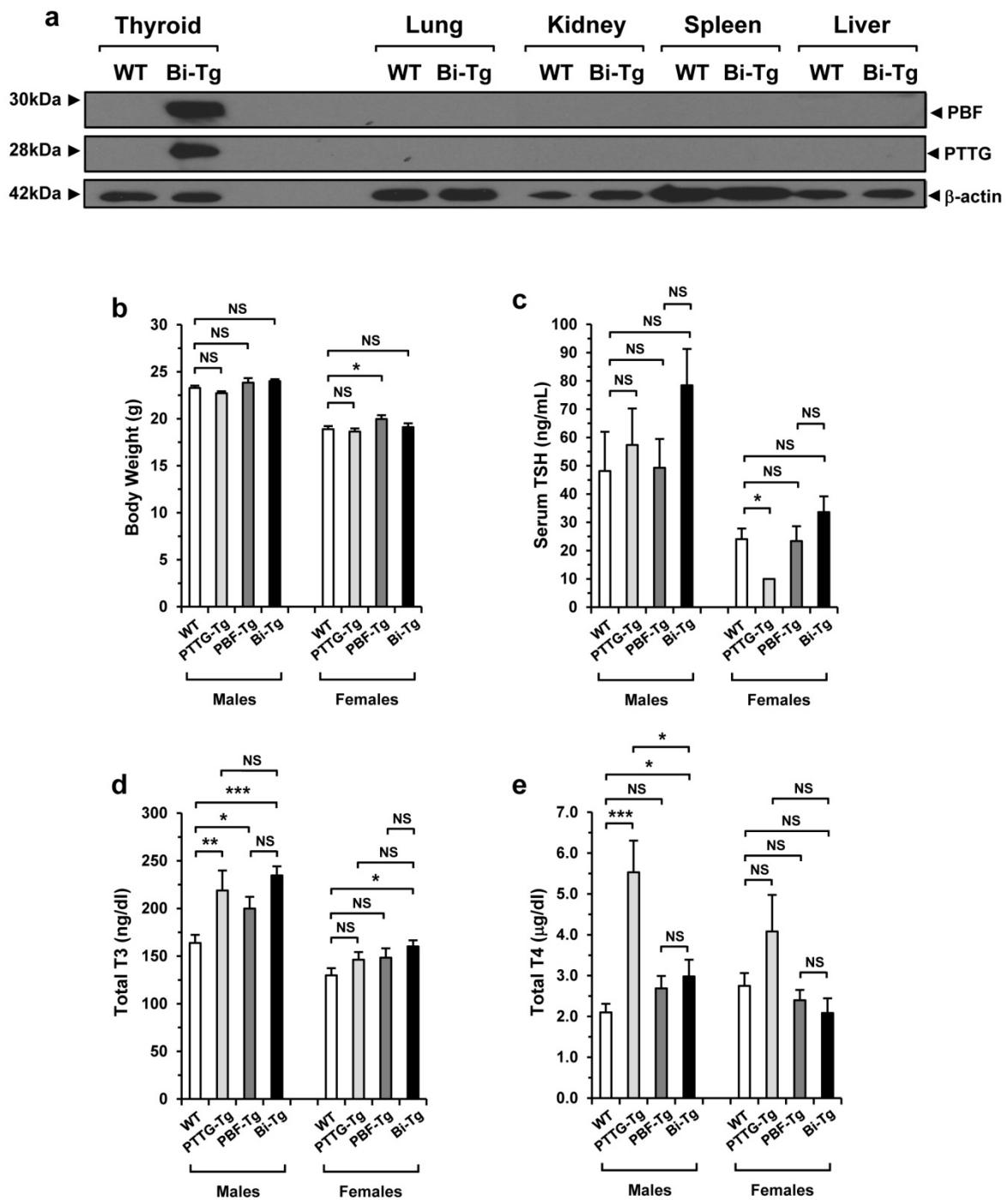
