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CORRECTION

Correction to: Castor1 overexpression regulates microglia M1/M2 polarization via inhibiting mTOR pathway

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The original online version of this article was revised. The authors would like to update and correct the Supplementary Information. The authors apologize for any inconvenience caused by the original errors.

Supplementary Information for
Castor1 overexpression regulates microglia M1/M2 polarization via inhibiting mTOR pathway

This file includes:
Extended Materials and Method

Supplementary Fig. 1–4

Supplementary Table 1–2

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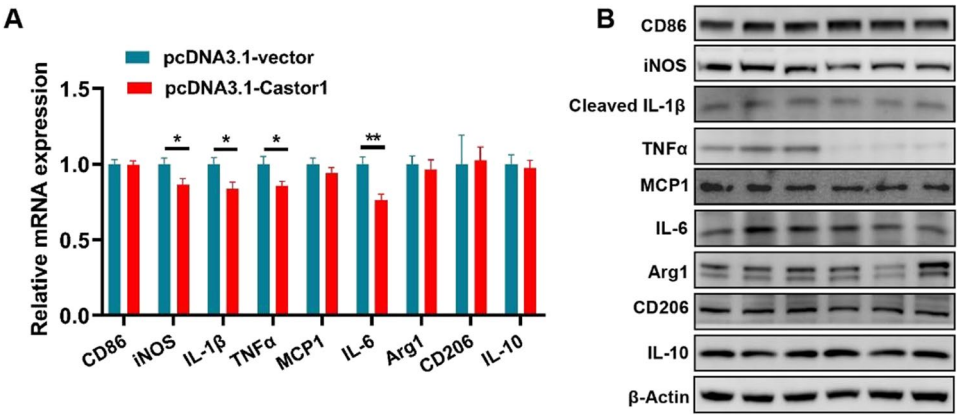
Extended Materials and Method

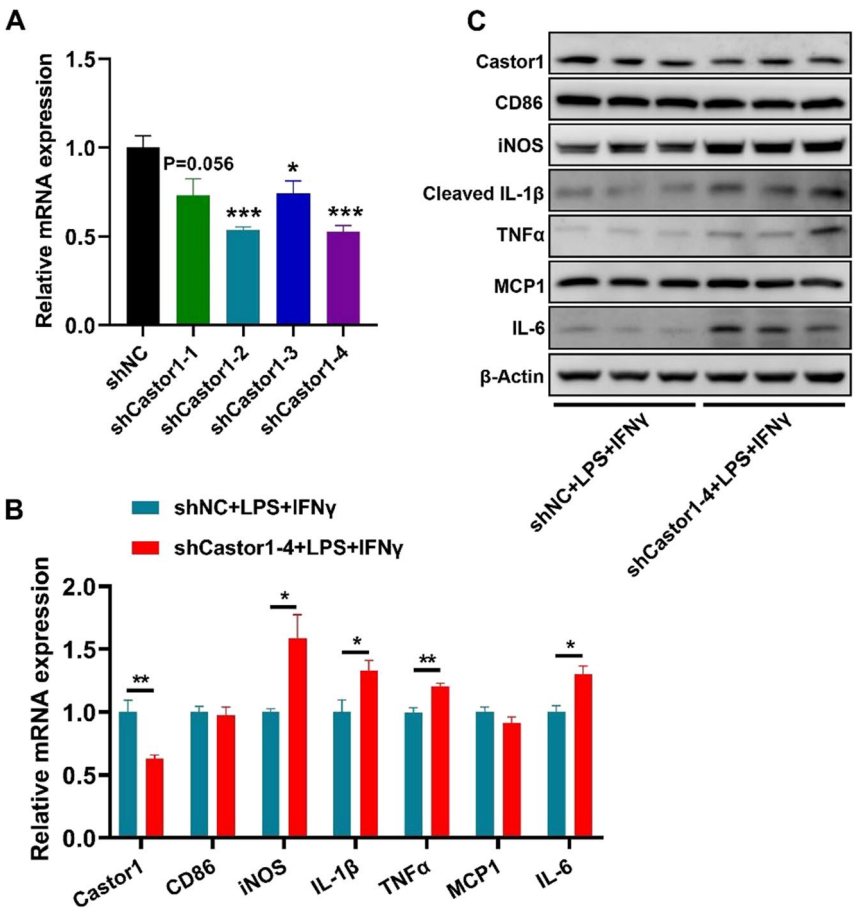
Isolation and culture of primary mouse microglia

