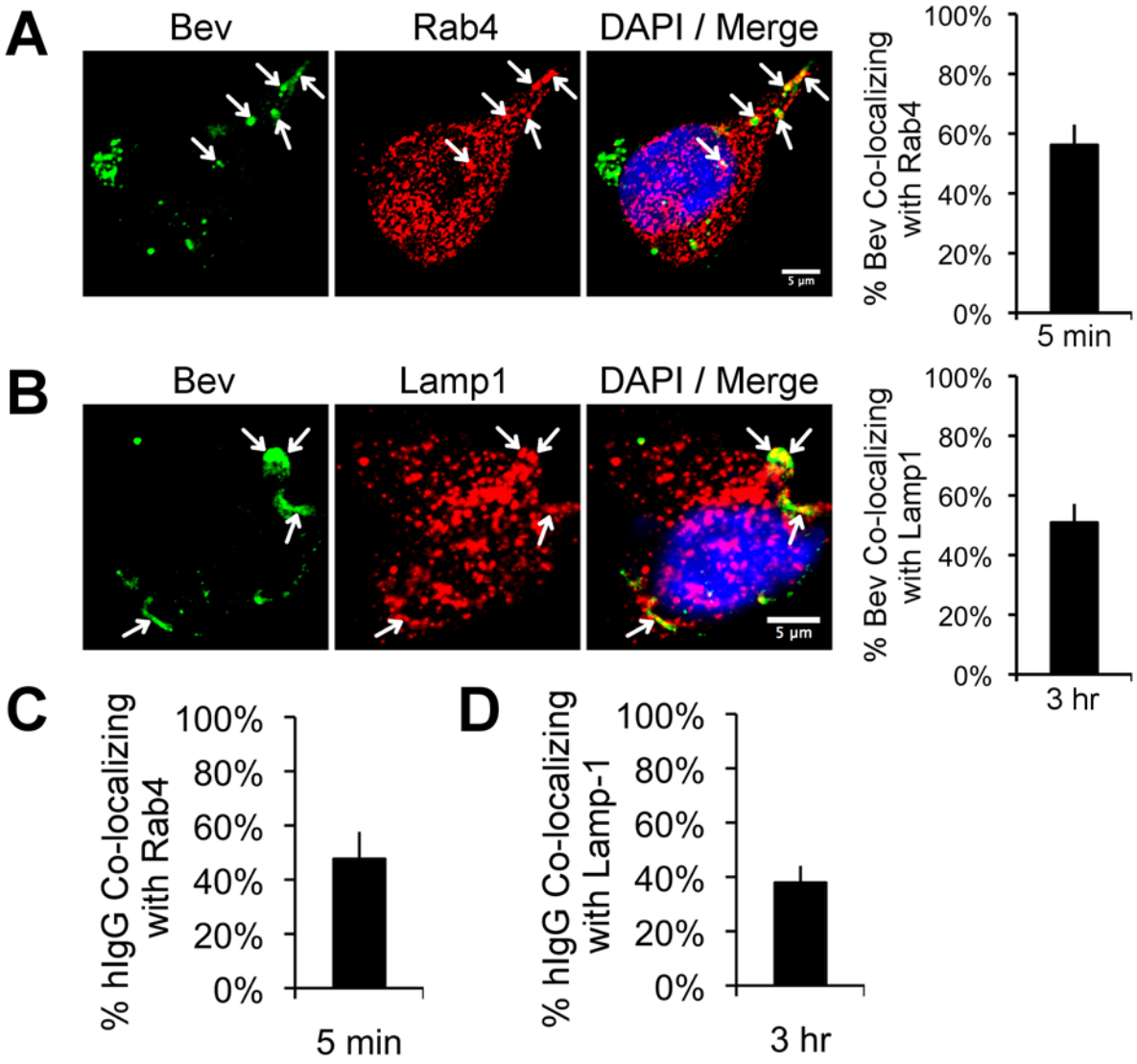
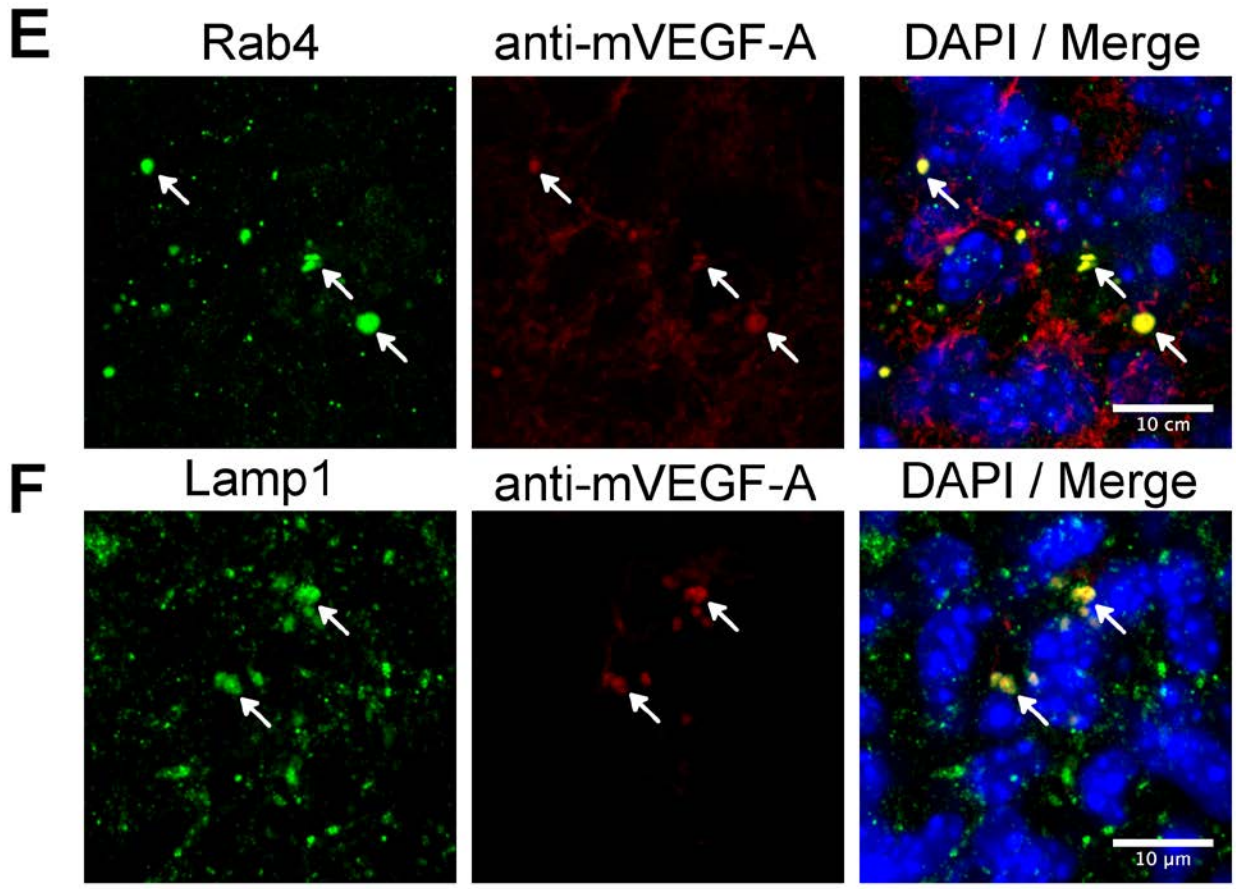
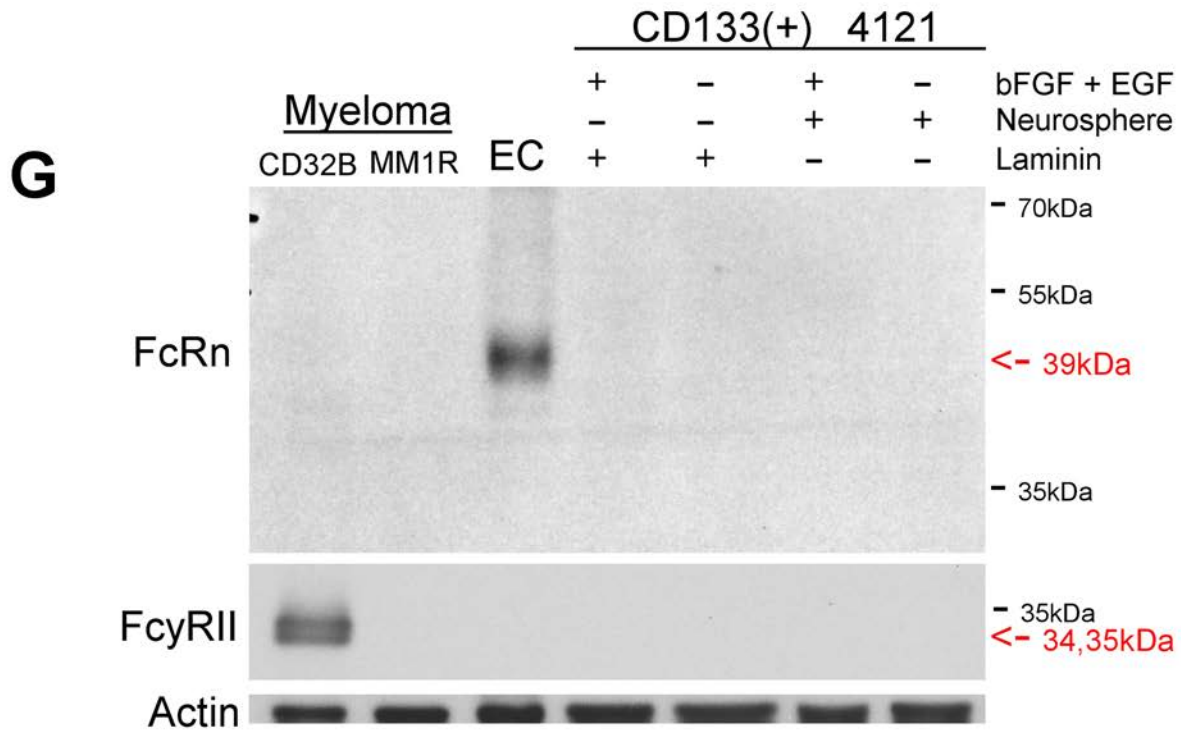
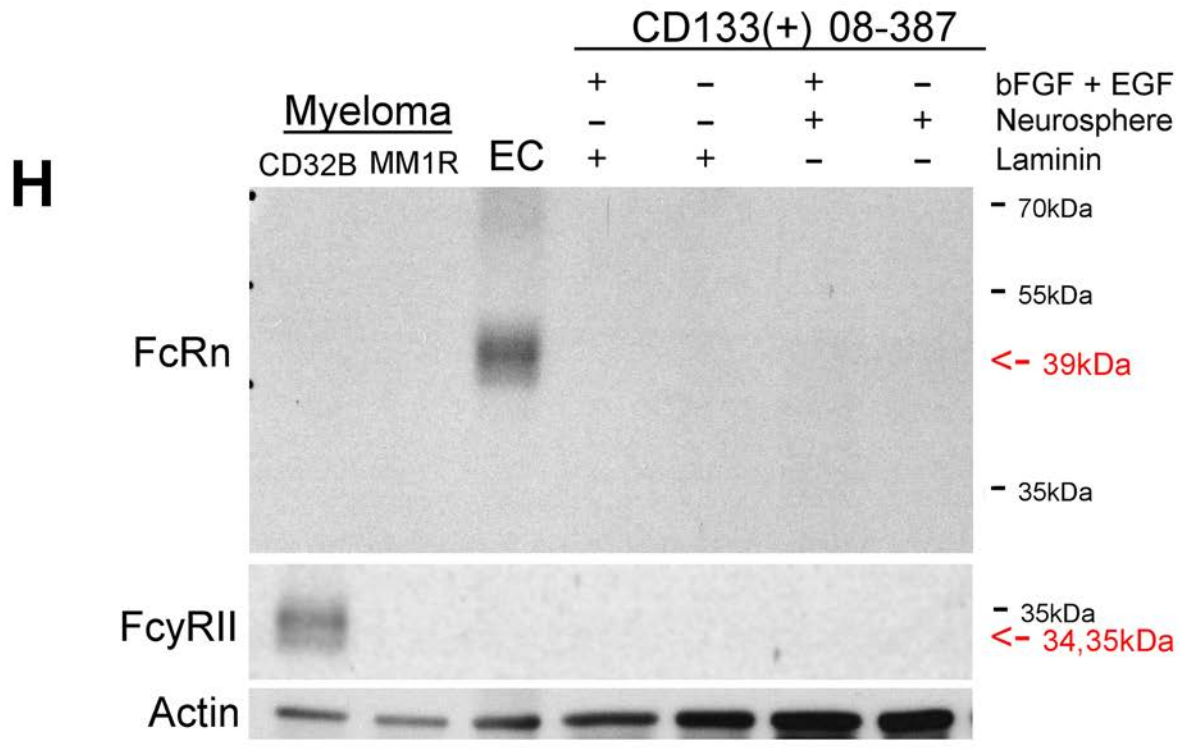
Suppl Figure 3




E
Rab4
anti-mVEGF-A
DAPI / Merge
10 cm
F
Lamp1
anti-mVEGF-A
DAPI / Merge
10 µm


G
CD133(+) 4121
+ − + − bFGF + EGF
− − + + Neurosphere
+ + − − Laminin
Myeloma
CD32B MM1R
EC
FcRn
70kDa
55kDa
<- 39kDa
35kDa
FcyRII
35kDa
<- 34,35kDa
Actin


H
CD133(+) 08-387
+ − + − bFGF + EGF
− − + + Neurosphere
+ + − − Laminin
Myeloma
CD32B MM1R
EC
FcRn
70kDa
55kDa
<- 39kDa
35kDa
FcyRII
35kDa
<- 34,35kDa
Actin

SFigure 3. Co-localization of different fractions of bevacizumab or human IgG (hIgG) with Rab4 or LAMP1 in CD133^{+} GBM cells in vitro, and of rat anti-mouse