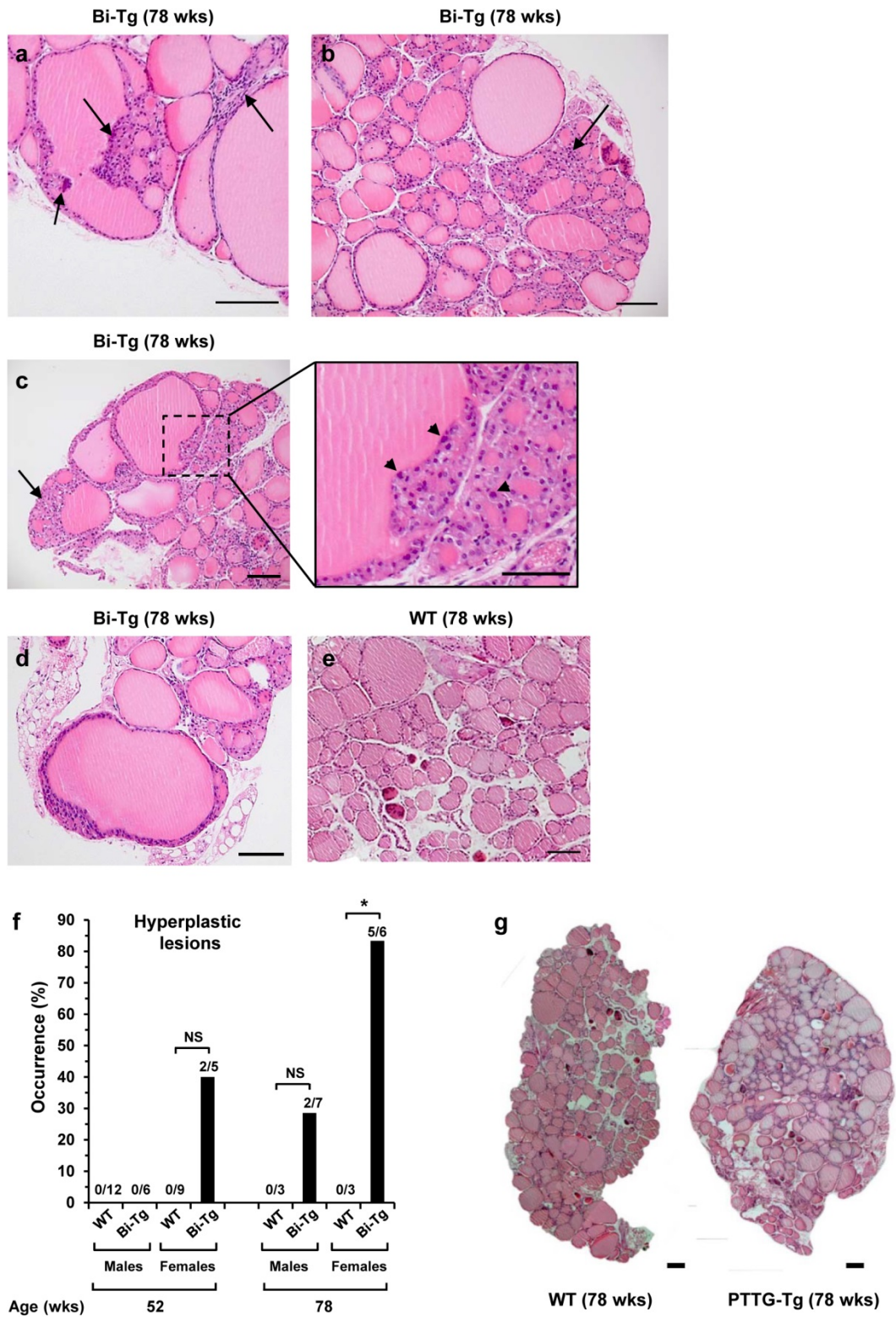


