Supplementary Material: Evolving E3 ligase towards recognising novel substrates for targeted protein degradation

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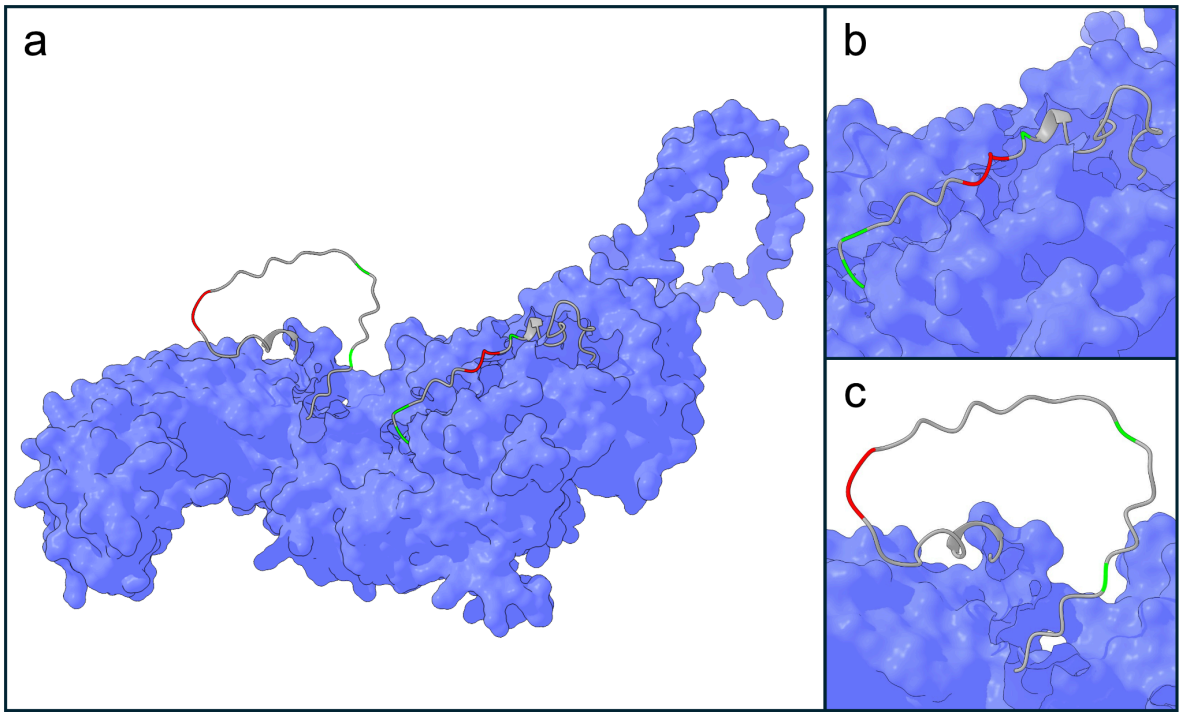
Supplementary Result 1: NLRP3 fragments

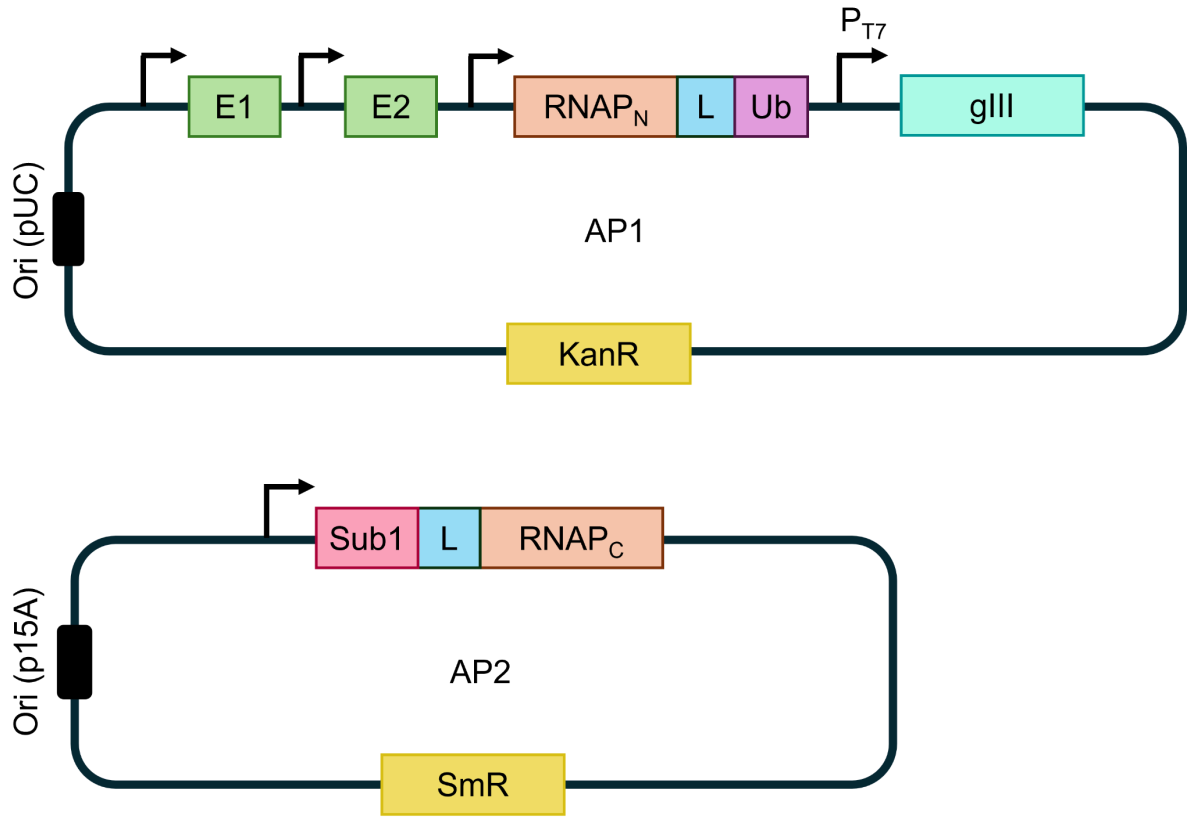
To minimise interference from NLRP3's size in the evolutionary system, peptide fragments containing the VXP motif, surrounding residues, and necessary lysines near the degron were designed. Designed NLRP3 fragments (underlined bases indicate possible VXP motifs): 191-KTKTCESP<u>VSP</u>IKMELLFDPDDEHSEPVH-220 and 684-LHNMPKEEEEEEKEGRHLDMVQC<u>VLP</u>SSSHAACSHG-719.

Supplementary Result 2: Disrupting the SIAH1/2 degron sequence

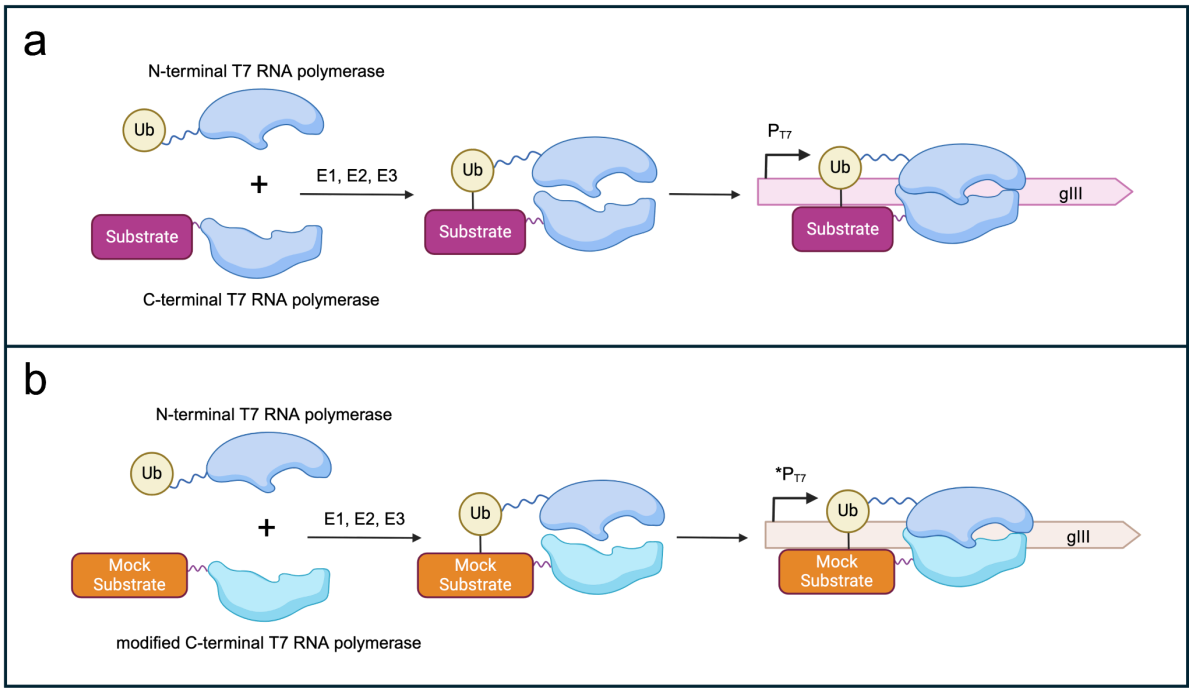
The native degron sequence of EGLN3 is FIADVEP. First, we mutated the Val residue at position 5 to Trp (V > W), expecting its bulky side chain to cause steric hindrance within SIAH's binding cleft. In parallel, we also mutated Pro residue at position 7 to Ala (P > A). Additionally, we modified degron positions 1 and 3 to resemble the native degron sequence of NLRP3 to prove that SIAH activity could be further optimised to recognise our final target. NLRP3 contains two VXP motifs: CESPVSP, and MVQCVLP. Since the second motif (MVQCVLP) is closer to a natural ubiquitination site, we introduced single amino acid substitutions in the native degron sequence of EGLN3 at position 1 (F > M), position 3 (A > Q), or both (F > M and A > Q) while keeping the VXP motif intact.



