

**Supplementary Figures and Table:**

**Regulator of G Protein Signaling 17 Represents a Novel Target for Treating  
Cisplatin Induced Hearing Loss**

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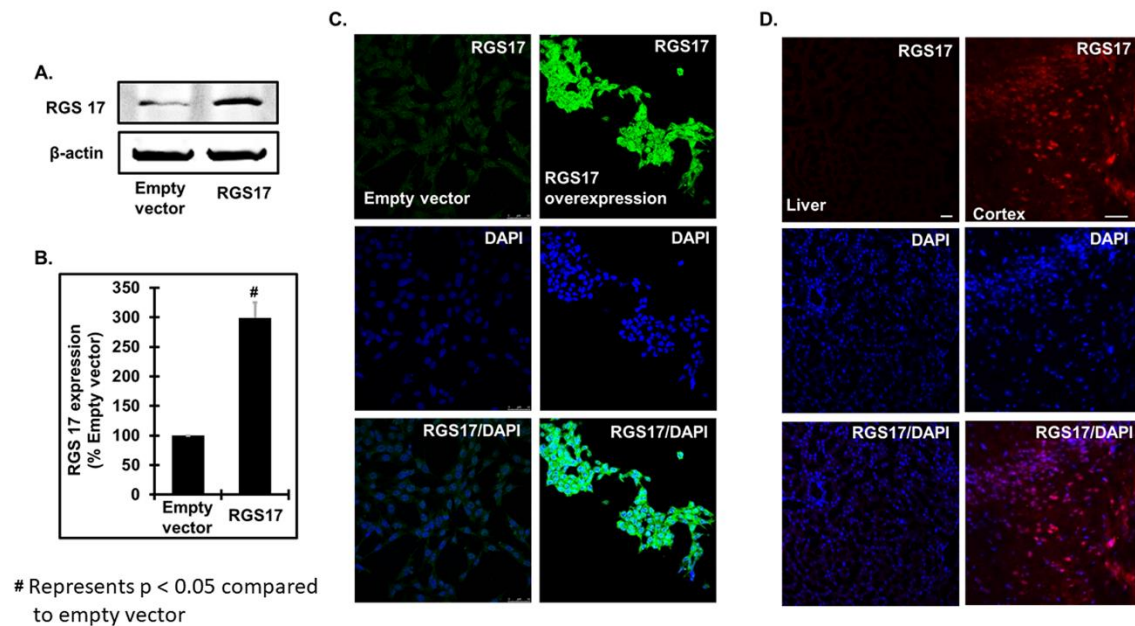
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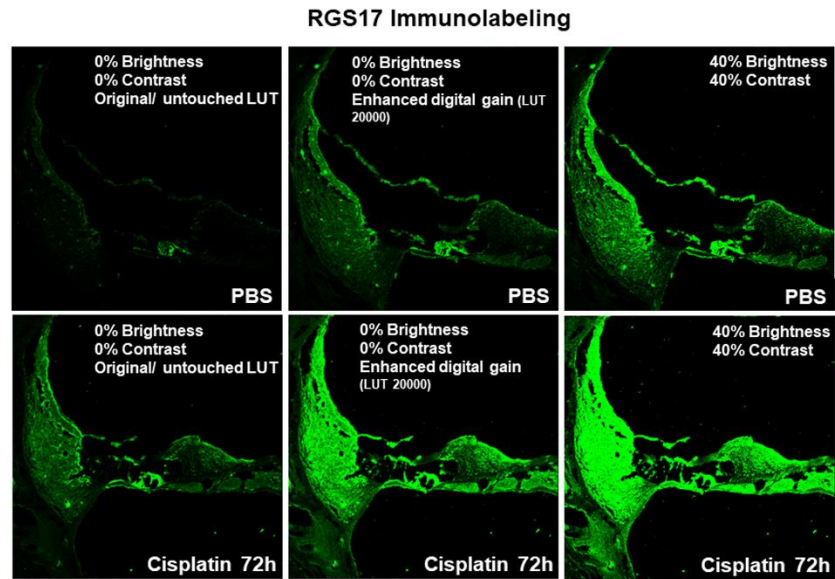
## Supplementary Figures:

