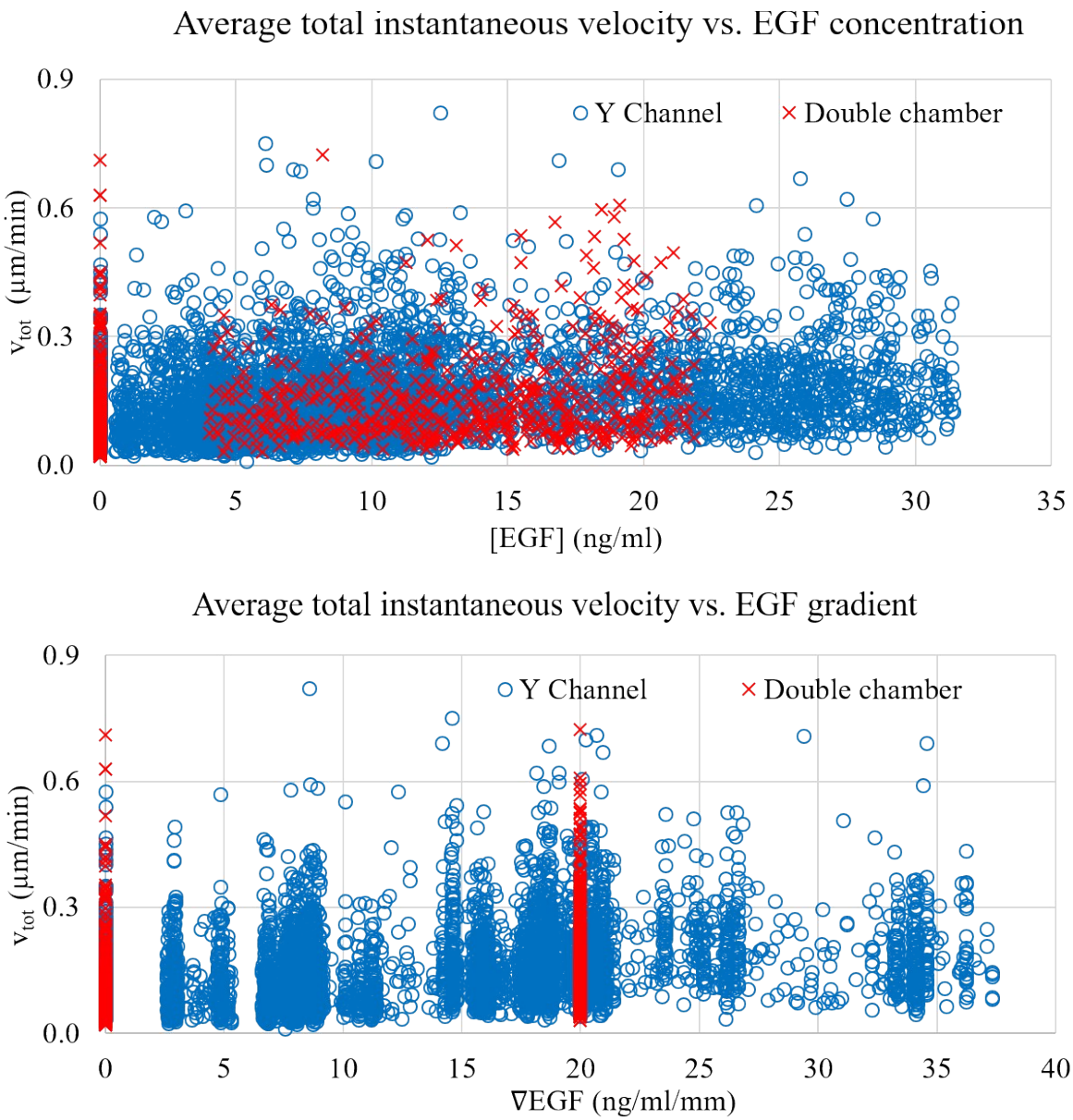
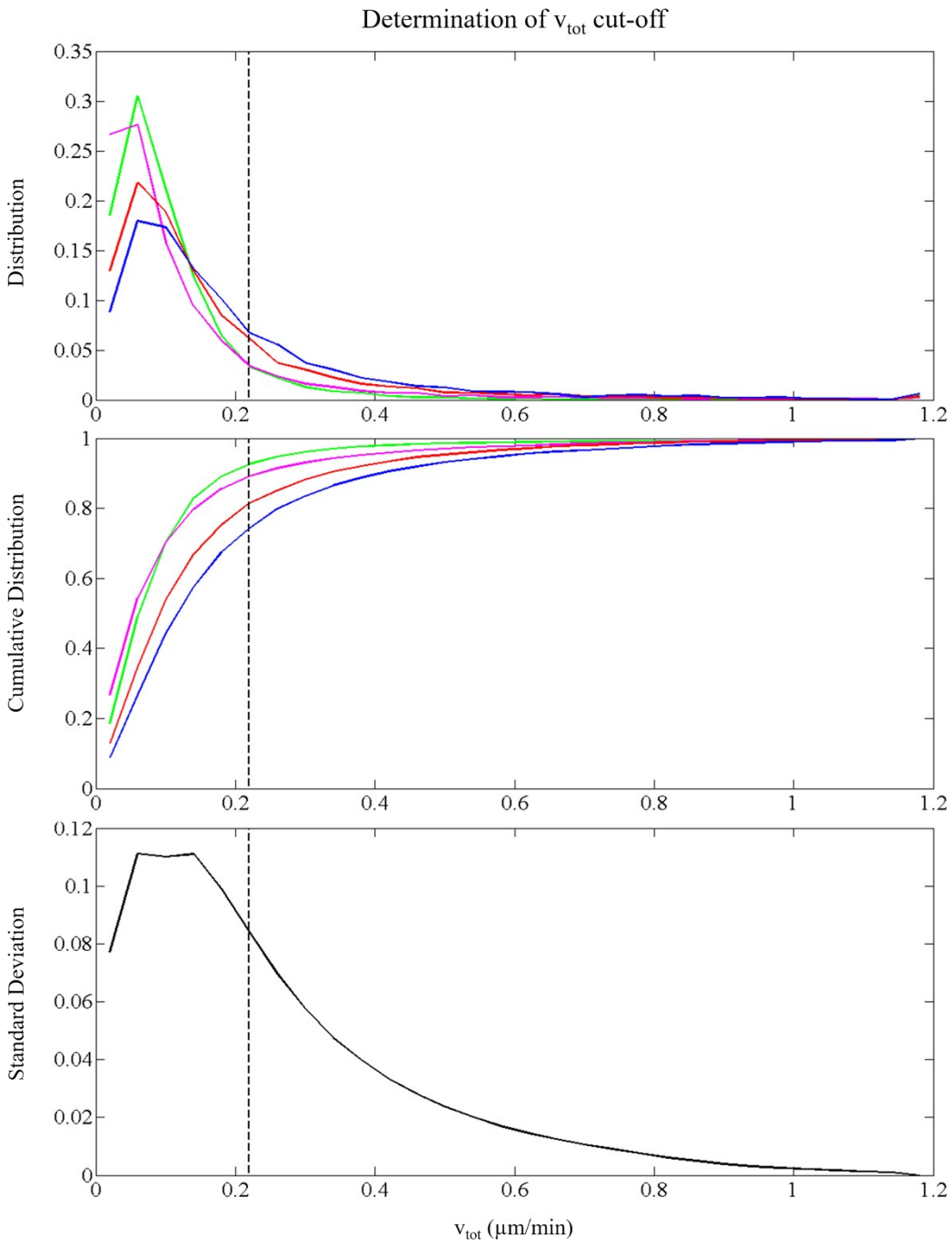
Electronic Supplementary Material (ESI) for Molecular BioSystems.

