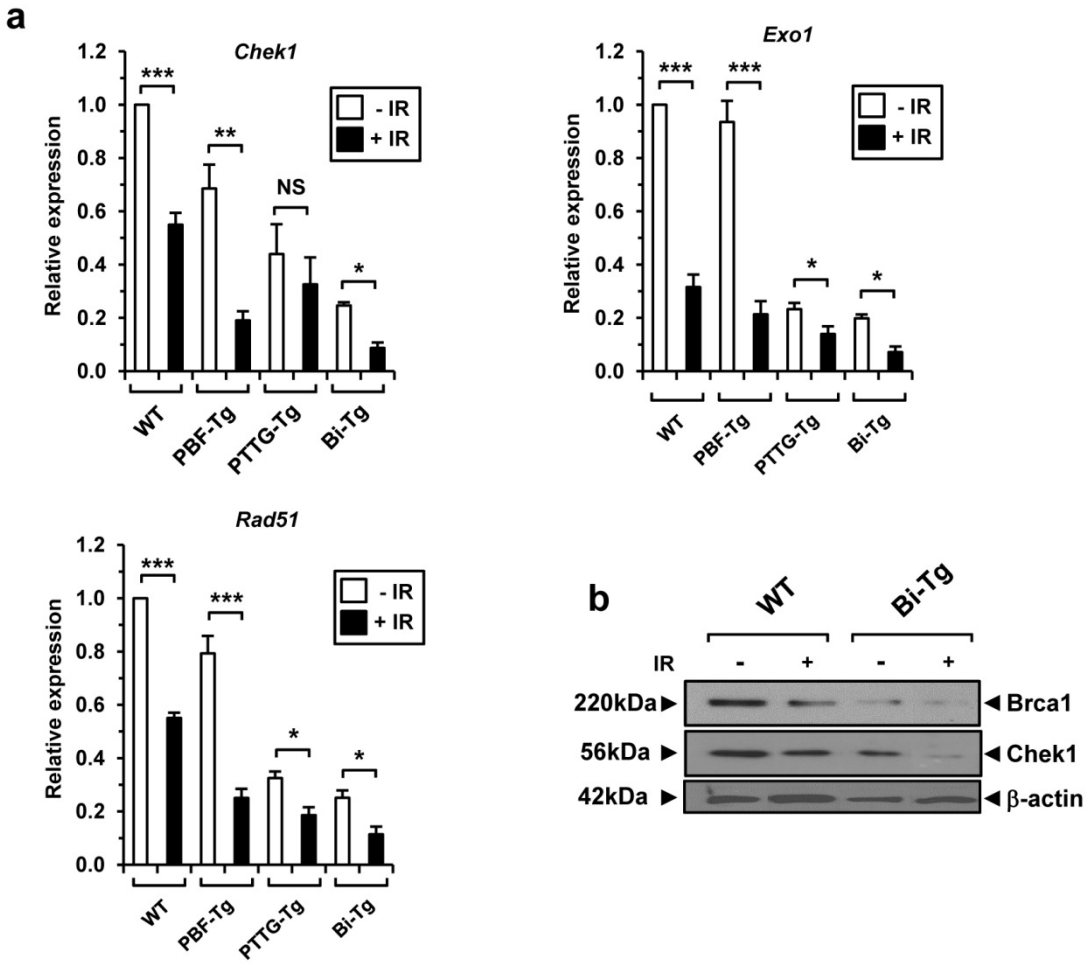
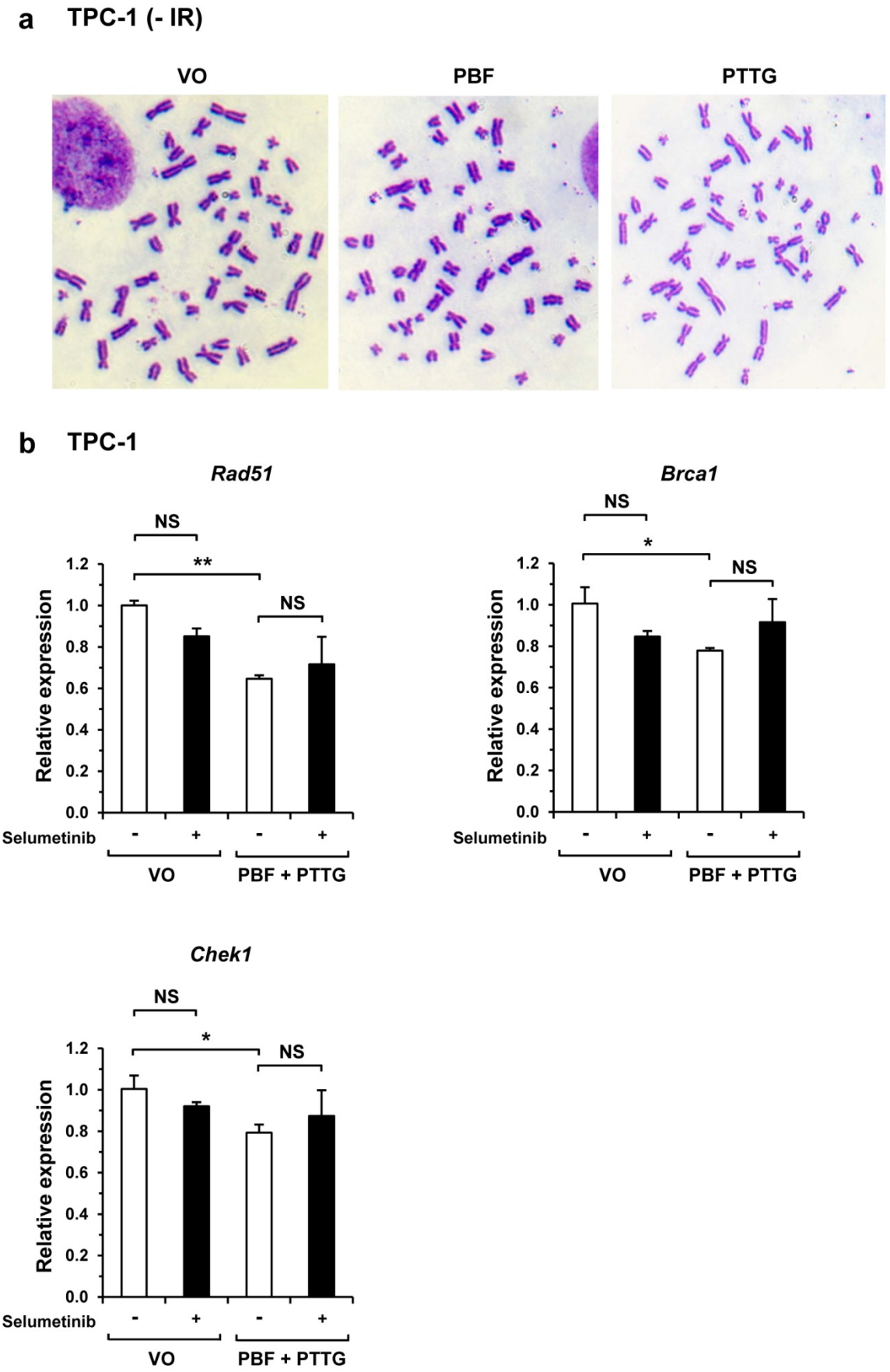
Supplementary Figure S8



Supplementary Figure S8. Effect of genotype and irradiation on DDR gene expression in primary thyrocytes. (**a**) Relative fold changes in *Chek1*, *Rad51* and *Exo1* expression in irradiated (+IR) thyrocytes of each genotype (WT, PBF-Tg, PTTG-Tg and Bi-Tg) versus non-irradiated (-IR) controls. (mean±s.e.m., n=4, unpaired two-tailed t-test) (NS, not significant; *P<0.05; **P<0.01; ***P<0.001). (**b**) Western blot analysis of Brca1 and Chek1 in WT and Bi-Tg primary thyrocytes either non-irradiated (-) or irradiated (+). Blot shown is representative from at least 3 independent experiments.

