Supplementary table 1. Sequence identity (%) at the amino acid level between different RaCIs

	RaCI2	**RaCI3**	**RaCI6**	**RaCI7**	**RaCI4**	**RaCI1**	**RaCI5**
RaCI2	100	41	38	42	40	42	38
RaCI3		100	71	73	44	35	36
RaCI6			100	76	47	36	38
RaCI7				100	45	35	37
RaCI4					100	38	35
RaCI1						100	40
RaCI5							100

Supplementary Table 2 Plasmids, primers and synthetic genes used in this study

Plasmid	Description	Restriction sites	Primers	Expression
pEpreS2-2	From Expres2ion Biotechnologies, Denmark			*Drosophila melanogaster* S2
pMJ41	Modified pExpres2-2 vector; Kozak-BIP-His6-NgoMIV	Acc65I/EcoRI	MJ41 + MJ42 (annealed and inserted)	
pMJ80	pMJ41 His6-RaCI1	NgoMIV/NotI		
pMJ85	pExpres2-2 RaCI2 (native signal peptide)	EcoRI/NotI		
pMJ81	pExpres2-2 RaCI3 (native signal peptide)	EcoRI/NotI		
pMJ82	pExpres2-2 RaCI4 (native signal peptide)	EcoRI/NotI		
pMJ84	pExpres2-2 RaCI5 (native signal peptide)	EcoRI/NotI		
pMJ83	pExpres2-2 RaCI7 (native signal peptide)	EcoRI/NotI		
pMJ100	pETM14 RaCI1 (no signal peptide)	NcoI/NotI		*Escherichia coli*
pMJ99	pETM14 RaCI2 (no signal peptide)	NcoI/NotI		
pMJ97	pETM14 RaCI3 (no signal peptide)	BspHI/NotI		
pMJ98	pETM14 RaCI4 (no signal peptide)	NcoI/NotI		
pMJ111	pETM14 - RaCI2 ΔC10 (for NMR experiments)		MJ127 + MJ128 (template = pMJ99)	
pMJ103	pETM14 - RaCI2 ΔN10		MJ87 + MJ88 (template = pMJ99)	
pMJ104	pETM14 - RaCI2 ΔC18		MJ149 + MJ150 (template pMJ99)	
pMJ15	pKLAC2-His10-OmCI wildtype[1]		MJ6 + MJ8	*Kluyveromyces lactis*
pMJ19	pKLAC2-His10-OmCI N78Q		MJ20 + MJ21 (template = pMJ15)	
pMJ27	pKLAC2-His10-OmCI N78Q/N102Q		MJ22 + MJ23 (template = pMJ19)	

Primer	Sequence	Description
MJ6	GCGCGGCGGCCGCCTAGCAGTCCTTGAGATGGGGAT	OmCI + stopcodon + NotI (rv)
MJ8	GGCCCCTCGAGAAAAGAATGGCTAGCCATCACCATCACCATCACCATCACCATCACTCCGGAGACAG CGAAAGCGACTGCACTG	XhoI + His10 + OmCI (fw)
MJ20	CGGTGATGATGACGTTTAAG*C***AAG**GCACAGACTGGGCTTCAACCG	OmCI - N78Q (fw)
MJ21	CGGTTGAAGCCCAGTCTGTGCC**TTG**CTTAAACGTCATCATCACCG	OmCI - N78Q (rv)
MJ22	GGTAACGGCAACCCTTGGT**CAA**CTAACCCAAAATAGGGAAGTGG	OmCI - N102Q (fw)
MJ23	CCACTTCCCTATTTTGGGTTAG**TTG**ACCAAGGGTTGCCGTTACC	OmCI - N102Q (rv)
MJ41	GTACCGCCACCATGAAGCTGTGCATCCTGCTGGCCGTGGTGGCCTTCGTGGGACTGAGCCTGGGAC ACCACCACCATCACCACGCCGGCG	Acc65I-Kozak-BIP-His6-NgoMIV-EcoRI
MJ42	AATTCGCCGGCGTGGTGATGGTGGTGGTGTCCCAGGCTCAGTCCCACGAAGGCCACCACGGCCAG CAGGATGCACAGCTTCATGGTGGCG	Acc65I-Kozak-BIP-His6-NgoMIV-EcoRI

