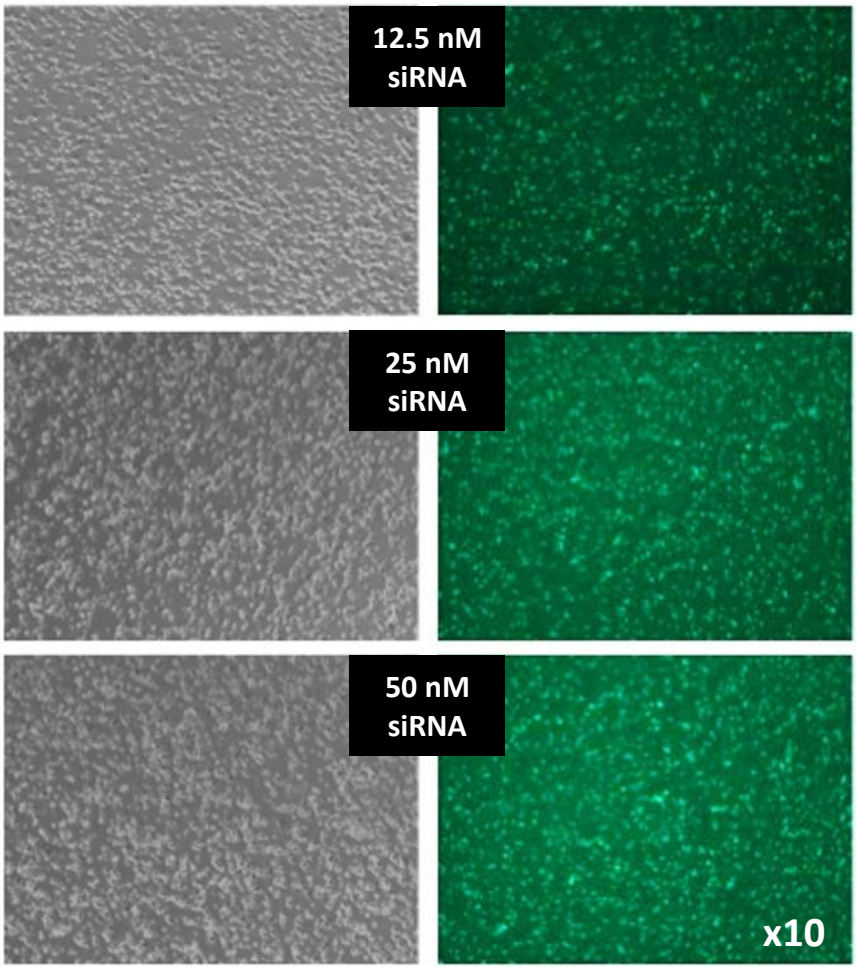
primary human monocytes: transfection of siRNA



VB-01LB-00, VB-01LB-01

- Test 3 siRNA concentrations 12.5, 25 and 50nM (25nM corresponds to the standard protocol)
- Fluorescence microscopy performed 24h post-transfection

Fig. 1: Comparative uptake of a FITC-labeled siRNA in primary monocytes transfected with Viromer® BLUE

Data from A. Wortmann, Philipps-University Marburg (Germany)

primary human monocytes: transfection of siRNA

Extract from Materials and Methods:

Gene silencing by siRNA

Small interfering RNA was delivered into total monocytes using Viromer technology according to the manufacturer's protocol (Viromer Green, Lipocalyx). Monocytes were transfected with either control or Hsp27/HSPB1 siRNA (SMARTpool, ON-TARGETplus HSPB1 siRNA, Dharmacon) for 24 h. Knockdown of Hsp27 was confirmed by Immuno- blotting.

VG-01LB-00, VG-01LB-01

Extract from Results:

Hsp27 modulates IL-1β production in monocytes

[…] Prior studies identified Hsp27 as an essential subunit of the AUF1 protein complex, which regulates ARE-mediated mRNA decay of cytokines, including IL-1β, in monocytic cells such as THP-1. We therefore assessed the protein levels of Hsp27 in freshly isolated monocyte subsets. Correlating with the higher IL-1β mRNA degradation rate, significantly more Hsp27 protein was detected in non-classical as compared to classical (1.8 ± 0.09 vs 0.29 ± 0.06) monocytes (Fig. 6a).

