Genetic variation in the *NEIL2* DNA glycosylase gene is associated with oxidative DNA damage in *BRCA2* mutation carriers

SUPPLEMENTARY MATERIALS

Supplementary Table 1: Genetic/allelic frequencies of the *NEIL2* variant rs804271 (G>T)

	GG		GT		TT		rs804271 G>T allelic frequency
	n	%	*n*	%	*n*	%	
FBOC*	66	**0.39**	75	**0.45**	25	**0.15**	**0.39**
BRCA1	17	**0.42**	18	**0.45**	5	**0.13**	**0.35**
BRCA2	20	**0.43**	20	**0.43**	6	**0.13**	**0.36**
Controls	29	**0.36**	37	**0.46**	14	**0.17**	**0.4**

* Familial breast and ovarian cancer patients. No significant differences were detected in the genetic frequencies among FBOC group. (Pearson Chi-squared test).

Supplementary Table 2: Regulatory motifs altered by the SNP (rs804271)

Element	Ref_value	Alt_value	Match on:
			Ref: AGGGGACGGAGGCCGCATGGGCCGCCGAGCCGGGAAATCTCCGCCCCCAGCTGGAGCGG
			Alt: AGGGGACGGAGGCCGCATGGGCCGCCGAGACGGGAAATCTCCGCCCCCAGCTGGAGCGG
E2F1	12.1	7.3	SSCGSSAAAH
SIN3A	11.2	8.4	GSNSCTSNSSNNSS
YY1	11.1	10.9	SSSNSSSSNNNSNSS

Modified from: http://archive.broadinstitute.org/mammals/haploreg/haploreg.php.

Supplementary Table 3: Gtex information summary, regarding *NEIL2* transcriptional up-regulation when rs804271 is present (30 different tissues)

Gene Symbol	SNP	Effect Size	[a]*P*-Value	Tissue
NEIL2	rs804271	0.52	6.80E-37	Nerve - Tibial
NEIL2	rs804271	0.36	5.90E-18	Heart - Atrial Appendage
NEIL2	rs804271	0.32	1.40E-17	Adipose - Subcutaneous
NEIL2	rs804271	0.31	4.50E-15	Artery - Tibial
NEIL2	rs804271	0.35	5.50E-15	Artery - Aorta
NEIL2	rs804271	0.6	1.70E-14	Ovary
NEIL2	rs804271	0.34	6.60E-13	Whole Blood
NEIL2	rs804271	0.26	2.10E-12	Thyroid
NEIL2	rs804271	0.24	2.60E-11	Muscle - Skeletal
NEIL2	rs804271	0.24	8.00E-11	Adipose - Visceral
NEIL2	rs804271	0.29	1.30E-10	Fibroblasts
NEIL2	rs804271	0.39	3.40E-09	Pituitary
NEIL2	rs804271	0.21	1.4E-08	Esophagus - Muscularis
NEIL2	rs804271	0.2	4.5E-08	Heart - Left Ventricle
NEIL2	rs804271	0.32	0.00000021	Colon - Sigmoid
NEIL2	rs804271	0.41	0.00000066	Vagina
NEIL2	rs804271	0.33	0.0000011	Adrenal Gland
NEIL2	rs804271	0.32	0.0000019	Brain - Caudate (basal ganglia)
NEIL2	rs804271	0.27	0.0000023	Artery - Coronary
NEIL2	rs804271	0.38	0.0000025	Brain - Cortex
NEIL2	rs804271	0.23	0.000021	Pancreas
NEIL2	rs804271	0.35	0.000023	Uterus
NEIL2	rs804271	0.19	0.000032	Stomach
NEIL2	rs804271	0.27	0.00028	Liver
NEIL2	rs804271	0.16	0.001	Lung
NEIL2	rs804271	0.28	0.0022	Spleen
NEIL2	rs804271	0.18	0.0029	Small Intestine - Terminal Ileum
NEIL2	rs804271	0.28	0.0033	LCLs
NEIL2	rs804271	0.24	0.024	Prostate
NEIL2	rs804271	0.09	0.026	Testis

[a]Nominal eQTL *p*-values were generated for each SNP-gene pair using a two-tailed *t* test, testing the alternative hypothesis that the beta (slope of the linear regression model) deviates from the null hypothesis of $\beta = 0$.

