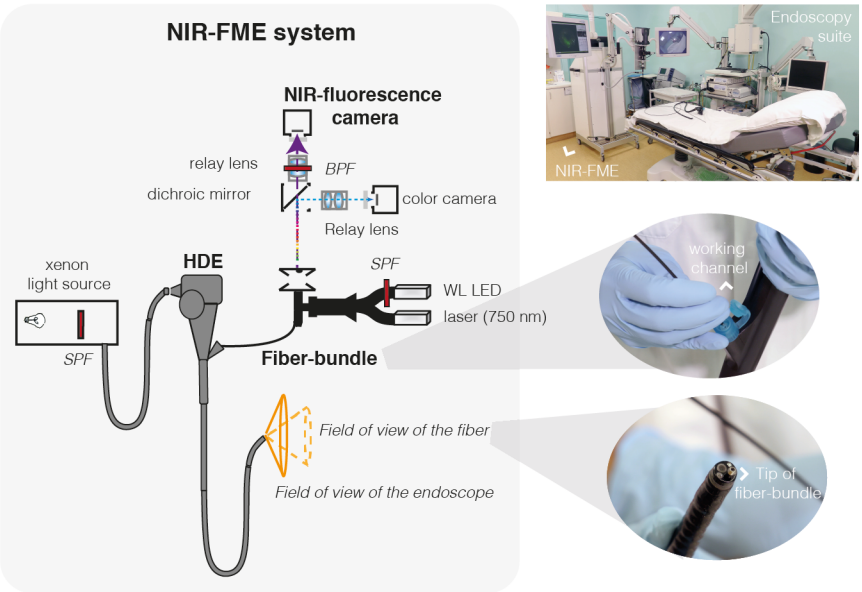
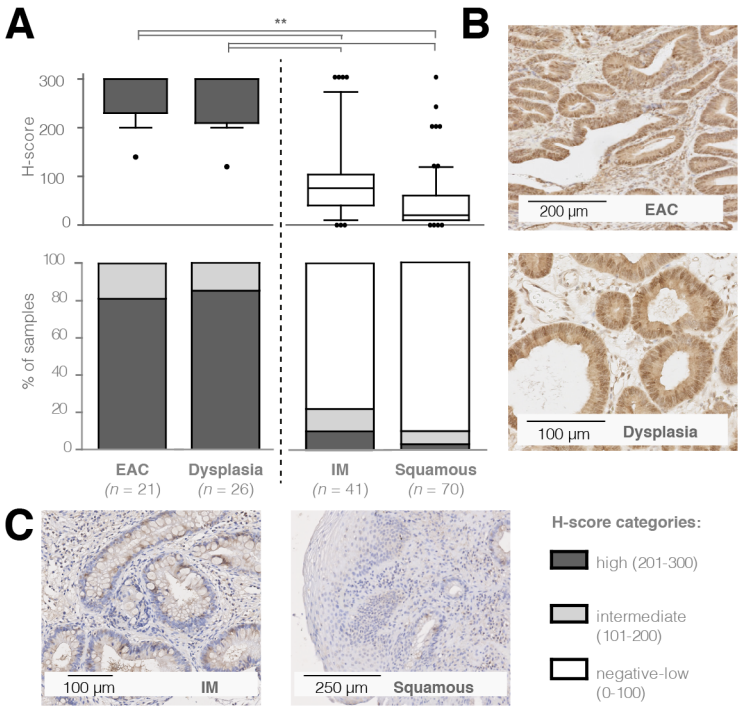
Supplementary table 1. Patient and lesion characteristics

Participants, no. (%)	**14**
- Male	12 (80%)
- Female	2 (20%)
- IV tracer administration	5 (36%)
- Topical tracer application	9 (64%)
Age, mean (range), years	**65.07** (48-77)
Body mass index (BMI), mean ± SD	**27.67** ± 5.52
BE segment mean (range), cm; Prague criteria	
Circumferential length (C)	**3.64** (0 – 10)
Maximum length (M)	**5.57** (1 – 13)
Focal lesion description	
no. (%); Paris classification	**17** *
• Protruding lesion (Is)	5 (29%)
• Elevated lesion (IIa)	3 (18%)
• Flat lesion (IIb)	8 (47%)
• Unidentifiable focal lesion during HD/NBI	1 (6%)
EMR sections, median (range), per patient	**4** (1 – 10)
Aberrant tissue identified with NIR-FME	
Fluorescent focal lesions, no. (%)	**16/17 (94%)** **
• IV tracer administration	4/5 (80%) **
• Topical tracer application	12/12 (100%)
Histological grade, no. (%)	
Focal lesions:	**17** *
- EAC	15 (88%)
- HGD	1 (6%)
- LGD	1 (6%)
Additional fluorescent areas, no. (%)	**5**
• IV tracer administration	0
• Topical tracer application	5
Histological grade, no. (%)	
Additional lesions:	**5**
- EAC	1
- HGD	1
- LGD	2
- Indefinite for dysplasia	1

