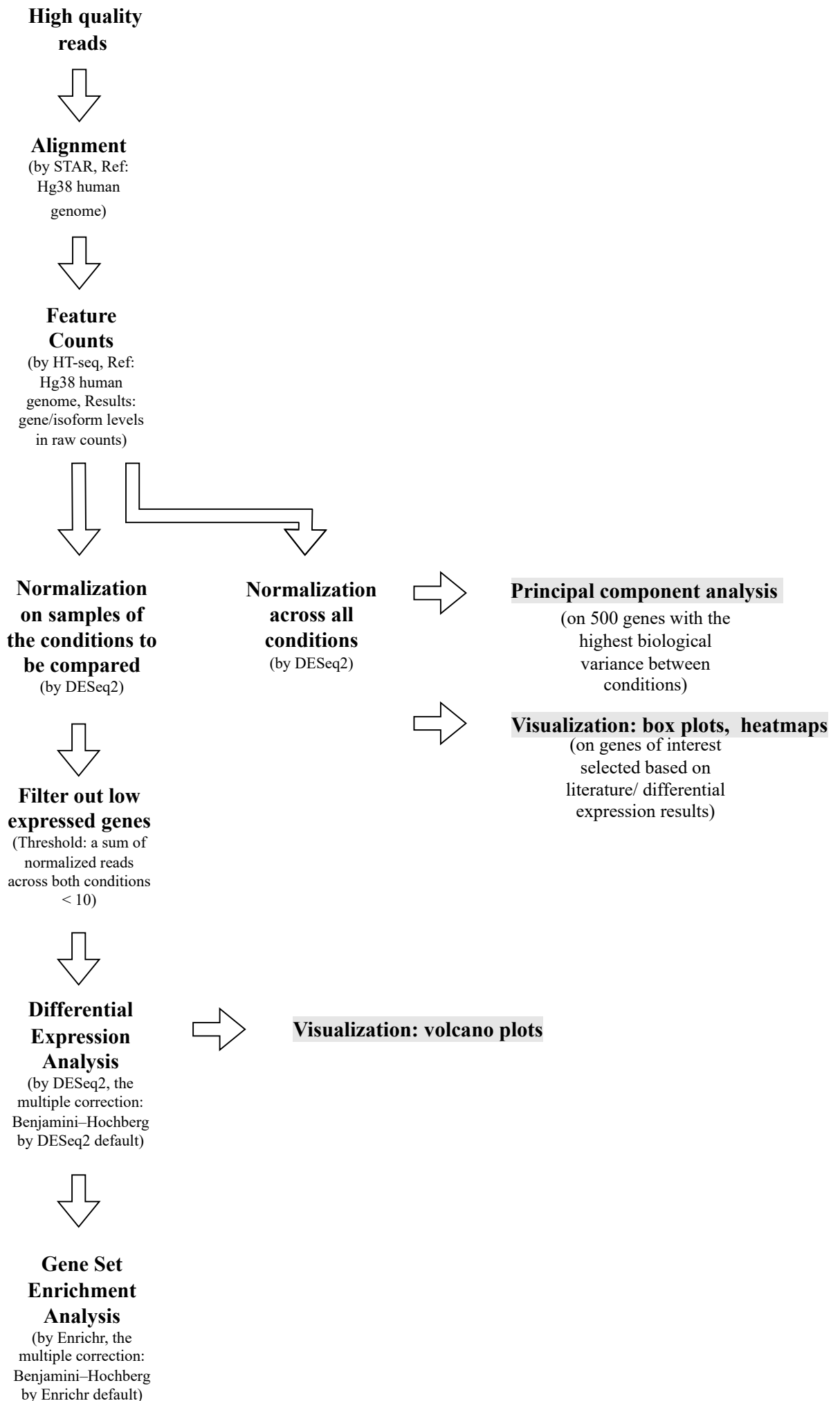


# Figure S1

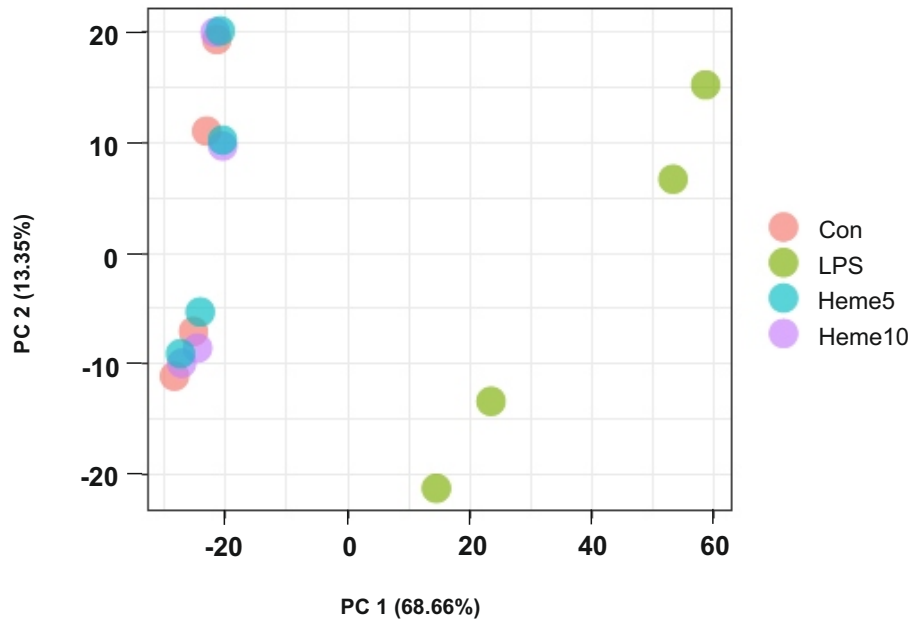


**Figure S1. Related to Figure 1**

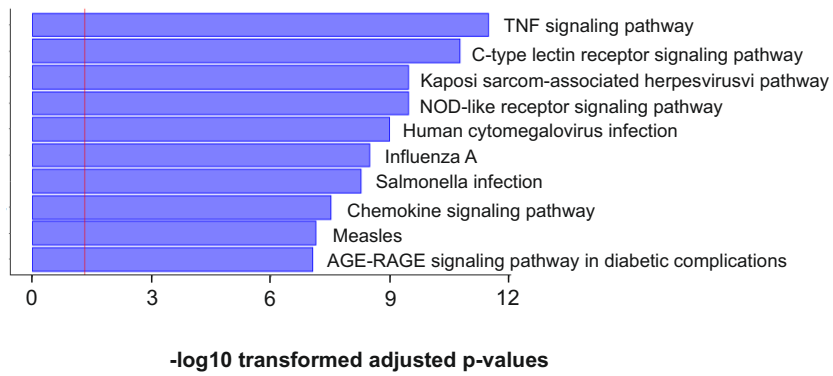
hMDMs were treated with heme (5  $\mu$ M, 10  $\mu$ M) and LPS (1  $\mu$ g/ml) alone for 6 h and then processed for bulk RNA-seq. Schematic illustrating the workflow of the bulk RNA-seq analysis.

