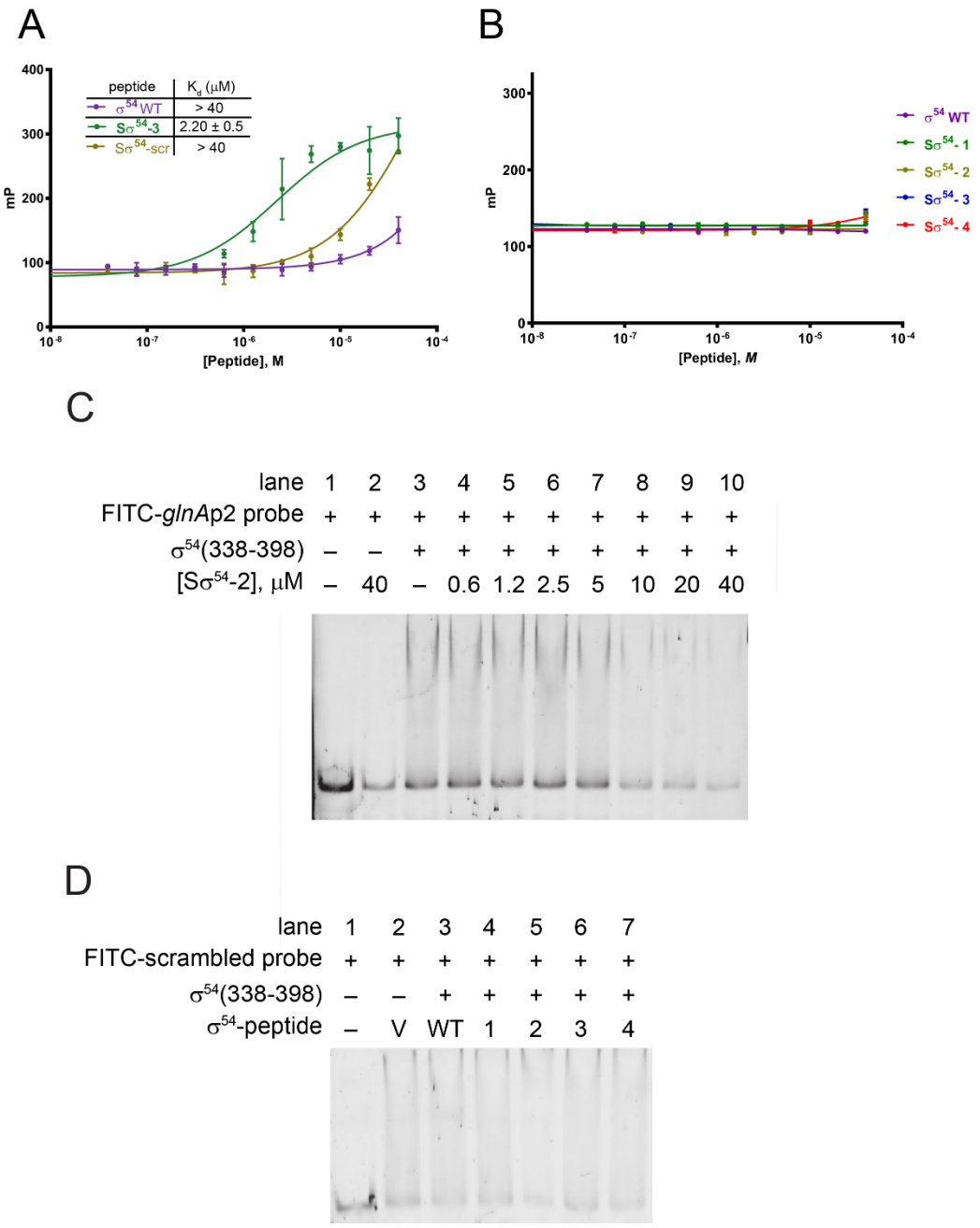
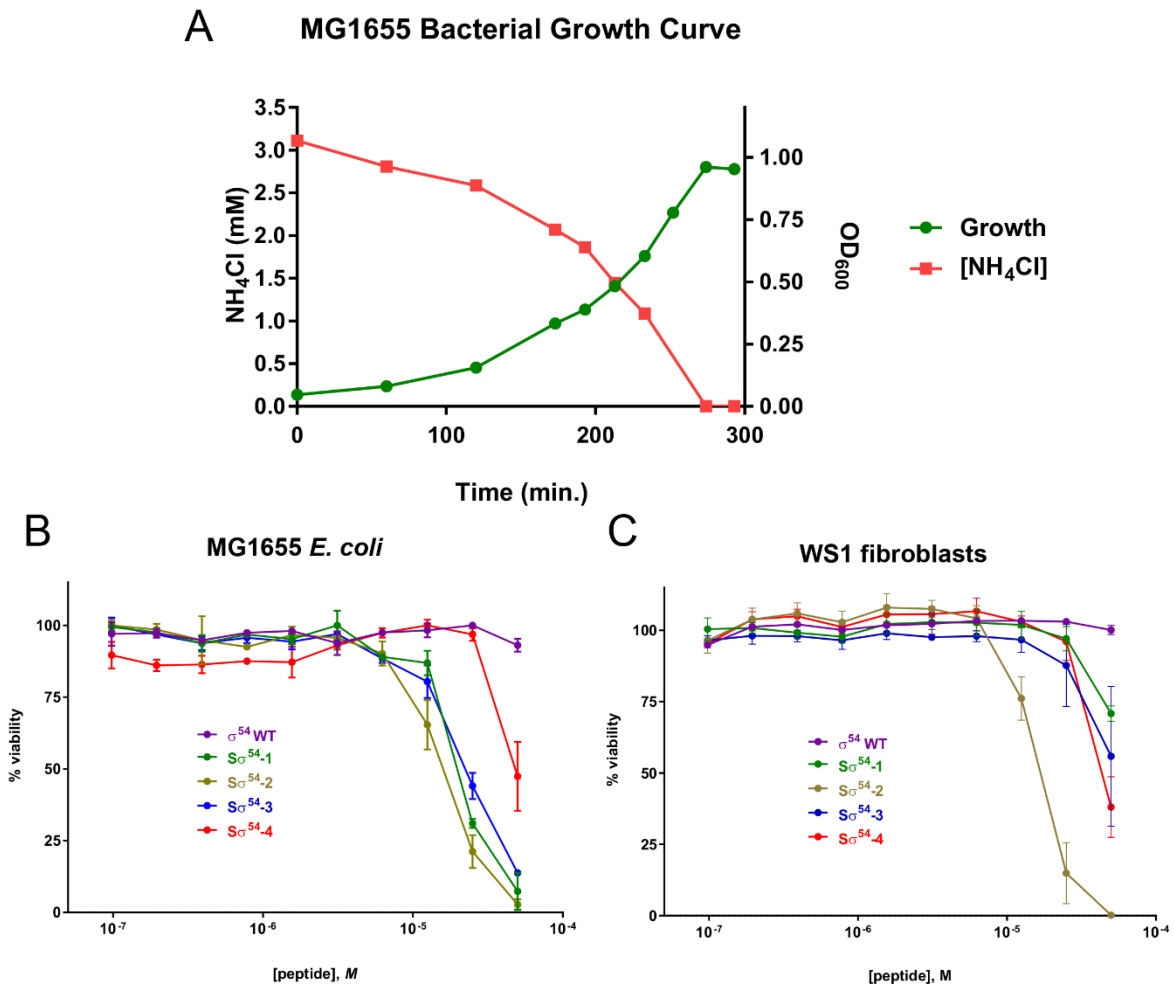
Supplemental Information
Supplemental Figures