Supplementary Table 4: List of lymphoblastoid cell lines (LCL)

LCL [a]	BRCA1 mutationb	Mutation typec	Exon	dAge	ers804271
06S179-L[1]	Wild type	-	-	31	GT
09S797-L[2]	Wild type	-	-	27	TT
10S889-L[3]	Wild type	-	-	20	GT
11S66-L[4]	Wild type	-	-	30	GT
11S534-L[5]	Wild type	-	-	50	GT
11S954-L	Wild type	-	-	35	GT
11S375-L	Wild type	-	-	23	GT
10S1202-L	c.5123C > A; p.Ala1708Glu	Missense	18	53	GG
10S890-L[3]	c.5123C > A; p.Ala1708Glu	Missense	18	25	GT
11S65-L[6]	c.5117G > A; p.Gly1706Glug	Missense	18	31	GT
11S67-L[6]	c.5117G > A; p.Gly1706Glu	Missense	18	34	GG
07S1291-L	c.3239T > A; p.Leu1080X	Nonsense/TRC	11	34	GT
09S798-L[2]	c.2410C > T; p.Gln804X	Nonsense/TRC	11	24	GG
09S546-L	c.212 + 1G > A; p.?	Splice/TRC	5	42	TT
11S376-L[7]	c.212 + 1G > A; p.?	Splice/TRC	5	39	GT
11S384-L[7]	c.212 + 1G > A; p.?	Splice/TRC	5	75	GT
09S491-L	c.815_824dup10; p.Thr276	Frameshift/TRC	11	24	GT
10S1177-L[4]	c.68_69delAG; p.Glu23	Frameshift/TRC	2	27	TT
10S44-L	c.4309delT; p.Ser1437	Frameshift/TRC	13	22	GG
11S1004-L[5]	c.981_982delAT; p.Cys328X	Frameshift/TRC	11	25	GT

[a]1–7 LCL from relatives of the same family (sisters or mother & daughter)

[b]Mutation nomenclature based on GenBank reference sequences NM_007294.3 with numbering starting at the A of the first ATG, following the journal guidelines (www.hgvs.org/mutnomen); p.?, unknown protein nomenclature (variant causing skipping of exon 5 of BRCA1)

