Title: Analysis of matched primary and recurrent BRCA1/2 mutation-associated tumors identifies recurrence-specific drivers

Supplementary Information Files

File name: Supplementary_information.pdf
Description: Supplementary Methods, Supplementary Tables, Supplementary Figures, Supplementary Figure Legends

File name: Supplementary_Data1.xlsx
Description: Clinical metadata and sequencing performed by tumor (Basser cohorts)

File name: Supplementary_Data2.xlsx
Description: Pathogenic mutations by sequencing type and by tumor group (primary-private, recurrence-private, and shared)

File name: Supplementary_Data3.xlsx
Description: Pathogenic TP53 mutations by sequencing type and tumor group

File name: Supplementary_Data4.xlsx
Description: Tumor mutational burden, HRD, and aneuploidy scores by tumor

File name: Supplementary_Data5.xlsx
Description: Gene set enrichment analysis from whole exome sequencing (mutations and copy number variation)

File name: Supplementary_Data6.xlsx
Description: MutSigCV results from primary and recurrent tumor cohorts

File name: Supplementary_Data7.xlsx
Description: Targeted sequencing metrics, summary and for BRCA1 and BRCA2 genes

File name: Supplementary_Data8.xlsx
Description: Somatic Loss of Function Mutations in BRCA1/2 by sequencing type and tumor group

File name: Supplementary_Data9.xlsx
Description: Results from all GISTIC analyses

File name: Supplementary_Data10.xlsx
Description: Subtractive analysis of GISTIC segments in primary vs. recurrent tumors

File name: Supplementary_Data11.xlsx
Description: PARP1 copy number in primary/recurrent and TCGA cohorts

File name: Supplementary_Data12.xlsx
Description: TCGA tumors by group

File name: Supplementary_Data13.xlsx
Description: Gene set enrichment analysis from RNA sequencing
Gene fusions (FusionInspector output) involving MALAT1 and immunoglobulin genes

BRCA2 isoform expression by tumor and clinical metadata used for survival analyses; Cox Proportional Hazards models

RT-qPCR results from validation of differential BRCA2 isoform expression; primer sequences
Supplementary Methods

Validation of differential BRCA2 transcript usage by quantitative reverse transcription PCR (RT-qPCR)

We validated expression of BRCA2-001/Short and BRCA2-201/Long by RT-qPCR in 20 primary and recurrent tumors with adequate RNA. For each sample, 1000 ng of RNA (without reverse transcription) was used as a genomic DNA contamination control (control sample) and another 1000 ng for reverse transcription and PCR amplification (experimental sample). Both control and experimental samples were treated with ezDNase (Invitrogen) to remove traces of genomic DNA (gDNA). Next, cDNA was generated from experimental RNA samples using Master Mix from the SuperScript IV VILO kit (Thermo Fisher); control samples were treated with the No RT Control mix from the same kit. Control samples did not undergo reversion transcription; otherwise, experimental and control samples were processed identically.

Quantitative PCR was performed on a QuantStudio Flex Real-Time PCR System (Applied Bio) using Power SYBR Green Master Mix (Thermo Fisher). GAPDH expression was used as the endogenous control. Three sets of primers were used to amplify and differentiate between BRCA2-001/Short and BRCA2-201/Long: one for the junction of Exon 26 and Exon 27 (Exon 26/27 primers), one for the 3’ end of Exon 27 (Exon 27 primers), and one located in the intronic region between Exon 27 and Exon 28 of the long variant (Intron 27 primers). Our RNA sequencing data was used to guide the primer design, which supported expression in the three regions selected. As we used RNA exome (capture-based) expression data, we could determine that the segment of Intron 27 that we used for the primer design was included in the BRCA2-201/Long isoform, but not whether other portions or the entirety of the intron was expressed. All primer sequences are reported in Supplementary Data 16 and depicted in Supplementary Figure 10b.

Five well were prepared for each sample on a 384-well plate: one for GAPDH expression in the experimental sample, three for each of the BRCA2 primers in the experimental
sample, and one for GAPDH expression in the control sample. Each well was prepared with 10µL SYBR Green Master Mix, 6µL PCR-grade water, 1µL 10 µM forward primer, 1µL 10 µM reverse primer, and 2 µL cDNA. The PCR reaction program was: 50 °C for 2 minutes, 90 °C for 10 minutes for denaturing, 40 cycles of amplification (95 °C for 15 seconds then 59 °C for 60 seconds), and a final step with 95°C for 15 seconds and 50°C for 2 minutes. Two 384-well plates were run separately, for a total of two replicates per sample.