# Figure S2

## A



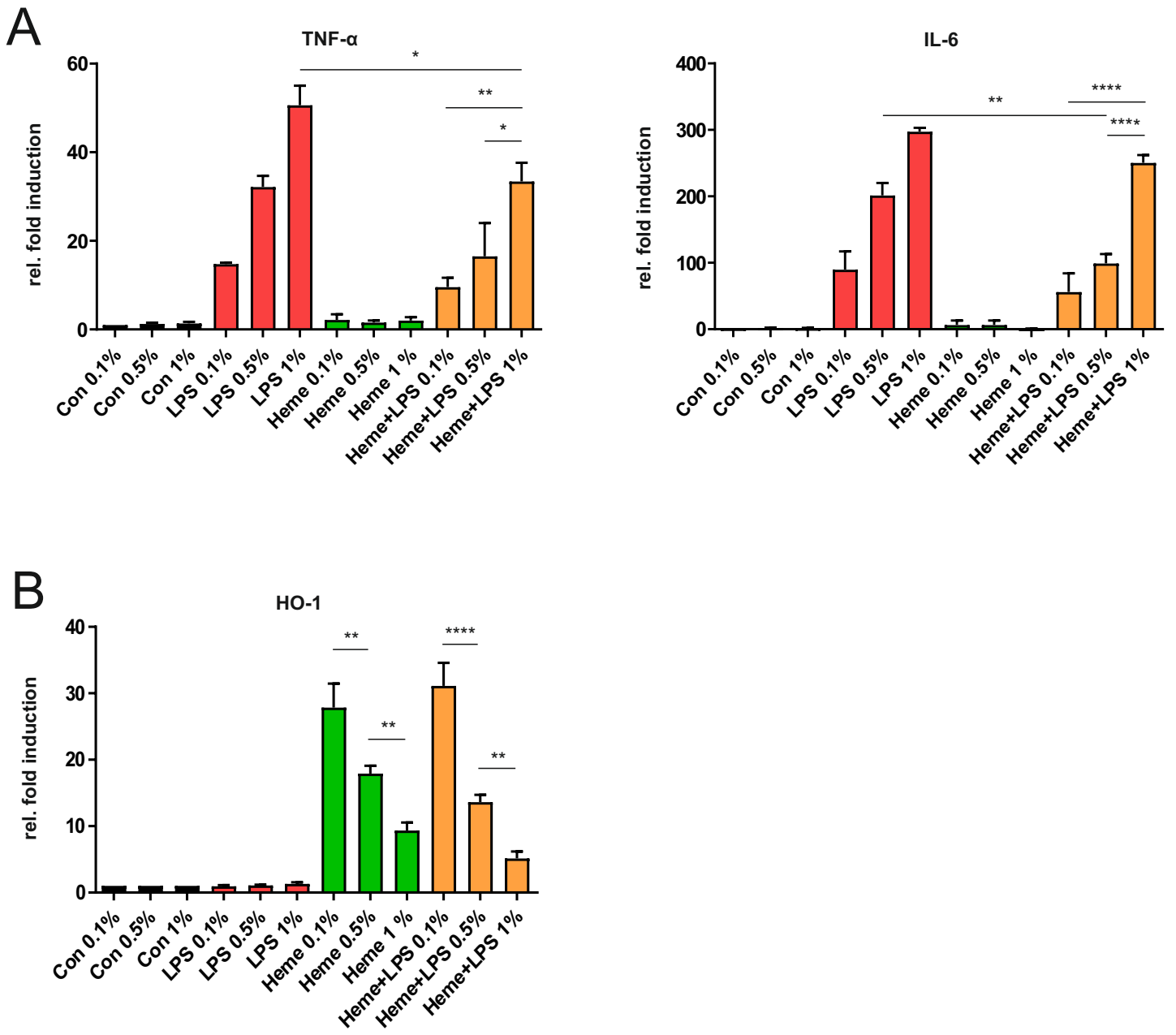
## B



## Figure S2. Related to Figure 1

hMDMs were treated with heme (5  $\mu$ M, 10  $\mu$ M) and LPS (1  $\mu$ g/ml) alone for 6 h and then processed for bulk RNA-seq. **(A)** PCA based on all identified genes. Samples were plotted based on the first 2 principal components (n = 4). P value < 0.01. **(B)** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of differentially expressed genes in heme vs LPS.

# Figure S3

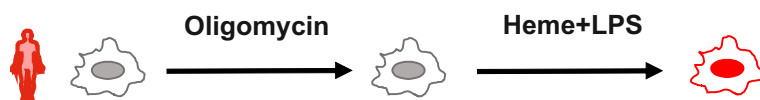


### Figure S3. Related to Figure 3

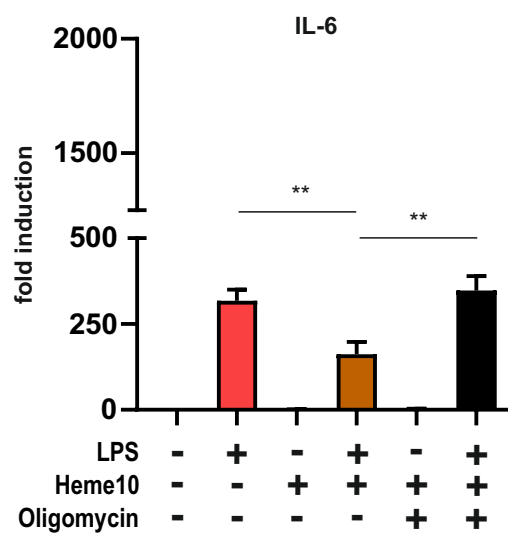
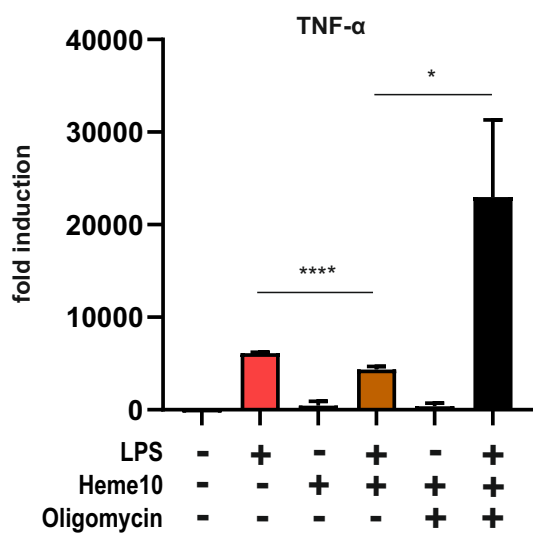
**(A-B)** hMDMs were treated with heme (10  $\mu$ M) and LPS (1  $\mu$ g/ml) alone or in combination for 16 h in different serum concentrations as indicated. Gene expression of TNF- $\alpha$ , IL-6 and **(C)** HO-1 were measured by RT-PCR. The values were normalized to the expression of GAPDH and the respective  $\Delta\Delta$ CT values are shown. The values are shown as mean of  $\pm$  SEM of two independent experiments. Statistical analysis was performed using one-way ANOVA with Tukey's test; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

# Figure S4

## A



## B



#### Figure S4. Related to Figure 5

**(A)** To study whether OXPHOS inhibition reverses the anti-inflammatory effect of heme in human macrophages, hMDMs were pre-treated with oligomycin (2  $\mu$ M) for 1 h followed by stimulation with heme (10  $\mu$ M) and LPS (1  $\mu$ g/ml) alone or in combination for 16 h. **(B)** Gene expression of TNF- $\alpha$  and IL-6 were measured by RT-PCR. The values were normalized to the expression of HPRT and the respective  $\Delta\Delta$ CT values are shown. The values represent mean  $\pm$  SEM of three independent experiments. Statistical analysis was performed using one-way ANOVA with Tukey's test or unpaired t-test; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001. Con, control; Oligo, oligomycin.