Supporting Information

3D micro-tissue models to analyze the effects of ultra-low dose LPS on vascular sprouting dynamics in the tumor microenvironment

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Number of pages: 6

Number of figures: 4

Number of tables: 0

Methods

TUNEL Apoptosis Assay

HBMECs were seeded on top of 5 mg/ml collagen hydrogels and exposed to the same treatment conditions as in the angiogenesis assay described previously. After 7 days of incubation, the samples were fixed in 4% formalin in PBS for one hour. The permeabilization solution, consisting of 0.1% Triton X-100 and 0.1% sodium citrate in PBS, was added to the samples for two minutes on ice. The samples were then rinsed twice in PBS. The manufacturer staining protocol for the In Situ Cell Death Fluorescein Kit (Sigma-Aldrich, St. Louis, MO) was followed. Briefly, for the positive control samples, the scaffolds were incubated in 250 U/ml DNase (Sigma-Aldrich, St. Louis, MO) in 50 mM Tris-HCl and 1 mg/ml BSA for 10 minutes at room temperature. For the negative control, only the label solution was added to the wells. For all other scaffolds, the staining solution was added to the samples for 1 hour at 37°C and protected from light. After incubation, the samples were rinsed twice in PBS and then imaged using a Zeiss LSM 800 Confocal Laser Scanning Microscope (Carl Zeiss Microscopy, Oberkochen, Germany).
