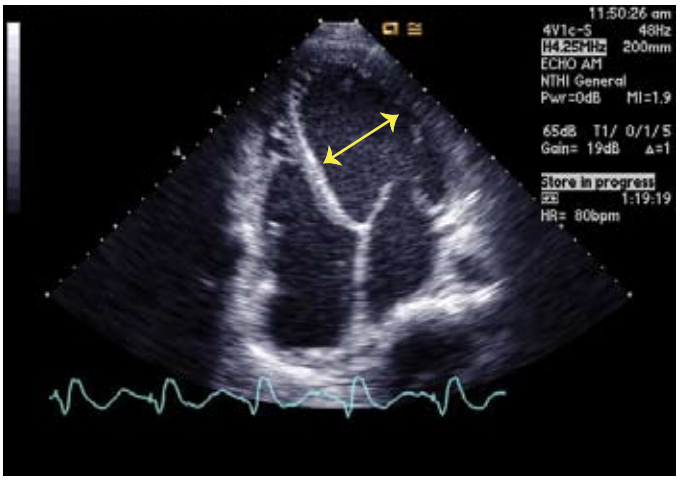
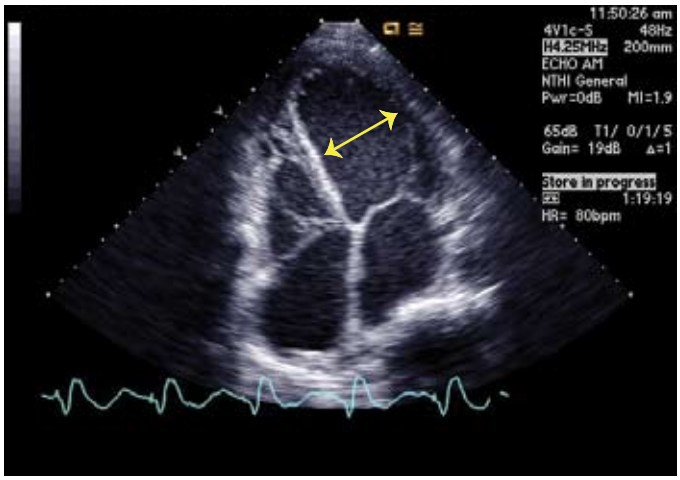
Failing Heart

