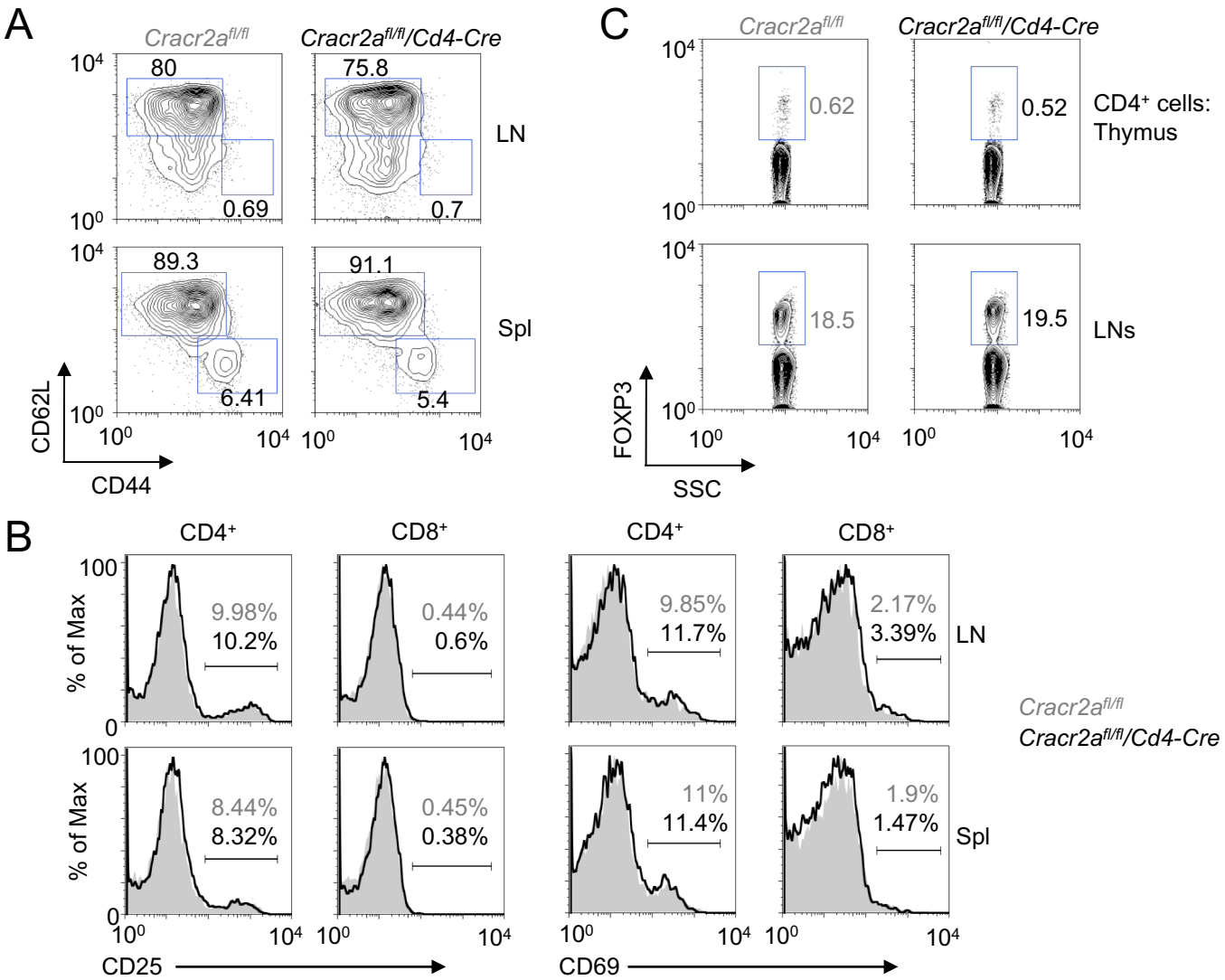
