

Supplementary information

Design and evaluation of a tripartite chemogenetic fluorescent reporter for visualizing ternary protein complexes

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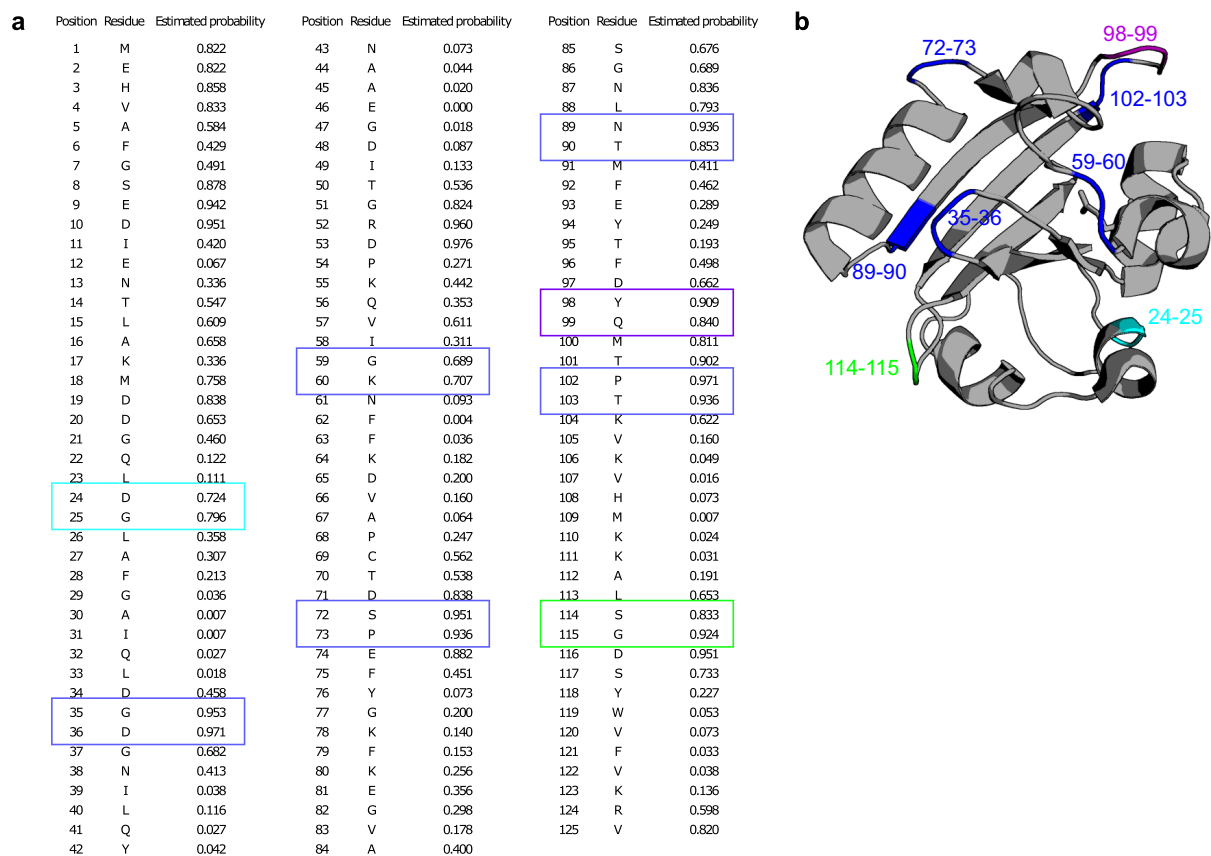
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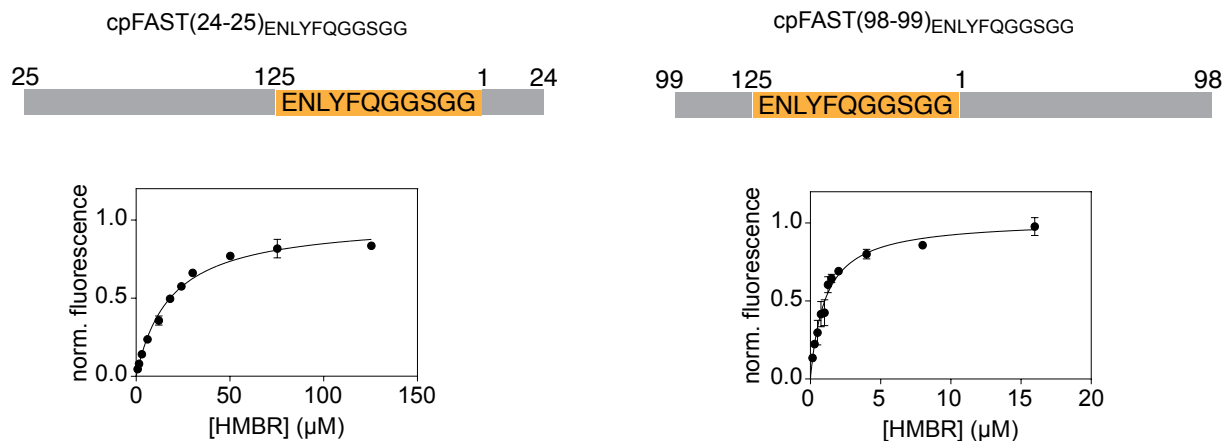
Supplementary Figs 1-9

