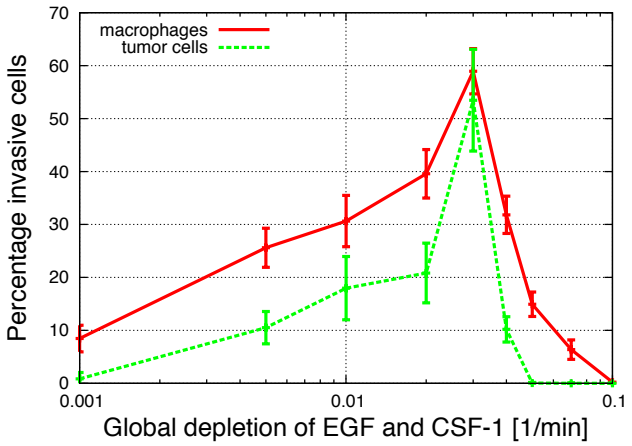

Fig. S-2 Increasing the global depletion of EGF and CSF-1 *in vitro*, results in a biphasic response. Initially, the number of invasive tumor cells and macrophages increased to a maximum around 0.03 min^{-1}. At higher global depletion rates, the number of invasive tumor cells and macrophages decreased to zero. When the global ligand depletion was too large, the macrophages could not detect the CSF-1 from the top because the CSF-1 concentration was below the detection threshold. See S4-Movie and S5-Movie for video with global depletion of 0.03 and 0.07 min^{-1} respectively.

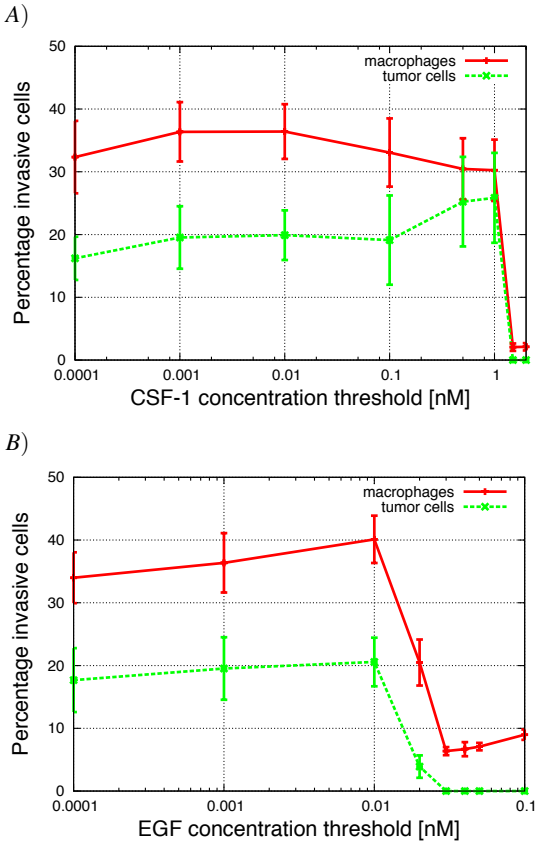

Fig. S-3 The concentration threshold is the concentration of signaling molecules around the cells needed for them to both start secreting their own signaling molecule and detect gradients. Increasing the A) CSF-1 concentration threshold and B) EGF concentration threshold results, in a decreasing number of invasive cells until no cells became invasive.

S2 Appendix: Supplemental Results

Changes in global depletion of EGF and CSF-1 *in vitro*

Our next step was to look at how the global depletion of the EGF and CSF-1 signals affected the invasiveness of the cells. Increasing the global depletion of EGF and CSF-1 resulted in a biphasic response in the number of invasive cells (Fig S-2). As the global depletion increased from zero, the percentage of invasive tumor cells and macrophages increased to a maximum. Increasing the depletion rate further resulted in a rapid decline in a number of invasive cells. When the global decay was low, the CSF-1 concentration built up close to the plate. As explained before, most macrophages could not detect an upwards CSF-1 gradient and thus very few cells invaded. As the global decay was increased, the CSF-1 gradient from the top was enhanced and more macrophages invaded followed by the tumor cells. However, when the decay was too high, the CSF-1 signal from the top was attenuated and the macrophages had difficulty detecting the gradient. At 0.1 min^{-1} global depletion rate neither macrophages nor tumor cells invaded.

