

Supplementary Images

Maximum intensity projections of the three image sets (panels 1-3) used to derive transcript counts summarised in main figures 5 and 6, and in supplementary table 2. Image set ids are denoted in supplementary table 2. CCE ES cells were differentiated for four days in aMEM 10% serum on Collagen type IV (Col IV). At day four, 20,000 cells were re-plated in a 6mm well on a Col IV coated glass slide and re-cultured for a further 24 hours in serum free medium (SFO3) and VEGF (30 ng/ml). The cells were then hybridised with probes against Cdh5 (Cy3), Gata2 (a488*, Cy3), Etv2 (Cy5), Tal1 (a488, Cy5), Runx1 (Cy3, Cy5) and Fli1 (a488, Cy3, Cy5) as described in the main text. Colours are: DAPI white, a488 blue, Cy3 green, Cy5 red, resulting in the following labelling: Cdh5 green, Gata2 cyan, Etv2 red, Tal1 magenta, Runx1 orange (~ish) and Fli1 whitish. Note that single coloured labelled transcripts give much stronger signals (eg, Cdh5 and Etv2) which tend to obscure the lower intensity combinatorially labelled transcripts when viewed by eye. This is especially true for Gata2 since the number of Gata2 transcripts is generally low. The images were derived from overlapping z-stacks merged using the dvreader application (<http://www.gitorious.org/dvreader>). Pixel dimensions are 107 x 107 nm. All three images were viewed with the same viewing parameters (i.e. brightness / contrast). *a488 = Alexa fluor 488. Original data and non-compressed tiff files of the projections are available from the authors upon request.