Supplementary Table 1

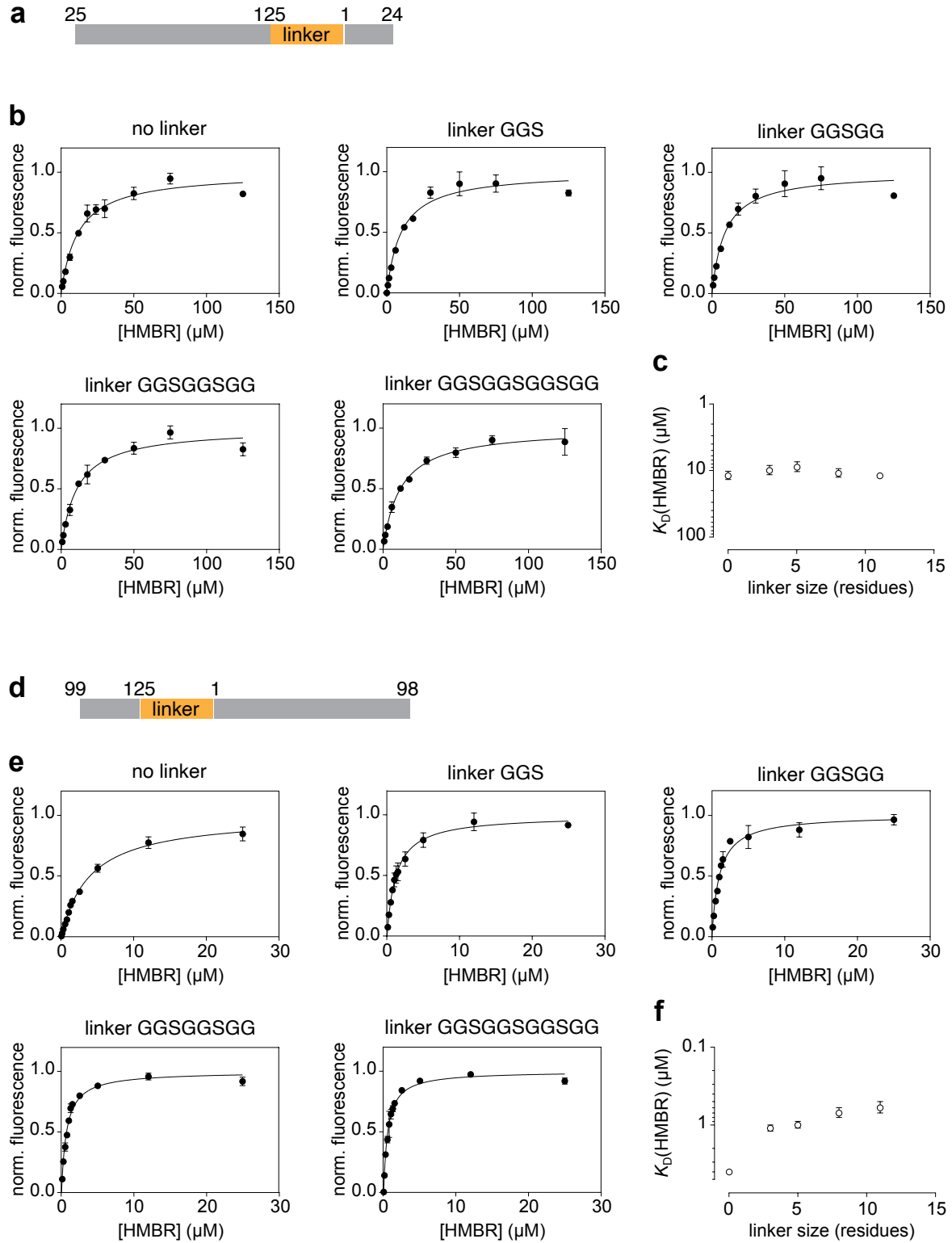
Supplementary references



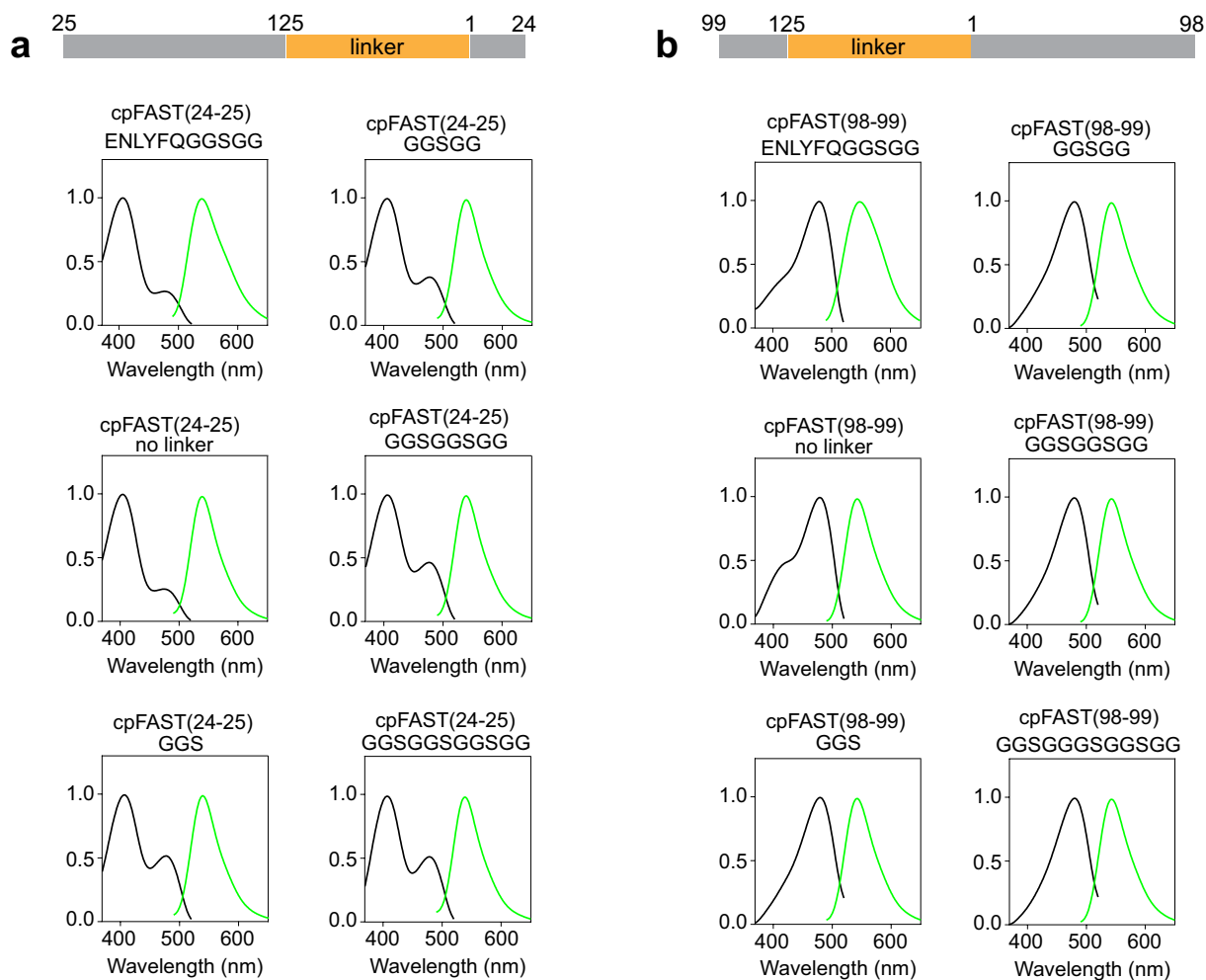
Supplementary Figure 1. Identification of the split sites for the design of putative circular permutations of FAST. **a** Probability for the circular permutation of the photoactive yellow protein PYP (PDB 1NWZ), the parental protein of FAST, estimated using CPred. **b** Split sites retained for the generation of circular permutations of FAST and further experimental analysis.



