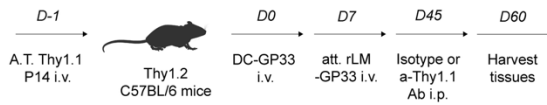
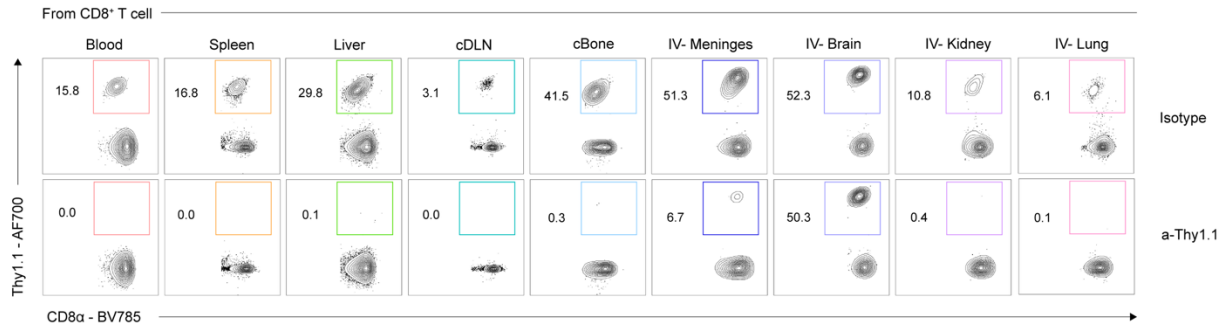


## Supplemental Figures.

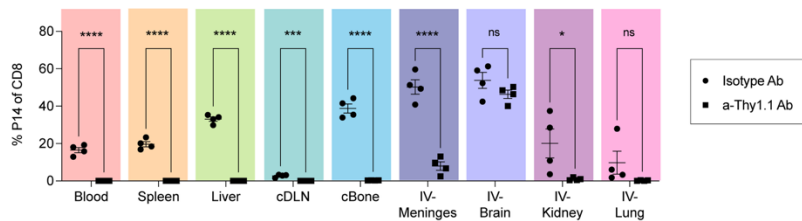
**A**



**B**

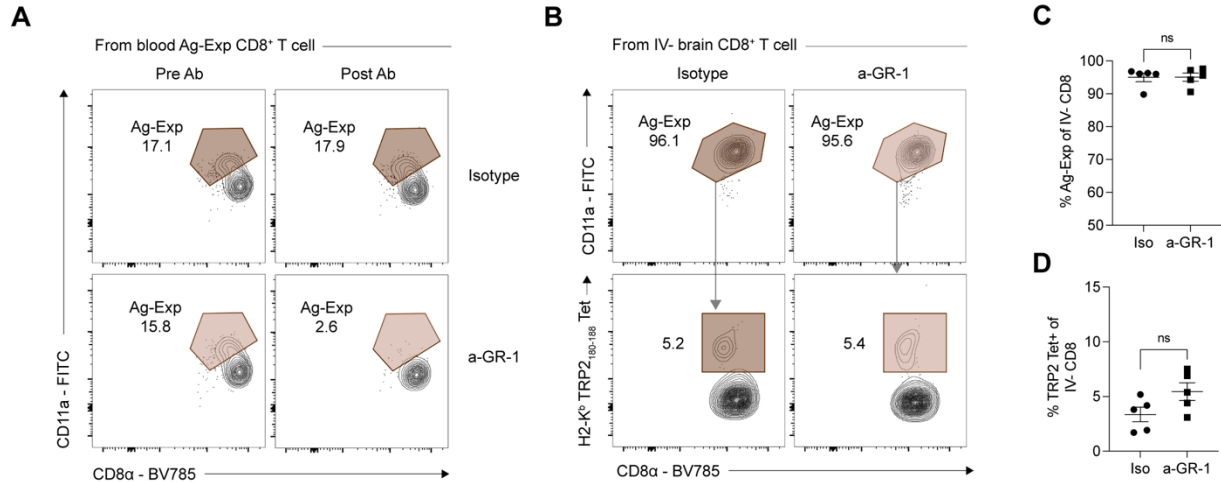


**C**

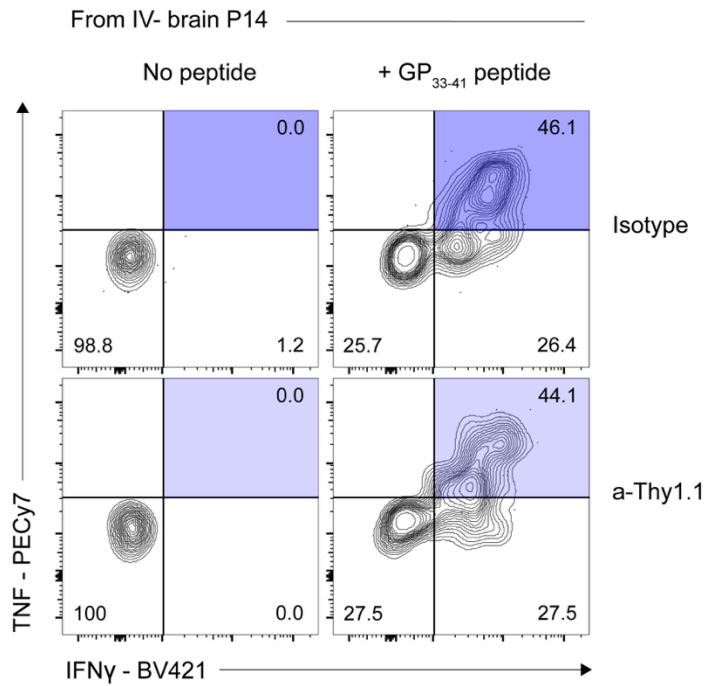
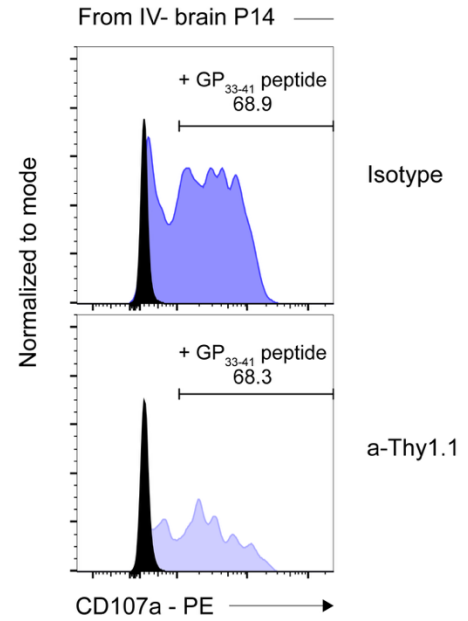


### Supplemental Figure 1. a-Thy1.1 antibody treatment systemically depletes TCR-tg T cells while preserving brain T<sub>RM</sub>.

