Data Supplement

Title: **A RGS7-CaMKII complex drives myocyte-intrinsic and -extrinsic mechanisms of chemotherapy-induced cardiotoxicity**

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Supplemental Materials and Methods

Drug treatment regimens

For chronic chemotherapy treatment, mice received multiple doses of doxorubicin (cumulative dose of 45 mg/kg i.p.; 9 mg/kg every other week), 5-FU (cumulative dose of 200 mg/kg i.p.; 40 mg/kg every other week), oxaliplatin (cumulative dose of 45 mg/kg i.p.; 9 mg/kg every other week), irinotecan (cumulative dose of 175 mg/kg i.p.; 35 mg/kg every other week), or saline over a period of 10 weeks. Mice on the acute treatment regimen received a single dose of doxorubicin (20 mg/kg i.p.), 5-FU (150 mg/kg i.p.), oxaliplatin (30 mg/kg i.p.), irinotecan (50 mg/kg i.p.), or saline administrated via i.p. injection. 9 weeks after the final drug dose mice were euthanized by cervical dislocation and blood/multiple tissues were collected for downstream analysis.

7,12-Dimethylbenza(α)anthracene-induced tumor generation

Mammary adenocarcinomas were generated in virgin female mice using the mammary-specific carcinogen 7,12-dimethylbenza(α)anthracene (DMBA). Mice received either DMBA (1 mg/20 g body weight) or vehicle control through oral gavage once per week for 5 consecutive weeks starting at 6 weeks of age. Mice were then mated continuously to provide the oscillating hormonal environment necessary to promote tumorigenesis in breast. The DMBA-treated group was monitored twice a week for mammary tumor generation by palpation. Mice were sacrificed once tumors reached a diameter of 0.3 cm. Mammary tissue from DMBA-treated mice and normal age-matched controls was excised, fixed with 4% paraformaldehyde and 5 μm paraffin-embedded sections were cut for immunohistochemical studies. The remaining tissue was frozen in liquid nitrogen and used for western blot analysis.

Cloning and construct generation

The full-length RGS7 and CaMKIIδ coding sequences were amplified by PCR from human blood cDNA according to our published method(1). RGS7 and CaMKIIδ deletion & point mutation sequences were generated by overlapping primer-based PCR amplification and cloned into the pEGFP-N1 vector or pCMV-HA-N vector, respectively. information regarding the primers utilized for all construct generation has been included in table S5.

To generate viral constructs for RGS7 overexpression *in vivo*, the full-length mouse RGS7 sequence was isolated from mouse brain and cloned into the PMD20 vector as above. The lentiviral vector for mRGS7 was generated via subcloning into the pLenti CMV Puro DEST cloning vector (Addgene, Watertown, MA, USA) and packaged using the pMD2.G VSV-G envelope expressing plasmid (Addgene) and psPAX2 (Addgene). Lentiviral particles were generated in AC-16 cells as per a standard protocol. 70 µL of lentivirus containing 2 X 10^8 particles of either mRGS7-Lenti or a control empty vector virus were packaged for delivery with Invivofectamine 3.0 (Thermo Fisher Scientific) and administered via intracardiac injection as described above. Mice received injections of saline or KN-93 (8 mg/kg, 2 doses every 4 days) beginning 15 days after viral injection. Animals were sacrificed 2 days after the final KN-93 injection and tissues isolated for downstream analyses.

Histology and immunohistochemistry

Paraffin-embedded formalin-fixed mouse and human heart tissue sections were stained with Hematoxylin and Eosin (H&E) or Masson trichome (Sigma, St. Louis, MO) to detect tissue architecture or collagen deposition, respectively. Regents were utilized as per the manufacturers' protocols. Immunohistochemical staining of both mouse and human tissue sections was performed as per a standard protocol(2). All reagents were utilized as per the manufacturers' instructions. For RGS7 and CaMKII staining, 7-10 sections were stained from each animal with 5 pictures randomly selected from each slide. The blue-stained collagen in tissue section image stained with Masson trichrome was processed using the "Threshold" tool of ImageJ software (NIH, USA) and the fraction of the total area that was stained blue was quantified. Average myocyte area was estimated by quantifying apparent myocyte area

with ImageJ. 7-10 sections were quantified per animal with 5 myocytes randomly selected per slide.

Cardiovascular Phenotyping

We used two-dimensional echocardiography to determine cardiac function. Using an ultrasound system (Vivid S5 system, GE Healthcare, USA) in M-mode, we measured the left ventricular parameters and ejection fraction on lightly sedated mice following drugs and/or RGS7shRNA.

Immunoblotting

Tissues were rapidly dissected from mice and flash frozen in liquid nitrogen. Tissue homogenates and cell lysates were prepared in RIPA buffer containing protease and phosphatase inhibitors (Sigma), quantified, and probed as previously described(2). Twenty µg of protein per sample was subjected to SDS-PAGE and immunoblotting using standard techniques. Immunoblots were developed using chemiluminescence method with HRP-labeled secondary antibodies. Antibody dilution and catalog information can be found in Table S2. Densitometric quantification of western blots was performed utilizing Image J software (NIH). Protein expression was normalized to loading control (β-Actin) and expressed relative to control conditions.

Murine ventricular cardiomyocyte (VCM), fibroblast (VCF), and endothelial cell (EC) isolation and culture

Primary ventricular cardiomyocytes (VCM) and fibroblasts (VCF) were isolated from 8–10-week-old adult mice according to a published protocol(3). Cells were transduced with lentiviral vectors encoding shRGS7 or control shRNA according to the manufacturer's instructions.

Cardiac ECs were isolated from 8-week-old mice hearts following a previously published protocol(4). Briefly, mice hearts were rinsed with chilled PBS thoroughly to remove

blood and minced into small pieces for EC culture. The pieces were transferred to 8 ml of DMEM containing 10 % FBS, 0.16 mg/ ml gentamycin, 4 mM l-glutamine and 0.3 units/ml of collagenase A and incubated at 37 °C for at least 60 minutes with periodical shaking. Cell clumps were separated initially through a sterile 18G needle, the cell population was filtered through a 30-µm filter, and the resultant single-cell suspension was washed thrice in DMEM. The solution was spun once more at 400×g for 6 minutes and cell pellet was collected. The cells were re-suspended in 5 ml of DMEM. Next, 8 µl of Dynabeads (Invitrogen) coated with a CD31 antibody was added to the cell suspension as per the manufacturer's instructions. After 15 minutes at room temperature, the unbound cells were removed from the mixture using a magnet. Dynabead-bound cells were further washed 5 times with DMEM, and immuno-selected cells were plated either on petri dishes or chamber slides with HiEndoXLTM endothelial cell expansion medium (Himedia) supplemented growth factors.

Culture of human cell lines

The human cardiomyocyte cell line AC16 (Merck, Darmstadt, Germany) was cultured in DMEM and 10% FBS (Gibco, Waltham, MA, USA) in a 37°C incubator at 5% CO_2. The human umbilical vein endothelial cells (HUVEC, Himedia) cell line was cultured in HiEndoXLTM endothelial cell expansion medium with 3% FBS in a 37°C incubator at 5% CO_2. Both breast cancer cell lines (MCF7 and MDA-MB-231) were cultured in DMEM with 10% FBS in a 37°C incubator at 5% CO_2. Cellartis Cardiomyocytes (iPSC CM, ChiPSC22) were procured from Takara Bioscience and cultured using Cellartis CM Culture Base (Y10063) in 37°C incubator at 5% CO2 with 10% FBS.

Drug treatment of cultured cells

Cells were treated with doxorubicin (3 µM, 16 hours), oxaliplatin (100 nM, 16 hours) or 5-FU (500 µM, 16 hours) in the presence or absence of pre-treatment with Ru360 (50µM, 1 h), cyclosporin A (0.2 µM, 45 minutes), or KN93 (50 µM, 1h) where indicated. For conditioned media experiments, cells were initially cultured in standard culture media until cells were 80–

85% confluent. The media was then replaced with serum-free DMEM, and drugs were added for 36 h. After replacing the media again and rinsing the cells, cells were incubated for an additional 8 h in drug/serum-free media. This “conditioned media” (CM) was collected from donor cells and then used to replace standard media on recipient cells. Recipient cells were collected for further processing after 12-16 h.

