Uric acid priming in human monocytes is driven by the AKT–PRAS40 autophagy pathway

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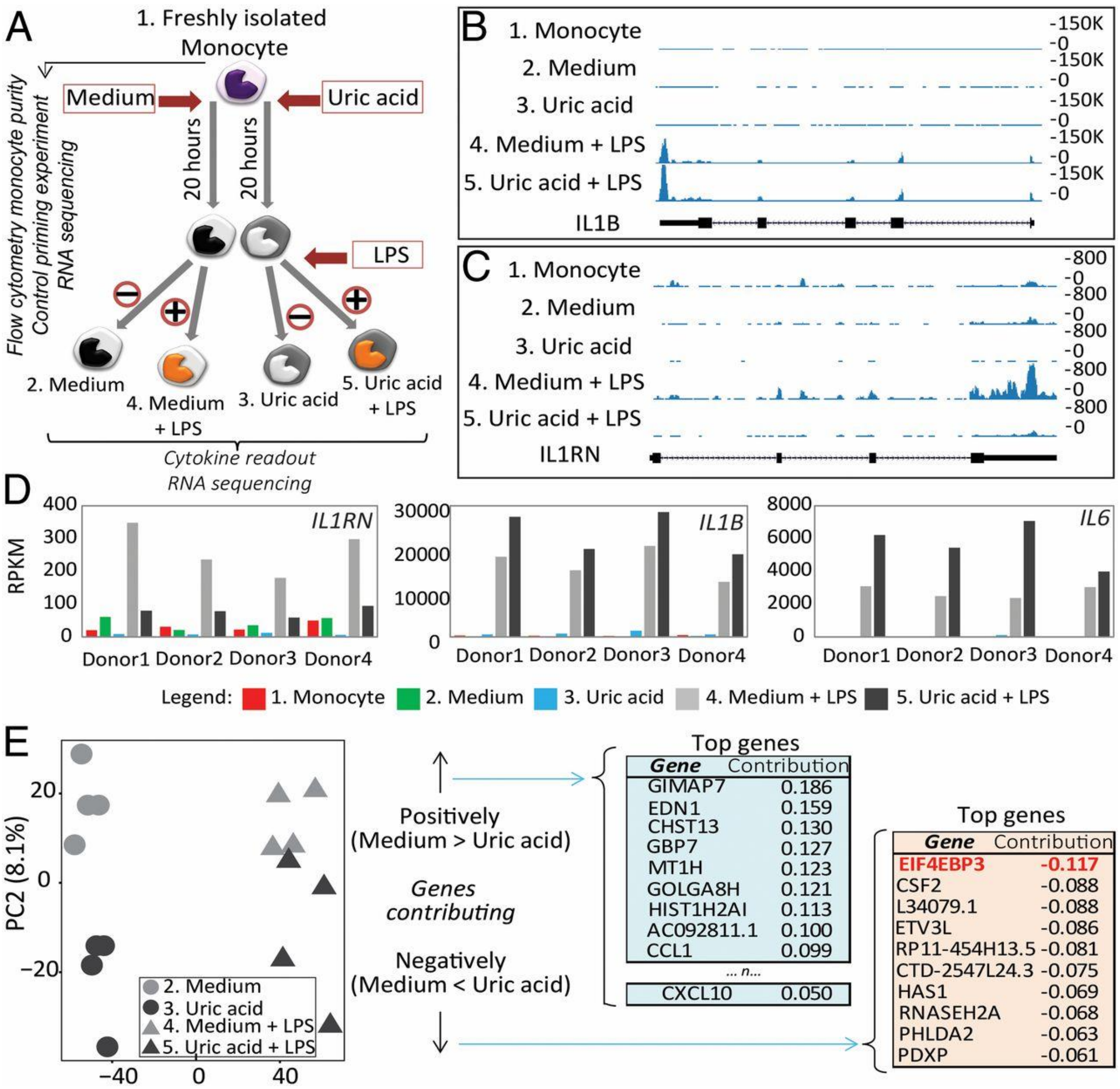
Rheumatology Journal Club

Background

- Uric acid is the end product of purine metabolism in humans (and other hominoids).
- Hyperuricemia, UA levels > 6.8mg/dL, can cause gouty arthritis.
- MSU crystals bind nod-like receptors and cause production of proinflammatory IL-1β.
- Hyperuricemia also known to be associated with a number of diseases involving an inflammatory component, e.g., atherosclerosis.
- Previous work by this group demonstrated that soluble uric acid primes PBMCs by stimulating IL-1β, Il-6 production, and lowering production of anti-inflammatory IL-1Ra.
- Here the authors are exploring these results in more detail, trying to ascertain the mechanism by which soluble UA is upregulating cytokine production.
- Appears to involve elements of the autophagy system.

Transcriptomic analysis in uric acid-primed monocytes.



