Supplementary Materials: NEK1 Phosphorylation of YAP Promotes its Stabilization and Transcriptional Output

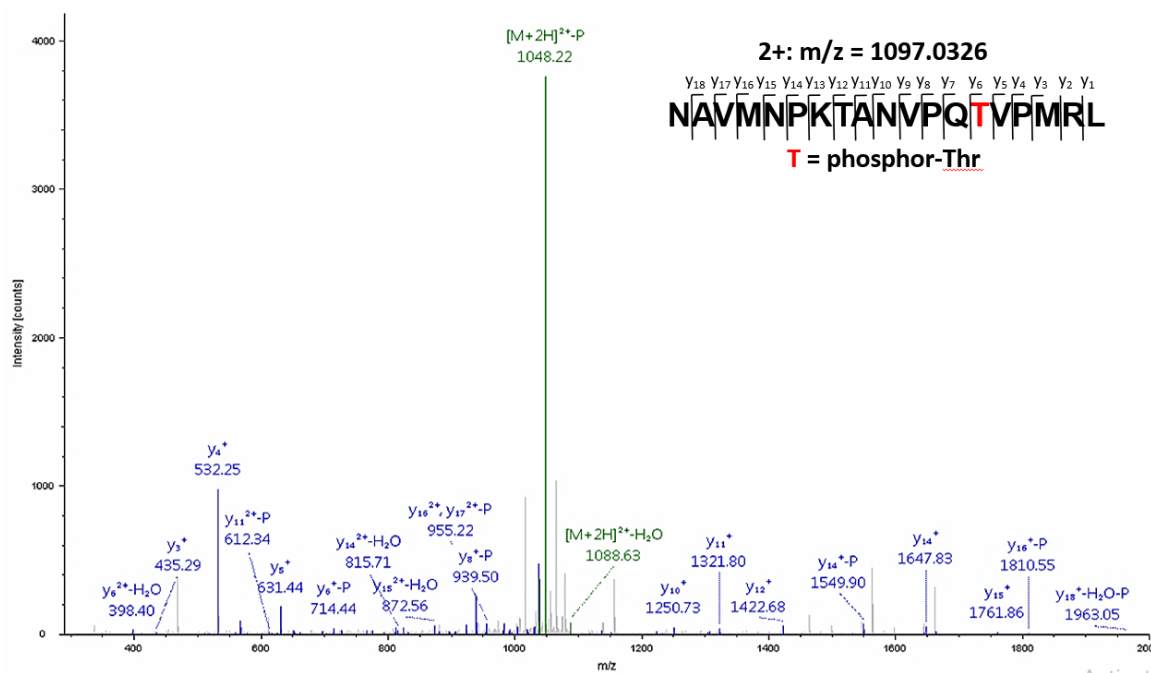**Md Imtiaz Khalil, Ishita Ghosh, Vibha Singh, Jing Chen, Haining Zhu and Arrigo De Benedetti**

Figure S1. MS/MS spectrum of the phosphor-peptide N70AVMNPKTANVPQTVPMRL88 to determine pT83.
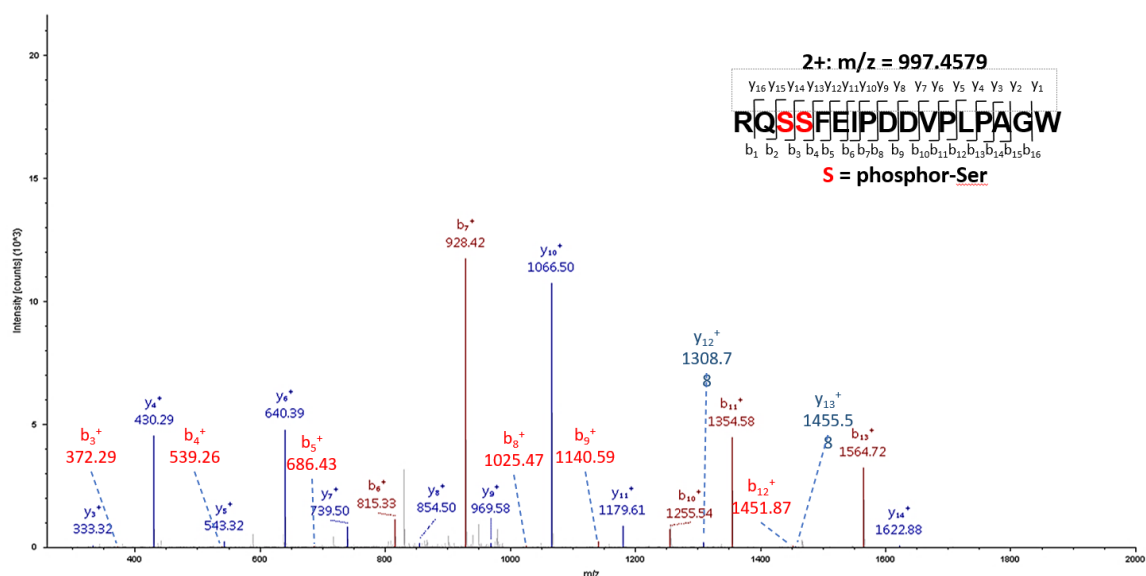

