SDC1

Immunostaining of tissue sections for light microscopy

Tissues were fixed in 4% (v/v) PFA then processed to paraffin wax on a Thermo Excelsior Tissue Processor. Serial sections were cut on a microtome at 5 μm,and mounted onto Polysine slides. For immunostaining, tissue sections were dewaxed in histolene and hydrated through graded ethanol.

Primary and secondary antibodies were used at the following dilutions: 1:10 rabbit anti human CD31 (Thermo Fisher Scientific), 1:100 Dako mouse anti human podoplanin clone D2-40 (Agilent Technologies), 1:300 Dako mouse anti human α-smooth muscle actin (Agilent Technologies), 1:100 rabbit anti human von Willebrand Factor (vWF) (Millipore), 1:200 goat anti rabbit-biotin (Vector), 1:200 goat anti mouse-biotin (Vector), 1:200 Dako rabbit anti mouse-biotin (Agilent Technologies). Isotype control: Dako mouse IgG (Agilent Technologies).

Washing was performed with 0.05% (v/v) Tween-20 in PBS. Antigen retrieval was undertaken using 10 mM tri-sodium citrate buffer pH 6.0 at 95°C for 20 minutes for all antibodies except smooth muscle actin. Endogenous peroxidase activity was blocked with 3% methanol in distilled water for 5 minutes. Protein blocking was performed at room temperature for 5 minutes using Thermo UltraVision Protein Block (Thermo Fisher Scientific), or alternatively for vWF immunostaining using 20% normal goat serum (Sigma Aldrich) in PBS/Tween-20 for 30 minutes. Tissues were incubated with primary antibody diluted in Dako Envision Flex diluent (Agilent Technologies) for CD31 and D2-40, 20% (v/v) normal goat serum (Sigma Aldrich) in PBS/Tween-20 for vWF, or Thermo Ultra Clean diluent alone for smooth muscle actin staining, at the dilutions specified, for 30-60 minutes at room temperature. To confirm

staining specificity isotype controls were performed using mouse IgG at the equivalent concentration. Biotinylated secondary antibodies were applied in the same diluent as the corresponding primary antibody, at the dilutions specified for 30 minutes.

Enzyme labeling was performed using Vector ABC Elite (Vector), or Dako horse radish peroxidase-streptavidin (Agilent Technologies) at 1:400 dilution for 30 minutes, and the colour developed by applying Dako DAB (Agilent technologies). Tissues were counterstained with Mayer's haematoxylin, dehydrated, and mounted in DPX (Merck).

Brightfield images were acquired on an Olympus BX61 microscope with a DPX71 colour camera.