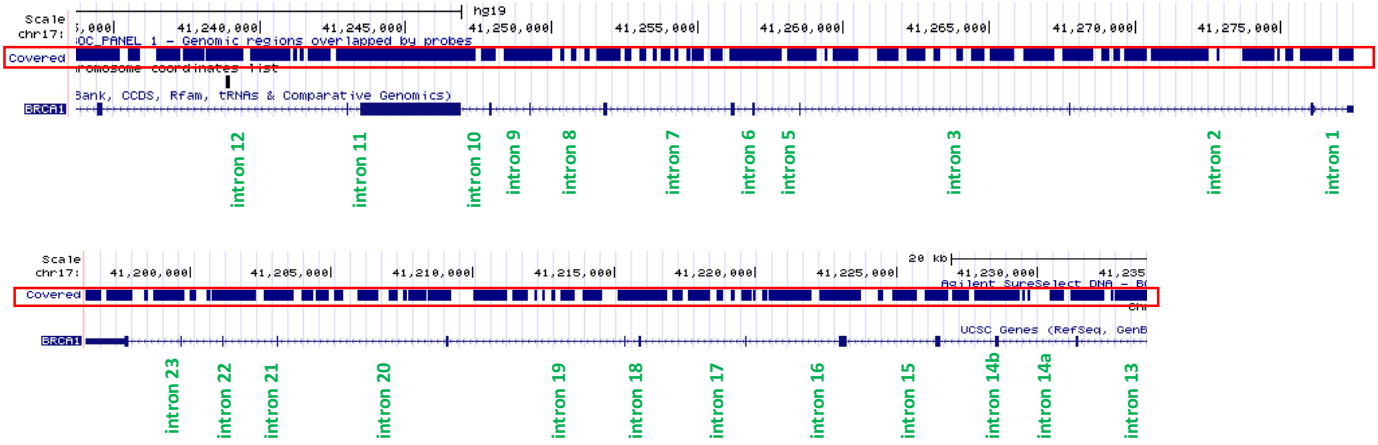
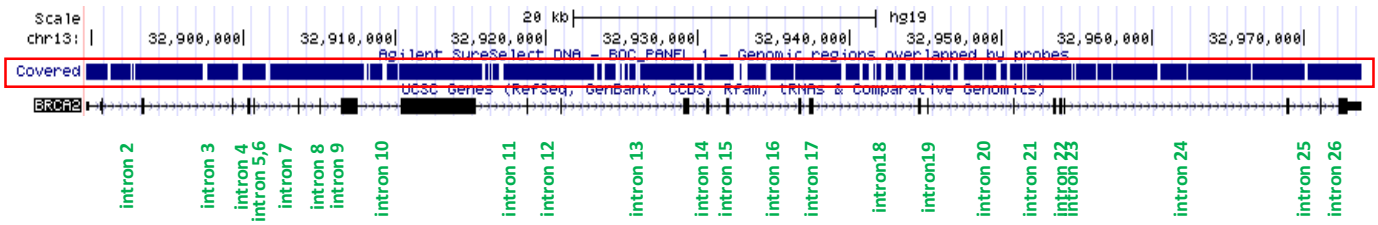
BRCA1

