EN-13-1646

Supplemental Figures 1-8

Supplemental Fig. 1. Short tandem repeat (STR) profiling of cell lines. Panels A-D show a copy of the Cell Line DNA Typing report and electropherogram obtained for each cell line used in this study following STR analysis by the accredited company DNA Diagnostics Company (DDC, Britannia House, London, UK). We observed 86-100% match of the specific allele markers for our cell lines with those reported in on-line STR analysis databases (e.g.*http://www.dsmz.de/services/services-human-and-animal-cell-lines/online-str analysis.html*) or in published studies [e.g. Schweppe RE *et al.,* (2008) JCEM 93(11): 4331-4341]. According to American Type Culture Collection (ATCC) a threshold of 75% identity and above is acceptable to consider a cell line as genuine. Therefore, our observed values of 100% (K1), 93.8% (TPC1), 88.2% (SW1736) and 86.1% (H1299) are sufficiently above this threshold to consider the cell lines used as genuine.

Supplemental Fig. 2. Establishing optimal irradiation dose and timing to initiate a p53 response in thyroid cells. A, Western blot analysis of p53 in K1 cells irradiated (+) with 15 Gy dose and p53 protein levels monitored at 0, 2, 8 or 24 h post-treatment compared to untreated (-) controls. Graph shows quantified p53 levels relative to β-actin for each condition. Highest p53 levels were observed at 8 h post-irradiation. B, Quantified p53 levels relative to β-actin in TPC1 cells irradiated with 0 to 40 Gy dose as indicated for 8 h (left), or irradiated (+) with 15 Gy dose and p53 protein levels monitored at 0, 2, 8 or 24 h post-treatment compared to untreated (-) controls (right). Western blots presented in Figure 1C were used for these quantifications. Highest p53 levels were observed with a 15 Gy dose at 8 h post-irradiation. C, Western blot analysis of p53 in K1 cells irradiated with 0 to 40 Gy dose as indicated for 8 h. Graph shows quantified p53 levels relative to β-actin for each condition. Highest p53 levels were observed with a 15 Gy dose. D, Relative mRNA levels of indicated p53-responsive gene in TPC1 cells following

irradiation with 15 Gy for 8 h (+IR) or untreated (-IR). Data presented as mean ± SE from 3 independent experiments. *, $P < 0.05$; ***, $P < 0.001$.

Supplemental Fig. 3. Proximity ligation assays (PLA) in thyroid cells. A, PLA assay to demonstrate PBF and p53 interaction by presence of red clusters in TPC1 cells transiently transfected for 24 h with plasmid expression vectors for p53 and HA-tagged PBF. Nuclear compartments were stained with Hoechst dye as indicated. Antibodies used were anti-HA.11 (Covance Research) and p53 (DO-1, Santa Cruz). B, Control experiment showing absence of red blobs in TPC1 cells when antibodies were omitted from the PLA assay. C, PLA assay to demonstrate specific PBF and p53 interaction by presence of red spots in K1 cells using same conditions as in (A). Scale bars: 10 μM.

Supplemental Fig. 4. Functional studies of PBF dysregulation in thyroid cells. A, TPC1 cells were transfected with either PBF-specific or control siRNA for 48 h and either irradiated (+IR) with a 15 Gy dose or untreated (-IR) prior to analysis of cell viability 24 h later. For each experiment cell viability was determined from n=5 per condition. Data presented as mean ± SE from 4 independent experiments. B, Analysis of caspase-3/7 activity in K1 cells transfected with either PBF-specific or control siRNA for 48 h and then irradiated with a 15 Gy dose (+IR) or untreated (-IR). For each experiment caspase-3/7 activity was determined from n=5 per condition at 24 h post-irradiation. Data presented as mean ± SE from 3 independent experiments. C, SW1736 cells were transfected with either vector only (VO) or PBF for 24 h and either untreated (-IR) or irradiated (+IR) with a 15 Gy dose. Cells were then replated and viability measured after 24 h. *, $P < 0.05$; **, $P < 0.01$; NS – not significiant.

Supplemental Fig. 5. Altered gene expression of DNA repair genes in irradiated PBF-Tg thyrocytes. Graph shows relative mRNA expression levels of 10 genes (≥ 1.5-fold; $P < 0.05$) following irradiation of PBF-Tg thyrocyte cultures. Fold changes are relative to gene expression levels in irradiated WT thyrocyte cultures. Data presented as mean ± SE from 3 independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS = not significant.

