

Supplementary information

Technical Resource

Mapping Thyroid Changes in Size and Position during Enlargement in Adult Mice with Hyperthyroidism

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Table S1. Thyroid sizes in native mice, and in 35-week immunized mice of various phases.

	native	T03-06	T06-09	T09-12	T13-15	T>15
N	10	7	15	11	13	14
Max (mm ³)	3.62	5.99	8.57	11.68	14.95	20.61
Min (mm ³)	2.30	4.22	6.19	9.21	12.36	15.08
means (mm ³)	2.92	5.25	7.26	10.59	13.82	16.95
SD	0.39	0.65	0.83	0.88	0.80	1.69
SEM	0.12	0.24	0.21	0.26	0.22	0.45

The ranges of thyroid volume include 3-6 (T03-06), 6.1-9 (T06-09), 9.1-12 (T9-12), 12.1-15 (T12-15), and >15 (T>15) in mm³.

Table S2. Background of mice used for sketching thyroid cartilage and experiment validation.

purposes	N	strains ¹	gender	means ± SD	
				age(weeks)	body weight (g)
sketching cartilage	6	BALB/c	female	42	23.07 ± 1.61
validation	6	C57 BL/6J	male	9.35 ± 0.25	23.62 1.06

1. The mice were purchased from Charles River Laboratory (Sulzfeld, Germany).

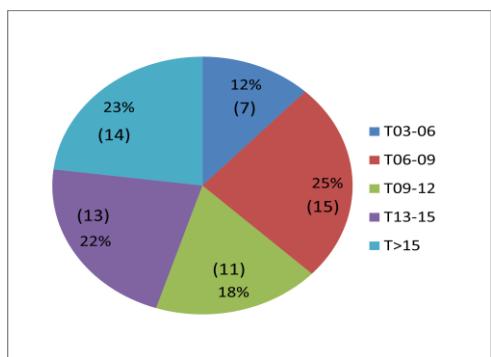


Figure S1. The distribution in thyroid volume of various phases developed during immunization of 35 w.

The ranges of the volume include 3-6 (T03-06), 6.1-9 (T06-09), 9.1-12 (T9-12), 12.1-15 (T12-15), and >15 (T>15) in mm³. The numerals in brackets represent the number of thyroids collected in the corresponding range of volume.

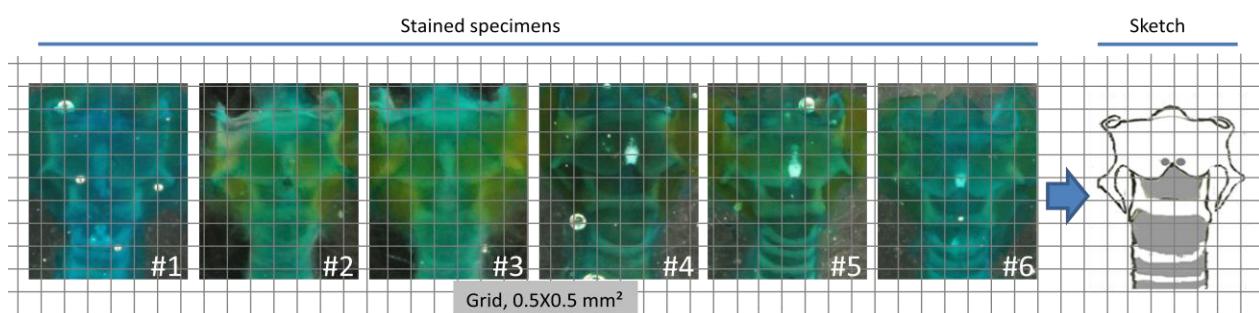


Figure S2. Stained specimens and a sketch of the skeleton of thyroid, cricoid, and tracheal cartilages. Six age-matched specimens randomly collected were subjected to Alcian blue staining and the stained specimens displayed a high similarity in cartilage skeleton. A skeleton was sketched using a coordinate or checked paper.

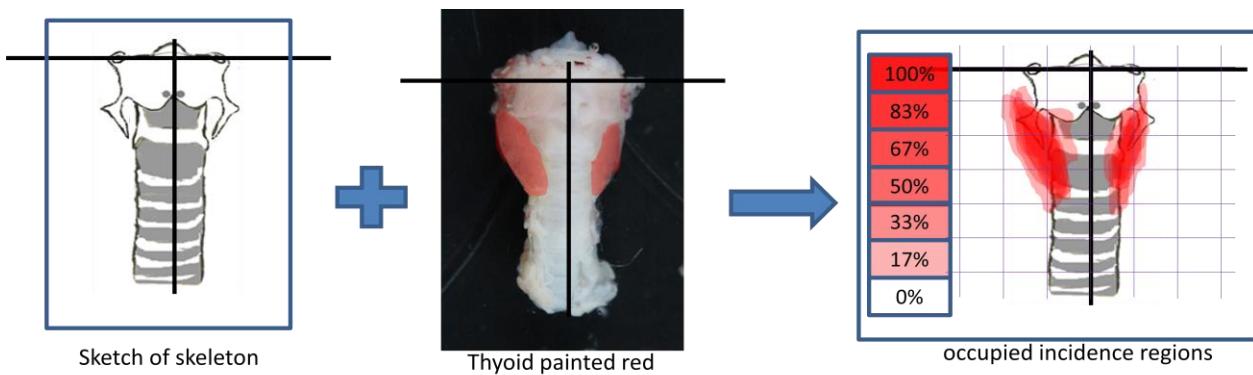


Figure S3. Protocol for drawing graphs of site-dependent incidence in front view. The sketch of the cartilage skeleton was superposed with red-painted thyroids, which led to the production of graphs of site-dependent incidence. Grid, 1X1 mm²