Changing the EGF and CSF-1 concentration detection thresholds *in vitro*

As mentioned in the model description, in order for the cells in the simulations to be able to sense gradients, polarize and secrete signaling molecules, the concentration of the signaling molecule needs to be above a set concentration threshold. Increasing the CSF-1 concentration detection threshold for the macrophages resulted in a nearly constant percentage of collected tumor cells and macrophages until a threshold of 1nM was reached. At detection thresholds above 1nM the number of collected cells decreased rapidly until no cells were collected (Fig S-3 A). At higher CSF-1 detection threshold, it took the macrophages longer to detect the CSF-1 signal from above, and that could have given the tumor cells more time to get closer and follow the macrophages upwards. Increasing the EGF detection threshold had a slightly different effect on invasiveness than incresing the CSF-1 threshold. Initially, the number of both invasive macrophages and tumor cells increased slightly as the EGF detection threshold was increased to 0.01 nM (Fig S-3 B). Further increase in the EGF threshold lead to a fast drop in the number of invasive cells. As before, the tumor cell–macrophage interaction helped the macrophages get out of the flat CSF-1 signal in the boundary region. However, for higher EGF thresholds, the tumor cells did not chemotact towards the macrophages and the macrophages got stuck in the boundary region and only around 8% of the macrophages invaded. This is similar to the situation in (Fig 3 B) where the EGF secretion was too low and there was no mechanical tumor cell macrophage interaction. At this point no tumor cells invaded because they could not detect the EGF signal.

Changing the external CSF-1 source from the media *in vitro*

The external source of CSF-1 in the experiments comes from the media located about 750–1,000 μm above the cells. To explore

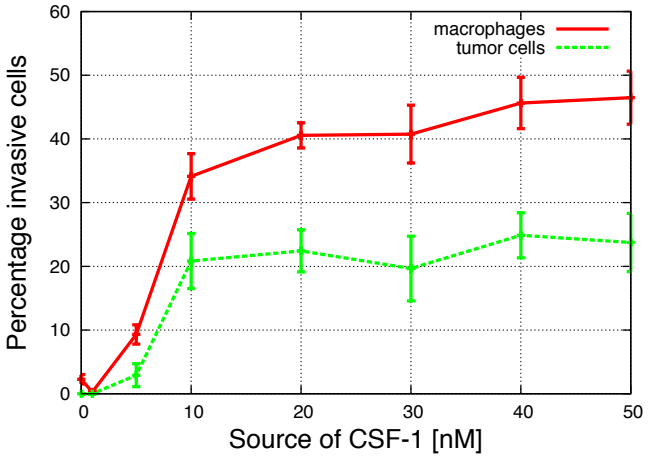

Fig. S-4 Increasing the concentration of CSF-1 in the media resulted in increased percentage of invasive cells. The media was located 750 μm above the cells. There was no further increase in the number of invasive cells as the CSF-1 concentration was increased past 40 nM mostly due to over-saturation of signal at the bottom.

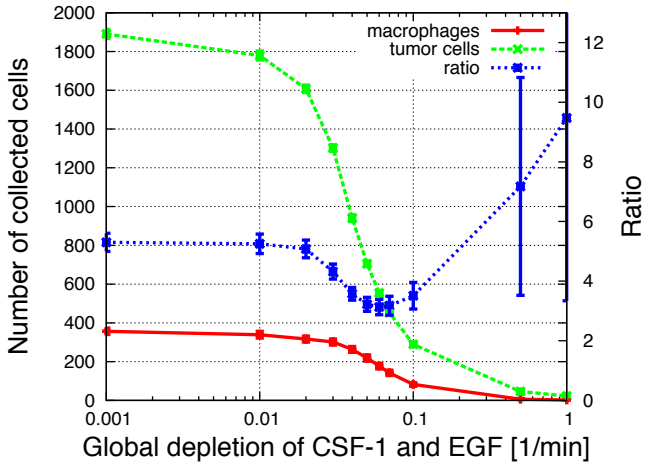

Fig. S-5 Increasing the global depletion of EGF and CSF-1 for the *in vivo* simulations resulted in a decrease in the number of tumor cells and macrophages collected in the needle. The larger global ligand depletion caused the CSF-1 concentration to be below threshold for cells that were located further away from the needle opening. See S9-Movie for GLD = 0.01 min^{-1}.

