September 6, 2021.

Report for:

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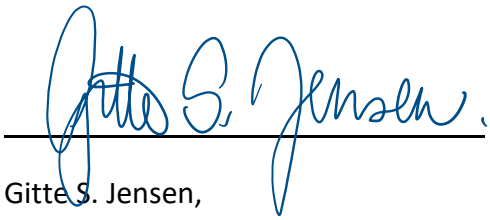
Report 175-004. Immune activation: Synergy of ingredients in the blend Quick Start.



Gitte S. Jensen,

Research director.

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1 Executive Summary

LifeSeasons has developed a novel nutraceutical blend 'Quick-Start' for immune support, including ingredients that have in past studies been associated with rapid immune activation in clinical trials. The goal for this study was to test the speed and magnitude of the activation by Quick-Start and to evaluate contributions from its 5 core ingredients:

- Proprietary extract from the nutritional yeast Saccharomyces cerevisiae,
- Vitamin C
- NutraMune,
- Zinc,
- ElderMune, a proprietary elderberry extract.

Model: As a cellular model for this work, we used freshly isolated peripheral blood mononuclear cells (PBMC) from a healthy blood donor.

Immune cell activation: The treatment of immune cells with Quick-Start resulted in an increase of the CD69 activation marker which translates to increased alertness towards recognizing and killing target cells (i.e., cancer cells and virally transformed cells). This was seen in parallel to a reduction in the expression of the growth factor receptor CD25, indicating that cells were focusing their effort on immune recognition instead of cell division. Please also refer to the overview tables for immune cell activation (Page 11).

Cytokines and growth factors: The treatment of immune cells with Quick-Start triggered rapid induction of multiple cytokines, chemokines, and growth factors already in 2 hours. Several cytokines showed a complex dose response to Quick-Start after

24 hours, suggesting strong and prolonged immune cell activation at higher doses and anti-inflammatory and regulating effects at lower doses. Please also refer to the overview tables for cytokine production (Page 26).

Synergy between ingredients: The nutraceutical formulation Quick-Start showed rapid and robust immune activating and modulating effects in vitro. The effects showed contributions from all 5 core ingredients. There were clear indications as to which ingredients contributed most robust effects to the overall synergistic effect of Quick-Start on human immune cells. The ingredients are listed below in the order of strong to weaker or more complex contributions to the overall effects of Quick-Start:

1. Saccharomyces cerevisiae
2. Vitamin C.
3. NutraMune
4. Zinc
5. ElderMune

Further work: The results warrant proceeding with validating repeats with the intent of writing a manuscript. The work reported here was done on cells from 1 healthy blood donor and validating repeats for the work described here is needed if you wish to pursue a peer reviewed publication.

Clinical testing is also warranted, and the results reported here has helped build confidence in an acute study design where we will evaluate rapid 2-hour efficacy of the blend in a randomized, cross-over, placebo-controlled cross-over trial in healthy people.

Report 175-004. Immune activation: Synergy of ingredients in the blend Quick Start.

2 Purpose

To compare the novel nutraceutical blend 'Quick-Start' to 5 of its key ingredients to help document synergistic effects of the blend.

3 Background

LifeSeasons Inc. has launched new products designed to support the immune system. One of the products is called **'Quick-Start'** and is designed to increase the immune alertness and responsiveness within 2 hours. This is based on previous research on one of the key immune modulating ingredients, EpiCor.[13]

The project reported here was planned based on the following needs:

- Scientific documentation that the blend triggers a rapid response to support the marketing claim associated with the product Quick Start: "***Response in 2 hours***";
- Verify synergy between ingredients in the blend;
- Establish foundational results, based on which further validation work can be planned, as we move towards publishing a scientific paper on the combined results.

The in vitro testing described here aimed at comparing the effects of the blend to each of its 5 key ingredients, specifically for immune cell activation and cytokine changes to pro- and anti-inflammatory cytokines, antiviral peptides, and restorative growth factors. This strategy was based on published work using similar in vitro methods to document synergy beyond what would be expected by a simple additive effect of the ingredients in a blend.[12]

4 Work Performed

4.1 Test Products

Table 1. Test products compared in this project.

Name	Handling	% in Quick-Start	Top dose in immune assay
Quick-Start	Aqueous	100%	2.000 g/L
ElderMune	Aqueous	3 36 T	0.067 g/L
NutraMune	Aqueous	16.8 %	0.336 g/L
Saccharomyces cerevisiae	Aqueous	16.8 %	0.336 g/L
Vitamin C: PureWayC	Aqueous	33.6 %	0.676 g/L
Zinc gluconate	Aqueous	0.5 %	0.010 g/L

4.2 Product handling

Products were supplied by LifeSeasons Inc. and shipped to NIS Labs. On each lab testing day, fresh stock solutions were prepared from each product in physiological saline, and the powders were allowed to rehydrate for 1 hour. Insoluble materials were removed by centrifugation followed by filtration. Serial dilutions were prepared in sterile saline in preparation for adding to cell cultures.

4.3 Tests performed

The products were tested and compared in a selected panel of lab assays to compare their immune-activating biological activities.

The testing included:

- Expression of the Very Early Activation antigen **CD69** on Natural Killer cells, NKT cells, T lymphocytes, and non-NK non-T cells;
- Expression of the receptor for the T cell growth factor Interleukin-2, **CD25**, on Natural Killer cells, NKT cells, T lymphocytes, and non-NK non-T cells;
- Production of a broad panel of 27 cytokines, chemokines, anti-viral peptides, and growth factors at **2 hours** and **24 hours** of immune cell culture.

5 Results

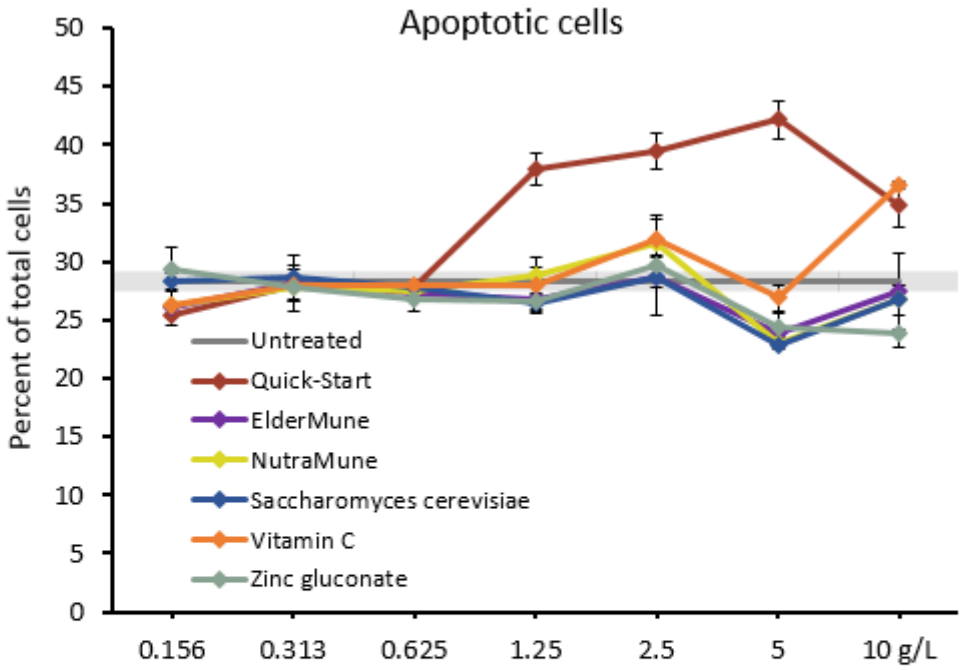
5.1 Cellular survival/viability – Preparation for further bioassay work

For the particular purpose of testing the activating effects of the test products on immune cell activation and cytokine production, an initial cell viability assay was needed as a preparatory step when starting work on the biological effects of complex natural products. The data generated from this testing helped identify the ***optimal dose range*** for the subsequent immune cell testing.

The products were tested at doses that matched their respective doses in the blend Quick-Start. The dose range for Quick-Start went from 0.156 g/L to 10 g/L, following standard procedures and aiming to cause some degree of cellular stress at the higher doses, based on which we could plan a dose range deemed an optimal upper dose range to aim for immune cell activation. Please note that some cellular stress is typically observed under conditions of robust immune cell activation due to the high demand on the cell metabolism.

Synopsis:

- Quick-Start caused cellular stress at the higher end of the dose range.
- This was in part due to the stress caused by the higher doses of vitamin C.
- All other test products were well tolerated by the immune cells used for the viability testing.
- The dose range for subsequent immune cell testing was decided upon as the range from 0.25 g/L to 2.0 g/L, thereby avoiding higher doses, but still including the dose of 1.25 g/L where some cellular stress was seen in cultured treated with Quick-Start, assuming some stress would be associated with robust cellular activation.



	0.156	0.313	0.625	1.25	2.5	5	10 g/L
Quick-Start	**			*	*	*	
ElderMune	**		(*)	*		**	
NutraMune	**		(*)			**	
Saccharomyces				*		**	
Vitamin C							**
Zinc gluconate						(*)	**

*Figure 1. Percent apoptotic cells when treated with test products. The averages + standard deviations of duplicate data points are shown for the products. For reference, the percent apoptotic cells of untreated control cultures is shown as a grey line. Statistical significance at different doses of each product when compared to untreated control cultures is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.*

5.2 Effect on immune activation

Many natural products have the capacity to activate immune cells and modulate the regulatory responses. Due to the gut mucosa containing a large volume of immune tissue in our body, consumed products get into contact with immune cells, such as antigen-presenting cells, regulatory cells, and immune active cells. Testing of natural products on immune cells harvested from peripheral blood is a model for some of the potential immune activating and modulating activities that a natural product may trigger upon consumption.

Human peripheral blood mononuclear cell (PBMC) cultures were used for this testing. A set of cultures were left untreated as negative control cultures for immune activation. Triplicate sets of cultures were treated with serial dilutions of the test product. The inflammatory bacterial lipopolysaccharide LPS from E. coli was used as a positive control for activation. The cultures were incubated for 24 hours, after which the cells and the culture supernatants were harvested and used to monitor the reactions in each culture. **The testing was performed on cells from a healthy blood donor. Since the results are promising, and there is a desire to move forward with manuscript writing for a peer-reviewed scientific publication, then additional validation repeats are needed before manuscript writing begins (separate project/budget**) – See the section below regarding "Further Work".

5.2.1 Immuno-staining

The cells were stained with a combination of monoclonal antibodies to monitor activation, and analyzed by multi-parameter flow cytometry, using an acoustic dual laser Attune flow cytometer. The analysis included fluorescent markers for **CD3**, **CD56**, **CD25**, and **CD69**. This combination allowed monitoring of changes to monocyte/macrophages, as well as activation of natural killer cells, NKT cells, and T lymphocytes.

The stained cells were analyzed by multi-parameter flow cytometry, using an acoustic dual laser Attune flow cytometer. During data analysis, the physical properties of different cell types allowed electronic gating on lymphocytes versus monocytes, so that the CD69 versus CD25 expression could be analyzed on these cell types separately. In addition, the lymphocyte fraction was divided into 4 separate subpopulations, based on whether cells were stained with CD3, CD56, both, or none.

This is based on established and published Reports for natural products research.[1] [2] [3] [4] [5] See also the figure below.

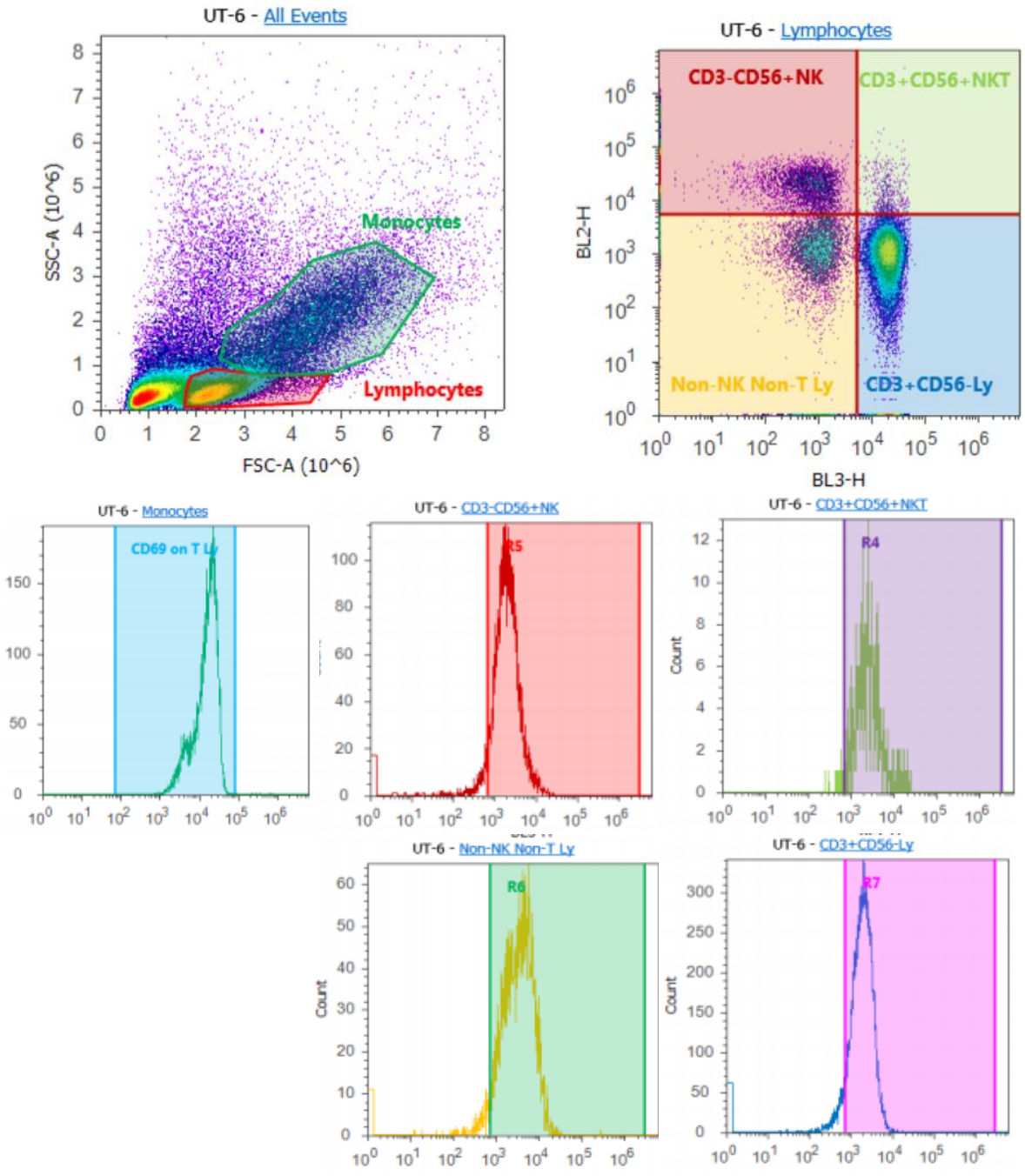


Figure 2. Flow cytometry data showing gates for lymphocytes, monocytes, and the four subsets of lymphocytes, allowing analysis of CD69 expression on all five cell types.

Below is an **overview table** for changes to immune cell activation status after 24 hours.

If it was clear that 1 or more ingredients were driving the response seen when cells were stimulated with Quick-Start, the ingredient is listed in brackets after the cell type. If multiple ingredients seem to contribute to Quick-Start's effect, the bracket indicates "multiple".

Table 2. Quick-Start's effects on immune cell activation.

Overview table: Quick-Start's effects on immune activation markers			
	Increase	Decrease	No Change
CD69	NK cells (Vit C)		
	NKT cells (Vit C, NutraMune)		
			T cells
	non-NK non-T cells		
	Monocytes (multiple)		
CD25	NK cells		
		NKT cells (Vit C, Zinc)	
		T cells (All)	
	non-NK non-T cells (Sacch)		
	Monocytes (Sacch)		

5.2.2 Changes to the CD69 activation marker

CD69 is the earliest inducible cell surface glycoprotein during lymphoid activation resulting in lymphocyte proliferation and cellular signaling. While CD69 plays an important role in immunity through the increase of lymphocyte proliferation and cellular signaling, it has recently been implicated in the immunomodulatory effects leading to the control of inflammation.[6]

- CD69 is rapidly induced in NK cells shortly after activation,[7] and its direct role in NK cytotoxicity has been demonstrated;[8]
- When human NK cells are co-cultured with K562 target cells, CD69 expression is upregulated, and the increase significantly correlated with NK cell activity, as measured by today's gold standard CD107 mobilization assay;[9]
- CD69 has the capacity to activate the NK cytolytic machinery in the absence of other NK–target cell adhesion molecule interactions; [10]
- **Importantly**: A direct and highly significant correlation between CD69 levels and NK cell activity was demonstrated by Clausen et al 2003,[11] in a study involving 14 breast cancer patients tested repeatedly during chemotherapy.
- Therefore, in our work on immunomodulating natural products, we have used CD69 staining for NK cell activation (and indicative of NK cell activity) in several published studies over the past 12 years, both in vitro[8] [12] [13] [14] [15] [16] [17] and in clinical studies.[18] [19]

Synopsis:

- Treatment of PBMC with Quick-Start resulted in an increase of the CD69 activation marker on NK cells, NKT cells, non-NK non-T cells, and monocytes.
    - The increase on CD69 expression on NKT cells was driven by the activation by Vitamin C.
- Treatment of PBMC with Quick-Start did not change the level of CD69 expression on T cells.

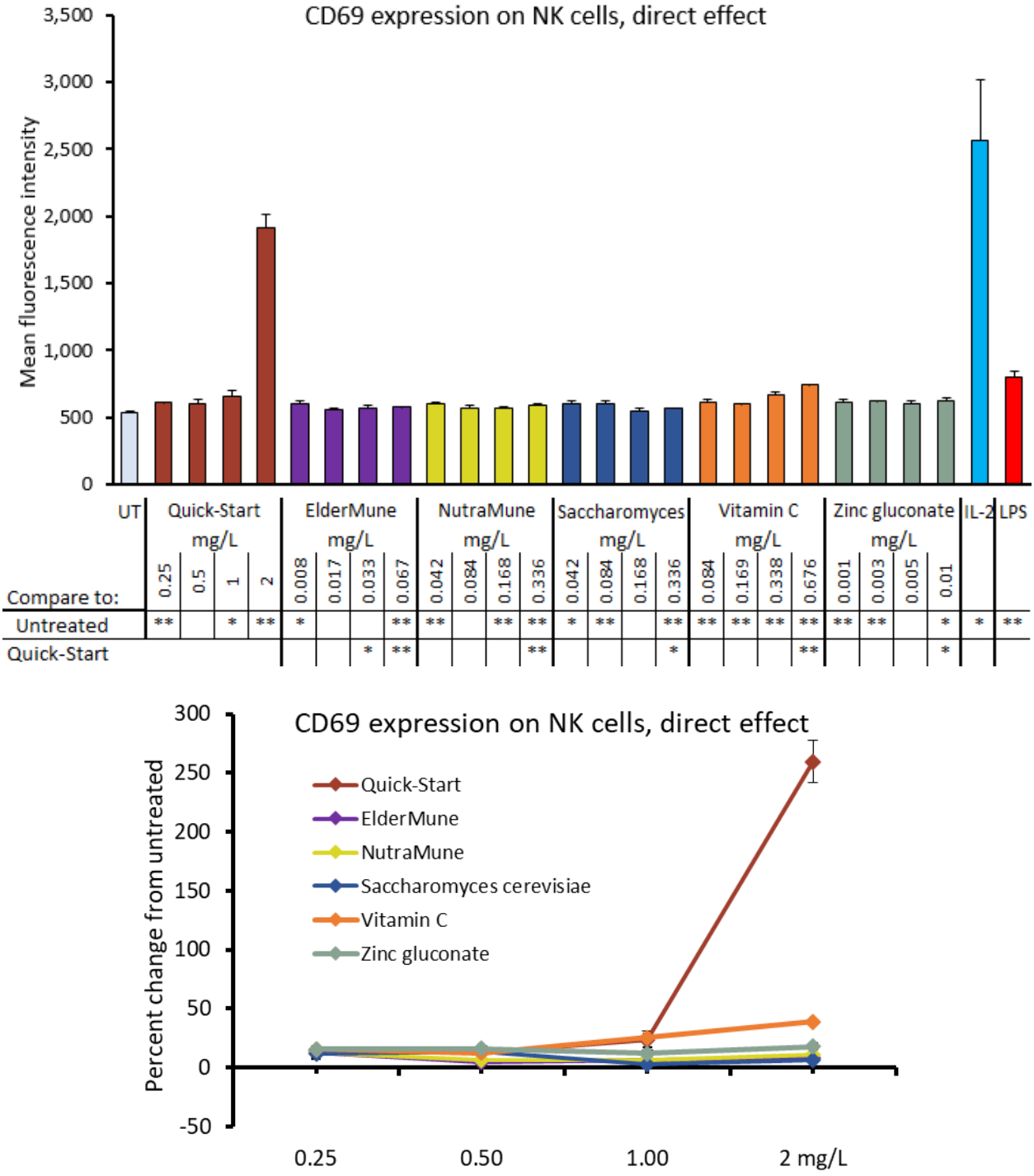
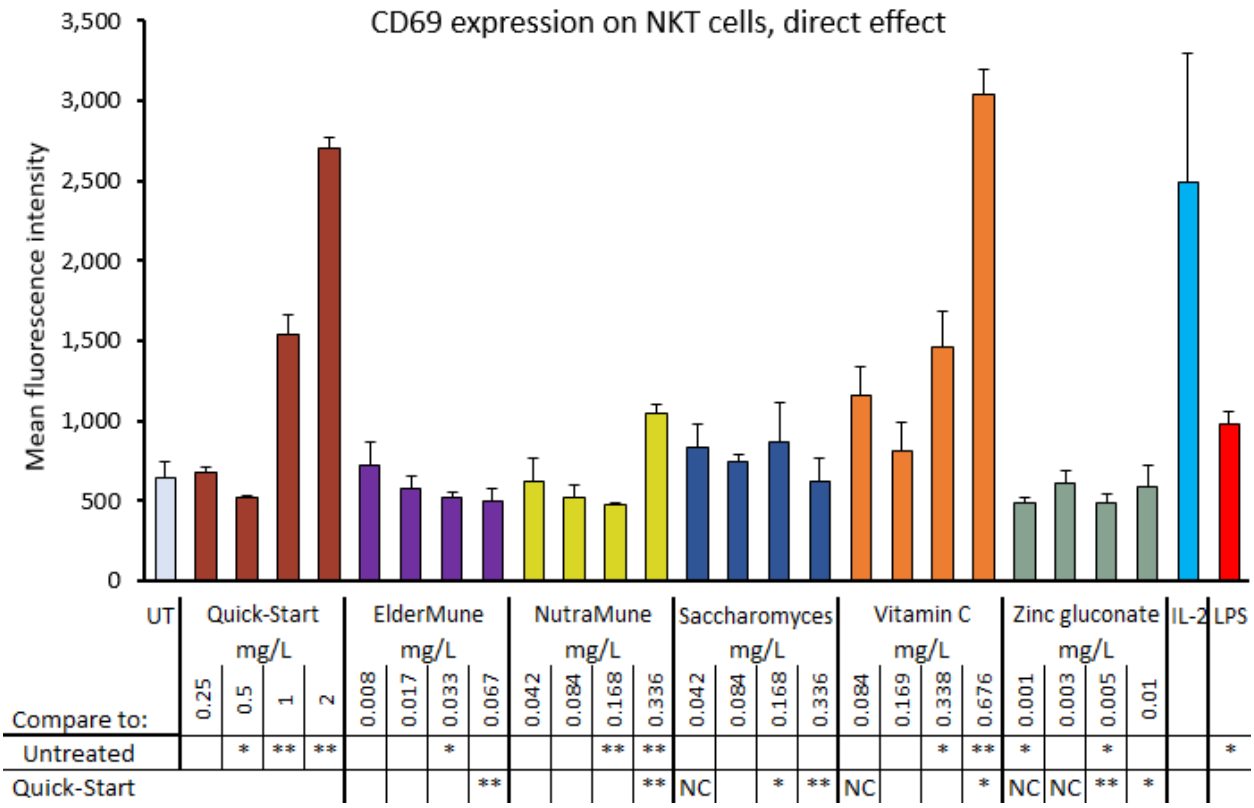


Figure 3. Expression of CD69 on NK cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated			*	**	**			*				**	**							*	**	*		*			*
Quick-Start									**				**	NC		*	**	NC			*	NC	NC	**	*		

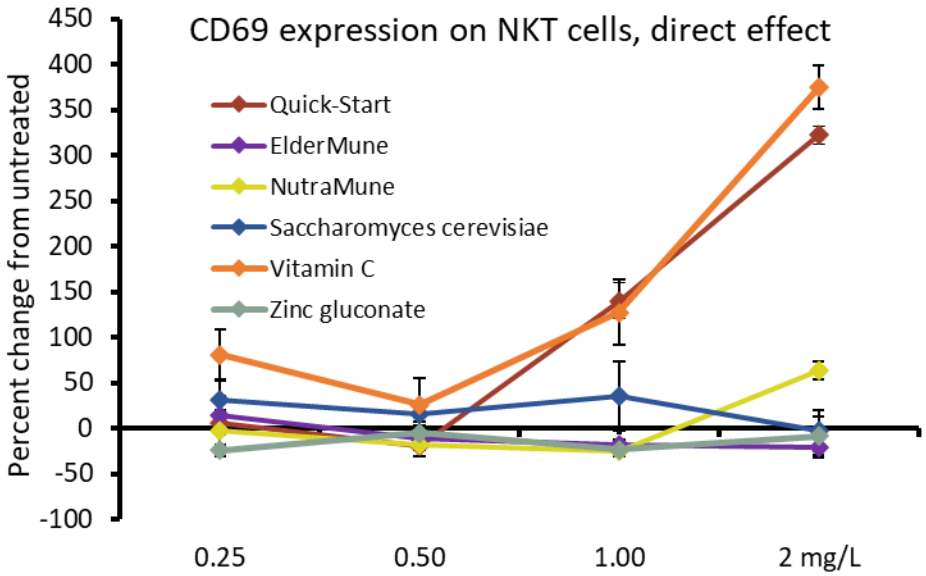


Figure 4. Expression of CD69 on NKT cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*

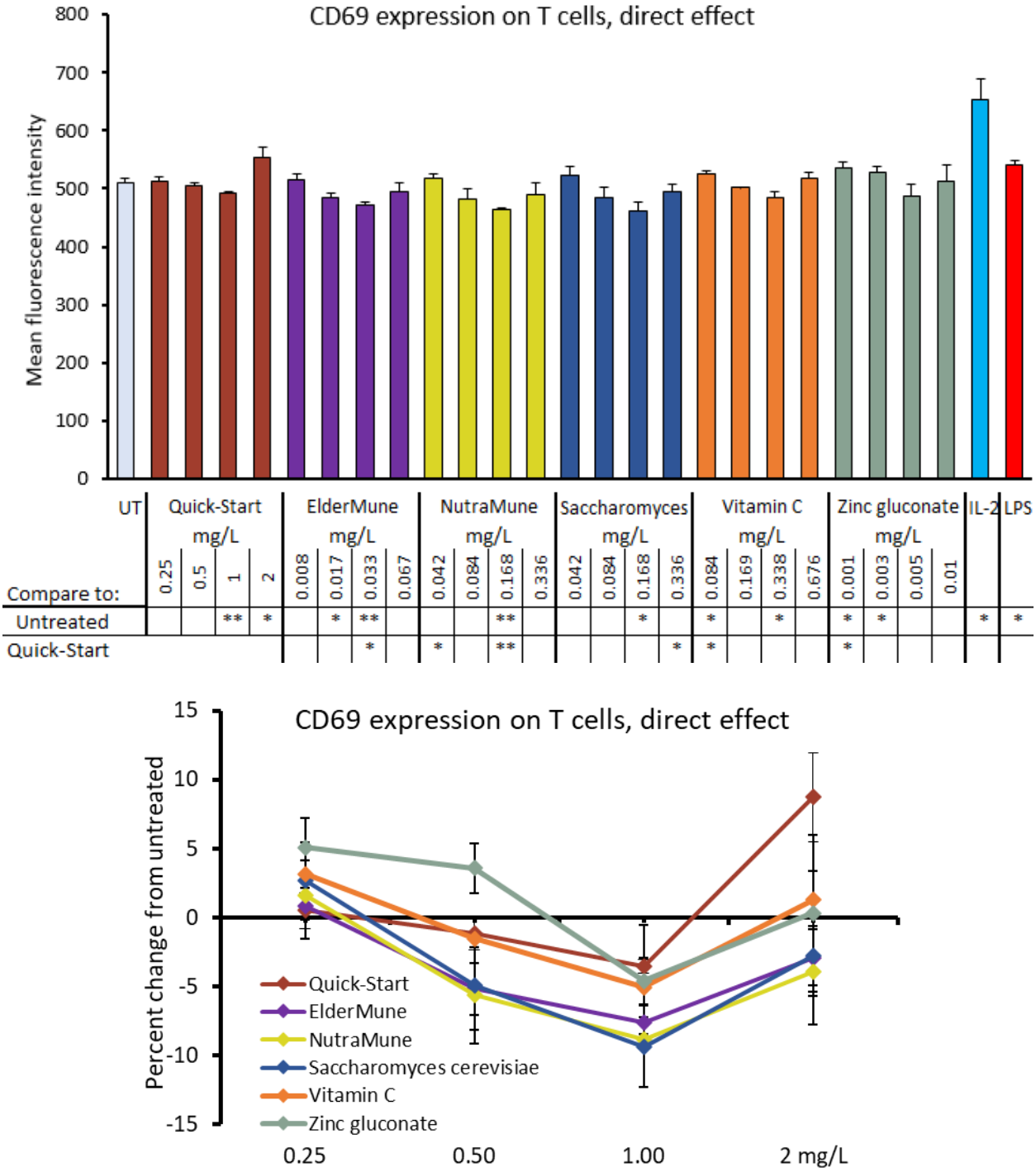
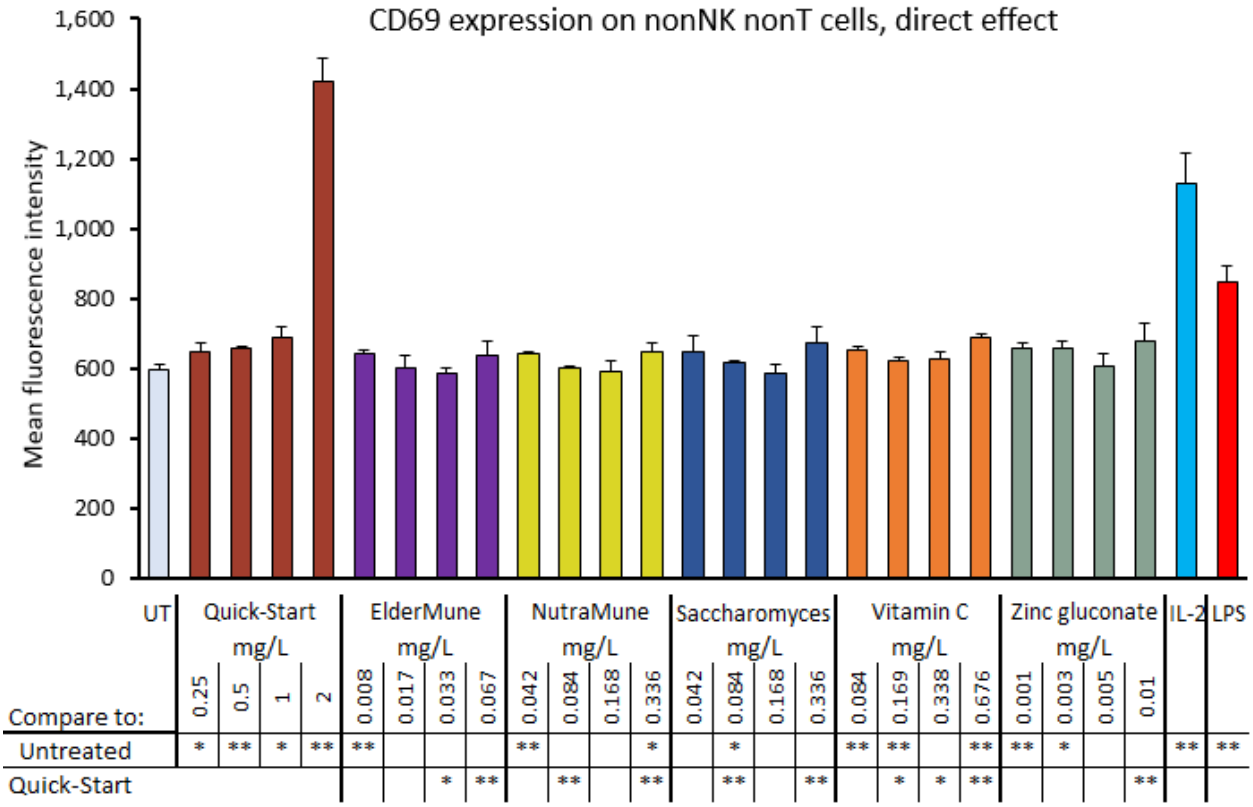


Figure 5. Expression of CD69 on T cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated		*	**	*	**	**				**			*		*			**	**		**	**	*			**	**
Quick-Start								*	**		**		**		**		**		*	*	**				**		

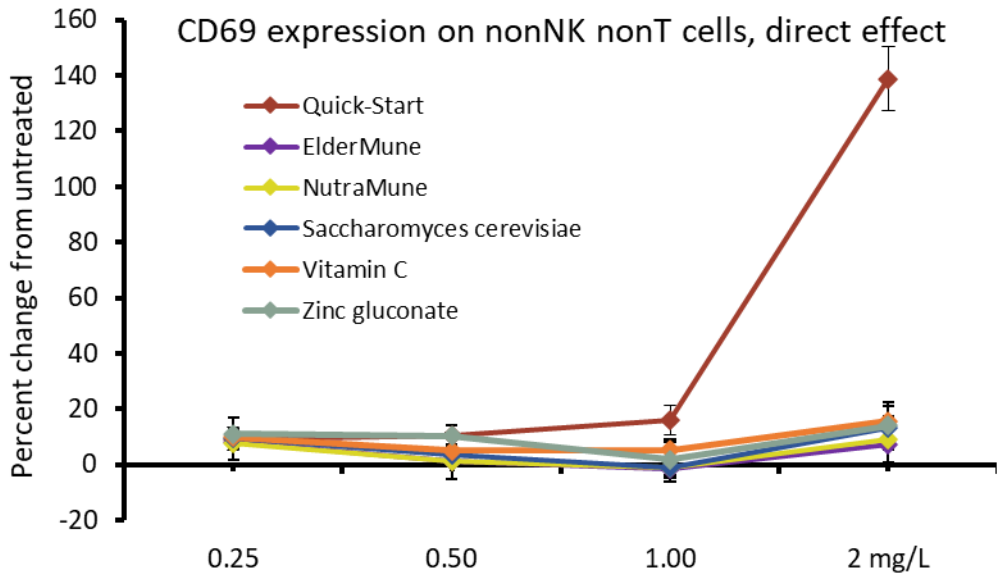
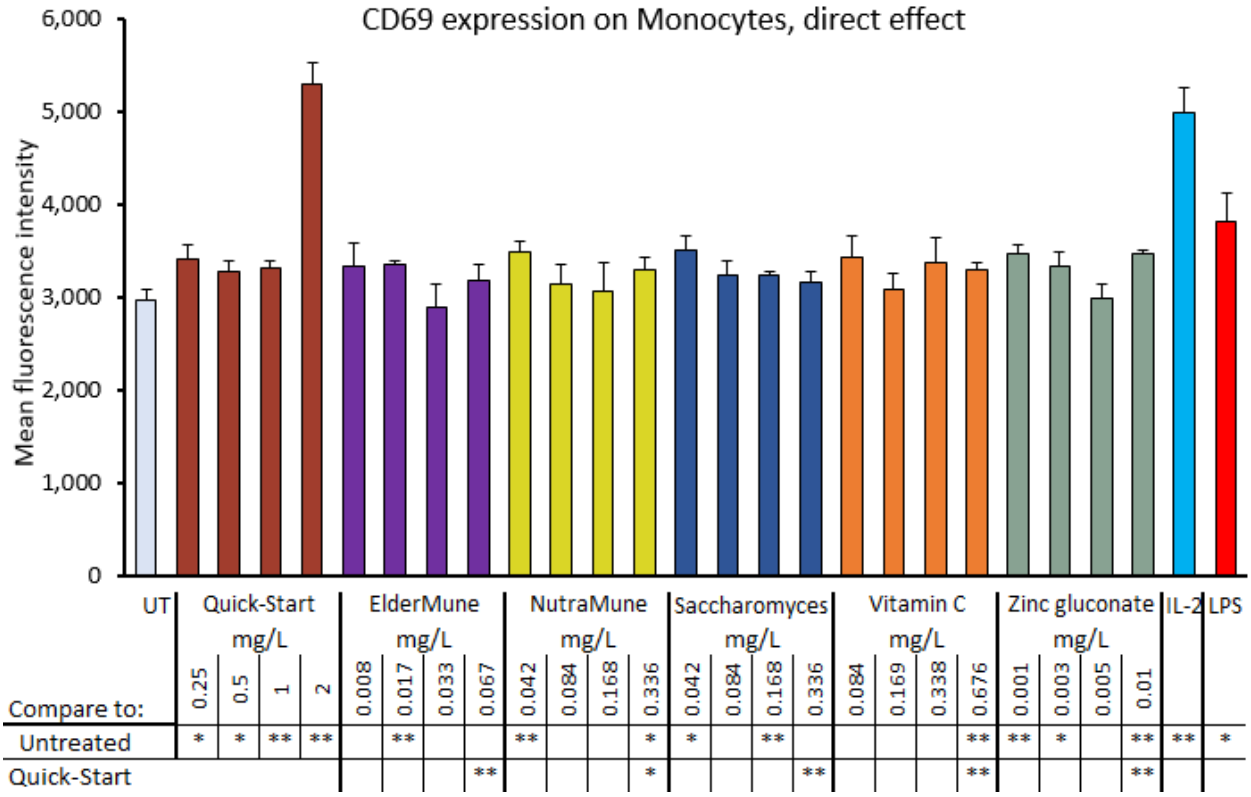


Figure 6. Expression of CD69 on nonNK nonT cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*



