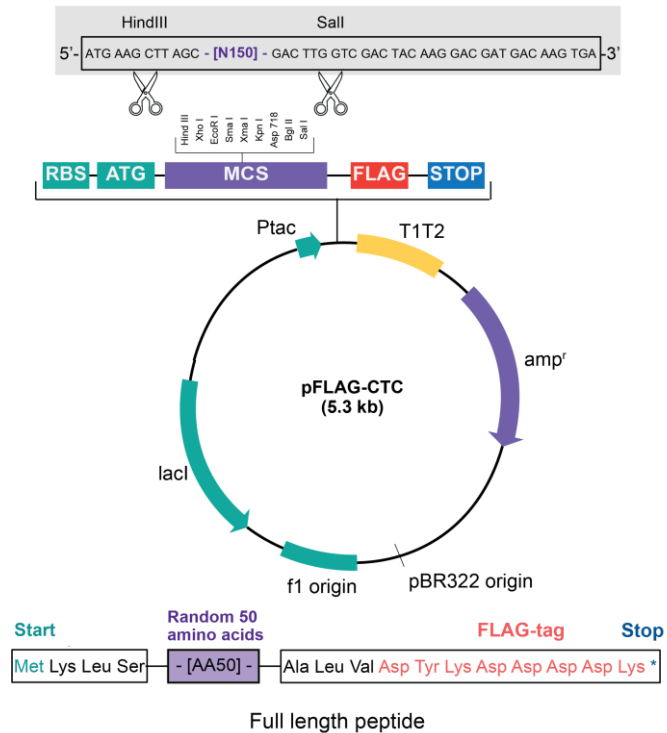


Supplementary files

Supplementary file S1

Expression vector and insert design. The IPTG inducible expression vector pFLAG-CTC was used to clone random sequence ORF inserts into the multiple cloning site (MCS) between the HindIII and SalI sites, as shown on top. The scheme of the full-length peptide product expressed upon induction is shown at the bottom.



Supplementary file S2

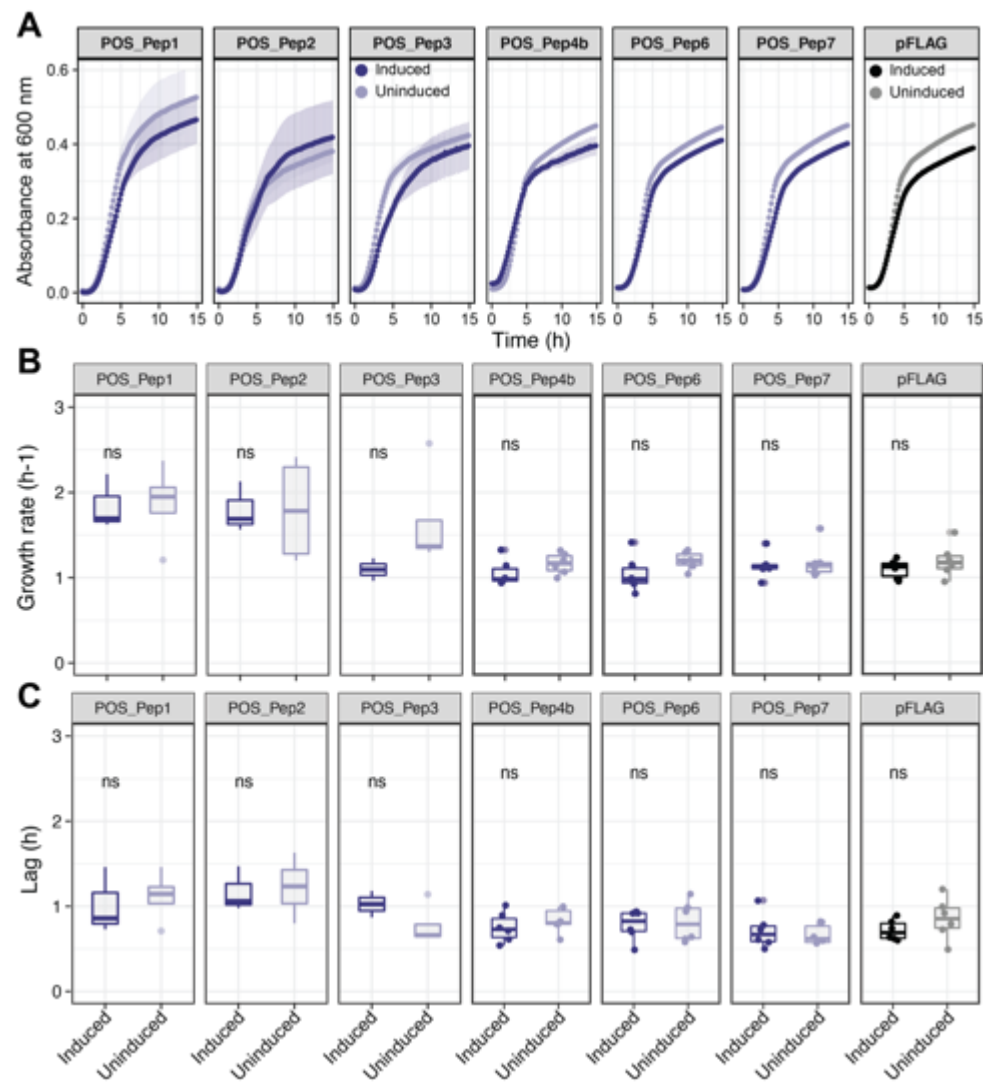
Sequences of clones in this study: coding parts of the inserts are shown with their translation products. Constant parts derived from the vector sequences are shaded in grey.

