Title: Obesity promotes breast epithelium DNA damage in BRCA mutation carriers

Authors: Priya Bhardwaj[1], Neil M. Iyengar[1,2], Heba Zahid[3], Katharine M. Carter[1], Dong Jun Byun[4], Man Ho Choi[4], Qi Sun[5], Oleksandr Savenkov[6], Charalambia Louka[1], Catherine Liu[1], Phoebe Piloco[1], Monica Acosta[1], Rohan Bareja[7], Olivier Elemento[7], Miguel Foronda[8], Lukas E. Dow[1,9], Sofya Oshchepkova[1], Dilip D. Giri[10], Michael Pollak[11], Xi Kathy Zhou[6], Benjamin D. Hopkins[12], Ashley M. Laughney[9], Melissa K. Frey[13], Lora Hedrick Ellenson[10], Monica Morrow[14], Jason A. Spector[15], Lewis C. Cantley[9], Kristy A. Brown[1,9*]

Affiliations:

[1]Department of Medicine, Weill Cornell Medicine; New York, USA.

[2]Department of Medicine, Memorial Sloan Kettering Cancer Center; New York, USA.

[3]Department of Medical Laboratory Technology, College of Applied Medical Science, Taibah University; Medina, Saudi Arabia.

[4]Center for Advanced Biomolecular Recognition, Korea Institute of Science and Technology, Seoul, Korea.

[5]Computational Biology Service Unit of Life Sciences Core Laboratories Center, Cornell University, Ithaca; USA.

[6]Department of Population Health Sciences, Weill Cornell Medicine; New York, USA.

[7]Caryl and Israel Englander Institute for Precision Medicine, Weill Cornell Medicine; New York, USA.

[8]Department of Neurology, Memorial Sloan Kettering Cancer Center; New York, USA.

[9]Sandra and Edward Meyer Cancer Center, Weill Cornell Medicine; New York, USA.

[10]Department of Pathology, Memorial Sloan Kettering Cancer Center; New York, USA.

[11]Departments of Medicine and Oncology, McGill University; Montreal, Canada.

[12]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai; New York, USA

[13]Department of Obstetrics & Gynecology, Weill Cornell Medicine; New York, USA

[14]Department of Surgery, Memorial Sloan Kettering Cancer Center; New York, USA.

[15]Laboratory of Bioregenerative Medicine & Surgery, Weill Cornell Medicine; New York, USA.

*Corresponding author. Email: kab2060@med.cornell.edu

One Sentence Summary: Elevated bodyweight is positively associated with DNA damage in breast epithelium of *BRCA* mutation carriers

Abstract:

Obesity is an established risk factor for breast cancer among women in the general population after menopause. Whether elevated bodyweight is a risk factor for women with a germline mutation in *BRCA1* or *BRCA2* is less clear due to inconsistent findings from epidemiological studies and lack of mechanistic studies in this population. Here, we show that DNA damage in normal breast epithelium of *BRCA* mutation carriers is positively correlated with body mass index and with biomarkers of metabolic dysfunction. Additionally, RNA-sequencing reveals significant obesity-associated alterations to the breast adipose

microenvironment of *BRCA* mutation carriers, including activation of estrogen biosynthesis, which impacts neighboring breast epithelial cells. We found that blockade of estrogen biosynthesis or estrogen receptor activity decreases DNA damage, whereas treatment with leptin or insulin increases DNA damage in *BRCA* heterozygous epithelial cells. Furthermore, we show that increased adiposity is associated with mammary gland DNA damage and increased penetrance of mammary tumors in *Brca1*+/- mice. Overall, our results provide mechanistic evidence in support of a link between bodyweight and breast cancer development in *BRCA* mutation carriers and suggests that maintaining a healthy bodyweight or pharmacologically targeting estrogen or metabolic dysfunction may reduce the risk of breast cancer in this population.

INTRODUCTION

Inheriting a pathogenic mutation in the DNA repair genes *BRCA1* or *BRCA2* is causally linked to the development of breast and ovarian cancer in women (*1, 2*). Although there is strong evidence linking obesity to the development of hormone receptor positive breast cancer after menopause in the general population (*3*), there are conflicting results in *BRCA* mutation carriers. Some studies have found that maintaining a healthy bodyweight or weight loss in young adulthood is associated with delayed onset of breast cancer (*4, 5*). Other studies have reported that adiposity or elevated bodyweight in adulthood is associated with increased cancer risk (*5-9*). Conversely, some reports indicate that increased body mass index (BMI) in young adulthood may have protective effects, and that risk is modified by menopausal status (*9-11*). The lack of clarity on the role of bodyweight and risk of breast cancer development in *BRCA* mutation

carriers limits the ability of clinicians to provide evidence-based guidance on prevention strategies beyond prophylactic surgical intervention.

Weight gain and obesity are often coupled with metabolic syndrome, insulin resistance, and significant changes to adipose tissue, including that of the breast microenvironment (*12-15*). Obesity-induced changes to breast adipose tissue includes dysregulation of hormone and adipokine balance, and increased production of inflammatory mediators (*16*). For example, estrogen biosynthesis is increased in obese breast adipose tissue due to overexpression of aromatase in adipose stromal cells which catalyzes the conversion of androgens to estrogen (*17-19*). Additionally, excessive expansion of adipocytes leads to hypoxia, lipolysis, and altered adipokine production including higher leptin to adiponectin ratio (*15, 20, 21*). These changes to the breast microenvironment may have important implications for breast carcinogenesis given that breast epithelial cells are embedded in this milieu and engage in epithelium-adipose crosstalk (*22*).

BRCA1 and BRCA2 are critical for their role in homologous recombination-mediated repair of DNA double strand breaks (*23*). Mutations in either *BRCA1* or *BRCA2* cause a defect in DNA repair which can lead to an accumulation of DNA damage and consequently, tumorigenesis (*24, 25*). Studies have linked obesity or metabolic syndrome to DNA damage, including in leukocytes (*26*), skeletal muscle (*27*), peripheral blood mononuclear cells (*28*), and in pancreatic β-cells (*29*), but no studies have examined the relationship between obesity and DNA damage in normal breast epithelial cells.

We show that BMI and markers of metabolic dysfunction are positively correlated with DNA damage in normal breast epithelium of women carrying a *BRCA* mutation, a finding that is extended to the fallopian tube of *BRCA* mutation carriers. RNA-sequencing of whole breast

tissue and of isolated breast epithelial organoids from *BRCA* mutation carriers, along with *ex vivo* and *in vitro* studies with *BRCA1* and *BRCA2* mutant primary tissues and cell lines, suggests several obesity-associated factors as possible drivers of DNA damage. Additionally, metformin, fulvestrant, leptin neutralizing antibodies and a PI3K inhibitor reduce damage induced by the obese breast microenvironment. *In vivo* studies in *Brca1* heterozygous knockout mice demonstrate that high fat diet-induced obesity leads to glucose intolerance in association with elevation in epithelial cell DNA damage and greater mammary tumor penetrance relative to mice fed a low fat diet. The data presented provide mechanistic evidence supporting an increased risk of breast cancer development in *BRCA* mutation carriers with elevated bodyweight and metabolic dysfunction, and importantly, provides clinically relevant strategies for risk reduction.

RESULTS

Obesity positively correlates with breast epithelial cell DNA damage in women carrying a mutation in *BRCA1* or *BRCA2*

To assess levels of DNA damage in normal breast epithelium in association with bodyweight in women carrying a *BRCA1* or *BRCA2* mutation, tissue microarrays were constructed from non-cancerous breast tissue obtained from 72 women undergoing mastectomy. The study population included *BRCA1* (n=43) and *BRCA2* (n=29) mutation carriers who had documented body mass index (BMI, kg/m^2) between 17.7 and 44.9 (median 23.7) at the time of surgery as shown in **Table 1**. When grouping the population by BMI category of lean (BMI $\leq$ 24.9 kg/m^2, n=46) or overweight/obese (BMI $\geq$ 25.0 kg/m^2, n=26), median age is significantly higher in the overweight/obese group compared to the lean group (44.5 vs 38.5, respectively, P=0.01). Additional clinical features elevated in the overweight/obese group compared to the

lean group include percent of subjects diagnosed with dyslipidemia (23.1% vs 2.2%, *P*=0.01) and with hypertension (23.1% vs 4.3%, *P*=0.02). The lean group also has a greater representation of pre-menopausal vs post-menopausal subjects compared to the overweight/obese group (*P*=0.04). Diagnosis of diabetes, race, presence of invasive tumor, tumor subtype and *BRCA*1 vs *BRCA*2 mutation were not significantly different between the two BMI groups (**Table 1**).

Immunofluorescence staining for the DNA double strand break marker γH2AX was performed with nuclear counterstain Hoechst to visualize the number of foci of DNA damage per epithelial cell (**Fig. 1A**). Among *BRCA1* and *BRCA2* mutation carriers, BMI was positively associated with breast epithelial cell DNA damage as quantified by # of γH2AX foci/100 cells (**Fig. 1B**). Age was also found to be significantly correlated with DNA damage (**Fig. 1C**). While this correlation diminished when adjusting for BMI (*P*=0.11, **Table 2**), BMI remained positively associated with DNA damage when adjusting for age (*P*=0.025, **Table 2**). Post-menopausal women were found to exhibit significantly higher levels of DNA damage compared to pre-menopausal women (**Fig. 1D**). Additionally, circulating levels of sex hormone binding globulin (SHBG), which binds estrogen to decrease its bioavailability, were negatively correlated with breast epithelial cell DNA damage (**Fig. 1E**). This negative association remains significant when adjusting for both BMI and age (*P*=0.047 and *P*=0.026, respectively, **Table 2**). Elevated BMI is often coupled to insulin resistance, a hallmark of metabolic dysfunction. Accordingly, fasting serum levels of insulin and HOMA2 IR were positively correlated with levels of breast epithelial cell DNA damage while glucose was not **(Fig. 1F-H)**. Insulin and HOMA2 IR retained significance after adjustments for either BMI or age (*P*<0.001 for both, **Table 2**). No correlation with DNA damage was observed for circulating biomarkers of inflammation including high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) or with crown-like structures

(CLS), a histological marker of local breast adipose inflammation (*30*) (**Fig 1. I-K**). These data indicate that among *BRCA* mutation carriers, elevated bodyweight is a risk factor for breast epithelial cell DNA damage. Furthermore, specific obesity-associated factors including insulin resistance and estrogen balance may be important drivers of this risk.

Elevated bodyweight is associated with significant differences in gene expression in breast adipose tissue and in breast epithelial cells of *BRCA* mutation carriers

To identify changes associated with obesity in breast epithelial cells and in the breast adipose microenvironment that may be linked to DNA damage, we conducted RNA-seq studies on both isolated primary breast epithelial cells and non-cancerous whole breast tissue obtained from *BRCA1* and *BRCA2* mutation carriers.

RNA-seq was conducted on breast tissue pieces obtained from lean (BMI $\leq$ 24.9kg/m^2, n=64) and overweight/obese (BMI $\geq$25 kg/m^2, n=67) *BRCA* mutation carriers. An unsupervised heatmap was constructed which shows general clustering of lean cases and clustering of overweight/obese cases by gene expression (**Fig. 2A**). 2329 genes were significantly upregulated by obesity and 1866 were significantly downregulated. Ingenuity Pathway Analysis (IPA) identified several pathways that were significantly altered in the overweight/obese cases which include pathways associated with obesity and metabolic dysfunction, such as “Phagosome Formation”, “LXR/RXR Activation”, “Tumor Microenvironment Pathway Activation”, and “Estrogen Biosynthesis” (**Fig. 2B**). A heatmap of genes involved in estrogen regulation shows a significant increase in many genes involved in the bioactivity, biosynthesis and activation of estrogens, including steroid sulfatase, 3βHSD1, AKR1C3, AKR1B15, 17βHSD1 and aromatase

(CYP19A1) (**Fig. 2C**). Conversely, gene expression of 17βHSD8, involved in estrogen inactivation, was significantly lower in overweight/obese relative to lean cases. Moreover, there were mixed effects of obesity on the expression of genes involved in estrogen catabolism to hydroxylated metabolites and neutralization by COMT.

To explore which changes in the breast microenvironment are associated with DNA damage in breast epithelial cells, we analyzed breast tissue pathway changes in relation to level of epithelial cell DNA damage quantified by γH2AX immunofluorescence staining (**Fig. 2D**; n=61). The level of epithelial cell DNA damage in each case was stratified by quartiles and breast tissue gene expression was compared in the highest quartile (Q4) relative to the lowest quartile (Q1), independent of BMI . The top 15 canonical pathways activated in Q4 vs Q1 breast tissue are shown (**Fig. 2D**) with several pathways being common to both DNA damage and BMI analyses (**Fig. 2D** vs **Fig. 2B**). Although the estrogen biosynthesis pathway was found to be activated in tissue from overweight/obese compared to lean cases (**Fig. 2B**, z-score=0.775, *P* value=1.14×10^{-6}), a stronger activation score is found when comparing Q4 vs Q1 (**Fig. 2D**, z-score=2.646, *P* value=2.7×10^{-3}), suggesting that tissue estrogen biosynthesis is highly correlated with level of breast epithelial cell DNA damage, irrespective of BMI.

Breast epithelial organoids were isolated from *BRCA* mutation carriers who were either lean (n=10) or overweight/obese (n=9) at the time of surgery. To validate and characterize the isolated epithelial organoids, immunofluorescence staining was conducted for cytokeratin 8 (CK8) and cytokeratin 14 (CK14), characteristic markers of luminal and basal epithelial cells, respectively, that are known to comprise the breast epithelium (**Fig. 2E**). 1144 genes were significantly upregulated in the setting of overweight/obesity and 537 were significantly downregulated compared to lean organoids. The top 20 canonical pathways identified by IPA as

183 regulated in the overweight/obese organoids are shown (**Fig. 2F**) and include activation of
184 pathways known to be associated with obesity, including "HIF1α signaling", "IL-8 signaling",
185 "ERK/MAPK signaling", and "PI3K/AKT signaling", among others.

186 Collectively, these RNA-seq studies show that *BRCA1* and *BRCA2* mutation carriers who
187 are overweight or obese have significantly altered breast epithelial cell and breast adipose
188 microenvironment gene expression compared with lean counterparts. Moreover, our data provide
189 rationale for further exploring whether estrogen is a driver of DNA damage in the breast.

190

191

192 **Crosstalk between epithelial cells and the breast adipose microenvironment**

193 Given the significant gene expression changes identified in *BRCA* heterozygous breast
194 adipose tissue and in breast epithelial cells in association with overweight/obesity, we next
195 investigated whether the breast adipose microenvironment drives gene expression in breast
196 epithelial cells. IPA Upstream Regulator tool was used to identify regulators of gene expression
197 differences in overweight/obese relative to lean organoids. To highlight endogenous factors that
198 may be responsible for driving gene expression changes, results were filtered to show the top 20
199 secreted factors. Among these factors, beta-estradiol (an estrogen) is the top predicted upstream
200 regulator (**Table 3**). A number of additional predicated upstream organoid regulators are
201 significantly upregulated in overweight/obese breast tissue, including several interleukins (IL2,
202 IL15, and IL5), TGFβ1, CSF1, ANGPT2, and WNT5A. Some factors, such as insulin, are known
203 to be elevated in obesity, but not produced locally in breast tissue and therefore do not have an
204 observed tissue gene expression level. These data suggest that some endogenously produced

205 factors in the overweight/obese breast microenvironment may interact with neighboring breast
206 epithelial cells to induce gene expression changes and DNA damage.

