Title: Intercellular adhesion molecule-1 augments myoblast adhesion and fusion through homophilic *trans*-interactions

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Supplemental Figures

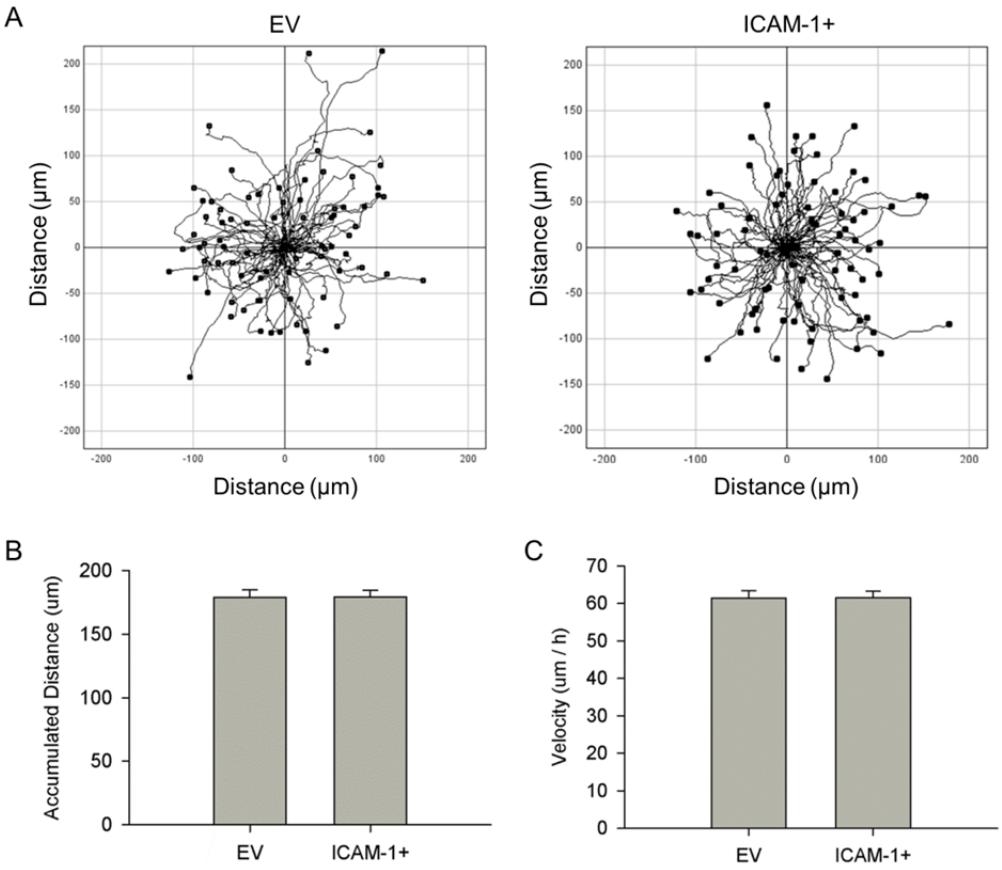

Figure S1. Myoblast motility. A) Migratory paths of EV and ICAM-1+ myoblasts for 3 h at 1 d of differentiation. B and C) Accumulated distance (B) traveled and velocity of movement (C) for EV and ICAM-1+ myoblasts. n=80 myoblasts per cell line in 4 independent experiments.

