nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Band intensities of Western Blots were detected by Odyssey® CLx Imaging system (Li-Cor Biosciences) and ImageStudioTM Lite v.5.2.5 software (LI-COR Biosciences).
Data analysis	BWA 0.5.9-r26-dev, MACS2 (v2.1.2), DiffBind (v3.0.15), Rseb (v0.3.2) R-package (https://github.com/sebastian-gregoricchio/Rseb), ChIPseeker (v1.26.2), GIGGLE (http://dbtoolkit.cistrome.org/), deeptools (v2.5.3), bedtools (v2.25.0), HISAT2 (v2.1.0), DESeq2 (v1.30.1), clusterProfiler (v3.18.1), msigdbr (v7.5.1), ggplot2 (v3.3.5), ImageStudio™ Lite v.5.2.5.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All mouse data generated or analysed during this study are included in this published article (and its supplementary information files). The in vitro data with human

cell lines are also available as Source Data. The ChIP-seq and RNA-seq data has been deposited to the GEO database (GSE260486). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE49 partner repository with the dataset identifier PXD049477. BC patients' RNA-seq data are deposited on the European Genome-Phenome Archive (EGA) under accession number EGAS00001004944

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Blood samples and tissue samples from female breast cancer patients were used in this study.
Reporting on race, ethnicity, or other socially relevant groupings	Samples were derived from the IRCCS Ospedale Policlinico San Martino, in Genoa (NCT05748704) and the Fondazione IRCCS Istituto Nazionale dei Tumori, Milano (NCT03454282). Since samples have been pseudonymized, we do not have information about race/ethnicity.
Population characteristics	Female breast cancer patients. Race/ethnicity are not described in the study
Recruitment	IThe NCT05748704 trial was conducted at the IRCCS Ospedale Policlinico San Martino (Genoa), between December 2022 and February 2024 and was approved by the Comitato Etico Regione Liguria. This trial consists of a single-arm phase I/II clinical study of a FMD with solid tumours who are candidates to receive active medical or radiotherapy treatment (or with medical treatment or radiotherapy already ongoing). Inclusion Criteria were as follows: • Written informed consent • Age > 18 years • Patients with solid or hematologic tumors undergoing active treatment, including patients who are preparing to start a new treatment with chemotherapeutic regimens, hormone therapies, other molecularly targeted therapies (including kinase inhibitors), biologics (including trastuzumab) , pertuzumab, cetuximab and bevacizumab) or inhibitors of immune checkpoints (eg Opdivo, Keytruda), ie patients in whom treatment is already underway; • ECOG performance status 0-1 • Adequate organ function • BMI >21 kg/m2 (with possibility to also enroll patients with 19<BMI<21 based on the judgement of the treating physician) • Low nutritional risk according to nutritional risk screening (NRS) Exclusion criteria were as follows: • Age > 65 • Diabetes mellitus; • Previous therapy with IGF-1 inhibitors; • BMI <19 kg/m2; • bioimpedance phase angle <5.0°; • medium/high nutritional risk according to NRS; • Any metabolic disorder that can affect gluconeogenesis or ability to adapt to fasting periods; • Treatment in progress with other experimental therapies. All patients signed an informed consent for participating in the study, as well as for the use of clinical and biological data for research purposes. No bias that can impact on the results applies to this study. The DigesT study (NCT03454282) trial was conducted between July 2018 and December 2020 and in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was approved by the Institutional Review Board (IRB) and the Ethics Committee of Fondazione IRCCS Istituto Nazionale dei Tumori Milan, (INT 157/17). All patients provided written informed consent before any study procedures, as well as for the use of clinical and biological data for research purpose. Patients with resectable breast cancer (cT1N0M0 stage or cT1cN1M0-cT2cN0M0 stages not requiring pre-operative systemic treatment at the judgment of the investigator) who are candidate to curative surgery were enrolled; Were also enrolled patients with melanoma, that were not included in this study. Inclusion Criteria were as follows: • Age ≥ 18 and ≤ 75 years. • Evidence of a personally signed and dated informed consent document (ICD) indicating that the patient has been informed of all pertinent aspects of the study before enrollment and FMD prescription. • Willingness and ability to comply with the FMD protocol, the scheduled visits, treatment plans, laboratory tests and other procedures. • Histologically confirmed diagnosis of invasive breast cancer candidate to curative surgery. • For breast cancer patients, any biological subgroup (including estrogen receptor-positive, HER2-positive, triple-negative breast cancer) will be admitted; HER2-positive tumors will be defined on the basis of an IHC score of 3, or a score of 2 with ISH evaluation indicative of gene amplification. • Availability of archival FFPE tissue blocks of primary breast cancer. • Presence of an Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1. • Presence of adequate bone marrow and organ function as defined by the following laboratory values: • ANC ≥ 1.5 x 109/l • platelets ≥ 100 x 109/l • hemoglobin ≥ 9.0 g/dl • calcium (corrected for serum albumin) within normal limits or ≤ grade 1 according to NCI-CTCAE version 4.03 if not clinically significant • potassium within the normal limits, or corrected with supplements • creatinine < 1.5 ULN

