JBC/2012/355917, REVISED. **SUPPLEMENTAL DATA**

LAPTM5 is a Positive Regulator of Pro-inflammatory signaling pathways in Macrophages

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Supplementary Figure S1. Endogenous expression of LAPTM5 in macrophages. (A) Confocal fluorescence analysis of RAW264.7 cells transfected with control or LAPTM5 siRNA. At 72h post transfection, cells were fixed and immunostained with anti-LAPTM5 antibody. Bar, 5 μm. **(B)** Knockdown of LAPTM5 using shRNA. Western blot analysis of RAW264.7 cell lines stably expressing control or LAPTM5 shRNA. Cell lysates were immunoblotted with anti-LAPTM5 or actin antibodies as a control for protein loading

Supplementary Figure S2. LAPTM5 does not co-localize with transferrin. RAW264.7 cells were serum-starved for 2h in DMEM and subsequently incubated with 50 μg/ml Alexa 488-labeled transferrin at 37ºC. After 60min uptake, excess transferrin was washed away and cells were fixed and immunostained with antibodies towards LAPTM5. Representative confocal images are shown. The merged panel shows an overlay of the two channels. Bar, 5 μm. Quantification of co-localization between LAPTM5 and transferrin was performed on randomly selected cells using Volocity 5.4.1 software and expressed as Colocalization Coefficient M2 = 0.221, r(20), $p>0.10$ (corresponding to the degrees of freedom (r) and level of significance (p)).

Supplementary Figure S3. A20 is upregulated in LAPTM5-deficient RAW264.7 cells. Stable RAW264.7 cells expressing control or LAPTM5 shRNA were stimulated with **(A)** 1μg/ml LPS or **(B)** 50 ng/ml TNFα for the indicated time points. Cells were lysed and analyzed by immunoblotting with antibodies towards A20 and actin as a control for protein loading. Note increased basal A20 expression in RAW264.7 cells expressing shRNA against LAPTM5 compared to control cells, indicated by the asterisks. Panels on the right show quantification of basal A20 protein levels relative to actin from control (white column) and LAPTM5 knockdown (black column) RAW264.7 cells. The graph indicates the increase of A20 protein in LAPTM5 knockdown relative to control cells and is presented as mean ± SD of three independent experiments.

Figure S1

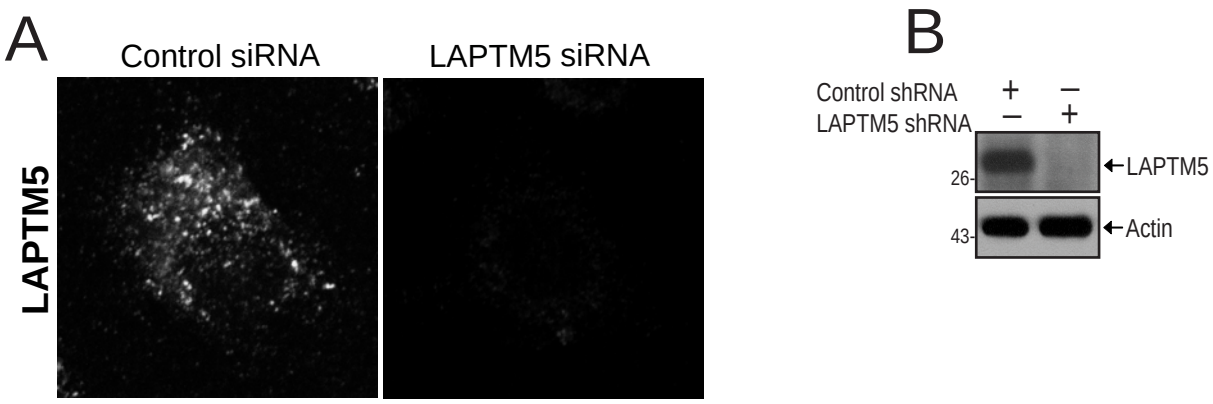

A
Control siRNA
LAPTM5 siRNA
LAPTM5
B
Control shRNA + −
LAPTM5 shRNA − +
26-
43-
LAPTM5
Actin

