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Artificial diets with selective restriction of amino acids and very low levels of lipids induce anticancer activity in mice with metastatic triple-negative breast cancer

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# These authors contributed equally to this work

1. SUPPLEMENTARY FIGURES AND TABLES

Citation: Guillén-Mancina, E.; Jiménez-Alonso, J.J.; Calderón-Montaño, J.M.; Jiménez-González, V.; Ortega-Díaz, P; Burgos-Morón, E.; López-Lázaro, M. Artificial diets with selective restriction of amino acids and very low levels of lipids induce anticancer activity in mice with metastatic triple-negative breast cancer. *Cancers* **2022**,

Academic Editor: Firstname Lastname

Received: date
Accepted: date
Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Table S1. Composition of artificial media. Concentrations in M0 (complete medium) and M1 (selective AA restricted medium) are shown in mg/L. Both artificial media were prepared from scratch in our laboratory. All solid ingredients were mixed in sterile water and sterilized by filtration through 0.2 μm membrane filters. Media were supplemented with FBS and penicillin/streptomycin.

Compound	**M0**	**M1**
Calcium chloride dihydrate	265	265
Magnesium sulfate	98	98
Ferric (III) nitrate	0.1	0.1
Potassium chloride	400	400
Sodium phosphate monobasic	109	109
Sodium chloride	6400	6400
D-glucose	4500	4500
Choline chloride	4	4
D -pantothenic acid hemicalcium salt	4	4
Folic acid	4	4
Nicotinamide	4	4
Pyridoxine hydrochloride	4	4
Thiamine hydrochlorie	4	4
Myo-inositol	7.2	7.2
D-biotin	0.2	0.2
Riboflavin	0.4	0.4
vitamin B12	0.005	0.005
Sodium bicarbonate	3700	3700
L-phenylalanine	192	192
L-histidine	76	76
L-lysine	235	235
L-threonine	160	160
L-Isoleucine	95	95
L-valine	235	235
L-leucine	533	533
L-tryptophan	21	21
L-methionine	53	53
L-glutamine	533	533
L-arginine	134	-
Glycine	90	-
L-alanine	90	-
L-aspartic acid	178	-
L-serine	42	-
L-tyrosine	90	-
L-cystine dihydrochloride	64	-
L-asparagine-1-hydrate	50	-
L-glutamic acid	20	-
L-proline	20	-

