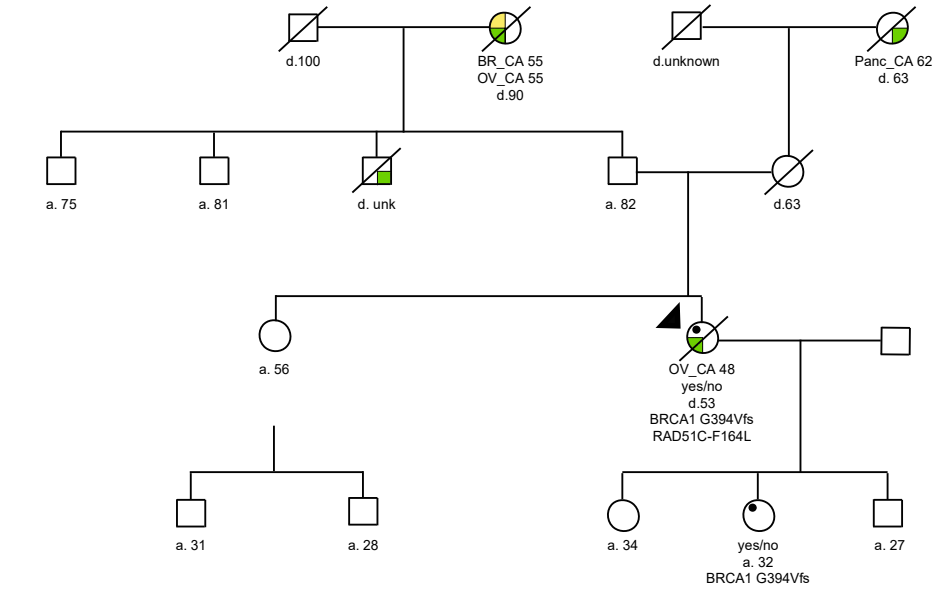
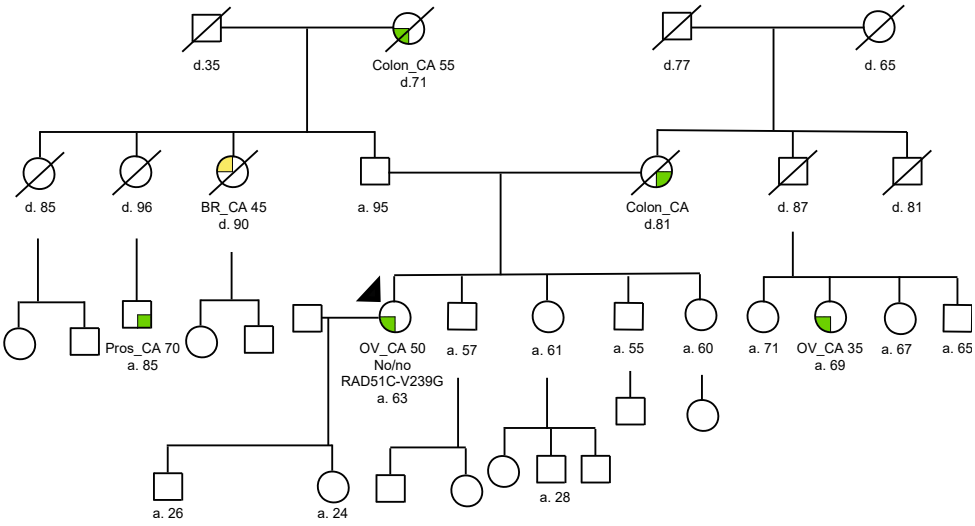