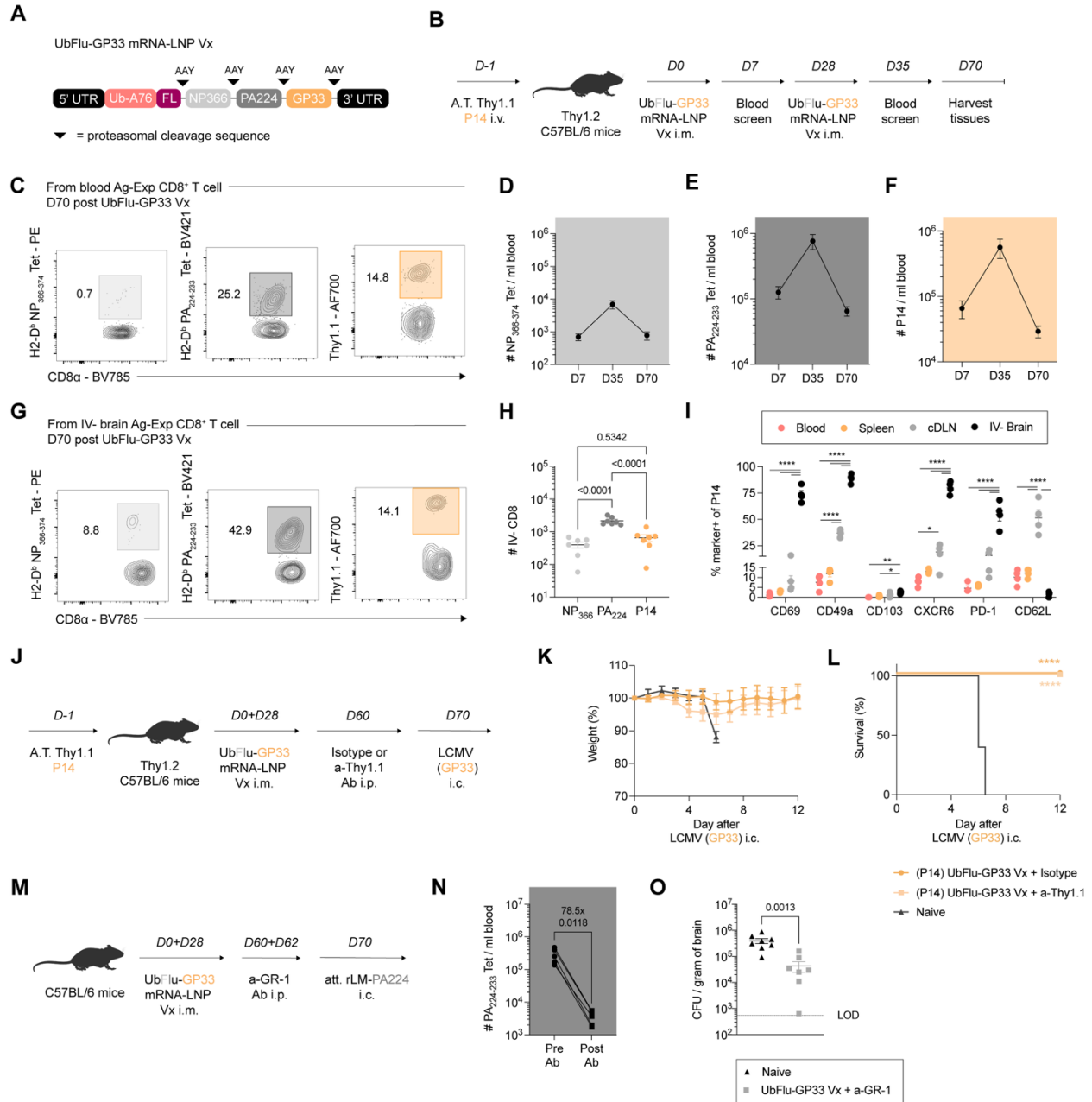
**(A)** Experimental design. Thy1.2 C57BL/6N mice were adoptively transferred with  $2 \times 10^4$  Thy1.1 P14 intravenously (i.v.) one day prior to i.v. injection with GP<sub>33-41</sub> peptide-pulsed, LPS-matured dendritic cells (DC-GP33). After DC prime, mice were boosted with attenuated recombinant *Listeria monocytogenes* expressing GP<sub>33-41</sub> (att. rLM-GP33). At a memory timepoint 45 days after DC prime, mice were treated with 2  $\mu$ g of isotype control or a-Thy1.1 antibody intraperitoneally (i.p.) to deplete P14 memory T cells in select tissues. Approximately 3 minutes prior to tissue harvest on D60, mice were injected intravenously with a fluorophore conjugated anti-CD45 antibody to distinguish P14 T cells in the vasculature (IV+) versus P14 T cells localized in tissues (IV-) at the time of isolation. **(B)** Representative flow plots of Thy1.1<sup>+</sup> CD8<sup>+</sup> T cells in the blood, spleen, liver, cervical draining lymph nodes (cDLN), cranial bone (cBone), IV- meninges, IV- brain, IV- kidney, and IV- lung following isotype or a-Thy1.1 antibody treatment. **(C)** Frequency of Thy1.1<sup>+</sup> P14 T cells in respective tissues following antibody treatments. Experiments in **(B-C)** show representative data from 1 independent experiment with n=4 mice per group. Statistical significance was determined by student's t-test. Graphs show the mean  $\pm$  s.e.m. with each symbol representing one mouse. Individual *p* values are summarized as: \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, \*\*\*\**p* < 0.0001. Graphical illustrations were created using BioRender (<https://biorender.com>).



**Supplemental Figure 2. a-GR-1 antibody treatment effectively depletes  $T_{CIRC}$  while preserving brain  $T_{RM}$ .** (A) Representative flow plot of CD11a<sup>hi</sup> Ag-Exp CD8<sup>+</sup> T cells in the blood of DC-rLM-TRP2 prime-boosted mice pre- and post- antibody treatment with isotype control or a-GR-1 antibodies. (B) Representative flow plot of Ag-Exp CD8<sup>+</sup> T cells and TRP2<sub>180-188</sub>-specific CD8<sup>+</sup> T cells in the IV- brain from DC-rLM-TRP2 prime-boosted mice 10 days following isotype or a-GR-1 antibody treatment. (C) Frequency of Ag-Exp CD8<sup>+</sup> T cells and (D) TRP2<sub>180-188</sub>-specific CD8<sup>+</sup> T cells in the IV- brain after antibody treatments. Experiments in (A) show representative data from 2 independent experiments with n=7-9 mice per group total. Experiments in (B-D) show representative data from 1 of 2 independent experiments with n=5 mice per group. Statistical significance was determined by student's t-test. Graphs show the mean  $\pm$  s.e.m. with each symbol representing one mouse.

