

## Supplementary Information

### Orthogonal Cas9 Proteins for RNA-Guided Gene Regulation and Editing

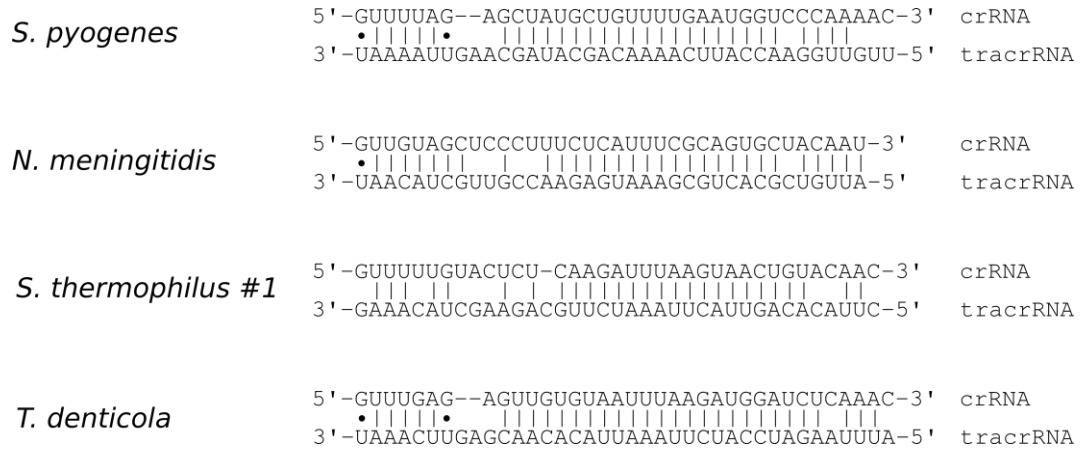
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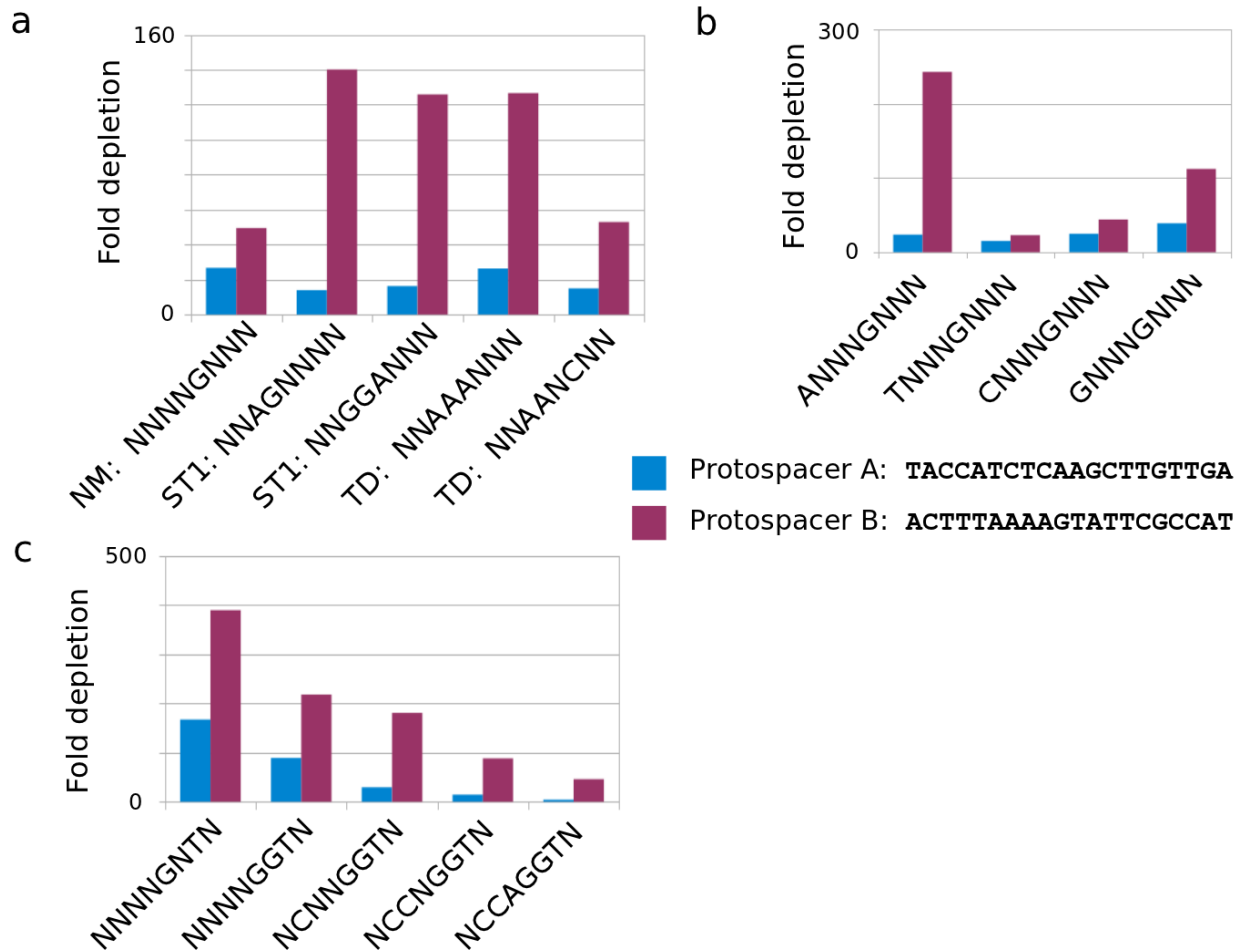
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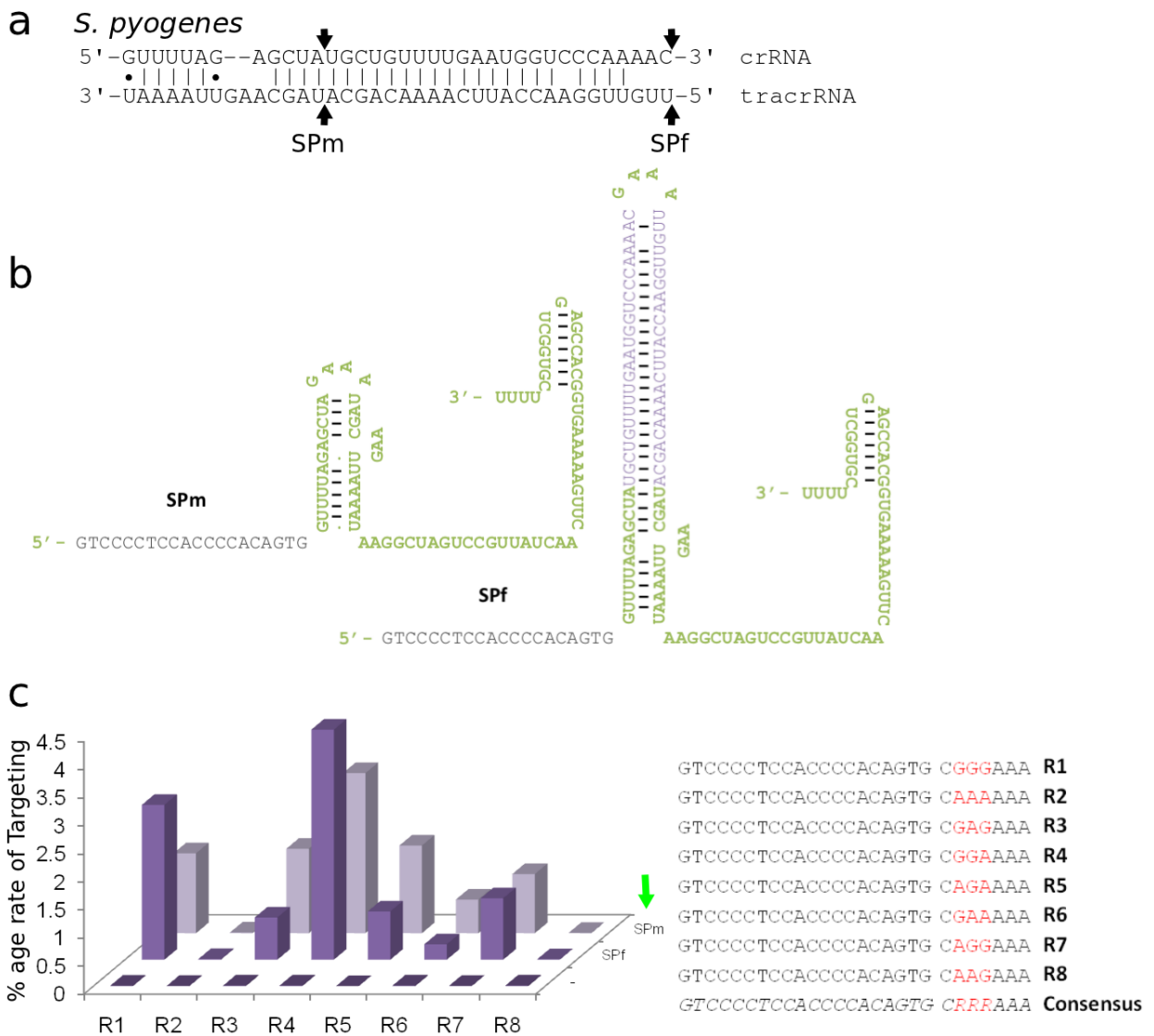
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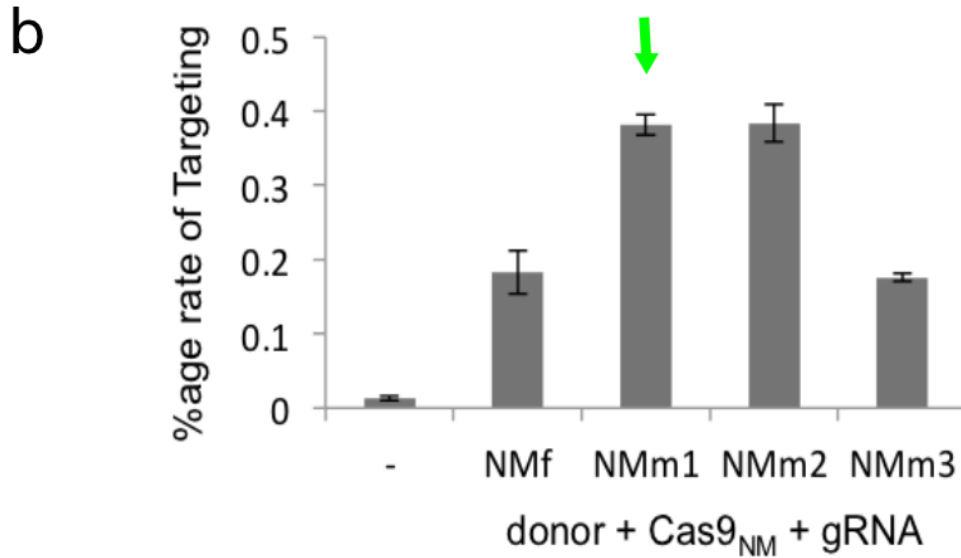
**Supplementary Figure 1** | crRNA-tracrRNA interactions for each type II system.



