

Supplementary material

Prohealth and anticancer activity of fungal fractions isolated from milk-supplemented cultures of *Pleurotus sajor-caju*

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Table S1. Biochemical composition of the cow whole milk and the studied extracts of *Pleurotus sajor-caju*.

Proteins [μg/mL]					
Milk	PSC1-I	PSC1-II	PSC2-I	PSC3-II	PSC4-II
523.3	238.3	508.3	802.9	654.2	550.5
±	±	±	±	±	±
5.3	36.7	9.1	16.6	12.4	38.4
Amino acids [μg/mL]					
Milk	PSC1-I	PSC1-II	PSC2-I	PSC3-II	PSC4-II
509.5	452.4	90.6	498.9	492.4	37.5
±	±	±	±	±	±
0.7	0.1	0.4	0.2	0.5	0.3
Carbohydrates [μg/mL]					
Milk	PSC1-I	PSC1-II	PSC2-I	PSC3-II	PSC4-II
69.5	42.0	19.7	188.6	117.9	54.4
±	±	±	±	±	±
0.1	0.2	0.4	0.8	0.5	0.2
Phenolic compounds [μg/mL]					
Milk	PSC1-I	PSC1-II	PSC2-I	PSC3-II	PSC4-II
0.0016	0.0447	0.0175	1.9068	0.1749	1.9532
±	±	±	±	±	±
0.0001	0.0004	0.0003	0.0121	0.0043	0.0029
Free radicals [%]					
Milk	PSC1-I	PSC1-II	PSC2-I	PSC3-II	PSC4-II
0.446	0.987	0.357	0.098	0.144	0.124
±	±	±	±	±	±
0.003	0.007	0.002	0.001	0.000	0.001

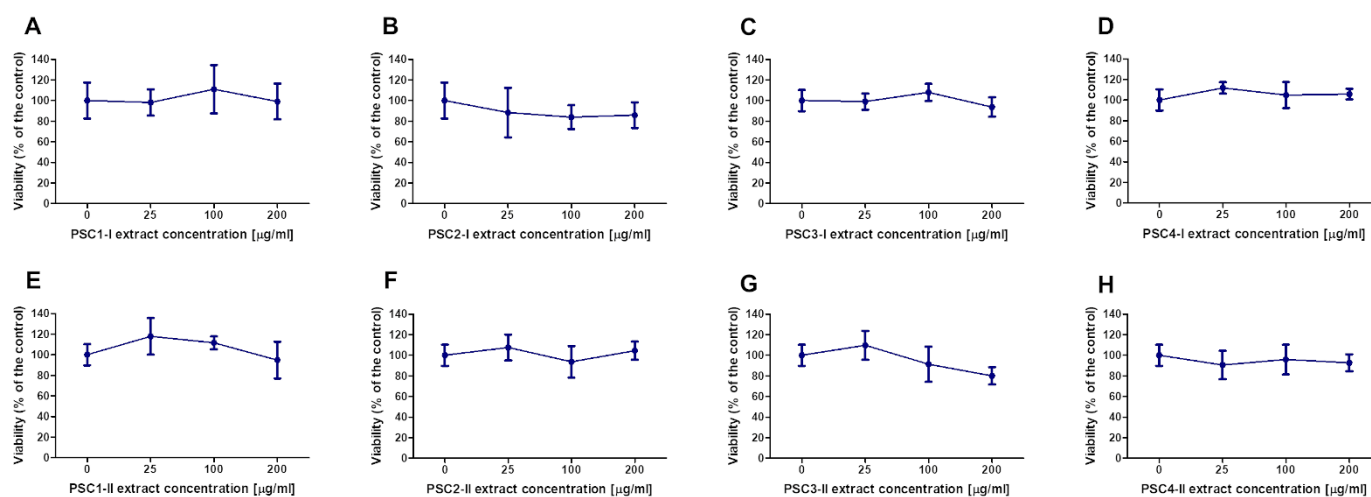


Figure S1. Results of screening PSC extracts on cells – cytotoxicity of PSC1-I (A), PSC2-I (B), PSC3-I (C), PSC4-I (D), PSC1-II (E), PSC2-II (F), PSC3-II (G), PSC4-II (H) on HT-29 cells measured with NR method. For the further analyses, PSC2-I, PSC3-II and PSC4-II were selected due to the strongest effect on HT-29 and the weakest cytotoxicity towards CCD 841 CoTr cell (not shown).

