

Supplementary materials

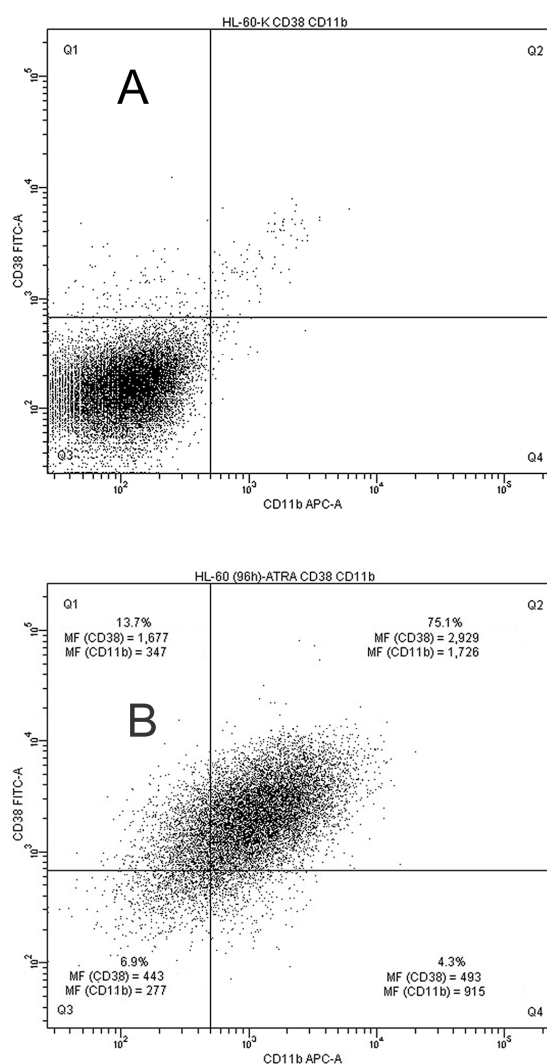
# Omics Technologies to Decipher Regulatory Networks in Granulocytic Cell Differentiation