Figure S2. MS/MS spectrum of the phosphor-peptide R161QSSFEIPDDVPLPAGW177 to determine pS163/pS164. It is noted that this phosphor-peptide was detected in all 3 sample. It is noted that the MS/MS spectrum cannot distinguish pS163 or pS164.
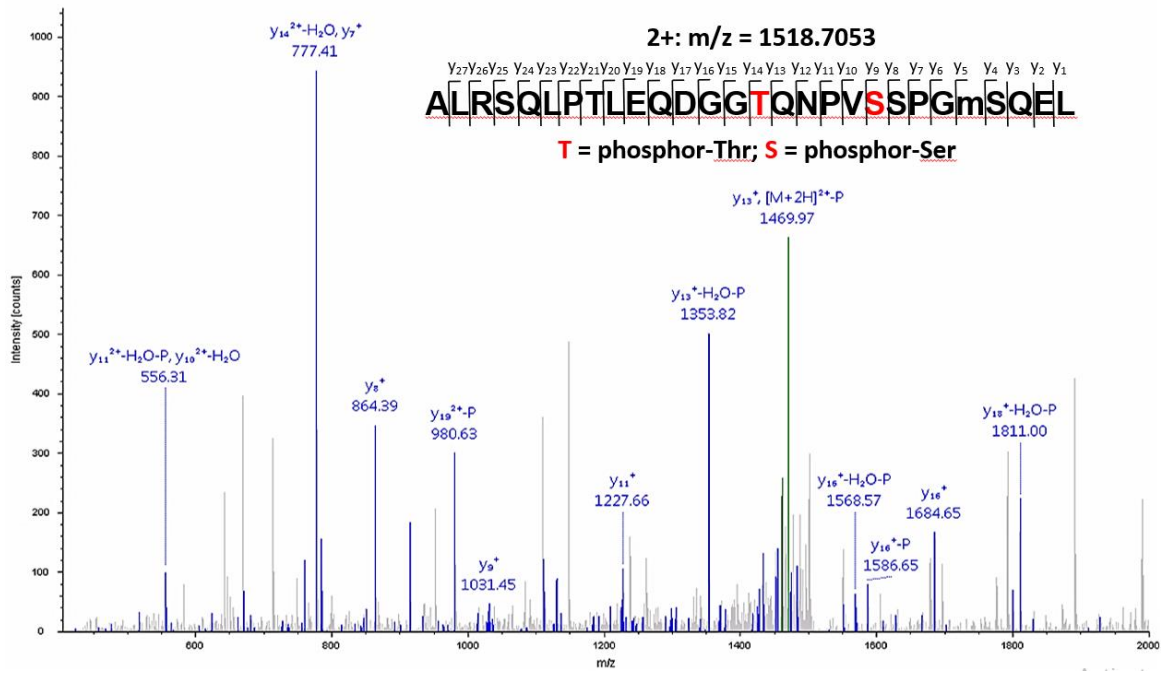
Figure S3. MS/MS spectrum of the phosphor-peptide A$_{347}$LRSQLPTLEQDGGTQNPVSSPGmSQEL$_{374}$ to determine pT361/pS366. It is noted that the MS/MS spectrum cannot distinguish pT361 or pS366.
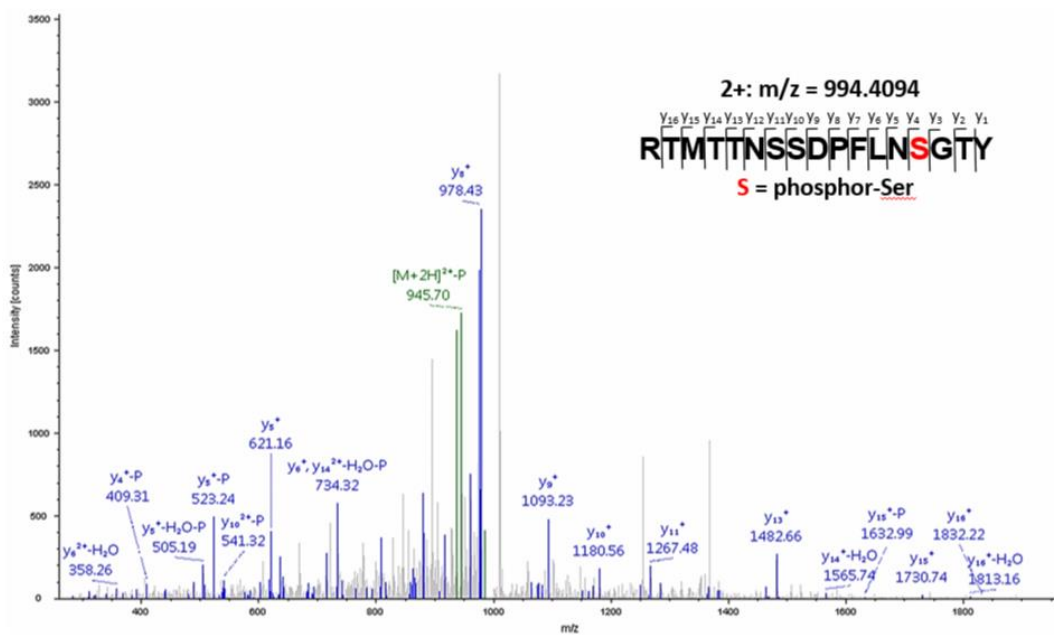

