SUPPLEMENTARY MATERIAL

Reagents used in study

Cell culture reagents. The following reagents were used in the course of the study: RPMI-1640, phosphate-buffered saline (PBS), Hank's Balanced Salt Solution (HBSS), fetal calf serum (FCS), hepes buffer, EDTA, sodium pyruvate, β-mercaptoethanol, penicillin, streptomycin and gentamycin were from Invitrogen (Carlsbad, CA). Collagenase 1a, DNase I, human serum, Brefeldin A, PMA and DL-Dithiothreitol were from Sigma-Aldrich (St. Louis, MO). DNase I and collagenase D were from Roche (Mannheim, Germany). Percoll and Ficoll-paque PLUS were from GE Healthcare (Uppsala, Sweden). Recombinant human (rh) IL-12, rhIL-15 and rhTL1a were from R&D systems (Minneapolis, MN), rhIL-18 was from MBL International (Watertown, MA). Inhibitors for MAPK p38 (SB203580) and PI3K (Ly294002) and Ionomycin were from Calbiochem (San Diego, CA). MHC-II (HLA-DR, DP, DQ, clone Tu39) blocking antibody and istotype control antibody (clone G155-178) were from BD biosciences (San Jose, CA).

Flow cytometry reagents. Phycoerythrin (PE)-Cy7-CD3 (clone SK7), Pacific blue (PB)/Allophycocyanin (APC)/BD Horizon™V500-CD4 (RPA-T4), PE-CD4 (L200), BD Horizon V450-CD8 (RPA-T8), PE-Cy7-CD45RO (UCHL1), BD Horizon V450 IFN-γ (B27), BD Horizon Brilliant Violet-CD161 (DX12), BD Horizon V450/APC-IgG1 isotype control, PE conjugated streptavidin and the APC-BrdU Flow kit were from BD biosciences. A700-CD4 (OKT4), PE-IL-15Rα (JM7A4) and PE-IgG2b isotype control were from BioLegend (San Diego, CA). Quantum dot (QD) 605-CD4 (S3.5), QD605-CD8 (3B5) and LIVE/DEAD® Fixable Near IR Dead Cell Stain Kit

were from Invitrogen. Fluorescein isothiocyanate (FITC)-IL-18Rα (H44), PE-FOXP3 (236A/E7) and PE/FITC-IgG1 isotype control were from eBioscience (San Diego, CA). PE-GM-CSF (BVD2-21C11), PE-TNF-α (cA2), PE-IL-6 (MQ2-13A5) for intracellular detection was from Miltenyi Biotech. PE-IgG1 isotype, polyclonal biotinylated anti-DR3 and control biotinylated goat IgG were from R&D Systems and PE-IgG2a isotype was from Beckman coulter Inc (Fullerton, CA). Biotinylated anti-goat IgG was from Jackson ImmunoResearch (West Grove, PA).

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. TL1a and IL-15, in the presence of IL-12/IL-18, dose dependently induce pro-inflammatory cytokine production in peripheral blood CD45RO$^+$CD4$^+$ T cells. (**A** and **B**) Peripheral blood CD45RO$^+$CD4$^+$ T cells (1x10^6 cells/ml, 200 µl/well) were cultured with IL-12 (2 ng/ml), IL-18 (50 ng/ml) and increasing concentrations of either IL-15 or TL1a as indicated and cytokine levels were determined in culture supernatants at (**A**) d 1 (**B**) d 4. Results are mean (SEM) from 3 (all titrations of IL-15 and IFN-γ titration of TL1a) or 7 (titration of TL1a (except IFN-γ)) biological replicates.