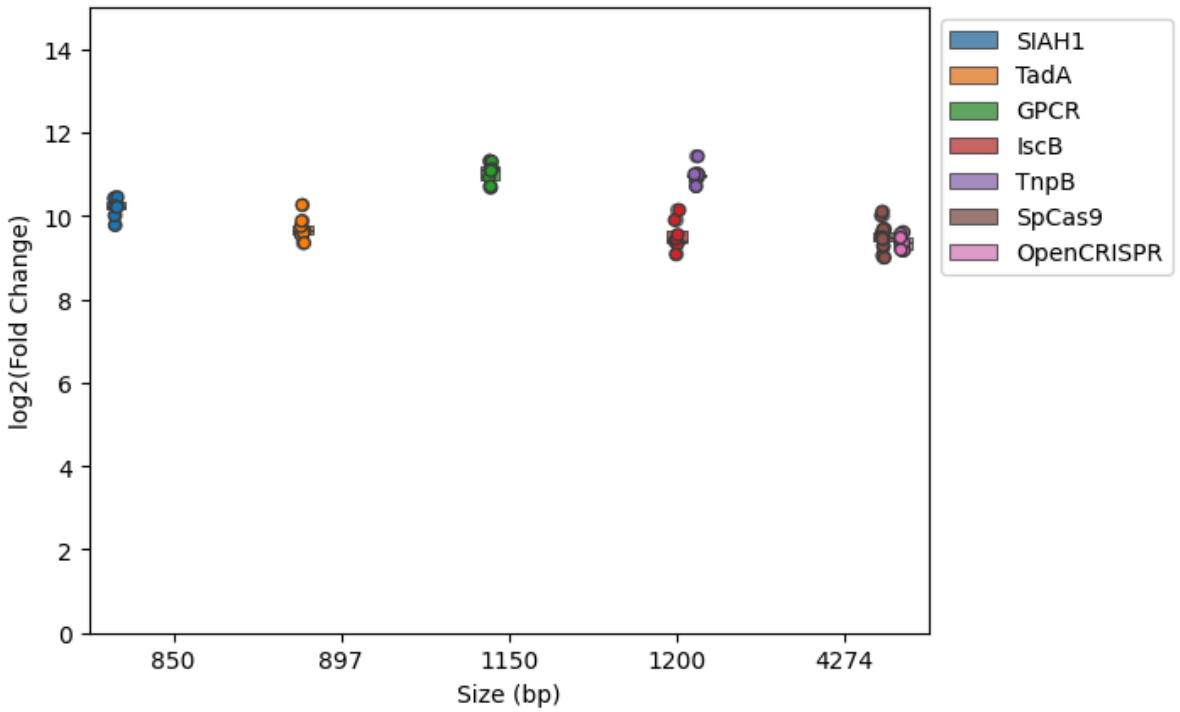
Supplementary Figure 1: NLRP3 structure prediction by AlphaFold (AlphaFold protein structure database: AF-Q96P20-F1-v4). (a) NLRP3 contains two VXP motifs: **(b)** 200-VSP-203 and **(c)** 707-VLP-710, shown in red. Proximal lysins exist near the VXP degrons (green). Two short fragments, containing the VXP motif and surrounding lysins, were chosen as peptide substrates from NLRP3 for the evolutionary system (grey).



Supplementary Figure 2: **Plasmid map of AP1 and AP2.** The plasmid maps illustrate the general structure of AP1 and AP2. **AP1** is based on the pTU2 backbone from the EcoFlex kit, which contains a pUC origin of replication (ori) for high-copy plasmid and a kanamycin resistance gene for selection. It encodes the following elements: E1, E2 and N-terminal RNAP linked to ubiquitin. The *gIII* gene is placed under the control of a T7 promoter. **AP2** is also derived from the pTU2 backbone but utilises a p15A ori for moderate-copy replication and carries a spectinomycin resistance gene for selection. It encodes the substrate fused to RNAP. The specific substrate, linker and promoter employed vary according to the experimental design.



Supplementary Figure 3: Selection logic for SIAH1/2-dependent *gIII* expression. (a) Split T7 RNAP subunits fused to ubiquitin or a canonical substrate of SIAH1/2. The presence of E1, E2 and E3 (SIAH1/2) should lead to the assembly of the T7 RNAP subunits and thereby *gIII* transcription under the control of a T7 promoter. **(b)** Potential off-target effects of the evolved SIAH1/2 could be selected against by punishing spurious ubiquitination of a mock substrate by E3 ligase. In a new AP1neg plasmid, a mutated version of the C-term RNAP subunit that recognises a modified T7 promoter sequence [1] is fused to a mock substrate. A non-functional *gIII* (here, mock *gIII*) is placed under the control of the modified T7 promoter (Supplementary Table 3). Recognition and subsequent ubiquitination of the mock substrate by the evolved E3 ligase leads to the expression of mock *gIII*. Consequently, the phage offspring are not able to propagate further. Figure created with BioRender.com.



Supplementary Figure 4: **Overnight propagation of phages of different genome sizes in S2060 containing all components of the system and strong constitutive expression (Strain: 1076-08-00).** Phages used in various independent PACE experiments that lacked E3 ligases were tested on our strain and showed propagation efficiency comparable to SIAH1 phages. Overnight propagation appears to be independent of phage genome size, possibly due to the toxicity of various proteins. These findings suggest that both split RNA polymerase parts fold into functional proteins and assemble at high rates in a nonspecific manner when expressed under constitutive pσ70 promoters, while ubiquitination-dependent RNA polymerase assembly likely occurs at much lower rates compared to nonspecific assembly.

Supplementary Table 1: Selected canonical SIAH1/2 substrates

Canonical substrate	Protein size (aa)	Degron	Ubiquitination site	Canonical E3 ligase
EGLN3	239	176-ADVEPIF-182	K(159,172)	SIAH1/2
EGLN1	426	69-VGP-72, 376-VQP-379*	K256	SIAH1/2
α-Synuclein	140	116-MPVDPDN-122	K(6,10,12,21,23,32,34)	SIAH1/2

Supplementary Table 2: Plasmids

Plasmid	Description	Reference
DP6	Drift plasmid DP6, expresses the genes dnaQ926, dam, seqA, emrR, ugi, and cda1 from an arabinose inducible promoter and gIII from a hybrid phage shock/Tet promoter	Addgene #140446
MP6	Mutagenesis plasmid MP6, expresses the genes dnaQ926, dam, seqA, emrR, ugi, and cda1 from an arabinose inducible promoter	Addgene #69669
pBP	Backbone vector with internal BsmBI removed by site-directed mutagenesis	Addgene #72947
pBP_BBa_B0034	Plasmid containing the RBS B0034 part (5' GTAC/3' CATA fusion), from the EcoFlex MoClo kit	Addgene #72980
pBP-J23108	Plasmid containing the J23108 standard iGEM promoter (5' CTAT/ 3' GTAC fusion), from the EcoFlex MoClo kit	Addgene #72964
pBP-L3S2P21	Plasmid containing the L3S2P21 terminator, from the EcoFlex MoClo kit	Addgene #72999
pBP-SJM910	Plasmid containing the SJM910 promoter (5' CTAT/3' GTAC fusion), from the EcoFlex MoClo kit	Addgene #72972
pBT114-splitC	Plasmid containing M13 genes I, IV, and VI	[2]
pBT29-splitD	Plasmid containing M13 genes II, V, VII, VIII, and IX	Addgene #122599
pES0001	Level 0 vector encoding human Ubiquitin-activating enzyme E1 (HsUba1). HsUba1 was excised from its corresponding DNA fragment using NdeI/SphI and ligated into pBP	This work
pES0002	Level 0 vector encoding Linker 3. Linker 3 was PCR-amplified from its corresponding DNA fragment using primers o024: 5'-atatcatatgggtctcaTAAACTGATTAAAGCAGCACA-3' and o025: 5'-atatggcatgcggtctctTATGCCTTGTGGACG-3' (lower case, restriction sites; upper case, annealing), digested with NdeI/SphI and ligated into pBP	This work
pES0003	Level 0 vector encoding N-term RNAP. N-term RNAP was excised from its corresponding DNA fragment using NdeI/SphI and ligated into pBP	This work
pES0004	Level 0 vector encoding Ubiquitin. Ubiquitin was excised from its corresponding DNA fragment using NdeI/SphI and ligated into pBP	This work
pES0005	Level 0 vector encoding pσ70 together with its RBS. Pσ70+RBS was PCR-amplified from its corresponding DNA fragment using primers o013: 5'-CATTAGTTACTGGCGCAC-3' and o014: 5'ACGAGTTCTGATCACAG-3', digested with NdeI/SphI and ligated into pBP	This work
pES0006	Level 0 vector encoding wheat Ubiquitin-activating enzyme E1 (TuUba1). TuUba1 was excised from its corresponding DNA fragment using NdeI/SphI and ligated into pBP	This work
pES0007	Level 0 vector encoding human Ubiquitin-conjugating enzyme E2 (UbcH5A). UbcH5A was excised from its corresponding DNA	This work

	fragment using NdeI/SphI and ligated into pBP	
pES0008	Level 0 vector encoding C-term RNAP. C-term RNAP was excised from its corresponding DNA fragment using NdeI/SphI and ligated into pBP	This work
pES0013	Level 0 vector encoding EGLN3. EGLN3 was excised from its corresponding DNA fragment using NdeI/SphI and ligated into pBP	This work
pES0015	Level 0 vector encoding α-Synuclein. α-Synuclein was excised from its corresponding DNA fragment using NdeI/SphI and ligated into pBP	This work
pES0017	Level 0 vector encoding T7 promoter. T7 promoter was excised from the annealing product of primers o005: 5'-tatgggtctcactatTAATACCGGTCACTATAGgtacagagaccgcatg-3' and o006: 5'-cggtctctgtacCTATAGTGACCGGTATTAatagtgagaccca-3' (lower case, restriction sites; upper case, annealing), digested with NdeI/SphI and ligated into pBP	This work
pES0021	Level 0 vector encoding Linker 2. Linker 2 was PCR-amplified from the annealing product of primers o022: 5'-tatgccGCCAGATCCGCCGGAGGT-3' and o023: 5'-taaaACCTCCGGCGGATCTGGCgg-3' (lower case, restriction sites; upper case, annealing), digested with NdeI/SphI and ligated into pBP	This work
pES0022	Level 0 vector encoding Linker 4. Linker 4 was PCR-amplified from its corresponding DNA fragment using primers o026: 5'-atatcatatgggtctcaTAAAGGAGGTAGTGCAGG-3' and o027: 5'-atatggcatgcggtctctTATGCCTCCACTACTCG-3' (lower case, restriction sites; upper case, annealing), digested with NdeI/SphI and ligated into pBP	This work
pES0027	Level 0 vector encoding gIII fused to luciferase. gIII-luciferase was PCR-amplified from pJC175e using primers o009: 5'-atatcatatgggtctcacataATGAAAAAATTATTATTCGCAATTCCT-3' and o016: 5'-atatggcatgcggtctcttcgATTAGGTATATTCCGTGTGGTACTTC-3' (lower case, restriction sites; upper case, annealing), digested with NdeI/SphI and ligated into pBP	This work
pES1001	Level 1 vector encoding N-term RNAP fused to ubiquitin with Linker 3 driven by pσ70. Assembled by Golden Gate assembly into pTU1-A-RFP backbone using plasmids pBP-L3S2P21, pES0002, pES0003, pES0004, and pES0005, digested with BsaI and ligated with T4 ligase.	This work
pES1002	Level 1 vector encoding HsUba1 driven by SJM910. Assembled by Golden Gate assembly into pTU1-B-RFP backbone using plasmids pBP_BBa_B0034, pBP-L3S2P21, pBP-SJM910, and pES0001, digested with BsaI and ligated with T4 ligase.	This work
pES1003	Level 1 vector encoding TuUba1 driven by SJM910. Assembled by Golden Gate assembly into pTU1-B-RFP backbone using plasmids pBP_BBa_B0034, pBP-L3S2P21, pBP-SJM910, and	This work

