Copper activates HIF-1α/GPER/VEGF signalling in cancer cells

Supplementary Material
Supplementary Fig. 1: CuSO₄ rescues the inhibitory effects of TEPA on hypoxia-induced transcription of HIF-1α, GPER and VEGF. In SkBr3 and HepG2 cells, the up-regulation of HIF-1α (A) and VEGF (B) mRNA expression induced by a treatment with 200 µM CuSO₄ for 8 hours is no longer evident in the presence of TEPA (50 µM) in normoxic condition. The up-regulation of HIF-1α (A) and VEGF (B) mRNA expression induced by hypoxia (2% O₂ for 8 hours) is no longer evident in the presence of TEPA (50 µM) but rescued culturing cells in the aforementioned hypoxic condition along with 200 µM CuSO₄, as determined by real-time PCR. Values are normalized to the 18S expression and shown as fold changes of mRNA expression induced by treatments also upon hypoxia respect to cells cultured with vehicle (-) in normoxic condition. (○), (●) indicate p <0.05 (C) In SkBr3 and HepG2 cells, the transactivation of a VEGF promoter plasmid (pVEGF) observed upon treatment with 200 µM CuSO₄ for 12 hours is no longer evident in the presence of TEPA (50 µM) in normoxic condition. The transactivation of a VEGF promoter plasmid (pVEGF) induced by hypoxia (2% O₂ for 12 hours) is no longer evident in the presence of TEPA (50 µM) but rescued culturing cells in the aforementioned hypoxic condition along with 200 µM CuSO₄. The luciferase activities were normalized to the internal transfection control and values of cells receiving vehicle (-) and cultured upon normoxia were set as 1-fold induction upon which the activities induced by treatments also in hypoxic conditions were calculated. Each data point represents the mean ± SD of three independent experiments performed in triplicate. (○), (●) indicate p <0.05 (D) In SkBr3 and HepG2 cells, the up-regulation of GPER mRNA expression induced by a treatment with 200 µM CuSO₄ for 8 hours is no longer evident in the presence of TEPA (50 µM) in normoxic condition. The up-regulation of GPER mRNA expression induced by hypoxia (2% O₂ for 8 hours) is no longer evident in the presence of TEPA (50 µM) but rescued culturing cells in the aforementioned hypoxic condition along with 200 µM CuSO₄, as determined by real-time PCR. Values are normalized to the 18S expression and shown as fold changes of mRNA expression induced by treatments also upon hypoxia respect to cells cultured with vehicle (-) in normoxic condition. (○), (●) indicate p <0.05 (E) In SkBr3 and HepG2 cells, the transactivation of a GPER promoter plasmid
(pGPER) observed upon treatment with 200 µM CuSO₄ for 12 hours is no longer evident in the presence of TEPA (50 µM) in normoxic condition. The transactivation of a GPER promoter plasmid (pGPER) induced by hypoxia (2% O₂ for 12 hours) is no longer evident in the presence of TEPA (50 µM) but rescued culturing cells in the aforementioned hypoxic condition along with 200 µM CuSO₄. The luciferase activities were normalized to the internal transfection control and values of cells receiving vehicle (−) and cultured upon normoxia were set as 1-fold induction upon which the activities induced by treatments also in hypoxic conditions were calculated. Each data point represents the mean ± SD of three independent experiments performed in triplicate. (○), (●) indicate p <0.05
Supplementary Fig. 2: HIF-1α and GPER are involved in the proliferative effects induced by CuSO₄. (A) MTT proliferation assays in SkBr3 cells transfected for 24 h with shRNA, shHIF-1α or shGPER and then treated for 48 hours with vehicle or 200 µM CuSO₄, as indicated. Efficacy of HIF-1α (B) and GPER (C) silencing in SkBr3 cells. (D) MTT proliferation assays in SkBr3 cells treated for 48 hours with vehicle, 200 µM CuSO₄ alone or in combination with TEPA (50 µM). Values are mean ± SD of three independent experiments. (*) p <0.05 for cells receiving vehicle versus CuSO₄ treatment.