NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	lines 157-166, page 6 a single animal
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	This study includes two distinct in vivo experiments using female NMRI-nu mice (8 weeks old). For the OXPHOS inhibition therapeutic study, 8 mice were used in total: 4 mice allocated to the vehicle control group and 4 mice to the Ama-treated group (n = 4 per group). For the chemoprevention study, 10 mice were used in total: 5 mice allocated to the vehicle control group and 5 mice to the prevention group (n = 5 per group). The total number of animals used across both experiments was 18. All animals were female due to the biological relevance of modeling breast cancer. The total number of experimental units (animals) in this specific experiment was 8 (4 No a priori sample size calculation was performed. The sample sizes (n = 4 per group for the therapeutic study and n = 5 per group for the chemoprevention study) were determined based on our laboratory's extensive experience (PMID: 30373788) with subcutaneous triple-negative breast cancer (TNBC) xenograft models, published literature for metabolic and prevention interventions, and the ethical principle of minimizing animal usage (Reduction) while maintaining sufficient statistical power to detect
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Inclusion and exclusion criteria were established a priori. For the therapeutic study, mice were included only if they successfully developed palpable subcutaneous tumors reaching a volume of ~100 mm³ prior to treatment initiation. For the chemoprevention study, all mice successfully undergoing early intervention before tumor implantation were included. Data points would be excluded only in cases of technical failure during measurement, premature death due to non-tumor-related causes, or if an There were no animal or data point exclusions in either of the two independent in vivo experiments. All allocated animals (4+4 in the therapeutic study and 5+5 in the chemoprevention study) successfully completed the study timeline, and 100% of the collected data points For all therapeutic analyses (tumor growth curves, body weight, and final tumor weight), the exact value of n was 4 for the vehicle group and 4 for the Amx group. For all chemoprevention analyses (tumor initiation delay, tumor volume monitoring, and final tumor weight), the exact value of n was 5 for the control group and 5 for the prevention group. This information is explicitly stated in the
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Randomisation was strictly used to allocate animals to control and treatment groups. For the therapeutic study, mice were randomised after tumors reached ~100 mm³ using a block stratification method based on baseline tumor volumes; this ensured that the average tumor size was equal across all groups at the start of treatment. For the chemoprevention study, healthy 8-week-old mice were randomly assigned to control or prevention groups prior to To minimise potential confounding factors, cages containing control and treated animals were distributed systematically and equally across different shelves of the holding racks to control for variations in light intensity, temperature, and humidity. Furthermore, during bi-weekly monitoring, the order of treatments, tumor volume measurements, and body weight recordings was alternated and randomised across cages each time, ensuring that measurements for one specific group were
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Blinding was partially applied during the study. The principal investigator who performed the animal handling and bi-weekly physical tumor measurements (caliper monitoring) was aware of the group allocations. However, to eliminate bias, the primary raw data (three-dimensional tumor measurements) were handed over to a co-author (PhD student) who independently processed the raw values, calculated the volumes using two alternative methodologies, and generated the final data plots shown in the Supplementary figures, without direct involvement in the
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	The following outcome measures were assessed in this study: 1) In vivo tumor growth dynamics, first calculated 3) digitally by measuring three-dimensional tumor volumes using caliper measurements, 2) tumor growth inhibition and relative body weight measurements, 3) Long-term preventive efficacy (in the chemoprevention study), assessed by tracking tumor initiation delay and tumor-free survival, 4) Ex vivo tumor burden, determined by measuring the final mass (weight) of excised tumors at the end of the study, 5) Methodological consistency., verified by independent processing of raw caliper values using two alternative mathematical volume formulas (results detailed in The primary outcome measure used to evaluate the hypothesis and determine the validity of the experimental groups was the longitudinal change in tumor volume (tumor growth rate) over the course of the treatment and prevention periods. Secondary outcome measures included changes in body weight (safety profile) and the final post-excision tumor
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Statistical analyses were performed using GraphPad Prism 8.0.1 (GraphPad Software). For in vivo tumor growth dynamics (both therapeutic and chemoprevention studies), longitudinal tumor volume data were analyzed using two-way ANOVA followed by Sidak's multiple-comparison post hoc test. Final excised tumor weights were analyzed using an unpaired Student's t-test. To ensure methodological transparency, statistical analyses were conducted and plotted for tumor volumes calculated by both three-dimensional and two-dimensional geometric formulas, with both bar diagrams and respective p-values included in the Supplementary data. Before applying parametric tests (two-way ANOVA and Student's t-test), the assumption of normal distribution was assessed for all groups using the Shapiro–Wilk normality test in GraphPad Prism. Homogeneity of variances was evaluated using Brown-Forsythe or F-tests. The data met the assumptions for parametric analysis (normally distributed with similar variances); therefore, no non-parametric transformations or alternative tests were required.
