

## 1 **1. Supplemental Materials and Methods**

2 *1.1. H&E Staining.* Surgical specimens of the thyroid and thymic tissues were paraffin-embedded for  
3 H&E staining and immunohistochemistry (IHC). Paraffin-embedded tissues sections (4  $\mu\text{m}$ ) were  
4 stained with hematoxylin (Polyscience, Inc. Warrington, PA) for 40 s and with eosin (Sigma-Aldrich,  
5 St Louis, MO) for 30 s. The tissue sections were examined under a light microscope (Olympus BX51;  
6 Olympus, Center Valley, PA) after mounting with Permount mounting medium (Fisher Scientific,  
7 Miami, FL).

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9 **2.1.** *Cell culture.* Human papillary thyroid (TPC-1) cell lines were kindly provided from Dr. Shunichi  
10 Yamashita and Dr. Norisato Mitsutake (Nagasaki University Graduate School of Biomedical Sciences,  
11 Nagasaki, Japan). TPC-1 and FRO cells in RPMI 1640 medium (Welgene, Daegu, Korea) supplemented  
12 with non-heat-inactivated 5% fetal bovine serum (FBS; Invitrogen, Grand Island, NY) and 1 $\times$   
13 antibiotics (Invitrogen) were grown.

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15 **2.1.** *Western blotting.* Grained thymus tissues or TPC-1 cells were lysated in radio-immunoprecipitation  
16 assay (RIPA) buffer (Sigma-Aldrich) containing protease inhibitor cocktail (Roche Diagnostics, Basel,  
17 Switzerland), and the insoluble pellets were removed by centrifugation at 8000  $\times$ g for 10 min at 4°C.  
18 Proteins in the cell lysates were quantified using BCA protein assay kits (Thermo Scientific, Palm  
19 Springs, CA). Twelve microgram of proteins mixed with polyacrylamide gel electrophoresis sample  
20 buffer (Invitrogen) and NuPAGE reducing agent (Invitrogen) was separated in 8% SDS-PAGE mini  
21 gels and transferred to PVDF membranes (GE healthcare life sciences, Marlborough, MA). Membranes  
22 were blocked in 5% skim milk for 1 hr at room temperature, and incubated with following primary  
23 antibodies for NIS (goat polyclonal antibody (sc-48052)) for 1.5 hr at room temperature or rabbit anti-  
24 GAPDH (Abclon, Guro-gu, Seoul, South Korea). The membranes were incubated in Horseradish

1 peroxidase (HRP)-conjugated anti-rabbit secondary antibodies (Invitrogen). Bound secondary  
2 antibodies were detected by using ECL detection reagents (Roche, Nutley, NJ).

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1 **2. Supplemental Figure Legends**

2 SUPPLEMENTAL FIGURE 1: Microscopic findings in human thymic tissues. Hematoxylin and eosin  
3 (H&E) staining was performed in the thymic tissues: representative 24-year-old (A), 39-year-old (B),  
4 46-year-old (C) and 55-year-old patient (D) images are shown. All images are magnified 40x, and the  
5 scale bars represent 500  $\mu\text{m}$ .

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7 SUPPLEMENTAL FIGURE 2: NIS protein expression in the thymus tissue by Western blot. The  
8 expression of NIS protein was examined by Western blot analysis. The expression of NIS protein is  
9 shown in representative thymus samples in the four age groups (20s, 30s, 40s, and over 50s groups).  
10 TPC-1 cells were used as a positive control for NIS expression. Glycosylated (87 - 110 kDa) NIS  
11 proteins and non-glycosylated (approximately 50 kDa) NIS proteins are indicated with black arrow and  
12 white arrows, respectively. GAPDH was used as an internal control.

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1 SUPPLEMENTAL TABLE 1: Expression of thyroid-related proteins in Hassall's corpuscles of the  
2 thymus according to the ages.

