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Supplemental Material to:

Guillermo Risso, Federico Pelisch, Berta Pozzi, Pablo Mammi, Matías Blaustein, Alejandro Colman-Lerner, and Anabella Srebrow

Modification of Akt by SUMO conjugation regulates alternative splicing and cell cycle

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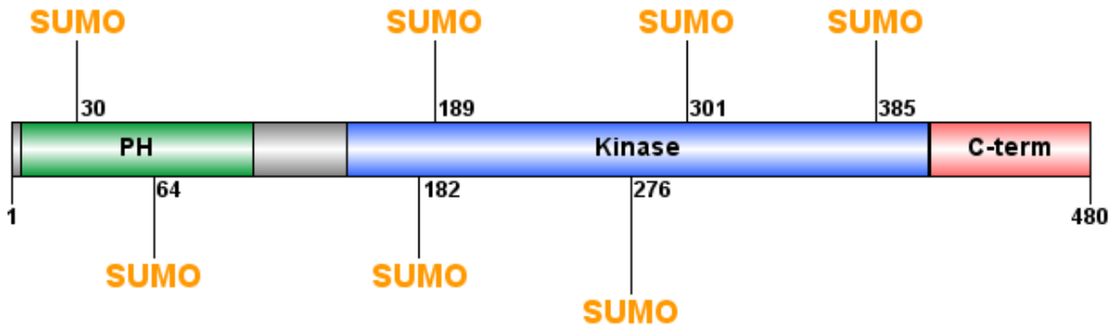
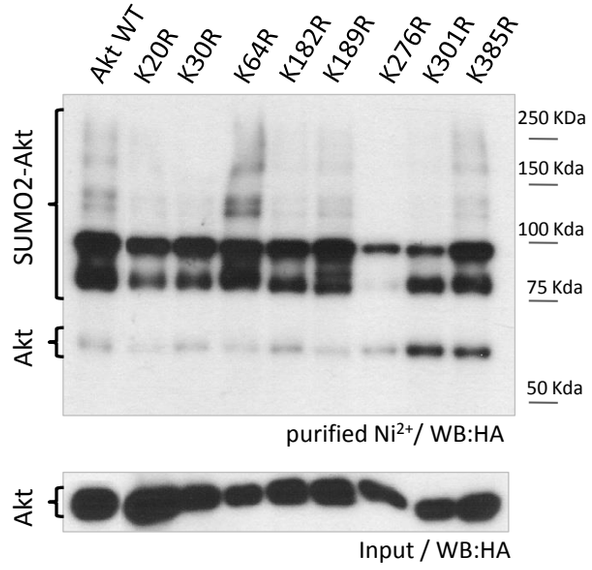
A

