Supplementary

A Genetic Approach for Cooperative Function of tRNA Mimicry Complex, eRF1/eRF3 in translation termination on the Ribosome

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SUPPLEMENTARY TABLE AND FIGURES LEGENDS

Table S1. List of *S. cerevisiae* strains used in this study.

Table S2. List of vectors and plasmids used in this study.

Table S3. List of primers used in this study.

Figure S1

A) Schematic drawing of domain structures and conserved regions between Pc-eRF3 and aEF1α. The conserved G-domain motifs are shown as black squares according to ref. 11.

B) Sequence alignment of Pc-eRF3 and its homologs. Muscle (67, 68) and ESPript (http://espript.ibcp.fr/ESPript/ESPript/index.php) (69) programs were used to generate the alignment with secondary structure of aEF1α. Sc-eRF3: *Saccharomyces cerevisiae* eRF3, Sp-eRF3: *Schizosaccharomyces pombe* eRF3, Hs-eRF3: human GSPT1, Ap-EF1a: *Aeropyrum pernix* EF1α, Ec-EFTu: *E. coli* EF-Tu, Ta-EFTu: *Thermus aquaticus* EF-Tu. The Pc-eRF3 mutation sites identified in this study are indicated by triangles.

Figure S2

Sequence alignment of Sc-eRF1 and its homologs. Muscle (67, 68) and ESPript (http://espript.ibcp.fr/ESPript/ESPript/index.php) (69) programs were used to generate the alignment and secondary structure prediction. Sp: *Schizosaccharomyces pombe*, Hs: human, aRF1: *Aeropyrum pernix* aRF1. The Sc-eRF1 cooperative mutation sites identified in this study are indicated by triangles.

Figure S3

Complementation analysis of eRF3 temperature sensitive strain (eRF3ts) of *S. cerevisiae* (YK21-02). Growth of the eRF3ts strain that had been transformed with the Pc-eRF3-mutant plasmids at restrictive temperature, 37℃, is shown. (-: no growth, +: weak growth, ++: good growth)

Figure S4

Yeast two-hybrid binding assay of Pc-eRF3c mutants against Sc-eRF1. Binding domain vector pGBT9 alone, or fused to Sc-eRF3c, Pc-eRF3c^{wt}, or its mutant genes were introduced into the AH109 strain together with pGAD424-Sc-eRF1, and growth of the transformants were examined on selection plates. (-: no binding, +: weak binding, ++: good binding) This figure is complete

data set of Figure 2B.

Figure S5

Complementation analysis of the double tet-OFF strain of *S. cerevisiae* (Y138) by Pc-eRF3c mutants. p416GPD vector, p416GPD-Sc-eRF3c, p416GPD-Pc-eRF3 c^{wt} and its mutants were introduced into Y138 in combination with p414GPD-Sc-eRF1, and growth of the transformants were examined in the presence of 7.5 μg/ml doxycycline. (-: no growth, +: weak growth, ++: good growth)

Figure S6

Yeast two-hybrid binding assay of Sc-eRF1 mutants against Pc-eRF3c^{wt}. Activation domain vector pGAD424 alone, or fused to Sc-eRF1 or its mutant genes were introduced into the AH109 strain together with pGBT9-Pc-eRF3c^{wt}, and growth of the transformants were examined on selection plates. (-: no binding, +: weak binding, ++: good binding) . This figure is complete data set of Figure 4C.

SUPPLEMENTARY REFERENCES

67. Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**(5):1792-1797.

68. Edgar, R.C. (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, (**5**) 113.

69. Gouet, P., Robert, X. and Courcelle E. (2003) "ESPript/ENDscript: extracting and rendering sequence and 3D information from atomic structures of proteins". Nucl. Acids Res. 31(13), 3320-3323

70. Kikuchi, Y., Shimatake, H. and Kikuchi, A. (1988) A yeast gene required for the G1-to-S transition encodes a protein containing an A-kinase target site and GTPase domain. Embo J, 7, 1175-1182.

