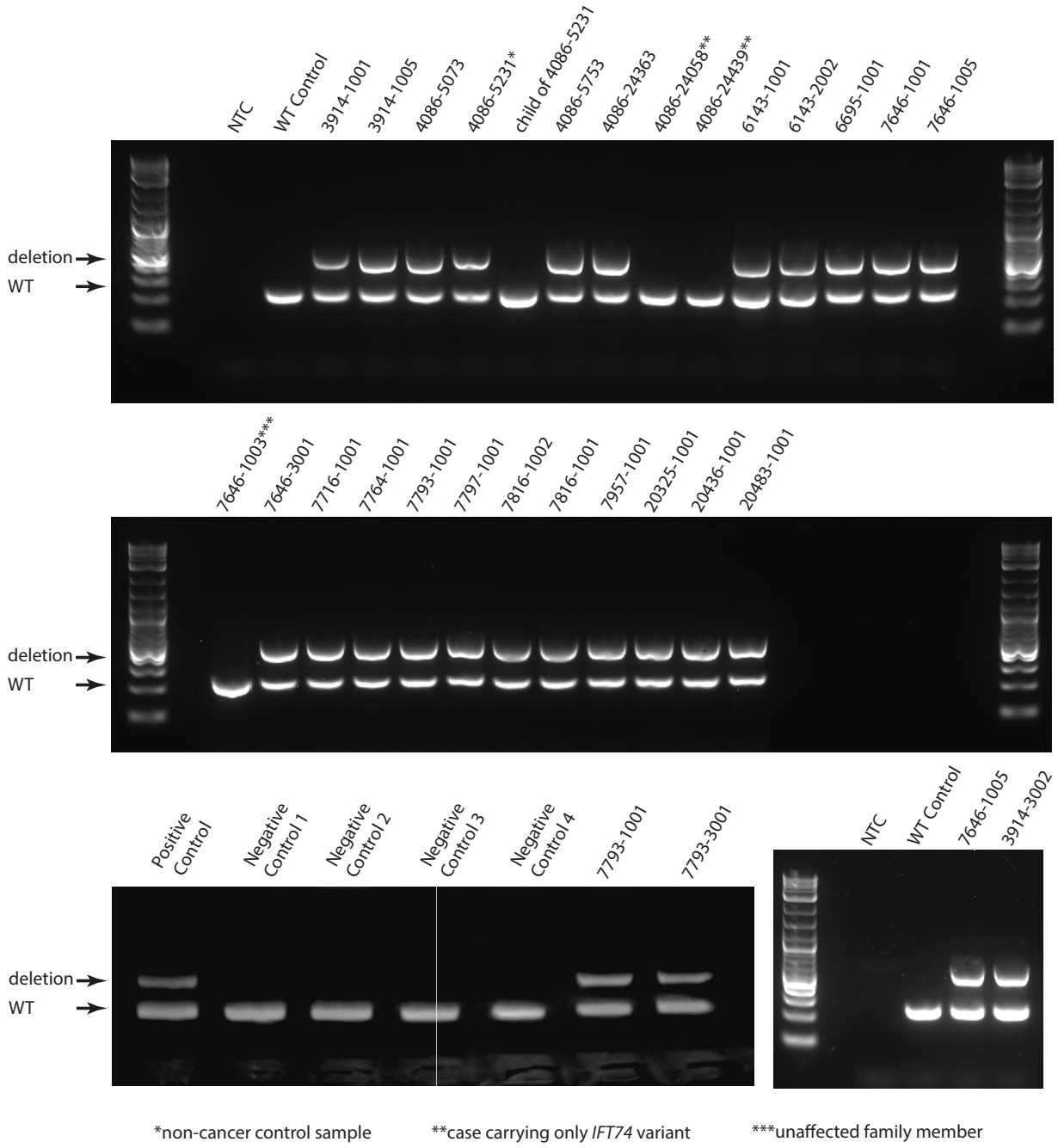
Supplementary Fig. 1: Genotyping of the 9p21 deletion using a multiplexed PCR assay

