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1 **TITLE** Mosaic variants in *TNFRSF1A*: An emerging cause of tumor necrosis
2 **factor receptor-associated periodic syndrome**

3

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24 **KEY WORDS** Autoinflammatory disease, TRAPS, *TNFRSF1A*, mosaic, post-zygotic, variant,
25 NGS, genetic counseling.

26 ***ABBREVIATIONS***

- 27 CRD: cysteine-rich domain
- 28 CRP: C-reactive protein
- 29 DD: intracellular death domain
- 30 gnomAD: genome aggregation database
- 31 HGVS: human genome variation society
- 32 NGS: next generation sequencing (i.e. deep targeted sequencing)
- 33 NSAIDs: non-steroidal anti-inflammatory drugs
- 34 PBMC: peripheral blood mononuclear cell
- 35 S-S: disulfide bridge
- 36 SAA: serum amyloid A
- 37 SAIDs: systemic autoinflammatory disorders
- 38 SP: signal peptide
- 39 TM: transmembrane region
- 40 TNFR: tumor necrosis factor receptor
- 41 TNFRSF1A: tumor necrosis factor receptor superfamily 1A
- 42 TRAPS: tumor necrosis factor receptor-associated periodic syndrome
- 43 VAF: variant allele fraction
- 44 WBC: white blood cells

45 **ABSTRACT**

46 **Objective:** To identify the molecular basis of a systemic autoinflammatory disorder (SAID)
47 evocative of tumor necrosis factor receptor-associated periodic syndrome (TRAPS).

48 **Methods:** (i) Deep next generation sequencing (NGS) through a SAID gene panel; (ii) variant
49 allele distribution in peripheral blood subpopulations; (iii) *in silico* analyses of mosaic variants
50 using TNFRSF1A crystal structure; (iv) review of the very rare *TNFRSF1A* mosaic variants
51 reported previously.

52 **Results:** In a 36-year-old man suffering from recurrent fever for 12 years, high-depth NGS
53 revealed a *TNFRSF1A* mosaic variant c.176G>A p.(Cys59Tyr) that Sanger sequencing failed to
54 detect. This mosaic variant displayed a variant allele fraction of 14% in whole blood; it affects
55 both myeloid and lymphoid lineages. The p.(Cys59Tyr), a recurrent germline pathogenic variant,
56 affects a crucial cysteine located in the first cysteine-rich domain (CRD1) and involved in a
57 disulfide bridge. Introduction of a tyrosine at this position is expected to disrupt the CRD1
58 structure. Review of the three previously reported *TNFRSF1A* mosaic variants revealed that they
59 are all located in a small region of CRD2 and that germinal cells can be affected.

60 **Conclusion:** This study expands the localization of *TNFRSF1A* mosaic variants to the CRD1
61 domain. Noticeably, residues involved in germline *TNFRSF1A* mutational hot spots can also be
62 involved in post-zygotic mutational events. Including our study, only four patients have been so
63 far reported with *TNFRSF1A* mosaicism highlighting the need for a high-depth NGS-based
64 approach to avoid the misdiagnosis of TRAPS. Genetic counseling has to consider the potential
65 occurrence of *TNFRSF1A* mosaic variants in germinal cells.

66 ***KEY MESSAGES***

- 67 • In sporadic cases with clinical suspicion of TRAPS, search for *TNFRSF1A* mosaic
68 variants is recommended.
- 69 • High-depth NGS is required for detection of low-level mosaicism.
- 70 • The risk to transmit a *TNFRSF1A* variant to the offspring also exists for mosaic variants.

71 **INTRODUCTION**

72 Tumor necrosis factor receptor (TNFR)-associated periodic syndrome (TRAPS) is a rare systemic
73 autoinflammatory disorder (SAID) caused by heterozygous germline variants in the tumor
74 necrosis factor receptor superfamily member 1A (*TNFRSF1A*) gene (1). TRAPS is characterized
75 by recurrent episodes of high-grade fever lasting from 1 to 3 weeks accompanied by abdominal
76 pain, arthralgia, migratory erythematous plaques and patches involving the limbs and often
77 associated with underlying myalgia and ocular manifestations (2–4). During febrile episodes,
78 patients have elevated serum levels of acute phase reactants such as C-reactive protein (CRP) and
79 serum amyloid A (SAA). AA amyloidosis is a severe complication of chronic untreated
80 inflammation of TRAPS patients (5). In most patients, disease-onset occurs in the first decade of
81 life, although around 10% of patients are diagnosed beyond the age of 30 (6,7).

