Supplementary Figure Legends

Figure S1. High YAP/TAZ expression in KS. (a) Specificity of the YAP/TAZ antibody by immunohistochemical staining. Tumors generated by vGPCR-infected SVEC cells with either control (upper panel) or YAP/TAZ knockdown (lower panel) were stained with the YAP/TAZ antibody. (b) YAP/TAZ expression in HIV-negative specimens. Immunohistochemical staining of YAP/TAZ was performed in HIV-negative KS samples. Sample ID was labeled on the lower left corner. (c) The expression of LANA and YAP/TAZ in KS samples. Representative tumors stained for LANA (top panels) and YAP/TAZ (bottom panels). LANA and YAP/TAZ expression was scored from 0 (weakest expression) to 4 (highest expression) depending on the signal strength. Sample ID is indicated on the lower left corner.

Figure S2. KSHV induces YAP/TAZ expression. (a) Expression of lytic genes in 293T cells infected with recombinant KSHV. (b) Protein lysates of 293T cells infected with or without KSHV were harvested and subjected to immunoblotting.

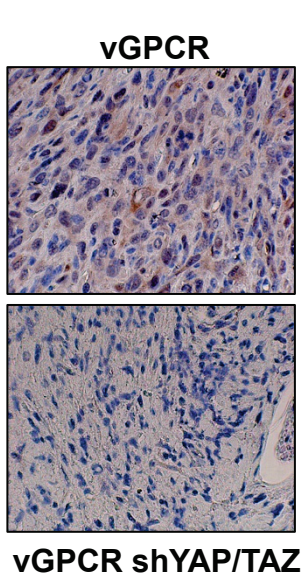
Figure S3. YAP and TAZ are activated by vGPCR. (a) The glycosylation of vGPCR. SVEC stable cells expressing HA-vGPCR were harvested and treated with a protein deglycosylation mix. (b) mRNA levels of YAP and TAZ was determined by quantitative RT-PCR. (c) YAP/TAZ were more stable in vGPCR expressing cells. MCF10A stable cells were treated with cycloheximide for the indicated times and the results were quantified (hours: hr). (d) The localization of vGPCR. HEK293A cells expressing either control or vGPCR were stained for subcellular localization of HA-vGPCR (green), TAZ (red), and DAPI (blue). (e) vGPCR activates a YAP/TAZ reporter. Control (CTL), vGPCR, or LPAR positive control plasmids were co-transfected with 5 X UAS-luciferase, Renilla, and Gal4-CTGF into HEK293A cells. Luciferase activity was measured and normalized.

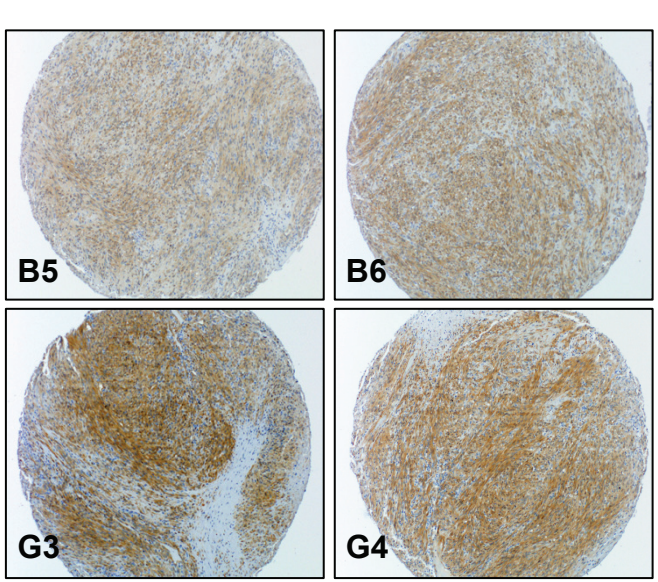
Figure S4. The mechanism of YAP/TAZ activation. (a, b) The activity of Lats2 and MST2 in vGPCR expressing cells. HEK293A cells were transfected with the indicated plasmids and serum-starved for 12 hours. Flag-Lats2 or Flag-MST2 was immunoprecipitated and an in vitro kinase assay was performed using either GST-YAP or GST-Mob as substrates. (c) MST2 does not affect vGPCR-induced TAZ expression. Overexpression of MST2 failed to suppress the effect of vGPCR on TAZ protein accumulation and CTGF expression.

