SUPPLEMENTARY INFORMATION

Targeting TACC3 induces immunogenic cell death and enhances T-DM1 response in HER2-positive breast cancer

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Running Title: Enhancing T-DM1 response by inducing immunogenic cell death

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Conflict of Interest

O. Sahin, B.C. and E.B. are the co-founders of OncoCube Therapeutics LLC. O. Sahin is the president of LoxiGen, Inc, and O. Sahin and E.B. are members of scientific advisory board of A2A Pharmaceuticals. The other authors declare no potential conflicts of interest.

Supplementary Methods

Western blotting

Protein isolation and Western blotting were done as previously described[1,2]. Briefly, RIPA buffer (150 mmol/L NaCl, 50 mmol/L Tris base pH 8.0, 1 mmol/L EDTA, 0.5% sodium deoxycholate, 1% NP40, 0.1% SDS, 1 mmol/L DTT, and 1 mmol/L Na3VO4) supplemented with protease and phosphatase inhibitor cocktails were used to isolate total protein lysate. Protein concentrations were measured using the BCA Protein Assay Reagent Kit (Thermo Fisher Scientific, MA, USA). Equal amounts of protein (15–20 µg) were separated using 10% or 12% SDS-PAGE gel. Separated proteins were transferred to PVDF membranes (Bio-Rad, CA, USA) using a Trans-Blot turbo transfer system (Bio-Rad, CA, USA) and incubated with primary antibodies listed in **Supplementary Table S3**. HRP-linked anti-mouse IgG (#7076) or anti-rabbit IgG antibodies (#7074) (Cell Signaling Technology, MA, USA) were used as secondary antibodies, and signals were detected by enhanced chemiluminescence (ECL) prime western blot detection reagent (Cytiva, MA, USA). Images were acquired using Image Lab Software (Biorad, CA, USA) or iBright Analysis Software (Thermo Fisher Scientific, MA, USA).

Inhibitor treatments

T-DM1 was obtained from Genentech under a material transfer agreement (OR-219728 and OR-224086A) and dissolved in 100% ddH_2O to yield a stock concentration of 2 mg/ml. BO-264 (synthesized as reported previously[13]), TC Mps1 (Tocris Biosciences, Bristol, UK), SPL-B (Axon MedChem, VA, USA), DM1 (MedChemExpress) were dissolved in 100% DMSO to yield a stock concentration of 10 mM. Trastuzumab was dissolved in 100% water to yield a stock concentration of 10 mg/ml. For cell viability assay, BT-474 WT and T-DM1R ($8x10^3$ cells/well), SK-BR-3 WT and T-DM1R, AU-565 cells ($6x10^3$ cells/well), MDA-MB-231 cells ($6x10^3$ cells/well), MDA-MB-157 and EMT6.huHER2 ($3x10^3$ cells/well) cells were seeded, and inhibitor treatments were performed at different concentrations. Cell viability was measured 72 hours after treatment with SRB (Sigma Aldrich) as described previously[3]. Annexin V/PI staining (Biolegend,

USA) was performed according to manufacturer's instructions using EMT6.huHER2 cells treated with 500 nM BO-264 and 5 µg/mL T-DM1, alone or in combination for 48 hours.

Gene knockout via CRISPR/Cas9-mediated gene editing and stable overexpression

To generate EMT6.huHER2 cells, the human HER2 sequence from the pLL-RSV-hHER2 vector that was kindly provided by Hasan Korkaya (Augusta University, GA, USA) was cloned into the pCDH-CMV vector. The sgRNAs targeting mouse TACC3 were designed and selected based on having high on-target (=high efficacy) and low off-target (=high specificity) activity using the CRISPick tool (Broad Institute). The sgRNA sequences targeting TACC3 in EMT6.huHER2 murine mammary tumor cell line are: 5'-CACCGAGTTTAAGGAGTCGGCCTGG-3' (sg#1) and 5'-CACCGCTGAGATCCTAAGAGCAGA-3' (sg#2). The designed sgRNAs were cloned into human lentiCRISPR v2 vector (Addgene, MA, USA). For lentiviral packaging, HEK293T cells were transfected with sgRNAs and the packaging plasmids, pMD2.G and psPAX2 (Addgene, MA, USA). Transduction of the cells was performed in the presence of 10 ug/ml polybrene, and selection of transduced cells was done using 1µg/ml puromycin. Subsequently, transduced cells were transferred to 96-well plates in 1:2 serially diluted for seeding single cell colonies[4].

Immunofluorescence staining

SK-BR-3 (150,000 cells /well) and BT-474 (300,000 cells/well) WT and T-DM1R, EMT6.huHER2 (270,000 cells/well) and AU-565 (200,000 cells /well) cells were plated on glass coverslip in 6-well cell culture plates and treated with 0.05, 4 µg/ml and doses 7.5 µg/ml of T-DM1 and or 500 nM BO-264 on the next day. Forty-eight hours after treatment, cells were fixed with 2% paraformaldehyde for 5 min. For examination of microtubule organization, cells were treated with T-DM1 for 24 hours. Later, they were blocked for 1 hour in blocking buffer (3%BSA in PBS) and incubated with primary antibodies (**Supplementary Table S3**) in blocking buffer for 1 hour at room temperature (RT). Antibodies against calreticulin was used at a dilution of 1:500, and alpha-tubulin at a dilution of 1:1000. Then, the cover slips were incubated with secondary antibodies in blocking buffer for 1 hour at RT in dark (1:1000 dilution).

Cells were also counter stained with DAPI for 5 min (0.01 μg/μl). Finally, slides were mounted using Prolong™ Glass mounting (Invitrogen, MA, USA) and examined using Zeiss LSM 880 confocal laser scanning microscope.

IHC staining in patient tumors

To determine the levels of infiltrated T cell lymphocytes into tumor microenvironment based on T-DM1 responsiveness before and after T-DM1 therapy, we performed IHC staining on tumor samples using CD3 (clone: LN10, Leica Biosystems), CD4 (clone: 4B12, Leica Biosystems) and CD8 (clone: 4B11, Leica Biosystems) primary antibodies. Antigen retrieval was done for 20 min with the EDTA buffer for CD3 and CD4 while it was 10 min with citrate buffer for CD8. Slides were incubated with CD3, CD4 and CD8 at a dilution of 1:300, 1:100 and 1:50 for 25 min at room temperature using Leica BOND-III IHC/ISH automated immune-stainer, respectively. Lymphoid tissue in tonsil was used as positive control tissues, while leiomyoma and placental tissue was used as negative control. CD3, CD4 and CD8 stained slides were digitally scanned via Nanozoomer digital scanner (Hamamatsu Photonics) and evaluated in one screen (by NDP.view2 software) to precisely give a ratio of all three of them in the peritumoral stroma. Antibody validation for TACC3 was performed using normal breast as negative and IDC tumor as positive control.

Quantification of CD3, CD4 and CD8 in peritumoral stroma

CD3, CD4 and CD8 percentages were calculated by dividing the positive cells by all the inflammatory cells in the peritumoral stroma of the tumor, including lymphocytes, macrophages, neutrophile leukocytes etc. Following formula is an example of percentage calculation: CD3 (%) = (number of CD3 positive cells / number all the inflammatory cells in peritumoral stroma) x 100

Multiplex IHC staining

Immunofluorescence staining of FFPE tumor slides were performed by deparaffinization at 60 °C for 1 hour, followed by rehydration in citrisolv for 5 min (3 times), 100% ethanol for 5 min (twice), 95% ethanol for 5 min (twice), deionized water for 5 min (twice). Antigen retrieval was done with Tris-EDTA pH=9 at

