

Fig. S1

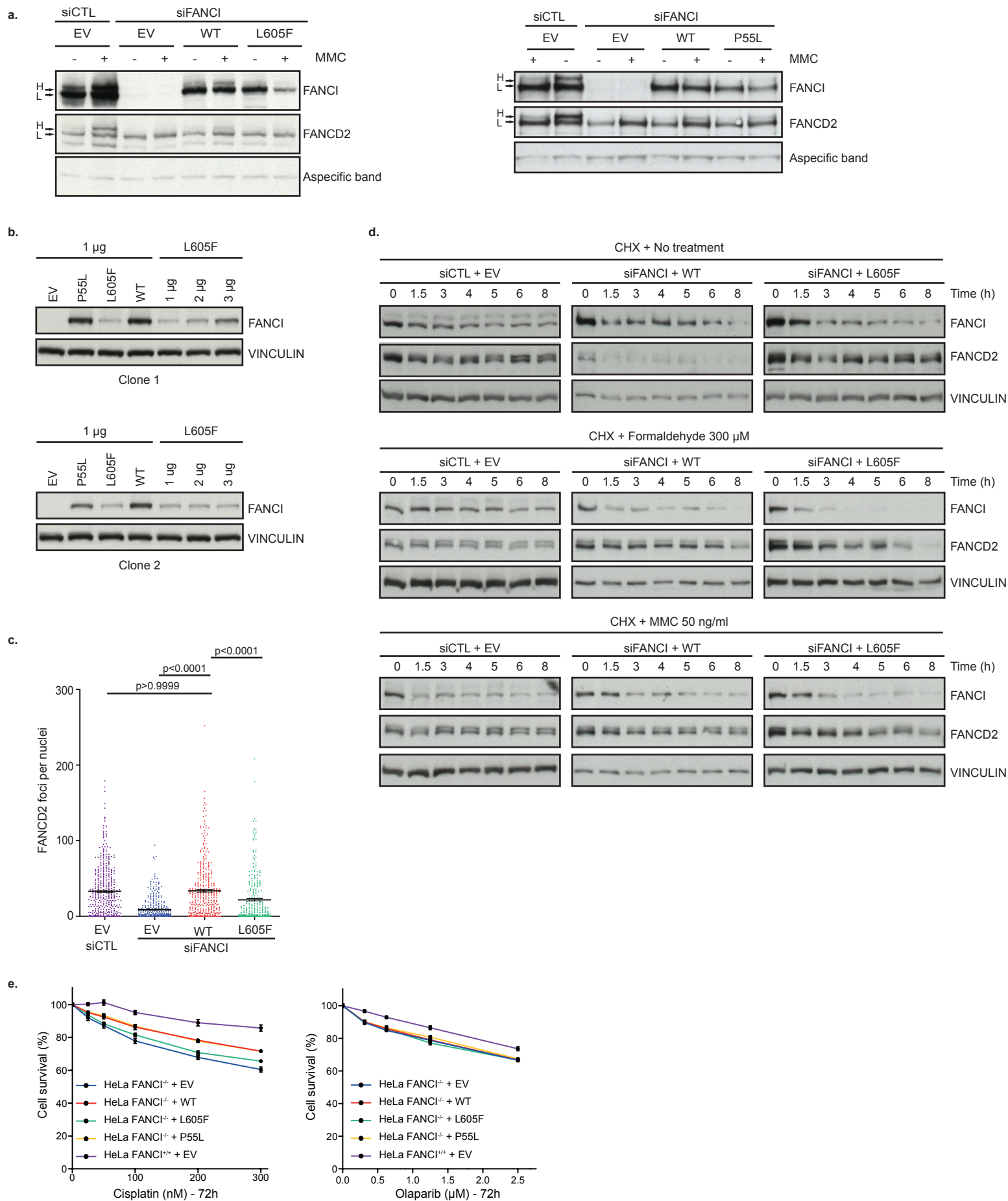
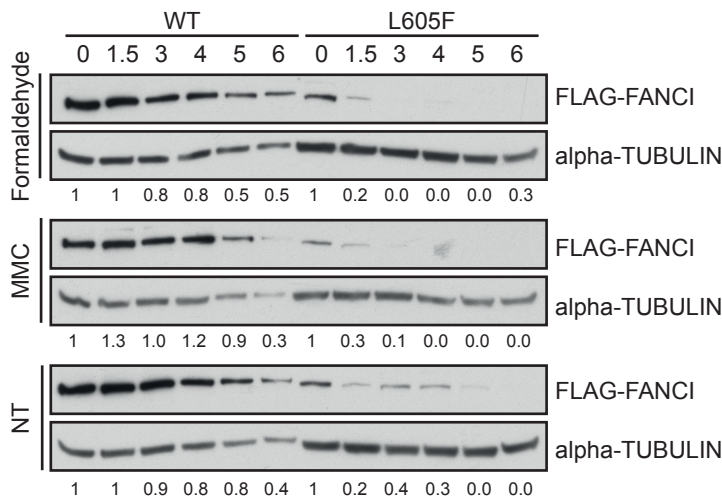


