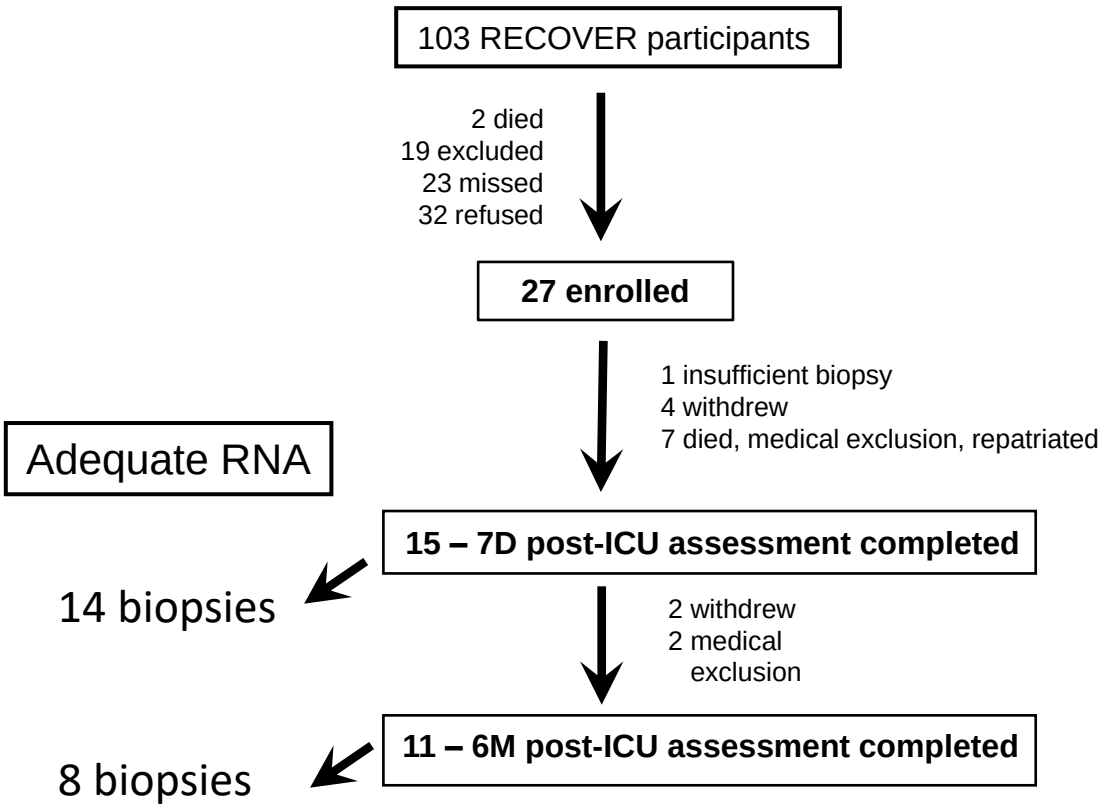
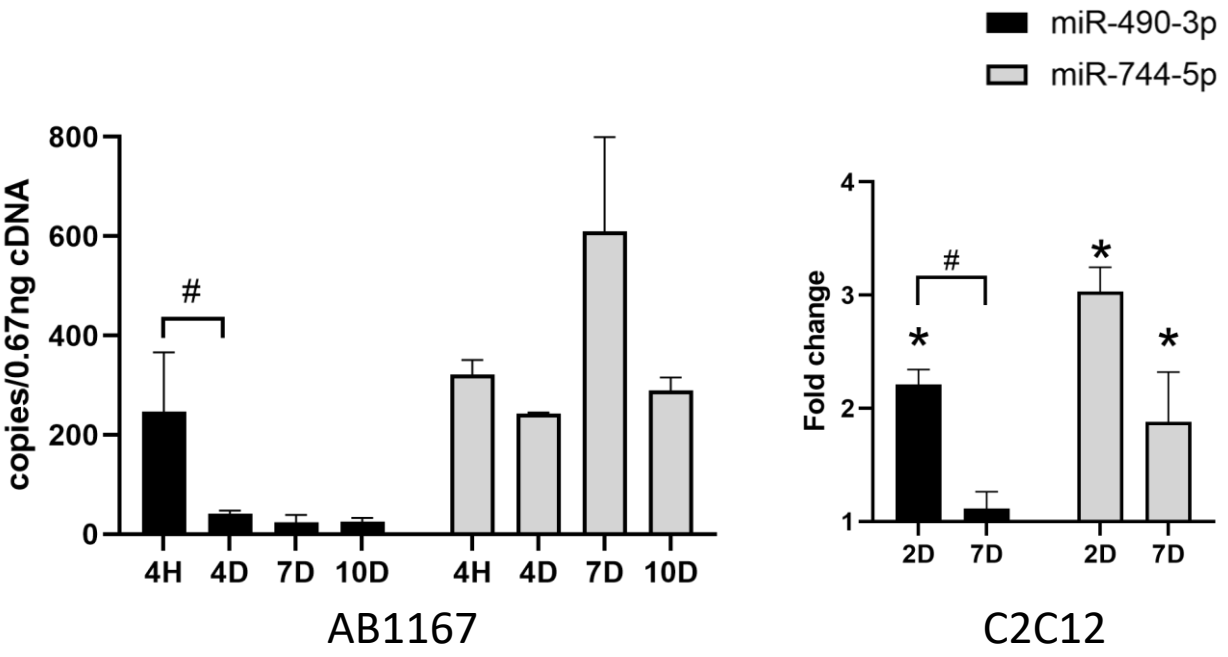
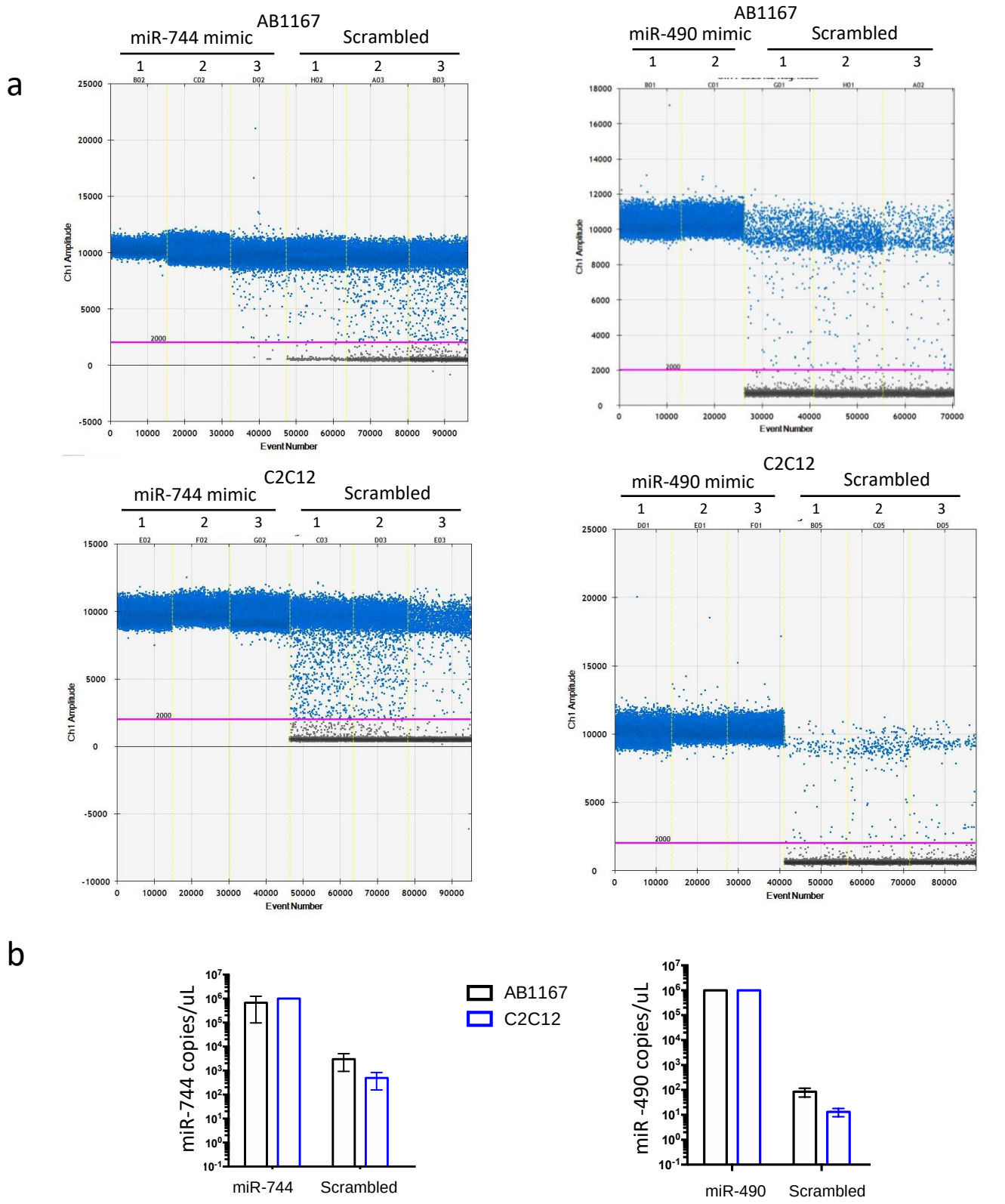


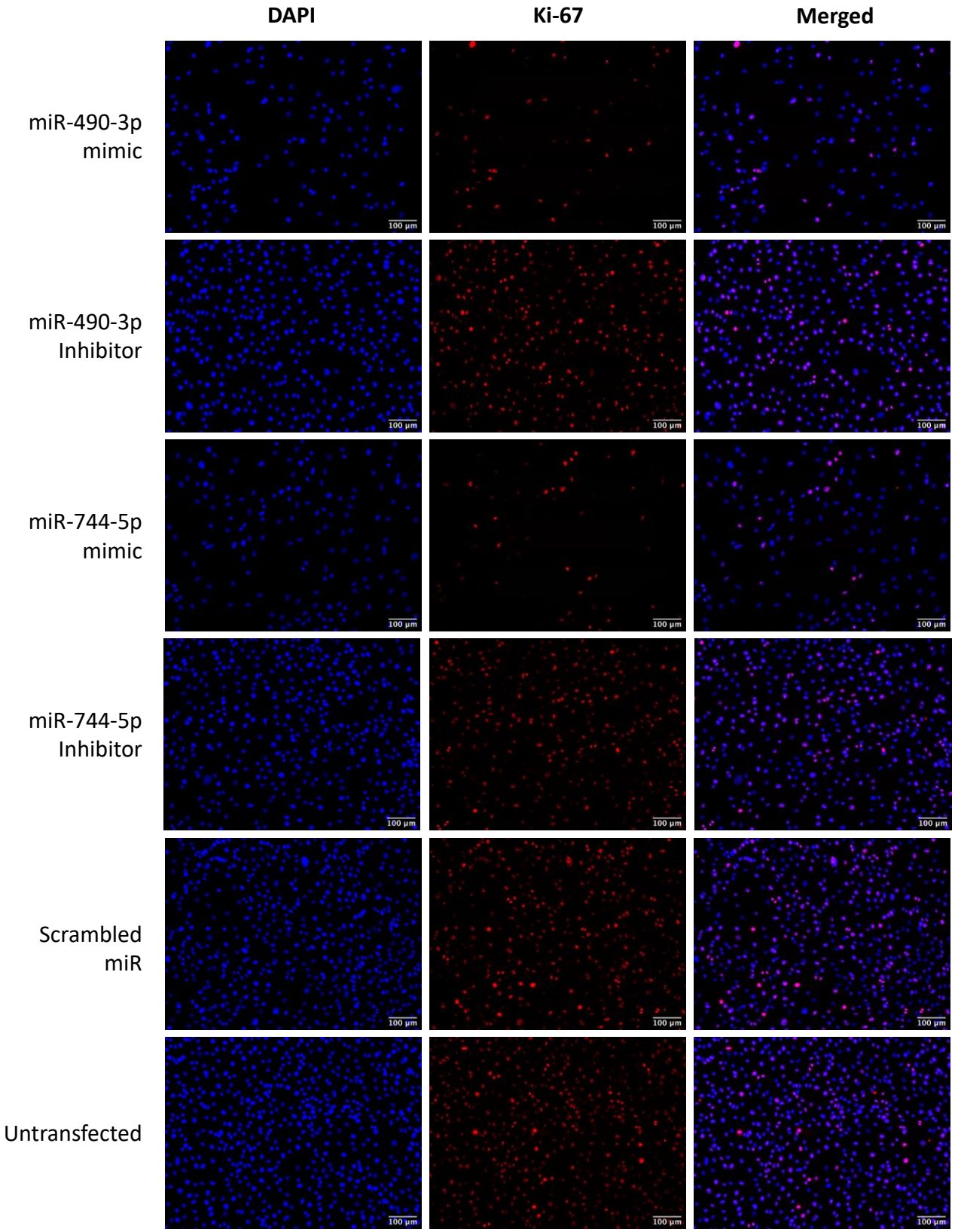
Supplementary Figure 1. ***Flow diagram of participant recruitment and study completion.*** Of 103 RECOVER trial patients recruited in 1 trauma and 2 medical-surgical ICUs at the University Health Network (UHN) and St. Michaels Hospital (SMH) Toronto, Canada, 59 eligible patients were invited to participate in the study. 27 patients consented to study enrollment. 12 patients died, were repatriated to another institution or withdrew prior to the 7D post-ICU assessment. 