Supplemental Methods 8:

8.1 Introduction

We analyzed several pathways that are generally altered in different cancer types, specifically the RAS/PI3K, RB, and p53 signaling pathways, as well as the homologous recombination (HR) pathway, which has germline as well as somatic alterations in ovarian cancer. For all pathway analyses, we used the set of cases (N=316) with complete data (mRNA expression, DNA copy-number, methylation, and protein mutations).

Figure S8.1 outlines the assessment approach used to determine whether a particular gene was altered or not altered in a particular sample. Our approach was based on first examining each gene across all samples, and binning each gene into one of four categories:

- Category 1: Gene is altered by mutations.
- Category 2: Gene is primarily altered by copy number alterations, and mRNA expression levels correlate with copy number changes.
- Category 3: Gene is epigenetically silenced.
- Category 4: Gene has evidence of a bimodal expression pattern, unrelated to copy number status.

As outlined in Figure S8.1, we then used different alteration criteria for each of the four categories. For example, for Category 2 genes, we classified each gene as a likely oncogene or tumor suppressor, and a gene was called altered in a specific sample if the gene was altered by a high level copy-number amplification or homozygous deletion (as defined by GISTIC, see

Supplemental Methods 5). Category 3 epigenetically silenced genes were defined by k-means clustering; for example, for *BRCA1*, we used k-means clustering on the two-dimensional space of DNA methylation and expression data to separate the epigenetically silenced group and the non-epigenetically silenced group of samples. Finally, for category 4 genes, alteration status was defined by relative expression compared to the expression distribution in tumor samples diploid in the particular gene, ≥ one standard deviation. In all categories, a gene was called altered if the gene contained a non-synonymous, somatic (or in the case of BRCA1/2, a germline) mutation in a protein-coding region.

A pathway was considered altered in a given sample, if at least one gene in the pathway was altered.

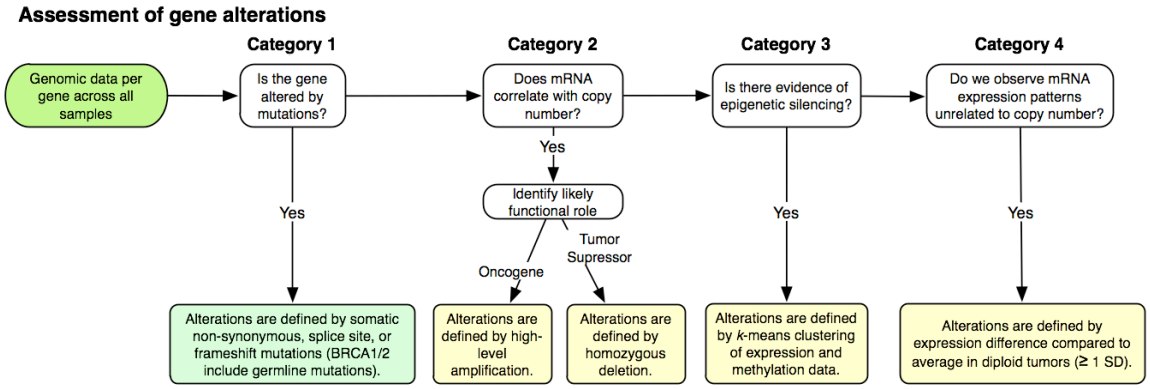

Figure S8.1. Assessment of gene alterations used in pathway analysis.

8.2. Cancer Pathways

TP53 pathway

For the TP53 protein, we observe a mutation rate of 87%. With the depth of coverage of TP53 with the hybrid capture and next generation sequencing approaches, it is possible and even likely that a subset of mutations in TP53 were missed raising the possibility that TP53 mutations are essentially universal. Samples with truncating TP53 mutations, i.e. nonsense, splice, and frame shift mutations (approximately one third of cases) have markedly lower TP53 expression than those with missense mutations or in-frame deletions (Figure S8.2), possibly caused by nonsense-mediated decay (NMD) of mRNA (17 samples with low expression are candidates for missed truncating mutations). Amplifications of *MDM2* and *MDM4* are uncommon, occurring in 4% and 3% of cases, respectively.

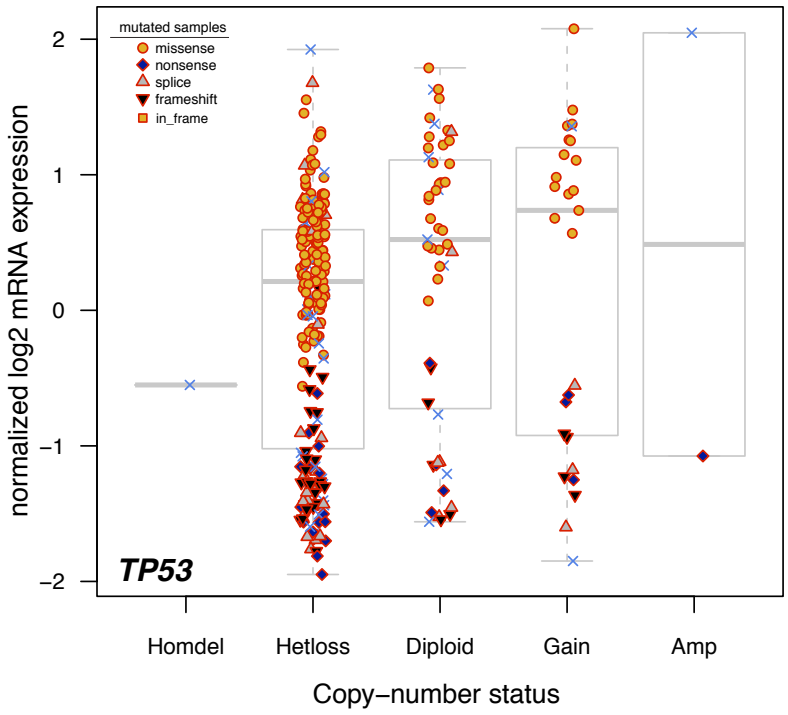

Figure S8.2: Truncating mutations of TP53 lead to markedly lower transcript levels, independent of copy-number status.

RB pathway

Amplification of *CCNE1* is one of the most common focal copy number change events in serous ovarian cancer, occurring at a frequency of 20%. *RB1*, immediately downstream of *CCNE1*, is deleted in 25 samples and mutated in an additional nine samples (10.8% of cases combined). As is the case with *PTEN* and *NF1* (see below), some of the *RB1* deletions are intragenic, i.e., do not affect the entire gene, and cases with intragenic deletions have low mRNA expression at the exon level but not the whole gene level (data not shown).

CDKN2A, a negative regulator of cyclins and cyclin-dependent kinases, is frequently altered in various types of cancer, typically by deletion or epigenetic regulation. In this data set, we observe a striking bimodal expression pattern, with approximately one third of the cases with very low or no expression (Figure S8.3). There is no evidence for *CDKN2A* promoter methylation in the samples with low expression. Low *CDKN2A* mRNA expression is mutually exclusive with *CCNE1* amplification and *RB1* deletion/mutation events (P = 4.726e-11, two-sided Fisher's Exact Test, Figure S8.4).

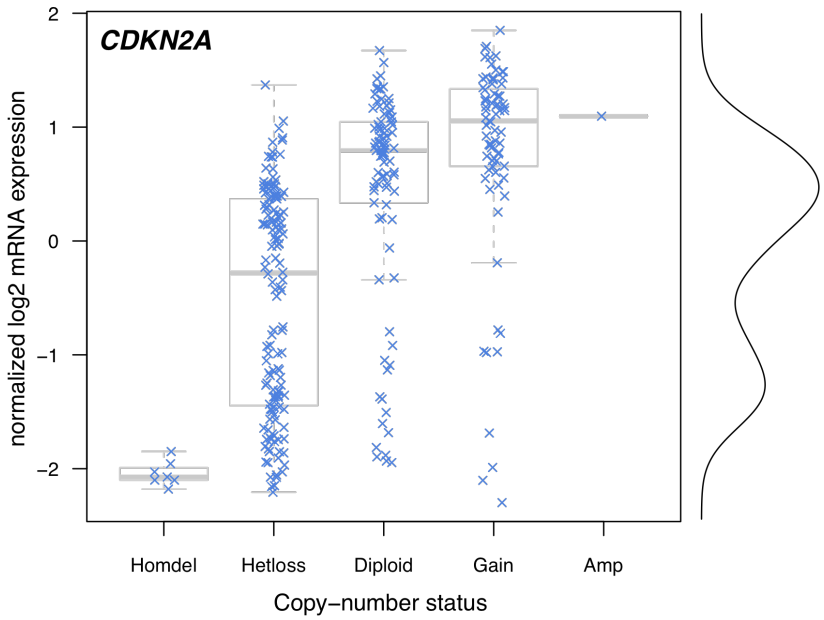

Figure S8.3: Bimodal expression pattern of *CDKN2A*.

