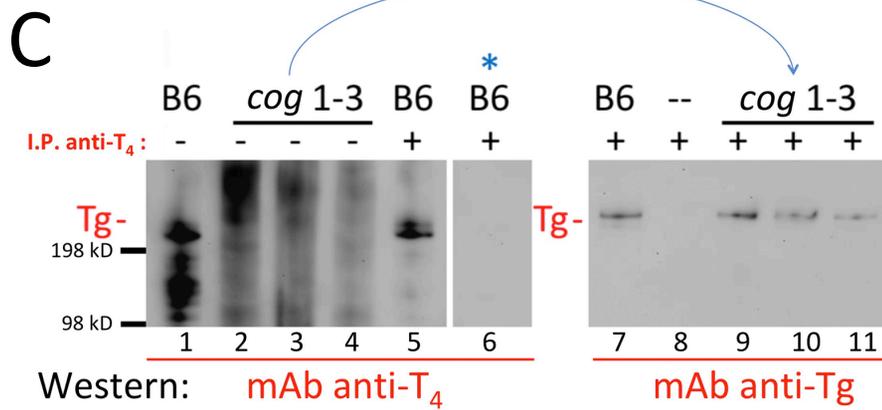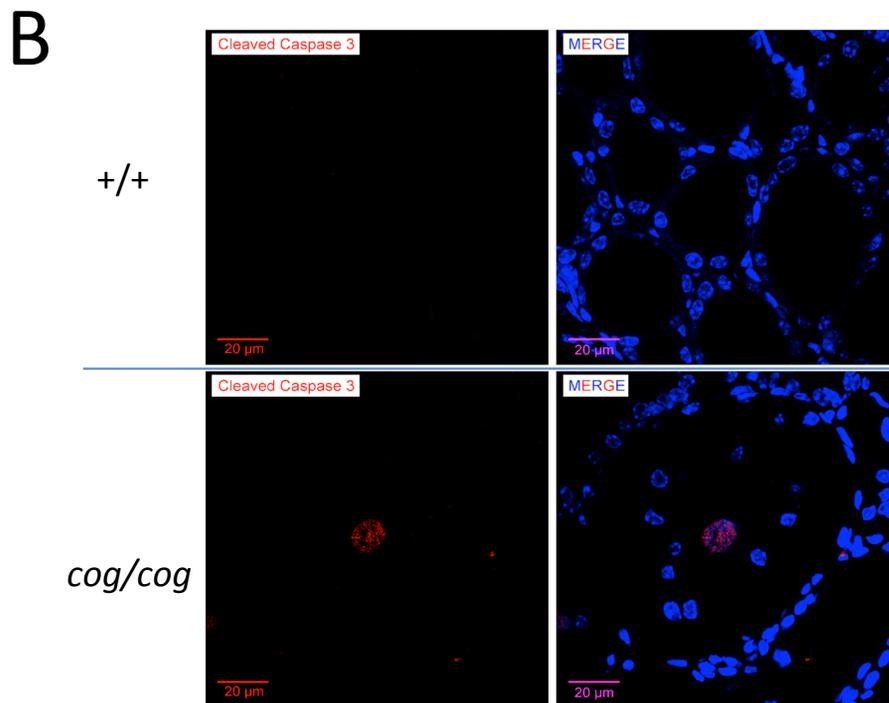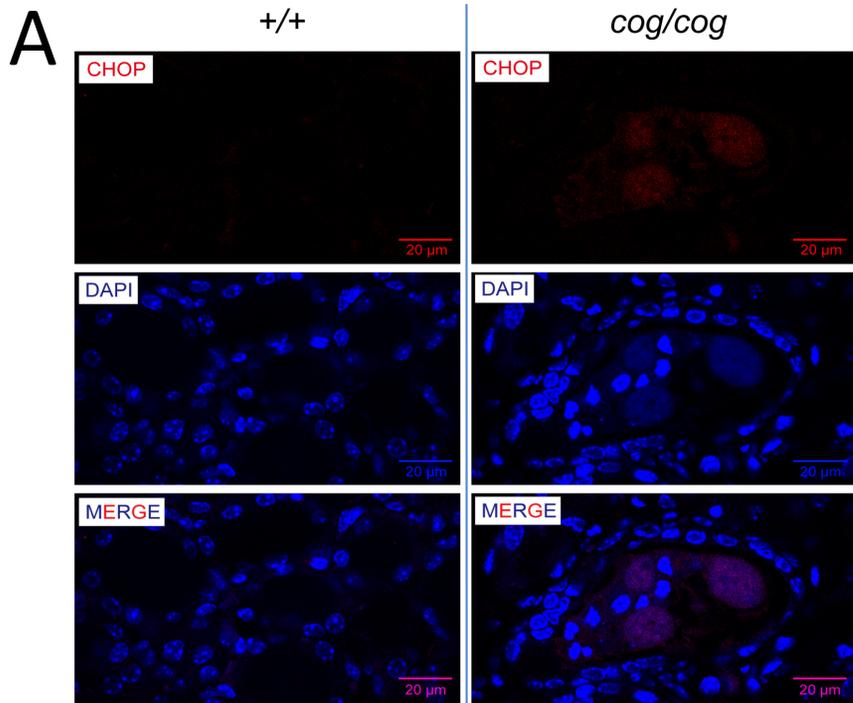**Supplemental Figures**