15 and 11 patients completed the 7D and 6M post-ICU analyses respectively. One muscle biopsy obtained at 7D and three taken at 6M did not provide sufficient RNA for miR expression profiling and were therefore excluded from the analysis. (7D = 7 days, 6M = 6 months)



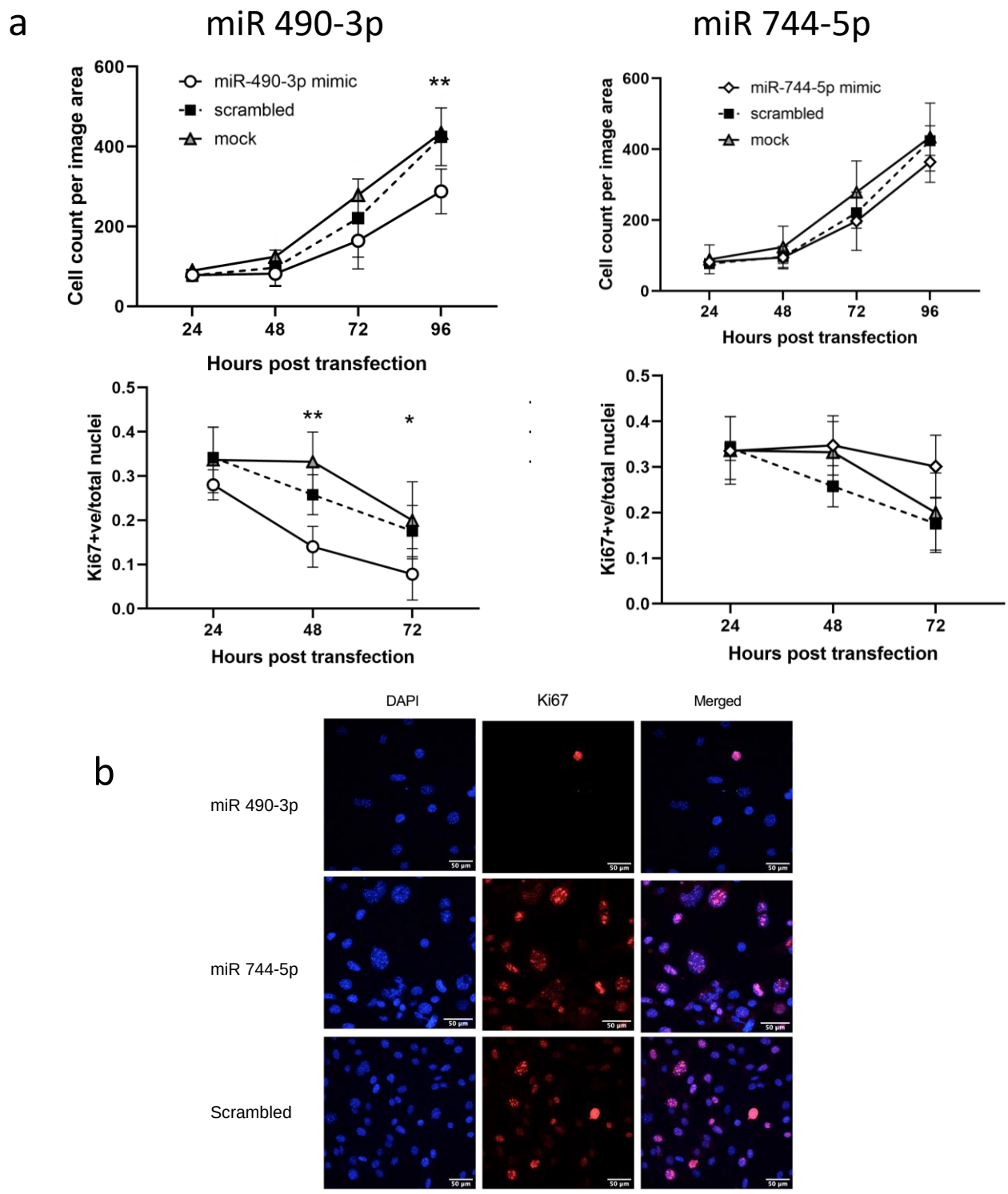
Supplementary Figure 2. ***miR-490-3p and mir-744-5p endogenous expression during myoblast proliferation and differentiation.*** Absolute copy number of miRs 490-3p and 744-5p in AB1167 human myoblasts (left panel) as determined by droplet digital PCR (ddPCR) and relative expression in C2C12 murine myoblasts (right panel) as determined by qPCR at serial time