

Supplementary Material

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humanVPAC2_CTerm	1 -SEVQCE	LKRKWR	SRCPTPSASRDYR	VCGSSFSRNG	SEGALQF	-----	HRGSRAQS	FLOTETS	SVI
mouseVPAC2_CTerm	1 -SEVQCE	LKRKWR	GLCLTQAGSRDY	RLHSWSMSRNG	SESALQI	-----	HRGSRTQS	FLQSETS	SVI
ratVPAC2_CTerm	1 -SEVQCE	LKRKWR	GLCLTQPGSRDY	RLHSWSMSRNG	SESALQI	-----	HRGSRTQS	FLQSETS	SVI
bovineVPAC2_CTerm	1 NSEVQSE	LRRRWR	GLWPSRPSARDY	RLHSSSISR	TGSDGALQ	-----	PRGSRAPS	LLQTE	TSVI
zebrafishVPAC2_CTerm	1 --EVOSE	LKRKWR	SLRLKRYIGRDY	RLHSSSISR	NGTENMAQF	-----	QRNTRAQ	SFLOTET	TVV
tasmaniandevilVPAC2_CTerm	1 -SEVQSE	LKRKWR	SLCLNQSMNRDY	RLHSSSISR	NGSEILHF	-----	HRNSRAHS	FLQTE	TVI
pigVPAC2_CTerm	1 -SEVQSE	LRRRWR	GLCPSRPSGRDY	RLHSSSISR	NGSEGALQV	-----	PRGSRAPS	FLQME	TSVI
turkeyVPAC2_CTerm	1 -SEVQCE	LKRKWR	SLCWKQTAGRDY	RLHSS-ISR	NGSEVSOL	-----	HRNSRAQS	FMOTET	TMI
rhesusmacaqueVPAC2_CTerm	1 -SEVQCE	LKRKWR	SRCPTPSTSRDY	RVCSSISR	NGSEGALQF	-----	HRGSRAQS	FLOTETS	SVI
opossumVPAC2_CTerm	1 -SEVQCE	LKRKWR	GLCLNQSMNRDY	RLHSSSISR	NGSEVLHF	-----	HRNSRAHS	FLQTE	TVI
frogVPAC2_CTerm	1 -TEVOGE	LKRKWR	SLDYIRYKHKDK	RMHSLTISR	NGSEGVQF	-----	HRDSRAQS	IMQTE	TMI
humanVPAC1_CTerm	1 -GEVQAE	LRRKWR	RWHLQVGLGWN	PKYRHP	SGGSNGATC	STOV	SMLTRV	SPGARR	SSFOAEV
mouseVPAC1_CTerm	1 -GEVQAE	LRRKWR	RWHLQVGLGWS	SKQHP	WGGSNGV	SCSTOV	SMLTRV	SPSARR	SSFOAEV
ratVPAC1_CTerm	1 -GEVQAE	LRRKWR	RWHLQVGLGWS	SKQHP	WGGSNGATC	STOV	SMLTRV	SPSARR	SSFOAEV
bovineVPAC1_CTerm	1 -GEVQAE	LRRKWR	RWHLQVGLGWD	PKYQHP	SAGSNGATC	STOV	SMLTRV	SPGAGR	SSFOAEV
zebrafishVPAC1_CTerm	1 -GEVQGE	IKRKWR	RWHLEFLGPD	TKYQHP	SMGSGN	GNFSTO	ISMLTR	SPKTRR	ASTCODE
tasmaniandevilVPAC1_CTerm	1 -GEVQAE	FKRKWR	RWHLEFLGPD	VKYHHP	SVGSGN	GNFSTO	ISMLTR	SPKDRR	SSFOAEV
pigVPAC1_CTerm	1 -GEVQAE	LRRKWR	RWHLQVGLGWD	SKYQHP	SGGSNGD	TCSTOV	SMLTRV	SPSARR	SSFOAEV
turkeyVPAC1_CTerm	1 -GEVQAE	LRRKWR	RWHLEFLGSD	MKYHHP	SLGSGN	TNFSTO	ISMLTK	SPKTRR	SSFOAEV
rhesusmacaqueVPAC1_CTerm	1 -GEVQAE	LRRKWR	RWHLQVGLGWN	PKYRHP	SGGSNSATC	STOV	SMLTRV	SPGARR	SSFOAEV
opossumVPAC1_CTerm	1 -GEVQAE	IKRKWR	RWHLEFLGSD	LKYHHP	SVGSGN	TNFSTO	ISMLTR	SPKDRR	SSFOAEV
frogVPAC1_CTerm	1 -GEVQCE	LKRKWR	RWHLEFLGSD	MKYHHP	SLGSGN	TNFSTO	ISMLTK	SPKTRR	SSFOAEV

