SI MATERIALS AND METHODS

Patients and samples. Subjects were prospectively enrolled at diagnosis. The protocol included peri-operative collection of tissue, blood, and peritoneal fluid. Longitudinal clinical data was also collected. Patients indicated as “BRCA germline positive” were found to have a deleterious mutation in either BRCA1 or BRCA2 by commercial testing (Myriad Genetics, Inc.) or by NGS (1). Patients indicated as “BRCA germline negative” either had a low-risk family history suggesting hereditary mutation was unlikely or were evaluated with negative findings. Peritoneal cytopathology for the 17 ovarian cancer samples included 6 positive, 2 atypical, and 9 negative. Peritoneal cytopathology was negative for all control patients. Tumors were surgically staged according to the International Federation of Obstetrics and Gynecology (FIGO) criteria. Data analysis was performed blinded, and all patient samples were de-identified. The 37 peritoneal fluid samples of this study included 7 ascites and 30 peritoneal washes. If the liquid portion of a peritoneal fluid sample exceeded 2 mL, the sample was centrifuged at 1,000g for 3 minutes and excess supernatant was removed before DNA extraction.

***TP53* mutation detection in primary lesions.** *TP53* mutations for most primary tumors had been previously established by NGS at a sequencing depth of ~300X as previously described (1), except in OvCA 01 - 03. For these 3 cancers (which were incidentally discovered after risk-reducing salpingo-oophorectomy), the microscopic lesion was isolated for DNA extraction via laser capture microdissection using a Veritus system (Arcturus) from consecutive formalin-fixed sections. DNA was extracted using the PicoPure DNA extraction kit (Arcturus) and *TP53* exons were then PCR amplified for Sanger sequencing and analyzed for clonal somatic mutation as previously described (2, 3).

***TP53* capture library.** Thirteen 5’ biotinylated 120 bp nucleotide xGen Lockdown Probes (IDT) were designed with 1x tiling to capture *TP53* exons 4-10 and associated splice junctions. Two consecutive rounds of capture yielded >98% reads mapping to the targeted gene.

Duplex Sequencing. DNA was sonicated, end-repaired, a-tailed, and ligated with Duplex Sequencing adaptors (SI Appendix Fig. S1) using AMPure XP bead cleanups after each step (1.2X volume beads in steps before ligation and 1.0X volume beads after ligation). After 5 rounds of PCR amplification and a 1.0X AMPure XP bead cleanup, one-half of the total PCR product was enriched for *TP53* exons 4-10 by two consecutive rounds of target capture with customized biotinylated oligonucleotides (IDT) and M-270 streptavidin beads (Life Technologies) followed by PCR amplification (4). In the final PCR reaction, an index sequence was added to each sample for multiplexing. Libraries were pooled and sequenced on an Illumina HiSeq2500. Lane percentage allocated to each sample was adjusted proportionally to the amount of starting DNA.

References:

1. Walsh T*, et al.* (2011) Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc Natl Acad Sci U S A* 108(44):18032-18037.
2. Swisher EM*, et al.* (2005) Tumor-specific p53 sequences in blood and peritoneal fluid of women with epithelial ovarian cancer. *Am J Obstet Gynecol* 193(3 Pt 1):662-667.

3. Norquist BM*, et al.* (2010) The molecular pathogenesis of hereditary ovarian carcinoma: alterations in the tubal epithelium of women with BRCA1 and BRCA2 mutations. *Cancer* 116(22):5261-5271.
4. Schmitt MW*, et al.* (2015) Sequencing small genomic targets with high efficiency and extreme accuracy. *Nat Methods* 12(5):423-425.

SI Appendix Table S1. Clinical information of patients with ovarian cancer

sample ID	germline BRCA mutation	age	meno-pausal status	race	history of chemo-therapy	preope-rative plasma CA125	primary anatomic site of cancer	FIGO clinical stage	histology	peritoneal fluid sample	peritoneal cyto-pathology
OvCA 01	BRCA1	46	pre	na	yes (breast cancer)	16	fallopian tube	0*	serous intraepithelial neoplasia	peritoneal wash	negative
OvCA 02	BRCA1	50	post	white	no	25	ovary	IA*	high-grade carcinoma	peritoneal wash	negative
OvCA 03	BRCA1	62	post	multi-racial	yes (breast cancer)	18	fallopian tube	IC*	serous intraepithelial neoplasia	peritoneal wash	positive
OvCA 04	negative	59	post	black	no	36	fallopian tube	IA	high-grade serous carcinoma	peritoneal wash	negative
OvCA 05	negative	85	post	white	no	248	ovary	IC	high-grade serous carcinoma	ascites	positive
OvCA 06	negative	47	pre	white	no	131	ovary	IIB	high-grade serous carcinoma	peritoneal wash	negative
OvCA 07	negative	65	post	white	no	not detecta-ble	ovary	IIC	high-grade serous carcinoma	ascites	negative
OvCA 08	BRCA1	50	pre	white	no	63	ovary	IIC	mixed endometrioid & serous carcinoma	ascites	positive
OvCA 09	negative	68	post	white	no	103	ovary	IIIB	high-grade serous carcinoma	peritoneal wash	negative
OvCA 10	negative	61	post	white	no	534	ovary	IIIB	high-grade serous carcinoma	peritoneal wash	negative
OvCA 11	negative	54	post	asian	no	851	ovary	IIIB	high-grade serous carcinoma	ascites	positive
OvCA 12	BRCA1	52	pre	white	no	2238	ovary	IIIC	high-grade serous carcinoma	ascites	negative
OvCA 13	BRCA2	62	post	white	no	11	fallopian tube	IIIC	high-grade serous carcinoma	peritoneal wash	atypical
OvCA 14	negative	76	post	white	no	2294	ovary	IIIC	high-grade serous carcinoma	peritoneal wash	positive
OvCA 15A†	BRCA1	42	post	na	yes (breast cancer)	209	ovary	na**	high-grade serous carcinoma	peritoneal wash	negative
OvCA 15B†	BRCA1	42	post	na	yes (breast cancer)	1579	ovary	Recu-rrence	high-grade serous carcinoma	ascites	positive
OvCA 16	negative	63	post	white	no	82	fallopian tube	Recu-rrence	high-grade serous carcinoma	peritoneal wash	atypical

