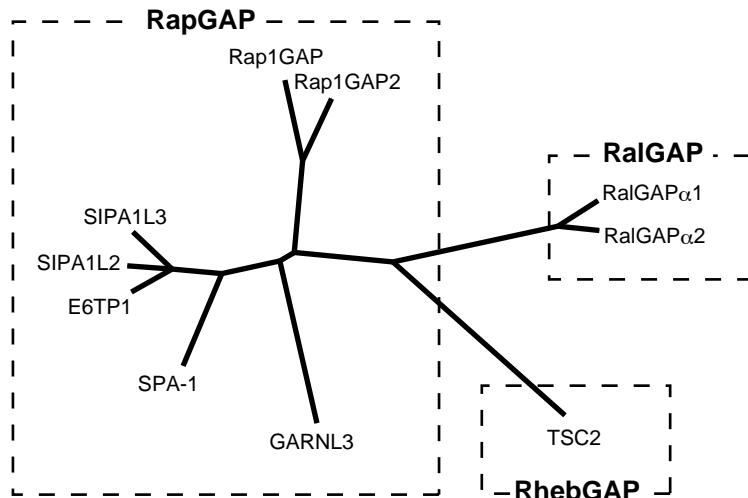


# Supplementary Figure 1

A

RapGAPs	Rap1GAP	<b>PKASRLLIVTFDEHVISNNFKFGVIVYQKLGQTS</b>	-EEEELFSTNEESPAFVEFLEFLGQKVKL	233
	Rap1GAP2	<b>PKASQMIVSYDEHEVNNTFKFGVIVYQKARQTL</b>	-EEEELFGNNEESPAFKEFLDLLGDTITL	285
	E6TP1	<b>PKVTEQLMKLDEOGLNYQQKVGIMYCKAGQST</b>	-EEEEMYNNESAGPAFEFLQLLGERVR	651
	SPA-1	<b>PKVPRTLTLDQEVVSVQRKVGILYCRAGQGS</b>	-EEEEMYNNQEAGPAFMQFLTLLGDVVRL	430
RalGAPs	RalGAP $\alpha$ 1	<b>EKLLRELRNLDSDRCRETHKIAVYVAEGQED</b>	-KHSILTNTGSGQAYEDFVAGLGWEVNL	375
	RalGAP $\alpha$ 2	<b>SKLLRELKNLDSDRCRETHKIAVYVAEGQED</b>	-KCSILSNERGSQAYEDFVAGLGWEVDL	1687
RhebGAP	TSC2	<b>QSFERSVQILDQIPSYDTHKIAVLYVGEQQS</b>	NSELAILSNEHGSYRYTEFLTGLGRIEL	1584
	Rap1GAP	<b>QDFKG---FRGGLDVTHGQTGTESVYCNFRNK</b>	EIMFHVSTKLPYTEGDAQQQLQRKRHIGN*	290
	Rap1GAP2	<b>QDFNG---FRGGLDVTHGQTGVEVYTTFRDR</b>	EIMFHVSTKLPFTDGDAQQQLQRKRHIGN	342
	E6TP1	<b>KGFKEK---YRAQOLDKTDTGTHSLYTTYKDYE</b>	EIMFHVSTMLPYTPNNKQQLLRKRHIGN	708
	SPA-1	<b>KGFES---YRAQOLDKTDTGTHSLYTTYQDH</b>	EIMFHVSTMLPYTPNNQQQLLRKRHIGN	430
	RalGAP $\alpha$ 1	<b>TNHCG---FMGGGLQK-NKSTGLTTPYFATSTVE</b>	EIVFHVSTRMPS-DSDDSLTKKLRLHGN	1950
	RalGAP $\alpha$ 2	<b>STHC---FMGGGLQK-NGSTGQTAPEYATSTVE</b>	EIVFHVSTRMPS-DSDDSLTKKLRLHGN	1742
	TSC2	<b>KDCQFDKVYLGGDV-CGEDQFTYCWHDDIMQAVFHIA</b>	TLMPPTKDVDKRCDKKRHLGN	1643
	Rap1GAP	<b>DIVAVVFQDEN-TPFVPDMIASNFLHAYVVV</b>	QAEGGGPDGPLYKVSVTARDDVPPFFGPPL	349
	Rap1GAP2	<b>DIVAITFQEEEN-TPFVPDMIASNFLHAYIVV</b>	QVETPGTETPSYKVSVTAREDVPTFGPPL	401
	E6TP1	<b>DIVTIVFQEPGAQPFSPKNI</b>	RSHFOHVFLVVRVHNPCSDSVCYSVAVTRSRDVPSFGPPI	768
	SPA-1	<b>DIVTIVFOEPGSKPFCPTTIRSHFOHVFLVVR</b>	RAHTPCTPHTTYRVAWSRTQDTPAFGPAL	490
	RalGAP $\alpha$ 1	<b>DEVHIVWSEHT-RDYRRGI</b>	IPTEFGDVLIVIYP---MKNHMFSIQIMKPEVPFFGPLF	2005
	RalGAP $\alpha$ 2	<b>DEVHIVWSEHS-RDYRRGI</b>	IPTAEGDVSIIIYP---MKNHMFFIAITKKPEVPFFGPLF	1797
	TSC2	<b>DFVSIVYNDSG-EDFKLGTIKGQENFVHVIVTP</b>	---LDYECNLVSLQCRKDMEGLVDT	1698
	Rap1GAP	<b>PDP-AVFRKCPFQEFLLTKLINAELYACY</b>	377	
	Rap1GAP2	<b>PSP-PVFKQKCPFREFLTKLTNAENACC</b>	429	
	E6TP1	<b>PKG-VTFPKSNVFRDFLLAKVINAENAAH</b>	796	
	SPA-1	<b>PAGGGPFAANADFRAFLAKALINGEQAAAG</b>	519	
	RalGAP $\alpha$ 1	<b>DGA---IVNGKVLPIMVRAATINASRALK</b>	2031	
	RalGAP $\alpha$ 2	<b>DGA---IVSKLLPSLVCATCINASRAVK</b>	1823	
	TSC2	<b>VAK---IVSDRNLFPVARQMALHANMASQ</b>	1724	

