

## SUPPLEMENTAL FILE

### **A dendritic cell population responsible for transglutaminase 2-mediated gluten**

### **antigen presentation in celiac disease**

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## **Supplemental Figure legends**

**Figure 1. HB-230-labeled active TG2 is observed in the small intestines of WT mice but not in TG2KO.**

(A) A schematic illustration of the Swiss-roll preparation of the ileum, distal jejunum, and Peyer's patches in the mouse small intestine. Representative images of Swiss-rolls from WT and TG2KO mice dosed with HB-230 2 h before sacrifice. White arrows in the WT image indicate HB-230 signals within the tissue. (B) WT mice received 10 mg/kg HB-230 orally 24 h before euthanasia. After sacrifice, the small intestine and MLN were collected, embedded, and snap-frozen separately. All images were taken using a Zeiss confocal microscope at 40x magnification.

**Figure 2. The percentage of HB-230<sup>+</sup> cells among macrophages in mice dosed with HB-230 or PBS.**

Small intestinal lamina propria cells were isolated from WT and TG2KO mice after receiving HB-230 or PBS 2 h before sacrifice. Viable CD45<sup>+</sup> immune cells were enriched and stained with antibodies. (A) Macrophages were gated on MHCII<sup>+</sup>F4/80<sup>+</sup> cells, and CD103<sup>+</sup> DCs were gated on F4/80<sup>-</sup>MHCII<sup>+</sup>CD11c<sup>+</sup>. No changes in overall populations of either cell type were observed in response to HB-230 treatment. (B) Among macrophages, HB-230 was gated based on the PBS control group (WT, n=7; TG2KO, n=7).

**Figure 3. Extracellular HB-230 colocalizes with collagen and smooth muscle actin.**

WT mice received HB-230 at a dose of 10 mg/kg via oral administration. Two hours after administration, tissues were collected, snap-frozen, and stained with antibodies. Images were captured and analyzed using the PhenoCycler system. (A) (Left) Images of a representative cryosection with extracellular HB-230 overlaid with labeled antibodies against TG2 protein and type I collagen. (Right) Magnified images of the lamina propria (Box 1) and muscularis (Box 2). (B) Extracellular HB-230 overlaid with TG2 protein and anti-PDGFR $\alpha$  antibody near the basement membrane of villi (Box 2). (C) A representative cryosection labeled with HB-230, antibody against TG2 protein, and antibodies against markers of non-immune cell compartments in the small intestine, including epithelial cells (E-cadherin), smooth muscle cells ( $\alpha$ SMA), lymphatic vessels (LYVE-1), and endothelial cells (CD31). On the right, % HB-230<sup>+</sup> cells among epithelial cells and endothelial cells. Each point represents a normalized quantity of E-cad<sup>+</sup>HB-230<sup>+</sup> cells in E-cad<sup>+</sup> cells or CD31<sup>+</sup>HB-230<sup>+</sup> cells in CD31<sup>+</sup> cells from 10 villi. For each mouse, two randomly picked regions with 10 villi were analyzed (WT, n=3). All representative images were captured using a confocal microscope at 20x magnification. Data represent mean  $\pm$  SEM.

**Figure 4. Gluten peptides reach MLNs following oral administration but are not presented by CD103<sup>+</sup> DCs to T cells.**

WT mice received 10 mg/kg of HB-298 orally for 2 h before sacrifice. After euthanasia, MLNs were collected, snap-frozen, and stained with anti-CD103 antibody. Some HB-298 is observed

in the MLN post-doing but does not colocalize with CD103<sup>+</sup> DCs. The image was taken using a confocal microscope at 40x magnification.

**Figure 5. Elevated extracellular HB-230 is observed in DR3.DQ2 mice when on an enhanced gluten diet.**

WT and DR3.DQ2 mice were given normal chow or specified diets for 14 days prior to receiving 10 mg/kg HB-230 orally. Two hours after administration, tissues were collected, snap-frozen, and stained with antibodies. (A) Elevated extracellular HB-230 was noted at the tips of the villi in DR3.DQ2 mice that received a high-gluten diet for 14 days. Five representative images from 2 to 3 mice were taken using a confocal microscope at 40x magnification. (B) The left image demonstrates that intracellular HB-230 counting using QuPath software (36) to identify HB-230<sup>+</sup> cells in each villus. The dot plot shows HB-230<sup>+</sup> cells per villus in each cohort (n=10). P values were determined using an unpaired Mann-Whitney two-tailed *t*-test. \*\*, *p* < 0.01. Data represent mean ± SEM.