Figure S2. TL1a and IL-15 induced cytokine production in the presence of IL-12/IL-18 is MHC-II independent and PI3K and MAPK p38 dependent. (**A**) MHC-II antibody (20 µg/ml) or isotype control antibody (20 µg/ml) was added to peripheral blood CD45RO$^+$CD4$^+$ T cell (1x10^6 cells/ml, 200 µl/well) cultures 1 h prior to addition of IL-12 (2 ng/ml), IL-18 (50 ng/ml), IL-15 (25 ng/ml) and TL1a (100 ng/ml). Cytokine levels were determined in culture supernatants at d 4. Results are mean (SEM) of 5 biological replicates. (**B**) MHC-II antibody inhibits a mixed lymphocyte reaction. PBMCs were cultured (2x10^6 cells/ml, 100 µl/well) in medium alone (white and black bars) or in presence of MHC-II (green bars) or isotype control (yellow bars) antibody. After 1 h PBMC from the same (negative control, white bar) or a second donor (2x10^6 cells/ml, 100 µl/well) was added to the cultures. ^{3}H thymidine was added to the cultures after 3 d and 18 h later cells were harvested and thymidine incorporation assessed according to Materials and Methods. Results are the mean (SEM) of 3 separate experiments. (C) The PI3K inhibitor Ly294002 (1µM), the

MAPK p38 inhibitor SB203580 (10μM) or DMSO (0.1%) was added to peripheral blood CD45RO$^+$CD4$^+$ T cell (1x10^6 cells/ml, 200 μl/well) cultures 1 h prior to addition of the indicated cytokines. Cytokine levels were determined in culture supernatants at d 1 (IFN-γ) or d 4. Results are mean (SEM) of 3 (IFN-γ) or 9 (other cytokines) biological replicates. Inh, inhibitor.

Figure S3. The activity of TL1a on CD45RO$^+$CD4$^+$ T cells is mediated through DR3. Peripheral blood CD45RO$^+$CD4$^+$ T cells (1x10^6 cells/ml, 200 μl/well) were cultured in medium alone (blue bars) or with IL-12 (2 ng/ml), IL-18 (50 ng/ml), IL-15 (25 ng/ml) and TL1a (100 ng/ml) as indicated in the absence (black bars) or presence of neutralizing DR3 Fab' (5 μg/ml, grey bars) or isotype control Fab' (5 μg/ml, white bars). Cytokine levels in culture supernatants were assessed after 1 d (IFN-γ) and 4 d (other cytokines). Results are the mean (SEM) of 4 (IFN-γ) or 14 (other cytokines) biological replicates. Iso, isotype.

Figure S4. Staining of DR3 transfectants. (**A**) DR3 Fab' or (**B**) polyclonal DR3 antibody was used to stain mock (grey line, unfilled histogram) or DR3 transfected HEK293 6E cells (black line, unfilled histogram). Shaded area represents DR3 transfected HEK293 6E cells without antibody (**A**) or stained with control goat IgG (**B**).

Figure S5. IL-15 dose dependently induces IL-10 production in CD45RO$^+$CD4$^+$ T cells. Peripheral blood CD45RO$^+$CD4$^+$ T cells (1x10^6 cells/ml, 200 μl/well) were cultured with IL-12 (2 ng/ml), IL-18 (50 ng/ml) and with increasing concentrations of either IL-15 or TL1a as indicated. Levels of IL-10 were determined in culture

