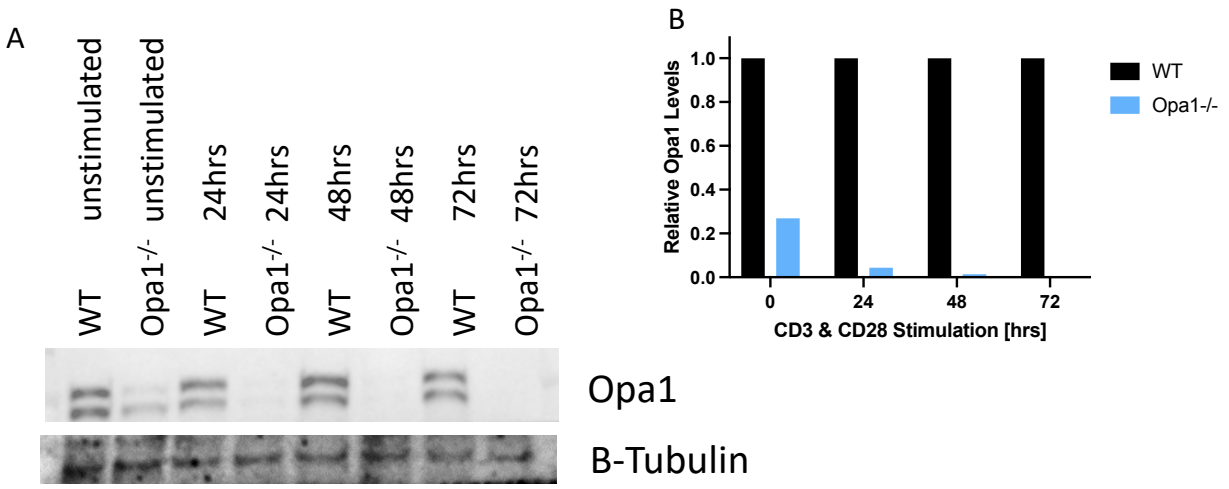
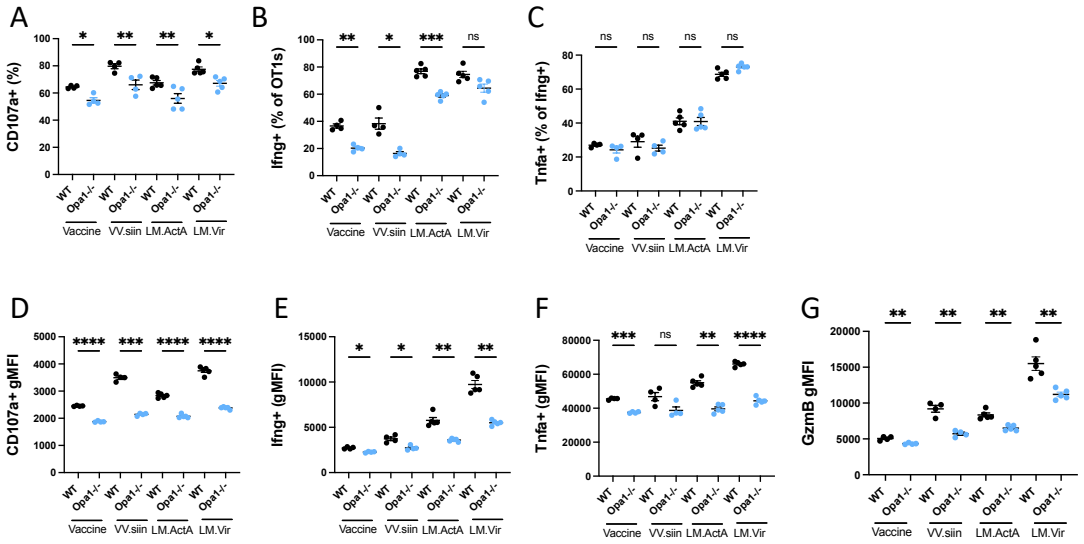


Supplemental Figure 1. Protein confirmation of Opa1 deletion

A) WT or Opa1$^{-/-}$ OT1s were isolated from B6 or mixed B6x129 background mice and stimulated with aCD3 (plate-bound; 10ug/mL) and aCD28 (2ug/mL) for indicated durations at 37C. Protein was isolated from 2-3 mice for each condition.
B) Protein quantified by densitometry of bands in (A)



Supplemental Figure 2. Opa1-deficient T cells are functionally impaired during the primary responses to infection and vaccination

