Figure S1

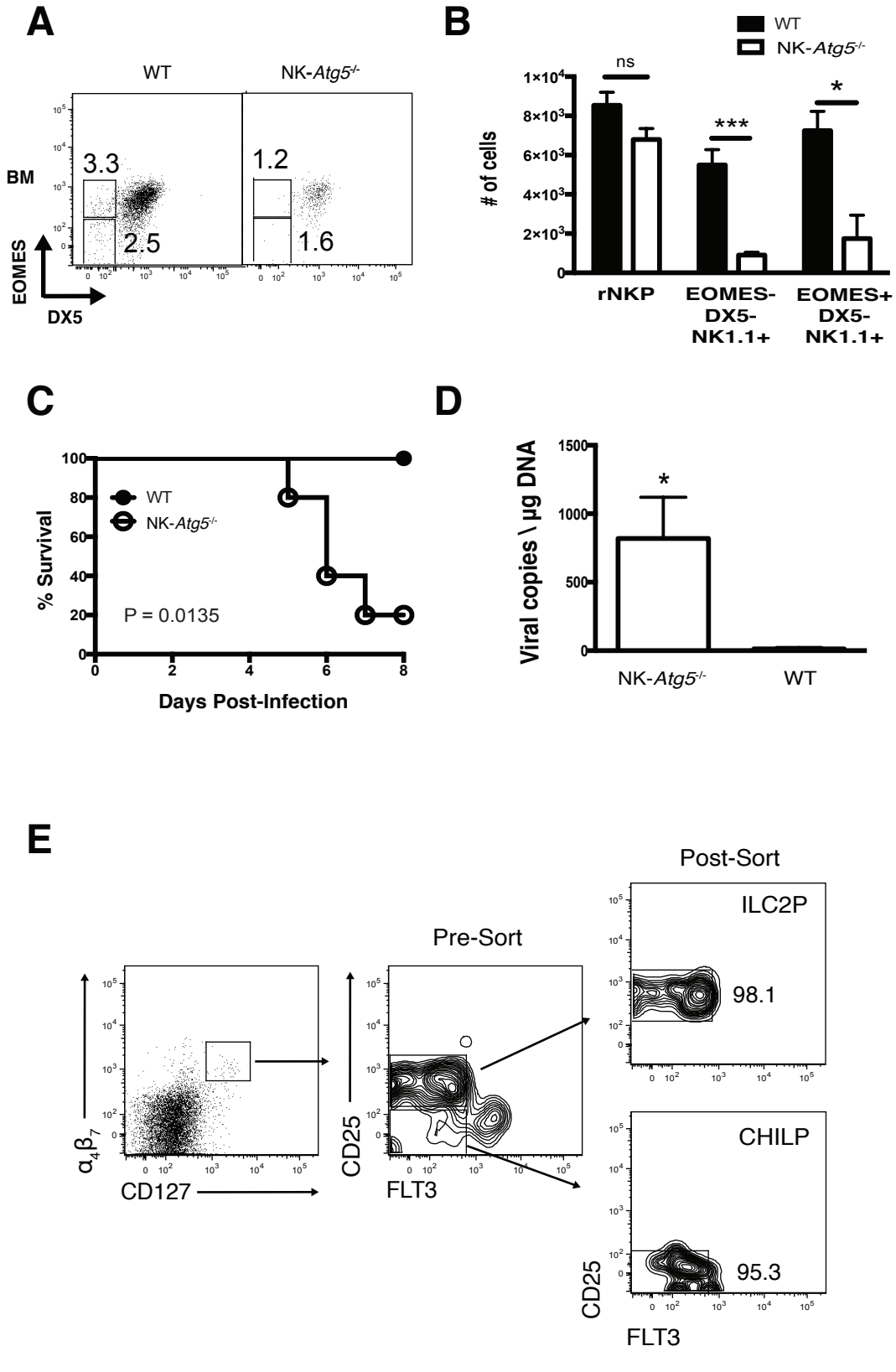

A
WT
NK-Atg5-/-
BM
3.3
2.5
1.2
1.6
EOMES
DX5
B
WT
NK-Atg5-/-
ns
***
*
# of cells
rNKP
EOMES-DX5-NK1.1+
EOMES+DX5-NK1.1+
C
WT
NK-Atg5-/-
% Survival
P = 0.0135
Days Post-Infection
D
Viral copies \ µg DNA
*
NK-Atg5-/-
WT
E
Pre-Sort
Post-Sort
α4β7
CD127
CD25
FLT3
ILC2P
98.1
CHILP
95.3

