Supplementary Information

Food Amyloid Fibrils as Safe Nutrition Ingredients: *In-vitro* and *In-vivo* Assessment

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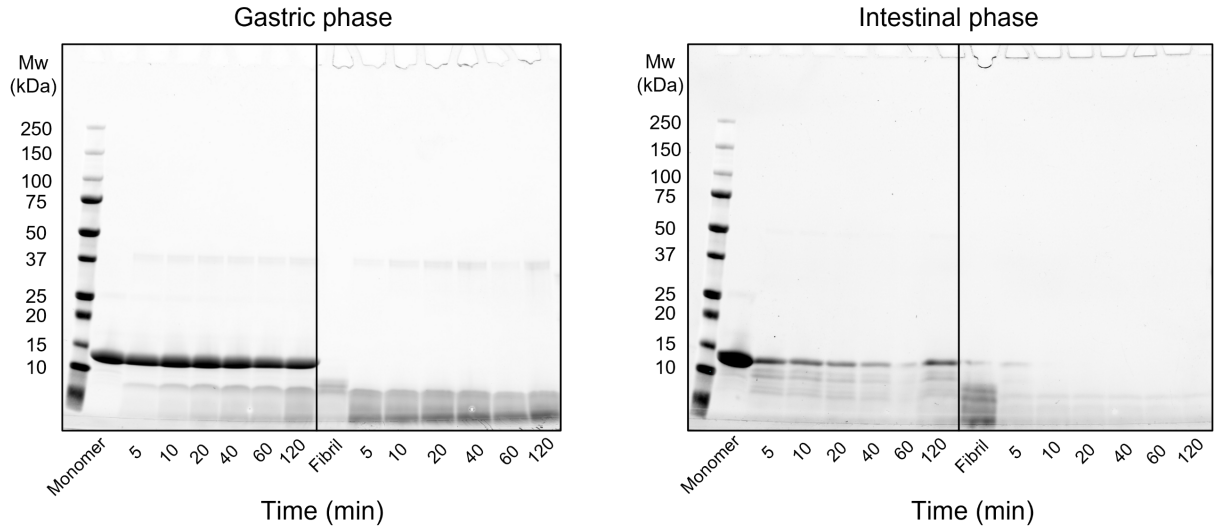

Figure S1. SDS-PAGE of the gastrointestinal digestion of lysozyme monomer and amyloid fibrils.

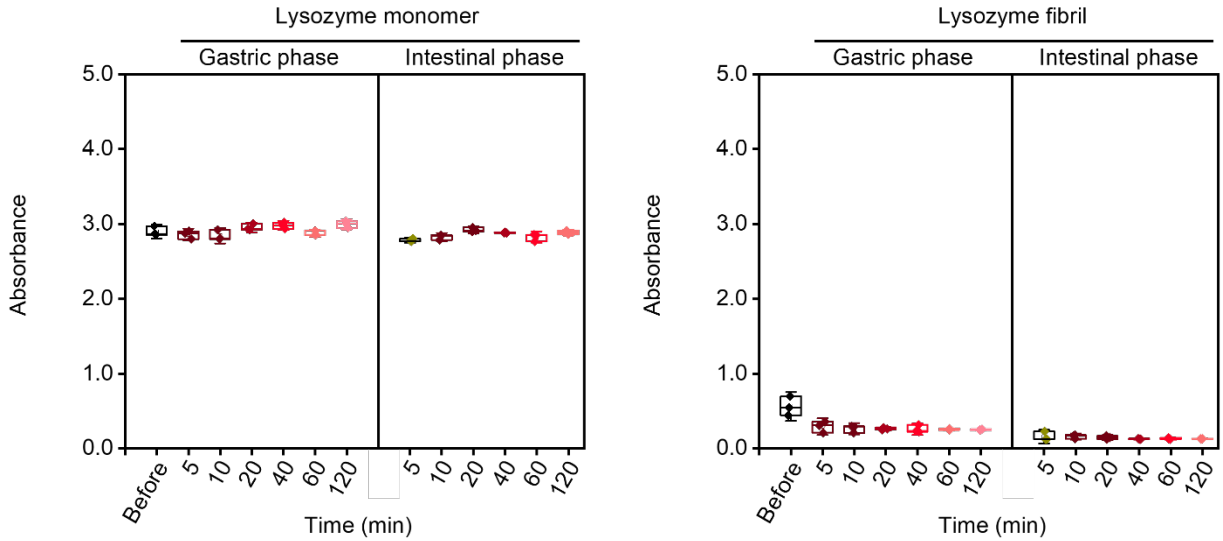

Figure S2. ELISA antigenicity assay of lysozyme monomer and amyloid fibrils over the course of gastrointestinal digestion.

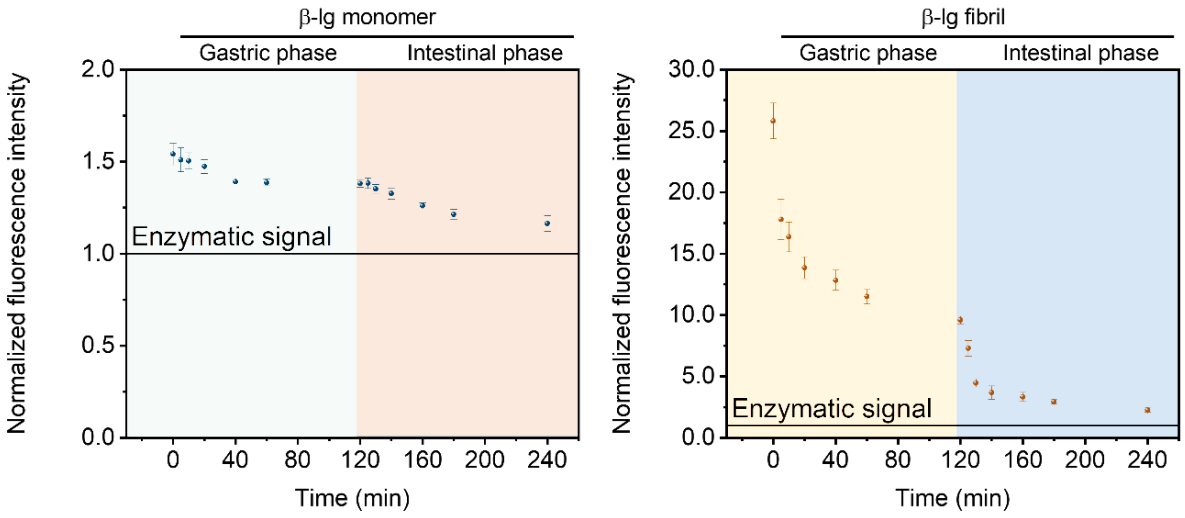

Figure S3. ThT fluorescence assay of β-lg monomer and amyloid fibrils over the course of gastrointestinal digestion. Enzymatic signal refers to the background contribution intensity from digestive enzymes.

