Supplementary Material

Optimizing fresh specimen staining for rapid identification of tumor biomarkers during surgery

Connor W. Barth[1], Jasmin M Schaefer[1], Vincent M. Rossi[4], Scott C. Davis[4§], Summer L. Gibbs[1,2,3§]

[1]Biomedical Engineering Department, [2]Knight Cancer Institute, [3]OHSU Center for Spatial Systems Biomedicine, Oregon Health & Science University, Portland, OR 97201

[4]Thayer School of Engineering, Dartmouth College, Hanover, NH 03755

[§]Corresponding Authors:

Scott C. Davis, Ph.D.
Thayer School or Engineering
Dartmouth College
Hanover, NH 03755
Email: Scott.C.Davis@Dartmouth.edu
Phone: 603-646-9684

Summer L. Gibbs, Ph.D.
Oregon Health & Science University
Collaborative Life Sciences Building
2730 SW Moody Ave, Mail Code: CL3SG
Portland, OR 97201
Email: gibbss@ohsu.edu
Phone: 503-494-8940

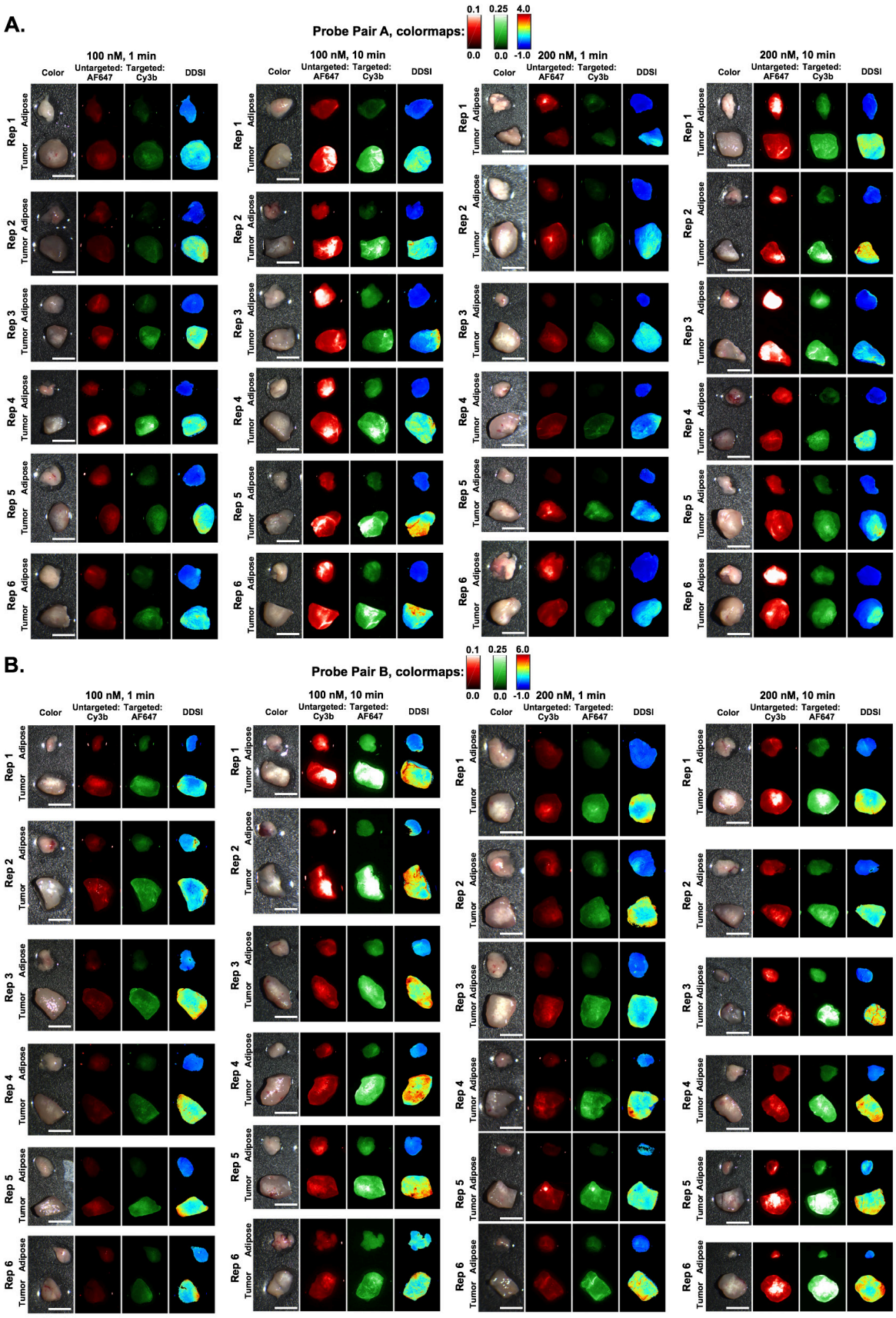
A.
Probe Pair A, colormaps:
0.1
0.25
4.0
0.0
0.0
-1.0
100 nM, 1 min
100 nM, 10 min
200 nM, 1 min
200 nM, 10 min
Color
Untargeted: AF647
Targeted: Cy3b
DDSI
Rep 1
Rep 2
Rep 3
Rep 4
Rep 5
Rep 6
Adipose
Tumor
B.
Probe Pair B, colormaps:
0.1
0.25
6.0
0.0
0.0
-1.0
100 nM, 1 min
100 nM, 10 min
200 nM, 1 min
200 nM, 10 min
Color
Untargeted: Cy3b
Targeted: AF647
DDSI
Rep 1
Rep 2
Rep 3
Rep 4
Rep 5
Rep 6
Adipose
Tumor