Fig. S1. The isoform with the p.L605F variant impairs FANCI stability and function.

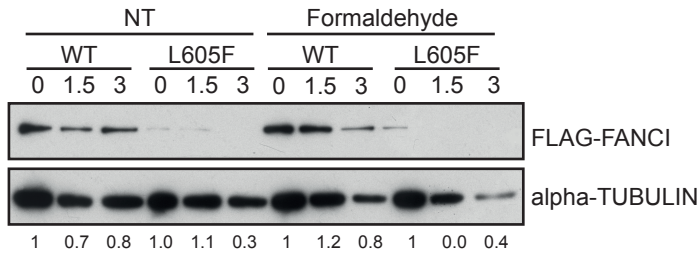
a) Western blots of HeLa FANCI^{+/+} cells transfected with siRNA control (siCTL) or targeting FANCI (siFANCI) and then complemented with Flag-FANCI constructs or empty vector (EV) and treated with 50 ng/ml MMC for 18 hours. The upper band, H, shows the ubiquitination of FANCI and FANCD2 after treatment. The lower band, L, corresponds to non-ubiquitinated FANCI or FANCD2. VINCULIN was used as a control. b) Western blot of HeLa FANCI^{-/-} cells from clones 1 and 2 were complemented with increasing amounts of FANCI p.L605F plasmid. c) Immunofluorescence of HeLa FANCI^{+/+} cells transfected with siRNA targeting FANCI and complemented with EV or Flag-FANCI siRNA resistant constructs. The number of FANCD2 foci in Flag positive cells after treatment with MMC (50 ng/ml, 18 hours) is shown. The upper and lower edge of the solid bars represents the SEM. The Kruskal-Wallis test was used to compare groups and the *P*-value is shown for each test. d) Western blots of HeLa FANCI^{+/+} cells transfected with siRNA targeting FANCI and complemented with Flag-FANCI siRNA resistant constructs and treated with CHX and either mock-treated or treated with damaging agents formaldehyde or MMC for different lengths of time. At each time point, whole cell extracts were analyzed by Western blot to assess protein levels. Experiment has been performed twice. e) Survival curves of HeLa FANCI^{-/-} cells from clone 2 that were complemented with constructs of Flag-FANCI variants or empty vector (EV) and plated in triplicate in a 96 well plate. Cell viability was monitored following cisplatin or olaparib treatments for 72 hours and was assessed by counting remaining nuclei. Experiments were performed in three biological replicates.

Fig. S2

a.



b.



c.