ΔCT values were calculated by subtracting the GAPDH CT value of the same sample from the CT value of BRCA2 primer sets. Samples were excluded from further analysis if they failed to meet the following criteria in both replicates: 1) CT values ≤ 35 for all wells containing experimental RNA (GAPDH and BRCA2 primers) and 2) CT values > 40 for contamination control RNA. ΔΔCT values were computed for the final cohort by normalizing ΔCT values to that of a single representative sample (Supplementary Data 16).

Construction of Tissue Microarrays (TMAs) containing primary and recurrent tumors

Twenty-three primary and recurrent BRCA1/2 mutation-associated tumors (a subset of the primary/recurrent cohort used throughout the study) were assembled into three tissue microarrays (TMAs). Each tissue microarray contained 4-11 1mm cores per tumor to account for tumor heterogeneity, including intratumoral, stromal, and lymphocytic-rich areas. TMA blocks were constructed, cut, stained, and imaged at the Pathology Clinical Service Center at the University of Pennsylvania. Normal liver, spleen, and kidney tissue from other patients were included as controls for antibody testing.

Immunohistochemistry (IHC) analysis of PARP1

Immunohistochemistry for PARP1 was performed on each TMA using standard laboratory protocols and the PARP (46D11) Rabbit mAb (Cell Signaling Technology, catalog #9532). Stained slides were imaged for quantification at 20x resolution. PARP1 nuclear positivity was
quantified for each core using H-score (0-300 scale), as determined by AN. Hematoxylin and
eosin (H&E) staining was used to assess tumor content in parallel; cores with inadequate tumor
per H&E were excluded by AN. We calculated an average PARP1 H-score for each tumor for
downstream analyses (range 3-10 evaluable cores per tumor).

Co-detection by Indexing (CODEX) coverslip preparation and staining
TMA slides were cut at 5µm and placed on previous coated poly-lysine-l coverslips for
CODEX37. We used a custom 40-plex breast and ovarian cancer specific antibody panel (see
Supplementary Table 2 for antibody list including dilutions). CODEX staining was performed
according to the manufacturer protocols, with one additional step to reduce background
fluorescence38. Briefly, tissue coverslips were submerged in bleaching solution (4.5% (w/v) H2O2
and 20mM NaOH in PBS) after antigen retrieval. While in solution, slides were incubated
between two LED lights (Aibecy A4 Ultra Bright 25,000 Lux LED Light) for 45min at room
temperature (RT). Bleaching solution was then replaced, and tissue coverslips were incubated
between lights for an additional 45min at RT. Coverslips were then washed four times in 1x PBS
for 3-5 minutes. Lastly, the tissue was placed in staining solution following the manufacturer’s
protocol.

CODEX Imaging
Imaging was performed using a Leica DMi8 inverted microscope with 4 filter cubes (DAPI:
Excitation: 359nm Emission: 457nm; RHOD: Excitation: 551nm, Emission: 573nm, Y5:
Excitation: 651, Emission: 671nm; Y7: Excitation: 756nm, Emission: 779nm), equipped with 20x
lens (numerical aperture: 0.8) and LAS X3.6.0.20104. Each core was imaged with a 20x
immersion objective in a 2x2 tiled acquisition, ~300nm/pixel resolution and 6 z-planes per tile.
CIM v1.29.0.364 was used to control Fluidics operation and set up cycle/marker information.
Processing of raw images from CODEX

Raw data were formatted and transferred using CIM version 1.29.0.364. We processed the data in order to perform cropping and stitching, flat field correction, drift-compensation, best focus definition and background subtraction. To remove out-of-focus light, image deconvolution was performed using Microvolution software (Microvolution LLC, California, USA). QPTIFFs were generated using processor v1.8.0.257 – dev. We generated 43-55 images per TMA (one per core), each consisting of 41 channels (40 antibodies plus 1 nuclear stain).