71. Mumberg, D., Muller, R. and Funk, M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene, 156, 119-122.

72. Fields, S. and Song, O. (1989) A novel genetic system to detect protein–protein interaction. Nature 340, 245–246.

Table S1. Yeast strains

Y40	MATα ade2D::hisG his3D200 leu2D0 lys2D0 met15D0 trp1D63 ura3D0 *SUP35::HIS3* HO::tTA-tetO (YDR172W/SUP35):: kanMX4	This work
YK21-02	MATα *gst1-1*(*sup35ts*)	(70)
Y138	MATα ade2D::hisG his3D200 leu2D0 lys2D0 met15D0 trp1D63 ura3D0 *SUP35::HIS3* HO::tTA-tetO (YDR172W/SUP35):: kanMX4 tTA-tetO (YBR143C/SUP45)::hphMX4	This work
Y133	MATα *can1-100 ade2-101* his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 sal2(sup35ts) HO::Rluc-[UGA]-Luc2	This work
Y134	MATα can1-100 ade2-101 his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 sal2(sup35ts) HO::Rluc-[UGG]-Luc2	This work
S13-I01	MATα ura3-1 ade2-1 leu2-3,112 sal4-2 (sup45ts) HO::Rluc-[UAA]-Luc2	(47)
S13-I03	MATα ura3-1 ade2-1 leu2-3,112 sal4-2 (sup45ts) HO::Rluc-[UAG]-Luc2	(47)
S13-I05	MATα ura3-1 ade2-1 leu2-3,112 sal4-2 (sup45ts) HO::Rluc-[UGA]-Luc2	(47)
S13-I07	MATα ura3-1 ade2-1 leu2-3,112 sal4-2 (sup45ts) HO::Rluc-[UGG]-Luc2	(47)
AH109	MATa *trp1-901 leu2-3 112 ura3-52 his3-200 gal4Δ gal80Δ* *LYS2*::$GAL1_{UAS}$-$GAL1_{TATA}$-HIS3 $GAL2_{UAS}$-$GAL2_{TATA}$-ADE2 *URA3*::$MEL1_{UAS}$-$MEL1_{TATA}$-lacZ, MEL1	CLONTECH

Table S2. Vectors and plasmids

S. cerevisiae expression vectors		
P416CYC	CYC promoter CEN6/ARSH4 URA3	(71)
P416GPD	GPD promoter CEN6/ARSH4 URA3	(71)
P414CYC	CYC promoter CEN6/ARSH4 TRP1	(71)
P414GPD	GPD promoter CEN6/ARSH4 TRP1	(71)
p416GPD-FLAG	p416GPD vector containing FLAG-tag gene for N-terminal fusion	This study
p416CYC-FLAG	p416CYC vector containing FLAG-tag gene for N-terminal fusion	This study
pGAD424	Two-hybrid DNA activation domain vector, GAL4AD (aa768-881)	(72) CLONTECH
pGBT9	Two-hybrid DNA binding domain vector, GAL4BD (aa1-147)	(72) CLONTECH

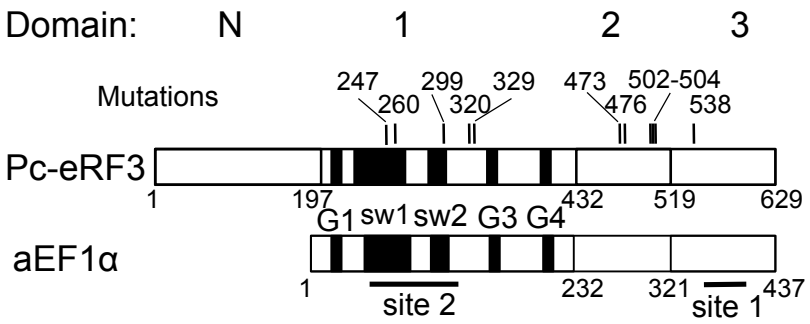
plasmids	
p416GPD-full-length Pc-eRF3	Pc-eRF3 coding gene (nt 1-1890) in p416GPD
p416GPD-Pc-eRF3c	Pc-eRF3 coding gene (nt 592-1890) with ATG codon in p416GPD
p416CYC-Sc-eRF3c	Sc-eRF3 coding gene (nt 760-2058) in p416CYC
p416GPD-Sc-eRF3c	Sc-eRF3 coding gene (nt 760-2058) in p416GPD
p416GPD-FLAG-full-length Pc-eRF3	Pc-eRF3 coding gene (nt 1-1890) in p416GPD-FLAG
p416GPD-FLAG-Pc-eRF3c	Pc-eRF3c coding gene (nt 592-1890) in p416GPD-FLAG
p416CYC-FLAG-Sc-eRF3c	Sc-eRF3c coding gene (nt 592-1890) in p416CYC-FLAG
p414GPD-Sc-eRF1	Sc-eRF1 in p414GPD
p416CYC-Sc-eRF1	Sc-eRF1 in p416CYC
pGBT9-Pc-eRF3c	Pc-eRF3c coding gene (nt 592-1890) in pGBT9
pGBT9-Pc-eRF3△1	Pc-eRF3 coding gene (nt 1294-1890) in pGBT9
pGBT9-Pc-eRF3c K349A	Pc-eRF3 coding gene (nt 592-1890) K349A in pGBT9
pGBT9-Sc-eRF3c	Sc-eRF3 coding gene (nt 760-2058) in pGBT9
pGAD424-Sc-eRF1	Sc-eRF1 in pGAD424