Abbreviations: OvCA: ovarian cancer; na: not available

*Microscopic cancers incidentally discovered at prophylactic surgery in women with germline BRCA mutations

**Disease not staged, as patient was receiving chemotherapy for previously diagnosed breast cancer

† Samples 15A and 15B correspond to primary and recurrence surgeries from the same patient

SI Appendix Table S2. Clinical information of control patients

patient ID	germline BRCA mutation	age at surgery	meno-pausal status	race	history of chemo-therapy	preope-rative plasma CA125	indication for surgery	benign surgical pathology	peritoneal fluid sample	peritoneal fluid cyto-pathology
CON 01	negative	68	post	white	no	12	large central pelvic mass arising from uterus	hernia sac	peritoneal wash	negative
CON 02	negative	67	post	white	no	10.5	adnexal mass with solid nodularity	mucinous cystadenoma	peritoneal wash	negative
CON 03	negative	79	post	white	no	26.4	cystsic mass arising from left ovary	leiomyomata	peritoneal wash	negative
CON 04	negative	55	post	white	no	7.9	complex right adnexal mass	mucinous cyst	peritoneal wash	negative
CON 05	negative	59	post	white	no	7	complex pelvic mass	struma ovarii	peritoneal wash	negative
CON 06	negative	73	post	white	no	not detected	persistent, large, complex pelvic mass	serous cystadenofribroma	peritoneal wash	negative
CON 07	negative	67	post	white	no	71	large, complex mass with nodularity & calcifications	brenner tumor, mucinous cystadenoma	peritoneal wash	negative
CON 08	negative	58	post	white	no	11.7	right adnexal mass	cystadenofibroma	peritoneal wash	negative
CON 09	negative	63	post	na	no	58	right cystic mass with internal debris	simple cyst	peritoneal wash	negative
CON 10	negative	63	post	white	no	7	ovarian mass w/ fluid filled structures, echogenic debris, thickened septa	struma ovarii, endometriosis	peritoneal wash	negative
CON 11	BRCA1	43	pre	white	no	8	prophylactic salpingo-oophorectomy for risk reduction	no gross or microscopic pathology	peritoneal wash	negative
CON 12	BRCA2	45	post	white	no	not detected	prophylactic salpingo-oophorectomy for risk reduction		peritoneal wash	negative
CON 13	BRCA1	41	pre	white	no	10	prophylactic salpingo-oophorectomy for risk reduction		peritoneal wash	negative
CON 14	BRCA2	46	pre	white	no	16	prophylactic salpingo-oophorectomy for risk reduction		peritoneal wash	negative
CON 15	BRCA2	45	pre	white	yes (breast cancer)	23	prophylactic salpingo-oophorectomy for risk reduction		peritoneal wash	negative
CON 16	BRCA1	33	pre	white	no	11	prophylactic salpingo-oophorectomy for risk reduction		peritoneal wash	negative
CON 17	BRCA1	37	pre	white	no	10	prophylactic salpingo-oophorectomy for risk reduction		ascites	negative
CON 18	BRCA2	50	post	white	yes (breast cancer)	11	prophylactic salpingo-oophorectomy for risk reduction		peritoneal wash	negative
CON 19	BRCA1	32	pre	white	no	5	prophylactic salpingo-oophorectomy for risk reduction		peritoneal wash	negative
CON 20	BRCA2	52	post	na	no	7	prophylactic salpingo-oophorectomy for risk reduction		peritoneal wash	negative

SI Appendix Table S3. Detection of ovarian cancer TP53 mutations in peritoneal fluid

sample ID	FIGO clinical stage	sequen -cing method	primary tumor TP53 mutation genomic coordinate (hg19)	amino acid change	mutation type	tumor TP53 mutation detected in matched peritoneal fluid?	total TP53 tumor mutant molecules detected	total DCS nucleotides (DCS depth) at tumor allele	tumor mutant allele frequency in matched peritoneal fluid
OvCA 01	0*	Sanger	Chr17:7578265 A>G	I195T	missense	yes	1	3421	2.92E-04
OvCA 02	IA*	Sanger	Chr17:7578203 C>T	V216M	missense	no	0	5829	not detected
OvCA 03	IC*	Sanger	Chr17: 7578437 G>A	Q165X	nonsense	yes	7269	14042	5.18E-01
OvCA 04	IA	NGS	Chr17:7578394 T>C	H179R	missense	yes	2	8671	2.31E-04
OvCA 05	IC	NGS	Chr17:7578461 C>A	V157F	missense	yes	164	3280	5.00E-02
OvCA 06	IIB	NGS	Chr17:7577124 C>T	V272M	missense	yes	1	23921	4.18E-05
OvCA 07	IIC	NGS	Chr17:7578403 C>T	C176Y	missense	yes	99	6376	1.55E-02
OvCA 08	IIC	NGS	Chr17:7578555 C>T	na	splice	yes	4	3672	1.09E-03
OvCA 09	IIIB	NGS	Chr17:7578442 T>C	Y163C	missense	yes	3	12440	2.41E-04
OvCA 10	IIIB	NGS	Chr17:7578526 C>T	C135Y	missense	yes	30	5238	5.73E-03
OvCA 11	IIIB	NGS	Chr17:7577548 C>T	G245S	missense	yes	22	1868	1.18E-02
OvCA 12	IIIC	NGS	Chr17:7577538 C>T	R248Q	missense	yes	275	10427	2.64E-02
OvCA 13	IIIC	NGS	Chr17:7577551 C>T	G244S	missense	yes	2	3733	5.36E-04
OvCA 14	IIIC	NGS	Chr17:7574003 G>A	R342X	nonsense	yes	12	1749	6.86E-03
OvCA 15A†	na	NGS	Chr17:7578275 G>A	Q192X	nonsense	yes	1	24737	4.04E-05
OvCA 15B†	Recu- rrence	NGS	Chr17:7578275 G>A	Q192X	nonsense	yes	3949	5762	6.85E-01
OvCA 16	Recu- rrence	NGS	Chr17:7576852 delC	na	indel	yes	2**	31027**	6.45E-05

