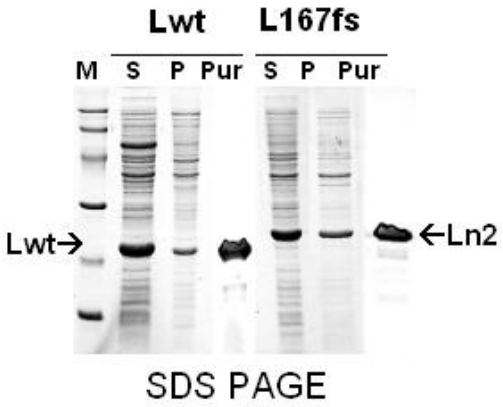


Supplemental S1

Figure S1. Expression and purification of the mutant L167fs. SDS-PAGE of the soluble (S) and insoluble (P) fractions of the homogenates of *E. coli* expressing Lwt and L167fs ferritins. Pur indicates the purified preparations.

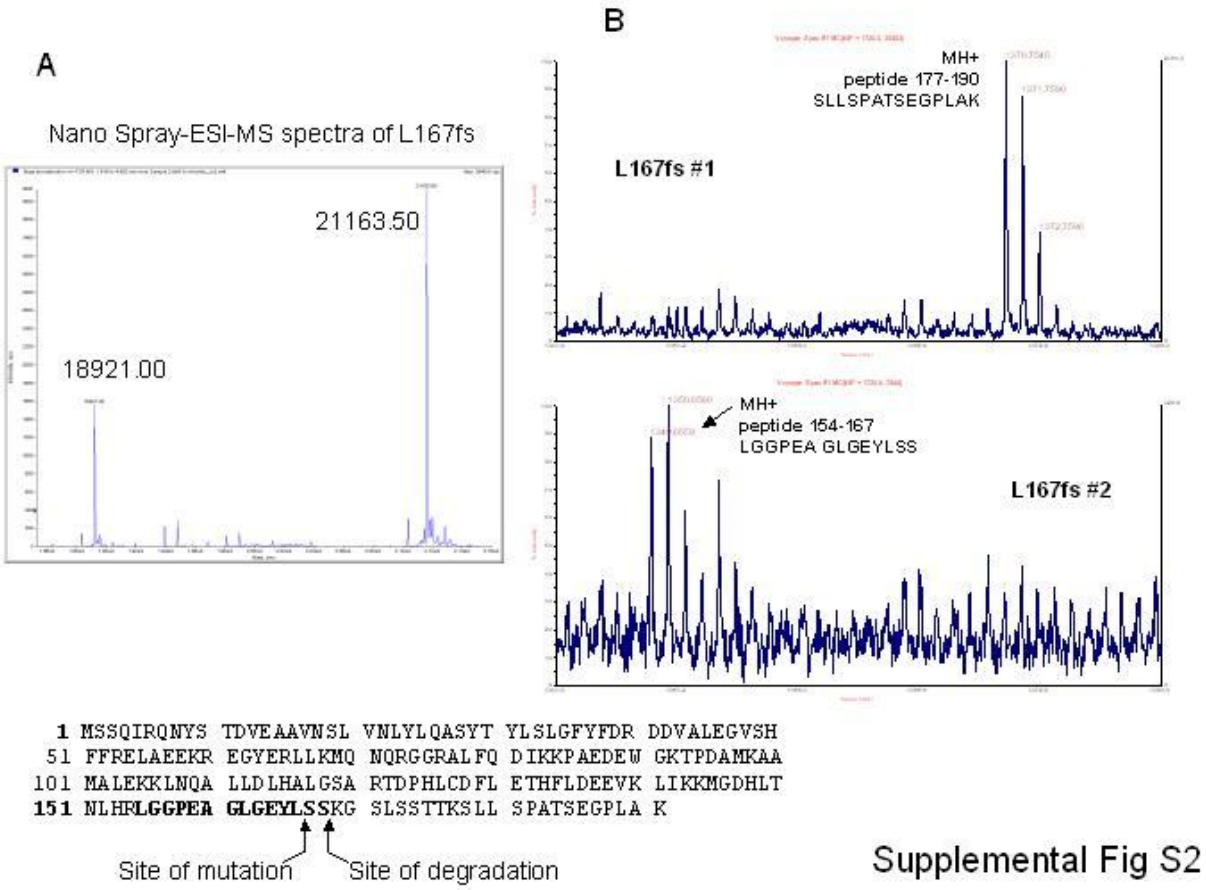
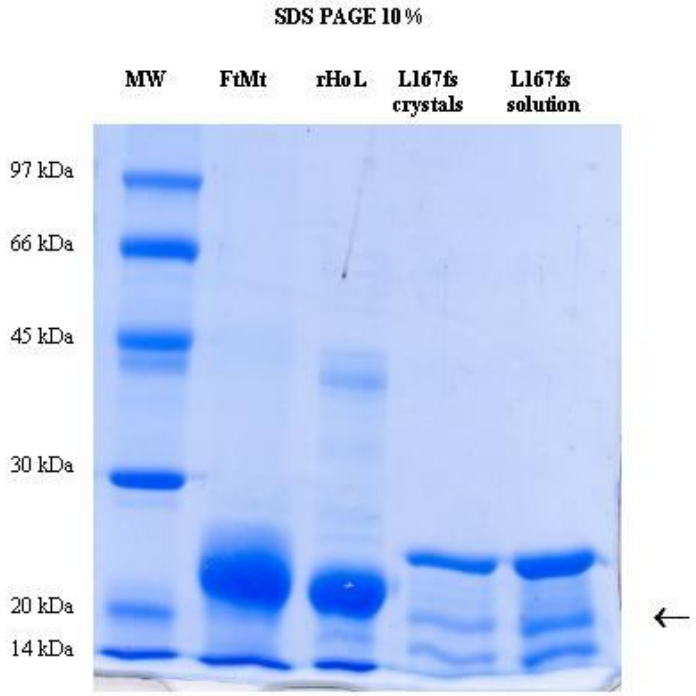


