SUPPORTING INFORMATION

Truncation of the constant domain drives amyloid formation by immunoglobulin light chains

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Running title: Amyloidogenicity of immunoglobulin light chain fragments

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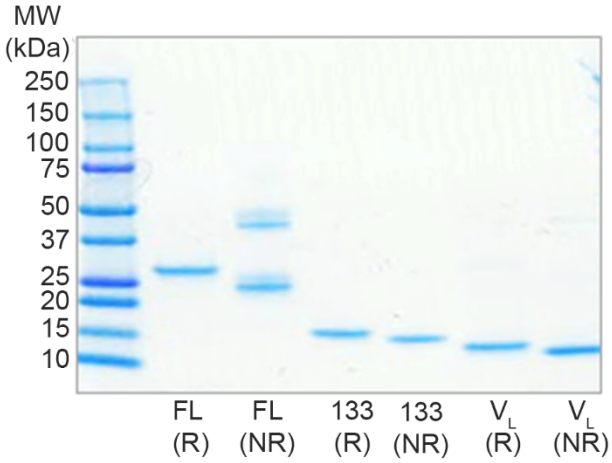
MATERIAL INCLUDED:

FIGURES S1-S6

A



B

	full-length AL55	133-AL55	V_L-AL55
Amino acid	217 (+ N-term Met)	133 (+ N-term Met)	111 (+ N-term Met)
Predicted MW (Da)	23306.63	14309.59	12290.35

Figure S1. Recombinant full-length AL55, V_L-AL55 and 133-AL55. (A) SDS 4-20 % PAGE analysis under reducing (R) and non-reducing (NR) conditions. The two bands visible for the full-length under non-reducing conditions correspond to the monomer (~ 25 kDa) and to the disulfide-linked dimer (~ 50 kDa). (B) main biochemical parameters of the three proteins calculated using the Expasy ProtParam tool. MW, molecular weight.

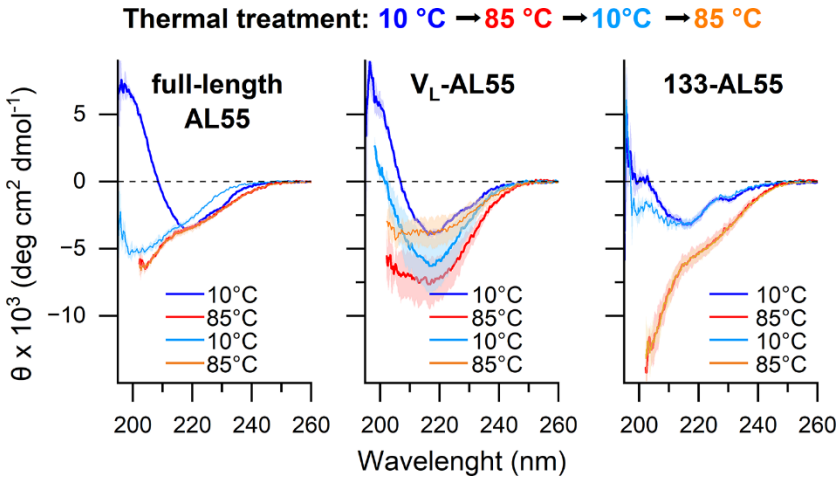


Figure S2. Assessment of the thermal unfolding reversibility of full-length and truncated AL55 species. Overlay of CD far UV spectra of each protein samples recorded during the first thermal treatment at 10°C (blue) and 85°C (red) respectively. Spectra recorded after cooling (light blue) and after a second thermal treatment (orange) are shown.

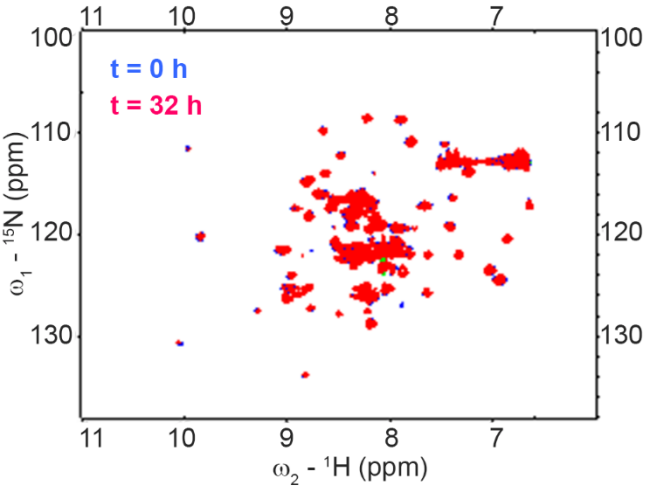


Figure S3. Conformational stability of 133-AL55 at 15 °C monitored by solution NMR. The "fingerprint" spectra, i.e. 2D [^{1}H-^{15}N] HSQC spectra, of uniformly [^{13}C, ^{15}N] labeled 133-AL55 at 0.468 mM in PBS pH 7.4 containing 5% D_2O were recorded immediately after dissolution (blue trace) and after 32 hours at 15 °C (red trace).

