LETTER TO THE EDITOR

Characterization of a novel *FLI1* mutation in a family with thrombocytopenia and other congenital malformations

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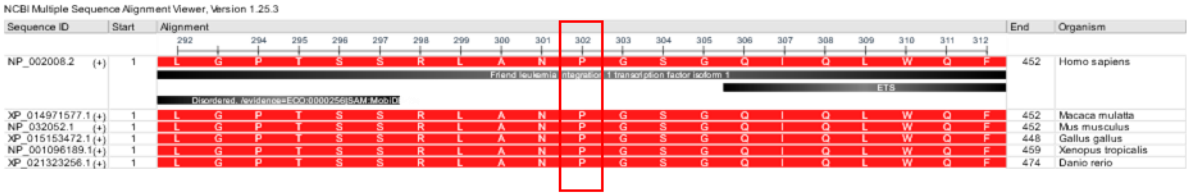
https://doi.org/10.3324/haematol.2024.287120

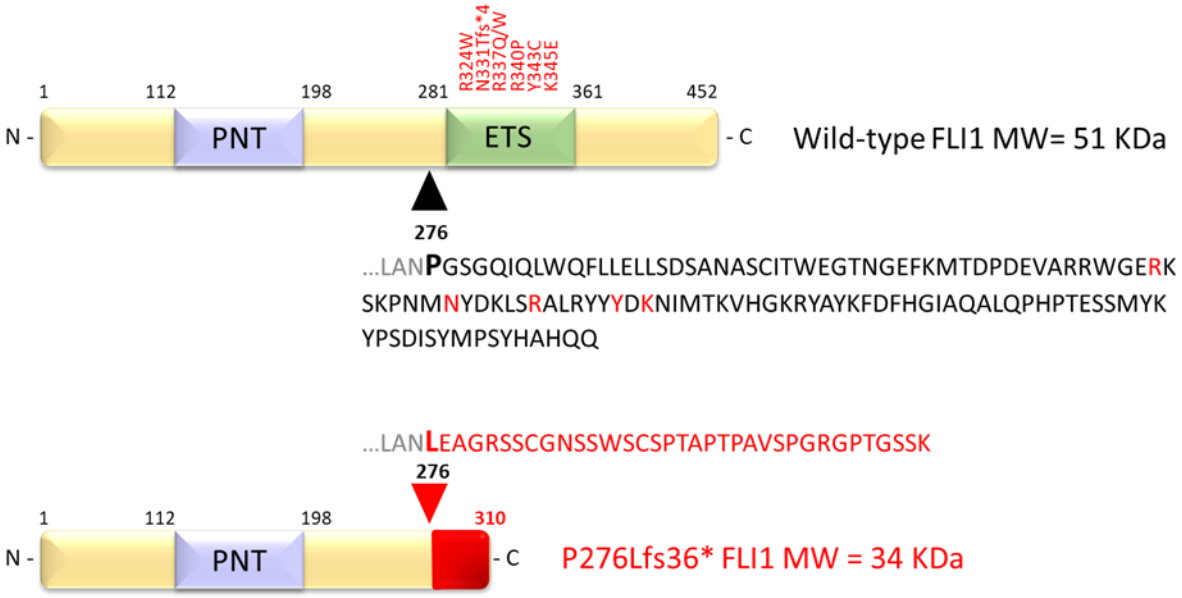
Received: December 23, 2024.
Accepted: May 26, 2025.
Early view: June 5, 2025.