82
83 TRAPS is caused mainly by missense variants in *TNFRSF1A*, a gene that encodes the 55-kD
84 TNF receptor, TNFRSF1A (also known as TNFR1, TNFR, CD120a, p55), a transmembrane
85 protein belonging to the large TNF-receptor superfamily. TNFRSF1A comprises a 29-residue N-
86 terminal signal peptide (SP), an extracellular domain (ECD), a transmembrane region (TM), and
87 a C-terminal cytoplasmic region with a characteristic intracellular death domain (DD) (**Figure**
88 **1A and 1B**). The extracellular domain of TNFRSF1A consists of four cysteine-rich domains
89 (CRDs) (**Figure 1A and 1B**). The ligand-binding pocket for TNF is mainly formed by CRD2 and
90 CRD3, while CRD1, which mediates receptor self-assembly (homotrimerization) in the absence
91 of ligand, is also called “the pre-ligand-binding assembly domain” (PLAD). CRD1 is an essential
92 domain for efficient TNF binding and receptor function (8,9).

93
94 TRAPS was initially described in familial forms of SAID transmitted as an autosomal dominant
95 disease (1,3,10). It also has been reported in sporadic cases, due to *de novo* variants either in
96 germline (12) or more recently, in mosaic state (13–15). Here, we report a 36-year-old patient
97 presenting recurrent febrile attacks for more than 12 years in whom high-depth next generation
98 sequencing (NGS) revealed a *TNFRSF1A* mosaic variant that Sanger sequencing failed to
99 identify. We discuss, in light of the previous reports, the diagnostic challenges and consequences
100 of somatic mosaic *TNFRSF1A* variants.

101 ***PATIENT AND METHODS***

102 Patient's clinical features were collected using a standardized form. Written informed consent
103 was obtained from the patient participating in the study for genetic tests according to French
104 legislation and the principles of the Declaration of Helsinki.

105

106 ***Molecular analyses***

107 Genomic DNA was extracted from the patient's peripheral whole blood as well as from patient's
108 monocytes, neutrophils, T and B cells isolated from peripheral blood mononuclear cells (PBMCs)
109 by positive selection using immunomagnetic microbeads (MACS, Miltenyi Biotec) as previously
110 described (16).

111 Sanger sequencing: *TNFRSF1A* exons 2-4 and their intronic junctions were amplified by
112 polymerase chain reaction (PCR) and sequenced using the Big Dye Terminator v3.1 sequencing
113 kit on an ABI 3730XL automated capillary DNA sequencer (Applied Biosystems, Foster City,
114 CA). Sequences were analyzed using the SeqScape software (Applied Biosystems). The current
115 nomenclature of *TNFRSF1A* variants, which differs by 29 amino acids from the former
116 nomenclature in which the variant numbering began after the N-terminal signal peptide of the
117 protein, follows the Human Genome Variation Society (HGVS) recommendations
118 (<https://www.hgvs.org/>) and is based on the cDNA sequence (NM_001065).

119 Deep-targeted next generation sequencing (NGS): The DNA samples were extracted and
120 sequenced following a deep targeted sequencing approach (sequencing depth: 500 to 1000X)
121 using a NextSeq500 (Illumina) platform as previously described (16). We used a custom targeted
122 capture that contains the coding regions and flanking intronic sequences of the main genes so far
123 implicated in SAIDs. All the results obtained through this targeted sequencing were confirmed by
124 Sanger sequencing.

125

126 ***TNFRSF1A crystal structure analysis***

127 The protein 3D structure was rebuilt using human crystals of the extracellular domain (ECD), the

128 transmembrane region (TM), and the intracellular death domain (DD) of the human TNFRSF1A
129 (Protein Data Bank accession numbers, 1EXT (ref: 8939750), 7K7A (ref: 33163490), 1ICH (ref:
130 11453696), respectively. The 3D structures were visualized with the Pymol software.

