Supporting Information

Barbash et al. 10.1073/pnas.1713074114

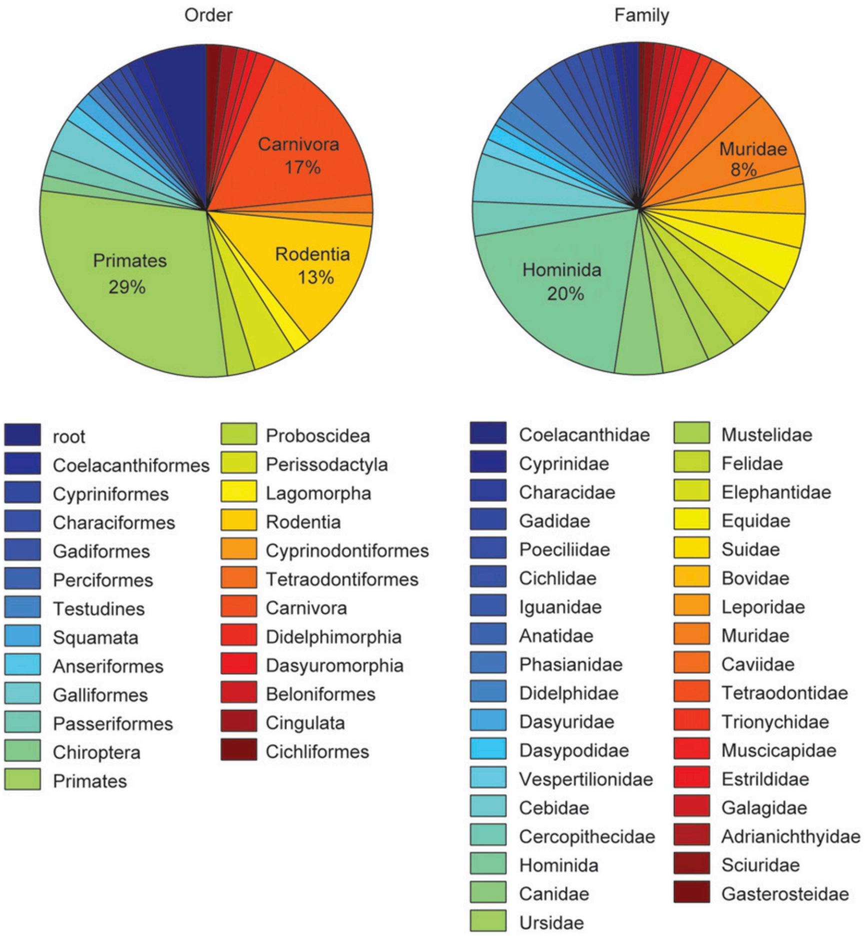

Fig. S1. Organisms order and family frequencies in the phylogenetic analysis. As noted, analysis was made for any protein pair that had an ortholog for at least one human GPCR and one human RAMP. Pie charts showing the frequencies in which organisms of any kind of order and family appeared in our analysis. As expected from our human-centered analysis, most organisms belong to the order of primates and the family of Hominidae.

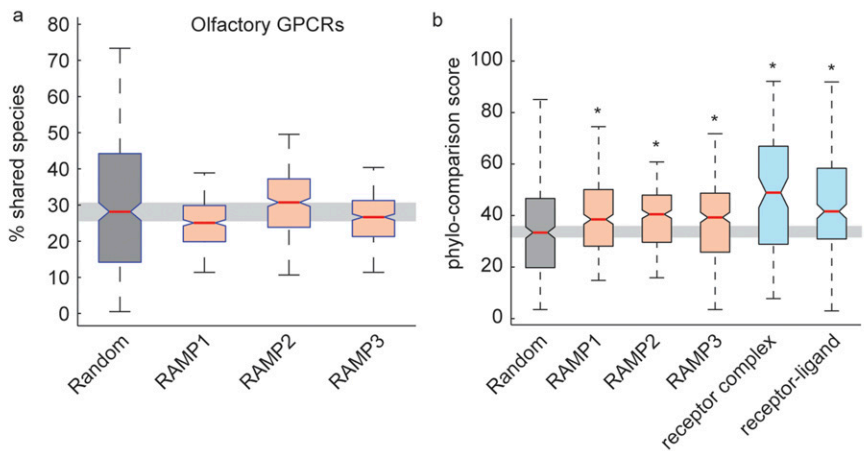

Fig. S2. (*A*) Boxplot (as in Fig. 1) of percent of shared species between olfactory GPCRs and each of the three RAMPs shows no difference from that expected by chance. (*B*) Boxplot (as in Fig. 1) for global phylogenetic score as calculated by phylo-comparison for all GPCRs.

