Supplemental Materials

Molecular Biology of the Cell

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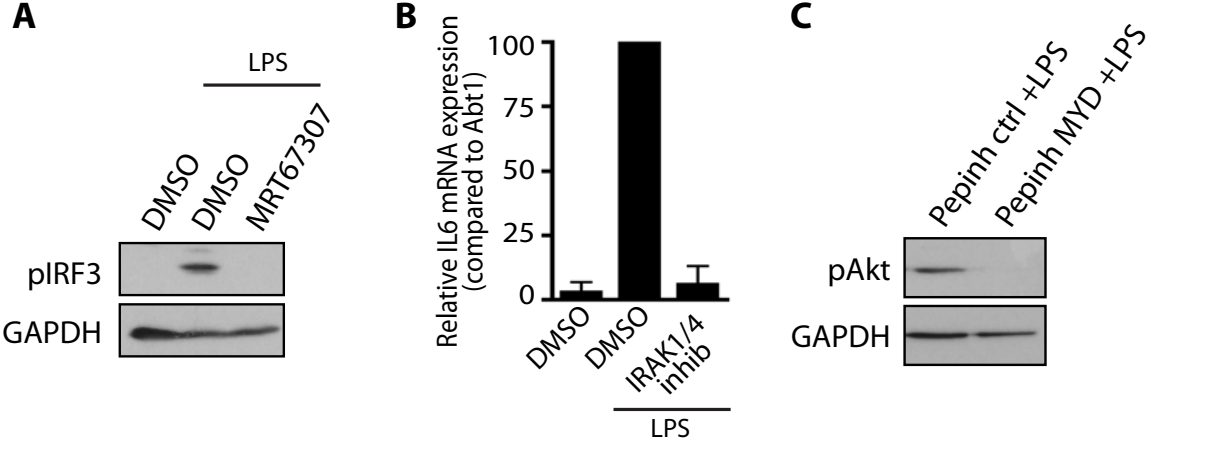
Supplementary Figure 1. MRT67307, IRAK1/4 inhibitor and Pepinh-MYD inhibit pathways downstream of their targets. (A) Western blot of RAW whole-cell lysates from conditions indicated above the lanes. Note that LPS induces phospho-IRF3, while pre-treatment with the TBK1 inhibitor MRT67307 followed by LPS stimulation blocked phosphorylation of IRF3, the downstream substrate of TBK1. GAPDH was probed as a loading control. (B) Relative expression of IL6 mRNA in RAW cells treated with DMSO as control or pre-treated for 20 min with DMSO or IRAK1/4 inhibitor followed by 2 h LPS. Note the strong induction of IL6 transcription with LPS and the block in IL6 transcription with the IRAK1/4 inhibitor. Data are mean ± SEM from 3 individual experiments where expression is relative to LPS condition (C) Western blot of RAW whole-cell lysates. Cells were incubated with either Pepinh-ctrl or Pepinh-MYD for 3 h followed by LPS stimulation for 2 h. MyD88 inhibition with Pepinh-MYD strongly blocks phosphorylation of Akt in response to LPS. GAPDH was probed as a loading control.