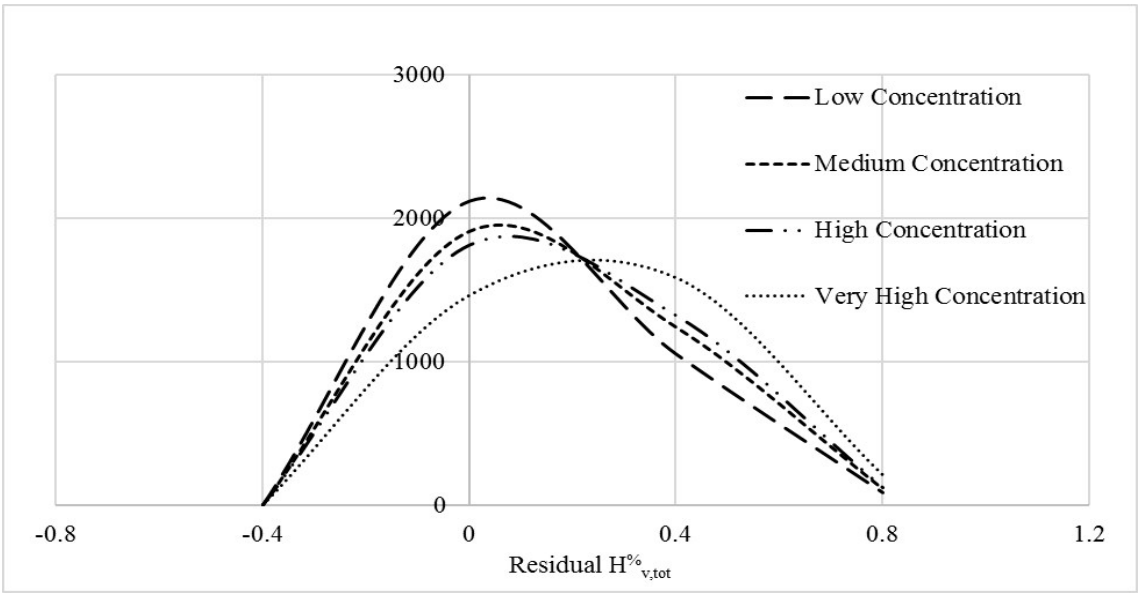
SUPPLEMENTARY FIGURES

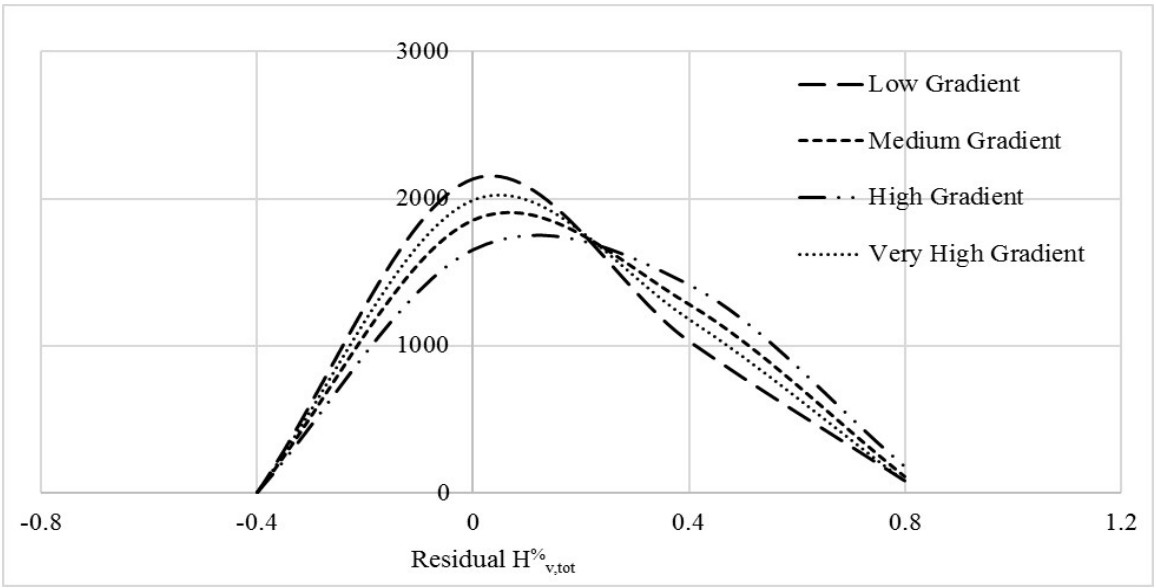


Supplementary Figure 1. Average instantaneous total velocity v_{tot} (µm/min) of individual cells vs **(top)** EGF concentration (ng/ml) and **(bottom)** EGF gradient (ng/ml/mm). Each point in the figures represents the result for a single cell. Cell velocity results obtained in the experiments with the double chamber (red, crosses) and y-channel (blue, circles) devices are shown together. As seen, results obtained with these two devices overlap very well. Although there is a noticeable upward trend with increasing ∇EGF, measured v_{tot} values are randomly distributed around a constant value at all [EGF] values.