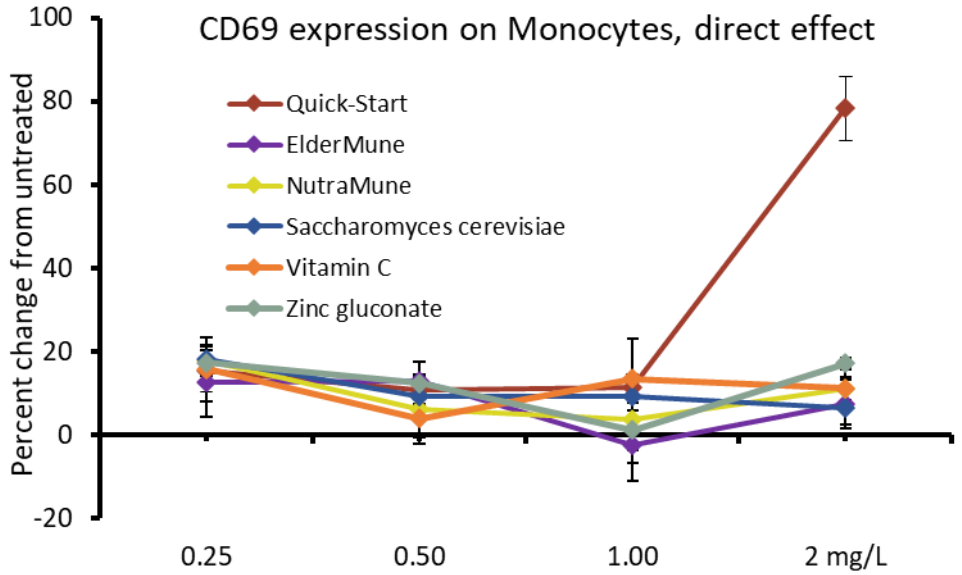


Figure 7. Expression of CD69 on Monocytes – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*

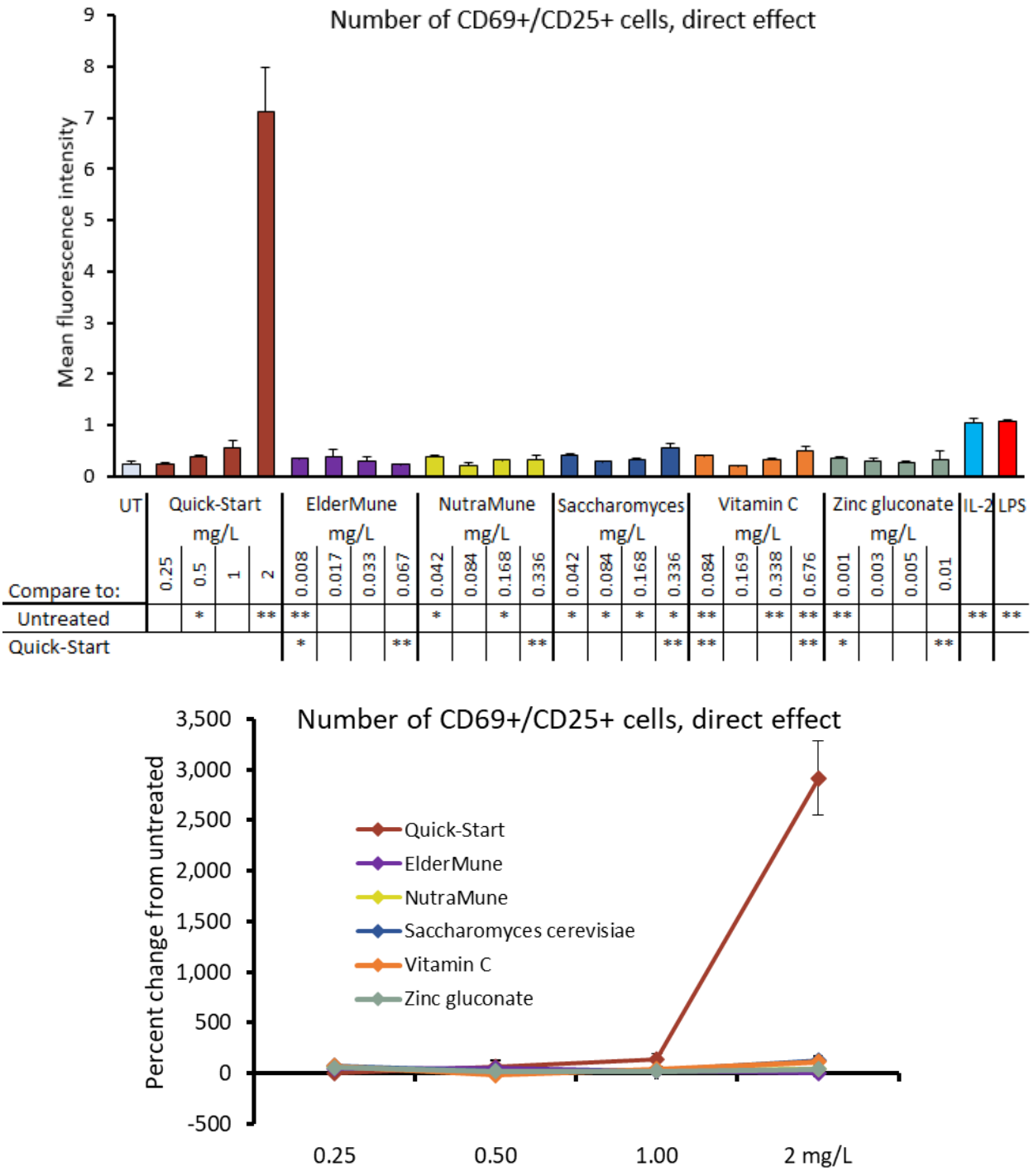


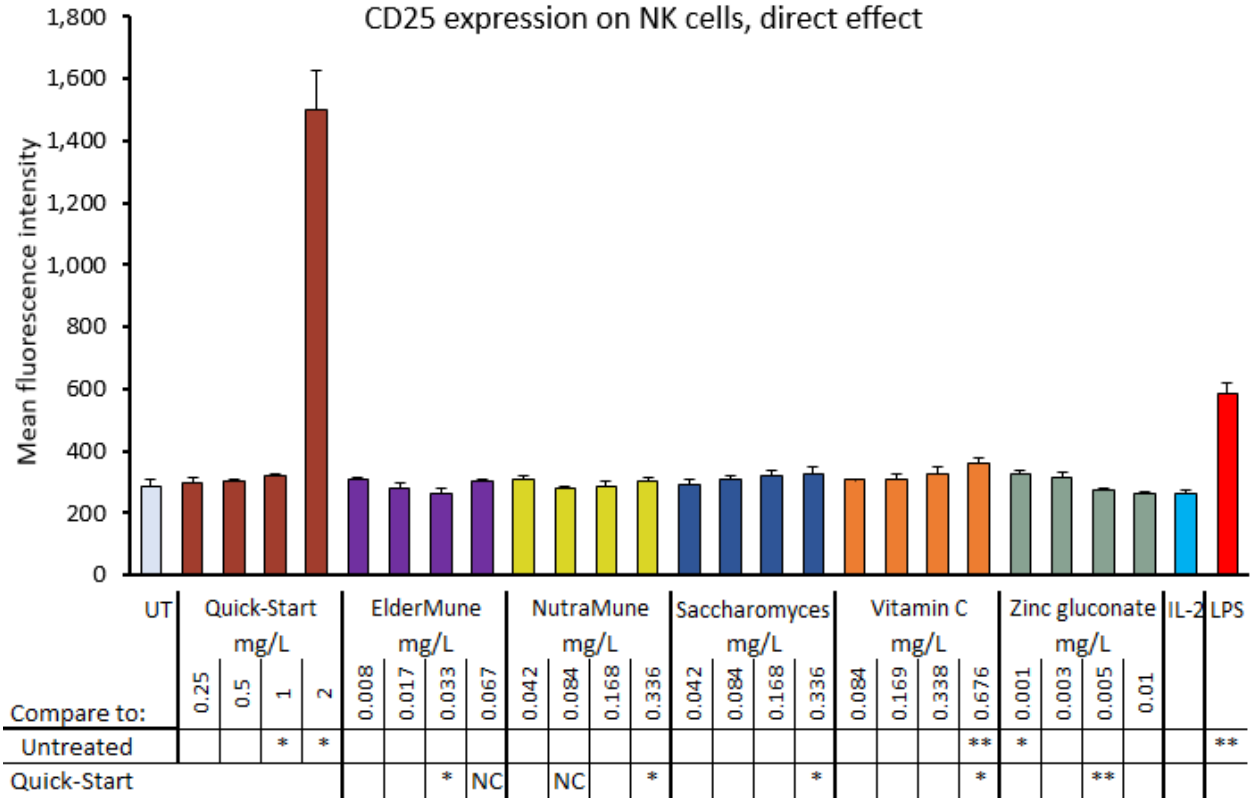
Figure 8. Number of CD69+/CD25+ cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*

5.2.3 Changes to the CD25 activation marker

CD25 is the receptor for the cytokine Interleukin-2 (IL-2) and is present on activated T cells and B cells. CD25 can also be expressed on NK and NKT cells, and in some cases shows an inverse correlation with CD69 expression. It has been shown that in some situations NK cells decide whether to enter a mode of proliferation with predominant expression of CD25, or to enter a highly cytotoxic killing mode, in which case they preferentially express CD69.

Synopsis:

- Treatment of PBMC with Quick-Start resulted in an increase of the CD25 activation marker on NK cells, non-NK non-T cells, and monocytes.
    - The increase on CD69 expression on non-NK non-T cells and monocytes was driven by the activation by *Saccharomyces cerevisiae*.
- Treatment of PBMC with Quick-Start resulted in a decrease in CD25 on NKT and T cells.
    - The decrease on NKT cells was driven by Vitamin C and zinc.
    - The decrease on T cells was associated with similar effects by all ingredients.



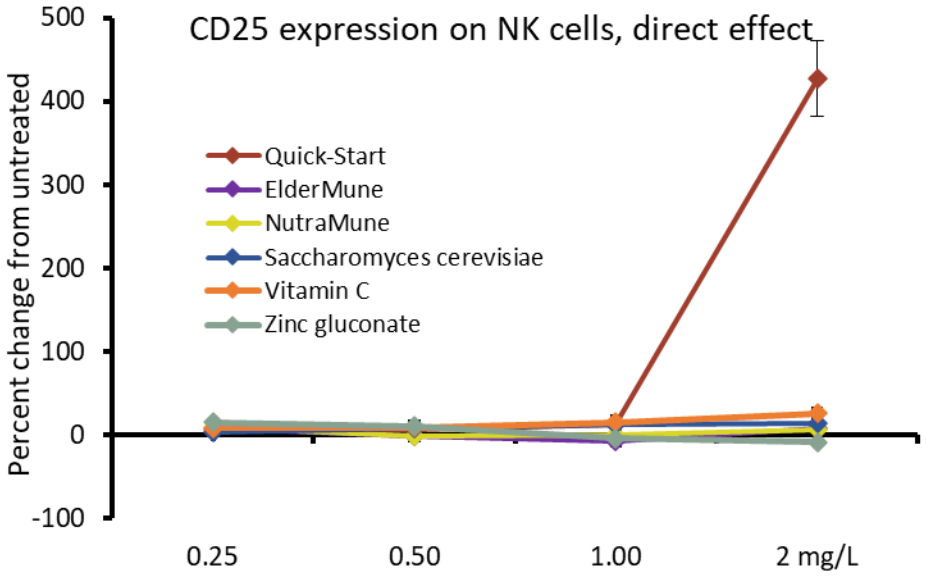
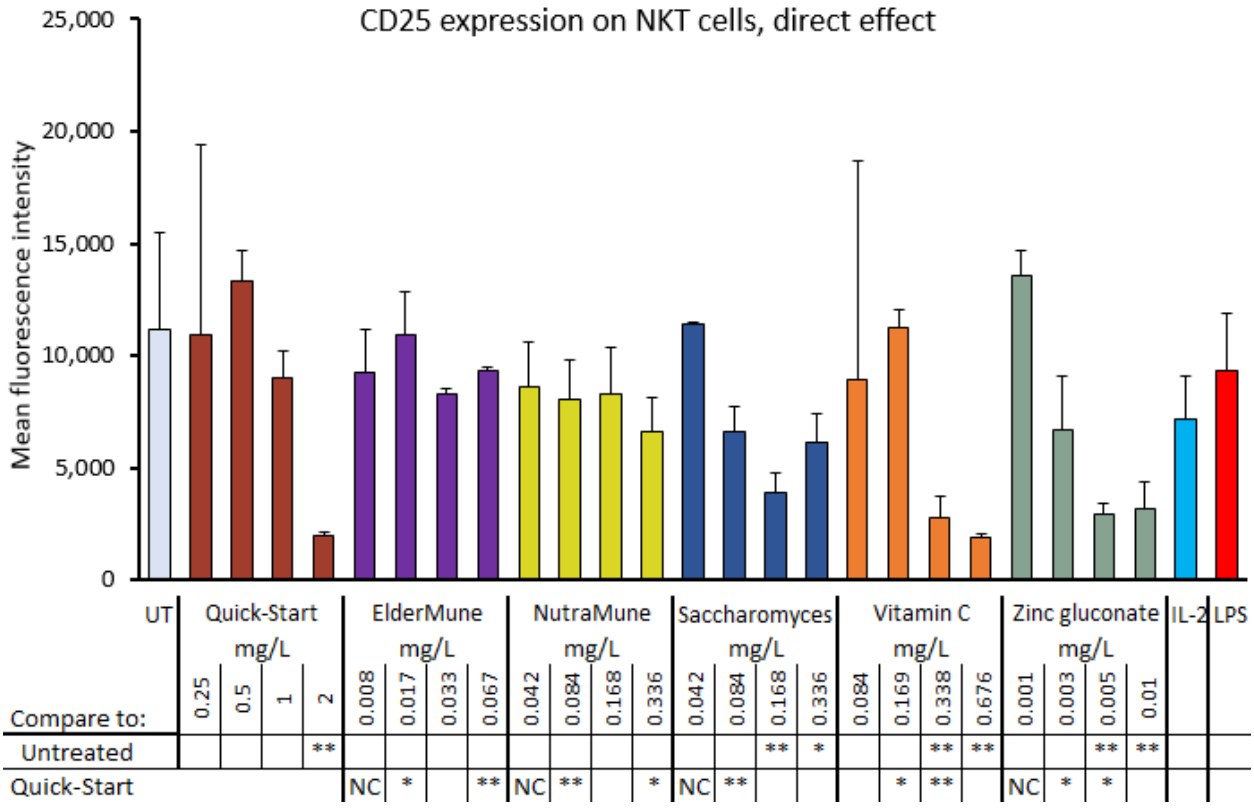


Figure 9. Expression of CD25 on NK cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*



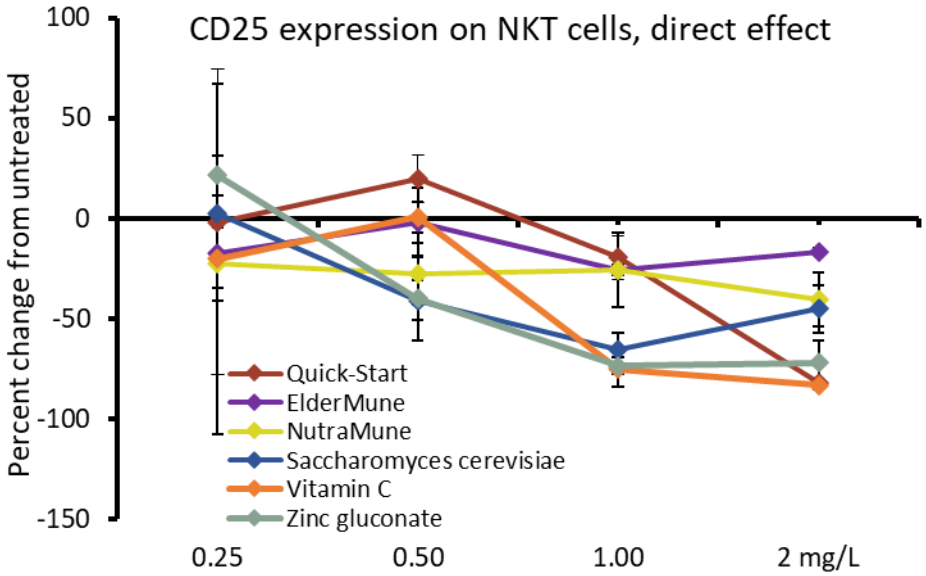


Figure 10. Expression of CD25 on NKT cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*

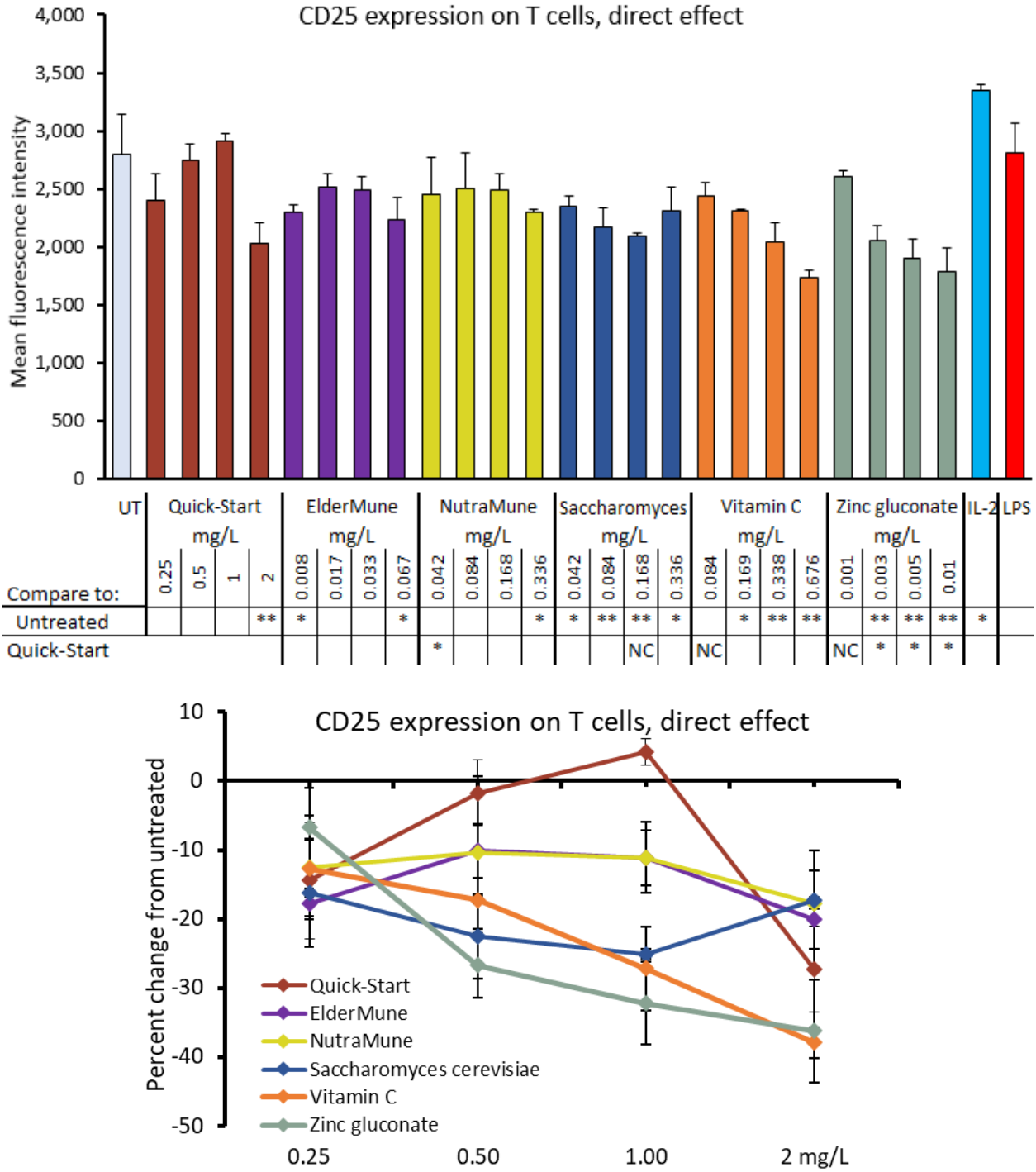
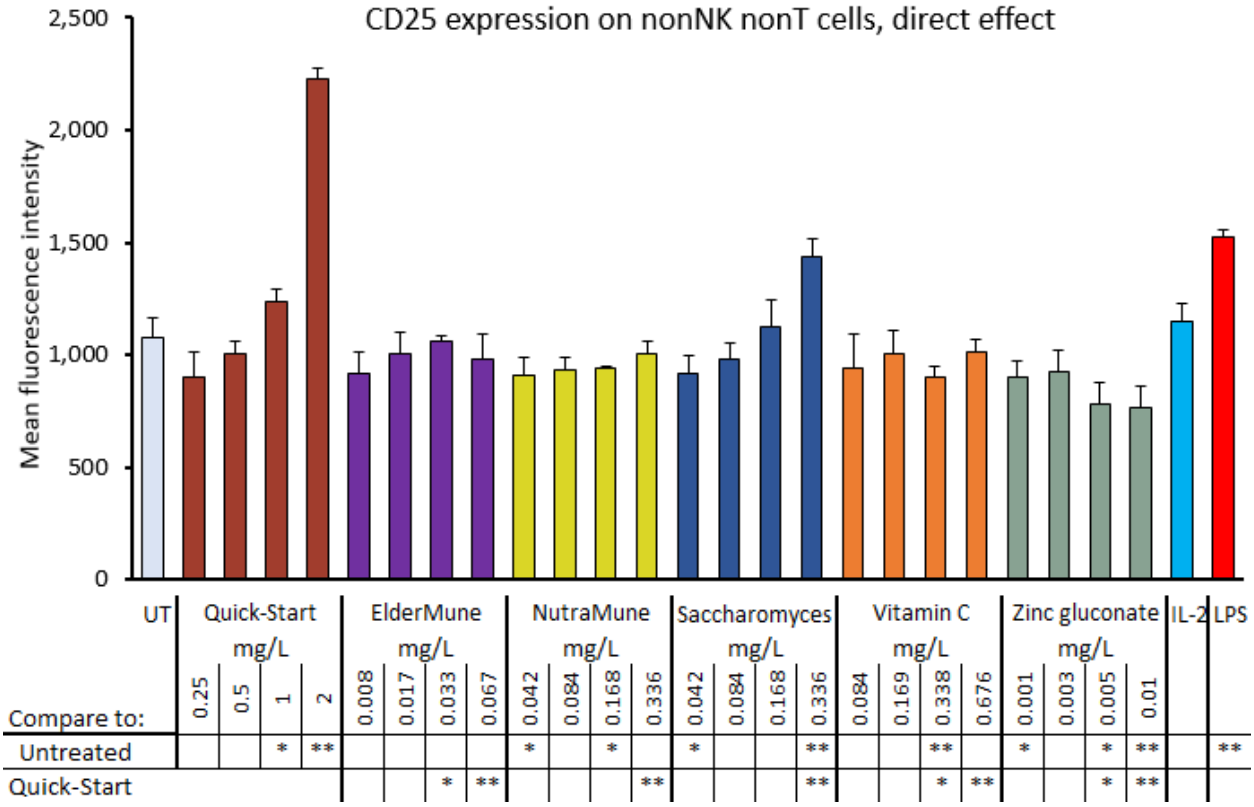


Figure 11. Expression of CD25 on T cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*



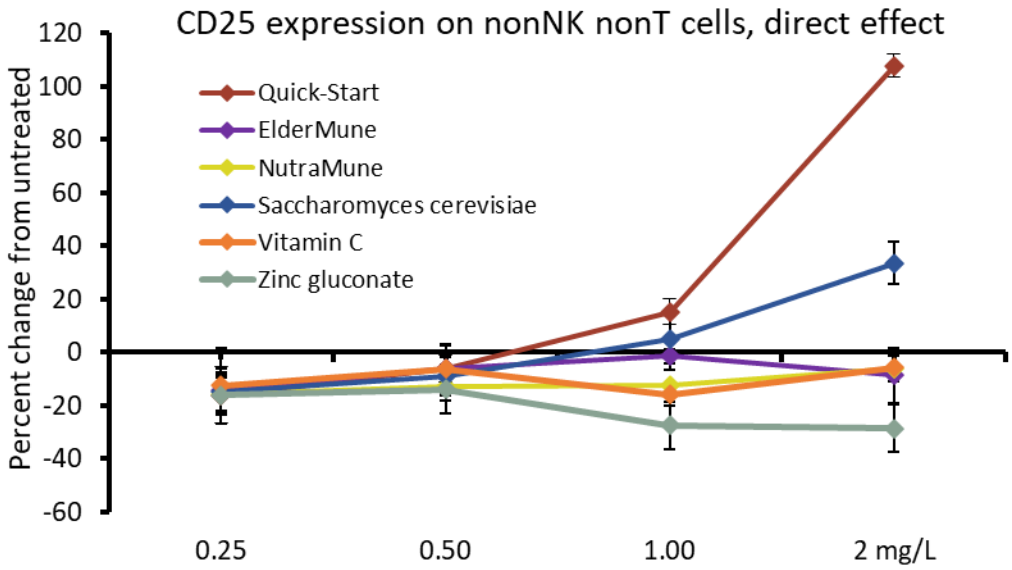
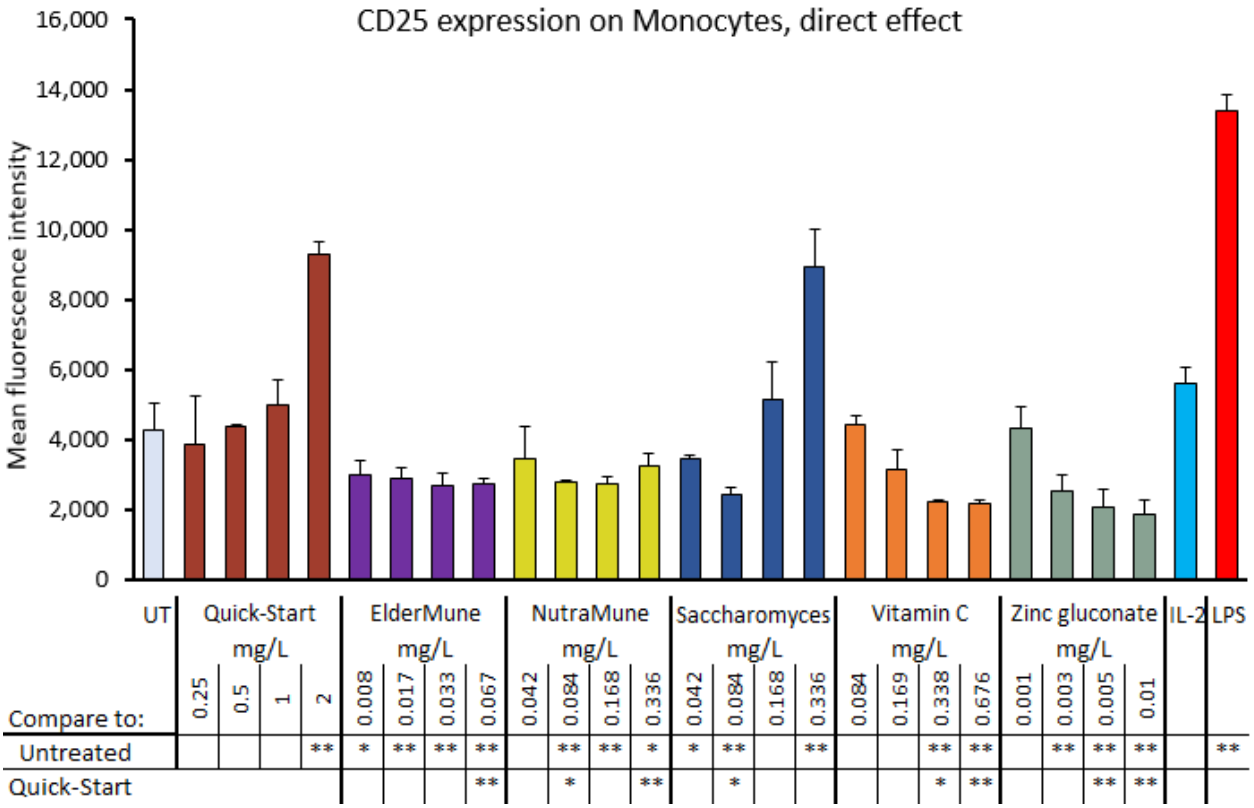


Figure 12. Expression of CD25 on nonNK nonT cells – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*



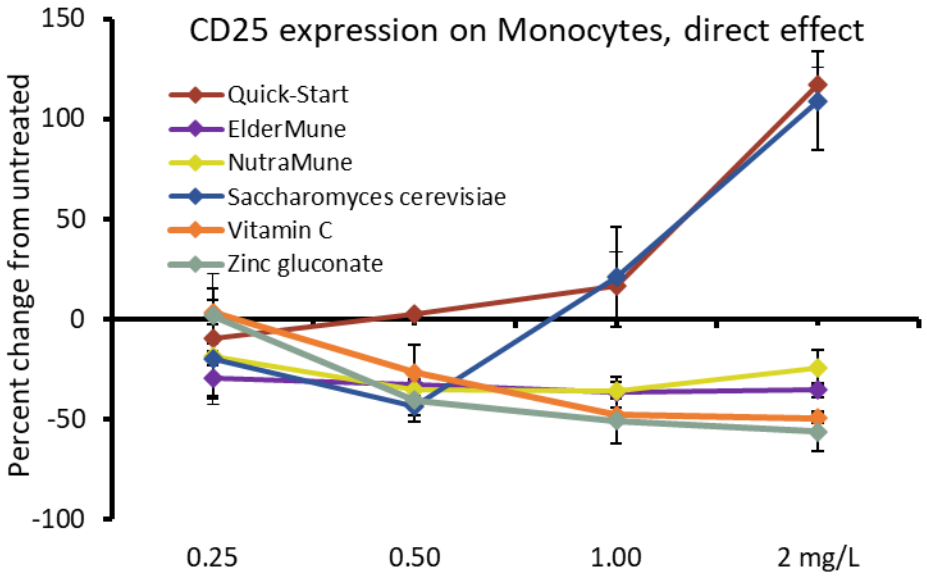


Figure 13. Expression of CD25 on Monocytes – direct effects. ***Top****: Raw data are shown as the average ± standard deviation of triplicate cultures. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom****: The percent change compared to untreated control cultures is shown for the 6 products.*

5.2.4 Cytokines and growth factors

The culture supernatants from each culture are used for testing of a broad panel of pro- and anti-inflammatory cytokines, anti-viral peptides, and regenerative growth factors, using a 27-plex Luminex magnetic bead array and the MagPix® multiplexing system.

The following markers are tested: IFN-gamma, IL-1beta, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic FGF, G-CSF, GM-CSF, IP-10, MCP-1 (MCAF), MIP-1alpha, MIP-1beta, PDGF-BB, RANTES, TNF-alpha, and VEGF.

The following cytokines were below levels of detection: IL-7, IL-12p70, IL-13, and IL-17.

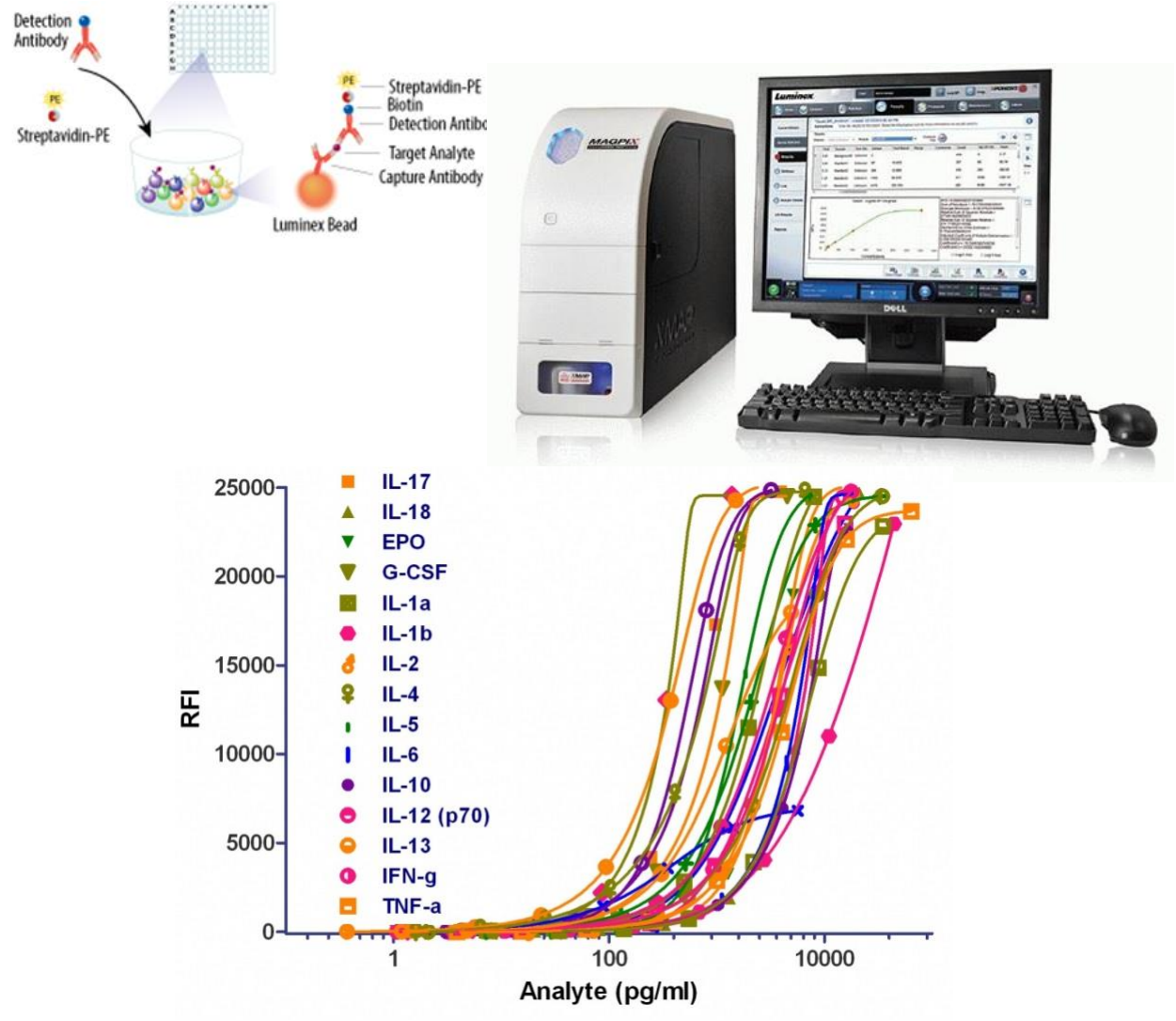


Figure 14. Luminex multiplex protein array using magnetic bead principles to capture and quantify multiple biomarkers in one small biological samples (in vitro or clinical).

The cytokines are classified into 4 groups of biomarkers, and this grouping is used in this report, starting with the overview table below, and continuing through the remainder of the report:

- Immune-activating pro-inflammatory cytokines,
- Anti-inflammatory cytokines,
- Regulating cytokines,
- Growth factors.

Below is an **overview table** for changes to the cytokines that were detectable in the culture supernatants at 2 hours, 24 hours, or both.

If it was clear that 1 or more ingredients were driving the response seen when cells were stimulated with Quick-Start, the ingredient is listed in brackets after the cytokine. If multiple ingredients seem to contribute to Quick-Start's effect, the bracket indicates "multiple".

Table 3. Quick-Start's effects on cytokine production.

Overview table: Quick-Start's effects on cytokine production					
	Increase at 2 hour	Decrease at 2 hour	Increase at 24 hours	Decrease at 24 hour	Complex dose response 24 hour
Immune-activating, pro-inflammatory cytokines	IFN-γ (Sacch)			IFN-γ	
	IL-1β (Sacch)		IL-1β (Sacch)		
	IL-5 (Sacch)		IL-5 (Sacch)		IL-5 biphasic (Sacch)
	IL-6 (Sacch)		IL-6 (Sacch)		
	IL-8 (Sacch)				IL-8 (Sacch)
	IL-17A (Sacch)				IL-17 (Sacch)
	Eotaxin (Sacch)				Eotaxin (Sacch)
	IP-10 (Sacch)			IP-10 (multiple)	
	MCP-1 (Sacch)			MCP-1	
	MIP-1α (Sacch)		MIP-1α (Sacch)		
	MIP-1β (Sacch)		MIP-1β (Sacch)		
		RANTES (multiple)			RANTES (Sacch)
	TNF-a (Sacch)				TNF-α
Anti-inflammatory cytokines	IL-1ra			IL-1ra	
			IL-10 (Sacch)		
Regulating cytokines	IL-2 (Zinc)		IL-2 (multiple)		
	IL-4 (Sacch)		IL-4 (Sacch)		
		IL-9 (multiple)			IL-9 biphasic (multiple)
Growth factors	bFGF (Sacch)				bFGF (Sacch)
	PDGF (Sacch)				PDGF (Sacch)
	VEGF (Sacch)		VEGF (Sacch)		
					GM-CSF (Sacch)
	G-CSF (Sacch)				G-CSF (Sacch)

Synopsis – 2-hour cytokine changes:

- There was a mild but highly significant increase in production of most immune-activating pro-inflammatory cytokines already after 2 hours in the cell cultures treated with Quick-Start. This effect was in most cases attributed to the effect by Saccharomyces. A few cytokines were also mildly increased by NutraMune, but to a lesser extent that the cytokine levels induced by Saccharomyces.
- Only 2 cytokines were showing a decrease when compared to untreated control cultures after 2 hours in incubation with Quick-Start. These 2 cytokines include the immune-activating pro-inflammatory cytokine RANTES and the regulating cytokine IL-9. This reduction was similar to the reduction seen for multiple ingredients and was not attributed to a single ingredient.
- The anti-inflammatory cytokine IL-1ra and 4 growth factors were also increased after only 2 hours in cell culture with Quick=Start.
- The regulating cytokines IL-1 and IL-4 were also increased after 2 hours. The increased IL-2 levels were similar to the increase seen for zinc, whereas the increase in IL-4 was similar to that seen for Saccharomyces.

