Supplementary Material

Arsenic Directs Stem Cell Fate by Imparting Notch Signaling into the Extracellular Matrix Niche

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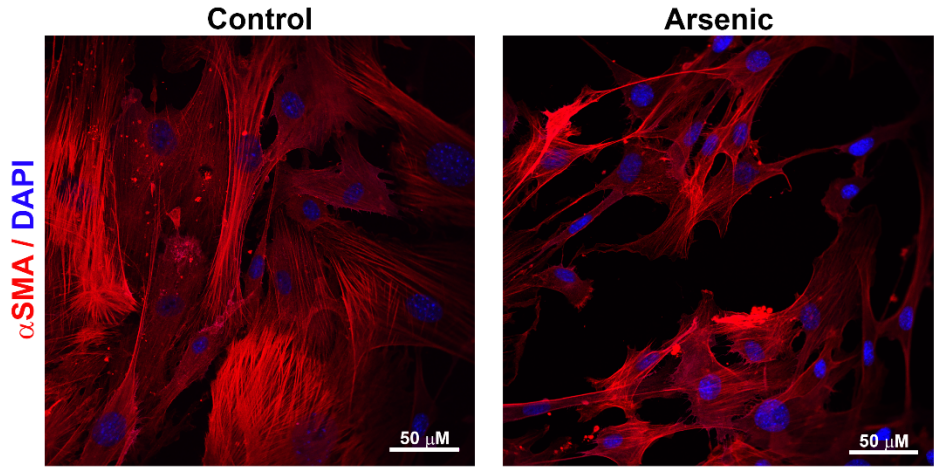
Supplementary Information:

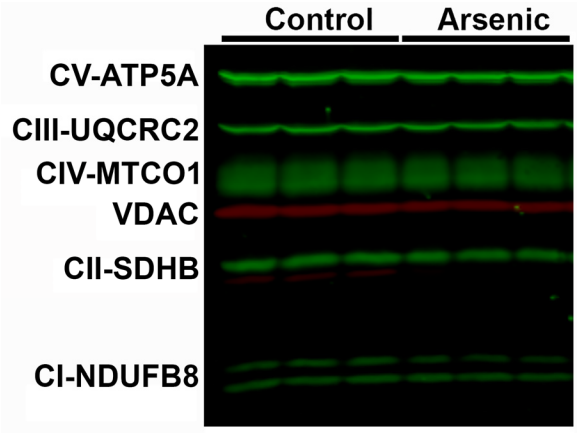
Supplemental Table 1: Antibodies used for imaging, functional assay, and immunoblotting

Primary Antibody	Host	Company	Catalogue No.	Dilution
Anti-Actin, α-Smooth Muscle	Mouse	Sigma	A2547	1:250
CD34 Antibody [EP373Y]	Rabbit	Abcam	ab81289	1:100
Anti-Desmin	Rabbit	Abcam	ab15200	1:1000
Anti-DLL4	Rabbit	Abcam	Ab7280	1:500
DLL4 blocking antibody	American hamster	eBiosciences / ThermoFisher	16-5948-82	1:100
Anti-PDGFR alpha [16A1]	Mouse	Abcam	ab96569	1:200
PDGF Receptor α D1E1E XP(R)	Rabbit	Cell Signaling	3174S	1:250
MyoD (G-1)	Mouse	Santa-Cruz	sc377460	1:800
Anti-activated Notch1	Rabbit	Abcam	Ab52301	1:200

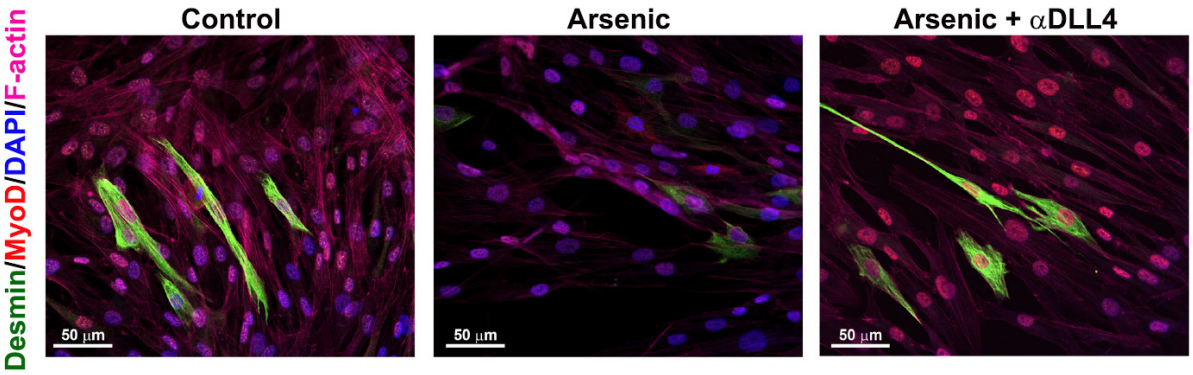
Secondary Antibody	Company	Catalogue No.	Dilution
Alexa Fluor 488 Donkey anti Rabbit IgG (H+L)	Invitrogen / Thermo Fisher Scientific	A21206	1:2000
Cy3 Conjugated Donkey anti Mouse Affinipure	Jackson ImmunoResearch	715-165-151	1:700
Alexa Fluor 647 Phalloidin	Invitrogen / ThermoFisher	A22287	1:40
Cy3 Goat anti Rabbit IgG (H+L)	Invitrogen / ThermoFisher Scientific	A10520	1:250
IRDye® 800CW Donkey anti-Mouse IgG	LI-COR Biosciences	926-32212	1:20,000
IRDye® 680LT Donkey anti-Rabbit IgG	LI-COR Biosciences	926-68023	1:20,000



Supplemental Figure 1: CTF Characterization: CTF were isolated from hind limb muscles of two mice in each group. The cells were fixed 24 hours after being plated in cultures and immunostained with antibody to αSMA. Confocal images were captured at 40x magnification. Greater than 95% of isolated cells were αSMA in all cultures.



Supplemental Figure 2: Effect of Arsenic on CTF mitochondrial respiratory protein expression: Respiratory protein expression measured by immunoblotting in the additional three replicate cultures of CTF^{ctr} and CTF^{ars} (first three replicates shown in Figure 2B). The abundances of respiratory complex proteins (green) and VDAC (red) from all six replicate cultures are given in Table 1.



Supplemental Figure 3: Effect of ECM DLL4 inhibition on hMuSC differentiation: CTF^{ctr} and CTF^{ars} were cultured for 3 days to elaborate ECM. The cells were removed and the ECM was incubated with an antibody that blocks DLL4/Notch binding. After rinsing to remove unbound antibody, hMuSC were seeded and cultured in differentiation medium for 2 days before fixing and immunofluoresent imaging for desmin (green) and nuclear MyoD (red) expression. Nuclei were stained with DAPI (blue) and F-actin was stained with Alexa Fluor 647-phalloidin (magenta). Images were captured at 40x magnification.