Supplemental Fig. 6. Validation of mRNA changes in focused array experiments. A, qPCR analysis of relative mRNA levels for *Mgmt*, *Rad51*, *Chek1* and *Tp53* following irradiation of either WT or PBF-Tg thyrocyte cultures as indicated. Data was normalized to adjust for effects of the PBF transgene on gene expression. B, Same data for *Mgmt* and *Rad51* as in (A) except all fold changes shown are relative to gene expression levels in non-irradiated WT thyrocytes. Data presented as mean ± SE from 4 independent experiments. **, $P < 0.01$; ***, $P < 0.001$; NS = not significant.

Supplemental Fig. 7. Elevated Rad6 expression in PBF-Tg thyroids. A, Representative images of Rad6 staining in thyrocytes in WT and PBF-Tg thyroids are shown. B, Representative images of Rad6 and PBF (HA) immunostaining of hyperplastic lesions (upper and lower panels) in PBF-Tg thyroid glands. A specific HA antibody was used to detect the HA-tagged PBF protein. C, Representative H&E stained image of a hyperplastic lesion in a PBF-Tg mouse thyroid. Scale bars: 100 µm.

Supplemental Fig. 8. Sequencing of p53 mRNA in human thyroid tumours. A, The *TP53* gene was sequenced to determine mutational status in 11 human thyroid tumours. Total mRNA was extracted from tumor tissue and p53 mRNA amplified to cDNA by OneStep RT-PCR (Qiagen). In total 4 different primer sets (P1-P4) were used to amplify the entire p53 mRNA. A representative agarose gel is shown demonstrating specific amplification using 3 different primer sets (P1-P3) with 3 different tumour RNA samples. L=DNA ladder. B, Details of primer used in p53 sequencing based on published sequences [Sjogren S *et al.,*(1996) J Natl Cancer Inst. 88(3-4):173-182]. C, Representative electropherogram trace showing the first 316 base pairs of the *TP53* gene. The ATG translational start site is indicated by an arrow. Sequencing was performed using an ABI3730 capillary sequencer (Functional Genomics and Proteomics, University of Birmingham). All eleven human thyroid tumours had wild-type p53 sequence.

Supplemental Figure 1

A H1299 cells

Cell Line DNA Typing Report

Report Date 9/25/13

DDC is accredited/ certified by AABB, CAP, ACLASS-International, ISO/IEC 17025, CLIA, NYSDOH & ASCLD/LAB-International.

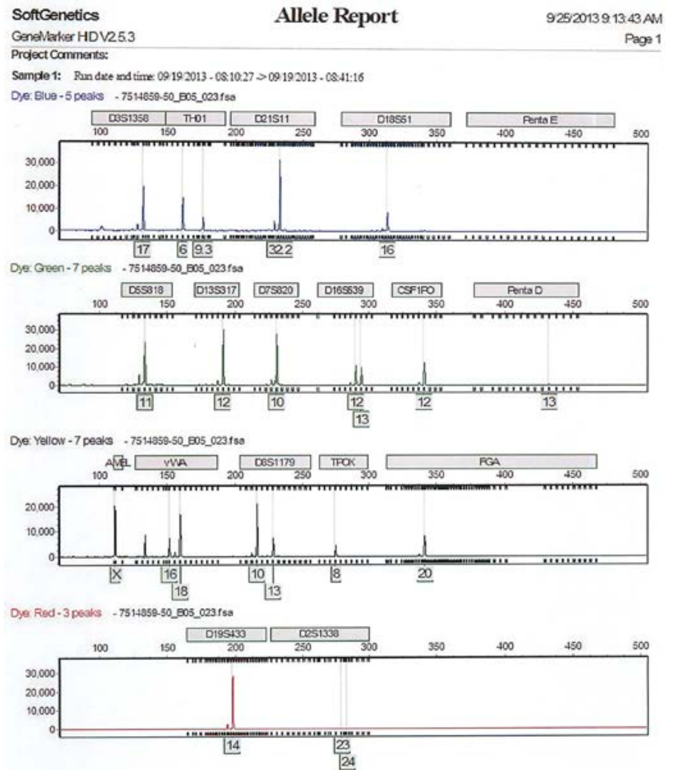
Case	7514859
Investigator	M. Read
Sample ID	H1299
DDC ID	7514859-50
Date Received	9/18/2013

Marker	Allele 1	Allele 2
D3S1358	17	
TH01	6	9.3
D21S11	32.2	
D18S51	16	
D5S818	11	
D13S317	12	
D7S820	10	
D16S539	12	13
CSF1PO	12	
Penta D	13	
AMEL	X	
vWA	16	18
D8S1179	10	13
TPOX	8	
FGA	20	
D19S433	14	
D2S1338	23	24

This DNA test was performed using the Promega PowerPlex 18 System.