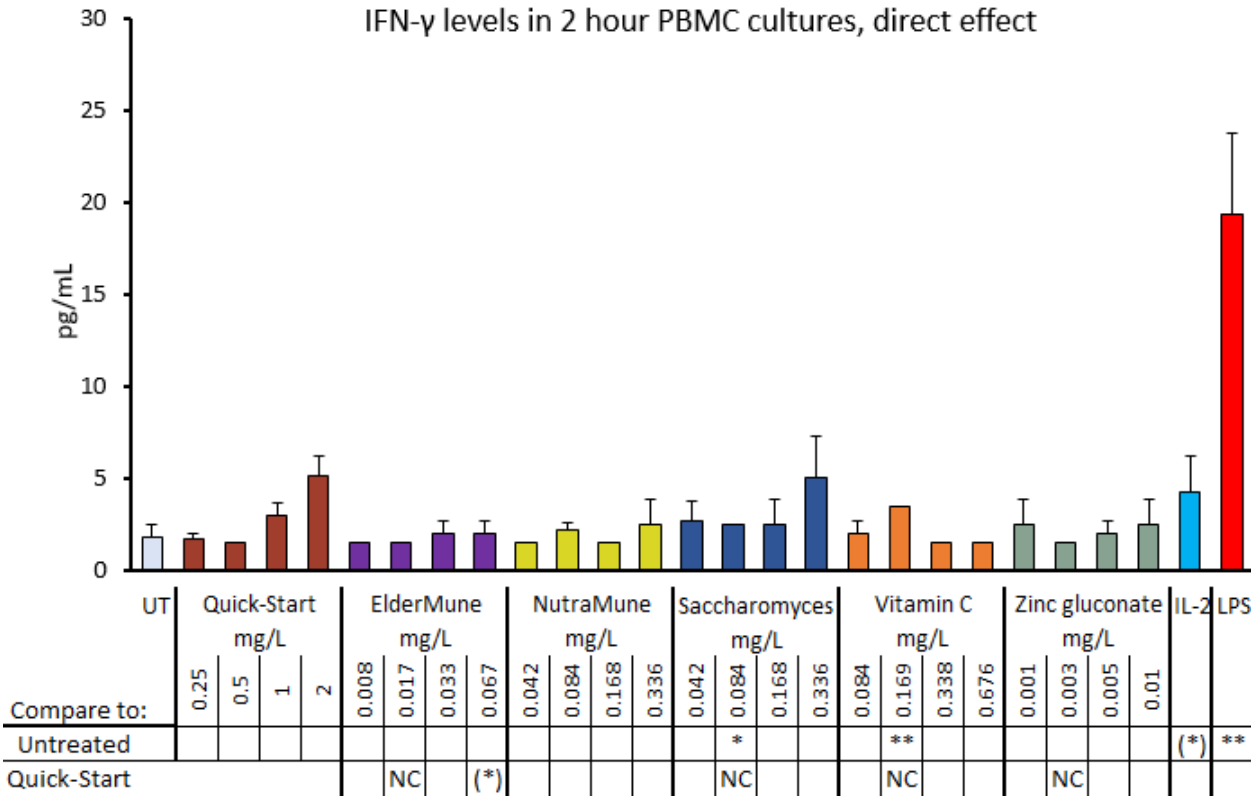
Synopsis – 24-hour cytokine changes:

- After 24 hours, a more diverse response was observed, where more than half the cytokines showed a biphasic dose response (including both increases and decreases depending on the dose of Quick-Start). For most biomarkers, the changes were similar to changes caused by Saccharomyces.
- 9 cytokines showed an increase above untreated control cultures.
- 4 cytokines showed a decrease after 24 hours. Including IFN-g, IP-10, MCP-1, and IL-1ra.

5.2.5 Immune-activating pro-inflammatory cytokines

Table 4. Pro-inflammatory cytokines/chemokines.

IFN-γ	Interferon gamma. Also called macrophage-activating factor. Associated with several autoinflammatory and autoimmune diseases.
IL-1β	Interleukin 1 beta. Produced by activated macrophages as a proprotein which is cleaved by caspase 1. Important mediator of inflammation.
IL-5	Interleukin 5. Key mediator in eosinophil activation (allergy).
IL-6	Interleukin 6. Mostly a pro-inflammatory cytokine. Inhibitor to IL-6 has been developed as drug for rheumatoid arthritis.
IL-8	Interleukin 8. Neutrophil chemotactic factor. Often associated with inflammation.
IL-12p70	Interleukin 12 (protein 70). Produced by activated antigen-presenting cells. Strong inducer of interferon gamma.
IL-13	Interleukin 13. Secreted by Th2 helper cells which are mediators of inflammation.
IL-17A	Interleukin 17A. Pro-inflammatory cytokine produced by activated T cells. High levels of this cytokine are associated with several inflammatory diseases that include rheumatoid arthritis, psoriasis, and multiple sclerosis.
Eotaxin	Eosinophil chemotactic protein (CCL11). Implicated in recruitment of cells to the lungs during an immune defense reaction. Plays a role in allergic airway responses.
IP-10	Interferon gamma-induced protein 10 (CXCL10). A key modulator of the interferon-gamma response. Chemoattraction for monocytes/macrophages, T cells, NK cells, and dendritic cells.
MCP-1	Monocyte chemotactic protein-1 (CCL2). Recruit cells to sites of inflammation produced by injury or infection.
MIP-1α	Macrophage Inflammatory Protein 1 alpha (CCL3). Produced by macrophages following stimulation with bacterial endotoxins. Crucial for immune responses to infection and inflammation. Activates neutrophils and induces the release of pro-inflammatory cytokines.
MIP-1β	Macrophage Inflammatory Protein 1 beta (CCL4). Proinflammatory. See MIP-1α description above.
RANTES	Regulated on Activation, Normal T cell Expressed and Secreted (CCL5). Chemotactic for T cells, eosinophils, and basophils. Plays active role in recruiting leukocytes into inflammatory sites.
TNF-α	Tumor necrosis factor alpha. Adipokine involved in systemic inflammation. Produced mainly by activated macrophages. Member of a group of cytokines that stimulate acute phase reaction.



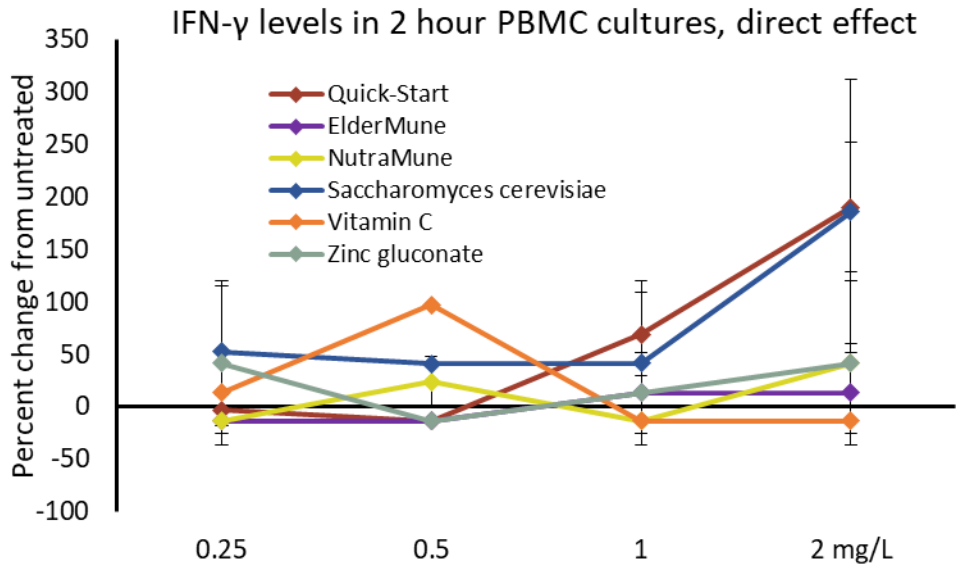
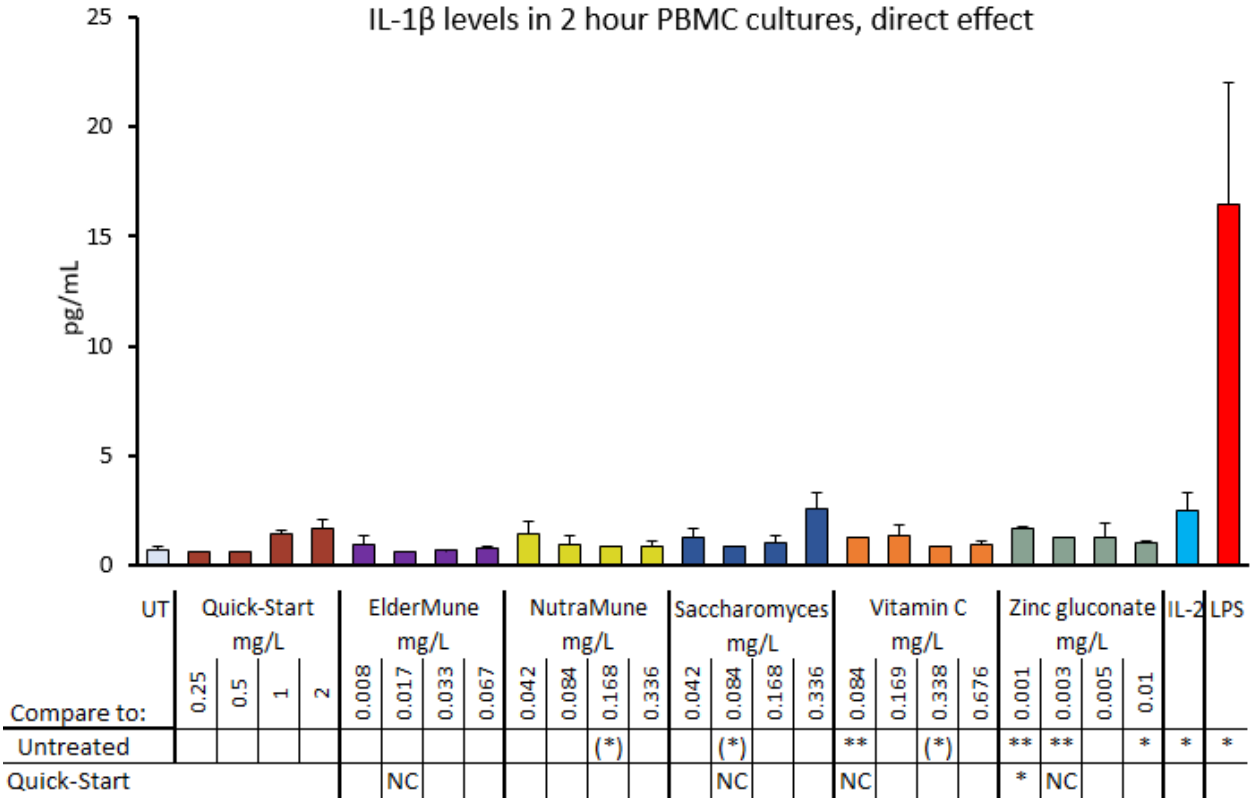


Figure 15. Interferon-gamma (IFN-γ) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated												(*)			(*)			**		(*)		**	**		*	*	*
Quick-Start							NC								NC			NC				*	NC				

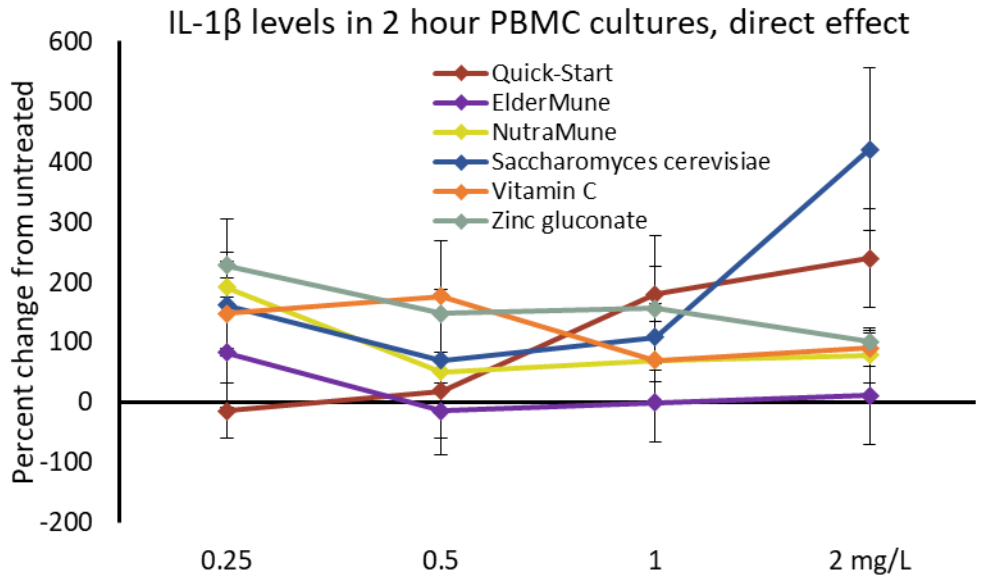
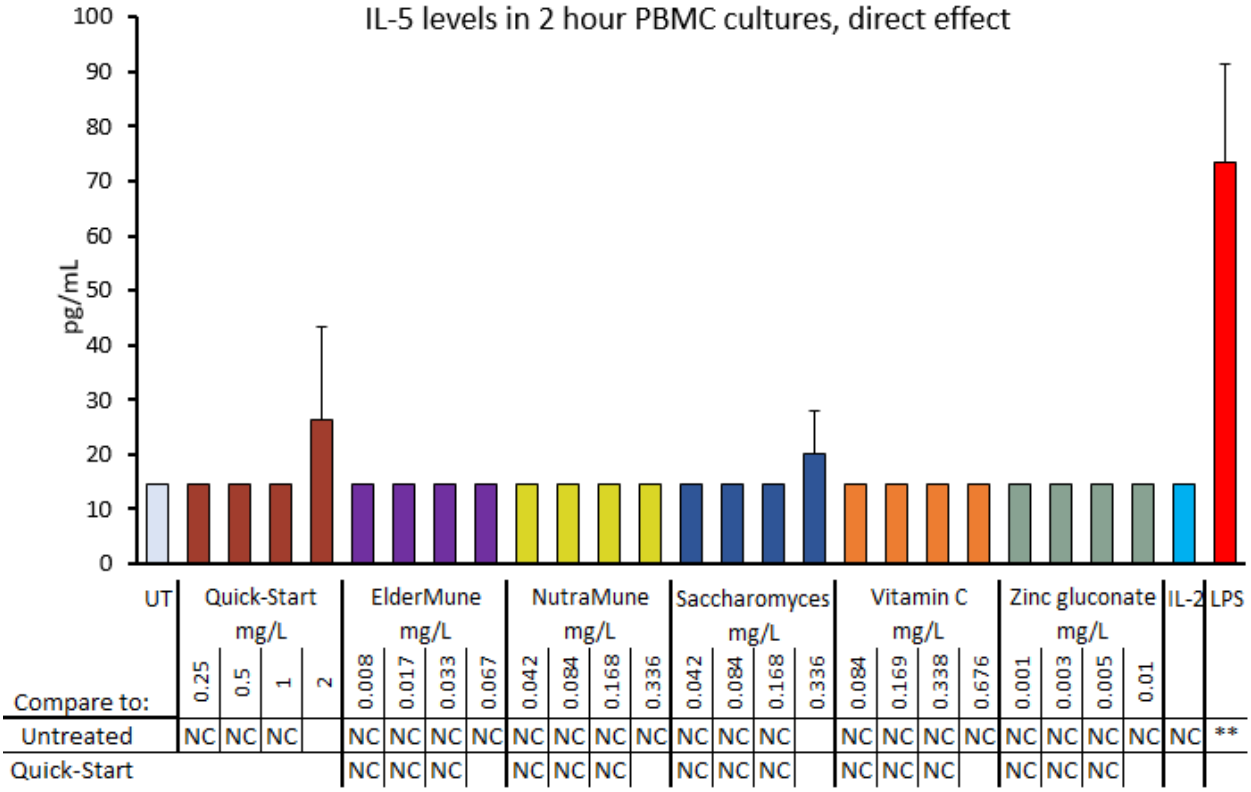


Figure 16 . Interleukin 1 beta (IL-1β) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	**
Quick-Start						NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC			

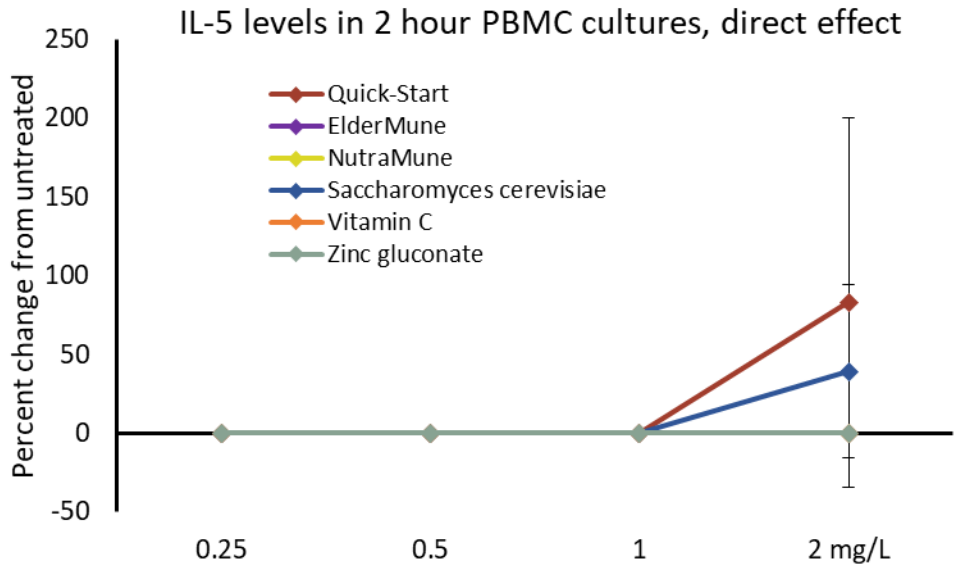
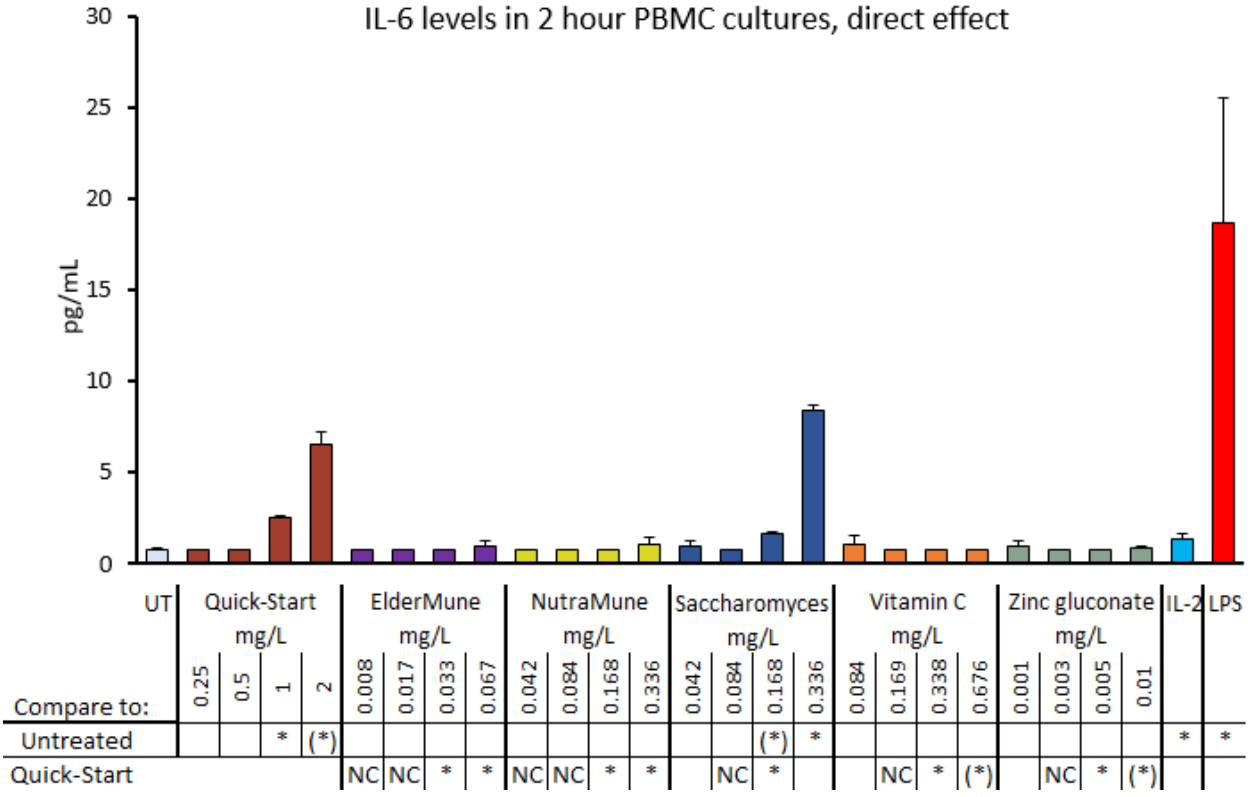


Figure 17. *Interleukin 5 (IL-5) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions.* ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05:* *, P<0.01: ** and NC when not calculated. ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



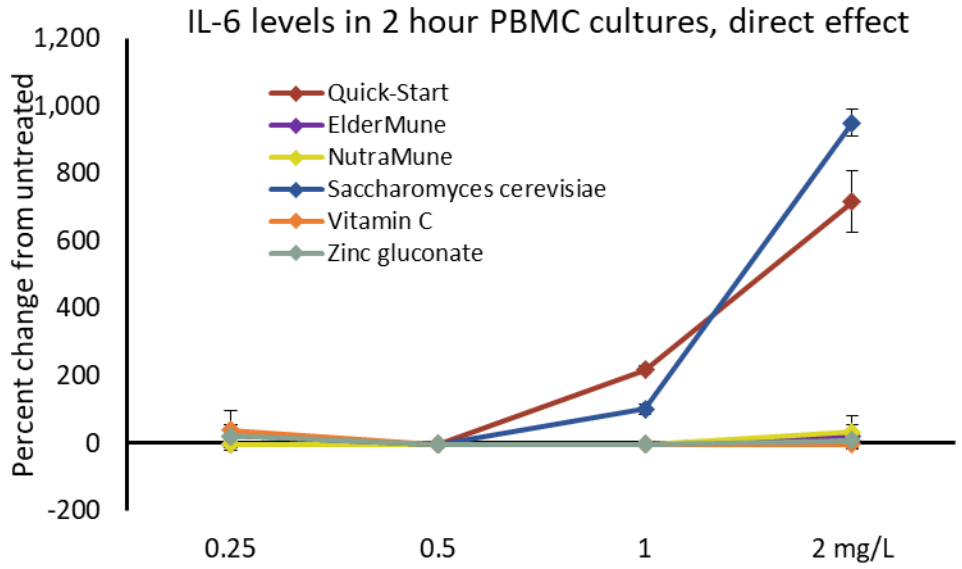
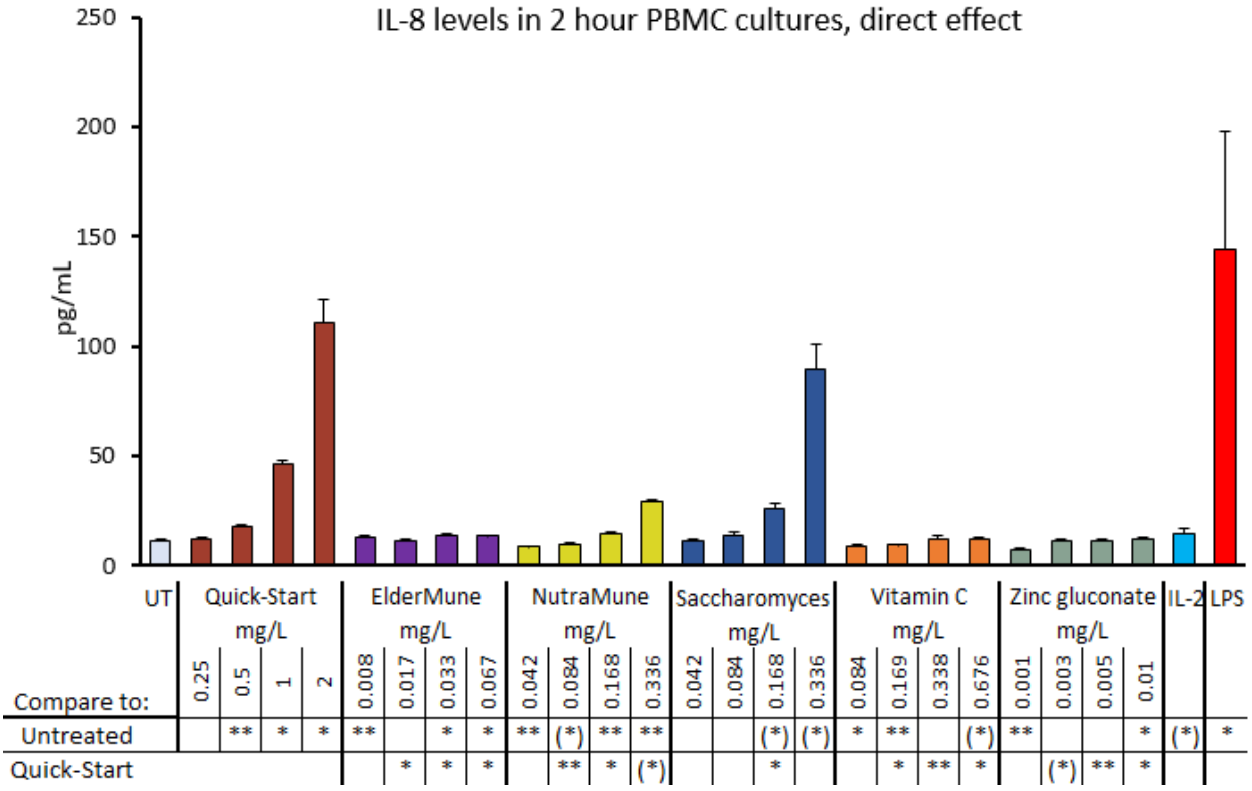


Figure 18. Interleukin 6 (IL-6) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



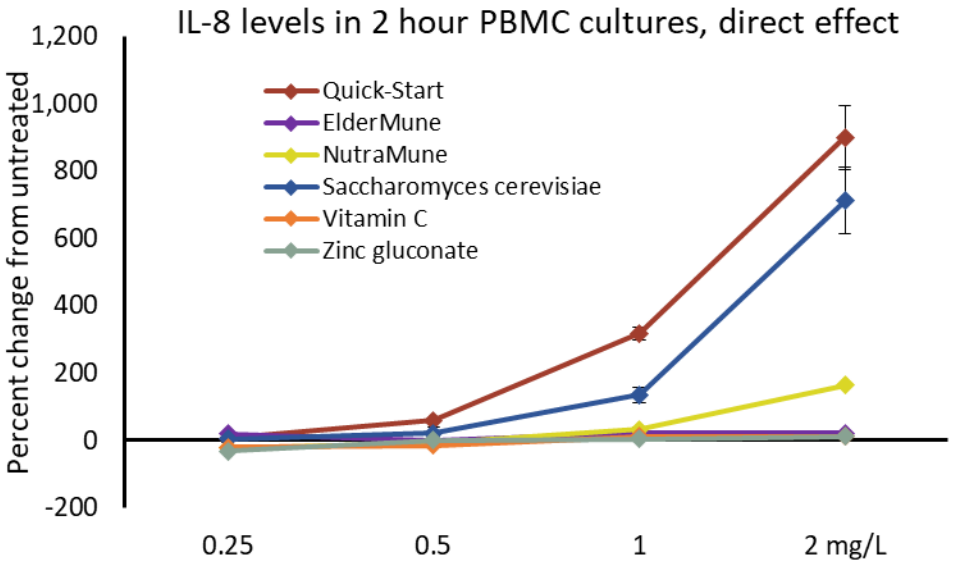
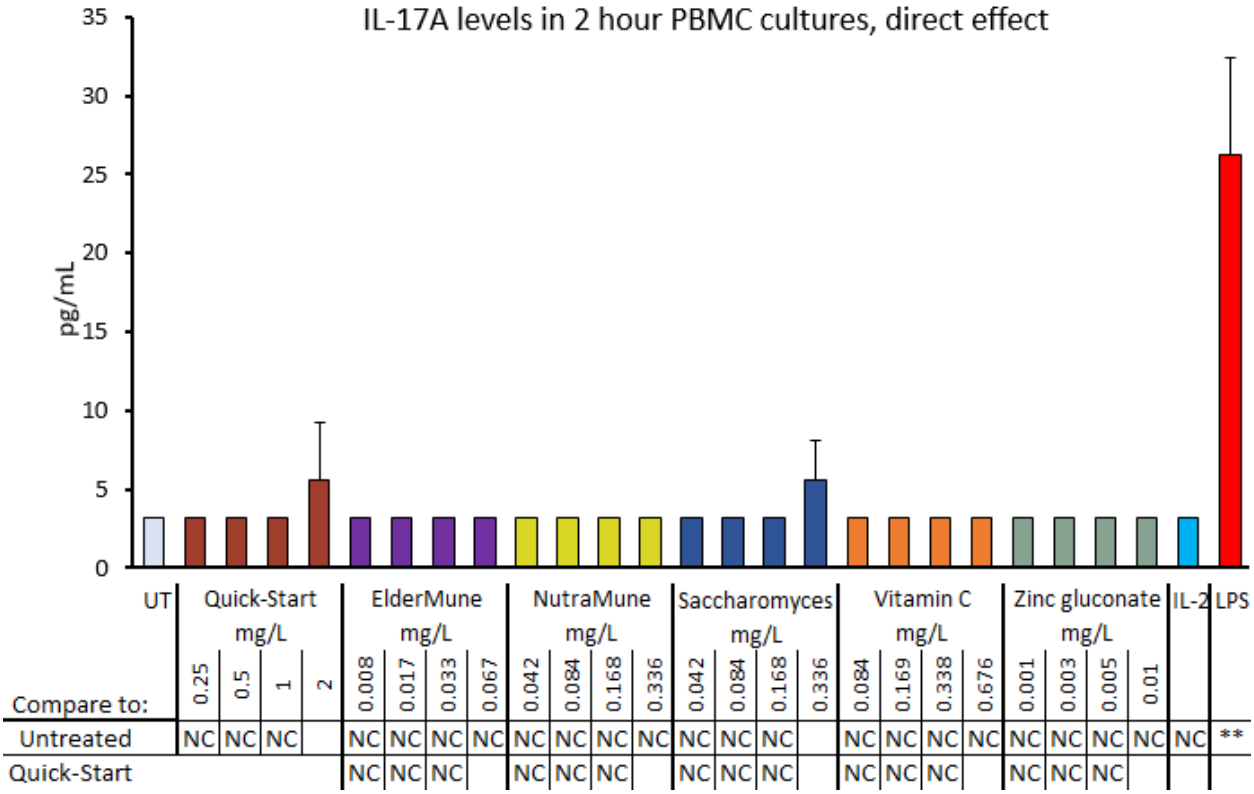


Figure 19. Interleukin 8 (IL-8) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	**
Quick-Start						NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC			

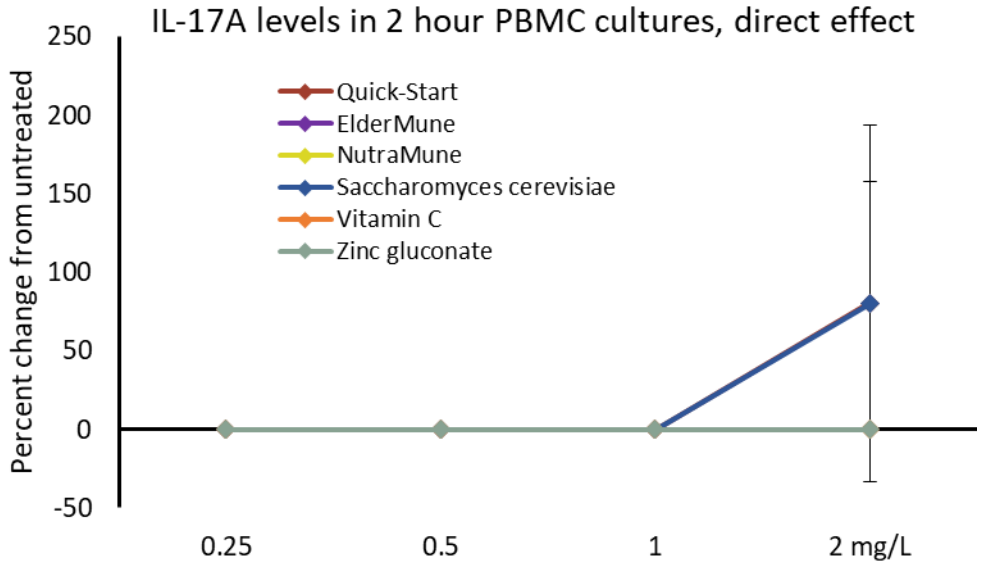
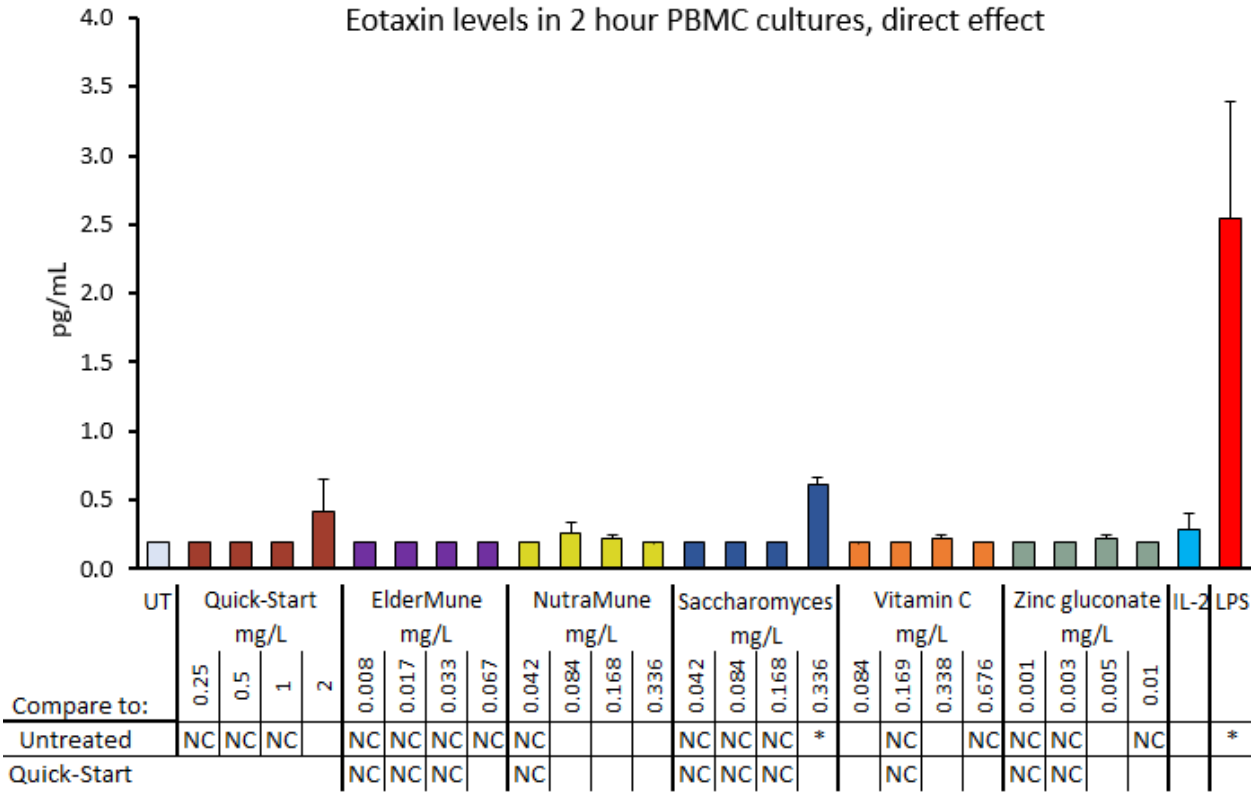


Figure 20. Interleukin 17A (IL-17A) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	NC	NC	NC	NC		NC	NC	NC	NC	NC				NC	NC	NC	*		NC		NC	NC	NC		NC		*
Quick-Start						NC	NC	NC		NC				NC	NC	NC			NC			NC	NC					