GRCh37/hg19

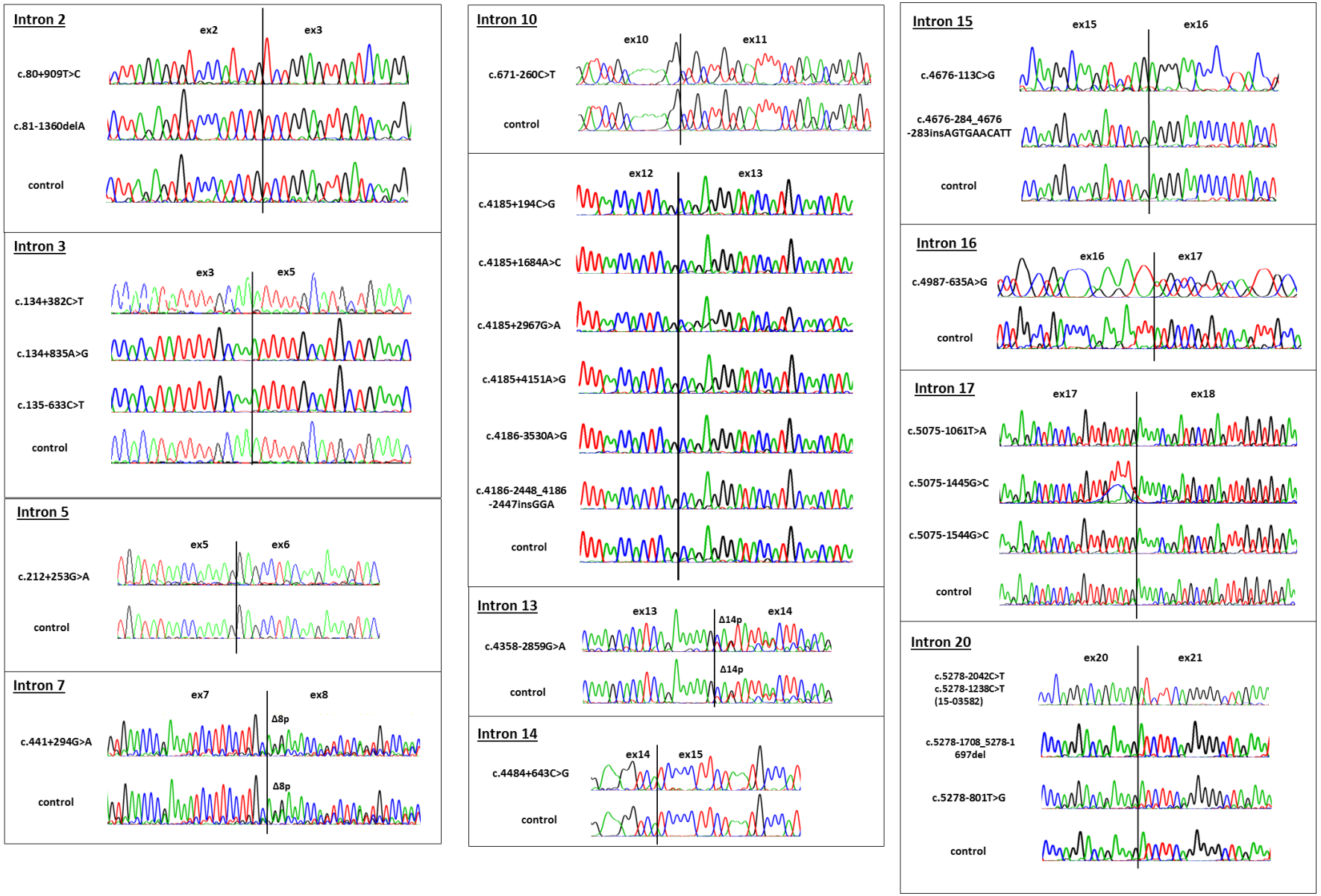


BRCA2

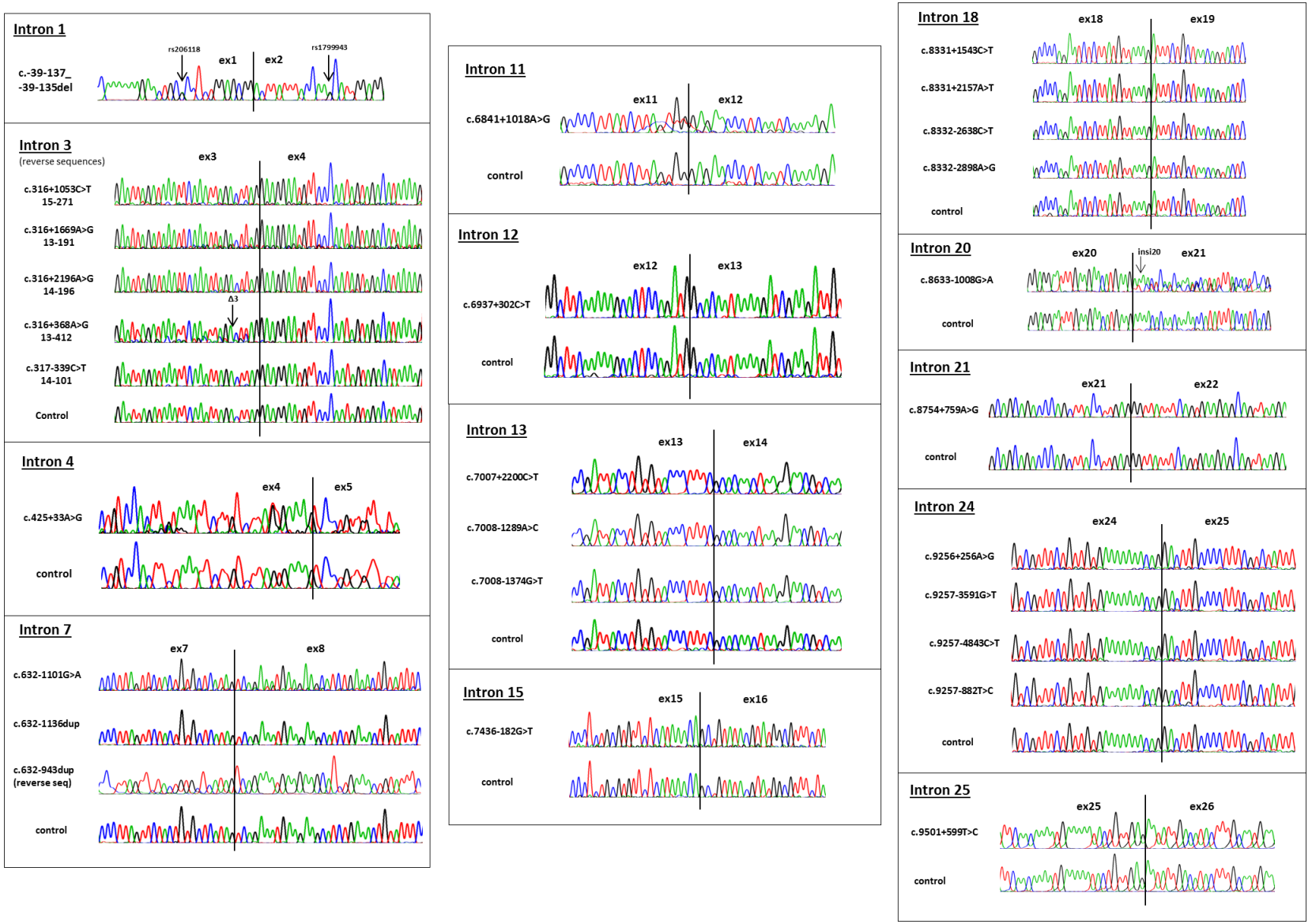
GRCh37/hg19



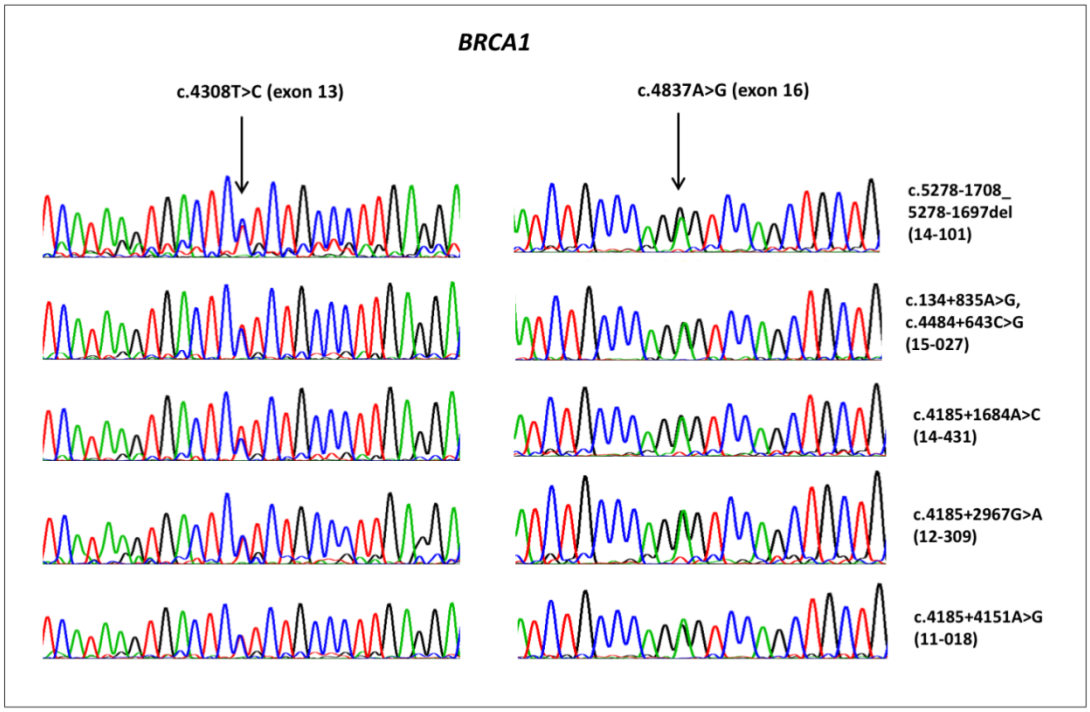
Supplementary Figure 1. Probe coverage over *BRCA1/2* genomic regions displayed using UCSC Browser. Region marked in red corresponds to a UCSC custom track showing a "dense" visualization of region captured by our probe design (BED files). Regions lacking coverage correspond to repetitive sequences or regions unsuitable for probe hybridization.

