

3D Gelatin-Chitosan hybrid Hydrogels combined with human Platelet Lysate highly support human Mesenchymal Stem Cell proliferation and osteogenic differentiation

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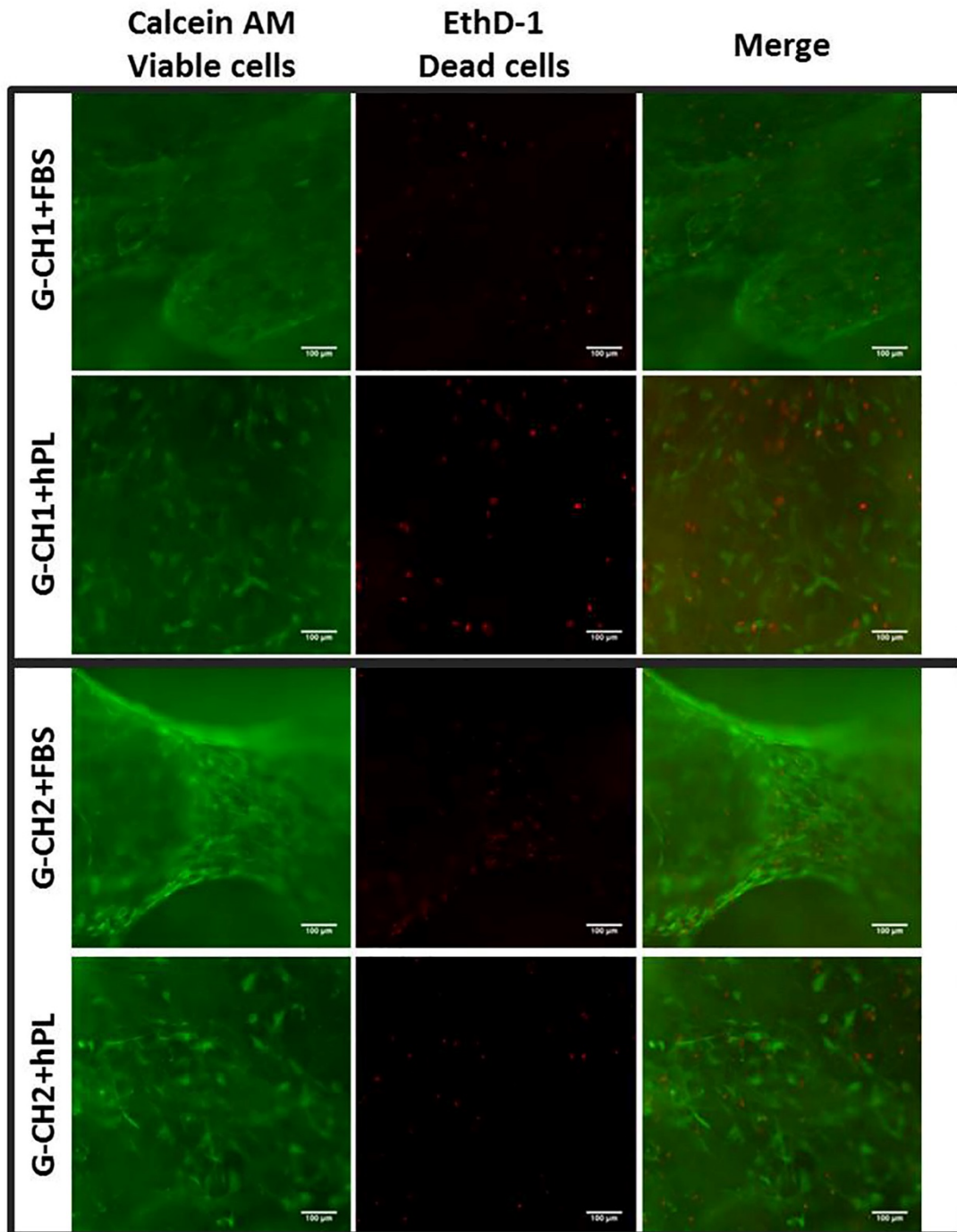
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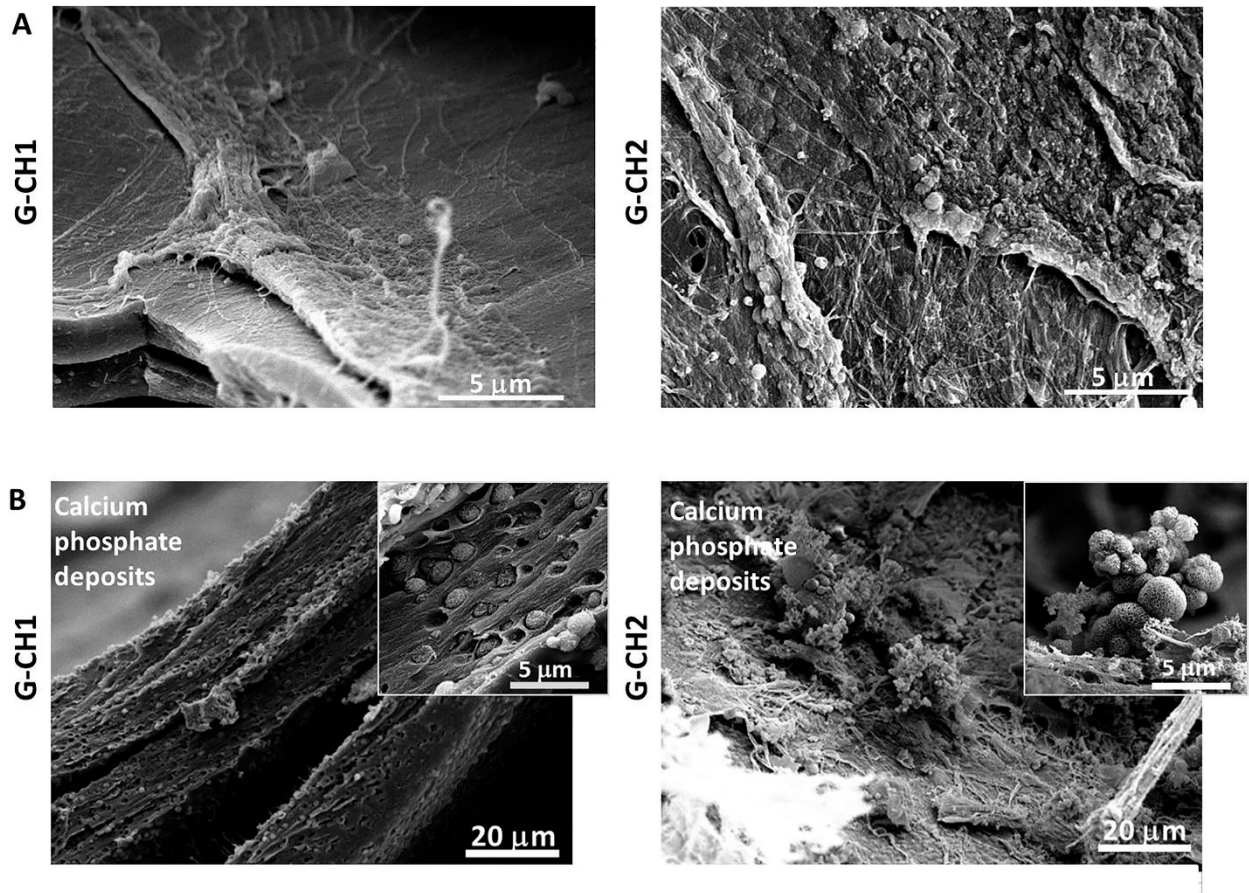
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Supplementary material