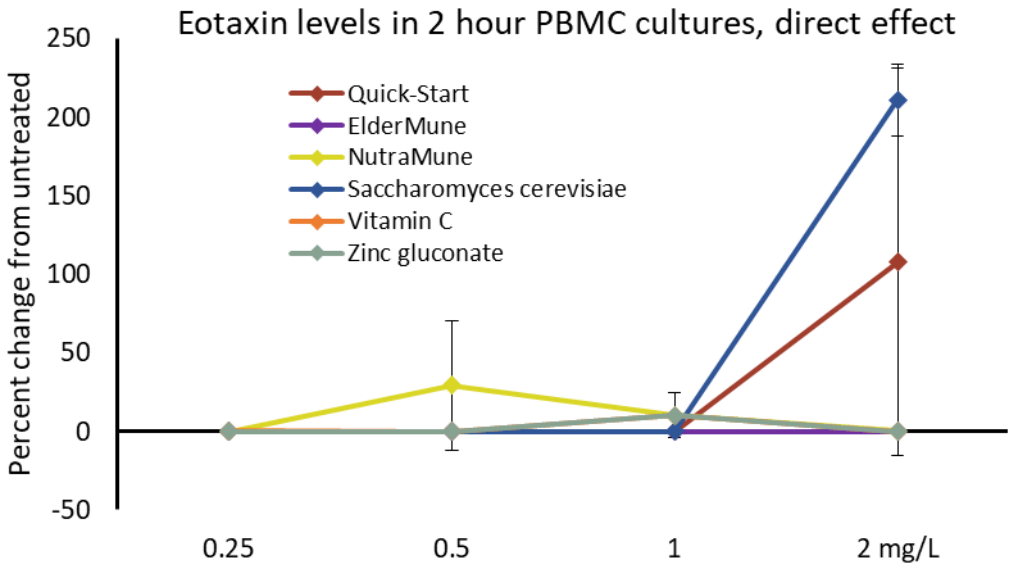
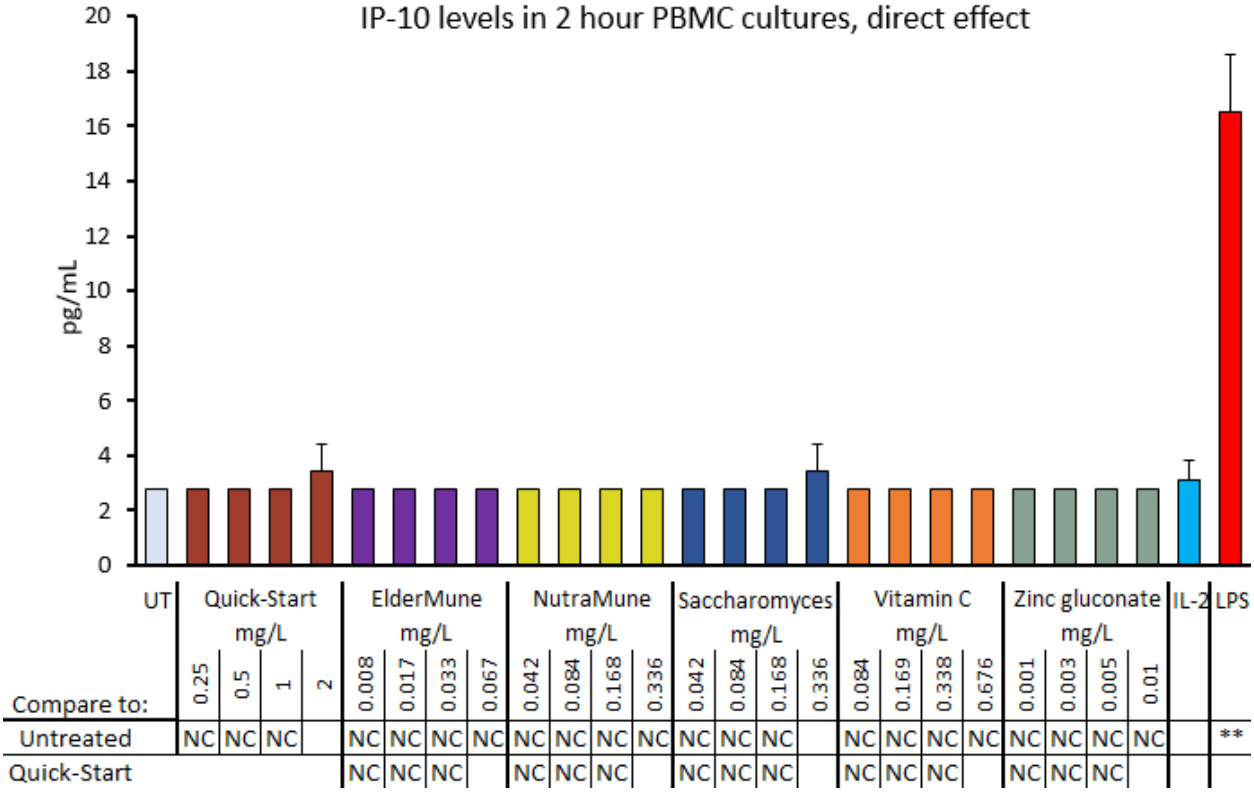


Figure 21. Eotaxin levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC		**
Quick-Start						NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC			

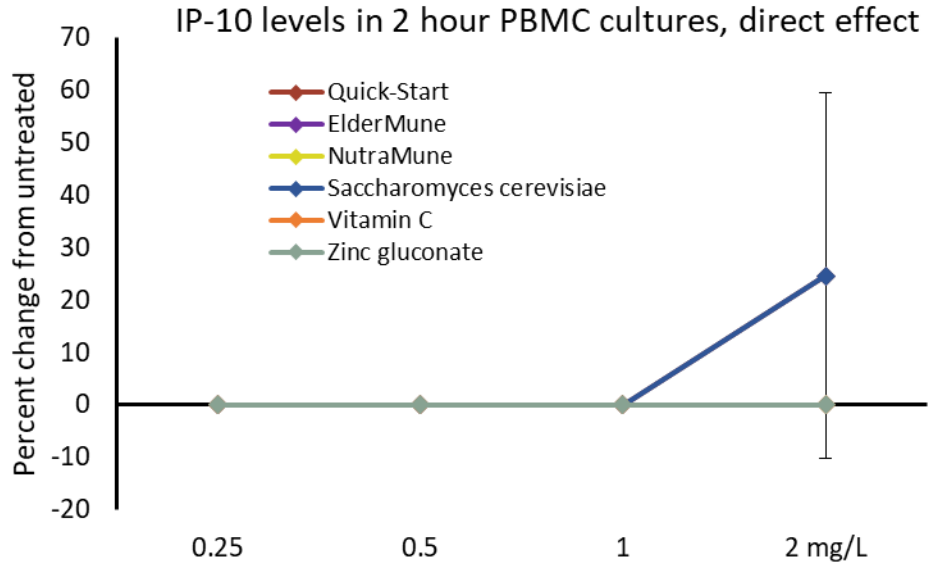
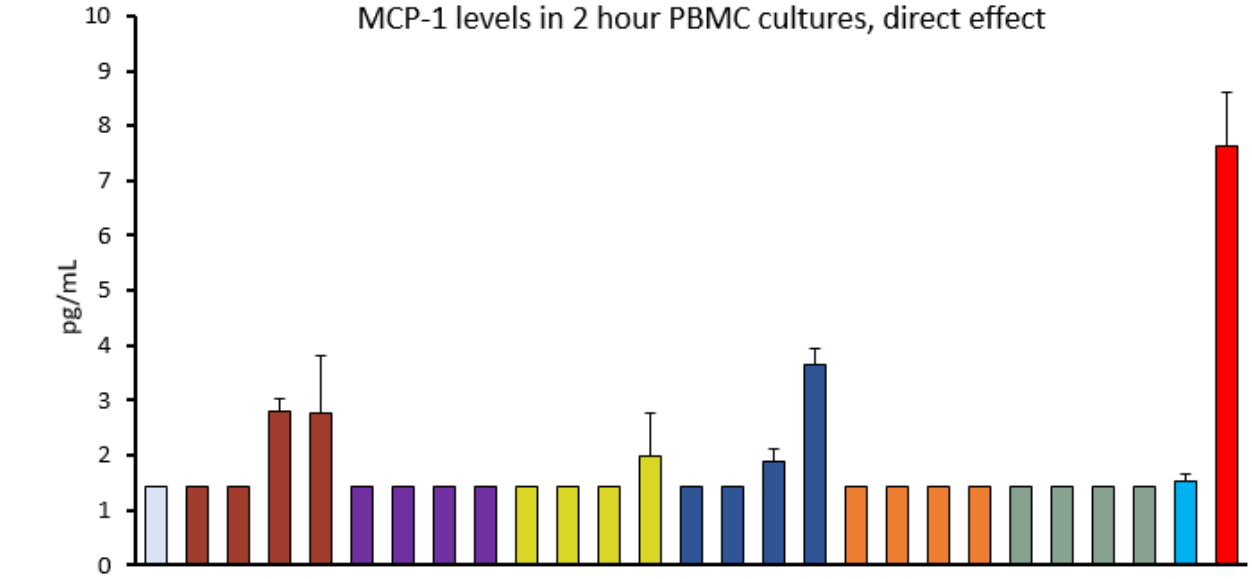


Figure 22. Interferon gamma-induced protein 10 (IP-10) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



Compare to:	UT	Quick-Start mg/L 0.25	Quick-Start mg/L 0.5	Quick-Start mg/L 1	Quick-Start mg/L 2	ElderMune mg/L 0.008	ElderMune mg/L 0.017	ElderMune mg/L 0.033	ElderMune mg/L 0.067	NutraMune mg/L 0.042	NutraMune mg/L 0.084	NutraMune mg/L 0.168	NutraMune mg/L 0.336	Saccharomyces mg/L 0.042	Saccharomyces mg/L 0.084	Saccharomyces mg/L 0.168	Saccharomyces mg/L 0.336	Vitamin C mg/L 0.084	Vitamin C mg/L 0.169	Vitamin C mg/L 0.338	Vitamin C mg/L 0.676	Zinc gluconate mg/L 0.001	Zinc gluconate mg/L 0.003	Zinc gluconate mg/L 0.005	Zinc gluconate mg/L 0.01	IL-2	LPS
Untreated	NC	NC	NC	(*)		NC	NC	NC	NC	NC	NC	NC		NC	NC		(*)	NC	NC	NC	NC	NC	NC	NC	NC		**
Quick-Start						NC	NC	(*)		NC	NC	(*)		NC	NC	(*)		NC	NC	(*)		NC	NC	(*)			

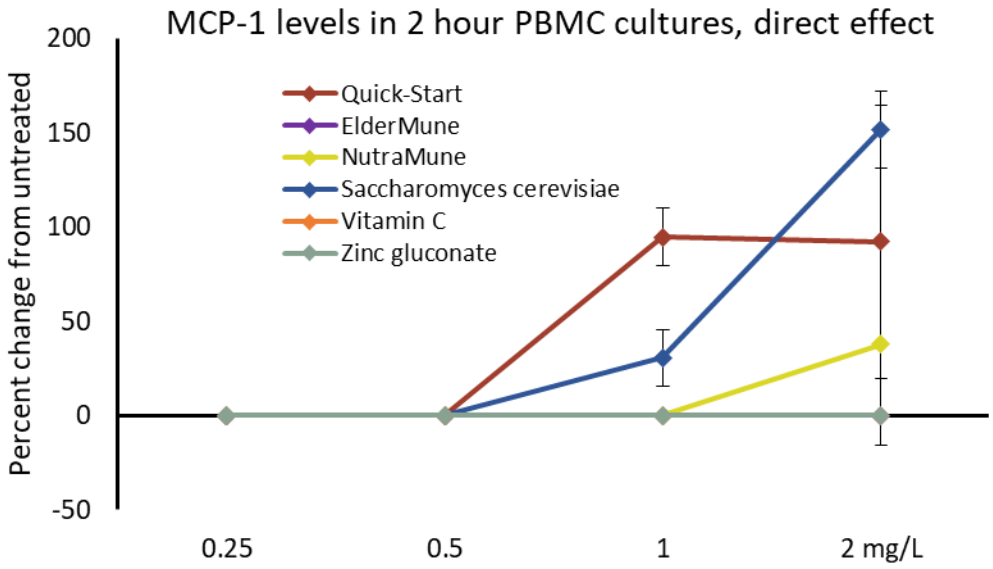


Figure 23. Monocyte chemotactic protein 1 (MCP-1) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.*
Bottom: *Results are shown as the average ± standard deviation of the percent change from untreated.*

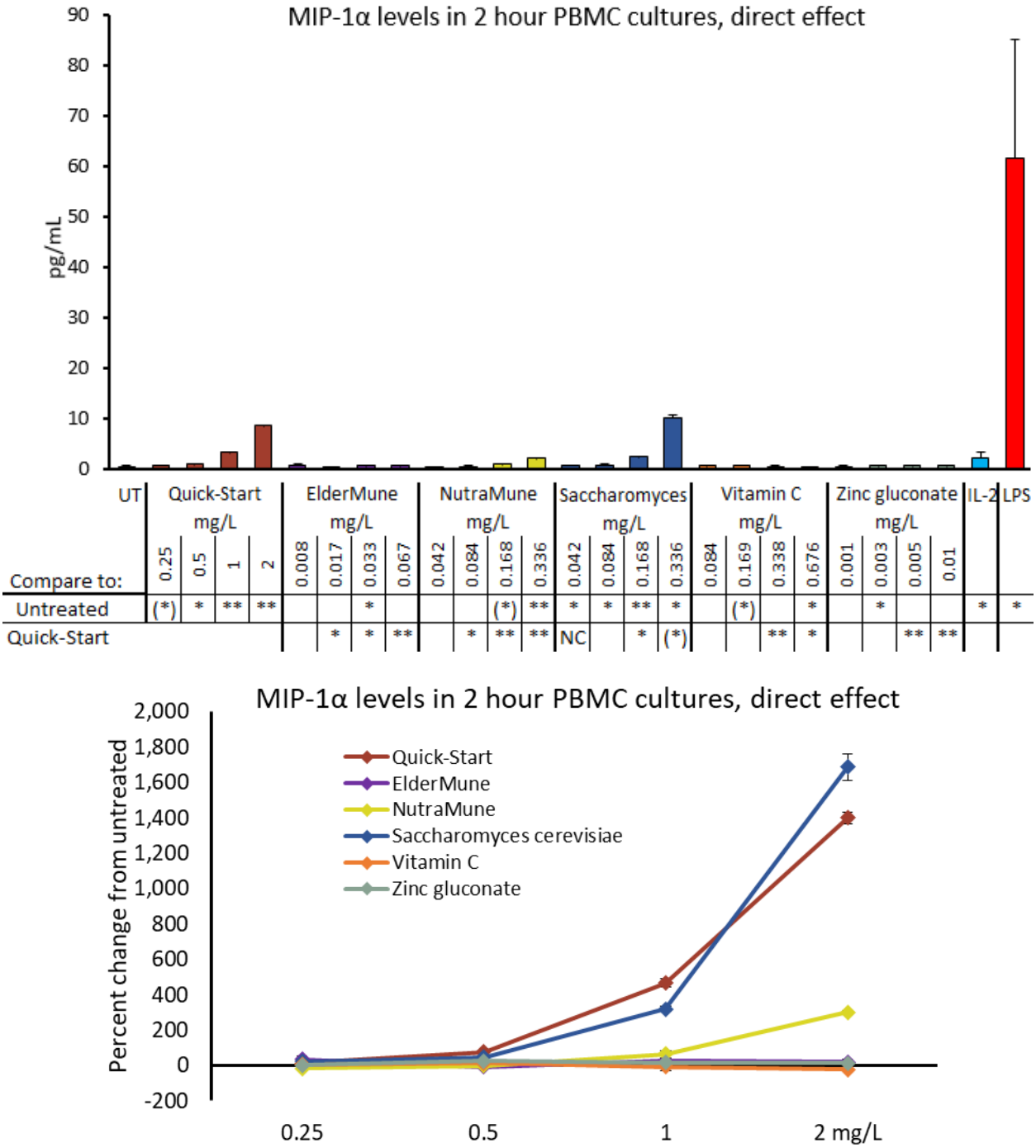


Figure 24. Macrophage inflammatory protein 1 alpha (MIP-1α) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*

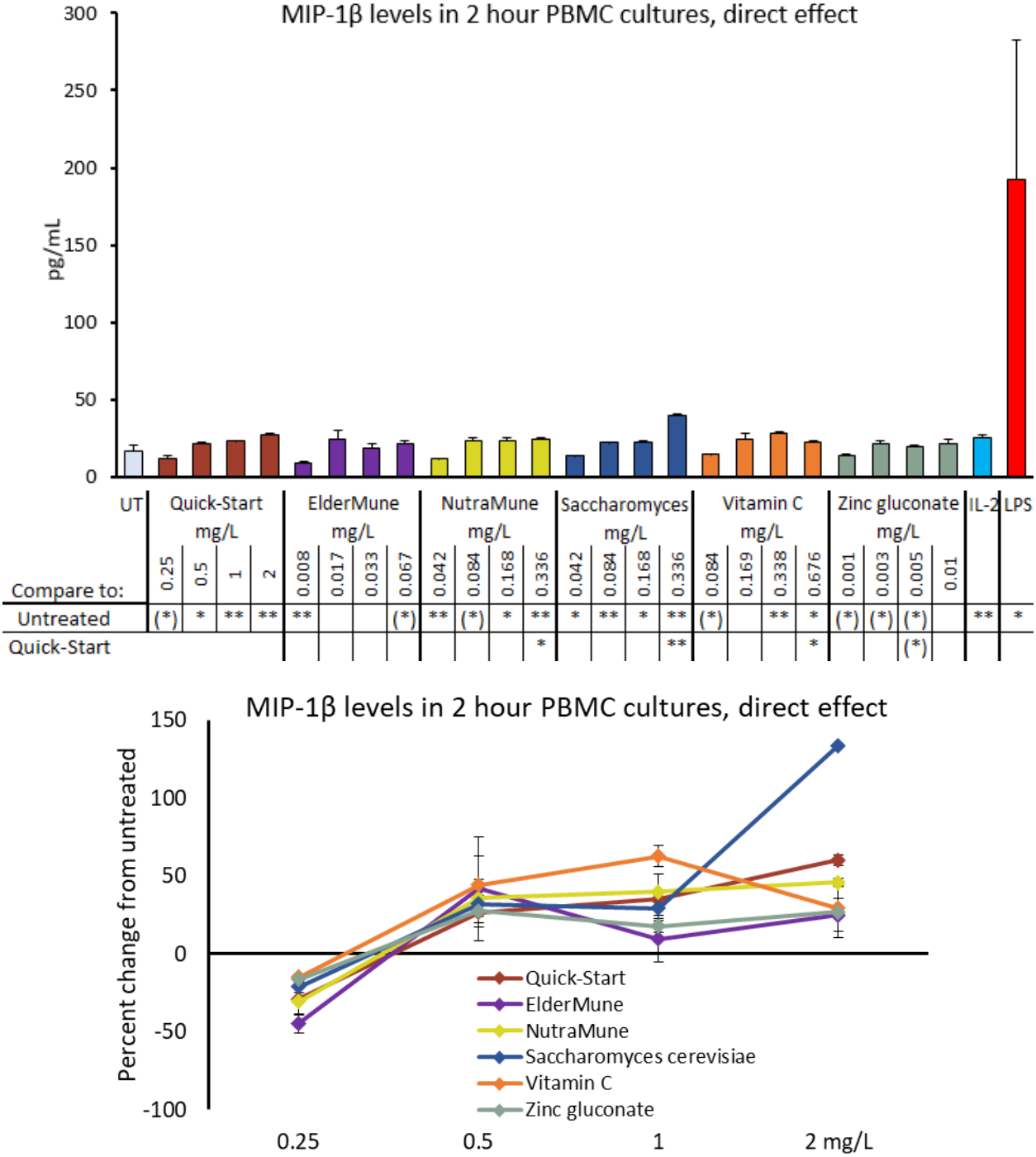
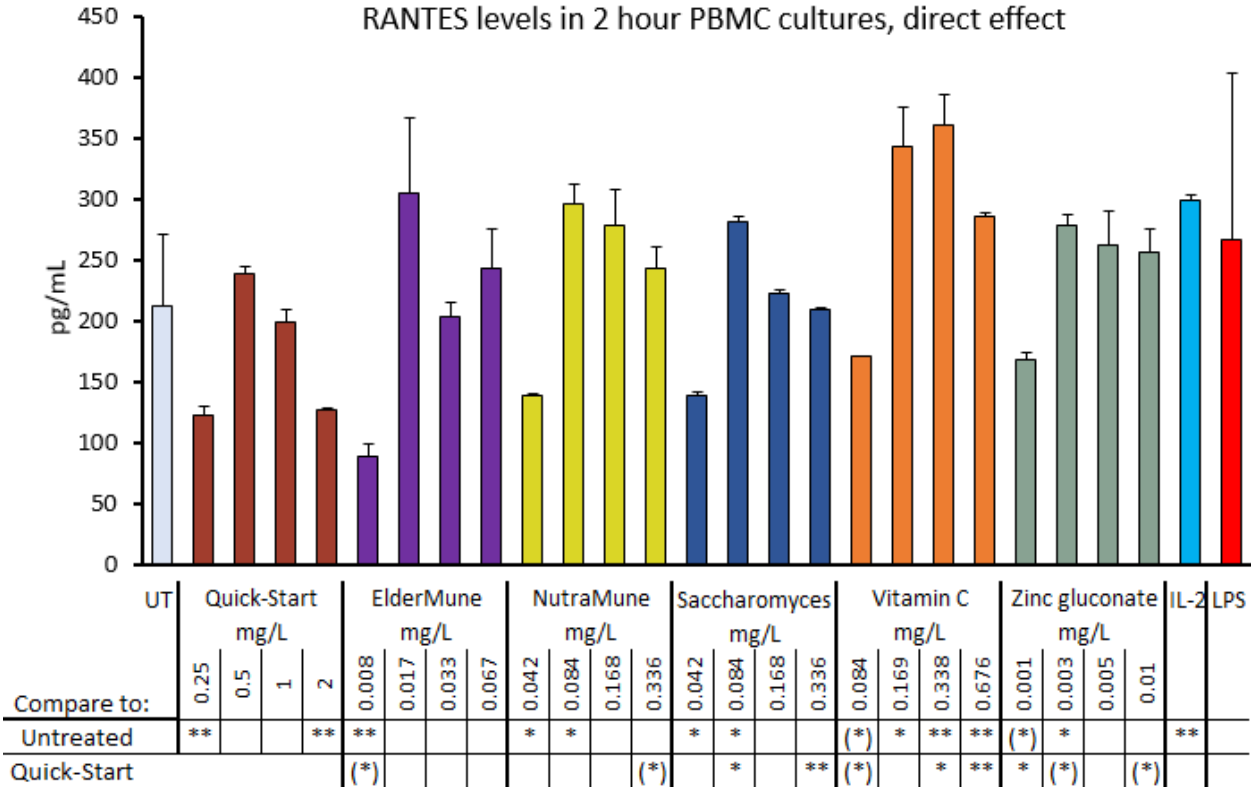


Figure 25. Macrophage inflammatory protein 1 beta (MIP-1β) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	**				**	**				*	*			*	*			(*)	*	**	**	(*)	*			**	
Quick-Start						(*)							(*)		*		**	(*)		*	**	*	(*)		(*)		

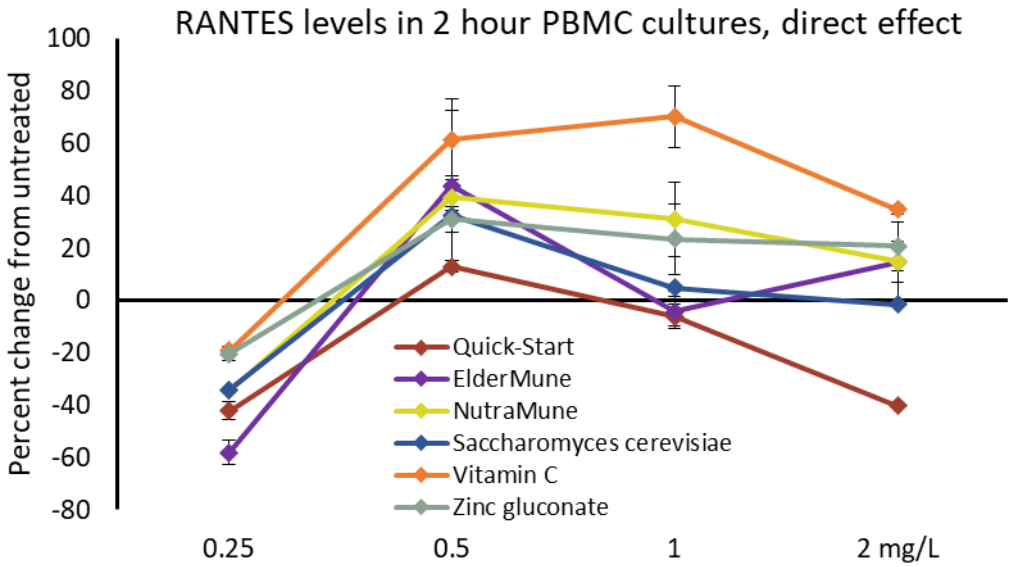
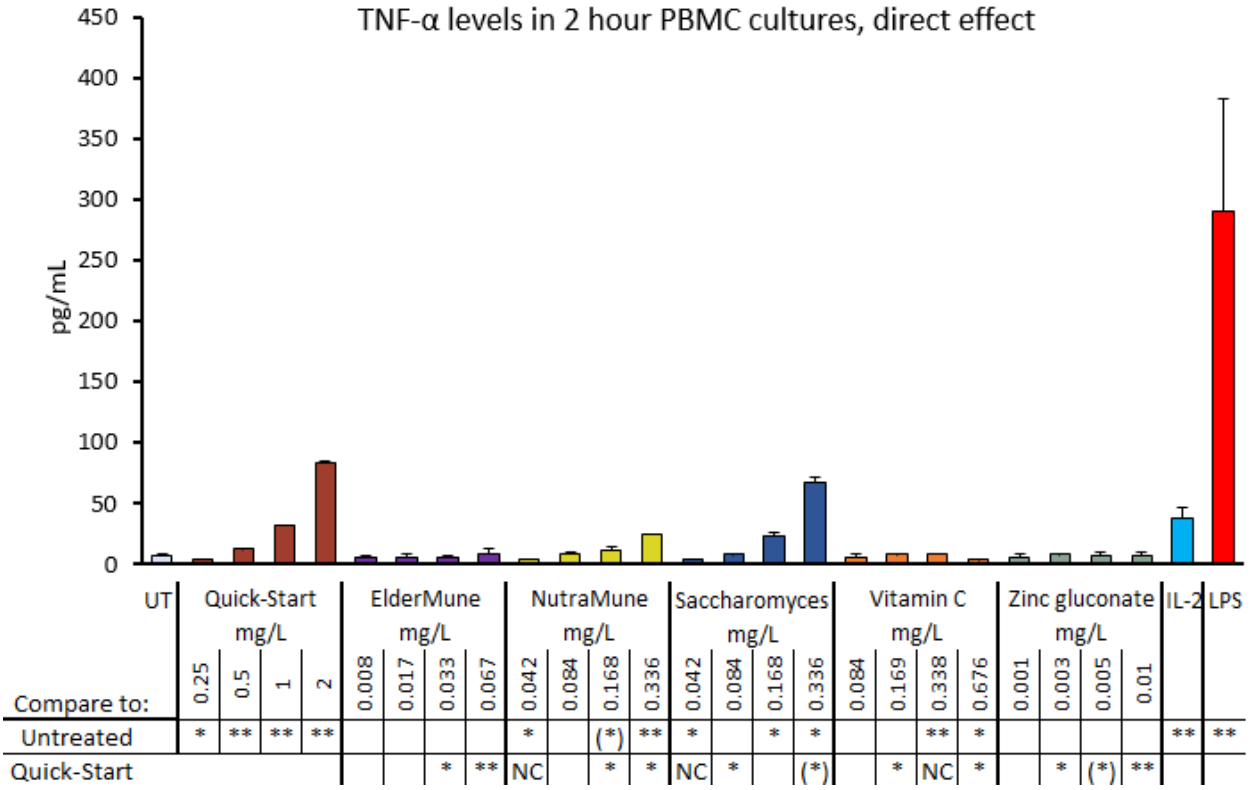


Figure 26. Regulated on activation, normal T cell expressed and secreted (RANTES) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions.
Top: *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated		*	**	**	**					*		(*)	**	*		*	*			**	*					**	**
Quick-Start								*	**	NC		*	*	NC	*		(*)		*	NC	*		*	(*)	**		

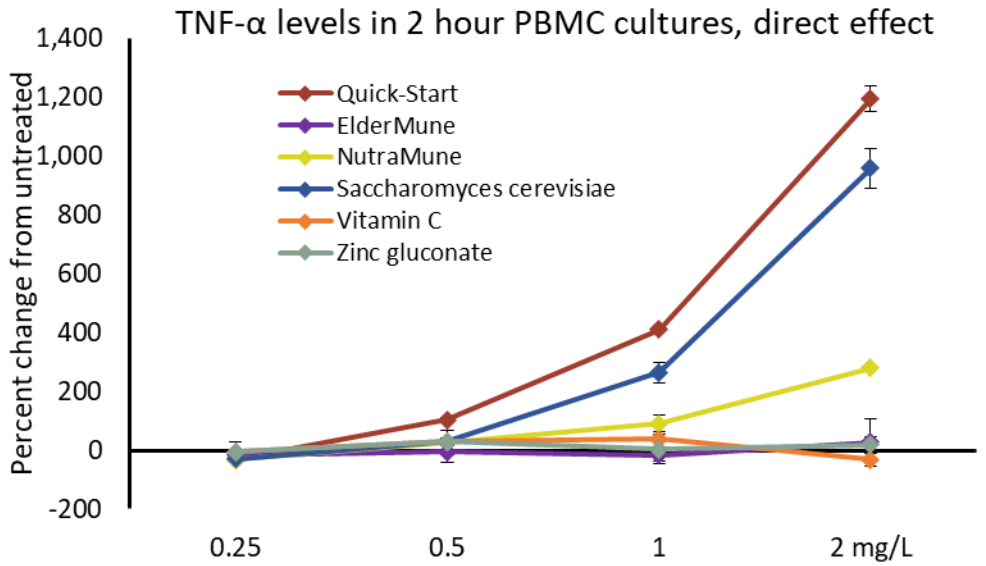
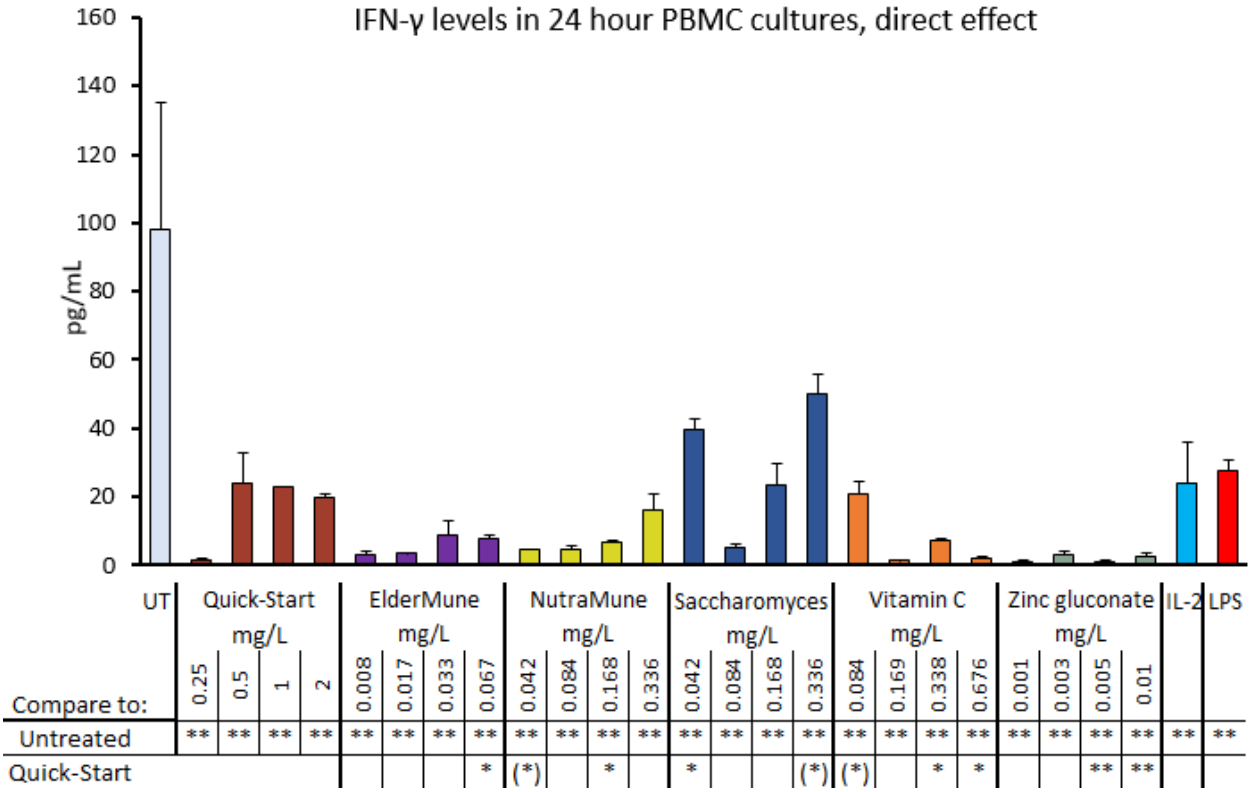


Figure 27. Tumor necrosis factor alpha (TNF-α) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.*
Bottom: *Results are shown as the average ± standard deviation of the percent change from untreated.*



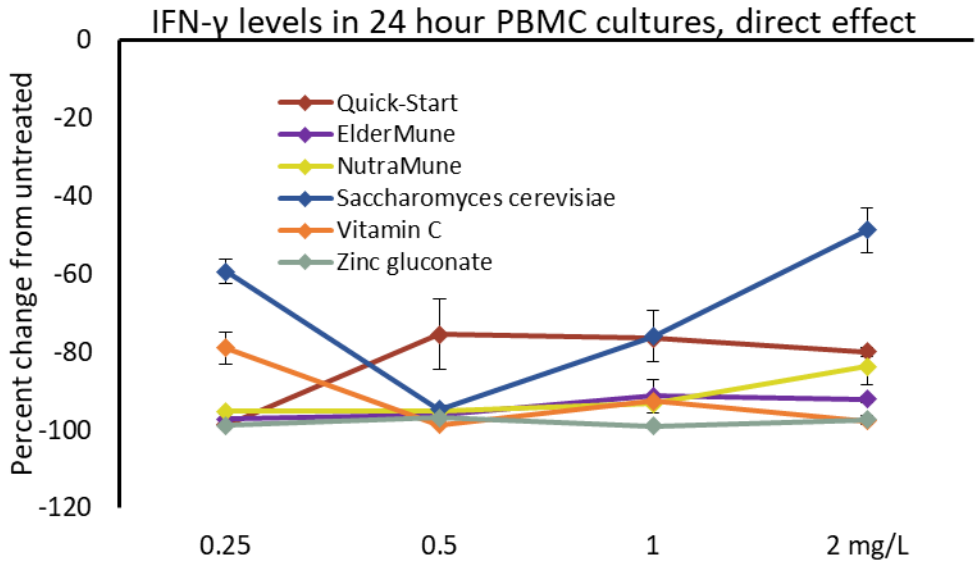
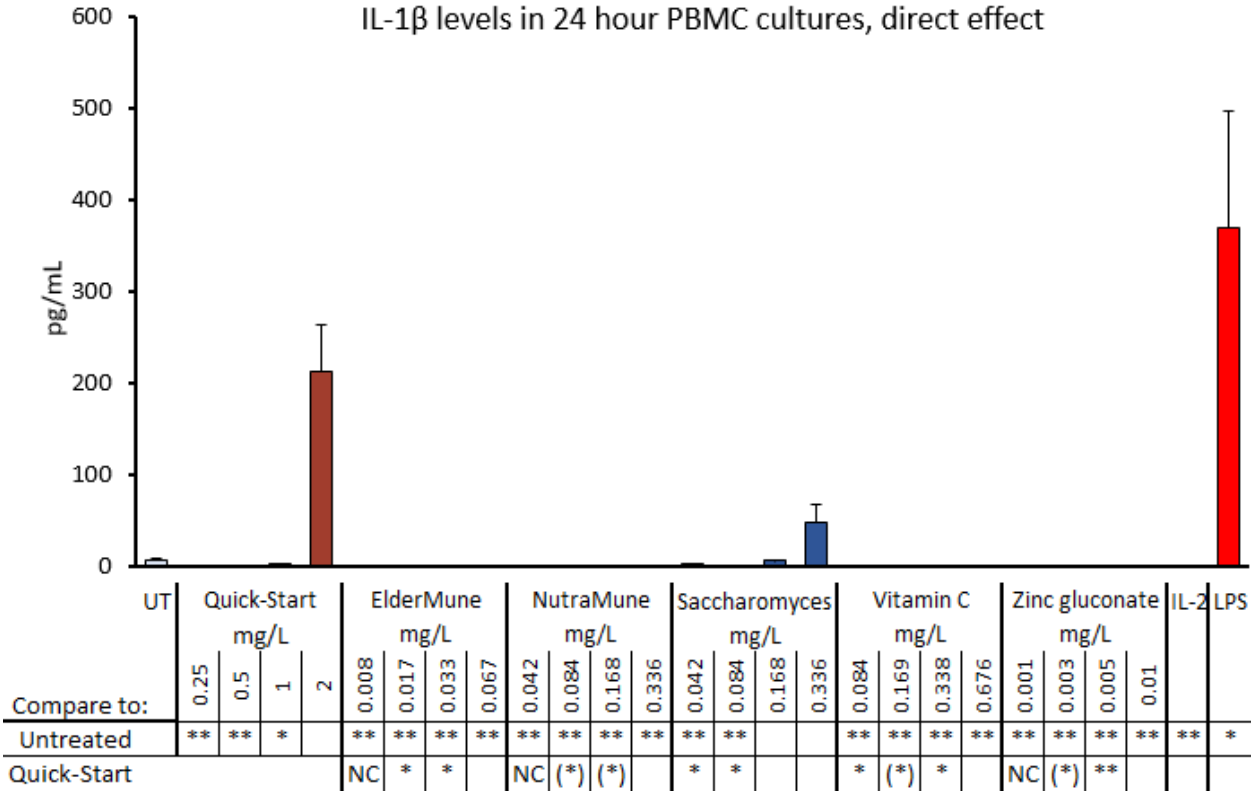


Figure 28. Interferon-gamma (IFN-γ) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	**	**	**	*		**	**	**	**	**	**	**	**	**	**			**	**	**	**	**	**	**	**	**	*
Quick-Start						NC	*	*		NC	(*)	(*)		*	*			*	(*)	*		NC	(*)	**			