B



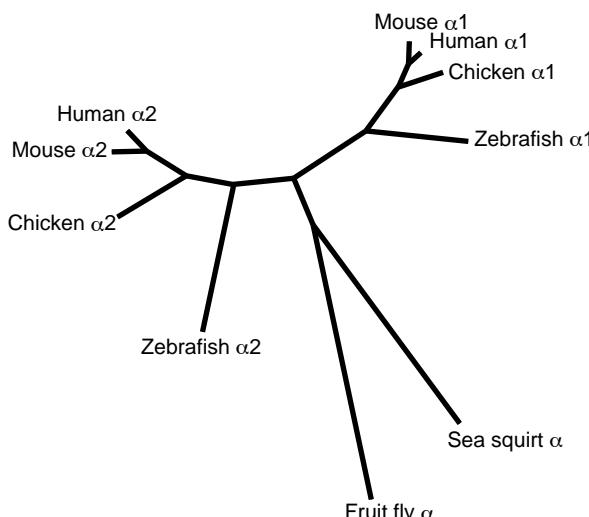
SUPPLEMENTARY FIGURE 1. **The asparagine thumb GAP family.** A, Amino acid sequence alignment of the catalytic domain of *H.sapiens* Rap1GAP (NP\_002876), Rap1GAP2 (NP\_001093868), SPA-1 (NP\_006738), E6TP1 (NP\_056371), TSC2 (NP\_000539), RalGAP $\alpha$ 1 (NP\_055805), and RalGAP $\alpha$ 2 (NP\_0655076). Black boxes and shaded boxes indicate >50% conserved and >50% similar residues, respectively. The black bar shows the catalytic helix of Rap1GAP. An asterisk marks the position of the catalytic asparagine. B, Phylogenetic tree of the asparagine thumb GAP family. Three putative RapGAPs, SIPA1L2 (NP\_065859), SIPA1L3 (NP\_055888), and GARNL3 (NP\_115669) are also included. The human asparagine thumb GAP family comprises 2 RalGAPs, 1 RhebGAP, and 7 probable RapGAPs.

