

**SUPPLEMENTARY FIG. S2.** Histopathological evidence of I-Tg defect: quantification. Follicles from five controls and seven Vps34<sup>cKO</sup> sections stained with I-Tg were counted for the presence of the thyroxine hormonogenic peptide. Results are expressed as a percentage of I-Tg-positive follicles (2712 control follicles and 3436 Vps34<sup>cKO</sup> were counted). As compared with control mice (black box:  $96\pm3\%$ ), only a quarter (red box;  $25\pm12\%$ ) of Vps34<sup>cKO</sup> follicles were positive for I-Tg (\*\*p=0.01 by Mann-Whitney nonparametric test). I-Tg, iodinated thyroglobulin.



**SUPPLEMENTARY FIG. S3.** Macroautophagy and chaperone-mediated autophagy. (**A**) Thyroid sections from control (left) and Vps34<sup>cKO</sup> (right) labeled for TOM20 (red), lysosomal LAMP-1 (green), and E-cadherin (white). Nuclei are labeled by Hoechst (presented in blue). In control and Vps34<sup>cKO</sup>, TOM20 shows a perinuclear localization without colocalization with LAMP-1 (no yellow), even though LAMP-1 signal is more widespread. (**B**) Thyroid sections from control (left) and Vps34<sup>cKO</sup> (right) labeled for thyro-globulin (red), LAMP-2A (green), and E-cadherin (white). Nuclei are labeled by Hoechst (shown in blue). In Vps34<sup>cKO</sup> sections, LAMP-2A signal is much stronger than in control thyroid, indicating up-regulation of chaperone-mediated autophagy.