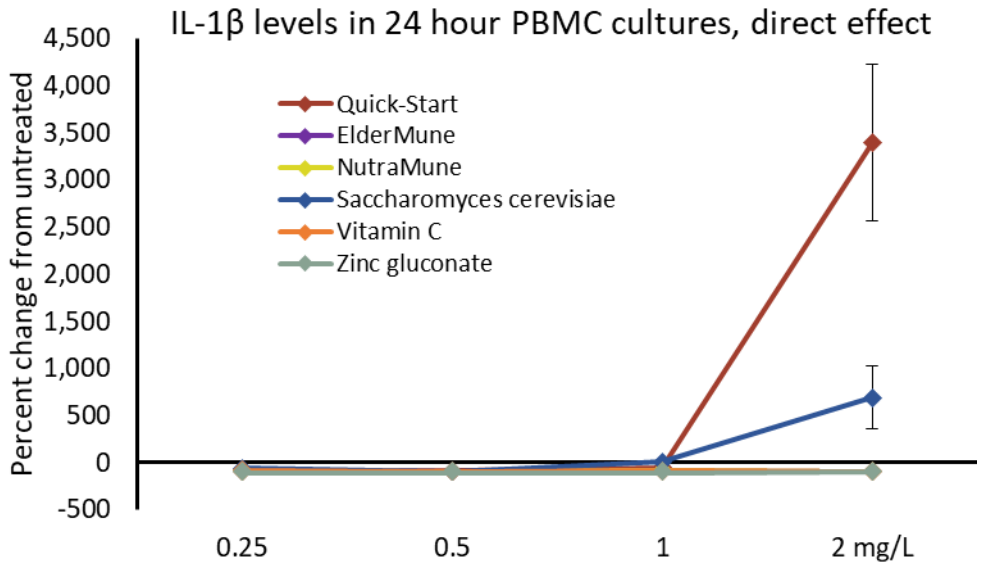
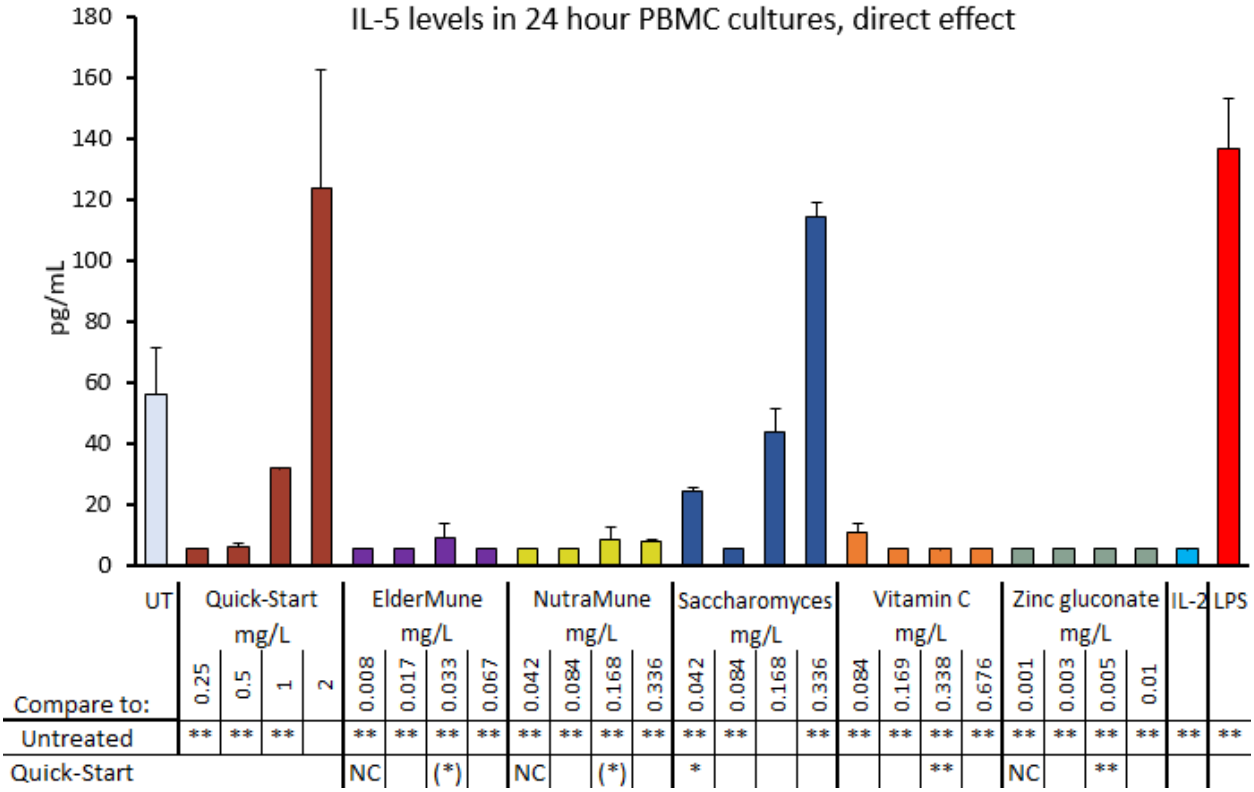


Figure 29. Interleukin 1 beta (IL-1β) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



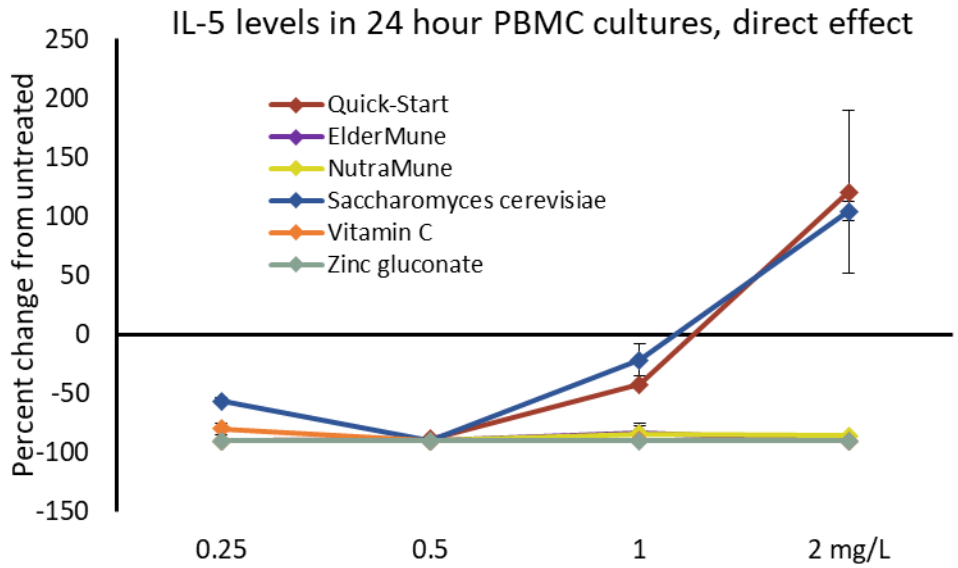
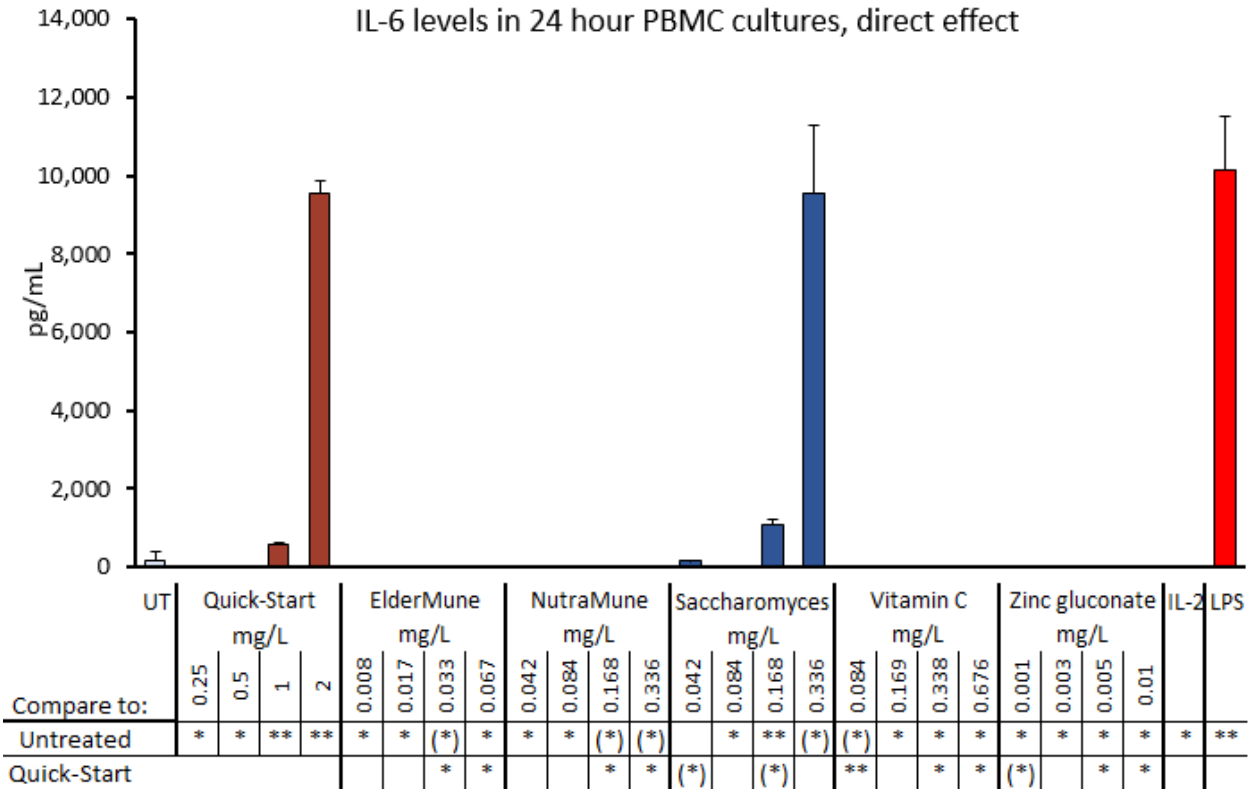


Figure 29. Interleukin 5 (IL-5) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	*	*	*	**	**	*	*	(*)	*	*	*	(*)	(*)		*	**	(*)	(*)	*	*	*	*	*	*	*	*	*	**
Quick-Start								*	*			*	*	(*)		(*)		**		*	*	(*)		*	*		

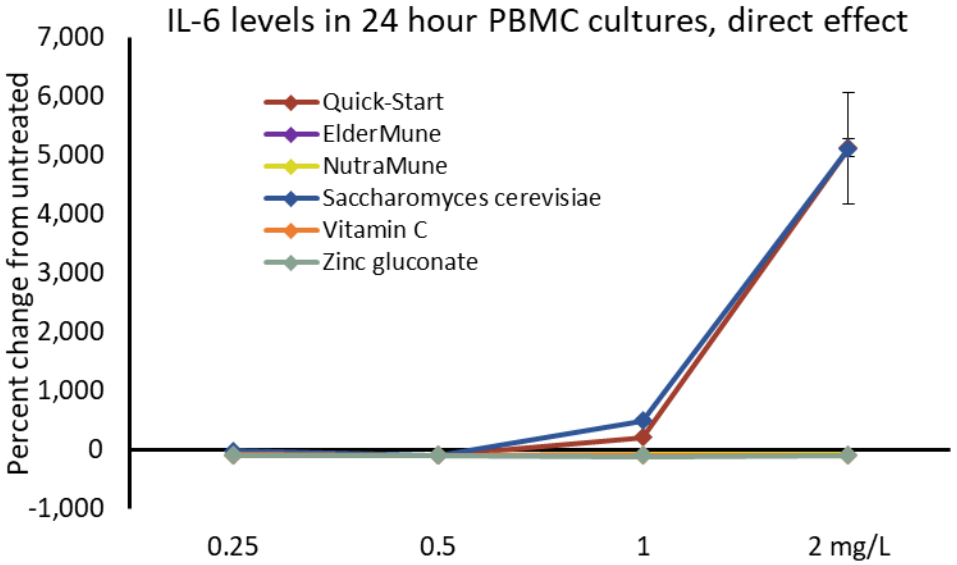


Figure 30. Interleukin 6 (IL-6) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*

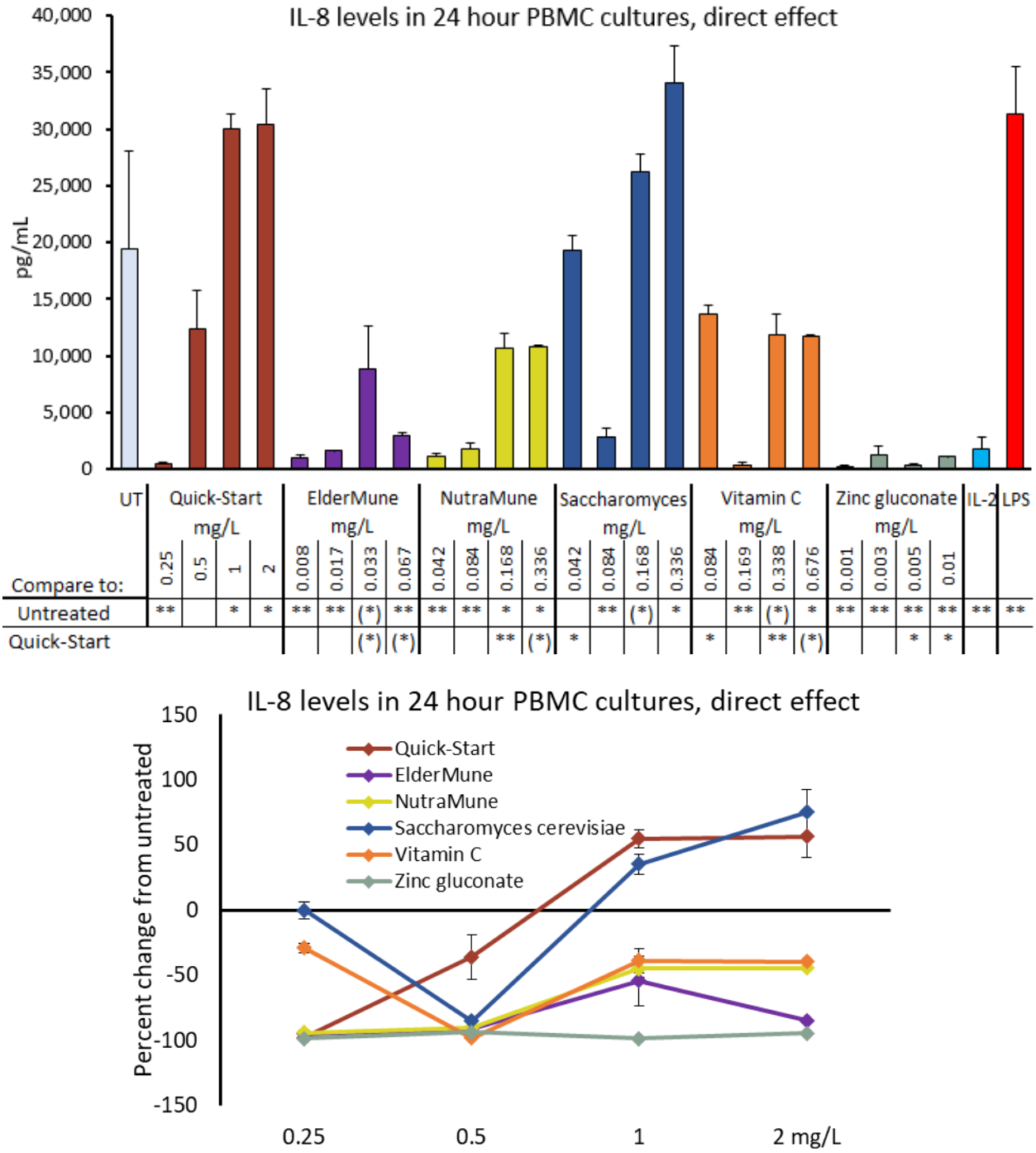
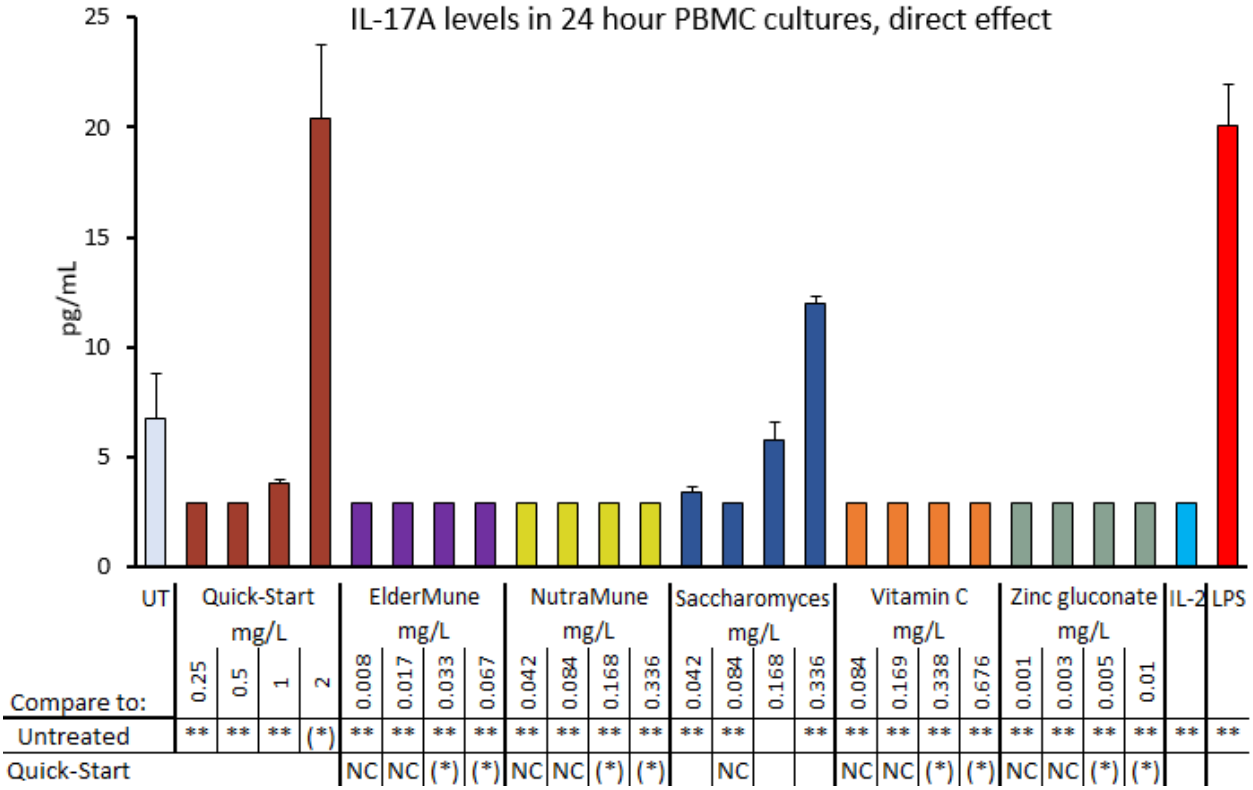


Figure 31. Interleukin 8 (IL-8) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



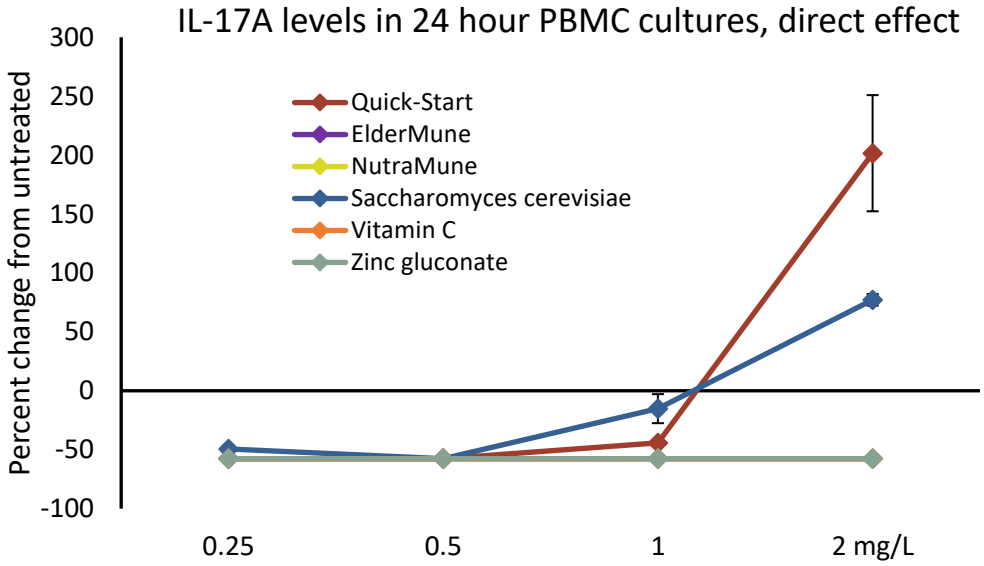
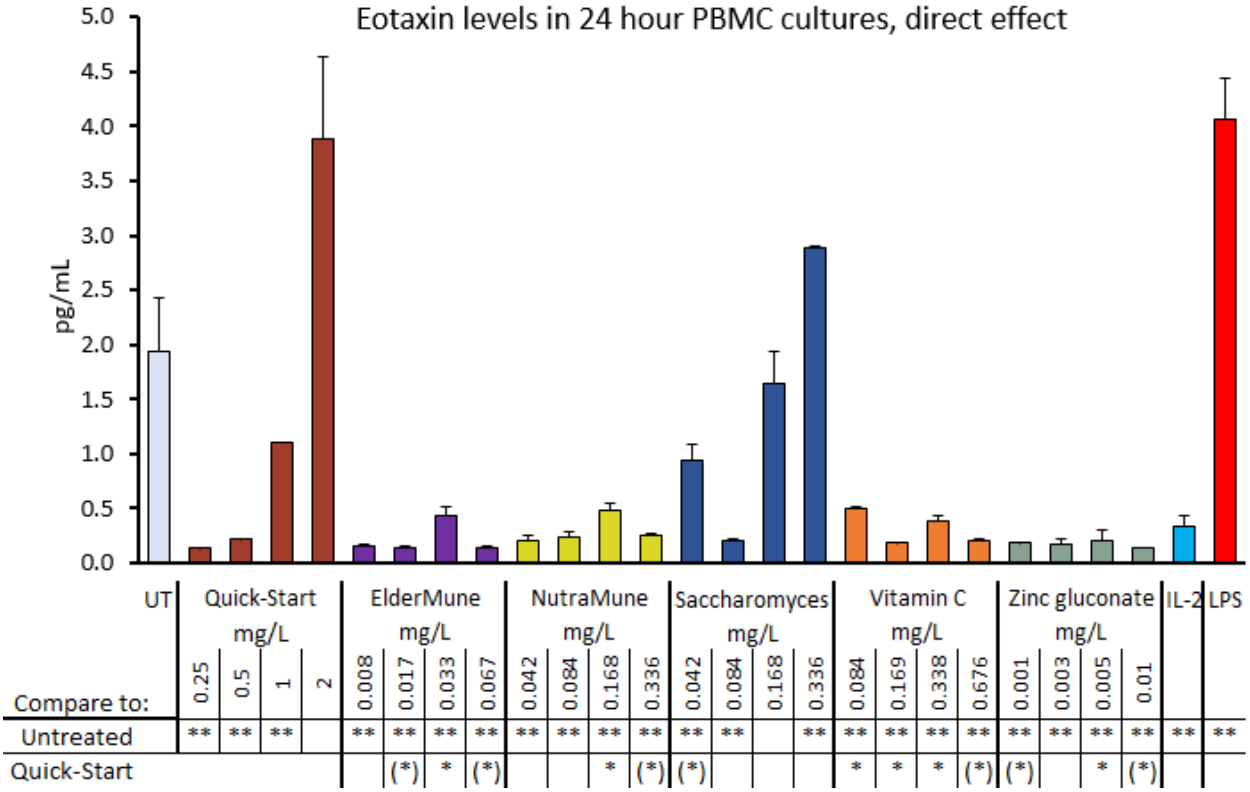


Figure 32. Interleukin 17A (IL-17A) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



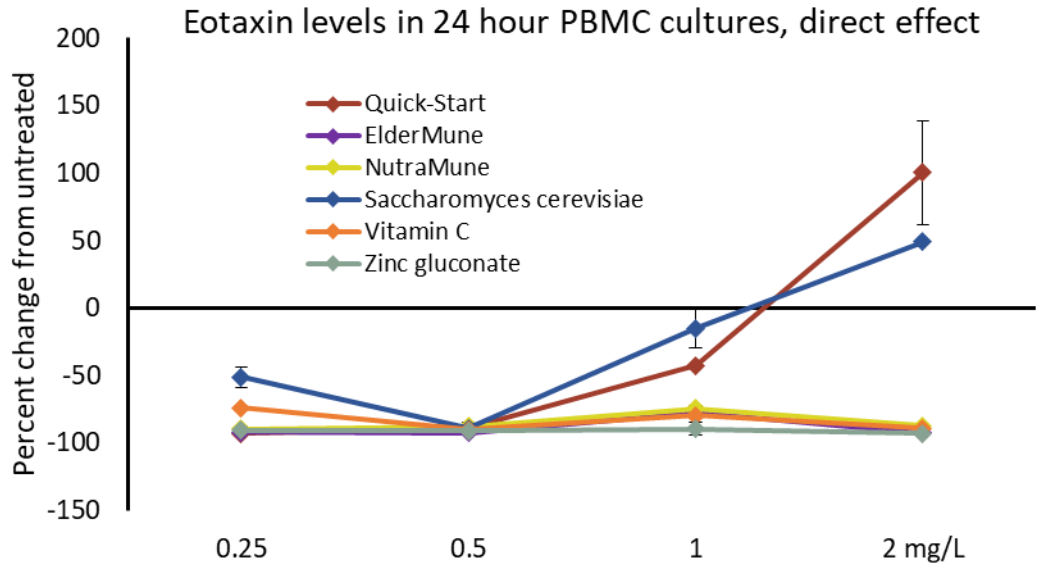
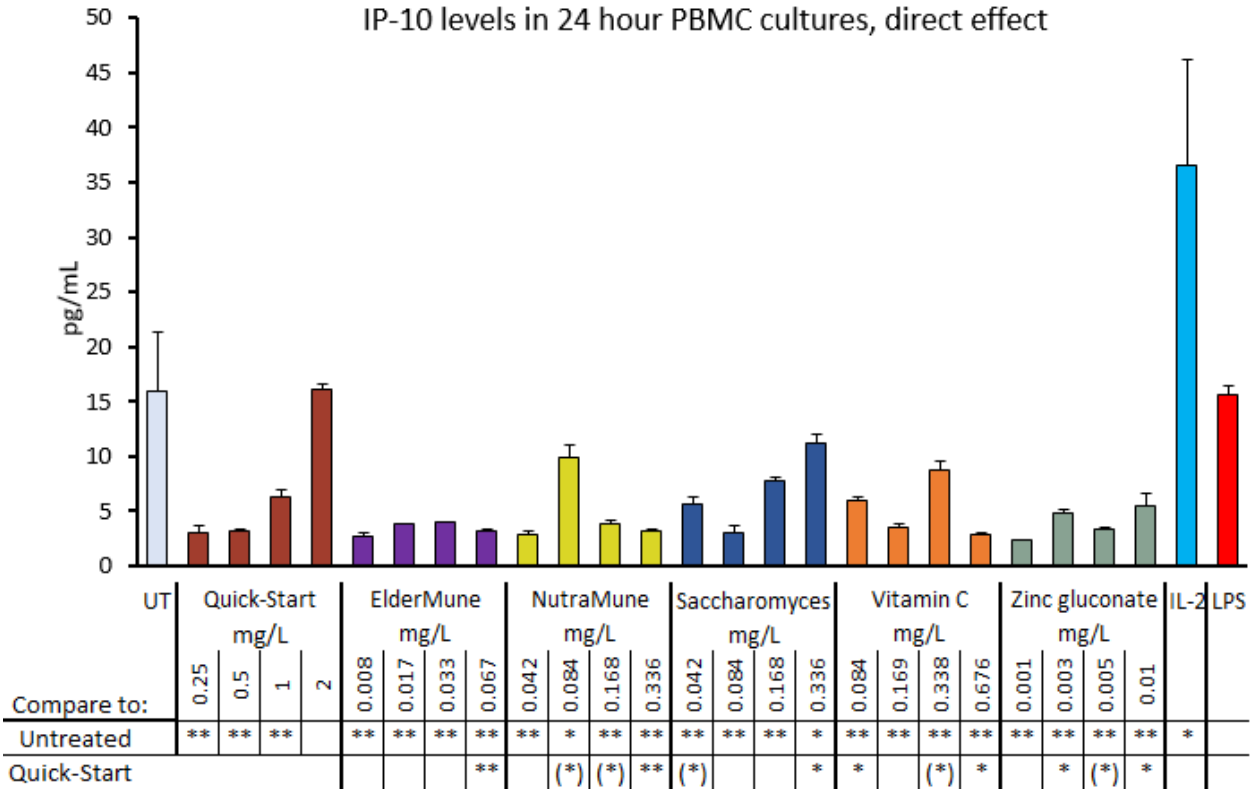


Figure 33. Eotaxin levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



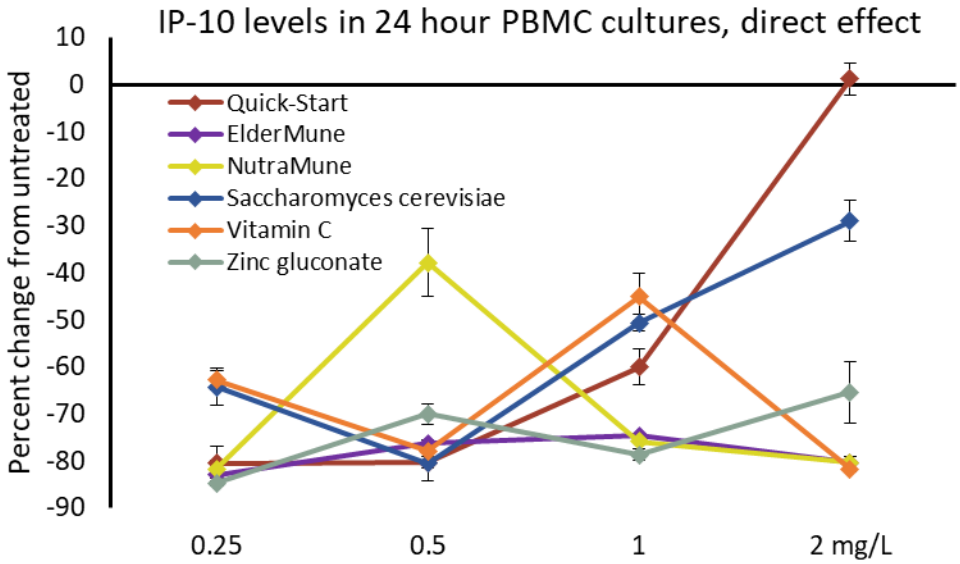


Figure 34. Interferon gamma-induced protein 10 (IP-10) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*

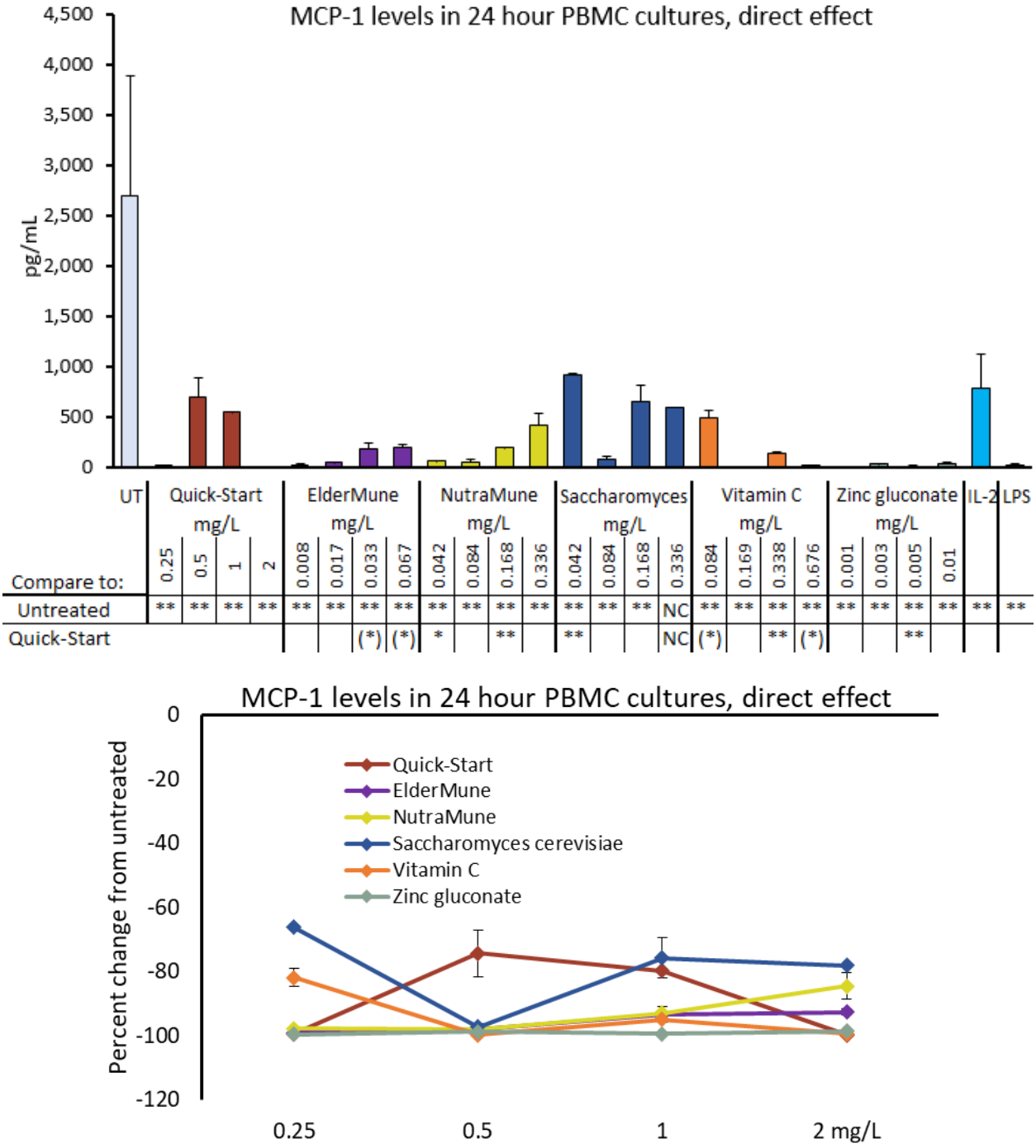
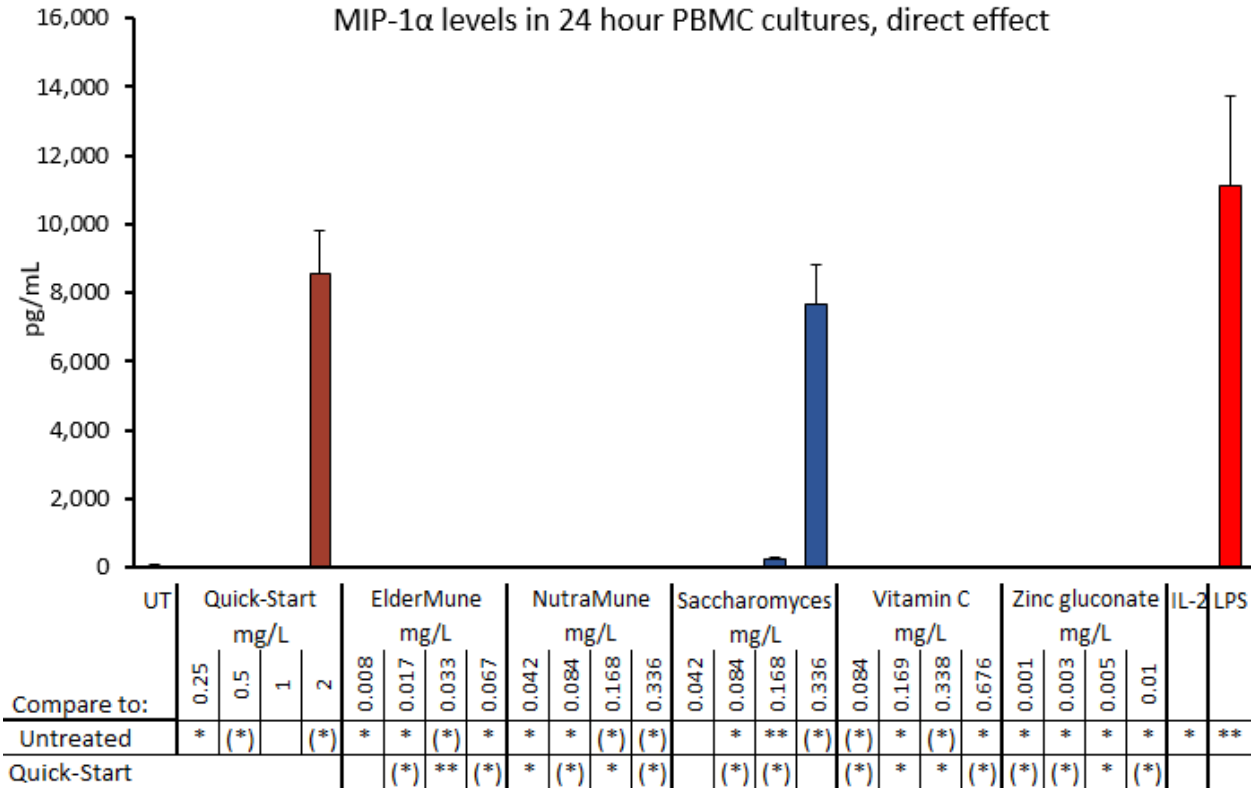


Figure 35. Monocyte chemotactic protein 1 (MCP-1) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	*	(*)		(*)	*	*	(*)	*	*	*	(*)	(*)		*	**	(*)	(*)	*	(*)	*	*	*	*	*	*	*	**
Quick-Start							(*)	**	(*)	*	(*)	*	(*)		(*)	(*)		(*)	*	*	(*)	(*)	(*)	*	(*)		

