

Supplementary Figure Legends

Supplementary Figure S5. A distinct transcriptional signature for DDR genes in Bi-Tg thyrocytes. **(a)** DDR genes down-regulated >1.5-fold (i.e. relative expression level ≤ 0.66) in Bi-Tg, PTTG-Tg and PBF-Tg thyrocytes vs WT controls as indicated (mean, $n=3$ arrays, unpaired two-tailed t -test, $P<0.05$). **(b)** Transcriptional signature of 79 DDR genes in PTTG-Tg (triangles), PBF-Tg (diamonds) and Bi-Tg (squares) thyrocytes (mean, $n=3$ arrays, ¹Kruskal-Wallis test, ²Mann-Whitney test) (NS, not significant; $*P=0.024$; $***P$ -values are shown). Red filled circle indicates a p53 target gene. Key features of transcriptional signature for Bi-Tg thyrocytes include: **(1)** significant repression of a subset of 41 DDR genes between Bi-Tg and PTTG-Tg with expression >0.8 (range 0.8-3.2 in PTTG-Tg; $***P=1.8 \times 10^{-5}$); **(2)** DDR genes repressed in Bi-Tg thyrocytes vs PBF-Tg and PTTG-Tg thyrocytes, including PMS1, GTF2H1, GADD45, MLH1, MGMT, GTF2H2, POLH, REV1, PARP2, DCLRE1A, PARP1, RAD51C, XRCC6, TERF1 and UNG; **(3)** significant down-regulation of DDR genes >1.5-fold ($n=31$ genes; $*P<0.05$) in Bi-Tg vs WT, and **(4)** significant repression of a subset of 38 DDR genes between Bi-Tg and PBF-Tg thyrocytes with expression <0.8 (range 0.15-0.8 in PTTG-Tg; $***P=9.42 \times 10^{-7}$). SRD5A2, TNP1 and RBBP4 not included from Figure 2a due to lack of expression in all 4 genotypes. **(c)** Order of DDR genes plotted in Figure 2b and c are shown.

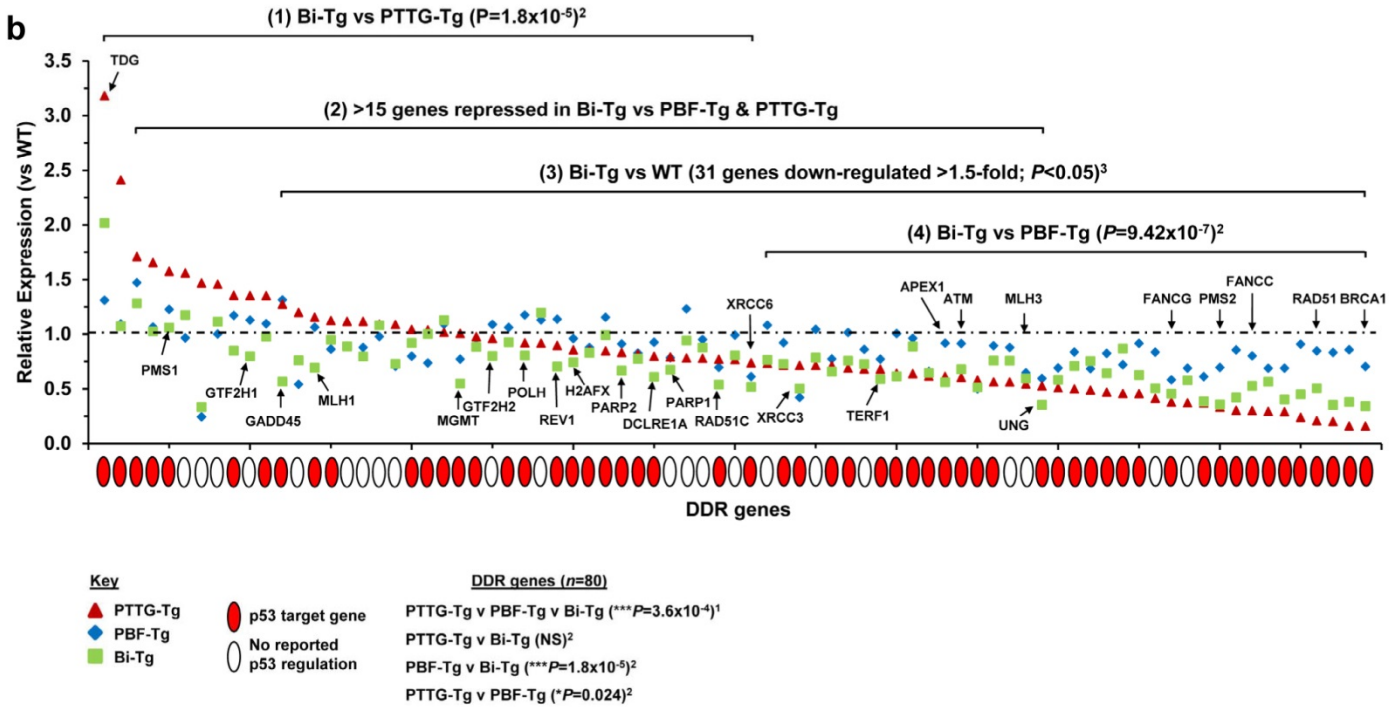
Supplementary Figure S6. Correlation of DDR transcriptional signature with p53 target gene status. **(a)** Transcriptional signature of subset of DDR genes in PTTG-Tg (triangles), PBF-Tg (diamonds) and Bi-Tg (squares) thyrocytes with relative expression >0.8 (range 0.8-3.2 in PTTG-Tg) (mean, $n=3$ arrays, ¹Kruskal-Wallis test, ²Mann-Whitney test, ³unpaired two-tailed t -test) (NS, not significant; $*P=0.018$; $**P=0.002$; $***P=1.8 \times 10^{-5}$). Filled circles (underneath) indicate the status of each DDR gene as a p53 target gene. p53 target gene categories include DDR genes regulated by a p53 response element (red), the p53-p21 pathway (orange), the p53-miRNA pathway (blue) and other pathways such as the p53-lincRNA-21 pathway and known interactions with transcription factors (black). Further information and literature evidence for p53 target gene categories is provided in Supplementary Table S1. **(b)** Same as (a) except but using subset of DDR genes with relative expression <0.8 (range 0.15-0.8 in PTTG-Tg) ($*P=0.017$; $***P$ -values are shown). DDR genes with relative expression <0.8 in PTTG-Tg were more likely to be regulated by the p53-miR-34 pathway (10/38 genes) than DDR genes in PTTG-Tg with expression >0.8 (2/41 genes; $P=0.011$; Fishers exact test). Expression cut-off value of 0.8 was chosen as it approximates to the mean expression value for all 79 DDR genes in the three transgene genotypes (i.e. 0.82 ± 0.04). SRD5A2, TNP1 and RBBP4 not included from Figure 2a due to lack of expression in all 4 genotypes. **(c)** Order of DDR genes plotted in panels (a) and (b) above.

