Appendix Information

Cellular mechanism of fibril formation from serum amyloid A1 protein

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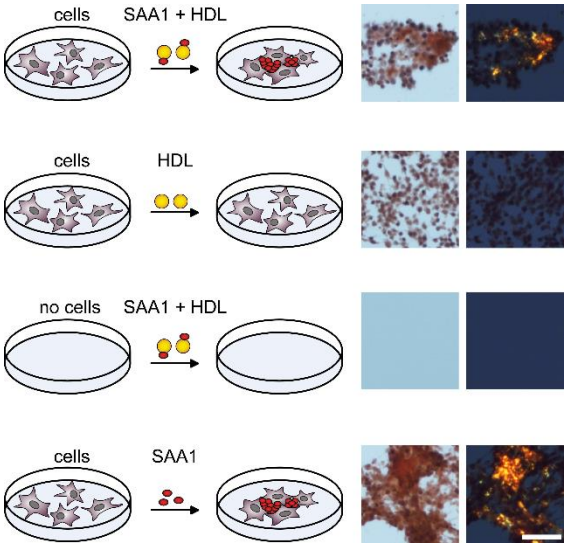
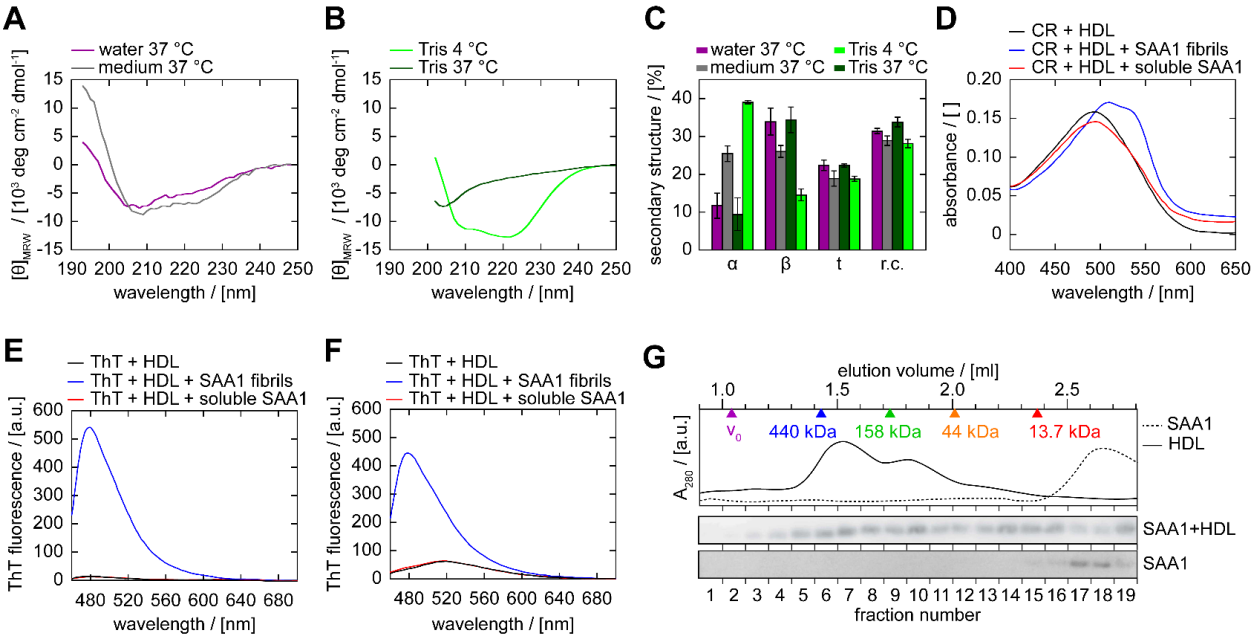
Appendix Figure S1



Appendix Figure S1. Fibril formation from SAA1 protein in the cell culture model.

Left: Schematic representation of the cell culture model using varied combinations of cells, SAA1 and HDL as indicated. Right: Bright field and dark field polarizing microscopy images of CR-stained images of cell cultures incubated with 0.5 mg/ml SAA1 and HDL for 6 days. Scale bar: 50 µm.

Appendix Figure S2



Appendix Figure S2. Characterization of SAA1 protein.

A-B CD spectra of 0.5 mg/ml SAA1 in water or cell culture medium without FBS recorded at 37 °C (A) and of 0.3 mg/ml SAA1 in Tris buffer (pH 8) at 4 °C and 37 °C (B).