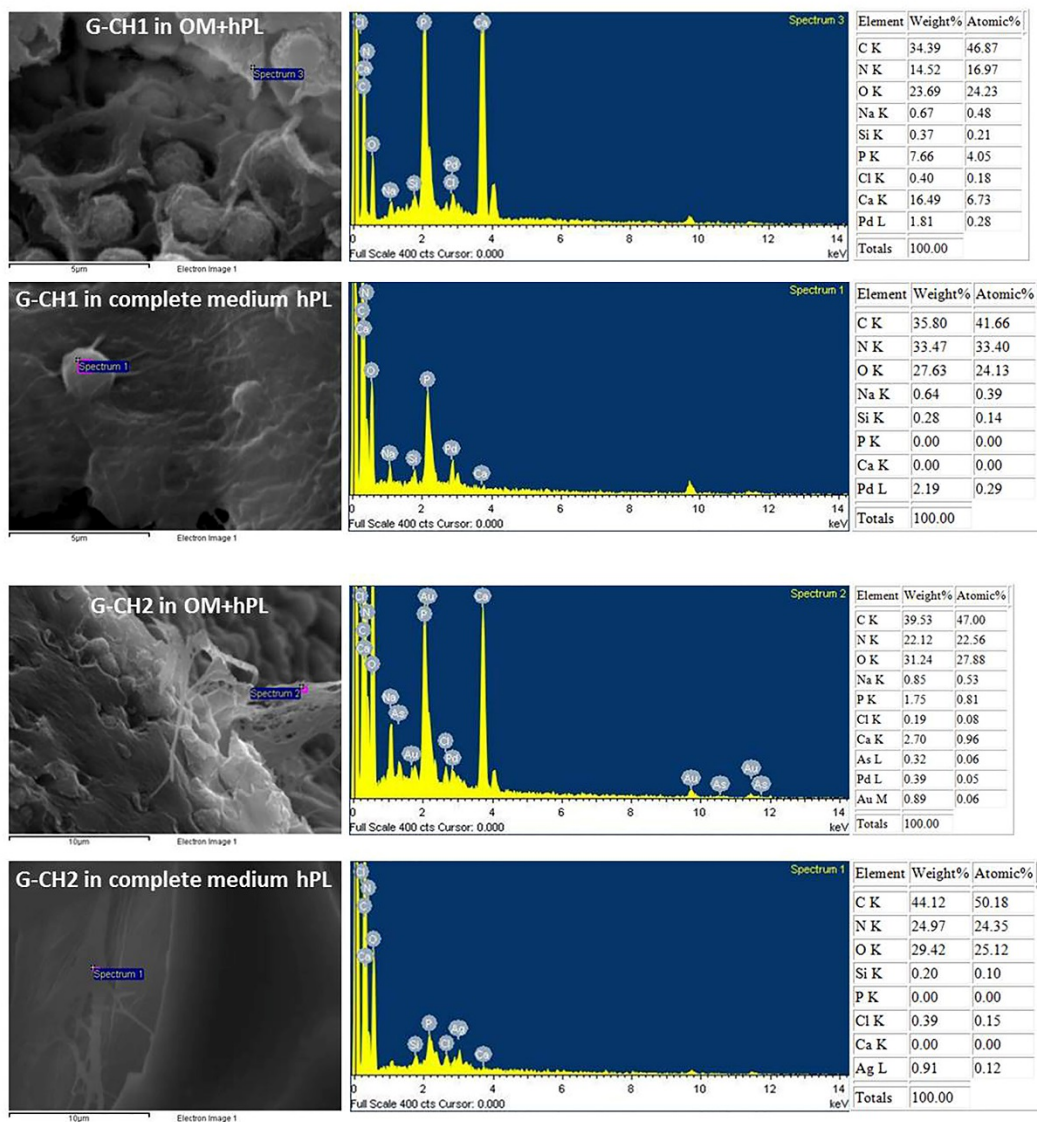


Supplementary Figure S1 Live/Dead staining of BM-hMSCs cultivated for 21 days in G-CH1 and G-CH2 hydrogels in the complete medium FBS or complete medium hPL. Scale bar: 100 μm .