Table S3. Primers

P363	5'-GGGATCCATATGAATTCAGAGGCAAACAACAGTGA-3'
P318	5'-GGGTCGACCTACTCCTCGGAGATAAGTT-3'
P362	5'-GGGATCCATATGTATGGAAAAGAACACGTAAATG-3'
P555	5'-GGGATCCATATGCCATTAATCATTCCTATTCA-3'
P549	5'-ATTGTAGCAATTAATGCAATGGATGATCCTAC-3'
P550	5'-GTAGGATCATCCATTGCATTAATTGCTACAAT-3'
P291	5'-GGGATCCATATGTTTGGTGGTAAAGATCAC-3'
P292	5'-GGCTCGAGTTACTCGGCAATTTTAACAAT-3'
P289	5'-GGGATCCATATGGATAACGAGGTTGAAAAA-3'
P306	5'-GGGTCGACTTAAATGAAATCATAGTCAGATCC-3'
P420	5'-GGTCTAGAACTAGTCCATGGACTACAAAGACGATG-3'
P421	5'-GGGGATCCTGTCGTCATCGTCTTTGTAGTCCATGG-3'

Figure S1

A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aEF1α
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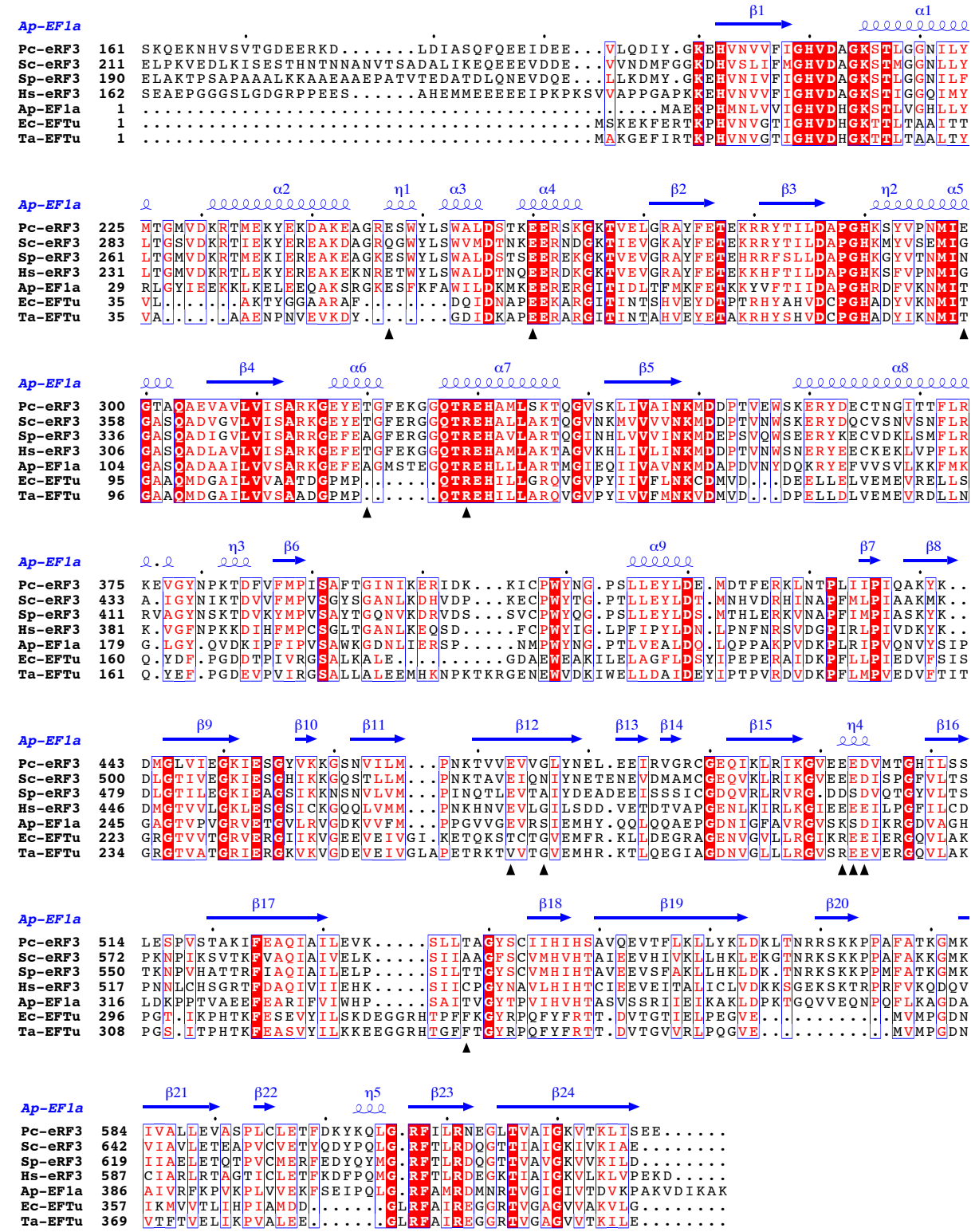
B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Sp-eRF3 190 ELAKTPSAPAAALKKAAEAAEPATVTEDATDLQNEVDQE...LLKDMY.GKEHVNIVFIGHVDAGKSTLGGNILF
Hs-eRF3 162 SEAEPGGGSLGDGRPPEES.....AHEMMEEEEEIPKPKSVVAPPGAPKKEHVNVVFIGHVDAGKSTIGGQIMY
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Ta-EFTu 1 .....MAKGEFIRTKPHVNVGTIGHVDHGKTTLTAALTY
Pc-eRF3 225 MTGMVDKRTMEKYEKDAKEAGRESWYLSWALDSTKEERSKGKTVELGRAYFETEKRRYTILDAPGHKSYVPNMIE
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Sp-eRF3 261 LTGMVDKRTMEKIERDAKEAGKESWYLSWALDSTSEERDKCKTVEVGRAYFETEHRRFSLIDAPGHKGYVTNMIN
