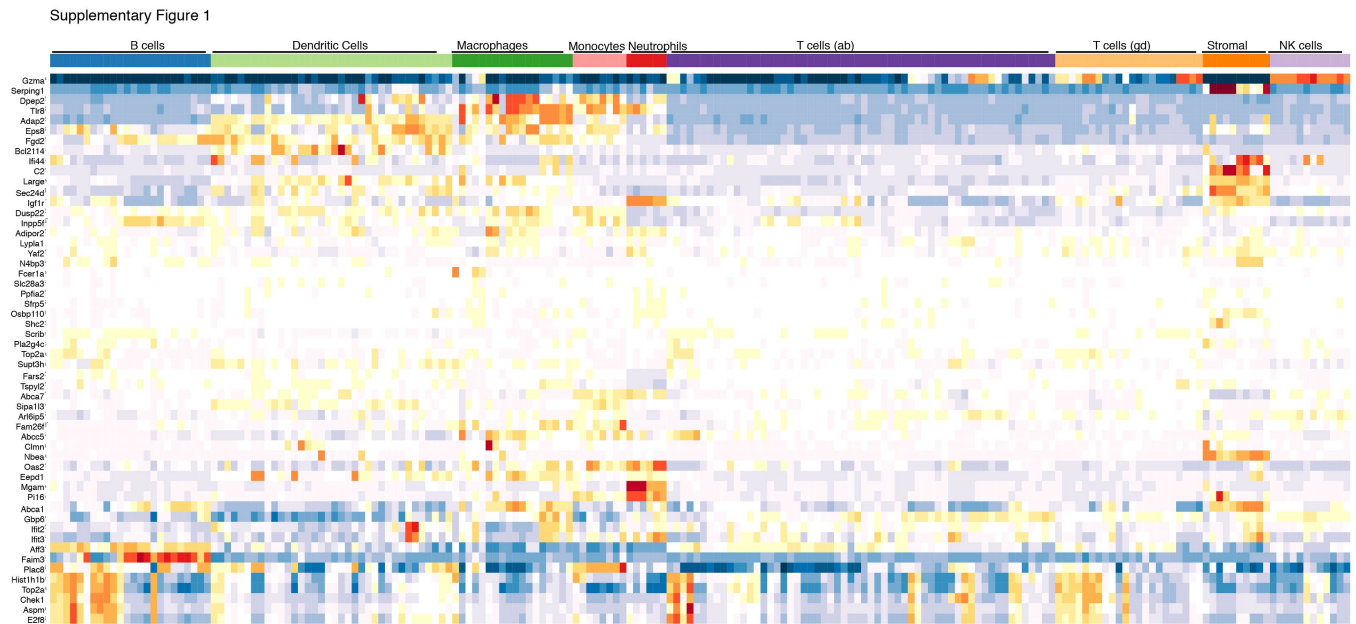


1 **Transcriptomic analysis reveals a previously unknown role for CD8<sup>+</sup> T-**  
2 **cells in rVSV-EBOV mediated protection**

3 Andrea Rivera<sup>1</sup>, Suhas Sureshchandra<sup>2</sup>, Andrea Marzi<sup>3</sup>, Heinz Feldmann<sup>3</sup>, Ilhem  
4 Messaoudi<sup>4\*</sup>