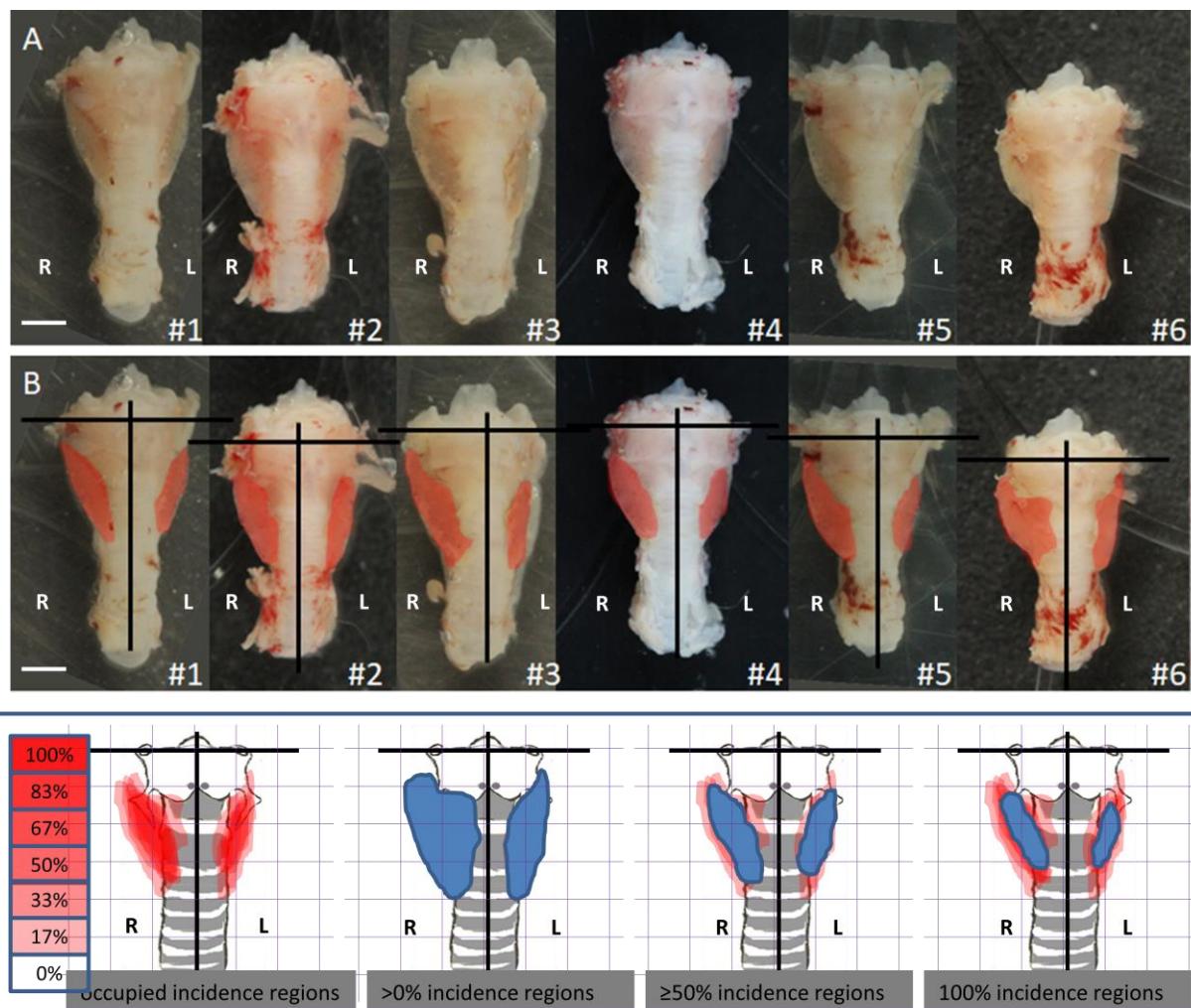


Figure S4. Graphs of site-dependent incidence of thyroid in native mice. A. Specimen collection. B. Thyroid painting. C. Incidence region of thyroid occupied. Grid, 1X1 mm²; Bars = 1 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

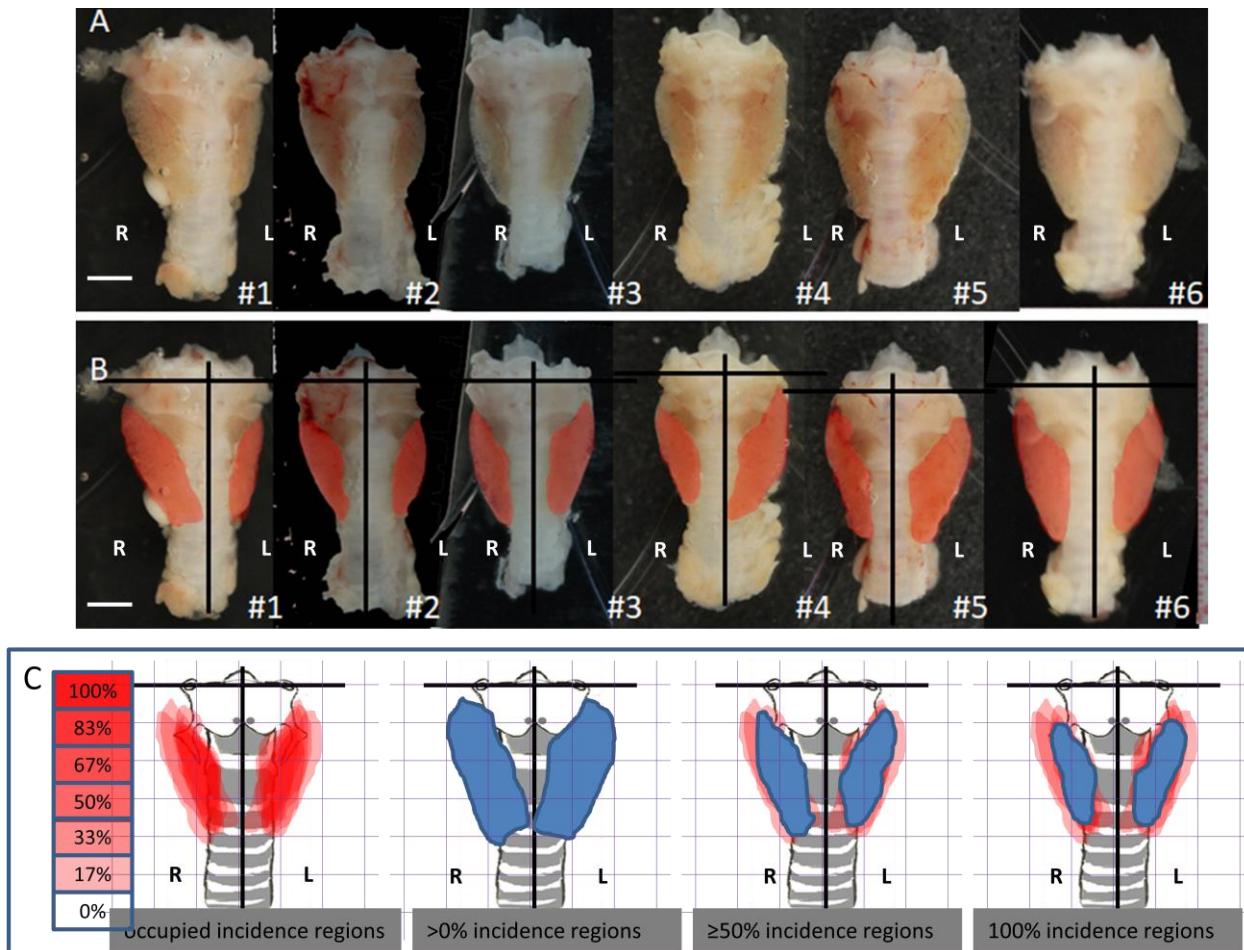


Figure S5. Graphs of site-dependent incidence of thyroid (T3-6) in an arrangement of 3.1-6 mm³. A.
 Specimen collection. B. Thyroid painting. C. Incidence region of thyroid occupied. Grid, 1X1 mm²; Bars = 1 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

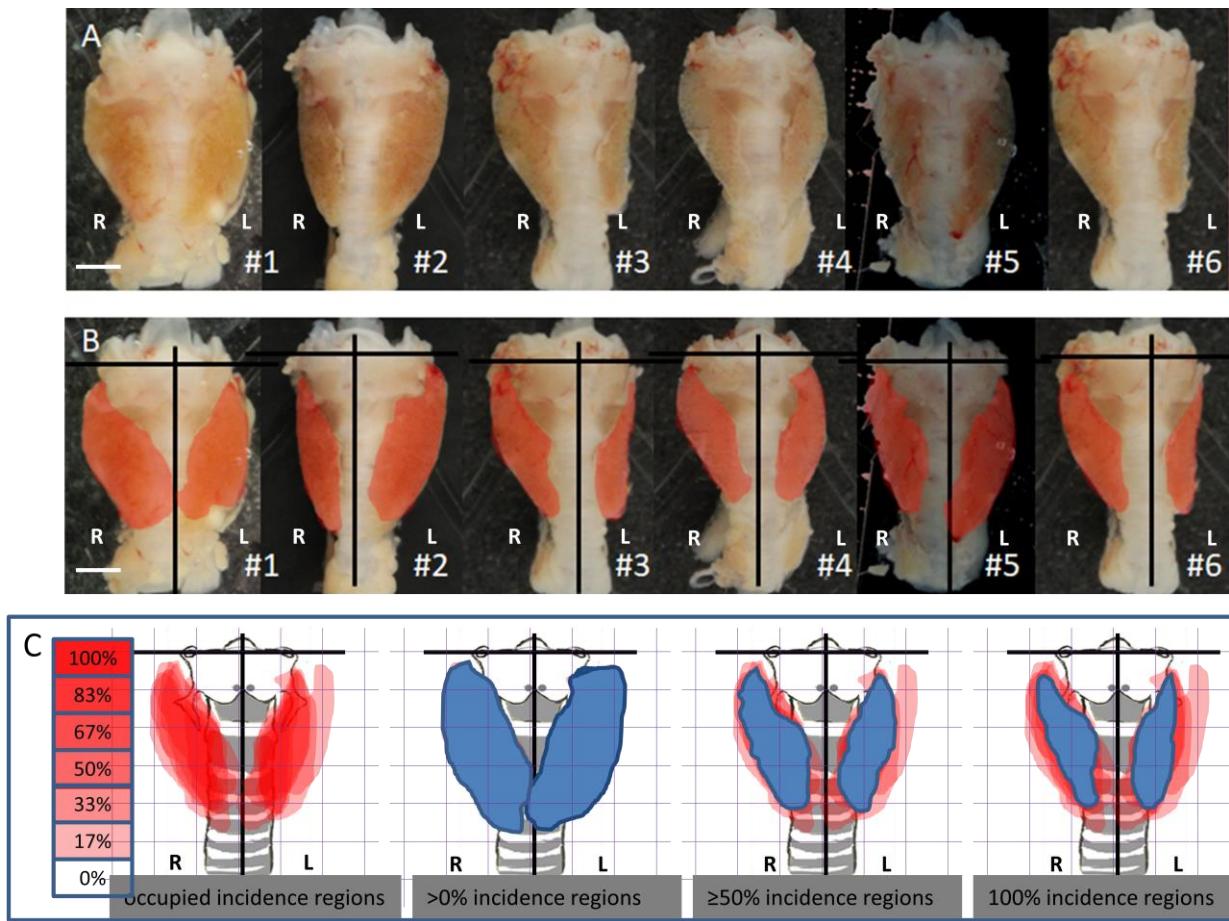


Figure S6. Graphs of site-dependent incidence of thyroid (T6-9) in an arrangement of 6.1-9 mm³. A. Specimen collection. B. Thyroid painting. C. Incidence region of thyroid occupied. Grid, 1X1 mm²; Bars = 1 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

