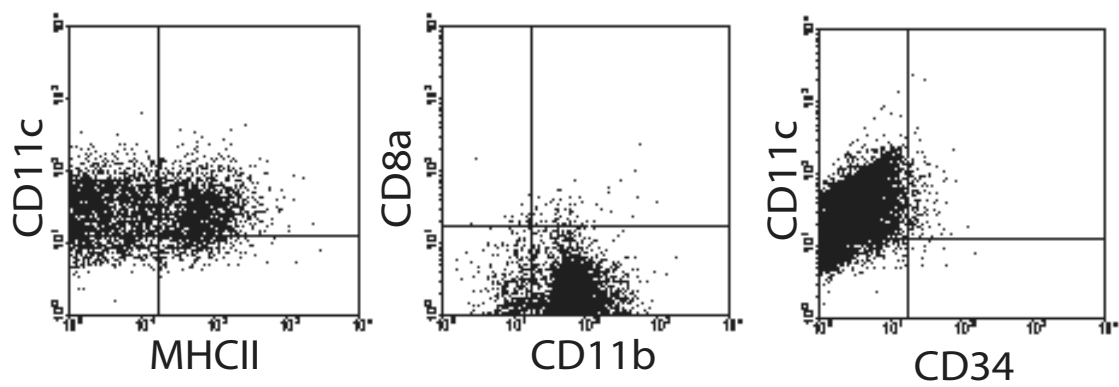
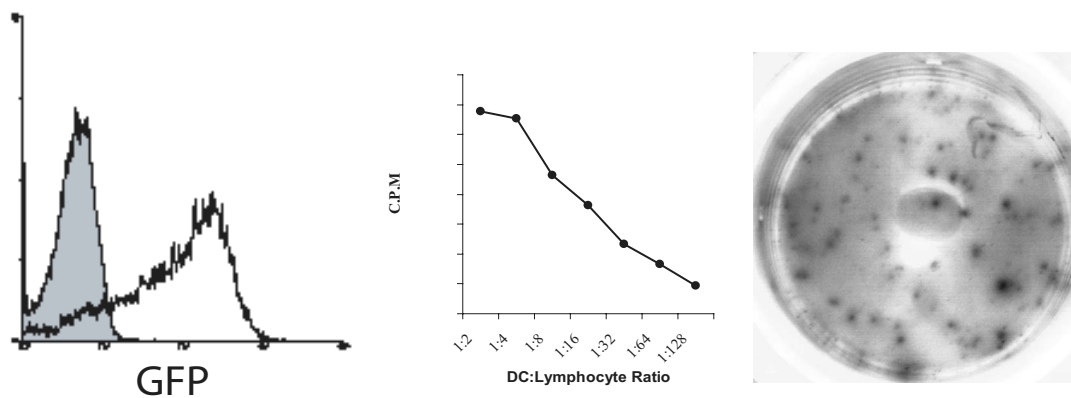