Abbreviations: OvCa, ovarian cancer; NGS, Next Generation Sequencing; DCS, Duplex Consensus Sequence; na, not applicable

*Microscopic cancers incidentally discovered at prophylactic surgery in women with germline BRCA mutations

**Numbers correspond to single strand consensus sequences (SSCS) instead of DCS. Mutation was not detected in DCS reads, but it was detected in 2 SSCS reads well above background artifact.

†Samples 15A and 15B correspond to primary and recurrence surgeries from the same patient

SI Appendix Table S4. Multivariate models for tumor-specific *TP53* mutant allele frequency in peritoneal fluid of women with ovarian cancer

	variable name	*estimate*	*SE*	*T-stat*	*p-value*
Model1	Ascites	0.008	0.003	2.608	0.023
	Positive cytology	0.001	0.003	0.338	0.741
Model2	Ascites	0.008	0.002	3.507	0.005
	Age	0.003	0.001	2.875	0.015
	Positive cytology	-0.002	0.003	-0.697	0.500
Model3	Ascites	0.007	0.002	3.568	0.004
	Age	0.003	0.001	2.642	0.023
	Germline BRCA mutation	0.002	0.002	0.659	0.523
Model4	Ascites	0.007	0.002	3.730	0.003
	Age	0.002	0.001	2.784	0.018
	Plasma CA-125	0.001	0.001	1.080	0.303
Model5	Ascites	0.008	0.002	3.565	0.005
	Age	0.003	0.001	2.972	0.014
	Future recurrence	0.002	0.002	0.954	0.363
Model6	Ascites	0.007	0.002	3.573	0.005
	Age	0.003	0.001	2.994	0.013
	Stage	-0.001	0.002	-0.589	0.569

Notes: Tumor mutant allele frequency and plasma CA-125 were log transformed. Stage comparison was done as early (FIGO 1 and 2) vs late (FIGO 3 or recurrence)

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Overview of Duplex Sequencing. (A) Ligation of Duplex Sequencing adaptors and PCR. Duplex Sequencing adaptors contain a molecular tag consisting of a randomized nucleotide sequence (represented as α (cyan) and β(orange)) and two universal priming sites (purple and green). DNA is fragmented (yellow) and ligated to Duplex Sequencing adaptors. PCR produces copies of both strands of DNA, which can be identified as αβ or βα. (B) Bioinformatic workflow of Duplex Sequencing. Colored dots represent mutations (green: true mutation; blue and purple: PCR or sequencing error; brown: first-round PCR error or DNA damage). Raw sequencing reads are grouped by "family" (αβ and βα) and a Single-Strand Consensus Sequence (SSCS) is determined. Mutations not present in all reads (e.g. blue and purple dots) are errors that are filtered out. The SSCSs from reciprocal families (αβ and βα) are paired and their sequences are compared to form a Duplex Consensus Sequence (DCS). Mutations present in only one SSCS (brown dot) are first-round PCR errors or artifacts of DNA damage and are filtered out. Only mutations present in both strands of DNA (green dot) are true mutations.

Fig. S2. Total "biological background" *TP53* mutations per sample is directly proportional to total Duplex Consensus Sequence (DCS) nucleotides sequenced. Each dot corresponds to a peritoneal fluid sample. "Biological background" *TP53* mutations are all *TP53* mutations in exons 4-10 detected by Duplex Sequencing except the tumor *TP53* mutation.

Fig. S3. Comparison of *TP53* mutations found as "biological background" in peritoneal fluid of women with and without ovarian cancer to the catalogue of clonal *TP53* mutations in ovarian serous cystadenocarcinomas in the International Agency for Research on Cancer (IARC) *TP53* database. The catalogue includes 890 non-synonymous *TP53* mutations, which were compared to 85 and 90 non-synonymous "biological background" *TP53* mutations in peritoneal fluid from women with ovarian cancer and controls, respectively. The mutation types were not statistically significantly different (Pearson Chi-Square p-value>0.05) between the three groups.

Fig. S4. Comparison of expected *TP53* mutations in exons 5-8 based on all possible single nucleotide substitutions (1567 total substitutions, as described in Petitjean *et al*, reference 23 in article) *vs*. observed *TP53* "biological background" mutations in exons 5-8 in peritoneal fluid of women with and without cancer (59 and 70 mutations, respectively).

Fig. S5. Characterization of *TP53* mutations found as "biological background" in peritoneal fluid of women with and without ovarian cancer and with and without hereditary *BRCA1/2* mutations (*BRCA*+/-) by mutation type (A) and pathogenicity of missense mutations (B). *TP53* activity associated with missense mutations was determined via "MUT-*TP53* 2.00" as described in Soussi T*. et al* (reference 24 in article).