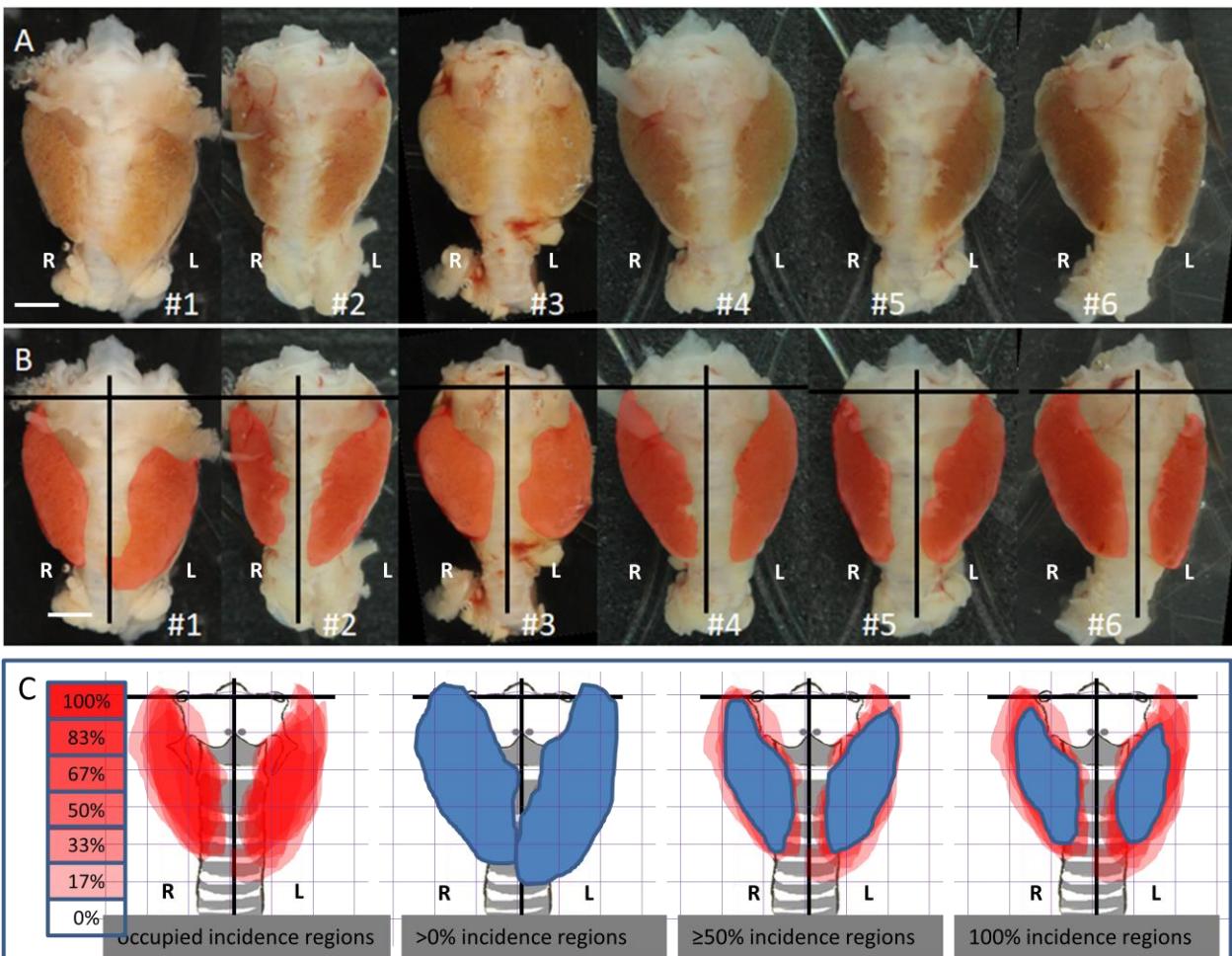


Figure S7. Graphs of site-dependent incidence of thyroid (T9-12) in an arrangement of 9.1-12 mm³. A. Specimen collection. B. Thyroid painting. C. Incidence region of thyroid occupied. Grid, 1X1 mm²; Bars = 1 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

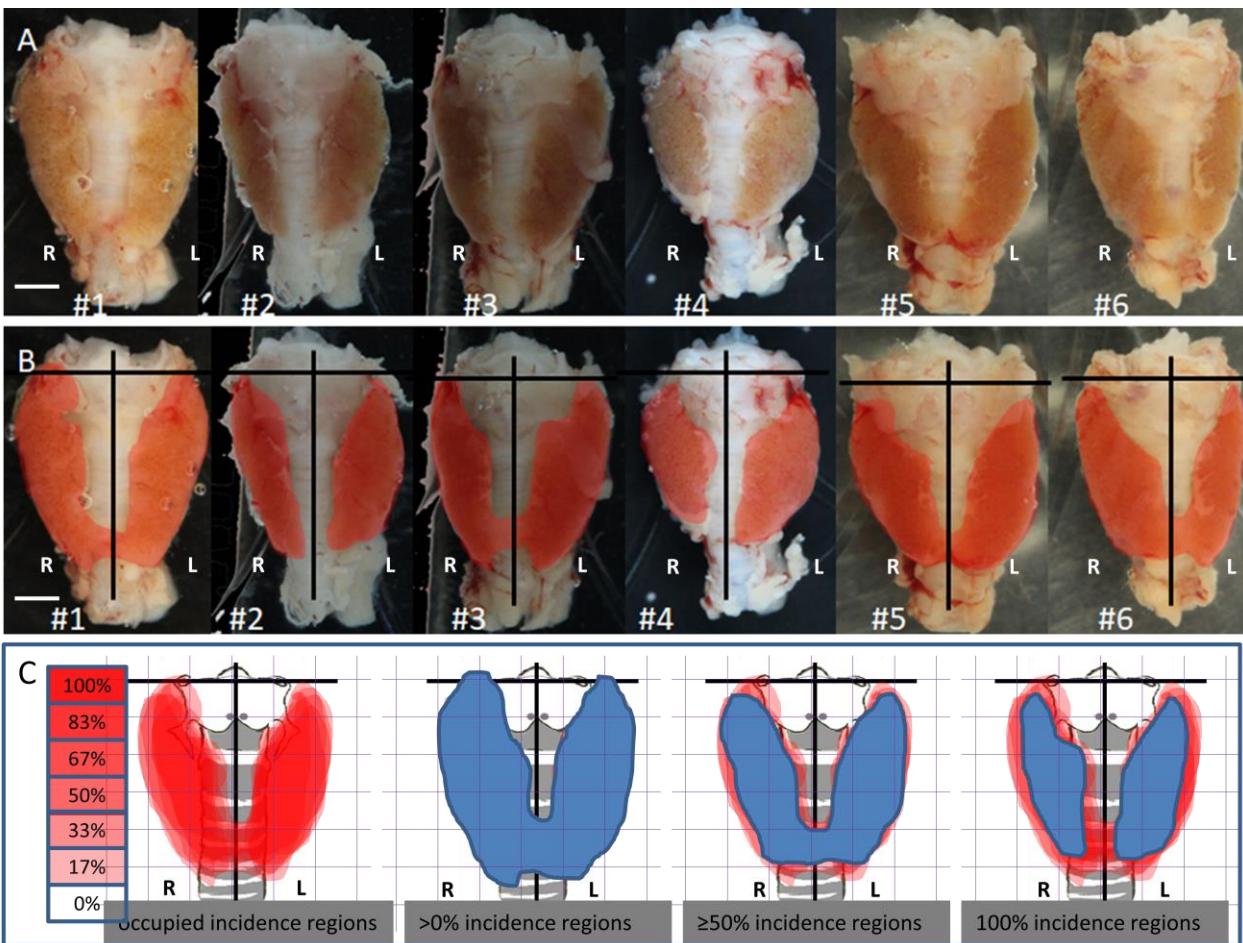


Figure S8. Graphs of site-dependent incidence of thyroid (T12-15) in an arrangement of 12.1-15 mm³. A. Specimen collection. B. Thyroid painting. C. Incidence region of thyroid occupied. Grid, 1X1 mm²; Bars = 1 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