VEGF-A IgG in an established tumor in the immune competent mouse model of GBM in vivo show similar trafficking patterns. **A-D**, CD133^{+} GBM cells (4121 in A&B; and 08-387 in C&D) were plated for 18 h as in Figure 3, followed by addition of bevacizumab (A&B) or hIgG (C&D) (250 µg/ml) for 5 min, the cells washed and fixed or the media replaced and the cells washed and fixed at 3 h. The cells were reacted with Alexa-488-anti-hIgG and anti-Rab4 or anti-LAMP1 antibody, followed by Alexa-594-conjugated secondary antibody, DAPI nuclear stain, and photographed by confocal microscopy. Arrows denote co-localization of bevacizumab (green) with Rab4 or LAMP1 (red) (A&B). The percent bevacizumab or hIgG co-localized with Rab4 or LAMP1 is plotted as the mean±SEM based on the Mander's coefficient at the indicated times. **E&F**, Administration of rat anti-mouse VEGF-A IgG to an established tumor in the immune competent mouse model of GBM results in highly similar trafficking of rat IgG in the perivascular tumor cells. Tumors were induced, and then once established treated with rat anti-mouse VEGF-A IgG for 2 weeks, followed by euthanasia, brain harvest and fixation, as described in Figure 1. Sections of tumor from four tumor-bearing mice were reacted with Alexa-488-anti-rat IgG (green), and anti-Rab4 or anti-LAMP1 antibody, and Alexa-594-conjugated secondary antibody (red), followed by DAPI nuclear stain and confocal microscopy. A fraction of the rat anti-mouse VEGF-A IgG is colocalized with Rab4 (yellow) (arrows) (E) and a fraction of the rat anti-mouse VEGF-A IgG is colocalized with LAMP1 (yellow) (arrows) (F). **G&H**, CD133^{+} GBM cells (4121 or 08-387), as well as the control of brain ECs (#422), were plated as

described in the legend for Figure 4G. MM.1R human myeloma cells transfected with FcγRIIB (CD32B) and plated in 10% FBS served as a positive control for expression of FcγRIIB. Detergent lysates (30 µg) of all cells were subjected to SDS-PAGE, and then western blotted with mouse anti-FcRn (Novus Biologicals #NBP2-42214), stripped and re-probed with goat anti-FcγRIIB (R&D Systems #AF1330), and then stripped and re-probed with goat anti-actin antibody (Santa Cruz #sc-1615).