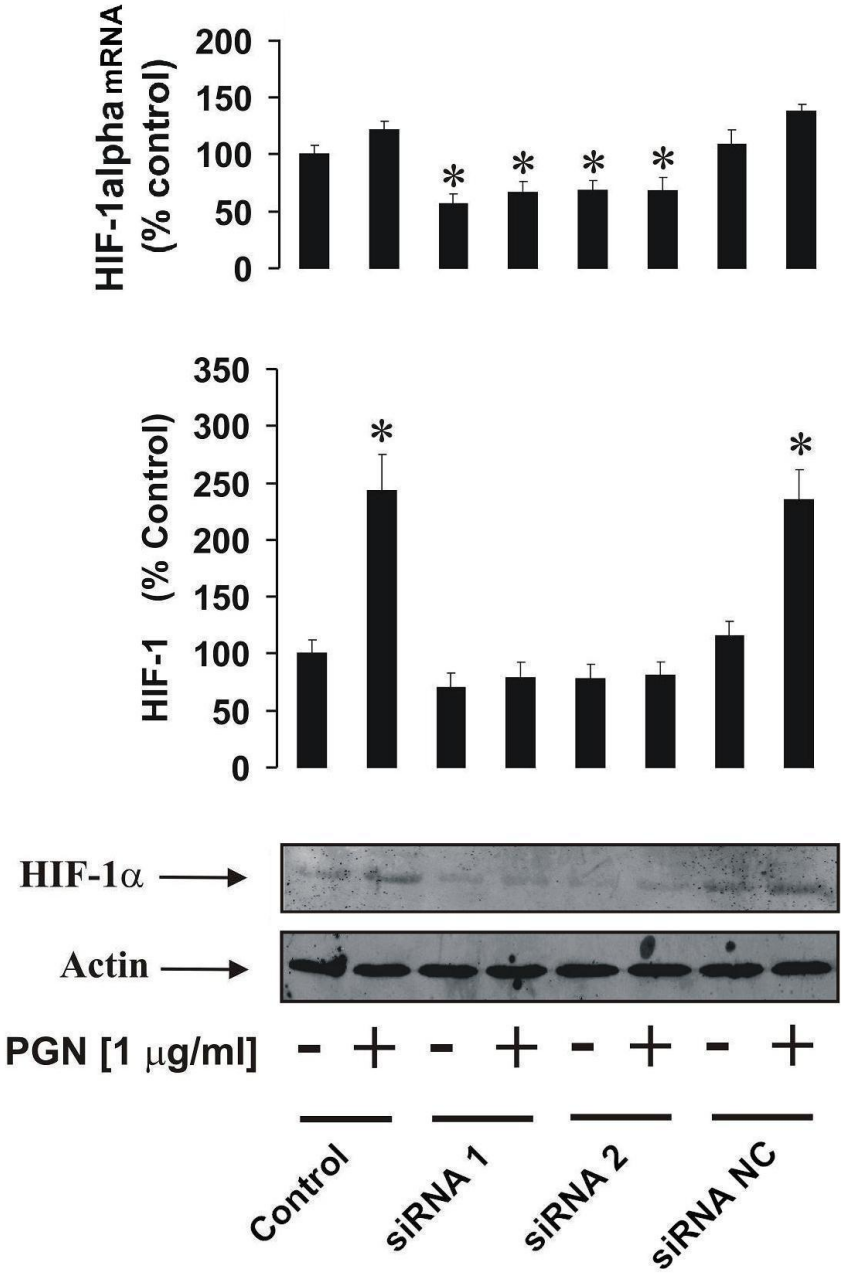
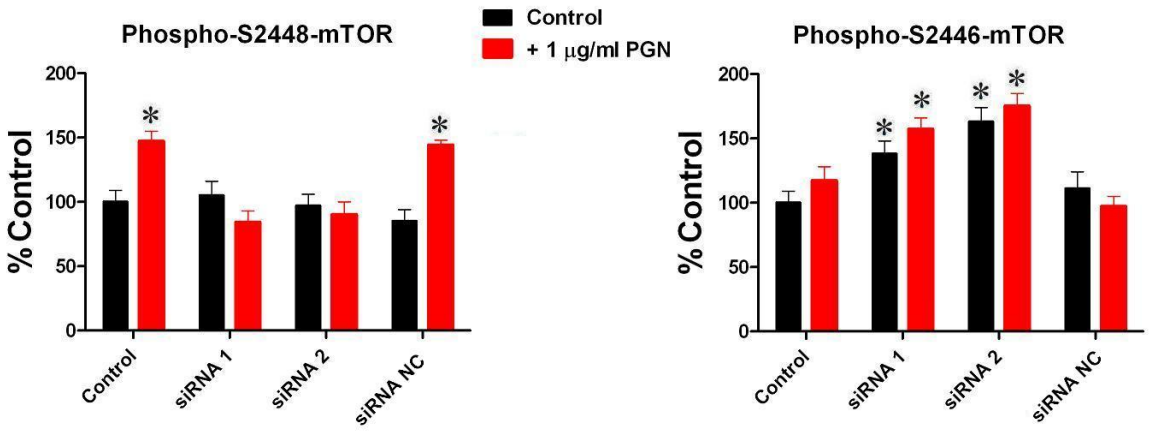
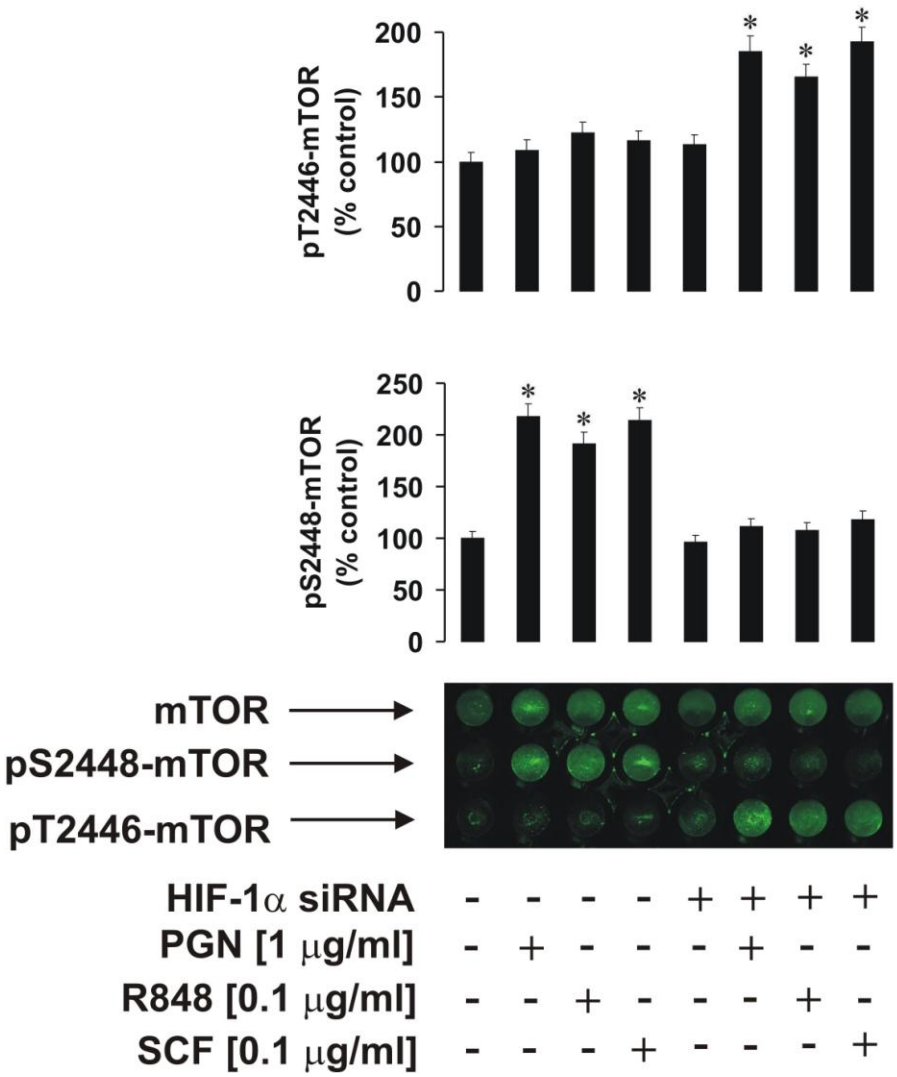
Supplementary information



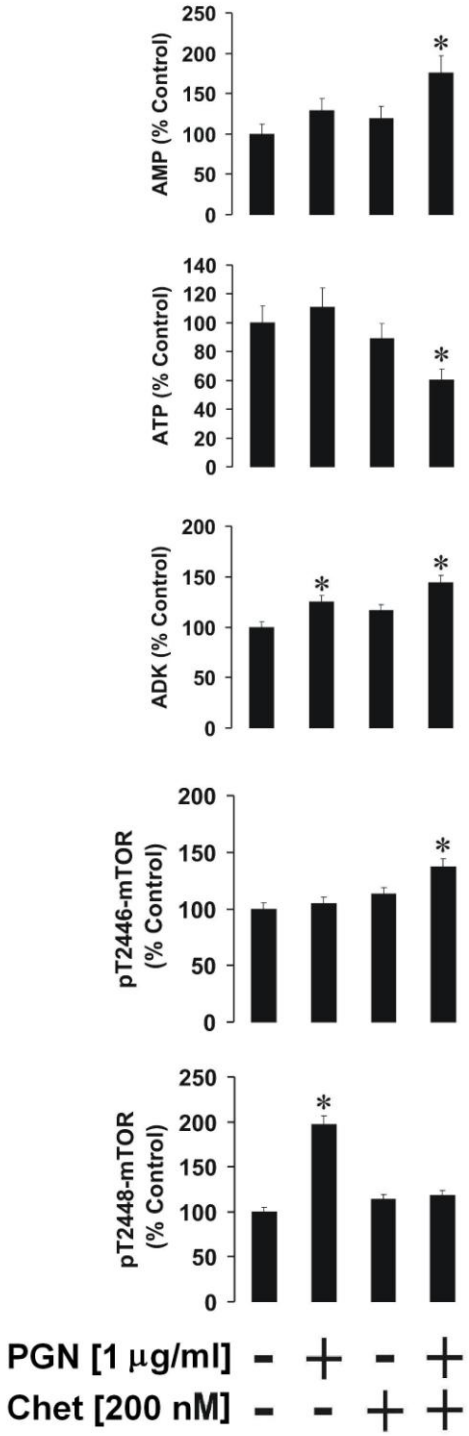
Supplementary figure 1. Effects of HIF-1α-specific and negative control siRNAs on PGN-induced HIF-1α accumulation in THP-1 cells. Normal THP-1 cells and those transfected with HIF-1α-specific siRNAs (siRNA 1 – used in all experiments, siRNA 2 – used as alternative sequence) or negative control siRNA (siRNA NC) were exposed to 1 μg/ml PGN for 4 h. HIF-1α accumulation was monitored by Western blot analysis and its mRNA levels by quantitative real-time PCR. Both HIF-1α-specific siRNAs (but not the negative control siRNA) abolished PGN-induced HIF-1α accumulation. Western blot data show one representative experiment of four that gave similar results and were quantitatively analysed. Other quantitative data are mean values ± S.D of at least three individual experiments; *$p < 0.01$ vs. control.



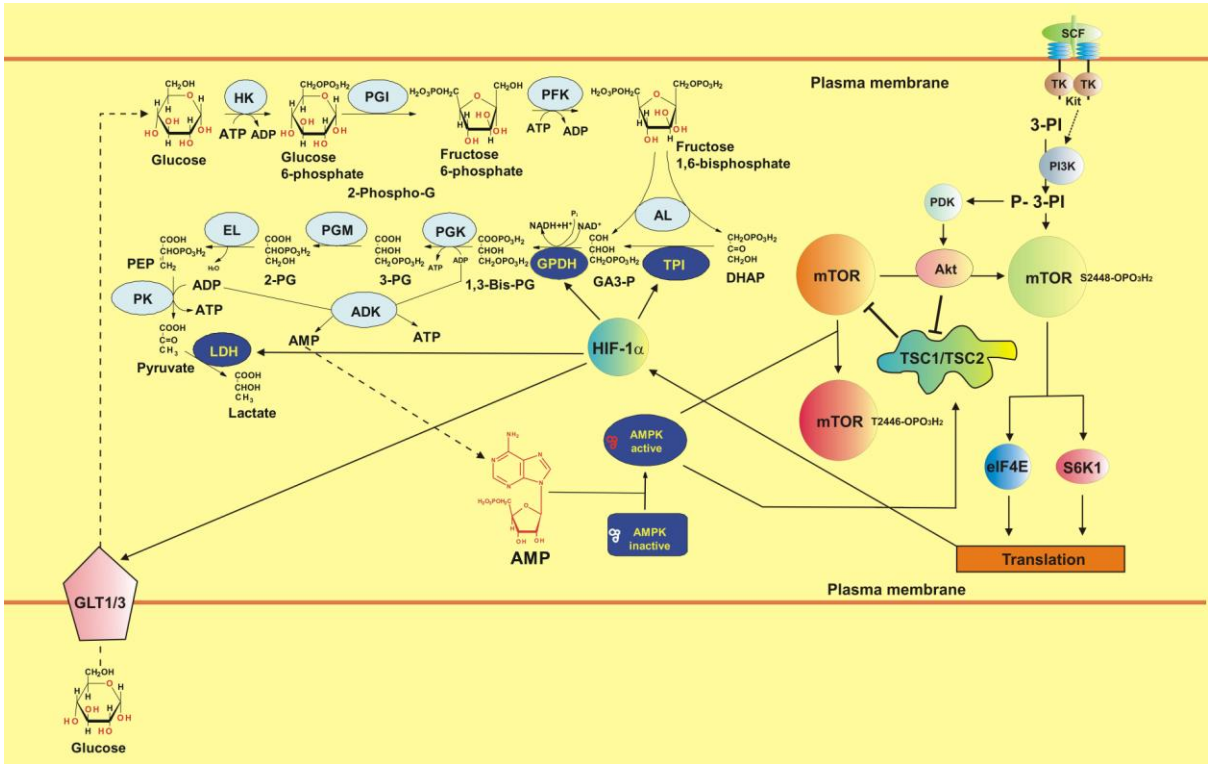
