

Supplementary material to:

**THE mTOR KINASE INHIBITOR RAPAMYCIN ENHANCES THE
EXPRESSION AND RELEASE OF PRO-INFLAMMATORY CYTOKINE
INTERLEUKIN 6 MODULATING THE ACTIVATION OF HUMAN
MICROGLIAL CELLS**

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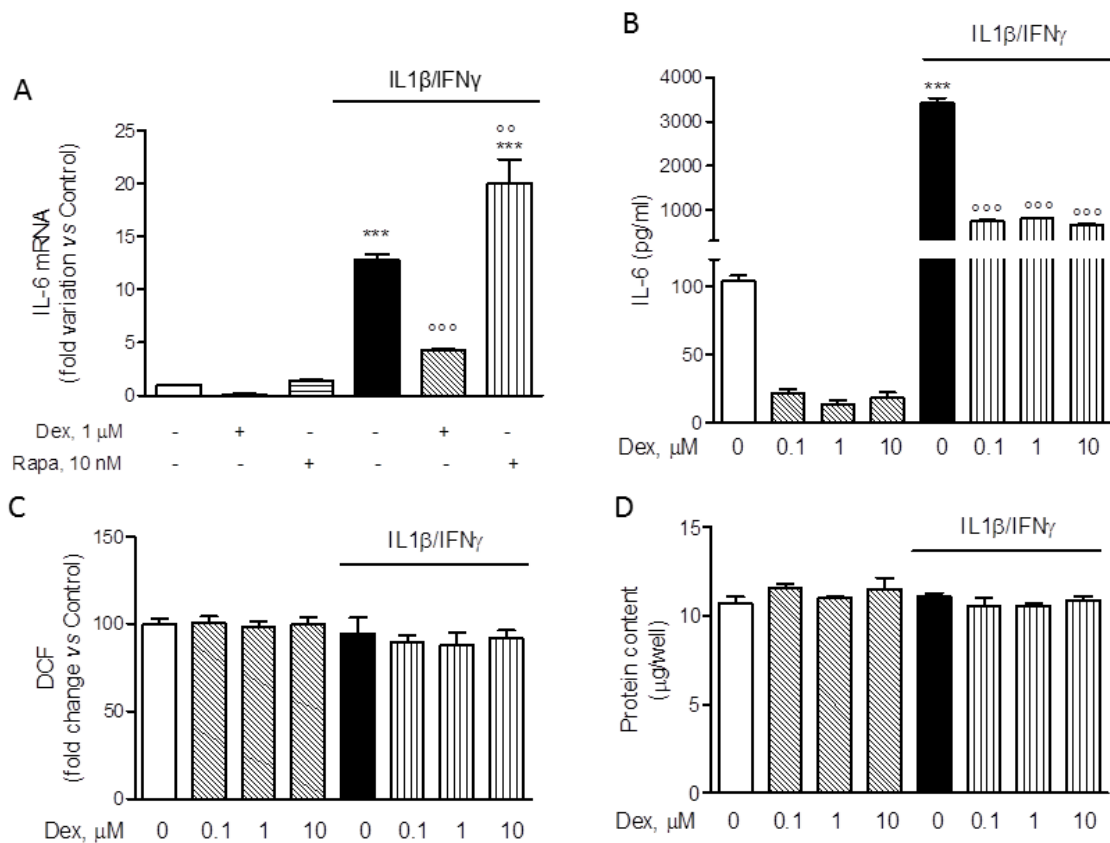
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Supplementary Figure 1: Effect of dexamethasone on HMC3 cell inflammatory activation and protein synthesis. (A-D) The effects of a well-known anti-inflammatory drug, dexamethasone (Dex) were evaluated on HMC3 proinflammatory activation. **(A)** Cells were treated in plain medium (Control) or stimulated with IL for 4 h. As indicated, 1 μM Dex and 10 nM RAPA (for comparison) were added at the beginning of the experiment. Total cytosolic RNA was prepared and used for Q-PCR analysis of IL-6 gene. Data are expressed as fold change versus Control, taken as calibrator for comparative quantitation analysis of mRNA levels. As shown in the graph, IL significantly increased IL-6 mRNA levels as early as after 4 h of incubation. Dex completely abolished IL-6 expression under basal conditions and significantly reduced the induction obtained with IL. On the other hand, RAPA significantly increased the stimulatory effect of IL (consistently with results reported at 24 h). Each sample was measured in triplicate, the experiment was repeated two times with similar results. Data are means ± SEM and were analyzed by one-way ANOVA followed by the Bonferroni's *post hoc* test. ***, $P < 0.001$, versus Control; °°, $P < 0.01$, versus IL. **(B-C)** Microglial cells were treated in plain medium (as Control) or stimulated with IL for 24 h. Dex in the concentration range of 0.1-10 μM was added at the beginning of the experiment. **(B)** The amount of IL-6 released in the incubation media by HMC3 cells, under the different experimental conditions, was measured. Consistently with mRNA data, IL significantly increased the release of IL-6 in the incubation media. In contrast to RAPA, Dex significantly reduced the stimulatory effect of IL (at any level of concentration tested). Data are shown as pg/ml. Data are means ± SEM (n=4). The experiment was repeated three times using cells from two different thaws with similar results. Data were analyzed by one-way ANOVA followed by the Bonferroni's *post hoc* test. ***, $P < 0.001$, versus Control; °°, $P < 0.001$, versus IL. **(C)** Reactive free radicals were assessed by the oxidation of H2DCF-DA (DCF) added to the cells at the end of the experiment. Data are expressed as fold change versus Control (set as 100 %). Data are means ± SEM (n=4). The experiment was repeated three times using cells from two different thaws with similar results. Data were analyzed by one-way ANOVA followed by the Bonferroni's *post hoc* test. **(D)** At the end of experiments in which ROS production was assessed **(C)**, cells were lysed in 200 mM NaOH and protein content was evaluated by the Bradford's method. Data are means ± SEM (n=4). The experiment was repeated three times using cells from two different thaws with similar results. Data were analyzed by one-way ANOVA followed by the Bonferroni's *post hoc* test.