Supplementary Information for
A platform for deep sequence-activity mapping and engineering antimicrobial peptides

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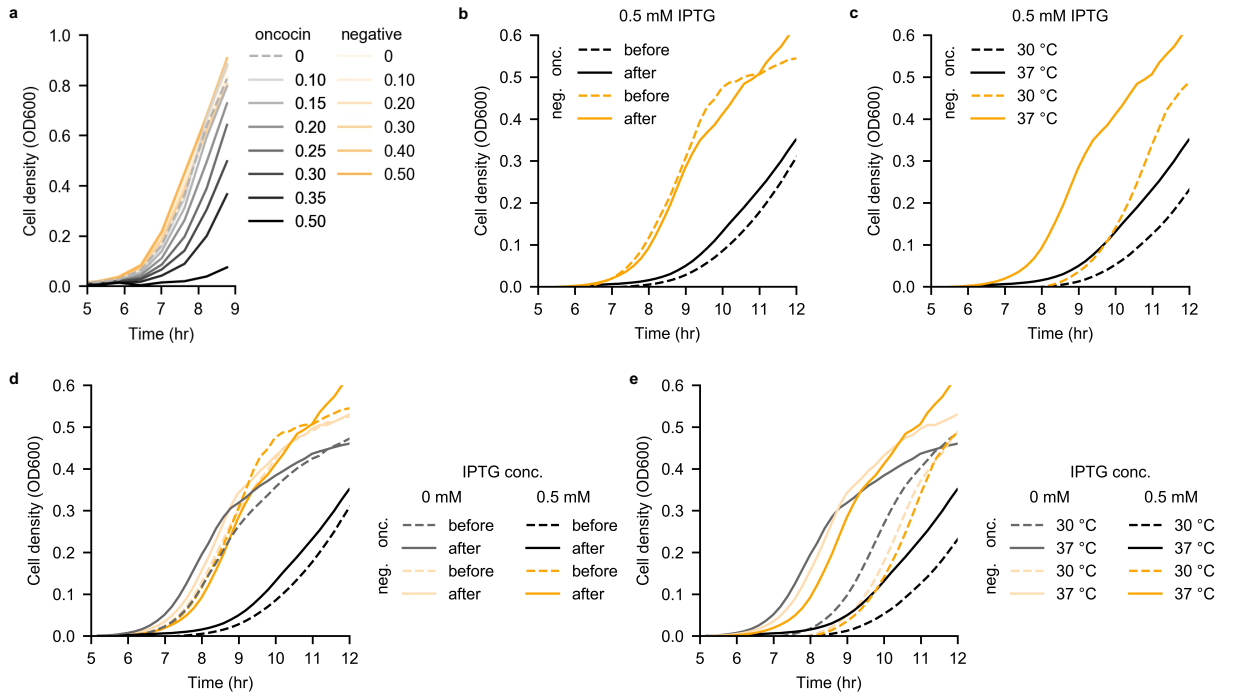
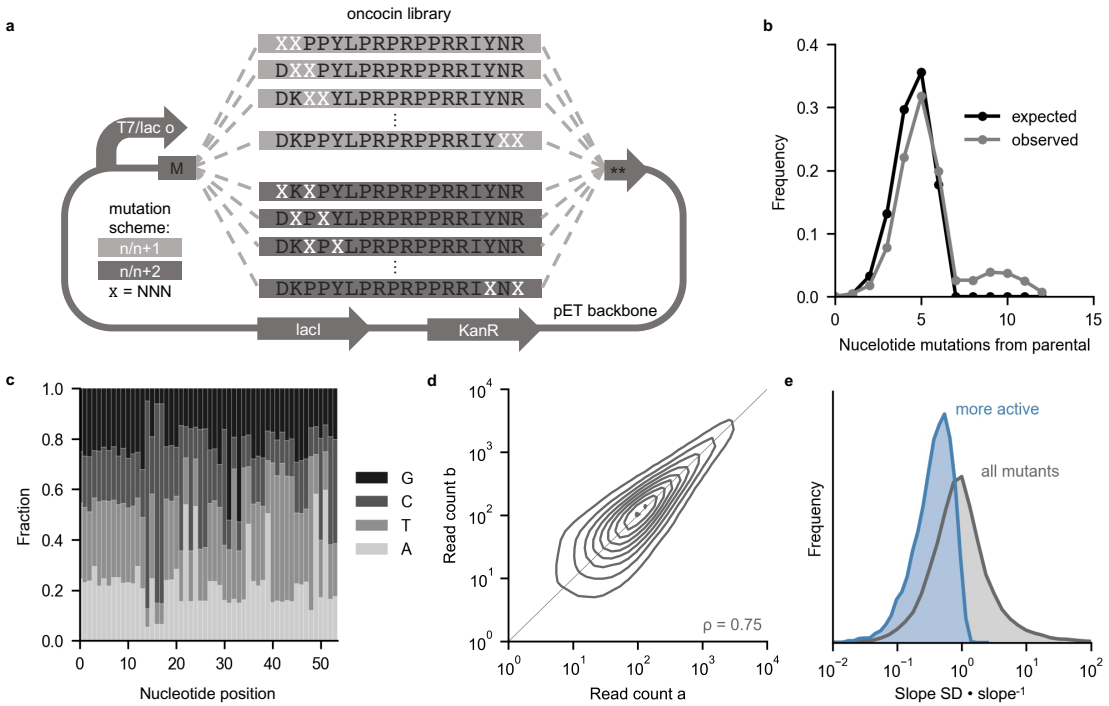
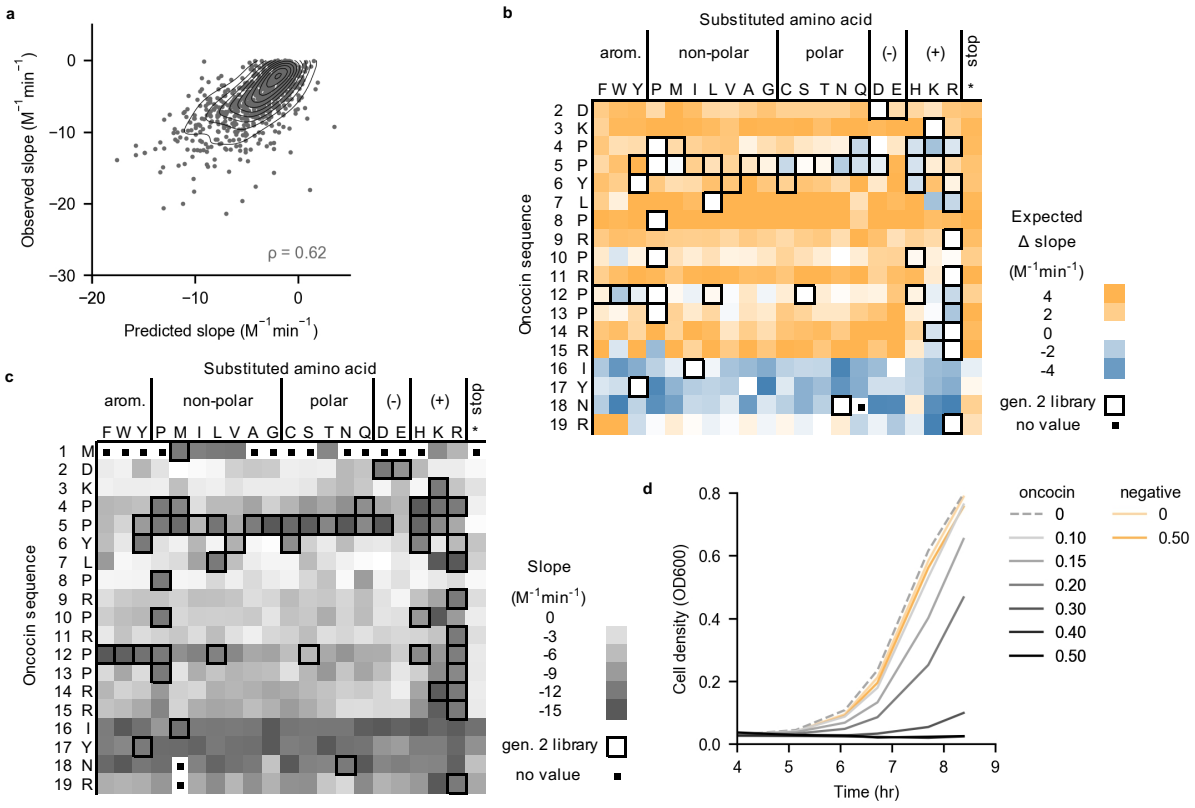


