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Supplemental information

miR-99a regulates CD4^{+} T cell differentiation and attenuates experimental autoimmune encephalomyelitis by mTOR-mediated glycolysis

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Supplementary Table 1. The sequences of primers.

Gene		Sequence
Actb	Primer (forward)	TTCCAGCCTTCCTTCTTGGG
	Primer (reverse)	TGTTGGCATAGAGGTCTTTACGG
Ifng	Primer (forward)	ATGAACGCTACACACTGCATC
	Primer (reverse)	CCATCCTTTTGCCAGTTCCTC
Tgfb	Primer (forward)	CTTCGACGTGACAGACGCT
	Primer (reverse)	GCAGGGGCAGTGTAAACTTATT
Il17a	Primer (forward)	AGCGTGTCCAAACACTGAG
	Primer (reverse)	CGCCAAGGGAGTTAAAGACTT
Hk2	Primer (forward)	TGATCGCCTGCTTATTCACGG
	Primer (reverse)	AACCGCCTAGAAATCTCCAGA
Pgam1	Primer (forward)	TCTGTGCAGAAGAGAGCAATCC
	Primer (reverse)	CTGTCAGACCGCCATAGTGT
Slc2a3	Primer (forward)	ATGGGGACAACGAAGGTGAC
	Primer (reverse)	GTCTCAGGTGCATTGATGACTC
Ldha	Primer (forward)	TGTCTCCAGCAAAGACTACTGT
	Primer (reverse)	GACTGTACTTGACAATGTTGGGA
Foxp3	Primer (forward)	CACCTATGCCACCCTTATCCG
	Primer (reverse)	CATGCGAGTAAACCAATGGTAGA
Pdcd1	Primer (forward)	TGGGGCCTAAGCCTATGTCT
	Primer (reverse)	CTCCCAAGGGTGGCTTTAGG
Icos	Primer (forward)	TGCTCCTGGCAGACATGAAG
	Primer (reverse)	TTGGTGAGTTCGCAGAGGAC
Tbx21	Primer (forward)	AACCGCTTATATGTCCACCCA
	Primer (reverse)	CTTGTTGTTGGTGAGCTTTAGC
Mtor	Primer (forward)	ATGCTATGGAGGTTACGGGTCTG
	Primer (reverse)	CCGCTTATTGCCTTTGGTATTTG

Supplementary Figures

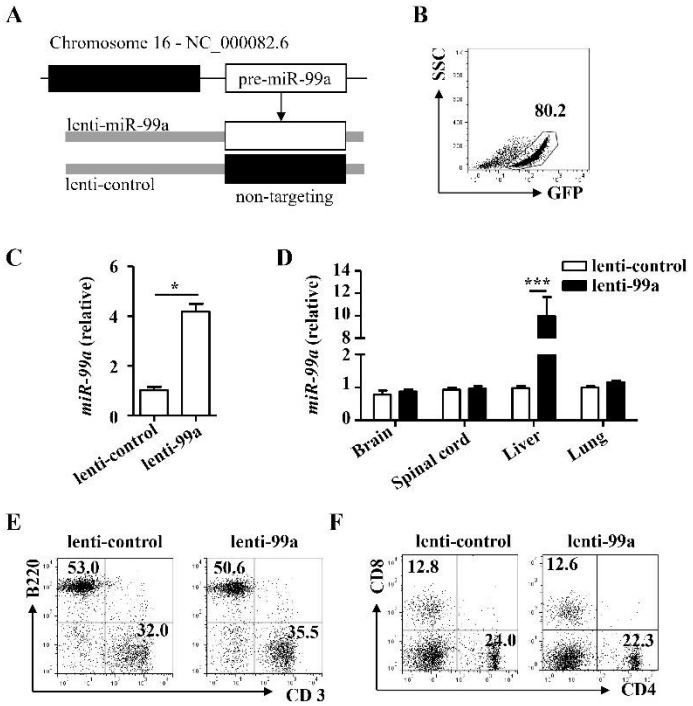

Figure S1. Overexpression of miR-99a in CD4$^+$ T cells and mice through by lentivirus infection. (A) miR-99a was encoded by pre-miR-99a which located after the 2810055G20Rik gene. Pre-miR-99a or an artificial sequence targeting none of the known proteins was cloned into a lentivirus vector to overexpress miR-99a or miR-control. (B-C) *In vitro* infection with lentiviruses led to stable exogenous gene expression with over 80% efficiency in purified CD4$^+$ T cells as indicated by GFP reporter fluorescence. The expression of miR-99a was analyzed by real-time PCR in infected CD4$^+$ T cells (n=5). (D) Real-time PCR analysis of miR-99a expression in the brain, spinal cord, liver and lung of the mice infected with lenti-99a or lenti-control (n=5). (E-F) Analysis of CD3e and B220 for determining total T and B lymphocytes, as well as CD4 and CD8 for identifying T helper cells and cytotoxic T cells in the spleen of mice infected with lenti-99a or lenti-control. The values are shown as the means ± S.E.M. *P < 0.05, ***P < 0.001 (Mann-Whitney U test and two-way ANOVA).

