

## Supplemental material

**Running title:** The development of an affordable, sustainable and efficacious plant-based immunomodulatory food ingredient based on bell pepper or carrot RG-I pectic polysaccharides

**Supplemental table S1.** Extraction yield and MW distribution of bell pepper-PS and carrot-PS after extraction in hot water (100°C, 90 min) and bicarbonate (pH 7.5-8, 100°C, 90 min).

material	extraction	Extraction yield (wt%)	Mw distribution			
			>110 kDa (wt%)	70-110 kDa (wt%)	40-70 kDa (wt%)	<40 kDa (wt%)
bell pepper-PS	water	9	12	16	21	51
	NaHCO <sub>3</sub>	12	18	13	27	42
carrot-PS	water	9	9	16	22	53
	NaHCO <sub>3</sub>	20	33	23	17	27

MW fractions corresponding to >110 kDa, 70-110 kDa and 40-70 kDa were collected and extraction yields measured. The remainder fraction was considered to have a MW <40 kDa.

**Supplemental table S2.** Secretome of in vitro whole blood assay.

	GM-CSF (pg/mL)	IFN- $\gamma$ (pg/mL)	IL-1 (pg/mL)	IL-2 (pg/mL)	IL-3 (pg/mL)	IL-4 (pg/mL)	IL-5 (pg/mL)	IL-6 (pg/mL)	IL-7 (pg/mL)	IL-8 (pg/mL)	IL-9 (pg/mL)	IL-10 (pg/mL)	IL-12 (pg/mL)	IL-13 (pg/mL)	IL-15 (pg/mL)	IL-17 (pg/mL)	IL-18 (pg/mL)	IL-21 (pg/mL)	IL-23 (pg/mL)	IL-27 (pg/mL)	IL-31 (pg/mL)	IL-33 (pg/mL)	IL-35 (pg/mL)	IL-36 (pg/mL)	IL-37 (pg/mL)	IL-38 (pg/mL)	IL-39 (pg/mL)	IL-40 (pg/mL)	IL-41 (pg/mL)	IL-42 (pg/mL)	IL-43 (pg/mL)	IL-44 (pg/mL)	IL-45 (pg/mL)	IL-46 (pg/mL)	IL-47 (pg/mL)	IL-48 (pg/mL)	IL-49 (pg/mL)	IL-50 (pg/mL)	IL-51 (pg/mL)	IL-52 (pg/mL)	IL-53 (pg/mL)	IL-54 (pg/mL)	IL-55 (pg/mL)	IL-56 (pg/mL)	IL-57 (pg/mL)	IL-58 (pg/mL)	IL-59 (pg/mL)	IL-60 (pg/mL)	IL-61 (pg/mL)	IL-62 (pg/mL)	IL-63 (pg/mL)	IL-64 (pg/mL)	IL-65 (pg/mL)	IL-66 (pg/mL)	IL-67 (pg/mL)	IL-68 (pg/mL)	IL-69 (pg/mL)	IL-70 (pg/mL)	IL-71 (pg/mL)	IL-72 (pg/mL)	IL-73 (pg/mL)	IL-74 (pg/mL)	IL-75 (pg/mL)	IL-76 (pg/mL)	IL-77 (pg/mL)	IL-78 (pg/mL)	IL-79 (pg/mL)	IL-80 (pg/mL)	IL-81 (pg/mL)	IL-82 (pg/mL)	IL-83 (pg/mL)	IL-84 (pg/mL)	IL-85 (pg/mL)	IL-86 (pg/mL)	IL-87 (pg/mL)	IL-88 (pg/mL)	IL-89 (pg/mL)	IL-90 (pg/mL)	IL-91 (pg/mL)	IL-92 (pg/mL)	IL-93 (pg/mL)	IL-94 (pg/mL)	IL-95 (pg/mL)	IL-96 (pg/mL)	IL-97 (pg/mL)	IL-98 (pg/mL)	IL-99 (pg/mL)	IL-100 (pg/mL)	IL-101 (pg/mL)	IL-102 (pg/mL)	IL-103 (pg/mL)	IL-104 (pg/mL)	IL-105 (pg/mL)	IL-106 (pg/mL)	IL-107 (pg/mL)	IL-108 (pg/mL)	IL-109 (pg/mL)	IL-110 (pg/mL)	IL-111 (pg/mL)	IL-112 (pg/mL)	IL-113 (pg/mL)	IL-114 (pg/mL)	IL-115 (pg/mL)	IL-116 (pg/mL)	IL-117 (pg/mL)	IL-118 (pg/mL)	IL-119 (pg/mL)	IL-120 (pg/mL)	IL-121 (pg/mL)	IL-122 (pg/mL)	IL-123 (pg/mL)	IL-124 (pg/mL)	IL-125 (pg/mL)	IL-126 (pg/mL)	IL-127 (pg/mL)	IL-128 (pg/mL)	IL-129 (pg/mL)	IL-130 (pg/mL)	IL-131 (pg/mL)	IL-132 (pg/mL)	IL-133 (pg/mL)	IL-134 (pg/mL)	IL-135 (pg/mL)	IL-136 (pg/mL)	IL-137 (pg/mL)	IL-138 (pg/mL)	IL-139 (pg/mL)	IL-140 (pg/mL)	IL-141 (pg/mL)	IL-142 (pg/mL)	IL-143 (pg/mL)	IL-144 (pg/mL)	IL-145 (pg/mL)	IL-146 (pg/mL)	IL-147 (pg/mL)	IL-148 (pg/mL)	IL-149 (pg/mL)	IL-150 (pg/mL)	IL-151 (pg/mL)	IL-152 (pg/mL)	IL-153 (pg/mL)	IL-154 (pg/mL)	IL-155 (pg/mL)	IL-156 (pg/mL)	IL-157 (pg/mL)	IL-158 (pg/mL)	IL-159 (pg/mL)	IL-160 (pg/mL)	IL-161 (pg/mL)	IL-162 (pg/mL)	IL-163 (pg/mL)	IL-164 (pg/mL)	IL-165 (pg/mL)	IL-166 (pg/mL)	IL-167 (pg/mL)	IL-168 (pg/mL)	IL-169 (pg/mL)	IL-170 (pg/mL)	IL-171 (pg/mL)	IL-172 (pg/mL)	IL-173 (pg/mL)	IL-174 (pg/mL)	IL-175 (pg/mL)	IL-176 (pg/mL)	IL-177 (pg/mL)	IL-178 (pg/mL)	IL-179 (pg/mL)	IL-180 (pg/mL)	IL-181 (pg/mL)	IL-182 (pg/mL)	IL-183 (pg/mL)	IL-184 (pg/mL)	IL-185 (pg/mL)	IL-186 (pg/mL)	IL-187 (pg/mL)	IL-188 (pg/mL)	IL-189 (pg/mL)	IL-190 (pg/mL)	IL-191 (pg/mL)	IL-192 (pg/mL)	IL-193 (pg/mL)	IL-194 (pg/mL)	IL-195 (pg/mL)	IL-196 (pg/mL)	IL-197 (pg/mL)	IL-198 (pg/mL)	IL-199 (pg/mL)	IL-200 (pg/mL)	IL-201 (pg/mL)	IL-202 (pg/mL)	IL-203 (pg/mL)	IL-204 (pg/mL)	IL-205 (pg/mL)	IL-206 (pg/mL)	IL-207 (pg/mL)	IL-208 (pg/mL)	IL-209 (pg/mL)	IL-210 (pg/mL)	IL-211 (pg/mL)	IL-212 (pg/mL)	IL-213 (pg/mL)	IL-214 (pg/mL)	IL-215 (pg/mL)	IL-216 (pg/mL)	IL-217 (pg/mL)	IL-218 (pg/mL)	IL-219 (pg/mL)	IL-220 (pg/mL)	IL-221 (pg/mL)	IL-222 (pg/mL)	IL-223 (pg/mL)	IL-224 (pg/mL)	IL-225 (pg/mL)	IL-226 (pg/mL)	IL-227 (pg/mL)	IL-228 (pg/mL)	IL-229 (pg/mL)	IL-230 (pg/mL)	IL-231 (pg/mL)	IL-232 (pg/mL)	IL-233 (pg/mL)	IL-234 (pg/mL)	IL-235 (pg/mL)	IL-236 (pg/mL)	IL-237 (pg/mL)	IL-238 (pg/mL)	IL-239 (pg/mL)	IL-240 (pg/mL)	IL-241 (pg/mL)	IL-242 (pg/mL)	IL-243 (pg/mL)	IL-244 (pg/mL)	IL-245 (pg/mL)	IL-246 (pg/mL)	IL-247 (pg/mL)	IL-248 (pg/mL)	IL-249 (pg/mL)	IL-250 (pg/mL)	IL-251 (pg/mL)	IL-252 (pg/mL)	IL-253 (pg/mL)	IL-254 (pg/mL)	IL-255 (pg/mL)	IL-256 (pg/mL)	IL-257 (pg/mL)	IL-258 (pg/mL)	IL-259 (pg/mL)	IL-260 (pg/mL)	IL-261 (pg/mL)	IL-262 (pg/mL)	IL-263 (pg/mL)	IL-264 (pg/mL)	IL-265 (pg/mL)	IL-266 (pg/mL)	IL-267 (pg/mL)	IL-268 (pg/mL)	IL-269 (pg/mL)	IL-270 (pg/mL)	IL-271 (pg/mL)	IL-272 (pg/mL)	IL-273 (pg/mL)	IL-274 (pg/mL)	IL-275 (pg/mL)	