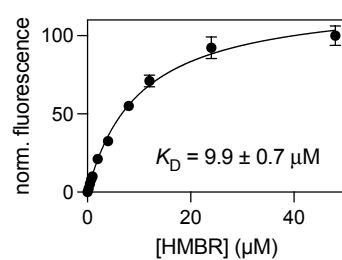
Supplementary Figure 2. Characterization of the fluorogen binding affinity of cpFAST(24-25)_{ENLYFQGGSGG} and cpFAST(98-99)_{ENLYFQGGSGG}. HMBR titration curves in pH 7.4 HEPES buffer (50 mM) containing NaCl (150 mM). The protein concentration was fixed to 0.1 μM. Data represent the mean ± SD of three experiments. Least-squares fit (line) gave the thermodynamic dissociation constant $K_{D,HMBR}$ provided in **Fig. 2a**. Source data are provided as a Source Data file.



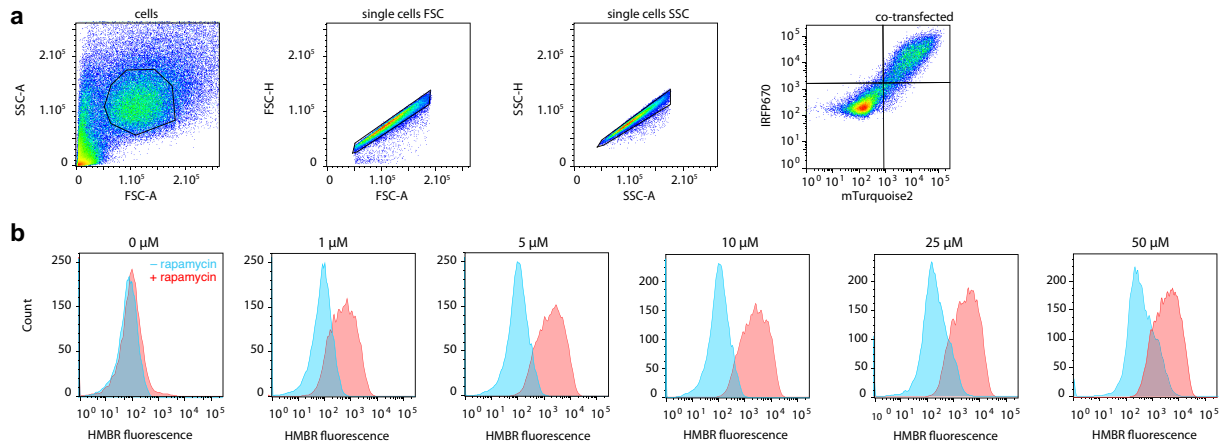
Supplementary Figure 3. Influence of the linker in cpFAST(24-25) and cpFAST(98-99).
a,d Schematic representation of cpFAST(24-25)_{linker} and cpFAST(98-99)_{linker}, respectively. **b,e** HMBR titration curves for the different circular permutations, in pH 7.4 HEPES buffer (50 mM) containing NaCl (150 mM). The protein concentration was fixed to 0.1 μM . Data represent the mean $\pm$ SD of three experiments. Least-squares fit (line) gave the thermodynamic dissociation constant $K_{D,\text{HMBR}}$ provided in **Fig. 2b**. **c,f** Evolution of HMBR dissociation constants in function of the linker size. Source data are provided as a Source Data file.



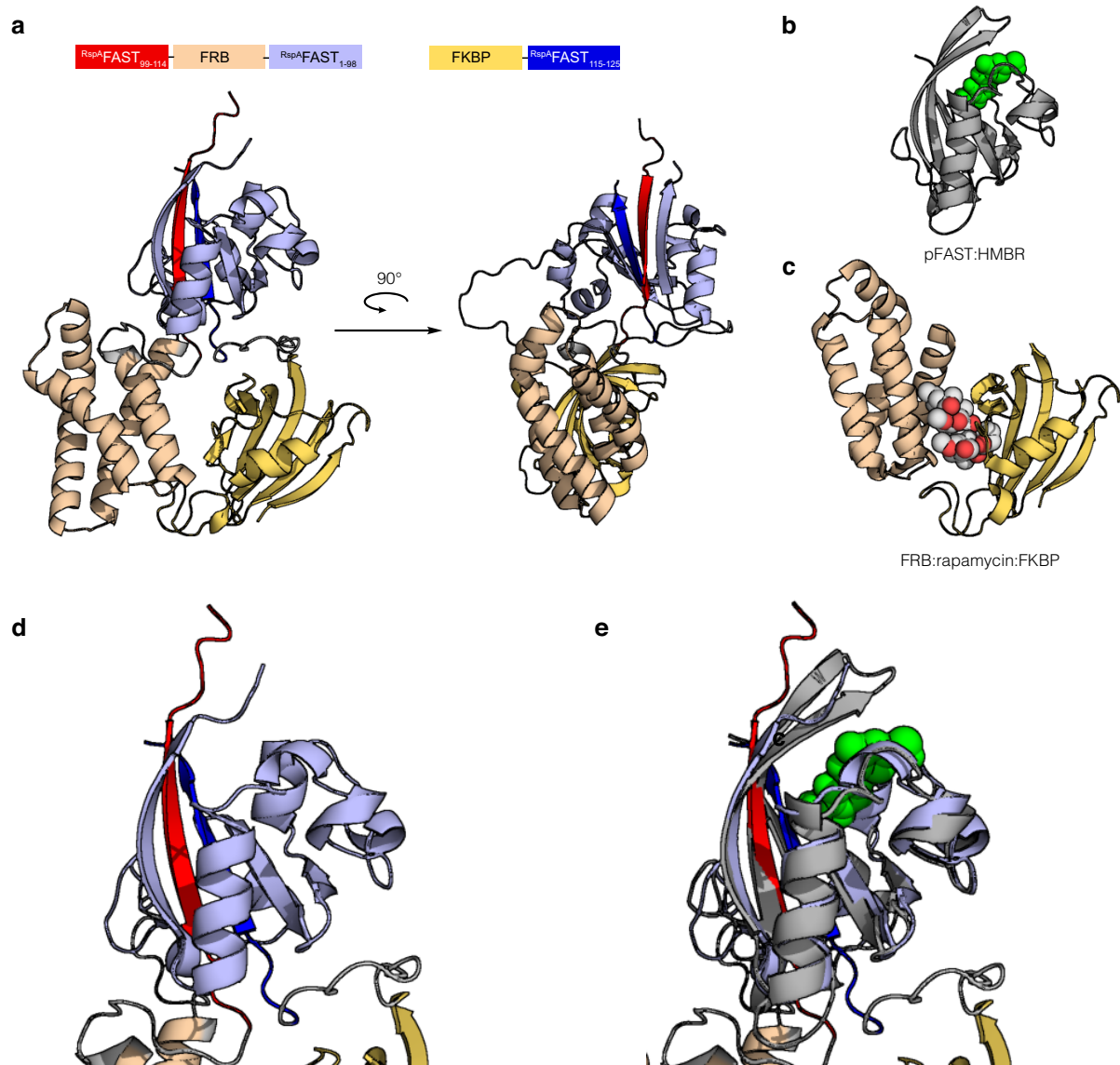
Supplementary Figure 4. Spectral properties of the cpFAST:HMBR assemblies. Normalized absorption (black) and emission (green) spectra of the cpFAST(24-25)_{linker} (**a**) and cpFAST(98-99)_{linker} (**b**) with HMBR. Spectra for ENLYFQGGSGG and other GGS linkers were recorded using 3 μ M and 1.5 μ M HMBR, respectively, and 40 μ M protein in pH 7.4 phosphate buffer saline at 25°C. Source data are provided as a Source Data file.