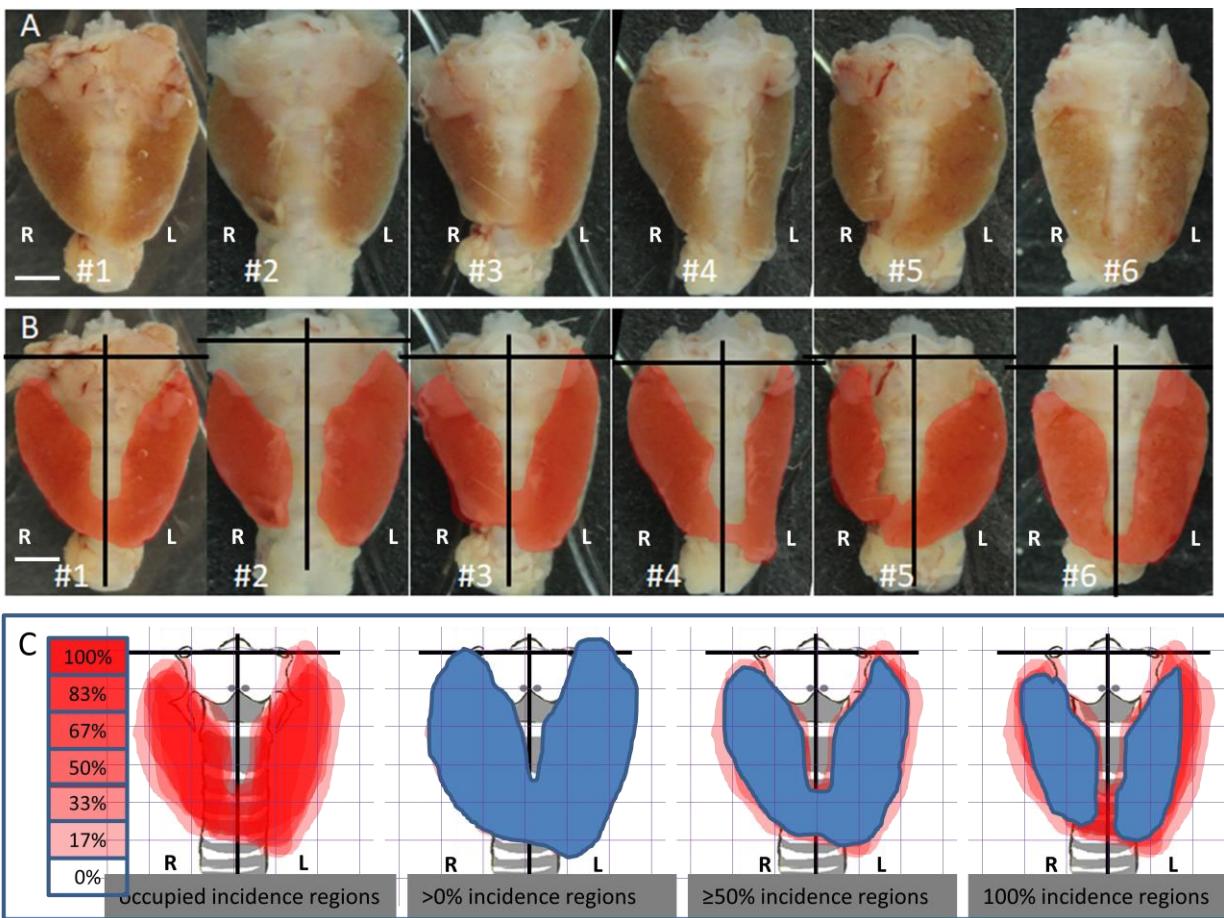


Figure S9. Graphs of site-dependent incidence of thyroid ($T>15$) in an arrangement of $>15 \text{ mm}^3$. A. Specimen collection. B. Thyroid painting. C. Incidence region of thyroid occupied. Grid, $1\times 1 \text{ mm}^2$; Bars = 1 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

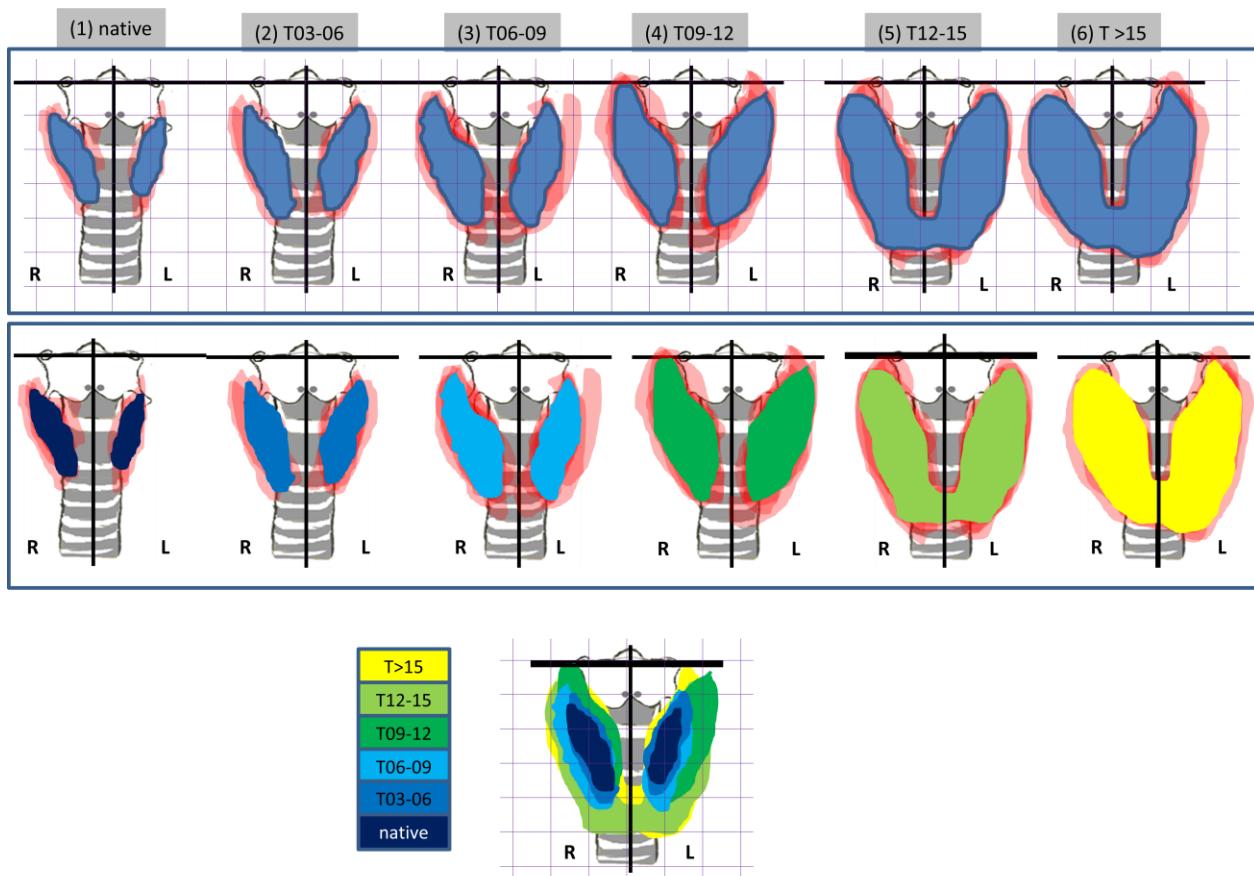


Figure S10. Phase-dependent topographs of thyroid of $\geq 50\%$ incidence regions. A. Graphs of $\geq 50\%$ incidence regions. B. Thyroid painting. C. Phase-dependent topographs. Grid, $1 \times 1 \text{ mm}^2$; R, right lobe; L, left lobe.

