Supplementary Online Information for

The proto-oncogene PBF/PTTG1IP regulates thyroid cell growth and represses radioiodide treatment

Short title: PBF/PTTG1IP Role in Thyroid Disease

Martin L. Read[1], Greg D. Lewy[1], Jim C.W. Fong[1], Neil Sharma[1], Robert I. Seed[1], Vicki E. Smith[1], Erica Gentilin[2], Adrian Warfield[3], Margaret C. Eggo[1], Jeffrey A. Knauf[4], Wendy E. Leadbeater[1], John C. Watkinson[3], Jayne A. Franklyn[1], Kristien Boelaert[1], and Christopher J. McCabe[1]

[1]School of Clinical and Experimental Medicine, Institute of Biomedical Research, University of Birmingham, B15 2TH, UK
[2]Section of Endocrinology, Department of Biomedical Sciences and Advanced Therapies, University of Ferrara, Via Savonarola 9, 44121 Ferrara, Italy
[3]University Hospitals Birmingham NHS Foundation Trust, Birmingham, B15 2TJ, UK
[4]Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

Supplementary Figure Legends

Supplementary Figure 1

Construction of the Tg-PBF-HA transgene. A, A 576 bp PCR fragment containing the human PBF gene with a 3' HA tag was subcloned into unique *Eco*RI-*Bam*HI restriction sites in plasmid pSG5. B, A 1.2 kb fragment containing the beta-globin intron and PBF-HA gene was excised using unique *Cla*I-*Bam*HI restriction sites and inserted into pBKS-Tg. C, The entire 3.5 kb Tg-PBF-HA transgene (containing the thyroglobulin promoter, beta-globin intron and PBF-HA gene) was excised from pTg-PBF-HA by digesting with *Spe*I and *Sal*I. D, The Tg-PBF-HA transgene was purified from 1.5% agarose gel by the QIAquick gel extraction kit (Qiagen) and subsequently purified with an Elutip tip before dilution in 1x TE to a final concentration of 5 ng/μl for microinjection. Genomic DNA was extracted from ear clippings of potential founders using the DNeasy Blood and Tissue kit (Qiagen) and the presence of the Tg-PBF-HA transgene identified by real-time RT-PCR analysis.

Supplementary Figure 2

Transgene copy number was determined for WT ($N = 7$), hemizygous PBF-$Tg^{+/-}$ ($N = 27$) and homozygous PBF-$Tg^{+/+}$ ($N = 20$) mice. Genomic DNA extracted from human liver tissue was used to calibrate PBF transgene copy numbers. Data presented as mean ± SE. Student's t test was used for statistical analysis.

Supplementary Figure 3

PBF transgene expression was detected in thyroids of PBF-Tg mice by Western blot analysis. There was no significant expression of the HA-tagged PBF protein in other major organs examined, including the liver, kidney and spleen, in either PBF-Tg (PBF) or WT mice using an anti-HA antibody at 1:1000 dilution.

Supplementary Figure 4

Effect of gender and age on thyroid weight in PBF-Tg mice. A, Mean thyroid weight of PBF-Tg mice (diamonds) up to 365 days old. Plotted trend line shows an r squared value of 0.9895, confirming a clear relationship between thyroid weight and mouse age. B, Thyroid weight, adjusted for total body weight, in age-matched PBF-Tg and WT mice up to 78 weeks. ***$P < 0.0001$ compared to age-matched WT mice. Numbers of mice analysed at each time point are shown. C, The mean weight of thyroid glands from female (F) PBF-Tg mice was significantly greater than that of male (M) PBF-Tg litter-mates at both 7 (11.3% weight increase, $P = 0.04$) and 52 (38.5% weight increase, $P = 0.0045$) weeks of age; N = 12-45. In contrast, there was no significant difference in thyroid weight between male and female PBF-Tg mice at 78 weeks of age, or between male and female WT mice at any age examined. NS- not significant.

Supplementary Figure 5

Thyroid macrofollicular lesions in PBF-Tg mice. A, Thyroid sections were assessed for the size of macrofollicular lesions in PBF-Tg and WT mice at 52 and 78 weeks of age in at least ten independent sectional planes per thyroid; N = 6 - 12 per genotype. The

diameter of thyroid follicles (major axis) was measured using ImageJ software. Representative images are shown of macrofollicular lesions in PBF-Tg thyroids from 52 (B) and 78 (C and D) week old mice compared to a WT thyroid (E). F, A composite image of an entire thyroid section from a 78 week old WT mouse with no evidence of either macrofollicular or hyperplastic lesions. Scale bars: 100 μm.

Supplementary Figure 6

Evidence of hyperplastic lesions in PBF-Tg thyroids. Representative images of hyperplasia in PBF-Tg thyroids from 52 (A and B) and 78 (C-H) week old mice are shown. There was no evidence of hyperplasia in WT thyroids. Scale bars: 100 μm.

Supplementary Figure 7

Serum T3 and T4 concentrations in PBF-Tg mice. There was no significant difference in total T3 and total T4 serum levels between PBF-Tg (N = 3-8) and WT mice (N = 5-10). Data presented as mean ± SE. Student's t test was used for statistical analysis. NS- not significant.