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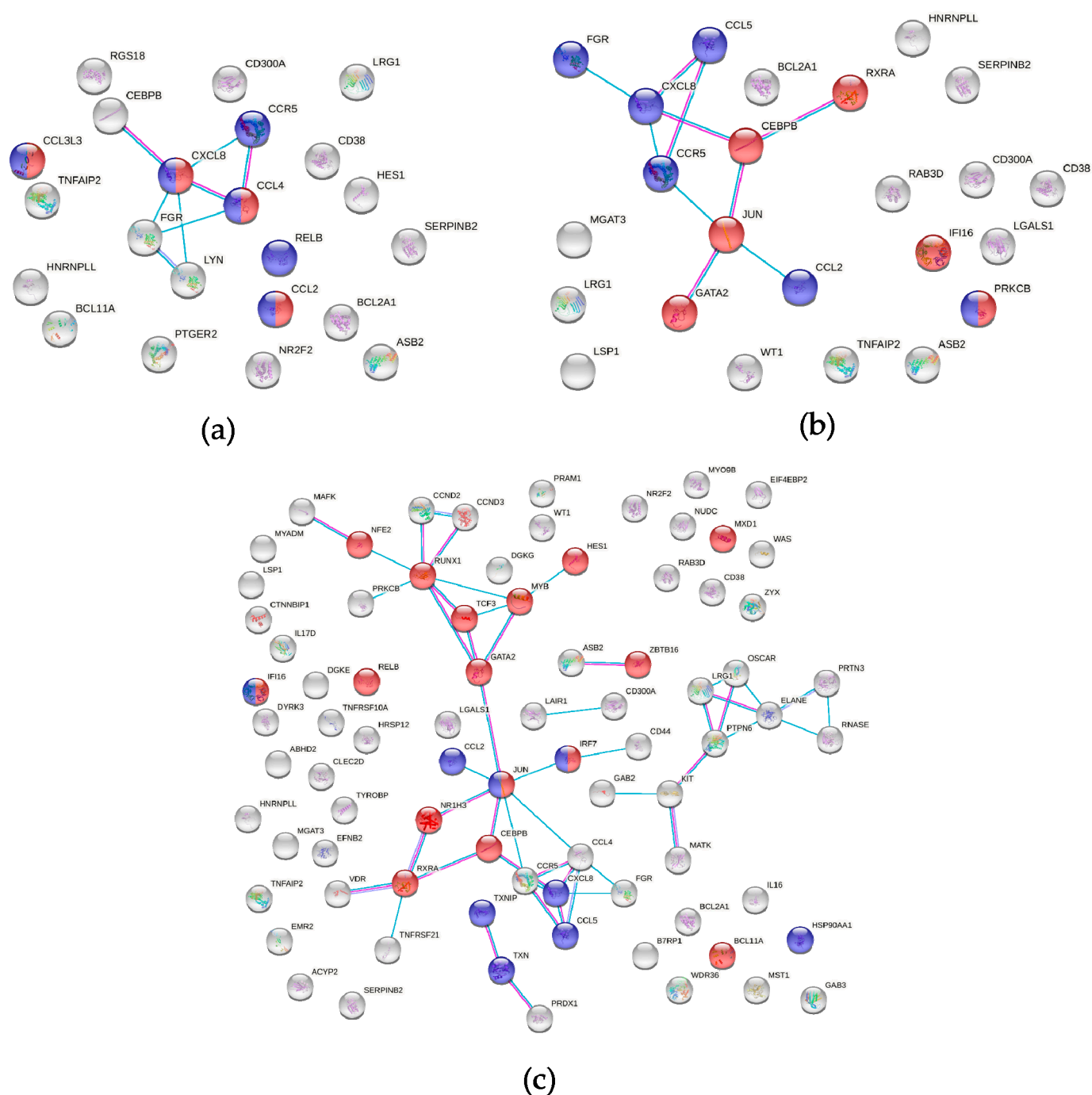
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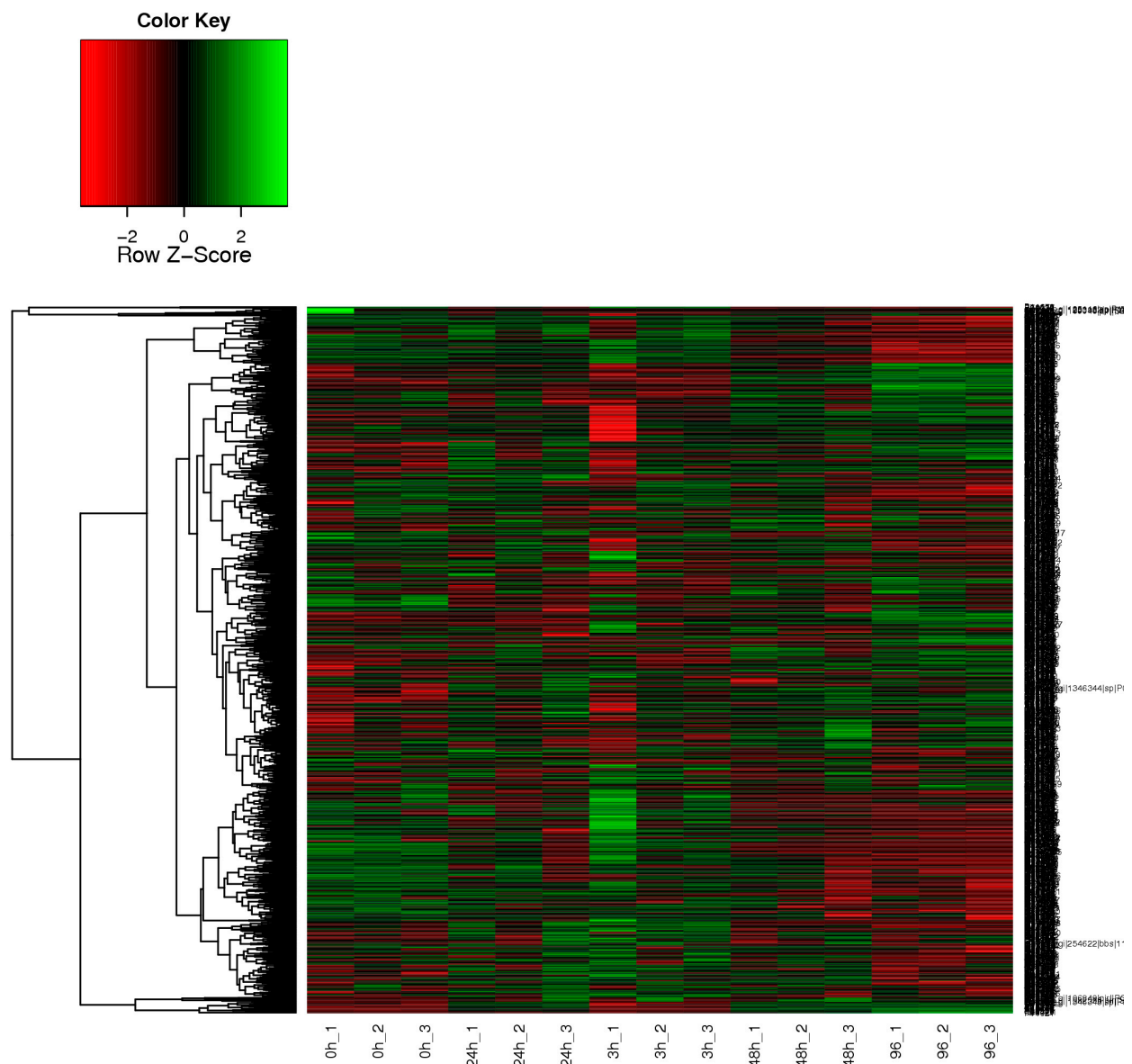
<sup>†</sup> These authors contributed equally to this work