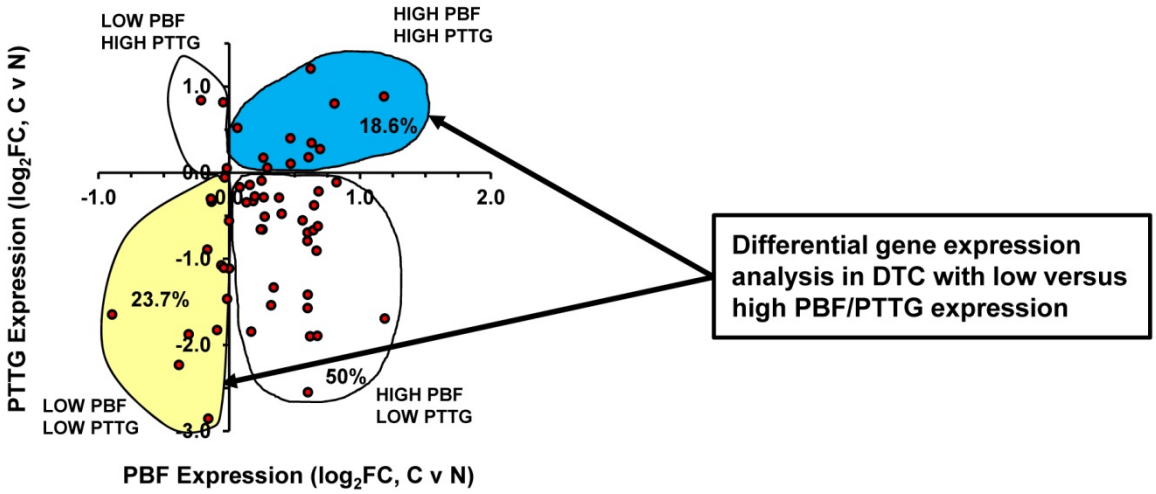
Supplementary Figure S9



Supplementary Figure S9. Chromosomal aberrations and DDR gene expression in TPC-1 cells. (**a**) Representative metaphase spreads of TPC-1 cells transfected with VO, PBF or PTTG for 48 h prior to analysis. (**b**) Relative fold changes in *Rad51*, *Brca1* and *Chek1* expression in TPC-1 cells transfected with either VO or PBF + PTTG and then incubated with 200 nM selumetinib (mean±s.e.m., n=3, unpaired two-tailed t-test) (NS, not significant; *P<0.05; **P<0.01).

Supplementary Figure S10

a **Matched tumour/normal TCGA thyroid dataset (*n*=59)**

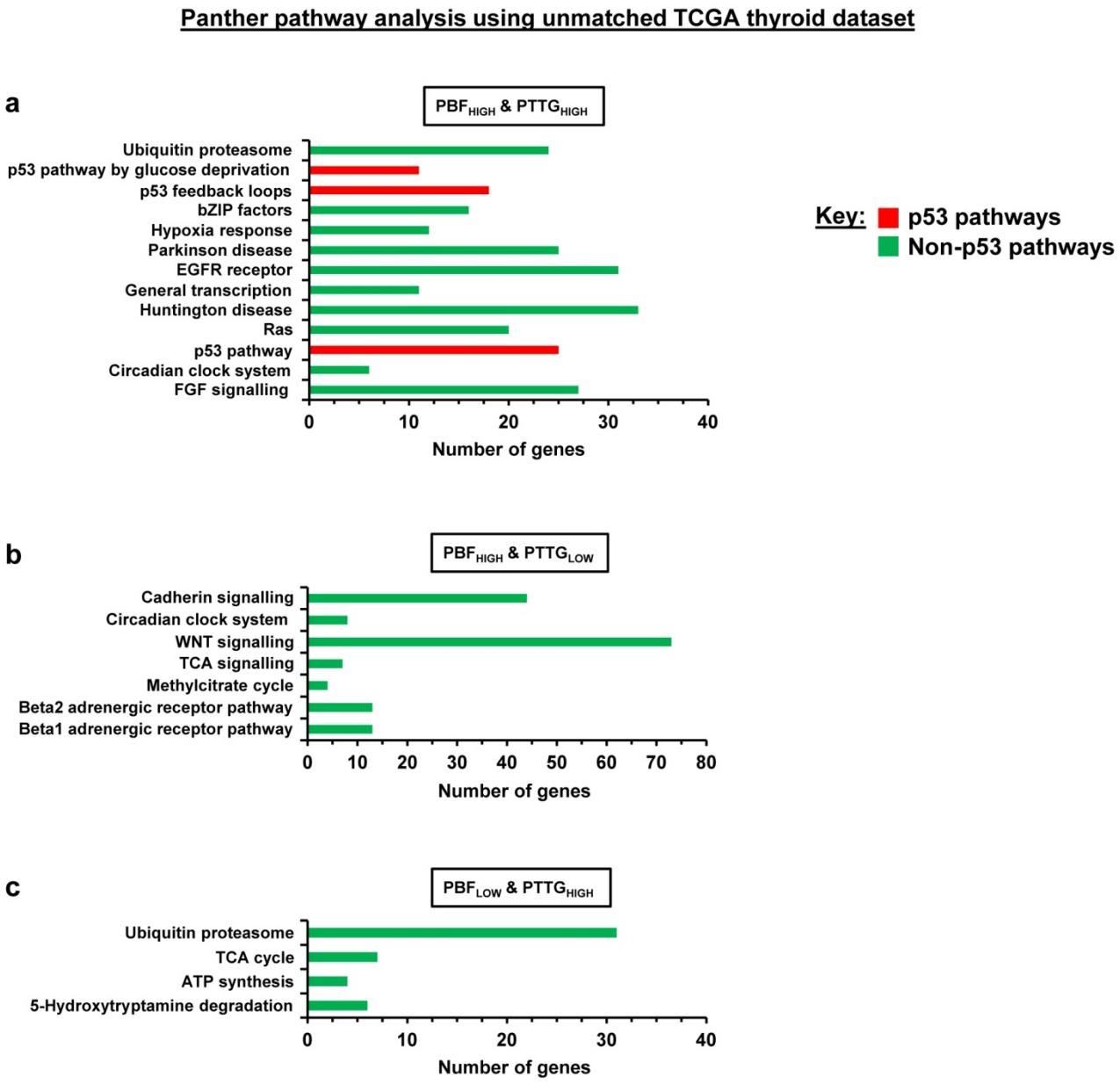


b

Panther Pathway (Genes 1-3000; *P*<0.005)	P-Value	Genes
p53 pathway feedback loops 2	0.0097	PDPK1, SMG1, MAPK11, MAPK14, PTENP1, PIK3CA, PIK3CB, PIK3C2A, PIK3C2B, PIK3C3, PPM1D, RRAS, RBL1, ATM, PRKDC, TALDO1, HRAS
p53 pathway	0.043	PDPK1, BAX, CREBBP, EP300, KAT2B, MYST3, MYST4, PERP, SIN3A, SMG1, SUMO1P3, SUMO2, PTENP1, PIK3CA, PIK3CB, PIK3C2A, PIK3C2B, PPM1D, ATM, WRN, PRKDC, SIRT1, SIRT6, SFN
REACTOME Pathway (Genes 1-3000 genes; *P*<0.005)	**P-Value**	**Genes**
DNA Repair	0.0039	APEX1, FANCM, MAD2L2, MRE11A, MPG, MGMT, REV1, REV3L, XRCC6, ALKBH2, CCNH, CDK7, ERCC1, ERCC4, MDC1, NTHL1, POLD4, POLR2A, POLR2F, POLR2G, POLR2I, POLR2J, POLR2K, POLR2L, RFC1, ATM, PRKDC, TP531
REACTOME Pathway (Genes 3001-6000 genes; *P*<0.05)	**P-Value**	**Genes**
DNA Repair	0.016	FANCC, RAD50, ALKBH3, BRCA1, BRCA2, ERCC6, ERCC8, GTF2H1, GTF2H3, GTF2H4, LIG3, NBN, POLB, POLH, POLD3, POLR2B, POLR2E, RFC2, RPA1, RPA2, RPA3, SMUG1, TCEA1, USP1, UBE2T

