

## Experimentally Validated NoLS (EVN) dataset

Accession	Protein Name	NoLS	Targets reporter protein to nucleolus <sup>a</sup>	References
NP_001012270	Survivin	MQRKPTIRRNLRRLRRK	GFP	[1]
NP_006161	p120	SKRLSSRARKRAAKRRLG	β-gal (but requires additional NLS)	[2]
NP_005336	HSP70	FKRKHKKDISQNKRAVRR	GFP	[3,4]
NP_937862	ING1b (NoLS-1)	DKPNSKRSRRQRNNENR	GFP	[5]
NP_937862	ING1b (NoLS-2)	TPKEKAKTSKKKKRSKAKA	GFP	[5]
NP_005238	FGF3	GKGVQPRRRRQKQSPDNLEP	N/A	[6]
NP_006618	Rpp29	RHKRKEKKKAKGLSARQRREL	GFP	[7]
NP_945316	PTHrP	GKKKKGKPGKRREQEKKRRRT	β-gal	[8]
NP_003778	NOLP	KEKIQAIIDSCRRQFPEYQERAR	N/A	[9]
NP_001002	S7	RRILPKPTRKSRTKNKQKRPR	N/A	[10]
NP_001034800	DEDD	LKRRRA	N/A	[11]
NP_001091059	Rpp38	KIKKLIPNPNKIRKPPKSKKATPK	GFP	[7]
NP_478102	ARF	QLRRPRHSHPTRARRCP	GFP	[12]
NP_003133	La	QESLNKWKSKGRRRFKGGKGNKA AQP GSGK GK	PTB-GFP	[13]
NP_005560	LIMK2	KKRTLKNDRKKR	GFP	[14]
NP_001997	FGF2	RSRYTWSYVALKR	GFP	[15]
NP_477352	PI4K230	SKKTNRGSQQLHKYMKRRTL	<i>soybean trypsin inhibitor</i>	[16]
NP_002383	MDM2	KKLLKRNK	<i>thioredoxin</i>	[17]
NP_003945	NIK	RKKRKKK	GFP	[18]
NP_078908	SAP30L	RRYKRHYK	N/A	[19]
NP_951038	HIC p40	GRCRRLANFPGRKRRRRR	GFP	[20]
NP_848927	LYRIC (NoLS-1)	KSKKKKKKKKQGE	GFP	[21]
NP_848927	LYRIC (NoLS-2)	KQIKKKKKARRET	GFP	[21]
NP_078805	Parafibromin (NoLS-1)	RRAATENIPVVRPDRK	GFP	[22]
NP_078805	Parafibromin (NoLS-2)	KKKQGCQRENETLIQRRK	GFP	[22]
NP_078905	MLF-1	MAPRGRRRPRPHRSEGARRSKNT LERTHS	GFP	[23]
NP_060239	G2E3	RKHDDCPNKYGEKKTKEK	N/A	[24]
NP_077289	Nop25	KRKHPRAAQDSKPPRAPRTSKAQ RRR	GFP fused to rat Nop25-NoLS	[25]
NP_039252	NRG1-beta	MSERKEGRGKGGKKKERGSK	GFP	[26]
NP_055318	DRIM	KKMKKHKHKNSEAKKRK	GFP	[27]
NP_849193	STT3-B	KQKYLSKKTTRKRGYIKNKLVFVK GKKISKKT V	GFP	[28]
NP_068810	RelA	EQPKQRGMRFYKCEGRSAGSIP GER	N/A	[29]
NP_112578	PAPA-1	HGHGVHKKKHKHKKHKKH	N/A	[30]
AAB60345	L1 ORF2	RLKIKGQRKIYQANGKQKK	N/A	[31]
AAH01024	Nucleostemin	KRPKLKASKRMTCHKRYKIQKKV REHHRKLRLEAKKQGHKKPRK	N/A	[32]
NP_002511	B23	QDLWQWRKSL	GFP	[33]
NP_937983	hTERT	MPRAPRCRAVRSLLR	GFP	[34]
NP_003277	DNA topo I	NKKKKPKKE	N/A	[35]
NP_796375	midnolin	QQKRLRRKARRDARGPYHWSPSR KAGRS	GFP	[36]
NP_004851	FXR2P	RPQRNRNRRRRNR	N/A	[37]
NP_000347	TCOF	KRKKDKEKKEKKKAKKASTKDSE SPSQKKKKKKTAEQTV	GFP	[38,39]
NP_004695	U3-55K	GQEHLRGRWWRIKEARNSVCIPLR RVPVPPAAGS	N/A	[40]
NP_150241	PML-I	DRPLVFFDLKIDN	GFP	[41]
NP_061940	GNL3L	MMKLRHKNKKPGEKSGHKKISW PYPQPAKQNGKKATSKVPSAPHFV HPN	GFP	[42]
NP_004251	RECQL4	KQAWKQKWRKK	GFP	[43]
NP_068778	Inh3	HRKGRRR	N/A	[44]