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Pc-eRF3 300 GTAQAEVAVLVISARKGEYETGFEKGGQTREHAMLSKTQGVSKLIVAINKMDDPTVEWSKERYDECTNGITTFLR
Sc-eRF3 358 GASQADVGVLVISARKGEYETGFERGGQTREHALLAKTQGVNKMVVVVNKMDDPTVNWSKERYDQCVSNVSNFLR
Sp-eRF3 336 GASQADIGVLVISARRGEFEAGFERGGQTREHAVLARTQGINHLVVVINKMDEPSVQWSEERYKECVDKLSMFLR
Hs-eRF3 306 GASQADLAVLVISARKGEFETGFEKGGQTREHAMLAKTAGVKHLIVLINKMDDPTVNWSNERYEECKEKLVPFLK
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Ta-EFTu 96 GAAQMDGAILVVSAADGPMP.....QTREHILLARQVGVPYIVVFMNKVDMVDD...DPELLDLVEMEVRDLLN
Ap-EF1a
Pc-eRF3 375 KEVGYNPKTDFVFMPISAFTGINIKERIDK...KICPWYNG.PSLLEYLDE.MDTFERKLNTPLIIPIQAKYK..
Sc-eRF3 433 A.IGYNIKTDVVFMPVSGYSGANLKDHVDP...KECPWYTG.PTLLEYLDT.MNHVDRHINAPFMLPIAAKMK..
Sp-eRF3 411 RVAGYNSKTDVKYMPVSAYTGQNVKDRVDS...SVCPWYQG.PSLLEYLDS.MTHLERKVNAPFIMPIASKYK..
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Ap-EF1a 179 G.LGY.QVDKIPFIPVSAWKGDNLIERSP.....NMPWYNG.PTLVEALDQ.LQPPAKPVDKPLRIPVQNVYSIP
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Ta-EFTu 308 PGS.ITPHTKFEASVYILKKEEGGRHTGFFTGYRPQFYFRTT.DVTGVVRLPQGVE.....MVMPGDN
Ap-EF1a
Pc-eRF3 584 IVALLEVASPLCLETFDKYKQLG.RFILRNEGLTVAIGKVTKLISEE.....
Sc-eRF3 642 VIAVLETEAPVCVETYQDYPQLG.RFTLRDQGTTIAIGKIVKIAE.....
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Figure S2

