Supplementary Data

Dll4-containing exosomes induce capillary sprout retraction ina 3D microenvironment

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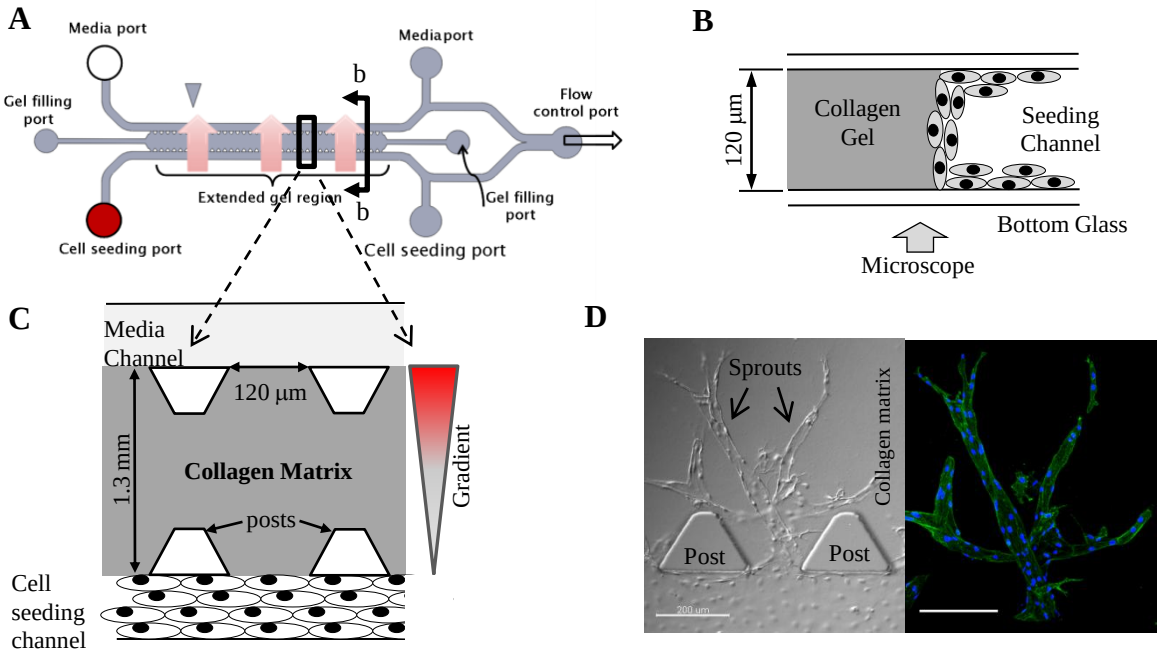


Figure S1. **Microfluidic device and 3D sprouting assay.** Microfluidic device provides a 3D environment for endothelial cell sprouting and morphogenesis. **A**) Layout of the microfluidic device consisting of a media channel connecting 2 media ports, a cell seeding channel, and a gel region in the middle. Arrays of trapezoidal posts cage collagen gel solution into the middle gel region, separating the gel from the media and cell seeding channels. Sprouting can be observed at 37 sites separated by the trapezoidal posts. **B**) Vertical view of cross-section b-b in A. Seeded HMVECs form a monolayer. Sprouts extend towards the collagen gel with 120 µm of depth. **C**) Details of the microfluidic device. Post spacing is 100-125 µm, whereas the width of the gel region is 1.3 mm. Diverse concentrations of VEGF applied to the two channels, the media and cell seeding channels, create a gradient of VEGF concentration over the gel region. **D**) DIC image of sprouting HMVECs 7 days after applying a VEGF gradient across the gel region. The sprouts grow from the monolayer towards the collagen gel region separated by the trapezoidal posts. HMVECs were subsequently fixed and stained for actin cytoskeleton (green) and nucleus (blue). Scale bar 200 µm.

