Supplementary Materials:

Table S1: Historical control data: Mean revertants per plate ± SD (Ames test), Table S2. Validation data: cyclophosphamide, Table S3. Historical control data (micronucleated reticulocytes), Figure S1A Pepsin Digestion of bovine serum albumin (BSA) at a ratio of 10 activity units per microgram test protein, Figure S1B Pepsin Digestion of bovine serum albumin (BSA) at a ratio of 1 activity unit per microgram test protein, Figure S2 Pepsin in SGF sampled for stability.

Bacterial Reverse Mutation Test (Ames Test)

Table S1. Historical control data: Mean revertants per plate ± SD (Ames test)[1]

				Plate Incorporation Method		Pre–Incubation Method	
Strain	Treatment	Dose	S9	Mean ± SD	Range	Mean ± SD	Range
TA1535	Sodium azide	1.5	–	618 ± 91	359 – 1192	622 ± 71	478 – 831
TA1537	ICR 191 Acridine	1	–	1136 ± 1437	119 – 6388	3227 ± 1227	875 – 5700
TA98	Daunomycin	6	–	938 ± 343	350 – 1500	602 ± 345	146 – 1227
TA100	Sodium azide	1.5	–	600 ± 126	394 – 1003	539 ± 166	138 – 904
E. Coli	MMS	2.5	–	634 ± 101	389 – 846	509 ± 143	313 – 808
TA1535	2–AA	10	+	267 ± 86	85 – 636	293 ± 64	64 – 391
TA1537	2–AA	10	+	280 ± 99	42 – 512	260 ± 107	112 – 541
TA98	2–AA	10	+	2321 ± 971	83 – 3915	2384 ± 938	506 – 3530
TA100	2–AA	10	+	2377 ± 806	976 – 4169	2388 ± 583	1308 – 3620
E. Coli	2–AA	10	+	125 ± 30	63 – 196	128 ± 29	60 – 188
TA1535	Sterile Water	N/A	–	13 ± 2	7 – 21	16 ± 3	8 – 23
TA1537	Sterile Water	N/A	–	12 ± 4	6 – 25	14 ± 5	5 – 23
TA98	Sterile Water	N/A	–	28 ± 8	16 – 49	29 ± 7	14 – 46
TA100	Sterile Water	N/A	–	130 ± 16	104 – 155	118 ± 18	83 – 143
E. Coli	Sterile Water	N/A	–	45 ± 7	29 – 57	46 ± 10	30 – 67
TA1535	Sterile Water	N/A	+	13 ± 1	9 – 20	12 ± 2	8 – 19
TA1537	Sterile Water	N/A	+	15 ± 3	8 – 28	14 ± 5	6 – 26
TA98	Sterile Water	N/A	+	29 ± 5	18 – 40	35 ± 7	23 – 50
TA100	Sterile Water	N/A	+	145 ± 14	116 – 170	125 ± 16	88 – 147
E. Coli	Sterile Water	N/A	+	59 ± 13	13 – 31	53 ± 9	36 – 76

[1]Historical Data maintained by PSL from 2015

SD, standard deviation; dose units μg/plate; range is min – max

In Vivo Mouse Erythrocyte Micronucleus Test (Flow Cytometry)

Table S2. Validation data: cyclophosphamide (from PSL)

% MN-RET	Mean ± SEM	
Treatment	M	F
0 mg/kg	0.17 ± 0.02	0.17 ± 0.02
1.5 mg/kg	0.23 ± 0.03	0.22 ± 0.04
5 mg/kg	0.28[a] ± 0.03	0.29[a] ± 0.02
15 mg/kg	0.57[b] ± 0.03	0.46[b] ± 0.05
40 mg/kg	1.33[b] ± 0.07	1.36[b] ± 0.29

[a]$p < 0.05$
[b]$p < 0.001$
% MN-RET, frequency (%) of positive CD71 micronucleated reticulocytes (i.e., micronucleated immature erythrocytes [MIEs]); SEM, standard error of mean; M, male; F, female

% RET	Mean ± SEM	
Treatment	M	F
0 mg/kg	1.74 ± 0.10	1.79 ± 0.17
1.5 mg/kg	2.36 ± 0.37	1.83 ± 0.23
5 mg/kg	1.39 ± 0.15	1.77 ± 0.13
15 mg/kg	1.48 ± 0.15	1.15 ± 0.14
40 mg/kg	0.81[a] ± 0.18	1.15 ± 0.36

[a]$p < 0.05$
% RET, frequency (%) of CD71 positive reticulocytes; SEM, standard error of mean; M, male; F, female

% MN-NCE	Mean ± SEM	
Treatment	M	F
0 mg/kg	0.12 ± 0.01	0.11 ± 0.01
1.5 mg/kg	0.11 ± 0.02	0.11 ± 0.01
5 mg/kg	0.10 ± 0.01	0.10 ± 0.01
15 mg/kg	0.15 ± 0.00	0.11 ± 0.01
40 mg/kg	0.12 ± 0.01	0.14 ± 0.02

% MN-NCE, frequency (%) of micronucleated normochromatic erythrocytes; SEM, standard error of mean; M, male; F, female

Table S3. Historical control data (from PSL).