points post plating. C2C12 miR expression was calculated as fold change ($2^{-\Delta\Delta CT}$ relative quantification method) between time points proliferation; baseline – 2 days (D) and differentiation; 2D-7D, relative to geometric mean of two reference RNA [snoRNA234 and RNU6b]. MiR-490-3p was expressed in early proliferative AB1167 and C2C12 myoblasts (4 hours [H], 2D) but then decreased with progression to differentiation (4D, 7D, 10D). miR-744-5p expression was consistent across all time points with no significant changes as cells moved from highly proliferative at 4H to fully differentiated at 7D and 10D post plating. (#$p<0.05$ between time points; *$p<0.05$ relative fold change; n= minimum of 4 experiments/cell line; cells plated in triplicate/experimental condition)



Supplementary Figure 3. ***Digital Droplet PCR (ddPCR) to demonstrate myoblast transfection***. To confirm miR transfection for all experiments, isolated RNA from miR-transfected (miR-490-3p or miR-744-5p) and control (scrambled) myoblast cultures was assayed using Taqman-based ddPCR, providing absolute quantitation. (a) ddPCR visual report for miR-744-5p (left panels) and miR-490-3p (right panels) in AB1167 (upper panels) and C2C12 (lower panels) transfected myoblasts 48 hours post transfection and (b) miR copy