

## **SUPPLEMENTAL MATERIAL**

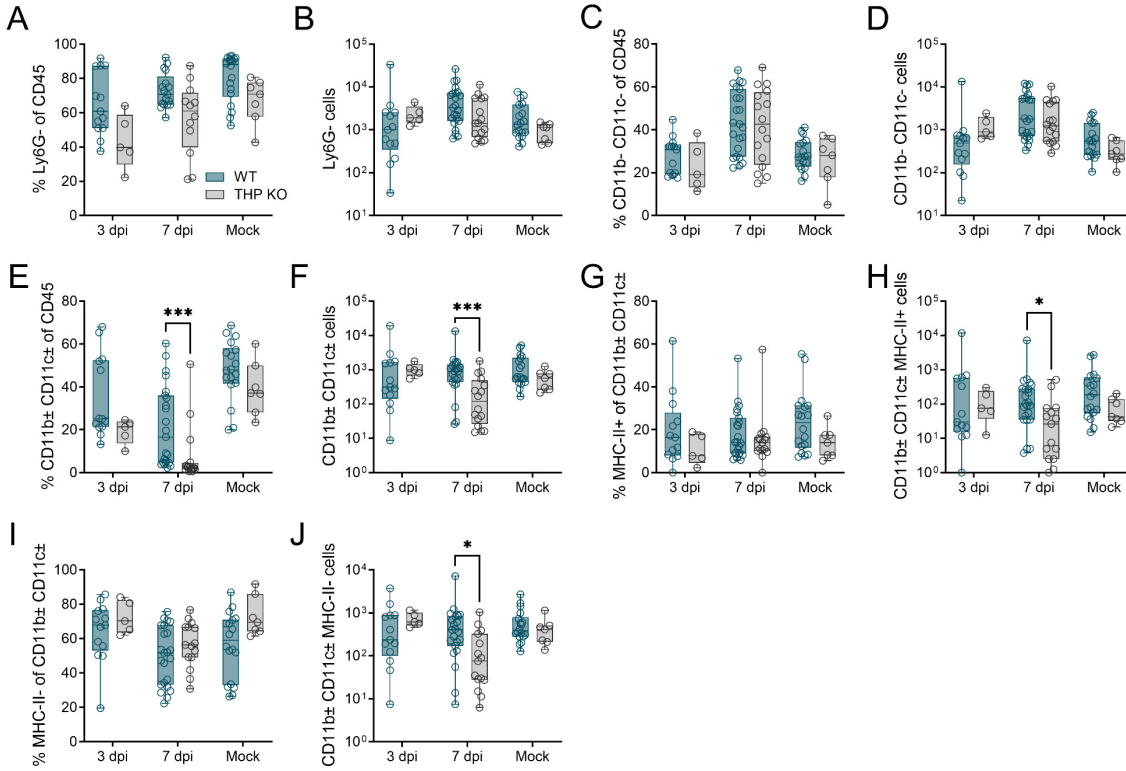
### **Tamm-Horsfall protein augments neutrophil NETosis during urinary tract infection**

