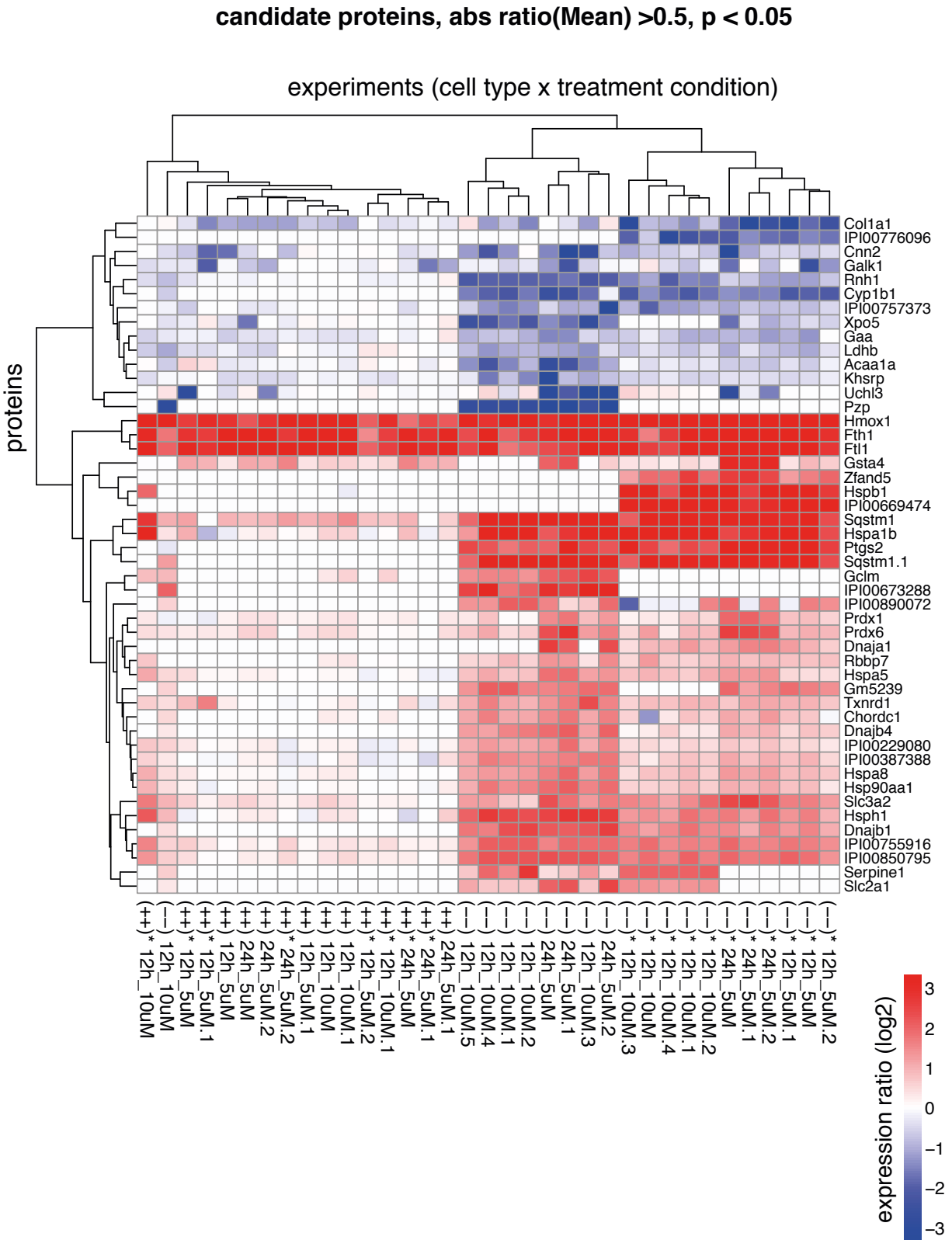
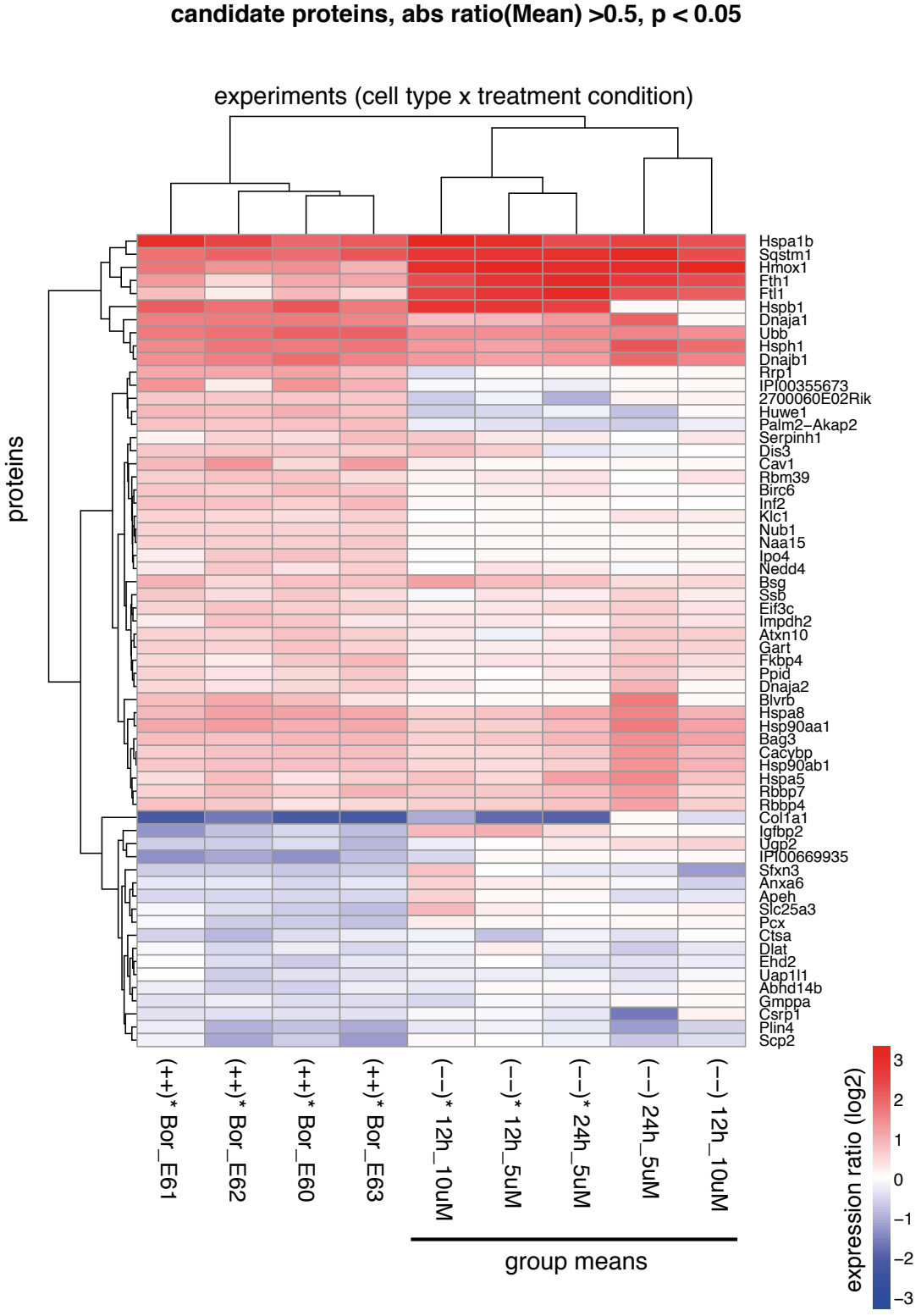
Supplementary Figure 1