Supplementary Figure 8

Whole lobe analyses of NIS expression in PBF-Tg mice. Representative images of NIS immunostaining in entire thyroid lobes from 26 (A and C) and 52 (B and D) week old PBF-Tg and WT mice are shown. Scale bars: 500 μm. Assessment of NIS immunostaining in entire thyroid lobes showed that NIS protein expression was both repressed and more heterogeneous in PBF-Tg thyroids compared to WT. Higher

magnification of NIS staining in PBF-Tg (E and F) and WT (G) thyroids are also shown. Scale bars: 100 μm.

Supplementary Figure 9

MAPK (pERK1/2) expression levels in PBF-Tg and WT thyroids. Western blot analysis did not show any significant difference in pERK1/2 expression in thyroid lysates from PBF-Tg and WT mice; $N = 4$.

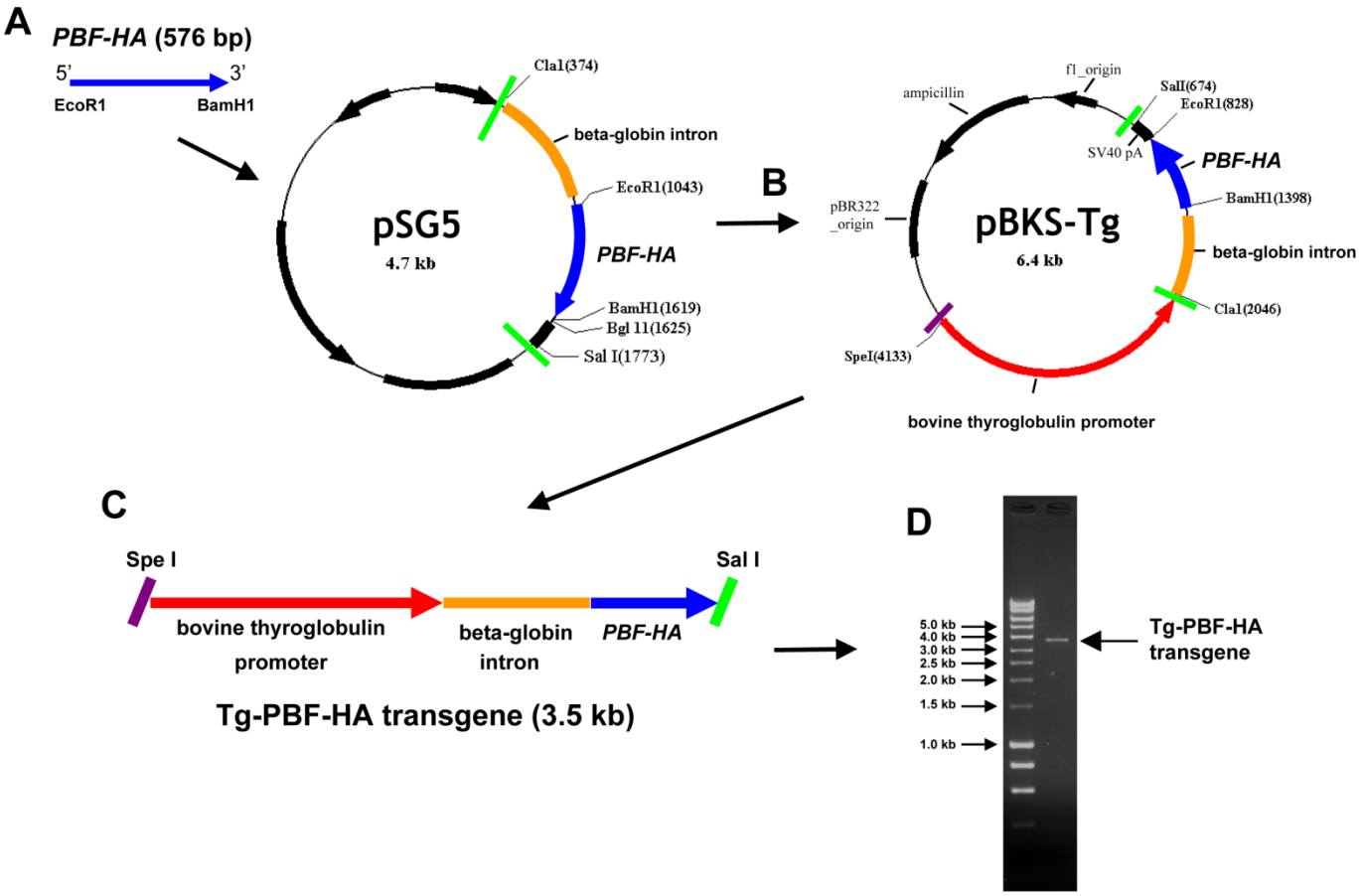
Supplementary Figure 10

Elevated cyclin D1 expression in hyperplastic lesions in PBF-Tg thyroids. A, Representative images are shown of cyclin D1 immunostaining in PBF-Tg and WT thyroids in 78 week old mice. B, A scatterplot showing the percentage of cyclin D1 positive cells in hyperplastic lesions identified in PBF-Tg thyroids. The mean value ± SE is shown. C, Arrows highlight enlarged nuclei present in a thyroid hyperplastic lesion immunostained with cyclin D1 in a 78-week old PBF-Tg mouse. D, Representative images of a hyperplastic lesion in a PBF-Tg thyroid from a 78-week old mouse stained with either cyclin D1 or H&E. Scale bars: 100 μm.