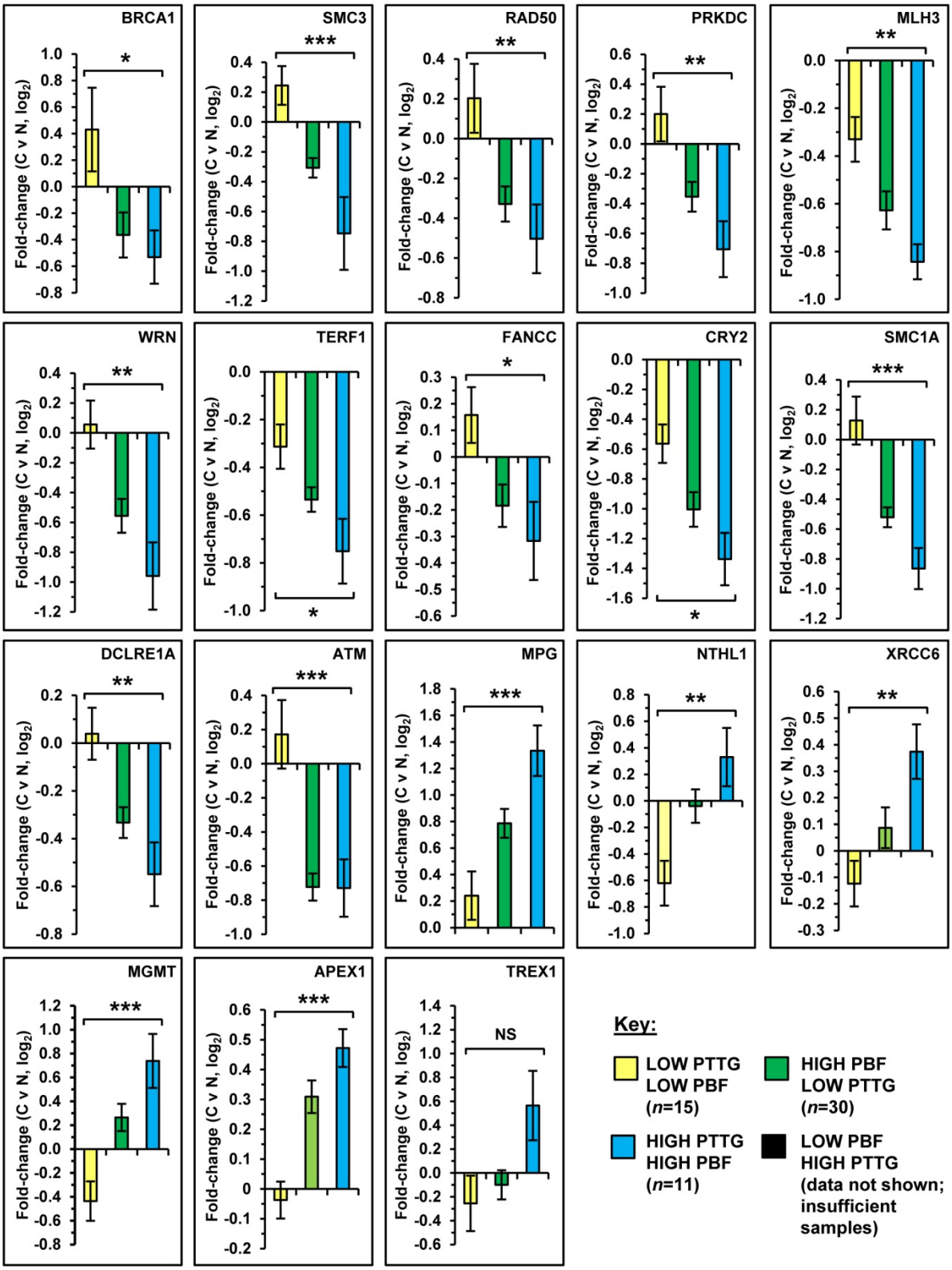
Supplementary Figure S10. Differentially expressed genes in human DTC with low vs high PBF/PTTG expression. (**a**) Scatterplot showing fold-change (FC) in PBF and PTTG expression in DTC vs matched normal samples (log_2, *n*=59, TCGA dataset). Shaded areas indicate DTC samples with low (yellow) and high (blue) PBF/PTTG expression. (**b**) Panther (upper) and Reactome (lower) analysis using DAVID to identify p53 pathway and DNA repair genes differentially expressed in low vs high PBF/PTTG-expressing thyroid tumours. Gene categories and their respective *P*-values are indicated (modified Fisher's exact test). Several genes (underlined) identified by DAVID were previously found to be significantly repressed in murine Bi-Tg thyrocytes (Figure 2a and Supplementary Figure S5a).

Supplementary Figure S11



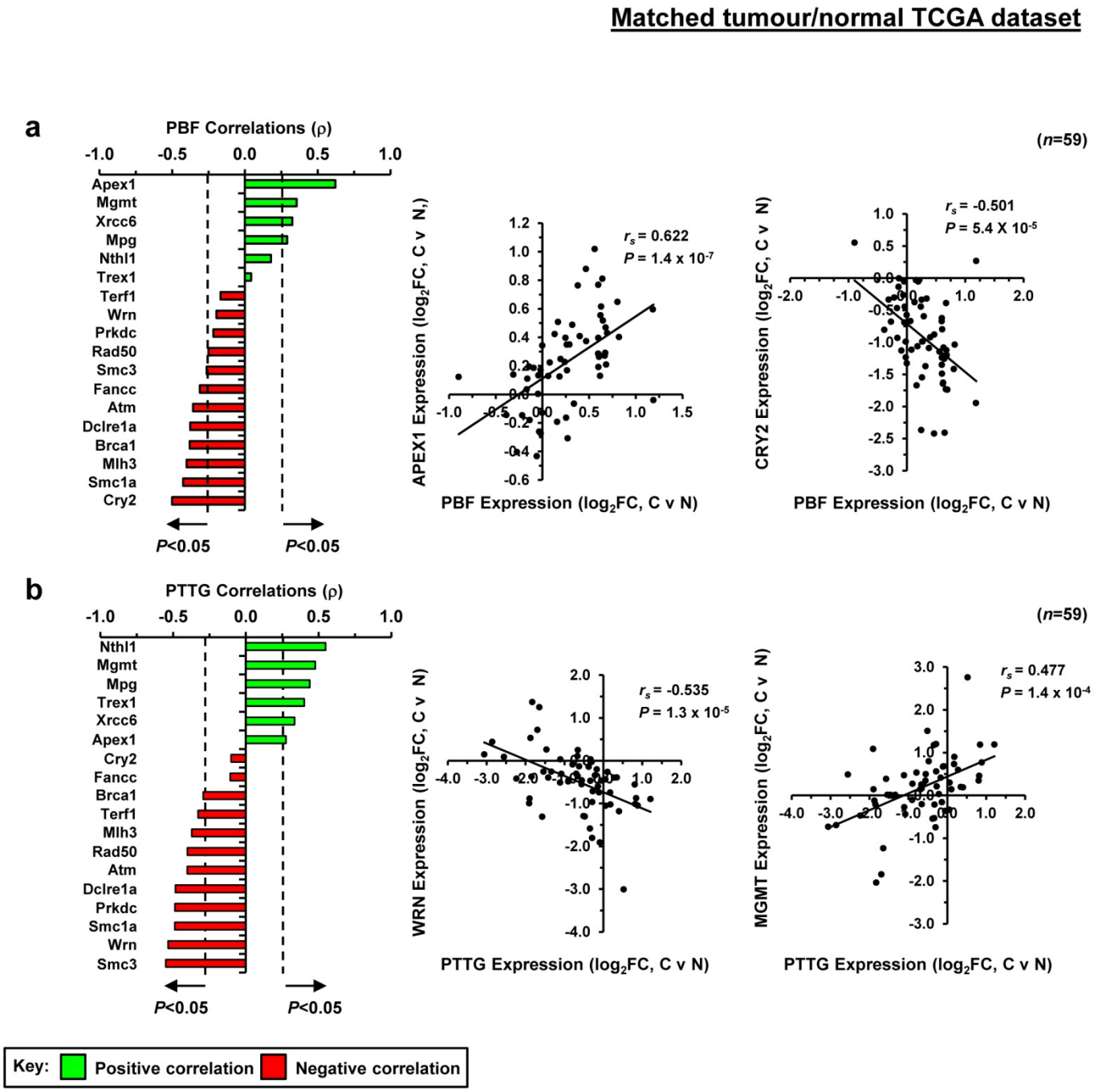
Supplementary Figure S11. Enrichment of p53 pathway genes identified by DAVID analysis is specific to DTC with elevated expression of PBF and PTTG. (**a**) DAVID analysis of differentially expressed genes in the unmatched TCGA dataset comparing DTC samples with high PBF/high PTTG tumoural expression (*n*=25) and low PBF/low PTTG tumoural expression (*n*=28). Gene categories enriched for p53 pathway genes are highlighted in red. $P<0.05$ for all subgroups (modified Fisher's exact test). (**b**) Same as (a) but comparing DTC samples with high PBF/low PTTG tumoural expression (*n*=19) and low PBF/low PTTG tumoural expression (*n*=28). (**c**) Same as (a) but comparing DTC samples with low PBF/high PTTG tumoural expression (*n*=7) and low PBF/low PTTG tumoural expression (*n*=28).