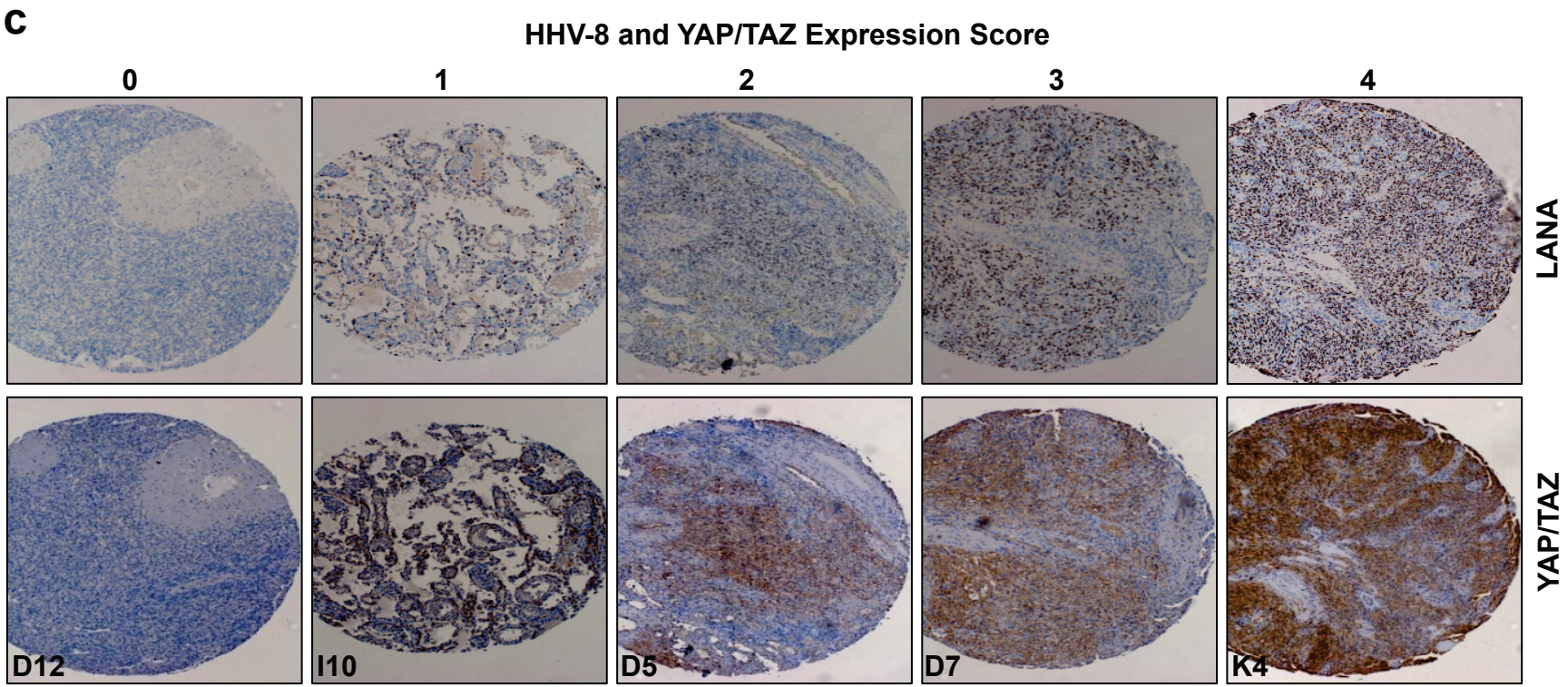
Figure S5. YAP/TAZ are required for cell migration induced by vGPCR. A transwell cell migration assay was conducted in MCF10A cells. Cells are stained with crystal violet.

Figure S6. YAP/TAZ are required for tumorigenesis induced by vGPCR. 1 x 10^6 SVEC cells were injected subcutaneously into the flanks of nude mice. Tumors were harvested 5 weeks after injection and weighed.