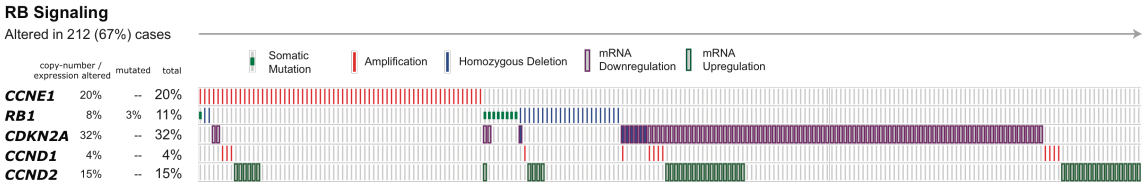

Figure S8.4: Alteration pattern in the RB signaling pathway. Each column represents an individual case; each row represents a gene. Only cases with RB signaling alterations (N=212) are shown. The percent altered is relative to N=316.

RAS/PI-3-Kinase-signaling

Various key members of the RAS/PI3K pathway are frequently altered by several different mechanisms in ovarian cancer[1]. The most commonly altered genes in the pathway are *PTEN* (homozygous deletion or mutation), *PIK3CA* (amplification or mutation), *KRAS* (amplification or mutation), *NF1* (homozygous deletion or mutation), as well as *AKT1* and *AKT2* (amplification) (Figure S8.5). Known activating mutations are observed in *PIK3CA* (two cases, E545A and H1047R), *KRAS* (two cases, both G12V), and *BRAF* (one case, N581S).

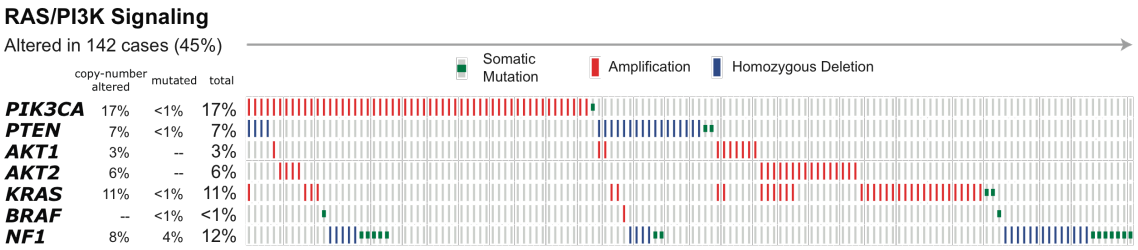

Figure S8.5: Alteration pattern in the RAS/PI3K signaling pathway. Each column represents an individual case; each row represents a gene. Only cases with RAS/PI-3-K signaling alterations (N=142) are shown. The percent altered is relative to N=316.

A fraction of the homozygous deletions of *PTEN* and *NF1* are intragenic, i.e. they only affect part of the gene. In these cases, we usually observe lower expression of the deleted exons than of the rest of the gene (Figure S8.6, A-C).

We also observed uncommon but focal amplification of *ERBB2* (4 cases, 1.3%) and *ERBB3* (12 cases, 3.8%) (Figure S8.6, D-E). While *ERBB2* expression is markedly increased with amplification, expression increase of *ERBB3* is only modest.

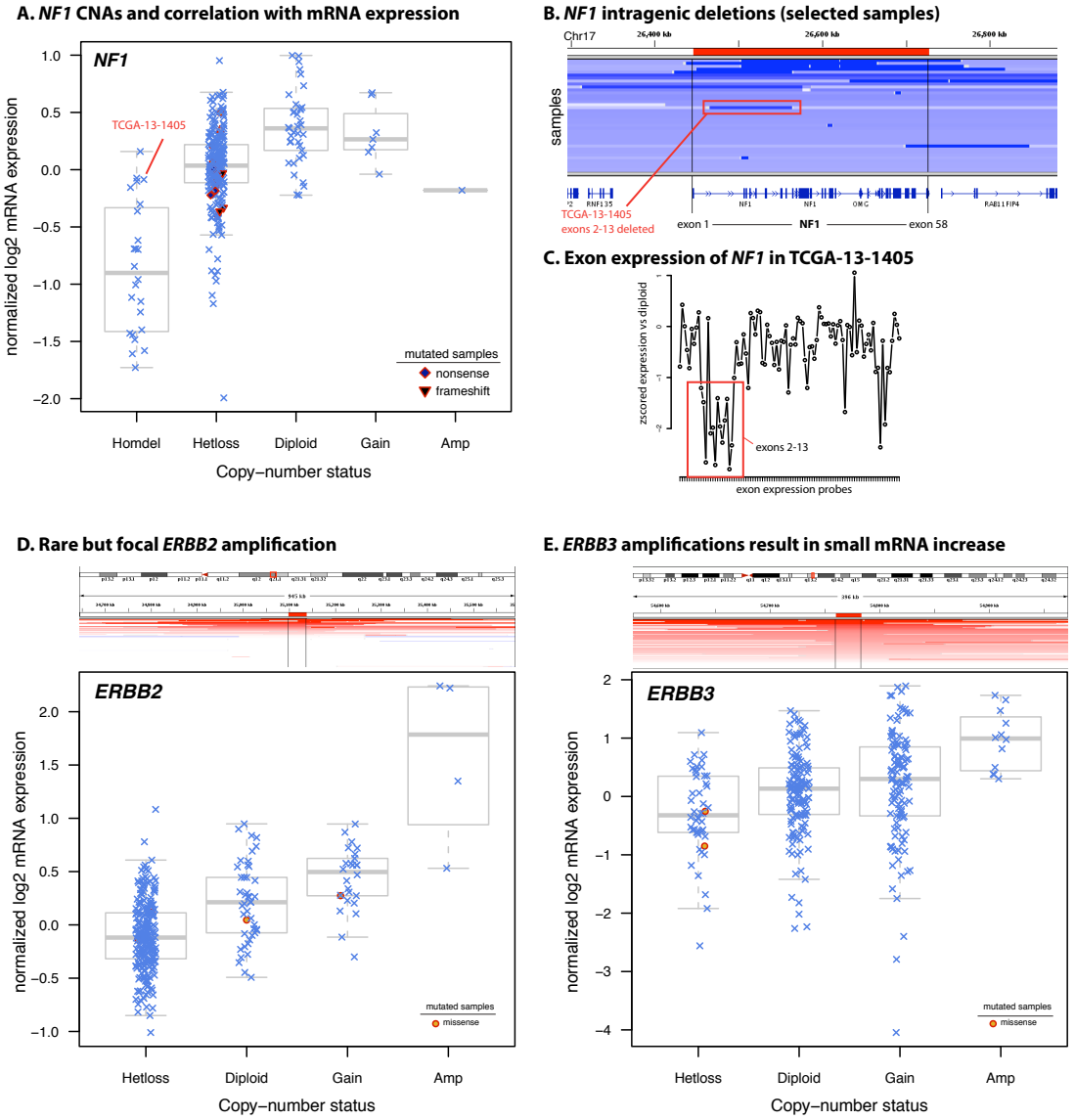

Figure S8.6: *NF1* Deletion and *ERBB2*/*ERBB3* Amplification. A) Correlation between *NF1* copy-number state and mRNA expression. Some samples with homozygous deletion of *NF1* do not have low mRNA expression, usually because they are only partially deleted, with possible full loss of function. **B)** Intragenic deletions of *NF1* are frequent, sometimes only affecting one exon. **C)** Sample TCGA-13-1405 has a deletion of exons 2-13 of *NF1*, and these exons show the lowest expression values across the gene. **D)** The few samples with focal, high-level amplification of *ERBB2* result in markedly increased mRNA expression. **E)** *ERBB3* expression is only modestly increased by gene amplification.

8.3 Homologous Recombination (HR)

Introduction

Approximately 10-15% of ovarian cancers appear to be hereditary, and the majority of these cases are due to germline mutations in *BRCA1* or *BRCA2*[1]. A subset of sporadic ovarian tumors appear to share distinctive DNA-repair defects with *BRCA1*/*BRCA2* germline mutation carriers, a phenomenon broadly described as "BRCAness"[2,3,4]. DNA-repair defects can be caused by germline or somatic alterations to the homologous recombination (HR) DNA repair pathway, including somatic mutation of *BRCA1*/*BRCA1* and epigenetic silencing of *BRCA1*, alterations to the core set of Fanconi Anemia genes, and additional genetic alterations to other key members of the HR pathway. For example, somatic mutations in *BRCA1* and *BRCA2* have previously been observed in sporadic ovarian cancer, but these events were considered relatively rare in ovarian cancer -- early studies have reported somatic mutation rates of 7-9% in *BRCA1* and 4% in *BRCA2*[5,6,7]. Additionally, *BRCA1* silencing via promoter hypermethylation has been reported in ovarian cancer[8,9], and recent studies have observed *BRCA1* hypermethylation in 18% of ovarian patients[10]. Other recent studies have identified EMSY amplification[11,12] and *FANCF* hypermethylation[13] as two additional means of inactivating the BRCA pathway in a broader spectrum of sporadic ovarian cancers.

Identifying ovarian cancer cases with defects in BRCA or the homologous recombination (HR) pathway is of increased clinical relevance due to the advent of new PARP inhibitors,[14,15] with potentially synthetic lethal effect when applied to cells with pre-existing defects in HR DNA repair. *In vitro* experiments have demonstrated that PARP inhibitors uniquely affect the survival of tumors cells with defects in HR, while leaving normal cells intact, and that *BRCA1* and *BRCA2* deficient cells are up to 1000 times more sensitive to the current set of PARP inhibitors[16,17]. Multiple PARP inhibitor drugs are currently in clinical trials in breast and ovarian cancer[14], and early Phase 1 and 2 trials in *BRCA1*/*BRCA2* mutation carriers appear promising[18,19]. High-throughput screening has also identified PARP sensitivity in cells deficient in other HR pathway members, including *RAD51*, *RAD54*, *DSS1*, *RPA1*, *NBS1*, *ATR*, *ATM*, *CHK1*, *CHK2*, *FANCD2*, *FANCA*, and *FANCC*[20]. *PTEN* deficiency has also been recently identified to cause homologous recombination defects in human tumor cells, and to sensitize tumor cells to PARP inhibitors[21]. Many investigators have therefore hypothesized that PARP inhibitors may be effective against a much larger group of tumors, beyond just BRCA1/BRCA2 mutation carriers[3,14,15].