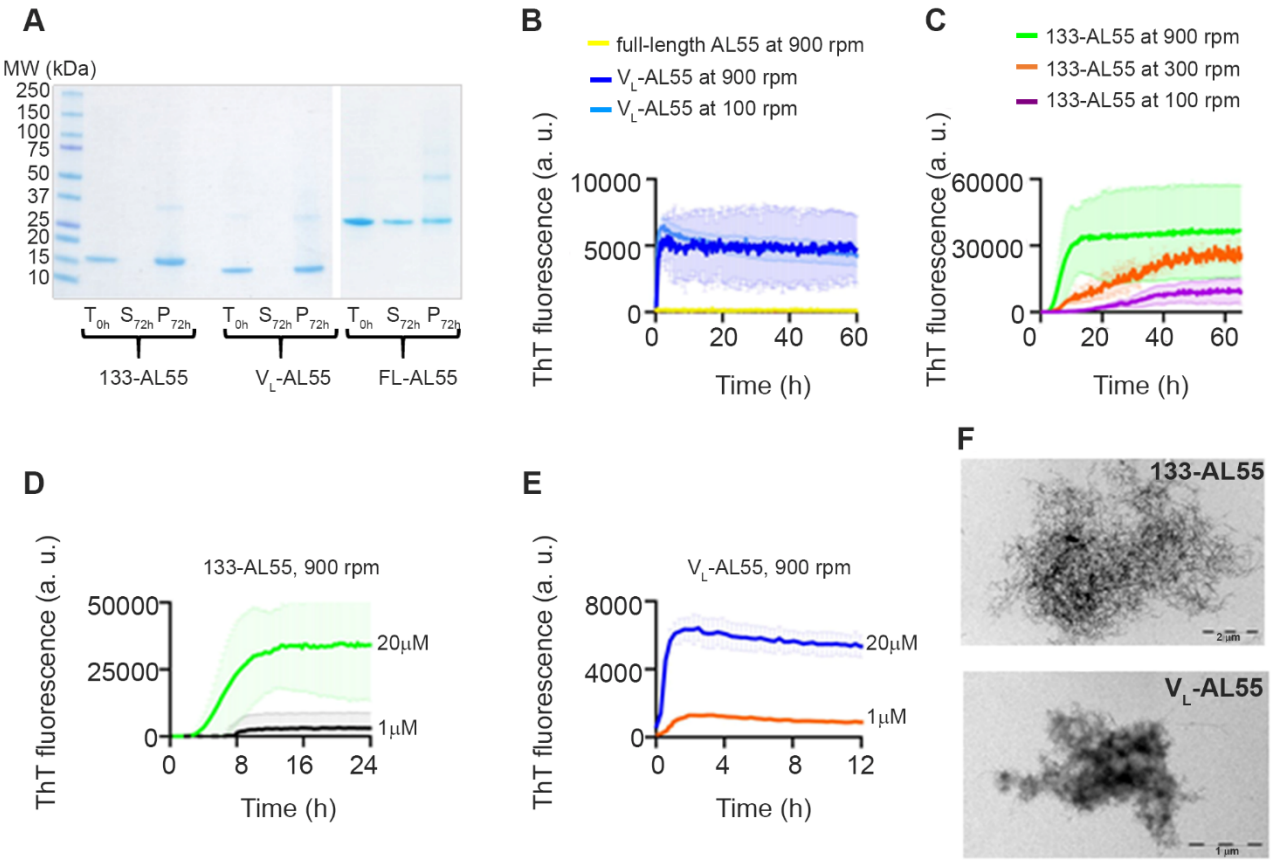


Figure S4. Fibrillogenesis of full-length AL55, V_L-AL55 and 133-AL55. (A) SDS 4-20 %-PAGE analysis under reducing conditions of pellets (P) separated by centrifugation from supernatants (S) after incubation of proteins for 72 h under agitation at 100 rpm. The starting material is indicated as T_{0h}. (B), fibrillogenesis of V_L-AL55 and full-length AL55 (20 μM) at 100 rpm and 900 rpm, monitored by ThT assay as described in Material and Methods. Shown V_L-AL55 at 900 rpm (blue), V_L-AL55 at 100 rpm (light blue); full-length AL55 at 900 rpm (yellow); full-length AL55 at 100 rpm not shown as it overlaps with the one at 900 rpm. (C), fibrillogenesis of 133-AL55 (20 μM) at 100 rpm (purple), 300 rpm (orange) and 900 rpm (green), monitored by ThT assay. (D), fibrillogenesis of 133-AL55 at 900 rpm at 20 μM (green) and 1 μM (black) respectively. (E), fibrillogenesis of V_L-AL55 