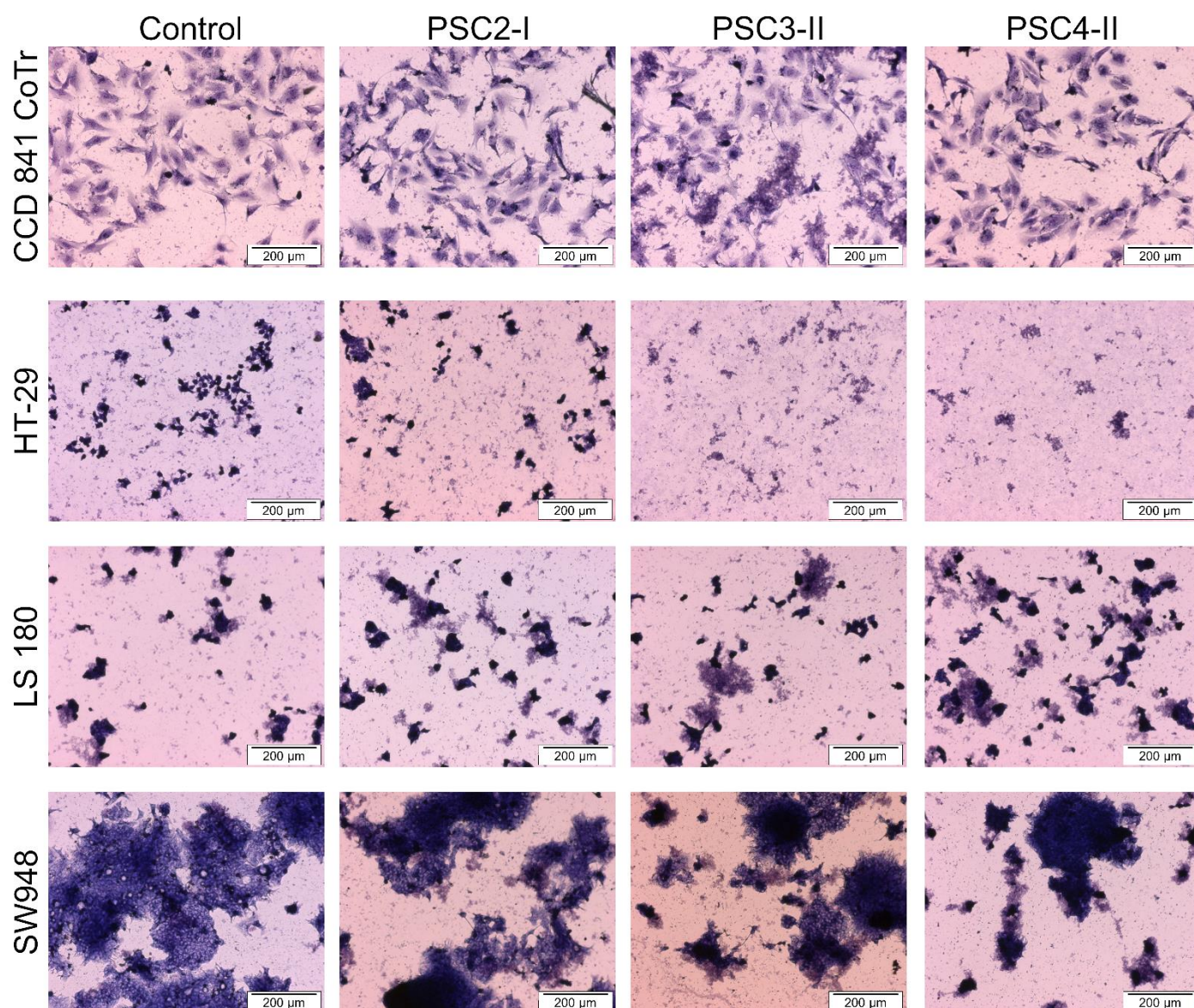


Figure S2. Anti-cancer effect of the PSC2-I, PSC3-II and PSC4-II extracts on CCD 841 CoTr, HT-29, LS 180 and SW948 cells: effect of the extracts on cell morphology – May-Grünwald- Giemsa staining.

