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Supplementary Information for

Dual Mechanisms of Post-transcriptional Regulation of Tet2 by Let-7 MicroRNA in Macrophages

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SI Methods

Western Blot

Western blot was performed as previously described (1). Cell lysate was separated by electrophoresis on 10% or 12% SDS-polyacrylamide gels and probed with specific Abs. The following antibodies were used to blot for the protein of interest: antibody against Tet1 (Invitrogen; PA5-49432), Tet2 (Cell Signaling Technology; 36449), Tet3 (Abcam; ab139805), SDHA (SC-27992; Santa Cruz Bio), SLC25A10 (PA3-840; Invitrogen) and antibody against β-actin (Sigma; A3854).

RNA extraction and RT real-time qPCR

RNA extraction, RT and qPCR were performed as previously described (2). We performed SYBR Green–based RT-qPCR for mRNA expressions of mouse IL-6, Tet1, Tet2 and Tet3 mRNA. Sequence-specific primers for qPCR are listed in Table S1. IL-6, Tet1, Tet2 and Tet3 mRNAs were normalized to β-actin.

siRNA Experiment

Control siRNA and SDHA siRNA were obtained from Santa Cruz Biotechnology. Control siRNA (sc-37007) consists of a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. SDHA siRNA (sc-61835) is a pool of three target-specific 19- to 25-nt siRNAs designed to knock down gene

expression. The siRNAs were prepared according to the transfection protocol for cell cultures from Santa Cruz Biotechnology.

Seahorse Analyzer

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were performed as previously described (2). $1e^6$ LPS-activated BMDMs/well were seeded into a 24-well plate, and the OCR and ECAR measurements were normalized to cell number.

Luciferase Reporter Assays

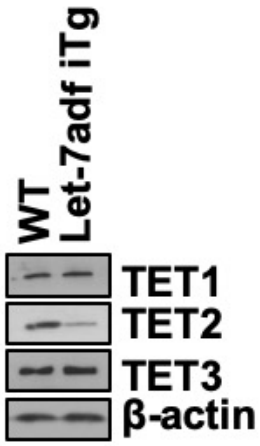
Luciferase Reporter assays to measure microRNA let-7d-based repression of target Tet2 3′ UTRs were performed as previously described (2). Luciferase activity was examined by the Dual-luciferase Reporter Assay System (Promega) and normalized to relative controls.

REFERENCES

(1) Chaudhuri AA, *et al.* (2012) Oncomir miR-125b regulates hematopoiesis by targeting the gene Lin28A. *Proc Natl Acad Sci U S A* 109(11):4233-4238.

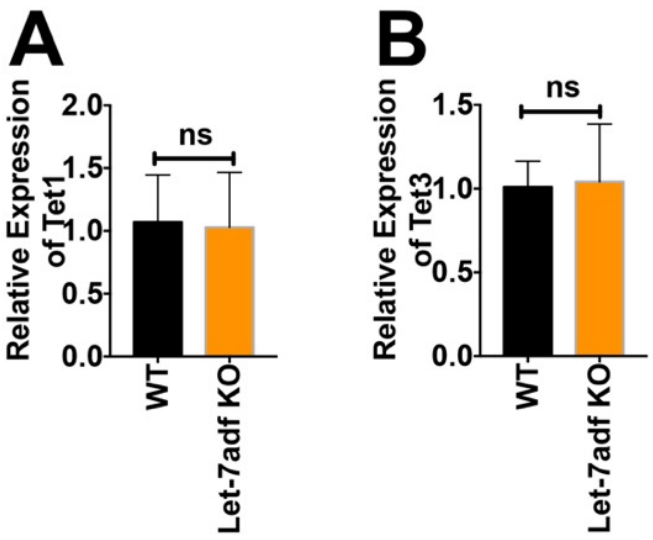
(2) Jiang S, Yan W, Wang SE, & Baltimore D (2018) Let-7 Suppresses B Cell Activation through Restricting the Availability of Necessary Nutrients. *Cell Metab*.