at 900 rpm, 20 μM (blue) and 1 μM (orange) respectively. All data shown as mean and SD of at least three independent experimental replicates. (F), TEM images of the aggregates by AL-133 and V_L-AL55, at low magnification. Scale bars are indicated.

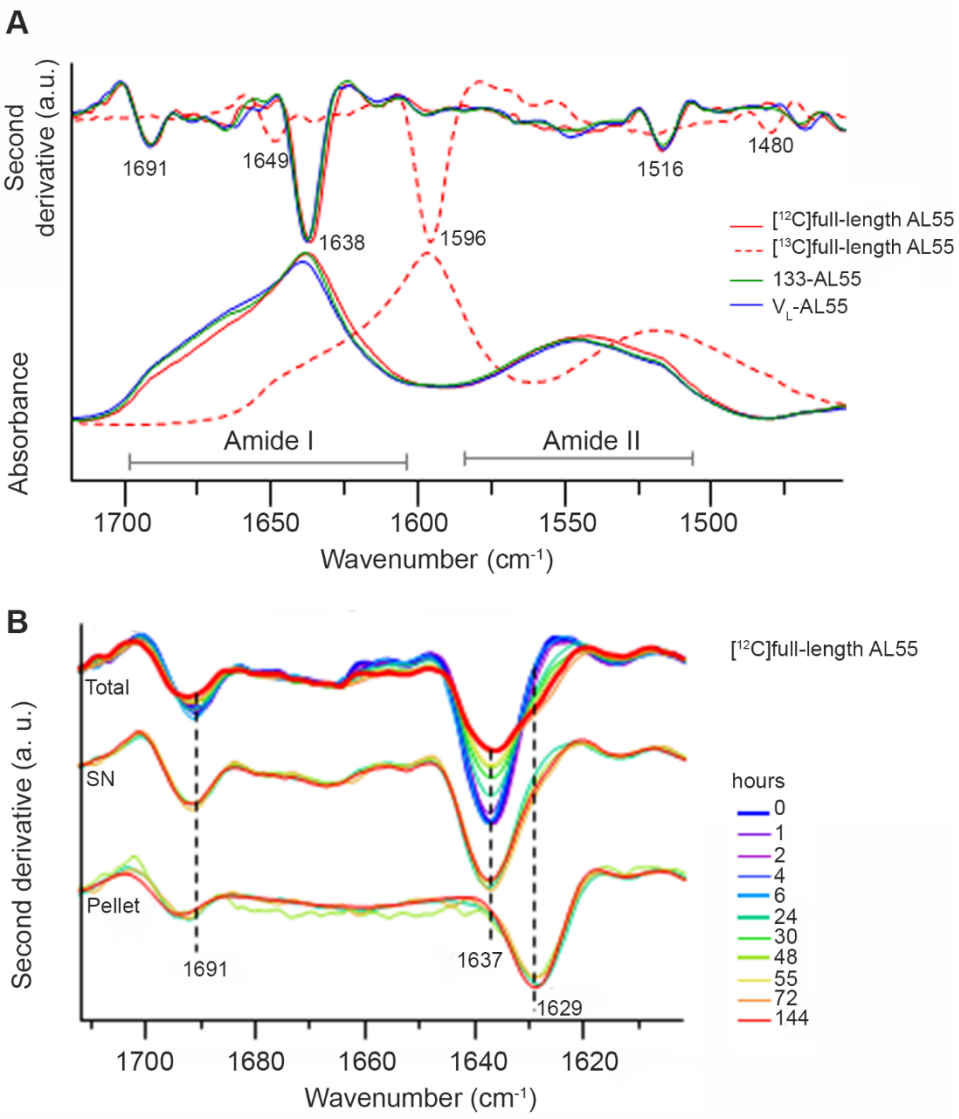


Figure S5. FTIR spectroscopy. (A), FTIR absorption spectra and second derivatives of the three native unlabeled [^{12}C] species and the labeled [^{13}C]full-length AL55, are shown with corresponding main peaks positions. (B), time course of unlabeled full-length AL55. Second derivatives of the absorption spectra collected at different incubation times, as indicated. Aliquots of each sample (20 µl) at 24, 48, 72 and 144 hours of incubation were centrifuged and corresponding FTIR spectra of supernatants (SN, middle spectra) and pellets (bottom spectra) were acquired. Representative spectra with the main peak positions are shown.