### Supplementary Figure 1:



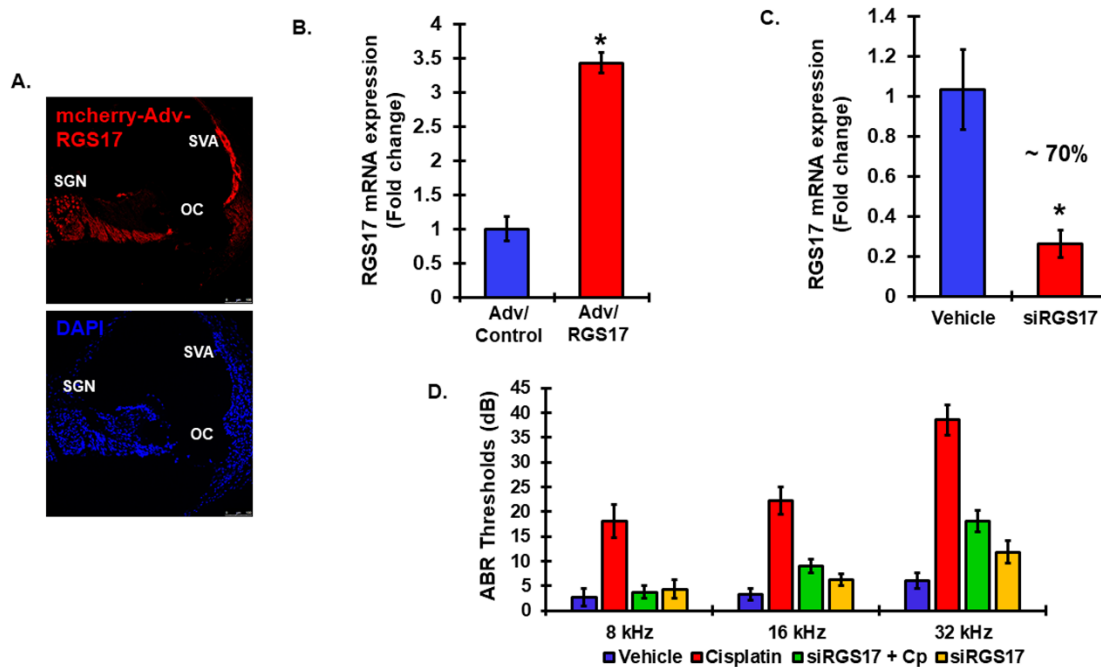
**Supplementary Figure 1.** Verification and validation of RGS17 antibody. **A.** UB\OC-1 cells were transfected with either empty vector (control) or RGS17 overexpression vector for 48-72 hours, cell lysates were prepared and immunoblotted using anti-RGS17 antibody. RGS17-transfected cells show more immunoblot reactivity when compared to the control. **B.** Graphical representation of western blot analysis shows significant increase in RGS17 expression in RGS17-transfected cells. **C.** Both RGS17-transfected cells and vector control cells were stained using RGS17 antibody. Immunofluorescence activity of RGS17-overexpressed cells is higher in intensity than control cells. **D.** Liver and cerebellum was harvested from C57/BL6 mice and fixed it using 4% paraformaldehyde. Tissues were cryo-sectioned and stained with anti-RGS17 antibody. Cortex shows higher intensity fluorescence of RGS17 compared to liver. (Scale 50  $\mu$ m).

## Supplementary Figure 2:



**Supplementary Figure 2.** Immunolabeling of mid-modiolar section of the rat cochlea showing modification of original untouched LUT images versus enhanced digital gain and enhanced brightness and contrast.

