

Supplementary Data

**NLRP1 inflammasome drives IL-18 in Familial Mediterranean Fever and MEFV variants**

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**Running Title:** NLRP1 is associated with MEFV

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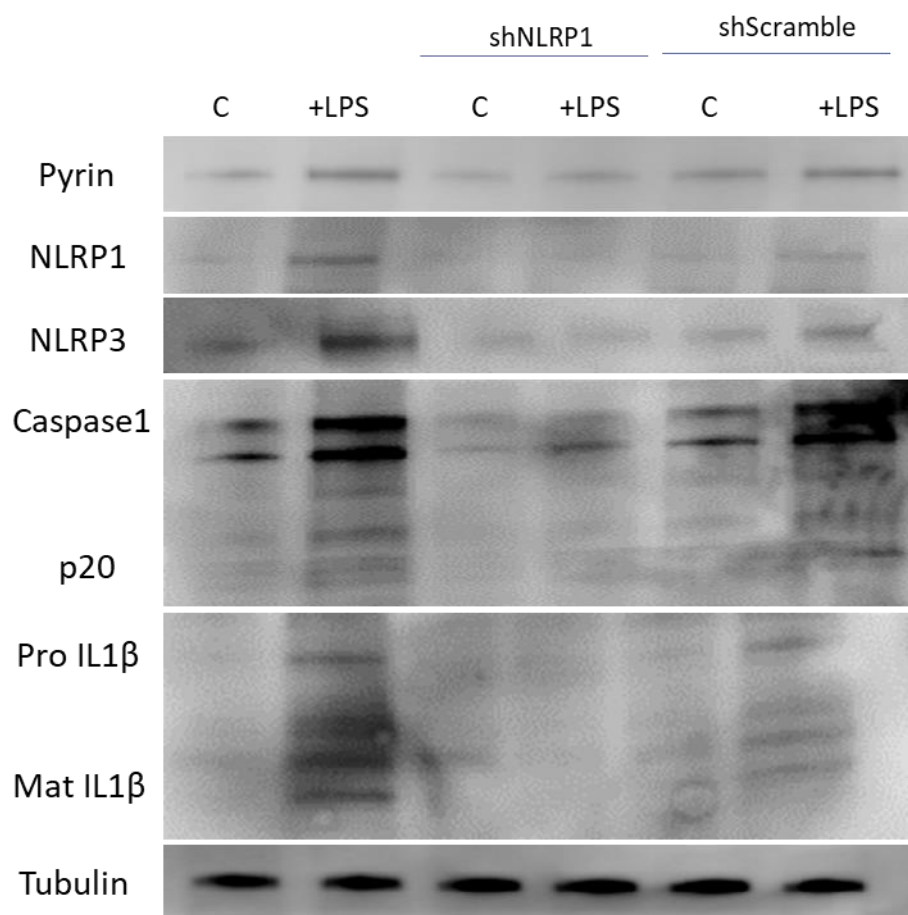
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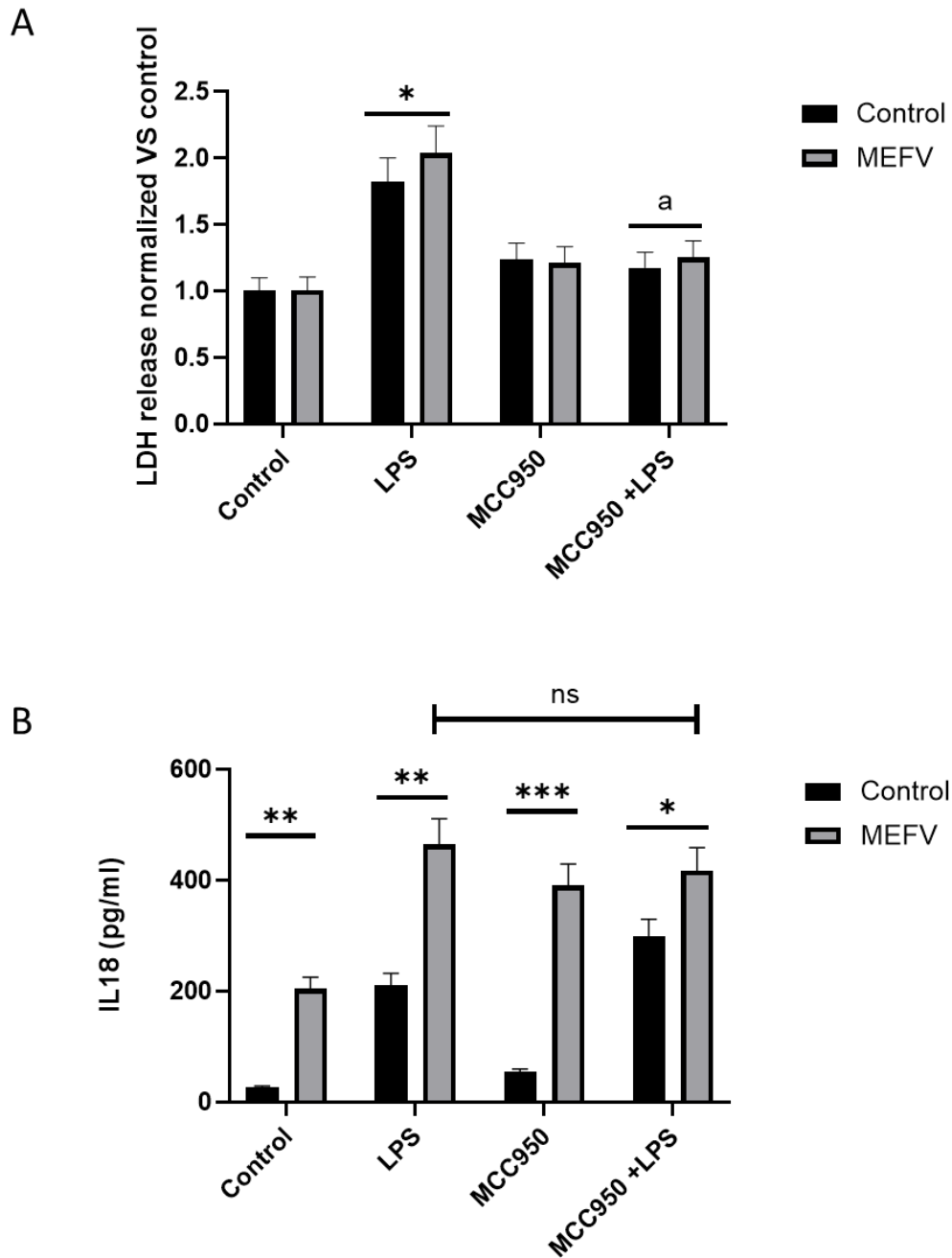
**Table 1.** Clinical characteristic and mutations in MEFV patients.