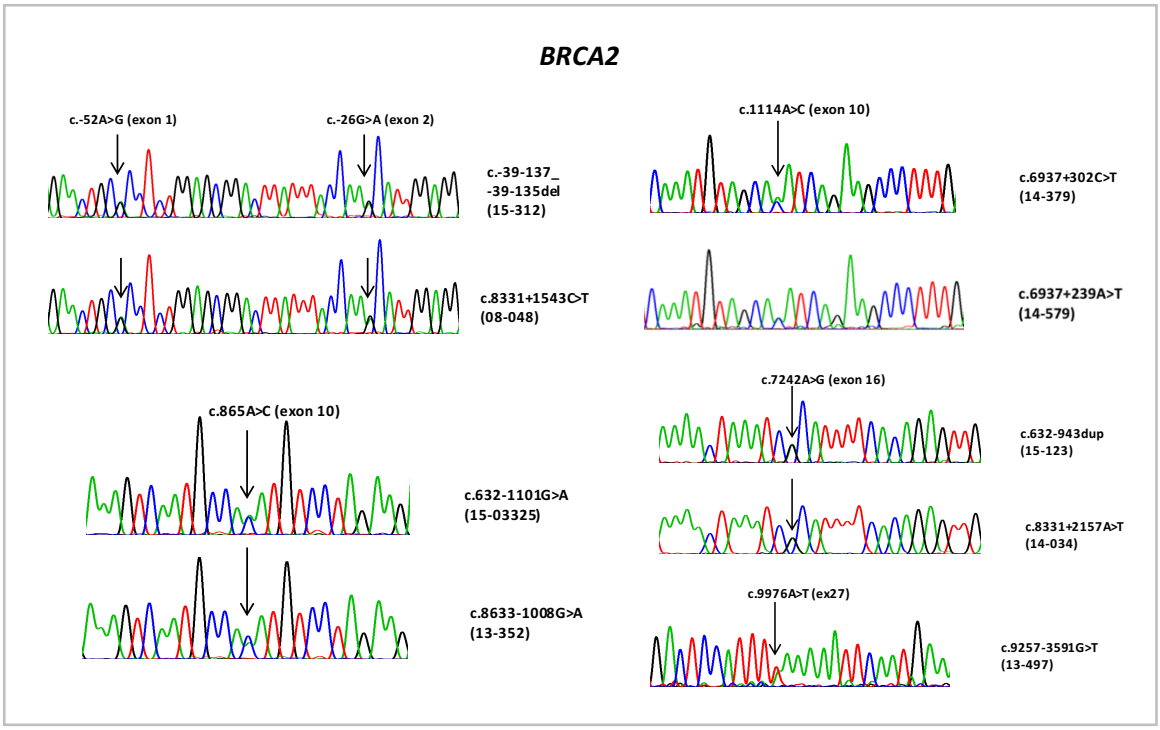


Supplementary Figure 2. Sanger electropherograms from *BRCA1* deep intronic variants characterization in patient RNA. Variants and one control for each sequence fragment are shown. Sequences covered exons adjacent to intron containing the variant. Full-length transcript was observed in all cases and no aberrant transcripts were detected. *BRCA1* alternative isoforms Δ8p and Δ14p were detected in carriers and controls when analyzing fragments involving exon 8 and exon 14, respectively.

