Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2016.

Supplementary Information

A Nanobiosensor for Dynamic Single Cell Analysis during Microvascular Self-Organization

S. Wang,[a, b] J. Sun,[b,c] D. D. Zhang,[d] and P. K. Wong[b,e,*]

[a] Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI 48109, USA
[b] Department of Aerospace and Mechanical Engineering, University of Arizona, Tucson, AZ 85721, USA
[c] Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801 USA
[d] Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ 85724, USA
[e] Departments of Biomedical Engineering, Mechanical Engineering and Surgery, The Pennsylvania State University, University Park, PA 16802, USA

*Corresponding author: Tel: +1- 814-863-5267; Fax: +1-814-863-0490
E-mail: *pak@engr.psu.edu*

Keywords: single cell analysis, locked nucleic acid, nanoparticle, gene expression, self-organization

Fig. S1

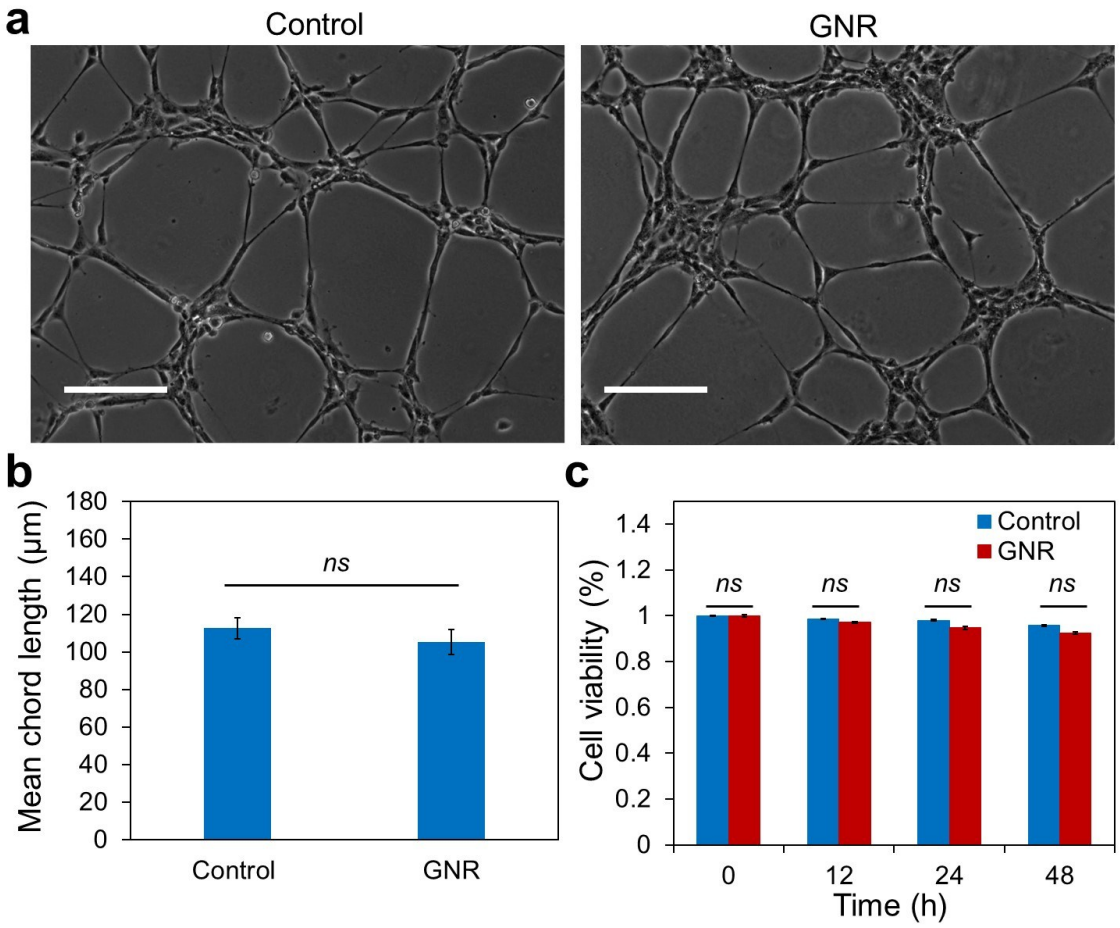

Fig. S1. The GNR-LNA nanobiosensor for investigating microvascular self-organization. (a) Microvascular networks with and without GNRs. Scale bars, 200 μm. Images are representative of three independent experiments. (b) The mean chord lengths of microvascular networks with and without GNR. (c) The viability of HUVECs with and without GNRs. Data represent over 100 cells in each group and are expressed as mean ± s.e.m. (n=5, ns, not significant; unpaired Student's *t*-test).