5

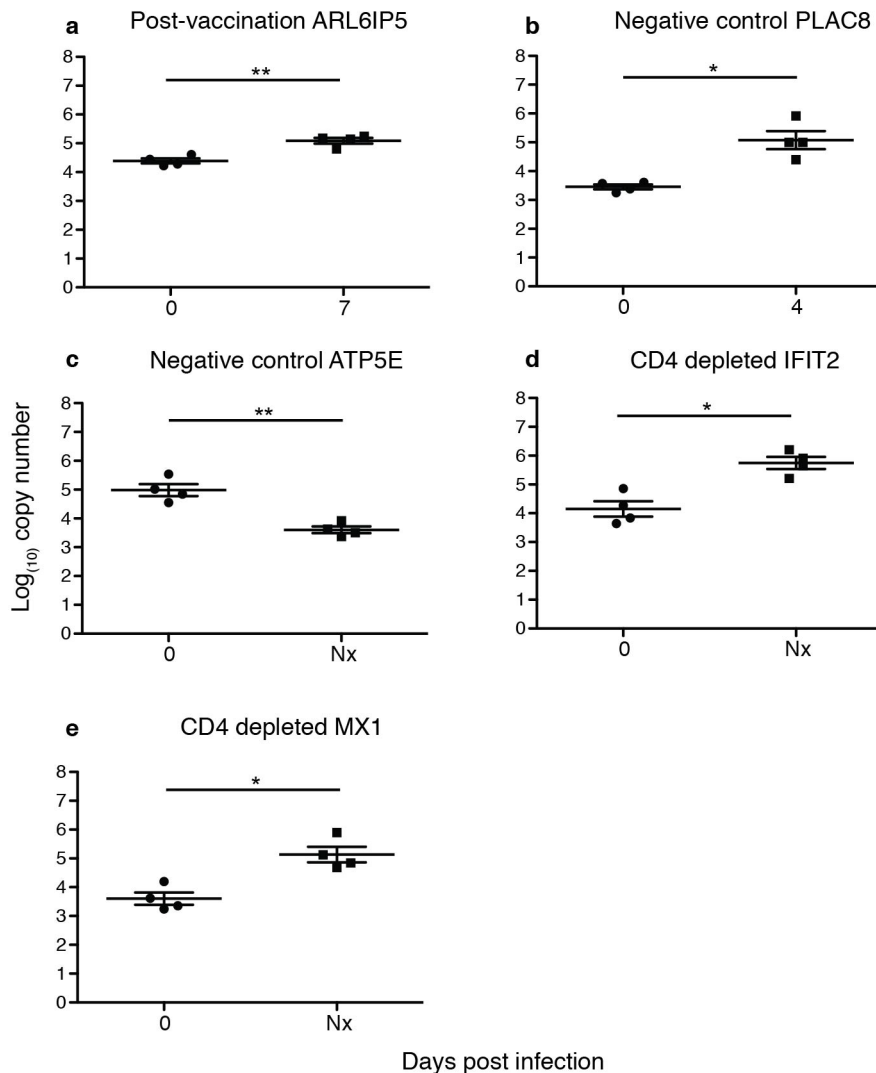
6 **Supplementary Information**



8 **Supplementary Figure 1. ImmGen analysis of DEGs 7 days post vaccination.**

9 Heatmap profile showing expression profile of genes differentially expressed after rVSV-  
10 EBOV vaccination across immune cell populations as predicted by ImmGen's  
11 MyGeneSet application. Each row designates a gene and corresponding microarray ID  
12 while each column represents a study. Red indicates high likelihood of expression while  
13 blue indicates low likelihood of expression within a given immune cell subset.

Supplementary Figure 2



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2 **Supplementary Figure 2. qRT-PCR validation of DEGs post-challenge.** qRT-PCR

3 expression levels of (a) ARL6IP5 in animals 7 days post vaccination with rVSV-EBOV-

4 GP; t-value=8.284; degrees of freedom (df) = 3. (b) PLAC8 in negative controls 4 dpi; t-

5 value=4.550; df=3. (c) ATP5E in negative controls at necropsy; t-value=6.299; df=3. (d)

6 IFIT2 in CD4-depleted animals at necropsy; t-value=3.341; df=3. (e) MX1 in CD4-

7 depleted animals at necropsy are shown; t-value=3.641; df=3. Expression of mRNA was