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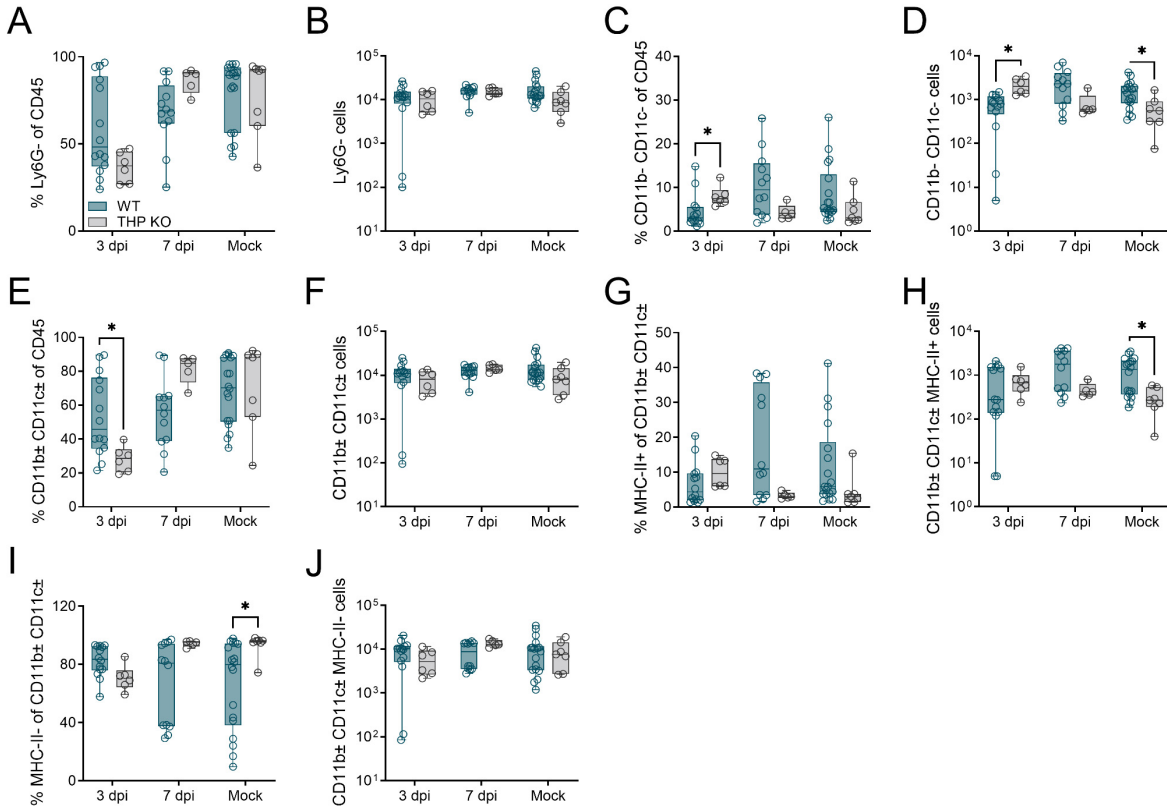
#### **Contents:**