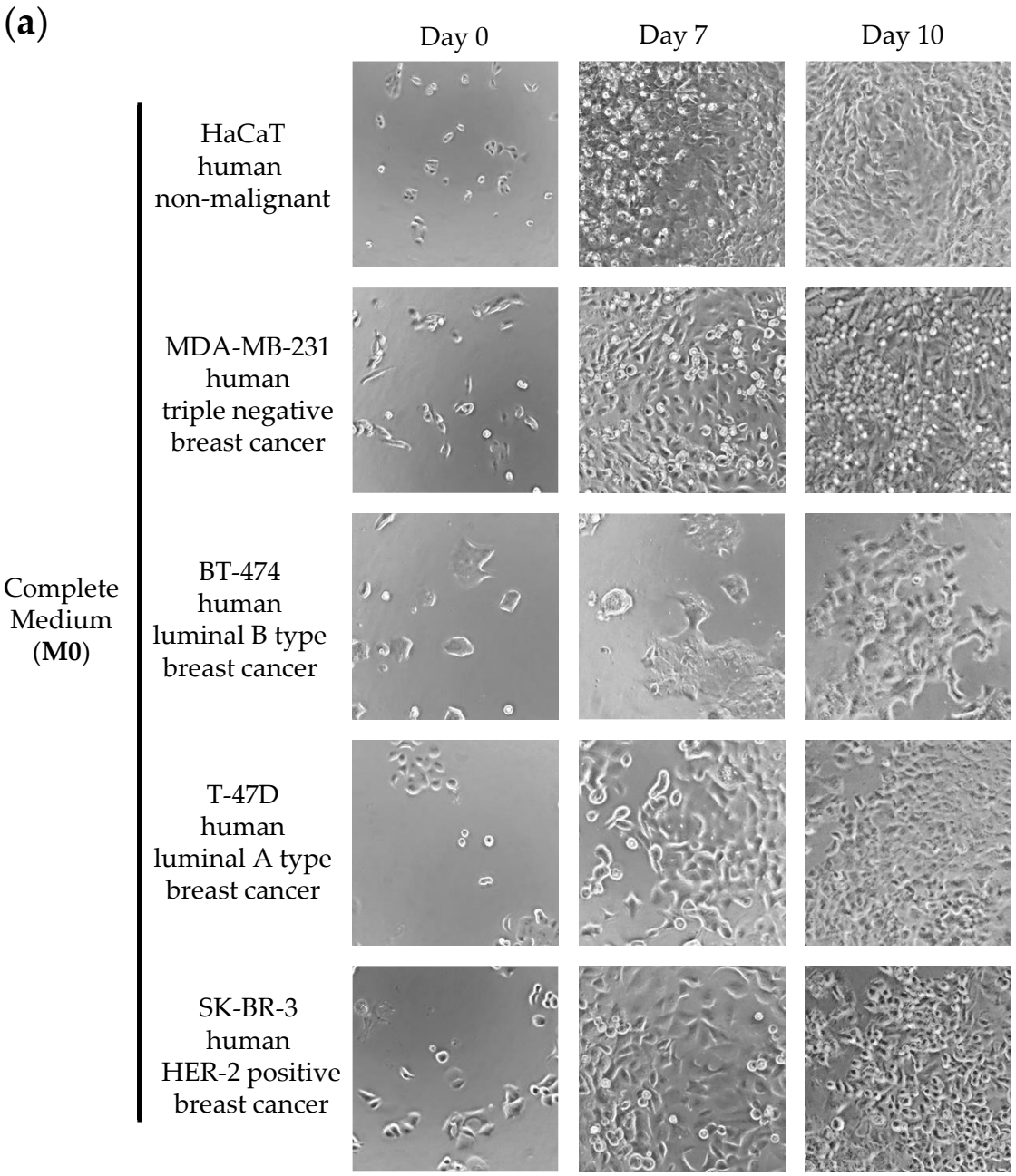

Figure S1. Evaluation of amino acid restriction on breast cancer cells and non-malignant cells. Cells were grown in a complete medium (**M0**) or in a medium lacking 10 AAs (**M1**) for 7 days followed by 3 days of recovery in DMEM medium. T-47D cells were cultured in RPMI 1640. Cells were monitored by microscopic visualization and photographed on days 7 and 10. Representative photographs at 10x magnification are shown. Cell viability was estimated with the resazurin assay on days 7 and 10. The percentage of cell viability is shown at the bottom right of the photographs when it was less than 20%. The detailed composition of M0 (**a**) and M1 (**b**) is shown in Table S1.