A key challenge is to determine the extent of BRCA defects in sporadic ovarian cancers, develop biomarkers for these defects and for the response to, e.g., PARP inhibitor therapy, and apply this knowledge to identify patients likely to benefit from PARP inhibition therapy.

Analysis of alterations in HR DNA repair processes

For the analysis of the homologous recombination (HR) and BRCA pathways, four levels of analysis were performed:

- First, a detailed analysis of BRCA1/2 mutations and epigenetic silencing of *BRCA1*.
- Second, a detailed analysis of well-annotated genes known to be involved in the canonical HR pathway. This includes, for example, the set of Fanconi Anemia genes, *C11orf30* (EMSY), *RAD51*, the DNA damage sensing genes *ATM* and *ATR* and *PTEN*.

- Third, a global, but less detailed assessment of approximately 40 other HR-related genes. Additional genes were derived from an extended literature and pathway search, and Gene Ontology annotation.

- Fourth, to investigate potential cross-talk with other genes and pathways, we compared the complete set of BRCA inactivation events to all recurrently altered copy number peaks, as defined by GISTIC, looking for trends in mutual exclusivity and co-occurrence.

BRCA Alterations

BRCA Mutations

BRCA1 is mutated in 37 of 316 cases (11.7%): Twenty-seven (8.5%) cases have germline mutations and 10 (3.2%) have somatic mutations (Table S8.1, Figure S8.7A). Thirteen of the observed *BRCA1* germline mutations correspond to the well-known 'founder' mutations 185/187delAG and 5382/5385insC, both of which have been extensively studied in Ashkenazi Jewish populations[22,23,24,25]. *BRCA2* is mutated in 29 of 316 cases (9.2%): Twenty (6.3%) cases have germline mutation and 9 cases (2.9%) have somatic mutations (Table S8.1, Figure S8.7B). Five of the observed *BRCA2* germline mutations correspond to the well-known 6174delT founder mutation[24,26]. Thirty of the 37 (81%) *BRCA1* mutations are accompanied by heterozygous loss of *BRCA1*, indicating that both alleles are inactivated, as predicted by Knudson's two-hit hypothesis for a tumor suppressor gene (Figure S8.8A). Twenty-one of the 29 (72.4%) *BRCA2* mutations are accompanied by heterozygous loss (Figure S8.8B). Eighty-eight percent of germline *BRCA1* mutations matched to existing records in the Breast Cancer Information Core (BIC) Database (http://research.nhgri.nih.gov/projects/bic/), compared to 40% for somatic mutations; similarly, 58% of germline *BRCA2* mutations matched to existing BIC records, compared to 30% for somatic mutations.

In total, *BRCA1* or *BRCA2* are mutated in 64/316 cases (20.3%, Table S8.3). This corresponds to a germline mutation rate of 14.6% and a somatic mutation rate of 6.0%. The observed mutation rates are within range of previous reports. For example, a 2010 study involving 235 women with ovarian cancer found germline and somatic mutation rates of approximately 11.5% and 7% respectively[4], and a 2005 U.S. based survey involving a total of 232 women found *BRCA1/2* germline mutations in 13.8% of all cases, and 14.8% of serous cases[27].

With the exception of two cases, *BRCA1* and *BRCA2* mutations are mutually exclusive, but the mutual exclusivity is not statistically significant (N=316 P = 0.5518, two-sided Fisher's exact test).

Table S8.1: *BRCA1* Mutations

***BRCA1* Germline Mutations, (sorted by nucleotide position)**

Case ID	Mutation Type	Mutation	Chromosome Location	NT Position†	Note	# of Records in BIC Database††	Copy Number Status
TCGA-10-0931	Frame Shift Deletion	p.E23fs	17:38529571-38529572	187	185/187DelAG Founder Mutation [23 24].	1980	Heterozygous Loss
TCGA-13-1408	Frame Shift Deletion	p.E23fs	17:38529571-38529572	187	185/187DelAG Founder Mutation [23 24].	1980	Heterozygous Loss
TCGA-23-1027	Frame Shift Deletion	p.E23fs	17:38529571-38529572	187	185/187DelAG Founder Mutation [23 24].	1980	Diploid
TCGA-23-1118	Frame Shift Deletion	p.E23fs	17:38529571-38529572	187	185/187DelAG Founder Mutation [23 24].	1980	Heterozygous Loss
TCGA-23-2078	Frame Shift Deletion	p.E23fs	17:38529571-38529572	187	185/187DelAG Founder Mutation [23 24].	1980	Heterozygous Loss
TCGA-23-2079	Frame Shift Deletion	p.E23fs	17:38529571-38529572	187	185/187DelAG Founder Mutation [23 24].	1980	Diploid
TCGA-13-0887	Frame Shift Deletion	p.C24fs	17:38529570-38529571	188	185/187DelAG Founder Mutation [23 24].	1980	Heterozygous Loss
TCGA-13-1494	Split Site SNP	e3-1	17:38512077-38512077	N/A		N/A	Heterozygous Loss
TCGA-13-0893	Frame Shift Insertion	p.R504fs	17:38499565-38499566	1627	1627Ins ATAAATTAAA	0	Heterozygous Loss
TCGA-13-0903	Frame Shift Deletion	p.R504fs	17:38499564-38499564	1629	DelC	2	Heterozygous Loss
TCGA-61-2109	Frame Shift Deletion	p.K654fs	17:38499113-38499113	2080	DelA	31	Heterozygous Loss
TCGA-04-1356	Frame Shift Deletion	p.N723fs	17:38498908-38498908	2285	DelC	0	Heterozygous Loss
TCGA-59-2348	Nonsense Mutation	p.E797*	17:38498685-38498685	2508	2508 G to T (Glu to Stop)	3	Heterozygous Loss
TCGA-13-1512	Frame Shift Deletion	p.D825fs	17:38498599-38498599	2594	DelC	55	Heterozygous Loss
TCGA-09-1669	Frame Shift Deletion	p.E1346fs	17:38497039-38497039	4154	DelA	50	Heterozygous Loss
TCGA-25-2392	Frame Shift Deletion	p.E1346fs	17:38497039-38497039	4154	DelA	50	Diploid
TCGA-24-2298	Frame Shift Insertion	p.Q1395fs	17:38496488-38496489	4302	4302InsTC.	1	Diploid
TCGA-24-1470	Frame Shift Deletion	p.T1677fs	17:38473195-38473198	5146	DelTAAC	1	Heterozygous Loss
TCGA-57-1582	Frame Shift Deletion	p.R1726fs	17:38468889-38468892	5296	DelGAAA	39	Gain
TCGA-09-2051	Frame Shift Insertion	p.Q1756fs	17:38462605-38462606	5385	5382/5385 insC Founder Mutation [24 25].	1063	Heterozygous Loss
TCGA-13-0883	Frame Shift Insertion	p.Q1756fs	17:38462605-38462606	5385	5382/5385 insC Founder Mutation [24 25].	1063	Heterozygous Loss
TCGA-23-1122	Frame Shift Insertion	p.Q1756fs	17:38462605-38462606	5385	5382/5385 insC Founder Mutation [24 25].	1063	Amplification
TCGA-23-2077	Frame Shift Insertion	p.Q1756fs	17:38462605-38462606	5385	5382/5385 insC Founder Mutation [24 25].	1063	Heterozygous Loss
TCGA-23-2081	Frame Shift Insertion	p.Q1756fs	17:38462605-38462606	5385	5382/5385 insC Founder Mutation [24 25].	1063	Heterozygous Loss
TCGA-25-2401	Frame Shift Insertion	p.Q1756fs	17:38462605-38462606	5385	5382/5385 insC Founder Mutation [24 25].	1063	Heterozygous Loss
TCGA-09-2045	Frame Shift Deletion	p.Q1779fs	17:38454735-38454735	5454	DelC	5	Heterozygous Loss
TCGA-61-2008	Nonsense Mutation	p.W1815*	17:38453208-38453208	5564	5564 G to A	0	Heterozygous Loss

BRCA1 Somatic Mutations, (sorted by nucleotide position)							
Case ID	**Mutation Type**	**Mutation**	**Chromosome Location**	**NT Position†**	**Note††**	**# of Records in BIC Database††**	**Copy Number Status**
TCGA-13-0804	Missense Mutation	p.C47W	17:38512070-38512070	260	260 C to G	0	Heterozygous Loss
TCGA-25-1625	Nonsense Mutation	p.E116*	17:38509760-38509760	465	465 G to T	0	Heterozygous Loss
TCGA-29-2427	Nonsense Mutation	p.L431*	17:38499782-38499782	1411	1411 T to G (Leu to Stop).	1	Heterozygous Loss
TCGA-25-1630	Frame Shift Deletion	p.A521fs	17:38499517-38499517	1676	1676DelG	0	Heterozygous Loss
TCGA-23-1026	Frame Shift Deletion	p.G813fs	17:38498636-38498636	2557	2557DelG	0	Heterozygous Loss
TCGA-25-1632	Frame Shift Insertion	p.S1216fs	17:38497425-38497426	3767	3767Ins AGAACTTA. Three 3767 InsA records recorded in BIC Database.	3	Heterozygous Loss
TCGA-13-1489	Frame Shift Insertion	p.N1265fs	17:38497279-38497280	3913	3913InsAA.	0	Heterozygous Loss
TCGA-04-1357	Nonsense Mutation	p.Q1538*	17:38479937-38479937	4731	4731 C to T	3	Diploid
TCGA-24-2035	Frame Shift Deletion	p.G1710fs	17:38469440-38469440	5248	5248DelG	0	Heterozygous Loss
TCGA-13-0730	Nonsense Mutation	p.R1835*	17:38451310-38451310	5622	5622 C to T (Arg to Stop)	63	Heterozygous Loss

† Nucleotide positions are reported in reference to *BRCA1* GenBank record U14680, as per The Breast Cancer Information Core Database (http://research.nhgri.nih.gov/projects/bic/).

