

Supplementary Table 1A. Effect of placebo on proportion of NK cell subsets in young and older cohort. Data are mean \pm SEM for n= 25 young subjects and n= 27 older subjects on placebo treatment. Data were analysed using a paired samples t –test (before vaccination) and using a Linear Mixed Model (after vaccination).

% of immune cells	Parent population	Young cohort						Older cohort					
		baseline	week 4	<i>P</i> -value	Week 6	Week 8	<i>P</i> -value	baseline	week 4	<i>P</i> -value	Week 6	Week 8	<i>P</i> -value
				Wk0vs4			LMM			Wk0vs4			LMM
Lymphocytes	PBMC	78.00 \pm 1.18	78.59 \pm 1.00	0.456	78.45 \pm 1.25	79.01 \pm 1.28	0.840	71.56 \pm 1.85	71.71 \pm 1.56	0.912	70.97 \pm 2.09	73.21 \pm 1.51	0.790
CD56 ⁻ CD16 ⁺	lymphocyte	5.99 \pm 0.94	5.57 \pm 0.71	0.496	5.27 \pm 0.74	4.91 \pm 0.58	0.120	8.511 \pm 0.92	7.99 \pm 0.68	0.254	8.60 \pm 0.98	8.79 \pm 0.83	0.114
CD3 ⁺ CD56 ⁺	lymphocyte	3.15 \pm 0.43	3.02 \pm 0.44	0.289	2.81 \pm 0.39	2.80 \pm 0.43	0.271	3.02 \pm 0.87	3.17 \pm 0.90	0.115	3.28 \pm 0.96	3.20 \pm 0.89	0.192
CD3 ⁺ CD56 ⁻	lymphocyte	67.67 \pm 1.91	67.49 \pm 1.90	0.892	68.35 \pm 1.35	71.43 \pm 1.20	0.468	69.87 \pm 1.44	69.39 \pm 1.28	0.713	68.92 \pm 1.35	70.28 \pm 1.30	0.782
CD3 ⁻ CD56 ⁺	CD3 ⁻ lymphocyte	16.91 \pm 1.26	16.40 \pm 1.65	0.121	16.33 \pm 1.72	14.87 \pm 1.35	0.141	24.28 \pm 1.25	24.74 \pm 1.84	0.835	23.12 \pm 1.75	25.23 \pm 1.83	0.335
CD56 ^{bright}	CD3 ⁻ CD56 ⁺	11.10 \pm 1.74	10.40 \pm 1.74	0.259	11.63 \pm 2.03	10.97 \pm 1.69	0.975	6.79 \pm 1.00	6.87 \pm 0.81	0.902	7.61 \pm 0.94	6.91 \pm 0.99	0.156
CD56 ^{bright} CD16 ⁻	CD3 ⁻ CD56 ⁺	9.84 \pm 1.62	9.33 \pm 1.60	0.327	10.03 \pm 1.72	9.92 \pm 1.61	0.443	5.68 \pm 0.87	5.82 \pm 0.71	0.819	6.35 \pm 0.84	5.79 \pm 0.89	0.571
CD56 ^{bright} CD16 ^{dim}	CD3 ⁻ CD56 ⁺	1.88 \pm 0.32	1.55 \pm 0.30	0.040	2.24 \pm 0.42	1.71 \pm 0.26	0.407	1.58 \pm 0.27	1.54 \pm 0.22	0.833	1.76 \pm 0.23	1.60 \pm 0.25	0.479
CD56 ^{dim}	CD3 ⁻ CD56 ⁺	88.16 \pm 1.80	88.97 \pm 1.78	0.205	87.57 \pm 2.10	88.15 \pm 1.76	0.936	92.67 \pm 1.04	92.59 \pm 0.84	0.911	91.82 \pm 0.98	92.56 \pm 1.04	0.090
CD56 ^{dim} CD16 ⁻	CD3 ⁻ CD56 ⁺	27.00 \pm 2.20	28.16 \pm 2.20	0.246	27.10 \pm 2.32	29.71 \pm 2.54	0.188	21.15 \pm 2.14	22.70 \pm 2.21	0.320	22.35 \pm 2.37	20.51 \pm 2.02	0.407
CD56 ^{dim} CD16 ⁺	CD3 ⁻ CD56 ⁺	61.30 \pm 3.16	60.95 \pm 3.11	0.759	60.62 \pm 3.06	58.67 \pm 3.41	0.343	71.58 \pm 2.69	69.93 \pm 2.55	0.388	69.54 \pm 2.87	72.11 \pm 2.69	0.583
CD3 ⁻ CD56 ⁺ CD57 ⁺	CD3 ⁻ CD56 ⁺	44.76 \pm 3.04	44.60 \pm 3.29	0.872	42.85 \pm 2.90	42.28 \pm 2.83	0.742	50.43 \pm 2.77	50.57 \pm 2.81	0.880	48.52 \pm 2.72	50.95 \pm 2.66	0.862
CD3 ⁺ CD56 ⁺ CD57 ⁺	CD3 ⁺ CD56 ⁺	40.40 \pm 3.30	37.97 \pm 3.45	0.050	39.53 \pm 3.48	35.79 \pm 3.36	0.126	50.18 \pm 3.62	48.56 \pm 3.97	0.362	48.65 \pm 4.06	50.88 \pm 4.04	0.145
CD3 ⁺ CD57 ⁺	CD3 ⁺	7.65 \pm 0.82	7.94 \pm 1.09	0.462	7.13 \pm 0.65	6.74 \pm 0.61	0.278	11.10 \pm 1.01	10.88 \pm 1.11	0.671	11.66 \pm 1.33	11.71 \pm 1.14	0.242
CD56 ^{dim} CD57 ⁻	CD56 ^{dim}	41.65 \pm 2.22	41.89 \pm 2.29	0.774	42.00 \pm 2.03	43.56 \pm 1.99	0.755	36.76 \pm 2.24	35.90 \pm 2.38	0.281	37.23 \pm 2.21	36.45 \pm 2.17	0.677
CD56 ^{dim} CD57 ⁺	CD56 ^{dim}	44.83 \pm 2.89	45.19 \pm 3.08	0.694	44.58 \pm 2.76	42.39 \pm 2.68	0.504	50.74 \pm 2.68	52.20 \pm 2.95	0.191	50.49 \pm 2.72	51.54 \pm 2.73	0.409
CD56 ^{dim} CD16 ⁺ CD57 ⁺	CD56 ^{dim} CD16 ⁺	55.24 \pm 2.89	55.86 \pm 3.04	0.450	55.62 \pm 2.93	54.20 \pm 2.69	0.407	59.02 \pm 2.70	60.68 \pm 2.99	0.054	58.90 \pm 2.88	59.26 \pm 2.72	0.334
CD56 ^{dim} CD16 ⁻ CD57 ⁺	CD56 ^{dim} CD16 ⁻	18.38 \pm 2.15	18.58 \pm 2.40	0.860	16.34 \pm 1.89	15.01 \pm 1.48	0.889	17.97 \pm 1.98	20.98 \pm 2.39	0.047	18.85 \pm 2.20	19.02 \pm 2.40	0.847

