anti-Necdin antibody, rabbit polyclonal (NC243), ChIP grade, KO-Validated

74-100　　100 µg

Shipping and Storage: Shipped at 4℃ or -20℃, and upon arrival, aliquot and store at -20℃.

Immunogen: Recombinant GST-fused mouse necdin (aa 83-325)

Form: Protein A affinity purified IgG. 2 mg/ml in PBS- with 50% glycerol. Filter-sterilized. No additive.

Validation of specific reactivity: Specificity of reaction has been validated with knock-out mice by western blot and IHC-F

Reactivity: React with mouse, rat, human, chicken

Applications:

1. Western blotting (1/1,000-1/3,000)
2. Immunohistochemistry、frozen section (1/500)
3. Immunocytochemistry (1/500)
4. Immunoprecipitation (1/100)
5. Chromatin Immunoprecipitation (1/100)
6. Immunoaffinity assay (Identification of Necdin interacting proteins by column conjugated with anti-Necdin antibody)

Background: Necdin (**n**eurally differentiated **e**mbryonal **c**arcinoma-**d**erived prote**in**) is a 325-amino acid residue protein encoded by a cDNA clone isolated from neurally differentiated mouse embryonal carcinoma cells (ref.1). **Necdin** is a potent growth suppressor that is expressed predominantly in postmitotic cells such as neurons and muscle cells. **Necdin** has been implicated in the pathogenesis of Prader-Willi syndrome, a human neurodevelopmental disorder associated with genomic imprinting. Furthermore, **necdin** binds to major transcription factors E2F1 and p53, and also to NEFA and nucleobindin, both of which are calcium-binding proteins involved in intracellular calcium homeostasis. From these findings **necdin** is suggested to target various factors involved in the regulation of cell proliferation and survival, and plays a key role in development and differentiation of subsets of neurons in the brain. An antibody (named NC243) against mouse **necdin** was raised in rabbit (ref.1) in the laboratory of Prof. K. Yoshikawa at Osaka Univ.

Data Link: Swiss-Prot P25233 (mouse), Q99608 (human)

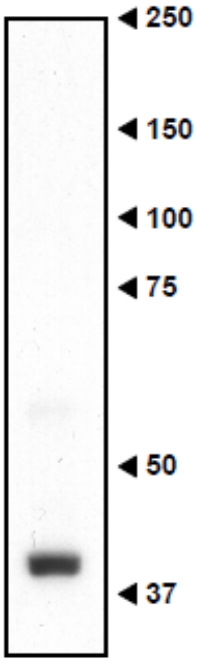

Fig.1.Western blotting of Necdin in the crude extract of mouse embryo.

The extract (20 μg protein)) was prepared from cerebral cortex of E 16.5 mouse embryo. The anti-Necdin antibody (NC143) was used at 1/3,000 dilution. As the secondary antibody, HRP conjugated goat anti-rabbit IgG was used at 1/20,00 dilution

Molecular mass of mouse Necdin is 37 kDa. The larger size reported here and elsewhere (see Ref) may reflect post-translational modifications such as ubiquitination and phosphorylation at several sites (Swiss-Prot)

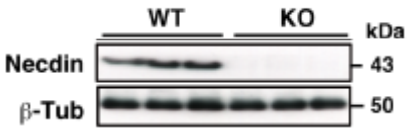

Fig.2 Validation of the anti-necdin antibody with knock-out mice.

Proteins in forebrain lysates from wild-type and necdin knock-out mouse embryos at E14.5 were analyzed by Western blotting. The primary antibody was used at 1/2,000 dilution. Each lane represents the extract from one littermate. Protein levels were normalized to β-tublin.

(Image from Minamido R et al. *PLoS One.* **9** (1) PMID: 24392139.)

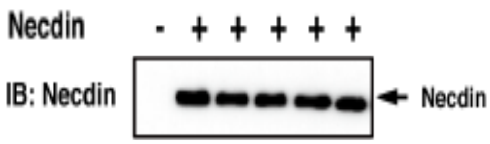

Fig.3 Immunoprecipitation of necdin

HEK293A cells were transfected with expression vectors for necdin (+). Cell lysates were immunoprecipitated and immunoblotted with anti-necdin antibody. HEK293A cell lysate (-) is a negative control.