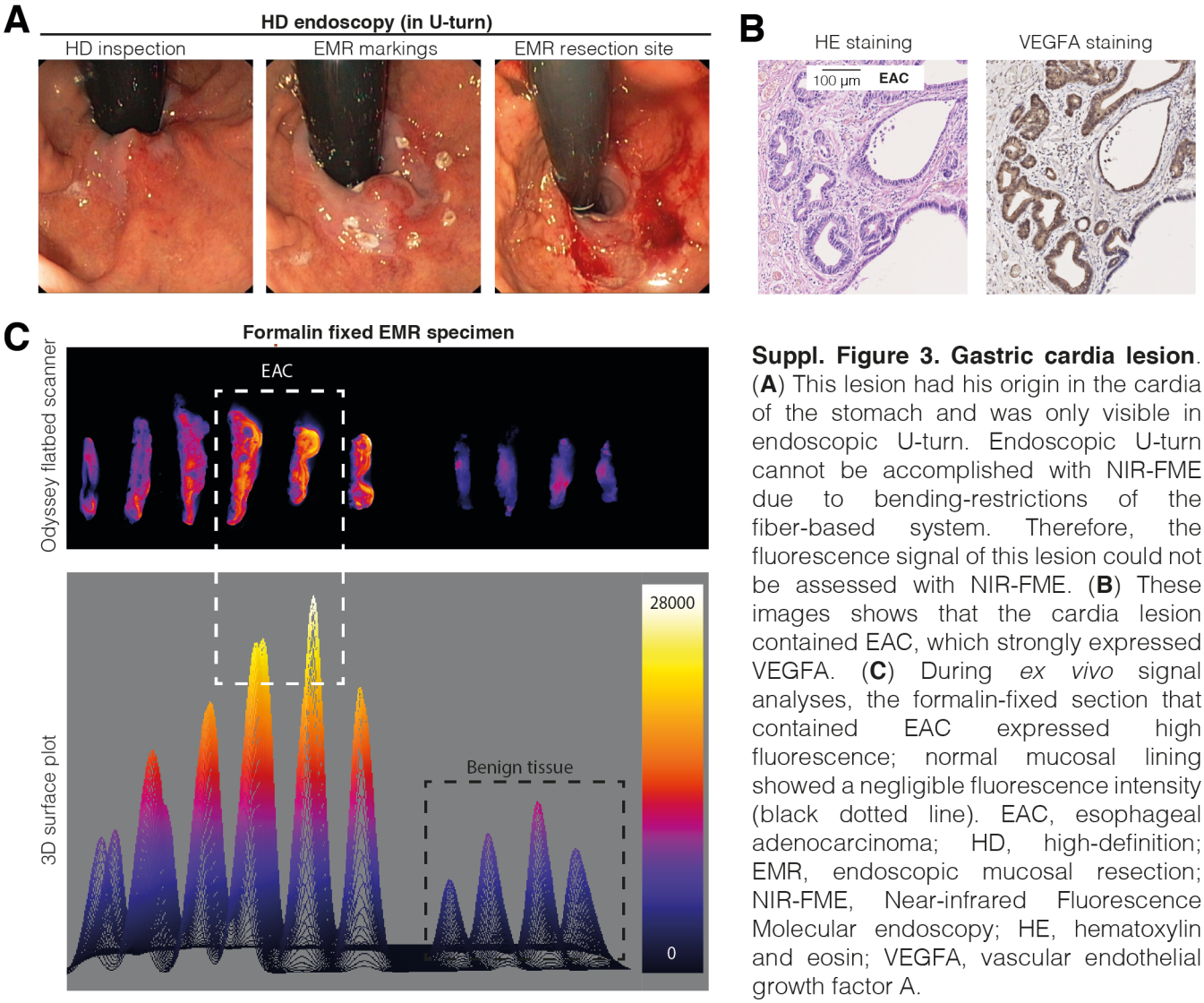
*NOTE. * In three out of 14 patients two separate focal lesions were present, resulting in 17 focal lesions in total. ** One lesion was not identifiable during IV NIR-MFE: gastric cardia lesion, only visible in U-turn, which is not feasible with NIR-FMEE system. Abbreviations: NIR-FME, near-infrared Fluorescence Molecular Endoscopy; HD, high-definition; NBI, narrow-band imaging; EAC, oesophageal adenocarcinoma; HGD, high-grade dysplasia; LGD, low-grade dysplasia; IV, intravenous.*