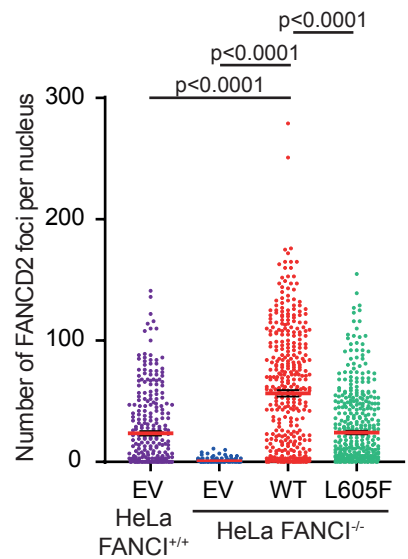
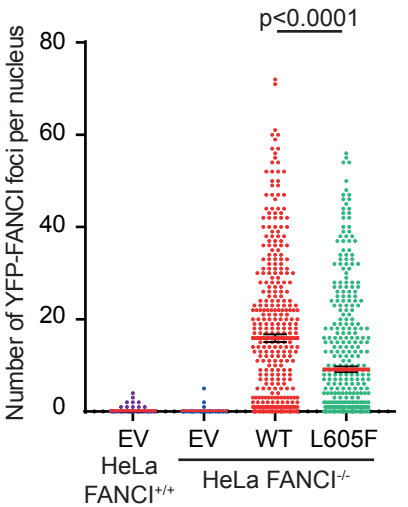
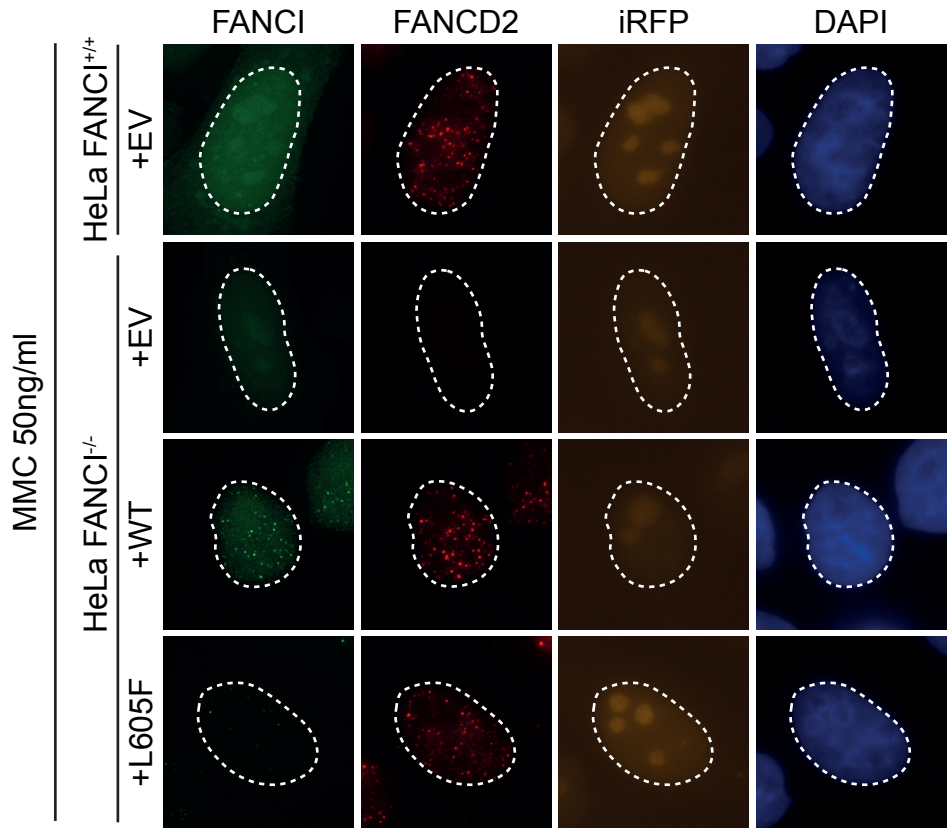
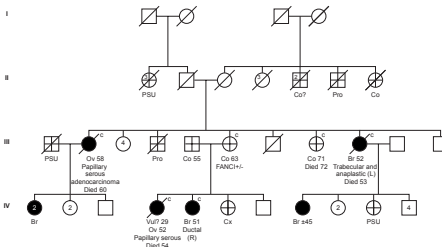
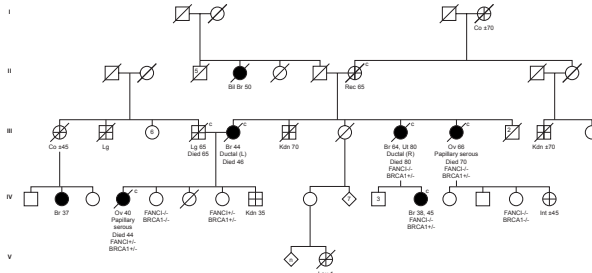


