

Supplementary Figure 1. Anti-viral effector CTL demonstrate unique miRNA profiles after *in vivo* differentiation (A) Heatmap of miRNA expression levels as assessed by miRNA microarray in sorted CD4⁺CD62L⁺ naïve and day 9 acute influenza virus infection effector OT-I T cells is shown. The 50 most upregulated and 50 most downregulated miRNA are represented relative to the total average expression for the given miRNA. (B) Venn diagram of mature miRNAs present exclusively or shared between naïve and effector OT-I CD8⁺ T cells. (C) Table of the 30 most upregulated and (D) 30 most downregulated miRNA relative to naïve expression levels in effector CTL.

Supplementary Figure 2

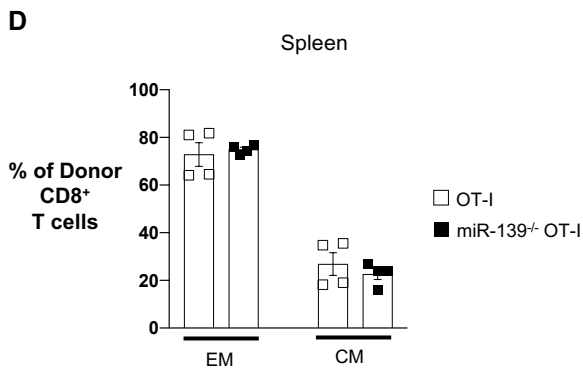
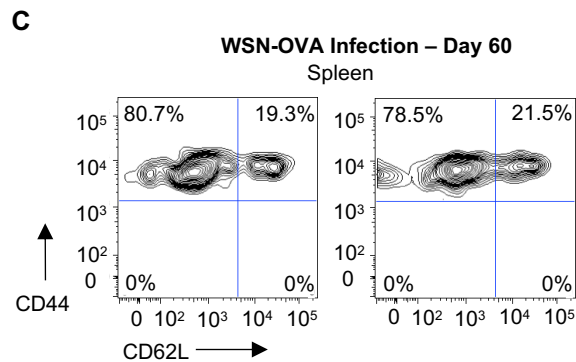
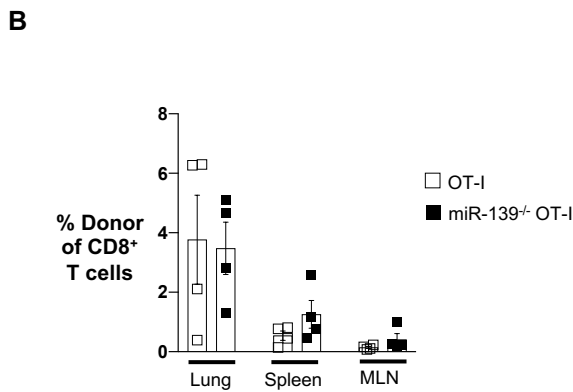
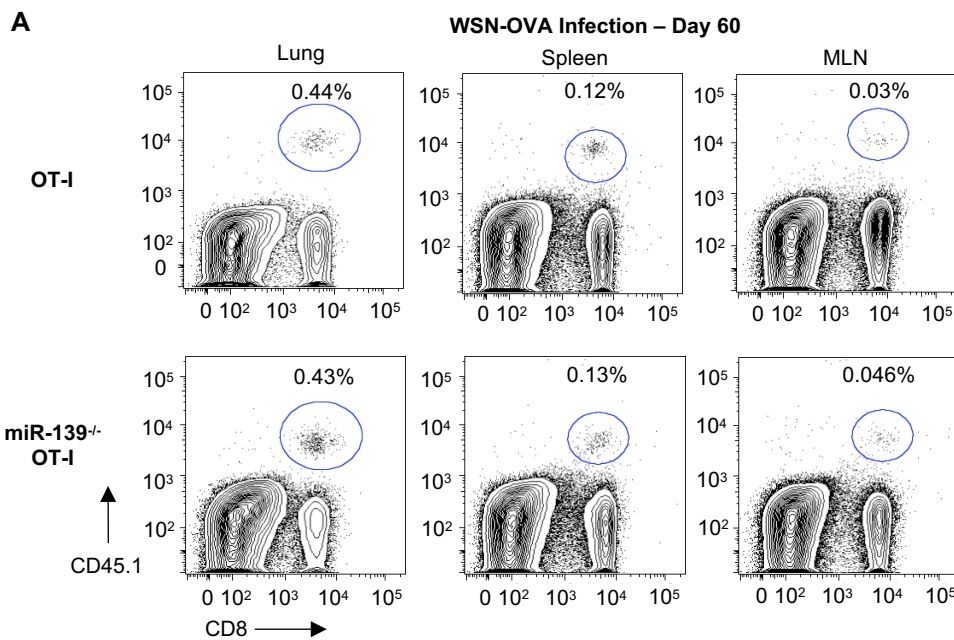
A