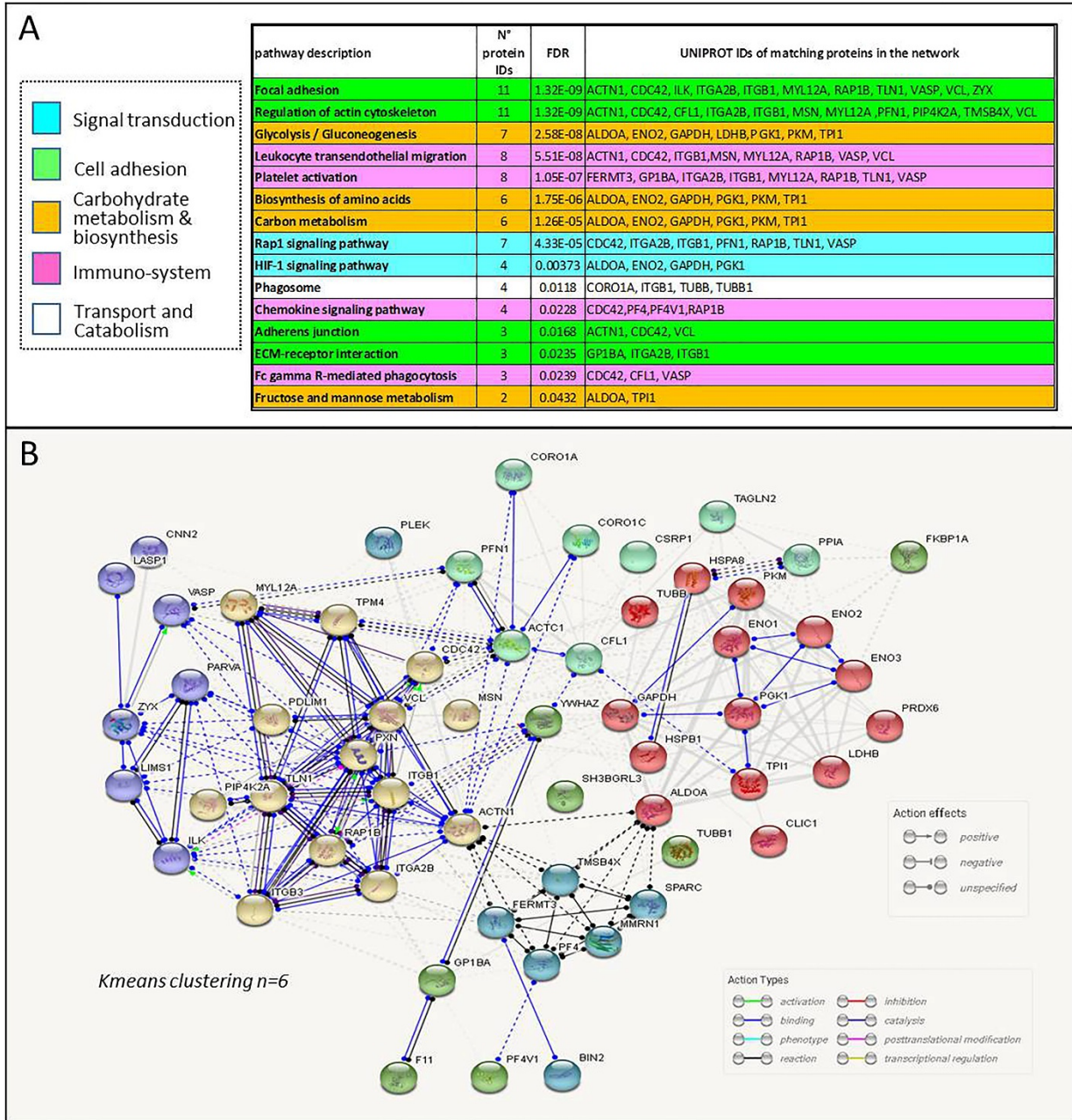
SEM-Day 21



Supplementary Figure S2 (A) SEM micrographs of BM-hMSCs cultivated in G-CH1 scaffold and G-CH2 scaffold in complete medium hPL w/o osteogenic differentiation stimuli at 21 days culture. Scale bar: 5 μm. **(B)** SEM micrographs of calcium phosphate deposition in G-CH1 and G-CH2 with BM-hMSCs at day 21 with osteogenic differentiation stimuli and hPL. Scale bars: 20 μm and 5 μm.



Supplementary Figure S3 Evaluation of calcium phosphate deposition with SEM-EDX of G-CH1 in OM with hPL , G-CH1 in complete medium hPL, G-CH2 in OM with hPL and G-CH2 in complete medium hPL at day 21. EDX spectra of treated sample area were reported.