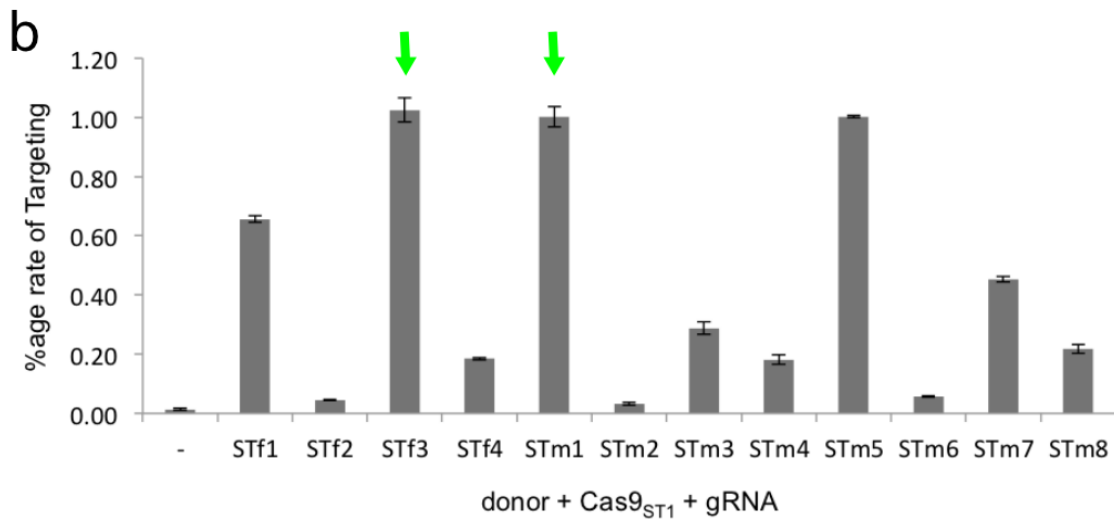
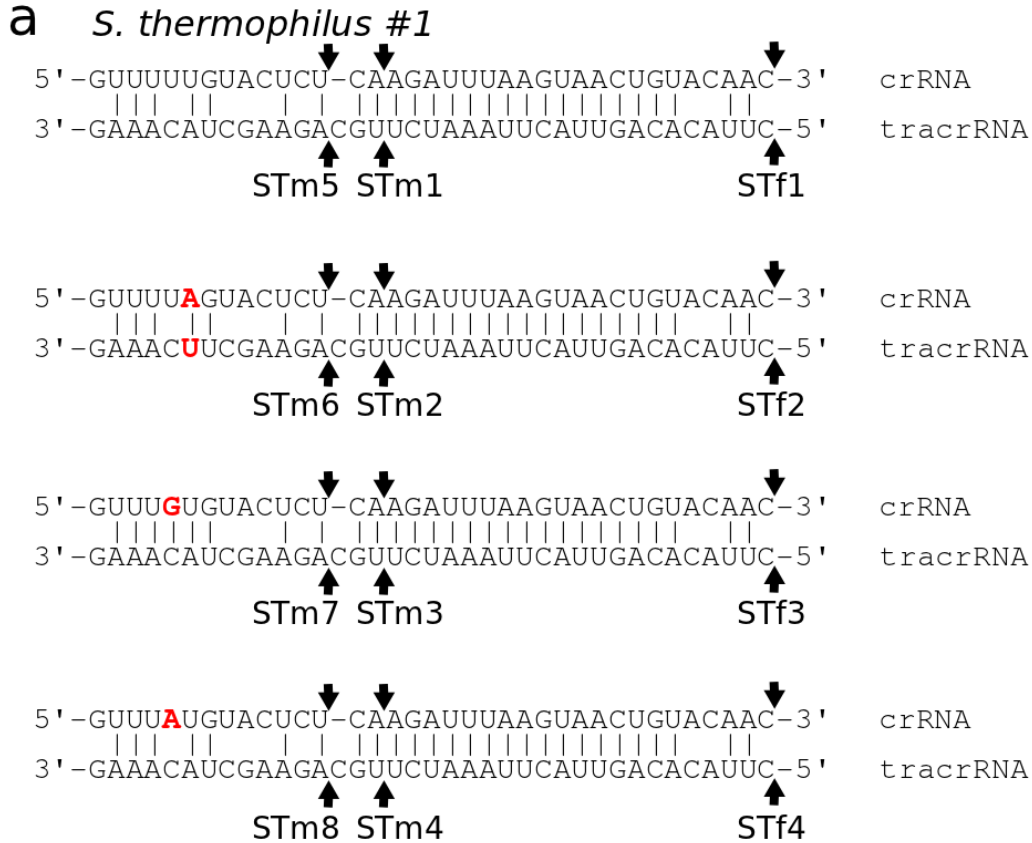
**Supplementary Figure 2** | Protospacer and PAM sequences in Cas9 recognition. (a) Relative depletion of the two protospacers differs by Cas9 protein. Sequences with moderate-activity PAMs, whose fold depletion is minimally biased by transformation efficiency or Cas9 inactivation, display markedly different relative depletion patterns for the different Cas9 proteins. In general, ST1 > TD > NM in relative depletion of protospacer B over protospacer A. (b) Relative protospacer depletion is influenced by bases in the PAM region. NM depletes both protospacers approximately equally for TNNNG, but favors protospacer 2 by 2-fold for CNNNG, 3-fold for GNNNG, and 10-fold for ANNNG. (c) Specific unfavored bases can dramatically reduce PAM recognition. Although NNNNGNT is depleted by ~160-fold with protospacer library 1, the addition of successive unfavored mutations reduces fold depletion to only 4-fold for NCCAGGT. Results are drawn from a single library selection experiment.