Mature miRNAs ≥ 2 fold change upregulated in effector CTL.				
Rank	Mature miR	Fold Change	p-value	q-value
1	miR-126a-3p	103.90	0.0130	0.0025
2	miR-182-5p	46.43	0.0001	0.0001
3	miR-183-5p	23.24	0.0001	0.0001
4	miR-195a-3p	14.08	0.0470	0.0077
5	miR-31-5p	12.38	0.0001	0.0001
6	miR-23a-5p	10.32	0.0001	0.0001
7	miR-143-3p	10.20	0.0098	0.0020
8	miR-145a-5p	10.09	0.0165	0.0030
9	miR-125b-5p	9.62	0.0001	0.0001
10	miR-27a-5p	9.24	0.0002	0.0001
11	miR-421-3p	7.59	0.0001	0.0001
12	miR-27b-5p	7.44	0.0001	0.0001
13	miR-146a-5p	6.92	0.0001	0.0001
14	miR-210-3p	5.98	0.0013	0.0004
15	miR-449a-5p	5.85	0.0013	0.0004
16	miR-7030-5p	5.80	0.0360	0.0062
17	miR-7666-3p	4.90	0.0002	0.0001
18	miR-183-3p	4.51	0.0077	0.0017
19	miR-301a-3p	4.43	0.0165	0.0030
20	miR-132-3p	3.74	0.0001	0.0001
21	miR-147-3p	3.65	0.0001	0.0001
22	miR-7648-3p	3.44	0.0017	0.0004
23	miR-6937-5p	3.30	0.0001	0.0001
24	miR-155-5p	3.20	0.0003	0.0001
25	miR-18a-5p	3.03	0.0001	0.0001
26	miR-6968-5p	3.02	0.0009	0.0003
27	miR-1934-3p	2.97	0.0038	0.0009
28	miR-21a-5p	2.90	0.0013	0.0004
29	miR-22-3p	2.88	0.0006	0.0002
30	miR-298-5p	2.84	0.0008	0.0003
31	miR-7221-3p	2.82	0.0001	0.0001
32	miR-6970-5p	2.82	0.0001	0.0001
33	miR-199a-3p	2.69	0.0009	0.0003
34	miR-199b-3p	2.69	0.0009	0.0003
35	miR-346-3p	2.66	0.0001	0.0001
36	miR-7081-5p	2.58	0.0017	0.0004
37	miR-3082-5p	2.58	0.0098	0.0020
38	miR-18b-5p	2.52	0.0001	0.0001
39	miR-7658-5p	2.49	0.0015	0.0004
40	miR-149-3p	2.46	0.0009	0.0003
41	miR-222-3p	2.46	0.0006	0.0002
42	miR-193a-5p	2.45	0.0002	0.0001
43	miR-330-3p	2.30	0.0023	0.0006
44	miR-671-5p	2.19	0.0139	0.0027
45	miR-3077-5p	2.16	0.0004	0.0002
46	miR-3110-3p	2.16	0.0059	0.0013
47	miR-212-3p	2.09	0.0374	0.0063
48	miR-501-3p	2.09	0.0046	0.0010
49	miR-6909-5p	2.03	0.0350	0.0061
50	miR-1224-5p	2.03	0.0098	0.0020
51	miR-6914-5p	2.02	0.0143	0.0027