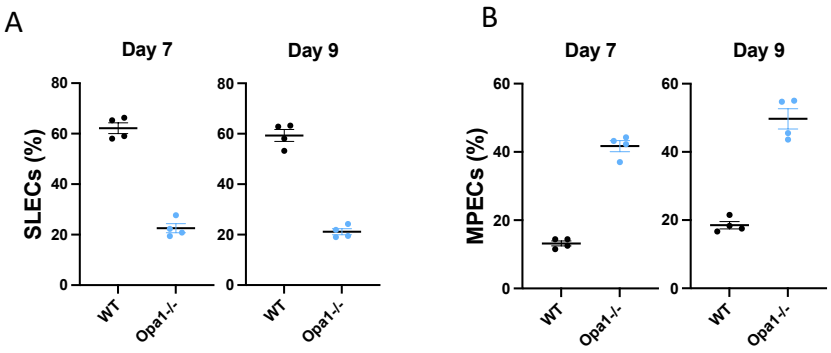
As in Figure 1, 500 WT and 500 Opa1$^{-/-}$ OT1s were transferred into mice followed by either vaccination or infection with VV.Siin, LM.ActA, or LM.Vir. Seven days following infectious or vaccine challenge, 4x10^{6} splenocytes were incubated in the presence of SIINFEKL, Brefeldin A, and aCD107a-APC for 6 hours at 37C.

A-C) The frequency of CD107a+ (A), Ifng+ (B), or Tnfa+ Ifng+ (C) cells.

D-G) gMFI of positive population for CD107a (D), Ifng (E), Tnfa (G), and Gzmb (G).

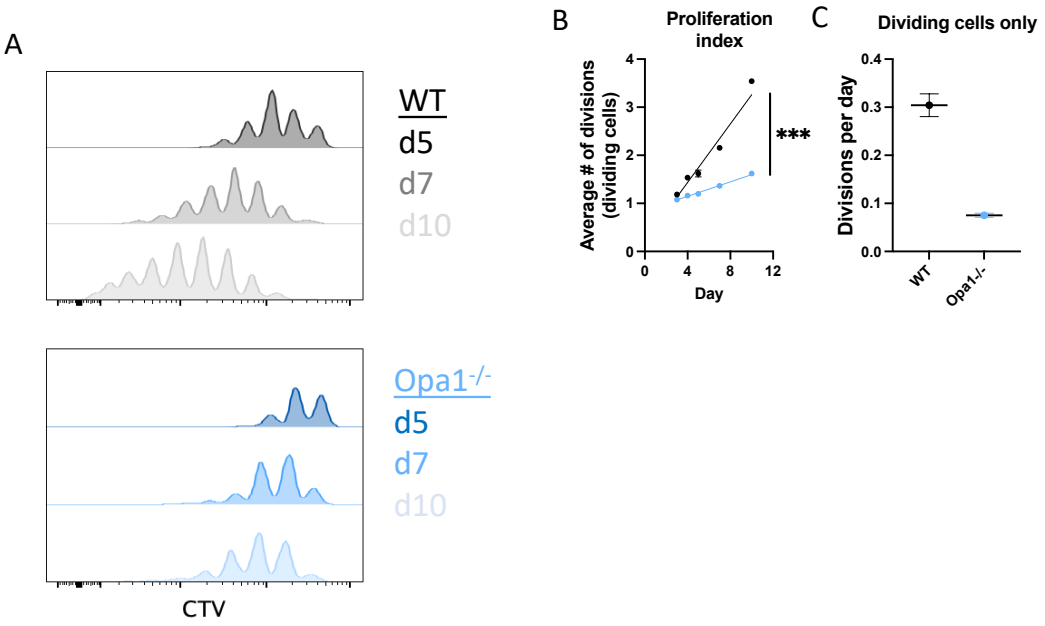
Data shown are means +/- SEM; n >= 4 mice per group, representative of 2-3 experiments. Significance was defined by two-tailed paired Student's t tests where *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

