Supplementary Table and Figure legends.

Table S1. Cell viability of *TRA1* deletion mutants.
Plasmids containing either wild-type 3xFlag-*TRA1* (pSHY690; ARS CEN *TRP1*) or the indicated Tra1 deletion derivatives of PSH690 were transformed into the *TRA1* shuffle strain (SHY785) and patched on 5-FOA containing plates to select for cells having lost the *TRA1*-containing *URA3* marked plasmid. Growth was assessed relative to wild-type where wild-type growth is denoted by (++++), while no growth is denoted by (-). Transformants were classified as dominant or recessive using growth tests shown in Fig S2 and data not shown.

Table S2. Cell growth phenotypes of viable *TRA1* mutants.
Viable *TRA1* mutants from Table S1 were compared with a wild type *TRA1* containing strain for growth on three types of synthetic media: 1) glucose complete media (GC) lacking tryptophan incubated at the indicated temperatures, 2) GC media containing 3 μg/ml SMM and lacking tryptophan, isoleucine, and valine, or 3) synthetic media containing 2% galactose and lacking tryptophan. Growth was assessed relative to wild-type where wild-type growth is denoted by (++++), while no growth is denoted by (-).

Table S3. Viability of *TRA1* PI3K domain site-specific mutants.
Plasmids encoding either wild-type *TRA1* (pSH690) or the indicated Tra1 single residue mutations were transformed into the *TRA1* shuffle strain (SHY785) and streaked onto 5-FOA containing plates to select for cells lacking the *TRA1*-encoding *URA3* marked plasmid. Growth was assessed related to wild-type where wild-type growth is denoted by (++++), while no growth is denoted by (-).

Table S4. RT-qPCR and CHIP primer sequences used in this study.

Figure S1. Domain and repeat sequence alignment of Tra1 and TRRAP.
An HMM alignment of Tra1 and TRRAP. The predicted secondary and tertiary

structures are shown above and below the sequence of Tra1 and TRRAP. Individual domain and repeat units are depicted as colored boxes using the same color scheme presented in Figure 1B. HEAT and TPR repeats are colored as orange and green boxes. Secondary structure for Tra1 and TRRAP were predicted by PSIPRED. H, helix, E, beta sheet, C, coil, -, gap. The amino acid position of Tra1 and TRRAP are indicated at the left side of the amino acid sequence.

Figure S2. Dominance growth test of *TRA1* deletion mutants. Plasmids containing either wild-type 3xFlag-*TRA1* (pSHY690; ARS CEN *TRP1*) or the indicated *TRA1* deletion derivatives of PSH690 were transformed into the *TRA1* shuffle strain (SHY785). Five fold serial dilution of each yeast strain were spotted on either glucose complete (GC) plates lacking uracil and tryptophan, or GC media containing 3 μg/ml SMM and lacking uracil and tryptophan. Growth was assessed relative to wild-type.

Supplementary Table 1. Cell viability of *TRA1* deletion mutants

Deletion	Residues	Domain[1]	Growth Phenotype (FOA)[2]	Dominance[3]
Δ1	5-87	H1-2	++++	R
Δ2	88-165	H3-4	+++	R
Δ3	166-235	H4	++++	R
Δ4	236-318	H5	++++	R
Δ5	319-399	H6-7	++	R
Δ6	400-500	H8-9	-	R
Δ7	501-559	DOR	++++	R
Δ8	560-653	H10	-	R
Δ9	654-731	H11	-	R
Δ10	732-820	H12-13	-	R
Δ11	821-908	H14-15	-	R
Δ12	909-987	H16	-	R
Δ13	988-1126	H17-19	-	R
Δ14	1127-1223	H20-21	-	R
Δ15	1224-1319	H22-23	-	R
Δ16	1320-1423	H24-25	-	R
Δ17	1424-1508	H26-27	++	R
Δ18	1509-1590	H28-29	++	R
Δ19	1591-1685	H29-30	-	R
Δ20	1686-1823	H31-33	-	R
Δ21	1824-1955	H34-36	++++	R
Δ22	1956-2050	H37-38	++++	R
Δ23	2051-2151	H39-41	++++	R
Δ24	2152-2230	H41-42	++	R
Δ25	2231-2325	H43-45	+	R
Δ26	2326-2436	H46-48	-	R
Δ27	2437-2552	H49-50	-	R
Δ28	2553-2614	H51-T1	-	R
Δ29	2615-2700	T2-4	-	R
Δ30	2701-2823	T5-7	-	R

[1] H, HEAT; T, TPR; PI3KN, N-lobe; PI3KC, C-lobe

[2]Growth compared to wildtype ++++

[3]Transformants were classified as domainant (D) or recessive (R)

