Figure S1

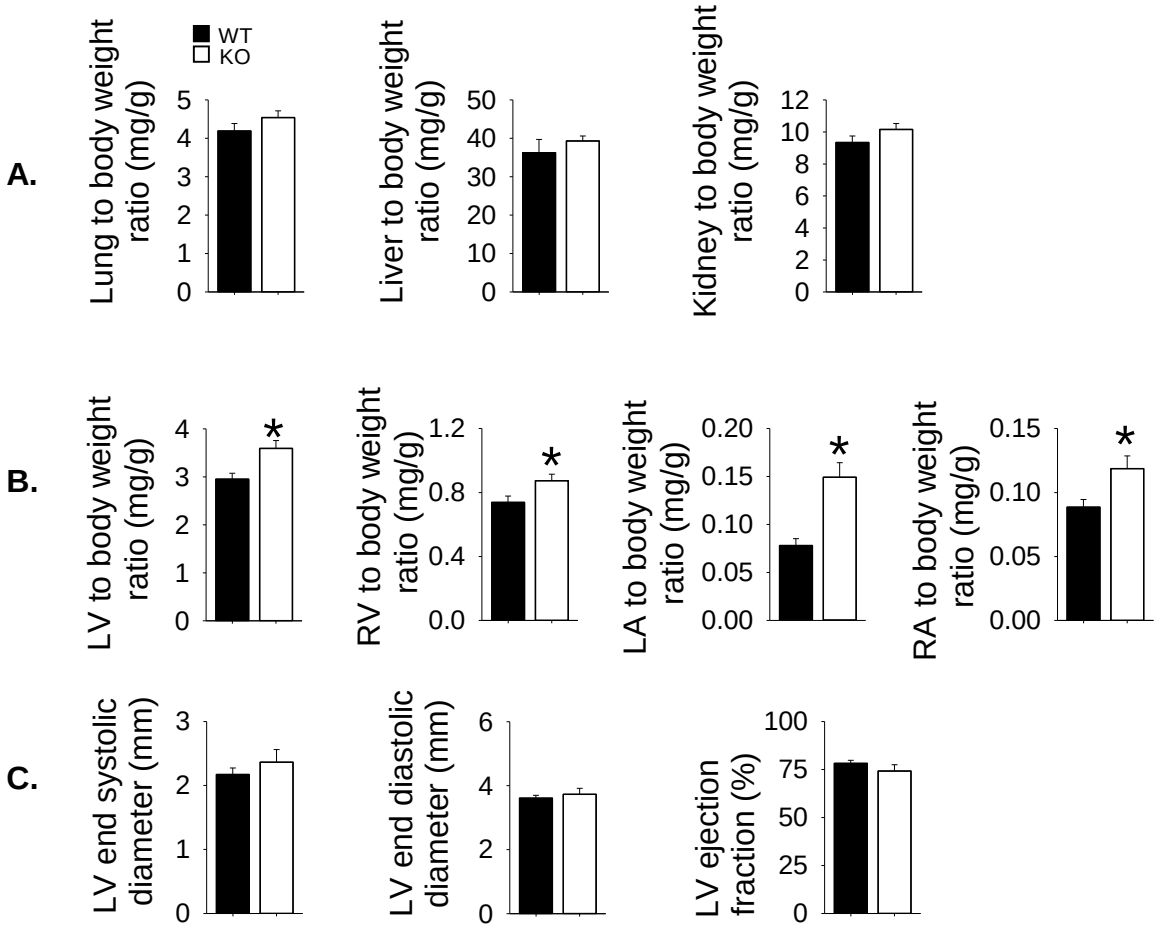


Figure S1 (A) Lung, liver and kidney weights to body weight ratio in WT and cADK$^{-/-}$ mice and **(B)** left and right ventricular and atrial weights relative to body weight (n=9 WT and 10 cADK$^{-/-}$)
(C) End systolic diameter, end diastolic diameter and ejection fraction of WT and cADK$^{-/-}$ mice were measured four weeks after the last tamoxifen injection by echocardiography. * indicates p<.05 relative to WT. (n= 7 WT and 6 cADK$^{-/-}$)

Figure S2

S2A (from figure 2)

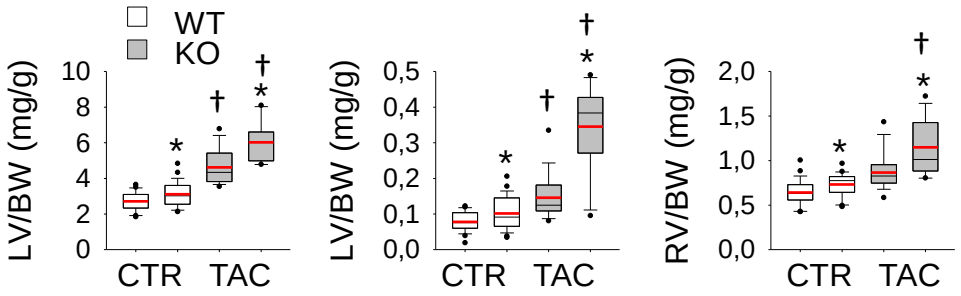


Figure S2A. **Cardiomyocyte ADK expression increases in response to LV pressure overload and attenuates pressure overload induced LV hypertrophy and fetal gene program.** (**A**) LV, (**B**) LA, and (**C**) RV weight to body weight ratios in WT and cADK$^{-/-}$ mice after 6 weeks of control or pressure overload (TAC) conditions. (n=26, 24, 17, and 17 for WT, ADK$^{-/-}$, WT-TAC, and ADK$^{-/-}$-TAC, respectively) red bars are mean, red bars are median.

S2B (from figure 3)

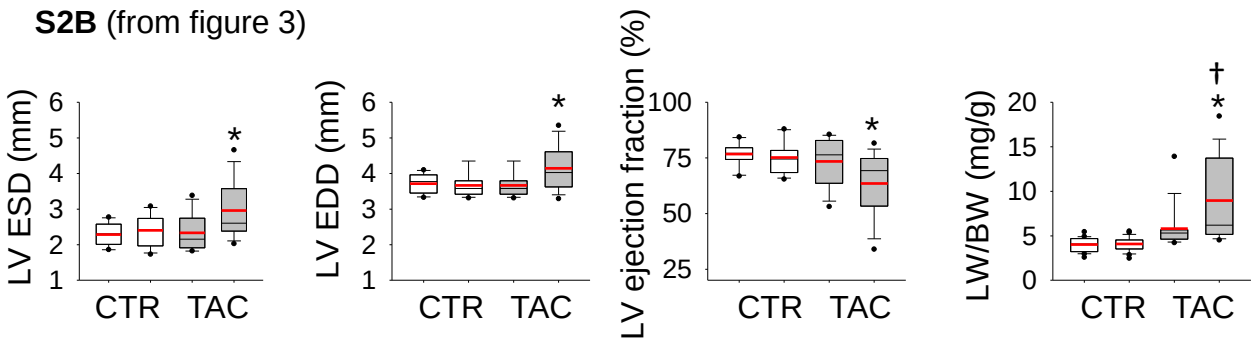


Figure S2B. Effects of cardiomyocyte ADK disruption on LV function during pressure overload (A) Echocardiography measurements of end systolic diameter (ESD), **(B)** end diastolic diameter (EDD) and **(C)** ejection fraction (EF) 6 weeks after sham or TAC surgery**.** (n=10, 11, 12, and 14 for WT. ADK$^{-/-}$, WT-TAC, and ADK$^{-/-}$-TAC, respectively) **(D)** Lung weight to body weight ratio in WT and cADK$^{-/-}$ mice 6 weeks after TAC (n= 26, 24, 17, and 17 for, WT, ADK$^{-/-}$, WT-TAC, and ADK$^{-/-}$-TAC, respectively). Red bars are mean, black bars are median.

