Sanos *et al*., **Commensal microflora and the transcription factor RORγt instruct the differentiation of interleukin 22-producing NKp46$^+$ cells residing in the intestinal lamina propria**

Supplementary Methods

LP isolation

Intestines were removed and placed in ice-cold PBS. After removal of the residual mesenteric fat tissue, PP were carefully excised, and the intestines and colons were opened longitudinally. The intestines were thoroughly washed in ice-cold PBS and cut into 1.5cm pieces, which were incubated in 5ml of 5mM EDTA in Hank's balanced salt solution (HBSS) without calcium or magnesium for 15-20 min at 37°C with slow rotation (100 r.p.m.). Following each incubation, the epithelial cell layer containing the IELs was removed by vortexing. The remaining tissue was collected and fresh EDTA solution was added. After the second EDTA incubation, the pieces were washed in HBSS and finely cut with a scalpel and placed in 5ml digestion solution containing 2% FCS, 0.5mg/ml each of collagenase D (Roche), DNaseI (Sigma) and Dispase (BD Biosciences). Digestion was performed by incubating the tissue pieces at 37°C for 20 min with slow rotation. After the initial 20 min, the solution was vortexed and passed through a 40 m cell strainer. The pieces were collected and reincubated in fresh digestion solution and the procedure was repeated a total of four times. Supernatants from all four digestions were then spun for 10min at 800×*g*. The cells were resuspended in 40% Percoll (GE Healthcare) and overlayed onto 5ml of 80% Percoll in a 15ml tube. Percoll gradient separation was performed by centrifugation for 20 min at 2500 r.p.m. at 25°C. LP lymphocytes were

collected at the interphase of the Percoll gradient, washed once and resuspended. The cells were used immediately.

Antibodies for immunofluorescence staining and flow cytometry

The following antibodies were used for flow cytometry experiments: NKp46 (29A1.4), NK1.1 (PK136), CD3ε (145-2C11), CD4 (L3T4), CD45.2 (104), CD45 (30F11), CD122 (TM-β1), CD127 (A7R34), CD117/c-kit (ACK2), CD51 (RMV-7), CD11b (M1/70), CD27 (LG.7F9), CD49b (DX5), CD69 (H1.2F3), NKG2D (CX5), NKG2A (20d5), KLRG1 (2F1), IL-22 (clone AM22.1), IFN-γ (XMG1.2), IL-17A (TC11-18H10), and IL-17F (18F10).

The following antibodies were used for immunofluorescence staining of tissue sections: polyclonal goat anti-mouse NKp46 (R&D Systems), polyclonal rabbit anti-GFP (Invitrogen), anti-Thy1.2 (53-2.1, eBioscience) and anti-Ly49G2 (4D11, eBioscience).

Real-time PCR

The following qPCR assays were used (all from Applied Biosystems): murine hypoxanthine guanine phosphoribosyl transferase 1 (mHPRT-1; Mm00446968_m1), murine IL-22 (mIL22; Mm00444241_m1), murine IL17A (mIL17A; Mm00439619_m1), murine IL-17F (mIL17F; Mm00521423_m1), murine RegIIIβ (mPap; Mm00440616_g1) and murine RegIIIγ (mReg3g; Mm00441127_m1). Each cDNA was amplified and measured in triplicates with 50-100ng cDNA per well in a

reaction volume of 15 l and the following cycle conditions: 2 min at 50°C, 10 min at 95°C, and then 15 s at 95°C and 60 s at 60°C for 40 cycles.