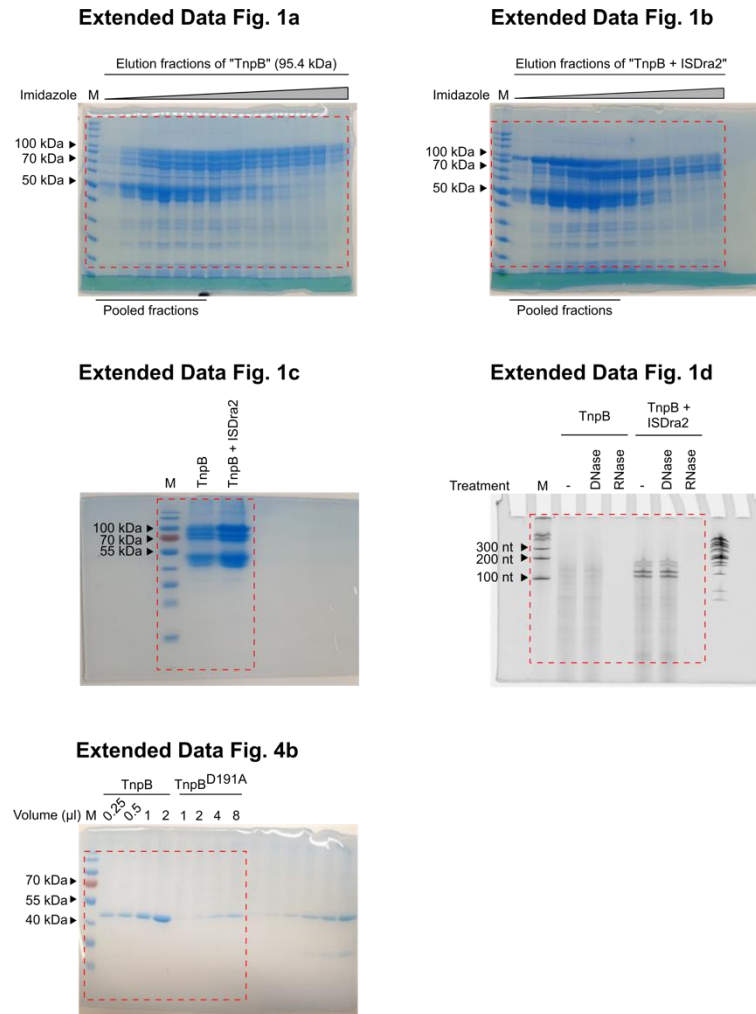
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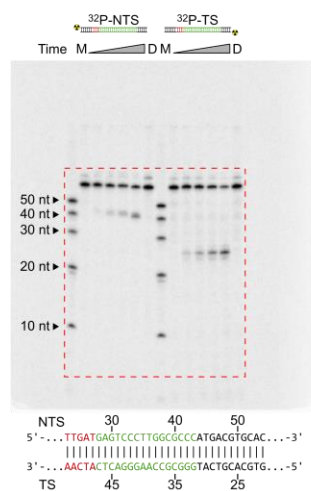
Fig. 2e**Fig. 2f****Fig. 3b**

Supplementary Fig. 1 | Uncropped images for the Main Text Figures. The red rectangles indicate the cropping location.

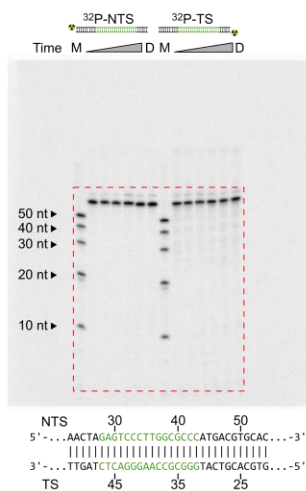


Supplementary Fig. 2 | Uncropped images for the Extended Data Figures. The red rectangles indicate the cropping location.

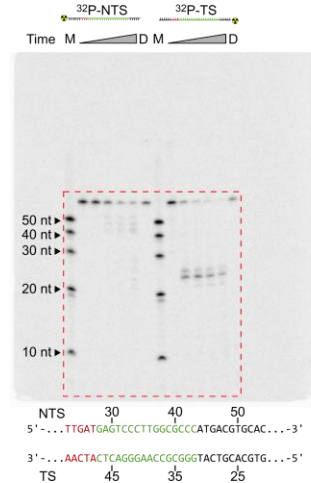
Extended Data Fig. 5a



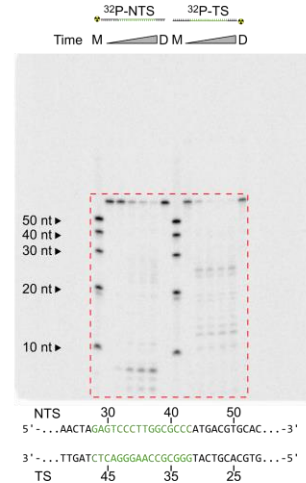
Extended Data Fig. 5b



Extended Data Fig. 6a



Extended Data Fig. 6b



Supplementary Fig. 2 | Uncropped images for the Extended Data Figures (continued). The red rectangles indicate the cropping location.