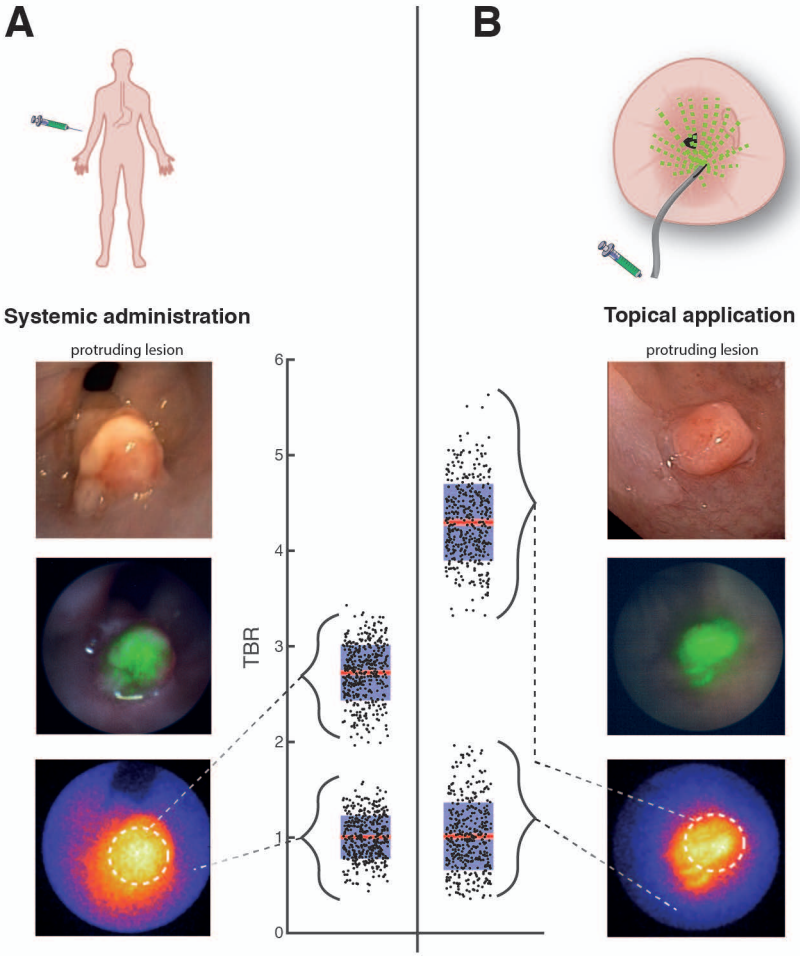
Suppl. Figure 1. Schematic overview of the NIR-FME system. This figure illustrates attachment of the fiber-bundle to an external ultra sensitive NIR fluorescence camera and insertion through the working channel of a clinical HD video-endoscope. EAC, esophageal adenocarcinoma; NIR, near-infrared; FME, fluorescence molecular endoscopy; HDE, high-definition