207

208 **Targeting estrogen in breast tissue from *BRCA* mutation carriers reduces epithelial cell**
209 **DNA damage**

210 Next, we conducted mechanistic studies to determine whether targeting estrogen
211 signaling or biosynthesis in breast tissue would lead to decreased levels of breast epithelial cell
212 DNA damage. We first conducted immunohistochemistry (IHC) staining to verify that normal
213 epithelium from *BRCA1* and *BRCA2* mutation carriers express the estrogen receptor (ERα).
214 Epithelial cells staining positively for ERα were found throughout the epithelium among carriers
215 of *BRCA1* or *BRCA2* mutations (representative images shown in **Fig. 3A, top row**). IF staining
216 was then conducted to visualize whether γH2AX foci co-localize with ERα positive cells.
217 Representative images are shown which highlight ERα positive cells frequently staining
218 positively for γH2AX foci (**Fig. 3A, bottom row**). Next, we tested whether disrupting estrogen
219 signaling through use of the drug fulvestrant, which degrades the estrogen receptor, would
220 impact levels of DNA damage in the breast. Breast tissue was obtained from *BRCA* mutation
221 carriers undergoing surgery (n=7) and were plated as explants in the presence of fulvestrant
222 (100μM) or vehicle for 24 hours (**Fig. 3B**). Explants were formalin fixed and sectioned for
223 assessment of breast epithelial cell DNA damage by IF staining. An approximately 32.5%
224 reduction in DNA damage was observed overall after treatment with fulvestrant (**Fig. 3C**).

225 Next, we hypothesized that targeting estrogen biosynthesis in the breast by
226 downregulating aromatase expression would lead to less estrogen exposure to the epithelial cells,
227 and consequently decreased DNA damage. In support of this hypothesis, RNA-seq data from
228 *BRCA1* and *BRCA2* mutation carriers showed a positive correlation between breast adipose

aromatase expression and level of breast epithelial cell DNA damage (**Fig. 3D**). Importantly, since aromatase expression is known to be upregulated in association with obesity, we conducted additional statistical analyses to adjust for BMI and found that aromatase remained independently positively correlated with DNA damage (P=0.037). To target estrogen biosynthesis, we utilized metformin, a widely used antidiabetic drug which has also been shown to decrease aromatase production in the breast via stimulation of AMP-activated protein kinase (AMPK) in adipose stromal cells (*31, 32*). Breast tissue obtained from *BRCA* mutation carriers (n=3) were plated as explants and treated with metformin (0-100μM) for 24 hours followed by IF assessment of breast epithelial cell DNA damage. A dose-dependent decrease in DNA damage was observed with significant differences after 75 and 100μM of metformin treatment (**Fig. 3E**). Since metformin is known to decrease aromatase expression in adipose stromal cells surrounding breast epithelial cells, we digested breast tissue to isolate the epithelial cells from their microenvironment (**Fig. 3B**) and treated them with metformin for 24 hours to determine if the presence of the breast microenvironment is required for the effect of metformin on DNA damage. Although there was a modest trend for reduction in DNA damage with increasing doses of metformin, these results were not significant (**Fig. 3F**). Consistently, tissue levels of estradiol (E2) were markedly reduced in breast explants after 24-hour metformin treatment in a dose-dependent manner (**Fig. 3G**). Additionally, testosterone and androstendione, which are converted to E2 and estrone (E1) by aromatase, respectively, were increased in explants following treatment with metformin while both E1 and E2 decreased (**Fig. 3H**). These data show that metformin treatment leads to decreased estrogen biosynthesis in breast tissue in association with reduction in epithelial cell DNA damage.

Local and systemic factors contribute to DNA damage in *BRCA1* and *BRCA2* heterozygous breast epithelial cells

Our data support a paracrine interaction between adipose tissue and breast epithelial cells. Having found a direct role for estradiol in mediating DNA damage in primary human tissues, we next explored the role of additional obesity-associated factors, including those present in breast adipose tissue conditioned media (CM), as well as recombinant leptin and insulin. To first investigate whether factors derived from breast adipose tissue have the ability to directly induce DNA damage in *BRCA* mutant breast epithelial cells, *BRCA1* heterozygous knockout MCF-10A cells were treated with CM from reduction mammoplasty or non-tumor quadrants of mastectomy tissue (**Fig. 4A**, n=36, BMI: 20.6-40.1 kg/m^2). Breast adipose CM treatment was positively correlated with DNA damage as a function of the patient's BMI, as measured by immunofluorescence of γH2AX foci (**Fig. 4B**). RNA-seq was performed in a subset of CM-treated samples (lean and obese, n=3/group). Results demonstrate that consistent with DNA damage measurements (**Fig. S1**), IPA analysis of differentially regulated genes in the obese CM treated cells relative to lean showed increased activation of functions associated with DNA damage and genomic instability including "Formation of micronuclei", "Chromosomal instability", and "Breakage of chromosomes". Alternatively, activation of functions associated with DNA repair were decreased, including "Repair of DNA" and "Checkpoint control" (**Table 4**).

To determine whether effects of CM on DNA damage were generalizable to *BRCA2* mutation carriers, a subset of CM cases were tested in MCF-10A cells carrying a heterozygous *BRCA2* mutation, generated using CRISPR-Cas9 gene editing (see Supplementary Materials and Methods). A positive correlation between BMI and DNA damage was also observed in these

cells (**Fig. 4C**). These studies demonstrate that factors secreted by breast adipose tissue directly stimulate DNA damage in breast epithelial cells. Furthermore, given the lack of estrogen receptor expression in MCF-10A cells (*33*), these studies also highlight the existence of additional factors beyond estrogen that may be contributing to DNA damage induction in the setting of obesity in *BRCA1* and *BRCA2* mutant breast epithelial cells.

The expression of leptin, known to be directly correlated with adiposity, is significantly higher in overweight/obese compared to lean breast tissue from *BRCA* mutation carriers (Table S1, log2FC= 0.61, P=3.48x10^{-6}). A number of studies have found leptin to have pro-mitogenic and anti-apoptotic effects in breast cancer cells (*34-37*). However, its effects on normal breast epithelial cells are less well characterized. Here, we treated both *BRCA1* and *BRCA2* heterozygous MCF-10A cells with leptin (400ng/mL) for 24 hours and found a significant induction of DNA damage in both cell lines (**Fig. 4D**) and in primary breast epithelial cells (**Fig. 4E**). Additionally, the ability of obese CM to induce DNA damage in *BRCA1* heterozygous breast epithelial cells is blocked when treating in the presence of a leptin neutralizing antibody (**Fig. 4F**).

Next, having identified insulin as positively correlated with DNA damage in tissue microarrays from *BRCA* mutation carriers, independent of BMI (**Fig. 1F, Table 2**), and as a top upstream regulator of gene expression in primary breast epithelial organoids from overweight/obese women (**Fig. 3A**), we conducted additional mechanistic studies to determine whether insulin can directly induce DNA damage. Treatment of *BRCA1* and *BRCA2* heterozygous knockout MCF-10A cells with insulin (100nM) for 24 hours resulted in a significant increase in DNA damage in both cell lines (**Fig. 4G**) and in primary breast epithelial cells (**Fig. 4H**). Both leptin and insulin have been shown to act via PI3K (*38, 39*). Treatment of

BRCA1 heterozygous breast epithelial cells with a PI3K inhibitor, BKM120 (1μM), was effective at reducing obese CM-induced DNA damage (**Fig. 4I**). These data show that factors produced locally by obese breast adipose tissue or elevated with metabolic dysfunction contribute to induction of DNA damage in *BRCA* heterozygous knockout breast epithelial cells.

High fat diet feeding is associated with elevated mammary gland DNA damage and early tumor penetrance in female *Brca1* heterozygous knockout mice

DNA damage is a known driver of chromosomal defects that can lead to cancer. However, whether obesity-associated elevation in breast epithelial cell DNA damage is linked to breast cancer penetrance in the setting of a heterozygous *BRCA* mutation has not been established. To investigate this question, we conducted preclinical studies utilizing mice that were developed to carry a whole-body heterozygous loss in *Brca1* (*Brca1*+/-) on a C57Bl/6 background. Four-week-old female *Brca1*+/- mice were randomized to receive low fat diet (LFD) or high fat diet (HFD) for 22 weeks to produce lean and obese mice, respectively (**Fig. 5A**). Mice fed HFD gained significantly more weight than LFD fed mice and weighed on average 34.1g vs 23.3g, respectively, at the time of sacrifice (**Fig. 5B**). Overall adiposity was also increased in association with HFD feeding as determined by greater accumulation of subcutaneous and visceral fat compared to the LFD group (**Fig. S2)**. To confirm that the HFD-fed mice exhibit altered metabolic homeostasis in our *Brca1*+/- model of diet-induced obesity, glucose tolerance tests were conducted after 21 weeks on experimental diets, highlighting delayed clearance of glucose from blood over 90 minutes post-intraperitoneal injection of glucose in the HFD group compared to LFD-fed mice (**Fig. 5C & D**). To determine whether changes observed in the mammary fat pad of *Brca1*+/- mice in response to feeding were

322 analogous to those seen in the breast tissue of women in relation to obesity, RNA-seq was
323 conducted on inguinal mammary fat pads from LFD and HFD mice harvested at sacrifice (**Table**
324 **S5**). IPA was used to identify activation of the top differentially regulated canonical pathways in
325 HFD mammary fat pads relative to LFD, results of which were juxtaposed with regulation of
326 these same pathways in human breast tissue from overweight/obese vs lean *BRCA* mutation
327 carriers. The top 20 canonical pathways regulated by obesity in the mouse mammary fat pad
328 show very similar regulation patterns compared to overweight/obese human breast tissue (**Fig.**
329 **5E**), suggesting that diet-induced obesity in our *Brca1*+/- mice can serve as a model system for
330 obesity in women carrying a *BRCA* mutation with respect to studies of the breast.
331 Similar to findings made in human breast tissue from *BRCA* mutation carriers, IF staining
332 for γH2AX of *Brca1*+/- mouse mammary glands at the time of sacrifice show that HFD-fed mice
333 have elevated levels of mammary gland DNA damage compared to LFD-fed mice (**Fig. 5F**).
334 Furthermore, there is a trend for a positive correlation between DNA damage and bodyweight
335 (irrespective of diet) (**Fig. 5G**) and a significant positive correlation between DNA damage and
336 mammary fat pad weight (**Fig. 5H)**, suggesting that level of adiposity may be a stronger
337 predictor of DNA damage in mammary epithelium compared to whole body weight.
338 Next, we examined whether elevation in mammary gland DNA damage is associated
339 with tumorigenesis. Female *Brca1*+/- mice were first made obese by HFD-feeding for 10 weeks
340 and then were implanted with a subcutaneous medroxyprogesterone acetate (MPA) pellet to
341 sensitize them to mammary tumor development upon exposure to three doses of the carcinogen
342 7,12-dimethylbenz[a]anthracene (DMBA) (**Fig. 5I**). Mammary tumors developed earlier in the
343 HFD group compared with the control LFD group (**Fig. 5J**). Additionally, 85.7% of mice in the

HFD group developed mammary tumors by the end of the 28-week surveillance period compared to 69.2% of mice in the LFD group (**Fig. 5K**).

Obesity is associated with DNA damage in the fallopian tube, but not ovary, of *BRCA* mutation carriers

In addition to elevated breast cancer risk, women carrying a *BRCA1* or *BRCA2* mutation have high lifetime risk for developing ovarian cancer (*1, 2*). Since weight gain is associated with increased risk of ovarian cancer in *BRCA* mutation carriers (*40*), we extended our studies in the breast to investigate the impact of elevated BMI on DNA damage in the ovarian epithelium as well as in epithelial cells of the fallopian tube (**Fig. 6**). IF staining for γH2AX was performed with nuclear counter stain Hoechst to quantify number of foci of DNA damage per epithelial cell in non-tumorous ovarian tissue and fallopian tube fimbria from women carrying a *BRCA1* or *BRCA2* mutation undergoing prophylactic salpingo-oophorectomy. In the ovarian epithelium, there was no increase in DNA damage in the overweight/obese cases (n=12) compared to the lean cases (n=21) (**Fig. 6A**, P=0.59). However, there was a significant increase in DNA damage observed in the epithelial cells of the fallopian tube from overweight/obese women (n=9) compared to lean women (n=17) (**Fig. 6B**, P=0.03).

DISCUSSION

The data presented here demonstrate that BMI is positively associated with DNA damage in normal breast epithelial cells in carriers of a mutation in *BRCA1 or BRCA2*. Beyond BMI, insulin and insulin resistance, as measured by HOMA2 IR, were independently associated with DNA damage, irrespective of BMI or age. Accordingly, it is possible that *BRCA* mutation

carriers who are defined as lean by BMI, but hyperinsulinemic ('metabolically obese'), may also be at risk for elevated levels of DNA damage and consequently, breast cancer development. Although previous studies have shown that inflammation can lead to DNA damage in both normal and cancerous cells in other tissues (*41-44*), our data do not support a link between local or systemic inflammation and breast epithelial cell DNA damage.

To our knowledge, this is the first study to conduct transcriptional profiling of non-cancerous breast tissue and isolated breast epithelial cells from overweight/obese vs lean *BRCA* mutation carriers. While several factors and pathways associated with metabolic dysfunction were shown to be upregulated in breast tissue and in epithelial cells, the identification of pathways related to estrogen biosynthesis (tissue) and signaling (epithelial cells) were of particular interest given the availability of clinically approved drugs that target estrogen. Additionally, previous *in vitro* studies showed that treatment with estrogen and estrogen metabolites induced DNA damage in *BRCA1* heterozygous breast epithelial cells (*45*), providing further rationale for exploring the role of estrogen as a mediator of obesity-induced DNA damage. Here, we show that fulvestrant, an estrogen receptor degrader, is effective at reducing epithelial cell DNA damage in breast tissue explants from *BRCA* mutation carriers. However, this drug is not currently approved for use in the prevention setting and the side effects may limit its future use for this purpose. Alternatively, metformin is widely prescribed in patients with type II diabetes and has an excellent safety profile which makes this drug an intriguing option for preventative use in *BRCA* mutation carriers with excess bodyweight. We show that metformin was effective at reducing breast epithelial cell DNA damage at clinically relevant concentrations primarily due to effects on the breast adipose microenvironment. Previous studies have shown that metformin decreases adipose stromal cell expression of aromatase through activation of

AMPK (*31, 32*). Our study extends these findings by demonstrating the downstream consequence of downregulation in aromatase through mass spectrometry studies which showed marked reduction in E2 in breast tissue after metformin treatment. In addition to reducing estrogen exposure, previous work has shown that metformin treatment reduces endogenous reactive oxygen species (ROS) and associated DNA damage (*46*) in a mammary epithelial cell line, providing an additional possible mechanism for the effects of metformin in our studies.