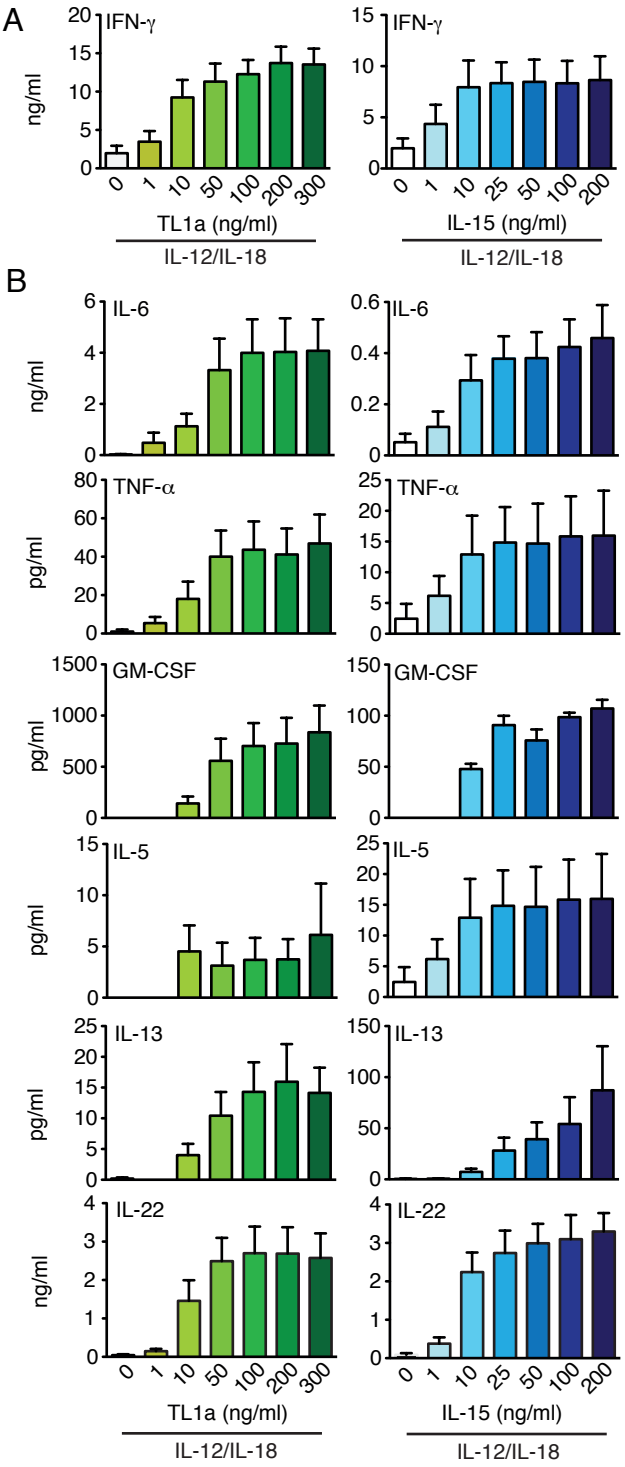
supernatants at d 4. Results are mean (SEM) from 3 (IL-15) or 7 (TL1a) biological replicates.

Figure S6. DR3 expression on intestinal CD4⁺ T cells. LPMCs were isolated from human small intestine by collagenase digestion or after placing finely cut tissue pieces in culture medium overnight and collecting cells from the culture medium ('walk-out' cells). DR3 expression was assessed directly after isolation or on collagenase isolated intestinal $CD4^+$ T cells that had been left in culture medium ($1x10^6$ cells/ml, 200 µl/well) for 2 d. Flow cytometry plots are gated on live, $CD3^+CD4^+$ cells. Results are showing 1 representative experiment of 3 performed. DR3 staining (black line, unfilled histogram), isotype control (shaded histogram)