Supplementary Table 1B. Effect of *B.longum* + GI-OS on proportion of NK cell subsets in young and older cohort. Data are mean \pm SEM for n= 26 young subjects and n= 23 older subjects on *B.longum* treatment. Data were analysed using a paired samples t –test (before vaccination) and using a Linear Mixed Model (after vaccination).

% of immune cells	Parent population	Young cohort						Older cohort					
		baseline	week 4	<i>P</i> -value Wk0vs4	Week 6	Week 8	<i>P</i> -value LMM	baseline	week 4	<i>P</i> -value Wk0vs4	Week 6	Week 8	<i>P</i> -value LMM
Lymphocytes	PBMC	80.60 \pm 1.23	80.37 \pm 1.10	0.769	79.78 \pm 1.13	79.96 \pm 1.15	0.325	71.90 \pm 1.36	69.64 \pm 1.84	0.054	71.74 \pm 1.97	70.08 \pm 1.74	0.335
CD56 ⁻ CD16 ⁺	lymphocyte	4.98 \pm 0.48	4.57 \pm 0.37	0.306	4.52 \pm 0.32	4.44 \pm 0.37	0.337	8.97 \pm 1.28	10.25 \pm 1.28	0.031	7.66 \pm 1.02	9.12 \pm 1.29	0.600
CD3 ⁺ CD56 ⁺	lymphocyte	4.23 \pm 1.18	3.46 \pm 0.98	0.213	3.61 \pm 1.02	3.54 \pm 1.03	0.608	5.42 \pm 1.66	5.57 \pm 1.64	0.557	5.40 \pm 1.8	4.94 \pm 1.23	0.226
CD3 ⁺ CD56 ⁻	lymphocyte	69.66 \pm 1.70	70.26 \pm 1.78	0.650	70.84 \pm 1.64	70.60 \pm 1.71	0.532	67.60 \pm 2.18	67.43 \pm 2.06	0.872	67.29 \pm 2.33	66.99 \pm 1.92	0.287
CD3 ⁻ CD56 ⁺	CD3 ⁻ lymphocyte	16.91 \pm 1.26	15.17 \pm 1.01	0.218	13.94 \pm 1.49	13.95 \pm 1.28	0.338	20.63 \pm 0.95	26.00 \pm 1.36	0.018	24.23 \pm 1.62	26.00 \pm 1.62	0.601
CD56 ^{bright}	CD3 ⁻ CD56 ⁺	8.70 \pm 1.08	9.96 \pm 1.41	0.126	10.16 \pm 1.13	9.22 \pm 1.31	0.500	7.02 \pm 1.03	6.35 \pm 0.98	0.250	6.89 \pm 1.05	7.84 \pm 1.10	0.700
CD56 ^{bright} CD16 ⁻	CD3 ⁻ CD56 ⁺	7.58 \pm 0.89	8.76 \pm 1.19	0.117	8.84 \pm 0.92	8.13 \pm 1.13	0.443	5.72 \pm 0.82	5.21 \pm 0.75	0.227	5.69 \pm 0.90	6.21 \pm 0.89	0.860
CD56 ^{bright} CD16 ^{dim}	CD3 ⁻ CD56 ⁺	1.74 \pm 0.30	1.86 \pm 0.33	0.339	2.00 \pm 0.31	1.70 \pm 0.29	0.840	1.74 \pm 0.29	1.62 \pm 0.34	0.516	1.61 \pm 0.29	2.12 \pm 0.37	0.407