Supplementary Table 1 continued

Deletion	Residues	Domain[1]	Growth Phenotype (FOA)[2]	Dominance[3]
Δ31	2824-2913	T7-10	-	R
Δ32	2914-3007	T10-12	+	R
Δ33	3008-3093	T13-14	-	R
Δ34	3094-3176	T15-16	-	R
Δ35	3177-3239	FRB	-	R
Δ36	3240-3345	FRB	-	R
Δ37	3346-3397	PI3KN	-	R
Δ38	3398-3536	PI3KN-C	-	R
Δ39	3537-3676	PI3KC	-	R
Δ40	3677-3710	PI3KC	-	R
Δ41	3711-3724	PI3KC-FATC	++	R
Δ42	3725-3744	FATC	-	R
Δ43	3486-3517	PI3KN-C	-	R
Δ44	3585-3600	PI3KC	-	R

[1] H, HEAT; T, TPR; PI3KN, N-lobe; PI3KC, C-lobe

[2]Growth compared to wildtype ++++

[3]Transformants were classified as domainant (D) or recessive (R)

Supplementary Table 2. Cell growth phenotypes of viable *TRA1* mutants

Deletion	Growth Phenotype[1]				
	(24°C)	(30°C)	(37°C)	(SMM)	(GAL)
Δ1	++++	++++	+++	+++	+++
Δ2	+++	+++	+++	+	+++
Δ3	++++	++++	+++	++	++++
Δ4	++++	++++	+++	+	++++
Δ5	++	++	-	-	++
Δ7	++++	++++	++++	++++	++++
Δ17	++	++	+	+	++
Δ18	+++	+++	++	+++	+++
Δ21	++++	++++	+++	+++	++++
Δ22	++++	++++	+++	+++	++++
Δ23	++++	++++	+++	+++	+++
Δ24	++	++	-	-	+
Δ25	+	+	-	N.D.	N.D.
Δ32	++	+	-	-	+
Δ41	+++	++	+	++	++
Y3407E	++++	++++	++++	++++	++++
R3414E	++++	++++	++++	++++	++++
R3424E	++++	++++	++++	++++	++++
V3462E	++++	++++	++++	++++	++++
K3477E	++++	++++	++++	++++	++++
F3480E	++++	++++	++++	++++	++++
K3528E	+++	+++	+++	+++	+++
W3542E	+++	+++	+++	+++	+++
M3563E	++++	++++	++++	++++	++++
H3570E	++++	++++	++++	++++	++++
E3618R	++++	++++	+++	++++	++++
I3640E	++++	++++	++++	++++	++++
E3658R	++++	++++	++++	++++	++++
W3674E	++++	++++	++++	++++	++++

[1]Growth compared to wildtype ++++

Supplementary Table 3. Cell viability of site-specific *TRA1* mutants

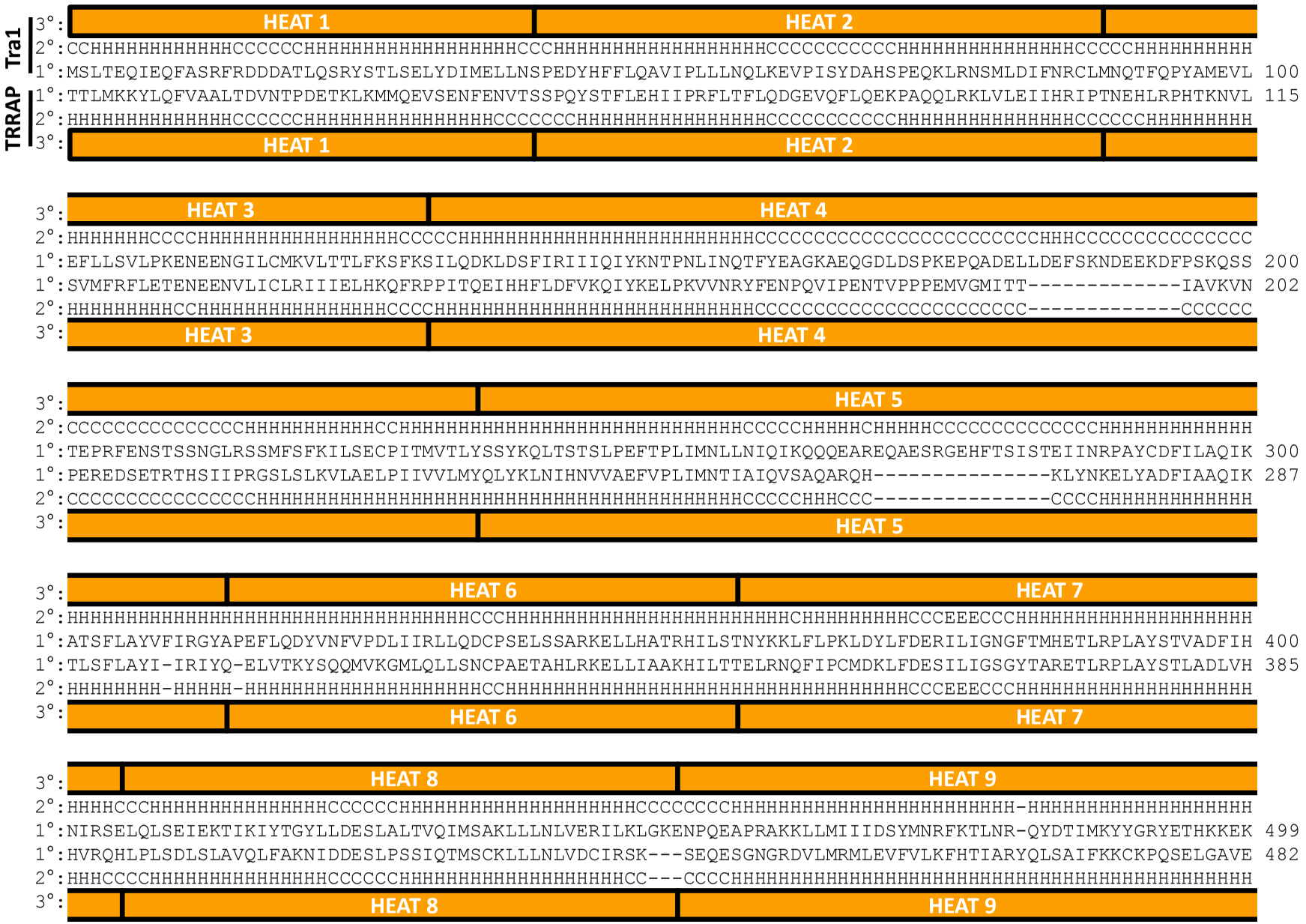
Residues	Domain[1]	Growth Phenotype (FOA)[2]
Y3407E	PI3KN	++++
R3414E	PI3KN	++++
R3424E	PI3KN	++++
K3432E	PI3KN	-
V3462E	PI3KC	++++
K3477E	PI3KC	++++
F3480E	PI3KC	++++
K3528E	PI3KC	+++
W3542E	PI3KC	+++
M3563E	PI3KC	++++
H3570E	PI3KC	++++
E3618R	PI3KC	++++
I3640E	PI3KC	++++
R3650E	PI3KC	-
E3658R	PI3KC	++++
W3674E	PI3KC	++++