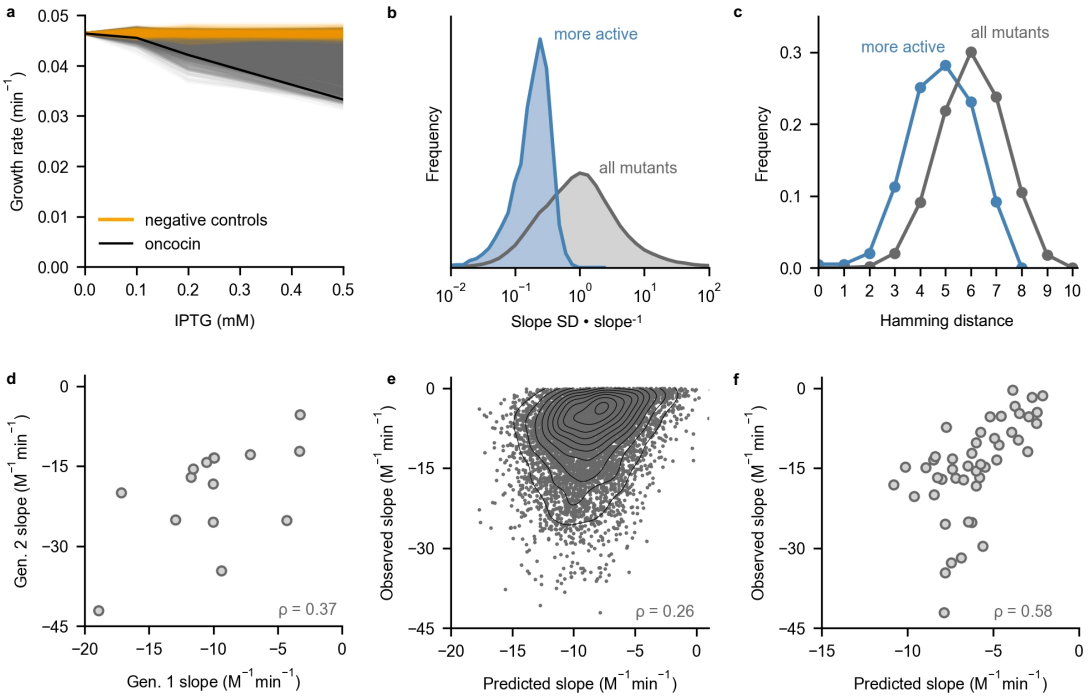
Supplementary Fig. 1. Assay development. **a**, Expression of oncocin (black) and negative control (vector expressing a start codon followed by five stop codons; orange) in T7 LysY I^q *E. coli* across varying induction levels revealed inducible depletion of oncocin. AMP production was induced at 37 ºC after transformation recovery. The legend indicates the concentration of IPTG inducer in mM. Uninduced oncocin was plotted with a dashed line to aid visualization. **b**, Induction start time did not substantially affect oncocin (black) depletion relative to negative control (orange). AMP production was induced with 0.50 mM IPTG before transformation recovery (dashed) or after recovery (solid). Cells were grown at 37 ºC. **c**, Lower induction temperature reduced depletion of oncocin (black) relative to negative control (orange). Cells were grown at 30 ºC (dashed) or 37 ºC (solid). AMP production was induced with 0.50 mM IPTG after the transformation recovery. **d**, **Supplementary Fig. 1b** with uninduced populations (light). **e**, **Supplementary Fig. 1c** with uninduced populations (light).