(Image from Minamido R et al. *PLoS One.* **9** (1) PMID: 24392139.)

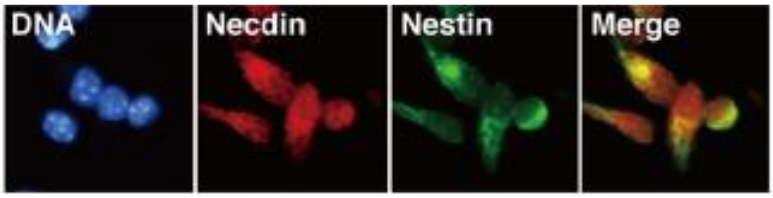

Fig 4. Immunofluorescence staining of necdin. Expression of necdin, and nestin in primary neural precursor cells (NPCs) from mouse neocortex. Primary NPCs were prepared from the neocortex at E14.5 and subjected to double-immunostaining for necdin and nestin. DNA was stained with Hoechst 33342. Necdin was immunostained with anti-necdin antibody (NC243) at 1/500 dilution and Nestin with anti-nestin antibody (ST1; BioAcademia 73-105)

(Images from Minamido R et al. *PLoS One.* **9** (1) PMID: 24392139.)

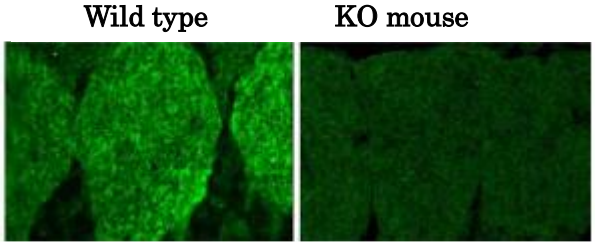

Fig.5 Immunohistochemistry of necdin : Validation of anti-necdin antibody (NC243) with KO-mouse.

Cryosections of cervical dorsal root ganglion tissues from wild-type (WT) and necdin-null (KO) mice at E14.5 were prepared and immunostained for necdin. Antibody was used at 1/500 dilution. As the secondary antibody, goat anti-rabbit IgG conjugated with Alexa Fluora 555 was used at 1/2,000.

Fig.6 Immunohistochemical staining of necdin in mouse forebrain.

E13.5 forebrain cryosections were immunostained for necdin. CX, Cortex; LV, lateral ventricle; LGE, lateral ganglionic eminence; SP, septum. The antibody was used at 1/500 dilution. As the secondary antibody, goat anti-rabbit IgG conjugated with Alexa Fluora 555 was used at 1/2,000.