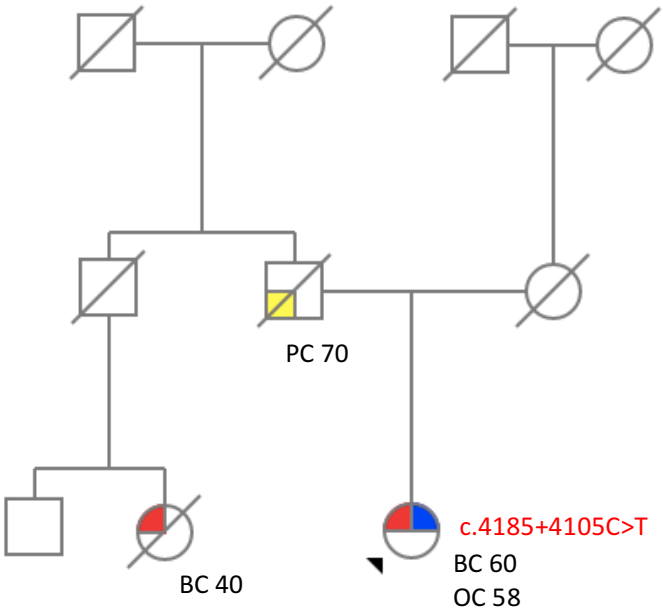


Supplementary Figure 3. Sanger electropherograms from *BRCA2* deep intronic variants characterization in patient RNA. Sanger from variants and one control for each fragment are shown. Sequences covered exons adjacent to intron containing the variant. Full-length transcript was observed in all cases and no aberrant transcripts were detected. *BRCA2* alternative isoforms Δ3 and ▼20A were detected when analyzing fragments involving exon 3 and exon 20, respectively.





Supplementary Figure 4. Representative examples of *BRCA1/2* allelic imbalance assessment by Sanger sequencing. Long RT-PCRs were designed to cover *BRCA1* and *BRCA2* exons containing informative exonic variants (marked with arrows), and Sanger sequencing was performed using internal primers.



Supplementary Figure 5. Family pedigree from *BRCA1* c.4185+4105C>T carrier. Family originally from Lleida (Catalonia). Proband (arrow head) was diagnosed with ovarian cancer at age 58 and with an infiltrating ductal breast carcinoma (BC) at age 60. A paternal cousin was also diagnosed with BC at age 40.