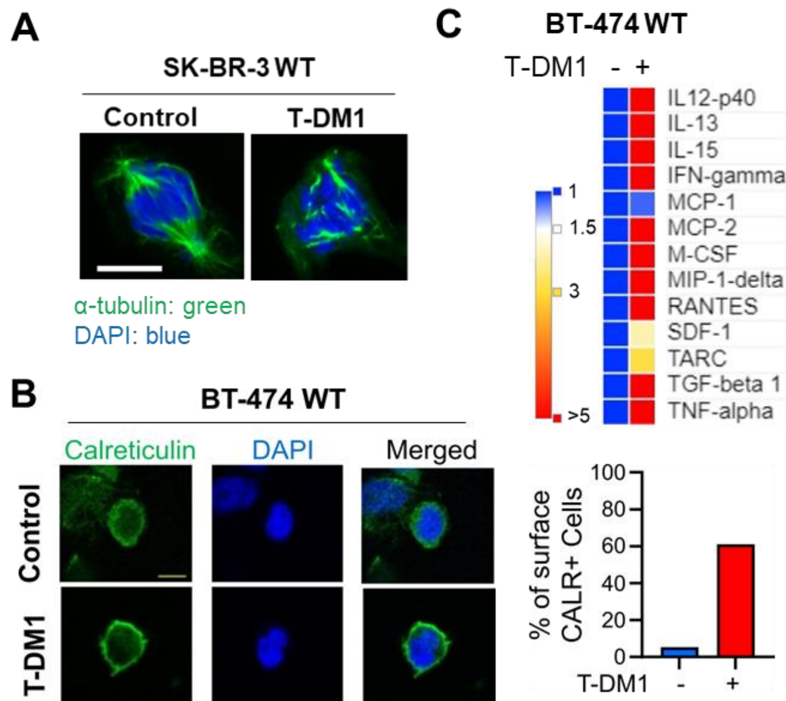
96 °C for 15 min, followed by cooling down to room temperature for 30 min, and washing with TBST for 5 min. Blocking was done at room temperature with Buffer W (IBA-Lifesciences) for 30 min. Optimized multiplex immunofluorescence was performed using the OPAL™ multiplexing method. OPAL™ is based on Tyramide Signal Amplification (TSA) using the Roche Ventana Discovery Ultra Automated Research Stainer (Roche Diagnostics, Indianapolis, IN). Tissues were stained with antibodies against CD11c, CD86, CD27, NK 1.1, CD4, CD8, Foxp3, CD25, CD3, p-eIF2α and HMGB1 (**Supplementary Table S3**) and the fluorescence signals were generated using the following fluorophores: Opal 480, Opal 520, Opal 570, Opal 620, Opal 690 and Opal 780 (Akoya Biosciences, Marlborough, MA). The stained slides were imaged at 20X magnification using the PhenoImager HT™ Automated Quantitative Pathology Imaging System (Akoya Biosciences, Marlborough, MA). Analyses of the images were done using inForm® Tissue Analysis Software (v[2.6.0], Akoya Biosciences, Marlborough, MA). By using software algorithm, cells were segmented and cell phenotypes (showing positive staining for the markers) are assigned to individual cells. Cell density analysis was performed by aggregating and consolidating the phenotypes, and then normalizing positive populations to each tissue area (mm2), using the PhenoptrReports Open Source R Package (https://akoyabio.github.io/phenoptrReports/index.html, Akoya Biosciences, Marlborough, MA).

Multiplex cytokine array

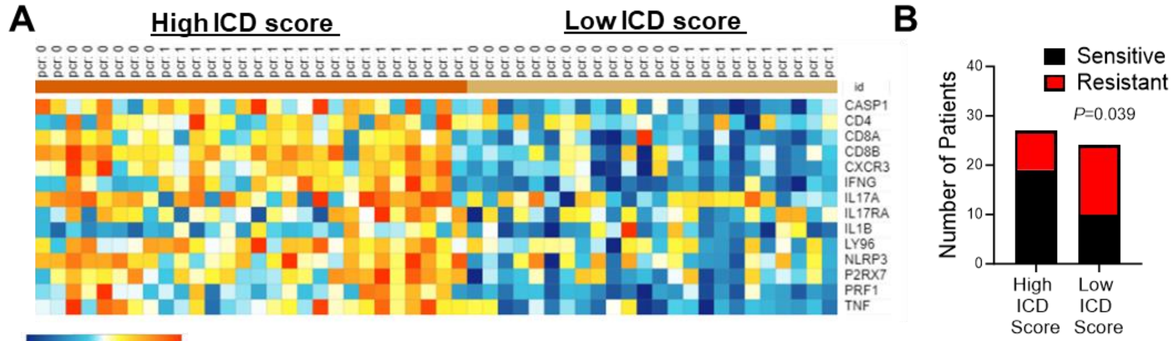
The multiplex cytokine analyses were performed using (1) supernatants of the DC and T cell co-cultures, and (2) serum samples collected from Fo5 tumors of mice treated with T-DM1 alone or in combination with BO-264 for a week were performed using the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corporation (Calgary, Alberta, Canada). Forty-five markers were simultaneously measured in the samples using Eve Technologies' Mouse Cytokine 45-Plex Discovery Assay® which consists of two separate kits; one 32-plex and one 13-plex (MilliporeSigma, Burlington, Massachusetts, USA). The assay was run according to the manufacturer's protocol. The 32-plex consisted of Eotaxin, G-CSF, GM-CSF, IFNγ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17, IP-10, KC, LIF, LIX, MCP-1, M-CSF, MIG, MIP-1α, MIP-1β, MIP-2, RANTES,

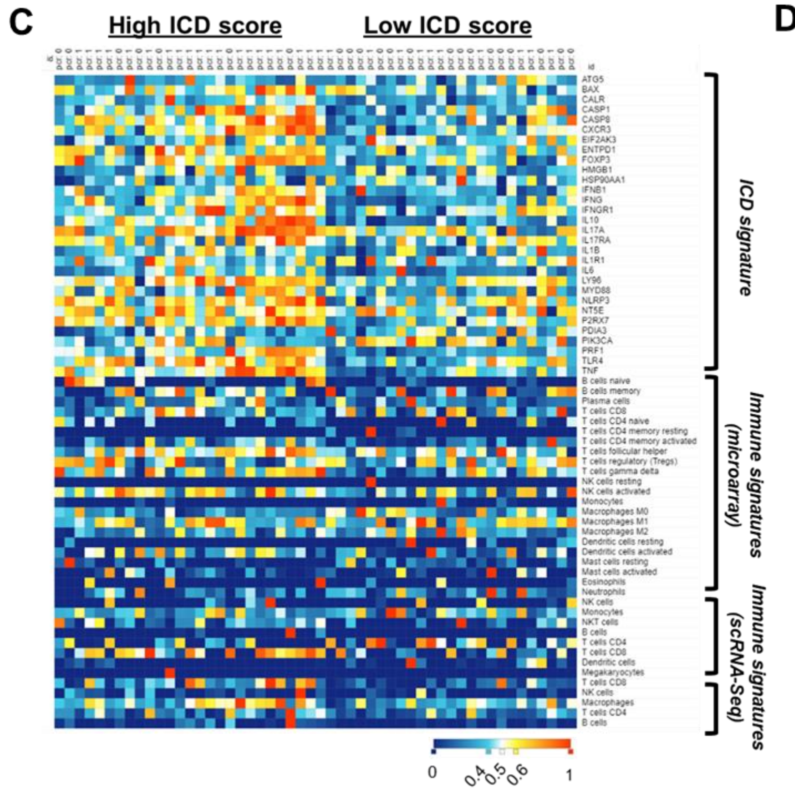
TNFα, and VEGF. The 13-plex consisted of 6Ckine/Exodus2, Erythropoietin, Fractalkine, IFNβ-1, IL-11, IL-16, IL-20, MCP-5, MDC, MIP-3α, MIP-3β, TARC, and TIMP-1.

Supplementary Figures



Supplementary Figure S1. T-DM1 induces mitotic arrest and ICD markers in T-DM1 sensitive breast cancer cells in a SAC-dependent manner. A IF staining of α-tubulin (green) in T-DM1 treated SK-BR-3 WT cell. Scale bar=10 µm. DAPI was used to stain the nucleus. **B** IF cell surface staining of calreticulin (green) in T-DM1 treated BT-474 WT cells. Scale bar=10 µm. Its quantification is provided on the right DAPI was used to stain the nucleus. **C** Chemokine array blot analysis inT-DM1 treated BT-474 WT cells.