	pES0006, digested with BsaI and ligated with T4 ligase.	
pES1004	Level 1 vector encoding UbcH5A driven by SJM910. Assembled by Golden Gate assembly into pTU1-C-RFP backbone using plasmids pBP_BBa_B0034, pBP-L3S2P21, pBP-SJM910, and pES0007, digested with BsaI and ligated with T4 ligase.	This work
pES1026	Level 1 vector encoding gIII-luciferase under the control of T7 promoter. Assembled by Golden Gate assembly into pTU1-D-RFP backbone using plasmids pBP_BBa_B0034, pBP-L3S2P21, pES0017, and pES0027, digested with BsaI and ligated with T4 ligase.	This work
pES1033	Level 1 vector encoding C-term RNAP fused to EGLN3 protein with Linker 4 driven by pσ70. Assembled by Golden Gate assembly into pTU1-A-RFP backbone using plasmids pBP-L3S2P21, pES0005, pES0008, pES0013, and pES0022, digested with BsaI and ligated with T4 ligase.	This work
pES1035	Level 1 vector encoding C-term RNAP fused to α-Synuclein with Linker 4 driven by pσ70. Assembled by Golden Gate assembly into pTU1-A-RFP backbone using plasmids pBP-L3S2P21, pES0005, pES0008, pES0015, and pES0022, digested with BsaI and ligated with T4 ligase.	This work
pES1072	Level 1 vector encoding HsUba1 driven by J23108. Assembled by Golden Gate assembly into pTU1-B-RFP using plasmids pBP_BBa_B0034, pBP-J23108, pBP-L3S2P21, and pES0001, digested with BsaI and ligated with T4 ligase.	This work
pES1074	Level 1 vector encoding UbcH5A driven by J23108. Assembled by Golden Gate assembly into pTU1-C-RFP using plasmids pBP_BBa_B0034, pBP-J23108, pBP-L3S2P21, and pES0007, digested with BsaI and ligated with T4 ligase.	This work
pES1076	Level 1 vector encoding C-term RNAP fused to EGLN3 protein with Linker 2 driven by J23108. Assembled by Golden Gate assembly into pTU1-A-RFP backbone using plasmids pBP-J23108, pBP-L3S2P21, pE0008, pES0013, and pES0021, digested with BsaI and ligated with T4 ligase.	This work
pES1097	Variant of pES1076 encoding the substitution F > M at position 1 of the degron motif. The EGLN3 sequence was PCR-amplified from pES1076 using primers o060 and o061 and recircularized with the KLD Enzyme Mix	This work
pES1098	Variant of pES1076 encoding the substitution A > Q at position 3 of the degron motif. The EGLN3 sequence was PCR-amplified from pES1076 using primers o062 and o063 and recircularized with the KLD Enzyme Mix	This work
pES1101	Variant of pES1076 encoding the substitution P > A at position 7 of the degron motif. The EGLN3 sequence was PCR-amplified from pES1076 using primers o067 and o068 and recircularized with the KLD Enzyme Mix	This work
pES1102	Variant of pES1076 encoding the substitution V > W at position 5 of the degron motif. The EGLN3 sequence was PCR-amplified	This work

	from pES1076 using primers o069 and o066 and recircularized with the KLD Enzyme Mix	
pES2008	Level 2 vector encoding N-term RNAP fused to ubiquitin driven by pσ70, HsUba1 driven by SJM910, UbcH5A driven by SJM910, and gIII-luciferase driven by T7 promoter. Assembled by Golden Gate assembly into pTU2-A-RFP backbone using plasmids pES1001, pES1002, pES1004, and pES1026, digested with BsmBI and ligated with T4 ligase.	This work
pES2009	Level 2 vector encoding N-term RNAP fused to ubiquitin driven by pσ70, TuUba1 driven by SJM910, UbcH5A driven by SJM910, and gIII-luciferase driven by T7 promoter. Assembled by Golden Gate assembly into pTU2-A-RFP backbone using plasmids pES1001, pES1003, pES1004, and pES1026, digested with BsmBI and ligated with T4 ligase.	This work
pES2037	Level 2 vector encoding HsUba1 driven by J23108, UbcH5A driven by J23108, and gIII-luciferase driven by T7 promoter. Assembled by Golden Gate assembly into pTU2-A-RFP KanR backbone using plasmids Dummy A, pES1026, pES1072, pES1074, and pES1095, digested with BsmBI and ligated with T4 ligase.	This work
pJC175e	Phage-responsive accessory plasmid that produces functional pIII in response to phage infection	Addgene #79219
pTU1-A-RFP	Level 1 Destination vector backbone for Position A from the EcoFlex MoClo kit	Addgene #72939
pTU1-B-RFP	Level 1 Destination vector backbone for Position B from the EcoFlex MoClo kit	Addgene. #72940
pTU1-C-RFP	Level 1 Destination vector backbone for Position C from the EcoFlex MoClo kit	Addgene #72941
pTU1-D-RFP	Level 1 Destination vector backbone for Position D from the EcoFlex MoClo kit	Addgene #72942
pTU1-E-RFP	Level 1 Destination vector backbone for Position E from the EcoFlex MoClo kit	Addgene #72944
pTU2-A-RFP	Level 2 Destination vector backbone for Position A from the EcoFlex MoClo kit	Addgene #74093
SIAH1-SP	Selection plasmid encoding SIAH1 and the rest of M13 phage genes, excluding gIII. Assembled by Golden Gate assembly using pBT114-splitC, pBT29-split D, and the POI with compatible restriction sites	This work
SIAH2-SP	Selection plasmid encoding SIAH2 and the rest of M13 phage genes, excluding gIII. Assembled by Golden Gate assembly using pBT114-splitC, pBT29-split D, and the POI with compatible restriction sites	This work
UN-SP / pBT100.164	Selection plasmid encoding TadA-7.10 and the rest of M13 phage genes, excluding gIII. Used as a negative control for phage propagation assays.	[2]

Supplementary Table 3: DNA Sequences used in this study: The lowercase letters represent the attachment sequences utilised for cloning purposes. The uppercase letters represent the coding sequences for the genes of interest employed in this study.

Description	Sequence	Source
a-Synuclein	atatcatatgggtctcagtacATGGATGTGTTTATGAAAGGCCTGTCAA AAGCCAAAGAAGGCGTGGTGGCGGCGGCGGAAAAAACCAA ACAGGGCGTGGCAGAAGCAGCGGGCAAAACCAAAGAAGGC GTGCTGTATGTGGGCAGCAAAACCAAAGAAGGCGTGGTGCA TGGCGTGGCGACCGTGGCGGAAAAAACGAAAGAACAGGTG ACCAACGTTGGCGGCGCGGTGGTGACCGGCGTGACCGCG GTGGCGCAGAAAACCGTGGAAGGTGCAGGCAGCATCGCCG CCGCGACCGGTTTTGTGAAAAAAGATCAGCTGGGCAAAAAC GAAGAAGGCGCGCCGCAGGAGGGCATTCTGGAAGATATGC CGGTGGATCCGGATAACGAAGCGTATGAAATGCCGAGCGAA GAAGGCTATCAGGATTATGAACCGGAAGCGggtaaaagagaccgc atgccatat	[3]
Dummy A	CTATAGAGACCTAAGAATAGTAATACAGGACCCGAATCGTTTC AGTTGCCTGGTCTCATGTT	This work
EGLN1	ATGGCGAATGATAGCGGCGGCCCGGGCGGCCCGAGCCCAA GCGAACGCGATCGTCAGTATTGCGAACTGTGCGGCAAAATG GAAAACCTGCTGCGCTGCTCCCGCTGCCGCAGTTCCTTTTA CTGTTGTAAGGAACATCAGCGCCAGGATTGGAAAAAACACAA ACTGGTGTGTCAGGGCTCCGAAGGTGCCCTGGGCCATGGT GTGGGCCCGCACCAGCATAGCGGCCCGGCGCCGCCGGCG GCGGTTCCGCCGCCGCGTGCGGGCGCACGCGAACCGCGT AAAGCGGCGGCGCGCCGCGATAACGCCAGCGGCGATGCGG CGAAAGGCAAAGTGAAAGCAAAACCGCCGGCGGATCCGGC CGCGGCCGCGAGCCCGTGTCGTGCGGCCGCCGGTGGCCA GGGCAGCGCGGTGGCTGCGGAAGCCGAACCGGGCAAAGA AGAACCGCCGGCGCGCAGCAGCCTGTTTCAGGAAAAAGCC AATCTGTATCCGCCGTCAAACACCCCGGGTGATGCCCTGAG CCCGGGCGGCGGCTTACGCCCGAACGGCCAGACCAAACCG CTGCCGGCGCTGAAACTGGCGCTGGAATATATTGTGCCGTG CATGAATAAACATGGCATTTGCGTGGTGGATGATTTTCTGGGT AAAGAAACCGGCCAGCAGATCGGCGATGAAGTGCGCGCCC TGCATGATACCGGCAAATTTACCGATGGCCAGCTGGTGAGCC AGAAAAGTGATAGCTCAAAAGATATTCGTGGCGATAAAATTAC CTGGATTGAAGGCAAAGAACCGGGCTGCGAAACCATTGGCC TGCTGATGAGCAGCATGGATGACCTGATTCGCCACTGCAATG GCAAACTGGGCAGCTATAAAATTAACGGTCGCACCAAAGCGA TGGTCGCGTGTTATCCGGGCAATGGTACCGGCTATGTGCGC CATGTGGATAACCCGAACGGCGATGGCCGCTGCGTTACCTG CATTTACTATCTGAACAAAGATTGGGACGCCAAAGTGAGCGG CGGCATTCTGCGCATCTTTCCGGAAGGCAAAGCACAGTTCG CGGATATTGAGCCGAAATTTGATCGCCTGCTGTTTTTCTGGA GCGATCGCCGTAATCCGCACGAAGTGCAGCCGGCGTACGC GACCCGCTATGCCATTACCGTGTGGTATTTTGATGCGGATGA ACGTGCGCGTGCCAAAGTGAAATATCTGACCGGCGAAAAAG GCGTGCGTGTGGAACTGAACAAACCGAGCGATAGCGTTGGC AAAGATGTGTTT	[4]
EGLN3	atatcatatgggtctcagtacATGCCGCTGGGCCATATTATGCGCCTGG ATCTGGAAAAAATTGCCCTGGAATATATCGTGCCGTGCCTGC ATGAAGTTGGCTTCTGCTATCTGGATAATTTTCTGGGCGAAGT AGTGGGCGACTGCGTGCTGGAACGTGTGAAACAGCTGCATT GTACCGGCGCGCTGCGCGACGGTCAGCTGGCGGGCCCGC GCGCGGGCGTGAGCAAACGTCATCTGCGCGGTGATCAGATT	[4]