number. Representative results are shown. miR expression was maintained for the duration of experiments (data not shown).

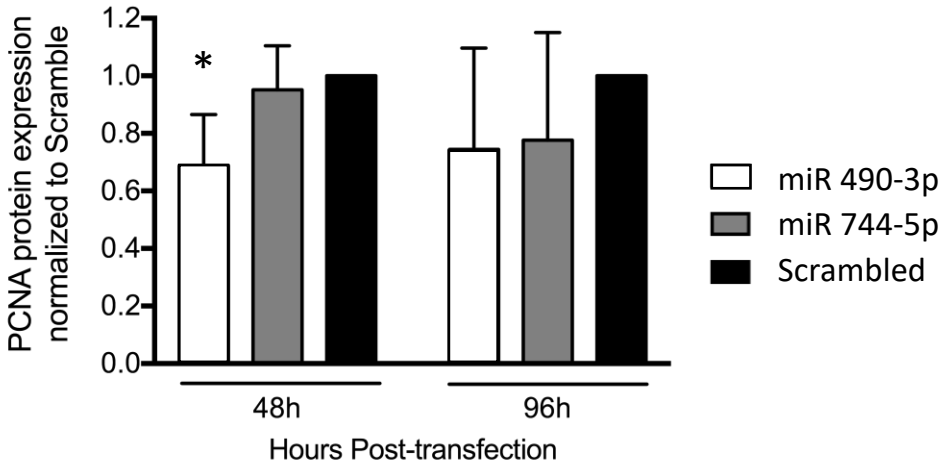


Supplementary Figure 4. ***miRs 490-3p and 744-5p are negative regulators of AB1167 human myoblast proliferation*****.** Representative confocal images of immunostaining for proliferation antigen Ki67 (red) in AB1167 myoblasts transfected with miR-490-3p mimic, miR-490-3p inhibitor, miR-744-5p mimic, miR-744-5p inhibitor, or scrambled negative control and untransfected cells, 72 hours post-transfection. Nuclei are stained with Dapi (blue). Image quantification is shown in Figure 3.

