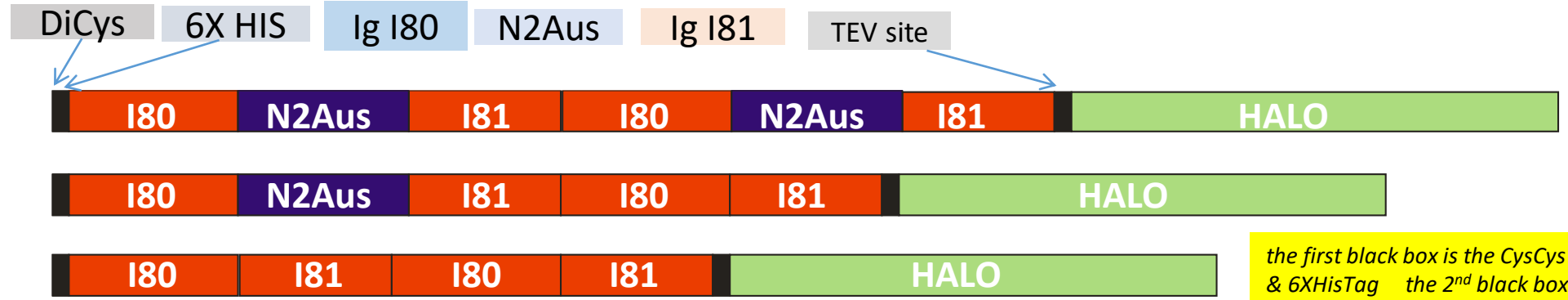


# A) 6XHis-2hN2Aus-Halo protein (construct name in pJexpress404 vector: 211904)

MACCHHHHHTKKAAVDGRLLFFVSEPQSIRVVEKTTATFIAKVGGDPIPNVKWTGKWRQLNQGGRVFIHQKGDEAKLEIRDTTKTDSGLYRC  
 VAFNEHGEIESNVNLQVDERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLKNVDPKEYEKYARMYGITDFRGLLQAFELLKQSQEEETHRL  
 EIEEIERSERDEKE **FEELVSFIQQRLSQTEPVTLIKDIENQT** VLKDNDAVFEIDIKINYPEIKLSWYKGTEKLEPSDKFEISIDGDRHTLRVKNCQLKDQ  
 GNYRLVCGPHIASAKLTVIETKKAAVDGRLLFFVSEPQSIRVVEKTTATFIAKVGGDPIPNVKWTGKWRQLNQGGRVFIHQKGDEAKLEIRDTTKT  
 DSGLYRCVAFNEHGEIESNVNLQVDERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLKNVDPKEYEKYARMYGITDFRGLLQAFELLKQS  
 QEEETHRLEIEEIERSERDEKEFEELVSFIQQRLSQTEPVTLIKDIENQTVLKDNDAVFEIDIKINYPEIKLSWYKGTEKLEPSDKFEISIDGDRHTLRVK  
 NCQLKDQGNRYRLVCGPHIASAKLTVIEEDLYFQSDNDGSEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPLFLHGNPTSSYVWRNIIPHVAPT  
 HRCIAPDLIGMGKSDKPDLYFFDDHVRFMDFIEALGLEEVVLVIHDWGSALGFHWAKRNPERVKGI AFMEFIRPIPTWDEWPEFARETFQAF  
 RTTDVGRKLIIDQNVFIEGTLPMGVVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVALVEEYMDWLHQSPVPKLLFWGTPGVLI  
 PPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPDIGSEIARWLSTLEISG

105.33kDa pI=5.09

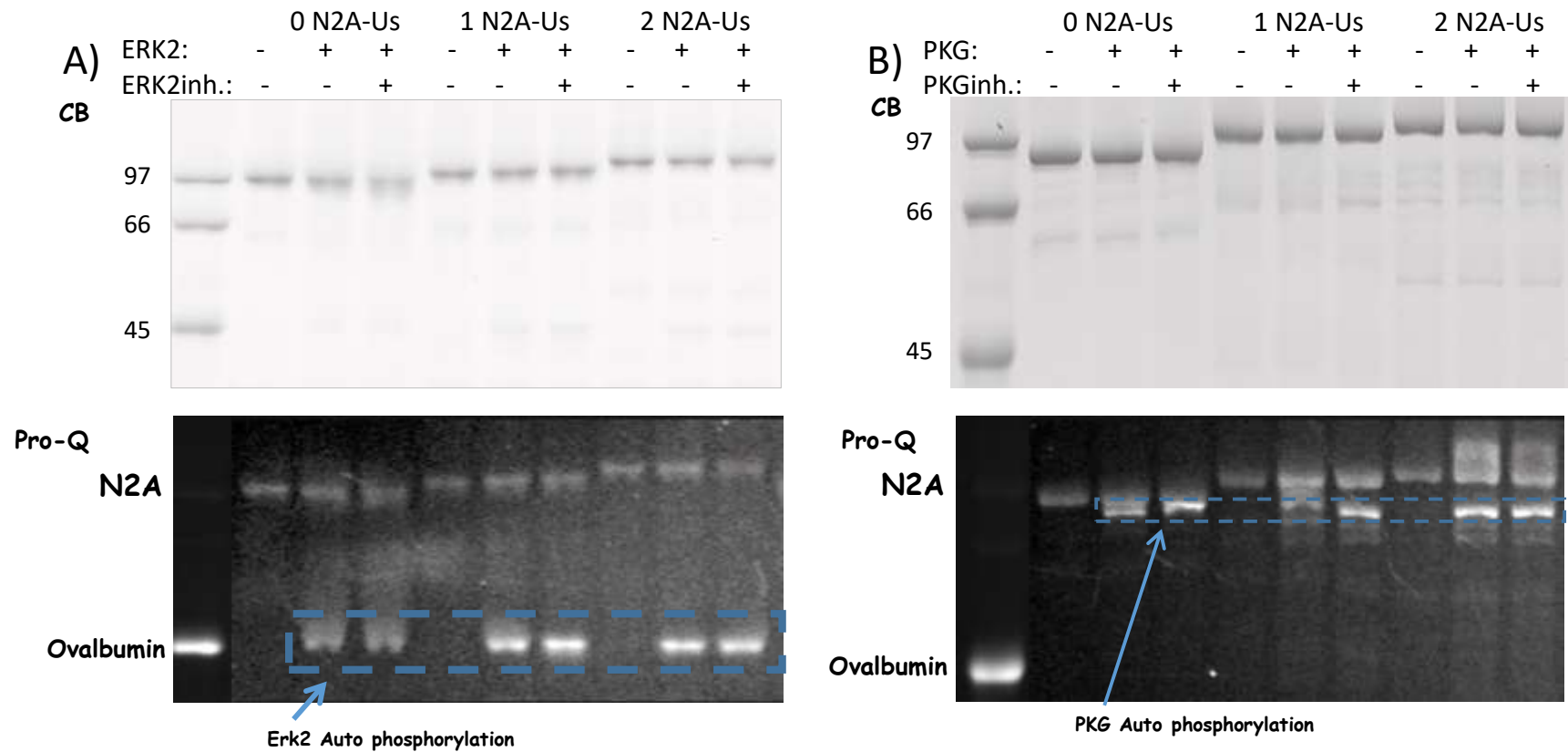


*the first black box is the CysCys & 6XHisTag the 2<sup>nd</sup> black box is the TEV protease site*

**Supplemental Figure S1. A)** Top: sequence of 2-N2A-U protein construct. Bottom: domain structure of 2-N2A-U, 1-N2A-U and 0-N2A-U constructs used in this study. The identified PKA site Ser-9895 (NP\_001254479.2) is shown in red. The molecular weight of these constructs is 105,3, 92.9 and 80.5 kDa. B) (Next page) Nucleotide sequence that was synthesized.