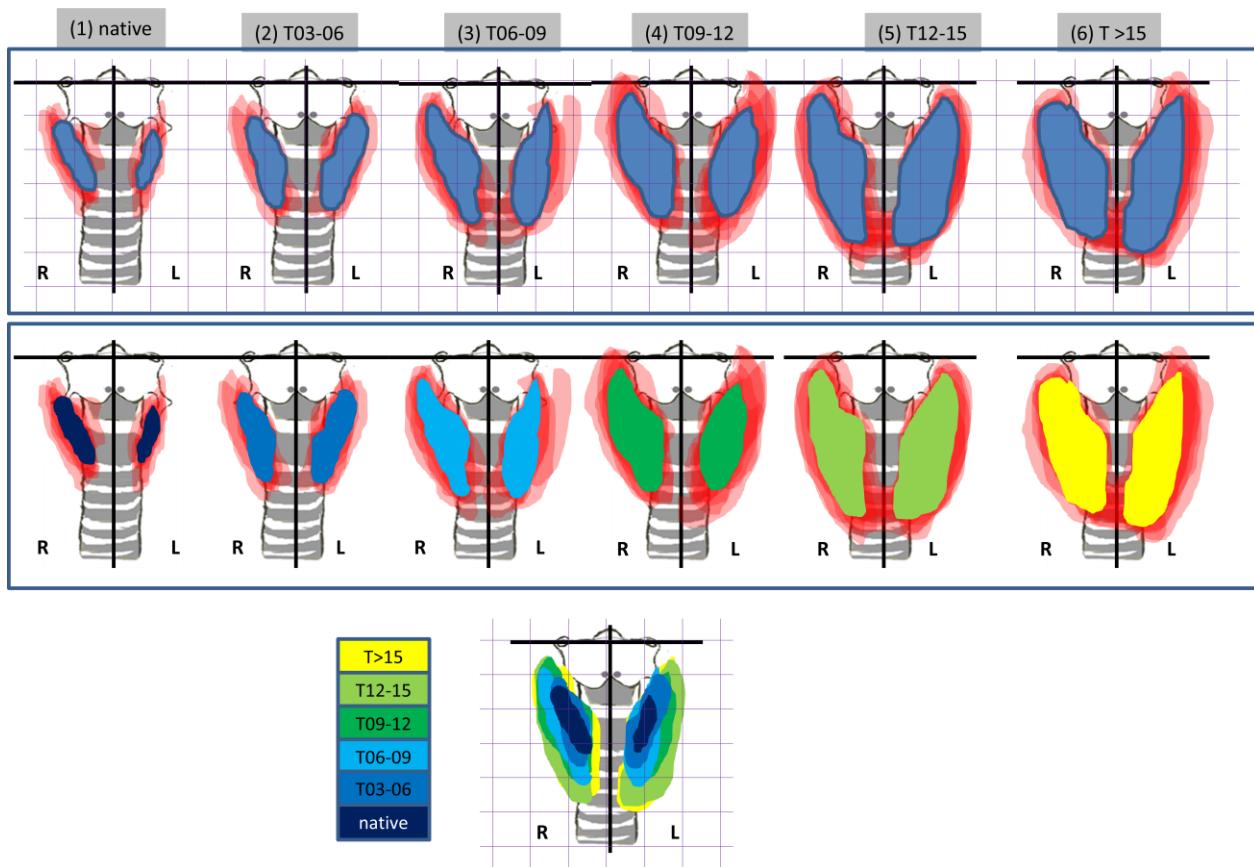


Figure S11. Phase-dependent topographs of 100% incident region in thyroid. A. Graphs of 100% incidence regions. B. Thyroid painting. C. Phase-dependent topographs. Grid, 1X1 mm²; R, right lobe; L, left lobe.

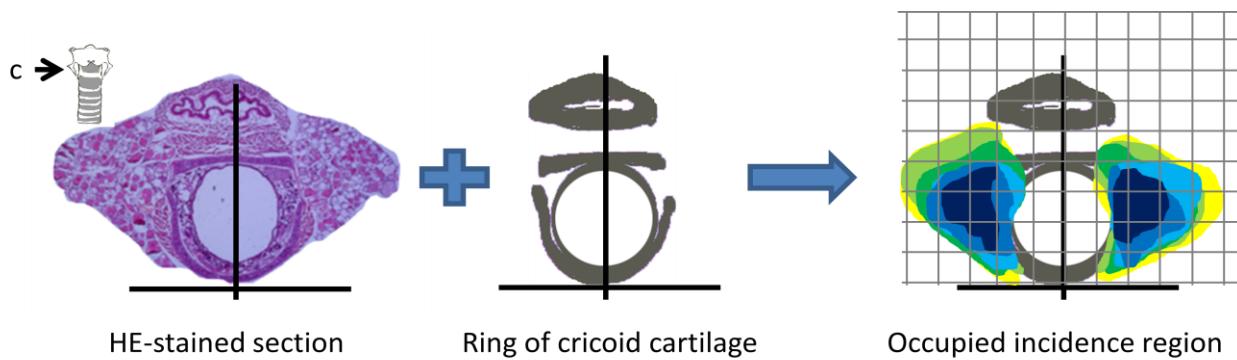


Figure S12. Protocol for drawing graph of site-dependent incidence in thyroid section. Grid, 0.4X0.4 mm²; “c” in the inset indicates the site for the sectioning plane.

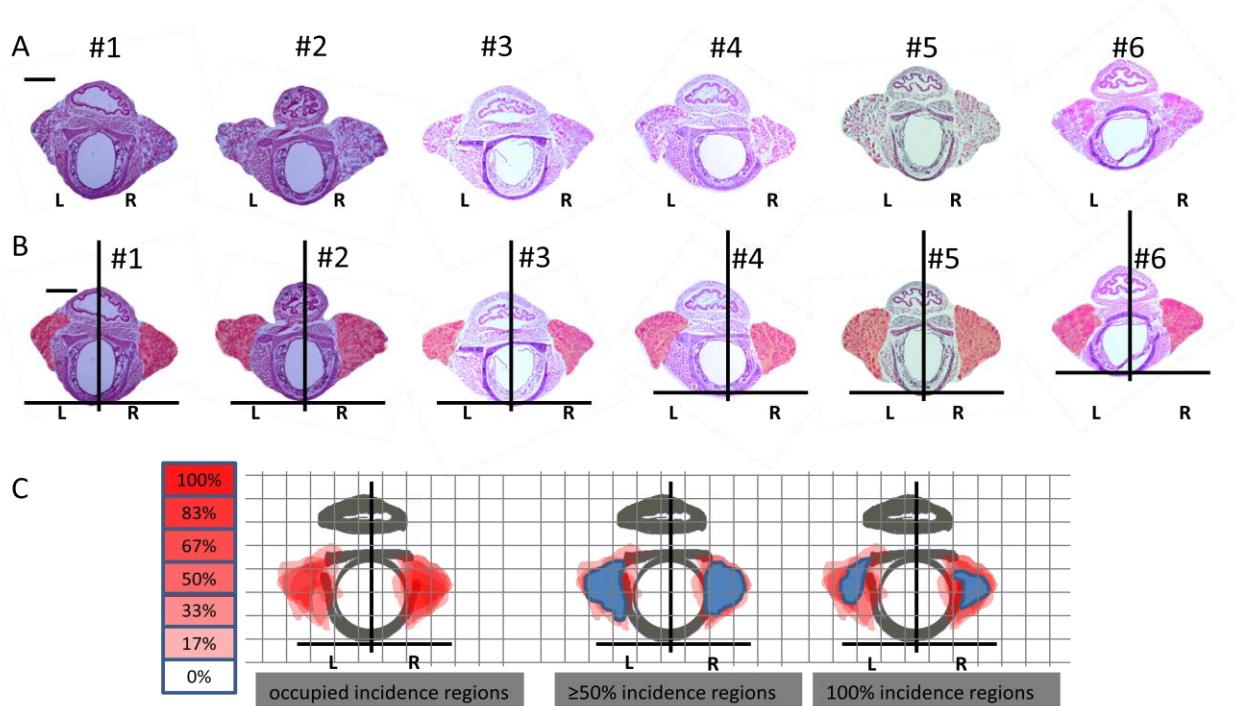


Figure S13. Graphs of site-dependent incidence of thyroid in sections from native mice. A. Section collection. B. Thyroid tissue painting. C. Incidence region of thyroid occupied in section. Grid, 0.4X0.4 mm²; Bars = 0.4 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