CODEX cell segmentation

Processed data (QPTIFF) was opened in Phenochart version 1.1.0. DAPI sensitivity was adapted in order to select individual core areas to train the segmentation algorithm. QPTIFF was then selected and loaded using InForm version 2.5.1. We adapted cell segmentation settings such as pixel intensity, nuclear component splitting, and expected cell size to detect cells with that varied in size and shape. To define single cell boundaries, we used three markers to identify tumor (TC) and immune cells (IC) and to distinguish between nuclei, cytoplasm and membrane: nuclear marker (DAPI), CD45 (IC), and PANCK (TC). All segmented areas were visually checked with ImageJ v1.53c to confirm appropriate segmentation.

CODEX marker quantification

We used ImageJ v1.53c with CODEX_MAV (version 1.5.0.8) plugin to visualize individual cores and merge cores by tumor. Samples were gated using CD45 to detect immune cells (IC), PANCK or Cytokeratin14 to detect tumor cells (TC), and Vimentin to detected stromal cells. Individual populations for each patient were then gated on RAD51, PD-1 and CTLA-4 positivity. Additionally, pixel intensity of each marker was determined for each population (normalized counts) using ImageJ MAV version 1.5.0.8. All gated cells of each population (such as PANCK+/RAD51+ cells) were visually assessed using ImageJ MAV. The percentages of
RAD51+, CTLA-4+, and PD-1+ cells per population (TC or IC) were calculated using the total cell number of each population. In regards to select RAD51+ cells, we only included cells with a nuclear RAD51 expression <1-2µm. We visualized groupwise percentages of cells with box plots generated with GraphPad Prism 6.0. Groupwise differences were assessed using two-sided Wilcoxon rank sum tests (α=0.05).
Supplementary Table 1: Genes included in targeted capture for high-depth sequencing, by coverage type.

| Exons+UTR Coverage | Whole Gene Coverage |
|--------------------|---------------------|
| ABL1               | DICER1              |
| ALK                | EIF5A2              |
| AQR                | EME1                |
| ARAF               | EME2                |
| ARID1A             | EPHA3               |
| ATM                | ERBB3               |
| ATR                | ERBB4               |
| AURKA              | ERCC2               |
| BAP1               | ERCC3               |
| BARD1              | ERCC4               |
| BLM                | ERCC6               |
| BRAF               | ESR1                |
| BRIP1              | FANCA               |
| C11ORF80           | FANCE               |
| CBX2               | FANC                  |
| CCND1              | FBXW7               |
| CCND2              | FGF1                |
| CCND3              | FGFR1               |
| CDK12              | FLT3                |
| CDK4               | FOXL2               |
| CDK6               | FRS2                |
| CDKN1A             | GNA11               |
| CDKN1B             | GNAQ                |
| CDKN1C             | GNAS                |
| CDKN2A             | GTF2H3              |
| CDKN2B             | HRAS                |
| CEBPA              | IGF1R               |
| CHEK1              | JAK2                |
| CHEK2              | JAK3                |
| CREBBP             | JARID2              |
| CRKL               | KDR                 |
| CSF1R              | KIT                 |
| CTNNB1             | KLHDC3              |
| CUL4A              | KMT2C               |
| DAB2               | KRAS                |
| DDB1               | LIG3                |
| DDR2               | MAP2K1              |
| EGFR               | MECT                |
| ERCC2              | MSH6                |
| ERCC3              | MSH4                |
| ERCC4              | MTROR               |
| ERCC6              | MUS81               |
| ESR1               | NBN                 |
| FANCA              | NF2                 |
| FBXW7              | NRAS                |
| FGF1               | OPCML               |
| FGFR1              | PALB2               |
| FLT3               | PAX8                |
| FOXL2              | PEG3                |
| FRS2               | PIK3R1              |
| GNA11              | PLAGL1              |
| GNAQ               | PMS2                |
| GNAS               | POLD1               |
| GTF2H3             | POLD2               |
| HRAS               | POLD3               |
| IGF1R              | POLD4               |
| JAK2               | POLK                |
| JARID2             | PRM1D               |
| KDR                | PPP2R1A             |
| KIT                | PRDM9               |
| KLHDC3             | PRKC1               |
| KMT2C              | PRKDC               |
| KRAS               | PRPF19              |
| LIG3               | RAB25               |
| MAP2K1             | RAD51               |
| MECT               | RAD51               |
| MSH6               | RAD51               |
| MSH4               | RAD52               |
| MTROR              | RAD54B              |
| MUS81              | RPA1                |
| NBN                | RPA2                |
| NF2                | RPS6KA2             |
| NOTCH2             | RSF1                |
| SETMAR             | SMARCA4             |
| SSBP1              | SMARCB1             |
| SPARC              | SPARC               |
| SRC                | SRC                 |
| SSBP1              | STK11               |
| SMAD2              | TERT                |
| SMAD4              | TRIP13              |
| SMCHD1             | TOP3A               |
| SMCHD1             | TOP3B               |
| STK11              | TRIP13              |
| TERT               | TRIP13              |
| TRIP13             | TRIP13              |
| TRIP13             | TRIP13              |
| TOP3A              | TRIP13              |
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**Supplementary Table 2: Antibodies used for CODEX.**

