Tissue-resident Eomes⁺ NK cells are the major innate lymphoid cell population in human infant intestine
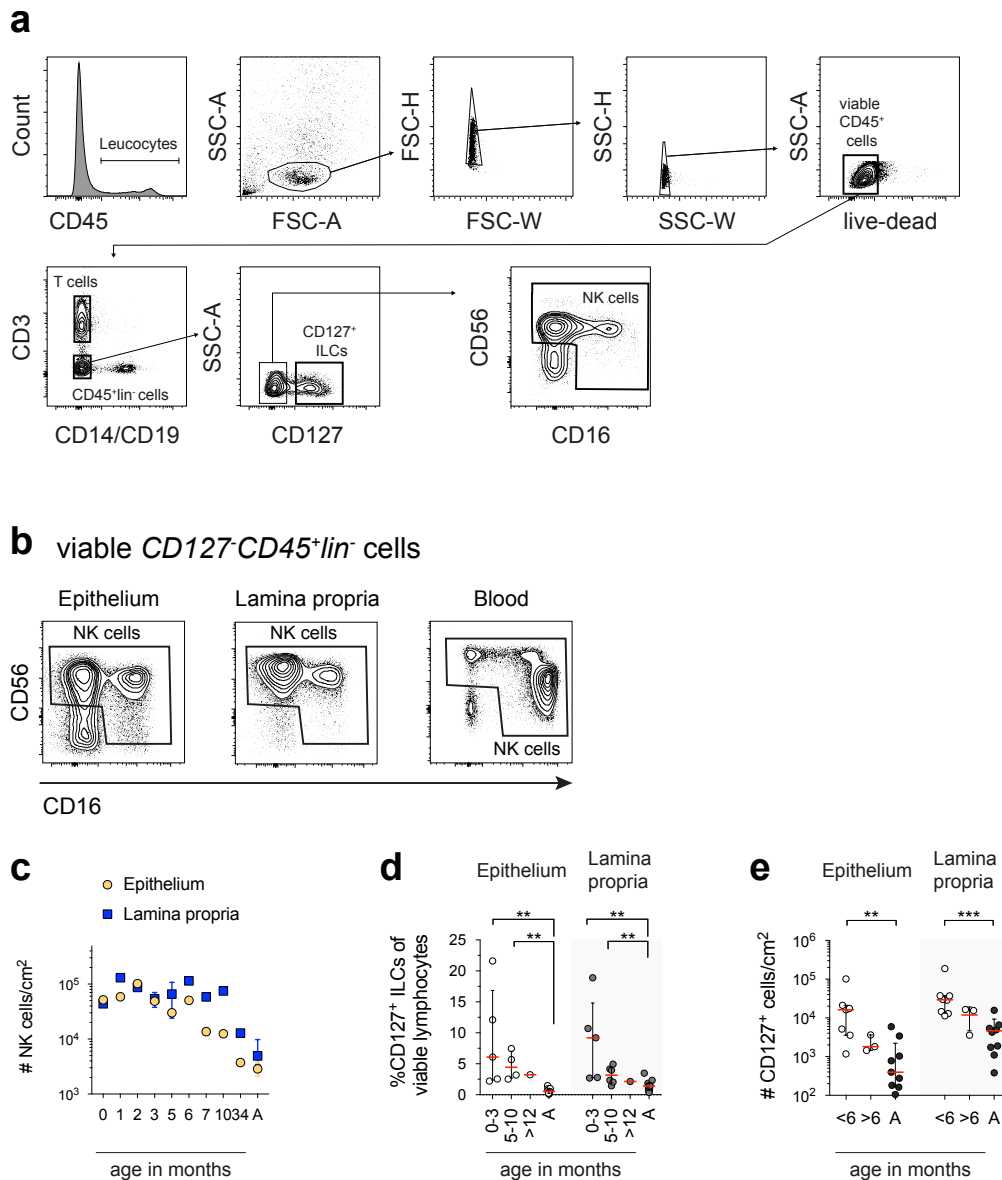
Sagebiel et al.

Supplementary Figures

a

b viable *CD127⁻CD45⁺lin⁻* cells

Supplementary figure 1 | ILC populations in intestines and blood

(**a**) Gating strategies for viable CD45⁺ cells (Fig. 1a, 2a), CD45⁺lin⁻ cells (Fig. 1b, 2b, 7c), T cells (Fig. 8), CD127⁺ ILCs (Fig. 7b, Supplementary Fig. 1d-e, 6) and NK cells (Fig. 2c-e, 3, 4, 5, 6, 7a, 7b, 8, Supplementary Fig. 1b-c, 2, 3, 4, 5). Fluorochromes may differ based on specific panel. (**b**) Representative flow diagrams of CD56 and CD16 expression by viable CD127⁻CD45⁺lin⁻ cells in intestinal epithelium (EP), lamina propria (LP) and peripheral blood (B) of infants. (**c**) Absolute NK cell numbers/cm² at different ages in EP (yellow circles) and LP (blue squares) in infants and adults (infant samples N=9 (EP) and N=11 (LP), adult samples N=8 (EP) and N=9 (LP)). (**d**) Frequencies of CD127⁺ ILCs of viable CD45⁺ lymphocytes for different age groups in EP and LP samples (infant samples N=10 (EP) and N=12 (LP), adult samples N=11 (EP and LP)). (**e**) Absolute CD127⁺ ILC numbers/cm² for different age groups in EP and LP samples (infant samples N=10 (EP) and N=11 (LP), adult samples N=9 (EP and LP)). Median frequencies indicated by red lines. Error bars define interquartile ranges between 75th and 25th percentiles. Statistical comparisons are Mann-Whitney U comparisons. Asterisks represent the following *p*-values: **p < 0.01, ***p < 0.001. A = Adult samples. FACS plots of representative donors are shown.