Diastole

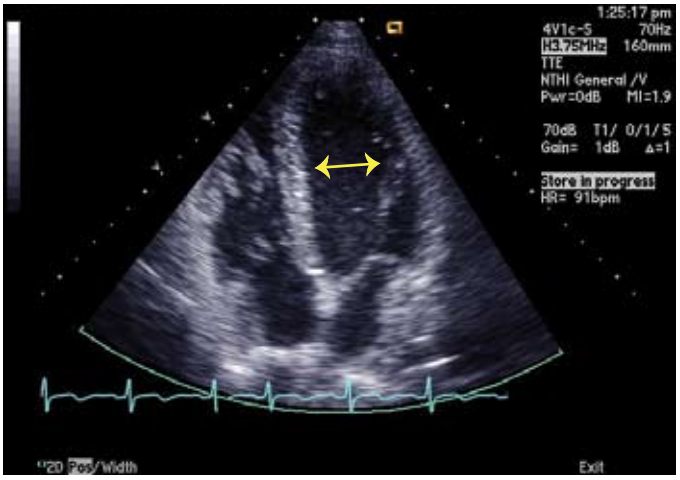


Systole

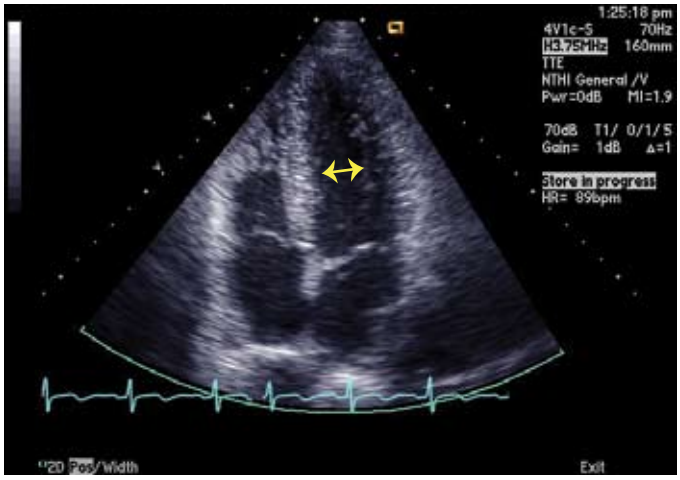


Non-Failing Heart

Diastole

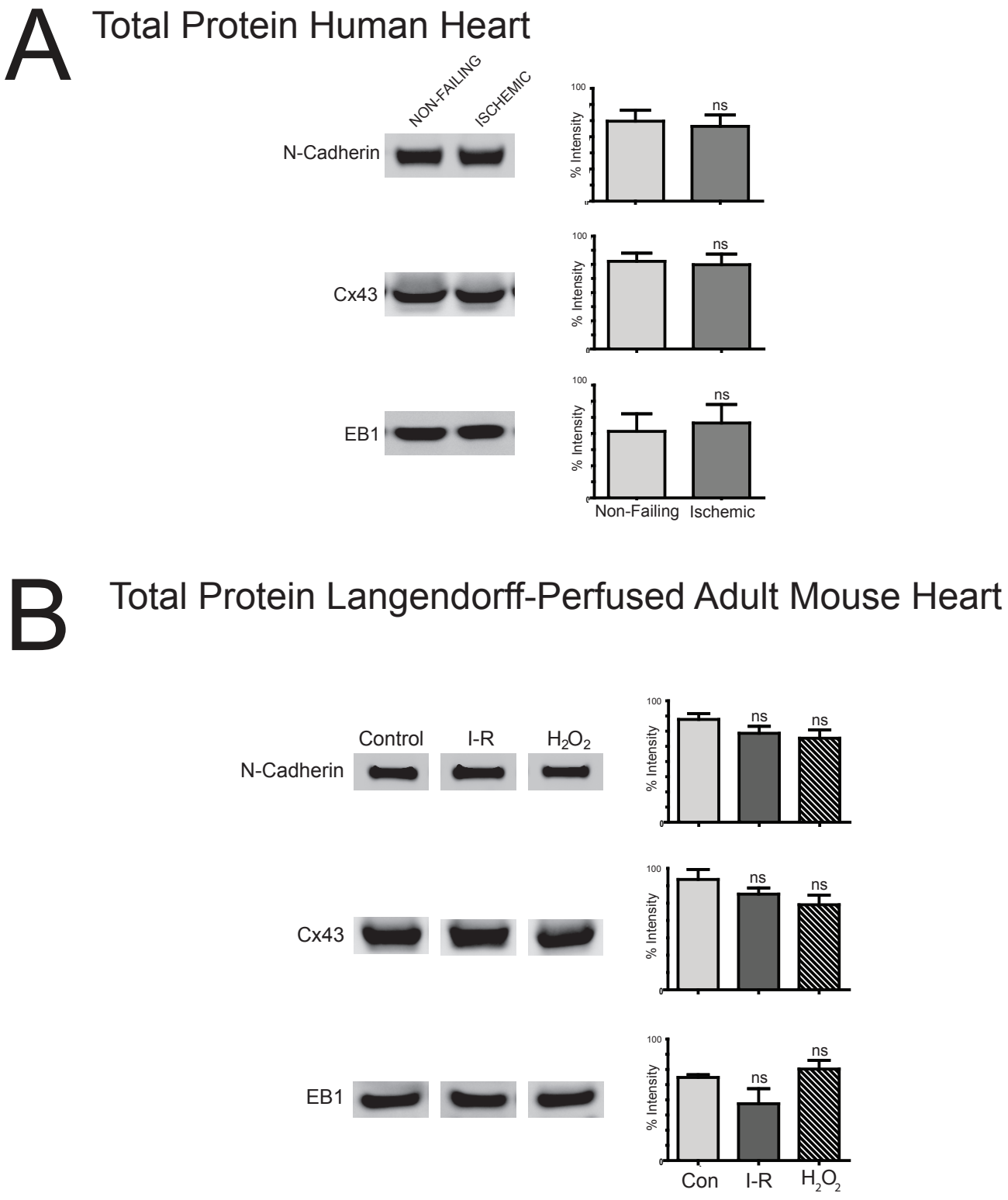


Systole



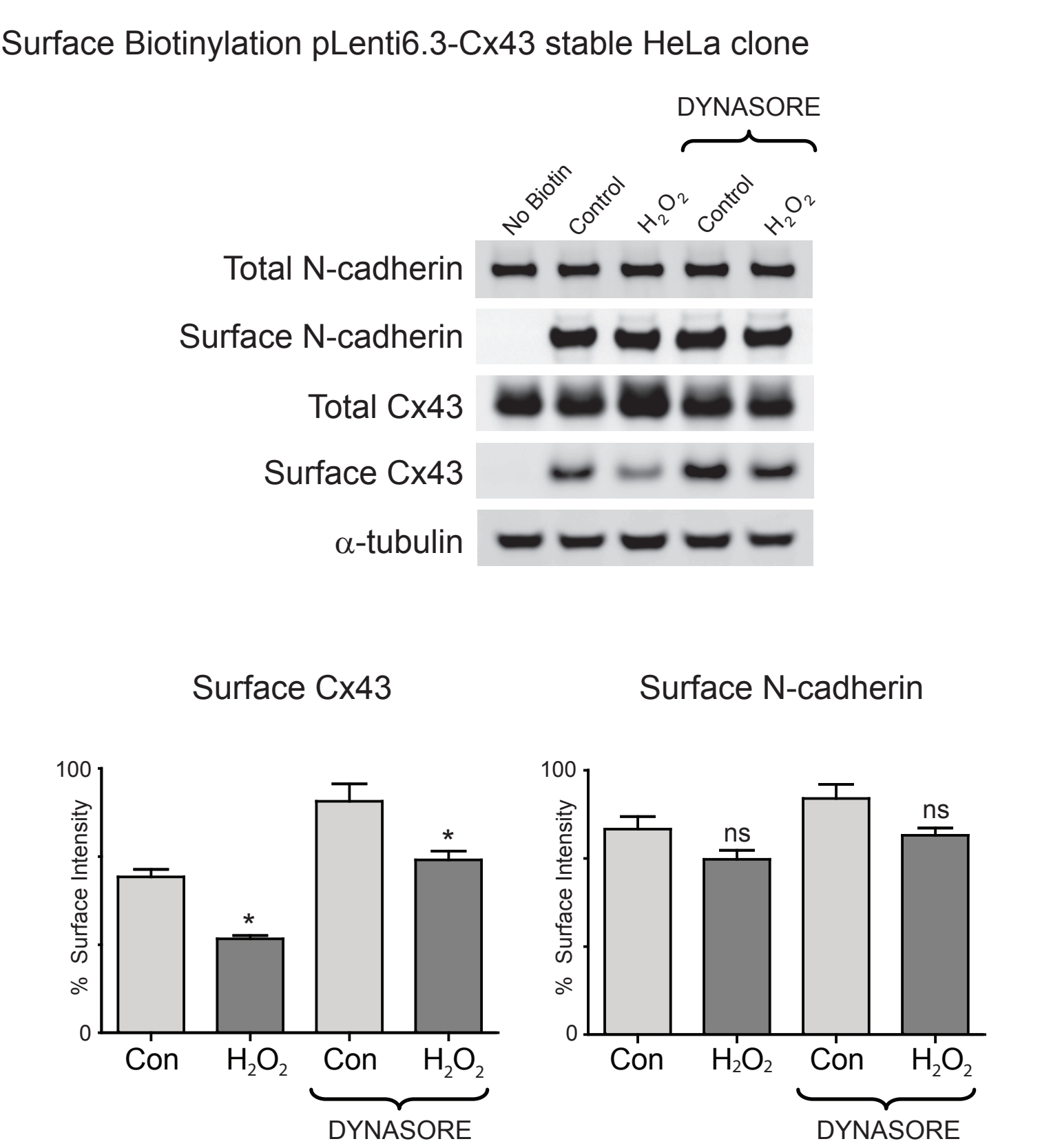
Supplemental Figure 1: Echocardiogram of failing and non-failing human hearts prior to explantation

Apical four chamber views during end-diastole (left column) and end-systole (right column) of failing and non-failing hearts prior to explantation. Left ventricular diameters indicated by arrows. Note full scale on the failing heart is 20 cm whereas full scale on the non-failing heart is 16 cm. Failing heart left ventricular end-diastolic volume index (LVEDVI) was 127 ml/m2 and left ventricular end-systolic volume index (LVESVI) was 103 ml/m2, respectively. Non-failing heart LVEDVI and LVESVI were 34 ml/m2 and 10 ml/m2 respectively.



