



Supplementary Figure 2. Production and testing of the Prox-1 antibodies. We subcloned cDNA encoding Prox-1 homeobox domain and prospero domain (amino acid residues 578-750 of human Prox-1) into the pGEX2t vector, and purified the GST-Prox-1 fusion protein from *E. coli* using glutathione Sepharose according to the manufacturer's instructions (Amersham, Piscataway, NJ). We immunized rabbits with the fusion protein according to a standard protocol and isolated Prox-1 specific antibodies from the serum using sequential columns with GST- and GST-Prox-1-coupled to vinylsulfone agarose resin (Sigma). **(a)** Immuno blotting using antibodies against Prox-1. The Prox-1 antibody detected a 85-kD band in the lysates from 293T cells transfected with Prox-1/pAMC. Lysates from pAMC vector transfected cells are shown as control. **(b)** Methanol fixed frozen sections of E17.5 mouse skin were stained with rat anti-mouse PECAM-1, rat anti-mouse podoplanin (PA2.26)¹ and rabbit anti-Prox-1 primary antibodies, and corresponding secondary donkey antibodies conjugated with TRITC or Cy2 (Jackson ImmunoResearch Laboratories). Note the expression of Prox-1 in podoplanin positive lymphatic vessels, which are also weakly positive for PECAM-1. Scale bars 200 μ m.

Reference

1. Scholl, F. G., Gamallo, C., Vilaró, S. & Quintanilla, M. Identification of PA2.26 antigen as a novel cell-surface mucin-type glycoprotein that induces plasma membrane extensions and increased motility in keratinocytes. *J. Cell Sci.* 112, 4601-4613 (1999).