Based on the samples received, I verify that the genetic data is correct as reported on 9/25/13

Thomas M. Reid, Ph.D.
Associate Laboratory Director



B TPC1 cells

Cell Line DNA Typing Report

Report Date 9/25/13

DDC is accredited/ certified by AABB, CAP, ACLASS-International, ISO/IEC 17025, CLIA, NYSDOH & ASCLD/LAB-International.

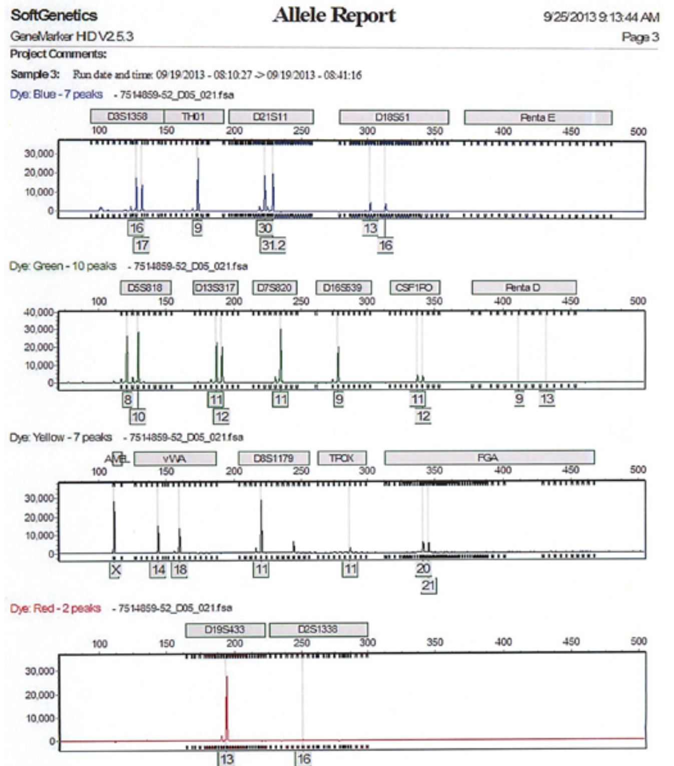
Case	7514859
Investigator	M. Read
Sample ID	TPC1
DDC ID	7514859-52
Date Received	9/18/2013

Marker	Allele 1	Allele 2
D3S1358	16	17
TH01	9	
D21S11	30	31.2
D18S51	13	16
D5S818	8	10
D13S317	11	12
D7S820	11	
D16S539	9	
CSF1PO	11	12
Penta D	9	13
AMEL	X	
vWA	14	18
D8S1179	11	
TPOX	11	
FGA	20	21
D19S433	13	
D2S1338	16	

This DNA test was performed using the Promega PowerPlex 18 System.

Based on the samples received, I verify that the genetic data is correct as reported on 9/25/13

Thomas M. Reid, Ph.D.
Associate Laboratory Director



Supplemental Figure 1

C K1 cells

Cell Line DNA Typing Report

Report Date 9/25/13

DDC is accredited/ certified by AABB, CAP, ACLASS-International, ISO/IEC 17025, CLIA, NYSDOH & ASCLD/LAB-International.

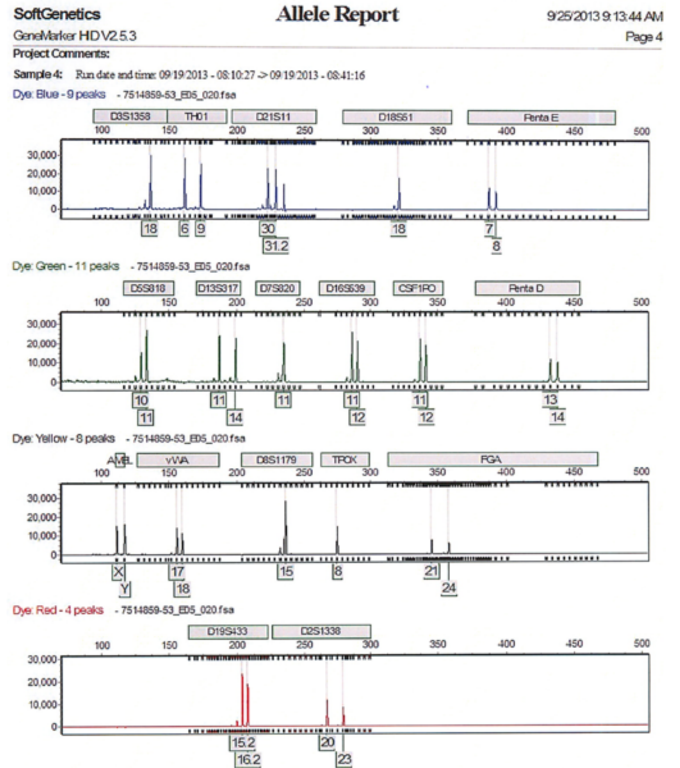
Case	7514859
Investigator	M. Read
Sample ID	K1
DDC ID	7514859-53
Date Received	9/18/2013