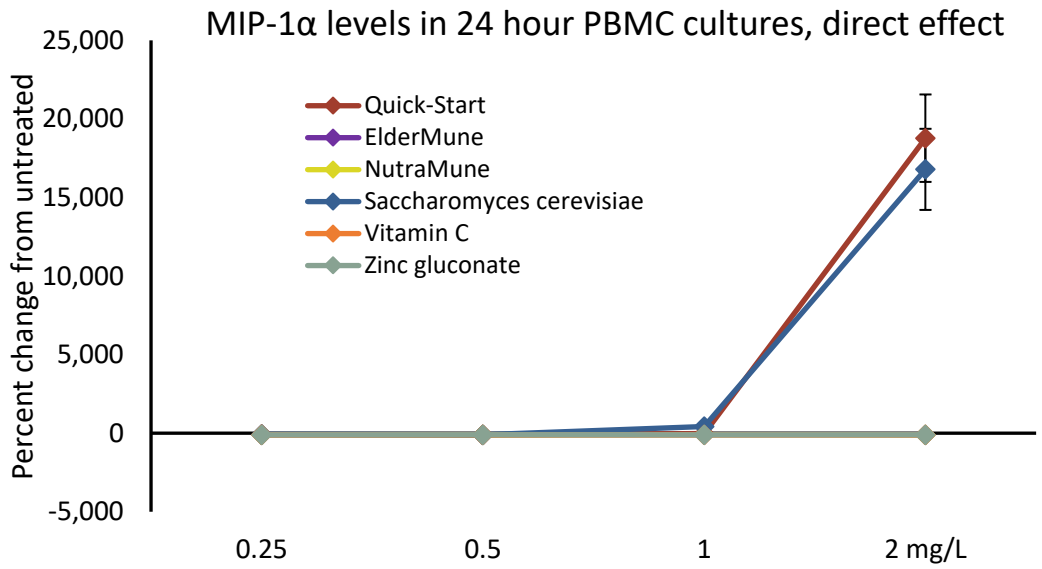
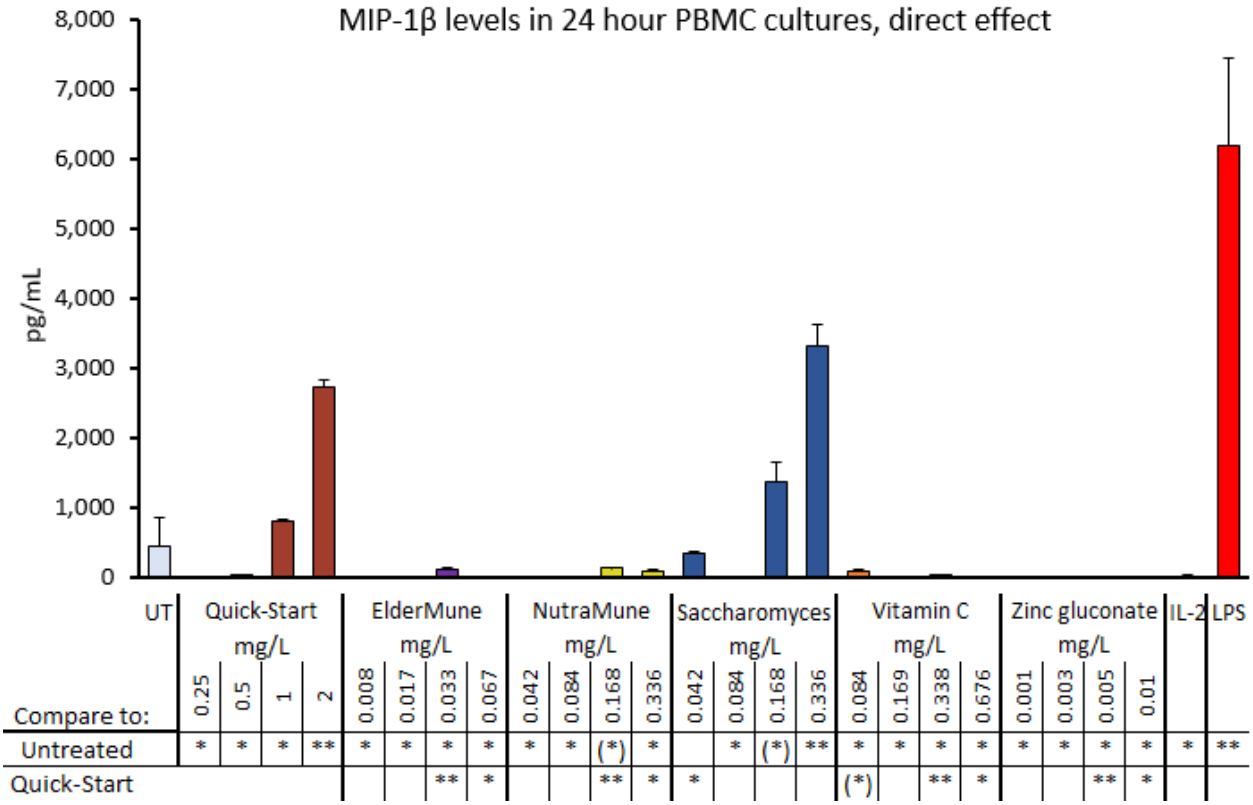


Figure 36. Macrophage inflammatory protein 1 alpha (MIP-1α) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



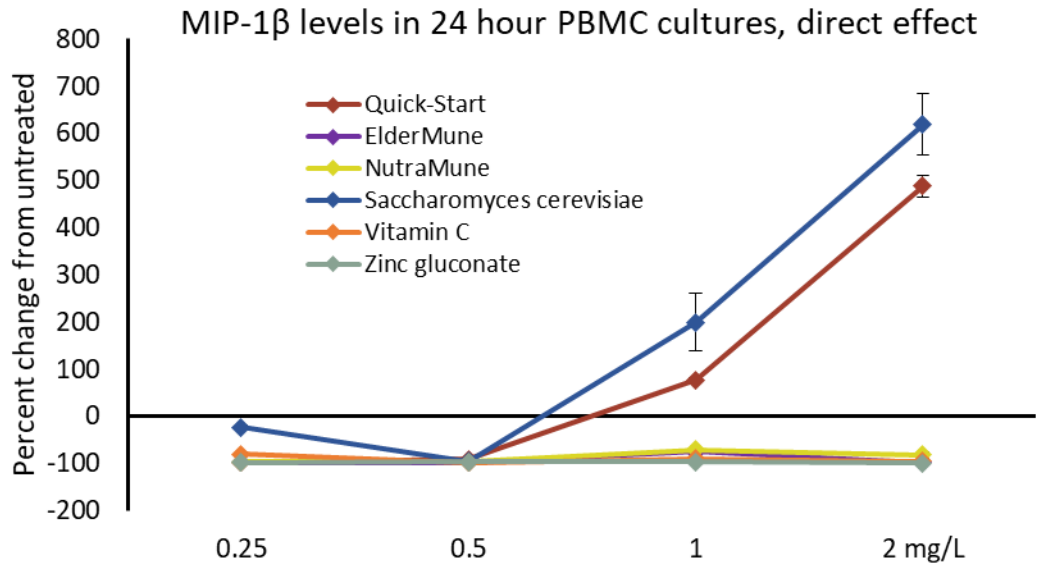
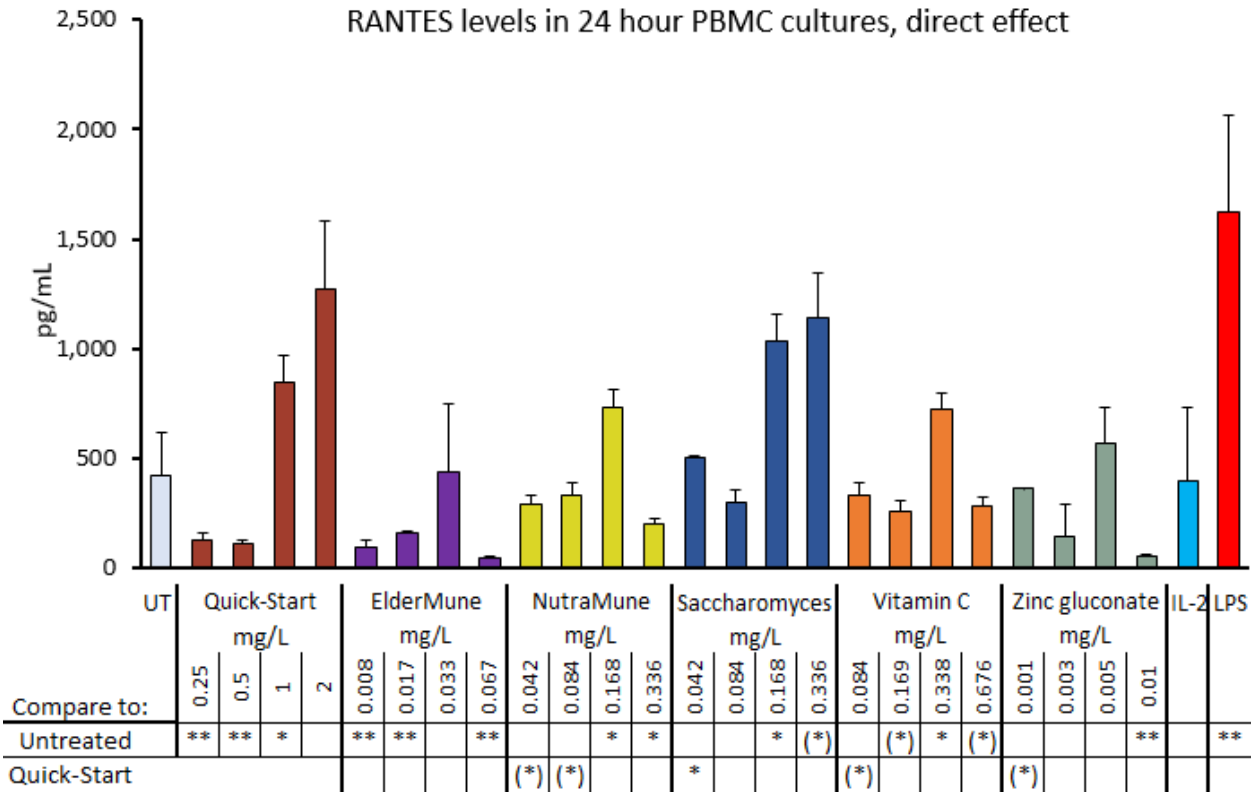


Figure 37. Macrophage inflammatory protein 1 beta (MIP-1β) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



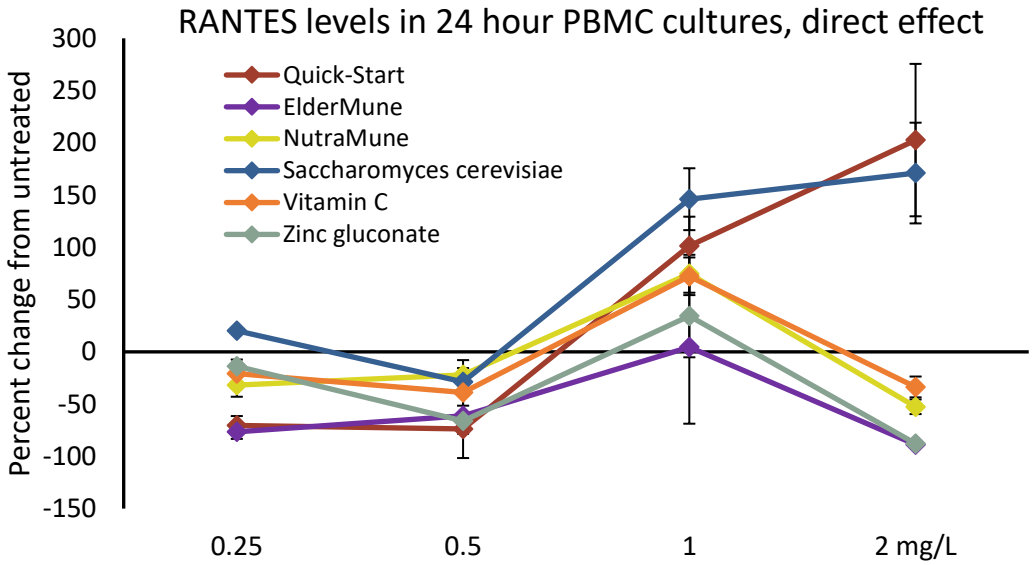
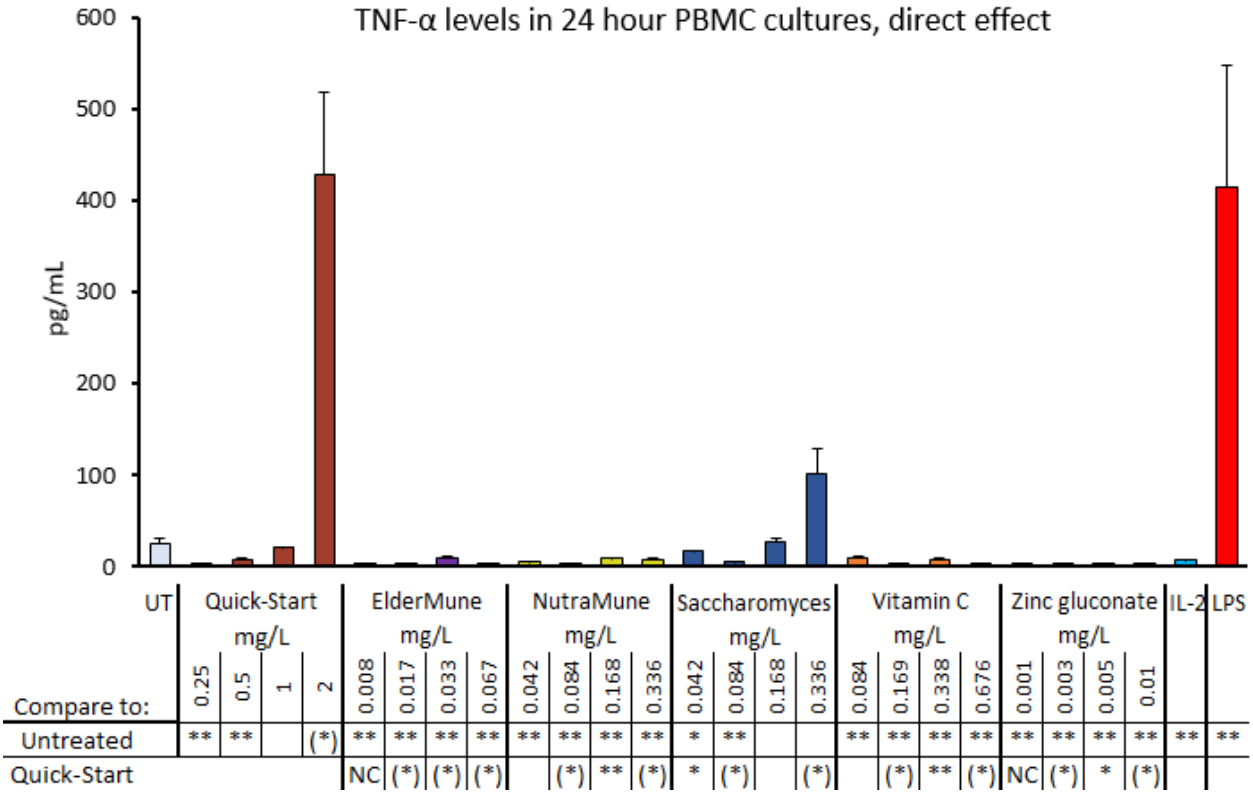


Figure 38. Regulated on activation, normal T cell expressed and secreted (RANTES) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions.
Top: *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	**	**	**		(*)	**	**	**	**	**	**	**	**	*	**			**	**	**	**	**	**	**	**	**	**
Quick-Start						NC	(*)	(*)	(*)		(*)	**	(*)	*	(*)		(*)		(*)	**	(*)	NC	(*)	*	(*)		

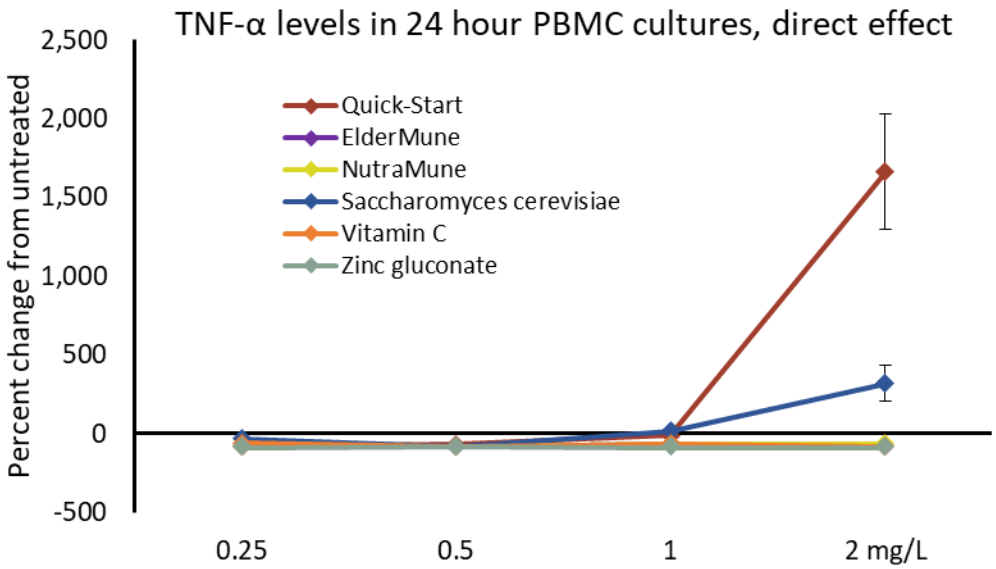
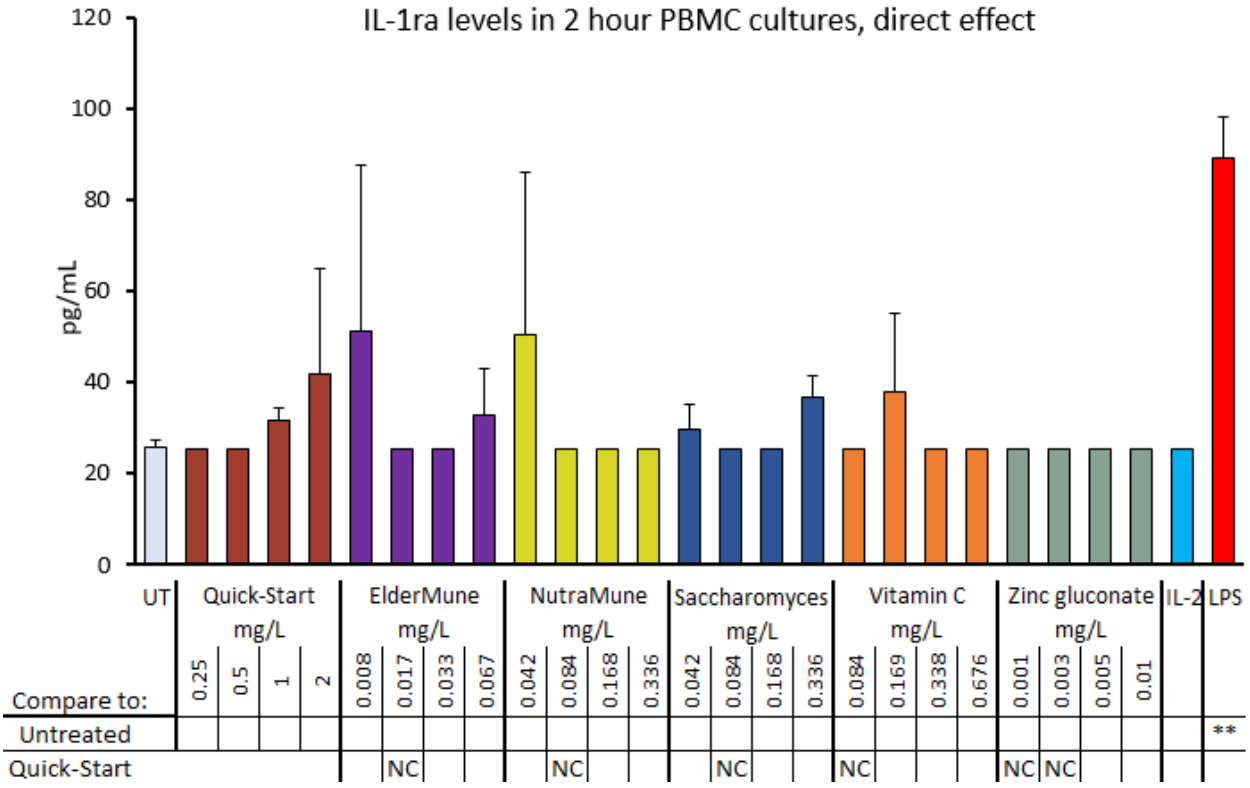


Figure 39. Tumor necrosis factor alpha (TNF-α) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*

5.2.6 Anti-inflammatory cytokines/chemokines

Table 5. Anti-inflammatory cytokines.

IL-1ra	Interleukin-1 receptor antagonist. Natural inhibitor of the pro-inflammatory effects of IL-1β.
IL-10	Interleukin 10. Anti-inflammatory cytokine but requires activation of cells to induce.



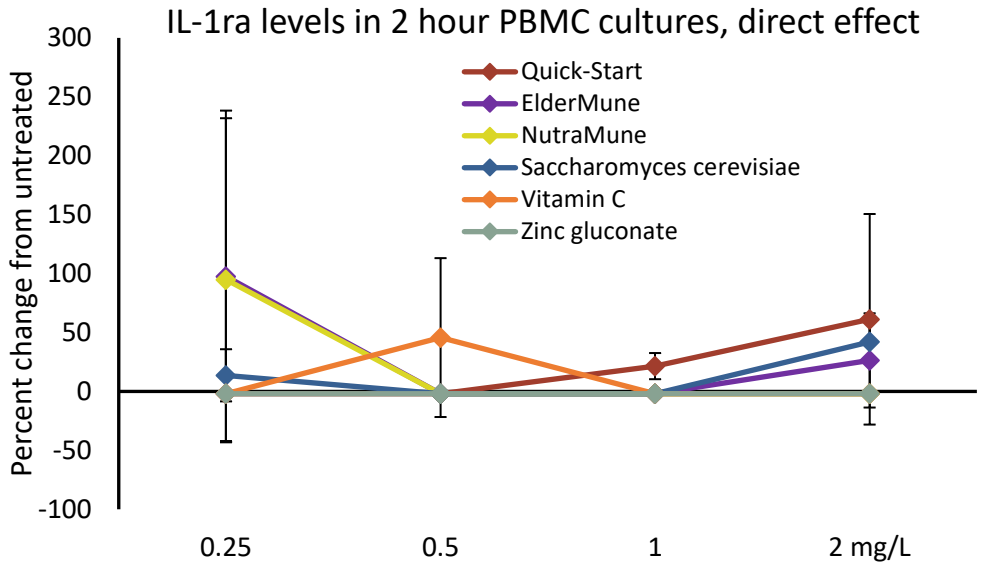
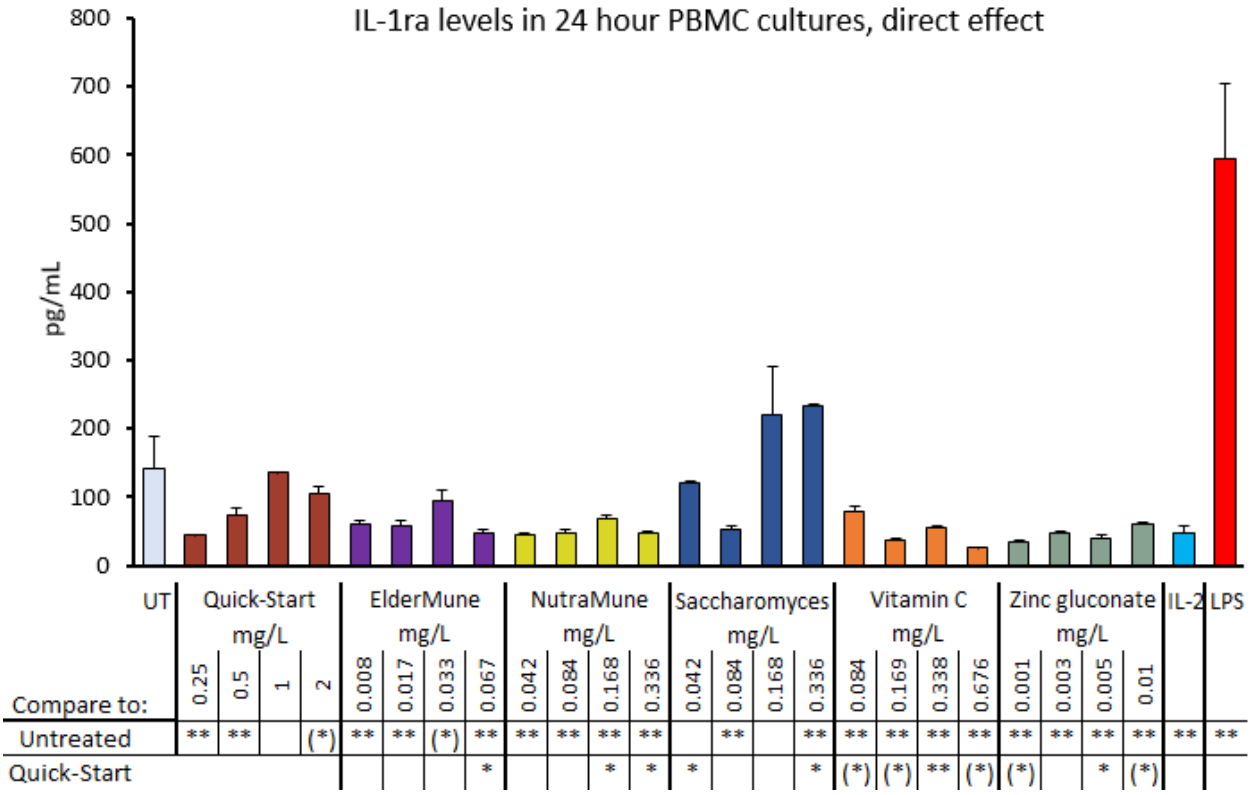


Figure 40. Interleukin 1 receptor antagonist (IL-1ra) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



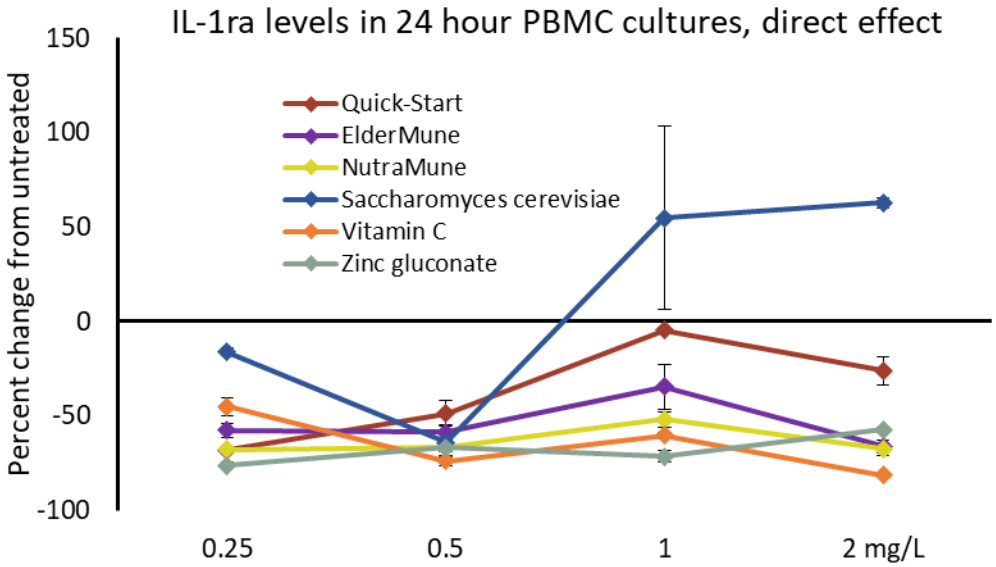
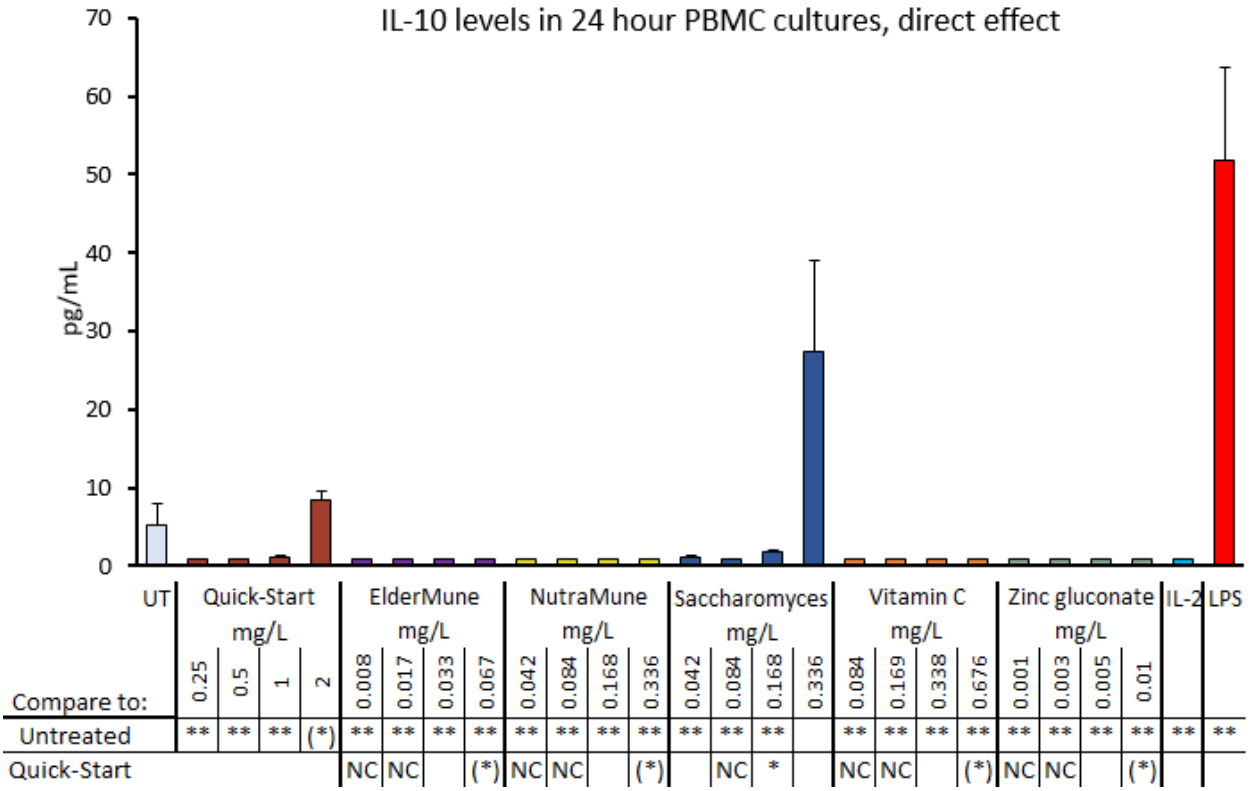


Figure 41. Interleukin 1 receptor antagonist (IL-1ra) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



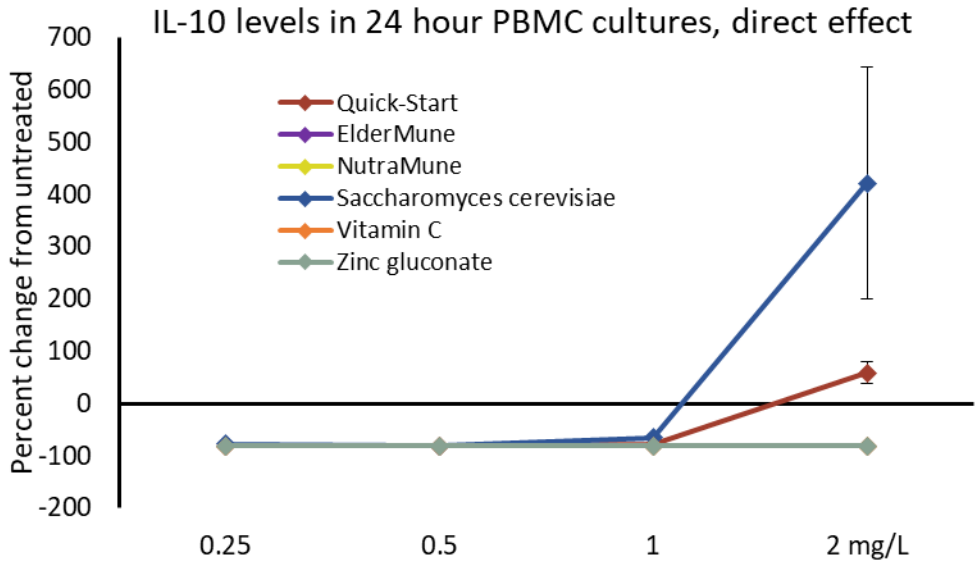
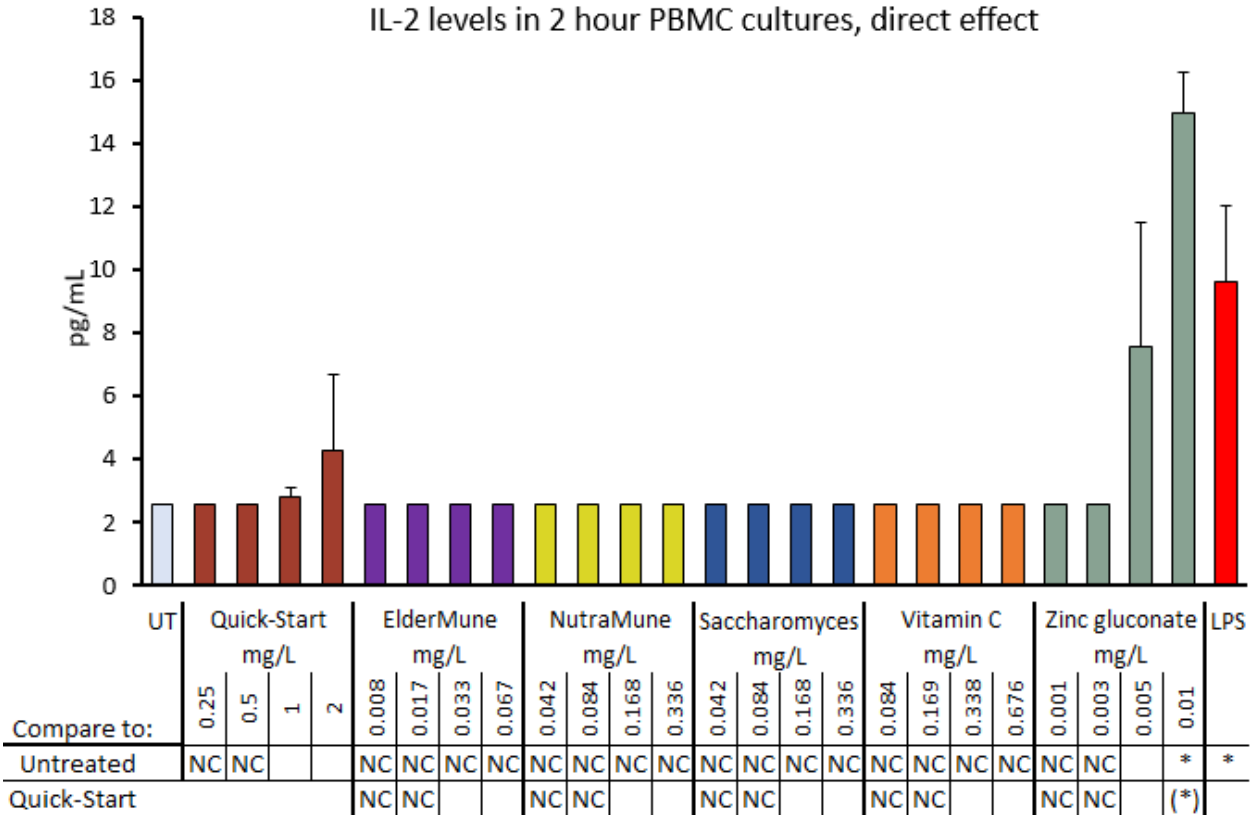


Figure 42. Interleukin 10 (IL-10) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*

5.2.7 Regulating cytokines

Table 6. Cytokines/chemokines with regulating properties.

IL-2	Interleukin 2. Necessary for the growth, proliferation, and differentiation of T cells. Part of the body's natural response to microbial infection. Also important for discriminating between "non-self" and "self".
IL-4	Interleukin 4. Induces naïve T cells (T0) to become Th2. Overproduction associated with allergies.
IL-7	Interleukin 7. Hematopoietic growth factor secreted by stromal cells in the bone marrow and thymus. Stimulates the differentiation of hematopoietic stem cells into lymphoid progenitor cells. Also stimulates proliferation of B cells, T cells and NK cells.
IL-9	Interleukin 9. Cytokine produced by T cells - particularly CD4+ helper cells. Identified as a candidate gene for asthma.
IL-15	Interleukin 15. Secreted by mononuclear phagocytes following infection by virus(es). Induces proliferation of NK cells.