2a



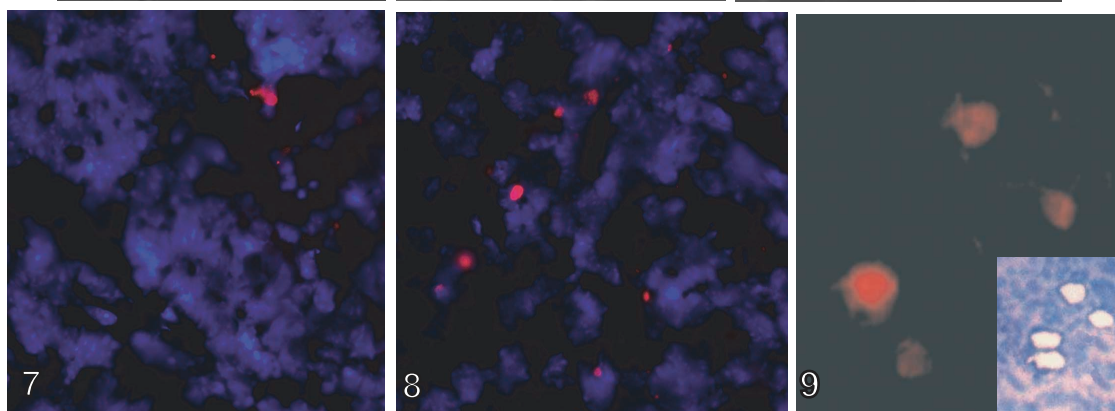
2b



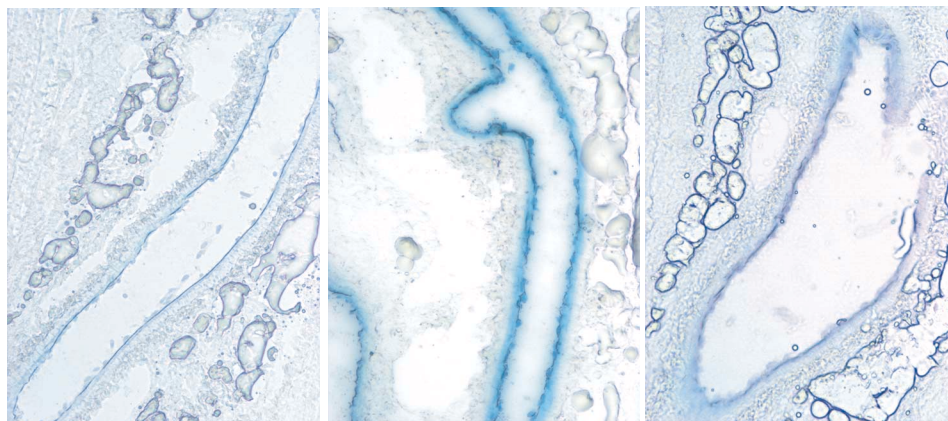
2c



2d



2e



Supplementary Figure 2

Bone marrow-derived DCs undergo endothelial specialization *in vitro*

(a) Flow cytometry analysis of DCs generated from flushed bone marrow cells after 8 days of culture with GM-CSF (20 ng/ml) *prior* to immunopurification with anti-CD11c and anti-CD34 mAb-loaded immunomagnetic beads. Cells exhibit a CD11c⁺CD11b⁺CD34⁻CD8 α ⁻MHC-II⁺ myeloid DC phenotype. (Note that examples of cells *following* positive CD11c and negative CD34 immunopurification are shown in main Fig. 2a). (b) **Left:** Immature DCs engulf tumor antigen. CD11c⁺ cells express GFP after overnight incubation with GFP⁺ apoptotic ID8 cells. Shaded: unpulsed DCs; open: pulsed DCs. **Middle:** Stimulation of T cell proliferation by bone marrow-derived CD11c⁺CD34⁻ immunopurified DCs. Significant proliferation, as measured by [³H]thymidine incorporation, is seen in non-adherent splenocytes from animals bearing ID8 tumors following incubation *ex vivo* with syngeneic bone-marrow derived DCs pulsed with apoptotic ID8 cells and matured with TNF- α . **Right:** Interferon- γ -secreting splenocytes are detected by ELISPOT analysis under the same circumstances. Thus, bone marrow-derived cells used in all experiments exhibit typical DC morphology, phenotype, and functional properties. (c) DC migration and aggregation underlies the formation of cords *in vitro*. Immature DCs cultured in the presence of conditioned media from Defb29 Vegf cells align with each other forming strings. Recruitment of additional cells results in cord formation. Migration of an index cell and integration into a string is shown at three different time points (See also movies). (d) To investigate the “endothelial-like switch” *in vivo*, 10⁶ immature CD34⁻CD11c⁺ DCs were stained with PKH26 red fluorescent cell linker, and subcutaneously injected around ID8-Vegf or ID8-Defb29 Vegf tumors. (**left**) A higher number of immature DCs was recruited by ID8-Defb29 Vegf tumors than by ID8-Vegf control tumors (**middle**) in all the specimens analyzed (n=10). (**right**) 150 red fluorescent cells per specimen were selectively microdissected from routinely prepared frozen sections. PKH26-labeled cells are noted within a resected tumor. Detail: Following laser-capture microdissection, tissue sections were stained by H&E. Note the site of microdissected cells. (e) Vascular lakes perfusable with i.v. administered Evans’ blue are noted in tumors injected with immature DCs.