[1] PI3KN, N-lobe; PI3KC, C-lobe

[2]Growth compared to wildtype ++++

Supplementary Table 4. RT-qPCR and ChIP primer sequences used in this study

Name	Method	Sequence (5' to 3')
ACT1-FP	RT-qPCR	TCAAAATGGCGTGAGGTAGAGA
ACT1-RP	RT-qPCR	GATGCATTATGGATCGTTGAACTC
GAL1-FP	RT-qPCR	ACTTGCACCGGAAAGGTTTG
GAL1-RP	RT-qPCR	GGTACATCACCCTCACAGAAGACTT
GAL7-FP	RT-qPCR	GGACCTCGCCTCGATTTTA
GAL7-RP	RT-qPCR	AATTCATCACCAGTCGCATTC
GAL3-FP	RT-qPCR	TTTATGGATGCTTACTACGCCAGAT
GAL3-RP	RT-qPCR	AACGTTCAATACCAGTTCCGATATC
HIS4-FP	RT-qPCR	GCACTGCCATTTTACCAAGTACTG
HIS4-RP	RT-qPCR	CTTGGTGGAGATGCAAACACA
ARG1-FP	RT-qPCR	CCAAGCCTTTGGATGTTTTCTT
ARG1-RP	RT-qPCR	ACGATCTTCTACAATATCGATTCTACCA
ARG4-FP	RT-qPCR	CCACTATTCGATTGCTTAACAACTG
ARG4-RP	RT-qPCR	CAGCTTCCATCTTTTCCTTATTTACA
RPL2B-FP	RT-qPCR	CGTGCTTTCCACAAGTACAGATTG
RPL2B-RP	RT-qPCR	CCACCGTGAGGGTGATCAA
RPS11B-FP	RT-qPCR	ACAACAGATACGAAAAGAGACACAAGA
RPS11B-RP	RT-qPCR	CACCAACTTGGACACGGAAA
RPS5-FP	RT-qPCR	TCAAGCACACTTTGGACATCATC
RPS5-RP	RT-qPCR	TTGGACCAGTGTTGGTGATAGC
GAL1-UAS-FP	ChIP	AGTAATACGCTTAACTGCTCATTGCT
GAL1-UAS-RP	ChIP	ACGCACGGAGGAGAGTCTTC
GAL1-ATG-FP	ChIP	TAACGTCAAGGAGAAAAAACTATAATGACT
GAL1-ATG-RP	ChIP	CCTTTGCGCTAGAATTGAACTCA
HIS4-UAS-FP	ChIP	TTGCGATACGATGGGTCATA
HIS4-UAS-RP	ChIP	GAGTCACTGTGCATGGGTTTA
HIS4-ATG-FP	ChIP	CAACTGCGCTGTGTAATAGTAATACAAT
HIS4-ATG-RP	ChIP	GCCAGATCATCAATTAACGGTAGA
RPL2B-UAS-FP	ChIP	ACAAACCCAGACAGTACACTATACA
RPL2B-UAS-RP	ChIP	GCGGGTTGGCGATTTCGAATAA
RPL2B-ATG-FP	ChIP	GTCATAAACTCACCAAGAAACCACA
RPL2B-ATG-RP	ChIP	GAGGAATCCTTCATAACATTTGCCA
POL1-ORF-FP	ChIP	TTTCTGCTGAGGTGTCTTATAGAATTCA
POL1-ORF-RP	ChIP	GCTTTGGGCCCATGCAT



