



Supplemental Material to:

**Linah Mahmoud, Fatma Al-Enezi, Maher Al-Saif,
Arjumand Warsy, Khalid SA Khabar, Edward G Hitti**

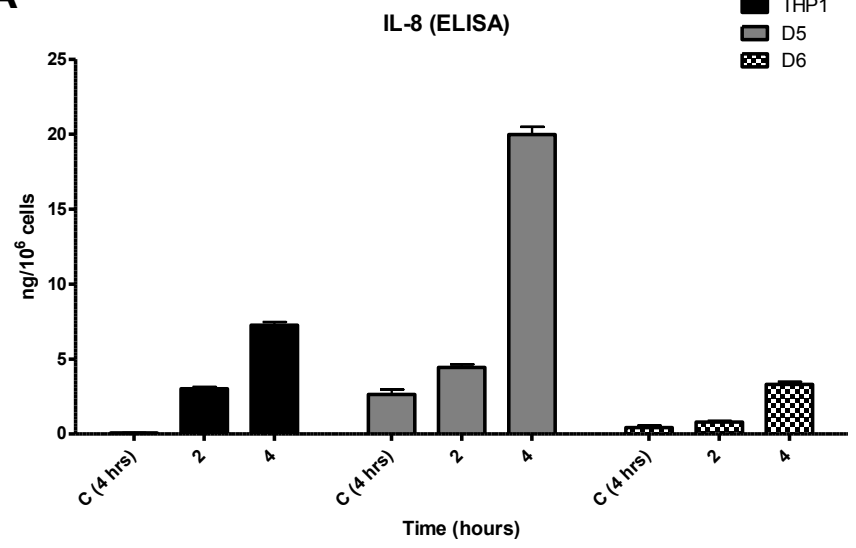
**Sustained stabilization of Interleukin-8 mRNA
in human macrophages**

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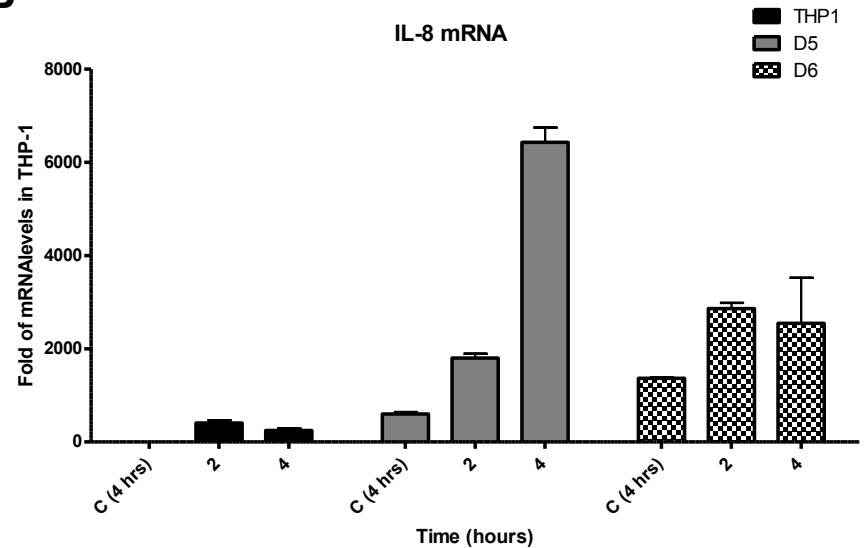
<http://dx.doi.org/10.4161/rna.27863>