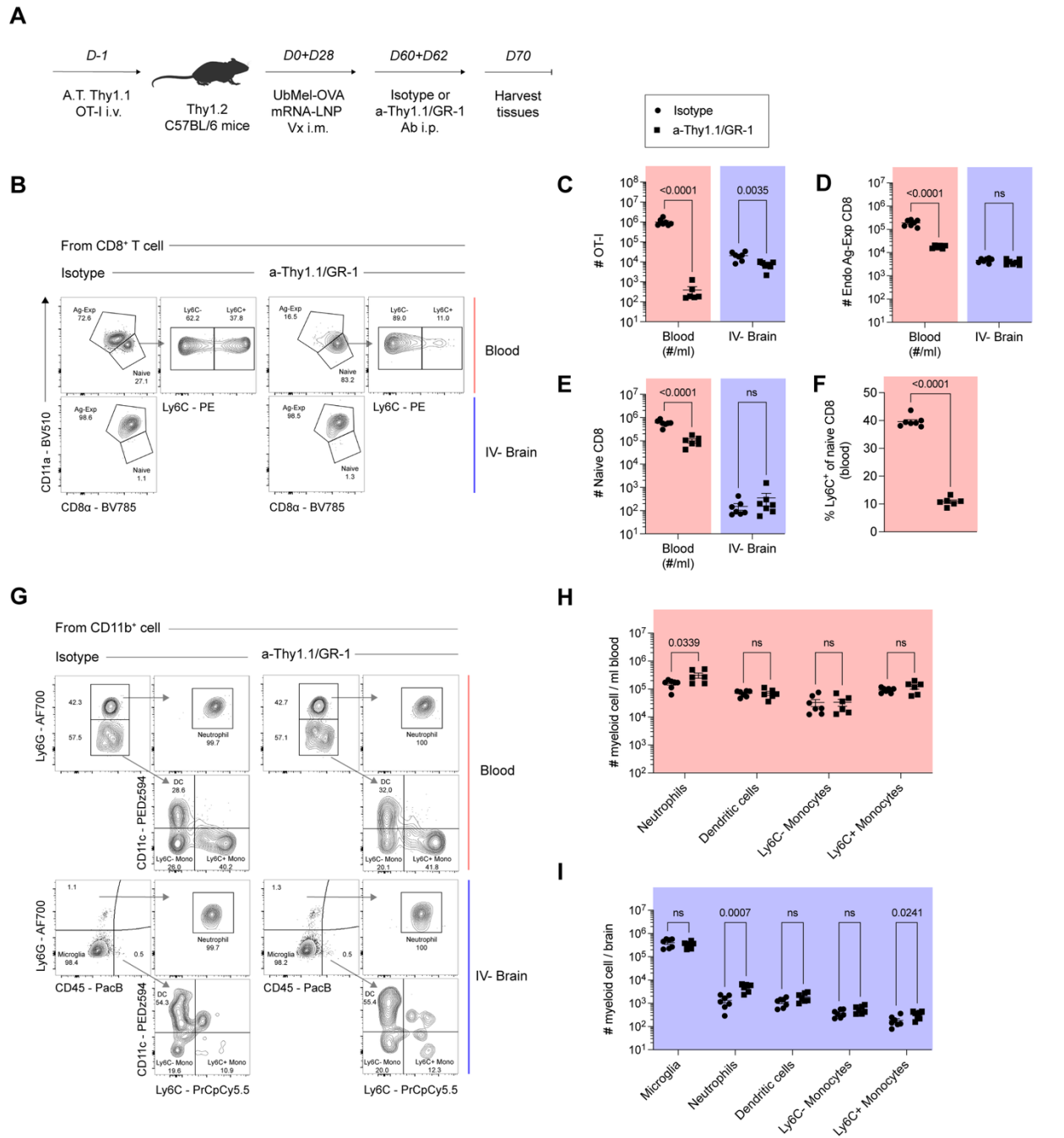
**A****B**

**Supplemental Figure 3. Representative gating of cytokine production and cytolytic capacity of brain T<sub>RM</sub> following ex vivo GP<sub>33-41</sub> peptide stimulation. (A)** Expression of IFN- $\gamma$  and TNF among IV- brain P14 following 5-6-hour ex vivo 200 nM GP<sub>33-41</sub> peptide stimulation or no peptide control in B16-GP33 and LCMV i.c. surviving mice previously treated with isotype or a-Thy1.1 antibodies. **(B)** Expression of CD107a among IV- brain P14 following peptide stimulation or no peptide control (black histograms).



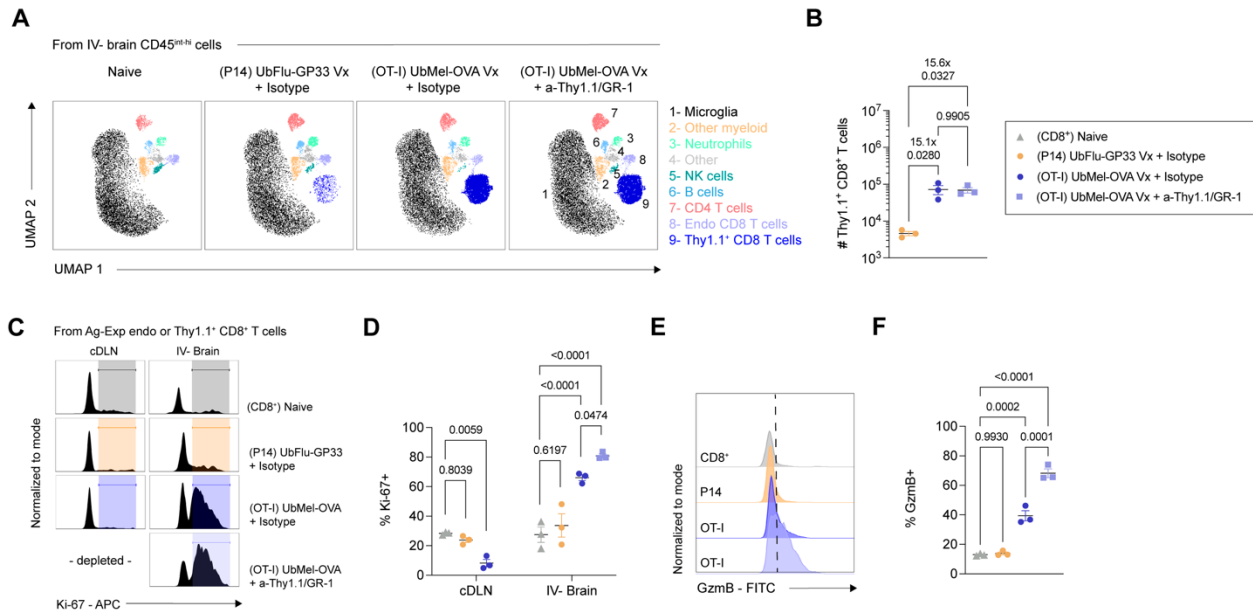
**Supplemental Figure 4. Peripheral mRNA-LNP vaccination generates protective virus-specific brain T<sub>RM</sub>.** (A) Construct design. Same as in Figure 5 but with NP<sub>366-374</sub>, PA<sub>224-233</sub>, and GP<sub>33-41</sub> coding sequences. (B) Thy1.2 C57BL/6N mice were adoptively transferred with  $2 \times 10^4$  Thy1.1 P14 i.v. and immunized one day later with  $5 \mu\text{g}$  UbFlu-GP33 mRNA-LNP vaccine (Vx) i.m. with boosting 28 days later. (C) Tetramer and Thy1.1<sup>+</sup> P14 virus-specific CD8<sup>+</sup> T cell staining. (D-F) Number of virus-specific CD8<sup>+</sup> T cells in blood across time. (G) Representative staining and (H) numbers of virus-specific CD8<sup>+</sup> T cells in the IV- brain. (I) T<sub>RM</sub>-associated marker expression among P14. (J) Mice were vaccinated as in (B), treated i.p. with isotype or anti-Thy1.1 antibody (Ab) to deplete P14 T<sub>CIRCM</sub>, and challenged i.c. with LCMV. (K) Weight loss and (L) survival after LCMV i.c. challenge. (M) C57BL/6N mice were immunized, treated with a-GR-1 Ab i.p. to deplete T<sub>CIRCM</sub>, and challenged i.c. with att. rLM-PA<sub>224-233</sub>. (N) Number of H2-D<sup>b</sup> PA<sub>224-233</sub> Tet<sup>+</sup> CD8<sup>+</sup> T cells / ml of blood. (O) rLM-PA<sub>224-233</sub> colony forming units (CFU) / gram of

