

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. A subset of TNBC cells undergo ferroptosis following glutathione depletion and

are sensitive to GPX inhibition. a, Relative viability of BT-549 cells incubated with 10 μ M BSO and the specified dose of deferiprone for 72 hours. Values were normalized to account for loss of viability associated with deferiprone. Error bars in this and the subsequent panel show standard error of the mean. **b**, Cell viability dose response curves for ML162 in 10 TNBC cells lines and the non-transformed mammary epithelial cell line MCF-10A. **c**, Relative mRNA expression of GPX4 measured by qPCR in the indicated cell lines. In **b** and **c**, non-ferroptotic and ferroptotic TNBC cell lines (as defined in **Fig 1f**) are colored turquoise and red, respectively.

Figure S2. Conjugated linolenic acids trigger ferroptosis in TNBC cells. a, Relative viability

dose response curves for α ESA in BT-549 cells co-treated with the specified dose of vitamin E (α -tocopherol) for 48 hours. Error bars denote standard deviation. **b**, Light micrographs of BT-549 cells treated with 25 μ M α ESA for 48 hours in the presence or absence of 20 μ M Z-VAD or 2 μ M fer-1. **c**, Relative viability of BT-549 cells incubated for 72 hours with either vehicle or 20 μ M α ESA and either 20 μ M Z-VAD, 50 μ M nec-1s, or 2 μ M fer-1. Errors bars show standard error of the mean. **d**, $\text{Log}_2(\text{IC}_{50})$ values for the indicated polyunsaturated fatty acid in MDA-MB-231 after 72 hours of incubation. Error bars show standard error of the mean. **e**, Cell viability dose response curves for arachidonic acid in BT-549 after 72 hours of treatment with or without 2 μ M fer-1. Error bars show standard deviation. Cell viability dose response curves for the noted conjugated linolenic acid in the presence or absence of 2 μ M fer-1 in **f**, MDA-MB-231 or **g**, BT-549 cells. Treatment time was 48 hours for BT-549 and 72 hours for MDA-MB-231. Error bars indicate standard error of the mean.

Figure S3. Markers for apoptosis and cell proliferation in tung oil-treated xenograft tumors.

Bar charts showing the percent of cells in orthotopic MDA-MB-231 xenograft tumors from mice treated with safflower oil (control) or tung oil that were positive for **a**, cleaved caspase-3 or **b**, Ki67. Error bars denote standard error of the mean.

Figure S4. Mechanism of ferroptosis by α ESA. a, Cell viability dose response curves for α ESA

in control and *Acs14*-deficient mouse embryonic fibroblast (Pfa1) cells with or without 2 μ M fer-1. Error bars show standard deviation. Relative mRNA expression of ACSL family members by qPCR in **b**, BT-549 and **c**, MDA-MB-231 72 hours after transfection with the indicated *ACSL* siRNA. Error bars depict standard error of the mean. BT-549 do not express *ACSL5* and neither BT-549 nor MDA-MB-231 express *ACSL6* at a level that could be quantified.

Figure S5. Quantitative LC/MS assessment of oxidized phospholipids altered by α ESA

treatment in BT-549 cells. Radar plot showing significant increases in the amount of **a**, mono- and **b**, di-oxygenated PE species after treatment with 25 μ M α ESA for 8 hours. Amount of phospholipid (pmol/ μ mol of total phospholipid) is shown in magenta for controls and blue for α ESA-treated cells for all radar plots. **c**, Bar charts showing the amounts of PE species that were significantly increased by α ESA treatment compared to vehicle-treated controls and decreased in α ESA-treated cells in which ACSL1 had been depleted compared to α ESA-treated cells. Data are based on three replicates and error bars show standard deviation. The color scheme shown in this panel applies to subsequent bar charts. **d**, Radar plot showing significant increases in oxidized CL species after treatment with α ESA. **e**, Bar charts showing the amounts of oxidized CL species that

were significantly increased by α ESA treatment and responsive to ACSL1 knockdown. **f**, Radar plot showing significant increases in oxidized PC species after treatment with α ESA. Amounts of **g**, a PC species and **h**, a PS species that were significantly increased by α ESA treatment and decreased by ACSL1 depletion.