Supplementary Figure S7. Expression levels of pChk1(Ser345) in thyroid tissue. Representative images of pChk1(Ser345) expression in wild-type (WT) and Bi-Tg thyroids in **(a)** 26 and **(b)** 78 week old mice. **(c)** Representative images of pChk1(Ser345) expression in a human differentiated thyroid tumour (middle and right panels) and normal thyroid tissue (left panel). Scale bars, 100 μm .

Supplementary Figure S5

a

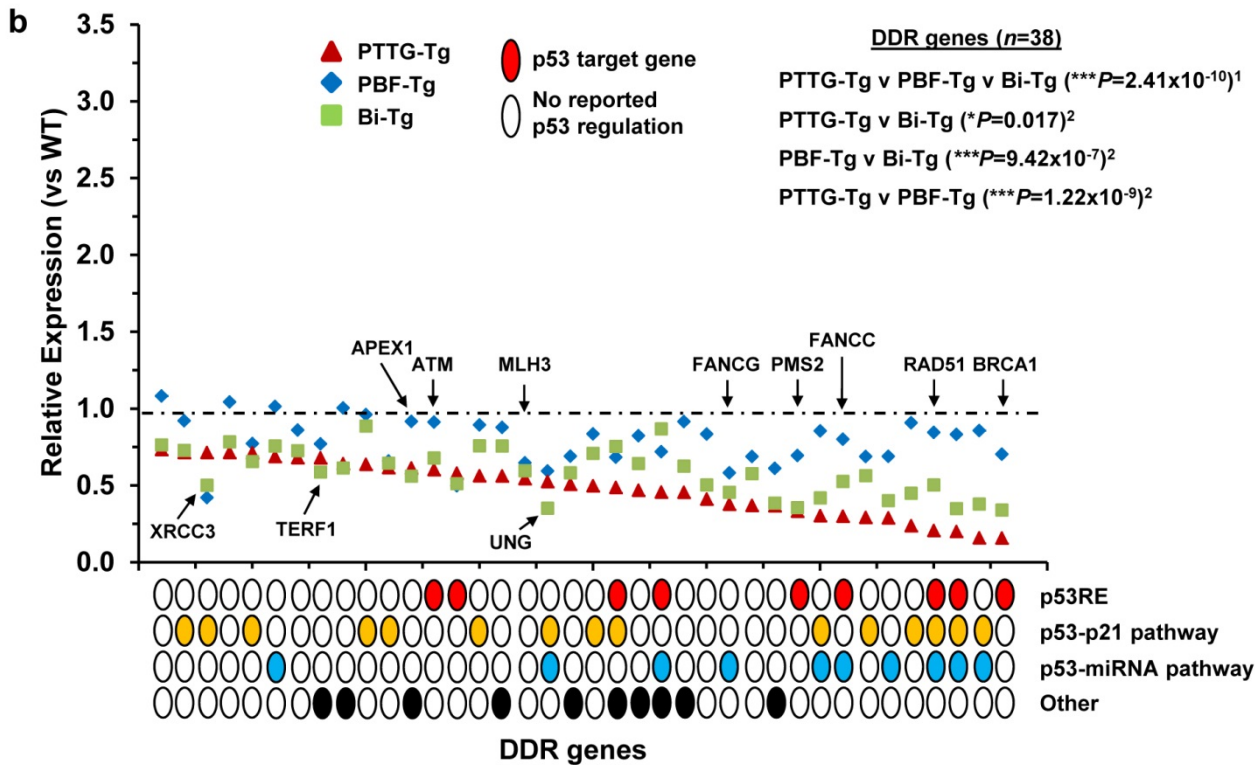
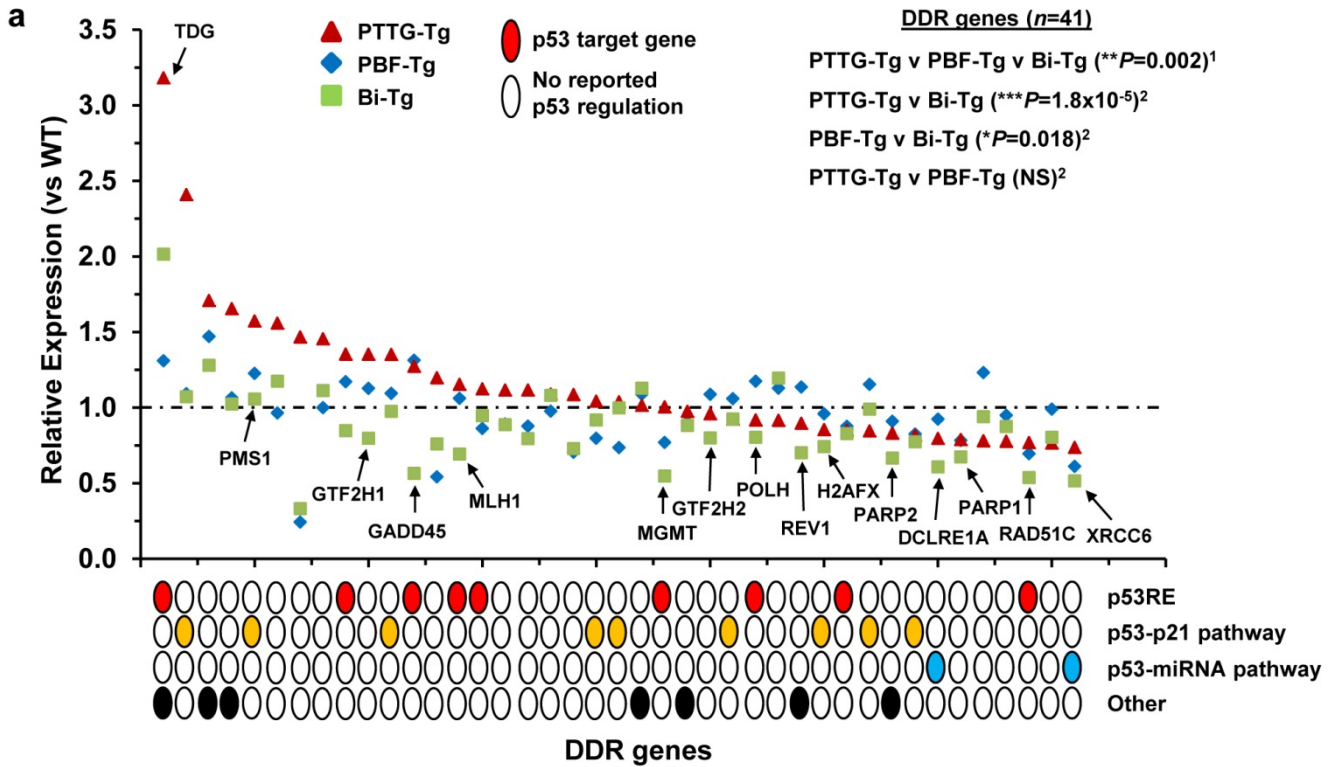
Genes down-regulated >1.5-fold in Bi-Tg thyrocytes ($P<0.05$) versus WT thyrocytes ($n=31$)
TRPC2, BRCA1, CHEK1, UNG, PMS2, EXO1, MBD4, CHAF1A, FEN1, POLE, FANCG, XRCC3, TREX1, MUTYH, XRCC6, FANCC, RAD51C, MGMT, APEX1, GADD45, RAD18, XPA, CRY2, TERF1, MLH3, DCLRE1A, MSH3, MPG, XRCC2, SMC3, PARP2
Genes down-regulated >1.5-fold in PTTG-Tg thyrocytes ($P<0.05$) versus WT thyrocytes ($n=29$)
BRCA1, EXO1, CHEK1, RAD51, POLE, CHAF1A, RAD18, FANCC, FEN1, PMS2, MBD4, XPA, FANCG, TREX, MPG, PTTG1, RAD50, POLD1, LIG1, CRY2, UNG, WRN, MUTYH, ATM, APEX1, XRCC2, PRKDC, MSH3
Genes down-regulated >1.5-fold PBF-Tg thyrocytes ($P<0.05$) versus WT thyrocytes ($n=10$)
TRPC2, XRCC3, MUTYH, RAD9B, FANCG, UNG, XRCC6, MBD4, MLH3, XRCC2