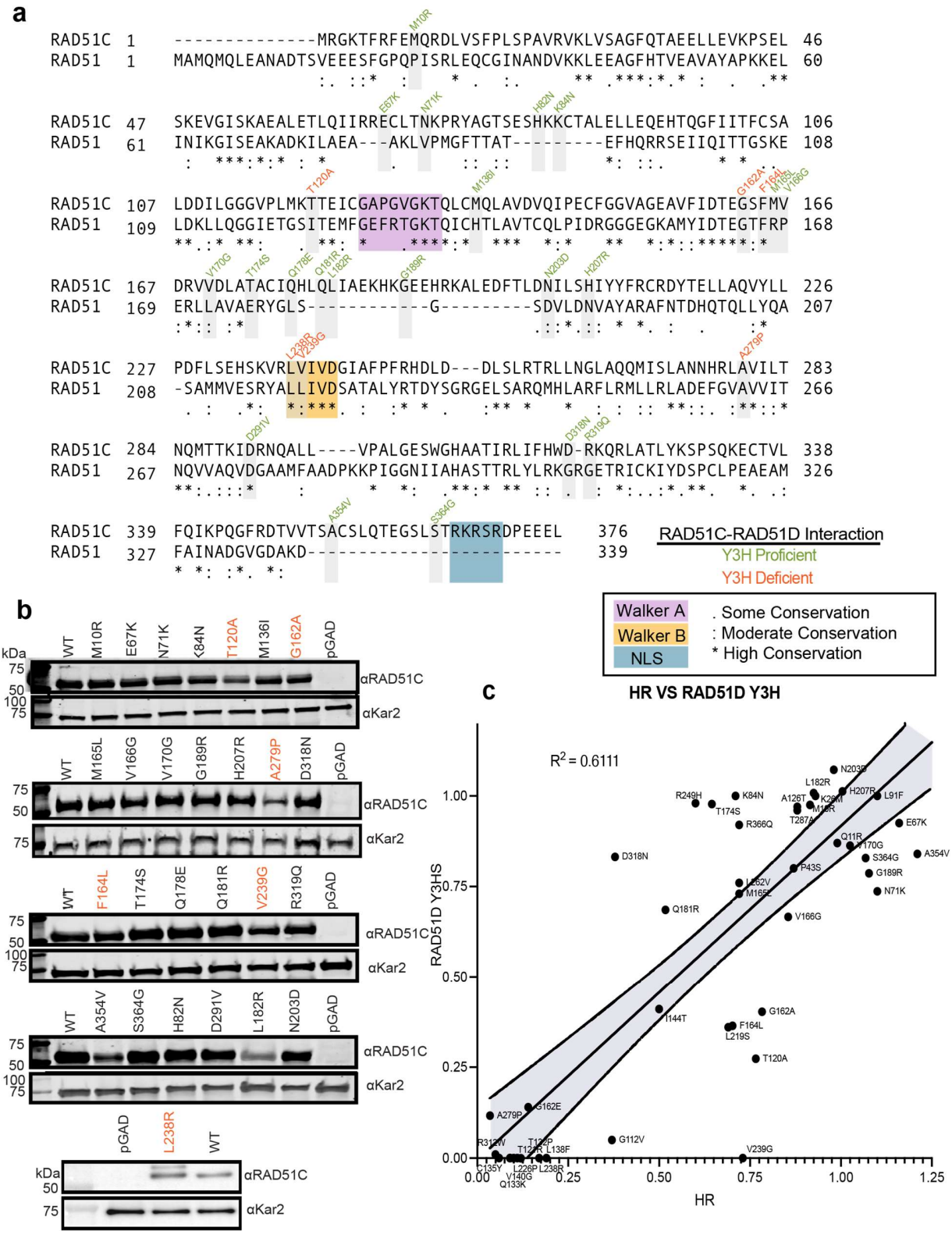
a RAD51C-F164L Family Pedigree