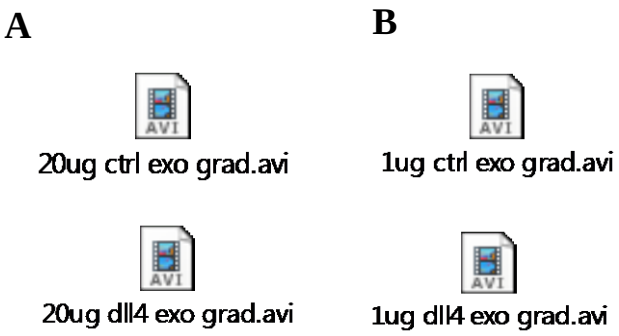


Figure S2. Dll4 exosomes cause retraction of tip cells and its filopodia at the leading edge of sprouts upon its application in the gradient. Time lapse video clips from HMVECs treated with exosomes for 9 hours were put together using Imaris software. Each set is a representative of one exosome concentration. **A)** 20 $\mu g ml^{-1}$, **B)** 1 $\mu g ml^{-1}$.

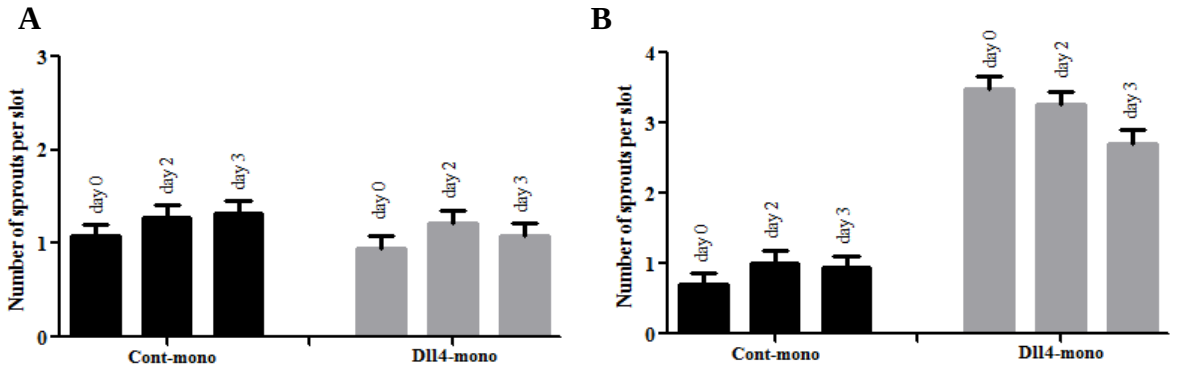


Figure S3. . Dll4 exosomes reduces the number of sprouts only when they are applied in the gradient form (media channel) at high concentration HMVECs were seeded and after formation of the monolayer they were conditioned with VEGF for three days to allow formation of the sprouts. At day three, images from all 37 slots were taken by light microscopy before addition of exosomes directly to the cells monolayer at different concentration. This is labeled as day 0 on the bars. Fresh medium with exosomes were added daily for the next two days and images were taken again on day 2 and day 3. The number of sprouts in each slots were counted from the images and plotted using Prism software. The experiment was performed at two different concentrations of exosomes. **A)** 1 μgmL^{-1}, and **B)** 50 μgmL^{-1}. There was no significant differences between day3 and day0 for control exosomes and Dll4 exosomes.