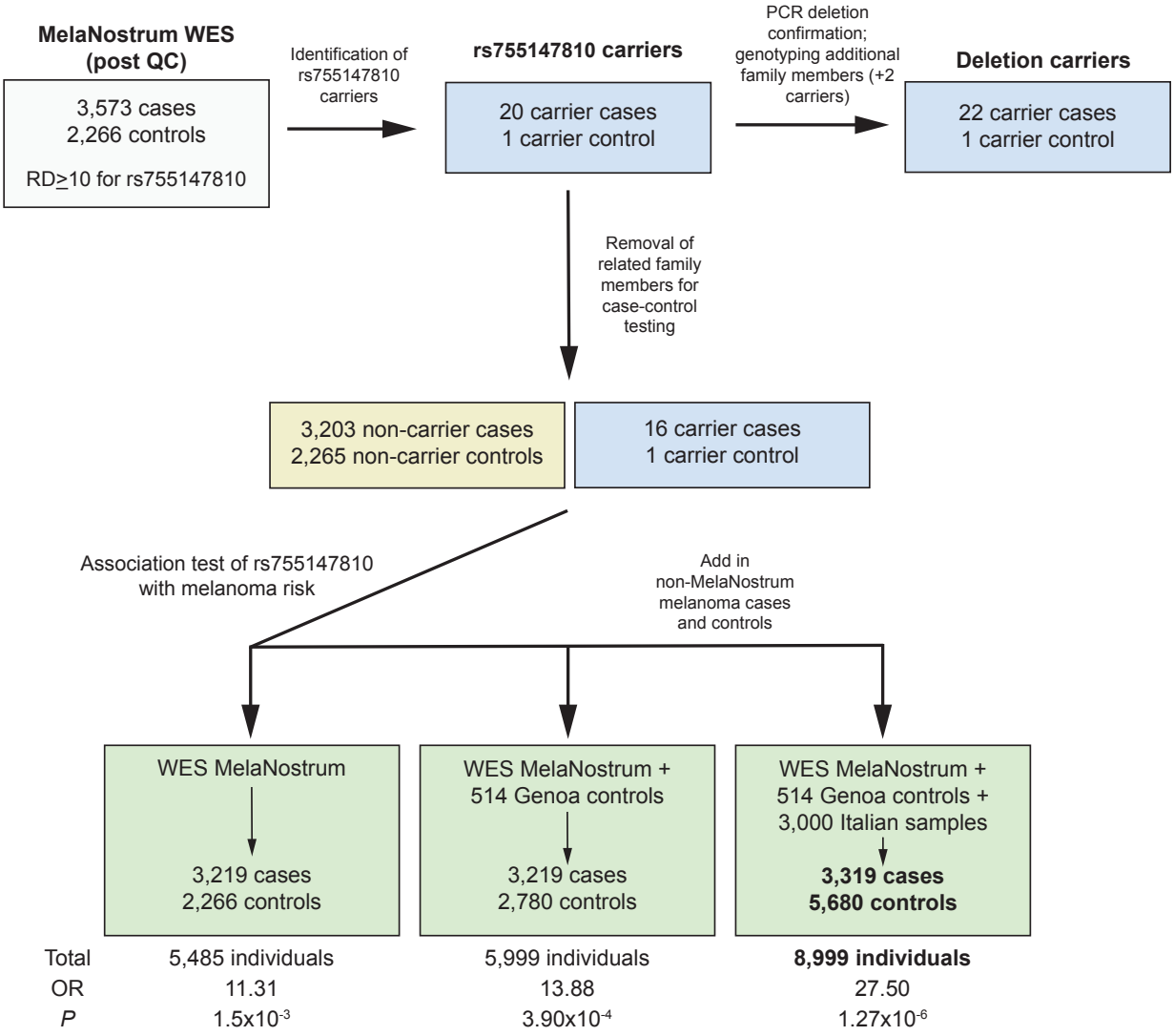


*non-cancer control sample **case carrying only *IFT74* variant ***unaffected family member

Multiplexed PCR assay was performed on genomic DNA of the samples from Cesena and Genoa, Italy. The upper band (593 bp) indicates the presence of the 9p21 deletion, while lower band (284 bp) indicates the presence of the wild-type (WT) allele. Genomic DNA from the C283T (melanocyte) cell line was used as WT control. NTC: no template

control. The deletion is present in all samples carrying the *MTAP* rs755147810 variant, but not in the two samples carrying only the *IFT74* rs375317034 variant.

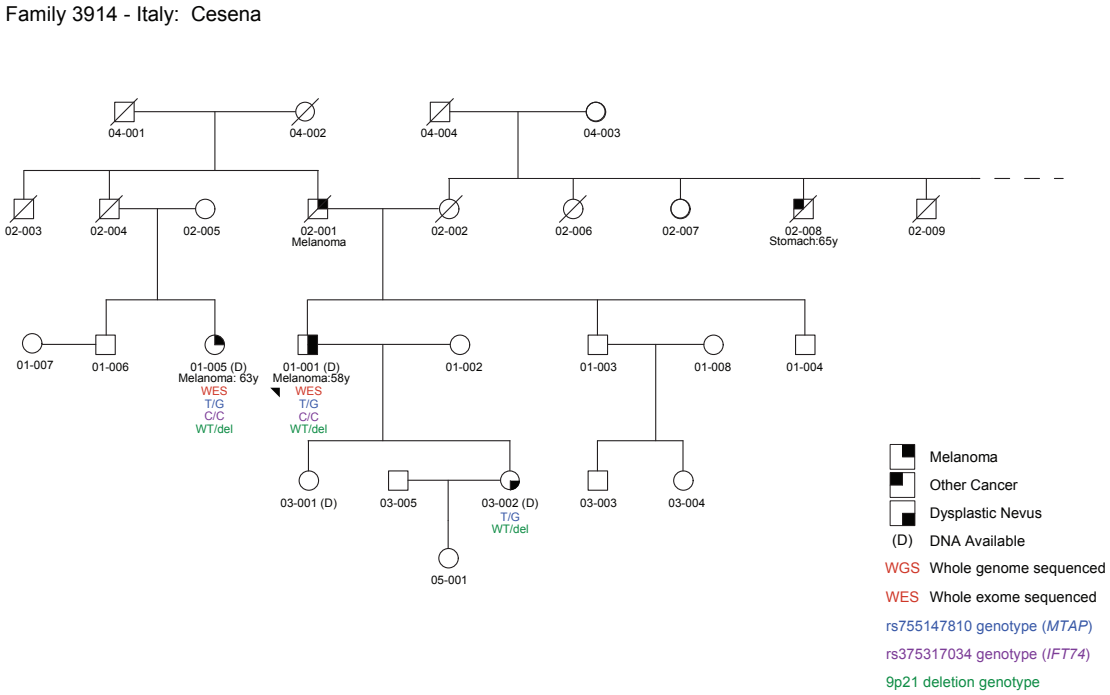
Supplementary Fig. 2: Flowchart summary of samples used for identification of additional carriers and association analysis