the effect that the CSF-1 source has on the invasiveness of cells, we systematically increased the CSF-1 source in the simulations (Fig S-4). As CSF-1 was increased from zero, the number of both invading tumor cells and macrophages increases. However, the increase in invasiveness was more rapid for macrophages. The higher the CSF-1 source, the less time it took for the macrophages to detect the CSF-1 gradient and thus some invaded sooner, often before a tumor cell could follow. Maximum cell invasion, 25% invasive tumor cells and 45% invasive macrophages, occured when the CSF-1 source was 40 nM. Above this CSF-1 concentration, the percentage of invasive cells remains roughly constant because of over-saturation of CSF-1 at the bottom due to the no flux boundary condition.

Changing global depletion of EGF and CSF-1 *in vivo*

In these *in vivo* simulations, the global ligand depletion, GLD, represented the natural removal of the ligand, the degradation of the ligand by soluble MMPs and perfusion (removal of ligand from fluid flow). Therefore, the benchmark global depletion in these simulations was higher than in the *in vitro* simulations. Increasing the GLD of both EGF and CSF-1 had little effect at first, but once GLD increased past 0.01 min^{-1} the number of collected tumor cells and macrophages decreased rapidly and went to zero for high GLD (Fig S-5). Increased GLD lead to increased attenuation of the EGF from the needle, so only cells that were close to the needle detected the signal, whereas for cells further away the concentration was below threshold. The tumor cell/macrophage ratio remained ~ 5 until GLD > 0.01 when it decreased to a minimum of 3 for GLD $= 0.05$ min^{-1}. Above GLD of 0.1 min^{-1} less than 10 macrophages were collected in the needle, thus the ratio could not be accurately determined because the variation was too large.

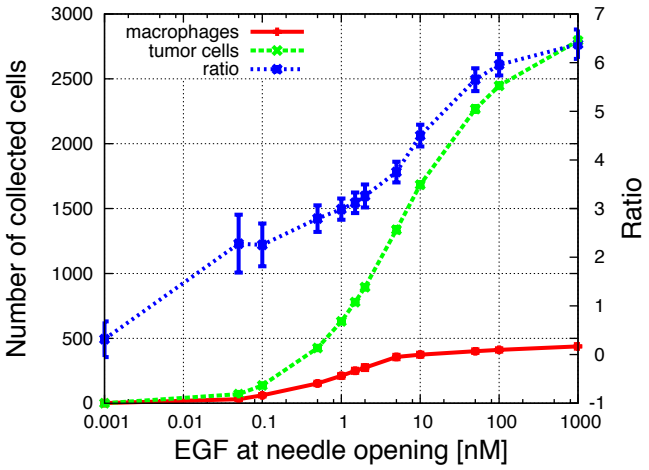

Fig. S-6 Increasing the concentration of EGF at the needle opening resulted in an increase in the number of tumor cells and macrophages collected in the needle. The number of collected tumor cells increased faster than the collected macrophages when the EGF concentration was increased, and thus the tumor cell/macrophage ratio increased.

Changing the EGF concentration in the needle *in vivo*

Increasing the EGF concentration at the needle opening in the simulations resulted in an increase in collected tumor cells (Fig S-6). The number of collected macrophages also increased because the CSF-1 signal from the tumor cells diffused outwards so the surrounding macrophages could follow the tumor cells to the needle. However, the number of collected tumor cells increased at a faster rate, and consequently the tumor cell/macrophage ratio increased. It should be noted that this is a semi-log plot so the ratio remains around 3 for EGF needle concentration between 0.5-1.3 nM. The higher concentration of EGF in the needle enabled

cells located further away from the needle opening to detect a gradient of EGF from the needle and chemotact towards it. Because the tumor cells secreted CSF-1, more macrophages also migrated towards the needle. As the macrophage population began to get depleted, the increase in number of collected macrophages was slower. When the EGF concentration in the needle was very high, many tumor cells left before macrophages had time to follow them.