**Supplemental Methods**

**Materials and reagents**

All reagents were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise stated. 12-HETE standard was obtained from Cayman Chemical (Ann Arbor, MI). Anti-12-LOX leukocyte-type (12-LOX-L) antibody was purchased from Abcam (Cambridge, MA). Anti-12-LOX platelet-type (12-LOX-P) antibody was purchased from Novus Biologicals (Littleton, CO). Anti-β-actin antibody was purchased from Sigma. Goat anti-rabbit and goat anti-mouse secondary antibodies were purchased from Invitrogen (Camarillo, CA). Human breast cancer MDA-MB-231 cells and mouse breast cancer 4T1 cells were obtained from the American Type Culture Collection (Manassas, VA). Serum or plasma glucose level was determined using a Glucose Assay Kit from Abcam.

**Animal studies**

All animal experiments were approved by The University of Texas MD Anderson Cancer Center Animal Care and Use Committee. All mice were maintained under a 12-hr light/dark cycle at controlled humidity and temperature (20-24°C). Mice of strain FVB/N-Tg(MMTVneu)202Mul/J were purchased from the Jackson Laboratory (Bar Harbor, Maine), bred and housed at the MD Anderson Cancer Center animal facility. At 5 weeks of age, the mice were randomized into one of four dietary groups (**Supplementary Table S1a**). Mice were monitored and weighted weekly. Ten days after breast tumors were found, the mice were euthanized, and mammary gland tumors were collected. Tumor tissues were either formalin fixed or flash frozen in liquid nitrogen and then stored at -80ºC for future analysis.

For the MDA-MB-231 orthotopic mouse model, 6- to 8-week-old mice were supplied from MD Anderson’s Department of Experimental Radiation Oncology and were allowed to acclimatize for 3 days prior to study initiation. Mice were fed an AIN-76 diet (Harlan Laboratories, Livermore, CA) and water *ad libitum*. MDA-MB-231 cells (1.5 × 106) were injected into the fourth abdominal fat pad (groin mammary fat)[1](#_ENREF_1). These mice were then randomized to control or sucrose diets (described below and listed in **Supplementary Table S1b**; n=5 per group). Tumor volume (V = D2xL/2; V: volume, D: width diameter, L: length diameter) and mouse weight were measured twice a week. Five weeks after inoculation, the mice were euthanized, and tumors were collected and weighed. Tumor tissues were either formalin fixed or flash frozen in liquid nitrogen and then stored at -80°C for future analysis.

For the 4T1 orthotopic model, BALB/c mice were purchased from Charles River Laboratories (Wilmington, MA) at 5 weeks old. They were acclimated for 1 week before being injected with 4T1 mouse breast cancer cells (10,000 cells per mouse) into their fourth mammary fat pad. As for the other models, mice were randomized to similar diet groups and fed one of four diets (**Supplementary Table S1b or S1c**) for 3 weeks or until the mice became moribund. Mice were then euthanized, and primary mammary gland tumors were collected and fixed as described previously. To examine lung metastases, lung tissues were fixed with Davidson fixative solution (Newcomer Lab Supply, Middleton, WI). Lung metastases were measured macroscopically or microscopically through examining the number of nodules in the lung. Similarly, blood was also collected by cardiac puncture, and serum and plasma were separated, then aliquoted into small vials and stored in a -80°C freezer until further analyses.

**Diets**

Both control and sucrose-enriched diets were made by Research Diet, Inc. (New Brunswick, NJ) and kept in the refrigerator upon arrival. They were composed of 15% fat by weight, which equates to 30% of energy per day for humans. The diets were modified based on AIN-93G dietary content. The amount of sucrose was increased from 0 g/kg (control) to 62.5 g/kg, 125 g/kg, 250 g/kg and 500 g/kg. For the last animal study, the sucrose diet contained 250 g/kg sucrose, the glucose diet contained 125 g/kg dextrose, the fructose diet contained 125 g/kg fructose and the glucose plus fructose diet contained 125 g/kg dextrose and 125 g/kg fructose.

**Eicosanoid analyses**

Frozen mammary tumor tissue or breast cancer cells (3x106) were analyzed for eicosanoid levels using a modification of a previously described method[2](#_ENREF_2). In brief, frozen tissues (approximately 20-25 mg) were homogenized in 500 µL PBS (0.1% Butylated hydroxytoluene and 1 mM EDTA) using a Precellys high-throughput tissue homogenizer coupled with a Cryolys cooling unit (Bertin Technologies, Rockville, MD). Eicosanoids were then extracted in hexane:ethyl acetate (1:1) and measured by the LC-MS/MS method using Agilent 1200 series HPLC pumps (Santa Clara, CA) coupled with tandem quadruple mass spectrometry (Agilent 6460 series). Eicosanoids of interest were chromatographically separated using a Kinetex 3-μm C18 2 × 100 mm analytic column (Phenomenex). The mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile using a linear gradient consisting of 20%B to 40%B for 4 min and then staying at 40%B for 8.5 min, followed by changing from 40%B to 90%B in 12 min. This was then increased to 98%B organic concentration over the next 5 min and kept at this condition for an additional 4 min to achieve chromatographic baseline resolution. The flow rate was 0.4 mL/min with a column temperature of 35°C. The mass spectrometer was operated in negative electrospray ionization mode with source and gas temperatures at 350°C for eicosanoid analysis. Eicosanoids were detected and quantified by multiple reaction mode (MRM) monitoring of the transitions of prostaglandins and their relevant internal standard (Cayman Chemical)[2](#_ENREF_2). Eicosanoid levels were normalized against tissue protein concentrations measured by a DC Protein Assay Kit (Bio-Rad, Hercules, CA).

**Western blotting**

Frozen tissues were homogenized in cell lysis buffer (Invitrogen) added with 1% protease inhibitor cocktails (Sigma). The homogenates were centrifuged at 14,000 rpm for 15 min, and then the supernatant was removed to a new vial. The protein concentration was quantified as described above. 10 µg of protein was separated by SDS-PAGE using a 2-12% resolving polyacrylamide gel and then transferred onto a PVDF membrane (Invitrogen). Primary antibodies against 12-LOX-L (1:1,000 in Tris-buffered saline with 0.05% Triton X-100 (TBS-T)), 12-LOX-P (1:1,000 in TBS-T) and actin (1:10,000 in TBS-T) were used. The membranes were incubated in primary antibodies at 4°C overnight followed by secondary antibodies against rabbit or mouse primary antibodies (1:5,000) at room temperature for 1 hr. The signaling was detected with SuperSignal West Dura Extended Duration Substrate (Thermo Scientific, Waltham, MA).

**Immunohistochemical analysis**

*Hematoxylin and Eosin (H&E) Staining* Tissue samples from mice were obtained at necropsy. Tissues were fixed in 4% paraformaldehyde and subsequently embedded in paraffin. Sections were stained with H&E using a standard protocol and imaged and analyzed by a pathologist.

**References**

1. Iorns, E.*, et al.* A new mouse model for the study of human breast cancer metastasis. *PLoS One* **7**, e47995 (2012).

2. Yang, P.*, et al.* Determination of endogenous tissue inflammation profiles by LC/MS/MS: COX- and LOX-derived bioactive lipids. *Prostaglandins Leukot. Essent. Fatty Acids* **75**, 385-395 (2006).