Fig. S2

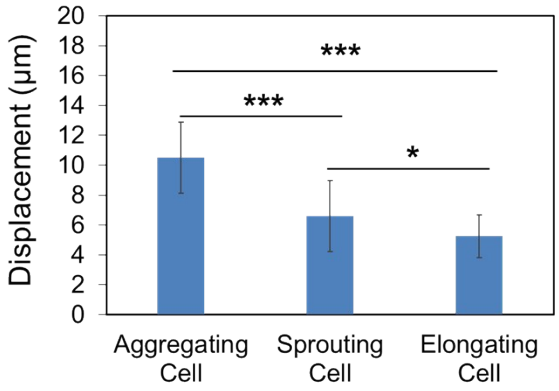

Fig. S2. The maximum displacement of cell subpopulations. Data represent over 100 cells in each group and are expressed as mean ± s.e.m. (n=3, **P<0.05, **P<0.01, ***P<0.001*; unpaired Student's *t*-test).

Fig. S3

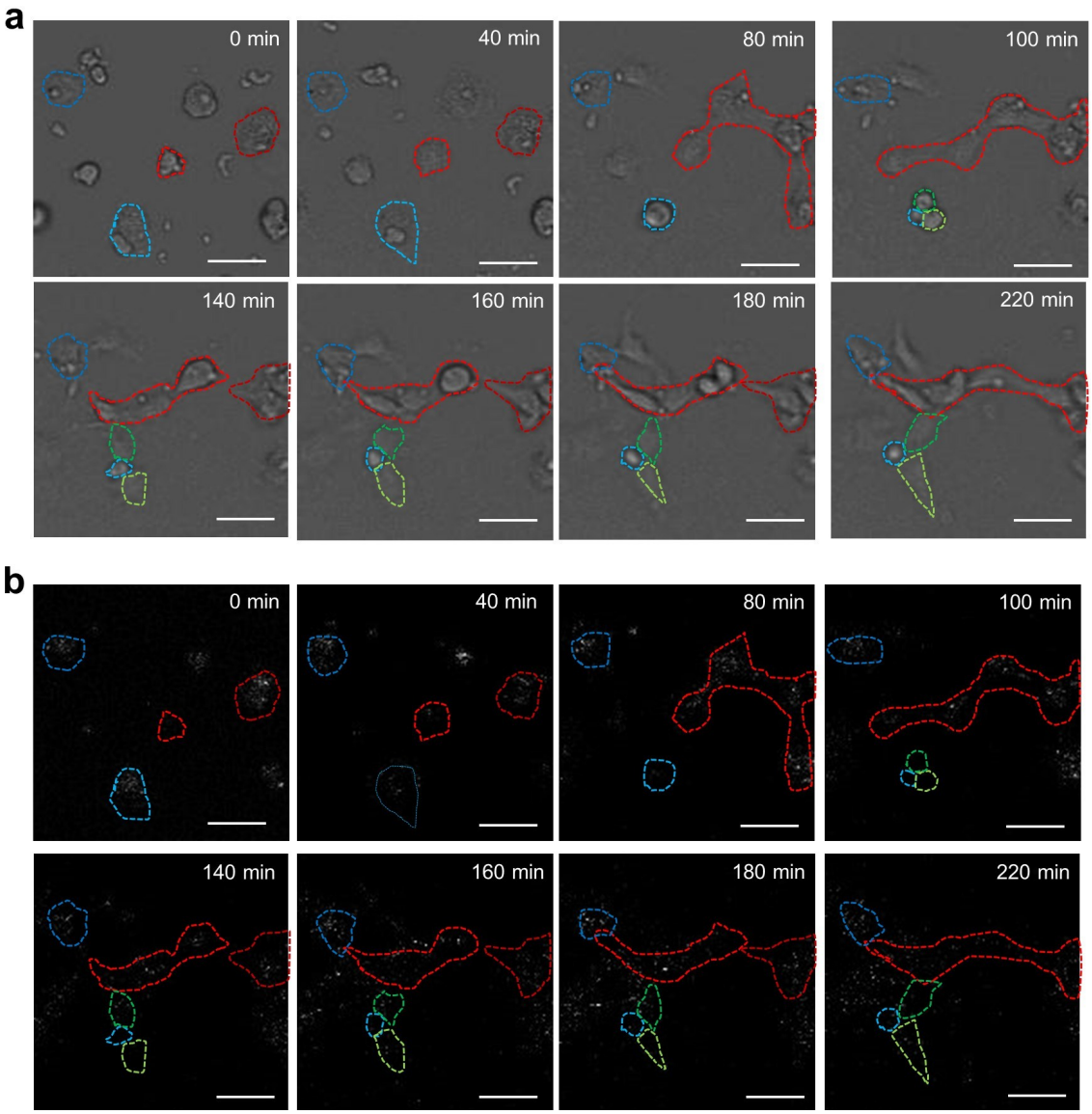

Fig. S3. Dynamic single cell analysis. (a) Bright-field images of endothelial cells during microvascular self-organization. (b) Fluorescence images of endothelial cells during microvascular self-organization. The cells were treated wtih random probes. Dotted lines indicdate the cell mophology. Scale bars, 50 μm. Images are representive from three independent experiments.