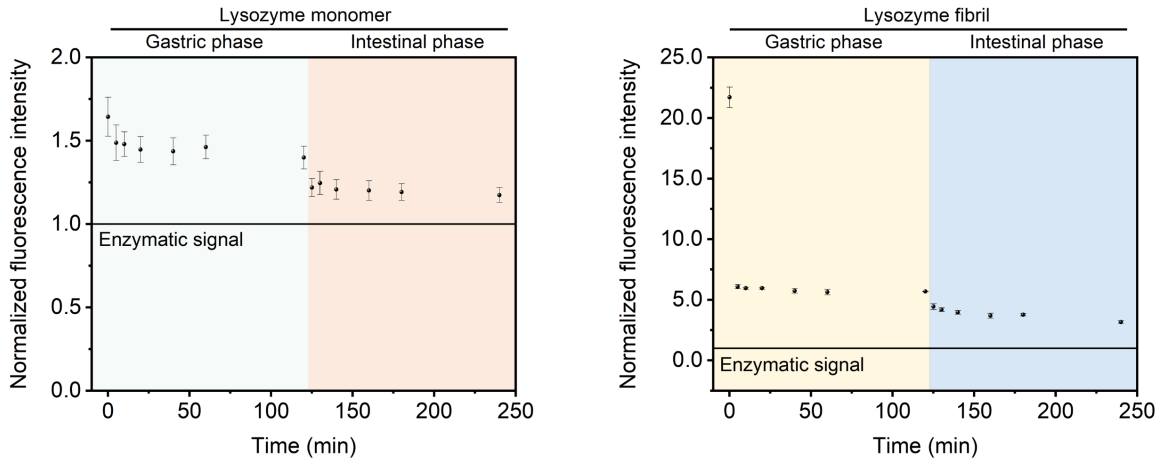

Figure S4. TO fluorescence assay of lysozyme monomer and amyloid fibrils over the gastrointestinal digestion. Enzymatic signal refers to the background contribution intensity from digestive enzymes.

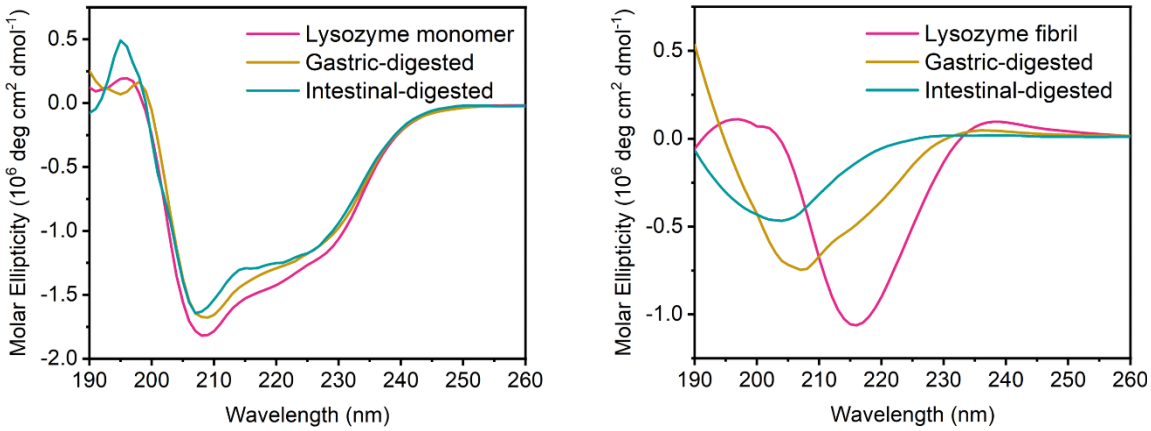

Figure S5. Circular dichroism spectra of lysozyme monomer and amyloid fibrils after gastric and intestinal digestion.

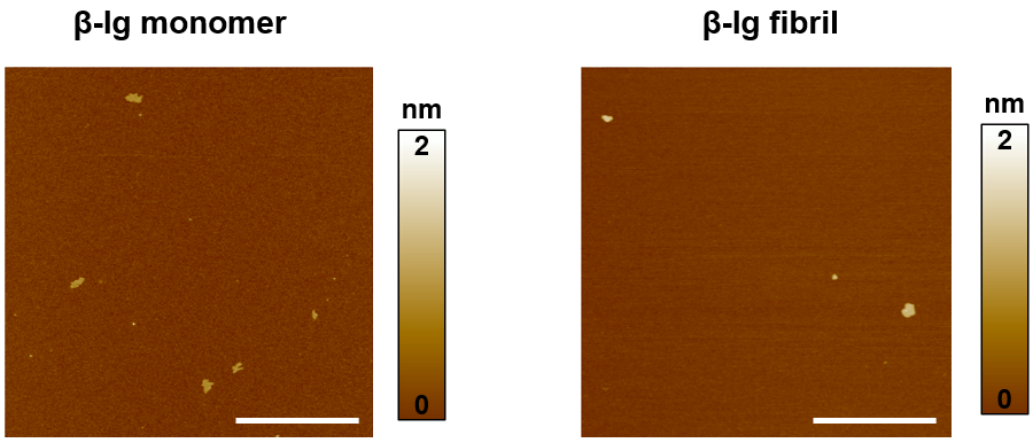

Figure S6. AFM images of intestinal-digested product of β-lg monomer and amyloid fibrils after 10 kDa filter. The scale bar is 1 μm.

