Supplementary Material

SUMO-Modification of Human Nrf2 at K^{110} and K^{533} Regulates Its Nucleocytoplasmic Localization, Stability and Transcriptional Activity

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Supplemental Figure 1: Putative SUMO-conjugation motifs in Nrf2. *A*. NIH's COBALT: Multiple Alignment Tool comparing amino acid sequences of Nrf2 from mouse (Accession # NP_035032.1) and human Nrf2 (Accession # NP_006155.2) Putative SUMO-conjugation motifs as predicated by SUMOplot are bold and italicized. Top two SUMO-conjugation motifs are underlined. *B.* Map of the Nrf2 domain structures showing the location of top two putative SUMO-acceptor Lysine residues predicted by SUMOplot in Nrf2 protein.

Supplemental Figure 1

A

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Human   1    MMDLELPPPGLPSQQDMDLIDILWRQDIDLGVSREVFDFSQRRKEYELEKQKKLEKERQEQLQKEQEKAFFAQLQLDEET  80
Mouse   1    MMDLELPPPGLQSQQDMDLIDILWRQDIDLGVSREVFDFSQRQKDYELEKQKKLEKERQEQLQKEQEKAFFAQFQLDEET  80

Human   81   GEFLPIQPAQHIQSETSGSANYSQVAHIPKSDALYFDDCMQLLAQTFPFVDDNEVSSATFQSLVPDIPGHIESPVFIATN  160
Mouse   81   GEFLPIQPAQHIQTDTSGSASYSQVAHIPKQDALYFEDCMQLLAETFPFVDDHE-------SLALDIPSHAESSVFTAPH  153

Human   161  QAQSPETSVAQVAPVDLDGMQQDIEQVWEELLSIPELQCLNIENDKLVETTMVPSPEAKLTEVD-NYHFYSSIPSMEKEV  239
Mouse   154  QAQSLNSSL-EAAMTDLSSIEQDMEQVWQELFSIPELQCLNTENKQLADTTAVPSPEATLTEMDSNYHFYSSISSLEKEV  232

Human   240  GNCSPHFLNAFEDSFSSILSTEDPNQLTVNSLNSDATVNTDFGDEFYSAFIAEPSISNSMPSPATLSHSLSELLNGPIDV  319
Mouse   233  GNCGPHFLHGFEDSFSSILSTDDASQLT--SLDSNPTLNTDFGDEFYSAFIAEPSDGGSMPSSAAISQSLSELLDGTIEG  310

Human   320  SDLSLCKAFNQNHPESTAEFNDSDSGISLNTSPSVASPEHSVESSSYGDTLLGLSDSEVEELDSAPGSVKQNGPKT-PVH  398
Mouse   311  CDLSLCKAFNPKHAEGTMEFNDSDSGISLNTSPSRASPEHSVESSIYGDPPPGFSDSEMEELDSAPGSVKQNGPKAQPAH  390

Human   399  SSGDMVQPLSPSQGQSTHVHDAQCENTPEKELPVSPGHRKTPFTKDKHSSRLEAHLTRDELRAKALHIPFPVEKIINLPV  478
Mouse   391  SPGDTVQPLSPAQGHSAPMRESQCENTTKKEVPVSPGHQKAPFTKDKHSSRLEAHLTRDELRAKALHIPFPVEKIINLPV  470

Human   479  VDFNEMMSKEQFNEAQLALIRDIRRRGKNKVAAQNCRKRKLENIVELEQDLDHLKDEKEKLLKEKGENDKSLHLLKKQLS  558
Mouse   471  DDFNEMMSKEQFNEAQLALIRDIRRRGKNKVAAQNCRKRKLENIVELEQDLGHLKDEREKLLREKGENDRNLHLLKRRLS  550

Human   559  TLYLEVFSMLRDEDGKPYSPSEYSLQQTRDGNVFLVPKSKKPDVKKN  605
Mouse   551  TLYLEVFSMLRDEDGKPYSPSEYSLQQTRDGNVFLVPKSKKPDTKKN  597
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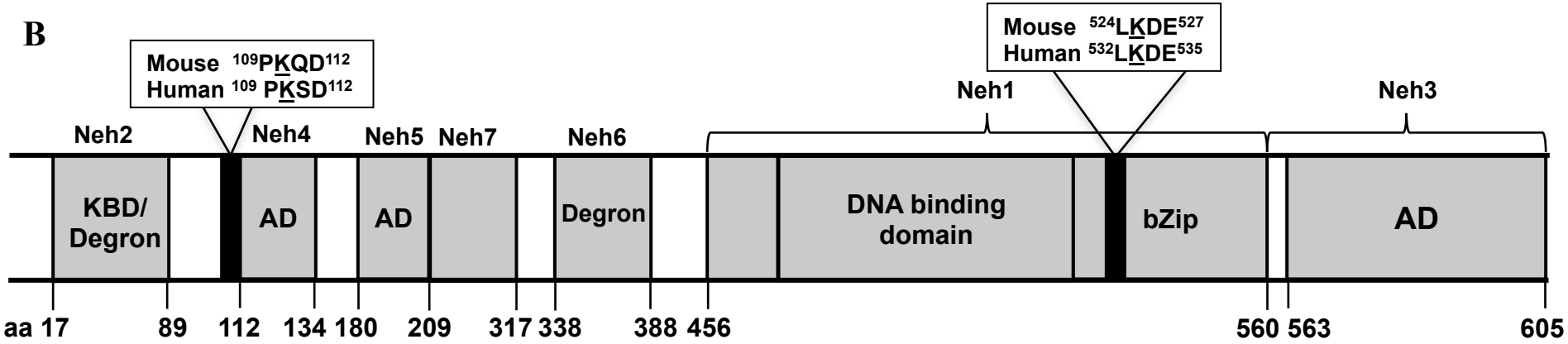
B



Supplemental Figure 2: SUMO-acceptor K residues are needed for transactivation of the hNrf2 and stability of HO1 protein. HEK293T cells were transfected with plasmid for either FLAG-tagged WT or mutant hNrf2 ($K^{110}R$, $K^{533}R$, or 2K) for 24 hrs. The transfected cells were treated with 100 μM Cycloheximide (CHX) for 0, 5, 15, 30, and 60 min. Total cell lysates were resolved on a 7.5% SDS-PAGE gel and analyzed by western blotting using anti-FLAG and anti-β-actin antibodies. *A*. Representative HO1 and Histone-H3 blots are shown. *B.* Densitometric quantitation of the blots in *A*. The levels of HO1 were normalized to those of Histone-H3. The normalized values at 0 min were designated as 100%. Subsequent normalized values were calculated as percentages of those at 0 min. ImageJ software was used for quantitation. The values plotted are means ± S.E (n=3).

Supplemental Figure 2