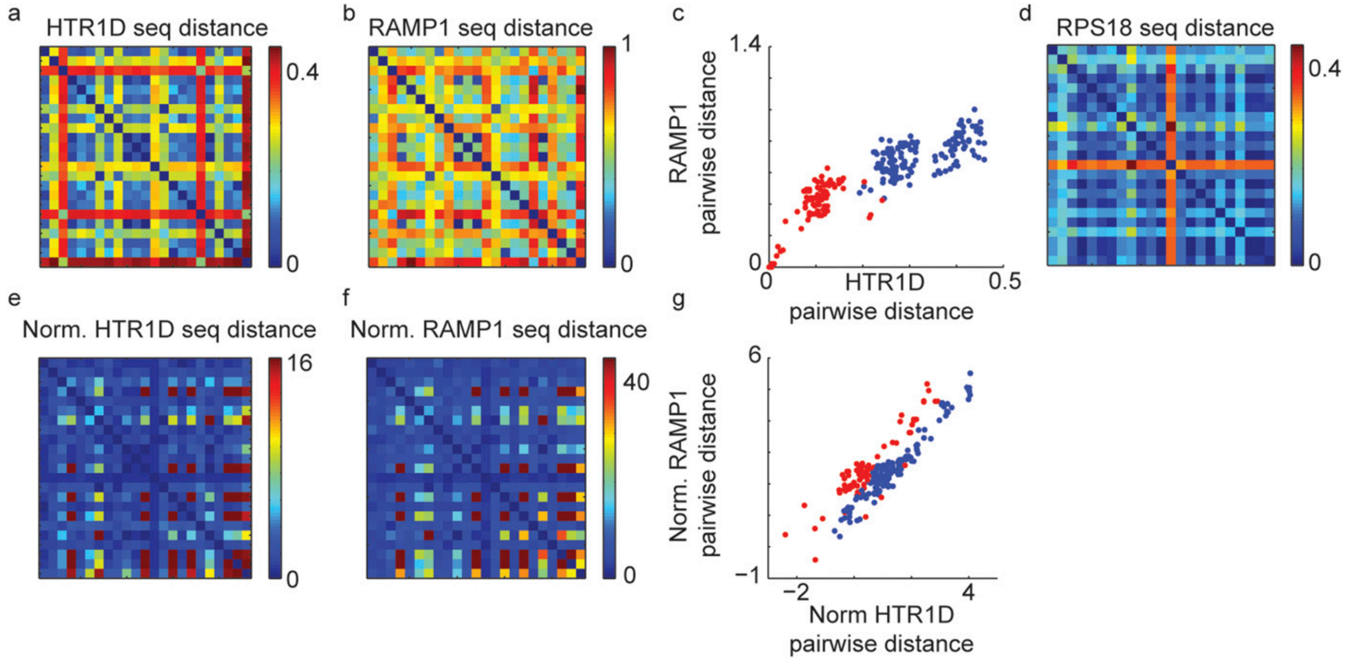

Fig. S3. Effect of normalization by 18S rRNA; HTR1D (a GPCR) and RAMP1 gene pairs as an example. (*A* and *B*) Color-coded matrices of phylogenetic resemblance based on amino acid sequence distance between organisms for (*A*) HTR1D and (*B*) RAMP1. (*C*) Scatter plot of between-species phylogenetic resemblance for RAMP1 versus HTR1D shows clusters of intraspecies (red) and interspecies (blue) family relationship. (*D*) The 18S rRNA distance matrix. (*E* and *F*) Color-coded 18S rRNA normalized (Norm.) matrices of phylogenetic resemblance for (*E*) HTR1D and (*F*) RAMP1. (*G*) Scatter plot, as in *C*, after normalization with 18S rRNA matrix. Note that, before normalization, interspecies and intraspecies relationships govern the correlation, while, after normalization, they do not.