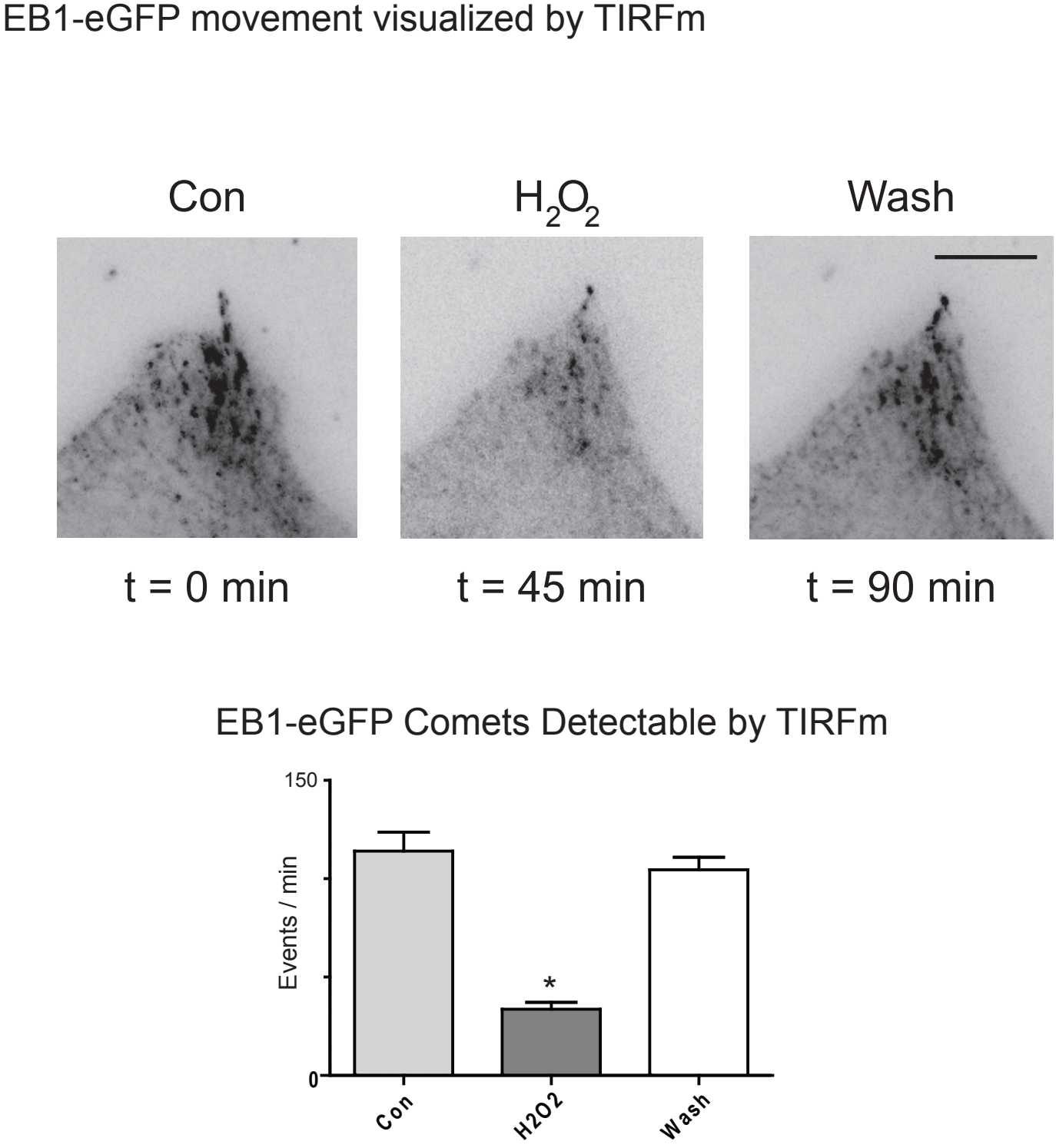
Supplemental Figure 2: Total protein levels of N-cadherin, Cx43, and EB1 in explanted human hearts and Langendorff-perfused adult mouse hearts

Ventricular tissue was weighed and homogenized in RIPA buffer before analysis by Western blot. Quantification was performed using Quantity One software (human tissue n=4; mouse tissue n=3) and statistical analysis performed using a one-way ANOVA with Bonferroni post-test. Human data is presented in (A) and mouse in (B). Error bars represent mean +/- SEM.