**Figure 6. Analysis of small intestinal samples from WT and DdVil-IL15 mice.**

WT and DdVil-IL15 mice were orally administered 10mg/kg HB-230 for 2 h before sacrifice, followed by staining with antibodies. (A) Representative confocal microscopic images of distal and proximal Swiss-rolled small intestine sections of WT or DdVil-IL15 mice 2 h post HB-230 administration (HB-230 in magenta; CD11c in green; nuclei in blue). (B, C) The dot plots show the percentage of HB230<sup>+</sup> cells within CD103<sup>+</sup>CD11b<sup>+</sup> cDC2 (B) and CD103<sup>+</sup>XCR1<sup>+</sup> cDC1 (C)

subsets. Statistical significance was determined using one-way ANOVA with Tukey's multiple comparison test; \*,  $p < 0.05$ . Data represent mean  $\pm$  SEM.

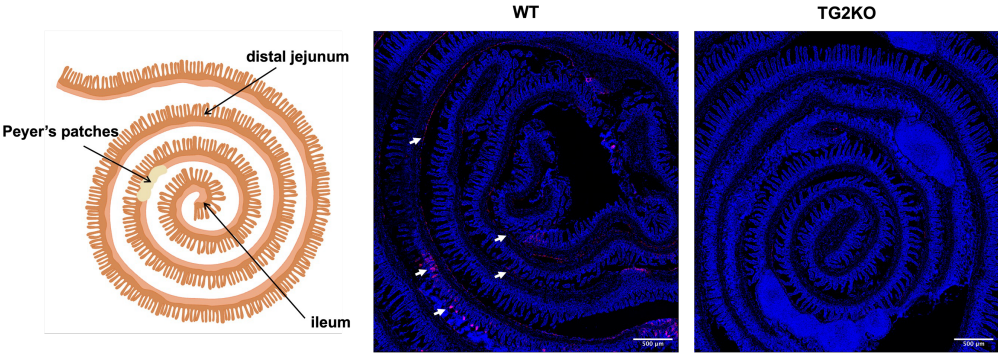
**Figure 7. Reovirus infection increased ECM-associated TG2 activity and HB-230 uptake by CD103<sup>+</sup>CD11b<sup>+</sup> cDC2.**

(A) Experimental schema of the reovirus infection model. WT and TG2KO mice were administered  $3 \times 10^8$  PFU of T1L reovirus or PBS via oral gavage 48 h before receiving 10 mg/kg HB-230. Two hours following HB-230 dosing, mice were sacrificed, and the small intestines were collected for analysis. (B) Representative confocal images of distal and proximal Swiss-rolled small intestine sections (HB-230 in magenta; CD11c in green; nuclei in blue). Intestinal lamina propria cells were isolated from T1L-infected mice, followed by staining with antibodies. The dot plot indicates the percentage of CD11c<sup>+</sup>MHCII<sup>+</sup>F4/80<sup>-</sup> DCs (C) and CD11c<sup>+</sup>MHCII<sup>+</sup>F4/80<sup>+</sup> macrophages (D) among total CD45<sup>+</sup> or HB-230<sup>+</sup>CD45<sup>+</sup> cells. CD103<sup>+</sup> DCs are subcategorized and analyzed for the percentage of HB230<sup>+</sup> among CD103<sup>+</sup>XCR1<sup>+</sup> cDC1 (E) or CD103<sup>+</sup>CD11b<sup>+</sup> cDC2 (F). Statistical significance was determined using two-way ANOVA with Turkey's multiple comparison test. (C, D) or one-way ANOVA with Tukey's multiple comparison test (E, F). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ . Data represent mean  $\pm$  SEM.