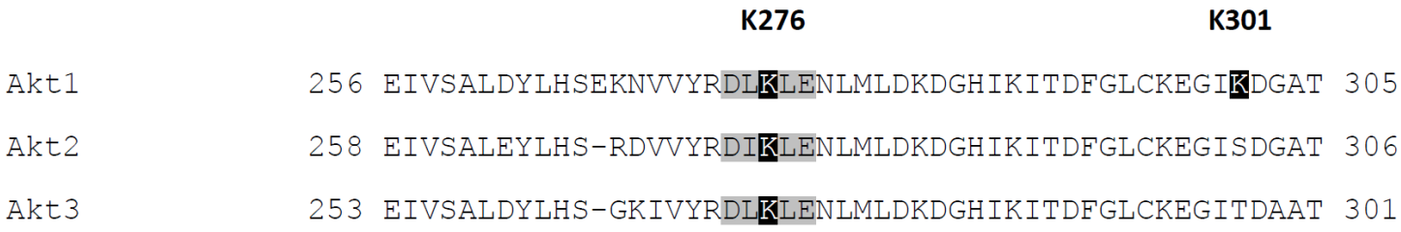


B



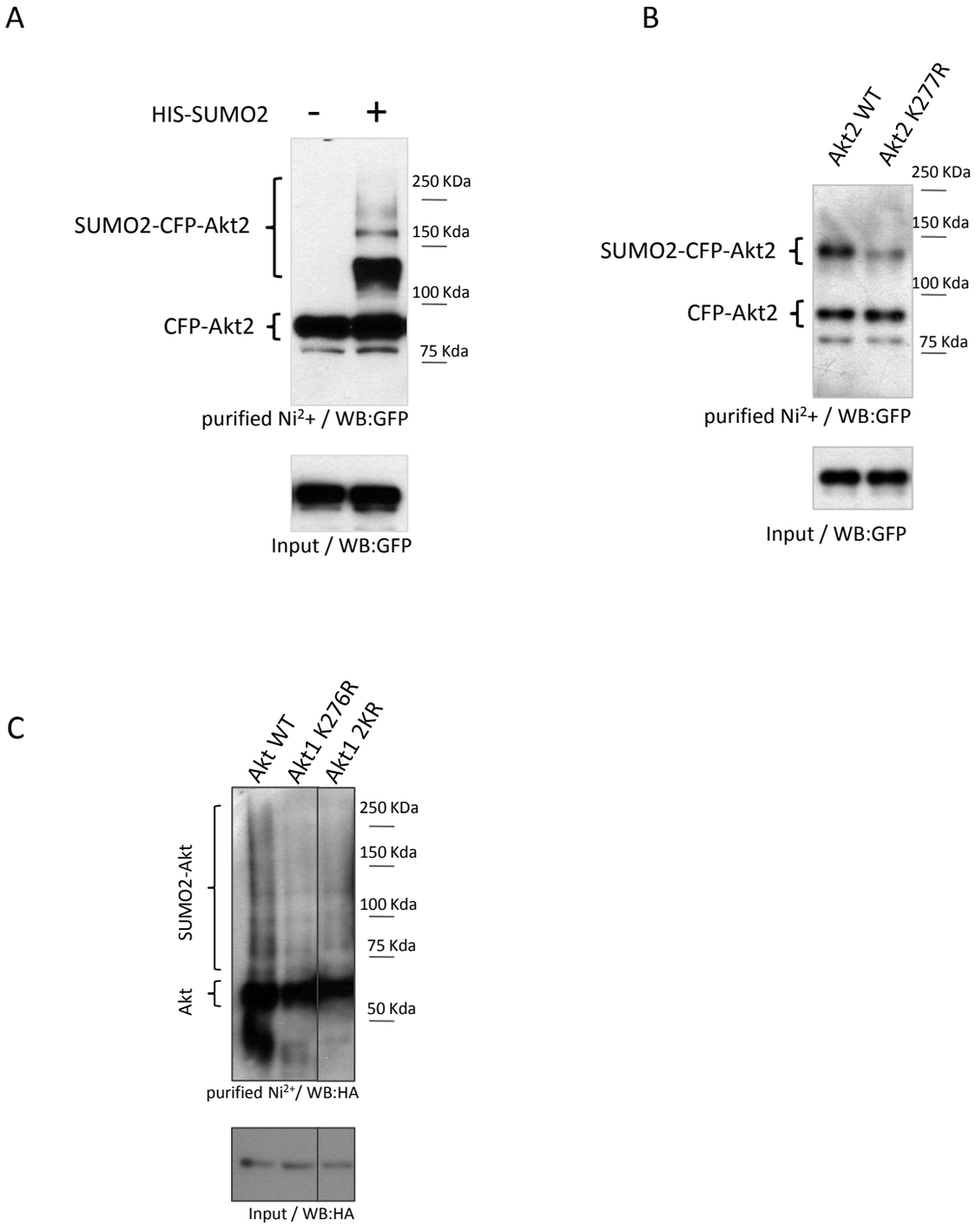
(A) Putative SUMO conjugation sites within Akt1, as predicted by *in silico* analysis. The scheme also shows the three Akt functional domains conserved in all members of the AGC-kinase family: an N-terminal pleckstrin homology (PH) domain, a central kinase domain, and a C-terminal regulatory domain. (B) HEK 293T cells were transfected with expression vectors for either wild type or different single mutants HA-Akt1 as indicated on the top of the panel. SUMO conjugation to HA-Akt was analyzed as indicated in figure 1.