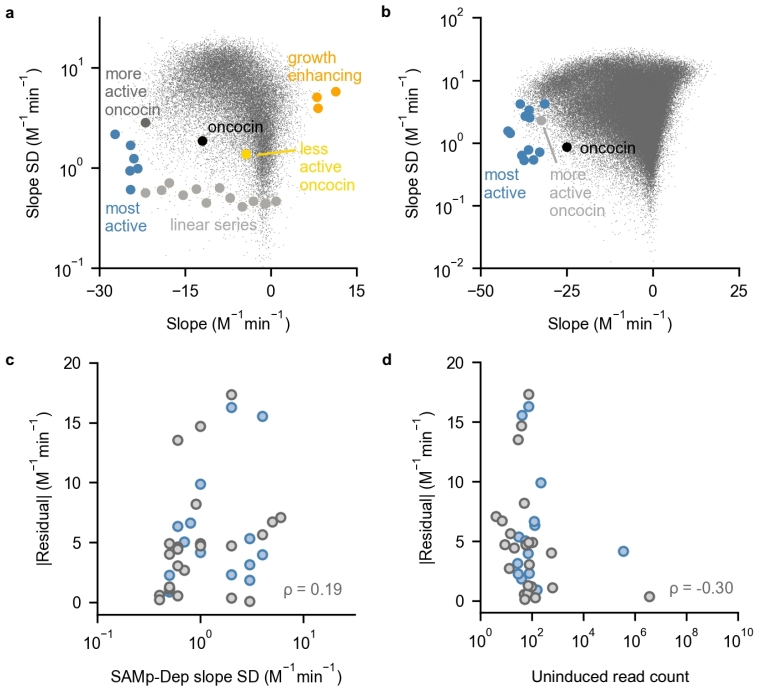
Supplementary Fig. 2. First-generation library design and deep sequencing analysis. **a,** The first-generation oncocin mutagenic library used an *n*/*n*+1 and *n*/*n*+2 mutation scheme. Degenerate NNN codons (X) cover all 64 codons encoding 20 amino acids. **b**, The observed nucleotide mutation distribution followed the expected binomial distribution with $n_{mutations} = 6$ and $p_{mutation} = 0.75$. The number of nucleotide mutations was calculated relative to the parental oncocin DNA sequence. **c**, Mutated codons displayed the expected nucleotide composition from NNN codons. **d**, Read counts for each induction condition correlated across replicates. Read count a and b were randomly selected replicates without replacement for each mutant at each induction condition and represented as a kernel density plot. ρ indicates Pearson correlation coefficient. **e**, Slope was reproducible for most mutants more active than parental (blue). Relative slope standard deviation was higher for the full set of mutants due to many mutants having slope near 0 $M^{-1}min^{-1}$. Slope standard deviation (SD) was from three SAMP-Dep replicates.



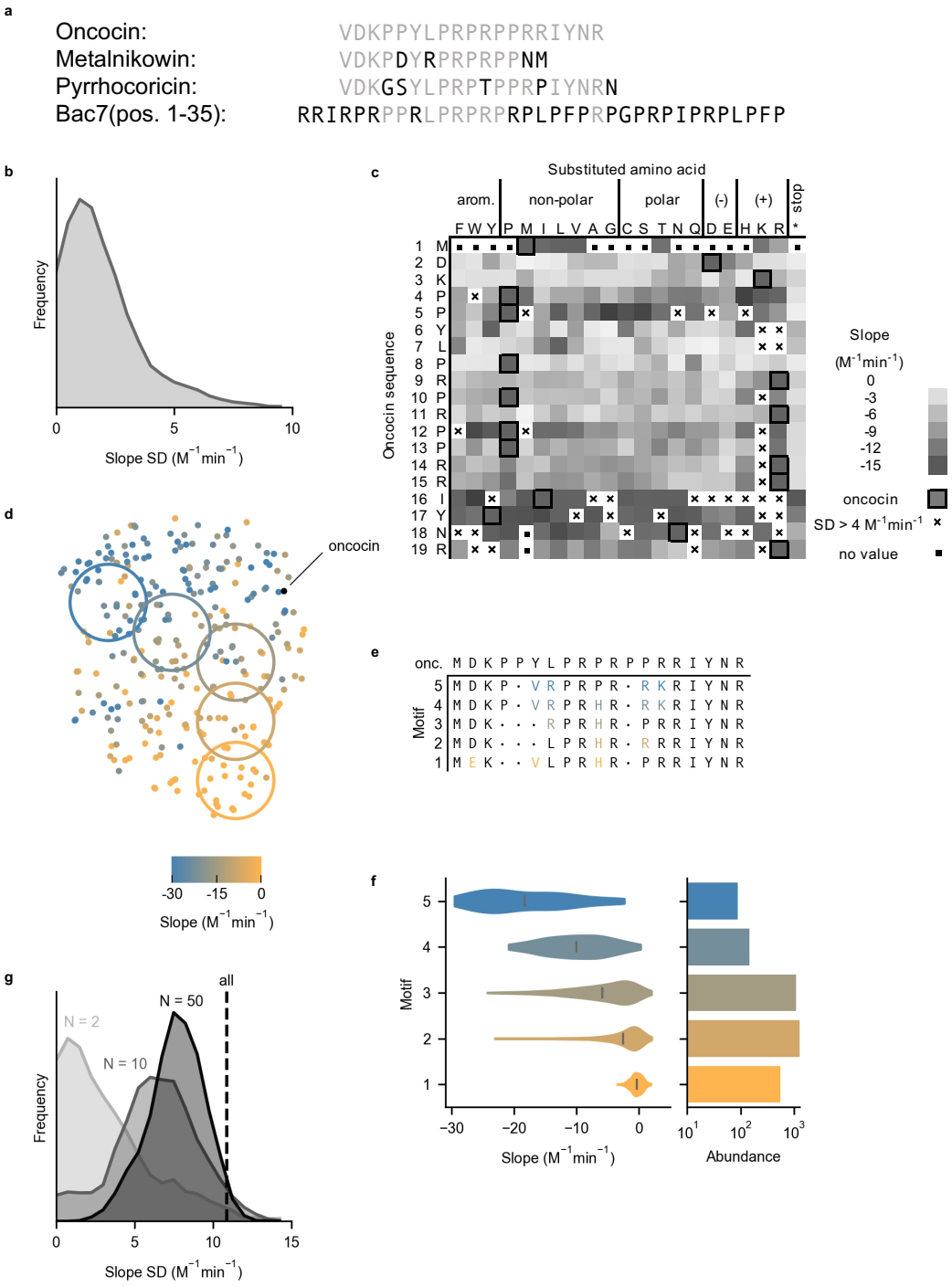
Supplementary Fig. 3. First-generation deep sequencing analysis empowers second-generation library design. **a**, An additive model trained on 80% of first-generation sequence-activity pairs predicted slope for the remaining 20% of first-generation sequences. All mutants were filtered for slope standard deviation < 2 $M^{-1}min^{-1}$. **b**, Coefficients from an additive model trained on 100% of first-generation sequence-activity pairs indicated expected slope contributions. A negative expected change in slope (blue) suggested the mutation enhanced activity broadly. Outlined residues were incorporated in the second-generation library corresponding to **Fig. 3a**. **c**, The single mutant slope heatmap indicated individual mutation effect on activity. Outlined residues were incorporated in the second-generation library corresponding to **Fig. 3a**. **d**, Expression of second-generation parental oncocin (black) and negative control (orange) in T7 LysY I^q *E. coli* across varying induction levels revealed inducible depletion of oncocin. AMP production was induced at 37 ºC after transformation recovery. The legend indicates the concentration of IPTG inducer in mM. Uninduced oncocin was plotted with a dashed line to aid visualization.