Figure S2

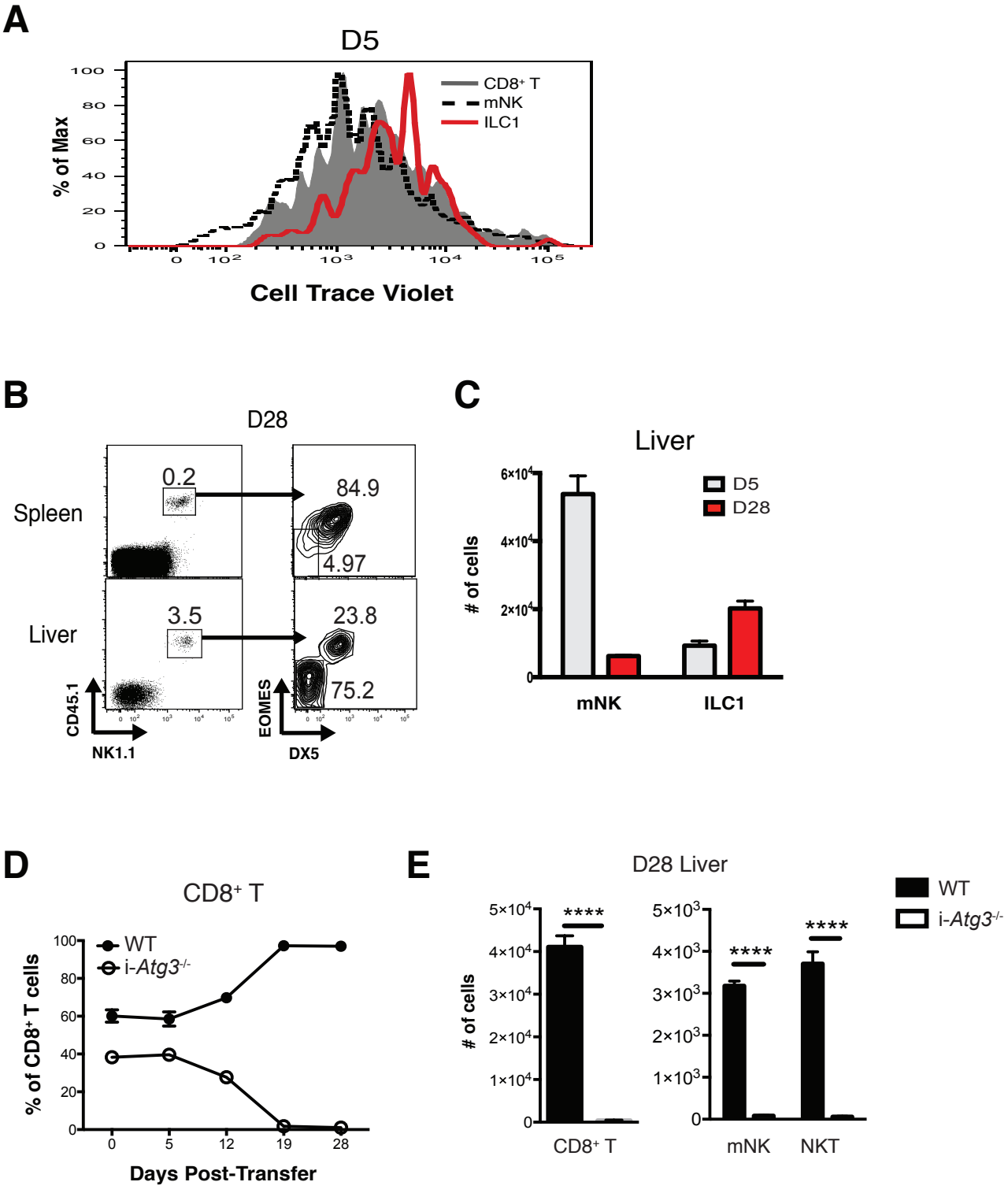

A
D5
CD8⁺ T
mNK
ILC1
% of Max
Cell Trace Violet
B
D28
Spleen
Liver
0.2
84.9
4.97
3.5
23.8
75.2
CD45.1
NK1.1
EOMES
DX5
C
Liver
D5
D28
# of cells
mNK
ILC1
D
CD8⁺ T
WT
i-Atg3-/-
% of CD8⁺ T cells
Days Post-Transfer
E
D28 Liver
WT
i-Atg3-/-
# of cells
****
CD8⁺ T
mNK
NKT