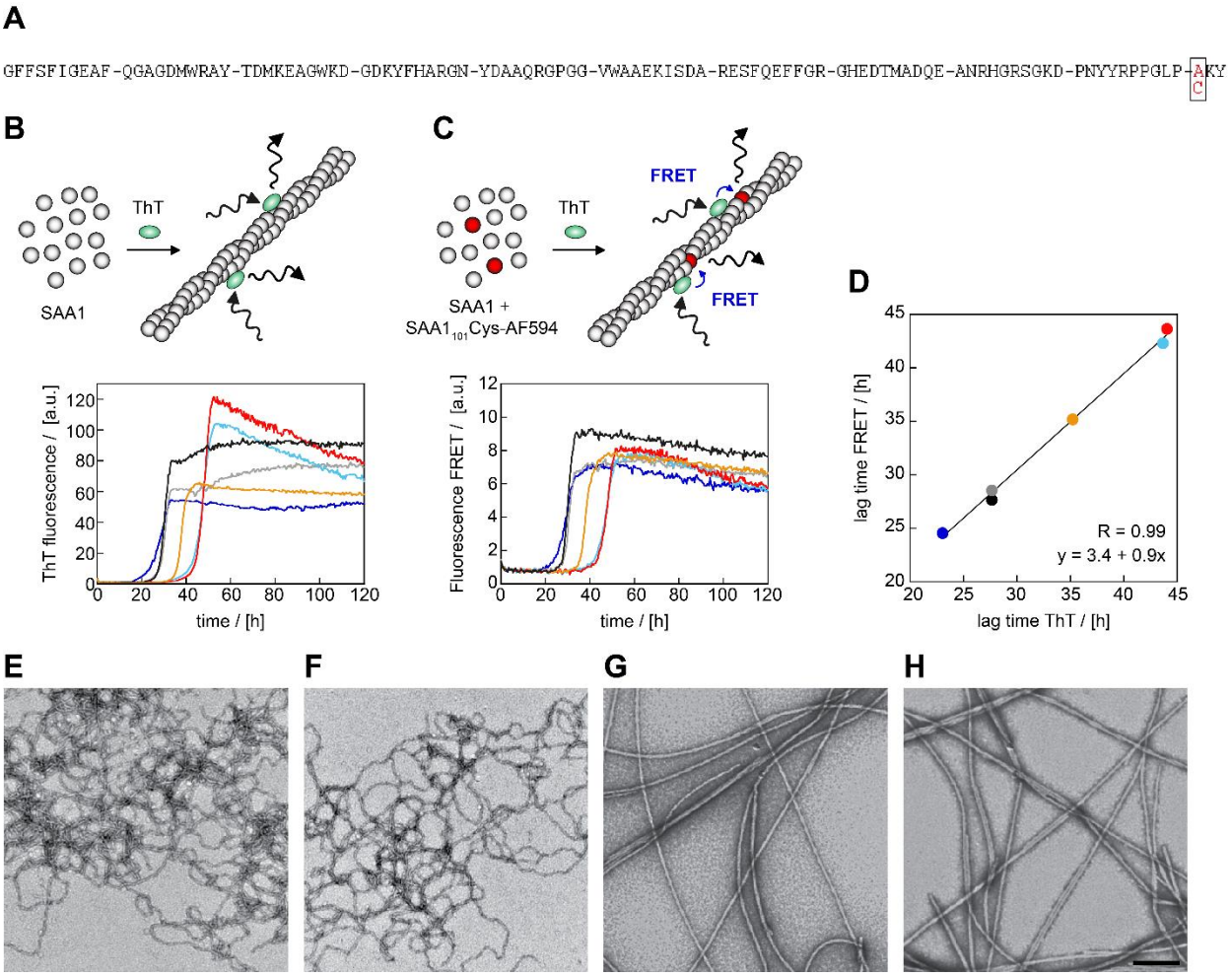
C Secondary structural composition of the samples analysed in panels (A) and (B), presenting the percentage of α-helix (α), β-sheet (β), β-turn (t) and random coil (r.c.) structure.

D-F CR absorption (D) and ThT fluorescence (E-F) spectra recorded of freshly dissolved (soluble) SAA1, SAA1 fibrils, HDL and dye as indicated. The spectra in panel (D-E) were recorded in Tris buffer (pH 8). The spectra in panel (F) were obtained with samples containing cell culture medium with FBS.

