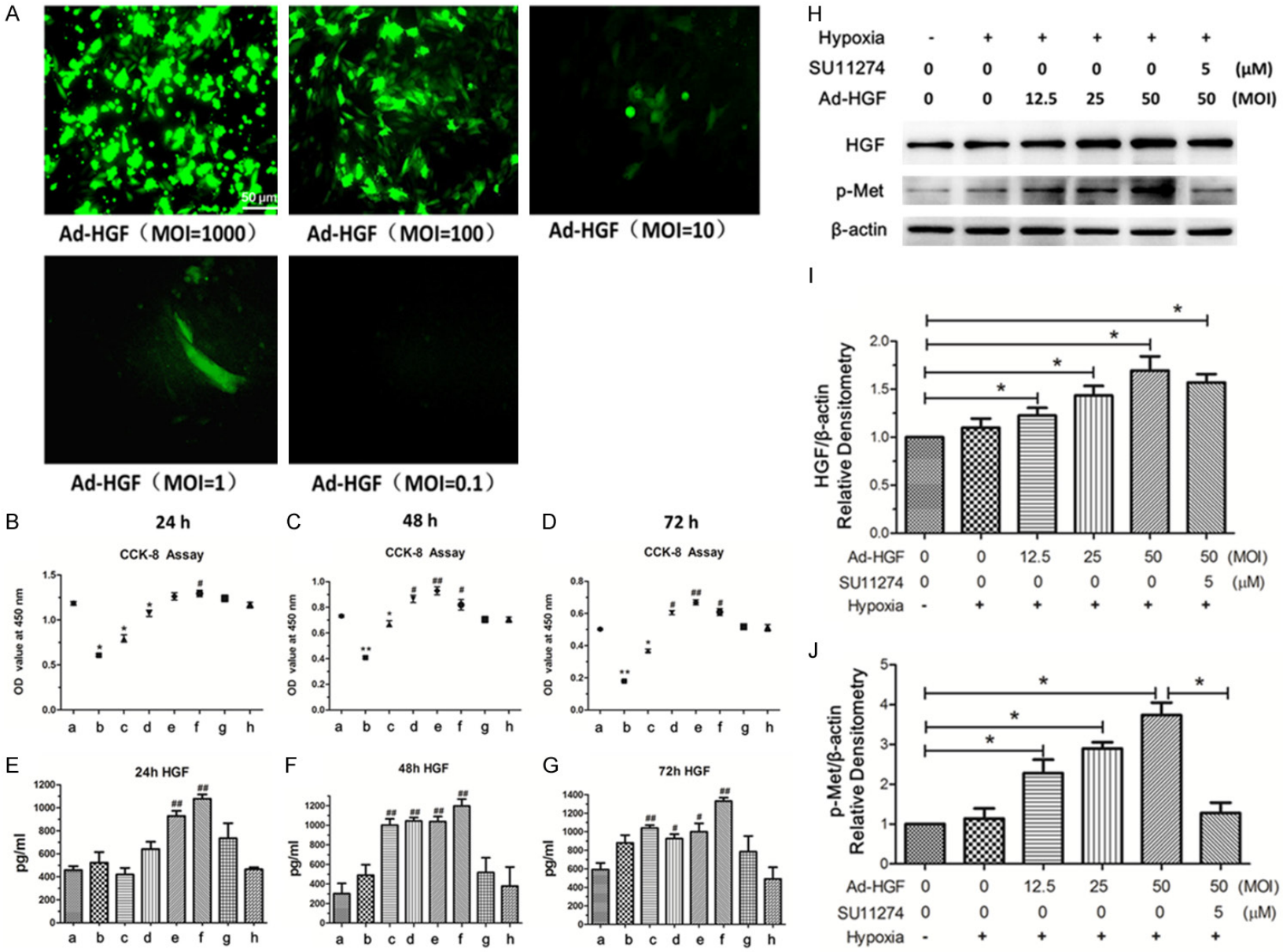
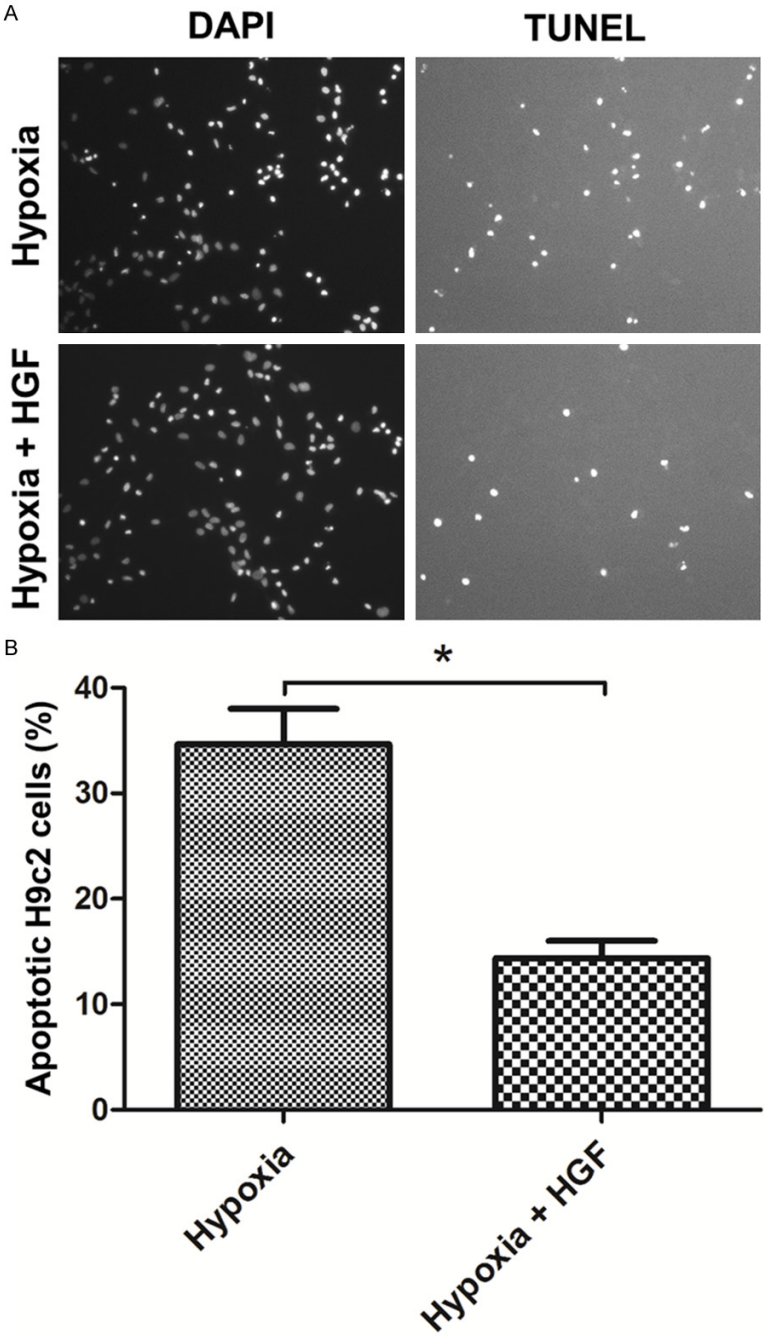
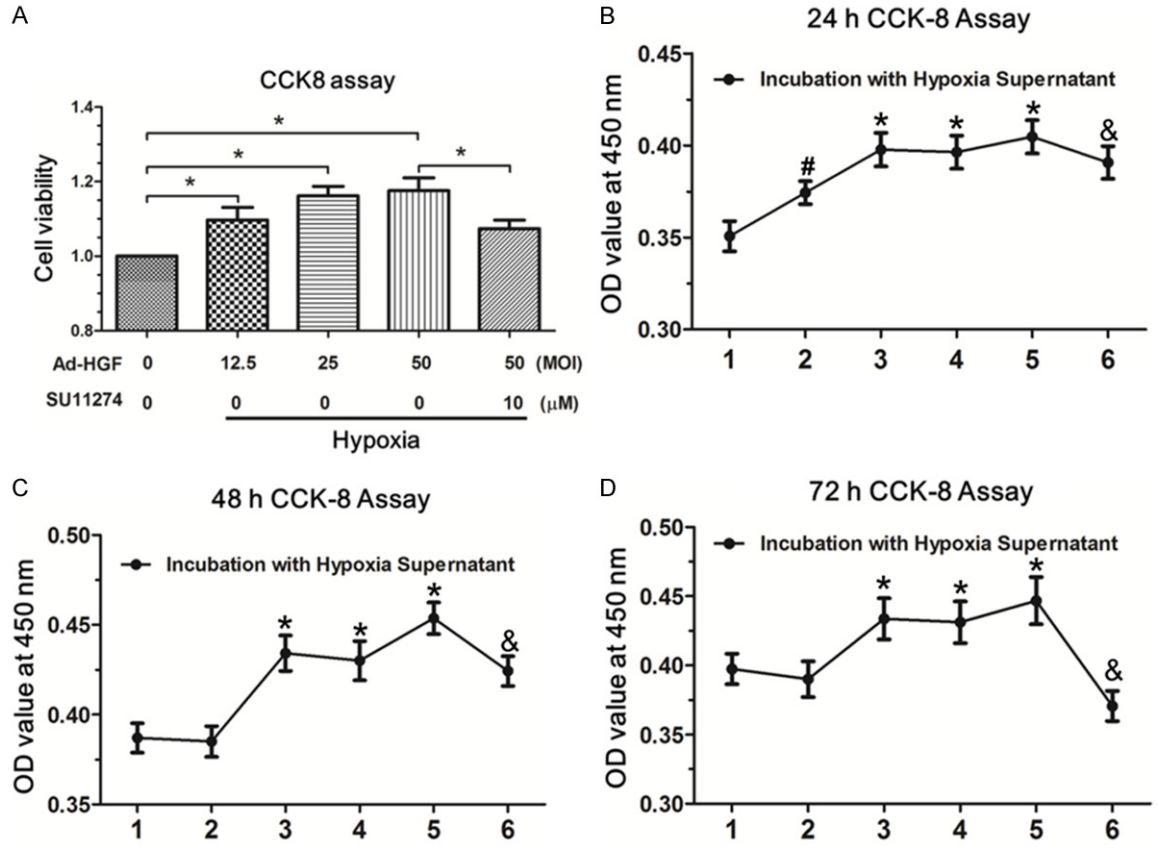

A
50 μm
Ad-HGF（MOI=1000）
Ad-HGF（MOI=100）
Ad-HGF（MOI=10）
Ad-HGF（MOI=1）
Ad-HGF（MOI=0.1）
B
24 h
C
48 h
D
72 h
CCK-8 Assay
OD value at 450 nm
a b c d e f g h
E
24h HGF
F
48h HGF
G
72h HGF
pg/ml
H
Hypoxia - + + + + +
SU11274 0 0 0 0 0 5 (μM)
Ad-HGF 0 0 12.5 25 50 50 (MOI)
HGF
p-Met
β-actin
I
HGF/β-actin
Relative Densitometry
Ad-HGF 0 0 12.5 25 50 50 (MOI)
SU11274 0 0 0 0 0 5 (μM)
Hypoxia - + + + + +
J
p-Met/β-actin
Relative Densitometry
Ad-HGF 0 0 12.5 25 50 50 (MOI)
SU11274 0 0 0 0 0 5 (μM)
Hypoxia - + + + + +

Supplementary Figure 1. Choose the appropriate multiplicity of infection (MOI) of Ad-HGF to infect the H9c2 cells. A. Different MOIs of Ad-HGF (1000, 100, 10, 1, 0.1 MOI) were used to infect the H9c2 cells with the same cell density for 24 hours and the GFP fluorescence conjugated was observed under the fluorescence microscope. The infection efficiency of Ad-HGF (1000 or 100 MOI) was high, but the number of