Supplementary Figure 1. Representative image showing selective detection of CD-31^{+} pixels using MetaMorph 7.7 imaging software. The original image is shown in Figure 6B.

Supplementary Figure 2. Original whole gel images of data depicted in Figure 1B. Lanes shown in Figure 1B are depicted by an asterisk. Gel 1: elution profile of Granzyme B/$VEGF_{121}$ from HEK-293T conditioned media. Purity greater than 95% was obtained. Gel 2: Asterisked SDS-PAGE lanes show GrB/$VEGF_{121}$ under reducing and non-reducing conditions, and show that the majority of the purified protein is a natural homodimer.

Supplementary Figure 3. Total VEGFR-2 expression assessed by Western blot analysis (top) and flow cytometry (bottom). Corresponding western blot lanes for VEGFR-2 and Actin are numerically indicated. VEGFR-2 expression in bEND.3 cells was tested using both methods, and has been previously reported as 2 x 10^5 receptors per cell[1].

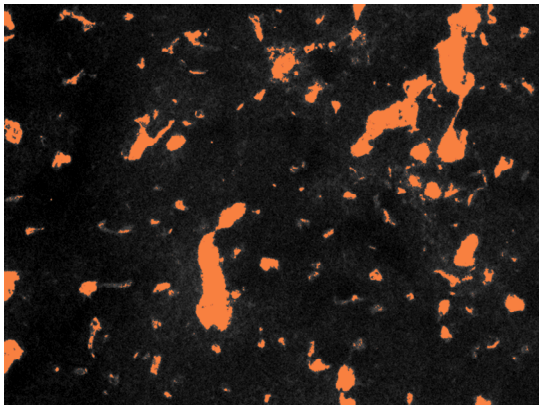
Supplementary Figure 4. Internalization of GrB/$VEGF_{121}$ into PAE/VEGFR-1 is much lower than into PAE/VEGFR-2 cells. Cells were treated with 20 nM GrB/$VEGF_{121}$ for 24 h, followed by removal of cell surface-bound protein as described in Methods. Cells were treated with mouse anti-GrB monoclonal antibody followed by a goat anti-mouse AF488 antibody.

1. Veenendaal LM, Jin H, Ran S, Cheung L, Navone N, Marks JW, Waltenberger J, Thorpe P, Rosenblum MG. In vitro and in vivo studies of a VEGF121/rGelonin chimeric fusion toxin targeting the neovasculature of solid tumors. Proc Natl Acad Sci U S A 2002;99:7866-71.

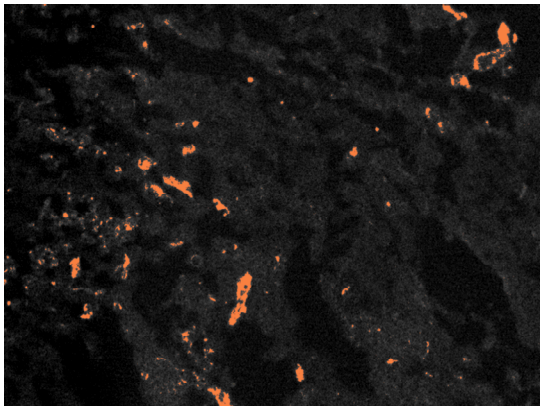
Quantitation of CD-31+ areas in PC-3 Tumors