D

Hacettepe cohort

pre (n=41) *post (n=18)*

% of CD3+ cells (150, 100, 50, 0)

Res Sens Res Sens

Pre-treatment (n=41)

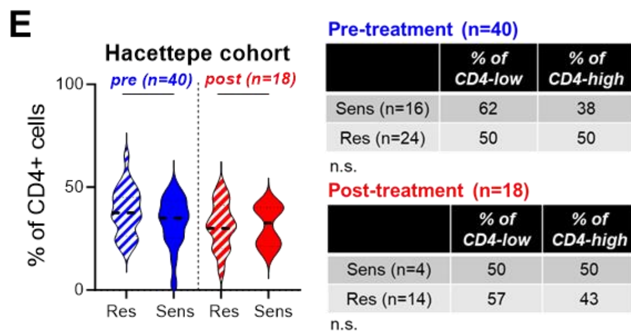
	% of CD3-low	*% of CD3-high*
Sens (n=17)	41	59
Res (n=24)	33	67

n.s.

Post-treatment (n=18)

	% of CD3-low	*% of CD3-high*
Sens (n=4)	50	50
Res (n=14)	57	43

n.s.



Pre-treatment (n=40)

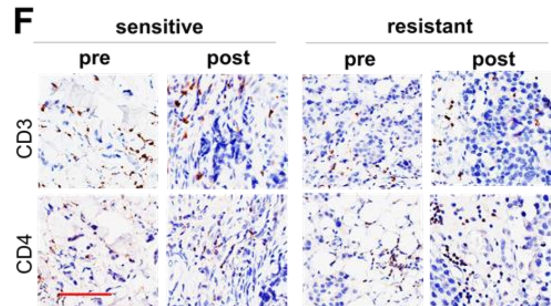
	% of CD4-low	*% of CD4-high*
Sens (n=16)	62	38
Res (n=24)	50	50

n.s.

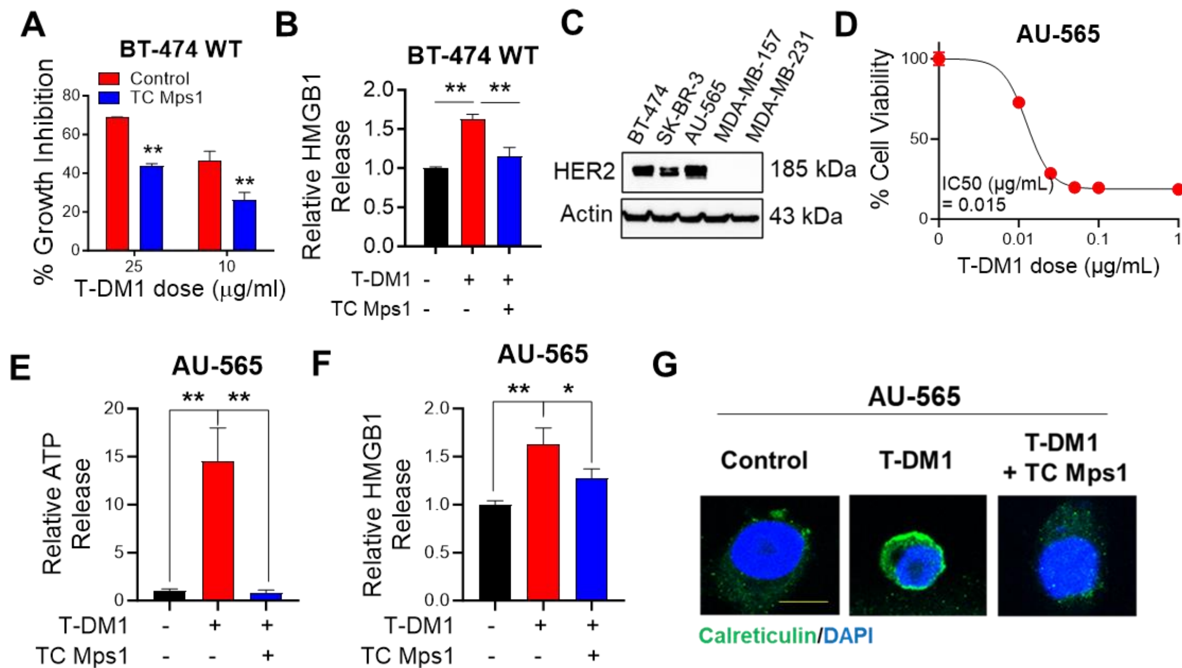
Post-treatment (n=18)

	% of CD4-low	*% of CD4-high*
Sens (n=4)	50	50
Res (n=14)	57	43

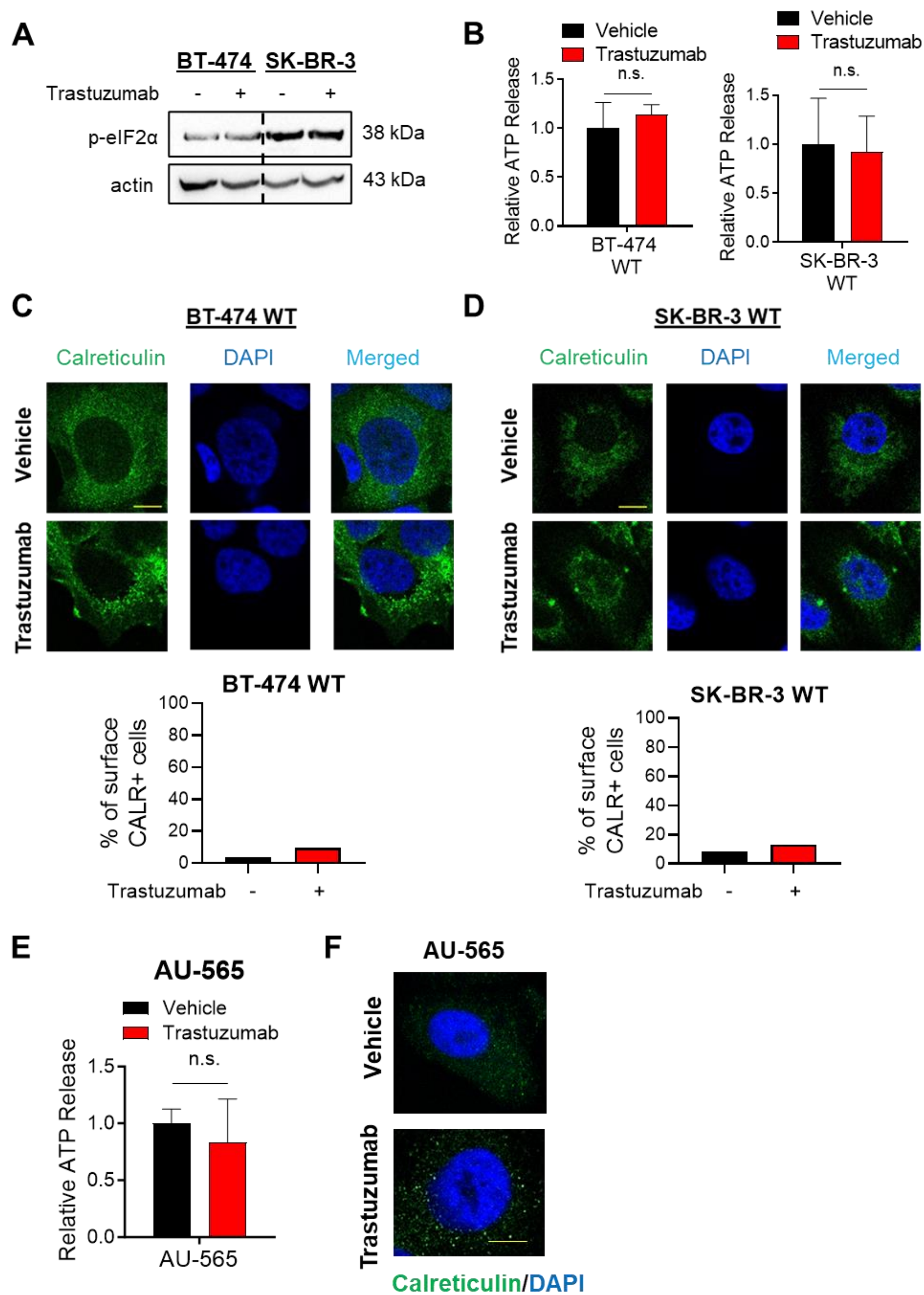
n.s.



