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### **Supporting Information**

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Cell Adhesion, Morphology, and Metabolism Variation via Acoustic Exposure within Microfluidic Cell Handling Systems

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**Figure S 1.** The process flow diagram depicting the preparation of cells, exposure to varying acoustic conditions and subsequent analysis methods.



**Figure S 2.** Flow velocity (i) and shear profiles (ii) in the cross section view of the channel 25  $\mu$ m deep channel at 10  $\mu$ L min<sup>-1</sup>(a), 25  $\mu$ m deep channel at 5  $\mu$ L min<sup>-1</sup> (b) and 50  $\mu$ m deep channel at 10  $\mu$ L min<sup>-1</sup> (c). The top view (d) of the flow velocity (i) and shear (ii) profile at the channel bend for a 25  $\mu$ m deep channel at 10  $\mu$ L min<sup>-1</sup> scenario.

# <u>MSC</u>



# <u>MG63</u>





**Figure S 3.** Acoustic exposure resulted in no observed changes in cell attachment phenotypes. Phase contrast images for MSC, MG63 and HaCaT cells across 24 h, 48 h and 72 h post-exposure. Labels of no cell attachment denote scenarios in which cells could not adhere to the growth substrate post-exposure and thus could not be assessed. Scale bar, 50  $\mu$ m.

**Table S 1.** Quantification of 24 h live cell percentage calculated from live/dead fluorescence staining, with data presented as mean  $\pm$  SD from triplicate samples passed through the 10  $\mu$ L min<sup>-1</sup>; 25  $\mu$ m high channel.

	MSC [%]	MG63 [%]	L929 [%]	HaCaT [%]
TCP Ctrl	96.9 ± 2	97.0 ± 4	96.5 ± 3	95.7 ± 2
Flow Ctrl	93.6 ± 3	97.7 ± 2	97.3 ± 2	96.9 ± 1
400 mV	-	96.3 ± 3	97.6 ± 2	96.7 ± 2
800 mV	-	-	96.3 ± 3	93.7 ± 4



**Figure S 4.** Cell count data at three time points for MSCs (a), MG63 (b), L929 (c) and HaCaT (d).

**Table S 2.** Quantification of 24 h live cell percentage calculated from live/dead fluorescence staining, with data presented as mean  $\pm$  SD from triplicate samples passed through the 10  $\mu$ L min<sup>-1</sup>; 50  $\mu$ m high channel.

	MSC [%]	MG63 [%]
TCP Ctrl	97.1 ± 4	97.7 ± 2
Flow Ctrl	95.9 ± 3	96.4 ± 4
400 mV	95.3 ± 5	97.3 ± 3
800 mV	7.03 ± 6	95.6 ± 5

**Table S 3.** Quantification of 24 h live cell percentage calculated from live/dead fluorescence staining, with data presented as mean  $\pm$  SD from triplicate samples passed through the 5  $\mu$ L min<sup>-1</sup>; 25  $\mu$ m high channel.

	L929 [%]	HaCaT [%]
TCP Ctrl	97.7 ± 3	95.7 ± 4
Flow Ctrl	96.7 ± 3	95.3 ± 2
400 mV	96.2 ± 4	93.7 ± 3
800 mV	-	94.2 ± 4



**Figure S 5.** Percentage difference in cell metabolic activity normalised to TCP for MSC (a), MG63 (b) based on results reported in Figure 6a and b (at 10  $\mu$ L min<sup>-1</sup> in a 50  $\mu$ m channel height) and for L929 (c) and HaCaT (d) based on results reported in Figure 6c and d (at 5  $\mu$ L min<sup>-1</sup> in a 25  $\mu$ m channel height).

**Table S 4.** Normalised range of metabolic activity highlighting the decreasing trend in range as cell stiffness increases (as shown in Figure 8a) based on results reported in (Figure S5) at a 400 mV acoustic exposure at 72 h.

	MSC [%]	MG63 [%]	L929 [%]	HaCaT [%]
Min	-18.18	-15.45	-1.49	10.19
Max	13.37	7.13	13.04	12.50
Range	31.55	22.57	14.53	2.31