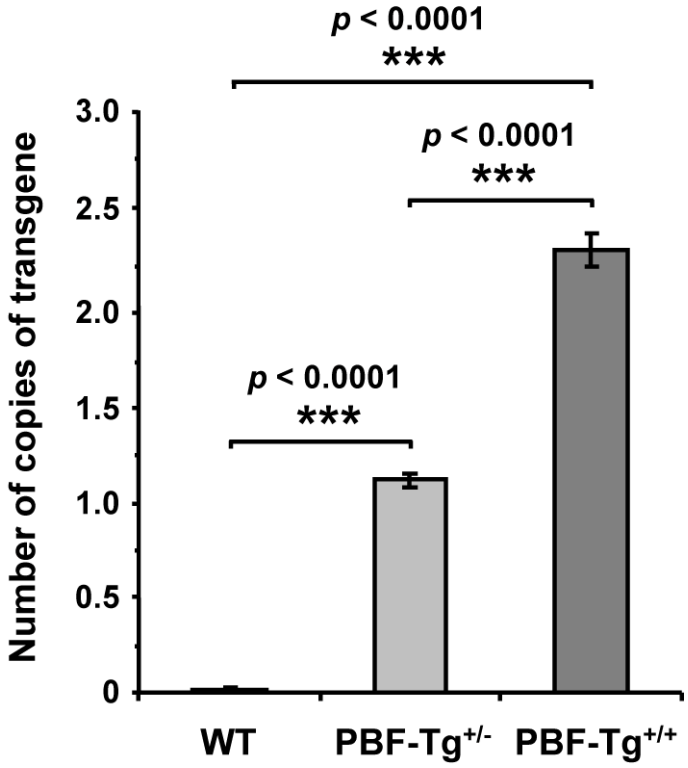
Supplementary Figure 11

Quantification of TSHR mRNA expression in primary cultures of human thyrocytes transfected with either PBF or Scr siRNA as indicated; $N = 5$. Data presented as mean ± SE. NS- not significant.