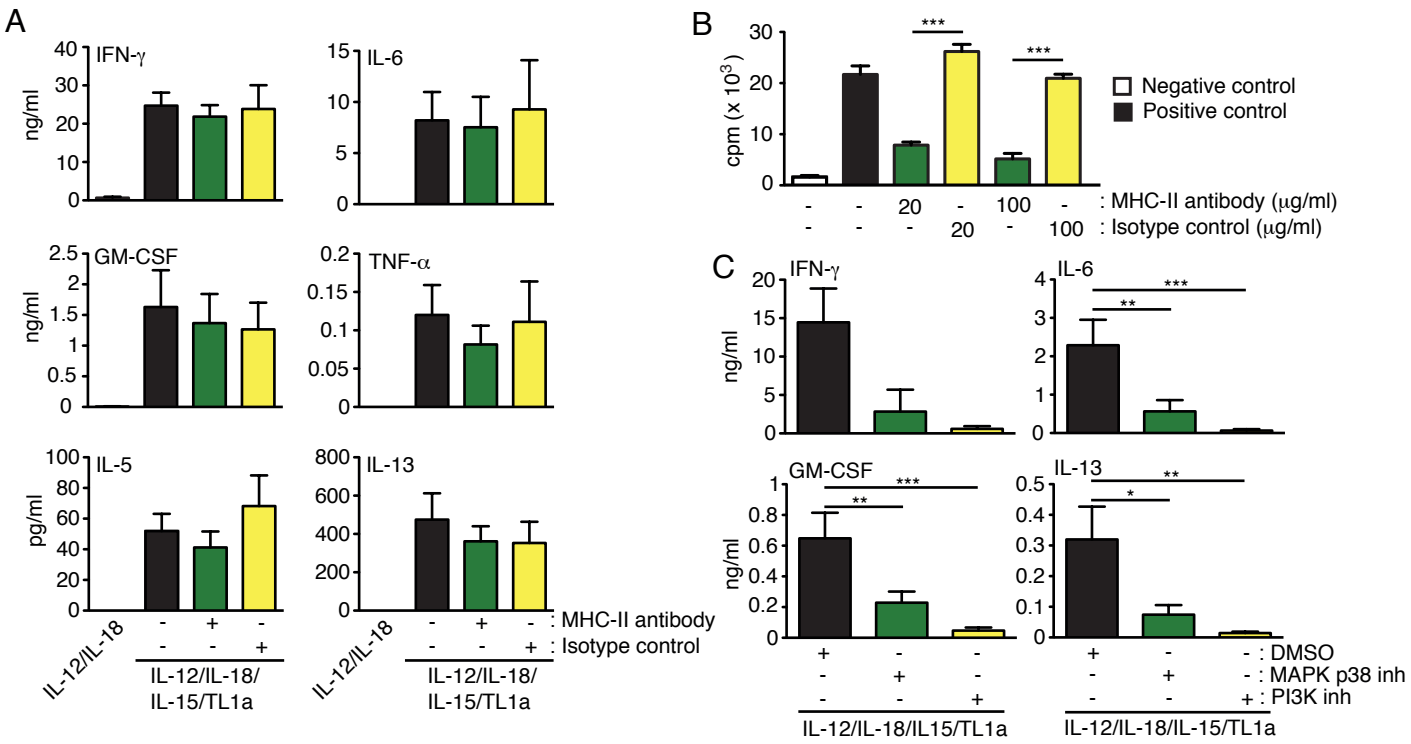
Figure S7. Intestinal lamina propria IL-18Rα⁺CD4⁺ T cells produce pro-inflammatory cytokines in response to TL1a and IL-15 in the presence of IL-12/IL-18. (**A**) MHC-II or isotype control antibody (20 µg/ml) was added to LPMCs cultures ($1x10^6$ cells/ml, 200 µl/well) 1 h prior to addition of the indicated cytokines; IL-12 (2 ng/ml), IL-18 (50 ng/ml), IL-15 (25 ng/ml) and TL1a (100 ng/ml) or medium alone (control). IFN-γ expression was assessed after 2 d by intracellular flow cytometry analysis. Plots are gated on $CD3^+CD4^+$ T cells. Results are from 1 representative experiment of 3 performed. (**B**) LPMCs ($1x10^6$ cells/ml, 200 µl/well) were incubated with medium alone (control) or the indicated cytokine cocktail for 2 d and Brefeldin A was added the last 4 h of culture. IL-6, GM-CSF and TNF-α expression was assessed by intracellular flow cytometry analysis. Plots are gated on $CD3^+CD4^+$ cells. Results are representative flow cytometry plots and numbers in plots represent the mean (SEM) of 3 independent experiments. FMO, fluorescence minus

one. (C) Proportion of $CD3^-$, $CD3^+CD8^-$, $CD3^+CD8^+$ cells (grey bars) and proportion of cytokine producing cells amongst live LPMC (black bars) after 2 d culture in medium alone or with IL-12 /Il-18/IL-15/TL1a. The results are the mean of 5 (IFN-γ) and 3 (other cytokines) biological replicates.

SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2



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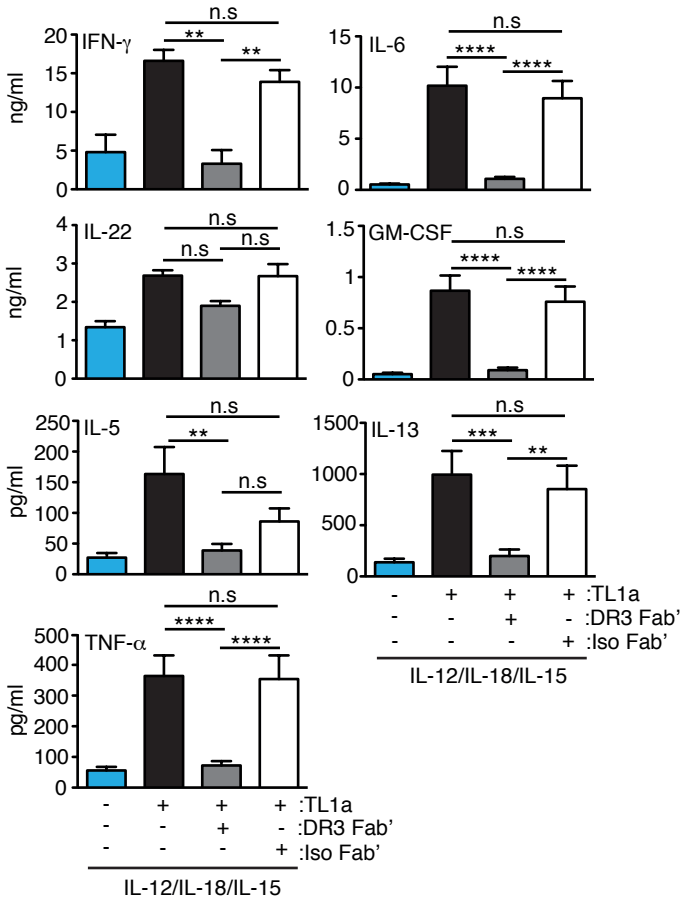

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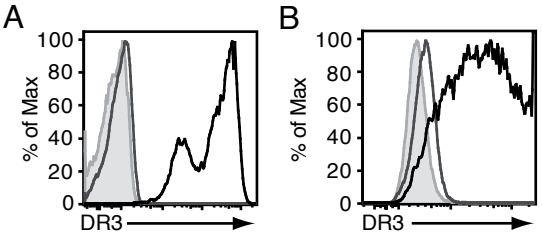

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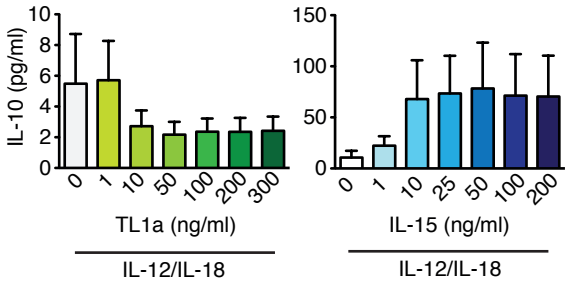
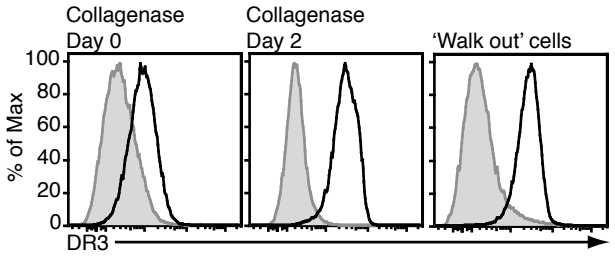


SUPPLEMENTARY FIGURE 6



SUPPLEMENTARY FIGURE 7