Figure S1. Adult CD4$^+$ LTi cells are a dominant innate source of IL-22 in the gut following *C. rodentium* infection, related to Figure 2. (A) C57BL/6 mice were infected with *C. rodentium* on day 0 and sacrificed on day 4. Frequency of ex vivo stimulated IL-22$^+$ innate cells gated as indicated in IEL of infected mice. All data are representative of 2 or more independent experiments with a minimum of 3-4 mice per group. (B) C57BL/6 mice were infected with *C. rodentium* on day 0 and sacrificed on days 2, 4 and 6. Cells from the mLN were briefly stimulated ex vivo and the frequency of IL-22$^+$ cells were examined in CD4$^+$, CD3$^+$, CD5$^+$ T cells, and (C) CD4$^+$, CD3$^-$, CD5$^-$ LTi cells. All data are representative of 2 independent experiments with a minimum of 3 mice per time point.

Figure S2. CD4$^+$ LTi cells are the dominant IL-23-responsive and IL-22-producing innate population in the spleen and IEL, related to Figure 3. (A) Frequency of CD90$^+$ CD4$^-$ cells in the IL-22$^+$ gated population of *Rag1*$^{-/-}$ splenocytes (SPL) subsequent to culturing with rIL-23 overnight; cells were also stained with anti- c-kit, NK1.1, NKp46 and CD11c antibodies (bold black lines) and corresponding isotype and negative control antibodies (solid grey histograms). (B) Frequency of IL-22$^+$ cells in naïve *Rag1*$^{-/-}$ mice IEL cultures following overnight incubation with rIL-23, and the frequency of CD90$^+$ CD4$^+$ and CD90$^+$ CD4$^-$ cells in the IL-22$^+$ gated population. All in vitro data are representative of 2 or more independent experiments with a minimum duplicate wells. (C) CD4$^+$ LTi cells were purified from naïve *Rag1*$^{-/-}$ splenocytes and stained with H&E. Scale bar, 10 μm.

Figure S3. CD4 mAb administration specifically depletes CD4$^+$ LTi cells, related to Figure 5. C57BL/6 *Rag1*$^{-/-}$ were administered an isotype control mAb, anti-CD90 mAb or anti-CD4 mAb starting on day 0, infected with *C. rodentium* on day 0, and sacrificed at day 10. (A) Frequency of CD4$^+$ CD90$^+$ cells in Lin$^-$ gated splenocytes (SPL) from antibody treated *Rag1*$^{-/-}$ mice. (B) Frequency of NK1.1$^+$ cells in splenocytes (SPL) from antibody treated *Rag1*$^{-/-}$ mice. (C) Frequency of NKp46$^+$ cells in IEL compartment from antibody treated *Rag1*$^{-/-}$ mice. All data are representative of 2 independent experiments with a minimum of 2 mice per time point.