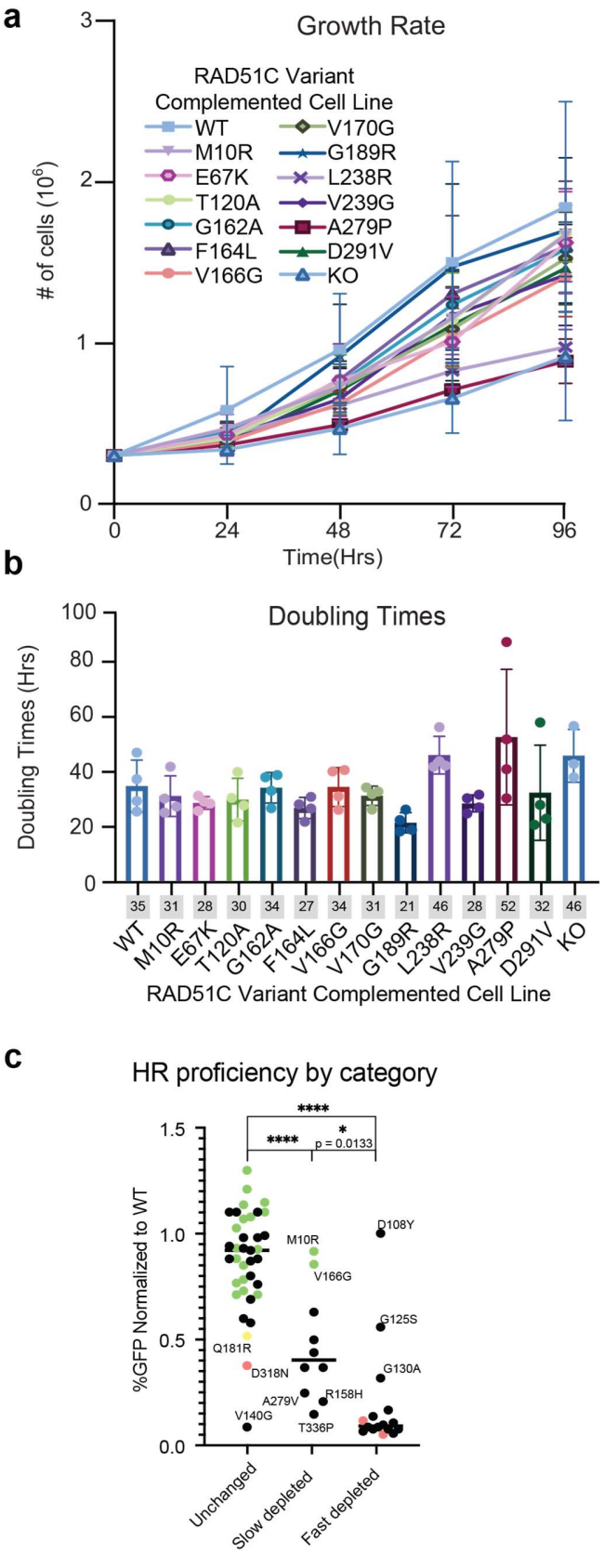
b RAD51C-V239G Family Pedigree



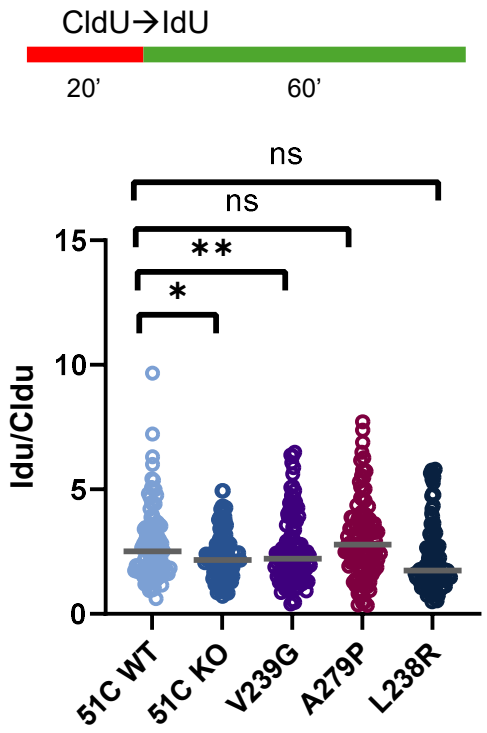
Supplementary Figure 1. RAD51C F164L and V239G variant inheritance and cancer occurrence. Pedigrees showing family inheritance of RAD51C-F164L **(a)** or RAD51C-V239G **(b)** and cancer occurrence. The patient of interest (detailed in **Supplementary Table 1)** is identified by the black arrow. Females are indicated with a circle and males are indicated with a square. Slashes indicate deceased individuals, and age of death is shown below by (d.). Cancer status is detailed below each individual as well as age of diagnosis and current age (a.). If available, BRCA allele status and *RAD51C* variant status is also indicated. Cancer type is indicated by the colored quadrant (top left yellow, unilateral breast cancer; bottom left green, ovarian cancer; bottom right green, cancer type other than breast or ovarian cancer). BRCA variant carriers are also indicated by a black dot.