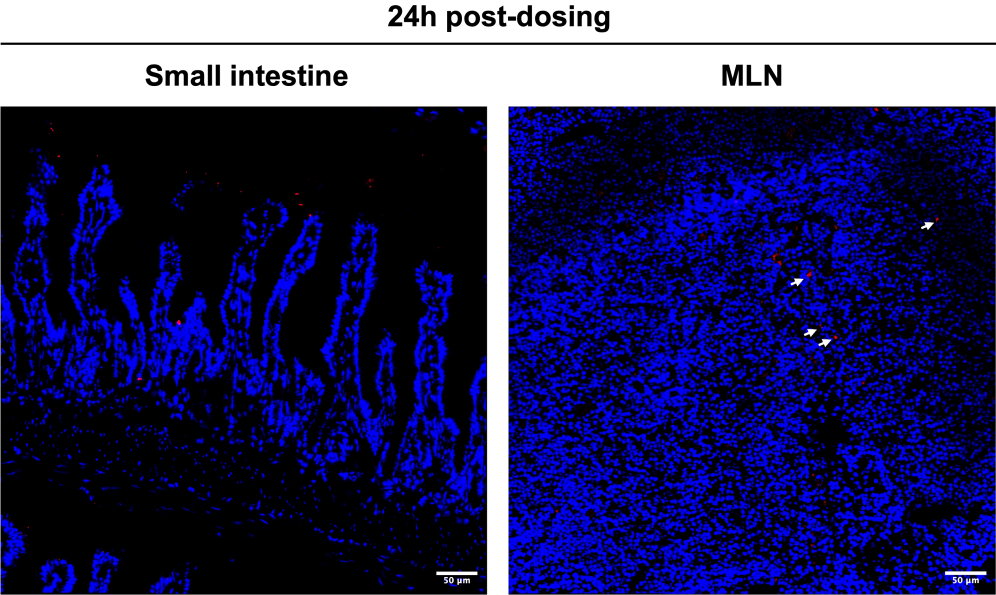
**Supplemental video** Time-lapsed, live cell imaging of BMDCs exposed to HB-230 for 90 min.