Marker	Allele 1	Allele 2
D3S1358	18	
TH01	6	9
D21S11	30	31.2
D18S51	18	
Penta E	7	8
D5S818	10	11
D13S317	11	14
D7S820	11	
D16S539	11	12
CSF1PO	11	12
Penta D	13	14
AMEL	X	Y
vWA	17	18
D8S1179	15	
TPOX	8	
FGA	21	24
D19S433	15.2	16.2
D2S1338	20	23

This DNA test was performed using the Promega PowerPlex 18 System.

Based on the samples received, I verify that the genetic data is correct as reported on 9/25/13

Thomas M. Reid, Ph.D.
Associate Laboratory Director



D SW1736 cells

Cell Line DNA Typing Report

Report Date 9/25/13

DDC is accredited/ certified by AABB, CAP, ACLASS-International, ISO/IEC 17025, CLIA, NYSDOH & ASCLD/LAB-International.

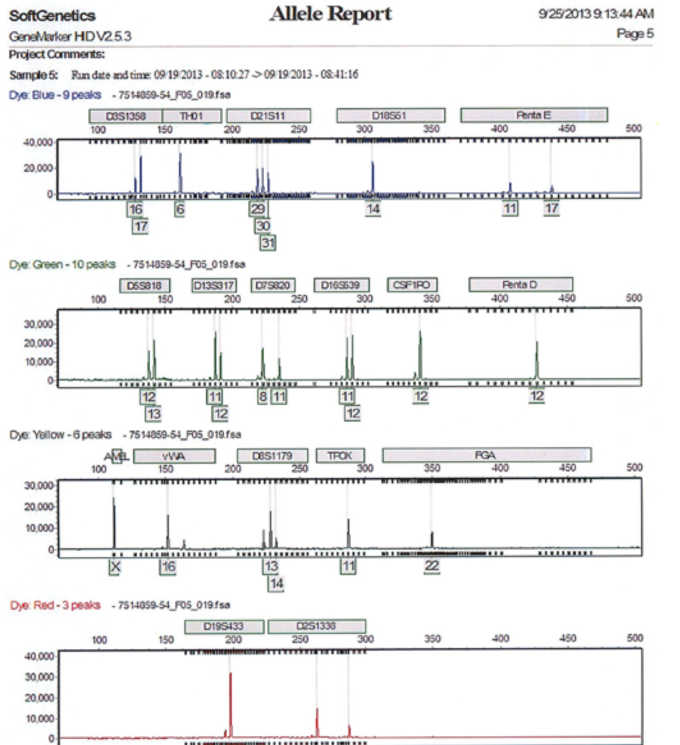
Case	7514859
Investigator	M. Read
Sample ID	SW1760
DDC ID	7514859-54
Date Received	9/18/2013

Marker	Allele 1	Allele 2	Allele 3
D3S1358	16	17	
TH01	6		
D21S11	29	30	31
D18S51	14		
Penta E	11	17	
D5S818	12	13	
D13S317	11	12	
D7S820	8	11	
D16S539	11	12	
CSF1PO	12		
Penta D	12		
AMEL	X		
vWA	16		
D8S1179	13	14	
TPOX	11		
FGA	22		
D19S433	14		
D2S1338	19	25	

This DNA test was performed using the Promega PowerPlex 18 System.

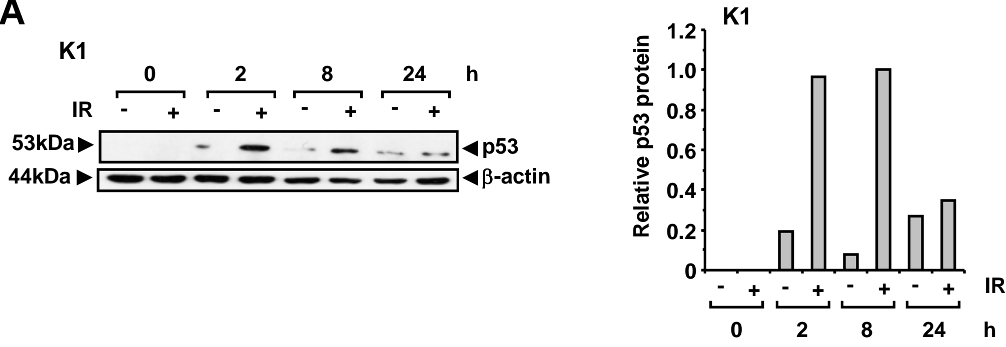
Based on the samples received, I verify that the genetic data is correct as reported on 9/25/13