CD56 ^{dim}	CD3 ⁻ CD56 ⁺	90.52 \pm 1.13	89.23 \pm 1.47	0.133	89.01 \pm 1.18	90.01 \pm 1.37	0.526	92.47 \pm 1.09	93.11 \pm 1.04	0.280	92.58 \pm 1.09	91.58 \pm 1.15	0.685
CD56 ^{dim} CD16 ⁻	CD3 ⁻ CD56 ⁺	30.52 \pm 2.06	32.23 \pm 2.01	0.078	32.72 \pm 1.92	34.82 \pm 2.33	0.109	19.25 \pm 1.75	19.66 \pm 2.29	0.712	19.97 \pm 2.14	19.12 \pm 1.95	0.470
CD56 ^{dim} CD16 ⁺	CD3 ⁻ CD56 ⁺	60.17 \pm 2.65	57.15 \pm 2.71	0.020	56.44 \pm 2.49	55.35 \pm 3.01	0.418	73.29 \pm 2.32	73.51 \pm 2.70	0.865	72.74 \pm 2.66	72.55 \pm 2.52	0.666
CD3 ⁻ CD56 ⁺ CD57 ⁺	CD3 ⁻ CD56 ⁺	47.48 \pm 2.34	45.26 \pm 2.15	0.087	44.44 \pm 2.34	44.76 \pm 2.40	0.742	50.40 \pm 2.57	51.87 \pm 2.69	0.162	51.28 \pm 2.50	51.05 \pm 2.66	0.862
CD3 ⁺ CD56 ⁺ CD57 ⁺	CD3 ⁺ CD56 ⁺	42.89 \pm 4.42	43.74 \pm 4.23	0.572	42.71 \pm 4.33	42.62 \pm 4.28	0.334	56.26 \pm 4.32	56.0 \pm 4.26	0.862	58.42 \pm 4.44	56.30 \pm 4.13	0.278
CD3 ⁺ CD57 ⁺	CD3 ⁺	9.00 \pm 0.91	9.15 \pm 1.07	0.795	9.45 \pm 1.12	9.25 \pm 1.02	0.295	12.36 \pm 1.68	12.97 \pm 1.79	0.508	11.84 \pm 1.82	12.61 \pm 1.63	0.501
CD56 ^{dim} CD57 ⁻	CD56 ^{dim}	39.99 \pm 1.54	41.41 \pm 1.28	0.183	42.03 \pm 1.47	43.39 \pm 1.74	0.942	34.7 \pm 2.12	34.65 \pm 2.18	0.956	35.07 \pm 2.11	34.60 \pm 2.09	0.620
CD56 ^{dim} CD57 ⁺	CD56 ^{dim}	46.15 \pm 2.21	44.62 \pm 1.78	0.240	43.75 \pm 2.11	42.86 \pm 2.31	0.938	54.68 \pm 2.61	55.24 \pm 2.70	0.587	55.31 \pm 2.60	54.73 \pm 2.59	0.415
CD56 ^{dim} CD16 ⁺ CD57 ⁺	CD56 ^{dim} CD16 ⁺	58.10 \pm 2.32	57.80 \pm 1.97	0.792	57.20 \pm 2.13	57.37 \pm 2.15	0.494	62.26 \pm 2.77	62.43 \pm 2.75	0.853	62.83 \pm 2.80	61.85 \pm 2.71	0.494
CD56 ^{dim} CD16 ⁻ CD57 ⁺	CD56 ^{dim} CD16 ⁻	17.67 \pm 1.51	16.87 \pm 1.41	0.522	16.64 \pm 1.44	15.99 \pm 1.41	0.705	21.33 \pm 2.59	22.19 \pm 3.02	0.444	22.10 \pm 2.93	22.24 \pm 2.74	0.614