Figure S6. Ferroptosis triggered by GPX4 inhibition is enhanced by α ESA in TNBC cells.

Relative cell viability dose response curves for ML162 in MDA-MB-231 cells and vehicle, 3 μ M α ESA, or 2 μ M fer-1. Cells were treated for 72 hours and error bars show standard error of the mean.

Figure S1

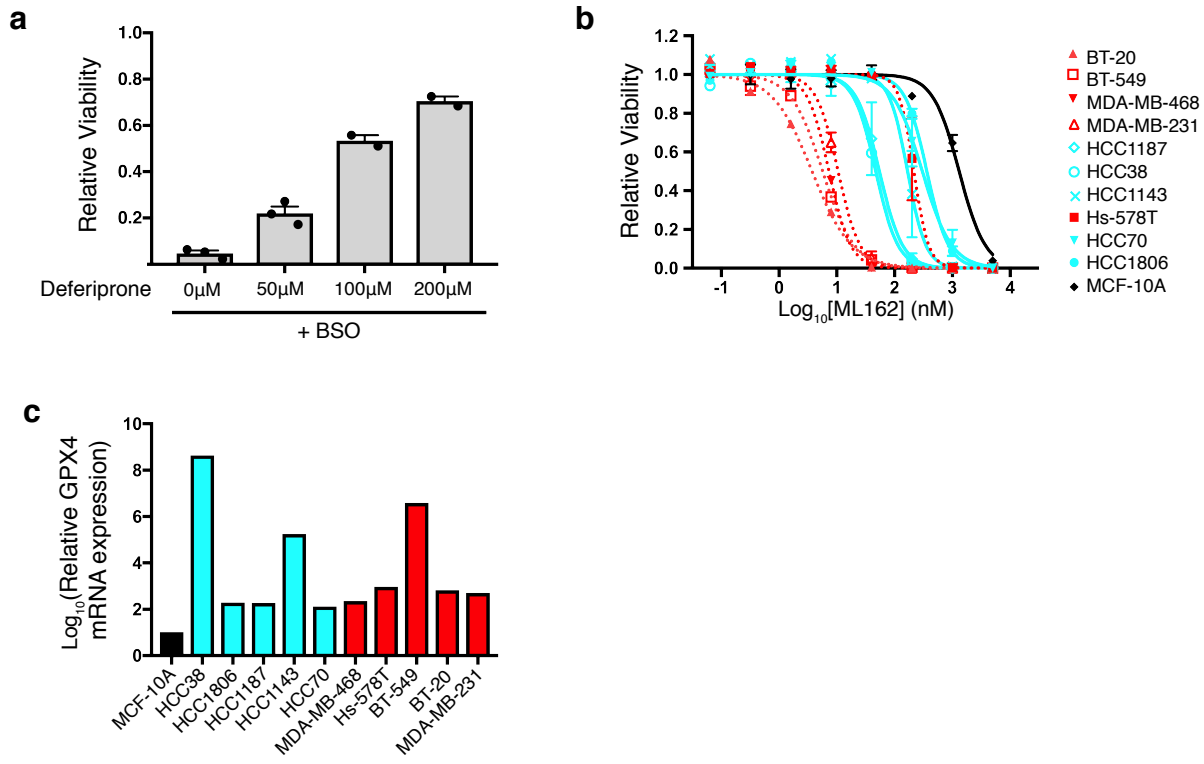


Figure S2

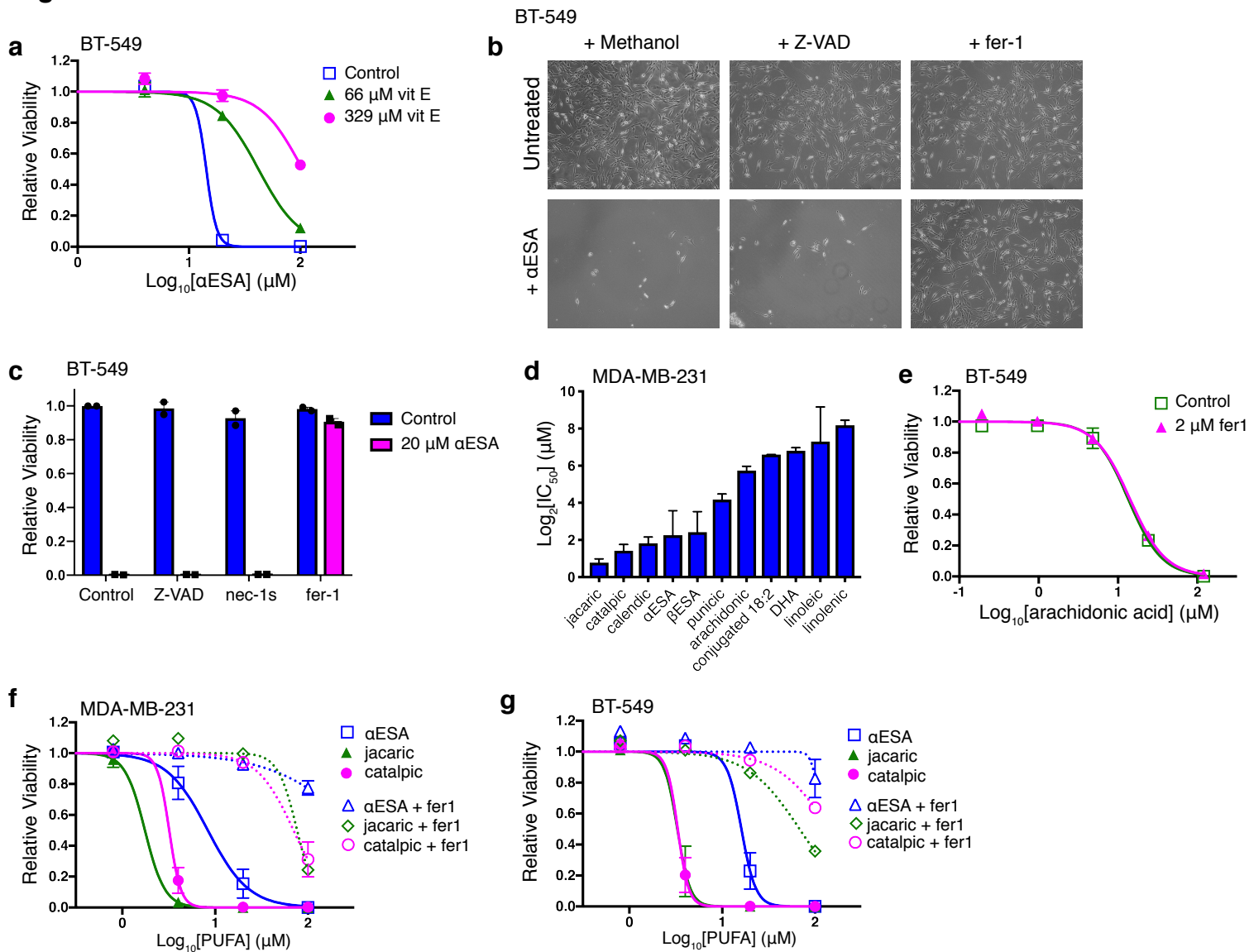


Figure S3

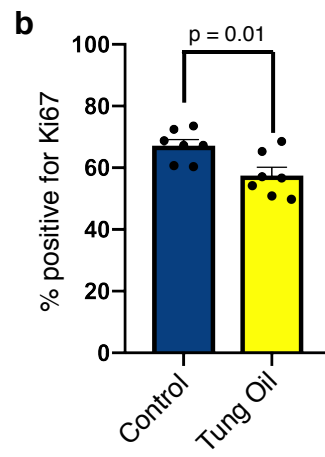
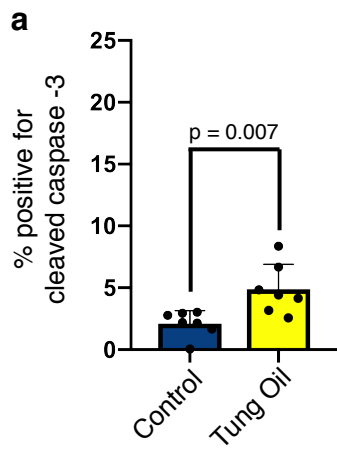