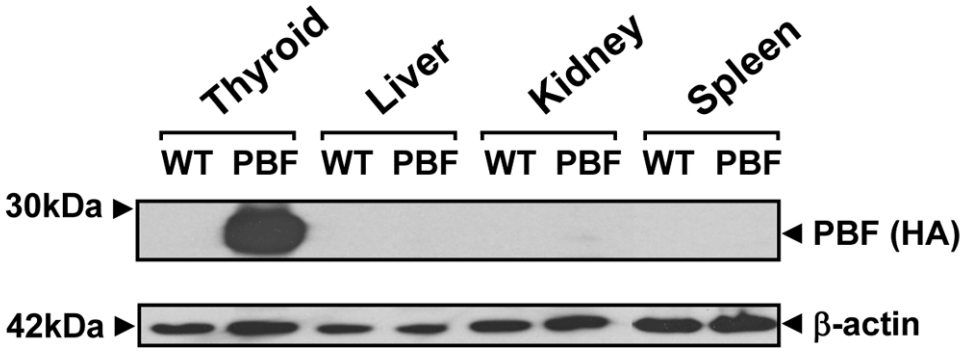
Supplementary Figure 1



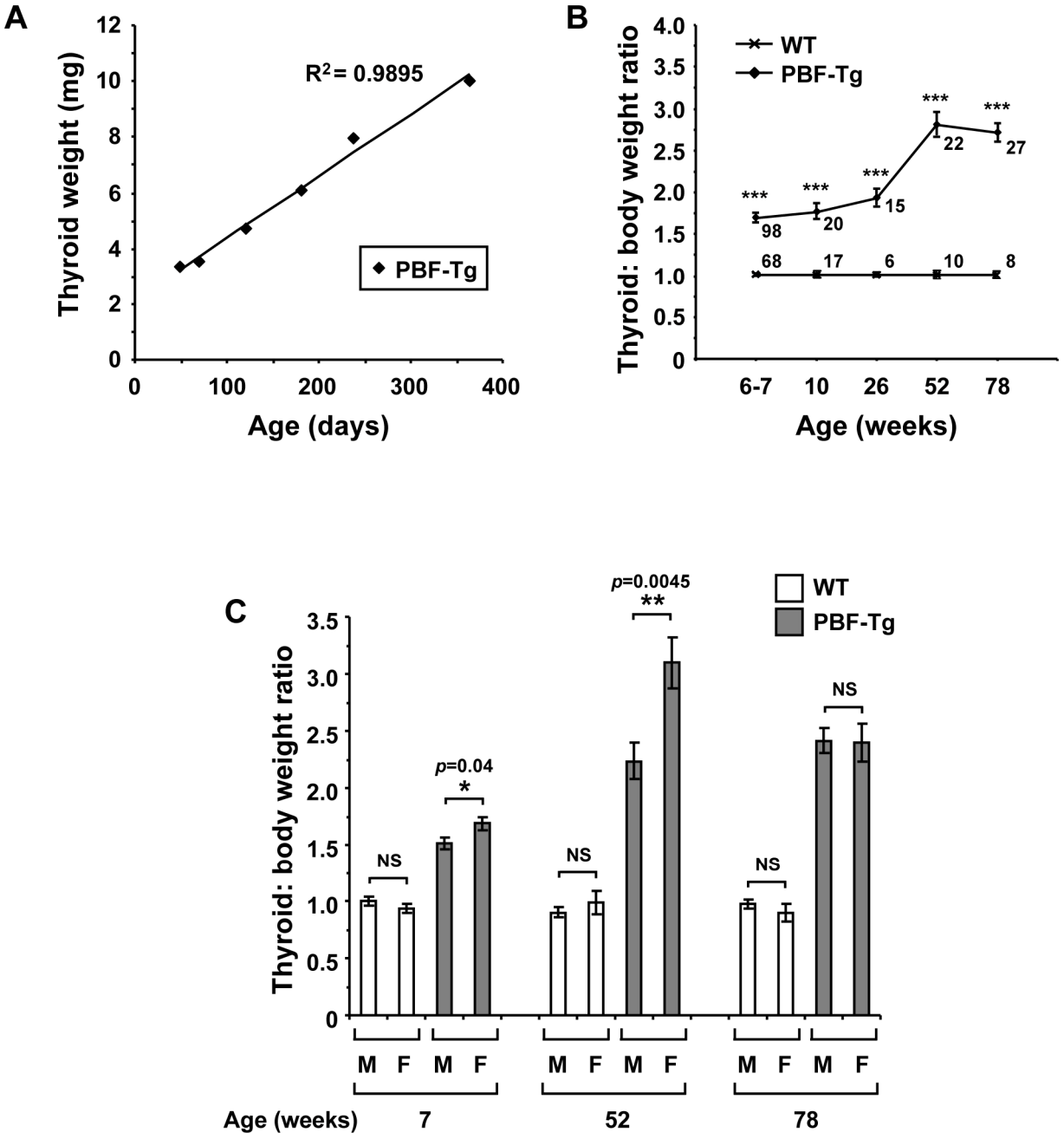
Supplementary Figure 2



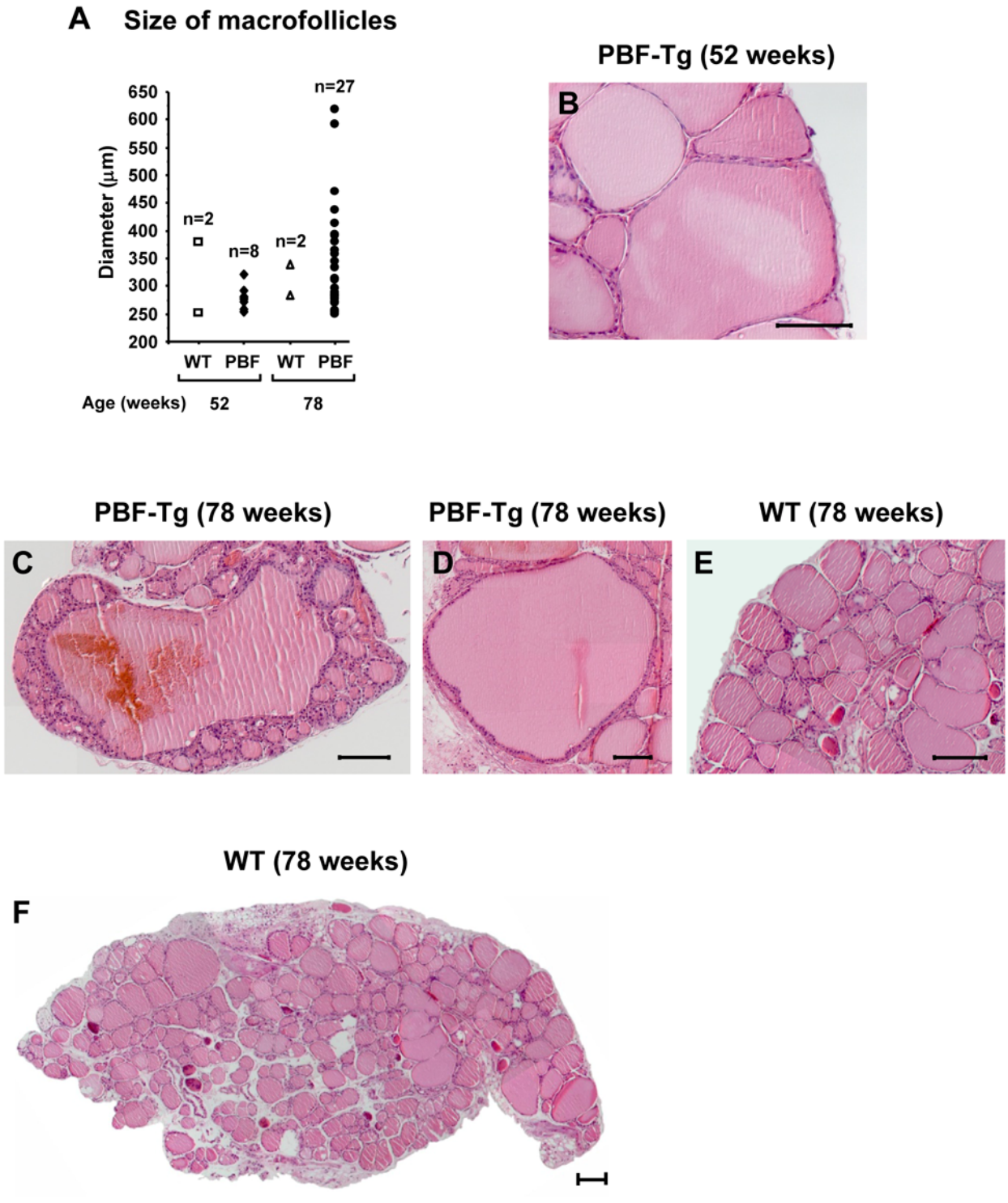