Supplementary Table 2A. NK cell activity before and after placebo treatment. Data are mean (± 2 SEM) for n= 21 and 28 subjects per group at four E/T ratios. Data were analysed using a paired samples t –test (before vaccination) and LMM (after vaccination). There were no statistically significant effects of the placebo treatment, either before or after vaccination.

Total NK cell activity												
Young cohort					Older cohort							
E/T ratio	baseline	week 4	week 6	week 8	Before vaccine	After vaccine	baseline	week 4	week 6	week 8	Before vaccine	After vaccine
					<i>P</i> - value Wk0vs4	<i>P</i> - value LMM					<i>P</i> - value Wk0vs4	<i>P</i> - value LMM
100:1	21.43 \pm 1.58	22.69 \pm 2.24	25.93 \pm 2.37	25.72 \pm 2.03	0.145	0.249	26.42 \pm 2.18	28.34 \pm 2.25	26.54 \pm 1.95	27.44 \pm 1.80	0.316	0.093
50:1	10.29 \pm 1.21	13.09 \pm 1.70	12.90 \pm 1.64	12.81 \pm 1.40	0.109	0.695	12.54 \pm 1.38	15.53 \pm 1.58	13.97 \pm 1.14	13.78 \pm 1.15	0.283	0.387
25:1	4.02 \pm 0.51	4.98 \pm 0.65	4.81 \pm 0.65	4.87 \pm 0.59	0.123	0.973	5.22 \pm 0.45	5.64 \pm 0.80	4.53 \pm 0.45	4.88 \pm 0.42	0.037	0.423
12.5:1	1.86 \pm 0.23	2.16 \pm 0.21	1.85 \pm 0.23	1.61 \pm 0.23	0.201	0.652	2.37 \pm 0.32	2.21 \pm 0.25	1.87 \pm 0.23	2.24 \pm 0.34	0.123	0.551