Supplemental Figure S1. Additional binding experiments.



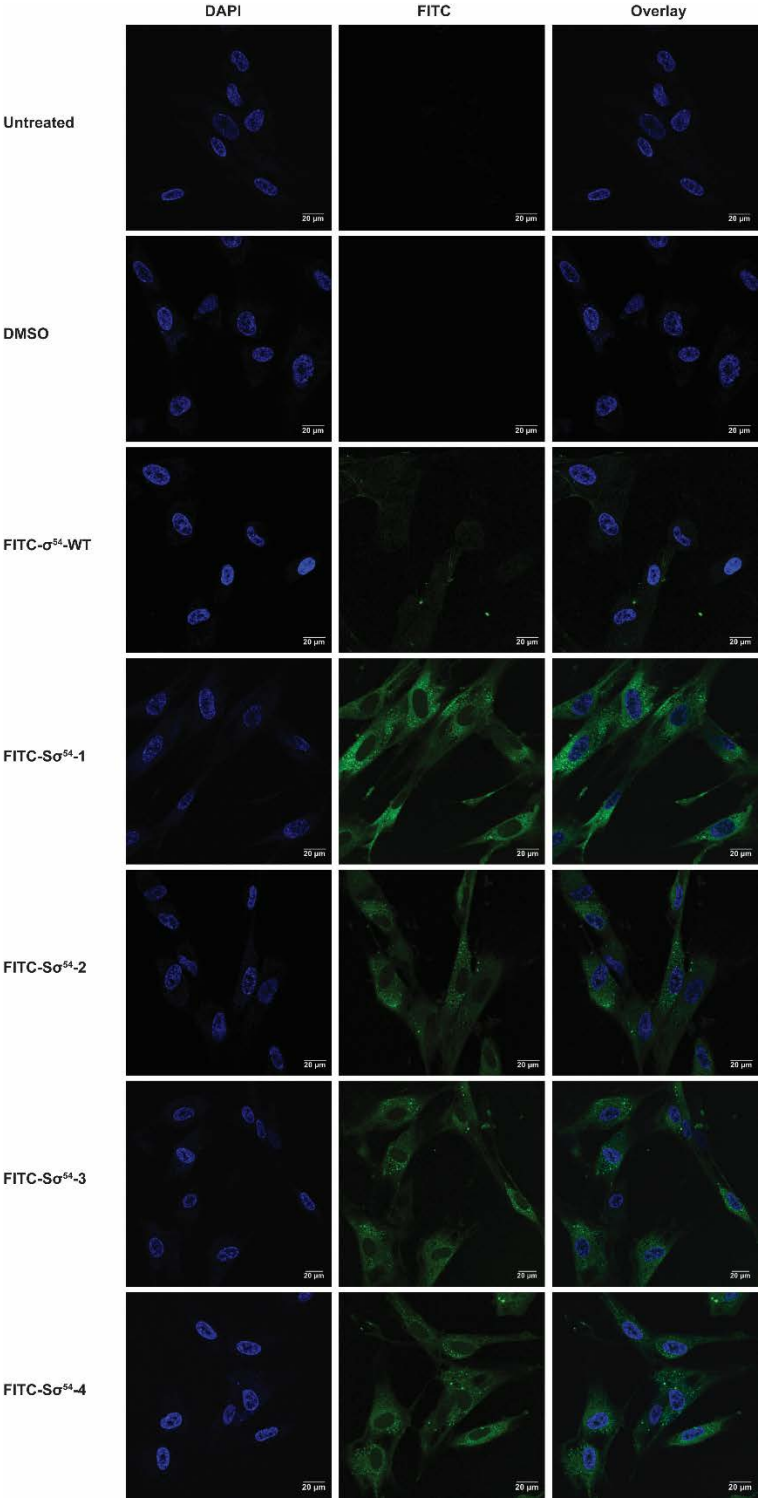
Supplemental Figure S1, related to Figure 2A. (A) Binding of scrambled peptide to *glnA*p2 DNA. Fluorescence polarization curves and accompanying dissociation constants of Sσ^{54} scrambled with FITC *glnA*p2 30mer. **(B)** Binding of Sσ^{54} peptides to scrambled *glnA*p2 DNA. **(C)** EMSA showing dose-responsive binding of Sσ^{54}-2 to *glnA*p2 DNA with displacement of σ^{54}(338-398). **(D)** EMSA showing lack of Sσ^{54} peptide binding to scrambled FITC-*glnA*p2 DNA.

Supplemental Figure S2. Growth conditions and effects of nitrogen depletion and peptide treatment on *E. coli*.



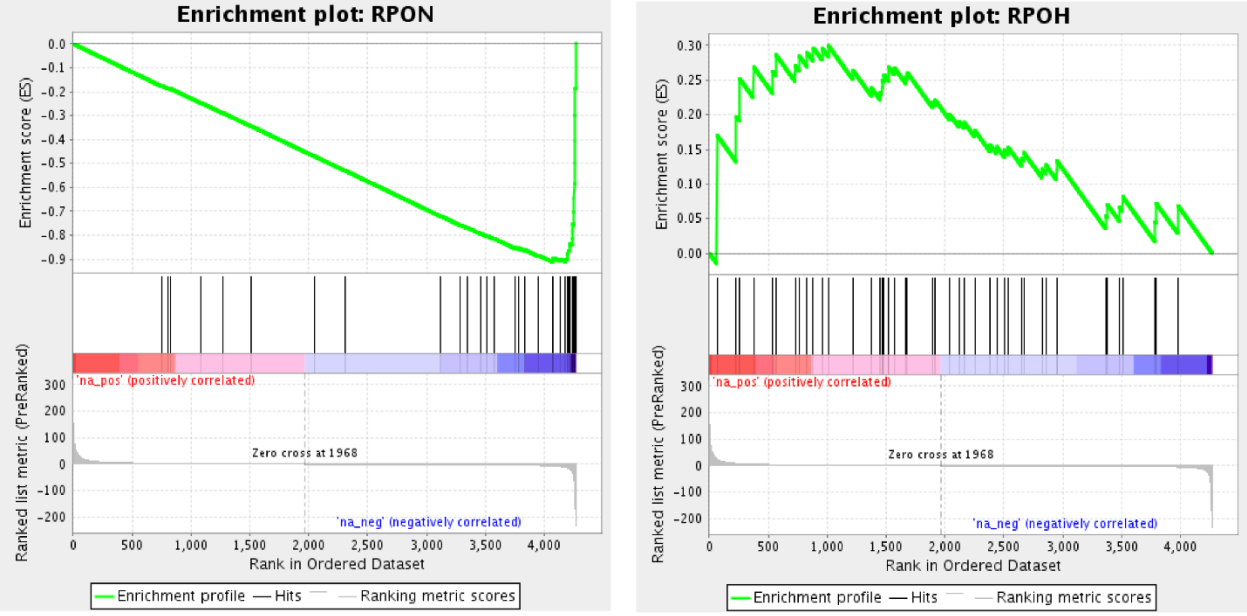
Supplemental Figure S2, related to Figure 4 and STAR Methods. (A) Gradual depletion of nitrogen in Gutnick minimal media. Quantification of ammonia concentration during growth of MG1655 *E. coli* in Gutnick minimal media with 3 mM NH_4Cl. **(B)** Viability of WS1 fibroblasts after 24 h treatment in Opti-MEM. **(C)** Viability of MG1655 *E. coli* after 1 h treatment in LB media.