†† Mutations were matched by nucleotide position and compared to existing mutation records in the Breast Cancer Information Core (BIC) Database (http://research.nhgri.nih.gov/projects/bic/) on August 30, 2010.

Table S8.2: *BRCA2* Mutations

BRCA2 Germline Mutations, (sorted by nucleotide position)							
Case ID	**Mutation Type**	**Mutation**	**Chromosome Location**	**NT Position†**	**Note††**	**# of Records in BIC Database††**	**Copy Number Status**
TCGA-24-0975	Splice Site SNP	e6+2	13:31798752-31798752	N/A		N/A	Heterozygous Loss
TCGA-24-2288	Frame Shift Deletion	p.V220fs	13:31801605-31801606	885	del TG	0	Heterozygous Loss
TCGA-13-0900	Frame Shift Deletion	p.N257fs	13:31803141-31803145	995	delCAAAT	1	Heterozygous Loss
TCGA-04-1367	Nonsense Mutation	p.E294*	13:31804495-31804495	1108	1108 G to T	0	Heterozygous Loss
TCGA-25-2404	Frame Shift Deletion	p.K343fs	13:31804640-31804640	1253	1253 DelA	0	Heterozygous Loss
TCGA-24-1463	Frame Shift Insertion	p.I605fs	13:31805420-31805421	2033	2033 InsA	0	Diploid
TCGA-24-1417	Frame Shift Deletion	p.N1706fs	13:31811604-31811607	5340	delAATA	0	Heterozygous Loss
TCGA-24-2024	Frame Shift Deletion	p.Y1710fs	13:31811620-31811623	5356	delTATG	0	Heterozygous Loss
TCGA-04-1336	Frame Shift Deletion	p.T1738fs	13:31811703-31811706	5439	delTACT	0	Heterozygous Loss
TCGA-13-0913	Frame Shift Deletion	p.E1857fs	13:31812061-31812065	5797	delGAAAC	0	Heterozygous Loss
TCGA-13-0886	Frame Shift Deletion	p.S1982fs	13:31812438-31812438	6174	6174delT Founder Mutation [24,26].	1087	Heterozygous Loss

TCGA-13-1498	Frame Shift Deletion	p.S1982fs	13:31812438-31812438	6174	6174delT Founder Mutation [24,26].	1087	Diploid
TCGA-13-1499	Frame Shift Deletion	p.S1982fs	13:31812438-31812438	6174	6174delT Founder Mutation [24,26].	1087	Heterozygous Loss
TCGA-24-2280	Frame Shift Deletion	p.S1982fs	13:31812438-31812438	6174	6174delT Founder Mutation [24,26].	1087	Heterozygous Loss
TCGA-59-2351	Frame Shift Deletion	p.S1982fs	13:31812438-31812438	6174	6174delT Founder Mutation [24,26].	1087	Heterozygous Loss
TCGA-13-0726	Nonsense Mutation	p.R2394*	13:31827170-31827170	7408	7408 A to T	5	Heterozygous Loss
TCGA-24-2293	Nonsense Mutation	p.R2520*	13:31828687-31828687	7786	7786 C to C	44	Diploid
TCGA-24-1562	Nonsense Mutation	p.K3326*	13:31870626-31870626	10204	10204 A to T	293	Diploid
TCGA-13-1512	Nonsense Mutation	p.K3326*	13:31870626-31870626	10204	10204 A to T.	293	Diploid
TCGA-23-1026	Nonsense Mutation	p.K3326*	13:31870626-31870626	10204	10204 A to T	293	Diploid

***BRCA2* Somatic Mutations, (sorted by nucleotide position)**

Case ID	Mutation Type	Mutation	Chromosome Location	Nucleotide Position†	Note	# of Records in BIC Database††	Copy Number Status
TCGA-04-1331	Nonsense Mutation	p.C711*	13:31808625-31808625	NT Position: 2361	2361 C to A	0	Heterozygous Loss
TCGA-13-0890	Frame Shift Deletion	p.S1230fs	13:31810178-31810178	NT Position: 3914	3914DelT	0	Heterozygous Loss
TCGA-23-1030	Missense Mutation	p.T1354M	13:31810553-31810553	NT Position: 4289	4289 C to T	11	Diploid
TCGA-13-0885	Frame Shift Deletion	p.K1406fs	13:31810708-31810711	NT Position: 4444	delAAAG	0	Heterozygous Loss
(2 mutations)	Frame Shift Deletion	p.E1407fs	13:31810710-31810713	NT Position: 4446	delAGAA	1	Heterozygous Loss
TCGA-24-1103	Missense Mutation	p.K1638E	13:31811404-31811404	NT Position: 5140	5140 A to G	0	Heterozygous Loss
TCGA-09-2050	Nonsense Mutation	p.S1882*	13:31812137-31812137	NT Position: 5873	5873 C to A.	28	Heterozygous Loss
TCGA-24-1555	Frame Shift Deletion	p.P2608fs	13:31834675-31834675	NT Position: 8049	8049DelT.	0	Heterozygous Loss
TCGA-13-1481	Frame Shift Deletion	p.S2697fs	13:31835426-31835441	NT Position: 8316	8315DelTGAGCGCAAATATATC.	0	Diploid
TCGA-23-1120	Frame Shift Deletion	p.P3278fs	13:31870481-31870481	NT Position: 10059	10059DelG.	0	Heterozygous Loss

† Nucleotide positions are reported in reference to *BRCA2* GenBank record U43746, as per The Breast Cancer Information Core Database (http://research.nhgri.nih.gov/projects/bic/).

†† Mutations were matched by nucleotide position and compared to existing mutation records in the Breast Cancer Information Core (BIC) Database (http://research.nhgri.nih.gov/projects/bic/) on August 30, 2010.

Table S8.3: BRCA Mutation Rates

Gene	Germline Mutation Rate	Somatic Mutation Rate	Total Mutation Rate
BRCA1	8.54%	3.16%	11.71%
BRCA2	6.33%	2.85%	9.18%
Both Genes	14.56%	6.01%	20.25%

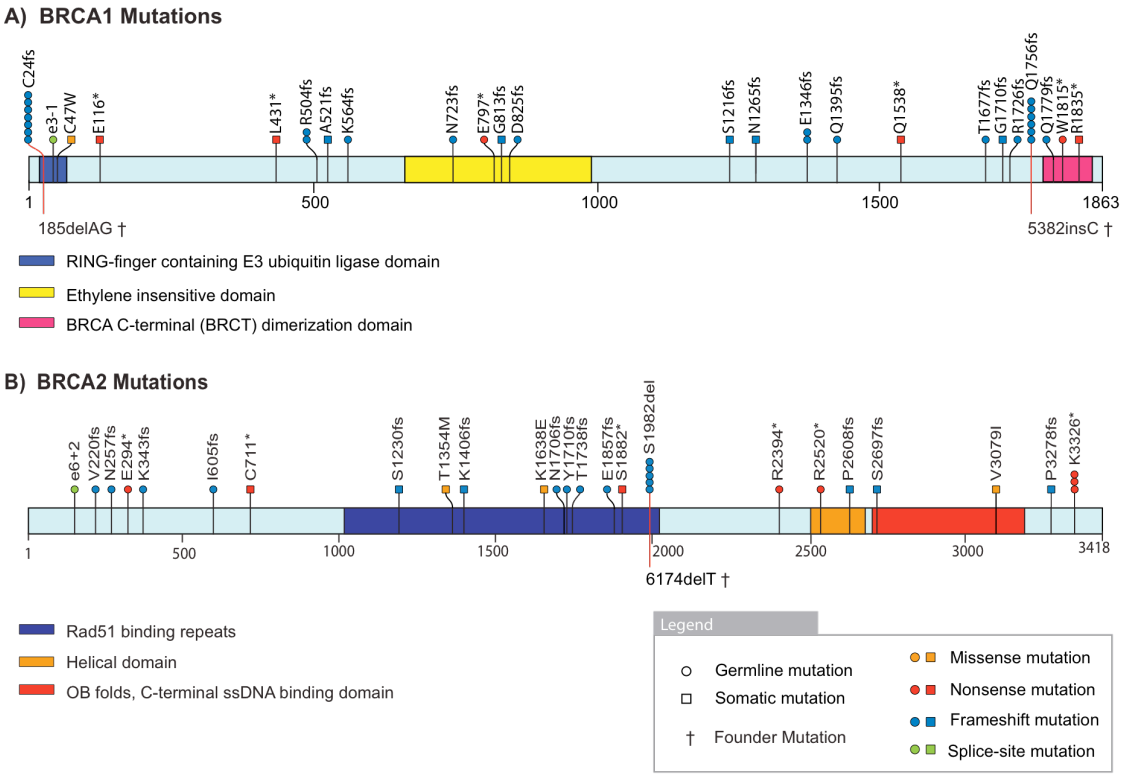

Figure S8.7: Summary of BRCA Mutations. All BRCA1/2 germline and somatic mutations are displayed along the protein domain structure. A) BRCA1 Mutations. Thirteen cases, all germline, have well-known BRCA1 founder mutations at 185/187delAG and 5382/5385insC. B) BRCA2 mutations. Five cases, all germline, have known BRCA2 founder mutations at 6174delT.