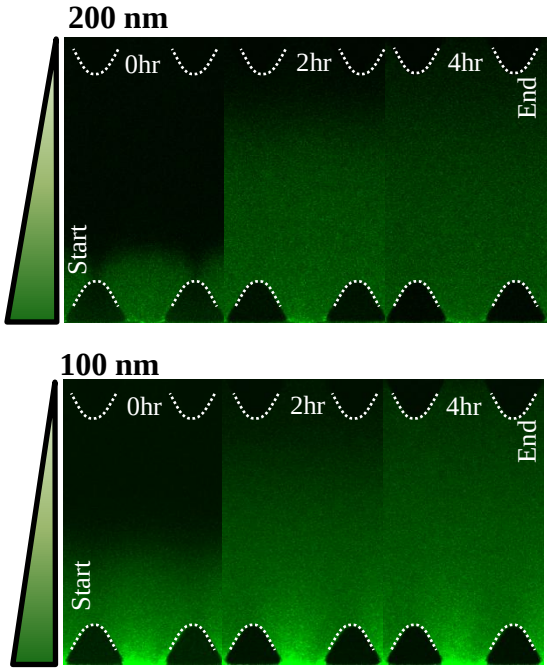


Figure S4. Collagen gel at 2.5 mg/ml is porous to 100 and 200 nm fluoresbrite®YG microspheres. Microspheres were diluted 100x in EGM-2 and were applied to the seeding channel of MFD. They were immediately subjected to time-lapse confocal microscopy and monitored for 6 hours with 15 minutes time intervals. After three hours, the beads reached the opposite channel diffusing through the collagen gel. Each panel represent the distribution of the beads across the collagen gel at the stated time point. Scale bar 200 μm.

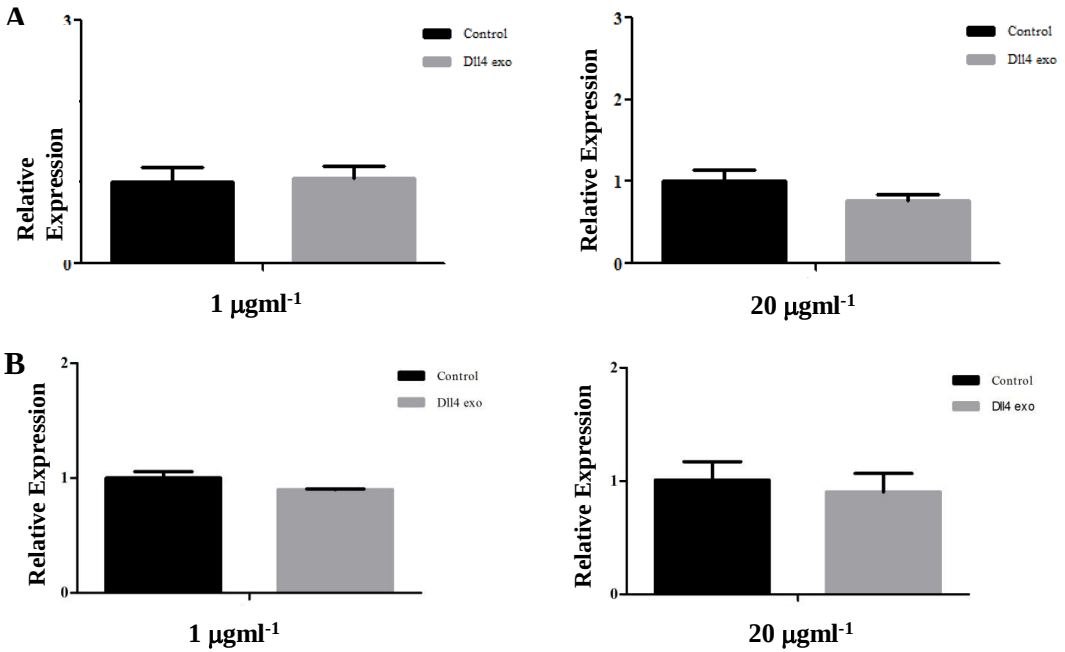


Figure S5. Dll4 exosomes have no significant effect on Nrp1 and Jag1 expression level. HMVECs were seeded in 6 well plates at a density of $2.5x10^5$ cells per well. Once they reached 80-90% confluency, they were starved for 2hours and conditioned with 1 μgmL^{-1} and 20 μgmL^{-1} exosomes and incubated for 8 hours. RNA extraction was followed at the end point and used to set up qRT-PCR. **A)** Nrp1, **B)** Jag1