Figure S3

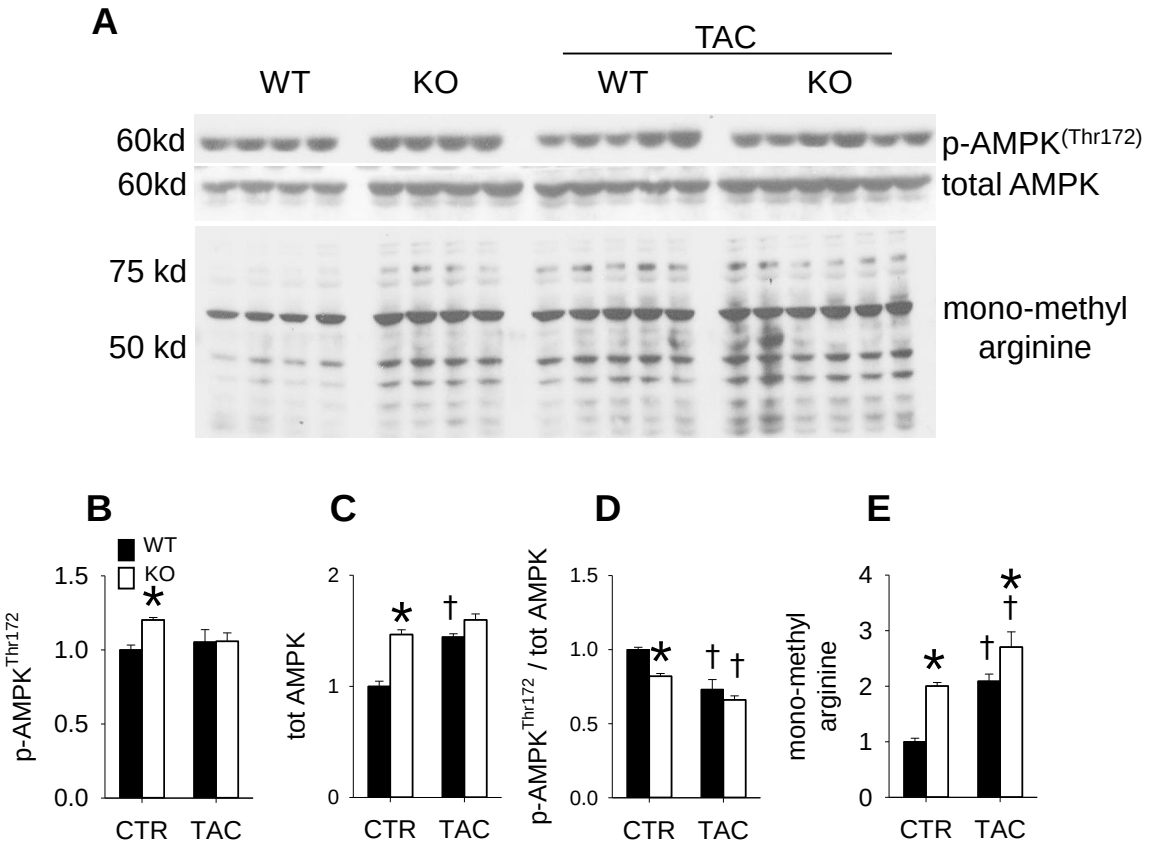


Figure S3 Western analysis of ventricular phospho-AMPKThr^{172} **(A, B)**, total AMPK alpha**(A,C)**, phospho/total AMPK **(A,D)** and mono-methlyated-arginines **(A,E)** in WT, cADK$^{-/-}$, WT-TAC, and cADK$^{-/-}$-TAC * indicates p<.05 relative to WT. †=p<.05 relative to sham control, same genotype. (n=4, 4, 5, and 6 for WT. cADK$^{-/-}$, WT-TAC, and cADK$^{-/-}$-TAC, respectively)

Figure S4

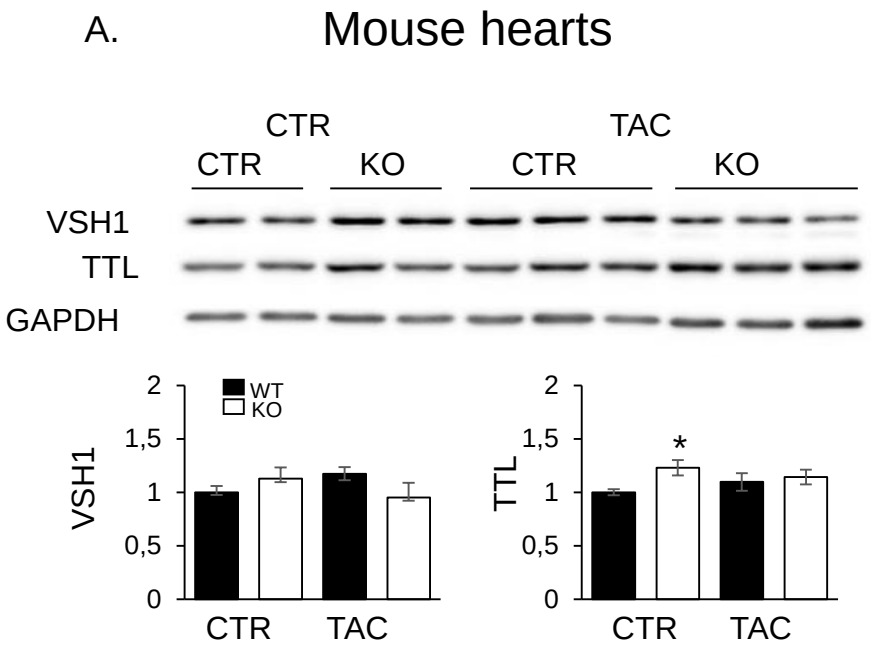


Figure S4 Heart lysates from CTR and cADK$^{-/-}$ mice under control and TAC conditions were analyzed by western blot for expression of vasohibin1 (VSH1) and tubulin tyrosine ligase (TTL). (n=4, 4, 5, and 6 for WT. cADK$^{-/-}$, WT-TAC, and cADK$^{-/-}$ TAC respectively; * indicates $p<.05$ as compared to WT under same conditions.)