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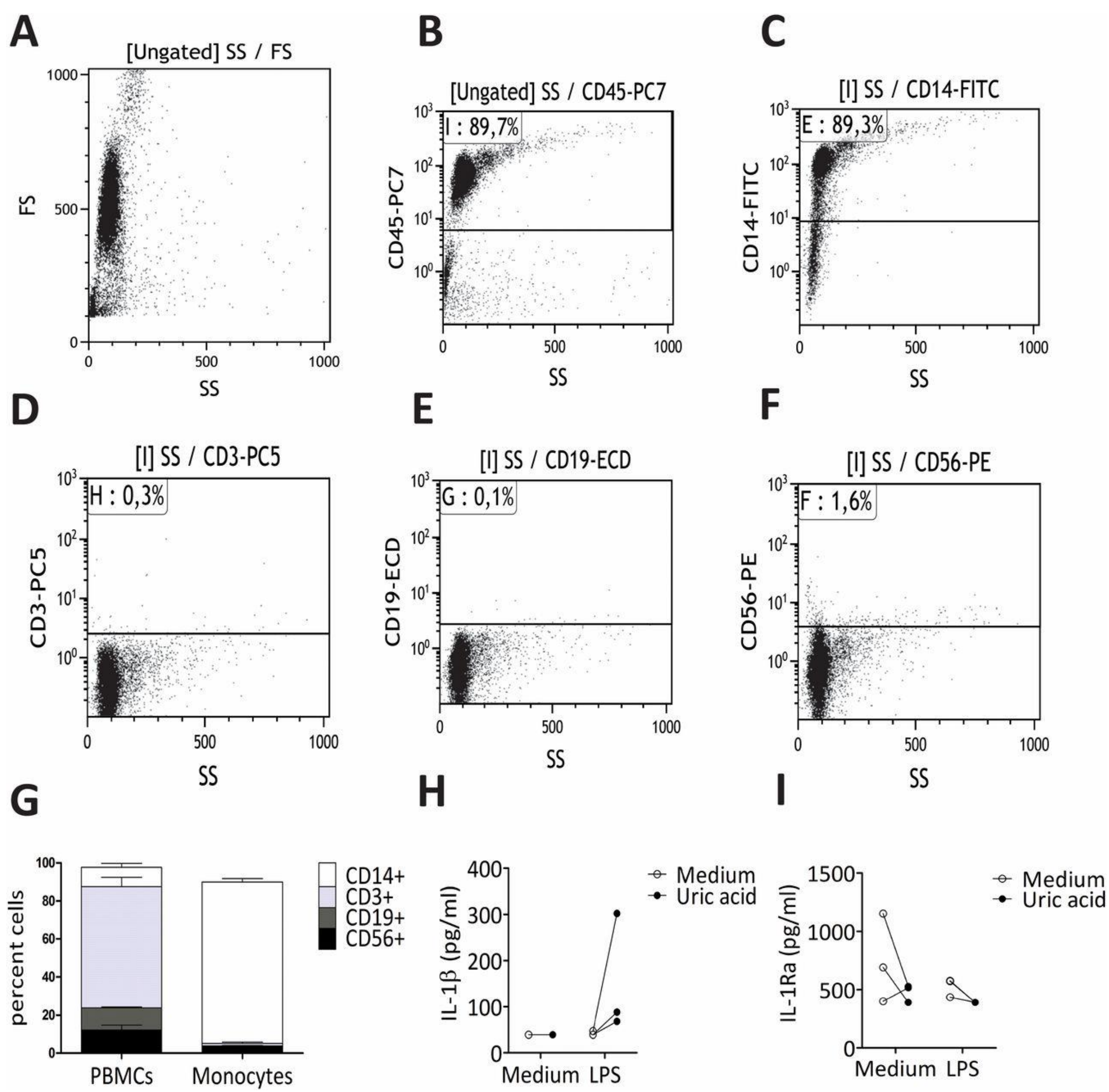
Flow cytometry and cytokine control data for negatively selected monocytes.



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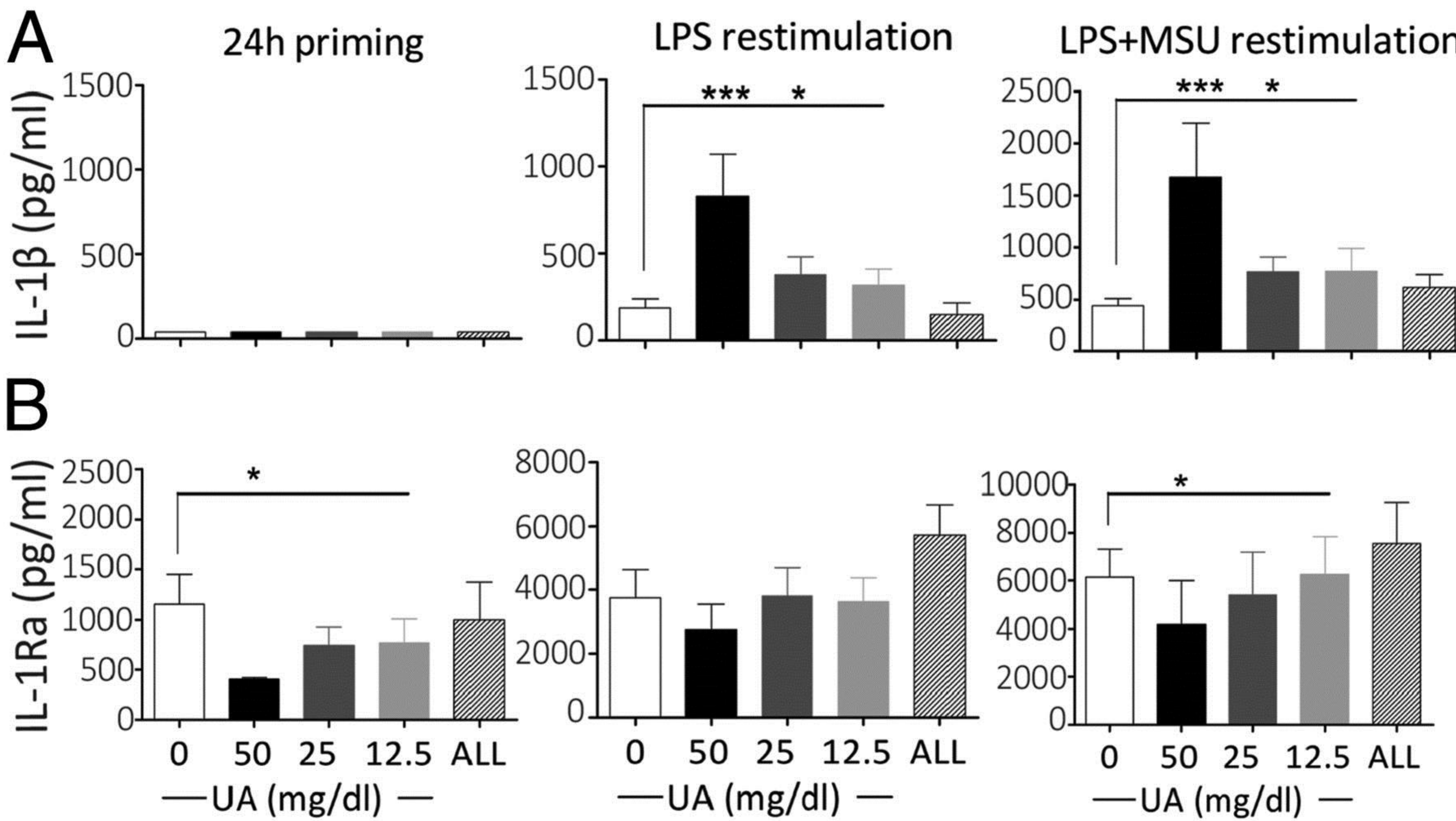
Enhanced IL-1β and diminished IL-1Ra production in uric acid-primed monocytes.