Thomas M. Reid, Ph.D.
Associate Laboratory Director



Supplemental Figure 2

A



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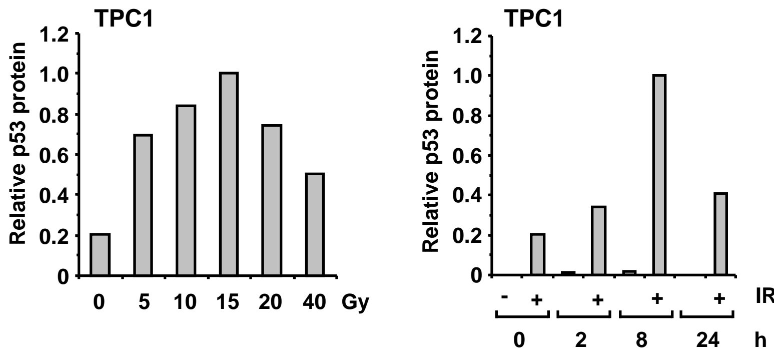

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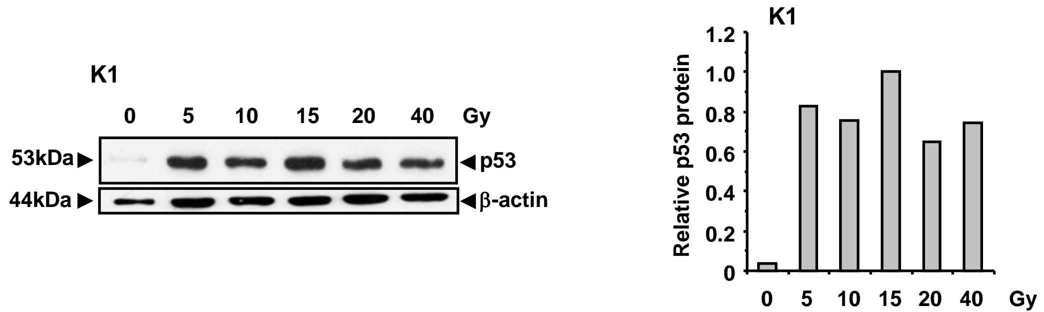

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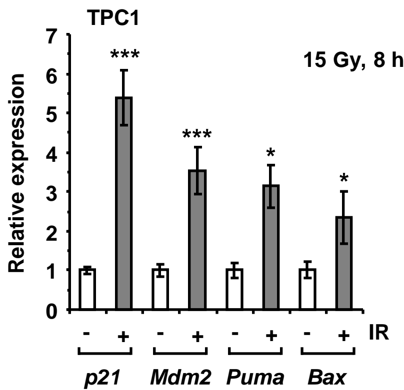
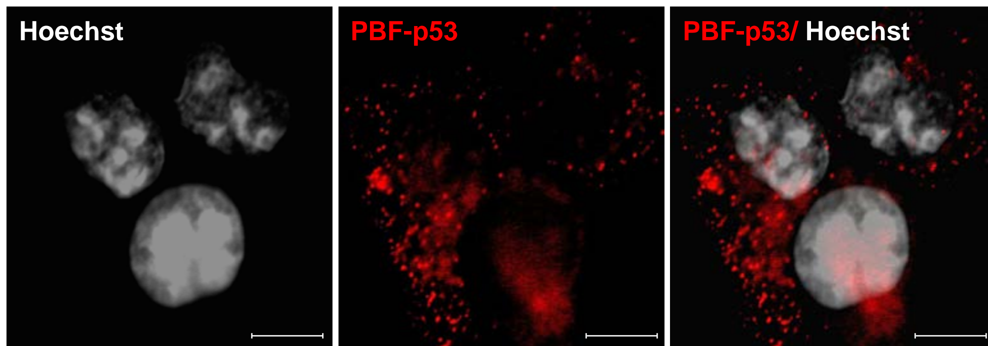


