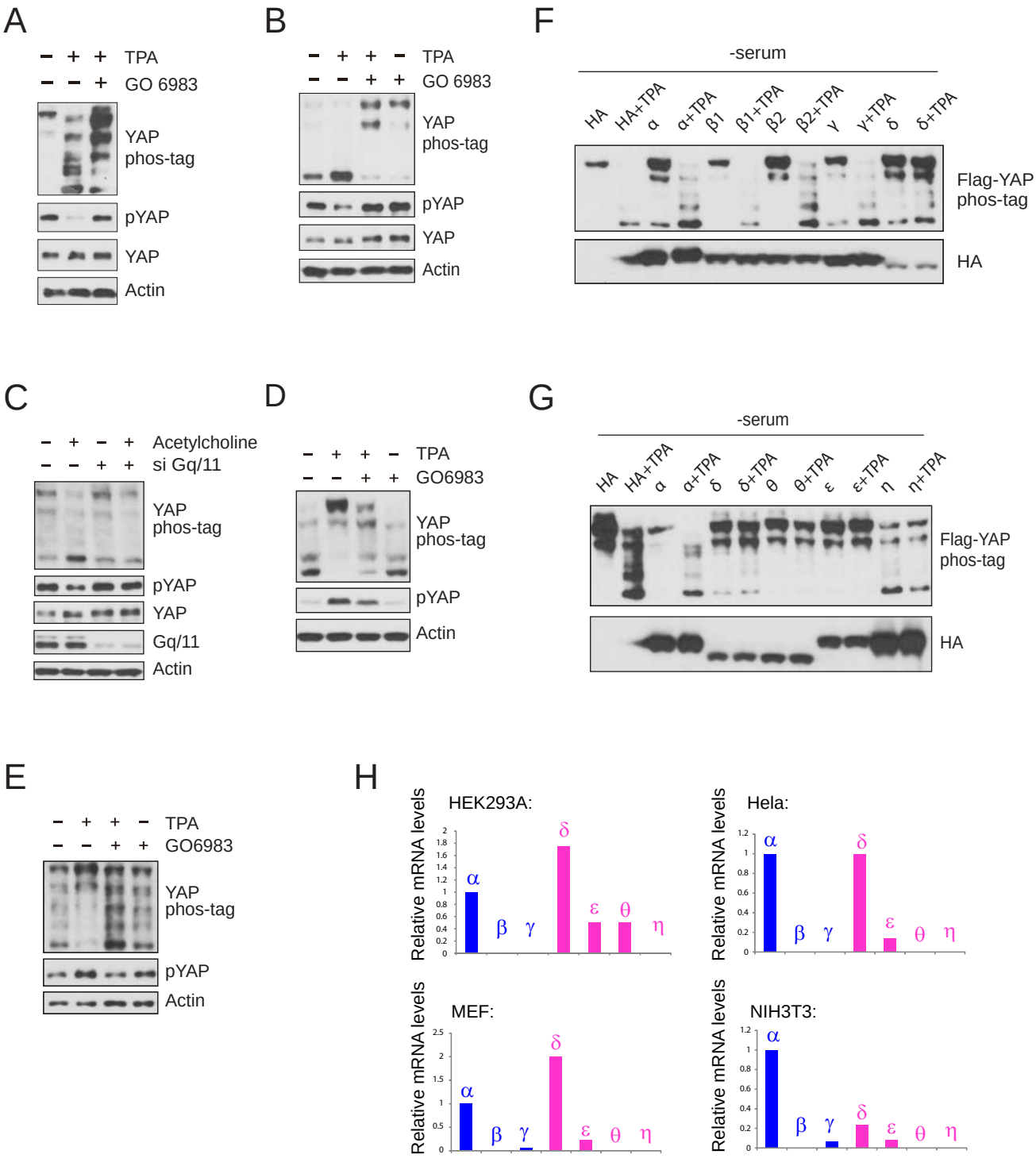
A
TPA
GO 6983
YAP phos-tag
pYAP
YAP
Actin
B
TPA
GO 6983
YAP phos-tag
pYAP
YAP
Actin
F
-serum
HA
HA+TPA
α
α+TPA
β1
β1+TPA
β2
β2+TPA
γ
γ+TPA
δ
δ+TPA
Flag-YAP phos-tag
HA
C
Acetylcholine
si Gq/11
YAP phos-tag
pYAP
YAP
Gq/11
Actin
D
TPA
GO6983
YAP phos-tag
pYAP
Actin
G
-serum
HA
HA+TPA
α
α+TPA
δ
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θ
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ε
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η+TPA
Flag-YAP phos-tag
HA
E
TPA
GO6983
YAP phos-tag
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Actin
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HEK293A:
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MEF:
NIH3T3:
Relative mRNA levels
α
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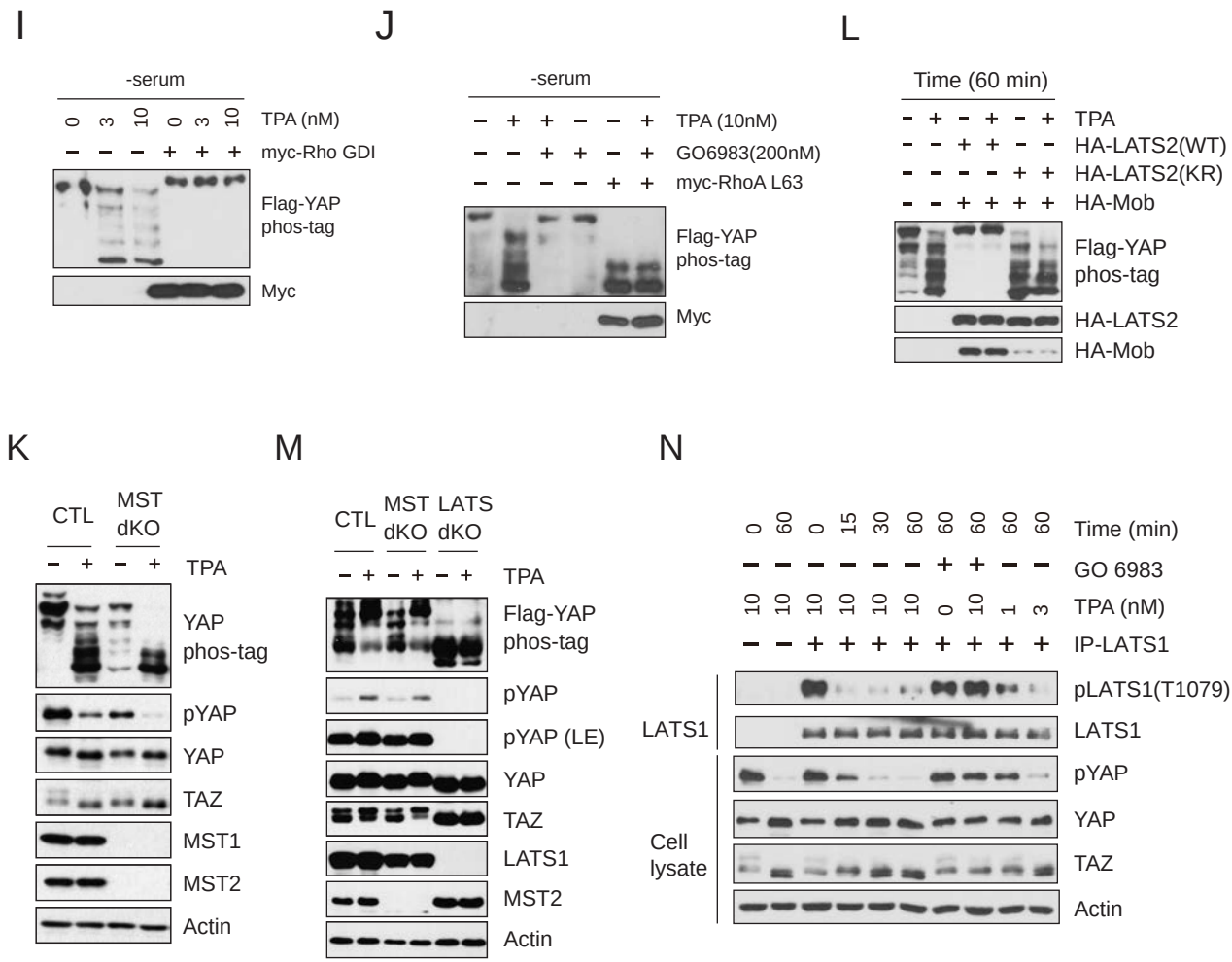
I
-serum
0 3 10 0 3 10
TPA (nM)
– – – + + +
myc-Rho GDI
Flag-YAP
phos-tag
Myc
J
-serum
– + + – – + TPA (10nM)
– – + + – + GO6983(200nM)
– – – – + + myc-RhoA L63
Flag-YAP
phos-tag
Myc
L
Time (60 min)
– + – + – + TPA
– – + + – – HA-LATS2(WT)
– – – – + + HA-LATS2(KR)
– – + + + + HA-Mob
Flag-YAP
phos-tag
HA-LATS2
HA-Mob
K
CTL
MST
dKO
– + – + TPA
YAP
phos-tag
pYAP
YAP
TAZ
MST1
MST2
Actin
M
CTL
MST
dKO
LATS
dKO
– + – + – + TPA
Flag-YAP
phos-tag
pYAP
pYAP (LE)
YAP
TAZ
LATS1
MST2
Actin
N
0 60 0 15 30 60 60 60 60 60 Time (min)
– – – – – – + + – – GO 6983
10 10 10 10 10 10 0 10 1 3 TPA (nM)
– – + + + + + + + + IP-LATS1
LATS1
Cell
lysate
pLATS1(T1079)
LATS1
pYAP
YAP
TAZ
Actin