Fig. S6. Mutation type of "biological background" *TP53* mutations found in individual peritoneal fluid samples from cases (A) and controls (B). Y-axis shows mutation counts. Abbreviations: OvCA, ovarian cancer; Con: control.

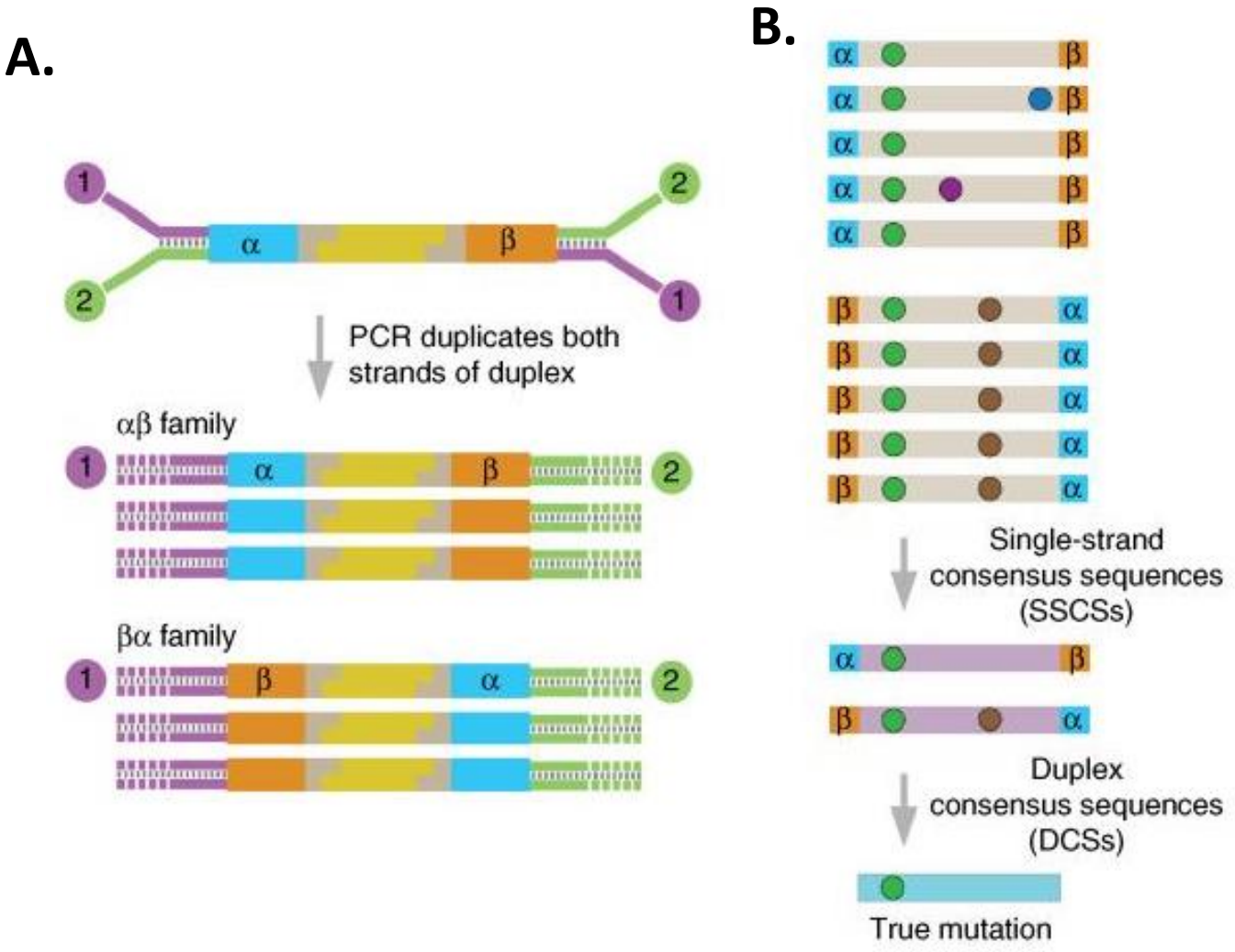
Fig. S7. Functional *TP53* activity associated with each "biological background" missense mutation in individual peritoneal fluid samples from cases (A) and controls (B). Y-axis shows mutation counts. Functional *TP53* activity was determined via "MUT-*TP53* 2.00" as described in Soussi T. *et al* (reference 24 in article). Abbreviations: OvCA, ovarian cancer; Con: control.

Fig. S8. Frequency of "biological background" *TP53* mutations in exons and introns of ovarian cancer and control peritoneal fluid samples. For each individual, the frequency of "biological background" *TP53* mutations was calculated as the number of non-tumor mutations divided by the total number of Duplex

Consensus Sequence nucleotides. This calculation was done separately for exons and introns. The plot shows the mean frequency of *TP53* “biological background” mutations for controls (blue) and ovarian cancers (red) in exons and introns. Error bars indicate the standard error of the mean. The p-values correspond to non-parametric comparisons of means between two groups using 2-tailed Mann-Whitney tests. Abbreviation: ns, non significant.

Fig. S9. Comparison of “biological background” *TP53* mutations found in peritoneal fluid (197 mutations) and peripheral blood (57 mutations). The fraction of mutations is indicated for categories of mutation type (A), pathogenicity (B), and spectrum (C). The mutations found in peritoneal fluid and peripheral blood are not statistically significantly different in terms of their type, pathogenicity, and spectrum (Pearson Chi-Square p-value>0.05 in A, B and C). *TP53* activity was determined via “MUT-*TP53* 2.00” as described in Soussi T. *et al* (reference 24 in article).