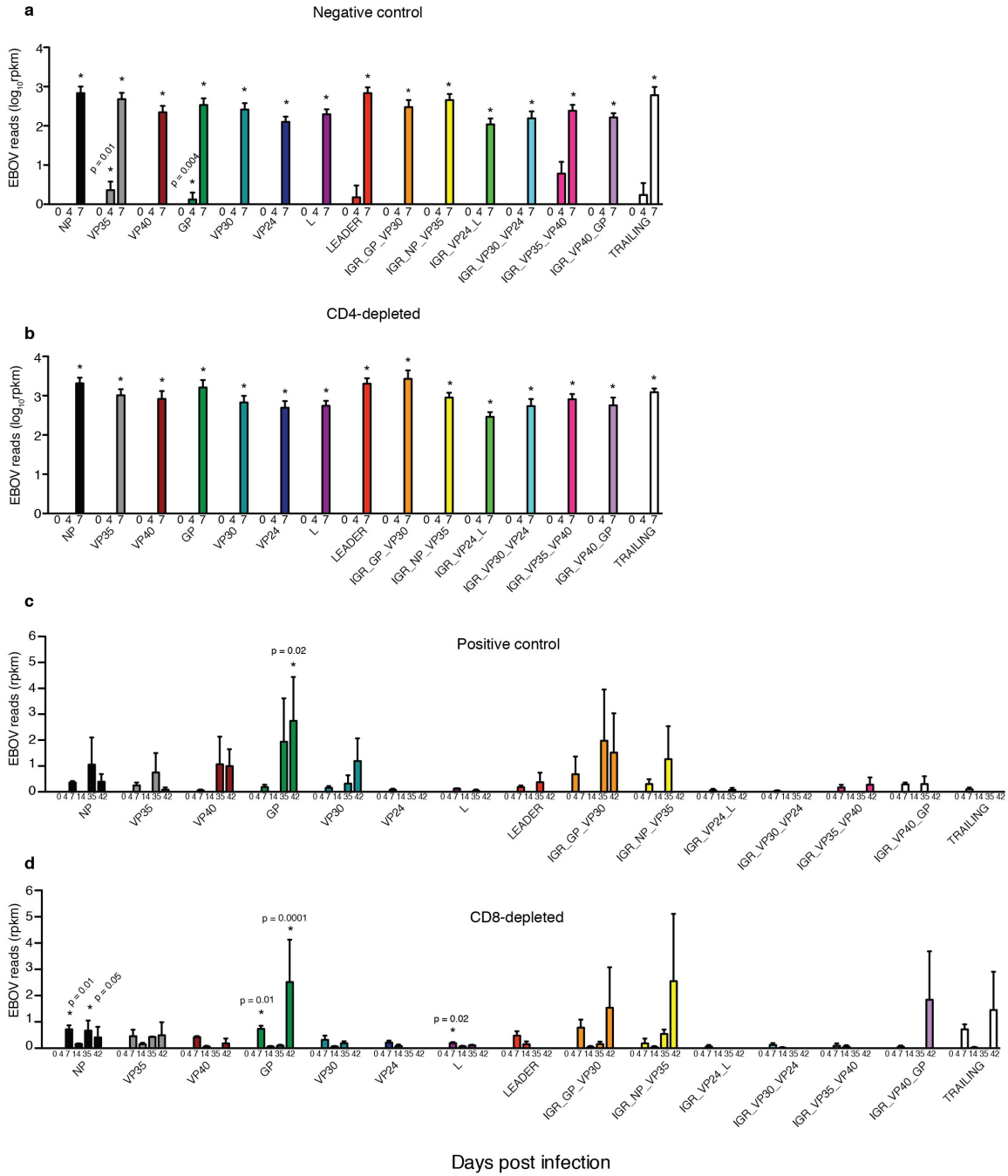
8 normalized to expression of RPL32. Changes in gene expression post infection were

1 determined using a paired two-sided t-test (n=4 in each group). Means  $\pm$  are shown. \*, P

2 < 0.05, \*\*, P < 0.01, \*\*\*, P < 0.001, \*\*\*\*, P < 0.0001).

