## Reduction of GPSM3 expression akin to the arthritis-protective SNP rs204989 differentially affects migration in a neutrophil model

BJ Gall<sup>1</sup>, AB Schroer<sup>1</sup>, JD Gross<sup>1</sup>, V Setola<sup>1,2</sup>, DP Siderovski<sup>1</sup>

<sup>1</sup>Department of Physiology & Pharmacology and

<sup>2</sup>Department of Behavioral Medicine & Psychiatry,

West Virginia University School of Medicine, Morgantown, WV, USA 26506-9229

**Table S1**: Counts of formazan-positive/-intermediate/-negative cells (out of 500 cells<br/>counted per condition; analysed in triplicate per experiment; n = 2 experiments)<br/>enumerated for the creation of Figure 2B's bar graphs.

							V	ehicle	treate	d								
NB4 line:	Formazan-Positive					Formazan-Intermediate						Formazan-Negative						
shRNA19.1	3	2	0	2	1	2	6	9	8	5	7	8	491	489	492	493	492	490
shRNA19.5	2	1	0	1	0	1	9	6	9	4	7	9	489	493	491	495	493	490
shRNA20.2	0	1	1	0	0	0	7	5	6	11	8	10	493	494	493	489	492	490
shRNA20.5	1	1	3	3	0	1	9	8	9	8	7	7	490	491	488	489	493	492
scrambled 2	3	0	0	2	1	0	5	6	8	8	10	6	492	494	490	490	489	494
scrambled 3	2	0	0	3	1	0	8	10	7	6	7	9	490	490	493	491	492	491
									reated									
NB4* line: Formazan-Positive							Formazan-Intermediate						Formazan-Negative					
shRNA19.1	447	448	438	451	457	447	48	40	53	35	39	46	5	12	9	14	4	7
shRNA19.5	462	455	463	454	453	450	31	36	32	38	37	48	7	9	5	8	10	2
shRNA20.2	451	453	455	461	466	462	40	41	34	31	26	31	9	6	11	8	8	7
shRNA20.5	465	460	459	451	451	464	30	28	32	40	41	30	5	12	9	9	8	6
scrambled 2	461	465	450	462	452	459	31	28	39	32	39	31	8	7	11	6	9	10
scrambled 3	452	463	460	454	458	450	40	31	31	34	35	40	8	6	9	12	7	10

Table S2: Sequences of shRNA hairpins used in this study.

shRNA	Sequence (5'>3')	<b>Target Region</b>
19	GCGAGATGGAACAGGGATTTACTCGAGTAAATCCCTGTTCCATCTCGC	3' UTR
20	AGAACAGCTTTACAGCACTATCTCGAGATAGTGCTGTAAAGCTGTTCT	CDS
22	CCACCACTCGGCCTTGGCGATCTCGAGATCGCCAAGGCCGAGTGGTGG	CDS
Scr	CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG	N/A



Figure S1. GPSM3 deficiency has no effect on NB4\* cell migration in the absence of chemoattractant in the lower chamber (panel A) and only a minimal effect in initial rate of migration towards 10% fetal bovine serum (panel B & inset). Real-time Transwell migration measured by fluorescence of migrated cells (RFU, relative fluorescence units) towards indicated lower-chamber contents by indicated NB4\* cell lines (after an initial 120 hours of ATRA differentiation). Inset bar-graph of initial rates: \*, p < 0.05 *vs* scrambled controls.



Figure S2. GPSM3 deficiency has negligible effects on the mRNA expression levels of CXCL8 receptors CXCR1 (panel A) and CXCR2 (panel B). Post-differentiated NB4\* cell line abundance of CXCR1 and CXCR2 mRNAs was measured by qRT-PCR and normalized to the scrambled 2 line; significance (p < 0.05) determined by Kruskal-Wallis test (unequal group variances, Brown-Forsythe p < 0.05) with Dunn's post-hoc. All data plotted as mean ± S.E.M. "blood", mRNA level measured from total RNA of human whole blood.



Figure S3. Dose-response analysis confirming that GPSM3 deficiency has no effect on NB4\* cell migration toward fMLP. Real-time Transwell migration towards indicated concentrations of fMLP over 34 minutes by indicated NB4\* cell lines (after 120 hours of ATRA differentiation).



**Figure S4**. **PCR confirmation of lack of** *Mycoplasma spp.* **and** *Acholeplasma spp.* **contamination**. PCR amplification of highly conserved 16S rRNA coding region (lower band) and internal control DNA (upper band) was performed using Lookout® Mycoplasma PCR Detection Kit (Sigma-Aldrich, St. Louis, MO), which is sensitive to 10 genomes per 1 µl. Performed by directly adhering to the manufacturer's instructions. <u>Lane 1</u>: (Positive control) PCR amplification of both the internal control (upper band) and *Mycoplasma orale* DNA fragments (lower band). <u>Lane 2</u>: (Negative control) PCR amplification of only the internal control, including HEK 293T cells previously tested to be free of *Mycoplasma spp.* contamination. <u>Lane 10</u>: (Media control) PCR amplification only the internal control from fresh media.