***Homo sapiens* Gβ2**	(200) VSGACDASIKLWDVR
***Mus musculus* Gβ2**	(200) VSGACDASIKLWDVR
***Rattus norvegicus* Gβ2**	(200) VSGACDASIKLWDVR
***Xenopus laevis* Gβ3**	(200) ISGACDASAKLWDVR
***Danio rerio* Gβ2**	(200) VSGACDASAKLWDIR
***Drosophila melanogaster* Gβ13F**	(200) VSGACDASAKLWDIR
***Caenorhabditis elegans* Gβ1**	(200) ISGACDASAKLWDIR
***Saccharomyces cerevisiae* Gβ**	(244) ASCGSDGYTYIWDSR
***Schizosaccharomyces pombe* Gβ**	(164) VTGGCDKLAKLWDLR
***Arabidopsis thaliana* Gβ**	(221) ISGSCDSTARLWDTR
***Oryza sativa* Gβ**	(222) VSGSCDATVRLWDIR

Figure S1, Related to Figure 1. G-protein β subunit contains conserved DWD box and Arg214 in human Gβ2 is invariably conserved. The DDB1-binding WD40 (DWD) box sequences of Gβ proteins from different organisms are compared. Gβ sequences compared include *Homo sapiens* (NCBI reference number: NP_005264.2), *Mus musculus* (NP_034442.1), *Rattus norvegicus* (NP_112299.1), *Xenopus laevis* (NP_001080686.1), *Danio rerio* (AAH91666.1), *Drosophila melanogaster* (NP_727907.1), *Caenorhabditis elegans* (AAK55963.1), *Saccharomyces cerevisiae* (EDN63545.1), *Schizosaccharomyces pombe* (BAA21396.1), *Arabidopsis thaliana* (AEE86380.1) and *Oryza sativa* (ADL27745.1). Arg214 in human Gβ2 that is critical for binding with DDB1 and the homologous Arg residues in other Gβ proteins are highlighted by red color. The numbers in the parentheses correspond to the positioning of the first amino acid in each Gβ proteins.