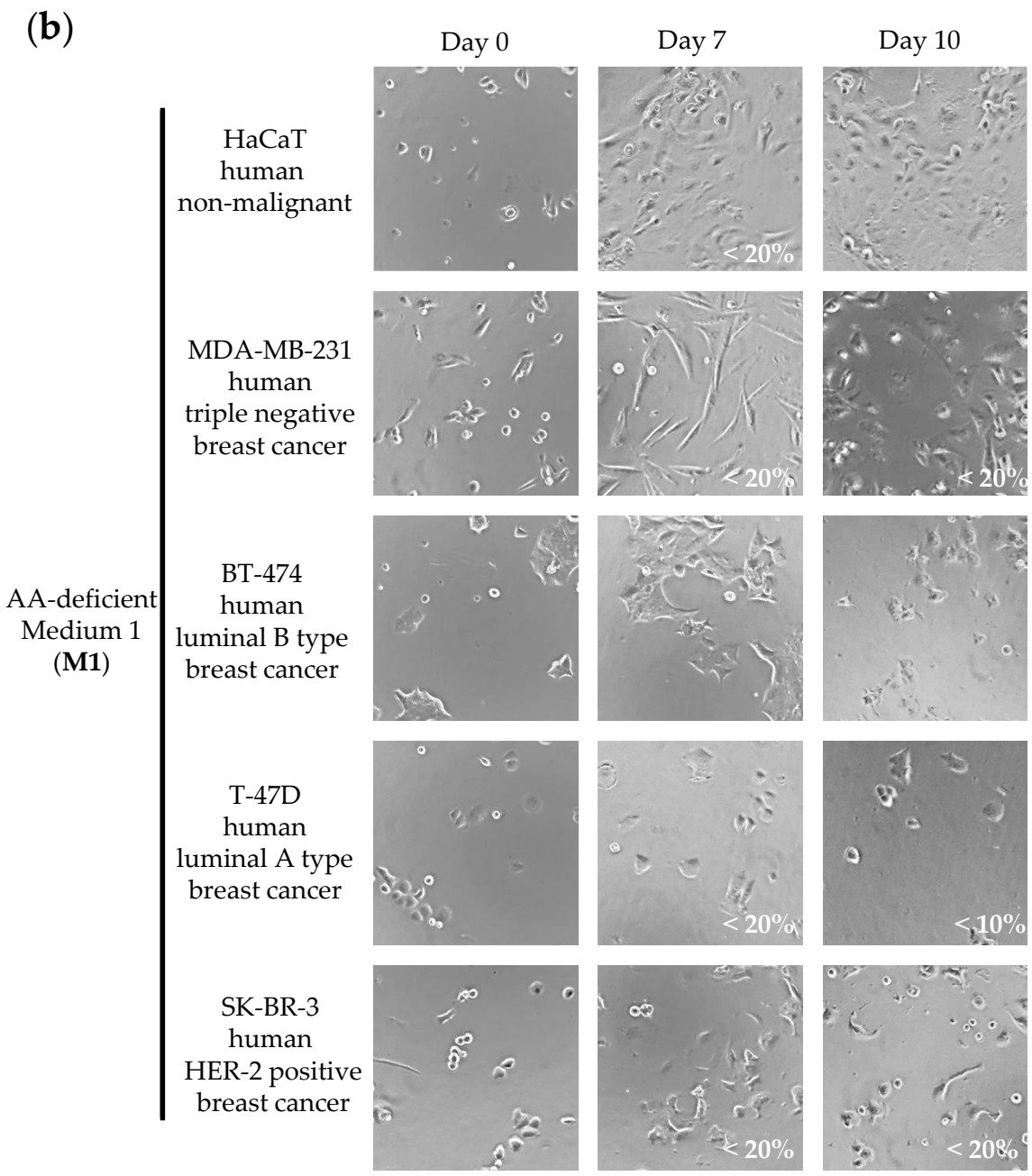

Figure S1 (cont). Evaluation of amino acid restriction on breast cancer cells and non-malignant cells. Cells were grown in a complete medium (**M0**) or in a medium lacking 10 AAs (**M1**) for 7 days followed by 3 days of recovery in DMEM medium. T-47D cells were cultured in RPMI 1640. Cells were monitored by microscopic visualization and photographed on days 7 and 10. Representative photographs at 10x magnification are shown. Cell viability was estimated with the resazurin assay on days 7 and 10. The percentage of cell viability is shown at the bottom right of the photographs when it was less than 20%. The detailed composition of M0 (**a**) and M1 (**b**) is shown in Table S1.