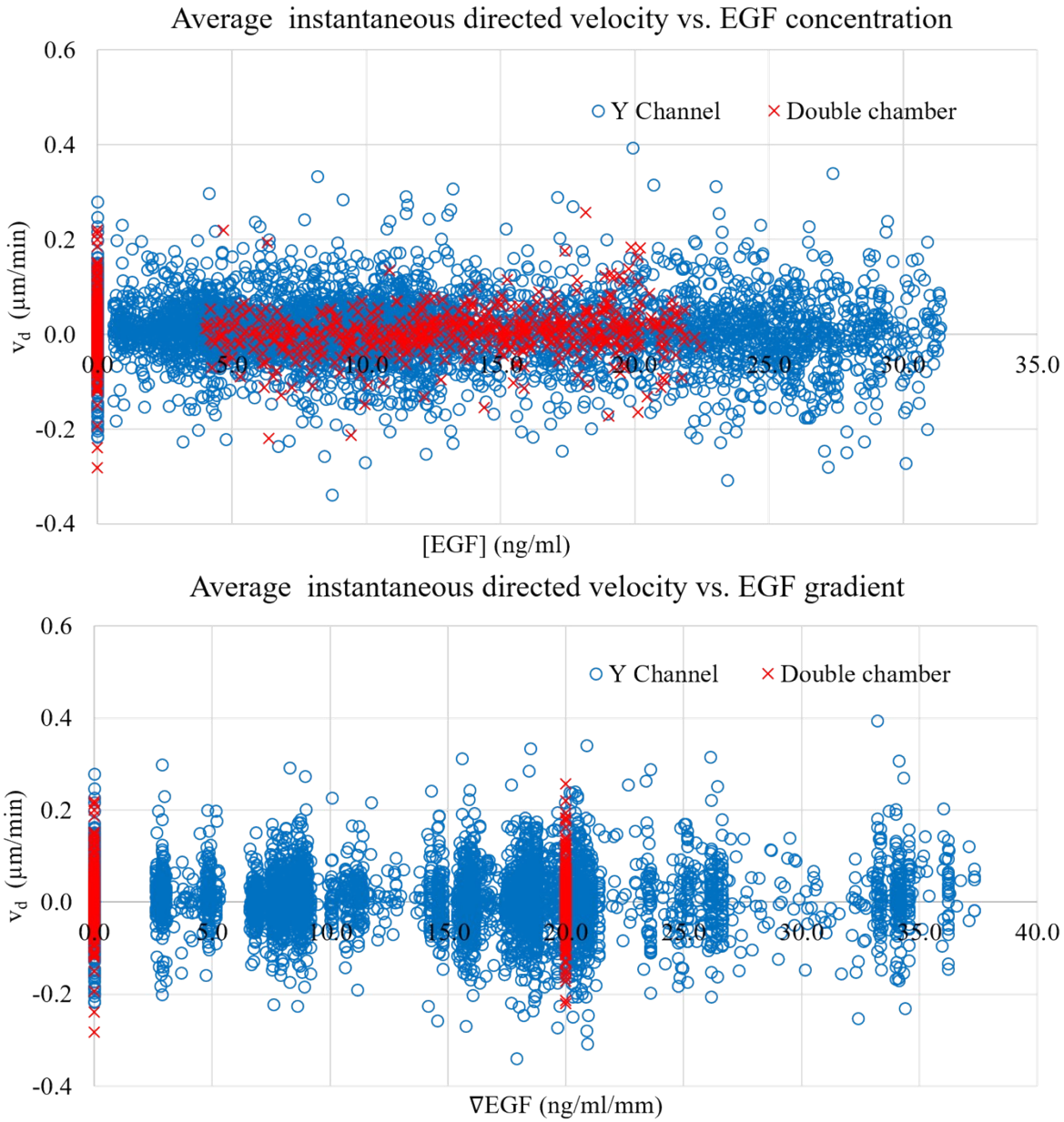


S**upplementary Figure 2.** Determination of v_{tot} cutoff from velocity histograms for four groups as described in text. Results for the groups are shown with different colored lines. **Top:** Histogram diagram for v_{tot}. **Middle:** Cumulative histogram diagram for v_{tot}, which is the integral of the distribution shown in the top plot. **Bottom:** Standard deviation of the distributions for the four groups. The cut-off value was taken as 0.22 µm/min which corresponded to an optimum variance and maximal separations in the distributions among the groups.