• blood uric acid < 10 mg/dl
• ALT and AST ≤ 2.5 x ULN
• total bilirubin < ULN except for patients with Gilbert syndrome who may only be included in the total bilirubin is < 3.0 x ULN or direct bilirubin < 1.5 x ULN
• Albumin > 3 g/dL
• Fasting glucose ≤ 200 mg/dl.
• Total Cholesterol ≤ 300 mg/dl.
• Triglycerides ≤ 300 mg/dl.

Female patients of childbearing potential must agree to sexual abstinence or to use two highly effective method of contraception throughout the study and for at least 30 days after the end of the FMD. Abstinence is only acceptable if it is in line with the preferred and usual lifestyle of the patient. Examples of contraceptive methods with a failure rate of < 1% per year include tubal ligation, male sterilization, hormonal implants, established, proper use of combined oral or injected hormonal contraceptives, and certain intrauterine devices. Alternatively, two methods (e.g., two barrier methods such as a condom and a cervical cap) may be combined to achieve a failure rate of < 1% per year. Barrier methods must always be supplemented with the use of a spermicide. A patient is of childbearing potential if, in the opinion of the Investigator, she is biologically capable of having children and is sexually active.

Female patients are not of childbearing potential if they meet at least one of the following criteria:
• Have undergone a documented hysterectomy and/or bilateral oophorectomy
• Have medically confirmed ovarian failure
• Achieved post-menopausal status, defined as: (≥ 12 months of non-therapy-induced amenorrhea) or surgically sterile (absence of ovaries) and have a serum FSH level within the laboratory's reference range for postmenopausal females.
Exclusion Criteria:
• Prior systemic treatment for breast cancer.
• Diagnosis of a concurrent malignancy other than breast cancer, or malignancy other than breast cancer diagnosed within 5 years of treatment enrollment, with the exception of adequately treated.
• Body Mass Index (BMI) < 20 Kg/m2.
• Anamnesis of alcohol abuse.
• Unintentional weight loss ≥ 5% in the last three months, unless the patient has a BMI > 25 Kg/m2 at study enrollment. Intentional weight loss is permitted if < 10% in the last three months and patient BMI is > 22 kg/m2.
• Severe heart, liver, pulmonary, kidney comorbidities.
• Current status of pregnancy or lactation, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test (> 5 mIU/mL).
• Active HBV or HCV infection.
• Severe infections within 4 weeks prior to FMD initiation, including, but not limited to, hospitalization for complications of infection, bacteremia, or severe pneumonia.
• Active autoimmune diseases that require systemic treatment (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs).
• History of recent diagnosis of hypothyroidism for which replacement therapy (eg., thyroxine) and blood endocrine profile are not stabilized yet.
• Established diagnosis of diabetes mellitus type I or diabetes mellitus type II that requires pharmacological treatment (including, but not limited to, insulin, insulin secretagogues and metformin).
• Severe impairment of the gastrointestinal (GI) function or GI disease that may alter the digestion and absorption of nutrients during the re-feeding phase (e.g. active ulcerative diseases of the stomach or intestine, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection).
• Known history of Human Immunodeficiency Virus (HIV) infection.
• Clinically significant heart disease and/or recent cardiac events including:
o history of angina pectoris, coronary artery bypass graft (CABG), symptomatic pericarditis, or myocardial infarction within 12 months prior to the start of study treatment;
o history of documented congestive heart failure (NYHA III-IV);
o documented cardiomyopathy.
• History of cardiac arythmias, (e.g. ventricular tachycardia, chronic atrial fibrillation), complete left bundle branch block, high grade AV block (e.g. bifascicular block, Mobitz type II and third degree AV block), supraventricular, nodal arrhythmias, or conduction abnormality in the previous 12 months.
• Uncontrolled hypertension defined by a Systolic Blood Pressure (SBP) ≥ 160 mmHg and/or Diastolic Blood Pressure (DBP) ≥ 100 mmHg, with or without anti-hypertensive medication.
• Known reduction of left-ventricular ejection fraction (LVEF) to less than 50%, as assessed by multigated radionuclide scintigraphic scan (MUGA) or echocardiography.
• Previous episodes of symptomatic hypotension causing unconsciousness.
• Baseline fasting plasma glucose ≤ 65 mg/dl.
• Ongoing therapy with systemic corticosteroids, or systemic corticosteroid therapy ≤ 2 weeks before study enrollment, or who have not recovered from side effects of such treatment. The following uses of corticosteroids are permitted: topical applications (e.g. for rash), inhaled sprays (e.g. for obstructive airways diseases), eye drops.
• Any serious medical or psychiatric illness that in the assessment of the investigator renders the patient not suitable for participation in this clinical study.

