Supporting Information

Disruption of the CD loop by Enzymatic Cleavage Promotes the Formation of Toxic Transthyretin Oligomers through a Common Transthyretin Misfolding Pathway

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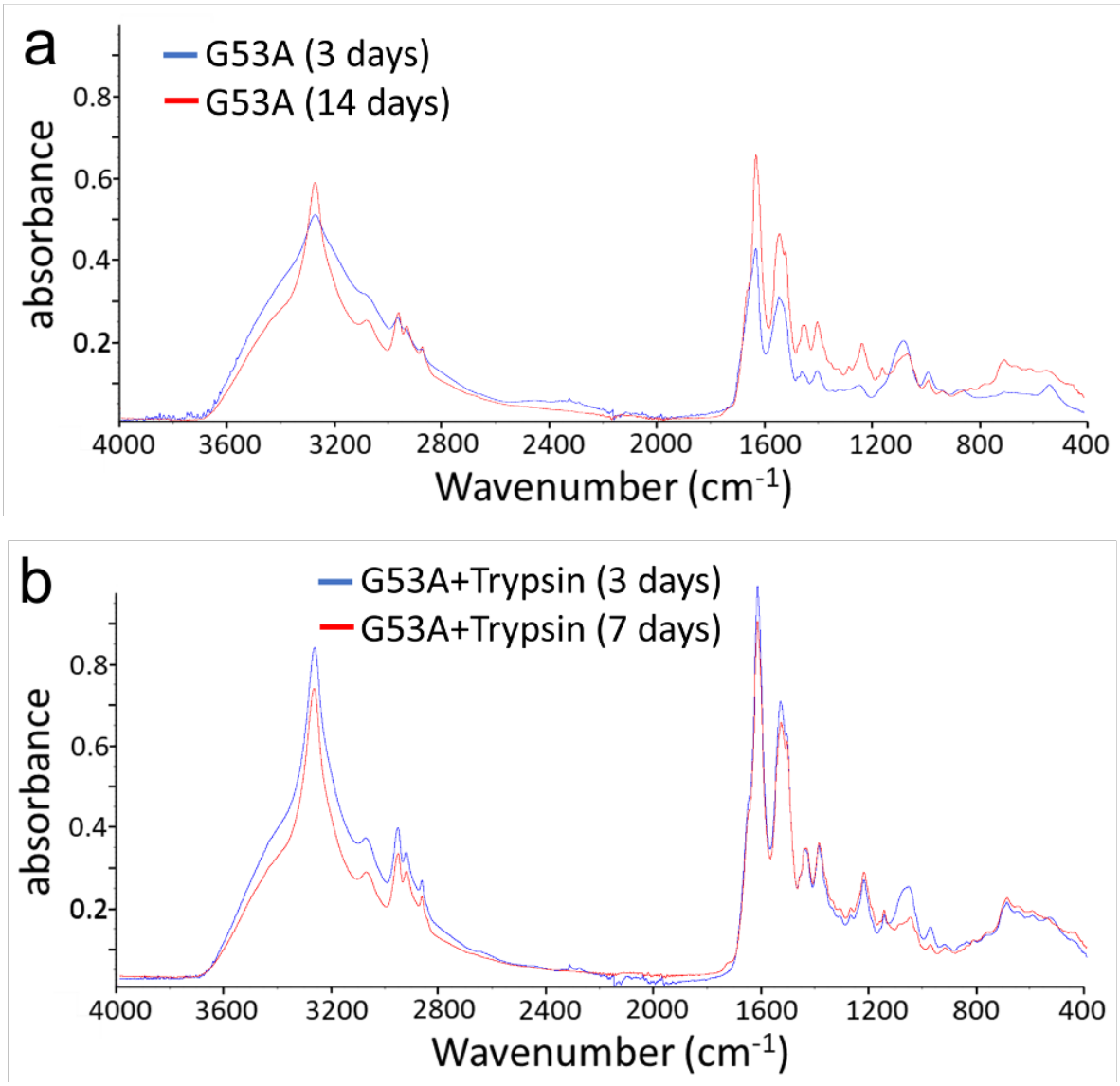

Figure S1. FT-IR spectra of the TTR aggregates formed at different incubation times in the absence (a) and presence (b) of trypsin. Approximately, 6 and 12 μg of the aggregates were used for the FT-IR spectra in (a) and (b), respectively.