131 In order to study *in silico* the structural changes involved by the p.(Cys59Tyr) *TNFRSF1A* variant
132 in the extracellular domain of TNFRSF1A, we used the corresponding human crystal structure
133 1EXT (A chain) available on Protein Data Bank (17) and PremPS
134 (<https://lilab.jysw.suda.edu.cn/research/PremPS/>) bioinformatics tool.

135

136 **RESULTS**

137 ***Disease phenotype***

138 The patient was a 36-year-old male, born to healthy non-consanguineous French parents, with no
139 family history of autoinflammatory or autoimmune disorders. He presented since the age of 24
140 years with recurrent episodes of fever (38-40C°) lasting for 1-2 weeks with spontaneous
141 resolution. The febrile attacks were accompanied by severe lower back pain and myalgia. During
142 his clinical course, the patient complained of recurrent thoracic pain and reported an acute
143 episode of pericarditis that lasted for several weeks and which was efficiently treated with
144 aspirin. He also developed migratory erythematous plaque eruptions localized at elbows and left
145 lower limb. From the age of 30, he also complained from recurrent attacks of abdominal pain.
146 The patient never displayed ophthalmological or neurological symptoms, nor lymphadenopathy
147 or organomegaly.

148 Laboratory tests performed during the febrile episodes showed elevated inflammatory biological
149 markers such as increased serum levels of CRP (171 mg/l) and leukocytosis (15,000 WBCs/ μ l).
150 Serum ferritin levels and kidney function tests were normal and extensive analyses of the
151 immunological and infectious profile were found to be negative. Between attacks, CRP levels
152 ranged from 6 to 36 mg/l. During the attacks, he was efficiently treated with analgesics and non-
153 steroidal anti-inflammatory drugs (NSAIDs).

154

155 ***Molecular diagnosis and 3D modelling of the TNFRSF1A regions surrounding the identified***
156 ***sequence variant***

157 With the aim of identifying the molecular basis of the patient's disease that was reminiscent of
158 TRAPS, Sanger sequencing of *TNFRSF1A* exons 2 to 4 (in which the great majority of the
159 variants are localized) was performed on DNA extracted from whole blood. No pathogenic
160 heterozygous *TNFRSF1A* variant was identified. To deepen the molecular investigations, the
161 DNA sample was re-analyzed by NGS using a panel containing the main genes so far implicated
162 in SAIDs. This high-depth sequencing approach enabled us to identify a somatic mosaic variant
163 in exon 2 of *TNFRSF1A* c.176G>A p.(Cys59Tyr) with a variant allele fraction (VAF) of 14% in
164 whole blood DNA. The p.(Cys59Tyr) variant is not described in the genome aggregation
165 database (gnomAD). Noticeably, the c.176G>A variant has already been reported as a recurrent
166 germline mutational event in TRAPS patients (7,18,19). Cys59 (also referred to as C30), has also
167 been involved in other germline missense pathogenic variants (C30R, C30S, and C30F)
168 (1,19,20). Located in the first cysteine-rich domain (CRD1) of *TNFRSF1A*, Cys59 is involved in
169 the second disulfide bridge (S-S2; Cys59-Cys72, also referred to as C30-C43) of CRD1 as shown
170 in the 3D structure of the protein (**Figure 1B** and **C-right panel**). Introduction at this position of
171 a tyrosine, an aromatic amino-acid, is expected to disrupt the CRD1 structure, by loss of the
172 disulfide bridge and by the establishment of several aromatic, polar and hydrophobic interactions
173 (**Figure 1C-left panel**).

174

175 ***Cellular distribution of TNFRSF1A mosaicism***

176 To study the cellular distribution of the *TNFRSF1A* mosaicism, different cell types were
177 subsequently isolated from the patient's PBMCs. The analyzed cells included monocytes,
178 neutrophils, B and T cells. Deep next generation sequencing showed a wide distribution of the
179 variant allele in the studied cells with average VAF of ~15% in myeloid cells (i.e. 15.8% in
180 monocytes and 14.1% in neutrophils) and ~9% in lymphoid cells (i.e. 8.9% in B cells and 9.6%
181 in T cells) (**Figure 2, lower panel**). Hindsight search for the *TNFRSF1A* variant c.176G>A p.
182 (Cys59Tyr) by Sanger sequencing revealed the presence of a superimposed sequence, mimicking
183 a background signal, but indeed corresponding to this variant (**Figure 2, upper Panel**).