Figure S2. Electron spray analysis of L167fs. A: A 5-month old L167fs preparation shown in Fig 2C was subjected to nanoSpray-ESI-MS analysis and deconvoluted mass spectrum is shown. It contained a component of 21163.5 Da corresponding to the full protein deleted of the N-terminal methionine (residues 2-191, L167fs#1), and a component of 18921.0 Da, corresponding to the truncated subunit (residues 2-168, L167fs#2). The bold characters indicated the sequence determined by MALDI-TOF analysis. B: The same preparation was loaded on a SDS-PAGE stained with Coomassie blue and supernatants from trypsin digestion of bands L167fs#1 and L167fs #2 were analyzed by MALDI_TOF MS. Mass spectrum of band L167fs#2 showed the signal MH+ 1349.65 amu that corresponds to semi-tryptic peptide 155-168 LGGPEAGLGEYLSS not present in MS spectrum of band L167fs #1. Signal MH+ 1370.75 amu corresponds to peptide 179-191 with sequence SLLSPATSEGPLAK at the C-terminal of L167fs, present in MS spectrum of sample L167fs#1 and not L167fs#2. Instead, only in MS spectrum of sample L167fs #1 signals at 1477.76 amu, 2239.14 amu and 1370.75 amu were present. Those signals can be assigned to the tryptic peptides 155-169; 155-178 and 179-191 at C-terminal of L167fs. Thus the 155-168 peptide results from the cleavage of L167fs after Ser168, two residues downstream the site of the mutation.

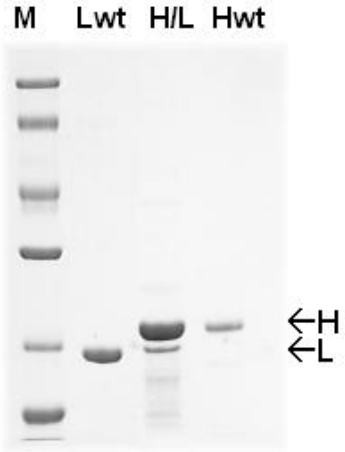


Supplemental S3

Figure S3. Integrity analysis of the crystallized L167fs protein by SDS-PAGE. L167fs crystals were washed and dissolved in water. L167fs solution is 9.89 mg/mL in 20 mM Tris-HCl pH 7.4, 0.1M sodium azide, 0.1M PMSF and 0.1M EDTA. MtF, human mitochondrial ferritin (Corsi *et al.* 2002) (MW 20948.3 Da), rHoL, recombinant horse L ferritin (Gallois *et al.* 1997) (MW 19830.4 Da), L167fs (MW 21 164.8 Da). The arrow indicates the position of the band which might correspond to the truncated mutant (MW 18922.3 Da). Theoretical MW are calculated for sequences without the initial methionine.