[c]-: Refers to the non-carrier control; TRC: Stands for truncating mutation

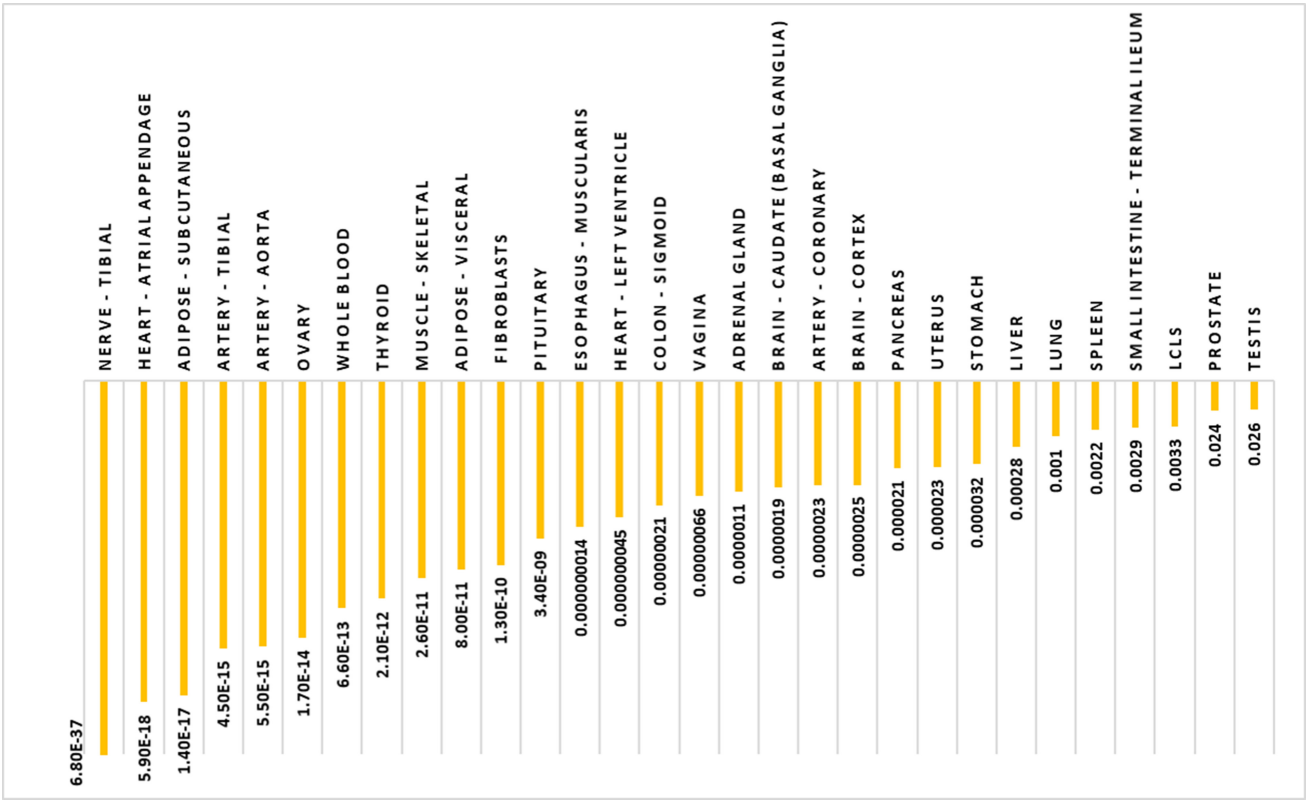
dAge of the woman at the time of extraction of the blood sample from which the LCL was established

[e]G>T indicate the polymorphism (rs804271).

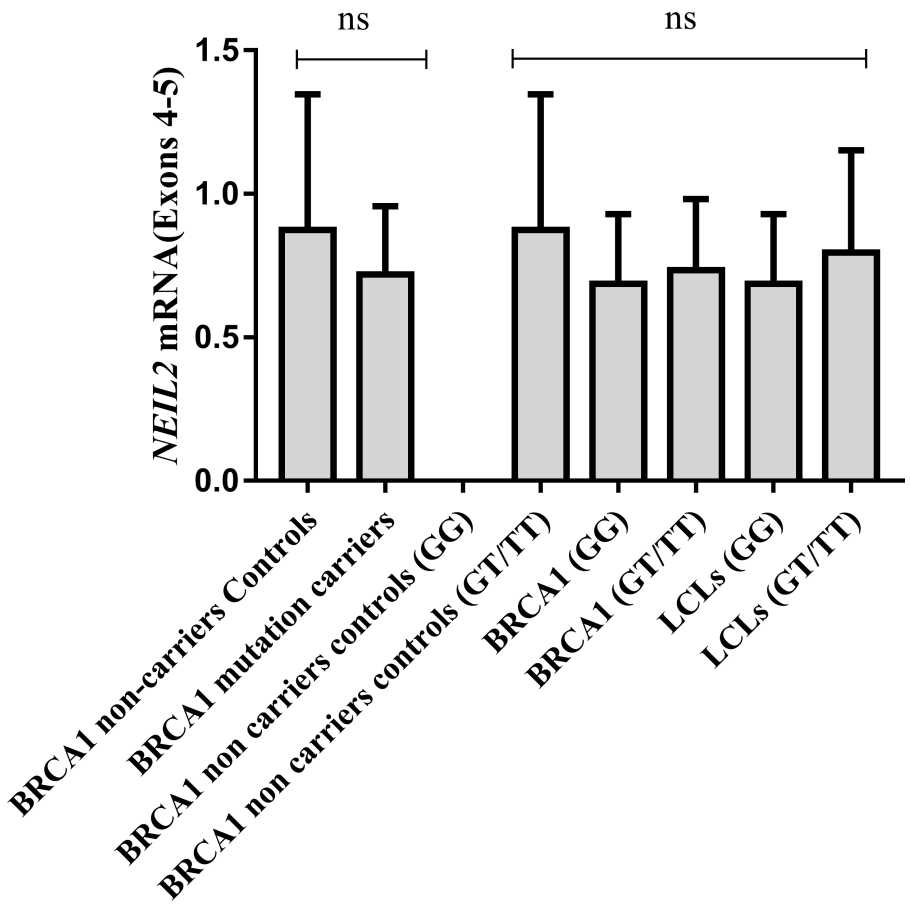
Supplementary Table 5: Lineal regression analysis in FBOC samples: Cancer effect on the studies variables