Epidemiological studies have reported decreased risk of breast cancer in *BRCA* mutation carriers in association with reduced estrogen exposure achieved via salpingo-oophorectomy surgery which diminishes ovarian estrogen production or through treatment with tamoxifen, an estrogen receptor antagonist in the breast (*47-49*). Our studies propose estrogen-mediated induction of DNA damage as a possible explanation for the protective effects observed by decreasing estrogen exposure in this population. Estrogen can induce DNA damage through various actions as reviewed by our group and others (*50, 51*), including through ligand binding to ERα which stimulates proliferation and potentially replication stress with ROS production as a byproduct of increased cellular respiration. Additionally, the metabolism of estrogen yields genotoxic metabolites, a process which produces ROS through redox cycling. These metabolites can directly interact with DNA to form adducts in an ER-independent manner. Given the multiple avenues through which estrogen can induced DNA damage in cells, additional studies are warranted to characterize the mechanisms of estrogen-induced DNA damage in breast epithelial cells from *BRCA* mutation carriers in the setting of obesity.

Interestingly, our RNA-seq analysis of *BRCA1* heterozygous MCF-10A cells treated with obese vs lean CM not only showed increased activation of pathways associated with DNA damage, but also downregulation of pathways associated with DNA repair. This raises the

414 possibility that obesity may affect DNA repair capacity, which would be especially detrimental
415 in cells already exhibiting defective DNA repair due to a mutation in *BRCA1* or *BRCA2*.
416 Additional studies exploring the relationship between obesity and DNA repair would be relevant
417 not only for *BRCA* mutation carriers, but also for the general population where obesity is
418 associated with increased breast cancer risk in post-menopausal women (*3*). Defective DNA
419 repair capacity would illuminate a novel mechanism through which obese non-carriers become
420 more susceptible to breast cancer.

421 Our *in vitro* studies demonstrate the ability of several obesity-associated factors,
422 including leptin and insulin, to cause DNA damage, suggesting a collective milieu of factors that
423 may contribute to the elevation in DNA damage observed in *BRCA* mutation carriers in
424 association with BMI. The ability of obese CM to induce damage in *BRCA1* heterozygous cells
425 was diminished when treating in the presence of an antibody or drug that inhibits leptin or insulin
426 signaling, respectively. Of note, since insulin signals through phosphatidylinositol 3-kinases
427 (PI3K), we utilized BKM120, a PI3K inhibitor, to disrupt insulin actions in the presence of obese
428 CM. It is possible that inhibiting PI3K signaling not only disrupted insulin signaling, but also
429 signaling of other factors associated with obesity that act through PI3K, including growth factors
430 or leptin, which collectively contributed to the observed decrease in DNA damage. Additionally,
431 growing evidence points to a role for the PI3K pathway in the DNA damage response, however,
432 these studies have been limited to cancer cells (*52-55*).

433 Our studies also show a link between obesity-induced DNA damage and tumor
434 development using a *Brca1*+/- mouse model of diet-induced obesity. HFD-fed mice exhibited
435 elevated mammary gland DNA damage in association with decreased latency and increased
436 overall penetrance of mammary tumors when exposed to the carcinogen DMBA. These data

suggest that the elevation in DNA damage that we observed in association with BMI in women carrying a *BRCA* mutation may also be associated with increased breast cancer penetrance. The extent to which data from this mouse model can be extrapolated to humans is somewhat limited given that we employed a carcinogen-indued tumor model, whereas in *BRCA* mutation carriers, tumors will arise after years of exposure to both endogenous and environmental factors, some of which will act as carcinogens.

Finally, our data show that obesity-associated DNA damage may not only be limited to the breast epithelium of *BRCA* mutation carriers. Although no increase in DNA damage was found in epithelial cells of the ovary in overweight/obese women undergoing prophylactic salpingo-oophorectomy, we did observe a significant increase in DNA damage in the epithelial cells of the fallopian tube in overweight/obese women. Our results are consistent with reports from recent years which point to the fallopian tube as the likely site of origin of ovarian cancer (*56, 57*), to be confirmed by ongoing clinical trials of risk-reducing salpingectomy with delayed oophorectomy, and also highlights a potential mechanism for the link between weight gain and ovarian cancer in this population.

A limitation of our study includes a cohort size of n=72 in our correlation study of DNA damage and BMI which prevented us from analyzing effects of BMI separately in *BRCA1* and *BRCA2* mutation carriers. Although both BRCA1 and BRCA2 are essential for DNA repair, their roles in the DNA damage response are not identical and each mutation is associated with different subtypes of tumor development. Larger studies assessing the relative effect of BMI on DNA damage in *BRCA1* and *BRCA2* mutation carriers separately could provide additional information to help personalize risk estimates. Additionally, levels of estrogens vary considerably during the menstrual cycle and impact proliferation of breast epithelial cells. Our

studies did not account for phase of menstrual cycle when assessing DNA damage which may have led to increased variability in our data, particularly considering our identification of estrogen as a mediator of obesity-induced epithelial cell DNA damage.

Many methodological challenges exist which explain the lack of consensus in epidemiological studies attempting to ascertain modifiers of breast cancer risk in *BRCA* mutation carriers, as reviewed by Milne & Antinou (*58*). Although a number of studies have associated bodyweight with increased risk of breast cancer as discussed earlier, the largest study to date to contradict these findings showed protective effects of BMI on pre-menopausal breast cancer risk in *BRCA* mutation carriers (*11*). Drawing definitive conclusions from this study is limited due to the utilization of subject-reported BMI at the time of study questionnaire which is subject to recall bias and utilization of calculated genetic BMI score which does not necessarily predict actual observed BMI and may be influenced by dietary and environmental factors. Additionally, a subset of the overweight/obese population may have received treatment for obesity-associated co-morbidities like diabetes which potentially confounds risk assessment if these treatments or medications reduce breast cancer risk. Overall, given the inconsistencies in reported data and significant challenges in assessing modifiers of breast cancer risk in this population, the consensus to date is that there is insufficient evidence to determine the effect of bodyweight on breast cancer risk in *BRCA* mutation carriers (*58-60*). Therefore, a strength of our study is the presentation of mechanistic experimental evidence which helps to elucidate the relationship between bodyweight and breast cancer risk in this population.

Additionally, our findings provide rationale for conducting clinical trials in overweight/obese *BRCA* mutation carriers to test the efficacy of pharmacological interventions that target metabolic health, weight and/or estrogens. In fact, identifying which obesity-related

factors need to be targeted for risk reduction, if not all, will have a meaningful impact on developing effective risk reduction strategies. Although recently reported results of the phase 3 randomized MA.32 trial (NCT01101438) found that addition of metformin to standard of care in non-diabetic patients with high-risk breast cancer did not significantly improve invasive disease-free survival vs placebo (*61*), it remains to be determined if metformin in the preventative setting would be effective at reducing risk of breast cancer, particularly among *BRCA* mutation carriers and those with metabolic dysfunction. Our studies point towards the potential of metformin in this setting, as it has been shown to reduce weight, as well as cause decreases in circulating levels of insulin, leptin and estrogens (*62-64*). These studies would help clarify whether accumulation of DNA damage over time is reversable or if targeted interventions prevent accumulation of further damage. Positive results would offer clinicians actionable evidence-based prevention strategies for patients in this high-risk population who opt to delay or forgo risk-reducing surgery.

MATERIALS AND METHODS

Study Design

The objective of this study was to gain insight into the role of obesity and metabolic dysfunction on breast cancer penetrance among carriers of germline mutations in *BRCA1* and *BRCA2* and to identify clinically relevant prevention strategies. Clinical samples including both archival tissues and prospectively collected tissues from *BRCA* mutation carriers, as well as cell lines engineered to carry a *BRCA1* or *BRCA2* heterozygous knockout mutation and *Brca1*+/- mouse models were utilized in support of this objective. All studies utilizing human tissues were

conducted in accordance with protocols approved by the Institutional Review Boards of Memorial Sloan Kettering Cancer Center (MSKCC) under protocol #10-040 and Weill Cornell Medicine under protocols #1510016712, 1004010984-01 and 1612017836. Informed consent from each subject was obtained by study investigators prior to tissue collection. Animal experiments were conducted in accordance with an approved Institutional Animal Care and Use Committee protocol (#2018-0058) at Weill Cornell Medicine.

Studies utilizing archival tissues were coded and DNA damage was analyzed in a blinded fashion. Studies utilizing prospectively collected tissues and *in vitro* treatment studies were not blinded, however, DNA damage was analyzed by immunofluorescence staining using methodology to limit bias as described in the section "*Confocal microscopy & quantification of γH2AX foci*" below. Sample size power calculations were performed for human breast tissue microarray construction (BMI vs DNA damage study) and in animal studies. Any sample exclusion criteria are described in the sections below or in the figure legends.

Human breast tissue microarray construction & study population

Archival paraffin blocks of embedded non-tumorous breast tissue were obtained from 72 women carrying a *BRCA1* (n=42) or *BRCA2* (n=30) mutation who had previously undergone prophylactic or therapeutic mastectomy at MSKCC from 2011-2016. **Table 1** describes the clinical characteristics of the study population which were extracted from electronic medical records. BMI was calculated using height and weight recorded prior to surgery (kg/m^2) and menopausal status was determined per criteria established by the National Comprehensive Cancer Network ((*65*). A pathologist reviewed hematoxylin & eosin-stained sections from each block to identify areas enriched in breast epithelium. Cores measuring 1.5mm in diameter from

identified epithelial areas of each case were incorporated into paraffin blocks for the construction of tissue microarrays. Each tissue microarray was constructed with cases representing an equal distribution of clinical characteristics, including *BRCA1* or *BRCA2* mutation status and BMI. Unstained sections were cut from each tissue microarray and used for quantification of breast epithelial cell DNA damage by immunofluorescence staining as described in the section below.

Assessment of DNA damage by immunofluorescence staining

To quantify epithelial cell DNA damage, immunofluorescence staining of the DNA double strand break marker γH2AX was conducted on human tissue sections, mouse mammary gland tissue sections, or plated cells. Antibodies/reagents that were used include: primary γH2AX (p Ser139) antibody (Novus Biologicals #NB100-74435 unless otherwise stated) at 1:300 dilution, Goat anti-Mouse Alexa Fluor 546 secondary antibody (Life technologies #A11030) at 1:1000 dilution, Hoechst 33342 nuclear stain (Santa Cruz Biotechnology #SC-495790) at 1:1000 dilution, CAS block (Life Technologies #008120), M.O.M (Mouse-on-Mouse) immunodetection kit (Vector Laboratories # BMK-2202), and ProLong Gold Antifade Mountant (Invitrogen # P36934). Full staining procedures for tissue sections, plated cells, and co-localization studies can be found in the Supplementary Materials and Methods.

Confocal microscopy & quantification of γH2AX foci

Tissue slides or plated epithelial cells stained with γH2AX and Hoechst were imaged using a Zeiss LSM 880 confocal microscope. Confocal settings were not changed across samples within each experiment. Areas to image were first selected based on identification of regions rich in breast epithelial cells as determined by Hoechst staining prior to viewing the γH2AX channel

554 to limit any potential bias in image selection. Images were exported to the image analysis
555 software Imaris (Oxford Instruments) for semi-automated quantification of γH2AX foci per 100
556 cells. Imaris analysis settings were programmed to identify and quantify total cell number in
557 each image and to identify number of γH2AX foci co-localizing with nuclei. All Imaris-analyzed
558 images were visually inspected by investigators to ensure appropriate identification of γH2AX
559 foci and exclusion of background staining. A minimum of 100 cells per case or condition were
560 analyzed and DNA damage was reported as # of γH2AX foci per 100 cells. Any sample with less
561 than 100 cells detected were excluded.

562

563 **Quantification of blood biomarkers**

564 Fasting blood was collected from patients prior to surgery. Serum was separated by
565 centrifugation, aliquoted, and stored at -80°C. Enzyme-linked immunosorbent assay was used to
566 measure serum levels of insulin (Mercodia, Uppsala, Sweden), hsCRP, glucose, SHBG, and IL-6
567 (R&D Systems, Minneapolis, MN) following the manufacturer's protocols.

568

569 **RNA-Seq studies & computational analysis:**

570 RNA-Sequencing (RNA-Seq) was conducted on samples in 4 studies including: breast
571 tissue from *BRCA* mutation carriers, isolated breast epithelial organoids from *BRCA* mutation
572 carriers, breast adipose tissue conditioned media (CM)-treated *BRCA*1 heterozygous MCF-10A
573 cells, and *Brca1*+/- mouse mammary fat pads. Details on RNA extraction, sequencing
574 methodology, and computational analyses can be found in the Supplementary Materials and
575 Methods.

Isolation of primary breast epithelial cells and breast explant studies

For *ex vivo* tissue explant studies and isolation of breast epithelial cells, breast tissue was obtained from women undergoing breast mammoplasty or mastectomy surgeries at Weill Cornell Medicine and MSKCC from 2017-2021. Surgical specimens were transferred from the operating room to a pathologist who evaluated the breast tissue to confirm that the tissue distributed for experimentation was normal and uninvolved with any quadrant where a tumor may have been present. The tissue was then brought to the laboratory and utilized in the experiments as described below.

Isolation of breast epithelial cells

Approximately 25mL of breast tissue was utilized in each organoid preparation with care taken dissect out overly fibrous areas or visible blood vessels. The tissue was finely minced and mixed with complete Ham's F12 media (Corning #10-080-CV, supplemented with 10% FBS and 1% penicillin/streptomycin) containing a digestion mix of 10mg/mL collagenase type 1 (Sigma Aldrich #C0130) and 10µg/mL hyaluronidase (Sigma Aldrich #H3506) in a total volume of 50mL. The tissue was digested overnight on a rotator at 37°C and then centrifuged. The supernatant containing free lipid and adipocytes was discarded and the pellet was washed and reconstituted with in media followed by incubation at 4°C for 1 hour to ensure inhibition of enzyme activities. After centrifugation, the pellet was treated with red cell lysis buffer (Sigma Aldrich #11814389001), pelleted, reconstituted in media, and then ran through a 100µM filter followed by 40µM filter. Breast epithelial organoids were collected from the top of the 40µM filter in mammary epithelial cell growth medium with added supplements (PromoCell #C-

21010). Isolated mammary epithelial organoids were snap frozen in liquid nitrogen for RNA extraction and RNA-sequencing or plated for *in vitro* studies.

Ex vivo metformin and fulvestrant explant studies

To examine the role of breast adipose tissue estrogen in mediation of DNA damage in *BRCA* mutant epithelial cells, breast explants were treated with drugs targeting estrogen signaling (fulvestrant) or production (metformin). 1 cm breast tissue explants were cut from breast tissue transferred after surgery and were plated in replicate in a 12-well dish. Metformin studies: Breast explants from n=3 subjects were cultured in complete Ham's F12 media (10% FBS, 1% penicillin/streptomycin) supplemented with either vehicle (methanol) or metformin hydrochloride (25-100µM, Sigma #PHR1084). Fulvestrant studies: Breast explants from n=7 subjects were cultured in basal mammary epithelial cell growth media + 0.1% BSA containing either vehicle (ethanol) or 100uM fulvestrant (Sigma #I4409).

After 24 hours of treatment at 37°C in a 5% CO_2 incubator, explants were snap frozen in liquid nitrogen and formalin fixed and paraffin embedded. Tissue sections were cut from each paraffin block for assessment of breast epithelial cell DNA damage by immunofluorescence staining.

