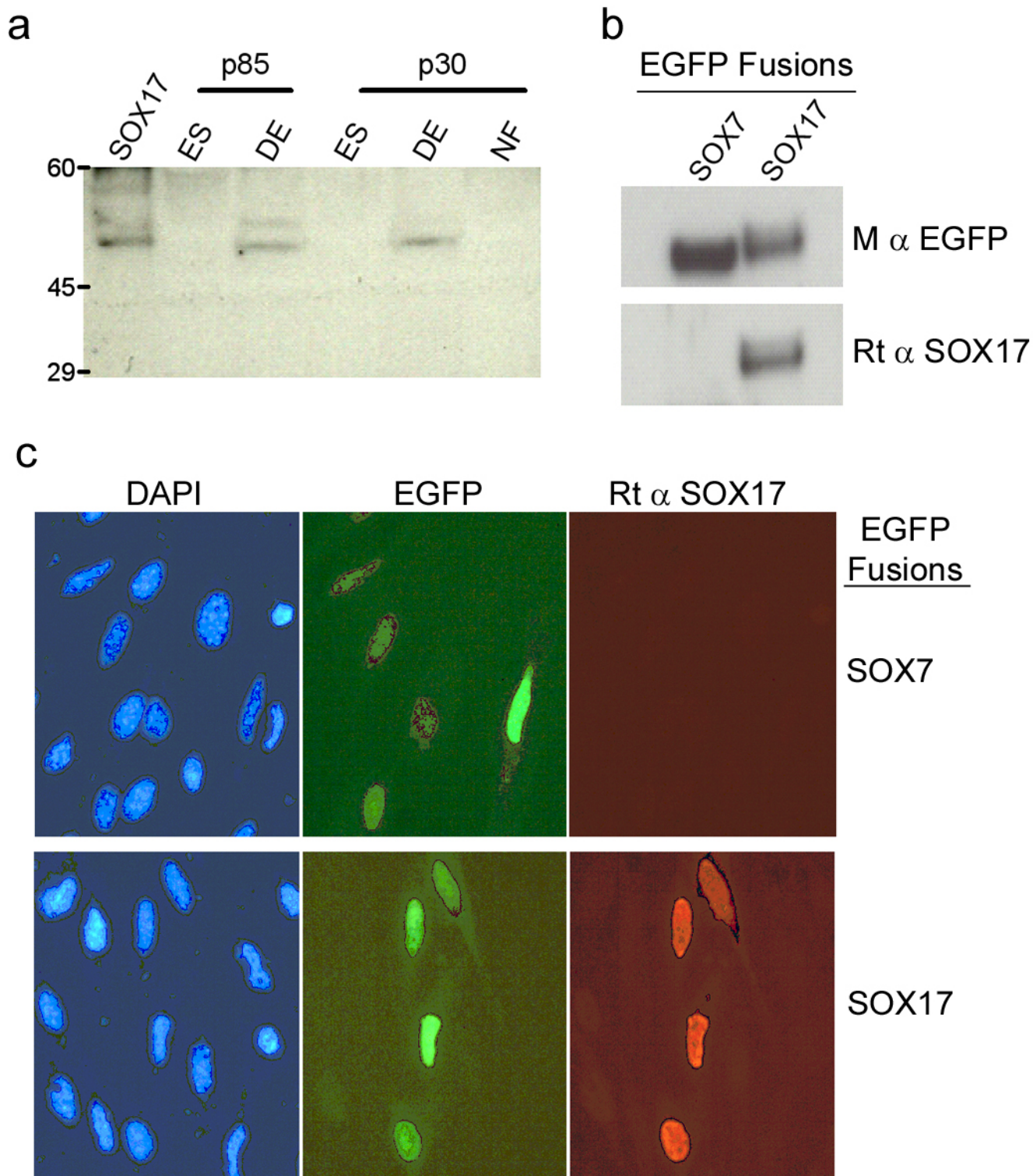


## Supplementary Fig. 1 Validation of the SOX17 anti-serum.



(a) Western blot analysis shows the presence of SOX17 in hESC-derived definitive endoderm. The first lane (SOX17) contains approximately 10  $\mu$ g of protein extract from MDX12 human fibroblasts transiently transfected with a pCMV-SOX17 expression vector (Origene), while the other lanes contain approximately 100  $\mu$ g of hESC extract. ES: undifferentiated hESC; DE: definitive endoderm; NF: HESC differentiated in low serum without ActivinA. DE cells generated from early (p30) and later (p85) passages of Cyt25 hESC appear to express similar amounts of SOX17. The 44 kDa SOX17 migrates at approximately 51 kDa under these conditions.

(b) and (c) The SOX17 antiserum does not detect the related SOX7 protein, as determined by western blot (b) and immunofluorescence (c) analyses. For these studies, MS1-V (ATCC # CRL-2460) cells were transiently transfected with expression vectors of SOX17-EGFP and SOX7-EGFP fusions (pEGFP-N1, Clontech).