Supporting Information

Synthesis and Characterization of Anti-EGFR Fluorescent Bead-Based Nanoparticles for Targeted Optical Imaging

Leslie W. Chan[1], Yak-Nam Wang[2], Lih Y. Lin[3], Melissa P. Upton[4], Joo Ha Hwang[5], Suzie H. Pun[1,]*

[1]Department of Bioengineering, University of Washington, Seattle, WA 98195

[2]Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington, Seattle, WA 98195

[3]Department of Electrical Engineering, University of Washington, Seattle, WA 98195

[4]Department of Pathology, University of Washington, Seattle, WA 98195

[5]Division of Gastroenterology, Department of Medicine, University of Washington, Seattle, WA 98195

Contents

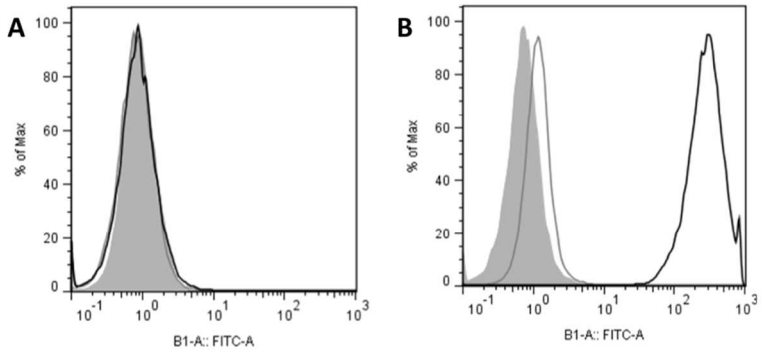


Figure S1 Evaluation of EGFR Expression in (A) H520 ($EGFR^-$) and (B) A431 ($EGFR^+$) cell lines using flow cytometry. Baseline fluorescence (shaded gray) in both cell lines were measured. Cells were treated with FITC-conjugated secondary antibody to determine background fluorescence (gray line). Cells were treated with M225 antibody and FITC-conjugated secondary antibody to evaluate homogeneity of EGFR expression (black line). H520 cells were negative for EGFR expression and A431 cells were positive for EGFR expression.