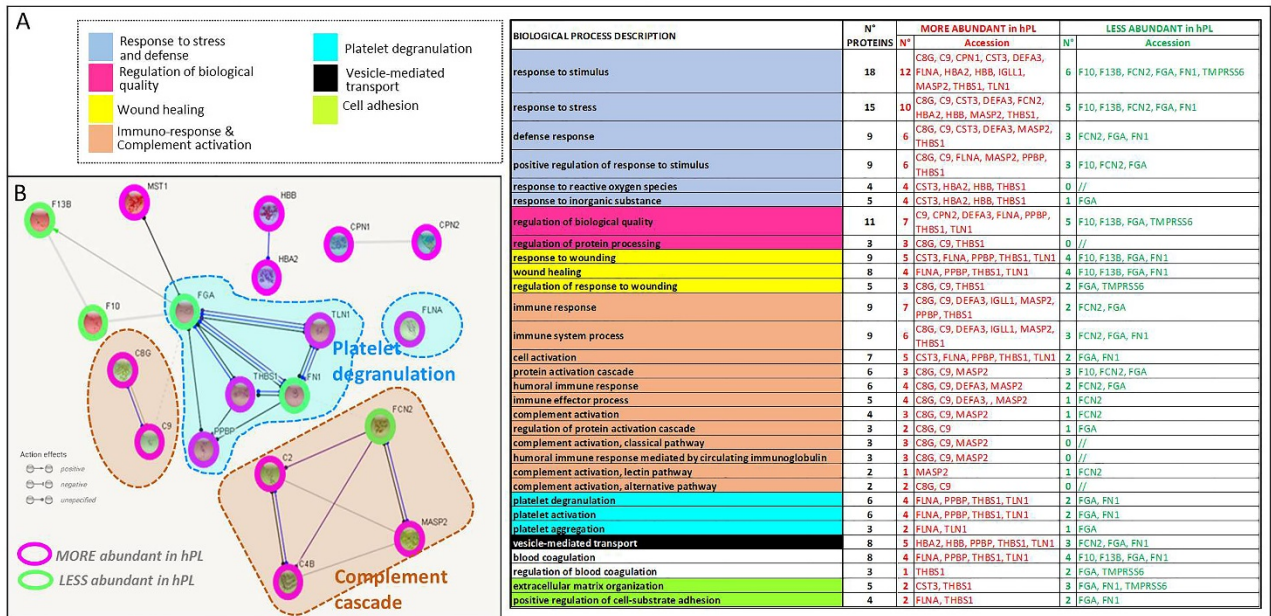
Supplementary Figure S4 Enrichment in KEGG pathways (**A**) and networks (**B**) based on STRING annotation tool (<https://string-db.org/>) applied on the list of 59 protein specific of only hPL independently from sample preparation method. Network edges are based on molecular action (Kmeans clustering n=6). FDR=False Discovery Rate.

UNIPROT Accession	Description	Protein Avg Mass (Da)	protein coverage (%)	Unique peptides	Average Normalized Area		RATIO (hPL/PPP)
					NOT depleted hPL	NOT depleted PPP	
P02775 CXCL7_HUMAN	Platelet basic protein	13894	19	2	1.65E+06	7.38E+04	22.4
P68871 HBB_HUMAN	Hemoglobin subunit beta	15998	32	2	1.81E+06	2.57E+05	7.0
P69905 HBA_HUMAN	Hemoglobin subunit alpha	15258	30	3	6.93E+06	1.14E+06	6.1
P0DOX2 IGA2_HUMAN	Immunoglobulin alpha-2 heavy chain	48935	32	2	3.95E+05	1.63E+05	2.4
P02751 FINC_HUMAN	Fibronectin	262622	26	47	3.24E+06	5.04E+06	0.6
P02671 FIBA_HUMAN	Fibrinogen alpha chain	94973	53	79	1.51E+07	2.84E+07	0.5
P01593 KVD33_HUMAN	Immunoglobulin kappa variable 1D-33	12848	38	2	8.54E+04	2.01E+05	0.4

Supplementary Figure S5 List of significant protein differences obtained comparing hPL and PPP in not depleted samples, based on Peaks Studio, applying the following filters: Fold change \geq 1.5; pANOVA $<$ 0.05; \geq 2unique peptides, in at least 4 of 12 replicates.

