

**A human multisubunit E3 ubiquitin ligase required for heterotrimeric  
G-protein  $\beta$ -subunit ubiquitination and downstream signaling**

Brian D. Young<sup>1,2</sup>, Jihui Sha<sup>1</sup>, Ajay A. Vashisht<sup>1,3</sup>, and James A. Wohlschlegel<sup>1,2\*</sup>

<sup>1</sup>Department of Biological Chemistry and <sup>2</sup>Molecular Biology Institute, David Geffen School of  
Medicine, UCLA, Los Angeles, California 90095

<sup>3</sup>Genomics Institute of the Novartis Research Foundation, San Diego, California 92121 (current address)

\*Corresponding author: James A. Wohlschlegel ([jwohl@mednet.ucla.edu](mailto:jwohl@mednet.ucla.edu))

## **SUPPORTING INFORMATION**

Figure S1. KCTD2 binds G $\beta$  in response to G-protein activation

Table S1. Spectral counting data for KCTD2/5/17 interactions with G-protein subunits

Table S2. Subset of interactors that differentially associate with GNB1-WT and GNB1-K15R/K23R

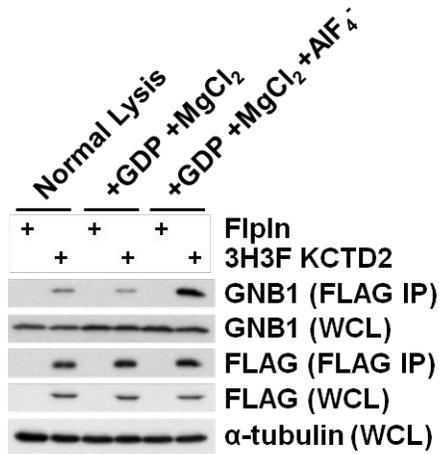
Table S3. List of cDNA plasmids

Table S4. List of PCR primers

Table S5. Peptide and protein identification tables and MSStats statistical outputs for MS analyses.

Supplemental PDF file containing uncropped images of Western blots

## SUPPORTING INFORMATION



**Figure S1: KCTD2 binds Gβ in response to G-protein activation**

HeLa control cells and cells stably expressing 3H3F-KCTD2 were lysed in the presence and absence of G-protein signaling activators (AlF<sub>4</sub><sup>-</sup>, GDP, and MgCl<sub>2</sub>). Immunoblots of whole-cell lysates (WCL) and anti-FLAG immunoprecipitations (FLAG IP) are shown. This is representative of two independent experiments.

**Table S1: Spectral counting data for the protein interactomes of the KCTD2/5/17 subfamily**

<i>Prey</i>	<i>Bait</i>			
	Control	KCTD2	KCTD5	KCTD17
KCTD2	0	335.75	21.75	44
KCTD5	0	27	181	86.5
KCTD17	0	49.75	62	526
CUL3	0	25.75	43.75	15.75
GNB1	0	153.75	42.5	4.25
GNB2	0	139	42.5	3.75
GNB4	0	83.5	28	2.5
GNG2	0	3.25	0	0
GNG4	0	2.75	0.25	0
GNG7	0	2.5	0.25	0
GNG12	0	16	5.5	0

Anti-HA immunopurifications (n=2) from control HEK-293 cells and cells stably expressing 3H3F-tagged KCTD proteins were analyzed by mass spectrometry as described in Figure 2B. Columns and rows represent different bait and prey, respectively. Average spectral counts for select prey are shown.

**Table S2: Subset of Proteins Differentially Associated with GNB1 wildtype or GNB1-K15R/K23R**

<b>Protein</b>	<b>Description</b>	<b>iLog2FC (K15R/K23R : WT)</b>	<b>iPvalue</b>
GRK3	Protein Kinase	-5.103	0.032
PRKCI	Protein Kinase	-3.403	0.022
YWHAH	Signaling Adapter	4.162	0.014
SLC39A11	Zinc Transporter	-4.465	0.010
SLC1A3	Amino Acid Transporter	-2.522	0.012
CLINT1	Membrane Trafficking	4.724	0.021
VPS18	Membrane Trafficking	-4.624	0.048

GNB1 Immunoprecipitates (n=2) from control HEK-293 cells and cells stably expressing 3H3F-tagged GNB1 wildtype or GNB1-K15R/K23R were analyzed by mass spectrometry and quantified by MS1-based label-free quantitation. Log2 fold-changes and the p-values after imputation of missing values were calculated by MSStats and ArtMS.

**Table S3: List of cDNA Plasmids**

<i>ORF</i>	<i>GenBank</i>	<i>Vendor</i>	<i>Catalog Number</i>
KCTD2	BC160142.1	GeneCopoeia	Y3428
KCTD5	BC007314.2	Dharmacon	MHS6278-202826872
KCTD17	BC031038.1	Dharmacon	MHS6278-202808680
GNAS	BC002722.2	Dharmacon	MHS6278-202829474
GNB1	BC005888.2	Dharmacon	MHS6278-202829088
GNG12	BC005940.1	Dharmacon	MHS6278-202839798
CUL3	BC092409.1	Dharmacon	MHS6278-202759865

**Table S4—List of PCR Primers**

<i>Primer</i>	<i>Sequence</i>
KCTD5 Forward	5'-GGG GAC <u>AAG TTT GTA CAA AAA AGC AGG CTC CGC</u> GGA GAA TCA CTG CGA G-3'
KCTD5 Reverse	5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA TCA CAT CCT TGA GCC TCG TTC-3'
KCTD17 Forward	5'-GGG GAC <u>AAG TTT GTA CAA AAA AGC AGG CTC CAG</u> GAT GGA GGC CGG GGA G-3'
KCTD17 Reverse	5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA TCA GAT GGG AAC CCC AAG TCC-3'
GNAS Forward	5'-GGG GAC <u>AAG TTT GTA CAA AAA AGC AGG CTC CGG</u> CTG CCT CGG GAA CAG T-3'
GNAS Reverse	5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA TTA GAG CAG CTC GTA CTG ACG-3'
GNB1 Forward	5'-GGG GAC <u>AAG TTT GTA CAA AAA AGC AGG CTC CAG</u> TGA GCT TGA CCA GTT ACG G-3'
GNB1 Reverse	5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA TTA GTT CCA GAT CTT GAG GAA GCT-3'
GNG12 Forward	5'-GGG GAC <u>AAG TTT GTA CAA AAA AGC AGG CTC CTC</u> CAG CAA AAC AGC AAG CAC C-3'
GNG12 Reverse	5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA CTA TAA GAT GAT GCA AGT TTT TTT ATC CTT-3'
CUL3 Forward	5'-GGG GAC <u>AAG TTT GTA CAA AAA AGC AGG CTC CTC</u> GAA TCT GAG CAA AGG CAC G-3'
CUL3 Reverse	5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA TTA TGC TAC ATA TGT GTA TAC TTT GCG-3'

Underlined and *italicized* sequences mark the attB1 and attB2 sequences, respectively.