Fig. S4

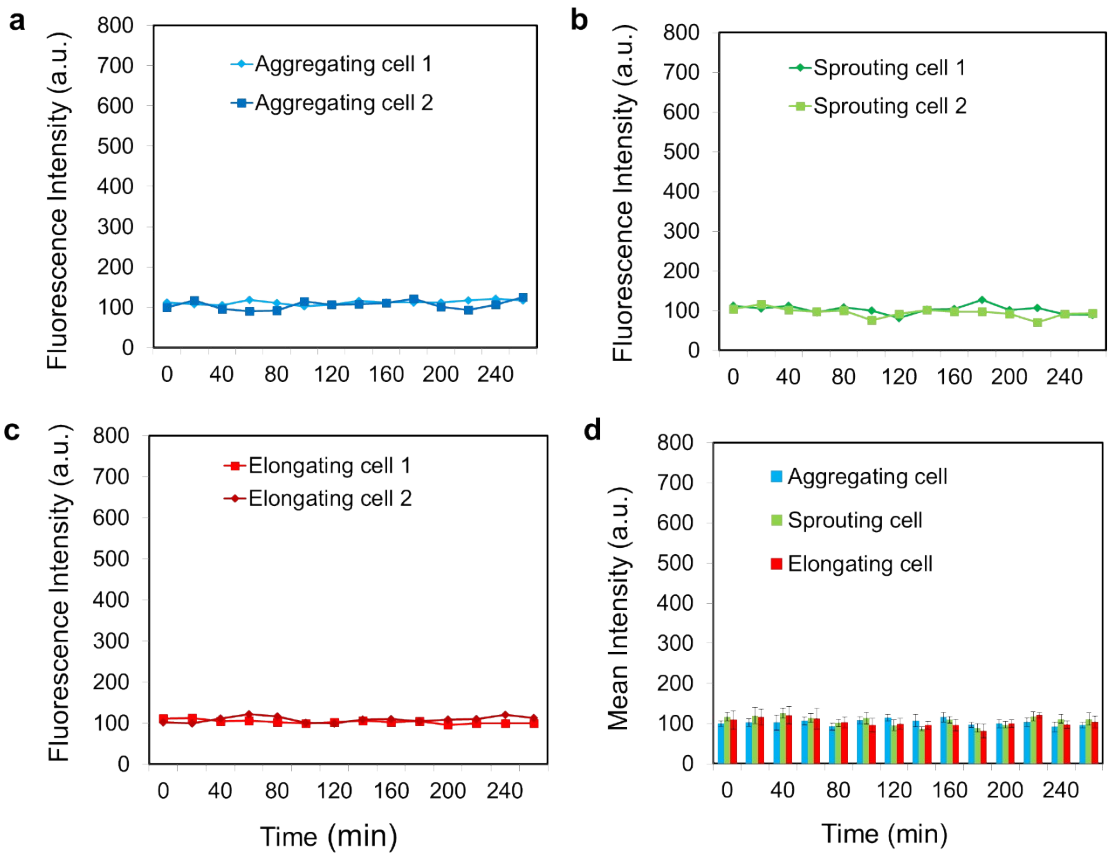

Fig. S4. The intensity of random probe during microvascular self-organization. (a-c) Tracking of fluorescence intensity of the random probe in representative (a) aggregating cells, (b) sprouting cells and (c) elongating cells. (d) Comparison of the average fluorescence intensities in cell subpopulations. Data represent over 100 cells in each group and expressed as mean ± s.e.m. (n=3).

