

Supplementary Information

Interaction between parasite-encoded JAB1/CSN5 and macrophage migration inhibitory factor proteins attenuates its proinflammatory function

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Protein	Gene ID	<u>Protein Identification Probability</u>	<u>Percent Coverage</u>
Ribosomal Proteins*	EHI_073600	100%	62%
Macrophage Migration Inhibitory Factor (MIF)	EHI_092370	100%	54%
Histone [#]	EHI_182990	100%	41%
Copine	EHI_140260	100%	38%
Ribonucleoprotein complex subunit 4	EHI_115300	100%	36%
JAB1/CSN5	EHI_050500	100%	33%
Molybdenum cofactor sulfurase	EHI_027700	100%	33%
Sulfate adenylyltransferase	EHI_197160	100%	32%
Inorganic pyrophosphatase	EHI_124880	100%	32%
Skp1	EHI_066770	100%	31%

Figure. S1 Top proteins detected by mass spectrometry. *Represents 40S ribosomal protein S15a. [#]Represents histone H2B.

Name	ID% w/ Hu-JAB1	Sim% w/ Hu-JAB1	Length (aa)	MW(Da)	IP
Human	100%	100%	334	37.5	6.1
<i>Entamoeba</i>	39%	61%	318	36.5	5.35
<i>Toxoplasma</i>	41%	54%	489	53.6	5.61
<i>Trypanosoma</i>	35%	53%	398	44.7	6.19
<i>Leishmania</i>	34%	53%	473	51.4	7.72
<i>Acanthamoeba</i>	71%	79%	311	34.8	5.87
<i>Naegleria</i>	75%	85%	310	34.8	6.56

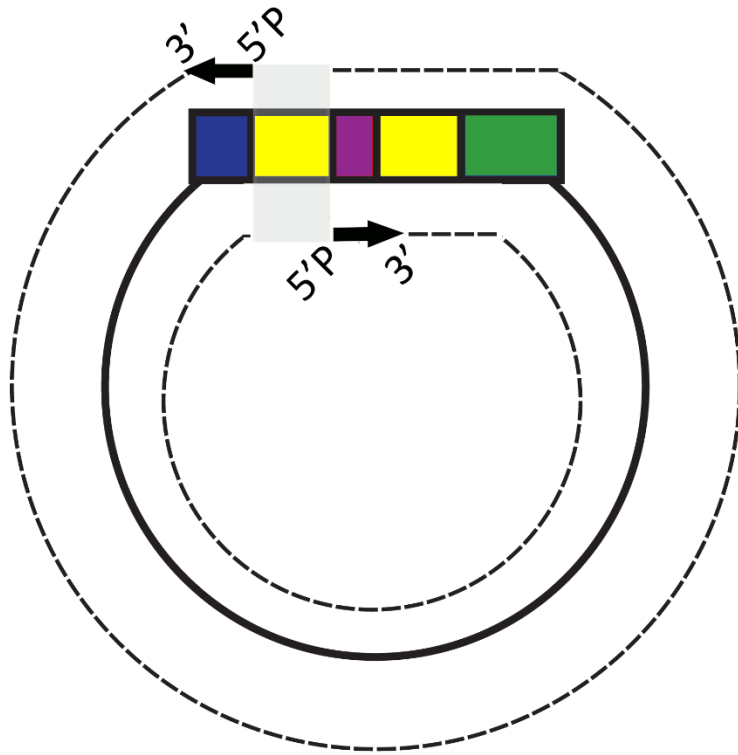
Figure. S2 Human JAB1/CSN5 (ENSG00000121022) orthologues in parasites: *Entamoeba histolytica* (EHI_050500), *Toxoplasma gondii* (TGARI_308590), *Trypanosoma Cruzi* (TcCLB.507083.60), *Leishmania donovani* (LBRM2903_160015100), *Acanthamoeba castellanii* (ACA1_074760), *Naegleria fowleri* (NF0060740). Listed are the level of their sequence identity and similarity with the human protein, length of amino acid sequence, molecular weight and isoelectric point.