SI Appendix, Fig. S1



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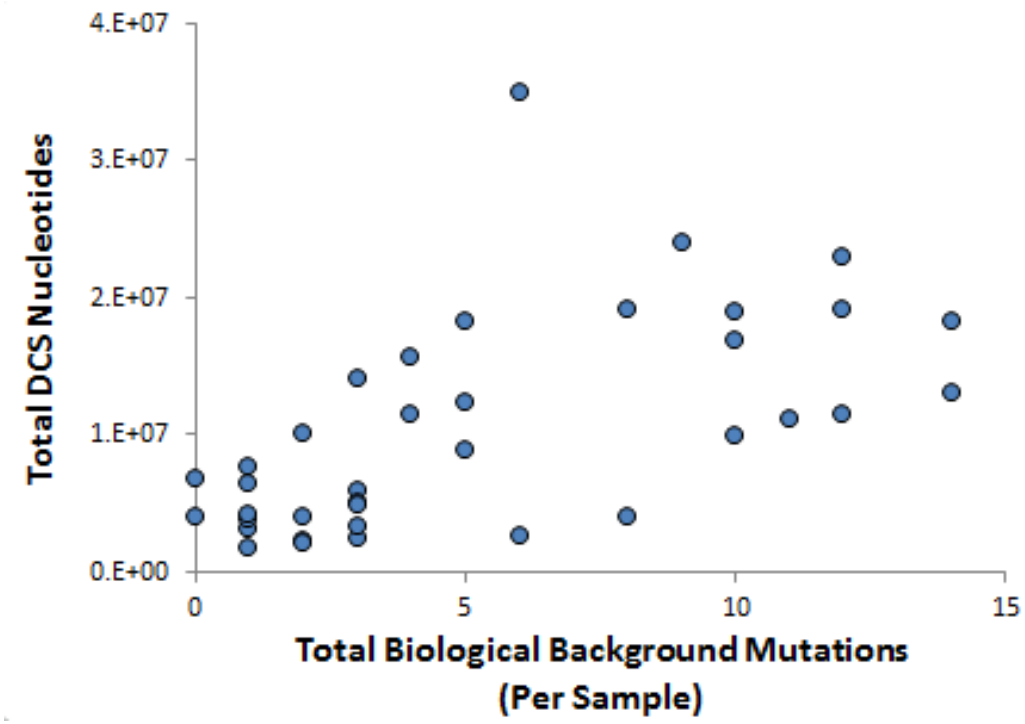
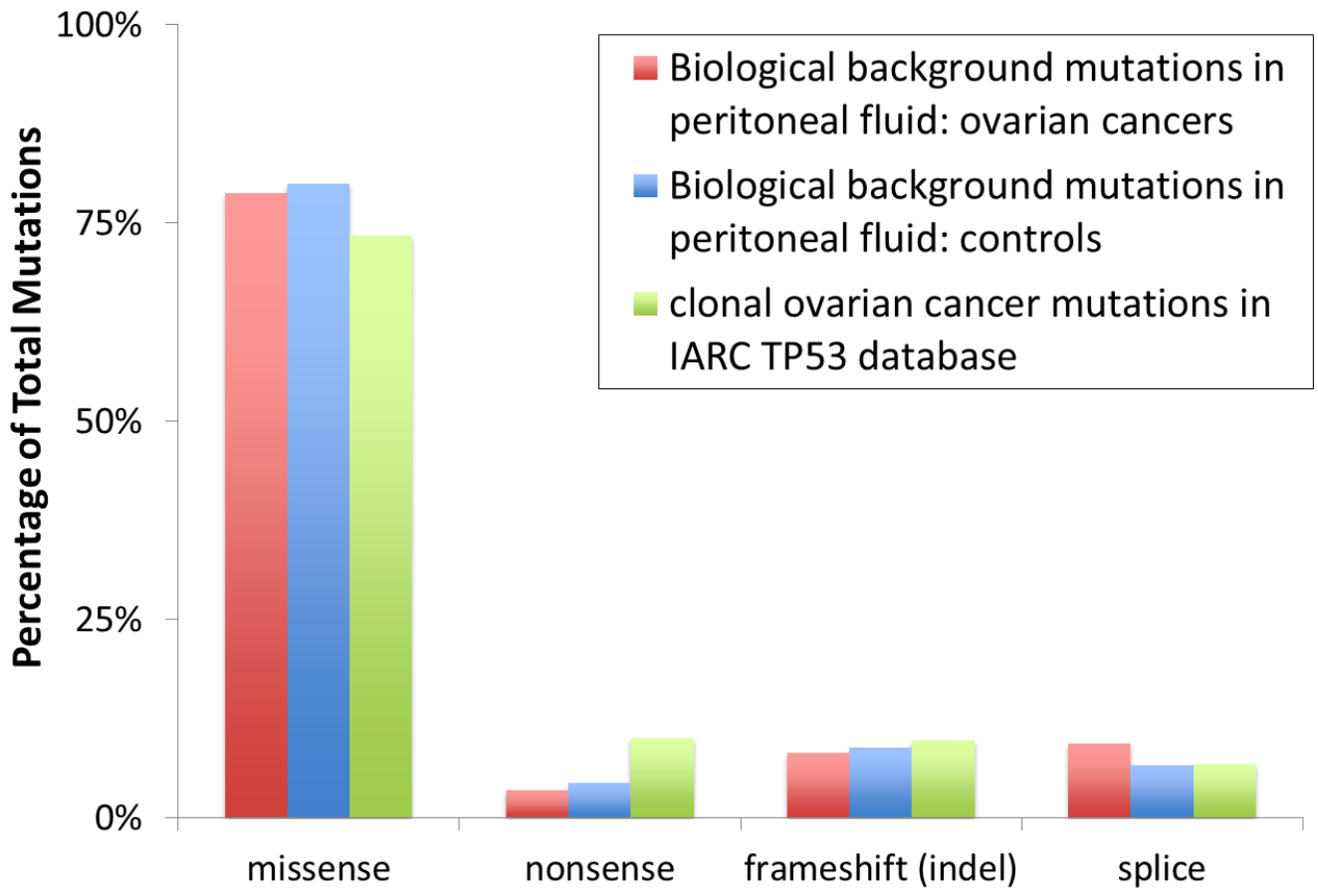