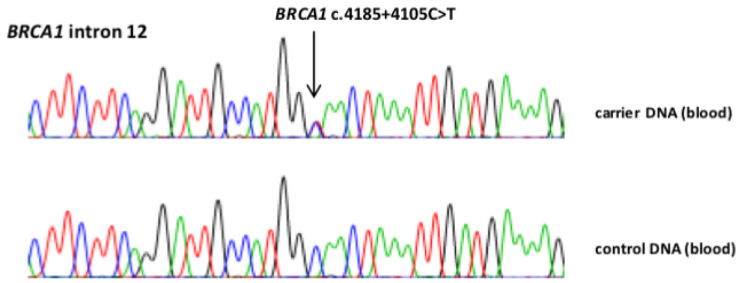
***BRCA1* c.80+909T>C**

***BRCA1* c.4185+4105C>T**

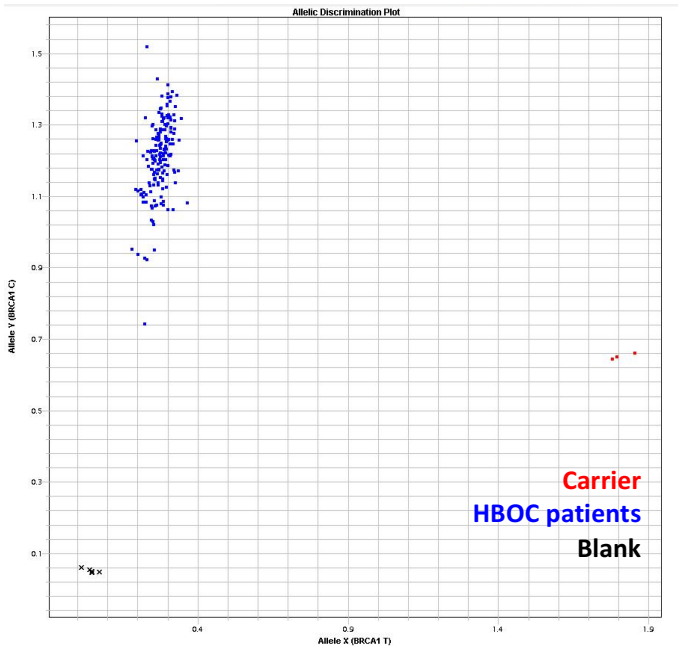
	***BRCA1* c.80+909T>C**		***BRCA1* c.4185+4105C>T**	
***In silico* tools**	**WT sequence**	**VAR sequence**	**WT sequence**	**VAR sequence**
SpliceSiteFinder-like [0-100]	75.6	79.7	75.9	78.5
MaxEntScan [0-12]	4.0	6.5	-	6.1
NNSPLICE [0-1]	0.8	1.0	-	0.9
GeneSplicer [0-24]	0.6	3.5	-	1.5
Human Splicing Finder [0-100]	85.9	87.9	-	82.3

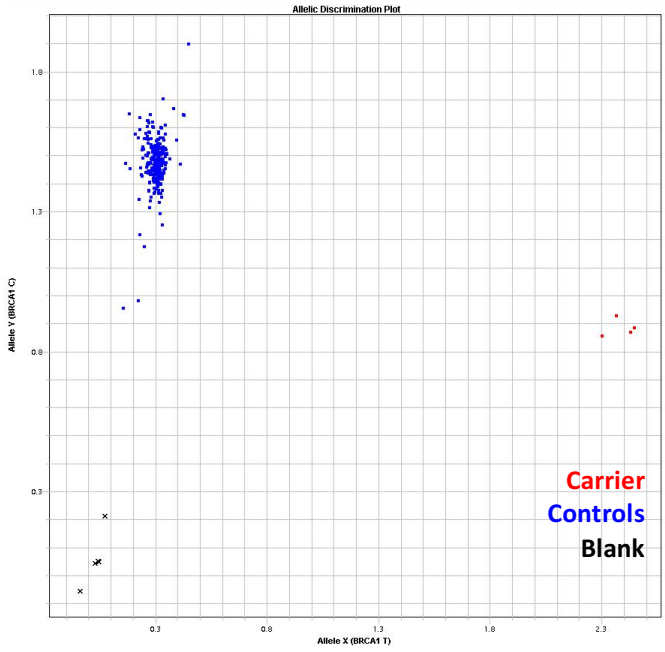
Supplementary Figure 6. *In silico* splicing analysis of *BRCA1* c.80+909T>C and c.4185+4105C>T variants. Predictions from wild-type sequences (WT) and variant sequences (VAR) were obtained from 5 tools (SSF-like, MES, NNSPLICE, GeneSplicer and HSF) using Alamut software v2.10. Score ranges from each tool are detailed between brackets. *BRCA1* c.80+909T>C variant increases the strength of a preexisting donor site, whereas *BRCA1* c.4185+4105C>T variant is predicted to activate a cryptic donor site (red circle) which is recognized by the splicing machinery together with a preexisting acceptor site located 114nt upstream (black circle).

A.