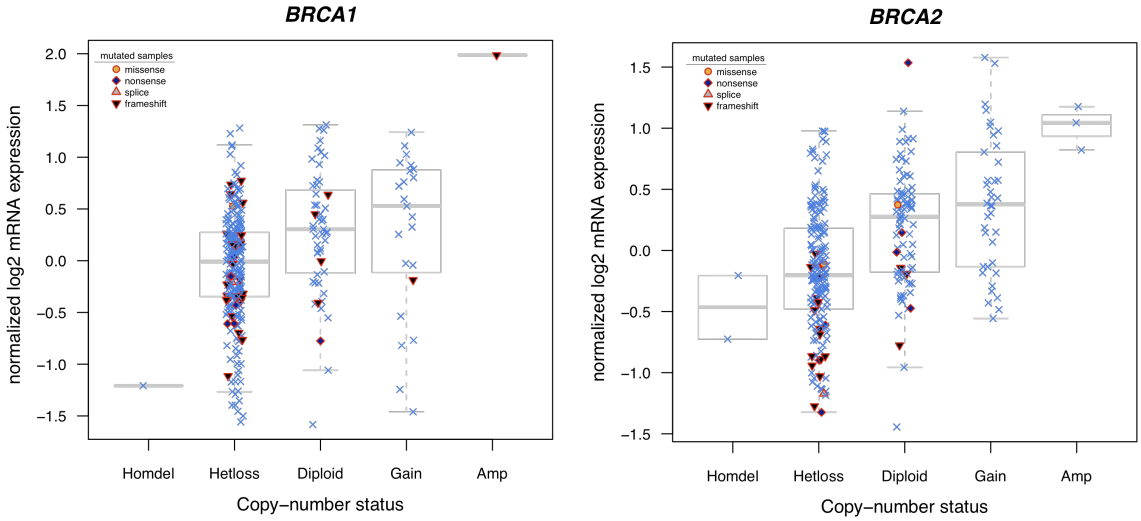

Figure S8.8: Heterozygous loss associated with BRCA1/2. A) Thirty of the 37 (81%) of BRCA1 mutations are accompanied by heterozygous loss; **B)** Twenty-one of the 29 (72.4%) of the BRCA2 mutations are accompanied by heterozygous loss.

Epigenetic Silencing of BRCA1

BRCA1 silencing via promoter hypermethylation has been reported previously in ovarian and breast cancer[8,9], and recent studies have reported *BRCA1* hypermethylation in 18% of ovarian patients[10].

As described in Supplemental Methods 7, we analyzed the relationship between DNA methylation and gene expression for nine different probes located in or near the *BRCA1* promoter region, and found statistically significant inverse correlations for four of the nine probes (cg19531713, cg19088651, cg08993267, cg04658354). The target CpG sites of those probes are located in the CpG island that contains the transcription start site of *BRCA1*. For each of the aforementioned four probes, we used *k*-means clustering on the two-dimensional space of DNA methylation and expression data to separate the epigenetically silenced group and the non-epigenetically silenced group of samples. Expression data were scaled to have the same range as DNA methylation data for the purpose of clustering. We then combined the calls from the four probes. Since data was lacking for some probes in some samples, we relied on the fraction of the four probes calling a particular sample in the hypermethylated group, rather than on a fixed number of probes. Samples with >50% consensus on belonging to the hypermethylated group across the four probes were classified as samples with silencing of *BRCA1* by promoter hypermethylation.

Using this method, we identified 34 of 316 cases (10.8%) with epigenetic silencing of *BRCA1*. Notably, epigenetic silencing of *BRCA1* is mutually exclusive of *BRCA1/2* mutations *(P* = 4.45 e-04, two-sided Fisher's exact test). This mutual exclusivity provides evidence of strong selective pressure to inactivate BRCA via either mutation or epigenetic silencing.

Analysis of the Core HR Pathway

Amplification of EMSY

Previous studies have identified amplification and overexpression of EMSY (*C11orf30*) as an alternative means by which tumors selectively inactivate the BRCA pathway. EMSY was discovered in a yeast two-hybrid screen with BRCA2, and the EMSY protein binds specifically to the transactivation domain in BRCA2[12]. An excess of EMSY can result in an inhibition of BRCA2 transcriptional activity, and overexpression of EMSY may eliminate selective pressure in sporadic breast and ovarian cancer to inactivate BRCA2[28]. The EMSY protein is also known to be co-located with *BRCA2* at chromosomal sites of DNA damage and to interact with proteins involved in the regulation of chromatin[29].

Previous studies have identified amplification of EMSY in 13% of sporadic primary breast cancer and 17% of high-grade sporadic ovarian cancer[2,11]. Ovarian tumors with EMSY amplification have been associated with significantly worse outcome[30]. However, in a multivariate analysis that included histological subtype, grade, stage, age and EMSY amplification as the covariates, only stage and age were significant prognostic predictors[30]. EMSY is located at 11q13, a region known to be amplified in multiple cancers, including breast, ovarian, head and neck, lung, and bladder cancer[12]. The amplicon is gene dense, and the region likely contains a cassette of genes rather than a single oncogene -- for example, in ovarian cancer, the amplicon tends to include several genes including EMSY, *LRRC32* (GARP), and *PAK1*[12].

For the unified case list (N=316), we identified 19 cases with EMSY amplification (GISTIC) and 6 cases with EMSY mutation (Figure S8.9). By this analysis, there is evidence for EMSY alteration in 7.9% of cases. However, we do not observe co-occurrence or mutual exclusivity between BRCA inactivation events (mutations plus methylation) and EMSY amplification and mutation ($P = 0.8248$, two-sided Fisher's Exact Test).

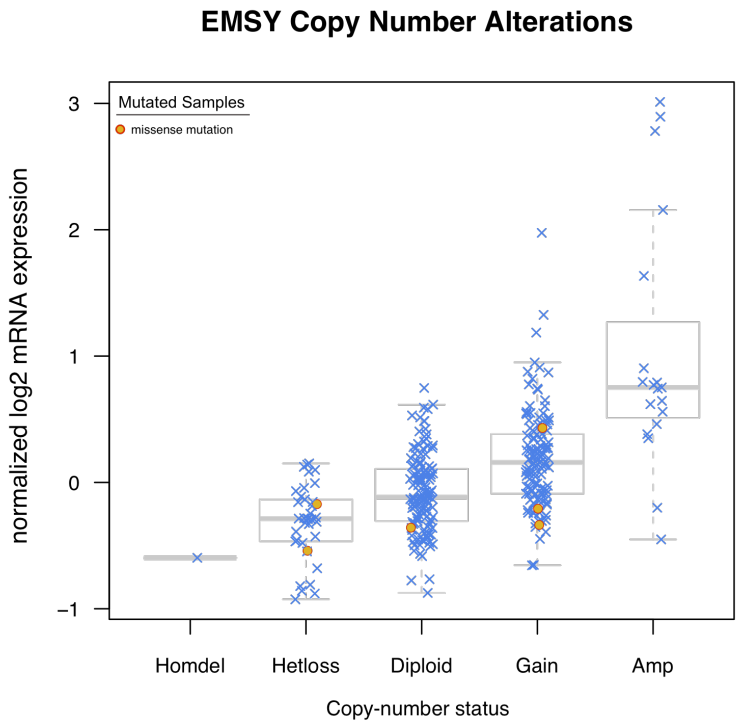

Figure S8.9: EMSY/C11orf30 Copy Number Alterations. Normalized log2 mRNA expression v. GISTIC copy number status for EMSY.

Absence of FANCF Hypermethylation

A number of recent studies have identified hypermethylation of FANCF as an alternative means of altering the BRCA pathway in sporadic cancers, including ovarian cancer [2]. For example, a 2008 study observed hypermethylation of FANCF in 13.2% of 53 ovarian tumors samples[13]. However, in the TCGA data, we observe no clear evidence of FANCF silencing by hypermethylation (Figure S8.10).