Figure S2

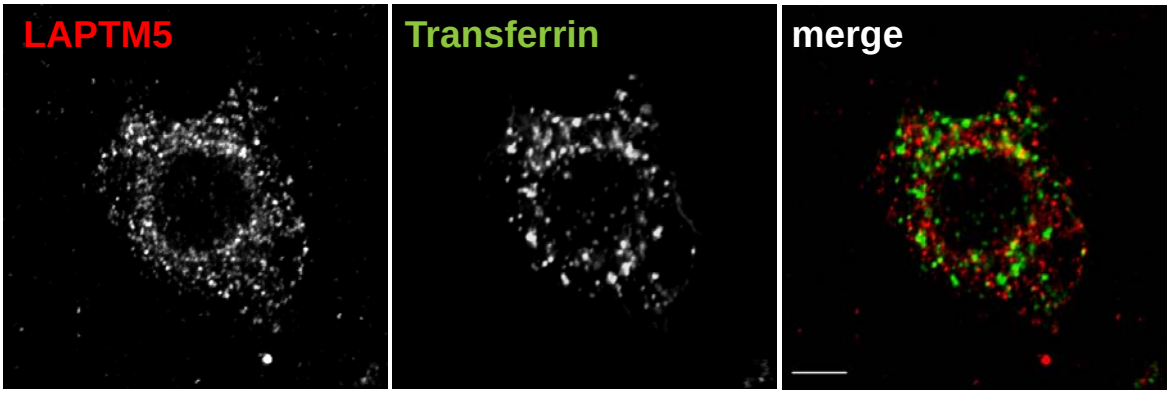
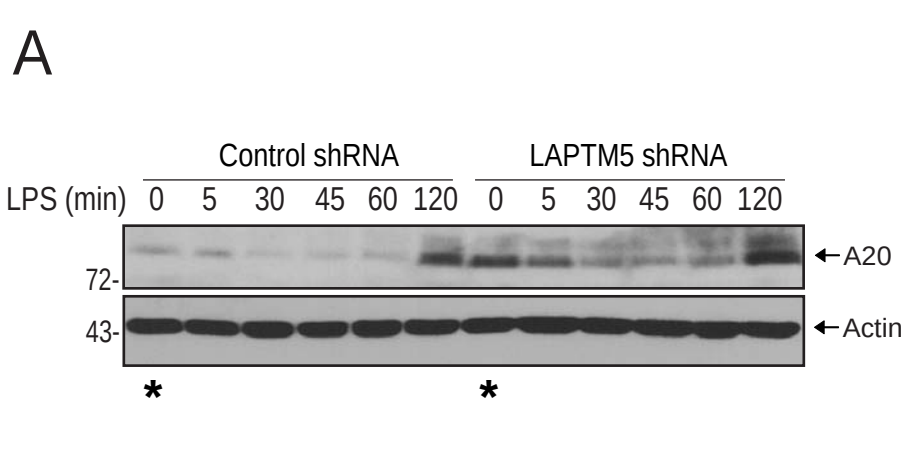
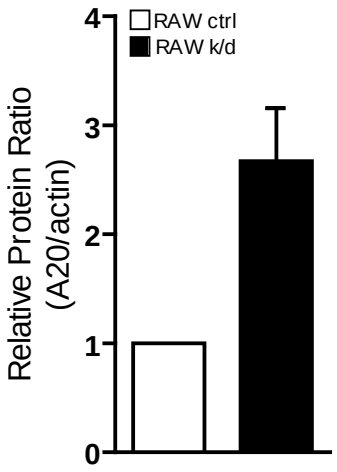
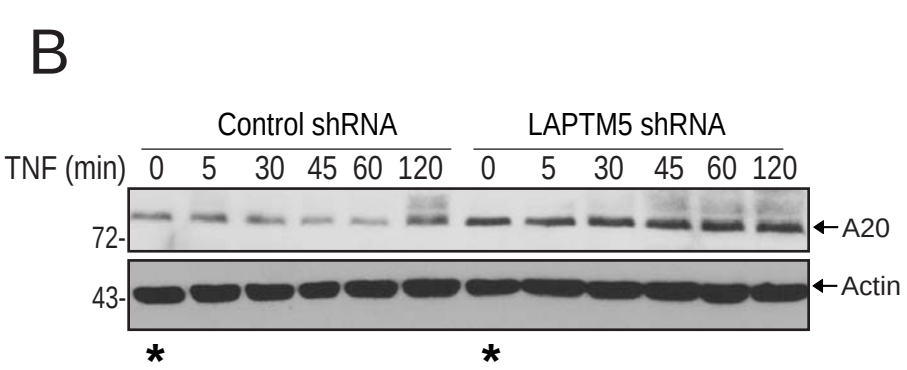
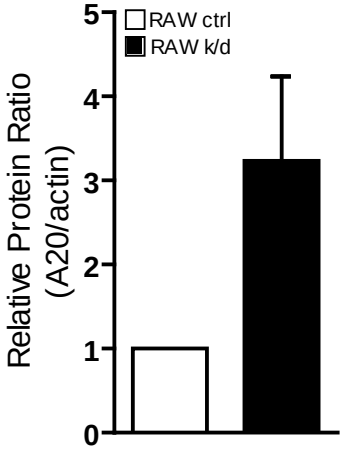


Figure S3


A
Control shRNA
LAPTM5 shRNA
LPS (min) 0 5 30 45 60 120 0 5 30 45 60 120
72-
43-
A20
Actin
*
*


RAW ctrl
RAW k/d
Relative Protein Ratio
(A20/actin)
0
1
2
3
4


B
Control shRNA
LAPTM5 shRNA
TNF (min) 0 5 30 45 60 120 0 5 30 45 60 120
72-
43-
A20
Actin
*
*


RAW ctrl
RAW k/d
Relative Protein Ratio
(A20/actin)
0
1
2
3
4
5