Ethics oversight

For the study NCT05748704, was approved by Comitato Etico Regione Liguria. For the study NCT03454282, was approved by the Institutional Review Board (IRB) and the Ethics Committee of Fondazione IRCCS Istituto Nazionale dei Tumori di Milano.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For our in vitro experiments, no statistical method was used to predetermine sample size, but our sample size were similar to those reported in several publication including ours (at least three technical replicates each experiment). For our in vivo experiments, we estimated sample size by PS (Power and Sample size calculation-Vanderbilt University) software considering a multifactorial variance analysis. By this approach we estimated that the minimum number of mice that was assigned to each treatment group in our in vivo experiments (typically n=5) would reach a power of 0.85. The Type I error probability associated with our tests of the null hypothesis was 0.05. For ChIP-seq and RNA-seq experiments , a minimal of n=3 was used for independent biological samples, to allow for t-test statistics. (doi: https://doi.org/10.1186/s13059-014-0550-8)
Data exclusions	No data were excluded
Replication	We confirm that all experiments were reproducible by repeating them several times and by using different lots of reagents, different stocks of cell lines and some experiments were also repeated and confirmed by other operators. All in vitro experiments were performed in, at least, 3 individual biological replicates, with at least 3 technical replicates per experiment. ChIP-seq, RNA-seq and proteomics were performed in, at least, 3 individual cell line or tumour samples. All IHC stainings were performed in, at least, 3 different tumour samples (except for GR staining of PDX 186). The immunofluorescence was performed 2 times, independently. The western blot was repeated 3 times, independently. In all cases, both individual and averaged results are shown.
Randomization	Samples and mice were assigned to the different experimental groups in a random fashion.
Blinding	Operators were unblinded with the exception of the pathologist who analyzed the samples from Fig. 2 and from Extended Data Fig. 1. Blinding during animal experiments was not possible because mice underwent a specific diet supply and daily treatment. Similarly, our clinical studies were one-arm studies and no blinding applied to these trials.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	ERα antibody (#06-935, Millipore, 5µg/IP) H3K27ac (#39133, Active Motif) --> https://www.activemotif.com/catalog/details/39133/histone-h3-acetyl-lys27-antibody-pab Glucocorticoid Receptor (GR, #12041S, Cell Signaling, 5µg/IP, 1:1000 for WB, 1:100 for IF) Progesterone Receptor (PR, #8757S, Cell Signaling, 5µg/IP) c-JUN (#9165S, Cell Signaling, 5µg/IP) β-actin (#MAB1501R, Millipore, 1:10000 for WB) Alexa Fluor™ 488 goat anti-rabbit IgG (#A-11008, ThermoFisher Scientific, 1:1000) Secondary antibodies donkey-α-mouse IRDye® 680RD (926-68073, LI-COR Biosciences, 1:10000) Secondary antibodies donkey-α-rabbit IRDye® 800CW (926-32213, LI-COR Biosciences, 1:10000) OmniMap anti-Rabbit HRP (760-4311, Ventana Medical systems)

KI-67 (5278384001, Ready-to-use, Roche Diagnostics/Ventana)
GR (for IHC, clone D6H2L, 1:600 dilution, 12041, Cell Signalling)
ERα (for IHC, clone SP1, Ready-to-use, 5278406001, Roche Diagnostics/Ventana)
PR (for IHC, clone 1E2 (Ready-to-Use, 527799001, Roche Diagnostics/Ventana)
Anti-Rabbit HQ (07017812001, Ventana Medical systems)
CD16/CD32 antibody (1:400, 553142, BD Bioscience)