Figure S1

A



B



C

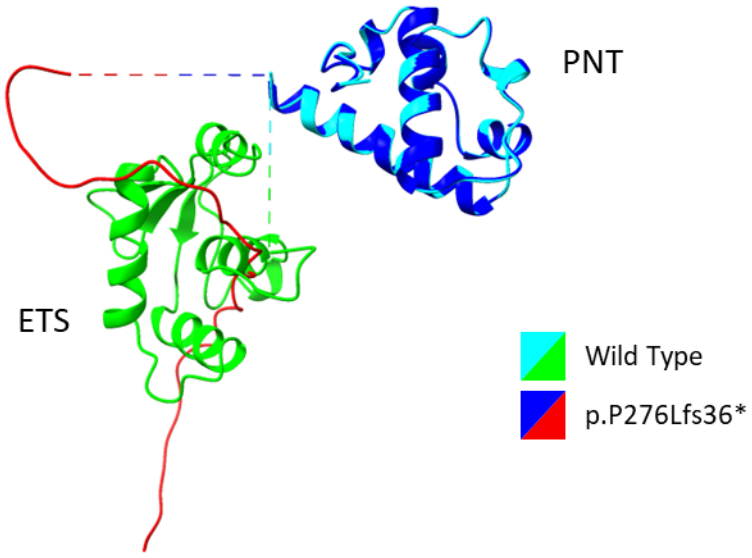


Figure S1. **Prediction of the impact of the mutation on FLI1 protein. A)** Aminoacidic conservation of P276 in different protein homologs across different species performed with the Constraint-based Multiple Alignment Tool (COBALT). **B)** Schematic representation of the sequence of the WT (51kDa) or p.P276Lfs36* truncated protein lacking the entire ETS domain (34kDa); (MW=molecular weight). The amino acid sequence of the frameshift product is indicated in red, compared to the wild-type sequence in black. The FLI1 functional domains are reported: PNT = pointed domain (residues 112-198, blue), ETS = E26 Transformation-Specific domain (residues 281-361, green). The FLI1-RT mutations identified so far, together with the involved aminoacids, are indicated in red in wild type FLI1. **C)** AlphaFold2 prediction of wild type (light blue-green) and mutant (dark blue-red) FLI1. Disordered regions (aminoacids 1-122, 202-266 and 374-452) visualization has been disabled to highlight the functional domains: the pointed domain (PNT, light or dark blue) and the ETS domain of wild type protein (green) or the neoynthesized frameshift product (red).