IL-276 (pg/mL)	IL-277 (pg/mL)	IL-278 (pg/mL)	IL-279 (pg/mL)	IL-280 (pg/mL)	IL-281 (pg/mL)	IL-282 (pg/mL)	IL-283 (pg/mL)	IL-284 (pg/mL)	IL-285 (pg/mL)	IL-286 (pg/mL)	IL-287 (pg/mL)	IL-288 (pg/mL)	IL-289 (pg/mL)	IL-290 (pg/mL)	IL-291 (pg/mL)	IL-292 (pg/mL)	IL-293 (pg/mL)	IL-294 (pg/mL)	IL-295 (pg/mL)	IL-296 (pg/mL)	IL-297 (pg/mL)	IL-298 (pg/mL)	IL-299 (pg/mL)	IL-300 (pg/mL)	IL-301 (pg/mL)	IL-302 (pg/mL)	IL-303 (pg/mL)	IL-304 (pg/mL)	IL-305 (pg/mL)	IL-306 (pg/mL)	IL-307 (pg/mL)	IL-308 (pg/mL)	IL-309 (pg/mL)	IL-310 (pg/mL)	IL-311 (pg/mL)	IL-312 (pg/mL)	IL-313 (pg/mL)	IL-314 (pg/mL)	IL-315 (pg/mL)	IL-316 (pg/mL)	IL-317 (pg/mL)	IL-318 (pg/mL)	IL-319 (pg/mL)	IL-320 (pg/mL)	IL-321 (pg/mL)	IL-322 (pg/mL)	IL-323 (pg/mL)	IL-324 (pg/mL)	IL-325 (pg/mL)	IL-326 (pg/mL)	IL-327 (pg/mL)	IL-328 (pg/mL)	IL-329 (pg/mL)	IL-330 (pg/mL)	IL-331 (pg/mL)	IL-332 (pg/mL)	IL-333 (pg/mL)	IL-334 (pg/mL)	IL-335 (pg/mL)	IL-336 (pg/mL)	IL-337 (pg/mL)	IL-338 (pg/mL)	IL-339 (pg/mL)	IL-340 (pg/mL)	IL-341 (pg/mL)	IL-342 (pg/mL)	IL-343 (pg/mL)	IL-344 (pg/mL)	IL-345 (pg/mL)	IL-346 (pg/mL)	IL-347 (pg/mL)	IL-348 (pg/mL)	IL-349 (pg/mL)	IL-350 (pg/mL)	IL-351 (pg/mL)	IL-352 (pg/mL)	IL-353 (pg/mL)	IL-354 (pg/mL)	IL-355 (pg/mL)	IL-356 (pg/mL)	IL-357 (pg/mL)	IL-358 (pg/mL)	IL-359 (pg/mL)	IL-360 (pg/mL)	IL-361 (pg/mL)	IL-362 (pg/mL)	IL-363 (pg/mL)	IL-364 (pg/mL)	IL-365 (pg/mL)	IL-366 (pg/mL)	IL-367 (pg/mL)	IL-368 (pg/mL)	IL-369 (pg/mL)	IL-370 (pg/mL)	IL-371 (pg/mL)	IL-372 (pg/mL)	IL-373 (pg/mL)	IL-374 (pg/mL)	IL-375 (pg/mL)	IL-376 (pg/mL)	IL-377 (pg/mL)	IL-378 (pg/mL)	IL-379 (pg/mL)	IL-380 (pg/mL)	IL-381 (pg/mL)	IL-382 (pg/mL)	IL-383 (pg/mL)	IL-384 (pg/mL)	IL-385 (pg/mL)	IL-386 (pg/mL)	IL-387 (pg/mL)	IL-388 (pg/mL)	IL-389 (pg/mL)	IL-390 (pg/mL)	IL-391 (pg/mL)	IL-392 (pg/mL)	IL-393 (pg/mL)	IL-394 (pg/mL)	IL-395 (pg/mL)	IL-396 (pg/mL)	IL-397 (pg/mL)	IL-398 (pg/mL)	IL-399 (pg/mL)	IL-400 (pg/mL)	IL-401 (pg/mL)	IL-402 (pg/mL)	IL-403 (pg/mL)	IL-404 (pg/mL)	IL-405 (pg/mL)	IL-406 (pg/mL)	IL-407 (pg/mL)	IL-408 (pg/mL)	IL-409 (pg/mL)	IL-410 (pg/mL)	IL-411 (pg/mL)	IL-412 (pg/mL)	IL-413 (pg/mL)	IL-414 (pg/mL)	IL-415 (pg/mL)	IL-416 (pg/mL)	IL-417 (pg/mL)	IL-418 (pg/mL)	IL-419 (pg/mL)	IL-420 (pg/mL)	IL-421 (pg/mL)	IL-422 (pg/mL)	IL-423 (pg/mL)	IL-424 (pg/mL)	IL-425 (pg/mL)	IL-426 (pg/mL)	IL-427 (pg/mL)	IL-428 (pg/mL)	IL-429 (pg/mL)	IL-430 (pg/mL)	IL-431 (pg/mL)	IL-432 (pg/mL)	IL-433 (pg/mL)	IL-434 (pg/mL)	