Figure S1


a
vGPCR
vGPCR shYAP/TAZ


b
B5
B6
G3
G4


c
HHV-8 and YAP/TAZ Expression Score
0
1
2
3
4
LANA
YAP/TAZ
D12
I10
D5
D7
K4

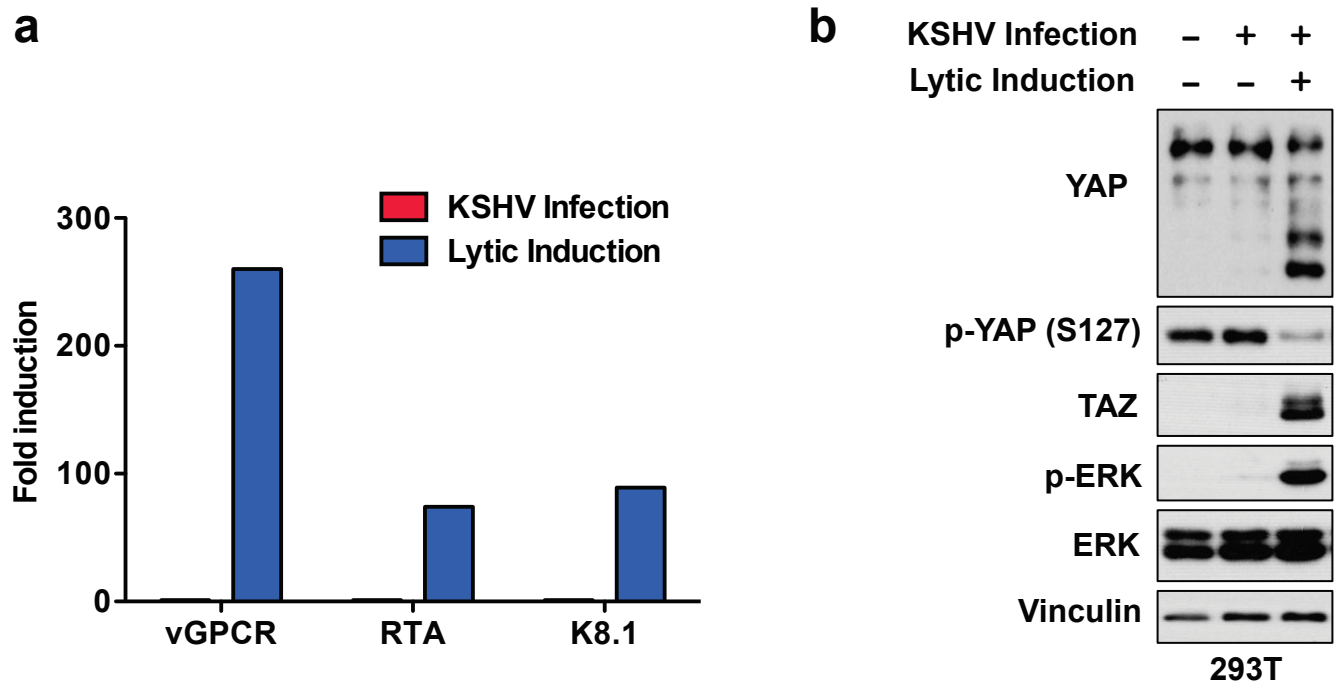

a
KSHV Infection
Lytic Induction
Fold induction
300
200
100
0
vGPCR
RTA
K8.1
b
KSHV Infection – + +
Lytic Induction – – +
YAP
p-YAP (S127)
TAZ
p-ERK
ERK
Vinculin
293T

