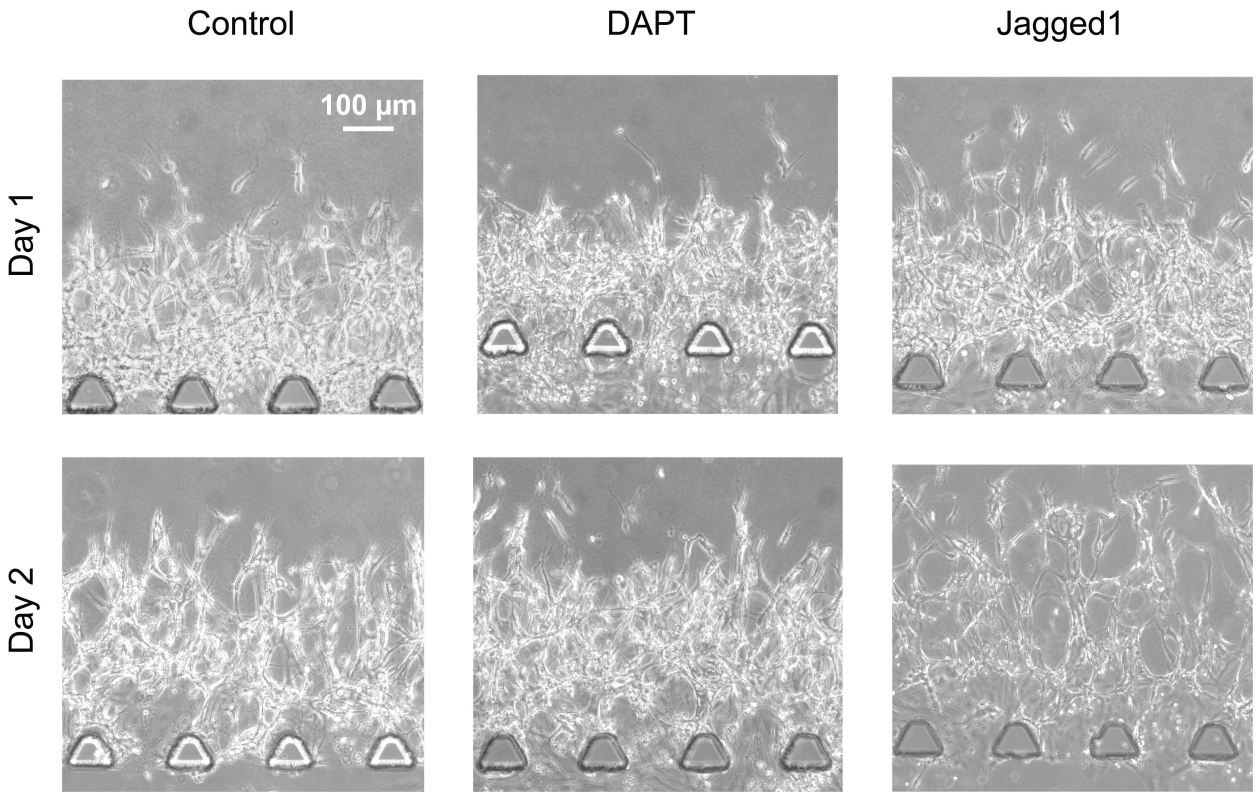
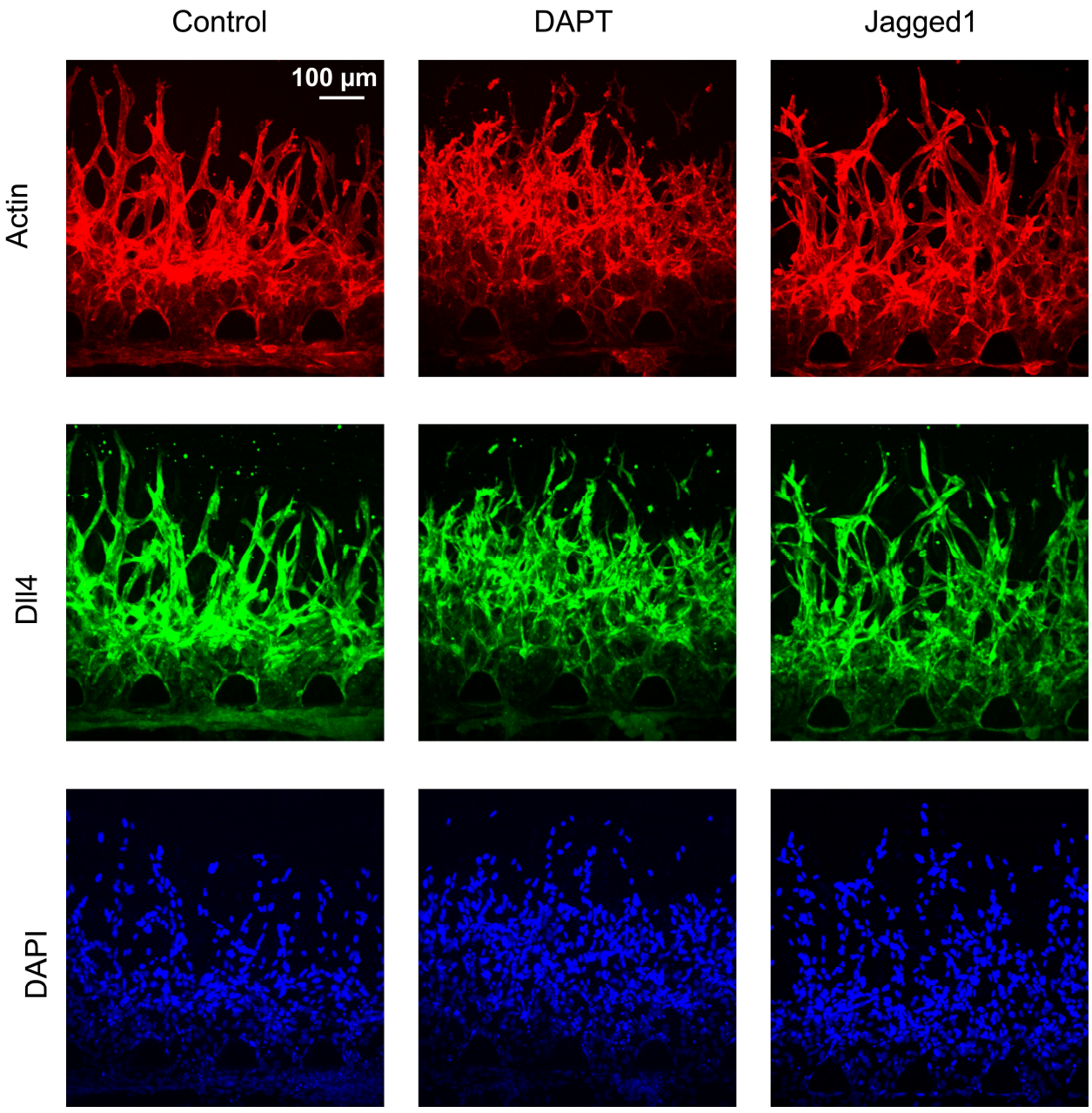
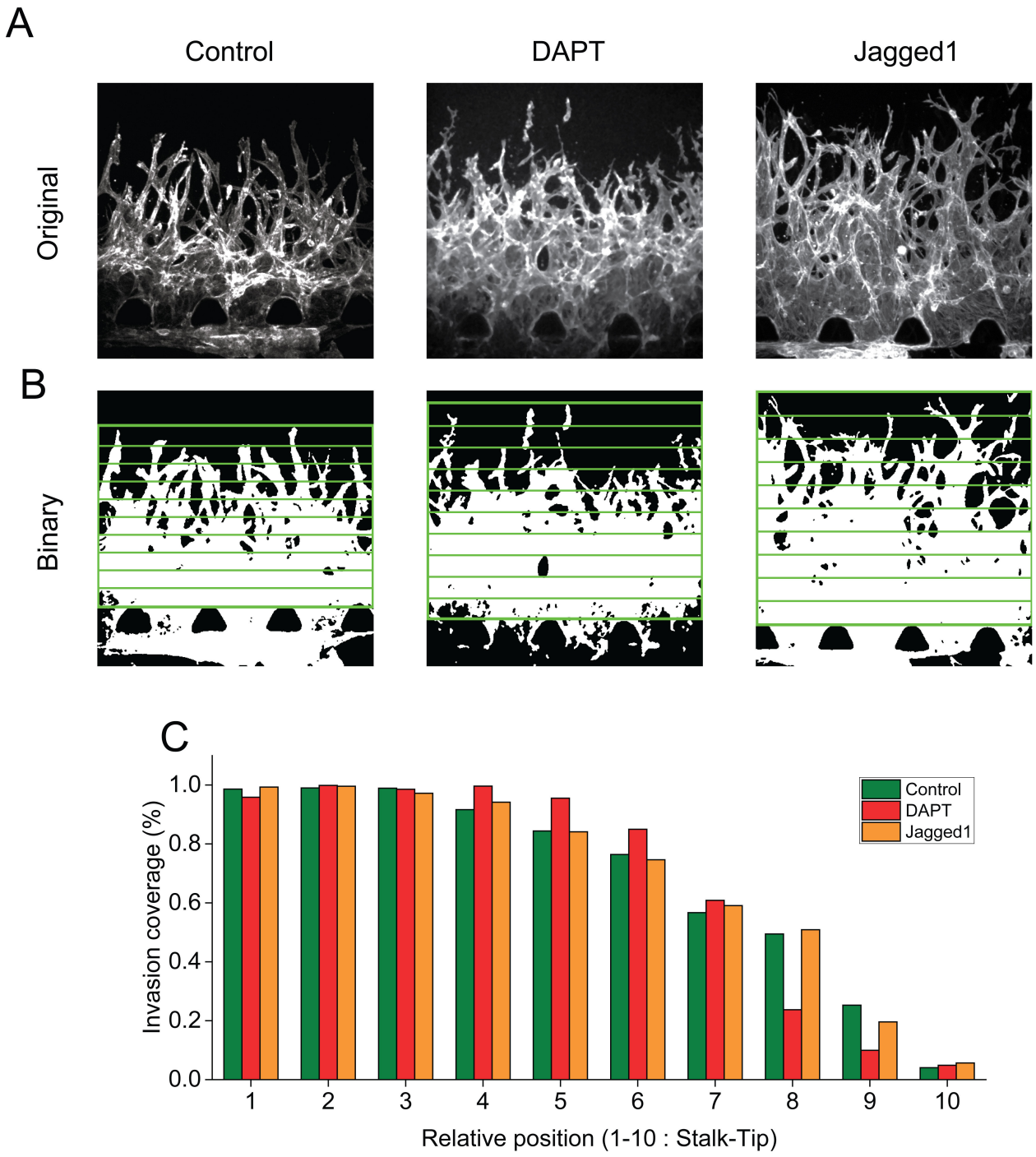
Supporting information



SI Figure 1. Representative phase-contrast images taken at Day 1 (top) and Day 2 (bottom) showing directional migration and angiogenic invasion of ECs in the fibrin gel. Samples were treated without (control) or with DAPT or Jagged1 as indicated. All images were recorded close to the gel interface within the endothelial channel.

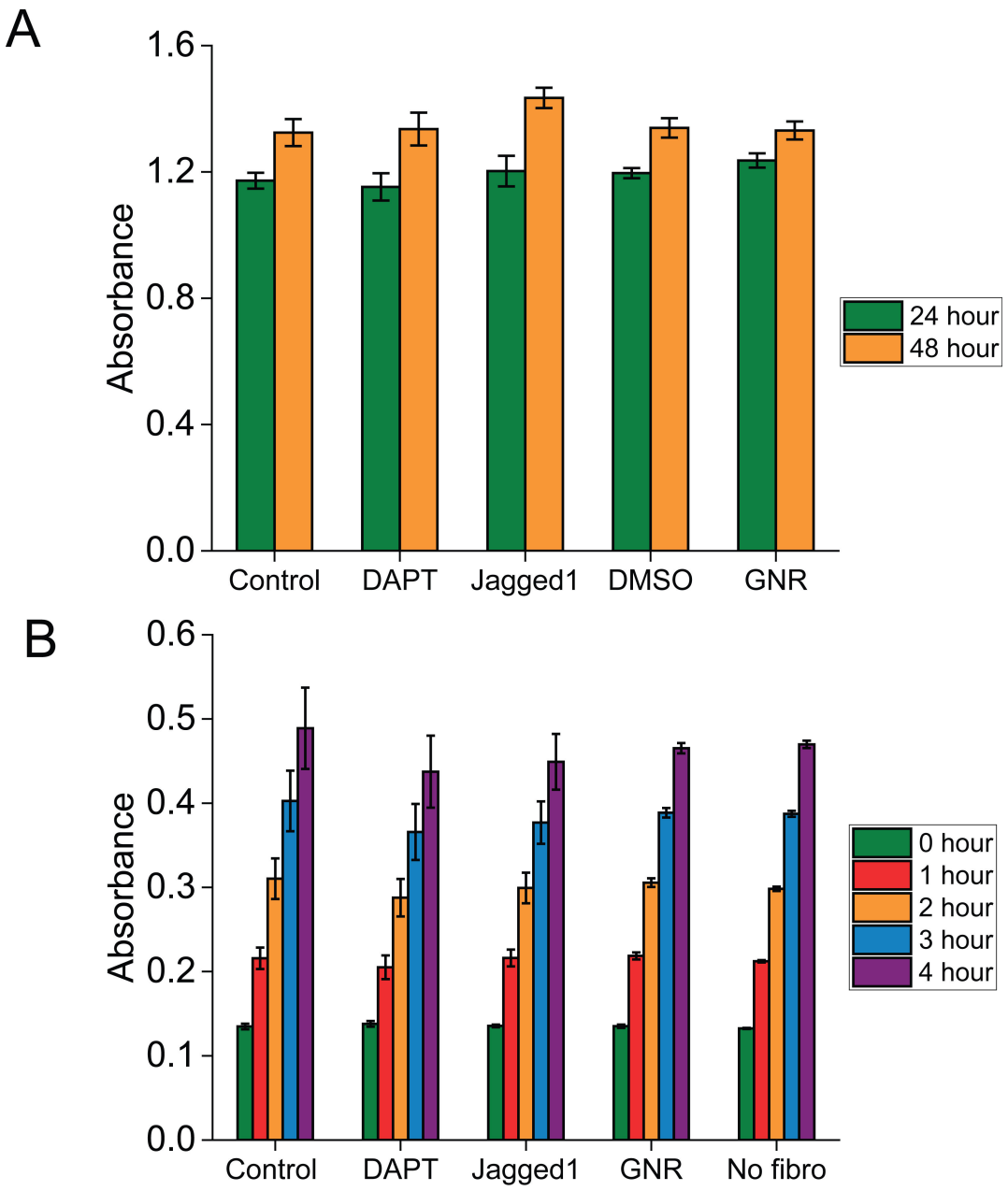


SI Figure 2. Representative confocal images in *z*-projection showing angiogenic morphogenesis of ECs treated without (control) or with 20 µM DAPT or 20 µM Jagged1 as indicated. Images were acquired 48 hr after initial cell seeding. Cells were stained for F-actin (red; top), Dll4 protein (green; middle) and nuclei (blue; bottom), as indicated.

