



Figure S3

**A:** Transmigration assay of immature DCs: cells were loaded in the upper chamber of a 5µm pore collagen coated-transwell assay in the presence of 5µM XestC or DMSO and counted after overnight migration. The median +/- standard deviation from 3 independent experiments is shown. The p-value was calculated with a paired t-test.

**B-F-H:** Analysis of immature DCs migrating in micro-channels in the presence of 5µM XestC or DMSO as control ( $n>260$  cells per condition from three independent experiments).

**B:** Median instantaneous speeds of immature DCs migrating in micro-channels in the presence of 5µM Xest C or DMSO ( $n>260$  cells from three independent experiments). Boxes illustrate 10-90 percentiles of values and whiskers represent the range of values. The Mann-Whitney t-test was applied for statistical analysis.

**C:** XestC does not induce DC maturation. Immature DCs were incubated 16 hours in the presence of 5µM of XestC or DMSO as control and analyzed for CD86 expression by flow cytometry. Values from one experiment are shown

**D:** Top panel, Flow Cytometry analysis of shScramble-(grey), shIP<sub>3</sub>R(1,3)A- (blue), shIP<sub>3</sub>R(2,3)B- (red) and shIP<sub>3</sub>R(1,3)C- (green) -silenced DCs showing no increase of CD86 surface expression upon lentiviral infection. One representative experiment out of three is shown.

Bottom panel, Flow Cytometry analysis showing the intracellular Ca<sup>2+</sup> levels of shScramble-(grey) and shIP<sub>3</sub>R(1,3)A- (blue), shIP<sub>3</sub>R(2,3)B- (red) and shIP<sub>3</sub>R(1,3)C-(green)-silenced DCs. One representative experiment out of three is shown.

**E:** One representative (out of three) transmigration assay of immature DCs silenced for IP<sub>3</sub>R(1,3)A and A' (blue), IP<sub>3</sub>R(2,3)B and B' (red) and IP<sub>3</sub>R(1,3)C and C' (green). The median of quadruplicates is shown.

**F:** Velocity fluctuations ( $\Delta V/V_0$ ) of immature DCs migrating in micro-channels. Boxes illustrate 10-90 percentiles of values and whiskers represent the range of values. The Mann Whitney test was applied for statistical analysis.

**G:** Percentage of cells changing direction during migration in micro-channels. A paired t-test was applied for statistical analysis.

**H:** Dot plot showing the frequency of changes in direction displayed by immature DCs that changed direction at least one time. The Mann Whitney test was applied for statistical analysis.

**I:** Scheme of the 2D-confined micro-fluidic device used in Figure 4. It is composed of a flat glass surface (bottom) and a PDMS ceiling (top) covered with 5 µm-high pillars. Both the bottom and the top are coated with fibronectin.