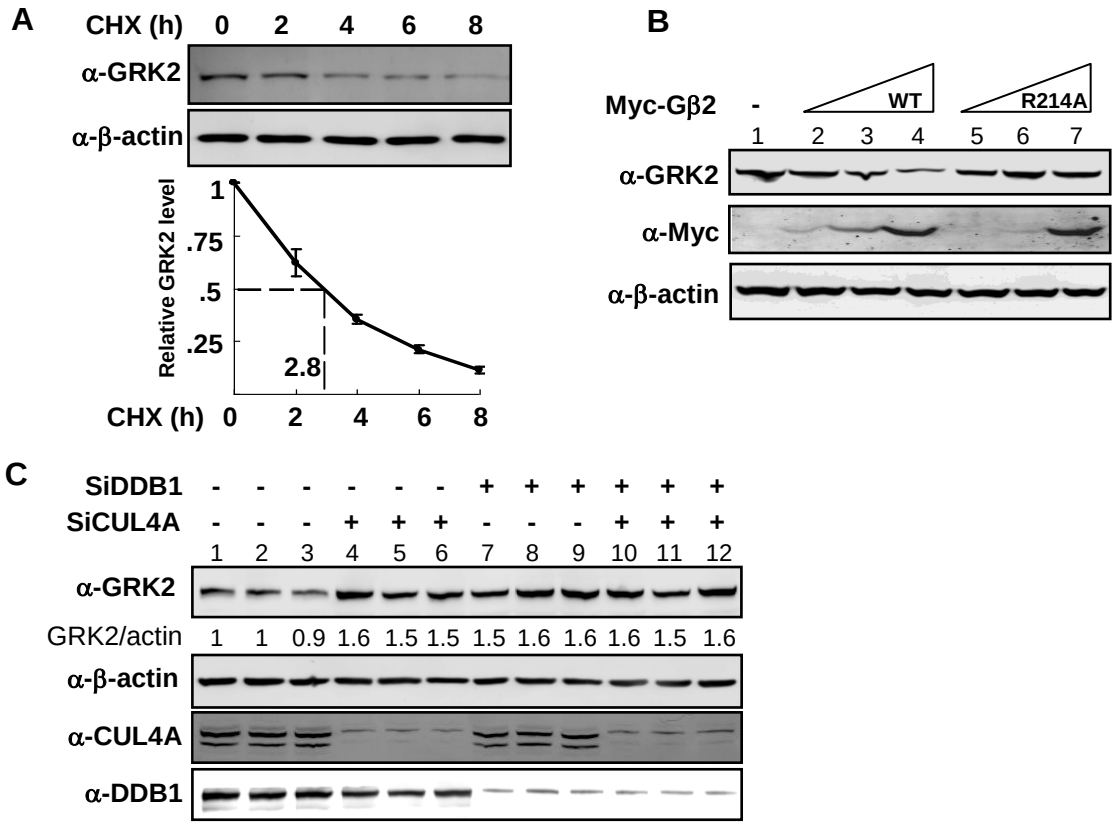

Figure S2, Related to Figure 3. CRL4^{Gβ2} E3 ligase ubiquitylates GRK2 and controls GRK2 protein levels.

(**A**) GRK2 is a relatively unstable protein. The half-life of endogenous GRK2 protein in HEK293 cells was determined by a cycloheximide-chase experiment. (**B**) Ectopic expression of the wild-type, but not DDB-binding deficient mutant R214A mutant Gβ2, decreases GRK2. HEK293 cells were transfected with different amount of plasmids expressing indicated proteins. The steady state level of ectopically expressed Myc-Gβ2 and endogenous GRK2 protein was determined by direct western blotting. (**C**) Knocking down *CUL4A* or *DDB1* increases GRK2. HEK293 cells were transfected in triplicate with siRNA oligonucleotides targeting either CUL4A or DDB1 individually or in combination. The GRK2 protein levels were determined by direct western blotting and normalized against β-actin.

