

**CDK1 promotes epithelial-mesenchymal transition and migration of head and neck squamous carcinoma cells via repressing  $\Delta$ Np63 $\alpha$ -mediated transcriptional regulation**

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## Legends for supplementary data

**Supplementary Table S1.** The list of identified  $\Delta$ Np63 $\alpha$ -interacting kinases.

HEK293T cells were transfected with Flag-tagged  $\Delta$ Np63 $\alpha$ . Cells were lysed using Cell lysis buffer for Western and IP (Beyotime) with Protease and phosphatase inhibitor cocktail for general use (Beyotime) at ice for 10 min. Lysates were cleared by centrifugation at 13,000 rpm for 15 min. Protein concentration was determined using the Bradford protein assay reagent (Coolaber). Then, 2 mg of total proteins from cell lysates were incubated with anti-Flag or normal mouse IgG as control at 4 °C for 8 h, and the immune complexes were precipitated with protein A+G-agarose at 4 °C for 2 h. The immunoprecipitates were washed with lysis buffer, separated by SDS-PAGE, and subjected to mass spectrometry (MS) as described previously<sup>1</sup>. The identified kinases were listed. MW, molecular weight. Calc. pI, calculated isoelectric point.

**Supplementary Figure S1.** The depiction of identified phosphorylated residues in  $\Delta$ Np63 $\alpha$  protein. The phosphorylated residues were depicted in  $\Delta$ Np63 $\alpha$ , according to the abovementioned mass spectrometry. TAD, transactivation domain; DBD, DNA binding domain; OD, oligomerization domain; SAM, sterile alpha motif; TID, trans-inhibition domain.

**Supplementary Figure S2.** The effects of mutations at the phosphor-sites on transactivity of  $\Delta$ Np63 $\alpha$ . The phospho-sites in DNA-binding domain (DBD) and trans-inhibition domain (TID) of  $\Delta$ Np63 $\alpha$  were substituted with alanine (A) to prevent the potential phosphorylation. The mutant and wild-type  $\Delta$ Np63 $\alpha$  constructs were subjected to luciferase reporter assay to assess the effects of these residues on  $\Delta$ Np63 $\alpha$ -mediated transcription. HEK293T cells were transfected with a mixture of

K14-Luc and TK-Renilla, plus  $\Delta$ Np63 $\alpha$  or its mutant plasmid as indicated. Firefly and Renilla luciferase activities were measured. The K14-Luc expression levels were normalized to Renilla activity and presented as means  $\pm$  SD (n = 3). Two-tailed *t*-test was used for comparison between two groups; \*, p < 0.05; \*\*, p < 0.01.

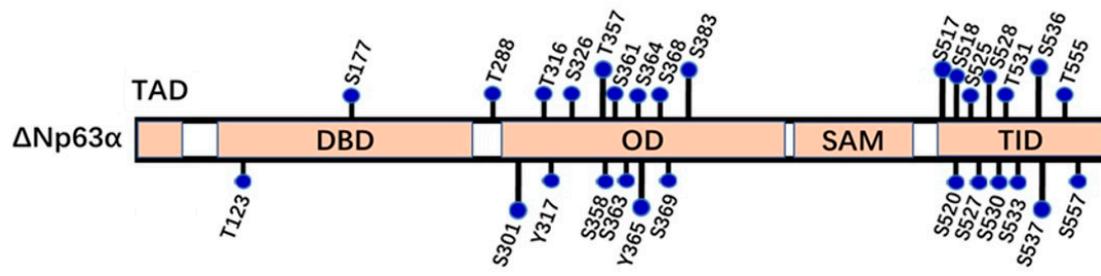
**Supplementary Figure S3.** The fragment of  $\Delta$ Np63 $\alpha$  containing T123 is a conservative motif fitting the consensus sequence for CDK1 modification. The fragment containing T123 fits the consensus sequence for CDK1 according to the prediction using Eukaryotic Linear Motifs (ELM, website: <http://elm.eu.org>).