Human	EMVVGWYHSHPGFGCWLSGVD
<i>Entamoeba</i>	ESIVGWYHSHPGFGCWLS SID
<i>Trypanosoma</i>	EKVVGWYHSHPGFGCWLSGED
<i>Leishmania</i>	ENVVGWYHSHPGFGCWLS SED
<i>Toxoplasma</i>	EMVVGWYHSHPGFGCWFSGTD
<i>Acanthamoeba</i>	EMVVGWYHSHPGFGCWLSGVD
<i>Naegleria</i>	EMVVGWYHSHPGFGCWLS SVD

Figure.S3 Alignment of the conserved JAMM motif of RPN11 from human (ENSG00000115233) and pathogenic parasites: *Entamoeba histolytica* (EHI_164750), *Toxoplasma gondii* (TGARI_269840), *Trypanosoma Cruzi* (TcCLB.503697.9), *Leishmania donovani* (LBRM2903_200012600), *Acanthamoeba castellanii* (ACA1_073080), *Naegleria fowleri* (NF0076640). Identical (green), conserved (blue), semi-conserved (pink), and non-conserved residues (red).

#	Sequence	Use
1	5' ACCCATGAAGCCTGCTTTTTTGTACAAA 3'	Δ3-50 <i>EhJAB1</i> (Δ1)
2	5' TGCTATGTTAGCAGCGTGGCA	
3	5' CTTTTTGAAATAATACGGATCTTTTTTCCACGG 3'	Δ51-133 <i>EhJab1</i> (Δ2)
4	5' CATAGCCATCCGAGCTATAAATGTTGG 3'	
5	5' ATACCAACCGCAAAACGGTTCITTTAAAACC 3'	Δ134-147 <i>EhJab1</i> (Δ3)
6	5' GTTGCAACCGAAAACTGCATCAGAGC 3'	
7	5' ATCAATACCGCTCAGCCAACATTTATAGCT 3'	Δ148-187 <i>EhJab1</i> (Δ4)
8	5' GGTTTTAAACCGCAGCAGAAAGCC 3'	
9	5' ATTCCGGAAAGGTACGAAAGGC 3'	Δ187-318 <i>EhJab1</i> (Δ5)
10	5' AAGACCCAGCTTTCTTGTACAAAGTG 3'	
11	5' ATGCCAGGGTATTAATCCAATATTCATGC3'	Δ247-318 <i>EhJab1</i> (Δ6)
12	5' AAGACCCAGCTTTCTTGTACAAAGTG 3'	
13	5' AACAAGTTTGTACAAAAAAGCAGGCTTC 3'	pDEST15- <i>EhJAB1</i> deletion screen
14	5' CACTTTGTACAAGAAAGCTGGGTCTTA 3'	
15	5' AAAAAAGGATCCATGGGTGAAGAGGCAGCATATA 3'	5'BamH1 - <i>EhJAB1</i> - 3' Xho1
16	5' AAAAAACTCGAGTTATTTGCCGGTCACTTTCTGA 3'	

Figure. S4 Primers used for making the *EhJAB1* deletion constructs by inverse PCR (#1-12), screening of deletion constructs (#13-14) and sub-cloning of *EhJAB1* in pGEX-4T1 vector (#15-16) are described.



Dpnl Digestion
(for clearing bacteria derived template)



Self ligation



Transformation into *E. coli*

Figure.S5 Schematic of the strategy and steps for making the deletion mutants of *EhJAB1*. Inverse PCR done with 5' phosphorylated primers.

