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Fig. S1: Synthesis of nanodrugs.

Step 1: Attachment of $mPEG_{5000}$-NH_2 (mPEG), H_2N-Leu-ethylester (LOEt) and 2-mercapto-1-ethylamine (MEA) yielding Preconjugate-2 through amide linkage after NHS activation of PMLA's pendant carboxylates. Step 2: Maleimide functionalized mAbs were conjugated by stable thioether bond. Thiol reactive AONs were attached to MEA through disulfide linkages. Optical agent (Alexa Fluor-680) was attached through thioether linkage. Excess thiols groups were masked using PDP.

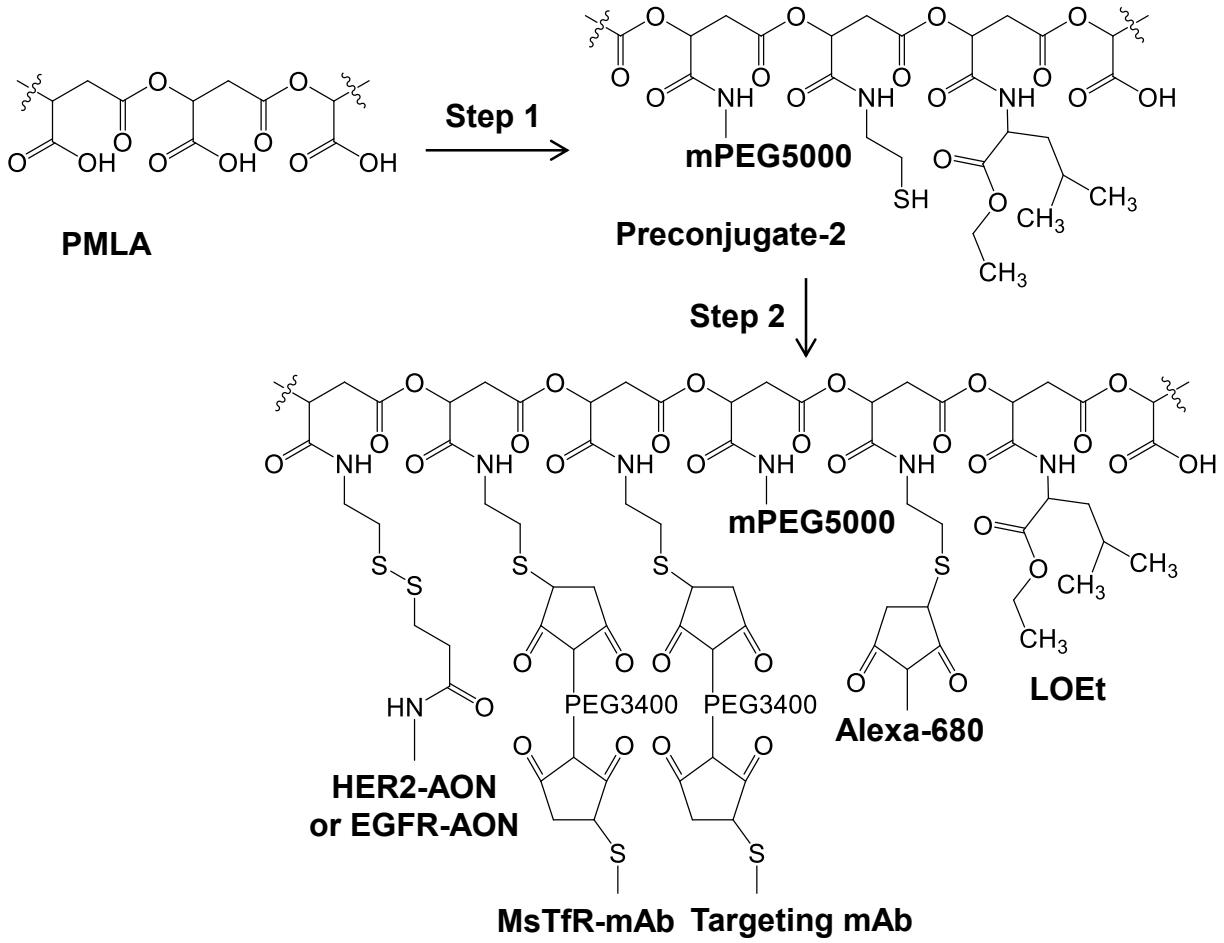


Fig. S2: **Targeting NIAs do not lose antigen-binding ability upon mAb conjugation.**