We identified deletion carriers in MelaNostrum WES data using rs755147810 in *MTAP* as a proxy for the deletion. We subsequently confirmed the deletion in rs755147810 carriers using a PCR-based assay, as well as genotyped additional members of families carrying the deletion (2 additional carriers). In order to facilitate case-control testing, we subsequently removed related individuals from the WES dataset, retaining only a single member per multi-case family. We then tested association of *MTAP* variant rs755147810 with melanoma risk in the Melanostrum WES dataset, as well as in larger datasets adding in additional Italian case and control samples. WES: Whole-exome sequencing; QC: quality control; RD: read depth; OR: odds ratio; *P*: Fisher test *P* values.

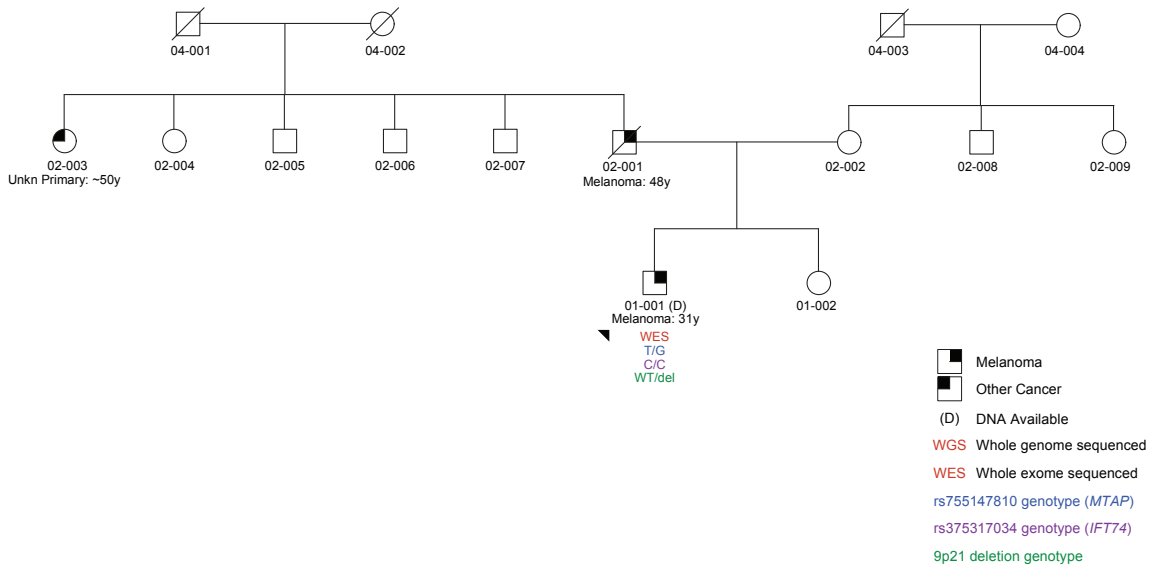
Supplementary Fig. 3: Pedigrees of 12 families carrying the deletion

