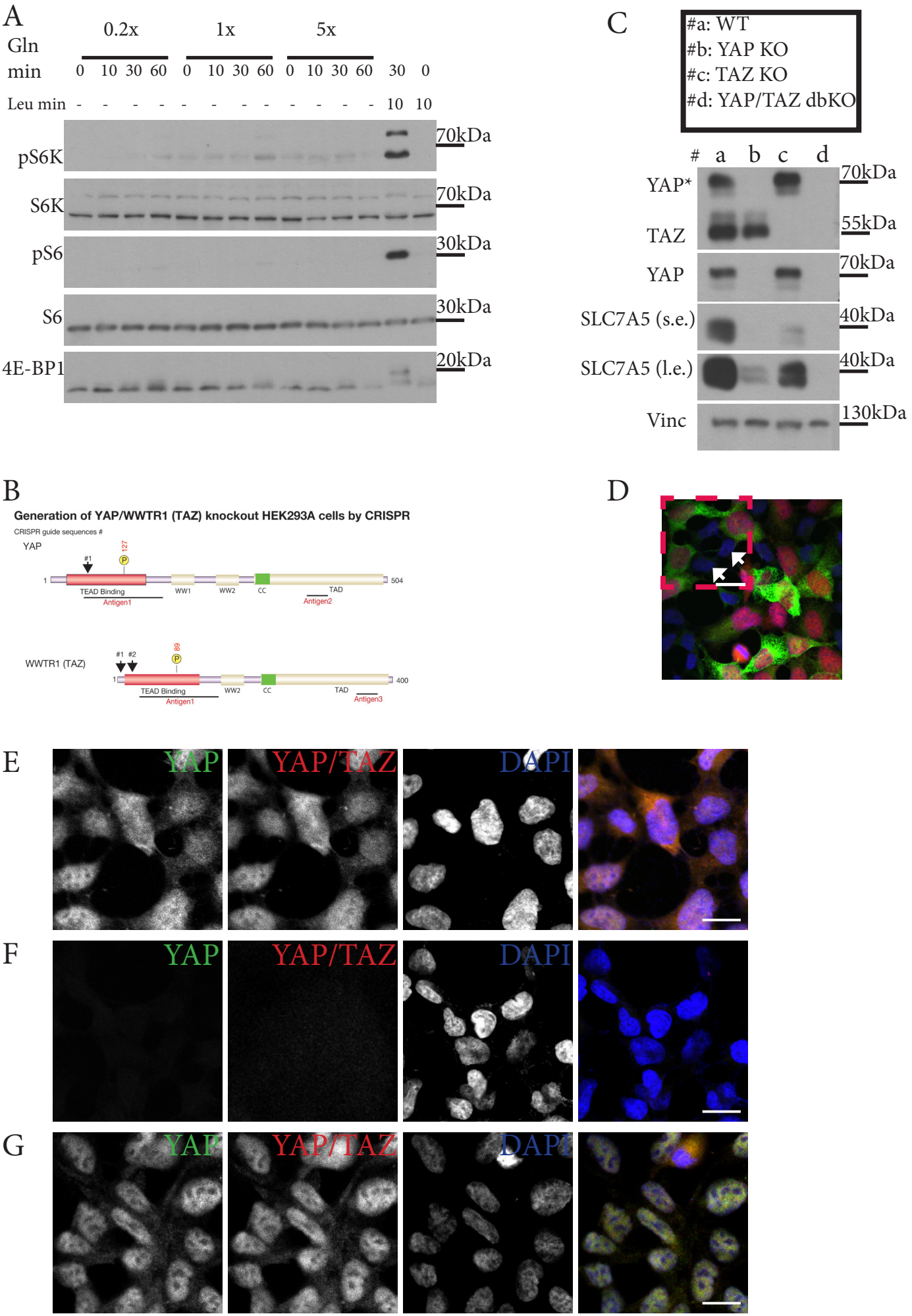
Sup figure 1


A
Gln
0.2x
1x
5x
min
0 10 30 60 0 10 30 60 0 10 30 60 30 0
Leu min - - - - - - - - - - - - 10 10
pS6K
S6K
pS6
S6
4E-BP1
70kDa
70kDa
30kDa
30kDa
20kDa
C
#a: WT
#b: YAP KO
#c: TAZ KO
#d: YAP/TAZ dbKO
a b c d
YAP*
TAZ
YAP
SLC7A5 (s.e.)
SLC7A5 (l.e.)
Vinc
70kDa
55kDa
70kDa
40kDa
40kDa
130kDa
B
Generation of YAP/WWTR1 (TAZ) knockout HEK293A cells by CRISPR
CRISPR guide sequences #
YAP
#1
127
1
504
TEAD Binding
Antigen1
WW1
WW2
CC
TAD
Antigen2
WWTR1 (TAZ)
#1 #2
89
1
400
TEAD Binding
Antigen1
WW2
CC
TAD
Antigen3
D
E
F
G
YAP
YAP/TAZ
DAPI

Supplementary information, Figure S1 (A) Glutamine is required for leucine to activate mTORC1. 293A wells were AA starved for 6 hours and stimulated with either glutamine (Gln) alone at different concentrations and time, leucine (Leu) alone, or glutamine followed by short time leucine stimulation. 1x denotes the concentration present in normal DMEM growth medium. **(B)** Design of CRISPR mediated YAP/TAZ dbKO cells. Schematic representation of YAP and TAZ; TAZ is encoded by the WWTR1 gene. Illustrated are protein motifs and domains known to be functionally important for the function of YAP and TAZ. WW domain, CC: coiled-coiled region, TAD: transactivation domain. The CRISPR guide sequences were designed to target the N-terminus of YAP or TAZ as illustrated. In addition, the location of the antigens used to raise the commercial abs against YAP and TAZ used in this study are also illustrated. These antibodies were used to verify the KO status during the screening to obtain clones and likewise used throughout this study reported here. Antigen 1 utilized in mice to raise antibodies (sc-101199) that both recognize YAP and TAZ. Antigen 2 utilized to generate the YAP specific antibodies (ab52771) is