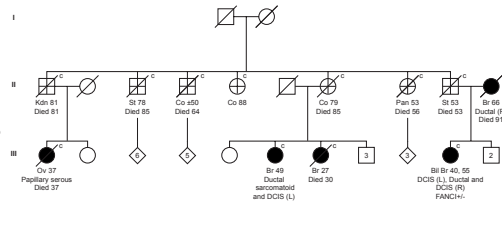
Fig. S2. The isoform with the p.L605F variant impairs FANCI stability in OC cell lines and FANCI localization to DNA damage in HeLa cells. a) Western blots of OVCAR-4 cells expressing Flag-FANCI wild type (WT) or p.L605F. Cells were treated with CHX and either mock-treated (NT) or treated with formaldehyde (300 μ M for the indicated times) or MMC (50 ng/ml for the indicated times). Alpha-tubulin was used as a loading control. b) Western blots of OVCAR-3 cells expressing Flag-FANCI wild type (WT) or p.L605F, treated with cycloheximide and either mock-treated or treated with formaldehyde (300 μ M for the indicated times). Alpha-tubulin was used as a loading control. c) Immunofluorescence analysis of wild-type HeLa or HeLa FANCI^{-/-} cells complemented with either YFP alone (EV), YFP-FANCI, or YFP-FANCI p.L605F constructs. iRFP was used as a transfection marker. The adjacent scatter plot shows the number of YFP-FANCI foci per nucleus or FANCD2 foci in iRFP-positive cells after treatment with MMC (50 ng/ml, 18 hours). Mean with SEM is represented. The Kruskal-Wallis test was used to compare groups and the P-value is shown for each test.

Fig. S3

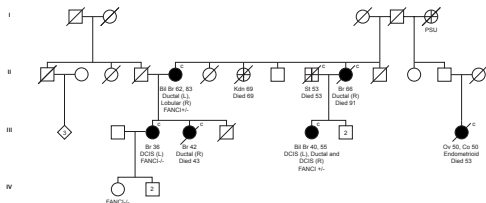
Family 1480

Family 1555
BRCA1 c.235A>G(T>A); p.S845N/V

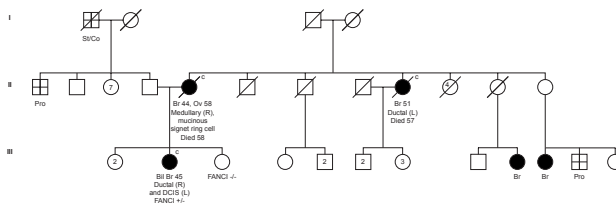
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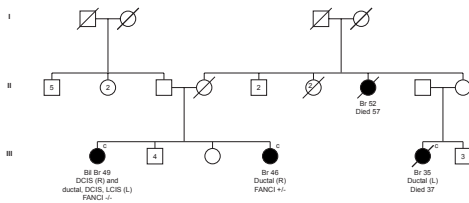
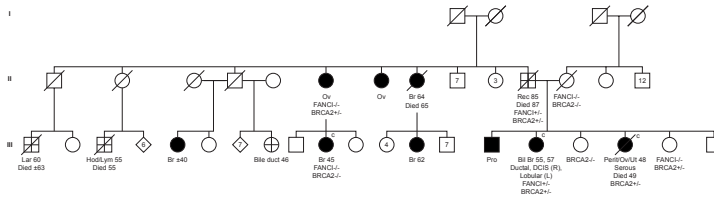
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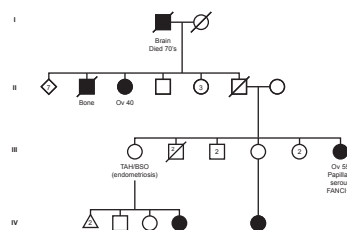
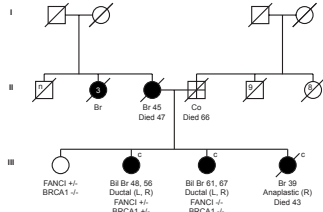
Family 1501