Supplementary Figure 3



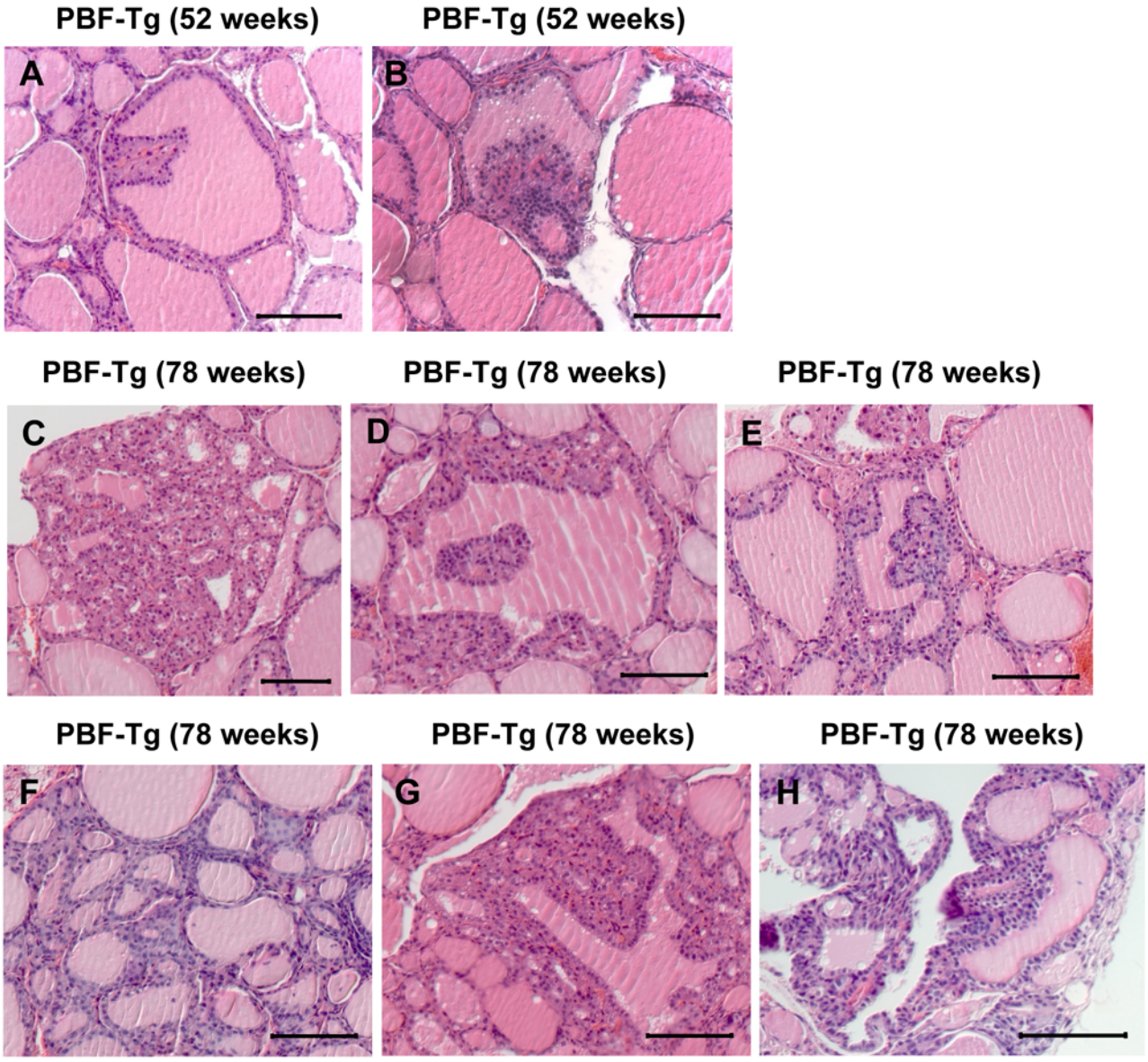
Supplementary Figure 4



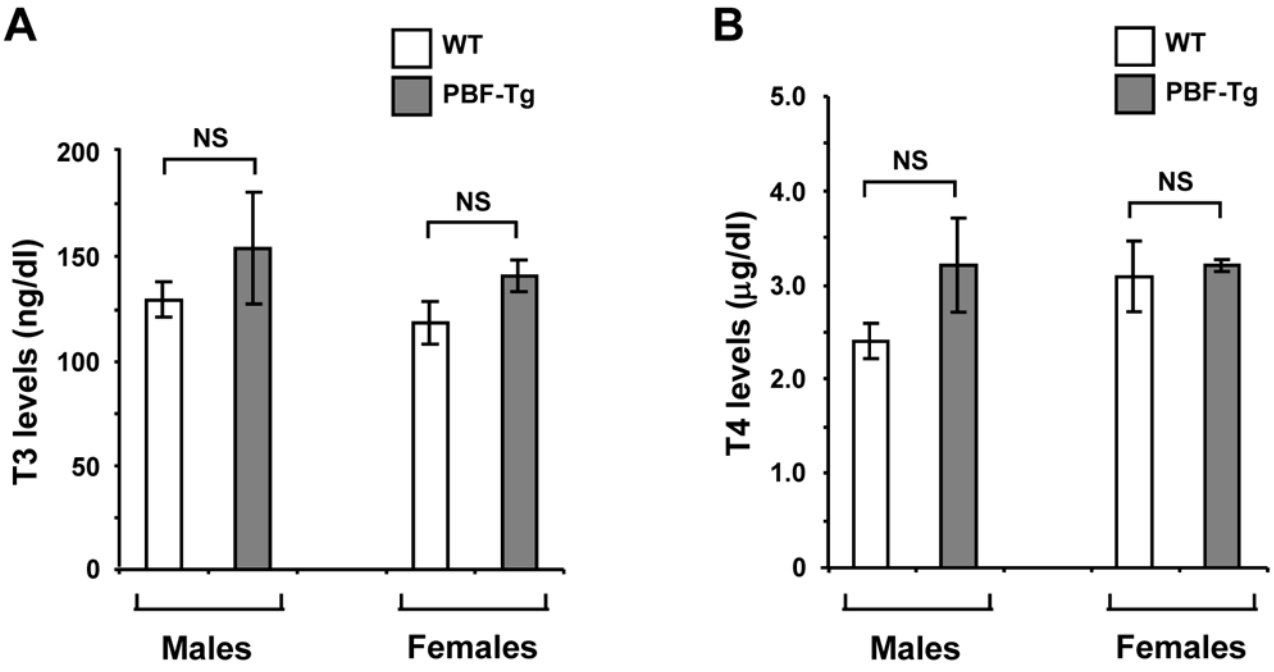
Supplementary Figure 5



Supplementary Figure 6