Supplementary Fig. 4. Second-generation deep sequencing analysis. All mutants used for training and predictive analyses were filtered for slope standard deviation < 2 $M^{-1}min^{-1}$. **a**, Growth rate versus induction level for a subset of 10,000 mutants indicated most mutants reduced activity while some enhanced activity. Negative controls (orange) and parental oncocin (black) were included for comparison. All mutants had error comparable to parental oncocin (slope standard deviation < 2 $M^{-1}min^{-1}$). **b**, Slope was reproducible for most mutants more active than oncocin (blue). Slope reproducibility for all mutants (grey) was higher due to many mutants having slope near 0 $M^{-1}min^{-1}$. Slope standard deviation (SD) was from three SAMP-Dep replicates. **c**, More active mutants than oncocin (blue) were more sequence-similar to oncocin relative to the entire library (grey). Hamming distance was calculated on an amino acid basis relative to oncocin. **d**, Second-generation slope correlated with first-generation slope for amino acid variants observed in both libraries. **e**, An additive model trained on 100% of first-generation mutants predicted slope for 20% of second-generation sequences. The same subset of second-generation mutant slopes was predicted in **Fig. 4d**. **f**, An additive model trained on 100% of first-generation mutants predicted slope of second-generation mutants that conformed to the first-generation library design.

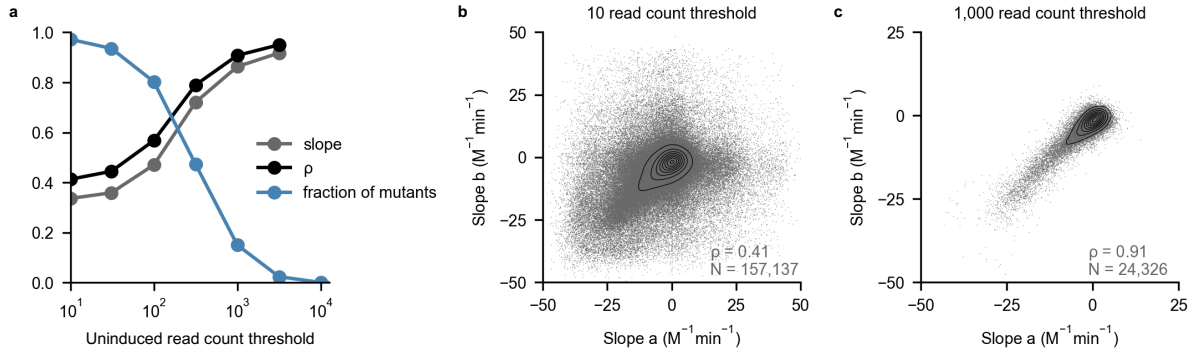


Supplementary Fig. 5. Clonal validation mutants and deviation from linear fit. **a**, First-generation mutants selected for clonal validation answered several questions. The linear series (grey) addressed SAMP-Dep sensitivity and correlation with clonal activity. The most potent mutants (blue) addressed SAMP-Dep utility. Synonymous oncocin mutants (dark grey and yellow) addressed codon effect on activity. Putative growth enhancing mutants (orange) with positive SAMP-Dep slopes addressed an unexpected growth-enhancing phenotype. SD represents standard deviation. **b**, Second-generation mutants with substantially enhanced SAMP-Dep slope (blue) were selected for clonal validation. A synonymous oncocin mutant (grey) was among the most potent mutants. **c**, Residuals for all first-generation (grey) and second-generation (blue) clonal mutants relative to SAMP-Dep slope standard deviation indicated a weak relationship. Slope standard deviation was from three SAMP-Dep replicates. **d**, Residuals for all first-generation (grey) and second-generation (blue) clonal mutants relative to uninduced read count indicated a weak relationship.

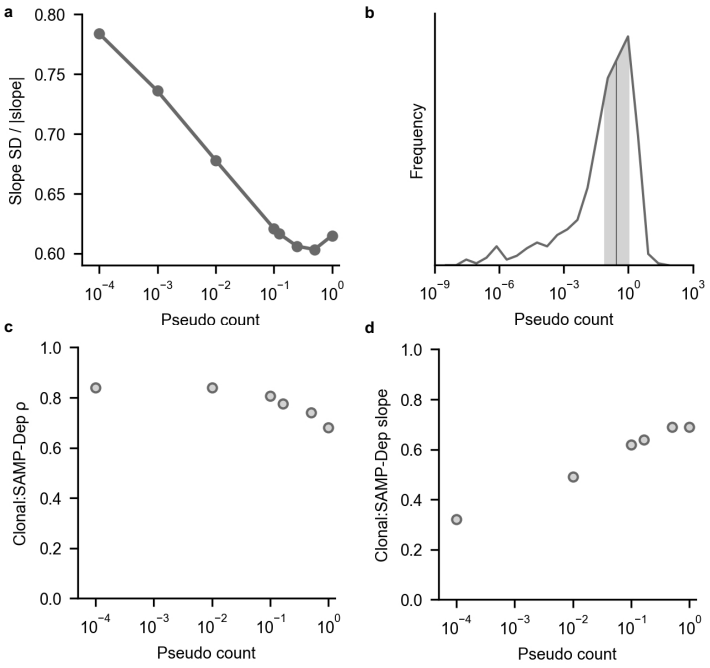


Supplementary Fig. 6. Additional first-generation sequence-function relationships. **a**, Oncocin homologs metalnikowin, pyrrhocoricin, and bac7 have highly similar sequences. **b**, The growth:induction SAMP-Dep slope was reproducible for most mutants in the single mutation slope heatmap (**Fig. 5a**). The slope standard deviation from three SAMP-Dep replicates is presented with kernel smoothing. **c**, The slope of single-site mutants, from

Fig. 5a, with standard deviation < 4 $M^{-1}min^{-1}$. **d**, Alternative trajectory across a two-dimensional representation of sequence space (**Fig. 5f**). **e**, Common motifs conserved within colored bins from **Supplementary Fig. 6d**. **f**, *Left*: Second-generation mutant slope distribution with motifs identified in **Supplementary Fig. 6e**. *Right*: Motif prevalence in the second-generation library. **g**, Slope standard deviation histograms for N = 2, 10, and 50 nearest neighbors by Euclidean distance supported clustering. The dashed line represents slope standard deviation of all 291 mutants. The slope standard deviation is presented with kernel smoothing.



Supplementary Fig. 7. Slope reproducibility increased with mutant abundance. **a**, Second-generation SAMP-Dep slope reproducibility across replicates increased with more stringent uninduced count thresholds. Slope (grey) and ρ (black) indicated the slope and Pearson correlation coefficient of SAMP-Dep slope between replicates. Fraction of mutants (blue) indicated the proportion that passed the uninduced read count threshold. **b**, Slope reproducibility was poor across replicates for mutants observed at least ten times when uninduced. **c**, Slope reproducibility was strong across replicates for mutants observed at least a thousand times when uninduced.