To investigate the involvement of Hsp27 in the regulation of IL-1β gene expression and production, we performed siRNA-mediated knockdown of Hsp27 in the total monocyte population […]. **The knockdown efficiency for Hsp27 was greater than 95%** (Fig. 6b, top panel).

We performed qPCR, immuno-blotting and ELISA to determine the effect of Hsp27 knockdown on IL-1β mRNA and protein levels and observed elevated IL-1β mRNA expression in Hsp27 knockdown monocytes compared to control siRNA transfected cells (Fig. 6c). Reduction in Hsp27 levels led to an increase of pro-IL-1β protein in LPS-treated monocytes by immuno-blotting (Fig. 6b). Pro-IL-1β was only observed in LPS-stimulated cells, demonstrating that the siRNA transfection itself did not activate the primary monocytes (Fig. 6b). When measured by ELISA, we detected ATP-mediated IL-1β release in LPS-stimulated monocytes, where Hsp27-knockdown cells released significantly more IL-1β (Fig. 6d and e). […]

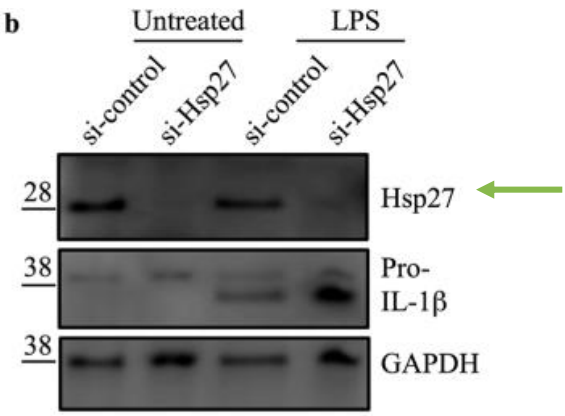
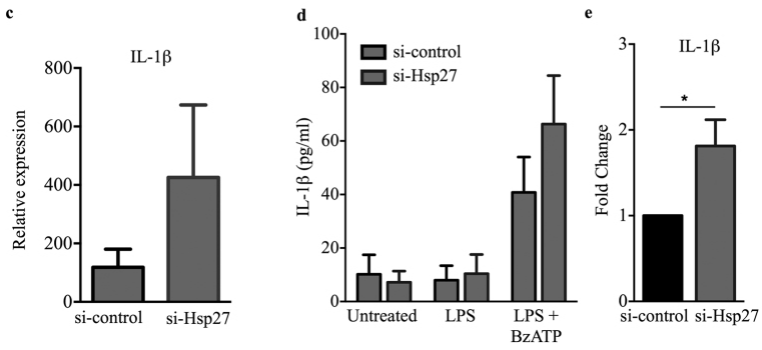
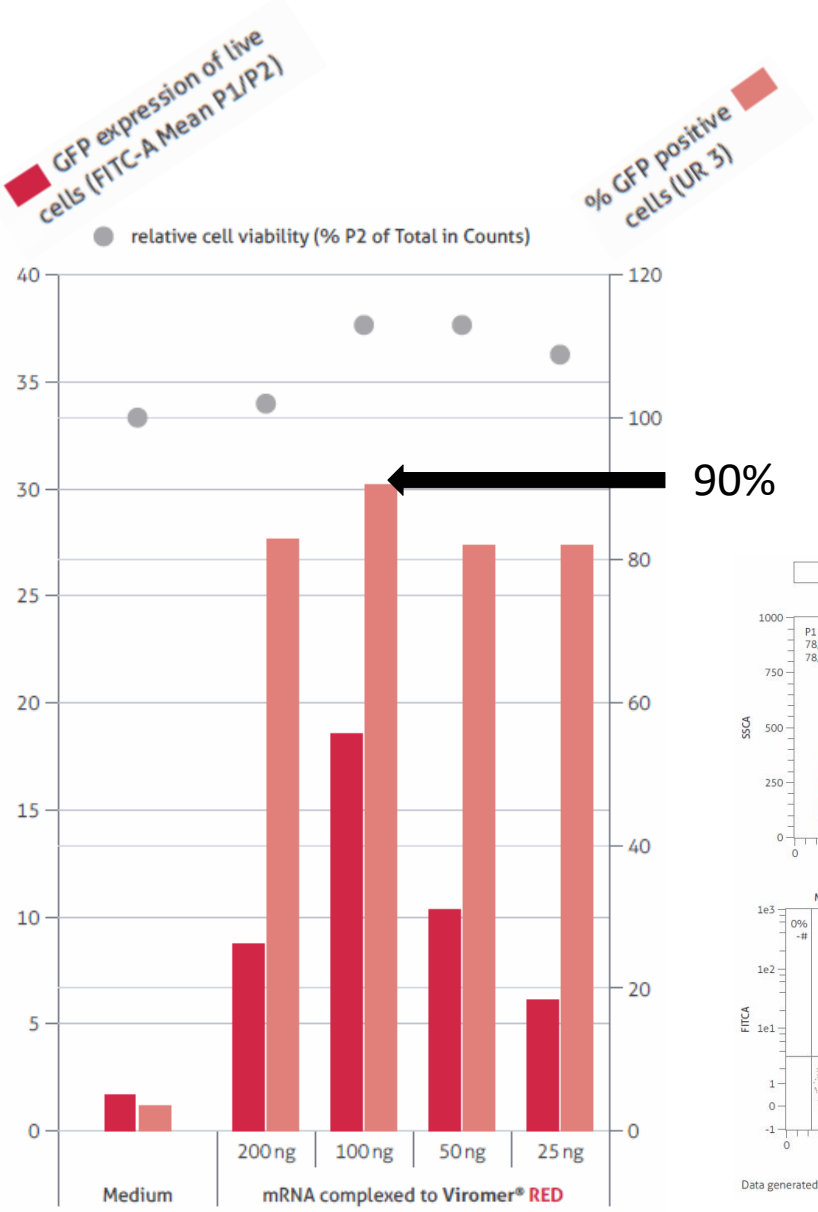