Fig. S1: *DDSI staining condition optimization full dataset.* All color, fluorescence, and DDSI images of tumor and adipose tissue pairs following staining using a range of dual-stain soak concentrations and incubation times for **(A)** probe pair A (Herceptin-Cy3b, DkRb-AF647) and **(B)** probe pair B (Herceptin-AF647, DkRb-Cy3b). All untargeted and targeted channel images are background corrected, normalized by their exposure time and calibration drop intensity, and displayed on equivalent color scales across staining conditions and probe pairs. DDSI images are displayed with equivalent color scales across staining conditions. Scale bars = 5 mm.

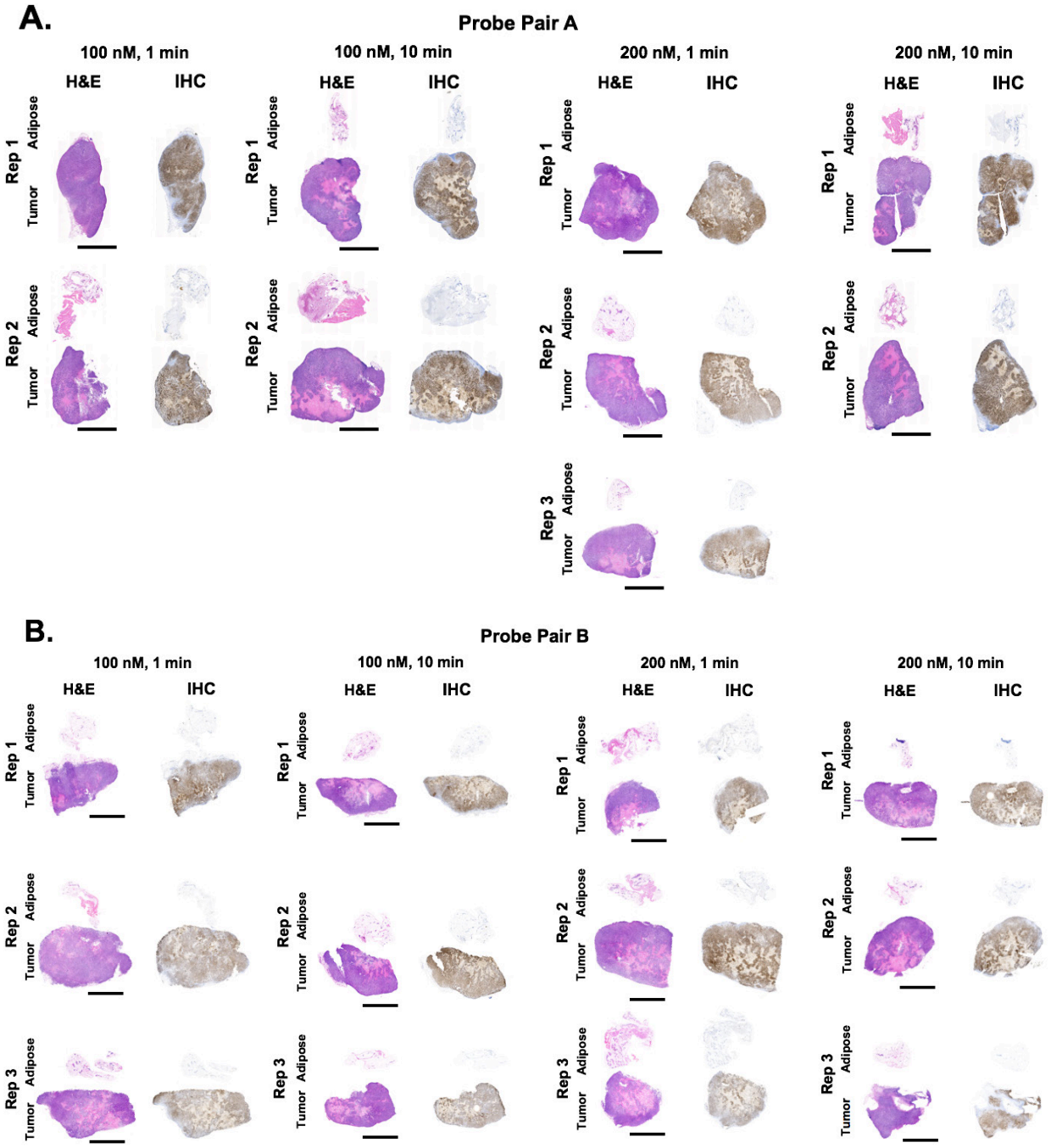

Fig. S2: *HER2 immunohistochemistry analysis.* All H&E and HER2 targeted IHC images of tumor and adipose tissue pairs following staining using a range of dual-stain soak concentrations and incubation times for **(A)** probe pair A (Herceptin-Cy3b, DkRb-AF647) and **(B)** probe pair B (Herceptin-AF647, DkRb-Cy3b). Images are acquired from serial cryosections of the same face imaged in whole specimen images. H&E = Hematoxylin & Eosin, IHC = immunohistochemistry. Scale bars = 5 mm.