Independent Variable	Dependent variable	β coeff	*p*-value
Cancer status	*NEIL2* mRNA	-0.23	0.091
	Telomere Oxidation	0.14	0.396

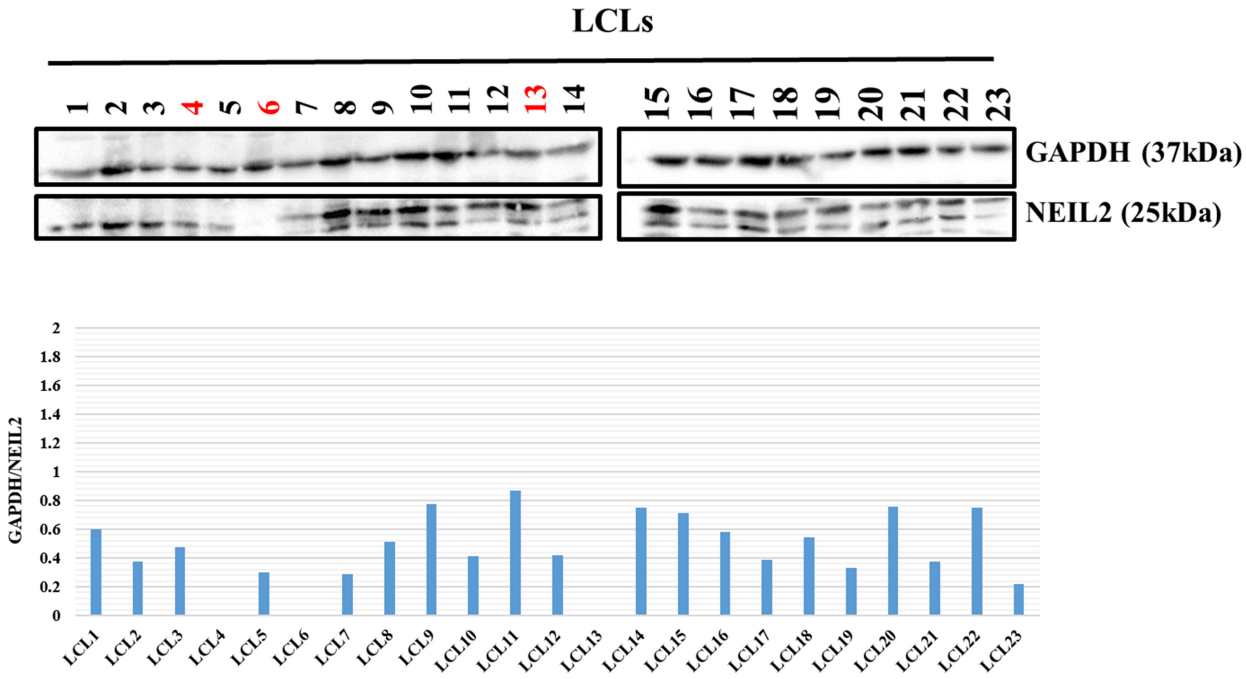
We included as dependent variables *NEIL2* mRNA levels, telomere oxidation. As independent variables, we included cancer status. β coefficients quantify how much the independent variable (cancer status) modifies the dependent variables, and it shows the direction of the modification.