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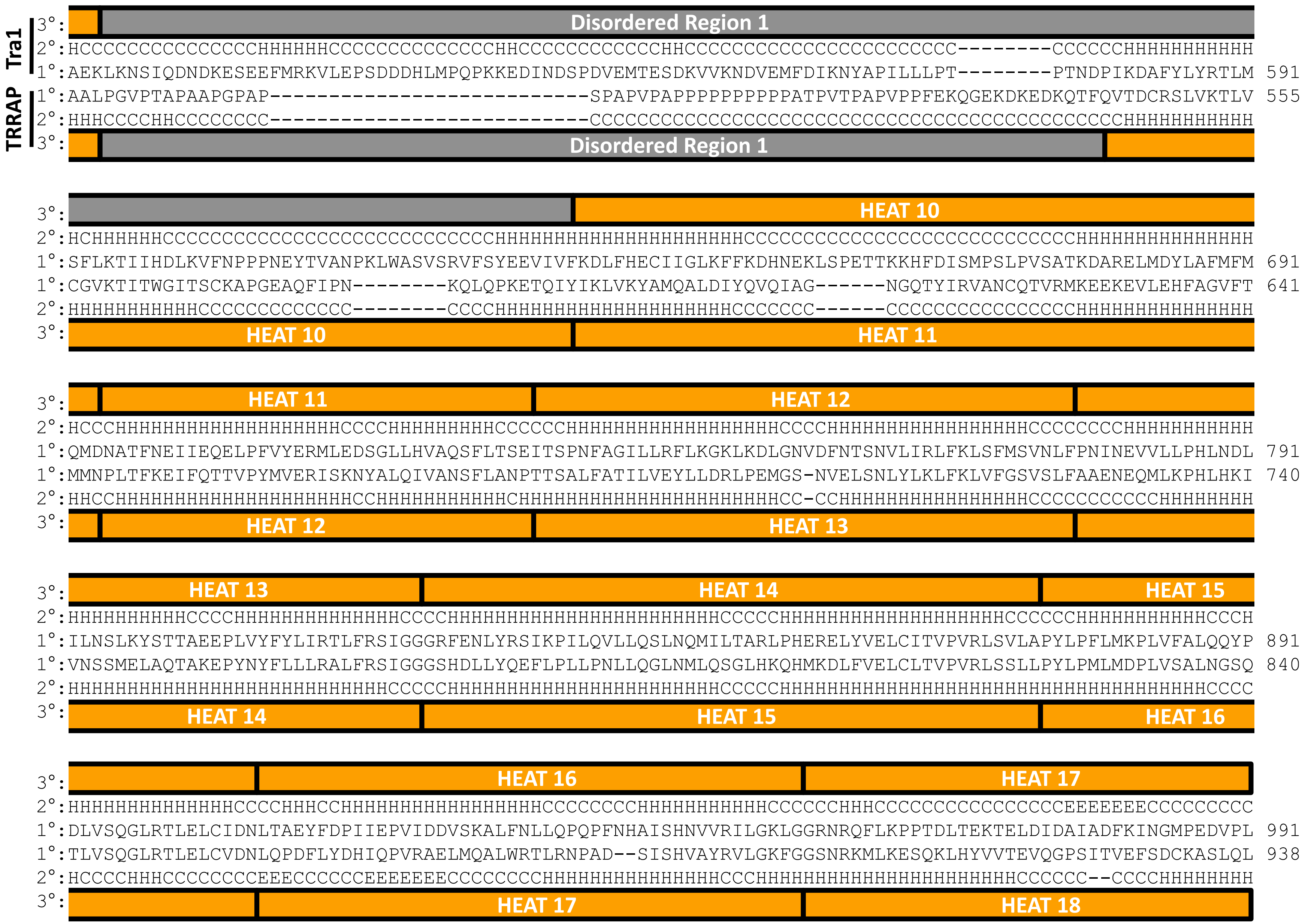