G SEC of 0.5 mg/ml soluble SAA1 and HDL in phosphate buffer (pH 7.4) and western blot of SEC fractions of 0.5 mg/ml SAA1 and HDL in cell culture medium and 0.5 mg/ml SAA protein in phosphate buffer using an anti-SAA1 antibody.

Data information: In (C), data are presented as mean ± s.d.

Appendix Figure S3



Appendix Figure S3. Fluorescently labelled SAA1$_{101}$Cys incorporates into fibrils in buffered solution.

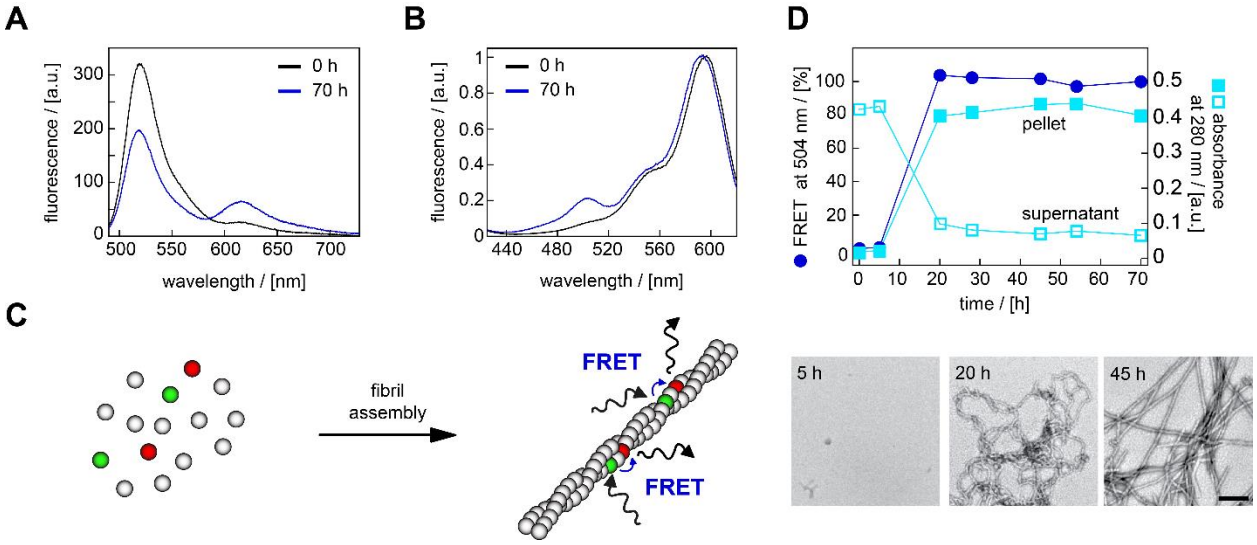
A Primary structures of SAA1 and SAA1$_{101}$Cys.

B-D Kinetics of SAA1 fibrillation monitored by ThT flourescence (EX/EM: 450/490 nm) (B) and FRET from ThT to AF594 (EX/EM: 450/620 nm) of a 49:1 mixture of SAA1 and SAA1$_{101}$Cys-AF594 (C). Six independent replicates are shown. The average lag time is 33.6 ± 8.1 h. The FRET channel was corrected for ThT fluorescence crosstalk. (D) Correlation plot of the lag times obtained with the two read outs. Colors in (B-D) refer to identical samples.

E-H TEM images of fibrils grown in 50 mM sodium phosphate pH 3 (E-F) or 10 mM Tris pH 8 (G-H) from 50 μM SAA1 (E, G) or a 49:1 mixture of SAA1 and $SAA1_{101}$Cys-AF594 (F, H) for 6 days at 300 rpm and 37°C. Scale bar: 200 nm.

Data information: The SAA1 concentration was 50 μM in these experiments.

Appendix Figure S4



Appendix Figure S4. Generation of a FRET-sensor of fibril formation.