(a) For flow cytometry, MDA-MB-468 cells overexpressing EGFR were trypsinized and washed with PBS supplemented with 2% FBS and incubated with contrast agent P/Gd-DOTA(10-12%)/cetuximab(0.12%)/MsTfR-mAb(0.12%)/Rhodamine(1%) with a final concentration of cetuximab at 2.5 μg/ml, for 30 min at 4^0C. PBS treated cells were included as negative control. Washed cells fixed with 2% paraformaldehyde were imaged using BD LSRII system at Cedars-Sinai Flow Cytometry Core and analyzed by the flow system software version 2.5.1. The shift (blue) was seen. **(b)** Corresponding results were obtained with BT-474 cells overexpressing HER2 after incubation with P/Gd-DOTA(10-12%)/trastuzumab(0.12%)/MsTfR-mAb(0.12%)/Alexa-680(1%), with a final concentration of trastuzumab at 2.5 μg/ml. PBS treated cells were included as negative control. In a separate control experiment in which cells had been pre-incubated with unlabeled monoclonal antibodies (cetuximab or trastuzumab), the shifts were not observed (data not shown).

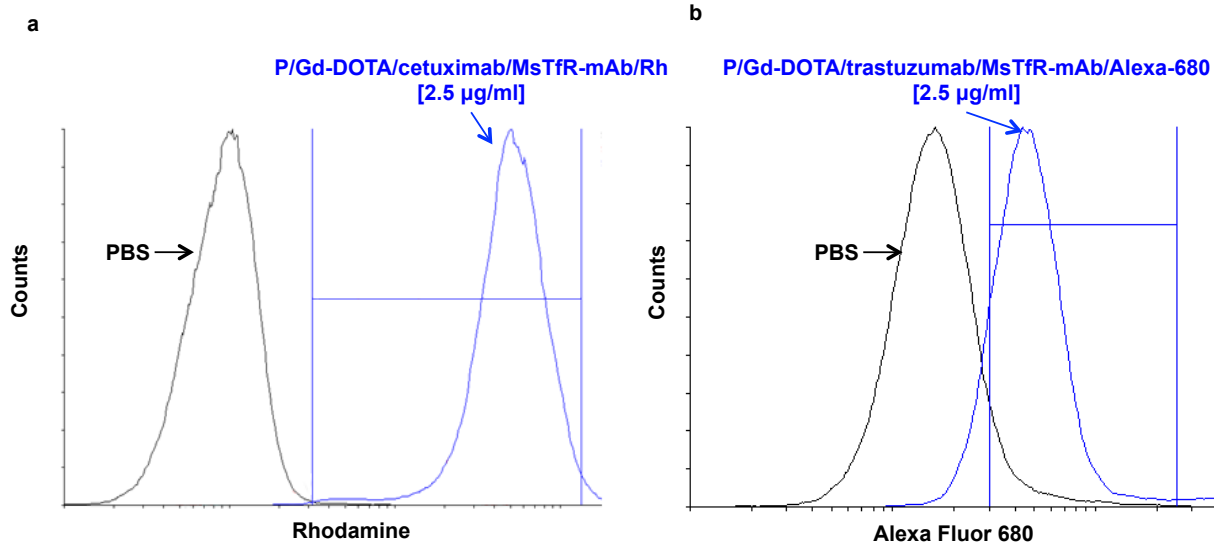
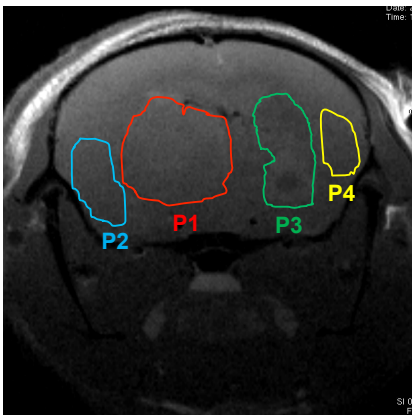


Fig. S3: Data acquisition for quantitative MRI analysis.