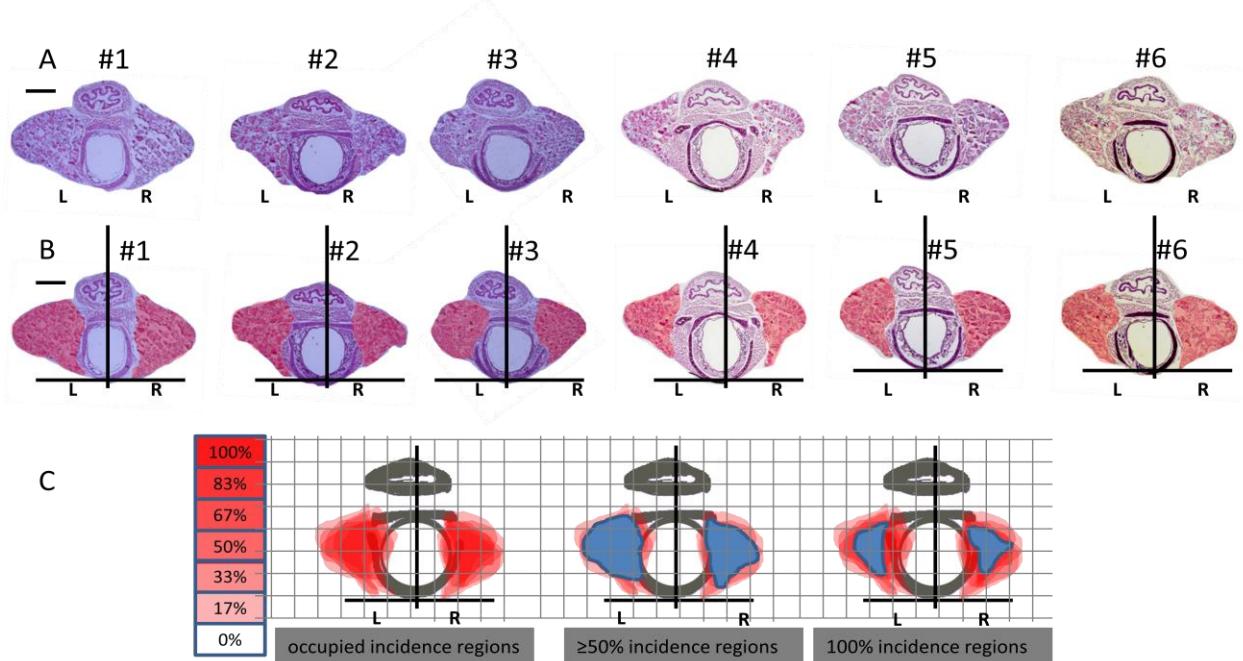


Figure S14. Graphs of site-dependent incidence of thyroid in sections from the mice of phase T03-06. A. Section collection. B. Thyroid tissue painting. C. Incidence region of thyroid occupied in section. Grid, 0.4X0.4 mm²; Bars = 0.4 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

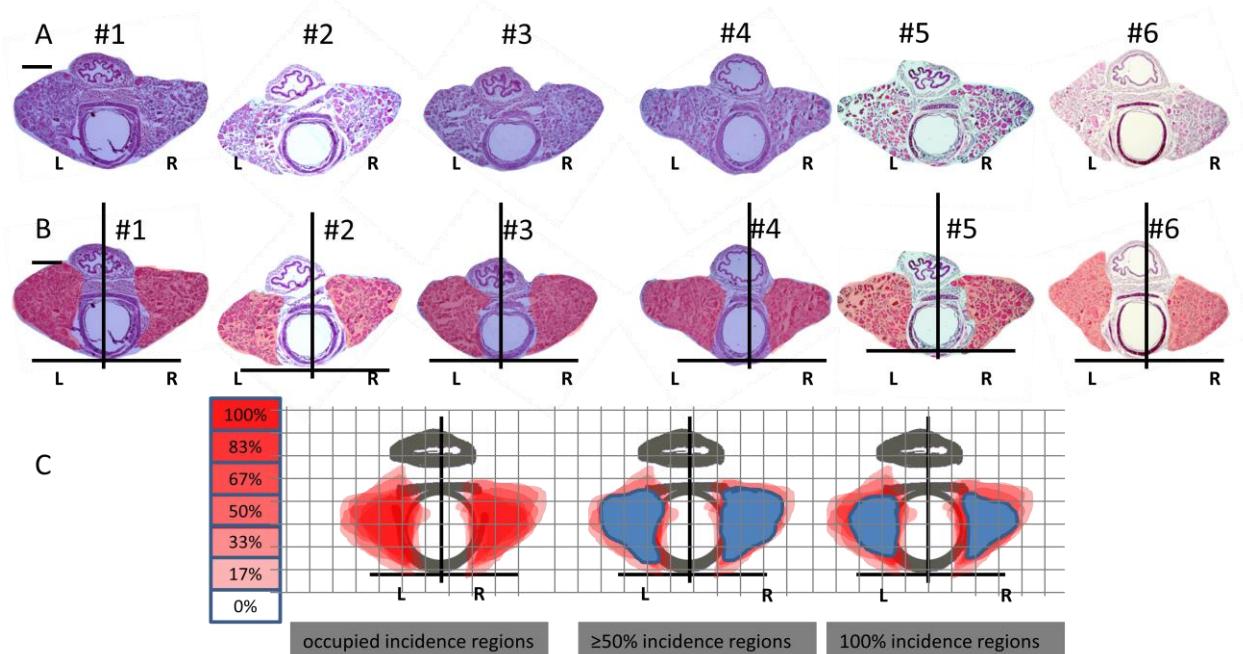


Figure S15. Graphs of site-dependent incidence of thyroid in sections from the mice of phase T06-09. A. Section collection. B. Thyroid tissue painting. C. Incidence region of thyroid occupied in section. Grid, 0.4X0.4 mm²; Bars = 0.4 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

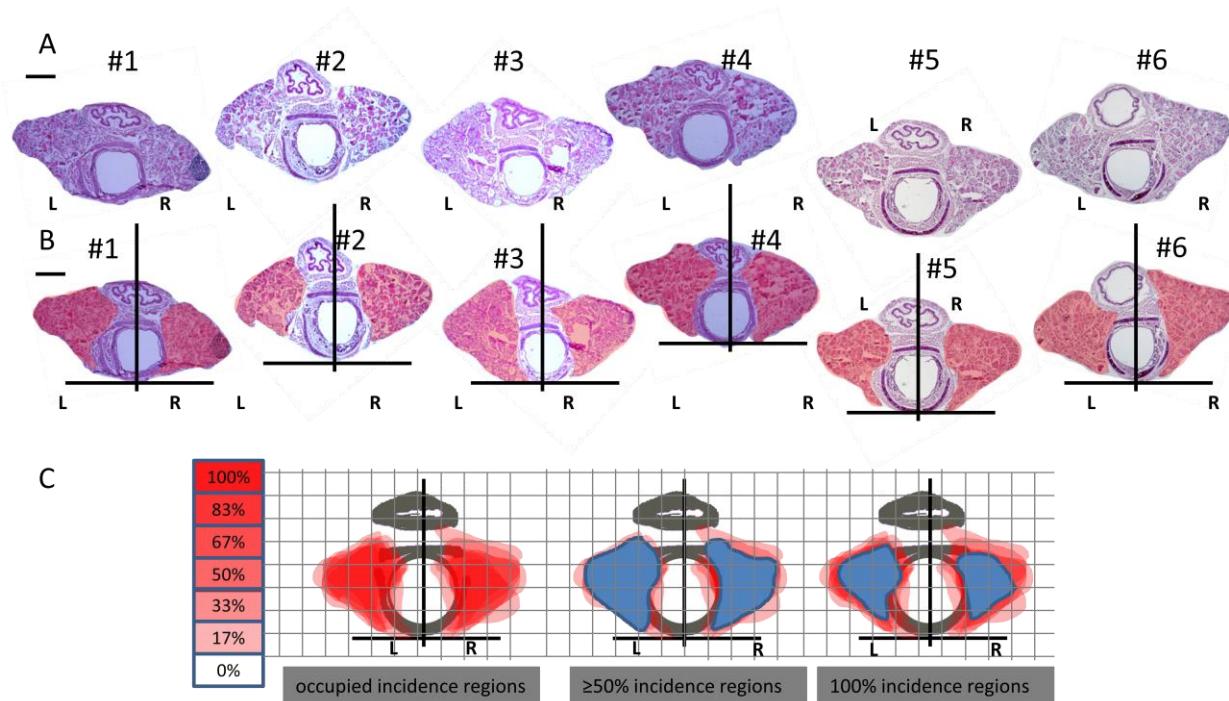


Figure S16. Graphs of site-dependent incidence of thyroid in sections from the mice of phase T09-12. A. Section collection. B. Thyroid tissue painting. C. Incidence region of thyroid occupied in section. Grid, 0.4X0.4 mm²; Bars = 0.4 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