Supplementary Figure S1



Supplementary Figure S1. Characterisation of Bi-Tg mice. **(a)** Western blot analysis demonstrating PBF and PTTG expression in thyroid glands harvested from Bi-Tg mice. No significant expression of HA-tagged PBF protein or FLAG-tagged PTTG was detected in other major organs, including lung, kidney, spleen and liver. Wild-type (WT) mice were used as controls. **(b)** Body weight (g) of male (WT, $n=14$; PTTG-Tg, $n=18$; PBF-Tg, $n=18$; Bi-Tg, $n=13$) and female (WT, $n=18$; PTTG-Tg, $n=18$; PBF-Tg, $n=18$; Bi-Tg, $n=12$) mice at 6 weeks of age (mean \pm s.e.m., unpaired two-tailed t -test) (NS, not significant; * $P<0.01$). **(c)** Serum TSH concentrations (ng/ml) in male and female transgenic mice (mean \pm s.e.m., $n=6$, unpaired two-tailed t -test) (NS, not significant; * $P<0.01$). Mouse serum concentrations were determined by Prof Samuel Refetoff (University of Chicago, Chicago, IL, USA). **(d)** Serum T3 concentrations (ng/dl) in 6 week old male (WT, $n=16$; PTTG-Tg, $n=6$; PBF-Tg, $n=15$; Bi-Tg, $n=6$) and female (WT, $n=15$; PTTG-Tg, $n=6$; PBF-Tg, $n=12$; Bi-Tg, $n=6$) transgenic mice (mean \pm s.e.m., unpaired two-tailed t -test) (NS, not significant; * $P<0.01$; ** $P<0.01$; *** $P<0.001$). **(e)** Serum T4 concentrations (μ g/dl) in 6 week old male (WT, $n=14$; PTTG-Tg, $n=6$; PBF-Tg, $n=6$; Bi-Tg, $n=6$) and female (WT, $n=15$; PTTG-Tg, $n=5$; PBF-Tg, $n=5$; Bi-Tg, $n=5$) transgenic mice (mean \pm s.e.m., unpaired two-tailed t -test) (NS, not significant; * $P<0.01$; *** $P<0.001$).

