

SFigure 5. Co-localization of a fraction of the bevacizumab-VEGF complex with Rab4 and a fraction with LAMP1 in CD133⁺ GBM tumor cells. CD133⁺ GBM cells (08-387) were plated for 18 h as in Figure 3. Bevacizumab (250 μg/ml) was incubated with biotinylated-rec-human-VEGF (100 ng/ml) for 1 h, and the mixture added to the cells for 5 min, the cells washed and fixed or the media replaced and the cells washed and fixed at 3 h. A&B, The cells were reacted with Alexa-488-anti-human IgG and anti-Rab4 or anti-LAMP1 antibody, and Alexa-594-conjugated secondary antibody, as well as Alexa-647-Streptavidin, followed by DAPI nuclear stain and confocal microscopy. Arrows denote bevacizumab (green), Rab4 or LAMP1 (red) and VEGF (magenta), as well as their co-localization (A&B). Triple labeling is indicated by the arrows in both A & B. C&D, The percent bevacizumab-VEGF complex co-localized with Rab4 (5 min) (C) or LAMP1 (3 h) (D) is plotted as the mean±SEM based on the Mander's coefficient. Scale bars denote 5-μm.