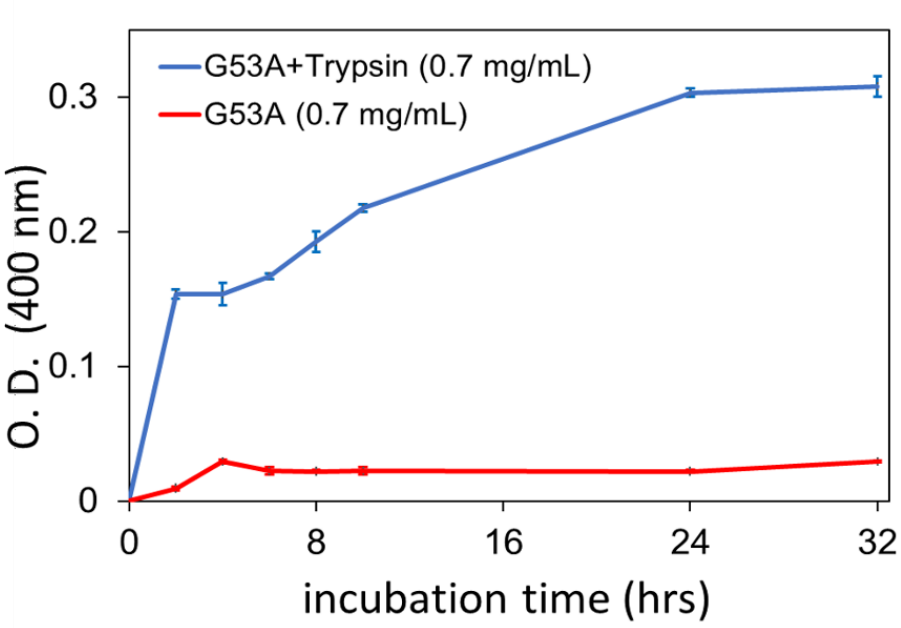

Figure S2. Aggregation kinetics of G53A TTR (0.7 mg/ml) in the presence and absence of trypsin. A ratio by weight of 200:1 protein to enzyme was used. Optical density (O. D.) was measured at 400 nm for turbidity of the samples.