c

Order of DDR genes in Figure 2b
TDG, RAD9, UBE2A, HUS1, PMS1, NTHL1, TRPC2, MIF, TRP53, GTF2H1, RAD1, GADD45A, RAD9B, MLH1, XPC, POLI, RAD17, RAD51L1, RAD52, RAD21, RAD23A, RBM4, MGMT, OGG1, GTFH2, BRCA2, POLH, XRN2, REV1, H2AFX, MSH2, WRNIP1, PARP2, XRCC1, DCLRE1A, PARP1, ERCC1, SUMO, RAD51C, MARE, XRCC6
Order of DDR genes in Figure 2c
MRE11A, POLD3, XRCC3, SLK, SMC3, TLK1, ATRX, TERF1, MSH3, PRKDC, XRCC2, APEX1, ATM, MUTYH, SMC1A, WRN, MLH3, UNG, CRY2, LIG1, POLD1, RAD50, PTTG1, MPG, TREX, FANCG, XPA, MBD4, PMS2, FEN1, FANCC, RAD18, CHAF1A, POLE, RAD51, CHEK1, EXO1, BRCA1

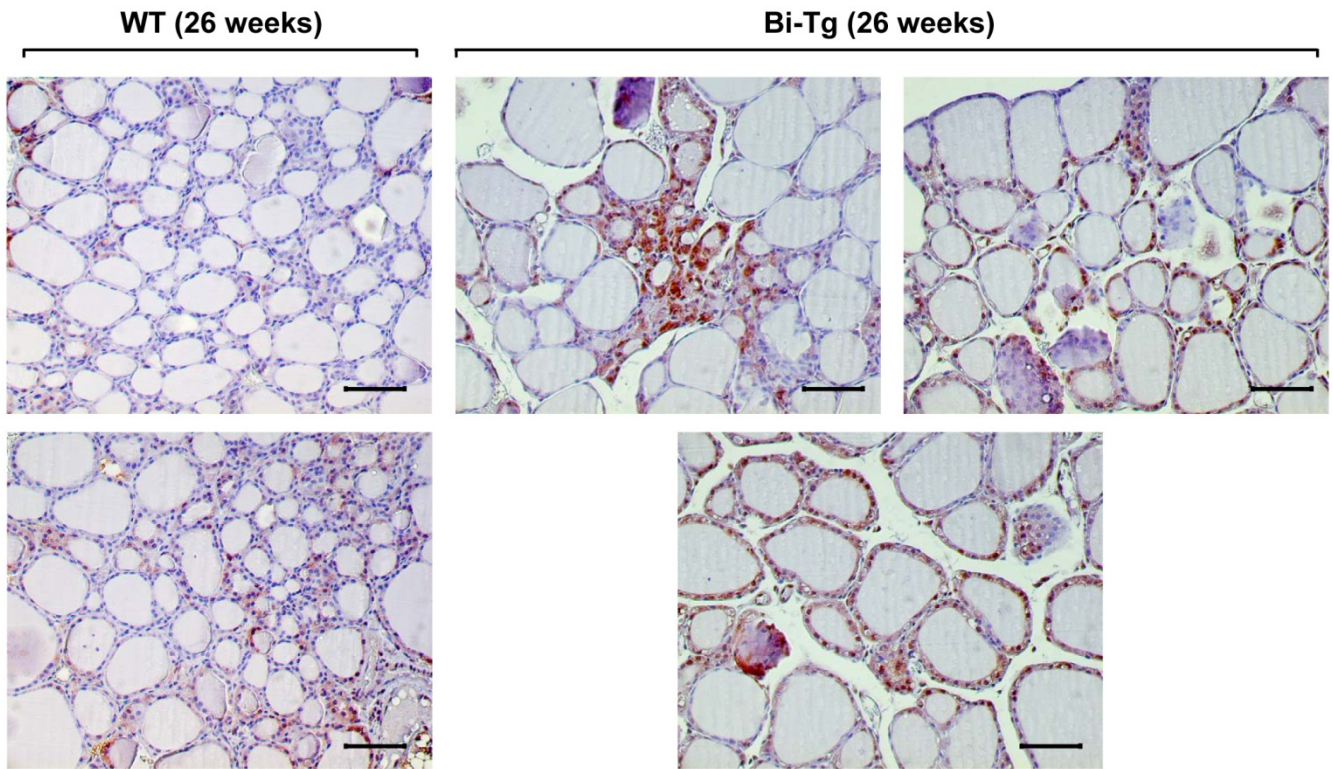
Supplementary Figure S6