Family 1522

Family 762
BRCA2 c.3170_3174delAGAAA; p.K1057Tfs*8

Family 1520

Family 1520
BRCA1 c.2125_2126insA; p.F709Yfs*3

Family 1023

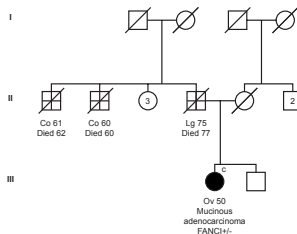
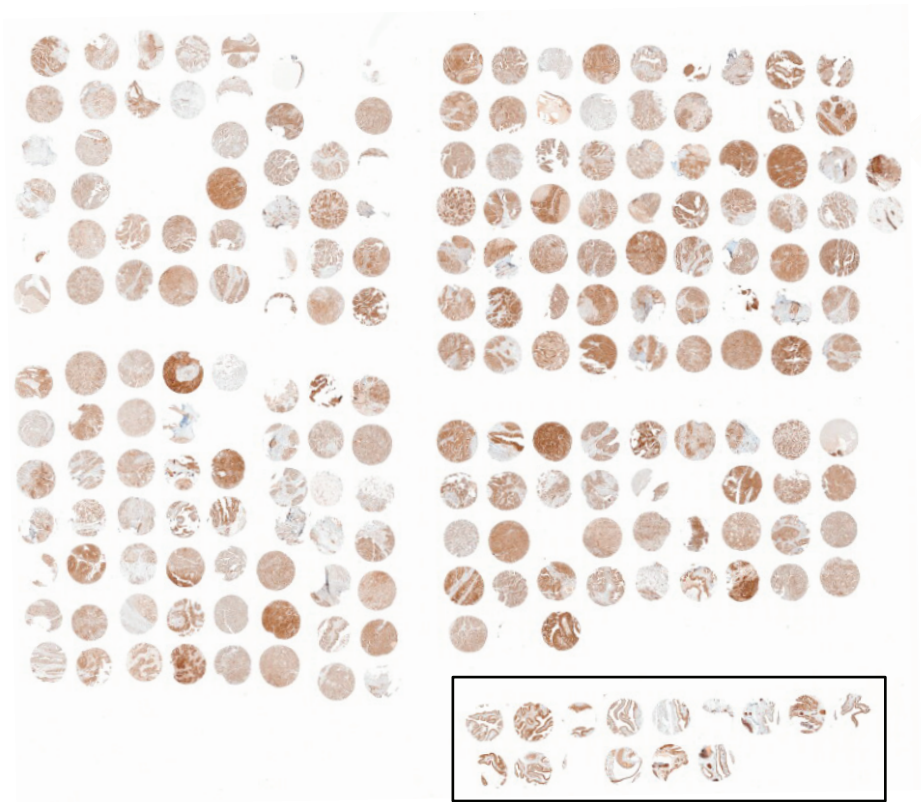


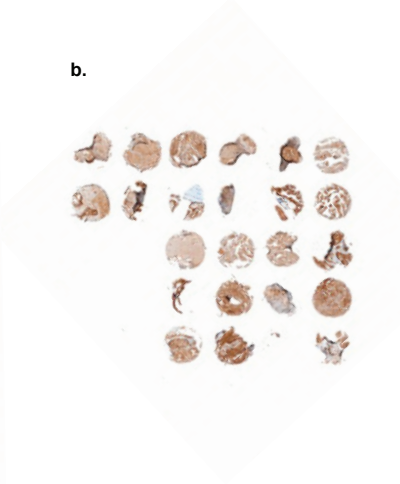
Fig. S3. Pedigrees of OC and BC families with *FANCI* c.1813C>T; p.L605F. Cancer type (Ov: ovarian, Leu: leukemia, Br: breast, Cx: cervical, Co: colon, Rec: rectal, St: stomach, Kdn: kidney, Pro: prostate, Lg: lung, Perit: peritoneum, Ut: uterine, Lar: larynx, Hod: Hodgkin's lymphoma, Lym: lymphoma, and PSU: primary site unknown), risk reducing surgery (TAH/BSO: total abdominal hysterectomy/bilateral salpingo-oophorectomy), and age at diagnosis is indicated. *BRCA1* and *BRCA2* carrier status is shown for pathogenic variant positive families, F762, F1055, F1520; all other families are *BRCA1* and *BRCA2* pathogenic variant negative. c next to a symbol denotes a confirmed cancer case. Pedigrees may have been truncated to protect anonymity.