Primary mouse microglia were isolated from 6 to 8-week-old male C57BL/6 J mice (Beijing SPF Biotechnology) as previously described with modifications (Cardona et al. 2006). Briefly, after perfusion, brains were diced into 2 mm pieces and then further homogenized using a 15-ml dounce homogenizer containing 3 ml of digestion cocktail (0.25% trypsin with 0.025 U/ml DNase I). Homogenized tissue was filtered through a 70 µm cell strainer to obtain a single cell suspension, centrifuged at 300×g for 5 min at 18 °C and resuspended in 37% Percoll (17–0891-02, GE Healthcare). Transfer the cell suspension (37% layer) to 15 ml conical tubes containing 4 ml of 70% Percoll at the bottom. Then on top of the 37% layer slowly pipette 4 ml of 30% Percoll, followed by 2 ml of PBS. Centrifuge the gradient 40 min at 300×g with no brake and ultimately the microglia was harvested from 70 to 37% interphase. Isolated cells were further cultured in six-well plates coated with poly-d-lysine. The DMEM/F12 culture medium was supplemented with 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 10 ng/mL murine recombinant macrophage colony stimulating factor (M-CSF, 51112-MNAH, Sino Biological). The culture medium was changed twice a week.

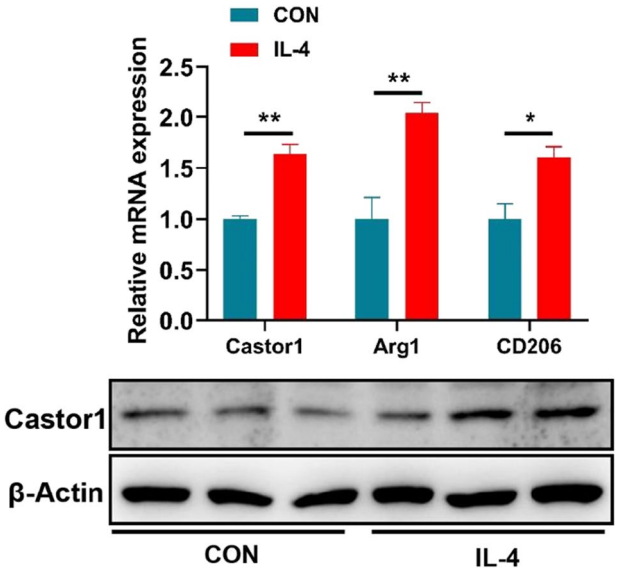
Supplementary Fig. 1. Castor1 overexpression inhibited M1 related genes expression. BV2 cells were transfected with pcDNA3.1-vector or pcDNA3.1-Castor1 plasmid for 24 h without any other stimulations and then the M1 or M2-related genes were examined. The results demonstrated that Castor1 overexpression inhibited the expression of some M1 related markers, including iNOS ($P < 0.05$), IL-β ($P < 0.05$), TNF-α ($P < 0.05$) and IL-6 ($P < 0.001$). However, there were no change in the expression of M2-related genes. Data are expressed as SEM, $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the pcDNA3.1-vector group.