Fig. S1. Detection of CHOP and cleaved caspase-3 in thyroid follicles of $TG^{cog/cog}$ mice. **A**. CHOP immunofluorescence (red) with DAPI counter-stain (blue) in thyroid glands of WT and $TG^{cog/cog}$ mice (n=3); scale bars = 20 µm. Note karyolysis: chromatin expansion with abnormally diminished DAPI staining in the nuclei that are positive for CHOP. **B**. Cleaved caspase-3 immunofluorescence (red) with DAPI counter-stain (blue) in thyroid glands of WT and $TG^{cog/cog}$ mice (n=3-4) ; scale bars = 20 µm. **C**. Thyroid homogenates from WT ("B6") and three independent $TG^{cog/cog}$ mice (*cog* 1-3) were analyzed either before or after immunoprecipitation with mAb anti-T_4, followed by immunoblotting with either the same antibody (lanes 1-6) or with mAb anti-Tg the recognizes an epitope in the region of Tg residues 1000-1100. In the immunoblotting of lane 6 (blue asterisk), 2 µg/mL of competitor T_4 was added as a specificity control. In lane 8, identical immunoprecipitation and immunoblotting was performed, but in the absence of thyroid homogenate. The Tg protein bearing T_4 as shown in lanes 9 – 11 are derived from the initial samples shown in lanes 2 – 4, respectively. Two additional $TG^{cog/cog}$ mice (total n=5) run on a separate gel yielded the same results.

Fig. S2. Dead and dying thyrocytes in the thyroid follicular lumina in human goitrous hypothyroidism caused by homozygous $TG^{W2346R/W2346R}$. **A.** H&E images from a surgical specimen of the thyroid gland of the patient; scale bars = 10 µm. **B-D**. Representative images of TUNEL staining (red) and immunofluorescence of T_4-containing protein (green) with DAPI counter-stain (blue) in the thyroid gland of the same patient; scale bars = 20 µm. The immunofluorescence was repeated 3 times.
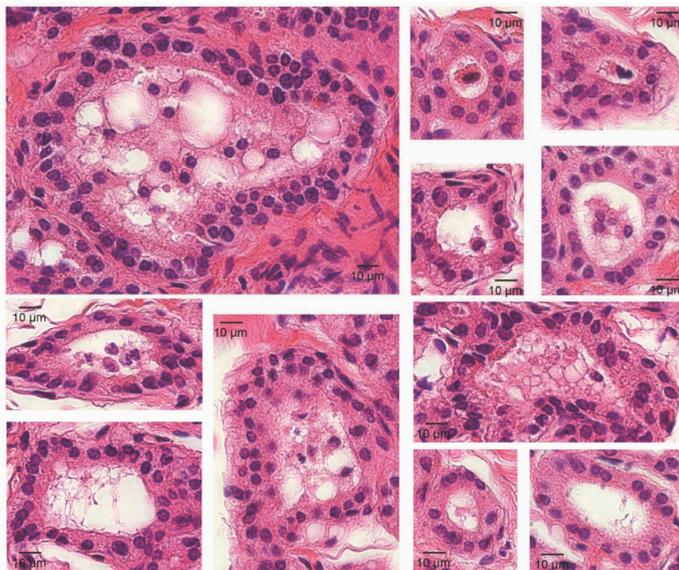