Supplementary Figure S2. Association of immune cell markers with ICD and T-DM1 response in patients. A Heatmap of a breast cancer-specific signature of ICD and its correlation with pCR in T-DM1+pertuzumab-treated patients from GSE194040. pCR: 1, sensitive; pCR: 0, resistant. **B** Chi-square analysis of sensitive vs. resistant tumors expressing low vs. high ICD scores from A. **C** Heatmap of the ICD signature and the immune cell markers obtained from microarray-based and scRNA-Seq-based analysis, and their correlation with pCR in T-DM1+pertuzumab-treated patients from GSE194040. **D** Percentage of CD3+ cells in sensitive (sens) vs. resistant (res) tumors collected pre- (n=41) and post-T-DM1 (n=18) treatment. Tables of the percentages of CD3-low or CD3-high tumors (based on average levels of CD3+ cells in each group) are given below and significance was calculated using Chi-square testing. **E** Percentage of CD4+ cells in sensitive (sens) vs. resistant (res) tumors collected pre- (n=40) and post-T-DM1 (n=18) treatment. Tables of the percentages of CD4-low or CD4-high tumors (based on average levels of CD4+ cells in each group) are given below and significance was calculated using Chi-square testing. **F** The representative images from D and E. Scale bar=150 µm. Data correspond to mean values ± standard deviation (SD). *P*-values are calculated with Chi square test.

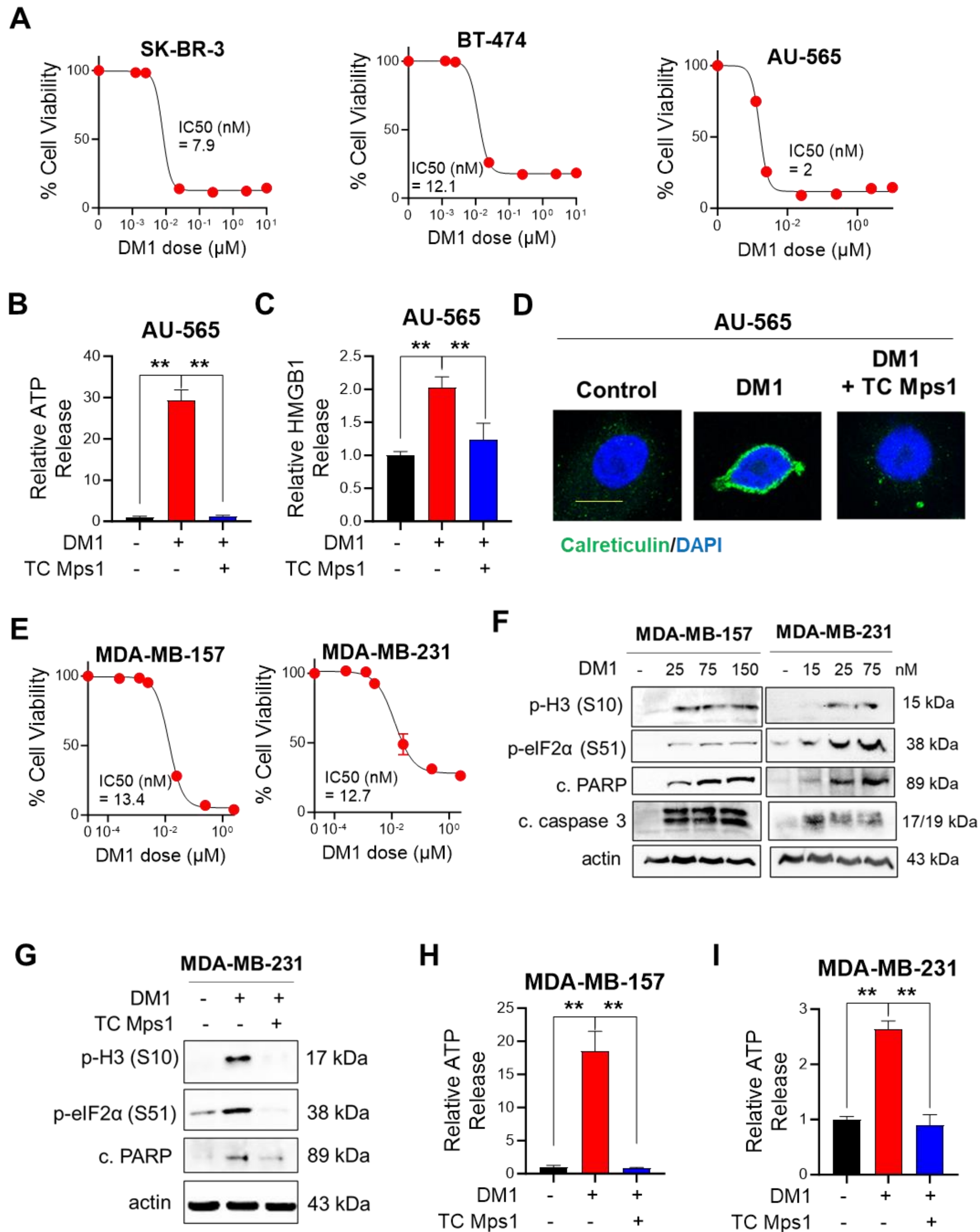


Supplementary Figure S3. T-DM1 induces mitotic arrest and ICD markers in T-DM1 sensitive breast cancer cells in a SAC-dependent manner. A Percent growth inhibition in BT-474 WT cells treated with T-DM1 alone or in combination with 1 μM TC Mps1 (Mps1 inhibitor) (n=3). **B** Relative HMGB1 release in BT-474 WT cells treated with T-DM1 alone or in combination with 1 μM TC Mps1 (n=3). **C** Western blot analysis of HER2 in HER2-high, and HER2-low cell lines. **D** Percent cell viability of AU-565 cells treated with increasing doses of T-DM1 for 3 days (n=4-6). **E, F** Relative ATP (E) and HMGB1 (F) release in AU-565 cells treated with T-DM1 (0.05 μg/mL) with or without TC Mps1 (n=3). **G** IF cell surface staining of calreticulin (green) in AU-565 cells treated with T-DM1 with or without TC Mps1. Scale bar=10 μm. Data correspond to mean values ± standard deviation (SD). *P*-values are calculated with the unpaired, two-tailed Student's t test. *, *P*<0.05; **, *P*<0.01.



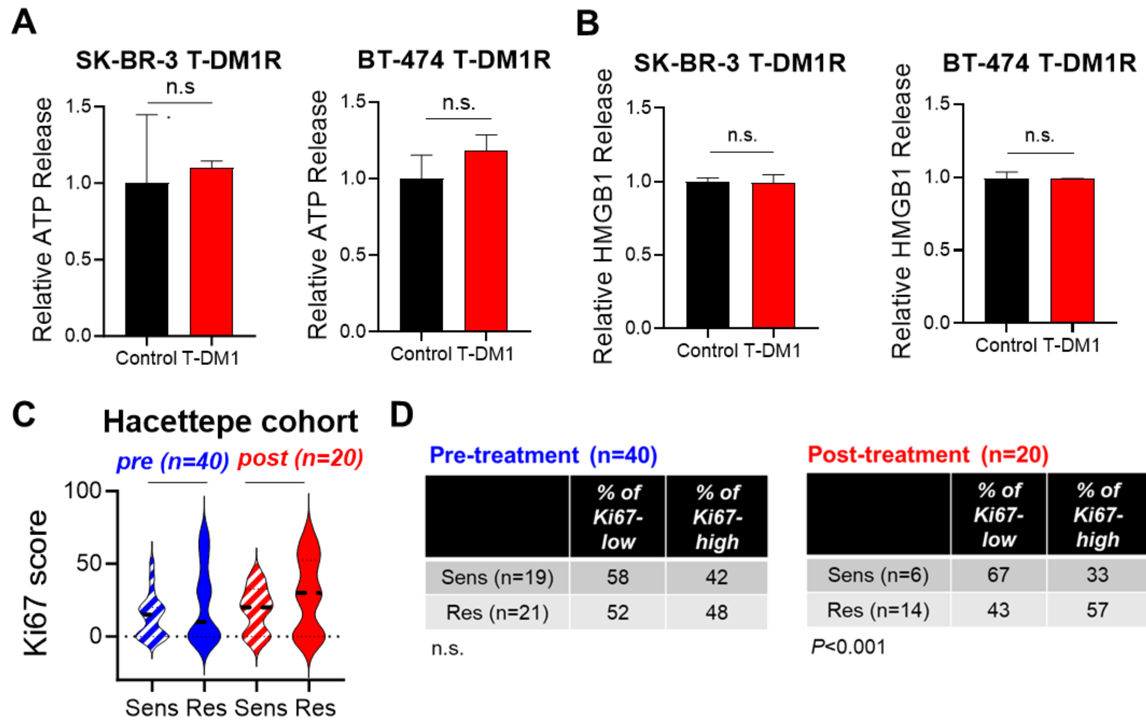
Supplementary Figure S4. Effects of trastuzumab on ICD in HER2+ BT-474, SK-BR-3 WT and AU-565 cells. A Western blot analysis of p-eIF2α in trastuzumab-treated SK-BR-3 and BT-474 WT cells. Actin