All experiments used B6 donor and recipient mice



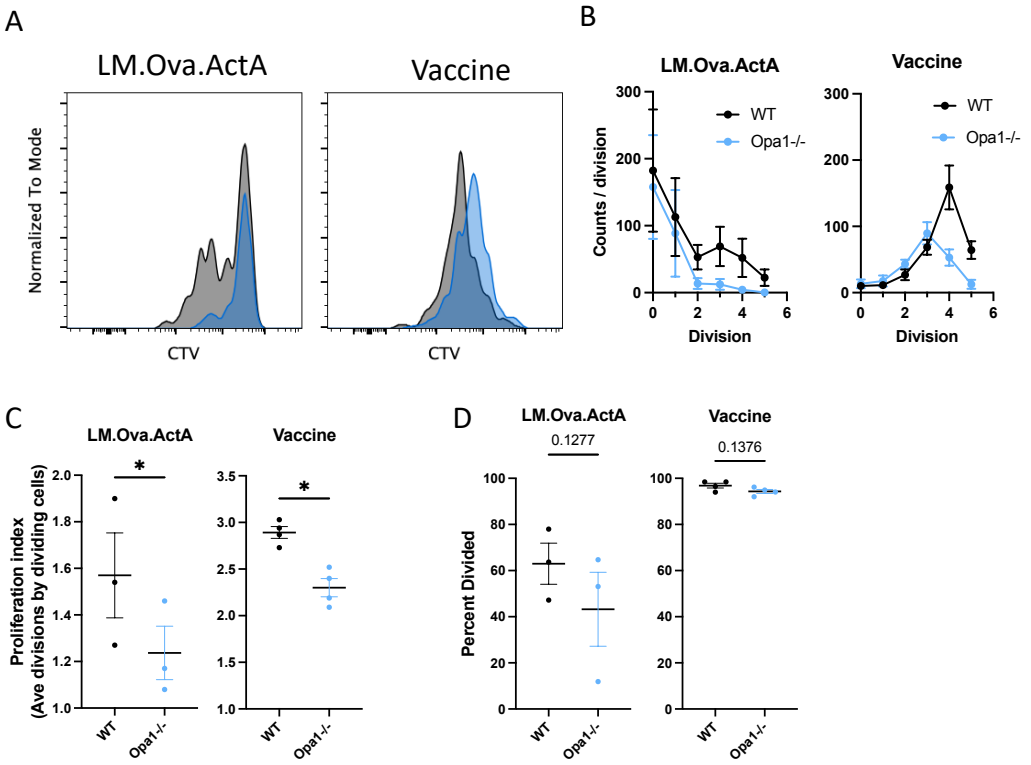
Supplemental Figure 3. Delayed peak does not impact effector and memory precursor subsets in Opa1$^{-/-}$ CD8 T cells

SLEC (A) and MPEC (B) frequencies of transferred OT1 T cells in the blood at either 7 or 9 days post LM.Ova.ActA infection.



Supplemental Figure 4. Proliferation modeling of lymphopenia-induced proliferation in WT and Opa1$^{-/-}$ OT1s

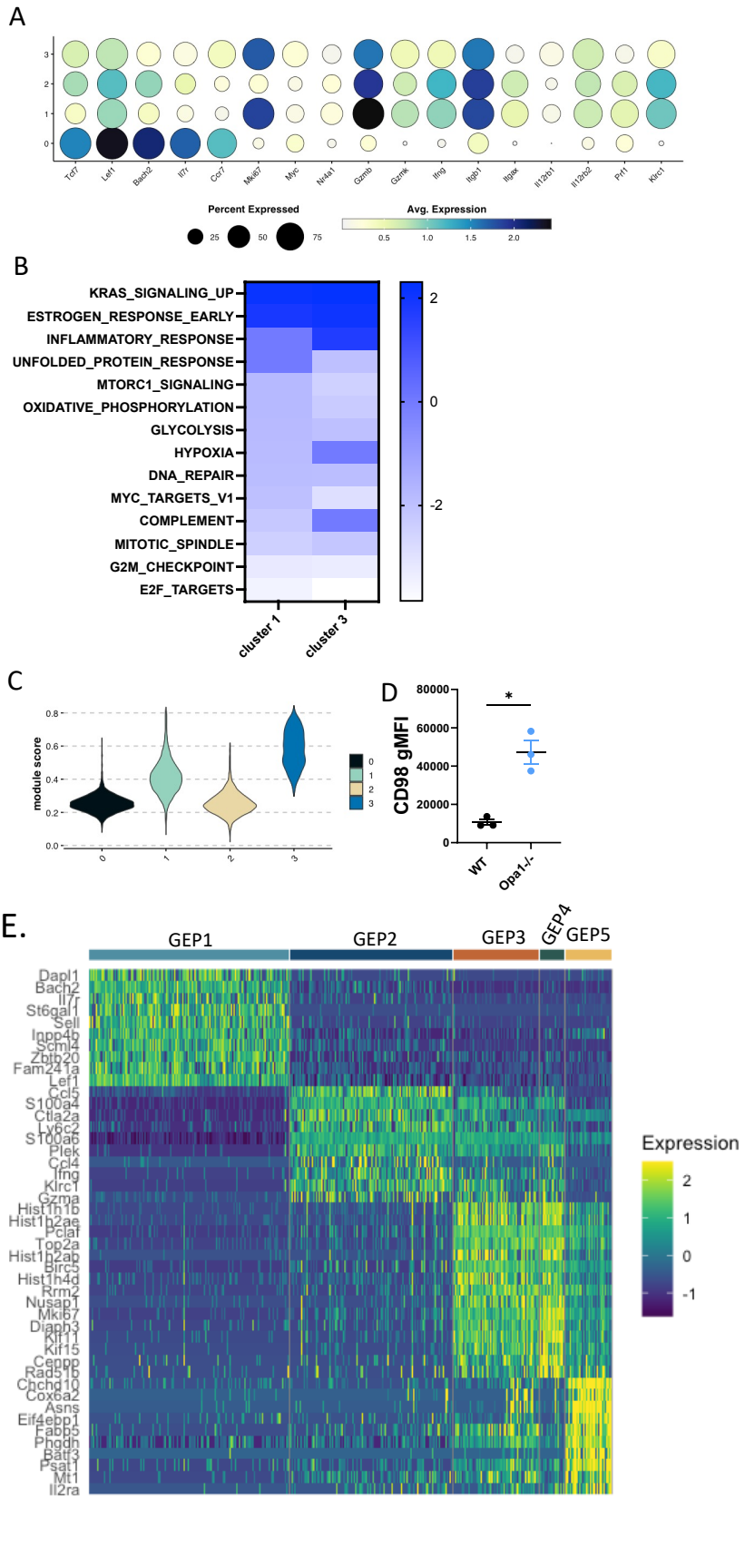
Mice were sub-lethally irradiated (600 rads) followed by co-transfer of 10^5 CTV-labeled WT and Opa1$^{-/-}$ OT1s. Splenocytes were harvested and analyzed by flow cytometry at indicated time points.
A) Representative flow plots of CTV for WT and Opa1$^{-/-}$ cells at various time points following
B) Average number of divisions completed by dividing cells at each day
C) Average divisions per day for cells in (B)