| Antibody            | Clone          | Dilution | Manufacturer   |
|---------------------|----------------|----------|----------------|
| Anti-human pATM     | EP1890Y        | 1:50     | Abcam          |
| Anti-human RAD51    | EPR4030(3)     | 1:50     | Abcam          |
| Anti-human PD-1     | D4W2J          | 1:100    | Cell Signaling |
| Anti-human BRCA1    | MS110          | 1:100    | Abcam          |
| Anti-human CTLA-4   | EPR1476        | 1:100    | Abcam          |
| Anti-human CD45     | 2B11+PD7/26    | 1:100    | Novusbio       |
| Anti-human PanCK    | AE-1/AE-3      | 1:200    | Akoya          |
| Anti-human Keratin 14 | Poly19053     | 1:200    | Akoya          |
| Anti-human H3-Ser28 | HTA28          | 1:200    | Akoya          |

**Supplementary Figures and Figure Legends**
Mutations from WES

Mutations from Targeted Sequencing

Mutation Type
- Heterozygous LoF mutation
- Heterozygous GoF mutation
- No mutation
- Not sequenced

Tumor OrIGIN
- Germline Mutation
- Tumor Type
- RB1 Status
- PTEN Status

Tumor Mutational Burden = # SNVs + Indels

Capture size (Mbp)
Supplementary Figure 1. Stratified analysis of mutations from whole exome and targeted sequencing. A. 67 paired primary and recurrent tumors from 27 patients sequenced by whole exome sequencing (WES, n=67) and high-depth targeted sequencing (n=44). Tumors are displayed in chronological order by patient, with the primary tumor at the top and latest recurrence at the bottom. LOH = BRCA1/2 allele-specific loss of heterozygosity. B. Somatic mutations from WES, by mutation type and zygosity. C. Somatic mutations from targeted sequencing, by mutation type and zygosity. Display in B and C is limited to genes with ≥ 1 mutation with alternative allele fraction ≥0.05 from targeted sequencing. D. Comparison of tumor mutational burden for primary/recurrent tumor pairs (n = 54 biologically independent tumor samples, comprising 2 samples/patient from 27 patients). For patients with multiple recurrences, one recurrence was chosen at random for comparison. Boxplot elements are as follows: median, center line; box limits, first and third quartiles (spanning the IQR, interquartile range); whiskers, 1.5x IQR in each direction; outliers plotted individually. Pairwise differences were determined by two-sided Wilcoxon signed rank test (α=0.05). NS = not significant.
a 6-1 (nonLOH; estimated cellularity 0.19)

b 6-2 (LOH; estimated cellularity 0.55)
Supplementary Figure 2. BRCA1 germline variants, B allele frequency, and copy number before and after a representative nonLOH to LOH transition. A. Location and variant allele fraction of BRCA1 germline variants in Patient 6’s primary breast tumor without allele-specific loss of heterozygosity LOH (top), pictured with B allele frequency and copy number across chromosome 17 (bottom). B. Location and variant allele fraction of BRCA1 germline variant in Patient 6’s first breast tumor recurrence with LOH (top), pictured with B allele frequency and copy number across chromosome 17 (bottom). For A and B, germline variants were called by VarDictJava and VarScan2. B allele frequency and copy number plots were generated in Sequenza.
Supplementary Figure 3. *BRCA1* germline variants, B allele frequency, and copy number before and after a representative LOH reversal. A. Location and variant allele fraction of *BRCA1* germline variants in Patient 20’s primary ovarian tumor with allele-specific loss of heterozygosity (LOH, top), pictured with B allele frequency and copy number across chromosome 17 (bottom). B. Location and variant allele fraction of *BRCA1* germline variant in Patient 20’s second ovarian tumor recurrence without LOH (top), pictured with B allele frequency and copy number across chromosome 17 (bottom). For A and B, germline variants were called by VarDictJava and VarScan2. B allele frequency and copy number plots were generated in Sequenza.
Supplementary Figure 4. Genome-wide measures of copy number variation in primary/recurrent cohort. A. Comparison of aneuploidy score for primary/recurrent tumor pairs (n = 54 biologically independent tumor samples, comprising 2 samples/patient from 27 patients).