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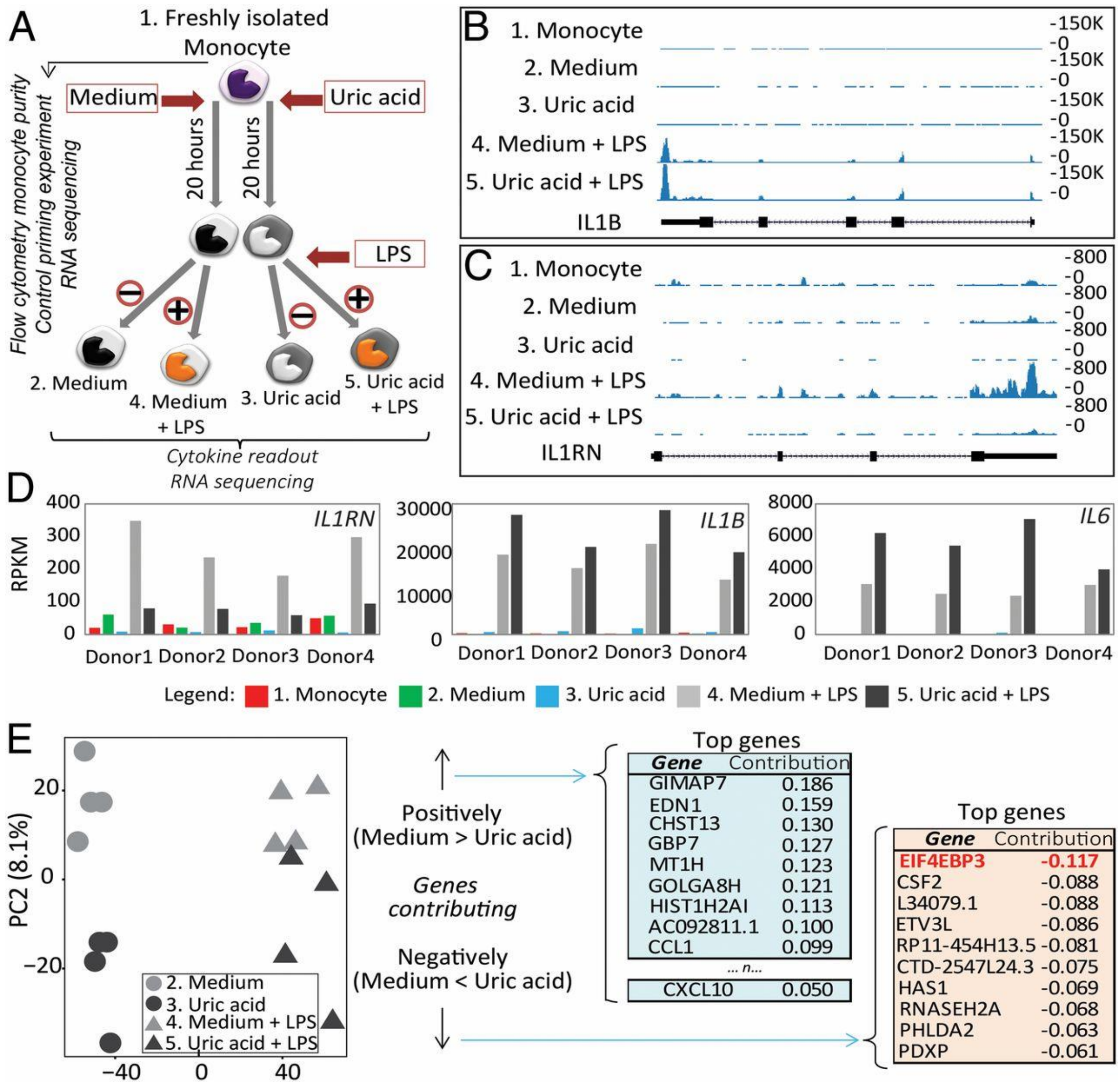

Gene	Contribution
GIMAP7	0.186
EDN1	0.159
CHST13	0.130
GBP7	0.127
MT1H	0.123
GOLGA8H	0.121
HIST1H2AI	0.113
AC092811.1	0.100
CCL1	0.099
... n...	
CXCL10	0.050

Top genes

Gene	Contribution
EIF4EBP3	-0.117
CSF2	-0.088
L34079.1	-0.088
ETV3L	-0.086
RP11-454H13.5	-0.081
CTD-2547L24.3	-0.075
HAS1	-0.069
RNASEH2A	-0.068
PHLDA2	-0.063
PDXP	-0.061