	1	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
1. NEG_Pep1	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2. NEG_Pep1_Stop	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3. NEG_Pep2	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4. NEG_Pep2_Stop	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5. NEG_Pep3	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
6. NEG_Pep3_Stop	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
7. NEG_Pep4	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
8. NEG_Pep4_Stop	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9. NEG_Pep5	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10. NEG_Pep5_Stop	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
11. NEG_Pep6	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12. NEG_Pep6_Stop	ATG	CTT	AGC	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

S2A: NEG clones and their STOP codon variants.

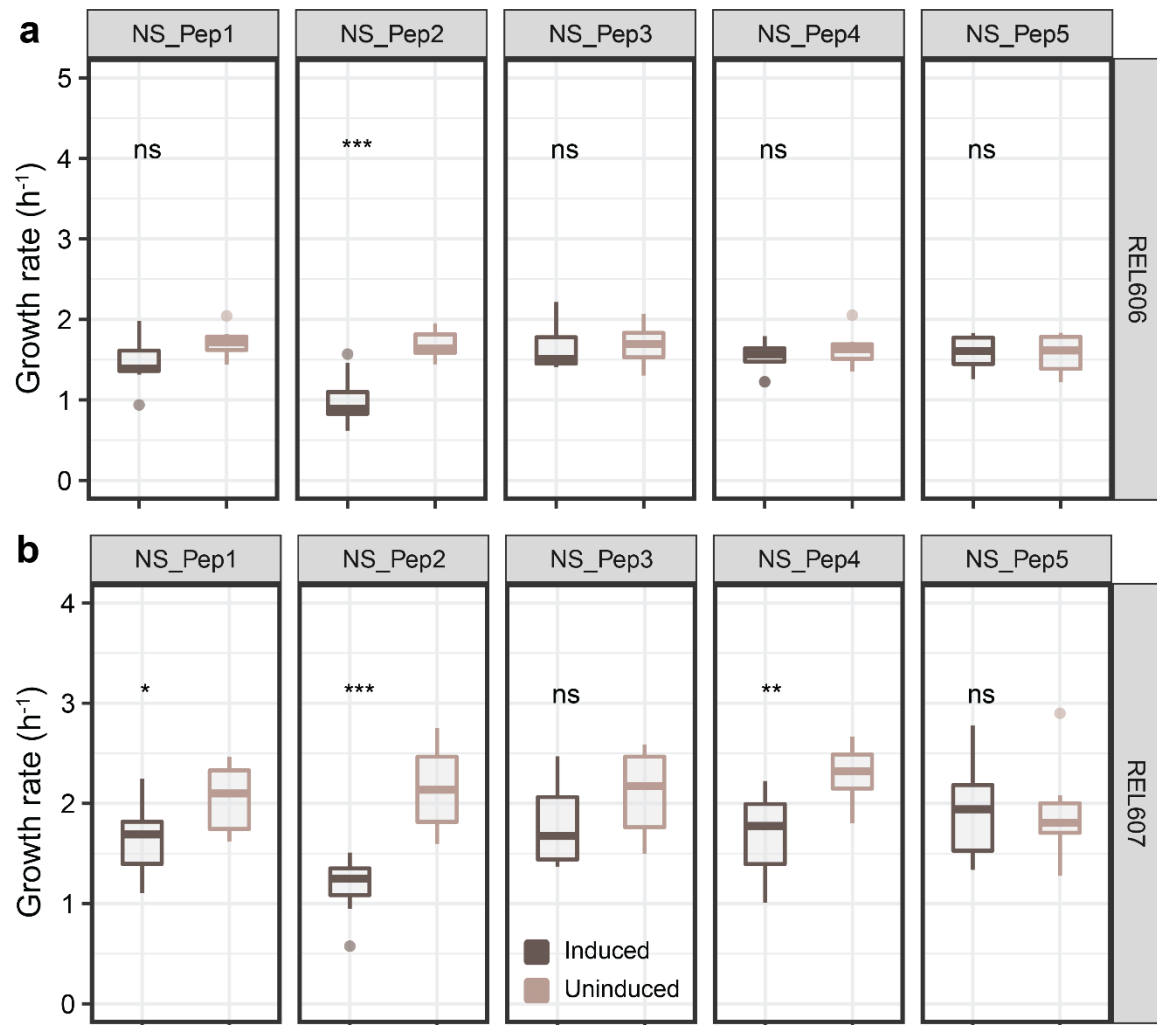
Growth rate comparisons for NEG peptide STOP clones. A) Growth curves without IPTG induction are depicted as light colours and with IPTG induction are in dark colours. OD600 was recorded every 10 min with at least five replicates for each clone and condition. Dots represent means and whiskers, which show the standard error of the mean (SEM) across replicates. B) Growth rate comparisons between the six clones after the lag. C) Lag time comparisons between the six NEG STOP clones, measured as the time from the start of the experiment until the start of the exponential growth. Student's t-test was performed with p-values as follows: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ns = $P > 0.05$.

Supplementary file S4



Growth rate comparisons for POS peptide clones compared to empty vector. A) Growth curves without IPTG induction are depicted as light colours and with IPTG induction are in dark colours. OD600 was recorded every 10 min with at least five replicates for each clone and condition. Dots represent means and whiskers, which show the standard error of the mean (SEM) across replicates. B) Growth rate comparisons between the six clones. C) Lag time comparisons between the six clones, measured as the time from the start of the experiment until the start of the exponential growth. Student's t-test was performed with p-values as follows: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ns = $P > 0.05$.

Supplementary file S5



Growth rate comparisons for NS peptide clones in two different backgrounds. A) Growth rate comparisons between the five clones in REL606. B) Growth rate comparisons between the five clones in REL607. Student's t-test was performed with p-values as follows: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ns = $P > 0.05$.

