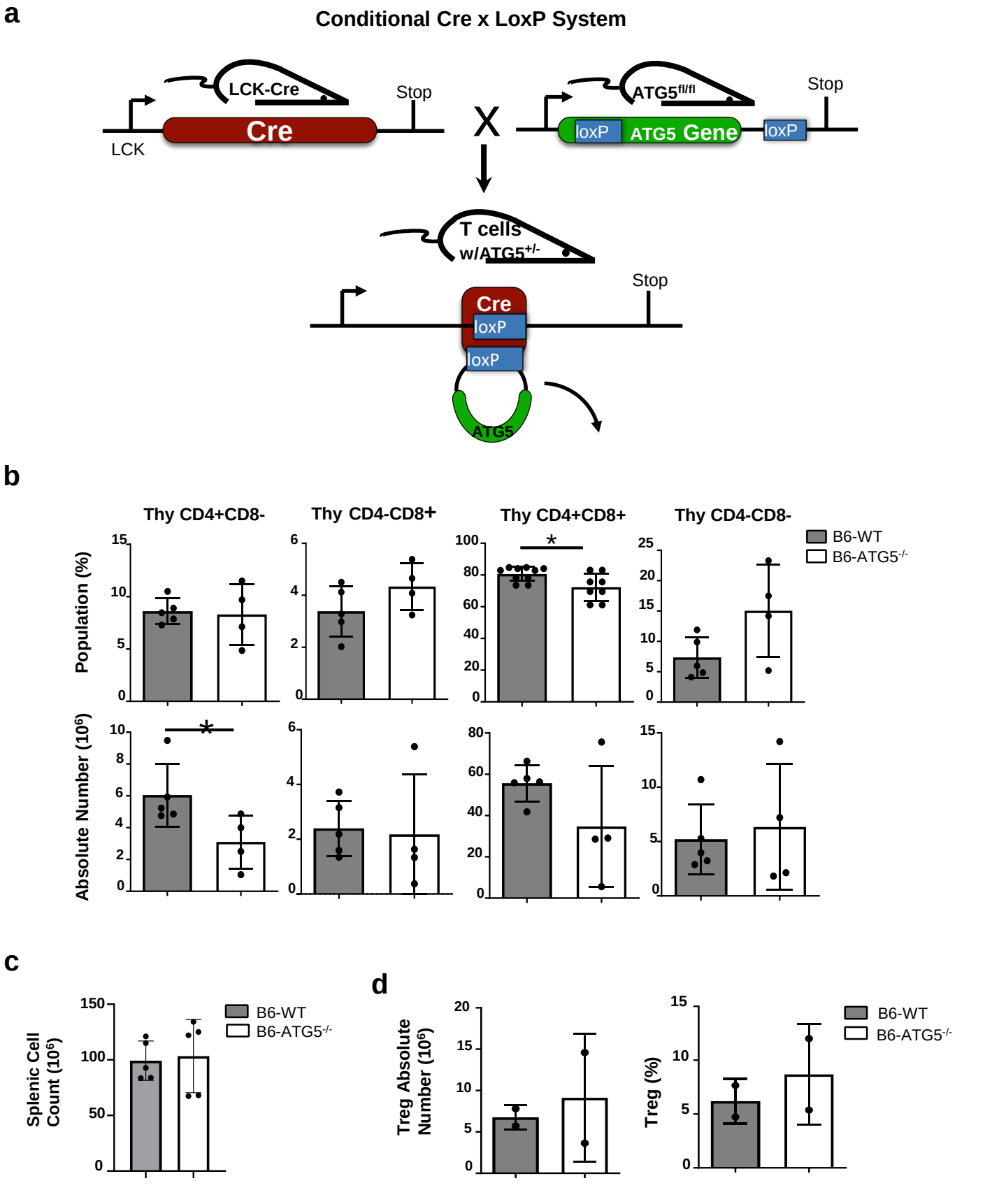
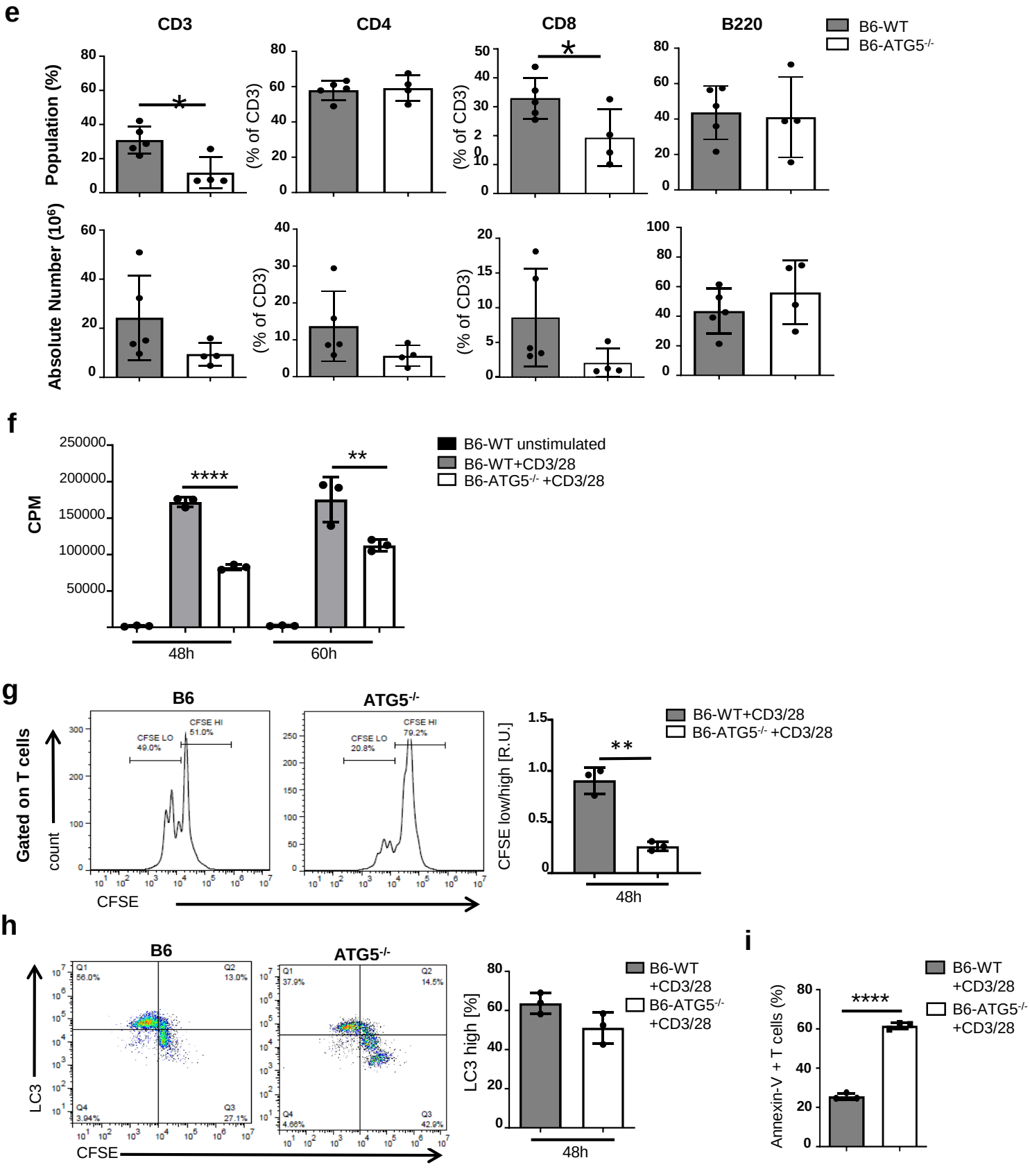
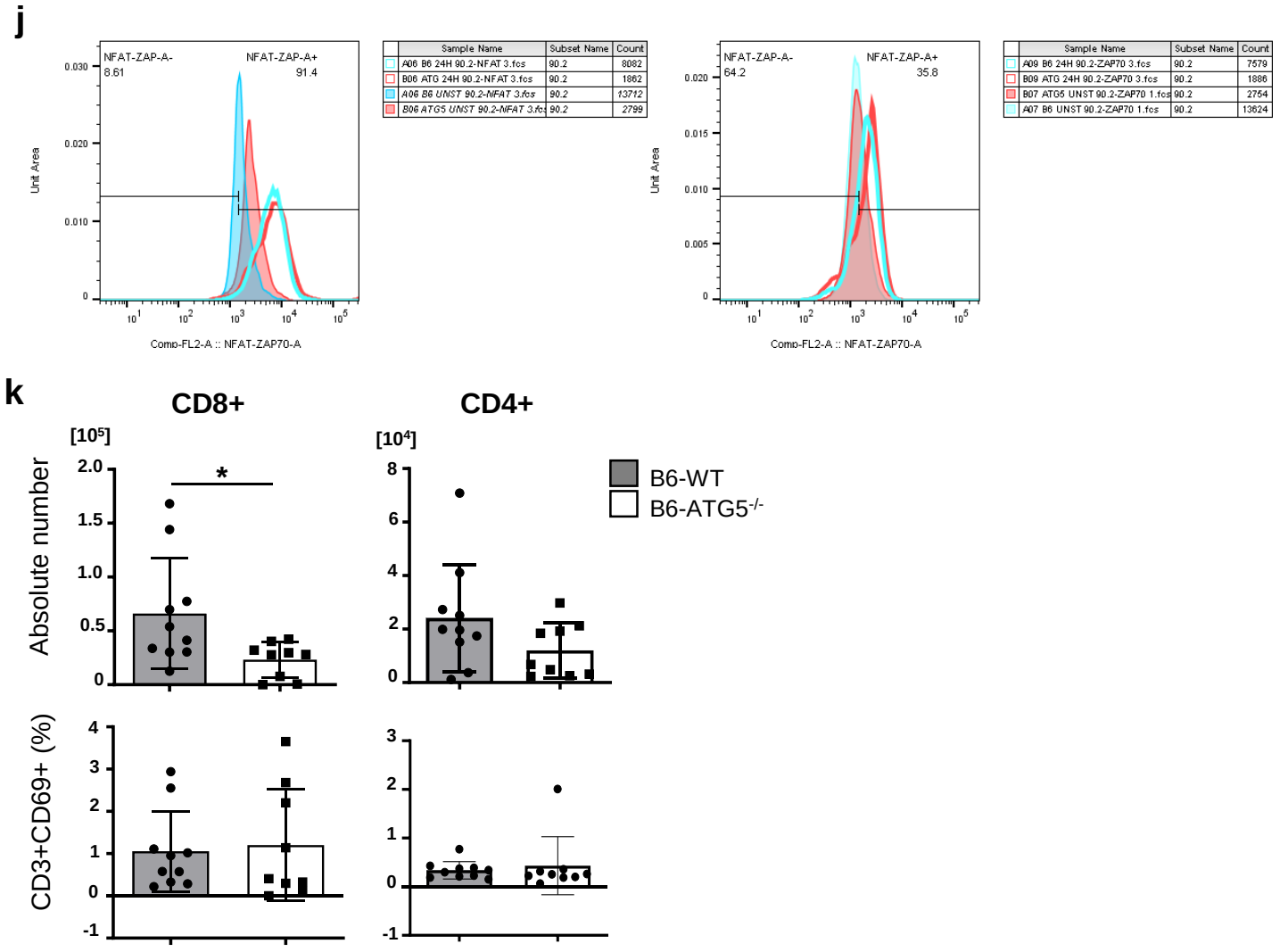

a
Conditional Cre x LoxP System
LCK-Cre
Stop
Cre
LCK
X
ATG5fl/fl
Stop
loxP
ATG5 Gene
loxP
T cells
w/ATG5+/-
Stop
Cre
loxP
loxP
ATG5
b
Thy CD4+CD8-
Thy CD4-CD8+
Thy CD4+CD8+
Thy CD4-CD8-
B6-WT
B6-ATG5-/-
Population (%)
Absolute Number (10^6)
*
*
c
Splenic Cell Count (10^6)
B6-WT
B6-ATG5-/-
d
Treg Absolute Number (10^6)
Treg (%)
B6-WT
B6-ATG5-/-


e
CD3
CD4
CD8
B220
B6-WT
B6-ATG5-/-
Population (%)
Absolute Number (10⁶)
(% of CD3)
f
CPM
B6-WT unstimulated
B6-WT+CD3/28
B6-ATG5-/- +CD3/28
48h
60h
g
B6
ATG5-/-
Gated on T cells
count
CFSE
CFSE LO 49.0%
CFSE HI 51.0%
CFSE LO 20.8%
CFSE HI 79.2%
CFSE low/high [R.U.]
