HOKKAIDO UNIVERSITY

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| Title | Extramammary Paget's disease patient-derived xenografts harboring ERBB2 S310F mutation show sensitivity to HER2-targeted therapies |
| Author(s) | Maeda, Takuya; Kitamura, Shinya; Nishihara, Hiroshi et al. |
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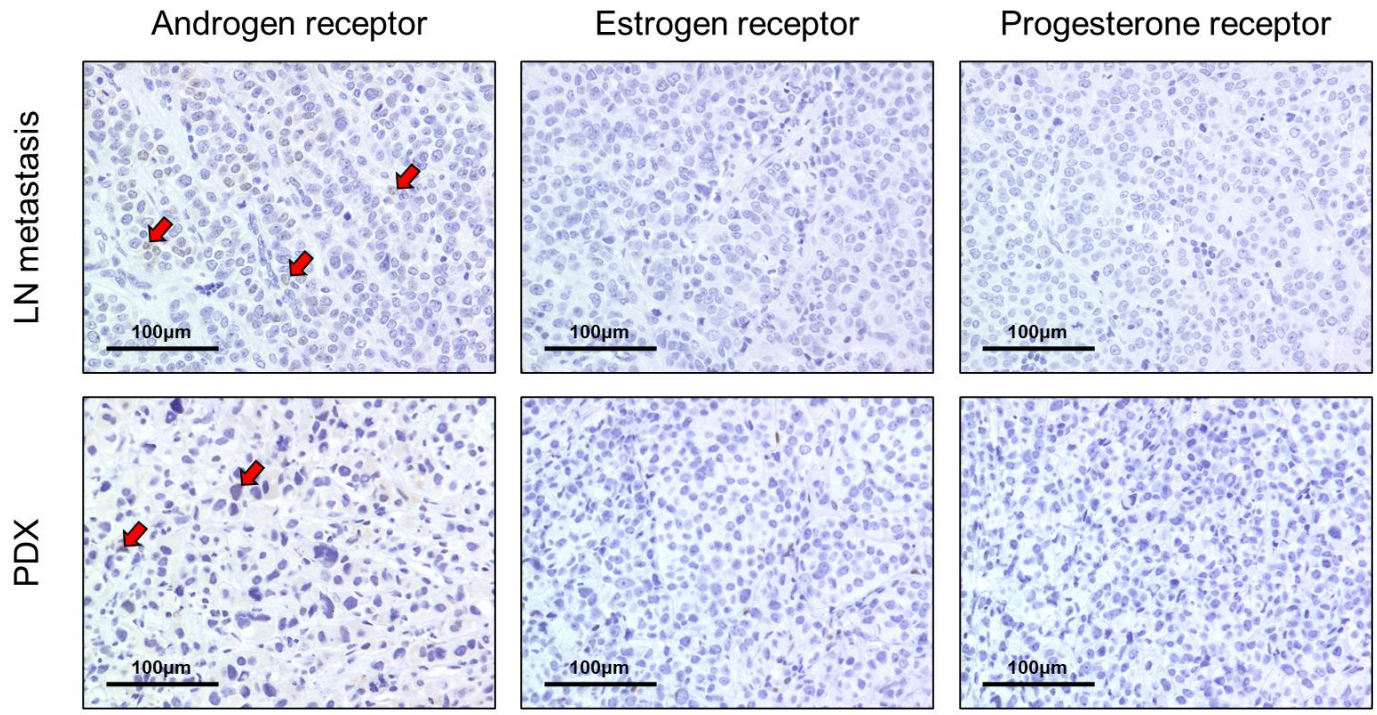
**Supplementary Table S1. Minor gene alterations**