	ACCTGGATTGGCGGCAACGAAGAAGGCTGCGAAGCGATTAG CTTCCTGCTGTCGCTGATTGATCGCCTGGTGCTGTATTGTGG CAGCCGCCTGGGCAAATATTATGTGAAAGAACGTAGCAAAGC CATGGTAGCGTGTTACCCGGGCAACGGTACCGGCTATGTTC GCCATGTGGATAATCCGAACGGCGATGGTCGCTGTATTACCT GCATTTACTACCTGAACAAAAACTGGGATGCCAAACTGCACG GCGGCATTCTGCGCATTTTTCCGGAAGGCAAAAGCTTTATTG CGGATGTGGAACCGATCTTCGATCGCCTGCTGTTCTTCTGGA GCGATCGCCGCAACCCGCATGAAGTCCAACCGAGCTACGCG ACCCGCTACGCCATGACCGTGTGGTACTTTGATGCGGAAGA ACGCGCGGAAGCGAAAAAAAAATTTCGCAACCTGACCCGCA AAACCGAAAGCGCCCTGACCGAAGATggtaaaagagaccgcatgcc atat	
gIII	atatcatatgggtctcacataATGAAAAAACTGCTGTTTGCCATTCCGC TGGTGGTGCCGTTTTATAGCCATAGCGCCGAAACCGTGGAAT CGTGCCTGGCGAAACCGCATACCGAAAACTCATTTACCAACG TGTGGAAAGATGATAAAACCCTGGATCGTTATGCGAATTACGA AGGCTGTCTGTGGAACGCCACCGGCGTTGTGGTGTGTACG GGCGATGAAACCCAGTGTTACGGTACCTGGGTCCCGATCGG CCTGGCGATTCCGGAAAATGAAGGTGGCGGCAGCGAGGGT GGCGGCAGCGAAGGCGGTGGCAGCGAAGGCGGCGGTACC AAACCGCCGGAATATGGCGATACCCCGATTCCGGGCTACAC CTATATTAATCCGCTGGATGGCACCTACCCGCCGGGCACCGA ACAGAATCCGGCGAATCCGAACCCGAGCCTGGAAGAAAGCC AGCCGCTGAACACCTTCATGTTTCAGAACAACCGCTTTCGCA ACCGGCAGGGCGCGCTGACGGTGTACACCGGCACCGTGAC CCAGGGCACCGATCCGGTGAAAACCTACTACCAGTATACCCC GGTGTCGAGCAAAGCGATGTATGATGCGTACTGGAATGGCA AATTTCGCGATTGCGCCTTCCATAGCGGATTTAATGAAGACC CGTTCGTTTGCGAATATCAGGGTCAGAGCAGCGATCTGCCG CAGCCGCCGGTGAATGCGGGCGGCGGCAGCGGCGGCGGC AGCGGCGGCGGCTCCGAAGGCGGCGGCAGTGAAGGCGGC GGCAGCGAAGGCGGCGGCAGCGAAGGCGGCGGCTCCGGC GGCGGCAGCGGCAGCGGCGATTTTGATTATGAAAAAATGGC GAATGCCAACAAAGGCGCCATGACCGAAAACGCGGACGAAA ACGCCCTGCAGAGCGATGCCAAAGGCAAACTGGATAGCGTA GCGACCGATTATGGCGCGGCGATTGACGGCTTTATCGGTGAT GTGAGCGGCCTGGCAAACGGTAACGGCGCCACCGGTGATT TTGCGGGCAGCAACAGCCAGATGGCGCAGGTGGGCGATGG CGATAACAGCCCGCTGATGAACAACTTTCGCCAGTACCTGCC GTCGCTGCCGCAGAGCGTGGAATGCCGCCCGTTTGTGTTCA GCGCGGGCAAACCGTACGAATTCAGCATTGATTGCGATAAAA TTAACCTGTTTCGTGGTGTCTTTGCGTTTCTGCTGTACGTGG CGACCTTTATGTATGTGTTTAGCACCTTCGCCAATATTCTGCG CAACAAAGAATCATAAtcgaagagaccgcatgccatat	[5]
HsUba1	atatcatatgggtctcacataATGTCTAGCTCCCCGCTGTCTAAAAAGC GCCGCGTTTCGGGCCCAGACCCGAAGCCGGGTTCTAACTG CTCCCCGGCCCAATCGGTGTTAAGTGAGGTCCCTAGCGTCC CCACCAATGGCATGGCGAAGAACGGCTCGGAAGCAGACATC GATGAAGGCCTGTACAGTCGTCAATTGTATGTCCTGGGCCAC GAGGCAATGAAACGTCTGCAGACCTCTAGCGTGCTGGTGTC GGGACTGCGCGGCCTGGGGGTAGAGATTGCTAAAAACATTA TCTTGGGCGGTGTTAAGGCTGTGACCCTCCACGATCAGGGT ACCGCACAGTGGGCCGACCTGTCCTCCCAGTTTTACTTGCG CGAAGAAGACATAGGCAAGAACCGTGCCGAAGTGAGTCAGC CACGTTTAGCGGAATTAAATAGTTATGTCCCAGTGACCGCGTA TACAGGCCCGCTGGTGGAAGATTTTCTGTCAGGCTTCCAGG TGGTCGTATTAACGAACACTCCCCTGGAAGACCAACTGCGT GTGGGTGAATTTTGTCATAATCGTGGCATCAAACTAGTAGTCG	UniProt P22314

	CCGATACACGTGGTCTGTTCGGTCAGTTATTCTGCGATTTTG GTGAGGAGATGATTTTAACGGATAGCAATGGCGAACAGCCAC TGTCTGCTATGGTGAGTATGGTTACGAAAGATAACCCCGGCG TGGTGACATGTCTCGATGAAGCGCGGCACGGGTTTGAATCT GGTGACTTCGTCAGTTTCAGTGAGGTACAGGGCATGGTGGA ACTAAATGGCAACCAGCCGATGGAAATAAAGGTTCTGGGTCC ATACACGTTTAGCATCTGCGATACGAGTAATTTTTCTGACTAC ATCCGTGGTGGCATCGTCAGCCAGGTTAAGGTACCGAAAAA AATCAGTTTCAAAAGCTTAGTGGCATCACTCGCGGAACCAGA TTTTGTTGTTACGGATTTTGCGAAATTTTCGAGGCCGGCGCA ACTTCATATTGGCTTTCAGGCACTGCATCAGTTTTGTGCCCA GCACGGTCGTCCGCCGCGTCCGCGCAATGAGGAAGACGCG GCGGAACTGGTAGCACTGGCGCAGGCGGTTAACGCACGTG CCCTTCCGGCGGTACAGCAGAATAACTTGGATGAAGACCTTA TTAGAAAACTGGCCTATGTCGCAGCTGGGGATCTGGCGCCT ATTAACGCCTTTATAGGGGGCTTGGCGGCCCAGGAAGTGAT GAAAGCTTGCTCCGGCAAATTTATGCCTATCATGCAGTGGTTA TATTTTGATGCGCTGGAATGCTTACCTGAGGACAAAGAGGTG CTAACGGAAGATAAATGTTTGCAGCGCCAGAATCGCTACGAC GGTCAGGTGGCCGTCTTCGGATCAGATCTGCAGGAAAAACT TGGCAAACAAAAATATTTTTTGGTAGGCGCGGGAGCAATCGG TTGCGAATTACTGAAAAACTTTGCCATGATTGGACTTGGTTGT GGCGAAGGTGGGGAAATTATTGTTACCGACATGGATACGATC GAAAAATCGAATCTGAACCGTCAGTTTTTATTTCGCCCATGG GATGTTACTAAATTGAAAAGCGATACTGCCGCGGCGGCCGTG CGTCAGATGAATCCGCACATTCGTGTCACCAGCCATCAGAAT CGTGTAGGGCCAGATACCGAACGGATTTACGATGATGACTTT TTCCAGAACCTGGACGGTGTCGCTAATGCCCTGGACAACGT TGACGCACGAATGTATATGGACAGACGGTGCGTCTATTACCG CAAACCCTTGCTGGAGAGCGGGACCCTGGGAACTAAAGGTA ATGTTCAGGTGGTGATACCATTTCTGACCGAATCTTATTCATC ATCTCAAGATCCCCCTGAAAAAAGTATTCCGATTTGTACACTT AAAAACTTCCCCAATGCCATCGAACACACACTCCAATGGGCT CGCGACGAATTCGAAGGTTTATTCAAACAACCTGCCGAGAAC GTGAACCAGTACCTGACCGATCCGAAATTCGTGGAGCGCAC GCTGCGTCTGGCAGGCACACAGCCGCTGGAGGTCTTGGAG GCTGTACAGCGCTCATTGGTTCTGCAACGCCCGCAAACTTG GGCGGATTGCGTCACATGGGCATGTCATCATTGGCATACCCA ATATAGCAACAATATCCGGCAGCTTTTGCACAACTTCCCGCC GGATCAGTTAACATCATCCGGTGCTCCTTTCTGGAGCGGTCC TAAACGTTGCCCGCATCCTCTGACCTTTGACGTCAACAACCC ACTTCATCTTGATTATGTGATGGCGGCAGCGAACCTGTTTGC GCAAACGTATGGCCTCACTGGGTCGCAAGATAGAGCAGCGG TCGCAACTTTCCTGCAATCTGTGCAAGTTCCGGAATTTACGC CCAAGTCAGGAGTAAAGATCCACGTTTCGGATCAGGAACTC CAGTCAGCAAATGCCAGCGTAGATGATTCGCGTCTGGAAGA ACTGAAAGCAACCCTCCCGTCCCCCGATAAACTTCCGGGATT TAAAATGTATCCGATCGACTTCGAGAAGGACGATGATTCGAAT TTTCACATGGATTTTATTGTGGCGGCCAGCAATCTGCGCGCA GAAAACTACGATATCCCGTCAGCCGATCGCCATAAGTCCAAA CTGATTGCGGGCAAAATTATTCCGGCTATTGCTACCACCACT GCCGCGGTTGTTGGTCTGGTGTGTCTGGAACTGTACAAAGT TGTTCAAGGCCATCGACAGCTGGATAGCTATAAAAACGGGTT TCTCAACCTAGCGCTCCCGTTCTTCGGTTTTTCCGAGCCGCT GGCTGCCCCGCGGCATCAGTATTACAATCAGGAATGGACCC TGTGGGATCGCTTCGAGGTGCAAGGCTTGCAACCGAATGGG GAGGAAATGACGCTCAAACAATTCCTGGACTATTTTAAAACC GAACATAAGCTTGAGATTACCATGTTGAGCCAGGGAGTGTCC ATGCTGTACAGCTTCTTCATGCCTGCCGCTAAATTAAAGGAA CGCCTGGACCAGCCAATGACCGAAATCGTAAGCCGCGTGAG	