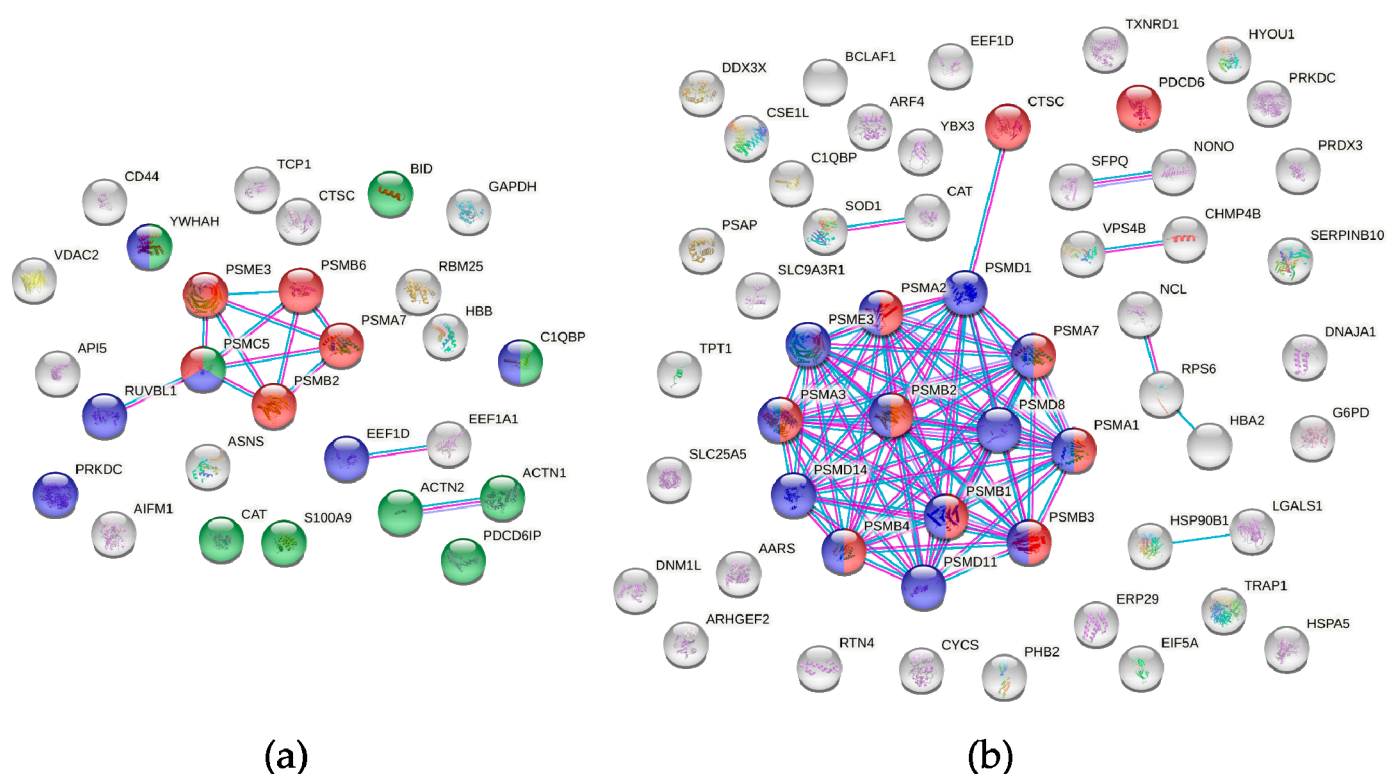
**Figure S1.** Evaluation of HL-60 cell line by the CD38 and CD11b expression level measured by flow cytometry: (a) untreated cells and (b) cells at 96 h after ATRA treatment.



