Legends for Supplementary Material

Supplemental Table 1. Physical properties of UC and in situ cell frequency quantifications. Volumetric cell densities are based on body-centered closed packing and face-centered closed packing models using the mean intracellular distance obtained from images of 10-micron thick sections. Within the Wharton's Jelly, there are 2 regions with significantly different cell distributions; region 1 immediately surrounds the vessels as shown in the schematic, while region 2 is the outer ring of the UC cross-section. Values are based on physical measurements taken from images of frozen sections (mean± standard deviation).

Supplemental Figure 1. Histological sections of human umbilical cord vein (A-C) and artery (D-F) stained with Alcian blue to show proteoglycan connective tissue (A,B,D,E) and hematoxylin and eosin (C,F). The tunica intima includes the endothelial lining of the lumen, and elastin connective tissue layer. Shown are the tunica media which includes smooth muscle and the perivascular region which immediately surrounds the vessels. The UC vessels lack a tunica adventitia and it is believed that the entire Wharton's Jelly is the connective tissue which may act as the adventitia. (G) Standard curve to determine the amount of dsDNA per gram whole UC tissue: 187,000 ng / gram. (H) Standard curve to determine the mean amount of DNA per UC cell; 16.1 pg/ cell.

Supplemental Figure 2. (A) MSC marker analysis on endothelial / dispase-isolated UC cells through 10 passages. Cells were examined for their expression of CD34, CD144, CD146, CD44, CD105, CD73 and CD90 by flow cytometry. Levels of CD144 and CD146 remain high in this endothelial cell population. **(B)** Cell proliferation through 10 passages, number of cumulative population doublings versus time, days (mean population doubling time = 27 hours).

Supplemental Figure 3. UCSCs exhibit osteogenic and chondrogenic activity. (A) After stimulation with bone morphogenic protein BMP4, more collagenase-isolated UCSCs express

the osteogenic marker alkaline phosphatase as compared to the prestimulated (day 1) and unstimulated cells (day 7). **(B)** Quantification of the mean area of ALP positivity supports (p = 0.04, t-test, -BMP4 vs. +BMP4 day 7) the qualitative observations. **(C)** UCSCs increase production of proteoglycans (detected by Alcian blue staining) and collagens (detected by Masson's Trichrome staining) after stimulation with chondrogenic media (day 10) which includes human TGF-β1, ITS mix and dexamethasone. Prior to stimulation (day 1), proteoglycan and collagen staining is low. **(D)** In comparison to UCSC-pellets at day 1, or day 10 without TGF-β stimulation, we detect significantly more relative amounts of ECM in pellets which received TGF-β stimulation ($p < 0.01$ in both cases). **(E)** Side-by-side comparisons of collagenase and dispase isolated cells in osteogenic and chondrogenic assays. UC cells expressing markers of MSCs show multilineage activity, however UC cells expressing endothelial markers do not show signs of osteogenic or chondrogenic activity after several attempted assays.

Supplemental Figure 4. MSC marker analysis on USCS grown in EGM2 and DMEM. Side-by-side comparisons of UCSCs marker profile after culture in EGM2 or DMEM; 4 days after initial seeding density of 800 cells/ $cm^{2.}$