MJ87	CTGTTCCAGGGGCCCATGAAAGATCAGTGTGCAAATG	RaCI2 ΔN10 (fw)
MJ88	CATTTGCACACTGATCTTTCATGGGCCCCTGGAACAG	RaCI2 ΔN10 (rv)
MJ127	CACCGAAAAGCACCACCACC**TAA**ACCGAACAGAGCTTTAATATG	RaCI2 ΔC10 (fw)
MJ128	CATATTAAAGCTCTGTTCGGT**TTA**GGTGGTGGTGCTTTTCGGTG	RaCI2 ΔC10 (rv)
MJ149	CACCTGTTATCTGCTGGCA**TAA**ACACCGAAAAGCACCACC	RaCI2 ΔC18 (fw)
MJ150	GGTGGTGCTTTTCGGTGT**TTA**TGCCAGCAGATAACAGGTG	RaCI2 ΔC18 (rv)

Synthetic gene[2]	**Sequence**
RaCI1 (pmJ80)	CGAATTGGCGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGAGCTCGCCGGCGAGGAAGTGAAGACCACCCCCATCCCCAACC ACCAGTGCGTGAACGCCACGTGCGAGCGCAAGCTGGATGCCCTGGGCAATGCCGTGATCACCAAGTGCCCACAGGGCTGCCTG TGCGTCGTGCGCGGAGCCAGCAATATCGTGCCCGCCAACGGAACCTGCTTCCAGCTGGCCACCACCAAGCCACCAATGGCCCC AGGCGATAACAAGGACAACAAGGAGGAGGAGTCCAACTGAGCGGCCGCGGTACCTGGAGCACAAGACTGGCCTCATGGGCCTT CCGCTCACTGC
RaCI2 (pMJ85)	CGAATTGAAGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGAGCTCGAATTCGCCACCATGAACGCCGTGACCGTGCTGGCCTT TACCGCCTTCGCCCTGATTGTGCACGATTGCTACAGCGAGGAGGCCAACACCACCCCCATCAGCGTGAAGGATCAGTGCGCCAA CGTGACCTGCCGCCGCACCGTGGATAATCGCGGCAAGCGCCACATCGATGGCTGCCCACCAGGATGCCTGTGCGTGCTGAAGG GCCCCGATAGCAAGGATAACCTGGATGGCACGTGCTACCTGCTGGCCACCACCCCAAAGAGCACCACCACCAGTACCGAGCAG AGCTTCAACATGGAGGAGTAGGCGGCCGCGGTACCTGGAGCACAAGACTGGCCTCATGGGCCTTCCTTTCACTGC
RaCI3 (pMJ81)	CGAATTGGCGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGAGCTCGAATTCGCCACCATGGCCGCCCTGAATGGCCTGGTGTT GCTGCTGCTGACCATCAGCGCCATGTTCATCAGCGAGTGCTACAGCAGCGGCGAGAGCCAGAGCATTCAGCGCAAGGGACAGT GCGAGGAAGTGATCTGCCACCGCAAGCTGAACCACCTGGGAGAGCGCGTGACCAGCGGATGCCCAACCGGATGCCTGTGCGTG ATCCGCGAGCCCGATAACGTGGACAACGCCAACGGAACCTGCTACGCCCTGATGAGCAGCACCACCACGACCACGACGACCCC AGATGGAACCACCACCTCCGAAGAGGAGGAGTAGGCGGCCGCGGTACCTGGAGCACAAGACTGGCCTCATGGGCCTTCCGCTC ACTGC
RaCI4 (pMJ82)	CGAATTGGCGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGAGCTCGAATTCGCCACCATGAGCGCCTTCAACATCTTCGCCCT GGTCGTGGTCGTGTGCGCCCTGATGATCAACGAGTGCTGCACCAGCCAGGAGCCCACCACCCCACTGAAGGCCGCCAGTCAGT GCAGCAACGTGAAGTGCCGCCGCCGCTTCGATCACCTGGGCAATAGTGTGACCGAGGGCTGCCCAAGCGGATGCCTGTGCGTG TACCAGGCCACCGGCTACAACCAGGAGGCCAACGGAACCTGCTACGAGCTGATGAAGACCTCGACCACCACGACGACCGAGGG AACCCCAGCCCAGTAGGCGGCCGCGGTACCTGGAGCACAAGACTGGCCTCATGGGCCTTCCGCTCACTGC