Supplementary Figures 1-6

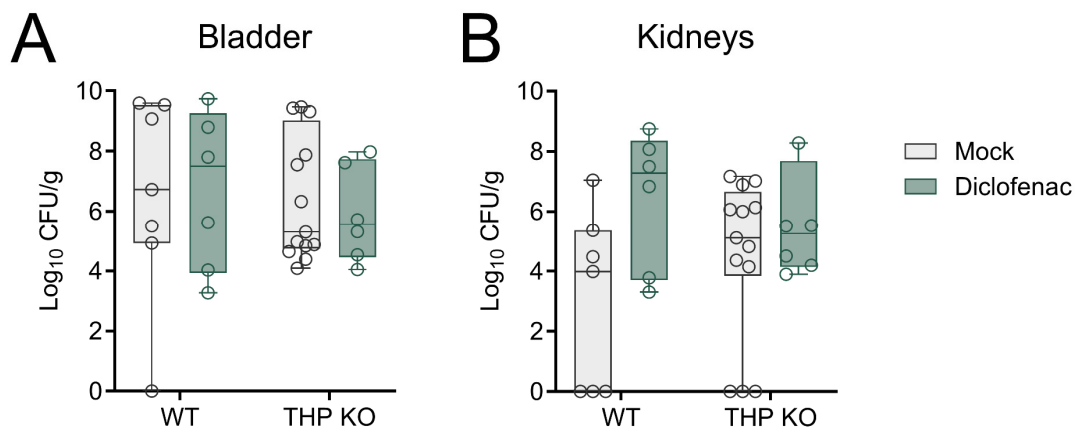
Supplementary Tables 1-2



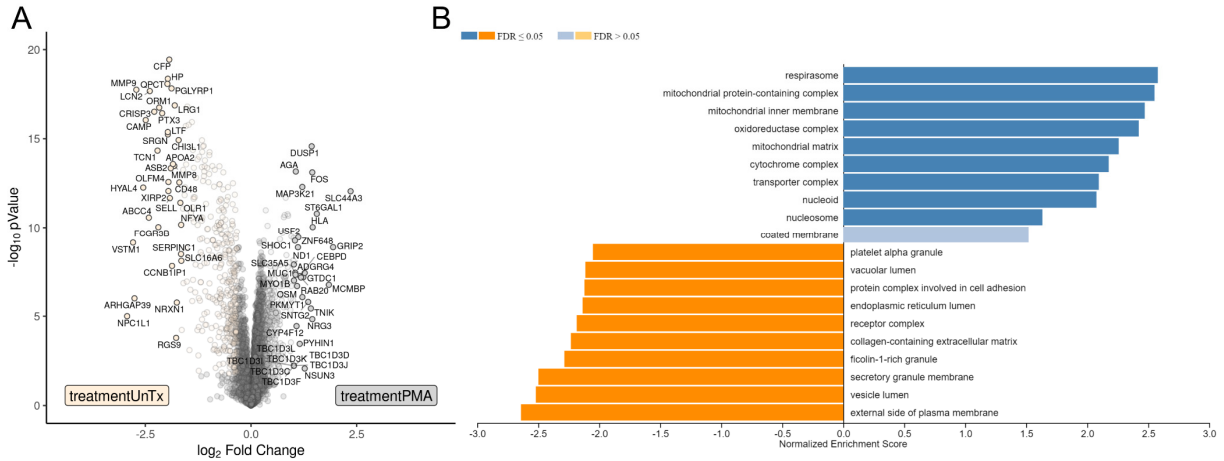
**Supplemental Figure 1. Impact of THP on other bladder immune populations during UTI.** Wild type (WT) and THP knockout (THP KO) mice were transurethally infected with 10<sup>8</sup> CFU of UPEC strain UTI89 or mock-infected as a control. Frequency (A) and total counts (B) of Ly6G<sup>+</sup> cells, frequency (C) and counts (D) of CD11b<sup>+</sup>CD11c<sup>-</sup> (lymphocytes) cells, and frequency (E) and counts (F) CD11b<sup>var</sup>CD11c<sup>var</sup> (myeloid) cells. Frequency (G) and total counts (H) of MHC-II<sup>+</sup> (antigen presenting cells) and frequency (I) and counts (J) of MHC-II<sup>-</sup> (other myeloid) cells. Frequency is expressed as a percentage of total bladder CD45<sup>+</sup> cells (A, C, E) or as a percentage of total myeloid (CD11b<sup>var</sup>CD11c<sup>var</sup>) cells (G, I). Experiments were performed at least two times with data combined,  $n = 5-23$ /group. Box and whisker plots extend from 25th to 75th percentiles, horizontal bars indicate median, and all points are shown. Data were analyzed by Mann-Whitney  $U$  test with Holm-Šidák's multiple comparisons correction. \* $P < 0.05$ , \*\*\* $P < 0.001$ . Supplemental data to **Figure 2**.