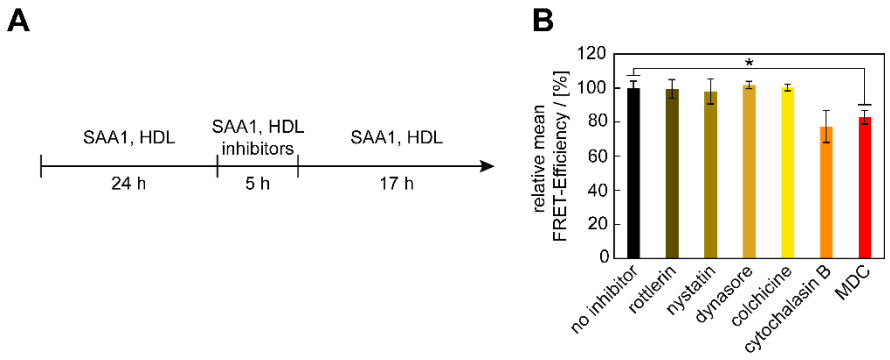
A-B Fluorescence emission (A, EX: 480 nm) and excitation spectra (B, EM: 630 nm) of a 48:1:1 mixture of SAA1, $SAA1_{101}$Cys-AF488 and $SAA1_{101}$Cys-AF594 before (black) and after incubation for 70 h at 37°C (blue).

C Schematic representation of the FRET sensor. Incubation of a 48:1:1 mixture of unmodified SAA1 (white), $SAA1_{101}$Cys-AF488 (green) and $SAA1_{101}$Cys-AF594 (red) leads to their co-assembly into fibrils. Scale bar: 100 nm.

D Time-dependent measurement of *in vitro* fibril formation (in the absence of cells) with a 48:1:1 mixture of SAA1, $SAA1_{101}$Cys-AF488 and $SAA1_{101}$Cys-AF594 measured by absorption at 280 nm as an increase in insoluble protein fraction after centrifugation (light blue squares) or the relative fluorescence intensity at 504 nm of the excitation spectrum arising from FRET (dark blue circles). 100% represents the maximum value of the measured FRET intensity. Unfilled squares: absorbance of the supernatant at 280 nm, filled squares: absorbance of the re-dissolved pellet at 280 nm. TEM images of aliquots taken from this reaction at different time points.

Data information: The SAA1 concentration was 50 μM in these experiments.

Appendix Figure S5



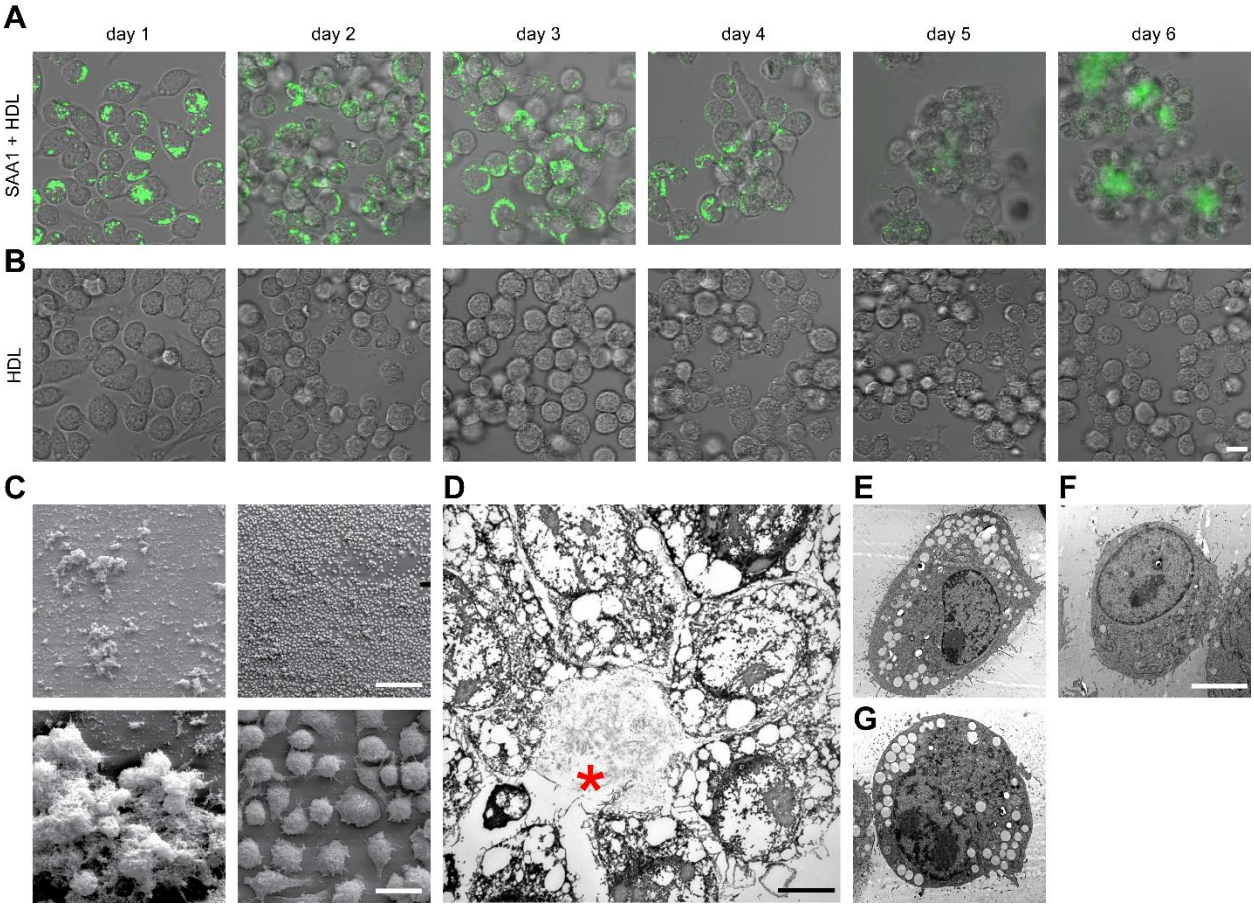
Appendix Figure S5. Analysis of the effect of the uptake inhibitors on intracellular fibril formation measured as FRET intensities.