a



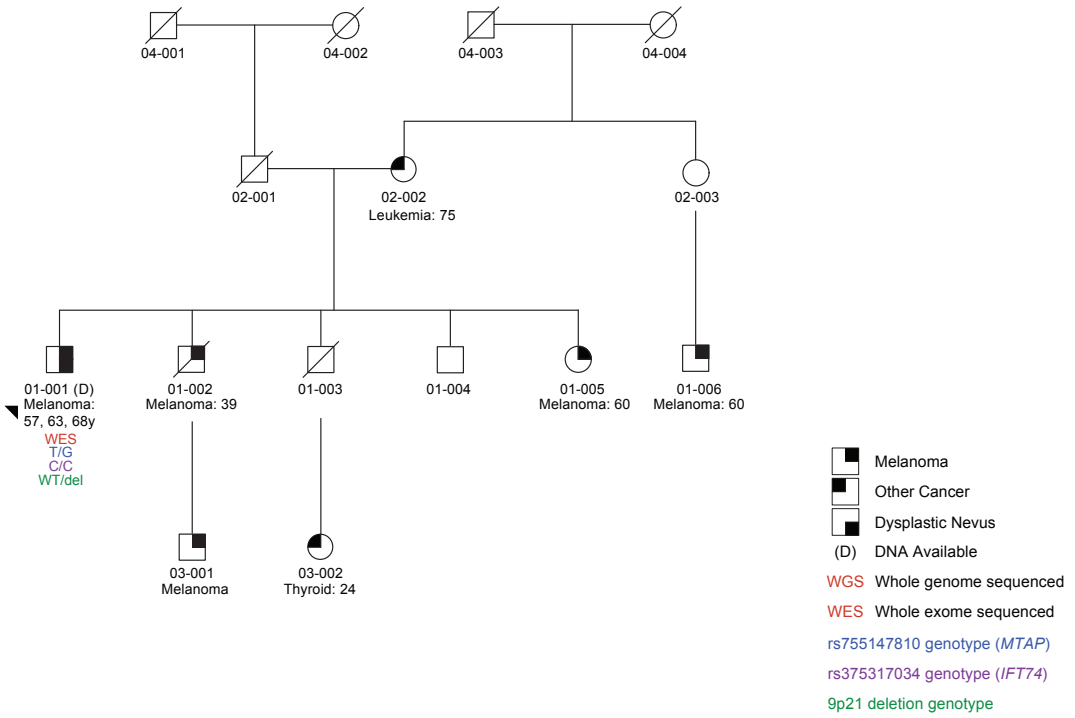
b

Family 7716 - Italy: Genoa



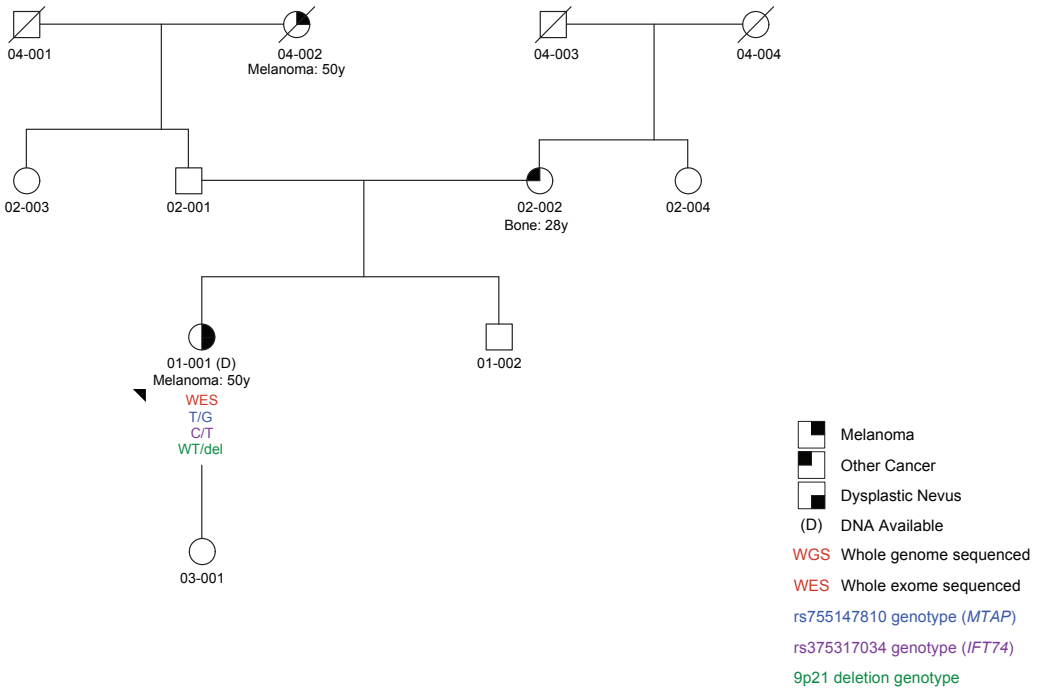
c

Family 20325 - Italy: Genoa



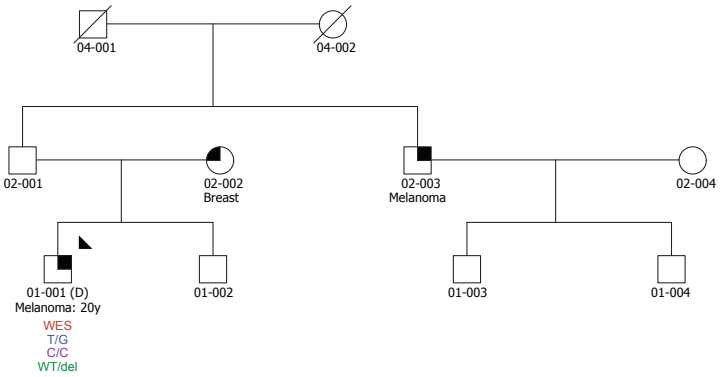
d

Family 7764 - Italy: Genoa



e

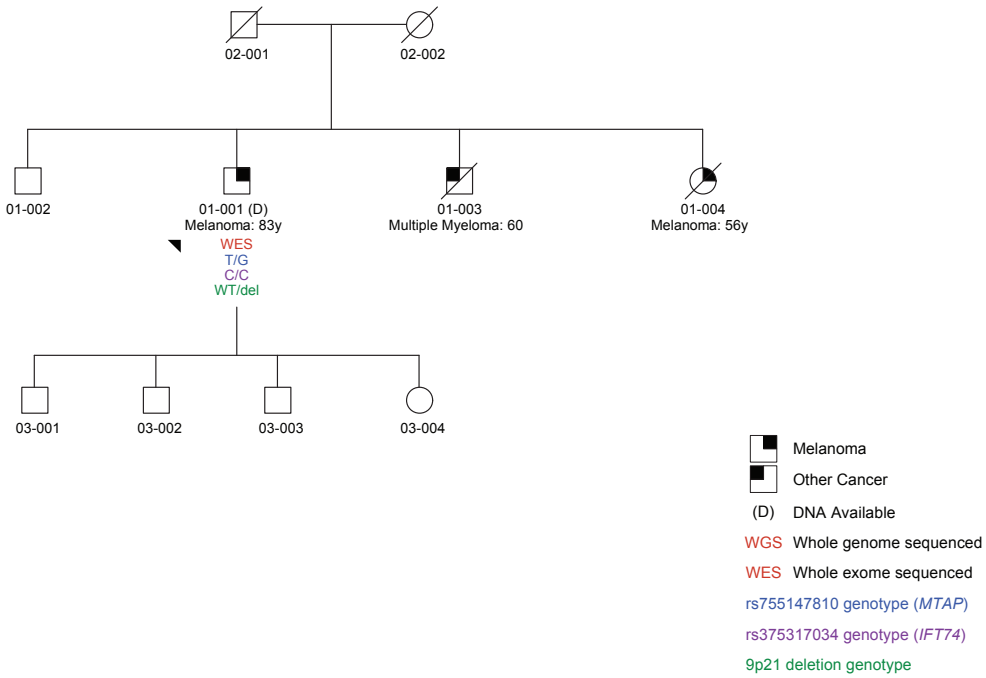
Family 6695 - Italy: Genoa



Melanoma
Other Cancer
(D) DNA Available
WGS Whole genome sequenced
WES Whole exome sequenced
rs755147810 genotype (*MTAP*)
rs375317034 genotype (*IFT74*)
9p21 deletion genotype