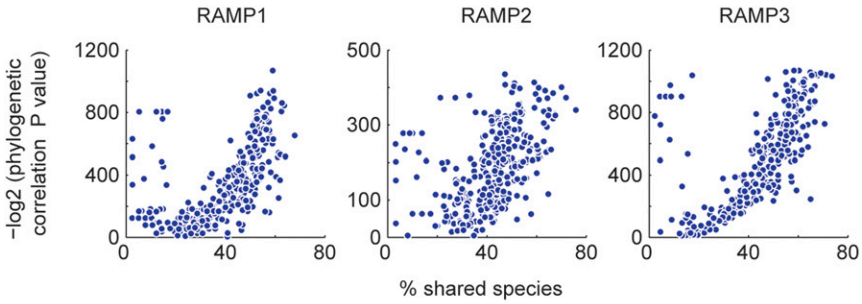

Fig. S4. Similar pattern of coevolution, across GPCR–RAMP pairs, based on percent shared species and correlation in phylogenetic trees, for all three RAMPs. Pearson's $r = 0.56$, $P = 2.51 \times 10^{-22}$ for RAMP1; $r = 0.48$, $P = 1.63 \times 10^{-20}$ for RAMP2; and $r = 0.61$, $P = 1.36 \times 10^{-25}$ for RAMP3.

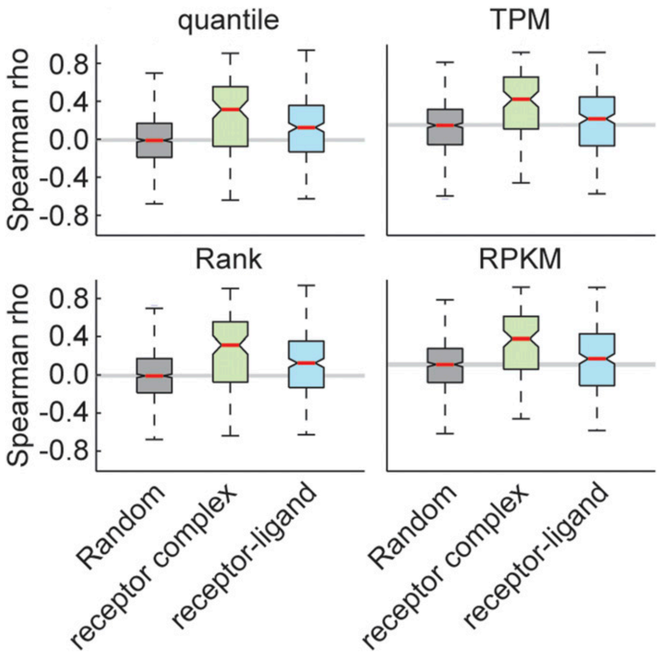

Fig. S5. Boxplot (as in Fig. 4) of coexpression, measured as Spearman correlation coefficient for four different methods for normalizing the expression data, show similar results.

Table S1. Forty-four organisms in which an orthologous gene was identified for at least one human GPCR and one human RAMP

Latimeria chalumnae
Danio rerio
Astyanax mexicanus
Gadus morhua
Xiphophorus maculatus
Oryzias latipes
Oreochromis niloticus
Anas platyrhynchos
Ficedula albicollis
Gallus gallus
Meleagris gallopavo
Sarcophilus harrisii
Dasypus novemcinctus
Callithrix jacchus
Macaca mulatta
Papio anubis
Gorilla gorilla gorilla
Chlorocebus sabaeus
Pan troglodytes
Pongo abelii
Homo sapiens
Canis familiaris
Ailuropoda melanoleuca
Mustela putorius furo
Felis catus
Loxodonta africana
Gasterosteus aculeatus
Equus caballus
Sus scrofa
Bos taurus
Oryctolagus cuniculus
Mus musculus
Rattus norvegicus
Cavia porcellus
Monodelphis domestica
Tetraodon nigroviridis
Pelodiscus sinensis
Anolis carolinensis
Otolemur garnettii
Takifugu rubripes
Spermophilus tridecemlineatus
Poecilia formosa
Myotis lucifugus
Taeniopygia guttata