Figure S1 B)

AAGGAGGTAAAAATGGCGTGCTGTACCACCATCATCATCACACTAAAAAGCTGCAGTTGACGGTCGTTTGTTCGTTAGCGAACCGCAGAGCATCCGCGTCGTTGAGAAAACCTACCGCAACGTTTCATTGC  
TAAGGTTGGTGGTGACCCGATTCCGAATGTTAAGTGGACGAAGGGCAAATGGCGCCAGCTGAACCAGGGCGGTGCTGTGTTTATTACCCAGAAAGGCGATGAGGGCAAGCTGGAAATTCGTGACACCACGAA  
AACGGATTCCGTTTTGTACCGCTGCGTTGCGTTCAATGAGCACGGCGAGATCGAGAGCAACGTCAATTTGCAAGTGGATGAGCGTAAAAACAAGAGAAAATTGAGGGCGATCTCCGTGCCATGCTGAAAAAG  
ACCCCGATCCTGAAAAAAGGTGCCGGTGAAGAAGAAGAGATCGATATCATGGAACCTGTTGAAGAATGTCGATCCGAAAAGAGTACGAAAAGTATGCTCGTATGTACGGTATTACGGACTTTCGCGGCCTGCTGCAA  
GCATTTGAATTGTTGAAACAAAGCCAGGAAGAAGAAACCCATCGTCTGGAGATCGAGGAAATTGAACGCAGCGAGCGGATGAAAAGGAGTTCGAAGAGTTAGTGAGCTTCATTACGCAGCGCTTGAGCCAG  
ACCGAGCCTGTTACCCTGATCAAAGATATTGAGAATCAAACCGTGCTGAAAGATAACGATGCCGTTTTTCGAGATCGACATCAAGATCAACTACCCGAAATCAAGCTGTCTTGGTATAAGGGTACGGAAAACTGG  
AGCCGTCGGATAAGTTCGAGATCAGCATTGATGGTGTATCGCCACACCCTGCGTGTTAAGAATTGCCAGCTGAAAGACCAGGGCAAACCTACCCGCTGGTCTGTGGTCCGCACATTGCGAGCGCGAAACTGACCGTG  
ATCGAAACGAAAAAGGCAGCGGTGGACGGTCGTGTTTTTCGTGTCCGAGCCGCAAAGCATCCGTGTGGTTCGAAAAGACCACCGCCACCTTTATCGCAAAGGTCGGTGGCGATCCGATCCCGAACGTCGAAGT  
GGACCAAGGGCAAGTGGCGTCAGCTGAATCAAGGTGGCCGCGTTTTTATCCATCAGAAAGGTGACGAGGCAAACCTGGAGATCCGTGACACGACTAAGACTGATAGCGGCCTGTATCGTTGCGTTGCATTTAAC  
GAGCACGGTGAGATTGAAAGCAATGTTAATCTGCAAGTTGACGAACGTAAAAAGCAAGAAAAAATCGAGGGCGACCTTCGTGCGATGCTGAAAAGACGCCGATCTTGAAGAAAGGCGCGGGCGAGGAAGA  
GGAAATCGACATCATGGAGCTGCTGAAAAACGTGGATCCGAAGGAGTATGAGAAGTACGCCCGCATGTATGGTATCACGGATTTCCGTGGTTTTGCTGCAGGCCTTCGAACTTTGAAACAGAGCCAGGAAGAAG  
AAACGCATCGCCTGGAAATCGAAGAGATCGAGCGTAGCGAACGCGATGAGAAGGAATTTCGAGGAGTTGGTTAGCTTTATCCAACAACGTCTGAGCCAAACCGAACCGGTCACGCTGATTAAGGACATCGAAAA  
CCAAACGGTCTTGAAAGACAACGATGCGGTGTTTGAATTGACATTAAGATTAACCTACCCAGAGATTAACCTGTCGTGGTACAAGGGCACCGAAAAGCTGGAACCGTCTGACAAATTTGAAATCTCCATCGACGG  
TGACCGCCACACGCTGCGTGTGAAAAACTGTCAACTGAAAGATCAAGGTAACCTATCGCCTGGTGTGCGGCCACATATTGCCAGCGCCAAGCTGACCGTCATTGAAGAGGATTTGTACTIONCCAGAGCGACAATGA  
TGGTAGCGAGATTGGCACCGGCTTCCCGTTTGATCCGCACTATGTTGAGGTCCTGGGTGAACGCATGCACTACGTTGATGTTGGTCCGCGTGACGGTACGCCGTTTTTGTTCCTGCACGGCAATCCGACCTCTAGC  
TACGTTTGGCGTAATATCATCCCGCACGTGGCGCCGACCCATCGTTGTATCGCGCCTGACCTGATTGGCATGGGTAAAAGCGACAAACCGGATCTGGGTTACTTCTTTGACGACCATGTGCGTTTCATGGATGCATT  
TATCGAAGCCTTGGGCTTGGAAGAGGTCGTGCTGGTTATTCATGACTGGGGTAGCGCTCTGGGCTTCACTGGGCGAAACGCAACCCGGAACCGGTGAAAGGTATCGCGTTCATGGAGTTCATTGCTCCGATCCC  
AACCTGGGACGAGTGGCCGAATTCGCGCGTGAACCTTCCAAGCGTTTCGTACCACCGATGTCGGTCGCAAGCTGATTATTGACCAAATGTGTTTCATTGAGGGTACCCTGCCGATGGGTGTGGTTCGTCCGCT  
GACCGAAGTCGAGATGGACCATTACCGTGAGCCGTTCTGAACCCAGTGATAGAGAACCTCTGTGGCGTTTTCCGAACGAACTGCCGATTGCGGGCGAGCCGGCAAACATCGTAGCGCTGGTTGAAAGAGTATA  
TGGATTGGCTGCATCAGAGCCCGTTCCGAAGCTGTTGTTCTGGGGTACTCCGGGTGTGCTGATTCCGCCGGCTGAGGCTGCCCGTCTGGCAAAAAGCCTGCCGAATTGCAAAGCCGTTGACATTGGCCCTGGT  
CTGAATCTGCTGCAAGAGGACAATCCGGACCTGATCGGCAGCGAGATCGCACGTTGGCTCAGCACGCTGGAATTAGCGGCTAA