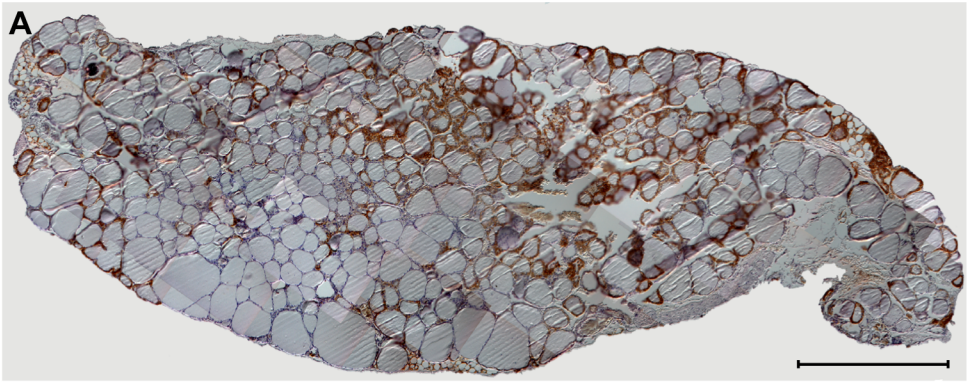


Supplementary Figure 7

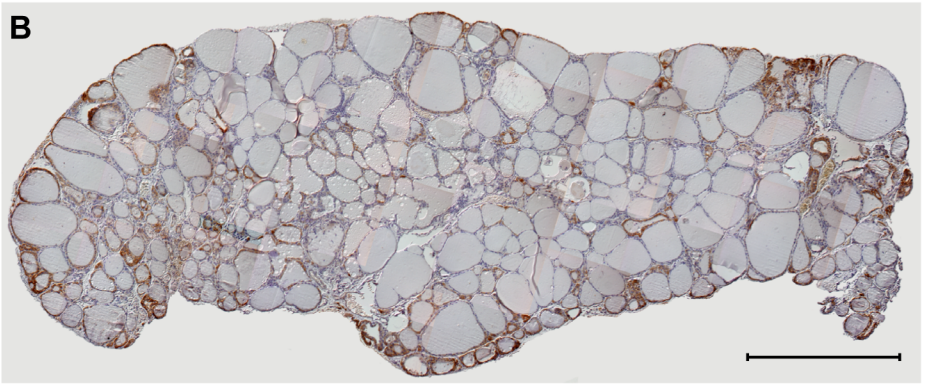


Supplementary Figure 8

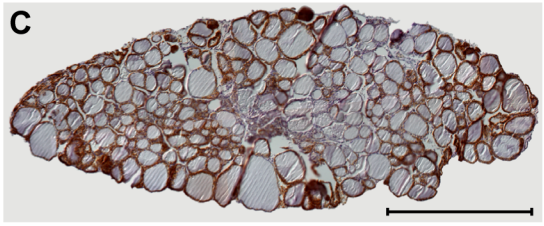
PBF-Tg (26 weeks)