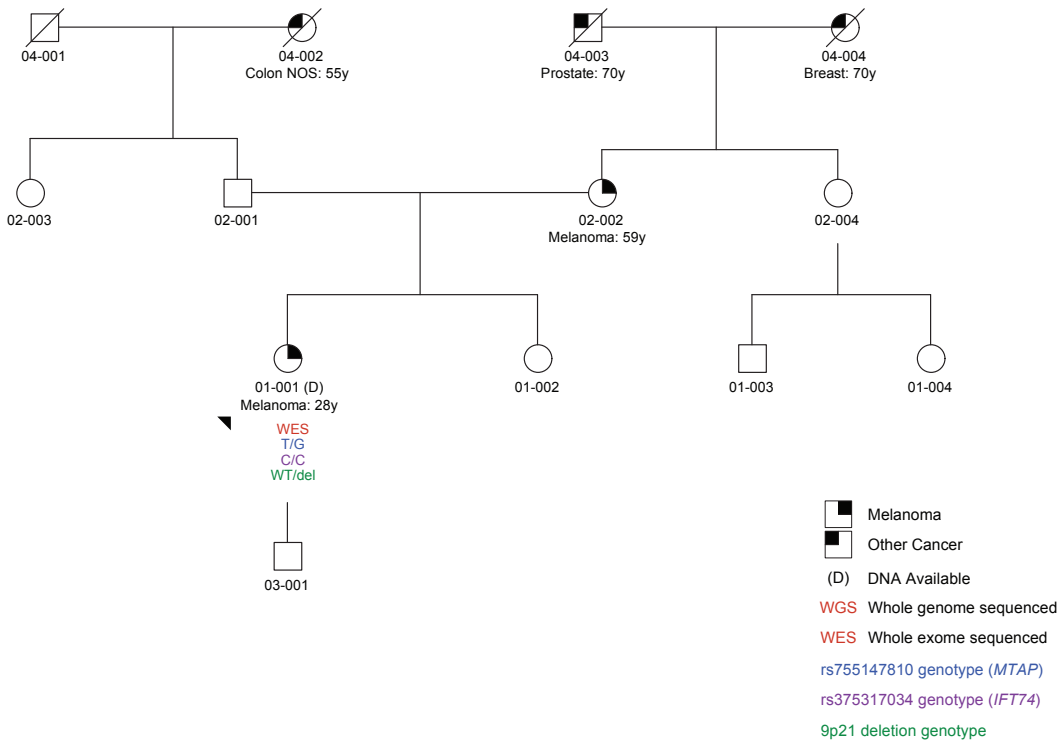
f

Family 7797 - Italy: Genoa



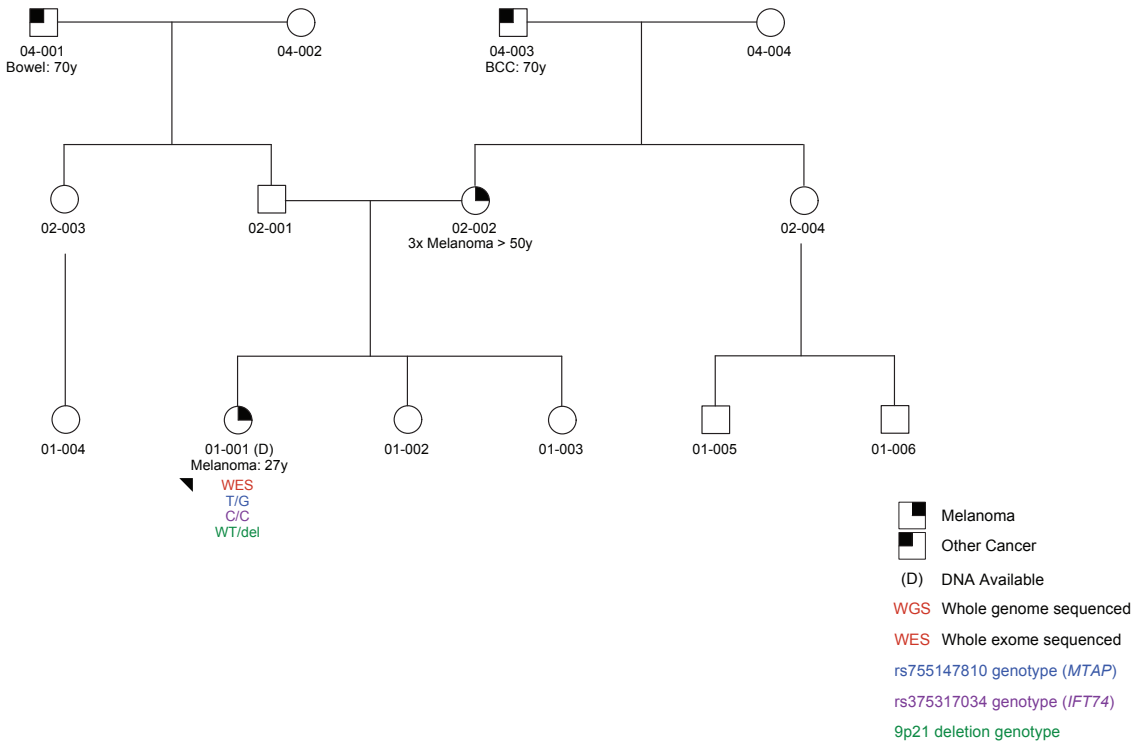
g

Family 7957 - Italy: Genoa



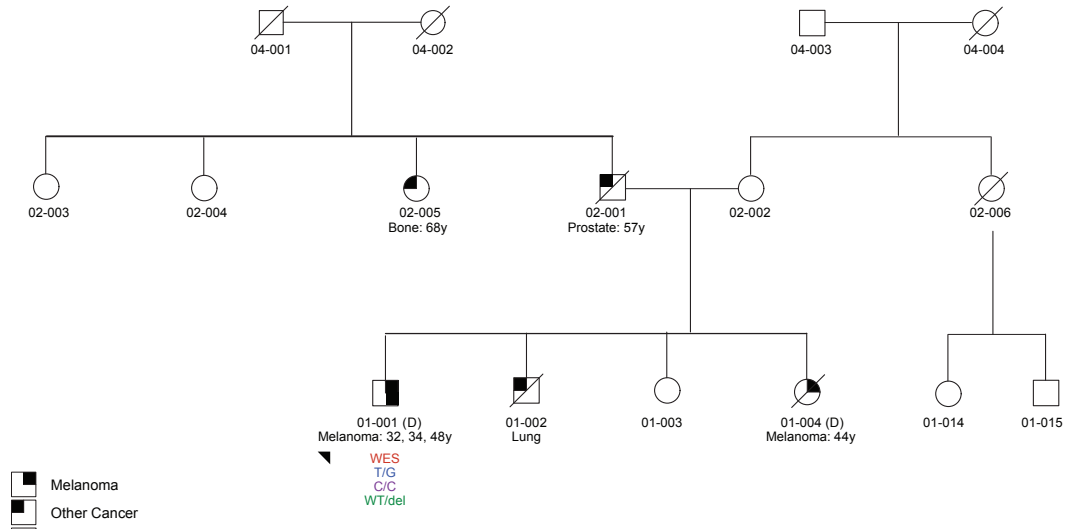
h

Family 20436 - Italy: Genoa



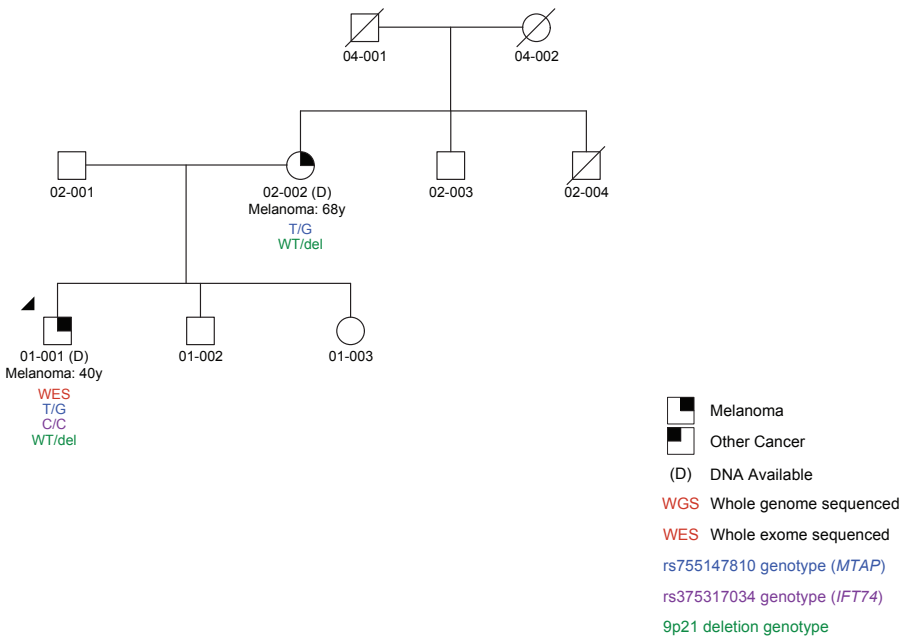
i

Family 20483 - Italy: Genoa



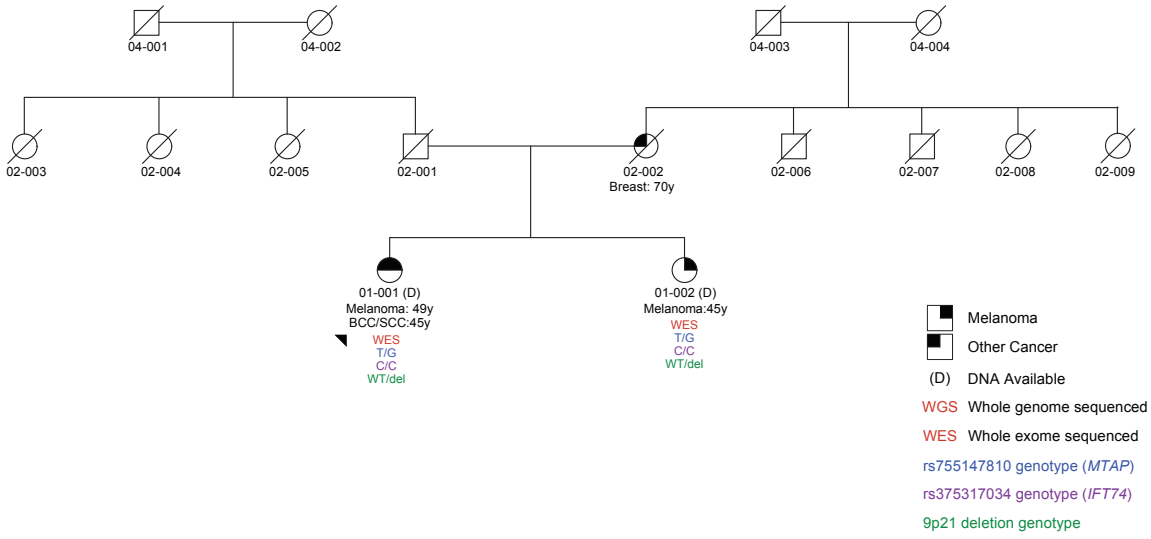
j

Family 6143 - Italy: Genoa

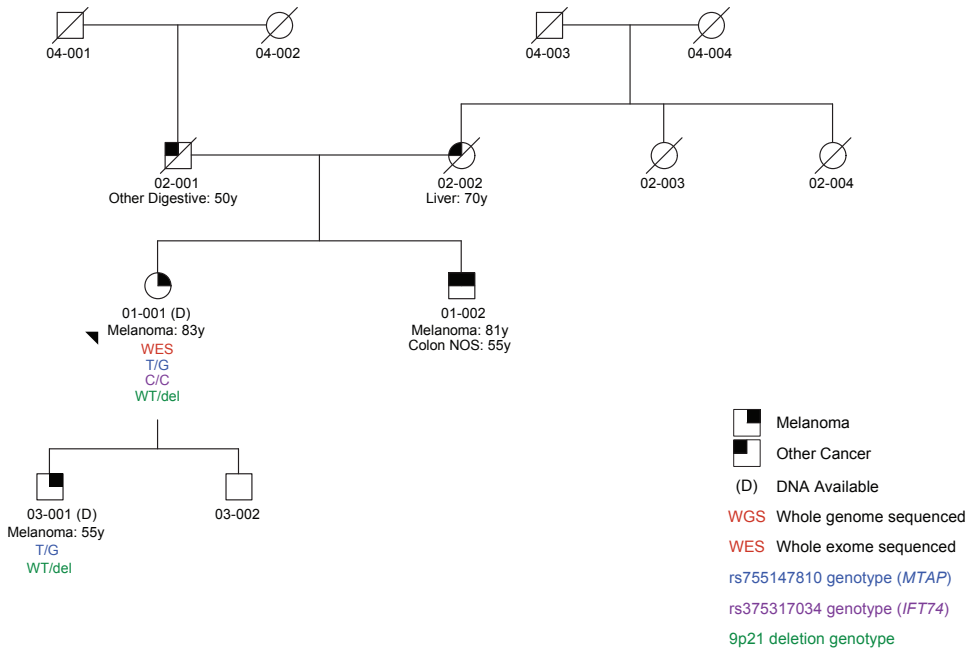


k

Family 7816 - Italy: Genoa



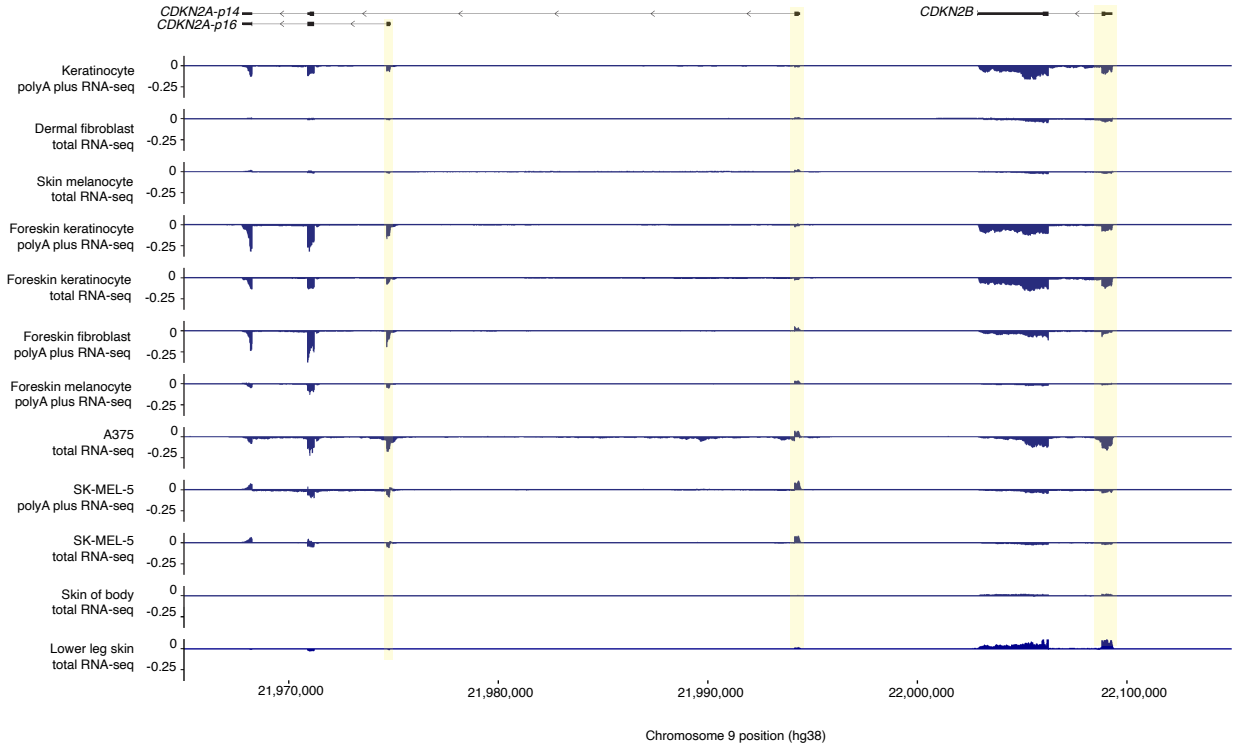
l



a, Pedigree of a three-case melanoma family from Cesena, Italy (Family 3914). **b**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 7716). **c**, Pedigree of a five-case melanoma