Table S2. Organisms in OMA groups of orthologous genes for the three RAMPs

RAMP1	RAMP2	RAMP3
Ailuropoda melanoleuca	*Ailuropoda melanoleuca*	*Ailuropoda melanoleuca*
Anas platyrhynchos	*Bos Taurus*	*Anas platyrhynchos*
Anolis carolinensis	*Callithrix jacchus*	*Anolis carolinensis*
Astyanax mexicanus	*Canis familiaris*	*Astyanax mexicanus*
Callithrix jacchus	*Cavia porcellus*	*Bos taurus*
Canis familiaris	*Chlorocebus sabaeus*	*Callithrix jacchus*
Cavia porcellus	*Choloepus hoffmanni*	*Canis familiaris*
Gallus gallus	*Dasypus novemcinctus*	*Cavia porcellus*
Chlorocebus sabaeus	*Erinaceus europaeus*	*Gallus gallus*
Danio rerio	*Felis catus*	*Dipodomys ordii*
Echinops telfairi	*Gorilla gorilla gorilla*	*Erinaceus europaeus*
Erinaceus europaeus	*Equus caballus*	*Felis catus*
Felis catus	*Homo sapiens*	*Ficedula albicollis*
Ficedula albicollis	*Loxodonta africana*	*Gadus morhua*
Gadus morhua	*Macaca mulatta*	*Gasterosteus aculeatus*
Gorilla gorilla gorilla	*Monodelphis domestica*	*Gorilla gorilla gorilla*
Equus caballus	*Nomascus leucogenys*	*Equus caballus*
Homo sapiens	*Ochotona princeps*	*Homo sapiens*
Latimeria chalumnae	*Pan troglodytes*	*Latimeria chalumnae*
Meleagris gallopavo	*Papio anubis*	*Loxodonta africana*
Mus musculus	*Sus scrofa*	*Macropus eugenii*
Mustela putorius furo	*Pongo abelii*	*Macaca mulatta*
Myotis lucifugus	*Procavia capensis*	*Meleagris gallopavo*
Nomascus leucogenys	*Pteropus vampyrus*	*Monodelphis domestica*
Ochotona princeps	*Oryctolagus cuniculus*	*Mus musculus*
Oreochromis niloticus	*Rattus norvegicus*	*Mustela putorius furo*
Oryzias latipes	*Ovis aries*	*Nomascus leucogenys*
Pan troglodytes	*Sorex araneus*	*Oreochromis niloticus*
Pelodiscus sinensis	*Tursiops truncatus*	*Ornithorhynchus anatinus*
Sus scrofa		*Oryzias latipes*
Poecilia Formosa		*Otolemur garnettii*
Pteropus vampyrus		*Pan troglodytes*
Rattus norvegicus		*Papio anubis*
Takifugu rubripes		*Poecilia formosa*
Tetraodon nigroviridis		*Pongo abelii*
Tursiops truncatus		*Rattus norvegicus*
Xenopus tropicalis		*Sarcophilus harrisii*
Xiphophorus maculatus		*Ovis aries*
		Spermophilus tridecemlineatus
		Taeniopygia guttata
		Takifugu rubripes
		Tursiops truncatus
		Xenopus tropicalis
		Xiphophorus maculatus