SI Appendix, Fig. S3



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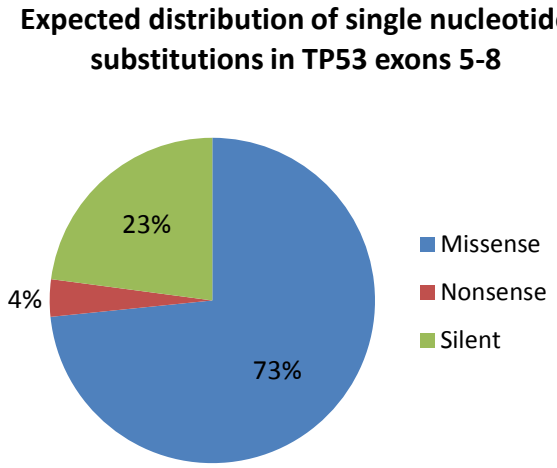

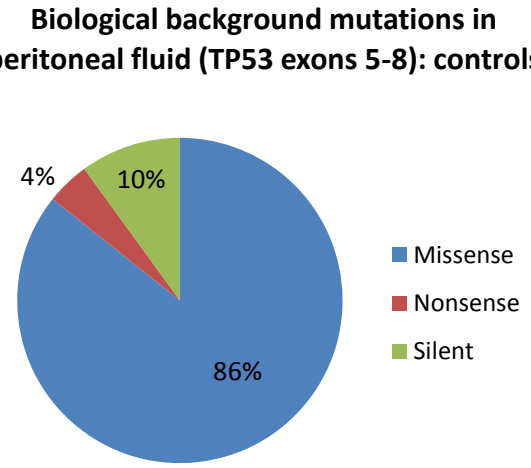

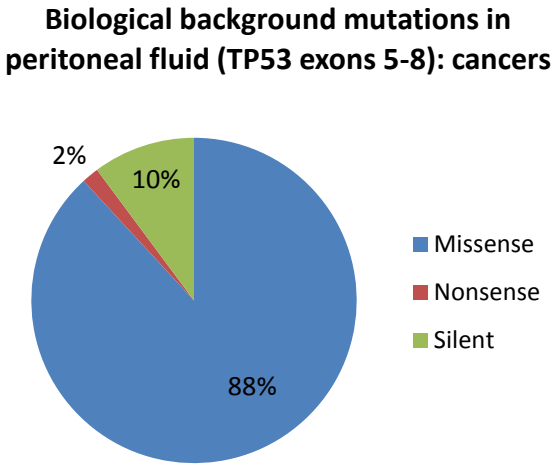

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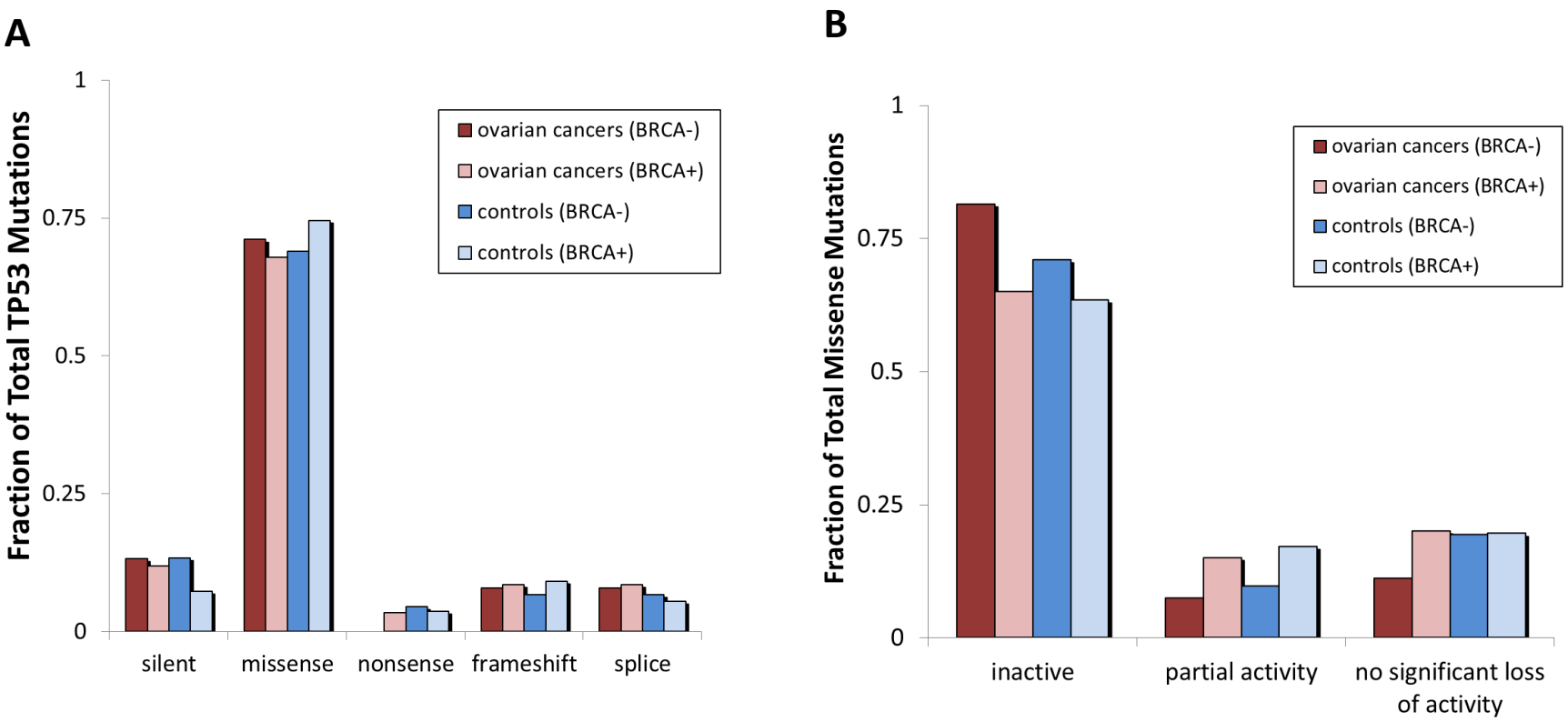