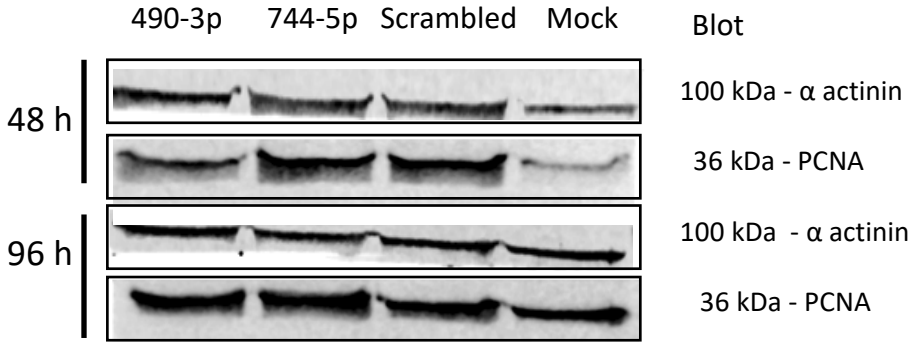


Supplementary Figure 5. ***miR-490-3p overexpression significantly reduces C2C12 myoblast proliferation*****.** (a) C2C12 myoblast counts and proportion of Ki67–positive nuclei at 24h (n=4), 48h (n=6), 72h (n=4) and 96h (n=4) post transfection with miR 490-3p (left panels), miR 744-5p (right panels) and negative controls (scrambled miR, mock transfection). (B) Representative confocal images of immunostaining for DAPI (blue) and proliferation antigen Ki67 (red) in C2C12 myoblasts transfected with miR 490-3p, miR 744-5p or scrambled miR at 48 hours post plating. miR-490-3p overexpression inhibits C2C12 myoblast proliferation compared to scrambled control, but miR-744-5p overexpression has no effect on C2C12 cells. (Data are mean +/-SD *p<0.05; **, p<0.01. Cells plated in triplicate/experimental condition).

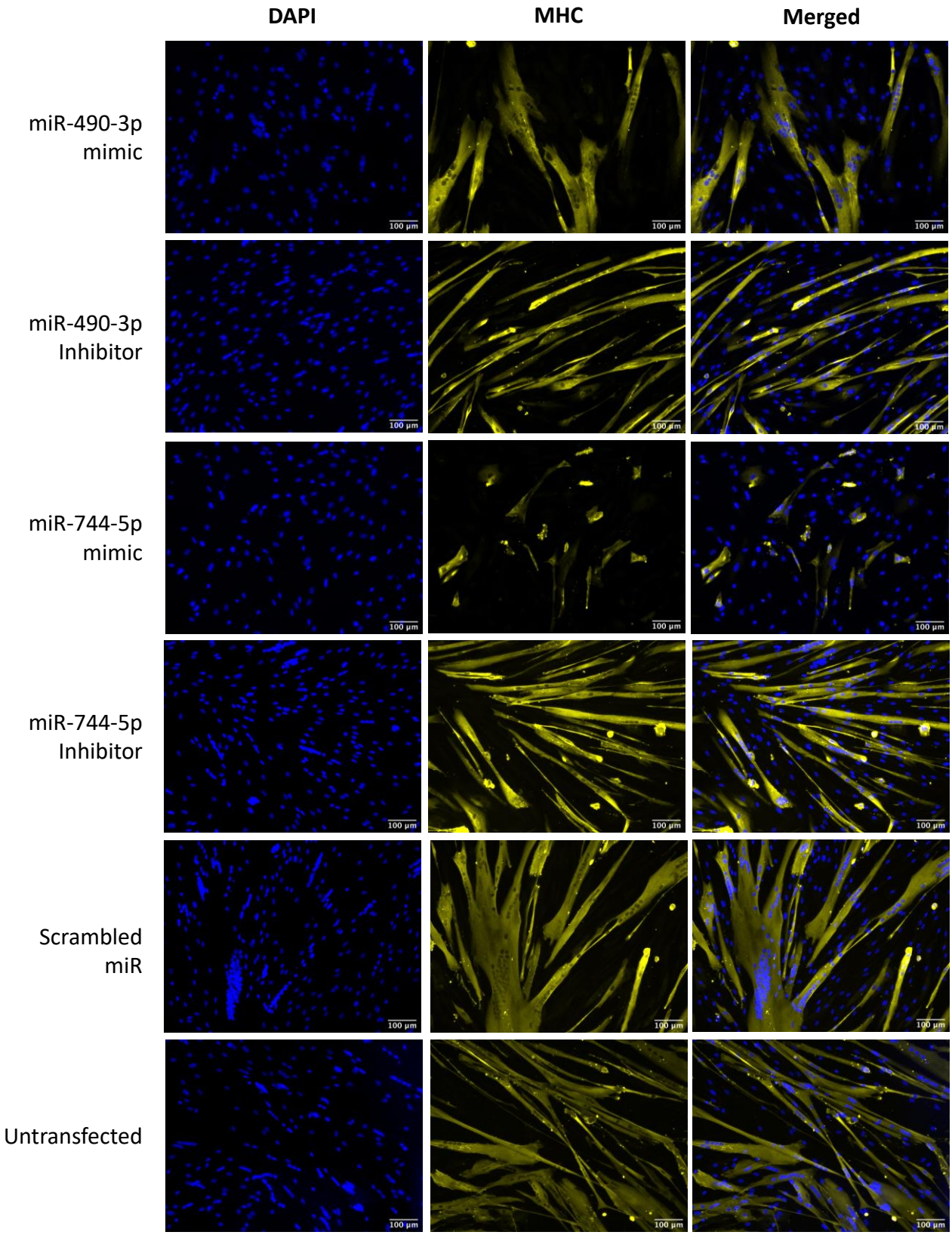
a



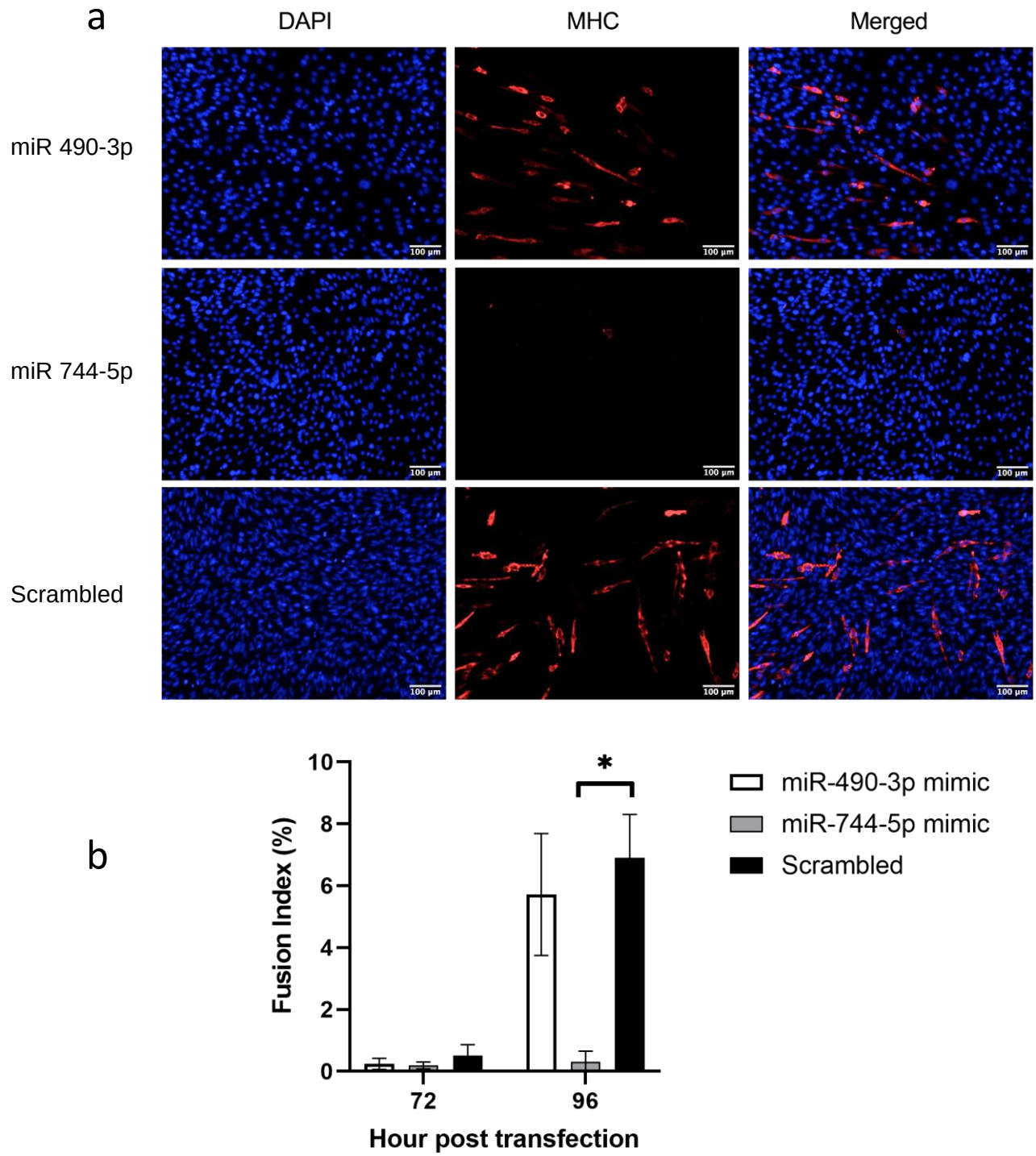
b