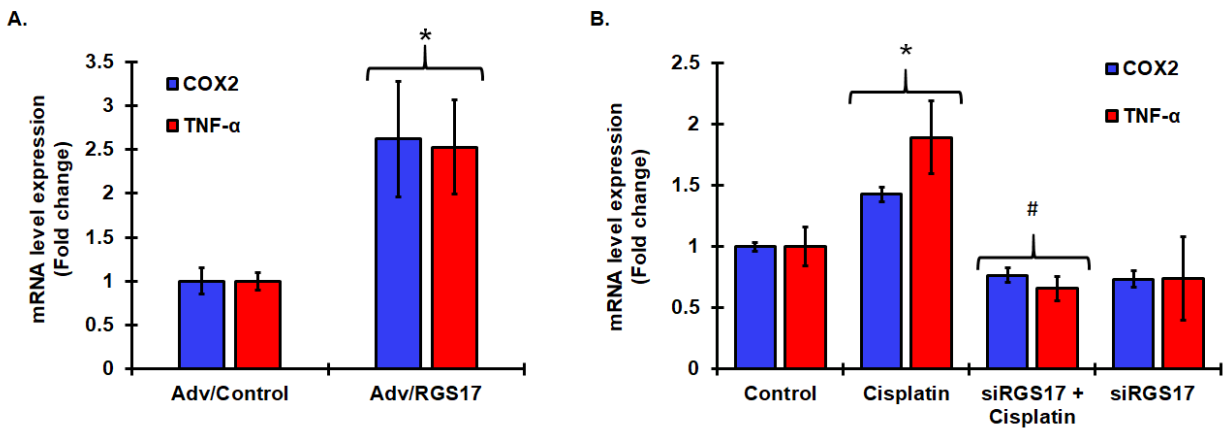
### Supplementary Figure 3:



**Supplementary Figure 3.** Quantification of overexpression and knockdown of *RGS17* and ABR thresholds of *RGS17* knockdown animals. Cochleae from treated male Wistar rats were processed for RNA isolation. Four group of animals: adenoviral vector control, *RGS17* overexpressing adenoviral vector (Adv), vehicle and siRGS17 treated groups. **A.** *RGS17* overexpressing adenoviral vector was tagged with mcherry. Rats were administered adenovirus overexpressing *RGS17* via trans-tympanic route. The cochleae were collected, fixed and processed for midmodiolar sectioning. They were stained with anti-mcherry (red) antibody to detect mcherry fluorescence which indicated *RGS17* expression after infection. **B.** Trans-tympanic administration of Adv overexpressing *RGS17* significantly increases the expression of *RGS17* mRNA level in the cochlea by more than 300% whereas **C.** siRGS17 administration significantly decreased *RGS17* mRNA level which was about 70% knockdown. Data represents mean fold change  $\pm$  SEM, N=6, \* denotes statistically significant difference from controls,  $p < 0.05$ . **D.** Post ABR results showed significant elevations in ABR threshold shifts with cisplatin at 8, 16 and 32 kHz. siRGS17 pretreatment significantly attenuated cisplatin-induced elevation in ABR threshold shifts. Data indicate mean  $\pm$  SEM. Asterisks, # $p < 0.01$  vs. vehicle, \* $p < 0.01$  vs. cisplatin, N>12, two-way ANOVA. TT: trans-tympanic; i.p: Intraperitoneal.