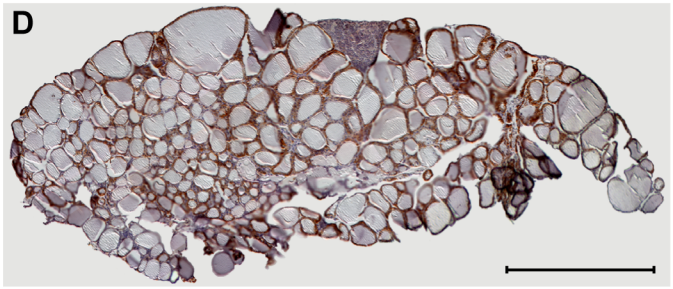
PBF-Tg (52 weeks)



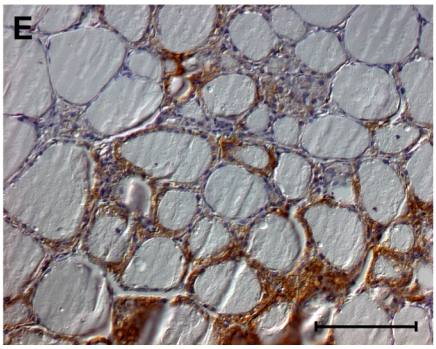
WT (26 weeks)



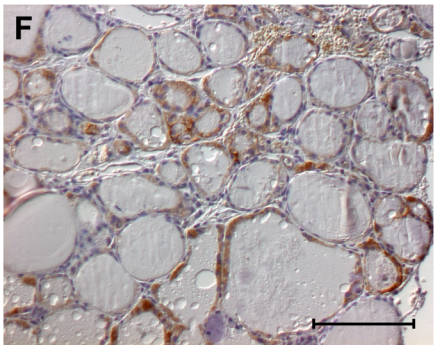
WT (52 weeks)



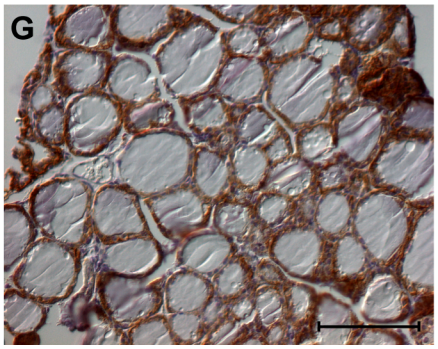
PBF-Tg (26 weeks)



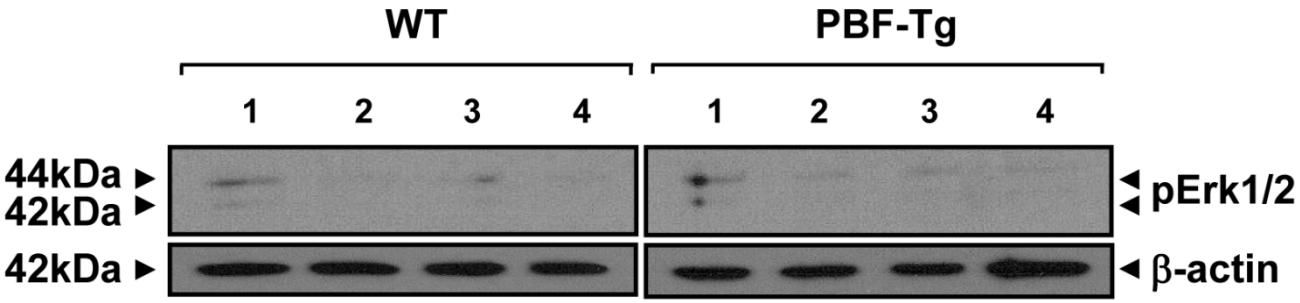
PBF-Tg (52 weeks)