UNIPROT Accession	Description	Protein Avg Mass (Da)	protein coverage (%)	Unique peptides	Average Normalized Area		RATIO (hPL/PPP)
					NOT depleted hPL	NOT depleted PPP	
P02775 CXCL7_HUMAN	Platelet basic protein	13894	40	4	3.58E+06	8.59E+04	41.7
P07996 TSP1_HUMAN	Thrombospondin-1	129383	3	2	1.02E+06	4.57E+04	22.3
P68871 HBB_HUMAN	Hemoglobin subunit beta	15998	50	4	5.16E+06	8.50E+05	6.1
P69905 HBA_HUMAN	Hemoglobin subunit alpha	15258	36	6	9.66E+06	2.59E+06	3.7
P06681 CO2_HUMAN	Complement C2	83268	7	6	1.56E+06	5.69E+05	2.7
P0C0L5 CO4B_HUMAN	Complement C4-B	192750	36	2	3.78E+05	1.42E+05	2.7
P59666 DEF3_HUMAN	Neutrophil defensin 3	10245	19	2	3.89E+05	1.54E+05	2.5
P21333 FLNA_HUMAN	Filamin-A	280737	5	7	1.11E+06	4.52E+05	2.5
P15814 IGLL1_HUMAN	Immunoglobulin lambda-like polypeptide 1	22963	11	2	2.28E+06	9.48E+05	2.4
P15169 CBPN_HUMAN	Carboxypeptidase N catalytic chain	52286	4	2	3.95E+05	1.88E+05	2.1
O00187 MASP2_HUMAN	Mannan-binding lectin serine protease 2	75702	6	3	2.03E+05	1.00E+05	2.0
P07360 CO8G_HUMAN	Complement component C8 gamma chain	22277	30	5	1.16E+06	6.43E+05	1.8
P01860 IGHG3_HUMAN	Immunoglobulin heavy constant gamma 3	41287	28	2	4.05E+06	2.39E+06	1.7
P02748 CO9_HUMAN	Complement component C9	63173	34	15	3.39E+06	2.02E+06	1.7
P22792 CPN2_HUMAN	Carboxypeptidase N subunit 2	60557	6	2	1.24E+06	7.49E+05	1.7
Q9Y490 TLN1_HUMAN	Talin-1	269765	2	4	1.85E+06	1.14E+06	1.6
P26927 HGFL_HUMAN	Hepatocyte growth factor-like protein	80320	11	4	5.45E+05	3.50E+05	1.6
P01034 CYTC_HUMAN	Cystatin-C	15799	19	2	1.90E+05	1.23E+05	1.5
P00742 FA10_HUMAN	Coagulation factor X	54732	6	3	6.97E+05	1.19E+06	0.6
P05160 F13B_HUMAN	Coagulation factor XIII B chain	75511	15	8	5.81E+05	1.06E+06	0.5
Q9Y6R7 FCGBP_HUMAN	IgGfc-binding protein	572021	1	4	1.90E+05	3.50E+05	0.5
Q8IU80 TMPS6_HUMAN	Transmembrane protease serine 6	90000	4	2	1.40E+06	2.67E+06	0.5
Q15485 FCN2_HUMAN	Ficolin-2	34001	8	2	3.74E+05	7.38E+05	0.5

Supplementary Figure S6 List of significant protein differences obtained comparing hPL and PPP in depleted samples, based on Peaks Studio, applying the following filters: Fold change \geq 1.5; pANOVA $<$ 0.05; \geq 2unique peptides, in at least 4 of 12 replicates.



Supplementary Figure S7 Enrichment in biological processes **(A)** and networks **(B)** based on STRING annotation tool (<https://string-db.org/>) applied on proteins resulted significantly varied in their abundance by label-free relative quantification. Network edges are based on molecular action (Kmeans clustering n=6).

[Insert Supplementary Table 1 rev]

Supplementary Table 1 Enrichment in biological process based on STRING annotation tool (<https://string-db.org/>) applied on the list of 59 proteins specific of only hPL independently from sample preparation method.