<sup>a</sup> Indicates whether this NoLS has been shown to target a reporter protein to the nucleolus when fused to it. The reporter protein chosen is reported.

## References

1. Song Z, Wu M (2005) Identification of a novel nucleolar localization signal and a degradation signal in Survivin-deltaEx3: a potential link between nucleolus and protein degradation. *Oncogene* 24: 2723-2734.
2. Valdez BC, Perlaky L, Henning D, Saijo Y, Chan PK, et al. (1994) Identification of the nuclear and nucleolar localization signals of the protein p120. Interaction with translocation protein B23. *J Biol Chem* 269: 23776-23783.
3. Dang CV, Lee WM (1989) Nuclear and nucleolar targeting sequences of c-erb-A, c-myc, N-myc, p53, HSP70, and HIV tat proteins. *J Biol Chem* 264: 18019-18023.
4. Knowlton AA (1999) Mutation of amino acids 246-251 alters nuclear accumulation of human heat shock protein (HSP) 72 with stress, but does not reduce viability. *J Mol Cell Cardiol* 31: 523-532.
5. Scott M, Boisvert FM, Vieyra D, Johnston RN, Bazett-Jones DP, et al. (2001) UV induces nucleolar translocation of ING1 through two distinct nucleolar targeting sequences. *Nucleic Acids Res* 29: 2052-2058.
6. Antoine M, Reimers K, Dickson C, Kiefer P (1997) Fibroblast growth factor 3, a protein with dual subcellular localization, is targeted to the nucleus and nucleolus by the concerted action of two nuclear localization signals and a nucleolar retention signal. *J Biol Chem* 272: 29475-29481.
7. Jarrous N, Wolenski JS, Wesolowski D, Lee C, Altman S (1999) Localization in the nucleolus and coiled bodies of protein subunits of the ribonucleoprotein ribonuclease P. *J Cell Biol* 146: 559-572.
8. Henderson JE, Amizuka N, Warshawsky H, Biasotto D, Lanske BM, et al. (1995) Nucleolar localization of parathyroid hormone-related peptide enhances survival of chondrocytes under conditions that promote apoptotic cell death. *Mol Cell Biol* 15: 4064-4075.
9. Ueki N, Kondo M, Seki N, Yano K, Oda T, et al. (1998) NOLP: identification of a novel human nucleolar protein and determination of sequence requirements for its nucleolar localization. *Biochem Biophys Res Commun* 252: 97-102.
10. Annilo T, Karis A, Hoth S, Rikk T, Kruppa J, et al. (1998) Nuclear import and nucleolar accumulation of the human ribosomal protein S7 depends on both a minimal nuclear localization sequence and an adjacent basic region. *Biochem Biophys Res Commun* 249: 759-766.
11. Stegh AH, Schickling O, Ehret A, Scaffidi C, Peterhansel C, et al. (1998) DEDD, a novel death effector domain-containing protein, targeted to the nucleolus. *Embo J* 17: 5974-5986.
12. Zhang Y, Xiong Y (1999) Mutations in human ARF exon 2 disrupt its nucleolar localization and impair its ability to block nuclear export of MDM2 and p53. *Mol Cell* 3: 579-591.
13. Horke S, Reumann K, Schweizer M, Will H, Heise T (2004) Nuclear trafficking of La protein depends on a newly identified nucleolar localization signal and the ability to bind RNA. *J Biol Chem* 279: 26563-26570.
14. Goyal P, Pandey D, Siess W (2006) Phosphorylation-dependent regulation of unique nuclear and nucleolar localization signals of LIM kinase 2 in endothelial cells. *J Biol Chem* 281: 25223-25230.
15. Sheng Z, Lewis JA, Chirico WJ (2004) Nuclear and nucleolar localization of 18-kDa fibroblast growth factor-2 is controlled by C-terminal signals. *J Biol Chem* 279: 40153-40160.
16. Kakuk A, Friedlander E, Vereb G, Jr., Lisboa D, Bagossi P, et al. (2008) Nuclear and nucleolar localization signals and their targeting function in phosphatidylinositol 4-kinase PI4K230. *Exp Cell Res* 314: 2376-2388.
17. Lohrum MA, Ashcroft M, Kubbutat MH, Vousden KH (2000) Identification of a cryptic nucleolar-localization signal in MDM2. *Nat Cell Biol* 2: 179-181.
18. Birbach A, Bailey ST, Ghosh S, Schmid JA (2004) Cytosolic, nuclear and nucleolar localization signals determine subcellular distribution and activity of the NF-kappaB inducing kinase NIK. *J Cell Sci* 117: 3615-3624.