## Supplementary Figure 2

A

	<i>Homo sapiens</i>	EKLLRELRLNLDQRQC	RETHKIAVFFVAEGQEDKHSIL	TNTGGSQAYEDFVAGLGWEVNLT	1896	
α1	<i>Mus musculus</i>	EKLLRELRLNLDQRQC	RETHKIAVFFVAEGQEDKYSIL	TNTGGSQAYEDFVAGLGWEVNLT	1848	
	<i>Gallus gallus</i>	EKLLRELRLNLDQRQC	RETHKIAVFFVAEGQEDKHSIL	TNTGGSQAYEDFVAGLGWEVNLT	1848	
	<i>Danio rerio</i>	EKLLRELRLNLDQRQC	RETHKIAVFFVAEGQEDKHSIL	SNTAGSQAYEDFVSGLGWEVNLT	1837	
α2	<i>Homo sapiens</i>	SKLLRELRLNLDQRQC	RETHKIAVFFIAEGQEDKCSILS	NERGSQAYEDFVAGLGWEVDLS	1688	
	<i>Mus musculus</i>	SKLLRELRLNLDQRQC	RETHKIAVFFIAEGQEDKCSILS	NERGSQAYEDFVAGLGWEVDLS	1725	
	<i>Gallus gallus</i>	SKLLRELRLNLDQRQC	RETHKIAVFFIAEGQEDKCSILS	NARGSQAYEDFVAGLGWEVDLS	1691	
	<i>Danio rerio</i>	SKLLRELRLNLDQRQC	RETHKIAVFFIGEGQEDKYSILS	NNTQGSQVYEDFVSGLGWEVNLA	1813	
	<i>Drosophila melanogaster</i>	EKLMRELRLNVDLQKCRE	THKMAVIYVAAGQEDKGSILRNT	SGSSTYEMFVSALGWEIDLE	1665	
	<i>Ciona intestinalis</i>	QNWPNFLLSHIPH	RETHKVAVLYIASQEDKLSILS	NTGGSKDYEVDVAGLGWEVDLK	2058	
				*		
α1	<i>Homo sapiens</i>	NHCGFMGGLOQKNKSTGLT	TPYFATSTVEVIFHVSTRMP	SDSDDSLTKKLRLHGNDEVHIV	1956	
	<i>Mus musculus</i>	NHCGFMGGLOQNRNSTGLT	TPYFATSTVEVIFHVSTRMP	SDSDDSLTKKLRLHGNDEVHIV	1908	
	<i>Gallus gallus</i>	NHCGFMGGLOQKNKSTGLT	TPYFATSTVEVIFHVSTRMP	SDSDDSLTKKLRLHGNDEVHIV	1908	
	<i>Danio rerio</i>	SHCGFMGGLOQNKSTGF	TPYFATSTVEVIFHVSTRMP	PDSDDSLTKKLRLHGNDEVHIV	1897	
α2	<i>Homo sapiens</i>	THCGFMGGLQRNGSTGQ	TAPYYATSTVEVIFHVSTRMP	SDSDDSLTKKLRLHGNDEVHIV	1748	
	<i>Mus musculus</i>	THCGFMGGLQRNGSTGQ	TAPYYATSTVEVIFHVSTRMP	SDSDDSLTKKLRLHGNDEVHIV	1785	
	<i>Gallus gallus</i>	THCGFMGGLQRNGSTGQ	TAPYYATSTVEVIFHVSTRMP	SDSDDSLTKKLRLHGNDEVHIV	1751	
	<i>Danio rerio</i>	THCGFMGGLQRNGSTGQ	TAPYYATSTVEVIFHVSTRMP	SDSDDSLTKKLRLHGNDEVHIV	1873	
	<i>Drosophila melanogaster</i>	THNGFEGGLP	RQCG-CGATAPYYATPFLEV	VYHVATRMPSDSEAMLLKTRHGNDEVHIV	1724	
	<i>Ciona intestinalis</i>	VHC	CGKLSQSDGSTGKSA	PYYATSTLEVIFHVATRFPTNV	TARNVKLKHGNDEVHIV	2118
α1	<i>Homo sapiens</i>	WSEHTRDYRRGIIPTEFG	DVLIVIYPMKHNHMF	SIQIMKKPEVPFFGPLFDGAIVNGKVL	2016	