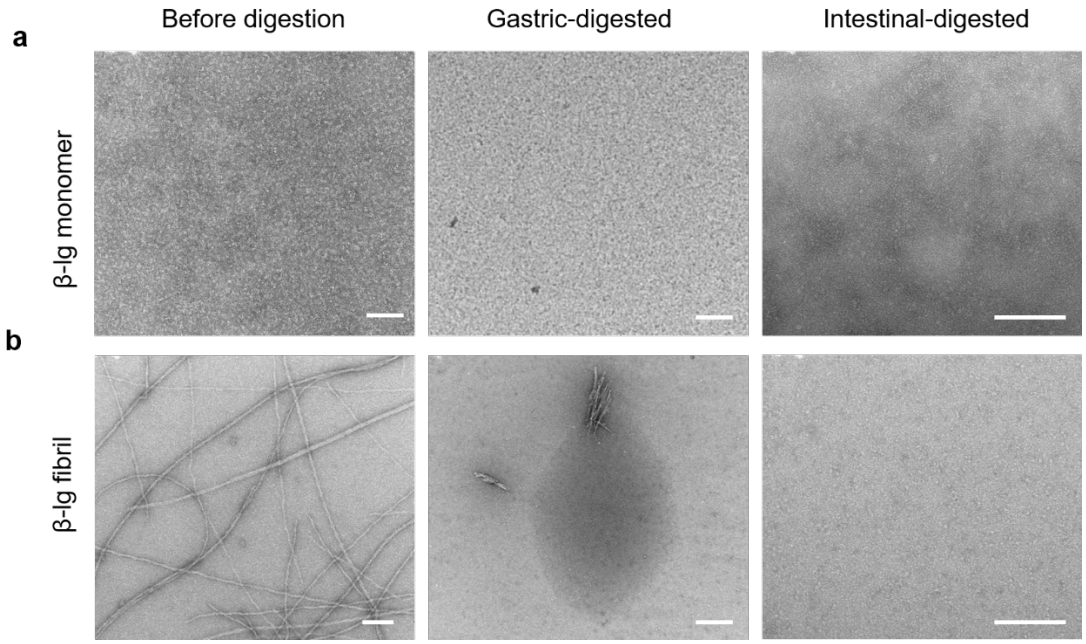

Figure S7. TEM images of β-lg monomer and amyloid fibrils before and after gastrointestinal digestion. β-lg monomer (a) and amyloid fibrils (b) before digestion, after gastric, and intestinal digestion. The scale bar is 100 nm.

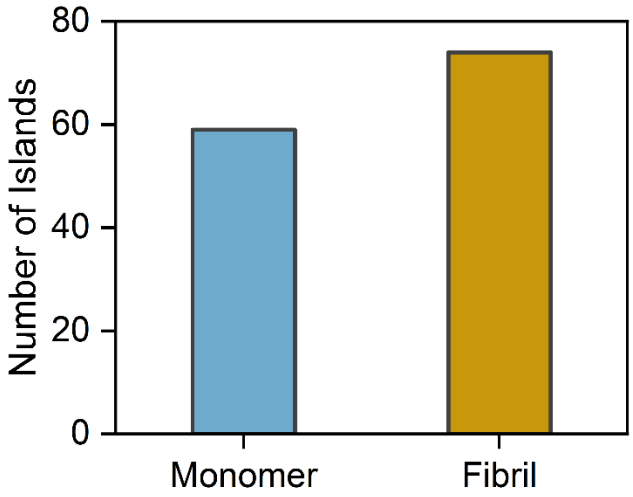

Figure S8. Statistical comparison of the quantity of nano-islands from β-lg monomer and amyloid fibril after intestinal digestion.

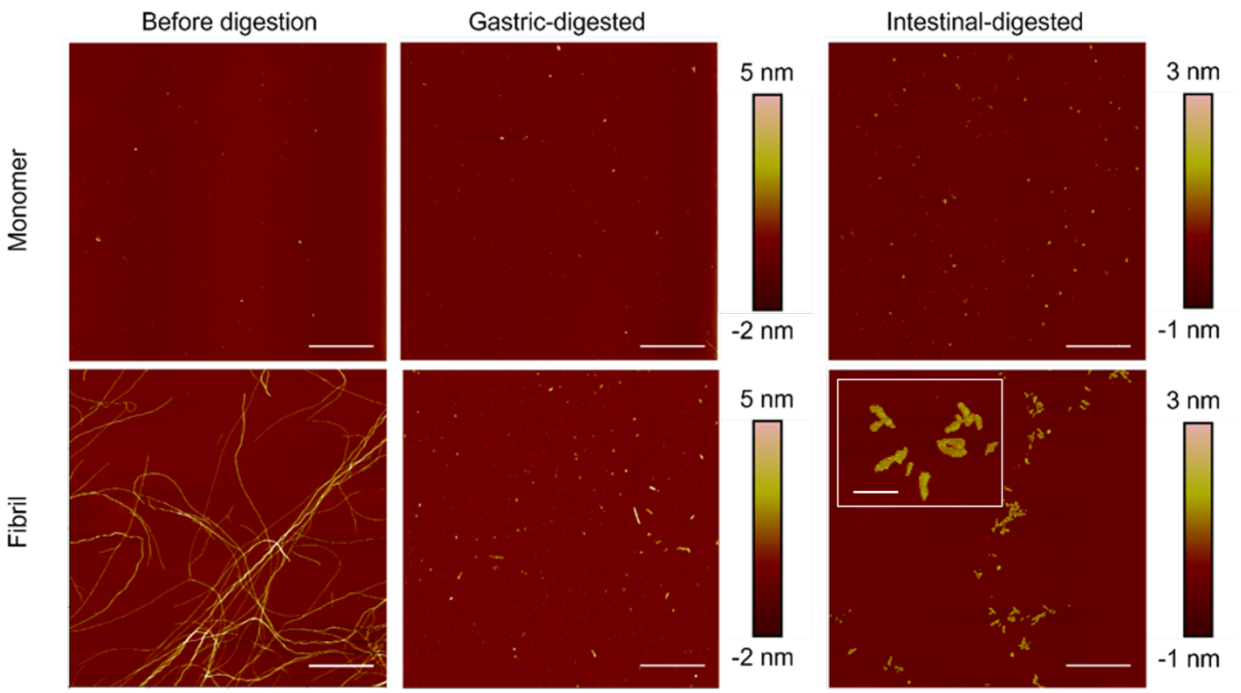

Figure S9. AFM images of lysozyme monomer and amyloid fibrils before digestion, after gastric digestion, and intestinal digestion. Scale bar represents 2 μm. Inset: magnified image of aggregates. Height of aggregates ranged between 1~1.5 nm. Inset scale bar represents 400 nm.