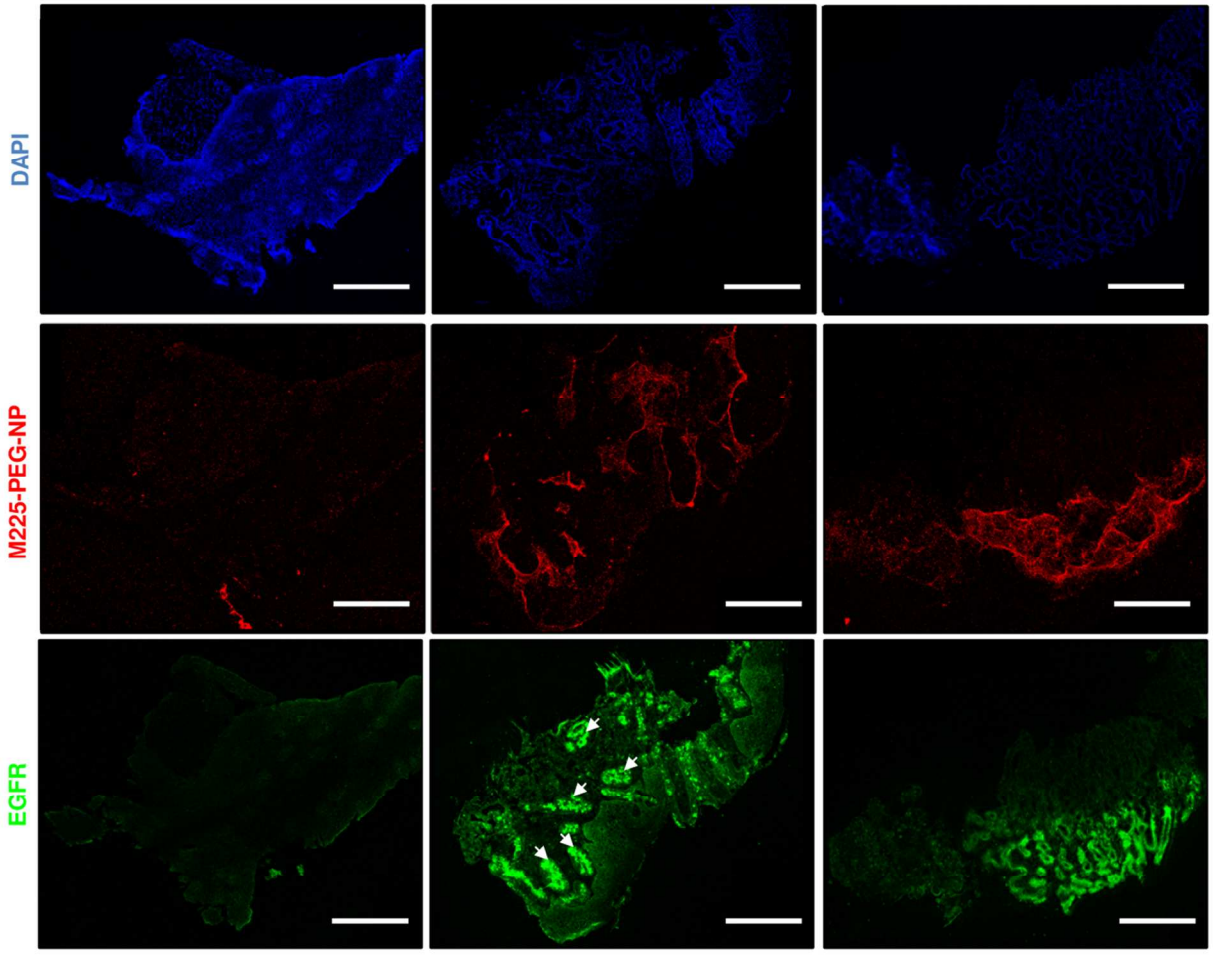