is used as a loading control here and in every Western blot. **B** Relative ATP release in trastuzumab-treated SK-BR-3 WT and BT-474 WT cells (n=4, 5). **C, D** IF cell surface staining of calreticulin (green) in trastuzumab-treated BT-474 WT (C) and SK-BR-3 WT (D) cells. The quantification of percentage of surface CALR-positive cells are given below. Scale bar=10 µm. **E** Relative ATP release in AU-565 cells treated with 1µg/mL trastuzumab (n=3). **F** Surface Calreticulin staining of AU-565 cells treated with 1µg/mL trastuzumab. Scale bar=10 µm. Data correspond to mean values ± standard deviation (SD). Significance was calculated with the unpaired, two-tailed Student's t test. n.s., not significant.



Supplementary Figure S5. Effect of the payload DM1 on ICD markers in HER2+ and HER2- cell lines. A Percent cell viability of HER2+ cells treated with increasing doses of DM1 for 3 days (n=4-6). **B**

C Relative ATP (B) and HMGB1 (C) release in AU-565 cells treated with DM1 (15 nM) with or without TC Mps1 (n=3). **D** IF cell surface staining of calreticulin (green) in AU-565 cells treated with DM1 with or without TC Mps1. Scale bar=10 µm. **E** Percent cell viability of HER2- cells treated with increasing doses of DM1 for 3 days (n=4-6). **F** Western blot analysis of mitotic arrest, apoptosis, and ICD markers in HER2- MDA-MB-157 and MDA-MB-231 cells treated with increasing doses of DM1. **G** Western blot analysis of mitotic arrest, apoptosis and ICD markers in MDA-MB-231 cells treated with 75 nM DM1 with or without TC Mps1. **H, I** Relative ATP release in MDA-MB-157 (H) and MDA-MB-231 (I) cells treated with DM1 with or without TC Mps1 (n=3). Data correspond to mean values ± standard deviation (SD). *P*-values are calculated with the unpaired, two-tailed Student's t test. **, $P<0.01$.



Pre-treatment (n=40)

	% of Ki67-low	% of Ki67-high
Sens (n=19)	58	42
Res (n=21)	52	48

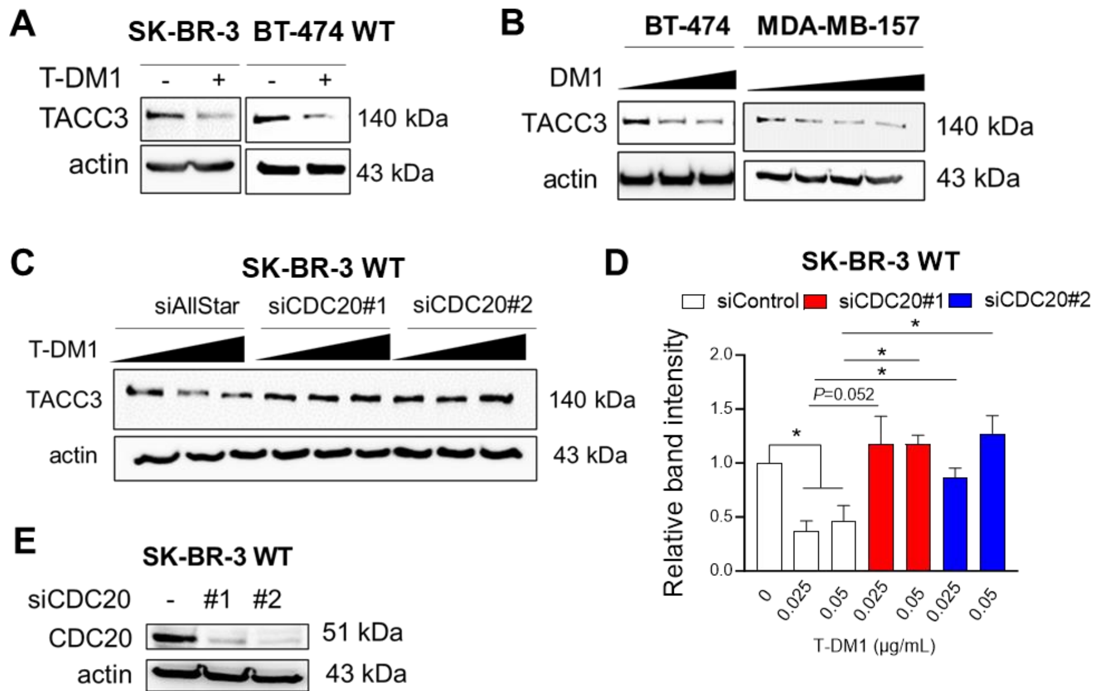
n.s.

Post-treatment (n=20)

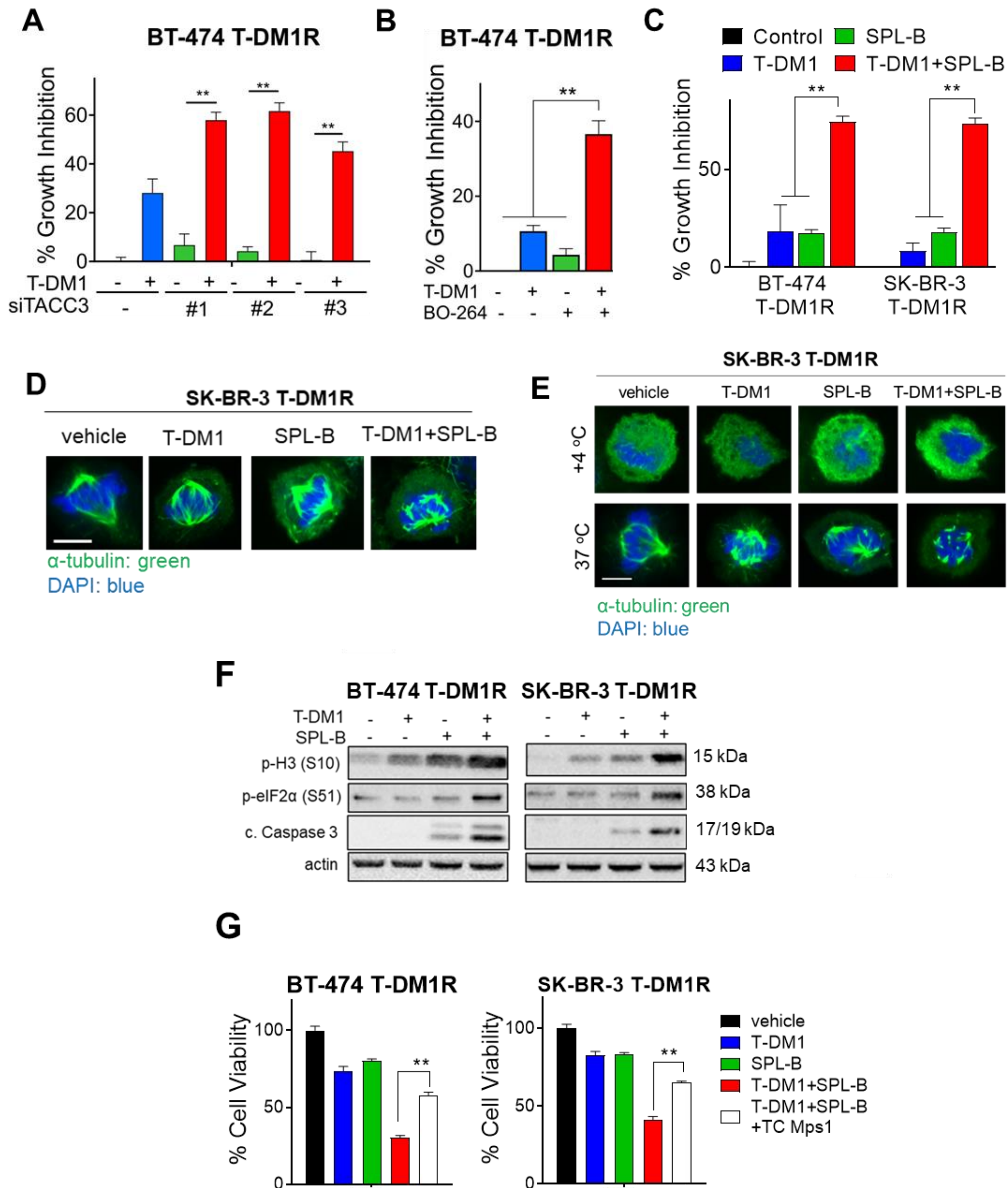
	% of Ki67-low	% of Ki67-high
Sens (n=6)	67	33
Res (n=14)	43	57