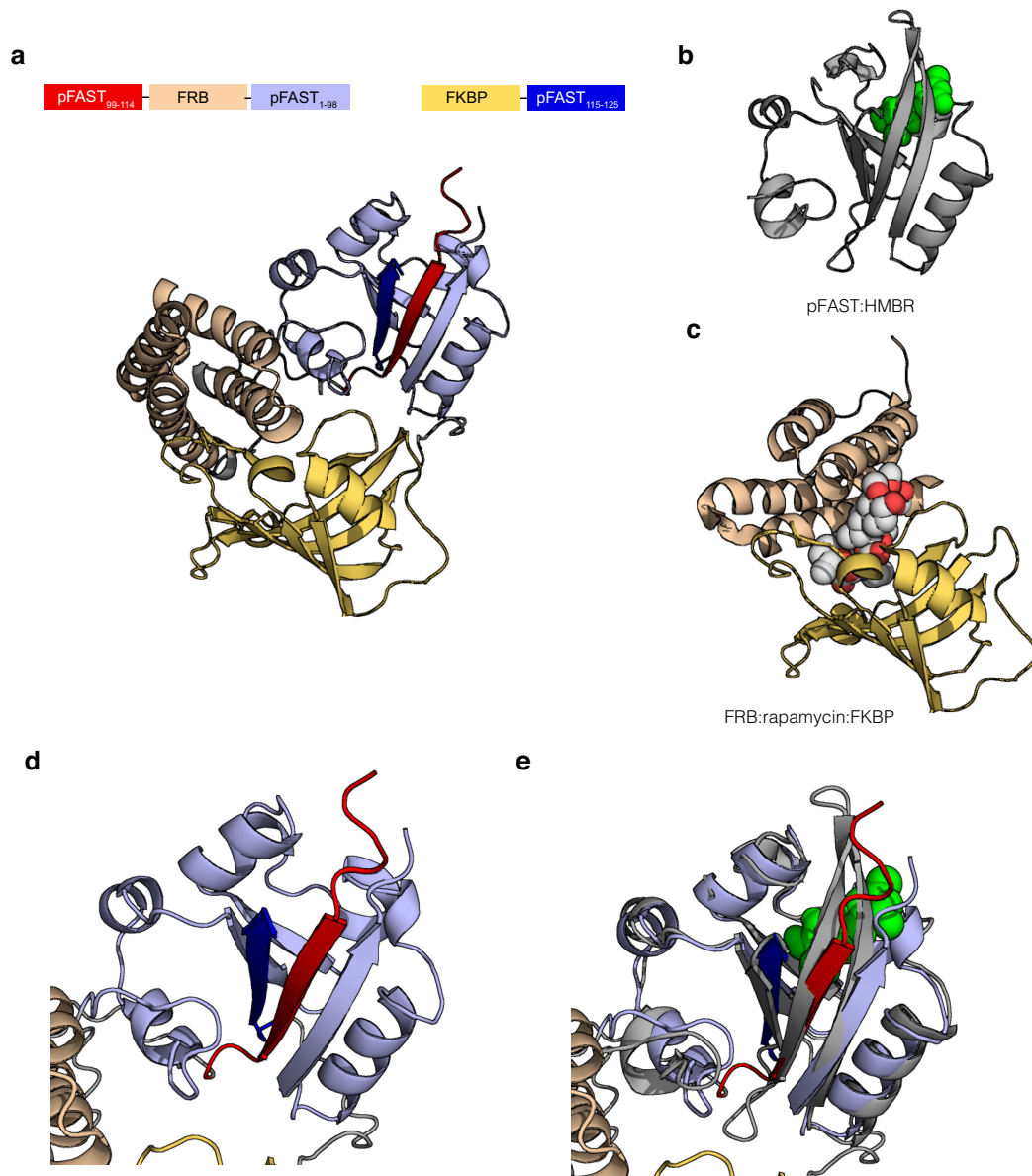
Supplementary Figure 5. Characterization of the fluorogen binding affinity of nanoFAST. HMBR titration curves for nanoFAST, in pH 7.4 phosphate buffer (50 mM) containing NaCl (150 mM). The protein concentration was fixed to 0.5 μM. Data represent the mean ± SD of three experiments. Least-squares fit (line) gave the indicated thermodynamic dissociation constant K_D . Source data are provided as a Source Data file.