| Sample characteristics | Minor gene alterations (VAF, %) | |
|---|---|---|
| **Patient's blood** | None | |
| **Patient's LN** | *TP53* A161T (50.4%) | |
| | *RB1* S780* (42.8%) | |
| | *NF1* D2545N (33.1%) | |
| **PDX (G1)** | *TP53* A161T (100%) | *KMT2D* G5087E (47.1%) |
| | *ALK* Q180H (17.2%) | *KMT2D* S2149T (45.7%) |
| | *ALK* R179L (16.0%) | *RB1* S780* (100.0%) |
| | *PTCH1* G1363S (19.7%) | *NF1* D2545N (47.5%) |
| | *PTCH1* A1353T (17.4%) | *CDK*12 S1288R (36.0%) |
| | *TSC1* A529S (70.2%) | *BCOR* R710S (18.1%) |
| | *TSC1* P513S (69.6%) | *KDM6A* I598V (23.4%) |
| | *NOTCH1* S1409N (90.2%) | *KDM6A* M754V (17.5%) |
| | *FGFR2* L92F (36.2%) | *KDM6A* T794P (17.5%) |
| | *FGFR2* L63M (41.0%) | |
| | *FGFR2* V60L (41.3%) | |
| | *FGFR2* A52V (41.9%) | |
| | *EGFR2* V49A (42.8%) | |
| **PDX (G2)** | *TP53* A161T (100%) | *KMT2D* G5087E (44.6%) |
| | *ALK* Q180H (12.2%) | *KMT2D* S2149T (37.5%) |
| | *ALK* R179L (12.3%) | *RB1* S780* (99.5%) |
| | *PTCH1* G1363S (19.7%) | *NF1* D2545N (50.3%) |
| | *PTCH1* A1353T (17.5%) | *CDK12* S1288R (19.8%) |
| | *TSC1* A529S (59.8%) | *KDM6A* T794P (26.8%) |
| | *NOTCH1* S1409N (72.5%) | |
| | *FGFR2* L92F (24.9%) | |
| | *FGFR2* L63M (25.8%) | |
| | *FGFR2* V60L (26.3%) | |
| | *FGFR2* A52V (26.8%) | |

