SUPPLEMENTARY MATERIAL

MATERIAL AND METHODS

Colony-Forming Efficiency at Various Seeding Densities

LECs were seeded in 60 mm dishes (21.1 cm²) at 300, 500, and 1000 cells/dish and cultured for 10 to 14 days. Cultured cells were stained with rhodamine B (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 30 min. Colony-forming efficiency (CFE) was calculated as CFE = number of colonies / number of inoculated cells. Five independent experiments were performed.

Measurement of Colony Cell Density

LECs were inoculated in two well chamber slides (10⁴ cells/ 4.2 cm²). To exclude the possible influence of colony size on the cell density, cultures were fixed after achieving the same approximate size of 1.4 mm diameter. Cells were fixed and stained as mentioned in the main text. Twenty colonies were photographed in each group, and colony perimeter was traced using the Photoshop software (Adobe Systems incorporated, San Jose, CA). Colony area and the number of nuclei were analyzed using the NIH/Image software (Scion Image, Scion corporation, Frederick, MD). Cell density in each colony was calculated as cell density = number of cell nuclei /colony size (mm²).