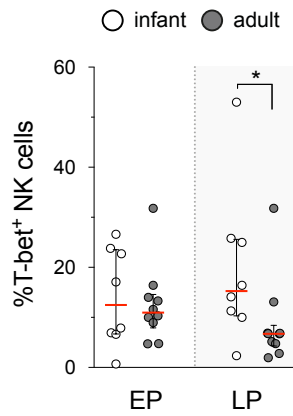| Epithelium	Lamina propria
CXCR6-PE-Cy7	CXCR6-PE-Cy7
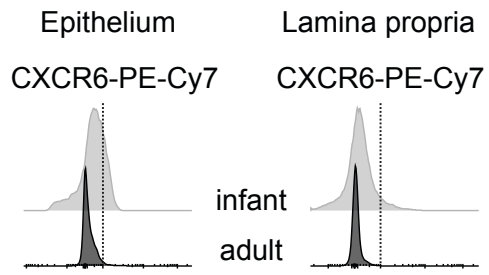
infant

adult

Supplementary figure 2 | CXCR6 expression by intestinal NK cells

Representative histogram overlay of CXCR6 expression by infant (gray) and adult (black) NK cells.
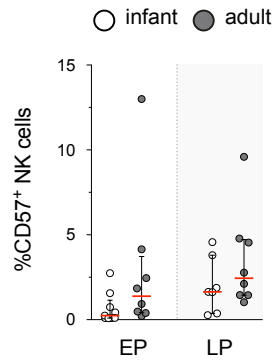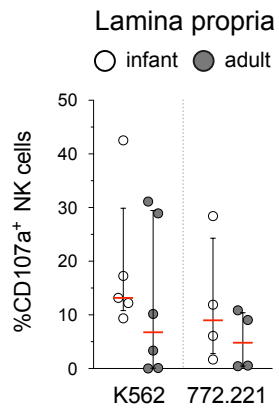
Supplementary figure 3 | Intestinal CD57+ NK cells are scarce

(**a**) Frequencies of CD57+ NK cells from epithelium (EP) and lamina propria (LP) of infants (white circles) and adults (dark circles) (infant samples N=9 (EP) and N=7 (LP), adult samples N=8 (EP and LP)). Median frequencies indicated by red lines. Error bars define interquartile ranges between 75th and 25th percentiles. Statistical comparisons are Mann-Whitney U comparisons.

Supplementary figure 4 | T-bet expression in infant and adult intestinal NK cells

