

Supplemental material for Neutrophils from a Patient with Papillon-Lefèvre Syndrome.

Supplemental Table 1

Differentially expressed azurophil granule proteins from a normal control (Ctl) versus patient samples (PLS). In total 1422 proteins including proteins flagged as contaminants and decoys were identified from the α , β_1 , β_2 and γ fractions by GeLC-MSMS. The list was subsequently filtered for the most interesting proteins by the following criteria: i) Must be present in the α -fraction, ii) Intensity must comprise $>0.1\%$ of total intensity, iii) Must peak in the a-fraction or at least have 75% of the intensity of the b1-fraction if peaking here (to account for the small contamination of the a-fraction in the β_1 -fraction). Listed are proteins that are at least 50% increase in Ctl relative to the PLS samples: Protein intensities for individual proteins were calculated as the sum of the peptide intensities for all peptides matched to the listed proteins. The number show the ratio of the protein intensities in the Ctl and PLS α -fractions for the listed protein. (4): Protein intensities for individual proteins were calculated as the sum of the peptide intensities for all peptides matched to the listed proteins. The number shows in % how much each protein in the Ctl α -fraction comprises of the summed intensities of all proteins in the Ctl α -fraction.

Supplemental Figure 1:

Subcellular fractionation of control and PLS neutrophils. Neutrophils (1.7×10^8) from a healthy control (Ctl) and the PLS patient (1.7×10^8) were cavitated and separated on a 3-layer Percoll density gradient. Fractions of 1 mL each were collected from the bottom of the gradients and assayed for marker proteins for each major granule subsets: MPO (azurophil granules), NGAL (specific granules), Gelatinase (gelatinase granules), Albumin (secretory vesicles), and HLA (Plasma membranes). The distribution is given in 1A. For each marker the value is given relative to its maximal value. These were as follows: MPO Ctl: 21.5 $\mu\text{g/mL}$, PLS: 31.9

µg/mL; NGAL Ctl: 2.8 µg/mL, PLS: 2.7 µg/mL; Gelatinase: Ctl: 8.7 µg/mL, PLS: 9.2 µg/mL; Alb Ctl: 2,6 µg/mL, PLS: 1.7 µg/mL; HLA Ctl: 175 arb.U/mL, PLS: 136 arb.U/mL.

Samples with peak activity were collected and pooled as follows: α-band (MPO-peak): fractions #1-8; β1-band (NGAL peak): #9-15; β2-band (gelatinase peak): #16-20. γ-band (Alb and HLA): #21-28. Percoll was removed by centrifugation and the biological material was resuspended in 200 µL PBS. The distribution of marker proteins in these fractions which were named α (azurophil granules), β1 (specific granules), β2 (gelatinase granules), and γ (Light membranes including secretory vesicles and plasma membranes), were determined by elisa and expressed relative to the amount present in control, which were as follows: MPO: 268 µg/mL; NGAL: 26 µg/mL; Gelatinase: 19.5 µg/mL; Alb: 25 µg/mL; HLA: 2033 arb U/mL.

Supplemental Figure 2.

Quantitative real time PCR.

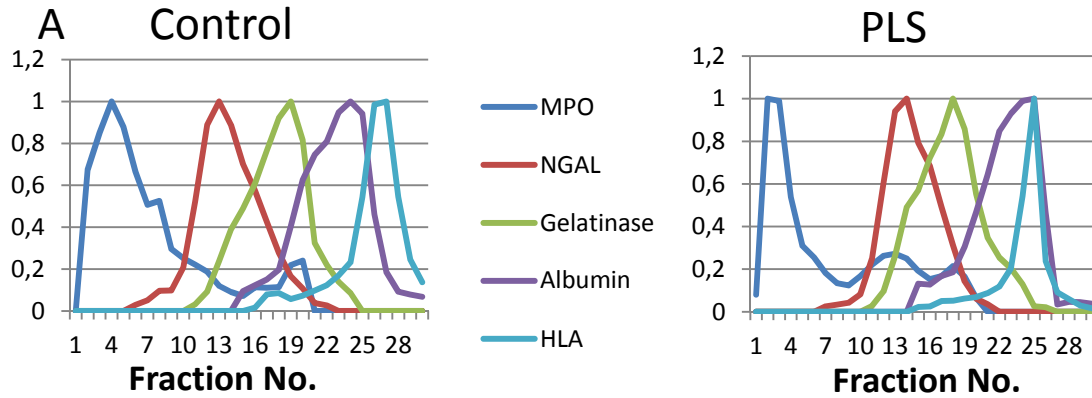
Bone marrow cells from a normal control and the PLS patient were separated on Lymphoprep and depleted of non-myeloid cells by MACS as described in Methods. Cells from the interphase which contain the neutrophil precursors up to meta-myelocytes were subjected to quantitative real time PCR as described in Methods. The mRNA for Myeloperoxidase (MPO), Neutrophil Elastase (ELANE), Cathepsin G (CTSG), Proteinase 3 (PRTN3) and Cathepsin C (CTSC), and NGAL (LCN2) was determined. Actin was run as an internal standard.

