

SAKAMACH Supplementary

## **Supplementary Materials**

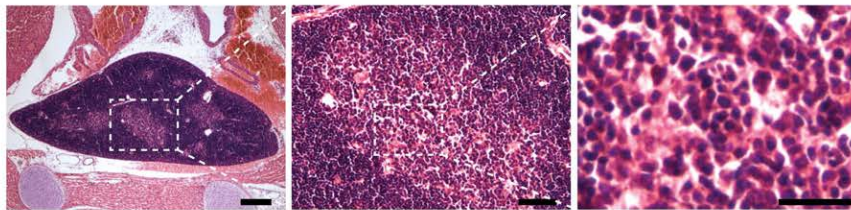
# **TAK1 Regulates Thymus Resident Macrophages By Protecting Lysosomal Integrity**

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**Supplementary Figures**

Sakamachi et al. Supplementary Figure S1

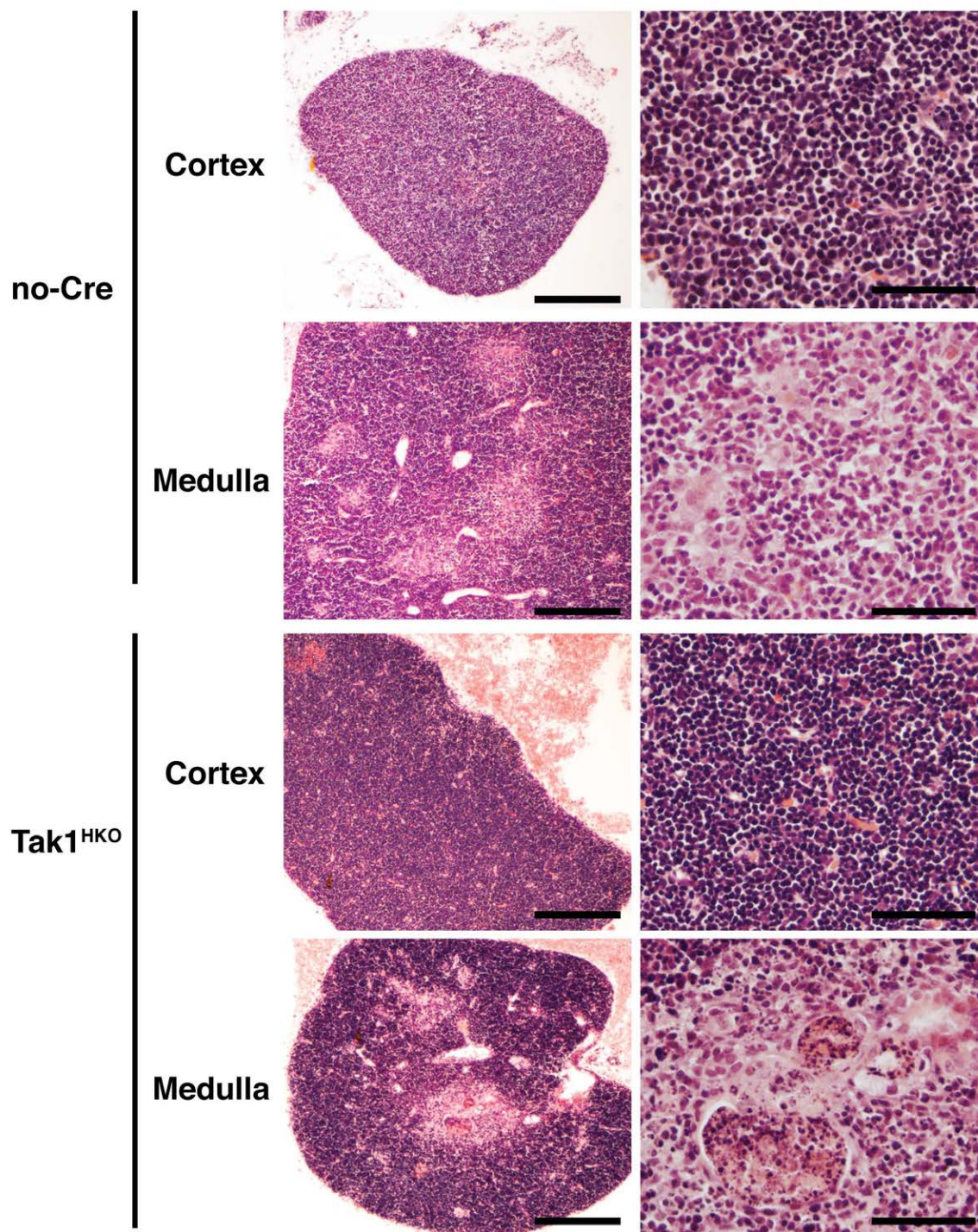
