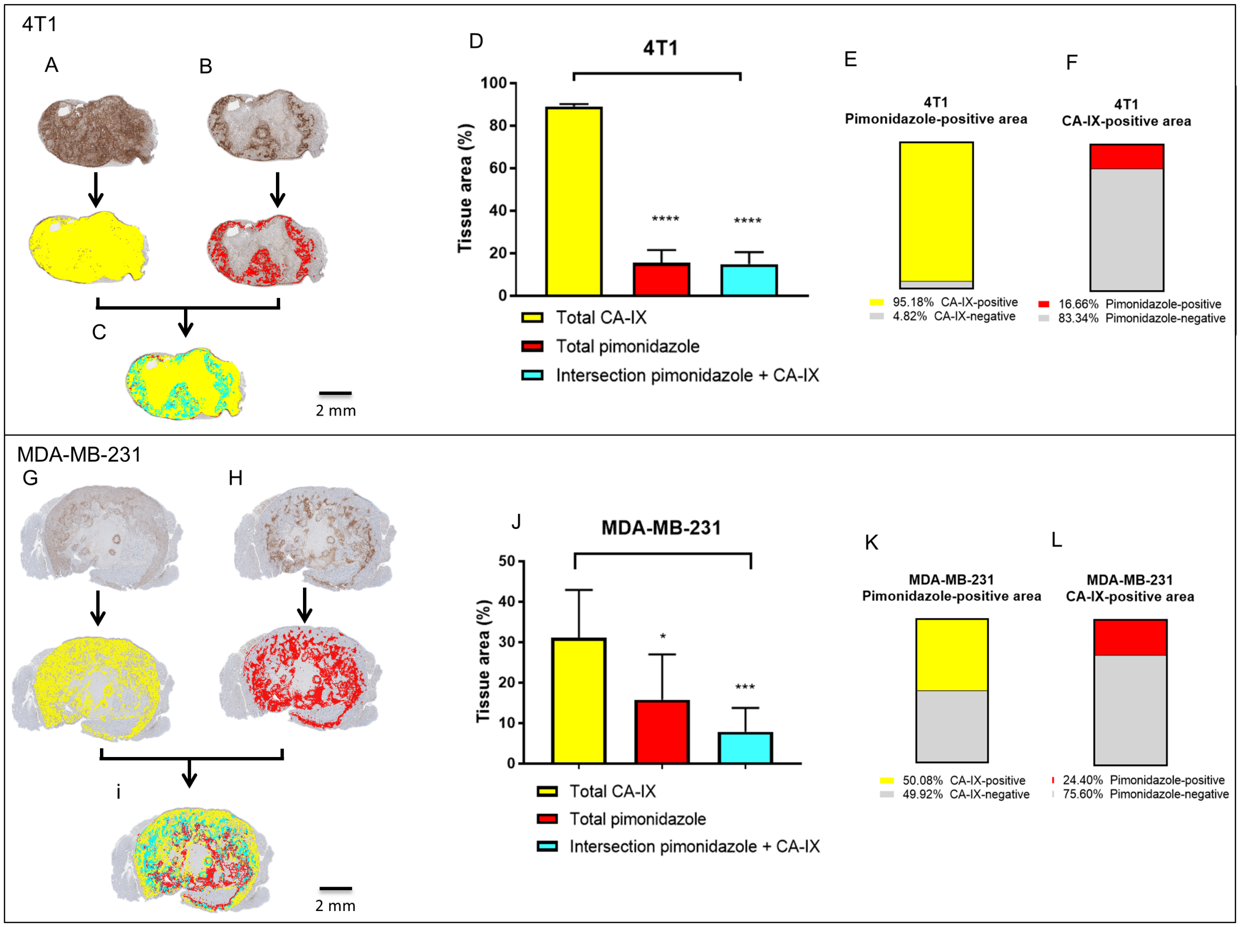
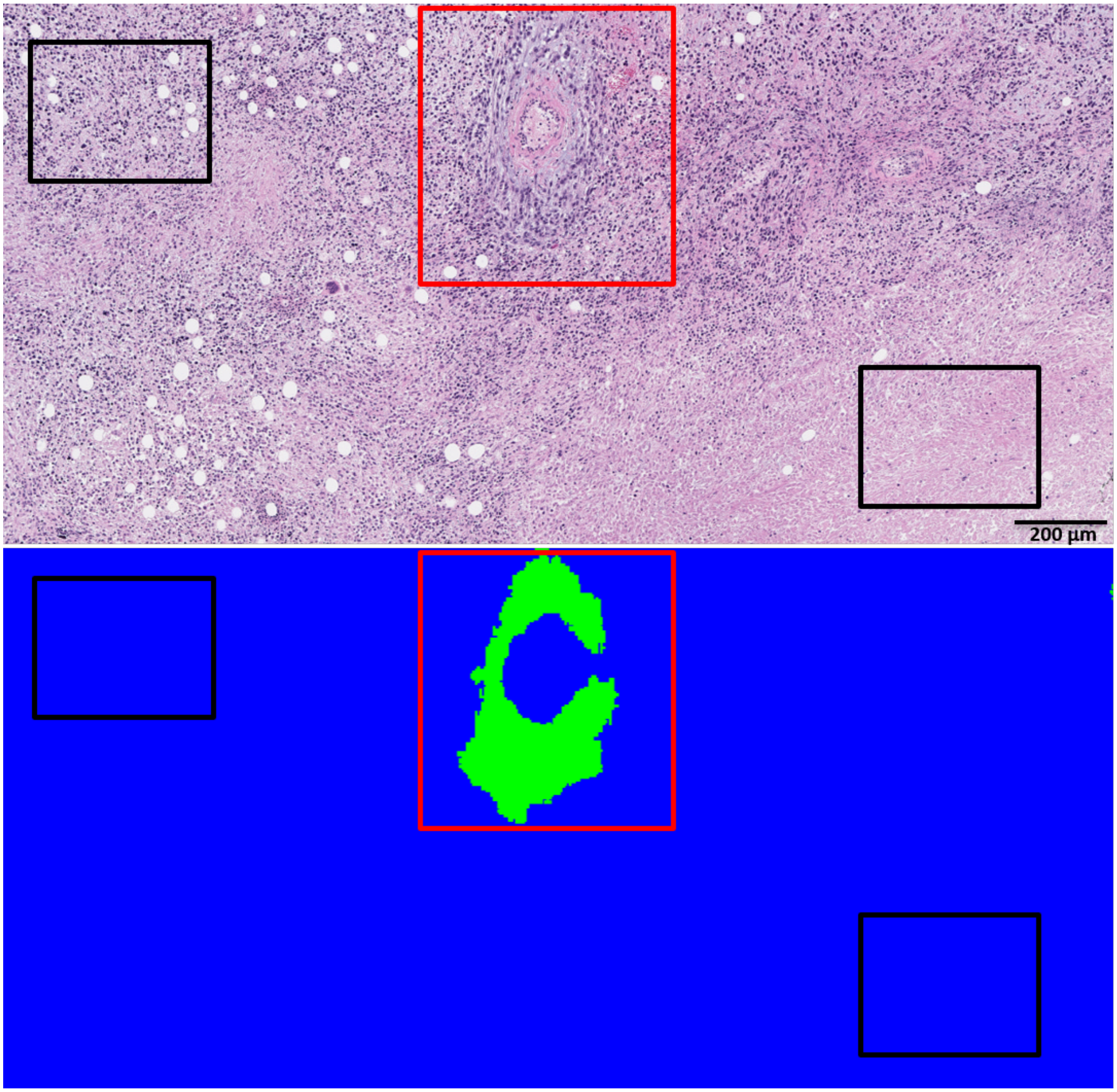
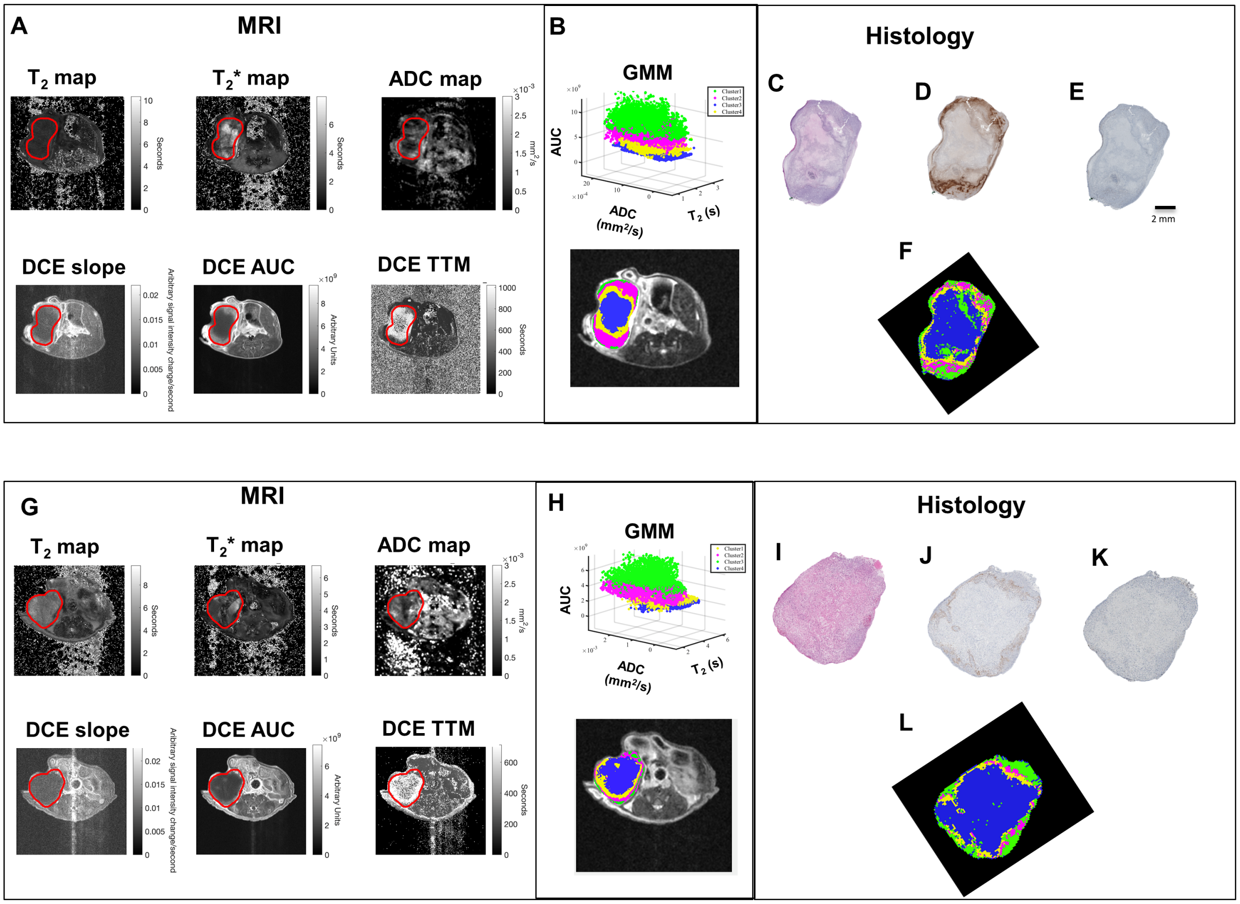
SUPPLEMENTARY FIGURES



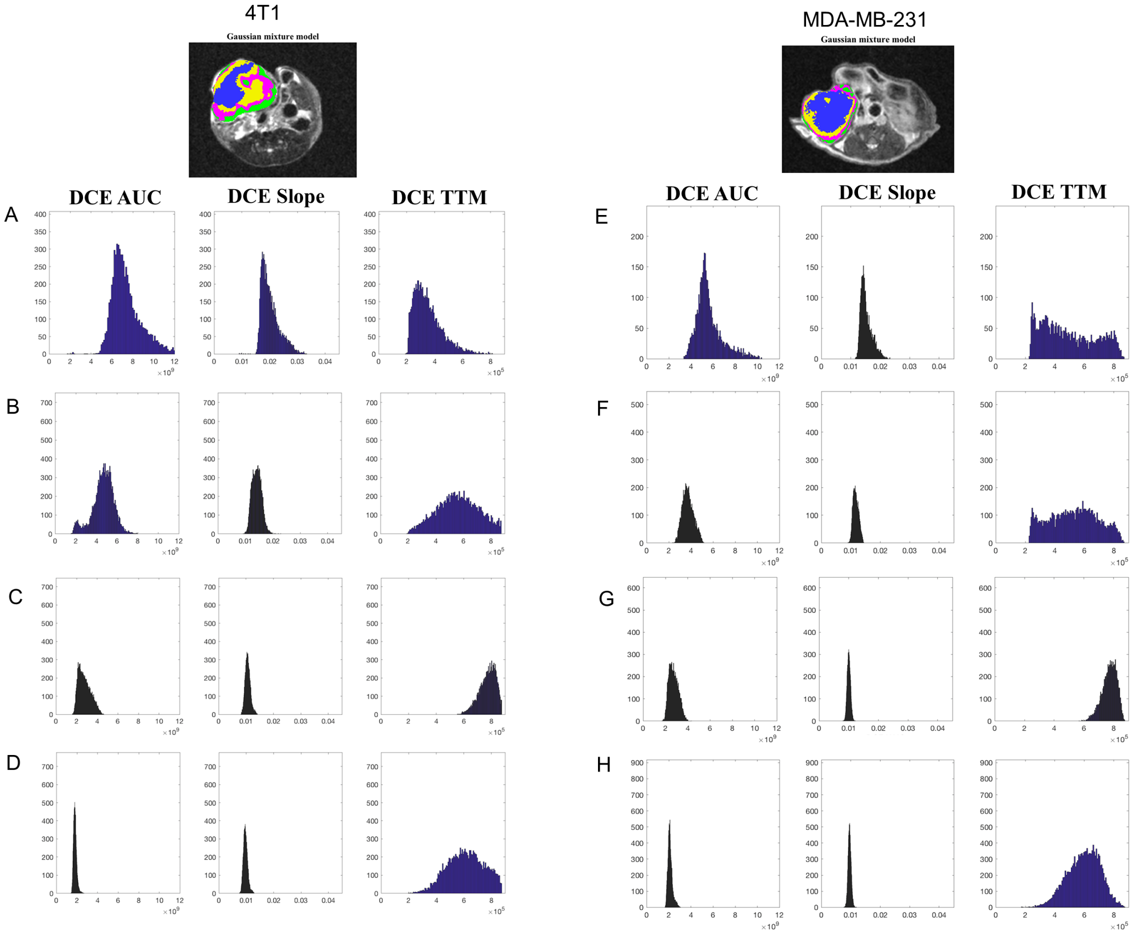
Supplementary Figure S1. Pimonidazole and carbonic anhydrase IX (CA-IX) staining patterns in 4T1 and MDA-MB-231 breast tumors. **(A and G)** Histological samples stained with CA-IX and respective masked images, showing the