Supplementary Table 1

Primer sequences used in this study

Name	Primer	Sequence
NEG_Pep1	Forward	ATGAAGCTTAGCGTTGGGAAACCGGATACATGGC
NEG_Pep1	Reverse	GTAGTCGACCAATGCCTCGAAGGCCCAAAGGTC
NEG_Pep1_STOP	Forward	ATGAAGCTT TAG GTTGGG TAA CCGG
NEG_Pep1_STOP	Reverse	GTAGTCGACCAATGCCTCGAAGG
NEG_Pep2	Forward	ATGAAGCTTAGCGCGGCTACCTGGGTCGCGAGTC
NEG_Pep2	Reverse	GTAGTCGACCAATGCATCTCGCGTACGCCTGTGG
NEG_Pep2_STOP	Forward	ATGAAGCTT TAG GCGGCT TAA TGGGT
NEG_Pep2_STOP	Reverse	GTAGTCGACCAATGCATCTCGCGTA

NEG_Pep3	Forward	ATGAAGCTTAGCTGTCCATTTCCGGATACCCATG
NEG_Pep3	Reverse	GTAGTCGACCAATGCACACACCCAGAAGACGTGC
NEG_Pep3_STOP	Forward	ATGAAGCTT TAG TGTCCA TA ACCGGATACCC
NEG_Pep3_STOP	Reverse	GTAGTCGACCAATGCACACACCC
NEG_Pep4	Forward	ATGAAGCTTAGCGTGTATATTCTTACGGTCCAGT
NEG_Pep4	Reverse	GTAGTCGACCAATGCCAGCGTGTTAGCCCGACGC
NEG_Pep4_STOP	Forward	ATGGAAGCTT TAG GTGTAT TA ACTTACGGTCCAGT
NEG_Pep4_STOP	Reverse	GTAGTCGACCAATGCCAGCGTG
NEG_Pep5	Forward	ATGAAGCTTAGCTCAGTTTGCATCCTTGTCTGG
NEG_Pep5	Reverse	GTAGTCGACCAATGCCCCGAGAGGGCTTGCGCTCT
NEG_Pep5_STOP	Forward	ATGAAGCTT TAG TCAGTT TA AATCCTTGTCTGG
NEG_Pep5_STOP	Reverse	GTAGTCGACCAATGCCCCGAGAGG
NEG_Pep6	Forward	ATGAAGCTTAGCAAAGTAGTTTATCGTCGCGCAG
NEG_Pep6	Reverse	GTAGTCGACCAATGCCGAGGTTACACAGACACTG
NEG_Pep6_STOP	Forward	ATGAAGCTT TAG AAAGTA TA AATATCGTCGCGC
NEG_Pep6_STOP	Reverse	GTAGTCGACCAATGCCGAGGTTACA
POS_Pep1	Forward	ATGAAGCTTAGCCGCGGTATTCACCTAGGTCGGA
POS_Pep1	Reverse	GTAGTCGACCAATGCGTCCAAAACCCAGTGT
POS_Pep2	Forward	ATGAAGCTTAGCTACTGGAATAGCTCTATGGCGT
POS_Pep2	Reverse	GTAGTCGACCAATGCGTCGGTATCAAACCGT
POS_Pep3	Forward	ATGAAGCTTAGCCCCGTCTCCTGGATTACGGTG
POS_Pep3	Reverse	GTAGTCGACCAATGCATAGCTTACCCCAGGC
POS_Pep4b	Forward	ATGAAGCTTAGCGTCATGCGTCCCATATCTCG
POS_Pep4b	Reverse	TCATCGTCCTTGTAGTCGACCAATGCTTAGCG
POS_Pep6	Forward	ATGAAGCTTAGCGAAGGTGGCCGCCGAT
POS_Pep6	Reverse	CATCGTCCTTGTAGTCGACCAATGCGGTACG
POS_Pep7	Forward	ATGAAGCTTAGCGTCAAATTTGCAAGGGTTGGGGTC
POS_Pep7	Reverse	TCATCGTCCTTGTAGTCGACCAATGCACCTC
Outer_pFLAG	Forward	GCATAATTCGTGTCGCTCAA
Outer_pFLAG	Reverse	AAAAGGGAATAAGGGCGACA