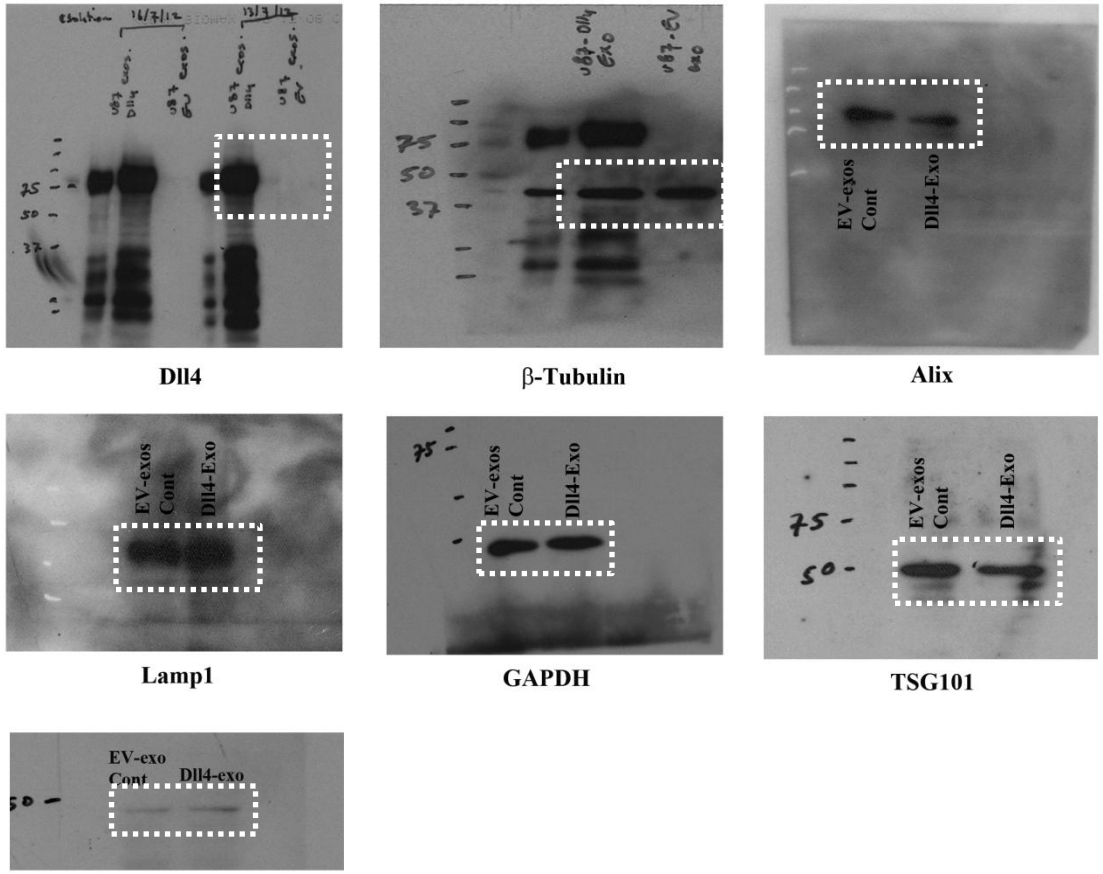


Figure S6. The original images of blots used in Figure 2B. The dotted box on each blot represent the cropping line.

