Supplementary Figure 1. Comparison of α3β1 and α6β4 integrin expression in 4T1 cell lines. Integrins α3 and α6 were immunoprecipitated from Triton X-100 lysates of cell surface-biotinylated cells. The locations of the α3, β1, α6, and β4 integrin subunits are indicated. All three cell lines expressed similar levels of α6β4 integrin, which appeared modest compared to the level of α3β1. The expression of α3β1 was reduced specifically in the α3si cells, as previously observed.

Supplementary Figure 2. BLI of spontaneous and experimental metastasis (color)

(**A**) Color version of Fig. 2A, showing growth of primary tumor in mammary fat pad on day 10 and day 31. (**B**) Ex-vivo BLI of spontaneous metastasis from fat pad to lung. Representative BLI images of lungs harvested at the time of euthanasia (days 31 or 35 after implantation of cells in mammary fat pad). (**C**) Color version of Fig. 4A showing BLI imaging on day 0, immediately after tail vein injection and on day 14, about halfway through the assay.

Supplementary Figure 3. Certain large primary mammary fat pad tumors exhibited substantial centralized zones of necrosis. Subgross photomicrograph of a primary mammary fat pad tumor bordered by viable neoplastic cells (**inset A**, bar = 20 μm) with a central zone pallor. Upon higher magnification this pallor is due to locally extensive coagulative necrosis (asterisk) and accumulation of necrotic cellular debris (arrows) (**inset B**, bar = 20 μm). HE, bar = 1 mm.

Supplementary Figure 4. Silencing α3 integrin leads to fewer pulmonary metastases following mammary fat pad injection. (**A-C**) Representative photomicrographs of lungs at 31 days post-injection of tumor cells. Metastatic sites are indicated by asterisks. (**A**) Wild type tumor. The inset shows a higher magnification of the diffuse intravascular neutrophils within the

pulmonary septal capillaries (arrows indicate examples) that were noted in all lung samples examined regardless of group. (**B**) α3si tumor. (**C**) α3Rx tumor. Bars in A-C = 100 μm (A, inset bar = 20 μm). (**D**) Histologic tumor nodule scores based on the number discrete tumor nodules within the pulmonary parenchyma (as described for Figure 6).

Supplementary Figure 5. RNAi-mediated silencing of RhoC has no impact on 4T1 cell metastatic colonization. (**A**) RhoC targeting constructs were cloned into pSIREN-RetroQ and used to create stably transduced, polyclonal populations of 4T1 cells. RhoC immunoblotting revealed near total silencing using the sh1, sh3, and sh4 constructs. (**B-D**) 5,000 4T1 WT, RhoC sh1 and RhoC sh3 were implanted in mammary fat pad, and total tumor burdens, tumor volumes, and lung metastatic burdens were quantified as described for wild type, α3si, and α3Rx cells in Materials and Methods. Neither RhoC silencing construct had any impact on the growth of the primary tumor or on spontaneous metastasis to lung. (**E**) RhoC silencing is maintained in vivo. Cells were explanted from the lungs of mice harboring wild type 4T1 cells and RhoC sh1 and sh3 4T1 cells. For each set, the lane marked "parental" corresponds to the original cell line implanted into mammary fat pad at the beginning of the assay, and the adjacent two lanes correspond to sublines recovered from lung explants at the end of the assay. RhoC immunoblotting confirmed that RhoC remained silenced in the sh1 and sh3 cells in vivo. The RhoC targeting sequences were *sh1*, 5´-ATAGCCGACATCGAAGTGGAT-3´;

sh2, 5´-TGAAACAGGAGCCGGTTCGAT-3´; *sh3*, 5´-TCTACGTGCCTACCGTCTTTG-3´, *sh4*, 5´-AACAGGAGCCGGTTCGATCTG -3´; & *sh5*, 5´- TCCGGAAGAATAAGCGCCGGA - 3´.

Supplementary Figure 6. Cox-2, matrix metalloproteinase, VEGF, and transendothelial migration assays. (**A**) Cox-2 immunoblot of 4T1 cell lysates. (**B**) VEGF ELISA assay performed on conditioned medium from 4T1 cell lines using a murine VEGF mini-ELISA development kit (PeproTech) according to manufacturer's directions. (**C**) Gelatin zymography of wild type, α3si, and α3Rx 4T1 cells. Conditioned medium from 4T1 cell cultures was collected,

mixed 1:1 with 2X non-reducing SDS PAGE sample buffer, and run on a 10% polyacrylamide gel containing 1 mg/ml gelatin. After incubation for 1 h in 50 mM Tris buffer (pH 7.4) containing 2% Triton X-100 to renature the gelatinases, the gel was developed at 37^{o}C for 18 h in 50 mM Tris (pH 7.4), 5 mM $CaCl_2$, 1% Triton X-100 at 37^{o}C, and then stained with Coomassie Brilliant Blue R 250. (**D**) Trans-endothelial migration assay performed as previously described (Drake et al, *Mol Biol Cell* 2009, **20**:2207–2217), except that human umbilical vein endothelial cells were used instead of human lung microvascular endothelial cells. Cells that migrated through the endothelial monolayer were measured by BLI.

Supplementary Figure 7. MDA-MB-231 breast cancer cells do not secrete laminin-332 or laminin-511 or depend on α3 integrin in short term proliferation assays. (**A**) EGW593.lu cells derived from a lung metastasis of MDA-MB-231 breast carcinoma were stained for LM-332 (monoclonal antibody 6F12 against laminin-β2) and LM-511 (monoclonal antibody 4B12 against laminin-α5) or with an isotype negative control antibody. For comparison, LM-332 and LM-511 expression in A431 epidermoid carcinoma cells is shown. (**B**) EGW593.lu cells were transduced with a control vector (CON) or vectors harboring shRNAs targeting α3 integrin (α3sh3 and α3sh4). Flow cytometry confirmed 90% silencing of α3 integrin in the α3sh3 cells and 70% silencing in the α3sh4 cells. Proliferation assays on LM-511, collagen I, or uncoated plates revealed similar growth rates for all three cell types as measured by WST assays.