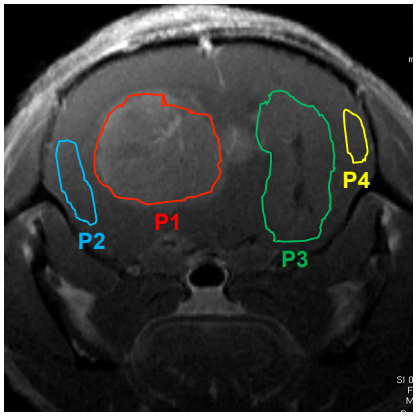
Representative images of a MRI brain scan for a mouse injected with P/Gd-DOTA/cetuximab/MsTfR-mAb/Alexa-680 are shown at 0, 20, 60, 120, and 180 min. For each time point, MRI scan intensities of whole brain were recorded, and up to 20 slices of 1 mm thickness were measured and 3-6 slices were used for quantitative evaluation. For each selected slice, multiple regions of interest (ROI) were selected within the perimeters of tumors (P1 and P3) and adjacent healthy brain (P2 and P4). ROIs for normal brain areas (blue and yellow) were randomly selected from the same slice as for the tumor area (red and green). Average intensity (per unit area) was calculated for each ROI individually. Intensities in tumor areas relative to intensities in normal brain tissue were plotted for the indicated times after injection of the contrast agents. Values are given under each panel in the example shown in Supplementary Figure 3. Mean ± SEM values calculated from at least 3 ROI are given in the figures of contrast enhancement as a function of time.

0 min



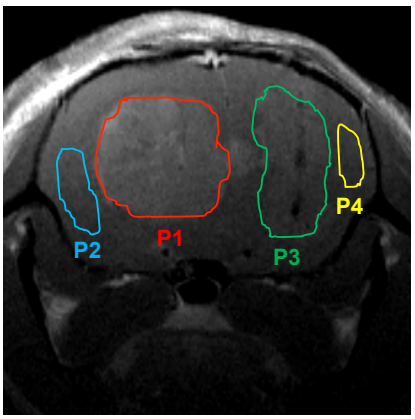
[(P1-P2)/P2]*100 = -1.8%
[(P3-P4)/P4]*100 = -1.4%

20 min



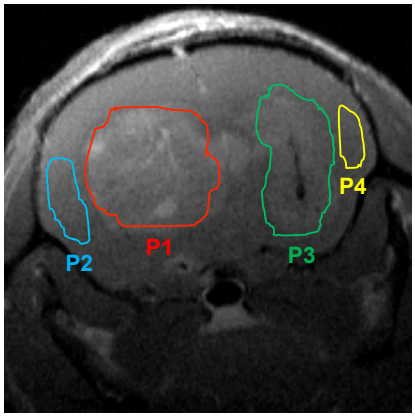
[(P1-P2)/P2]*100 = 47%
[(P3-P4)/P4]*100 = 0.4%

60 min



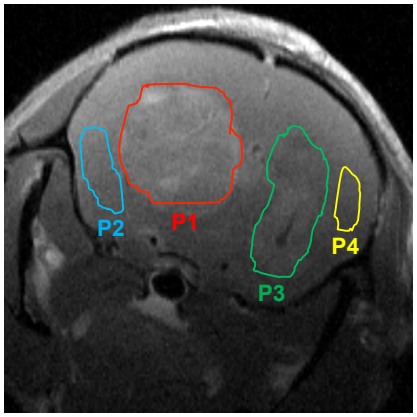
[(P1-P2)/P2]*100 = 62.3%
[(P3-P4)/P4]*100 = 1.1%

120 min



[(P1-P2)/P2]*100 = 42.7%
[(P3-P4)/P4]*100 = 0.3%

180 min



[(P1-P2)/P2]*100 = 29.7%
[(P3-P4)/P4]*100 = -0.4%

Fig. S4: Control IgG-carrying nanoconjugate does not show cell/cytoplasmic delivery of AON to metastatic brain tumors *in vivo*.

Fluorescence microscopy of brain cryostat sections after I.V. injection of nanoconjugate P/mPEG(5%)/LOEt(40%)/EGFR-AON-Lissamine(2%)/IgG(0.2%)/Alexa-680(1%) *in vivo*. The PMLA platform was labeled with Alexa-680 (green). EGFR-AONs were labeled with Lissamine (red). Vessels were revealed by immunostaining for vWF (arrows, magenta). Eight hours after I.V. injection of control nanodrug, mice were euthanized and organs were harvested after intra-arterial total body PBS perfusion. No signal for PMLA or AON was detected in the brain tumor area or the normal brain tissue of mice bearing xenografts with non-targeted nanoconjugate with control IgG. Bar = 25 μm.

