## **Supplementary materials**





**Supplementary Figure 1 Relative expression of** *TNFA, TNFR1* **and** *TNFR2 mRNA* **in AGS and HGC-27 gastric cancer cell lines.** The graph shows that both cell lines express *TNFA* and *TNFR1* mRNA, whereas *TNFR2* expression was very low in the HGC-27 (RQ = 0.0001) relative to AGS cell line (RQ = 1.0). *ACTB* and *GAPDH* were used for normalization of mRNA quantification.



Supplementary Figure 2 Relative gene expression of *TNFA* in the AGS cell line after treatment with *H. pylori* extract. After the incubation time of 4, 6 and 24 hours and in the *H. pylori* extract (eHP) volumes of 50 and 100  $\mu$ L, *TNFA* expression was higher for the eHP volume of 100  $\mu$ L with 6 hours incubation (RQ = 9.56). For 24 hours treatment, *TNFA* expression was less than one. From these data, the incubation time of 6 hours was chosen. *ACTB* and *GAPDH* were used for normalization of mRNA quantification. Control was AGS cell line, maintained with complete culture medium added with water in the same volume used in eHP.



Supplementary Figure 3 Relative gene expression of *TNFA* in the AGS cell line after treatment with *H. pylori* extract. After the incubation time of 6 hours and in the volumes of 100, 150 and 200  $\mu$ L the data show a progressive increase in *TNFA* expression for the eHP volumes of 100 (RQ = 2.04) and 150  $\mu$ L (RQ = 3.59) compared to control. Reduced expression of *TNFA* was observed in the treatment with 200  $\mu$ L of eHP (RQ = 1.42). *ACTB* and *GAPDH* were used for normalization of mRNA quantification. Control was AGS cell line maintained with complete culture medium added, with water in the same volume used in eHP.







**Supplementary Figure 4 Relative expression of TNF-α signaling pathway genes.** Expression levels in non-silenced AGS, downregulated AGS for TNFR1 (shTNFR1) and downregulated AGS for TNFR2 (shTNFR2) comparing the conditions without treatment (control-C) and treated with extract of *H. pylori* (eHP). A: Expression levels in non-silenced AGS; B: Downregulated AGS for TNFR1 (shTNFR1); C: Downregulated AGS for TNFR2 (shTNFR2). Bars

represent the mean  $\pm$  SD from three independent events. Dotted line indicates relative quantification: RQ = 1.0. Statistically significant difference: <sup>a</sup>*P*≤0.05; <sup>b</sup>*P*≤0.01; <sup>c</sup>*P*≤0.001.





Supplementary Figure 5 Relative expression of miRNAs miR-19a, miR-34a, miR-103a, miR-130a and miR-181c. Expression levels in non-silenced AGS, downregulated AGS for TNFR1 (shTNFR1) and downregulated AGS for TNFR2 (shTNFR2) comparing the conditions without treatment (control-C) and treated with extract of *H. pylori* (eHP). A: Expression levels in non-silenced AGS; B: Downregulated AGS for TNFR1 (shTNFR1); C: Downregulated AGS for TNFR2

(shTNFR2). Bars represent the mean  $\pm$  SD from three independent events. Dotted line indicates relative quantification: RQ = 1.0. Statistically significant difference: <sup>a</sup>*P* ≤ 0.05.



DOI: 10.3748/wjg.v0.i0.0000 Copyright ©The Author(s) 2022.

**Supplementary Figure 6 Cell cycle distribution analysis.** Number of cells in the G0/G1, S and G2/M phases of the cell cycle in non-silenced AGS, downregulated AGS for TNFR1 (shTNFR1) and downregulated AGS for TNFR2 (shTNFR2) comparing the conditions without treatment (control-C) and treated with extract of *H. pylori* (eHP). A: cell cycle in non-silenced AGS; B: Downregulated AGS for TNFR1 (shTNFR1); C: Downregulated AGS for TNFR2 (shTNFR2). Bars represent the mean ± SD from three independent events.



**Supplementary Figure 7 Apoptosis distribution analysis**. Number of cells in the early apoptosis and late apoptosis in non-silenced AGS, downregulated AGS for TNFR1 (shTNFR1) and downregulated AGS for TNFR2 (shTNFR2) comparing the conditions without treatment (control-C) and treated with extract of *H. pylori* (eHP). A: Number of cells in the early apoptosis; B: Late apoptosis. Bars represent the mean ± SD from three independent events.