Supplementary Table 1 | Plasmids used in this study.

Plasmid name	Description	Figures	Link
pTK120- ISDra2-TnpB	TnpB expression plasmid (TwinStrep-10×His-MBP-TEV-TnpB)	1c-d, Extended Data Fig. 1	https://benchling.com/s/seq-wOnPpT0TgQJseCavvnaR
pTWIST- ISDra2	Plasmid containing complete ISDra2 transposon system	-	https://benchling.com/s/seq-A70Ed8hYrquvmBH2Zw0A
pGD3	pTWIST-ISDra2 construct containing TnpA deletion	1c-d, Extended Data Fig. 1	https://benchling.com/s/seq-S4BsxOuWtIs1mosJHjPb
pTK151	TnpB expression plasmid (6×His-MBP-TEV-TnpB-StrepTag II)	2d, Extended Data Fig. 4	https://benchling.com/s/seq-Rd1yxYkgDNoDHL5aZRwD
pTK152	TnpB D191A expression plasmid (6×His-MBP-TEV-TnpB (D191A)-StrepTag II)	2d, Extended Data Fig. 4	https://benchling.com/s/seq-LA9lcMvtjVZI4udtfyCt
pGB71	reRNA expression plasmid	2d, Extended Data Fig. 4	https://benchling.com/s/seq-Mkw73LLrFgtP3V5fWpkQ
pGB74	TnpB and reRNA expression construct used for plasmid interference assay in <i>E. coli</i>	3a-b	https://benchling.com/s/seq-X2A1t5z7VY19ARY3ffQ7
pGB75	TnpB D191A and reRNA expression construct used for plasmid interference assay in <i>E. coli</i>	3a-b	https://benchling.com/s/seq-jEol8nNlxvKp5XAPLpbW
pGB76	TnpB E278A and reRNA expression construct used for plasmid interference assay in <i>E. coli</i>	3a-b	https://benchling.com/s/seq-mQIFsmbzdS5WSGIbdDTu
pETDuet-1	Empty vector used for plasmid interference assay in <i>E. coli</i>	3a-b	https://benchling.com/s/seq-dWuzfZLfOTaEx2zx9nFv
pTZ57	7N plasmid library	2a-c, Extended Data Fig. 2	https://benchling.com/s/seq-5vm5J2YMrqofoxGYzNAj
pGB77	Plasmid for TnpB and reRNA expression in <i>E. coli</i> targeting 7N plasmid library (16 nt guide)	2a-c	https://benchling.com/s/seq-WQGHHuLIWZEIB960BGVm

pGB78	Plasmid for TnpB and reRNA expression in <i>E. coli</i> targeting 7N plasmid library (20 nt guide)	Extended Data Fig. 2	https://benchling.com/s/seq-WSC3bcDdfc55gYVgfopZ
pGB72	TnpB target plasmid with TAM sequence used for <i>in vivo</i> plasmid interference and <i>in vitro</i> activity experiments	2e-g, 3a-b	https://benchling.com/s/seq-SC1ze8ARFSbNhpCDwGKv
pGB73	TnpB target plasmid without TAM sequence used for <i>in vitro</i> activity experiments	2f	https://benchling.com/s/seq-b9XdVGsl4g3tUK3dVgkq
pSG4K5	Non-target plasmid used for <i>in vivo</i> plasmid interference and <i>in vitro</i> activity experiments	3a-b	https://benchling.com/s/seq-oyan25ZovFhAOLSYUEuk
pRZ122	Plasmid for TnpB and non-targeting reRNA expression in human cells	4a-b	https://benchling.com/s/seq-ZcyeLQKncrXYuOOBrxwI
pRZ123	Plasmid for TnpB and reRNA expression in human cells, targeting <i>EMX1-1</i>	4a-c	https://benchling.com/s/seq-A7J0kVZQAhu0G0dvocYA
pRZ124	Plasmid for TnpB and reRNA expression in human cells, targeting <i>EMX1-2</i>	4a-b	https://benchling.com/s/seq-5Myy239GbPkHFjliZSJP
pRZ125	Plasmid for TnpB and reRNA expression in human cells, targeting <i>HPRT1</i>	4a-b	https://benchling.com/s/seq-gbyVUxBIWAnaVmYThZtV
pRZ126	Plasmid for TnpB and reRNA expression in human cells, targeting <i>AGBL1-1</i>	4a-b	https://benchling.com/s/seq-vaf08y4lbzDTSIgCDF3C
pRZ127	Plasmid for TnpB and reRNA expression in human cells, targeting <i>AGBL1-2</i>	4a-b	https://benchling.com/s/seq-yZ3derrT6fAOPmDJNC1o

Supplementary Table 2 | Sequences of the TnpB protein variants used in this study.