Fig. S1. Related to Fig. 4. Amino acid sequence alignment of VPAC2's and VPAC1's C-termini. Amino acid sequences were aligned using CLUSTALW and shaded using BOXSHADE such that identical amino acids are shaded black, and conserved residues are shaded in gray. The last 44 amino acids (highlighted in green) of VPAC2's C-terminus differ significantly from those of VPAC1.

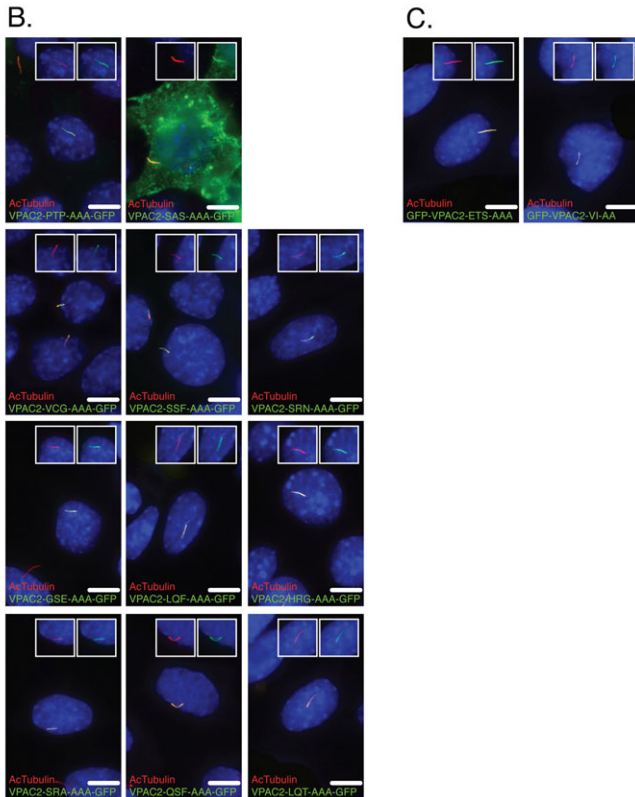


Fig. S2. Related to Fig. 5. (A) Schematic of VPAC2. The last 44 amino acids of VPAC2 C-terminus is shown in letters. (B) Representative images of mutated residues that did not affect ciliary targeting. IMCD3 cells expressing VPAC2-GFP harboring alanine mutations were serum starved to induce ciliogenesis. (C) ETSVI at the end of VPAC2's C terminus, a potential PDZ binding domain, is not important for ciliary targeting. IMCD3 cells expressing GFP-VPAC2 harboring alanine mutations were serum starved to induce ciliogenesis. (B,C) Cells were immunostained for acetylated α -tubulin (red). DNA was labeled with mounting medium containing 4',6-diamidino-2-phenylindole (DAPI). Scale bars: 5 μ m.