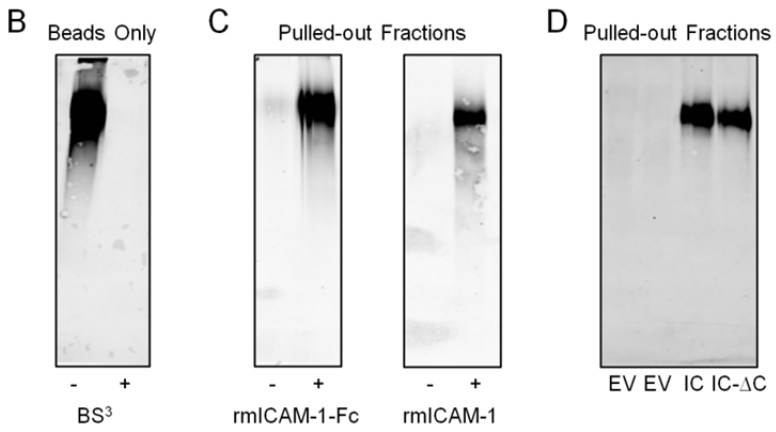

Figure S2. Western blot for ICAM-1 in beads coated with rmICAM-1-Fc. Images depict the entire lane (~200-25 kDa) for samples presented in Figure 1B, C and D. The ICAM-1 band appeared at ~110 kDa. B) BS^3 was used to covalently link rmICAM-1-Fc to beads. C) Beads crosslinked with ICAM-1 were incubated with PBS-T, rmICAM-1-Fc or rmICAM-1. ICAM-1 was detected in pulled-out fractions via western blotting. D) Western blots for ICAM-1 in pulled-out fractions of EV, ICAM-1+ (IC), and ICAM-1-ΔC (IC-ΔC) myoblasts.