Supplementary Figure S12



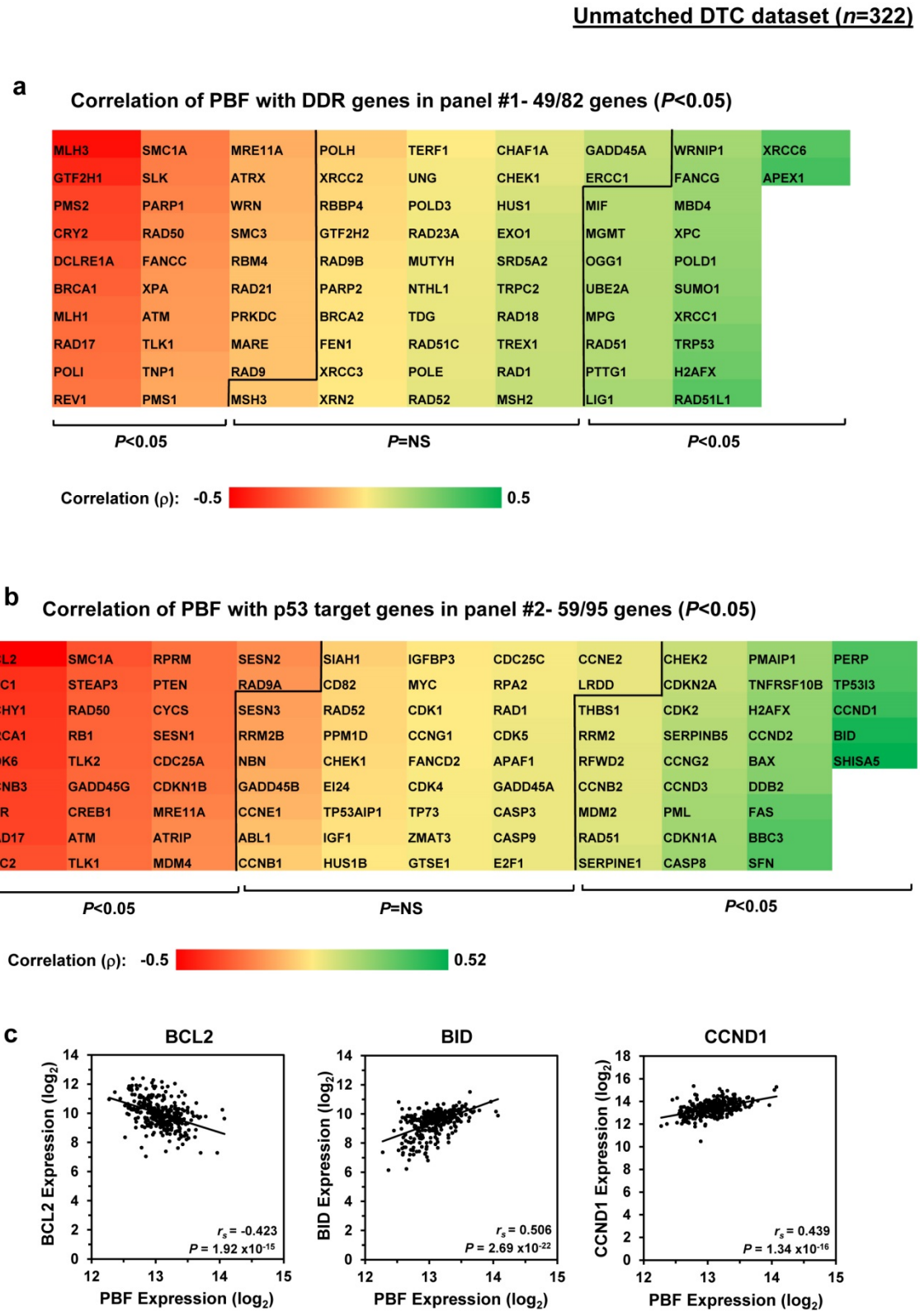
Supplementary Figure S12. Modulation of DDR genes in DTC with different subsets of PBF and PTTG expression. Fold-change in expression for a panel of 18 DDR genes is shown using matched thyroid tumour and normal samples with low PBF/low PTTG (*n*=15, yellow), high PBF/low PTTG (n=30, green) and high PBF/high PTTG (*n*=11, blue) expression (mean log_2FC±s.e.m., Kruskal-Wallis test) (NS, not significant; **P*<0.05; ***P*<0.01; ****P*<0.001). Gene expression data for DTC samples with low PBF/high PTTG was not shown due to insufficient samples. The relative fold-change in expression for DTC samples with high PBF/high PTTG was significantly greater than the subset with high PBF/low PTTG for 8 DDR genes (i.e. SMC3, PRKDC, WRN, SMC1A, MPG, MGMT, APEX1, TREX1; *P*<0.05; Mann-Whitney test; 1-tailed).

Supplementary Figure S13



Supplementary Figure S13. Correlation of DDR genes with PBF and PTTG expression in human DTC. (**a-b**) Correlation of PBF (a) and PTTG (b) expression with 18 DDR genes in matched DTC/normal samples (*n*=59). *P* and ρ values were calculated using Spearman's correlation tests. Representative scatterplots showing significant correlations for fold-change (FC) in *Apex1*, *Cry2*, *Wrn* and *Mgmt* expression with either PBF (a) or PTTG (b) are shown on right.

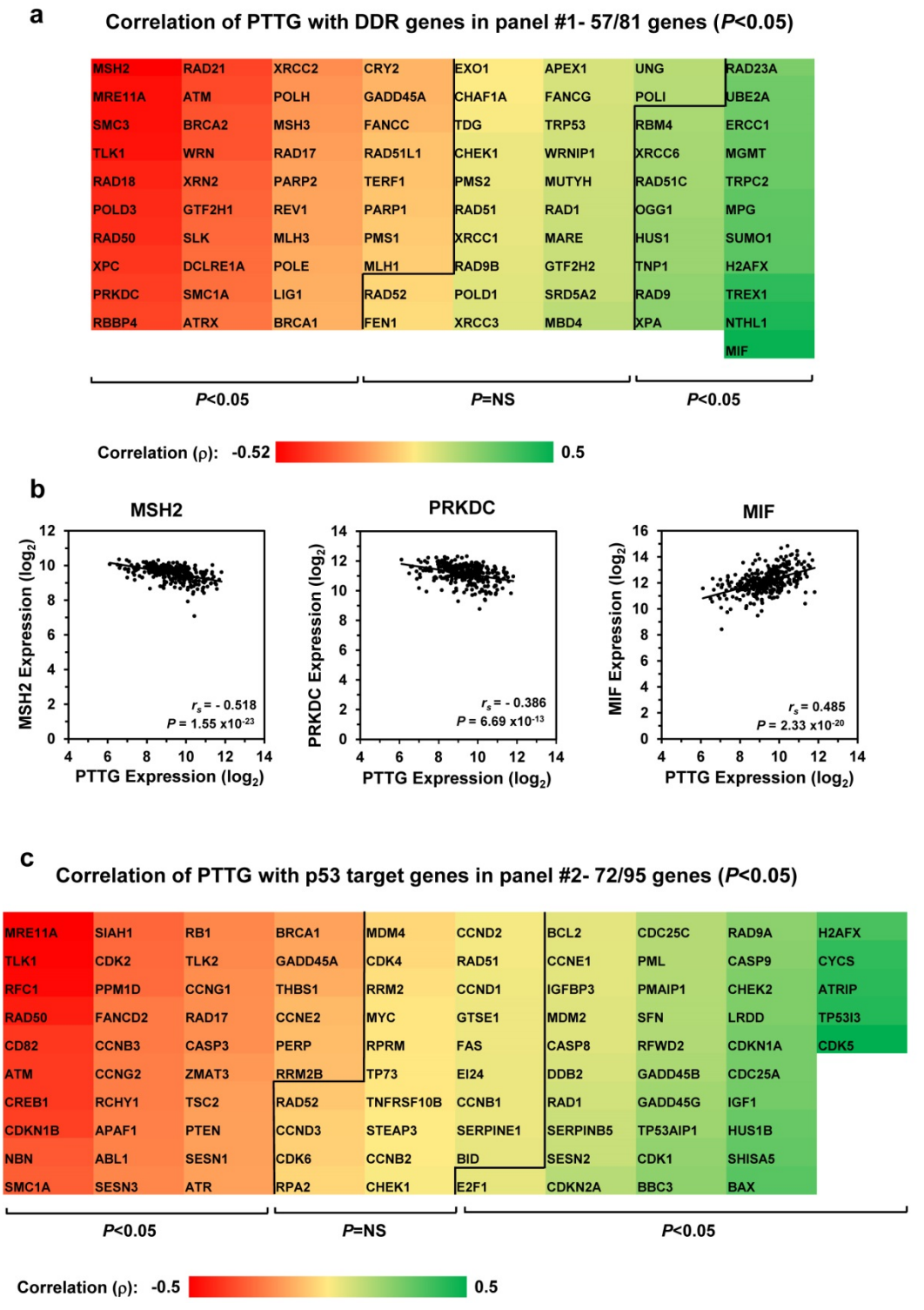
Supplementary Figure S14



Supplementary Figure S14. Correlation of PBF expression in DDR and p53 target gene panels. (**a**) Heatmap showing relative correlation values (ρ) for PBF expression with a panel (#1) of 82 DDR genes using TCGA data (*n*=322 unmatched DTC samples). Significant correlations (**P*<0.05) were observed with PBF for ~60% of DDR genes (*n*=49/82 genes). *P* and ρ values were calculated using the Spearman's correlation test. (**b**) Heatmap showing relative correlation values (ρ) for PBF expression with a panel (#2) of 95 p53 target genes using TCGA data (*n*=322 unmatched DTC samples). Significant correlations (**P*<0.05) were observed with PBF for 62% of p53 target genes (*n*=59/95 genes; Spearman's correlation test). (**c**) Representative scatterplots showing significant correlations for expression of *BCL2*, *BID* and *CCND1* with PBF (*n*=322; Spearman's correlation test). Further information on gene panel #1 and #2 is provided in Supplementary Table S1.