Table S3. Receptor–ligand gene pairs used in the analysis

Receptor gene	Ligand gene	Receptor gene	Ligand gene	Receptor gene	Ligand gene	Receptor gene	Ligand gene
NOTCH1	JAG1	IL2RG	IL4	FGFR2	FGF18	BMPR1B	BMP8
NOTCH2	JAG1	IL13RA1	IL4	FGFR3	FGF18	BMPR2	BMP8
NOTCH3	JAG1	IL13RA2	IL13	FGFR4	FGF18	ACVR1	BMP8
NOTCH4	JAG1	IL4R	IL13	FGFR1	FGF19	ACVR2	BMP8
NOTCH2	JAG2	IL2RG	IL13	FGFR2	FGF19	ACVR2B	BMP8
NOTCH3	JAG2	CSF2RB	IL3	FGFR3	FGF19	BMPR1B	BMP10
NOTCH4	JAG2	CSF2RB	IL5	FGFR4	FGF19	BMPR2	BMP10
NOTCH1	DLL1	CSF2RB	CSF2	NTRK1	NGFB	ACVR1	BMP10
NOTCH2	DLL1	IFNGR2	IFNG	NGFR	NGFB	ACVR2	BMP10
NOTCH3	DLL1	IL10RB	IL10	NGFR	BDNF	ACVR2B	BMP10
NOTCH4	DLL1	IL17R	IL17	NTRK1	NTF3	BMPR1B	BMP15
NOTCH2	DLL3	IL12RB2	IL12A	NTRK2	NTF3	BMPR2	BMP15
NOTCH3	DLL3	IL12RB2	IL12B	NTRK3	NTF3	ACVR1	BMP15
NOTCH4	DLL3	IFNAR2	IFNA4	NGFR	NTF3	ACVR2	BMP15
NOTCH2	DLL4	IFNAR1	IFNA1	NTRK1	NTF5	ACVR2B	BMP15
NOTCH3	DLL4	IFNAR2	IFNA1	NTRK2	NTF5	ACVR1B	INHBA
NOTCH4	DLL4	IFNAR2	IFNA5	NTRK3	NTF5	ACVR2	INHBA
EPHA2	EFNA1	IFNAR2	IFNA16	NGFR	NTF5	ACVR2B	INHBA
EPHA3	EFNA1	IFNAR2	IFNA8	PDGFRB	PDGFB	ACVR1B	INHBB
EPHA4	EFNA1	IFNAR2	IFNA14	FLT1	VEGF	ACVR2	INHBB
EPHA5	EFNA1	IFNAR2	IFNA21	KDR	VEGF	ACVR2B	INHBB
EPHA7	EFNA1	IFNAR2	IFNA2	NRP1	VEGF	ACVR1B	INHBC
EPHA8	EFNA1	IFNAR2	IFNA13	NRP2	VEGF	ACVR2	INHBC
EPHA2	EFNA2	IFNAR2	IFNA6	NRP1	VEGFB	ACVR2B	INHBC
EPHA3	EFNA2	IFNAR2	IFNA7	FLT4	VEGFC	ACVR1B	INHA
EPHA4	EFNA2	IFNAR2	IFNA10	FLT4	FIGF	ACVR2	INHA
EPHA5	EFNA2	IFNAR1	IFNA17	NRP1	PGF	ACVR2B	INHA
EPHA7	EFNA2	IFNAR2	IFNA17	EGFR	EGF	CCXCR1	SCYC1
EPHA8	EFNA2	IFNAR2	IFNW1	EGFR	TGFA	CCXCR1	SCYC2
EPHA2	EFNA3	IFNAR2	IFNB1	EGFR	AREG	CX3CR1	SCYD1
EPHA3	EFNA3	IL1R2	IL1A	EGFR	BTC	CCR3	SCYA11
EPHA4	EFNA3	IL1R2	IL1B	ERBB3	BTC	GPR9	SCYA11
EPHA5	EFNA3	IL18RAP	IL18	ERBB4	BTC	CCR3	SCYA24
EPHA7	EFNA3	FGFR1	FGF1	ERBB4	NRG1	CCR1	SCYA23
EPHA8	EFNA3	FGFR2	FGF1	ERBB3	NRG1	CCR1	SCYA14
EPHA2	EFNA4	FGFR3	FGF1	ERBB2	NRG1	CCBP2	SCYA14
EPHA3	EFNA4	FGFR4	FGF1	EGFR	DTR	CCR3	SCYA26
EPHA4	EFNA4	FGFR1	FGF2	ERBB4	DTR	CCR1	SCYA3
EPHA5	EFNA4	FGFR2	FGF2	EGFR	EREG	CCR5	SCYA3
EPHA7	EFNA4	FGFR3	FGF2	ERBB4	EREG	CCR5	SCYA4
EPHA8	EFNA4	FGFR4	FGF2	IL6ST	IL11	CCR8	SCYA4
EPHA2	EFNA5	FGFR1	FGF3	IL6R	IL6	CCR1	SCYA15
EPHA3	EFNA5	FGFR2	FGF3	IL6ST	IL6	CCR3	SCYA15
EPHA4	EFNA5	FGFR1	FGF4	IL6ST	LIF	CCR8	SCYA1
EPHA5	EFNA5	FGFR2	FGF4	LIFR	CTF1	CCR7	SCYA21
EPHA7	EFNA5	FGFR3	FGF4	IL6ST	CNTF	GPR9	SCYA21
EPHA8	EFNA5	FGFR4	FGF4	LIFR	CNTF	CCR1	SCYA5
EPHB2	EFNB1	FGFR1	FGF5	IL6ST	OSM	CCR3	SCYA5
EPHB3	EFNB1	FGFR2	FGF5	LIFR	OSM	CCR4	SCYA5
EPHB4	EFNB1	FGFR3	FGF5	KIT	SCGF	CCR5	SCYA5
EPHB6	EFNB1	FGFR1	FGF6	FLT3	FLT3LG	FY	SCYA5
EPHA4	EFNB1	FGFR2	FGF6	CSF1R	CSF1	CCR2	SCYA2
EPHB2	EFNB2	FGFR4	FGF6	PTPRZ1	MDK	FY	SCYA2
EPHB3	EFNB2	FGFR2	FGF7	PTPRB	MDK	CCR2	SCYA8
EPHB4	EFNB2	FGFR2	FGF8	PTPRB	PTN	CCR3	SCYA8
EPHB6	EFNB2	FGFR3	FGF8	KIAA0468	PTN	CCR5	SCYA8
EPHA4	EFNB2	FGFR4	FGF8	TGFBR1	TGFB1	CCR1	SCYA8
EPHB2	EFNB3	FGFR1	FGF9	TGFBR2	TGFB1	CCBP2	SCYA8
EPHB3	EFNB3	FGFR2	FGF9	TGFBR3	TGFB1	GPR2	SCYA27
EPHB4	EFNB3	FGFR3	FGF9	TGFBR2	TGFB2	CCR1	SCYA7
EPHB6	EFNB3	FGFR4	FGF9	TGFBR3	TGFB2	CCR2	SCYA7
EPHA4	EFNB3	FGFR1	FGF10	TGFBR2	TGFB3	CCR3	SCYA7
TNFRSF1A	TNF	FGFR2	FGF10	TGFBR3	TGFB3	CCR5	SCYA7