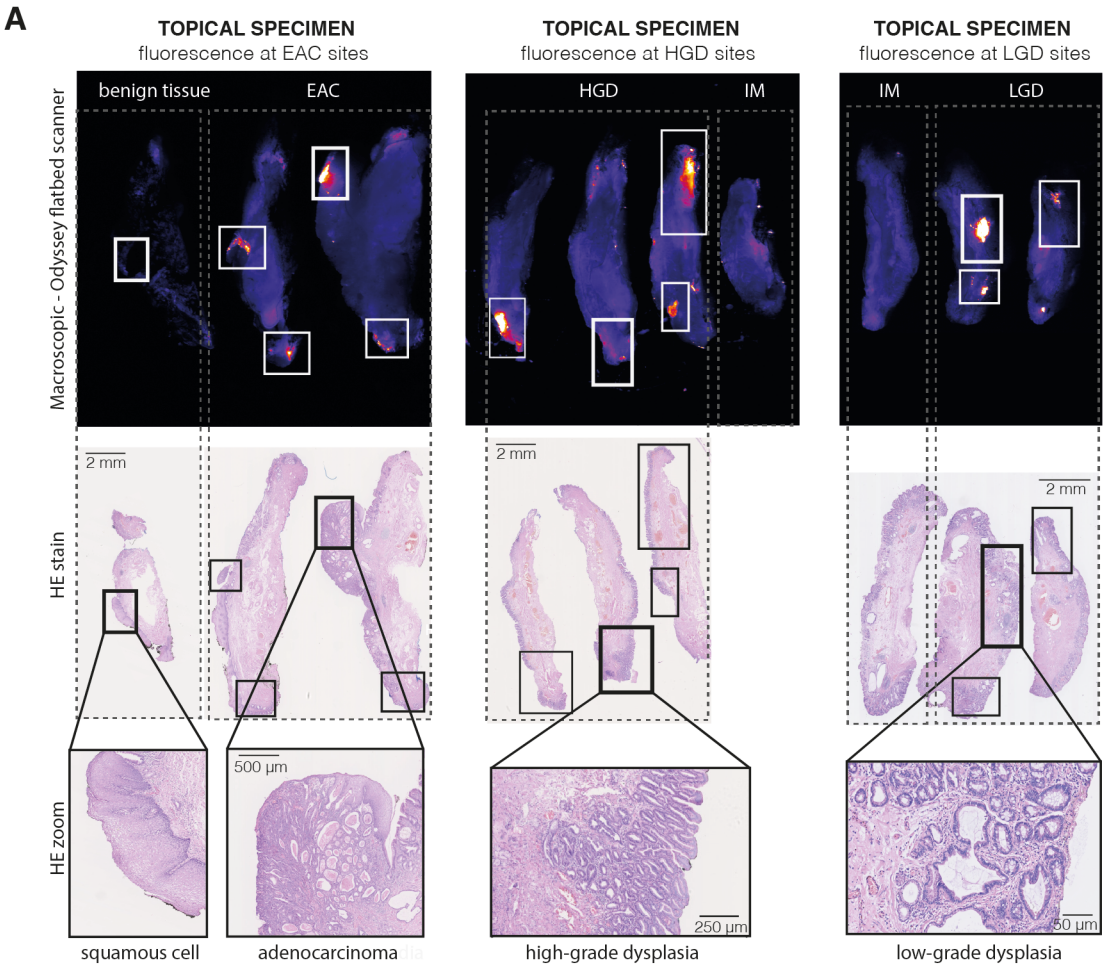
Suppl. Figure 2. *Ex vivo* target validation: anti-VEGFA IHC. Box plot (median, 10-90 percentile) and bar graph, both presenting VEGFA IHC results (H-score) in esophageal samples, including esophageal adenocarcinoma, dysplasia, intestinal metaplasia and normal squamous epithelium. The representative images illustrate the significant difference in VEGFA-staining intensities (brown); the high staining intensity observed in the aberrant lesions (EAC and dysplasia) is in clear contrast with the low to negative stained benign tissue (incl. IM and squamous epithelium). IHC, immunohistochemistry, EAC, esophageal adenocarcinoma; IM, intestinal metaplasia. $^{**}P < 0.0001$ (Kruskal-Wallis test).



Suppl. Figure 3. Gastric cardia lesion. (**A**) This lesion had his origin in the cardia of the stomach and was only visible in endoscopic U-turn. Endoscopic U-turn cannot be accomplished with NIR-FME due to bending-restrictions of the fiber-based system. Therefore, the fluorescence signal of this lesion could not be assessed with NIR-FME. (**B**) These images shows that the cardia lesion contained EAC, which strongly expressed VEGFA. (**C**) During *ex vivo* signal analyses, the formalin-fixed section that contained EAC expressed high fluorescence; normal mucosal lining showed a negligible fluorescence intensity (black dotted line). EAC, esophageal adenocarcinoma; HD, high-definition; EMR, endoscopic mucosal resection; NIR-FME, Near-infrared Fluorescence Molecular endoscopy; HE, hematoxylin and eosin; VEGFA, vascular endothelial growth factor A.



Suppl. Figure 4. Imaging statistics of topical vs. systemic FME signal. This figure illustrates the *in vivo* TBR for the different labeling approaches. (**A**) The systemic injection of bevacizumab-800CW lead to a TBR of 2.75 +- 0.29 (**B**) The topical application resulted in a TBR of 4.30 +- 0.41 (mean +- std) prior to rinsing. The images are normalized to match a mean of 1 in the surrounding tissue region of interest. The scatted plot shows the statistics for the surrounding tissues (bottom cluster) and tumor region (upper cluster). The black dots represent single intensity values. The red lines show the mean for each group. The one standard deviation area is colored in blue. The data is jittered and subsampled for visualization. TBR, tumor-to-background ratio.



Suppl. Figure 5. Fluorescence in topical tracer specimen. A clear difference in the amount of tracer left in the topical tracer based specimens after formalin fixation (**A:** only superficial spots), compared to the systemic tracer based specimens can be observed (**B:** fluorescence within the deeper tissue layers). Since the amount of tracer present within the systemic specimen is much higher, enough signal is left to accomplish *ex vivo* analyses. What can be appreciated from this figure is that, despite only a limited amount of fluorescent tracer remained in the topical tracer based specimens following fixation, the fluorescent spots that are still present are within the setions that contained aberrant tissue, located at sites of dysplastic and neoplastic cells, therefore signifying the specificty of the tracer (indicated with rectangles; confirmed by HE). The benign sections (squamous, IM) did not contain these high fluorescent spots. EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia; LGD, low-grade dysplasia; IM, intestinal metaplasia; HE, haematoxylin and eosin.