B. Comparison of Homologous Recombination Deficiency (HRD) score for primary/recurrent tumor pairs (n = 54 biologically independent tumor samples, comprising 2 samples/patient from 27 patients). C-E. Comparison of individual HRD metrics for primary/recurrent tumor pairs (n = 54 biologically independent tumor samples, comprising 2 samples/patient from 27 patients): loss of heterozygosity (HRD-LOH, C), non-telomeric allelic imbalance (HRD-NTAI, D), and large scale state transitions (HRD-LST, E). For patients with multiple recurrences, one recurrence was chosen at random for statistical comparisons in plots A-E. Boxplot elements are as follows: median, center line; box limits, first and third quartiles (spanning the IQR, interquartile range); whiskers, 1.5x IQR in each direction; outliers plotted individually. Pairwise differences were determined by paired two-sided Wilcoxon signed rank test (α=0.05). F. GISTIC qplot for 90% confidence interval deletions in primary tumors. G. GISTIC qplot for 90% confidence interval deletions in recurrences. For F and G, all highlighted genes have residual q<0.05. NS = not significant.
Supplementary Figure 5. GISTIC results by tumor type in primary/recurrent cohort. A. GISTIC qplot for 90% confidence interval amplifications in (primary and recurrent) breast tumors. B. GISTIC qplot for 90% confidence interval amplifications in (primary and recurrent) ovarian tumors. C. GISTIC qplot for 90% confidence interval deletions in (primary and recurrent) breast tumors. D. GISTIC qplot for 90% confidence interval deletions in (primary and recurrent) ovarian tumors. For A-D, all highlighted genes have residual q<0.05.
TCGA PanCancer Breast and Ovarian patients with matched tumor/normal WES
n=864 breast
n=327 ovarian

Remove tumors that failed QC
n=6 breast
n=15 ovarian

Identify tumors with a pathogenic germline mutation in BRCA1 or BRCA2
(see Methods)

BRCA1/2 germline mutation-associated tumors (gBRCA1/2 group)
n=37 Breast
n=69 Ovarian

For tumors without gBRCA1/2 mutation:
Remove tumors with somatic BRCA1/2 loss via somatic mutation, deletion, low expression, or BRCA1 promoter methylation
(see Methods)
n=44 breast
n=35 ovarian

Remove tumors with germline or somatic mutations in HR-related genes
(see Supplementary File 7)
n=119 breast
n=9 ovarian

Remove tumors that failed Sequenza
n=1 HR-WT Breast
n=1 gBRCA1/2 Ovarian

Tumors proficient for homologous recombination genes (HR-WT group)
n=657 Breast
n=198 Ovarian

b

TCGA Tumor Group

Percent of Tumors (%)

PARP1 Copy Number Status
- Deletion
- Loss
- Neutral
- Gain
- Amplification
- No CN reported for locus