Figure S1

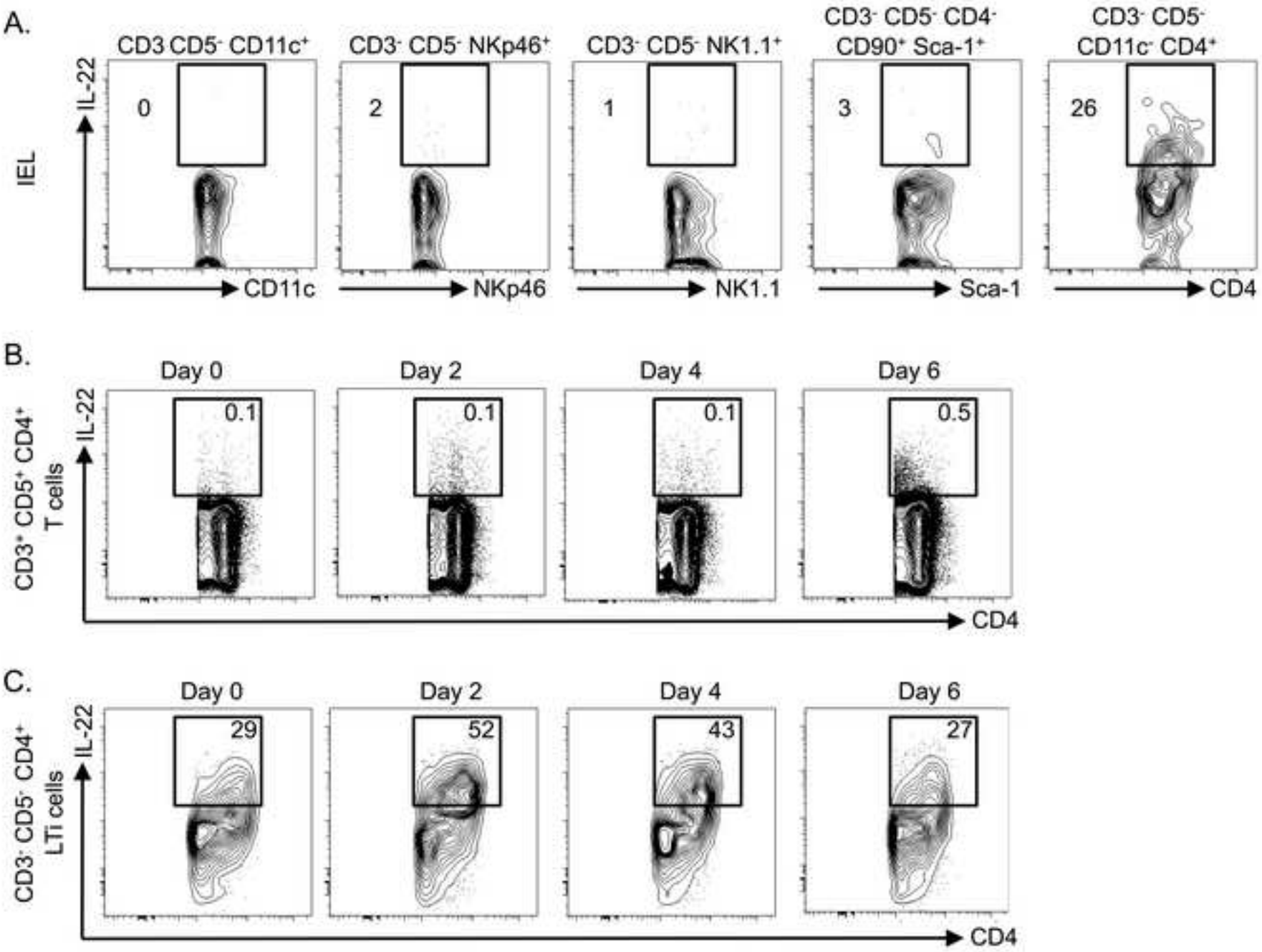

A.
CD3 CD5- CD11c+
CD3- CD5- NKp46+
CD3- CD5- NK1.1+
CD3- CD5- CD4- CD90+ Sca-1+
CD3- CD5- CD11c- CD4+
IEL
IL-22
0
2
1
3
26
CD11c
NKp46
NK1.1
Sca-1
CD4
B.
Day 0
Day 2
Day 4
Day 6
CD3+ CD5+ CD4+ T cells
IL-22
0.1
0.1
0.1
0.5
CD4
C.
Day 0
Day 2
Day 4
Day 6
CD3- CD5- CD4+ LTi cells
IL-22
29
52
43
27
CD4