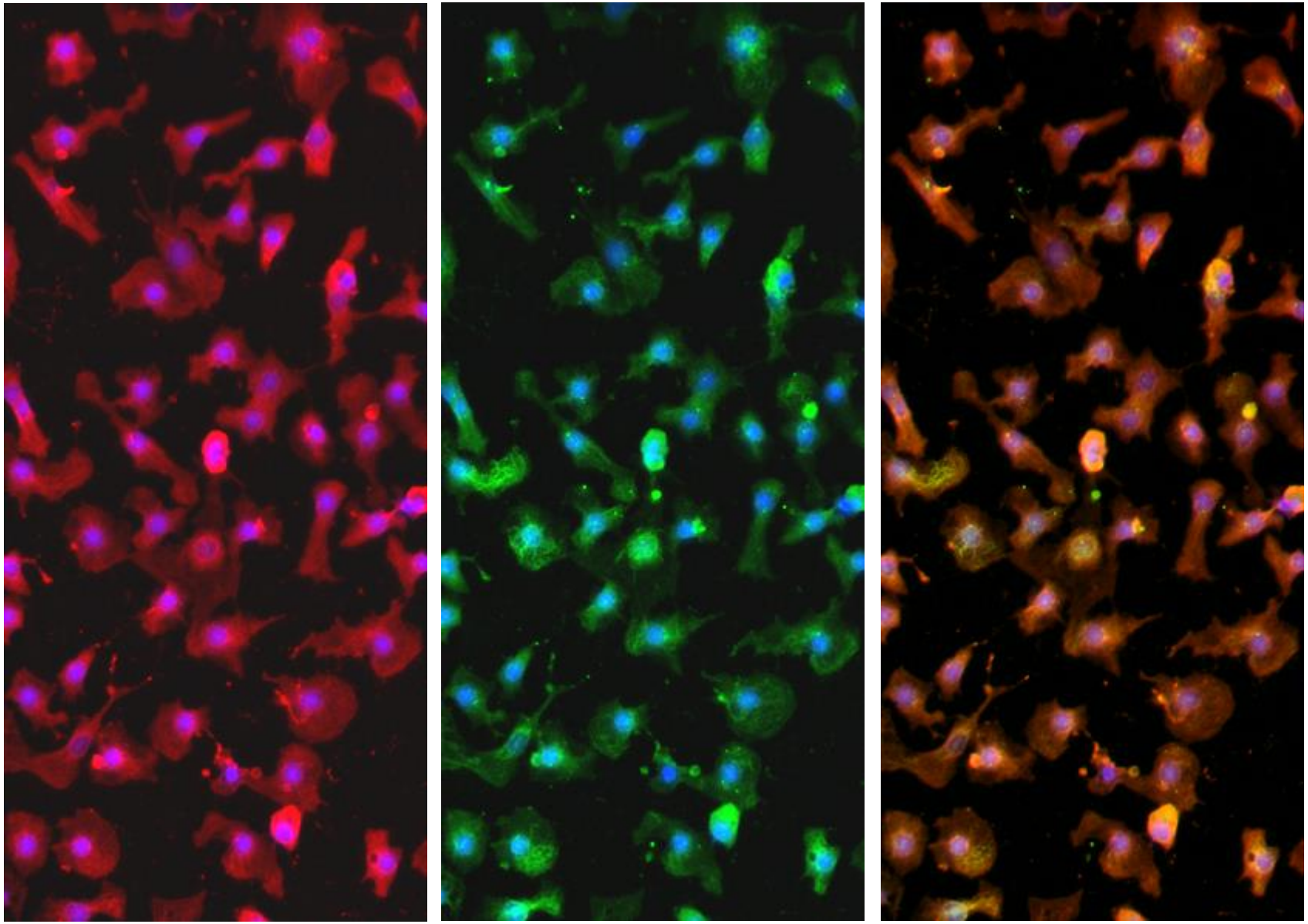
Figure S5A

Untreated cardiomyocytes

α-Tubulin

Glu-Tubulin

α-Tubulin
Glu-Tubulin



Online supplement

Expanded Methods

Echocardiography and LV hemodynamics*.** Mice were anesthetized with 1.5% isoflurane and echocardiographic images were obtained using a Visual Sonics high resolution Vevo 770 system as previously described (Khairallah et al., 2012). LV diameter, shortening fraction and wall thickness were measured from 2-D guided short-axis M-Mode views of the LV. ***Evaluation of aortic pressure. Mice were anesthetized with *1.5% isoflurane*. A 1.2 Fr. pressure catheter (Scisense Inc. Ontario Canada) was introduced into the right common carotid artery for aortic pressure measurement and then advanced into LV for measurement of LV systolic and end-diastolic pressures, and positive and negative LV dP/dt_{max}. Data represent the mean of at least 10 beats of recording during stable hemodynamic conditions.

Neonatal Cardiomyocyte culture: NRVMs were isolated from 2-4 day-old Sprague-Dawley rats by enzymatic digestion and separated from non-muscle cells on a discontinuous Percoll gradient as previously described(Fassett et al., 2009). Myocytes were plated on plastic dishes in serum-containing DMEM (1 $x10^5$ cells/cm^2), incubated for 24-48 hrs to allow attachment and spreading, followed by incubation in serum free DMEM for 24 hours. Hypertrophy was induced by addition of 50µM phenylephrine. ADK expressing adenovirus was prepared as previously described(Luo et al., 2007) using the mouse cDNA encoding the ADK-long (nuclear localized) isoform. For adenoviral infection, cardiomyocytes were infected with 2 MOI/cell ADK or β-Gal expressing virus for 24 hours, after which media was changed prior to treatment. Cells were treated with 2-chloroadenosine (5µM) or adenosine (10µM). 1 µM adenosine deaminase inhibitor, pentostatin (Tocris), was included during adenosine treatment. *Collection of triton soluble and insoluble fractions from NRVMs* was carried out as previously described(Fassett et

al., 2009).

MT isolation: Microtubule isolation and subcellular fractionation: Microtubule fractions were isolated from mouse ventricles in glycerol based microtubule stabilization buffer as previously described (Fassett et al., 2009; Tsutsui et al., 1993). Briefly, ventricles were pulverized into powder under liquid nitrogen, then immediately homogenized for 15 seconds in room temperature microtubule stabilization buffer (Tsutsui et al., 1993). Lysates were centrifuged at 100,000 g for 20 minutes at room temperature, and non-polymerized tubulin “Free” was collected in the supernatant. To depolymerize microtubules, the pellet was resuspended and incubated for 2 hours at 0 degrees C in microtubule destabilization buffer. After centrifuging for 20 minutes at 100,000g (Tsutsui et al., 1993), the supernatant was collected as the cold sensitive “MT” fraction. To collect cold resistant, stabilized MTs or MT fragments linked to membranes, the remaining pellet was dissolved and resuspended by pipetting and vortexing in triton x-100 lysis buffer (20mM Tris-HCl, 150mM NaCl, 1mM EDTA, 10% glycerol, 1% triton x-100, ph 7.5 + protease inhibitor cocktail (Roche)) followed by centrifugation at 4 degrees C at 14000g for 10 minutes. This step was repeated, and the combined supernatants were saved as the “membrane” fraction. The remaining pellet, containing tubulin associated with the triton insoluble cytoskeleton, was resuspended in 2x SDS lysis buffer and saved as the “CSK” fraction. BCA assay was used to measure protein concentration. The cold released microtubule sample volume was based on the corresponding free fraction. Equal amounts of protein from free (cytosolic), membrane, and cytoskeletal fractions were loaded for western blot analysis.

Immunostaining: NRVMs were fixed in -20°C methanol for 10 minutes, washed once in PBS,

permeabilized with 0.1%triton x-100 in PBS for 5 minutes, blocked for 1 hour at RT in blocking buffer (2% FCS in PBS + .02% sodium azide) then incubated overnight in 1/500 dilution of mouse anti-alpha tubulin (Sigma Aldrich. St Louis, MO) and rabbit anti-detyrosinated tubulin (Abcam; Cambridge, UK) in blocking buffer. The next day, cells were washed 2 times in PBS, then incubated in 1/600 dilutions of donkey anti-mouse alexa fluor 555, or donkey anti-rabbit, alexa fluor 480 (Thermo Scientific; Waltham, MA) for 1 hour. DNA was stained using DAPI (1/10,000).

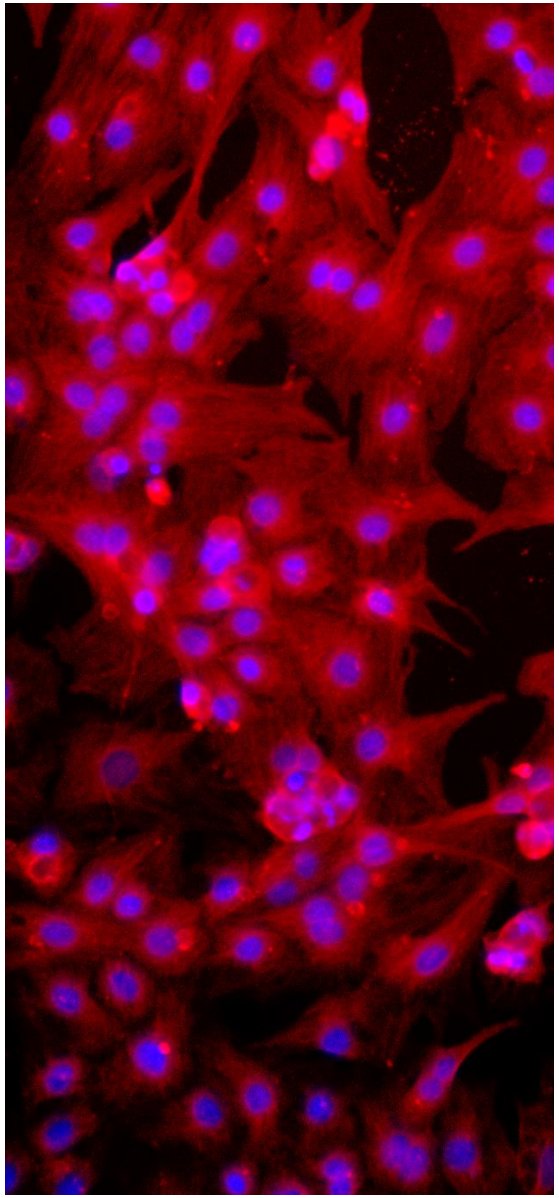
Quantification of detyrosinated versus total tubulin in NRVMs ImageJ software was used to quantify immunofluorescence from alpha tubulin and detyrosinated tubulin stained cardiomyocytes after the indicated treatments. Unsharp mask, background subtraction and thresholding were used to distinguish microtubules from background stain of free tubulin. The area of α-tubulin in a cell was traced and area measured. The same cell trace was then restored on the image of detyrosinated tubulin, and detyrosinated tubulin area was divided by the area of the total tubulin. The exposure time and processing of the images for quantitation was performed exactly the same for each different treatment.

Fassett, J.T., Xu, X., Hu, X., Zhu, G., French, J., Chen, Y., and Bache, R.J. (2009). Adenosine regulation of microtubule dynamics in cardiac hypertrophy. Am J Physiol Heart Circ Physiol *297*, H523-532.
Khairallah, R.J., Shi, G., Sbrana, F., Prosser, B.L., Borroto, C., Mazaitis, M.J., Hoffman, E.P., Mahurkar, A., Sachs, F., Sun, Y.*, et al.* (2012). Microtubules underlie dysfunction in duchenne muscular dystrophy. Sci Signal *5*, ra56.
Luo, J., Deng, Z.L., Luo, X., Tang, N., Song, W.X., Chen, J., Sharff, K.A., Luu, H.H., Haydon, R.C., Kinzler, K.W.*, et al.* (2007). A protocol for rapid generation of recombinant adenoviruses using the AdEasy system. Nature protocols *2*, 1236-1247.
Tsutsui, H., Ishihara, K., and Cooper, G.t. (1993). Cytoskeletal role in the contractile dysfunction of hypertrophied myocardium. Science *260*, 682-687.