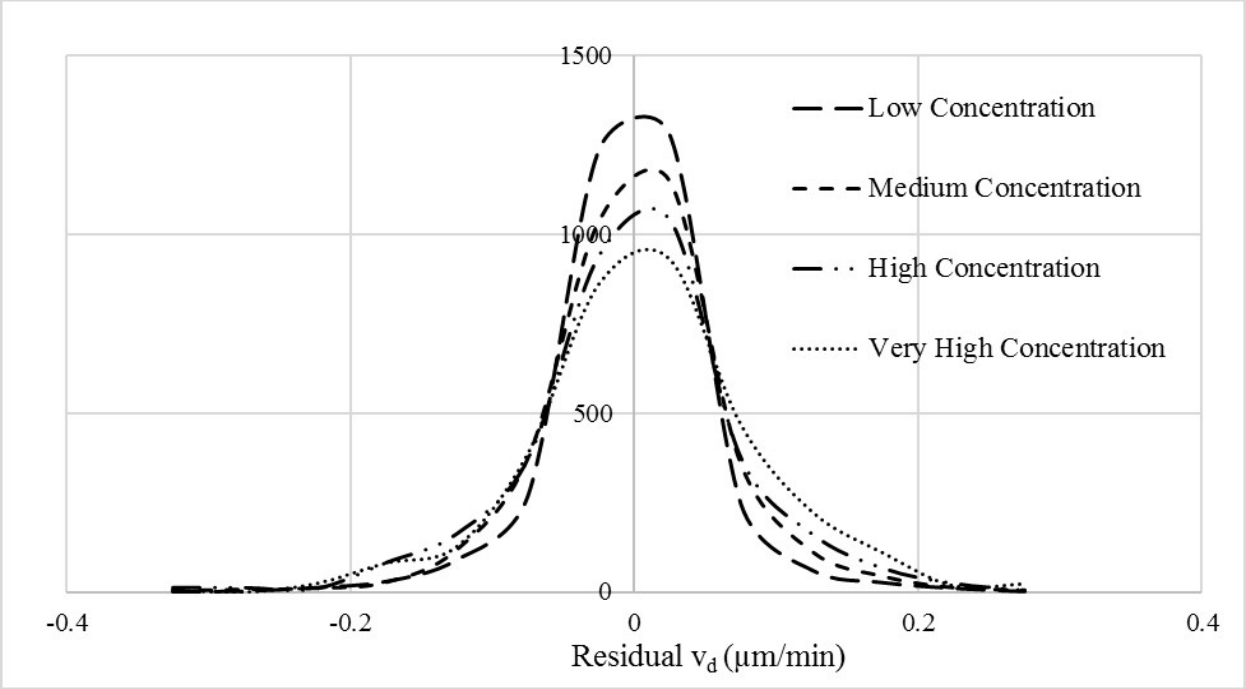


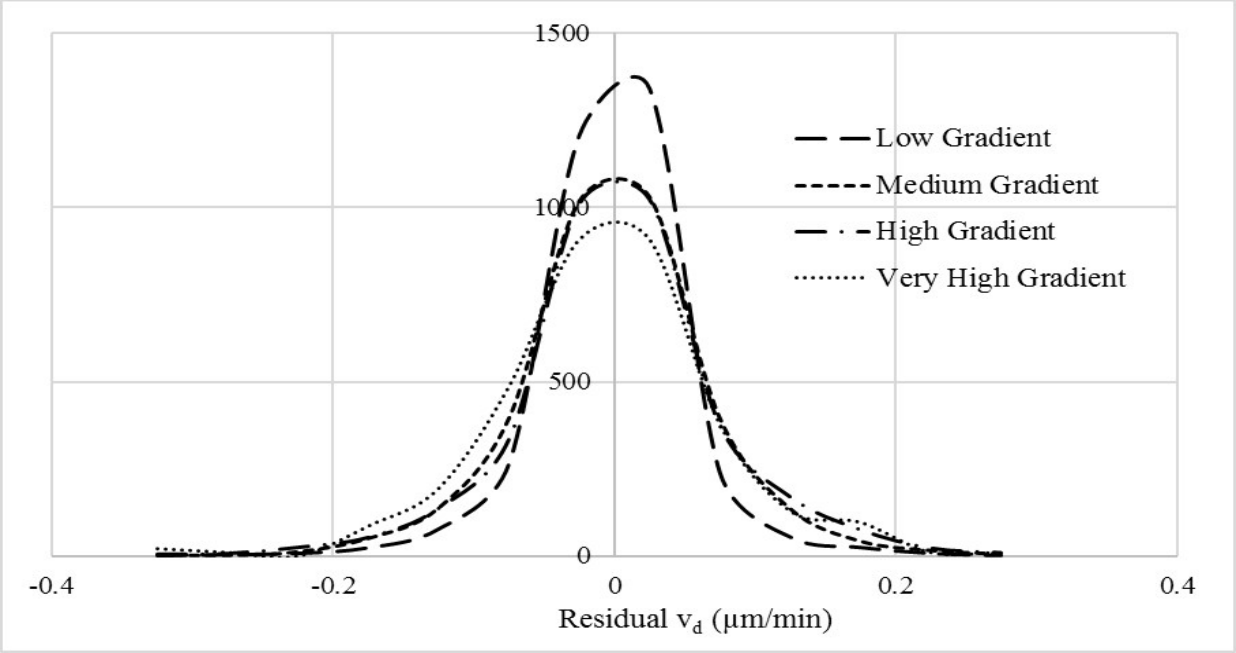


Supplementary Figure 3. Histogram of the instantaneous total velocity above cut-off $H^{\%}_{v,tot}$ residual distributions for the four cell groups (described in main text) which were defined based on the levels of cells' exposure to (top) EGF concentration and (bottom) EGF gradient. Histograms are normalized separately for each group. Apart from widening of the residuals at high ligand levels, we find that the residual distributions for the groups are very similar to each other with the same peak positions and distribution shapes further confirming unbiased model fit