Supplementary information, Figure S1 Conventional and novel PKC oppositely regulate the Hippo-YAP pathway. **(A and B)** TPA induces YAP dephosphorylation in various cell lines. Serum starved Hela cells (A) or Human U251MG glioma cells (B) were treated with 10nM TPA and/or 200nM PKC inhibitor GO6983 as indicated. **(C)** Acetylcholine activates YAP via Gq/11. U251MG cells were transfected with either control siRNA or a pool of siRNAs for Gq and G11. Cells were grown in serum containing medium for 48h followed by serum free medium for 16h. Cells were stimulated with 5uM acetylcholine for 1h and lysed for Western Blots. **(D and E)** TPA stimulates YAP phosphorylation in MEF cells (D) and A549 cells (E). Cells were cultured in medium with 10% serum for 36h. Cells were pretreated with GO6983 for 30min and then stimulated with TPA for 1h. **(F)** Conventional PKCs has minor effect on YAP phosphorylation. 500ng of individual HA-PKC isoform and 2ng Flag-YAP were co-transfected into HEK293A cells. 24h after transfection, cells were serum starved for 12h and stimulated with 10nM TPA for 1h. Cells were lysed for Western Blot. **(G)** The novel PKC promotes YAP phosphorylation. Experiments were performed the same as described in panel (F) except novel PKC plasmids were used in the transfection. (**H**) Absolute mRNA quantification of PKC isoforms in different cell lines. First, each individual PKC isoform fragment (the exact same primers used here and the late RT-PCR) was amplified by PCR, purified, and quantified. Second, the quantified PKC fragment was diluted serially for quantitative PCR to obtain a standard curve of Ct value against the known PKC fragment quantity. Therefore, a standard quantitative curve was established for each PKC isoform. Third, the absolute amount of each individual PKC isoform in each cell line was calculated by plotting the Ct value (obtained for