A Schematic representation of the experimental time line.

B Cells were exposed to a 48:1:1 mixture of SAA1, $SAA1_{101}$Cys-AF488, $SAA1_{101}$Cys-AF594 (total SAA1 concentration 50 µM), HDL and the respective inhibitor. The mean FRET intensity was determined by calculating the mean value of all pixels in one FRET image. The inhibitor-free sample was set to 100% (n = 3).

Data information: The SAA1 concentration was 50 µM in these experiments. In (B), data are presented as mean ± s.d. * $p < 0.05$ (Student's t-test).

Appendix Figure S6



Appendix Figure S6. Amyloid formation and cell clustering.

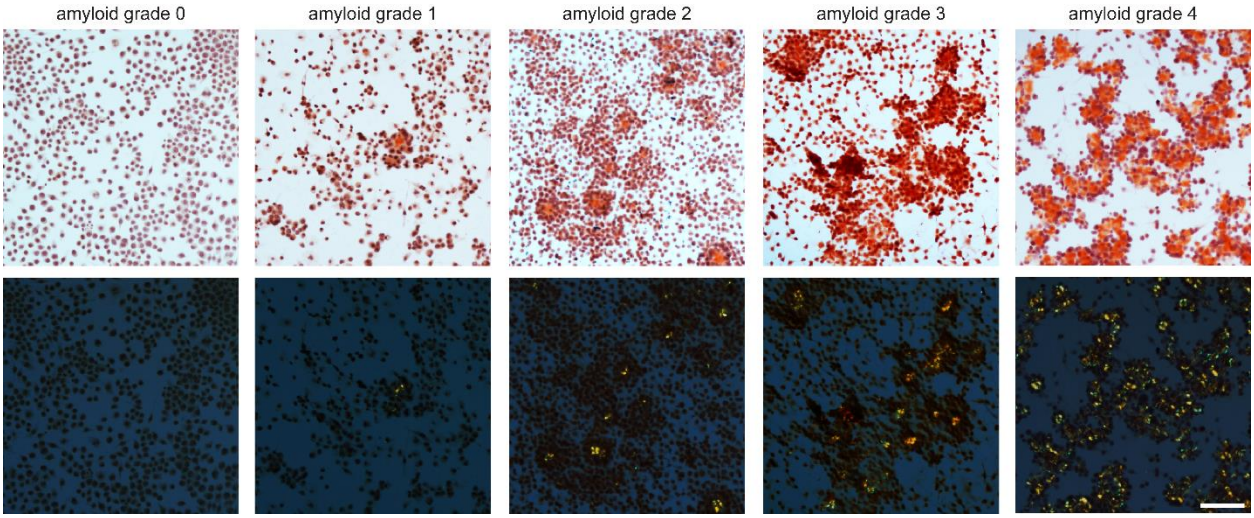
A-B LSM images of J774A.1 cells exposed to 0.5 mg/ml SAA1, 0.01 mg/ml SAA1-AF488 and HDL (A) or HDL only (B) up to 6 days. Scale bar: 10 µm.

