SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Lack of effect of probe on control blood from healthy volunteers. Peripheral blood samples from healthy volunteers were processed in a manner identical to that from patients (as described in the Material and Methods), and exposed to the Probe. The purification process results in mostly peripheral blood mononuclear cells (PBMCs), and which was exposed to probe and Hoechst stain (which stains all nuclei) and serially imaged via live-cell microscopy. **A.** Counts of cells from eight different live-cell imaging sessions, with the graph indicating the number of Hoechst-positive and green fluorescent protein (GFP, resulting from the Probe) cells. **B.** Representative images of the live-cell microscopy, showing images taken 12, 24 and 48 hours after exposure to Probe exposure, and which demonstrated lack of GFP expression in the PBMCs (but which readily took up the Hoechst stain).

Supplementary Figure 2. Representative scattergrams of cell counts from blood obtained from normal healthy volunteers and glioma patients and processed via CTC assay. Representative scattergrams are shown of blood collected and processed from a control subject (left panel) or a patient with glioma (right panel). Both blood samples were processed utilizing our standard CTC assay workflow described in the Materials and Methods section. Cell size (in microns) is plotted on the x-axis and fluorescence intensity (GFP, standard deviations above the background intensity) is plotted in the y-axis. The hashed box in both panels highlights the size and intensity parameters used to determine a CTC. Counted cells considered to be CTCs are located

within the hashed boxes and delineated by red squares. Counted cells not considered to be normal cells are delineated by blue squares with a red outline.

Supplementary Figure 3. Verification of the specificity of Anti-EGFR antibody. C8161 and Mel624 melanoma cells known to express and be deficient for EGFR, respectively, were harvested for western blotting (left panel), and immunofluorescence (right panel). The western blots were probed for EGFR (Cell Signal) or Ran (loading control, Cell Signal), indicating the specificity of the antibody for detecting EGFR. The immunofluorescence likewise confirmed that specificity of the antibody for EGFR-expressing cells.

Supplementary Figure 4. Detection of EGFR-expressing CTCs from a patient with EGFR over-expressing high grade glioma. Peripheral blood from a patient with a WHO grade IV primary glioma that showed amplification of EGFR was exposed to the probe, and imaged for GFP and EGFR expression. All cells that showed GFP also stained for EGFR on immunofluorescence indicating complete concordance. In contrast, the surrounding white blood cells showed neither GFP nor EGFR. DAPI is taken up in the nuclei of all cells, including both WBCs and CTCs.

Supplementary Figure 5. Comparison of EGFR-expressing CTCs from additional patients with EGFR over-expressing high grade glioma and EGFR (-) glioma tumors. Peripheral blood from patients with a WHO grade IV primary glioma that showed amplification of EGFR (patients 2 and 3) were exposed to the probe, and imaged for GFP and EGFR expression. All cells that showed GFP also stained for EGFR on immunofluorescence from these patients indicating complete concordance.

Peripheral blood samples from the cohort of patients with WHO grade IV primary glioma that showed no amplification of EGFR (representative patient 4, EGFR(-)) were also exposed to the probe, and imaged for GFP and EGFR expression. In agreement with the primary surgical pathology sample, no CTCs identified (via GFP signal) co-stained for EGFR on immunofluorescence from these patients. Representative EGFR, DAPI, and GFP channel images of detected CTCs are shown as indicated.

Supplementary Figure 6. Glioma CTCs show lack of EpCAM expression. Blood samples from the patient described in Figure 3 and Supplemental Figure 3 ("Patient 1") were stained for EpCAM, along with blood samples from two other patients with gliomas that lack EGFR expression. None of the CTCs from these patients showed EpCAM expression.