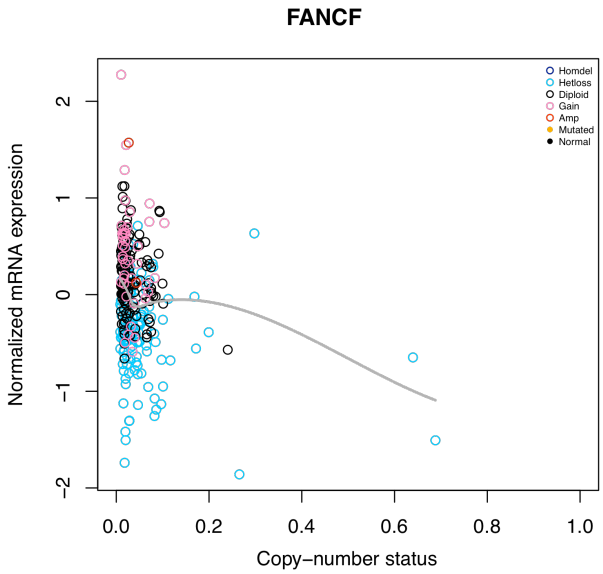

Figure S8.10: DNA methylation beta values v. normalized log2 mRNA expression levels for FANCF. We observe no clear evidence of hypermethylation of FANCF.

Homozygous Deletions of PTEN

PTEN deficiency has been identified to cause homologous recombination defects in human tumor cells, and to sensitize tumor cells to PARP inhibitors[21]. However, the exact role of PTEN in homologous recombination and DNA repair remains controversial and an area of active research[31]. DNA copy-number analysis identifies a focal deletion region at 10q23.31 (q-value: 5.41E-11), which includes only *PTEN*. This corresponds to 21 cases (6.7%) of *PTEN* homozygous deletion, each of which is associated with down-regulation at the mRNA level (Figure S8.11). We also observe two somatic mutations in *PTEN*. However, we do not observe co-occurrence or mutual exclusivity between BRCA inactivation events (mutations plus methylation) and PTEN homozygous deletion and mutation (P = 0.3607, two-sided Fisher's Exact Test).

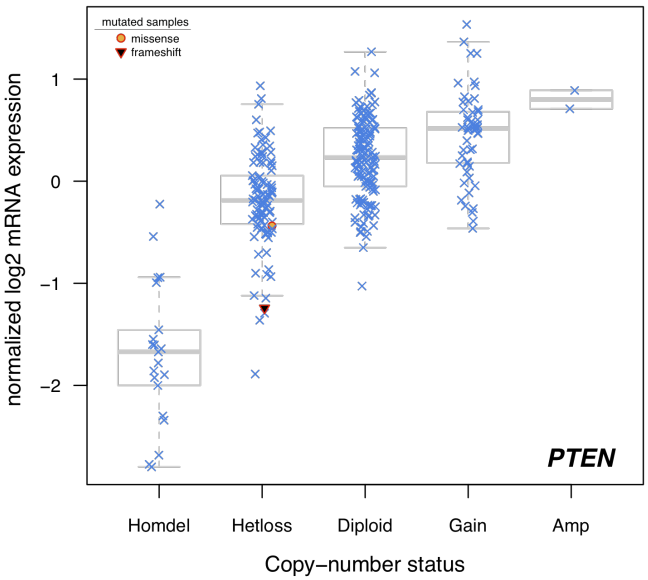

Figure S8.11: PTEN Copy Number Alterations. PTEN is homozygously deleted in 21 cases (6.65%), and homozygous deletions are associated with down-regulation at the mRNA level. N=316 cases.

Fanconi Anemia and Other Core HR Genes

Table S8.4 provides mutation and copy number alteration rates for other well-annotated genes known to be involved in homologous recombination (HR), derived from literature curation[32,33,34,35]. A fingerprint of the complete set of HR genes is provided in Figure S8.12. Due to the low mutation rates observed in the Fanconi Anemia genes, we do not observe co-occurrence or mutual exclusivity between BRCA inactivation events (mutations plus methylation) and Fanconi Anemia mutations ($P = 0.7834$, two-sided Fisher's Exact Test).

Table S8.4: Analysis of other Core Members of the HR Pathway

Fanconi Anemia Genes, Total Mutation Rate: 5.06%

Gene Symbol	Entrez Gene ID	*In Vitro* Sensitivity to PARPi*	Number of Samples Mutated (N=316)	% of Samples Mutated (N=316)	Copy Number Alterations†
C19orf40	91442		0	0.00%	7.91%
FANCA	2175	Yes	3	0.95%	2.85%
FANCB	2187		0	0.00%	0.00%
FANCC	2176	Yes	2	0.63%	1.58%
FANCD2	2177	Yes	1	0.32%	0.95%
FANCE	2178		1	0.32%	2.53%
FANCF	2188		0	0.00%	0.63%
FANCG	2189		1	0.32%	0.00%
FANCI	55215		2	0.63%	1.58%
FANCL	55120		2	0.63%	1.58%
FANCM	57697		1	0.32%	0.95%
PALB2	79728		4	1.27%	0.63%

Core HR RAD Genes, Total Mutation Rate: 1.58%

Gene Symbol	Entrez Gene ID	*In Vitro* Sensitivity to PARPi*	Number of Samples Mutated (N=316)	% of Samples Mutated (N=316)	Copy Number Alterations†
RAD50	10111		2	0.63%	1.27%
RAD51	5888	Yes	1	0.32%	1.27%
RAD51C	5889		0	0.00%	0.63%
RAD51L1	5890		0	0.00%	2.22%
RAD51L3	5892		0	0.00%	0.95%
RAD52	5893		0	0.00%	7.28%
RAD54B	25788		0	0.00%	4.11%
RAD54L	8438		2	0.63%	5.38%

DNA damage response genes involved in HR, Total Mutation Rate: 2.22%

Gene Symbol	Entrez Gene ID	*In Vitro* Sensitivity to PARPi*	Number of Samples Mutated (N=316)	% of Samples Mutated (N=316)	Copy Number Alterations†
ATM	472	Yes	4	1.27%	1.27%
ATR	545	Yes	2	0.63%	3.80%
CHEK1	1111	Yes	0	0.00%	3.48%
CHEK2	11200	Yes	1	0.32%	1.90%

**In Vitro* Sensitivity to PARPi based on: [20].

† Copy number rates include amplifications and homozygous deletions as determined by GISTIC copy-number analysis.

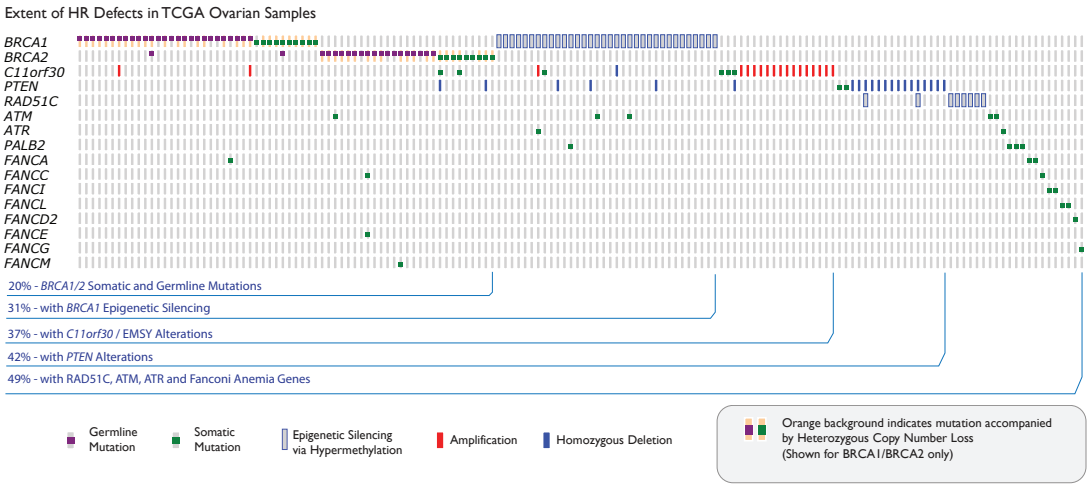

Figure S8.12: Genomic Fingerprint of HR Pathway Alterations. Each column represents an individual case; each row represents a gene. Only cases with HR defects (N=154) are shown. Copy number alterations are only shown for EMSY and *PTEN*. While it is not yet clear if all of these HR defects result in a sufficient decrease in homologous recombination to result in sensitization to PARP inhibitors, our findings indicate that HR defects occur in a substantial fraction of sporadic ovarian tumors. We therefore suggest comprehensive profiling of these molecular alterations in ongoing and future clinical trials of PARP inhibitors.

Extended HR Analysis

To extend the analysis beyond well-annotated genes involved in HR, a more global, but less detailed analysis was performed on 42 other potentially relevant genes. These additional genes were derived from an extended literature and pathway search, and Gene Ontology annotation. More specifically, the list was derived from the ATM/BRCA pathway from BioCarta, the Homologous Recombination Repair pathway from Reactome[36,37], and Gene Ontology GO:0000724: double-strand break repair via homologous recombination. The complete list of genes analyzed, along with mutation rates and GISTIC copy number analysis is provided in Table S8.5. Within the larger gene set, we observe only very low mutations rates. For example, the Bloom syndrome gene (*BLM*) participates in genome maintenance, is essential for BRCA1 function[38] and is mutated in four cases. Additionally, several genes including *BCL2L1*, *OBFC2B* and *RBBP8* appear within relatively narrow recurrent regions of amplification, as defined by GISTIC copy number analysis.

