Supplemental Materials

Identification of PKC activation as a novel mechanism for RGS2 protein up-regulation through phenotypic screening of natural product extracts

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Supplemental Methods

16S rDNA PCR Amplification, Cloning, and Sequencing

1 mL of the ISP2 culture of *actinomycete* strain *Streptomyces manzanensis* (12610-H1) was centrifuged and supernatant were discarded. The genomic DNA was extracted using the Wizard SV Genomic DNA purification kit (Promega catalog # A1120) as described by the manufacturer's protocol. The 16S rDNA was amplified from the isolated genomic DNA by PCR using the universal primers FC27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and RC1492 (5′-TACGGCTACCTTGTTACGACTT-3′).(Rainey et al., 1996) The reaction mixtures consisted of 25 to 50 ng of gDNA (1.0 µL), ExTaq PCR buffer (2.5 µL), 10 µM deoxynucleoside triphosphate mixture (4.0 µL), 50 pmol of each primer FC27 (0.6 µL) and RC1492 (0.6 µL), ExTaq polymerase (0.6 µL), master mix solution (dNTP, $MgCl_2$, 2.0 µL), DMSO (0.6 µL) and H_2O (13.7 µL) for a total volume of 25 µL. The reaction was performed using a TechGene thermal cycler with the following PCR reaction, initial denaturation for 30 s at 98ºC, 30 cycles of amplification with 30 sec at 95ºC, 30 s at 50ºC, and 90 s at 72ºC, and a final elongation of 7 min at 72ºC. PCR products were purified using the Wizard® SV Gel DNA Recovery Kit (Promega catalog # A9282). The purified

PCR products were modified to add a 3'-A overhang using Taq DNA polymerase. Reaction mixtures consisted of 3 µL of PCR product, 5 µL of 2X Taq buffer, 1 µL of T4-ligase were ligated into the 1 µL pGEM®-T Easy vector (Promega). The resulting pGEM-16S rDNA construct was transformed into chemically competent *E. coli* DH-5α cells, cultured on LB-ampicillin plates (50 µL Amp/mL LB from 5 mg/mL ampicillin stock) and spread over with 40 µL of X-gal (20 mg/mL in DMSO) and 40 µL IPTG (100 mM in sterile H_2O) followed by an overnight incubation at 37ºC for α-complementation. After incubation, single white colonies were picked and were inoculated in 3 mL of LB media in sterile culture tubes (×6). All the tubes were incubated at 37ºC shaker for overnight growth. Plasmid DNA was isolated and sequenced using the primers T7 and SP6.

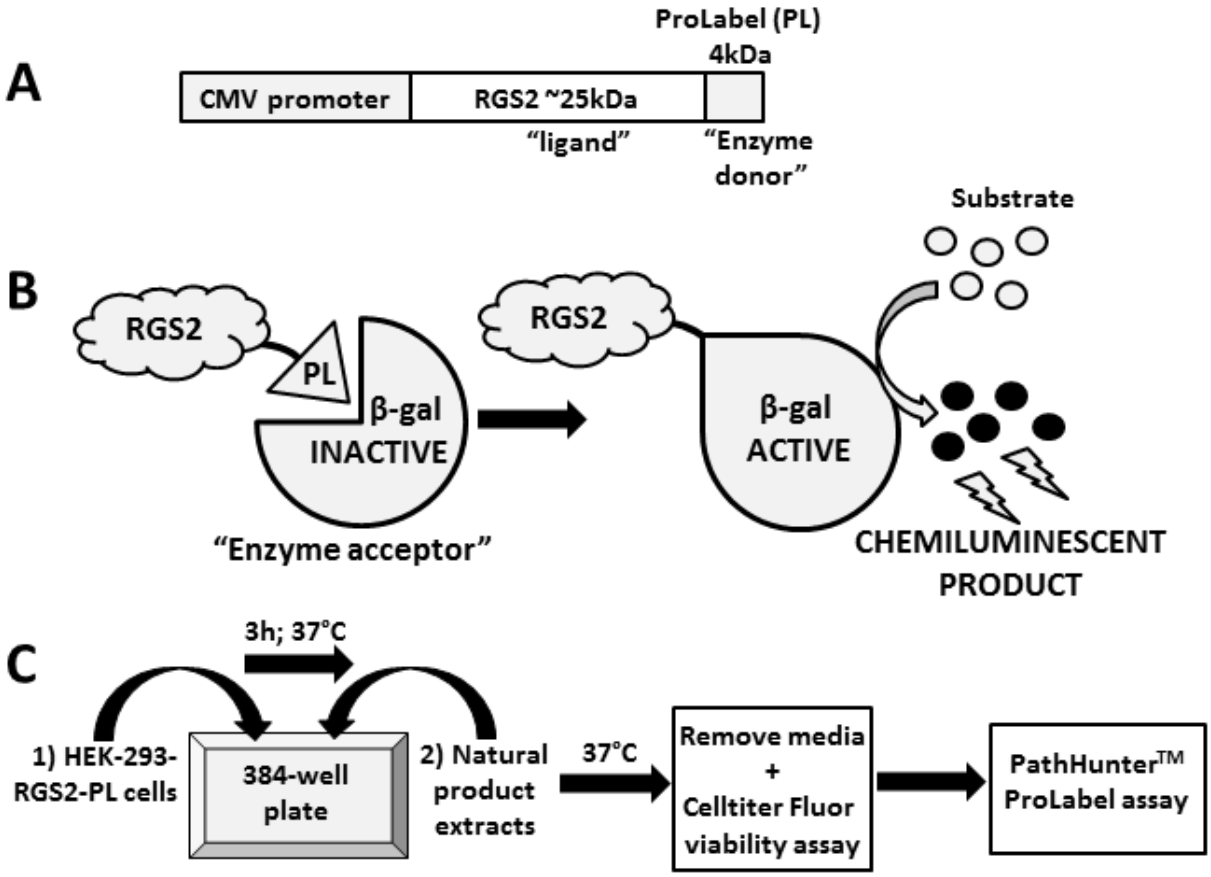
Phylogenetic Analysis of Streptomyces manzanensis