Supplementary Fig. 8. A pseudo count of 10^{-1} accurately quantified potent mutant activity while maintaining low slope error. **a**, Pseudo counts between 0.1 and 1 reduced relative error, average slope standard deviation (SD) relative to average slope, for mutants assigned a pseudo count. **b**, Mutants with monotonically decreasing read count with inducer concentration and not observed at full induction were extrapolated to predict read count at 0.50 mM IPTG. The median pseudo count was 0.3 (vertical line), and values ranging from 0.1 to 1 (shaded) were common. **c, d**, The relationship between clonally measured slope and SAMP-Dep slope (**Fig. 4a**) was calculated with different values for pseudo counts. **c,** Clonal and SAMP-Dep slopes correlated better with a low pseudo count, indicating high pseudo counts underestimated potent mutant activity. **d**, Clonal:SAMP-Dep slope ratio increased with larger pseudo counts, indicating SAMP-Dep slope was inaccurately large for low pseudo counts.

Supplementary Table 1 | Validation results

DNA name*	Peptide name	Generation	Clonal slope ($M^{-1}min^{-1}$)	SAMP-Dep slope ($M^{-1}min^{-1}$)	MIC (μM)
clone 1	A	2	-42 ± 2	-42.0 ± 2	10 ± 1
clone 2		2	-35 ± 5	-41 ± 1	
clone 3	E	2	-30 ± 5	-38.0 ± 0.6	22 ± 3
clone 4		2	-28.8 ± 0.4	-36.1 ± 0.8	
clone 5		2	-27 ± 1	-36 ± 3	
clone 6		2	-25 ± 2	-32.8 ± 0.7	
clone 7		2	-24 ± 2	-37.4 ± 0.5	
clone 8	D	2	-24 ± 2	-36 ± 3	21 ± 4
clone 9		2	-23.4 ± 0.4	-34.5 ± 0.5	
clone 10	B	2	-23 ± 2	-31 ± 4	15 ± 2
clone 11		2	-22 ± 2	-32 ± 2	
clone 12	C	1	-20 ± 2	-24.5 ± 0.6	19 ± 4
clone 13	F	2	-20 ± 3	-37 ± 3	60 ± 9
parental onc.	oncocin	2	-19 ± 4	-25 ± 1	25 ± 5
clone 14		1	-16 ± 1	-19.1 ± 0.6	
clone 15		1	-13 ± 5	-22 ± 3	
clone 16		1	-10 ± 5	-25 ± 2	
clone 17		1	-9 ± 2	-23 ± 1	
clone 18		1	-8 ± 1	-11.3 ± 0.5	
parental onc.	oncocin	1	-8 ± 1	-12 ± 2	25 ± 5
clone 19		2	-8 ± 1	-38 ± 4	
clone 20		1	-8 ± 2	-17.8 ± 0.7	
clone 21		1	-7 ± 2	-24.7 ± 0.9	
clone 22		1	-7 ± 2	-4 ± 1	
clone 23		1	-5 ± 1	-9.0 ± 0.6	
clone 24		1	-4.8 ± 0.4	-13.0 ± 0.6	
clone 25		1	-4 ± 1	-15.4 ± 0.5	
clone 26		1	-2 ± 1	-5.0 ± 0.4	
clone 27		1	-1.5 ± 0.4	8 ± 5	
clone 28		1	-1 ± 1	-1.0 ± 0.4	
clone 29		1	-1 ± 1	-3.1 ± 0.5	
clone 30	G (negative)	1	-0.6 ± 0.4	0.8 ± 0.5	>80
clone 31		1	-0.2 ± 0.4	-7.2 ± 0.5	
clone 32		1	0 ± 1	8 ± 4	
clone 33		1	0 ± 1	-24 ± 1	
clone 34		1	0.6 ± 0.2	-27 ± 2	
negative		2	0.6 ± 0.2	0 ± 4	
clone 35		1	0.7 ± 0.2	-21.0 ± 0.6	
negative		1	0.7 ± 0.4	-2 ± 2	
clone 36		1	1.0 ± 1	12 ± 6	

* DNA and peptide sequences in **Supplementary Table 6**

Supplementary Table 2 | Key Resources.

Reagent	Source
Bacterial Strains	
E. coli, MC1061 F-	Lucigen
E. coli, T7 express LysY/I^q	NEB
E. coli, 5-alpha	NEB
Critical commercial assays	
300-bp Paired-end (2x300 PE) MiSeq with v3 chemistry	Illumina
50-bp Paired-end (2x50 PE) HiSeq 2500 with v4 chemistry	Illumina
150-bp Paired-end (2x150 PE) NovaSeq 6000 with SP flow cell	Illumina
Oligonucleotides	
pET oncocin V1M insert: TTAAGAAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGT CCCCGCCCCCCACGTCGCATTTACAACCGCTGATAAGATCCCACCA TCACCATCAT	IDT
pET true negative control insert: TTAAGAAGGAGATATACATAATGTGATAATGATAATGAGATCCCACC ATCACCATCAT	IDT
BbvCI recognition site insert: GCCGCAAGGAATGGTGCATGCCTCAGCCATGCAAGGAGATGGCGC CC	IDT
First-generation one-pot saturation mutagenesis primers, see **Supplementary Table 3**	IDT

Illumina sequencing primers, see **Supplementary Table 4**	IDT
Second-generation library primers, see **Supplementary Table 5**	IDT
Clonal validation library primers, see **Supplementary Tables 6** and **7**	IDT
Peptides	
Peptide validation library, see **Supplementary Table 6**	Genscript

Supplementary Table 3 | First-generation one-pot saturation mutagenesis oligos

Degenerate codon positions	Sequence
2/3	ATAATTTTGTTTAACTTTAAGAAGGAGATATACATAATGNNNNNNCCAC CGTATTTACCACGTCCCCGCCCC
3/4	AGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATAATGGATNNN NNNCCGTATTTACCACGTCCCCGCCCC
4/5	TTTAACTTTAAGAAGGAGATATACATAATGGATAAGNNNNNNTATTTAC CACGTCCCCGCCCCCCACG
5/6	AAGAAGGAGATATACATAATGGATAAGCCANNNNNNTTACCACGTCCCC GCCCCCCACG
6/7	ATACATAATGGATAAGCCACCGNNNNNNCCACGTCCCCGCCCCCCACGT C
7/8	AAGGAGATATACATAATGGATAAGCCACCGTATNNNNNNCGTCCCCGCC CCCCACG
8/9	AGGAGATATACATAATGGATAAGCCACCGTATTTANNNNNNCCCCGCCC CCCACGTCGC
9/10	GGAGATATACATAATGGATAAGCCACCGTATTTACCANNNNNNCGCCCC CCACGTCGCATTT
10/11	GAGATATACATAATGGATAAGCCACCGTATTTACCACGTNNNNNNCCCC CACGTCGCATTTACAA
11/12	GATAAGCCACCGTATTTACCACGTCCCNNNNNNCCACGTCGCATTTACA ACCGCTGATAA