brain tissue with limit of detection (LOD) noted. Experiments in **(A-H; O)** show concatenated data from 2 independent experiments with n=7-15 mice per group total. Experiments in **(K-L)** show concatenated data from 3 independent experiments with n=7-10 mice per group total. Experiments in **(I; N)** show data from 1 of 2 independent experiments with n=4-5 mice. Statistical significance was determined by Student's t-test, paired t-test, one-way ANOVA with Tukey's multiple comparison's test, or log-rank test for survival curves compared to naïve mice. Graphs show the mean  $\pm$  s.e.m. with each symbol representing one mouse. Individual  $p$  values are noted on respective graphs or are otherwise summarized as: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Graphical illustrations were created using BioRender (<https://biorender.com>).

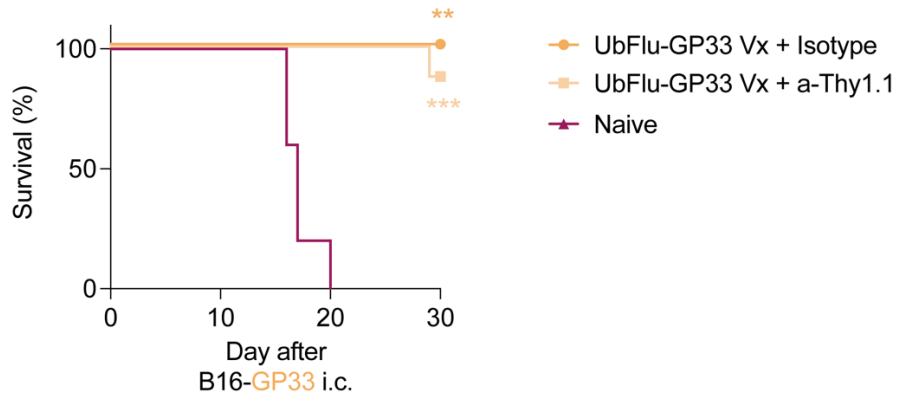


**Supplemental Figure 5. Combination a-Thy1.1/GR-1 antibody depletion impact on CD8<sup>+</sup> T cell and myeloid cell compartments. (A)** Experimental design. Thy1.2 C57BL/6N mice were adoptively transferred with  $10^4$  Thy1.1 OT-I i.v. and immunized one day later with  $5 \mu\text{g}$  UbMel-OVA mRNA-LNP vaccine (Vx) i.m. Mice were boosted 28 days later with  $5 \mu\text{g}$  UbMel-OVA mRNA-LNP vaccine ipsilaterally i.m. At 60 and 62 days after initial immunization, mice were treated i.p. with isotype control antibody or a combination of  $2 \mu\text{g}$  a-Thy1.1 /  $200 \mu\text{g}$  a-GR-1 antibodies to broadly deplete TCR-tg and endogenous  $T_{\text{CIRC}}$ . After 70 days, tissues were harvested. **(B)** Representative gating of antigen-experienced (Ag-Exp) and naïve CD8<sup>+</sup> T cells in the blood and IV- brain following antibody depletion. **(C)** Number of OT-I, **(D)** endogenous Ag-Exp CD8<sup>+</sup> T cells, and **(E)** naïve CD8<sup>+</sup> T cells in the blood and IV- brain of antibody treated

