Supplementary Material

Iron-mediated oxidative stress induces PD-L1 expression via activation of c-Myc in lung adenocarcinoma

Anna Martina Battaglia†1, Alessandro Sacco†1, Ilenia Aversa2, Gianluca Santamaria3, Camillo Palmieri2, Cirino Botta4, Roberto De Stefano5, Maurizio Bitetto6, Lavinia Petriaggi1, Emanuele Giorgio1, Concetta Maria Faniello1, Francesco Costanzo1,7, Flavia Biamonte\*1,7

**\*Correspondence:** Flavia Biamonte, flavia.biamonte@unicz.it

## Supplementary Figures

**Supplementary Figure 1.** Apoptotic cell death determined using Annexin V/PI flow cytometry assay in A549 and H460 cell lines upon administration of 250µM ferlixit for 24h.

Immagine che contiene testo, diagramma, Parallelo, schermata

Descrizione generata automaticamente

**Supplementary Figure 2.** **(A)** qRT-PCR of *PD-L1* mRNA levels in A549 and H460 cells upon administration of 250μM ferlixit for 24h or additionally treated with 200μM trolox for 6h. *ACTB* was used as housekeeping gene. **(B)** Western blot analysis of HIF-1α, p-mTOR/mTOR, p-STA3/STA3, p-ERK/ERK, NF-κB, Nrf2, c-JUN, p-S6/S6 protein expression in A549 and H460 cell lines upon administration of 250µM ferlixit for 24h alone or in combination with 200µM trolox for 6h. LAMIN and ɣ-TUB were used as a normalization control for nuclear and cytosolic protein quantification, respectively. **(C)** Real-time PCR of PD-L1 expression in A549 and H460 cells untreated or treated with ferlixit (250μM for 24h) and upon c-Myc silencing (48h). *ACTB* was used as housekeeping gene. Results are presented as mean ± SD from three independent experiments. \**p*-value < 0.05; \*\* *p*-value < 0.01.