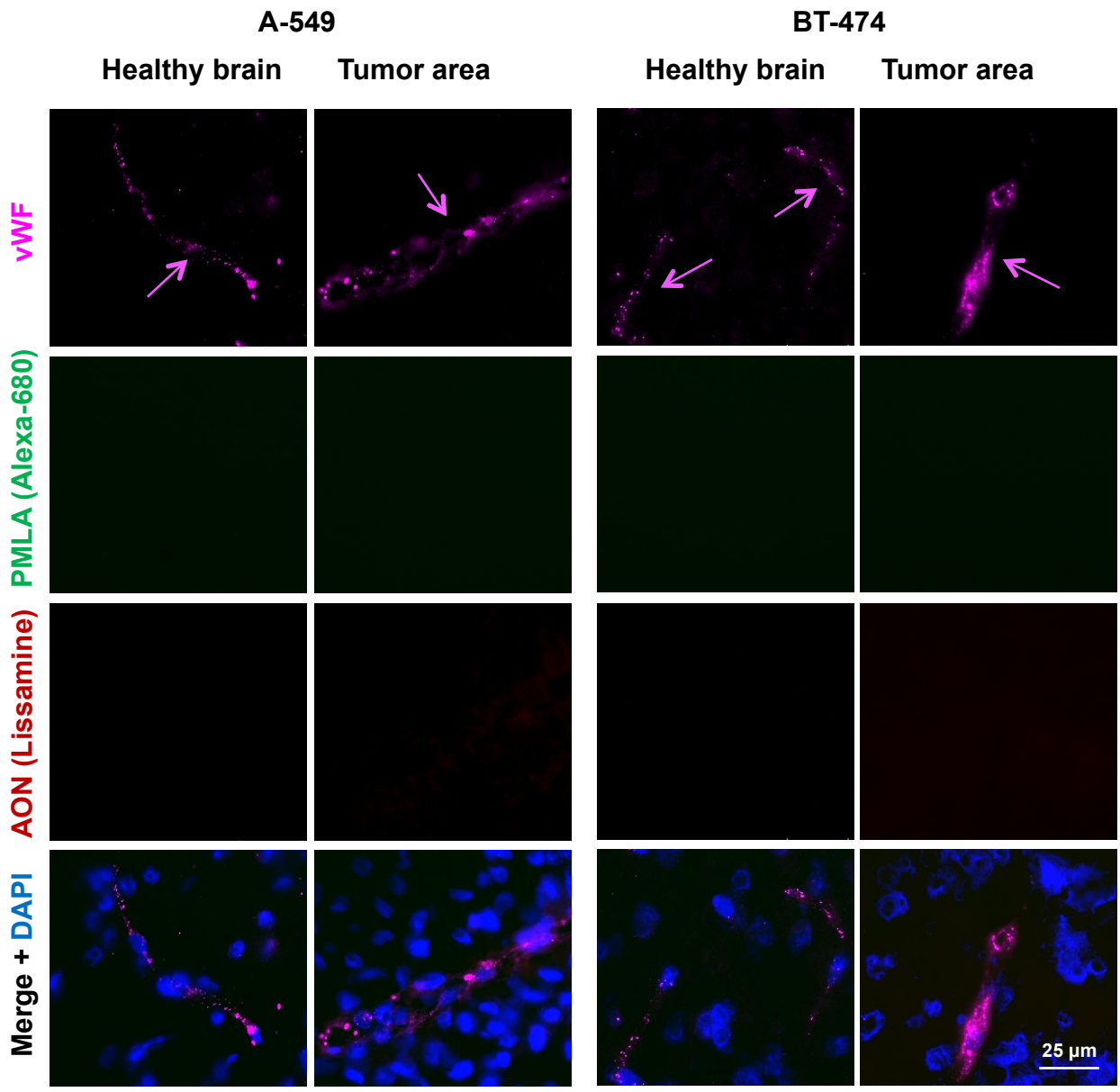
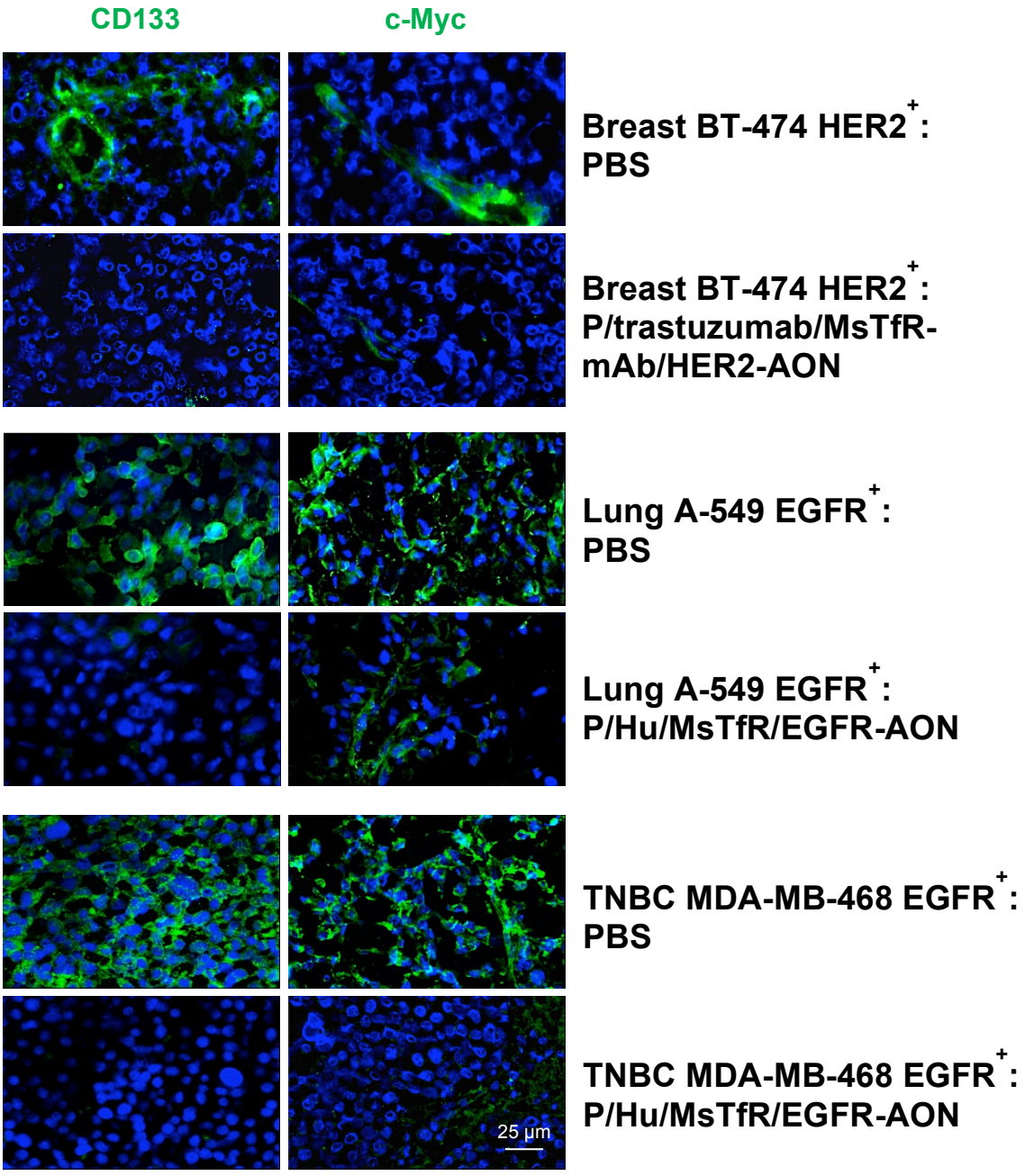