Figure S4. MS/MS spectrum of the phosphor-peptide R$_{375}$TMTTNSSDPFLN**S**GTY$_{391}$ to determine pS388.

Fig1A_LNCaP_YAP-orig-quantitated

	LNCaP				+NEK1-T141A				+NEK1-wt			
	-	+	-	+	-	+	-	+	-	+	-	+
	-	-	+	+	-	-	+	+	-	-	+	+

YAP

63 KDa

Cl-YAP

48KDa

AU-YAP	1	0.63	0.58	0.53	0.43	0.61	0.53	0.35	1.33	0.79	1.27	0.74
AU-Cl-YAP	1	0.49	3.73	7.39	3.31	5.64	5.90	4.47	0	0	0	0

Fig1B_ NEK1/YAP co-IP

	Input		NEK-IP		IgG-IP	
THD	-	+	-	+	-	+

75KDa

YAP

63KDa

Cl-YAP

48KDa

YAP	1	1.06	-	1.67	1.47	0.04	0.01
Cl-YAP	1	0.98	-	3.14	3.95	0.06	0.03

Fig1B_ NEK1/TLK1 co-IP

	Input		NEK-IP		IgG-IP	
THD	-	+	-	+	-	+

100KDa

TLK1

75KDa

63KDa

TLK1	1	0.59	1.82	1.23	0.04	0.05

Fig1C_NT1_YAP

	C	THD	J54

63KDa

YAP

Cl-YAP

48KDa

GAPDH

YAP	1	0.67	0.54
Cl-YAP	1	4.16	4.20
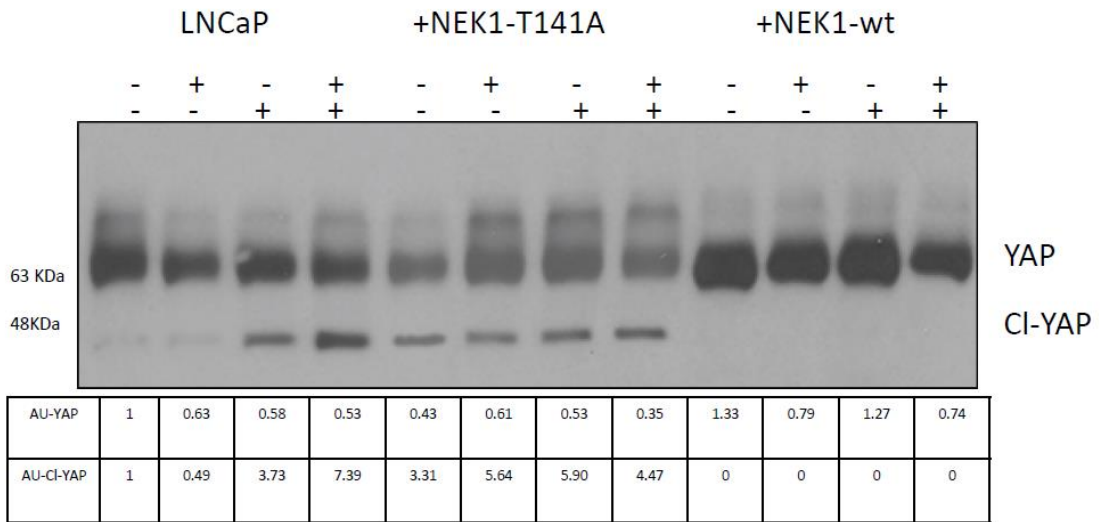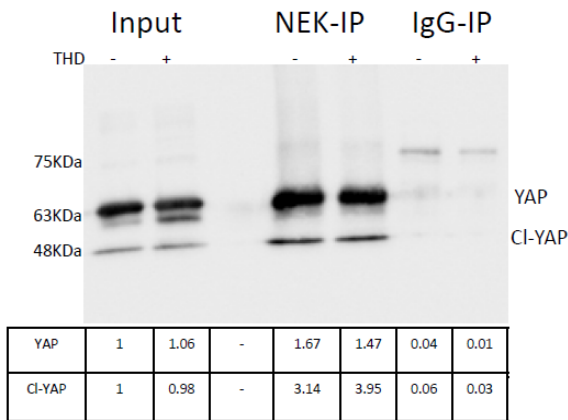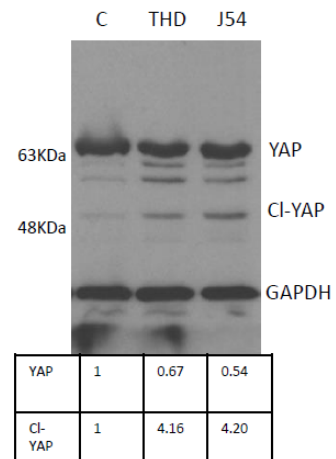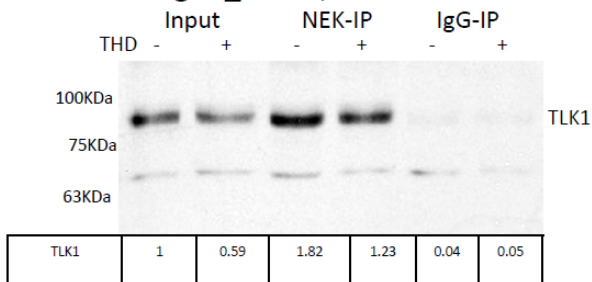
Figure 2A (Left Panel): NT1 NEK1 KO