# Supplemental Figure 1

**A**

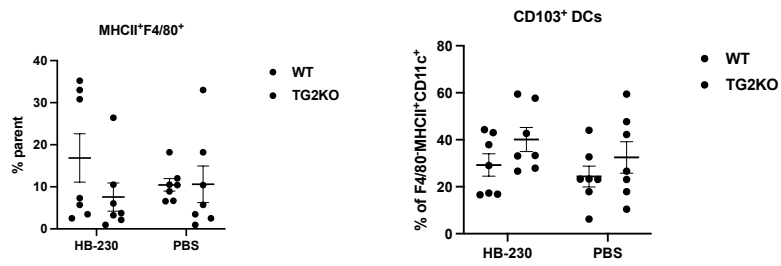


**B**

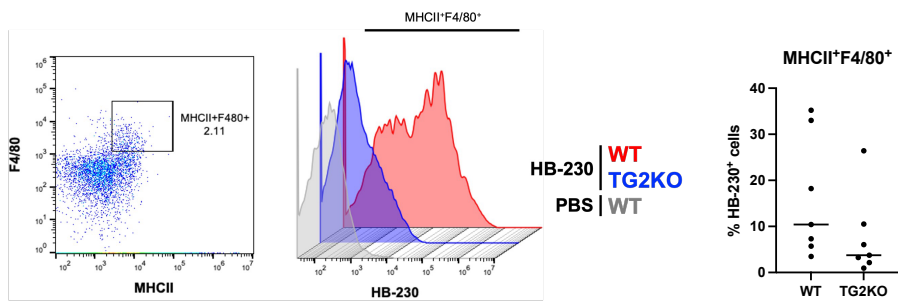


## Supplemental Figure 2

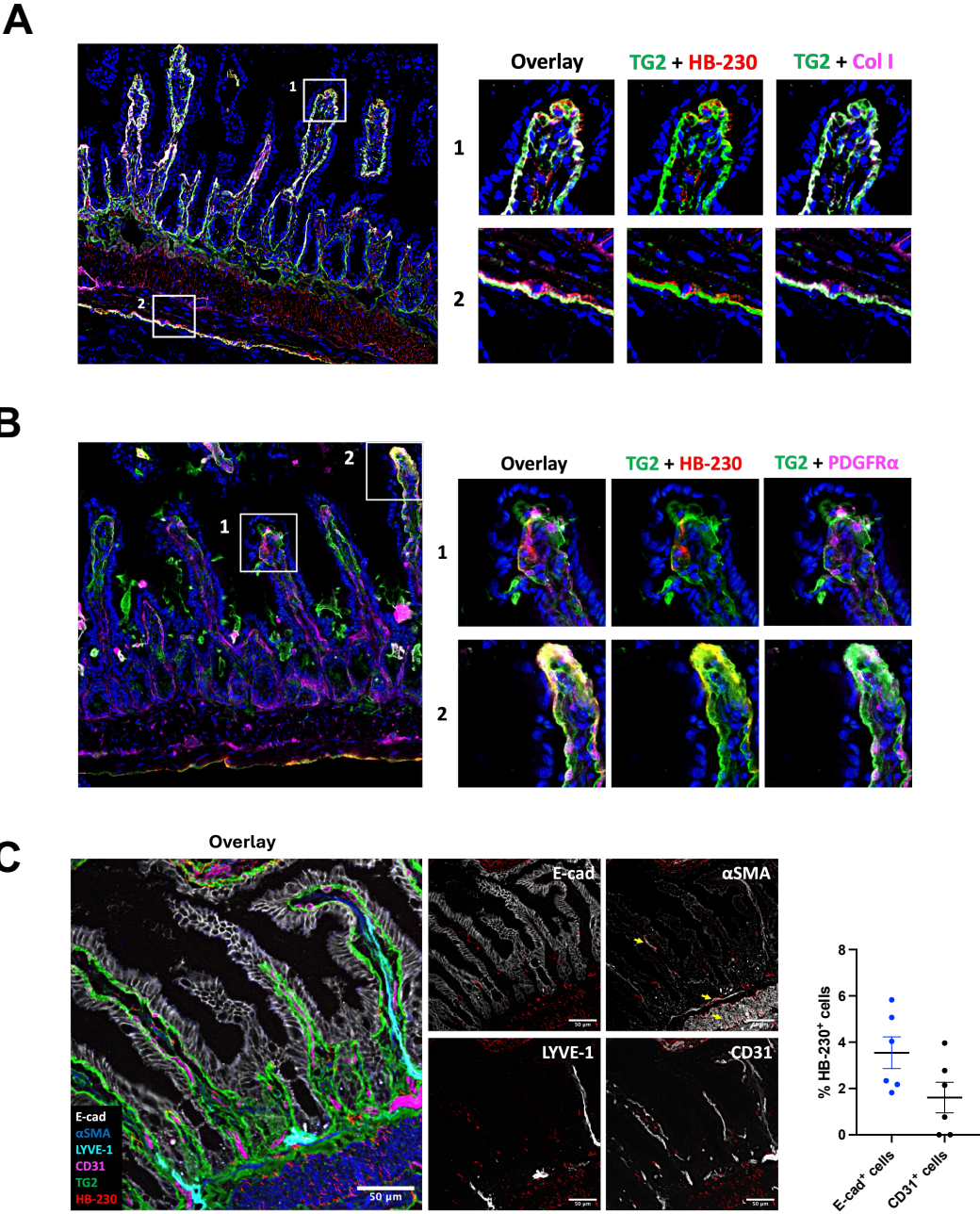
**A**



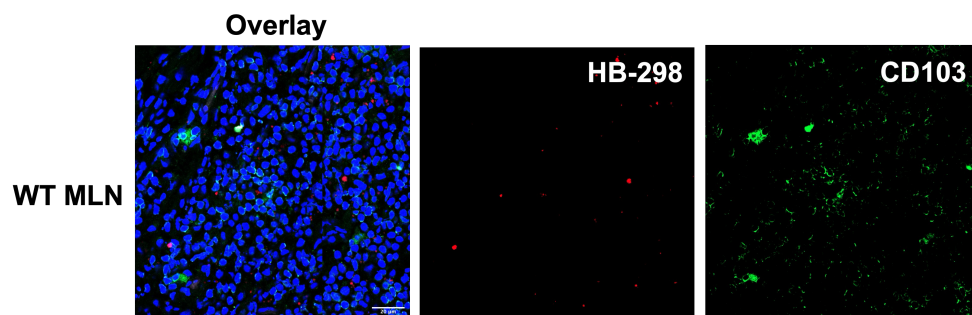
**B**



# Supplemental Figure 3

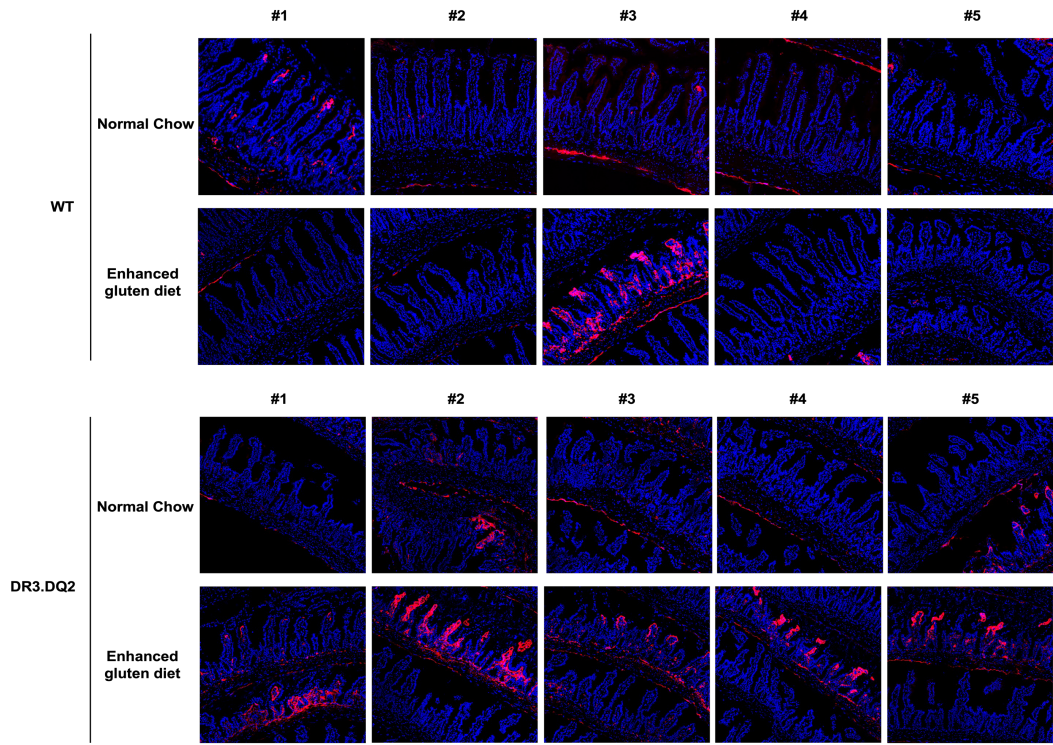


