**SUPPLEMENTAL** **FIGURE AND TABLE LEGENDS**

**Figure S1. Expression of Notch ligands and receptors in macrophages during in vitro differentiation and activation.** A, Monocytes from normal mice were cultured in the presence of GM-CSF for 4 days to differentiate into BMDMs, and the expression of Notch ligands (Dll1, Dll3, Dll4, Jagged1, Jagged2) and receptors (Notch1 - 4) was determined by qRT-PCR. B, BMDMs from normal mice were stimulated with PBS, LPS+IFN-γ or IL-4. The expression of Notch ligands and receptors was determined by qRT-PCR. Bars, mean ± SD (n = 3); \*, *P* < 0.05; \*\*, *P* < 0.01.

**Figure S2. Activation of Notch signaling in macrophages repressed the growth of transplanted B16F melanoma tumors.** A, NICcA and control (Ctrl) mice were inoculated s.c with 3 × 106 of B16F cells. Tumors were dissected on day 14 after the inoculation and photographed. Tumor weights and tumor sizes were measured and compared. B, Single cell suspensions were prepared from the tumors, and analyzed by FACS after staining as indicated.

**Figure S3. Activation of Notch signaling by conditional NIC expression in macrophages did not change the number of macrophages, but reduced the number of MDSCs and increased the number of CD8+ T-cells in transplanted tumors.** A,Ctrl and NICcA mice were inoculated s.c with LLC cells. Tumors were collected 24 days after the inoculation, and tumor-infiltrating macrophages were analyzed by FACS after the indicated staining. B, The numbers of CD11b+F4/80+ macrophages in tumors were compared. C, D, Tumor-infiltrating cells were analyzed by FACS after the indicated staining for MDSCs (C) and T-cells (D).Tumor-infiltrating MDSCs (CD11b+Ly6G+) and cytotoxic T-cells (CD8+) were quantitatively compared (Figure 1C). Bars, mean ± SD (n = 8); ns, not significant.

**Figure S4. miR-125a expression in BM cells and macrophages.** Monocytes from normal mice were cultured in the presence of GM-CSF for 4 days in the presence of GSI or DMSO, and miR-125a expression was determined by qRT-PCR (n = 3). Bars, mean ± SD; \*, *P* < 0.05; \*\*, *P* < 0.01.

**Figure S5. miR-125a is a downstream molecule to Notch signaling in macrophages.** A, Three siRNAs targeting Notch1 were designed and synthesized. The efficiency of the siRNAs was determined by transfection followed by qRT-PCR. B, BMDMs from normal mice were stimulated with PBS, LPS+IFN-γ or IL-4 in the presence of Ctrl, siRNA1, or siRNA2. The expression of iNOS, IL-12, TNF-, and MR was determined by qRT-PCR. C, Monocytes from normal mice were cultured in the presence of GM-CSF for 4 days in the presence of Ctrl, siRNA1, or siRNA2 to Notch1, and the expression of miR-125a and miR-99b was determined by qRT-PCR. Bars, mean ± SD (n = 3); \*, *P* < 0.05; \*\*, *P* < 0.01.

**Figure S6. Activation of Notch signaling with immobilized mD1R in macrophages promoted M1 polarization.** Purified mD1R protein was coated on cultured dishes. BMDMs from normal mice were then seeded and cultured in the presence of LPS+IFN-γ. The expression of Hes1 and Hey1 (A), iNOS, IL-12, TNF-, and MR (B) was determined by qRT-PCR. Bars, mean ± SD (n = 3); \*, *P* < 0.05; \*\*, *P* < 0.01.

**Figure S7. Characterization of the miR-125a gene and its enhancer.** A, 5’-RACE was performed to amplify the 5’ sequence of *Spaca6* mRNA using Primer 1 and Primer 2 (C). The amplified fragment (439 bp) is indicated by the arrow. M, DNA markers (from the top: 5000, 3000, 2000, 1500, 1000, 750, 500, 200, and 100 bp). B, The sequence of the 439-bp fragment amplified by 5’-RACE. The bold letters indicate the translated sequence, with the predicted amino acid sequence shown below the nucleotide sequence. The putative initiation codon is marked in red. The underlined sequence is Primer 2. The splicing boundary between Exon 1’ and Exon 1 is indicated.C,Schematic representations of *Spaca6* (upper) and *Spaca6A* (lower). The thin lines represent introns, the open boxes represent untranslated regions, and the filled boxes represent putatively translated regions. The exons of *Spaca6A* are enumerated according to those of *Spaca6*, with the newly identified exon designated as Exon 1’. The first intron of *Spaca6A* contains binding sites of RBP-J and YY1, and a miRNA cluster including miR-99b/let-7e/miR-125a.