NEK1 1.0 0.06 0.17 0.20 0.24 0.12 0.12 0.04 0.15 0.04 0.08 0.34 0.06 0.08
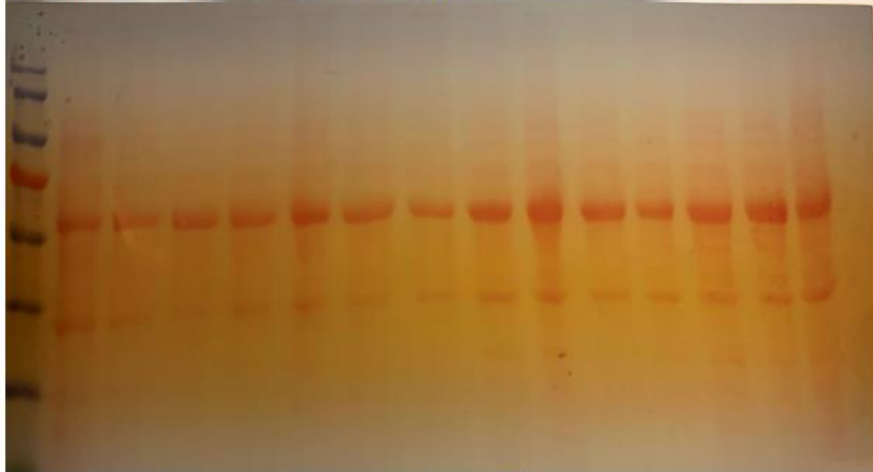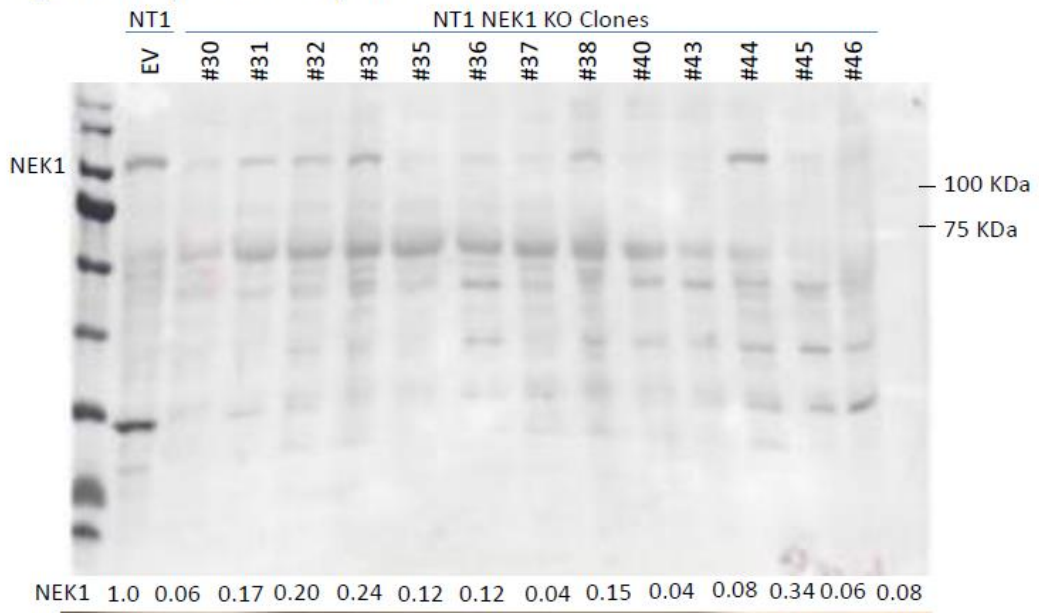
Ponceau S.