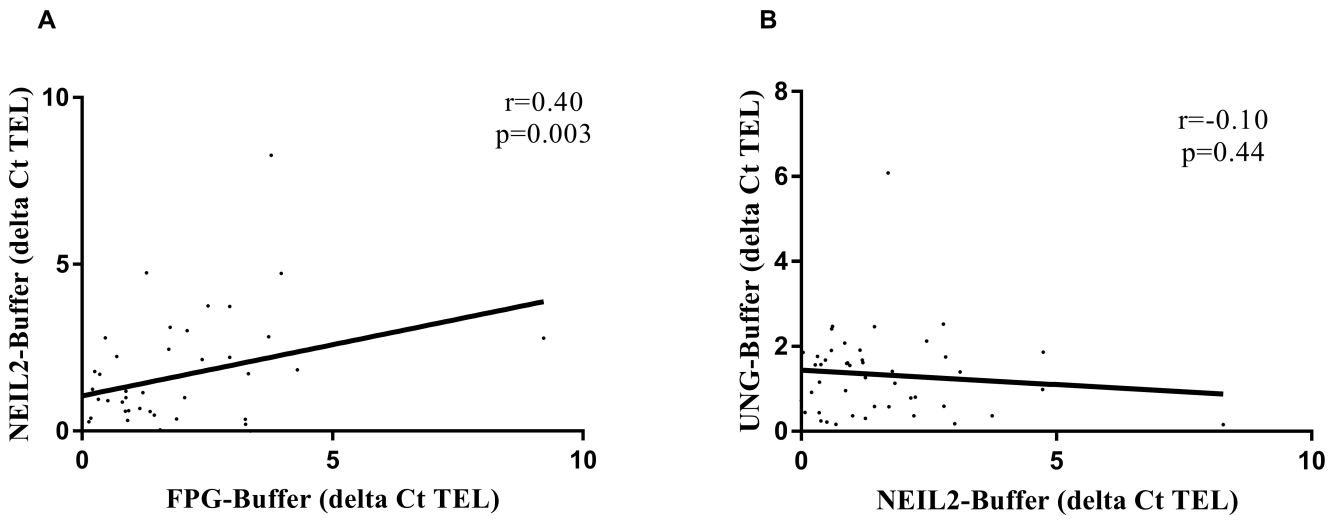
Supplementary Figure 1: Association p-values for all tissues in which the SNP has a significant effect increasing NEIL2 mRNA levels. https://www.gtexportal.org/home/snp/rs804271



