Supplementary Material for: Powers et al "A rare Ashkenazi Jewish predominant *TP53* mutation confers risk of multiple cancers"

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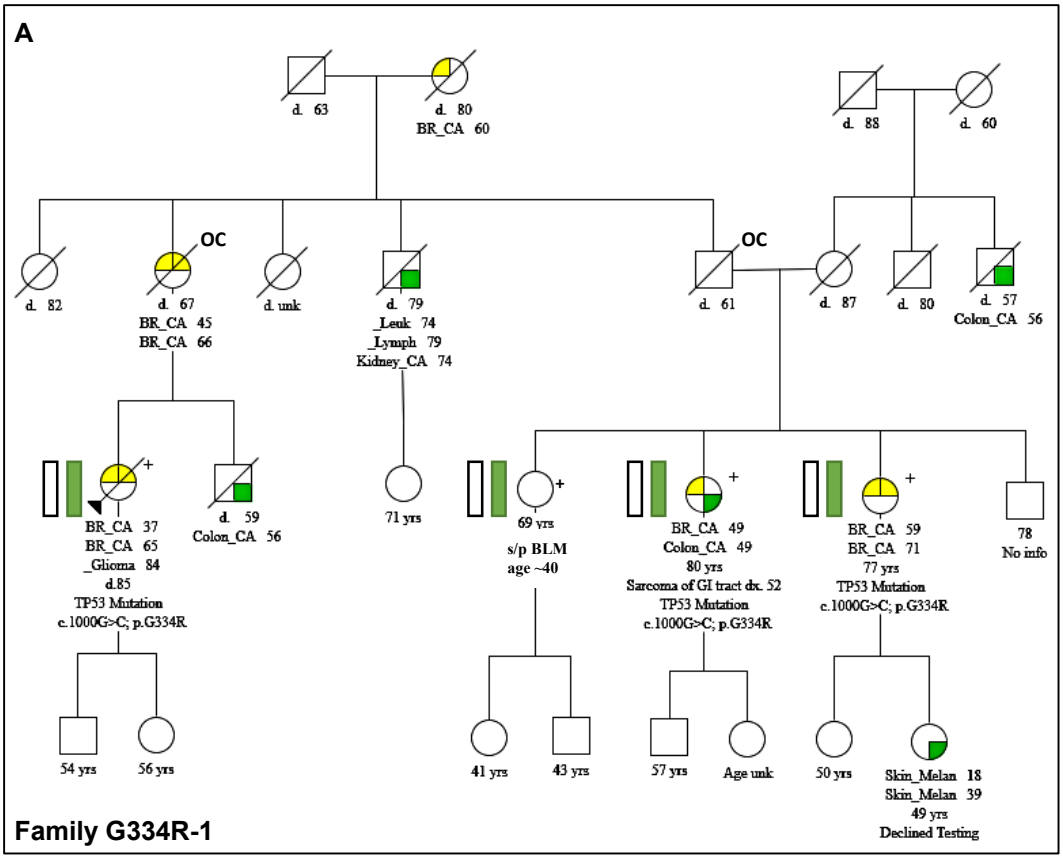
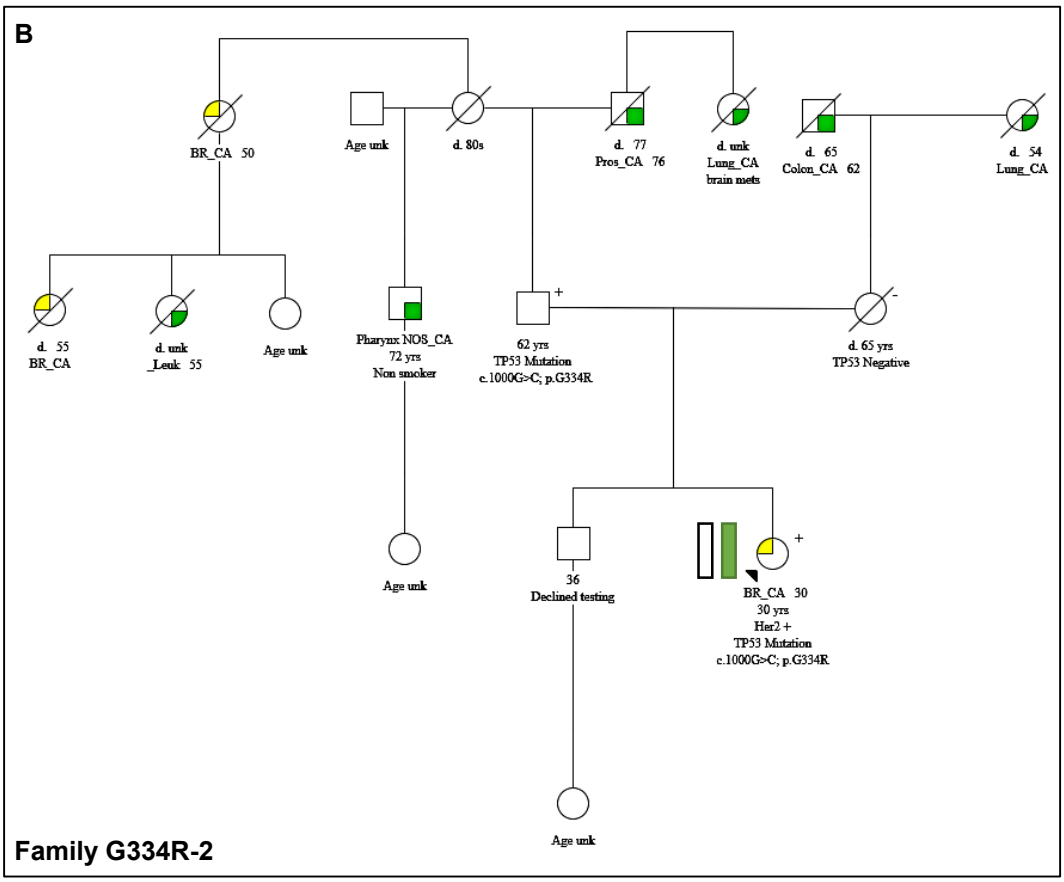
Supplementary Tables are saved as Excel documents