Generation of RGS7 KO AC-16 cells using CRISPR/Cas9

Guide RNA (gRNA) targeting human RGS7 gene exon17 were designed using tools available from Integrated DNA technologies (IDT, Newark, NJ, USA). High on target and low off target gRNAs were chosen without a PAM sequence, cloned into the PX459 CRISPR system plasmid (Addgene) using standard methods as we previously described(2) and confirmed via sequencing. The resulting construct was transfected into AC-16 cells using lipofectamine 3000 (Thermo Fisher). Cells were re-plated 48 hours post-transfection and subjected to puromycin selection. After 14 days, puromycin selected colonies were plated at 1 cell/well. 21 colonies were picked, and each colony was pelleted down separately for subsequent genomic DNA isolation by phenol/chloroform/isoamyl alcohol extraction for sequencing and protein detection by western blotting. We successfully knocked out RGS7 in one colony (#11). The T7 endonuclease 1 (T7E1) mismatch detection assay was used for validation (Figure S1).

Immunoprecipitation

AC16 cells (3×10^6) were lysed, and protein concentration measured via BCA protein assay. 200 µg of protein was equilibrated in IP lysis buffer (50 mM Tris, 5 mM EDTA, 250 mM NaCl and 0.1% Triton X-100) and bait antibodies (GFP, RGS7, CaMKII, or control mouse IgG) for 12 hours on a rotor at 4°C. 30 µl of Protein G sepharose beads (Abcam) were pre-cleared, equilibrated and then added to lysate. After a 2-hour incubation, bead slurries were centrifuged and washed 3X with IP buffer. Immunocomplexes were eluted in non-reducing

laemmli buffer at 95°C and subjected to SDS-PAGE and immunoblotting with prey antibody (CaMKII or RGS7).

Measurement of ROS generation

ROS generation was estimated in the tissues and primary cells using the cell-permeable oxidation-sensitive probe, CM-H_2DCFDA as described previously(5). Briefly, cells were harvested by centrifugation, washed three times with ice-cold PBS, re-suspended in PBS and incubated with 5 μM CM-H_2DCFDA (Sigma) for 20 minutes at 37°C. After incubation cells were again washed and lysed in PBS with 1% Tween 20. ROS level was determined at the ratio of dichlorofluorescein excitation at 480 nm to emission at 530 nm. Cell lysates and cardiac tissue lysates were used to measure apoptosis and caspase-3 activity using ELISA kits according to manufacturer's protocols. The CM-H_2DCFDA assay is utilized as a general oxidative stress indicator and not as a detector of a specific oxidant due to known limitations of the probe(6).

ELISAs and enzymatic assays

A summary of commercially available kits used to measure superoxide dismutase (SOD)and caspase-3 activity, levels of mitochondrial Ca^{2+}, cell death (apoptosis; cytoplasmic histone-associated DNA fragments), Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), ALT, AST, and triglycerides is available in Table S3. Cells/tissues/samples were harvested, and samples processed according to the manufacturer's instructions.

Cell viability

The MTT reduction assay was used to monitor cell viability. 5×10^4 cells/well were seeded in 48 well plates with DMEM + 10% FCS. The constructs were transfected into the cells using lipofectamine and cells were harvested after 36 h. The MTT (Sigma) solution was prepared at 1 mg/mL concentration in medium without phenol red, and 200 μL of MTT solution was added into each well. The cells were incubated for 2 h at 37 °C. 200 μL of DMSO was then

added into each well for solubilization of the formed formazan crystals. The optical density of the wells was determined at a wavelength of 550 nm (Biotek Instruments).

Clonogenic survival assay

MCF7 and MDA-MB-231 cells were cultured and transfected with GFP constructs encoding either RGS6 or RGS7 or vector control. Colony formation was assayed using crystal violet (Sigma) staining as per a standard protocol. 36 h after transfection, cells were sorted, GFP-labeled cells were plated again at a density of 100 cells/plate, and cells were incubated at 37°C with growth medium replaced every 3 days. After 10 days, colonies were fixed with methanol and stained with 0.5% crystal violet (1:1 methanol to water).

YASARA Homology modelling of RGS7 & In-silico Molecular docking and Molecular Dynamics (MD) Simulations

As there is no reported experimental X-ray or solution NMR structure of RGS7 in the international protein data bank (PDB) repository (https://www.rcsb.org), the3D structural model of RGS7 was generated using YASARA (http://www.yasara.org).The amino-acid sequence of RGS7 was used as an input (in FASTA format) and the default homology modelling macro of YASARA structure was executed to generate the structural model of RGS7. The binding modes of RGS7 in complex with CaMKII were generated using the inbuilt VINA docking method. The highest energy RGS7-CaMKII complex was further evaluated for solution stability under biological conditions by performing 160 nanosecond molecular dynamics (MD) simulation in explicit water solvent using YASARA Dynamics software (20.7.4.W.64 employing the AMBER14 force field). The MD snapshots were saved every 250 ps and different trajectories were generated during the MD simulations and analyzed using YASARA macro “md_analysis.mcr”. Energy terms were calculated using AMBER14 force field parameters. For the analysis of amino acid residues present on the protein-protein interaction interface, the solvent accessible surface area (SASA) calculations were performed using the InterProSurf Webserver

(http://curie.utmb.edu/usercomplex.html)(7). The analysis involves the SASA calculation of residues in the complex and in the isolated subunits; the residues showing significant change in the SASA value were considered most likely to be on the interaction interface. The CaMKII-RGS7 complex structure with highest binding energy was energy minimized (first using YASARA software) and used as an input for the SASA calculation.

Histological characterization of chemotherapy patient samples

To stratify patient samples based on cardiac health, tissues from chemotherapy patients were stained with Masson Trichrome (as above) to detect fibrotic remodeling. In collaboration with a pathologist, sections were scored on the following scale: 0-1, no fibrosis or a very small ischemic scar; 2, moderate sized ischemic scar; 3, moderate ischemic scar and collagen deposition in the extracellular space; 4, widespread collagen deposition. Samples with scores ≤ 1 were categorized as “chemotherapy -fibrosis” and samples with scores ≥2 were categorized as chemotherapy +fibrosis”.“Control” samples were taken from individuals with no history of cancer or chemotherapy treatment as well as no known cardiac issues. An effort was made to ensure controls contained an equal male/female ratio (~50%) and age range (50-80) as samples isolated for the “chemotherapy -fibrosis” and “chemotherapy +fibrosis” group. The cause of death and any/all co-morbid conditions are provided for all individuals in Table S6.

Additional statistical analysis information

A student’s t-test was used to compare between groups for datasets with only 2 groups (1A-B, 2B, 4C, 7A-B, S2A-B, S3A, S5B, S6B, S8A-B). For these datasets nominal p-values are provided and no adjustment was performed. For the remaining datasets with more than 2 groups either a one-way ANOVA (1 variable; 1D, 2A, 2D-F, 3E-F, 4A, S2C, S3C-D, S5C-E, S7A, Table S1) or two-way ANOVA (2 variables; 2C, 3G-I, 4F-K, 5A-E, 6B-E, 7C-G, S3B, S3E, S5G, S6A, S6C-D, S7B, S9A-C) was used and a post-hoc adjustment was made using

the Bonferroni method to correct for multiple comparisons. For datasets analyzed in this manner, adjusted p-values are provided.