P<0.001

Supplementary Figure S6. Effect of T-DM1 on ICD markers in T-DM1 resistant breast cancer cell lines, and association of patient characteristics with TACC3 post-T-DM1 therapy. A, B Relative ATP (A) and HMGB1 (B) release from T-DM1R cells treated with T-DM1 (n=3). **C** Ki67 score in sensitive (sens) vs. resistant (res) pre (n=40) or post-treatment (n=20) samples from Hacettepe cohort. **D** Table of the percentages of Ki67-low or Ki67-high tumors (based on median levels of Ki67 score in each group). Data correspond to mean values ± standard deviation (SD). Significance was calculated with the unpaired, two-tailed Student's t test for A, B and using Chi-square test for D. n.s., not significant.

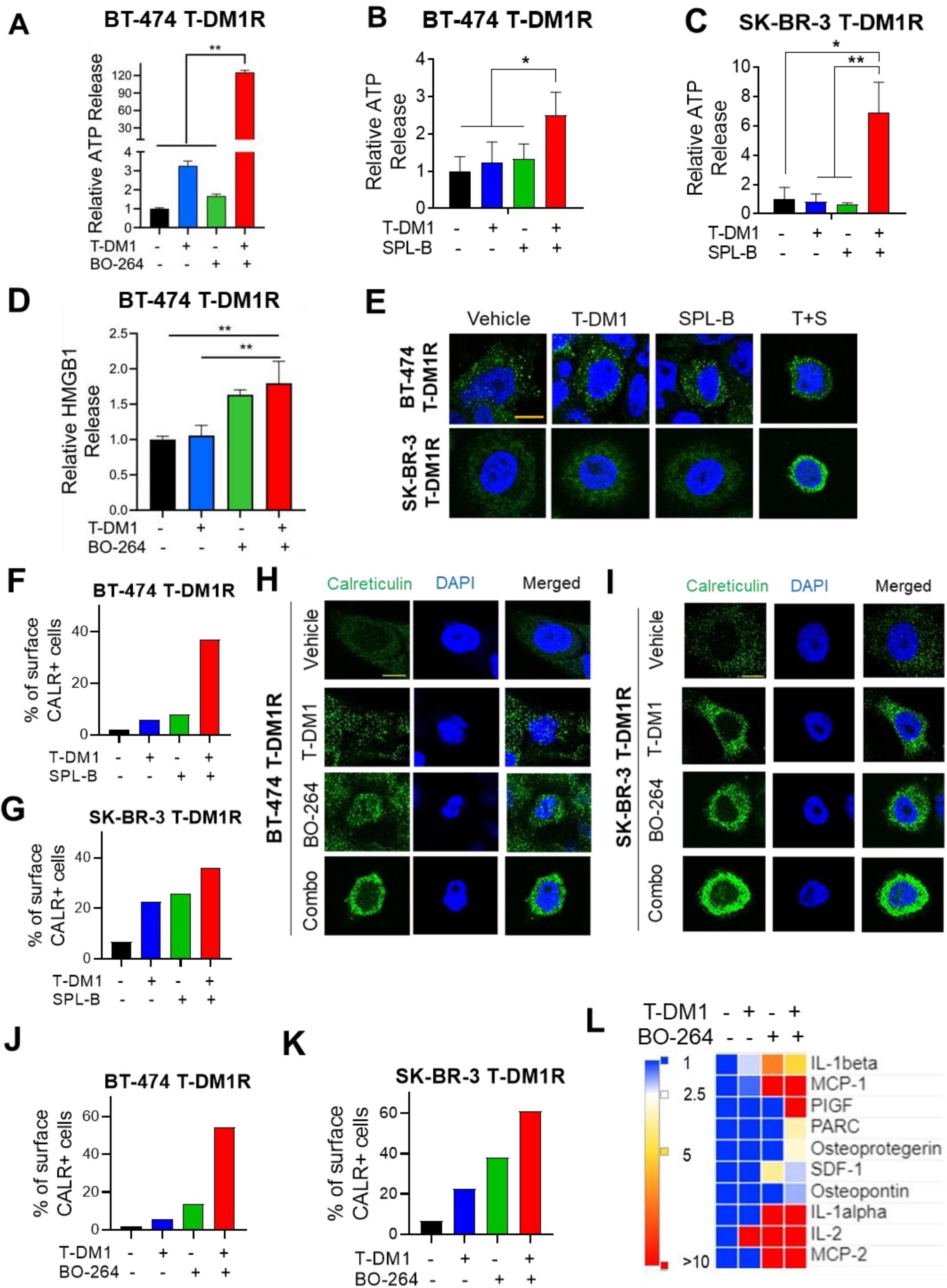


Supplementary Figure S7. T-DM1 induces APC/Cdc20-mediated TACC3 degaradation. A Western blot analysis of TACC3 in SK-BR-3 and BT-474 WT cells treated with T-DM1. **B** Western blot analysis of TACC3 in DM1-treated BT-474 and MDA-MB-157 cells. **C** Western blot analysis of TACC3 in TDM1-treated SK-BR-3 cells transfected with 2 different CDC20 siRNAs. **D** Relative band intensity from C, showing average of two independent experiments. **E** Western blot analysis of CDC20 to confirm siRNA-mediated knockdown. Data correspond to mean values ± standard deviation (SD). *P*-values are calculated with the unpaired, two-tailed Student's t test. *, $P<0.05$.