Supplementary Information Text



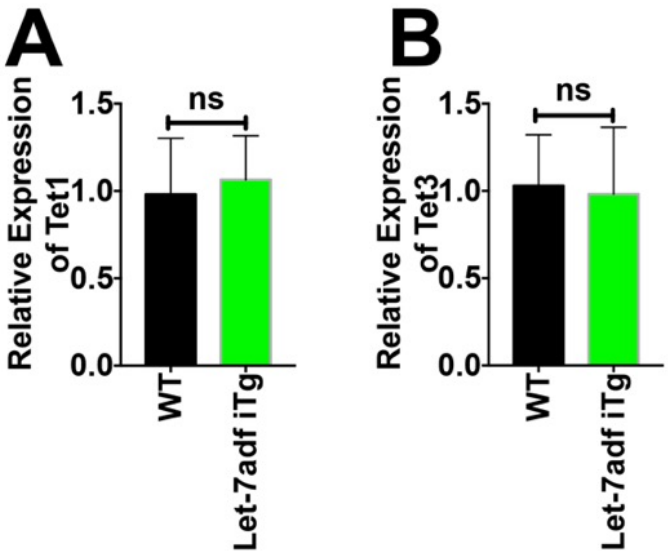
Supplemental Figure S1. Let-7adf iTg represses TET2 protein expression in macrophages.

The protein expressions of TET1, TET2 and TET3 in BMDMs from WT or the let-7adf cluster iTg mice obtained by Western blot.



Supplemental Figure S2. Let-7adf KO is dispensable to Tet1 and Tet3 transcriptional levels in macrophages.

(A-B) The Let-7adf cluster KO/WT BMDMs were stimulated by LPS for 24 hours. The Tet1 (A) and Tet3 (B) mRNA levels were examined by qPCR.

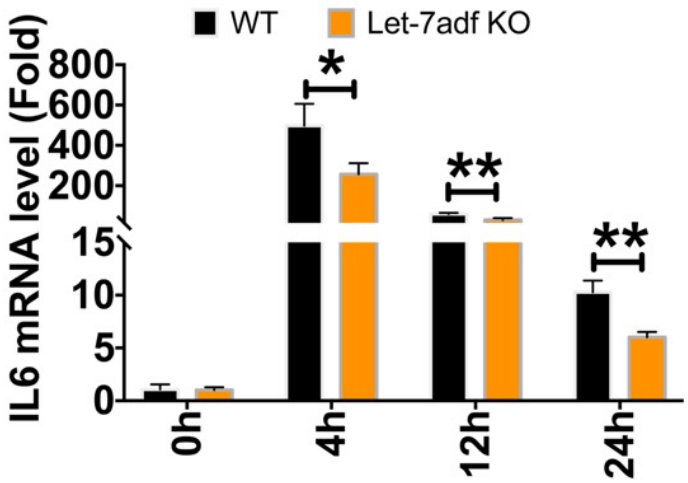


Supplemental Figure S3. Let-7adf iTg is dispensable to Tet1 and Tet3 mRNA levels in macrophages.

(A-B) The Let-7adf cluster iTg/WT BMDMs were stimulated by LPS for 24 hours. The Tet1 (A) and Tet3 (B) mRNA levels were examined by qPCR.

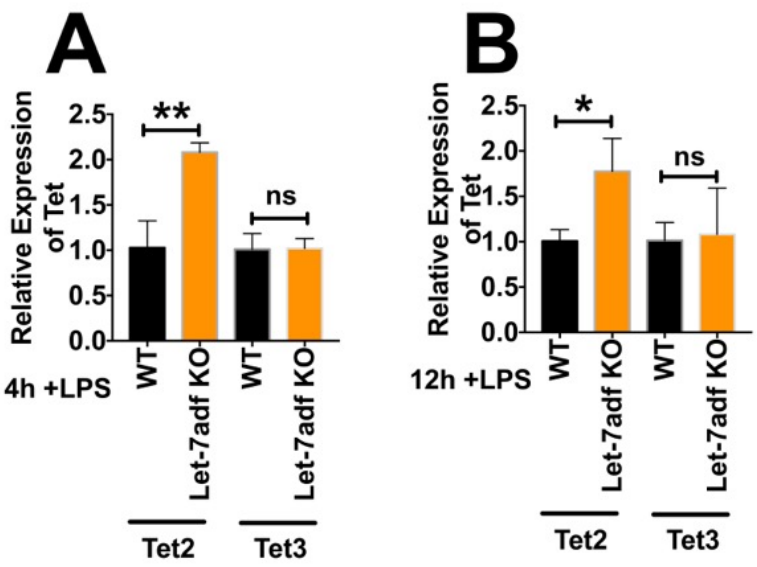
Supplemental Figure S4. Validation of siRNA of Tet2.