## Supplementary Figure 4:

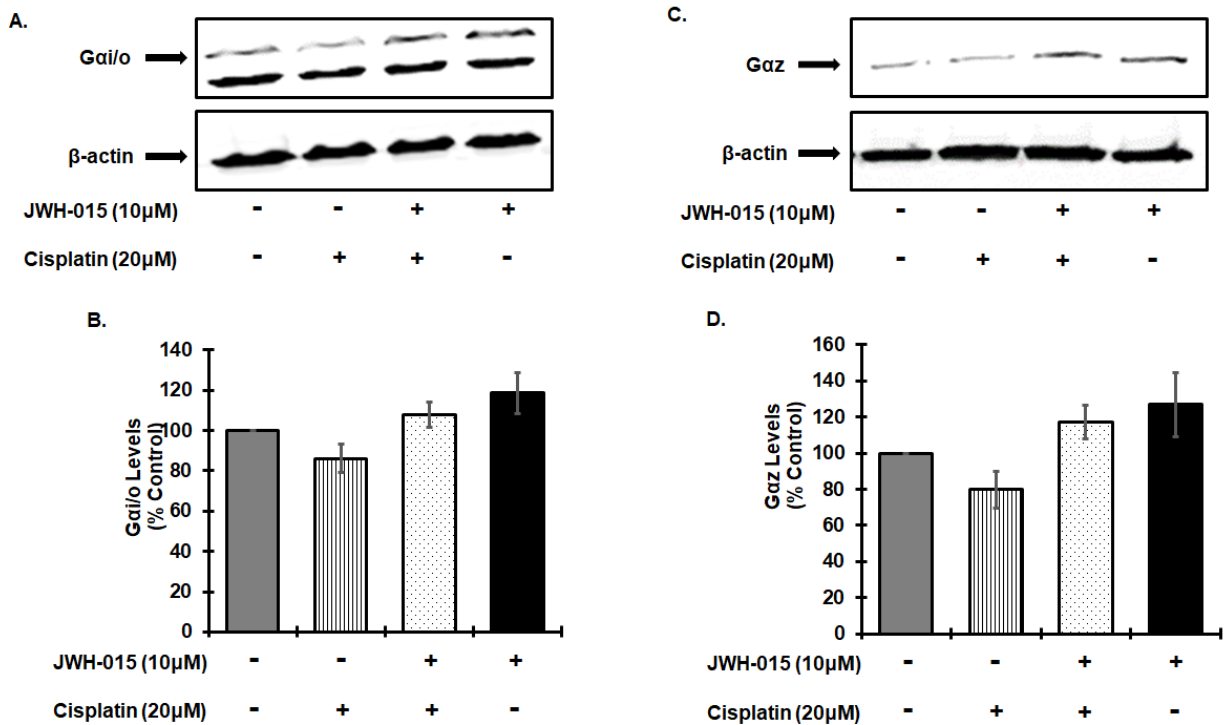
Supplementary figure 4



**Supplementary Figure 4.** Overexpression of *RGS17* in the cochlea elevates inflammatory mediators whereas knockdown of *RGS17* attenuates it. RNA collected from the animals described in fig. was used to conduct RT-qPCR for inflammatory genes. **A.** Cochleae overexpressing *RGS17* significantly increased expression of *COX2* and *TNF- $\alpha$*  as compared to control vector group. **B.** Cisplatin treated rats showed significant increase in *COX2* and *TNF- $\alpha$*  expression in the cochleae as compared to vehicle treated rats whereas siRGS17 pretreated animals significantly decreased mRNA expression of these inflammatory genes as compared to cisplatin treated cochleae. Data indicate fold change in the mRNA levels  $\pm$  SEM,  $N \geq 4$ , statistical significance # $p < 0.05$  vs vehicle and \* $p < 0.05$  vs cisplatin group, two-way ANOVA.

## Supplementary Figure 5:

Supplemental figure 5



**Supplementary Figure 5.** Activation of CB2R increases the expression of *Gα* proteins *Gai/o* and *Gaz* expression *in vitro*. UB/OC1 cells were pre-treated with either vehicle (PBS) or JWH-015 (10μM) for 30 min followed by cisplatin (20μM) treatment for 24 hours and cell lysates were prepared. The levels of *Gai/o* and *Gaz* was analyzed using western blot analysis. Normalization of these *Gα* protein was performed against beta-actin. **A and B.** Western blot analysis showed cisplatin treatment decreased *Gai/o*, but JWH-015 pretreatment induced the levels of *Gai/o*. **C and D.** Similarly, western blot analysis showed cisplatin decreased *Gaz*, whereas pretreatment with JWH-015 increased the levels of *Gaz*. Data represents mean ± SEM, N=3.

