Supplementary Materials

Spectroscopic Photoacoustic Molecular Imaging of Breast Cancer

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Supplemental Tables

Supplemental Table 1. Human B7-H3 Immunohistochemical Staining Score Summary. Individual and composite immunohistochemical (IHC) scores of B7-H3 staining of normal breast tissue, benign, precursor, and malignant breast lesions presented as the mean ± standard deviation. *P < 0.01.

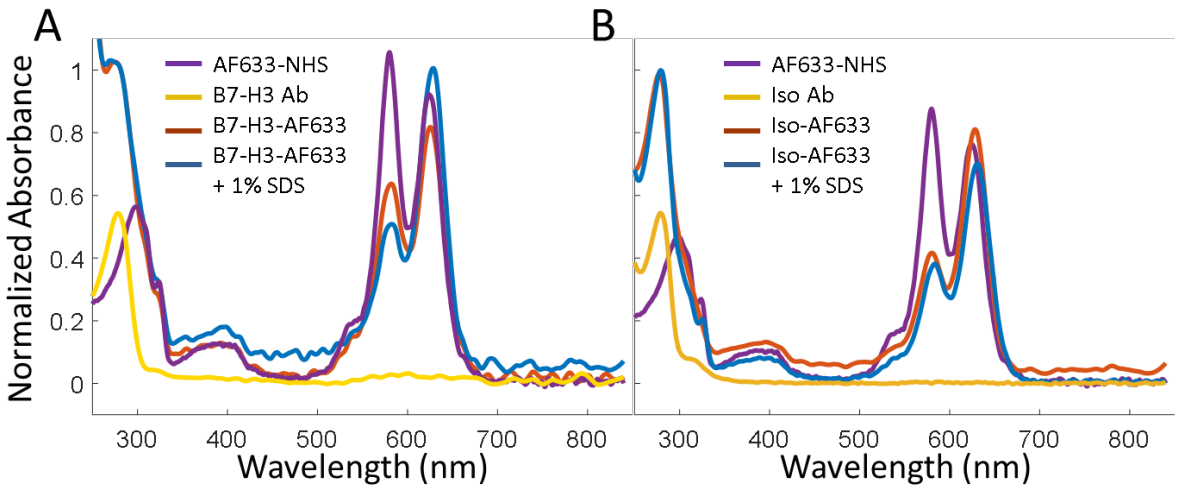
Histology	Subtype	n	Composite IHC Score Mean ± SD	Combined Grouping IHC Scores Mean ± SD
Normal Breast Tissue	--	53	0.96 ±1.82	1.40 ± 2.10
Benign and Precursor Breast Lesions	Adenosis	5	4.00 ± 4.00	
	ADH	2	0.00 ± 0.00	
	ALH	6	1.50 ± 1.975	
	ApoM	5	1.60 ± 3.58	
	CCL	75	1.267 ± 1.68	
	DCIS	13	2.77 ± 3.09	
	FA	1	4.00 ± 0.00	
	FEA	9	2.11 ± 1.83	
	NPFCC	2	2.00 ± 2.83	
	Radial scar	2	0.00 ± 0.00	
	UDH	9	2.68 ± 2.40	
Breast Cancer	Luminal A	40	7.17 ± 3.78	8.8 ± 3.69*
	Luminal B	15	9.80 ± 3.43	
	Her2	20	9.55 ± 3.33	
	Triple negative	22	10.55 ± 2.91	

Supplemental Table 2. Spectroscopic photoacoustic molecular B7-H3 signal and fluorescence ICG signal presented by individual experimental time points.