Fig. S4

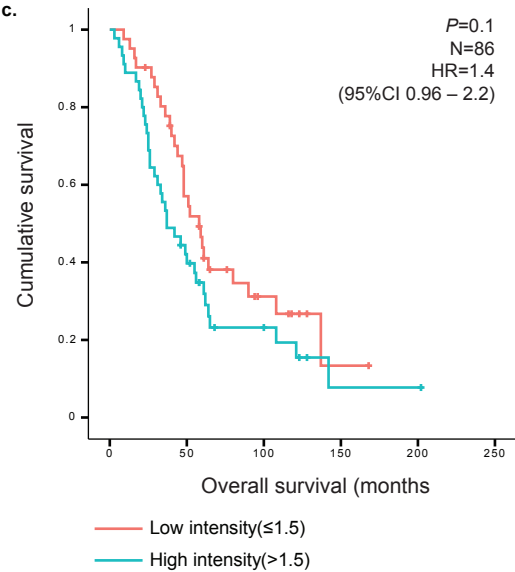
a.



b.



c.



d.

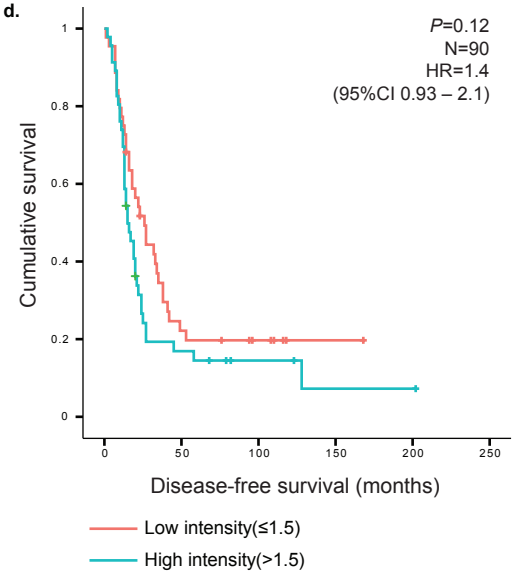


Fig. S4. Immunohistochemical analyses of FANCI protein expression of tissue microarrays (TMAs) from (a) HGSC cases and normal fallopian tube (a, black box) and (b) *FANCI* c.1813C>T OC carriers tissue cores. TMAs were stained with anti-FANCI antibody on the same slide. Kaplan-Meier survival curve of HGSC cases for overall (c) and disease-free (d) survival (in months) as measured in the epithelial cell component. Cases included in the analyses had received only adjuvant taxol and carboplatin chemotherapy.

Fig. S5

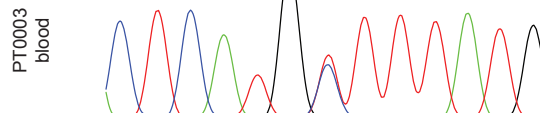
a.

FANCI c.1813C>T

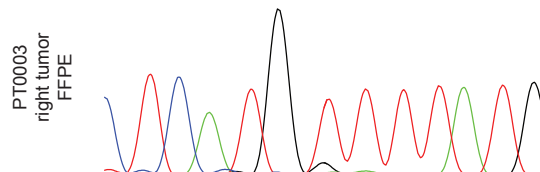


FANCI genotype

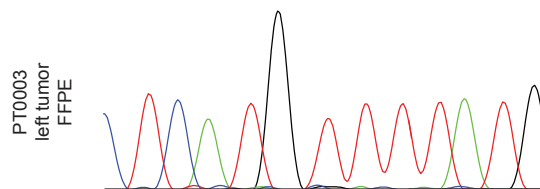
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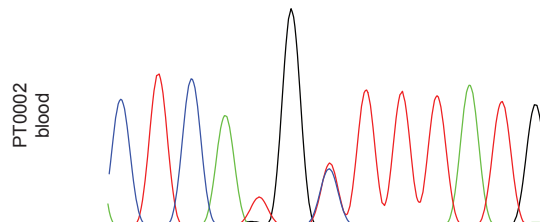
C;T



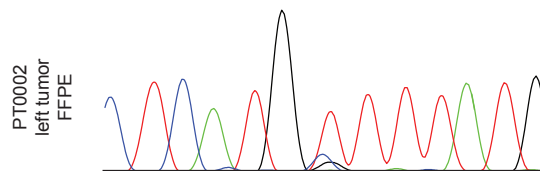
T;T



T;T



C;T



T;T

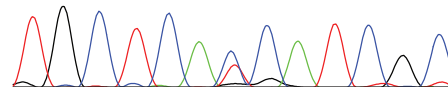
b.