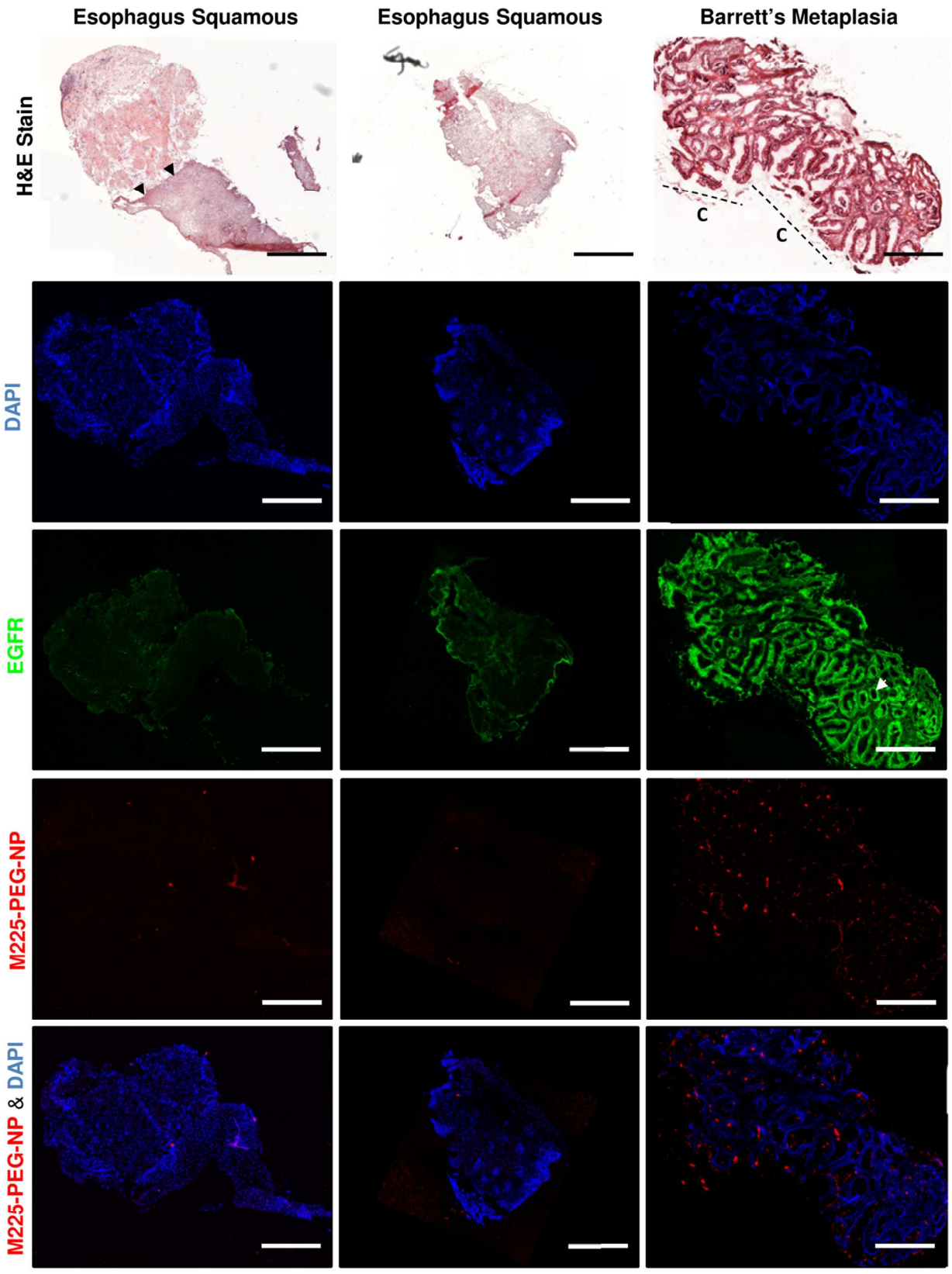
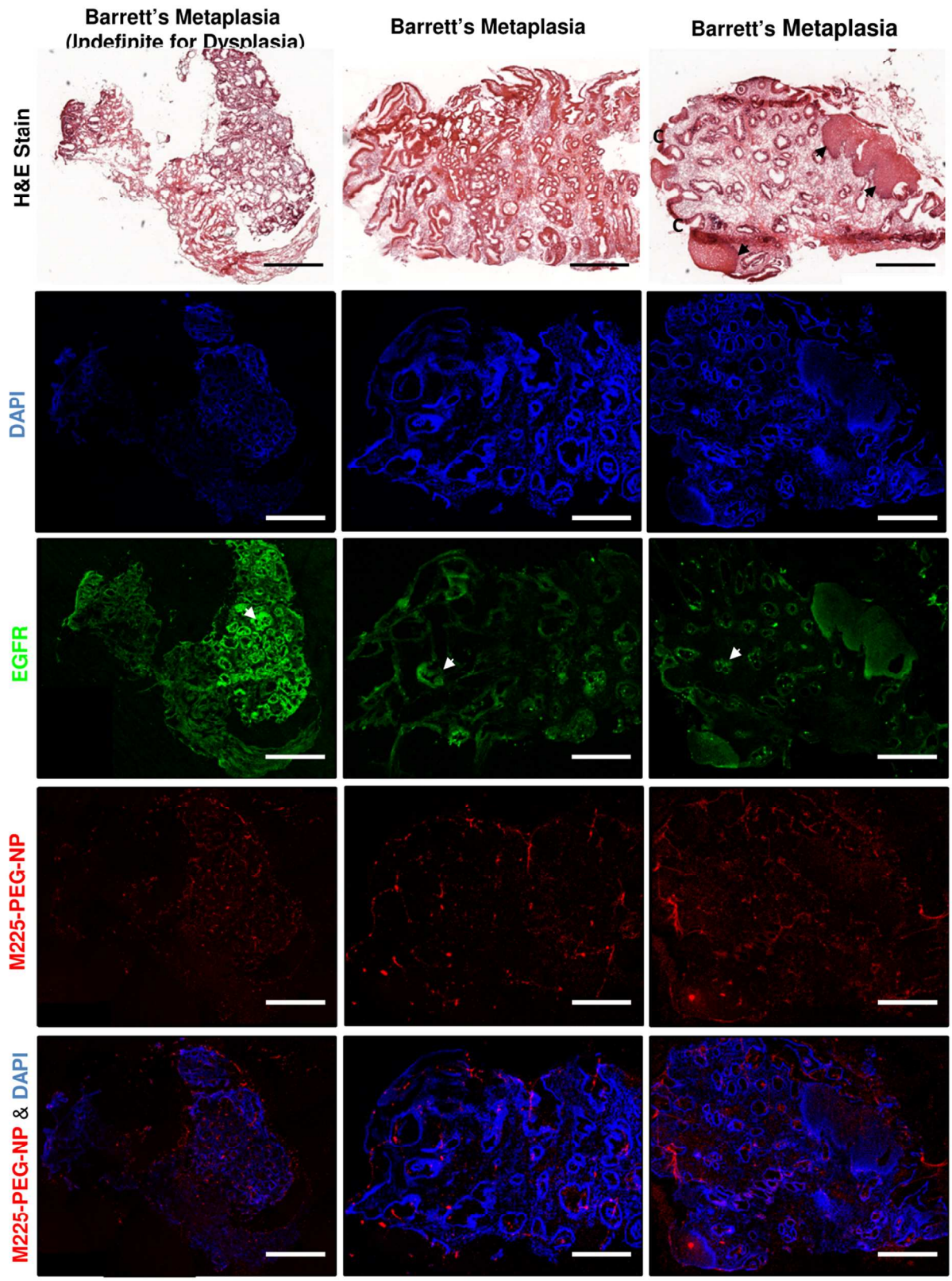