References

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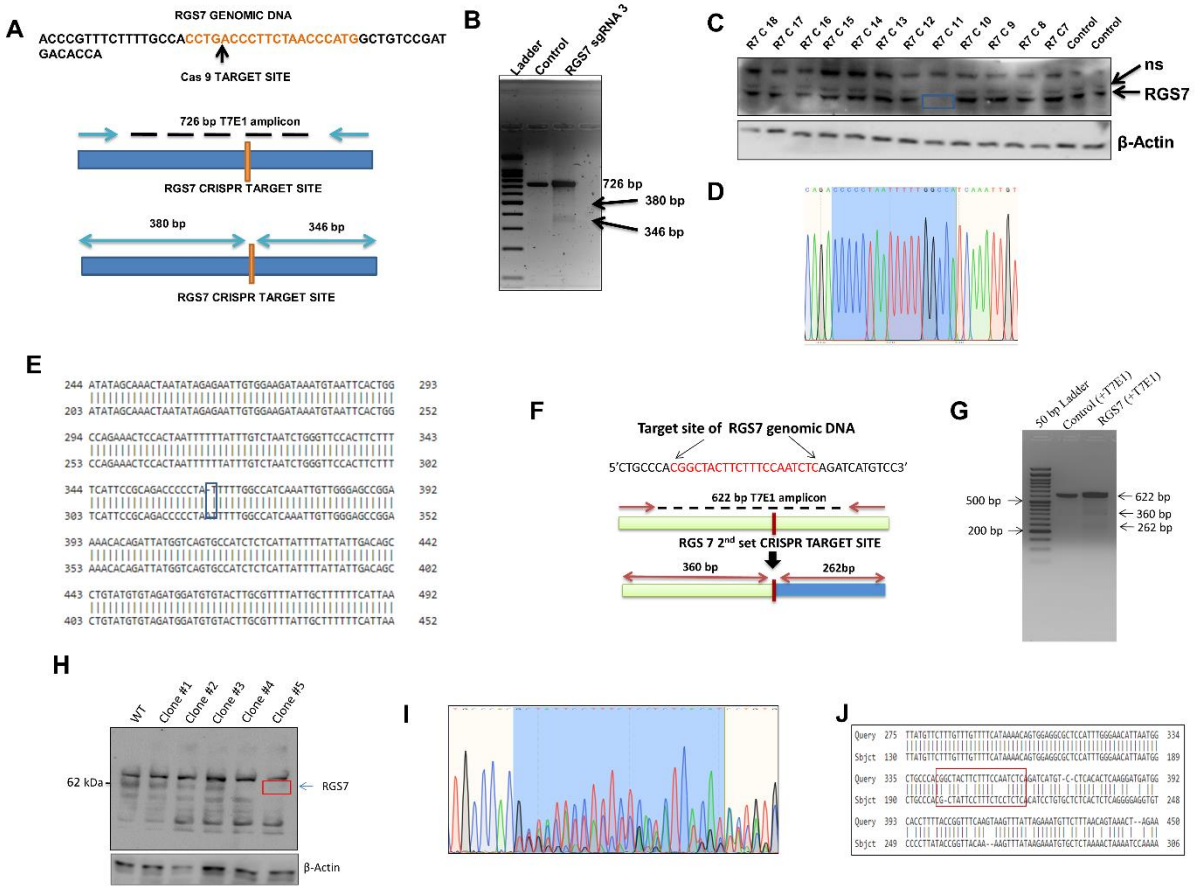

Figure S1 - Validation of RGS7 knockout in AC-16 cells via CRISPR/Cas9-dependent genomic excision. (A-E) CRISPR Clone #1. (F-J) CRISPR Clone #2. (A, F) Design of excision site in the *RGS7* gene overlaid with predicted products from T7E1 validation assay. (B, G) Results from T7E1 assay depicting expected 380 and 346 base pair (bp) or 320 bp and 262 bp products for knockout lines #1 and #2, respectively. (C, H) Validation of CRISPR/Cas9 clones 7-18 (C) and 1-5 (H) demonstrating successful gene knockout in clones 5 and11. (D, I) Sequencing chromatogram from clone 11 and 5 and (E, J) blast alignment highlight a single base pair deletion in clone 11 and multi-bp deletion in clone 5.

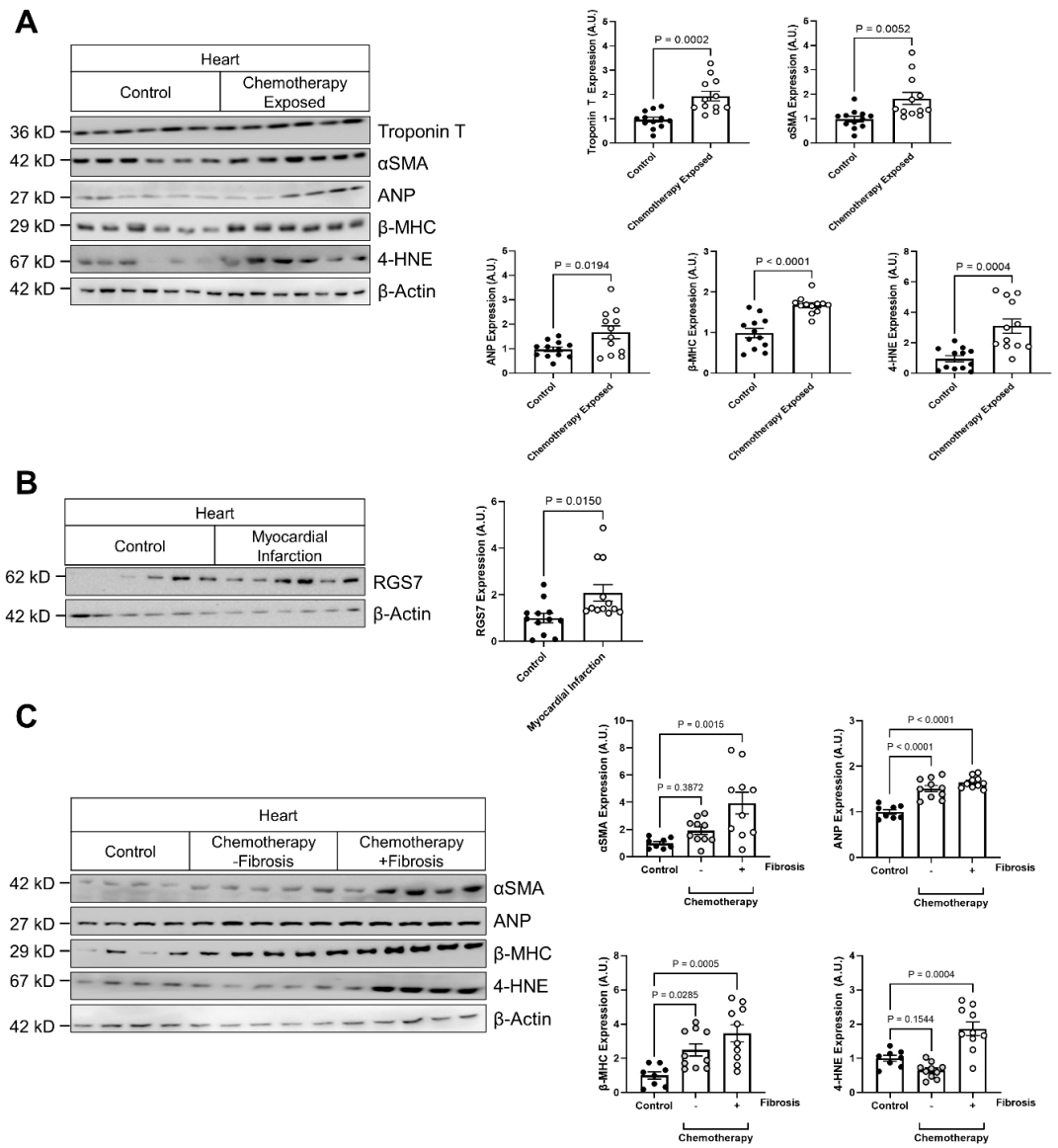

Figure S2 – Molecular characterization of cardiac tissue samples from chemotherapy-treated or control patients. (A) Immunoblotting for troponin T, regulator of cardiac contractility β myosin heavy chain (β-MHC), the myofibroblast marker α smooth muscle actin (αSMA), marker of lipid peroxidation and oxidative stress 4-Hydroxynonenal (4-HNE) and heart failure prognostic factor atrial natriuretic peptide (ANP) in heart samples from chemotherapy-treated or control patients (n=10). (B) Immunoblotting for RGS7 in heart samples from MI or control patients (n=12). (C) Samples from chemotherapy-exposed patients were stratified based on cardiac health and classified into 2 groups: no fibrosis and fibrosis. Troponin T, αSMA, ANP, βMHC, and 4-HNE immunoreactivity were determined via western blotting in cardiac tissue (n=8-10). β-Actin serves as a loading control for western blots. Immunoblots are accompanied by a densitometric quantification wherein expression is normalized to the corresponding control group. Data were analyzed by student's t-test or one-way ANOVA with

Sidak's post-hoc test. Exact *P* values are provided on graphs. Data are presented as mean ± SEM.