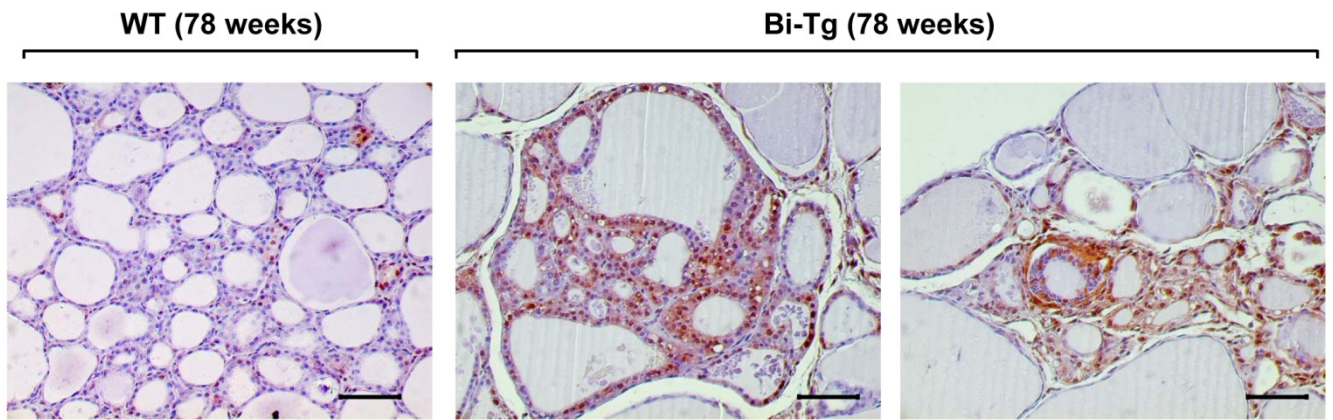
c Order of DDR genes. *Upper-* TDG, RAD9, UBE2A, HUS1, PMS1, NTHL1, TRPC2, MIF, TRP53, GTF2H1, RAD1, GADD45, RAD9B, MLH1, XPC, POLI, RAD17, RAD51L1, RAD52, RAD21, RAD23A, RBM4, MGMT, OGG1, GTFH2, BRCA2, POLH, XRN2, REV1, H2AFX, MSH2, WRNIP1, PARP2, XRCC1, DCLRE1A, PARP1, ERCC1, SUMO, RAD51C, MARE, XRCC6
Lower- MRE11A, POLD3, XRCC3, SLK, SMC3, TLK, ATRX, TERF1, MSH3, PRKDC, XRCC2, APEX1, ATM, MUTYH, SMC1A, WRN, MLH3, UNG, CRY2, LIG1, POLD1, RAD50, PTTG1, MPG, TREX1, FANCG, XPA, MBD4, PMS2, FEN1, FANCC, RAD18, CHAF1A, POLE, RAD51, CHEK1, EXO1, BRCA1

Supplementary Figure S7

a pChk1(Ser345)



b pChk1(Ser345)



c pChk1(Ser345)