	CAAACGAAAACTGGGTCGACACGTGCGCGCGCTTGTTCTCG AACTGTGTTGCAATGATGAAAGTGGGGAGGACGTTGAGGTG CCGTATGTTCGGTACACGATTCGTTAAtcgaagagaccgcatgccatat	
KanR	ATGAGCCACATTCAGCGTGAAACCAGCTGCAGCCGTCCGCG CCTGAACAGCAACATGGATGCGGATCTGTATGGCTATAAATG GGCCCGCGATAATGTTGGCCAGAGCGGCGCGACCATTTATC GCCTGTATGGTAAACCGGATGCGCCGGAACTGTTTCTGAAA CATGGCAAAGGCAGCGTGGCCAACGATGTGACCGATGAAAT GGTGCGTCTGAACTGGCTGACGGAATTCATGCCGCTGCCGA CCATTAAACACTTTATTCGCACGCCGGATGATGCGTGGCTGC TGACCACCGCAATTCCGGGCAAAACCGCGTTTCAGGTGCTG GAAGAATACCCGGATAGCGGTGAAAATATTGTGGATGCGCTG GCGGTGTTTCTGCGTCGCCTGCACAGCATCCCGGTCTGCAA CTGTCCGTTTAATAGCGATCGTGTGTTCCGCCTGGCGCAGG CACAGAGCCGCATGAACAACGGCCTGGTGGATGCGAGCGAT TTTGACGATGAACGTAATGGCTGGCCGGTGGAACAGGTGTG GAAAGAAATGCACAAACTGCTGCCGTTTAGCCCGGATAGCG TGGTGACCCATGGCGATTTCAGCCTGGATAACCTGATCTTTG ATGAAGGCAAACTGATTGGCTGTATTGATGTGGGCCGCGTG GGCATTGCCGATCGCTATCAAGATCTGGCCATTCTGTGGAAC TGCCTGGGCGAATTCAGCCCGAGCCTGCAAAAACGCCTGTT TCAGAAATATGGCATTGACAACCCGGATATGAATAAACTGCAG TTTCACCTGATGCTGGATGAATTCTTTTGA	Addgene #204045
Linker 1	GGTGGCAGCGGGAGCGGCTCGTCG	[1]
Linker 2	ACCTCCGGCGGATCTGGC	[1]
Linker 3	CTGATTAAAGCAGCACAGCGGGCCCGTGAGGCCGAACGCG ATTTAGCTGCGGCGGTTGCTCAGGCGGCAGCCGGGCAGGC CGTGCCACGCGCGGCGCGTCCGCAA	[6]
Linker 4	GGCGGCAGCGCCGGAAGTGGCTCCGGTGCAGGGTCGGGT TCAGGTGGTAGCGCTGGTTCCTCTGGTTCAAGCGGCGCGAG TAGTGGA	[6]
Luciferase	ATGAAATTTGGAAACTTTTTGCTTACATACCAACCTCCCCAAT TTTCCCAAACAGAGGTAATGAAACGTTTGGTTAAATTAGGTCG CATCTCTGAGGAGTGTGGTTTTGATACCGTATGGTTACTGGA GCATCATTTCACGGAGTTTGGTTTGCTTGGTAACCCTTATGTC GCTGCTGCATATTTACTTGGCGCGACTAAAAAATTGAATGTAG GAACTGCCGCTATTGTTCTTCCCACAGCCCATCCAGTACGCC AACTTGAAGATGTGAATTTATTGGATCAAATGTCAAAAGGACG ATTTCGGTTTGGTATTTGCCGAGGGCTTTACAACAAGGACTT TCGCGTATTCGGCACAGATATGAATAACAGTCGCGCCTTAGC GGAATGCTGGTACGGGCTGATAAAGAATGGCATGACAGAGG GATATATGGAAGCTGATAATGAACATATCAAGTTCCATAAGGTA AAAGTAAACCCCGCGGCGTATAGCAGAGGTGGCGCACCGGT TTATGTGGTGGCTGAATCAGCTTCGACGACTGAGTGGGCTG CTCAATTTGGCCTACCGATGATATTAAGTTGGATTATAAATACT AACGAAAAGAAAGCACAACTTGAGCTTTATAATGAAGTGGCT CAAGAATATGGGCACGATATTCATAATATCGACCATTGCTTATC ATATATAACATCTGTAGATCATGACTCAATTAAAGCGAAAGAGA TTTGCCGGAAATTTCTGGGGCATTGGTATGATTCTTATGTGAA TGCTACGACTATTTTTGATGATTCAGACCAAACAAGAGGTTAT GATTTCAATAAAGGGCAGTGGCGTGACTTTGTATTAAAAGGA CATAAAGATACTAATCGCCGTATTGATTACAGTTACGAAATCAA TCCCGTGGGAACGCCGCAGGAATGTATTGACATAATTCAAAA AGACATTGATGCTACAGGAATATCAAATATTTGTTGTGGATTTG AAGCTAATGGAACAGTAGACGAAATTATTGCTTCCATGAAGCT CTTCCAGTCTGATGTCATGCCATTTCTTAAAGAAAAACAACGT TCGCTATTATATTATGGCGGTGGCGGTAGCGGCGGTGGCGG	[7]

	TAGCGGCGGTGGCGGTAGCGGCGGTGGCGGTAGCAAATTT GGATTGTTCTTCCTTAACTTCATCAATTCAACAACTGTTCAAG AACAGAGTATAGTTCGCATGCAGGAAATAACGGAGTATGTTG ATAAGTTGAATTTTGAACAGATTTTAGTGTATGAAAATCATTTT TCAGATAATGGTGTTGTCGGCGCTCCTCTGACTGTTTCTGGT TTTCTGCTCGGTTTAACAGAGAAAATTAAAATTGGTTCATTAA ATCACATCATTACAACTCATCATCCTGTCCGCATAGCGGAGGA AGCTTGCTTATTGGATCAGTTAAGTGAAGGGAGATTTATTTTA GGGTTTAGTGATTGCGAAAAAAAAGATGAAATGCATTTTTTTA ATCGCCCGGTTGAATATCAACAGCAACTATTTGAAGAGTGTTA TGAAATCATTAACGATGCTTTAACAACAGGCTATTGTAATCCA GATAACGATTTTTATAGCTTCCCTAAAATATCTGTAAATCCCCA TGCTTATACGCCAGGCGGACCTCGGAAATATGTAACAGCAAC CAGTCATCATATTGTTGAGTGGGCGGCCAAAAAAGGTATTCC TCTCATCTTTAAGTGGGATGATTCTAATGATGTTAGATATGAAT ATGCTGAAAGATATAAAGCCGTTGCGGATAAATATGACGTTGA CCTATCAGAGATAGACCATCAGTTAATGATATTAGTTAACTATA ACGAAGATAGTAATAAAGCTAAACAAGAGACGCGTGCATTTAT TAGTGATTATGTTCTTGAAATGCACCCTAATGAAAATTTCGAAA ATAAACTTGAAGAAATAATTGCAGAAAACGCTGTCGGAAATTA TACGGAGTGTATAACTGCGGCTAAGTTGGCAATTGAAAAGTG TGGTGCGAAAAGTGTATTGCTGTCCTTTGAACCAATGAATGA TTTGATGAGCCAAAAAAATGTAATCAATATTGTTGATGATAATA TTAAGAAGTACCACACGGAATATACCTAA	
Mock gIII	ATGAAAAAATTATTATTCGCAATTCCTTTAGTTGTTCCTTTCTAT TCTCACTCCGCTGAAACTGTTCATCACCATCACCATCACGCT GAAACTGTTGAAAGTTGTTTAGCAAAACCCCATACAGAAAATT CATTTACTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTA CGCTAACTATGAGGGCTGTCTGTGGAATGCTACAGGCGTTGT AGTTTGTACTGGTGACGAAACTCAGTGTTACGGTACATGGGT TCCTATTGGGCTTGCTATCCCTGAAAATGAGGGTGGTGGCTC TGAGGGTGGCGGTTCTGAGGGTGGCGGTTCTGAGGGTGGC GGTACTAAACCTCCTGAGTACGGTGATACACCTATTCCGGGC TATACTTATATCAACCCTCTCGACGGCACTTATCCGCCTGGTA CTGAGCAAAACCCCGCTAATCCTAATCCTTCTCTTGAGGAGT CTCAGCCTCTTAATACTTTCATGTTTCAGAATAATAGGTTCCG AAATAGGCAGGGGGCATTAACTGTTTATACGGGCACTGTTAC TCAAGGCACTGACCCCGTTAAAACTTATTACCAGTACACTCCT GTATCATCAAAAGCCATGTATGACGCTTACTGGAACGGTAAAT TCAGAGACTGCGCTTTCCATTCTGGCTTTAATGAGGATCCATT CGTTTGTGAATATCAAGGCCAATCGTCTGACCTGCCTCAACC TCCTGTCAATGCTGGCGGCGGCTCTGGTGGTGGTTCTGGTG GCGGCTCTGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGA GGGTGGCGGCTCTGAGGGAGGCGGTTCCGGTGGTGGCTCT TCCCAAATGGCTCAAGTCGGTGACGGTGATAATTCACCTTTA ATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGT TGAATGTCGCCCTTTTGTCTTTGGCGCTGGTAAACCTTACGA GTTCAGTATCGACTGCGATAAGATCAACCTGTTCCGCGGTGT CTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTATTTTC TACGTTTGCTAACATACTGCGTAATAAGGAGTCTTAA	[8]
NLRP3 Substrate 191-220	atatcatatgggtctcagtacATGAAAACCAAAACCTGCGAAAGCCCG GTGAGCCCGATTAAAATGGAACTGCTGTTTGATCCGGATGAT GAACATAGCGAACCGGTGCATTAAggtaaaagagaccgcatgccatat	This work
NLRP3 Substrate 648-719	atatcatatgggtctcagtacATGCTGCATAATATGCCGAAAGAAGAAG AAGAAGAAGAAAAAGAAGGCCGCCATCTGGATATGGTGCAG TGCGTGCTGCCGAGCAGCAGCCATGCCGCGTGCAGCCATG GCTAAggtaaaagagaccgcatgccatat	This work