Fig. S3. Screening for T_4-containing protein in $TG^{rdw/rdw}$ rats. **A.** A sampling of thyroid follicles from H&E images of the thyroid glands of $TG^{rdw/rdw}$ rats (n=4) ; scale bars = 20 µm, showing dying and dead thyrocyte ghosts at different stages, in the follicle lumen. **B**. Low power immunofluorescence demonstrating specificity of T_4-containing protein (green; with DAPI counter-stain in blue) in the thyroid gland (*upper panels*) *versus* the parotid gland (*lower panels*) from a $TG^{rdw/rdw}$ rat (gland

architecture shown in insets at left, scale bars = 20 µm). The immunofluorescence was repeated 3 times ; scale bars = 50 µm. **C**. Low power immunofluorescence of T_4-containing protein (green; with DAPI counter-stain in blue) in thyroid glands of $TG^{rdw/rdw}$ rats (n=3-5) either untreated (*upper panels*) or treated with propylthiouracil chow (PTU for 27 d, *lower panels*) ; scale bars = 200 µm..

Fig. S4. Thyrocyte proliferation, and model of thyroxine synthesis in congenital hypothyroidism from mutant *TG*. **A**. Ki67 immunohistochemistry of the thyroid gland of WT (n=4) and $TG^{rdw/rdw}$ rats (n=6) as well as $TG^{cog/cog}$ mice (n=2); scale bars = 50 µm.   There are essentially no Ki67-positive nuclei detectable in the WT control thyroid.   **B**. Schematic cartoon of thyroid hormone synthesis under normal conditions, and in congenital goiter with bi-allelic *TG* mutation.
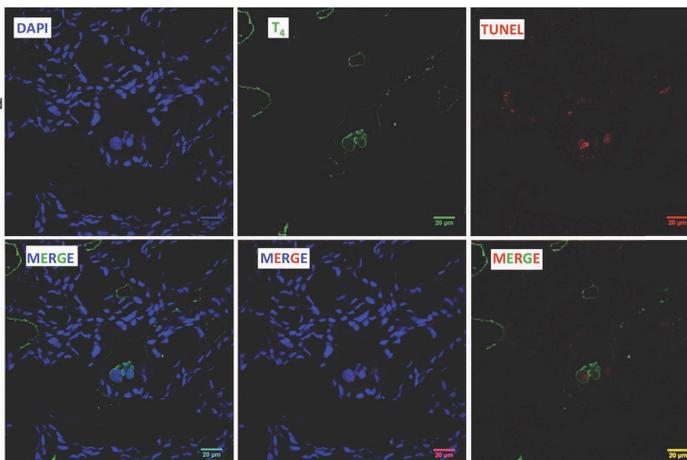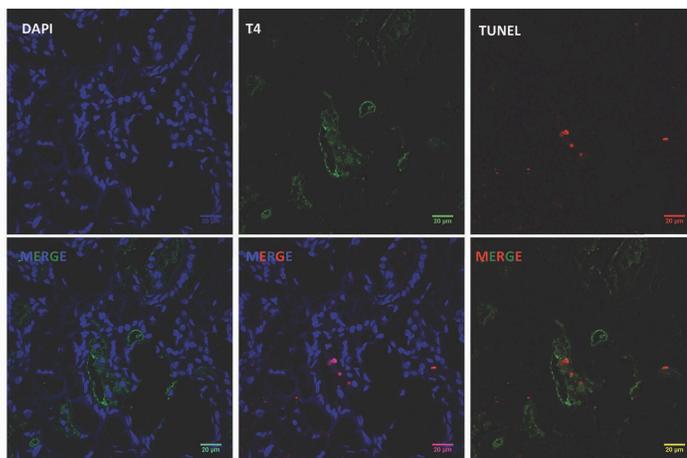
A