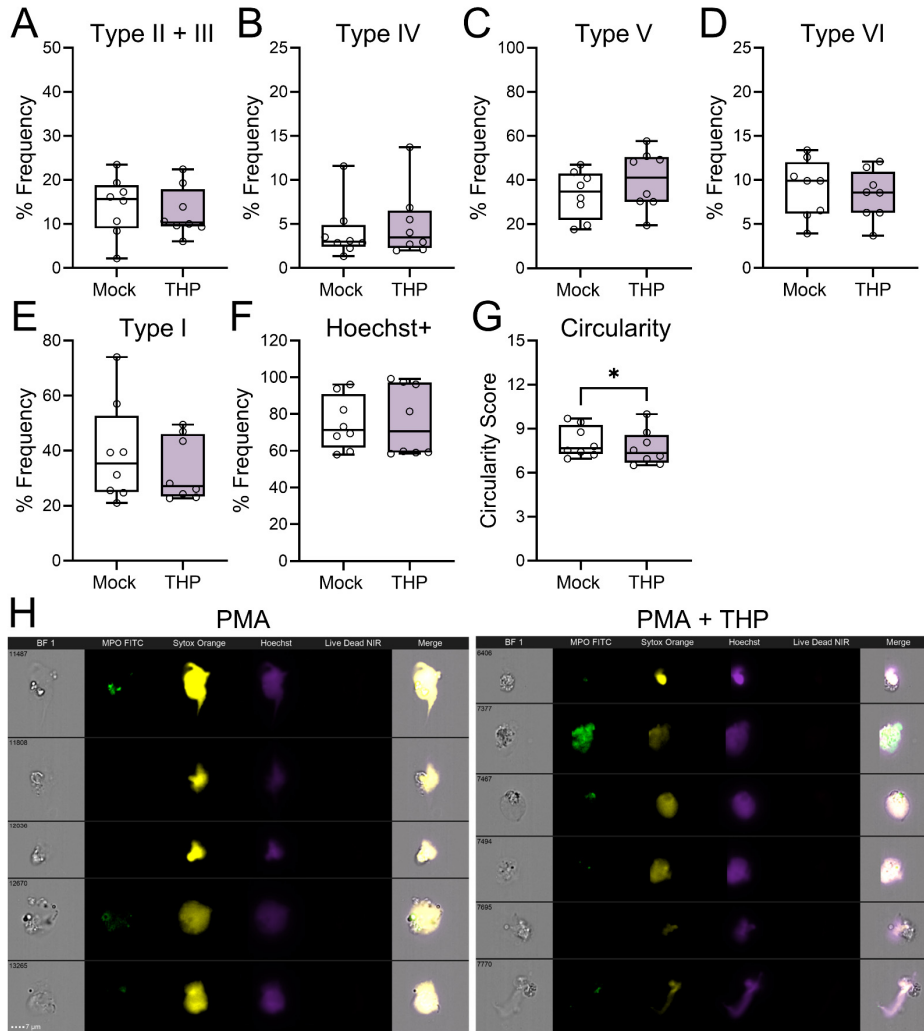
**Supplemental Figure 2. Impact of THP on other kidney immune populations during UTI.** Wild type (WT) and THP knockout (THP KO) mice were transurethrally infected with  $10^8$  CFU of UPEC strain UT189 or mock-infected as a control. Frequency (**A**) and total counts (**B**) of Ly6G- cells, frequency (**C**) and counts (**D**) of CD11b-CD11c- (lymphocytes) cells, and frequency (**E**) and counts (**F**) CD11b<sup>var</sup>CD11c<sup>var</sup> (myeloid) cells. Frequency (**G**) and total counts (**H**) of MHC-II+ (antigen presenting cells) and frequency (**I**) and counts (**J**) of MHC-II- (other myeloid) cells. Frequency is expressed as a percentage of total kidney CD45+ cells (A, C, E) or as a percentage total myeloid (CD11b<sup>var</sup>CD11c<sup>var</sup>) cells (G, I). Experiments were performed at least two times with data combined,  $n = 5-18$ /group. Box and whisker plots extend from 25th to 75th percentiles, horizontal bars indicate median, and all points are shown. Data were analyzed by Mann-Whitney  $U$  test with Holm-Šidák's multiple comparisons correction. \* $P < 0.05$ . Supplemental data to **Figure 2**.