NK cell activity on per cell basis												
Young cohort					Older cohort							
E/T ratio	baseline	week 4	week 6	week 8	Before vaccine	After vaccine	baseline	week 4	week 6	week 8	Before vaccine	After vaccine
					<i>P</i> - value Wk0vs4	<i>P</i> - value LMM					<i>P</i> - value Wk0vs4	<i>P</i> - value LMM
100:1	1.62 \pm 0.24	1.84 \pm 0.29	2.18 \pm 0.40	2.43 \pm 0.39	0.173	0.657	1.30 \pm 0.15	1.38 \pm 0.17	1.38 \pm 0.15	1.28 \pm 0.15	0.209	0.868
50:1	0.78 \pm 0.15	0.96 \pm 0.17	1.13 \pm 0.24	1.21 \pm 0.19	0.061	0.761	0.63 \pm 0.08	0.76 \pm 0.10	0.72 \pm 0.08	0.65 \pm 0.08	0.524	0.778
25:1	0.33 \pm 0.07	0.34 \pm 0.06	0.38 \pm 0.07	0.43 \pm 0.06	0.463	0.690	0.25 \pm 0.03	0.29 \pm 0.07	0.24 \pm 0.03	0.22 \pm 0.02	0.919	0.608
12.5:1	0.14 \pm 0.03	0.15 \pm 0.02	0.17 \pm 0.04	0.14 \pm 0.03	0.652	0.977	0.11 \pm 0.02	0.10 \pm 0.01	0.09 \pm 0.01	0.10 \pm 0.01	0.795	0.725

Supplementary Table 2B. NK cell activity before and after the treatment with *B. longum* + GI-OS. Data are mean (± 2 SEM) for n=32 and 25 subjects per group at four E/T ratios. Data were analysed using a paired samples t-test (before vaccination) and LMM (after vaccination). There were no statistically significant effects of treatment, either before or after vaccination.

Total NK cell activity												
Young cohort						Older cohort						
E/T ratio	baseline	week 4	week 6	week 8	Before vaccine	After vaccine	baseline	week 4	week 6	week 8	Before vaccine	After vaccine
					<i>P</i> - value Wk0vs4	<i>P</i> - value LMM					<i>P</i> - value Wk0vs4	<i>P</i> - value LMM
100:1	25.05 \pm 2.19	25.32 \pm 1.80	27.23 \pm 1.85	29.82 \pm 2.28	0.894	0.249	27.64 \pm 2.55	31.79 \pm 2.77	31.28 \pm 2.86	32.97 \pm 2.09	0.015	0.093
50:1	12.94 \pm 1.65	13.31 \pm 1.36	13.37 \pm 1.29	14.05 \pm 1.47	0.698	0.695	12.96 \pm 1.34	15.40 \pm 1.78	16.54 \pm 1.40	16.03 \pm 1.26	0.095	0.387
25:1	3.98 \pm 0.49	4.91 \pm 0.58	4.48 \pm 0.53	4.98 \pm 0.62	0.006	0.973	4.95 \pm 0.53	4.94 \pm 0.66	5.56 \pm 0.59	6.20 \pm 0.68	0.996	0.423
12.5:1	1.69 \pm 0.21	1.77 \pm 0.26	1.55 \pm 0.22	1.89 \pm 0.27	0.696	0.652	2.10 \pm 0.29	2.28 \pm 0.24	2.56 \pm 0.35	2.19 \pm 0.32	0.533	0.551

NK cell activity on per cell basis												
Young cohort						Older cohort						
E/T ratio	baseline	week 4	week 6	week 8	Before vaccine	After vaccine	baseline	week 4	week 6	week 8	Before vaccine	After vaccine
					<i>P</i> - value Wk0vs4	<i>P</i> - value LMM					<i>P</i> - value Wk0vs4	<i>P</i> - value LMM
100:1	1.97 \pm 0.28	2.29 \pm 0.36	2.22 \pm 0.33	2.54 \pm 0.42	0.183	0.657	1.22 \pm 0.16	1.33 \pm 0.18	1.41 \pm 0.17	1.42 \pm 0.16	0.203	0.868
50:1	1.04 \pm 0.19	1.19 \pm 0.20	1.03 \pm 0.12	1.19 \pm 0.21	0.275	0.761	0.58 \pm 0.08	0.63 \pm 0.11	0.76 \pm 0.09	0.68 \pm 0.08	0.497	0.778
25:1	0.33 \pm 0.05	0.44 \pm 0.08	0.35 \pm 0.05	0.42 \pm 0.08	0.021	0.690	0.21 \pm 0.03	0.19 \pm 0.03	0.25 \pm 0.03	0.25 \pm 0.03	0.606	0.608
12.5:1	0.14 \pm 0.03	0.15 \pm 0.03	0.11 \pm 0.02	0.17 \pm 0.03	0.585	0.977	0.09 \pm 0.01	0.09 \pm 0.01	0.11 \pm 0.02	0.09 \pm 0.01	0.850	0.725