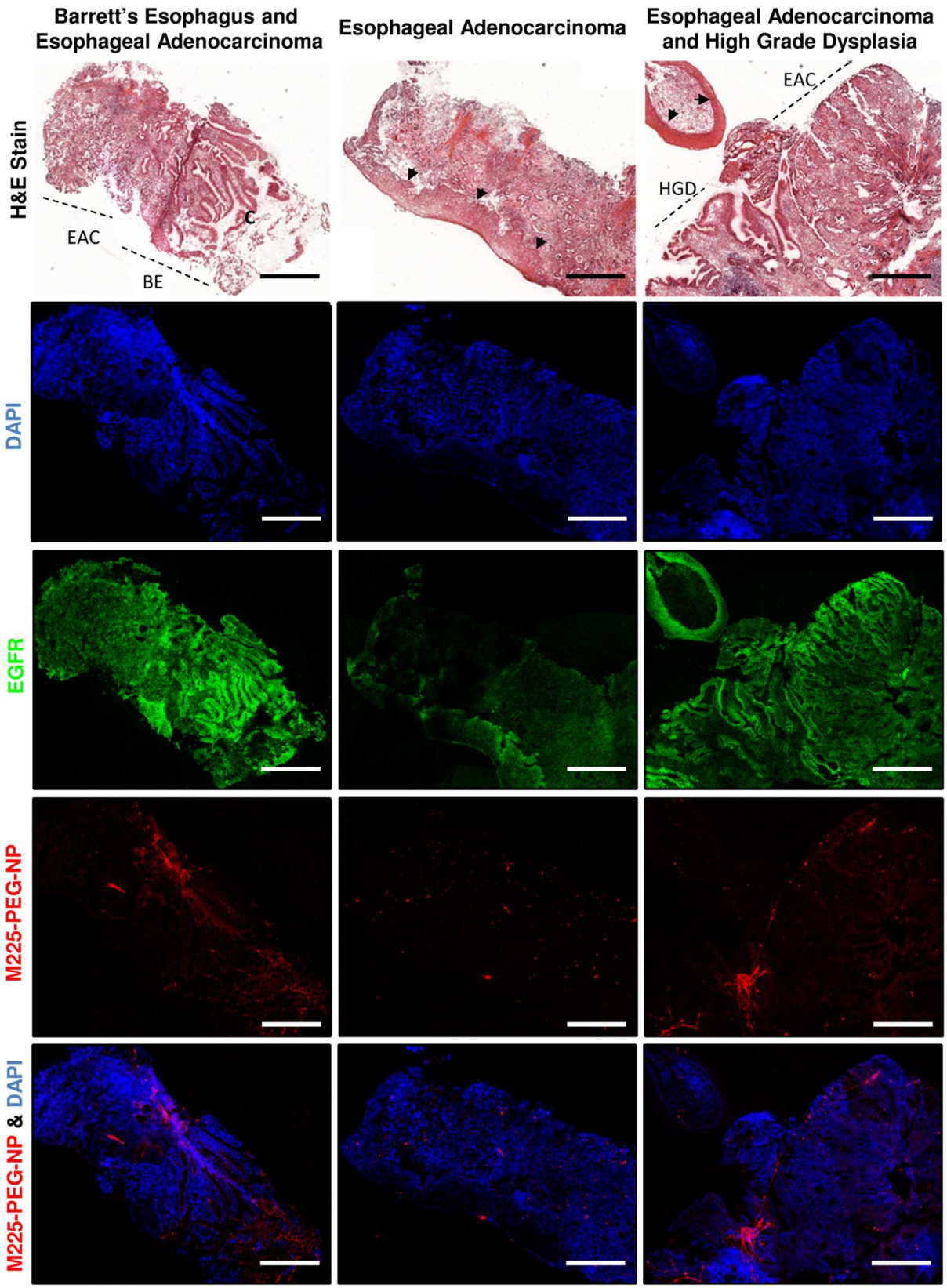