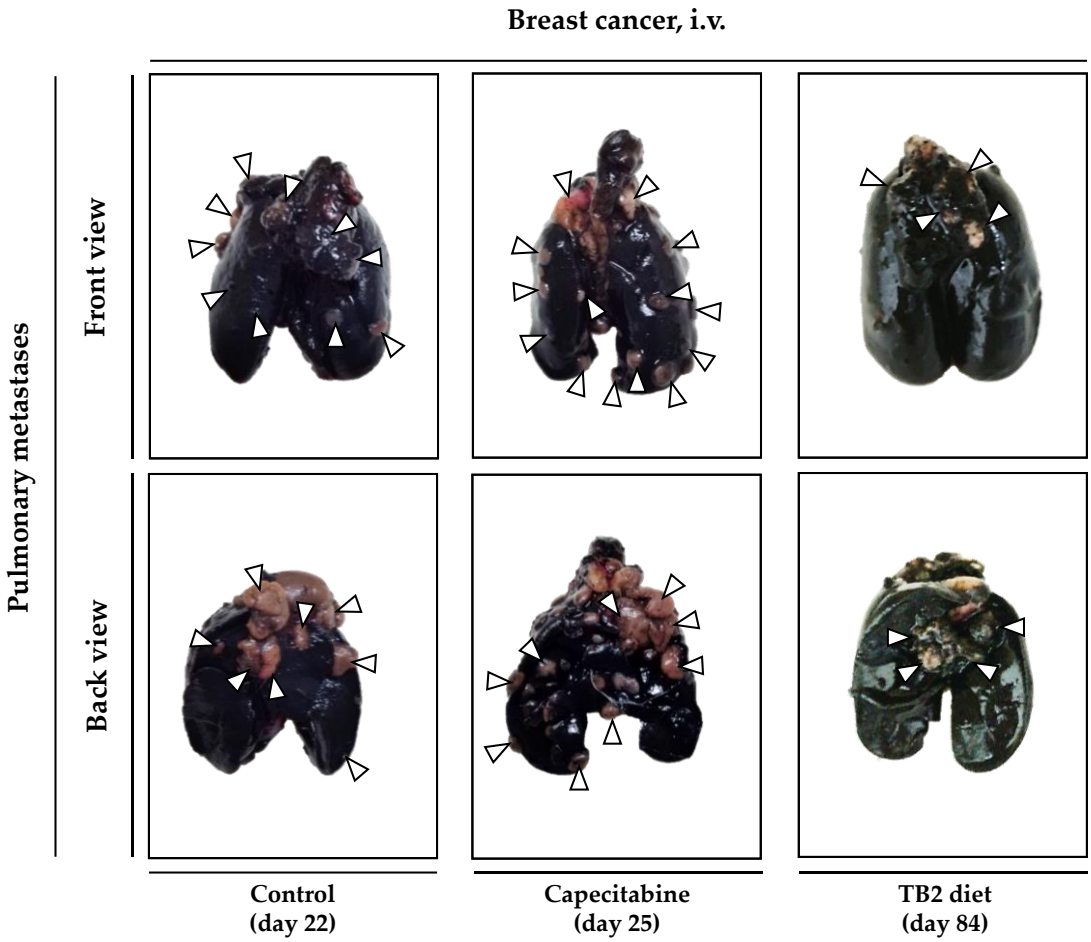

Figure S2. Lung photographs at the time of sacrifice of mice with TNBC treated with capecitabine or diet TB2. In this model, 100 000 4T1 murine breast cancer cells were inoculated in the tail vein of immunocompetent BALB/c mice. After 8 days, mice were treated with capecitabine (450 mg/kg/day, 7/7 schedule, 3 cycles), with diet TB2 (normal diet was replaced by diet TB2 for 4 weeks), or were left untreated (control, normal diet). Mice were euthanized by cervical dislocation when signs of disease progression were apparent. After sacrifice, lungs were excised and stained with India ink (tumors show a white appearance and normal lung parenchyma appears black). Each mouse was sacrificed at different time points, when symptoms of advanced disease were patent. The day of sacrifice is shown in brackets.