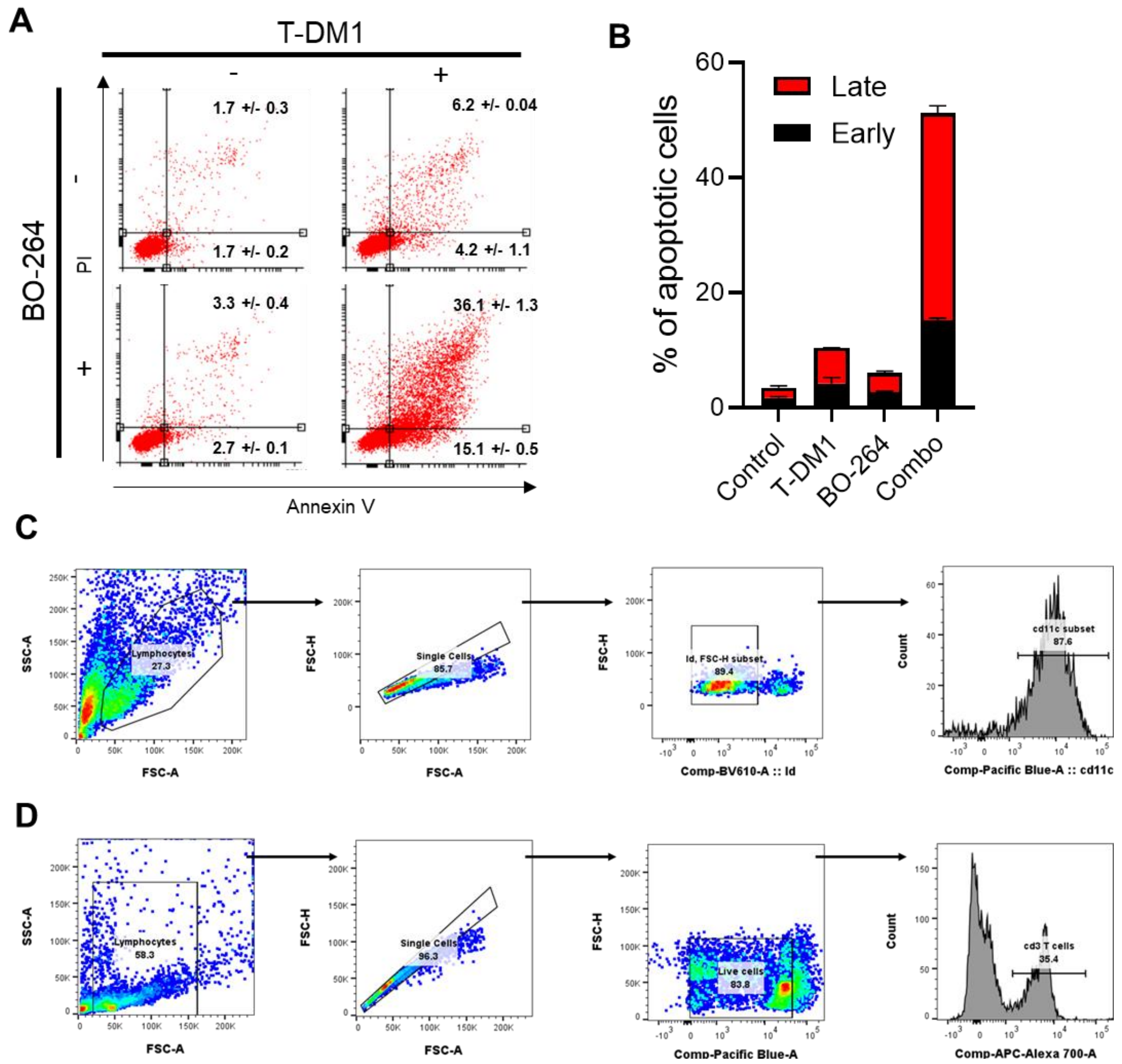


Supplementary Figure S8. TACC3 inhibition overcomes T-DM1 resistance in a SAC-dependent manner. **A** Percent growth inhibition in BT-474 T-DM1R cells transfected with siTACC3 and treated with 15 µg/mL T-DM1 (n=4-6). **B** Percent growth inhibition in BT-474 T-DM1R cells treated with T-DM1 alone or in combination with 1 µM TACC3 inhibitor (BO-264) (n=4-6). **C** Percentage growth inhibition in BT-474 and SK-BR-3 T-DM1R cells treated with T-DM1 alone or combination with 1.3 µM and 1.1 µM

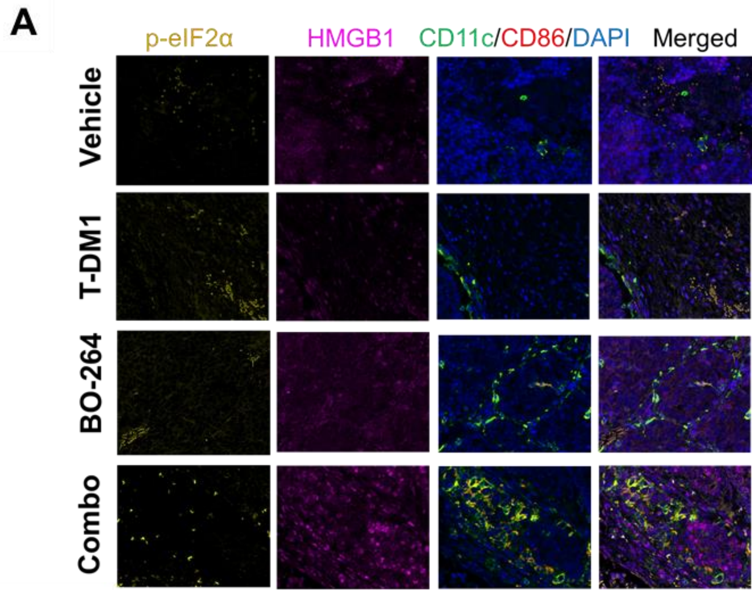
of the TACC3 inhibitor, SPL-B, respectively (n=3). **D** IF staining of α-tubulin (green) in SK-BR-3 T-DM1R cells treated with T-DM1 or SPL-B alone or combination. Scale bar=10 µm. **E** Microtubule polymerization assay in SK-BR-3 T-DM1R cells treated with T-DM1 or SPL-B alone or combination. Scale bar=10 µm. **F** Western blot analysis of mitotic arrest, apoptosis and ICD markers in T-DM1R cells treated with T-DM1 alone or in combination with SPL-B. **G** Percentage cell viability in BT-474 and SK-BR-3 T-DM1R cells treated with combination therapy with or without TC Mps1 (n=4, 5). Data correspond to mean values ± standard deviation (SD). *P*-values are calculated with the unpaired, two-tailed Student's t test. **, $P<0.01$.

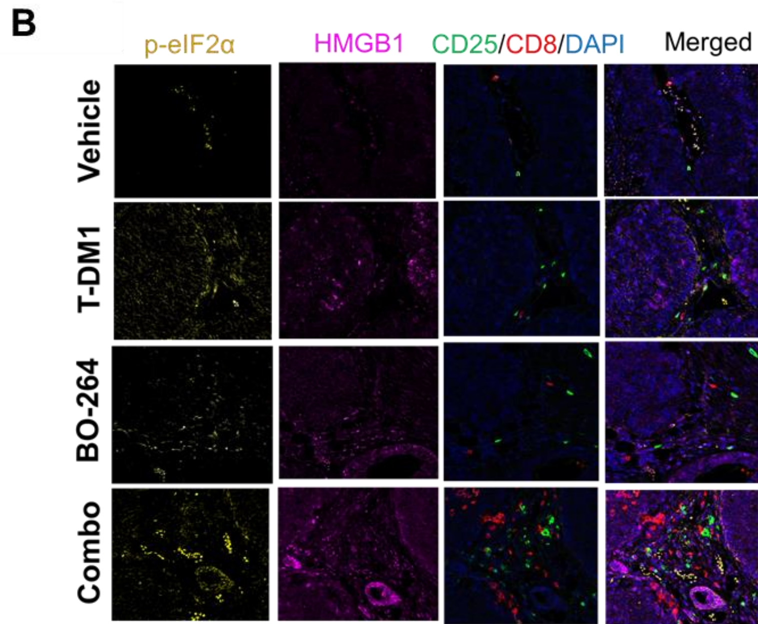

A
BT-474 T-DM1R
Relative ATP Release
T-DM1
BO-264
B
BT-474 T-DM1R
Relative ATP Release
T-DM1
SPL-B
C
SK-BR-3 T-DM1R
Relative ATP Release
T-DM1
SPL-B
D
BT-474 T-DM1R
Relative HMGB1 Release
T-DM1
BO-264
E
Vehicle
T-DM1
SPL-B
T+S
BT-474 T-DM1R
SK-BR-3 T-DM1R
F
BT-474 T-DM1R
% of surface CALR+ cells
T-DM1
SPL-B
G
SK-BR-3 T-DM1R
% of surface CALR+ cells
T-DM1
SPL-B
H
Calreticulin
DAPI
Merged
BT-474 T-DM1R
Vehicle
T-DM1
BO-264
Combo
I
Calreticulin
DAPI
Merged
SK-BR-3 T-DM1R
Vehicle
T-DM1
BO-264
Combo
J
BT-474 T-DM1R
% of surface CALR+ cells
T-DM1
BO-264
K
SK-BR-3 T-DM1R
% of surface CALR+ cells
T-DM1
BO-264
L
T-DM1
BO-264
1
2.5
5
>10
IL-1beta
MCP-1
PIGF
PARC
Osteoprotegerin
SDF-1
Osteopontin
IL-1alpha
IL-2
MCP-2