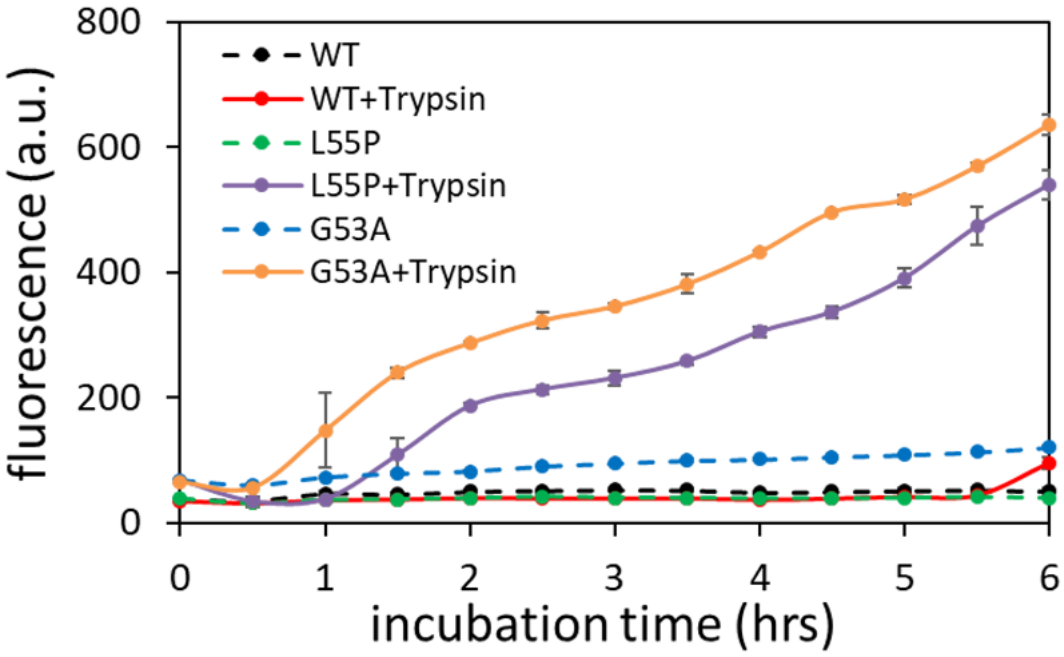

Figure S3. Aggregation kinetics of WT, G53A and L55P TTR (0.3 mg/ml) in the presence and absence of trypsin. A ratio by weight of 200:1 protein to enzyme was used. ThT fluorescence emission was monitored every 30 min at 482 nm with excitation of 440 nm

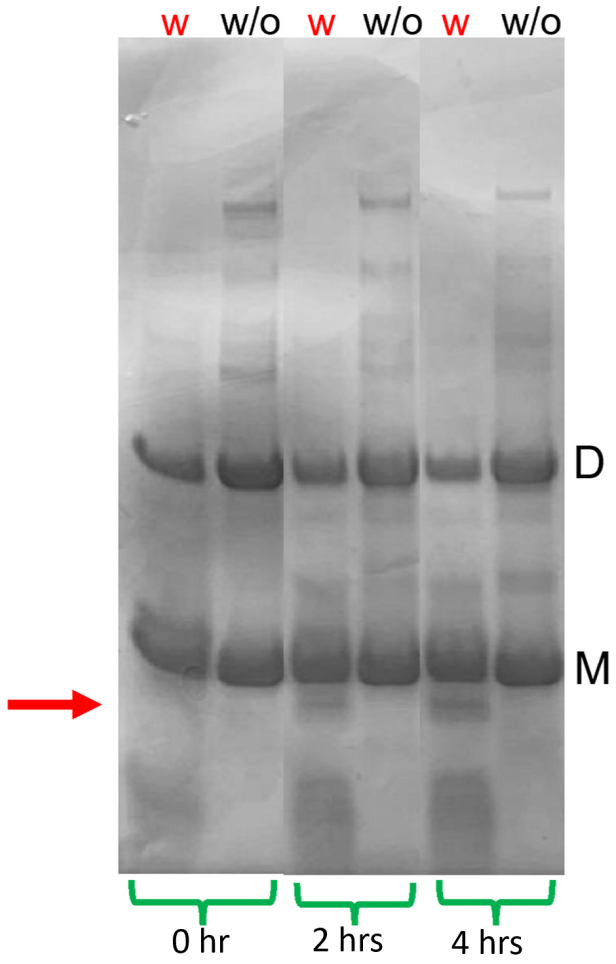

Figure S4. SDS-PAGE of the G53A TTR (0.3 mg/ml) in the presence (w) and absence (w/o) of trypsin incubated at different times (0 hr, 2 hrs, and 4 hrs). The red arrow indicates the TTR fragment.

