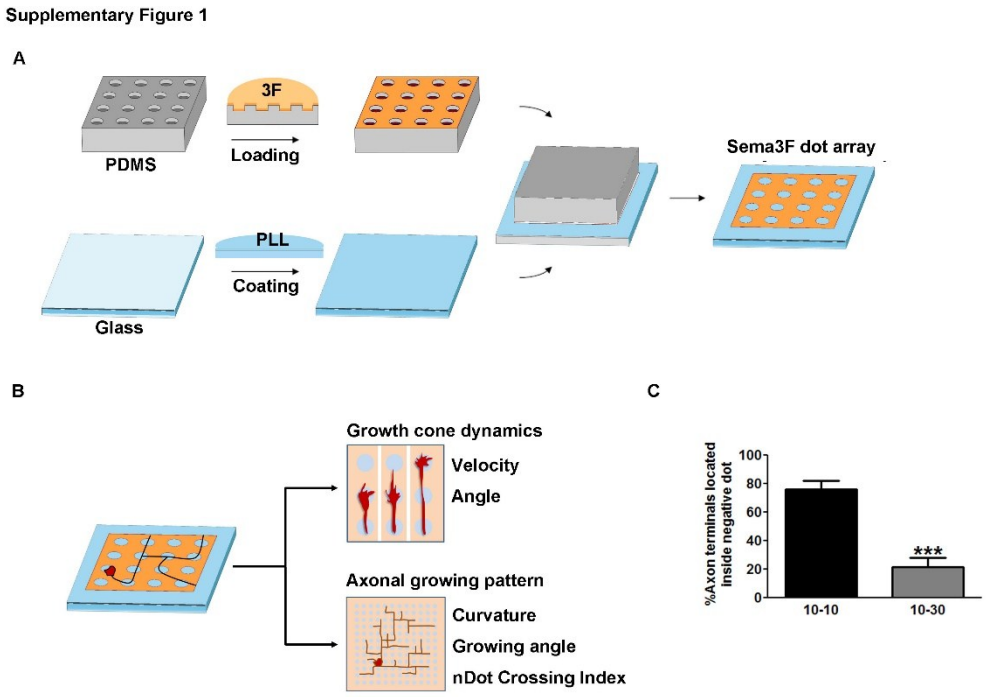
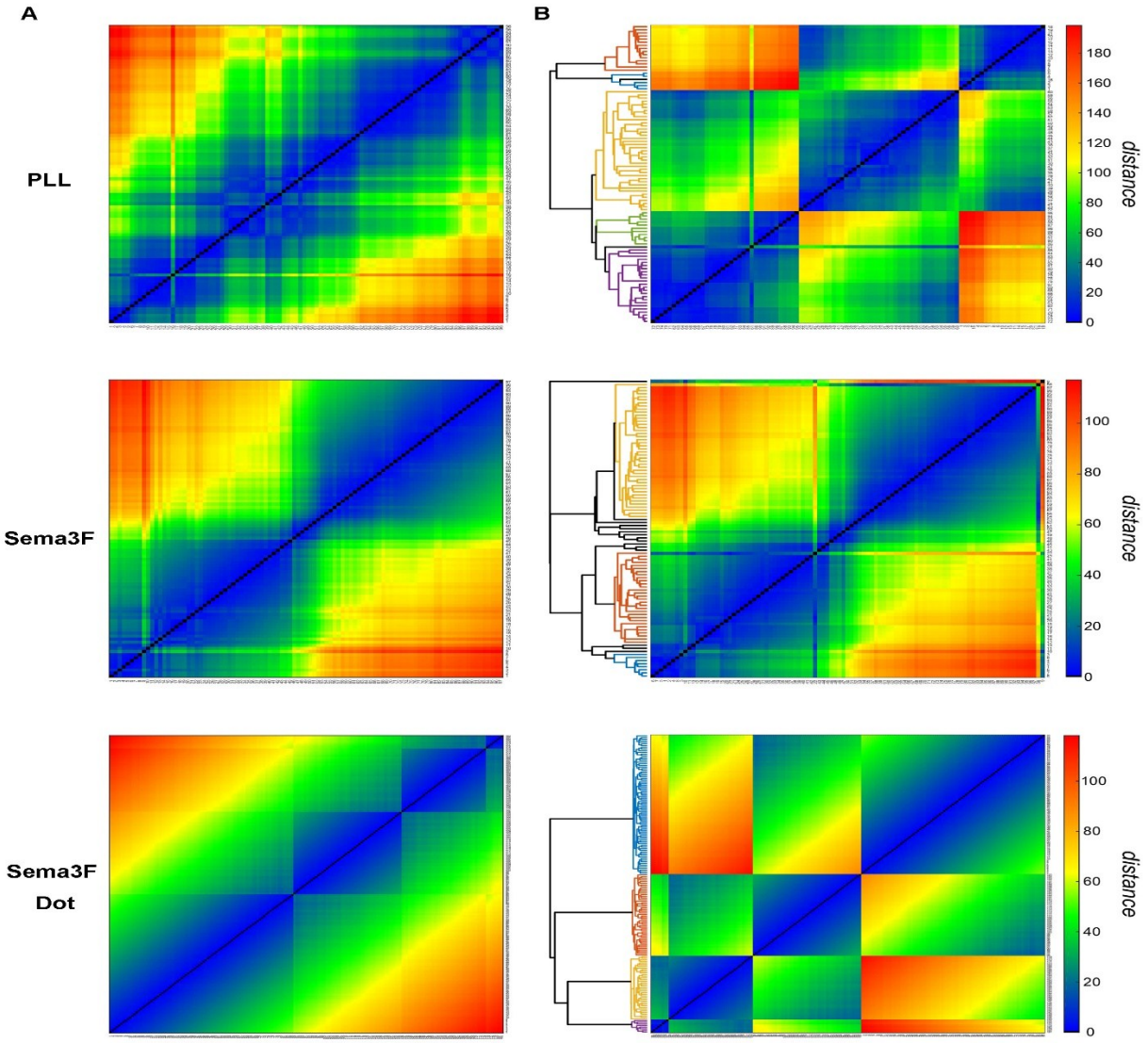
Electronic Supplementary Material (ESI) for Lab on a Chip.