Fig. S5

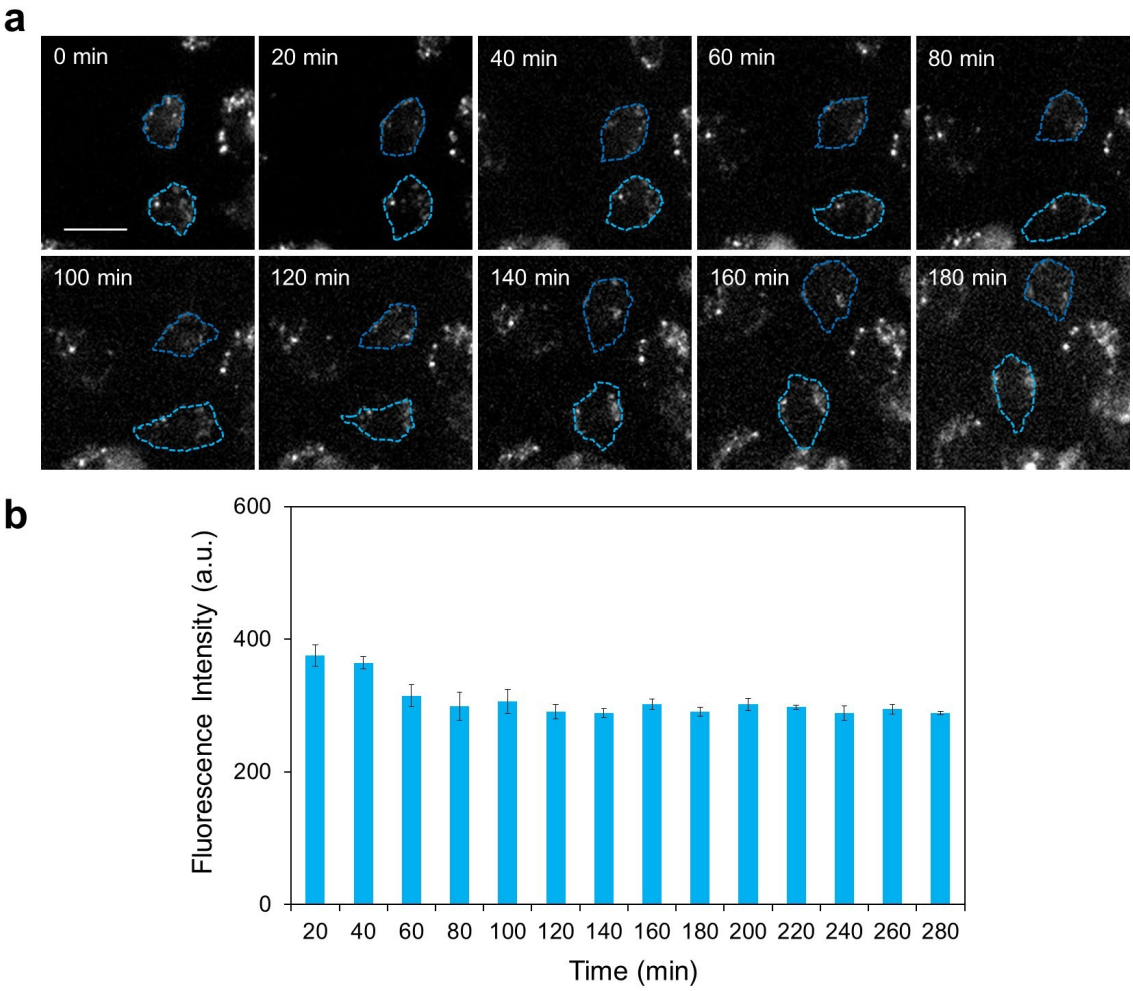

Fig. S5. Dynamic tracking of aggregating cells. (a) Dll4 mRNA tracking of aggregating cells during microvascular structure formation. (b) Average intensity of Dll4 mRNA expression of aggregting cells. Data represent over 100 cells and expressed as mean ± s.e.m. (n=3).