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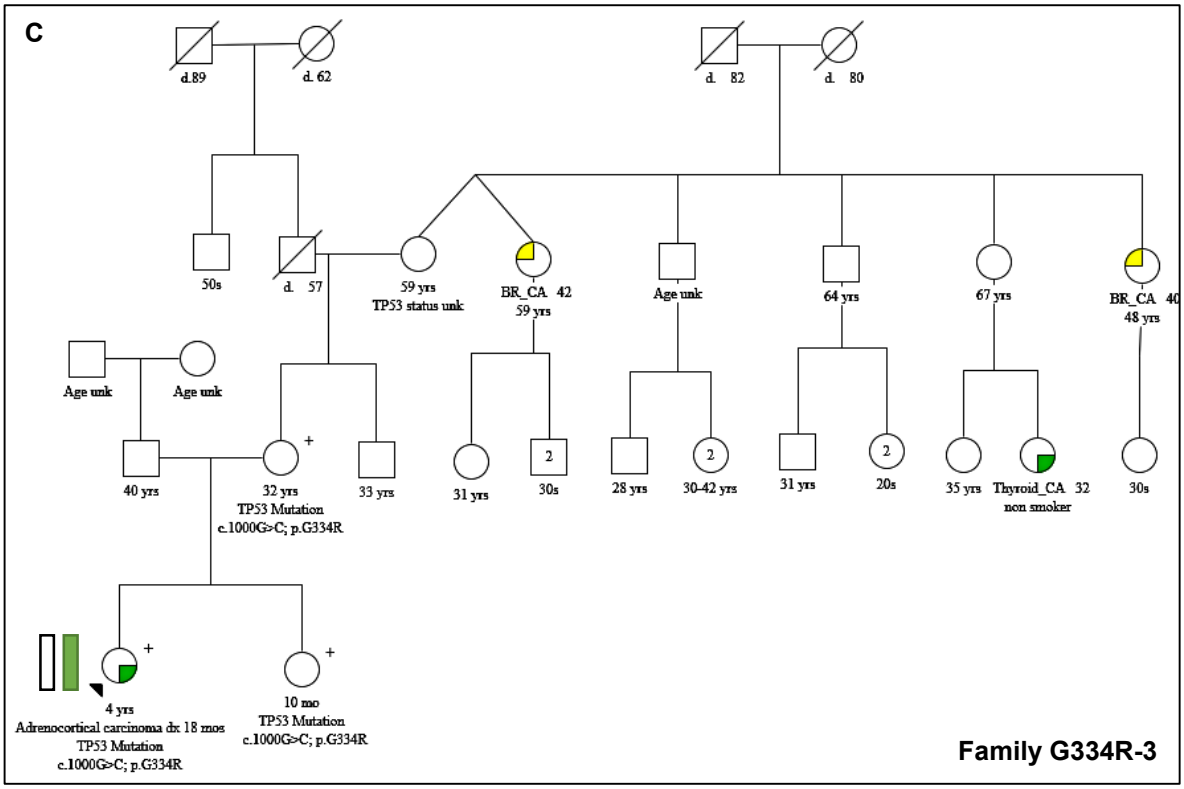

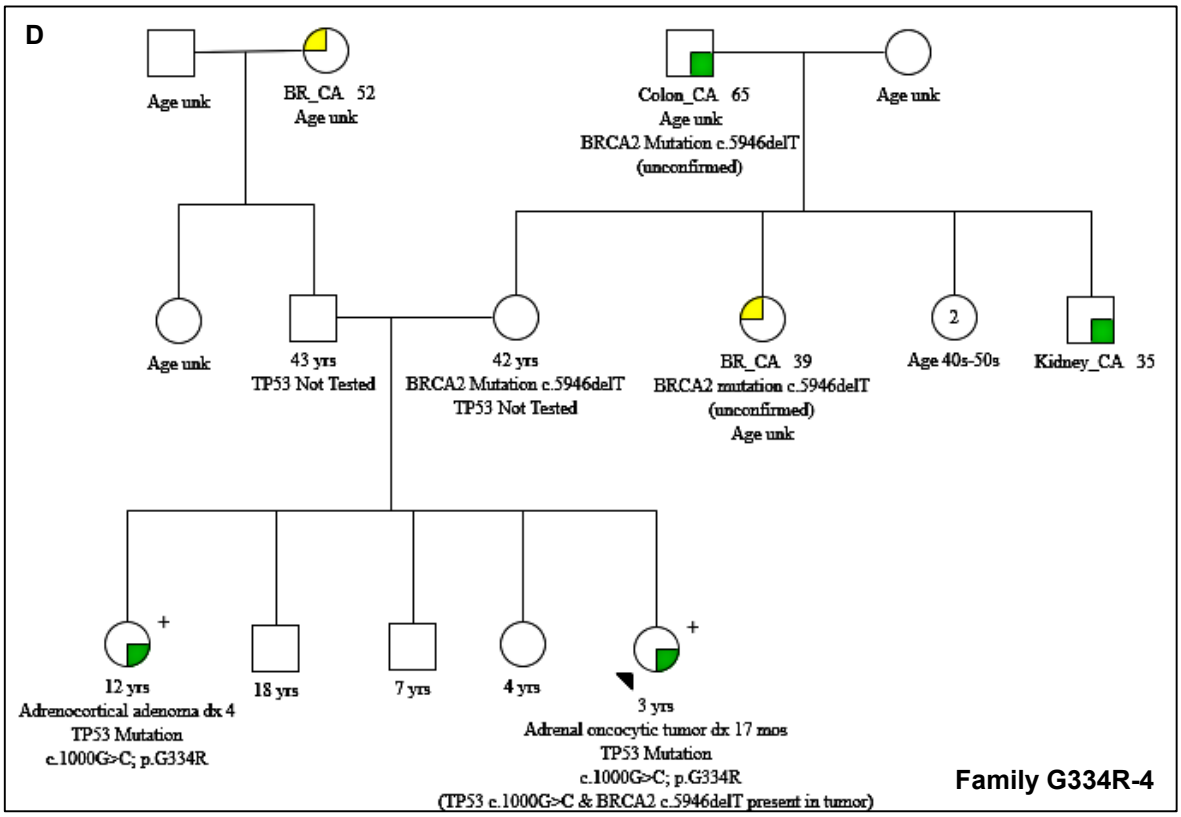

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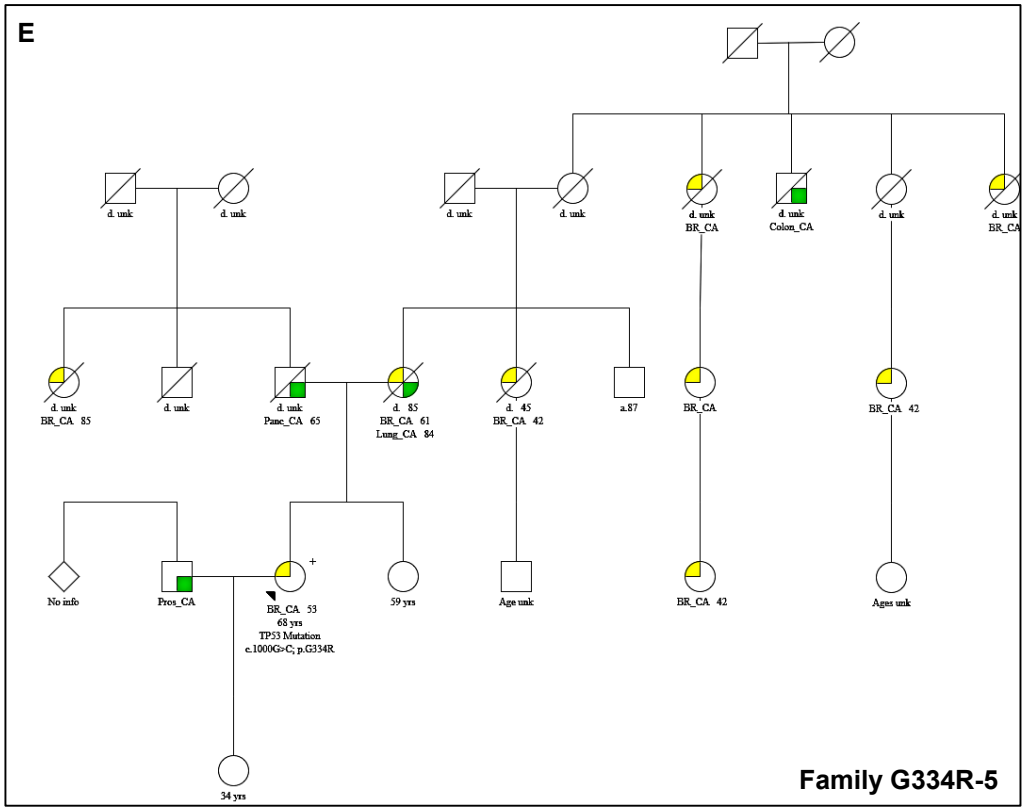

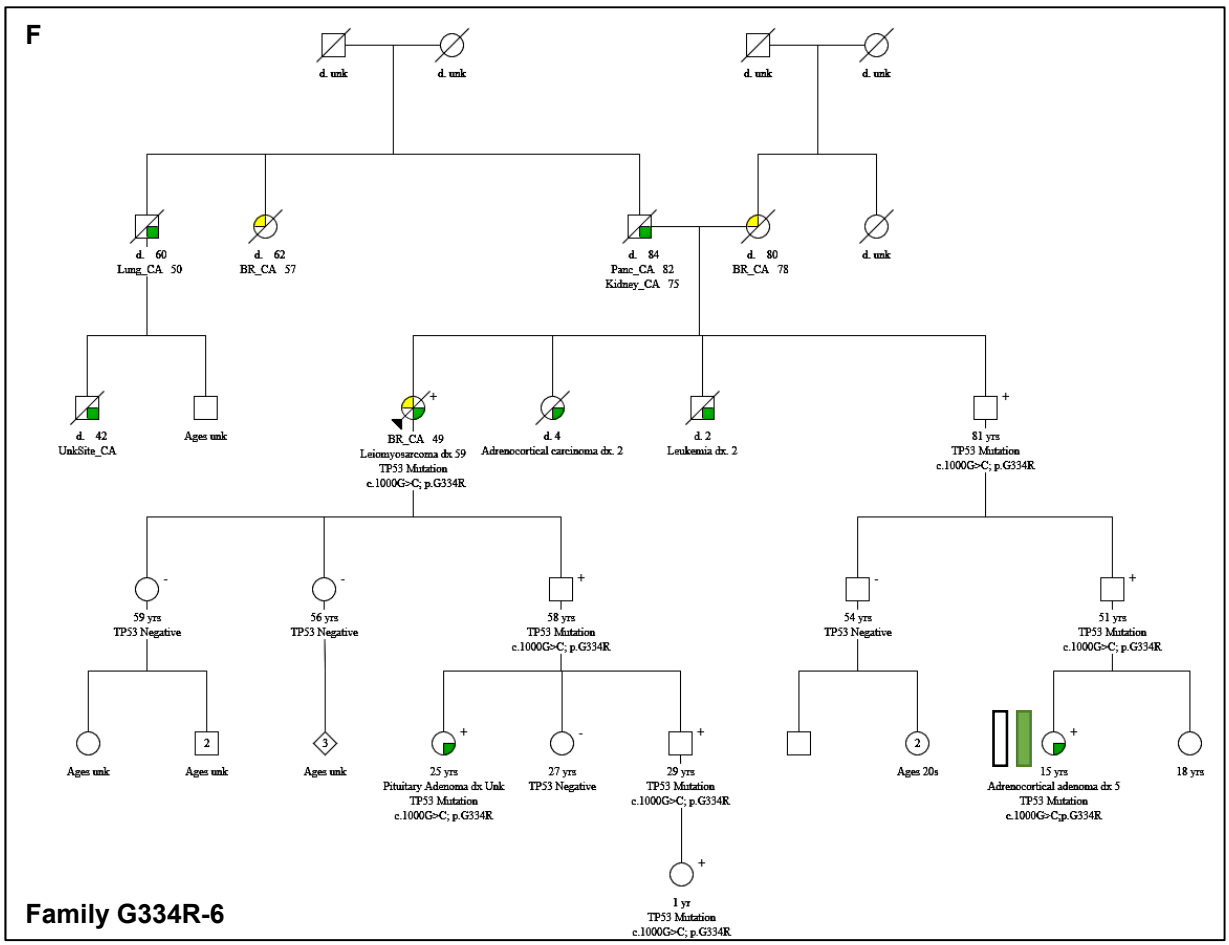

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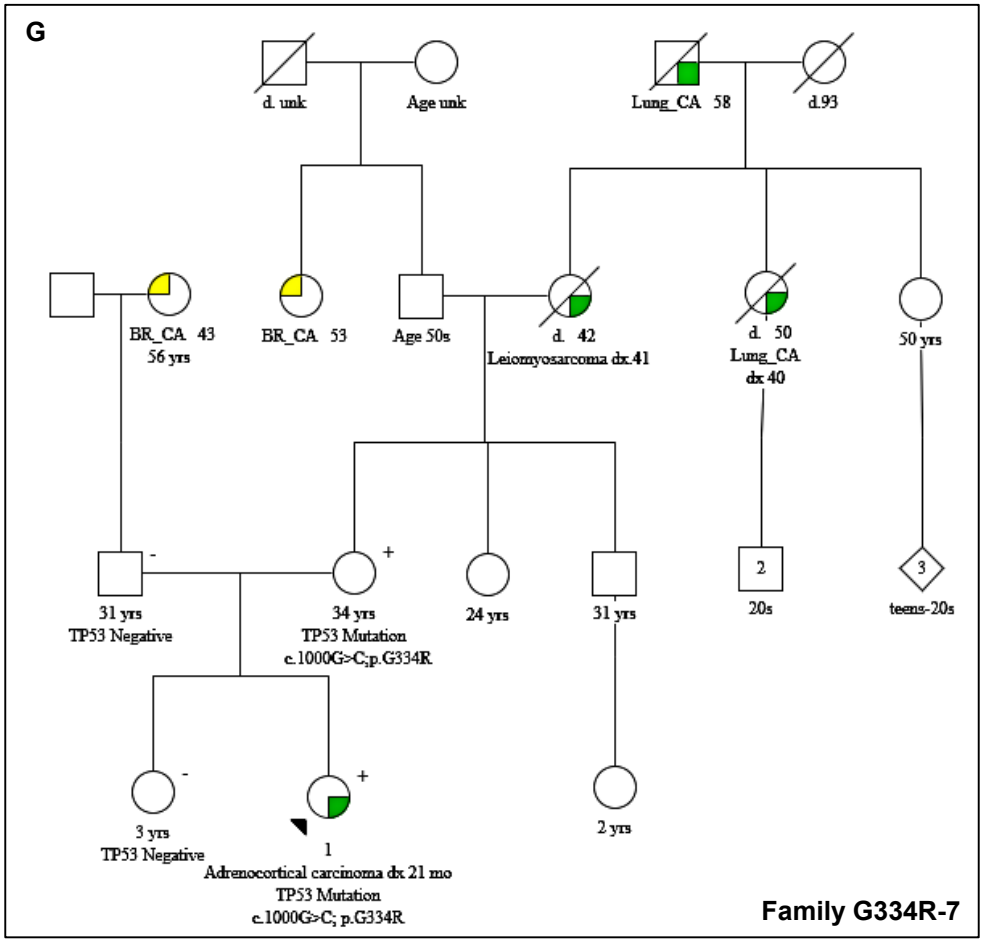

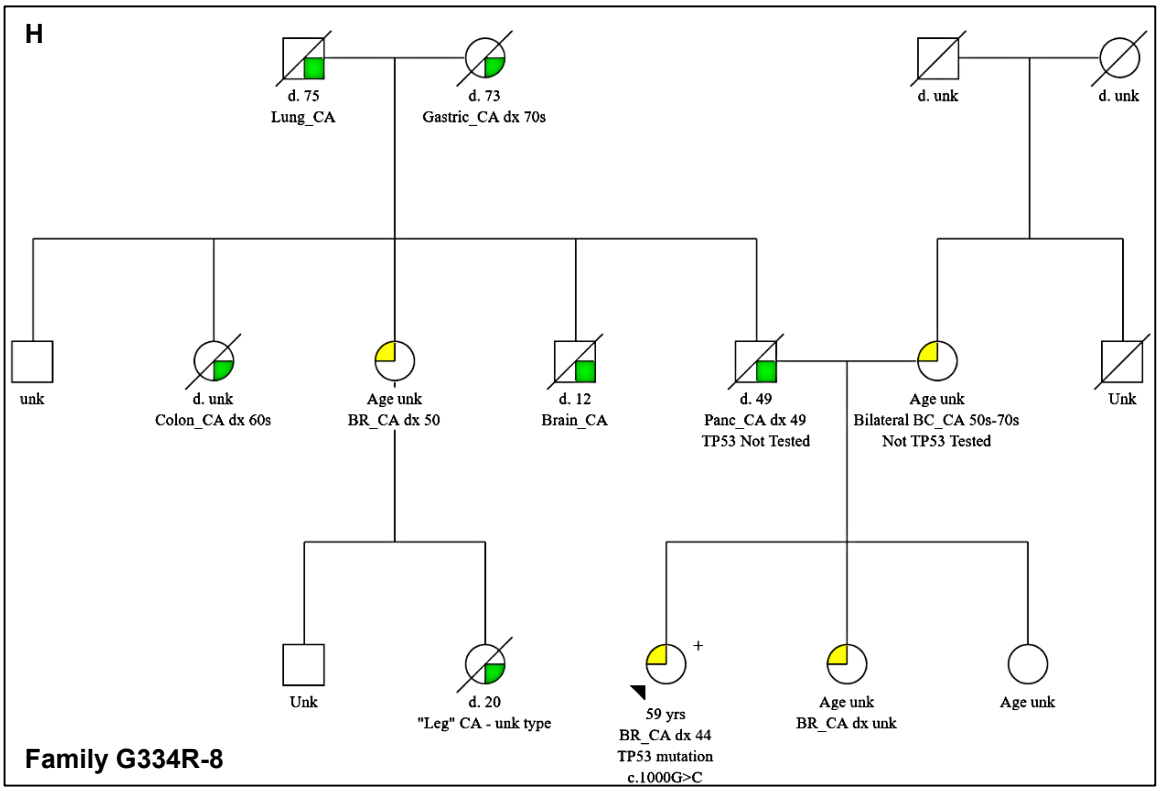