Validation

ERα antibody (#06-935, Millipore) --> https://www.merckmillipore.com/NL/en/product/Anti-Estrogen-Receptor-Antibody,MM_NF-06-935. Tested in https://doi.org/10.1371/journal.pone.0215340
H3K27ac (#39133, Active Motif) --> https://www.activemotif.com/catalog/details/39133/histone-h3-acetyl-lys27-antibody-pab
Glucocorticoid Receptor (GR, #12041S, Cell Signaling) --> https://www.cellsignal.com/products/primary-antibodies/glucocorticoid-receptor-d6h2l-xp-rabbit-mab/12041
Progesterone Receptor (PR, #8757S, Cell Signaling) --> https://www.cellsignal.com/products/primary-antibodies/progesterone-receptor-a-b-d8q2j-xp-rabbit-mab/8757
c-JUN (#9165S, Cell Signaling) --> https://www.cellsignal.com/products/primary-antibodies/c-jun-60a8-rabbit-mab/9165
β-actin (#MAB1501R, Millipore) --> https://www.merckmillipore.com/NL/en/product/Anti-Actin-Antibodyclone-C4,MM_NF-MAB1501R
Alexa Fluor™ 488 goat anti-rabbit IgG (#A-11008, ThermoFisher Scientific) --> https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008
Secondary antibodies donkey-α-mouse IRDye® 680RD (926-68073, LI-COR Biosciences) --> https://www.licor.com/bio/reagents/irdye-680rd-donkey-anti-mouse-igg-secondary-antibody
Secondary antibodies donkey-α-rabbit IRDye® 800CW (926-32213, LI-COR Biosciences) --> https://www.licor.com/bio/reagents/irdye-800cw-donkey-anti-rabbit-igg-secondary-antibody
CD16/CD32 antibody (553142, BD Bioscience) --> https://www.bdbiosciences.com/en-pl/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-rat-anti-mouse-cd16-cd32-mouse-bd-fc-block.553142?tab=citations_references

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The MCF7, T47D and HEK293T cell lines were purchased from the American Type Culture Collection (ATCC). TSAE1 cell line was a kind gift from Clare Isacke at ICR London.
Authentication	Authentication was performed by short tandem repeat profiling (Eurofins Genomics)
Mycoplasma contamination	Cell lines were subjected to regular Mycoplasma testing, by qPCR detection (MyTaq HS Red mix (bioline Bio-25047)) of 16S rRNA derived from several mycolplasma species.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used in our study belongs to the Commonly misidentified lines.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Six/eight-week-old female athymic nude mice (purchased from Envigo, Italy) were used in the experiments at the Animal Facility of the IRCCS Ospedale Policlinico San Martino (Genoa). 8-week-old female NSG mice (Jackson Laboratory) and BALB/c mice (Jackson Laboratory) were used in experiments performed at the Animal facility of the Netherlands Cancer Institute (NKI, Amsterdam).
Wild animals	The study did not involve wild animals.
Reporting on sex	All mice were female
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	Italian Istituto Superiore di Sanità and Organismo preposto al Benessere Animale (OPBA) of the IRCCS Ospedale Policlinico San Martino (Genoa, Italy) and Animal Ethics Committee of the Netherlands Cancer Institute.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Clinical trials NCT05748704 (IRCCS Ospedale Policlinico San Martino, Genoa, Italy) and NCT03454282 (Fondazione IRCCS Istituto Nazionale dei Tumouri, Milan, Italy).
Study protocol	Information on NCT05748704 trial can be obtained at: https://clinicaltrials.gov/ct2/show/NCT05748704. Information on NCT03454282 trial can be obtained at: https://clinicaltrials.gov/ct2/show/NCT03454282.