184 ***Review of the previously reported TNFRSF1A mosaic variants***

185 To better assess the impact of *TNFRSF1A* mosaic variants in TRAPS, we reviewed the three
186 previously reported patients with a mosaic pathogenic variant in this gene (**Table 1**). The first
187 reported patient carried a *de novo TNFRSF1A* gonosomal mosaic variant (c.255_278del,
188 p.Ser86_Glu93del) with a VAF of ~21% in whole blood. He presented from early adolescence
189 recurrent febrile episodes that were lasting for 2 weeks and accompanied with both clinical and
190 biological inflammation (**Table 1**). The variant was detected at different fractions in the studied
191 tissues which included myeloid cells (19%), lymphocytes (20-30%), hair (4%), nails (11%) and
192 sperms (16%). The patient was efficiently treated with Anakinra (13).

193 The second reported patient carried a *de novo TNFRSF1A* missense somatic mosaic variant
194 (c.265T>C, p.Phe89Leu) with a VAF of ~30% in whole blood. He presented since the age of 54
195 with an intermittent daily fever over a period of 3-4 weeks and accompanied by systemic
196 inflammation and highly elevated CRP levels with leukocytosis (18,500 WBC/ μ l) (**Table 1**).
197 Kidney function tests were normal. The variant was detected in the patient's buccal swab, natural
198 killer (NK) cells, neutrophils, and B lymphocytes with an estimated VAF ranging from 25 to
199 45%, but with no variant detected in monocytes, T cells and hair roots. The administration of
200 Canakinumab which followed the identification of the molecular defect showed complete clinical
201 and biological remission (14).

202 In addition, a *TNFRSF1A* postzygotic missense variant (c.269C>A, p.Thr90Asn) with a very low
203 VAF of ~1.3% in whole blood was identified in a patient for whom phenotypic data are not
204 available (15). All these three mosaic variants were found to be clustered in a small region of the
205 CRD2 domain and involved residues Phe89-Thr90 (15). Noticeably, the Phe89 and Thr90
206 residues have already been involved in recurrent germline missense pathogenic events in TRAPS
207 patients (7,21,22). Modelling of *TNFRSF1A* 3D structure showed that these variants are
208 localized within the first loop of the CRD2 domain, a 16-residue-long loop formed by the first
209 disulfide bridge of CRD2 between Cys84 and Cys99 residues (S-S1 of CRD2, also referred to as
210 C55-C70) (**Figure 1B**).

211

212 ***DISCUSSION***

213 Post-zygotic mosaicism, defined by the presence of two or more cell populations with different
214 genotypes in the same individual, is an emerging field in systemic autoinflammatory diseases
215 adding to the complexity of monogenic disorders and raising new challenges from both clinical
216 and molecular viewpoints.

217 Here we illustrate the diagnostic challenges in relation with a *TNFRSF1A* mosaic variant in a 36-
218 year-old patient with no family history of autoinflammatory disease, presenting with recurrent
219 febrile episodes accompanied by systemic inflammatory manifestations. Despite broad biological
220 and radiological investigations carried out for more than 10 years, the underlying etiology
221 remained unknown. Although the clinical presentation of the patient was suggestive of TRAPS,
222 Sanger sequencing of *TNFRSF1A* failed to identify the causative variant. A NGS-based high-
223 depth sequencing analysis revealed a somatic mosaic pathogenic variant in *TNFRSF1A*
224 c.176G>A p. (Cys59Tyr) with a VAF of ~14% in whole blood, enabling us to establish the
225 diagnosis of TRAPS. The c.176G>A variant occurred on a CpG dimer in keeping with the fact
226 that a significant excess of variants occurs in CpG dinucleotides (23) leading to *de novo* pre-
227 zygotic (germline) or post-zygotic (mosaic) variants. Already known as a *TNFRSF1A* recurrent
228 germline variant, c.176G>A could also be a hot spot for mosaic variants. Moreover, despite the
229 advances in NGS-based sequencing technologies (including high-depth targeted NGS) that
230 allows identification of mosaic variants, only three patients with *TNFRSF1A* mosaic variants
231 have been reported so far suggesting that this diagnosis may be underreported.