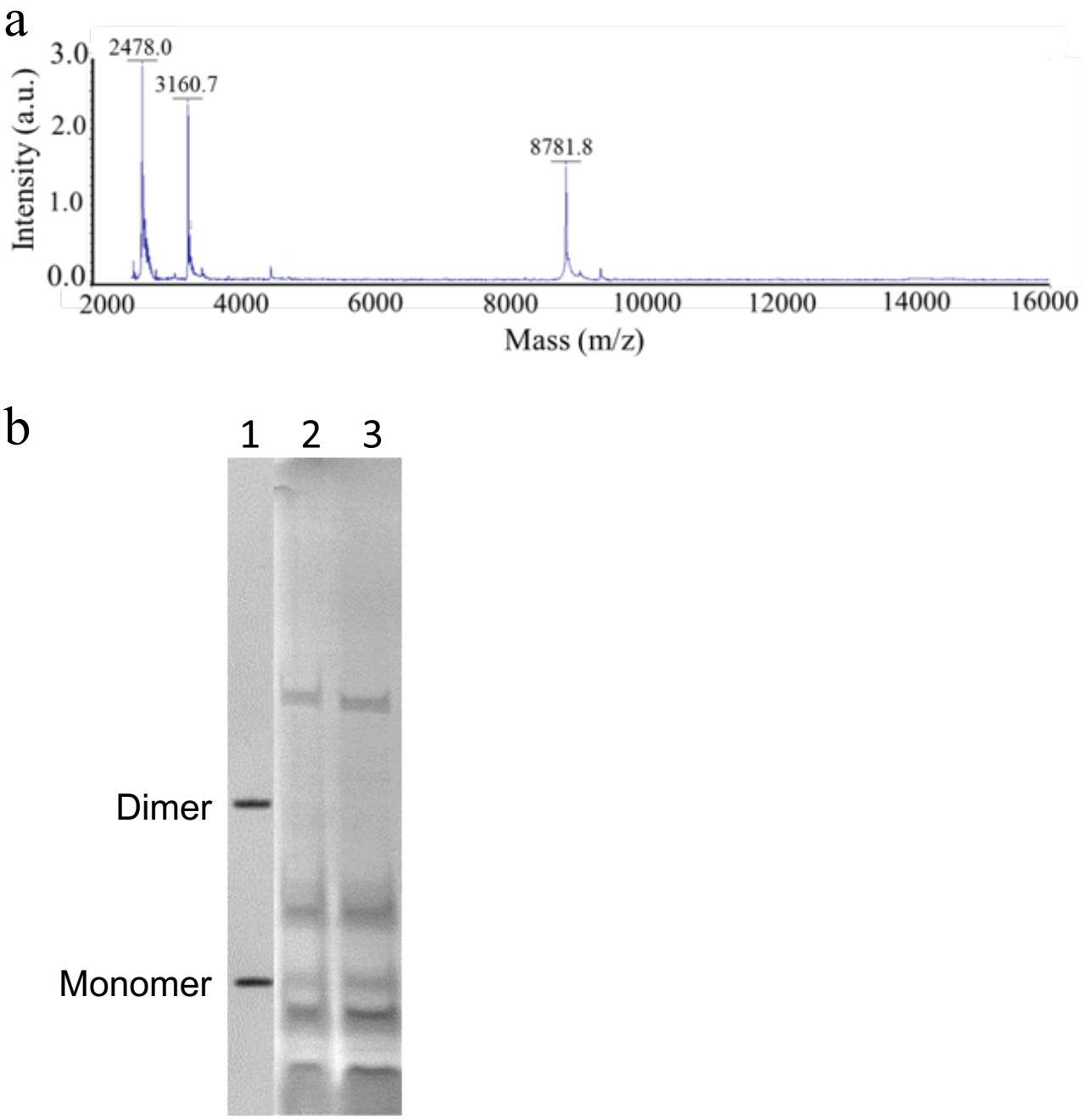

Figure S5. (a) MALDI spectrum of the G53A TTR solution (0.3 mg/ml) incubated for 8 hrs in the presence of trypsin. The molecular weight of 8781.8 corresponds to the C-terminal fragment (49 – 127). (b) SDS-PAGE of the G53A TTR precipitates collected after 7 days of incubation: Lane 1: G53A native state, Lane 2: precipitates dissolved with SDS sample buffer, Lane 3: precipitates dissolved with 8M urea.