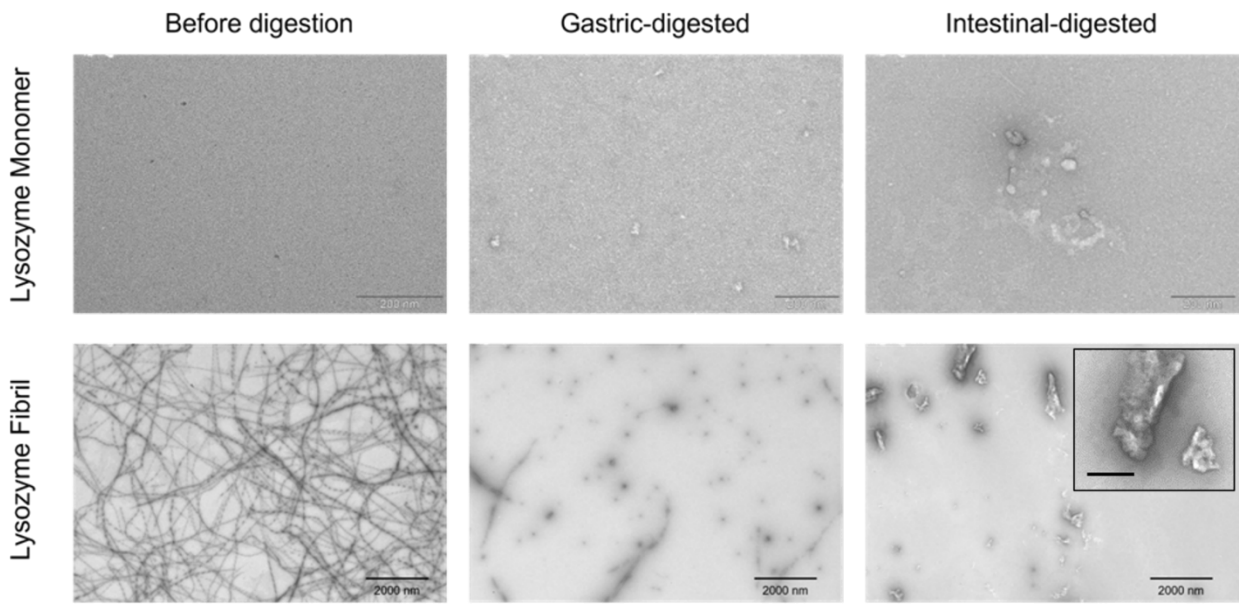

Figure S10. TEM images of lysozyme amyloid fibrils and their gastrointestinal digested products. Scale bars for monomer and amyloid fibrils represent 200 nm and 2 μm, respectively. Inset: magnified image of aggregates. Inset scale bar represents 400 nm.

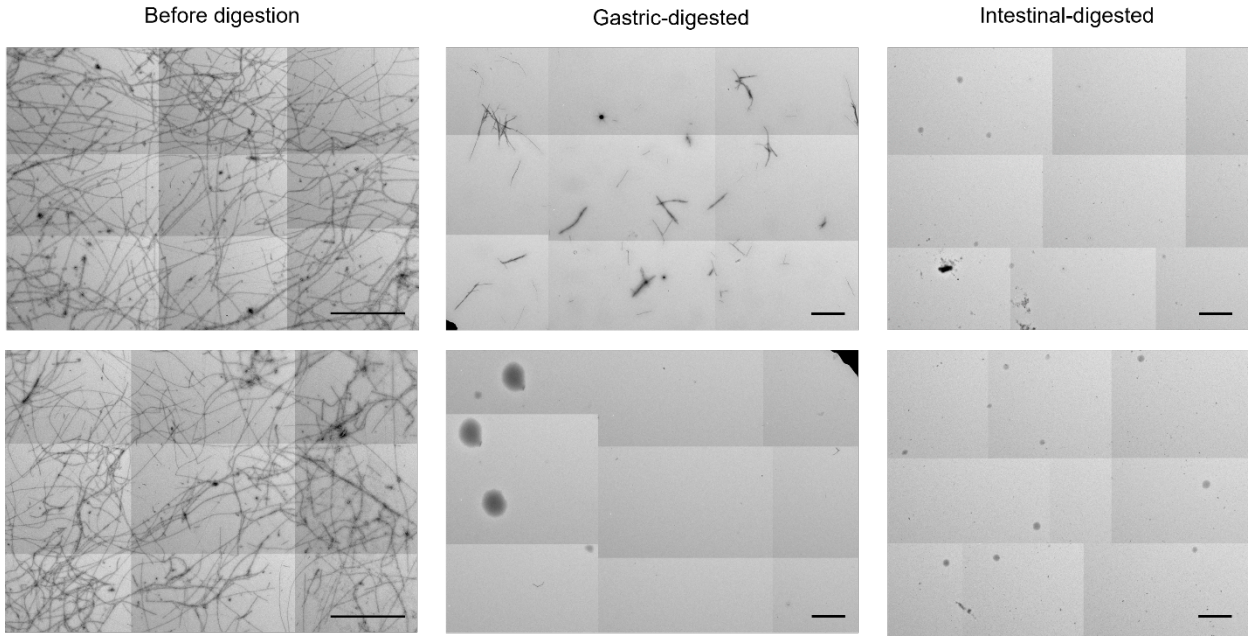

Figure S11. Collated overview TEM images of β-lg amyloid fibrils before digestion, after gastric digestion, and intestinal digestion. Scale bar represents 2 μm.

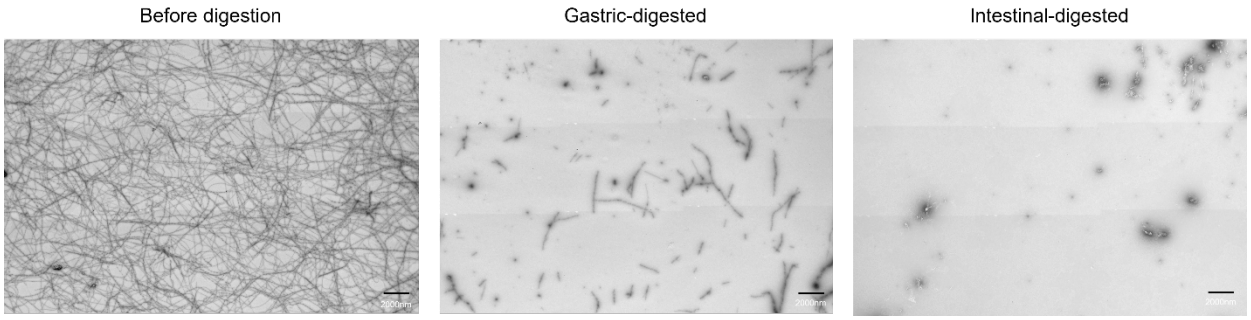

Figure S12. Collated overview TEM images of the gastrointestinal digested products of lysozyme amyloid fibrils. Scale bar represents 2 μm.