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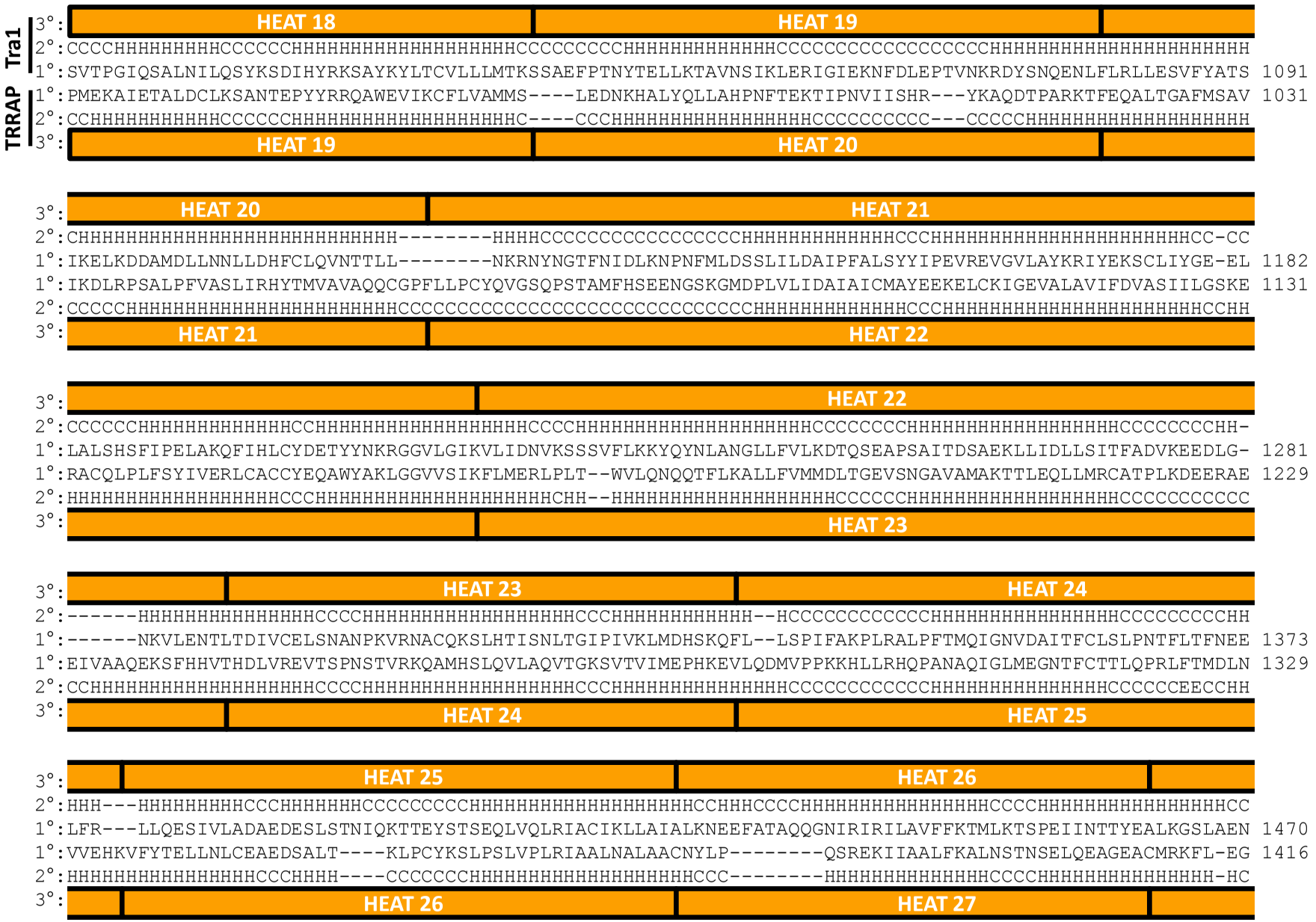