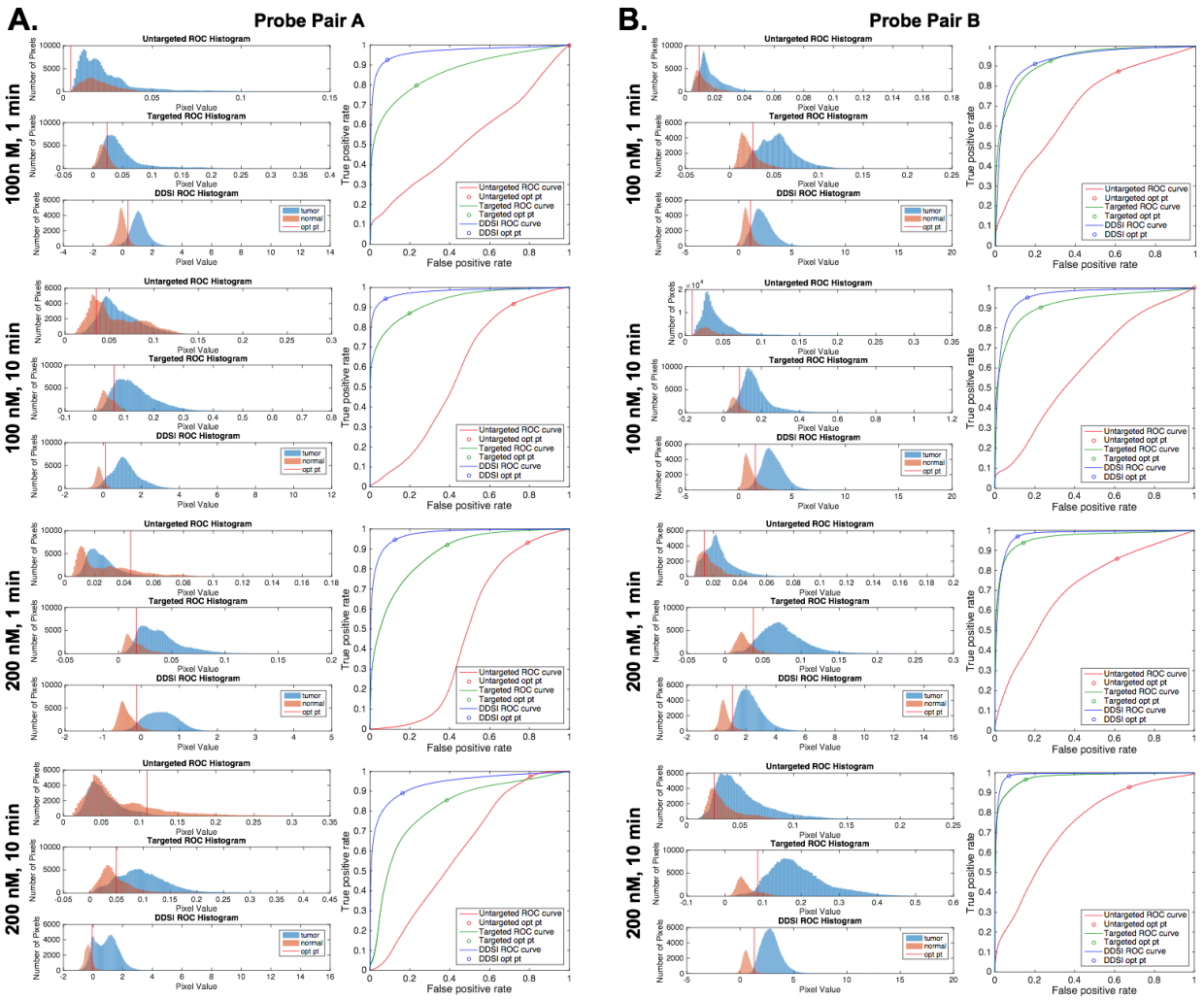

Fig. S3: *ROC analysis of staining condition optimization dataset*. Tumor and normal tissue pixel intensity histograms, ROC curves, and AUC values for untargeted, targeted, and DDSI image tumor vs. normal adipose tissue differentiation following staining using a range of dual-stain soak concentrations and incubation times for **(A)** probe pair A (Herceptin-Cy3b, DkRb-AF647) and **(B)** probe pair B (Herceptin-AF647, DkRb-Cy3b). Optimal points determined from ROC analysis are displayed on each ROC curve and as a vertical line on each pixel value histogram. ROC = receiver operator characteristic, AUC = area under curve, opt pt = optimal point.