TnpB

MIRNKAFVRLYPNAAQTELINRTLGSARFVYNHFLARRIAAYKESGKGLTYGQTSSSELTLKQAEETSWLSEVDKFALQNSLKNLETAYKNFFRTVKQSGKKVGFPRFRKKRTGESYRTQFTNNNIQIGEGRLKLPKLGWVKTKGQQDIQGKILNVTVRRIHEGHYEASVLCVEIPIYLPAAPKFAAGVDVGKDFAIKDFVDFKHEQNPKYYRSTLKRRLKAQQTLSRRKKGSARYGKAKTKLARIHKRIVNKRQDFLHKLTTSLVREYEIIGTEHLKPDNMRKNRRLALSISDAGWGEFIRQLEYKAAWYGRVSKVSPYFPSSQLCHDCGFKNPEVKNLAVRTWTCPCNCGETHDRDENAALNIRREALVAAGISDTLNAHGGYVRPASAGNGLRSENHATLVV*

TwinStrep-10×His-MBP-TEV (cleavage site)-TnpB

MGGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKSMGGSHHHHHHHHHHGMASMKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRLFVGGYQAQSGLLAEITPDKAFQDKLYPFTWDVAVRYNGKLIAYPIAVEALSILYNKDLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKHKHMNADTDYSIAEAFNKGGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNLGLIEENLYFQSNAGGGGMIRNKAFVRLYPNAAQTELINRTLGSARFVYNHFLARRIAAYKESGKGLTYGQTSSSELTLKQAEETSWLSEVDKFALQNSLKNLETAYKNFFRTVKQSGKKVGFPRFRKKRTGESYRTQFTNNNIQIGEGRLKLPKLGWVKTKGQQDIQGKILNVTVRRIHEGHYEASVLCVEIPIYLPAAPKFAAGVDVGKDFAIKDFVDFKHEQNPKYYRSTLKRRLKAQQTLSRRKKGSARYGKAKTKLARIHKRIVNKRQDFLHKLTTSLVREYEIIGTEHLKPDNMRKNRRLALSISDAGWGEFIRQLEYKAAWYGRVSKVSPYFPSSQLCHDCGFKNPEVKNLAVRTWTCPCNCGETHDRDENAALNIRREALVAAGISDTLNAHGGYVRPASAGNGLRSENHATLVV*

6×His-MBP-TEV (cleavage site)-TnpB-StrepTag II

MGGSHHHHHHGMASMKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRLFVGGYQAQSGLLAEITPDKAFQDKLYPFTWDVAVRYNGKLIAYPIAVEALSILYNKDLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKHKHMNADTDYSIAEAFNKGGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNLGLIEENLYFQSNAGGGGMIRNKAFVRLYPNAAQTELINRTLGSARFVYNHFLARRIAAYKESGKGLTYGQTSSSELTLKQAEETSWLSEVDKFALQNSLKNLETAYKNFFRTVKQSGKKVGFPRFRKKRTGESYRTQFTNNNIQIGEGRLKLPKLGWVKTKGQQDIQGKILNVTVRRIHEGHYEASVLCVEIPIYLPAAPKFAAGVDVGKDFAIKDFVDFKHEQNPKYYRSTLKRRLKAQQTLSRRKKGSARYGKAKTKLARIHKRIVNKRQDFLHKLTTSLVREYEIIGTEHLKPDNMRKNRRLALSISDAGWGEFIRQLEYKAAWYGRVSKVSPYFPSSQLCHDCGFKNPEVKNLAVRTWTCPCNCGETHDRDENAALNIRREALVAAGISDTLNAHGGYVRPASAGNGLRSENHATLVVSGWSHPQFEK*