RaCI5 (pMJ84)	CGAATTGGCGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGAGCTCGAATTCGCCACCATGAACGCCGTGATCGTGCTGTGCGT GACCATCAGCGCCGTGCTGATCCACCAGTGCTACAGTACCGCCGAGGCCACCCTGAGCATCAACGGCGGCGATATGTGCATCG AGAAGACCTGCAACCGCTCCATCGATGCCGCCGGAAAGAAAGTGATCGCCGGATGCCCAGGCGGCTGCCTGTGCGTGTTCAAC GTGTCCGATGTGACCTACCCCGCCAACGGCACGTGCTATCAGCTGGCCACCACCACGACCAATCGCCCAGGCGCCGTGATGGA GCGCGAGCGCTAAGCGGCCGCGGTACCTGGAGCACAAGACTGGCCTCATGGGCCTTCCGCTCACTGC
RaCI7 (pMJ83)	CGAATTGGCGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGAGCTCGAATTCGCCACCATGGCCGCCCTGAATGGCCTGGTGTT GCTGCTGCTGACCATCAGCGCCATGTTCATCAGCGAGTGCTACAGCAGCGGCGAGAGCCAGAGCATTCAGCGCAAGGGACAGT GCGAGGAAGTGACCTGCCACCGCACCCTGAATCACCTGGGAGTGGCCGTGACCAGCGGATGCCCAAGTGGATGCCTGTGCGTG ATCAGCGCCCCAGATAGCGCCGTGAACGTGAACGGCACGTGCTACCAGCTGATGGGCTCCACCAGTACCACCACCAGCAGCAC CCCAAGCAGCGAGGATCAGGAGTAGGCGGCCGCGGTACCTGGAGCACAAGACTGGCCTCATGGGCCTTCCGCTCACTGC
RaCI1 (pMJ100)	CGAATTGAAGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGAGCTCCCATGGAAGAAGTTAAAACCACCCCGATTCCGAATCATC AGTGTGTTAATGCAACCTGTGAACGTAAACTGGATGCACTGGGTAATGCAGTTATTACCAAATGTCCGCAGGGTTGTCTGTGTGTT GTTCGTGGTGCAAGCAATATTGTTCCGGCAAATGGCACCTGTTTTCAGCTGGCAACCACCAAACCGCCTATGGCACCGGGTGATA ATAAAGATAACAAAGAAGAAGAAAGCAATTAAGCGGCCGCGGTACCTGGAGCACAAGACTGGCCTCATGGGCCTTCCTTTCACTG C
RaCI2 (pMJ99)	GGCCCCCATGGAGGAAGCAAATACCACCCCGATTAGCGTTAAAGATCAGTGTGCAAATGTTACCTGTCGTCGTACCGTTGATAAT CGTGGTAAACGTCATATTGATGGTTGTCCGCCTGGTTGTCTGTGTGTTCTGAAAGGTCCGGATAGCAAAGATAATCTGGATGGCA CCTGTTATCTGCTGGCAACCACACCGAAAAGCACCACCACCAGTACCGAACAGAGCTTTAATATGGAAGAGTAAGCGGCCGCGC GCG
RaCI3 (pMJ97)	GGCCCTCATGAGCGGTGAAAGCCAGAGCATTCAGCGTAAAGGTCAGTGTGAAGAAGTTATCTGTCATCGTAAACTGAATCATCTG GGTGAACGTGTTACCAGCGGTTGTCCGACCGGTTGTCTGTGTGTTATTCGTGAACCGGATAATGTGGATAATGCAAATGGCACCT GTTATGCACTGATGAGCAGCACCACCACAACCACCACGACACCGGATGGTACAACCACCTCTGAAGAAGAAGAATAAGCGGCCG CGCGCG
RaCI4 (pMJ98)	GGCCCCCATGGCACAAGAACCGACCACACCGCTGAAAGCAGCAAGCCAGTGTAGCAATGTTAAATGTCGTCGTCGTTTTGATCAT CTGGGTAATAGCGTTACCGAAGGTTGTCCGAGCGGTTGTCTGTGTGTTTATCAGGCAACCGGTTATAATCAAGAAGCAAACGGCA CCTGTTATGAGCTGATGAAAACCAGTACCACCACCACAACCGAAGGTACACCGGCACAGTAAGCGGCCGCGCGCG

[1]Amplified from pMETαC-OmCI (Nunn, M.A. et al., *J. Immunol* **174**, 2084-2091, 2005).
[2]Synthetic fragments were subcloned into constructs that are indicated between brackets.
Restriction sites are underlined.

Mutations introduced with primers are in bold.
Fw = forward primer, rv = reverse primer.