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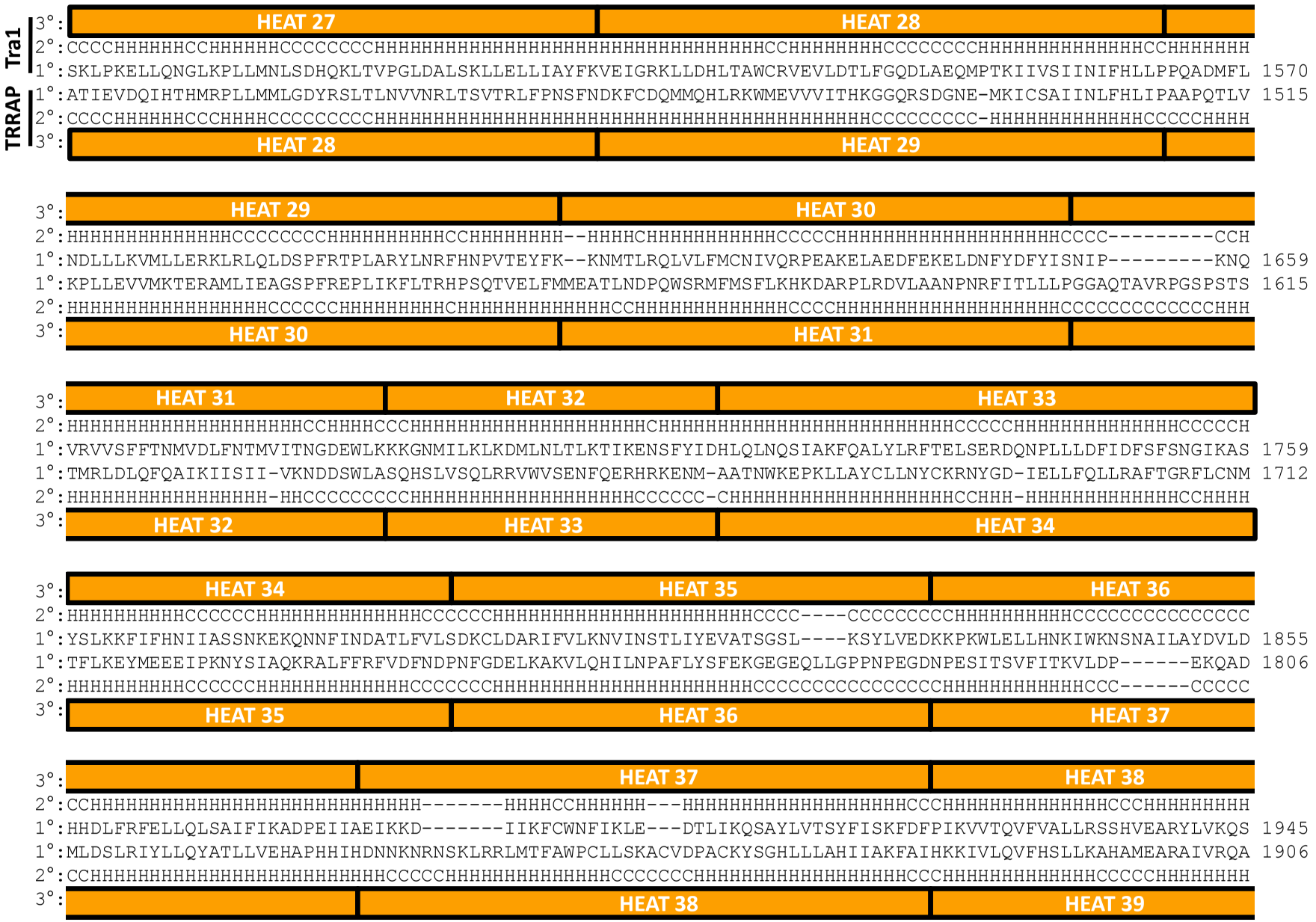

Knutson & Hahn, Figure S1

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TRRAP 1°:MAILTPAVPARMEDGHQ--MLTHWTRKIIVEEGHTVPQLVHILHLIVQHFKVYYPVRHHLVQHMVSAMQRLGFTPSVTIEQRRLAVDLSEVVIKWELQRI 2004
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      3°: [          |              Disordered Region 2                |       HEAT 41        ]
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      1°:KDQQPDSDMDPNSSGEGVNSVSSSIKRGLSVDSAQEVKRFRTATGAISAVFGRSQSLPGADSLLAKPIDKQHTDTVVNFLIRVACQVNDNTNTAGSPGEV 2104
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      1°:TSELVMLSLELVKTRLAVMSMEMRKNFIQAILTSLIEKSPDAKILRAVVKIVEEWVKNNSPMAANQTPTLREKSILLVKMMTYIEKRFPEDLELNAQFLD 2402
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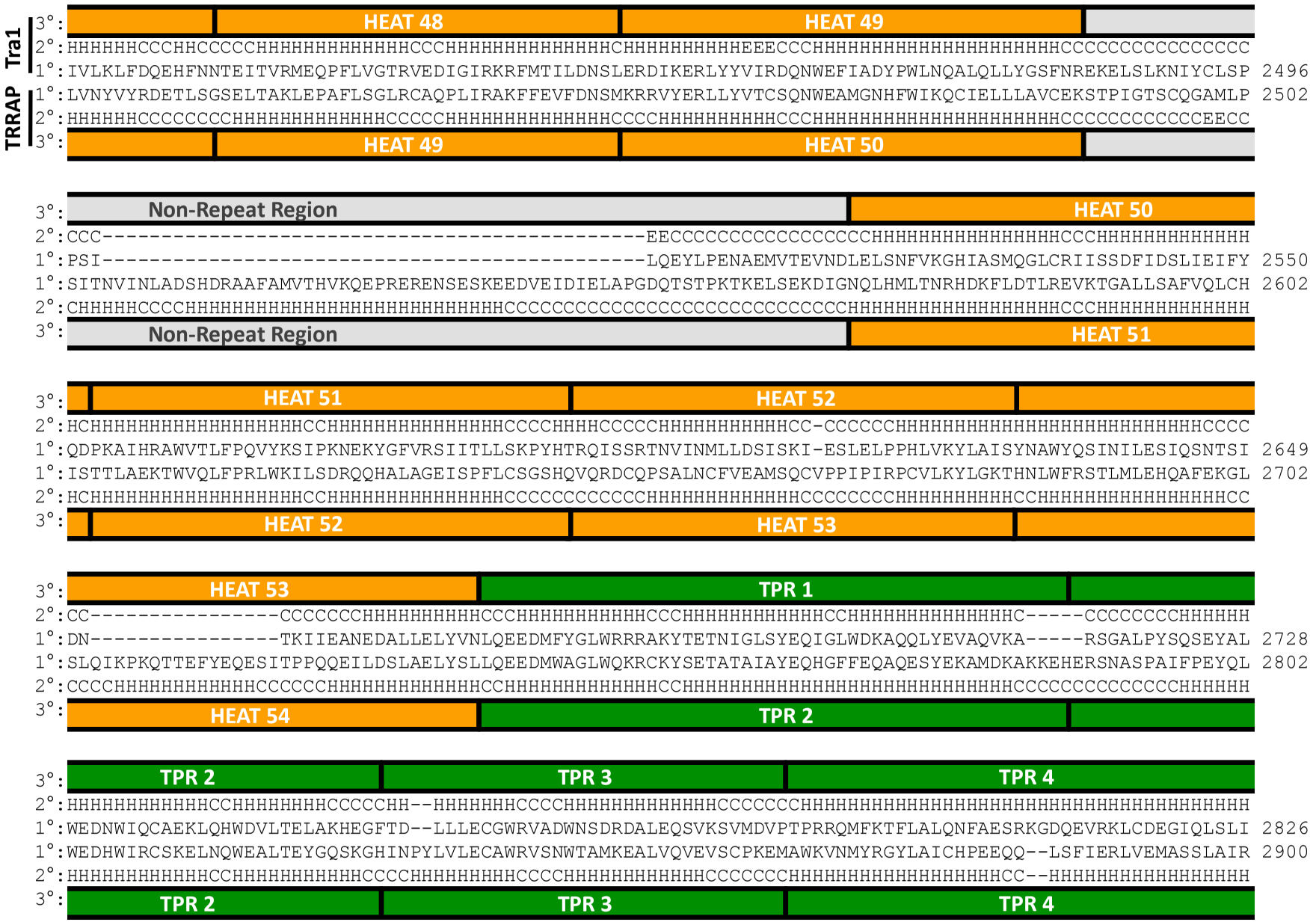
Knutson & Hahn, Figure S1