232 It should be noted that the low-depth sequencing (about 20X to 100X) used in whole genome or
233 exome sequencing does not allow identification of low-level mosaicism. High-depth NGS (500-
234 1000X) is necessary to detect low-level mosaicism. However, detection of very low VAF (below
235 1-2%) remains challenging. To do so, specific deep sequencing method using single-molecule
236 molecular inversion probes (smMIP) was requested in a previously reported patient carrying a
237 *TNFRSF1A* mosaic variant (15).

238 The tissue distribution of the variant *TNFRSF1A* allele raises important questions on the
239 mechanism leading to mosaicism and the impact of such mosaicism on genetic counseling. Based
240 on the body parts and tissues that harbor the variant cells and the potential for transmission to

241 offspring, mosaicism can be divided into three types: the germline mosaicism (also known as
242 gonadal mosaicism); the somatic mosaicism; and the gonosomal mosaicism which is a
243 combination of both germline and somatic mosaicism (24). Notably, different tissue distributions
244 of the variant alleles were observed in two of the three previously studied patients. In the first
245 patient, who developed the disease at early adolescence, an in-frame mosaic *TNFRSF1A* deletion
246 (c.255_278del, p.Ser86_Glu93del) was detected with a VAF of ~21% in whole blood but also in
247 all tissues tested (VAF ranging from 4 to 30%) (13). In the second patient, who had an adult-
248 onset TRAPS, a *TNFRSF1A* mosaic missense variant (c.265T>C, p.Phe89Leu) was detected in
249 whole blood (VAF of 30%) and more precisely in neutrophils, NK cells, B lymphocytes and
250 buccal cells, but not in monocytes, T cells or hair samples (14). In our patient, in whom the VAF
251 was of 14% in whole blood, the variant allele was detected in two cell types of myeloid origin
252 (i.e. 15.8% in monocytes and 14.1% in neutrophils) as well as in lymphoid cells (i.e. 9.6% in T
253 cells and 8.9% in B cells). Taken together, these distributions are in favor of an early embryonic
254 *TNFRSF1A* mutational event and underline that genetic counseling should take into account the
255 fact that mosaic variants are not restricted to the disease-target cells (i.e. leukocytes in TRAPS)
256 and may be present in other cell lineages including germinal lineage. Interestingly, the
257 phenotypic severity of TRAPS does not appear to be correlated with the *TNFRSF1A* variant, the
258 germinal or mosaic status or the VAF of mosaic variants (1,7,13,14,18–20).

259 Our study extends the region targeted by mosaic variants from a small region (Phe89-Thr90) of
260 the CRD2 domain (15) to Cys59 located in the CRD1 domain. Among the six highly conserved
261 cysteine residues found in CRD1, Cys59 is believed to play a crucial role in the structure and
262 function of the receptor (1,20) and, according to our study, the p.(Cys59Tyr) pathogenic variant
263 is expected to disrupt the protein structure.

264 In conclusion, in sporadic cases of autoinflammatory disorders with long lasting episodes of fever
265 and no germline *TNFRSF1A* variant, search for a somatic mosaic variant in this gene should be
266 done by high-depth NGS. *TNFRSF1A* mosaic pathogenic variants can be located not only in the
267 CRD2 domain but also in CRD1 and the affected residues belong to the germline mutational
268 hotspots. As for genetic counseling, the stage of variant occurrence is of prime importance: while
269 germline variants have a one-in-two risk of being passed on to the next generation, this risk is
270 lower in case of post-zygotic variants and exists only if the variant affects the gonadal cells.