%MN-RET	Mean ± SEM		Control limits	
Treatment	M	F	M	F
Vehicle	0.16 ± 0.01	0.17 ± 0.01	-0.01 – 0.34	-0.07 – 0.41
CP 40	1.56 ± 0.08	1.49 ± 0.12	-0.12 – 3.24	-1.04 – 4.02

% MN-RET, frequency (%) of positive CD71 micronucleated reticulocytes (i.e., micronucleated immature erythrocytes [MIEs]); M, male; F, female

%RET	Mean ± SEM		Control limits	
Treatment	M	F	M	F
Vehicle	2.21 ± 0.15	1.90 ± 0.08	-0.80 – 5.22	0.27 – 3.54
CP 40	0.69 ± 0.05	0.81 ± 0.06	-0.29 – 1.68	-0.54 – 2.17

% RET, frequency (%) of CD71 positive reticulocytes; M, male; F, female

%MN-NCE	Mean ± SEM		Control limits	
Treatment	M	F	M	F
Vehicle	0.12 ± 0.00	0.11 ± 0.01	0.03 – 0.22	-0.04 – 0.26
CP 40	0.12 ± 0.00	0.13 ± 0.00	0.02 – 0.23	0.04 – 0.22

% MN-NCE, frequency (%) of micronucleated normochromatic erythrocytes; M, male; F, female

Pepsin Digestion

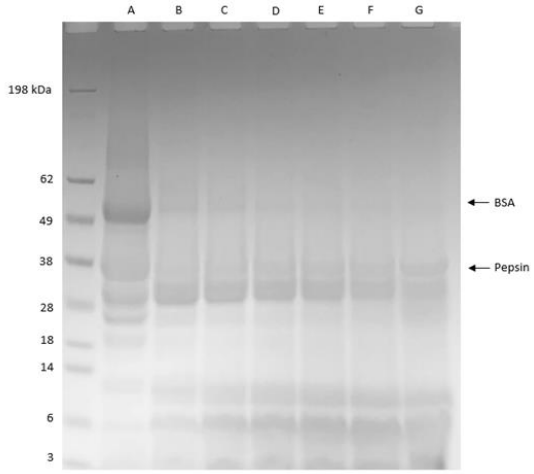

Figure S1A Pepsin Digestion of bovine serum albumin (BSA) at a ratio of 10 activity units per microgram test protein. Silk fibroin at 350 μg run with pepsin in SGF sampled at various time points. Lanes A-G contain samples of silk fibroin digested with pepsin at 0 (Lane A), 2 (Lane B), 5 (Lane C), 10 (Lane D), 20 (Lane E), 30 (Lane F), and 60 (Lane G) minute intervals.

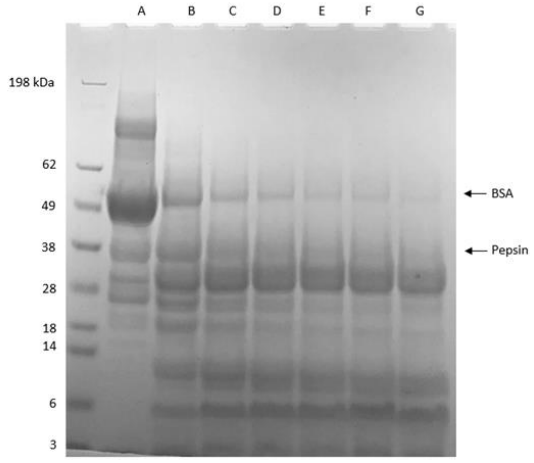

Figure S1B Pepsin Digestion of bovine serum albumin (BSA) at a ratio of 1 activity unit per microgram test protein. Silk fibroin at 350 μg run with pepsin in SGF sampled at various time points. Lanes A-G contain samples of silk fibroin digested with pepsin at 0 (Lane A), 2 (Lane B), 5 (Lane C), 10 (Lane D), 20 (Lane E), 30 (Lane F), and 60 (Lane G) minute intervals.

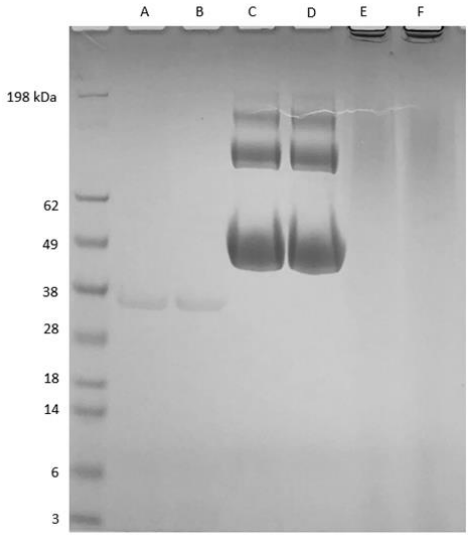

Figure S2 Pepsin in SGF sampled for stability at 0 min. (Lane A) and 60 min. (Lane B). BSA in SGF sampled for stability at 0 min. (Lane C) and 60 min. (Lane D). Silk fibroin in SGF sampled for stability at 0 min. (Lane E) and 60 min. (Lane F).