Supporting Information

Agadjanian et al. 10.1073/pnas.0901531106

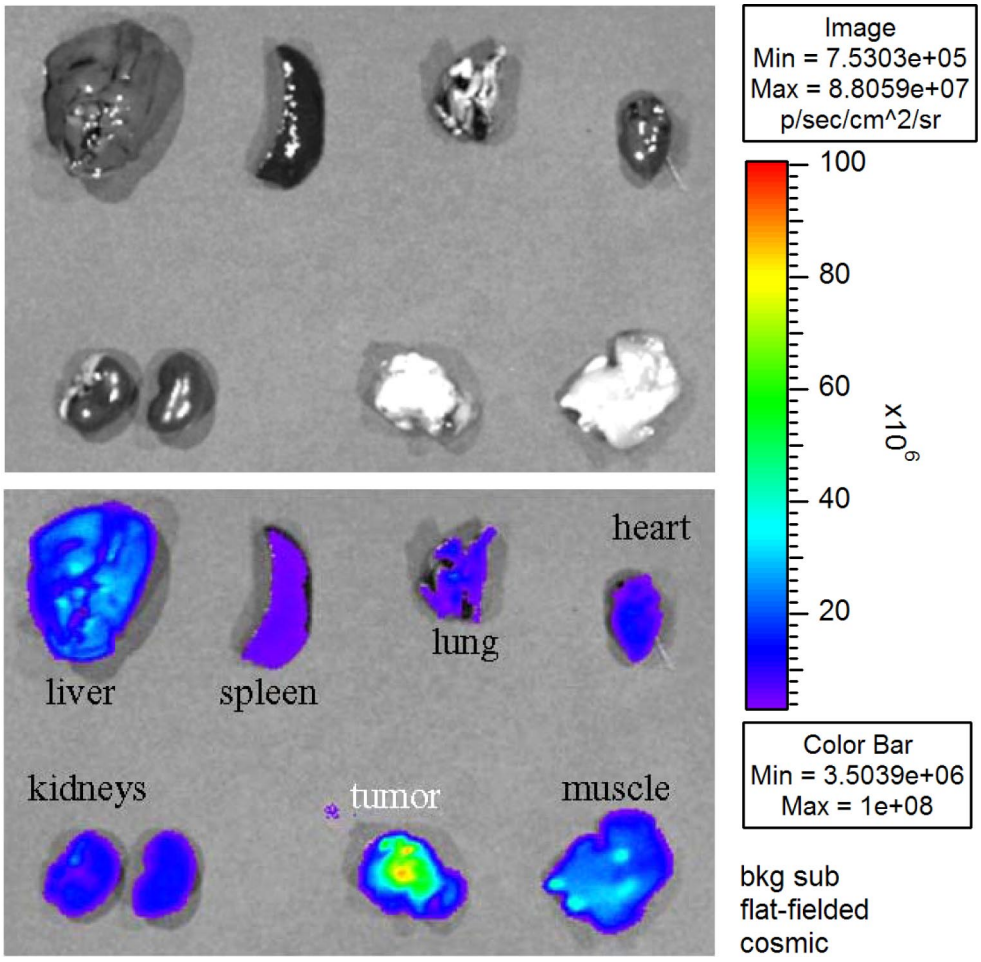

Fig. S1. Preferential accumulation of GFP-Her at HER2$^+$ tumors. Female nude mice bearing ≈250–300 mm^3 HER2$^+$ tumors (generated 3–4 weeks earlier by s.c. injection of 1×10^7 human MDA-MB-435 cells) were injected with GFP-Her (3 nmol) via the tail vein. Tissues were harvested at 3.5 hr after injection and visualized using a Xenogen small-animal imager. Blue pseudocoloring indicates no fluorescence, whereas GFP intensity is reflected by a color value shift toward red in the color bar.