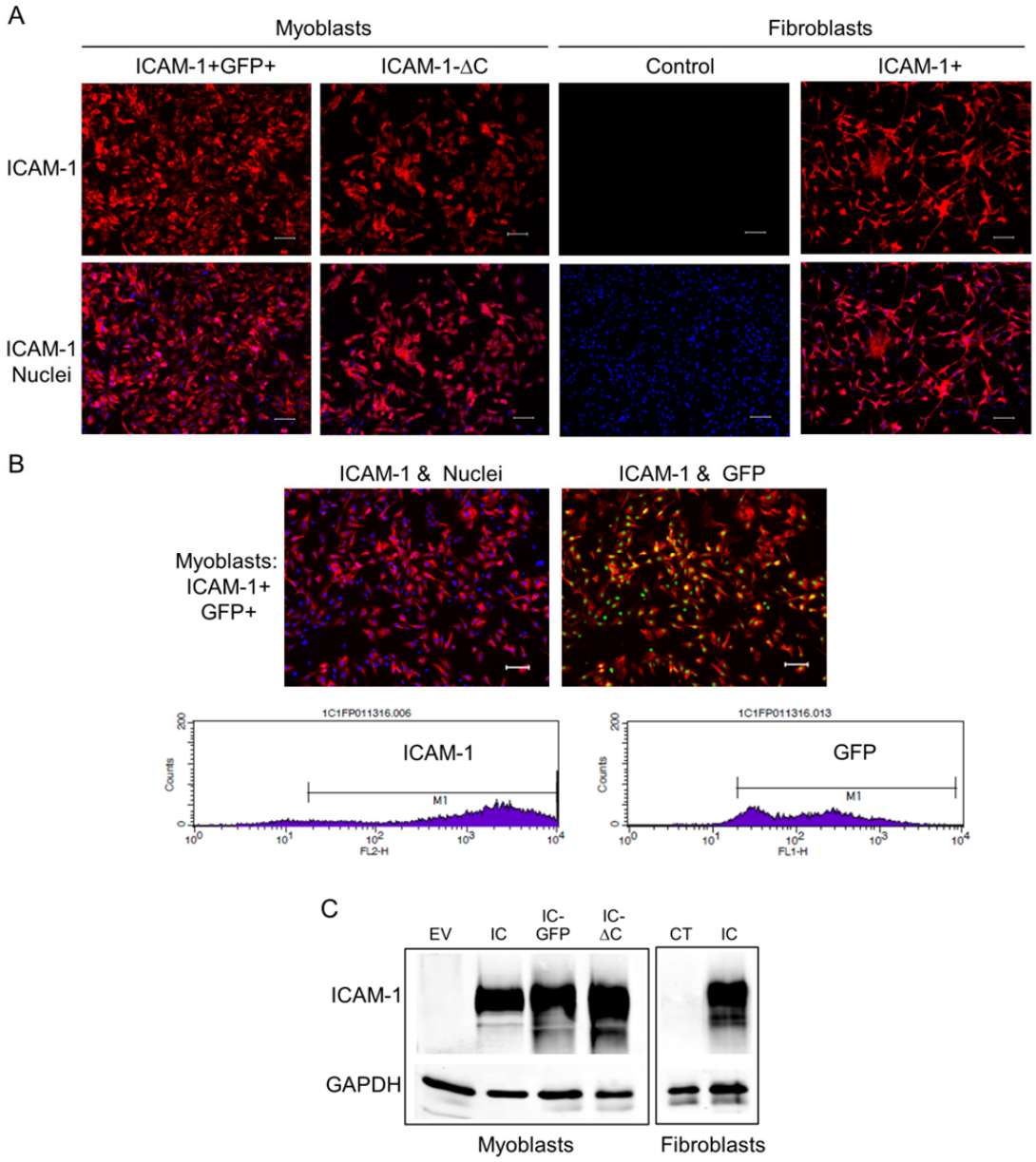

Figure S3. Transfection efficiency. A) Representative images of ICAM-1 (red) and nuclei (blue) in ICAM-1+GFP+ and ICAM-1-ΔC myoblasts, as well as control fibroblasts and ICAM-1+ fibroblasts (Scale bar = 100 μm). B) Representative images of GFP (green) and nuclei (blue) in ICAM-1+GFP+ myoblasts. Quantitative analysis revealed that 90-95% of ICAM-1+GFP+ myoblasts had nuclear localization of GFP (Scale bar = 100 μm). C) Representative western blot for ICAM-1 and GAPDH (loading control) in cell lines of myoblasts and fibroblasts. EV = empty vector, IC = ICAM-1+, IC-GFP = ICAM-1+nucGFP+, IC-ΔC = ICAM-1-ΔC, and CT = control fibroblasts.