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272

273 **Authors' contribution:** Study design: IG and SA. Sample collection and clinical description:

274 GG. Data analysis: FDM, WP, BC. Tables and figures: EA, CL, EEK, JD. Data interpretation:

275 IG, ML, EA, CL. Writing of the manuscript: EA, IG and SA. Critical proofreading of the

276 manuscript and final approval of the submitted version: IG, SA, GG, SAK, ML, EA, CL, EEK,

277 JD, BC, FDM, WP.

278

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281

282 **Conflict of interest:** The authors have no conflict of interest to declare.

283

284 **Data availability statement:** The data are available upon request from the corresponding author.

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364

365 Table (1): Clinical and molecular characteristics of TRAPS patients with somatic mosaic
 366 *TNFRSF1A* variants.

	Current study	Rowczenio et al. (13)	Kontzias et al. (14)	Kant et al. (15)
<i>TNFRSF1A</i> genotype				
HGVS sequence name	c.176G>A	c.255_278del	c.265T>C	c.269C>A
HGVS protein name	p.(Cys59Tyr)	p.(Ser83_Glu93del)	p.(Phe89Leu)	p.(Thr90Asn)
Former nomenclature	C30Y	S54_E64del	F60L	T61N
VAF (% in whole blood)	14%	21%	30%	1.1-1.4%
Reported in germline state	Yes (7, 18, 19)	No	Yes (7, 21, 22)	Yes (7)
gnomAD (allele frequency)	0	0	0	0
Variant classification*	Pathogenic	Likely pathogenic	Pathogenic	Likely Pathogenic
Age at disease onset (Y)	24	Early childhood	54	NA
Age at diagnosis (Y)	36	41	60	NA
Gender	M	M	M	NA
Origin	France	United Kingdom	Caucasian	NA
Recurrent episodes	Yes (6 episodes)	10-12/Y	Every 7-8 weeks	NA
Duration (Weeks)	1-2	2	3-4	NA
Main symptom(s)	Recurrent fever and severe lower back pain	Recurrent fever and severe abdominal pain	Intermittent daily fever	NA
Thoracic symptoms	Acute pericarditis, recurrent thoracic pain	Pleuritic chest pain	No	NA
Neurological symptoms	No	Headache	Headache	NA
Cutaneous lesions	Migratory erythematous plaque eruptions at elbows and left lower limb	Generalized erythema	Erythematous non pruritic rash at trunk	NA
Lymphadenopathy	No	Cervical (painless)	Cervical (painless)	NA
Ocular symptoms	No	Red eye	Bilateral episcleritis	NA
Other symptoms	No	Night sweats and late-onset puberty	No	NA
CRP levels (mg/l) (during one of the attacks)	171	87	210	NA
Family history	No	No	No	NA
Treatment	NSAIDs	Anakinra	Canakinumab	NA

HGVS: Human Genome Variation Society, VAF: variant allele fraction, gnomAD: genome aggregation database, Y: year, NSAIDs: non-steroidal anti-inflammatory drugs, CRP: C-reactive protein, NA: not available, *according to *Infervers* database (25) (<https://infervers.umai-montpellier.fr/web/search.php?n=2>).

367 **Figure Legends:**

368 **Figure 1. *TNFRSF1A* variants and their impact at the protein level**

369 (a) *TNFRSF1A* protein domain-organization diagram. Location in the extracellular domain of the
370 protein of the c.176G>A p.(Cys59Tyr) pathogenic variant identified in this study (in red) and the
371 previously reported *TNFRSF1A* mosaic variants (Ser86_Glu93del, Phe89Leu and Thr90Asn) (in
372 black). SP: Signal peptide, CRD: Cysteine-rich domain, TM: transmembrane region, and DD:
373 intracellular death domain.

374 (b) **Upper part:** The *TNFRSF1A* 3D structure includes the four cysteine-rich domains (CRD);
375 CRD1 (blue), CRD2 (purple), CRD3 (green) and CRD4 (yellow) belonging to the extracellular
376 domain, the transmembrane region (TM) (dark red) and the intracellular death domain (DD)
377 (grey). The Cys59 residue (in red) involved in the sequence variant identified in this study is
378 located in CRD1. The previously reported mosaic variants are localized within the first loop of
379 CRD2 (in black). **Lower part:** An illustrative scheme of the amino acid sequence encompassing
380 the three disulfide bridges of CRD1 (S-S1, S-S2 and S-S3, in cyan) and the first disulfide bridge
381 of CRD2 (S-S1, in purple).