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Species: Mouse (Mus musculus). Strain: NMRI-nu (athymic nude mice). Sex: Female (strictly selected due to the biological relevance to human breast cancer modeling). Age at the start of the study: 8 weeks old. Average body weight of the animals at the time of tumor cell implantation was approximately 22–24 grams. Provenance: The animals were obtained from an authorized commercial breeder (The Jackson Laboratory). Immune status: Athymic, T-cell deficient immunodeficient mice, making them suitable for human tumor cell xenografting. Genetic modification status: Spontaneous genetic mutation (Foxn1nu) resulting in hairlessness and lack of a functional thymus. The animals were
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Human triple-negative breast cancer stem-like cells (1 × 10⁶ cells) were suspended in PBS and mixed with Matrigel (1:1 volume ratio) for enhanced engraftment. Mice received subcutaneous injections into the right flank of female NMRI-nu mice. For drug administration, the OXPHOS inhibitor IACS was dissolved in the animals' drinking water to achieve a final target dose of 100 mg/kg. The control groups (both therapeutic and chemoprevention) received standard drinking water (vehicle) without any pharmacological additives. To minimize animal suffering and ensure ethical compliance at the end of the experimental endpoint, all animals were humanely euthanized by CO2 inhalation in strict accordance with institutional guidelines, and tumors were surgically excised using standard sterile The timeline differed between the two independent experimental units: 1) Therapeutic study (n=4 per group): Treatment with Ama (100 mg/kg) or vehicle via drinking water was initiated only AFTER tumors reached a palpable volume of ~100 mm³. The treatment was administered twice weekly and maintained continuously until the study endpoint. 2) Chemoprevention study (n=5 per group): Treatment with Ama (100 mg/kg) or vehicle was initiated EIGHT (8) DAYS prior to tumor cell implantation and was maintained continuously twice weekly throughout the entire post-injection period to evaluate the delay in tumor initiation. All animal procedures were performed in the specific pathogen-free (SPF) animal facility at Gebze Technical University. Prior to the start of any experimental procedures or cell injections, all mice underwent a mandatory acclimatization period of 7 days following their arrival from the breeder facility. This period allowed the animals to adapt to the local environment and stable housing conditions (12-hour light/dark cycle, controlled temperature of To investigate the therapeutic potential of OXPHOS inhibition in a clinically relevant model, we incorporate a well-established, reproducible, and technically invasive model to treat highly-negative breast tumor (TNBC) growth dynamics in vivo. 2) The inclusion of Matrigel was necessary to provide an extracellular matrix scaffold that supports the initial survival and engraftment of the cancer stem cells. 3) Tumor volume monitoring, twice weekly) was selected based on previously published pharmacokinetic and efficacy data for the specific OXPHOS inhibitor in nude models. 4) The oral administration via drinking water was utilized as a non-invasive method to minimize animal stress and ensure consistent dosing. 5) The chemoprevention model and the pre-implantation chemoprevention model was designed specifically to differentiate the effects of OXPHOS inhibition on already developed tumor masses versus its efficacy during the early
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Summary and descriptive statistics are comprehensively reported for each experimental group across all in vivo studies. For both the therapeutic (n=4 per group) and chemoprevention (n=5 per group) studies, longitudinal data (tumor growth curves and body weight tracking) as well as endpoint metrics (final excised tumor weight) are presented as the mean value ± standard error of the mean (mean ± SEM). The independent replications and technical replicates for the corresponding in vitro screening (mammosphere formation, two-gen co-culture assays, and oxidative stress analysis) are likewise displayed While formal statistical effect sizes (such as Cohen's d) were not explicitly calculated, the magnitude of the therapeutic and preventive efficacy is clearly defined by reporting the exact p-values for all major comparisons and the percentage of tumor growth inhibition (TGI). In the therapeutic model, Ama treatment led to a statistically significant reduction in tumor volume compared to the vehicle group ($P < 0.09$, two-way ANOVA). In the chemoprevention model, the early OXPHOS inhibition resulted in a highly significant delay in tumor initiation and reduction in final tumor burden ($P < 0.01$, two-way ANOVA). Respective p-values and statistical significance thresholds for all individual data points and bar diagrams are explicitly indicated