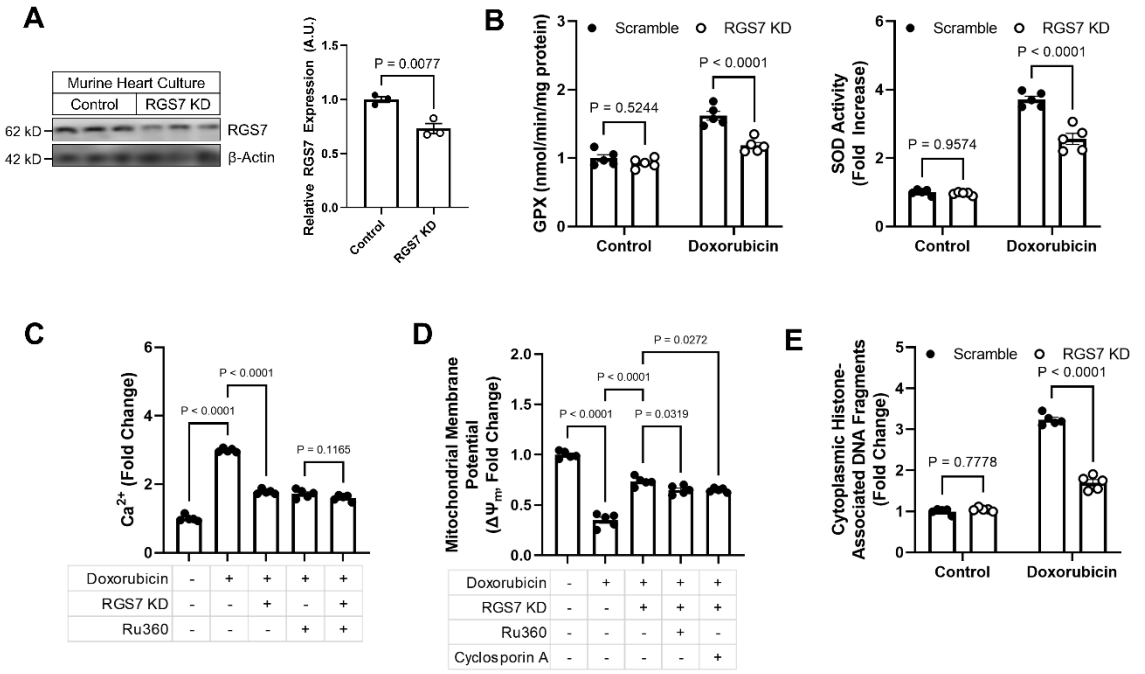

Figure S3 – A second RGS7 shRNA similarly impacts doxorubicin-dependent myocyte toxicity. (B) Verification of RGS7 knockdown in total heart cultures treated with scramble or RGS7-directed shRNA (n=3). (B-E) Control (scramble shRNA) or RGS7 KD VCM were treated with doxorubicin (3 μM, 16 hours) ± pre-treatment mitochondrial calcium uniporter inhibitor Ru360 (50 μM, 1 h) or mitochondrial permeability transition pore (mPTP) blocker cyclosporin A (0.2 mM, 45 minutes) where indicated. (B) Glutathione peroxidase (GPX) activity (n=5) and superoxide dismutase (SOD) activity (n=5). (C) Mitochondrial Ca^{2+} flux (n=5). (D) Mitochondrial membrane potential ($\Delta\psi_M$; n=5). (E) Apoptosis (cytoplasmic histone-associated DNA fragments; n=5). β-Actin serves as a loading control for western blots. Immunoblots are accompanied by a densitometric quantification wherein expression is normalized to the corresponding control group. Data were analyzed by student's t-test or one- or two-way ANOVA with Sidak's post-hoc test. Exact *P* values are provided on graphs. Data are presented as mean ± SEM.

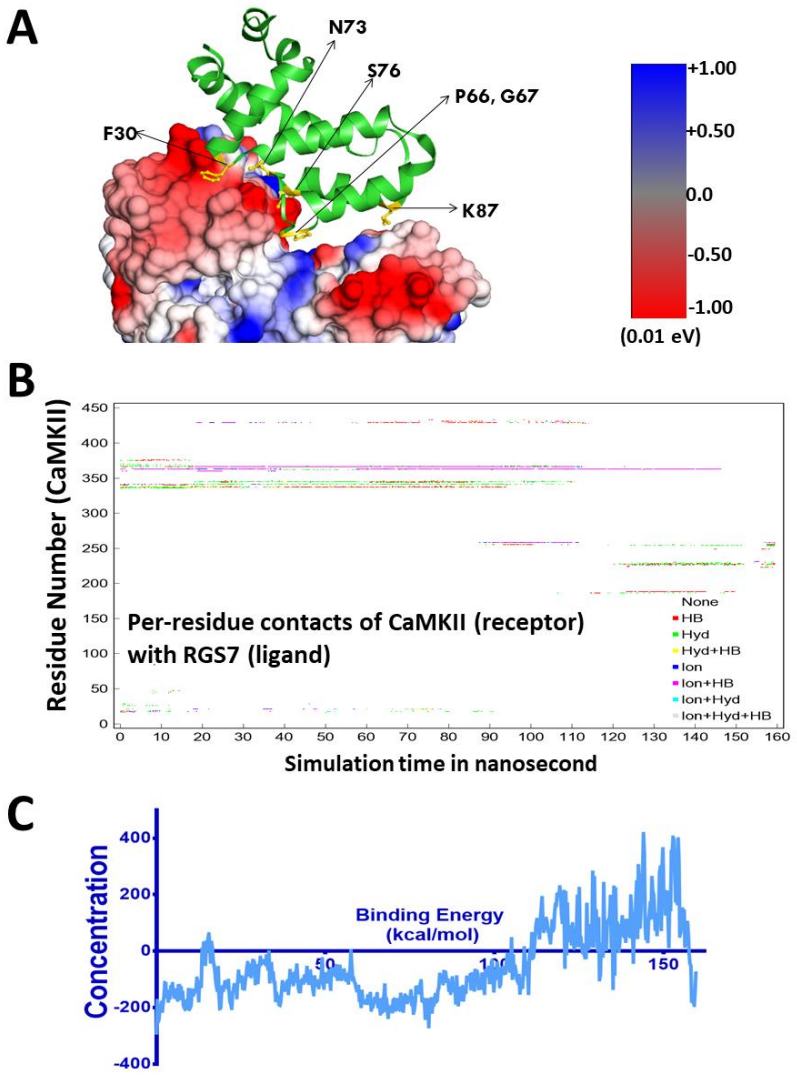

Figure S4 – Modeling of the RGS7/CaMKII complex. Solvent accessible surface area (ASA) calculations were performed using InterProSurf Webserver (http://curie.utmb.edu/usercomplex.html). The SASA values of complex residues were compared to those of monomeric structures of CaMKII and RGS7 to identify residues in the protein-protein interaction interface. (A) The best docking poses of RGS7 with CaMKII selected after cluster analysis based on the highest binding energy of the complex. (B) The plot showing the types of binding contacts formed by CaMKII receptor residues with the RGS7 protein as a function of simulation time. Shown here are three types of contacts: ionic interactions (in blue), hydrophobic contacts (in green) and hydrogen bonds (in red). Mixtures of these three colors can show up if a certain residue is involved in more than one type of contact with the ligand. (C) Plot showing profiling of RGS7 (ligand) energy of binding (BE) with CaMKII receptor structure evaluated as a function of simulation time using YASARA macro named “md_analyzebindenergy.mcr”. Note, the values estimated are often larger than the expected binding energy values as the calculation in YASARA does not include intermolecular VdW interaction energies.