Phylogenetic analysis was conducted in GENEIOUS pro version 4.8.4 available from http://www.geneious.com. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed.

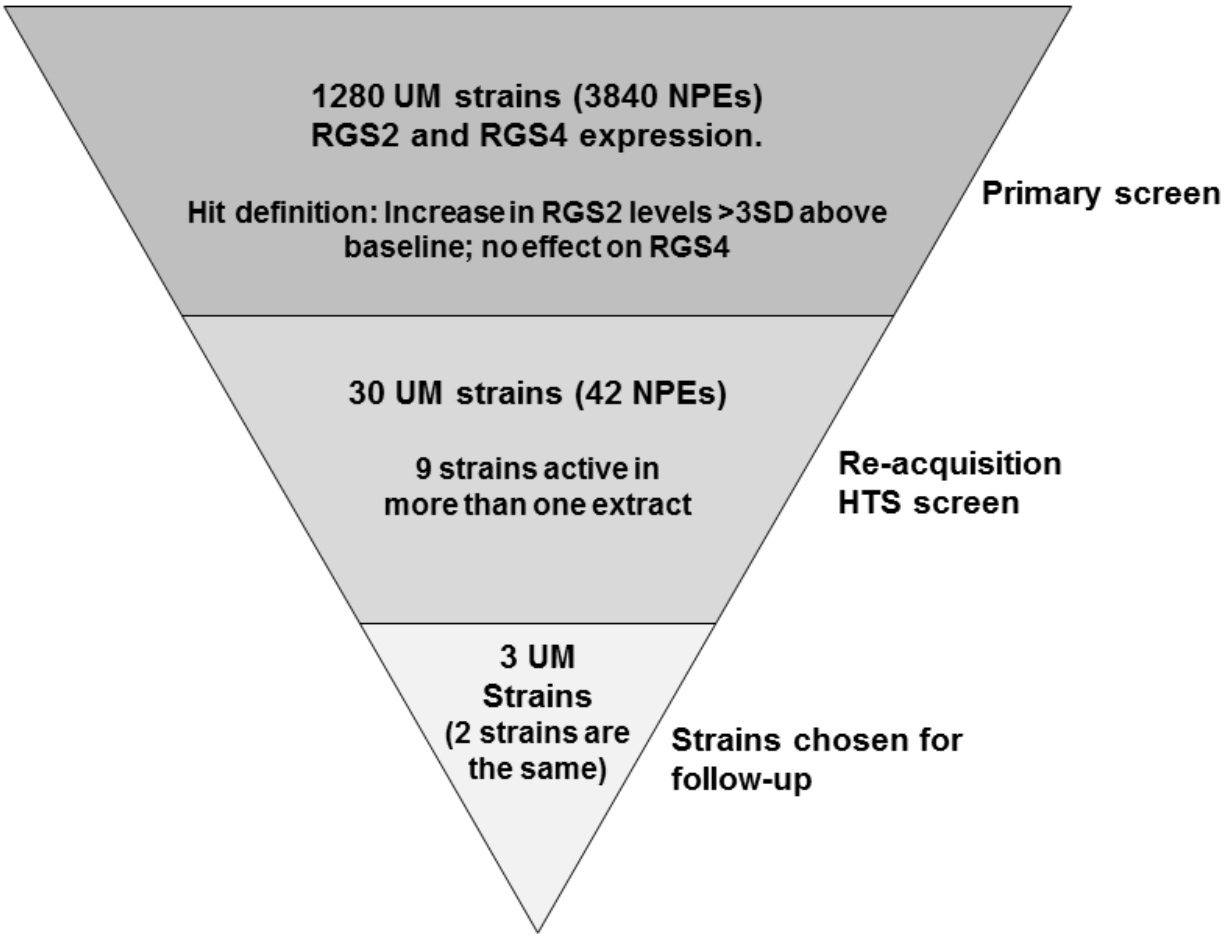
Supplemental References

Rainey FA, Ward-Rainey N, Kroppenstedt RM and Stackebrandt E (1996) The genus Nocardiopsis represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam. nov. *International journal of systematic bacteriology* **46**(4): 1088-1092.