www.landesbioscience.com/journals/rnabiology/article/27863/

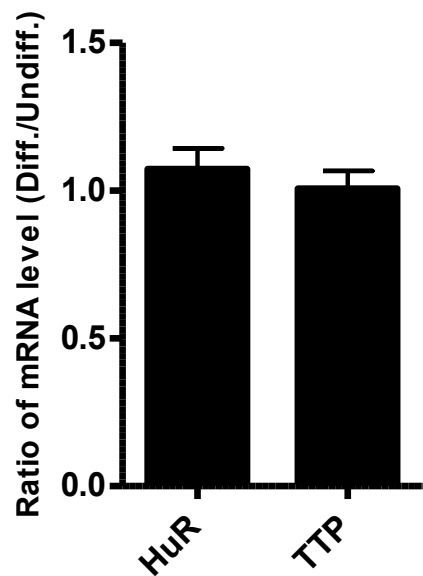
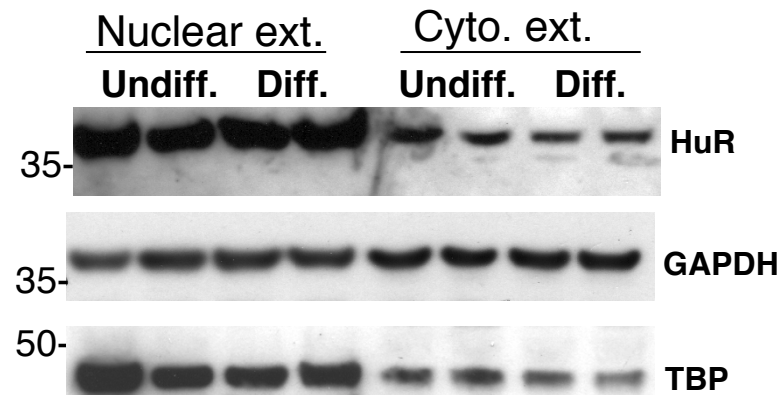
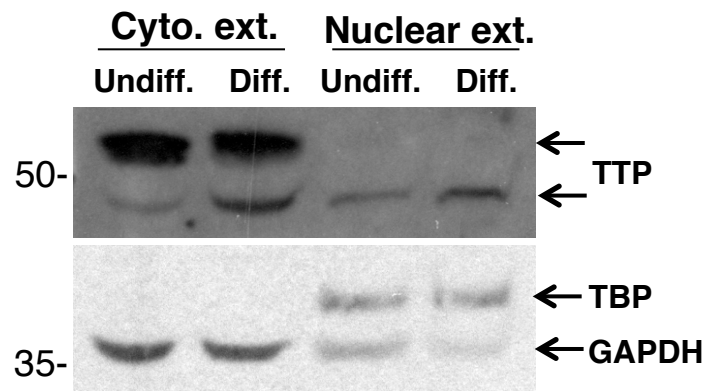
A