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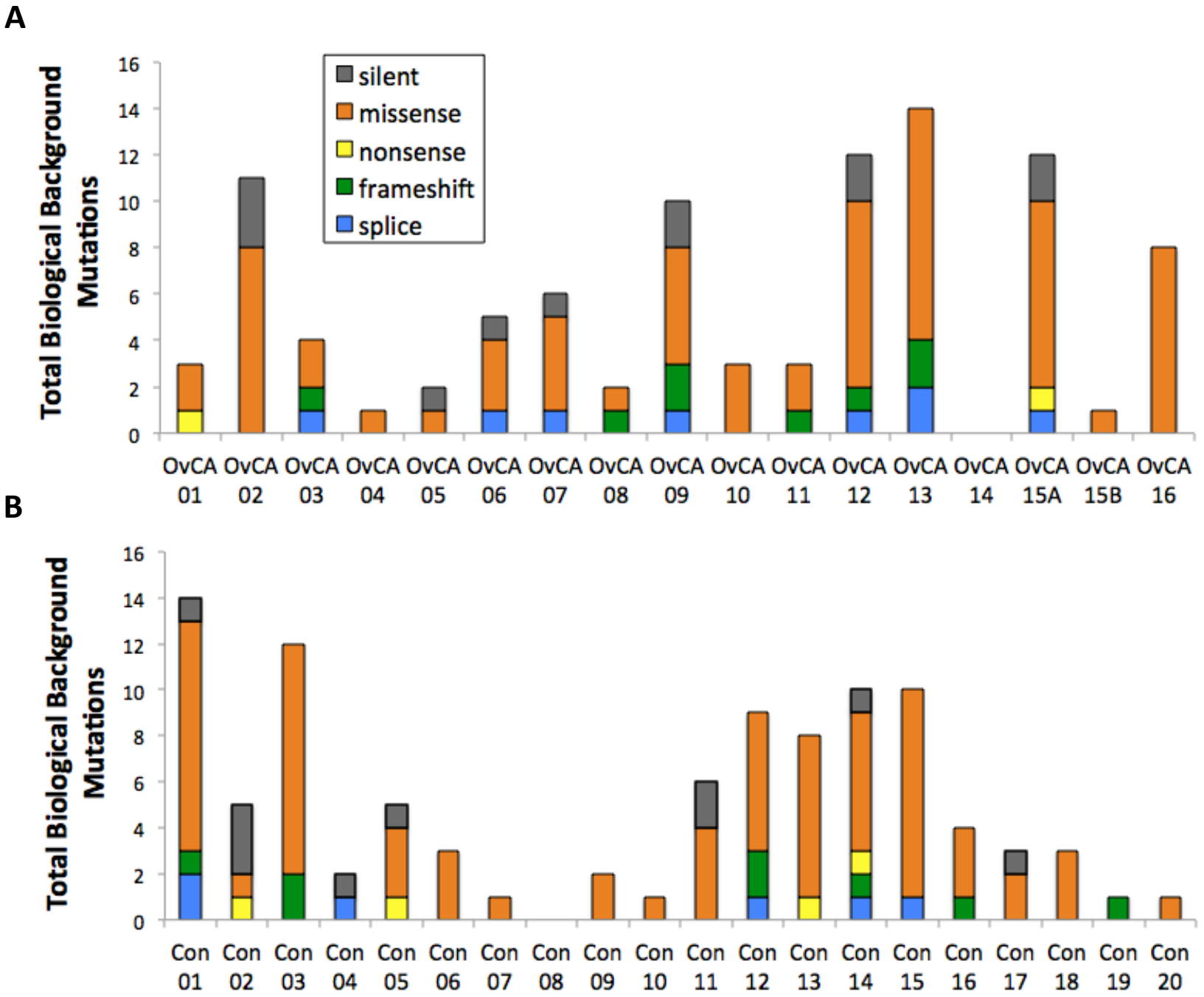