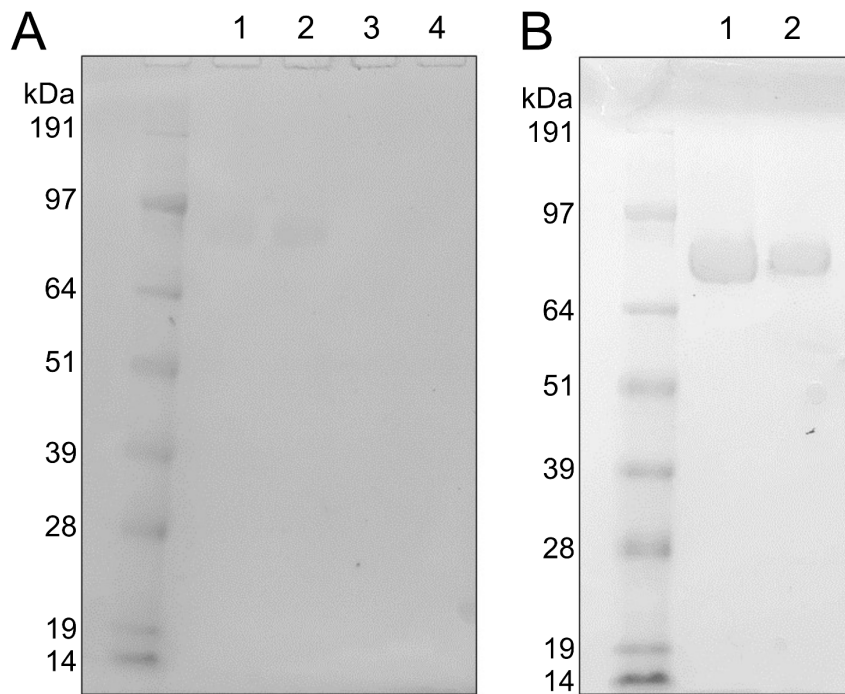
**Supplemental Figure 3. Cox-2 inhibitor Diclofenac does not alter bacterial burdens in WT or THP KO mice.** Wild type (WT) and THP knockout (THP KO) mice were transurethrally infected with  $10^8$  CFU of UPEC strain UTI89. To inhibit COX-2, mice were administered diclofenac in the drinking water beginning on day 0 and maintained through day 6 post-infection. Bladder (**A**) and kidney (**B**) UPEC burdens at 7 days post-infection. Experiments were performed at least two times with data combined,  $n = 6-13$ /group. Box and whisker plots extend from 25th to 75th percentiles, horizontal bars indicate median, and all points are shown. Data were analyzed by two-way ANOVA with Sidak's multiple comparisons test and all comparisons were determined not significant ( $P > 0.05$ ). Supplemental data to **Figure 2**.



**Supplemental Figure 4. PMA stimulation alters the neutrophil proteome.** Peripheral human neutrophils were isolated, pretreated with 50  $\mu\text{g}/\text{mL}$  THP, and stimulated with phorbol 12-myristate 13-acetate (PMA) for 2.5 hours. Volcano plot (A) and gene set enrichment analysis (B) of differentially identified proteins in untreated vs. PMA stimulated samples. Experiments were performed as part of one independent experiment,  $n = 4$  donors. Differential proteins were identified via  $\text{Log}_2$  fold change  $>1.25$  and moderated t-test followed by multiple-hypothesis testing correction using the Benjamini–Hochberg procedure with a false discovery rate adjusted  $P < 0.05$ . GSEA was performed with a gene set minimum of 10, a gene set maximum of 500, 2,000 permutations using the gene ontology cellular component gene sets. Supplemental data to proteomic analyses in **Figure 6**.



**Supplemental Figure 5. Additional effects of THP on cellular morphologies as determined by imaging flow cytometry.** Peripheral human neutrophils were isolated, pretreated with THP and mock-stimulated (A-G) or stimulated with phorbol 12-myristate 13-acetate (PMA) for 2.5 hours (H). Cells were stained with anti-MPO FITC, Sytox Orange (non-membrane permeable nucleic acid dye), Hoechst (membrane-permeable nucleic acid dye), and Live/Dead stain (non-membrane permeable amine-reactive dye) and visualized for fluorescence and brightfield (BF) images on an imaging flow cytometer. Frequency of NETs and NET precursors (Type III and II) (A), NETs fragments (Type IV) (B), dead cells with condensed nuclei (Type V) (C), dead cells with decondensed nuclei (Type VI) (D), and live cells (Type I) (E). (F) Frequency of Hoechst+ cells. (G) Circularity scores (degree of the Hoechst mask deviation from a circle, with lower values having more deviation) across groups. (H) Additional representative images of Type III NETs from PMA and PMA + THP treatment groups showing variable levels of MPO staining across groups. Experiments were performed in eight independent experiments with data combined,  $n = 8$  donors. Box and whisker plots extend from 25th to 75th percentiles, horizontal bars indicate median, and all points are shown (A-G). Data were analyzed by Mann-Whitney  $U$  test (A-G). \* $P < 0.05$ . Supplemental data to **Figure 9**.