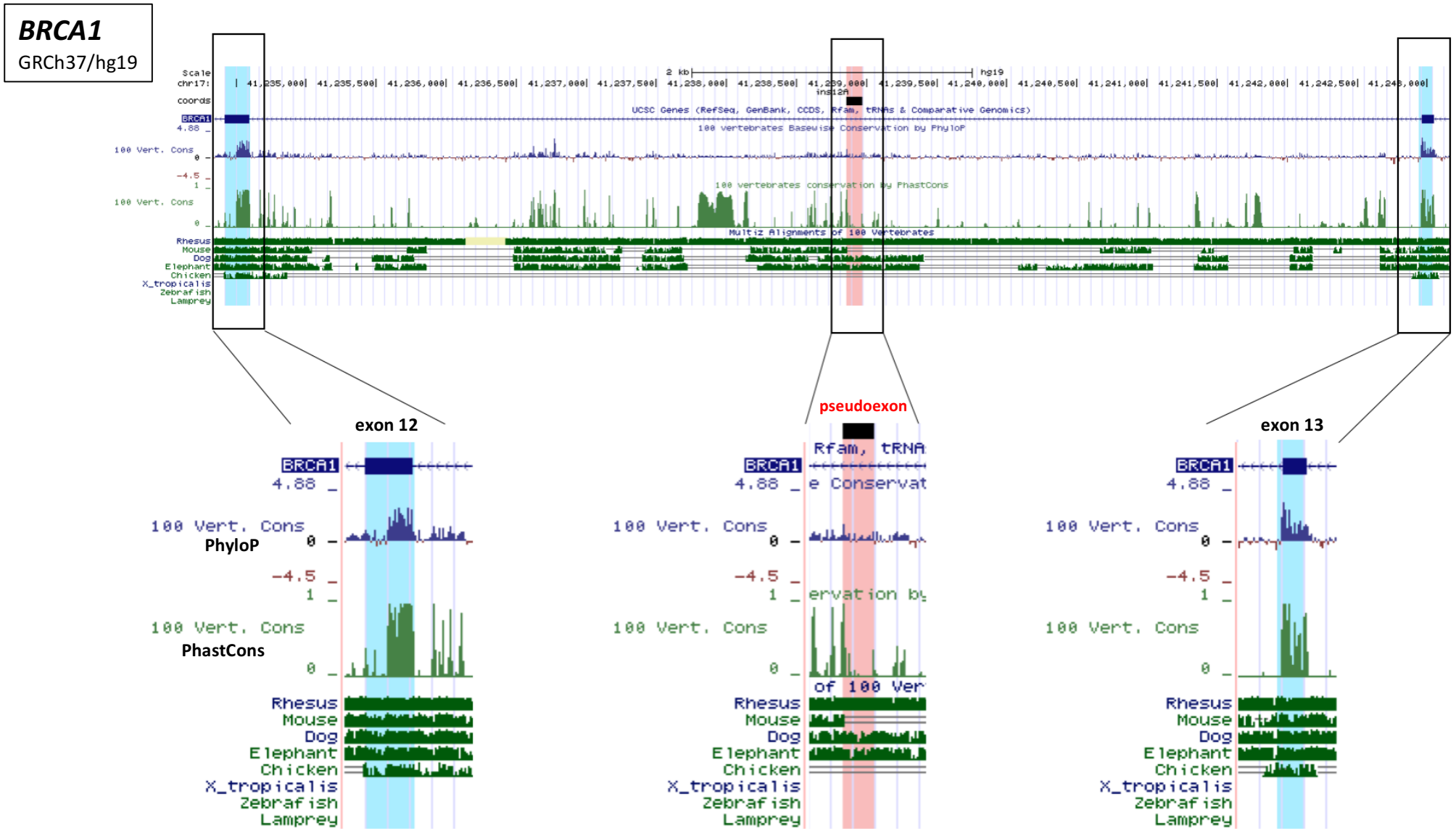


B.