	20s	30s	40s	Over 50s	Total
	n = 5	n = 6	n = 5	n = 6	n = 22
NIS	59%	66.7%	80%	88%	71.2%
TSHR	70%	97.5%	51.1%	100%	79.7%
TPO	0%	14.2%	14.6%	27.5%	14.1%
Tg	0%	0%	0%	0%	0%

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4 The expression of NIS, TSHR, TPO, and Tg was detected in the thymic tissues by  
5 immunohistochemistry (IHC). The positive Hassall's corpuscles (%) were analyzed in the four age  
6 groups: 20s, 30s, 40s, and over 50s groups.

7 The positively stained and total Hassall's corpuscles were counted in the thymic tissues, and expressed  
8 as the percentage of positive Hassall's corpuscles.

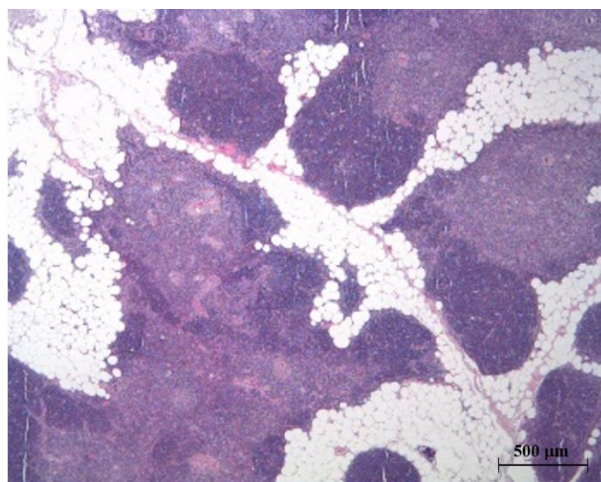
1 SUPPLEMENTAL TABLE 2: Expression of thyroid-related proteins in thymocytes of the thymus  
 2 according to the ages.

	20s	30s	40s	Over 50s	Total
	n = 5	n = 6	n = 5	n = 6	n = 22
NIS	1 (20%)	2 (33.3%)	1 (20%)	1 (16.7%)	5 (22.7%)
TSHR	2 (40%)	5 (83.3%)	3 (60%)	5 (83.3%)	15 (68.2%)
TPO	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tg	1 (20%)	0 (0%)	0 (0%)	0 (0%)	1 (4.5%)

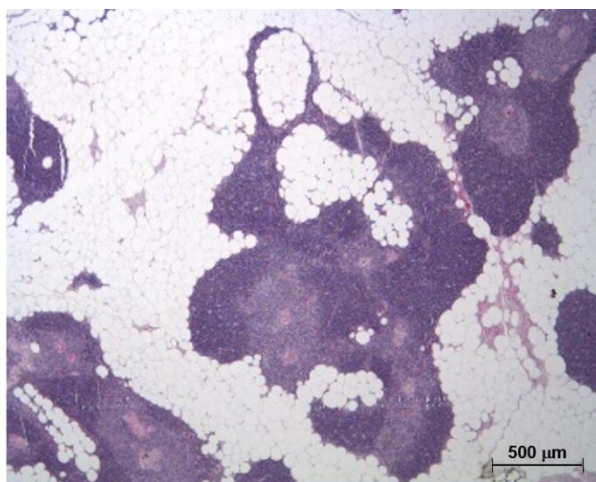
3  
 4 The expression of NIS, TSHR, TPO, and Tg was detected in the thymic tissues by  
 5 immunohistochemistry (IHC). The positive sample number and the positive percentages in thymocytes  
 6 of the thymic samples were analyzed in the four age groups: 20s, 30s, 40s, and over 50s groups.  
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1 SUPPLEMENTAL FIGURE 1

