Supplementary Figure 1. (A) Correlation analysis for Nrf2 nuclear protein expression and Nrf2 mRNA in AML samples (circles). (B) Correlation analysis for Nrf2 nuclear protein expression and ROS activity in AML samples (squares). (C) Correlation analysis for Nrf2 mRNA and Keap1 mRNA in AML samples (triangles). (D) Correlation analysis for Nrf2 mRNA and κB binding activity in AML samples (crosses). (E) Correlation analysis for Nrf2 protein and Keap1 protein in AML samples (diamonds). (F) Correlation analysis for Nrf2 protein and p65 protein in AML samples (plus)
Supplementary Figure 2. (A) Primer sequences and PCR product for mutational analysis. (B) Nrf2 promoter sequence. Translation start site is underlined.
Supplementary Figure 3. Calibration curve for MFI and representative histograms in AML cells (A) THP-1 cells, AML#20 and AML#22 were treated with increasing doses of H2O2 for 15 minutes, washed with PBS and incubated with 10μM of H2DCFDA for 15 min. Cells were then assessed for H2DCFDA oxidation using flow cytometry and a calibration curve calculated. (B) THP-1 cells, AML#20 and AML#22 cells washed with PBS and incubated with 10 μM of H2DCFDA for 15 min. Cells were then assessed for H2DCFDA oxidation using flow cytometry and representative histograms shown. (C) THP-1 cells were pretreated with 5 and 10 μM of NAC for 1 hour followed by 125 μM of H2O2 for 15 minutes washed with PBS and incubated with 10 μM of H2DCFDA for 15 min. Cells were then assessed for H2DCFDA oxidation using flow cytometry. (D) THP-1 cells and AML#20 were treated with H2O2 for 4 hours and whole cell protein extracted. Extracts were separated by SDS-PAGE and Western blot analysis was conducted for Nrf2 total protein. Blots were reprobed with β-actin to confirm equal sample loading.
Supplementary Figure 4. Nrf2 target mRNA and NF-κB target mRNA in human AML. A. Nrf2 target genes (GCLM, NQO1, Keap1), as well as NF-κB target genes (IkBα and FLIP) mRNA gene expression in AML cells compared to CD34+ control cells. (AML#2 and AML#6 have high Nrf2 expression and AML#4 and AML#9 have low Nrf2 expression) (B) GCLM and NQO1 mRNA levels in AML cells (AML#4 and AML#6) compared to control cells in response BAY-11-7082 at 5 µM and 10 µM.
Supplementary Figure 5. Nuclear and cytosolic distribution of NF-κB/p65 and Nrf2 in healthy CD34+ control cells and primary human AML cells. Primary CD34+ cells, AML cells and the leukemic monocyte cell line THP-1 were analysed for sub-cellular p65 (green) and Nrf2 (red) localisation by immunocytochemistry. DAPI nuclear stain is shown in blue.
Supplementary Figure 6. Nuclear localisation of p65 and Nrf2 in THP-1 in response to BAY 11-7082, LPS and BAY-11-7082 and LPS treatment. (A) THP-1 was analysed for sub-cellular p65 (green) and Nrf2 (red) localisation by immunocytochemistry in response to BAY 11-7082, LPS, or both. DAPI nuclear stain is shown in blue. (B) Using the images captured, p65 and Nrf2 localisation in the nucleus was calculated as a percentage of total localisation. Bars indicate the mean ± SEM from at least 8 cells per sample (statistical significance calculated by Two-way ANOVA, * P ≤ 0.05).
Supplementary Figure 7. LPS increases Nrf2 mRNA expression by NF-κB. Primary monocytes were treated with LPS with and without BAY 11-7082 (10 µM) for various indicated time points, then RNA was extracted and analyzed for Nrf2 mRNA and GAPDH mRNA expression.
Supplementary Figure 8. Lentivirus mediated Nrf2-targeted knockdown with GFP tag. Visualisation of human AML patient cells infected with either GFP-tagged Nrf2-targeted or control miRNA constructs, then colony formation tested. Microscopic analysis of colonies derived from AML cells transduced with miRNA targeted Nrf2-GFP or control construct, as indicated, and then treated with cytarabine and daunorubicin.
Supplementary Figure 9. Silencing Nrf2 enhances chemotherapy-induced reduction of AML cell colony formation. AML cells and control cells transduced with either miRNA targeted Nrf2-GFP or non-silencing construct for 4 days and then treated with 0.5 µM cytarabine and 0.2 µM daunorubicin and 20 nM of bortezomib. The colony forming assay was performed as described. In all panels values indicate the mean ± SEM from 3 independent experiments (* statistical significance, P ≤ 0.05, between the different treatment groups).