VAF: variant allele frequency

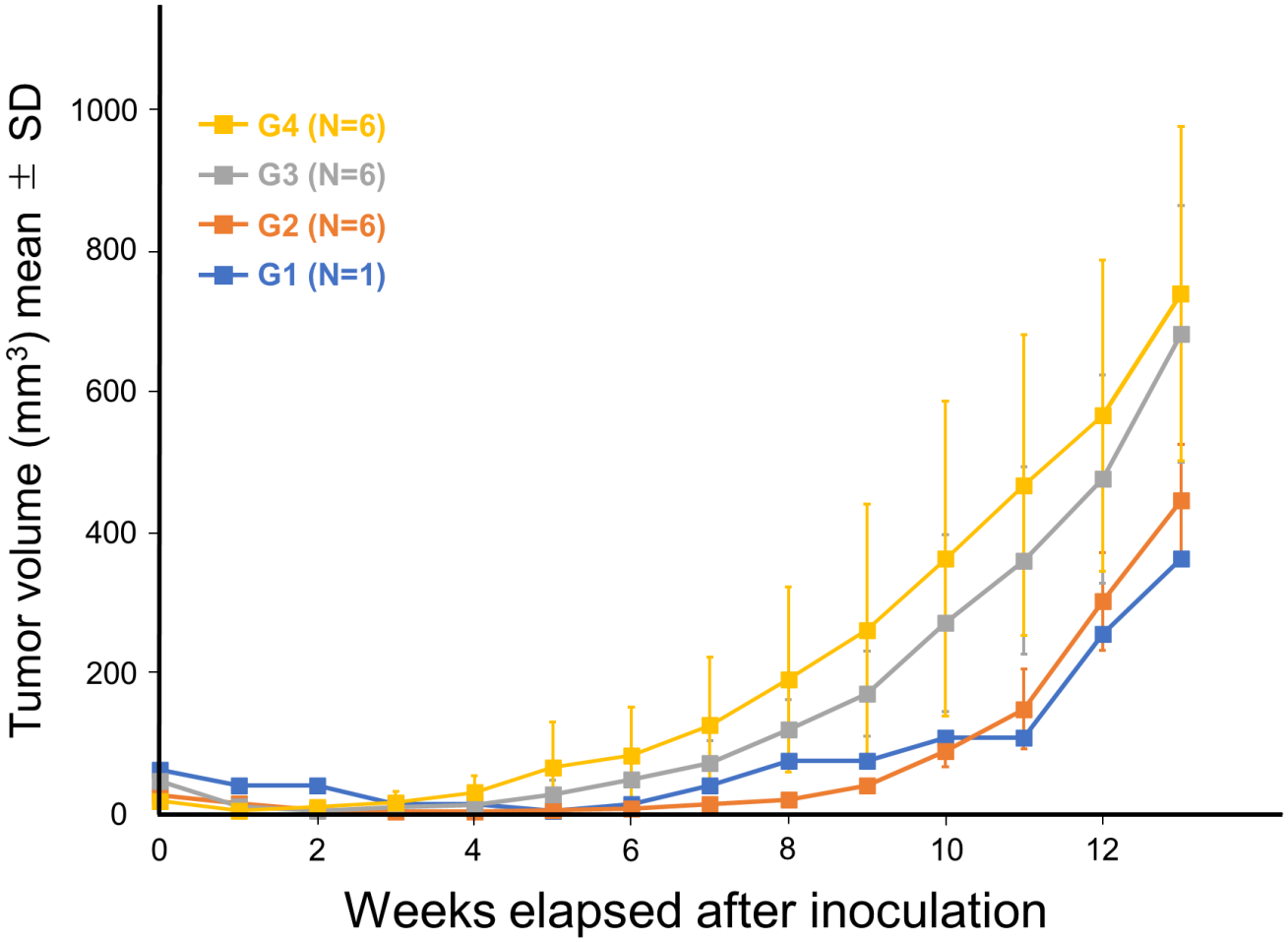
**Supplementary Table S2. List of next-generation sequencing targeting comprehensive cancer-associated genes (human comprehensive cancer panel v2, QIAGEN)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ABL1 | BUB1B | DDR2 | FGFR2 | IDH2 | MEN1 | PDGFRA | SMARCA4 |
| AKT1 | CARD11 | DICER1 | FGFR3 | IKZF1 | MET | PHF6 | SMARCB1 |
| AKT2 | CBL | DNMT3A | FH | IL6ST | MLH1 | PIK3CA | SMO |
| ALK | CBLB | ECT2L | FLCN | IL7R | MSH2 | PIK3R1 | SPOP |
| AMER1 | CD79A | EGFR | FLT3 | JAK1 | MSH6 | PMS2 | SRC |
| APC | CD79B | EP300 | FUBP1 | JAK2 | MTOR | PPP2R1A | STK11 |
| AR | CDC73 | EPCAM | GATA1 | JAK3 | MUTYH | PRDM1 | SUFU |
| ARID1A | CDH1 | ERBB2 | GATA2 | KDM6A | MYC | PRKAR1A | TERT |
| ARID2 | CDK12 | ERBB3 | GATA3 | KDR | MYD88 | PTCH1 | TNFAIP3 |
| ASXL1 | CDK4 | ERBB4 | GNA11 | KIT | NF1 | PTEN | TNFRSF14 |
| ATM | CDKN2A | ERCC5 | GNAQ | KLF6 | NF2 | PTPN11 | TP53 |
| ATRX | CHEK2 | ESR1 | GNAS | KMT2D | NFE2L2 | RAC1 | TSC1 |
| BAP1 | CIC | EZH2 | GPC3 | KRAS | NFKBIA | RB1 | TSC2 |
| BCL6 | CREBBP | FAM46C | GRIN2A | MAP2K1 | NOTCH1 | RET | TSHR |
| BCOR | CRLF2 | FANCA | H3F3A | MAP2K2 | NOTCH2 | ROS1 | U2AF1 |
| BRAF | CSF1R | FANCD2 | HIST1H3B | MAP2K4 | NPM1 | SDHB | VHL |
| BRCA1 | CTNNB1 | FANCE | HNF1A | MAP3K1 | NRAS | SETD2 | WT1 |
| BRCA2 | CYLD | FAS | HRAS | MAP4K3 | PALB2 | SF3B1 | XPC |
| BRIP1 | DAXX | FBXO11 | HSPH1 | MDM2 | PAX5 | SLC7A8 | ZNF2 |
| BTK | DDB2 | FBXW7 | IDH1 | MED12 | PBRM1 | SMAD4 | ZRSR2 |