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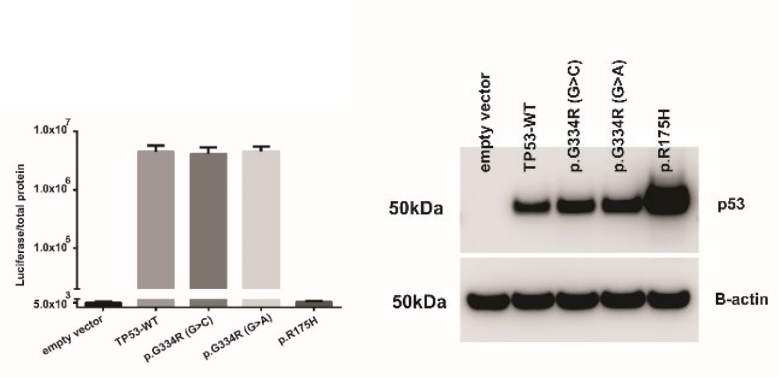
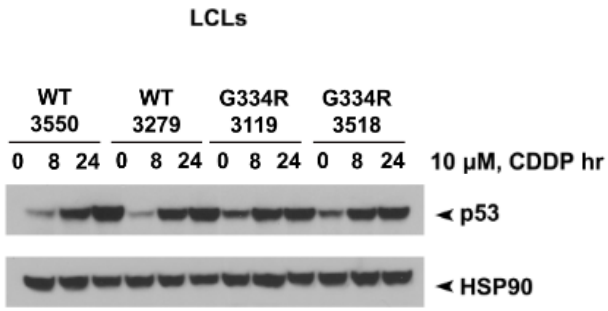
Supplementary Figure S1: Full Pedigrees from TP53 c.1000G>C;p.G334R clinically ascertained families. Fam ID allows reference to Table 1. **A)** Fam ID 1, full pedigree for family G334R-1 shown in Figure 