B

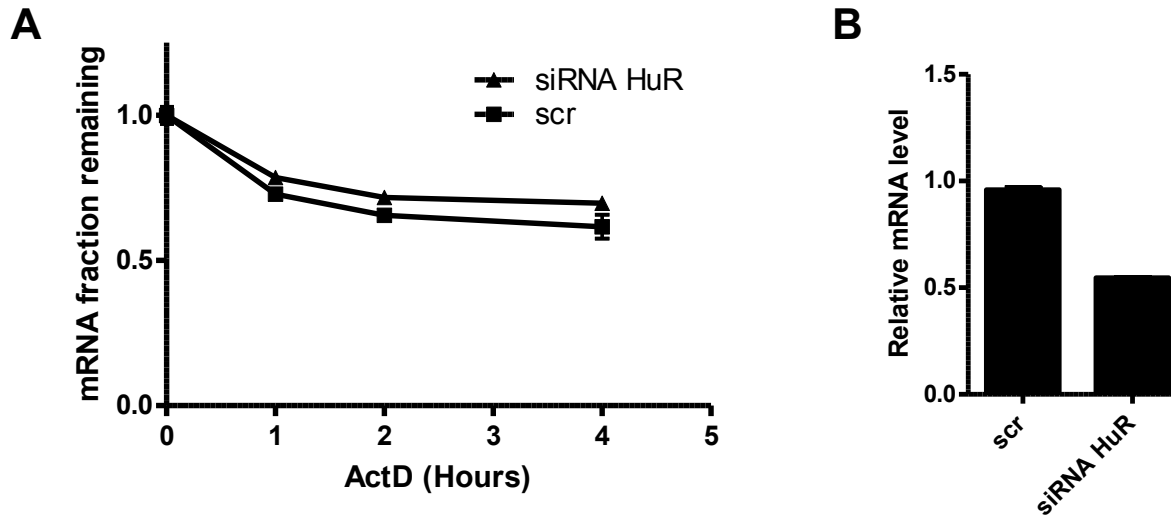


Supplementary Figure 1. Protein release and IL-8 mRNA levels in primary human monocytes and response to LPS
10⁶ primary monocytes from three healthy donors were seeded overnight in non-adherent cell culture plates (Costar 3471, Ultra Low Attachment), the following day the cells were washed and the medium was replaced, control cells were left untreated for 4 hours (C (4hrs)) or treated with 1µg/ml LPS for two and four hours. THP-1 cells were used as control and treated similarly. **(A)** Supernatants were collected and assayed for IL-8 using specific ELISA. **(B)** Total RNA was prepared from the cells and Real Time PCR was performed to quantify IL-8 mRNA levels. D: healthy human donor

A**B****C**

Supplementary Figure 2. TTP and HuR mRNA and protein levels in undifferentiated and PMA differentiated THP-1 cells. 10^6 THP-1 cells were left untreated (Undiff. for undifferentiated) or treated with 20 ng/ml PMA for 24 hours (Diff.). (A) Total RNA was extracted and TTP and HuR mRNA levels were quantified by RT-Real Time-PCR, shown is the ratio of relative mRNA level in PMA-differentiated cells to the levels in untreated THP-1 cells. Taqman primer sets for TTP, HuR were purchased from applied biosystems. (B) and (C) Nuclear and cytoplasmic protein lysate fractions of THP-1 cells were separated using the Compartmental Protein Extraction Kit from Biochain according to the manufacturer's protocol. Equal amounts (~25 μ g) of nuclear and cytoplasmic protein extracts (Nuclear ext. and Cyto. ext.) were subjected to electrophoresis on 10% polyacrylamide-SDS gels followed by transfer to nitrocellulose membranes (Hybond ECL, Amersham Biosciences). Membranes were sequentially hybridized with antibodies for HuR (B) or TTP (C) and the cytoplasmic and nuclear controls GAPDH and TATA binding protein (TBP). For HuR, each protein extract was loaded on two lanes. At least two bands of TTP are detected probably due to differential phosphorylation, as previously described for TTP protein in THP-1 cells.¹ Signal detection was performed with ECL Western blotting detection reagents (Amersham). Protein molecular weight markers were used to verify the size of the proteins. All antibodies were purchased from Abcam.

1. Brooks SA, Connolly JE, Diegel RJ, Fava RA, Rigby WF. Analysis of the function, expression, and subcellular distribution of human tristetraprolin. *Arthritis Rheum* 2002; 46:1362-70.



Supplementary Figure 3. HuR siRNA knock down and IL-8 mRNA stability in PMA-differentiated THP-1 cells. 10^7 THP-1 cells were transfected with 200 nM scramble (scr) (sense: GGCCAUGUAUACGCGGUUCdTdT, anti-sense GAACCGCGUAUACAUGGCCdTdT) or HuR siRNA (sense GCCUGUUCAGCAGCAUUGGdTdT, anti-sense CCAAUGCUGCUGAACAGGCdTdT) using lipofectamin LTX as stated in material and methods. Next day the transfection medium was replaced with serum supplemented RPMI 1640 medium and the cells were incubated for 24 hours and the cells were split in four 3 cm plates, 20 ng/ml PMA was added and the cells were left to differentiate overnight. **(A)** Actinomycin-D chase experiments and qPCR were performed, Graph prism software was used to plot the decay curves of IL-8 mRNA **(B)** The GAPDH relative level of HuR mRNA was assessed by qPCR in scr and HuR siRNA transfected cells.