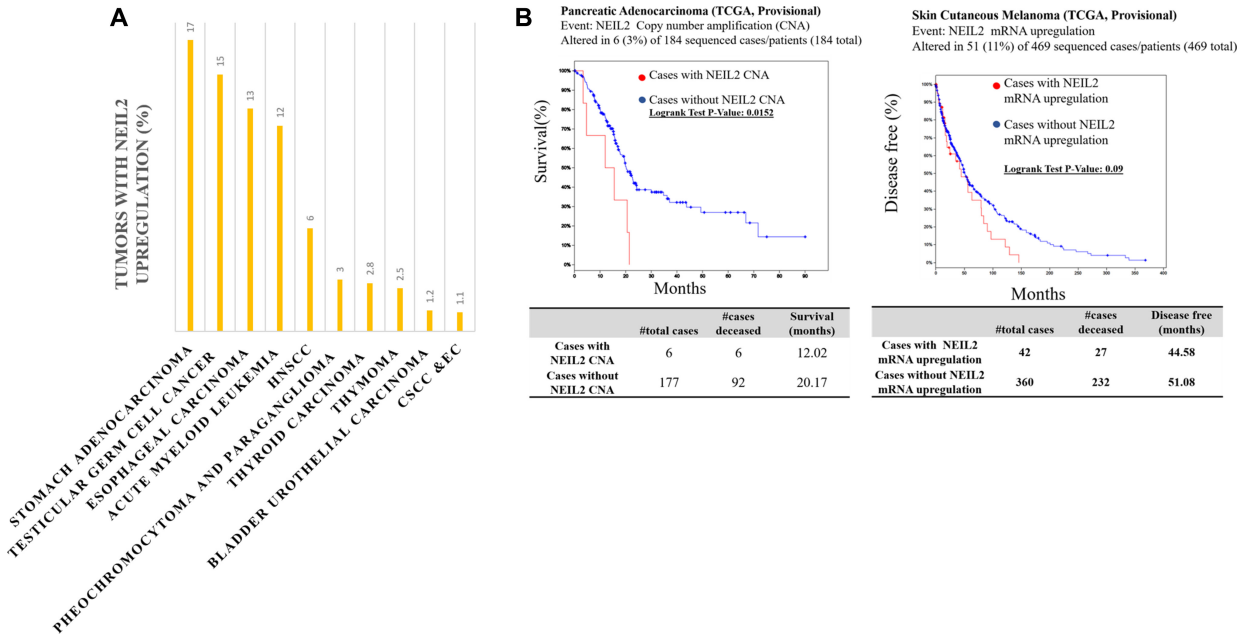
Supplementary Figure 2: Comparative analysis of NEIL2 mRNA expression according BRCA mutational status in LCLs series (BRCA1 mutation carriers are compared with non-carrier controls). Comparative analysis of NEIL2 mRNA expression according the SNP status ((carriers (GT/TT) Vs non-carriers (GG)) among LCL groups (BRCA1 mutation carriers and non-carrier controls). Bars represent the mean and the standard deviation for each group. Unpaired student t test was used to test for potential significant differences between means. No significant differences were found in any case.



Supplementary Figure 3: NEIL2/GAPDH immunoblotting. Variation of NEIL2 protein levels (25KDa) relative to GAPDH (37kDa) as reference housekeeping gene in different LCLs (1–23). In red 3 LCLs (4,6,13) that could not be included in the final analysis.



Supplementary Figure 4: (**A**) Correlation analysis between "NEIL2-lesions"and "FPG-lesions". (**B**) Correlation analysis between "UNG-lesions" and the relative amount of "uracil-lesions". Spearman test, was used to test whether correlation was significant. When *p*-value when ($p < 0.05$).



	#total cases	#cases deceased	Survival (months)
Cases with NEIL2 CNA	6	6	12.02
Cases without NEIL2 CNA	177	92	20.17

	#total cases	#cases deceased	Disease free (months)
Cases with NEIL2 mRNA upregulation	42	27	44.58
Cases without NEIL2 mRNA upregulation	360	232	51.08

Supplementary Figure 5: (**A**) Study of *NEIL2* mRNA expression among 10 different tumor types from TCGA. We selected only those studies in which *NEIL2* mRNA upregulation was mutually exclusive with *NEIL2* mRNA downregulation (none sequenced tumors). Head and Neck Squamous Cell Carcinoma (HNSCC)/ Cervical Squamous Cell Carcinoma & Endocervical Adenocarcinoma (CSCC &EC) (**B**) Two examples extracted from two independent tumor types studies from TCGA in which the event, *NEIL2* mRNA upregulation or copy number amplification, has a prognostic value in terms of overall survival (months) or disease free survival (months).