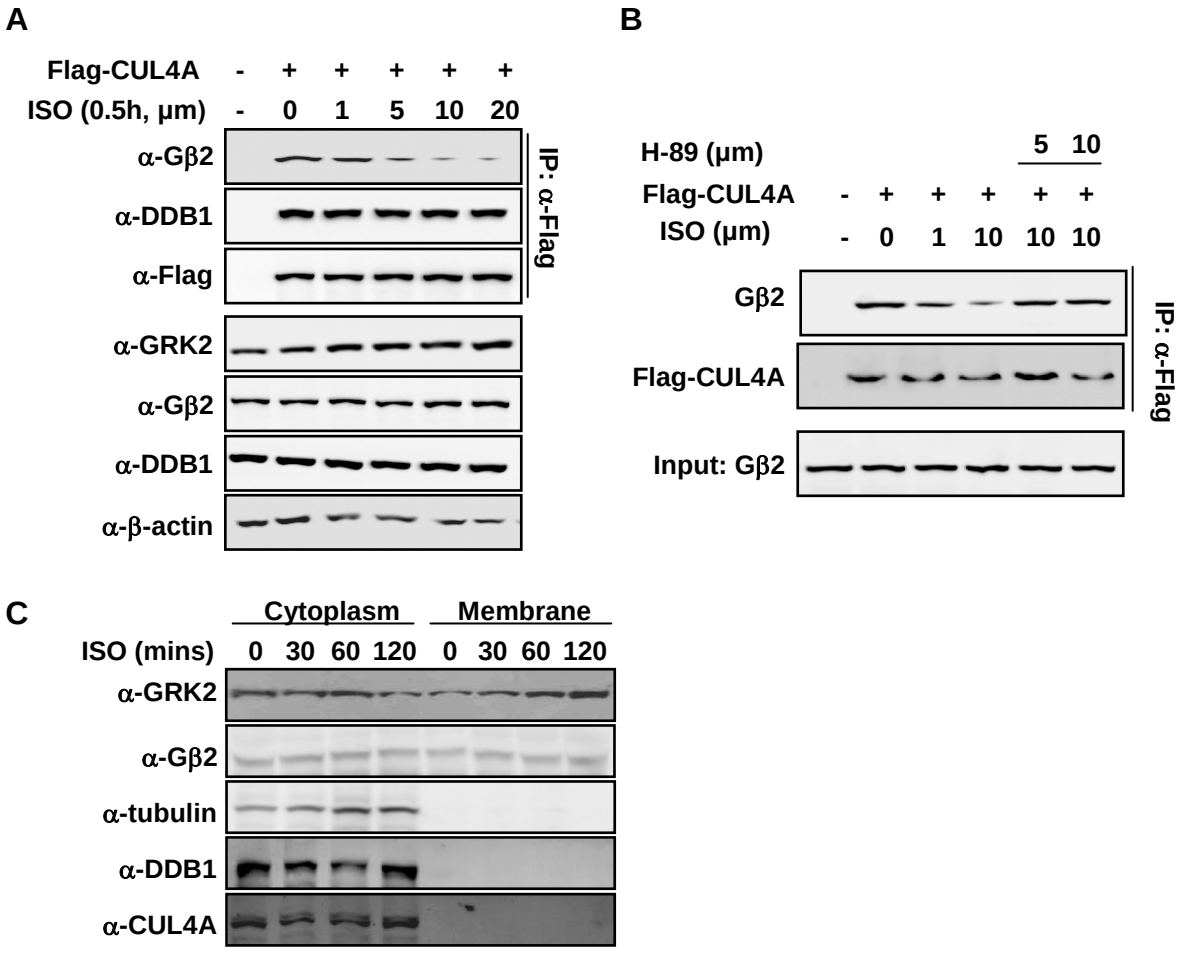

Figure S3, Related to Figure 4. Activation of GPCR disrupts Gβ2 binding to DDB1. (**A**) Gβ2-DDB1 binding is decreased by β-AR agonist, ISO, in a dose-dependent manner. HEK293 cells were transfected with plasmids expressing Flag-CUL4A, 24 hours after transfection, cells were treated with ISO with indicated concentrations for 30 minutes. The levels of individual proteins and the protein-protein interactions were determined by IP and Western analyses using indicated antibodies. (**B**) PKA inhibitor H-89 blocks ISO effect to dissociate Gβ2-DDB1. HEK293 cells were transfected with Flag-CUL4A plasmids. 24 hours after transfection, cells were treated with different concentrations of ISO and H-89, followed by IP and Western analysis. (**C**) ISO stimulates the membrane localization of GRK2. HEK293 cells were treated with ISO as indicated length of time and cytoplasmic and membrane fractions were isolated, followed by Western blotting analysis using indicated antibodies.

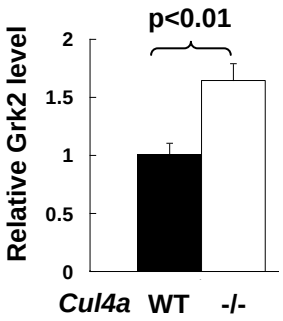

Figure S4, Related to Figure 6. Relative Grk2 protein level in Cul4a wild-type or null male mice. 6 wild-type and 6 *Cul4a*$^{-/-}$ male mice were dissected, and then Grk2 levels was detect in the heart. The GRK2 protein levels were determined by direct western blotting and normalized against tubulin. P values were performed with unpaired, 2-tailed Student's t-test (Excel software).

Protein	p (pep)	xc score	MW(Da)
Gβ2	9.48E-08	10.18	37307.1
TCPZ	1.11E-16	120.36	57987.7
TCPD	4.44E-15	136.29	57887.9
TCPH	6.66E-15	178.32	59329.0
PFD3	1.11E-14	60.22	22643.5
TCPB	2.11E-14	190.30	57452.3
TCPQ	2.66E-14	180.26	59582.6
TCPG	1.83E-13	178.32	60495.4
PFD5	3.42E-13	60.32	17317.0
Gαi3	3.62E-13	60.34	40506.3
TCPA	3.96E-13	180.32	60305.7
TCPE	5.41E-13	158.32	59632.9
Gαi1	9.31E-13	90.27	40335.3
Gαi2	2.02E-12	50.27	40425.1
CH60	1.01E-11	50.29	61016.5
Gγ12	1.91E-11	10.22	8001.2
PAIRB	4.28E-11	10.24	44938.5
HS90B	1.05E-10	10.21	83212.2
Gγ7	1.33E-10	10.21	7517.0
TCPW	2.15E-10	38.19	57728.6
Gα13	2.39E-10	70.25	44021.7
Gγ4	2.55E-10	10.15	8383.2
PFD4	5.65E-10	30.25	15304.6
KCTD5	8.15E-10	20.27	26076.1
ATPA	1.26E-09	10.17	59713.7
Gα11	2.39E-09	60.25	42096.6
GNAQ	5.38E-09	30.24	41441.0
PFD6	8.93E-09	60.24	14573.8
MPCP	1.16E-08	38.24	40068.8
Gβ1	1.36E-08	30.19	37353.0
HAT1	1.90E-08	10.19	49480.8
PRKDC	2.10E-08	40.22	468786.9
DOPP1	2.80E-08	10.18	27013.2
PFD2	3.92E-08	40.25	16637.6
IMB1	4.82E-08	10.17	97108.2
PHLP	5.28E-08	30.15	34259.7
CUL4A	8.94E-08	20.19	87624.4
TBB1	1.34E-07	20.19	50294.6
Gγ2	1.73E-07	10.16	7845.0
SYFA	2.32E-07	10.21	57527.6
S10A9	2.70E-07	10.20	13233.5
AT1A1	3.60E-07	10.17	112824.1
LPPRC	3.65E-07	20.21	157804.6
RLA2	5.68E-07	10.19	11657.9
XPOT	9.27E-07	10.15	109893.4
Gγ10	1.53E-06	10.19	7200.7
RLA1	1.67E-06	10.17	11506.7
TBB4	2.65E-06	8.24	49553.9
KCTD2	2.77E-06	20.15	28568.3
Gα12	3.91E-06	8.15	44251.5