Figure S4

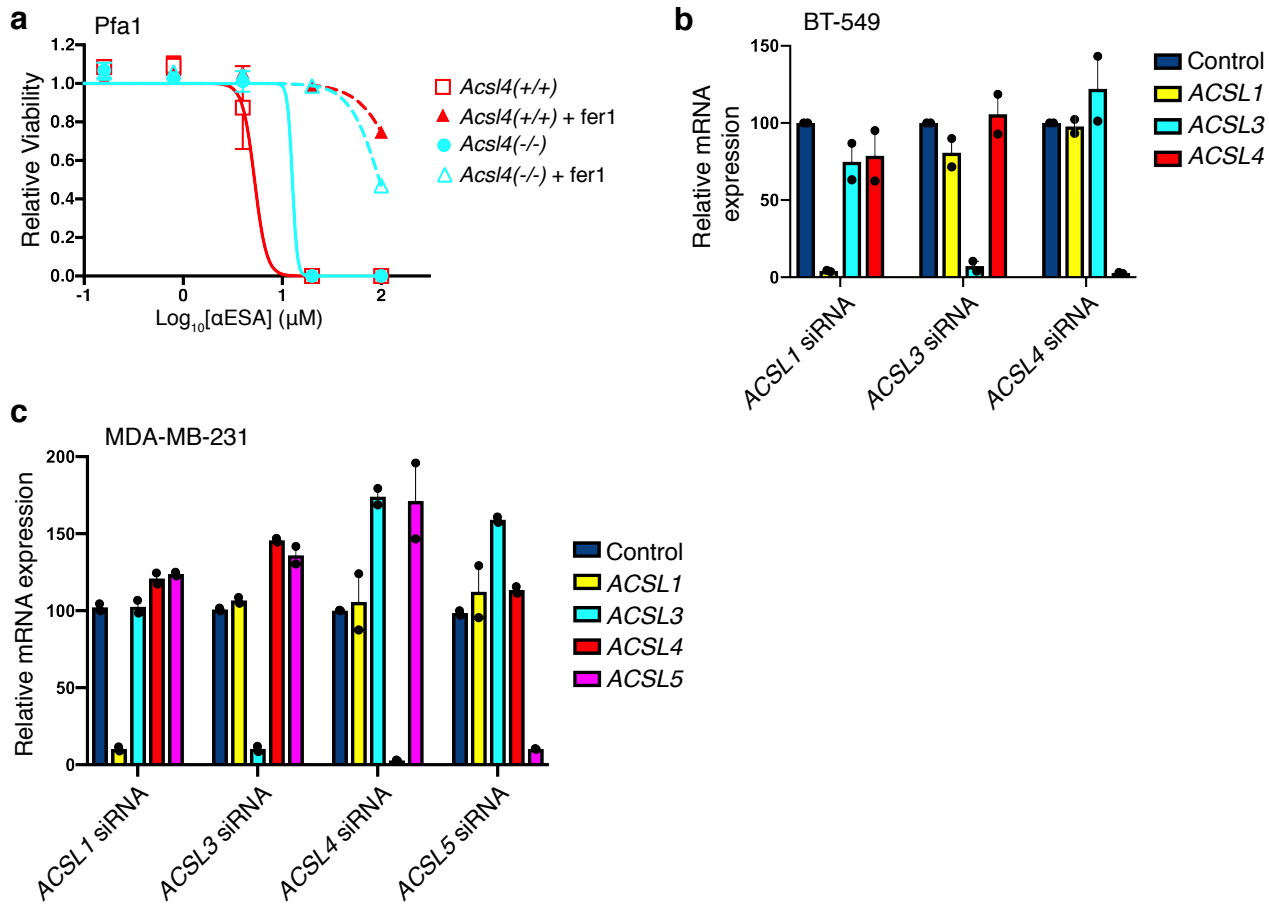