Table S8.5: Analysis of Other Potential HR Genes

Gene Symbol	Entrez Gene ID	# of Samples Mutated (N=316)	% of Samples Mutated (N=316)	Within GISTIC Peak (Amp/Del; Total Number of Genes within Peak appear in brackets)
BBC3	27113	0	0.00%	Deletion (323)
BCL2	596	0	0.00%	
BCL2L1	598	0	0.00%	Amplification (2)
BLM	641	4	1.27%	Amplification (62)
BTBD12	84464	2	0.63%	
DMC1	11144	0	0.00%	
EME1	146956	0	0.00%	
EME2	197342	0	0.00%	
ERCC4	2072	1	0.32%	
GEN1	348654	2	0.63%	
GIYD1	548593	0	0.00%	
H2AFX	3014	0	0.00%	Deletion (269)
HUS1	3364	1	0.32%	
LIG1	3978	0	0.00%	Deletion (323)
MDC1	9656	2	0.63%	
MDM2	4193	0	0.00%	
MRE11A	4361	0	0.00%	
MUS81	80198	1	0.32%	
NBN	4683	0	0.00%	
OBFC2A	64859	0	0.00%	
OBFC2B	79035	1	0.32%	Amplification (18)
PCNA	5111	0	0.00%	
PMAIP1	5366	0	0.00%	
POLD1	5424	1	0.32%	
POLD2	5425	2	0.63%	
POLD3	10714	1	0.32%	
POLD4	57804	0	0.00%	
RAD1	5810	1	0.32%	Amplification (80)
RAD17	5884	0	0.00%	Deletion (51)
RAD9A	5883	0	0.00%	
RBBP8	5932	1	0.32%	Amplification (11)
RPA1	6117	2	0.63%	
RPA2	6118	1	0.32%	Deletion (188)
RPA3	6119	0	0.00%	Deletion (84)
RTEL1	51750	0	0.00%	Amplification (39)
SHFM1	7979	0	0.00%	
TEX15	56154	4	1.27%	
TP53BP1	7158	4	1.27%	
TREX1	11277	0	0.00%	
UBE2N	7334	0	0.00%	Deletion (375)
XRCC2	7516	0	0.00%	Amplification (92)
XRCC3	7517	1	0.32%	

Survival Analysis of Cases with HR Defects

Previous studies have observed better outcome in BRCA-positive patients, including longer tumor-free intervals between relapses, and improved overall survival[39]. Previous studies have also observed shorter overall survival for patients with *BRCA1* hypermethylation[9].

In the TCGA ovarian data, we observe mutual exclusivity between *BRCA1* epigenetic silencing and *BRCA1/2* mutations (see above), and we therefore focused our survival analysis on comparing three patients groups: *BRCA1* epigenetically silenced, *BRCA1/2* mutated, and BRCA Wildtype (WT). Within the complete data set (N=316), we observe differences in age between the three groups (P = 0.01576, Kruskal Wallis Test). Post-hoc pairwise comparisons show differences between BRCA mutated and BRCA WT (57.74 years versus 61.84 years, Bonferroni adjusted P = 0.061, Wilcoxon signed-rank test). Univariate survival analysis of BRCA status shows divergent outcome for the two types of events, with BRCA mutated cases exhibiting better overall survival (OS) than BRCA wild-type (median OS 66.5 *versus* 41.9 months, P = 3.08 e-04, log-rank test, Figure S8.13), and *BRCA1* epigenetically silenced cases exhibiting similar survival to BRCA1/2 WT (median OS 41.5 *versus* 41.9 months, P = 0.69, log-rank test, Figure S8.13). In a multivariate survival analysis of BRCA mutated versus BRCA WT cases, mutation status and age were significant prognostic predictors (BRCA mutation status, P = 0.00375, Age, P = 0.02742). We therefore observe evidence of selective pressure to alter BRCA genes via distinct genetic mechanisms, but statistically significant differences in outcomes for patients. Sequencing additional samples will allow further exploration in the distinct outcome patterns seen in *BRCA1 versus BRCA2* and germline *versus* somatic events.

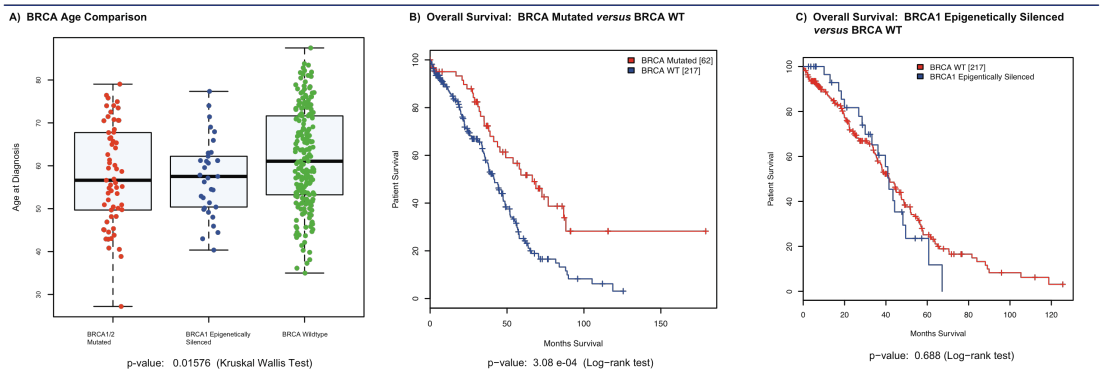

Figure S8.13: BRCA survival analysis. A) BRCA age comparison for the three BRCA categories analyzed. **B)** Kaplan-Meier curve comparing the survival of patients with BRCA mutation *versus* BRCA wild-type (WT). **C)** Kaplan-Meier curve comparing the survival of patients with BRCA1 epigenetic silencing *versus* BRCA wild-type (WT).

Effect of BRCA inactivation on genome stability

We investigated the effect of *BRCA1*/*BRCA2* mutations and *BRCA1* silencing on the overall level of DNA copy-number alterations. We computed the fraction of the genome that is not diploid for each case, and found that BRCA-altered cases to not exhibit increased levels of copy-number

alterations (Figure S8.14). The result is similar when using the number of breakpoints in the DNA copy-number profiles (data not shown).

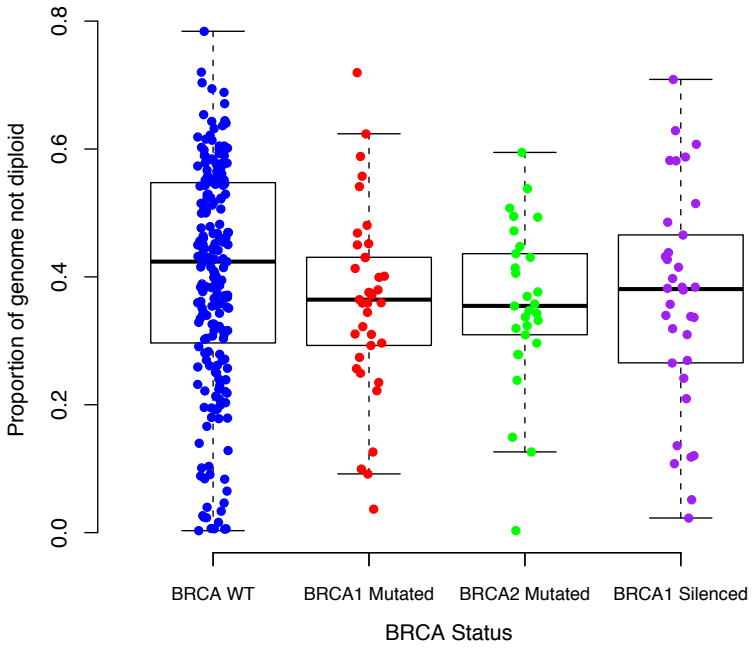

Figure S8.14: Cases with BRCA-alterations do not exhibit increased genomic instability.

Correlation of BRCA inactivation with recurrently altered copy number peaks in other genomic regions

To investigate potential cross-talk with other genes and pathways, we looked for potential correlations of BRCA inactivation events (mutation plus methylation, 98 samples, see above) with significantly altered copy number events as reported by GISTIC analysis (63 peaks of amplification and 50 peaks of deletion).

For each GISTIC peak, we defined the set of samples that is affected by the DNA copy-number alteration. We only considered samples as altered if at least half of the genes in the region are affected by homozygous deletion or high-level amplification. Each peak-associated set of samples was then tested for enrichment and depletion in BRCA inactivation by a two-tailed Fisher's exact test. Significant correlations were selected after Benjamini-Hochberg correction for false discovery (FDR < 5%) (Table S8.6).

We found a significant enrichment of BRCA inactivation for *MYC* amplified cases (49.0% of BRCA altered cases have *MYC* amplification *versus* 24.3% of BRCA wild type cases, FDR-adjusted $P = 0.002$, Table S8.6). *CCNE1* amplified cases show significant depletion of BRCA alteration (8.2% of BRCA altered cases have *CCNE1* amplification *versus* 25.7% of BRCA wild type cases, FDR adjusted $P = 0.009$). Unlike CCNE1, cases with alterations in *RB1* and *CDKN2A* (the other two main genes in the RB pathway, see 8.2. Cancer Pathways above), had overlap with BRCA alterations ($P = 0.18$ and $P = 0.6$, respectively, two-sided Fisher's Exact Test).