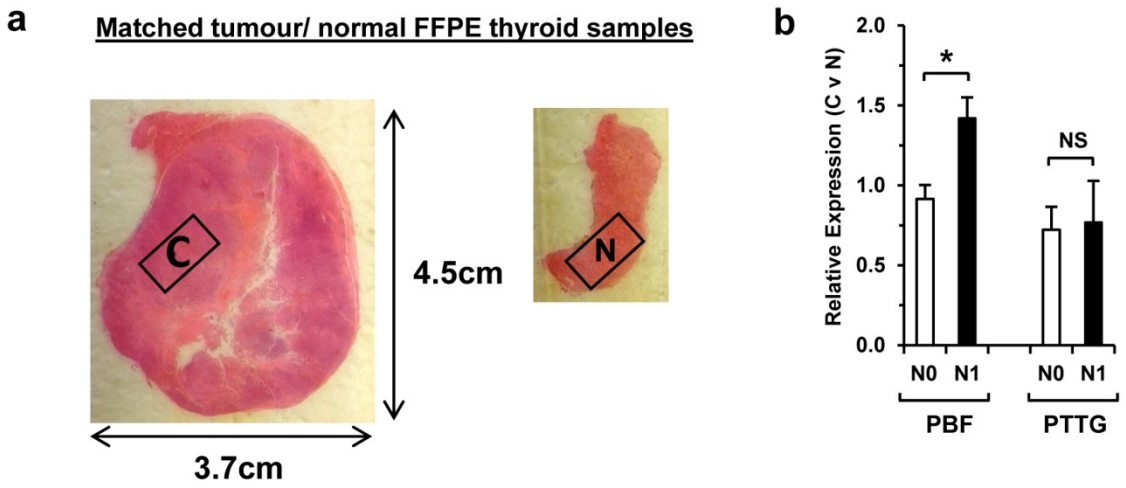
Supplementary Figure S15

Unmatched DTC dataset (*n*=322)



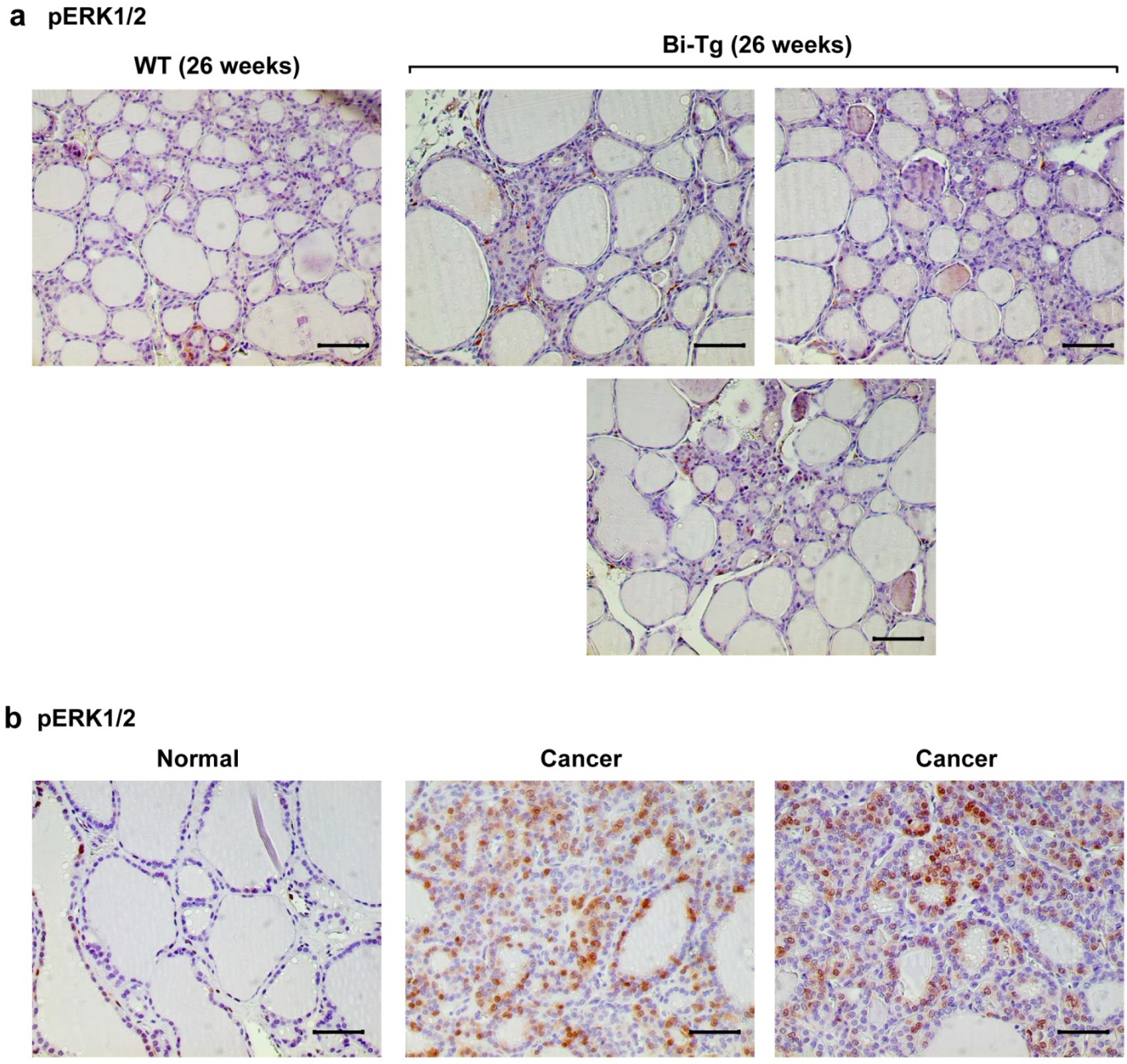
Supplementary Figure S15. Correlation of PTTG expression in DDR and p53 target gene panels. (**a**) Heatmap showing relative correlation values (ρ) for PTTG expression with a panel (#1) of 81 DDR genes using TCGA data (*n*=322 unmatched DTC samples). Significant correlations (**P*<0.05) with PTTG were observed for 70.4% of DDR genes (*n*=57/81 genes). *P* and ρ values were calculated using the Spearman's correlation test. (**b**) Representative scatterplots showing significant correlations for expression of *MSH2*, *PRKDC* and *MIF* with PTTG (*n*=322; Spearman's correlation test). (**c**) Heatmap showing relative correlation values (ρ) for PTTG expression with a panel (#2) of 95 p53 target genes using TCGA data (*n*=322 unmatched DTC samples). Significant correlations (**P*<0.05) with PTTG were observed for 75.8% of p53 target genes (*n*=72/95 genes; Spearman's correlation test). Further information on gene panel #1 and #2 is provided in Supplementary Table S1.

Supplementary Figure S16



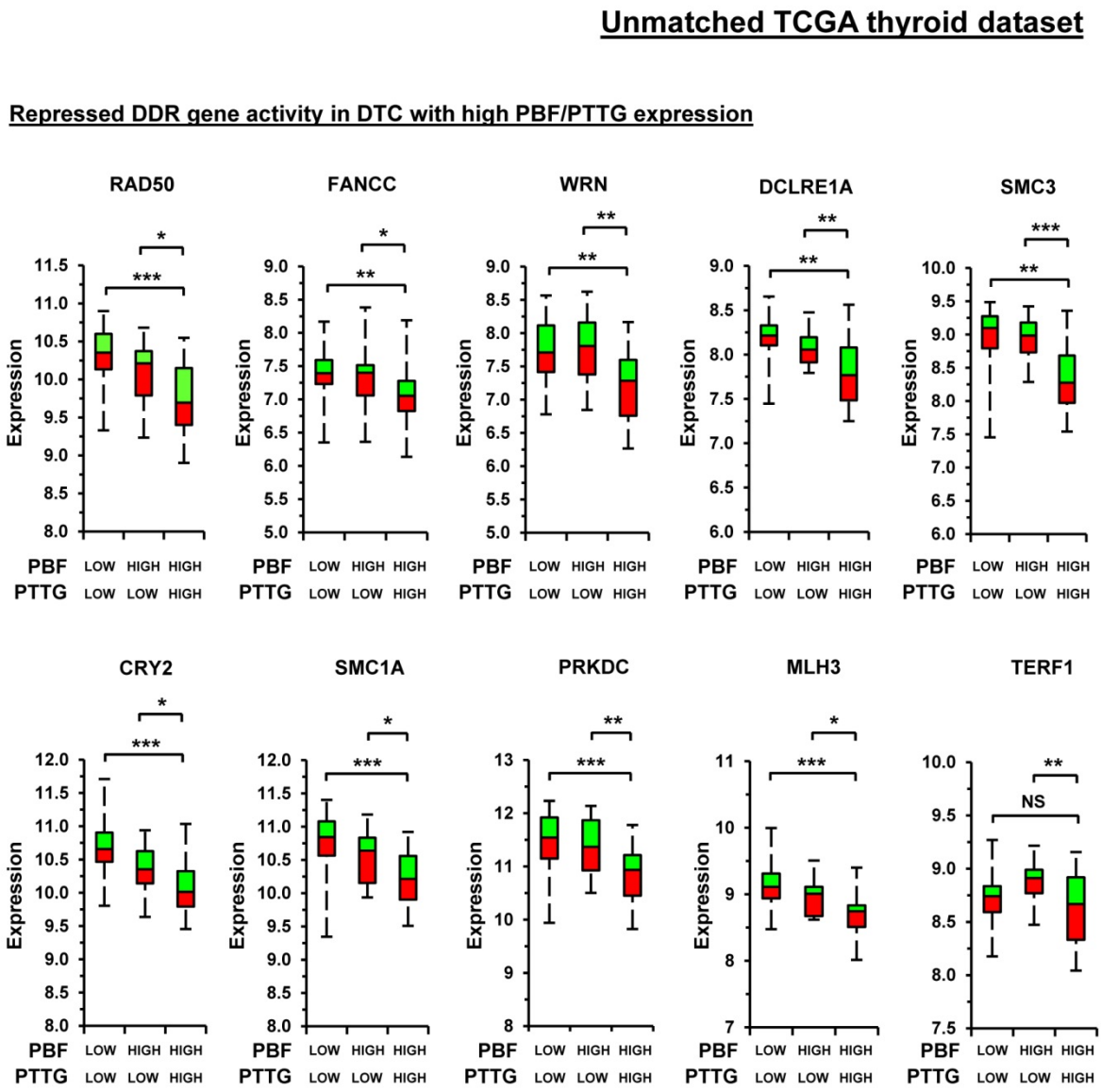
Supplementary Figure S16. Association of metastatic DTC with increased PBF expression. (**a**) Representative H&E stained images of matched FFPE DTC (C) and normal (N) thyroid tissue. Boxed areas represent boundaries of tissue used for RNA extraction. (**b**) Quantification of relative PBF (N0, *n*=27; N1, *n*=7) and PTTG (N0, *n*=23; N1, *n*=6) mRNA expression in metastatic DTC (N1) and non-metastatic DTC (N0) relative to matched normal tissue (mean±s.e.m., two-tailed Mann-Whitney test) (NS, not significant; **P*<0.05).