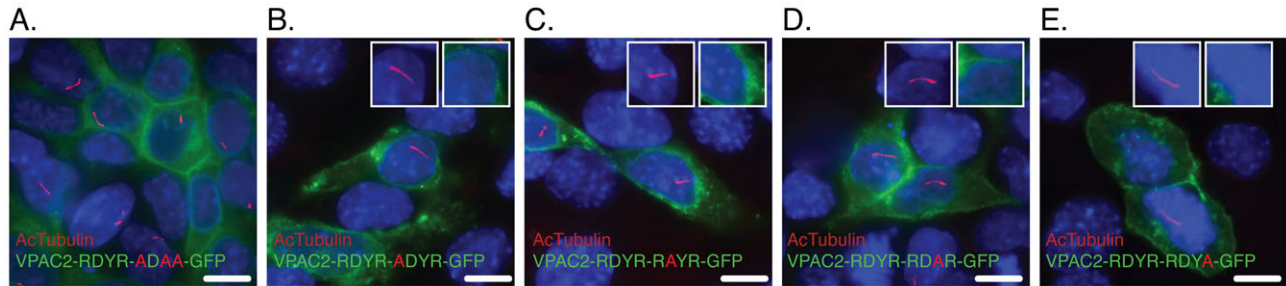


Fig. S3. Related to Fig. 5. (A) VPAC2 harboring RDYR→ADAA mutation in the C-terminus fails to target to cilia in IMCD3 cells. IMCD3 cells were transduced with lentivirus vectors expressing VPAC2-GFP with RDYR→ADAA mutation and serum starved. Cells were then immunostained for acetylated α -tubulin (AcTubulin, red). (B–E) Single mutations in the RDYR motif abolish ciliary targeting of VPAC2 in IMCD3 cells. IMCD3 cells were transfected with plasmids expressing VPAC2-GFP harbouring RDYR→ADYR (D), RDYR→RAYR (E), RDYR→RDAR (F), and RDYR→RDYA (G) in the C terminus and were immunostained for acetylated α -tubulin (AcTubulin, red). Insets show unmerged images of the region around cilia. DNA was labeled with mounting medium containing DAPI (A–E). Scale bars: 5 μ m.

Table S1. List of GPCRs tested.

GPCR family	GPCRs tested
Adhesion family (11)	BAI2, BAI3, CD97, ELTD1, EMR1, EMR2, GPR56, GPR64, GPR126, GPR133, LPHN1
Glutamate family (6)	GABBR2, GPR5C5B, GPRC5C, GPRC5D, GRM3, GRM4
Rhodopsin family (with known ligands, 58)	AGTR1, C3AR1, C5AR1, CCR1, CCR2, CCR6, CHRM1, CHRM2, CHRM3, CHRM5, CXCR6, CXCR7, CYSLTR1, DARC, EDNRA, EDNRB, F2R, F2RL1, FFAR1, FFAR2, FFAR3, FPR1, FPR3, FSHR, GHSR, GNRHR, GPER, HTR1B, HTR1D, LGR5, LTB4R, MAS1, MAS1L, MC1R, MC2R, MC3R, MRAP, <u>NPFFR2</u> , NMUR2, NPBWR2, OXGR1, P2RY2, P2RY4, P2RY5, P2RY6, P2RY8, P2RY10, P2RY12, P2RY13, P2RY14, PTAFR, RXFP1, RXFP3, TACR3, TRHR, TSHR, VN1R2, VN1R3
Rhodopsin family (orphan, 33)	GPR3, GPR4, GPR17, GPR18, GPR20, GPR21, GPR22, GPR34, GPR35, GPR37, GPR45, GPR52, GPR55, GPR63, GPR65, GPR68, GPR75, GPR78, GPR82, GPR83, GPR84, GPR85, GPR87, GPR107, GPR108, GPR119, GPR137B, GPR141, GPR146, GPR162, GPR171, GPR173, GPR177, GPR183
Secretin family (14)	CALCR, CALCRL, CRHR1, <u>CRHR2</u> , <u>GHRHR</u> , GIPR, GLP1R, GLP2R, PAC1, PTHR1, PTHR2, SCTR, VPAC1, VPAC2

All GPCRs are from human origin except NPFFR2, CRHR2 and GHRHR (underlined) are from mouse origin.