CA-IX-positive pixels in yellow; **(B and H)** Histological samples stained with pimonidazole and respective masked images, showing the pimonidazole-positive pixels in red; **(C and I)** Masked image combining CAI-IX (yellow) and pimonidazole (red). Cyan represents the intersection of markers, that is, pixels positive for both CA-IX and pimonidazole. **(D and J)** Quantification of tissue area (%) for each marker. p-values were obtained using one-way analysis of variance (ANOVA) followed by the Tukey test for comparison of mean values between regions (*$p<0.05$; ***$p<0.001$; ****$p<0.0001$) (n=6 histological samples for 4T1 and n=8 histological samples for MDA-MB-231). Graphs represent mean and SD. **(E and K)** Quantification of CA-IX-positive and CA-IX-negative areas in the pimonidazole-positive area. **(F and L)** Quantification of pimonidazole-positive and pimonidazole-negative areas in the CA-IX-positive area.



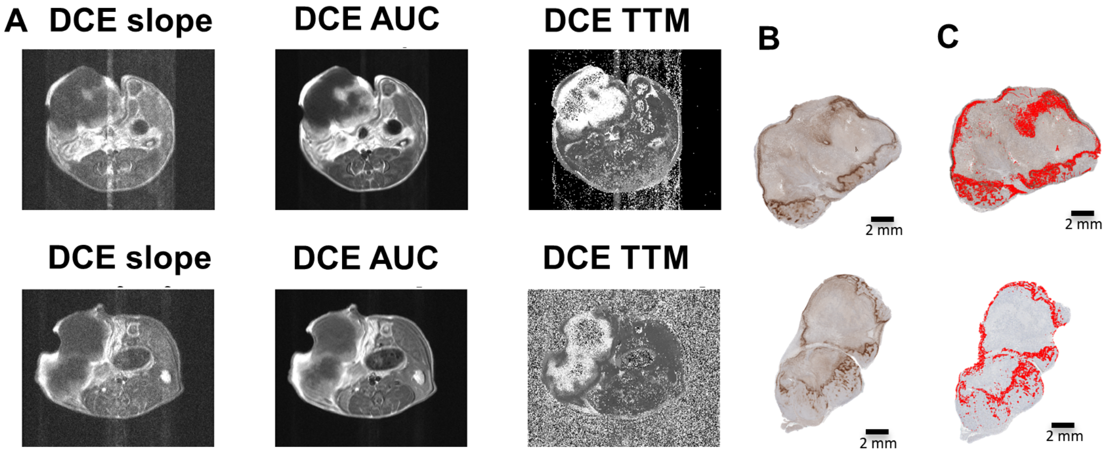
Supplementary Figure S2. Breast tumor stained with hematoxylin & eosin (H&E), showing different stages of necrosis in the black squares, which were masked as non-viable regions. Red square represents an area of viable tumor cells, surrounding a blood vessel.