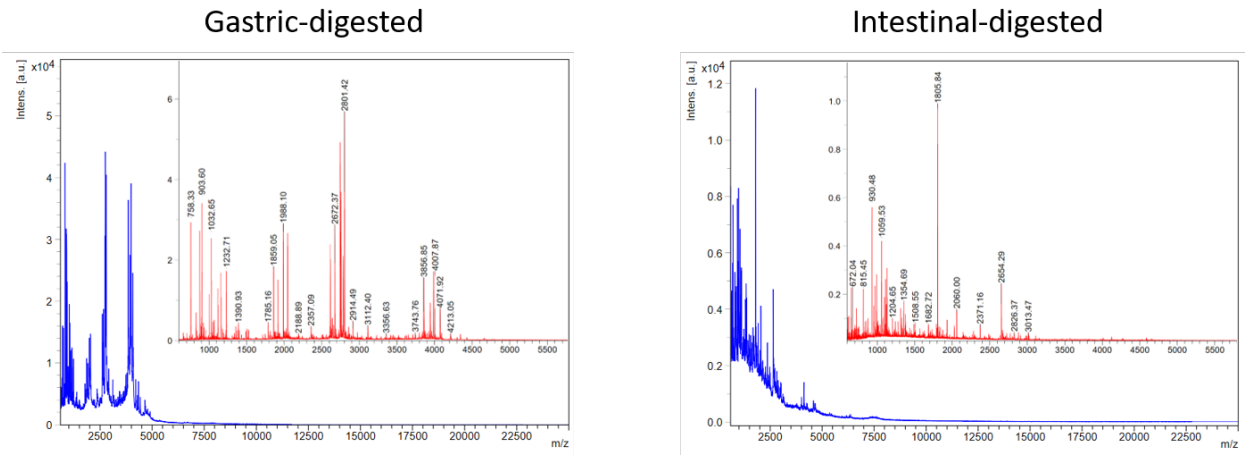

Figure S13. MALDI-MS spectra of 10 kDa filter permeate of gastric- and intestinal digested β-lg amyloid fibrils.

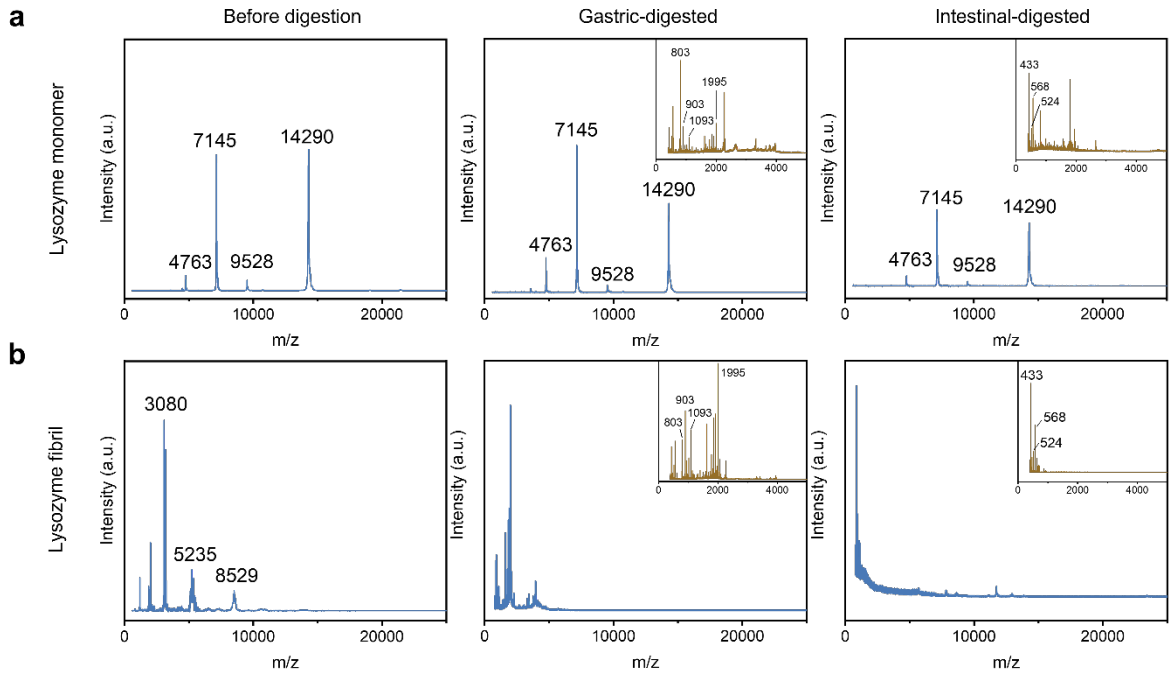

Figure S14. MALDI-MS spectra of lysozyme monomer (a) and amyloid fibrils (b) digested products. The blue plots were obtained in linear mode in the range of 0.5 to 25 kDa, and the inserted plots were recorded in reflection mode in the range of 0.4 to 5 kDa.

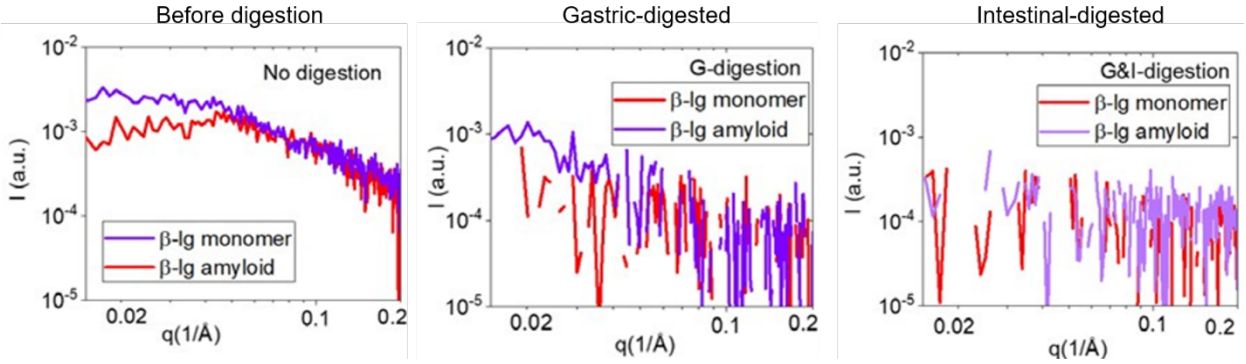

Figure S15. Background subtracted SAXS profiles of β-lg monomer and amyloid fibril before digestion, after gastric digestion, and intestinal digestion.

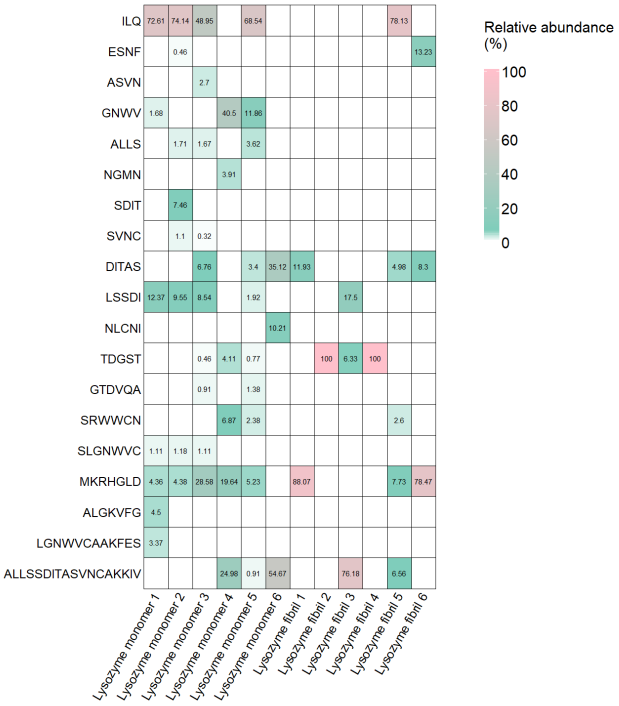

Figure S16. Identified digested polypeptides of lysozyme monomer and fibrils in intestine of mice after 4 h oral administration. Color scale represents the intensity of the relative abundance of digested polypeptides.