## Supplemental Figure 4

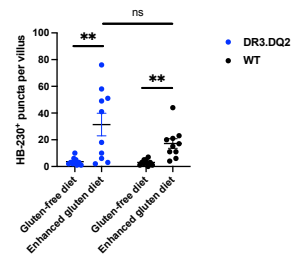
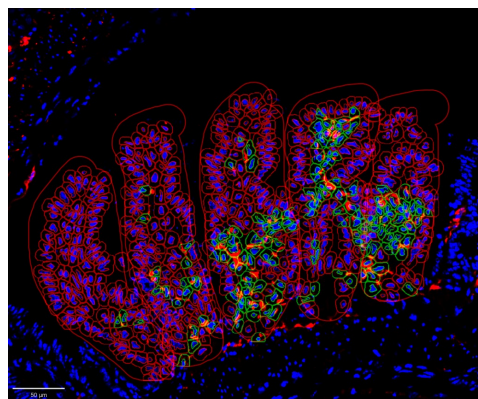


# Supplemental Figure 5

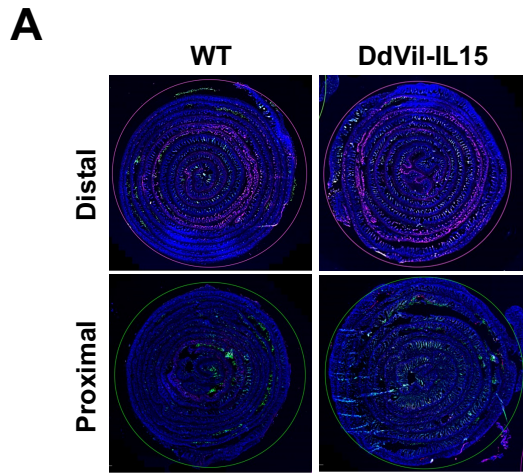
## A



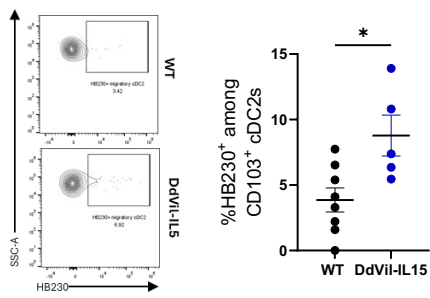
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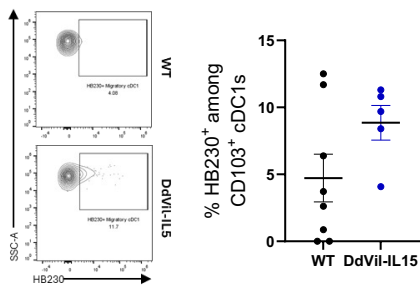
## Supplemental Figure 6