The protein expression of TET2 from siRNA-control and siRNA-Tet2 treated BMDMs obtained by Western blot.



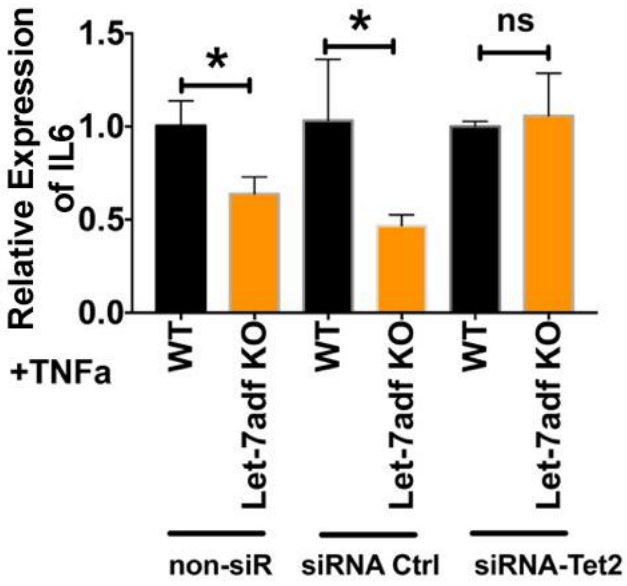
Supplemental Figure S5. Loss of Let-7adf maintains lower expression of IL-6.

The Let-7adf cluster KO/WT BMDMs were stimulated by LPS following a time course. The IL-6 mRNA levels were examined by qPCR.



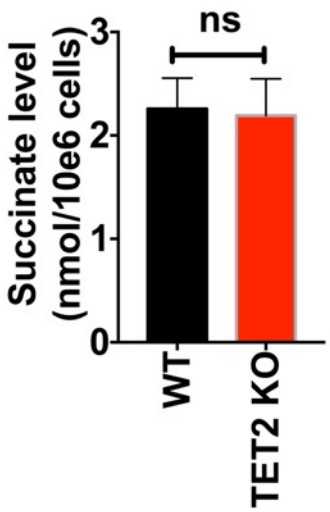
Supplemental Figure S6. Loss of Let-7adf maintains higher expression of Tet2, but not Tet3.

The Let-7adf cluster iTg/WT BMDMs were stimulated by LPS following 4h (A) and 12h (B). The Tet mRNA levels were examined by qPCR.



Supplemental Figure S7. Let-7adf contributes to the regulation of Tet2/IL-6 following the TNFa stimuli.

The Let-7adf cluster KO/WT BMDMs were stimulated by TNFa. The IL-6 mRNA levels were examined by qPCR.



Supplemental Figure S8. Tet2 mildly represses succinate accumulation in macrophages.

Succinate levels in Tet2 KO/WT macrophages were examined by 24 hours.

Table S1, related to qPCR in Experimental Procedures

IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Tet1	AGGAAAATGGGAACCCAAAC	TCTCAGAAAGGTCGCTTGGT
Tet2	AGGTTCTCAACGAGCAGGAA	TGAGATGCGGTACTCTGCAC
Tet3	GGCATGGCCTACACTTCATT	TTTTAGGATGGGCGTGTTTC
β-actin	AGGTGTGCACCTTTTATTGGTCTCAA	TGTATGAAGGTTTGGTCTCCCT