Article

Quantification of Trastuzumab–HER2 Engagement In Vitro and In Vivo

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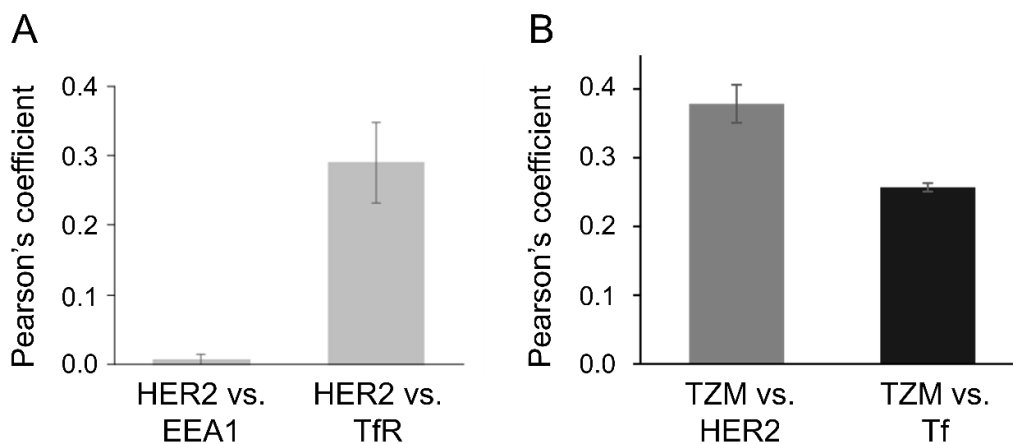
Supplemental Material

Figure S1. Colocalization analysis of HER2 and TZM with endocytic markers. (A) AU565 cells were subjected to immunostaining with anti-HER2, anti-EEA1 and anti-TfR as described in Figure 1A. Pearson coefficient was calculated on images shown in Figure 1A. (B) AU565 cells were subjected to 24 h uptake with 5 µg/mL TZM-AF700 and 5 µg/mL Tf-AF568 and processed for immunostaining for HER2 as shown in Figure 1B. Pearson coefficient was calculated on images shown in Figure 1B. Collected z-stacks were analyzed for colocalization in Imaris (Bitplane) software, as described in [26]. Data presented as mean and standard deviation, n=3-5.
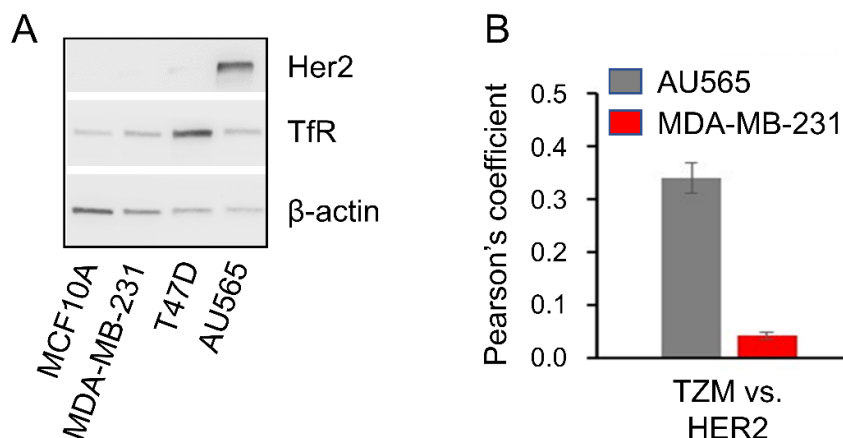
Figure S2. (A) Representative Western blot of HER2 and TfR expression in breast cancer cells and non-cancerous MCF10A cells. (B) AU565 and MDA-MB-231 cells were subjected to 24 h uptake with 20 µg/mL TZM-AF700 and processed for immunostaining for HER2. Pearson coefficient was calculated on images shown in Figure 2B. Collected z-stacks were analyzed for colocalization in Imaris (Bitplane) software, as described in [26]. Data presented as mean and standard deviation, n=5.
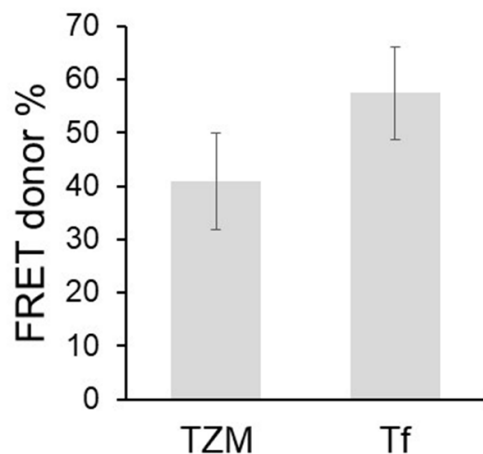

Figure S3. Comparison of NIR TZM-HER2 and Tf-TfR FLIM FRET in AU565 cells. Data presented as mean and standard deviation, n=10 points from five separate ROIs.
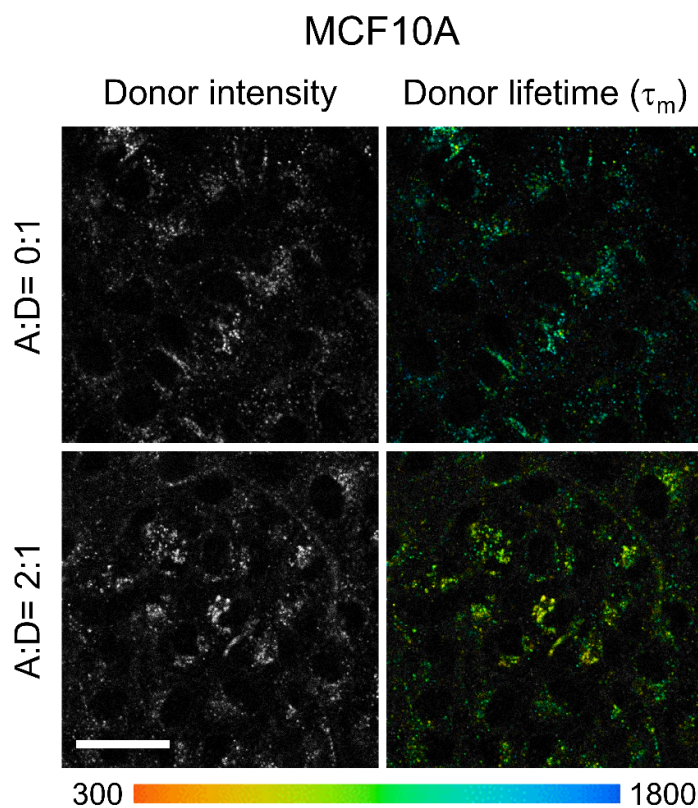

Figure S4. Representative TCSPC images of fluorescence intensity and mean lifetime map (τ_m) in MCF10A cells treated with TZM-AF700 (Acceptor: Donor =0:1), TZM-AF700 and TZM-AF750 (A:D=2:1) pseudo-color range= 500-1,800 ps. Photon counts were insufficient for adequate fitting analysis.