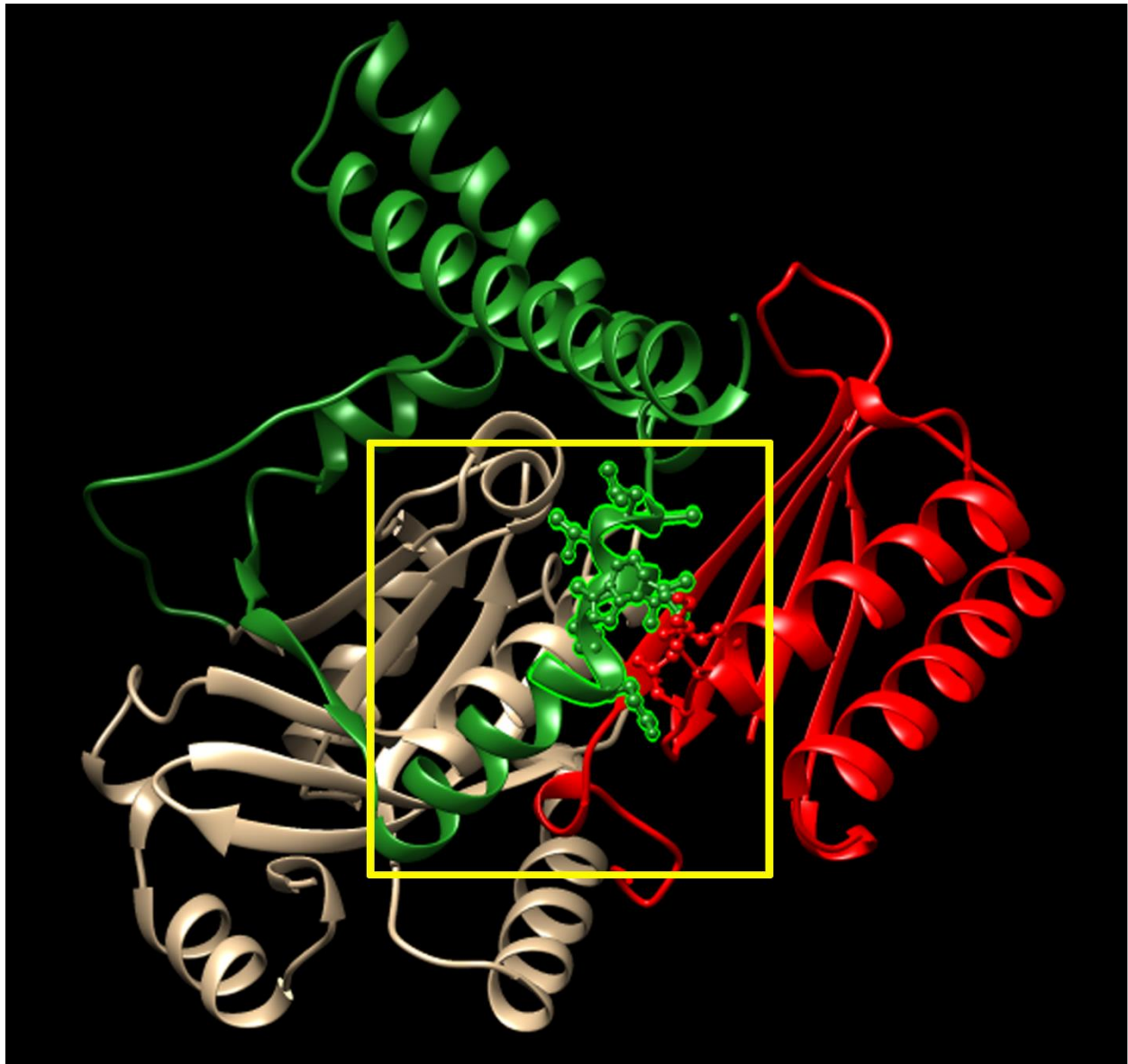


Figure.S6 Predicted *EhMIF-EhJAB1* interaction. *EhMIF* (red), *EhJAB1* C-terminal region (green) and rest of the *EhJAB1* protein (grey). The yellow box illustrates the C-terminal region of $\Delta 6$ *EhJAB1* mutant predicted to bind to *EhMIF*, and predicted *EhMIF* binding domain (64-69 aa) to *EhJAB1*.

