**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 that 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 T₄-containing protein (green; with DAPI counter-stain in blue) in thyroid glands of TG<sup>rdw/dw</sup> 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.** Throcyte 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<sup>rdw/dw</sup> rats (n=6) as well as TG<sup>cog/cog</sup> 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.
Zhang et al., Supplemental Fig. S2
A sampling of thyroid follicles from H&E images of the thyroid glands of TGrdw/rdw rats (6-10 wk), showing dying and dead thyrocyte ghosts at different stages, in the follicle lumen.
Normal Thyroid
Iodinated Tg in the follicle lumen

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

Zhang et al., Supplemental Fig. S4