3

Supplementary Figure 3



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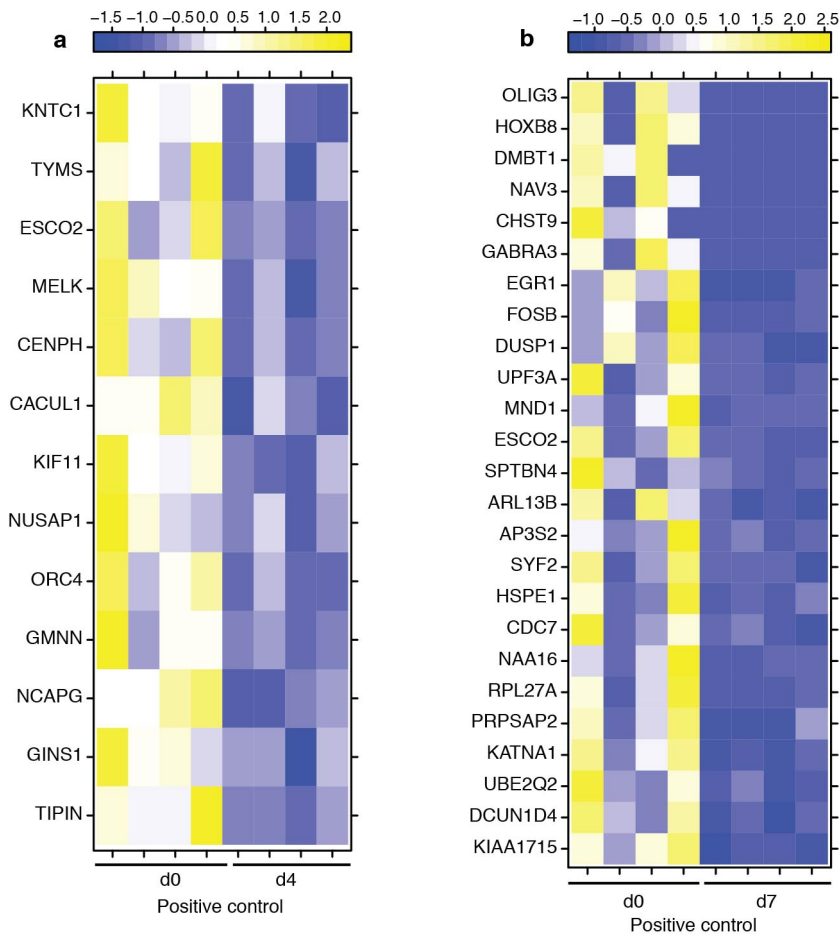
3 **Supplementary Figure 3. EBOV reads mapping to open reading frames and**

4 **intergenic regions. RPKM normalized RNA-Seq reads mapping to each EBOV open**

1 reading frame (ORF), intergenic region (IGR), as well as leader and trailing sequences at  
2 each time point in (a) negative controls, (b) CD4-depleted animals, (c) Positive controls  
3 and (d) CD8 depleted animals; n=4 in all groups. FDR adjusted p-Value was obtained  
