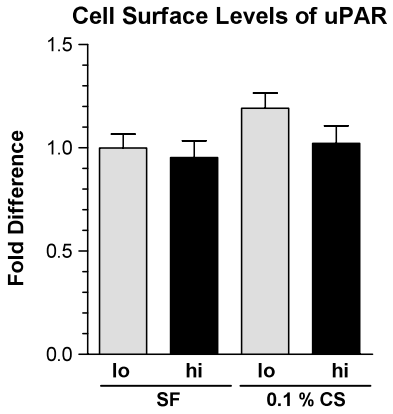

Figure W1. Levels of uPAR at the cell surface of PC-lo/diss and PC-hi/diss cells. Cell surface levels of uPAR on PC-lo/diss (gray bars) and PC-hi/diss (black bars) monolayers were determined using whole-cell ELISA PC-lo/diss and PC-hi/diss cells were seeded at equal density into 96-well plates precoated with 10 μg/ml type I collagen in serum-free AIM-V medium (Gibco, Grand Island, NY) or AIM-V medium supplemented with 0.1% chicken serum. After 24 hours, the cells were washed, fixed in 2% paraformaldehyde, and blocked with 1% bovine serum albumin in PBS–0.05% Tween 20. Primary mAb 3936 against uPAR (American Diagnostica Inc., Stamford, CT) was added at 2 μg/ml in blocking solution for 1 hour at room temperature, followed by incubation with anti-mouse HRP (Bio-Rad) at 0.5 μg/ml for 1 hour. After washing, ABTS substrate was added, and the optical density was determined at 405 nm. The background readings from control IgG wells were subtracted from the data. Fold differences in uPAR levels were calculated in comparison to PC-lo/diss cells incubated without serum (100%). Data are presented as means ± SEM from triplicate wells.

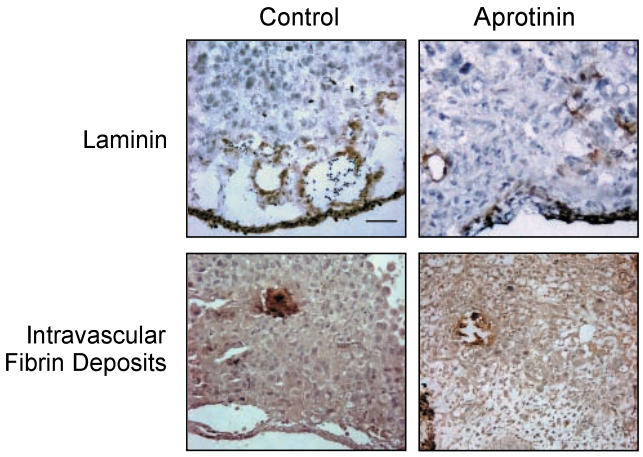

Figure W2. Immunohistochemical analysis of laminin and fibrin within primary CAM tumors. Frozen tissue sections from PC-hi/diss tumors, treated with aprotinin or buffer (vehicle control), were immunostained with mAb "31 or 31-2" recognizing avian laminin (top). Positive laminin immunostaining (brown) was associated with medium-to-large blood vessels in both control and aprotinin-treated tumors. Formalin-fixed paraffin-embedded sections were stained with anti-fibrin antibody to visualize fibrin clots, which were rare in both control and aprotinin-treated tumors and were only observed intravascularly (bottom). Images were captured at an original magnification of ×200. Scale bar, 25 μm.

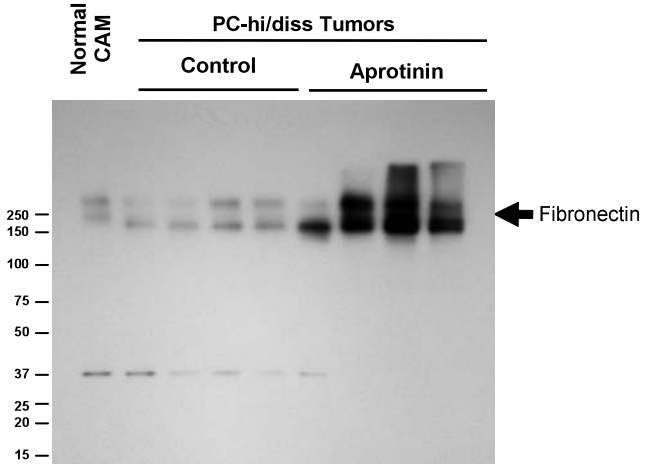

Figure W3. Accumulation of fibronectin in aprotinin-treated PC-hi/diss CAM tumors. Normal CAM was harvested from the embryos on day 16 of incubation. PC-hi/diss primary tumors, treated with aprotinin or buffer control, were harvested 6 days after cell grafting on the CAM. Harvested tissues were washed and lysed. Lysate samples containing 20 μg of protein were separated by SDS-PAGE under nonreducing conditions, transferred to a membrane support, and probed with mAb–B3/D6 to detect avian fibronectin. The positions of molecular weight markers (kDa) are indicated on the left.

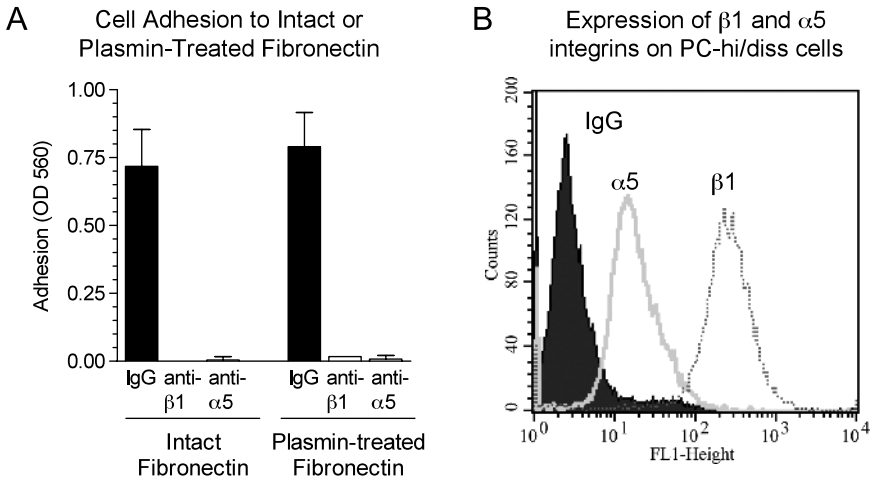

Figure W4. (A) PC-hi/diss cells express functional $\alpha_5\beta_1$ integrin, mediating adhesion to fibronectin. Multiwell plates were coated for 1.5 hours with 10 μg/ml fibronectin, which was left intact or treated with 40 nM plasmin overnight at 37°C. After plate washing and blocking with 1% bovine serum albumin, PC-hi/diss cells were allowed to adhere for 45 minutes in the presence of function-blocking antibodies against the β_1 or α_5 integrin subunits. Nonadherent cells were washed out, and adherent cells were fixed and stained with crystal violet. Cell-incorporated dye was extracted, and absorption, proportional to the cell number, was measured at 560 nm. Data are presented as means ± SEM of absorbance from triplicate wells. (B) Cell surface expression of the α_5 and β_1 integrin subunits in PC-hi/diss cells was determined by FACS analysis in comparison to control IgG. The cells were incubated for 1 hour with 3 μg/ml primary antibodies at 4°C, followed by incubation with fluorescein isothiocyanate–conjugated secondary anti-mouse antibodies (Sigma).