Supplementary Figure 6. Flow cytometry analysis. **a** Gating strategy. Side versus forward scatter (SSC-A vs FSC-A) density plot was used to identify cells based on size and granularity. Secondly, forward scatter height (FSC-H) vs. forward scatter area (FSC-A) density plot and then side scatter height (SSC-H) vs side scatter area (SSC- A) plot were used to select single cells. Co-transfected cells were then selected by using the signal of IRFP670 and mTurquoise2 transfection reporters. **b** Representative fluorescence analysis. HEK293T cells co-expressing the FK506-binding protein (FKBP) fused at the N-terminus of $R_{spA}FAST_{99-125}$ and the FKBP-rapamycin-binding domain of mammalian target of rapamycin (FRB) fused to the C-terminus of $R_{spA}FAST_{1-98}$ were treated without (blue) or with (red) 500 nM of rapamycin, and with 1, 5, 10, 25 or 50 μ M of HMBR and then analyzed by flow cytometry.



Supplementary Figure 7. AlphaFold-assisted topology choice. **a** AlphaFold¹ prediction of the RspA-FAST₉₉₋₁₁₄-FRB-RspA-FAST₁₋₉₈ / FKBP-RspA-FAST₁₁₅₋₁₂₅ interaction. **b** Model of the assembly pFAST:HMBR (ref. 2). **c** Structure of the FRB:rapamycin:FKBP complex (PDB 3FAP). **d** zoom on the assembled tripartite RspA-splitFAST. **e** zoom on the assembled tripartite RspA-splitFAST with superimposed pFAST:HMBR (pFAST in grey, HMBR in green).



Supplementary Figure 8. AlphaFold-assisted topology choice. **a** AlphaFold¹ prediction of the pFAST₉₉₋₁₁₄–FRB–pFAST₁₋₉₈ / FKBP–pFAST₁₁₅₋₁₂₅ interaction. **b** Model of the assembly pFAST:HMBR (ref. 2). **c** Structure of the FRB:rapamycin:FKBP complex (PDB 3FAP). **d** zoom on the assembled tripartite split pFAST. **e** zoom on the assembled tripartite split pFAST with superimposed pFAST:HMBR (pFAST in grey, HMBR in green).

a

FAST/1-125 1 MEHVAFGSEDIENTLAKMDDGQLDGLAFGAIQLDGDGNILQYNAAEGDITGRDPKQVIGKNFF 63
RspA-FAST/1-125 1 METVRFGGDDIENSLAKMDDKALDKLAFGAIQLDGNKILHYNAAEGTITGRDPKQVIGKNFF 63
pFAST/1-125 1 MEHVAFGSEDIENTLANMDDQLDRLAFGVQLDGDGNILLYNAAEGDITGRDPKQVIGKNFF 63

FAST/1-125 64 KDVAPGTDSPFEYGFKEGVASGNLNTMFEWMIPTSRGPTKVKVHMKKALS GDSYWFVVKRV 125
RspA-FAST/1-125 64 TDVAPGTQSKFEQGRFKEGVQKGD LNTMFEWMIPTSRGPTKVKVHMKKAMT GDSFWIFVKRL 125
pFAST/1-125 64 KDVAPGTDTPFEYGFKEGAASGNLNTMFEWTIPTSRGPTKVKVHLKKALS GDRYWFVVKRV 125

split site 98-99 split site 114-115

b

	FAST	RspA-FAST	pFAST
full-length	$K_{D,HMBR} = 0.13 \mu M$ (see ref 3)	$K_{D,HMBR} = 0.03 \mu M$ (see ref 4)	$K_{D,HMBR} = 0.01 \mu M$ (see ref 3)
bipartite 114-115	$EC_{50,-interaction} (HMBR) = 18 \mu M$ (see ref 4)	$EC_{50,-interaction} (HMBR) = 100 \mu M$ (see ref 4)	$EC_{50,-interaction} (HMBR) = 0.5 \mu M$ (see ref 5)
	$EC_{50,+interaction} (HMBR) = 3.1 \mu M$ (see ref 4)	$EC_{50,+interaction} (HMBR) = 1.0 \mu M$ (see ref 4)	$EC_{50,+interaction} (HMBR) = 0.5 \mu M$ (see ref 5)
bipartite 98-99 (See Fig. 3)	$EC_{50,-interaction} (HMBR) = 31 \mu M$	$EC_{50,-interaction} (HMBR) = 270 \mu M$	
	$EC_{50,+interaction} (HMBR) = 7.5 \mu M$	$EC_{50,+interaction} (HMBR) = 3.0 \mu M$	
tripartite (with 2 proteins) (See Fig. 4)		$EC_{50,-interaction} (HMBR) > 500 \mu M$	$EC_{50,-interaction} (HMBR) > 500 \mu M$
		$EC_{50,+interaction} (HMBR) = 123 \mu M$	$EC_{50,+interaction} (HMBR) = 12 \mu M$
		$EC_{50,-interaction} (HBR-2.5DM) > 500 \mu M$	$EC_{50,-interaction} (HBR-2.5DM) = 57 \mu M$
		$EC_{50,+interaction} (HBR-2.5DM) = 8.1 \mu M$	$EC_{50,+interaction} (HBR-2.5DM) = 1.2 \mu M$
tripartite (with 3 proteins) (See Fig. 5)			$EC_{50,-interaction} (HBR-2.5DM) = 91 \mu M$
			$EC_{50,+interaction} (HBR-2.5DM) = 0.31 \mu M$

Supplementary Figure 9. FAST variants and their bipartite and tripartite versions. a Alignment of the sequences of FAST, RspA-FAST and pFAST with the split sites 98-99 and 114-115 indicated. **b** Summary of the properties of their full-length, bipartite 114-115, bipartite 98-99 and tripartite versions. For the full-length proteins, the thermodynamic dissociation constants of the complex with the fluorogen HMBR ($K_{D,HMBR}$) are provided. For the bipartite and tripartite systems, the effective concentration for half maximal complementation in cells is given, either in the absence ($EC_{50,-interaction}$) or the presence ($EC_{50,+interaction}$) of an interaction. The fluorogens used are specified in brackets. ³⁻⁵