Fig. S6

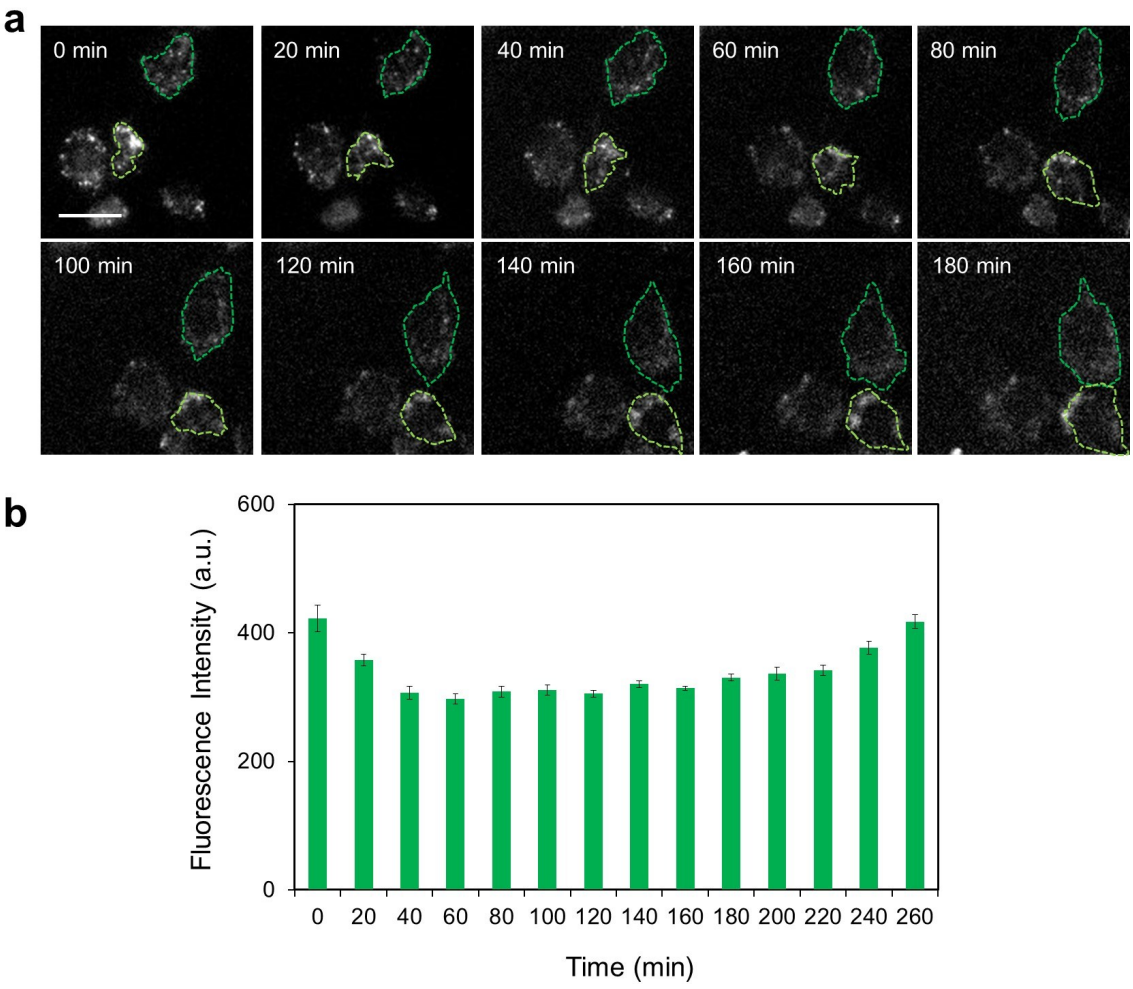

Fig. S6. (a) Dll4 mRNA tracking of sprouting cells during microvascular structure formation. (b) Average intensity of Dll4 mRNA expression of aggregting cells. Data represent over 100 cells and expressed as mean ± s.e.m. (n=3).