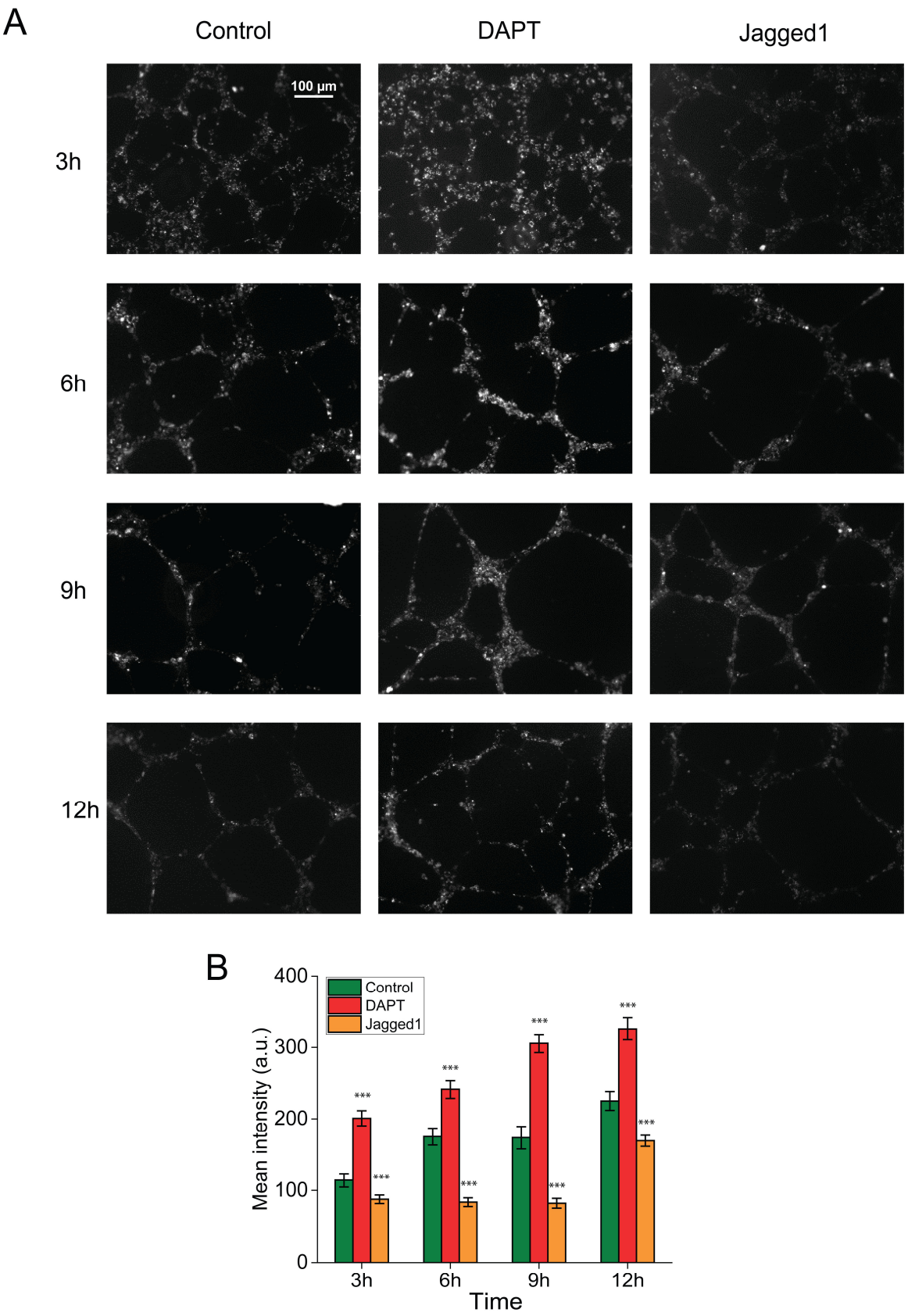


SI Figure 3. Methodology for quantification of spatial patterns of angiogenic invasion area coverage (%). (A) Confocal images showing angiogenic sprouts stained for F-actin. Samples were treated without (control) or with DAPT or Jagged1 as indicated. Images were acquired 48 hr after initial cell seeding. (B) Representative confocal images showing 10 sub-regions of equal areas between supporting posts and leading tip cells (labeled as 1 - 10 : Tip - stalk). (C)

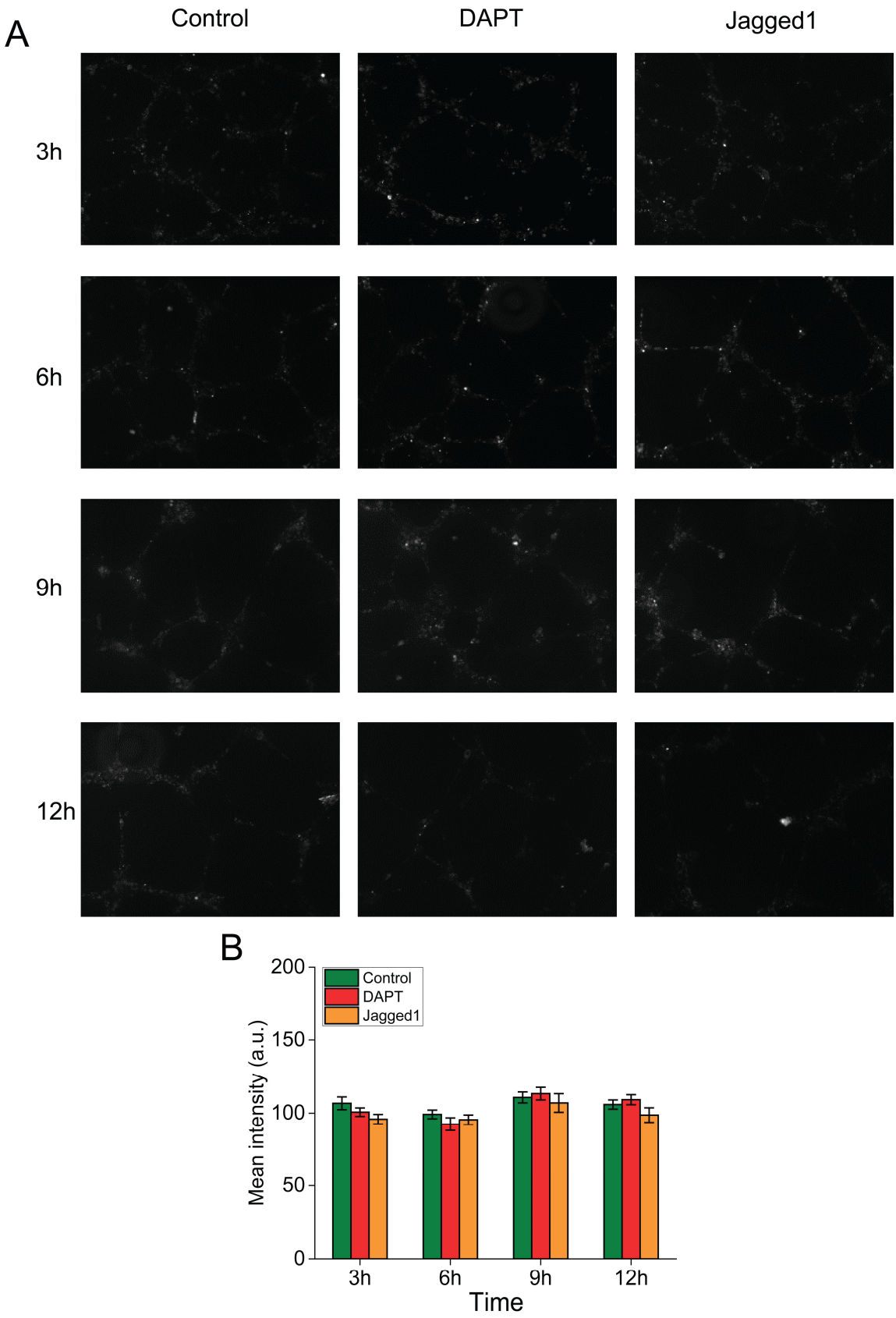
Quantification of angiogenic invasion area coverage (%) in the 10 sub-regions between supporting posts and leading tip cells under different conditions as indicated. Invasion coverage percentage was calculated using a custom designed image analysis algorithm (see Materials and Methods).



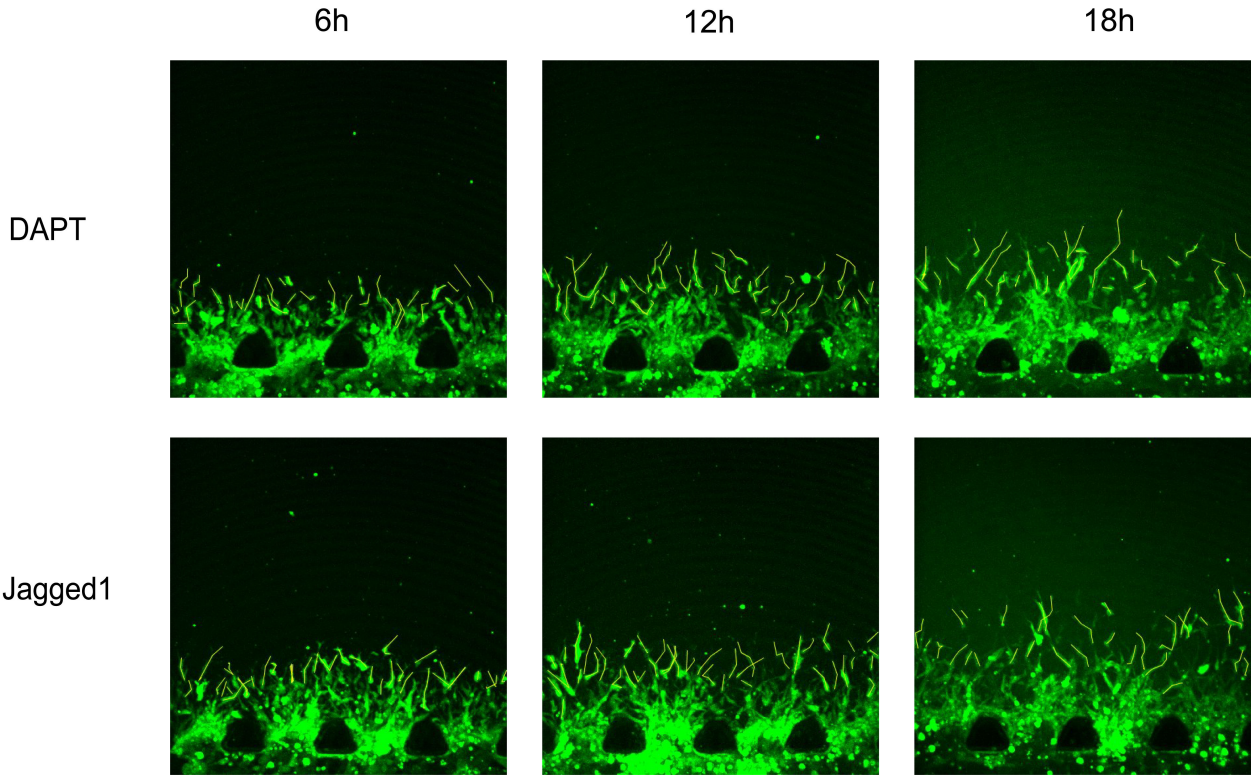
SI Figure 4. Bar plots showing toxicity (A) and proliferation (B) assays performed using the Cell Counting Kit-8 (CCK-8) (see Materials and Methods), with results revealing no significant toxic effect or influence on proliferation of ECs when treated with DAPT, Jagged1, DMSO or gold nanorod (GNR).