382 (c) *In silico* prediction of structural changes involved by the p.(Cys59Tyr) variant using PremPS
383 tool. **Right Panel:** Cys59 is involved in the second disulfide bridge (S-S2) of CRD1 domain. **Left**
384 **panel:** Cysteine-to-tyrosine substitution results in disruption of the disulfide bridge and in
385 establishment of a bulky cluster formed by the dense interaction between the tyrosine residue and
386 the surrounding amino acids.

387 Red balls indicate oxygen molecule; nitrogen atoms are in blue, sulfur atoms are in yellow and
388 carbon atoms are in grey. Dashed lines stand for the interacting bonds between the amino acids;
389 Polar interactions are in cyan, hydrophobic interactions are in blue and carbonyl interactions are
390 in orange.

391

392 **Figure 2. *TNFRSF1A* DNA sequencing in different cell types**

393 **Upper panel:** Sanger electrophoregrams showing the identified mosaic *TNFRSF1A* variant
394 c.176G>A p.(Cys59Tyr) (red). The red arrow shows the point mosaic variant (c.176G>A) which

395 is revealed by overlapping peaks of the WT nucleotide (G in black) and the variant one (A in
396 green). **Lower panel:** Fraction of the variant allele and ratio of the variant reads to the total
397 number of reads identified by a deep-targeted sequencing approach.