**Figure S2.** STRING interaction analysis (a) for 22 differentially expressed genes (DEGs) of MCD group (3h) enriched in interactions (PPI enrichment p-value = 0.00595), red and blue nodes shows DEGs (MCD group) that have been annotated by Molecular function (GO) as belonging to "chemokine activity" group (FDR = 5.70e-05) and by KEGG database as members of "Chemokine signaling pathway" group (FDR = 6.38e-08), respectively; (b) for 24 DEGs (MCD group, 24 h) enriched in interactions (PPI enrichment p-value = 0.00684), red and blue nodes shows DEGs (MCD group) that have been annotated by Molecular function (GO) as belonging to "transcription factor binding" group (FDR = 9.86e-06), respectively; and by KEGG database as members of "Chemokine signaling pathway" group (FDR = 9.86e-06), respectively; (c) for 81 DEGs (MCD group, 96 h) enriched in interactions (PPI enrichment p-value = 5.31e-07), red and blue nodes shows DEGs (MCD group) that have been annotated by Molecular function (GO) as belonging to "proximal promoter sequence-specific DNA binding" group (FDR = 1.08e-07) and by KEGG database as members of "NOD-like receptor signaling pathway" group (FDR = 3.39e-06), respectively. All networks were enriched using the intersection of 8612 genes present on all platforms as the background, along with evidence from experimental protein-protein interactions (PPI) (purple lines), and curated (turquoise blue lines) databases. The network was built based on the highest confidence (0.9).



**Figure S3.** Heatmap of protein expression. Expression color coded from low (dark red) to high (dark green). The figure reflects clusterization of proteins identified at 0, 3, 24, 48 and 96 h after ATRA treatment in 3 biological replicates.



**Figure S4.** STRING interaction analysis of differentially expressed proteins (DEPs) assigned to group “programmed cell death” and/or “regulation of cell death” at 3 and 96 h after ATRA treatment; (a) 27 DEPs at 3 h after ATRA treatment enriched in interactions (PPI enrichment p-value = 0.000248), red, blue, and green nodes shows DEPs that have been annotated by Molecular function (GO) as belonging to “signaling receptor binding” group (FDR = 0.0045), and to “transcription factor binding” group (FDR = 0.0048), as well as by KEGG database as members of “Proteasome” pathway (FDR = 3.75e-07), respectively; (b) 54 DEPs at 96 h after ATRA treatment enriched in interactions (PPI enrichment p-value = 1.0e-16), red and blue nodes shows DEPs that have been annotated by Molecular function (GO) as belonging to “endo-peptidase activity” group (FDR = 9.44e-06) and by KEGG database as members of “Proteasome” pathway (FDR = 1.38e-20), respectively.

**Table S6.** Transcription factors (TFs) that are responsible for the change in gene expression of Myeloid Cells Differentiation (MCD) group MCD at time points 3, 24 and 96 hours in the course of differentiation of HL-60 cells. TFs found also as potential regulators of differentially expressed proteins (see Table S8, below) are shown in bold. TFs demonstrating altered expression during ATRA-induced differentiation of HL-60 cells are underlined.

TF gene name, 3 h	TF gene name, 24 h	TF gene name, 96 h
	AHR ARNT	AHR ARNT
BACH1	BACH1	
		<u>HIC1</u> HIF1A
	HSF2 ILF3	HSF2
ILF3		
MAFB MAFG MAFK	MAFB MAFG MAFK	
MEF2A MEF2D	MEF2A MEF2D	MAZ
<u>NFATC1</u> NFATC2 NFATC3 NFATC4 NFE2 NFE2L1 NFE2L2 NFE2L3	<u>NFATC1</u> NFATC2 NFATC3 NFATC4 NFE2 NFE2L1 NFE2L2 NFE2L3 NFKB1 NFKB2 NKX2-5  RELA	