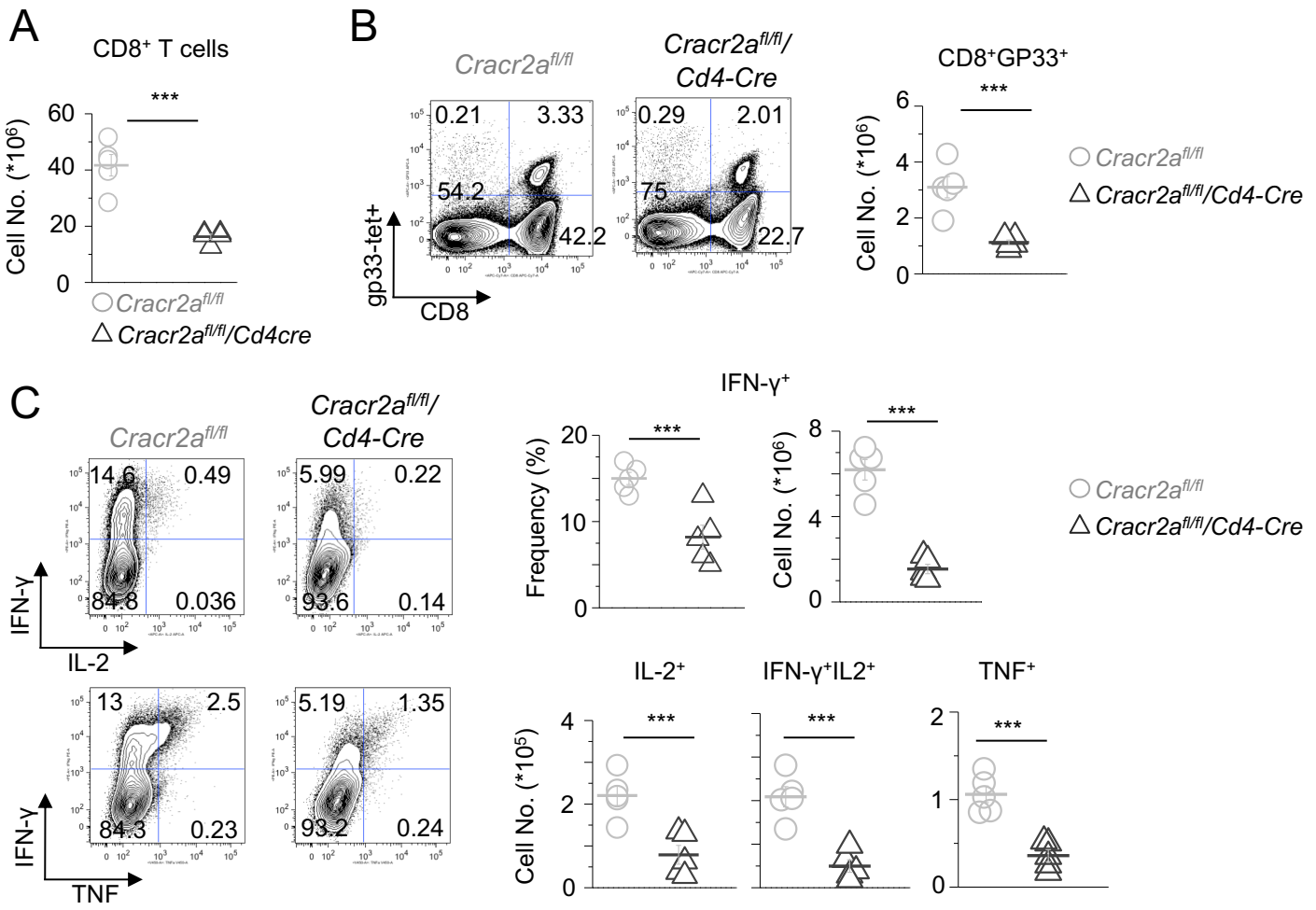