each PKC from each cell line) on the standard curve. Fourth, the amount of PKC alpha in each cell line was normalized as one and the relative amount of each individual PKC were shown in the plot. Conventional PKCs are colored in blue and novel PKCs are colored in pink. (**I**) YAP activation by TPA is repressed by RhoGDI. Cells were co-transfected with indicated plasmids. 24h after transfection, cells were serum starved for 12h and then stimulated with TPA for 1h. (**J**) GO6983's inhibitory effect on TPA-induced YAP activation is reversed by constitutively active RhoA Q63L. myc-RhoA Q63L was transfected into HEK293A cells. After 12h of serum withdrawal, cells were treated TPA and/or GO6983.

(**K**) Mst1/2 are not required for TPA induced YAP dephosphorylation in HEK293A cells. Mst1/2 double knockout HEK293A cells were serum starved for 12h and TPA stimulated for 1h and YAP phosphorylation was measured. (**L**) LATS overexpression suppresses the effects of TPA on YAP activation. Indicated plasmids were co-transfected into HEK293A cells. 24h after transfection, cells were serum starved for 12h and stimulated with 10nM TPA for 1h. Cell lysates were analyzed by Western Blots. (**M**) LATS1/2, but not MST1/2, are required for TPA's effect on YAP phosphorylation in MEF cells. MST1/2 or LATS1/2 double knockout MEF cells were cultured in the presence of serum for 12h followed by 1h of TPA stimulation. Cells were collected for Western Blots. (**N**) TPA inactivates LATS kinase in HEK293A cells. Serum starved cells were stimulated with different concentrations of TPA for various time durations as indicated. Endogenous LATS1 was immunoprecipitated for Immunoblotting with phosphoLATS antibody.