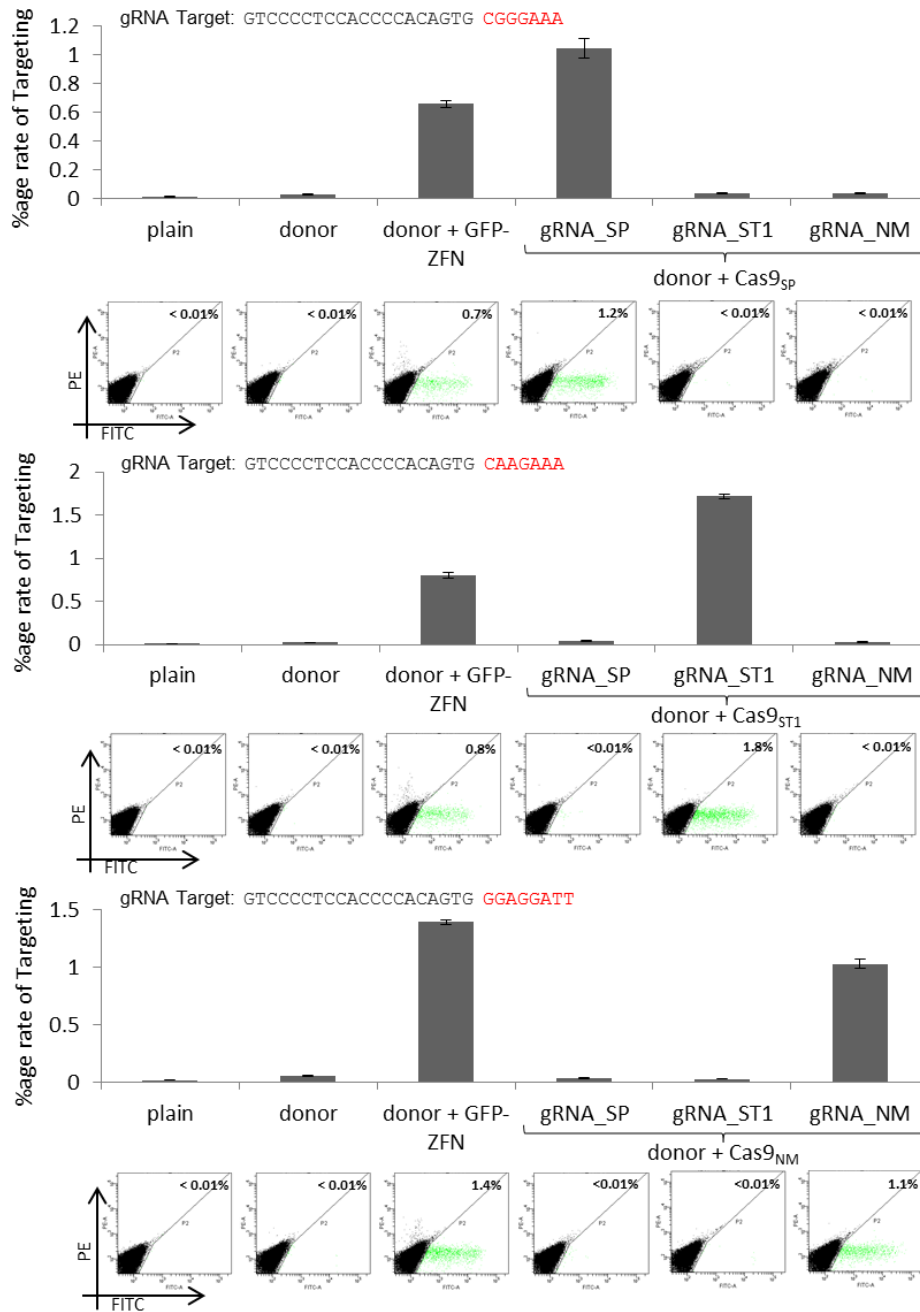
**Supplementary Figure 3 | SP sgRNAs and editing efficiencies.** (a) sgRNA truncation points for SP. (b) Fully drawn sgRNAs corresponding to the above. (c) Profile of SP activity in human cells with each sgRNA with different PAMs (n=3 biological replicates). SPm is the traditional sgRNA and was used in future experiments (green arrow).



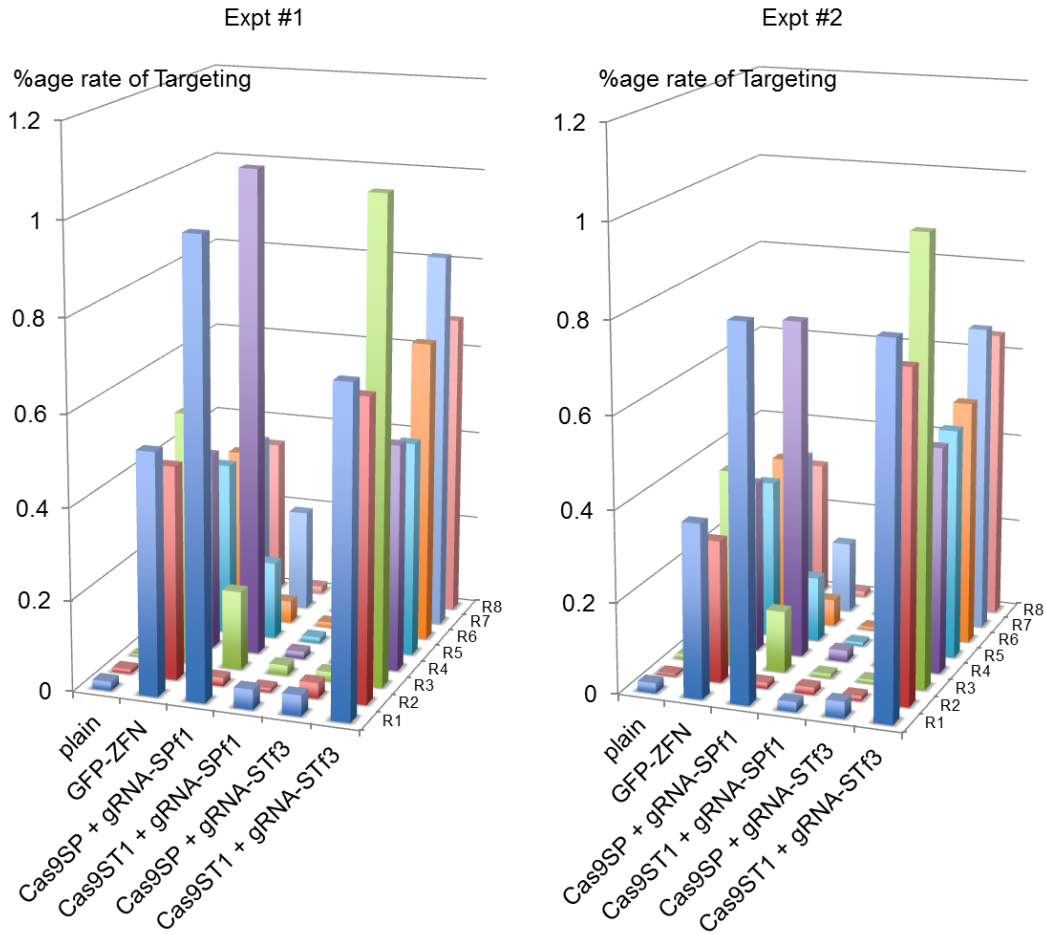
**Supplementary Figure 4** | NM sgRNAs and editing efficiencies. (a) Truncation points for NM sgRNAs. (b) NM activity in human cells with each sgRNA and a GGAGGATT PAM. NMm1 was selected for use in future experiments (green arrow). Error bars represent the standard deviation of three biological replicates.



**Supplementary Figure 5** | ST1 sgRNAs and editing efficiencies. (a) Truncation points and point mutations for ST1 sgRNAs. (b) ST1 activity in human cells with each sgRNA and a CAAGAA PAM. STf3 was used for the experiments illustrated in Supplementary Figure 8; all other experiments utilized STm1 (green arrows). Error bars represent the standard deviation of three biological replicates.