Supplementary Figure S17



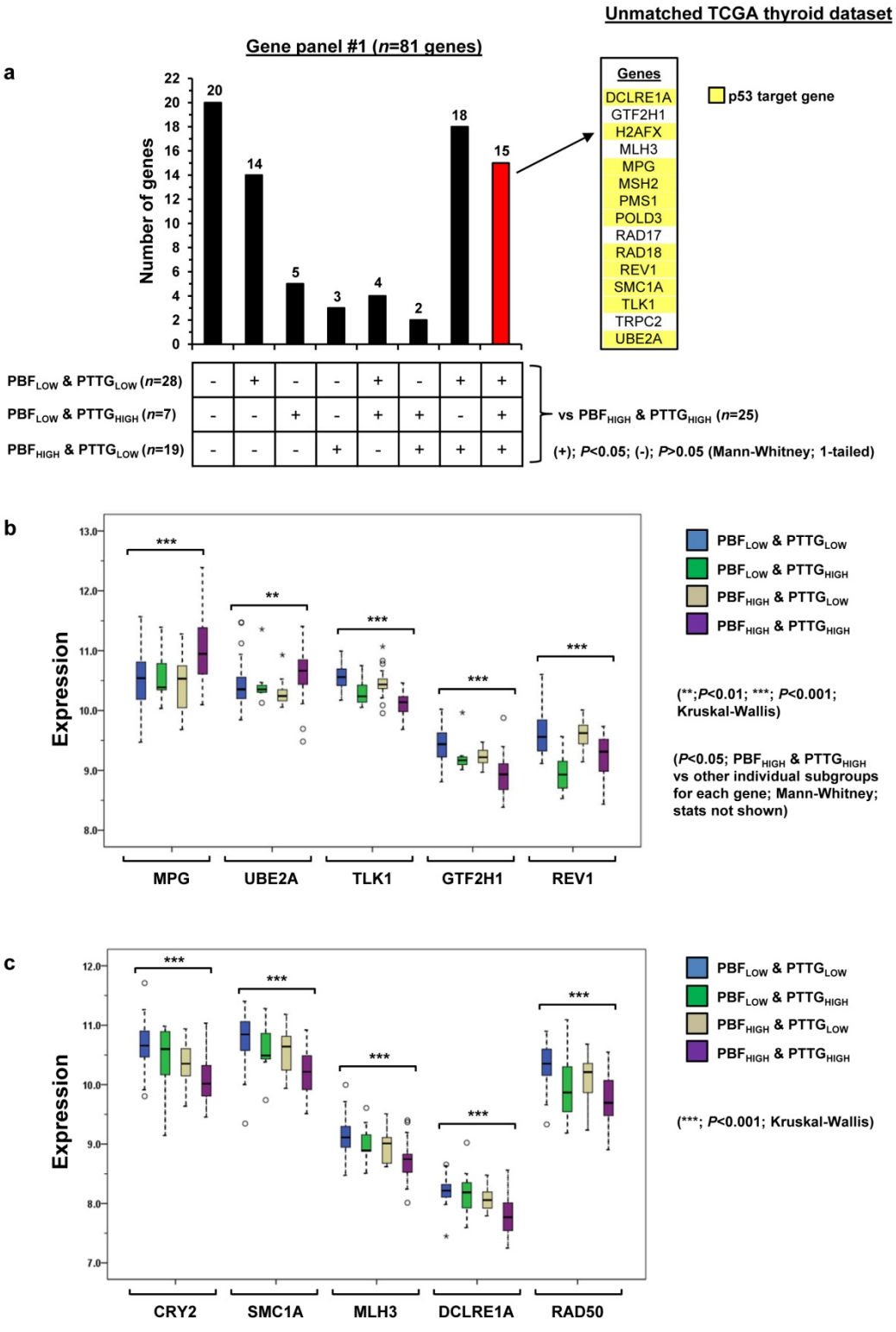
Supplementary Figure S17. Expression of pERK1/2 in thyroid tissue. (**a**) Representative images of pERK1/2 expression in wild-type (WT) and Bi-Tg thyroids in 26 week old mice. (**b**) Representative images of pERK1/2 expression in a human differentiated thyroid tumour (middle and right panels) and normal thyroid tissue (left panel). Scale bars, 100 μm.

Supplementary Figure S18



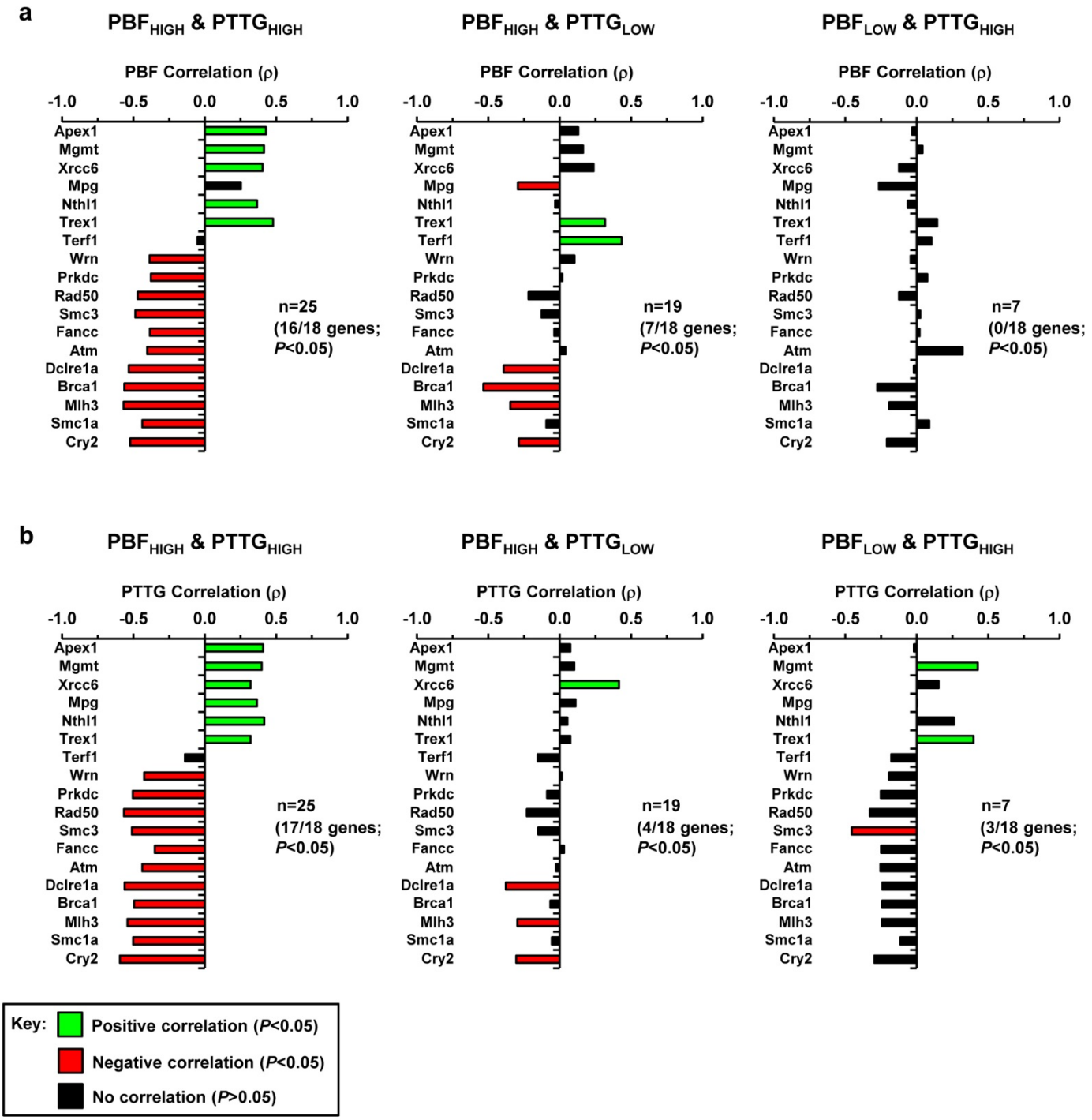
Supplementary Figure S18. Repressed DDR gene activity in DTC with high PBF/PTTG expression. Box-whisper plots showing relative expression of 10 DDR genes in unmatched DTC with different subsets of PBF and PTTG expression. *P*-values were determined by the Mann-Whitney test (median, PBF/PTTG expression groups: low/low, *n*=28; high/low, *n*=19; high/high, *n*=25) (**P*<0.05; ***P*<0.01; ****P*<0.001). DTC samples with low PBF/high PTTG expression were not used due to insufficient numbers for meaningful and comparative analysis. Non-parametric analysis of DTC samples comparing the 3 subsets of PBF/PTTG expression also showed a significant difference for all 10 DDR genes (***P*<0.01, Kruskal-Wallis test, analysis not shown).

