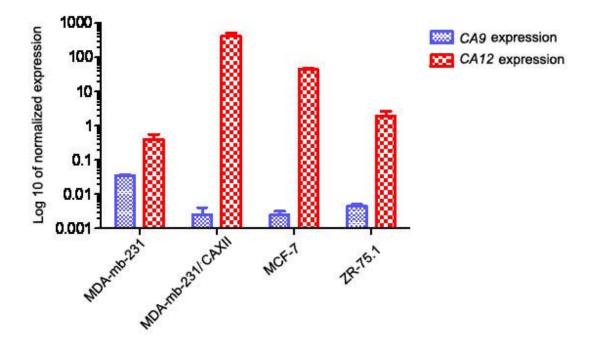
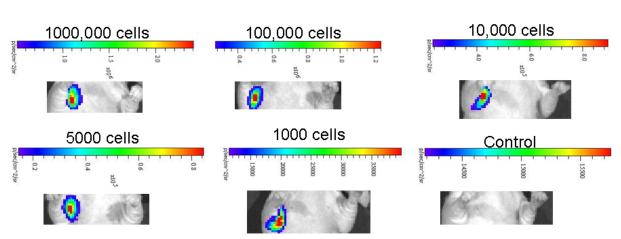


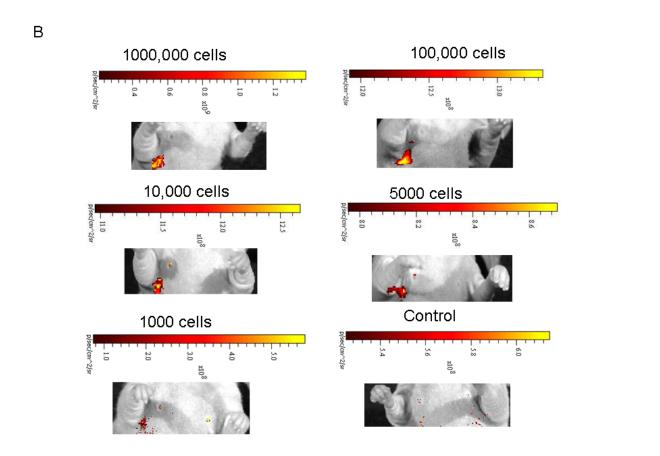
Supplementary Figure S3



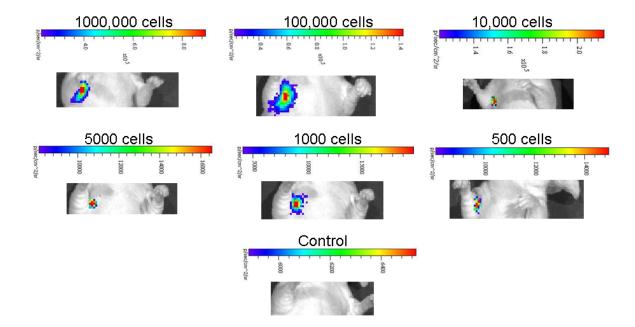


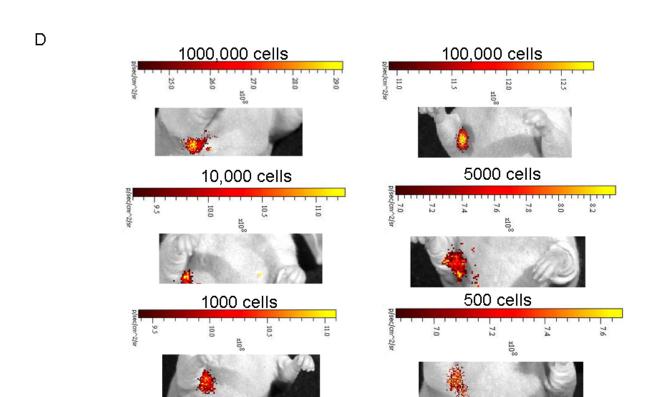
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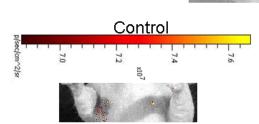