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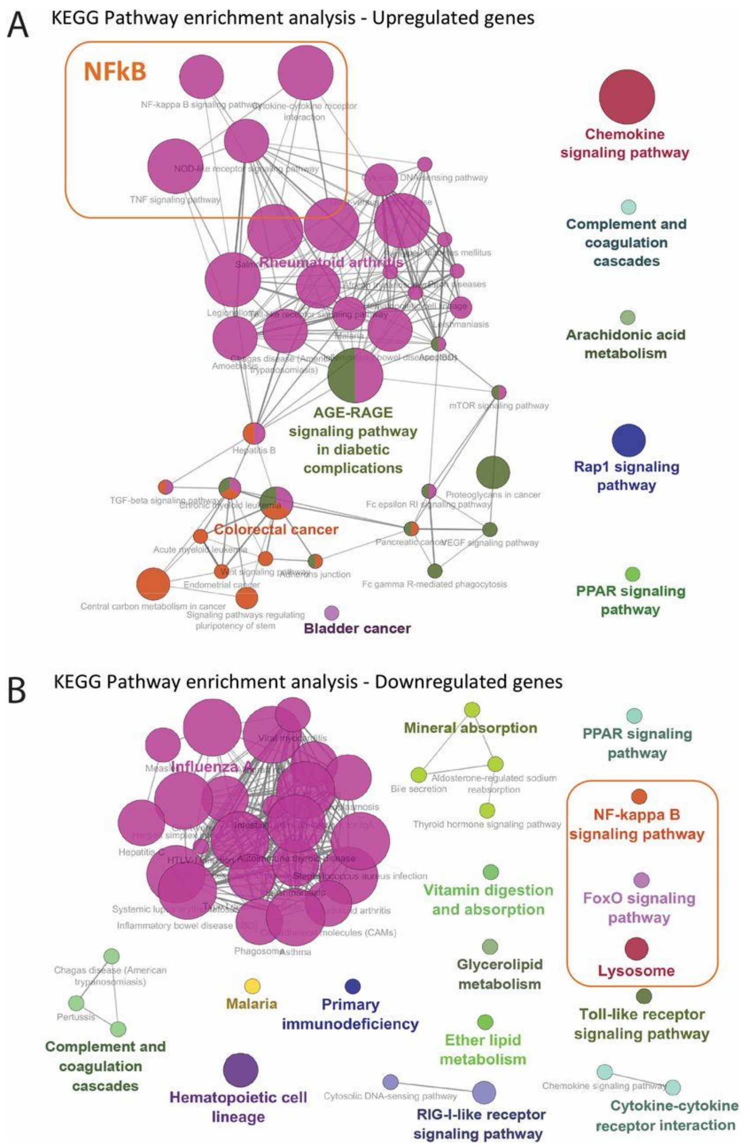
Pathway enrichment analysis was performed using significantly regulated genes and top 100 contributors to PC2 (Fig. 2E).



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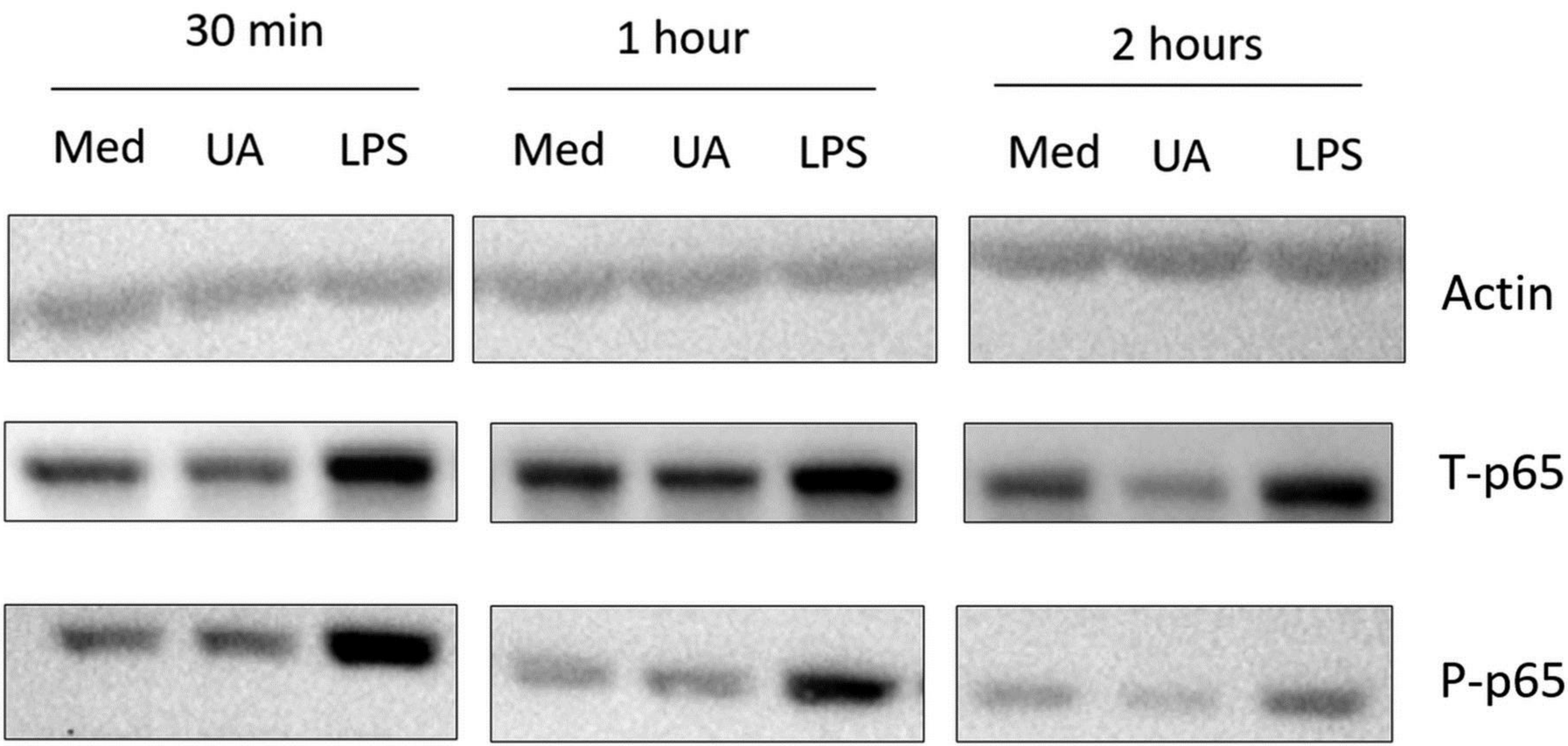
Western blot of the p65 component of NF-κB in uric acid priming.