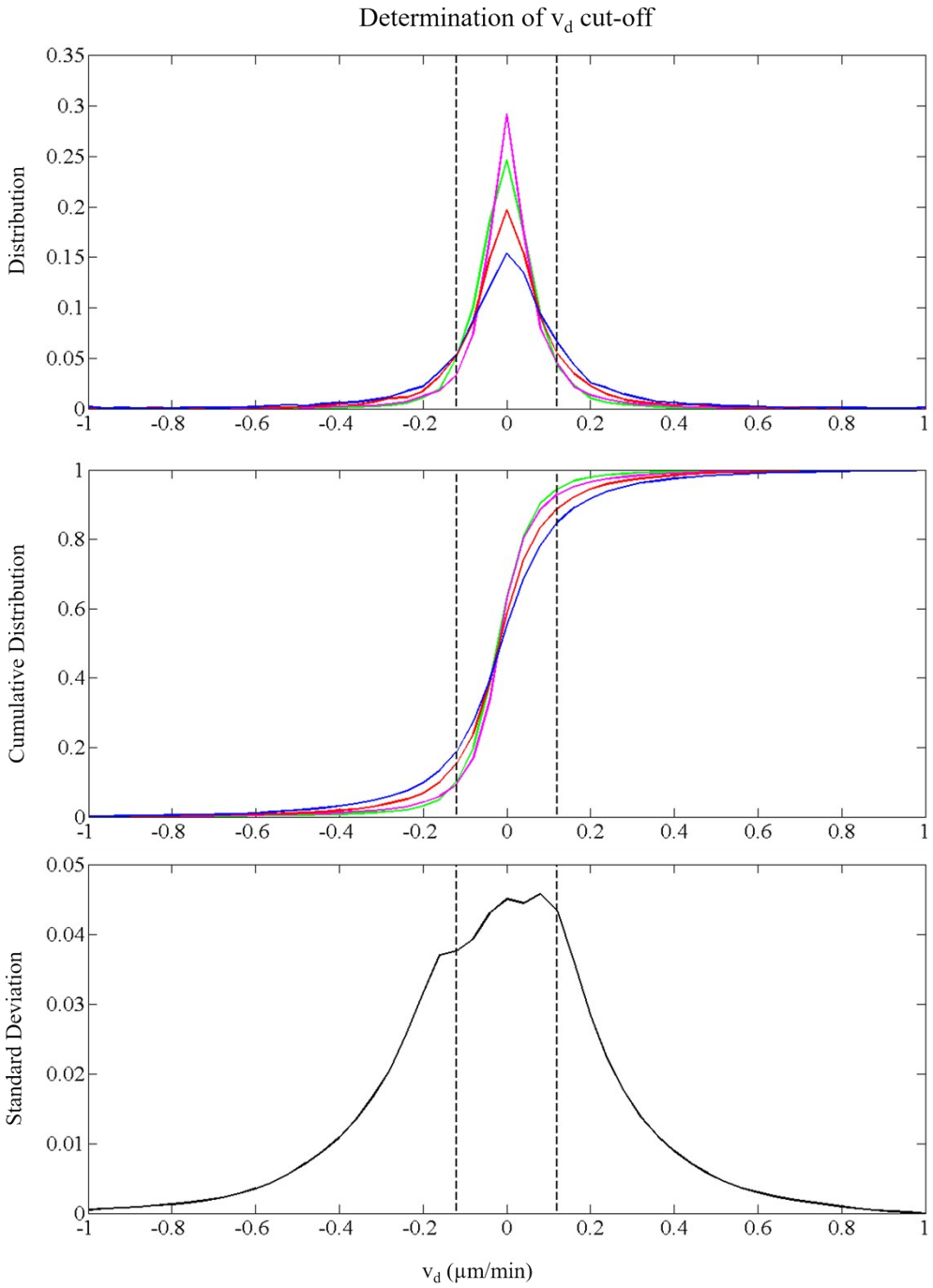


Supplementary Figure 4. Average instantaneous directed velocity v_d (µm/min) of individual cells vs. **(top)** EGF concentration (ng/ml) and **(bottom)** EGF gradient (ng/ml/mm). Each point in the figures represents the result for a single cell. Cell velocity results obtained in the experiments with the double chamber (red, crosses) and y-channel (blue, circles) devices are shown together. As seen, results obtained with these two devices overlap very well. Although there is a noticeable upward trend with increasing ∇EGF, measured v_d values are randomly distributed around zero at all [EGF] values.

