

Supplemental figures: Supplemental figure 1: The identification of tumor associated macrophages in mammary tumors in live animals.

Multiphoton microscopy of Texas red -dextran labeled macrophages (red) in living *MMTV-PyMT/Tie2-GFP* tumors (green endothelial cells) was performed to observe the location of macrophages relative to blood vessels. Macrophages were observed in the stroma subluminal to endothelial cells lining the blood vessels in an en face optical section. The collagen matrix (blue) is imaged by second harmonic generated polarized light. Scale Bar = 25 μ m.

Supplemental figure 2: FACS analysis shows that texas red-labeled cells express macrophage specific markers.

The graph on the left is FACS analysis of cells sorted from a tumor isolated from a *cfms-GFP/PyMT* mouse 2 hours after tail vein injection of Texas red dextran. In this case monocytes and macrophages are GFP labeled and only macrophages are expected to be texas red labeled. As the upper right quadrant shows, a large number of red-labeled cells express GFP. The lower left quadrant is nonfluorescent cells and the lower right quadrant is GFP-labeled monocytes.

The graph on the right is FACS analysis of cells isolated from a non-GFP expressing tumor from a *PyMT* mouse, preinjected 2 hours prior with texas red-dextran and then, prior to sorting, incubated with fitc-labeled anti-F4/80. In this case macrophages are expected to be both fitc and texas red labeled. As the upper right quadrant shows at least 97% of the red labeled cells express the macrophage specific marker F4/80. Together this analysis confirms that all macrophages are phagocytosing texas red dextran.

Supplemental figure 3: Peri-vascular macrophages are sites of EGF-and CSF1-dependent intravasation in PyMT mammary tumors.

Images from a time-lapse sequence, taken at 10 min intervals, of a cluster of macrophages (red) on a blood vessel in a live animal with a CAG-CAT-EGFP /*MMTV-PyMT* generated tumor (green tumor cells) demonstrate that macrophages (ϕ) are sites of entry of carcinoma cells into blood vessels (arrow shows direction of movement of green carcinoma cell). Intravasation is not observed along vessel walls without macrophages (e.g., asterisk). Transverse section of the vessel is outlined by the solid line in the first frame. Scale bar = 25 μ m.

Supplemental movie 1: Tumor cells move in association with macrophages *in vivo*.

A 30 minute time-lapse sequence of GFP-labeled tumor cells (green) in a PyMT generated mammary tumor (CAG-CAT-EGFP/ *MMTV-PyMT*) in a living animal moving in association with a macrophage cluster containing two macrophages (red) near a vessel. Still images are in figure 3c.

Supplemental movie 2: Macrophages define the sites of tumor cell entry into the blood.

A 30 minute time-lapse sequence of a cluster of macrophages (red) on a blood vessel in a live animal with a CAG-CAT-EGFP /*MMTV-PyMT* generated tumor (green tumor cells) demonstrate that macrophages are sites of entry of carcinoma cells into blood vessels. The macrophage is seen at the top edge of the vessel in the time lapse. Approximately three quarters of the way through the time lapse a

tumor cell is seen protruding into the vessel at the site of the macrophages. Still images are shown in supplementary figure 3.

Supplemental movie 3: Tumor cells and macrophages move together at the point of entry of tumor cells into a blood vessel.

A 30 minute time lapsed sequence of a tumor cell (green) entering into a blood vessel. A macrophage (red) can be seen moving into the same location as the tumor cell as the movie progresses. Still images are shown in figure 4a.

Supplemental movie 4: Intravasation results in entry of intact tumor cells into the blood flow.

A time-lapse movie taken at one minute intervals shows a tumor cell entering a blood vessel in a CAG-CAT-EGFP/ *MMTV-PyMT* generated tumor (green tumor cells). Upon entry into the vessel the cell is rapidly moved along by the blood flow at 20m/min indicating that it is moved passively in the flow. This velocity of blood flow is typical of vessels in mammary tumors. Scale bar = 25 μ m.