IL-435 (pg/mL)	IL-436 (pg/mL)	IL-437 (pg/mL)	IL-438 (pg/mL)	IL-439 (pg/mL)	IL-440 (pg/mL)	IL-441 (pg/mL)	IL-442 (pg/mL)	IL-443 (pg/mL)	IL-444 (pg/mL)	IL-445 (pg/mL)	IL-446 (pg/mL)	IL-447 (pg/mL)	IL-448 (pg/mL)	IL-449 (pg/mL)	IL-450 (pg/mL)	IL-451 (pg/mL)	IL-452 (pg/mL)	IL-453 (pg/mL)	IL-454 (pg/mL)	IL-455 (pg/mL)	IL-456 (pg/mL)	IL-457 (pg/mL)	IL-458 (pg/mL)	IL-459 (pg/mL)	IL-460 (pg/mL)	IL-461 (pg/mL)	IL-462 (pg/mL)	IL-463 (pg/mL)	IL-464 (pg/mL)	IL-465 (pg/mL)	IL-466 (pg/mL)	IL-467 (pg/mL)	IL-468 (pg/mL)	IL-469 (pg/mL)	IL-470 (pg/mL)	IL-471 (pg/mL)	IL-472 (pg/mL)	IL-473 (pg/mL)	IL-474 (pg/mL)	IL-475 (pg/mL)	IL-476 (pg/mL)	IL-477 (pg/mL)	IL-478 (pg/mL)	IL-479 (pg/mL)	IL-480 (pg/mL)	IL-481 (pg/mL)	IL-482 (pg/mL)	IL-483 (pg/mL)	IL-484 (pg/mL)	IL-485 (pg/mL)	IL-486 (pg/mL)	IL-487 (pg/mL)	IL-488 (pg/mL)	IL-489 (pg/mL)	IL-490 (pg/mL)	IL-491 (pg/mL)	IL-492 (pg/mL)	IL-493 (pg/mL)	IL-494 (pg/mL)	IL-495 (pg/mL)	IL-496 (pg/mL)	IL-497 (pg/mL)	IL-498 (pg/mL)	IL-499 (pg/mL)	IL-500 (pg/mL)
Apple pectin	16	1	0.00	0.02	0.48	0.50	1.0	46	28	1	7	0	3	177	0.0	2	0	4	1907	20	0	5	2.73	0.01	0	3.2	42	80	0.4	298	185	37	>500																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
bpRG-I	21	252	0.04	2.08	4.08	0.79	5.4	92	164	146	6	25	313	450	6.4	76	25	1999	4750	149	0	82	6.20	0.02	17	3.2	90	239	5.4	315	196	36	>100																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
cRG-I	22	148	0.02	1.14	3.51	0.65	3.7	75	159	160	21	23	284	317	3.4	63	17	338	3477	162	0	67	5.37	0.02	12	3.2	84	143	3.9	296	181	35	>50																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
b-glucan	20	2	0.00	0.01	0.44	0.47	1.4	51	120	18	14	0	13	161	0.0	7	0	4	1730	85	0	19	2.34	0.00	0	3.0	50	180	2.8	271	169	32	>10																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
LPS/SEB	34	15643	0.04	1.44	4.20	0.65	370	92	103	19	5	25	205	292	5.4	314	842	3147	4700	397	104	13380	5.18	0.06	9	3.3	77	105	2.5	278	179	34	>5																																																																																																																																																																																																																																																																																																																																																																																																																																																																							