Protein	p (pep)	xc score	MW(Da)
Gβ2	3.75E-07	10.15	37307.1
TCPZ	1.00E-30	188.37	57987.7
TCPB	1.11E-15	200.27	57452.3
TCPD	4.44E-15	198.28	57887.9
TCPG	3.32E-13	266.31	60495.4
PFD5	1.21E-12	50.32	17317.0
TCPE	9.21E-12	208.27	59632.9
H2AV	1.76E-11	10.28	13500.5
ATD3A	3.47E-11	30.24	71324.8
DNJA1	5.34E-11	10.26	44839.5
SCO2	4.22E-10	20.22	29791.4
GRK2	9.83E-10	250.26	79574.0
Gαi1	7.34E-09	28.23	40335.3
ADT1	1.02E-08	28.17	33043.2
AL1B1	1.35E-08	20.19	57202.3
TCPW	1.35E-08	38.19	57728.6
IRS4	1.49E-08	80.23	133684.7
HSP7C	2.15E-08	80.25	70854.4
RT36	2.17E-08	10.16	11459.0
PFD6	2.51E-08	30.24	14573.8
ECH1	2.82E-08	30.29	35793.4
PFD3	3.60E-08	50.23	22643.5
RCN2	4.10E-08	10.26	36853.7
HELZ	4.44E-08	20.20	218832.5
AT1A1	4.72E-08	10.20	112824.1
RL19	4.77E-08	10.24	23451.3
M1IP1	5.30E-08	10.21	20188.9
PFD2	5.33E-08	30.21	16637.6
FA29A	5.67E-08	10.21	108552.7
HNRH1	6.98E-08	20.23	49198.4
ADT2	1.34E-07	30.22	32874.2
DLDH	1.38E-07	30.23	54116.0
DHSA	1.40E-07	10.18	72645.4
TBB4	1.47E-07	20.23	49553.9
Gβ1	1.49E-07	50.17	37353.0
CYFP1	1.56E-07	10.21	145088.6
NPM	2.48E-07	20.21	32554.9
DDX3X	3.26E-07	20.23	73198.1
PFD4	3.60E-07	20.18	15304.6
Gαi3	4.49E-07	20.22	40506.3
MPCP	4.50E-07	20.20	40068.8
BAG2	4.58E-07	10.19	23757.2
PFD1	4.65E-07	30.20	14201.5
PARP1	6.89E-07	10.14	113012.4
EFTU	7.47E-07	20.19	49510.2
ANKH1	9.61E-07	10.17	269288.3
GFAP	1.04E-06	10.16	49849.7
Gγ12	1.84E-06	10.17	8001.2
Gα11	1.63E-05	10.17	42096.6
Gγ10	3.25E-04	10.12	7200.7

Table S1, Related to Figure 2. Top 50 Protein identified in Gβ2 wild-type (left) or R214A mutant (right) complex. Peptides found from the 'homo sapiens' database in descending order of score, showing peptide scores (XC) and probability (P (pep)). P (pep): Displays the probability value for the peptide

only if the unified search file was loaded. XC: Displays the cross-correlation value between the observed peptide fragment mass spectrum and the one theoretically predicted. More information about P(pep) and XC were described in An Approach to Correlate Tandem Mass Spectral Data of Peptides with Amino Acid Sequences in a Protein Database; Eng, J.K., McCormick, A.L., and Yates, J.R., III; (1994) J. Am. Soc. Mass Spectrometry 5, 976-989.