FIGURE LEGENDS FOR SUPPLEMENTARY FIGURES

Supplementary Figure 1: Human specific IHC on xenografts

Human specific anti-nucleoli IHC staining to prove human origin (scale bar 100 µM). Normal mouse stomach mucosa served as negative control (no nuclear staining, background staining due to the use of the secondary anti-mouse signal staining boost).

Supplementary Figure 2: Chemotherapy of normal gastric organoids

(A-E) Cell viability assay after treatment with 5-FU (A), oxaliplatin (B), irinotecan (C), epirubicin (D) and docetaxel (E) in varying concentrations. Analysis for oxaliplatin, irinotecan, epirubicin and docetaxel treatment was performed after 24 h, 5-FU treatment was analysed after 72 h. Values were normalised to untreated control organoids of the same patient.

Supplementary Figure 3: Chemotherapy of gastric cancer organoids

(A-E) Representative pictures of human cancer organoids and three normal organoid lines (DD379N, DD392N, DD399N) treated with 5-FU (A), oxaliplatin (B), irinotecan (C), epirubicin (D) and docetaxel (E) with 3 different concentrations. Responding organoids typically started to shrink, became dark and disaggregated (scale bar 100 µm).

Supplementary Figure 4: Chemotherapy of classical 2D gastric cancer cell lines

(A-E) Cell viability assay of AGS, KATO III, Snu1 and Snu5 after treatment with 5-FU (A), oxaliplatin (B), irinotecan (C), epirubicin (D) and docetaxel (E) in indicated concentrations. Analysis for oxaliplatin, irinotecan, epirubicin and docetaxel was performed after 24 h, 5-FU was analysed after 72 h of treatment. Values were normalised to untreated cells.

Supplementary Figure 5: Genetic features of gastric cancer organoids and corresponding primary tumours

(A). View on major chromosomal rearrangements in DD109. From the outside to the inside: known oncogenes (green: missense, black: nonsense, red: amplifications, blue: deletions), chromosomes, coding variants represented as small squares (green: missense, black: stop or splice site, red: frameshift), copy number variations (CNVs) as a scatterplot and as gained (red) or lost (blue) regions as well as interchromosomal (red) and intrachromosomal (blue) rearrangements bigger than 1 Mb. (B). Cancer organoid lines and their corresponding primary tumours were assigned to the microsatellite instable (MSI), genomic stable (GS) and chromosomal instable (CIN) subtypes. Of note, only DD191 and DD107 primary cancers could be analysed by whole genome sequencing, mutations in DD282 and DD109 needed to be analysed by Sanger sequencing. Represented are somatic copy number aberrations (SCNA), microsatellite instability status (MSI), Epstein-Barr-Virus status (EBV), prominent genes and

mutational features. Statuses are indicated as high (red), low (orange), and stable (light grey) and presence or absence of features in black or white, respectively. (C) Sanger sequencing results of primary tumour DNA from DD109 and DD282. Conflicts were resolved with ambiguity nucleotides. All analysed mutations present in the organoids except for ARID1A Gln1365* could be confirmed in the primary tumour.

Supplementary Figure 6: Trastuzumab treatment in combination with 5-FU

Combined treatment with trastuzumab plus 5-FU using different concentrations of each drug. Cell viability assay for DD107 (A) and DD109 (B) after 72 h of combined treatment. Values were normalised to untreated control organoids of the same patient.

Supplementary Figure 7: Genotyping of mouse corpus organoids

(A) Adenoviral infection of organoids. Successful infection was observed via the GFP fluorescence signal (scale bar 100 µM). (B) Genotyping PCR: For primers and genetic background of mice see Supplementary Methods.