Data collection	In the NCT05748704 clinical trial, patients were enrolled and data were collected at the Department of Internal Medicine and Medical Specialties of the University of Genoa (Viale Benedetto XV 6, 16132 Genoa, Italy), Day Service, Room 1, between December 2022 and February 2024. In the NCT03454282 clinical trial, patients were enrolled and data were collected at the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Onco-hematology Department, Medical Oncology 1 Unit, between July 2018 and December 2020.
Outcomes	In the NCT05748704 clinical trial, primary outcomes are the effects of the FMD regimen on the circulating levels of factors with pro- or anti-oncogenic activity (including insulin, IGF1, IGFBP1, IGFBP3, leptin, adiponectin, IL-6, TNF-alpha, IL1beta) as well as the effect of FMD cycles on leukocyte subpopulations with a role in tumor growth control, such as regulatory T cells, "myeloid-derived suppressor cells" (MDSCs) as well as NK cells, and its stem cell pool (e.g. e.g. hematopoietic stem cells, endothelial stem cells, mesenchymal stem cells). These biological parameters will be evaluated at the first, sixth and twelfth cycles of MF. Secondary outcomes are: - feasibility, measured as the percentage of prescribed diet consumed and intake of any extra food and monitored through the compilation of a food diary or via phone calls during the MF cycle - the effect of the MF on the microbioma composition. - clinical responses measured by CT, MRI or by blood chemistry tests, dosing of tumor markers and / or molecular biology tests in the case of prostate tumors or hematologic tumors (e.g. PSA in patients affected by prostate cancer, BCR / Abl mRNA in the case of patients undergoing treatment with kinase inhibitors for CML; CM in the case of patients undergoing treatment for multiple myeloma). -long-term efficacy (progression-free survival, overall survival). - effect of MF on circulating (blood) growth factors and adipokines, glucose, ketone bodies, and other metabolic parameters. In NCT03454282, the primary outcomes were the measuring of absolute and relative changes in PBMCs before and after the FMD. For the secondary outcomes, please consult https://clinicaltrials.gov/ct2/show/NCT03454282

Plants

Seed stocks	/
Novel plant genotypes	/
Authentication	/

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	The ChIP-seq and RNA-seq data has been deposited to the GEO database (GSE260486, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE260486).
Files in database submission	Paired-end fastq (*_R1/R2.fastq.gz) and bigWigs (*.bw): MCF7_Control_ER_Rep1 MCF7_Control_ER_Rep2 MCF7_Control_ER_Rep3 MCF7_TMX_ER_Rep1 MCF7_TMX_ER_Rep2 MCF7_TMX_ER_Rep3 MCF7_Fasting_ER_Rep1 MCF7_Fasting_ER_Rep2 MCF7_Fasting_ER_Rep3 MCF7_TMXplusFasting_ER_Rep1 MCF7_TMXplusFasting_ER_Rep2 MCF7_TMXplusFasting_ER_Rep3 MCF7_TMXplusFasting_ER_Rep4 MCF7_TMXplusFasting_ER_Rep5 MCF7_TMXplusFasting_ER_Rep6 MCF7_Control_H3K27ac_Rep1 MCF7_Control_H3K27ac_Rep2 MCF7_Control_H3K27ac_Rep3 MCF7_TMX_H3K27ac_Rep1 MCF7_TMX_H3K27ac_Rep2 MCF7_TMX_H3K27ac_Rep3

MCF7_Fasting_H3K27ac_Rep1
MCF7_Fasting_H3K27ac_Rep2
MCF7_Fasting_H3K27ac_Rep3
MCF7_TMXplusFasting_H3K27ac_Rep1
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MCF7_Control_GR_Rep2
MCF7_Control_GR_Rep3
MCF7_TMX_GR_Rep1
MCF7_TMX_GR_Rep2
MCF7_TMX_GR_Rep3
MCF7_Fasting_GR_Rep1
MCF7_Fasting_GR_Rep2
MCF7_Fasting_GR_Rep3
MCF7_TMXplusFasting_GR_Rep1
MCF7_TMXplusFasting_GR_Rep2
MCF7_TMXplusFasting_GR_Rep3
MCF7_Control_cJUN_Rep1
MCF7_Control_cJUN_Rep2
MCF7_Control_cJUN_Rep3
MCF7_TMX_cJUN_Rep1
MCF7_TMX_cJUN_Rep2
MCF7_TMX_cJUN_Rep3
MCF7_Fasting_cJUN_Rep1
MCF7_Fasting_cJUN_Rep2
MCF7_Fasting_cJUN_Rep3
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MCF7_TMXplusFasting_cJUN_Rep2
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MCF7_Control_PR_Rep2
MCF7_Control_PR_Rep3
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MCF7_TMX_PR_Rep2
MCF7_TMX_PR_Rep3
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MCF7_Fasting_PR_Rep2
MCF7_Fasting_PR_Rep3
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MCF7_TMXplusFasting_PR_Rep2
MCF7_TMXplusFasting_PR_Rep3
MCF7_Control_INPUT
MCF7_TMX_INPUT
MCF7_Fasting_INPUT
MCF7_TMXplusFasting_INPUT