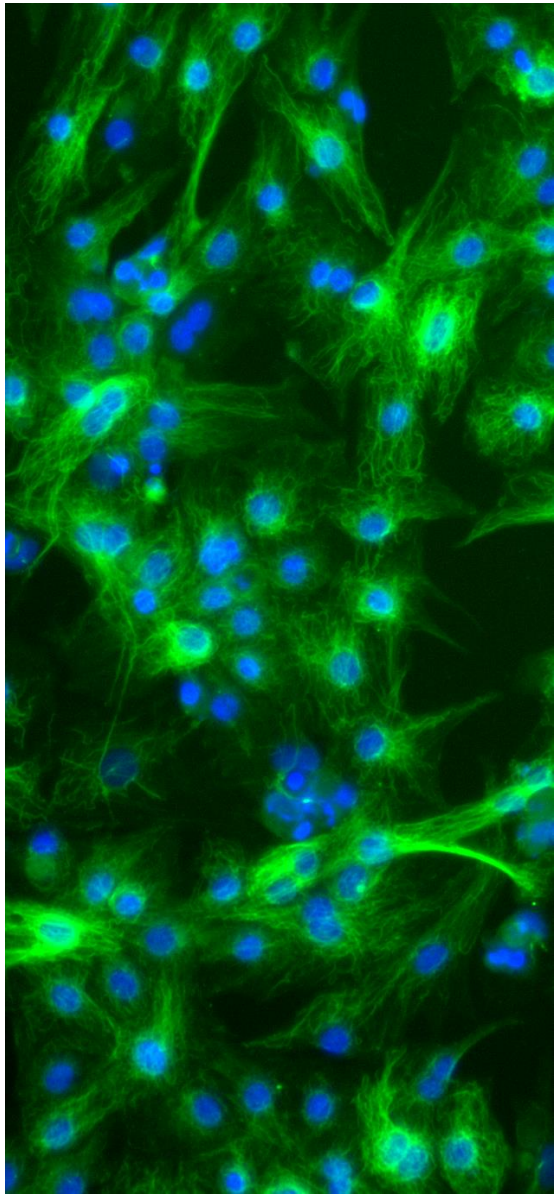
Figure S5B

Phenylephrine
treated cardiomyocytes

α-Tubulin



Glu-Tubulin



α-Tubulin
Glu-Tubulin

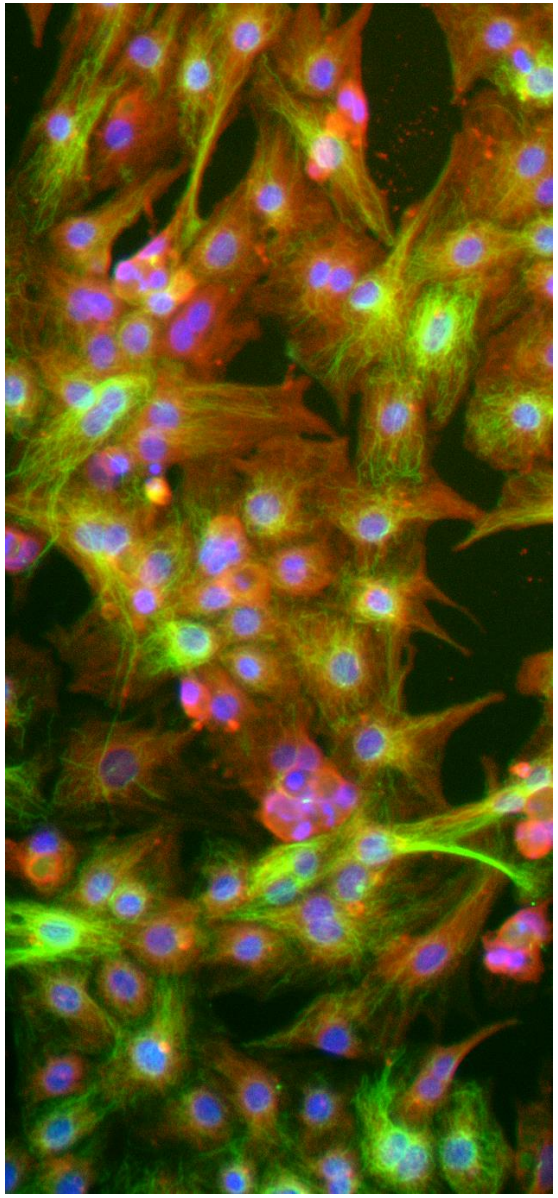
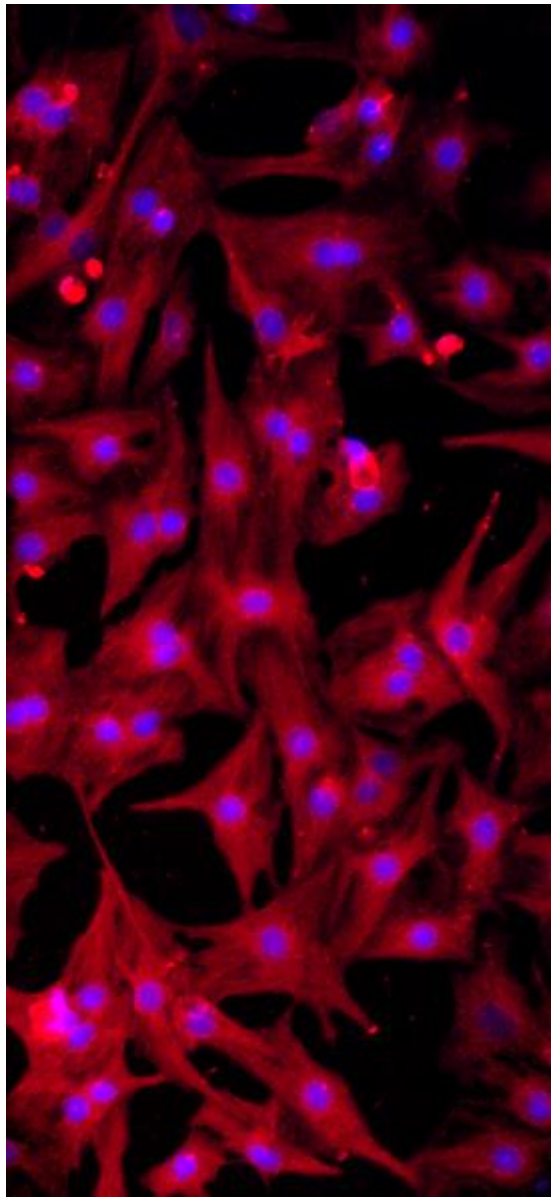


Figure S5C

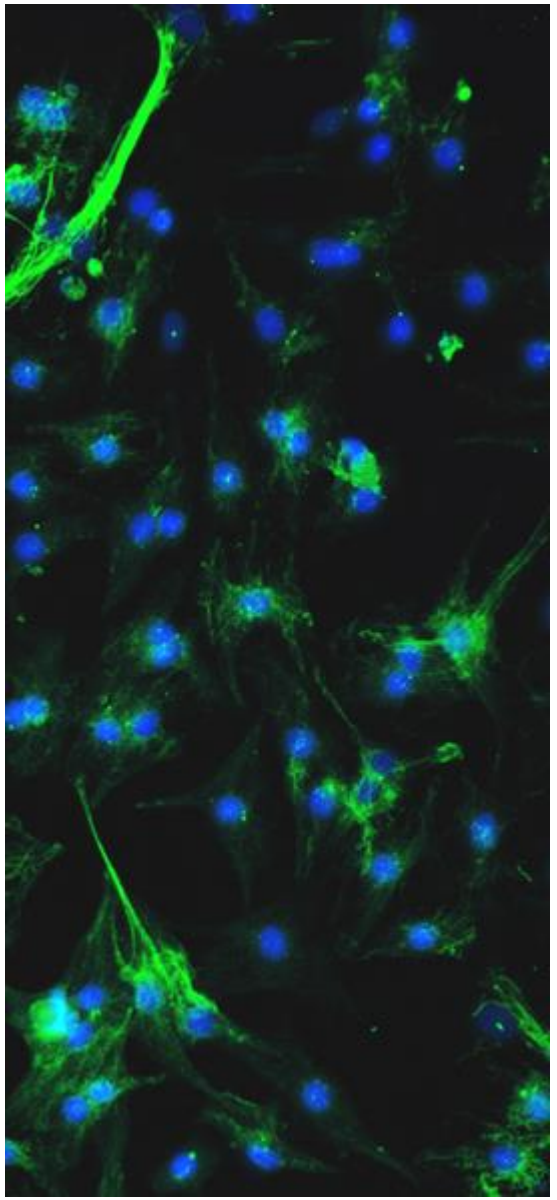
Phenylephrine + CADO
treated cardiomyocytes