Figure 2A (Right Panel): YAP1 level in NT1 NEK1 KO cells

		NT1 NEK1 KO Clones						
WT	EV	#35	#36	#37	#40	#43	#45	#46

YAP

— 75KDa

— 60KDa

YAP	1	0.77	0.21	0.07	0.18	0.22	0.24	0.19	0.35

Ponceau S.
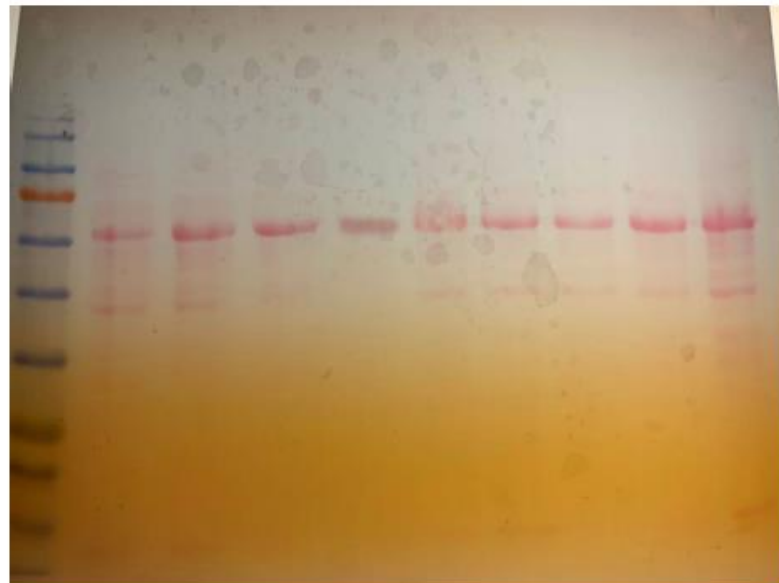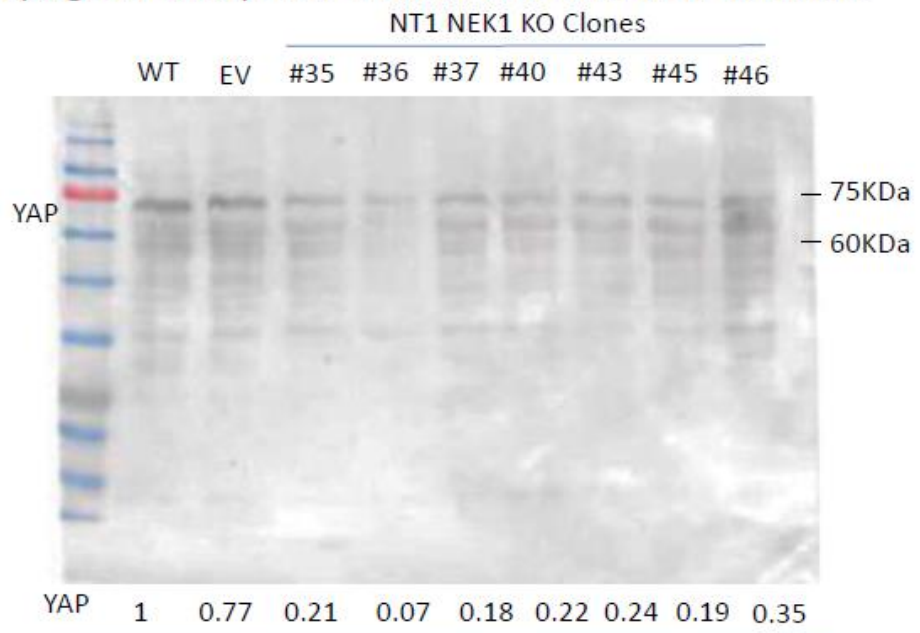
Figure 2C left panel:
YAP level upon THD treatment