**Supplemental Figure 2. ERK2 and PKG do not phosphorylate the N2A construct.** The top shows the coomassie blue (CB) stained gels and the bottom, Pro-Q Diamond phosphoprotein stained gel. The left lane was loaded with molecular weight standards, the bottom one of which is ovalbumin, which is a phosphoprotein (as obtained from the supplier), showing that the Pro-Q Diamond stain works well. Additionally, Pro-Q Diamond staining reveals that ERK2 and PKG are phosphorylated. The N2A constructs have a weak background Pro-Q Diamond stain that does not increase and scale with the number of N2A-Ues elements.

Human DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIERSEKDEKEFEELVSFTIQQRLSQTEP

Macaca DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIERSEKDEKEFEELVSFTIQQRLSQTEP

Chimp DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIERSEKDEKEFEELVSFTIQQRLSQTEP

Mouse DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSERDEKEFEELVAFITQQRITQTEP

Rat DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSERDEKEFEELVSFTIQQRLITQTEP

Rabbit DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLITQTEP

Cow DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLSQTEP

Cat DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLSQTEP

Bat DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLSQTEP

Shrew DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLSQTEP

Elephant DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLSQTEP

Horse DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFELLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLSQTEP

Pig DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFEMLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLSQTEP

Dog DERKKQEKIEGDLRAMLKKTPIILKKGAGEEEEIDIMELLLKNVDPKEYEYKYARMYGITDFRGLLQAFEMLKQSQEEETHRLEIEEIEKSEKDEKEFEELVSFTIQQRLSQTEP

\*\*\*\*\*.\* \*\* . \*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*\*\*\*\*\*.\*

**Supplemental Figure S3. Sequence alignment of N2A-Uts in a range of mammalian species.** The identified PKA phosphosite Ser-9895 (NP\_001254479.2) is shown in red. This serine is a threonine in mouse, rat and rabbit but is conserved in the other species. (\*) fully conserved residue. (: ) conservation between groups of strongly similar properties. (.) conservation between groups of weakly similar properties. Identity: of full shown sequence is 90%; Conserved: 95%. Source of sequences: Human: Q8WZ42.4; Macaca: XP\_028686722.1; Chimp: NP\_001316540.2; Mouse: A2ASS6.1; Rat: XP\_017456475.1; Rabbit: XP\_017198704.1; Cow: XP\_024835650.1; Cat: XP\_023115277.1; Bat: XP\_023599196.1; Shrew: XP\_027628916.1; Elephant: XP\_023398816.1; Horse: XP\_023477870.1; Pig: XP\_020931560.1; Dog: XP\_022270508.1