Table S3. Cont.

Receptor gene	Ligand gene	Receptor gene	Ligand gene	Receptor gene	Ligand gene	Receptor gene	Ligand gene
TNFRSF1B	TNF	FGFR3	FGF10	BMPR1B	BMP2	CCR2	SCYA13
LTBR	TNF	FGFR4	FGF10	BMPR2	BMP2	CCR3	SCYA13
TNFRSF7	TNFSF7	FGFR1	FGF11	ACVR1	BMP2	CCR1	SCYA13
TNFRSF1B	LTA	FGFR2	FGF11	ACVR2	BMP2	CCR5	SCYA13
TNFRSF14	LTA	FGFR3	FGF11	ACVR2B	BMP2	CCR4	SCYA17
LTBR	LTA	FGFR4	FGF11	BMPR1B	BMP3	CCR8	SCYA17
TNFRSF8	TNFSF8	FGFR1	FGF12	BMPR2	BMP3	CCR6	SCYA20
LTBR	LTB	FGFR2	FGF12	ACVR1	BMP3	CCR7	SCYA19
TNFRSF5	TNFSF5	FGFR3	FGF12	ACVR2	BMP3	CCR9	SCYA25
TNFRSF6	TNFSF6	FGFR4	FGF12	ACVR2B	BMP3	CCR4	SCYA22
TNFRSF6B	TNFSF6	FGFR1	FGF12B	BMPR1B	BMP4	IL8RA	IL8
TNFRSF10B	TNFSF10	FGFR2	FGF12B	BMPR2	BMP4	IL8RB	IL8
TNFRSF10C	TNFSF10	FGFR3	FGF12B	ACVR1	BMP4	FY	IL8
TNFRSF10D	TNFSF10	FGFR4	FGF12B	ACVR2	BMP4	IL8RA	SCYB6
TNFRSF11B	TNFSF10	FGFR1	FGF13	ACVR2B	BMP4	IL8RB	SCYB6
TNFRSF11B	TNFSF11	FGFR2	FGF13	BMPR1B	BMP5	GPR9	MIG
TNFRSF14	TNFSF14	FGFR3	FGF13	BMPR2	BMP5	CXCR4	SDF1
TNFRSF6B	TNFSF14	FGFR4	FGF13	ACVR1	BMP5	GPR9	SCYB11
LTBR	TNFSF14	FGFR1	FGF14	ACVR2	BMP5	IL8RB	SCYB5
TNFRSF12	TNFSF12	FGFR2	FGF14	ACVR2B	BMP5	IL8RA	SCYB5
TNFRSF18	TNFSF18	FGFR3	FGF14	BMPR1B	BMP6	GPR9	SCYB10
IL8RB	GRO2	FGFR4	FGF14	BMPR2	BMP6	IL8RB	PPBP
IL8RB	GRO3	FGFR1	FGF16	ACVR1	BMP6	FY	PPBP
BLR1	SCYB13	FGFR2	FGF16	ACVR2	BMP6	IL8RB	GRO1
IL2RB	IL2	FGFR3	FGF16	ACVR2B	BMP6	FY	GRO1
IL2RG	IL2	FGFR4	FGF16	BMPR1B	BMP7		
IL2RG	IL7	FGFR1	FGF17	BMPR2	BMP7		
IL2RG	IL9	FGFR2	FGF17	ACVR1	BMP7		
IL15RA	IL15	FGFR3	FGF17	ACVR2	BMP7		
IL2RB	IL15	FGFR4	FGF17	ACVR2B	BMP7		
IL2RG	IL15	FGFR1	FGF18	BMPR1A	BMP8		