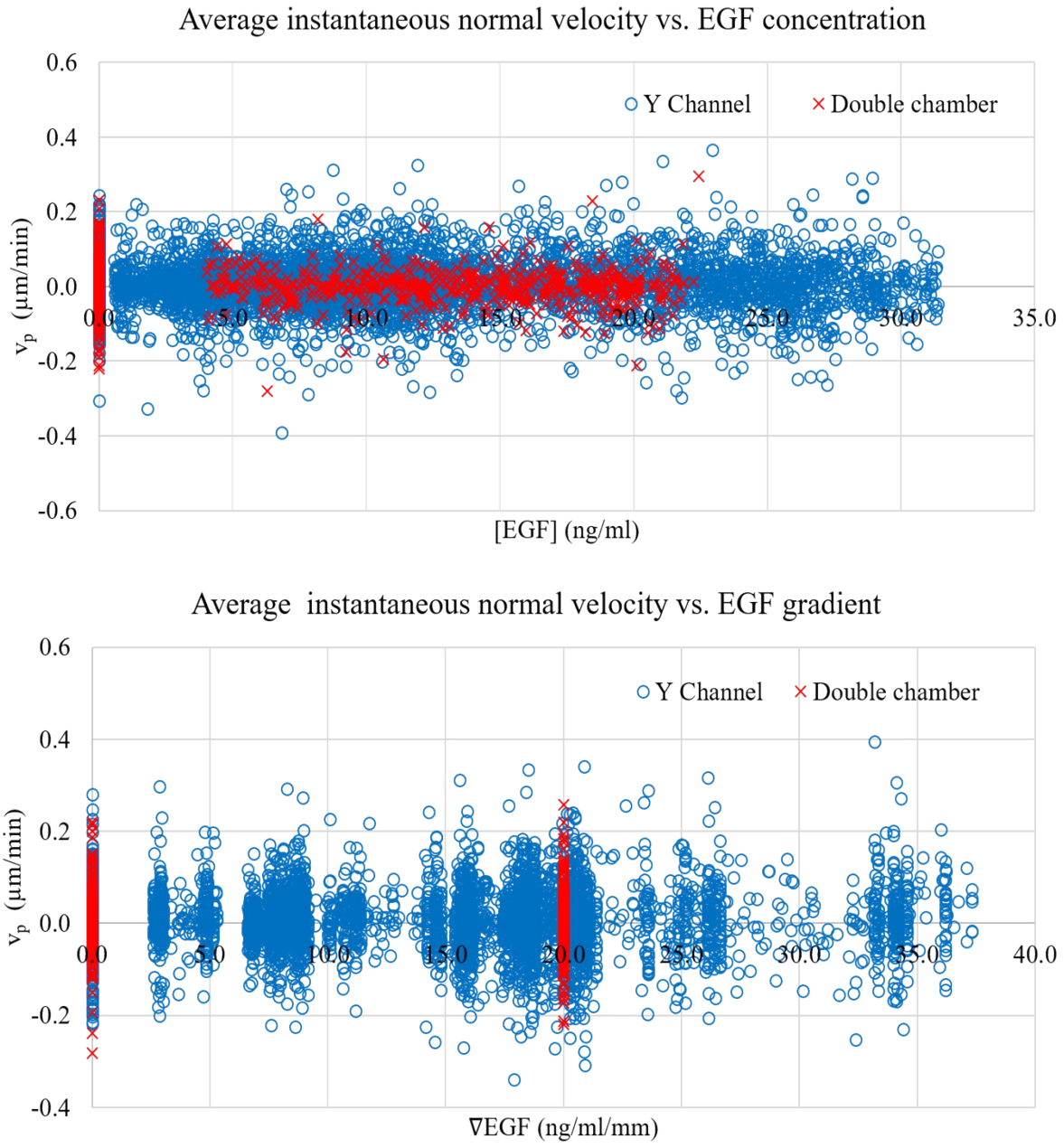




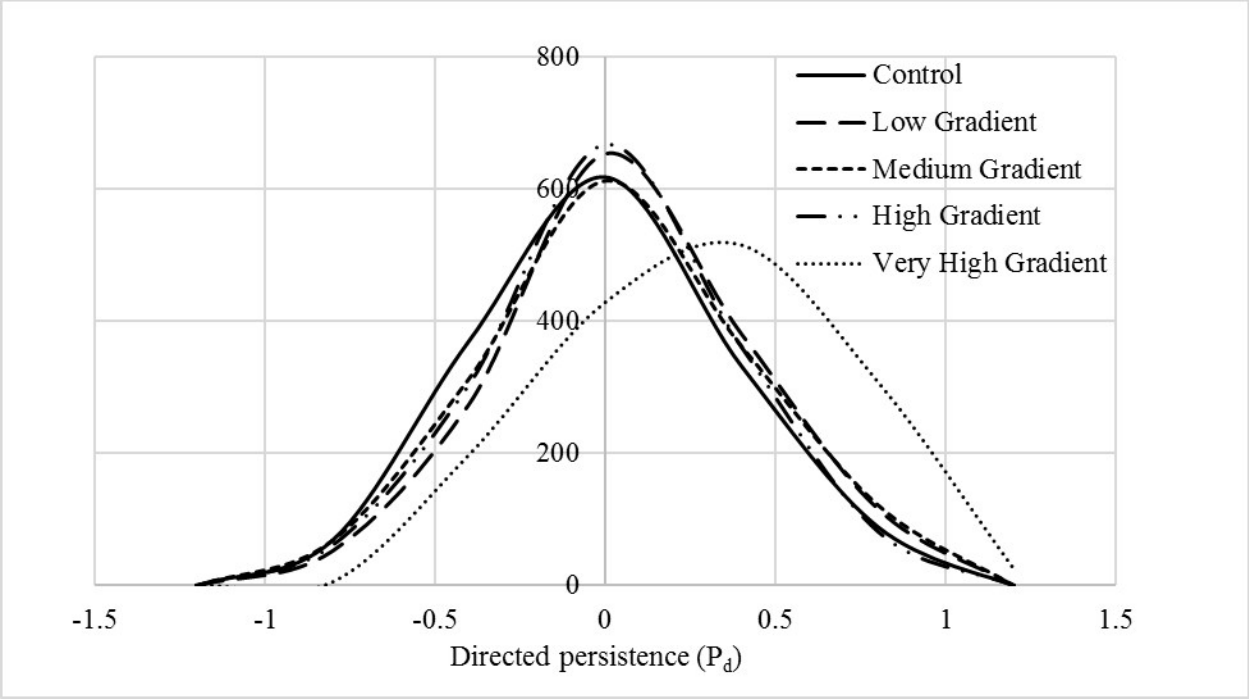
Supplementary Figure 5. Histogram of the average directed velocity v_d residual distributions for the four cell groups (described in the main text) which were defined based on the levels of cells' exposure to (top) EGF concentration and (bottom) EGF gradient. Histograms are normalized separately for each group. We find that the residual distributions for the groups are very similar to each other with the same peak positions and distribution shapes further confirming unbiased model fit.