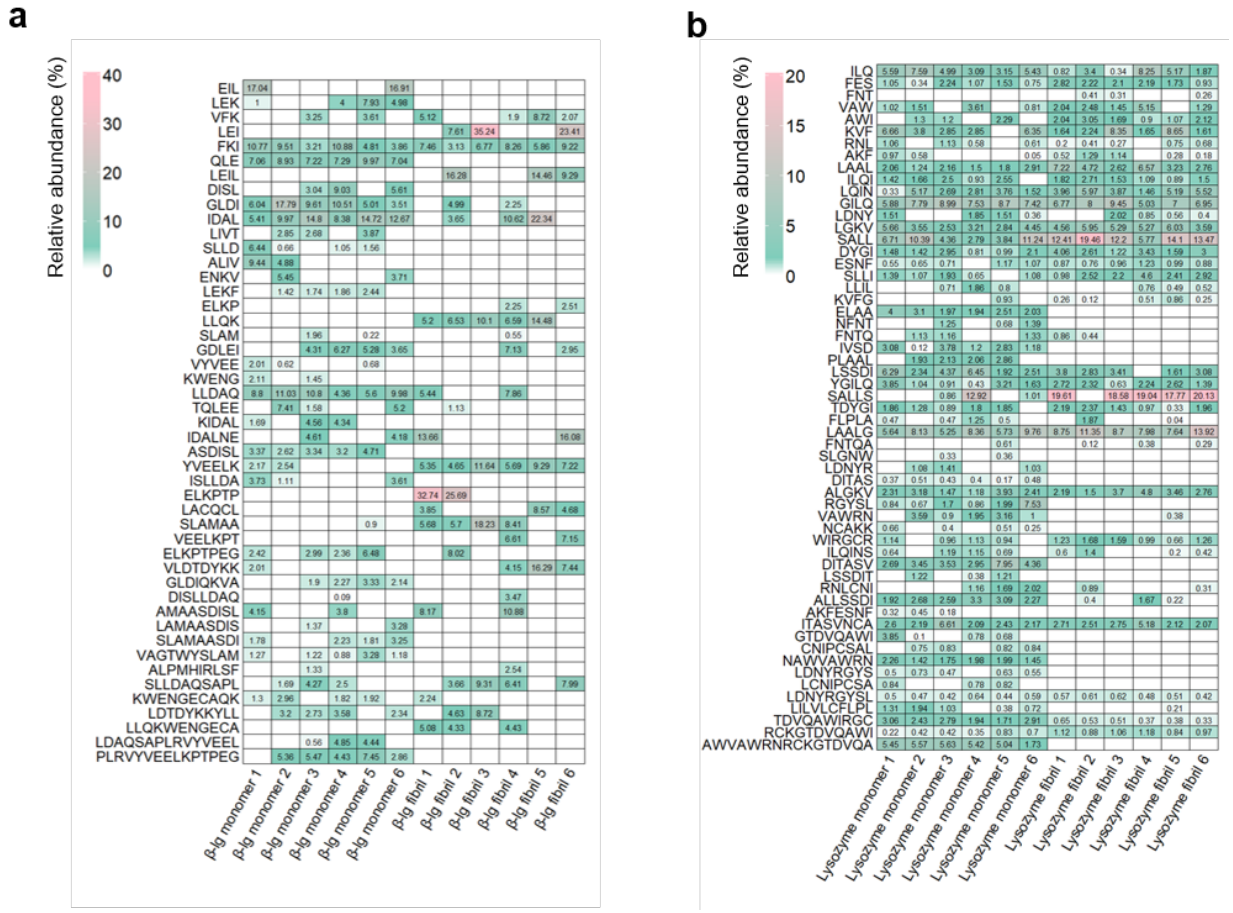

Figure S17. Identified digested polypeptides of β-lg (a) and lysozyme (b) monomer and fibrils in colon of mice after 4 h oral administration. Color scale represents the intensity of the relative abundance of digested polypeptides.