B6-WT+CD3/28
B6-ATG5-/- +CD3/28
48h
h
B6
ATG5-/-
LC3
CFSE
Q1 56.0%
Q2 13.0%
Q4 3.94%
Q3 27.1%
Q1 37.9%
Q2 14.5%
Q4 4.66%
Q3 42.9%
LC3 high [%]
B6-WT +CD3/28
B6-ATG5-/- +CD3/28
48h
i
Annexin-V + T cells (%)
B6-WT +CD3/28
B6-ATG5-/- +CD3/28



SFig. 2 Phenotypic analysis of ATG5$^{-/-}$ knockout animals. (a) Schematic of the Conditional Cre-LoxP system **(b)** B6-WT and B6-ATG5$^{-/-}$ mice thymic profile. Whole spleens were analyzed by flow cytometry to phenotypic analysis of cell populations between B6-WT and B6-ATG5$^{-/-}$ mice. **(c)** Whole spleen cellularity from B6-WT and B6-ATG5$^{-/-}$ mice **(d)** Treg profiles **(e)** Lymphocytic cell populations and absolute numbers **(f)** CD90.2-positive B6-WT and B6-ATG5$^{-/-}$ T cells were purified, before being transferred to a stimulation plate previously coated with anti-CD3/CD28. Unstimulated T cells serve as controls. T cell proliferation based on ^{3}H-thymidine incorporation was analyzed at 48 and 60 hours. The data in CPM are the mean ± SEM of triplicates from 2 independent experiments. **(g)** T cells were labeled with CFSE prior to stimulation with anti-CD3/CD28 and analyzed for CFSE dilution at 48 hours. Left panel: representative histograms of CFSE dilution. Right panel: mean of the ratios CFSE^{low}/ CFSE^{high} ± SEM of triplicates. Data are representative of 2 independent experiments. **(h)** B6-WT and B6-ATG5$^{-/-}$ T cells were sorted, labeled with CFSE and stimulated with CD3/28 as in Supplemental Figure 2g. At 48h, they were harvested, stained intracellular for LC3 and analyzed by FACS. Left panel: representative figures gated on T cells for CFSE$^{high/low}$ and LC3$^{high/low}$ populations. Right panel: the graph represents the mean percentage of LC3^{high} CD90.2-positive T cells ± SEM of triplicates **(i)** After CD3/28 stimulation for 48h, activated B6-WT and B6-ATG5$^{-/-}$ T cells were stained with Annexin-V and analyzed by flow cytometry. (i) Data is representative of 2 independent experiments. **(j)** Histogram overlays of flow cytometry data at 24 hr time point analysis of Left Panel: NFAT Right Panel: Zap-70. These are representative histograms from 3 independent experiments. **(k)** Donor T cells were analyzed for activation markers 7 days post BMT transplant. *P< 0.05; **P< 0.01; ***P< 0.001; ****P< 0.0001.