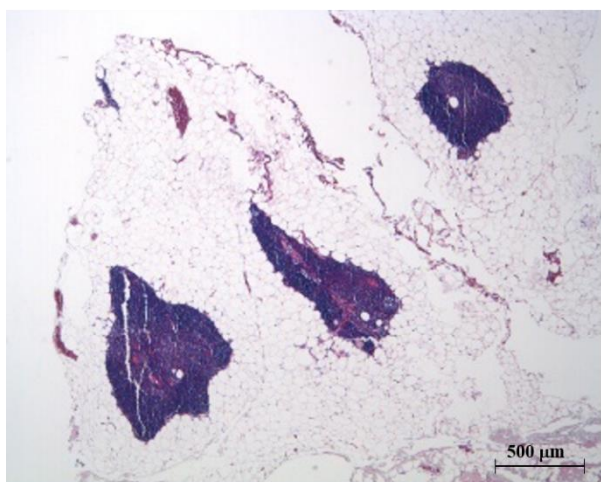
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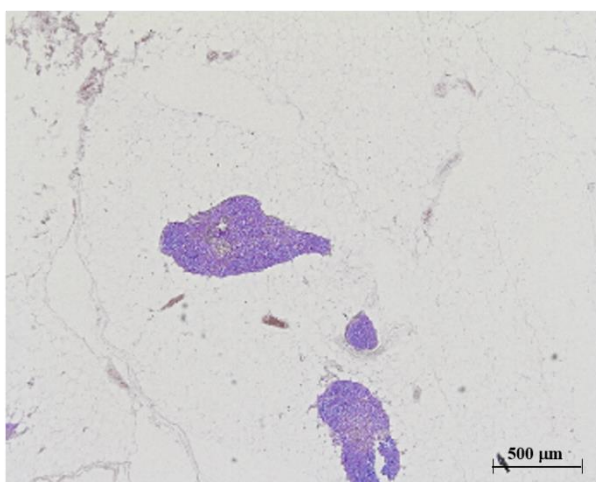
(a)



(b)



(c)



(d)

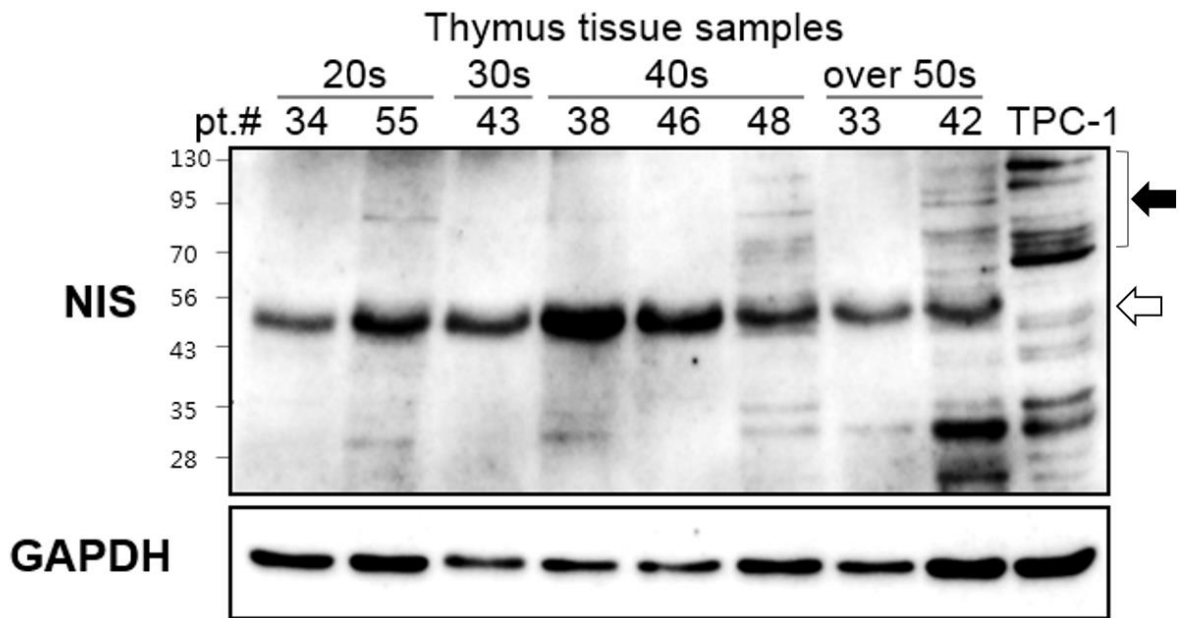
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1 SUPPLEMENTAL FIGURE 2

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