pσ70+RBS	TTTACAGCTAGCTCAGTCCTAGGTATAATGCTAGCAAAGAGGAGAA	[9]
RNAP C-term (CCG)	atatcatatgggtctcacataAAAGCGTTTATGCAGGTGGTTGAGGCCGATATGCTGAGTAAAGGCCTGCTGGGCGGCGAAGCCTGGTCGAGCTGGCATAAAGAAGATTCGATTCACGTTGGCGTCCGCTGTATTGAAATGCTGATTGAAAGCACCGGCATGGTAAGCCTGCATCGCCAGAACGCCGGCGTGGTGGGCCAGGATAGCGAAACCATTGAACTGGCGCCGGAATATGCCGAAGCCATTGCGACCCGTGCGGGCGCCCTGGCAGGCATCAGCCCGATGTTTCAGCCGTGCGTGGTGCCGCCGAAACCGTGGACCGGCATTACGGGCGGTGGCTATTGGGCGAACGGTCGCCGCCCGCTGGCCCTGGTGCGTACCCACAGCAAAAAAGCACTGATGCGCTACGAAGATGTCTACATGCCGGAAGTGTATAAAGCGATTAACATTGCCCAGAACACCGCGTGGAAAATTAATAAAAAAGTGCTGGCAGTGGCGAACGTGATCACCAAATGGAAACACTGTCCGGTCGAAGATATTCCGGCGATTGAACGTGAAGAACTGCCGATGAAACCGGAAGACATTGATATGAACCCTGAAGCGCTGACCGCATGGAAACGCGCGGCGGCCGCGGTGTACCGTAAAGATAAAGCGCGCAAAAGCCGTCGCATCAGCCTGGAATTCATGCTGGAACAGGCGAATAAATTCGCGAACCATAAGGCGATTTGGTTCCCGTACAATATGGATTGGCGCGGCCGCGTGTATGCGGTGAGCATGTTTAATCCGCAGGGCAATGATATGACCAAAGGCCTGCTGACCCTGGCGAAAGGCAAACCGATTGGCAAAGAAGGCTATTATTGGCTGAAAATTCATGGCGCGAACTGTGCGGGTGTGGATAAAGTTCCGTTTCCGGAACGTATTAAATTTATTGAAGAAAACCATGAAAATATTATGGCGTGCGCCAAAAGCCCGCTGGAAAATACGTGGTGGGCGGAACAGGATAGCCCGTTCTGCTTTCTGGCGTTTTGCTTCGAATATGCGGGTGTGCAGCACCACGGCCTGAGCTATAACTGCAGCCTGCCGCTGGCATTTGATGGTAGCTGTAGCGGCATTCAGCATTTTTCAGCGATGCTGCGTGATGAAGTAGGCGGCCGCGCGGTGAACCTGCTGCCGAGCGAAACCGTTCAGGATATTTACGGCATTGTGGCCAAAAAAGTGAATGAAATTCTGCAGGCCGATGCGATTAACGGCACCGATAATGAAGTGGTGACCGTCACCGATGAAAATACCGGCGAAATTAGCGAAAAAGTGAAACTGGGCACCAAAGCGCTGGCAGGCCAGTGGCTGGCCTATGGCGTGACCCGTAGCGTGACCAAACGTAGCGTTATGACCCTGGCTTACGGCAGCAAAGAATTTGGCTTTCGCCAGCAGGTGCTGGAAGACACCATTCAGCCGGCGATTGACAGCGGCAAAGGCCTGATGTTTACCCAGCCGAACCAGGCGGCCGGCTATATGGCGAAACTGATCTGGGGAAAGCGTGTCAGTGACCGTTGTGGCAGCCGTGGAAGCGATGAACTGGCTGAAAAGCGCGGCAAAACTGCTGGCGGCGGAAGTGAAAGATAAAAAAACCGGTGAAATTCTTCGTAAACGCTGCGCGGTGCATTGGGTGACCCCGGATGGCTTTCCGGTGTGGCAAGAATATAAAAAACCGATTAAAACCCGCGTGCATATTATGTTTCTGGGTCAATTTGAAATGCAGCCGACCATTAACACCAACAAGGATAGCGAAATTGATGCACGTAAACAGGAAAGCGGCATTGCGCCGAACTTTGTACATAGCCAGGATGGCAGCCATCTGCGCAAAACCGTAGTGTGGGCCCATGAAAAATATGGCATTGAATCGTTTGCGCTGATTCACGATAGCTTCGGCACCATTCCGGCCGATGCGGCGAATCTGTTCAAAGCCGTGCGCGAAACCATGGTGGATACCTACGAAAGCTGCGACGTGTTAGCGGATTTCTATGATCAGTTTGCCGATCAGCTGCACGAAAGCCAGCTGGATAAAATGCCGGCGCTGCCGGCCAAAGGCAACCTGAATCTGCGCGATATTCTGGAAAGCGATTTTGCGTTTGCCTAAtcgaagagaccgcatgccatat	[10]
RNAP C-term modified (GAC)	AAAGCGTTTATGCAGGTGGTTGAGGCCGATATGCTGAGTAAAGGCCTGCTGGGCGGCGAAGCCTGGTCGAGCTGGCATAAAGAAGATTCGATTCACGTTGGCGTCCGCTGTATTGAAATGCTGATTGAAAGCACCGGCATGGTAAGCCTGCATCGCCAGAACGCC	[1]

	GGCGTGGTGGGCCAGGATAGCGAAACCATTGAACTGGCGC CGGAATATGCCGAAGCCATTGCGACCCGTGCGGGCGCCCT GGCAGGCATCAGCCCGATGTTTCAGCCGTGCGTGGTGCCG CCGAAACCGTGGACCGGCATTACGGGCGGTGGCTATTGGG CGAACGGTCGCCGCCCGCTGGCCCTGGTGCGTACCCACAG CAAAAAAGCACTGATGCGCTACGAAGATGTCTACATGCCGGA AGTGTATAAAGCGATTAACATTGCCCAGAACACCGCGTGGAA AATTAATAAAAAAGTGCTGGCAGTGGCGAACGTGATCACCAA ATGGAAACACTGTCCGGTCGAAGATATTCCGGCGATTGAACG TGAAGAACTGCCGATGAAACCGGAAGACATTGATATGAACCC TGAAGCGCTGACCGCATGGAAACGCGCGGCGGCCGCGGTG TACCGTAAAGATAAAGCGCGCAAAAGCCGTCGCATCAGCCT GGAATTCATGCTGGAACAGGCGAATAAATTCGCGAACCATAA GGCGATTTGGTTCCCGTACAATATGGATTGGCGCGGCCGCG TGTATGCGGTGAGCATGTTTAATCCGCAGGGCAATGATATGA CCAAAGGCCTGCTGACCCTGGCGAAAGGCAAACCGATTGG CAAAGAAGGCTATTATTGGCTGAAAATTCATGGCGCGAACTG TGCGGGTGTGGATAAAGTTCCGTTTCCGGAACGTATTAAATT TATTGAAGAAAACCATGAAAATATTATGGCGTGCGCCAAAAGC CCGCTGGAAAATACGTGGTGGGCGGAACAGGATAGCCCGTT CTGCTTTCTGGCGTTTTGCTTCGAATATGCGGGTGTGCAGCA CCACGGCCTGAGCTATAACTGCAGCCTGCCGCTGGCATTTG ATGGTAGCTGTAGCGGCATTCAGCATTTTTCAGCGATGCTGC GTGATGAAGTAGGCGGCCGCGCGGTGAACCTGCTGCCGAG CGAAACCGTTCAGGATATTTACGGCATTGTGGCCAAAAAAGT GAATGAAATTCTGCAGGCCGATGCGATTAACGGCACCGATAA TGAAGTGGTGACCGTCACCGATGAAAATACCGGCGAAATTAG CGAAAAAGTGAAACTGGGCACCAAAGCGCTGGCAGGCCAG TGGCTGGCCTATGGCGTGACCCGTAGCGTGACCAAACGTAG CGTTATGACCCTGGCTTACGGCAGCAAAGAATTTGGCTTTCG CCAGCAGGTGCTGGAAGACACCATTCAGCCGGCGATTGACA GCGGCAAAGGCCTGATGTTTACCCAGCCGAACCAGGCGGC CGGCTATATGGCGAAACTGATCTGGGAAAGCGTGTCAGTGA CCGTTGTGGCAGCCGTGGAAGCGATGAACTGGCTGAAAAG CGCGGCAAAACTGCTGGCGGCGGAAGTGAAAGATAAAAAAA CCGGTGAAATTCTTCGTAAACGCTGCGCGGTGCATTGGGTG ACCCCGGATGGCTTTCCGGTGTGGCAAGAATATAAAAAACCG ATTCAGACCCGCCTGAACCTGATGTTTCTGGGTCAATTTCGC CTGCAGCCGACCATTAACACCAACAAGGATAGCGAAATTGAT GCACATAAACAGGAAAGCGGCATTGCGCCGAACTTTGTACAT AGCCAGGATGGCAGCCATCTGCGCAAAACCGTAGTGTGGGC CCATGAAAAATATGGCATTGAATCGTTTGCGCTGATTCACGAT AGCTTCGGCACCATTCCGGCCGATGCGGCGAATCTGTTCAA AGCCGTGCGCGAAACCATGGTGGATACCTACGAAAGCTGCG ACGTGTTAGCGGATTTCTATGATCAGTTTGCCGATCAGCTGC ACGAAAGCCAGCTGGATAAAATGCCGGCGCTGCCGGCCAAA GGCAACCTGAATCTGCGCGATATTCTGGAAAGCGATTTTGCG TTTGCCTAA	
RNAP N-term	atatcatatgggtctcagtacATGAACACCATTAATATTGCGAAAAATGA TTTCAGCGATATTGAACTGGCGGCCATTCCGTTTAATACCCTG GCCGATCACTATGGCGAACGCAGCGCGCGTGGCCAGCTGG CGCTGGAACATGAAAGCTACGAAATGGGCGAAGCGCGCTTT CGCAAAATGTTTGAACGCCAGCTGAAAGCCGGCGAAGTGGC GGATAATGCGGCGGCGAAGCCGCTGATTACCACCCTGCTGC CGAAAATGATTGCGCGCATTAACGATTGGTTTGAAGAAGTTA AAGCAAAACGTGGCCGCCGCCCGACCGCGTTCCAGTTTCTG AAAGAAATTAAACCGGAAGCGGTGGCATATATCACCATTAAAA CCAGCCTGGCcTGCCTGACCAGCGCGGATAACACCACCGTG CAGGCGGTCGCGTCGGCGATTGGCCGCACCATTGAAGATGA	[1]