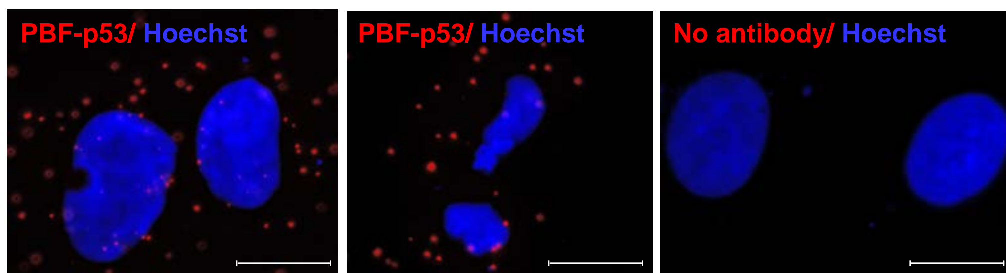
Supplemental Figure 3

A TPC1 cells

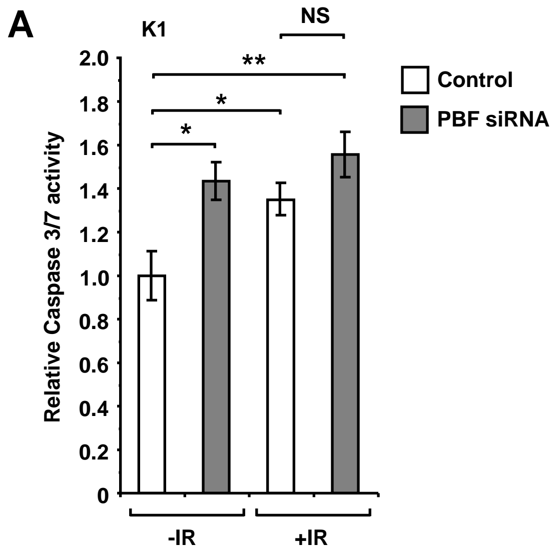


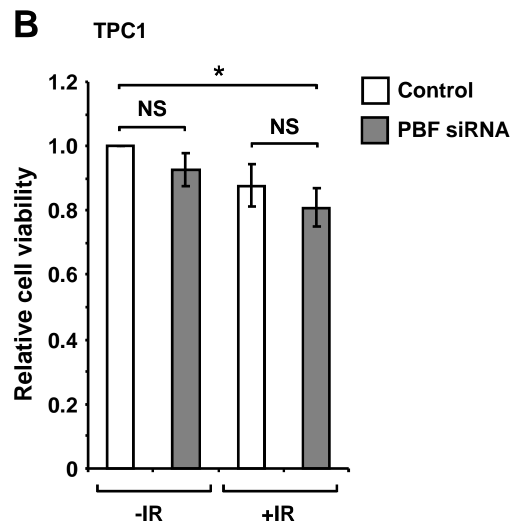
B TPC1 cells

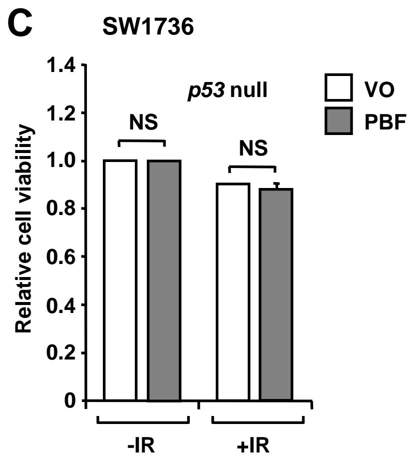
C K1 cells



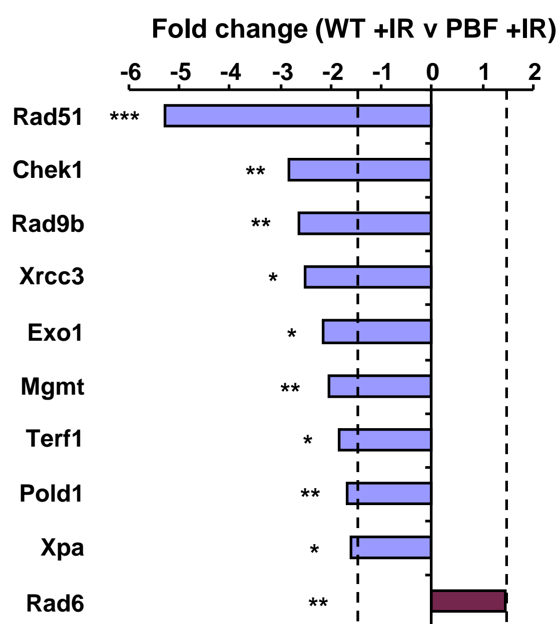
Supplemental Figure 4



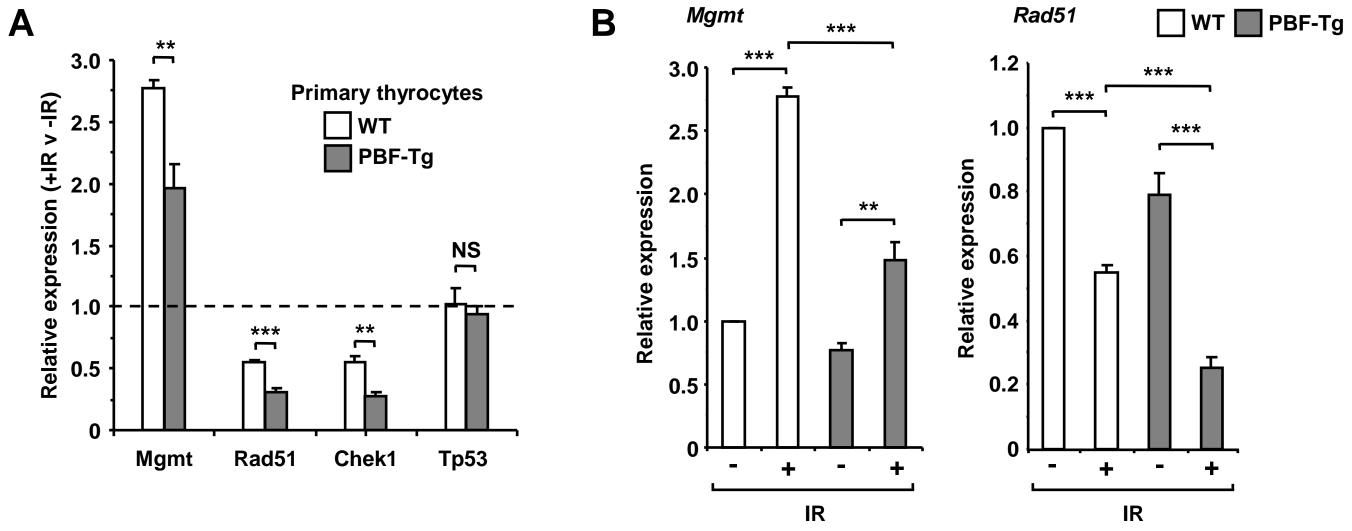




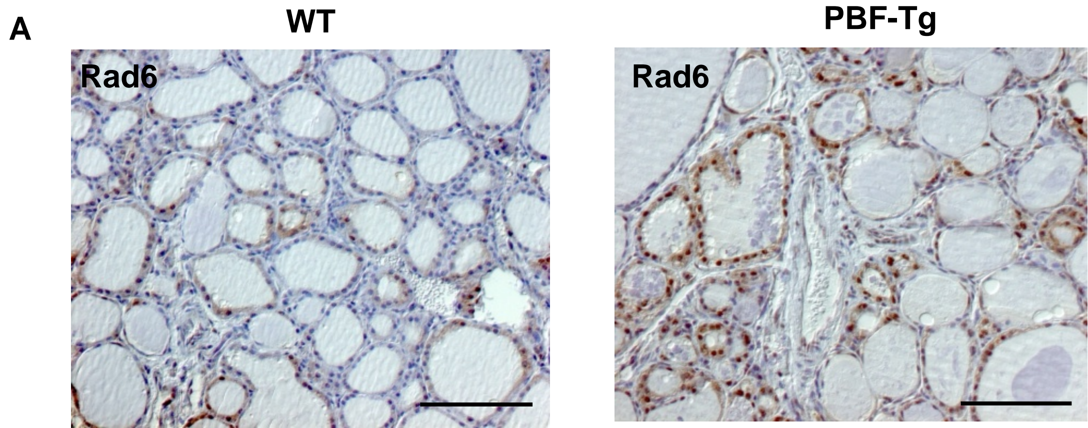