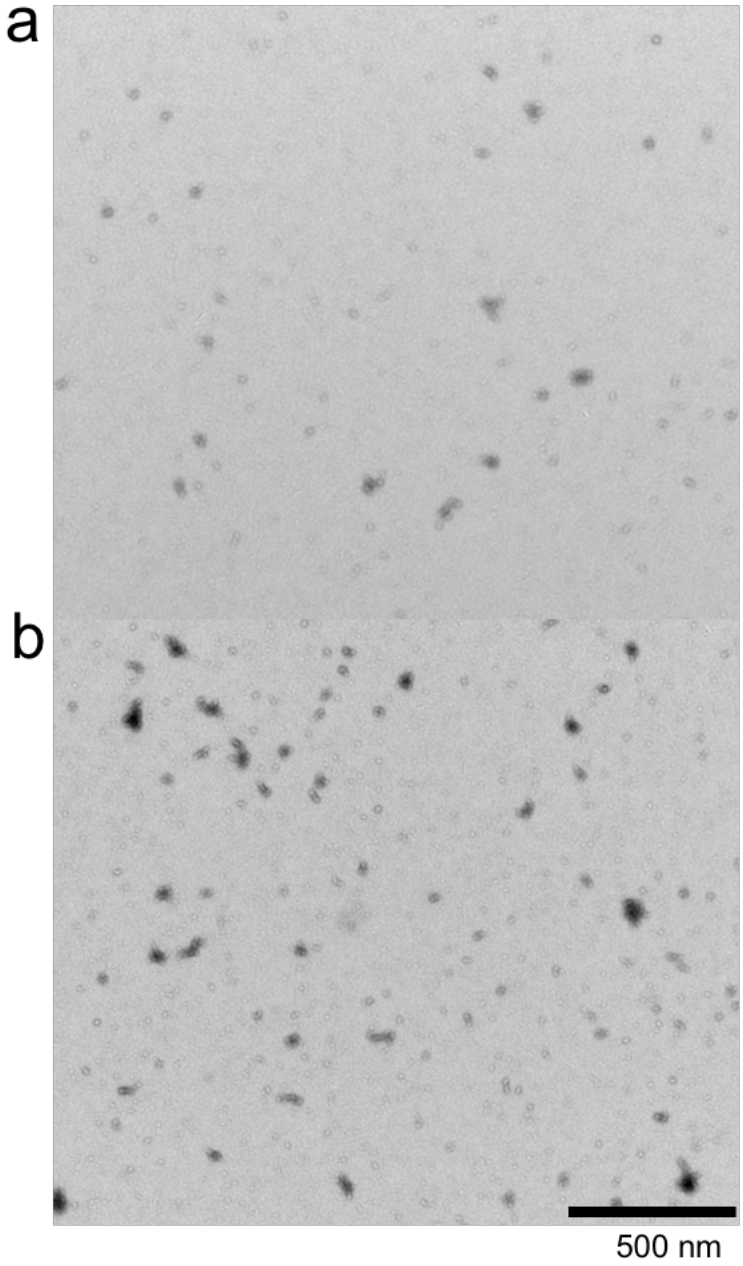

Figure S6. TEM images of G53A TTR (0.5 mg/ml) incubated for two hours at 37 ºC in the absence (a) and presence (b) of trypsin.