Supplementary figure 2. Effects of HIF-1α-specific and negative control siRNAs on PGN-induced mTOR 2448/T2446 phosphorylation in THP-1 cells. Normal THP-1 cells and those transfected with HIF-1α-specific siRNA or negative control siRNA were exposed to 1 μg/ml PGN for 4 h. mTOR 2448/T2446 phosphorylation was monitored by ELISA Quantitative data are mean values ± S.D. of at least three individual experiments. *$p < 0.01$ *vs.* control.



Supplementary figure 3. Effects of HIF-1α silencing on ligand-induced mTOR 2448/T2446 phosphorylation in THP-1 cells. Normal THP-1 cells and those transfected with HIF-1α-specific siRNA were exposed to the indicated concentrations of PGN, R848 and SCF for 4 h. mTOR 2448/T2446 phosphorylation was measured by in-cell Western according to the Li-Cor protocol. The image shown is from one experiment representative of three that gave similar results. Quantitative data are mean values ± S.D. of at least three individual experiments. *$p < 0.01$ *vs.* control.



Supplementary figure 4. Chetomin downregulates PGN-induced mTOR phosphorylation at S2448. THP-1 cells were exposed to PGN for 4 h with or without 2 h pre-treatment with HIF-1 transcriptional inhibitor chetomin. pS2448 and pT2446 mTOR were measured by ELISA. ADK activity, as well as ATP/AMP intracellular contents, were detected as outlined in Materials and Methods. Quantitative data are mean values ± S.D. of at least three individual experiments. *$p < 0.01$ *vs.* control.



Supplementary figure 5. Crucial involvement of HIF-1 in tracking mTOR activity during biological responses of mammalian myeloid cells (SCF is used as an example). SCF induces mTOR phosphorylation by the PI3K/Akt pathway and includes direct mTOR S2448 phosphorylation, which is also associated with downregulatory phosphorylation of the Tuberous sclerosis proteins 1 and 2 (TSC1/TSC2). Inactivation of the TSC1/TSC2 allows mTOR activation. Active mTOR initiates translation of key signalling proteins including HIF-1α. This is achieved by activation of eukaryotic initiation factor 4E (eIF4E) and S6 kinase 1 (S6K1). HIF-1 controls cellular adaptation to low oxygen availability, high energy consumption and inflammatory stress. It upregulates glycolysis