SUPPLEMENTAL MATERIAL

Fogelstrand et al., http://www.jem.org/cgi/content/full/jem.20082845/DC1

Figure S1. Flow cytometry analyses of VSMC CD98 in the CA. The right CA was perfused with saline, collected, and digested with a collagenase solution (10 mg/ml) for 10 min at 37°C. The adventitia was physically removed, and the remaining intima + media were further digested to a single-cell suspension in a solution of 10 mg/ml collagenase and 2.5 mg/ml elastase; labeled with a cocktail of PE–anti-CD98, FITC–anti-CD18, and FITC–anti-CD31 antibodies; and analyzed on a flow cytometer. To analyze CD98 expression in VSMCs, leukocytes (CD18 positive) and endothelial cells (CD31 positive) were excluded. (left) Cells derived from three pooled injured Slc3a2<sup>fl/fl</sup> CAs. (right) Cells derived from three pooled uninjured Slc3a2<sup>fl/fl</sup> CAs. Data are representative of two independent experiments. R1, CD18- and CD31-negative cells in injured CAs; R2, CD18- and CD31-negative cells in uninjured CAs.
Figure S2. Morphology of different vessels and organs from Slc3a2<sup>fl/fl</sup>SM22α-Cre mice and Slc3a2<sup>fl/fl</sup> controls. Tissue samples were collected, fixed for 3 h in 3.7% formaldehyde, embedded in paraffin, and sectioned. Paraffin sections were stained with hematoxylin/eosin or immunostained for the SMC marker SM22α (brown staining in CA and aorta). The different vessels and organs are depicted in the figure. Bars: (CA and femoral artery) 50 μm; (aorta and inferior vena cava) 200 μm; (other organs) 500 μm. Representative images from three mice in each group are shown.
Figure S3. CD98 expression in the dorsal aorta of Slc3a2<sup>fl/fl</sup>SM22α-Cre embryos and Slc3a2<sup>fl/fl</sup> controls at embryonic day 13.5. Frozen sections of the dorsal aorta at the level of the future proximal descending aorta were immunostained with anti-CD98 (green; left), isotype control antibodies for CD98 (green; middle), and anti-SM22α (SMC marker; red; right). The nuclei (right) are stained blue with DAPI. Lumens are facing up. Arrows are pointing at the border between the vessel wall and the lumen. Note the staining of Sm22α+ VSMC (less than blood cells in the lumen), which is reduced but not completely eliminated in the Slc3a2<sup>fl/fl</sup>SM22α-Cre embryo. Results are representative of seven Slc3a2<sup>fl/fl</sup>SM22α-Cre and two Slc3a2<sup>fl/fl</sup> embryos. Bar, 50 μm.

Figure S4. Morphology and smooth muscle α-actin immunostaining of cultured mouse VSMCs. (A) Morphology of cultured VSMCs at passage 5. VSMCs were isolated from the thoracic aorta from Slc3a2<sup>fl/fl</sup> mice and cultured in DMEM with 10% serum in uncoated 75-cm<sup>2</sup> culture flasks. Note the VSMC characteristic hill-and-valley growth pattern. (B) Cellular expression of smooth muscle α-actin in cultured VSMCs. VSMCs at passage 5 were trypsinized, fixed in 0.2% formaldehyde, and permeabilized with 0.5% saponin/0.5% BSA in PBS, and immunostained for smooth muscle α-actin and analyzed on a flow cytometer (continuous line, FITC-conjugated anti-smooth muscle α-actin; dotted line, FITC-conjugated isotype control). The experiment was repeated twice with identical results. Bar, 100 μm.