THD   -   +

YAP

— 75KDa

YAP   1   0.28

β-tubulin

— 45KDa
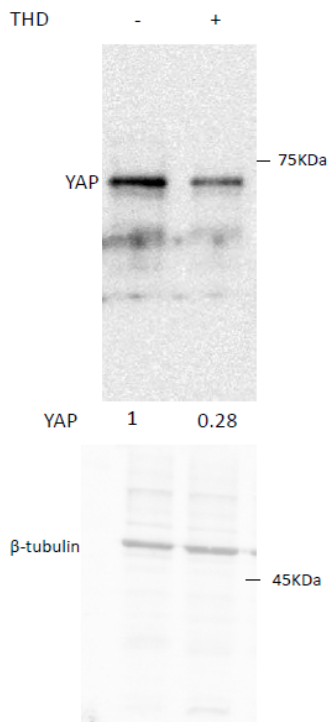
Figure 2C Right panel:
pYAP level upon THD treatment

THD   -   +

pYAP S127

— 75KDa

— 60KDa

pYAP   1   8.2

β-tubulin

— 45KDa
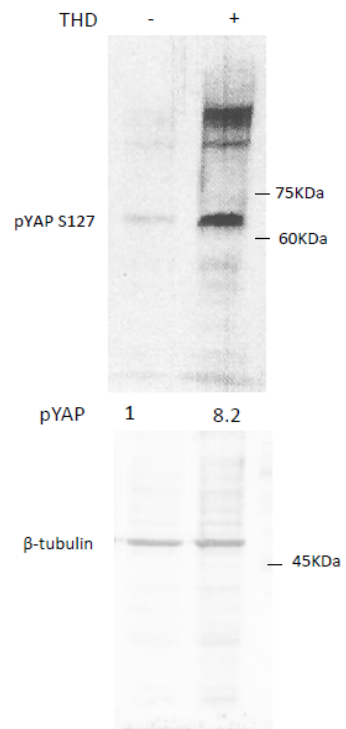
Figure 2D left panel:
TLK1 knockdown with shRNA