	AGCGCGCTTCGGCCGTATTCGCGATCTGGAAGCGAAACATT TCAAAAAAAACGTGGAAGAACAGCTGAATAAACGCGTGGGC CACGTTTATAAAtaaaagagaccgcatgccatat	
SIAH1	atatcatatggctcttctagtATGAGCCGTCAGACCGCGACCGCGCTGC CGACGGGCACCAGCAAATGCCCGCCGAGCCAGCGTGTGCC GGCGCTGACCGGCACGACCGCGAGCAACAATGATCTGGCG TCGCTGTTTGAATGCCCGGTTTGTTTTGATTATGTTCTGCCGC CGATTCTGCAGTGCCAGAGCGGTCACCTGGTGTGCAGCAAT TGCCGCCCGAAGCTGACCTGCTGCCCGACCTGCCGCGGCC CGCTGGGCAGCATTCGCAACCTGGCCATGGAAAAAGTGGCG AACTCGGTGCTGTTTCCGTGCAAATATGCCTCGAGCGGCTG TGAAATTACGCTGCCGCATACCGAAAAAGCGGATCATGAAGA ACTGTGCGAATTTCGCCCGTACAGCTGCCCGTGCCCGGGCG CGAGCTGCAAATGGCAGGGTAGCCTGGATGCGGTGATGCC GCATCTGATGCATCAGCATAAAAGCATTACCACCCTGCAGGG TGAAGATATTGTGTTCCTGGCCACCGATATTAACCTGCCGGG CGCGGTGGATTGGGTTATGATGCAGTCATGCTTTGGCTTTCA TTTTATGCTGGTGCTGGAAAAACAGGAAAAATACGACGGCCA TCAGCAGTTCTTTGCGATTGTGCAGCTGATTGGCACCCGCAA ACAGGCAGAAAACTTCGCGTATCGCCTGGAACTGAACGGCC ATCGTCGCCGTCTGACCTGGGAAGCCACCCCGCGCAGCATT CACGAAGGTATTGCCACCGCCATTATGAATAGCGATTGCCTG GTGTTTGATACCTCGATTGCGCAGCTGTTTGCGGAAAACGG CAACCTGGGTATTAATGTGACCATTAGTATGTGCTAAggcagaag agcgcatgccatat	[11]
SIAH2	atatcatatggctcttctagtATGAGCCGCCCGAGCAGCACCGGTCCG AGCGCGAATAAACCGTGCAGCAAACAGCCGCCGCCGCAGC CGCAGCATACCCCGAGCCCGGCCGCCCCGCCGGCCGCGG CGACGATTAGCGCGGCCGGCCCGGGCTCGAGCGCCGTGCC GGCAGCAGCGGCGGTGATTAGCGGCCCGGGCGGCGGCGG CGGCGCGGGCCCGGTGAGCCCGCAGCATCATGAACTGACC AGCCTGTTTGAATGTCCGGTGTGCTTCGATTATGTCCTGCCG CCGATTCTGCAGTGCCAGGCGGGCCATCTGGTGTGCAATCA GTGCCGTCAGAAACTGAGCTGCTGCCCGACCTGCCGTGGC GCCCTGACCCCGAGCATCCGTAACCTGGCGATGGAAAAAGT GGCGAGCGCGGTTCTGTTTCCGTGCAAATATGCCACCACCG GCTGCAGCCTGACCCTGCACCACACCGAAAAACCGGAACAT GAAGATATTTGCGAATACCGCCCTTATAGCTGTCCGTGTCCG GGCGCCAGCTGCAAATGGCAGGGTAGCCTGGAAGCCGTGA TGTCACATCTGATGCACGCGCATAAATCAATTACGACCTTACA GGGCGAAGATATTGTTTTTCTGGCGACCGATATTAATCTGCC GGGCGCCGTGGATTGGGTGATGATGCAGAGCTGTTTCGGCC ATCACTTTATGCTGGTGCTGGAAAAACAGGAAAAATACGAAG GTCATCAGCAGTTCTTTGCGATTGTGCTGTTAATTGGCACCC GCAAACAGGCAGAAAACTTTGCCTACCGCCTGGAACTGAAC GGTAATCGCCGTCGCCTGACCTGGGAAGCGACCCCGCGTA GCATTCACGATGGCGTGGCGGCAGCGATTATGAATAGCGATT GCCTGGTGTTTGATACCGCGATCGCGCATCTGTTTGCGGATA ATGGCAACCTGGGCATTAATGTGACCATTTCTACCTGCTGCC CGTAAggcagaagagcgcatgccatat	[12]
SmR	ATGAGGGAAGCGGTGATCGCCGAAGTATCGACTCAACTATCA GAGGTAGTTGGCGTCATCGAGCGCCATCTCGAACCGACGTT GCTGGCCGTACATTTGTACGGCTCCGCAGTGGATGGCGGCC TGAAGCCACACAGTGATATTGATTTGCTGGTTACGGTGACCG TAAGGCTTGATGAAACAACGCGGCGAGCTTTGATCAACGAC CTTTTGGAAACTTCGGCTTCCCCTGGAGAGAGCGAGATTCT CCGCGCTGTAGAAGTCACCATTGTTATGTACGACGACATCAT TCCGTGGCGTTATCCAGCTAAGCGCGAACTGCAATTTGGAG	[13]

	AATGGCAGCGCAATGACATTCTTGCAGGTATCTTCGAGCCAG CCACGATCGACATTGATCTGGCTATCTTGCTGACAAAAGCAA GAGAACATAGCGTTGCCTTGGTAGGTCCAGCGGCGGAGGAA CTCTTTGATCCGGTTCCTGAACAGGATCTATTTGAGGCGCTA AATGAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGC TGGCGATGAGCGAAATGTAGTGCTTACGTTGTCCCGCATTTG GTACAGCGCAGTAACCGGCAAAATCGCGCCGAAGGATGTCG CTGCCGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCA GCCCGTCATACTTGAAGCTAGACAGGCTTATCTTGGACAAGA AGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAAT TTGTCCACTACGTGAAAGGCGAGATCACCAAGGTAGTCGGC AAATAA	
T7 Promoter CGG	TAATACCGGTCACTATAG	[[10]
TuUba1	atatcatatgggtctcacataATGCTGCCCCGCAAGCGGGAAATCGTC GCCGGCGAAGTCGAAGACTTGCAGAAAAAGACCCGCGCCG GGGAGGGCGAGGTCACGAGGGAAGAAGGCGATGCAGCCAT GGCGGGGCGCGGCAACGAGATCGACGAGGACCTGCACAG CCGCCAGCTCGCCGTGTATGGGCGCGAGACAATGAAACGC CTCTTTGGCTCCAACGTCCTCGTGAGTGGACTGCAGGGTCT GGGTGCTGAAATCGCAAAAAACCTTGTCCTTGCGGGTGTCA AAAGCGTAACCTTGCATGATGATGGTAACGTGGAACTGTGGG ACTTATCAAGCAACTTCTTCCTGTCGGAGAATGATGTTGGGC AAAACCGTGCGCAAGCTTGTGTACAGAAATTACAAGAACTGA ACAATGCTGTTCTGGTGAGTGCCTTAACCGGCGATTTGACCA AAGAACACCTGTCTAAATTCCAGGCCGTTGTATTCACCGATAT CAGCTTAGATAAAGCGATTGAATTTGATGATTATTGCCACAGC CAACAGCCACCGATTGCGTTCATCAAATCTGAAGTTCGTGGC CTTTTTGGCAGTGTTTTTTGTGATTTTGGTCCTGAATTTACGG TTTTGGATGTGGATGGCGAAGAACCGCATACAGGAATTGTGG CATCAATCAGCAATGACAATCCAGCACTTGTATCTTGTGTGGA CGATGAACGTCTGGAGTTTCAGGATGGTGATCTAGTTGTGTT TTCGGAAGTCCATGGAATGACGGAGCTGAATGATGGCAAAC CACGCAAAGTTAAAAATGCACGTCCGTATTCTTTCTTCCTCGA AGAAGACACTTCCTCATTTGGCGCATACGTTCGTGGCGGTAT TGTAACCCAGGTAAAACCACCGAAAGTTATTAAATTCAAACCG TTAAAAGAGGCCATGTCAGAGCCGGGAGAATTTCTCATGAGT GATTTCTCCAAATTTGAACGGCCGCCGTTACTGCATTTGGCA TTCCAAGCGTTGGATAAGTTTCGTACTGAGTTGAGCCGTTTC CCTGTTGCGGGGTCCACCGATGATGTGCAACGCGTGATTGA ATATGCGATTAGCATTAATGATACGCTGGGAGATCGTAAACTG GAAGAAATTGACAAAAAGCTGCTGCATCATTTTGCCAGTGGC AGCCGCGCGGTTCTGAATCCGATGGCGGCGATGTTTGGTGG TATTGTGGGTCAGGAAGTAGTGAAAGCTTGCTCAGGGAAATT TCATCCGCTGTATCAGTTCTTCTATTTTGATTCTGTCGAGAGC CTCCCGGTTGACCCCTTGGAACCTGGTGATTTGAAACCGAA GAACAGTCGTTATGATGCGCAAATCAGCGTATTTGGCTCGAA GCTGCAAAACAAACTGGAAGAAGCAAAAATCTTTATGGTGGG TTCTGGTGCACTGGGCTGTGAGTTCTTAAAAAACCTGGCACT AATGGGTATTTCTTGCAGCCAGAATGGAAATCTGACTCTGAC AGATGATGATGTGATTGAAAAGAGTAATCTGAGCCGCCAATTT TTATTTCGTGACTGGAACATTGGCCAACCTAAATCAACAGTTG CGGCGACCGCTGCGATGGTAATTAATCCGAAACTTCATGTCG AAGCCCTTCAGAACCGCGCAAGTCCTGAGACTGAAAATGTG TTTAATGATGCCTTCTGGGAAAACCTTGATGCTGTGGTCAAT GCCCTGGACAATGTTACCGCAAGAATGTACATAGACTCCAGA TGTGTATATTTCCAGAAACCACTGTTGGAAAGCGGCACCCTG GGTGCGAAATGCAATACCCAGATGGTCATCCCTCACCTAACA	[6], codon-optimized