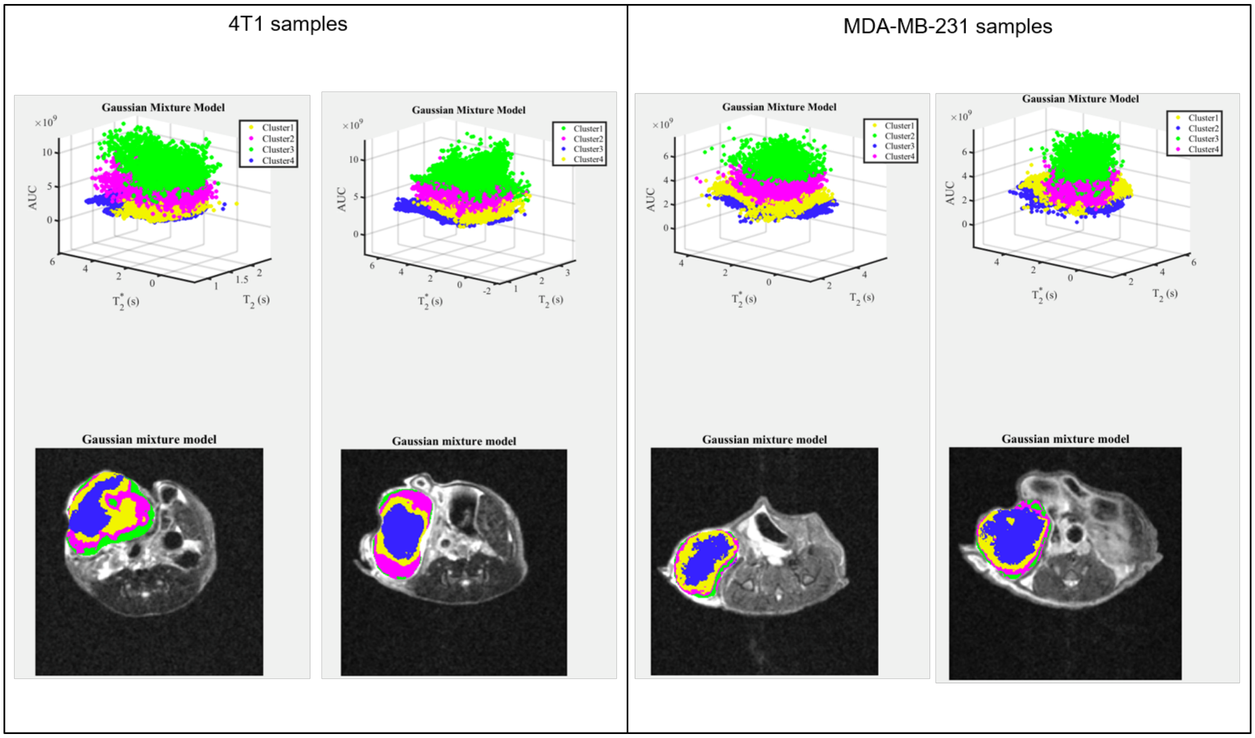
Supplementary Figure S3. Representative examples of the corresponding habitat maps from MRI and histology. 4T1 tumor is shown in (A) and MDA-MB-231 is shown in (G). (A and G) Six MRI parameter maps were obtained [T_2-map, T_2*-map, Apparent diffusion coefficient (ADC), Slope, Area under the curve (AUC) and time to max (TTM)]. Field of view 30 X 30 mm^2; image size 256 X 256; Tumor volume of interest (VOI) is shown in red. **(B and H)** These six MRI parameter maps were clustered by using a Gaussian Mixture Model (GMM) to create the Habitat Maps.. Green cluster shows high enhancement in DCE and the blue cluster shows low enhancement in DCE. Magenta and yellow Clusters show moderate enhancement in DCE. Histological images ((**C and I**) H&E; (**D and J**) pimonidazole; (**E and K**) CD31) were used to create a downsampled ground truth habitat-map from histology (**F and L**) in the same resolution of MR images. Ground truth habitat maps are delineated by viable cells region in green, non-viable cells regions in blue and hypoxic areas in viable or non-viable regions in magenta and yellow, respectively.