No shRNA   shTLK1   No shRNA   shTLK2

TLK1

— 100KDa

— 75KDa

TLK1   1   0.24   0.54   1   0.18   0.82

β-tubulin

— 60KDa

— 50KDa
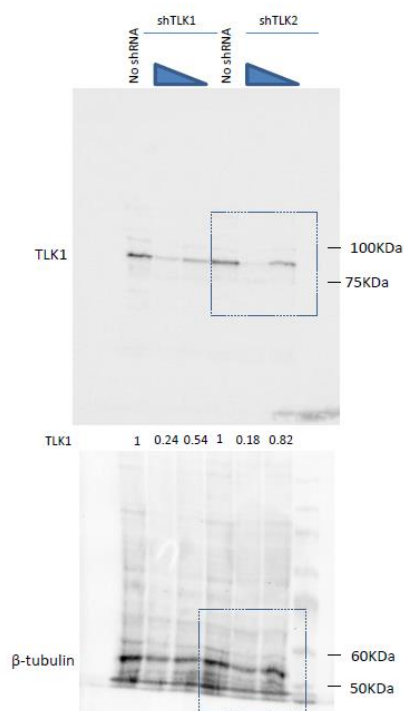
Figure 2D right panel: pNEK1 level in TLK1 KD cells

No shRNA   shTLK1   No shRNA   shTLK2

pNEK1

— 140KDa

— 100KDa

pNEK1   1   0.57   1   0.15   0.03

NEK1

— 100KDa
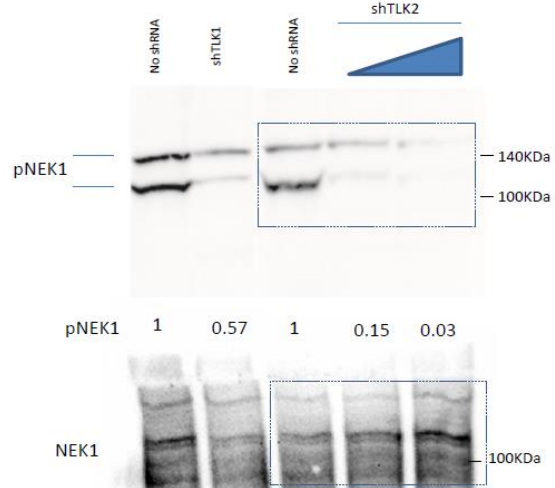
Figure 3C: Radioactive IVK of YAP1

NEK1	+	-	+	-
MK5	-	+	-	+
YAP1	-	-	+	+

100KDa

60KDa

| YAP | 0 | 0 | 1 | 0.09 |

GST-YAP1

His-NEK1

His-MK1

Coomassie Staining
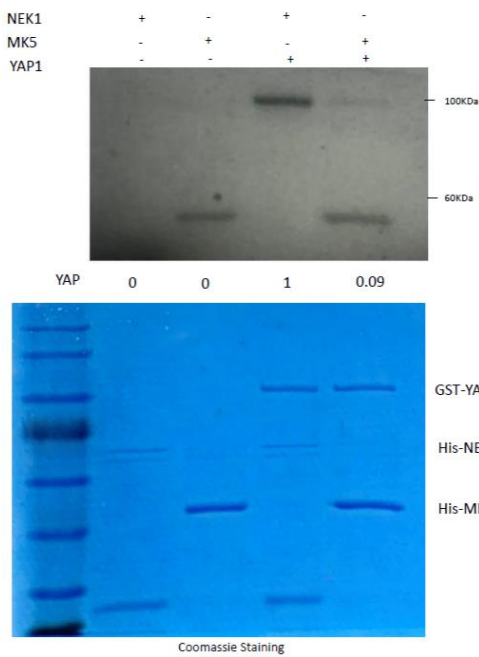
Figure 3D: IVK of YAP1 for MS determination