**Supplementary Figure 6** | Orthogonal gene editing in human cells. Editing efficiencies are depicted for (a) SP (b) ST1 and (c) NM. Activity was measured using a homologous recombination-mediated GFP repair assay and quantified by flow cytometry (plots from Fig. 6 are reproduced here). Included for comparison is a ZFN cleaving an adjacent target sequence. sgRNAs are those indicated in Supplemental Figures 3-5. Error bars represent the standard deviation of three biological replicates.



**Target Sites**

GTCCCCTCCACCCACAGTG	CGGGAAA	R1
GTCCCCTCCACCCACAGTG	CAAAAAA	R2
GTCCCCTCCACCCACAGTG	CGAGAAA	R3
GTCCCCTCCACCCACAGTG	CGGAAAA	R4
GTCCCCTCCACCCACAGTG	CAGAAAA	R5
GTCCCCTCCACCCACAGTG	CGAAAAA	R6
GTCCCCTCCACCCACAGTG	CAGGAAA	R7
GTCCCCTCCACCCACAGTG	CAAGAAA	R8
GTCCCCTCCACCCACAGTG	CRRRAAA	Consensus

**Supplementary Figure 7** | Orthogonality arises primarily from sgRNAs, not PAMs. Both enzymes can target the same sequence for certain PAMs, demonstrating that specificity arises from orthogonal sgRNAs, not just orthogonal PAMs. Each data point is the average of three biological replicates.



**Supplementary Table 1** | List of plasmids available from Addgene.

<b>Construct</b>	<b>Addgene ID</b>	<b>Description</b>
DS-SPcas	48645	Bacterial SP Cas9 expression
DS-NMcas	48646	Bacterial NM Cas9 expression
DS-ST1cas	48647	Bacterial ST1 Cas9 expression
DS-TDcas	48648	Bacterial TD Cas9 expression
PM-SP!TA	48649	Expresses crRNA targeting SP to protospacer A
PM-SP!TB	48650	Expresses crRNA targeting SP to protospacer B
PM-NM!TA	48651	Expresses crRNA targeting NM to protospacer A
PM-NM!TB	48652	Expresses crRNA targeting NM to protospacer B
PM-ST1!TA	48653	Expresses crRNA targeting ST1 to protospacer A
PM-ST1!TB	48654	Expresses crRNA targeting ST1 to protospacer B
PM-TD!TA	48655	Expresses crRNA targeting TD to protospacer A
PM-TD!TB	48656	Expresses crRNA targeting TD protospacer B
DS-SPcasN-	48657	Bacterial nuclease-null SP Cas9 expression
DS-NMcasN-	48658	Bacterial nuclease-null NM Cas9 expression
DS-ST1casN-	48659	Bacterial nuclease-null ST1 Cas9 expression
DS-TDcasN-	48660	Bacterial nuclease-null TD Cas9 expression
SK-YFP-SPNM-B	48661	Bacterial SP and NM reporter: protospacer B
SK-YFP-ST1-B	48662	Bacterial ST1 reporter: protospacer B
SK-YFP-TD-B	48663	Bacterial TD reporter: protospacer B
SK-YFP-NM-A	48664	Bacterial NM reporter: protospacer A
SK-YFP-ST1-A	48665	Bacterial ST1 reporter: protospacer A
SK-YFP-TD-A	48666	Bacterial TD reporter: protospacer A
EE-SP!gIII	48667	Bacterial SP Cas9 targeted to filamentous phage gene III
M-SPCas	48668	Mammalian SP Cas9 expression
M-ST1Cas	48669	Mammalian ST1 Cas9 expression
M-NMCas	48670	Mammalian NM Cas9 expression
M-SPm-sgRNA	48671	Mammalian SP sgRNA SPm
M-ST1-sgRNA	48672	Mammalian ST1 sgRNA STm1
M-NM-sgRNA	48673	Mammalian NM sgRNA NMm1
M-SPn-VP64	48674	Mammalian nuclease-null SP-VP64 expression
M-ST1n-VP64	48675	Mammalian nuclease-null ST1-VP64 expression
M-NMn-VP64	48676	Mammalian nuclease-null NM-VP64 expression
M-tdTom-SP	48677	Mammalian activation reporter: SP PAM
M-tdTom-ST1	48678	Mammalian activation reporter: ST1 PAM
M-tdTom-NM	48679	Mammalian activation reporter: NM PAM

## Supplementary Table 2 | sgRNA sequence designs for SP, ST1, and NM.

SP_chimera_trnc	GUUUUAGAGCUAGAAAUAGCAAGUUAAAA UAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUU GUUUUAGAGCUAUGCUGUUUUGAAUGGUCCCAAAAC
SP_chimera_full	GAAAUUGUUGGAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGCUUUUUU
SP_gRNA_f1	GTCCCCTCCACCCACAGTG GTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAAC GAAATTGTTGGAACCATTCAAAACAGCATAGCAAGTTAAAAAATAAGGCTAGTCCGTTATCAACTTGA AAAAGTGGCACCGAGTCCGGTGCTTTTTT
ST_chimera_trnc	GUUUUUGUACUCUCAGAAAUGCAGAAGCUACAAA GAUAAGGCUUCAUGCCGAAAUCAACACCCUGUCAUUUUUAUGGCAGGGUGUU GUUUUUGUACUCUCAAGAUUUUAAGUAACUGUACAAC
ST_chimera_full	GAAACUUACACAGUUACUAAAUCUUGCAGAAGCUACAAAAGAUAAAGGCUUCAUGCCGAAAUCAACA CCCUGUCAUUUUUAUGGCAGGGUGUU
ST_gRNA_t1	GTCCCCTCCACCCACAGTG GTTTTGTACTCTCAGAAATGCAGAAGCTACAAA GATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_t2	GTCCCCTCCACCCACAGTG GTTTTGTACTCTCAGAAATGCAGAAGCTTCAAA GATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_t3	GTCCCCTCCACCCACAGTG GTTTTGTACTCTCAGAAATGCAGAAGCTACAAA GATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_t4	GTCCCCTCCACCCACAGTG GTTTTGTACTCTCAGAAATGCAGAAGCTACAAA GATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_t5	GTCCCCTCCACCCACAGTG GTTTTGTACTCTGAAAAGAAGCTACAAA GATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_t6	GTCCCCTCCACCCACAGTG GTTTTGTACTCTGAAAAGAAGCTTCAAA GATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_t7	GTCCCCTCCACCCACAGTG GTTTTGTACTCTGAAAAGAAGCTACAAA GATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_t8	GTCCCCTCCACCCACAGTG GTTTTGTACTCTGAAAAGAAGCTACAAA GATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_f1	GTCCCCTCCACCCACAGTG GTTTTGTACTCTCAAGATTTAAGTAACTGTACAAC GAAACTTACACAGTTACTTAAATCTTGCAGAAGCTACAAAAGATAAGGCTTCATGCCGAAATCAACA CCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_f2	GTCCCCTCCACCCACAGTG GTTTTGTACTCTCAAGATTTAAGTAACTGTACAAC GAAACTTACACAGTTACTTAAATCTTGCAGAAGCTTCAAAAGATAAGGCTTCATGCCGAAATCAACA CCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_f3	GTCCCCTCCACCCACAGTG GTTTTGTACTCTCAAGATTTAAGTAACTGTACAAC GAAACTTACACAGTTACTTAAATCTTGCAGAAGCTACAAAAGATAAGGCTTCATGCCGAAATCAACA CCCTGTCATTTTATGGCAGGGTGTTTTTTT
ST_gRNA_f4	GTCCCCTCCACCCACAGTG GTTTTGTACTCTCAAGATTTAAGTAACTGTACAAC GAAACTTACACAGTTACTTAAATCTTGCAGAAGCTACAAAAGATAAGGCTTCATGCCGAAATCAACA CCCTGTCATTTTATGGCAGGGTGTTTTTTT

NM\_chimera\_trnc GUUGUAGCUCUUUCUCGAAAAGAGAACCGUUGCUACAAU  
AAGGCCGUCUGAAAAGAUGUGCCGCAACGCUCUGCCCUUAAAAGCUUCUGCUUUAAAGGGGC