Bed files (peaks):
MCF7_Control_ER_Rep1_chr.narrowPeak
MCF7_Control_ER_Rep2_chr.narrowPeak
MCF7_Control_ER_Rep3_chr.narrowPeak
MCF7_TMX_ER_Rep1_chr.narrowPeak
MCF7_TMX_ER_Rep2_chr.narrowPeak
MCF7_TMX_ER_Rep3_chr.narrowPeak
MCF7_Fasting_ER_Rep1_chr.narrowPeak
MCF7_Fasting_ER_Rep2_chr.narrowPeak
MCF7_Fasting_ER_Rep3_chr.narrowPeak
MCF7_TMXplusFasting_ER_Rep1_chr.narrowPeak
MCF7_TMXplusFasting_ER_Rep2_chr.narrowPeak
MCF7_TMXplusFasting_ER_Rep3_chr.narrowPeak
MCF7_TMXplusFasting_ER_Rep4_chr.narrowPeak
MCF7_TMXplusFasting_ER_Rep5_chr.narrowPeak
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MCF7_Control_H3K27ac_Rep2_chr.narrowPeak
MCF7_Control_H3K27ac_Rep3_chr.narrowPeak
MCF7_TMX_H3K27ac_Rep1_chr.narrowPeak
MCF7_TMX_H3K27ac_Rep2_chr.narrowPeak
MCF7_TMX_H3K27ac_Rep3_chr.narrowPeak
MCF7_Fasting_H3K27ac_Rep1_chr.narrowPeak
MCF7_Fasting_H3K27ac_Rep2_chr.narrowPeak
MCF7_Fasting_H3K27ac_Rep3_chr.narrowPeak
MCF7_TMXplusFasting_H3K27ac_Rep1_chr.narrowPeak

MCF7_TMXplusFasting_H3K27ac_Rep2_chr.narrowPeak
MCF7_TMXplusFasting_H3K27ac_Rep3_chr.narrowPeak
MCF7_TMXplusFasting_H3K27ac_Rep4_chr.narrowPeak
MCF7_TMXplusFasting_H3K27ac_Rep5_chr.narrowPeak
MCF7_TMXplusFasting_H3K27ac_Rep6_chr.narrowPeak
MCF7_Control_GR_Rep1_chr.narrowPeak
MCF7_Control_GR_Rep2_chr.narrowPeak
MCF7_Control_GR_Rep3_chr.narrowPeak
MCF7_TMX_GR_Rep1_chr.narrowPeak
MCF7_TMX_GR_Rep2_chr.narrowPeak
MCF7_TMX_GR_Rep3_chr.narrowPeak
MCF7_Fasting_GR_Rep1_chr.narrowPeak
MCF7_Fasting_GR_Rep2_chr.narrowPeak
MCF7_Fasting_GR_Rep3_chr.narrowPeak
MCF7_TMXplusFasting_GR_Rep1_chr.narrowPeak
MCF7_TMXplusFasting_GR_Rep2_chr.narrowPeak
MCF7_TMXplusFasting_GR_Rep3_chr.narrowPeak
MCF7_Control_cJUN_Rep1_chr.narrowPeak
MCF7_Control_cJUN_Rep2_chr.narrowPeak
MCF7_Control_cJUN_Rep3_chr.narrowPeak
MCF7_TMX_cJUN_Rep1_chr.narrowPeak
MCF7_TMX_cJUN_Rep2_chr.narrowPeak
MCF7_TMX_cJUN_Rep3_chr.narrowPeak
MCF7_Fasting_cJUN_Rep1_chr.narrowPeak
MCF7_Fasting_cJUN_Rep2_chr.narrowPeak
MCF7_Fasting_cJUN_Rep3_chr.narrowPeak
MCF7_TMXplusFasting_cJUN_Rep1_chr.narrowPeak
MCF7_TMXplusFasting_cJUN_Rep2_chr.narrowPeak
MCF7_TMXplusFasting_cJUN_Rep3_chr.narrowPeak
MCF7_Control_PR_Rep1_chr.narrowPeak
MCF7_Control_PR_Rep2_chr.narrowPeak
MCF7_Control_PR_Rep3_chr.narrowPeak
MCF7_TMX_PR_Rep1_chr.narrowPeak
MCF7_TMX_PR_Rep2_chr.narrowPeak
MCF7_TMX_PR_Rep3_chr.narrowPeak
MCF7_Fasting_PR_Rep1_chr.narrowPeak
MCF7_Fasting_PR_Rep2_chr.narrowPeak
MCF7_Fasting_PR_Rep3_chr.narrowPeak
MCF7_TMXplusFasting_PR_Rep1_chr.narrowPeak
MCF7_TMXplusFasting_PR_Rep2_chr.narrowPeak
MCF7_TMXplusFasting_PR_Rep3_chr.narrowPeak

Genome browser session
(e.g. UCSC)

