



Supplementary Figure 3. Postfixation incubation of NRCMs in media causes failure of NRCM nuclei to stain with Topro. (A) NRCM culture fixed and incubated in 4% PFA and stained for α -sarcomeric actinin (red) and Topro (teal). GFP fluorescence was scanned as a control (green). All NRCMs are Topro positive. (B) NRCM culture fixed in 4% PFA then incubated in media for 6 days at 37°C and stained for α -sarcomeric actinin (red) and Topro (teal). GFP fluorescence was scanned as a control (green). None of the NRCMs are Topro positive. (C) NRCM culture fixed in 4% PFA then incubated in media with GFP plus CSCs for 6 days at 37°C then stained for α -sarcomeric actinin (red) and Topro (teal). GFP fluorescence was scanned (green). None of the GFP-negative NRCMs are Topro positive (white arrows) and all of the GFP positive CSCs are Topro positive (yellow arrows). CSC: Cardiac stem cell; GFP: Green fluorescent protein; NRCM: Neonatal rat cardiomyocyte; PFA: Paraformaldehyde.