Collection of breast adipose tissue conditioned media

Conditioned media (CM) was generated from breast tissue obtained from n=36 women with BMIs that range from lean to obese (20.6 – 49.1 kg/m^2). Ten 1 cm explant pieces of breast adipose tissue were cut from each case with a focus on fatty areas containing no visible blood vessels. The pieces were weighed and placed on a 10cm dish with 10mL of basal (phenol red

free, serum free, and supplement mix free) mammary epithelial cell growth media (PromoCell #C-21215) containing 0.1% BSA. The explants were incubated at 37°C for 24 hours. After incubation the breast adipose tissue CM was collected and centrifuged at 300xg. The supernatant was aliquoted and stored -80°C for use in *in vitro* treatment studies.

In vitro **studies in MCF-10A cells**

Non-cancerous breast epithelial cell line MCF-10A carrying a *BRCA1* heterozygous mutation (185delAG/+) was purchased from Horizon Discovery and have been previously described (*66*). MCF-10A cells carrying a *BRCA2* heterozygous mutation (6174delT/+) were generated in-house using CRISPR/Cas9 gene editing (additional details provided in the Supplementary Materials and Methods). Cells were cultured in DMEM/F12 (Invitrogen #11330-032) supplemented with 5% FBS, 1% penicillin/streptomycin and the following growth factors: 20ng/mL EGF, 0.5mg/mL hydrocortisone, 100ng/mL cholera toxin, and 10µg/mL insulin (all purchased from Sigma Aldrich). Cells were serum starved for 16 hours prior to treatments.

In CM studies, CM was thawed on ice from each case and diluted to a final concentration of 25% CM. In leptin studies, cells were treated with 400ng/mL of human recombinant leptin (Sigma #L4146). In leptin neutralization studies, obese CM was pre-incubated with a leptin neutralizing antibody (Lep ab, 13.3µg/mL, Fisher Scientific #AF398) for 1 hour at 4°C and then cells were treated with lean or obese CM alone or obese CM + Lep ab. In insulin studies, cells were treated with 100nM insulin (Sigma #I1882). To block insulin signaling, cells were pre-treated with the PI3K inhibitor BKM120 (1uM, MedChemExpress #HY-70063) for 1 hour and then then treated with obese CM + BKM120. All treatments were conducted in replicates or

644 triplicates for 24 hours unless otherwise stated. After treatment, all wells were fixed with ice cold
645 methanol followed by γH2AX IF staining.

646

647 **_Brca1_+/- mouses studies**

648 *Generation of Brca1+/- mice*

649 To determine if obesity impacts mammary gland DNA damage and tumor penetrance in
650 the setting of a *Brca* mutation, *Brca1* heterozygous (*Brca1*+/-) mice were generated on a
651 C57BL/6 background as described in the Supplementary Materials and Methods.

652

653 *Diet-induced obesity & mammary gland DNA damage*

654 At 4 weeks of age, 24 female *Brca1*+/- mice were randomized to one of two groups
655 (n=12/gp). One group was fed 10 kcal% low fat diet (LFD, 12450Bi, Research Diets) and the
656 second group was fed 60 kcal% high fat diet (HFD, D12492i, Research Diets) *ad libitum* for 22
657 weeks until sacrifice. One week prior to sacrifice all mice were fasted overnight for 12 hours and
658 underwent glucose tolerance tests to confirm obesity-induced metabolic dysfunction as
659 previously described. In brief, baseline glucose measurements were taken from tail vein blood
660 drop collection using a handheld glucose meter (Bayer Contour). Mice then received an
661 intraperitoneal injection of 1g/kg glucose and tail vein blood glucose levels were recorded at 15-
662 30 minute intervals over 90 minutes. Following the final measurement respective experimental
663 diets were re-started *ad libitum* for an additional week prior to sacrifice. Mice were euthanized
664 via CO_2 inhalation and mammary gland tissue was collected and snap frozen (inguinal fat pads)
665 for RNA-Seq or fixed (thoracic fat pads) in 10% neutral buffered formalin overnight prior to
666 paraffin embedding and sectioning for histological assessment of DNA damage.

667

668 *MPA/DMBA tumor model*

669 To investigate how obesity impacts mammary gland tumor development in *Brca1*+/-
670 mice the same diet-induced obesity model as described above was utilized. At 4 weeks of age, 27
671 female *Brca1*+/- mice were randomized to one of two groups (n=13-14/gp). One group was fed
672 LFD and the second group was fed HFD for the duration of the study. At 14 weeks of age (after
673 10 weeks on experimental diets) all mice were surgically implanted with a 40mg
674 medroxyprogesterone acetate (MPA) pellet (90-day continuous release, Innovative Research of
675 America, #NP-161) placed subcutaneously. At 15, 16, and 17 weeks of age all mice were dosed
676 with 1mg/22g bodyweight of the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) delivered
677 by oral gavage in corn oil once per week for 3 consecutive weeks. Mammary tumor development
678 and growth were monitored weekly by palpating all 5 mammary gland pairs and recording tumor
679 presence and size with caliper measurements for 28 weeks following the last dose of DMBA.
680 Mice were euthanized at the end of the 28-week surveillance period or earlier based on ethical
681 endpoints, including tumor burden reaching 1.5cm. Mice that did not recover from pellet
682 implantation surgery or displayed morbidity unrelated to mammary tumors were excluded from
683 the study.

684

685 **Quantitative steroid analysis in breast explants**

686 Quantification of steroid levels (E2, E1, testosterone, and androstendione) in snap frozen
687 breast adipose tissue explants treated with metformin was performed using gas chromatography-
688 mass spectrometry (GC-MS)-based steroid profiling as previously described (*67, 68*). Detailed
689 protocol included in the Supplementary Materials.

690

691 **Statistical analysis**

692 To assess significant differences in baseline clinical characteristics and categorical
693 variables the Fisher exact test was used. To test the strength of correlation between DNA damage
694 and continuous variables, nonparametric Spearman's rank correlation coefficient was used with
695 two-tailed *P* value to determine significance of correlations. A multivariable linear model was
696 used to test the association between the level of DNA damage and clinical characteristics
697 adjusting for BMI or age. Two-tailed Mann Whitney test was performed on clinical data testing
698 significant differences between two groups. Two-tailed student's t-test was used *in vitro*
699 treatment studies and in mouse studies comparing two groups. All results were performed using
700 R (version 4.0.5) or GraphPad Prism 9. Results with a *P*-value < 0.05 were considered
701 statistically significant.

702

703 **Supplementary Materials**

704 Materials and Methods

705 **Fig. S1.** Breast adipose conditioned media from obese women stimulate more DNA damage in
706 *BRCA1*+/- MCF-10A cells compared to conditioned media from lean women.

707 **Fig. S2.** *Brca1*+/- mice fed high fat diet have significantly greater accumulation of body fat
708 compared to *Brca1*+/- mice fed low fat diet

709 **Fig. S3.** Generation of MCF-10A cells carrying a *BRCA*2 heterozygous mutation

710 **Data file S1:** Tables S1-5: Full list of differentially expressed genes in presented RNA-seq
711 studies (multi-tab Excel file).

712 **Data file S2:** Original data for experiments presented

713 References (*69-80*)

714
715
716 **References**

717 1. K. B. Kuchenbaecker, J. L. Hopper, D. R. Barnes, K.-A. Phillips, T. M. Mooij, M.-J.
718 Roos-Blom, S. Jervis, F. E. Van Leeuwen, R. L. Milne, N. Andrieu, D. E. Goldgar, M. B.
719 Terry, M. A. Rookus, D. F. Easton, A. C. Antoniou, L. Mcguffog, D. G. Evans, D.
720 Barrowdale, D. Frost, J. Adlard, K.-R. Ong, L. Izatt, M. Tischkowitz, R. Eeles, R.
721 Davidson, S. Hodgson, S. Ellis, C. Nogues, C. Lasset, D. Stoppa-Lyonnet, J.-P. Fricker,
722 L. Faivre, P. Berthet, M. J. Hooning, L. E. Van Der Kolk, C. M. Kets, M. A. Adank, E.
723 M. John, W. K. Chung, I. L. Andrulis, M. Southey, M. B. Daly, S. S. Buys, A. Osorio, C.
724 Engel, K. Kast, R. K. Schmutzler, T. Caldes, A. Jakubowska, J. Simard, M. L.
725 Friedlander, S.-A. Mclachlan, E. Machackova, L. Foretova, Y. Y. Tan, C. F. Singer, E.
726 Olah, A.-M. Gerdes, B. Arver, H. Olsson, Risks of Breast, Ovarian, and Contralateral
727 Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *JAMA* **317**, 2402-2416 (2017).
728 2. S. Chen, G. Parmigiani, Meta-Analysis of BRCA1 and BRCA2 Penetrance. *Journal of*
729 *Clinical Oncology* **25**, 1329-1333 (2007).
730 3. B. Lauby-Secretan, C. Scoccianti, D. Loomis, Y. Grosse, F. Bianchini, K. Straif, Body
731 Fatness and Cancer — Viewpoint of the IARC Working Group. *New England Journal of*
732 *Medicine* **375**, 794-798 (2016).
733 4. M. C. King, J. H. Marks, J. B. Mandell, G. New York Breast Cancer Study, Breast and
734 ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* **302**,
735 643-646 (2003).

5. J. Kotsopoulos, O. I. Olopade, P. Ghadirian, J. Lubinski, H. T. Lynch, C. Isaacs, B. Weber, C. Kim-Sing, P. Ainsworth, W. D. Foulkes, A. Eisen, P. Sun, S. A. Narod, Changes in body weight and the risk of breast cancer in BRCA1 and BRCA2mutation carriers. *Breast Cancer Research* **7**, (2005).

6. E. Bruno, A. Oliverio, A. Paradiso, A. Daniele, S. Tommasi, D. A. Terribile, A. Filippone, M. Digennaro, B. Pilato, K. Danza, D. Guarino, C. Rossi, M. M. Rossi, E. Venturelli, M. Giussani, B. Peissel, P. Pasanisi, Lifestyle Characteristics in Women Carriers of BRCA Mutations: Results From an Italian Trial Cohort. *Clin Breast Cancer* **21**, e168-e176 (2021).

7. A. Nkondjock, A. Robidoux, Y. Paredes, S. A. Narod, P. Ghadirian, Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. *Breast Cancer Res Treat* **98**, 285-294 (2006).

8. S. Abbas, A. Siddique, N. Shahid, R. T. Khan, W. Fatima, Breast cancer risk associated with BRCA1/2 variants in the Pakistani population. *Breast Cancer* **26**, 365-372 (2019).

9. P. Manders, A. Pijpe, M. J. Hooning, I. Kluijt, H. F. Vasen, N. Hoogerbrugge, C. J. van Asperen, H. Meijers-Heijboer, M. G. Ausems, T. A. van Os, E. B. Gomez-Garcia, R. M. Brohet, Hebon, F. E. van Leeuwen, M. A. Rookus, Body weight and risk of breast cancer in BRCA1/2 mutation carriers. *Breast Cancer Res Treat* **126**, 193-202 (2011).

10. S. J. Kim, T. Huzarski, J. Gronwald, C. F. Singer, P. Møller, H. T. Lynch, S. Armel, B. Y. Karlan, W. D. Foulkes, S. L. Neuhausen, L. Senter, A. Eisen, C. Eng, S. Panchal, T. Pal, O. Olopade, D. Zakalik, J. Lubinski, S. A. Narod, J. Kotsopoulos, P. Ainsworth, L. Bordeleau, N. Tung, E. Friedman, W. Meschino, C. Snyder, K. Metcalfe, E. Warner, B. Rosen, R. Demsky, J. N. Weitzel, K. Panabaker, F. Couch, S. Manoukian, B. Pasini, M.

B. Daly, L. Steele, H. Saal, T. Fallen, M. Wood, W. Mckinnon, E. Lemire, A. E. Chudley, K. Serfas, C. Elser, S. T. Vadaparampil, O. Ginsburg, C. A. Cullinane, J. L. Blum, T. Ross, C. Mauer, A. Kwong, C. Cybulski, J. Mccuaig, D. Rayson, C. Isaacs, Prospective evaluation of body size and breast cancer risk among BRCA1 and BRCA2 mutation carriers. *International Journal of Epidemiology* **47**, 987-997 (2018).

11. F. Qian, S. Wang, J. Mitchell, L. Mcguffog, D. Barrowdale, G. Leslie, J. C. Oosterwijk, W. K. Chung, D. G. Evans, C. Engel, K. Kast, C. M. Aalfs, M. A. Adank, J. Adlard, B. A. Agnarsson, K. Aittomäki, E. Alducci, I. L. Andrulis, B. K. Arun, M. G. E. M. Ausems, J. Azzollini, E. Barouk-Simonet, J. Barwell, M. Belotti, J. Benitez, A. Berger, A. Borg, A. R. Bradbury, J. Brunet, S. S. Buys, T. Caldes, M. A. Caligo, I. Campbell, S. M. Caputo, J. Chiquette, K. B. M. Claes, J. Margriet Collée, F. J. Couch, I. Coupier, M. B. Daly, R. Davidson, O. Diez, S. M. Domchek, A. Donaldson, C. M. Dorfling, R. Eeles, L. Feliubadaló, L. Foretova, J. Fowler, E. Friedman, D. Frost, P. A. Ganz, J. Garber, V. Garcia-Barberan, G. Glendon, A. K. Godwin, E. B. Gómez Garcia, J. Gronwald, E. Hahnen, U. Hamann, A. Henderson, C. B. Hendricks, J. L. Hopper, P. J. Hulick, E. N. Imyanitov, C. Isaacs, L. Izatt, Á. Izquierdo, A. Jakubowska, K. Kaczmarek, E. Kang, B. Y. Karlan, C. M. Kets, S.-W. Kim, Z. Kim, A. Kwong, Y. Laitman, C. Lasset, M. Hyuk Lee, J. Won Lee, J. Lee, J. Lester, F. Lesueur, J. T. Loud, J. Lubinski, N. Mebirouk, H. E. J. Meijers-Heijboer, A. Meindl, A. Miller, M. Montagna, T. M. Mooij, P. J. Morrison, E. Mouret-Fourme, K. L. Nathanson, S. L. Neuhausen, H. Nevanlinna, D. Niederacher, F. C. Nielsen, R. L. Nussbaum, K. Offit, E. Olah, K.-R. Ong, L. Ottini, S. K. Park, P. Peterlongo, G. Pfeiler, C. M. Phelan, B. Poppe, N. Pradhan, P. Radice, S. J. Ramus, J. Rantala, M. Robson, G. C. Rodriguez, R. K. Schmutzler, C. G. Hutten Selkirk, P. D.

Shah, J. Simard, C. F. Singer, J. Sokolowska, D. Stoppa-Lyonnet, C. Sutter, Y. Yen Tan, R. M. Teixeira, S. H. Teo, M. B. Terry, M. Thomassen, M. Tischkowitz, A. E. Toland, K. M. Tucker, N. Tung, C. J. Van Asperen, K. Van Engelen, E. J. Van Rensburg, S. Wang-Gohrke, B. Wappenschmidt, J. N. Weitzel, D. Yannoukakos, M. H. Greene, M. A. Rookus, D. F. Easton, G. Chenevix-Trench, A. C. Antoniou, D. E. Goldgar, O. I. Olopade, T. R. Rebbeck, D. Huo, Height and Body Mass Index as Modifiers of Breast Cancer Risk in BRCA1/2 Mutation Carriers: A Mendelian Randomization Study. *JNCI: Journal of the National Cancer Institute* **111**, 350-364 (2019).