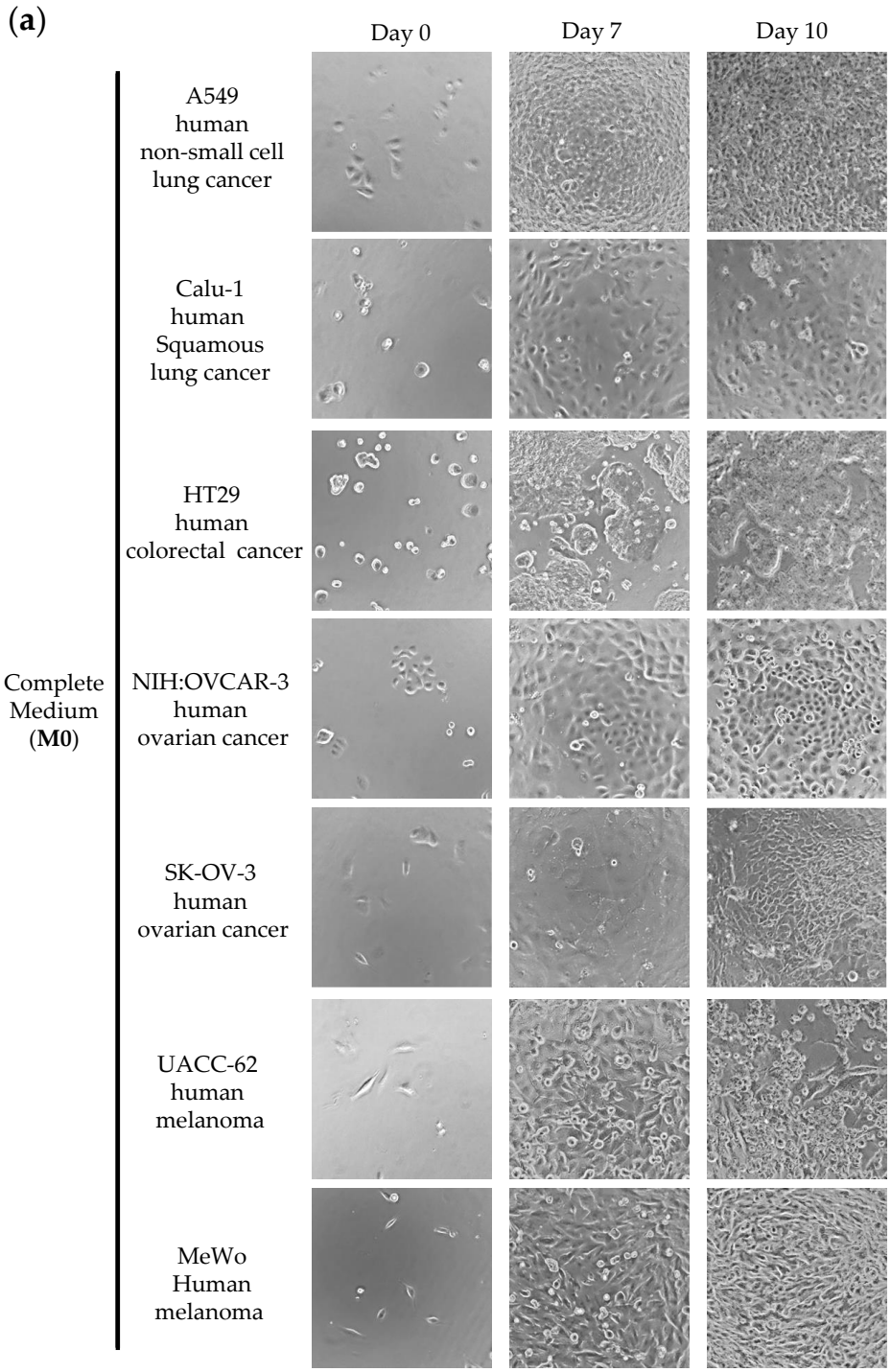

Figure S3. Evaluation of amino acid restriction on a panel of human cancer cell lines of different tissue origin. Cells were grown in a complete medium (**M0**) or in a medium lacking 10 AAs (**M1**) for 7 days followed by 3 days of recovery in DMEM medium. Calu-1, NIH:OVCAR-3 and UACC-62 cells that were cultured in RPMI 1640. Cells were monitored by microscopic visualization and photographed on days 7 and 10. Representative photographs at 10x magnification are shown. Cell viability was estimated with the resazurin assay on days 7 and 10. The percentage of cell viability is shown at the bottom right of the photographs when it was less than 20%. The detailed composition of M0 (**a**) and M1 (**b**) is shown in Table S1.