TP53 c.559+C>T



T G C T C A C C A T C G C

PT0003 left tumor FF



TP53 c.659A>G



G T G C C C T A T G A G C C

PT0002 right tumor FF

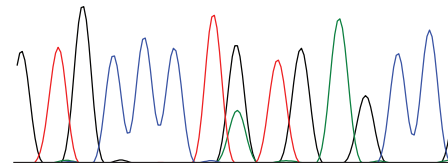
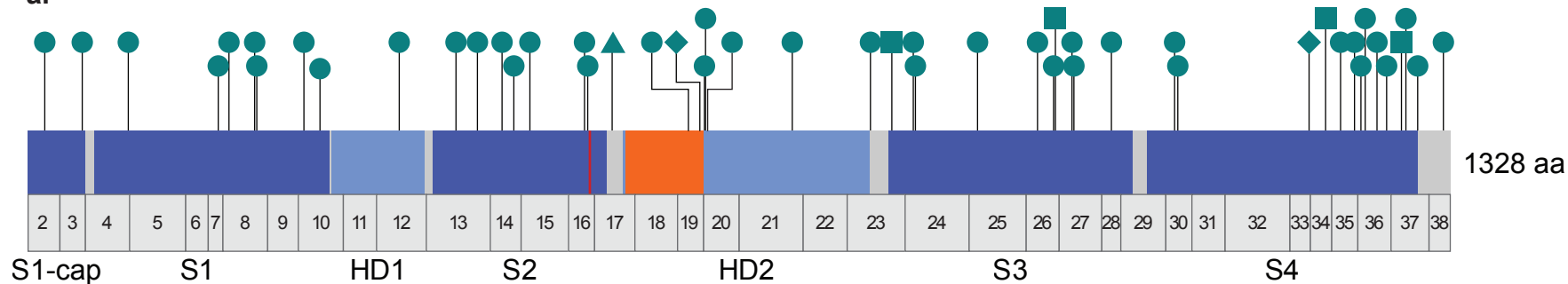


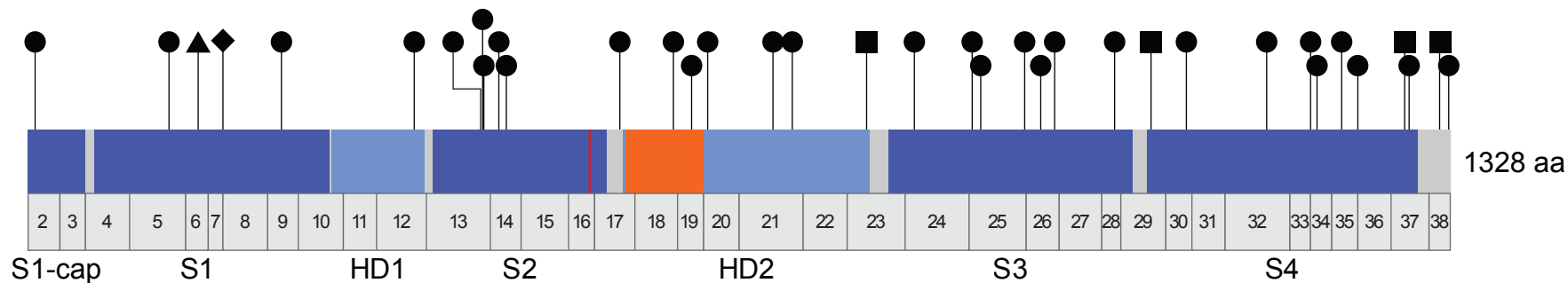
Fig. S5. Identification of *FANCI* c.1813C>T and *TP53* variants in French Canadian HGSC cases. a) DNA sequencing chromatogram showing the region containing c.1813C>T corresponding to DNA obtained from (top to bottom): PT0003 blood showing the heterozygous variant, PT0003 FFPE right tumor showing loss of the WT allele, PT0003 FFPE left tumor showing loss of the WT allele, PT0002 blood showing the heterozygous variant, and PT0002 FFPE left tumor showing loss of the WT allele. The forward sequence is shown. B) DNA sequencing chromatogram showing the region containing identified *TP53* variants corresponding to (top to bottom): PT0003 FF left tumor showing *TP53* variant c.559+1C>T and PT0002 FF right tumor showing *TP53* variant c.659A>G. The reverse sequence is shown. FFPE: formalin-fixed paraffin-embedded tumor tissue; FF: fresh frozen tumor tissue.