YAP1 NEK1 MK5 YAP1+NEK1 YAP1+MK5

GST-YAP1

His-NEK1

His-MK5

Excised bands

Coomassie Staining

Figure 3E: YAP1 pTyr determination

| YAP1 | + | + |
| NEK1 | - | + |

pYAP1 pTyr

pNEK1 Tyr
Auto-phosphorylation

WB

| pYAP1 pTyr | 1 | 17.71 |

GST-YAP1

His-NEK1

Ponceau S
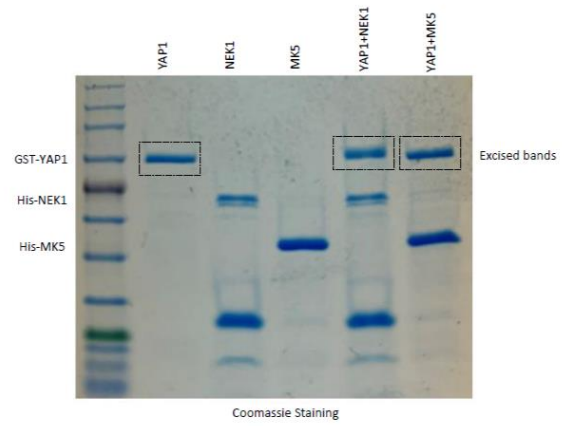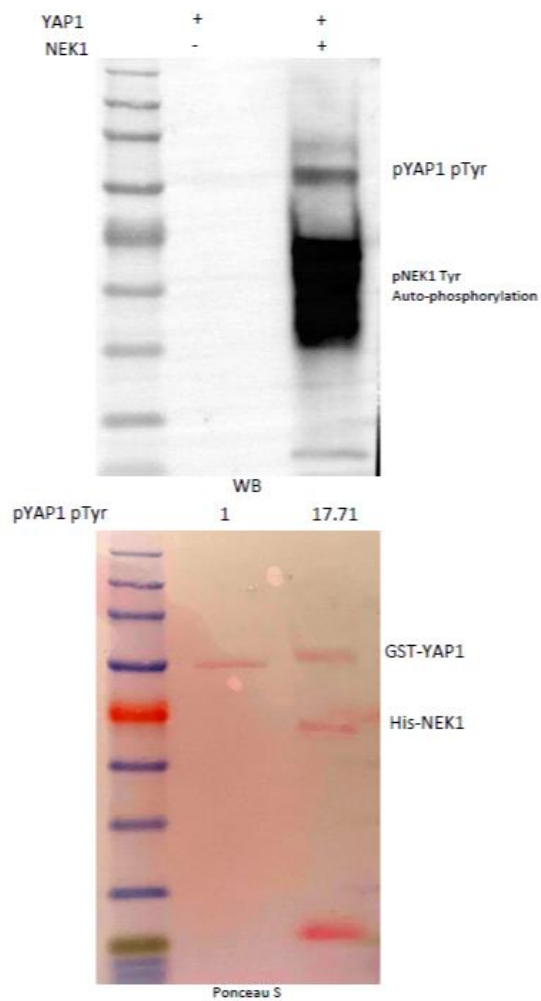
Figure S5. Densitometry of the original uncropped blots and gel images with their respective numbers from the main figures.

Table S1. Assigned phosphorylated sites.

Phosphorylated Sites	Peptides	YAP1	YAP1 + NEK1	YAP1 + MK5
T83	N_{70}AVMNPKTANVPQ**T**VPMRL$_{88}$	N	Y	N
* S163/S164	R_{161}Q**SS**FEIPDDVPLPAGW$_{177}$	Y	Y	Y
* T361/S366	A_{347}LRSQLPTLEQDGG**T**QNPV**S**SPGmSQEL$_{374}$	N	Y	N
S388	R_{375}TMTTNSSDPFLN**S**GTY$_{391}$	N	Y	N
* S406/Y407	H_{392}SRDESTDSGLSMS**SY**$_{407}$	N	Y	N
T493	A_{491}A**T**KLDKESFL$_{501}$	N	Y	N

* Cannot tell which site is preferred based on the MS/MS spectrum.