Efficacy Treatment

Saline



GrB/VEGF$_{121}$



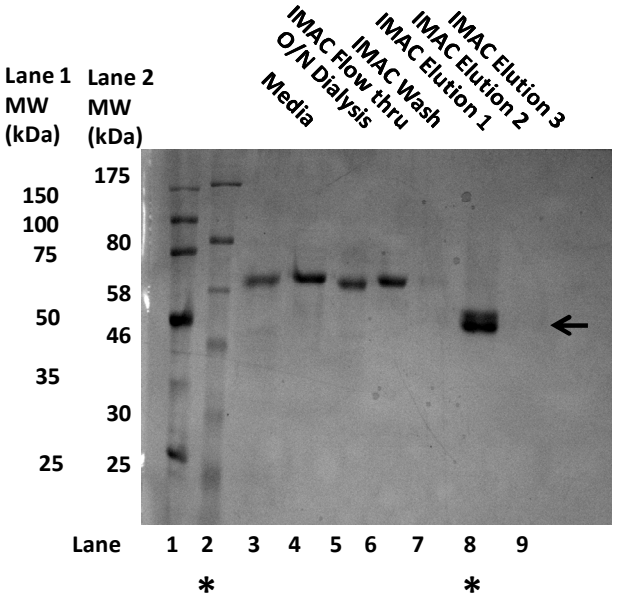
Supplementary Figure 1

Expression and Purification of GrB/VEGF$_{121}$ from HEK-293T Cells

* Lanes shown in Figure 1

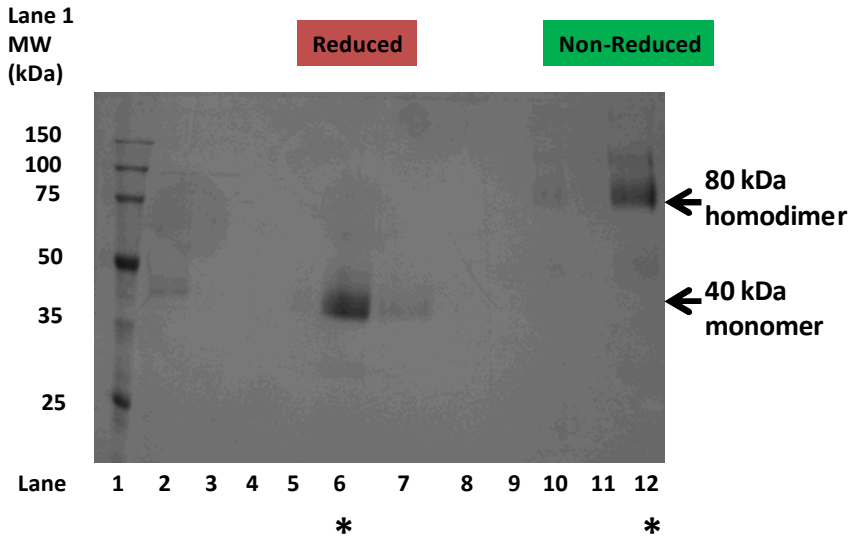
Gel 1

Purification of Pro-GrB/VEGF$_{121}$ on Co-IMAC

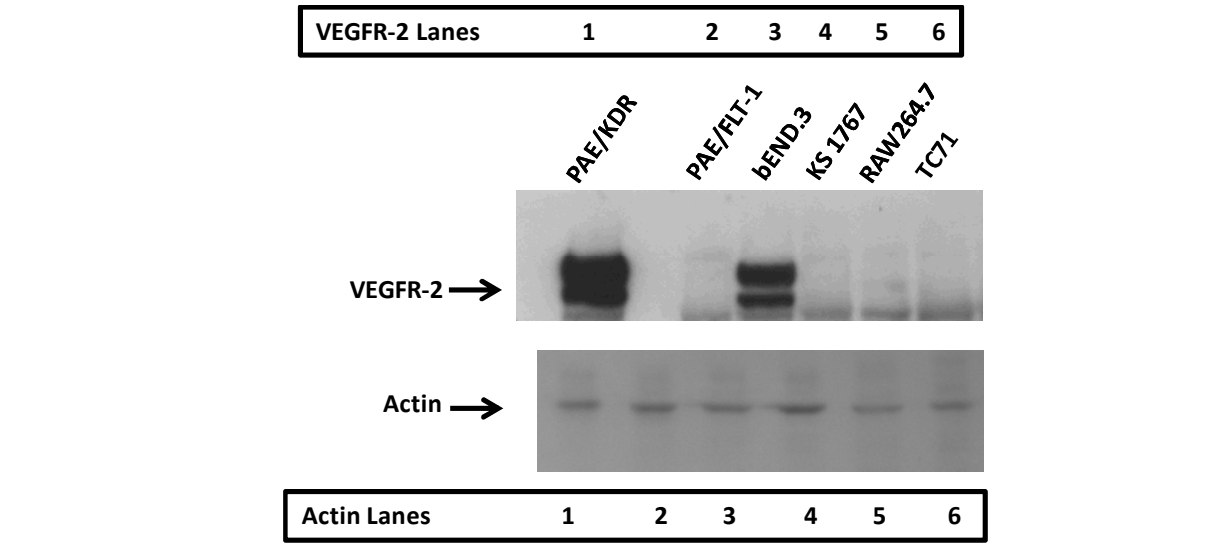