dead cells after infection increased. B-D. CCK-8 assay was further used to assess the cell activity after infection of Ad-HGF with various MOIs. a. control, b. MOI=1000, c. MOI=200, d. MOI=100, e. MOI=50, f. MOI=25, g. MOI=12.5, h. MOI=6.125. The infection for 24, 48 or 72 hours' CCK-8 assay showed the cell activity with Ad-HGF (1000, 200, 100 MOI) was at least once significantly decreased when compared with the control. E-G. The HGF levels in supernatant after Ad-HGF (1000, 200, 100, 50, 25, 12.5, 6.125 MOI) infection for 24, 48 or 72 hours were determined by the ELISA assay. Combined the above assays, we chose the 50, 25, 12.5 MOI of Ad-HGF for the following experiments. Data are expressed as mean ± SD, n=3, *P<0.05 vs. the control, **P<0.01 vs. the control, #P<0.05 vs. the control, ##P<0.01 vs. the control. H. Western blot analyzed the protein levels of HGF and p-Met in H9c2 cells under hypoxia after Ad-HGF (12.5, 25, 50 MOI) or SU11274 (10 μM) treatment. β-actin was used as loading control. I, J. The bar graphs showed the statistical analysis of the above protein levels. Data are shown as mean ± SD, n=3, *P<0.05.