**Supplementary Table 1: PCR Primers Sequences Used**

|                      |         |                                      |
|----------------------|---------|--------------------------------------|
| Rodent-TNF- $\alpha$ | Forward | 5'-CAGACCCTCACACTCAGATCA-3'          |
| Rodent-TNF- $\alpha$ | Reverse | 5'-TGAAGAGAACCTGGGAGTAGA-3'          |
| Rodent-COX-2         | Forward | 5'-TGATCGAAGACTACGTGCAAC-3'          |
| Rodent-COX-2         | Reverse | 5'-GTACTCCTGGTCTTCAATGTT-3'          |
| Rodent-NOX3          | Forward | 5'-ACC AGG CAA TTC ACA TAG CT -3'    |
| Rodent-NOX3          | Reverse | 5'-CCA CAG AAG AAC ACG CCA A -3'     |
| Rodent-KIM1          | Forward | 5'- ATGTGCTTGTCACCACCAG -3'          |
| Rodent-KIM1          | Reverse | 5'-TTCAAGTCTTCATTTCAGGCC -3'         |
| Rodent-STAT1         | Forward | 5'-CAT GGA AAT CAG ACA GTA CCT -3'   |
| Rodent-STAT1         | Reverse | 5'-TCT GTA CGG GAT CTT CTT GGA -3'   |
| Rodent-STAT3         | Forward | 5'-CAG CCA AAC TCC CAG ATC AT -3'    |
| Rodent-STAT3         | Reverse | 5'-ACC CAG ATT GCC CAA AGA TAG -3'   |
| Rodent-RGS17         | Forward | 5'-GAA GTC TTG TCC TGG TCT CA -3'    |
| Rodent-RGS17         | Reverse | 5'-TTG ATC ACC TCT CTA ACT CG -3'    |
| Rodent-iNOS          | Forward | 5'-CAT TCT ACT ACT ACC AGA TC-3'     |
| Rodent-iNOS          | Reverse | 5'- ATG TGC TTG TAA CCA CCA G-3'     |
| Rodent-SOD           | Forward | 5'-GCC TCA GCA ATG TTG TGT C -3'     |
| Rodent-SOD           | Reverse | 5'-TCA GAT TGT TCA CGT AGG TC -3'    |
| Rodent-NrF2          | Forward | 5'-CTC TCT GGA GAC GGC CAT GAC T -3' |
| Rodent-NrF2          | Reverse | 5'-CTG GGC TGG GGA CAG TGG TAG T -3' |
| Rodent-GNAZ          | Forward | 5'-GAC TCC ATC TGC AAC AAC AAC -3'   |
| Rodent-GNAZ          | Reverse | 5'-TTC CTC GTA CGT GTT CTG AC-3'     |
| Rodent-GAPDH         | Forward | 5'-ATG GTG AAG GTC GGT GTG AAC-3'    |
| Rodent-GAPDH         | Reverse | 5'-TGT AGT TGA GGT CAA TGA AGG -3'   |

**Supplementary Information:**

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## Regular and Supplementary Figures – Full Gel Images

Figure 2A:

- Fluorescence western blotting technique was used to detect protein band
- Images were taken using Odyssey Imaging system by LI-COR biosciences

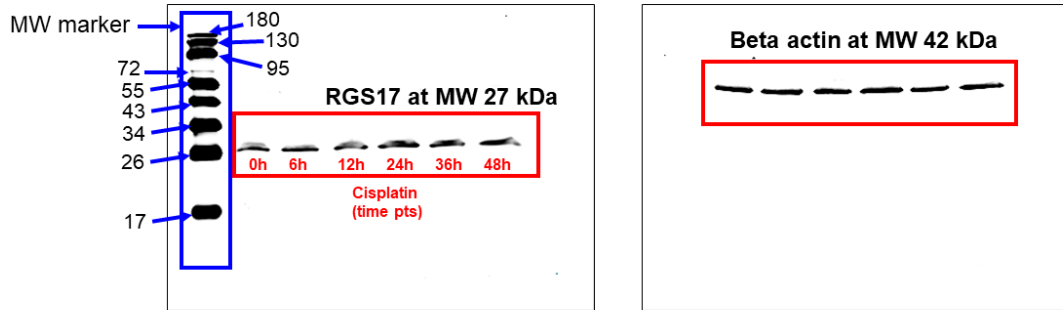


Figure 2B:

- RGS17 protein was detected via Chemiluminescence western blotting technique
- Beta actin protein was detected via Fluorescence western blotting technique using IR dye 800 channel (green color)