Supplemental Figure S3. Confocal microscopy of WS1 human fibroblasts exposed to FITC-σ^{54} peptides.



Supplemental Figure S3, related to STAR methods. Confocal fluorescence microscopy of WS1 fibroblasts treated with FITC-σ^{54} peptides. Scale bars represent 20 μm.

Supplemental Figure S4. Gene set enrichment analysis plots.



Supplemental Figure S4. Gene set enrichment plots, related to main figure 4C and STAR Methods.. (left) RpoN regulon, **(right)** RpoH regulon.

Supplemental Tables

Supplemental Table S1. Characterization of peptides used in this study. Related to Figure 1.

Peptide	Sequence[1]	ESI calcd[2]	ESI found	^{t}R[3]
Ac-WT σ^{54}	Ac-βAla-FKVARRTVAKYREML-NH_2	990.5	990.9	8.25 min
Ac-Sσ^{54}-1	Ac-βAla-FKVARRTZAKYREBX-NH_2	1021.6	1021.7	12.09 min
Ac-Sσ^{54}-2	Ac-βAla-FKVXRRTXAKYREBL-NH_2	1021.6	1021.9	12.62 min
Ac-Sσ^{54}-3	Ac-βAla-ZKVARRTXAKYREBL-NH_2	1004.6	1004.6	12.14 min
Ac-Sσ^{54}-4	Ac-βAla-XKVAXRTVAKYREBL-NH_2	955.0	955.4	13.88 min
Ac-Sσ^{54}-scr	Ac-βAla-RVLXEFKXRBTARYK-NH_2	1021.6	1022.0	10.06 min

[1] Non-proteogenic amino acids: B = norleucine, Z = (*R*)-2-(7'-octenyl)-alanine, X = (*S*)-2-(4'-pentenyl)-alanine.
[2] ESI/MS calculated and found for $([M+2H]/2)^{+}$.
[3] C-18 reverse phase HPLC retention time (^{t}R) for peptides run on a 40-minute gradient of 15-95% acetonitrile/water with 0.1% formic acid.

Supplemental Table S2. DNA oligos and primers used in this study. Related to Figure 2 and Figure 4.

Name	Sequence (5' -> 3')
FITC *glnA*P2 30mer Forward	/5FluorT/AGTTGGCACAGATTTCGCTTTATCTTTTTT
FITC *glnA*P2 30mer Reverse	/5FluorT/AAAAAAGATAAAGCGAAATCTGTGCCAACT
FITC *glnAP*2 30mer-scrambled Forward	/5FluorT/GTGCTATTATTCCGTTATCGTTGTTACTAT
FITC *gln*AP2 30mer-scrambled Reverse	/5FluorT/ATAGTAACAACGATAACGGAATAATAGCAC
6FAM-*glnA*p2 218mer TOP	/56-FAM/AGCGCAAATCAACAAACTTCACTTCGTG
6FAM-*glnA*p2 218mer BOTTOM	/56-FAM/AGCCCTTTTGCACGATGGTGC
*glnA*p2 218mer TOP	AGCGCAAATCAACAAACTTCACTTCGTG
*glnA*p2 218mer BOTTOM	AGCCCTTTTGCACGATGGTGC
cysG 3 Forward	ATTCCGTTCTCGGTGGTTCC
cysG 3 Reverse	CCAGCGTCTGTTTTTCTGCC
yeaG 1 Forward	AGGTCTGGCCGTCAATTCTG
yeaG 1 Reverse	CATTCTGCGCGTAGTGTTCG
pspA 10 Forward	GCAGGAAAAAGCCGAACTGG
pspA 10 Reverse	CTTTCTTCATGCGTGCCAGC
nac 3 Forward	CTGGATACACCAGCCACAGG
nac 3 Reverse	GGCATTATCGGGGCAAGTCT
glnA 4 Forward	TACGGATAGACGCAGAACGG
glnA 4 Reverse	AAACGTGAGTTCTGGGGGTG