Supplementary Figure S19



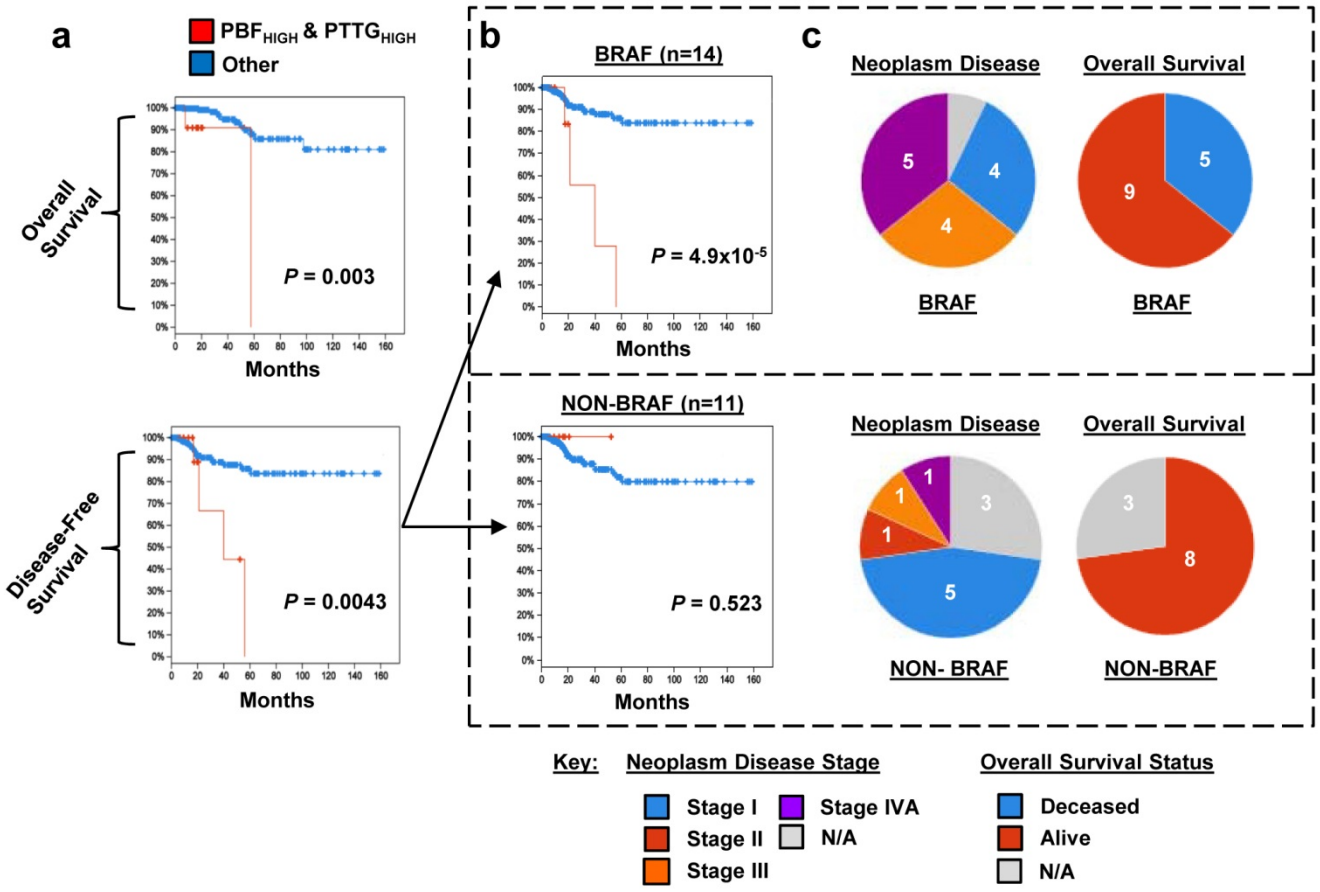
Supplementary Figure S19. DDR gene expression in unmatched DTC samples with different PBF/PTTG populations. (**a**) Graph summarises the number of DDR genes in panel #1 with expression levels significantly different in DTC with high PBF/high PTTG tumoural expression (*n*=25) compared to the other three DTC populations [(i.e. low PBF/low PTTG (*n*=28), low PBF/high PTTG (*n*=7) and high PBF/low PTTG (*n*=19)]. Expression of 15 DDR genes in DTC with high PBF/high PTTG expression were significantly different than in the 3 other DTC populations as indicated. Of these 15 DDR genes, 11 were identified as p53 target genes and are highlighted in yellow (see Supplementary Table S1) (***P*<0.05, Mann-Whitney test; 1-tailed). (**b-c**) Example box-whisper plots for the indicated genes (i.e. MPG, UBE2A, TLK1, GTF2H1, REV1, CRY2, SMC1A, MLH3, DCLRE1A and RAD50) in DTC with different PBF/PTTG expression subsets as indicated (***P*<0.01; ****P*<0.001; Kruskal-Wallis test).