19. Viiri KM, Korkeamaki H, Kukkonen MK, Nieminen LK, Lindfors K, et al. (2006) SAP30L interacts with members of the Sin3A corepressor complex and targets Sin3A to the nucleolus. *Nucleic Acids Res* 34: 3288-3298.
20. Thebault S, Basbous J, Gay B, Devaux C, Mesnard JM (2000) Sequence requirement for the nucleolar localization of human I-mfa domain-containing protein (HIC p40). *Eur J Cell Biol* 79: 834-838.
21. Thirkettle HJ, Girling J, Warren AY, Mills IG, Sahadevan K, et al. (2009) LYRIC/AEG-1 is targeted to different subcellular compartments by ubiquitylation and intrinsic nuclear localization signals. *Clin Cancer Res* 15: 3003-3013.
22. Hahn MA, Marsh DJ (2007) Nucleolar localization of parafibromin is mediated by three nucleolar localization signals. *FEBS Lett* 581: 5070-5074.
23. Suzuki H, Arakawa Y, Ito M, Saito S, Takeda N, et al. (2007) MLF1-interacting protein is mainly localized in nucleolus through N-terminal bipartite nuclear localization signal. *Anticancer Res* 27: 1423-1430.
24. Brooks WS, Banerjee S, Crawford DF (2007) G2E3 is a nucleo-cytoplasmic shuttling protein with DNA damage responsive localization. *Exp Cell Res* 313: 665-676.
25. Fujiwara T, Suzuki S, Kanno M, Sugiyama H, Takahashi H, et al. (2006) Mapping a nucleolar targeting sequence of an RNA binding nucleolar protein, Nop25. *Exp Cell Res* 312: 1703-1712.
26. Golding M, Ruhrberg C, Sandle J, Gullick WJ (2004) Mapping nucleolar and spliceosome localization sequences of neuregulin1-beta3. *Exp Cell Res* 299: 110-118.
27. Liu J, Du X, Ke Y (2006) Mapping nucleolar localization sequences of 1A6/DRIM. *FEBS Lett* 580: 1405-1410.
28. Caron E, Cote C, Parisien M, Major F, Perreault C (2006) Identification of two distinct intracellular localization signals in STT3-B. *Arch Biochem Biophys* 445: 108-114.
29. Stark LA, Dunlop MG (2005) Nucleolar sequestration of RelA (p65) regulates NF-kappaB-driven transcription and apoptosis. *Mol Cell Biol* 25: 5985-6004.
30. Kuroda TS, Maita H, Tabata T, Taira T, Kitaura H, et al. (2004) A novel nucleolar protein, PAPA-1, induces growth arrest as a result of cell cycle arrest at the G1 phase. *Gene* 340: 83-98.
31. Goodier JL, Ostertag EM, Engleka KA, Seleme MC, Kazazian HH, Jr. (2004) A potential role for the nucleolus in L1 retrotransposition. *Hum Mol Genet* 13: 1041-1048.
32. Tsai RY, McKay RD (2002) A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells. *Genes Dev* 16: 2991-3003.