NM\_chimera\_full GUUGUAGCUCUUUCUCAUUUCGCAGUGCUACAAU  
GAAA AUUGUCGCACUGCGAAAUGAGAACCGUUGCUACAAU AAGGCCGUCUGAAAAGAUGUGCCG  
CAACGCUCUGCCCUUAAAAGCUUCUGCUUUAAAGGGGC

NM\_gRNA\_t1 GTCCCCTCCACCCACAGTG GTTGTAGCTCCCTTTCTCGAAAGAGAACCGTTGCTACAAT  
AAGGCCGTCTGAAAAGATGTGCCGCAACGCTCTGCCCTTAAAGCTTCTGCTTTAACGGGCTTT  
TTTT

NM\_gRNA\_t2 GTCCCCTCCACCCACAGTG GTTGTAGCTCCCTTTCTCGAAAGAGAACCGTTGCTACAAT  
AAGGCCGTCTGAAAAGATGTGCCGCAACGCTCTGCCCTTAAAGCTTCTGCTTTAACGGGCTTT  
TTTT

NM\_gRNA\_t3 GTCCCCTCCACCCACAGTG GTTGTAGCTCCCGAAACGTTGCTACAAT  
AAGGCCGTCTGAAAAGATGTGCCGCAACGCTCTGCCCTTAAAGCTTCTGCTTTAACGGGCTTT  
TTTT

NM\_gRNA\_f1 GTCCCCTCCACCCACAGTG GTTGTAGCTCCCTTTCTCATTTTCGAGTGCTACAAT  
GAAAATTGTGCGACTGCGAAATGAGAACCGTTGCTACAATAAGGCCGTCTGAAAAGATGTGCCG  
CAACGCTCTGCCCTTAAAGCTTCTGCTTTAAGGGGCTTTTTTT

**FINAL gRNA LIST (ordered as gBlocks)**

SP\_gRNA\_t1 TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGC  
AGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGA  
GATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAG  
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GTCCCCTCCACCCACAGTG  
GTTTTGTACTCTCAGAAATGCAGAAGCTACAAAGATAAGGCTTCATGCCGAAATCAACACCCCT  
GTCATTTTATGGCAGGGTGTTTTTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT

ST\_gRNA\_t2 TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGC  
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GTCCCCTCCACCCACAGTG  
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ST\_gRNA\_t3  
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ST\_gRNA\_t4  
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GTCCCCTCCACCCACAGTG  
GTTTATGTA CTCTCAGAAATGCAGAAGCTACAAAGATAAGGCTTCATGCCGAAATCAACACCCCT  
GTCATTTTATGGCAGGGTGTTTTTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT

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ST\_gRNA\_t6  
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GTCCCCTCCACCCACAGTG  
GTTTTAGTACTCTGAAAAGAAGCTTCAAAGATAAGGCTTCATGCCGAAATCAACACCCCTGTCAT  
TTTATGGCAGGGTGTTTTTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT

ST\_gRNA\_t7  
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GATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAAG  
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CCGTAACCTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC  
GTCCCCTCCACCCACAGTG  
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TTTATGGCAGGGTGTTTTTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT

ST\_gRNA\_t8  
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CCGTAACCTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC  
**GTCCCCTCCACCCACAGTG**  
**GTTTATGTACTCTGAAAAGAAGCTACAAAGATAAGGCTTCATGCCGAAATCAACACCCGTGCAT**  
**TTTATGGCAGGGTGTTTTTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

NM\_gRNA\_t1  
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CCGTAACCTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC  
**GTCCCCTCCACCCACAGTG**  
**GTTGTAGCTCCCTTTCTCGAAAGAGAACCGTTGCTACAATAAGGCCGTCTGAAAAGATGTGCCG**  
**CAACGCTCTGCCCTTAAAGCTTCTGCTTTAACGGGCTTTTTTT**  
**CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

NM\_gRNA\_t2  
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GATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAAG  
TAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTA  
CCGTAACCTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC  
**GTCCCCTCCACCCACAGTG**  
**GTTGTAGCTCCCTTTCTGAAAGAACCGTTGCTACAATAAGGCCGTCTGAAAAGATGTGCCGCAAC**  
**GCTCTGCCCTTAAAGCTTCTGCTTTAACGGGCTTTTTTT**  
**CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

NM\_gRNA\_t3  
TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGC  
AGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGA  
GATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAAG  
TAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTA  
CCGTAACCTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC  
**GTCCCCTCCACCCACAGTG**  
**GTTGTAGCTCCCGAACGTTGCTACAATAAGGCCGTCTGAAAAGATGTGCCGCAACGCTCTGCC**  
**CCTTAAAGCTTCTGCTTTAACGGGCTTTTTTT**  
**CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

SP\_gRNA\_f1  
TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGC  
AGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGA  
GATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAAG  
TAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTA  
CCGTAACCTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC  
**GTCCCCTCCACCCACAGTG**  
**GTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACGAAATTGTTGGAACCATTCAAAACAGCA**  
**TAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT**  
**TT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

ST\_gRNA\_f1

TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGC  
AGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGA  
GATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAAG  
TAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTA  
CCGTAACCTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC  
**GTCCCCTCCACCCACAGTG**  
**GTTTTGTACTCTCAAGATTTAAGTAACTGTACAACGAAACTTACACAGTTACTTAAATCTTGC**  
**AGAAGCTACAAAGATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTTATGGCAGGGTGT**  
**TTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

ST\_gRNA\_f2

TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGC  
AGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGA  
GATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAAG  
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**GTCCCCTCCACCCACAGTG**  
**GTTTTAGTACTCTCAAGATTTAAGTAACTGTACAACGAAACTTACACAGTTACTTAAATCTTGC**  
**AGAAGCTTCAAAGATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTTATGGCAGGGTGT**  
**TTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

ST\_gRNA\_f3

TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGC  
AGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGA  
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CCGTAACCTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC  
**GTCCCCTCCACCCACAGTG**  
**GTTTGTGACTCTCAAGATTTAAGTAACTGTACAACGAAACTTACACAGTTACTTAAATCTTGC**  
**AGAAGCTACAAAGATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTTATGGCAGGGTGT**  
**TTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

ST\_gRNA\_f4

TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGC  
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**GTTTATGACTCTCAAGATTTAAGTAACTGTACAACGAAACTTACACAGTTACTTAAATCTTGC**  
**AGAAGCTACAAAGATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTTATGGCAGGGTGT**  
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NM\_gRNA\_f1

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**GTCCCCTCCACCCACAGTG**  
**GTTGTAGCTCCCTTTCTCATTTTCGAGTGCTACAATGAAAATTGTGCGACTGCGAAATGAGAAC**  
**CGTTGCTACAATAAGGCCGTCTGAAAAGATGTGCCGCAACGCTCTGCCCTTAAAGCTTCTGCT**  
**TTAAGGGCTTTTTTT CTAGACCCAGCTTTCTTGTACAAAGTTGGCATT**

### Supplementary Table 3 | Library amplification and sequencing primers.

Library amplification forward primers:

AATGATACGGCGACCACCGAGATCTACACCCTGCGGAAGCCGTTCTCGATGGACGAccgctttgacct  
agaattcatttac

AATGATACGGCGACCACCGAGATCTACACGGAAGGTAGGGAAGTAAAGTGGTGGATGTGgcttt  
gacctacatagcagaact

Library amplification reverse primers:

CAAGCAGAAGACGGCATAACGAGATATTACTCGGACGGACAGACGGGcctctagcacgcgt  
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CAAGCAGAAGACGGCATAACGAGATCGCTCATTGACGGACAGACGGGcctctagcacgcgt  
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CAAGCAGAAGACGGCATAACGAGATTCCGCGAAGACGGACAGACGGGcctctagcacgcgt  
CAAGCAGAAGACGGCATAACGAGATTCTCGCGCGACGGACAGACGGGcctctagcacgcgt  
CAAGCAGAAGACGGCATAACGAGATAGCGATAGGACGGACAGACGGGcctctagcacgcgt

read 1 sequencing primers:

CCTGCGGAAGCCGTTCTCGATGGACGACCGCTTTGACCTAGAATTCATTTAC  
GGAAGGTAGGGAAGTAAAGTGGTGGATGTGgctttgacctacatagcagaact

read 2 sequencing primer:

seq\_primer\_r\_psp1\_psp2 GACGGACAGACGGGcctctagcacgcgt

index read sequencing primer:

seq\_primer\_index\_psp1\_psp2 acgcgtgctagaggcCGTCTGTCCGTC

## **Supplementary Note 1 | Depletion biases in Cas9 library experiments**

The library-based selection strategy employed for PAM-finding imposes apparent minimum and maximum fold depletion values. For sequences that are barely depleted, the noise in the transformation efficiency of the two libraries overwhelms the weak depletion signals. For sequences that are efficiently depleted, the fold depletion value is primarily determined by the very small number of survivors. In most cases, these are not due to authentic survival events in the face of Cas9 pressure. Rather, they are the result of low-frequency Cas9, tracrRNA, or crRNA mutations leading to Cas9 inactivation and consequent target escape. Consequently, any analysis of target-dependent activity differences should focus on moderately depleted sequences.