12/13	GCCACCGTATTTACCACGTCCCCGCNNNNNNCGTCGCATTTACAACCGCTGATAAG
13/14	ACCGTATTTACCACGTCCCCGCCCCNNNNNNCGCATTTACAACCGCTGATAAGATC
14/15	CGTATTTACCACGTCCCCGCCCCCCANNNNNNATTTACAACCGCTGATAAGATCCCAC
15/16	CCACGTCCCCGCCCCCCACGTNNNNNNTACAACCGCTGATAAGATCCC
16/17	CGTCCCCGCCCCCCACGTCGCNNNNNNAACCGCTGATAAGATCCCACC
17/18	ACGTCCCCGCCCCCCACGTCGCATTNNNNNNCGCTGATAAGATCCCAC
18/19	CCCCGCCCCCCACGTCGCATTTACNNNNNNTGATAAGATCCCACCATCACCATC
2/4	TTTGTTTAACTTTAAGAAGGAGATATACATAATGNNNAAGNNNCCGTATTTACCACGTCCCCGCCCCCCA
3/5	GTTTAACTTTAAGAAGGAGATATACATAATGGATNNNCCANNNTATTTACCACGTCCCCGCCCCCCAC
4/6	CTTTAAGAAGGAGATATACATAATGGATAAGNNNCCGNNNTTACCACGTCCCCGCCCCCCAC
5/7	TTAACTTTAAGAAGGAGATATACATAATGGATAAGCCANNNTATNNNCCACGTCCCCGCCCCCCACG
6/8	GATATACATAATGGATAAGCCACCGNNNTTANNNCGTCCCCGCCCCCCACGTCGCATTT
7/9	GGAGATATACATAATGGATAAGCCACCGTATNNNCCANNNCCCCGCCCCCCACGTC

8/10	GAAGGAGATATACATAATGGATAAGCCACCGTATTTANNNCGTNNNCGCCCCCCACGTCGCATTT
9/11	GGAGATATACATAATGGATAAGCCACCGTATTTACCANNNCCCNNNCCCCCACGTCGCATTTACA
10/12	AATGGATAAGCCACCGTATTTACCACGTNNNCGCNNNCCACGTCGCATTTACAACCGCTG
11/13	TAAGCCACCGTATTTACCACGTCCCNNNCCCNNNCGTCGCATTTACAACCGCTGATAAG
12/14	GCCACCGTATTTACCACGTCCCCGCNNNCCANNNCGCATTTACAACCGCTGATAAGATC
13/15	CCGTATTTACCACGTCCCCGCCCCNNNCGTNNNATTTACAACCGCTGATAAGATCCC
14/16	TTACCACGTCCCCGCCCCCCANNNCGCNNNTACAACCGCTGATAAGATCCC
15/17	CCACGTCCCCGCCCCCCACGTNNNATTNNNAACCGCTGATAAGATCCCACC
16/18	CGTCCCCGCCCCCCACGTCGCNNNTACNNNCGCTGATAAGATCCCACCATC
17/19	ACGTCCCCGCCCCCCACGTCGCATTNNNAACNNNTGATAAGATCCCACCATCAC

Supplementary Table 4 | Illumina sequencing primers

Name	Sequence
FA_{G1}	TTTCCCTACACGACGCTCTTCCGATCT[1 to 3 N] AGAAGGAGATATACATAATG
RA_{G1}	GTTCAGACGTGTGCTCTTCCGATCT[1 to 3 N]GGTGGGATCTTATCA
FA_{G2}	TTTCCCTACACGACGCTCTTCCGATCT[4 to 8 N] AGAAGGAGATATACATATGGA
RA_{G2}	GTTCAGACGTGTGCTCTTCCGATCT[4 to 8 N]TGGTGGGATCCTCAT
FB	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTT
RB	CAAGCAGAAGACGGCATACGAGAT[Barcode]GTGACTGGAGTTCAGACG TGTGCTCTTCC

Supplementary Table 5 | Second-generation library primers

Primer name	Sequence
$Fw1_{G2}$	ATATACATATGGAMAAAADGKVCYRCCKTCCGCGTCMTCGTYSGC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw2_{G2}$	ATATACATATGGAMAAAADGKVCYRCCKTCCGCGTCMTCGTYWCC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw3_{G2}$	ATATACATATGGAMAAAADGMHSYRCCKTCCGCGTCMTCGTYSGC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw4_{G2}$	ATATACATATGGAMAAACMMKVCYRCCKTCCGCGTCMTCGTYSGC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA

$Fw5_{G2}$	ATATACATATGGAMAAACMMMHSYRCCKTCCGCGTCMTCGTYSGC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw6_{G2}$	ATATACATATGGAMAAACMMKVCYRCCKTCCGCGTCMTCGTYWCC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw7_{G2}$	ATATACATATGGAMAAAADGMHSYRCCKTCCGCGTCMTCGTYWCC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw8_{G2}$	ATATACATATGGAMAAACMMMHSYRCCKTCCGCGTCMTCGTYWCC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw9_{G2}$	ATATACATATGGAMAAAADGKVCGTGCKTCCGCGTCMTCGTYSGC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw10_{G2}$	ATATACATATGGAMAAAADGKVCGTGCKTCCGCGTCMTCGTYWCC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw11_{G2}$	ATATACATATGGAMAAAADGMHSGTGCKTCCGCGTCMTCGTYSGC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw12_{G2}$	ATATACATATGGAMAAACMMKVCGTGCKTCCGCGTCMTCGTYSGC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw13_{G2}$	ATATACATATGGAMAAACMMMHSGTGCKTCCGCGTCMTCGTYSGC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw14_{G2}$	ATATACATATGGAMAAACMMKVCGTGCKTCCGCGTCMTCGTYWCC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw15_{G2}$	ATATACATATGGAMAAAADGMHSGTGCKTCCGCGTCMTCGTYWCC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA
$Fw16_{G2}$	ATATACATATGGAMAAACMMMHSGTGCKTCCGCGTCMTCGTYWCC SAARACGTATTTACAACCGTTAATGAGGATCCCACCA

Fw negative$_{G2}$	ATATACATATGGAMTAAADGTAGYRCTAGCCGTAACMTTAGYSGT AGARATAAATTTAGAACTAGTAATGAGGATCCCACCA
Fw oncocin$_{G2}$	ATATACATATGGACAAACCACCGTACCTTCCGCGTCCTCGTCCGC CAAGACGTATTTACAACCGTTAATGAGGATCCCACCA
Common Rv$_{G2}$	TGGTGGGATCCTCATTAACGGTTGTAAATAC
XbaI extension	TTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATA CATATGGAMAAAC

