Supplementary Material
Livana Soetedjo et al. doi: 10.1242/bio.20134747

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.

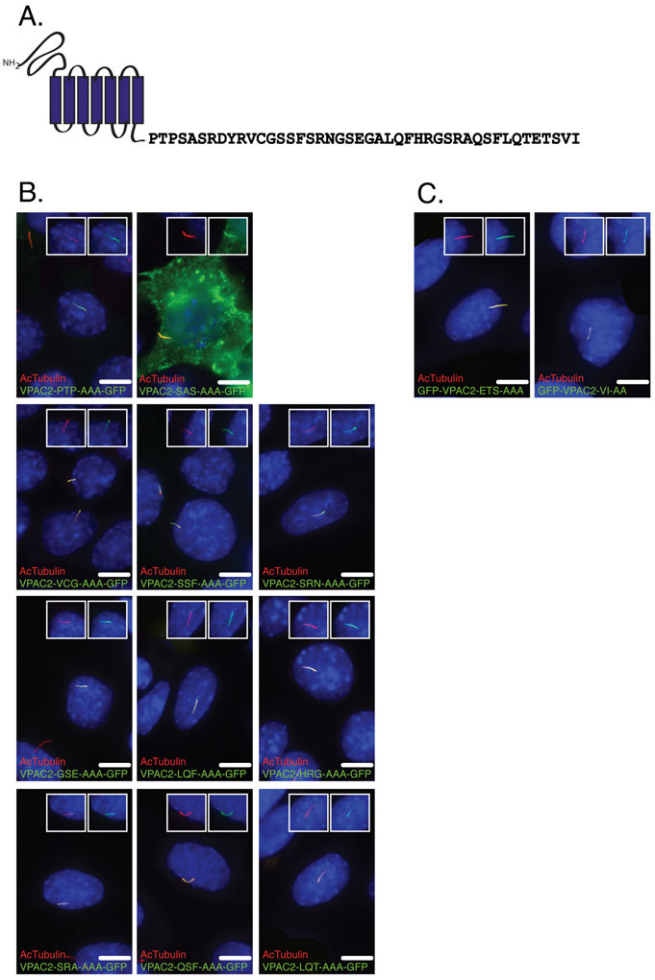

Fig. S2. Related to Fig. 5. (**A**) Schematic of VPAC2. The last 44 amino acids of VPAC2 C-terminus is shown in letters. (**B**) Representative images of mutated residues that did not affect ciliary targeting. IMCD3 cells expressing VPAC2-GFP harboring alanine mutations were serum starved to induce ciliogenesis. (**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-phenylinodole (DAPI). Scale bars: 5 µm.

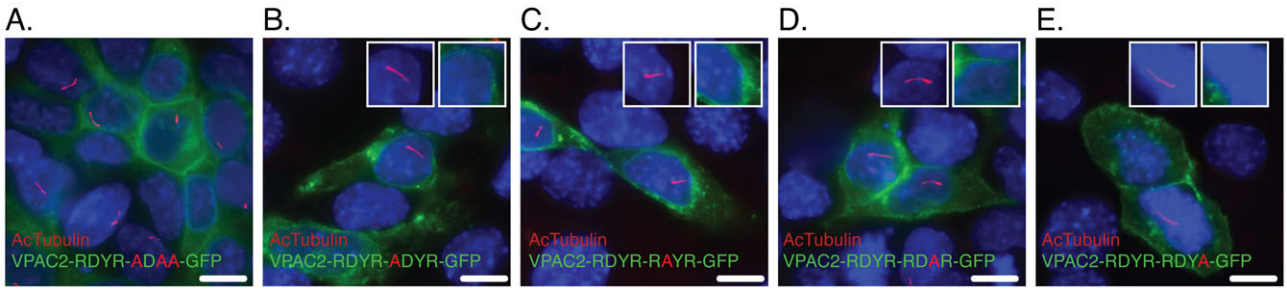

Fig. S3. Related to Fig. 5. (**A**) VPAC2 harboring RDYR→ADAA mutation in the C-terminus fails to target to cilia in IMCD3 cells. IMCD3 cells were transduced with lentivirus vectors expressing VPAC2-GFP with RDYR→ADAA mutation and serum starved. Cells were then immunostained for acetylated α-tubulin (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 μm.

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, NPFFR2, 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, CRHR2, GHRHR, 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 CD8α chimeric constructs.

	Primers
Full length C terminus (forward)	GGAACCGTCGACAGTGAGGTGCAGTGCGAG
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	CCTGAAGAATACCAGTGAGCTTGGTCAGTCAGTGGCCAAAACCAAGCTCAGCCTGGTATTCTTC
VPAC2 shRNAmiR #2 (nt2126) forward primer	TGCTGTAGAGAACAACCTTGGGCTGAGTTTTGGCCACTGACTGACTCAGCCCAGTTGTTCTCTA
VPAC2 shRNAmiR #2 (nt2126) reverse primer	CCTGTAGAGAACAACTGGGCTGAGTCAGTCAGTGGCCAAAACTCAGCCCAAGGTTGTTCTCTAC
BBS2 shRNAmiR (nt744) forward primer	TGCTGAGAAAGGGCATAACCAAACCGGTTTTGGCCACTGACTGACCGGTTTGGATGCCCTTTCT
BBS2 shRNAmiR (nt744) reverse primer	CCTGAGAAAGGGCATCCAAACCGGTCAGTCAGTGGCCAAAACCGGTTTGGTTATGCCCTTTCTC
Tubby shRNAmiR #1 (nt1043) forward primer	TGCTGAGATGATGCCTTCTGAGGGTTGTTTTGGCCACTGACTGACAACCCTCAAGGCATCATCT
Tubby shRNAmiR #1 (nt1043) reverse primer	CCTGAGATGATGCCTTGAGGGTTGTCAGTCAGTGGCCAAAACAACCCTCAGAAGGCATCATCTC
Tubby shRNAmiR #2 (nt1046) forward primer	TGCTGAGAAGATGATGCCTTCTGAGGGTTTTGGCCACTGACTGACCCTCAGAACATCATCTTCT
Tubby shRNAmiR #2 (nt1046) reverse primer	CCTGAGAAGATGATGTTCTGAGGGTCAGTCAGTGGCCAAAACCCTCAGAAGGCATCATCTTCTC
EmGFP-shRNAmiR forward primer	GCGCGAATTCACCGGTCGCCACCATGGTGAGCAAGGGCG
EmGFP-shRNAmiR reverse primer	TCCTCTCGAGTGCGGCCAGATCTGG

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

	Primers
GAPDH forward primer	AACCTGCCAAGTATGATGACATCA
GAPDH reverse primer	TGTTGAAGTCACAGGAGACAACCT
VPAC2 forward primer	CCATCTCTGTGCTGGTCAAG
VPAC2 reverse primer	AGGTAGGCCAGGAAACACCT
BBS2 forward primer	AGAACTGAGCAGTCCCCTGA
BBS2 reverse primer	AAATGAGTACTGCCCGGATG
Tubby forward primer	AAGAGCCGCTGATGGTACAG
Tubby reverse primer	CTTGGTCTTCTTGGCTGAGG