6×His-MBP-TEV (cleavage site)-TnpB (D191A)-StrepTag II

MGGSHHHHHHGMASMKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRLFVGGYQAQSGLLAEITPDKAFQDKLYPFTWDVAVRYNGKLIAYPIAVEALSILYNKDLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKHKHMNADTDYSIAEAFNKGGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNLGLIEENLYFQSNAGGGGMIRNKAFVRLYPNAAQTELINRTLGSARFVYNHFLARRIAAYKESGKGLTYGQTSSSELTLKQAEETSWLSEVDKFALQNSLKNLETAYKNFFRTVKQSGKKVGFPRFRKKRTGESYRTQFTNNNIQIGEGRLKLPKLGWVKTKGQQDIQGKILNVTVRRIHEGHYEASVLCVEIPIYLPAAPKFAAGVAVGKDFAIKDFVDFKHEQNPKYYRSTLKRRLKAQQTLSRRKKGSARYGKAKTKLARIHKRIVNKRQDFLHKLTTSLVREYEIIGTEHLKPDNMRKNRRLALSISDAGWGEFIRQLEYKAAWYGRVSKVSPYFPSSQLCHDCGFKNPEVKNLAVRTWTCPCNCGETHDRDENAALNIRREALVAAGISDTLNAHGGYVRPASAGNGLRSENHATLVVSGWSHPQFEK*

Supplementary Table 3 | TnpB targeted sequences in 7N library and dsDNA cleavage experiments.

Target plasmid	Sequence 5'→3' (TAM, Target)	Figures
pTZ57	NNNNNNNAGTTGACCCAACGTCG	2a-c
pTZ57	NNNNNNNAGTTGACCCAACGTCGCCGG	Extended Data Fig. 2
pGB72	TTGATGAGTCCCTTGGCGCCC	2e-g, 3a-b
pGB73	AACTAGAGTCCCTTGGCGCCC	2f

Supplementary Table 4 | Oligonucleotides used for *in vitro* cleavage assays.

Description	Sequence 5'→3' (TAM, Target)	Figures
Target (NTS)	GCACCTTACTGCAAGGTAGCGCTT GAT GAGTCCCTTGGCGCCCATG ACGTGCACAATCTAGATGCATCAGCTGC	Extended Data Fig. 5a, Extended Data Fig. 6a
Target (TS)	GCAGCTGATGCATCTAGATTGTGCACG T CATGGGCGCCAAGGGACT CATCA AGCGCTACCTTGCAGTAAGGTGC	Extended Data Fig. 5a, Extended Data Fig. 6a
Target (NTS)	GCACCTTACTGCAAGGTAGCGGAACTAGAGTCCCTTGGCGCCCATG ACGTGCACAATCTAGATGCATCAGCTGC	Extended Data Fig. 5b, Extended Data Fig. 6b
Target (TS)	GCAGCTGATGCATCTAGATTGTGCACG T CATGGGCGCCAAGGGACT C TAGTTCCGCTACCTTGCAGTAAGGTGC	Extended Data Fig. 5b, Extended Data Fig. 6b
Marker	GCACCTTACTGCAAGGTAGCGTATTTAAGTTGACCCAACGTCGCCG GCGT	Extended Data Fig. 5, Extended Data Fig. 6
	GCACCTTACTGCAAGGTAGCGTATTTAAGTTGACCCAACG	
	GCACCTTACTGCAAGGTAGCGTATTTAAGT	
	GCACCTTACTGCAAGGTAGC	
	GCACCTTACT	

Supplementary Table 5 | Primers used in human genome editing assay. Blue and red fonts represent Read1 and Read2 sequences, respectively, required for Illumina sequencing. Black font indicates the sequence used for amplification of the genomic locus.

Description	Sequence (5'→3')
Primary PCR <i>EMX1-1</i> (forward)	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTACAAACGGC AGAAGCTGG
Primary PCR <i>EMX1-1</i> (reverse)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCGTGGC AATGCGCC
Primary PCR <i>EMX1-2</i> (forward)	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCCGTGTCTC CAATCTCCC
Primary PCR <i>EMX1-2</i> (reverse)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCATAGGG AAGGGGGACAC
Primary PCR <i>HPRT1</i> (forward)	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGATGATGAA CCAGGTTATGAC
Primary PCR <i>HPRT1</i> (reverse)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCATGAG GAATAAACACCC
Primary PCR <i>AGBL1-1</i> (forward)	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATGTTTCCTCA CAAAGAGAGTC
Primary PCR <i>AGBL1-1</i> (reverse)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTTGATAA TGAATGGCTACTC
Primary PCR <i>AGBL1-2</i> (forward)	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTGTTGGCTC AAACACCAG
Primary PCR <i>AGBL1-2</i> (reverse)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCTATGGT GAATTGTCAAGC

Supplementary Table 6 | TnpB targeted sequences in human genome editing experiment.

Target	Target sequence (5'→3')
Non-targeting (NT)	AGTTGACCCAACGTCGCCGG
<i>EMX1-1</i>	GTGATGGGAGCCCTTCTTCT
<i>EMX1-2</i>	GCATTTCTGTTTAAATTTAT
<i>HPRT1</i>	TTATTTTGCATACCTAATCA
<i>AGBL1-1</i>	TGTTGGCTCAAACACCAGAT
<i>AGBL1-2</i>	AATGAATGGCTACTCTAACC