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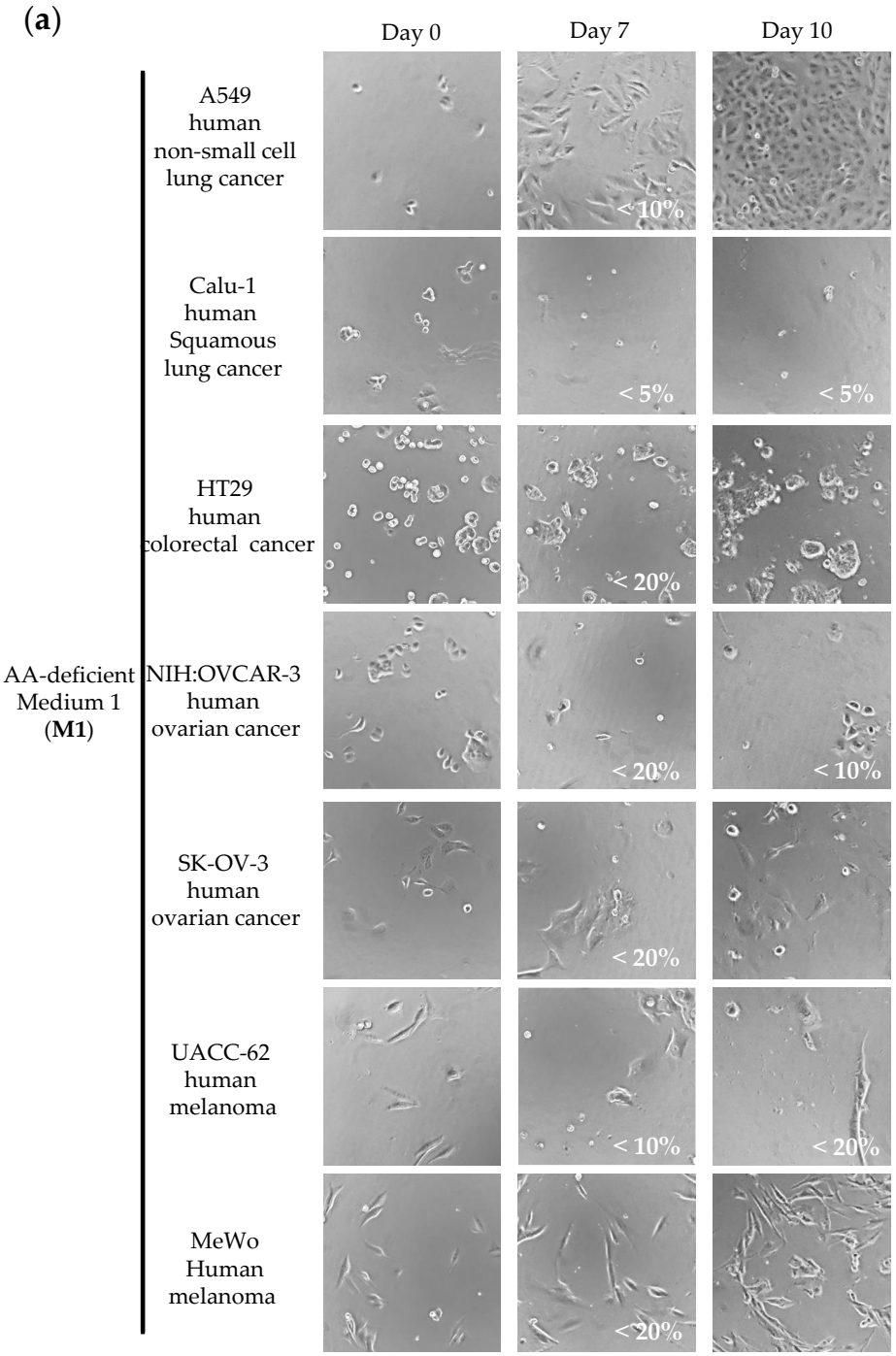