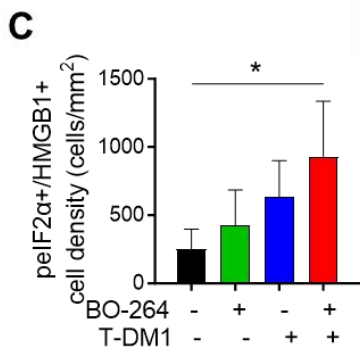
Supplementary Figure S9. Inhibition of TACC3 in combination with T-DM1 restores ICD markers in T-DM1R cells *in vitro*. A Relative ATP release from BT-474 T-DM1R cells treated with T-DM1 alone or in combination with BO-264 (n=4). **B, C** Relative ATP release from BT-474 (B) and SK-BR-3 (C) T-DM1R cells treated with T-DM1 alone or in combination with SPL-B (n=3, 4). **D** Relative HMGB1 release from BT-474 T-DM1R cells treated with T-DM1 alone or in combination with BO-264 (n=3). **E** IF cell surface staining of calreticulin (green) in T-DM1R cells treated with T-DM1 alone or in combination with SPL-B. Scale bar=10 μm. **F, G** The quantification of percentage of surface CALR-positive cells from E. **H, I** IF cell surface staining of calreticulin (green) in T-DM1R cells treated with T-DM1 alone or in combination with BO-264. Scale bar=10 μm. **J, K** The quantification of percentage of surface CALR-positive cells from H, I. **L** Cytokine array analysis showing relative changes in secreted cytokines among single agent- and combination-treated SK-BR-3 T-DM1R cells. Data correspond to mean values ± standard deviation (SD). *P*-values are calculated with the unpaired, two-tailed Student's t test. *, $P<0.05$; **, $P<0.01$.

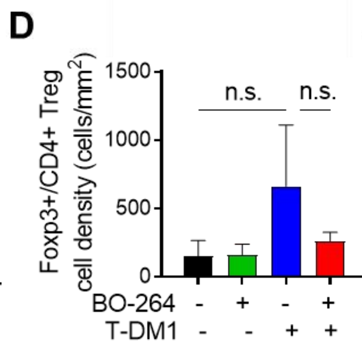


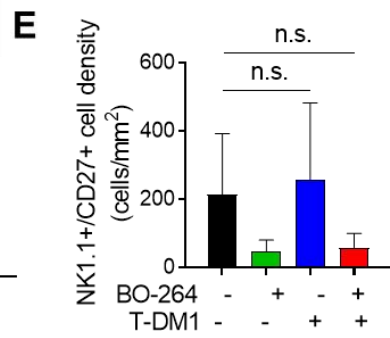
Supplementary Figure S10. T-DM1 combination with BO-264 induces apoptosis in T-DM1 resistant EMT6.huHER2 murine mammary tumor cell line with human HER2, and the gating strategy. A, B Annexin V/PI staining of EMT6.huHER2 cells treated with T-DM1 or BO-264 or their combination (A) and its quantification (B) (n=2). **C, D** Gating strategy for CD80/CD86 (C) and CD8/CD25 double positive cells (D) for flow cytometry analysis. Data for B correspond to mean values ± standard deviation (SD).


A
p-eIF2α
HMGB1
CD11c/CD86/DAPI
Merged
Vehicle
T-DM1
BO-264
Combo


B
p-eIF2α
HMGB1
CD25/CD8/DAPI
Merged
Vehicle
T-DM1
BO-264
Combo


C
peIF2α+/HMGB1+
cell density (cells/mm2)
*
1500
1000
500
0
BO-264 - + - +
T-DM1 - - + +


D
Foxp3+/CD4+ Treg
cell density (cells/mm2)
n.s.
n.s.
1500
1000
500
0
BO-264 - + - +
T-DM1 - - + +


E
NK1.1+/CD27+ cell density
(cells/mm2)
n.s.
n.s.
600
400
200
0
BO-264 - + - +
T-DM1 - - + +

Supplementary Figure S11. Multiplex staining of ICD markers and immune cells in MMTV.f.huHER2#5 tumors. A, B Multiplex staining of ICD markers (p-eIF2α and HMGB1) together with DC maturation markers (A) or T cell activation markers (B). **C** Quantification of p-eIF2α and HMGB1-positive cells. **D, E** Quantification of Treg (D) and NK (E) cell densities within MMTV.f.huHER2#5 tumors from mice treated with T-DM1 alone or in combination with BO-264 (n=3). Data correspond to mean values ± standard deviation (SD). Significance was calculated with the unpaired, two-tailed Student's t test. *, $P<0.05$; n.s., not significant.

Supplementary Tables

Supplementary Table S1. Patient characteristics of the Hacettepe cohort (provided as a separate excel file).

Supplementary Table S2. Sequences of the siRNAs used.

Gene Name	NCBI Gene ID	Company	Catalog number	sequence
TACC3	10460	Dharmacon	D-004155-02-0005	GAGCGGACCUGUAAAACUA
TACC3	10460	Dharmacon	D-004155-03-0005	GAACGAAGAGUCACUGAAG
TACC3	10460	Dharmacon	D-004155-04-0005	UCUCUUAGGUGUCAUGUUC
CDC20	991	Dharmacon	D-003225-10-0002	GCACAGUUCGCGUUCGAGA
CDC20	991	Dharmacon	D-003225-12-0002	GGGCCGAACUCCUGGCAAA

Supplementary Table S3. List of antibodies used in Western blot (WB), immunofluorescence (IF) and flow cytometry (FC) experiments.

Antibody	Provider	Catalog number	WB dilution	IF dilution	FC dilution
Beta-actin	Santa Cruz	sc-8432	1:10000	-	-
TACC3	Santa Cruz	sc-376883	1:1000	-	-
PARP	Cell Signaling	9542S	1:1000	-	-
eIF2α	Santa Cruz	sc-133132	1:1000	-	-
Phospho-eIF2α (Ser51)	Cell Signaling	9721	1:1000	1:100	-
Phospho-Histone H3 (Ser10)	Cell Signaling	9701	1:1000	-	-
Cleaved caspase 3	Cell Signaling	9664	1:1000	-	-
Cleaved PARP	Cell Signaling	5625	1:1000	-	-
PARP	Cell Signaling	9542	1:1000	-	
Cyclin B1	Cell Signaling	4138	1:1000	-	-
CDC20	Cell Signaling	4823	1:1000	-	-
HMGB1	ThermoScientific	PA1-16926	-	1:50	-
CD11c	Cell Signaling	97585	-	1:50	-
CD86	Cell Signaling	19589	-	1:50	-
CD27	Abcam	ab175403	-	1:100	-
NK 1.1	Cell Signaling	39197	-	1:50	-
CD4	Abcam	ab183685	-	1:100	-
CD8	Cell Signaling	98941	-	1:50	-
Foxp3	Cell Signaling	12653	-	1:50	-
CD25	Cell Signaling	39475	-	1:50	-
CD3	Cell Signaling	85061	-	1:50	-
Calreticulin (Calregulin)	Santa Cruz	sc-373863	-	1:500	-
Alpha-tubulin	Santa Cruz	sc-32293	-	1:1000	-
Anti-mouse IgG	Cell Signaling	7076	1:3000	-	-
Anti-rabbit IgG	Cell Signaling	7074	1:3000	-	-

Alexa Fluor® 488 anti-mouse	Life Technologies	A-11001	-	1:1000	-
Alexa Fluor® 647 anti-rabbit	Life Technologies	A-31573	-	1:1000	-
DAPI	Life Technologies	D1306	-	1:5000	-
CD3 (Alexa Alexa Fluor® 488)	Biolegend	100321	-	-	1:500
CD8 (PE/Cy7)	Biolegend	100722	-	-	1:500
CD80 (APC)	eBioscience	17-0801-82	-	-	1:500
CD86 (PE/Cy7)	Biolegend	105103	-	-	1:500
CD25 (Brilliant Violet 510)	Biolegend	102042	-	-	1:500

Supplementary References

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2. Saatci, O., Borgoni, S., Akbulut, O., Durmus, S., Raza, U., Eyupoglu, E., et al. Targeting PLK1 overcomes T-DM1 resistance via CDK1-dependent phosphorylation and inactivation of Bcl-2/xL in HER2-positive breast cancer. *Oncogene* 2018;37:2251-2269.
3. Vichai, V.,Kirtikara, K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc* 2006;1:1112-1116.
4. Lin, S.C., Haga, K., Zeng, X.L.,Estes, M.K. Generation of CRISPR-Cas9-mediated genetic knockout human intestinal tissue-derived enteroid lines by lentivirus transduction and single-cell cloning. *Nat Protoc* 2022;17:1004-1027.