Figure S2 Confocal images of *ex vivo* labeling of EGFR in human esophageal tissue sections using M225-PEG-NP (middle row) and conventional immunohistochemistry (bottom row). DAPI and M225-PEG-NP channels are shown as overlayed composites in Figure 5 and split here into individual channels.


Esophagus Squamous
Esophagus Squamous
Barrett's Metaplasia
H&E Stain
C
C
DAPI
EGFR
M225-PEG-NP
M225-PEG-NP & DAPI


Barrett's Metaplasia
(Indefinite for Dysplasia)
Barrett's Metaplasia
Barrett's Metaplasia
H&E Stain
C
C
DAPI
EGFR
M225-PEG-NP
M225-PEG-NP & DAPI


Barrett's Esophagus and Esophageal Adenocarcinoma
Esophageal Adenocarcinoma
Esophageal Adenocarcinoma and High Grade Dysplasia
H&E Stain
C
EAC
BE
EAC
HGD
DAPI
EGFR
M225-PEG-NP
M225-PEG-NP & DAPI

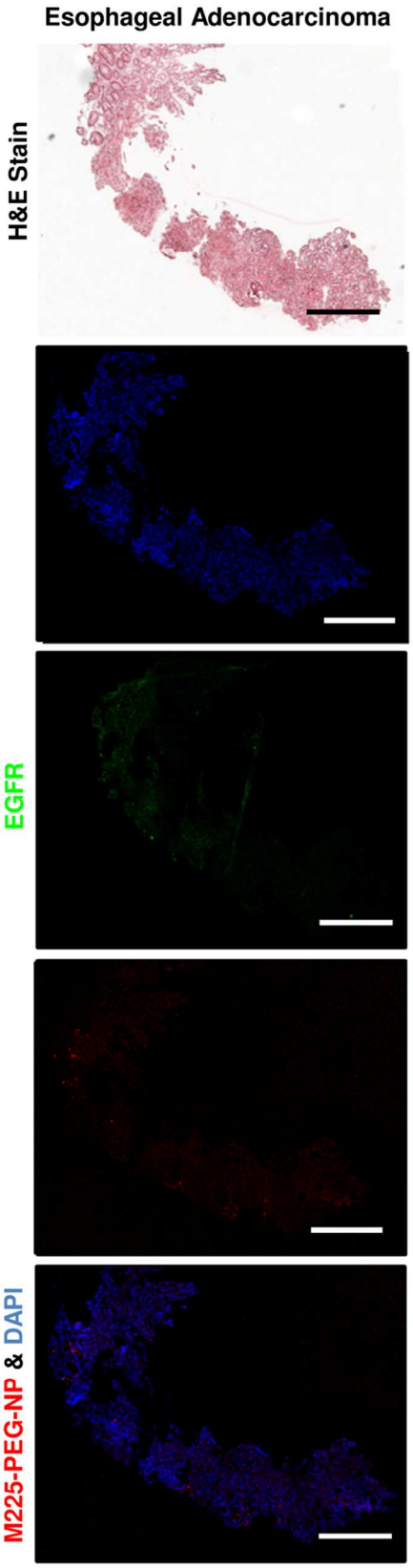

Esophageal Adenocarcinoma
H&E Stain
EGFR
M225-PEG-NP & DAPI

Figure S3 *Ex vivo* staining of esophagus squamous (SQ), Barrett's metaplasia (BE), and esophageal adenocarcinoma (EAC) biopsy sections with hematoxylin and eosin (top row),DAPI (second row), M225 antibody and Alexa Fluor 488 goat anti-mouse IgG (third row), M225-PEG-NP (fourth row), and M225-PEG-NP 900 with DAPI counterstain (bottom row). Standard immunohistochemistry (third row) showed high EGFR expression in the glands (white arrows), columnar epithelium (C), and basal epithelium (black arrows) in BE tissue sections. M225-PEG-NP bound to the same corresponding areas in adjacent tissue sections. Scale bar, 500 μm.