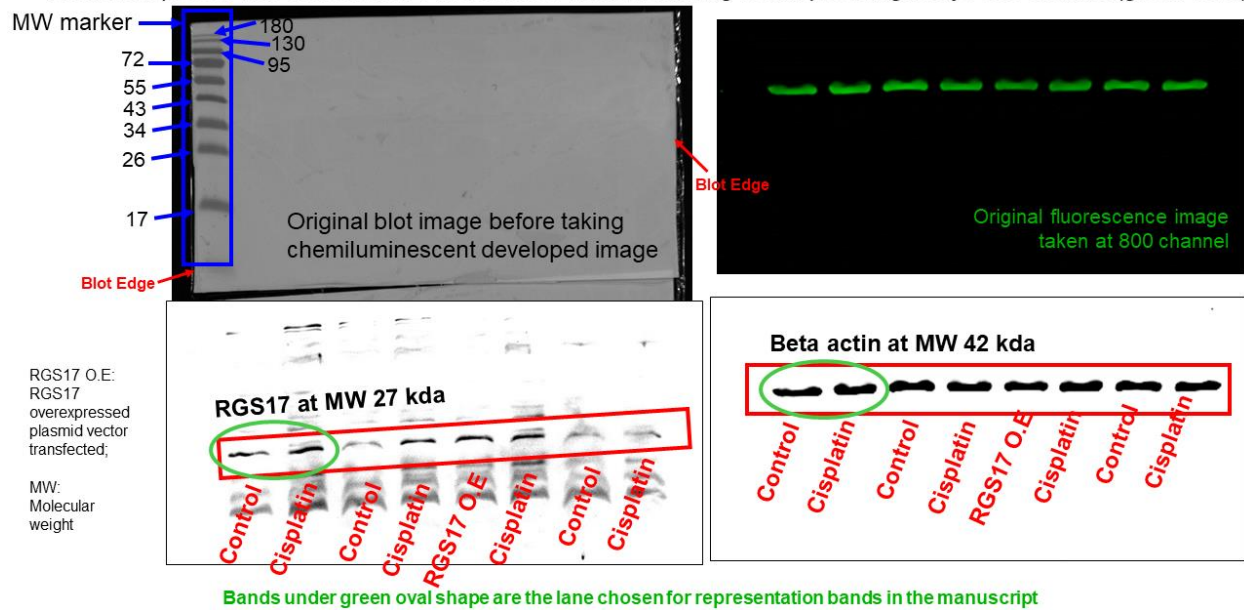
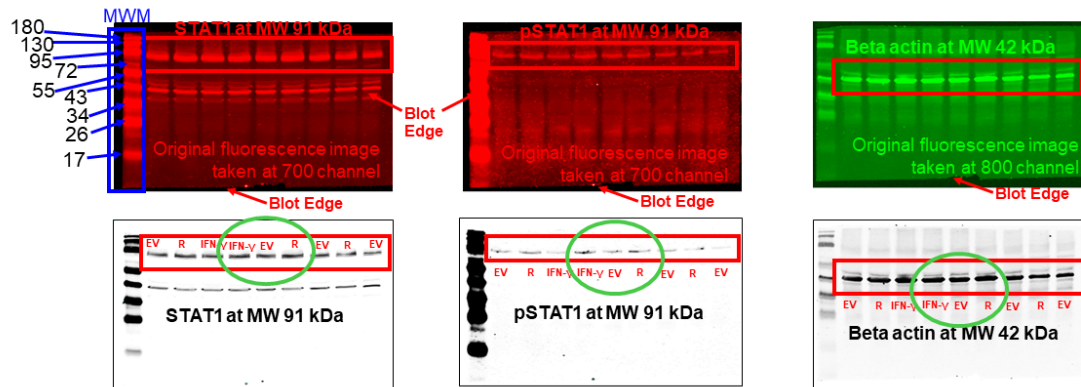


Figure 6A:

- Blot images were taken via Odyssey Imaging system by LI-COR biosciences
- Fluorescence western blotting technique was used to detect bands
  - pSTAT1 and STAT1 were detected at 700 channel
  - Beta actin was detected at 800 channel