B

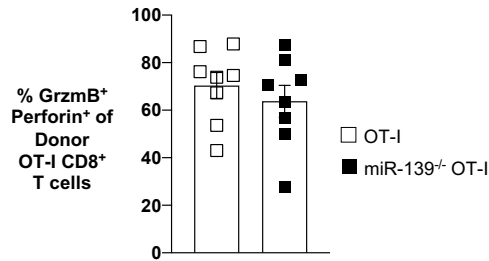
Mature miRNAs ≥ 2 fold change downregulated in effector CTL.									
Rank	Mature miR	Fold Change	p-value	q-value	Rank	Mature miR	Fold Change	p-value	q-value
1	miR-669c-5p	-11.09	0.0001	0.0001	51	miR-140-5p	-2.74	0.0286	0.0100
2	miR-181a-5p	-10.86	0.0001	0.0001	52	miR-674-5p	-2.70	0.0001	0.0001
3	miR-192-5p	-10.77	0.0001	0.0001	53	miR-29b-3p	-2.61	0.0046	0.0019
4	miR-466a-3p	-9.71	0.0004	0.0002	54	miR-328-3p	-2.58	0.0445	0.0137
5	miR-466e-3p	-9.71	0.0004	0.0002	55	miR-30e-3p	-2.44	0.0436	0.0137
6	miR-466b-3p	-9.12	0.0020	0.0010	56	miR-361-5p	-2.38	0.0001	0.0001
7	miR-466c-3p	-9.12	0.0020	0.0010	57	miR-30c-5p	-2.31	0.0004	0.0002
8	miR-466p-3p	-9.12	0.0020	0.0010	58	let-7a-5p	-2.29	0.0047	0.0019
9	miR-467c-5p	-7.85	0.0001	0.0001	59	miR-423-5p	-2.28	0.0190	0.0069
10	miR-466d-3p	-7.34	0.0004	0.0002	60	miR-17-3p	-2.28	0.0001	0.0001
11	miR-194-5p	-7.05	0.0005	0.0003	61	miR-200c-3p	-2.27	0.0015	0.0002
12	miR-139-5p	-6.89	0.0001	0.0001	62	miR-150-3p	-2.27	0.0001	0.0001
13	miR-26b-5p	-6.83	0.0242	0.0086	63	miR-6988-3p	-2.24	0.0350	0.0133
14	miR-28a-3p	-6.64	0.0001	0.0001	64	miR-466m-5p	-2.22	0.0334	0.0133
15	miR-30e-5p	-6.53	0.0002	0.0002	65	miR-669m-5p	-2.22	0.0334	0.0133
16	miR-669a-3p	-6.48	0.0001	0.0001	66	miR-19b-3p	-2.10	0.0015	0.0002
17	miR-669o-3p	-6.48	0.0001	0.0001	67	miR-30c-1-3p	-2.08	0.0455	0.0133
18	miR-378a-5p	-6.41	0.0006	0.0003	68	miR-92a-3p	-2.07	0.0001	0.0001
19	miR-339-5p	-6.21	0.0001	0.0001	69	miR-467d-5p	-2.05	0.0058	0.0002
20	miR-466c-5p	-6.21	0.0087	0.0033					
21	miR-342-5p	-6.17	0.0001	0.0001					
22	miR-466h-5p	-5.92	0.0067	0.0026					
23	miR-342-3p	-5.88	0.0001	0.0001					
24	miR-181c-5p	-5.45	0.0001	0.0001					
25	miR-378a-3p	-5.43	0.0001	0.0001					
26	miR-467a-5p	-5.06	0.0005	0.0003					
27	miR-128-3p	-4.67	0.0026	0.0012					
28	miR-181b-5p	-4.64	0.0001	0.0001					
29	miR-3068-3p	-4.58	0.0019	0.0010					
30	miR-30b-5p	-4.12	0.0002	0.0002					
31	miR-1843a-5p	-4.03	0.0407	0.0131					
32	miR-467d-3p	-3.99	0.0033	0.0015					
33	let-7b-5p	-3.93	0.0001	0.0001					
34	miR-7a-1-3p	-3.72	0.0062	0.0025					
35	miR-7033-5p	-3.55	0.0310	0.0107					
36	miR-669a-5p	-3.52	0.0025	0.0011					
37	miR-669p-5p	-3.52	0.0025	0.0011					
38	miR-1839-5p	-3.50	0.0007	0.0004					
39	miR-26a-5p	-3.46	0.0001	0.0001					
40	miR-151-5p	-3.31	0.0003	0.0002					
41	miR-30d-5p	-3.28	0.0001	0.0001					
42	miR-200b-3p	-3.23	0.0124	0.0046					
43	let-7f-5p	-3.20	0.0426	0.0136					
44	miR-150-5p	-3.20	0.0001	0.0001					
45	miR-1843b-5p	-3.03	0.0001	0.0001					
46	let-7d-3p	-2.98	0.0081	0.0031					
47	miR-320-3p	-2.94	0.0001	0.0001					
48	miR-29a-3p	-2.87	0.0008	0.0005					
49	miR-28a-5p	-2.87	0.0009	0.0005					
50	miR-151-3p	-2.86	0.0036	0.0015					

Supplementary Figure 2. MiRNA expression profiling of effector CTL All miRNAs upregulated (B) or downregulated (C) greater than 2-fold relative to naïve CD8⁺ T cells; FDR = 0.05, p ≤ 0.05 and q ≤ 0.05.