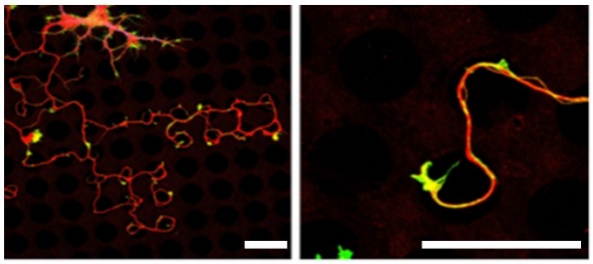

**Supplementary Figure 1.** Summary of experimental system and analytical tools. (A) Illustration of a 3F dot array system. Repulsive signal Sema3F are overlaid on a polydimethylsiloxane stamp and the stamp was applied to a PLL-coated glass. Sema3F is printed outside the dot, and dots are coated only with permissive substrate, poly-l-lysine without Sema3F. (B) An overview of analytical tools used in the present study. (C) Percentage of axon terminals located inside the dot in the 10-10 and 10-30 dot arrays.

Supplementary Figure 2



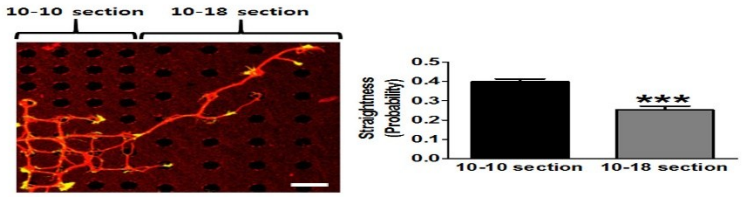

**Supplementary Figure 2.** Clustering of axon movement. Examples of the distance heat map (A) for a growth cone trajectory of each group in Fig. 1G, and reordered heat map (B). Each cluster was differently colored as shown in the dendrogram. Numbers indicate time sequences and were reordered by the clustering.

**Supplementary Figure 3**

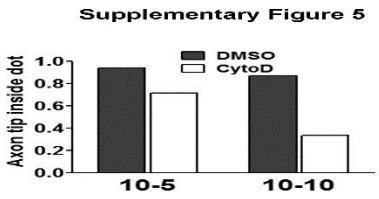


**Supplementary Figure 3.** Axonal morphology of neurons grown in 10-17 dot arrays. Neurons were stained with Tuj1 antibody and fluorescence-labeled phalloidin. Scale bars: 30 μm.