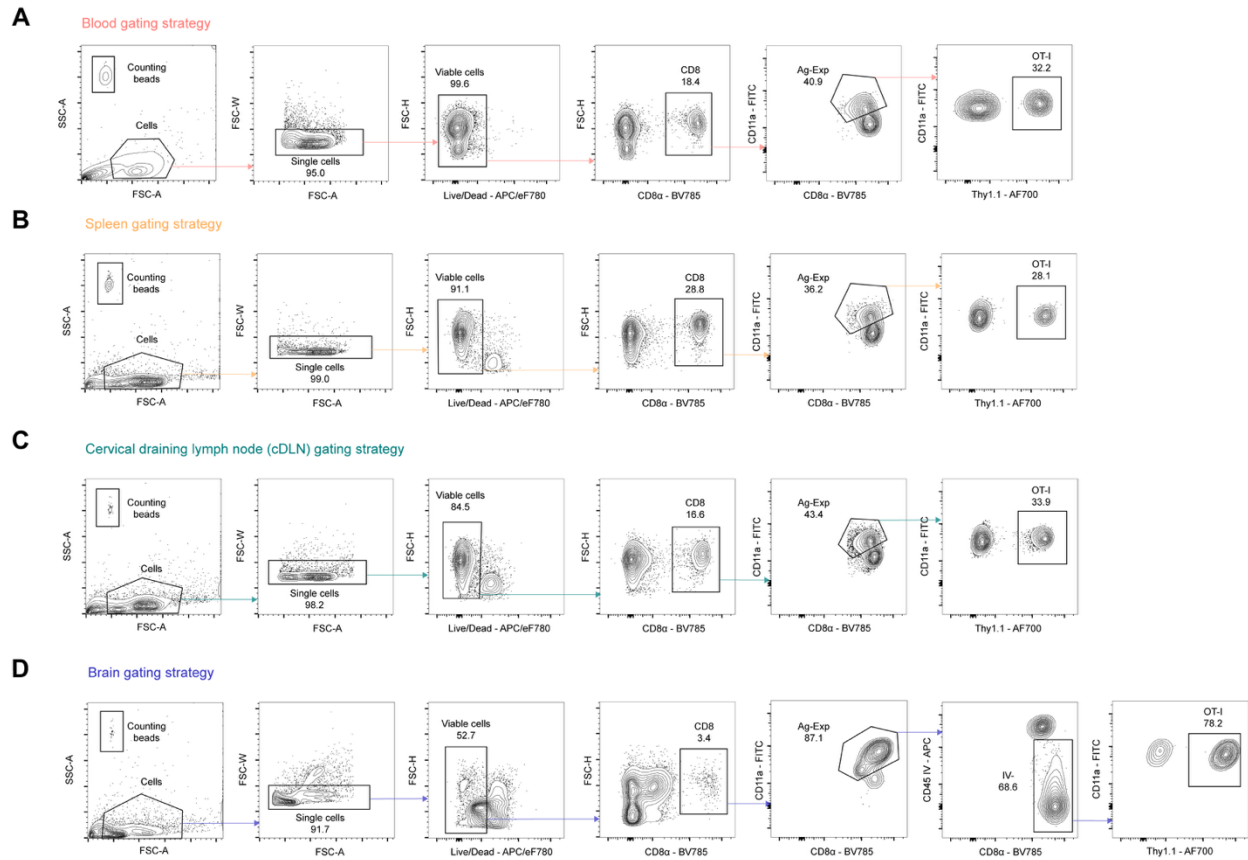
hosts. **(F)** Frequency of Ly6C<sup>+</sup> naïve CD8<sup>+</sup> T cells in the blood. **(G)** Representative gating of CD11b<sup>+</sup> myeloid cells in the blood and IV- brain of antibody treated hosts. **(H)** Number of neutrophils, dendritic cells, Ly6C<sup>-</sup> monocytes, and Ly6C<sup>+</sup> monocytes in the blood of antibody treated hosts. **(I)** Same as (H) but for IV- brain samples encompassing microglia populations. Experiments in **(A-I)** show concatenated data from 2 independent experiments with n=7-8 mice per group total dependent on tissue type. Statistical significance was determined by Student's t-test. Graphs show the mean  $\pm$  s.e.m. with each symbol representing one mouse. Individual *p* values are noted on respective graphs. Graphical illustrations were created using BioRender (<https://biorender.com>).



**Supplemental Figure 6. Tumor-specific brain T<sub>RM</sub> exhibit enhanced proliferative and cytolytic capacities acutely after tumor challenge.** (A) Uniform manifold approximation and projection (UMAP) representations of 45,000 downsampled IV- brain CD45<sup>int-hi</sup> cells per group concatenated from n=3 representative mice via flow cytometry 7 days after GL261-QUAD-Luc i.c. challenge. (B) Number of IV- Thy1.1<sup>+</sup> CD8<sup>+</sup> T cells in the brains of UbFlu-GP33 or UbMel-OVA vaccinated mice. (C) Representative histograms and (D) gMFI of Ki-67 expression among endogenous CD8<sup>+</sup> or Thy1.1<sup>+</sup> CD8<sup>+</sup> T cells in the cDLN or IV- brain of naïve or vaccinated mice. (E) Representative histograms and (F) gMFI of granzyme B (GzmB) expression among CD8<sup>+</sup> or Thy1.1<sup>+</sup> CD8<sup>+</sup> T cells in the IV- brain of naïve or vaccinated mice. Experiments in (A-F) show data from 1 independent experiment. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparison's test. Graphs show the mean ± s.e.m. with each symbol representing one mouse. Individual *p* values are noted on respective graphs.