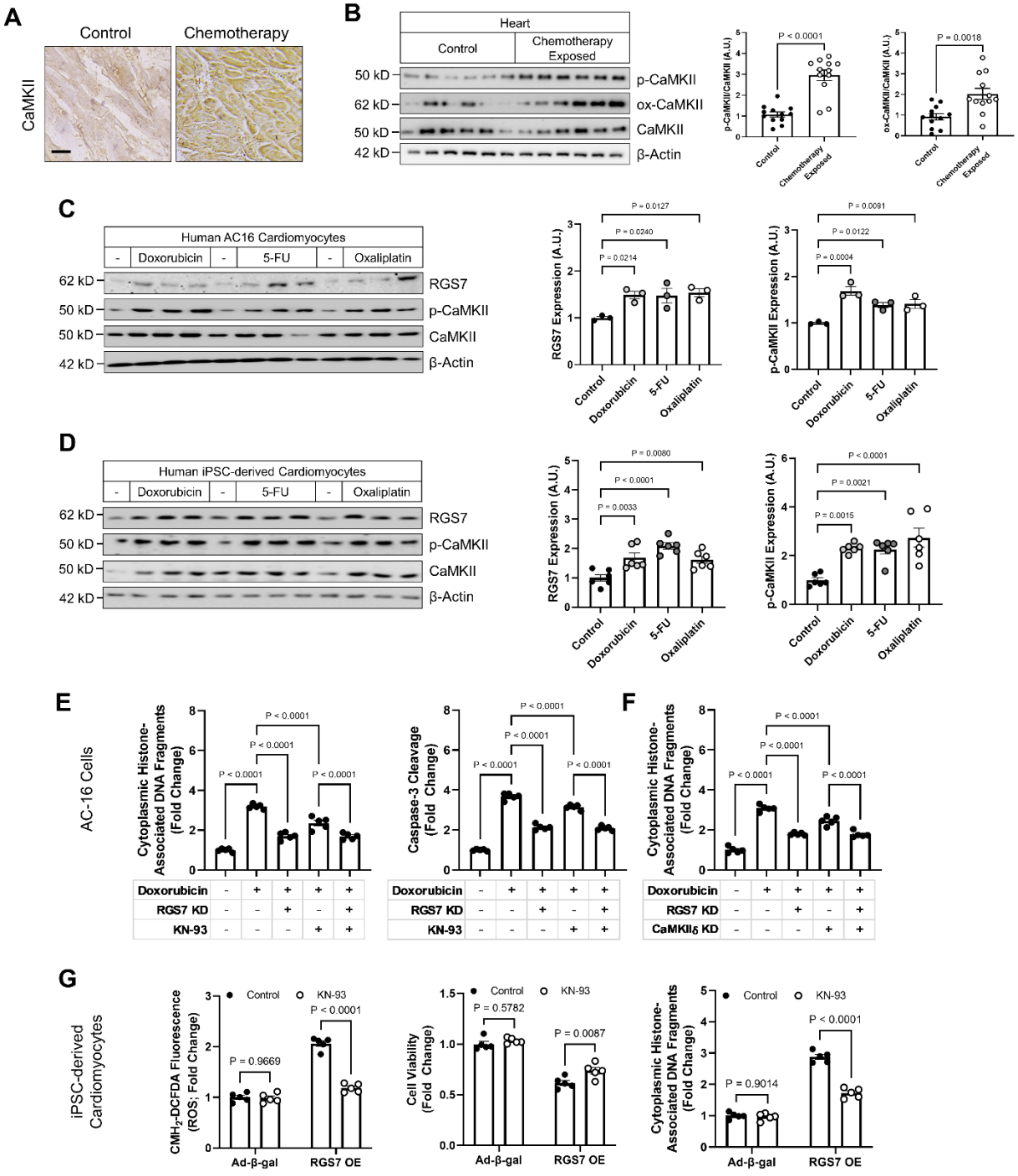

Figure S5 – CaMKII is up-regulated in human tissue and cells exposed to cancer chemotherapeutics and required for RGS7-dependent cytotoxicity. (A) Representative cardiac staining for CaMKII in a control or chemotherapy exposed patient [scale bar = 100 μm]. (B) Immunoblotting for phosphorylated and oxidized CaMKII protein expression in heart tissue from chemotherapy exposed patients or matched controls (n=12). (C) Human AC-16 cardiomyocytes or (D) iPSC-derived cardiomyocytes were treated with doxorubicin (3 μM, 16 hours), 5-FU (500 mM, 16 hours), or oxaliplatin (0.06 mM, 16 hours) and immunoblotting

performed probing for RGS7, p-CaMKII, and ox-CaMKII (n=3). (E-F) Control (scramble shRNA) or RGS7 KD AC-16 cells were treated with doxorubicin (3 μM, 16 hours) ± pre-treatment with (E) CaMKII inhibitor KN-93 (50 μM, 1 h) or (F) introduction of shRNA against the δ isoform of CaMKII. (E) Apoptosis (cytoplasmic histone-associated DNA fragments; n=5) and caspase-3 cleavage (n=5). (F) Apoptosis (cytoplasmic histone-associated DNA fragments; n=5). (G) iPSC-derived cardiomyocytes were transfected with RGS7-GFP or control plasmid (vector)± pre-treatment with CaMKII inhibitor KN-93 (50 μM, 1 h). CM-H_2-DCFDA fluorescence (total ROS; n=5), cell viability (n=5) and apoptosis (cytoplasmic histone-associated DNA fragments; n=5) were measured. β-Actin serves as a loading control for western blots. Immunoblots are accompanied by a densitometric quantification wherein expression is normalized to the corresponding control group. Data were analyzed by student's t-test or one- or two-way ANOVA with Sidak's post-hoc test. Exact *P* values are provided on graphs. Data are presented as mean ± SEM.

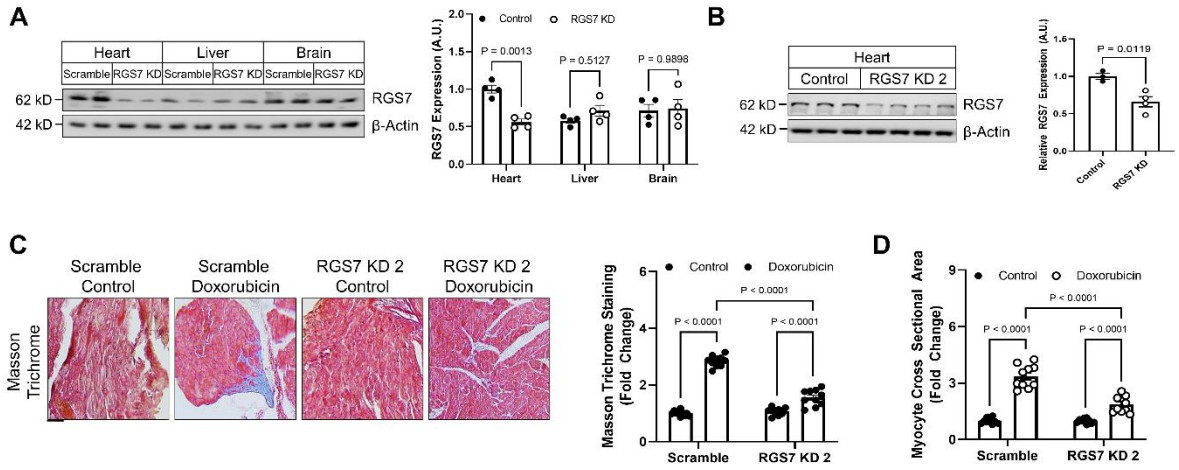

Figure S6– A second RGS7 shRNA protects against doxorubicin-induced cardiotoxicity *in vivo*. Scramble or RGS7-targeted shRNA was administered via intracardiac injection. Where indicated, mice were treated with doxorubicin (cumulative dose of 45 mg/kg i.p.) or saline control over 8 weeks. Tissues samples collected 1 week later for biochemical and histological analyses. (A) RGS7 expression in heart, liver, and brain tissue. (B) Verification of RGS7 knockdown in heart. (C) Representative images depicting Masson Trichrome staining (fibrosis) in doxorubicin treated control and cardiac RGS7-KD mice [scale bar = 100 μm]. Quantification of fibrotic area (blue stain) from Masson Trichome images (n=10) is also provided. (D) Quantification of myocyte area from H&E images (n=10). β-Actin serves as a loading control for western blots. Immunoblots are accompanied by a densitometric quantification wherein expression is normalized to the corresponding control group. Data were analyzed by student's t-test or two-way ANOVA with Sidak's post-hoc test. Exact *P* values are provided on graphs. Data are presented as mean ± SEM.

