

Supplementary Material

The Caspase-1-EGR4 axis drives acute myeloid leukemia progression by orchestrating macrophage repolarization

Yi Qian^{1,2}, Yue Chen¹, Zu-Xi Feng¹, Xiao-Feng Zhu², Li Zhang¹, Hao Xiong^{1,2}, Xiang-hui Zhang⁵, Jun Bai³, Yan-hong Li³, Yu-xian Wang³, Lijuan Li^{1,3,4*}, Liansheng Zhang^{1,3,4*}

Lijuan Li, Department of Hematology, The Second Hospital and Clinical Medical School, Lanzhou University, Lanzhou City 730030, China. E-mail: lilijuan1232025@163.com.

Liansheng Zhang, Department of Hematology, The Second Hospital and Clinical Medical School, Lanzhou University, Lanzhou City 730030, China. E-mail: doctorzhanglsh@sina.com.

This file includes: Original gels; Supplementary Table S1- S2.

Figure1C

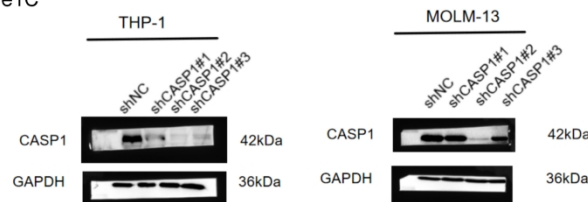


Fig.1. Efficient CASP1 KD in THP-1 and MOLM-13 cells. (C) Evaluation of CASP1-KD efficiency by western blot .

Figure 3A

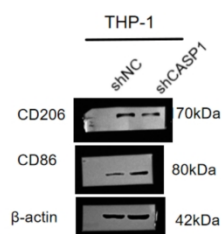


Figure 3B

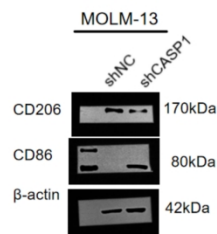


Fig.3. CASP1 KD in AML cells alters macrophage polarization at the protein level. (A,B) Representative western blot results showing the expression of M1 (CD86) and M2 (CD206) markers in macrophages treated with conditioned medium (CM) from shCASP1 or shNC AML cells.

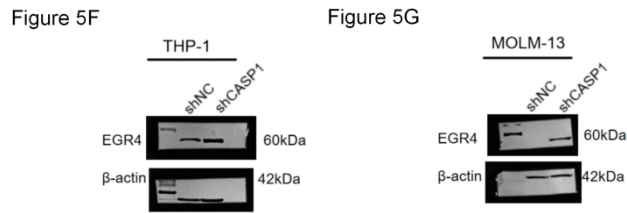


Fig.5. Transcriptomic profiling identifies EGR4 as a key transcriptional target upregulated by CASP1 KD in AML cells.(F,G) EGR4 protein expression was assessed by western blot in THP-1 and MOLM-13 cells.

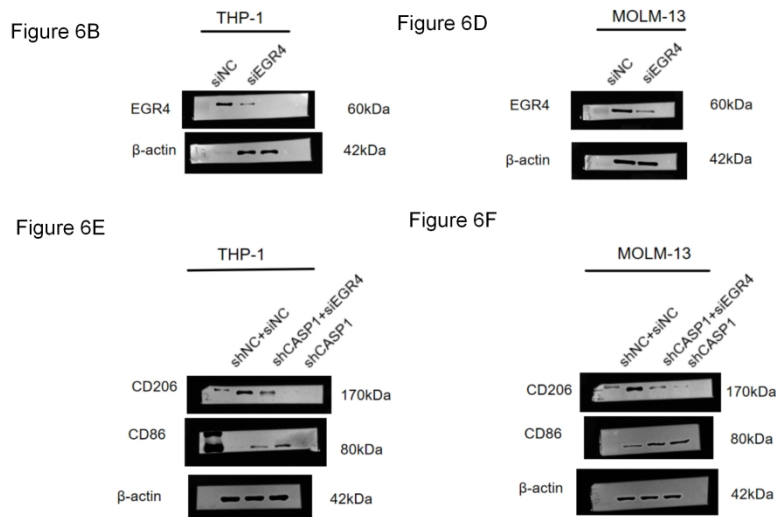


Fig. 6. EGR4 KD rescues the macrophage polarization shift induced by CASP1 KD.(B,D) Representative immunoblot of EGR4 protein expression. (E,F) Representative immunoblots for CD206 and CD86 in macrophages following stimulation with CM from the indicated AML cell groups.