Genomics and transcriptomics data access details:
- GSE260486 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE260486)

Methodology

Replicates

For each condition, a minimum of 3 samples were sequenced

Sequencing depth

All the samples were paired-end sequenced

sample n_peaks FRiP M_reads_mapped
MCF7 xenograft_control (no treatments)_Replicate 1_ER 29741 0.078 61.5
MCF7 xenograft_control (no treatments)_Replicate 2_ER 33150 0.095 49.7
MCF7 xenograft_control (no treatments)_Replicate 3_ER 13670 0.039 55.6
MCF7 xenograft_Tamoxifen treated_Replicate 1_ER 47243 0.138 62.3
MCF7 xenograft_Tamoxifen treated_Replicate 2_ER 9365 0.022 58.1
MCF7 xenograft_Tamoxifen treated_Replicate 3_ER 34206 0.098 53.1
MCF7 xenograft_ Fasting_Replicate 1_ER 46901 0.147 56.0
MCF7 xenograft_ Fasting_Replicate 2_ER 47993 0.148 76.2
MCF7 xenograft_ Fasting_Replicate 3_ER 37040 0.102 70.4
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 1_ER 72054 0.257 67.0
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 2_ER 49417 0.156 61.8
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 3_ER 61939 0.203 64.2
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 4_ER 40273 0.147 48.8
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 5_ER 711 0.003 38.2
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 6_ER 25818 0.087 35.0
MCF7 xenograft_control (no treatments)_Replicate 1_H3K27ac 57810 0.538 43.2
MCF7 xenograft_control (no treatments)_Replicate 2_H3K27ac 49081 0.636 51.7
MCF7 xenograft_control (no treatments)_Replicate 3_H3K27ac 57808 0.604 66.2
MCF7 xenograft_Tamoxifen treated_Replicate 1_H3K27ac 53674 0.675 68.2
MCF7 xenograft_Tamoxifen treated_Replicate 2_H3K27ac 63146 0.562 59.7
MCF7 xenograft_Tamoxifen treated_Replicate 3_H3K27ac 61864 0.588 70.1
MCF7 xenograft_ Fasting_Replicate 1_H3K27ac 60350 0.568 55.1

MCF7 xenograft_ Fasting_Replicate 2_H3K27ac 62978 0.571 67.8
MCF7 xenograft_ Fasting_Replicate 3_H3K27ac 59070 0.538 52.7
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 1_H3K27ac 52298 0.634 59.9
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 2_H3K27ac 57696 0.579 58.9
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 3_H3K27ac 54060 0.587 67.9
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 4_H3K27ac 62138 0.594 61.1
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 5_H3K27ac 69495 0.414 49.8
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 6_H3K27ac 63469 0.498 55.5
MCF7 xenograft_control (no treatments)_Replicate 1_GR 6514 0.007 35.6
MCF7 xenograft_control (no treatments)_Replicate 2_GR 5452 0.015 34
MCF7 xenograft_control (no treatments)_Replicate 3_GR 357 0.051 42.8
MCF7 xenograft_Tamoxifen treated_Replicate 1_GR 820 0.003 37.9
MCF7 xenograft_Tamoxifen treated_Replicate 2_GR 3944 0.007 36.8
MCF7 xenograft_Tamoxifen treated_Replicate 3_GR 1312 0.002 24.7
MCF7 xenograft_ Fasting_Replicate 1_GR 16421 0.039 46.7
MCF7 xenograft_ Fasting_Replicate 2_GR 22725 0.059 39.1
MCF7 xenograft_ Fasting_Replicate 3_GR 21758 0.053 51.8
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 1_GR 9669 0.02 29.9
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 2_GR 33000 0.099 41.1
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 3_GR 2447 0.005 34
MCF7 xenograft_control (no treatments)_Replicate 1_c-JUN 44567 0.078 23.9
MCF7 xenograft_control (no treatments)_Replicate 2_c-JUN 44402 0.135 34.3
MCF7 xenograft_control (no treatments)_Replicate 3_c-JUN 12203 0.087 36.2
MCF7 xenograft_Tamoxifen treated_Replicate 1_c-JUN 9248 0.017 34.5
MCF7 xenograft_Tamoxifen treated_Replicate 2_c-JUN 42874 0.145 30.7
MCF7 xenograft_Tamoxifen treated_Replicate 3_c-JUN 49230 0.088 24.5
MCF7 xenograft_ Fasting_Replicate 1_c-JUN 28022 0.087 39.1
MCF7 xenograft_ Fasting_Replicate 2_c-JUN 36738 0.131 36.6
MCF7 xenograft_ Fasting_Replicate 3_c-JUN 30904 0.096 22.4
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 1_c-JUN 32444 0.067 20.1
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 2_c-JUN 50206 0.208 36.3
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 3_c-JUN 33115 0.084 33.6
MCF7 xenograft_control (no treatments)_Replicate 1_PR 10825 0.019 35.1
MCF7 xenograft_control (no treatments)_Replicate 2_PR 21080 0.043 37.2
MCF7 xenograft_control (no treatments)_Replicate 3_PR 14426 0.033 43.6
MCF7 xenograft_Tamoxifen treated_Replicate 1_PR 5939 0.011 31.7
MCF7 xenograft_Tamoxifen treated_Replicate 2_PR 27179 0.056 38.8
MCF7 xenograft_Tamoxifen treated_Replicate 3_PR 4298 0.008 27.9
MCF7 xenograft_ Fasting_Replicate 1_PR 26274 0.055 53.3
MCF7 xenograft_ Fasting_Replicate 2_PR 39145 0.083 52.7
MCF7 xenograft_ Fasting_Replicate 3_PR 29032 0.068 44.9
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 1_PR 21180 0.06 23.9
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 2_PR 50226 0.113 54.8
MCF7 xenograft_ Fasting + Tamoxifen_Replicate 3_PR 21069 0.044 40.5
MCF7 xenograft_control (no treatments)_INPUT N.A N.A 60.3
MCF7 xenograft_Tamoxifen treated_INPUT N.A N.A 38.7
MCF7 xenograft_ Fasting_INPUT N.A N.A 55.9
MCF7 xenograft_ Fasting + Tamoxifen_INPUT N.A N.A 62.3