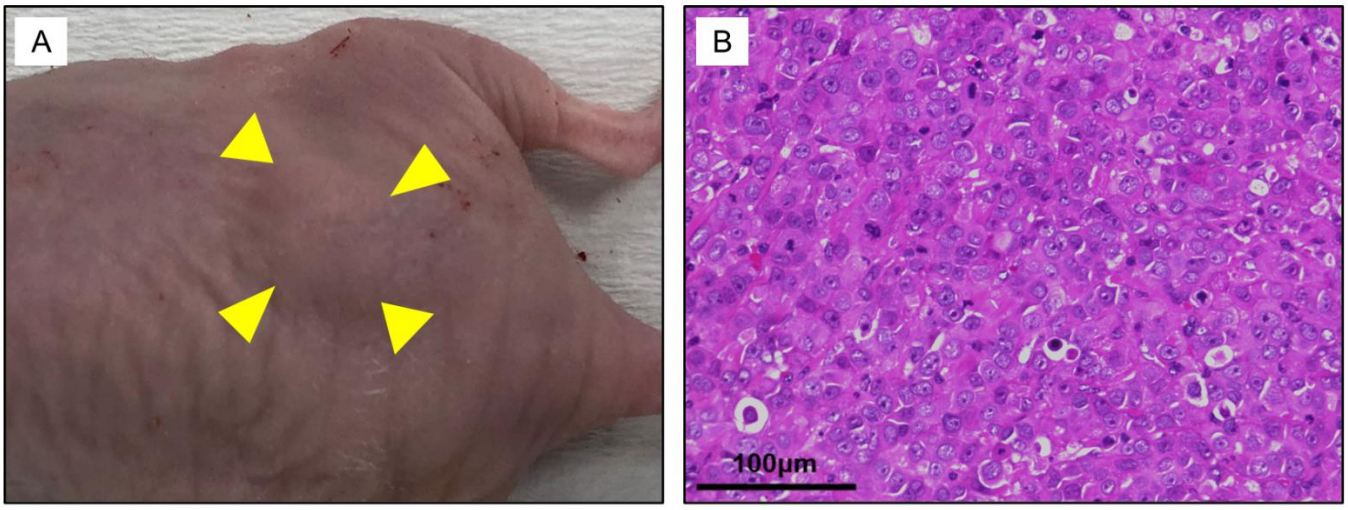

**Supplementary Figure S1. Immunohistochemistry images for androgen, estrogen and progesterone receptors.**

Both the tumor cells in the metastatic lymph node and the xenografted tumor cells were weakly positive for androgen receptor, and were negative for estrogen and progesterone receptors. Scale bar = 100 μm.




