JCB Online Supplemental Material

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Supplemental materials and methods

Endo180 siRNA

The following small interfering (siRNA) oligonucleotides were used: Endo180 siRNA oligonucleotides 5′CCCAACGUCUUCCUCAUCUdTdT3′ and 3′AGAUGAGGAAGACGUUGGGdTdT5′; reversed Endo180 (control) siRNA oligonucleotides 5′UCUACUCCUUCUGCAACCCdTdT3′ and 5′GGGUAGCAGAAGGAGUAGAdTdT3′. Annealed siRNA oligonucleotides (0.5 nmol/ml) were transfected into MDA-MB-231 cells seeded on Matrigel®-coated coverslips or culture dishes (30–50% confluent) with 100 μM Oligofectamine™ (Invitrogen) in Opti-MEM™ reduced serum medium (Invitrogen). Cells were incubated at 37°C for 4 h and washed twice with PBS before the addition of starvation media (DME + 0.1% FCS).

Cell migration/chemotaxis assay

Images of cells were digitally recorded at a time-lapse interval of 10 min for 5 h. Where indicated cells were incubated for 1 h prior and during the course of the assay with 10 μg/ml purified mAb. Migratory speed values are given as the mean speed for all cells analyzed over the 5-h period ±SEM and any statistical differences were determined by paired *t* test. In our determination of directionality of cells the method of analysis was designed to discount any bias caused by differences in cell speed between treatment groups. For the determination of directionality a horizon distance was calculated to be the distance passed by 50% or 85% of cells in a direct line from their starting point. Each of these cells was then assigned an angle, which defined the direction from the starting point of the trajectory to the point at which it first crossed the horizon. The 50% or 15% of trajectories that never reached the horizon were eliminated. These directional data were summarized in a circular histogram showing the number of cells lying within each 18° interval. The Rayleigh test for unimodal clustering of directions was applied to the data and $P > 0.05$ was the criterion for rejecting the null hypothesis that the directions had a uniform random distribution (i.e., a random motile response). In the case of significant unimodal clustering, the mean direction and its 95% confidence interval were represented as a red arrow and a green sector on the circular histogram. This method allowed for nonbiased calculation of cell directionality up a chemotactic gradient that was independent of migratory speed. Strong directionality was measured in the event that analysis of 50% and 85% of the total cell population indicated a positive unimodal clustering of directions. Weak or partially inhibited directionality was measured in the event when analysis of 85%, but not 50%, of the total cell population indicated a positive unimodal clustering of directions. No directionality or totally inhibited directionality was measured when analysis of both 50% and 85% of the total cell population indicated a uniform random distribution.