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01	
Untreated		NC	NC			NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC		*	*
Quick-Start						NC	NC			NC	NC			NC	NC			NC	NC			NC	NC		(*)	

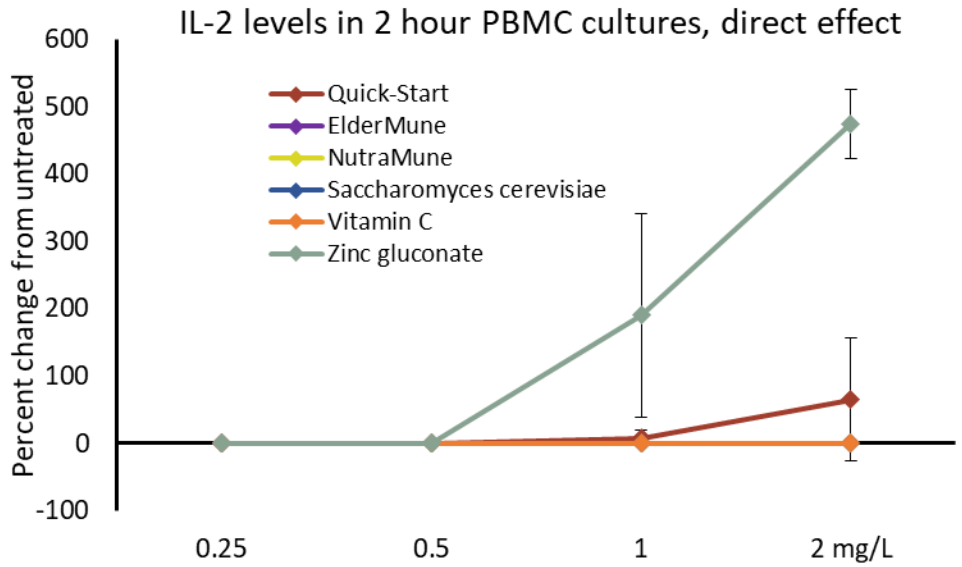
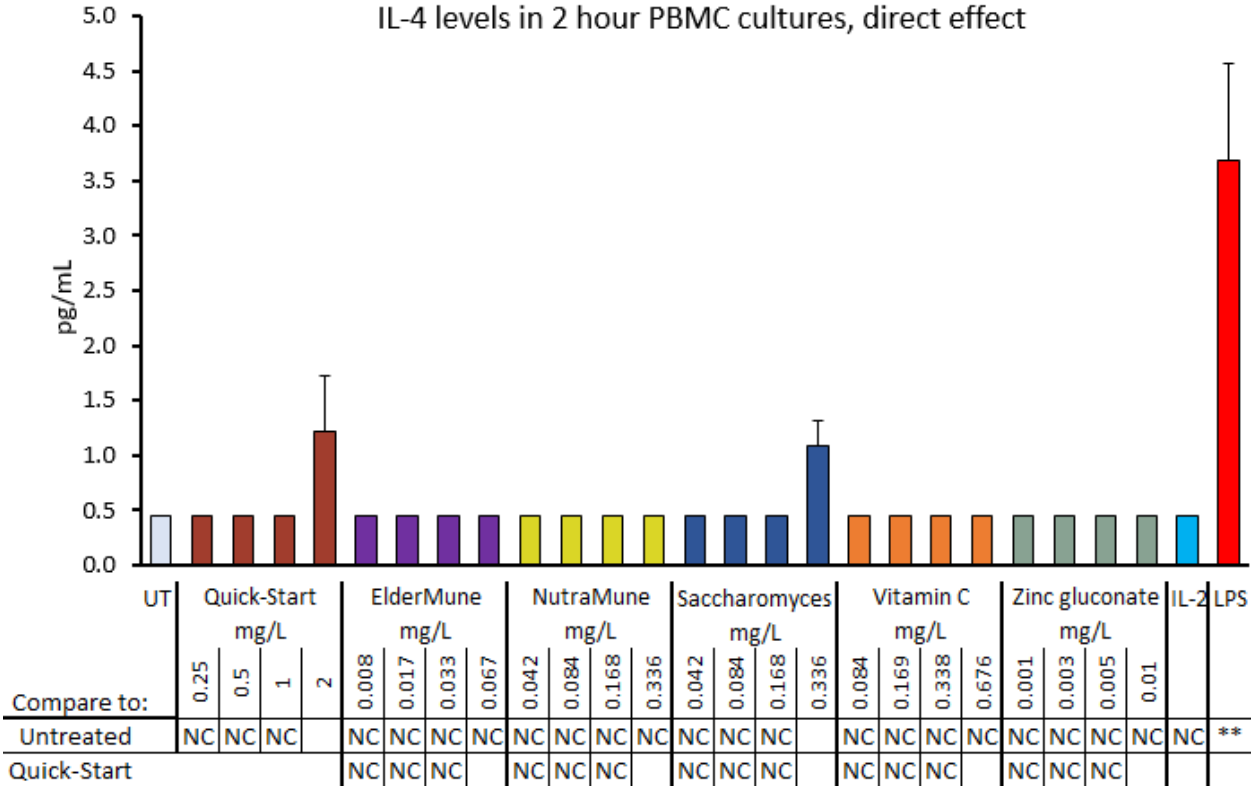


Figure 43. Interleukin 2 (IL-2) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	**
Quick-Start						NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC			

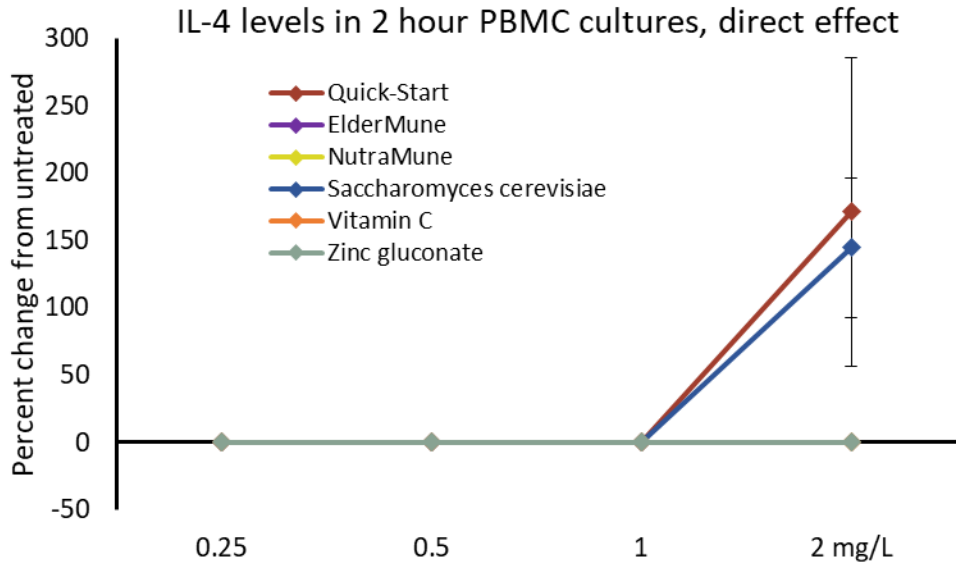
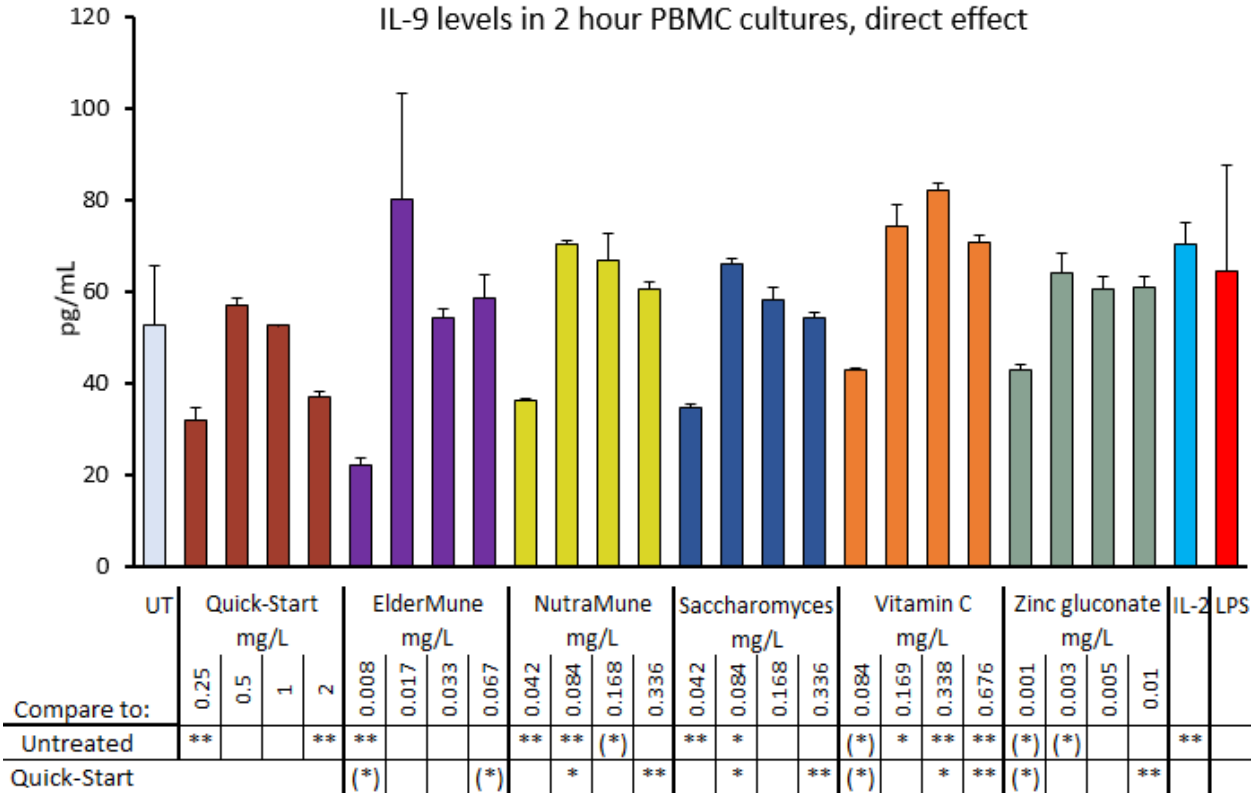


Figure 44. Interleukin 4 (IL-4) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	**				**	**				**	**	(*)		**	*			(*)	*	**	**	(*)	(*)			**	
Quick-Start						(*)			(*)		*		**		*		**	(*)		*	**	(*)			**		

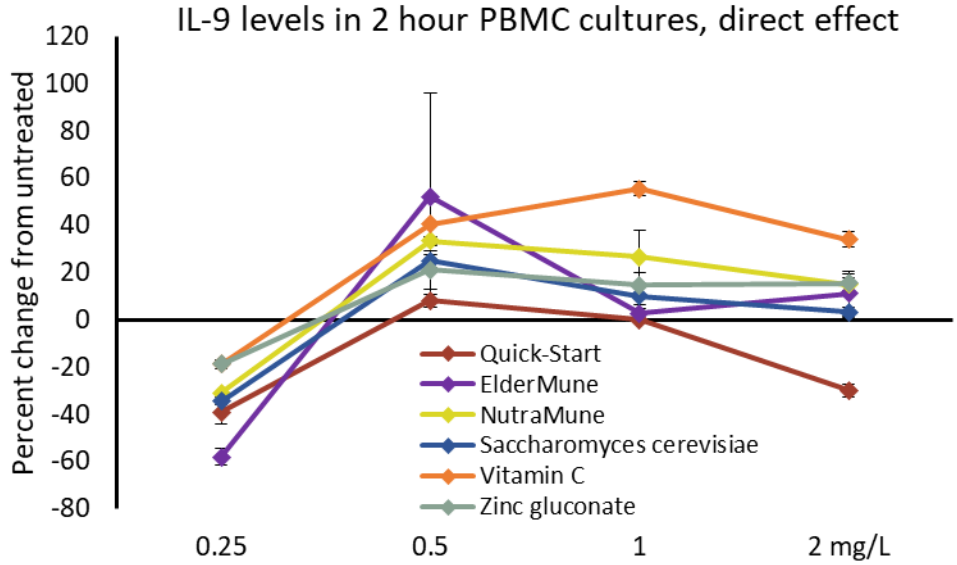
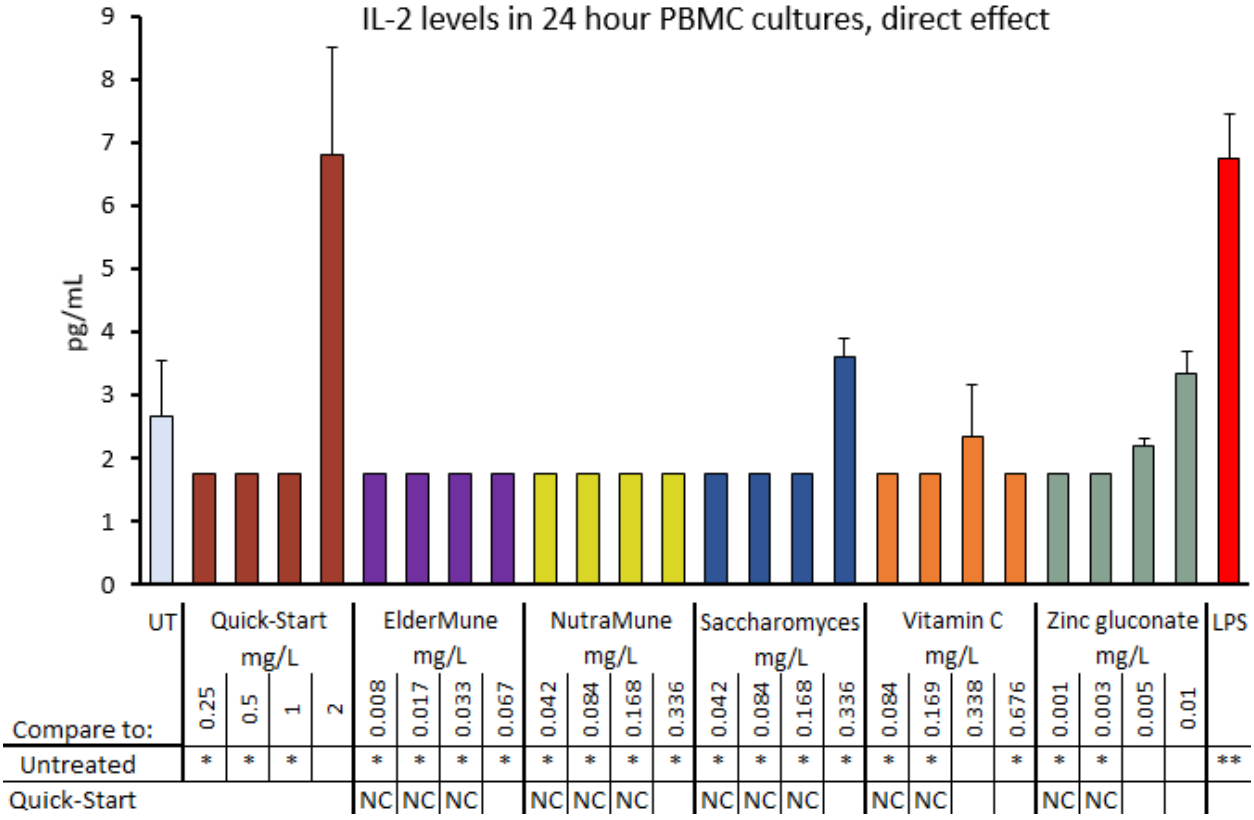


Figure 45. Interleukin 9 (IL-9) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01	
Untreated		*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*			**
Quick-Start						NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC			NC	NC			

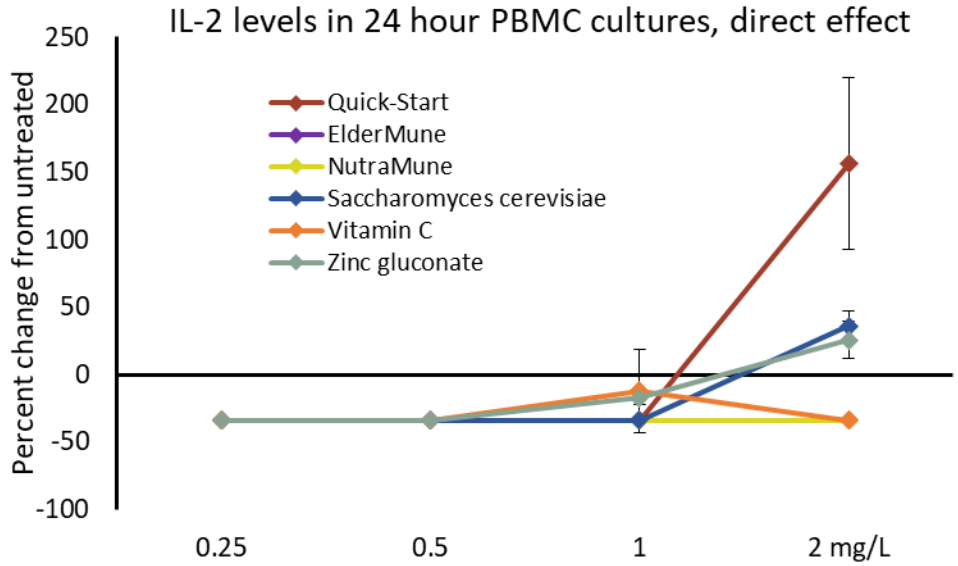
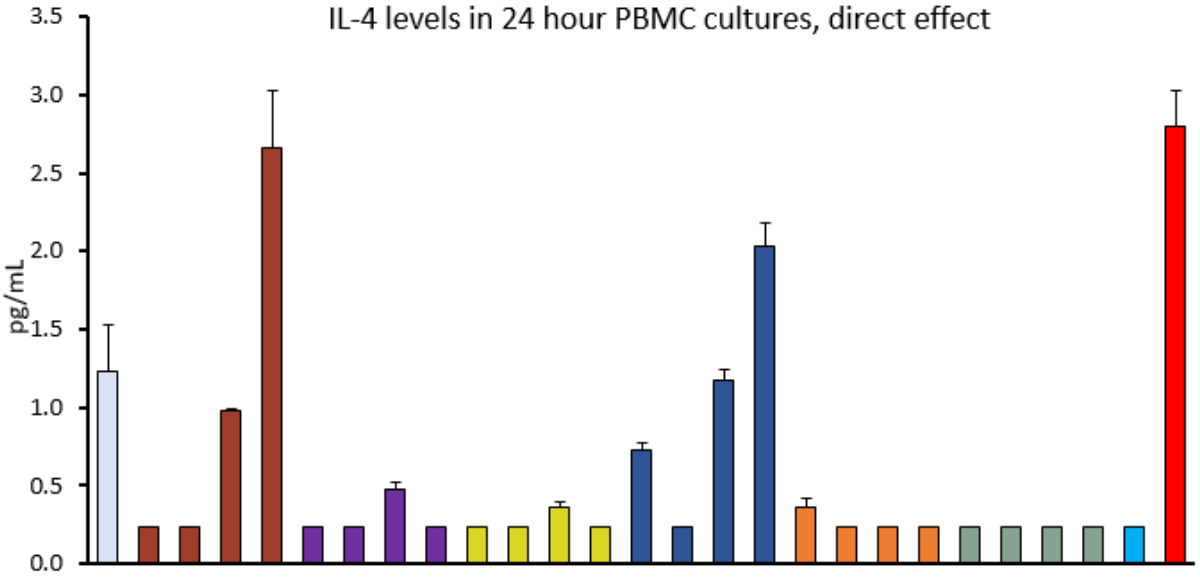


Figure 47. Interleukin 2 (IL-2) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	**	**	**	(*)	(*)	**	**	**	**	**	**	**	**	**	**		*	**	**	**	**	**	**	**	**	**	**
Quick-Start						NC	NC	*	(*)	NC	NC	*	(*)	*	NC				NC	**	(*)	NC	NC	**	(*)		

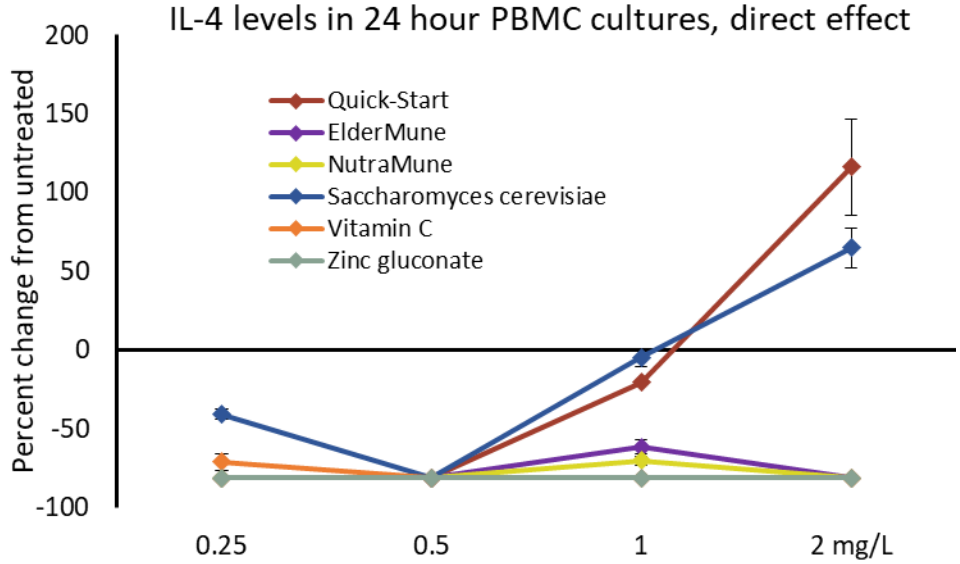
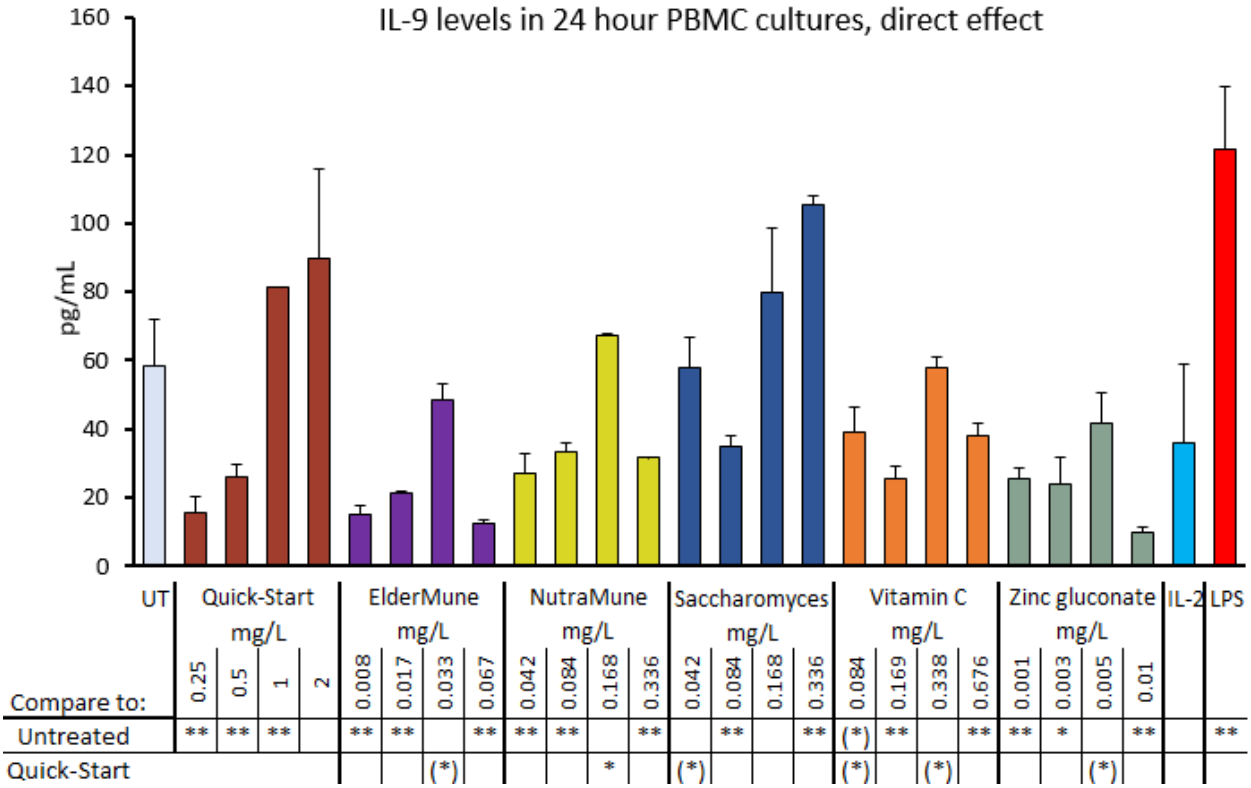


Figure 48. Interleukin 4 (IL-4) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



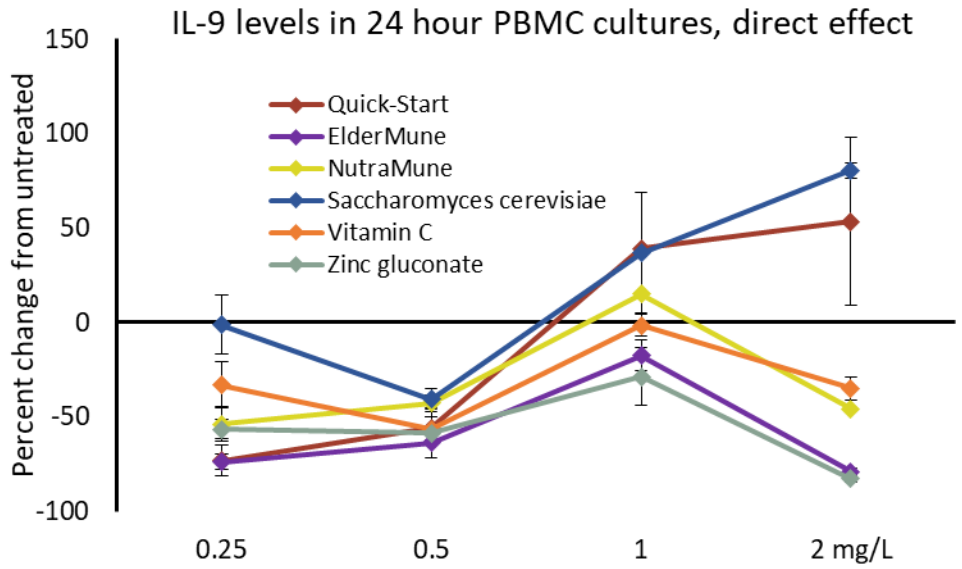
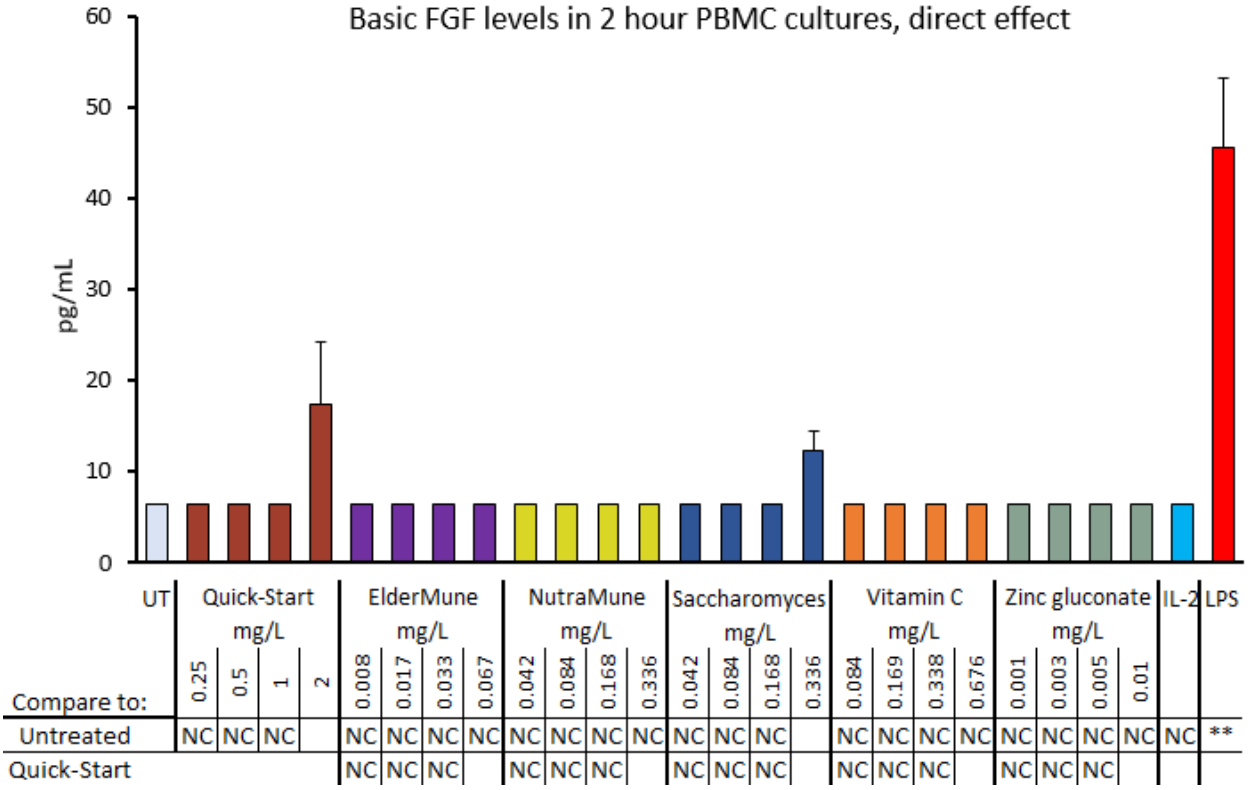


Figure 469. Interleukin 9 (IL-9) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*

5.2.8 Growth factors

Table 8. Growth Factors.

Basic FGF	Basic Fibroblast Growth Factor. Important in angiogenesis and wound healing.
PDGF-BB	Platelet-Derived Growth Factor Subunit Beta. Involved in angiogenesis and is a potent mitogen for cells of mesenchymal origin. Important for wound healing.
VEGF	Vascular Endothelial Growth Factor. Stimulates vasculogenesis and angiogenesis. Serum concentration of VEGG is high in bronchial asthma and diabetes mellitus.
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor. Secreted by macrophages, T cells, mast cells, NK cells, endothelial cells, and fibroblasts. Leukocyte growth factor that stimulates stem cells to produce granulocytes and monocytes. It is part of the immune/inflammatory cascade by leading to the activation of monocytes.
G-CSF	Granulocyte Colony-Stimulating Factor. Promotes proliferation of neutrophils. Known to mobilize endogenous stem cells involved in reparative and regenerative functions.



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated		NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	**
Quick-Start						NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC			

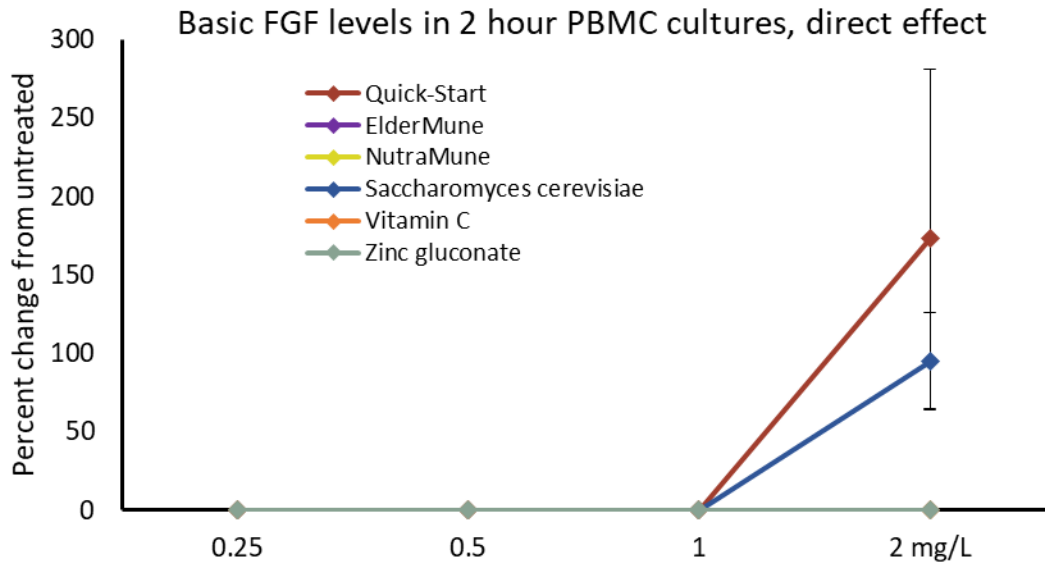
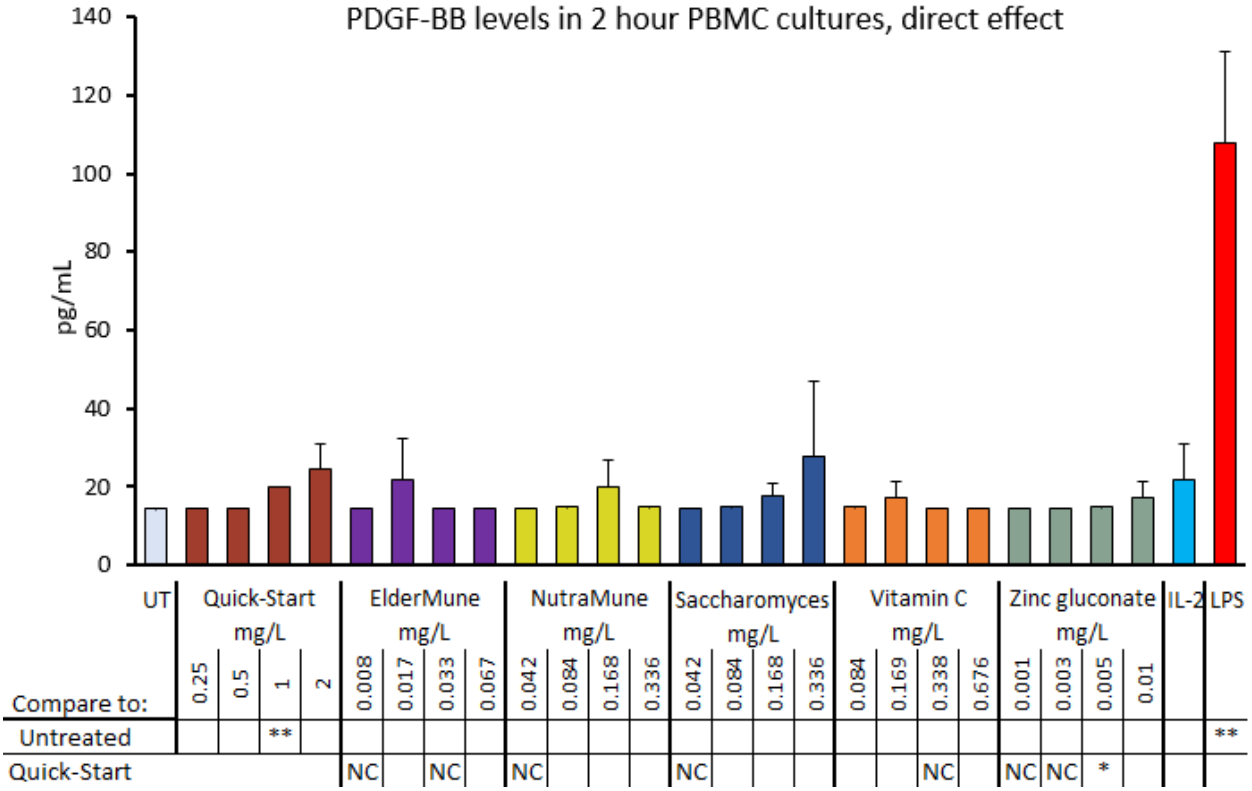


Figure 50. Basic fibroblast growth factor (Basic FGF) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



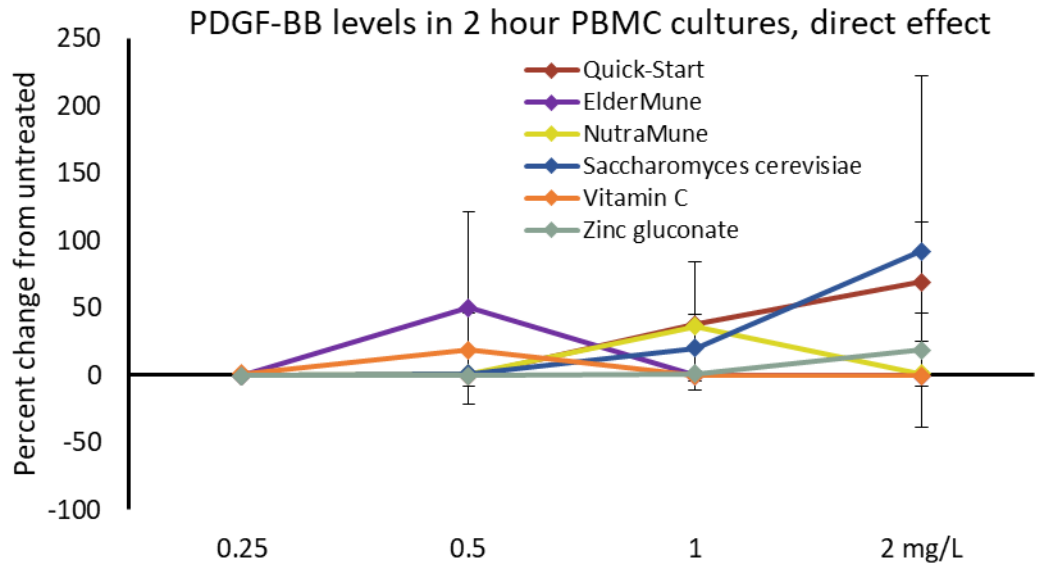
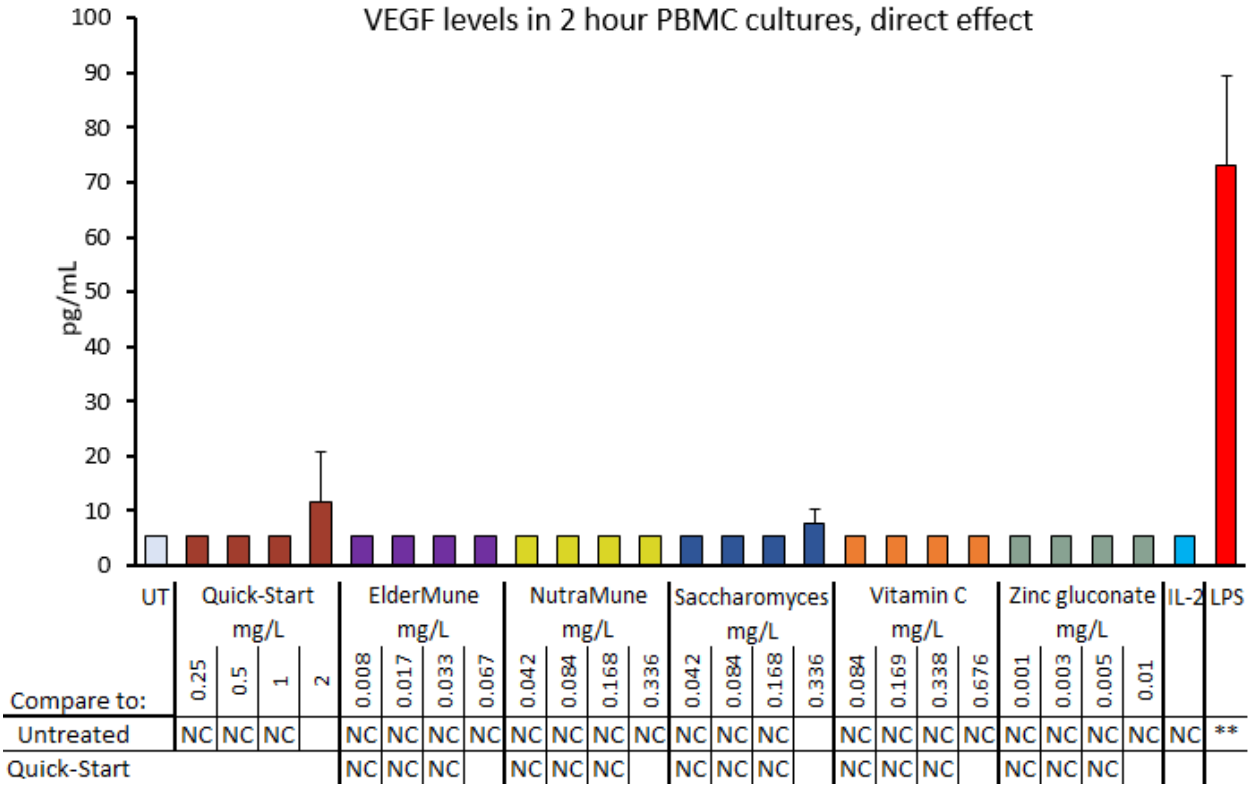


Figure 5147. Platelet-derived growth factor subunit beta (PDGF-BB) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated		NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC		NC	NC	NC	NC	NC	NC	NC	NC	NC	**
Quick-Start						NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC		NC	NC	NC			