Full-length gels and blots:

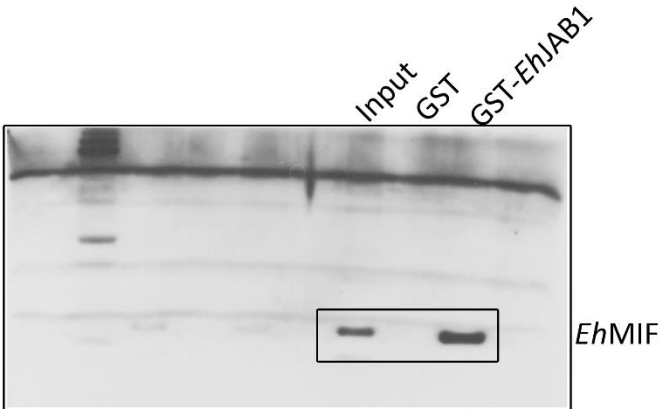


Figure 2C

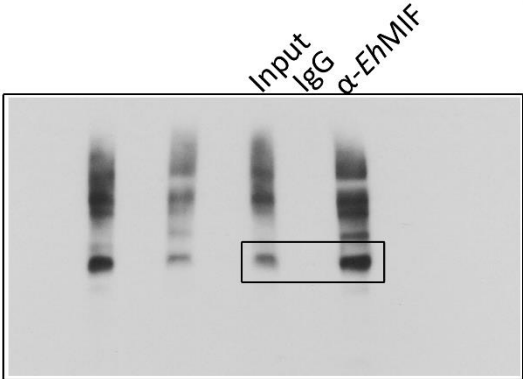


Figure 2E Top Panel

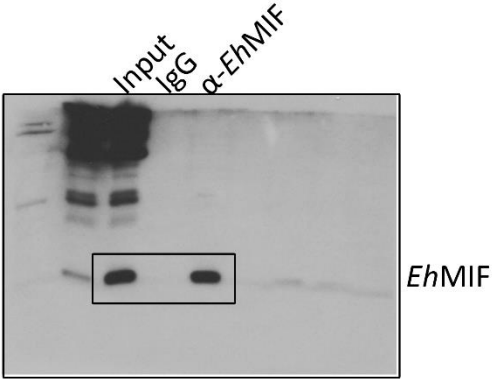
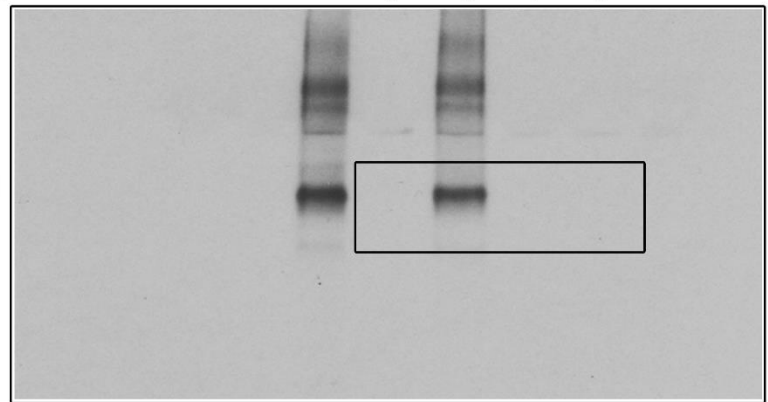