Figure S3

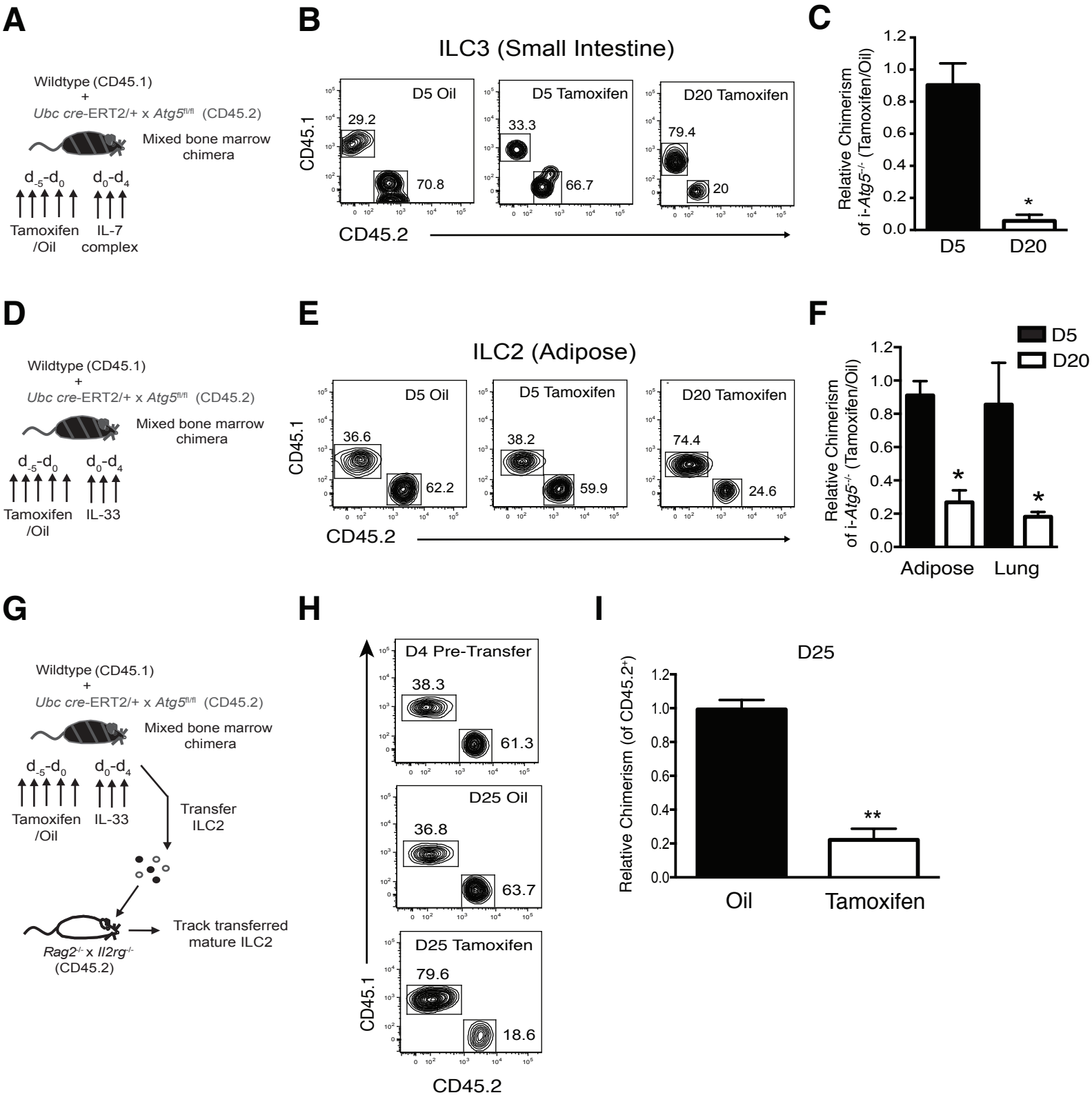
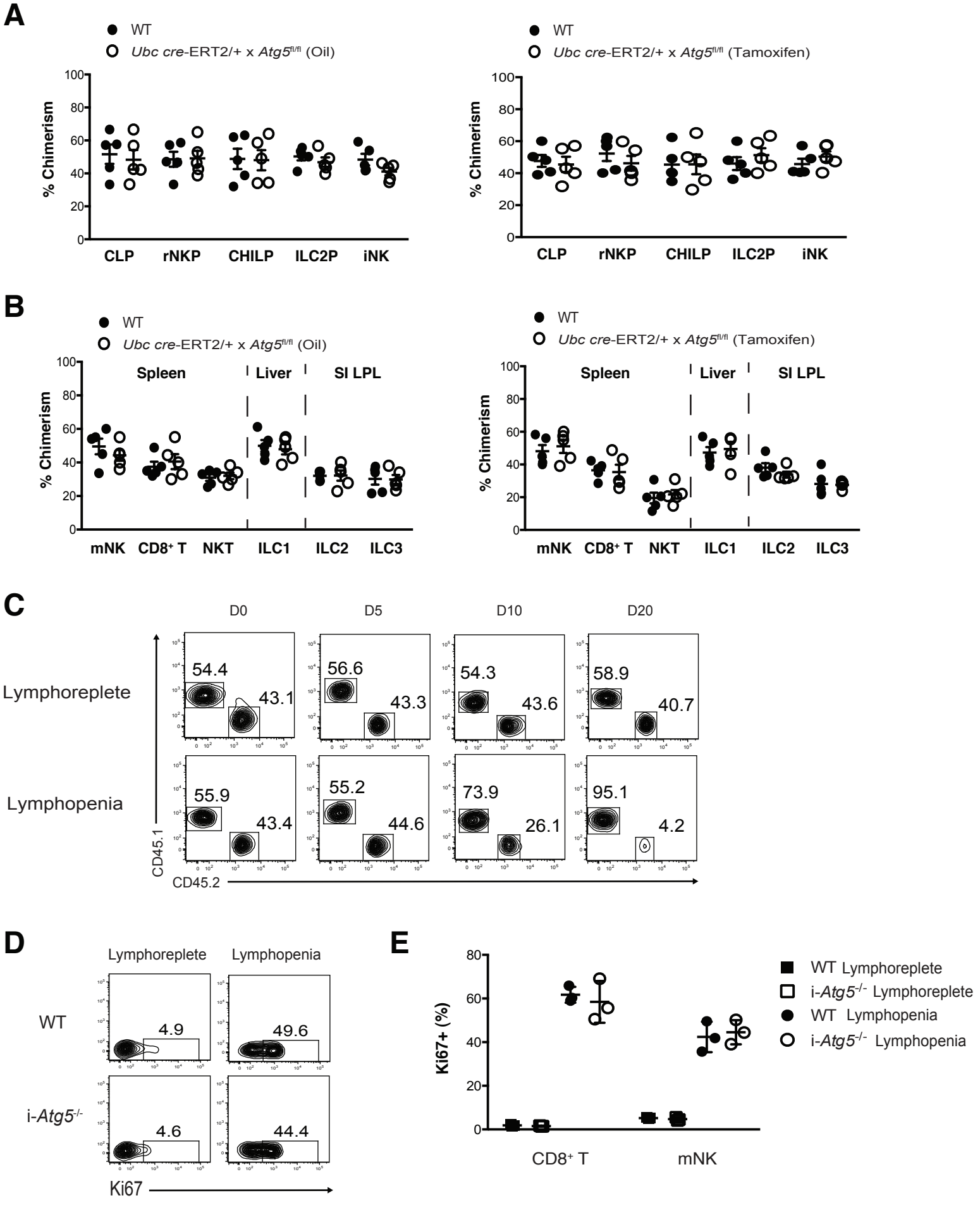