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73 **Figure S3 (cont.).** Evaluation of amino acid restriction on a panel of human cancer cell lines of dif-
74 ferent tissue origin. Cells were grown in a complete medium (**M0**) or in a medium lacking 10 AAs
75 (**M1**) for 7 days followed by 3 days of recovery in DMEM medium. Calu-1, NIH:OVCAR-3 and
76 UACC-62 cells that were cultured in RPMI 1640. Cells were monitored by microscopic visualization
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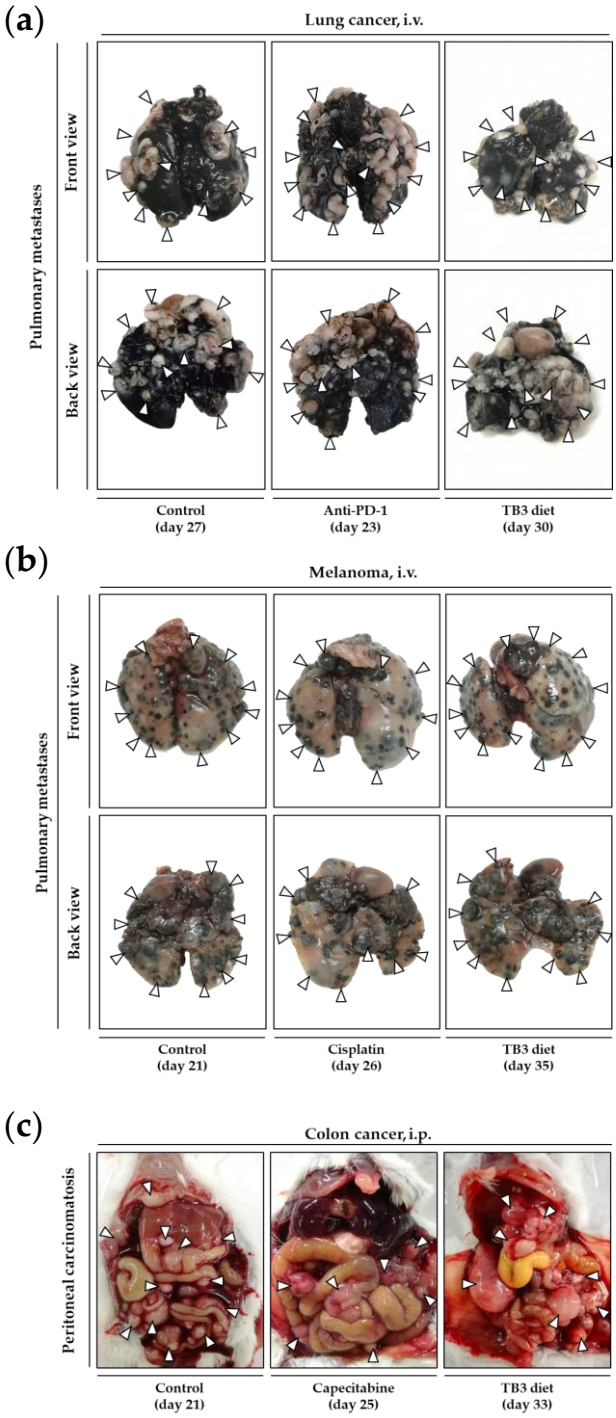

Figure S4. Photographs at the time of sacrifice of mice with different types of metastatic cancers treated with diet TB3. In these models, mice were treated with diet TB3 (normal diet was replaced by diet TB3 for 6 weeks), with a standard anticancer drug, or were left untreated (control, normal diet). In the lung cancer model (**a**), the lungs were excised and stained with India ink (tumors show a white appearance and normal lung parenchyma appears black). In the melanoma model (**b**), representative unstained lung photographs are shown (tumors show a black appearance due to their high production of melanin). In the peritoneal colon cancer model (**c**), representative photographs of the peritoneal cavity are shown. The day of sacrifice is shown in brackets.