4 following *EdgeR* analysis. In both negative control and CD4-depleted animals, (\*)  
5 denotes a p-Value < 0.0001.

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Supplementary Figure 4



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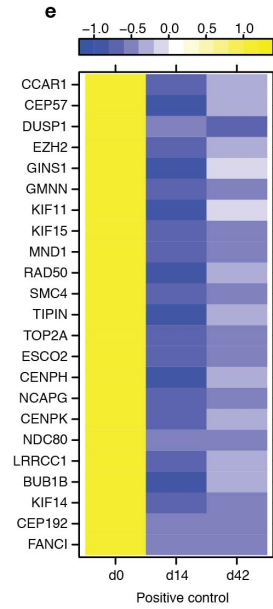
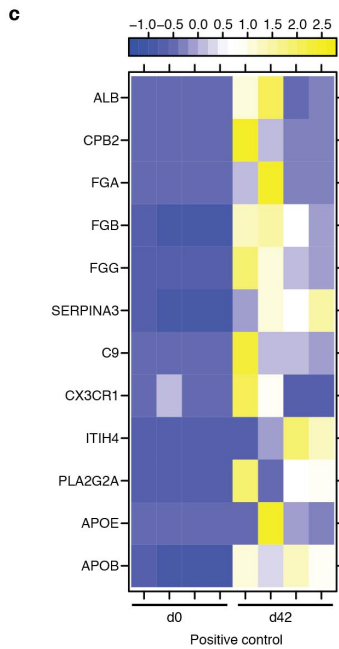
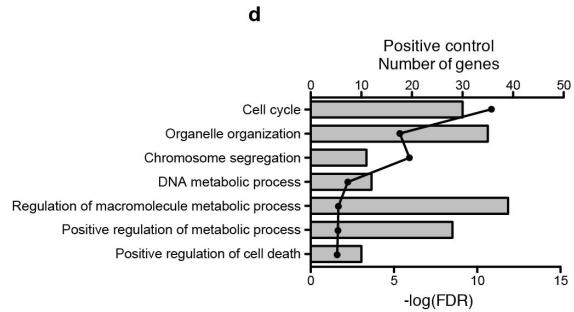
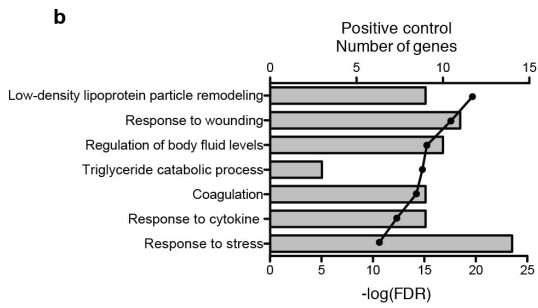
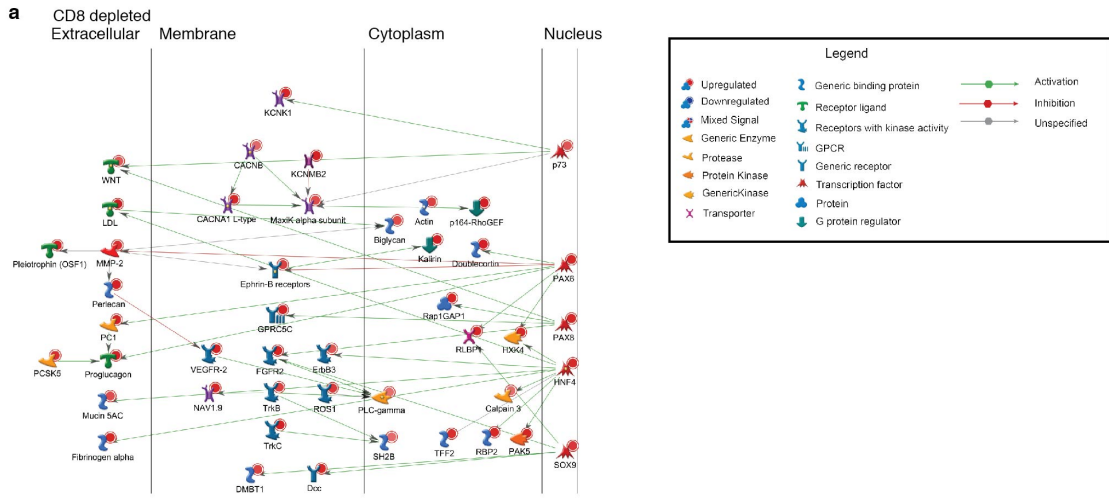
3 **Supplementary Figure 4. Downregulated genes in positive control animals. (a)**