Antibodies

ERα antibody (#06-935, Millipore, 5µg/IP)
H3K27ac (#39133, Active Motif, 5µg/IP)
Glucocorticoid Receptor (GR, #12041S, Cell Signaling, 5µg/IP)
Progesterone Receptor (PR, #8757S, Cell Signaling, 5µg/IP)
c-JUN (#9165S, Cell Signaling, 5µg/IP)

Peak calling parameters

MAPPING:
bwa mem -M -t {threads} -p hg38 sample_R1.fastq.gz 2> | samtools view -Sbu - | samtools sort -m 2G -T sample -@ {threads} -O bam - > sample.bam

PEAK calling
macs2 callpeak -t target.bam -c input.bam -f BAM -g 2861343702 -q 0.01 --outdir outdir --name sample_peaks

Data quality

Reads were filtered based on mapping quality (MAPQ ≥ 20), and duplicate reads were marked with Picard MarkDuplicates (v2.19.0). MACS2 (v2.1.2) was used to perform peak calling over input ChIP-seq samples; only peaks with a q-value < 0.01 were retained.

Software

Reads were aligned to the human genome build GRCh38 using BWA 0.5.9-r26-dev. Reads with a mapping quality (MAPQ) < 20 were removed from further analysis. Enrichment over input control was determined using MACS2. Only peaks not overlapping with the ENCODE Hg38/GRCh38 blacklisted regions, were retained.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	BC nodules were macrodissected from TSAE1-bearing mice and processed to generate single-cell suspensions using the Tumour Dissociation Kit (Miltenyi Biotec, cat.no. 130-096-730) in conjunction with the gentleMACS Octo Dissociator, following the manufacturers' protocols. The cell pellet of in vivo samples were incubated with anti-CD16/CD32 antibody (BD Bioscience) and stained with the antibodies against surface markers following standard procedures. Samples were fixed with the eBioscience fixation and permeabilization kit (Invitrogen), and stained for intracellular markers.
Instrument	Samples were acquired using a five-laser Aurora spectral flow cytometer (Cytek Biosciences)
Software	FlowJo v10 software.
Cell population abundance	Not applicable as no sorts were performed.
Gating strategy	To determine the populations of myeloid and lymphoid cells, we first used SSC, FSC and Zombie-NIR (live/dead dye) gating to select for single cell and live cell events. Immune cell populations were then identified by their high expression levels of CD45. The following populations were then identified from the immune cell compartment: lymphoid cells (CD45+ CD11b-). Myeloid cells (CD45+ CD11b+). From myeloid cells, we gated neutrophils (CD45+ CD11b+ Ly6G+), non-classical monocytes (CD45+ CD11b+ Ly6G- Ly6C-) and dendritic cells (CD45+ F4/80- CD11chigh MHCIIhigh).

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.