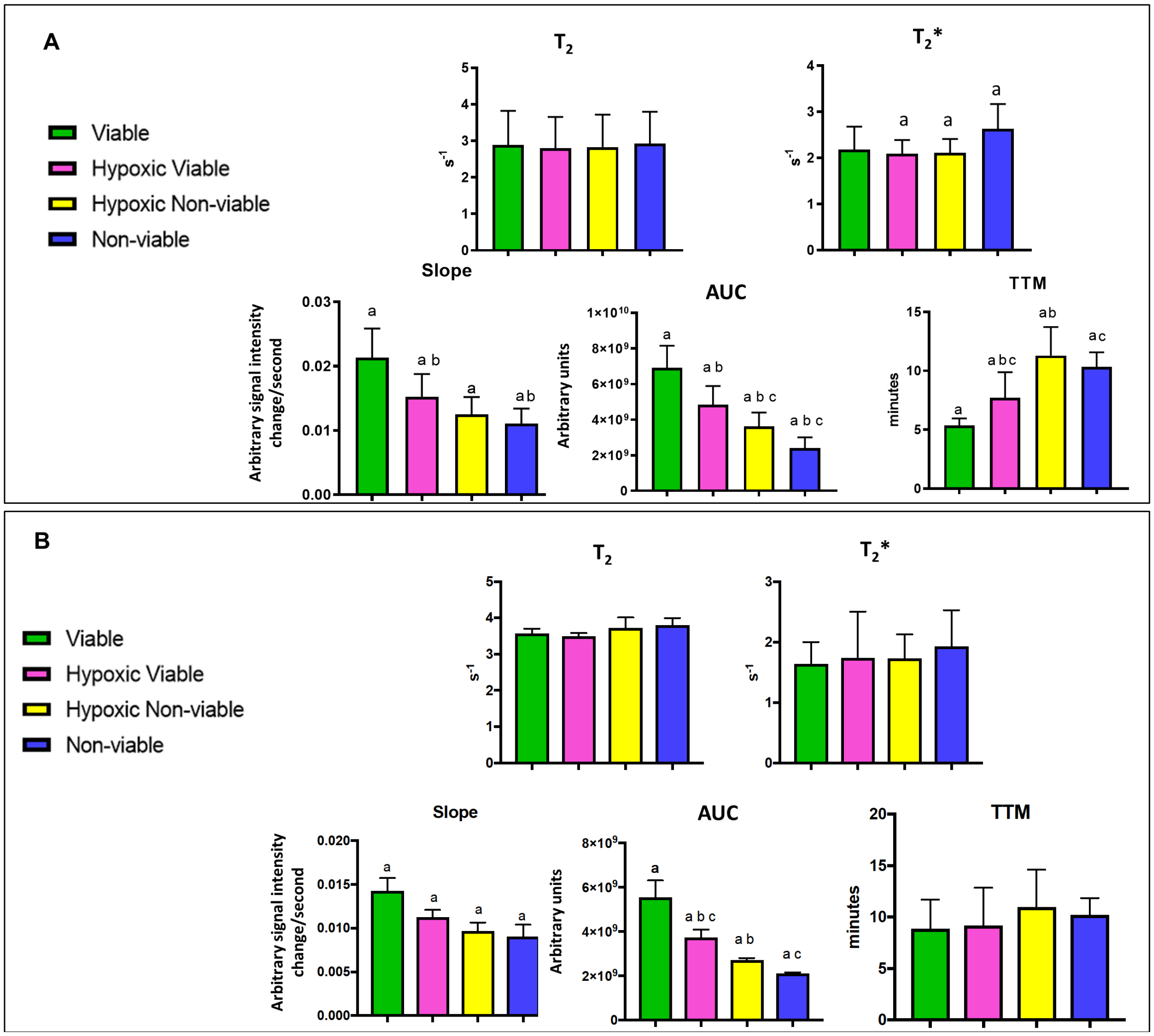
Supplementary Figure S4. Histograms showing the pixels values distribution of Area under the curve (AUC), slope and time to max (TTM) from DCE-MRI in each cluster of representative MRI habitat maps for 4T1 and MDA-MB-231 tumor samples (field of view 30 X 30 mm^2; image size 256 X 256). **(A and E)** Clusters green classified as viable tumor cell regions; **(B and F)** Clusters magenta, classified as hypoxic viable; **(C and G)** Clusters yellow, classified as hypoxic non-viable and **(D and H)** Clusters blue, classified as non-viable tumor cell regions.