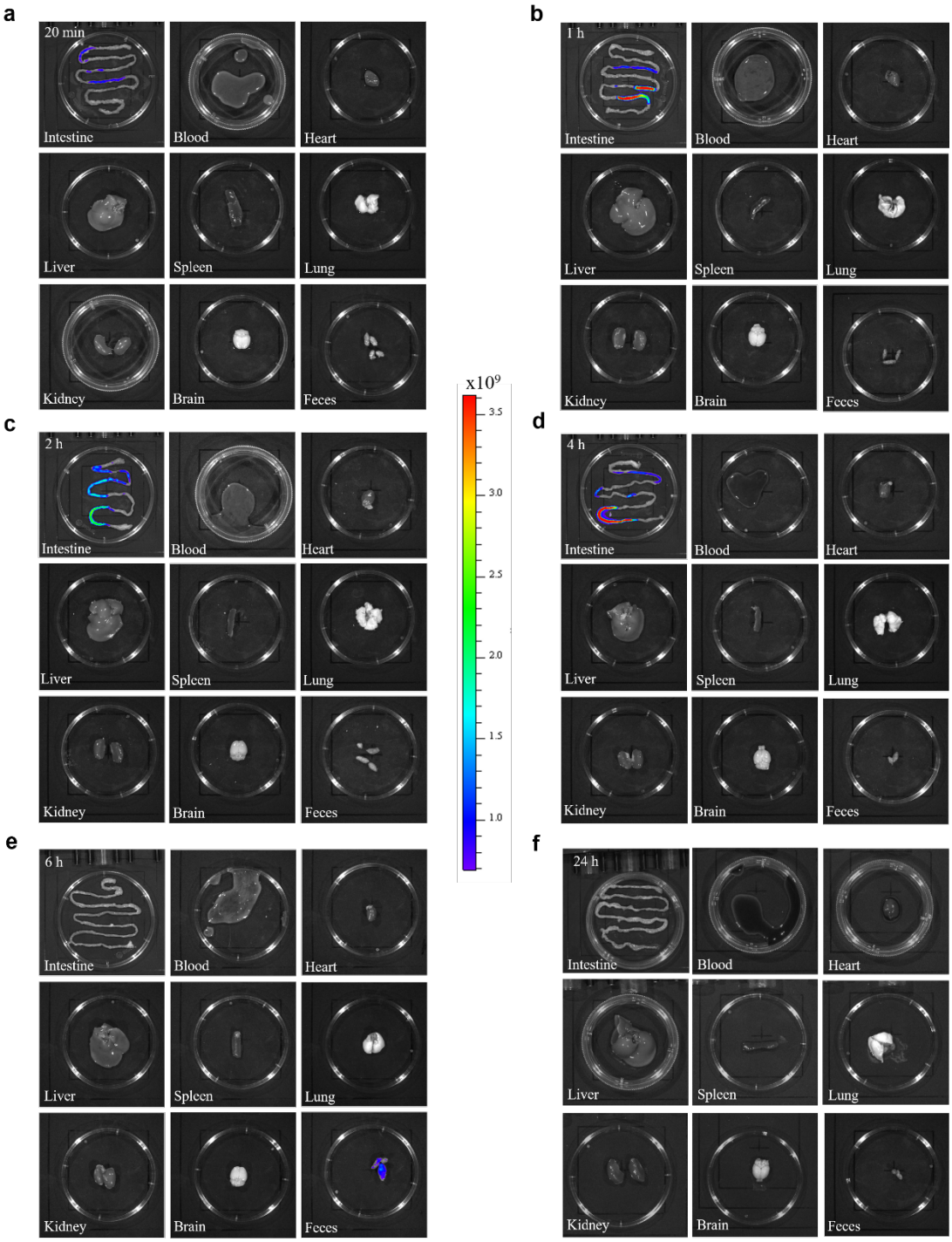

Figure S18. Fluorescence images of 5-DTAF-labeled β-lg fibril distribution in main organs and feces of mice after 20 min (a), 1h (b), 2 h (c), 4 h (d), 6 h (e), and 24 h (f) of oral administration.

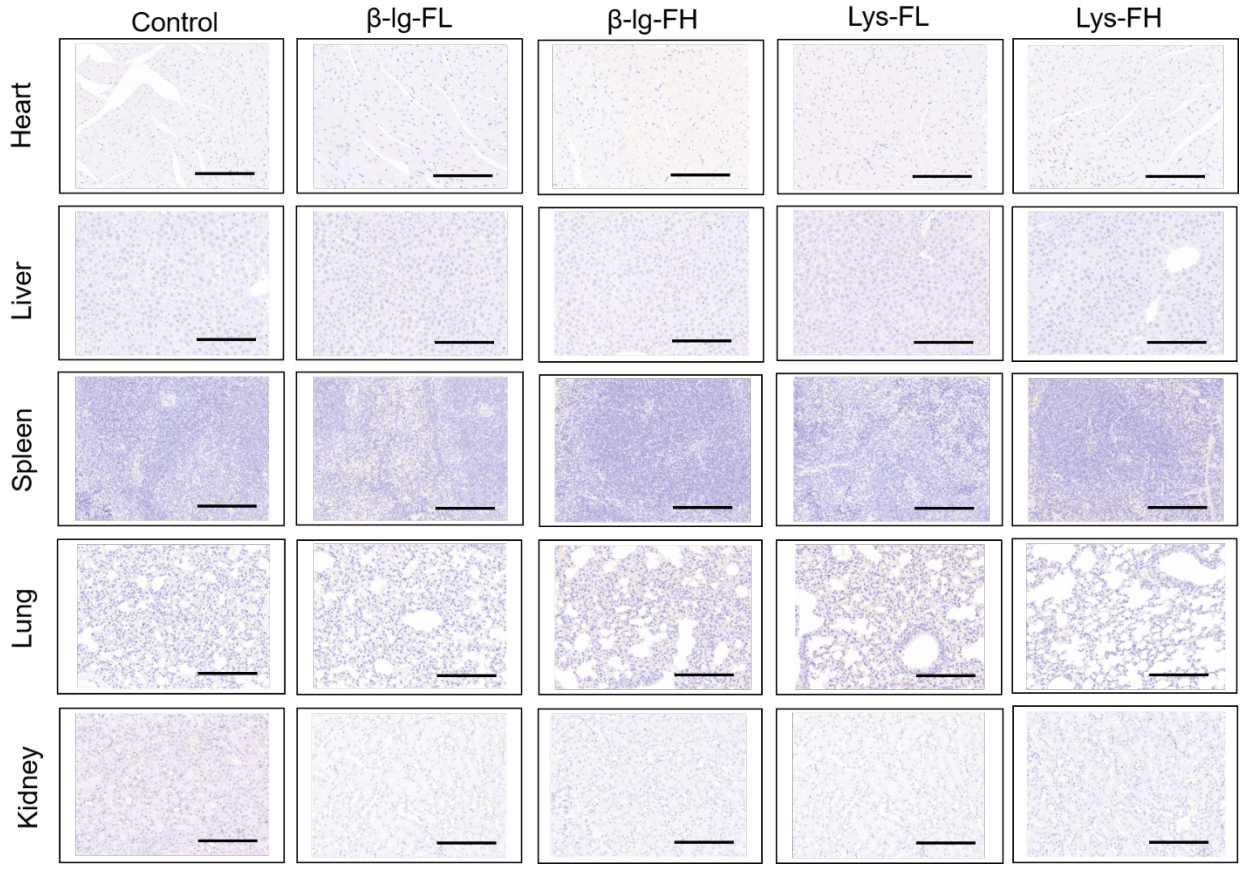

Figure S19. Micrographs of CR-stained major organ tissue sections from different mice groups after 30 d of feeding food amyloid (where F stands for fibril, H and L stand for high and low dosage). Scale bars represent 200 μm.