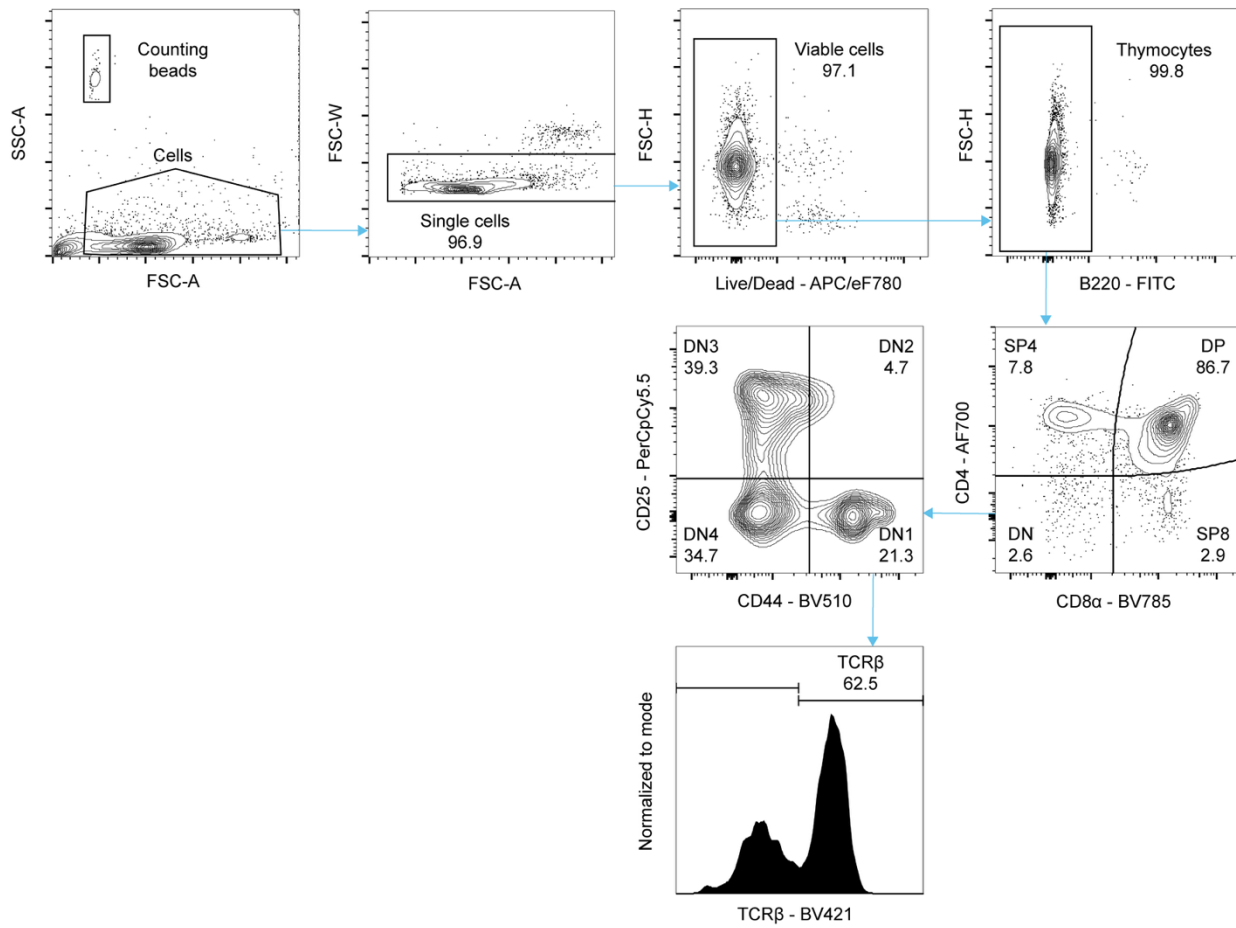
**A****B**

**Supplemental Figure 7. T<sub>RM</sub>-mediated protection against intracranial malignancy occurs independently of a peripheral T cell compartment.** (A) Experimental design. Thy1.2 TCR $\beta$ KO C57BL/6N mice were adoptively transferred with  $2 \times 10^4$  Thy1.1 P14 i.v. and immunized one day later with  $2.5 \mu\text{g}$  UbFlu-GP33 mRNA-LNP vaccine i.m. Mice were ipsilaterally boosted 28 days later. At 58 days after initial immunization, mice were treated i.p. with  $2 \mu\text{g}$  of isotype control or a-Thy1.1 antibody to deplete P14 T<sub>CIRC</sub>. After 65 days, mice were injected i.c. with B16-GP33 cells. (B) Kaplan-Meier survival curves of mice injected with B16-GP33 i.c. Experiments in (B) show data from 1 of 2 independent experiments with  $n=5-8$  mice. Statistical significance was determined by log-rank test for survival curves compared to naïve mice. Individual  $p$  values are summarized as: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Graphical illustrations were created using BioRender (<https://biorender.com>).



**Supplemental Figure 8. Representative gating strategy for CD8<sup>+</sup> T cell populations in tissues.** (A) Representative gating strategy for Ag-Exp CD8<sup>+</sup> T cells and/or TCR-tg CD8<sup>+</sup> T cells in the blood, (B) spleen, (C) cervical draining lymph nodes, and (D) IV- brain.

### Thymus gating strategy



**Supplemental Figure 9. Representative gating strategy for thymocytes.** Representative gating strategy for thymocyte characterization.

## Supplemental Tables.

**Supplemental Table 1. Key resource table.**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
BV785 anti-mouse CD8 $\alpha$ (53-6.7)	BioLegend	Cat #100750; RRID: AB_2562610
AlexaFluor700 anti-mouse CD90.1 (OX-7)	BioLegend	Cat #202528; RRID: AB_1626241
PerCPCy5.5 anti-mouse CD90.1 (OX-7)	BioLegend	Cat #202516; RRID: AB_961437
FITC anti-mouse CD90.1 (OX-7)	BioLegend	Cat #202504; RRID: AB_1595653
FITC anti-mouse CD11a (2D7)	BioLegend	Cat # 101106; RRID: AB_312779
BV510 anti-mouse CD11a (2D7)	BD Biosciences	Cat #740110; RRID: AB_2739868
PE anti-mouse V $\alpha$ 2 (B20.1)	BioLegend	Cat #127808; RRID: AB_1134183
APC anti-mouse CD45 (30-F11)	BioLegend	Cat #103112; RRID: AB_312977
Pacific Blue anti-mouse CD45 (30-F11)	BioLegend	Cat #103126; RRID: AB_493535
Pacific Blue anti-mouse CD45.2 (104)	BioLegend	Cat #109820; RRID: AB_492872
BV421 anti-mouse CD45.2 (104)	BioLegend	Cat #109832; RRID: AB_2565511
PE/CF594 anti-mouse CD69 (H1.2F3)	BD Biosciences	Cat #562455; RRID: AB_11154217
BB700 anti-mouse CD49a (Ha31/8)	BD Biosciences	Cat #742164; RRID: AB_2861198
PE anti-mouse CD103 (2E 7)	BioLegend	Cat #121406; RRID: AB_1133989
PECy7 anti-mouse CXCR6 (SA051D1)	BioLegend	Cat #151119; RRID: AB_2721670
BV421 anti-mouse PD-1 (29F.1A12)	BioLegend	Cat #135221; RRID: AB_2562568
PE anti-mouse PD-1 (29F.1A12)	BioLegend	Cat #135206; RRID: AB_1877231
BV510 anti-mouse CD62L (MEL-14)	BioLegend	Cat #104441; RRID: AB_2561537
PE anti-mouse CX3CR1 (SA011F11)	BioLegend	Cat #149006; RRID: AB_2564315
BV421 anti-mouse IFN- $\gamma$ (XMG1.2)	BioLegend	Cat #505830; RRID: AB_2563105
PECy7 anti-mouse TNF (MP6-XT22)	BioLegend	Cat #506324; RRID: AB_2256076
PE anti-mouse CD107a (1D4B)	BioLegend	Cat #121612; RRID: AB_2134487
AlexaFluor700 anti-mouse CD4 (GK1.5)	eBioscience	Cat #56-0041-82; RRID: AB_493999
FITC anti-mouse CD4 (H129.19)	BioLegend	Cat #130308; RRID: AB_1279237

