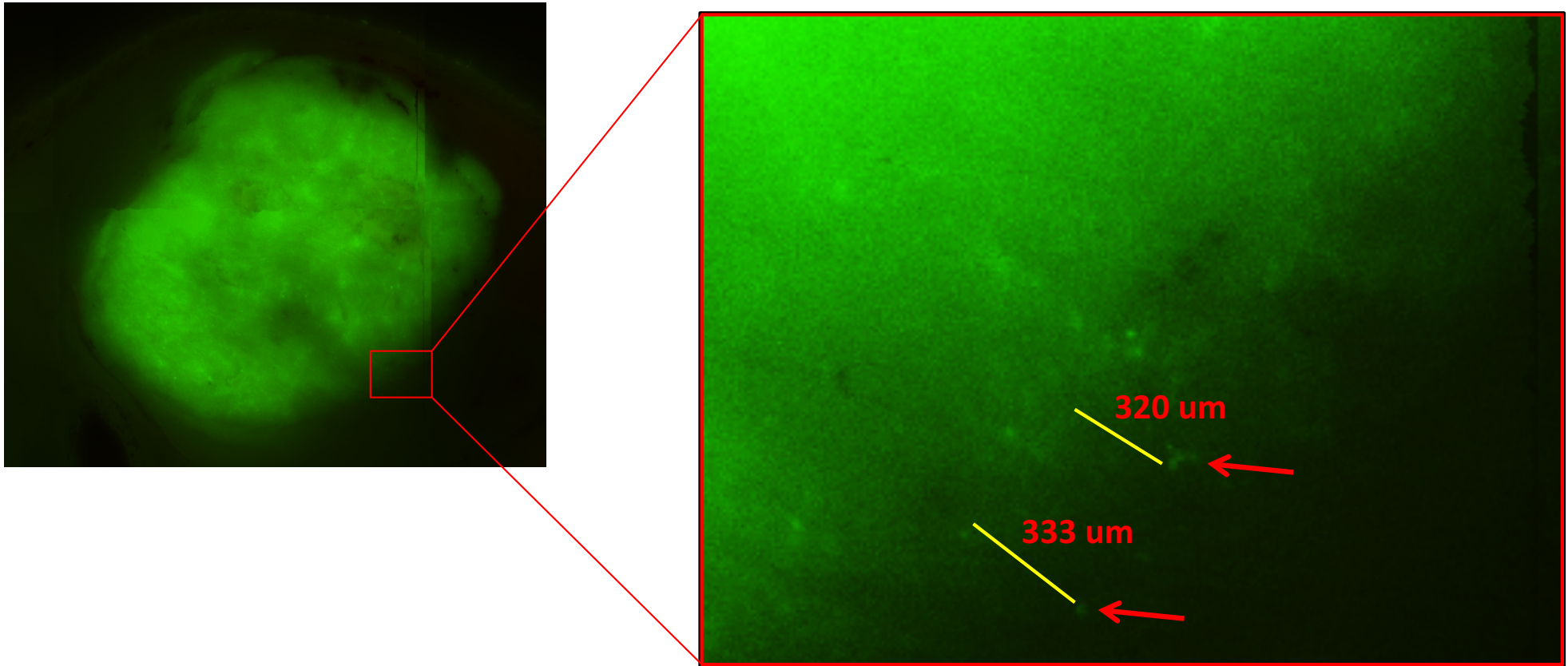


## A. Cryo image of PC3pip GFP tumor

## B. Zoom in of the tumor



**Figure S2: Cryoimaging detects dispersed PC3pipGFP cells.** (A) Cryoimage of intra-muscle PC3pipGFP model in mice. (B) Enlargement of the red box in (A). Micro dispersions of the cells (red arrow) away from the tumor mass (distance in yellow) was observed. Images were acquired using the CryoViz™ imaging system (BioInVision, Inc., Cleveland, Ohio). The optics included a liquid crystal RGB filter, a DAPI/FluoroGold Longpass fluorescence filter (Exciter: AT350/50x, Dichroic/Beam Splitter: T400lp, Emitter: ET425lp, Chroma, Rockingham, VT), and a low-noise monochrome camera. Mice were imaged in high resolution mode with a  $5.1 \mu\text{m} \times 5.1 \mu\text{m}$  in-plane pixel size and  $40 \mu\text{m}$  section thickness. The corresponding numbers for the lower resolution mode were  $10.1 \mu\text{m} \times 10.1 \mu\text{m}$  in-plane pixel size and  $40 \mu\text{m}$  section thickness. Raw image data from the CryoViz™ were processed to generate 3D color anatomical brightfield and molecular fluorescence volumes using the CryoViz™ Preprocessor software (BioInVision, Inc., Cleveland, Ohio). CryoViz™ Cell Detector software (BioInVision, Inc., Cleveland, Ohio) was subsequently employed to automatically detect tumor and migrating cells from the fluorescence whole mouse volumes. For 3D graphics, the main or unresected tumor as well as migrating cells in the fluorescence volumes were segmented out semi-automatically and rendered along with a true color brightfield volume rendering (n=1).