Fig. S7

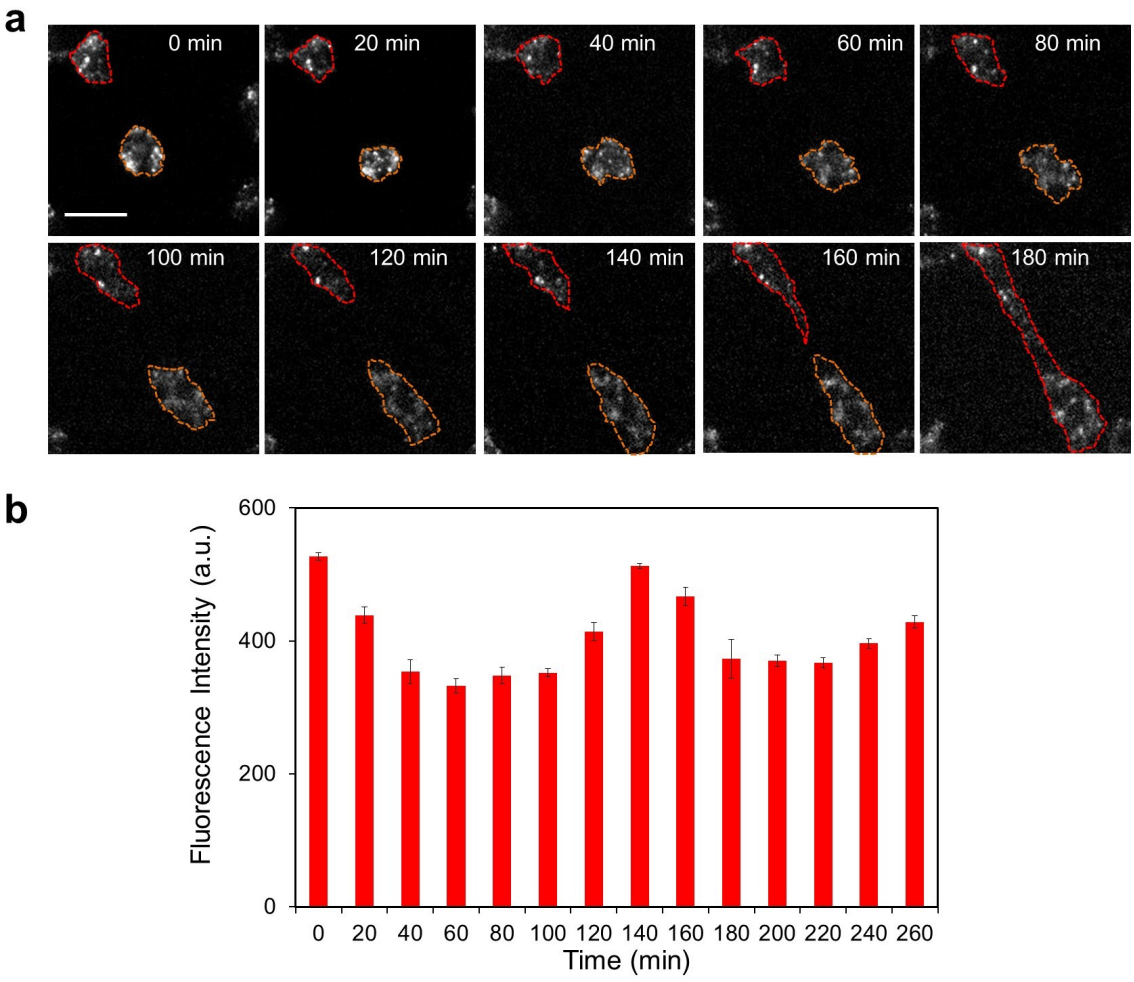

Fig. S7. (a) Dll4 mRNA tracking of aggregating cells during microvascular structure formation. (b) Average intensity of Dll4 mRNA expression of elongating cells. Images are representative of three independent experiments. Data represent over 100 cells and expressed as mean ± s.e.m. (n=3).