33. Nishimura Y, Ohkubo T, Furuichi Y, Umekawa H (2002) Tryptophans 286 and 288 in the C-terminal region of protein B23.1 are important for its nucleolar localization. *Biosci Biotechnol Biochem* 66: 2239-2242.
34. Yang Y, Chen Y, Zhang C, Huang H, Weissman SM (2002) Nucleolar localization of hTERT protein is associated with telomerase function. *Exp Cell Res* 277: 201-209.
35. Mo YY, Wang C, Beck WT (2000) A novel nuclear localization signal in human DNA topoisomerase I. *J Biol Chem* 275: 41107-41113.
36. Tsukahara M, Suemori H, Noguchi S, Ji ZS, Tsunoo H (2000) Novel nucleolar protein, midnolin, is expressed in the mesencephalon during mouse development. *Gene* 254: 45-55.
37. Tamanini F, Kirkpatrick LL, Schonkeren J, van Unen L, Bontekoe C, et al. (2000) The fragile X-related proteins FXR1P and FXR2P contain a functional nucleolar-targeting signal equivalent to the HIV-1 regulatory proteins. *Hum Mol Genet* 9: 1487-1493.
38. Fujioka H, Ariga T, Horiuchi K, Ishikiriya S, Oyama K, et al. (2008) Detection of a novel silent deletion, a missense mutation and a nonsense mutation in TCOF1. *Pediatr Int* 50: 806-809.
39. Winokur ST, Shiang R (1998) The Treacher Collins syndrome (TCOF1) gene product, treacle, is targeted to the nucleolus by signals in its C-terminus. *Hum Mol Genet* 7: 1947-1952.
40. Pluk H, Soffner J, Luhrmann R, van Venrooij WJ (1998) cDNA cloning and characterization of the human U3 small nucleolar ribonucleoprotein complex-associated 55-kilodalton protein. *Mol Cell Biol* 18: 488-498.
41. Condemine W, Takahashi Y, Le Bras M, de The H (2007) A nucleolar targeting signal in PML-I addresses PML to nucleolar caps in stressed or senescent cells. *J Cell Sci* 120: 3219-3227.

42. Rao MR, Kumari G, Balasundaram D, Sankaranarayanan R, Mahalingam S (2006) A novel lysine-rich domain and GTP binding motifs regulate the nucleolar retention of human guanine nucleotide binding protein, GNL3L. *J Mol Biol* 364: 637-654.
43. Woo LL, Futami K, Shimamoto A, Furuichi Y, Frank KM (2006) The Rothmund-Thomson gene product RECQL4 localizes to the nucleolus in response to oxidative stress. *Exp Cell Res* 312: 3443-3457.
44. Huang HS, Pozarowski P, Gao Y, Darzynkiewicz Z, Lee EY (2005) Protein phosphatase-1 inhibitor-3 is co-localized to the nucleoli and centrosomes with PP1gamma1 and PP1alpha, respectively. *Arch Biochem Biophys* 443: 33-44.