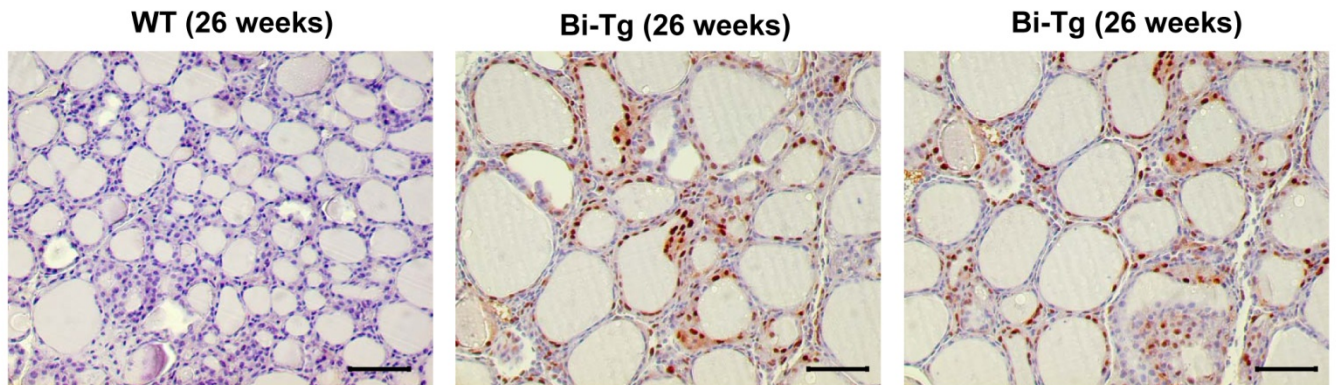
Supplementary Figure S2



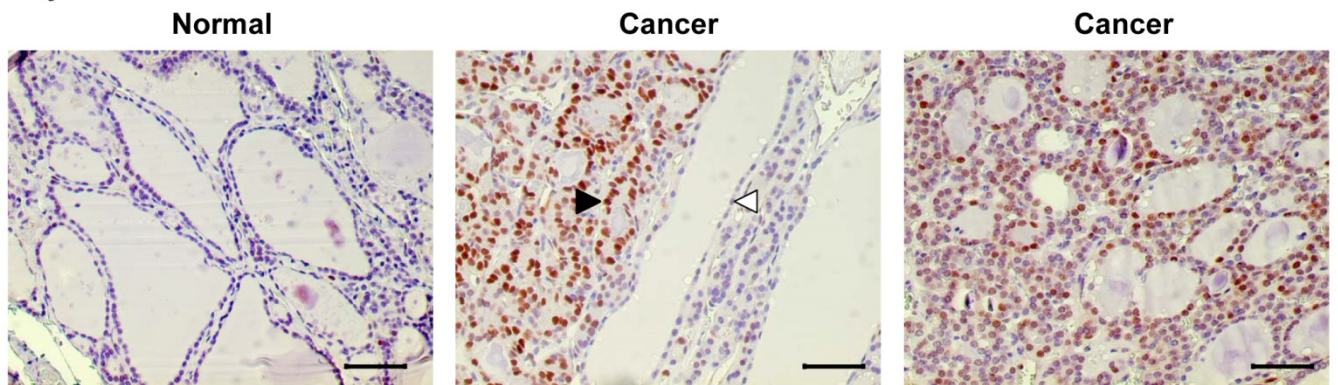
Supplementary Figure S2. Thyroid hyperplasia in Bi-Tg mice. (a-e) Representative H&E stained images showing hyperplastic lesions (arrows, a-c) and a macrofollicular lesion (d) in thyroids harvested from 78-week old Bi-Tg mice compared to normal follicles in WT thyroid (e). Enlarged nuclei indicative of proliferating or invading cells were present in hyperplastic lesions (black arrowheads, magnified image in c). (f) Occurrence of hyperplastic lesions in at least 10 independent sectional planes per Bi-Tg or WT thyroid ($n=3-12$ per genotype as indicated) (NS, not significant; $*P<0.05$; Fisher's exact test). (g) Composite image of an entire thyroid lobe from a 78-week-old WT (left) and PTTG-Tg (right) mouse. Scale bars, 100 μm .