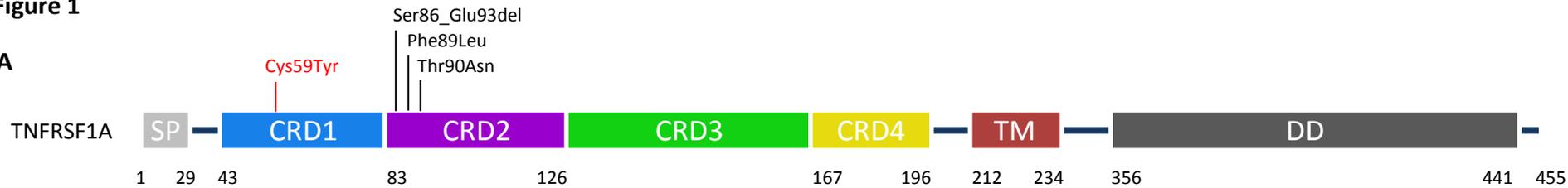
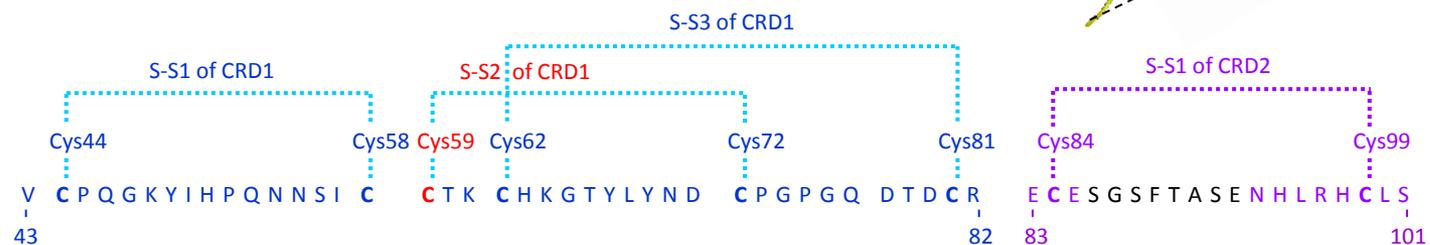
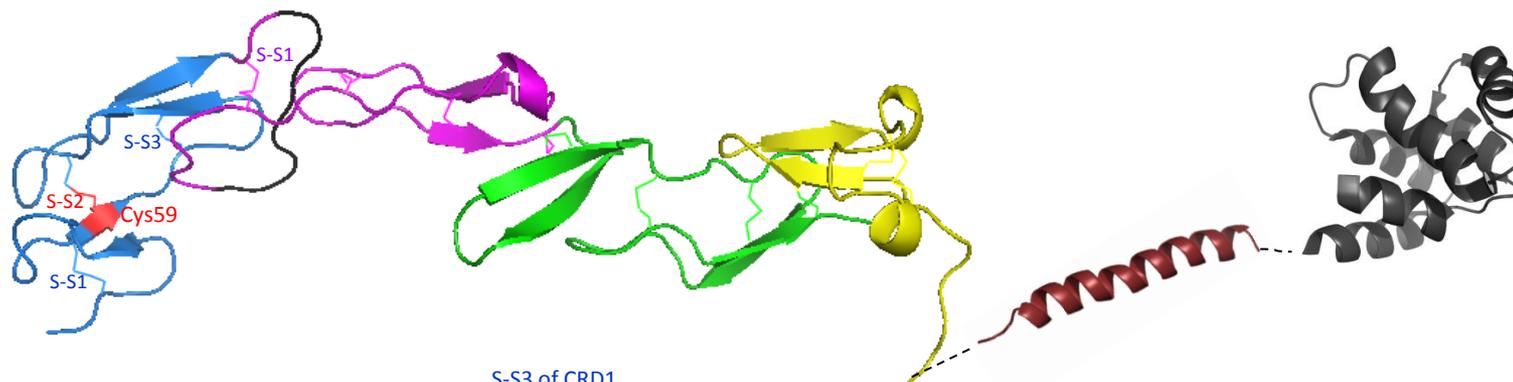
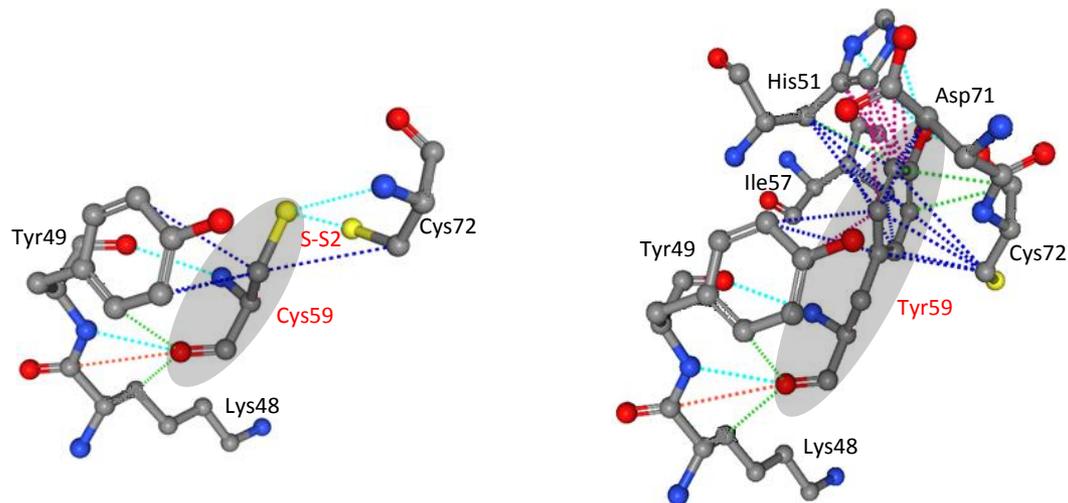
Figure 1**A****B****C**

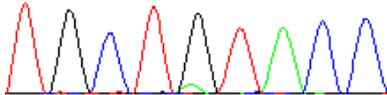
Figure 2

c.176G>A
p.(Cys59Tyr)

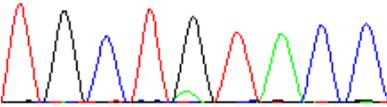
Cys58 Cys59 Thr60

T G C T G T A C C

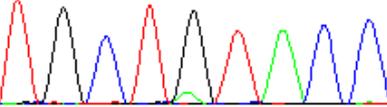
Peripheral blood



Monocytes



Neutrophils



B lymphocytes



T lymphocytes



Variant allele fraction
(variant reads / total reads)

Peripheral blood

14% (125/811)

Monocytes

15.8% (80/864)

Neutrophils

14.1% (59/368)

B lymphocytes

8.9% (130/889)

T lymphocytes

9.6% (85/899)