References: This antibody has been described in ref.1 and used in ref.1-14

1. Niinobe M *et al.* Cellular and subcellular localization of necdin in fetal and adult mouse brain. *Dev Neurosci* (2000) **22**: 310-319 PMID: 10965153. **WB, IHC-F (mouse)**
2. Taniguchi N. et al The Postmitotic Growth Suppressor Necdin Interacts with a Calcium-binding Protein (NEFA) in Neuronal Cytoplasm. J Biol Chem. 2000 Oct 13;275(41):31674-81. PMID:10915798. **WB, IP, IHC-F, Immunoaffinity column (mouse)**
3. Kobayashi M. et al. Ectopic Expression of Necdin Induces Differentiation of Mouse Neuroblastoma Cells. J Biol Chem. 2002 Nov 1;277(44):42128-35. PMID:12198120 **WB, IP (mouse)**
4. Tcherpakov M. et al. The p75 Neurotrophin Receptor Interacts with Multiple MAGE Proteins. J Biol Chem. 2002 Dec 20;277(51):49101-4. PMID:12414813 **WB, IF (rat)**
5. Andrieu A. et al. Expression of the Prader-Willi gene Necdin during mouse nervous system development correlates with neuronal differentiation and p75NTR expression. Gene Expr Patterns. 2003 Dec;3(6):761-5. PMID:14643685 **IHC (mouse), KO Validation**
6. Kuwajima T *et al* Necdin interacts with the Msx2 homeodomain protein via MAGE-D1 to promote myogenic differentiation of C2C12 cells. *J Biol Chem* (2004) **279**: 40484-40493 PMID: 15272023. **WB, IP, IF, IHC (mouse)**
7. Brunelli S. et al. Msx2 and necdin combined activities are required for smooth muscle differentiation in mesoangioblast stem cells. Circ Res. 2004 Jun 25;94(12):1571-8. PMID:15155529. **IHC-F (mouse)**
8. Hoek K. et al. Expression Profiling Reveals Novel Pathways in the Transformation of Melanocytes to Melanomas. Cancer Res. 2004 Aug 1;64(15):5270-82. PMID:15289333. **WB, IF (human)**
9. Kuwako K. et al Disruption of the Paternal Necdin Gene Diminishes TrkA Signaling for Sensory Neuron Survival. J Neurosci. 2005 Jul 27;25(30):7090-9. PMID:16049186 **WB,IP, IHC-F, Immunoaffinity assay, (mouse), KO-Validation for WB and IHC-F**
10. Goldfine AB. et al. Necdin and E2F4 Are Modulated by Rosiglitazone Therapy in Diabetic Human Adipose and Muscle Tissue. Diabetes. 2006 Mar;55(3):640-50. PMID:16505226. **WB, IF, (mouse)**
11. Kuwajima T et al. Necdin promotes GABAergic neuron differentiation in cooperation with Dlx homeodomain proteins. J Neurosci. (2006) **26**(20):5383-92. PMID:16707790 **WB, IHC-F, IP, Immunoaffinity assay, (mouse)**
12. Andrieu D. et al. Sensory defects in Necdin deficient mice result from a loss of sensory neurons correlated within an increase of developmental programmed cell death. BMC Dev Biol. 2006 Nov 20;6:56. PMID: 17116257. **WB (mouse)**
13. López-Sánchez N. et al. Single *mage* gene in the chicken genome encodes CMage, a

protein with functional similarities to mammalian type II Mage proteins. Physiol Genomics. 2007 Jul 18;30(2):156-71. PMID: 17374844 **IF/IC, IHC-F (mouse)**

14. Kurita M *et al* Necdin downregulates Cdc2 expression to attenuate neuronal apoptosis. *J Neurosci* (2006)**26**: 12003-12013 PMID: 17108174. **WB, ChIP, IF, IHC-F (mouse), KO-Validation for WB**
15. Kubota Y. et al. Necdin restricts proliferation of hematopoietic stem cells during hematopoietic regeneration. Blood. 2009 Nov 12;114(20):4383-92. PMID:19770359 **IF (mouse)**
16. Morillo SM. et al. Nerve Growth Factor-Induced Cell Cycle Reentry in Newborn NeuronsIs Triggered by p38MAPK-Dependent E2F4 Phosphorylation. Mol Cell Biol. 2012 Jul;32(14):2722-37. PMID:22586272. **WB, IP, (chicken)**
17. Minamide R *et al* Antagonistic Interplay between Necdin and Bmi1 Controls Proliferation of Neural Precursor Cells in the Embryonic Mouse Neocortex. PLoS One. (2014) **9** (1) PMID: 24392139. **WB, IF, IP (mouse). Validated with KO mice for WB and IHC-F**
18. Fujimoto I. *et al.* Necdin controls EGFR signaling linked to astrocyte differentiation in primary cortical progenitor cells. Cell Signal. 2016 Feb;28(2):94-107. PMID: 26655377 **WB, IP, (mouse)**
19. Hasegawa K. *et al.* Promotion of mitochondrial biogenesis by necdin protects neurons against mitochondrial insults. Nat Commun. 2016 Mar 14;7:10943. PMID: 26971449. **WB, IHC-F, (mouse) Validated with KO mice for WB**