1A; **B)** Fam ID 2, G334R-2; **C)** Fam ID 3, G334R-3; **D)** Fam ID 4, full pedigree for family G334R-3 shown in Figure 1C; **E)** Fam ID 5, G334R-5; **F)** Fam ID 6, full pedigree for family G334R-6 shown in Figure 1C; **G)** Fam ID 7, G334R-7; **H)** Fam ID 8, G334R-8. Shaded upper left quadrant represents a breast cancer (BR_CA) diagnosis, shaded upper right quadrant represents a second primary breast cancer, shaded bottom right quadrant represents a cancer diagnosis other than breast cancer(s). For non-breast cancer diagnoses, tumor type is listed on pedigree. Abbreviations: a.: alive at age n; d.: deceased at age n; dx: diagnosed; mets: metastases; mos: months; No info: no information; unk: unknown; yrs: years. Cancer abbreviations: BR_CA: breast cancer; Colon_CA: Colon cancer; Gastric_CA: Gastric cancer; GI: gastrointestinal; Kidney_CA: Kidney cancer; Leuk: Leukemia; Lung_CA: Lung cancer; Lymph: Lymphoma; Skin_Melan: Melanoma; Panc_CA: Pancreatic cancer; Pharynx NOS_CA: Cancer of pharynx at site unspecified; Pros_CA: Prostate cancer; Thyroid_CA: Thyroid cancer; UnkSite_CA: unknown site of primary cancer. Probands indicated with an arrowhead. Individuals that tested positive for TP53 c.1000G>C;p.G334Rdenotedwith “+”. Obligate carriers denoted with "oc". The shared common haplotype depicted in Main Figure 1 is represented by a green bar in the pedigrees for families G334R-1, -2, -3, -6.