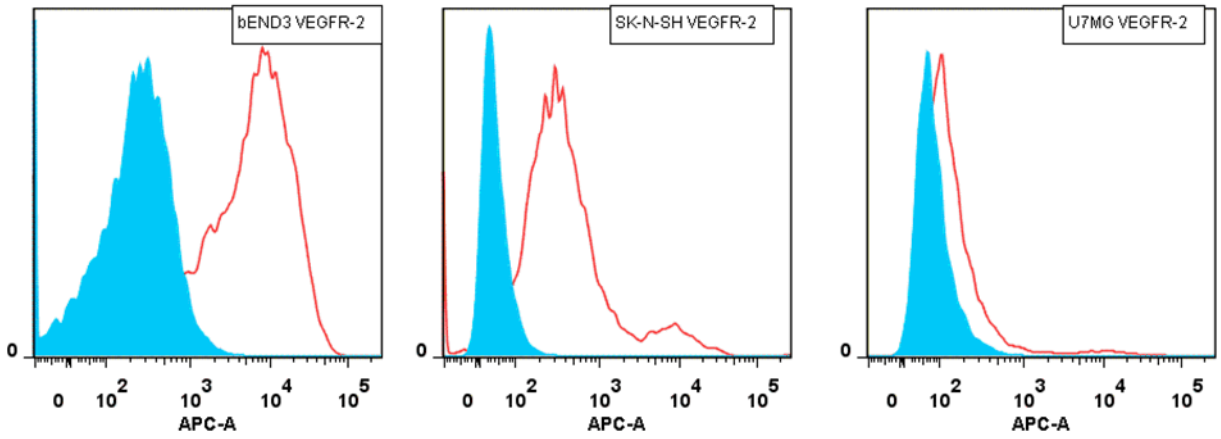
Gel 2

GrB/VEGF$_{121}$ following removal of the His$_6$ Tag by Enterokinase



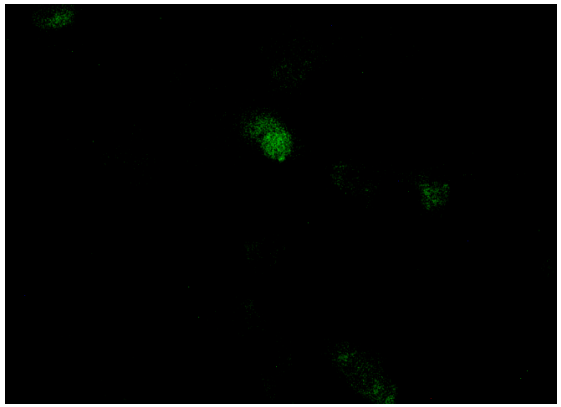
Supplementary Figure 2



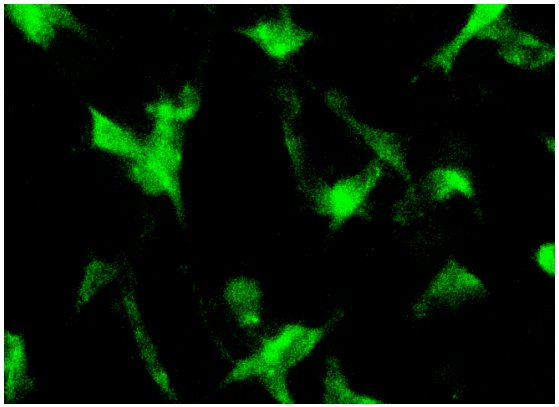


Supplementary Figure 3

PAE/VEGFR-1, GrB/$VEGF_{121}$, 24 h



PAE/VEGFR-2, GrB/$VEGF_{121}$, 24 h



Supplementary Figure 4