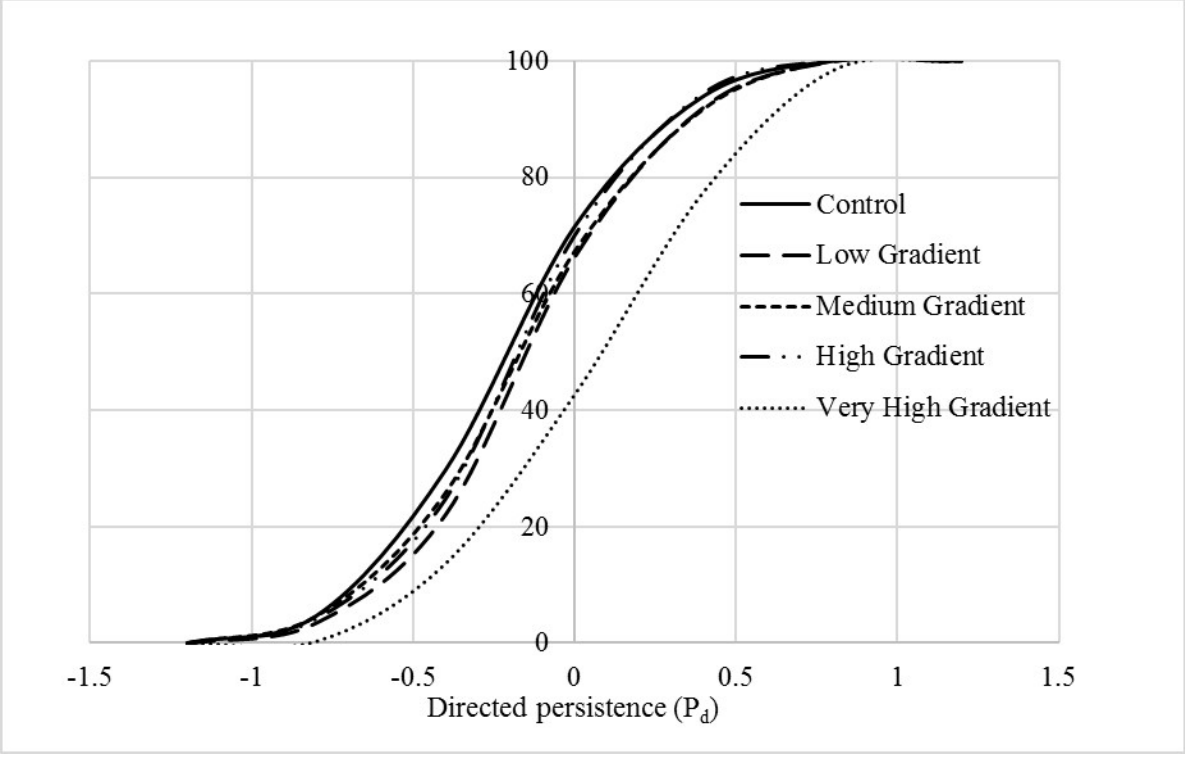


Supplementary Figure 6. Determination of v_d cutoff from velocity histogram of four groups as described in the text. Results for the groups are shown with different colored lines. **Top:** Histogram diagram for v_d. **Middle:** Cumulative histogram diagram for v_d, which is the integral of the distribution shown in the top plot. **Bottom:** Standard deviation of the distributions for the four groups. The cut-off value was taken as ±0.12 μm/min which corresponded to an optimum variance and maximal separations in the distributions among the groups.



Supplementary Figure 7. Average instantaneous directed velocity v_p normal to the gradient (µm/min) for individual cells vs **(top)** EGF concentration (ng/ml) and **(bottom)** EGF gradient (ng/ml/mm). Each point in the figures represents the result for a single cell. Cell velocity results obtained in the experiments with the double chamber (red, crosses) and y-channel (blue, circles) devices are shown together. As seen, results obtained with these two devices overlap very well. There is no noticeable trend with increasing ∇EGF or [EGF] and measured v_p values are randomly distributed around zero at all [EGF] and ∇EGF values.





Supplementary Figure 8. Normalized histogram (**top**) and cumulative histogram (i.e., percentage of cells with persistence smaller or equal than a certain persistence; **bottom**) distribution of persistence P_d without any EGF stimulation (control) and exposure to different levels of EGF gradient stimulation. Control and four gradient groups were defined as in Figure 6 in the main text.