Supplementary Table 6 | Clonal validation library sequences and primers

DNA name*	Peptide name	Generation	DNA sequence	Peptide sequence	Forward primer*	Reverse primer*
clone 1	A	2	ATGGACAAACCAAACCGCCTTCCGCGTCCTCGTCCGCCAAGACGTATTTACAACCGT	MDKPNRLPRPRPPRRIYNR	2fw4	2rv1
clone 2		2	ATGGACAAAAGGCCGGTGCGTCCGCGTCCTCGTTTCCCAAGACGTATTTACAACCGT	MDKRPVRPRPRFPRRIYNR	2fw14	2rv11
clone 3	E	2	ATGGACAAACCCTCCTACCTTCCGCGTCCTCGTCTCCCAAGACGTATTTACAACCGT	MDKPSYLPRPRLPRRIYNR	2fw11	2rv9
clone 4		2	ATGGACAAACCACCGTGCCGTCCGCGTCCTCGTTGGCCAAGACGTATTTACAACCGT	MDKPPCRPRPRWPRRIYNR	2fw10	2rv8
clone 5		2	ATGGACAAAAGGCCCTACCGTCCGCGTCCTCGTCCGCCAAAACGTATTTACAACCGT	MDKRPYRPRPRPPKRIYNR	2fw6	2rv4
clone 6		2	ATGGACAAACCCCCGTACCGTCCGCGTCCTCGTTGGCGAAGACGTATTTACAACCGT	MDKPPYRPRPRWRRRIYNR	2fw12	2rv10
clone 7		2	ATGGACAAACCATGCCACCGTCCGCGTCCTCGTTACCGAAGACGTATTTACAACCGT	MDKPCHRPRPRYRRRIYNR	2fw9	2rv7
clone 8	D	2	ATGGACAAACCACCCTACCGTCCGCGTCCTCGTTACCGAAAACGTATTTACAACCGT	MDKPPYRPRPRYRKRIYNR	2fw5	2rv3
clone 9		2	ATGGACAAAAGGCCCTACCTTCCGCGTCCTCGTTTCCGAAAACGTATTTACAACCGT	MDKRPYLPRPRFRKRIYNR	2fw8	2rv6
clone 10	B	2	ATGGACAAACACCCGCACCGTCCGCGTCCTCGTTACCGAAGACGTATTTACAACCGT	MDKHPHRPRPRYRRRIYNR	2fw13	2rv7
clone 11		2	ATGGACAAACCCCCGTACCTTCCGCGTCCTCGTCCGCCAAGACGTATTTACAACCGT	MDKPPYLPRPRPPRRIYNR	2fw2	2rv1
clone 12	C	1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCCACGTCGCATTGGGAACAGA	MDKPPYLPRPRPPRRIGNR	1fw19	1rv11
clone 13	F	2	ATGGACAAACCAACCCGCCTTCCGCGTCCTCGTCGGCCAAAACGTATTTACAACCGT	MDKPTRLPRPRRPKRIYNR	2fw3	2rv2
parental onc.	oncocin	2	ATGGACAAACCACCGTACCTTCCGCGTCCTCGTCCGCCAAGACGTATTTACAACCGT	MDKPPYLPRPRPPRRIYNR	2fw1	2rv1
clone 14		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCCACGGAGGATTTACAACCGC	MDKPPYLPRPRPPRRIYNR	1fw11	1rv6
clone 15		1	ATGGATAAGCCACCGTATTTACCACGGCCCCGGCCCCCCACGTCGCATTTACAACCGC	MDKPPYLPRPRPPRRIYNR	1fw17	1rv1
clone 16		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCAGACGTCGTCGCATTTACAACCGC	MDKPPYLPRPRRRRRIYNR	1fw24	1rv4
clone 17		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCAATAGGCGTCGCATTTACAACCGC	MDKPPYLPRPRNRRRIYNR	1fw22	1rv8
clone 18		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCCGCCACGTCGCATTTACAACCGC	MDKPPYLPRPRPPRRIYNR	1fw7	1rv1
parental onc.	oncocin	1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCCACGTCGCATTTACAACCGC	MDKPPYLPRPRPPRRIYNR	1fw16	1rv1
clone 19		2	ATGGAAAAACCCACCCGCCTTCCGCGTCCTCGTCACCGAAAACGTATTTACAACCGT	MEKPTRLPRPRHRKRIYNR	2fw7	2rv5
clone 20		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCCACGTCGCCCTCGTAACCGC	MDKPPYLPRPRPPRPRNR	1fw10	1rv5
clone 21		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCGGGTGCGTCGCATTTACAACCGC	MDKPPYLPRPRRVRRIYNR	1fw20	1rv8
clone 22		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCCTCCAAGACGCATTTACAACCGC	MDKPPYLPRPRPPRRIYNR	1fw18	1rv10
clone 23		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCCACGTCGCATTTACACCCGC	MDKPPYLPRPRPPRRIYTR	1fw6	1rv3
clone 24		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCTTTGTTCGTCGCATTTACAACCGC	MDKPPYLPRPRFVRRIYNR	1fw8	1rv4
clone 25		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCATGAAACGTCGCATTTACAACCGC	MDKPPYLPRPRMKRRIYNR	1fw9	1rv1
clone 26		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCTTACCAGGGCGCATTTACAACCGC	MDKPPYLPRPRLPGRIYNR	1fw4	1rv2
clone 27		1	ATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCCATTATGGATTTACAACCGC	MDKPPYLPRPRPPLWIYNR	1fw15	1rv9
clone 28		1	ATGGAGGTTCCACCGTATTTACCACGTCCCCGCCCCCCACGTCGCATTTACAACCGC	MEVPPYLPRPRPPRRIYNR	1fw2	1rv1
clone 29		1	ATGGATAAGCCACCGTATTTACCATCACCCATTCCCCCACGTCGCATTTACAACCGC	MDKPPYLPSPIPPRRIYNR	1fw3	1rv1
clone 30	G (negative)	1	ATGGGCGAGCCACCGTATTTACCACGTCCCCGCCCCCCACGTCGCATTTACAACCGC	MGEPPYLPRPRPPRRIYNR	1fw1	1rv1
clone 31		1	ATGGATAAGCCACCGTATTTTCCACGTCCCCGCCCCCCCACGTCGCATTTACAACCGC	MDKPPYFPRPRPPRRIYNR	1fw5	1rv1
clone 32		1	ATGGATAAGCCACCGTATTTACCACGTCCCGTGCCCGTGCGTCGCATTTACAACCGC	MDKPPYLPRPVPVRRIYNR	1fw14	1rv8
clone 33		1	ATGGATAAGCCACCGTATTTACCACGTACAATACCCCCACGTCGCATTTACAACCGC	MDKPPYLPRTIPPRRIYNR	1fw21	1rv1
clone 34		1	ATGGATAAGCCACCGTATGGTCCAGCTCCCCGCCCCCCCATTGTGGATTTACAACCGC	MDKPPYGPAPRPPLWIYNR	1fw23	1rv12
negative		2	ATGGAMTAGADGTAGYRCTAGCCGTAGCMTTAGYSGTAGARATAGATTTAGAACTAG	M•	see table _	see table _
clone 35		1	ATGGATAAGCCATGTTATCTTCCACGTCCCCGCCCCCCAGTATGGATTTACAACCGC	MDKPCYLPRPRPPVWIYNR	1fw12	1rv7
negative		1	ATGTGATAATGATAATGA	M	see table _	see table _
clone 36		1	ATGGATAAGCCACCGTATTTACCAGTTTTCCGCCCCCCACGTCGCATTTACAACCGC	MDKPPYLPVFRPPRRIYNR	1fw13	1rv1