For STAT1 blot



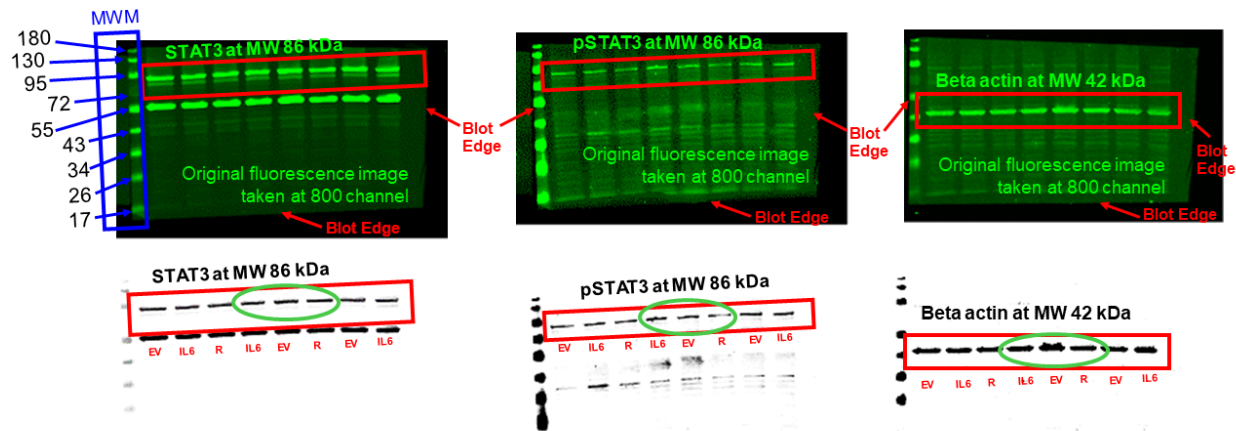
Bands under green oval shape are the lane chosen for representation bands in the manuscript

R: RGS17 overexpressed plasmid vector transfected; EV: Empty vector transfected MWM: Molecular weight marker

Figure 6A continued:

- Blot images were taken via Odyssey Imaging system by LI-COR biosciences
- Fluorescence western blotting technique was used to detect bands
  - pSTAT3, STAT3 and beta actin were detected at 800 channel

For STAT3 blot

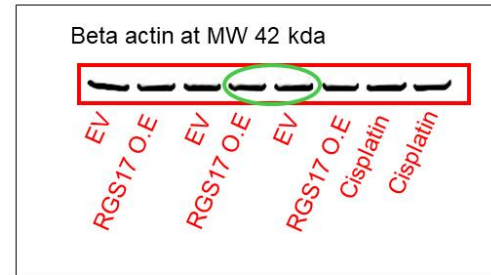
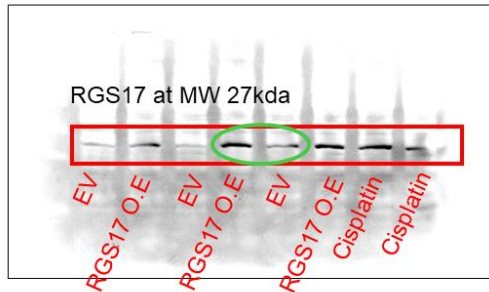
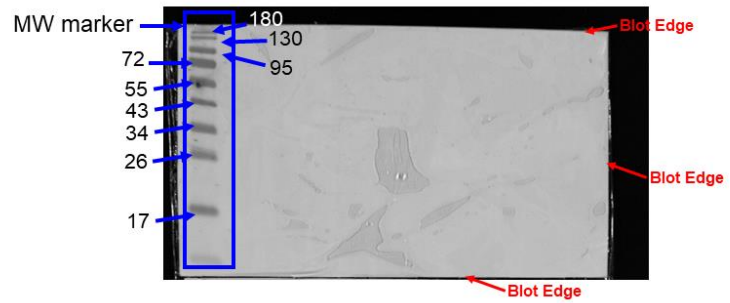


Bands under green oval shape are the lane chosen for representation bands in the manuscript