family from Genoa, Italy (Family 20325). **d**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 7764). **e**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 6695). **f**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 7797). **g**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 7957). **h**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 20436). **i**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 20483). **j**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 6143). **k**, Pedigree of a two-case melanoma family from Genoa, Italy (Family 7816). **l**, Pedigree of a three-case melanoma family from Genoa, Italy (Family 7793). For all pedigrees, DNA availability, diagnoses of melanoma, dysplastic nevi, and other cancers are noted along with age of onset for cancer cases. Cases with whole-genome sequencing (WGS) and whole-exome sequencing (WES) data, genotype for rare variants in *MTAP* (rs755147810) and *IFT74* (rs375317034) as well as a 100 kb non-coding deletion on chromosome band 9p21 (9p21 deletion) are noted. Four families

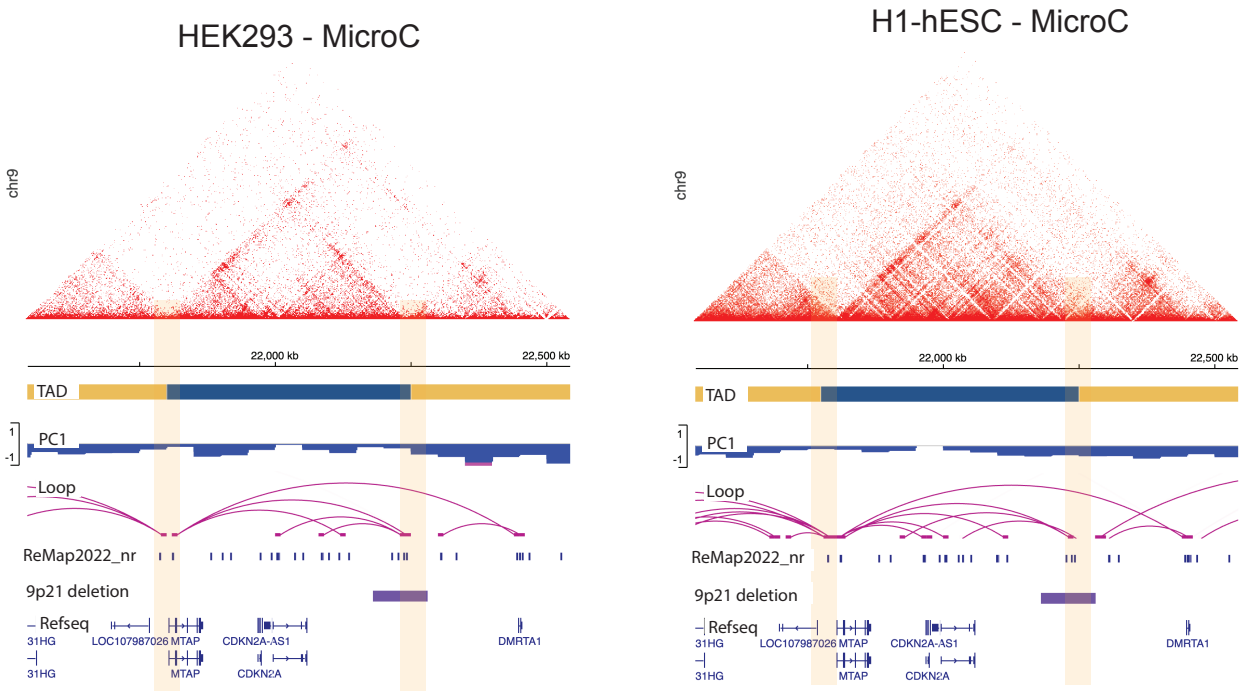
(Cesena pedigree 3914; Genoa pedigree 7793, 7816 and 6143) showed cosegregation of the 9p21 deletion and melanoma.

Supplementary Fig. 4: AlphaGenome predictions for effects of the 9p21 deletion on *CDKN2A* and *CDKN2B* transcription