Figure S2

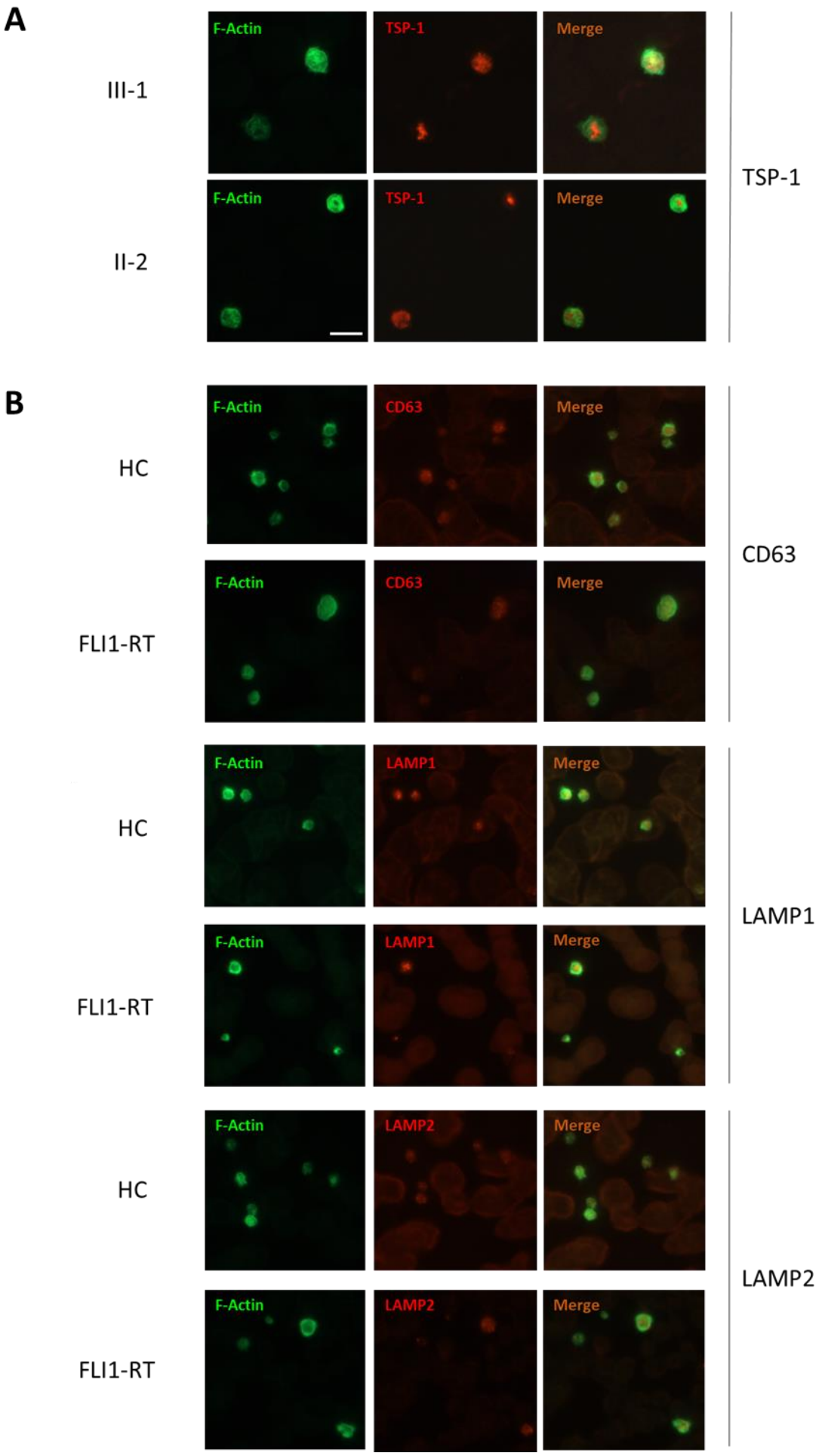

A
III-1
II-2
F-Actin
TSP-1
Merge
TSP-1
B
HC
FLI1-RT
F-Actin
CD63
Merge
CD63
HC
FLI1-RT
F-Actin
LAMP1
Merge
LAMP1
HC
FLI1-RT
F-Actin
LAMP2
Merge
LAMP2

Figure S2. Platelet phenotype of patients. A) Immunofluorescence staining of alpha granule marker thrombospondin 1 (TSP1, red, ab85762, Abcam) on proband (III-1) or her mother (II-2) blood smears. Platelets were identified by labeling for F-actin (green, Alexa Fluor 488 Phalloidin, A12379, Thermo Fisher Scientific). In the proband, the platelet at the bottom left shows typical confluence of alpha granules to form a single giant, irregularly shaped alpha granule, compared to the platelet at the top right which presents a normal, diffuse distribution of TSP1-positive alpha granules. **B)** Representative images of delta granules assessed by immunofluorescence staining of specific markers CD63 (556019, BD Pharmingen), LAMP-1 (sc-18821, Santa Cruz), and LAMP-2 (sc-18822, Santa Cruz) in platelets from healthy controls (HC) or III-1 and II-2 (FLI1-RT), showing a moderate reduction in delta granule content in patients compared to healthy controls.