*vav-Cre Tak1<sup>flox/+</sup>*



**Figure S1. Hematopoietic-specific heterozygous deficiency of *Tak1* does not cause abnormality in the thymus**

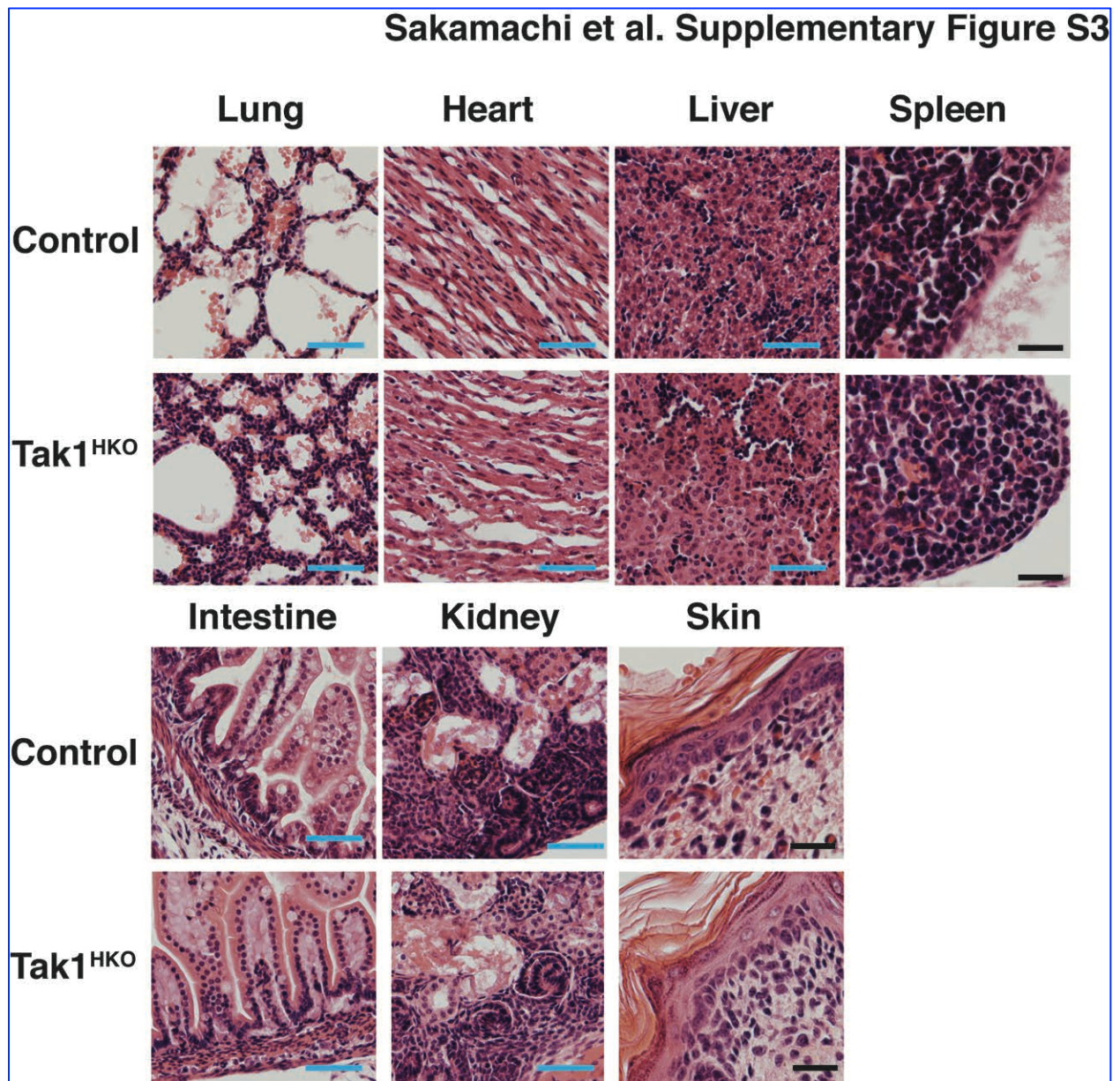
H&E staining of E18.5 *vav-Cre Tak1<sup>flox/+</sup>* thymus. Scale bars, 200  $\mu\text{m}$  (left panel), 50  $\mu\text{m}$  (middle panel), 20  $\mu\text{m}$  (right panel).