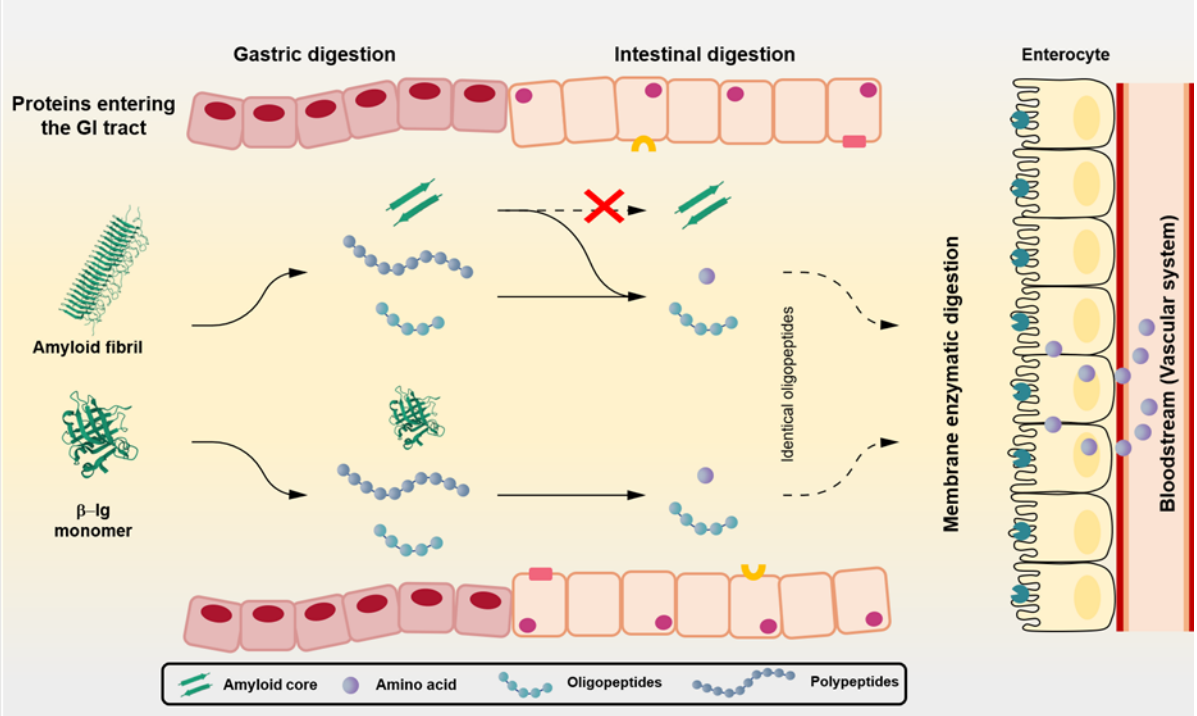

Figure S20. Schematic model of β-lg monomer and amyloid fibrils after entering in the gastrointestinal tract.

Table S1. Relative motility of *C. elegans* fed with monomer, amyloid fibrils, and *in-vitro* digested amyloid fibrils of β-lg and lysozyme.

Days of adulthood	Control			β-lg monomer	β-lg monomer digested	β-lg fibril	β-lg fibril digested	Lysozyme monomer	Lysozyme monomer digested	Lysozyme fibril	Lysozyme fibril digested	Digest buffer control
3	1	1	1	1	1	1	1	1	1	1	1	1
5	0.995	1.060	1.072	0.982	1.017	1.371	1.094	1.148	1.118	1.078	1.123	1.164
7	0.859	0.972	0.997	0.819	0.977	1.089	0.953	1.029	0.951	1.027	1.047	1.045
10	0.631	0.754	0.795	0.672	0.740	0.838	0.861	0.825	0.767	0.918	0.783	0.693
12	0.579	0.640	0.578	0.481	0.556	0.795	0.590	0.682	0.557	0.887	0.669	0.490
14	0.805	0.821	0.865	0.565	0.689	0.810	0.738	0.708	0.792	0.939	0.688	0.775
16	0.605	0.731	0.763	0.639	0.595	1.074	0.615	0.697	0.611	1.087	0.761	0.684
18	0.399	0.544	0.671	0.535	0.363	1.188	0.508	0.531	0.598	0.944	0.721	0.484
20	0.562	0.459	0.594	0.559	0.418	0.926	0.439	0.433	0.469	0.742	0.712	0.286
21	0.415	0.254	0.344	0.389	0.284	0.930	0.385	0.389	0.370	0.838	0.405	0.272
22	0.247	0.090	0.076	0.284	0.149	0.834	0.363	0.290	0.243	0.590	0.471	0.189
23	0.160	0.038	0.060	0.136	0.201	0.424	0.118	0.128	0.099	0.512	0.286	0.180
24	0.135	0.008	0.020	0.096	0.048	0.408	0.047	0.096	0.031	0.229	0.151	0.063
25	0.062	0.003	0.014	0.089	0.082	0.403	0.157	0.149	0.066	0.266	0.194	0.039

Table S2. Number of Q35 aggregates per worm fed with monomer, amyloid fibrils, and *in-vitro* digested amyloid fibrils of β-lg and lysozyme.

Control	β-lg monomer 1.5 mg/ml	β-lg monomer 0.25mg/ml	β-lg fibril 1.5 mg/ml	β-lg fibril 0.25mg/ml	β-lg fibril digested 0.25mg/ml	Lysozyme fibril 0.25mg/ml	Lysozyme fibril 1.5 mg/ml	Lysozyme digested 0.25mg/ml	Lysozyme monomer 0.25mg/ml	Lysozyme monomer 1.5 mg/ml
10	4	5	6	9	6	7	14	10	5	3
5	8	6	7	9	7	2	11	7	8	7
9	12	12	8	10	9	9	6	2	6	17
6	12	4	7	10	6	7	7	10	8	6
5	8	9	9	7	6	2	7	4	2	5
9	11	13	10	2	9	11	16	8	7	8
6	11	9	14	8	11	6	10	10	10	8
10	1	5	9	11	3	9	11	5	13	11
7	3	7	10	9	7	6	10	12	7	12
9	9	7	11	10	4	8	9	15	4	7
3	8	3	6	8	6	12	7	7	9	20
2	9	9	9	10	6	6	7	13	8	9
6	9	15	6	6	12	7	9	6	5	5
8	8	11	9	4	14	3	12	9	8	10
12	10	10	10	8	6	5	7	2	9	3
3	12	6	8	7	7	10	10	6	6	9
7	12	6	9	8	7	10	10	8	10	6
13	2	13	12	11	6	8	12	3	2	8
5	6	7	7	15	8	6	6	6	9	7
7	6	7	7	6	9	14	9	6	5	7
5	11	4	7	8	9	8	13	7	1	7
5	4	5	6	9	14	9	11	6	9	7
5	17	13	8	8	8	11	9	7	7	5
4	5	8	11	18	7	6	10	12	5	5
8	6	7	13	8	6	6	5	6	9	7
7	12	9	9	6	7	8	9	5	9	19
7	13	7	16	5	7	4	9	7	7	9
6	4	4	11	7	9	6	9	8	5	6
9	5	5		6	6	10	11	8	4	7
12	7			6	9	6	15	6	7	5
14				8		7	9	10		
10				13			11			
11				15			15			
8				9						
3										
3										
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11										
8										
5										

Table S3. Detection of peptides in mice serum after 1h digestion*

Sequence	Length	Mass	Location
MHIRLSFNPTQ	11	1342.6816	161-171
KIDALNE	7	801.42323	99-105

*No peptides benchmarked against sequences of β-lg and lysozyme were detected in mice serum after 1h digestion. The only two identified oligopeptides reported in Table S3 which could match β-lg sequences were found in the blood serum of all mice groups: mice fed with β-lg monomers, mice fed with β-lg amyloids and control mice. All mice were starved for 24 h prior to administration. This demonstrates that the presence of these two oligopeptide sequences is not coming from exogenous β-lg amyloids diet.