Table S4. Gene pairs that are subunits in the same receptor

Receptor		Receptor		Receptor		Receptor	
Gene 1	Gene 2	Gene 1	Gene 2	Gene 1	Gene 2	Gene 1	Gene 2
NOTCH1	NOTCH2	EPHB2	EPHB6	IL13RA2	IL2RG	BMPR1A	ACVR2B
NOTCH1	NOTCH3	EPHB2	EPHA4	IFNGR1	IFNGR2	BMPR1B	BMPR2
NOTCH1	NOTCH4	EPHB3	EPHB4	IFNAR1	IFNAR2	BMPR1B	ACVR1
NOTCH2	NOTCH3	EPHB3	EPHB6	FGFR1	FGFR2	BMPR1B	ACVR2
NOTCH2	NOTCH4	EPHB3	EPHA4	FGFR1	FGFR3	BMPR1B	ACVR2B
NOTCH3	NOTCH4	EPHB4	EPHB6	FGFR1	FGFR4	BMPR2	ACVR1
EPHA1	EPHA3	EPHB4	EPHA4	FGFR2	FGFR3	BMPR2	ACVR2
EPHA1	EPHA4	EPHB6	EPHA4	FGFR2	FGFR4	BMPR2	ACVR2B
EPHA1	EPHA5	TNFRSF1A	LTBR	FGFR3	FGFR4	ACVR1	ACVR2
EPHA1	EPHA7	TNFRSF1B	LTBR	NTRK1	NGFR	ACVR1	ACVR2B
EPHA1	EPHA8	TNFRSF1A	TNFRSF14	NTRK1	NTRK2	ACVR2	ACVR2B
EPHA2	EPHA3	TNFRSF1B	TNFRSF14	NTRK1	NTRK3	ACVR1B	ACVR2
EPHA2	EPHA4	TNFRSF14	LTBR	NTRK2	NTRK3	ACVR1B	ACVR2B
EPHA2	EPHA5	TNFRSF6	TNFRSF6B	NTRK3	NGFR	CCR3	GPR9
EPHA2	EPHA7	TNFRSF10A	TNFRSF10C	FLT1	NRP2	CCR7	GPR9
EPHA2	EPHA8	TNFRSF10A	TNFRSF10D	KDR	NRP1	CCR1	CCR4
EPHA3	EPHA4	TNFRSF10A	TNFRSF11B	KDR	NRP2	CCR1	FY
EPHA3	EPHA5	TNFRSF10B	TNFRSF10C	NRP1	NRP2	CCR3	CCR4
EPHA3	EPHA7	TNFRSF10B	TNFRSF10D	EGFR	ERBB3	CCR3	CCR5
EPHA3	EPHA8	TNFRSF10B	TNFRSF11B	EGFR	ERBB4	CCR3	FY
EPHA4	EPHA5	TNFRSF10C	TNFRSF10D	ERBB4	ERBB2	CCR4	CCR5
EPHA4	EPHA7	TNFRSF10C	TNFRSF11B	ERBB3	ERBB2	CCR4	FY
EPHA4	EPHA8	TNFRSF10D	TNFRSF11B	IL6R	IL6ST	CCR5	FY
EPHA5	EPHA7	TNFRSF14	TNFRSF6B	CNTFR	LIFR	CCR2	FY
EPHA5	EPHA8	TNFRSF6B	LTBR	OSMR	LIFR	CCR2	CCR5
EPHA7	EPHA8	IL2RA	IL2RG	PTPRZ1	KIAA0468	CCR2	CCBP2
EPHB1	EPHB3	IL2RB	IL2RG	PTPRB	KIAA0468	CCR3	CCBP2
EPHB1	EPHB4	IL15RA	IL2RB	TGFBR1	TGFBR3	CCR5	CCBP2
EPHB1	EPHB6	IL15RA	IL2RG	TGFBR2	TGFBR3	IL8RA	IL8RB
EPHB1	EPHA4	IL4R	IL13RA1	BMPR1A	BMPR2	IL8RA	FY
EPHB2	EPHB3	IL2RG	IL13RA1	BMPR1A	ACVR1	IL8RB	FY
EPHB2	EPHB4	IL13RA2	IL4R	BMPR1A	ACVR2		