a
RAD51C 1 --------------MRGKTFRFEMQRDLVSFPLSPAVRVKLVSAGFQTAEELLEVKPSEL 46
RAD51 1 MAMQMQLEANADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGFHTVEAVAYAPKKEL 60
RAD51C 47 SKEVGISKAEALETLQIIRRECLTNKPRYAGTSESHKKCTALELLEQEHTQGFIITFCSA 106
RAD51 61 INIKGISEAKADKILAEA---AKLVPMGFTTAT---------EFHQRRSEIIQITTGSKE 108
RAD51C 107 LDDILGGGVPLMKTTEICGAPGVGKTQLCMQLAVDVQIPECFGGVAGEAVFIDTEGSFMV 166
RAD51 109 LDKLLQGGIETGSITEMFGEFRTGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRP 168
RAD51C 167 DRVVDLATACIQHLQLIAEKHKGEEHRKALEDFTLDNILSHIYYFRCRDYTELLAQVYLL 226
RAD51 169 ERLLAVAERYGLS------------G---------SDVLDNVAYARAFNTDHQTQLLYQA 207
RAD51C 227 PDFLSEHSKVRLVIVDGIAFPFRHDLD---DLSLRTRLLNGLAQQMISLANNHRLAVILT 283
RAD51 208 -SAMMVESRYALLIVDSATALYRTDYSGRGELSARQMHLARFLRMLLRLADEFGVAVVIT 266
RAD51C 284 NQMTTKIDRNQALL----VPALGESWGHAATIRLIFHWD-RKQRLATLYKSPSQKECTVL 338
RAD51 267 NQVVAQVDGAAMFAADPKKPIGGNIIAHASTTRLYLRKGRGETRICKIYDSPCLPEAEAM 326
RAD51C 339 FQIKPQGFRDTVVTSACSLQTEGSLSTRKRSRDPEEEL 376
RAD51 327 FAINADGVGDAKD---------- 339
RAD51C-RAD51D Interaction
Y3H Proficient
Y3H Deficient
Walker A
Walker B
NLS
. Some Conservation
: Moderate Conservation
* High Conservation
b
kDa
WT
M10R
E67K
N71K
K84N
T120A
M136I
G162A
pGAD
αRAD51C
αKar2
M165L
V166G
V170G
G189R
H207R
A279P
D318N
F164L
T174S
Q178E
Q181R
V239G
R319Q
A354V
S364G
H82N
D291V
L182R
N203D
L238R
c
HR VS RAD51D Y3H
R² = 0.6111
RAD51D Y3HS
HR
0.00
0.25
0.50
0.75
1.00
1.25
N203D
L182R
H207R
L91F
R249H
K84N
A126T
K26M
M10R
T174S
T287A
R366Q
E67K
Q11R
V170G
D318N
A354V
S364G
P43S
G189R
L62V
M165L
N71K
Q181R
V166G
I144T
G162A
F164L
L219S
T120A
A279P
G162E
R319W
T122P
T122R
L138F
G112V
V239G
C135Y
V140G
Q133K
L226P
L238R

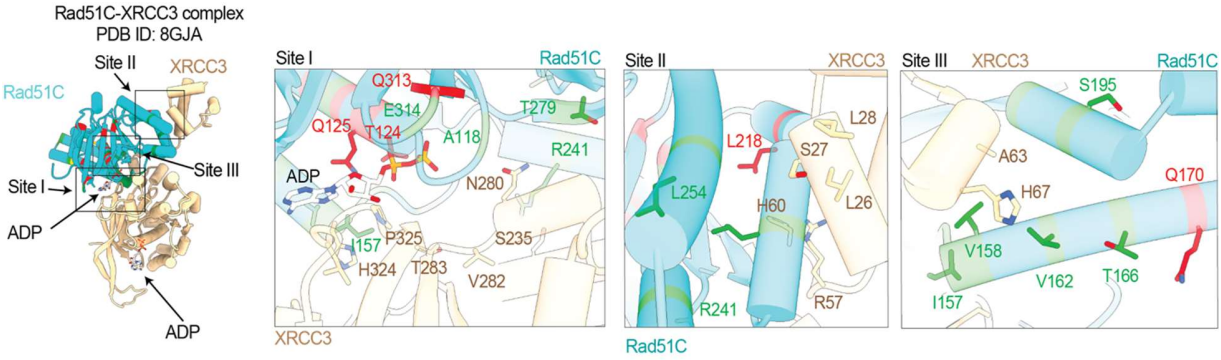
Supplementary Figure 2. Comparison of RAD51 conservation, protein expression, and yeast-3-hybrid among RAD51C ovarian cancer variants with deficient interaction with RAD51D. (a) Four out of five RAD51C-RAD51D interaction deficient variants moderately or highly conserved with RAD51 by multiple sequence alignment. Variants are indicated above the alignment, with ATPase motifs labeled in purple (Walker A) and gold (Walker B) and the nuclear localization sequence labeled in light blue. Conservation is indicated with an asterisk (*) for high conservation and a colon (:) for moderate conservation (conserved within amino acid groups of strongly similar properties) and a period (.) for some conservation (conserved within amino acid groups of weakly similar properties). RAD51D interaction capabilities are indicated in red (deficient) or green (proficient). **(b)** RAD51C ovarian cancer variants examined in **Fig. 1c** are expressed in yeast. Western blot analysis of wild-type RAD51C, the variant of interest, or an empty plasmid performed. RAD51C protein levels were analyzed using an αRAD51C antibody and equal protein loading was determined using the loading control Kar2 (αKar2). The experiments were performed in duplicate. **(c)** RAD51C variant HR proficiency results from this study and Prakash et al. compared against the yeast-three-hybrid (Y3H) RAD51C-RAD51D interaction results. The average relationship is represented by the black line, with 95% confidence intervals in green. R^2 and Pearson correlation values are shown. Note that results for L27P and Q178E have been excluded due to lack of protein expression.


