SUPPLEMENTARY FIGURES



Supplementary Figure 1. HO-1 inhibition decreases Gas6 production inU266 cells. (A, B) mRNA expression of HO-1 and Gas6 in U266 cells were measured by qRT-PCR. β -actin was used as a control. Cells were treated with ZnPPIX for 24 h. (C–E) Western blot and semiquantitative analysis of HO-1 and Gas6 protein levels in U266 cells-treated with ZnPPIX for 24 h. β -actin was used as a loading control. Data are expressed as mean \pm SD (n = 4). **P*< 0.05 vs. untreated control group (0 μ M). (F, G) HO-1 and Gas6 mRNA levels in U266 cells were measured by qRT-PCR after transfection with empty vector (EV) and HO-1 siRNA. (H–J) Western blot analysis was performed to detect the protein expression of HO-1 and Gas6 in HO-1 knockdownU266 cells. (K, L) Immunofluorescence staining was performed to visualize Gas6 expression using a primary rabbit antibody against Gas6, and followed by Alexa Fluor 555-conjugated secondary antibody. The endogenous Gas6 was shown in red. Nuclei were stained with DAPI (blue). The scale bars represent 100 μ m. Data are expressed as mean \pm SD (n = 4). **P*< 0.05 vs. U266; [#]*P*< 0.05 vs. U266-EV.



Supplementary Figure 2. HO-1 inhibition downregulates Gas6 expression in RPMI8226cells. (A, B) mRNA expression of HO-1 and Gas6 in RPMI8226cells were measured by qRT-PCR. (C–E) Western blot and semi-quantitative analysis of HO-1 and Gas6 protein levels in RPMI8226 cells-treated with ZnPPIX for 24 h. (F, G) HO-1 and Gas6 mRNA levels in RPMI8226 cells were measured by qRT-PCR after transfection with empty vector (EV) and HO-1 siRNA. (H–J) The effects of HO-1 knockdown on Gas6 protein expression level was shown in RPMI8226 cells. Data are expressed as mean \pm SD (n = 4). **P*< 0.05 vs. RPMI8226; #P< 0.05 vs. RPMI8226-EV.