Supplementary Material

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Co-culture with Intestinal Epithelial Organoids Allows Efficient Expansion and Motility Analysis of Intraepithelial Lymphocytes

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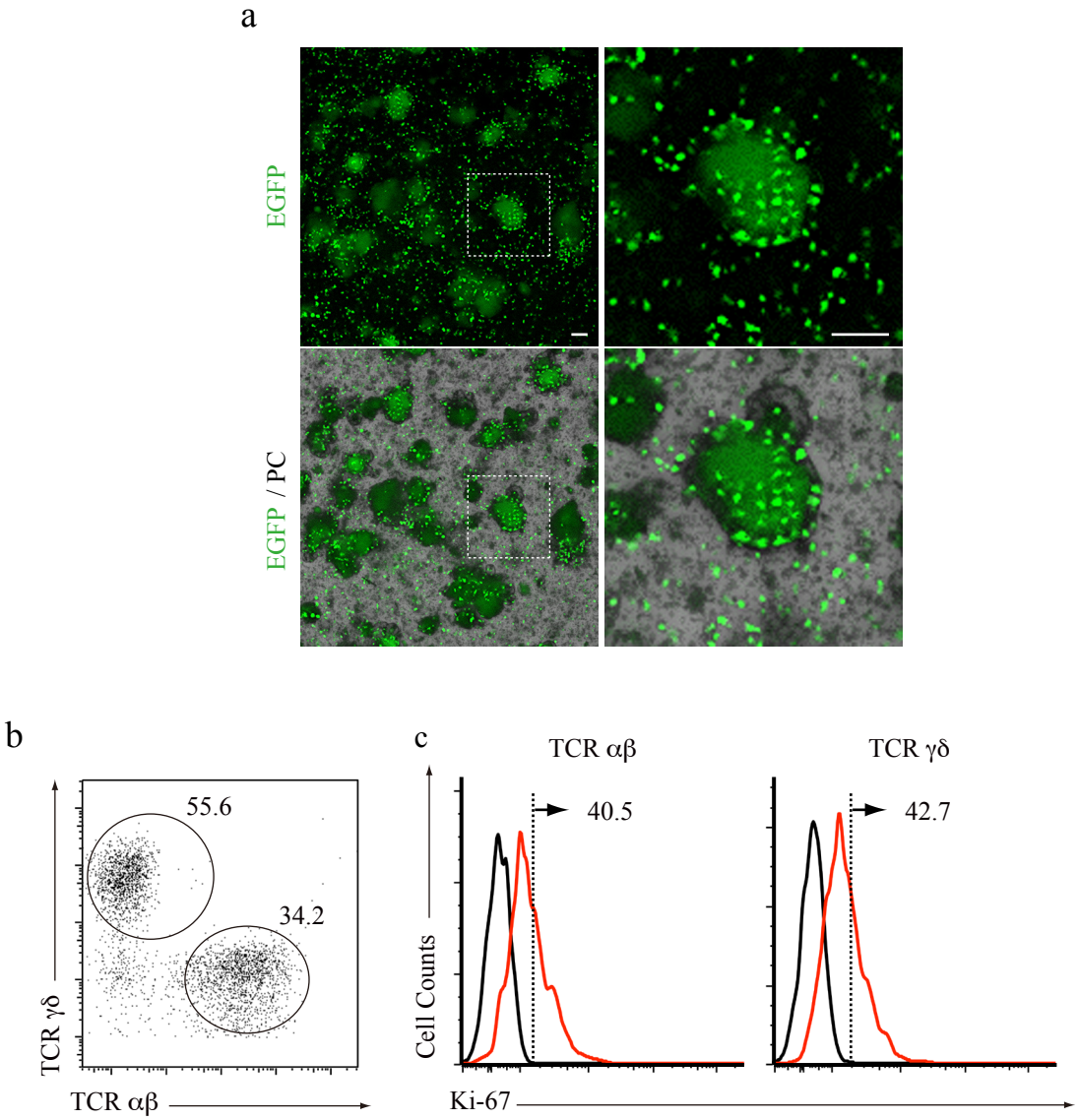
The supplementary material contains 1 figure and 3 movies.

Supplementary Movie 1
A single-plane imaging of IEL dynamics shown in Fig. 3a.
IELs from EGFP-tg mice and IECs from wild-type mice were co-cultured, and cells were visualized by single-plane time-lapse imaging at 20 sec intervals for 2 hours on Day 3. EGFP signals were shown with Hoechst 33342 staining. The time after the start of imaging is shown on the left.

Supplementary Movie 2
A multi-plane imaging of IEL dynamics shown in Fig. 3b.
IELs and IECs were separately isolated from EGFP-tg mice and H2B-mCherry mice, respectively. On Day 3 of co-culture, multi-plane time-lapse imaging was performed for 10 min at 20 sec intervals. Obtained images of EGFP and mCherry signals were shown as the maximum intensity projection of z-stacks. Scale bar shows 50 μm.

Supplementary Movie 3
A representative video showing the motility analysis of whole IELs in co-culture.
IELs and IECs were separately isolated from H2B-EGFP mice and wild-type mice, respectively. On Day 3 of co-culture, multi-plane time-lapse imaging was performed for 10 min at 30 sec intervals. Three-dimensional cell tracking was performed using an image processing software (Imaris). The trajectories of individual nuclei of IELs (yellow spheres) in 4D images are incorporated into the video.



Supplementary Fig. 1 IELs can be maintained for 14 days with proportional expansion of αβT and γδT subsets.
a IELs and IECs were separately isolated from EGFP-tg mice and wild-type syngeneic mice, respectively, and co-cultured in the presence of IL-2/IL-7/IL-15 in addition to the factors required for organid growth. Images of the co-culture on Day 15 are shown with fluorescent images of IELs (EGFP) and their merged ones with phase contrast (PC) images. High magnification views of the area within dotted squares are shown on the right. Scale bars show 100 mm.**b** IELs recovered from co-culture on Day 15 were analyzed by flow cytometry for their surface expression of TCRαβ and TCRγδ. **c** Expression of Ki-67 in IELs recovered from co-culture on Day 15 was analyzed by intracellular staining and flow cytometry. αβT IELs and γδT IELs were gated and Ki-67 expression was separately analyzed. For **b** and **c**, representative flow cytometry plots from three independent experiments performed on different donors are shown.