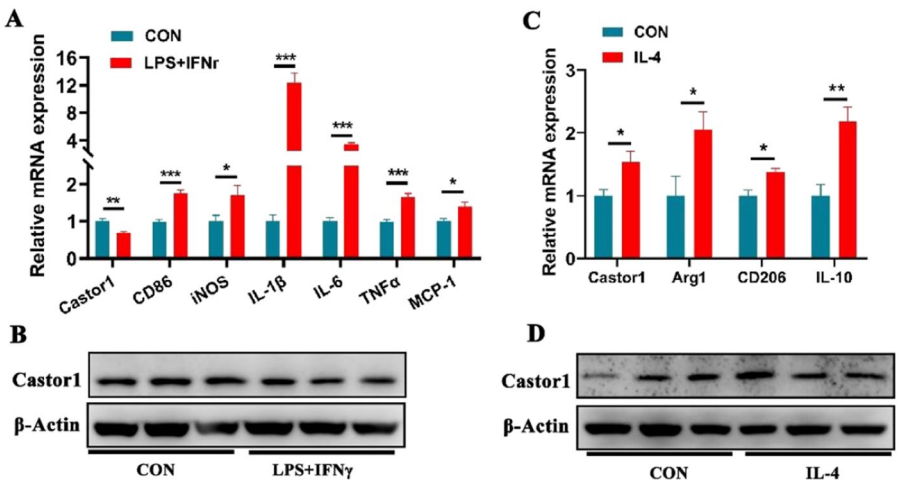
Supplementary Fig. 2. Castor1 knockdown promotes the transcription of M1-related genes. Castor1 knockdown was performed using Castor1-targeted shRNA cloned into the plasmid. qPCR analysis revealed that Castor1 expression was decreased 73% by shCastor1–1 ($P = 0.056$), 54% by shCastor1–2 ($P < 0.001$), 74% by shCastor1–3 ($P < 0.05$), and 53% by shCastor1–4 ($P < 0.001$) separately compared to the shNC. To further investigate the effect of Castor1 knockdown on the M1 polarization, BV2 cells were transfected with shNC or shCastor1–4 for 12 h and then treated with LPS/IFN-γ for 12 h. Compared with shNC groups, there was a significant increase in the expression of of M1-related genes such as iNOS ($P < 0.01$), IL-β ($P < 0.01$), TNF-α ($P < 0.001$) and IL-6 ($P < 0.01$). Data are expressed as SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to the shNC group.



Supplementary Fig. 3. The Expression of Castor1 in IL-4 treated BV2 cells. BV2 cells were treated with 20 ng/mL IL-4 for 12 h. The expression of M2-related markers and Castor1 was detected. The data shown that Castor1 expression was significantly upregulated in M2 polarization conditions. Data are expressed as SEM, $*P < 0.05$, $**P < 0.01$compared to the CON group.



Supplementary Fig. 4. The Expression of Castor1 in LPS/IFN-γ or IL-4 treated primary cultured microglia cells. Primary microglia cells were treated with 100 ng/mL LPS concurrent with 10 ng/mL IFN-γ or 20 ng/mL IL-4 for 12 h. The expression of M1- or M2-related markers and Castor1 was detected. The results shown that Castor1 expression was significantly decreased in M1 polarization conditions and upregulated in M2 polarization conditions. Data are expressed as SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to the CON group.



Supplementary Table 1. The sequence used for shCastor1

	sequence (5′–3′)
shNC	CTTACGCTGAGTACTTCGA
shCastor1–1	GCTGTTGACATCTCTGCTTAC
shCastor1–2	GCCCGTTACTGGAGACGA TTC
shCastor1–3	GCTGGTGATGAACGTATC TCA
shCastor1–4	GGATGAGGAGGGCTTTAA AGA

Supplementary Table 2. Primer sequence used for qPCR analysis

Gene	Primer sequence (5′–3	
CD86	Forward:	ACGATGGACCCCAGATGCACCA
	Reverse:	GCGTCTCCACGGAAACAGCA
iNOS	Forward:	GGCAGCCTGTGAGACCTTTG
	Reverse:	TGCATTGGAAGTGAAGCGTTT
IL-β	Forward:	CCTGCAGCTGGAGAGTGTGGAT
	Reverse:	TGTGCTCTGCTTGTGAGGTGCT
TNF-α	Forward:	AGCCCACGTCGTAGCAAACCAC
	Reverse:	AGGTACAACCCATCGGCTGGCA
IL-6	Forward:	AGGAGACTTCACAGAGGATACC
	Reverse:	GAATTGCCATTGCACAACTCTT
MCP-1	Forward:	GCATCCACGTGTTGGCTCA
	Reverse:	CTCCAGCCTACTCATTGGGATCA
Arg1	Forward:	TTAGGCCAAGGTGCTTGCTGCC
	Reverse:	TACCATGGCCCTGAGGAGGTTC
CD206	Forward:	TCAGCTATTGGACGCGAGGCA
	Reverse:	TCCGGGTTGCAAGTTGCCGT
IL-10	Forward:	GGCAGAGAACCATGGCCCAGAA
	Reverse:	AATCGATGACAGCGCCTCAGCC
β-Actin	Forward:	CTCTGGCTCCTAGCACCATGAAGA
	Reverse:	GTAAAACGCAGCTCAGTAACAGTCCG

Reference

Cardona AE, Huang D, Sasse ME, Ransohoff RM (2006) Isolation of murine microglial cells for RNA analysis or flow cytometry. Nat Protoc 1(4):1947–1951. https://doi.org/10.1038/nprot.2006.327