Supplemental Figure 5. Delayed kinetics of Opa1$^{-/-}$ response 48 hours after infection and vaccination
5x10^5 CTV-labeled WT and Opa1$^{-/-}$ OT1s were transferred into mice followed by LM.ActA infection or vaccination and analyzed by flow cytometry 48 hours later.

A) Representative flow plots of CTV for WT and Opa1$^{-/-}$ cells for each immune response.
B) Cells quantified per division for each response.
C) Average number of divisions by dividing cells .
D) Percent of the original population that has divided at least once.

Data shown are means +/- SEM; n >= 3 mice per group, representative of 2 experiments using B6 donors and recipients. Significance was defined by two-tailed paired Student's t tests where *p < 0.05.


A
Tcf7
Lef1
Bach2
Il7r
Ccr7
Mki67
Myc
Nr4a1
Gzmb
Gzmk
Ifng
Itgb1
Itgax
Il12rb1
Il12rb2
Prf1
Klrc1
Percent Expressed
25
50
75
Avg. Expression
0.5
1.0
1.5
2.0
B
KRAS_SIGNALING_UP
ESTROGEN_RESPONSE_EARLY
INFLAMMATORY_RESPONSE
UNFOLDED_PROTEIN_RESPONSE
MTORC1_SIGNALING
OXIDATIVE_PHOSPHORYLATION
GLYCOLYSIS
HYPOXIA
DNA_REPAIR
MYC_TARGETS_V1
COMPLEMENT
MITOTIC_SPINDLE
G2M_CHECKPOINT
E2F_TARGETS
cluster 1
cluster 3
2
0
-2
C
module score
0.8
0.6
0.4
0.2
0.0
0
1
2
3
D
CD98 gMFI
80000
60000
40000
20000
0
*
WT
Opa1-/-
E.
GEP1
GEP2
GEP3
GEP4
GEP5
Dapl1
Bach2
Il7r
St6gal1
Sell
Inpp4b
Scml4
Zbtb20
Fam241a
Lef1
Ccl5
S100a4
Ctla2a
Ly6c2
S100a6
Plek
Ccl4
Ifng
Klrc1
Gzma
Hist1h1b
Hist1h2ae
Pclaf
Top2a
Hist1h2ap
Birc5
Hist1h4d
Rrm2
Nusap1
Mki67
Diaph3
Kif11
Kif15
Cenpp
Rad51b
Chchd10
Cox6a2
Asns
Eif4ebp1
Fabp5
Phgdh
Batf3
Psat1
Mt1
Il2ra
Expression
2
1
0
-1

Supplemental Figure 6. Cluster defining genes in scRNAseq analysis

A) DotPlot showing the differentially expressed genes in each cluster. Size of dot is indicative of the percent of cells in the cluster expressing the gene while the color indicates the average expression.
B) fGSEA on ranked genes for clusters 1 and 3 using gene sets in the MSigDB Hallmark Gene series (mh.all.v2023)
C) Violin plots of module scores per cluster, derived from Hallmark Myc V1 and V2 gene sets.
D) cell surface CD98 expression on WT and Opa1-/- T cells 5 days post LM.ActA infection.
E) Frequency of cells expressing the top 10 genes expressed per GEP.