**Fig. S1.** Culture channel after cell seeding. Images of representative sections along the length of the culture channel are presented for two different seeding densities (lower in panel A and higher in panel B). Approximate positions of the images are indicated by the red squares highlighting the culture channel in the microbioreactors representations. In both cases it can be noticed how only few cells were adhering into the small lateral channels, connecting the flow and culture channels.
Fig. S2. Effect of surface forces and adhering cells on the gradient shape. The presented results refer to simulations performed using the experimental flow rate and Wnt3a diffusion coefficient. We simulated the behavior of the microbioreactor under three different conditions: A. absence of the surface forces related component, B. presence of the surface forces related component and absence of adhering cells (as in the manuscript), C. presence of both components, considering a 20 microns high (over-dimensioned) cell layer adhering to the bottom of the channels. Surface forces play a significant role in determining the shape of the gradient (panels A and B) while the cross section reduction due to the presence of cells doesn’t significantly affect the formation of the desired concentration gradient (panels B and C).
Fig. S3. Activation of the canonical β-catenin pathway in response to constant levels of Wnt3a. Left panels: A375-BARVS cells were cultured in 96 well plates at different concentrations of Wnt3a conditioned medium; numbers indicate % dilution of conditioned medium. Top panels: activation of the β-catenin pathway shown by fluorescent Venus signal, and merged images of Venus signal and nuclear Hoechst stain for each of the Wnt3a concentrations tested. Merged of Venus signal and bright field images are also shown. Images were taken after 12 hours of exposure to the ligand. Bar graph: Venus expression assessed by quantitative image processing was proportionate to the concentration of Wnt3a until a certain threshold.
**Fig. S4.** Quantification of the fluorescence expression along the length of the culture channel. From the analysis of two different microbioreactors within the same experimental run, we obtained the reported histograms, presenting data of the average fluorescence levels (Mean Grey Value, as given by ImageJ) of each channel section (top, mid and bottom) and position across the width of the culture channel (left, mid and right), with the same notation used in the main manuscript. The fluorescence value of the background was subtracted from each data point.