BV510 anti-mouse B220 (RA3-6B2)	BioLegend	Cat #103248; RRID: AB_2650679
FITC anti-mouse B220 (RA3-6B2)	eBioscience	Cat #11-0452-82; RRID: AB_465054
AlexaFluor700 anti-mouse Ly6G (1A8)	BioLegend	Cat #127622; RRID: AB_10643269
PE anti-mouse Ly6C (HK1.4)	BioLegend	Cat #128008; RRID: AB_1186132
PerCpCy5.5 anti-mouse Ly6C (HK1.4)	BioLegend	Cat #128012; RRID: AB_1659241
PEdazzle594 anti-mouse CD11c (N418)	BioLegend	Cat #117347; RRID: AB_2563654
PECy7 anti-mouse CD11b (M1/70)	BioLegend	Cat #101216; RRID: AB_312799
PE anti-mouse NK1.1 (PK136)	BioLegend	Cat #108708; RRID: 108708
PE anti-mouse NKp46 (29A1.4)	BioLegend	Cat #137604; RRID: AB_2235755
BV510 anti-mouse CD44 (IM7)	BioLegend	Cat #103043; RRID: AB_2561391
PerCpCy5.5 anti-mouse CD25 (PC61)	BioLegend	Cat #102030; RRID: AB_893291
BV421 anti-mouse TCR $\beta$ (H57-597)	BioLegend	Cat #109230; RRID: AB_10933263
PE anti-mouse CD5 (53-7.3)	BioLegend	Cat #100608; RRID: AB_312737
PECy7 anti-mouse CD69 (H1.2F3)	BioLegend	Cat #104512; RRID: AB_493564
AF647 anti-mouse CD31 (MEC13.3)	BioLegend	Cat #102516; RRID: AB_2161029
APC anti-mouse Ki-67 (SolA15)	Thermo Fisher	Cat #17-5698-82; RRID: AB_2688057
FITC anti-mouse Granzyme B (GB11)	BioLegend	Cat #515403; RRID: AB_2114575
BV510 anti-mouse TIM-3 (5D12)	BD Biosciences	Cat #747625; RRID: AB_2744191
APC/eF780 fixable viability stain	BD Biosciences	Cat #565388; RRID: AB_2869673
H2-D <sup>p</sup> TRP <sub>1455-463</sub>	Harty lab	NA
H2-K <sup>p</sup> TRP <sub>2180-188</sub>	Harty lab	NA
H2-D <sup>p</sup> GP100 <sub>25-33</sub>	Harty lab	NA
H2-D <sup>p</sup> NP <sub>366-374</sub>	Harty lab	NA
H2-D <sup>p</sup> PA <sub>224-233</sub>	Harty lab	NA
H2-K <sup>p</sup> OVA <sub>257-264</sub>	Harty lab	NA
$\alpha$ -Thy1.1 (19E12)	BioXCell	Cat #BE0214; RRID: AB_2687700
$\alpha$ -GR-1 (NIMP-R14)	BioXCell	Cat #BE0320; RRID: AB_2819047
$\alpha$ -PD-L1 (10F.9G2)	BioXCell	Cat #BE0101; RRID: AB_10949073
<b>Bacterial and virus strains</b>		
<i>Listeria monocytogenes</i> expressing -OVA <sub>257-264</sub> , attenuated ( $\Delta$ actA, $\Delta$ inlB-deficient)	Harty lab	NA

<i>Listeria monocytogenes</i> expressing -GP <sub>33-41</sub> , attenuated ( $\Delta$ actA, $\Delta$ inlB-deficient)	Harty lab	NA
<i>Listeria monocytogenes</i> expressing -TRP <sub>2180-188</sub> , attenuated ( $\Delta$ actA, $\Delta$ inlB-deficient)	Harty lab	NA
<i>Listeria monocytogenes</i> expressing -PA <sub>224-233</sub> , attenuated ( $\Delta$ actA, $\Delta$ inlB-deficient)	Harty lab	NA
Lymphocytic choriomeningitis virus (LCMV) strain Armstrong	Harty lab	NA
<b>Chemicals, peptides, and recombinant proteins</b>		
ACK lysis buffer	Harty lab	NA
Vitalyse	CMDG	Cat #WBL0100
Collagenase D	Sigma Aldrich	Cat #11088866001
Collagenase II	Sigma Aldrich	Cat #17101015
Liver digestion buffer	Gibco	Cat #17703034
DNase I	Sigma Aldrich	Cat #D4513-1VL
Percoll	GE Healthcare	Cat #17-0891-01
Hepes	Gibco	Cat #15630080
DPBS	Gibco	Cat #14190144
RPMI	Gibco	Cat #11875093
DMEM	Gibco	Cat #11965092
HBSS	Gibco	Cat #14025092
FACS Buffer	Harty lab	NA
0.25% Trypsin-EDTA	Gibco	Cat #25200056
TrypLE™ Express Enzyme	Gibco	Cat #12604013
Nuclease-free water	Thermo Fisher	Cat #AM9937
Brefeldin A	BioLegend	Cat #420601
24.2G Fc Block	Harty lab	NA
FACS Buffer	Harty lab	NA
Cytofix Fixation Buffer	BD Bioscience	Cat #554655
Igopal	Sigma Aldrich	Cat #56741
Tryptic Soy Broth	BD Bioscience	Cat #BA-257107.06
Methylcellulose	Sigma Aldrich	Cat #M7027
Low melting point agarose	Promega	Cat #V2111
ProLong™ Gold Antifade Mountant with DAPI	Thermo Fisher	Cat #P36935
Lipopolysaccharide	Sigma Aldrich	Cat # L8274
Triton™ X-100	Sigma Aldrich	Cat #X100
VivoGlo™ D-luciferin	Promega	Cat #P1043
Ψ UTP	Thermo Fisher	Cat #N-1019-1
5-M CTP	Thermo Fisher	Cat #N-1014-1
LipidFlex lipid formulation	PreciGenome	Cat #PG-SYN-LF1ML
SM-102 cationic lipid	MedChemExpress	Cat #HY-134541
TRP <sub>1455-463</sub>	Global Peptide	TAPDNLGYM
TRP <sub>2180-188</sub>	Global Peptide	SVYDFFVWL
GP <sub>10025-33</sub>	Global Peptide	KVPRNQDWL
OVA <sub>257-264</sub>	Global Peptide	SIINFEKL
NP <sub>366-374</sub>	Global Peptide	ASNENMETM
PA <sub>224-233</sub>	Global Peptide	SSELENFRAYV
GP <sub>33-41</sub>	Global Peptide	KAVYNFATC
Streptavidin-APC	Thermo Fisher	Cat #S868