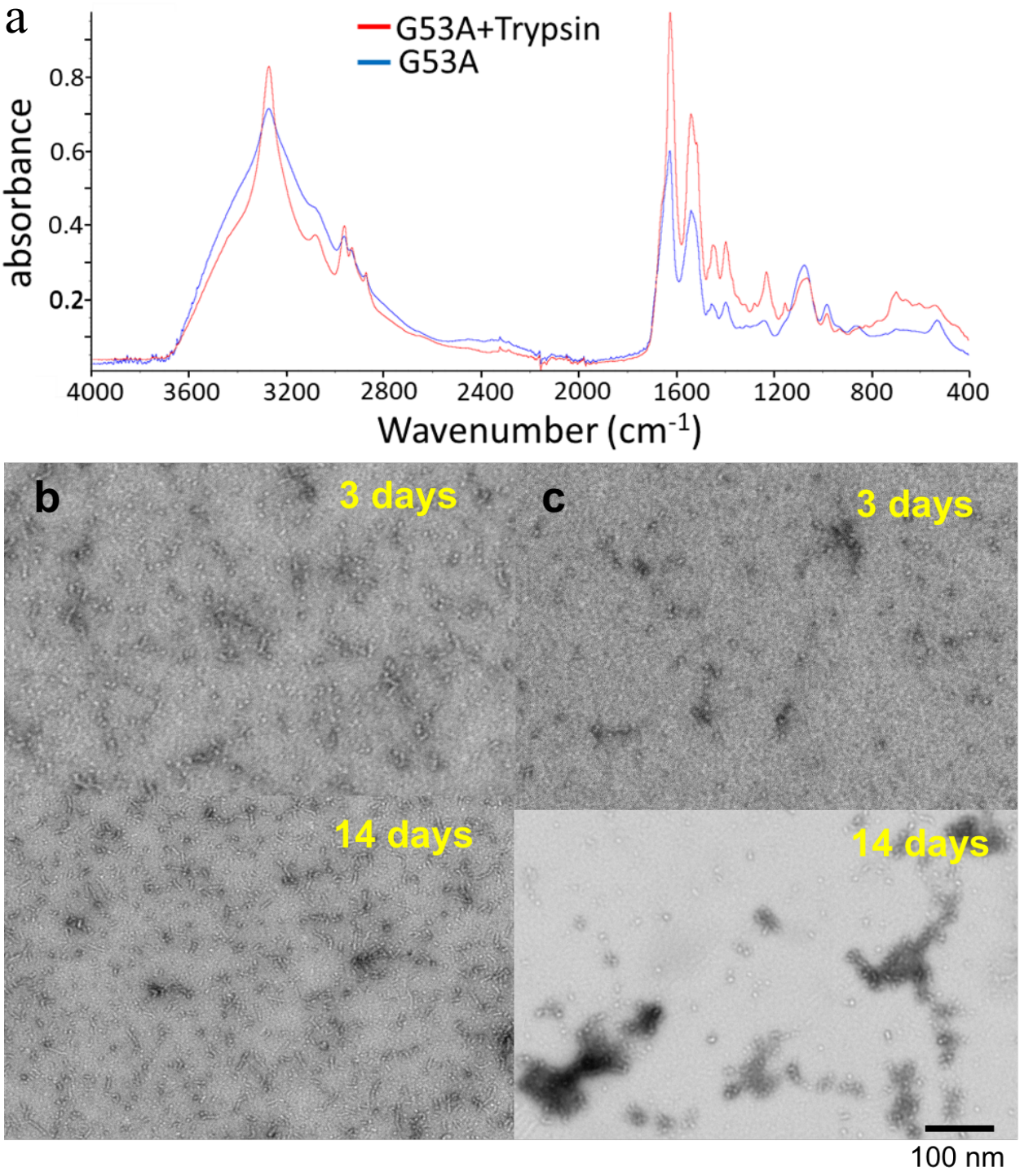

Figure S7. (a) FTIR spectra of G53A TTR amyloids obtained in the absence (blue) and presence (red) of trypsin after 3 days of incubation. TEM images of the G53A TTR amyloids formed in the absence (b) and presence (c) of trypsin. The protein precipitates were collected by centrifugation after 3 and 14 days of incubation.