**Figure S8. The sequence of the first intron enhancer of *Spaca6A*.** The nucleotides are numbered with the 1st nucleotide of the first intron of *Spaca6A* (as determined by the alignment of the RACE-amplified fragment sequence with the genomic *Spaca6* gene sequence [NC\_000083.6]) as 1. The putative transcription factor recognition sites are marked in red and underlined. The pri-miR-99b, pri-let-7e, and pri-miR-125a sequences were underlined.

**Figure S9. miR-125a enhanced phagocytosis of differentially activated macrophages.** BMDMs derived from normal mice were transfected with miR-125a mimics or control (Ctrl) oligonucleotides and stimulated with PBS, LPS+IFN-γ or IL-4. Then, macrophages (1 × 106) were co-cultured with *E. coli* (1 × 107) that had been transformed with a GFP-expressing vector for 2 h and subsequently photographed under a fluorescence microscope after extensive rinsing (A). Macrophages containing engulfed bacteria (green dots) were counted, and the numbers of bacteria per macrophage were quantified and compared (n = 6) (B). Bars, mean ± SD; \*, *P* < 0.05; \*\*, *P* < 0.01.

**Figure S10. Notch signaling regulated M1 and M2 macrophage polarization.** A, BM monocytes from RBP-JcKO and control (Ctrl) mice were cultured in the presence of GM-CSF for 7 days to generate BMDMs. The cells were then stimulated with PBS, LPS+IFN-γ or IL-4 for 24 h. The iNOS, IL-12, TNF-α and MR expression levels were determined using quantitative RT-PCR (n = 3). B,BM monocytes from NICcA and control (Ctrl) mice were cultured and analyzed as in (A). Bars, mean ± SD; \*, *P* < 0.05; \*\*, *P* < 0.01.

**Figure S11. miR-125a regulated M1 macrophages through targeting FIH1.** A,Thesequence of the 3’-UTR of FIH1 was aligned with the seed sequence of miR-125a. The recognized sequences (nucleotides 302 ~ 309 and 1177 ~ 1184) are marked in red. B, The efficiency of the siRNA targeting FIH1 was determined by transfection and qRT-PCR. C, FIH1 regulated M1 macrophage polarization by promoting iNOS expression through the inhibition of Hif-1α. iNOS expression was induced under hypoxic conditions (left). siFIH1 promoted iNOS expression in M1 macrophages (right). D,HeLa cells were transfected with combinations of miR-125a mimics (0, 50, or 100 ng), pcDNA-Hif-1α, and pGL3-HRE or pGL3-pro. Luciferase activity in cell lysates was determined 24 h after transfection (n = 3). Bars, mean ± SD; \*, *P* < 0.05; \*\*, *P* < 0.01.

**Figure S12. miR-125a repressed M2 macrophages through targeting IRF4.** A,Thesequence of the 3’-UTR of IRF4 was aligned with the seed sequence of miR-125a. The recognized sequence (nucleotides 352 ~ 358) are marked in red. B,siRNA for IRF4 reduced the IRF4 and MR mRNA levels in M2 macrophages (n = 3). C, ChIP assay. Cross-linked chromatin fragments from IL-4-treated BMDMs were immunoprecipitated with Ig or anti-IRF4 antibody. DNA fragments encompassing the PU.1-binding sites were amplified by PCR (n = 3). Lower panel, structures of the MR promoter and enhancer. Exons are indicated by filled boxes. PU.1 recognition sites are indicated by filled ellipses. Nucleotides are numbered, with the transcription initiation site as +1. D,IRF4 transactivated the MR enhancer, as shown by luciferase reporter assay (n = 3). E,IRF4 promoted MR enhancer activity in cooperation with PU.1 (n = 3). F, HeLa cells were transfected with miR-125a mimics (0, 50, or 100 ng) and pGL3-MR or pGL3-pro. Luciferase activity in cell lysates was determined 24 h after transfection (n = 4).Bars, mean ± SD; \*, *P* < 0.05, \*\*; *P* < 0.01; \*\*\*, *P* < 0.001.

**Figure S13. Self-amplification of miR-125a expression through RYBP/YY1.** A,Thesequence of the 3’-UTR of RYBP was aligned with the seed sequence of miR-125a. The recognized nucleotides (nucleotides 2051 ~ 2057) are marked in red. B, RAW264.7 cells were transfected with pFlag-RYBP or control, and the expression of miR-125a and miR-99b was determined by qRT-PCR (n = 4). Bars, mean ± SD; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001.

**Figure S14. A model of the Notch signaling-mediated regulation of TAMs through miR-125a.** Notch signaling is required for the differentiation of TAMs (20). After differentiation, in the presence of Notch activation, macrophages upregulate miR-125a, which could self-amplify its own expression, leading to enhanced M1 and diminished M2 polarization through FIH1-Hif-1 and IRF4, respectively. This population of macrophages exhibits anti-tumor activity. On contrary, If Notch signaling is absent, miR-125a will be reduced, resulting in TAM phenotype that promotes tumor growth.

**Table S1:** Primers and oligonucleotides used for PCR.

**Table S2:** Antibodies and related reagents used in this study.