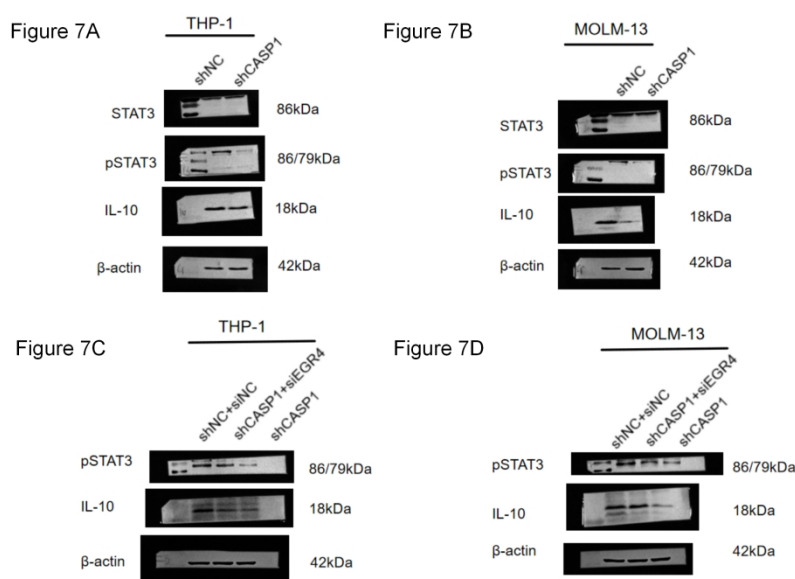


Fig.7. Mechanism of the CASP1-EGR4 axis through the IL-10/STAT3 pathway in macrophages. (A, B) Assessment of IL-10 and p-STAT3 by western blot in macrophages treated with CM from (A) THP-1 or (B) MOLM-13 cells expressing shNC or shCASP1, revealing a coordinate decrease following CASP1 KD. (C,D) Assessment of pathway recovery by western blot, showing partial restoration of IL-10 and p-STAT3 in macrophages after CM treatment from (C) THP-1 or (D) MOLM-13 models with dual CASP1 KD and EGR4 rescue.

Supplementary Table S1. Primer sequences for qPCR analysis of macrophage polarization marker genes.

primers	sequences
β-actin Forward	5'CCTGTACGCCAACACAGTGC3'
β-actin Reverse	3'ATACTCCTGCTTGCTGATCC5'
IL-10 Forward	5'GACTTTAAGGGTTACCTGGGTTG3'
IL-10 Reverse	3'TCACATGCGCCTTGATGTCTG5'
IL-4 Forward	5'CCAACTGCTTCCCCCTCTG3'
IL-4 Reverse	3'TCTGTTACGGTCAACTCGGTG5'
TGF-β Forward	5'GGCCAGATCCTGTCCAAGC3'
TGF-β Reverse	3'GTGGGTTTCCACCATTAGCAC5'
Arg1 Forward	5'CTGCTCATCTATACACGGTTACC3'
Arg1 Reverse	3'CCAGTCCGTCAACATCAAACT5'
IL-6 Forward	5'ACTCACCTCTTCAGAACGAATTG3'
IL-6 Reverse	3'CCATCTTTGGAAGGTTTCAGGTTG5'
IL-1β Forward	5'ATGATGGCTTATTACAGTGGCAA3'
IL-1β Reverse	3'GTCGGAGATTTCGTAGCTGGA5'
IL-12 Forward	5'CCTTGCACTTCTGAAGAGATTGA3'
IL-12 Reverse	3'ACAGGGCCATCATAAAAGAGGT5'

TNF- α Forward	5'CCTCTCTCTAATCAGCCCTCTG3'
TNF- α Reverse	3'GAGGACCTGGGAGTAGATGAG5'

Supplementary Table S2. Antibody specifications. All antibodies used in this study are specific for human antigens.

antibodies	Host Species	catalog number	vendor
anti-CASP1	Rabbit	ab207802	abcam,England
anti-EGR4	Rabbit	ab198197	abcam,England
anti- CD206	Rabbit	MA5-32498	Invitrogen, USA
anti- CD86	Rabbit	SJ20-00	HUABIO, China
anti- STAT3	Rabbit	SY24-08	HUABIO, China
anti- pSTAT3	Rabbit	SZ43-01-08	HUABIO, China
anti- Ki67	Rabbit	SR00-02	HUABIO, China
anti- EGR4	Rabbit	HA500445	HUABIO, China
anti- IL-10	Rabbit	JE59-71	HUABIO, China
anti- β - actin	Rabbit	6609-1-Ig	HUABIO, China
anti-GAPDH	Mouse	60004-1-Ig	Proteintech, China
Goat anti-Mouse	Mouse	SA00001-1	Proteintech, China
Goat anti-Rabb	Rabbit	SA00001-2	Proteintech, China
anti-human CD163-PE	Mouse	12-1639-42	Invitrogen, USA
anti-human CD86-PE	Mouse	12-0869-42	Invitrogen, USA