Supplementary Figure 2. HGF treatment decreased the apoptotic H9c2 cells under hypoxia. A. Fluorescence microscope analysis of the TUNEL and DAPI staining in H9c2 cells showed hypoxia increased the percent of apoptotic cells which was significantly attenuated by HGF (80 ng/ml). B. The bar chart displayed the statistical analysis of the percent of the apoptotic cells. Data are expressed as mean ± SD, n=3, *P<0.05.



Supplementary Figure 3. Treatment of Ad-HGF or its cell supernatant increased the activity of H9c2 cells. A. CCK-8 assay evaluated the activity of H9c2 cells under hypoxia after the indicated treatment. Ad-HGF dose-dependently increased the cell activity under hypoxia when compared to the control group, which was blocked by SU11274. Representative images were shown. B-D. The activities of H9c2 cells treated with the hypoxia supernatant for 24, 48 or 72 hours were determined by CCK-8 assay. Treatment groups: 1. Supernatant of H9c2 cells in normoxic group, 2. Supernatant of H9c2 cells under hypoxia for nine hours, 3. Supernatant of H9c2 cells under hypoxia for nine hours after infected with Ad-HGF (12.5 MOI), 4. Supernatant of H9c2 cells under hypoxia for nine hours after infected with Ad-HGF (25 MOI), 5. Supernatant of H9c2 cells under hypoxia for nine hours after infected with Ad-HGF (50 MOI), 6. Supernatant of H9c2 cells under hypoxia for nine hours after treated with Ad-HGF (50 MOI) and SU11274 (10 μM). The results revealed the supernatant of H9c2 cells under hypoxia after infected with Ad-HGF significantly enhanced the cell activity when compared with the control and the group of hypoxia supernatant without Ad-HGF infection. The hypoxia supernatant without Ad-HGF infection markedly increased the cell activity in 24 hours' incubation than the control, but showed no significant difference between them in later 48 or 72 hours' incubation. Data are expressed as mean ± SD, n=3, *P<0.05 vs. control, $^{\#}$P<0.05 vs. the control, $^{\&}$P<0.05 vs. the group 5.