	GAAAACTATGGGGCGTCACGCGATCCGCCGGAAAAACAGGC ACCGATGTGCACTGTACATTCATTTCCGCATAACATTGATCAT TGCCTAACCTGGGCGCGCTCGGAGTTTGAAGGTTTACTGGA GAAGACTCCCACGGAAGTAAATGCTTTCCTGTCAAATCCTAC GACCTACATTAGTGCAGCACGAACTGCGGGTGATGCACAGG CTCGCGATCAACTGGAACGTGTTATTGAGTGTCTGGACCGC GACAAATGCGAAACTTTTCAGGATTCTATTACCTGGGCCCGT CTGAAGTTTGAGGATTATTTTTCCAACCGTGTGAAACAGCTG ACGTTTACGTTCCCGGAAGACTCGATGACCAGCAGCGGTGC GCCGTTTTGGTCTGCTCCGAAACGGTTCCCGCGACCTGTGG AGTTCTCGTCCAGTGATCAGAGTCAGCTTAGCTTTATTTTGG CTGCTGCAATCTTGCGAGCGGAAACTTTTGGTATACCCATAC CGGAGTGGGCCAAAACCCCAAACAAACTGGCGGCTGAAGC GGTGGACAAAGTGATTGTCCCGGATTTTCAACCAAAGCAGG GGGTGAAAATCGTTACAGATGAAAAAGCCACGAGTCTGTCGT CTGCGAGCGTTGACGACGCGGCTGTCATTGAAGAACTGATT GCCAAGTTAGAAGAAGTTTCCAAAACACTGCCGTCAGGGTT CCACATGAACCCGATCCAGTTTGAAAAAGATGATGACACAAA CTTCCACATGGATGTGATCGCGGGCTTTGCCAACATGCGTG CGAGAAATTACAGCATTCCGGAAGTGGACAAATTAAAGGCCA AATTTATAGCCGGCCGCATCATCCCAGCGATCGCCACCTCCA CCGCGATGGCCACGGGCCTCGTCTGCCTTGAGCTTTATAAA GCCCTGGCTGGTGGACACAAGGTGGAAGACTACCGCAACA CGTTTGCAAACCTCGCAATCCCTCTGTTCTCGATTGCCGAAC CGGTTCCACCCAAAACCATCAAACACCAGGAATTATCGTGGA CGGTCTGGGACCGTTGGACCGTGACGGGCAATATCACGCTG AGGGAACTCCTGGAGTGGCTCAAAGAAAAAGGCCTGAACGC GTACAGCATTTCCTGTGGCACCTCGCTGCTGTACAACTCCAT GTTCCCCCGTCACAAAGAACGGCTTGACCGAAAGGTAGTTG ATGTTGCCCGTGAGGTGGCCAAGATGGAGGTGCCCTCTTAC CGGCGTCATCTGGACGTCGTGGTGGCGTGCGAGGATGACG ACGATAATGATGTCGACATCCCACTGGTGTCGGTGTACTTCC GCTAAatcgaagagaccgcatgccatat	
UbcH5A/ UBE2D1	atatcatatgggtctcacataATGGCGCTGAAACGCATTCAGAAAGAAC TGAGCGATCTGCAGCGCGATCCGCCGGCGCATTGCAGCGC GGGCCCGGTGGGCGATGATCTGTTTCATTGGCAGGCGACCA TTATGGGTCCGCCGGATAGCGCGTATCAGGGCGGCGTGTTT TTTCTGACCGTGCATTTTCCGACCGATTACCCGTTCAAACCG CCGAAAATTGCCTTTACCACCAAAATTTATCATCCGAATATTAA TAGCAACGGCAGCATCTGCCTGGATATTCTGCGCAGCCAGT GGAGCCCGGCGCTGACCGTTAGCAAAGTGCTGCTGAGCATT TGCAGCCTGCTGTGTGACCCGAACCCGGATGATCCGCTGGT GCCGGATATTGCGCAGATTTACAAAAGCGATAAAGAAAAATAT AACCGTCACGCCCGTGAATGGACCCAGAAATACGCGATGTA Atcgaagagaccgcatgccatat	UniProt P51668
Ubiquitin	atatcatatgggtctcacataATGCAAATCTTCGTGAAAACTCTGACCG GTAAGACCATCACGCTGGAAGTTGAGCCGAGCGACACAATA GAGAATGTCAAAGCCAAGATTCAAGATAAAGAAGGCATTCCG CCAGATCAGCAGCGCTTGATCTTTGCGGGAAAACAGCTGGA AGATGGTCGTACCCTGAGTGACTATAACATTCAGAAAGAATC CACGCTTCATCTGGTACTCCGCTTACGGGGCGGGTAAtcgaag agaccgcatgccatat	[6], codon-optimized

Supplementary Table 4: Primers

Oligo ID	Description	Sequence
o005	T7 Promoter CGG F	tatgggtctcactatTAATACCGGTCACTATAGgtacagagaccgcatg
o006	T7 Promoter CGG R	cggtctctgtacCTATAGTGACCGGTATTAatagtgagaccca
o009	gIII F	atatcatatgggtctcacataatgaaaaaattattattcgcaatTCCT
o013	Ps70 + RBS F	CATTAGTTACTGGCGCAC
o014	Ps70 + RBS R	ACGAGTTCTGATCACAG
o016	Luciferase from pJC175e R	ATATGGCATGCGGTCTCTTCGATTAGGTATATTCCGTGTGGTACTTC
o017	Sequencing Level 0,1 F	CTATAAAAATAGGCGTATCACG
o018	Sequencing all Levels R	CTGATTCTGTGGATAACCGTAT
o019	Sequencing Level 2 F	GAATTCGCGGCCGCTTCTAGA
o022	L2 F	tatgccGCCAGATCCGCCGGAGGT
o023	L2 R	taaaACCTCCGGCGGATCTGGCgg
o024	L3 F	atatcatatgggtctcaTAAACTGATTAAAGCAGCACA
o025	L3 R	atatggcatgcggtctctTATGCCTTGTGGACG
o026	L4 F	atatcatatgggtctcaTAAAGGAGGTAGTGCAGG
o027	L4 R	atatggcatgcggtctctTATGCCTCCACTACTCG
o060	EGLN3_MIADVEP F	ATGATTGCGGATGTGGAACCGATCT
o061	EGLN3_MIADVEP R and MIQDVEP R	GCTTTTGCCTTCCGGAAAAATGCG
o062	EGLN3_FIQDVEP F	CAGGATGTGGAACCGATCTTCGA
o063	EGLN3_FIQDVEP R	AATAAAGCTTTTGCCTTCCGGAAAAATGC
o066	EGLN3_FIADWEP R	ATCCGCAATAAAGCTTTTGCCTTCCGGA
o067	EGLN3_FIADVEA F	GCGATCTTCGATCGCCTGCTG
o068	EGLN3_FIADVEA R	TTCCACATCCGCAATAAAGCTTTTGC
o069	EGLN3_FIADWEP F	TGGGAACCGATCTTCGATCGCCT
o101	Sequencing KanR start	TGCTGGATGAATTCTTTTGA
oLS-1662	SP F	CACTGTTCATCTGTCCTCTTTC
oLS-1663	SP R	CGACCTGCTCCATGTTACTTAG
oLS670	Sequencing SP	GCAACTATCGGTATCAAGC

Supplementary Table 5: Bacterial strains

Cell line	Description	Source
DH5α	Genotype F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(rK-mK+), λ–	18265017, ThermoFisher Scientific
S2060	Derived from DH10β, genotype F' proA+B+ Δ(lacIZY) zzf::Tn10 lacIQ1 PN25-tetR luxCDE Ppsp(AR2) lacZ luxR Plux groESL / endA1 recA1 galE15 galK16 nupG rpsL ΔlacIZYA araD139 Δ(ara,leu)7697 mcrA Δ(mrr-hsdRMS-mcrBC) proBA::pir116 araE201 ΔrpoZ Δflu ΔcsgABCDEFG ΔpgaC λ–	Addgene #105064
S2208	Strain S2060 transformed with plasmid pJC175e	This work

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