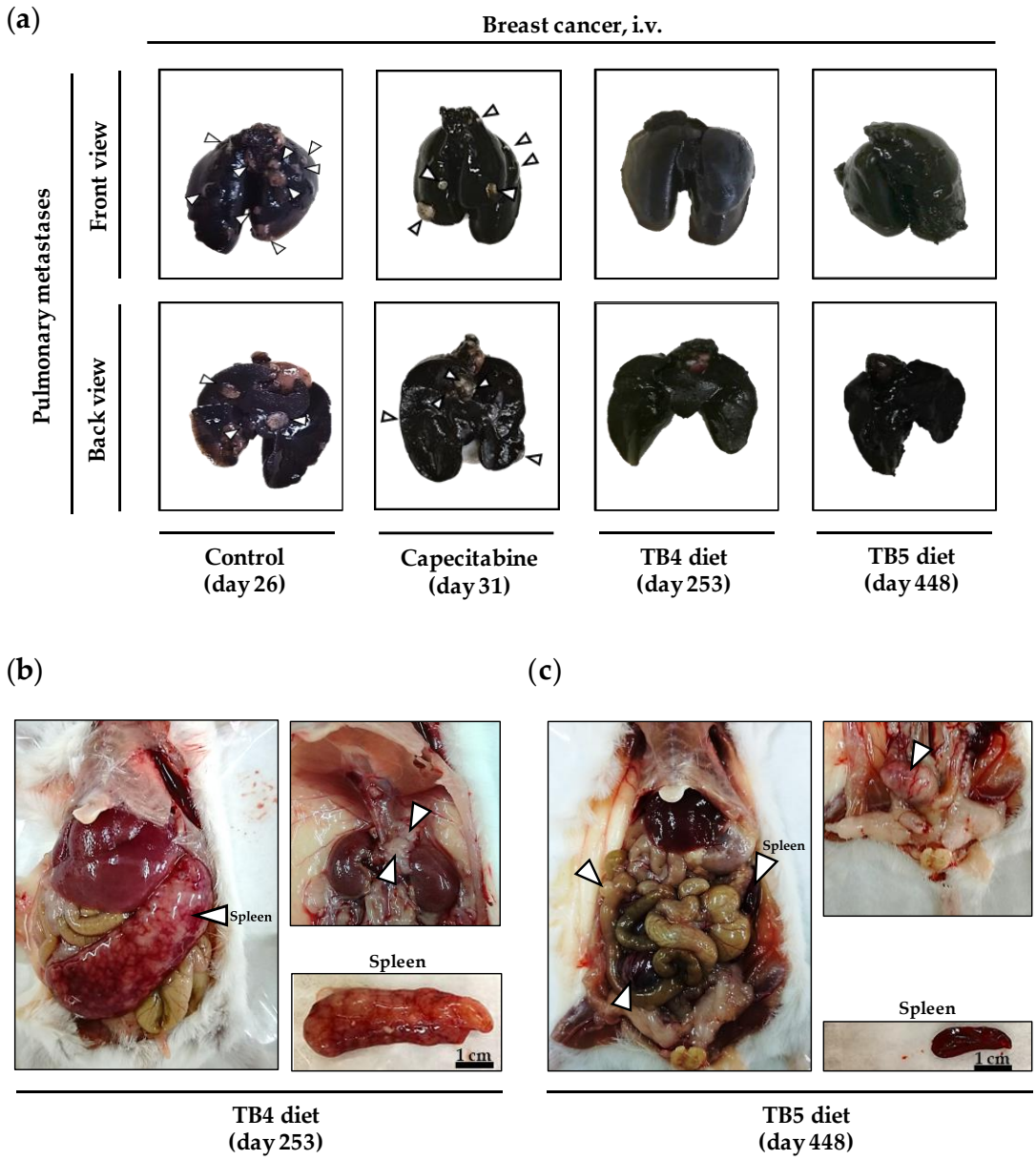

Figure S5. Autopsy images of mice treated with diets TB4 and TB5. In this model, 100 000 4T1 murine breast cancer cells were inoculated in the tail vein of immunocompetent BALB/c mice. After 8 days, mice were treated with capecitabine (450 mg/kg/day, 7/7 schedule, 3 cycles), with diet TB4 or TB5 (normal diet was replaced by TB4 or TB5 for 4 weeks), or were left untreated (control, normal diet). Mice were sacrificed at different time points, when symptoms of advanced disease were patent. The day of sacrifice is shown in brackets. (**a**) Lung photographs at the time of sacrifice of mice with TNBC. After sacrifice, lungs were excised and stained with India ink (tumors show a white appearance and normal lung parenchyma appears black). (**b**) Photographs of the peritoneal cavity and spleen of mice treated with diet TB4 that was sacrificed on day 253. (**c**) Photographs of the peritoneal cavity and spleen of mice treated with diet TB5 that was sacrificed on day 448. See main text for details.