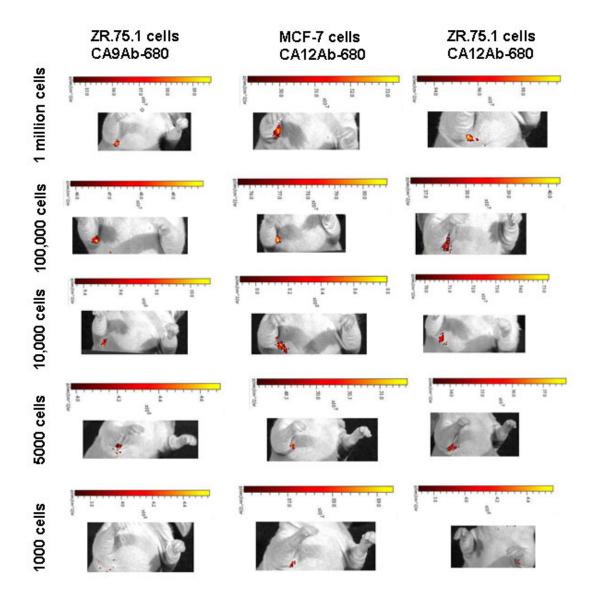


С









Supplementary Figure Legends

- **Fig. S1.** DNA microarray expression profiles of *CEACAM6*, *CXCL9*, *CXCL10* and *MMP9* mRNA in patient and unaffected tissue samples. Data are represented as mean ± s.d. Note the Log 10 scale. Asterisk: significant difference between breast tumor and normal breast, or diseased lymph nodes and normal lymph nodes with P values inset into the graphs.
- **Fig. S2.** ICC of CAXII expression (green) in engineered MDA-mb-231 cells but not in parental. Cell nuclei are stained blue.
- Fig. S3. qRT-PCR analysis of CA9 and CA12 expression in MDA-mb-231, MDA-mb-231/CAXII, MCF-7 and ZR-75.1 cells. CA9 expression in MDA-mb-231 is about 7 times more than ZR-75.1 cells, while its expression is very low in MCF-7 cells. CA12 expression in MDA-mb-231/CAXII (engineered cells) is about 10 and 20 times more than CA12 expression in MCF-7 cells and ZR-75.1 cells, respectively. Data are represented as mean \pm s.d. Note the Log 10 scale.
- **Fig. S4.** Ultrasound guided injection of cells into axillary node (yellow arrow). Needle is indicated by white arrow. (A) Pre-injection, (B) injection and (C) post-injection.
- **Fig. S5.** Sensitivity of CA9Ab-680 and CA12Ab-680 for detection of tumor cells in axillary lymph node. A range in number of MDA-mb-231 cells were injected into the axillary lymph node of nude mice. (A,C) Luciferase bioluminescence imaging 4 hours after implantation of the cells; MDA-mb-231/Luc (A) and MDA-mb-231/Luc expressing CAXII (C). (B,D) Agents were injected into the MFP proximal to the axillary nodes and fluorescence images acquired 24 hours after injection. Panel B shows mice carrying CA9Ab-680 agent and panel D shows mice carrying CA12Ab-680 agent. As few as 1000 and 500 cells were detected by CA9Ab-680 and CA12Ab-680, respectively. Note that

signal was not detected in a sham injected node (control with no cells). Exposure time is different for every image and the units are p/sc/cm2/sr. For fluorescence studies, the signals were autofluorescence background subtracted.

Fig. S6. Sensitivity of CA9Ab-680 and CA12Ab-680 for detection of tumor cells with different levels of *CA9* and *CA12* expression implanted into the axillary lymph node. A range in number of ZR-75.1/Luc cells (*CA9* and *CA12* low expressing cells, Fig. S5), MCF-7/Luc cells (*CA12* moderate expressing cells, Fig. S5) were injected into the axillary lymph node of nude mice using ultrasound guided injection. After confirmation of accurate implantation into the ALN by bioluminescence imaging, CA9Ab-680 and CA12Ab-680 were injected into the MFP proximal to the axillary nodes and fluorescence images acquired 24 hours after injection. About 5000 ZR-75.1 cells were detected by CA9Ab-680, and as few as 1000 MCF-7/Luc cells and 5000 ZR-75.1/Luc cells were detected by CA12Ab-680. Exposure time is different for every image and the normalized units are p/sc/cm2/sr. For fluorescence studies, the signals were autofluorescence background subtracted.