Case	Age	Sex	Mutation	Clinical presentation	Treatment
1	13	Male	c.383_391dupAGGGGAACG (p.Glu128_Asn130dup)	Fever (41°C max.), abdominal pain, oral aphthous, pharyngitis, headache, inflamed eyelid, psoriasis plaques	Paracetamol, ibuprofen, amoxicillin, stilsone, colchicine 1 mg/12h
2	49	Female	c.383_391dupAGGGGAACG (p.Glu128_Asn130dup)	Abdominal pain, fever (37,5°C max.), arthralgia, myalgia	Colchicine 500 mcg/24h
3	32	Female	p.Gly632Ser Heterozygote	Abdominal pain, fever (37,5°C max.), arthralgia, myalgia, oral aphthous	Paracetamol, metamizol, colchicine 500 mg/12h
4	16	Female	p.Met694Val Heterozygote	EBV infection, familial haemophagocytic lymphohistiocytosis, asthenia, nausea, fever (39°C máx.), arthralgia, myalgia, pharyngitis, abdominal pain	Metamizol, septrin forte, dexamethasone 1 mg/12h, Intravenous immunoglobulin, rituximab 550 mg/week
5	6	Male	p.Glu148Gln Homozygote p.Pro369Ser/p.Arg408Gln	Abdominal pain, diarrhea, fever (39,8°C	Ibuprofen, ciprofloxacin,

				max.), arthralgia, oral aphthous, pharyngitis, dactylitis, blepharitis, suppurative otitis	stilsone, colchicine 500 mcg/24h
6	31	Female	p.Glu148Gln Heterozygote p.Pro369Ser/p.Arg408Gln	Abdominal pain, chest pain, arthralgia, fever (37,5°C max.), global rash	Prednisone, colchicine 1,5 mg/24h, tocilizumab sc 162 mg/week
7	50	Male	p.Met694Val Homozygote	Abdominal pain, fever (37,5°C max.), arthralgia, myalgia	Ibuprofen, Colchicine 2 mg/12h
8	9	Male	p.Pro369Ser/p.Arg408Gln	Abdominal pain, arthralgia, fever (40°C max.), global rash, pharyngitis, oral aphthous, conjunctivitis, blepharitis	Stilsone, colchicine 500 mcg/12h, anakinra 150 mg sc/24h
9	51	Female	p.Pro369Ser/p.Arg408Gln	Abdominal pain, arthralgia, arthritis, myalgias, fever (38,5°C max.)	Colchicine 500 mcg/24h



**Supplementary Figure 1.** NLRP1 siRNA positive and Scramble controls. THP-1 cells were treated with either NLRP1 or scramble siRNA (25 pmol siRNA plus 250  $\mu$ l Lipofectamine RNAiMAX per well in a six-well plate) for 72 hours. Following this, the cells were stimulated with LPS for 4 hours. Western blot analysis revealed a significant reduction in NLRP1 and related proteins in the respective siRNA-treated cells.