Concentrations per mediator are indicated as average of 3 donors. Color-coded according to stimulation index (SI) vs unstimulated control (PBS) with scale on the right.

**Supplemental table S3.** Impact of bpRG-I and cRG-I on the microbiota composition at the family level (relative abundance) in batch cultures (48h) with human fecal inoculum.

Phylum	Family	Inoculum	Base medium	bpRG-I	cRG-I
Actinobacteria	Coriobacteriaceae	0.5%	0.5%	2.1%*	0.2%*
Bacteroidetes	Bacteroidaceae	29.3%*	44.7%	54.6%*	69.2%*
	Porphyromonadaceae	0.4%	0.6%	0.9%*	0.2%*
	Rikenellaceae	6.3%*	1.1%	1.1%	0.5%*
Firmicutes	Acidaminococcaceae	0.7%*	9.2%	10.7%	11.2%*
	Erysipelotrichaceae	0.3%*	0.1%	0.4%*	0.1%*
	Lachnospiraceae	33.5%*	18.9%	15.7%*	13.6%*
	Ruminococcaceae	28.6%*	3.7%	6.3%*	2.3%*
Proteobacteria	Alcaligenaceae	0.2%*	2.8%	1.2%*	0.5%*
	Desulfovibrionaceae	0%*	0.4%	0.1%*	0%*
	Enterobacteria	0%*	17.9%	6.9%*	2.2%*

Statistical differences within each family versus the base medium incubation, as calculated with a 2-sided student t-test, are indicated by an asterisk ( $p \leq 0.05$ ). Only families with abundances of at least 0.1% in one of the treatments are shown.