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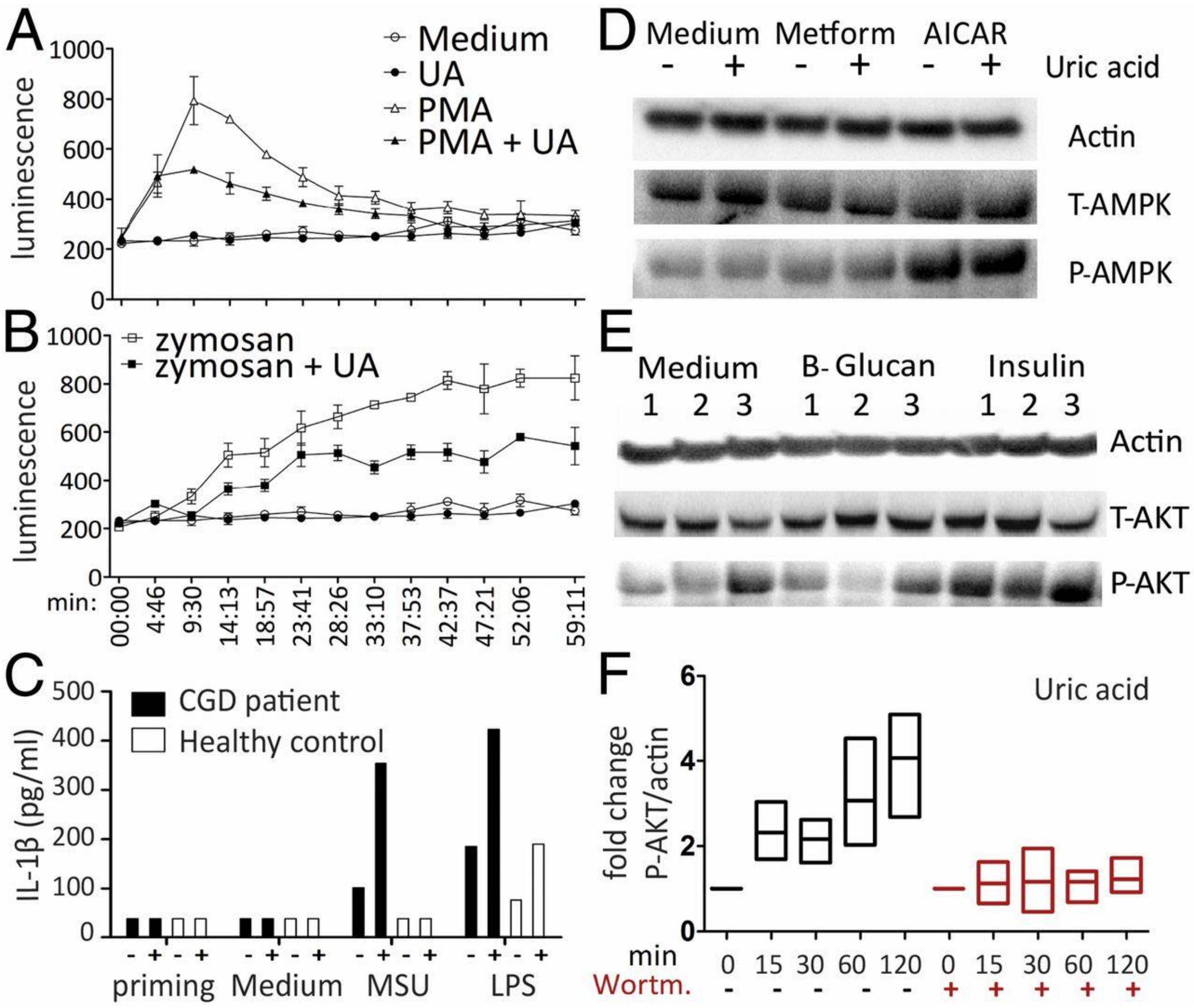
Phosphorylation of AKT and lack of ROS or AMPK induction by uric acid.



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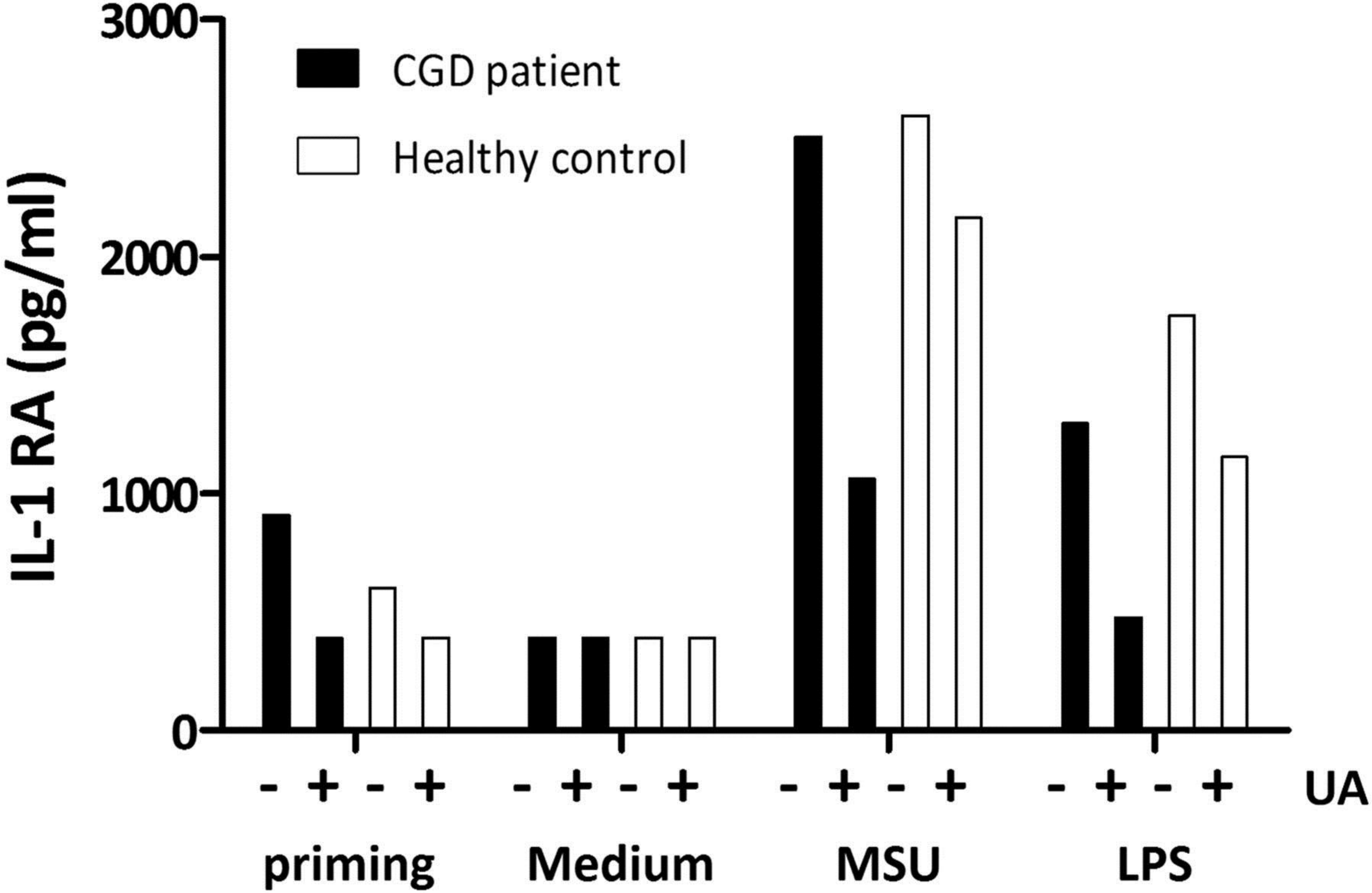
PBMCs of a CGD patient and control were isolated, and 0.5 × 106 cells were stimulated per well with 300 μg/mL MSU or 10 ng/mL LPS after priming with medium or uric acid, and IL-1RA levels were measured by ELISA.