	<i>Mus musculus</i>	WSEHTRDYRRGIIPTEFG	DVLIVIYPMKHNHMF	SIQIMKKPEVPFFGPLFDGAIVNGKVL	1968	
	<i>Gallus gallus</i>	WSEHTRDYRRGIIPTEFG	DVLIVIYPMKHNHMF	SIQIMKKPEVPFFGPLFDGAIVNGKVL	1968	
	<i>Danio rerio</i>	WSEHSRDYRRGVIPTEF	GDVLIVIYPMKHNHMF	SIQIKKPEVPFFGPLFDGAIVDGTIL	1957	
α2	<i>Homo sapiens</i>	WSEHSRDYRRGIIPTA	FGDV	SIIYPMKHNHMFIAITKKPEVPFFGPLFDGAIVSGKLL	1808	
	<i>Mus musculus</i>	WSEHSRDYRRGIIPTA	FGDV	SIIYPMKHNHMFITITKKPEVPFFGPLFDGAIVSGKLL	1845	
	<i>Gallus gallus</i>	WSEHNRNYRRGIIP	FGDV	IYPMKHNHMFIEIMKKPEVPFFGPLFDGAIVTAKLL	1811	
	<i>Danio rerio</i>	WSEHTRDYRRGVIPTDF	GDVLVIIYPMKHNHMF	VQIMKKPQVPFFGPLFDGAIVTGELLP	1933	
	<i>Drosophila melanogaster</i>	WSEHNRDYRRDILPTEF	CDVLIVVYPLRNGLFRV	TVNRKPEVPWFGPLANEVVSGACLA	1784	
	<i>Ciona intestinalis</i>	WSEHHRDYRRGIIPTEFG	DVIVIYPLRHGLFRV	QIIKKTEVPPYFGPLFDGAIVNARVLP	2178	
α1	<i>Homo sapiens</i>	IMVRATAINASRAL	K	2031		
	<i>Mus musculus</i>	IMVRSTA	INASRAL	1983		
	<i>Gallus gallus</i>	IMVRATA	INASRAL	1983		
	<i>Danio rerio</i>	TVVRATA	INASRAL	1872		
α2	<i>Homo sapiens</i>	SLVCATCINASRAV	K	1823		
	<i>Mus musculus</i>	SLICATCINASRAV	K	1860		
	<i>Gallus gallus</i>	SLICATCINASRAV	K	1826		
	<i>Danio rerio</i>	SLVRATCINASRAV	K	1948		
	<i>Drosophila melanogaster</i>	TLIRATAINASRT	KR	1799		
	<i>Ciona intestinalis</i>	VLVRETA	INAGRAKR	2193		

B



**SUPPLEMENTARY FIGURE 2. Comparison of RalGAP $\alpha$  homologues.** A, Amino acid sequence alignment of the catalytic domain of RalGAP $\alpha$  from various species: *H.sapiens* α1, *M.musculus* α1 (NP\_064378), *G.gallus* α1 (XP\_421244), *D.rerio* α1 (XP\_684334), *H.sapiens* α2, *M.musculus* α2 (NP\_001028520), *G.gallus* α2 (XP\_419313), *D.rerio* α2 (XP\_001920508), *D.melanogaster* α (NP\_651516), and *C.intestinalis* α (XP\_002123952). An asterisk marks the position of the asparagine corresponding to the catalytic asparagine of Rap1GAP. Invertebrates such as fruit fly and sea squirt possess a single gene for the α subunit. B, Phylogenetic tree of RalGAP $\alpha$  homologues.