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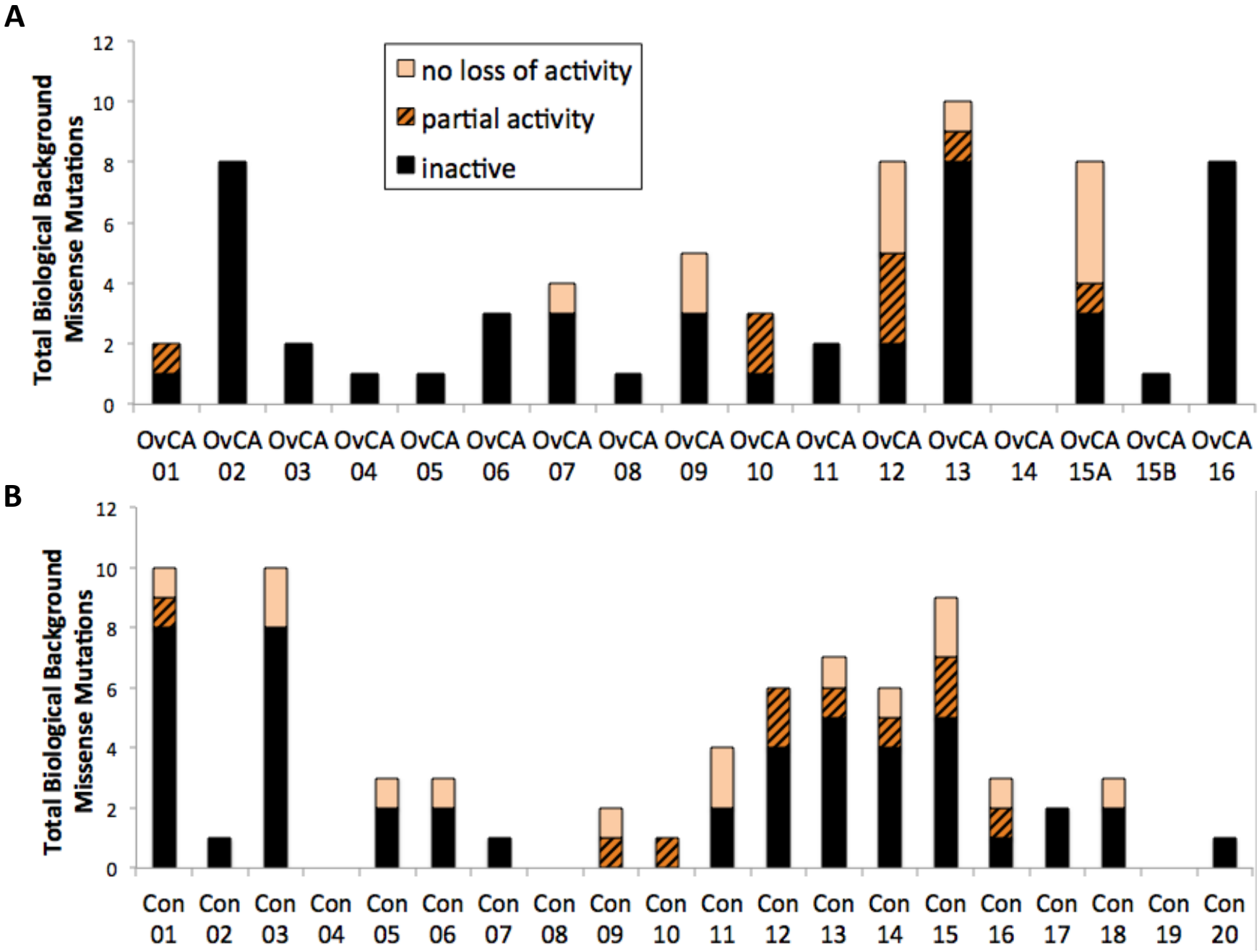

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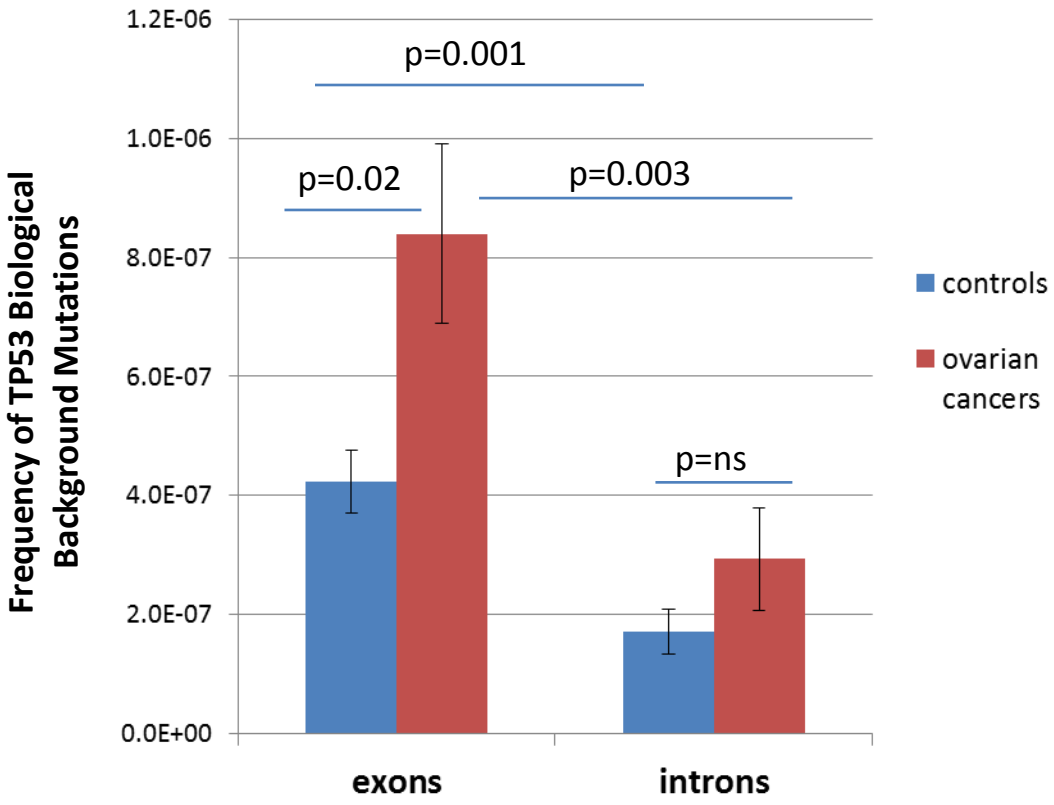

SI Appendix, Fig. S9