Supplementary Figure 6. ***miR 490-3p overexpression significantly reduces C2C12 myoblast proliferation.*** C2C12 myoblasts were transfected with miR-490-3p or miR-744-5p and scrambled negative control. 48 (n=3) or 96 (n=3) hours post transfection protein lysates were separated by SDS-PAGE, transferred to nitrocellulose and immunoblotted for proliferation cell nuclear antigen (PCNA) and α-actinin (loading control). (a) Images were acquired and Western blot band intensities quantified with Biorad GelDoc Easy Imager and ImageLabs software. PCNA expression was normalized to α-actinin for each miR treatment. Fold change of miR 490-3p and miR 744-5p transfected myoblasts was expressed relative to scrambled miR. (b) Representative Western Blot at 48h post-transfection. miR-490-3p constitutive expression inhibits C2C12 myoblast proliferation, but miR-744-5p overexpression has no effect. (Data are mean +/- SEM, *p<0.05. Cells plated in triplicate/experimental condition).



Supplementary Figure 7. ***miR-744-5p is a negative regulator of AB1167 human myoblast differentiation.*** Representative immunofluorescence images of miR-490-3p mimic, miR-490-3p inhibitor, miR-744-5p mimic, miR-744-5p inhibitor, or scramble-miR transfected and untransfected AB1167 myoblasts at 8 days post-transfection, immunostained for Myosin Heavy Chain (MHC, yellow) and DAPI (blue to indicate nuclei) to detect differentiation and fusion to myotubes. Image quantification is shown in Figure 4. MiR-744-5p negatively regulates myoblast differentiation, but miR-490-3p has no effect.



Supplementary Figure 8*. miR 744-5p overexpression decreases C2C12 differentiation*
(a) Representative images of miR-490-3p, miR-744-5p, and scrambled miR transfected C2C12 cells at 96h post-transfection, stained for myosin heavy chain (MHC, red) and DAPI (blue, nuclei) to detect myoblasts differentiating to myotubes. (b) Fusion Index at 72 (n=3) and 96 (n =3) hours post-transfection. MiR-744-5p significantly reduced the fusion index compare to scrambled control. Mir-490-3p constitutive expression had no effect. (Fusion index = percentage of nuclei in fused myotubes [MHC positive cells with 2 or more nuclei] relative to the total number of nuclei/image. Data are mean +/- SD, *P<0.05. Cells are plated in triplicate/experimental condition)