**Supplementary Figure S2. EMPD-PDX-H1 can be stably passaged.**

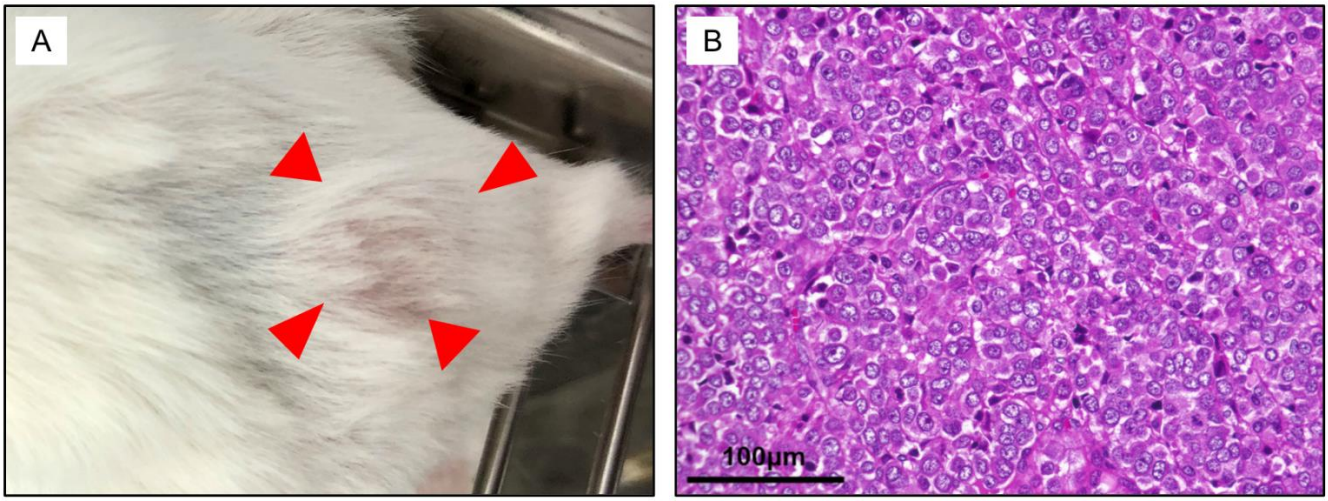
Tumor growth curves of EMPD-PDX-H1 (G1-G4). We measured the tumor volume once a week. The x-axis indicates time after inoculations (weeks); the y-axis indicates the tumor volume (long axis x short axis$^2$)/2 ($mm^3$).




**Supplementary Figure S3. EMPD-PDX-H1 can be transplanted into *nu/nu* mice.**

A. The transplantation of xenografted tumors into *nu/nu* mice was successful. The yellow arrowheads indicate the transplanted tumor.

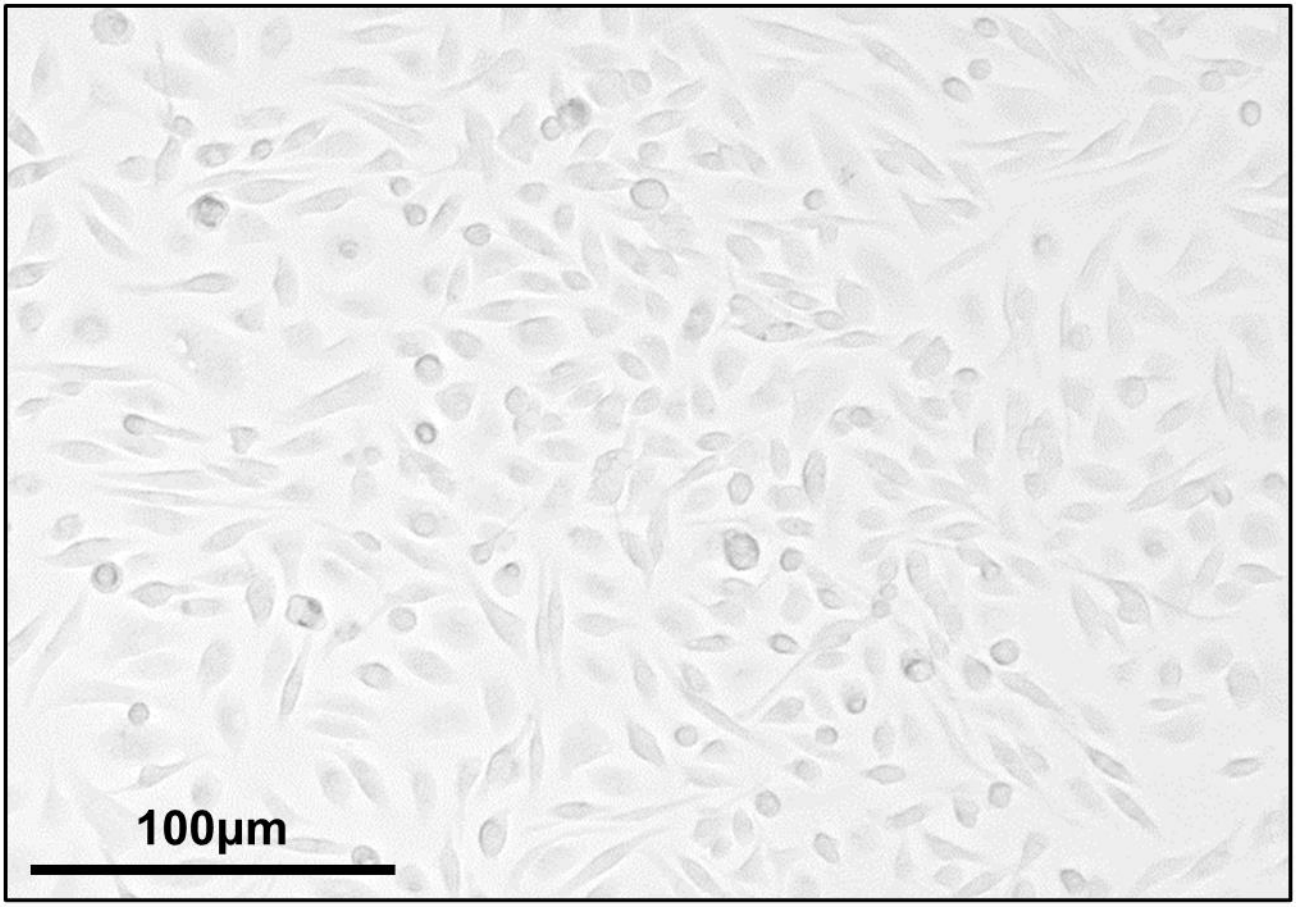
B. Histopathological image of a tumor removed from a *nu/nu* mouse. (HE staining, scale bar = 100 μm).