Streptavidin-PE	Thermo Fisher	Cat #S866
Streptavidin-FITC	Thermo Fisher	Cat #S869
Streptavidin-BV421	BioLegend	Cat #405225
<b>Critical commercial assays</b>		
CountBright™ Absolute Counting Beads, for flow cytometry	Thermo Fisher	Cat #C36950
FoxP3 / Transcription Factor Staining Kit	Tonbo / Cytex	Cat #SKU TNB-0607-KIT
CD11c Microbeads UltraPure	Miltenyi Biotec	Cat #130-125-835
ARCA T7 mRNA Synthesis Kit	NEB	Cat #E2065
RNeasy Mini Kit	Qiagen	Cat #74104
Quant-iT™ Ribogreen® Assay Kit	Thermo Fisher	Cat #R11490
<b>Experimental models: Cell lines</b>		
B16 F10 melanoma cell line	Harty lab	NA
B16-OVA F10 melanoma cell line	Lyse Norian lab (University of Alabama)	NA
B16-GP33 F10 melanoma cell line	Ryan Zander lab (University of Iowa)	NA
GL261-quad cassette-luciferase glioblastoma cell	Aaron Johnson lab (Mayo Clinic)	NA
<b>Experimental models: Organisms/strains</b>		
C57BL/6N	Charles River	000027
OT-I	Jackson Laboratories	003831
P14	Jackson Laboratories	037394
Thy1.1	Jackson Laboratories	000406
OT-I Thy1.1	Harty Lab	NA
P14 Thy1.1	Harty Lab	NA
TCRβKO	Jackson Laboratories / Butler Lab	002118
<b>Recombinant DNA</b>		
pMRNAxp mRNAExpress vector for in vitro transcription	System Bio	Cat #MR000PA-1
<b>Software and algorithms</b>		
FlowJo v10.10.1 (with Downsample and UMAP plug-ins)	FlowJo, LLC	<a href="https://www.flowjo.com/solutions/flowjo/">https://www.flowjo.com/solutions/flowjo/</a>
Xenogen Living Image software	Caliper Life Sciences	<a href="https://www.revvyty.com/product/spectrum-200-living-image-v4series-1-128113">https://www.revvyty.com/product/spectrum-200-living-image-v4series-1-128113</a>
Adobe Illustrator v24.0.1	Adobe	<a href="https://www.adobe.com/products/illustrator.html">https://www.adobe.com/products/illustrator.html</a>
Prism 10.1.1	GraphPad Software	<a href="https://www.graphpad.com/features">https://www.graphpad.com/features</a>
<b>Other</b>		
70 μm Filters	Thermo Fisher	Cat #22-363-548
Ethicon Perma-Hand Silk Suture, Size 4-0, 18"	Thermo Fisher	Cat #683G
Ethicon Coated Vicryl Suture, Size 5-0, 18"	Thermo Fisher	Cat #J493G
Vetbond	3M	Cat #B00016067
Hamilton 25 μL Microliter Syringe Model 702 N, Cemented Needle, 22s gauge, 2 in, point style 2	Hamilton	Cat #80400

BD 1 mL Micro-Fine™ IV Insulin Syringe	Thermo Fisher	Cat #14-829-1A
BD 1 mL TB Syringe	Thermo Fisher	Cat #309623
Vernier calipers	Thermo Fisher	Cat #S90343

**Supplemental Table 2. mRNA vaccine amino acid and coding sequences.**

<b>Vaccine components</b>	
Coding sequences	<p>Kozak sequence: <b>GCCACC</b></p> <p>Ub-A76:  <b>ATGCAGATCTTCGTGAAGACCCTGACCGGCAAGACCATCACCCCTGGAG  GTGGAGCCCAGTGACACCATCGAGAACGTGAAGGCCAAGATCCAGGA  TAAAGAGGGCATCCCCCTGACCAGCAGAGGCTGATCTTTGCCGGCA  AGCAGCTGGAAGATGGCCGCACCCTCTCTGATTACAACATCCAGAAAG  AGTCAACCCTGCACCTGGTCCTCCGTCTGAGGGGTGCC</b></p> <p>Flexible linker: <b>GTAGGAAAAGGTGGTTCAGGAGGC</b></p> <p>Proteasome recognition spacers: GCTGCTTAT</p> <p>TRP1<sub>455-463</sub>: <b>ACTGCTCCAGACAACCTGGGATACATG</b></p> <p>TRP2<sub>180-188</sub>: <b>AGTGTTTATGATTTTTTTGTGTGGCTC</b></p> <p>GP100<sub>25-33</sub>: <b>AAAGTACCCAGAAACCAGGACTGGCTT</b></p> <p>OVA<sub>257-264</sub>: <b>AGTATAATCAACTTTGAAAACTG</b></p> <p>NP<sub>366-374</sub>: <b>GCTTCCAATGAAAATATGGAGACTATG</b></p> <p>PA<sub>224-233</sub>: <b>TCCAGCCTTGAAAATTTAGAGCCTATGTG</b></p> <p>GP<sub>33-41</sub>: <b>AAAGCTGTGTACAATTCGCCACCATG</b></p>
Amino acid sequence	<p>Ub-A76:  <b>MQIFVKLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDG  RTLSDYNIQKESTLHLVLRLRGA</b></p> <p>Flexible linker: <b>VGKGGSGG</b></p> <p>Proteasome recognition spacers: AAY</p> <p>TRP1<sub>455-463</sub>: <b>TAPDNLGYM</b></p> <p>TRP2<sub>180-188</sub>: <b>SVYDFVWL</b></p> <p>GP100<sub>25-33</sub>: <b>KVPRNQDWL</b></p> <p>OVA<sub>257-264</sub>: <b>SIINFEKL</b></p> <p>NP<sub>366-374</sub>: <b>ASNENMETM</b></p> <p>PA<sub>224-233</sub>: <b>SSELENFRAYV</b></p> <p>GP<sub>33-41</sub>: <b>KAVYNFATM</b></p>
<b>UbMel-OVA vaccine</b>	
Coding sequence	<p><b>GCCACCATGCAGATCTTCGTGAAGACCCTGACCGGCAAGACCATCACCC  CTGGAGGTGGAGCCCAGTGACACCATCGAGAACGTGAAGGCCAAGAT  CCAGGATAAAGAGGGCATCCCCCTGACCAGCAGAGGCTGATCTTTG  CCGGCAAGCAGCTGGAAGATGGCCGCACCCTCTCTGATTACAACATCC  AGAAAGAGTCAACCCTGCACCTGGTCCTCCGTCTGAGGGGTGCCGTA  GGAAAAGGTGGTTCAGGAGGCGCTGCTTATACTGCTCCAGACAACCTG  GGATACATGGCTGCTTATAGTGTGTTTATGATTTTTTTGTGTGGCTCGCTG  CTTATAAAGTACCCAGAAACCAGGACTGGCTTGTGCTTATAGTATAAT  CAACTTTGAAAACTGGCTGCTTATTAG</b></p>

Amino acid sequence	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDG RTLSDYNIQKESTLHLVLRRLRGAVGKGGSGAAYTAPDNLGYMAAYSVD FFVWLAAYKVPRNQDWLAAYSIINFEKLAAY
<b>UbFlu-GP33 vaccine</b>	
Coding sequence	GCCACCATGCAGATCTTCGTGAAGACCCTGACCGGCAAGACCATCACC CTGGAGGTGGAGCCCAGTGACACCATCGAGAACGTGAAGGCCAAGAT CCAGGATAAAGAGGGGCATCCCCCTGACCAGCAGAGGCTGATCTTTG CCGGCAAGCAGCTGGAAGATGGCCGCACCCTCTCTGATTACAACATCC AGAAAGAGTCAACCCTGCACCTGGTCCTCCGTCTGAGGGGTGCCGTA GGAAAAGGTGGTTCAGGAGGCGCTGCTTATGCTTCCAATGAAAATATG GAGACTATGGCTGCTTATTCCAGCCTTGAAAATTTAGAGCCTATGTG GCTGCTTATAAAGCTGTGTACAATTCGCCACCATGGCTGCTTATTAG
Amino acid sequence	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDG RTLSDYNIQKESTLHLVLRRLRGAVGKGGSGAAYASNENMETMAAYSSLE NFRAYVAAYKAVYNFATMAAY