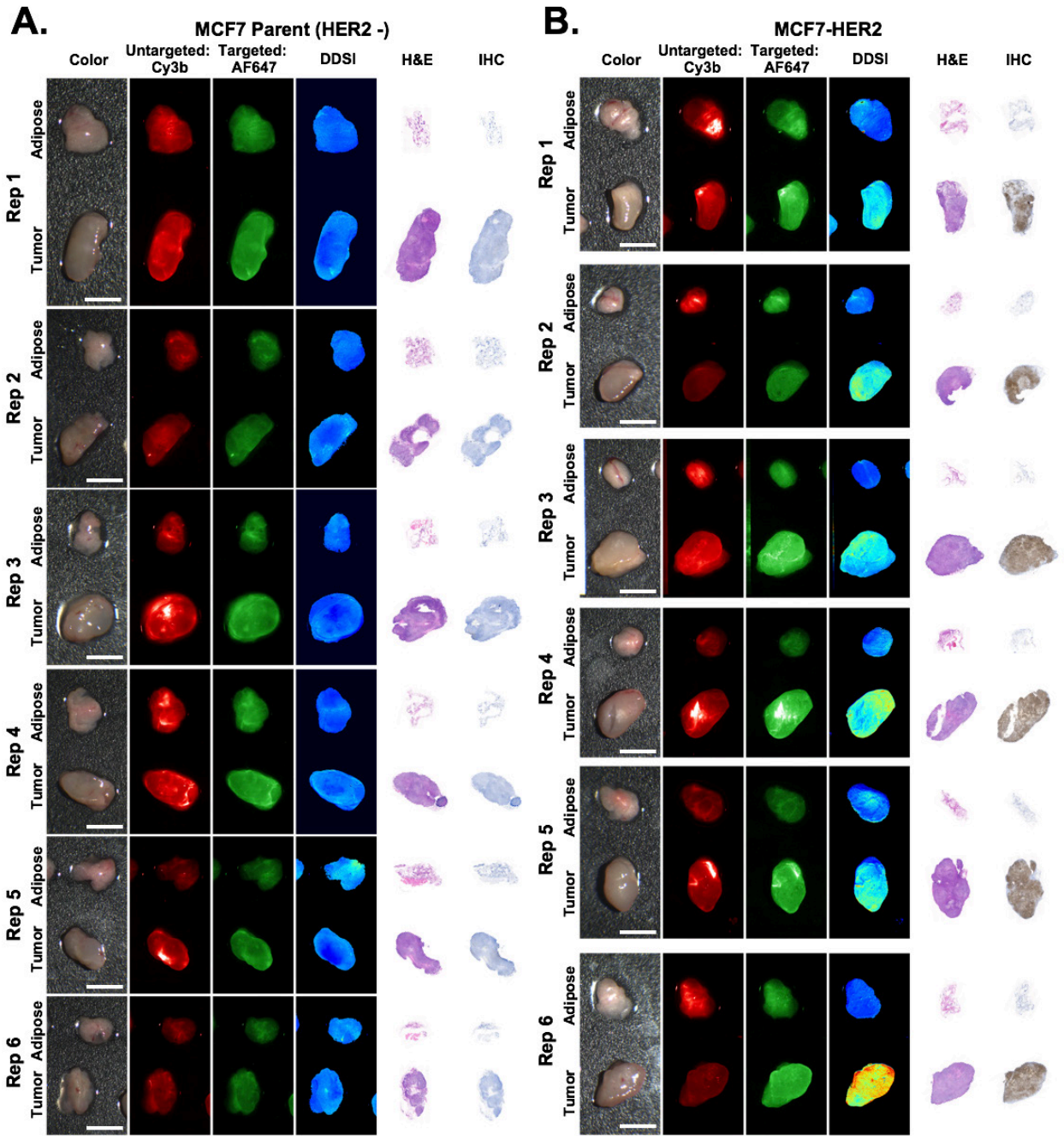

Fig. S4: *HER2 + and HER2 - testing dataset DDSI staining and IHC validation full dataset*. **(A)** All color, fluorescence, DDSI, H&E, and HER2 targeted IHC images of MCF7 – HER2 and MCF7 parent line tumor and adipose tissue pairs following staining using the ideal staining condition (Probe pair B, 200 nM concentration, 1 min incubation time). All untargeted and targeted channel images are background corrected, normalized by their exposure time and calibration drop intensity, and displayed on equivalent color scales. DDSI images are displayed with equivalent color scales. H&E and IHC images are acquired from serial cryosections of the same face imaged in the whole specimen images. H&E = Hematoxylin & Eosin, IHC = immunohistochemistry. Scale bars = 5 mm.