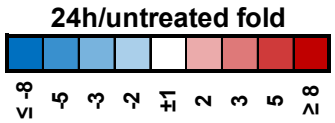
Supplementary Figure S2: Constructs encoding p53 wild-type (TP53-WT), p53-R175H (TP53-R175H), and p53-G334R (c.1000G>A and c.1000G>C) were transiently transfected into p53 deficient Saos-2 cells. Transcriptional activity was assessed using a promoter-reporter assay. Left panel shows the relative p53 luciferase activity. Right panel shows a Western blot analysis of corresponding p53 protein expression. Data represent three independent experiments, with each experiment comprising three biologic replicates.



Supplementary Figure S3: p53 levels after treatment with 10uM cisplatin (CDDP). Cell lysates from two *TP53* wildtype lymphoblastoid cell lines (WT LCLs), lines 3550 and 3279 and from two *TP53* p.G334R mutant LCLs, lines 3119 and 3518 were treated with 10uM CDDP for eight and 24 hours.

Relative changes at 24 hours after Nutlin treatment

WT p53		p.G334R		G334R/WT		WT p53		p.G334R		G334R/WT		
fold	FDR	fold	FDR	fold	FDR	c1	c2	c3	c4	c3	c4	Gene
12	0%	10.7	0%	-1.12	100%							*SESN1*
11.1	0%	10.4	0%	-1.07	100%							*MDM2*
10.7	0%	10.6	0%	-1.01	100%							*CDKN1A*
8.74	0%	7.58	0%	-1.15	90%							*FDXR*
4.31	0%	4.47	0%	1.04	100%							*RRM2B*
4.24	0%	3.14	0%	-1.35	62%							*XPC*
4.23	0%	4.49	0%	1.06	100%							*ZMAT3*
3.19	0%	3.37	0%	1.05	100%							*TNFRSF10B*
2.73	0%	3.72	0%	1.36	18%							*DDB2*
2.68	0%	2.21	0%	-1.21	100%							*BAX*
2.65	0%	2.02	0%	-1.31	100%							*RPS27L*
2.38	0%	2.44	0%	1.03	100%							*AEN*
2.31	0%	2.6	0%	1.13	100%							*CCNG1*



Supplementary Figure S4: Heatmap showing fold activation of a canonical p53 signature at 24 hours post nutlin with wildtype versus mutant lymphoblastoid cell lines. WT p53 "fold" is the average counts of the indicated genes in the WT LCLs 3550 (c1) and 3279 (c2) at 24 hours after Nutlin treatment compared to baseline counts for the lines. Similarly p.G334R "fold" is the average counts of the indicated genes in the mutant LCLs 3119 (c3) and 3518 (c4) at 24 hours after Nutlin treatment compared to baseline for the lines. G334R/WT "fold" is a ratio of WT p53 fold to p.G334R fold. The right half of the figure shows a heatmap of the technical replicates for each line.