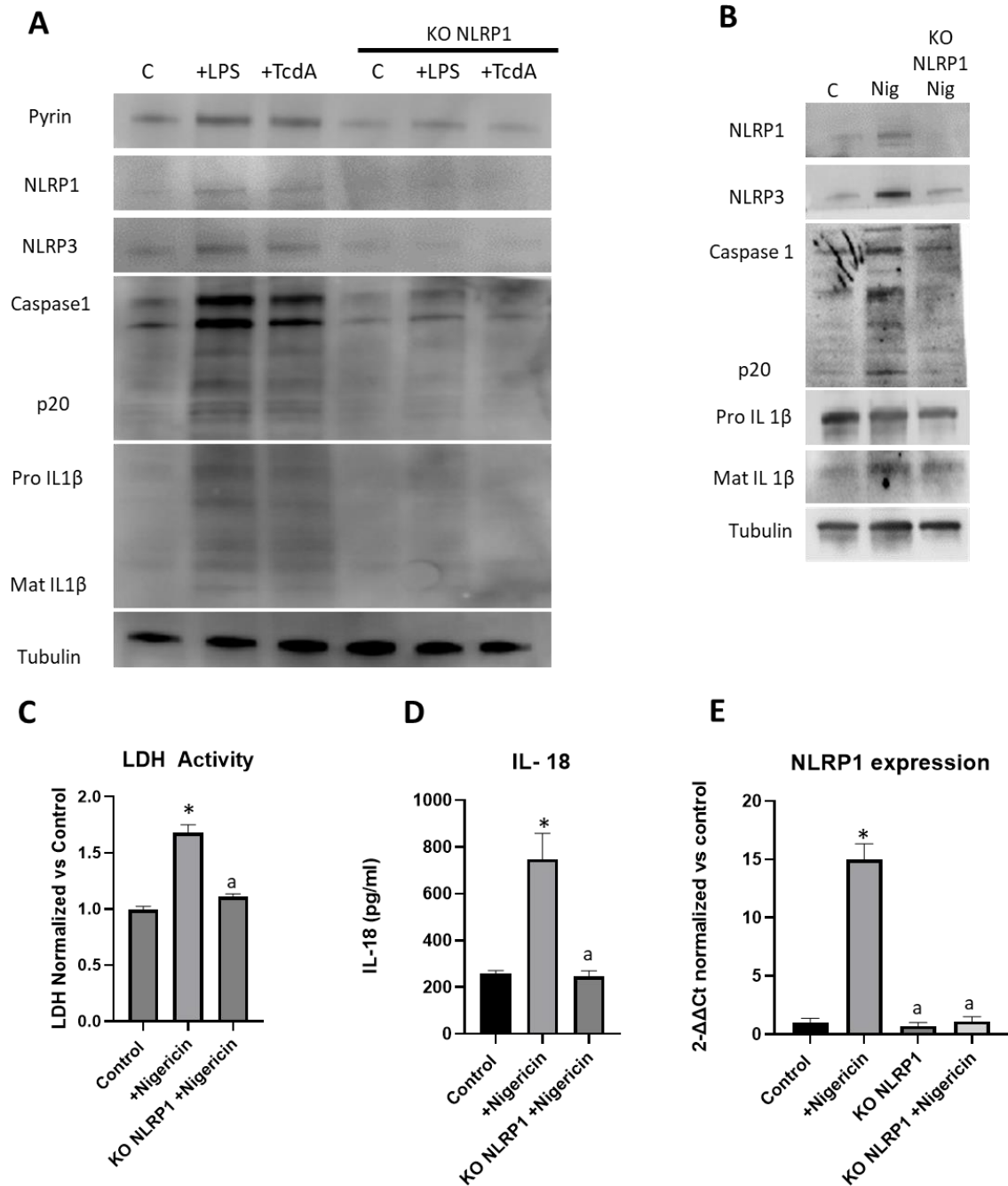
**Supplementary Figure 2. Effect of NLRP3 Inhibitor MCC950 on Patient-Derived PBMCs.**

Peripheral blood mononuclear cells (PBMCs) from patients were treated with the NLRP3 inhibitor MCC950 to assess its efficacy in the context of MEFV-related disease.

**A. LDH release following MCC950 treatment:** LPS stimulation resulted in elevated LDH release in both control and patient cells, which was mitigated by MCC950 treatment.

**B. IL-18 release levels measured by ELISA:** Cells with MEFV mutations exhibited high

baseline IL-18 release, which further increased following LPS stimulation or MCC950 treatment..\*  $p < 0.05$ , <sup>a</sup>  $p < 0.05$  (LPS *vs.* MCC950), ns = non-significant. Control samples were obtained from healthy subjects. n= 3



**Supplementary Figure 3.** NLRP1 CRISPR KO testing. THP1 cells were silenced according to the Materials and Methods section, then study stimulus (TcdA 100ng/ml for 3 hours or LPS at 1 mg/ml for 4 hours) were tested (A). Also, nigericin 10μl for 30' was used as strong NLRP1 and NLRP3 activator. NLRP1 KO reduced protein

expression (B), LDH release (C), IL-18 release (D) and NLRP1 mRNA expression (E).

\*  $p < 0.05$ , a  $p < 0.05$  (Nigericin vs. Nigericin+ KO NLRP1). n=3.