12. S. M. Grundy, Obesity, Metabolic Syndrome, and Cardiovascular Disease. *The Journal of Clinical Endocrinology & Metabolism* **89**, 2595-2600 (2004).

13. K. Sun, C. M. Kusminski, P. E. Scherer, Adipose tissue remodeling and obesity. *Journal of Clinical Investigation* **121**, 2094-2101 (2011).

14. H. Sell, C. Habich, J. Eckel, Adaptive immunity in obesity and insulin resistance. *Nat Rev Endocrinol* **8**, 709-716 (2012).

15. P. Bhardwaj, K. A. Brown, Obese Adipose Tissue as a Driver of Breast Cancer Growth and Development: Update and Emerging Evidence. *Front Oncol* **11**, 638918 (2021).

16. K. A. Brown, Metabolic pathways in obesity-related breast cancer. *Nature Reviews Endocrinology* **17**, 350-363 (2021).

17. E. R. Simpson, S. R. Davis, Minireview: Aromatase and the Regulation of Estrogen Biosynthesis—Some New Perspectives. *Endocrinology* **142**, 4589-4594 (2001).

18. X. Wang, E. R. Simpson, K. A. Brown, Aromatase overexpression in dysfunctional adipose tissue links obesity to postmenopausal breast cancer. *The Journal of Steroid Biochemistry and Molecular Biology* **153**, 35-44 (2015).

19. H. Zahid, K. Subbaramaiah, N. M. Iyengar, X. K. Zhou, I. C. Chen, P. Bhardwaj, A. Gucalp, M. Morrow, C. A. Hudis, A. J. Dannenberg, K. A. Brown, Leptin regulation of the p53-HIF1alpha/PKM2-aromatase axis in breast adipose stromal cells: a novel mechanism for the obesity-breast cancer link. *Int J Obes (Lond)* **42**, 711-720 (2018).

20. F. Liu, J. He, H. Wang, D. Zhu, Y. Bi, Adipose Morphology: a Critical Factor in Regulation of Human Metabolic Diseases and Adipose Tissue Dysfunction. *Obesity Surgery* **30**, 5086-5100 (2020).

21. K. A. Brown, Metabolic pathways in obesity-related breast cancer. *Nat Rev Endocrinol* **17**, 350-363 (2021).

22. N. Marino, R. German, X. Rao, E. Simpson, S. Liu, J. Wan, Y. Liu, G. Sandusky, M. Jacobsen, M. Stoval, S. Cao, A. M. V. Storniolo, Upregulation of lipid metabolism genes in the breast prior to cancer diagnosis. *npj Breast Cancer* **6**, (2020).

23. K. Yoshida, Y. Miki, Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Science* **95**, 866-871 (2004).

24. A. R. Venkitaraman, Cancer Susceptibility and the Functions of BRCA1 and BRCA2. *Cell* **108**, 171-182 (2002).

25. E. Kowalska, S. A. Narod, T. Huzarski, S. Zajaczek, J. Huzarska, B. Gorski, J. Lubinski, Increased Rates of Chromosome Breakage in BRCA1 Carriers Are Normalized by Oral Selenium Supplementation. *Cancer Epidemiology, Biomarkers & Prevention* **14**, 1302-1306 (2005).

826 26. M. Zaki, W. Basha, H. T. El-Bassyouni, S. El-Toukhy, T. Hussein, Evaluation of DNA
827 damage profile in obese women and its association to risk of metabolic syndrome,
828 polycystic ovary syndrome and recurrent preeclampsia. *Genes Dis* **5**, 367-373 (2018).
829 27. C. M. Dungan, B. D. Peck, R. G. Walton, Z. Huang, M. M. Bamman, P. A. Kern, C. A.
830 Peterson, In vivo analysis of γH2AX+ cells in skeletal muscle from aged and obese
831 humans. *The FASEB Journal* **34**, 7018-7035 (2020).
832 28. J. Fieres, M. Fischer, C. Sauter, M. Moreno-Villanueva, A. Burkle, P. H. Wirtz, The
833 burden of overweight: Higher body mass index, but not vital exhaustion, is associated
834 with higher DNA damage and lower DNA repair capacity. *DNA Repair (Amst)* **114**,
835 103323 (2022).
836 29. V. S. Y. Tay, S. Devaraj, T. Koh, G. Ke, K. C. Crasta, Y. Ali, Increased double strand
837 breaks in diabetic β-cells with a p21 response that limits apoptosis. *Scientific Reports* **9**,
838 (2019).
839 30. P. G. Morris, C. A. Hudis, D. Giri, M. Morrow, D. J. Falcone, X. K. Zhou, B. Du, E.
840 Brogi, C. B. Crawford, L. Kopelovich, K. Subbaramaiah, A. J. Dannenberg,
841 Inflammation and Increased Aromatase Expression Occur in the Breast Tissue of Obese
842 Women with Breast Cancer. *Cancer Prevention Research* **4**, 1021-1029 (2011).
843 31. K. A. Brown, N. I. Hunger, M. Docanto, E. R. Simpson, Metformin inhibits aromatase
844 expression in human breast adipose stromal cells via stimulation of AMP-activated
845 protein kinase. *Breast Cancer Research and Treatment* **123**, 591-596 (2010).
846 32. N. U. Samarajeewa, S. Ham, F. Yang, E. R. Simpson, K. A. Brown, Promoter-specific
847 effects of metformin on aromatase transcript expression. *Steroids* **76**, 768-771 (2011).

33. H. D. Soule, T. M. Maloney, S. R. Wolman, W. D. Peterson, Jr., R. Brenz, C. M. McGrath, J. Russo, R. J. Pauley, R. F. Jones, S. C. Brooks, Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. *Cancer Res* **50**, 6075-6086 (1990).

34. C. N. Perera, H. G. Chin, N. Duru, I. G. Camarillo, Leptin-regulated gene expression in MCF-7 breast cancer cells: mechanistic insights into leptin-regulated mammary tumor growth and progression. *Journal of Endocrinology* **199**, 221-233 (2008).

35. N. K. Saxena, P. M. Vertino, F. A. Anania, D. Sharma, Leptin-induced Growth Stimulation of Breast Cancer Cells Involves Recruitment of Histone Acetyltransferases and Mediator Complex to CYCLIN D1 Promoter via Activation of Stat3. *Journal of Biological Chemistry* **282**, 13316-13325 (2007).

36. A. L. Strong, J. F. Ohlstein, B. A. Biagas, L. V. Rhodes, D. T. Pei, H. A. Tucker, C. Llamas, A. C. Bowles, M. F. Dutreil, S. Zhang, J. M. Gimble, M. E. Burow, B. A. Bunnell, Leptin produced by obese adipose stromal/stem cells enhances proliferation and metastasis of estrogen receptor positive breast cancers. *Breast Cancer Research* **17**, (2015).

37. N. Yin, D. Wang, H. Zhang, X. Yi, X. Sun, B. Shi, H. Wu, G. Wu, X. Wang, Y. Shang, Molecular Mechanisms Involved in the Growth Stimulation of Breast Cancer Cells by Leptin. *Cancer Research* **64**, 5870-5875 (2004).

38. H.-K. Park, R. S. Ahima, Leptin signaling. *F1000Prime Reports* **6**, (2014).

39. B. D. Hopkins, M. D. Goncalves, L. C. Cantley, Insulin–PI3K signalling: an evolutionarily insulated metabolic driver of cancer. *Nature Reviews Endocrinology* **16**, 276-283 (2020).

40. S. J. Kim, J. Lubinski, T. Huzarski, P. Moller, S. Armel, B. Y. Karlan, L. Senter, A. Eisen, W. D. Foulkes, C. F. Singer, N. Tung, L. Bordeleau, S. L. Neuhausen, O. I. Olopade, C. Eng, J. N. Weitzel, R. Fruscio, S. A. Narod, J. Kotsopoulos, G. Hereditary Ovarian Cancer Clinical Study, Weight Gain and the Risk of Ovarian Cancer in BRCA1 and BRCA2 Mutation Carriers. *Cancer Epidemiol Biomarkers Prev* **30**, 2038-2043 (2021).

41. F. Colotta, P. Allavena, A. Sica, C. Garlanda, A. Mantovani, Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* **30**, 1073-1081 (2009).

42. L. M. Coussens, Z. Werb, Inflammation and cancer. *Nature* **420**, 860-867 (2002).

43. L. B. Meira, J. M. Bugni, S. L. Green, C.-W. Lee, B. Pang, D. Borenshtein, B. H. Rickman, A. B. Rogers, C. A. Moroski-Erkul, J. L. Mcfaline, D. B. Schauer, P. C. Dedon, J. G. Fox, L. D. Samson, DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. *Journal of Clinical Investigation*, (2008).

44. O. Kiraly, G. Gong, W. Olipitz, S. Muthupalani, B. P. Engelward, Inflammation-Induced Cell Proliferation Potentiates DNA Damage-Induced Mutations In Vivo. *PLOS Genetics* **11**, e1004901 (2015).

45. K. I. Savage, K. B. Matchett, E. M. Barros, K. M. Cooper, G. W. Irwin, J. J. Gorski, K. S. Orr, J. Vohhodina, J. N. Kavanagh, A. F. Madden, A. Powell, L. Manti, S. S. Mcdade, B. H. Park, K. M. Prise, S. A. Mcintosh, M. Salto-Tellez, D. J. Richard, C. T. Elliott, D. P. Harkin, BRCA1 Deficiency Exacerbates Estrogen-Induced DNA Damage and Genomic Instability. *Cancer Research* **74**, 2773-2784 (2014).

46. C. Algire, O. Moiseeva, X. Deschênes-Simard, L. Amrein, L. Petruccelli, E. Birman, B. Viollet, G. Ferbeyre, M. N. Pollak, Metformin Reduces Endogenous Reactive Oxygen Species and Associated DNA Damage. *Cancer Prevention Research* **5**, 536-543 (2012).

47. N. D. Kauff, S. M. Domchek, T. M. Friebel, M. E. Robson, J. Lee, J. E. Garber, C. Isaacs, D. G. Evans, H. Lynch, R. A. Eeles, S. L. Neuhausen, M. B. Daly, E. Matloff, J. L. Blum, P. Sabbatini, R. R. Barakat, C. Hudis, L. Norton, K. Offit, T. R. Rebbeck, Risk-Reducing Salpingo-Oophorectomy for the Prevention of BRCA1- and BRCA2-Associated Breast and Gynecologic Cancer: A Multicenter, Prospective Study. *Journal of Clinical Oncology* **26**, 1331-1337 (2008).

48. S. A. Narod, J. S. Brunet, P. Ghadirian, M. Robson, K. Heimdal, S. L. Neuhausen, D. Stoppa-Lyonnet, C. Lerman, B. Pasini, P. de los Rios, B. Weber, H. Lynch, G. Hereditary Breast Cancer Clinical Study, Tamoxifen and risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers: a case-control study. Hereditary Breast Cancer Clinical Study Group. *Lancet* **356**, 1876-1881 (2000).

49. T. R. Rebbeck, H. T. Lynch, S. L. Neuhausen, S. A. Narod, L. Van'T Veer, J. E. Garber, G. Evans, C. Isaacs, M. B. Daly, E. Matloff, O. I. Olopade, B. L. Weber, Prophylactic Oophorectomy in Carriers of BRCA1 or BRCA2 Mutations. *New England Journal of Medicine* **346**, 1616-1622 (2002).

50. C. E. Caldon, Estrogen Signaling and the DNA Damage Response in Hormone Dependent Breast Cancers. *Frontiers in Oncology* **4**, (2014).

51. P. Bhardwaj, C. C. Au, A. Benito-Martin, H. Ladumor, S. Oshchepkova, R. Moges, K. A. Brown, Estrogens and breast cancer: Mechanisms involved in obesity-related

development, growth and progression. *The Journal of Steroid Biochemistry and Molecular Biology* **189**, 161-170 (2019).

52. T. T. Huang, E. J. Lampert, C. Coots, J. M. Lee, Targeting the PI3K pathway and DNA damage response as a therapeutic strategy in ovarian cancer. *Cancer Treat Rev* **86**, 102021 (2020).

53. A. Juvekar, H. Hu, S. Yadegarynia, C. A. Lyssiotis, S. Ullas, E. C. Lien, G. Bellinger, J. Son, R. C. Hok, P. Seth, M. B. Daly, B. Kim, R. Scully, J. M. Asara, L. C. Cantley, G. M. Wulf, Phosphoinositide 3-kinase inhibitors induce DNA damage through nucleoside depletion. *Proc Natl Acad Sci U S A* **113**, E4338-4347 (2016).

54. R. J. Cardnell, Y. Feng, L. Diao, Y. H. Fan, F. Masrorpour, J. Wang, Y. Shen, G. B. Mills, J. D. Minna, J. V. Heymach, L. A. Byers, Proteomic markers of DNA repair and PI3K pathway activation predict response to the PARP inhibitor BMN 673 in small cell lung cancer. *Clin Cancer Res* **19**, 6322-6328 (2013).

55. A. Juvekar, L. N. Burga, H. Hu, E. P. Lunsford, Y. H. Ibrahim, J. Balmana, A. Rajendran, A. Papa, K. Spencer, C. A. Lyssiotis, C. Nardella, P. P. Pandolfi, J. Baselga, R. Scully, J. M. Asara, L. C. Cantley, G. M. Wulf, Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. *Cancer Discov* **2**, 1048-1063 (2012).

56. J. Ducie, F. Dao, M. Considine, N. Olvera, P. A. Shaw, R. J. Kurman, I.-M. Shih, R. A. Soslow, L. Cope, D. A. Levine, Molecular analysis of high-grade serous ovarian carcinoma with and without associated serous tubal intra-epithelial carcinoma. *Nature Communications* **8**, (2017).

57. S. I. Labidi-Galy, E. Papp, D. Hallberg, N. Niknafs, V. Adleff, M. Noe, R. Bhattacharya, M. Novak, S. Jones, J. Phallen, C. A. Hruban, M. S. Hirsch, D. I. Lin, L. Schwartz, C. L. Maire, J.-C. Tille, M. Bowden, A. Ayhan, L. D. Wood, R. B. Scharpf, R. Kurman, T.-L. Wang, I.-M. Shih, R. Karchin, R. Drapkin, V. E. Velculescu, High grade serous ovarian carcinomas originate in the fallopian tube. *Nature Communications* **8**, (2017).

58. R. L. Milne, A. C. Antoniou, Modifiers of breast and ovarian cancer risks for BRCA1 and BRCA2 mutation carriers. *Endocrine-Related Cancer* **23**, T69-T84 (2016).

59. T. M. Friebel, S. M. Domchek, T. R. Rebbeck, Modifiers of Cancer Risk in BRCA1 and BRCA2 Mutation Carriers: A Systematic Review and Meta-Analysis. *JNCI: Journal of the National Cancer Institute* **106**, dju091-dju091 (2014).

60. A. M. Coletta, S. K. Peterson, L. A. Gatus, K. J. Krause, S. M. Schembre, S. C. Gilchrist, B. Arun, Y. N. You, M. A. Rodriguez-Bigas, L. L. Strong, K. H. Lu, K. Basen-Engquist, Diet, weight management, physical activity and Ovarian & Breast Cancer Risk in women with BRCA1/2 pathogenic Germline gene variants: systematic review. *Hereditary Cancer in Clinical Practice* **18**, (2020).