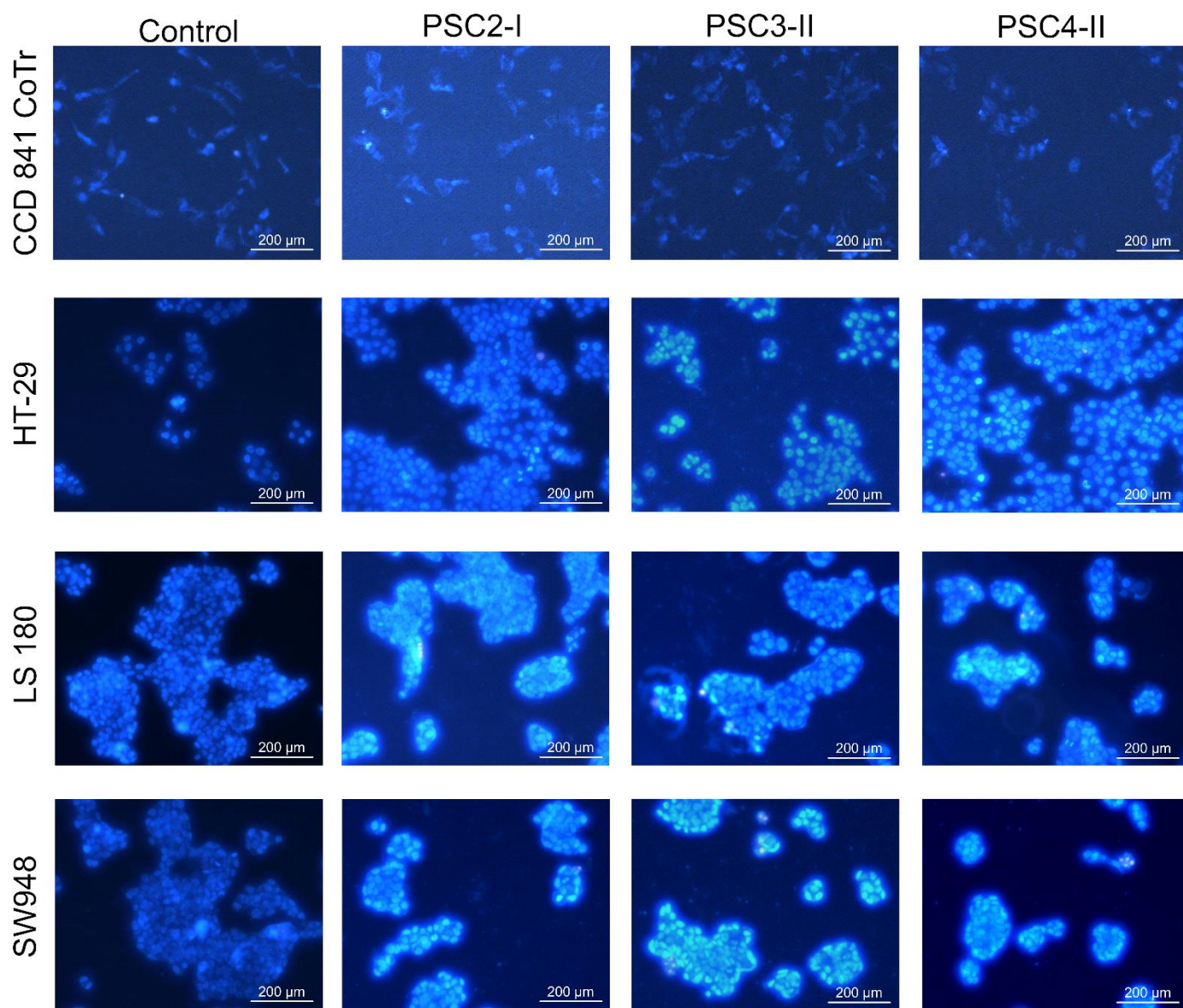


Figure S3. Anti-cancer effect of the PSC2-I, PSC3-II and PSC4-II extracts on CCD 841 CoTr, HT-29, LS 180 and SW948 cells: pro-apop-totic effect of the extracts.