α-Tubulin

Glu-Tubulin



Glu-Tubulin



α-Tubulin
Glu-Tubulin

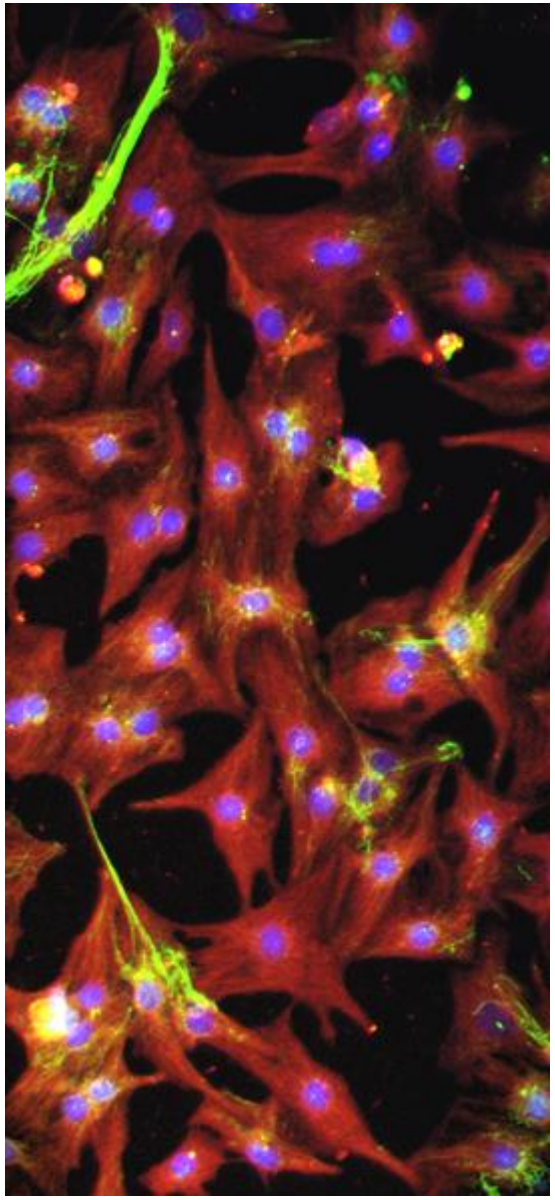


Figure S5D

Phenylephrine + CADO + A1R antag. (DPCPX; 5µM) treated cardiomyocytes

α-Tubulin

Glu-Tubulin

α-Tubulin
Glu-Tubulin

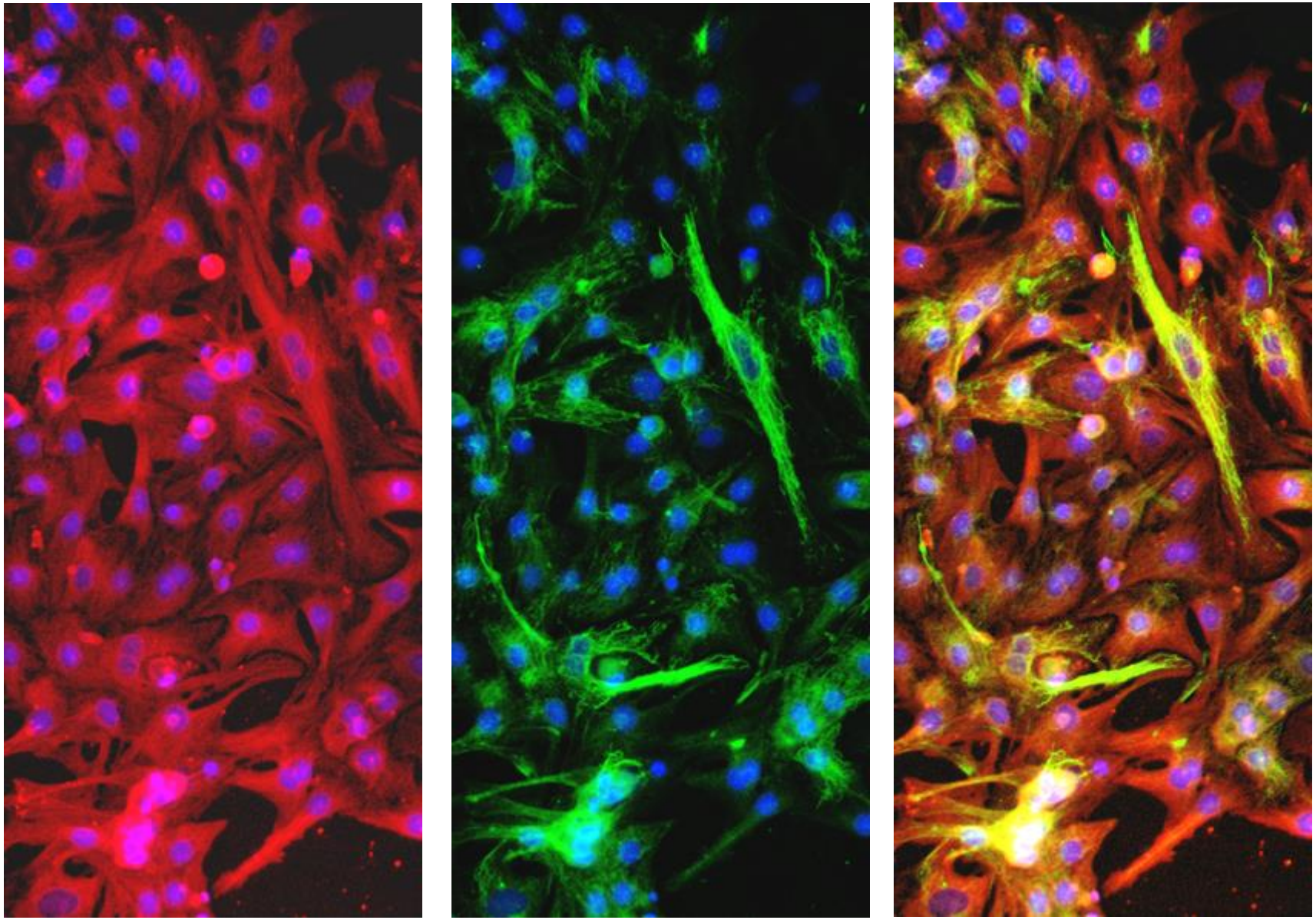
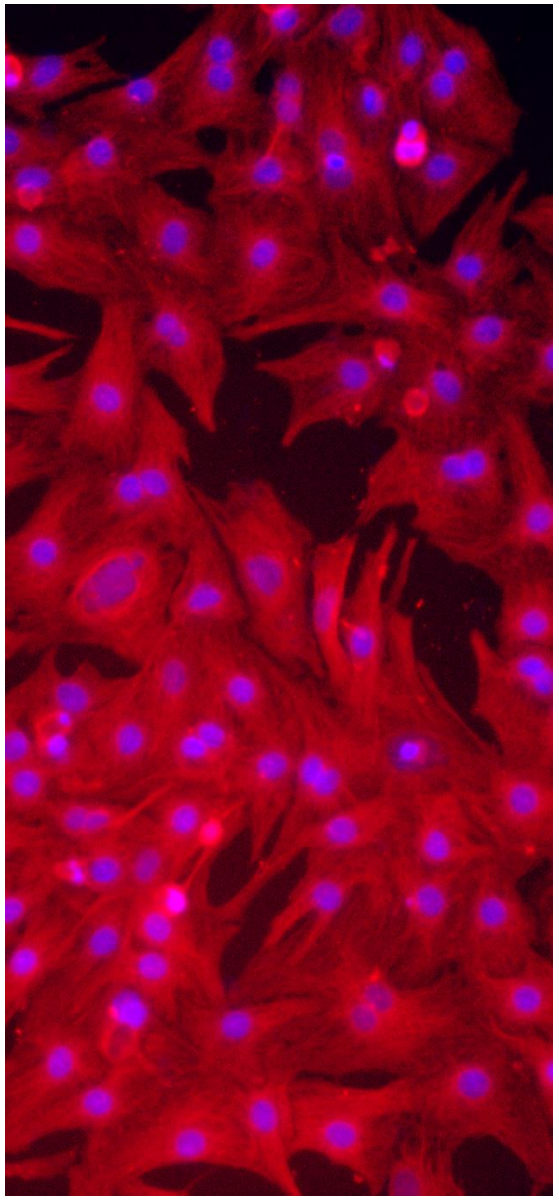