A
Wildtype (CD45.1)
+
Ubc cre-ERT2/+ x Atg5fl/fl (CD45.2)
Mixed bone marrow chimera
d-5-d0
d0-d4
Tamoxifen /Oil
IL-7 complex
B
ILC3 (Small Intestine)
D5 Oil
D5 Tamoxifen
D20 Tamoxifen
29.2
70.8
33.3
66.7
79.4
20
CD45.1
CD45.2
C
Relative Chimerism of i-Atg5-/- (Tamoxifen/Oil)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
D5
D20
D
Wildtype (CD45.1)
+
Ubc cre-ERT2/+ x Atg5fl/fl (CD45.2)
Mixed bone marrow chimera
d-5-d0
d0-d4
Tamoxifen /Oil
IL-33
E
ILC2 (Adipose)
D5 Oil
D5 Tamoxifen
D20 Tamoxifen
36.6
62.2
38.2
59.9
74.4
24.6
CD45.1
CD45.2
F
D5
D20
Relative Chimerism of i-Atg5-/- (Tamoxifen/Oil)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Adipose
Lung
G
Wildtype (CD45.1)
+
Ubc cre-ERT2/+ x Atg5fl/fl (CD45.2)
Mixed bone marrow chimera
d-5-d0
d0-d4
Tamoxifen /Oil
IL-33
Transfer ILC2
Track transferred mature ILC2
Rag2-/- x Il2rg-/-
(CD45.2)
H
D4 Pre-Transfer
38.3
61.3
D25 Oil
36.8
63.7
D25 Tamoxifen
79.6
18.6
CD45.1
CD45.2
I
D25
Relative Chimerism (of CD45.2+)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
Oil
Tamoxifen