C SEM images of J774A.1 cells incubated with 1 mg/ml SAA1 and HDL (left) or without SAA1 and HDL (right) for 6 days. Upper scale bar: 200 µm. Lower scale bar: 20 µm.

D TEM image of a 70 nm section of a cell cluster within a culture incubated with 1 mg/ml SAA1 and HDL for 5 days showing an extracellular core (red asterisk) surrounded by dead cells. Scale bar: 5 µm.

E-G TEM images of a 70 nm section of J774A.1 cells incubated with 1 mg/ml SAA1 and HDL (E), without SAA1 or HDL (F) or with only HDL only (G) for 1 day. Scale bar: 5 µm.

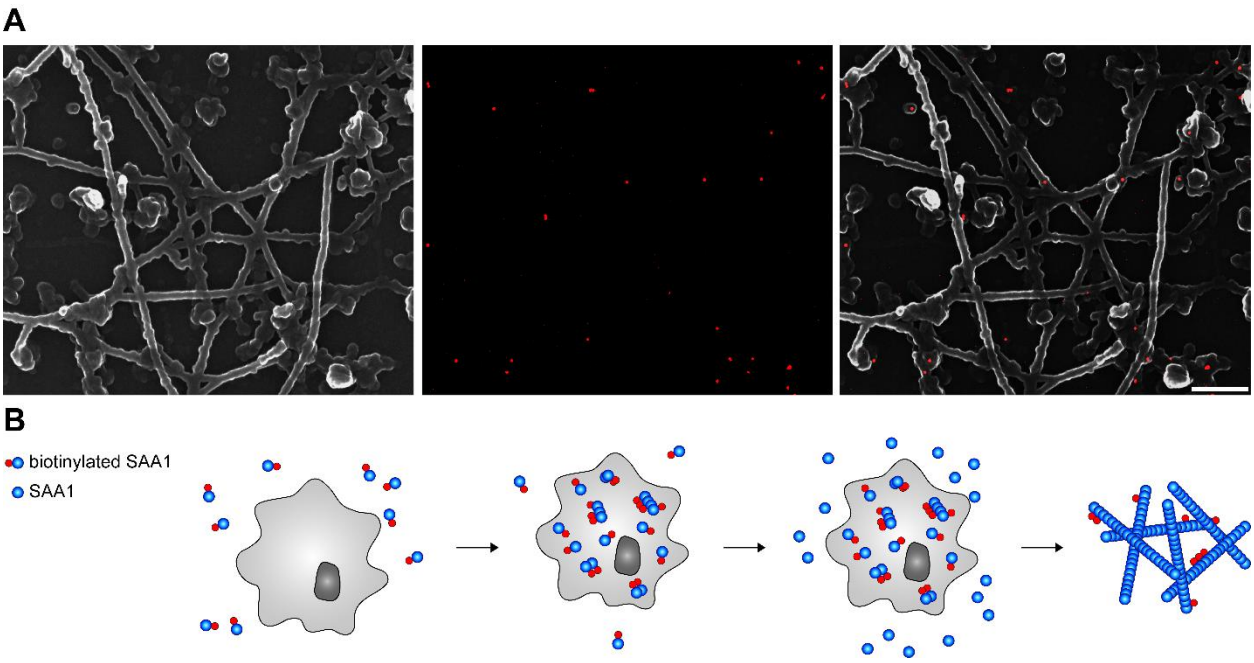
Appendix Figure 7



Appendix Figure S7. Amyloid grading by CR green birefringence.

Representative bright field (top) and dark field polarizing microscopy images (bottom) of extracellular amyloid deposits graded as: 0, no amyloid deposits; 1, few and isolated amyloid deposits; 2, many isolated amyloid deposits, but no coalescent areas of deposits; 3, amyloid deposits with substantial coalescence of the areas; 4, very extensive and large-sized amyloid deposits. Scale bar: 100 µm.

Appendix Figure S8



Appendix Figure S8. Intracellular SAA1 becomes released into the extracellular space.

A SEM images of an amyloid deposit which was labelled with 10 nm gold-conjugated streptavidin. Cells were pulsed with 1 mg/ml SAA1, 0.2 mg/ml biotinylated SAA1 and HDL for 1 day and chased by 1 mg/ml unlabelled SAA1 and HDL for another 4 days. Left: Secondary electron signal showing the fibril surface. Middle: Backscattered electron signal showing the distribution of gold particles. Right: Merged image. Scale bar: 200 nm.

B Schematic representation of the experimental set up.