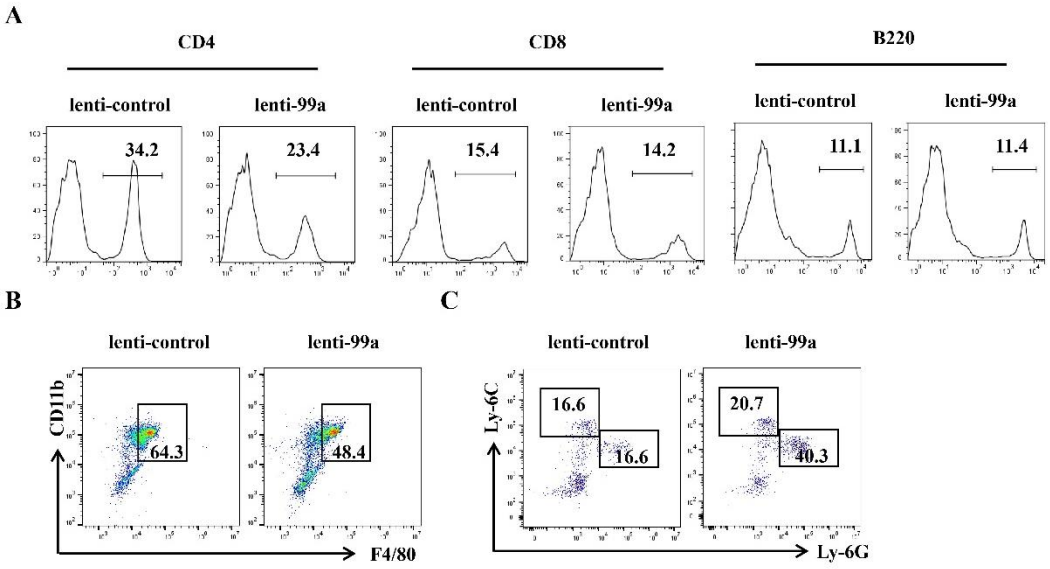

Figure S2. miR-99a suppresses immune cell infiltration in the CNS of the EAE mice. Flow cytometry of $CD4^+$ T cells, $CD8^+$ T cells, B cells (B220), macrophages ($CD11b^+F4/80^+$), M-MDSCs ($CD11b^+Ly\text{-}6C^{high}Ly\text{-}6G^{low}$) and PMN-MDSCs, （$CD11b^+Ly\text{-}6C^{low}Ly\text{-}6G^{high}$) obtained from the CNS of lenti-99a or lenti-control EAE mice(14 days after immunization).

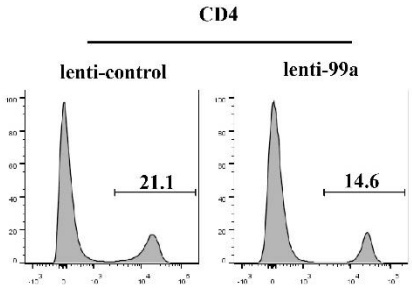

Figure S3. miR-99a suppresses $CD4^+$ T cells in the DLNs of the EAE mice. Flow cytometry of $CD4^+$ T cells obtained from the DLNs of the lenti-99a or lenti-control EAE mice (14 days after immunization).

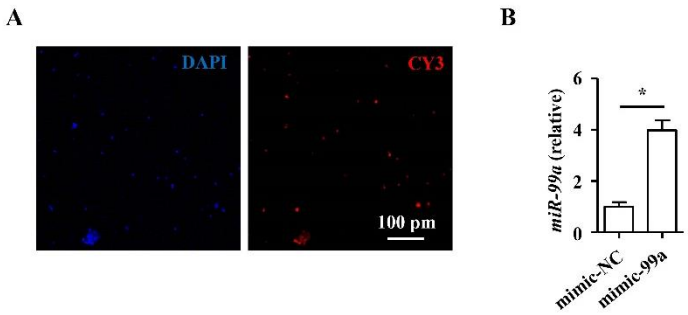

Figure S4. Overexpression of miR-99a in CD4$^+$ T cells by nucleofection. Naive CD4$^+$ T cells were nucleofected with miRNA mimic control (mimic-NC) or miRNA mimic miR-99a (mimic-99a) labeled by CY3. (A) Cells were stained with DAPI for 3 hours after nucleofection, and fluorescence images are shown. Scale bar, 100 pm. (B) Cells were activated by anti-CD3/CD28 antibodies 3 hours after nucleofection. miR-99a expression was detected by real-time PCR within 48 hours of nucleofection (n=4). The values are shown as the means ± S.E.M. *P < 0.05 (Mann-Whitney U test).

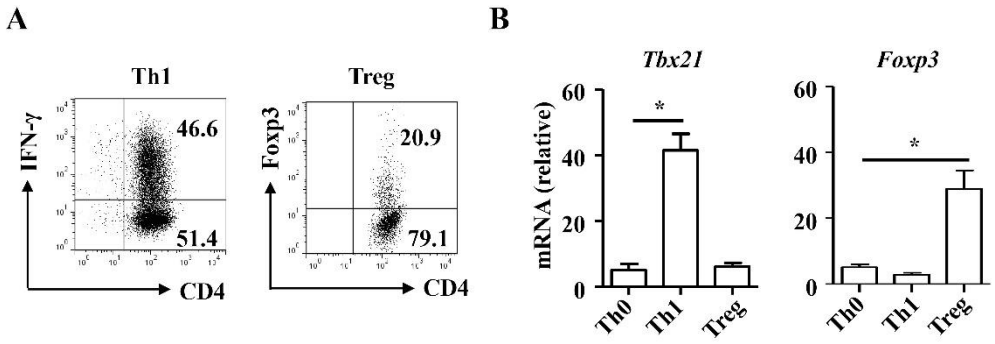

Figure S5. *In vitro* differentiation system for Th1 and Treg cells. Naive CD4$^+$ T cells were freshly isolated and cultured for 4 days under Th0, Th1 and Treg differentiation conditions. (A) Crucial lineage-specific cytokines were detected by flow cytometry. (B) Transcription factors were analyzed by real-time PCR (n = 4). The values are shown as the means ± S.E.M. *P < 0.05 (one-way ANOVA).