Table S2. Oligonucleotides used in this study to construct the CD8 α chimeric constructs.

	Primers
Full length C terminus (forward)	GGAACCGTCGACAGTGAGGTGACAGTGCAG
Truncated C terminus – from PTPSAS (forward)	GGAACCGTCGACCCGACCCCGTCCGCGAGC
Full length/truncated C terminus (reverse)	GTTGCTCGAGGATGACCGAGGTCTCCGTTTG

Table S3. Oligonucleotides used in this study to construct the lentivirus constructs encoding shRNAmiRs.

	Primers
VPAC2 shRNAmiR #1 (nt 718) forward primer	TGCTGAAGAATACCAGGCTGAGCTTGGTTTTGGCCACTGACTGACCAAGCTCACTGGTATTCTT
VPAC2 shRNAmiR #1 (nt718) reverse primer	CCTGAAGAATACCAGTGAAGCTTGGTCAGTCAGTGGCCAAAACCAAGCTCAGCCTGGTATTCTTC
VPAC2 shRNAmiR #2 (nt2126) forward primer	TGCTGTAGAGAACAACCTTGGGCTGAGTTTTGGCCACTGACTGACTCAGCCCAGTTGTTCTCTA
VPAC2 shRNAmiR #2 (nt2126) reverse primer	CCTGTAGAGAACAACCTGGGCTGAGTCAGTCAGTGGCCAAAACCTCAGCCCAAGGTTGTTCTCTAC
BBS2 shRNAmiR (nt744) forward primer	TGCTGAGAAAGGGCATAACCAAACCGTTTTGGCCACTGACTGACCGGTTTGGATGCCCTTTCT
BBS2 shRNAmiR (nt744) reverse primer	CCTGAGAAAGGGCATCCAAACCGGTCAGTCAGTGGCCAAAACCGTTTTGGTTATGCCCTTTCTC
Tubby shRNAmiR #1 (nt1043) forward primer	TGCTGAGATGATGCCTTCTGAGGGTTGTTTTGGCCACTGACTGACAACCCTCAAGGCATCATCT
Tubby shRNAmiR #1 (nt1043) reverse primer	CCTGAGATGATGCCTTCTGAGGGTTGTCAGTCAGTGGCCAAAACAACCCTCAGAAGGCATCATCTC
Tubby shRNAmiR #2 (nt1046) forward primer	TGCTGAGAAAGATGATGCCTTCTGAGGGTTTTGGCCACTGACTGACCCTCAGAACATCATCTTCT
Tubby shRNAmiR #2 (nt1046) reverse primer	CCTGAGAAGATGATGTTCTGAGGGTCAGTCAGTGGCCAAAACCTCAGAAGGCATCATCTTCTC
EmGFP-shRNAmiR forward primer	CGCGAATTCACCGGTCGCCACCATGGTGAGCAAGGGCG
EmGFP-shRNAmiR reverse primer	TCCTCTCGAGTGC GGCCAGATCTGG

Table S4. Oligonucleotides used in this study for RT-qPCR.

	Primers
GAPDH forward primer	AACCTGCCAAGTATGATGACATCA
GAPDH reverse primer	TGTTGAAGTACAGGAGACAACCT
VPAC2 forward primer	CCATCTCTGTGCTGGTCAAG
VPAC2 reverse primer	AGGTAGGCCAGGAAACACCT
BBS2 forward primer	AGAACTGAGCAGTCCCCTGA
BBS2 reverse primer	AAATGAGTACTGCCCGGATG
Tubby forward primer	AAGAGCCGCTGATGGTACAG
Tubby reverse primer	CTTGGTCTTCTTGCTGAGG