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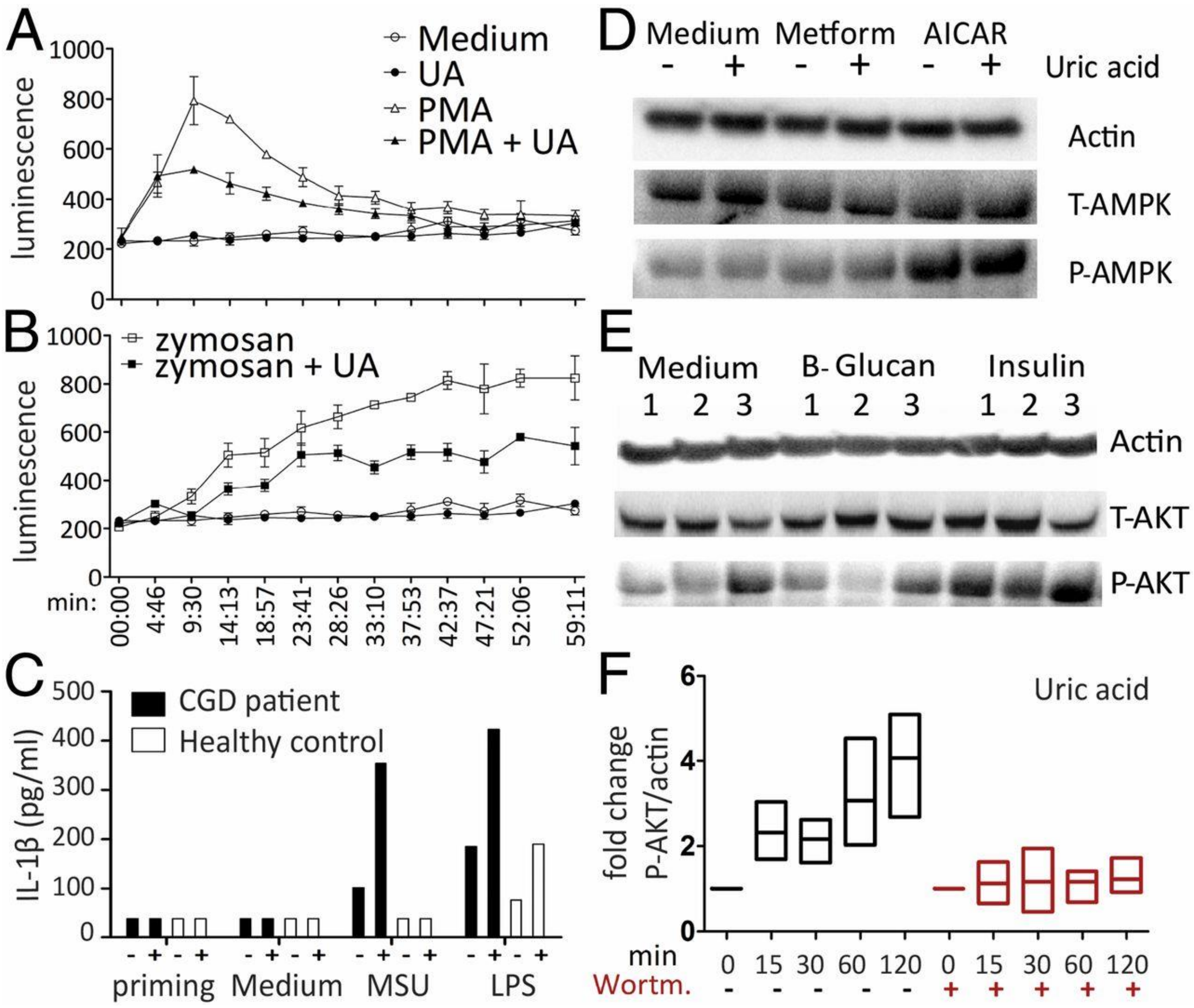