**Supplementary Figure S4. Cryopreserved EMPD-PDX-H1 can be inoculated.**

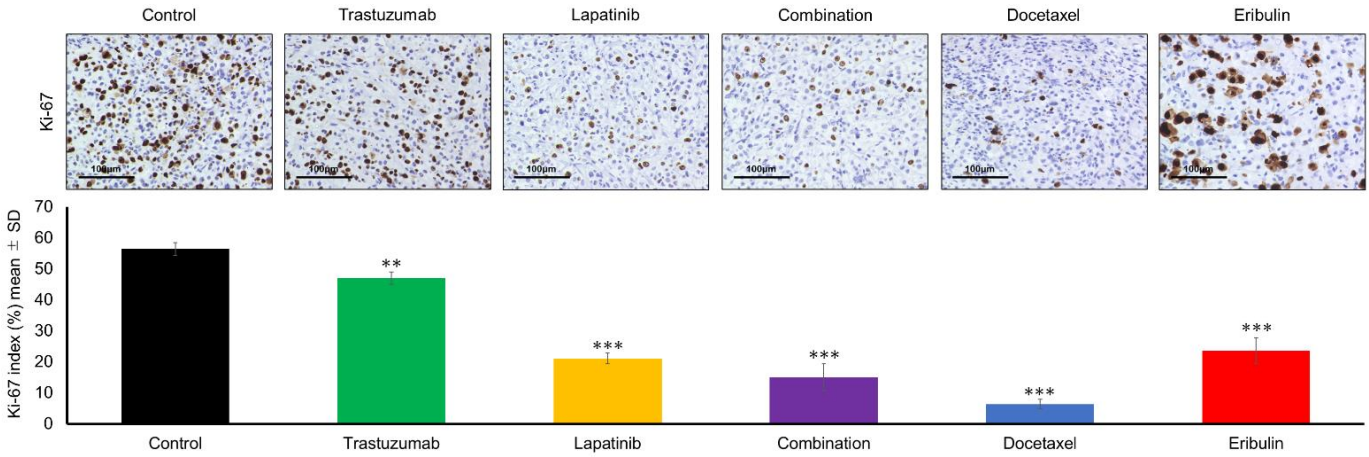
A. The inoculation of cryopreserved tumors into the NOD/Scid mice was successful. The red arrowheads indicate the transplanted tumor.

