



CD31

DAPI







A

В





Lung



А

в

Control IgG



anti-α4



С





400X

1000X











Supplementary Figure 1: Individual images of homing of CD34+ cells to tumor periphery. Five micron cryosections of the tumors from animals injected with CD34+ cells (red) were fixed in acetone, then incubated with DAPI (blue), rat anti-mouse CD31 and goat anti-rat FITC antibodies (green) and photographed at magnification 200X. Arrowheads indicate CD34+ cells. Arrows indicate CD31 positive blood vessels. Bar= 50μ m.

Supplementary Figure 2: Expression and function of integrin β 1 and β 7 in human CD34+ cells.

(A) FACs profiles for integrin $\beta 1$ and $\beta 7$ integrins on circulating CD34cells. FACS profile in gray represents fluorescence of isotype control antibody. Percent positive cells is indicated by number in right hand corner of each profile. (B) Average number of CD34- cells adhering to endothelial cell monolayers in the presence of medium, anti- $\beta 1$, anti- $\alpha v\beta 5$ and rsVCAM per 200X microscopic field +/-SEM. Asterisks indicate P< 0.01.

Supplementary Figure 3: Integrin expression and adhesion profiles of human CD34cells

(A) FACs profiles for integrin $\alpha 4\beta 1$, $\alpha 5\beta 1$, $\alpha \nu \beta 3$ and $\beta 2$ integrins on circulating CD34- cells. FACS profile in gray represents fluorescence of isotype control antibody. Percent positive cells is indicated by number in right hand corner of each profile. (B) Average number of CD34- cells adhering to endothelial cell monolayers in the presence of medium, anti- $\alpha 4\beta 1$, anti- $\alpha 5\beta 1$,

anti- $\alpha\nu\beta$ 5, anti- $\alpha\nu\beta$ 3, anti- β 2 and rsVCAM per 200X microscopic field +/-SEM. Asterisks indicate P< 0.01. (C-D) Average number of CD34- cells adhering to CS-1 fibronectin or (D) rsVCAM in the presence of medium, anti- $\alpha4\beta$ 1 or isotype matched control antibody (cIgG, anti- $\alpha\nu\beta$ 5) per 200X microscopic field +/-SEM. Asterisks indicate P< 0.01.

Supplementary Figure 4: Mouse lung tissue from CD34+ cell injected animals

Subcutaneous Lewis lung carcinoma (LLC) tumors and lung tissue from mice injected with 0.5 X 10⁶ CMTMR labeled CD34- cells, CMTMR labeled CD34+ cells or GFP+ α 4 β 1+ Chinese hamster ovary cells (CHO α 4+). Note that many human CMTMR labeled cells (red, arrowheads) are present in tumors but few are present in lungs. No GFP+ α 4+ CHO cells are present in tumors while many GFP+ cells are present in lungs.

Supplementary Figure 5: CD34+ cell contribution to angiogenesis is integrin α4β1 dependent

(A) Cryosections from mice with subcutaneous HT29 colon carcinoma tumors that were injected with CMTMR labeled CD34+ cells (red) and treated with saline, anti-human integrin $\alpha 4\beta 1$ and anti-human $\alpha v\beta 5$ for five days. After five days, animals were injected with fluorescent lectin *Bandeira simplicifolia* to highlight blood vessels (green). (B) Average number +/- SEM of CMTMR positive CD31+ blood vessels per 200x microscopic field for each treatment

group. (C) Cryosections from mice with subcutaneous Lewis lung carcinoma tumors that were injected with CMTMR labeled CD34+ cells (red) and sacrificed seven days later. Expression of Ki67 (green) in CMTMR (red) cells that formed CD31+ blood vessels (blue) in vivo.

Supplementary Figure 6: Lin- cell contribution to angiogenesis

Red (anti-CD31, arrows), green (EGFP, arrowheads) and blue (DAPI) color panels and merged images of cryosections of the periphery or center of LLC tumors from mice injected with EGFP+Lin- cells at 200X magnification. Peripheral tumor sections from animals were treated with saline, isotype matched, control anti-integrin antibodies (cIgG) or anti- α 4 β 1 antibodies are shown in the left three columns, while central tumor sections from saline treated mice are shown in the right column. EGFP+ blood vessels are yellow.

Supplementary Figure 7: Tie2LacZ positive blood vessels

Cryosections from Tie2LacZ mouse brain, liver, and spleen were stained to detect beta-galactosidase (blue) and counterstained with nuclear fast red. Images were captured at 400X, 600X and 1000X magnification.

Supplementary Figure 8: LacZ+ cell contribution to angiogenesis

Green (beta-galactosidase), red (CD31), blue (DAPI) and merged images of cryosections of bFGF saturated Matrigel from mice transplanted with Tie2LacZ bone marrow and treated with saline, anti- α 4 β 1 or control isotype matched anti-integrin $\beta 2$ antibodies (cIgG). Cryosections were immunostained for beta-galactosidase to detect LacZ expressing cells (green), anti-CD31 to detect blood vessels (red) and DAPI to detect nuclei (blue). LacZ+/CD31+ vessels are yellow (arrows).

Supplementary Figure 9: Immunostaining for LacZ+ cell contribution to angiogenesis

Green (beta-galactosidase), red (CD31), blue (DAPI) and merged images of cryosections of Tie2LacZ mouse brain at 400 and 1000X. Cryosections were immunostained for beta-galactosidase to detect LacZ expressing cells (green), anti-CD31 to detect blood vessels (red) and DAPI to detect nuclei (blue). LacZ+/CD31+ vessels are yellow.