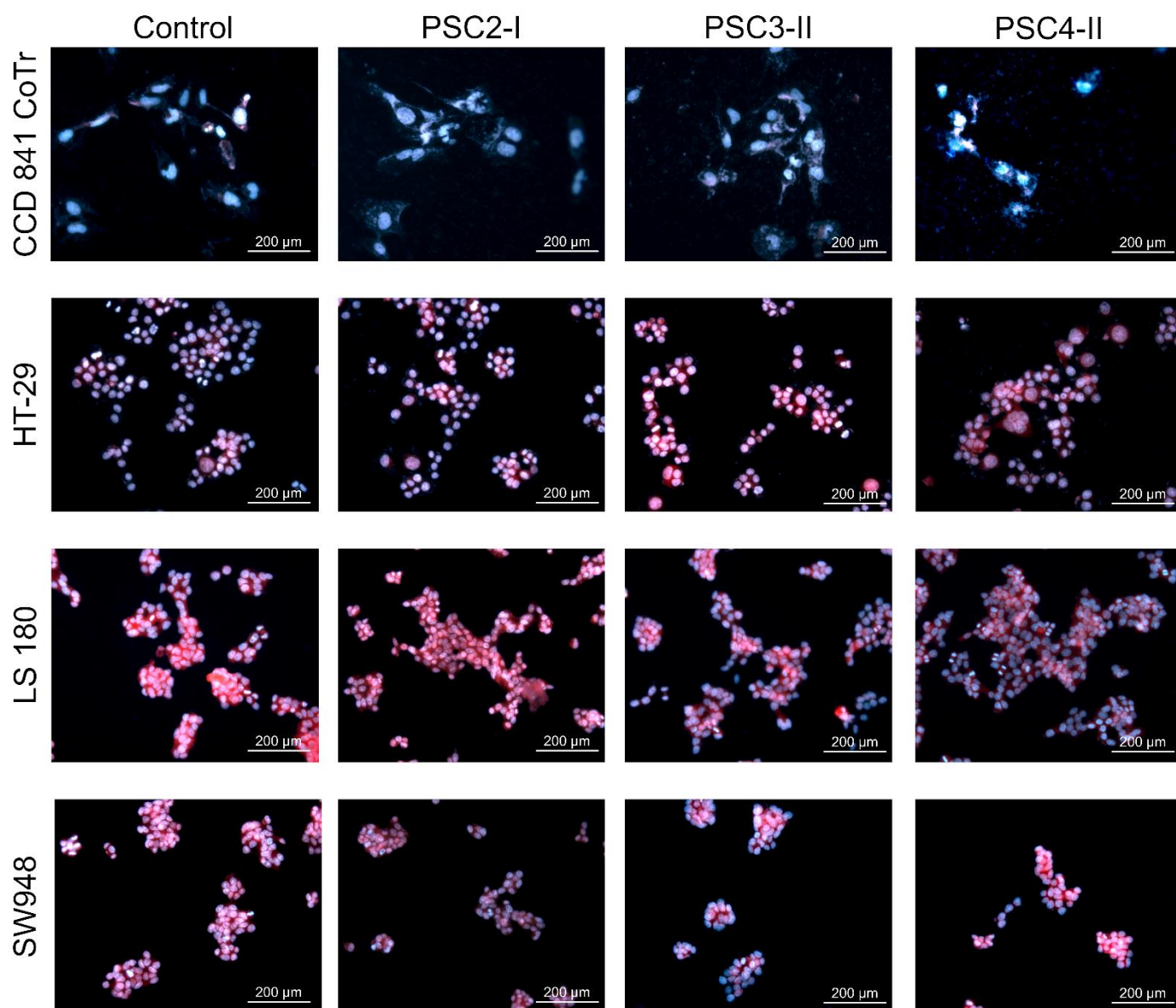


Figure S4. Anti-cancer effect of the PSC2-I, PSC3-II and PSC4-II extracts on CCD 841 CoTr, HT-29, LS 180 and SW948 cells: effect on the morphology of the cells and level of the proteins measured with DAPI/SR101 staining.