B. 10 weeks after transplantation, the enlarged tumors were extracted and histopathologically analyzed. (HE staining, scale bar = 100 μm)



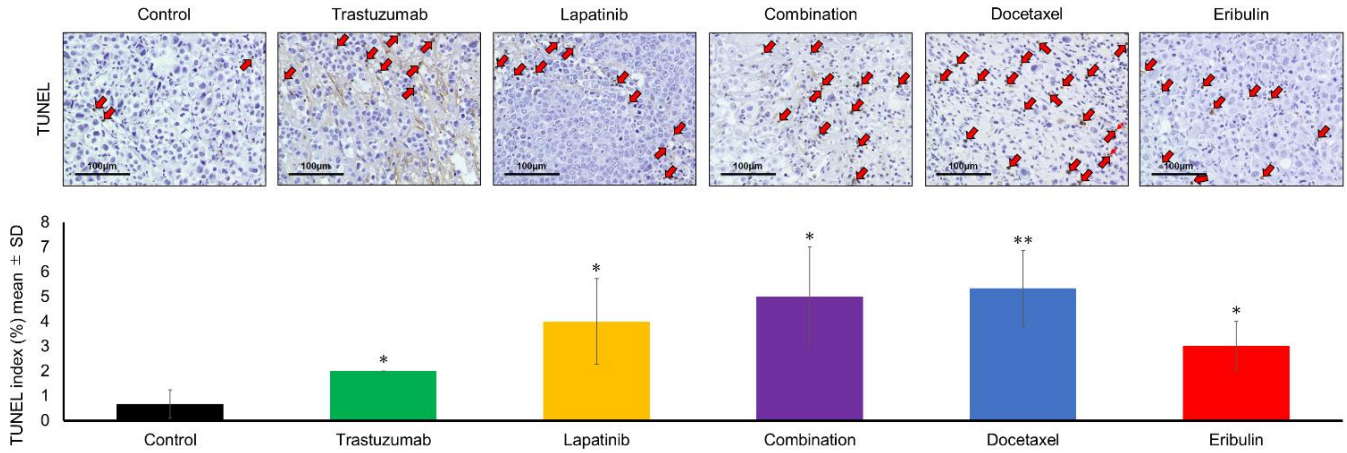

**Supplementary Figure S5. Primary culture cells originating from EMPD-PDX-H1.**

Tumor tissue from EMPD-PDX-H1 mice (3rd generation) was minced and directly plated onto dishes coated with type I collagen in a medium of RPMI containing 10% FBS. At three weeks, the primary tumor cells were proliferated and photographed (scale bar = 100 μm).




**Supplementary Figure S6. HER2-targeted therapies and cytotoxic agents are effective against the xenografted tumors (Ki-67 staining).**

For evaluation of therapeutic efficacy, treated tumors were extracted at day 28 in the targeted therapy groups (the trastuzumab, lapatinib and combination groups), and at day 10 in the chemotherapy groups (the docetaxel and eribulin groups). (Upper) Representative images of Ki-67 staining in control and treated EMPD-PDX-H1 tumors. (Lower) Treatment efficacy was evaluated based on Ki-67 expression. The y-axis indicates the percentage of Ki-67 positive cells. The results are presented as means, with the error bars representing the SD. **: $P < 0.01$, ***: $P < 0.001$.




**Supplementary Figure S7. HER2-targeted therapies and cytotoxic agents are effective against the xenografted tumors (TUNEL assay).**

For evaluation of therapeutic efficacy, treated tumors were extracted at day 28 in the targeted therapy groups (the trastuzumab, lapatinib and combination groups), and at day 10 in the chemotherapy groups (the docetaxel and eribulin groups). (Upper) Representative images of TUNEL assay in control and treated EMPD-PDX-H1 tumors. Red arrows indicate TUNEL-positive tumor cells. (Lower) Treatment efficacy was evaluated based on the levels of TUNEL positivity. The y-axis indicates the percentages of TUNEL-positive cells. The results are presented as means, with the error bars representing the SD. *: $P < 0.05$, **: $P < 0.01$.