A

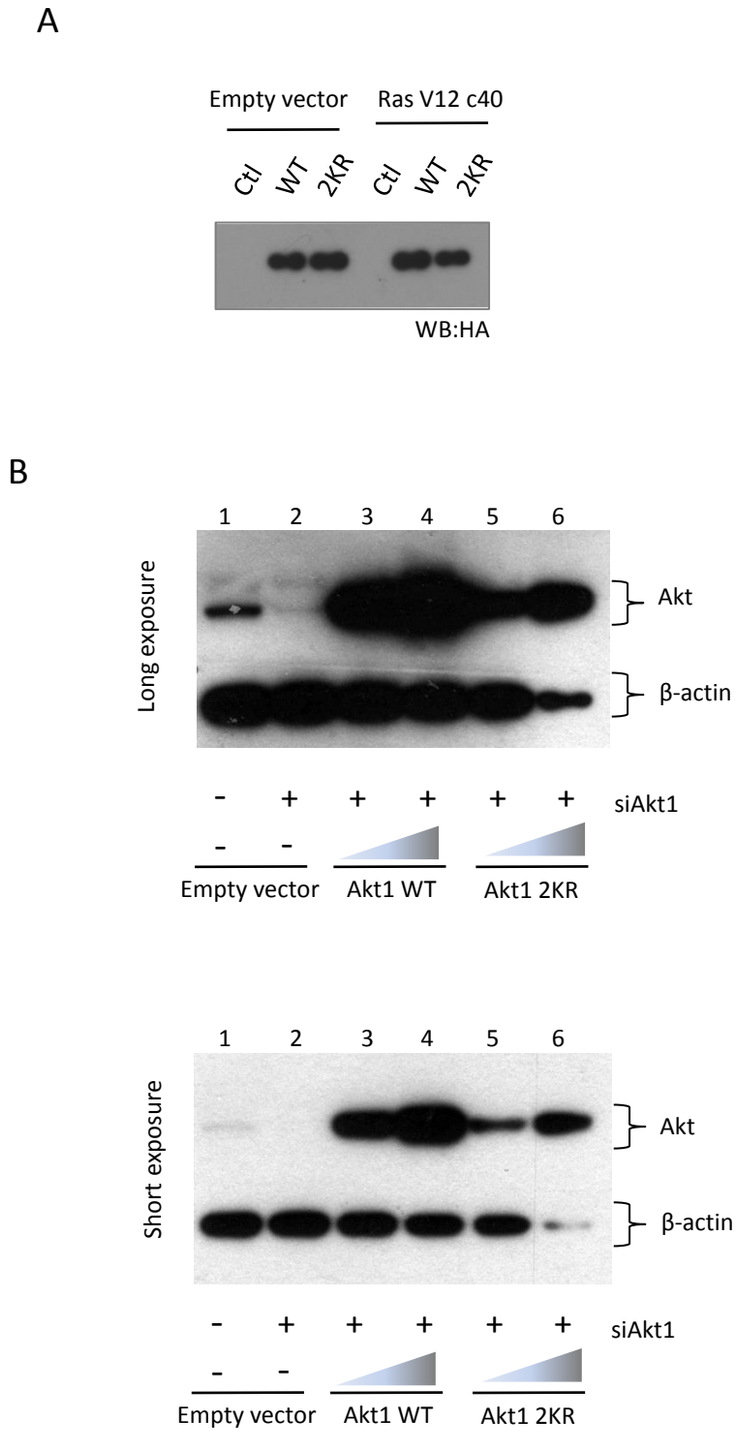


B

(A) Alignment of the primary sequence corresponding to part of the kinase domain of the three mammalian Akt isoforms (Akt1, Akt2 and Akt3) showing the conserved consensus SUMOylation motif (grey boxes) surrounding lysine (K) 276 of Akt1, K277 of Akt2 and K272 of Akt3, as well as K301 in Akt1 (black boxes). (B) Alignment of the primary sequence corresponding to part of the kinase domain of Akt1 isoform from different species.



(A) HEK 293T cells were transfected with CFP-Akt2 and Ubc9 expression vectors, with or without 6xHis-SUMO2. After 48 h, cells were lysed, an aliquot of the lysate was taken as input and the reminder was subject to denaturing Ni^{2+} affinity chromatography. Both fractions were analyzed by western blot with an anti-GFP antibody. (B) HEK 293T cells were transfected either with wild type (WT) or K277R CFP-tagged Akt2 expression vectors, together with 6xHis-tagged SUMO2 and Ubc9 expression vectors. Cells lysates were subject to denaturing Ni^{2+} affinity chromatography and analyzed by western blot with an anti-CFP antibody. (C) HeLa cells were transfected either with wild type (WT), K276R, or the double mutant K276R/K301R ("2KR") HA-tagged Akt1 expression vectors, together with 6xHis-tagged SUMO2 and Ubc9 expression vectors. Cells lysates were subject to denaturing Ni^{2+} affinity chromatography and analyzed by western blot with anti-HA antibody. Different lanes from the same gel were put together as indicated by the dividing line.



(A) Expression levels of the different HA-Akt variants corresponding to figure 4B, analyzed by western blot with anti-HA antibody. (B) Expression levels of endogenous Akt1 (lanes 1 and 2) and over-expressed HA-Akt1 variants (lanes 3 to 6) corresponding to figure 4C, analyzed by western blot with anti-Akt antibody. Upper and lower panels correspond to different exposure times of the same blot to properly visualize siRNA-knock down efficiency (lanes 1 and 2). The same membrane was simultaneously blotted with anti-βactin antibody as a loading control.