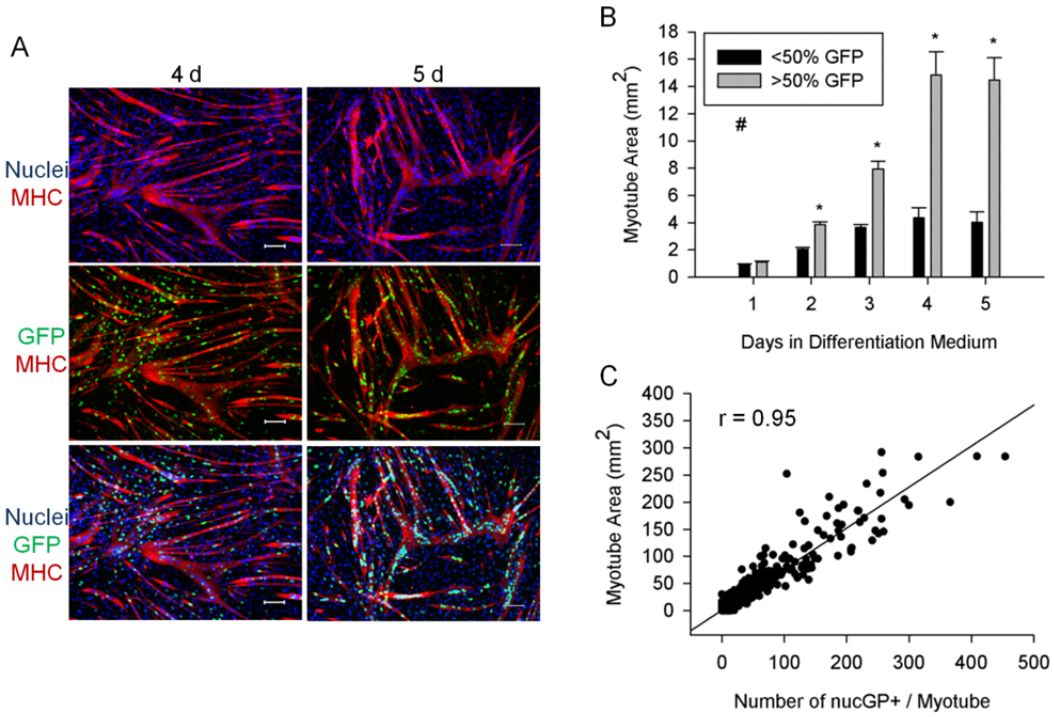

Figure S4. ICAM-1-ICAM-1 interactions in myotube hypertrophy. EV and ICAM-1+nucGFP+ myoblasts were mixed in equal number, and myotube area was quantified through 5 d of differentiation. A) Images of DAPI+ nuclei (blue) of EV and ICAM-1+nucGFP+ myoblasts, MHC (red), and nuclei of ICAM-1+nucGFP+ myoblasts (green) at 4 and 5 d of differentiation (scale bar = 100 μm). B) Quantitative analysis of myotube area for myotubes that contained nuclei primarily from ICAM-1+nucGFP+ (>50% GFP+; n=2827) or EV myoblasts (≤50% GFP+; n= 2944). # = higher for >50% GFP+ compared to ≤50% GFP+ throughout 5 d of differentiation (main effect for cell line; $p < 0.001$). * = higher for >50% GFP+ compared to ≤50% GFP+ at indicated day of differentiation (interaction effect; $p < 0.001$). n =4-6 replicates at each day of differentiation. C) Scatter plot of the number of number of GFP+ nuclei within a hybrid myotube at 2-5 d of differentiation vs. myotube area (n=5337 myotubes). A high Pearson-product moment correlation was observed ($r=0.95$; $p<0.001$).

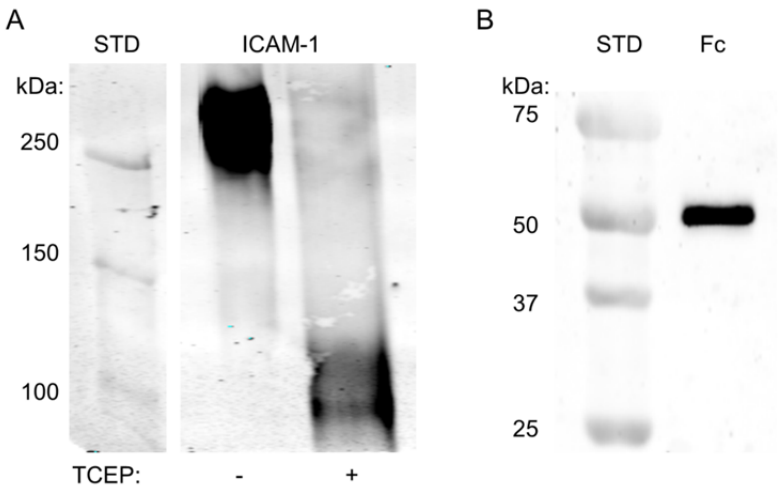

Figure S5. Wells coated with rmICAM-1-Fc and rhIgG1-Fc. A) Representative western blot for ICAM-1 in wells coated with rmICAM-1-Fc. rmICAM-1-Fc bound to wells was collected in non-reducing or reducing sample buffer, with TCEP serving as the reducing agent. Reducing conditions resulted in a large band shift in ICAM-1, demonstrating the dimeric state of rmICAM-1-Fc. B) Representative western blot for Fc in wells coated rhIgG1-Fc. Wells coated with rhIgG1-Fc were collected in non-reducing sample buffer.