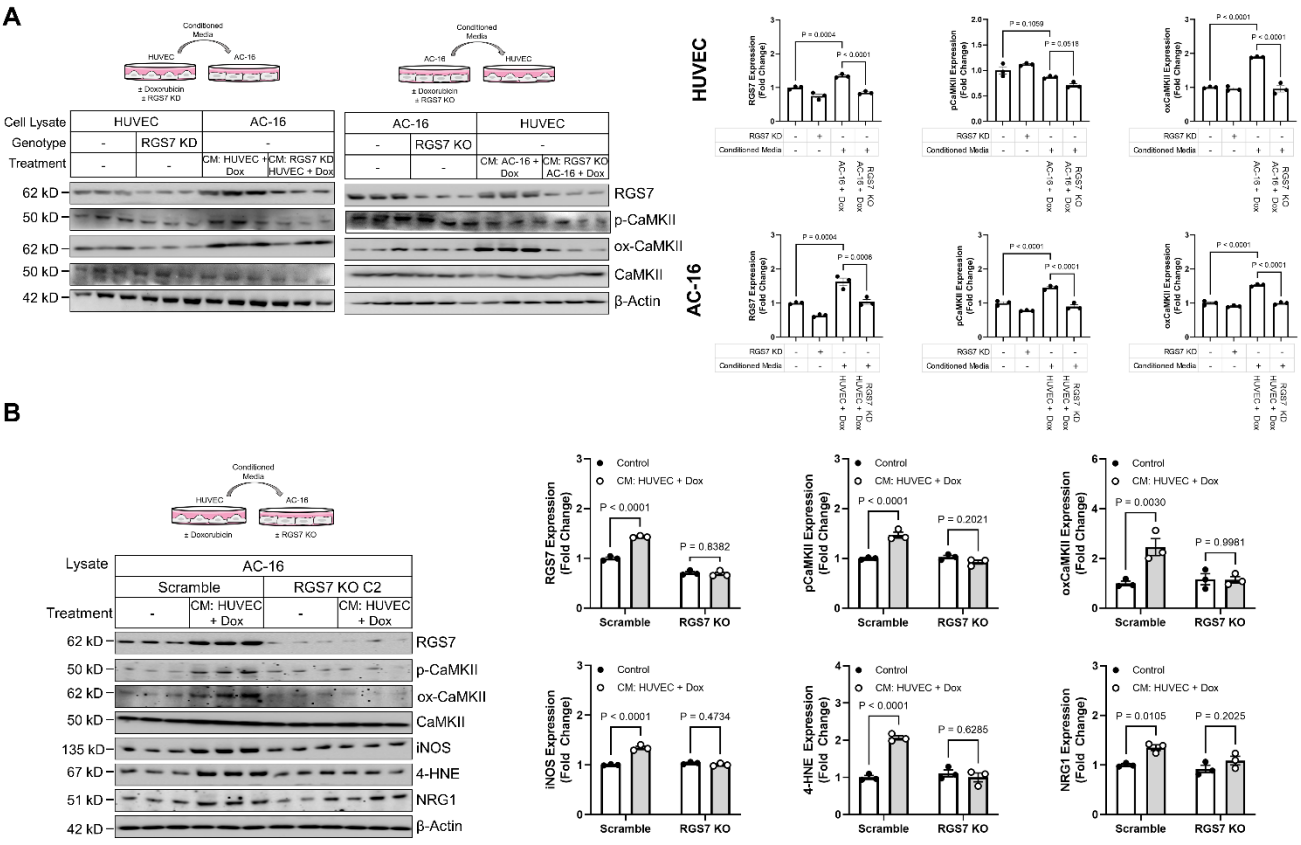

Figure S7 – RGS7 mediates pathological cross talk between EC and VCM following chemotherapy exposure. (A) Control, RGS7 KO AC-16, or RGS7 KD HUVEC cells were treated with doxorubicin (3 μM, 16 hours). Culture media was removed and added to HUVEC or AC-16 cultures, respectively. Immunoblotting was performed to detect RGS7, p-CaMKII, and ox-CaMKII (n=3). (B) Human HUVEC endothelial cells were treated with doxorubicin (3 μM, 24 hours). The culture media was removed and added to control or RGS7 KO AC-16 cardiomyocytes (clone #2). Immunoblotting was performed to detect RGS7, p-CaMKII, ox-CaMKII, iNOS, NRG1, and 4-HNE.β-Actin serves as a loading control for western blots. Immunoblots are accompanied by a densitometric quantification wherein expression is normalized to the corresponding control group. Data were analyzed by one- or two-way ANOVA with Sidak's post-hoc test. Exact *P* values are provided on graphs. Data are presented as mean ± SEM.

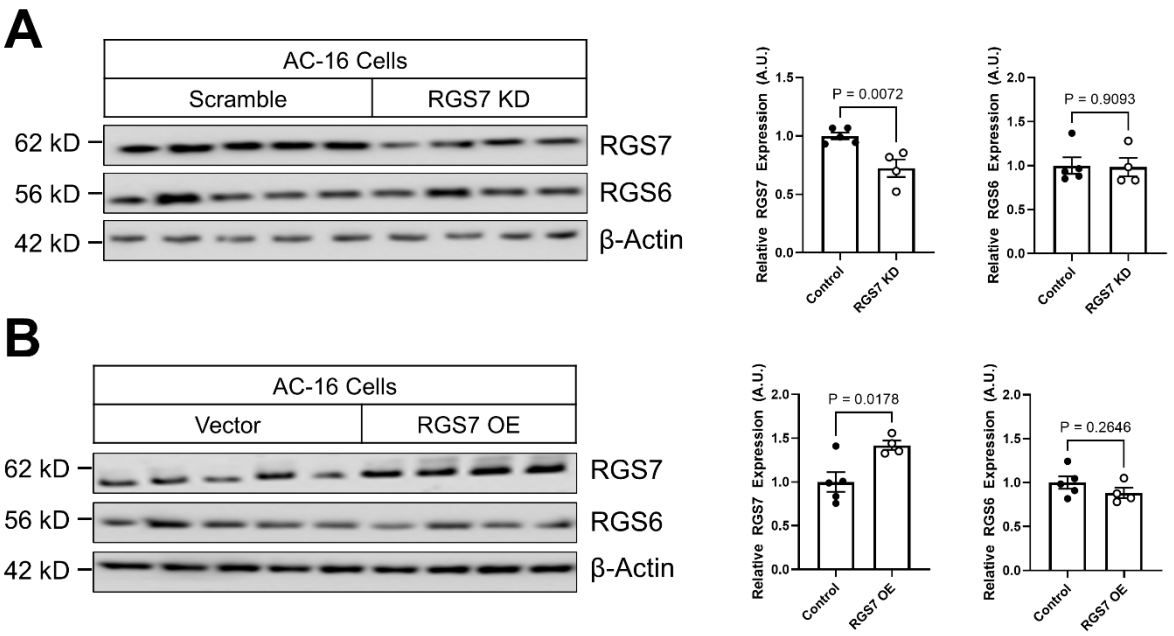

Figure S8 – Altering RGS7 expression fails to impact expression of RGS6. AC-16 cells were transfected with (A) scramble or RGS7-targeted shRNA or (B) RGS7-GFP or vector control. Immunoblotting was performed to detect expression of RGS7 and RGS6 (n=4-5). β-Actin serves as a loading control for western blots. Immunoblots are accompanied by a densitometric quantification wherein expression is normalized to the corresponding control group. Data were analyzed by student's t-test test. Exact *P* values are provided on graphs. Data are presented as mean ± SEM.