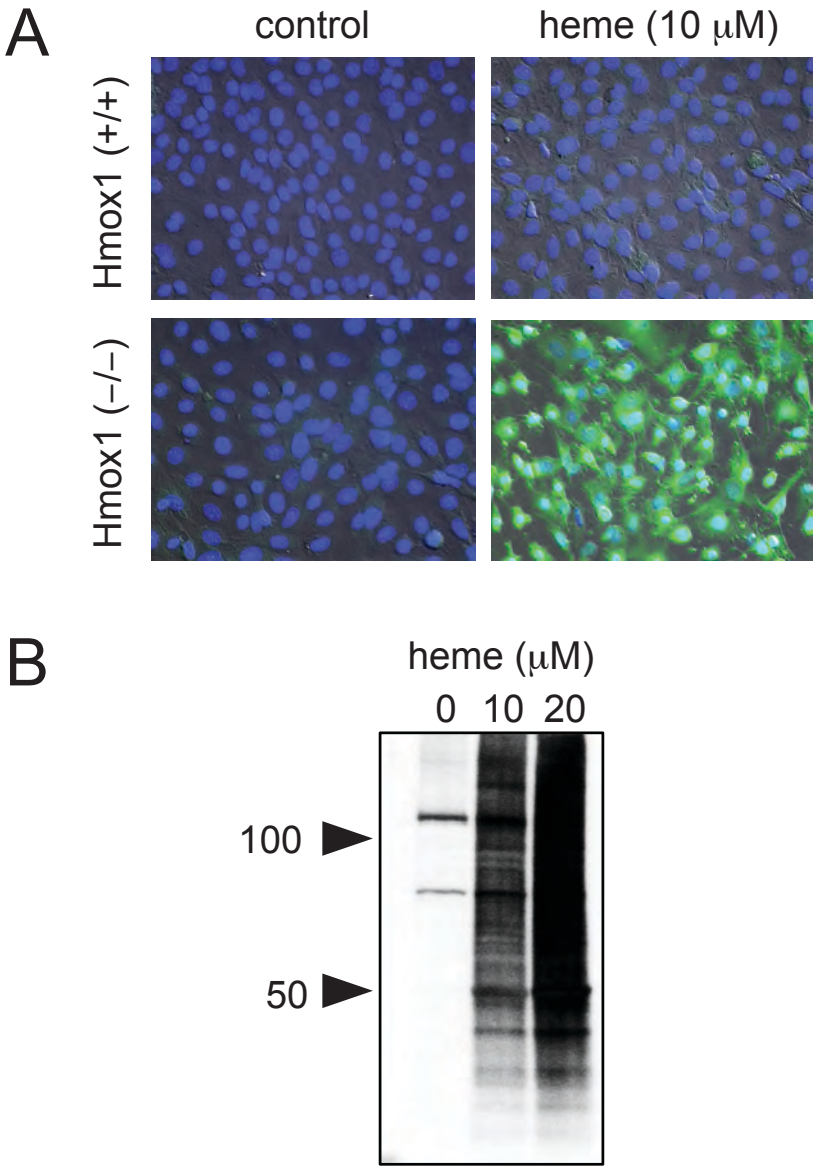
Supplementary Figure 1
Hierarchical clustering analysis of all proteins that have been identified as differentially expressed in any of the tested conditions. Each column in the plot represents data from an individual SILAC experiment. The plot includes all proteins with an absolute expression ratio of > 0.5 (log2) in treated compared to matched untreated control cells ($p < 0.05$). red: protein expression is higher in treated cells, blue: protein expression is lower in treated cells.