Figure S2

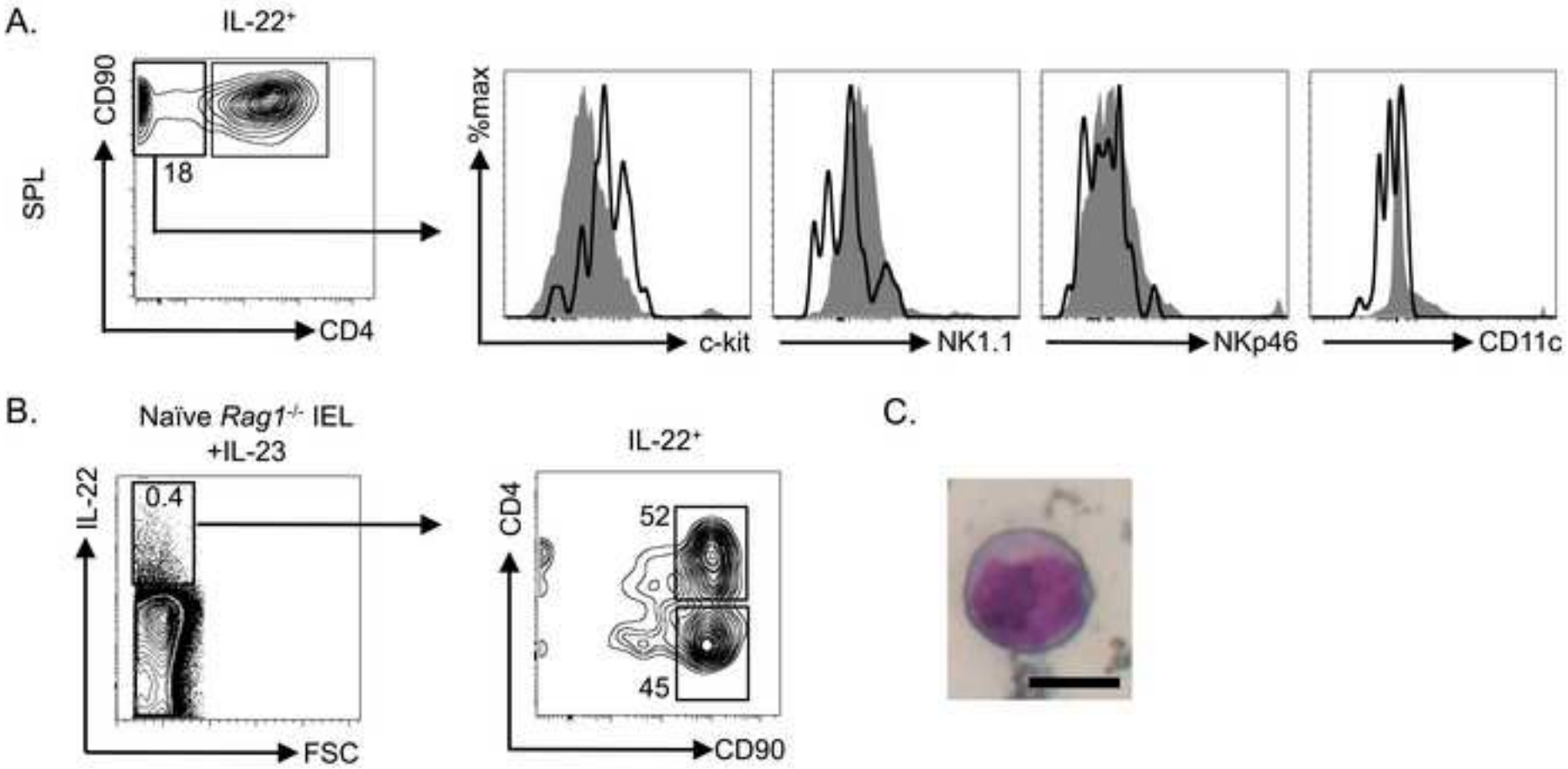
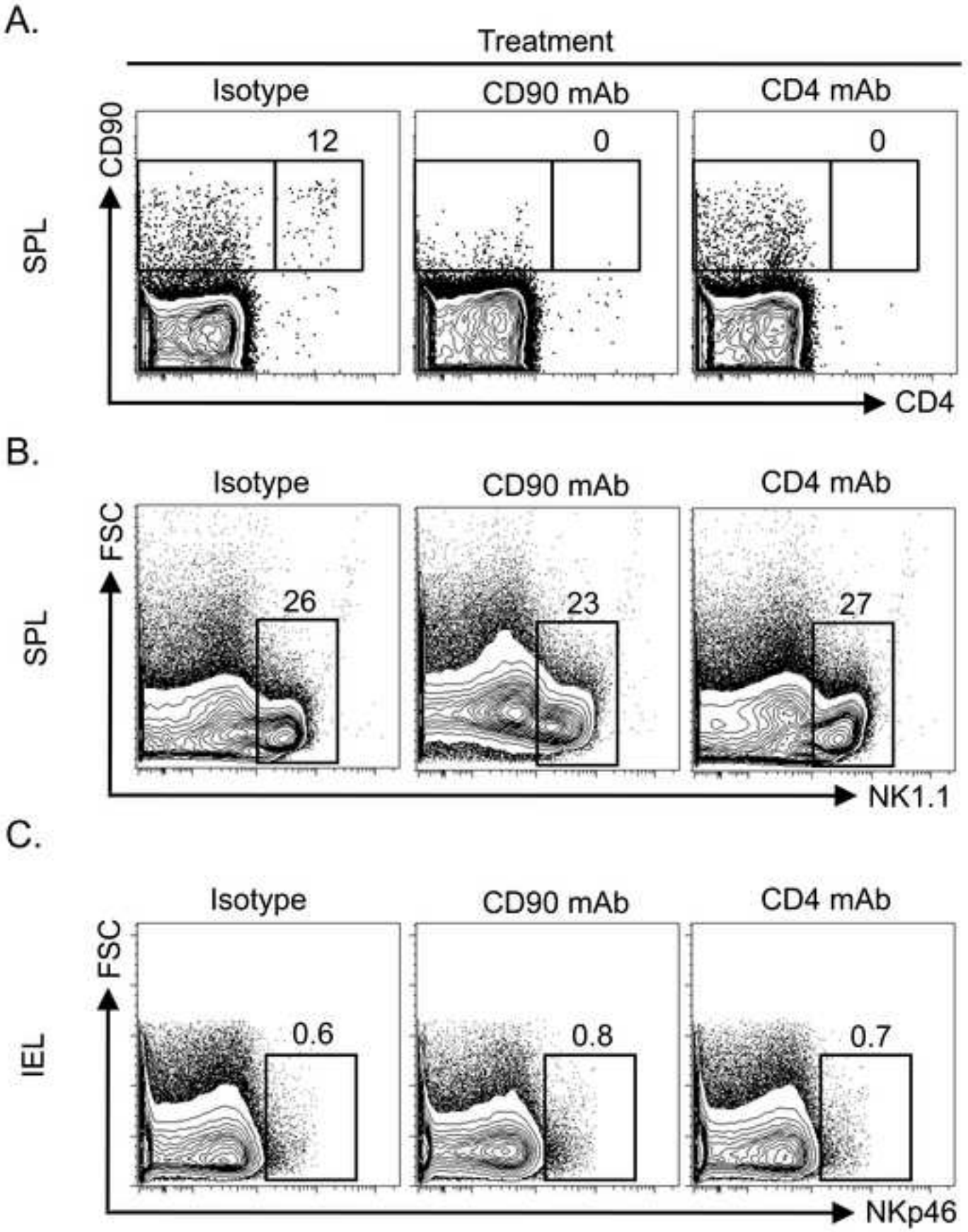

A.
IL-22+
SPL
CD90
18
CD4
%max
c-kit
NK1.1
NKp46
CD11c
B.
Naïve Rag1-/- IEL
+IL-23
IL-22
0.4
FSC
IL-22+
CD4
52
45
CD90
C.

Figure S3


A.
Treatment
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CD4 mAb
CD90
SPL
12
0
0
CD4
B.
Isotype
CD90 mAb
CD4 mAb
FSC
SPL
26
23
27
NK1.1
C.
Isotype
CD90 mAb
CD4 mAb
FSC
IEL
0.6
0.8
0.7
NKp46