Supplementary Figure S20



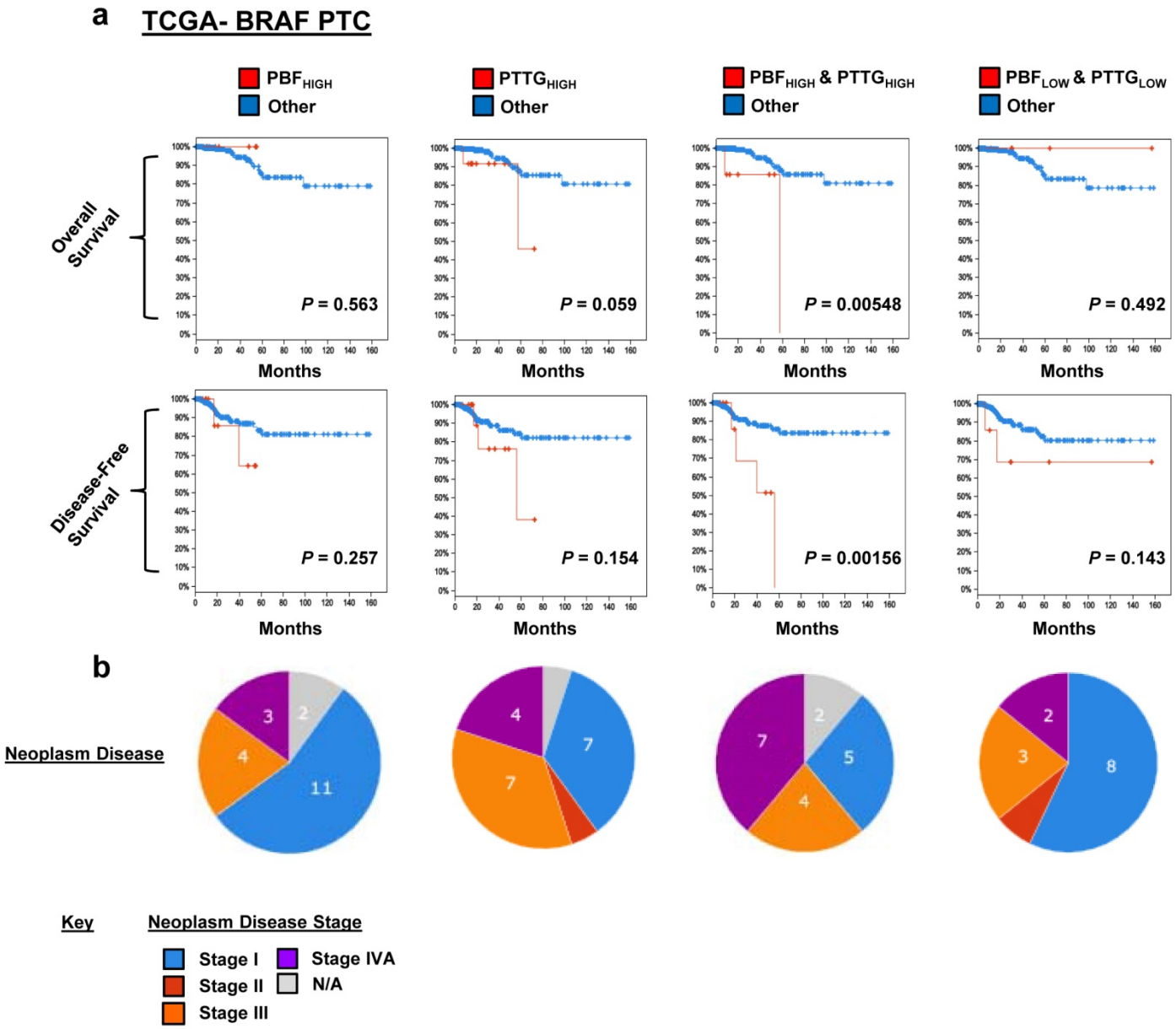
Supplementary Figure S20. Correlation of DDR genes in unmatched DTC samples with different PBF/PTTG populations. (**a**) Correlation of PBF expression with 18 DDR genes in unmatched DTC with both high PBF/high PTTG (n=25) and low PBF/low PTTG expression (n=28; 53 DTC samples in total). P and ρ values were calculated using Spearman's correlation tests; *P<0.05. Correlations were further determined in DTC with both high PBF/low PTTG and low PBF/low PTTG expression (middle panel; n=19; 47 DTC samples in total), as well as in DTC with both low PBF/high PTTG and low PBF/low PTTG expression (right panel; n=7; 35 DTC samples in total). (**b**) Same as (a) but correlation of PTTG expression was determined for 18 DDR genes in DTC samples. The majority of correlations were unique to DTC with high PBF/high PTTG expression (i.e. 11/16 genes for PBF and 14/17 genes for PTTG). Although in a few cases overexpression of one proto-oncogene appeared responsible for the correlation. There was significant correlation of PTTG, for instance, with three genes (MGMT, SMC3 and TREX1) in the low PBF/high PTTG population as well as in the high PBF/high PTTG population. In addition, there was a significant correlation of PBF with five genes (BRCA1, CRY2, DCLRE1A, MLH3 and TREX1) in the high PBF/low PTTG population as well as in the high PBF/high PTTG population.

Supplementary Figure S21



Supplementary Figure S21. PBF and PTTG expression with BRAF status is predictive of clinical outcome. (**a**) TCGA clinical data showing overall survival (upper) and disease-free survival (lower) curves for DTC with high PBF and PTTG expression (*n*=25) compared to all DTC cases (*n*=255). *P*-values were determined using the log-rank test. (**b**) Disease-free survival curves for BRAF-mutant (*n*=14) and non-BRAF mutant DTC (*n*=11) with high PBF and PTTG expression. *P*-values were determined using the log-rank test. (**c**) Pie charts summarize the neoplasm disease stage and overall survival status of DTC with high PBF and PTTG expression and either mutant BRAF (upper) or non-mutant BRAF (lower).

Supplementary Figure S22

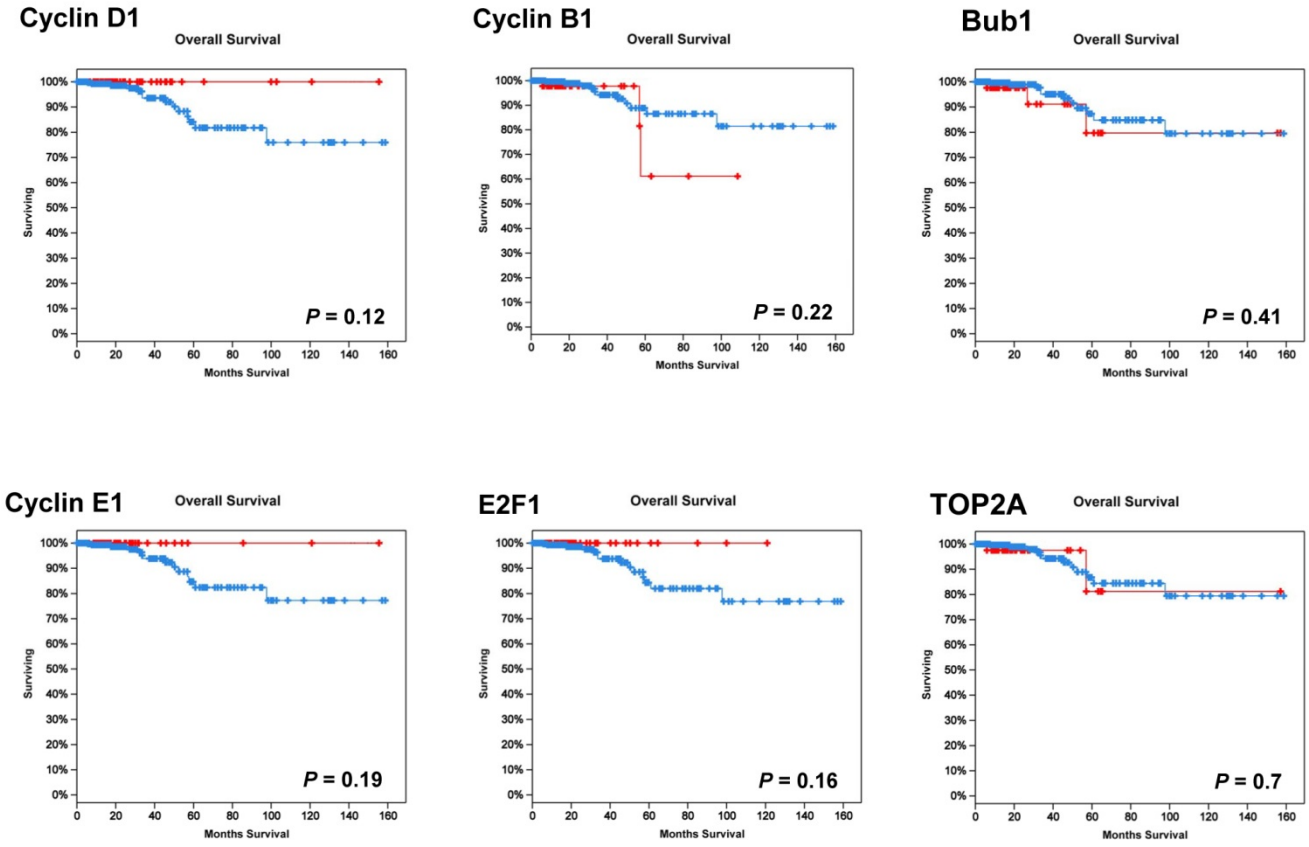


c **Most frequently associated mutations in BRAF-mutant DTC with high PBF/PTTG expression**

Frequency	*Gene*
16.7%	USP9X: Ubiquitin Specific Peptidase 9, X-Linked
11.1%	NUP93: Nucleoporin 93kDa
11.1%	SPTA1: Spectrin, Alpha, Erythrocytic 1
11.1%	COL5A1: Collagen, Type V, Alpha 1

Supplementary Figure S22. Reduction in patient survival with BRAF-mutant DTC and high PBF/PTTG tumour expression. (**a**) TCGA clinical data showing overall survival (upper) and disease-free survival (lower) curves for BRAF-mutant DTC of different PBF and PTTG expression subsets compared to all DTC cases. *P*-values were determined by the log-rank test (expression subsets: high PBF (*n*=20), high PTTG (*n*=20), high PBF/high PTTG (*n*=18) and low PBF/low PTTG (*n*=14). (**b**) Pie charts summarize the neoplasm disease stage of BRAF-mutant DTC with different PBF and PTTG expression subsets. (**c**) Table shows most frequently associated mutations (>10% incidence) in BRAF-mutant DTC with high PBF and PTTG expression.

Supplementary Figure S23



Supplementary Figure S23. Lack of association between TCGA patient survival outcome and a panel of cellular proliferation markers. TCGA clinical data showing overall survival curves for DTC with high tumoural expression (i.e. Q4- upper quartile of gene expression in unmatched TCGA cases, *n*=65) of six proliferation markers compared to all DTC cases (*n*=255). Proliferation markers analysed were cyclin D1, cyclin B1, bub1, cyclin E1, e2f1 and top2a as indicated. *P*-values were determined by the log-rank test.