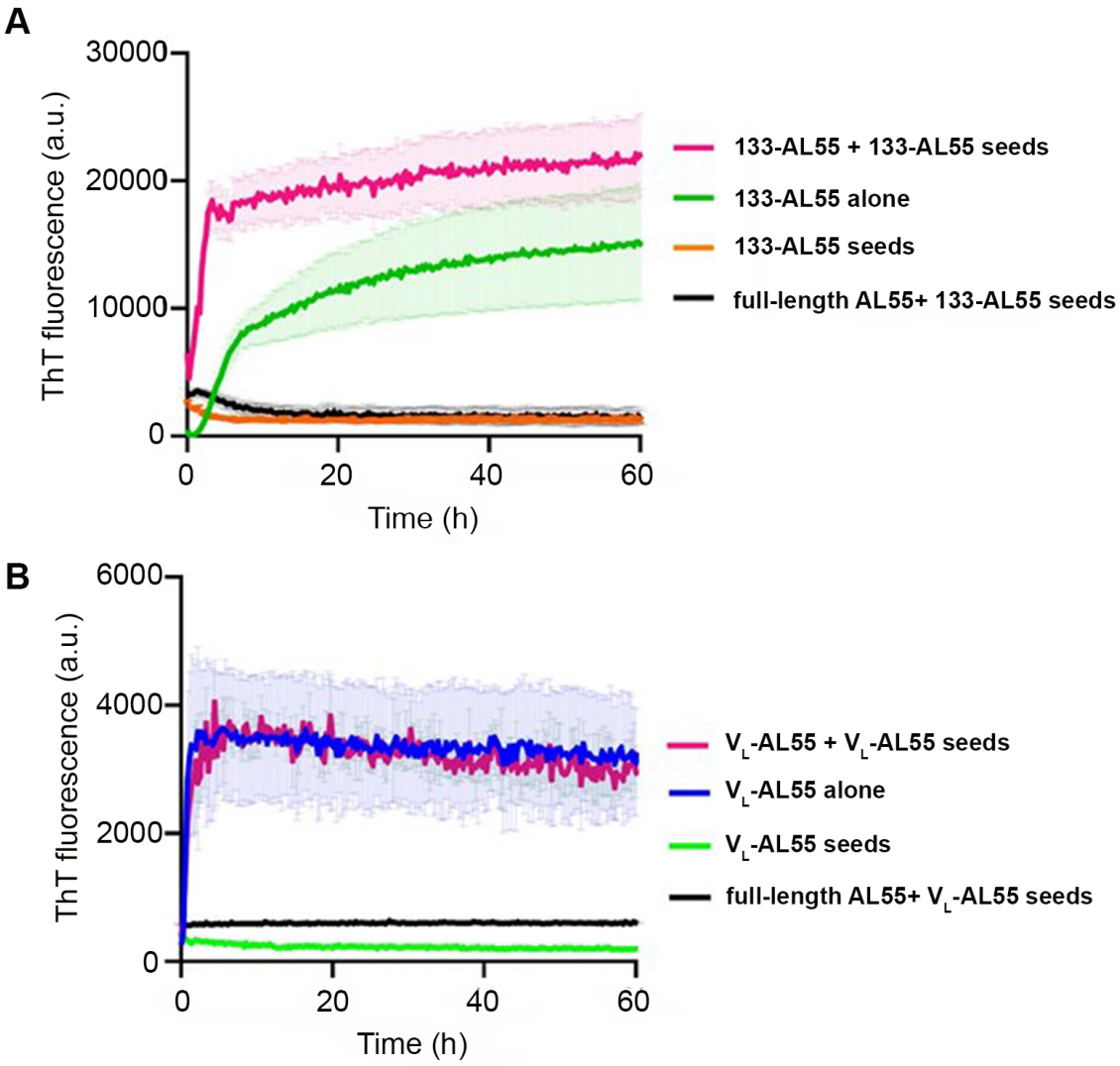


Figure S6. Seeding and cross-seeding experiments, performed by incubating soluble proteins with preformed aggregates by either 133-AL55 (A) or V_L-AL55 (B). Data are shown as mean and standard deviation of three independent experiments.