A-549
BT-474
Healthy brain
Tumor area
Healthy brain
Tumor area
vWF
PMLA (Alexa-680)
AON (Lissamine)
Merge + DAPI
25 µm

Fig. S5: Stem cell marker expression is markedly decreased in BM of three different tumors upon nanoconjugate treatment.

Tumor tissue sections were immunostained for CD133 and c-Myc (green) with DAPI counterstain (blue). In BT-474 $HER2^+$ BM both markers were expressed mostly in the vascular cells (top panels), and the staining was greatly diminished upon treatment with P/trastuzumab/MsTfR-mAb/HER2-AON nanodrug. In $EGFR^+$ A-549 lung cancer BMs (middle panels) and MDA-MB-468 TNBC BM (bottom panels), both markers were positive in many cells. After treatment with P/Hu/MsTfR-mAb/EGFR-AON nanodrug, the staining intensity and the number of positive cells were markedly diminished, confirming nanodrug affect on cancer stem cells.


CD133
c-Myc
Breast BT-474 HER2+: PBS
Breast BT-474 HER2+: P/trastuzumab/MsTfR-mAb/HER2-AON
Lung A-549 EGFR+: PBS
Lung A-549 EGFR+: P/Hu/MsTfR/EGFR-AON
TNBC MDA-MB-468 EGFR+: PBS
TNBC MDA-MB-468 EGFR+: P/Hu/MsTfR/EGFR-AON
25 µm