Oxygen tension plays a critical role in the hematopoietic microenvironment *in vitro*

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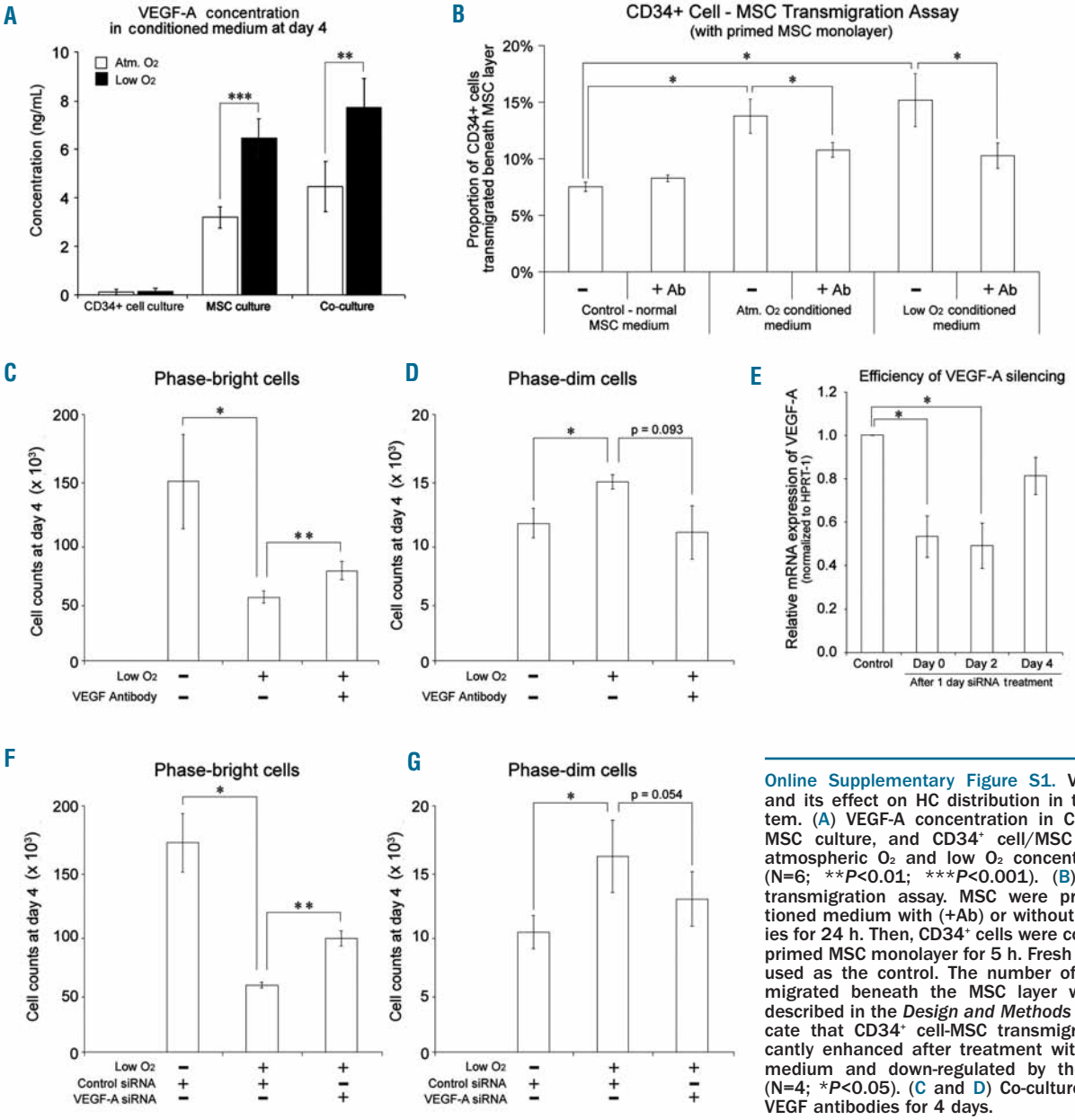

Online Supplementary Figure S1. VEGF-A expression and its effect on HC distribution in the co-culture system. (A) VEGF-A concentration in CD34⁺ cell culture, MSC culture, and CD34⁺ cell/MSC co-culture under atmospheric O_2 and low O_2 concentrations on day 4 (N=6; $^{**}P<0.01$; $^{***}P<0.001$). (B) CD34⁺ cell-MSC transmigration assay. MSC were primed with conditioned medium with (+Ab) or without (−) VEGF antibodies for 24 h. Then, CD34⁺ cells were co-cultured with the primed MSC monolayer for 5 h. Fresh MSC medium was used as the control. The number of CD34⁺ cells that migrated beneath the MSC layer was measured as described in the *Design and Methods* section. Data indicate that CD34⁺ cell-MSC transmigration was significantly enhanced after treatment with the conditioned medium and down-regulated by the VEGF blockade (N=4; $^{*}P<0.05$). (C and D) Co-culture assay that used VEGF antibodies for 4 days. (C) Number of phase-bright (PB) cells HC on the MSC surface) and (D) phase-dim (PD) cells (HC beneath the MSC layer) in atmospheric O_2 co-culture and low O_2 co-culture with VEGF antibodies were measured on day 4 (N=4; $^{*}p< 0.05$; $^{**}P<0.01$). (E) Efficiency of silencing VEGF-A expression by siRNA. MSC were treated with VEGF-A siRNA for 1 day. VEGF-A expression was measured by real-time reverse transcriptase polymerase chain reaction on day 0 (1 day after silencing), day 2, and day 4. Data are normalized to the negative control of siRNA treatment (N=3; $^{*}P<0.05$). (F and G) Co-culture assay with VEGF-siRNA-treated MSC. (F) Number of phase-bright cells and (G) phase-dim cells in atmospheric O_2 co-culture and low O_2 co-culture with siRNA-treated MSC on day 4 (N=6; $^{*}P<0.05$; $^{**}P<0.01$).