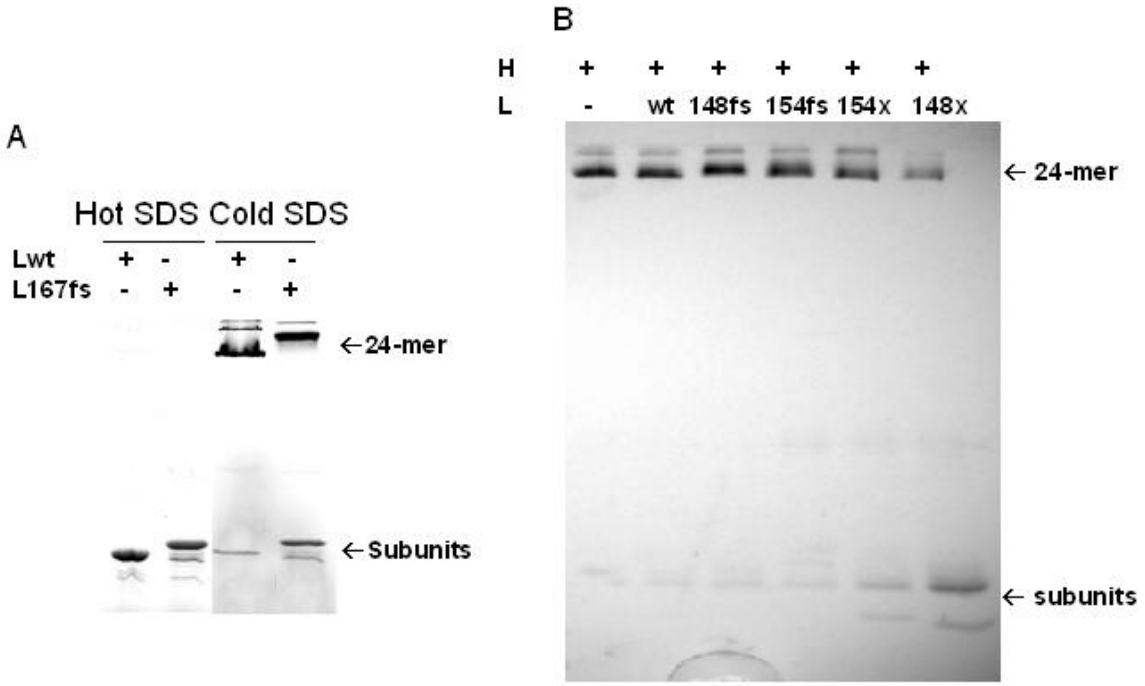
Corsi, B., Cozzi, A., Arosio, P., Drysdale, J., Santambrogio, P., Campanella, A., Biasiotto, G., Albertini, A. & Levi, S. Human mitochondrial ferritin expressed in HeLa cells incorporates iron and affects cellular iron metabolism. *J. Biol. Chem.* **277** (2002), pp. 22430–22437.

Gallois, B., Langlois d'Estaintot, B., Michaux, M.A., Dautant, A., Granier, T., Précigoux, G., Soruco, J.A., Roland, F., Chavez-Alba, O., Herbas, A & Crichton, R.R. X-ray structure of recombinant horse L-chain apoferritin at 2.0 Å resolution: implications for stability and function. J. B. I. C. (1997) 2, 360-367.



Supplemental S4

Figure S4. Heteropolymers expressed by the bicistronic vector pET-H/Lwt. The ferritin subunits expressed by the bicistronic vector (H/L) were analyzed on SDS-PAGE and compared with the recombinant Hwt and Lwt homopolymers. The heteropolymer contained about 90% H chain and 10% L chain. M: molecular weight markers. The arrows indicate H and L subunits.



Supplemental S5

Figure S6. Stability analysis of L167fs homopolymer and of the heteropolymers. The purified proteins were analyzed by discontinuous SDS-PAGE to separate the assembled 24-mer ferritin shells from the disassembled subunits. A: Analysis of Lwt and L167fs homopolymers treated with 1% SDS before (cold SDS) or after heating at 100°C (hot SDS). B: similar analysis of the indicated heteropolymers which were incubated with 1% SDS at room temperature without heating.