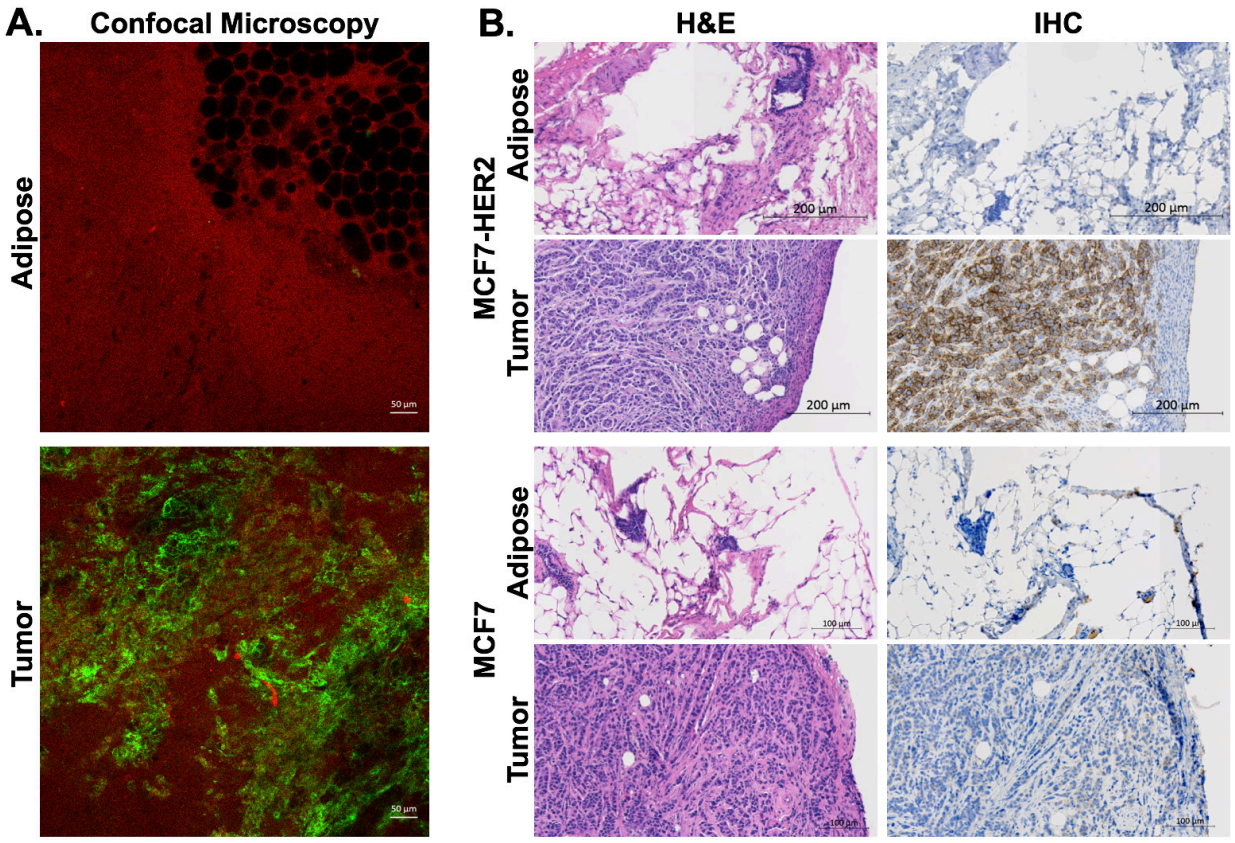

Fig. S5: *High resolution confocal microscopy and HER2 immunohistochemical analysis.* **(A)** Representative high resolution fluorescence confocal images of fresh MCF7-HER2 tumor and adipose tissue specimens imaged following completion of the ideal DDSI staining methodology (Probe pair B, 200 nM concentration, 1 min incubation time). Images were taken from the same cut face used for macroscopic images. Red channel = Cy3b fluorescence, Green channel = AF647 fluorescence. **(B)** Representative high resolution H&E and HER2 targeted IHC images of MCF7 – HER2 and MCF7 parent line tumor and adipose tissue pairs following staining using the ideal staining condition (Probe pair B, 200 nM concentration, 1 min incubation time). H&E and IHC images are acquired from serial cryosections of the same face imaged in the whole specimen images. H&E = Hematoxylin & Eosin, IHC = immunohistochemistry.