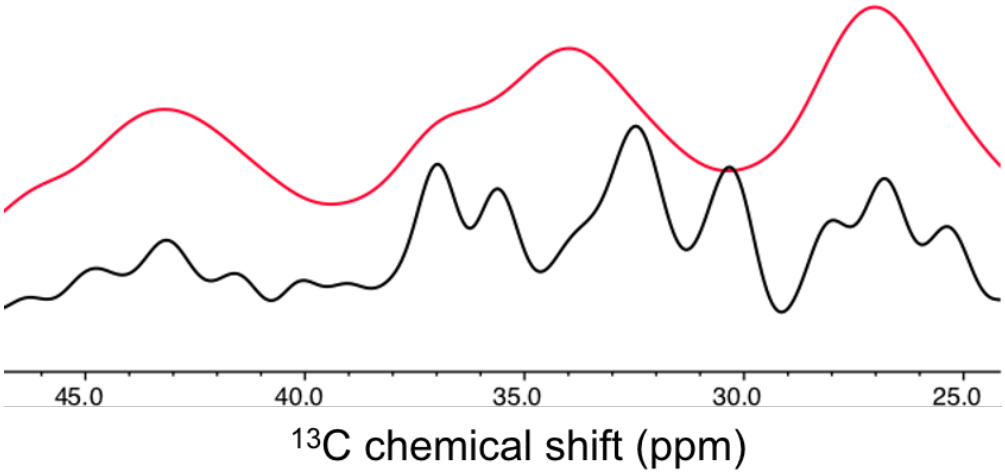

Figure S8. One-dimensional slices along 55 ppm in the 2D DARR spectra for the G53A TTR oligomers obtained in the presence (black) and absence (red) of trypsin.

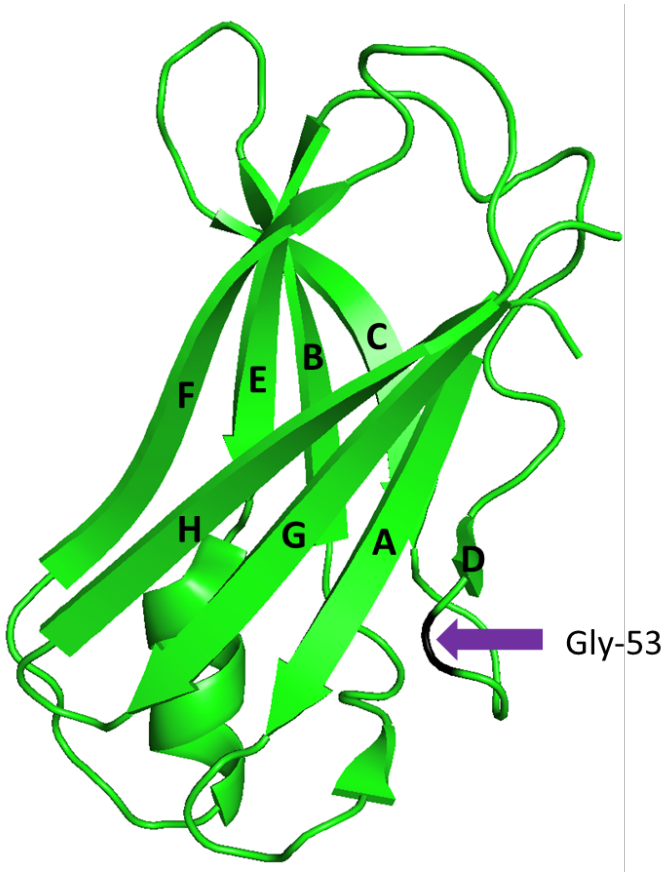

Figure S9. Crystal structure (PDB: 1F41) of monomeric subunit in WT TTR. The arrow above indicates the tight turn on G53 residue in the CD loop.

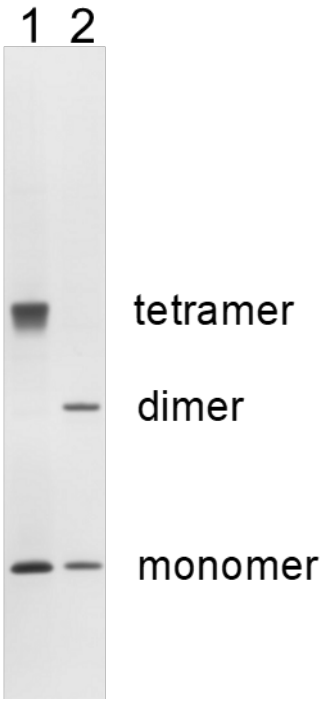

Figure S10. SDS-PAGE of purified WT TTR (lane 1) and G53A TTR (lane 2).