by inducing the expression of key glycolytic enzymes – TPI, GPDH and LDH. In addition, HIF-1 enhances glucose uptake by upregulating the expression of glucose transporters 1/3 (GLT1/3). Absence of active HIF-1 leads to activation of ADK which converts two ADP molecules into one ATP and one AMP. Thus, increased amounts of intracellular AMP activate AMPK, which controls downregulatory T2446 phosphorylation of mTOR and prevents its S2448 phosphorylation (this blocks its kinase activity).

Additional abbreviations used: HK – hexokinase, PGI – phosphoglucose isomerase, PFK – phosphofructokinase, AL – Aldolase, TPI – Triose-phosphate isomerase, GPDH – glyceraldehyde-3-phosphate dehydrogenase, PGK – phosphoglycerate kinase, PGM – phosphoglycerate mutase, EL – enolase, PK – pyruvate kinase, LDH – lactate dehydrogenase, DHAP – dihydroxyacetone phosphate, GA3-P – glyceraldehyde-3-phosphate, 3-PG – 3-phosphoglycerate, 2-PG – 2-phosphoglycerate, PEP – phosphoenolpyruvate, GLT1/3 – glucose transporters 1/3, TSC1/TSC2 – Tuberous sclerosis proteins 1 and 2, PDK – P-3-PI-dependent kinase.