

E08-03-0283 Peek

Supplemental Figure 1. MKN28 cells express the transcriptional repressor, Kaiso. MKN28 cells were fixed and incubated with an anti-Kaiso antibody or PBS alone, followed by incubation with an AlexaFluor-488 antibody and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as projection images of a Z-stack. Kaiso, green; nuclei, red. 630X magnification. (A) Staining with a mouse monoclonal anti-Kaiso antibody (11D) and anti-mouse AlexaFluor-488 secondary antibody. (B) Staining with a rabbit polyclonal anti-Kaiso antibody and anti-rabbit AlexaFluor-488 secondary antibody.

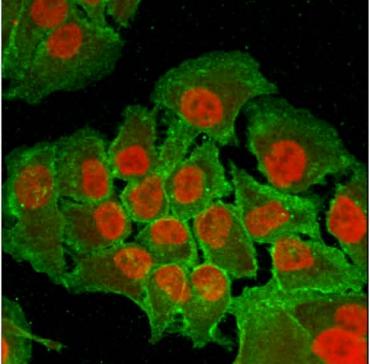
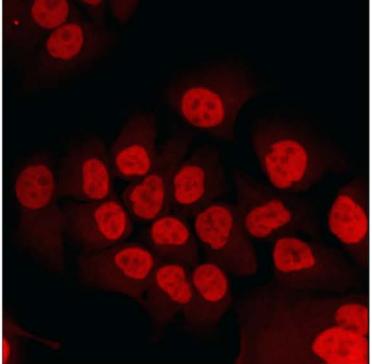
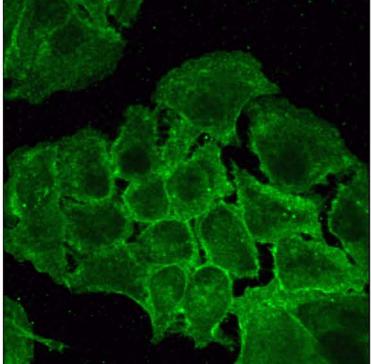
Supplemental Figure 2. *H. pylori* infection or increases in MKN28 cell confluence over time did not contribute to changes in total or subcellular p120 levels. (A) MKN28 cells were co-cultured with the *H. pylori* *cag*⁺ strain 7.13 (MOI=100) or medium alone. At defined time points, RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. Data represent fold *p120* mRNA expression in cells co-cultured with *H. pylori* versus medium alone from experiments performed on at least 3 occasions. Error bars, SEM. (B) MKN28 cells were cultured with medium alone. At defined time points, total protein was extracted, subjected to subcellular fractionation, and analyzed by Western blot using an anti-p120 antibody. The graph represents densitometric analysis of multiple Western blot repetitions performed on at least 3 occasions. Error bars, SEM.

Supplemental Figure 3. Exogenous expression of p120 does not significantly relieve Kaiso-mediated transcriptional repression in uninfected cells. MKN28 cells were

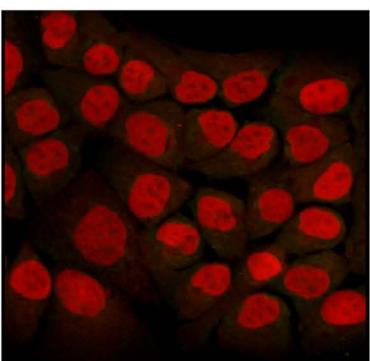
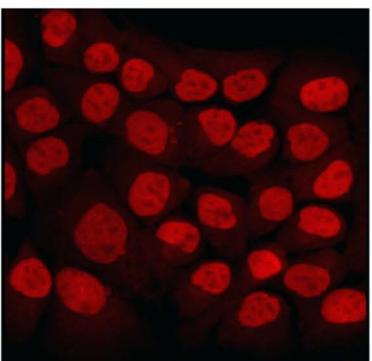
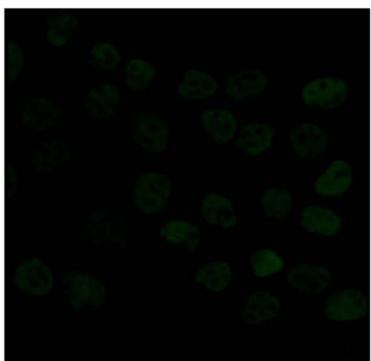
cotransfected with 100 ng 4xKBS-pGL3, 4 ng phRL Null, and 100 ng of the empty vector pCMV or 100 ng p120-pCMV for 12 hours, and the medium subsequently replaced. After 48 hours, cells were harvested and luciferase activity was determined in a dual channel luminometer. Data are represented as relative light units (firefly luciferase vs. renilla luciferase) for experiments performed on at least three occasions. Error bars, SEM.

A)

Kaiso
11D



AlexaFluor
m488 only



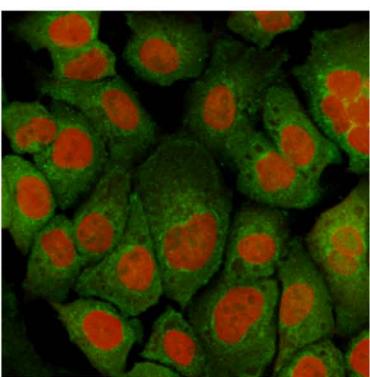
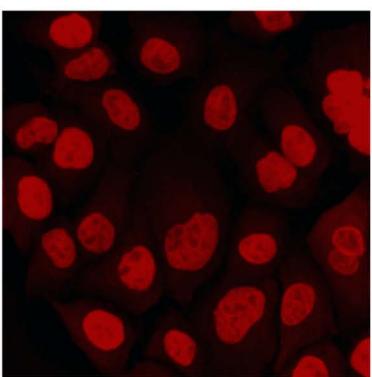
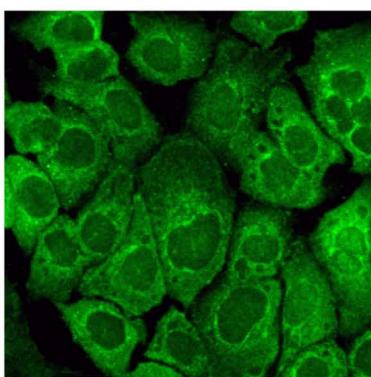
Kaiso