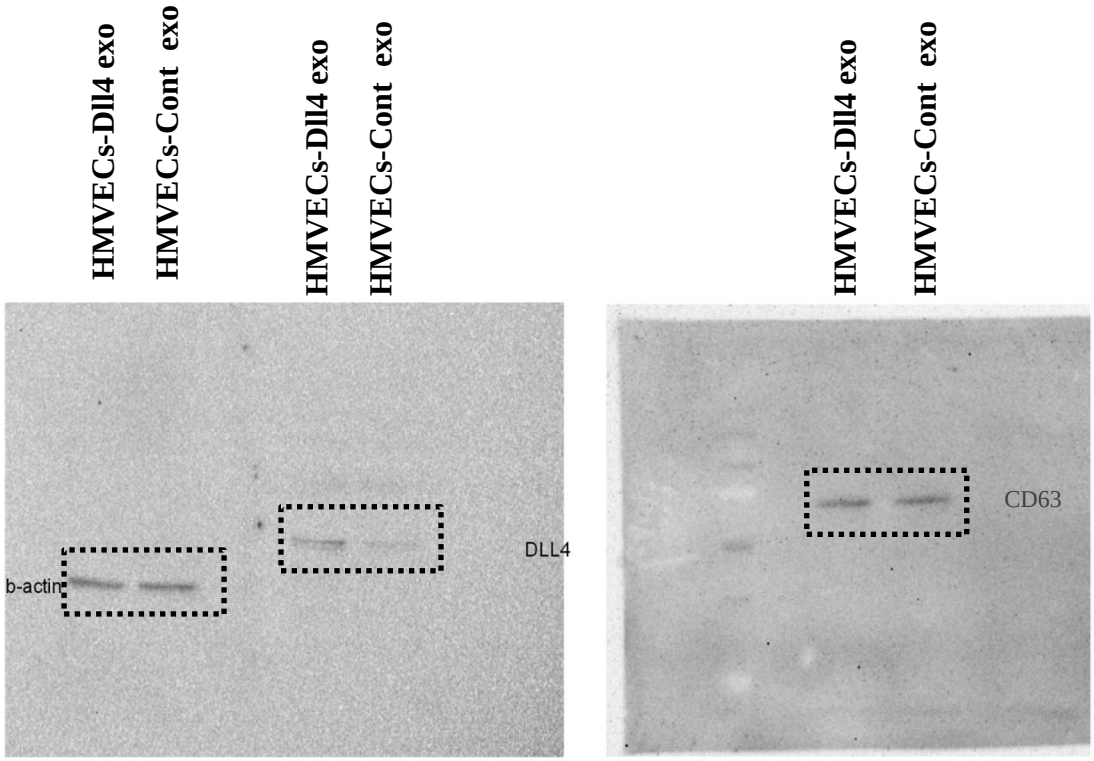


Figure S7. The original images of blots used in Figure 2C. The dotted box on each blot represent the cropping line.