+/+	cog/cog
CHOP	CHOP
20 μm	20 μm
DAPI	DAPI
20 μm	20 μm
MERGE	MERGE
20 μm	20 μm

B

	+/+
Cleaved Caspase 3	MERGE
20 μm	20 μm

	cog/cog
Cleaved Caspase 3	MERGE
20 μm	20 μm

C

	B6	cog 1-3	B6	B6 *	B6	--	cog 1-3
I.P. anti-T₄ :	−	− − −	+	+	+	+	+ + +

Tg-
198 kD
98 kD

Tg-

1 2 3 4 5 6   7 8 9 10 11

Western: mAb anti-T₄   mAb anti-Tg

Zhang et al., Supplemental Fig. S1

Panel A

TUNEL+
T4+
DAPI

Human thyroid
section

DAPI | T₄ | TUNEL
MERGE | MERGE | MERGE

Panel B

TUNEL+
T4+
DAPI

Human thyroid
section

DAPI | T4 | TUNEL
MERGE | MERGE | MERGE

Panel C

TUNEL+
T4+
DAPI

Human thyroid
section

DAPI | T4 | TUNEL
MERGE | MERGE | MERGE

Panel D

Zhang et al.,
Supplemental
Fig. S2

A

B

rdw/rdw Thyroid gland

T₄ DAPI MERGE

rdw/rdw Parotid gland

T₄ DAPI MERGE

C

T₄ DAPI

rdw/rdw

T₄ DAPI

rdw/rdw treated with PTU

Zhang et al., Supplemental Fig. S3

A

+/+   *rdw/rdw*   *cog/cog*

50 µm   50 µm   50 µm
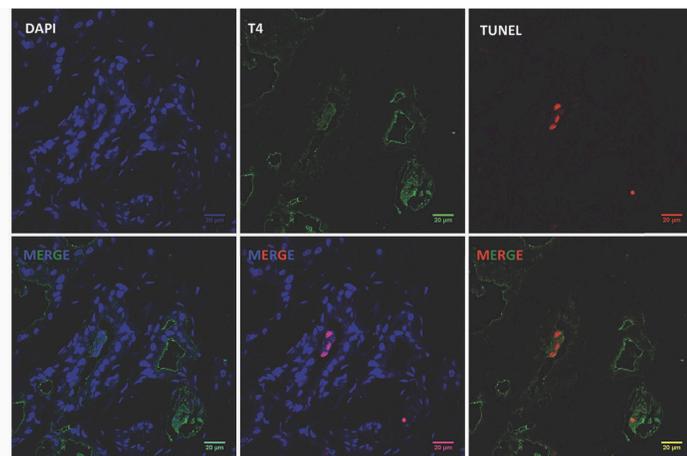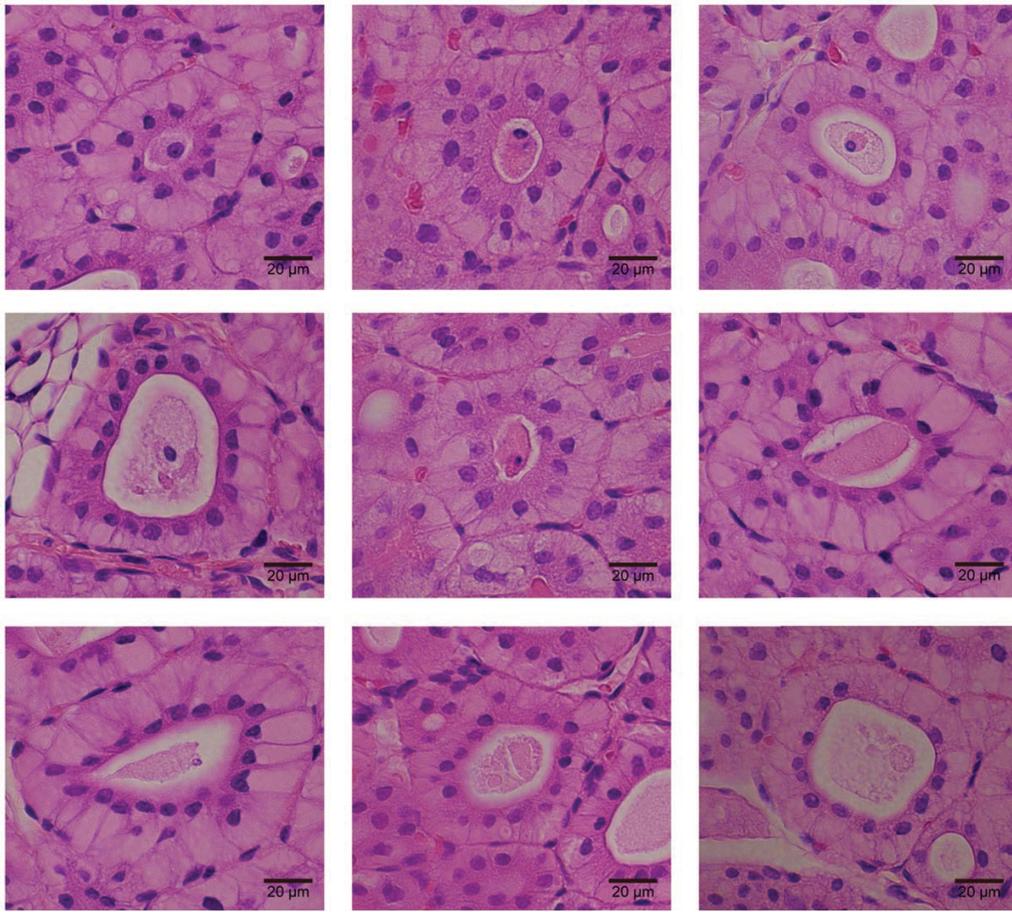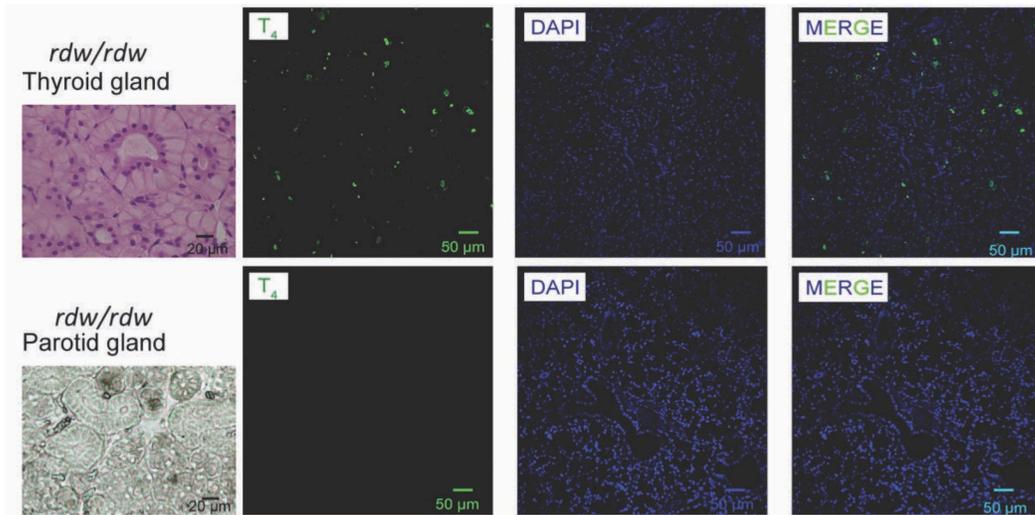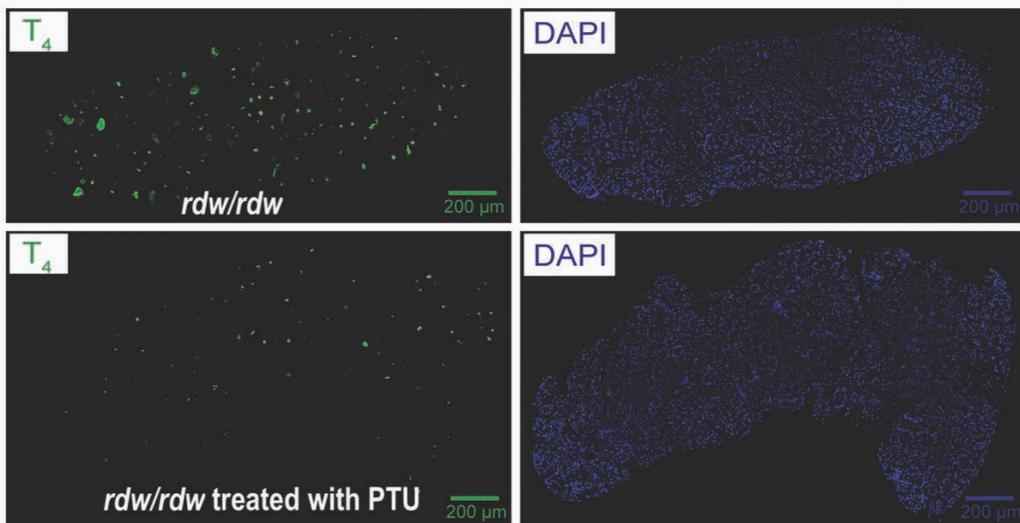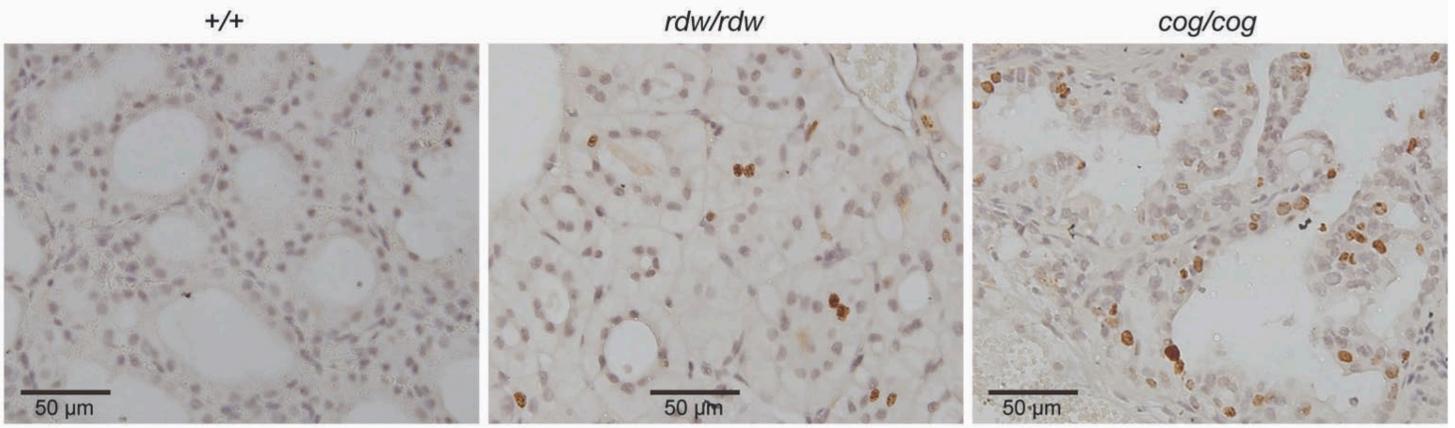
B