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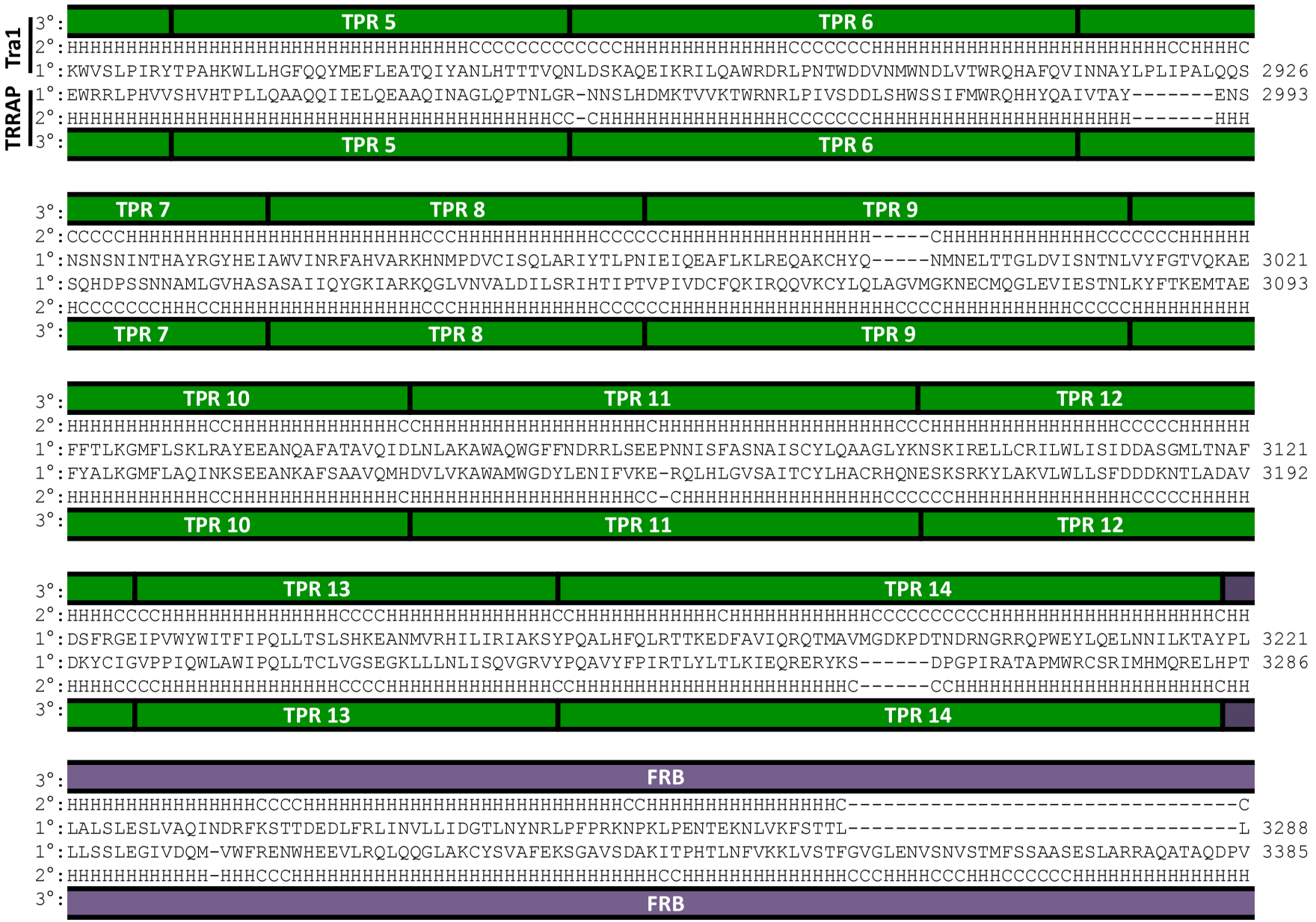