61. P. J. Goodwin, B. E. Chen, K. A. Gelmon, T. J. Whelan, M. Ennis, J. Lemieux, J. A. Ligibel, D. L. Hershman, I. A. Mayer, T. J. Hobday, J. M. Bliss, P. Rastogi, M. Rabaglio-Poretti, S. D. Mukherjee, J. R. Mackey, V. G. Abramson, C. Oja, R. Wesolowski, A. M. Thompson, D. W. Rea, P. M. Stos, L. E. Shepherd, V. Stambolic, W. R. Parulekar, Effect of Metformin vs Placebo on Invasive Disease–Free Survival in Patients With Breast Cancer. *JAMA* **327**, 1963 (2022).

62. P. J. Goodwin, R. J. O. Dowling, M. Ennis, B. E. Chen, W. R. Parulekar, L. E. Shepherd, M. J. Burnell, R. Vander Meer, A. Molckovsky, A. Gurjal, K. A. Gelmon, J. A. Ligibel,

D. L. Hershman, I. A. Mayer, T. J. Whelan, T. J. Hobday, P. Rastogi, M. Rabaglio-Poretti, J. Lemieux, A. M. Thompson, D. W. Rea, V. Stambolic, Effect of metformin versus placebo on metabolic factors in the MA.32 randomized breast cancer trial. *NPJ Breast Cancer* **7**, 74 (2021).

63. P. J. Goodwin, W. R. Parulekar, K. A. Gelmon, L. E. Shepherd, J. A. Ligibel, D. L. Hershman, P. Rastogi, I. A. Mayer, T. J. Hobday, J. Lemieux, A. M. Thompson, K. I. Pritchard, T. J. Whelan, S. D. Mukherjee, H. I. Chalchal, C. D. Oja, K. S. Tonkin, V. Bernstein, B. E. Chen, V. Stambolic, Effect of metformin vs placebo on and metabolic factors in NCIC CTG MA.32. *J Natl Cancer Inst* **107**, (2015).

64. I. Pimentel, B. E. Chen, A. E. Lohmann, M. Ennis, J. Ligibel, L. Shepherd, D. L. Hershman, T. Whelan, V. Stambolic, I. Mayer, T. Hobday, J. Lemieux, A. Thompson, P. Rastogi, K. Gelmon, D. Rea, M. Rabaglio, S. Ellard, M. Mates, P. Bedard, L. Pitre, T. Vandenberg, R. J. O. Dowling, W. Parulekar, P. J. Goodwin, The Effect of Metformin vs Placebo on Sex Hormones in Canadian Cancer Trials Group MA.32. *J Natl Cancer Inst* **113**, 192-198 (2021).

65. W. J. Gradishar, B. O. Anderson, S. L. Blair, H. J. Burstein, A. Cyr, A. D. Elias, W. B. Farrar, A. Forero, S. H. Giordano, L. J. Goldstein, D. F. Hayes, C. A. Hudis, S. J. Isakoff, B. M. Ljung, P. K. Marcom, I. A. Mayer, B. McCormick, R. S. Miller, M. Pegram, L. J. Pierce, E. C. Reed, K. E. Salerno, L. S. Schwartzberg, M. L. Smith, H. Soliman, G. Somlo, J. H. Ward, A. C. Wolff, R. Zellars, D. A. Shead, R. Kumar, P. National Comprehensive Cancer Network Breast Cancer, Breast cancer version 3.2014. *J Natl Compr Canc Netw* **12**, 542-590 (2014).

982 66. H. Konishi, M. Mohseni, A. Tamaki, J. P. Garay, S. Croessmann, S. Karnan, A. Ota, H.
983 Y. Wong, Y. Konishi, B. Karakas, K. Tahir, A. M. Abukhdeir, J. P. Gustin, J. Cidado, G.
984 M. Wang, D. Cosgrove, R. Cochran, D. Jelovac, M. J. Higgins, S. Arena, L. Hawkins, J.
985 Lauring, A. L. Gross, C. M. Heaphy, Y. Hosokawa, E. Gabrielson, A. K. Meeker, K.
986 Visvanathan, P. Argani, K. E. Bachman, B. H. Park, Mutation of a single allele of the
987 cancer susceptibility gene BRCA1 leads to genomic instability in human breast epithelial
988 cells. *Proc Natl Acad Sci U S A* **108**, 17773-17778 (2011).
989 67. S. Han, T. Baba, S. Yanai, D. J. Byun, K. I. Morohashi, J. H. Kim, M. H. Choi, GC-MS-
990 based metabolic signatures reveal comparative steroidogenic pathways between fetal and
991 adult mouse testes. *Andrology* **9**, 400-406 (2021).
992 68. J. Y. Moon, K. M. McNamara, J. J. Lee, B. C. Chung, H. Sasano, M. H. Choi, Improved
993 detectability of sex steroids from frozen sections of breast cancer tissue using GC-triple
994 quadrupole-MS. *J Steroid Biochem Mol Biol* **178**, 185-192 (2018).
995 69. N. L. Bray, H. Pimentel, P. Melsted, L. Pachter, Near-optimal probabilistic RNA-seq
996 quantification. *Nat Biotechnol* **34**, 525-527 (2016).
997 70. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for
998 RNA-seq data with DESeq2. *Genome Biol* **15**, 550 (2014).
999 71. A. Dobin, C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M.
1000 Chaisson, T. R. Gingeras, STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**,
1001 15-21 (2013).
1002 72. H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis,
1003 R. Durbin, S. Genome Project Data Processing, The Sequence Alignment/Map format
1004 and SAMtools. *Bioinformatics* **25**, 2078-2079 (2009).

73. C. Trapnell, A. Roberts, L. Goff, G. Pertea, D. Kim, D. R. Kelley, H. Pimentel, S. L. Salzberg, J. L. Rinn, L. Pachter, Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* **7**, 562-578 (2012).

74. T. Derrien, R. Johnson, G. Bussotti, A. Tanzer, S. Djebali, H. Tilgner, G. Guernec, D. Martin, A. Merkel, D. G. Knowles, J. Lagarde, L. Veeravalli, X. Ruan, Y. Ruan, T. Lassmann, P. Carninci, J. B. Brown, L. Lipovich, J. M. Gonzalez, M. Thomas, C. A. Davis, R. Shiekhattar, T. R. Gingeras, T. J. Hubbard, C. Notredame, J. Harrow, R. Guigo, The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res* **22**, 1775-1789 (2012).

75. X. Liu, H. Holstege, H. van der Gulden, M. Treur-Mulder, J. Zevenhoven, A. Velds, R. M. Kerkhoven, M. H. van Vliet, L. F. Wessels, J. L. Peterse, A. Berns, J. Jonkers, Somatic loss of BRCA1 and p53 in mice induces mammary tumors with features of human BRCA1-mutated basal-like breast cancer. *Proc Natl Acad Sci U S A* **104**, 12111-12116 (2007).

76. H. Liu, C. J. Murphy, F. A. Karreth, K. B. Emdal, F. M. White, O. Elemento, A. Toker, G. M. Wulf, L. C. Cantley, Identifying and Targeting Sporadic Oncogenic Genetic Aberrations in Mouse Models of Triple-Negative Breast Cancer. *Cancer Discov* **8**, 354-369 (2018).

77. F. Schwenk, U. Baron, K. Rajewsky, A cre-transgenic mouse strain for the ubiquitous deletion of loxP-flanked gene segments including deletion in germ cells. *Nucleic Acids Res* **23**, 5080-5081 (1995).

78. A. Katti, M. Foronda, J. Zimmerman, B. Diaz, M. P. Zafra, S. Goswami, L. E. Dow, GO: a functional reporter system to identify and enrich base editing activity. *Nucleic Acids Res* **48**, 2841-2852 (2020).

79. M. P. Zafra, E. M. Schatoff, A. Katti, M. Foronda, M. Breinig, A. Y. Schweitzer, A. Simon, T. Han, S. Goswami, E. Montgomery, J. Thibado, E. R. Kastenhuber, F. J. Sanchez-Rivera, J. Shi, C. R. Vakoc, S. W. Lowe, D. F. Tschaharganeh, L. E. Dow, Optimized base editors enable efficient editing in cells, organoids and mice. *Nat Biotechnol* **36**, 888-893 (2018).

80. J. Y. Moon, K. M. McNamara, J. J. Lee, B. C. Chung, H. Sasano, M. H. Choi, Improved detectability of sex steroids from frozen sections of breast cancer tissue using GC-triple quadrupole-MS. *J Steroid Biochem Mol Biol* **178**, 185-192 (2018).

Acknowledgments:

We thank Dr. David Otterburn and Dr. Leslie Cohen, Department of Surgery, and Dr. Paula Ginter, Department of Pathology, at Weill Cornell Medicine for facilitating access to reduction mammoplasty tissue. Studies were conducted with the support and facilities provided by the Microscopy and Image Analysis Core Facility, Genomics Resources Core Facility, and the Research Animal Resource Center at Weill Cornell Medicine (New York, NY). All schematics were created in BioRender.

Funding:

National Cancer Institute of the National Institutes of Health grant 1R01CA215797 (KAB)

National Cancer Institute of the National Institutes of Health grant F31CA236306 (PB)

Anne Moore Breast Cancer Research Fund (KAB)

National Breast Cancer Foundation grant ECF-16-004 (KAB)

National Center for Advancing Translational Sciences of the National Institutes of Health grant KL2-TR-002385 (MKF)

National Institute of General Medical Sciences of the National Institutes of Health grant T32GM007739 to the Weill Cornell/Rockefeller/Sloan Kettering Tri-Institutional MD-PhD Program (MA)

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or other funding agencies.

Author contributions:

Conceptualization: KAB, NMI
Methodology: KAB, PB, NMI, HZ, DJB, MHO, QS, RB, MF, LED, DDG, XKZ
Investigation: PB, HZ, KMC, DJB, CL, CL, PP, MA, MF, SO, MP, BH, MKF
Formal analysis: KAB, PB, QS, OS, RB, XKZ
Resources: KAB, MHC, OE, AML, LHE, MM, JAS, LCC
Funding acquisition: KAB, PB
Project administration: KAB
Supervision: KAB
Writing – original draft: PB, KAB
Writing – review & editing: All authors

Competing interests:

NMI receives consulting fees from Novartis, Pfizer, and Seattle Genetics; Honoraria from Curio Science, Cardinal Health, OncLive, IntrinsiQ Health; Research funding (to institution) from Novartis, SynDevRx, National Cancer Institute, American Cancer Society, Breast Cancer Research Foundation, and the Conquer Cancer Foundation. LED is a scientific advisor and holds equity in Mirimus Inc. and has received consulting fees and/or honoraria from Volastra Therapeutics, Revolution Medicines, Repare Therapeutics, Fog Pharma, and Frazier Healthcare Partners. BDH is a founder and consultant for Faeth Therapeutics.

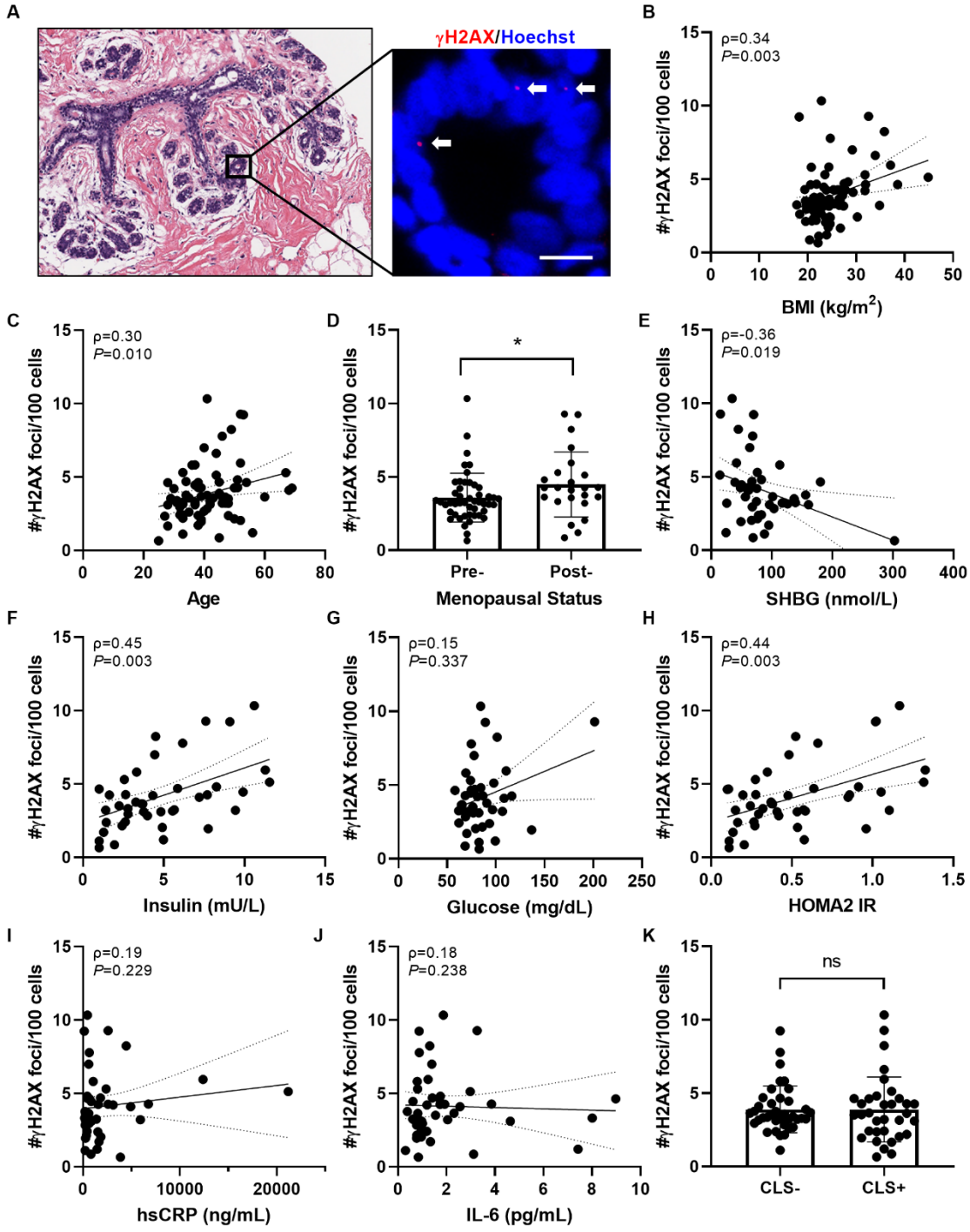

Fig.1

Fig. 1. BMI and additional clinical characteristics are positively correlated with DNA damage in breast epithelium of women carrying a *BRCA* mutation

(**A**) Representative image of tissue microarray section of normal breast epithelium shown by H&E stain (left) and by IF staining (right) for γH2AX (red, arrows) co-localizing with Hoechst (blue), scale bar=10μM. (**B-C**) Correlation between epithelial cell DNA damage as measured by #γH2AX foci/100 cells with clinical characteristics including BMI and age. (**D**) Average DNA damage in the study population grouped by menopausal status: pre-menopausal, n=48 and post-menopausal, n=24. (**E-J**) Epithelial cell DNA damage correlated with circulating serum biomarkers in a subset of the study population with available fasting serum at the time of surgery (n=43). (**K**) Average DNA damage in the study population when grouped by those exhibiting histological breast adipose tissue inflammation defined as presence of crown-like structures (CLS) vs those with no CLS present (i.e. CLS- vs CLS+). Two-tailed Mann Whitney test was used to determine significant differences in grouped comparisons and data is presented as mean +/- SD. and Correlation between variables were assessed by Spearman's rank correlation coefficient (ρ). Associated *P* value and ρ are shown for continuous variables with 95% confidence intervals. **P*<0.05; ns, not significant; n=72 unless otherwise stated.