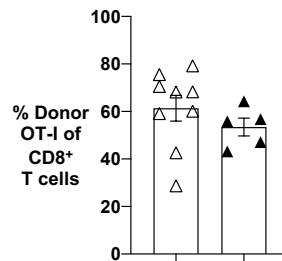


Supplementary Figure 3. Memory CD8⁺ T cell development is not regulated by miR-139 (A) Representative FACS plots of CD45.1⁺ donor OT-I and miR-139^{-/-} OT-I cells in the lungs, spleen, and mediastinal lymph nodes of C57Bl/6 recipient host mice 60 days post-infection with WSN-OVA influenza virus. (B) Frequency of CD45.1⁺ donor T cells within the CD8⁺ T cell compartment of the respective tissues. (C) Representative FACS plots of effector memory (EM)(CD44⁺CD62L⁻) and central memory (CM)(CD44⁺CD62L⁺) frequency within the donor OT-I CD8⁺ T cells in the spleen of mice 60 days post-infection with WSN-OVA influenza virus are shown. (D) Frequency of EM and CM donor OT-I CD8⁺ T cells in the spleen is shown. All figures are representative of 4 mice per group from two independent experiments. For figures B and D, error bars represent the standard error of the mean (SEM).

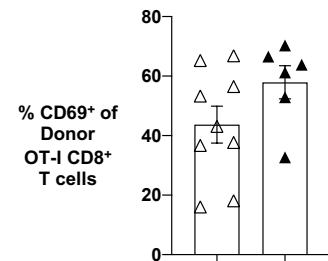
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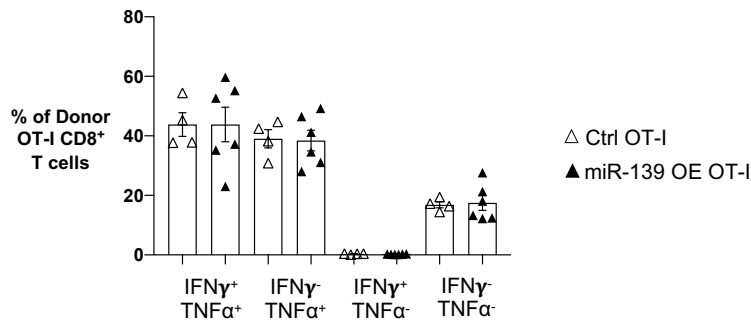
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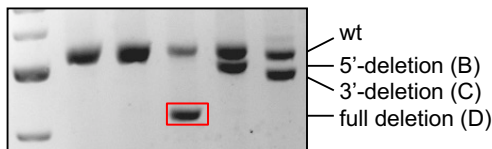
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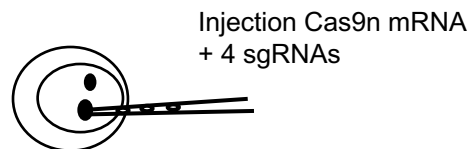
D



E



F



G



Supplementary Figure 4. (A) Dot plot of the frequency of Granzyme B and perforin double-positive cells within the lung donor OT-I populations in a competition experiment with WSN-OVA influenza virus infection at 10 days post-infection. (B) Dot plot of the frequency of cytokine producing scrambled control or miR-139 OE donor OT-I cells from the lungs of WSN-OVA influenza virus infected mice 10 days post-infection. (C) Dot plot of the frequency of scrambled control or miR-139 OE donor OT-I cells within the CD8⁺ T cell compartment in the lungs of WSN-OVA influenza virus infected mice 10 days post-infection. (D) Dot plot of the frequency of CD69⁺ scrambled control or miR-139 OE donor OT-I cells in the lungs of WSN-OVA influenza virus infected mice 10 days post-infection. Representative of 4-7 mice per group from two independent experiments. (E) Representative DNA gel electrophoresis showing full deletion of miR-139 in miR-139^{-/-} mice. (F) Image of the insertion of Cas9n mRNA and 4 sgRNAs into a C57Bl/6 embryo. (G) Schematic for the deletion of miR-139. For figures A-D, error bars represent the standard error of the mean (SEM).