Sakamachi et al. Supplementary Figure S2

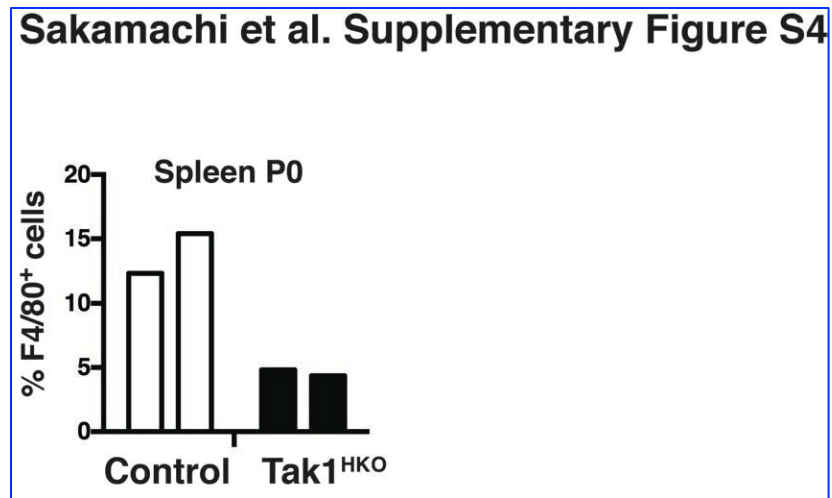


**Figure S2. H&E staining of thymus**

H&E staining of P0 no-Cre and Tak1<sup>HKO</sup> thymus. Two different positions (cortex and medulla) from one thymus of no-Cre or Tak1<sup>HKO</sup> mouse are shown. Scale bars, 200 μm (left panels), 50 μm (right panels).



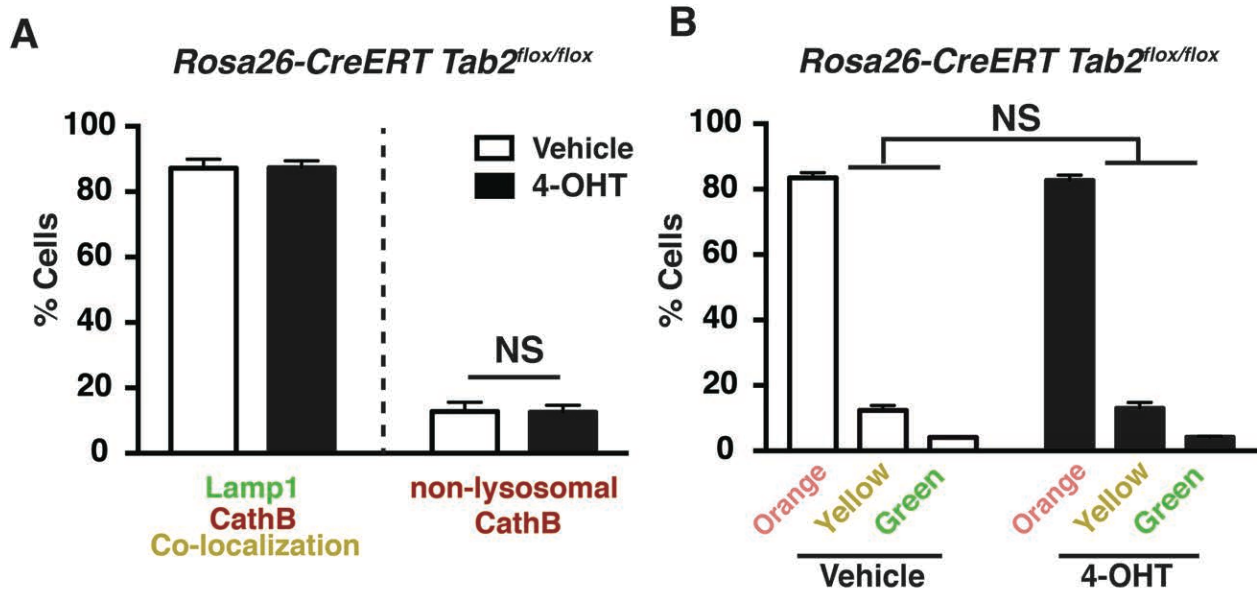
**Figure S3. H&E staining of the lung, heart, liver, spleen, intestine, kidney and skin.** Five controls including no-Cre and Tak1-Het and three Tak1<sup>HKO</sup> mice at P0 were observed. Representative H&E staining images of control and Tak1<sup>HKO</sup> tissues are shown. Scale bars, 50  $\mu$ m (blue), 20  $\mu$ m (black). The Tak1<sup>HKO</sup> lungs exhibited impaired inflation, while other tissues of control and Tak1<sup>HKO</sup> were indistinguishable.



**Figure S4. Spleen macrophages are diminished by *Tak1* deletion.**

Control and Tak1<sup>HKO</sup> spleens (n = 2 each) at P0 were analyzed by immunofluorescence staining using anti-F4/80 antibody (macrophages) and DAPI (all nuclei). F4/80<sup>+</sup> cells in DAPI stained cells (more than 800 nuclei per animal from at least three randomly chosen images) were quantified.

## Sakamachi et al. Supplementary Figure S5

**Figure S5. 4-OHT or Cre expression does not cause lysosomal injury**

*Rosa26-CreERT Tab2<sup>flox/flox</sup>* BMDMs were treated with vehicle or 0.3  $\mu$ M 4-OHT for 5 days. (A) Lysosomal architecture was visualized by staining using anti-lamp1 and anti-cathepsin B (CathB; red) antibodies, and quantified. Means  $\pm$  SD; NS, not significant (unpaired two tailed Student-t test). (B) Lysosomal function was determined by incubating cells in acridine orange at 4 days post 4-OHT treatment. Orange staining indicates normal functional lysosomal pH (around 3.5), and yellow or green staining indicates increased lysosomal pH. Means  $\pm$  SD; NS, not significant (one-way ANOVA).