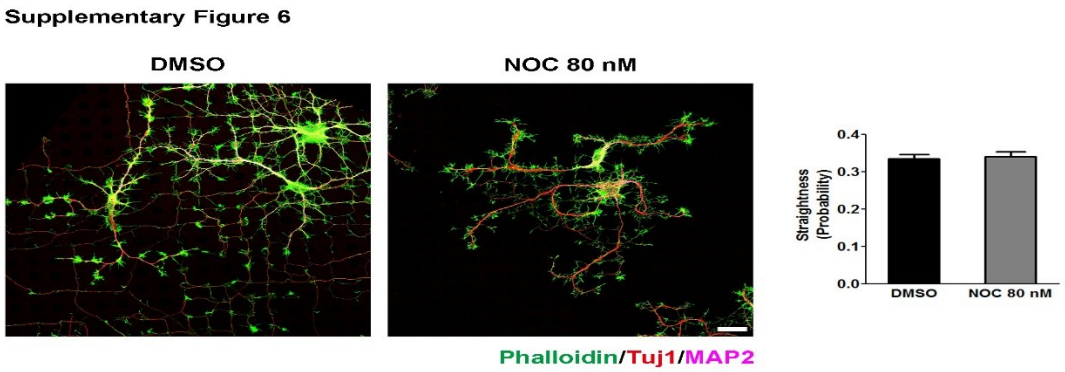
**Supplementary Figure 4**




**Supplementary Figure 4.** Axonal morphology and the probability of straightness from angle distribution analysis of neurons grown on the Sema3F micropattern with alternating sections of 10-10 and 10-18 dot arrays. Scale bar: 30 μm, ***: $p<0.001$ compared with "10-10 section" group.

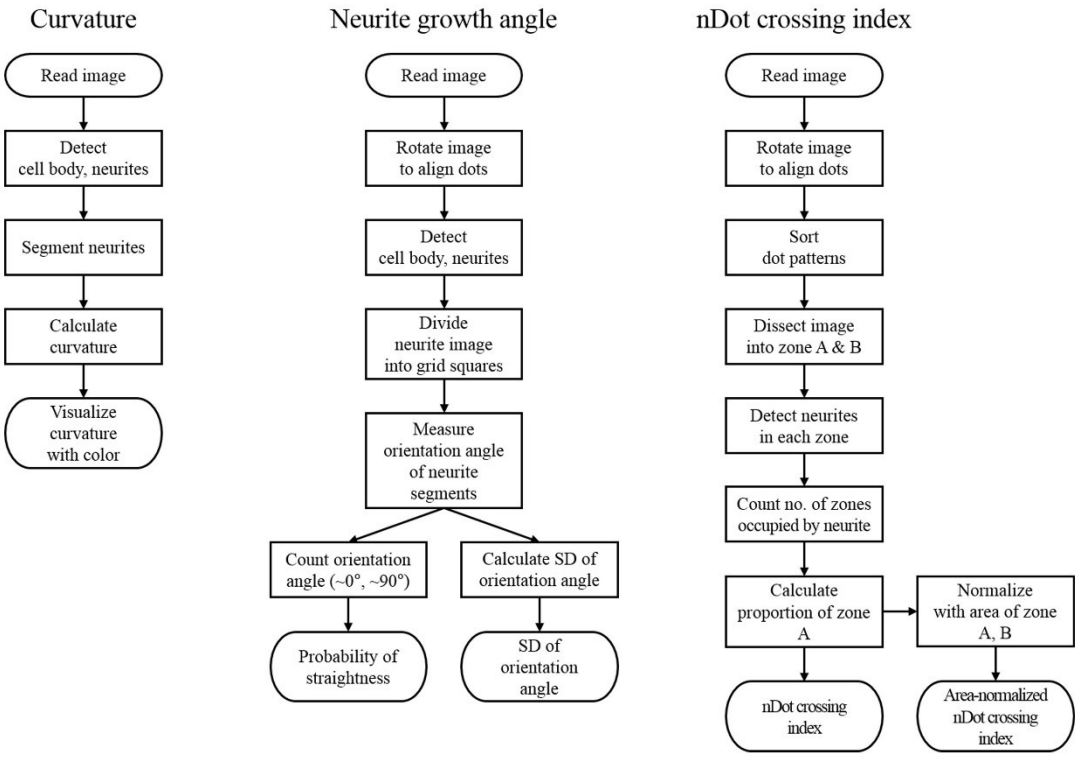



**Supplementary Figure 5.** The relative probability of axon tips located inside the dot was measured for each dot array.




