**LEGEND of the SUPPLEMENTAL FIGURES**

**Supplemental Figure 1**. Genomic structure of the TPO-CRE-ER and loxP BRAF alleles. A. TPO-CRE-ER transgene.The transgene was made by combining a 6.3 kb fragment of the human thyroperoxidase (TPO) promoter to the bacteriophage CRE recombinase, which was fused bi-cistronically with a fragment of the human estrogen receptor. The position of the forward and reverse screening primers for TPO and CRE is shown above the transgene. B. Mutated BRAF allele flanked by loxP sites. The mouse BRAF locus that has integrated the targeting DNA fragment containing the mutated exon 15 but has not undergone CRE recombination is shown at the top. The same locus after CRE recombinase is shown at the bottom: it now allows the expression of the mutated exon 15 (striped box), which codes for the valine to glutamic acid change at codon 600.

**Supplemental Figure 2**.Breeding strategy to obtain the desired NOD.H2h4\_TPO-CRE-ER\_BRAFV600E genotype. A. Backcrossing of the TPO-CRE-ER transgenic mice and BRAFv600Eknock-in mice (originally made on the C57BL6 background) onto the NOD.H2h4 background, to confer susceptibility to autoimmune thyroiditis.

B. Intercrossing performed to obtain the mice used in the study. TPO-CRE-ERtransgenic mice (which are hemizygous for CRE, as indicated by the empty rectangle) were intercrossed to homozygous floxed BRAFV600E mice. The first filial generation (F1) yields a progeny where 50% of the pups are hemizygous for CRE and heterozygous for the floxed BRAFV600E allele. This progeny is then mated back to homozygous floxed BRAFV600E mice. The second filial generation (F2) yields a progeny where 25% of the pups have the desired genotype (NOD.H2h4\_TPO-CRE hemizygous-ER\_BRAFV600E homozygous).

**Supplemental Figure 3**. Methods used to screen the mice used in the study. A and B. PCR genotyping to detect the TPO-CRE-ER transgene (left) or BRAFV600E knock-in (right) alleles.A.0.8% agarose gel run with 100 bp DNA ladder. Presence of the TPO-CRE-ER transgene results in an amplicon of about 1,180 bp. B.2% agarose gelrunwith 100 bp DNA ladder: presence of the BRAFv600Ea single amplicon of 307 bp indicates a homozygous mouse, two amplicons of 307 bp and 185 bp a heterozygous mouse, a single amplicon of 185 bp a wild type mouse. Positive control, negative control, and water only control were included in each reaction.

C and D. Representatives RT-PCR images showing cDNA derived from total thyroid RNA amplified using PCR primers specific for CRE (left) or TPO (right). C. Amplification curve showing PCR product detected at 27 cycles to assess the CRE mRNA expression. D. and at 17 cycles to assess the TPO mRNA expression. The horizontal lines indicated the detection threshold.

E through H. Gating strategy to select the Ak haplotype of the mouse MHC class II locus, a haplotype that is known to confer susceptibility to thyroiditis. Blood cells were first gated on side (SSC) and forward (FSC) scatter (panel E), then cells with low scatter (typical of mononuclear hematopoietic origin) were gated for expression of CD45 (panel F). CD45 positive cells were finally gated for expression of MHC class I (Kb) and class II (Ak) surface molecules. G. Mouse heterozygous for K and B. H. Mouse homozygous for K.

**Supplemental Figure 4**. Gating strategy used to quantify mouse thyroperoxidase (TPO) antibodies by flow cytometry. Chinese Hamster Ovary (CHO) cells stably transfected with mouse thyroperoxidase cDNA were first incubated with mouse sera. Cells were then stained with a goat antibody conjugated with FITC directed against mouse IgG. A. Cells were gated on side (SSC) and forward (FSC) scatters, then on the FITC channel. B. Staining profile of a sample where no mouse serum was added (blank). C. Staining obtained when a normal mouse serum, not containing TPO antibodies, was added (negative control). D. Staining obtained using serum from a mouse that developed TPO antibodies (positive control).

**Supplemental Figure 5.** Gating strategy used to analyze mouse thyroid. The digested thyroid gland, or lobe, was stained and gated first on CD45 to identify immune cells (A). CD45+ cells positive for Live/Dead Aqua were excluded (B). Doublets were excluded using both Forward Scatter (C) and Side Scatter (D). CD19+ B cells and CD3+ T cells were separated (E). CD 19+ positive cells were further characterized in terms of expression of IgM and IgD (G). CD3+ T cells were subdivided in CD8+ and CD4+ cells (F). CD8+ cells were further analyzed to assess expression of CD44 and CD62L. The data depicted was produced staining the spleen of a NOD. H2h4. The demarcation between positive and negative signals was determined using fluorescence minus one (FMO) controls.

**Supplemental Figure 6.** Density ofmacrophages (F4/80 expressing cells) in the thyroid glands of tamoxifen injected mice from the concomitant (A), pre-existing (B), or no iodine-enhanced (C) thyroidits groups. Only scattered macrophages are seen in the thyroid, with no significant difference among the three experimental groups. D) Positive control showing a high magnification (40X) an isolated macrophage in the muscle mass surrounding the thyroid gland.

**Supplemental Figure 7.** Expression of thyroid differentiation markers PAX8 (Figure A, E, I, M, Q, U) and TTF-1 (Figure B, F, J, N, R, V), cell proliferation Ki67 (Figure C, G, K, O, S, W), and apoptosis marker cleaved caspase 3 (Figure D, H, L, P, T, X ) in the three experimental groups (concomitant, pre-existing and no iodine-enhanced thyroidits).