Figure S3

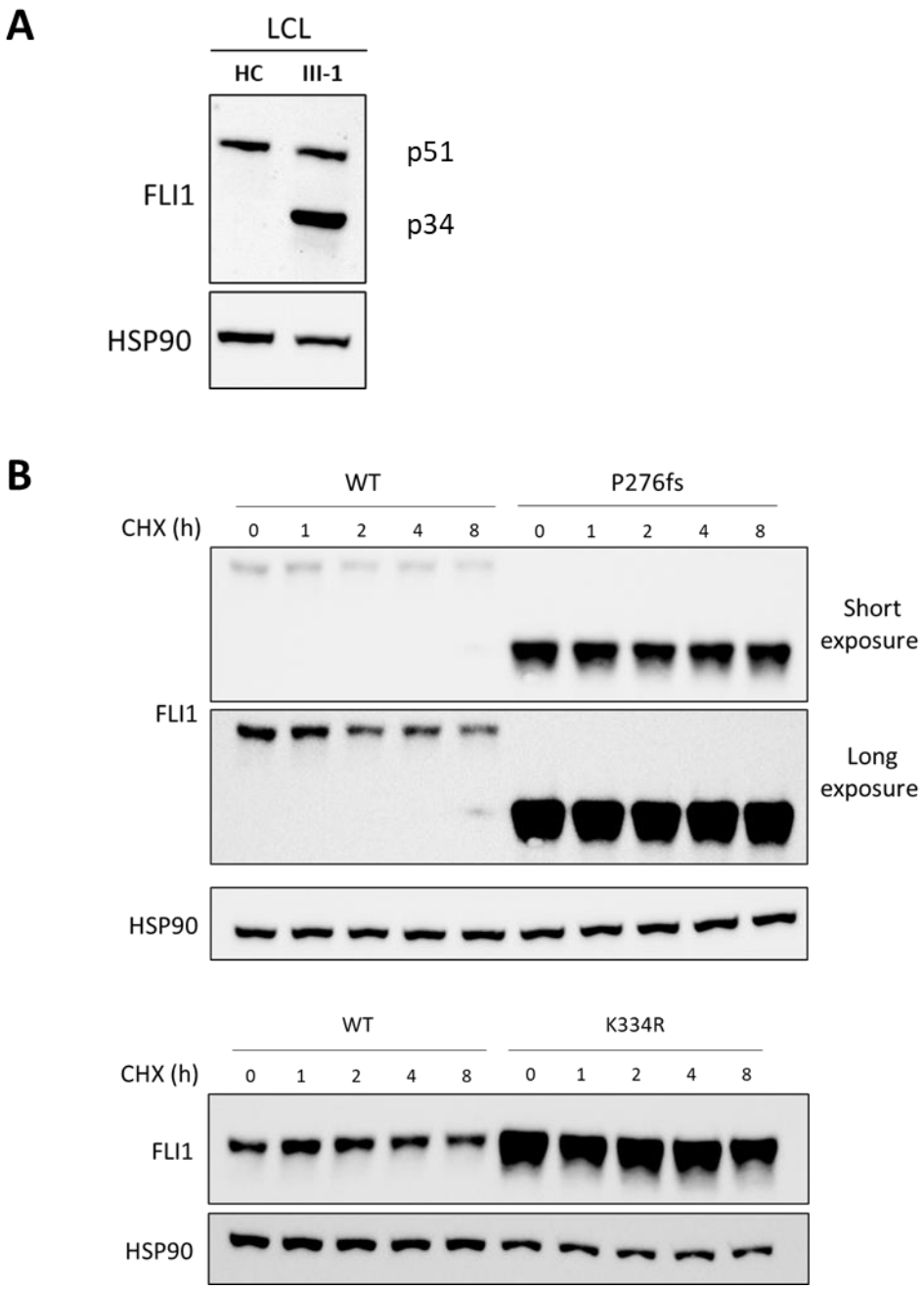


Figure S3. Mutant FLI1 (P276Lfs36*) expression and stability. A) Immunoblotting analysis using N-terminus FLI1 antibody (ab124791, Abcam) of whole-cell lysates from lymphoblastoid cell lines (LCL) established from proband (III-1) or a healthy control individual (HC). HSP90 (sc-13119, Santa Cruz) was used as loading control. **B)** Mutant FLI1 stability in HEK293T cells overexpressing the WT, P276Lfs36*, or K334R forms of FLI1. Immunoblotting analysis using N-terminus FLI1 antibody (ab124791, Abcam) of whole-cell lysates after treatment with cycloheximide (CHX) 50µg/mL at different time points (0,2,4,and 8 hours). HSP90 (sc-13119, Santa Cruz) was used as loading control.