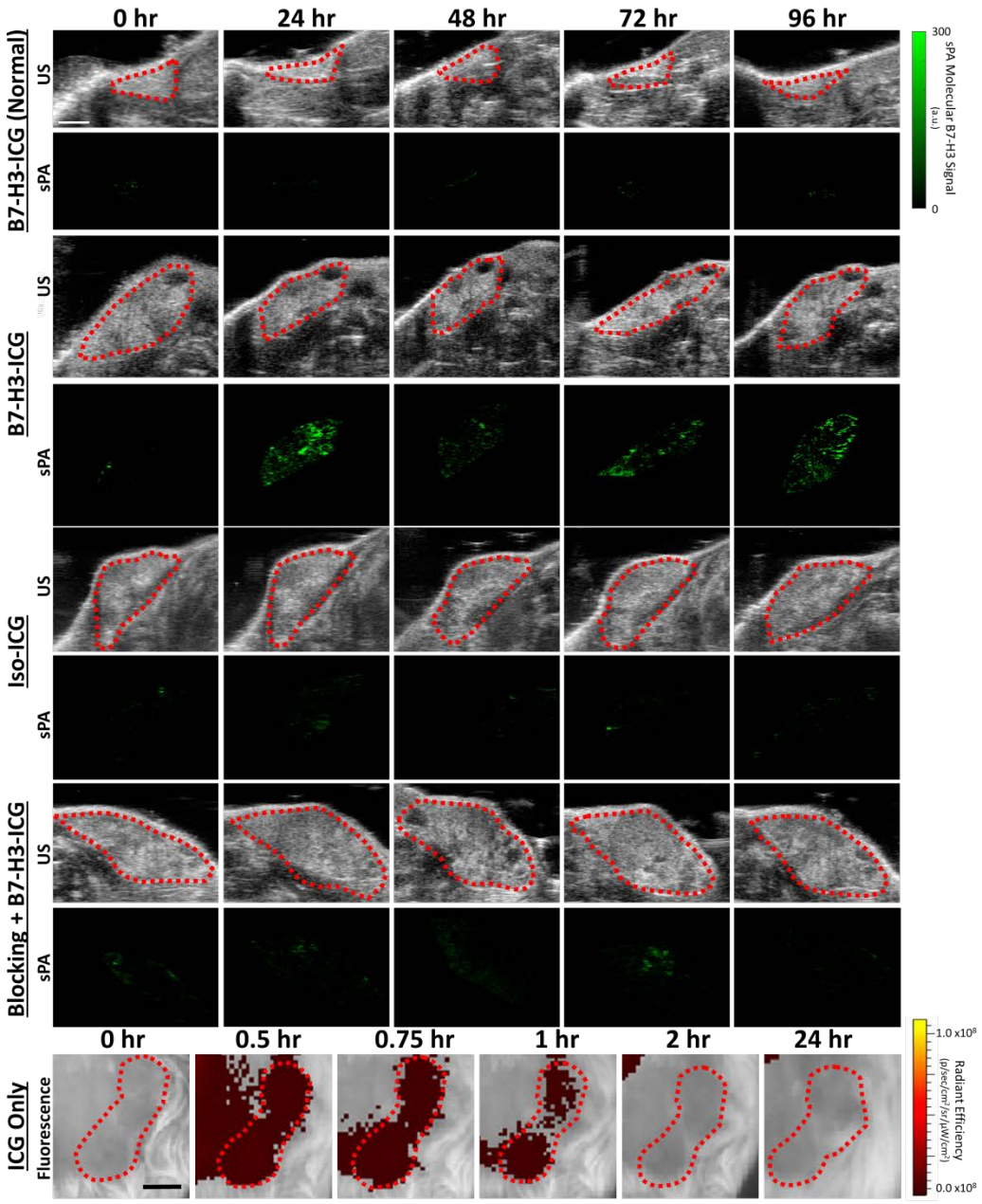
Spectroscopic Photoacoustic Imaging						
	Mean (95% CI), Interquartile Range					
Test Condition	0 hr	24 hr	48 hr	72 hr	96 hr	Combined
B7-H3-ICG (n=80)	**1.0** (1.0, 1.0), 1.0 - 1.0	**3.80*** (2.94, 4.66), 1.65 - 4.65	**2.29*** (1.71, 2.87), 0.77-2.93	**2.65*** (1.96, 3.34), 0.70-3.25	**3.27*** (2.43, 4.12), 1.00-3.88	**3.01*** (2.63, 3.38), 1.00-3.70
B7-H3-ICG (Norma) (n=60)	**1.0** (1.0, 1.0), 1.0 - 1.0	**1.37*** (1.19, 1.54), 0.88-1.96	**1.13** (0.89, 1.37), 0.61-1.54	**1.07** (0.84, 1.30), 0.40 - 1.68	**0.97** (0.80, 1.15), 0.50-1.40	**1.13** (1.03, 1.23), 0.6-1.6
Blocking+B7-H3-ICG (n=20)	**1.0** (1.0, 1.0), 1.0 - 1.0	**2.00*** (1.34, 2.66), 1.08-2.45	**0.86** (0.55, 1.17), 0.36-1.36	**0.92** (0.55, 1.29), 0.31-1.67	**1.14** (0.55,1.74), 0.41-1.50	**1.22** (0.97, 1.48), 0.40-1.79
Iso-ICG (n=30)	**1.0** (1.0, 1.0), 1.0 - 1.0	**1.2** (0.9, 1.5), 0.7-1.6	**1.2** (0.8, 1.5), 0.5-1.8	**1.4** (1.0-1.7), 0.7-2.0	**1.2** (0.8, 16), 0.5-1.4	**1.24** (1.08, 1.40), 0.5-1.75
Fluorescence	**0 hr**	**0.5 hr**	**0.75 hr**	**1 hr**	**2 hr**	**24 hr**
Free ICG (n=20)	**1.01** (0.97,1.04), 0.90-1.10	**1.07*** (1.02,1.12), 1.00-1.10	**1.10*** (1.05, 1.15), 1.10-1.20	**1.09*** (1.05,1.14), 1.03-1.18	**1.12*** (1.07,1.17), 1.10-1.20	**1.03** (0.99, 1.06), 1.00-1.10

Statistical significance (P < 0 .01) compared to the 0 hr time point is denoted with and asterisks (*).

