SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Effect of Hyaluronic Acid on Tight Junction Proteins Expression and Permeability Across Uninjured HLMVEC. (A) Incubation of uninjured HLMVEC with either HMW or LMW HA had no significant effect on protein permeability over 24 h. Data are presented as mean ± SD. N = 12. (B) By Western Blot analyses, incubation of uninjured HLMVEC with either HMW or LMW HA has no significant effects on the expression of the tight junctions, ZO-1 or VE-cadherin. Data are presented as mean ± SD. N = 3. Graphs were created using GraphPad Prism 8.4.3 for OS X (GraphPad Software, San Diego, California USA, www.graphpad.com).

Supplementary Figure 2. Effect of Cytomix With or Without Hyaluronic Acid on AKT Phosphorylation. (A) By Western blot, phosphorylation of AKT (pAKT) was significantly increased at 4 h following the addition of cytomix. Addition of HMW HA but not LMW HA further increased pAKT levels. Data are presented as mean ± SD. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; N = 3. (B) Neutralization of CD44 with an inhibitory antibody (IM7) partially blocked the increase in pAKT/AKT ratio following addition of HMW HA but not LMW HA administration. Data are presented as mean ± SD. *P<0.05; N = 3. Graphs were created using GraphPad Prism 8.4.3 for OS X (GraphPad Software, San Diego, California USA, www.graphpad.com).

Supplementary Figure 3. Effect of Cytomix With or Without Hyaluronic Acid on Caspase 3 Activation. (A) By Western blot, cytomix increased the activation of caspase 3 at 4 h. The addition of both HMW and LMW HA decreased this activation of caspase 3. Data are presented as mean ± SD. **P<0.01; ****P<0.0001; N = 3. (B) Neutralization of CD44 with an inhibitory antibody (IM7) blocked the inhibitory effect of HMW HA but not LMW HA on caspase 3 activation. Data are presented as mean ± SD. *P<0.05; N = 3. For Western blots of caspase 3, the blots were cut prior to the hybridization with the antibodies due to the significantly higher intensity of the pro-caspase 3 band compared to the activated form. Graphs were created using GraphPad Prism 8.4.3 for OS X (GraphPad Software, San Diego, California USA, www.graphpad.com).
Supplemental Figure 4. Effect of Cytomix With or Without Hyaluronic Acid on HAS2-AS1 and HMGA2 expressions. (A) Cytomix increased HAS2-AS1 mRNA levels over 24 h. Addition of LMW HA decreased HAS2 mRNA expression at 4 and 12 h. Data are presented as mean ± SD. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; N = 6. Individual P values are for direct comparison between two groups. (B) Cytomix increased HMGA2 mRNA levels over 24 h. Addition of LMW HA decreased HMGA2 mRNA expression at 4 h compared with cytomix group. Data are presented as mean ± SD. **P<0.01; ***P<0.001; ****P<0.0001; N = 6. Individual P values are for direct comparison between two groups. (C) Western blot for HMGA2 protein. Cytomix increased HMGA2 over 24 h in HLMVEC. At 4 h, HMW HA and LMW HA decreased HMGA2 whereas at 12 h, HMW HA further increased it. Data are presented as mean ± SD. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; N = 3. (D) Simultaneous addition of anti-CD44 Ab (IM7) altered HMGA2 regulation following injury. At 4h, neutralization of CD44 with an inhibitory antibody blocked the inhibitory effect of HMW HA but not LMW HA on HMGA2 expression. Interestingly, at 12 and 24 h, CD44 neutralization blocked the increase in HMGA2 following injury with both HMW HA or LMW HA. Data are presented as mean ± SD. *P<0.05; **P<0.01; N = 3. Graphs were created using GraphPad Prism 8.4.3 for OS X (GraphPad Software, San Diego, California USA, www.graphpad.com).