Figure S4


A
WT
Ubc cre-ERT2/+ x Atg5fl/fl (Oil)
% Chimerism
CLP
rNKP
CHILP
ILC2P
iNK
WT
Ubc cre-ERT2/+ x Atg5fl/fl (Tamoxifen)
B
Spleen
Liver
SI LPL
mNK
CD8+ T
NKT
ILC1
ILC2
ILC3
C
D0
D5
D10
D20
Lymphoreplete
Lymphopenia
54.4
43.1
56.6
43.3
54.3
43.6
58.9
40.7
55.9
43.4
55.2
44.6
73.9
26.1
95.1
4.2
CD45.1
CD45.2
D
Lymphoreplete
Lymphopenia
WT
i-Atg5-/-
4.9
49.6
4.6
44.4
Ki67
E
Ki67+ (%)
WT Lymphoreplete
i-Atg5-/- Lymphoreplete
WT Lymphopenia
i-Atg5-/- Lymphopenia
CD8+ T
mNK

Supplemental Figure Legends

Figure S1, related to Figure 1 *Atg5* is required for group 1 ILC development (A) Representative flow cytometric plots show Eomes and DX5 expression of $Lin^-NK1.1^+$ cells and (**B**) absolute numbers of refined NK precursor (rNKP; $Lin^-NK1.1^-CD122^+CD127^+DX5^-$), immature NK (iNK; $Lin^-NK1.1^+Eomes^+DX5^-$), and $Lin^-NK1.1^+Eomes^-DX5^-$ cells in the bone marrow of NK-*Atg5*$^{-/-}$ mice or littermate WT controls. (**C**) NK-*Atg5*$^{-/-}$ mice or littermate WT controls were infected with MCMV i.p. and survival was determined. (**D**) Viral titers from the blood on day 4 after infection are shown for mice infected with MCMV. (Lin^- refers to $TCR\beta^-CD19^-CD3\varepsilon^-Ly6G^-TER119^-TCR\gamma\delta^-$ cells). (**E**) WT mice (CD45.1 x CD45.2) were lethally irradiated and reconstituted with an equal number of bone marrow cells from WT (CD45.1) and *Ubc*$^{Cre\text{-}ERT2}$ x *Atg5*$^{fl/fl}$ (CD45.2) mice. Bone marrow was harvested from chimeric mice and ~1,000 CHILP ($Lin^-NK1.1^-DX5^-FLT3^-CD127^+\alpha_4\beta_7^+CD25^-$) and ~3,000 ILC2P ($Lin^-NK1.1^-DX5^-FLT3^-CD127^+\alpha_4\beta_7^+CD25^+$) cells were sorted to >95% purity using the gating strategy shown. Data are representative of two independent experiments, with n=5 per time point. Samples were compared using an unpaired, two-tailed Student's t test, and data presented as the mean ± s.e.m. (∗p < 0.05, ∗∗∗p < 0.001, ns = not significant).