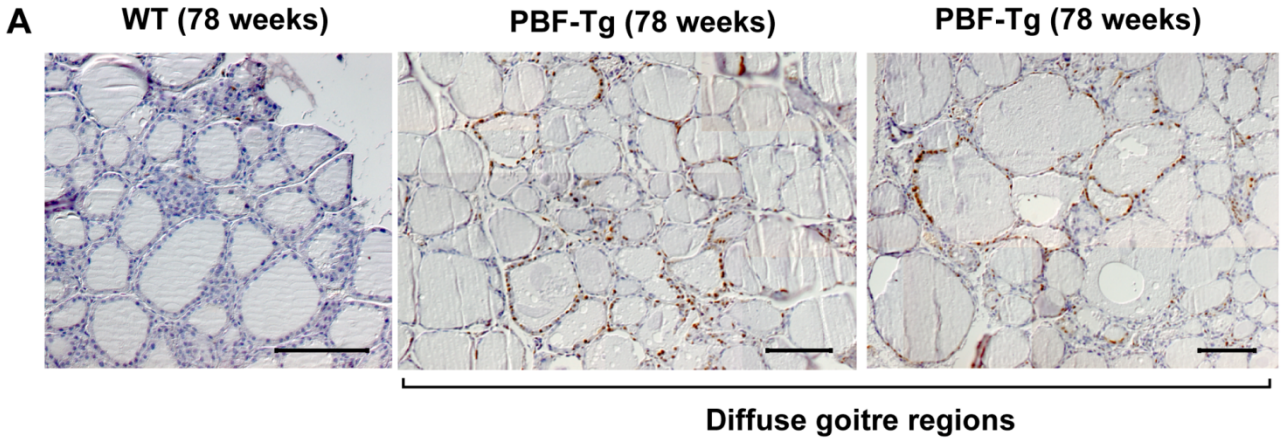
WT (26 weeks)

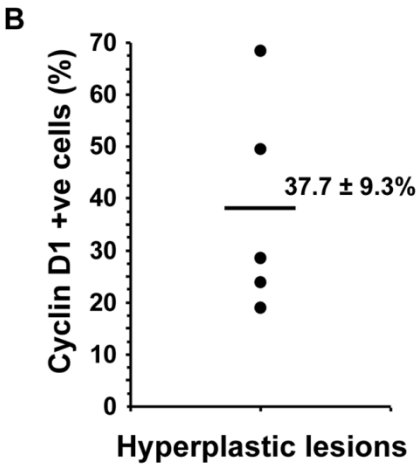


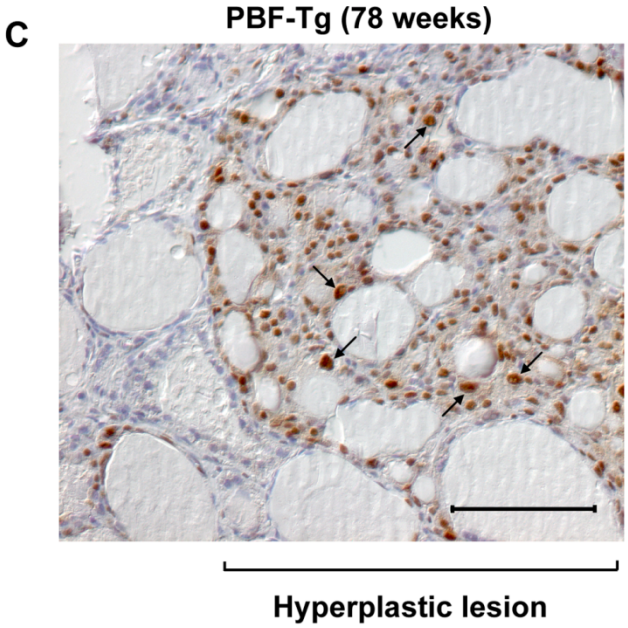
Supplementary Figure 9

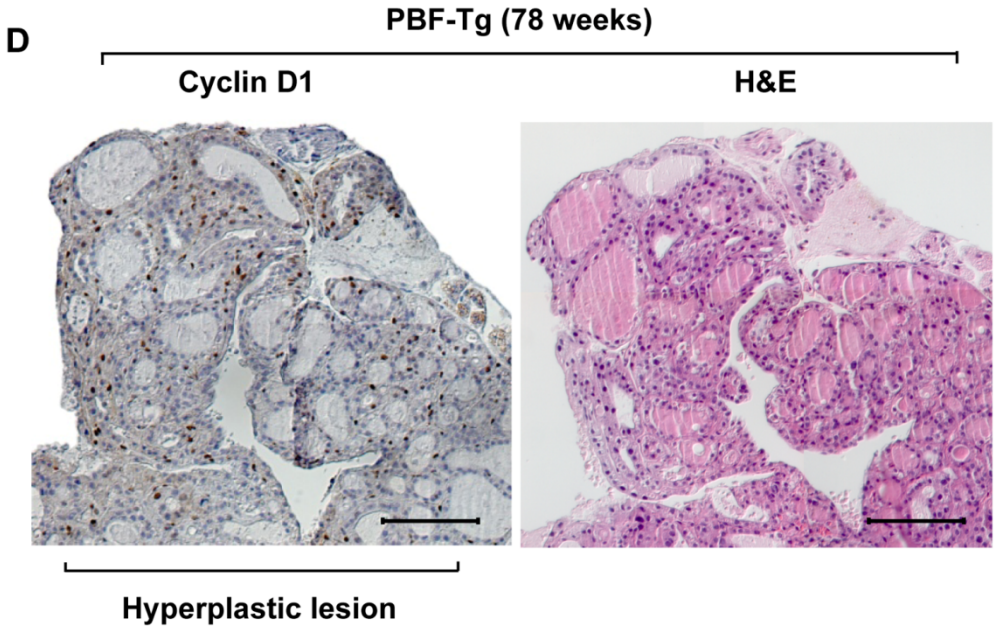


Supplementary Figure 10









Supplementary Figure 11