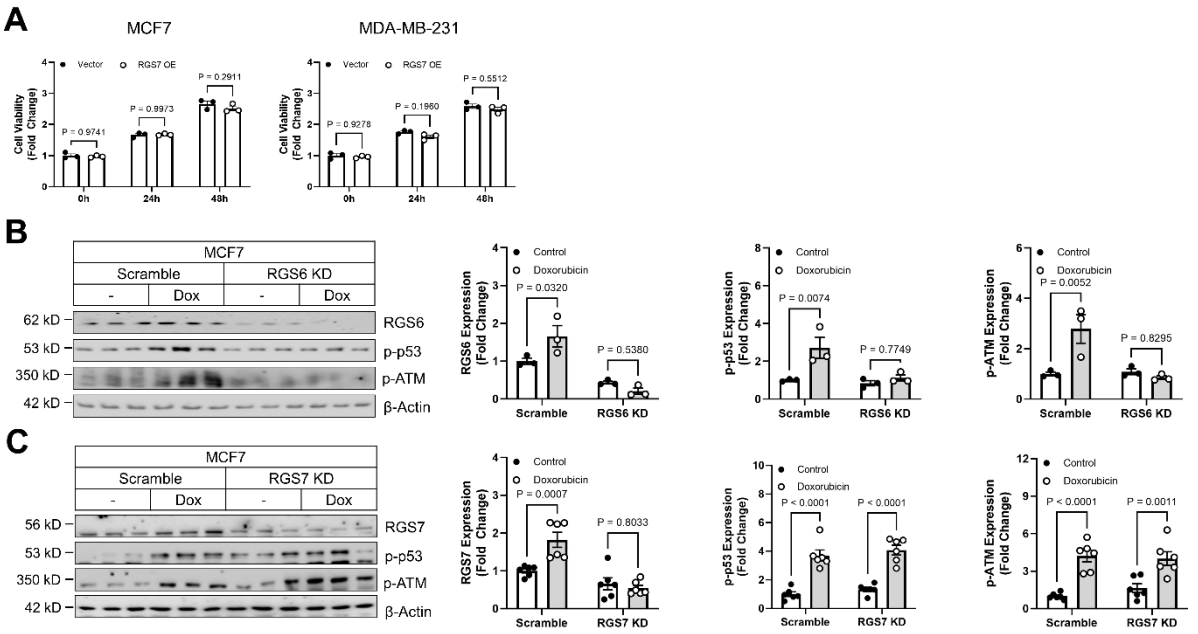

Figure S9–RGS7 fails to impact survival or induction of cell death signaling in breast cancer cells. (A) MCF7 and MDA-MB-231 cells were transfected with vector control or RGS7-GFP and cell survival monitored over a 48-hour period (n=3). MCF7 cells were treated with doxorubicin (2 µM, 12 h) following introduction of scramble or (B) RGS6- (n=3) or (C) RGS7-targed shRNA (n=6). RGS6/7, p-p53, and p-ATM expression were determined via western blot. Immunoblots are accompanied by a densitometric quantification wherein expression is normalized to the corresponding control group. Data were analyzed by two-way ANOVA with Sidak's post-hoc test. Exact *P* values are provided on graphs. Data are presented as mean ± SEM.

Table S1– Demographic and clinical cardiac parameters of human chemotherapy patients. Post-mortem heart tissue samples were scored by a pathologist for fibrotic remodeling. Samples with scores ≤ 1 were categorized as "chemotherapy -fibrosis" and samples with scores ≥2 were categorized as "chemotherapy +fibrosis". Data are presented as mean ± standard deviation (SD). Data were analyzed by one-way ANOVA with the Bonferroni post-hoc adjustment.

	Control	Chemotherapy -Fibrosis	Chemotherapy +Fibrosis	*P* value
Patients (*N*)	9	12	12	-
Male	4	5	4	-
Female	5	7	8	-
Age (years)	56.8 ± 12.9	65.3 ± 6.6	63.0 ± 2.8	0.19
Fibrosis Score	0 ± 0	0 ± 0	2.9 ± 0.7	**<0.0001**
Ejection Fraction (%)	-	28.9 ± 2.8	30.0 ± 4.2	0.48
Chemotherapy Duration (days)	-	130 ± 55.4	132.3 ± 31.9	0.91
Time since chemotherapy cessation (days)	-	0 ± 0	86.3 ± 68.6	**0.0003**

Table S2: Clinical Data for chemotherapy-treated heart autopsy samples: controls (Con) 1-9; chemotherapy patients without detectable fibrosis (C-F) 1-12; and chemotherapy patients with detectable fibrosis (C+F) 1-12. All "chemotherapy patients" had a history of chemotherapy with regimens containing 5-FU, an anthracycline and/or oxaliplatin.

Patient ID	Age	Sex	Cancer	Treatment received	Co-morbid conditions	Cause of death	Fibrosis Score
Con 1	55	M	N/A	N/A	Arthritis	Kidney failure	0
Con 2	54	M	N/A	N/A	Mild Asthma	Ischemic stroke	0
Con 3	62	F	N/A	N/A	-	Drowning	0
Con 4	65	F	N/A	N/A	-	Road accident	0
Con 5	56	M	N/A	N/A	Diabetes	Haemorrhagic stroke	0
Con 6	52	F	N/A	N/A	Eczema	Hepatolenticular degeneration	0
Con 7	71	M	N/A	N/A	Asthma	Suicide (by hanging)	0
Con 8	75	F	N/A	N/A	Fatty liver	Ischemic stroke	0
Con 9	51	F	N/A	N/A	Anxiety	Suicide (by hanging)	0
C-F1	66	M	GI	Folfox + Bavacizumab	Asthma	Metastasis	0
C-F2	54	F	Breast	Anthracycline	Mild depression	Metastasis	0
C-F3	69	F	Breast	Anthracycline	Eczema	Metastasis	0

C-F4	63	F	GI	Fluoropyrimidine	Mild Lupus	Metastasis	0
C-F5	67	F	Breast	Anthracycline	Psoriasis	Metastasis	0
C-F6	75	F	Breast	Anthracycline+ Capecitabine	Mild diabetes	Metastasis	0
C-F7	61	M	Colorectal	Folfox	Mild hypertension	Metastasis	0
C-F8	78	M	GI	Folfox + Bavacizumab	Fatty liver	Metastasis	0
C-F9	62	F	Breast	Anthracycline	Mild depression	Metastasis	0
C-F10	61	M	Colorectal	Folfox+ Panitumumab	Mild anxiety	Metastasis	0
C-F11	67	F	Breast	Anthracycline	Fibromyalgia	Metastasis	0
C-F12	60	M	Colorectal	Folfox + Oxaliplatin	Fatty liver	Metastasis	0
C+F1	61	F	Colorectal	Folfox	Mild diabetes	Myocardial infarction	2
C+F2	57	F	Breast	Anthracycline	Fatty liver	Atrial fibrillation	2
C+F3	63	F	Breast	Anthracycline	Mild depression	Myocardial infarction	3
C+F4	61	M	GI	Fluoropyrimidine	Asthma	Myocardial infarction	3
C+F5	72	F	Breast	Anthracycline	Fatty liver	Heart failure	3
C+F6	71	F	Breast	Anthracycline	Diverticulitis	Myocarditis & Heart failure	4
C+F7	66	M	Colorectal	Folfox	Diabetes	Angina & myocardial infarction	3
C+F8	59	M	GI	Folfox + Bavacizumab	Arthritis	Ventricular tachycardia & heart failure	3
C+F9	65	M	Colorectal	Folfox	COPD	Atrial fibrillation	3
C+F10	57	F	Breast	Anthracycline	Diabetes	Myocardial infarction	2
C+F11	61	F	Colorectal	Folfox + Capecitabine	Anxiety	Myocardial infarction	4
C+F12	63	F	Breast	Anthracycline	Fibromyalgia	Myocardial infarction	3

Table S3: Reagent List

Company	Location	Reagent
Sigma Chemical	St. Louis, MO, USA	N-acetyl cysteine (NAC) Ru360 Cyclosporin A Crystal Violet CM-H_2DCFDA DMBA 5-FU Diphenyleneiodonium chloride (DPI)