Table S2. Concentration of the NO secreted by CCD 841 CoTr, HT-29, LS 180 and SW948 cells after incubation with PSC2-I extract, with and without pre-incubation with LPS.

		PSC2-I			
		0 µg/ml	25 µg/ml	100 µg/ml	200 µg/ml
CCD 841 CoTr	LPS(-)	0.000	0.000	0.030	0.147
		±	±	±	±
		0.000	0.000	0.052	0.127
	LPS(+)	0.000	0.000	0.022	0.044
		±	±	±	±
		0.000	0.000	0.038	0.038
HT-29	LPS(-)	0.383	0.748	0.597	0.769
		±	±	±	±
		0.037	0.274	0.074	0.074
	LPS(+)	0.617	0.642	0.801	0.761
		±	±	±	±
		0.126	0.069	0.182	0.069
LS180	LPS(-)	0.451	0.411	0.492	0.532
		±	±	±	±
		0.035	0.070	0.121	0.070
	LPS(+)	0.564	0.623	0.702	0.662
		±	±	±	±
		0.068	0.068	0.000	0.068
SW948	LPS(-)	0.483	0.695	0.568	0.949
		±	±	±	±
		0.161	0.127	0.127	0.127
	LPS(+)	0.556	0.735	0.735	1.015
		±	±	±	±
		0.069	0.120	0.120	0.069

Table S3. Concentration of the NO secreted by CCD 841 CoTr, HT-29, LS 180 and SW948 cells after incubation with PSC3-II extract, with and without pre-incubation with LPS.

		PSC3-II			
		0 µg/ml	25 µg/ml	100 µg/ml	200 µg/ml
CCD 841 CoTr	LPS(-)	0.000	0.000	0.000	0.000
		±	±	±	±
		0.000	0.000	0.000	0.000
	LPS(+)	0.000	0.000	0.000	0.000
		±	±	±	±
		0.000	0.000	0.000	0.000
HT-29	LPS(-)	0.383	0.297	0.340	0.468
		±	±	±	±
		0.037	0.000	0.197	0.074
	LPS(+)	0.617	0.562	0.483	0.721
		±	±	±	±
		0.126	0.069	0.000	0.119
LS180	LPS(-)	0.451	0.411	0.208	0.370
		±	±	±	±
		0.035	0.185	0.140	0.121
	LPS(+)	0.564	0.702	0.427	0.505
		±	±	±	±
		0.068	0.312	0.068	0.136
SW948	LPS(-)	0.483	0.355	0.271	0.440
		±	±	±	±
		0.161	0.147	0.073	0.127
	LPS(+)	0.556	0.496	0.456	0.536
		±	±	±	±
		0.069	0.120	0.069	0.069

Table S4. Concentration of the NO secreted by CCD 841 CoTr, HT-29, LS 180 and SW948 cells after incubation with PSC4-II extract, with and without pre-incubation with LPS.

		PSC4-II			
		0 µg/ml	25 µg/ml	100 µg/ml	200 µg/ml
CCD 841 CoTr	LPS(-)	0.000	0.000	0.074	0.104
		±	±	±	±
		0.000	0.000	0.127	0.111
	LPS(+)	0.000	0.000	0.000	0.022
		±	±	±	±
		0.000	0.000	0.000	0.038
HT-29	LPS(-)	0.383	0.554	0.597	0.683
		±	±	±	±
		0.037	0.000	0.074	0.124
	LPS(+)	0.617	0.681	0.721	1.357
		±	±	±	±
		0.126	0.069	0.119	0.383
LS180	LPS(-)	0.451	0.411	0.492	0.653
		±	±	±	±
		0.035	0.070	0.000	0.070
	LPS(+)	0.564	0.780	0.702	0.780
		±	±	±	±
		0.068	0.180	0.312	0.068
SW948	LPS(-)	0.483	0.398	0.568	0.630
		±	±	±	±
		0.161	0.147	0.127	0.090
	LPS(+)	0.556	0.656	0.576	0.895
		±	±	±	±
		0.069	0.069	0.183	0.069