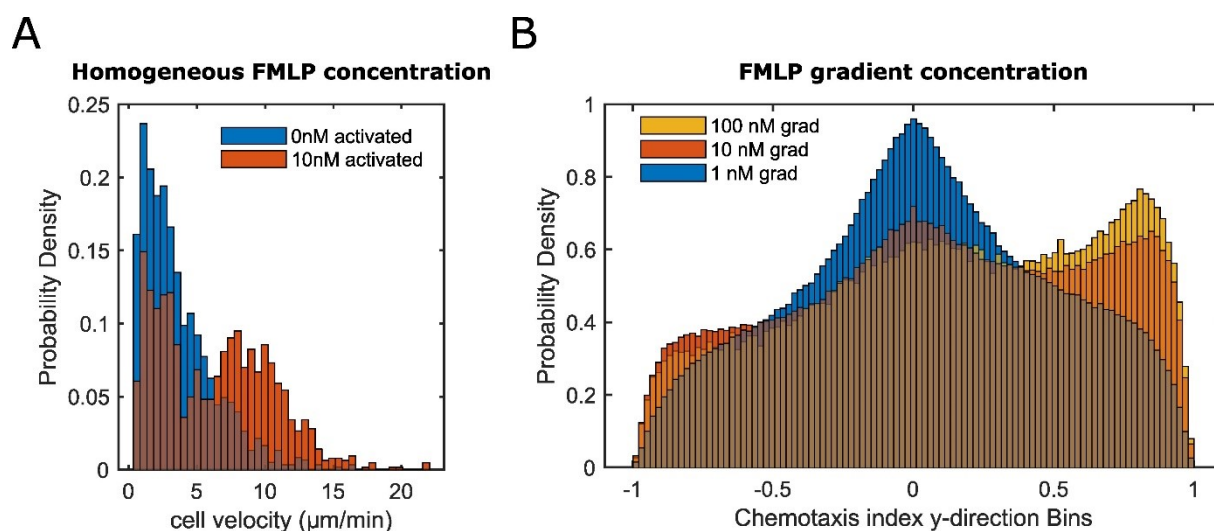


**Supplementary Figure S1** : Gradient experiment performed using the canal design shown in Figure 1D. **A** - Left - Fluorescence intensity of fluorescein in the channels at the beginning of the experiment. Initially (at  $t = 0$  h), the source chamber is filled with fluorescein in water (or the studied protein in cell media), the test chamber is filled with water (or cells in media), and the sink chamber is filled with water (or cell media). Middle - Fluorescence intensity of fluorescein in the channels after 1h of the gradient experiment (with a diffusion sequence duration ( $\tau_{\text{diff}} = 5$  min)). Right - Fluorescence intensity during the refill sequence, where the source chamber is refilled with fluorescein in water and the sink chamber with water, while the test chamber remains unchanged, maintaining a chemical gradient (scale bar: 100  $\mu\text{m}$ ). **B** - Design of the canal shown in Figure 1D. The canal is divided into three parts: sink (green), source (red), and middle (purple), separated by long microvalves. These microvalves act like microwalls when inflated (closed), and when deflated (open), the three parts form a single continuous canal.



**Supplementary Figure S2**: **A** - Probability density of the cell velocity for activated cells (roundness  $< 0.8$ ) in a homogeneous FMLP concentration of 0nM (blue) and 10nM (orange). **B** - Probability density of the chemotaxis index in the y-direction (aligned with the gradient) for an FMLP gradient of 0-1nM (blue), 0-10nM (orange), and 0-100nM (yellow).