Supplementary Figure 1. SEM image of FF (A) and PF (B) surfaces after 28 days differentiation. White arrow indicates groove direction; yellow arrow indicates secondary cell/ECM alignment. Scale bar =  $10 \mu m$ .

Supplementary Figure 2. Additional images illustrating secondary alignment of cells on PF after 28 days of differentiation. Top, 10x (A) and 20x (B) phase contrast images. Bottom, enlarged version of Figure 7F showing actin (red) and nuclei (green). White arrow indicates groove direction; yellow arrow indicates secondary cell alignment. Scale bars =  $200 \mu m$  (A),  $100 \mu m$  (B,C).

## **Supplementary Methods**

## **Scanning Electron Microscopy (SEM)**

Samples were fixed in 4% paraformaldehyde for 30 minutes and then dehydrated in a series of graded ethanol solutions. The dehydrated samples were punched from the bottom of the 24-well culture plates and affixed to a specimen stub with carbon tape. The cells and films were then sputtered with gold and imaged on a JEOL JSM-840 scanning electron microscope.