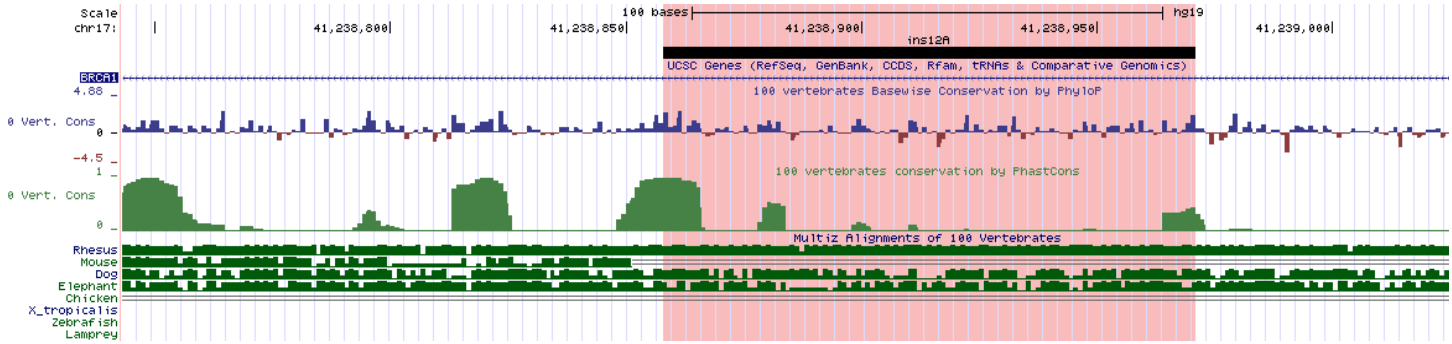


Supplementary Figure 7. Sanger confirmation of *BRCA1* c.4185+4105C>T (A) and Genotyping of Spanish HBOC patients and controls (B). A total of 1,030 HBOC patients with uninformative *BRCA1/2* results and 327 Spanish controls were genotyped at *BRCA1* c.4185+4105C>T. Allelic discrimination plots are shown. Variant carrier identified in this study (in red) was used as positive control.

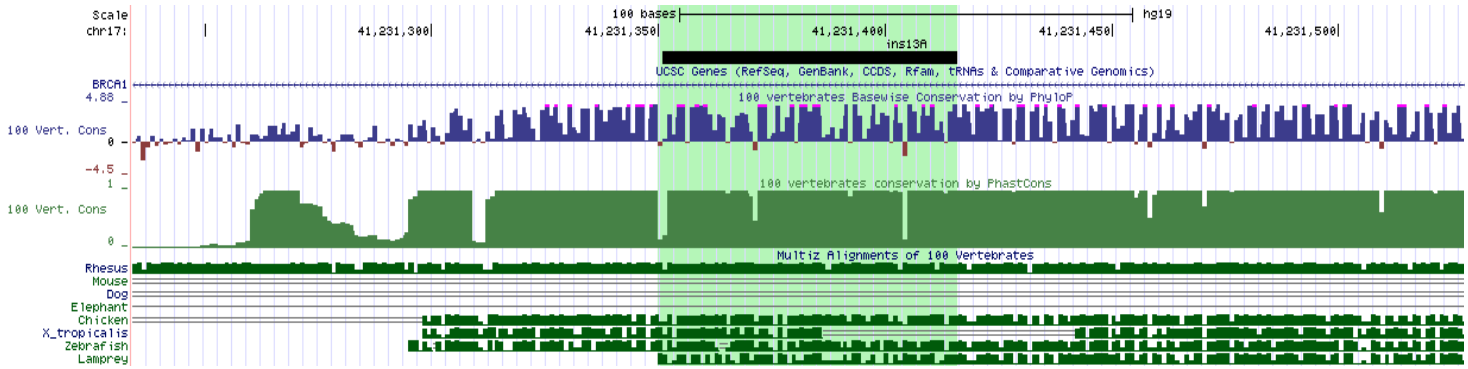


Supplementary Figure 8. Nucleotide conservation comparison between *BRCA1* pseudoexon (in red) and functional exons 12 and 13 (in blue). Sequences were compared across 100 vertebrate species using PhyloP, PhastCons and Multiz Alignment tools. Genome assembly GRCh37/hg19 was used and data was visualized in UCSC genome browser (https://genome.ucsc.edu/).

***BRCA1* ▼12A** (r.4185_4186ins4185+3990_4185+4103) (114nt)
chr17:41,238,971-41,238,858



***BRCA1* ▼13A** (r.4357_4358ins4358-2785_4358-2719) (66nt)
chr: 41,231,416 - 41,231,351



Supplementary Figure 9. Conservation analysis of pseudoexons included in the aberrant transcript *BRCA1*▼12A (in red) and the alternative isoform *BRCA1*▼13A (in green), respectively. Sequences were compared across 100 vertebrate species using PhyloP, PhastCons, and Multiz Alignment tools. Genome assembly GRCh37/hg19 was used and data was visualized in UCSC genome browser (https://genome.ucsc.edu/).