**Supplemental Figure 6. Representative protein gels of THP isolated from mouse and human urine.** (A) Polyacrylamide protein gel of THP isolated from pooled mouse urine as described in Methods. Lane 1: WT mock-infected, Lane 2: WT UPEC-infected, Lane 3: THP KO mock-infected, Lane 4: THP KO UPEC-infected. THP was visualized as a single band at ~ 85 kDa in lanes 1 and 2. (B) Polyacrylamide protein gel of THP isolated from pooled human urine as described in Methods. Lane 1: pooled batch #1, Lane 2: pooled batch #2. THP was visualized as a single band at ~ 85 kDa in lanes 1 and 2.

**Supplemental Table 1. Comparative N-glycan MALDI-tof analyses of THP during UPEC infection.** N-glycan MALDI-tof peaks of THP purified from urine of WT mice that were either mock-infected or UPEC-infected. Data represent one MALDI-tof analysis of pooled purified THP harvested from two independent experiments. Supplemental data to **Figure 3**.

m/z peak	WT Mock	WT UPEC	% change
2244.459	1.93	1.31	-47.1
2431.552	4.24	3.59	-18.1
2605.648	8.40	9.00	6.6
2792.735	2.47	2.75	10.2
2966.841	14.28	17.17	16.9
3241.968	4.00	4.46	10.1
3416.062	8.41	13.05	35.6
3777.244	17.42	17.32	-0.5
4226.453	14.22	16.24	12.5
4587.622	24.64	15.11	-63.1

**Supplemental table 2. Differentially abundant proteins between THP-treated and mock-treated human neutrophils in unstimulated and PMA-stimulated conditions.** Supplemental data to **Figure 6**.