Fig. 2: Knock-down of Hsp27 gene expression in primary monocytes transfected with Viromer® GREEN



Hadadi, Eva, et al. "Differential IL-1β secretion by monocyte subsets is regulated by Hsp27 through modulating mRNA stability." ***Scientific Reports*** **6 (2016).**

primary human monocytes: transfection of mRNA



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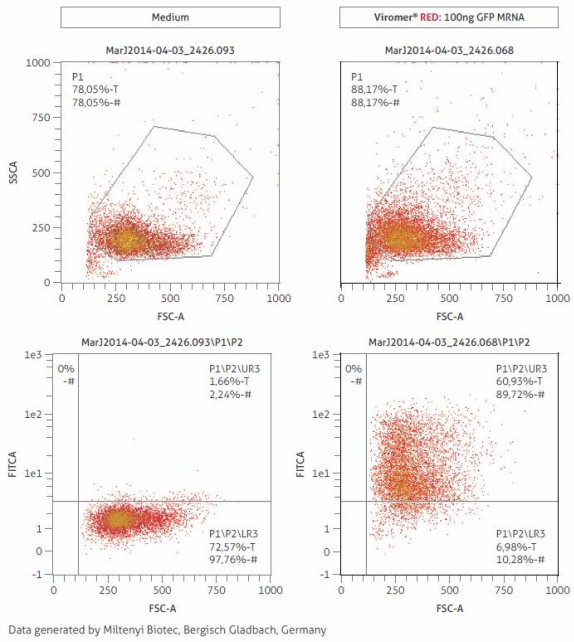

Fig. 3: Overexpression of GFP in primary human monocytes transfected with GFP-encoding mRNA by using Viromer® RED

- CD14 purified monocytes from PBMCs (buffy coat)
- 40,000 cells/96-well
- Test 4 mRNA amounts: 25, 50, 100 and 200ng/well (100ng corresponds to the standard protocol)
- GFP detection via MACSQuant

Data from Miltenyi Biotec, Bergisch Gladbach (Germany)