Fig. S8

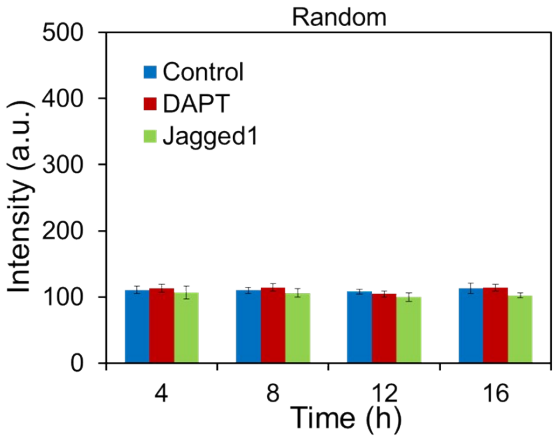

Fig. S8. Mean fluorescent intensity comparison of random probe in microvascular networks. Data represent over 100 cells in each group and are expressed as mean ± s.e.m. (n=5).

Fig. S9

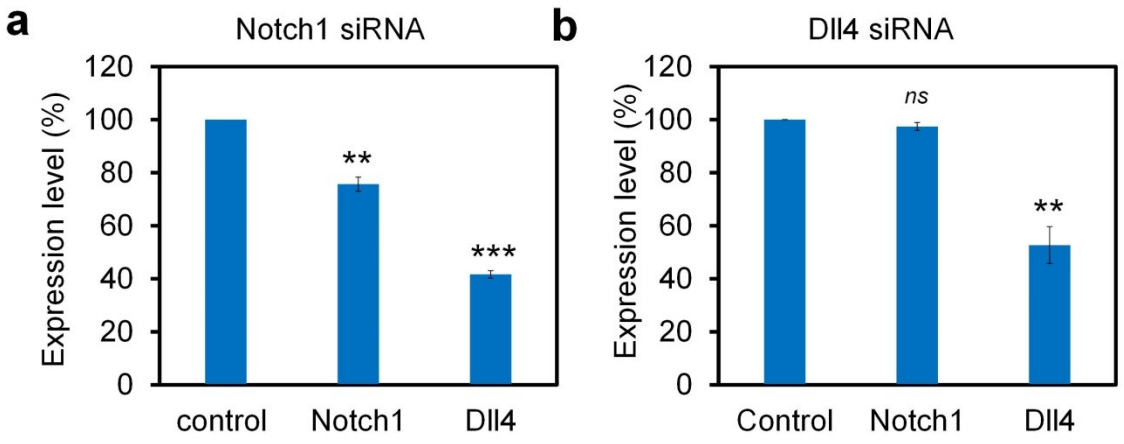

Fig. S9. Quantification of western blot results of Notch1 and Dll4 expression in HUVEC networks with (a) DII4 siRNA and (b) Notch1 siRNA. Data are representative from three independent experiments. (n=3, **P<0.05, **P<0.01, ***P<0.001*; unpaired Student's *t*-test).

Electrical Supplementary Material (ESI) for Nanoscale.

Tab. S1

Tab. S1. LNA probes and synthetic DNA target sequences

Name		Sequence (5'-3')	Fluorophore
Dll4	Probe	+AA+GG+GC+AG +TT+GG+AG+AG+GG+TT	/56-FAM
	Target	AACCCTCTCCAACTGCCCTT	
Random	Probe	+AC+GC+GA+CA+AG+CG+CA+CC+GA+TA	/56-FAM
	Target	TATCGGTGCGCTTGTCGCGT	

* + represents LNA monomer

Electrical Supplementary Material (ESI) for Nanoscale.

Tab. S2

Tab. S2. Kolmogorov–Smirnov two sample test results

	Dll4 mRNA		Random	
Treatment	DAPT	Jag1	DAPT	Jag1
h	1	1	0	0
p-value	1.57E-22	4.41E-15	0.556	0.2606
statistic (*D*)	0.7	0.57	0.11	0.14