a
Growth Rate
RAD51C Variant
Complemented Cell Line
WT
M10R
E67K
T120A
G162A
F164L
V166G
V170G
G189R
L238R
V239G
A279P
D291V
KO
# of cells (10^6)
Time(Hrs)
b
Doubling Times
Doubling Times (Hrs)
35 31 28 30 34 27 34 31 21 46 28 52 32 46
RAD51C Variant Complemented Cell Line
c
HR proficiency by category
****
*
****
p = 0.0133
%GFP Normalized to WT
M10R
V166G
D108Y
G125S
G130A
R158H
A279V
V140G
T336P
Q181R
D318N
Unchanged
Slow depleted
Fast depleted

Supplementary Figure 3. Growth and comparison of HR analysis of RAD51C ovarian cancer variants of interest expressed in RAD51C knockout U2OS cells. (a) The growth rates of *RAD51C* variant expressing cells. The growth rate of *RAD51C* KO cell line and RAD51C wild-type complemented or variant expressing cells were analyzed for cell number every 24 hours for four days. Growth of each cell line was measured using a coulter counter and logged over 96 hours and average growth of four experiments was graphed with standard deviations plotted. The experiment was repeated four times per cell line. **(b)** The doubling times of *RAD51C* variant expressing cells. Doubling times were calculated from the 24-hour log-phase period determined in **(a)**. Experiments were repeated four times for every variant cell line; in the *RAD51C*-knockout cell line, the experiment was performed three times. Average doubling time was plotted with standard deviations. **(c)** Comparison of growth to HR proficiency from [26]. Unchanged indicates a variant that has WT growth whereas a slow depleted variant has intermediate growth and a fast depleted variant is inviable. Variants analyzed in this study for HR proficiency are color coded in green (proficient), red (deficient), and yellow (intermediate). The average HR proficiency within the variants categorized as unchanged is significantly higher than the slow-depleted and fast-depleted variants. Statistical significance determined by t-test is indicated (**** indicates $p < 0.0001$). Outliers are indicated.



Supplementary Figure 4. The replication fork speed of RAD51C KO and V239G complemented cells is reduced. *RAD51C* CRISPR/Cas9 U2OS cells were complemented with the WT *RAD51C* or the indicated variant and DNA replication fork progression was determined. The cells were first pulsed with CldU for 20 min followed by IdU for 60 min.
Each data point represents Idu/Cldu ratio for one fiber measurement. From left to right, n = 204, 205, 205, 218, or 218 fibers analyzed from two experiments. Bars represent median of each data set. Statistical significance was determined by Kruskal-Wallis with multiple Dunn's comparison and is indicated (ns is not significant, * p = 0.0375, ** p = 0.0079).



Supplementary Figure 5. Location of conserved RAD51C variants on Alvinella pompejana CX3 structure. The Rad51C-XRCC3 structure ([36]:PDB ID:8GJA) with the indicated human RAD51C ovarian cancer identified variants are shown. Site I indicates residues that were identified in the ATPase region, Sites II and III are at the interface with XRCC3. Red indicates an HR deficient variant whereas green indicates an HR proficient variant. Note that *Alvinella pompejana* residues T124, Q125, Q170, L218, and Q313 correspond to human T132, Q133, Q178, L226, and D318, respectively.

Supplementary Table 1. Clinical information for ovarian cancer patients with either RAD51C-F164L, L238R, or V239G variants. The variant is indicated as well as the nucleotide change (Nuc. Change) and the prior classification status. The allele origin including variant allele frequency (VAF) is shown. The age of diagnosis, diagnosis, BRCA status, as well as treatment history and clinical outcomes are shown.

Variant	Nuc. Change	Classification	Allele Origin	Age at diagnosis	Diagnosis	BRCA Status	Treatment	Outcome
F164L	492T>G	Likely Benign (Myriad)	Germline	48	IIIC - Papillary serous cystadenocarcinoma	+	• carbo/taxol • bevacizumab • olaparib • carbo/gem	4 years Died - 2017
L238R	713T>G	VUS	Likely Germline, 99% VAF	54	Platinum resistant HGSOC, stage unknown	-	• cisplatin/taxotere • carbo/taxotere • carbo/gem/taxol • niraparib • CHK1 inhibitor (prexasertib) phase 2 trial	Recurrent HGSOC, improved with prexasertib before trial withdrawal, current status unknown
V239G	716T>A	VUS	Germline	50	IIIC - Papillary serous cystadenocarcinoma	-	• IV carbo/taxol • IP cisplatin	13 years Currently NED

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