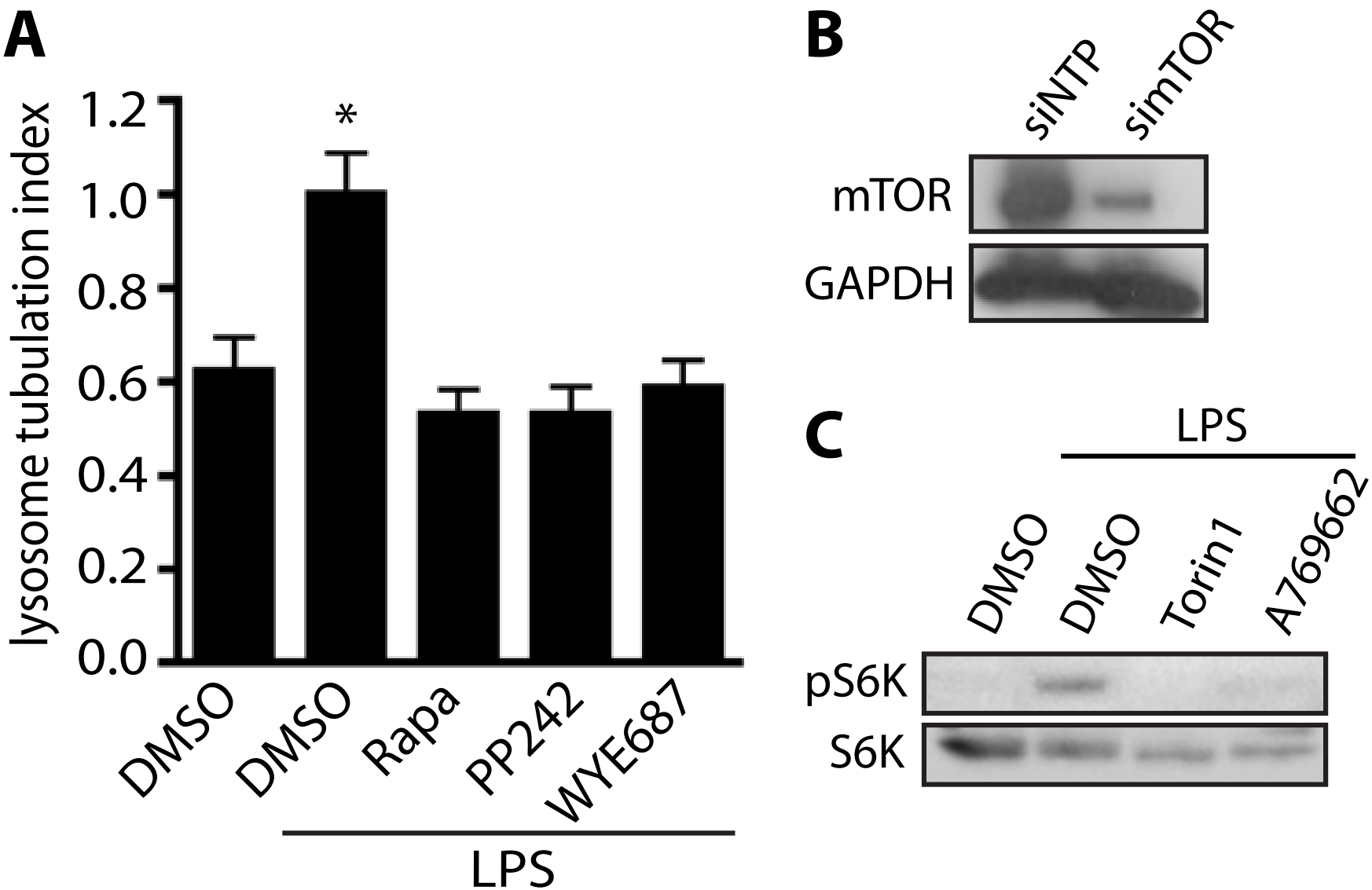
Supplementary Figure 2. mTOR activity is required for lysosome tubulation. (A) Multiple mTOR inhibitors block lysosome tubulation. Quantification of lysosome tubulation in RAW cells either treated with DMSO as control or pre-treated for 20 min with DMSO, 1 μM rapamycin, 200 nM PP242 or 50 nM WYE687 followed by 100 ng/mL LPS for 2 h to induce lysosome tubulation. Data are mean ± SEM of three independent experiments based on 25-30 cells per condition per experiment. Data was statistically analysed using a one-way ANOVA, followed by Tukey's post-hoc test. An asterisk (*) indicates a significant difference ($p < 0.0001$) between DMSO+LPS and DMSO alone. (B) Western blot of RAW whole-cell lysates showing that siRNA suppresses mTOR protein levels by at least 60% (simTOR) relative to non-targeting siRNA oligonucleotides (siNTP). GADPH was probed as a loading control. (C) Western blot of RAW whole-cell lysates showing that treatment of RAW cells with 100 μM of the AMPK activator A769662 inhibits mTOR. Note the lack of pS6K in A769662 + LPS condition. Total S6K was probed as a loading control.

Supplementary Figure 3. mTOR inhibition does not affect microtubules or basal lysosomal motility. (A) Microtubules of RAW cells were stained by immunofluorescence using antibodies against α-tubulin. Cells were either untreated, treated with 100 ng/mL LPS or 100 nM torin1 or both for 2 hours. (B) Lysosome track length was manually followed in control (DMSO) and torin1-treated cells over a period of 1 min and then the lysosome speed was calculated. Each dot is an individual lysosome, with a total of 55 and 44 lysosomes from 15 cells across three independent experiments. We could not observe a difference in resting lysosome motility between control and mTOR-inhibited cells.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3