Supplementary Figure S3

a Cyclin D1



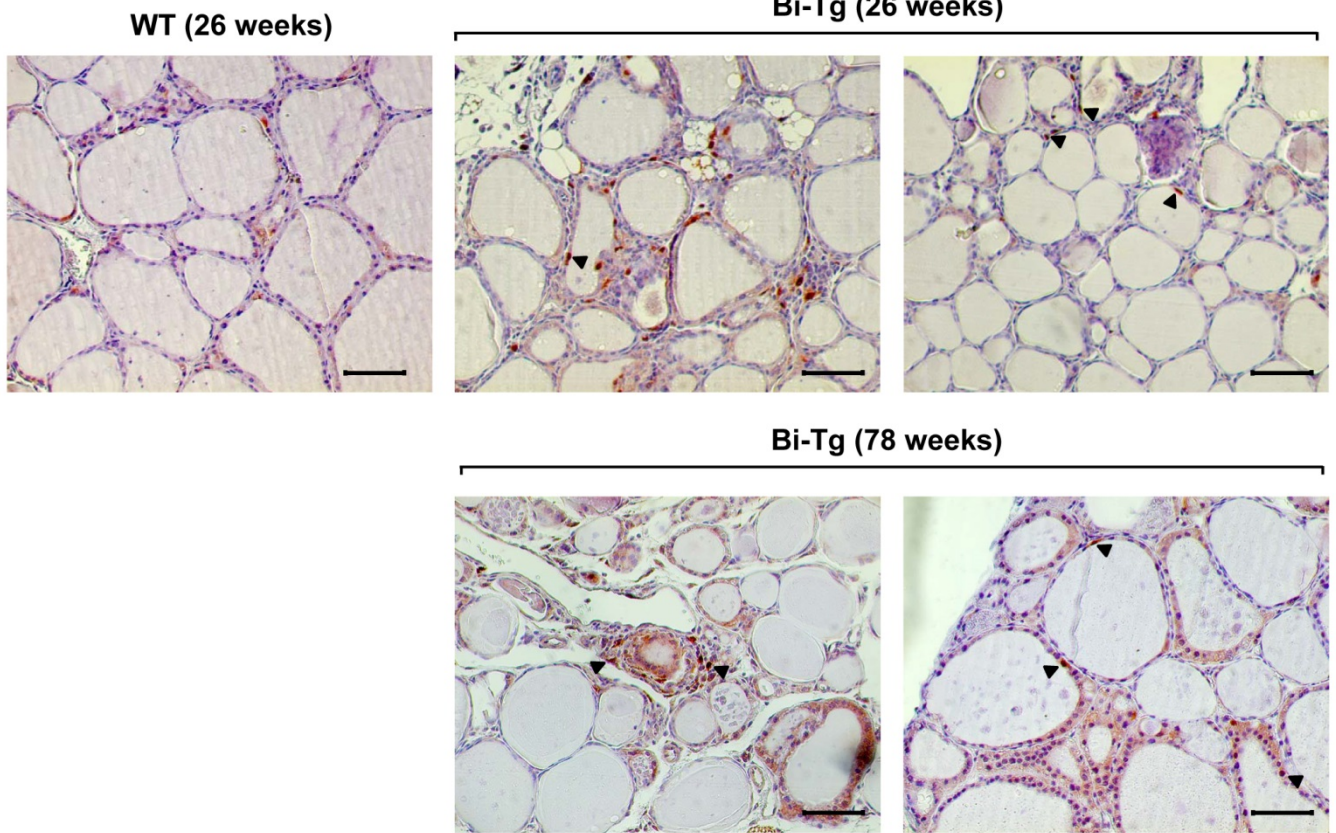
b Cyclin D1



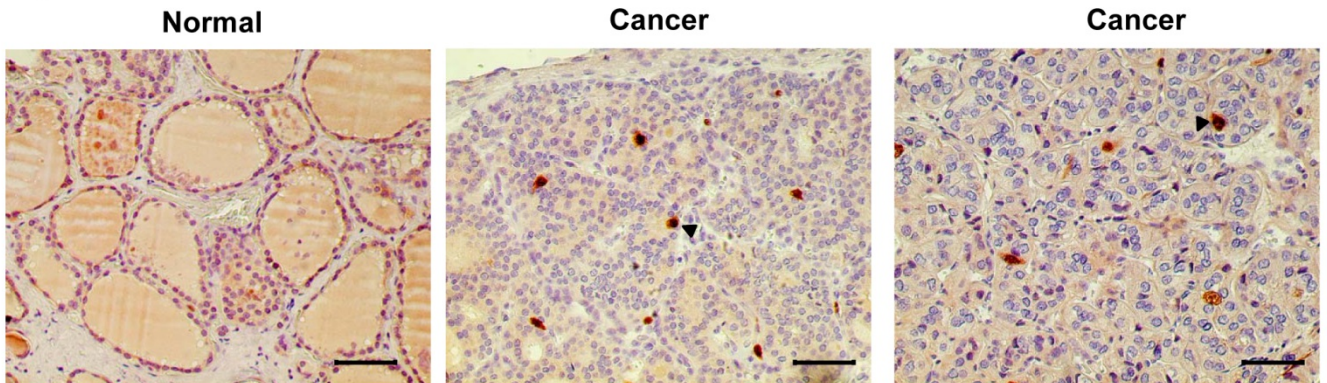
Supplementary Figure S3. Cellular proliferation in Bi-Tg thyroids and human thyroid cancer. **(a)** Representative images of cyclin D1 expression in wild-type (WT) and Bi-Tg thyroids in 26 week old mice. **(b)** Representative images of cyclin D1 expression in a human differentiated thyroid tumour (right panel) and normal thyroid tissue (left panel). Middle panel shows cyclin D1 expression in malignant cells (black arrowhead) adjacent to normal thyrocytes (white arrowhead). Scale bars, 100 μ m.

Supplementary Figure S4

a Cyclin A



b Cyclin A



Supplementary Figure S4. Expression levels of cyclin A in thyroid tissue. (a) Representative images of cyclin A expression in wild-type (WT) and Bi-Tg thyroids in 26 (upper) and 78 (lower) week old mice. (b) Representative images of cyclin A expression in a human differentiated thyroid tumour (middle and right panels) and normal thyroid tissue (left panel). Black arrowheads indicate intense nuclear expression of cyclin A. Scale bars, 100 μ m.