# The effects of T-DXd on the expression of HLA class I and chemokines CXCL9/10/11 in HER2-overexpressing gastric cancer cells

Shotaro Nakajima,<sup>1,2</sup> Kosaku Mimura,<sup>1,3</sup> Takuro Matsumoto,<sup>1</sup> Aung Kyi Thar Min,<sup>1</sup> Misato Ito,<sup>1</sup> Hiroshi Nakano,<sup>1</sup> Prajwal Neupane,<sup>1</sup> Yasuyuki Kanke,<sup>1</sup> Hirokazu Okayama,<sup>1</sup> Motonobu Saito,<sup>1</sup> Tomoyuki Momma,<sup>1</sup> Yohei Watanabe,<sup>1</sup> Hiroyuki Hanayama,<sup>1</sup> Suguru Hayase,<sup>1</sup> Zenichiro Saze,<sup>1</sup> and Koji Kono<sup>1,†</sup>

<sup>1</sup>Department of Gastrointestinal Tract Surgery,

<sup>2</sup>Department of Obesity and Inflammation Research,

<sup>3</sup>Department of Blood Transfusion and Transplantation Immunology,

Fukushima Medical University School of Medicine, Fukushima, Japan

#### <sup>†</sup>Corresponding author: Koji Kono

Department of Gastrointestinal Tract Surgery, Fukushima Medical University School of Medicine,

1 Hikariga-oka, Fukushima city, Fukushima 960-1295, Japan

Tel.:+81-24-547-1259; Fax.: +81-24-547-1980

E-mail: kojikono@fmu.ac.jp

Running title: The effect of T-DXd on HER2-positive GC

#### **Supplementary Figure Legends**

## Supplementary Figure S1. The effect of trastuzumab on the expression of HLA class I in HER2-positive GC cells.

(A) Cell surface expression of HER2 (*left*) and HLA class I (*right*) in HER2-positive GC cell lines treated with several concentrations of trastuzumab or 100 ng/ml IFN- $\gamma$  for 72 h (n=3). Representative histograms were shown. Values are shown as means ± SEM. \**P* <0.05, \*\**P* <0.01, \*\*\*\**P* <0.001, \*\*\*\**P* <0.0001. (B) Western blot analysis of the indicated molecules in HER2-positive GC cell lines treated with several concentrations of trastuzumab for 72 h.Values are shown as means ± SEM. \**P* <0.05, \*\*\**P* <0.001, \*\*\*\**P* <0.0001.

## Supplementary Figure S2. The effect of irinotecan on the expression of HLA class I in HER2-positive GC cells.

Cell surface expression of HER2 and HLA class I in HER2-positive GC cell lines treated with several concentrations of trastuzumab or 100 ng/ml IFN- $\gamma$  for 72 h (n=3). Representative histograms were shown. Values are shown as means ± SEM. \*\*\*P <0.001, \*\*\*\*P <0.0001.

### Supplementary Figure S3. The effect of T-DXd on the expression of HLA class I in HER2negative GC cells.

The expression of HER2 (*left*) and HLA class I (*right*) in HER2-negative GC cell lines treated with several concentrations of T-DXd or 100 ng/ml IFN- $\gamma$  for 72 h (n=3). Representative histograms were shown. Values are shown as means  $\pm$  SEM. \**P* <0.05, \*\*\**P* <0.001, \*\*\*\**P* <0.0001.

# Supplementary Figure S4. The effect of irinotecan on mRNA expression of *CXCL9/10/11* in HER2-positive GC cells.

mRNA expression of *CXCL9*, *CXCL10*, and *CXCL11* in NCI-N87 cells treated with or without 10 or 50  $\mu$ M irinotecan for 72 h (n=3). Values are shown as means  $\pm$  SEM. \*\**P* <0.05, \*\*\**P* <0.001.

#### Supplementary Figure S5. Schematic representation of the effect of T-DXd on GC.

T-DXd moderately increases HLA class I expression in HER2-positive GC cells. T-DXd also triggers the expression of CXCL9/10/11 in HER2-positive GC cells, which might recruit tumor-infiltrating lymphocytes into GC tissues. CXCL; C-X-C motif chemokine ligand, GC; gastric cancer, HER2; human epidermal growth factor-2, HLA; human leukocyte antigen, IFN- $\gamma$ ; interferon- $\gamma$ , T-DXd; trastuzumab deruxtecan, TIL; tumor-infiltrating lymphocytes

Supplementary Figure S6. Full-length blots shown in figures and supplementary figures.













Akt

Akt

Fig. 2B\_MKN7



Akt

Supplementary Fig. S1B\_NCI-N87

#### Supplementary Fig. S1B\_OE19 \_\_\_\_ ==== -----HER2 HER2 Phospho-ERK Phospho-ERK \_\_\_\_\_ -------------HLA-ABC **HLA-ABC ERK** ERK ==== ==== Phospho-Akt Phospho-Akt β**-actin** \_\_\_\_ $\beta$ -actin ====== ====:

Akt

Akt

#### Supplementary Fig. S1B\_MKN7



Akt