

## Supplementary Material

### Profiling of Gastric Cancer Cell-Surface Markers to achieve Tumor-Normal Discrimination

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### Supplementary Methods

#### Cohort Selection

| Age at surgery (years) | Stage T | Stage N | Stage M | AJCC | Grade                 |
|------------------------|---------|---------|---------|------|-----------------------|
| 65                     | T2      | N1      | M0      | 2A   | Undifferentiated      |
| 67                     | T4a     | T3b     | M0      | 3C   | Poorly differentiated |
| 68                     | T4a     | T3b     | M0      | 3C   | Poorly differentiated |
| 60                     | T3      | N3a     | M0      | 3B   | Poorly differentiated |
| 71                     | T2      | N1      | M0      | 2A   | Well differentiated   |

|    |     |     |    |    |                           |
|----|-----|-----|----|----|---------------------------|
| 70 | T4a | N3a | M1 | 4  | Moderately differentiated |
| 71 | T4a | T3b | M1 | 4  | Poorly differentiated     |
| 45 | T2  | N1  | M0 | 2A | Poorly differentiated     |
| 71 | T2  | N1  | M0 | 2A | Moderately differentiated |
| 39 | T4a | T3b | M1 | 4  | Poorly differentiated     |
| 75 | T2  | N1  | M0 | 2A | Moderately differentiated |
| 67 | T4a | N3a | M1 | 4  | Moderately differentiated |
| 84 | T1b | N0  | M0 | 1A | Moderately differentiated |
| 72 | T4  | N1  | M1 | 4A | Poorly differentiated     |

**Figure S1.** Patient and tumor characteristics involving Age of patient at which surgery occurred, American Joint Committee on Cancer (AJCC 7<sup>th</sup> Edition) Staging, Tumor (T), Node (N), Metastasis (M) Classification, and Tumor Grade from Singapore Gastric Cancer Consortium (SGCC) data.

*\*Note: One patient sample had two tumor/ normal pairs taken from two different tumors, accounting for a total of fifteen samples.*

#### Immunohistochemistry (IHC) and Quantitative analysis

Tissue microarray (TMA) samples were obtained from US Biomax Inc., Rockville, USA consisted of gastric adenocarcinoma (HStm-Ade090PG-01; normal distant tissue, normal adjacent tissue and tumor, n=29, 3 cores/case) and early gastric adenocarcinoma (US Biomax, HStm-Ade150CS1-01; T1N0M0=26, T1N1M0=4, T2N0M0=35 and matched normal adjacent tissue, 2 cores/case). These were immunostained with antibodies against CEACAM5 (II-7, Dako), CEACAM6 (9A6, Abcam), EpCAM (C-10, Santa Cruz) and CA72-4 (CC49, Santa Cruz) following a multiplex fluorescent immunohistochemistry protocol according to manufactures instruction (OPAL 5-color IHC Kit, PerkinElmer).

The Vectra 2 multispectral automated imaging system was used for acquisition and image analysis. Acquisition and image analysis were done with the Vectra 2 multispectral automated imaging system (PerkinElmer) and inForm 2.0 image analysis software. Briefly, multispectral images of stained tissue were unmixed using predefined spectra of each fluorophore used, to obtain pure images for each marker without interference from autofluorescence. A tissue segmentation algorithm was next employed to recognise tissue architecture to define normal gastric tissue regions from tumor regions (**Figure S2 in Supplementary Material**). Subsequently, a cell segmentation algorithm was used to define cell boundaries on the basis of nuclear counterstaining, aided by the membrane staining of markers of interest, where possible. Intensities of each marker was quantitated in the membrane-cytoplasm region on a cellular basis, and the mean value from all segmented cells was used as a descriptive value for each normal or tumor region.

## Supplementary Figure

### Figure Legends

**Figure S2.** Multispectral imaging. A) Pure images for each marker without interference from autofluorescence were obtained from unmixed multispectral images of stained tissue with predefined spectra of each fluorophore. B) A tissue segmentation algorithm was employed to recognise tissue architecture to define normal gastric tissue regions

from tumor regions, and a cell segmentation algorithm was used to define cell boundaries on the basis of nuclear counterstaining.