The observed tendency towards mutual exclusivity between BRCA inactivation and CCNE1 amplification prompted us to reevaluate the previously reported poor survival associated with *CCNE1*-amplification[40,41]. In evaluating the full case set, we observe worse outcome for CCNE1 amplified cases, in line with previous studies (P = 0.0718, Log Rank Test, Figure S8.15A). However, if we remove all BRCA inactivated cases, and examine survival differences in CCNE1 amplified cases within BRCA WT cases only, significant worse outcome is no longer detectable (*P* = 0.24, log-rank test, Figure S8.15B), suggesting that the previously reported survival difference can be explained by the better survival of BRCA-mutated cases.

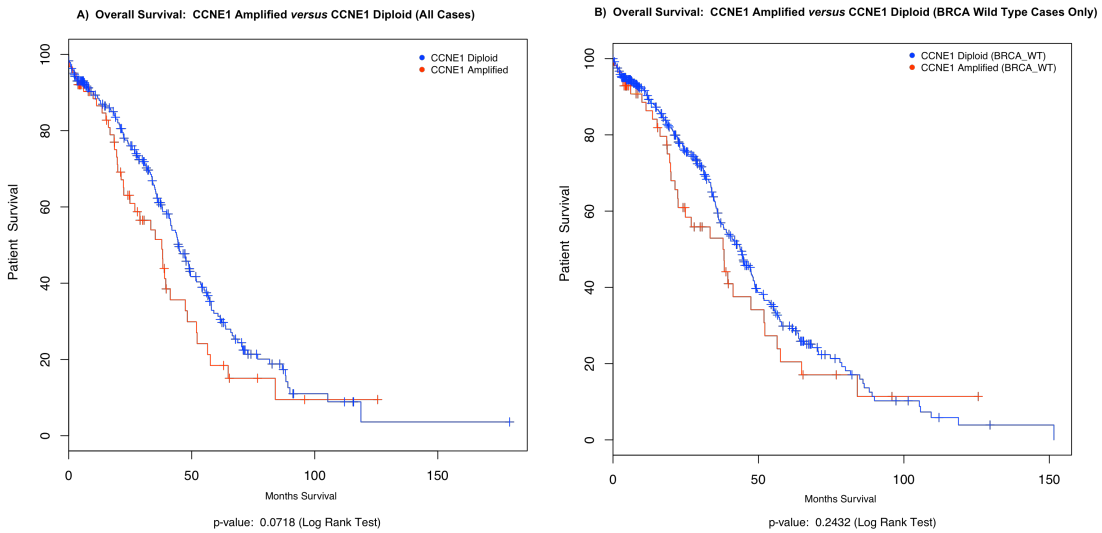

Figure S8.15: Overall Survival for CCNE1 amplified cases. Survival of CCNE1 amplified cases is compared to CCNE1 wild type cases: Among all cases **(A)**, and among BRCA wild type cases only **(B)**.

Table S8.6: Correlation between BRCA alterations and DNA copy-number events: Each peak is identified by its corresponding cytoband, and the regions are marked as either amplified (AMP) or deleted (DEL). The number of co-occurring cases with BRCA altered and BRCA wild type cases are in columns "BRCA Altered" and "BRCA WT" respectively. Fisher's p-values are reported (only regions with p<0.05 are in the table) with the corresponding FDR-corrected values. The red box highlights regions with significant enrichment/depletion after FDR correction.

GISTIC Region	Alteration	BRCA Altered	BRCA WT	Fisher's exact test	FDR	Relation	Genes in the regions
8q24.21	AMP	48	53	2.51E-05	0.00203	Co-oc.	MYC PVT1
19q12	AMP	8	56	0.00023	0.00963	Mut.Ex	CCNE1
8q24.3	AMP	37	42	0.00069	0.01877	Co-oc.	ZNF7 ZNF623 SHARPIN VPS28 PUF60 COMMD5 HSF1 GRINA DGAT1 GPAA1 EXOSC4 PYCRL CYC1 FAM83H GPR172A TSTA3 LRRC14 ADCK5 ZNF34 BOP1 ZC3H3 RPL8 PPP1R16A ZNF251 EEF1D CPSF1 MAF1 TIGD5 KIAA1688 ZNF707 PLEC1 NRBP2 ZNF696 FBXL6 SCRIB SLC39A4 MFSD3 OPLAH TOP1MT KIFC2 RECQL4 NFKBIL2 NAPRT1 RHPN1 C8ORFK29 ZFP41 MAPK15 PARP10 KIAA1875 GPT MGC70857 GLI4 ZNF517 SCXB FOXH1 SPATC1 MAFA SCRT1 LY6H CYHR1 C8orf30A C8orf51 GSDMD EPPK1 BREA2 C8orf31 GPIHBP1 LRRC24 C8orf73 MIR661 HEATR7A MIR937 MIR939 SCXA LOC100130274
19p13.13	AMP	3	32	0.00163	0.03290	Mut.Ex	CCDC130 TRMT1 STX10 CC2D1A PRKACA ZSWIM4 IER2 ASF1B NFIX RFX1 IL27RA CACNA1A NANOS3 RLN3 PODNL1 LYL1 C19orf53 C19orf57 MRI1 SAMD1 DCAF15 NACC1 LOC113230 PALM3 MIR181C MIR23A MIR24-2 MIR27A MIR181D
1q21.2	AMP	1	21	0.00349	0.05653	Mut.Ex	SETDB1 ARNT TARS2 VPS72 GOLPH3L PRUNE PIP5K1A LYSMD1 ENSA SCNM1 LASS2 CDC42SE1 MCL1 FAM63A SEMA6C HORMAD1 BNIPL MLLT11 TMOD4 ANXA9 CTSS ADAMTSL4 GABPB2 TNFAIP8L2 CTSK ECM1 RPRD2 C1orf56
19p12	AMP	1	17	0.01623	0.219	Mut.Ex	ZNF431 ZNF430 ZNF100 ZNF429 ZNF708 ZNF85 ZNF714 ZNF43 ZNF493 ZNF738 LOC641367
19p13.2	AMP	3	24	0.01731	0.20036	Mut.Ex	KEAP1 TYK2 EIF3G MRPL4 CDC37 KRI1 FDX1L QTRT1 DNM2 PPAN ATG4D ILF3 DNMT1 SLC44A2 AP1M2 ICAM3 CDKN2D RAVER1 PDE4A ICAM5 ICAM1 ICAM4 P2RY11 ANGPTL6 RDH8 COL5A3 S1PR2 S1PR5 C19orf66 LOC147727 C3P1 SNORD105 PPAN-P2RY11 MIR638 SNORD105B ZGLP1
4q13.3	AMP	0	11	0.02022	0.20474	Mut.Ex	COX18 ANKRD17 MTHFD2L BTC AREG ADAMTS3 RASSF6 EREG IL8 CXCL2 CXCL3 AFP AFM CXCL5 NPFFR2 ALB EPGN CXCL1 PF4 PF4V1 SLC4A4 GC CXCL6 PPBPL2 PPBP PPBPL1
18q11.2	AMP	0	12	0.02096	0.18864	Mut.Ex	TAF4B KCTD1
18q12.1	AMP	0	12	0.02096	0.18864	Mut.Ex	KIAA1012 RNF138 DSG2 FAM59A B4GALT6 DSC2 RNF125 DSG1 MEP1B DSC3 DSC1 DSG4 DSG3 TTR MCART2
3q29	AMP	20	23	0.02162	0.15922	Co-oc.	NCBP2 LSG1 WDR53 PAK2 OPA1 DLG1 LRCH3 RNF168 PPP1R2 FYTTD1 KIAA0226 LOC152217 SENP5 PCYT1A RPL35A PIGX ATP13A3 LMLN SDHALP2 BDH1 TMEM44 HRASLS TNK2 IQCG MUC20 TFRC PIGZ FAM43A MFI2 FGF12 MUC4 LRRC33 HES1 APOD ATP13A5 ZDHHC19 LRRC15 TM4SF19 LOC348840 ATP13A4 GP5 CPN2 ACAP2 UBXN7 MGC2889 C3orf34 C3orf59 C3orf21 OSTalpha FBXO45 LOC220729 TCTEX1D2 C3orf43 SDHALP1 MIR570 FAM157A MIR922 LOC100128023 LOC100131551
14q11.2	AMP	1	15	0.02692	0.18169	Mut.Ex	METT11D1 ZNF219 NDRG2 FLJ10357 SLC39A2 TPPP2 RNASE13 RNASE7 RNASE8 RNASE3 RNASE2 C14orf176
6q27	DEL	4	1	0.03363	0.20953	Co-oc.	FAM120B TBP PDCD2 PSMB1 FGFR1OP PHF10 SFT2D1 MLLT4 WDR27 BRP44L QKI PARK2 RNASET2 TCTE3 RPS6KA2 PACRG CCR6 DLL1 TTLL2 KIF25 UNC93A PDE10A DACT2 LOC441177 TCP10 FRMD1 PRR18 SMOC2 T GPR31 THBS2 C6orf123 C6orf70 C6orf208 C6orf176 LOC154449 C6orf118 LOC285796 C6orf120 TCP10L2 C6orf122 C6orf124 HGC6.3
8q24.12	AMP	30	44	0.04580	0.26502	Co-oc.	DEPDC6 COL14A1

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