Figure 2E Bottom Panel

50nM *EhJAB1*^B
 0.5uM *EhJAB1*
 1uM *EhJAB1*
 400nM *EhMIF*

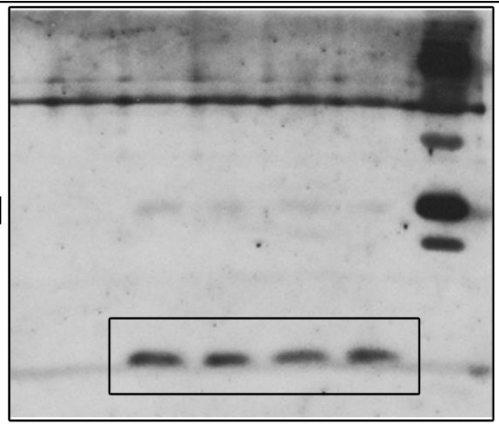
-	+	+	+
-	-	+	-
-	-	-	+
+	+	+	+

Figure 2F- Top Panel



EhJAB1^B

Figure 2F-Bottom Panel



EhMIF

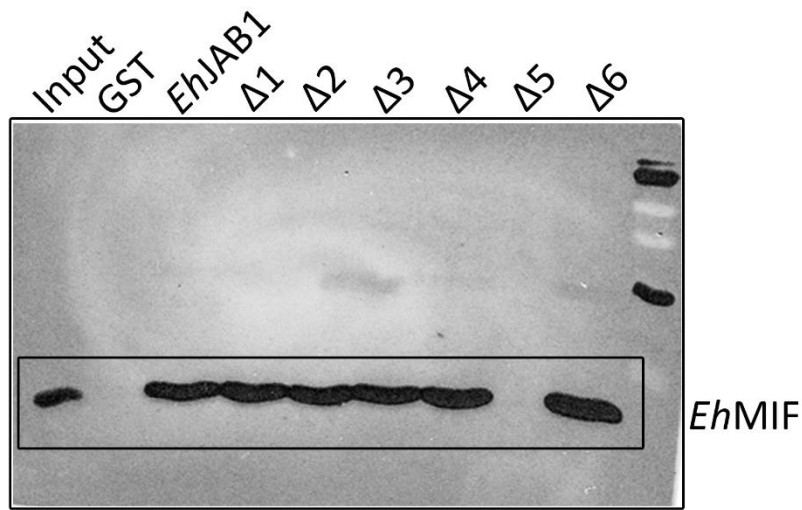