Fig. S6

a.



b.



Domain

Solenoid domain

Helical domain

Ubiquitination site, K523

S/TQ cluster

Sample

Ovarian cancer

Controls

Variant effect

○ Missense

□ Nonsense

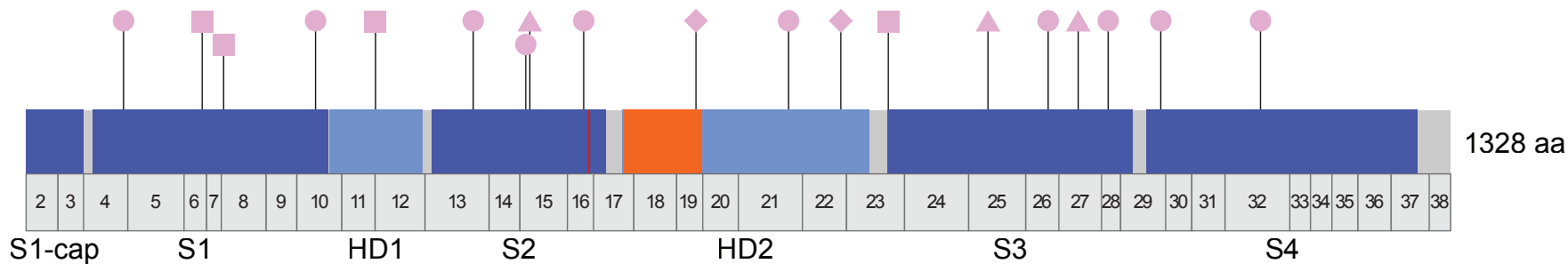
△ Frameshift

◇ Splicing

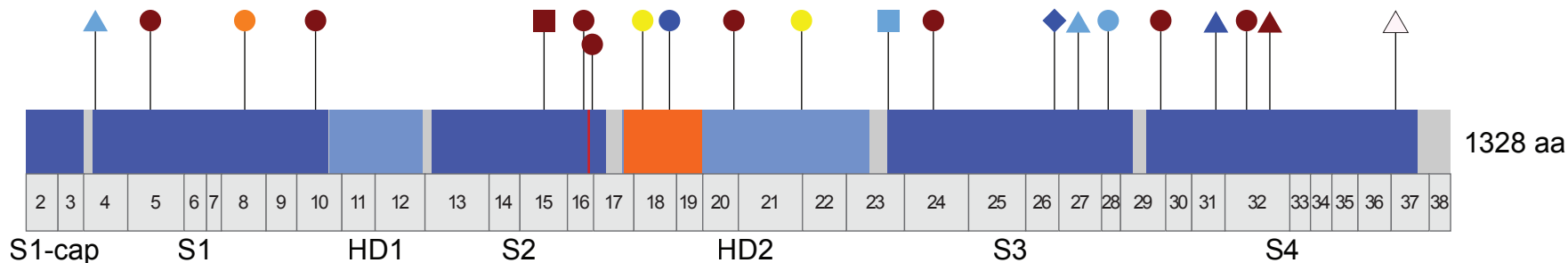
Fig. S6. Schemata of *FANCI* gene showing rare variants (VAF<1%) reported in (a) OC (b) and controls from the literature. FANCI domains were adapted from pfam (<https://pfam.xfam.org>). *FANCI* exon locations adapted from University of California Santa Cruz Genome Browser (<https://genome.ucsc.edu>).

Fig. S7

a.



b.



Domain

- Solenoid domain
- Helical domain
- Ubiquitination site, K523
- S/TQ cluster

Sample

- Breast cancer
- Prostate cancer
- Head and neck carcinoma
- Acute myeloid leukemia
- Sporadic sarcoma
- Colorectal cancer
- Malignant pleural mesothelioma

Variant effect

- Missense
- Nonsense
- Frameshift
- Splicing

Fig. S7. Schemata of *FANCI* gene showing rare variants (VAF<1%) reported in the literature in (a) BC (b) and other cancers. FANCI domains were adapted from pfam (<https://pfam.xfam.org>). *FANCI* exon locations adapted from University of California Santa Cruz Genome Browser (<https://genome.ucsc.edu>).