Table S5. Phylogenetic and coexpression measures for validated GPCR-RAMP interacting pairs

		Coexpression		Shared species		Phylogenetic			
GPCR	Interacting RAMP	C.C.	Rank	Percent	Rank	C.C.	C.C. Rank	C.P.	C.P. Rank
CALCRL	RAMP1	0.2	106	43.66	151	0.93	62	0	1 (18 zeros)
CALCRL	RAMP2	0.83	2	32.85	276	0.9	88	7.39E-115	27
CALCRL	RAMP3	0.82	1	52.11	151	0.94	35	0	1 (55 zeros)
CALCR	RAMP1	0.1	178	33.92	227	0.86	198	8.66E-63	213
CALCR	RAMP2	0.07	196	57.14	50	0.83	235	6.47E-70	136
CALCR	RAMP3	0.17	165	44.64	211	0.89	206	2.49E-144	202
CASR	RAMP1	−0.2	695	55.73	46	0.91	122	2.3E-232	25
CASR	RAMP3	−0.06	600	68.33	20	0.94	54	0	1 (55 zeros)
CRHR1	RAMP2	−0.4	756	NA	NA	NA	NA	NA	NA
GCGR	RAMP2	0.22	143	36.84	260	0.83	238	8.72E-29	273
GPER1	RAMP3	0.07	274	61.40	71	0.95	24	0	1 (18 zeros)
PTH1R	RAMP2	0.37	82	41.5	221	0.8	265	1.03E-61	166
PTH2R	RAMP3	−0.15	282	55.55	129	0.93	57	4.78E-211	137
SCTR	RAMP3	0.37	79	37.25	274	0.93	76	3.7E-117	221
SCTR	RAMP3	0.37	75	37.25	254	0.92	82	3.68E-117	220
VIPR1	RAMP1	−0.16	336	21.05	299	0.99	1	6.51E-38	269
VIPR1	RAMP2	0.09	189	8.82	335	0.87	175	2.26E-84	79
VIPR1	RAMP3	0.01	215	13.04	329	0.9	173	2.14E-272	86
VIPR2	RAMP1	0.73	1	10.25	330	0.99	1	6.51E-38	269
VIPR2	RAMP2	0.3	115	6.25	338	0.87	175	2.26E-84	79
VIPR2	RAMP3	0.34	92	8.88	333	0.9	173	2.14E-272	86

Measures are based on refs. 2, 4, 5, and 23. C.C., correlation coefficient; C.P., correlation *P* value; NA, cases of GPCR−RAMP pairs that had less than five shared species. As noted in *Results*, phylogenetic measures were only calculated for GPCR−RAMP pairs that had more than five shared species.

Dataset S1. Analyzed GPCRs, excluding olfactory GPCRs, including gene information, gene family information and the full measurements reported in the study

Dataset S1

Shown are GPCRs that were represented in both the OMA and GTEx databases and, in a separate spread sheet, those that were only represented in the GTEx database.