Figure S5

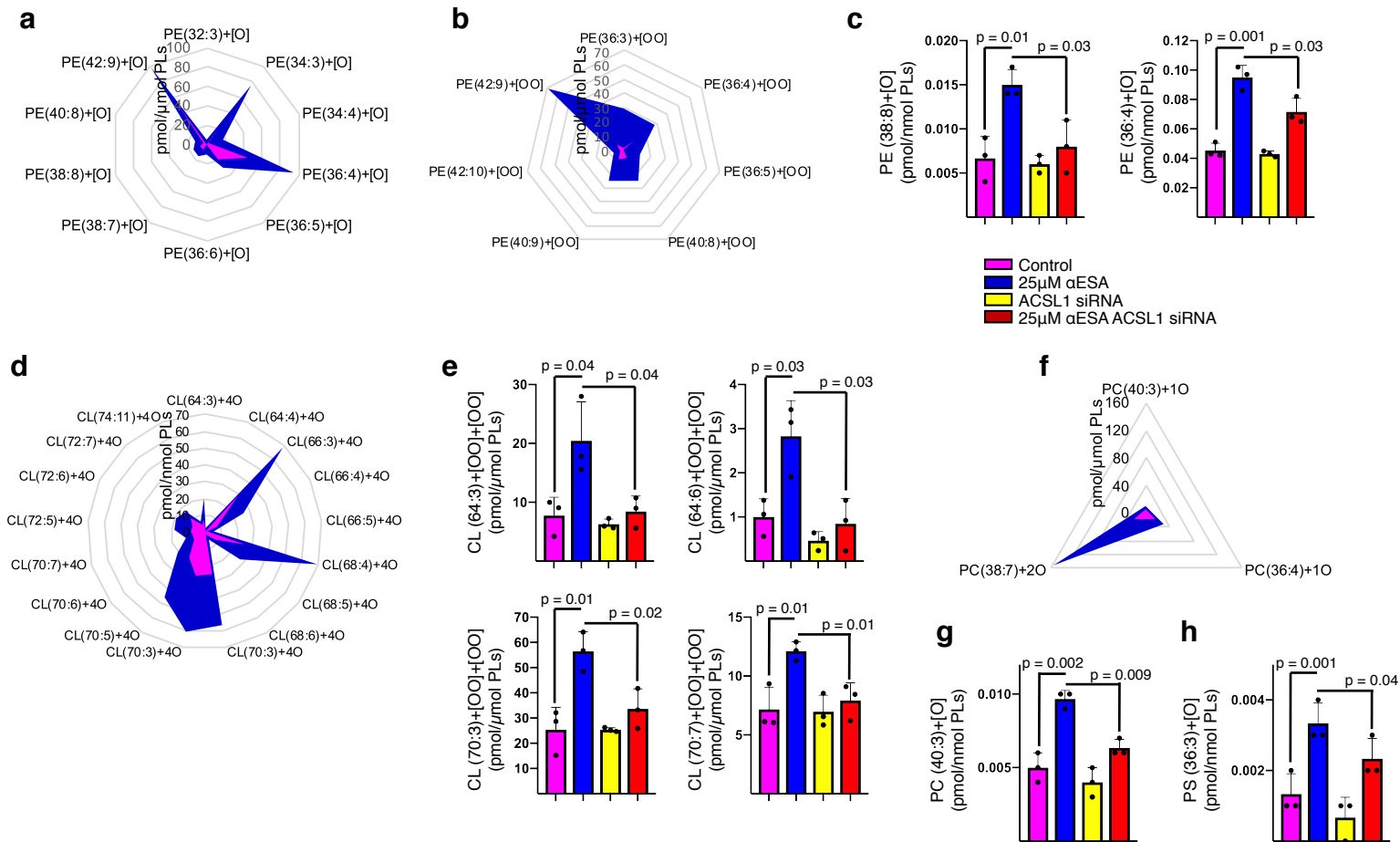


Figure S6