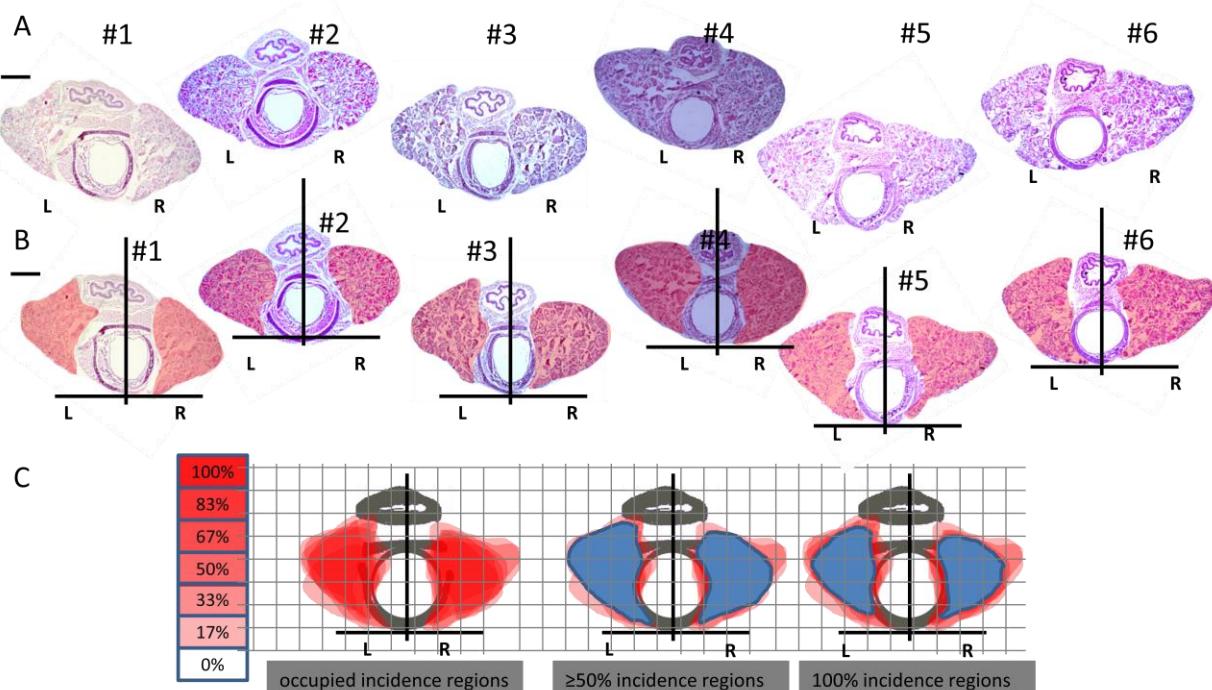


Figure S17. Graphs of site-dependent incidence of thyroid in sections from the mice of phase T12-15. A. Section collection. B. Thyroid tissue painting. C. Incidence region of thyroid occupied in section. Grid, 0.4X0.4 mm²; Bars = 0.4 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

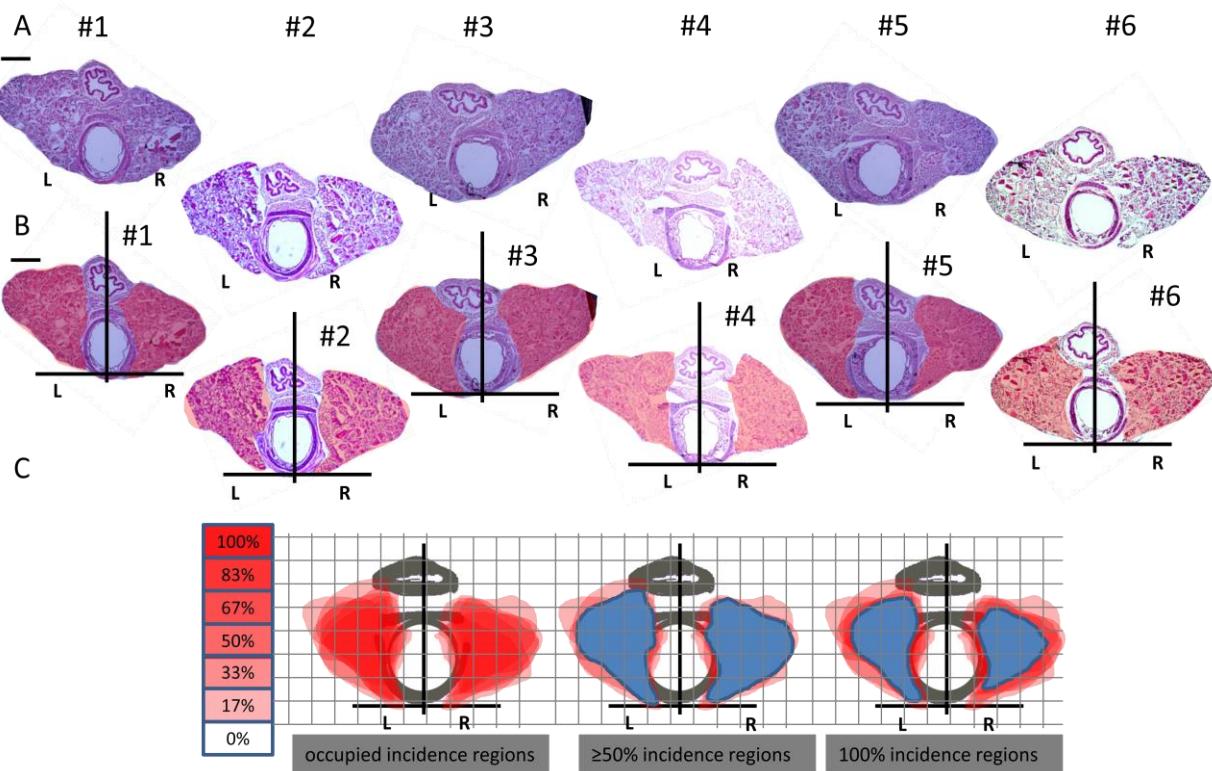


Figure S18. Graphs of site-dependent incidence of thyroid in sections from the mice of phase >15. A. Section collection. B. Thyroid tissue painting. C. Incidence region of thyroid occupied in section. Grid, 0.4X0.4 mm²; Bars = 0.4 mm; the target regions are indicated with blue; R, right lobe; L, left lobe.