**Supplementary Figure S4.**  $\Delta$ Np63 $\alpha$  directly binds to promoters of BPAG1 and N-cadherin. **(A)**, The binding site of  $\Delta$ Np63 $\alpha$  (S1) is contained in the BPAG1 promoter as reported previously<sup>2</sup>. TSS, transcription start site. **(B)**, There are 3 putative binding sites of  $\Delta$ Np63 $\alpha$  (S1, S2 and S3) in the N-Cadherin promoter as predicted with JASPAR online software (<https://jaspar.genereg.net/analysis>). TSS, transcription start site. **(C)**, Chromatin immunoprecipitation (ChIP) assays were performed in UM1 cells stably overexpressing Flag- $\Delta$ Np63 $\alpha$  or Flag- $\Delta$ Np63 $\alpha$  plus CDK1, with anti-Flag. DNA samples precipitated with either anti-Flag or mock IgG, as well as equivalent input, were subjected to polymerase chain amplification (PCR) to detect fragments of BPAG1 and N-cadherin promoters.

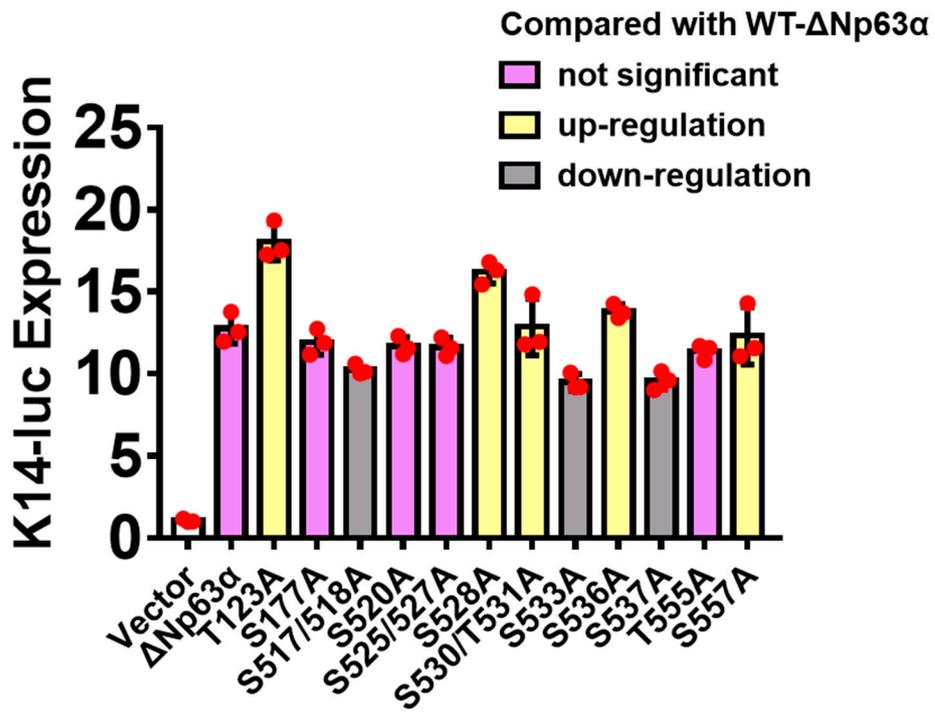
**Supplementary Table S1.**

| <b>Name</b>    | <b>Description</b>                               | <b>MW<br/>(KDa)</b> | <b>Calc. pI</b> | <b>Score</b> |
|----------------|--|---------------------|-----------------|--------------|
| AURKB          | Aurora kinase B                                  | 39.3                | 9.29            | 2.45         |
| CDK1           | Cyclin-dependent kinase 1                        | 34.1                | 8.4             | 35.6         |
| CDK4           | Cyclin-dependent kinase 4                        | 33.7                | 7.01            | 14.04        |
| CDK16          | Cyclin-dependent kinase 16                       | 55.7                | 7.62            | 13.26        |
| CHK2           | Checkpoint kinase 2                              | 60.9                | 5.91            | 162.33       |
| CK1 $\alpha$ 1 | Casein kinase I isoform alpha                    | 38.9                | 9.57            | 24.24        |
| CK1 $\delta$   | Casein kinase I isoform delta                    | 47.3                | 9.74            | 40.09        |
| CK1 $\epsilon$ | Casein kinase I isoform epsilon                  | 47.3                | 9.66            | 42.7         |
| CK2 $\alpha$ 1 | Casein kinase II subunit alpha                   | 45.1                | 7.74            | 21.25        |
| CK2 $\alpha$ 2 | Casein kinase II subunit alpha'                  | 41.2                | 8.56            | 16.59        |
| CK2 $\beta$    | Casein kinase II subunit beta                    | 24.9                | 5.55            | 22.49        |
| JAK1           | Janus tyrosine kinase 1                          | 133.2               | 7.55            | 17.3         |
| MAP3K7         | Mitogen-activated protein kinase kinase kinase 7 | 67.2                | 7.11            | 12.37        |
| MAPK1          | Mitogen-activated protein kinase 1               | 41.4                | 6.98            | 70.53        |
| RIOK1          | RIO serine/threonine kinase 1                    | 65.5                | 6.19            | 57.61        |
| SRPK2          | SRSF protein kinase 2                            | 77.5                | 4.97            | 5.52         |
| STK38          | Serine/threonine-protein kinase 38               | 54.2                | 7.15            | 205.52       |

