**Supplementary material to:**

**Haploinsufficiency of A20 impairs protein-protein intaractome and leads into caspase-8-dependent enhancement of NLRP3 inflammasome activation**

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**SUPPLEMENTARY MATERIALS AND METHODS**

***Genetic analysis***

Whole-exome sequencing was performed in the index patients (PII-1) using a SureSelect Clinical Research Capture Exome kit (Agilent, Santa Clara, CA, USA). The sequencing was performed using HiSeq 1500 Rapid run (Illumina, San Diego, CA, USA) in the Genomics Unit of the Institute for Molecular Medicine Finland (FIMM), as previously described1. The WES data were analyzed using a version 2.7 of the in-house developed analysis pipeline for quality control and variant identification (VCP)2 as described in more detail in *Trotta et al, 2018*3.

The frequency filtering was based on data from Genome Aggregation Database (http://gnomad.broadinstitute.org/;) and the *SISu* project (<http://sisu.fimm.fi/>)4 5. The rare variants affecting the coding regions were filtered based on the predicted consequences at the transcript level, with the selection of frameshift, nonsense, splicing and missense variants. The variants were evaluated according to the ACMG Standards and Guidelines6. Variant confirmation was performed using PCR amplification of genomic DNA and capillary electrophoresis using the ABI-3730XL DNA Analyzer and BigDye Terminator Cycle Sequencing kit (Applied Biosystem, Foster City, CA, USA). The same method was used for screening familial mutations in available family members.

***Creating A20 inducibly expressing Flp-In 293 T-REx cell lines***

The Flp-In293 T-REx cells (Invitrogen, Life Technologies) were cultured in DMEM (1.0 g/L glucose; D5523) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 50 mg/mL penicillin and 50 mg/mL streptomycin (all from Sigma).

Mutagenesis primers were TNFAIP3\_K91X\_forward 5’-GAAGTCCGGTAGCTTGTGGCGCTGAAAACGAAC-3’ and TNFAIP3\_K91X\_reverse 5’-CGCCACAAGCTACCGGACTTCTCGACACCAGT-3’.

**SUPPLEMENTARY TABLE 1 : Primer list for quantitative real-time PCR.** (F, forward primer; R, reverse primer)

|  |  |  |
| --- | --- | --- |
| **Primer** | **Sequence 5' -> 3'** | **Gene** |
| ASC\_F | TTGGACCTCACCGACAAGC | apoptosis-associated speck like protein containing a CARD domain |
| ASC\_R | ATGTCGCGCAGCACGTTA |
| B2M\_F | GAGTATGCCTGCCGTGTGAA | beta-2 microglobulin (housekeeping) |
| B2M\_R | TGCGGCATCTTCAAACCTCC |
| CASP1\_F | ATCCCACAATGGGCTCTGTTT | caspase-1 |
| CASP1\_R | CTCTTTCAGTGGTGGGCATCT |
| IFIT2\_F | GCCGAACAGCTGAGAATTGC | interferon induced protein with tetratricopeptide repeats 2 |
| IFIT2\_R | AGGCCAGTAGGTTGCACATTG |
| IFNB1\_F | GAGCTACAACTTGCTTGGATTCC | interferon beta 1 |
| IFNB1\_R | CAAGCCTCCCATTCAATTGC |
| IL1B\_F | TGGCAATGAGGATGACTTGT | interleukin-1 beta |
| IL1B\_R | GGAAAGAAGGTGCTCAGGTC |
| IL18\_F | TCAACTCTCTCCTGTGAGAACAAA | interleukin-18 |
| IL18\_R | GTCCTGGGACACTTCTCTGAAA |
| MX1\_F | CGAGATGTCCCGGATCTGACT | MX dynamin like GTPase 1 |
| MX1\_R | CACCACCAGGCTGATTGTCT |
| NLRP3\_F | CAACTGCAACCTCACGTCAC | NLR family pyrin domain containing 3 |
| NLRP3\_R | ACGGTCAGCTCAGGCTTTTC |
| RPLP0\_F | GAAATCCTGAGTGATGTGCAGC | ribosomal protein lateral stalk subunit P0 (housekeeping) |
| RPLP0\_R | TCGAACACCTGCTGGATGAC |
| TNF\_F | TGCTGCACTTTGGAGTGATCG | tumor necrosis factor alpha |
| TNF\_R | ATCTCTCAGCTCCACGCCATT |

**FIGURE LEGENDS**

**Supplementary figure 1.** Schematic illustration of the used affinity purification mass spectrometry (AP-MS) analysis for identification of stable protein-protein interactions and proximity labeling analysis with BioID for transient and close-proximity interactions.

**Supplementary figure 2. Inflammasome-dependent and –independent proinflammatory cytokine secretion in PBMCs of *TNFAIP3* p.(Lys91\*) carriers.** The experiment shown in Fig. 4, performed in commercial serum-free monocyte-macrophage culture media, was repeated here (A-C) using RPMI 1640 containing 10 % FBS. PBMCs from *TNFAIP3* p.(Lys91\*) mutation carriers, patients 1 (II-1, female, 30 years old) and 2 (III-1, female, 8 years old), were compared to sex- and age-matched controls 1 and 2, respectively**.** See the experimental details from Fig. 4 legend.

**Supplementary figure 3.** **Plasma IL-18 levels and baseline expression of inflammasome pathway components in *TNFAIP3* p.(Lys91\*) carrier PBMCs.** Samples from *TNFAIP3* p.(Lys91\*) mutation carriers, patients 1 (II-1, female, 30 years old) and 2 (III-1, female, 8 years old), were compared to sex- and age-matched controls 1 and 2, respectively**.** (A) Levels of circulating IL-18 in plasma were measured by ELISA. **B)** Relative gene expression in PBMCs was analyzed by quantitative PCR and normalized against housekeeping gene expression (arbitrary units).

**REFERENCES**

1. Trotta L, Hautala T, Hamalainen S, et al. Enrichment of rare variants in population isolates: single AICDA mutation responsible for hyper-IgM syndrome type 2 in Finland. *Eur J Hum Genet* 2016;24(10):1473-8. doi: 10.1038/ejhg.2016.37 [published Online First: 2016/05/05]

2. Sulonen AM, Ellonen P, Almusa H, et al. Comparison of solution-based exome capture methods for next generation sequencing. *Genome Biol* 2011;12(9):R94. doi: 10.1186/gb-2011-12-9-r94 [published Online First: 2011/10/01]

3. Trotta L, Martelius T, Siitonen T, et al. ADA2 deficiency: Clonal lymphoproliferation in a subset of patients. *J Allergy Clin Immunol* 2018;141(4):1534-37 e8. doi: 10.1016/j.jaci.2018.01.012 [published Online First: 2018/02/03]

4. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536(7616):285-91. doi: 10.1038/nature19057 [published Online First: 2016/08/19]

5. Lim ET, Wurtz P, Havulinna AS, et al. Distribution and medical impact of loss-of-function variants in the Finnish founder population. *PLoS Genet* 2014;10(7):e1004494. doi: 10.1371/journal.pgen.1004494 [published Online First: 2014/08/01]

6. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17(5):405-24. doi: 10.1038/gim.2015.30 [published Online First: 2015/03/06]