gBRCA1/2 Breast (n=37)
HR-WT Breast (n=657)
gBRCA1/2 Ovarian (n=69)
HR-WT Ovarian (n=198)
Supplementary Figure 6. Analysis of PARP1 copy number variation in TCGA cohorts. A. Strategy for grouping The Cancer Genome Atlas (TCGA) breast and ovarian tumors into those with germline BRCA1/2 mutations (gBRCA1/2) and “Homologous Recombination-Wild Type” (HR-WT) groups for analysis. Tumors were excluded on the basis of somatic BRCA1/2 loss or mutations in genes required for homologous recombination. B. PARP1 copy number by tumor in selected TCGA breast and ovarian cohorts. Groupwise differences in average copy number were determined by two-sided t-test (α=0.05). Copy number (CN, from Sequenza) was binned as follows: Deletion, CN=0; Loss, CN=1; Neutral, CN=2-3; Gain, CN=4-5; Amplification, CN≥6. NS = not significant.
Supplementary Figure 7. Sample relatedness identified by RNA sequencing. A. Distribution of $\log_2$ (counts per million) across cohort of samples used for RNA sequencing. B. Dendrogram of sample relatedness across RNA sequencing cohort, by tumor type and estrogen receptor (ER) status. C. Principal components (PC) analysis of RNA sequencing cohort.
Supplementary Figure 8. Differential gene expression in tumors vs. normal tissue. A.
Differential gene expression in primary breast tumors vs. normal breast tissue from BRCA1/2
mutation carriers. B. Differential gene expression in primary ovarian tumors vs. normal fallopian
tube tissue from BRCA1/2 mutation carriers. For A and B, a positive Log₂(fold change) indicates
genes with increased expression in primary tumors. Adjusted p values were computed based on
linear modeling of mean-variance trends (limma). C. Hallmark Gene Sets enriched in genes with
increased expression in primary and recurrent breast tumors compared to normal breast tissue
from BRCA1/2 mutation carriers. D. Hallmark Gene Sets enriched in genes with increased
expression in primary and recurrent ovarian tumors compared to normal fallopian tube from
BRCA1/2 mutation carriers. For C and D, all gene sets had adj. p<0.05. NES = normalized
enrichment score.
Supplementary Figure 9. PARP1 expression and copy number status in primary and recurrent tumors. PARP1 copy number status and expression across all tumors with whole exome and RNA sequencing, and all normal samples with RNA-seq. Groupwise differences in average copy number were determined by Kruskal-Wallis test, followed by Dunn’s test with Bonferroni correction ($\alpha=0.05$, **p<0.0001). NS = not significant.
Supplementary Figure 10. Validation of differential BRCA2 isoform expression by quantitative reverse transcription PCR (RT-qPCR). A. RNA binding protein (ELAVL1/HuR and PABPC1) prediction for BRCA2 transcripts based on RNA immunoprecipitation sequencing (RIP-seq) in GM12878 (Human B cells). ENST00000380152 refers to BRCA2-001/Short; ENST00000544455 refers to BRCA2-201/Long. B. Regions targeted by three sets of primers for detection of BRCA2-001/Short and BRCA2-201/Long by RT-qPCR. The regions were designed based on the RNA exome (capture-based) expression data. Color coding denotes whether primers were designed to detect both isoforms (black) or specifically BRCA2-201/Long (blue). C. Detection of BRCA2 transcript regions by RT-qPCR, compared to the BRCA2 isoform expression initially assessed by RNA-seq. Relative expression was normalized to GAPDH expression in one representative sample (n = 2 technical replicates from 20 biologically independent tumor samples in 14 patients; see Supplementary Methods). Boxplot elements are as follows: median, center line; box limits, first and third quartiles (spanning the IQR, interquartile range); whiskers, 1.5x IQR in each direction; outliers plotted individually. Groupwise differences in expression were assessed separately for each primer set by Kruskal-Wallis test (α=0.05). NS = not significant.
Median OS 86.8 (95%CI 58.6−132) vs 99.5 (95%CI 86.8−135) Months

BRCA2-001/Short Status
Expressed in Primary and/or Recurrent Ovarian Tumor
Not Expressed in Any Ovarian Tumor