Because the PAM-finding selection was run overnight, surviving plasmids were forced to evade Cas9-mediated cutting for an extended period, resulting in a highly stringent selection capable of picking up subtle base preferences in the PAM recognition process. By the same token, high stringency may have caused it to miss differences between highly active PAM sequences. For example, our analysis found that NM Cas9 display high activity with PAMs of NNNNGANN, NNNNGTTN, and NNNNGNNT, all of which were depleted as efficiently as NNNNGATT (Figure 2D). While all of these PAMs are more than sufficient for effector cleavage, they may differ for other Cas9-mediated activities. In principle, such differences between highly active sequences can be detected using the depletion assay by modulating the selection stringency, e.g. by reducing the time from transformation to plasmid isolation for sequencing. Alternatively, they may be assayed in the context of the activity of interest.

Finally, our experiments were run using two different protospacers. The various Cas9 proteins differed in their average activity levels on these sequences, exhibiting nonlinear interactions between protospacer and PAM. Other protospacers may exhibit activity differences that could affect the functional PAM by rendering it either more or less stringent.



## Supplementary Note 2 | Expressing novel Cas9 orthologs in bacteria

Characterizing a new Cas9 ortholog is complicated by the requirement for tracrRNA, crRNA, and a PAM sequence downstream of the protospacer. Our sequencing-based library depletion method will empirically detail the required PAM, but still requires advance knowledge of the tracrRNA and crRNA and their directionality. These can normally be discovered by identifying the repeat sequences within the CRISPR locus and searching for a nearby sequence that is expected to base-pair with the repeat; this is normally the tracrRNA. Searching for promoters and terminators on either side of the CRISPR locus and tracrRNA usually suffices to identify directionality; in ambiguous cases, a comparison with existing repeats is often helpful. The entire tracrRNA cassette, including the native promoter and terminator, should be copied wholesale into the expression vector. If there are multiple terminators downstream and it is unclear precisely where the tracrRNA ends, it is safer to include all of them. To compensate for possible differences in promoter strength between the native species and *E. coli*, we prefer to clone the tracrRNA cassette downstream of the corresponding cas9 gene such that the two are polycistronic. In the event that there is a promoter within the tracrRNA cassette that would transcribe backwards towards cas9 (this is often the native cas9 promoter), we insert the lambda t1 terminator to mitigate transcription in that direction only. Of the orthologs characterized in this study, the lambda t1 terminator was only required for NM.

Producing a desired crRNA for PAM-finding simply requires insertion of the desired spacer sequence (ideally at least 20 bp) upstream of the repeat identified in the CRISPR locus. We have determined that there is no need to insert an additional repeat upstream of the spacer and none of the targeting plasmids used in this study feature such a design, but if it is done the spacing should match that of the native CRISPR locus. RNA secondary structures involving the spacer sequence should be minimized if at all possible.

### Supplementary Note 3| Sequences of cas9 genes for bacterial experiments.

In bacteria, the native coding sequence of SP was utilized for nuclease studies, while the human-optimized version was employed for transcriptional repression. ST<sub>1</sub>, NM, and TD utilized human-optimized genes for both.

#### >SPcas

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#### >SPcas<sub>m4</sub>

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```

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**Supplementary Note 4** | For the wild-type Cas9 two versions were created: one bearing a single NLS on the C-term of the protein, and another bearing a 3XNLS on the C-term of the protein. Both versions showed comparable activity, with the former used for experiments in the manuscript.

Sequences of the nuclease-null Cas9\_VP64 activator constructs are based on the quadruple mutants detailed in the Online Methods. For each ortholog two versions were created: one bearing a NLS on the C-term of the protein, and another bearing an NLS on both the C-term and N-term of the protein. Both versions showed comparable activity, with the former used for experiments in the manuscript.

(NLS and VP64 domains are highlighted).

>Cas9<sup>SP</sup>

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>Cas9<sup>SP</sup>3XNLS

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>Cas9<sup>SP</sup> VP64  
m4

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>Cas9<sup>SP</sup> VP64<sub>m4</sub><sub>N</sub>

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AGCCTGTTCAAGACCGAGGACATCACCAGCGCCTTGAAGGACCGCAAGGAGGAGTTCAGGAGATCCTGGAGGCCCTGCTGAAGCAGCATCAGCTCGACA  
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CCACTACGGCAAGAAGAACCAGGAGAGAAGATCTACCTGCCTCTATCCCGCCGACGAGATCCGCAACCCCGTGGTGTGCGCGCCCTGAGCCAG  
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GCAAGGAGATCGAGAAGCGCAGGAGGAGAACCGCAAGGACCGGAGGAGGCCCGGCAAGTTCGCGAGTACTTCCCAACTTCGTGGGCGAGCC  
CAAGAGCAAGGACATCTGAAGTGCAGCTGTACGAGCAGCAGCAGCGCAAGTGCCTGTACAGCGGCAAGGAGATCAACCTGGGCCGCTGAACGAG  
AAGGGCTACGTGGAGATCGACACCGCCTGCCCTTACGCCGACCTGGGACGACAGCTTCAACAACAAGGTGCTGGTGTGGGCGAGCGAGAACCAGA  
ACAAGGGCAACAGACCCCTACGAGTACTTCAACGGCAAGGACAACAGCCGCGAGTGGCAGGAGTTCAGGCCCGCGTGGAGACCGAGCCCTTCCC  
CCGACGCAAGAAGCAGCGCATCTGCTGCAGAAGTTCGACGAGGAGCTTCAAGGAGCGCAACCTGAACGACACCCGCTACGTGAACCGCTTCCCTG  
TGCCAGTTCGTGGCCAGCCGATGCGCTGACCGGCAAGGGCAAGAAGCGGCTGTTCGCCAGCAACGGCCAGATCACCAACCTGCTGCGCGGCTTCT

GGGGCCTGCGCAAGGTGCGCGCCGAGAACGACCGCCACCACGCCCTGGACGCCGTGGTGGCTGCAGCACCGTGGCCATGCAGCAGAAGATCAC  
CCGCTTCGTGCGCTACAAGGAGATGAACGCCTTCGACGGTAAACCATCGACAAGGAGACCGGCGAGGTGCTGCACCAGAAGACCCACTTCCCCCAG  
CCCTGGGAGTTCTTCGCCCGAGGTGATGATCCGCGTGTTCGGCAAGCCCGAGCCGAGTTCGAGGAGGCCGACACCCCGGAGAAGGTGC  
GCACCCTGCTGGCCGAGAAGCTGAGCAGCCGCCCTGAGGCCGTGCACGAGTACGTGACTCCTCTGTTCTGAGCCCGCCCCAACCCGAAGATGAG  
CGGTCAGGGTCACATGGAGACCGTGAAGAGCGCCAAGCGCCTGGACGAGGGCGTGAGCGTGTGCGCGTGCACCCTGACCCAGCTGAAGCTGAAGGAC  
CTGGAGAAGATGGTGAACCGCGAGCGCGAGCCCAAGCTGTACGAGGCCCTGAAGGCCCGCCTGGAGGCCCAAGGACGACCCCGCCAAAGCCCTCG  
CCGAGCCCTTCTACAAGTACGACAAGGCCGGAACCCGACCCAGCAGGTGAAGGCCGTGCGCGTGGAGCAGGTGCAGAAGACCGGCGTGTGGGTGCG  
CAACCACAACGGCATCGCCGACAACGCCACCATGGTGCAGCTGGACGTGTTTCGAGAAGGGCGACAAGTACTACCTGGTGCCTACTACAGCTGGCAG  
GTGGCCAAAGGATCCTGCCCGACCGCGCCGTGGTGCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACAGCTTCAACTTCAAGTTACGCC  
TGCACCCCAACGACCTGGTGGAGGTGATCACCAGAAGGCCCGCATGTTCCGGTACTTCGCCAGCTGCCACCGCGGCAACCCGCAACATCAACATCCG  
CATCCACGACCTGGACCAAGAATCGGCAAGAACGGCATCCTGGAGGGCATCGGCGTGAAGACCGCCCTGAGCTTCCAGAAGTACCAGATCGACGAG  
CTGGGCAAGGAGATCCGCCCTGCCCTGAAGAAGCGCCCTCCTGTGCGCAGCAGGGCTGACCCCAAGAAGAAGAGGAAGGTGTGA