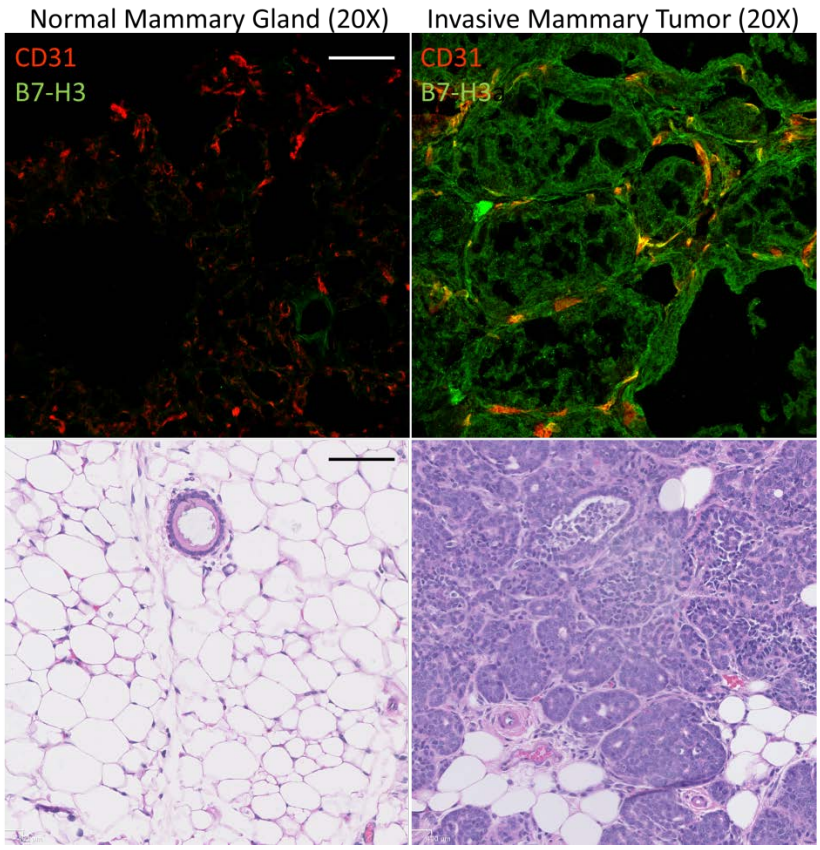
Supplemental Figures



Supplemental Figure 1. Absorbance Spectra of Antibody-AF633 Dye Conjugates. A. Absorbance spectra of both the B7-H3 antibody and AF633-NHS before conjugation, the B7-H3-AF633 conjugate, and the B7-H3- AF633 conjugate in 1% SDS for unquenched absorption signal. Ab-dye conjugates are normalized at 280 nm. Dye is normalized to the antibody-dye conjugate at 400 nm. **B.** Absorbance spectra of the isotype control antibody and AF633-NHS before conjugation, the Iso- AF633 conjugate, and the Iso-AF633 conjugate in 1% SDS for unquenched absorption signal. Antibody-dye conjugates are normalized at 280 nm. Dye is normalized to the antibody-dye conjugate at 400 nm. The specific (B7-H3) and isotype control antibody conjugates show similar absorbance spectra.



Supplemental Figure 2. Spectroscopic Photoacoustic Molecular Imaging of B7-H3 in Normal Breast Tissue and in Breast Cancer. B-mode ultrasound images were used to select the desired ROI (red dash lines) over the mammary glands to be used with the sPA imaging algorithm. Note strong B7-H3-targeted sPA molecular imaging signal in breast cancer compared to normal tissue. Iso-ICG, the blocked B7-H3-ICG condition, and free ICG show no tumor accumulation at. Scale bar in in ultrasound and sPA images represents 2 mm and in fluorescence image represents 1 mm. Note that free ICG was only imaged with fluorescence rapid clearance of ICG prohibiting sPA imaging.



Supplemental Figure 3. Immunohistochemistry and Histopathological Micrographs of Murine Mammary Tissues Stained for B7-H3 and CD31 Markers. Normal murine mammary gland stained for B7-H3 (green), and CD31 (red), showed little to no staining for the B7-H3 marker. Breast cancer showed strong B7-H3 expression on both vascular endothelial cells (CD31, red; co-localization shown in yellow) and tumor epithelium and stroma. Scale bars represent 80 µm.