Normal Thyroid
Iodinated Tg in the follicle lumen

ER
WT-Tg

Golgi
WT-Tg

WT-Tg
IODINATION
T_4 SYNTHESIS
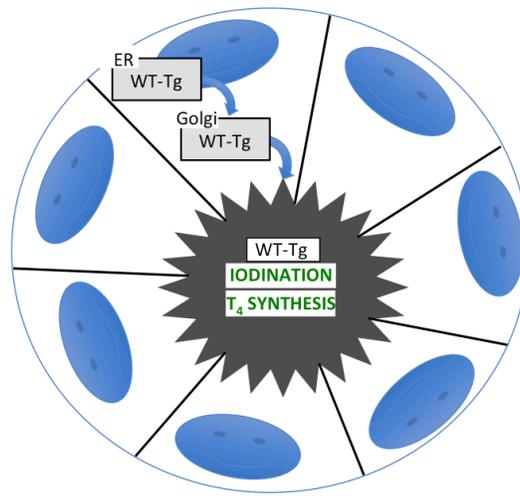
Homozygous mutant *TG* (e.g., *rdw/rdw* rat)
Tg entrapped in engorged ER; protein
delivered to follicle lumen via cell death

ER
rdwTg

ER
rdwTg

ER

ER
rdwTg

ER
rdwTg

rdwTg

ER
rdwTg

rdwTg

IODINATION
T_4 SYNTHESIS

ER

ER
rdwTg

rdwTg

Zhang et al.,
Supplemental
Fig. S4