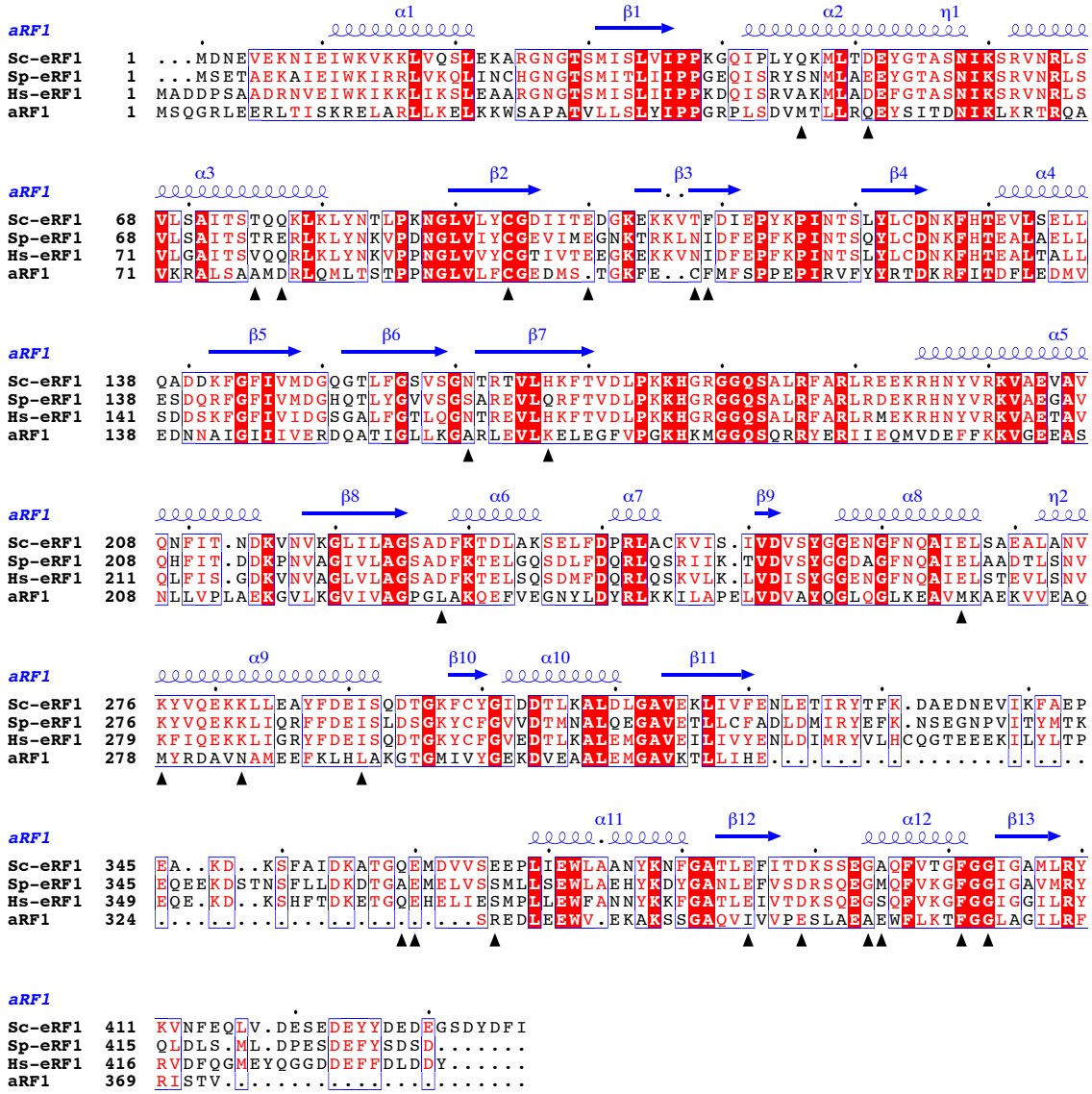
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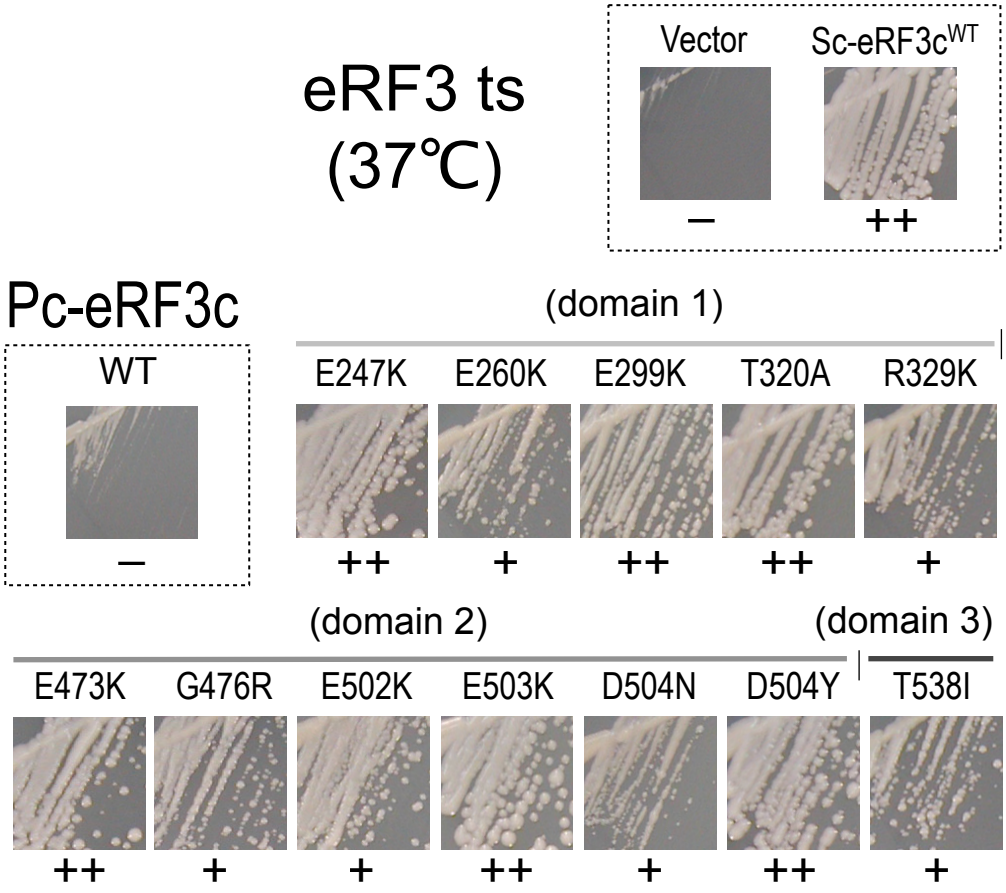
Figure S3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++