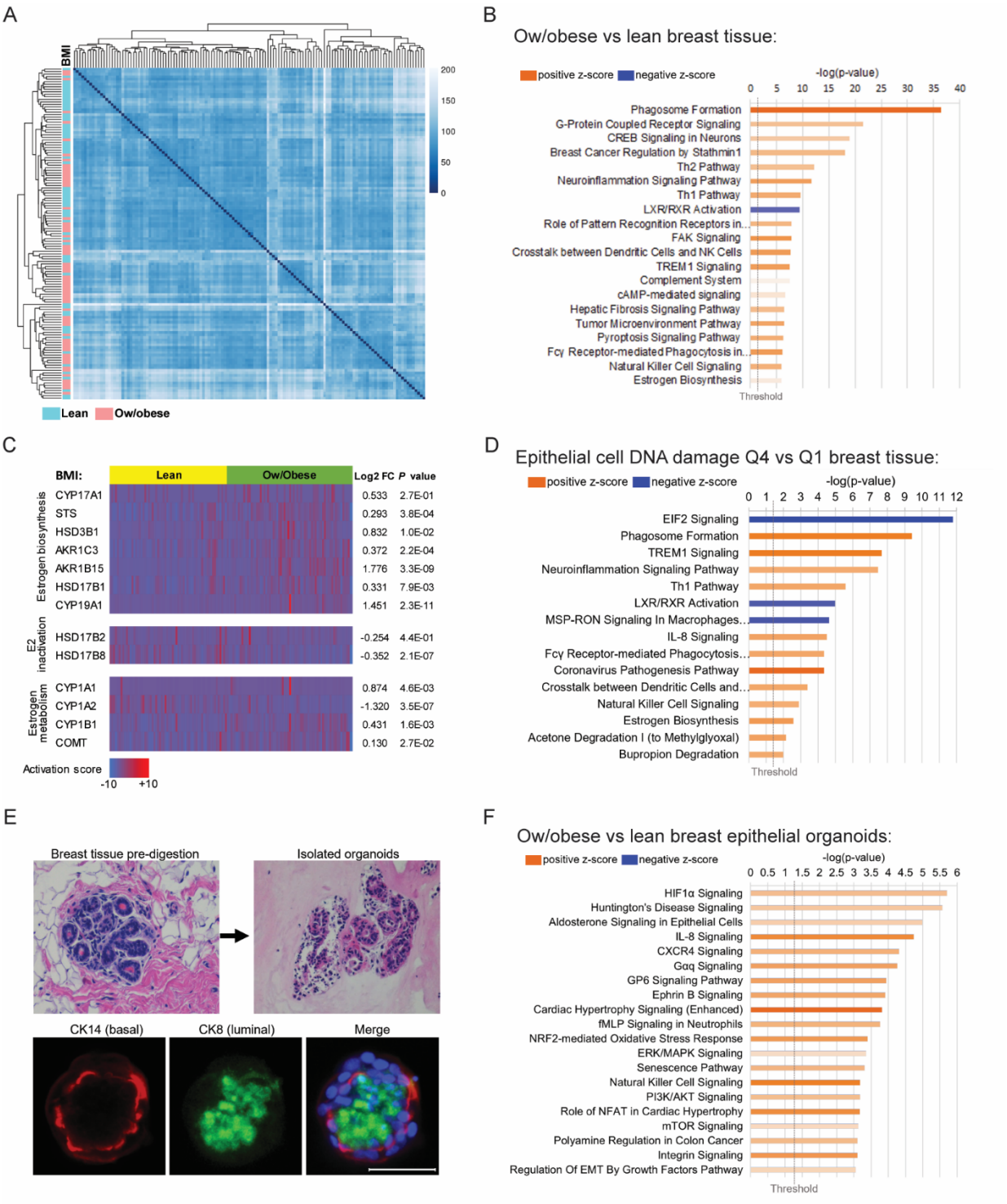

Fig. 2

Fig. 2. Elevated bodyweight is associated with significant changes in gene expression in breast adipose tissue and in breast epithelial cells of *BRCA* mutation carriers

(**A**) Unsupervised heatmap of whole breast tissue gene expression by RNA-seq in *BRCA* mutation carriers identified by BMI category of lean (n=64, blue) or overweight/obese (n=67, pink). (**B**) IPA analysis of RNA-Seq data showing activation (z-score) of the top 20 canonical pathways regulated in breast tissue from overweight/obese *BRCA* mutation carriers compared to lean carriers with an absolute value z-score of >0.5. (**C**) Heatmap of RNA-seq gene expression data generated from breast tissue of *BRCA* mutation carriers grouped by BMI category of lean (yellow) or overweight/obese (Ow/obese, green) showing selected genes associated with estrogen biosynthesis, estradiol (E2) inactivation, and estrogen metabolism. Corresponding gene expression (log2FC) and *P* values are shown in Ow/obese relative to lean tissue. (**D**) DNA damage in breast epithelial cells was quantified in tissue sections from n=61 patients from whom corresponding whole breast tissue RNA-seq data was also available. The cases were stratified by quartile of DNA damage and the breast tissue gene expression from cases with the highest level of DNA damage (quartile 4, Q4) were compared to cases with the lowest level (quartile 1, Q1) of DNA damage. Top 15 canonical pathways regulated in Q4 vs Q1 with an absolute value z-score of >2.0 are shown. (**E**) Representative H&E-stained images of a breast tissue section before digestion and epithelial organoids after isolation are shown. Organoids stain positively for luminal marker cytokeratin 8 (CK8, green) and basal marker cytokeratin 14 (CK14, red) as shown by IF staining merged with Hoechst (blue). Scale bar= 50μM. (**F**) IPA analysis of RNA-seq gene expression data showing activation of the top 20 canonical pathways regulated in primary breast epithelial organoids from of overweight/obese (Ow/obese) *BRCA* mutation carriers (n=9) relative to lean carriers (n=10) with an absolute value z-score of >1.0 is shown. The length of the bars on all canonical pathway graphs are determined by the Fisher's Exact Test *P* value with entities that have a -log(p-value) >1.3 shown.

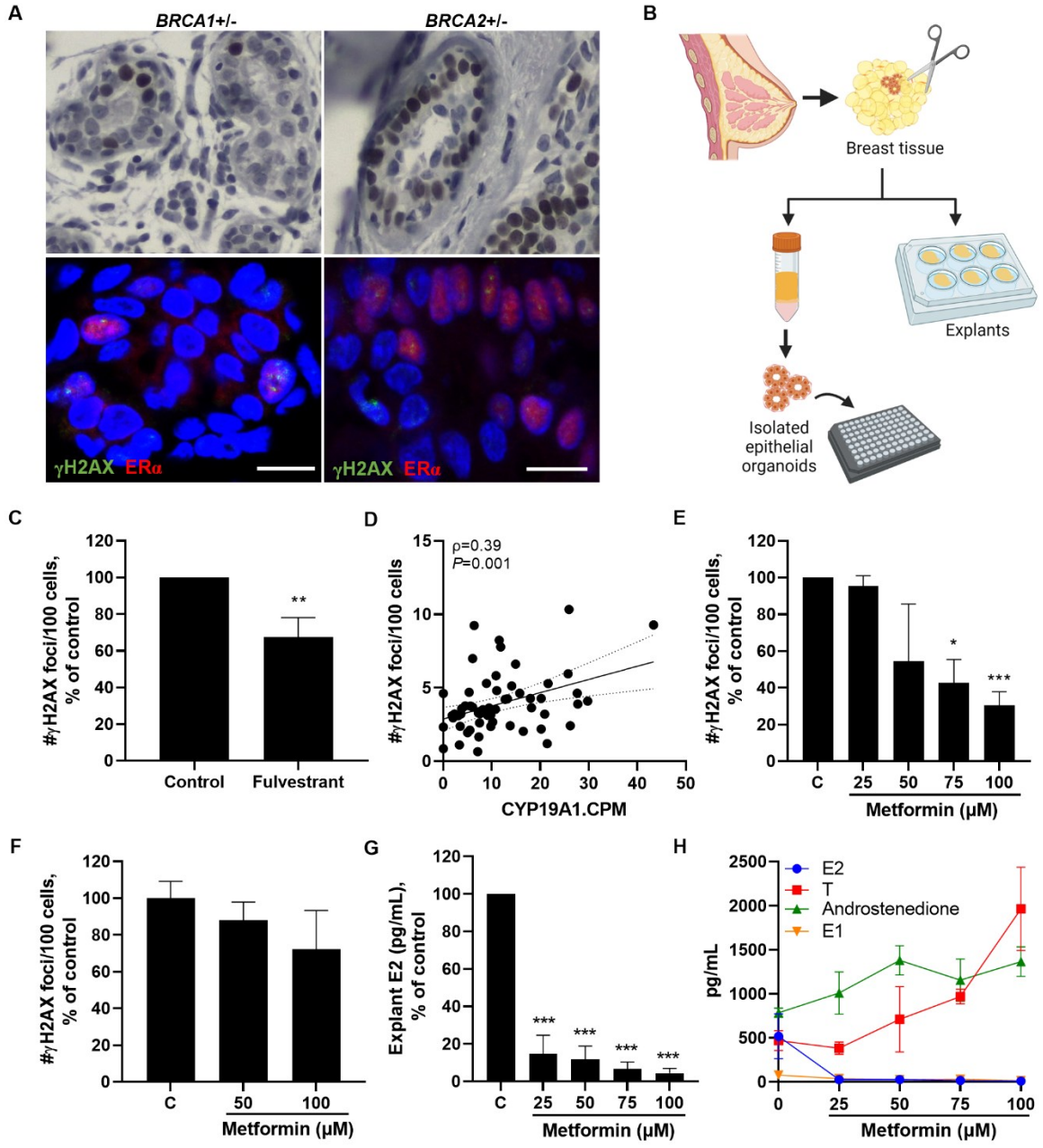

Fig. 3

Fig. 3. Targeting estrogen signaling or production in breast tissue decreases epithelial cell DNA damage in in women carrying a mutation in *BRCA1* or *BRCA2*

(**A**) Representative IHC staining of ERα expression in breast epithelium from carriers of a *BRCA1* or *BRCA2* mutation (top panel). Representative IF staining showing co-localization of #γH2AX foci (green) with ERα positive cells (red) (bottom panel), scale bar=10μM. (**B**) Experimental schematic showing collection of breast tissue and plating of explants or isolation of primary breast epithelial organoids for treatment studies. (**C**) Breast epithelial cell DNA damage assessed by IF (#γH2AX foci/100 cells) in e*x vivo* breast adipose tissue explants from *BRCA* mutation carriers treated with fulvestrant (100nM) for 24 hours (pooled average of n=7 patients). (**D**) Aromatase expression in breast tissue from *BRCA* mutation carriers (RNA-seq counts per million, CPM) correlated with level of breast epithelial cell DNA damage in corresponding tissue sections (n=61). Spearman's rank correlation coefficient (ρ) and associated *P* value are shown with 95% confidence intervals. (**E**) Breast epithelial cell DNA damage in e*x vivo* breast adipose tissue explants from *BRCA* mutation carriers treated with metformin (0-100μM) for 24 hours (pooled average of n=3 patients). (**F**) DNA damage in isolated primary breast epithelial cells from *BRCA* mutation carriers treated with metformin (0-100μM) for 24 hours (representative of n=2 experiments). (**G**) Average 17β-estradiol (E2) levels and (**H**) overlay of E2, testosterone (T), androstenedione, and estrone (E1) levels in e*x vivo* breast adipose explants after 24-hour treatment with metformin (pooled average of n=3 patients). Student's t-test was used to determine significant differences from control unless otherwise stated. Data is presented as mean +/- SEM. **P* <0.05, ***P* <0.01, ****P*<0.001.

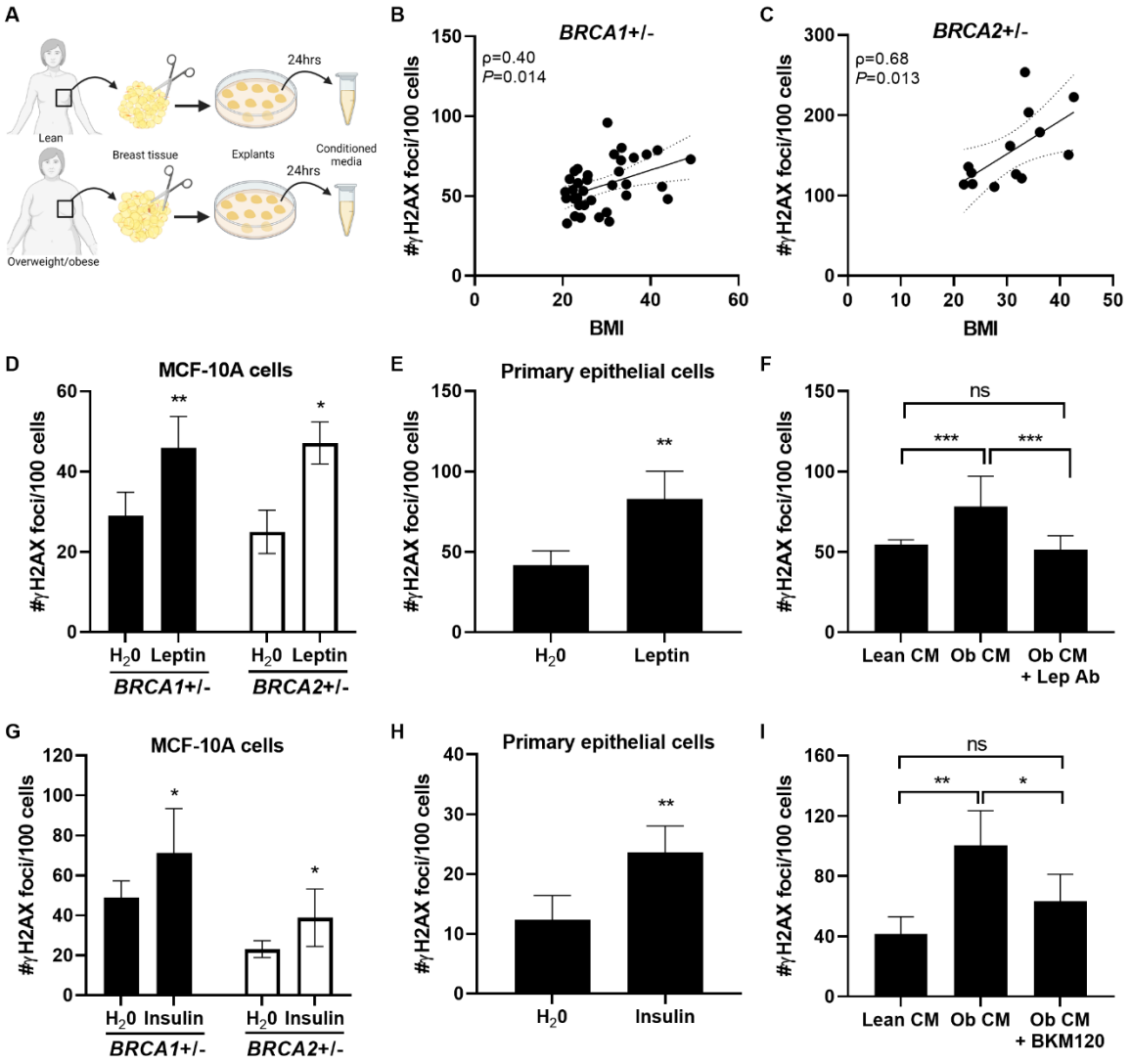

Fig. 4

Fig. 4. Obesity-induced changes to the local breast adipose microenvironment promotes DNA damage in *BRCA1* and *BRCA2* heterozygous breast epithelial cells