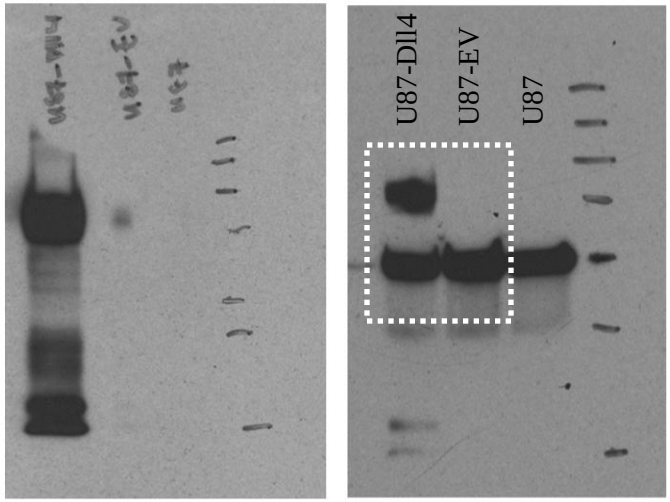
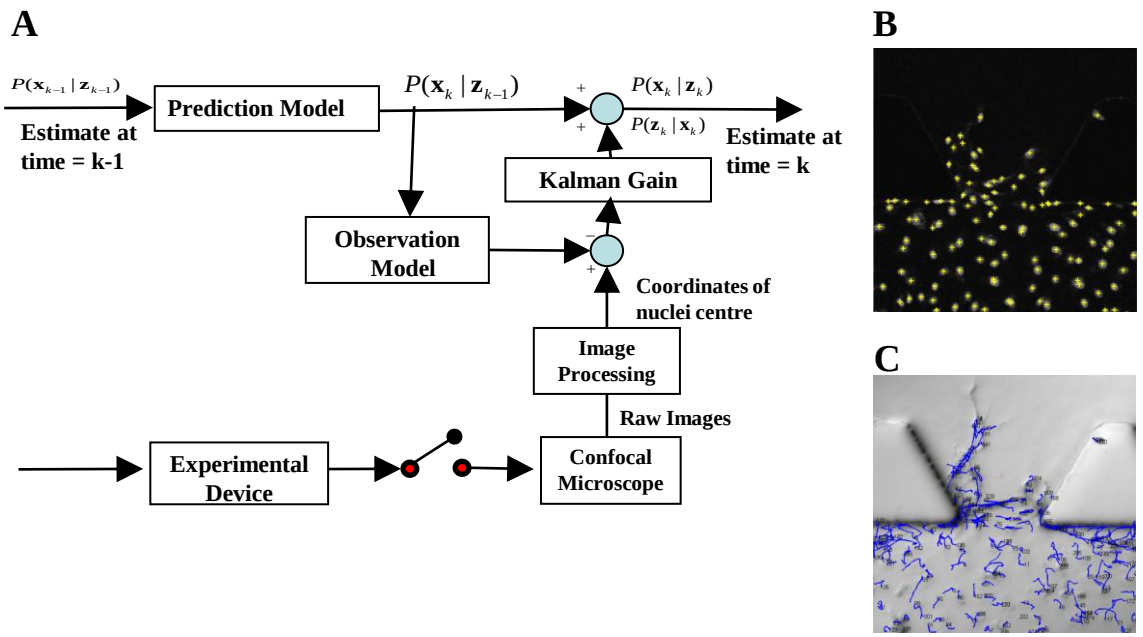


Figure S8. The original images of blots used in Figure 2C. The dotted box on the blot represent the cropping line. The blot was probed with anti-Dll4 antibody first and then with α-tubulin.

Supplementary Method



Description of image processing workflow: The tracking system, shown in Figure (A), consists of image processing, data association, and filtering. Image processing is applied to segment and label cell regions for each frame of time series images to determine the position coordinates of each nucleus center. To keep track of each nucleus in the time sequence, data association is performed to associate each nucleus position to the one in the previous time frame of images. The states estimated (the position and velocity of the nuclei) are propagated across time frames through a process model and corrected with the nuclei positions segmented at each time frame. The amount of correction is determined by the Kalman gain. The result is an estimate updated with information from all past estimates. **Image Processing for Cell Detection:** Nuclei candidates are segmented from the fluorescence images. We convolve each image with a Gaussian kernel, followed by a Laplacian operator to improve nuclei region contrast. K-means clustering was then applied to classify the image pixels into either cell or background regions. These cell pixels are clustered based on connected components clustering . A cluster may contain more than one nuclei to do partial area overlap or close cell contact. To separate these nuclei, we apply level sets on the clusters. The nuclei candidates are parameterized by the centroid of each cluster shown in Figure B.

Figure S1. Overview of modules for automated image processing. A) From experiments in micro-fluidic assays, 3D confocal images are acquired at 30 minute intervals with 20x objective. The centroid of each nuclei candidate is extracted in the image processing module. Nuclei tracks are created for each nuclei candidate segmented from the initial image acquired. Each nuclei centroid segmented from images later in the acquisition is associated with existing nuclei tracks. Matched candidates are updated to an existing track via Kalman filtering. New tracks are created for unmatched candidates. **B)** A maximum intensity projection of a 3D confocal image of stained nuclei is shown by the white blobs. The segmented nuclei centroids are labeled with yellow '+'. **C)** Nuclei centroid trajectories are obtained from our automated image processing software. An example of these trajectories are displayed by the blue lines against its corresponding transmitted light image in the xy plane.