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T320A
R329K
–
++
+
++
++
+
(domain 2)
(domain 3)
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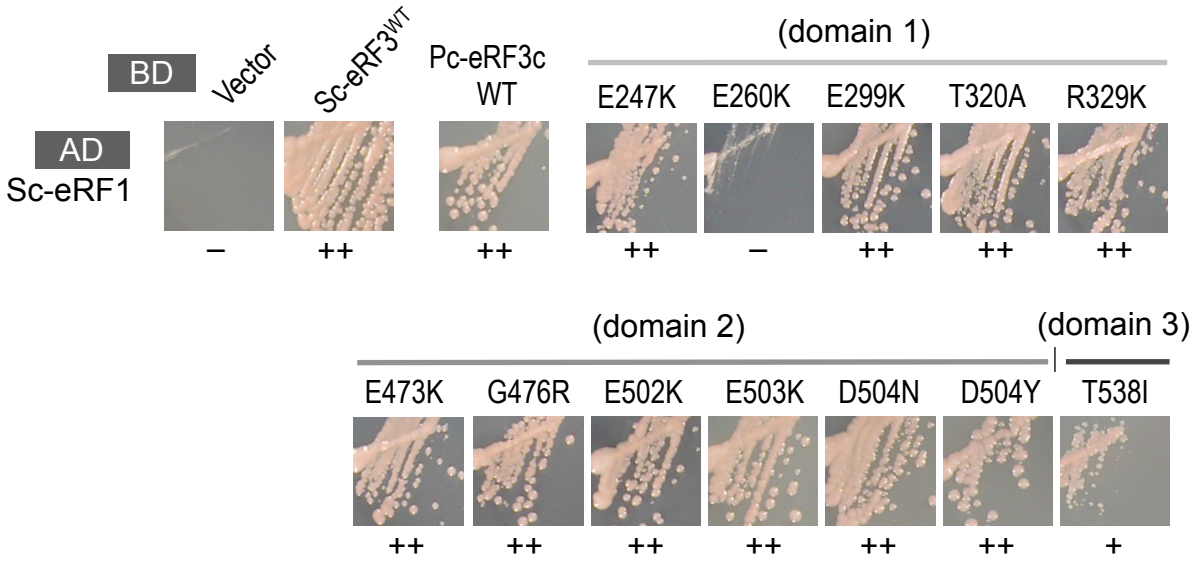
+