Figure S3

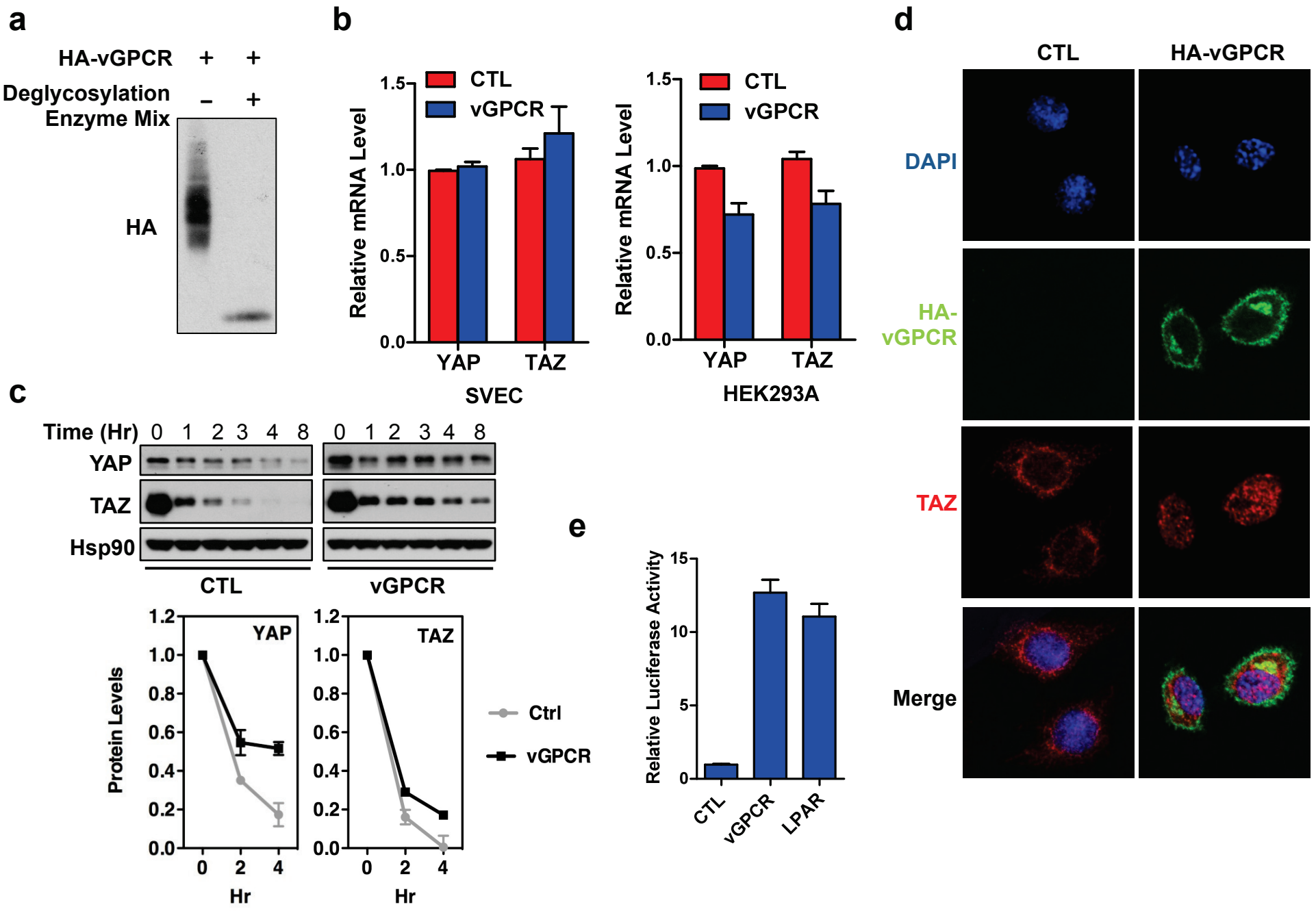

a
HA-vGPCR + +
Deglycosylation Enzyme Mix – +
HA
b
Relative mRNA Level
CTL
vGPCR
YAP
TAZ
SVEC
HEK293A
c
Time (Hr) 0 1 2 3 4 8 0 1 2 3 4 8
YAP
TAZ
Hsp90
CTL
vGPCR
Protein Levels
Hr
Ctrl
vGPCR
d
CTL
HA-vGPCR
DAPI
HA-vGPCR
TAZ
Merge
e
Relative Luciferase Activity
CTL
vGPCR
LPAR