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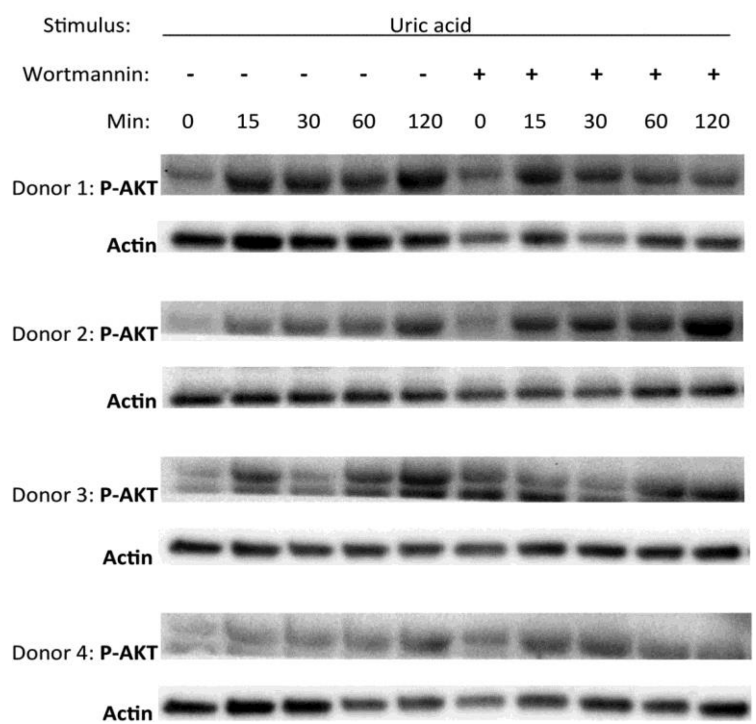
The 106 monocytes were treated with 50 mg/dL uric acid for increasing durations in the presence or absence of 100 nM wortmannin in four donors.

Uric acid		P-AKT/Actin			
Wortmannin	**Min**	Donor 1	Donor 2	Donor 3	Donor 4
-	**0**	1,00	1	1,00	1,00
-	**15**	1,97	1,69	3,04	2,58
-	**30**	2,62	1,95	1,61	2,47
-	**60**	2,25	2,03	3,44	4,54
-	**120**	3,44	2,68	5,06	5,10
+	**0**	1,00	1,00	1,00	1,00
+	**15**	1,63	1,51	0,66	0,72
+	**30**	1,94	1,65	0,46	0,62
+	**60**	1,24	1,34	1,41	0,69
+	**120**	1,28	1,72	0,92	0,99



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Negatively selected monocytes were primed with or without uric acid for 24 h, followed by 7 min of short exposure to high concentrations of 1 μg/mL LPS. Cells were lysed and lysates were exposed to phosphokinase array spotted membranes to evaluate the downs...

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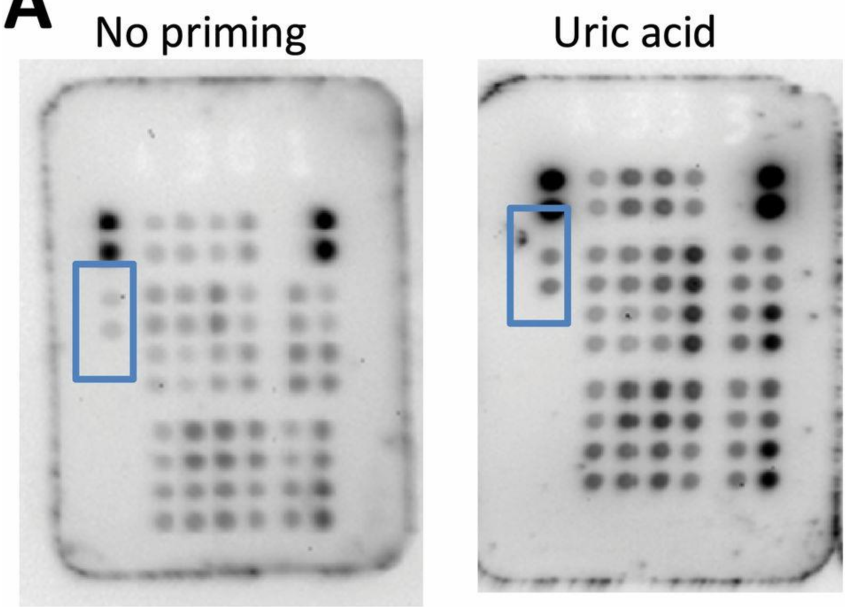

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Kinase	Site	%change
Fyn	Y420	**-77**
HcK	Y411	**-76**
p27	T198	**-73**
GSK-3 alpha/beta	S21/S9	**42**
ERK 1/2	T202/Y204, T185/Y187	**54**
PRAS40	T246	**72**

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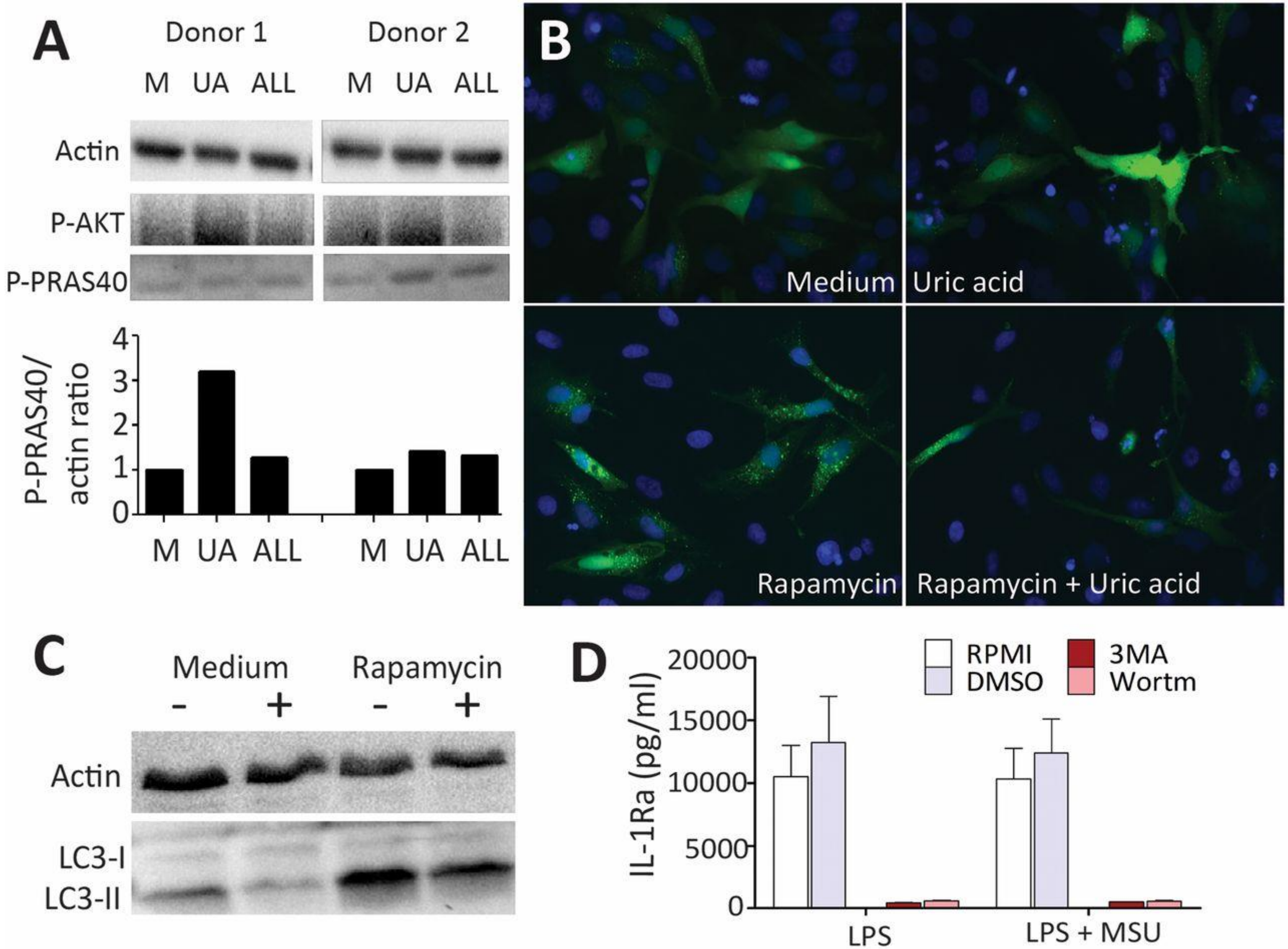
Identification of PRAS40 as intermediate in AKT signal transduction to inhibit autophagy in uric acid-primed cells.