Supplemental Figure 5

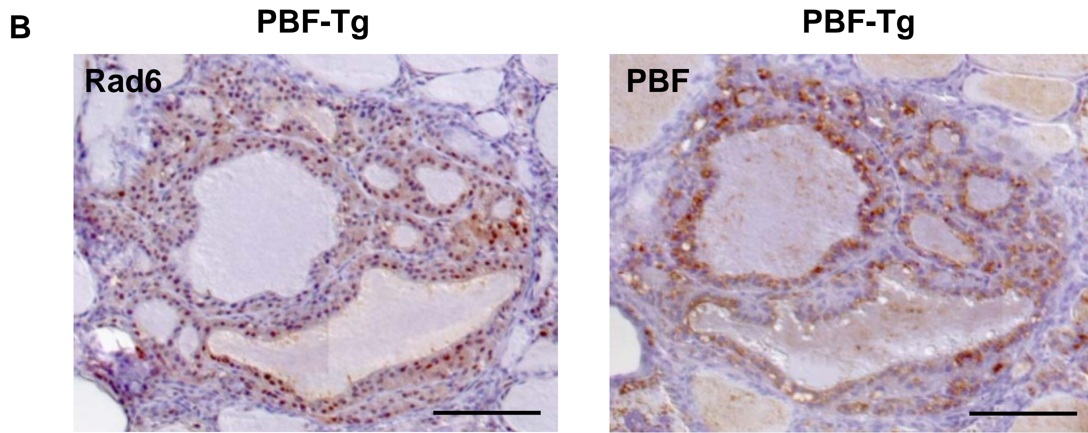


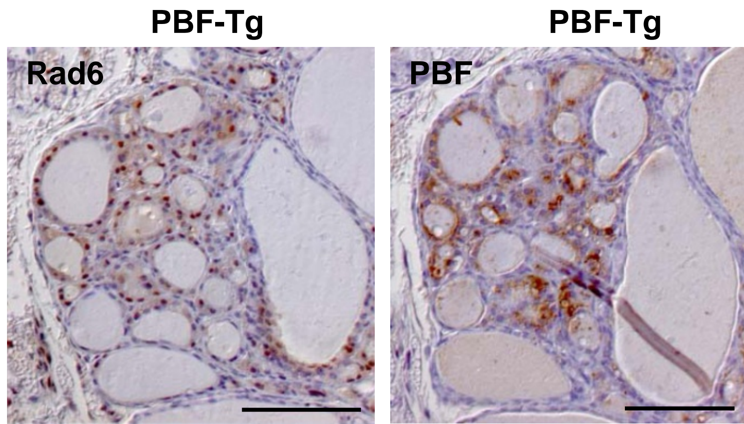
Supplemental Figure 6

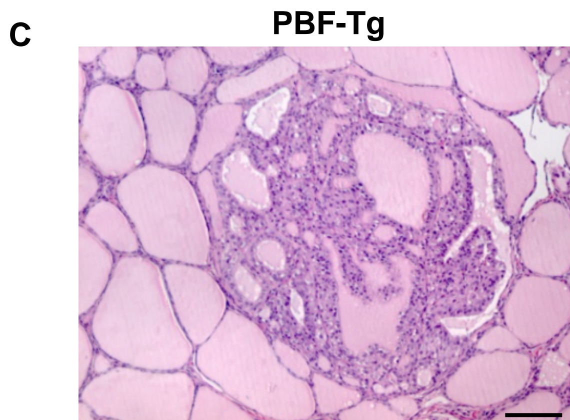


Supplemental Figure 7



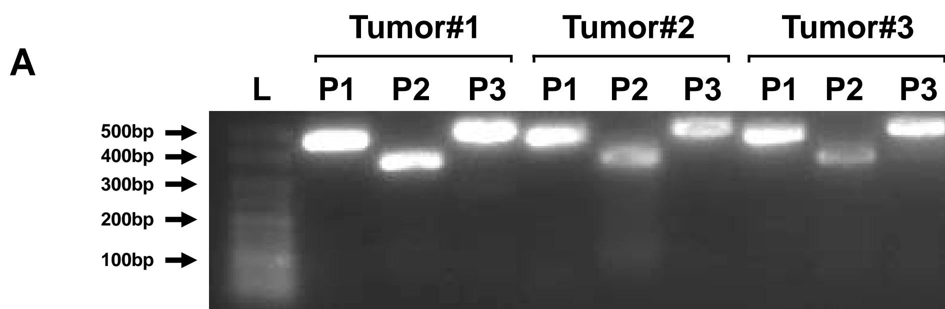






Supplemental Figure 8

A



B **Primers used in p53 sequencing**

	Primer pairs (5’ to 3’)	Direction	p53 exons amplified	PCR product length
P1	GACACGCTTCCCTGGATTGG GCAAAACATCTTGTTGAGGGC	F R	1, 2, 3 and 4 (parts of 5)	451 bp
P2	GTTTCCGTCTGGGCTTCTTG GTACAGTCAGAGCCAACCTCAGG	F R	5 and 6 (parts of 4 and 7)	367 bp
P3	GGCCCCTCCTCAGCATCTTA CAAGGCCTCATTCAGCTCTCG	F R	6, 7, 8 and 9 (parts of 5 and 10)	481 bp
P4	GGCGCACAGAGGAAGAGAATC GCACACCTATTGCAAGCAAGG	F R	9, 10 and 11 (part of 8)	443 bp

C