Figure S4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–
++
++
++
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++
++
++
(domain 2)
(domain 3)
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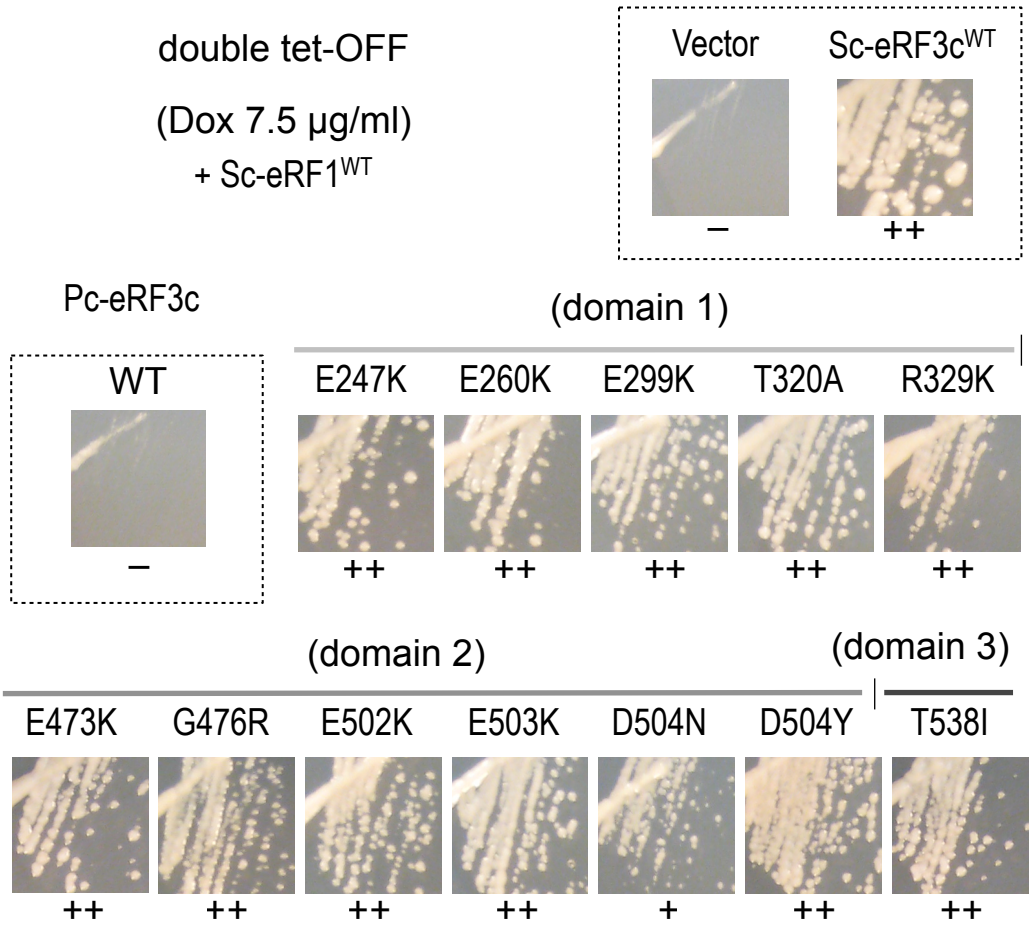
++
++
+