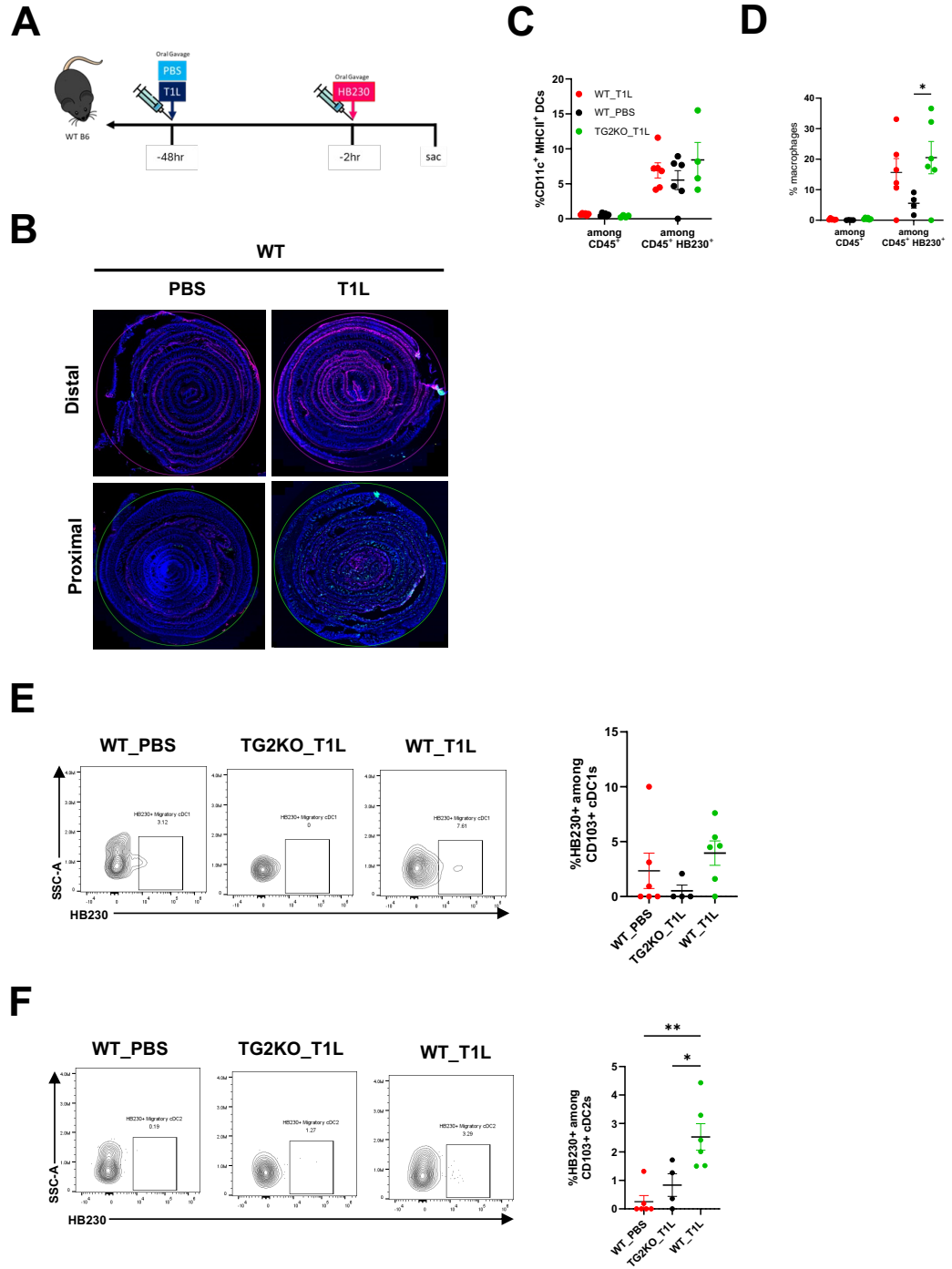
**B**



**C**



# Supplemental Figure 7



## Supplementary video