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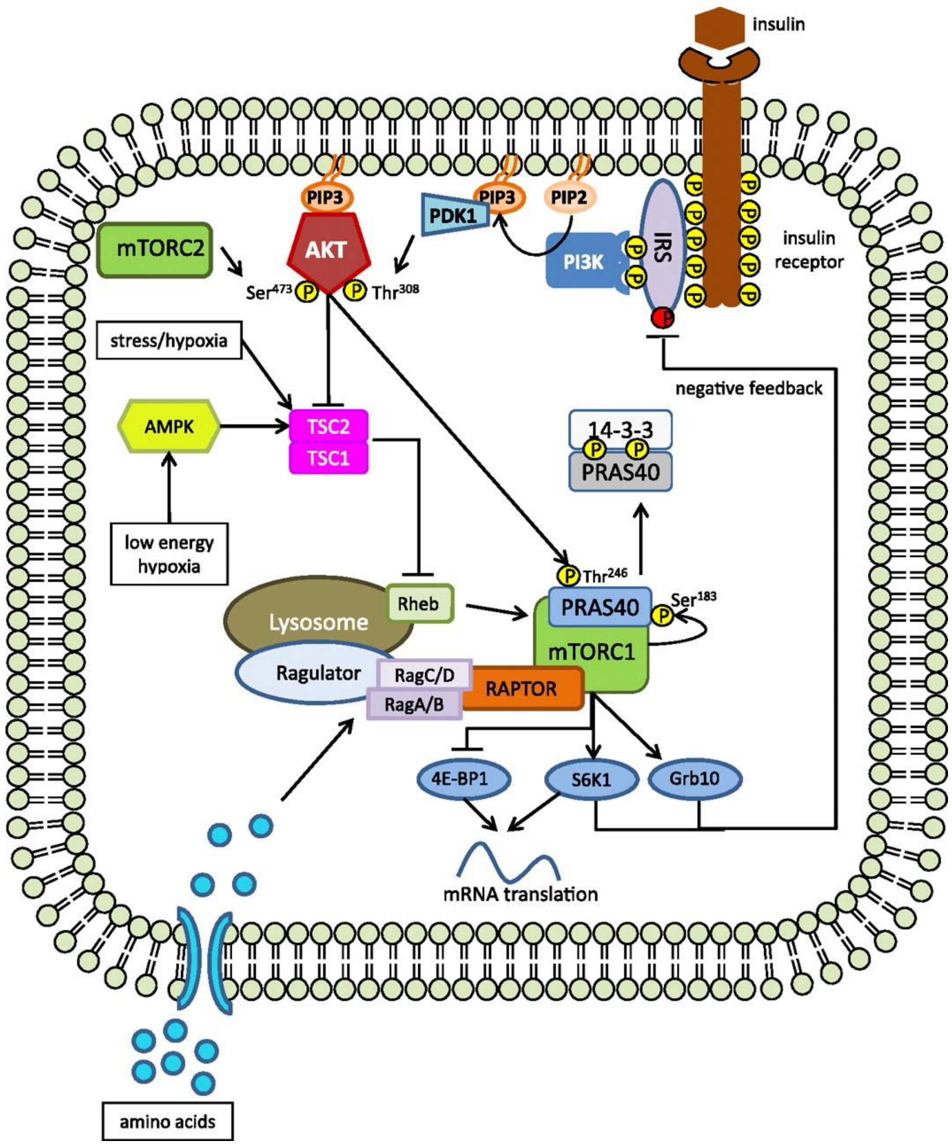
Take homes

- The authors have suggested a autophagy mechanism for the increased proinflammatory and decreased anti-inflammatory cytokine production which occurs in response to uric acid.
- Apparently the mechanism is via the AKT signaling pathway.
  - Pathway analysis implicated mTOR signaling (downstream of AKT).
  - A top hit from the RNA-seq experiment was overexpressed in uric acid treated cells and involved mTOR.
  - Uric acid exhibited modulatory effects on several components of the AKT pathway, i.e., AKT, PRAS40, autophagosome formation.
- Uric acid stimulated monocytes may inhibit autophagy.
  - This seems to be manifested by known downstream effects on increased production of IL-1β, and decreased anti-inflammatory IL1Ra.

Regulation of PRAS40 phosphorylation by Akt and mTORC1.



Claudia Wiza et al. Am J Physiol Endocrinol Metab
2012;302:E1453-E1460

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