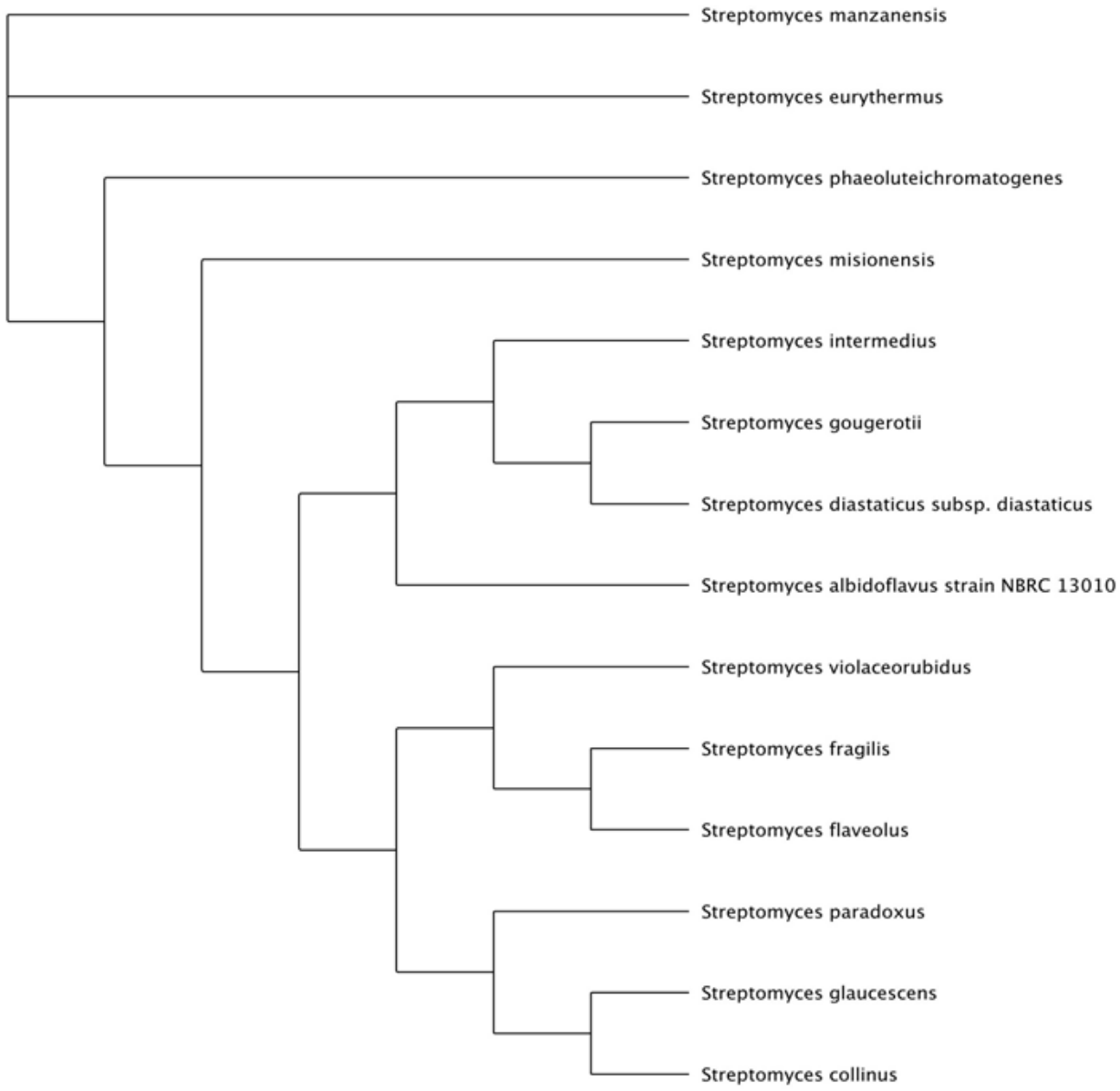
Supplemental figures



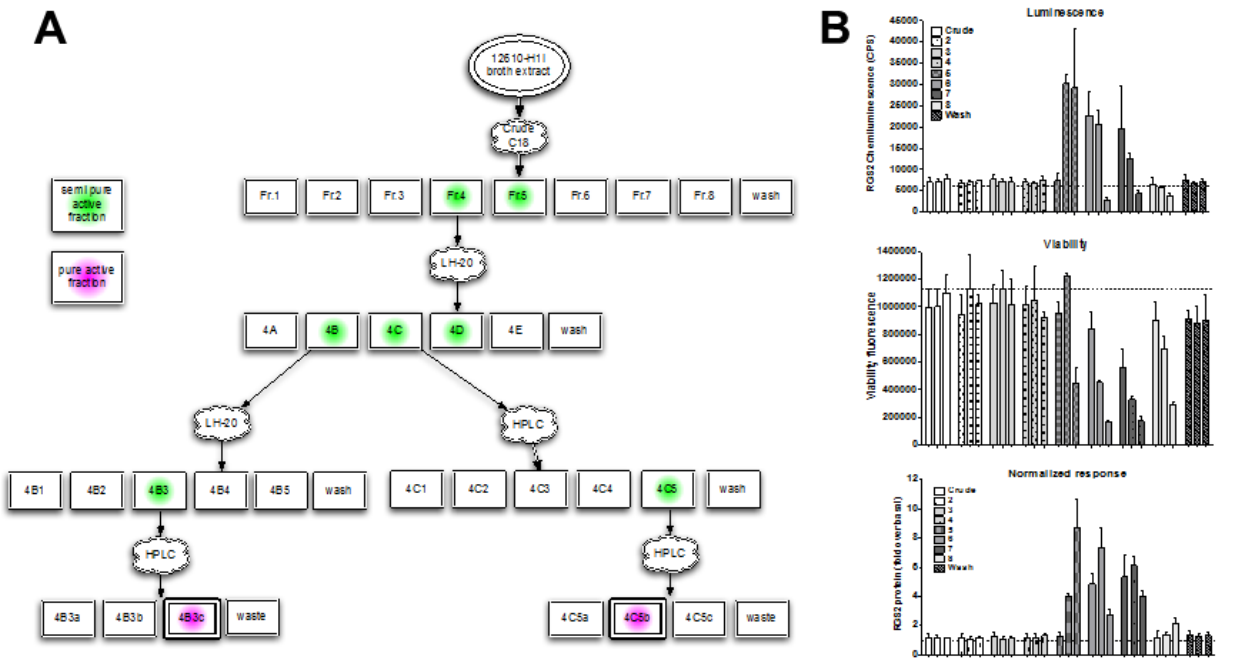
Supplemental Figure 1. Schematic presentation of the screening strategy to identify natural products that increase RGS2 protein levels. A. Human RGS2 was cloned into the pCMV-C3 vector (DiscoveRx) in-frame with the 4kDa ProLabel, a small fragment of β-galactosidase, under control of the CMV promotor. **B.** The PathHunter™ ProLabel assay is a β-galactosidase complementation assay, where excess inactive enzyme and substrate is added. The ProLabel tag on RGS2 forms active enzyme that can hydrolyze the added substrate to a chemiluminescent product that is proportional to the amount of RGS2. **C.** Flowchart of screening assay setup.



Supplemental Figure 2. Decision tree of high-throughput screen follow-up. 3840 natural product extracts (NPE), representing 1280 strains were screened against both RGS2 and RGS4 using the PathHunter™ ProLabel assay. Hits were defined as NPEs that increase RGS2 >3S.D. above baseline and had no effect on RGS4. The screen resulted in 42 hits, representing 30 different strains. Another criterion was added that priority was given to strains represented by more than one NPE, resulting in 9 hit strains. 3 of these were chosen for follow-up based on the ranking in the primary screen. 2 of these were collected in the same location and were identical. These will be described in an upcoming study. The third strain was chosen for the current study for follow-up.



Supplemental figure 3. Phylogenetic Analysis of *Streptomyces manzanensis* using Geneious Pro.



Supplemental Figure 4. Schematic presentation of bioassay guided fractionation and purification of the natural product extract strain *Streptomyces manzanensis*. **A.** Methodology used to fractionate natural product extracts **B.** Examples of bioassay results on fractions using the PathHunter™ ProLabel assay (top) for analysis of RGS2 protein levels. Results were normalized (bottom) to viability (center).