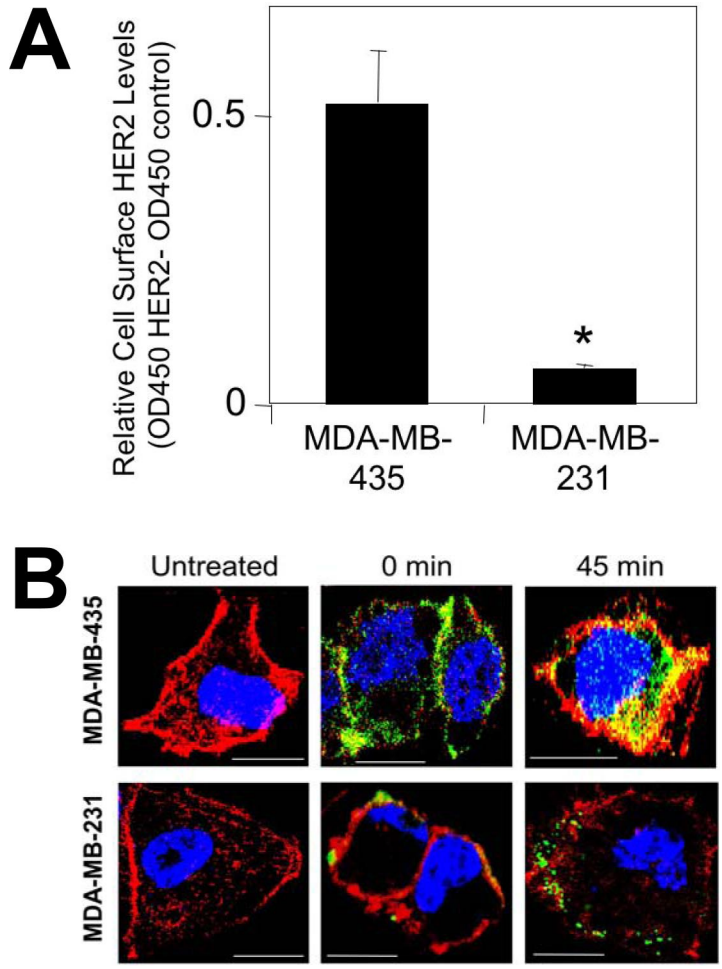

Fig. S2. Targeting of HerPBK10 protein. (*A*) Relative HER2 cell surface levels on MDA-MB-435 ($HER2^+$) and MDA-MB-231 ($HER2^-$) human tumor cell lines. Cells growing on 96-well plates were incubated with anti-HER2 subunit antibodies followed by HRP-conjugated secondary antibodies using standard ELISA procedures. Relative cell numbers were measured by crystal violet staining and quantified by measuring crystal violet absorbance at 590 nm. Relative subunit levels are reported as the ELISA signal of each cell population normalized by the relative cell number, or Abs 450 nm/590 nm. *, $P = 0.016$, as determined by 2-tailed unpaired *t* test. (*B*) Binding and uptake of HerPBK10 in $HER2^+$ and $HER2^-$ cell lines. At 2 days after plating on coverslips in 24-well dishes, cells were incubated with 5 μg of HerPBK10 per well in buffer A on ice for 1 hr to promote receptor binding but not internalization, then washed twice with buffer A to remove free protein, and incubated at 37 °C for the indicated time points to promote endocytosis. At each time point, separate wells of cells were washed with PBS/Mg 3 times and then fixed in 4% PFA for 15 min at room temperature and processed for immunohistochemistry as described by Rentsendorj, et al. [Rentsendorj A, et al. (2006) Typical and atypical trafficking pathways of Ad5 penton base recombinant protein: Implications for gene transfer. *Gene Ther* 13:821–836]. Images were captured using a Leica laser-scanning confocal fluorescence microscope (Leica Microsystems) Red, actin; Blue, nucleus; Green, HerPBK10. (Scale bars, $\approx$10 μm.)