(**A**) Experimental schematic showing the collection of breast adipose tissue conditioned media (CM) from lean and overweight/obese women. (**B**) MCF-10A cells were treated with CM for 24 hours. DNA damage assessed by IF (#γH2AX foci/100 cells) is shown correlated with BMI in *BRCA1*+/- (n=36 CM cases) and (**C**) *BRCA2*+/- (n=13 CM cases) MCF-10A cells. Spearman's

rank correlation coefficient (ρ) and associated *P* value are shown along with 95% confidence intervals. (**D**) DNA damage in *BRCA1*+/- and *BRCA2*+/- MCF-10A cells and in (**E**) primary *BRCA1*+/- breast epithelial cells treated with leptin (400ng/µl) for 24 hours. (**F**) DNA damage in *BRCA1*+/- MCF-10A cells after 24-hour treatment with lean CM, obese (ob) CM, or ob CM in the presence of a leptin neutralizing antibody (Lep Ab). (**G**) DNA damage in *BRCA1*+/- and *BRCA2*+/- MCF-10A cells and in (**H**) primary *BRCA2*+/- breast epithelial cells treated with insulin (100nM) for 24 hours. (**I**) DNA damage in *BRCA1*+/- MCF-10A cells after 24-hour treatment with lean CM, ob CM, or ob CM in the presence of PI3K inhibitor BKM120 (1µM). Student's t-test was used to determine significant differences in (**D-I)**. All experiments in MCF-10A cells were conducted a minimum of two times with representative results from one experiment shown. Data in primary cells were generated from cells treated in triplicate. Data is presented as mean +/- SD. **P* <0.05, ***P* <0.01, ****P* <0.001, ns= not significant.

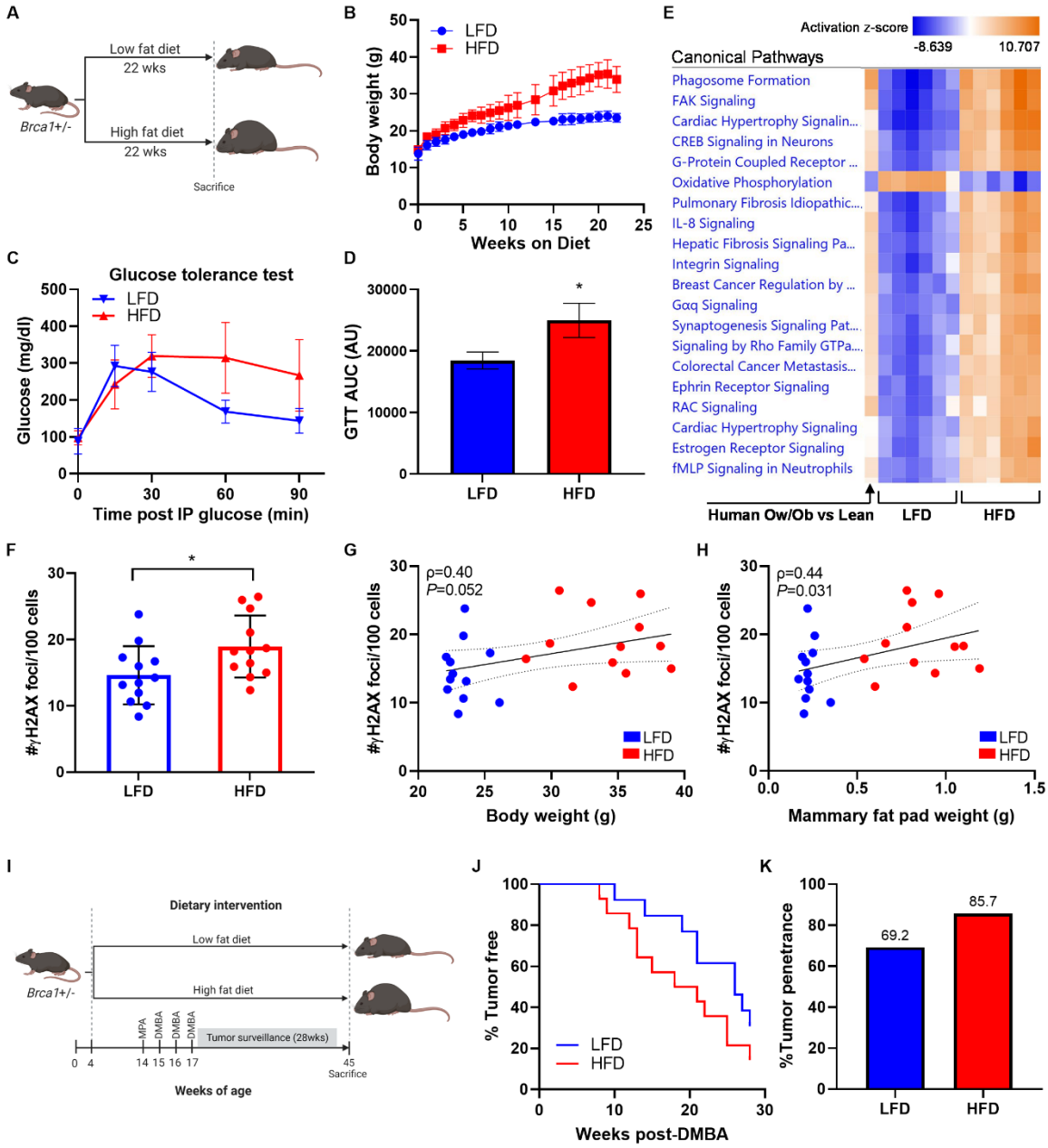

Fig. 5

Fig. 5. High fat diet feeding leads to elevated mammary gland DNA damage in association with increased mammary tumor penetrance and decreased tumor latency in *Brca1*+/- mice

(**A**) Experimental schematic of diet-induced obesity in female *Brca1*+/- mice (n=12/gp). (**B**) Average body weight of mice fed low fat diet (LFD) or high fat diet (HFD) over 22 wks. (**C**) Glucose tolerance test conducted one week prior to sacrifice and (**D**) area under curve (AUC) calculation for each group (mean +/- SEM). (**E**) RNA-Seq was conducted on whole mammary fat pad tissue from HFD and LFD mice (n=6/gp). Activation of top 20 canonical pathways regulated in mammary fat pads from HFD mice compared to LFD mice are shown adjacent to corresponding pathway regulation in breast tissue from overweight (Ow)/obese vs lean women carrying a *BRCA* mutation (n=64-67/gp). (**F**) DNA damage assessed by IF (#γH2AX foci/100 cells) in mammary glands at the time of sacrifice. (**G**) Correlation between mammary gland DNA damage and mouse body weight and (**H**) mammary fat pad weight among all mice. Spearman's rank correlation coefficient (ρ) and associated *P* values are shown along with 95% confidence intervals. (**I**) Experimental schematic of MPA/DMBA-induced tumorigenesis model in female *Brca1*+/- mice randomized to LFD or HFD groups (n=13-14/gp). (**J**) Mammary tumor development in LFD and HFD mice shown as % of mice tumor free over the 28-week surveillance period. (**K**) Overall mammary tumor penetrance at the end of the surveillance period shown as % of mice in each group that developed a mammary tumor. Student's t-test was used to determine significance unless otherwise stated. Data is presented as mean +/- SD unless otherwise stated. *P <0.05.

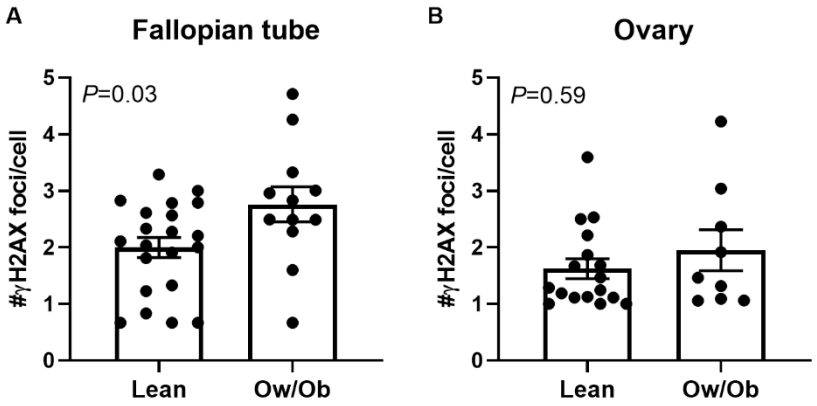

Fig. 6

Fig. 6. BMI is associated with DNA damage in the fallopian tube but not ovary

(**A**) DNA damage assessed by IF (#γH2AX foci/cell) in epithelial cells of the ovary and in (**B**) epithelial cells of fallopian tube fimbriae in *BRCA* mutation carriers grouped by BMI category of lean (n=17-21/gp) or overweight (Ow)/obese (Ob) (n=9-12). Two-tailed Mann Whitney test was used to determine significant differences (*P* value) between groups. Data is presented as mean +/-SEM.

Table 1. Baseline characteristics of study population based on BMI category

Variables	All (n = 72)	Lean (n = 46)	Overweight/Obese (n = 26)	*P*
BMI, median (range)	23.7 (17.7-44.9)	21.8 (17.7-24.7)	28.8 (25.3-44.9)	<0.01
BRCA mutation, n (%)				0.46
BRCA1	42 (58.3%)	29 (63.0%)	14 (53.8%)	
BRCA2	30 (41.7%)	17 (37.0%)	12 (46.2%)	
Age, median (range)	40 (25-69)	38.5 (25-60)	44.5 (28-69)	0.01
Diabetes, n (%)				0.36
No	71 (98.6%)	46 (100.0%)	25 (96.2%)	
Yes	1 (1.4%)	0 (0%)	1 (3.8%)	
Dyslipidemia, n (%)				0.01
No	65 (90.3%)	45 (97.8%)	20 (76.9%)	
Yes	7 (9.7%)	1 (2.2%)	6 (23.1%)	
Hypertension, n (%)				0.02
No	64 (88.9%)	44 (95.7%)	20 (76.9%)	
Yes	8 (11.1%)	2 (4.3%)	6 (23.1%)	
Menopausal status, n (%)				0.037
Pre-	48 (66.7%)	35 (76.1%)	13 (50.0%)	
Post-	24 (33.3%)	11 (23.9%)	13 (50.0%)	
Race, n (%)				0.19
Asian	1 (1.4%)	1 (2.2%)	0 (0.0%)	
Black	2 (2.8%)	2 (4.3%)	0 (0.0%)	
Other	2 (2.8%)	0 (0.0%)	2 (7.7%)	
White	59 (81.9%)	36 (78.3%)	23 (88.5%)	
Missing	8 (11.1%)	7 (15.2%)	1 (3.8%)	
Invasive tumor present, n (%)				1
No	40 (55.6%)	26 (56.5%)	14 (53.8%)	
Yes	32 (44.4%)	20 (43.5%)	12 (46.2%)	
Tumor subtype, n(%)				1
HR+	23 (31.9%)	15 (32.6%)	8 (30.8%)	
HER2+	1 (1.4%)	1 (2.2%)	0 (0.0%)	
TNBC	10 (13.9%)	6 (13.0%)	4 (15.4%)	
N/A	38 (52.8%)	24 (52.2%)	14 (53.8%)	

Abbreviations: BMI, body mass index; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer

Table 2. Association of clinical features and blood biomarkers with DNA damage, adjusting for age or BMI

Variables	Correction	*P*	Correction	*P*
BMI			Age	0.025
Age	BMI	0.115		
SHBG (nmol/L)	BMI	0.047	Age	0.026
Insulin (mU/L)	BMI	<0.001	Age	<0.001
HOMA2 IR	BMI	<0.001	Age	<0.001

Abbreviations: BMI, body mass index; SHBG, steroid hormone binding globulin, HOMA2 IR, homeostatic model assessment 2 for insulin resistance

Table 3. Predicted upstream regulators of gene expression differences in breast epithelial organoids from overweight/obese *BRCA* mutation carriers relative to lean carriers and associated gene expression in whole breast tissue

Organoid upstream regulator	Predicted activation state	Activation z-score	*P*-value of overlap	Breast tissue log2FC	*P*-value
beta-estradiol	Activated	4.728	2.2E-10	see Fig. 2C	
IL2	Activated	3.402	3.1E-02	0.563	2.3E-01
GDF2	Activated	3.217	4.9E-03	-0.081	9.8E-01
IL15	Activated	3.152	1.5E-03	0.299	4.1E-05
TNFSF11	Activated	3.125	3.2E-02	-0.757	9.7E-02
Insulin	Activated	3.113	6.1E-03		
IL4	Activated	3.016	1.9E-03	-0.25	7.4E-01
TGFB1	Activated	2.942	6.0E-09	0.455	2.2E-08
hydrogen peroxide	Activated	2.839	3.1E-03		
IL3	Activated	2.674	7.6E-04	-0.122	9.7E-01
CSF1	Activated	2.602	8.9E-03	0.35	1.4E-06
Lh	Activated	2.598	1.7E-03		
dinoprost (PGF2α)	Activated	2.569	2.9E-02		
IL5	Activated	2.496	5.5E-03	0.173	6.9E-01
ATP	Activated	2.443	8.7E-03		
MDK	Activated	2.433	2.9E-02	-0.34	4.4E-03
AGT	Activated	2.345	4.2E-03	-0.56	9.6E-04
ANGPT2	Activated	2.329	1.1E-03	0.38	9.2E-05
WNT5A	Activated	2.292	1.7E-03	0.184	1.3E-01
pyruvic acid	Activated	2.156	1.5E-03		

Table 4. Activation of diseases or functions associated with DNA damage or DNA repair in *BRCA1*+/- epithelial cells treated with breast adipose tissue condition media derived from obese women relative to lean women

Categories	Diseases or functions annotation	*P*-value	Predicted activation state	Activation z-score	# Molecules
Cellular Assembly and Organization	Formation of micronuclei	2.53E-06	Increased	2.756	9
DNA Replication, Recombination & Repair	Chromosomal aberration	5.37E-06	Increased	2.853	31
	Chromosomal instability	2.43E-08	Increased	2.603	19
	Breakage of chromosomes	2.88E-05	Increased	2.488	11
Cell Cycle; DNA Replication, Recombination & Repair	Checkpoint control	1.99E-06	Decreased	-2.756	15
	Spindle checkpoint	9.33E-07	Decreased	-2.035	12
DNA Replication, Recombination & Repair	Repair of DNA	4.36E-09	Decreased	-3.334	47
	Double-stranded DNA break repair of tumor cell lines	9.94E-06	Decreased	-2.241	14
	Metabolism of DNA	2.10E-10	Decreased	-2.09	54