Figure S5


double tet-OFF
(Dox 7.5 μg/ml)
+ Sc-eRF1WT
Vector
Sc-eRF3cWT
–
++
Pc-eRF3c
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WT
E247K
E260K
E299K
T320A
R329K
–
++
++
++
++
++
(domain 2)
(domain 3)
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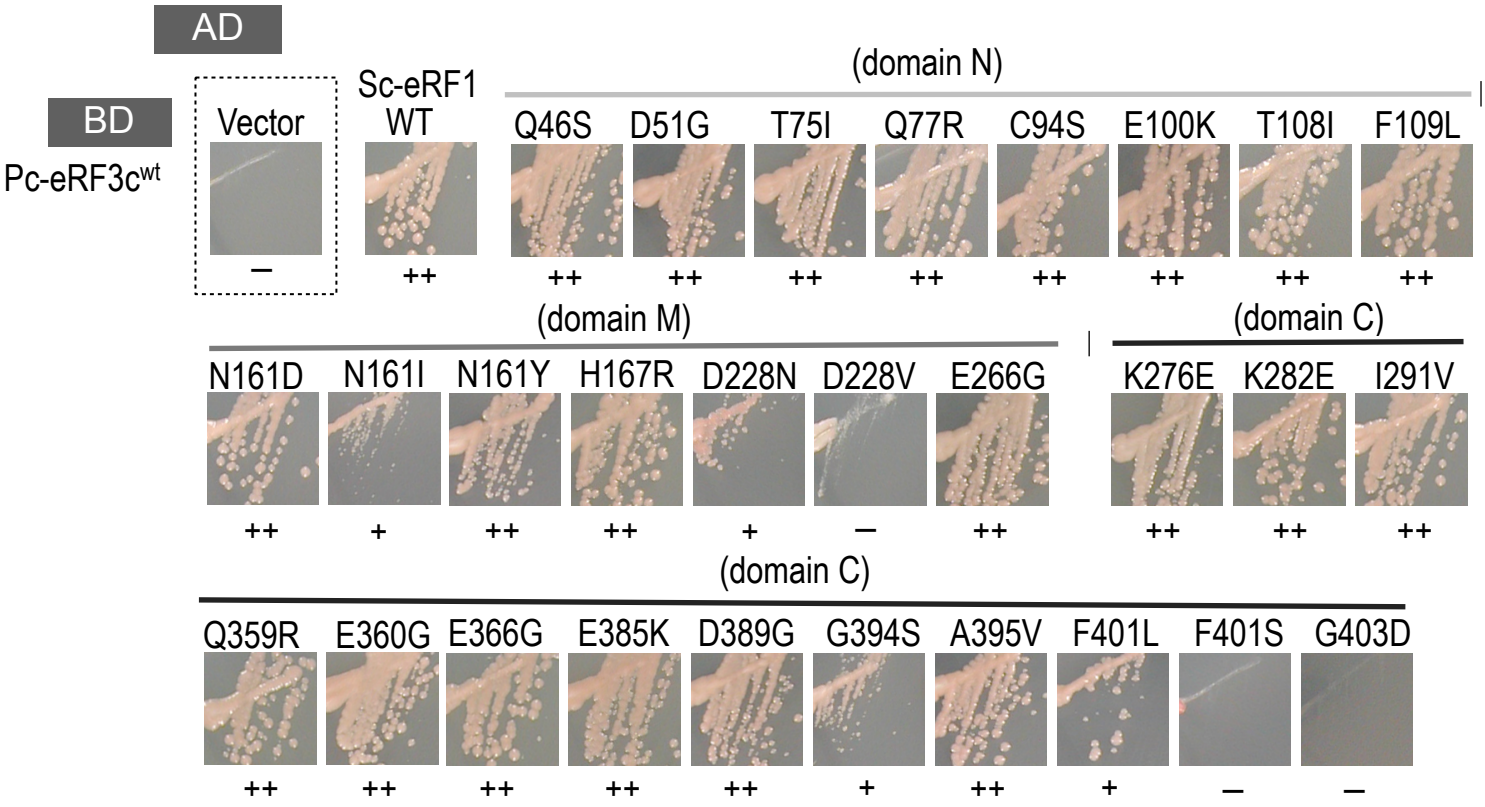
++
++
++
++
+
++
++

Figure S6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++ ++ ++ ++ ++ ++ ++ ++
(domain M)
N161D N161I N161Y H167R D228N D228V E266G
++ + ++ ++ + – ++
(domain C)
K276E K282E I291V
++ ++ ++
(domain C)
Q359R E360G E366G E385K D389G G394S A395V F401L F401S G403D
++ ++ ++ ++ ++ + ++ + – –