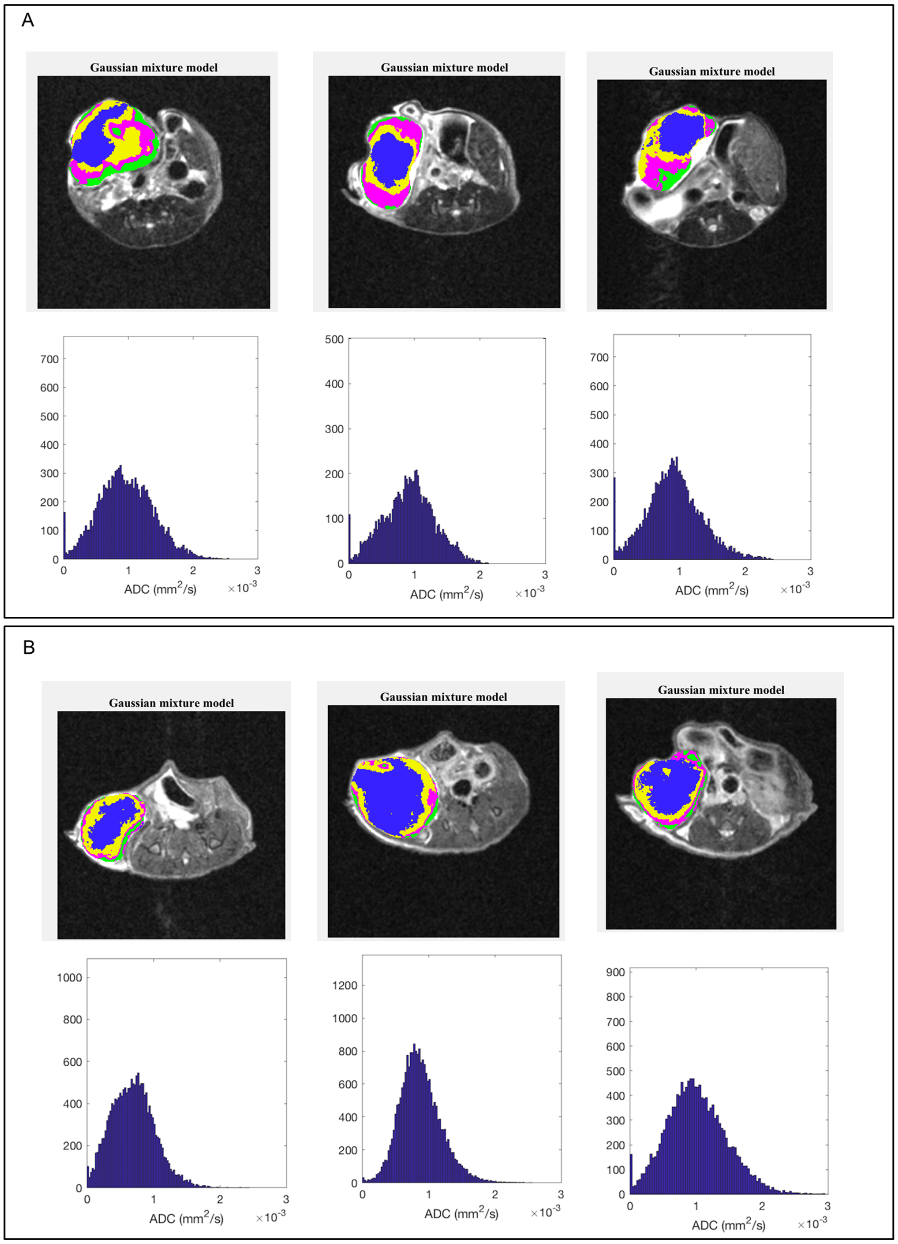
Supplementary Figure S5. Representative examples showing that the DCE-MRI maps have visual similarity with pimonidazole staining in breast samples. **(A)** DCE-MRI derived maps [Slope, Area under the curve (AUC) and time to max (TTM)] (field of view 30 X 30 mm^2; image size 256 X 256). **(B)** Histological slices stained with pimonidazole and masked images showing the pimonidazole-positive pixels in **(C).**



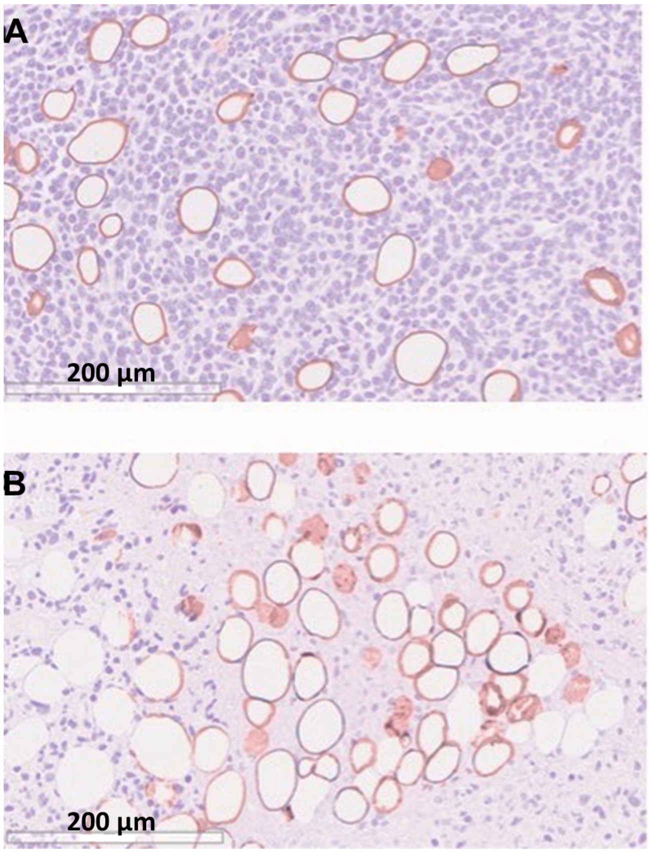
Supplementary Figure S6. Representative examples of MRI habitat maps, which were created by clustering five MRI-parameters [T_2-map, T_2*-map, Slope, Area under the curve (AUC) and time to max (TTM)] using a Gaussian Mixture Model (GMM) Expectation-Maximization algorithm. Field of view 30 X 30 mm^2; image size 256 X 256. Note that these maps are from the same tumors/slices shown in figure 4 and supplementary fig. S3 are visually similar the maps created by clustering six parameters, which also included the ADC maps.



Supplementary Figure S7. Mean values of each parameter from habitat maps generated by clustering five MRI-parameters [T_2-map, T_2*-map, Slope, Area under the curve (AUC) and time to max (TTM)]. Mean values of each parameter were compared between clusters (habitats) for the **(A)** 4T1 (n=12 samples) and **(B)** MDA-MB-231 (n=4 samples) tumor samples. Graphs represent mean and SD. p-values were obtained using one-way analysis of variance (ANOVA) followed by the Tukey test for comparison of mean values between regions (a-c indicate $p<0.05$ between groups).



Supplementary Figure S8. Histograms showing the distribution of the Apparent diffusion coefficient (ADC) values in the cluster classified as non-viable tumor cells (blue) in the MRI habitat map. Field of view 30 X 30 mm^2; image size 256 X 256. **(A)** Representative examples of 4T1 and **(B)** MDA-MB-231 tumor samples.



Supplementary figure S9. Representative images of the immunohistochemistry for perilipin-1 in breast tumor samples. **(A)** Positive stained adipocytes in 4T1 and **(B)** MDA-MB-231 breast tumor samples. Breast tumor cells were negative for perilipin-1. 20X magnification.