		KN-93
Addgene	Watertown, MA, USA	pLenti CMV Puro DEST cloning vector pMD2.G VSV-G envelope expressing plasmid psPAX2
Abcam	Cambridge, UK	Masson Trichrome Stain Protein A/G sepharose Doxorubicin Oxaliplatin Thiazolyl blue tetrazolium bromide (MTT) 10X RIPA 50X DAB Chromogen& DAB Substrate ECL Detection kit Irinotecan Bradford reagent Protease inhibitor cocktail Phosphatase inhibitor cocktail
Thermo Fisher Scientific	Waltham, MA, USA	Phusion Hot Start II High-Fidelity PCR Master Mix (F-565S) Platinum™ Super FiII PCR Master Mix FBS (Gibco) RNA later Trypsin EDTA 0.05% and 0.25% Alexafluor conjugates DNA Ladder (50, 100, 1000 kb) TaqManRNAase OptiMEM, MEM, DMEM Glycogen Dream Taq
Invitrogen	Carlsbad, CA, USA	Superscript III RT/Platinum Taq Mix Lipofectamine 3000 Invivofectamine 3.0 Platinum Taq DNA Polymerase High Fidelity Verso cDNA synthesis Kit
Takara Bio	Kyoto, Japan	pMD20-T vector
Sisco Research Laboratory	Mumbai, India	Hematoxylin/Eosin Acrylamide Aprotinin PMSF RNAase inhibitor Protein ladder 6X loading dye Ethidium bromide DTT 50X TAE Bovine Serum Albumin DMSO Ponceau Red Stain Xylene 4% Formaldehyde
Himedia	Mumbai, India	Puromycine dihydrochloride Collagenase type II & type IV Ampicillin sodium Proteinase K Amphotericine B
		PBS D-Mannitol
New England Biolabs	Ipswich, MA, USA	Restriction Enzymes: XhoI, HindIII-HF, BglII, EcoRI-HF, BsmB1, AgeI
Santa Cruz Biotechnology	Dallas, TX, USA	shRNA for RGS7, CaMKIIδ

Table S4: Antibody List

Company	Location	Antibody	Catalog #	Dilution

Abcam	Cambridge, UK	CaMKII	ab29	WB (1:1000), IHC (1:200), IP (1:100)
		pCaMKII	ab6640	WB (1:1000)
		Mouse Secondary-HRP	ab97023	WB (1:2000), IHC (1:500)
		Rabbit Secondary-HRP	ab97051	WB (1:2000), IHC (1:500)
		Cardiac Troponin T	ab8295	WB (1:1000), IHC (1:500)
		4-HNE	ab46545	WB (1:1000)
		iNOS	ab3523	WB (1:1000)
		RGS6	ab128943	WB (1:1000)
		ANP	ab189921	WB (1:1000)
		B-Actin	ab8227	WB (1:1000)
Cell Signaling Technology	Danvers, MA, USA	α-SMA	19245S	WB (1:1000)
		GFP	2956	WB (1:1000), IP (1:200)
		p-ATM	4526	WB (1:1000)
		p-p53	9284	WB (1:1000)
		Col1α	72026	WB (1:1000)
		pAKT	9271	WB (1:1000)
		AKT	9272	WB (1:1000)
		βMHC	MA1-26180	WB (1:1000)
		NRG1	PA5-34648	WB (1:1000)
Thermo Fisher Scientific	Waltham, MA, USA	FSTL1	20182-1-AP	WB (1:1000), IHC (1:250)
		RGS7	PA5-77000	WB (1:1200), IP (1:200), IHC (1:500)
GeneTex	Irvine, CA, USA	Ox-CaMKII	GTX36254	WB (1:1000)
Santacruz Biotechnology	Texas, USA	TGFβ1	sc-130348	WB (1:000)
Millipore	Burlington, MA, USA	Gβ5	ABS1062	WB (1:800)

Table S5: Assay Kit List

Company	Location	Assay	Catalog #
Abcam	Cambridge, UK	Ca^{2+} Flux Assay Kit	ab102505
		Mitochondrial isolation Kit	ab110170
		Mitochondrial Membrane Potential Assay Kit	ab113852
		Terminal deoxynucleotidyltransferasedUTP Nick-End Labelling (TUNEL) kit	ab206386
Roche	San Francisco, CA, USA	Cell Death Detection Kit	C755B93
Erba Mannheim	London, UK	ALT	120207
		AST	120204
		Triglycerides	120211
Biovision	Milpitas, MA, USA	SOD	K106-100
		Caspase-3	K335

TableS6: Cell Line List

Company	Location	Cell Line	Catalog #	Culture Conditions
Merck & Co.	Kenilworth, NJ, USA	AC-16	SCC109	37°C incubator at 5% CO_2 in DMEM + 10% FBS
Himedia	Mumbai, India	HUVEC	CL002	37°C incubator at 5% CO_2 in HiEndoXLTM endothelial cell expansion medium + 3% FBS
Takara	Kusatsu, Japan	Cellartis Cardiomyo	ChiPSC22	37°C incubator at 5% CO_2 in Cellartis CM Culture Base (Y10063)

		cytes (iPSC CM)		+ 10% FBS
National Centre for Cell Science (NCCS)	Pune, India	MCF7	-	37°C incubator at 5% CO_2 in DMEM + 10% FBS
National Centre for Cell Science (NCCS)	Pune, India	MDA-MB-231	-	37°C incubator at 5% CO_2 in DMEM + 10% FBS

Table S7: Primer List

#	**Names of Primer**	**Sequence of Primer**
1	RGS7 Human isoform 4 full length in pEGFP-N1Forward NheI	5'ATAGCTAGCATGGCCCAGGGGAATAAT3'
2	RGS7 Human isoform 4 full length in pEGFP-N1Reverse BglII	5'TATAGATCTTTAGTAAGACTGAGCAA3'
3	RGS7 Human Isoform 4 deletion 251-469aa Forward	5'ATAGCTAGCATGGCCCAGGGGAATAAT3'
4	RGS7 Human Isoform 4 deletion 251-469aa Reverse	5'TATAGATCTAGTTTCTGGTGTGGGTG3'
5	RGS7 Human Isoform 4 deletion 1-136aa Forward	5'ATAGCTAGCATGCAAAACAAGGCACG3'
6	RGS7 Human Isoform 4 deletion 1-136aa Reverse	5'TATAGATCTTTAGTAAGACTGAGCAA3'
7	RGS7 Human Isoform 4 deletion 256-315aa overlapping Forward	5'CTCCAACAGAACTTGAGGCAAGCAAAGAACC3'
8	RGS7 Human Isoform 4 deletion 256-315aa overlapping Reverse	5'TTGCCTCAAGTTCTGTTGGAGGTTTAGTTTC3'
9	RGS7 Human Isoform 4 deletion 327-446aa overlapping Forward	5'CCAGCAGAGGCTTCTACAGGCAAAGAA3'
10	RGS7 Human Isoform 4 deletion 327-446aa overlapping Reverse	5'CCTGTAGAAGCCTCTGCTGGCTCGGTT3'
11	RGS7 Human Isoform 4 point mutation N398V overlapping Forward	5'AGTGCTATTGTCTTGGATTC3'
12	RGS7 Human Isoform 4 point mutation N398V overlapping Reverse	5'GGAATCCAAGACAATAGCA3'
13	RGS7 Mouse isoform III full length in pEGFP-N1Forward SalI	5'ATAGTCGACATGGCTCAGGGAAATAA 3'
14	RGS7 Mouse isoform III full length in pEGFP-N1Reverse BamHI	5'TATGGATCCTAACAGGTTAGTGCTGG3'
15	CaMKIID Human Main Forward N-HA EcoRI	5'ATAGAATTCATGGCTTCGACCACAACCTG3'
16	CaMKIID Human Main Reverse N-HA XhoI	5'ATACTCGAGTTAGATGTTTTGCCACAAAG3'
17	CaMKIID Oxidation mutant (M281/282V) Human overlapping Reverse	5'CCTGTCTGTGCACCACGGAAGCA3'
18	CaMKIID Oxidation mutant (M281/282V) Human overlapping Forward	5'ACTGTTGCTTCCGTGGTGCACAG3'
19	CaMKIID Phosphorylation mutatnt(T287A) Human overlapping Reverse	5'AAGCAGTCTACAGCCTCCTG3'

20	CaMKIIDPhsophorylation mutant (T287A) Human overlapping Forward	5’ATGATGCACAGACAGGAGGCTGTAGA3’
21	CaMKIID (Oxi/Phospho) Human overlapping Reverse	5’AGTCTACAGCCTCCTGTCTGTGCACCACGGAAGCA3’
22	CaMKIID (Oxi/Phospho) Human overlapping Forward	5’GTTGCTTCCGTGGTGCACAGACAGGAGGCTGTAGA3’