**Supplementary Figure 6.** Effect of microtubule stability on the growth pattern of axons in 10-10 dot array. Representative images of neurons and growth cones treated with DMSO and 80 nM nocodazole (NOC) in 10-10 dot array. The probability of straightness from angle distribution analysis. Scale bar: 30 μm.

**Supplementary Figure 7**




**Supplementary Figure 7.** Flowchart for analysis of curvature, neurite growth angle and nDot crossing index. Analysis was performed using MATLAB software and ImageJ.

**Supplementary Table 1. DIVs of cultured neurons and sample sizes used for analysis**

| Figure No. | DIV of neurons | # of Samples |
|---|---|---|
| Fig. 1B | 3 → 4 | None (28), 3F (40) |
| | 3 → 4 | None (24), 3F dot (66) |
| Fig. 1D | 3 or 4 | 69 |
| Fig. 1H | 3 → 4 | Clusters: None (23), 3F (15), 3F dot (42) |
| | | Unclustered: None (14), 3F (9), 3F dot (26) |
| Fig. 2A | 3 | 20 ~ 25 |
| Fig. 2B | 3 | 15 ~ 25 |
| Fig. 3 and Fig. 4 | 9 | 5 (155), 10 (32), 15 (18), 20 (19), 25 (19), 30 (30) |
| | | # of segment for curvature analysis: 18070, 5361,2430,2177, 3098, 2945 |
| | | # of segment for angle analysis: 33420, 9060, 4193, 3602, 5914, 7316 |
| Fig.3C and 3D | 3 → 4 | 15 ~ 45 |
| Fig. 5A | 3 | Vehicle (31), CytoD (62) |
| Fig. 5C | 8 | 10-5 DMSO (23), CytoD (9) |
| | | # of segment for angle analysis: 5509, 6504 |
| | | 10-10 DMSO (19), CytoD (13) |
| | | # of segment for angle analysis: 5757, 6211 |
| Fig. 6A | 3 | Vehicle (25), 8-Br-cAMP (30), 8-Br-cGMP (28) |
| Fig. 6C | 8 | Vehicle (25), 8-Br-cAMP (23), 8-Br-cGMP (17) |
| | | # of segment for angle analysis: 8389, 8264, 7529 |
| Fig. 6D | 8 | Vehicle (22), Rolipram (22), MBMQ (38) |
| | | # of segment for angle analysis: 22321, 8357, 10109 |
| Fig. 7 | 8 | -L1 (22), +L1 (17) |
| | | # of segment for angle analysis: 4899, 2645 |
| Suppl Fig. 1C | 4 | 10-10 (55), 10-30 (48) |
| Suppl Fig. 4 | 8 | 14 axons |
| Suppl Fig. 5 | 8 | 40 for each group |
| Suppl Fig. 6 | 8 | DMSO (21), NOC (45) |
| | | # of segment for angle analysis: |

| | | |
|---|---|---|
| | | 2433, 793 |

**Supplementary Movie 1.** Axonal elongation in 10-10 dot array. Images were acquired every 5 min for 270 min. Red fluorescent dot array images and differential interference contrast (DIC) images of an axon were merged.

**Supplementary Movie 2 and 3.** Axonal elongation in 10-10 dot array. Images were acquired every 14 min for 44 hrs.

**Supplementary Movie 4.** Axonal elongation in 10-30 dot array. Images were acquired every 10 min for 40 hrs.

**Supplementary Movie 5.** Axonal elongation with treatment of Cytochalasin D in a 10-10 dot array. Images were acquired every 1 h for 11 h.

**Supplementary Table 1.** DIVs of cultured neurons and sample sizes used for analysis