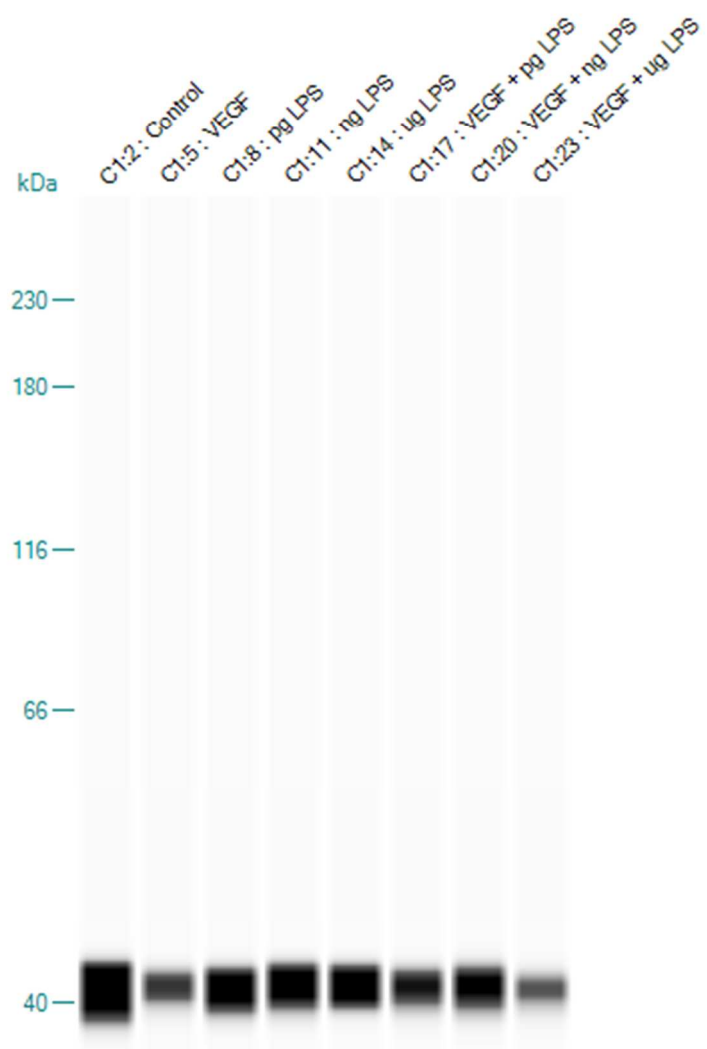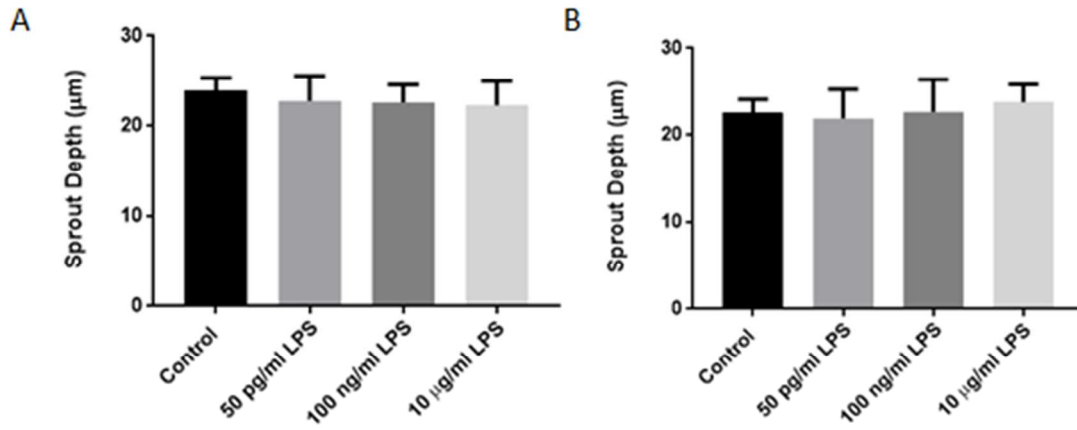
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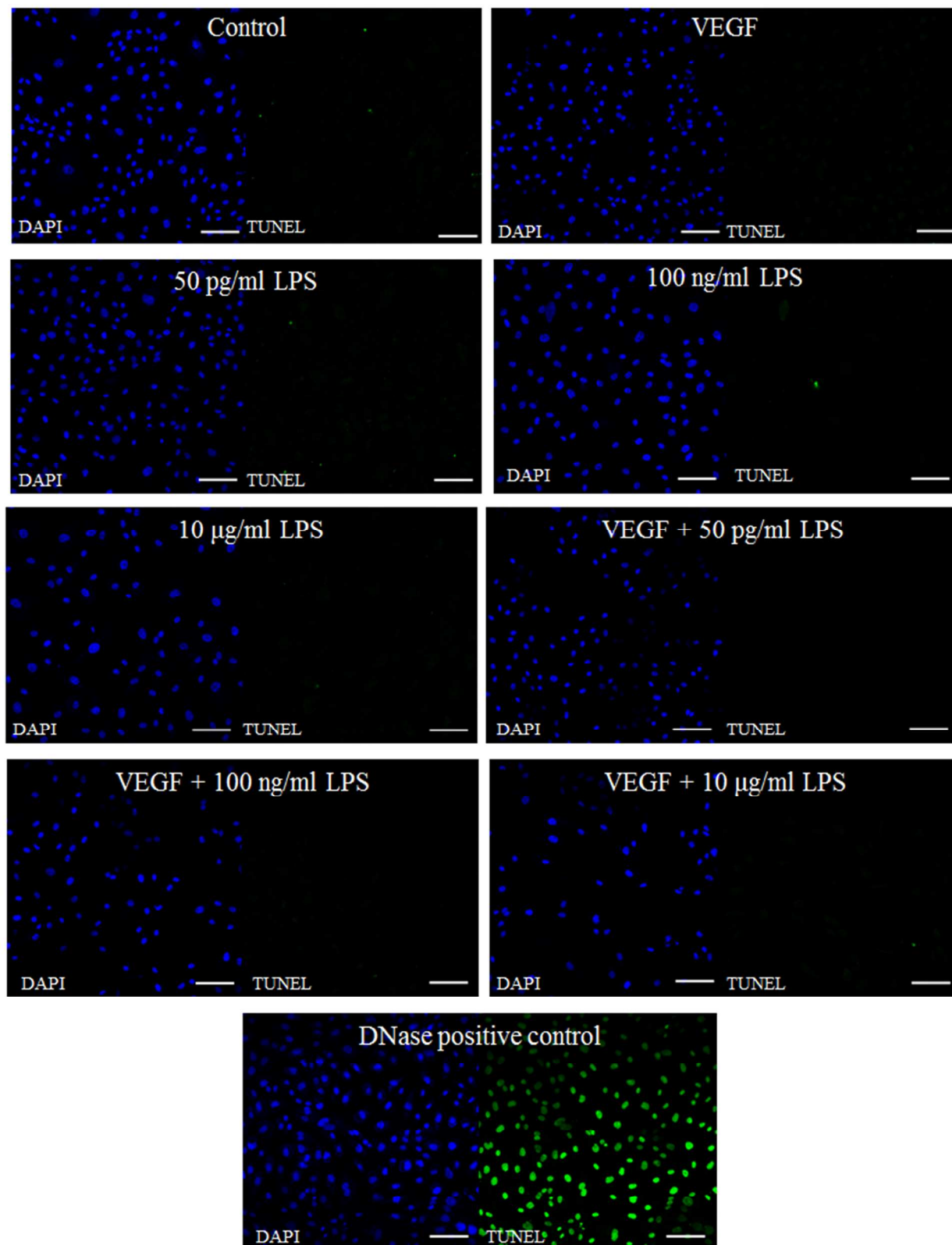
Supplementary Figure 1. Images of TUNEL apoptosis (green) and nuclei (blue) stains. There was no evidence of apoptosis among any of the treatment groups. Scale bar = 100 μm.
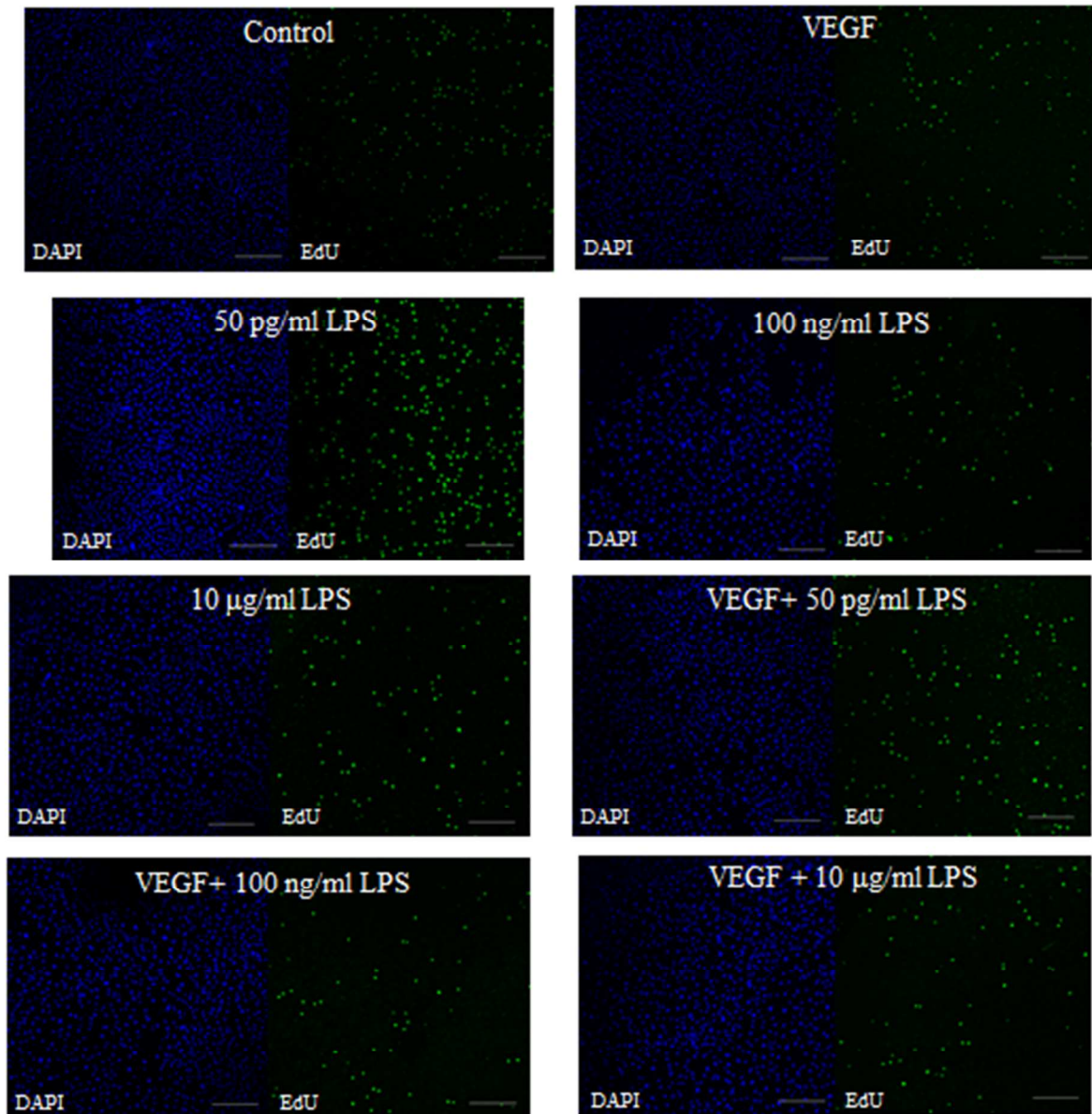
Supplementary Figure 2. Images taken after using the EdU proliferation assay. Cells are labeled with EdU (proliferating) and DAPI (all cells). Scale bar = 500 μm.

Supplementary Figure 3. Western blots of the system control, GAPDH.

Supplementary Figure 4. Sprout depth of primed HBMECs in a collagen hydrogel containing U251 cells after (A) 3 and (B) 7 days.