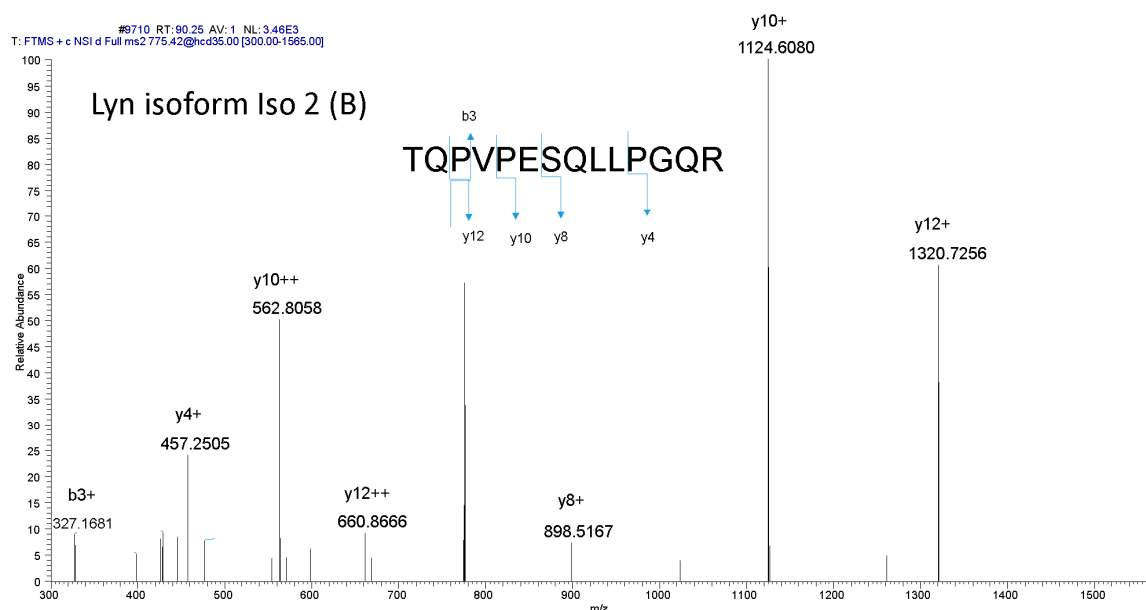
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	<u><b>RXRA</b></u> RXRB SMAD3	
SMAD3		
<u>SREBF1</u> SREBF2 <b>SRF</b>	<u>SREBF1</u> SREBF2 <b>SRF</b>	<b>SRF</b>
<u>TCF3</u>		<u>TCF3</u> TFAP2C TFCP2 <u><b>VDR</b></u> <u>WT1</u> ZBTB7A
	<u><b>VDR</b></u> <u>WT1</u> ZBTB7A	<u>ZBTB7B (c-Krox)</u>

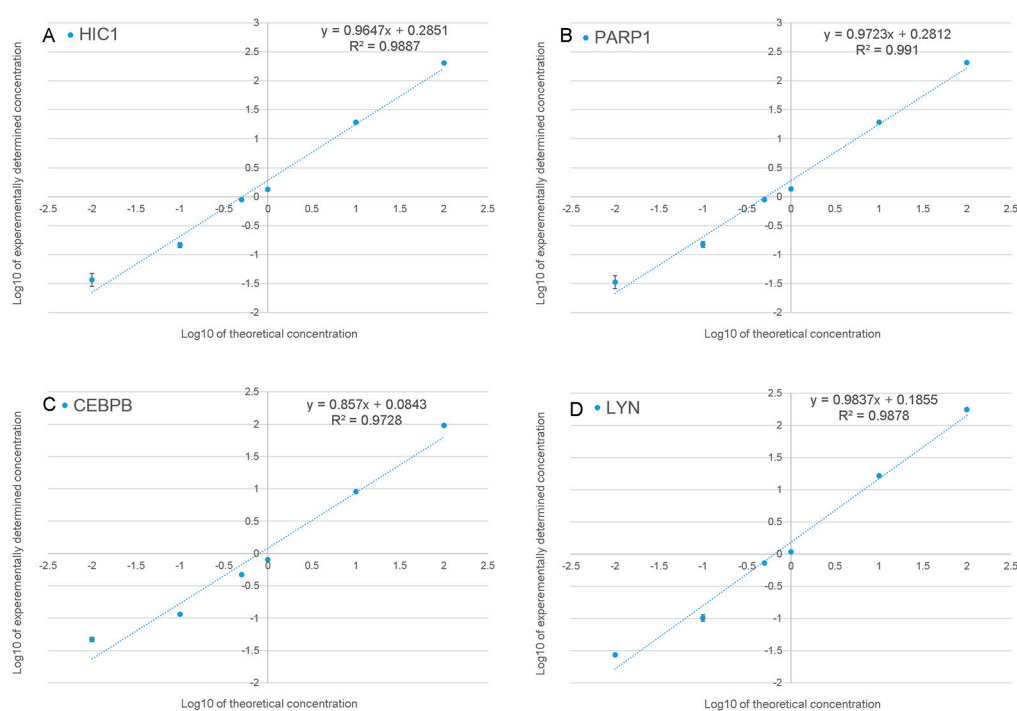
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**Table S7.** Transcription factors (TFs) possibly regulating protein expression during ATRA-induced HL-60 cell differentiation at time points 3 h, 24 h, 48 h, and 96 h. Common TF that also may be regulators of differentially expressed transcripts are shown in bold. TFs demonstrating altered expression during ATRA induced differentiation of HL-60 cells are underlined.