A
Control
DAPT
Jagged1
100 μm
3h
6h
9h
12h
B
Control
DAPT
Jagged1
Mean intensity (a.u.)
400
300
200
100
0
***
3h
6h
9h
12h
Time

SI Figure 5. Single-cell Dll4 mRNA expression analysis for EC networks cultured on 2D Matrigel substrates. (A) Representative fluorescence images showing networks of ECs without (control) or with treatments with DAPT or Jagged1 as indicated. Images were recorded at 3, 6, 9 and 12 hr after initial cell seeding. ECs were incubated with Dll4 GNR-LNA probes for 4 hr before cell seeding. (B) Mean fluorescence intensity of ECs showing Dll4 mRNA expression under different conditions as indicated. DAPT treatment upregulated Dll4 mRNA expression while Jagged1 treatment inhibited Dll4 mRNA expression. Data are obtained from > 40 cells in each condition and are expressed as mean ± S.E.M. from $n = 4$ substrates. *P*-values were calculated using two-sample *t*-test with respect to control. *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.005$.


A
Control
DAPT
Jagged1
3h
6h
9h
12h
B
Control
DAPT
Jagged1
Mean intensity (a.u.)
200
150
100
50
0
3h
6h
9h
12h
Time

SI Figure 6. Negative control using random GNR-LNA probes. (A) Representative fluorescence images showing networks of ECs under different conditions as indicated. Images were recorded at 3, 6, 9 and 12 hr after initial cell seeding. ECs were incubated with random GNR-LNA probes for 4 hr before cell seeding. (B) Mean fluorescence intensity of ECs under different conditions as indicated. This data confirmed that random GNR-LNA probes could not detect Dll4 mRNA expression. Data are obtained from > 40 cells in each condition and are expressed as mean ± S.E.M. from $n = 4$ substrates.



SI Figure 7. Confocal images showing fluorescence signals from GNR-LNA nanobiosensors for detection of Dll4 mRNA in ECs during angiogenic sprouting in the fibrin hydrogel under different conditions at different time points as indicated.