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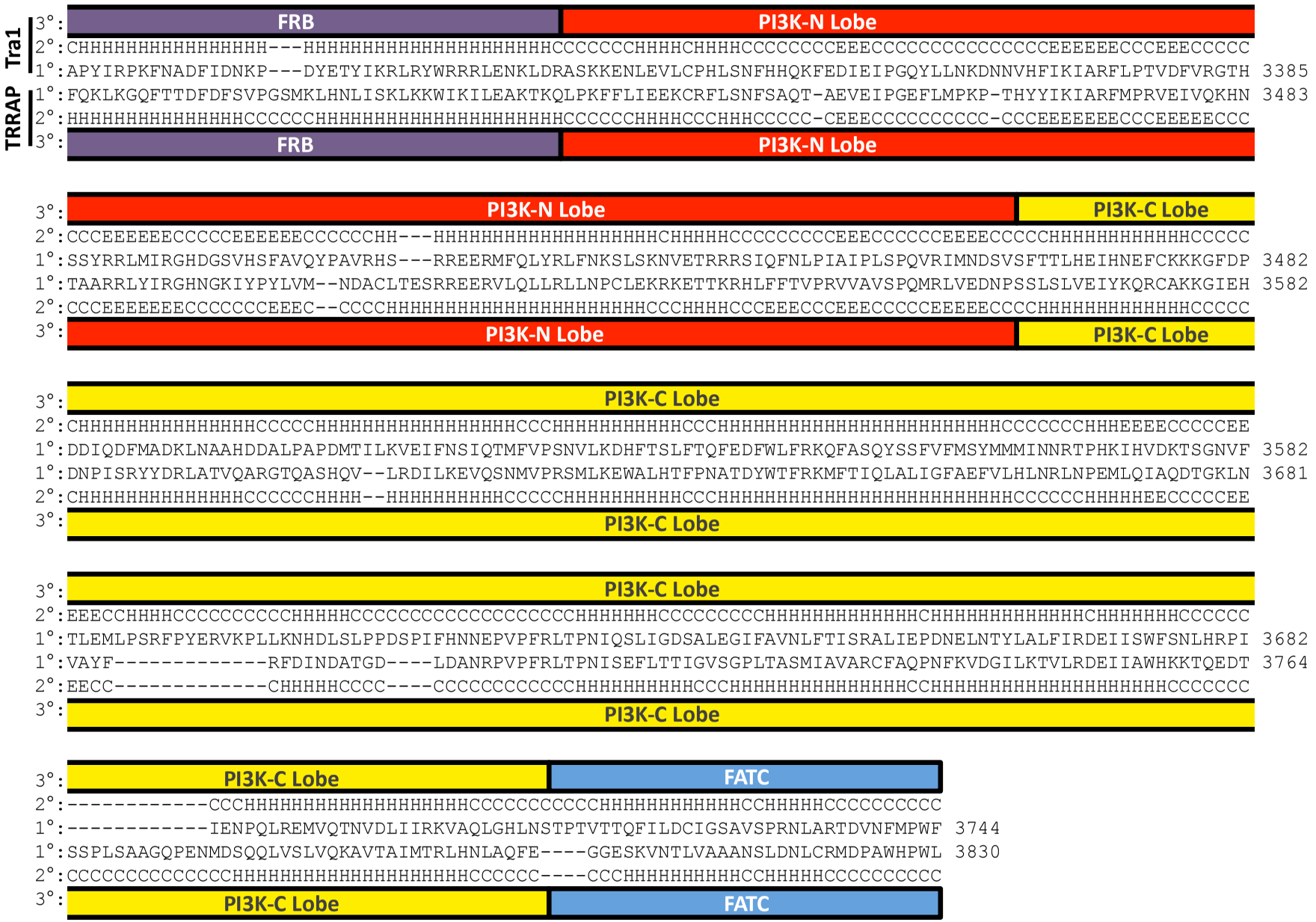

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Knutson & Hahn, Figure S2