Supplemental Figure 3: Surface levels of Cx43 but not N-cadherin are reduced in the presence of oxidative stress with or without endocytosis inhibition

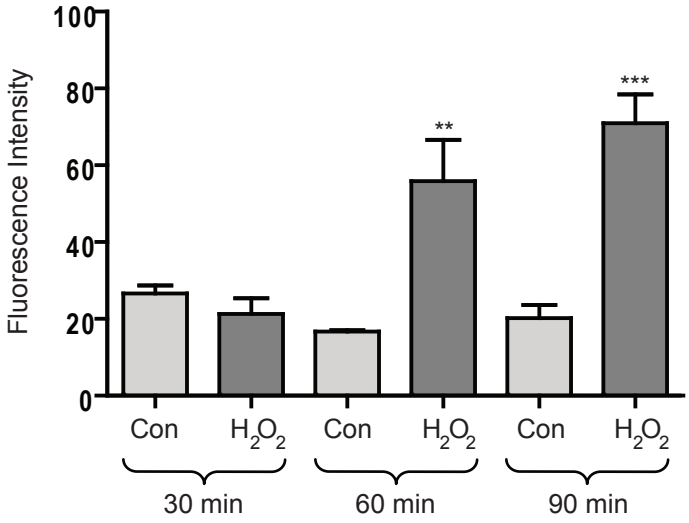
A stable HeLa cell line expressing Cx43 was incubated for 16 h in the presence or absence of 200 μM H_2O_2 prior to surface protein biotinylation and pulldown. Western blot analysis of input lysates and pulldowns of biotinylated surface proteins subjected to SDS-PAGE on the same gel. Endocytosis was inhibited using 80 μM Dynasore. Quantification was undertaken using Quantity One software (n=3) and statistical analysis performed using a one-way ANOVA with Bonferroni post-test. Error bars represent mean +/- SEM, $*P < 0.05$



Supplemental Figure 4: Oxidative stress reversibly reduces numbers of EB1-tipped microtubules approaching the plasma membrane in neonatal mouse ventricular myocytes

Neonatal mouse ventricular myocytes were transduced with lentivirus encoding EB1-eGFP and cultured for 72 h prior to imaging by TIRFm for 2 min. Images are projections of number of events over the full timecourse before, during (45 min), and after (45 min) exposure to 200 μM H_2O_2. Original magnification of images, x100. Scale bars: 10 μm. Data are representative of three separate experiments and statistical analysis was performed using a one-way ANOVA with Bonferroni post test. Error bars represent mean +/- SEM *$P < 0.05$

Dihydroethidium ROS assay



Supplemental Figure 5: Reactive oxygen species are induced in HeLa cells within 60 min exposure to hydrogen peroxide as detected by Dihydroethidium

HeLa cells were plated on glass-bottomed dishes and exposed to 200 μM H_2O_2 for the times indicated above. Dihydroethidium (DHE) was added 30 min prior to fluorescent microscopy analysis. Average nuclear red fluorescence intensities were determined and presented in the bar chart above. For statistical analysis a one-way ANOVA with Bonferroni post-test was employed. Error bars represent mean +/- SEM, $^{**}P < 0.01$, $^{***}P < 0.0001$

Parameters of Microtubule Dynamic Instability

	Control	+/- SEM	H_2O_2	+/- SEM
Mean Growth Rate (nm/sec)	208.8	11.04	75.14	9.02
Mean Shortening Rate (nm/sec)	-249.6	36.70	-131.20	25.30
Catastrophe Freq (s^{-1})	0.06		0.12	
Rescue Freq (s^{-1})	0.11		0.09	
% Time spent growing	58.80		33.54	
% Time spent shortening	33.33		46.14	
% Time spent pausing	7.91		20.33	

Supplemental Table 1: Parameters of perturbed microtubule dynamics in the presence or absence of oxidative stress

HeLa cells were transfected with α-tubulin-eGFP and spinning disk confocal microscopy was employed to capture 5 min timecourses 18 h post transfection. H_2O_2 was added to a final concentration of 200 μM 45 min prior to imaging where appropriate. Individual microtubules were tracked using ImageJ and the MTrackJ plugin.