Tissue Origin
Germline Mutation
Sample Type
BRCA2-001/Short
BRCA2-201/Long

Breast
Ovary
BRCA1
BRCA2
Primary Tumor

Transcripts per Million

Median OS 86.8 (95%CI 58.6−132) vs. 99.5 (95%CI 86.8−135) Months
p=0.233
Supplementary Figure 11. Differential isoform usage of BRCA2 in a validation cohort of BRCA1/2 mutation-associated primary tumors. A. BRCA2 isoform usage by sample type in an independent cohort of 42 primary breast and ovarian tumors from BRCA1/2 mutation carriers. B. Overall Survival (OS) curve for patients that expressed BRCA2-001/Short in any (primary or recurrent) ovarian tumor compared to those that did not. Survival proportions and p-value were calculated using a Cox proportional hazards model tested for significant associations with estrogen receptor status, age at diagnosis, BRCA1 vs. BRCA2 mutation, and tumor stage at diagnosis; and adjusted for patient recurrent status (α=0.05, see Methods).
Supplementary Figure 12. PARP1 expression in primary/recurrent tumors by immunohistochemistry (IHC). A. Image of PARP1 IHC in tissue microarray (TMA) of primary and recurrent breast tumors. B. Image of PARP1 IHC in TMA of primary and recurrent ovarian tumors (1/2). C. Image of PARP1 IHC in TMA of primary and recurrent ovarian tumors (2/2). For A-C, images were taken at 0.5x magnification. D. Representative images of individual TMA cores from ovarian tumors with low PARP1 expression (left, H-score = 125), medium PARP1 expression (middle, H-score = 200), and high PARP1 expression (right, H-score = 300). Images were taken at 7-7.2x. Images are representative of 23 total tumors for which PARP1 IHC was performed.
Supplementary Figure 13. Comparisons of DNA damage markers and immune checkpoint proteins in paired primary/recurrent tumors by CODEX. A. Representative image of cell cycle and DNA damage markers in breast and ovarian primary and recurrent tumors. Images are representative of 23 tumors for which CODEX was performed. B. Expression of markers for cell type, DNA damage response, and immune checkpoints in primary and recurrent breast tumors from five patients. C. Expression of markers for cell type, DNA damage response, and immune checkpoints in primary and recurrent ovarian tumors from four patients. D. Expression of markers for cell type, DNA damage response, and immune checkpoints in primary and recurrent ovarian tumors from four additional patients. For B-D, tumor and stromal cells are displayed separately for each specimen; PANCK, Keratin14, and CD45 were used for gating of tumor and stroma (see Supplementary Methods). Tumors are numbered in chronological order following the convention [Patient] - [Tumor number] such that 16-1 is Patient 16’s primary tumor, 16-2 the first recurrence, etc. For clinical metadata from individual tumors, see Supplementary Data 1. TMA = Tissue Microarray.
Supplementary Figure 1. Comparisons of RAD51 and immune checkpoint protein expression in primary and recurrent tumors by CODEX. A. Percent of RAD51+ tumor cells in primary vs. recurrent breast tumors. B. Percent of RAD51+ tumor cells in primary vs. recurrent ovarian tumors. C. Percent of CTLA-4+ cells in primary vs. recurrent breast tumors. D. Percent of CTLA-4+ cells in primary vs. recurrent ovarian tumors. E. Percent of PD-1+ cells in primary vs. recurrent breast tumors. F. Percent of PD-1+ cells in primary vs. recurrent ovarian tumors. For A-F, sample sizes were as follows: n=3 primary breast tumors, n=4 recurrent breast tumors, n=8 primary ovarian tumors, n=8 recurrent ovarian tumors. Boxplot elements are as follows: median, center line; box limits, first and third quartiles (spanning the IQR, interquartile range); whiskers, 1.5x IQR in each direction; outliers plotted individually. Groupwise differences were determined by two-sided Wilcoxon rank sum test ($\alpha=0.05$). NS = not significant.
**a** BRCA1 germline mutation

**Somatic reversion mutation**
(in-frame deletion)

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**Patient 20 Germline**

BRCA1 c.G4069T

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**Pt 20 Primary Ovarian Tumor**

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**Pt 20 Recurrence 1**

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**Pt 20 Recurrence 2**

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**Pt 20 Recurrence 3**

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**Pt 20 Recurrence 4**

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Refseq Genes

BRCA1
Supplementary Figure 15. Somatic reversion of *BRCA1* after PARPi in Patient 20. A. Graphical representation of in-frame somatic deletion of germline nonsense mutation in *BRCA1* (mutation pictured is not Patient 20’s actual germline mutation). B. Integrative Genomics Viewer (IGV) tracks from whole exome sequencing of germline and tumor DNA from patient 20. For each sample, forward strand alignment and coverage tracks are displayed around the site of the patient’s germline *BRCA1* c.G4069T (p.E1357X) mutation. G>T mutation appears as C>A because *BRCA1* lies on reverse strand. Pt = patient.