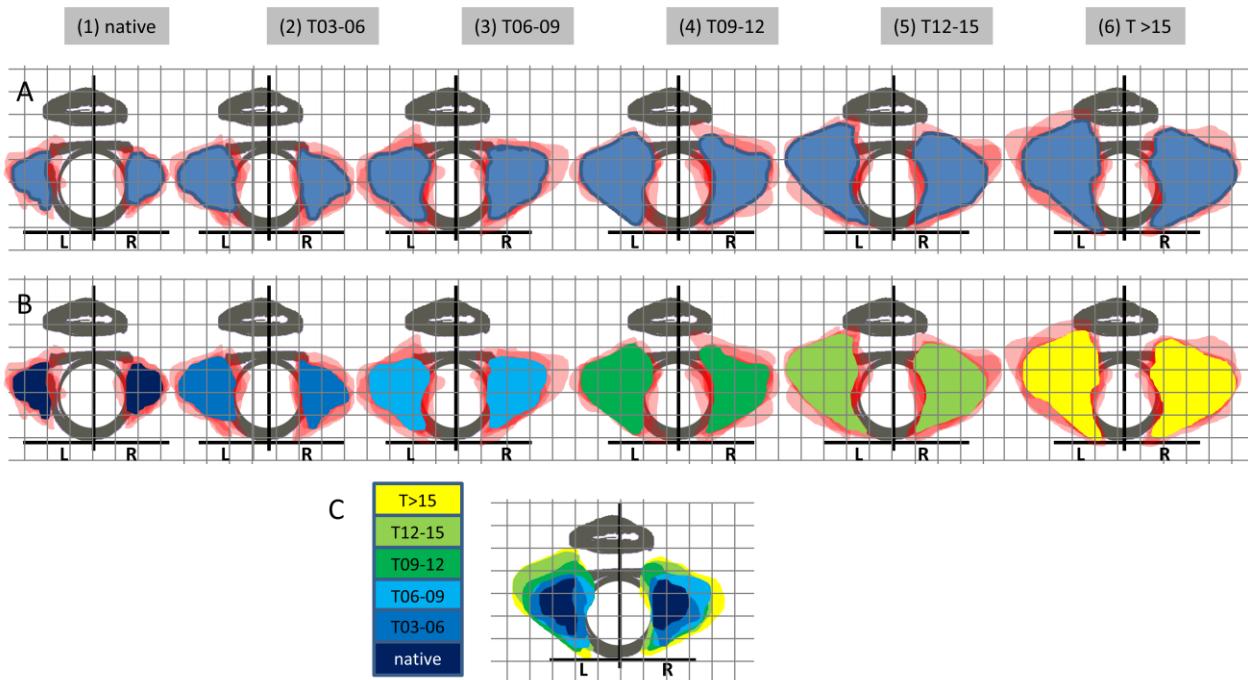


Figure S19. Phase-dependent topograph of thyroid of ≥50% incidence regions. A. Graphs of ≥50% incidence regions. B. Thyroid painting. C. Phase-dependent topographs. Grid, 0.4X0.4 mm²; R, right lobe; L, left lobe.

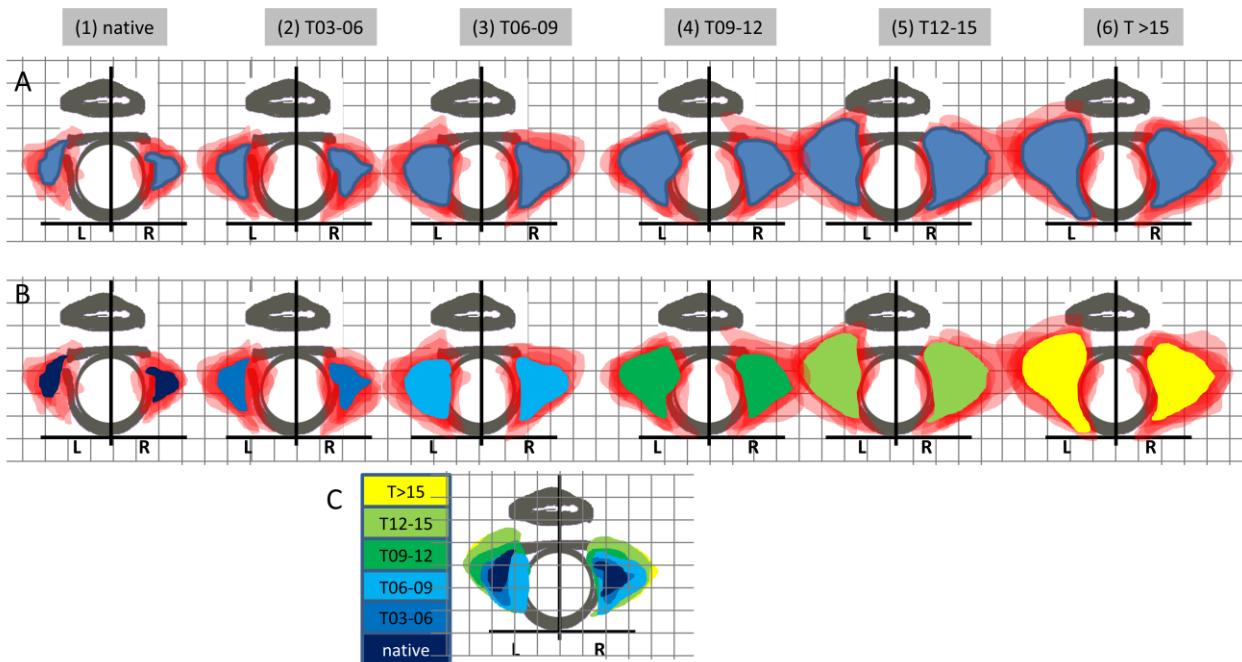


Figure S20. Phase-dependent topograph of thyroid of $\geq 50\%$ incidence regions. A. Graphs of $\geq 50\%$ incidence regions. B. Thyroid painting. C. Phase-dependent topographs. Grid, $0.4 \times 0.4 \text{ mm}^2$; R, right lobe; L, left lobe.

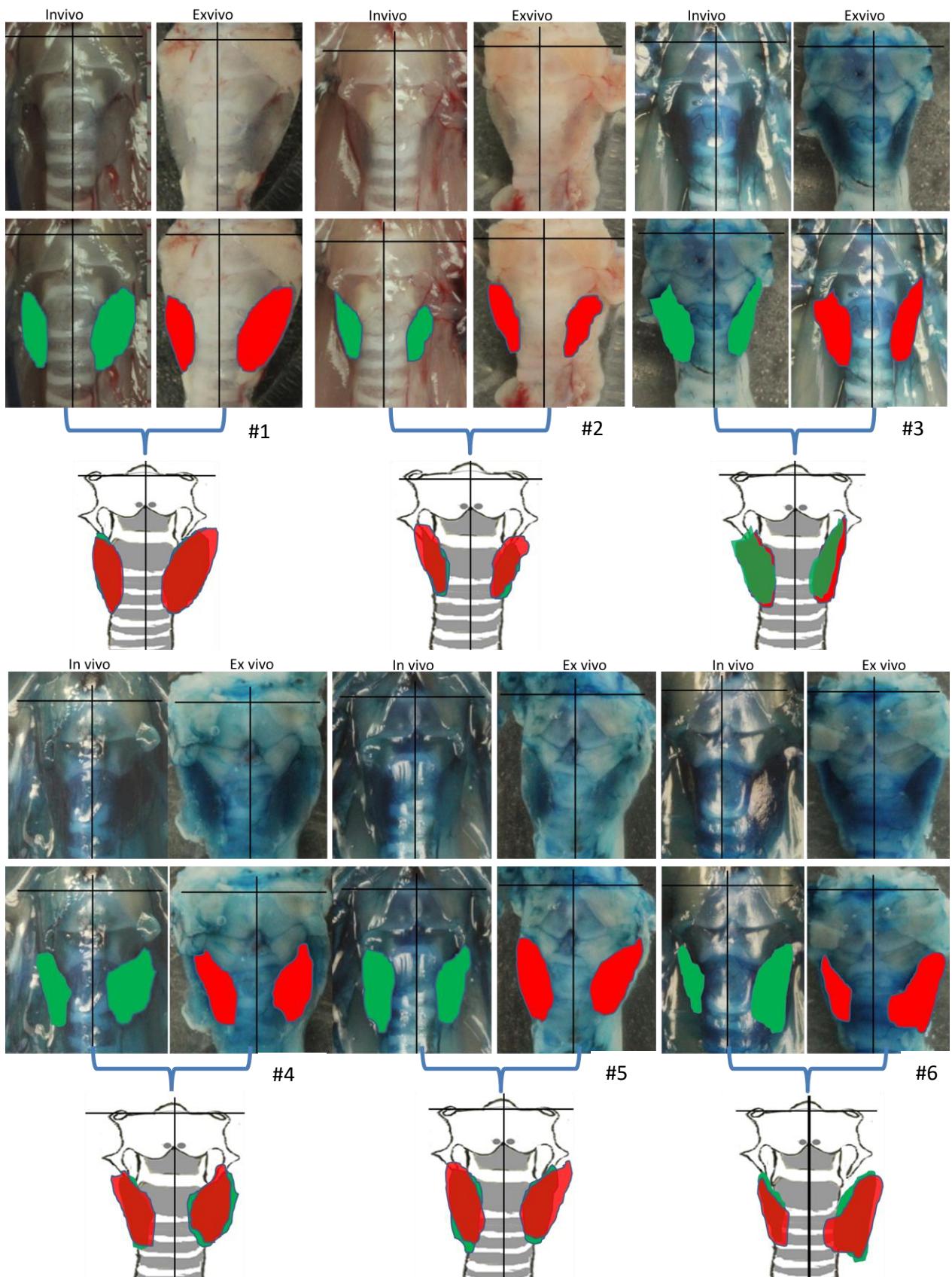


Figure S21. Validation of protocol by comparison of thyroid size and position between in vivo and ex vivo. The thyroids were marked with green (in vivo) or red (ex vivo) and their images were merged. Thyroids #3-6 were intracardially perfused with Evans Blue.