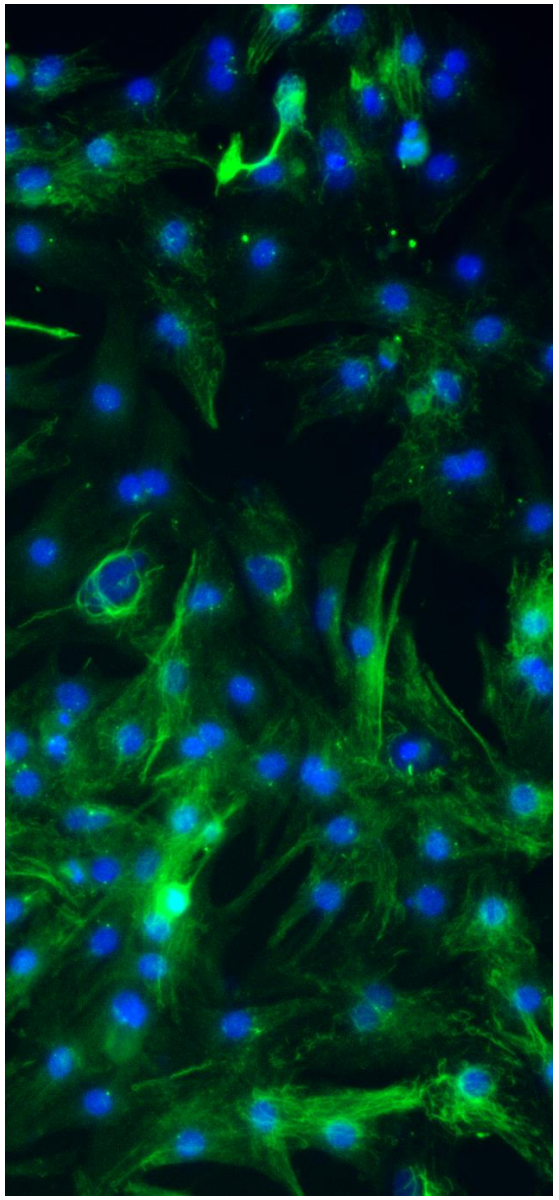
Figure S5E

Phenylephrine + CADO + A2A antag(zm-241,385; 5µM)
treated cardiomyocytes

α-Tubulin



Glu-Tubulin



α-Tubulin
Glu-Tubulin

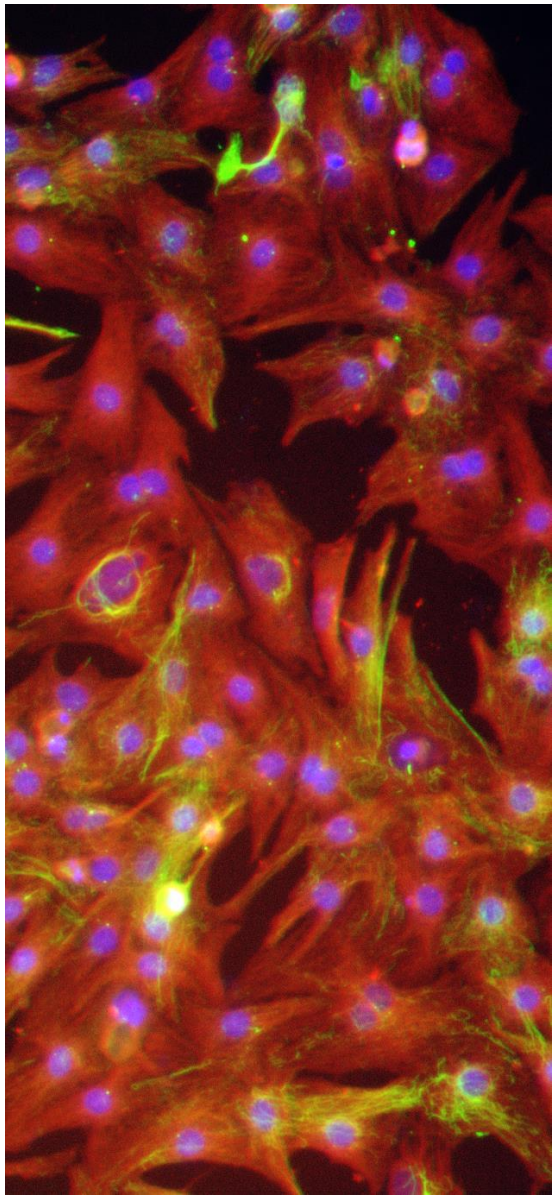


Figure S5F

Phenylephrine + CADO + A2B antag (MRS1754; 5μM) treated cardiomyocytes

α-Tubulin

Glu-Tubulin

α-Tubulin
Glu-Tubulin

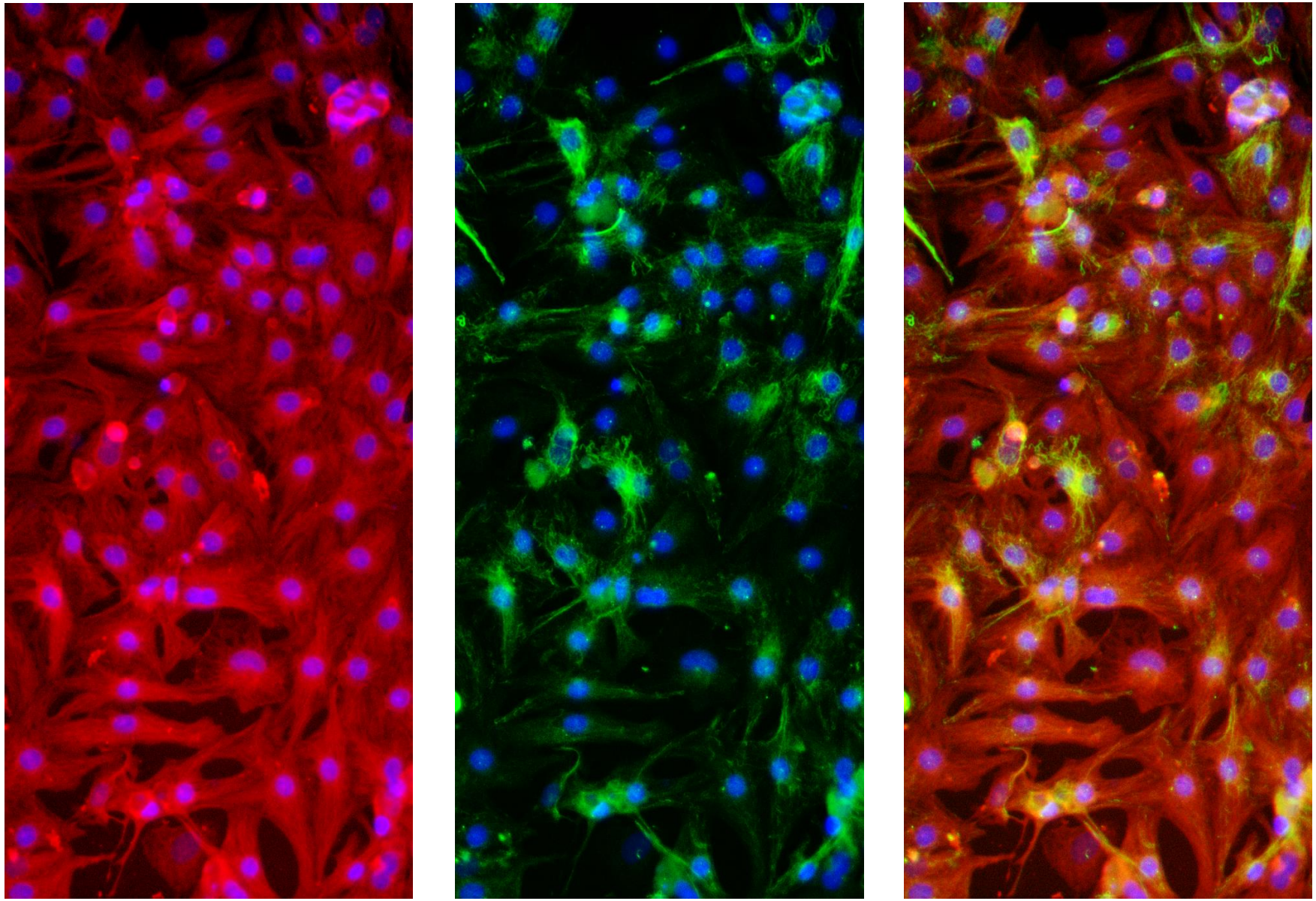
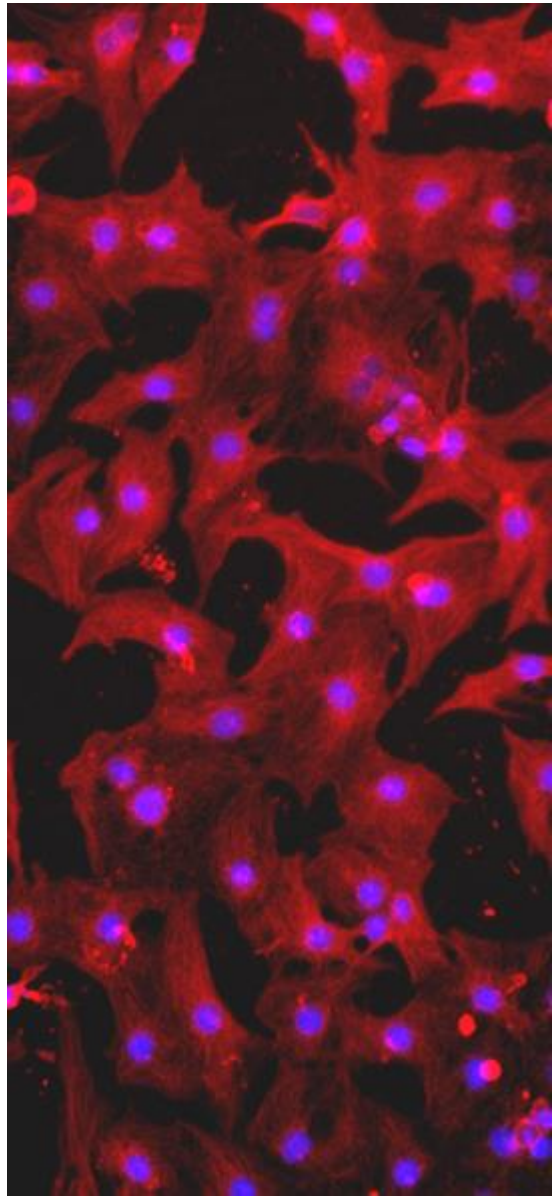