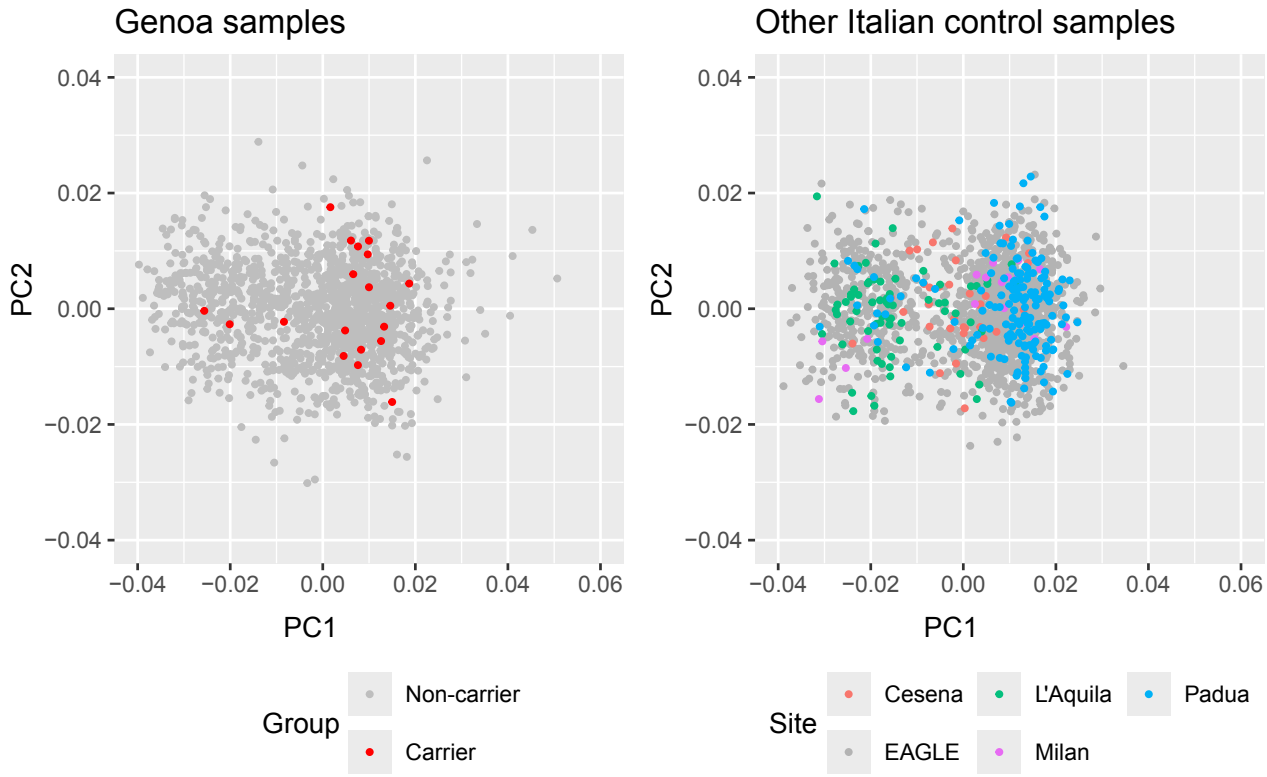
Predicted effects of the 9p21 deletion on *CDKN2A* and *CDKN2B* transcription using the deep learning model AlphaGenome. The 9p21 deletion is associated with reduced predicted RNA-seq signal in all three exons of the *CDKN2A* p16 transcript and *CDKN2B*, but not the exon unique to *CDKN2A* p14 (exon 1a), in many cell types and tissues, including two melanoma cell lines (A375 and SK-MEL-5). The y-axis represents the difference between predicted transcriptional coverage for the alternative and reference alleles (ALT – REF, *e.g.* reduced expression is reflected by negative values). Promoter regions for p16, p14, and *CDKN2B* are highlighted in yellow.

Supplementary Fig. 5: CTCF binding and HiC interactions across 9p21



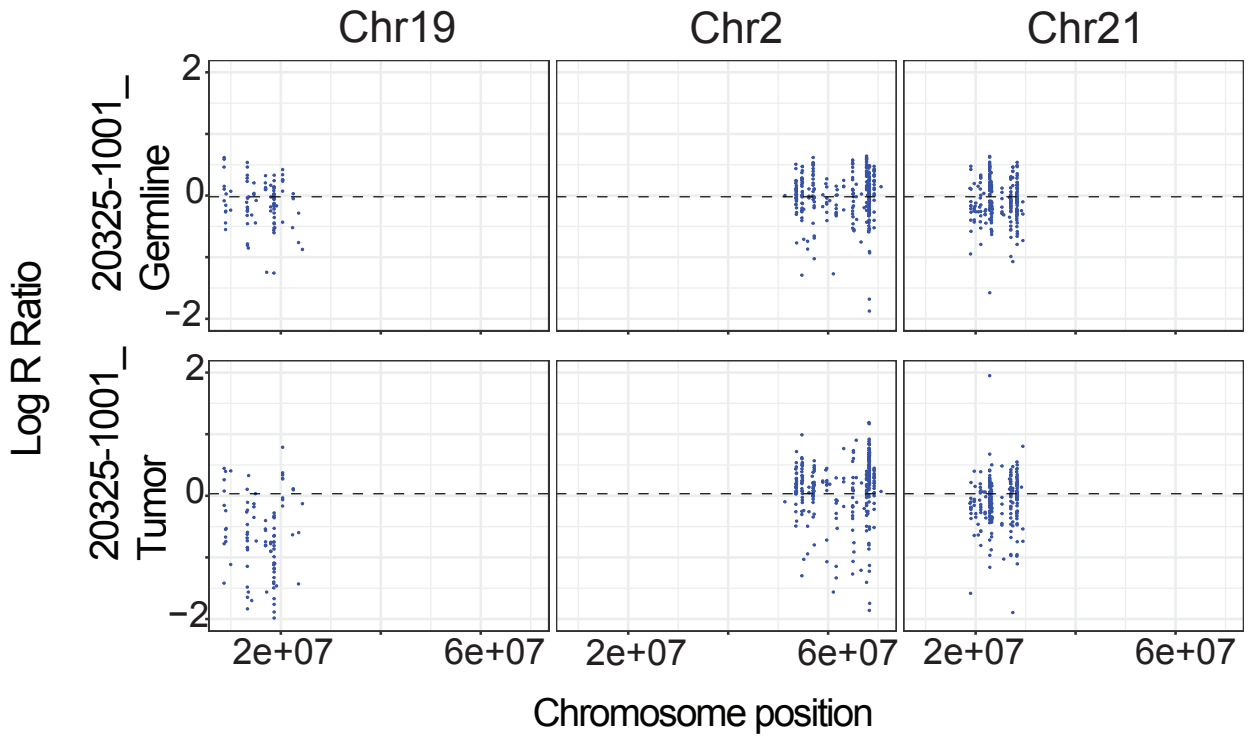
Three-dimensional (3D) organization of the 9p21 region showing the spatial organization of the 9p21 locus with two spanning topologically associating domains (TADs, highlighted in light orange) in the cell lines HEK293 and H1-hESC. Chromatin interactions within the region were displayed as a heatmap in triangle mode. PC1 displays principal components of A/B chromatin compartments, which indicates A (active) and B (inactive). Chromatin loops were derived from Capture Hi-C data. CTCF non-redundant (nr) peaks (ReMap2022_nr) were downloaded from ReMap2022 (PMID: 34751401) and filtered to only select peaks present in the corresponding cell line (HEK293 or hESC). All datasets, except the CTCF nr peak track and the 9p21 deletion annotation track, were provided in the 3D Genome Browser hosted by Northwestern University (https://3dgenome.fsm.northwestern.edu/).

Supplementary Fig. 6: Principal components analysis displays similarity in genetic components of Genoa samples and other Italian controls



Principal components analysis (PCA) was performed using imputed genotyping array data. PC values for principal components 1 and 2 (PC1 and PC2 display overlapping of samples originated from Genoa and other Italian control samples.

Supplementary Fig. 7: Log R Ratio (LRR) for SNPs in control regions of the tumor sequencing assay



Log R Ratio plot displays deletion in chr19 for 20325-01001_Tumor sample