(**a**) Frequencies of T-bet+ NK cells from epithelium (EP) and lamina propria (LP) of infants (white circles) and adults (dark circles) (infant samples N=8 (EP and LP), adult samples N=10 (EP and LP)). Median frequencies indicated by red lines. Error bars define interquartile ranges between 75th and 25th percentiles. Statistical comparisons are Mann-Whitney U comparisons. Asterisks represent the following *p*-values: **p* < 0.05.
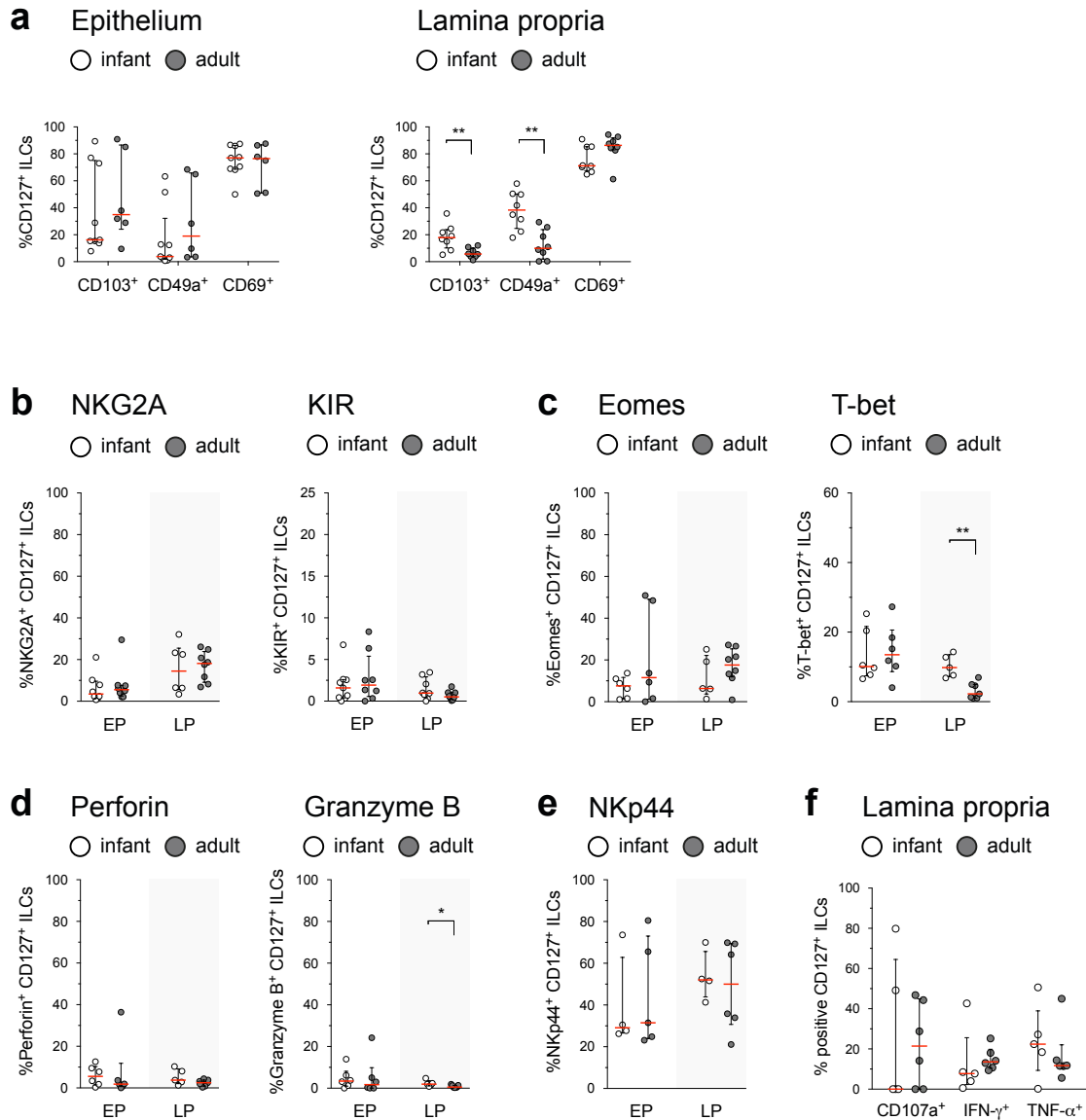
Supplementary figure 5 | Degranulation of intestinal NK cells upon stimulation

(**a**) Frequencies of infant (white circles) and adult (dark circles) CD107a^{+} NK cells from lamina propria (LP). Cells were stimulated for 6 hours in the presence of K562 or 772.221 target cells (effector/target-ratio: 1:5) (infant samples N=5 (K562) and N=4 (772.221), adult samples N=6 (K562) and N=4 (772.221)). Median frequencies indicated by red lines. Error bars define interquartile ranges between 75th and 25th percentiles. Statistical comparisons are Mann-Whitney U comparisons.

Supplementary figure 6 | Phenotypic characteristics of intestinal CD127⁺ ILCs

(**a**) Frequencies of infant (white circles) and adult (dark circles) CD103⁺, CD49a⁺ or CD69⁺ CD127⁺ ILCs in epithelium (EP) and lamina propria (LP) tissues (infant samples N=9 (EP) and N=8 (LP), adult samples N=6 (EP) and N=8 (LP)). (**b**) Frequencies of infant (white circles) and adult (dark circles) NKG2A⁺ or KIR⁺ CD127⁺ ILCs from EP and LP tissues (infant EP samples N=8 (NKG2A) and N=9 (KIR), infant LP samples N=6 (NKG2A) and N=8 (KIR), adult samples N=8 (EP and LP)). (**c**) Frequencies of infant (white circles) and adult (dark circles) Eomes⁺ or T-bet⁺ CD127⁺ ILCs from EP (infant EP samples N=6 (Eomes and T-bet), infant LP samples N=5 (Eomes and T-bet), adult EP samples N=6 (Eomes and T-bet), adult LP samples N=8 (Eomes) and N=7 (T-bet)). (**d**) Frequencies of infant (white circles) and adult (dark circles) perforin⁺ or granzyme B⁺ CD127⁺ ILCs from EP and LP tissues (infant samples N=6 (EP) and N=5 (LP), adult samples N=6 (EP) and N=7 (LP)). (**e**) Frequencies of infant (white circles) and adult (dark circles) NKp44⁺ CD127⁺ ILCs from EP and LP (infant samples N=4(EP and LP), adult samples N=5 (EP) and N=6 (LP)). (**f**) Frequencies of LP CD107a⁺, IFN-γ⁺ or TNF-α⁺ CD127⁺ ILCs in infants (white circles) and adults (dark circles). Cells were stimulated for 6 hours in the presence of PMA and ionomycin (infant samples N=5, adult samples N=6). Median frequencies indicated by red lines. Error bars define interquartile ranges between 75th and 25th percentiles. Statistical comparisons are Mann-Whitney U comparisons. Asterisks represent the following *p*-values: *p < 0.05, **p < 0.01.