Supplementary Figure S1



Supplementary Figure S2

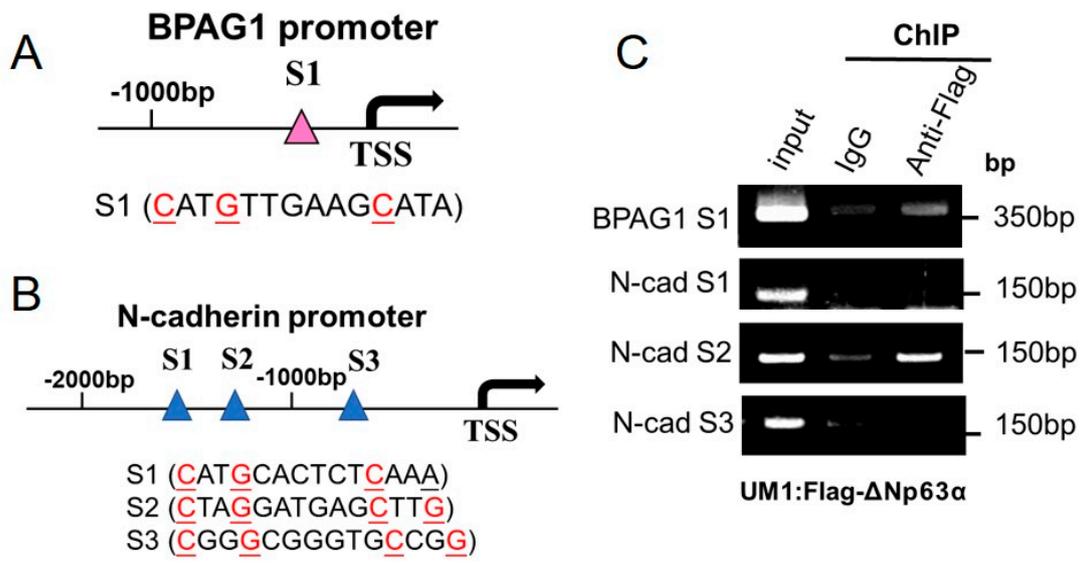


Supplementary Figure S3

**Consensus sequence for CDK1 modification: SP or TP**

|                       |                                 |
|-----------------------|---------------------------------|
| <i>Homo</i> ΔNp63α    | 118 QIKVM <b>TP</b> PPQGAVI 131 |
| <i>Mus</i> ΔNp63α     | 118 QIKVM <b>TP</b> PPQGAVI 131 |
| <i>Rat</i> ΔNp63α     | 118 QIKVM <b>TP</b> PPQGAVI 131 |
| <i>Xenopus</i> ΔNp63α | 118 QIKVM <b>TP</b> PPQGAVI 131 |

Supplementary Figure S4



**References:**

1. Peng, B. L.; Li, W. J.; Ding, J. C.; He, Y. H.; Ran, T.; Xie, B. L.; Wang, Z. R.; Shen, H. F.; Xiao, R. Q.; Gao, W. W.; Ye, T. Y.; Gao, X.; Liu, W., A hypermethylation strategy utilized by enhancer-bound CARM1 to promote estrogen receptor alpha-dependent transcriptional activation and breast carcinogenesis. *Theranostics* **2020**, *10* (8), 3451-3473.
2. Osada, M.; Nagakawa, Y.; Park, H. L.; Yamashita, K.; Wu, G.; Kim, M. S.; Fomenkov, A.; Trink, B.; Sidransky, D., p63-specific activation of the BPAG-1e promoter. *J Invest Dermatol* **2005**, *125* (1), 52-60.