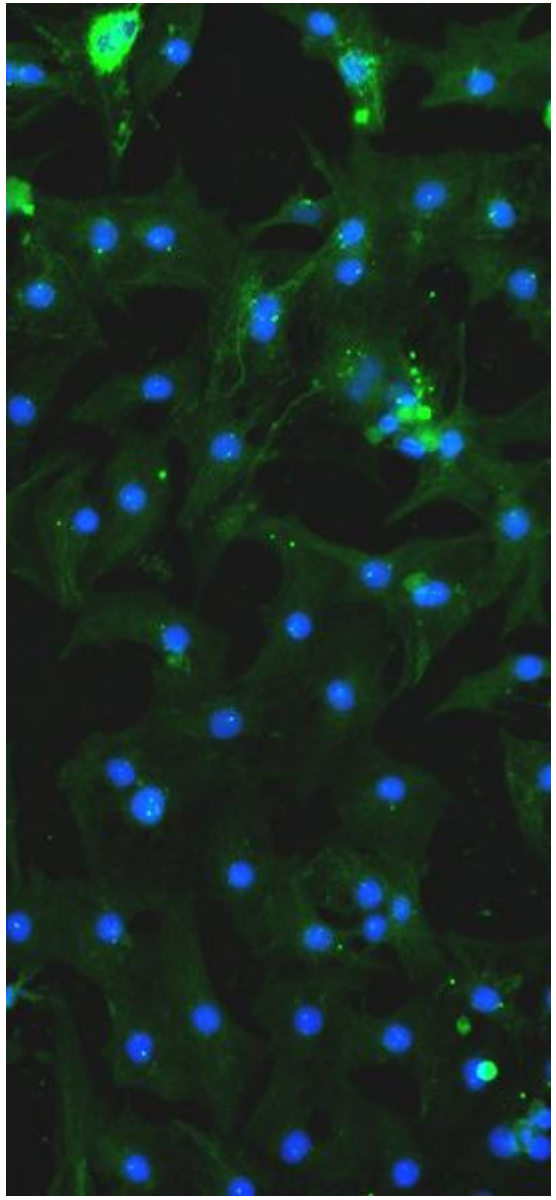
Figure S5G

Phenylephrine + CADO + A3 antag (MRS1191; 5μM) treated cardiomyocytes

α-Tubulin

Glu-Tubulin





α-Tubulin
Glu-Tubulin

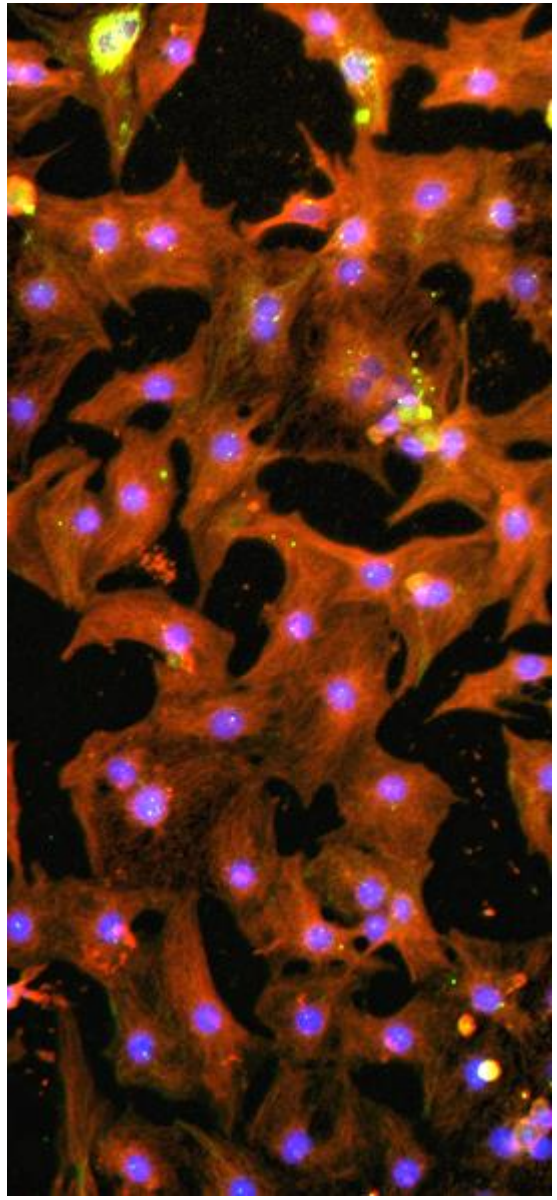
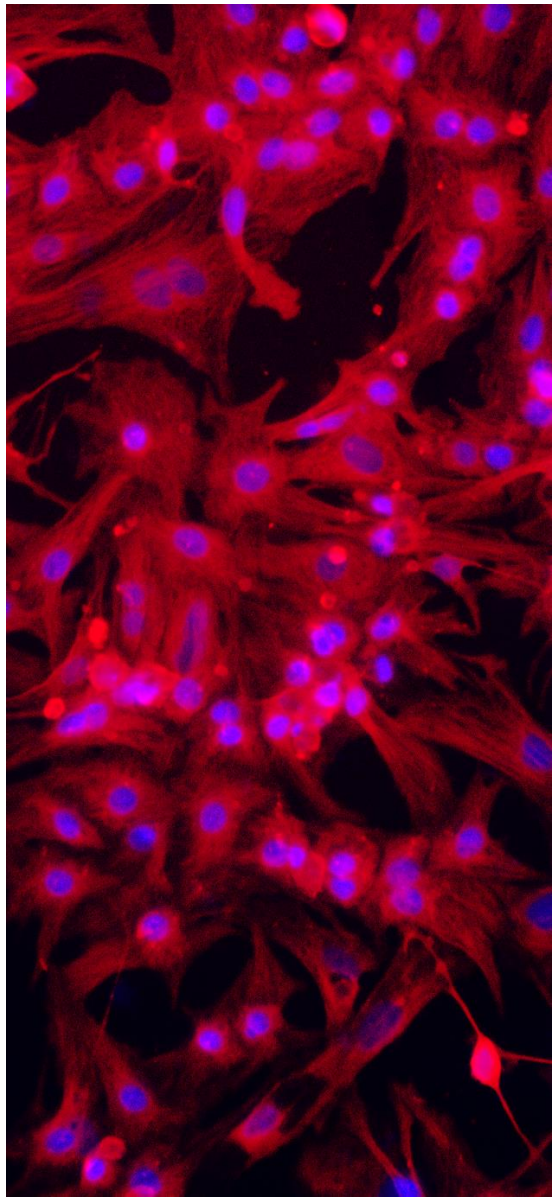