4 Heatmap of genes downregulated 4 dpi in positive control animals that map to “Mitotic

5 Cell Cycle Process”; each column represents 1 animal in the positive control group. **(b)**

6 Heatmap of all 25 genes downregulated 7 dpi in vaccinated animals.

Supplementary Figure 5



1 **Supplementary Figure 5. DEGs upregulated 35 dpi in CD8-depleted animals and 42**  
2 **dpi in positive control animals.** (a) Network depicting direct interactions of genes  
3 upregulated 35 dpi in CD8-depleted animals that map to “Signaling”. (b) Bar graph  
4 depicting the most statistically significant GO terms enriched among up-regulated genes  
5 positive control animals 42 dpi (c) Heatmap of genes upregulated 42 dpi in positive  
6 control animals mapping to “Response to Cytokine” and “Coagulation”; each column  
7 represents 1 animal in the positive control group. (d) Bar graph depicting the most  
8 statistically significant GO terms enriched among down-regulated genes positive control  
9 animals 42 dpi. (e) Heatmap of down-regulated genes 42 dpi in positive control animals  
10 mapping to “Cell Cycle”; each column shows median transcript RPKM counts of all  
11 animals in the positive control group at each day.  
12



1 **Supplementary Table 1. Composition of DEGs**  
 2

<b>Up-regulated 4 dpi</b>				
<b>Category</b>	<b>Negative control</b>	<b>CD4-depleted</b>	<b>Positive control</b>	<b>CD8-depleted</b>
Human homolog	552	115	1	8
ncRNA	97	2	0	0
uncharacterized	74	9	1	9
<b>Down-regulated 4 dpi</b>				
<b>Category</b>	<b>Negative control</b>	<b>CD4-depleted</b>	<b>Positive control</b>	<b>CD8-depleted</b>
Human homolog	950	307	37	2
ncRNA	13	5	63	1
uncharacterized	224	68	12	1
<b>Up-regulated 7 dpi</b>				
<b>Category</b>	<b>Negative control</b>	<b>CD4-depleted</b>	<b>Positive control</b>	<b>CD8-depleted</b>
Human homolog	1013	2074	49	174
ncRNA	36	26	3	32
uncharacterized	444	361	11	137
<b>Down-regulated 7 dpi</b>				
<b>Category</b>	<b>Negative control</b>	<b>CD4-depleted</b>	<b>Positive control</b>	<b>CD8-depleted</b>
Human homolog	814	1174	25	83
ncRNA	29	79	76	6
uncharacterized	246	365	31	52
<b>Up-regulated 14 dpi</b>				
<b>Category</b>	<b>Positive control</b>		<b>CD8-depleted</b>	
Human homolog	32		54	
ncRNA	3		15	
uncharacterized	4		8	
<b>Down-regulated 14 dpi</b>				
<b>Category</b>	<b>Positive control</b>		<b>CD8-depleted</b>	
Human homolog	295		179	
ncRNA	72		14	
uncharacterized	123		83	
<b>Up-regulated 35 dpi</b>				
<b>Category</b>	<b>Positive control</b>		<b>CD8-depleted</b>	
Human homolog	1		502	
ncRNA	0		31	
uncharacterized	0		305	
<b>Down-regulated 35 dpi</b>				
<b>Category</b>	<b>Positive control</b>		<b>CD8-depleted</b>	
Human homolog	0		536	
ncRNA	0		7	

uncharacterized	2	101
<b>Up-regulated 42 dpi</b>		
<b>Category</b>	<b>Positive control</b>	<b>CD8-depleted</b>
Human homolog	18	117
ncRNA	2	7
uncharacterized	5	27
<b>Down-regulated 42 dpi</b>		
<b>Category</b>	<b>Positive control</b>	<b>CD8-depleted</b>
Human homolog	84	352
ncRNA	15	87
uncharacterized	170	136

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**Supplementary Table 2.** Gene ontology enrichment of positive control animals 14 dpi

DPI	Up-regulated	# DEG	FDR adjusted pValue	Down-regulated	# DEG	FDR adjusted pValue
14	Glycogen catabolic process	2	6.959E-02	Cell cycle	77	1.445E-21
	Regulation of metabolic process	19	6.959E-02	Organelle organization	103	2.529E-14
	Biological regulation	25	2.927E+00	Chromosome segregation	24	1.534E-11
	Positive regulation of metabolic process	12	1.010E-01	Cellular macromolecule metabolic process	159	1.753E-11
	System development	13	1.306E-01	Metabolic process	197	3.395E-07
	Regulation of macromolecule metabolic process	15	1.306E-01	Viral transcription	12	2.191E-06

1 **Supplementary Table 3.** Gene ontology enrichment of CD8-depleted animals 14 and 35  
 2 dpi

DPI	Up-regulated	# DEG	FDR adjusted pValue	Down-regulated	# DEG	FDR adjusted pValue
14	Positive regulation of macrophage derived foam cell differentiation	7	5.404E-09	Cell cycle	37	3.341E-06
	Triglyceride catabolic process	7	1.205E-07	Organelle organization	59	3.341E-06
	Regulation of body fluid levels	14	1.060E-05	Chromosome organization	27	2.678E-05
	Coagulation	12	1.365E-05	DNA metabolic process	23	1.059E-04
	Lymphocyte activation	11	1.365E-05	RNA processing	19	1.866E-03
	Response to virus	8	1.439E-04	Macromolecule metabolic process	92	3.134E-03
	Response to wounding	13	1.447E-04	Cellular protein localization	26	3.228E-03
	Immune system process	21	6.011E-04	Cellular response to stress	33	3.260E-03
35	System development	236	4.277E-23	Organelle organization	213	3.511E-37
	Cell development	127	5.278E-18	Cell cycle	132	1.852E-34
	Regulation of localization	149	5.168E-17	Cellular macromolecule metabolic process	306	2.859E-23
	Cell differentiation	183	4.963E-14	Cell division	62	2.097E-21
	Regulation of ion transport	61	1.063E-13	Chromosome organization	84	1.183E-18
	Single-organism process	437	1.345E-13	Cellular metabolic process	332	3.671E-14
	Circulatory system development	71	9.951E-12	Protein metabolic process	201	8.241E-13
	Signaling	231	4.958E-10	Cellular response to stress	107	5.467E-12
	Chemotaxis	59	1.851E-09	DNA metabolic process	57	1.988E-09

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