Supplementary Figure 1. Conditional deletion of *Cracr2a* does not affect expression of T cell activation markers and development of Tregs in vivo
(**A**) Naïve and effector CD4⁺ T cell populations in the lymph nodes (LN) and spleens (Spl) of *Cracr2a*$^{fl/fl}$ or *Cracr2a*$^{fl/fl}$*/Cd4-Cre* mice determined using surface staining for CD62L and CD44.
(**B**) Representative flow plots showing expression of CD25 (left two panels) and CD69 (right two panels) markers in CD4⁺ and CD8⁺ T cells from lymph nodes and spleens of *Cracr2a*$^{fl/fl}$ or *Cracr2a*$^{fl/fl}$*/Cd4-Cre* mice as judged by surface staining.
(**C**) Representative flow plots showing frequencies of regulatory T cells (CD4⁺FOXP3⁺) from the thymi and lymph nodes of *Cracr2a*$^{fl/fl}$ or *Cracr2a*$^{fl/fl}$*/Cd4-Cre* mice. Data in all the panels are representative of at least three independent animals.



Supplementary Figure 2. CRACR2A deficiency impairs CD8⁺ T cell responses to acute infection with LCMV

(**A**) Number of splenic CD8⁺ T cells on day 8 after LCMV-Armstrong infection of *Cracr2a*$^{fl/fl}$ or *Cracr2a*$^{fl/fl}$*/Cd4-Cre* mice. Each symbol represents data obtained from an independent animal.

(**B**) Representative flow plots showing the frequency of LCMV-specific IAb-gp33 tetramer-positive CD8⁺ T cells (gp33-tet). Graph shows absolute numbers of gp33-tetramer-positive LCMV-specific CD8⁺ T cells from independent animals.

(**C**) Representative flow plots showing the frequency and numbers of cells producing IFN-γ vs. IL-2, or IFN-γ vs. TNF on day 8 after LCMV infection following ex vivo stimulation for 5 hours with gp33 peptide. Graphs show quantification of frequency or numbers of IFN-γ⁺CD8⁺ T cells (top) and absolute numbers of IFN-γ⁺, IFN-γ⁺IL-2⁺ or TNF⁺ CD8⁺ T cells (bottom) from independent animals. The LCMV infection data are representative of three independent experiments with 4-6 animals in each experiment.

*** $p < 0.0005$.

Supplementary Table 1. List of primers and shRNAs used in this study

Gene name	Forward Primer	Reverse Primer	Comments
hCRACR2A_shRNA1 (mature antisense)	ATACACCTTCTCTTCATGGCG		In pLKO.1 vector Silencing of all isoforms
hCRACR2A_shRNA2 (mature antisense)	TTTCAGCTTGGTATTCTCAGC		In pLKO.1 vector Silencing of all isoforms – gave best knockdown effect
hCRACR2A_shRNA3 (mature antisense)	ATCACTTTCATCTTCCAACAC		In pLKO.1 vector Silencing of all isoforms
hCRACR2A_shRNA4 (mature antisense)	TTTGCTCCTGAGTCTCCTTCT		In pLKO.1 vector Silencing of all isoforms
hCRACR2A_shRNA5 (mature antisense)	AAGTGACTAAATCCAGTAGTG		In pLKO.1 vector Silencing of all isoforms
CRACR2A – genotyping – Flox allele	CTA TTC ACA GTT GCC ATT TCT GC	GAT TGG AGG TGA TCC TGC AA	195 bp –WT allele, 350 bps – Flox allele
CRACR2A genotyping – KO allele	CTA TTC ACA GTT GCC ATT TCT GC	TGC ATC CTC TAG TCA TTT ACC TAG	255 bps – KO allele
Integrin α4	GATGCTGTTGTTGTACTTCGG	ACCACTGAGGCATTAGAGAGC	For qPCR
CCR5	CGAAAACACATGGTCAAACG	TTCCTACTCCCAAGCTGCAT	For qPCR
CXCR3	TGCTAGATGCCTCGGACTTT	ATAAGACGGATGGCCTTGTG	For qPCR
Integrin αL	CCAGACTTTTGCTACTGGGAC	GCTTGTTCGGCAGTGATAGAG	For qPCR
CCR6	CCTCACATTCTTAGGACTGGAGC	GGCAATCAGAGCTCTCGGA	For qPCR
IL-23R	ACACTGGGAAGCCTACCTACA	AGCTTGGACCCATACCAAATA	For qPCR
GM-CSF	TTTACTTTTCCTGGGCATTG	TAGCTGGCTGTCATGTTCAA	For qPCR
GATA-3	CTCGGCCATTCGTACATGGAA	GGATACCTCTGCACCGTAGC	For qPCR
IFN-γ	ACTGGCAAAAGGATGGTG	GTTGCTGATGGCCTGATT	For qPCR
T-bet	CAACAACCCCTTTGCCAAAG	TCCCCCAAGCAGTTGACAGT	For qPCR
IL-17A	CTCCAGAAGGCCCTCAGACTA	AGCTTTCCCTCCGCATTGACA	For qPCR
RORγ	CACGGCCCTGGTTCTCAT	CAGATGTTCCACTCTCCTCTTCTCT	For qPCR
Runx1	ACT TCC TCT GCT CCG TGC TA	CGC GGT AGC ATT TCT CAG TT	For qPCR
Runx3	CTC CAG CCC GAG ACT ACA AG	AGG GAG GGA GAG AAA GTC CA	For qPCR