Supplementary Figure 2



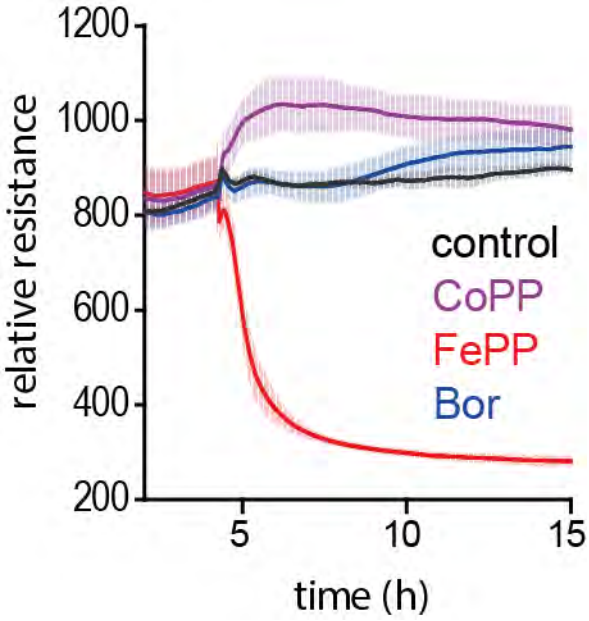
Supplementary Figure 2
Hierarchical clustering analysis of all proteins that have been identified as differentially expressed in heme treated Hmox1 (-/-) cells and bortezomib (10 pM) treated Hmox1 (+/+) MEF cells, respectively. The heme response data include all data from heme treated Hmox1 (-/-) MEF cells shown in Figure 2 that were included into this analysis as the five condition group means. The bortezomib data represent data from 4 independent SILAC experiments. The plot includes all proteins with an absolute expression ratio of > 0.5 (log2) in treated compared to matched untreated control cells ($p < 0.05$). red: protein expression higher in treated cells, blue: protein expression lower in treated cells.

Supplementary Figure 3



Supplementary Figure 3: Heme triggers accumulation of lipid peroxide modified proteins
(A) Hmox1 (–/–) and Hmox1 (+/+) MEF were incubated in serum free DMEM with 10 μM linoleamide alkyne in the absence (control) or presence of heme (10 μM) for 6 h. Protein adducts with linoleamide oxidation products (green) were then detected by fluorescence microscopy after reaction with Alexa-488 azide. Images were acquired using a Zeiss Axioscope microscope at an original magnification of 200 × (blue: nuclei/DAPI). **(B)** Formation of cellular protein-lipid-adducts in heme-treated Hmox1 (–/–) MEF detected by click-chemistry facilitated biotinylation of cell lysates and subsequent SDS-PAGE with Western blot detection by HRP streptavidin.

Supplementary Figure 4



Supplementary Figure 4: Toxicity of heme, CoPP and bortezomib
Monolayer integrity of confluent Hmox1 (–/–) MEF was analyzed with an ECIS® instrument as a measure of cytotoxicity over time during incubation with FePP (10 µM), CoPP (10 µM) or bortezomib (100 pM). The declining relative electrical resistance in the cultures treated with FePP indicates cell damage, which did nor occur during incubation with CoPP and bortezomib (data indicate mean ± SD of four biologic replicates).