Nuclei

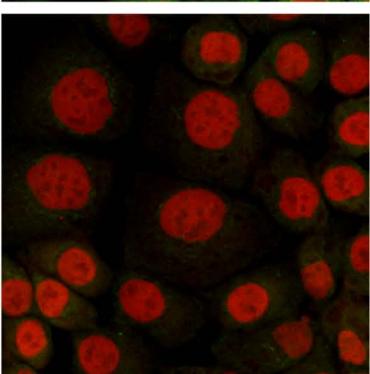
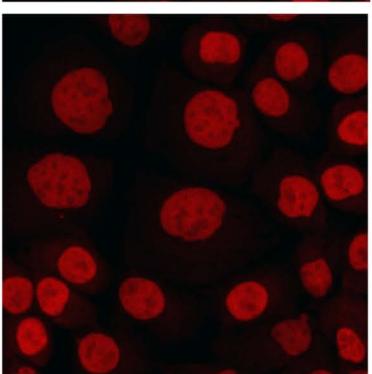
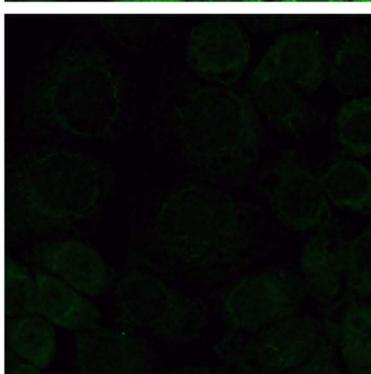
Merge

B)

Kaiso
11D



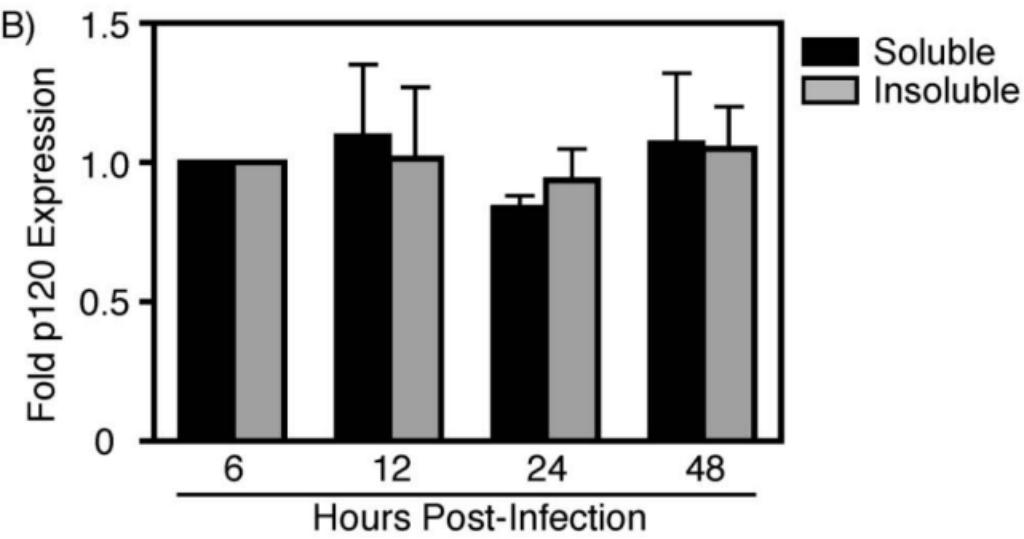
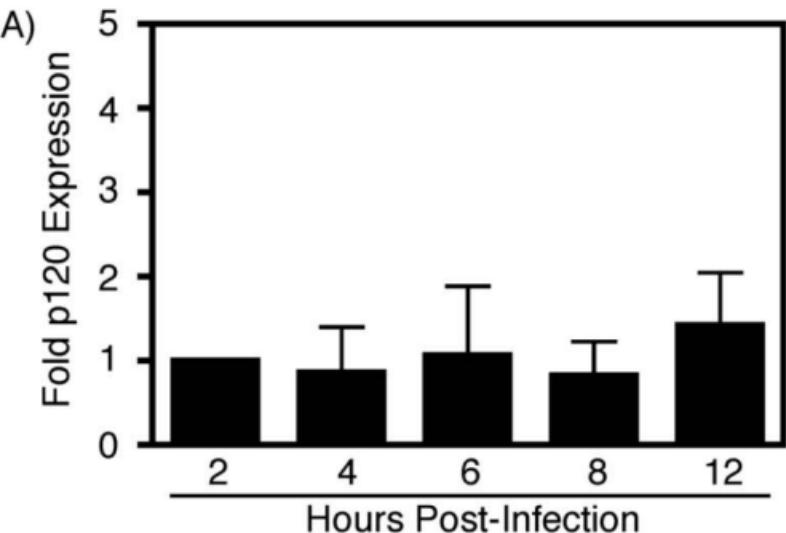
AlexaFluor
r488 only



Kaiso

Nuclei

Merge



Relative light units