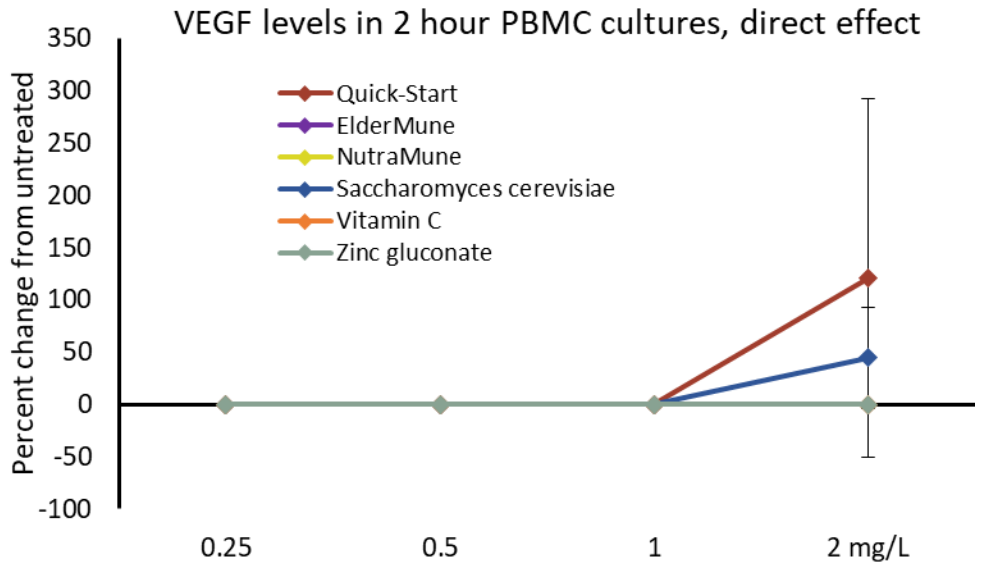
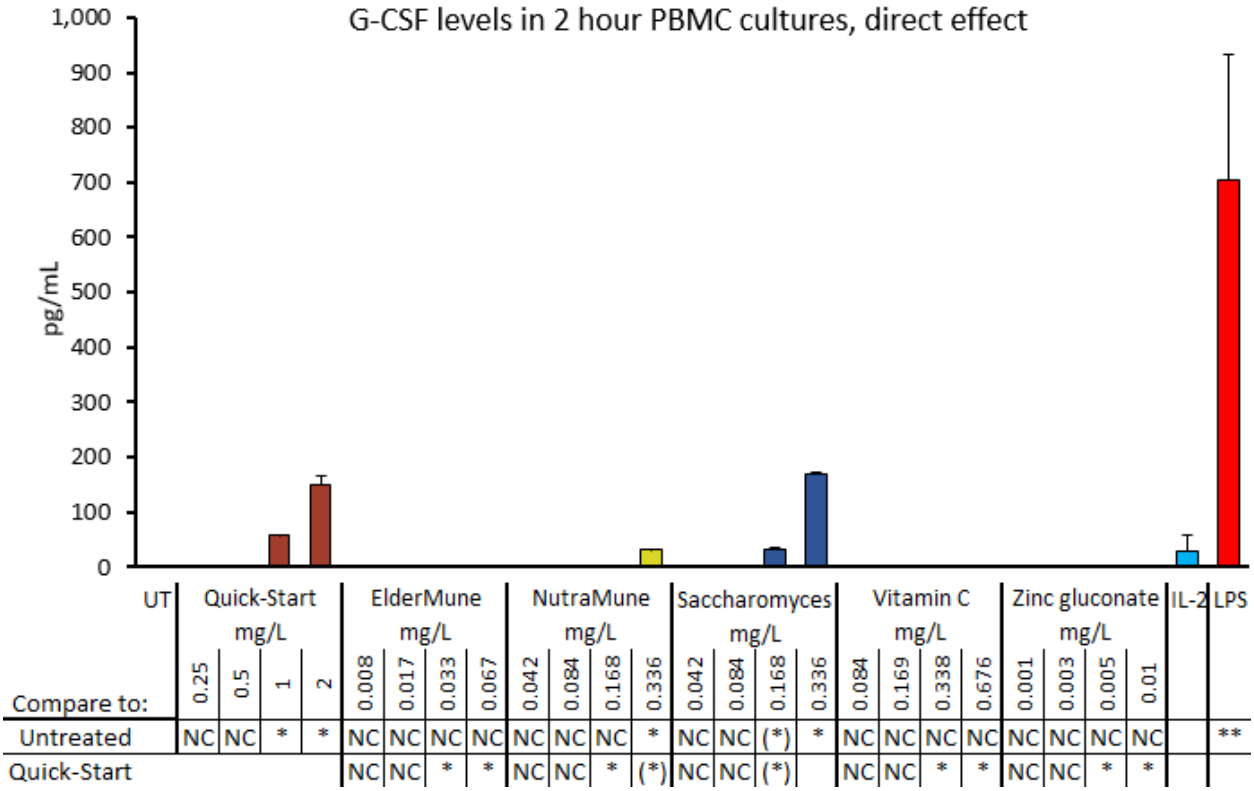


Figure 52. Vascular endothelial growth factor (VEGF) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



Compare to:	UT	Quick-Start mg/L 0.25	Quick-Start mg/L 0.5	Quick-Start mg/L 1	Quick-Start mg/L 2	ElderMune mg/L 0.008	ElderMune mg/L 0.017	ElderMune mg/L 0.033	ElderMune mg/L 0.067	NutraMune mg/L 0.042	NutraMune mg/L 0.084	NutraMune mg/L 0.168	NutraMune mg/L 0.336	Saccharomyces mg/L 0.042	Saccharomyces mg/L 0.084	Saccharomyces mg/L 0.168	Saccharomyces mg/L 0.336	Vitamin C mg/L 0.084	Vitamin C mg/L 0.169	Vitamin C mg/L 0.338	Vitamin C mg/L 0.676	Zinc gluconate mg/L 0.001	Zinc gluconate mg/L 0.003	Zinc gluconate mg/L 0.005	Zinc gluconate mg/L 0.01	IL-2	LPS
Untreated	NC	NC	*	*	NC	NC	NC	NC	NC	NC	NC	*	NC	NC	(*)	*	NC	NC	NC	NC	NC	NC	NC	NC		**
Quick-Start					NC	NC	*	*	NC	NC	*	(*)	NC	NC	(*)		NC	NC	*	*	NC	NC	*	*		

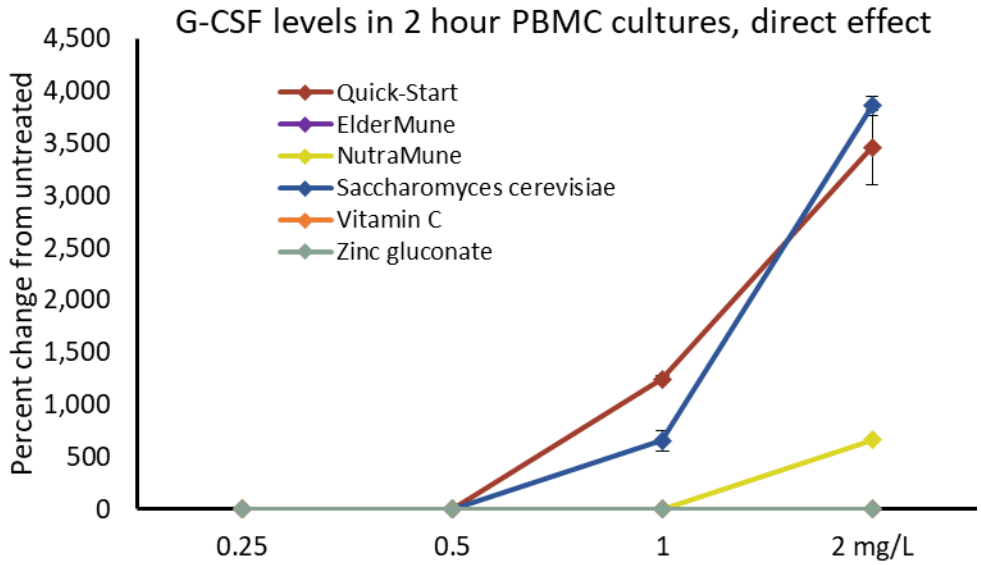
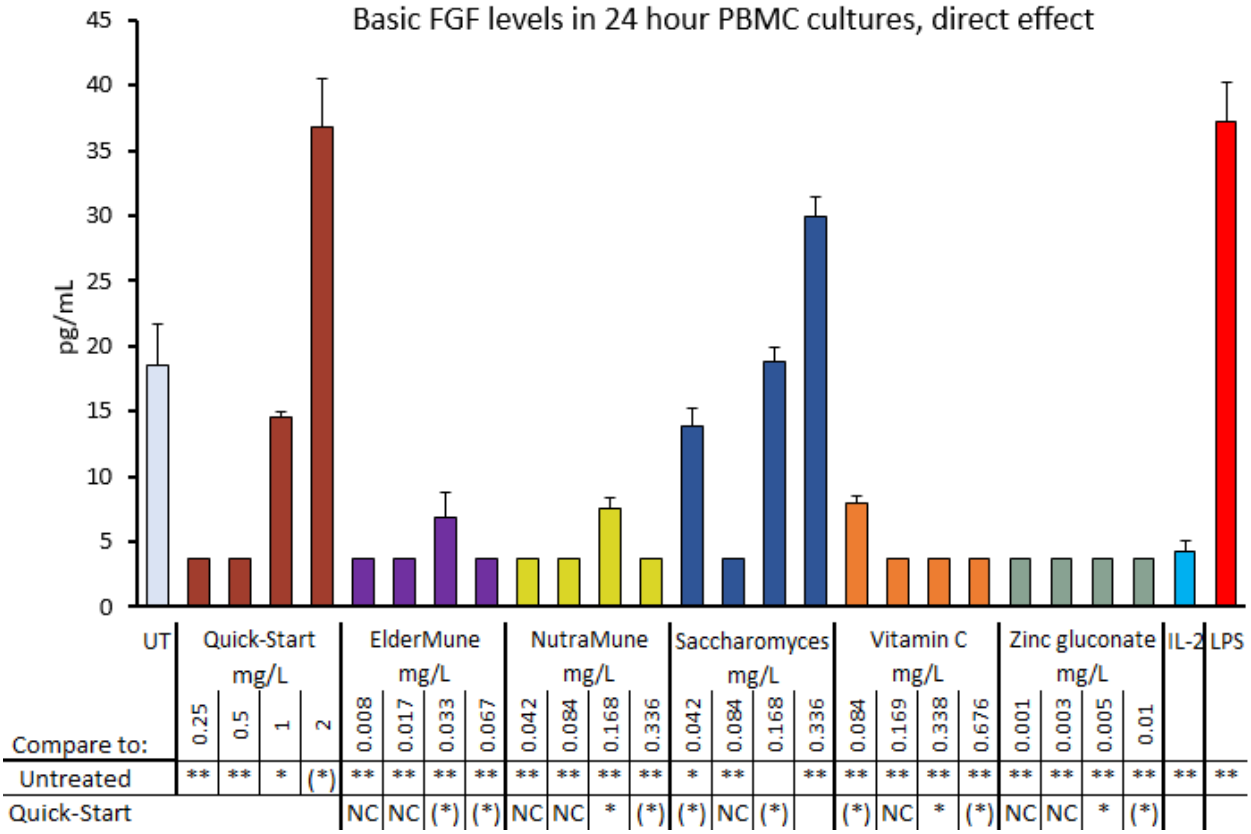


Figure 53. Granulocyte colony-stimulating factor (G-CSF) levels in 2-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.*
Bottom: *Results are shown as the average ± standard deviation of the percent change from untreated.*



	UT	Quick-Start mg/L				ElderMune mg/L				NutraMune mg/L				Saccharomyces mg/L				Vitamin C mg/L				Zinc gluconate mg/L				IL-2	LPS
Compare to:		0.25	0.5	1	2	0.008	0.017	0.033	0.067	0.042	0.084	0.168	0.336	0.042	0.084	0.168	0.336	0.084	0.169	0.338	0.676	0.001	0.003	0.005	0.01		
Untreated	**	**	**	*	(*)	**	**	**	**	**	**	**	**	*	**		**	**	**	**	**	**	**	**	**	**	**	**
Quick-Start						NC	NC	(*)	(*)	NC	NC	*	(*)	(*)	NC	(*)		(*)	NC	*	(*)	NC	NC	*	(*)			

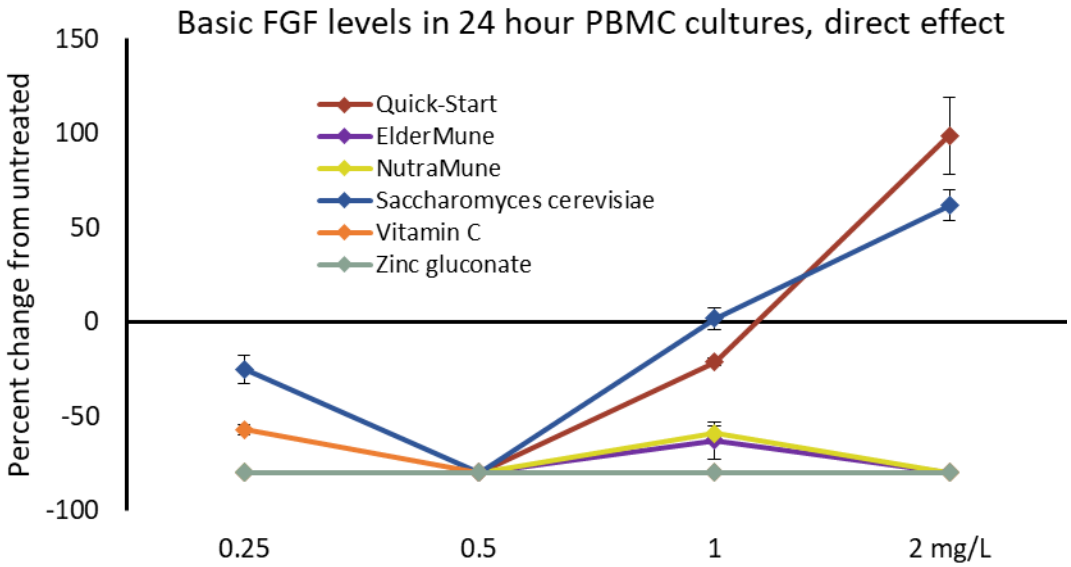
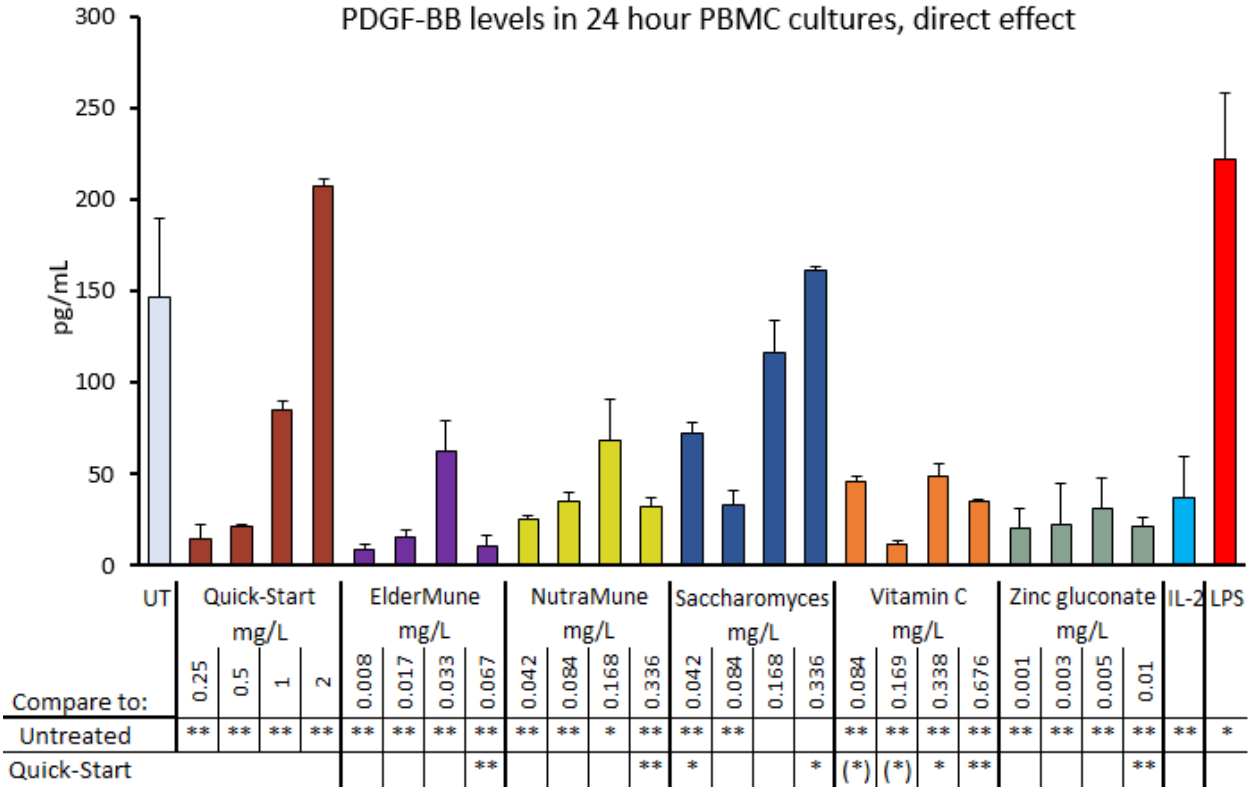


Figure 54. Basic fibroblast growth factor (Basic FGF) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



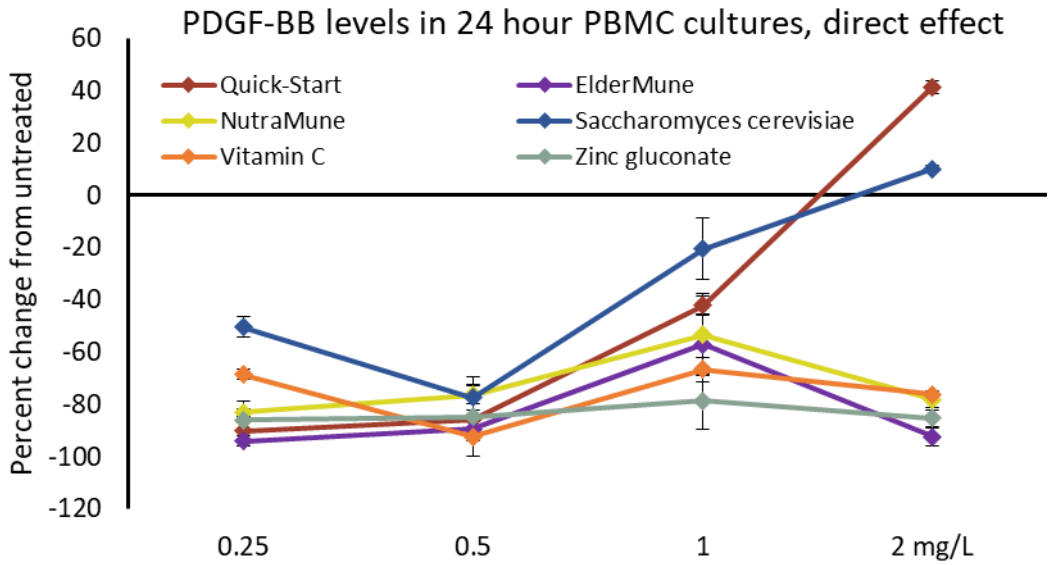
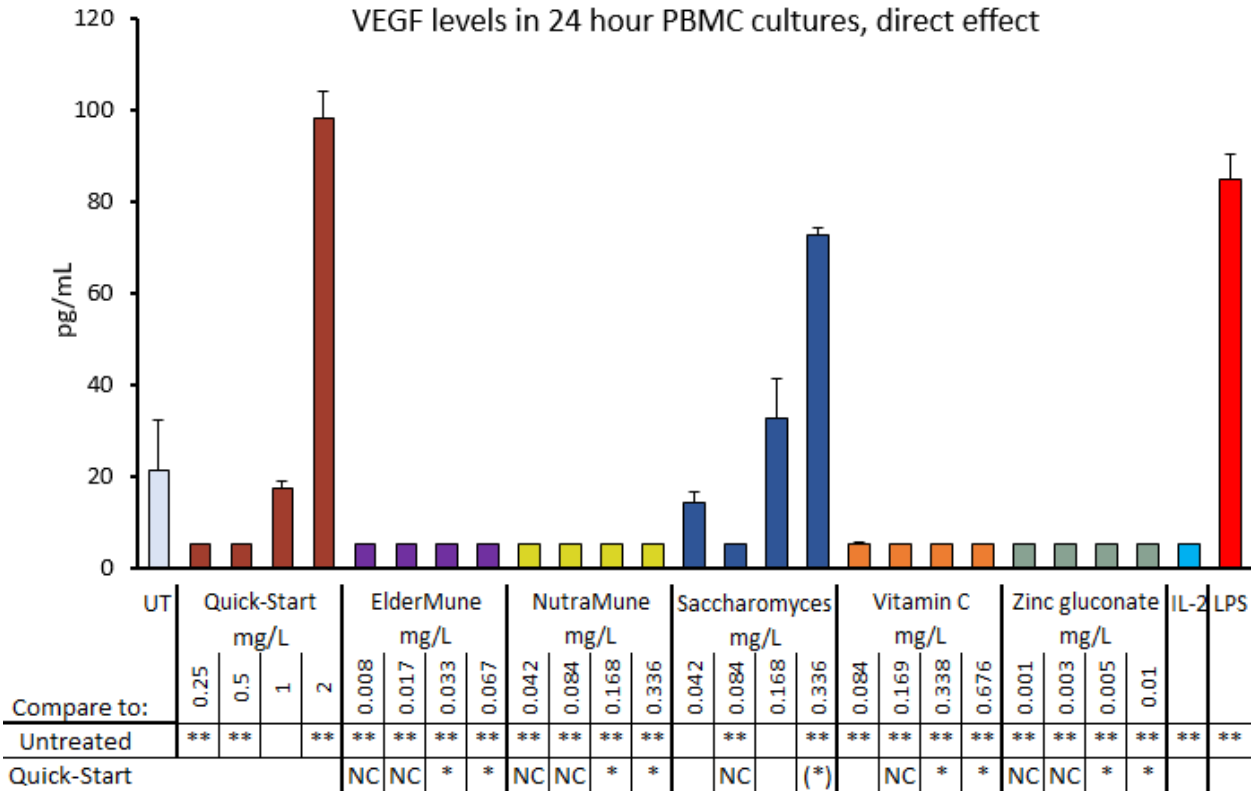


Figure 55. Platelet-derived growth factor subunit beta (PDGF-BB) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: * and P<0.01: **.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



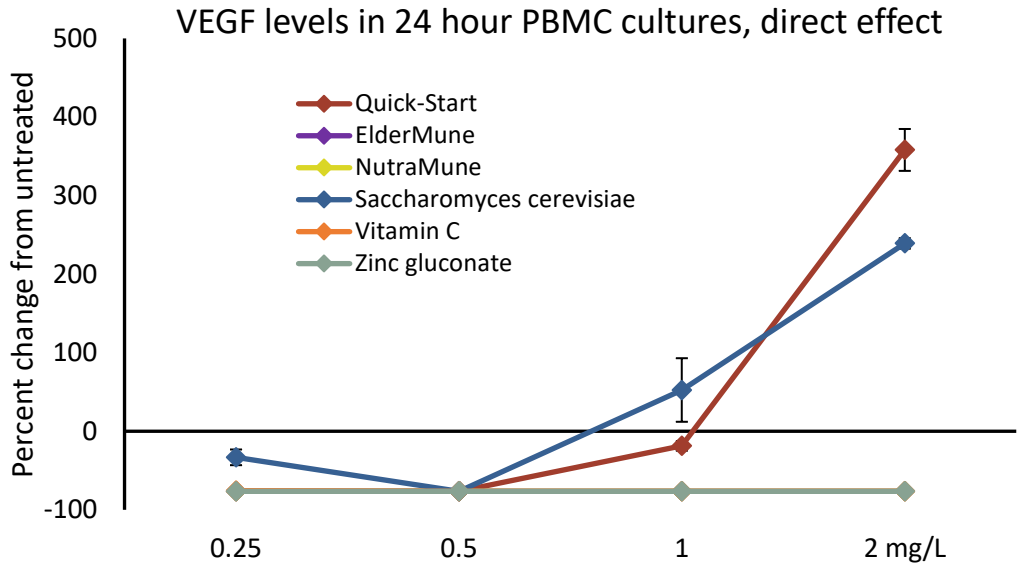
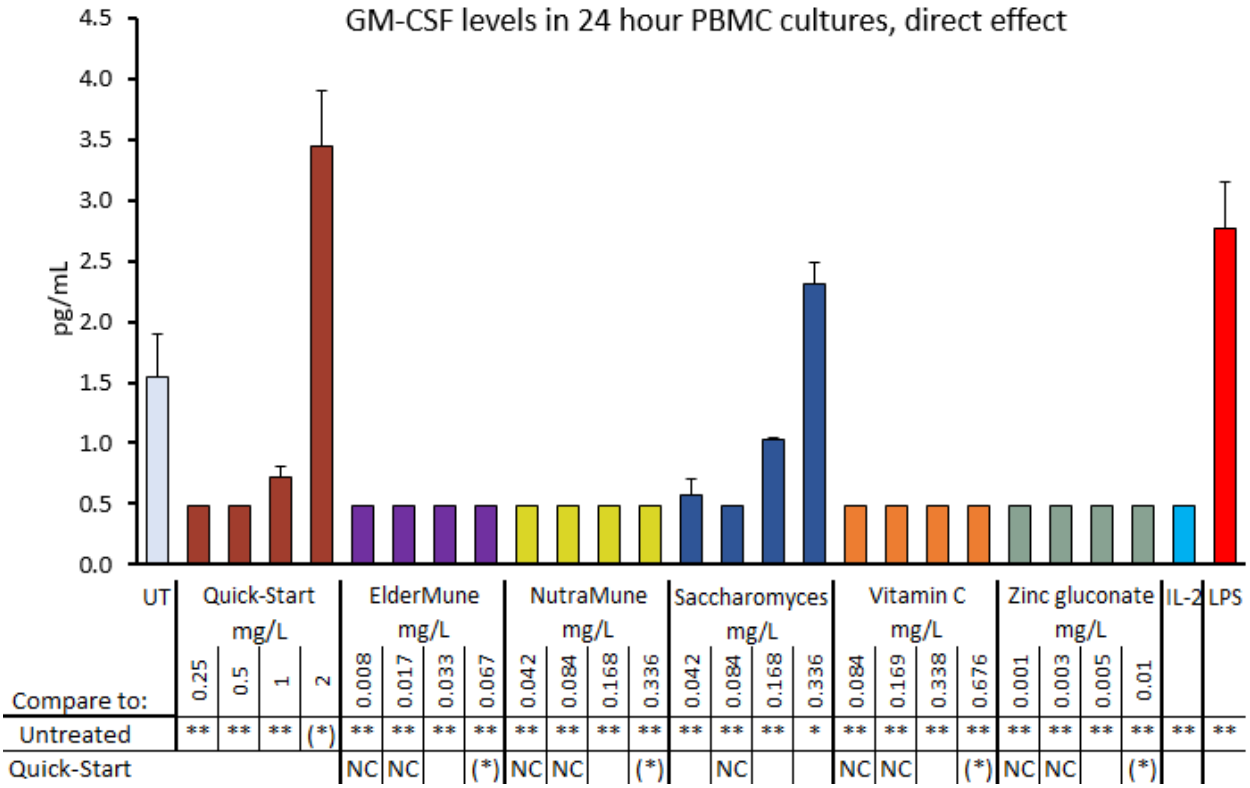


Figure 56. Vascular endothelial growth factor (VEGF) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.*
Bottom: *Results are shown as the average ± standard deviation of the percent change from untreated.*



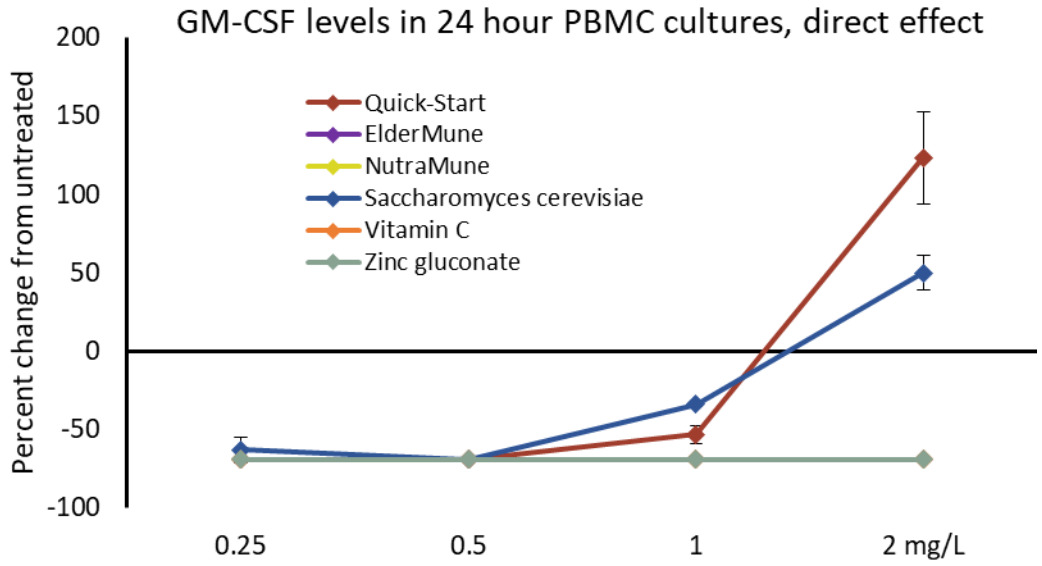
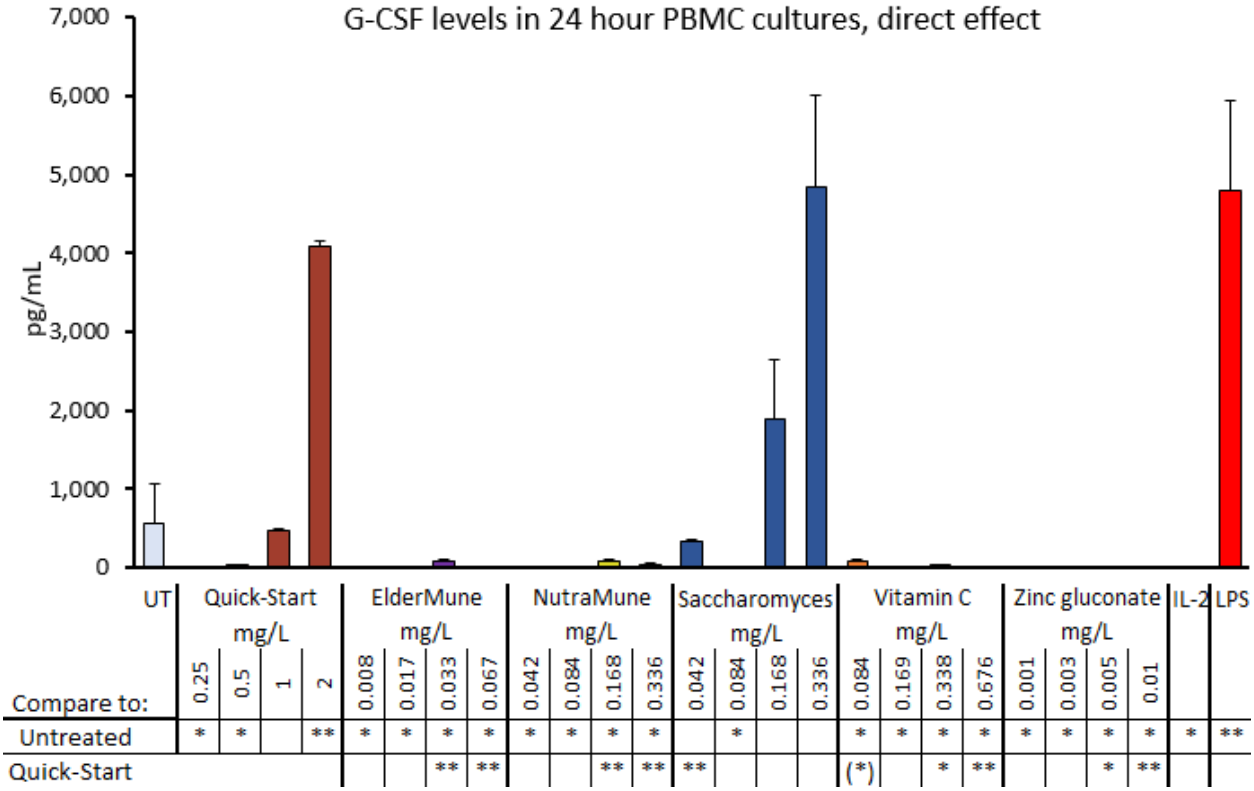


Figure 57. Granulocyte-macrophage colony-stimulating factor (GM-CSF) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*



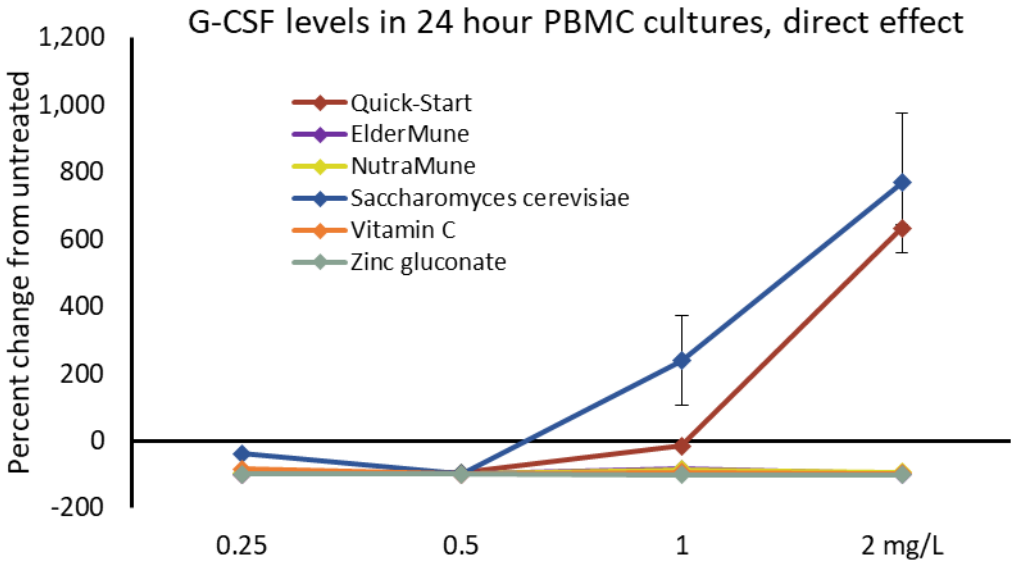


Figure 58. Granulocyte colony-stimulating factor (G-CSF) levels in 24-hour PBMC cultures treated with the test products under normal culture conditions. ***Top:*** *Results are shown as the average ± standard deviation of duplicate samples. Statistical significance at different doses is indicated by asterisks, when P<0.10: (*), P<0.05: *, P<0.01: ** and NC when not calculated.* ***Bottom:*** *Results are shown as the average ± standard deviation of the percent change from untreated.*

6 Conclusions

The nutraceutical formulation **Quick-Start** showed rapid and robust immune activating and modulating effects in vitro. The effects showed contributions from all **5 core ingredients** that were tested in parallel.

Model

As a cellular model for this work, we used freshly isolated peripheral blood mononuclear cells (PBMC) from a healthy blood donor. The PBMC cellular fraction contains multiple types of immune cells, designed to collaborate to produce a meaningful immune response to potential pathogens. The cell types include NK cells, NKT cells, T lymphocytes, and a broadly defined group of cells that did not stain for NK cell or T cell markers (non-NK non-T cells), and including dendritic cells, B lymphocytes, and stem cells.

Immune cell activation

The treatment of immune cells with Quick-Start resulted in an increase of the CD69 activation marker on NK cells, NKT cells, non-NK, non-T cells, and monocytes. This increased expression on NK cand NKT cells translate to increased alertness towards recognizing and killing target cells (i.e., cancer cells and virally transformed cells). The increased CD69 expression was seen in parallel to a reduction in CD25 expression, indicating that the NK cells were in a higher state of cytotoxicity and a reduced state of cell proliferation, focusing the effort on immune recognition.

Cytokines and growth factors

The treatment of immune cells with Quick-Start triggered rapid induction of multiple cytokines, chemokines, and growth factors already after 2 hours. Later, in the cell culture period, the initial burst of cytokine production changed, and some cytokines continued to be produced at a high level, whereas other cytokines were reduced compared to untreated control cultures. Several cytokines showed a complex dose response to Quick-Start after 24 hours, suggesting strong and prolonged immune cell activation at higher doses and anti-inflammatory and regulating effects at lower doses.

Synergy between ingredients

The effects of Quick-Start were showing contributions from all 5 ingredients that we tested in parallel. However, there were clear indications as to which ingredients contributed the most robust effects to the overall synergistic effect of Quick-Start on human immune cells:

- **Saccharomyces** was the leading ingredient, showing the most robust contribution, and showing an effect for the most cytokine biomarkers.

    - This included multiple immune-activating pro-inflammatory cytokines, one anti-inflammatory cytokine, and several growth factors.
    - The stem-cell related growth factor G-CSF was rapidly up-regulated by Saccharomyces, which is promising for a possible rapid effect after consumption stem cell release and reparative functions.
- **Vitamin C** was the second-leading ingredient for induction of immune cell activation.
    - Vitamin C specifically contributed to effects on the activation marker CD69 on NK and NKT cells.
    - Vitamin C also contributed to the rapid 2-hour increase in multiple cytokines, including MIP-1β, RANTES, and IL-9.
- **NutraMune** contributed to a mild increase in expression of CD69 on immune cells.
- **Zinc** contributed to the downregulation of the CD25 T cell growth factor receptor on NKT cells and monocytes, further enhancing the shift in cellular function from cell division to cell alertness. Zinc also contributed to the induction of multiple cytokines.
- **ElderMune** contributed to regulating effects of several cytokines with bi-phasic dose responses, including IL-9 and RANTES, suggesting a more complex regulating role for this elderberry extract in the overall effects of the blend Quick-Start.

7 Further work

The following strategy may be implemented:

1. **In vitro validation:** The results reported here show unique synergy between the ingredients in Quick-Start. The work was performed on cell from 1 healthy blood donor, and validation repeats are needed if you wish to pursue the writing of a manuscript for peer-review.
2. **Additional in vitro immune cell testing**: The effects of Quick-Start and its 5 leading ingredients will be tested in the laboratory in the context of bacterial and viral inflammatory challenges. **Note: It may be ideal to combine points 1 and 2 for a more comprehensive manuscript.**
3. **Clinical testing**: The rapid efficacy of the blend will be documented in a small, randomized, cross-over, placebo-controlled cross-over trial in healthy people. This may

be done in a modular fashion, starting with 4 participants, and expanding with more people to strive for a data set suitable for manuscript writing. **Note: If the data are of sufficient volume and quality this may warrant a second, stand-alone manuscript on Quick-Start**

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