>Cas9<sup>NM</sup><sub>3</sub>XNLS

gccaccATGGCCGCTTCAAGCCCAACCCCATCAACTACATCCTGGGCTGGACATCGGCATCGCCAGCGTGGGCTGGCCATGGTGGAGATCGACG  
AGGACGAGAACCCCATCTGCCTGATCGACCTGGGTGTGCGCGTGTTCGAGCGCGCTGAGGTGCCCAAGACTGGTGACAGTCTGGCTATGGCTCGCCG  
GCTTGTCTCGCTGTGTTCCGGCGCCTTACTCGCCGGCGCGCTACCCGCTTCTGCGCGCTCGCCGCTGCTGAAGCGCGAGGGTGTGCTGACGGTGC  
GACTTCGACGAGAAGCCGCTGATCAAGAGCCTGCCAACACTCCTTGGCAGCTGCGCGCTGCCGCTTGGAGCCGAGTACTCCTCTGGAGTGA  
GCGCCGTGCTGCTGACCTGATCAAGCACCGCGCTACTTACGCCAGCGCAAGAACGAGGGCGAGACCGCCGACAAGGAGCTGGGTGCTCTGCTGAA  
GGGCGTGGCCGACAACGCCACGCCCCTGCAGACTGGTACTTCCGCACTCCTGCTGAGCTGGCCCTGAACAAGTTCGAGAAGGAGAGCGGCCACATC  
CGCAACCAGCGCGCGACTACAGCCACACCTTCAGCCGCAAGGACCTGCAGGCCGAGCTGATCCTGCTGTTTCGAGAAGCAGAGGAGTTCGGCAACC  
CCCAGTGTAGCGCGCCCTGAAGGAGGGCATCGAGACCCTGCTGATGACCCAGCGCCCGCCTGAGCGCGACGCGCTGCAGAAGATGCTGGGCCA  
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GAGCAGGGCAGCGAGCGCCCTGACCGACACCGAGCGCGCCACCTGATGGACGAGCCCTACCGCAAGGCAAGCTGACCTACGCCAGGCCCGCA  
AGTGTCTGGGTCTGGAGGACACCGCCTTCTTCAAGGGCTGCGCTACGGCAAGGACAACGCGGAGGCCAGCACCTGATGGAGATGAAGGCCATACCA  
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CCACTACGGCAAGAAGAACACCGAGGAGAAGATCTACCTGCCTCTATCCCGCCGACGAGATCCGCAACCCCGTGGTGTGCGCGCCCTGAGCCAG  
GCCCGAAGGTGATCAACGGCGTGGTGCAGCGCTACGGCAGCCCGCCGCTCCACATCGAGACCCCGCGAGGTGGCCCAAGAGCTTCAAGGACC  
GCAAGGAGATCGAGAAGCGCCAGGAGGAGAACCAGGACCGCATCCAGCCGAGATCCTGAGGCGCAAGTTCGCGGAGTACTTCCCAACTTCTGGGCGAGCC  
CAAGAGCAAGGACATCCTGAAGTTCGCGCTGTACGAGCAGCAGCACGGCAAGTGCCTGTACAGCGCAAGGAGATCAACCTGGGCCGCTGAACGAG  
AAGGGCTACGTGGAGATCGACACGCCCCCTGCCCTTACGCCGACCTGGGACGACAGCTTCAACAACAAGGTGCTGGTGTGGCGAGCGAGAACCAGA  
ACAAGGGCAACCAGACCCCTACGAGTACTTCAACGGCAAGGACAACAGCCGCGAGTGGCAGGAGTTCAGGCCCGCTGGAGACCAGCCGCTTCCC  
CCGACGCAAGAAGCAGCAGGAGTGTGCTGCGAGAAGTTCGACGAGGACCGCTTCAAGGAGCGCAACTTGAACGACACCCGCTACGTGAACCGCTTCCG  
TGCCAGTTCGTGGCCGACCGCATGCGCTGACCGGCAAGGGCAAGAAGCGCGTGTTCGCCAGCAACGGCCAGATCACCACCTGCTGCGCGGCTTCT  
GGGCGCTGCGCAAGGTGCGCGCCGAGAACGACCGCCACACCGCCCTGGACCGCGTGGTGGTGGCTGCAGCACCGTGGCCATGCAGCAGAAGATCAC  
CCGCTTCGTGCGCTACAAGGAGATGAACGCCTTCGACGGTAAACCATCGACAAGGAGACCGGCGAGGTGCTGCACCAGAAGACCCACTTCCCCCAG  
CCCTGGGAGTTCCTTCGCCAGGAGGTGATGATCCGCGTGTTCGGCAAGCCCGAGTTCGAGGAGGCCGACACCCCGGAGAAGCTGC  
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CTGGAGAAGATGGTGAACCGCGAGCGCGAGCCCAAGCTGTACGAGGCCCTGAAGGCCCGCCTGGAGGCCCAAGGACGACCCCGCCAAAGCCCTTCG  
CCGACCCCTTCTACAAGTACGACAAGGCCGGAACCCGACCCAGCAGGTGAAGGCCGTGCGCGTGGAGCAGGTGCAGAAGACCGGCGTGTGGGTGCG  
CAACCACAACGGCATCGCCGACAACGCCACCATGGTGCAGCTGGACGTGTTTCGAGAAGGGCGACAAGTACTACCTGGTGCCTACTACAGCTGGCAG  
GTGGCCAAAGGATCCTGCCCGACCGCGCCGTGGTGCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACAGCTTCAACTTCAAGTTACGCC  
TGCACCCCAACGACCTGGTGGAGGTGATCACCAGAAGGCCCGCATGTTCCGGTACTTCGCCAGCTGCCACCGCGGCAACCCGCAACATCAACATCCG  
CATCCACGACCTGGACCAAGAATCGGCAAGAACGGCATCCTGGAGGGCATCGGCGTGAAGACCGCCCTGAGCTTCCAGAAGTACCAGATCGACGAG  
CTGGGCAAGGAGATCCGCCCTGCCCTGAAGAAGCGCCCTCCTGTGCGCGCGGCCGAGATCCAAAAAGAAGAGAAAGGTAGATCCAAAAAGA  
AGAGAAAGGTAGATCCAAAAAGAAGAGAAAGGTAGATACGGCCGATAG

>Cas9<sup>NM</sup><sub>m4</sub> VP64

gccaccATGGCCGCTTCAAGCCCAACCCCATCAACTACATCCTGGGCTGGCCATCGGCATCGCCAGCGTGGGCTGGCCATGGTGGAGATCGACG  
AGGACGAGAACCCCATCTGCCTGATCGACCTGGGTGTGCGCGTGTTCGAGCGCGCTGAGGTGCCCAAGACTGGTGACAGTCTGGCTATGGCTCGCCG  
GCTTGTCTCGCTGTGTTCCGGCGCCTTACTCGCCGGCGCGCTACCCGCTTCTGCGCGCTCGCCGCTGCTGAAGCGCGAGGGTGTGCTGACGGTGC  
GACTTCGACGAGAAGCCGCTGATCAAGAGCCTGCCAACACTCCTTGGCAGCTGCGCGCTGCCGCTTGGAGCCGAGTACTCCTCTGGAGTGA  
GCGCCGTGCTGCTGACCTGATCAAGCACCGCGCTACTTACGCCAGCGCAAGAAGTACTACCTGGTGCCTACTACAGCTGGCAG  
GTGGCCAAAGGATCCTGCCCGACCGCGCCGTGGTGCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACAGCTTCAACTTCAAGTTACGCC  
TGCACCCCAACGACCTGGTGGAGGTGATCACCAGAAGGCCCGCATGTTCCGGTACTTCGCCAGCTGCCACCGCGGCAACCCGCAACATCAACATCCG  
CATCCACGACCTGGACCAAGAATCGGCAAGAACGGCATCCTGGAGGGCATCGGCGTGAAGACCGCCCTGAGCTTCCAGAAGTACCAGATCGACGAG  
CTGGGCAAGGAGATCCGCCCTGCCCTGAAGAAGCGCCCTCCTGTGCGCGCGGCCGAGATCCAAAAAGAAGAGAAAGGTAGATCCAAAAAGA  
AGAGAAAGGTAGATCCAAAAAGAAGAGAAAGGTAGATACGGCCGATAG



**Supplementary Software 1** | The patternProp.py script returns total reads and fraction of reads for each 1-base derivative of an input PAM.