Gene ID	Gene Symbol	Gene Description	Log <sub>2</sub> Fold-Change Unstimulated <sup>a</sup> (p-value) <sup>c</sup>	Log <sub>2</sub> Fold-Change Stimulated <sup>b</sup> (p-value)
7369	UMOD <sup>d</sup>	uromodulin	2.894 <b>(2.14E-12)</b>	2.497 <b>(3.77E-11)</b>
347	APOD <sup>d</sup>	apolipoprotein D	2.112 <b>(2.14E-12)</b>	1.200 <b>(6.47E-09)</b>
55902	ACSS2	acyl-CoA synthetase short-chain family member 2	0.684 <b>(7.73E-08)</b>	0.466 <b>(1.27E-05)</b>
23506	BICRAL	GLTSCR1-like	0.669 <b>(0.039)</b>	0.032 (0.962)
3959	LGALS3BP <sup>d</sup>	lectin, galactoside-binding, soluble, 3 binding protein	0.624 <b>(2.70E-09)</b>	0.533 <b>(2.62E-08)</b>
3827	KNG1 <sup>d</sup>	kininogen 1	0.612 <b>(7.79E-07)</b>	0.485 <b>(1.58E-05)</b>
2731	GLDC	glycine dehydrogenase (decarboxylating)	0.539 <b>(0.017)</b>	0.445 (0.052)
259	AMBP <sup>d</sup>	alpha-1-microglobulin/bikunin precursor	0.488 <b>(3.02E-05)</b>	0.647 <b>(1.47E-06)</b>
220963	SLC16A9	solute carrier family 16, member 9	0.485 <b>(4.93E-04)</b>	0.364 <b>(0.005)</b>
3561	IL2RG	interleukin 2 receptor, gamma	0.456 <b>(3.02E-05)</b>	0.325 <b>(0.001)</b>
10621	POLR3F	polymerase (RNA) III (DNA directed) polypeptide F, 39 kDa	0.422 <b>(0.030)</b>	0.184 (0.445)
952	CD38	CD38 molecule	0.382 <b>(0.017)</b>	0.142 (0.487)
55647	RAB20	RAB20, member RAS oncogene family	0.554 <b>(0.046)</b>	0.088 (0.848)
440275	EIF2AK4	eukaryotic translation initiation factor 2 alpha kinase 4	0.352 <b>(1.66E-06)</b>	0.201 (0.001)
107987285	LOC107987285	uncharacterized protein	0.344 <b>(0.042)</b>	0.030 (0.928)
8225	GTPBP6	GTP binding protein 6 (putative)	0.221 (0.380)	0.433 <b>(0.030)</b>
1521	CTSW	cathepsin W	0.216 (0.605)	-0.704 <b>(0.011)</b>
3576	CXCL8	chemokine (C-X-C motif) ligand 8	0.204 (0.409)	0.406 <b>(0.038)</b>
54810	GIPC2	GIPC PDZ domain containing family, member 2	0.167 (0.523)	0.410 <b>(0.030)</b>
9698	PUM1	pumilio RNA-binding family member 1	0.160 (0.515)	0.392 <b>(0.028)</b>
722	C4BPA	complement component 4 binding protein, alpha	0.109 (0.807)	-0.529 <b>(0.019)</b>

91272	BOD1	bioorientation of chromosomes in cell division 1	0.104 (0.666)	0.354 <b>(0.018)</b>
678	ZFP36L2	ZFP36 ring finger protein-like 2	0.103 (0.510)	0.357 <b>(0.002)</b>
8031	NCOA4	nuclear receptor coactivator 4	0.055 (0.805)	0.328 <b>(0.003)</b>
966	CD59	CD59 molecule, complement regulatory protein	0.050 (0.760)	0.369 <b>(1.42E-04)</b>
1774	DNASE1L1	deoxyribonuclease I-like 1	0.045 (0.768)	0.332 <b>(2.02E-04)</b>
5150	PDE7A	phosphodiesterase 7A	0.041 (0.906)	0.425 <b>(0.003)</b>
79080	CCDC86	coiled-coil domain containing 86	0.024 (0.974)	0.545 <b>(0.030)</b>
1871	E2F3	E2F transcription factor 3	-0.018 (0.958)	0.400 <b>(0.002)</b>
197258	FCSK	fucokinase	-0.069 (0.713)	0.385 <b>(0.001)</b>
129446	XIRP2	xin actin binding repeat containing 2	-0.085 (0.768)	-0.528 <b>(0.001)</b>
7915	ALDH5A1	aldehyde dehydrogenase 5 family, member A1	-0.348 <b>(0.031)</b>	-0.094 (0.696)
10422	UBAC1	UBA domain containing 1	-0.378 <b>(0.017)</b>	0.048 (0.861)
10193	RNF41	ring finger protein 41, E3 ubiquitin protein ligase	-0.482 <b>(0.038)</b>	-0.104 (0.783)

<sup>a</sup>Fold-change between THP-treated and mock-treated samples in unstimulated conditions.

<sup>b</sup>Fold-change between THP-treated and mock-treated samples in PMA-stimulated conditions.

<sup>c</sup>Differential proteins were identified via Log<sub>2</sub> fold change >1.25 and moderated 2-tailed t-test followed by multiple-hypothesis testing correction using the Benjamini-Hochberg procedure with a false discovery rate adjusted  $P < 0.05$ . Proteins meeting these criteria are indicated by p-values in bold text.

<sup>d</sup>Detected in purified THP by mass spectrometry. See **Supplemental Table 4**.