raised in rabbit. Antigen 3 utilized to raise antibodies against TAZ (V386, from Cell signaling). The KO clones were likewise also sequence verified. **(C)** Verification of CRISPR mediated YAP, TAZ and YAP/TAZ db KO 293A cells by western. Cell lysates were prepared from cell lines denoted in the box. * denotes antibody (sc-101199) that recognizes both YAP and TAZ, raised against antigen 1 in Figure S1B. YAP and TAZ can be distinguished by their different apparent molecular weight. YAP specific antibody used here is (ab52771), raised against antigen 2, see sup Figure S1B. The membranes were in addition also probed against SLC7A5 and

Vinculin (Vinc), Vinc serves as a loading control. **(D)** YAP and TAZ are necessary for activation of mTORC1 by Gln/Leu. Full size image of Gln/Leu stimulated cells from Figure 1B. Red box denotes region depicted in Figure 1B. White arrow-heads highlight the same YAP/TAZ dbKO cells as in Figure 1B, and scale bar is also retained from Figure 1B. Red and green denote the YAP and pS6 signals, respectively, and blue the DAPI signal. **(E)** Specificity verification of YAP antibodies and YAP/TAZ antibodies. WT 293A cells were processed for immunofluorescence, and co-labeled with antibodies against YAP (ab52771) raised in rabbit, and YAP/TAZ Abs (sc-101199) raised in mouse. The cells were furthermore stained with DAPI. **(F)** Y/T dbKO cells were processed for immunofluorescence as cells in **D**. **(G)** LATS1/2 dbKO cells were processed as cells depicted in **E**, note the increased nuclear localization of both YAP and TAZ compared to WT cells in panel **E**. Samples and images in Figure **E-G** were processed in parallel and images were acquired with the same laser and microscope settings to verify abs specificity. Scale bars in **E-G** are 20 μm.