Supplementary Table 1. Plasmids used in this study

Plasmid	ORF	ORF sequence
pAG398	FAST- ENLYFQGGSGG- FAST-MYC	atggaaacatgtcgccttcggcagcgaggatcgcgaacactctggctaaagatggatgacgggcaactggacggcttggcattcggggccatt caactggacggggacggtaatatctccagtaacaatgcagctgaaggtgacataaactggacgtgactcctaaacaaagtattgccaagaatttc ttcaaaagatgttgcctccggccacggattcactgcagttttacgggaagttcaaaagaggcggtgctccggcaacctcaacactatgttcgaa tggtgatgataccgacaagcagagggcccaacaaaggttaaaatcctatgaagaagctctgtctggagactcttattgggtgtttgtgaagaga gtcgaacacgtgtattttcaggggcggtccggcgcggaacacgttgcctttggctctgaggtatcgaaaaatcgcgtggccaaaatggatgac ggcagctggatggcctggcattcggagccatacagctttgacggcgatggaaacatcctccagtaacaacggcgagggtgacattaccggt cgcatccaaaacaggttatttggcaagaactctttaaaggacgttgcacccgggactgactctccgaattttacggaaaagttcaaggaaagg gtggcatctggcaacttataacaatgtttgaatggatgattctactagttagaggacaaacaaaggtgaagatccacatgaagaagccttg tccggcgactcctactcgggtctttgtgaaacgggtgggactccgaacaaagcttatttctgaagaggactgttaa
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pAG479	HIS-FAST ⁹⁹⁻¹²⁵ - GGSGG-FAST ₁₋₉₈	atgggcagcagccatcatcatcatcatcacagcagcgccctggtgcccgcggcgagccatagtgctagcatgacgagggcccaacaaaggt AAATTCATATGAAGAAAGCTCTGCTCGGAGACTCTTATTGGGTGTTTGTGAAGAGAGTGGCGGCTccggcggtccggcggtccggcggtc TCTGAGGATATCGAAATACGCTTGCCCAAAATGGATGACGCTCAGCTGGATGGCCTGGCATTCGGAGCCATACAGCTTGACGGCGATGGAAC ATCTCCAGTACAAACCGCCGGAGGGTGACATTACCGGTCCGCATCCAAACAGGTTATTGGCAAGAACTCTTTAAGGACGTTTGACCCGGG ACTGACTCTCCGAATTTTACGGAAGTTCAAGGAAGGGTGGCATCTGGCAATCTTAATACAATGTTTGAATGGATGATTCTCTACT

pAG481	HIS-FAST ⁹⁹⁻¹²⁵⁻ GGs-FAST ₁₋₉₈	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcggcagccatatggctagcatgAGCAGAGGCCCAACCAAGGTA AAAaTTCATATGAAGAAAGCTCTGTCTGGAGACTCTTATTGGGTGTTTGTGAAGAGAGTcGggcggtccGAACACGTTGCCCTTTGGCTCTGAG GATATCGCAAAATACGCTGGCCAAAATGGATGACGGTCAGCTGGATGGCTGGCATTCCGAGGCCATACAGCTTGACGGCGATGGAACATCCTCAGTACAA CAGTACAAACGCCGGGAGGTGACATTACCGGTGCGGATCCAAACAGGTTATTGGCAAGAACTCTTTAAGGACGTTGCACCCGGGACTGAC TCTCCCAATTTTACGGAAAGTTCAAGGAAGGGTGGCATCTGCCAATCTTAATACAATCTTTGAATGGATGATTCTCACT
pAG483	HIS-FAST ⁹⁹⁻¹²⁵⁻ FAST ₁₋₉₈	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcggcagccatatggctagcatgAGCAGAGGCCCAACCAAGGTA AAAaTTCATATGAAGAAAGCTCTGTCTGGAGACTCTTATTGGGTGTTTGTGAAGAGAGTcGAACACGTTGCCCTTTGGCTCTGAGGATATCGAA AATACGCTGGCCAAAATGGATGACGGTCAGCTGGATGGCTGGCATTCCGAGGCCATACAGCTTGACGGCGATGGAACATCCTCAGTACAA GCCGCGGAGGGTGACATTACCGGTGCGGATCCAAACAGGTTATTGGCAAGAACTCTTTAAGGACGTTGCACCCGGGACTGACCTCCCGAA TTTTACGGAAAGTTCAAGGAAGGGTGGCATCTGCCAATCTTAATACAATCTTTGAATGGATGATTCTCACT
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pAG802	MYC-FKBP-FAST ⁹⁹⁻ 125-IRES-HA- iRFP670	atg[REDACTED]gaattccggagtgccaggtggaaccatctccccaggagacggcgccaccttccccaaagcgc ggccagacgtgcgtggtgcatcacacgggagctgtgaagatggaaagaaatttgattctccccgggacgaaacaaagccctttaaagtttatg ctaggcaagcaggaggtgatccgaggttggaagaagggttgcacagatgagtggtgcagagagccaaactgactatctccagattat gcatatggtgccactgggcccaggcatctccaccacatgccactctcgtcttcgatgtggagcttctaaaaactggaagaatccggagga ggcggcagcggcgagggggatccagcaggggacacccaagggtcaaggtgcacatgaagaagccctttccggtgacagcatattgggtctt gtgaacgggtgtaactcgaggactacaaggcagcagcgaagccgggagtcgcccctctcccccccccttaacgttactgctggccga agcgccttgaataaaggccggtgtgcgtttgtctatatgttattttccaccatattgcgctcttttggcaatgtgaggccggcgaacccggc cgtctctttgacgacattctcagggtcttcccccttcgccaaaggaatgcaaggtctgtggaatgctgtggaaggaagcattctcgt gaagctctttgaagacaaacacgtctgtagcgacctttgcaggcagcggaacccccaccttggcgacaggtgcctctggcgccaaaagcga cgtgtataagatacacactgcaaaaggcgcacacccccagtgccacgttgtgagttggatagttgtggaagagatcgaatggtctcctcaagc gtattcaacaagggtgtgaaggtgcccagaaggtaccaccattgtatgggatctgatctggggcctcggtgcacatgctttacatgtgtttag tcgaggttataaaacgtcttagcccccggaacccaggggagctggttttctttgaaaaacacgatataataggccacaacctgcgac gtaccatacagatgttccagattacgctgaattcattggcgctgaaggtcgactcactcctcgatcgagacgatccacatccccggcag cattcagcgtgcggtgctgctgtagcctgcagcgcgagcggtgcggatcacgcgcattacggaataatgcggcgctcttttggacgcga aactccgcggtcggtgagctactcgcgattactctggcgagacgcaagcccatgcgctgcgcaacgcctggcgagctcttcgatccaaa ggcagcggcgtgatctcgtgtggcgagcggcctgacgcgcgacacttcgaacatcactgcactgcagctgacggtacatgcgatcga gttcgagcctgcggcgccgaacaggcgcgaactcgtcgcgtgacgcgcgagatcatcgcgcgcaacaaagacgtgaagtcgctgcgaaga gatggccgcagcgtgcccgcgtatctgcaggcgatgctcggctatcacgcgctgatgttgtaccgcttcggcgacgacgctccgggatggt gatcgcgaggcggaagcgcagcagcctcgagagctttctcgttcagcactttccggcgctcgctggtccgcgacgagcgcggtcactgtact gaagaacgcgatccgctggtctcggatttcgcgcggcatcagcagccgagatggtgccgagcagcagcctccggcgcgcgctcgatctgtc gttcgcgacactgcgcagcatctcgcctgcacatctcgaatttctcgggaacatggcgctcagcgcctcgatgctcgctgcgatcattga cggcagcgtatggggtgatctgtcattcattacgagccgctgcgctgcgatggcgagcgcgttcggcgccgaattgttcgcgcgact cttatcgtgcacttcacgcgcgccccaccacaagc
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--	--