R: RGS17 overexpressed plasmid vector transfected; EV: Empty vector transfected MWM: Molecular weight marker

Supplementary fig 1A:

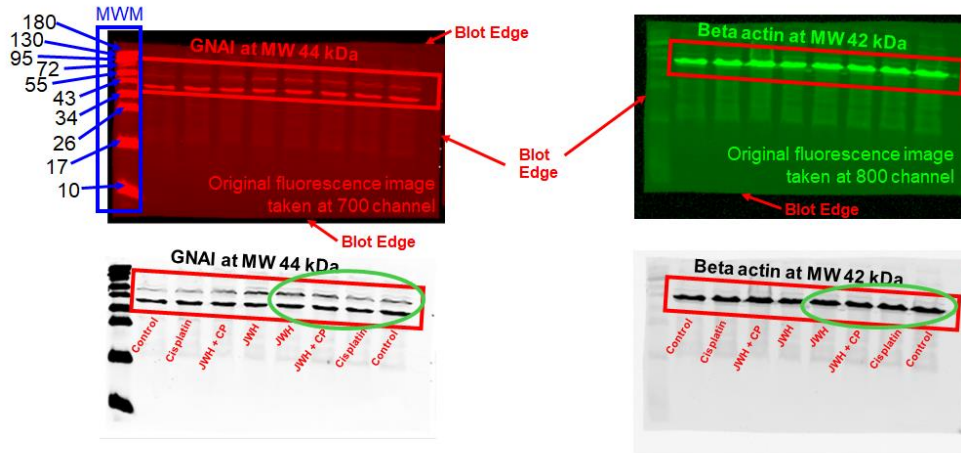
- RGS17 protein detected via Chemiluminescence western blotting technique
- EV: empty vector transfected UBOC1 cells
- RGS17 O.E: RGS17 overexpressed plasmid vector transfected



Bands under green oval shape are the lane chosen for representation bands in the manuscript

Supplementary Figure 5A:

- Blot images were taken via Odyssey Imaging system by LI-COR biosciences
- Fluorescence western blotting technique was used to detect bands
  - G alpha i (GNAI) were detected at 700 channel
  - Beta actin were detected at 800 channel

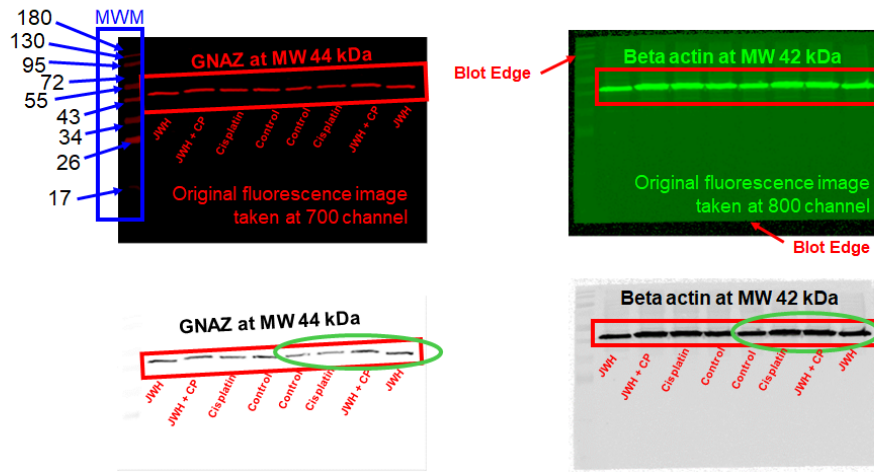


Bands under green oval shape are the lane chosen for representation bands in the manuscript

Cp: Cisplatin, MWM: Molecular weight marker

Supplementary Figure 5C:

- Blot images were taken via Odyssey Imaging system by LI-COR biosciences
- Fluorescence western blotting technique was used to detect bands
  - G alpha z (GNAZ) were detected at 700 channel
  - Beta actin were detected at 800 channel



**Bands under green oval shape are the lane chosen for representation bands in the manuscript**

Cp: Cisplatin, MWM: Molecular weight marker; Predicted molecular weight of GNAZ 41kDa observed around 44.