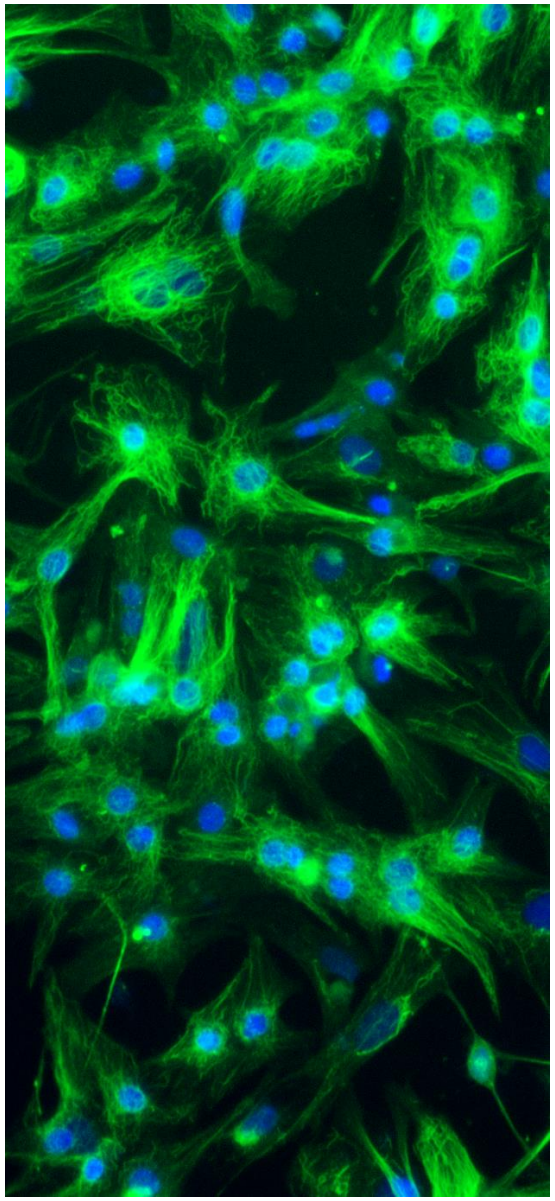
Figure S5H

Phenylephrine + CADO + ITU (0.3µM)

α-Tubulin



Glu-Tubulin



α-Tubulin

Glu-Tubulin

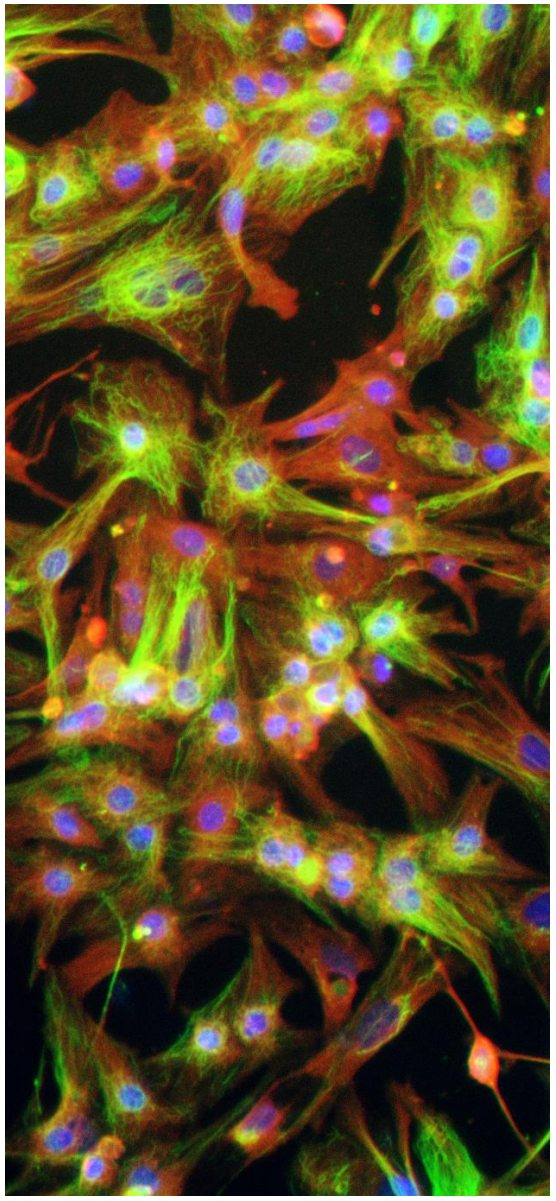


Figure S5 (A-H) Immunofluorescence of alpha tubulin (red) and detyrosinated tubulin (glu-tubulin, green) in NRVMs treated for 48 hours with vehicle**(A)**, PE (50µM) **(B)**, PE + CADO (5µM) **(C)**, PE+ CADO + A1 antagonist DPCPX (5µM) **(D)**, PE + CADO + A2A antagonist ZM-241,385 (5µM) **(E)**, PE + CADO + A2B antagonist MRS-1754 (5µM) **(F)**, PE + CADO + A3 antagonist MRS-1191 (5µM) **(G)**, or PE + CADO + iodotubercidin (ITU; 0.3µM) **(H)**.

Figure S6

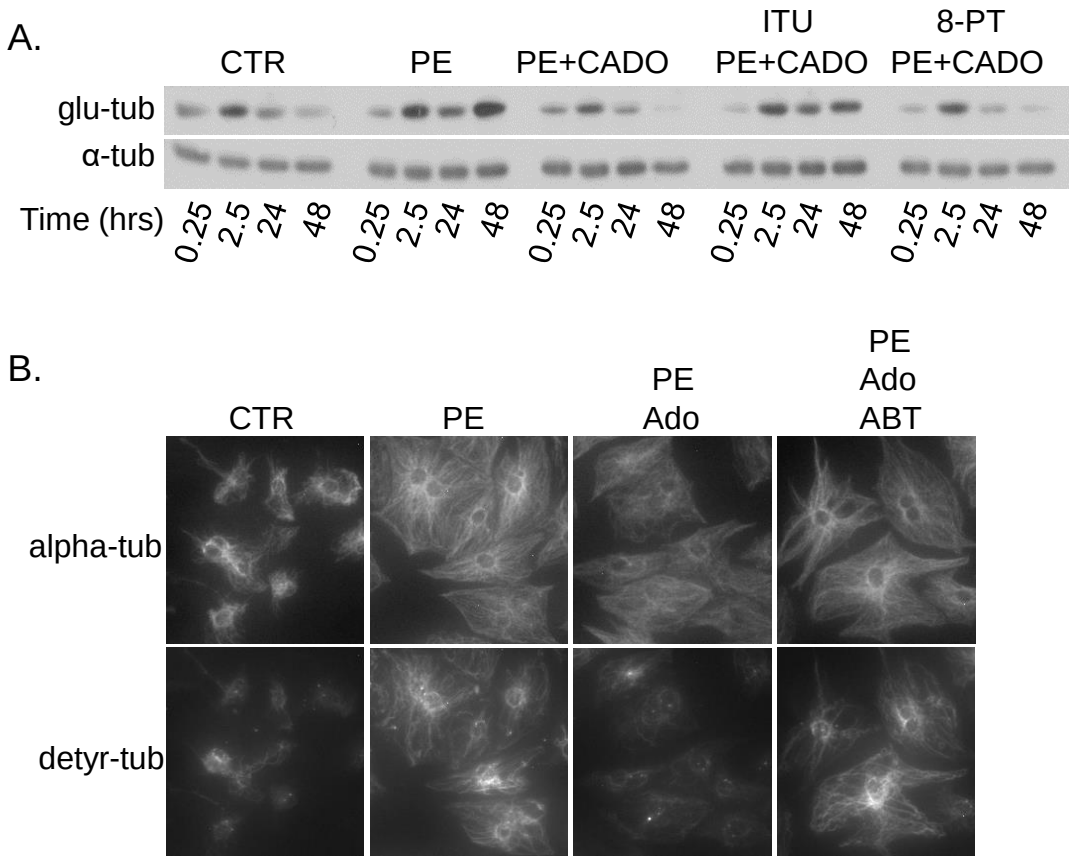


Figure S6 A) NRVMs treated with vehicle, PE (50μM), PE + CADO (5μM), PE + CADO + iodotubercidin (ITU; 0.3 μM), or PE + CADO + 8-PT (10 μM), and lysates were collected at the indicated time points and examined by western blot for α-tubulin or glu-tubulin. **B)** NRVMs were treated for 48 hours with vehicle, PE (50μM), PE + ADO (10μM) or PE+ ADO + ABT-702 (0.3 μM). Media was replaced after 24 hours with the same treatments. After 48 hours, cells were methanol fixed and microtubules were examined by immunofluorescent staining of α-tubulin and detyrosinated tubulin (glu-tubulin).