Figure S2, related to Figure 3. Liver ILC1s undergo homeostatic proliferation during lymphopenia (A) WT (CD45.1) liver lymphocytes were labeled with Cell Trace Violet and adoptively transferred into *Rag2*$^{-/-}$ *x Il2rg*$^{-/-}$ (CD45.2) recipients. Representative histogram shows Cell Trace Violet fluorescence of indicated adoptively transferred cells harvested from recipient livers on day 5 after transfer. (**B**) Percentages and (**C**) absolute numbers of adoptively transferred liver mNK and ILC1 cells harvested from the liver at indicated time points after transfer **(D,E)** WT:i-*Atg3* mixed bone marrow chimeras were injected i.p. with 1mg of tamoxifen (i-*Atg3*$^{-/-}$) daily for 5 days and splenocytes were adoptively transferred into *Rag2*$^{-/-}$ *x Il2rg*$^{-/-}$ hosts. (**D**) Percentages of adoptively transferred WT or i-*Atg3*$^{-/-}$ $CD8^+$ T cells in the blood at indicated time points post-transfer and (**E**) absolute numbers of indicated lymphocytes from the liver of recipients on d28 post-transfer. Data are representative of two independent experiments, with n=3-4 per group. Samples were compared using an unpaired, two-tailed Student's t test, and data presented as the mean ± s.e.m. (∗∗∗∗p < 0.0001).

Figure S3, related to Figure 3. *Atg5* is required for the survival of mature ILC2 and ILC3s following cytokine-induced proliferation *in vivo*. (A-C) Briefly, WT:i-*Atg5* mixed bone marrow chimeras were injected i.p. with either control oil or 1mg of tamoxifen daily for 5 days followed by three i.p. injections of rhIL-7/anti-IL-7 complex every other day (**A**) Schematic of experiment (**B**) Representative flow cytometric plots and (**C**) relative chimerism (tamoxifen treated chimerism of $CD45.2^+$/oil treated chimerism of $CD45.2^+$) of i-*Atg5*$^{-/-}$ ILC3s harvested from the small intestine on indicated days post tamoxifen/oil treatment. (**D-F**) WT:i-*Atg5* mixed bone marrow chimeras were injected i.p. with either control oil or 1mg of tamoxifen daily for 5 days followed by three i.p. injections of rmIL-33 every other day (**D**) Schematic of experiment (**E**) Representative flow cytometric plots and (**F**) relative chimerism of i-*Atg5*$^{-/-}$ ILC2s harvested from indicated peripheral organs on d5 or d20 post tamoxifen/oil treatment. (**G-I**) WT:i-*Atg5* mixed bone marrow chimeras were injected i.p. with either control oil or 1mg of tamoxifen daily for 5 days followed by three i.p. injections of rmIL-33 every other day and adipose ILC2s were adoptively transferred into *Rag2*$^{-/-}$ *x Il2rg*$^{-/-}$ recipients i.p. (**G**) Schematic of experiment (**H**) Representative flow cytometric plots and (**I**) relative chimerism (normalized to control oil chimerism) of control oil or tamoxifen treated i-*Atg5* ILC2s harvested from the adipose tissue on d21 following transfer. Data are representative of two independent experiments, with n=3 per cohort. Samples were compared using an unpaired, two-tailed Student's t test, and data presented as the mean ± s.e.m. (∗p < 0.05, ∗∗p < 0.01).

Figure S4, related to Figure 3. *Atg5* is not required for the survival of precursors or mature lymphocytes in lymphoreplete mice. (A,B) WT:i-*Atg5* mixed bone marrow chimeras were injected i.p. with either oil (as a control) or 1mg of tamoxifen daily for 5 days, and percent chimerism was measured in (**A**) bone marrow precursors and (**B**) peripheral mature lymphocytes after 21 days. Indicated cell types are identified as CLP ($Lin^-NK1.1^-FLT3^+CD127^+$), NKT cells ($TCR\beta^+NK1.1^+CD3\varepsilon^+$), $CD8^+$ T cells ($TCR\beta^+CD8^+CD3^+$), or as mentioned previously. (**C-E**) WT:i-*Atg5* mixed bone marrow chimeras were injected i.p. with 1mg of tamoxifen daily for 5 days**. (C)** Representative flow cytometric plots of mNK cells

in the blood of either tamoxifen treated i-*Atg5*$^{-/-}$ mixed bone marrow chimeras (lymphoreplete) or adoptively transferred tamoxifen treated i-*Atg5*$^{-/-}$ mNK cells in the blood of *Rag2*$^{-/-}$ *x Il2rg*$^{-/-}$ recipients (lymphopenia) on indicated time points post tamoxifen treatment (**D**) Representative flow cytometric plots show Ki67 intranuclear staining and (**E**) quantification of Ki67^{+} WT and i-*Atg5*$^{-/-}$ mNK cells on d5 following tamoxifen treatment. Data are representative of two independent experiments, with n=3-5 per group.