Usage: python patternProp.py [PAM] file.fastq

```
#!/usr/bin/env python
import sys
from Bio import SeqIO
pattern=sys.argv[1]
for arg in sys.argv[2:]:
    A=[0,0,0,0,0,0,0,0]
    T=[0,0,0,0,0,0,0,0]
    C=[0,0,0,0,0,0,0,0]
    G=[0,0,0,0,0,0,0,0]
    pA=[]
    pT=[]
    pC=[]
    pG=[]
    for rec in SeqIO.parse(arg, "fastq"):
        is_fit=1
        for j,p in enumerate(pattern):
            if str(p)!='N' and str(p)!=str(rec.seq[j]):
                is_fit=0
        if is_fit:
            for i,b in enumerate(str(rec.seq)):
                if b=='A':
                    A[i]=A[i]+1
                elif b=='T':
                    T[i]=T[i]+1
                elif b=='C':
                    C[i]=C[i]+1
                elif b=='G':
                    G[i]=G[i]+1
                else:
                    z=0
    for p in xrange(0,8,1):
        sum=A[p]+T[p]+C[p]+G[p]
        pA.append(float(A[p])/float(sum))
        pT.append(float(T[p])/float(sum))
        pC.append(float(C[p])/float(sum))
        pG.append(float(G[p])/float(sum))
    print arg+' absolute'+pattern
```

```

print '\p      1      2      3      4      5      6      7      8'
print 'A '+str(A[0])+ ' '+str(A[1])+ ' '+str(A[2])+ ' '+str(A[3])+ ' '+str(A[4])+
'+str(A[5])+ ' '+str(A[6])+ ' '+str(A[7])
print 'T '+str(T[0])+ ' '+str(T[1])+ ' '+str(T[2])+ ' '+str(T[3])+ ' '+str(T[4])+
'+str(T[5])+ ' '+str(T[6])+ ' '+str(T[7])
print 'C '+str(C[0])+ ' '+str(C[1])+ ' '+str(C[2])+ ' '+str(C[3])+ ' '+str(C[4])+
'+str(C[5])+ ' '+str(C[6])+ ' '+str(C[7])
print 'G '+str(G[0])+ ' '+str(G[1])+ ' '+str(G[2])+ ' '+str(G[3])+ ' '+str(G[4])+
'+str(G[5])+ ' '+str(G[6])+ ' '+str(G[7])
print arg+' percent'
print '\p      1      2      3      4      5      6      7      8'
print 'A '+str(pA[0])+ ' '+str(pA[1])+ ' '+str(pA[2])+ ' '+str(pA[3])+ ' '+str(pA[4])+
'+str(pA[5])+ ' '+str(pA[6])+ ' '+str(pA[7])
print 'T '+str(pT[0])+ ' '+str(pT[1])+ ' '+str(pT[2])+ ' '+str(pT[3])+ ' '+str(pT[4])+
'+str(pT[5])+ ' '+str(pT[6])+ ' '+str(pT[7])
print 'C '+str(pC[0])+ ' '+str(pC[1])+ ' '+str(pC[2])+ ' '+str(pC[3])+ ' '+str(pC[4])+
'+str(pC[5])+ ' '+str(pC[6])+ ' '+str(pC[7])
print 'G '+str(pG[0])+ ' '+str(pG[1])+ ' '+str(pG[2])+ ' '+str(pG[3])+ ' '+str(pG[4])+
'+str(pG[5])+ ' '+str(pG[6])+ ' '+str(pG[7])

```



**Supplementary Software 2** | The patternProp3.py script returns fraction of total reads for each 1-base derivative of an input PAM.

Usage: python patternProp3.py [PAM] file.fastq

```
#!/usr/bin/env python
#Take pattern as arg1, fg as arg2 and bg as arg3
import sys
from Bio import SeqIO
pattern=sys.argv[1]
A=[0,0,0,0,0,0,0,0,0]
T=[0,0,0,0,0,0,0,0,0]
C=[0,0,0,0,0,0,0,0,0]
G=[0,0,0,0,0,0,0,0,0]
pA=[]
pT=[]
pC=[]
pG=[]
for rec in SeqIO.parse(sys.argv[2], "fastq"):
    is_fit=1
    for j,p in enumerate(pattern):
        if str(p)!='N' and str(p)!=str(rec.seq[j]):
            is_fit=0
    if is_fit:
        for i,b in enumerate(str(rec.seq)):
            if b=='A':
                A[i]=A[i]+1
            elif b=='T':
                T[i]=T[i]+1
            elif b=='C':
                C[i]=C[i]+1
            elif b=='G':
                G[i]=G[i]+1
            else:
                z=0
        Abg=[0,0,0,0,0,0,0,0,0]
        Tbg=[0,0,0,0,0,0,0,0,0]
        Cbg=[0,0,0,0,0,0,0,0,0]
        Gbg=[0,0,0,0,0,0,0,0,0]
        pAbg=[]
        pTbg=[]
        pCbg=[]
```

```

pGbg=[]
for rec in SeqIO.parse(sys.argv[2], "fastq"):
    for i,b in enumerate(str(rec.seq)):
        if b=='A':
            Abg[i]=Abg[i]+1
        elif b=='T':
            Tbg[i]=Tbg[i]+1
        elif b=='C':
            Cbg[i]=Cbg[i]+1
        elif b=='G':
            Gbg[i]=Gbg[i]+1
        else:
            z=0
for p in xrange(0,8,1):
    sumbg=Abg[p]+Tbg[p]+Cbg[p]+Gbg[p]
    pAbg.append(float(Abg[p])/float(sumbg))
    pTbg.append(float(Tbg[p])/float(sumbg))
    pCbg.append(float(Cbg[p])/float(sumbg))
    pGbg.append(float(Gbg[p])/float(sumbg))
for p in xrange(0,8,1):
    sumbg=Abg[p]+Tbg[p]+Cbg[p]+Gbg[p]
    pA.append(float(A[p])/float(sumbg))
    pT.append(float(T[p])/float(sumbg))
    pC.append(float(C[p])/float(sumbg))
    pG.append(float(G[p])/float(sumbg))
print str(sys.argv[1])+' percentFGs/total'
print 'b\p      1      2      3      4      5      6      7      8'
print 'A '+str(pA[0])+ ' '+str(pA[1])+ ' '+str(pA[2])+ ' '+str(pA[3])+ ' '+str(pA[4])+ ' '+str(pA[5])+
      '+str(pA[6])+ ' '+str(pA[7])
print 'T '+str(pT[0])+ ' '+str(pT[1])+ ' '+str(pT[2])+ ' '+str(pT[3])+ ' '+str(pT[4])+ ' '+str(pT[5])+
      '+str(pT[6])+ ' '+str(pT[7])
print 'C '+str(pC[0])+ ' '+str(pC[1])+ ' '+str(pC[2])+ ' '+str(pC[3])+ ' '+str(pC[4])+ ' '+str(pC[5])+
      '+str(pC[6])+ ' '+str(pC[7])
print 'G '+str(pG[0])+ ' '+str(pG[1])+ ' '+str(pG[2])+ ' '+str(pG[3])+ ' '+str(pG[4])+ ' '+str(pG[5])+
      '+str(pG[6])+ ' '+str(pG[7])

```