Supplemental table 1

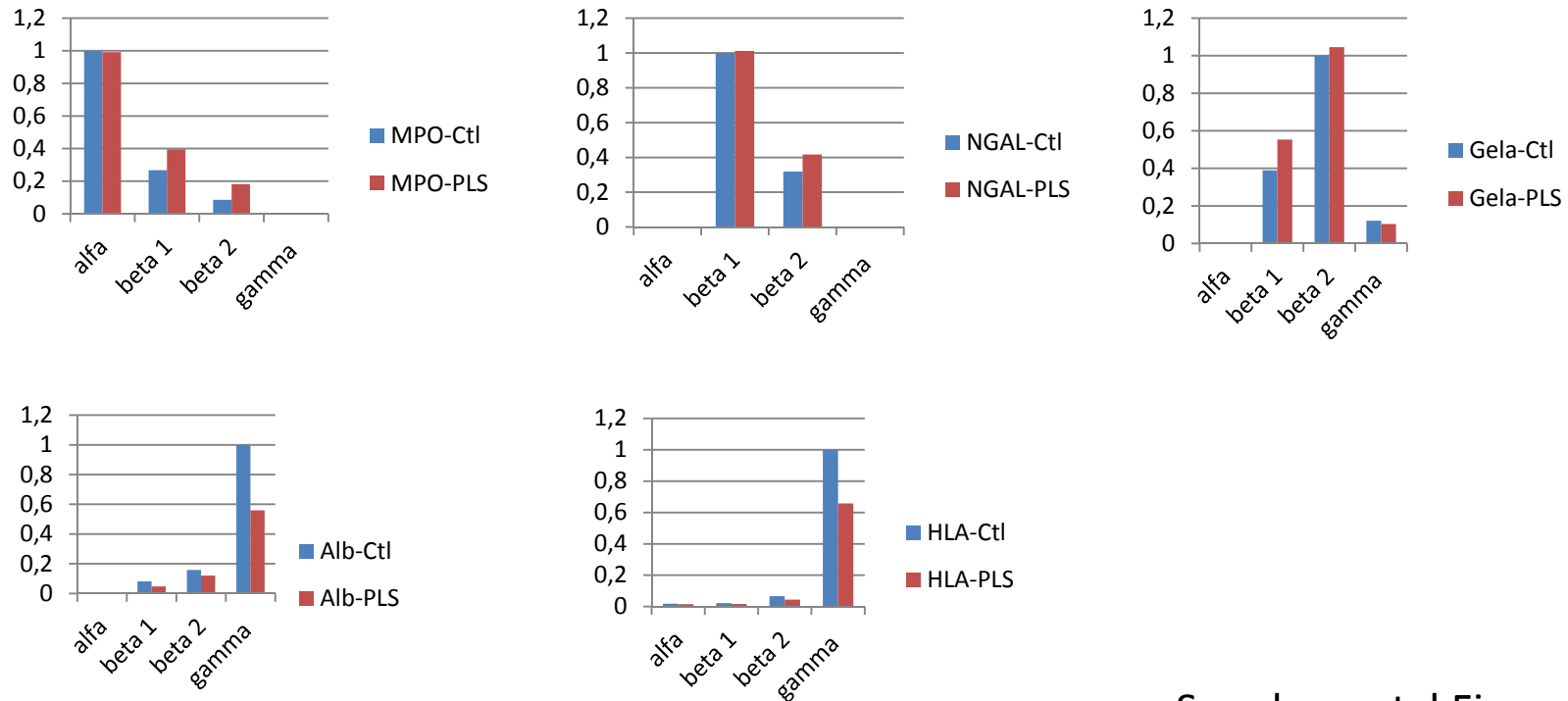
Differentially expressed azurophil granule proteins in normal samples (Ctl) versus patient samples (PLS)

Protein IDs	Gene Name	Protein name	Ratio(Ctl/PLS)	Fraction (in %) of the total protein intensity from the α -fraction
IPI00328415	CYB5R3	NADH-cytochrome b5 reductase 3	138.6	0.27
IPI00028064	CTSG	Cathepsin G	40.1	10.21
IPI00409684	NCKAP1	Nck-associated protein 1	26.0	0.45
IPI00013163	MNDA	Myeloid cell nuclear differentiation antigen (5)	16.2	0.12
IPI00016339	RAB5C; RABL	Ras-related protein Rab-5C	12.4	0.28
IPI00027769	ELA2; ELANE	Neutrophil elastase	9.71	1.87
IPI00019345	RAP1A	Ras-related protein Rap-1A	9.26	0.61
IPI00645452	TUBB	Tubulin beta-7 chain	7.02	0.27
IPI00006690	EPER; EPO	Eosinophil peroxidase	6.90	14.40
IPI00005778	PRG3	Proteoglycan 3	5.89	2.04
IPI00019449	RNASE2	Non-secretory ribonuclease	5.72	1.46
IPI00027409	MBN; PRTN3	Proteinase 3,	5.26	1.68
IPI00010133	CORO1A	Coronin-1A; 40 kDa protein	4.97	0.24
IPI00022246	AZU1	Azurocidin	4.83	5.94
IPI00010341	MBP; PRG2	Bone marrow proteoglycan	4.68	3.24
IPI00219825	PSAP	Prosaposin	1.74	0.14
IPI00019038	LYZ	Lysozyme	1.73	5.09
IPI00023728	GGH	Gamma-glutamyl hydrolase	1.64	0.15
IPI00025427	ECP	Eosinophil cationic protein	1.56	3.97

Subcellular fractionation of control and PLS neutrophils



B Characterization of fractions used for proteome analysis and Western blotting



Supplemental Figure 1

Supplemental Figure 2