Plasmid	ORF	ORF sequence
pAG1127	MYC-RspA-FAST ⁹⁹⁻¹¹⁴ - FRB-FAST ¹⁻⁹⁸ - IRES-HA- mTurquoise2	atggaacaaaagcttatttctgaagaggacttgaattcagcagaggcccccaccasggtgaaggtgcacatgaagaagggccatgaccagcggc gggggaggtcccgaggcgaggcagcatgtgcatgaaggcctggaagaggcatctcggtttgtactttggggaaggaacgtgaaagcgatg tttgaggtgctggagcccttgcatgctatgatggaacggggccccagactctgaaggaacatctttaatcaggccctatggtcgagattta atggagggcccaagagtggtgcaggaagtacatgaaatcagggaatgtcaaggacctcaccacaagcctgggacctctattatcatgtgttcga cgaatctcaagcaggtctccggaggaggcgccgagcgccggagggggatccatggagacgctgagattccggcgcgacgacatcgagaacac ctggccaaagatggacgacaaggccctggacaagctggccttcggcgccatccagctggacggcaacggcaagatcatccactacaacgcggcc gagggcacacatcaccggcagagaccaccaagacgctgatcggaagaactttctccacgacgtggcccccgccacacagcaaggtttccag ggcagattcaaggaggcgctgcagaaggcgacactgaacacacatgttcgagtggtgatccccacacactcaggagactacaaggacgacgac gacaagccccgggatccgcctctccctccccccccctaacgttactggccgaagcgcttggaataaaggccggtgtgcgtttgtctatatg ttattttccaccatattgccgtcttttggcaatgtgaggggccggaacccctggccctgtctcttgacgagcatctcagggtctttccct ctcgccaaaggaatgcaaggtctgttgatgtcgtgaaggagcagttctcttggaagctctcttggaagcaaaacacgtctgtagcgacctt tgacggcagcggaacccccacactggcgacaggtgctctcgccgcaaaagccacgtgtataagatcacactgcaaaaggcgccacaacccccag tgccactgtgtgagttggtatgtgtggaagagtcacaaatggctctctcacaagcgtattcaacaaggggctgaaggtgcccagaaggtacc cattgtatgggactgtatctgggctcggtgcacatgctttacatgtgtttagtcgaggttaaaaaaacgtctaggcccccgcaaccaggg gacgtgtgttttctttgaaaaacacgatgataatggccacaacacatgcgatctaccatacagatgttccagattacgctgaattcattgt gagcaaggcgagaggtgttccacgggtgtgtcccatctggtcgagctggagcgcgacgttaaacggccacaagtttcagcgtgtccggcga ggcgaggcgatgccacctacggcaagctgacccctgaagtctcatctgacacacggcgaagctggccgtgcccacccctcgtgacacac ctgtctctgggctgcaagcttgcgcctaccccgcacacatgaagcagcagactttccaaagtcgcacatcccgcaaggtctacgtcca ggagcgacacatctctcaaggacgacgccaactacaagaccggcgccaggtgaagttcgaggcgacacccctggtgaacgcgcatcgagct gaaggcgatcgacttcaaggaggacgccaacatctggggcacaagctggagtaaacactctcagcgacaacgtctatatccacggcgacaa gcagaagaacggcatcaaggccaacttcaagatccggcacaacacatcgaggacggcggtcgatcgagcactaccagcagaacacccc catcgcgacggccccctgctgctgccgacacacactacctgagcaccacgtccaagctgagcaagaaccccaacgagaagcgcatcacat ggtcctgctggagttcgtgacggcgccggggtacactctcgccatggacgagctgtacaagttaa
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pAG1265	MYC -bFos(Δ179-193)-pFAST ¹¹⁵⁻¹²⁵ -IRES-HA-IRFP670	ATGGAACAAAGCTTATTCTGAAGAGGACTTgaattcggctgctgagcagtcacatcggtcgctcggttaaagtgtgaacaactgtccccggaagaggaaagagaacgctgcacatccgctggaacgttaacaaatggcgagcgaaatggcgttaaccgctgctggaactgacgcagaccctgcagcggaacacgcagctggaagacgaaatccgcgaggttcatctctggcggaacacgctcgcgctgcaaaatccccgaacacgtgggttccggaggagggcgagcgaggagggatccGGTGACAGATATTGGGTCTTTGTGAACGGGTGTAACTCGAGGACTACAAGGACGACGACGACAAGCCCGGATccgcccctctccctcccccccttaacgttactggcgaagcgcgttggaaataaggccggtgtgctgttctctatattgttttccaccatattgcgcttcttggcaatgtgagggcccggaacactggccctgtctcttgacgagcattctagggtcttccctctctgcgaaggaatgcaaggtctgttgaatgtcgtgaaggaagcagctctcttgaagacaaacaacgtctgtagcgaccccttgcagggcagcggaacccccacacgtggcgacaggtgcctctgcggccaaaagcaacgttatcaagatacacctgcaaaaggcggaacacccccagctgccaagttgtgagttgtgaaagagtgcaaatggctctcaagcttatcaacaagggtctgaagagtgccagaagtgtaaccattgtatgggatctgatctgggctcggtgcacatgtgttcatatgttttagtcgaggttaaaaaaaacgttagcccccgaaacacgagggcgagctgttttcccttgaaaaaacacgatgatataatgttGGCCAcaaccATGcgatcgTACCCATAACGATGTCCAGATTACGCTgaattcatggcgctgaaggtcgatctcaactctcgatcgcgagccgatccacatccccggcagcattcagcgtgcccgtgctgctgagcctgcgacgcgagcggtgcggatcagcgcatcaggaataatggcgcgcttcttggacgcaaaactcccgcggtgctgagctactcgccgattacttcggcgagacggaagcccatgcgctgcgcaacgcaactggcgagcttccgatccaaagcgacccggcgctgatcttcggttggcgcgacggcctgacccggcgacacttcgacatctcaactgcacatgcgatcgaggtacatcgatcatcgaattcgagctcgcgcgccgaacagcgccgaacactcgctcgggctgacgcgagatcgatcgaggtgcgcgctatctgcagggatgctcggtatcacgcgctgagttgttgaacgcttcgcggagcagcggtccgggatggatgcggcgagggcgagcgacactcgagacttctcggtcagcacttctccggtcctcgctggtccgcagcgagcggtcactgtacttgaagaacgcgatccgctggtctcggtatcgcgcgcatcagcagcgagatcggtcccgcgacgcagcgcctccggcgccgcgctcgatctgtgttcgcgcacactgcgcgacatctgcgcctgccatctcgaaattctcggaacatggggctcagcgctcgagctcgatgtcgtgcatcattgagggcagctatgggattgtatcattgtcatcattacgagcgcgctgcccgtgcgatggcgagcgctgcggcgaaatgttcgcgacttcttctatcgctcacttcaacgcgcgcggcggaatgttcgcgacttccacgttcaacggcgcccccaccacaacgc
pAG1713	H2B-pFAST ⁹⁹⁻¹¹⁴ -P2A-bJUN- pFAST ¹⁻⁹⁸ -IRES-HA-mTurquoise2	ATGCCCGAACCTCGGAAGTCAGCGCCCGCTCCCAAAAAGGCTCTAAAAAAGCTGTCCGCAAGACCCAGAAGAAGGGGATAAGAAAAAGCGTAAGACACAGGAAGAGAGTATACGCCATTTACGTGTACAAAGTACTAAAAAAGTCCACCCGGACATGGCATCTCTCTCAAGGCGATGGCATTATGAACCTATTGTAAACGACATCTTCGAGCGCATCGCGGAGAAGCGTCGCGCTGGCGCATTACAAACAGCGCTCCACTATCACATCCCGGAGATCCAGACGGCGCTGCGCTGCTCCTGCCCGGAGAACTGGCCAAACACGCTGTGTCTGAGGGCAACAAAGCCGTGACCACTACACCACTCCCAAGACCGGGACCAACCAAGTCAAGTGCACATTGAAGAAAGCCCTTTTCagcgcgggggagggctccggagggcggagggcagcGGAAGCGGAGCTACTAATCTCAGCGCTGTGAAGCAGCGCTGGAGAGAGAACCTTGACCTAagcgagagggaaagcgcatgagaacacgcgatcgctgctccaagtgcgggaaaggaagctggagcgagatcgcccggttagaggaataagtgaaacacttgaaagcgcaaaactccgagctggcgtccacggccaacatgctcaggggaacaggtggcacagctttaaacagaaagtcatgaacacagcttaacagttgggtgcaaacatgctaacgcagcagttgcaaacgtttTccggaggagggcgagcgggcgagggggatccATGGAGCATGTTGCTCTTGGCAGTGAGGACATCGAGAACACTCTGGCCAATATGGACGACGAACAACCTGGATAGTTGGCTTTGGCGTAATTCAGCTCGATGGTGACGGGAATATCTGCTGTAATGCTGCTGAAGGGGACATCACTGGCAGAGATCCCAACACAGGTGATTGGGAAGAACTTCTTCAAGGATGTTGCACCTGGAACGGATACTCCGAGTTTACCGCAAAATCAAGGAAGCGCAGCGTCAGGGAATCGAACACCATGTTTCAATGGACGATACCGACATAACTCGAGGACTACAAGGACGACGACGACAAGCCCGGATccgcccctctccctcccccccttaacgttactggcgaagcgcgttggaaataaggccggtgtgctgttctctatattgttatcttccaccatattgcgctcttttggcaatgtgagggcccggaacacgtggccgtctcttggacgacattcctagggtcttccctctcgccaaaggaatgcaaggtctgttgaatgtcgtgaaggaagcagttctcttggaagcttcttgaagacaaacacgtctgtagcgaccccttgcagggcagcggaacccccacactggcgacaggtgcctctgcggccaaagccaagctgtataagatacacactgcgaaggcggaacacccacgtgccacgttgtgagttggatagtttggaaagagtgcaaatggctctctcaagcgtattcaacaaggggtgaaggtgcccagaaggtaccccatgtatgggatctgatctggggctcggtgcacatgcttcaatgtgttagtcgaggttaaaaaaacgtctagcccccggaacacacggggagcgtgggttctcttgaaaaaacacgatgataatggCCAcaaccATGcgatcgTACCCATACGATGTCCAGATTACGCTgaattcatggtgagcaagggcgaggagctgttccacggggtggtgcccatctggtcgagctggagcgagctaaacggccacaagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgacctgaagttcatctgcaccacggcaagctgcccgtgcccgtgcccacccctcgtgaccacctgtctcgggcgctgctctgcgcgctaccccgacacatgaagcagcagcacttctcaagtcggcatgcccgaaggtcactgcaggaagcgacacatctcttcaaggagcagcgcaactacaagacccgcgcgaggtgaagttcgagggcgacacccctggtgaacgcgatcgagctgaaggagatcgacttcaaggaggaagcgcaacatcctggggcaacagctggagtacaactacttcagcgcaacagcttatataccgcgcgacaagcagaagaacggcatcaaggccaacttcaagatccggcaacacatcgaggacggcggtgcagctgcgcgacacactaccagcagaacaccccatcgcgcgagggcccgctgctgctgcccgaacacactacctgagcaccagctccaagctgagcaagaccccaacgagaagcgcatcacatggtcgtgaggtctgtgaggtctgtgacggatgataaggtaa

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