Figure 4C

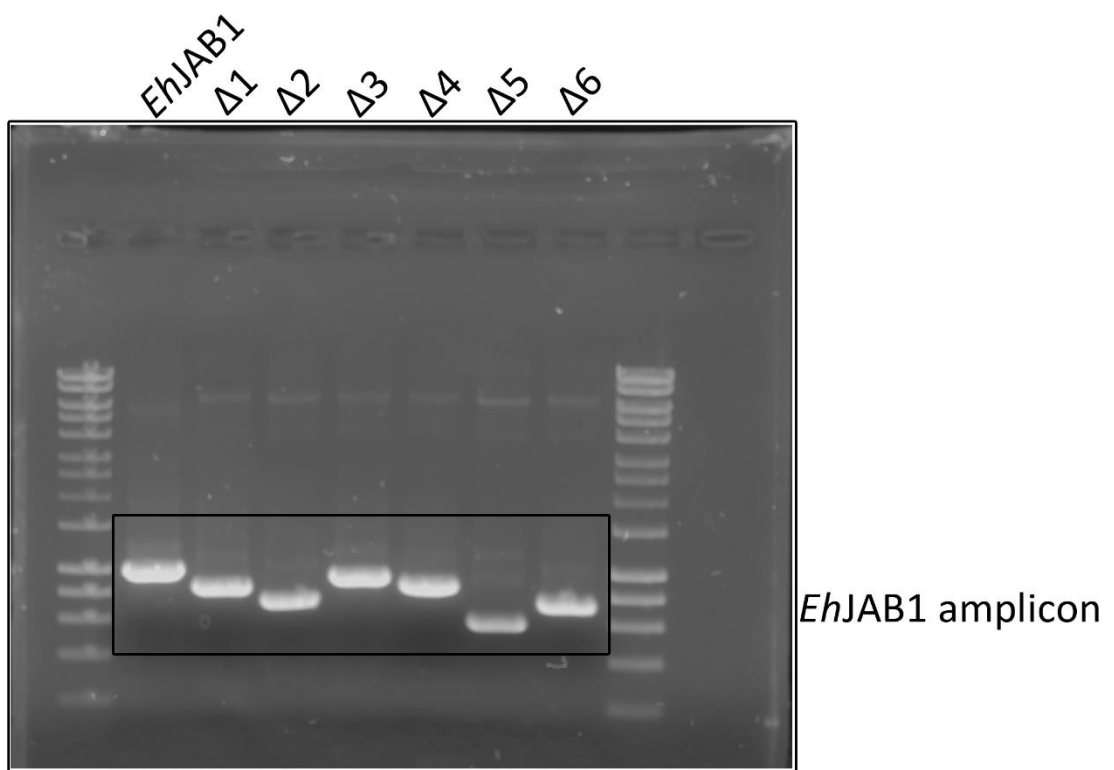


Figure 4D

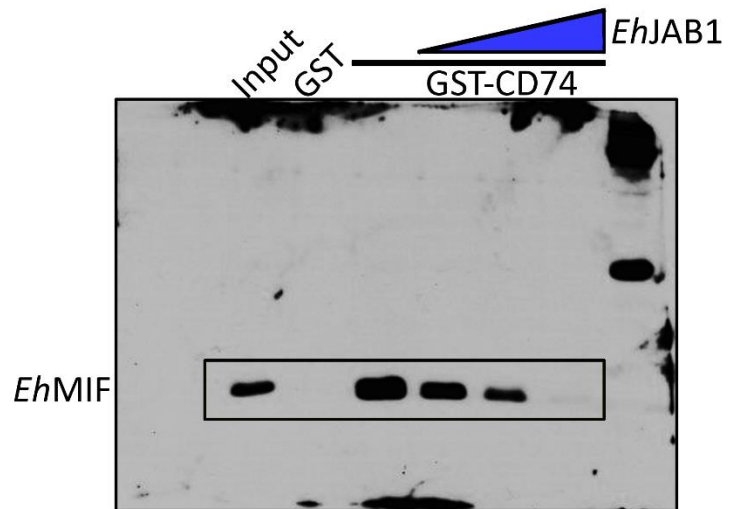


Figure 5A

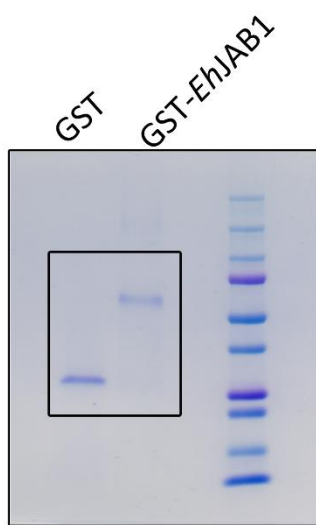


Figure 2B

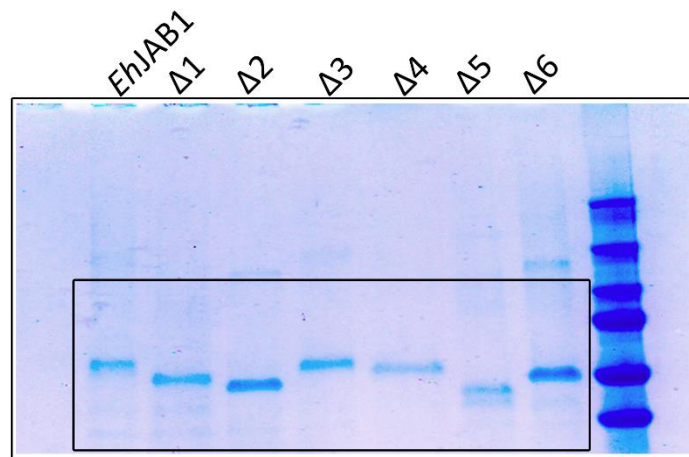


Figure 4C

Figure 3A and 3B raw data