**Supplemental table S4.** Inclusion and exclusion criteria in proof-of-concept human study with bpRG-I.

*Inclusion criteria*

- Apparently healthy, Caucasian, males and females, aged 20–65 years at study start
- Body mass index (BMI) of 18.5 to 29.9 kg/m<sup>2</sup>
- No disorders that may interfere with the study conduct or evaluation, e.g. current or previous respiratory, cardiovascular or metabolic diseases, chronic (auto)-immune, gastrointestinal disorders, infections etc.
- Values within the normal reference range at screening of leucocytes, lymphocytes and monocytes
- No clinically relevant deviations and/or no deviations > 2 x ULN (upper limit of normal) in other laboratory values at screening
- Readiness to comply with all specific study procedures, in particular:
  - Consumption of the investigational product (IP) during the entire study
  - Maintaining the habitual diet, with the exception regarding the required dietary restrictions throughout the study to be complied with
  - Accepting the collection of fecal samples for evaluation of fecal microbiome
  - Accepting blood draws to evaluate immune response
  - Filling in health questionnaires
- Women of childbearing potential: negative pregnancy testing ( $\beta$ -HCG (beta subunit of human chorionic gonadotropin) test in urine) at screening and readiness to use reliable contraception methods for the trial duration
- Prerequisite for the participation in the study was a written informed consent by the participant following written and oral information by the investigator regarding nature, purpose, possible benefit and possible risks of the clinical study

*Exclusion criteria*

- Previously known hypersensitivity to any component of the investigational product and/or to bell pepper
- Known severe acute or chronic immunological disorders such as acquired immune deficiency syndrome (AIDS) (or human immunodeficiency virus (HIV) positive)
- Malignant disease within the last 5 years prior to screening
- Known diagnosed allergies (including any food allergy)
- Untreated or unstable hypertension (>140/90 mmHg)
- Mastopathy and/or menstrual disorders
- Any surgery or vaccination within the last 3 months prior to screening and during the study
- Intake of antibiotics, systemic corticosteroids, immune suppressants, psychotherapeutic treatment that may affect the blood count within the last 3 months prior to screening (except for nonsteroidal anti-inflammatory drugs and other potentially anti-inflammatory local analgesics  $\geq 2$  days prior to screening and during study and/or intake of any treatment and/or supplementation influencing the immune system during the study (except for nonsteroidal anti-inflammatory drugs and other potentially anti-inflammatory analgesics  $\geq 2$  days prior to study visits)
- Regular use of statins
- Reported, unexplainable weight loss or gain >2 kg in the last month before screening
- Intense sporting activities >10 h/week before screening and during the study
- Participation in night shift work or reported irregular sleep-wake pattern 2 weeks before screening and during the study
- Reported dietary restrictions such as consuming a medically prescribed diet, slimming diet or vegetarian diet in the last 3 months prior to screening or during the study
- Reported total consumption of >10 units (tablets, capsules, sachets etc.) of vitamin supplements containing  $\beta$ -carotene, vitamin C, E or zinc in the last 3 months prior to screening or any such supplementation during the study
- Reported consumption of probiotic foods or drinks in the last month prior to screening or during the study
- Reported alcohol consumption of >2 units/day (1 unit equals approximately 250 mL of beer, 100 mL of wine or 35 mL of spirits)
- Reported history of abuse of drugs or medication in the last 6 months prior to screening
- Regular consumption of tobacco
- Blood donation or transfusion in the last 2 months prior to screening or during the study
- Participation in another study in the last 2 months before screening or during the study
- Participation in the present study of a person living in the same household as the subject

- Plans to travel abroad during the period of the entire study
- Inability to comply with study procedures (e.g. due to language difficulties etc.)
- Pregnancy or lactation
- Any other reason deemed suitable for exclusion, per investigator's judgment

**Supplemental table S5.** Baseline demographic and anthropometric data of the participants.

Parameter	Total n=57	Placebo n=20	0.3 g /day (low dose) n=19	1.5 g / day (high dose) n=18
Age	49.0 ± 12.0	48.0 ± 12.9	46.2 ± 13.5	52.5 ± 8.2
Female (#/%)	39/68.4%	14/70.0%	13/68.4%	12/66.7%
Height (cm)	171.5 ± 9.1	171.2 ± 9.3	173.4 ± 9.3	170.0 ± 8.8
Weight (kg)	70.8 ± 11.6	70.2 ± 11.5	70.9 ± 11.4	71.3 ± 12.4