TF gene name, 3h	TF gene name, 24h	TF gene name, 48h	TF gene name, 96h
ARNT	ARNT		
CUX1 (CDP)	CUX1 (CDP)		
	<b>EP300</b>		<u>GATA2</u>
	<u>HIC-1</u>		
	HSF1	HSF1	
		<b>HSF2</b>	<b>HSF2</b>
<u>IRF7</u>		LMO2	
			<b>MAX</b>
	NFYC	NFYC	NFYC
NKX31 (NKX3A)			NKX31 (NKX3A)
NR1H3			
PBX3	PBX3	PBX3	
POU2F1	POU2F1	POU2F1	
	<b>PPARA</b>	<b>PPARA</b>	
RARA (NR1B1)			RBPJ (RBPJK)
		RFXANK	
		<u>RUNX2 (AML3)</u>	
<u>RXRA</u>	STAT1	STAT1	STAT1
		SRF	
	<u>VDR</u>	<u>VDR</u>	<u>VDR</u>
	YY1		
ZNF384			



**Figure S5.** High-resolution annotated MS2 spectrum of LYN isoform B-specific peptide TQPVPESQLLPGR.



**Figure S6.** Calibration curves plotting of experimentally determined concentrations versus theoretical concentrations of the target analyte using isotopically labeled and label-free synthetic standard peptide (a) LEEAAPPSPDPR (HIC1), (b) TLGDFAAEYAK (PARP1), (c) VLELTAENER (CEBPB), and (d) TQPVPESQLLPGR (LYN). Isotopically labeled synthetic peptide was added to concentration of 12.5 fmol/ $\mu$ l into serial dilutions (0.01, 0.1, 0.5, 1, 10, 100 fmol/ $\mu$ l) of label-free synthetic peptide. Calibration curve is presented in Log10 scale.



**Table S8.** Transition for SRM method (QqQ TSQ Vantage (Thermo Scientific, USA), isotopically-labeled amino acid is shown in bold. +—singly-charged ion; ++—doubly-charged ion

Peptide sequence	Precursor ion m/z	Fragment ion m/z	Collision energy
TLGDFAAEYAK	593.293 <sup>++</sup>	971.4469 <sup>+</sup> 799.3985 <sup>+</sup> 581.293 <sup>+</sup>	20.7
TLGDFAAEYAK (heavy)	597.3001 <sup>++</sup>	979.4611 <sup>+</sup> 807.4127 <sup>+</sup> 589.3072 <sup>+</sup>	
LEEAAAPSDPFR	664.8277 <sup>++</sup>	815.4046 <sup>+</sup> 718.3519 <sup>+</sup> 514.2508 <sup>+</sup>	22.8
LEEAAAPSDPFR (heavy)	666.8312 <sup>++</sup>	819.4117 <sup>+</sup> 722.359 <sup>+</sup> 514.2508 <sup>+</sup>	
VLELTAENER	587.3091 <sup>++</sup>	961.4585 <sup>+</sup> 832.4159 <sup>+</sup> 719.3319 <sup>+</sup>	20.5
VLELTAENER (heavy)	592.3133 <sup>++</sup>	971.4668 <sup>+</sup> 842.4242 <sup>+</sup> 729.3401 <sup>+</sup>	
TQPVPESQLLPGQR	775.4203 <sup>++</sup>	1320.7270 <sup>+</sup> 1124.6058 <sup>+</sup> 570.3358 <sup>+</sup>	26.2
TQPVPESQLLPGQR (heavy)	778.9289 <sup>++</sup>	457.2518 <sup>+</sup> 1327.7442 <sup>+</sup> 1131.6230 <sup>+</sup> 577.3530 <sup>+</sup> 457.2518 <sup>+</sup>	26.2