* Primer sequences in **Supplementary Table 7**

Supplementary Table 7 | Clonal validation library primers

Primer name	Sequence
1fw1	AAGGAGATATACATAATGGGCGAGCCACCGTATTTACCACGTCCCCGCCCCCCACGTCGCATTTACAACC
1fw2	AAGGAGATATACATAATGGAGGTTCCACCGTATTTACCACGTCCCCGCCCCCCACGTCGCATTTACAACC
1fw3	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCATCACCCATTCCCCCACGTCGCATTTACAACC
1fw4	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCTTACCAGGGCGCATTTACAACC
1fw5	AAGGAGATATACATAATGGATAAGCCACCGTATTTTCCACGTCCCCGCCCCCCACGTCGCATTTACAACC
1fw6	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCACGTCGCATTTACACCC
1fw7	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCCGCCACGTCGCATTTACAACC
1fw8	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCTTTGTTCGTCGCATTTACAACC
1fw9	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCATGAAACGTCGCATTTACAACC
1fw10	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCACGTCGCCCTCGTAACC
1fw11	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCCACGGAGGATTTACAACC
1fw12	AAGGAGATATACATAATGGATAAGCCATGTTATCTTCCACGTCCCCGCCCCCCAGTATGGATTTACAACCGCTG
1fw13	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCAGTTTTCCGCCCCCCACGTCGCATTTACAACC
1fw14	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCGTGCCCGTGCGTCGCATTTACAACC
1fw15	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCCATTATGGATTTACAACCGCTG
1fw16	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCACGTCGCATTTACAACC
1fw17	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGGCCCCGGCCCCCACGTCGCATTTACAACC
1fw18	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCCTCCAAGACGCATTTACAACC
1fw19	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCCCCCACGTCGCATTGGGAACA
1fw20	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCCGGGTGCGTCGCATTTACAACC
1fw21	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTACAATACCCCCACGTCGCATTTACAACC
1fw22	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCAATAGGCGTCGCATTTACAACC
1fw23	AAGGAGATATACATAATGGATAAGCCACCGTATGGTCCAGCTCCCCGCCCCCCATTGTGGATTTACAACCGCTG
1fw24	AAGGAGATATACATAATGGATAAGCCACCGTATTTACCACGTCCCCGCAGACGTCGTCGCATTTACAACC
1rv1	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATGCGACGT
1rv2	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATGCGCCCT
1rv3	GGTGATGGTGGGATCTTATCAGCGGGTGTAAATGCGACGT
1rv4	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATGCGACGA
1rv5	GGTGATGGTGGGATCTTATCAGCGGTTACGAGGGCGACGT
1rv6	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATCCTCCGT
1rv7	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATCCATACT
1rv8	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATGCGACGC
1rv9	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATCCATAAT
1rv10	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATGCGTCTT
1rv11	GGTGATGGTGGGATCTTATCATCTGTTCCCAATGCGACGT
1rv12	GGTGATGGTGGGATCTTATCAGCGGTTGTAAATCCACAAT
2fw1	AAGGAGATATACATATGGACAAACCACCGTACCTTCCGCGTCCTCGTCCGCCAAGACGTA
2fw2	AAGGAGATATACATATGGACAAACCCCCGTACCTTCCGCGTCCTCGTCCGCCAAGACGTA
2fw3	AAGGAGATATACATATGGACAAACCAACCCGCCTTCCGCGTCCTCGTCGGCCAAAACGTA
2fw4	AAGGAGATATACATATGGACAAACCAAACCGCCTTCCGCGTCCTCGTCCGCCAAGACGTA
2fw5	AAGGAGATATACATATGGACAAACCACCCTACCGTCCGCGTCCTCGTTACCGAAAACGTA
2fw6	AAGGAGATATACATATGGACAAAAGGCCCTACCGTCCGCGTCCTCGTCCGCCAAAACGTA
2fw7	AAGGAGATATACATATGGAAAAACCCACCCGCCTTCCGCGTCCTCGTCACCGAAAACGTA
2fw8	AAGGAGATATACATATGGACAAAAGGCCCTACCTTCCGCGTCCTCGTTTCCGAAAACGTA
2fw9	AAGGAGATATACATATGGACAAACCATGCCACCGTCCGCGTCCTCGTTACCGAAGACGTA
2fw10	AAGGAGATATACATATGGACAAACCACCGTGCCGTCCGCGTCCTCGTTGGCCAAGACGTA
2fw11	AAGGAGATATACATATGGACAAACCCTCCTACCTTCCGCGTCCTCGTCTCCCAAGACGTA
2fw12	AAGGAGATATACATATGGACAAACCCCCGTACCGTCCGCGTCCTCGTTGGCGAAGACGTA
2fw13	AAGGAGATATACATATGGACAAACACCCGCACCGTCCGCGTCCTCGTTACCGAAGACGTA
2fw14	AAGGAGATATACATATGGACAAAAGGCCGGTGCGTCCGCGTCCTCGTTTCCCAAGACGTA
2rv1	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTCTTGGCGGACGAG
2rv2	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTTTTGGCCGACGAG
2rv3	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTTTTCGGTAACGAG
2rv4	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTTTTGGCGGACGAG
2rv5	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTTTTCGGTGACGAG
2rv6	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTTTTCGGAAACGAG
2rv7	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTCTTCGGTAACGAG
2rv8	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTCTTGGCCAACGAG
2rv9	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTCTTGGGAGACGAG
2rv10	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTCTTCGCCAACGAG
2rv11	GTGATGGTGGGATCCTCATTAACGGTTGTAAATACGTCTTGGGAAACGAG