Figure S4

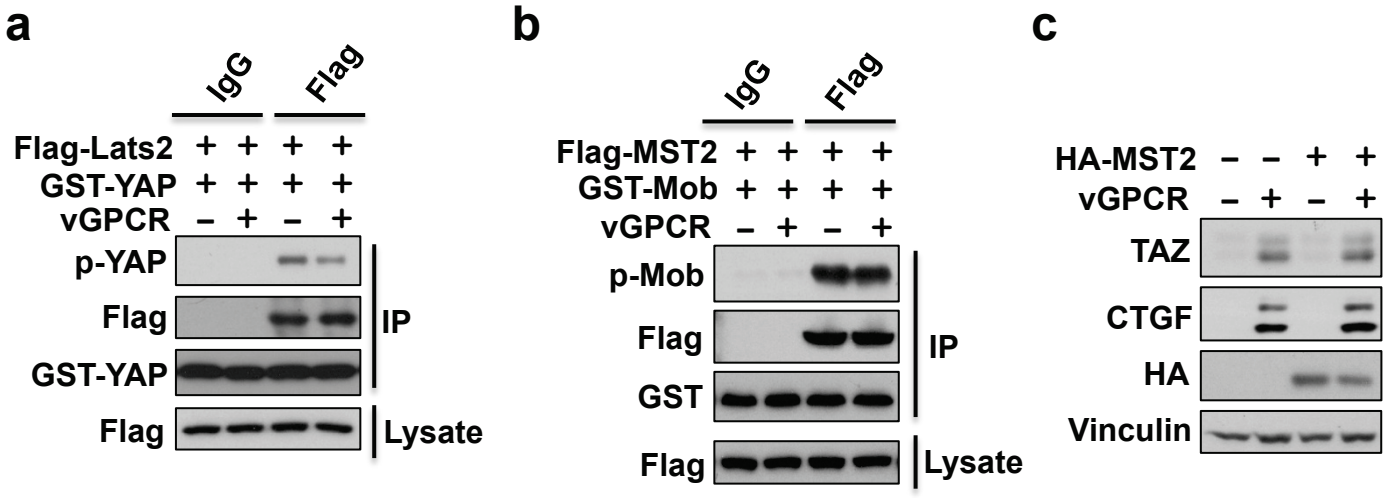

a
IgG
Flag
Flag-Lats2 + + + +
GST-YAP + + + +
vGPCR – + – +
p-YAP
Flag
GST-YAP
Flag
IP
Lysate
b
IgG
Flag
Flag-MST2 + + + +
GST-Mob + + + +
vGPCR – + – +
p-Mob
Flag
GST
Flag
IP
Lysate
c
HA-MST2 – – + +
vGPCR – + – +
TAZ
CTGF
HA
Vinculin

Figure S5

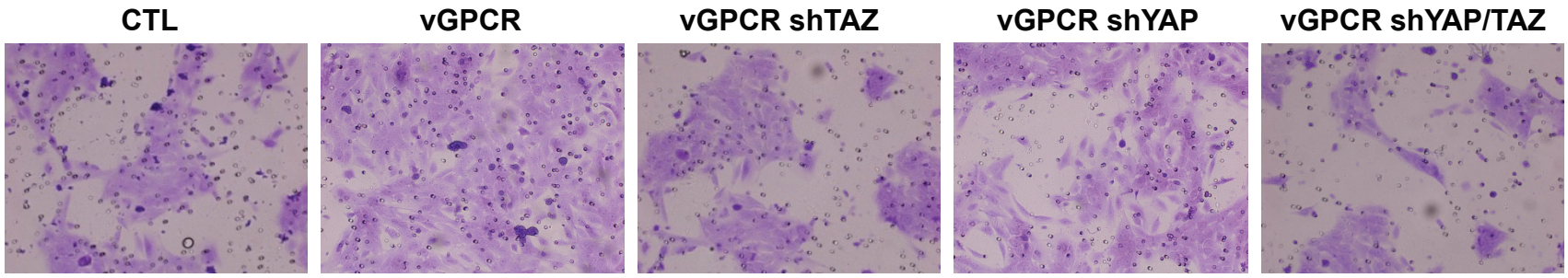


Figure S6