LRP4 LDLα Repeats of Astrocyte Enhances Dendrite Arborization of the Neuron

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Research

Keywords: LRP4, LDLα repeats, primary culture, Golgi staining, dendrite arborization

Posted Date: October 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-84416/v1

Version of Record: A version of this preprint was published on December 10th, 2020. See the published version at https://doi.org/10.1186/s13041-020-00708-z.

LRP4 LDLα repeats of astrocyte enhances dendrite arborization of the neuron

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Abstract

Low-density lipoprotein receptor-related protein 4 (LRP4) is essential for inducing the neuromuscular junction formation in muscle fibers, and LRP4 plays a role of dendritic development and synaptogenesis in the central nervous system (CNS). As a member of the low-density lipoprotein receptor family, LRP4 contains an enormously large extracellular region possessing multiple LDLα repeats in the N-terminal. LRP4 only with extracellular domain acts as a similar mechanism of full-length LRP4 in muscles to stimulate acetylcholine receptor clustering. In this study, we elucidated that LDLα repeats of LRP4 maintained the body weight and survival rate. Dendritic branches of the neurons in Lrp4-null mice with LRP4 LDLα repeats residue were more than in Lrp4-null mice without residual LRP4 domain. Supplement with conditioned medium from LRP4 LDLα over expression cells, primary culture neurons achieved strong dendritic arborization ability. In addition, astrocytes with LRP4 LDLα repeats residue could promoted the dendrite arborization of neurons in primary co-cultured system. These observations

signify that LRP4 LDLα repeats play an underlying prominent role in dendrite arborization.

Keywords: LRP4, LDLα repeats, primary culture, Golgi staining, dendrite arborization

Introduction

Synapses formed between neurons and target cells are the basis of brain function. Synaptic transmission is critical for thinking, learning, memory and response to environmental changes. Synaptic dysfunction involves various neuropsychiatric diseases, including autism, schizophrenia, epilepsy and addiction. LRP4 plays a vital role in neuromuscular junction (NMJ) presynaptic differentiation [1-3]. Biochemical studies have confirmed that LRP4 is a key regulator for NMJ formation [4]. It is the receptor of agrin and specifically binds to neural agrin, which promotes NMJ development [1, 3-6]. LRP4 contains an extracellular domain (ECD), intracellular domain (ICD) and transmembrane domain. ECD domain has eight LDLα domains, four β-propeller domains, and a domain for O-linked oligosaccharide modification. LRP4 ECD/ECD mice form partially functional NMJs, expressing only the ECD, without the transmembrane and ICD domain [7]. This suggested that the transmembrane domain and ICD are not required for NMJ formation [5, 8, 9], and LRP4 ECD domain plays a main role in the formation of NMJ.

LRP4 is mainly expressed in the hippocampus, olfactory bulb, cerebellum, and neocortex, especially in the postsynaptic membrane of the hippocampus [10-12]. Previous studies indicate that LRP4 is a protein present at the postsynaptic density of pyramidal neurons [12, 13]. LRP4

mRNA is present in the hippocampal dentate gyrus granulosa cell layer and hippocampal pyramidal cell layer. LRP4 plays a critical role in the central nervous system of adults, including hippocampal synaptic plasticity, maintenance of excitatory synaptic transmission, fear regulation, and long-term enhancement [13, 14]. LRP4 knockout can affect the synaptic plasticity and cognitive function of mice [13, 15]. LRP4 expressed has been shown to regulate glutamatergic synaptic transmission by regulating the release of adenosine triphosphate(ATP) in astrocytes [11]. The role of LRP4 extracellular segment in the brain is currently unclear.

In this study, two types of muscle-rescued Lrp4-null mice were used to identify LRP4 loss of function, which were muscle-rescued $Lrp4^{LacZ}$ mice (mr-$Lrp4^{LacZ}$) (RRID: MMRRC_048465 -UCD) and muscle-rescued $Lrp4^{mitt}$ mice (mr-$Lrp4^{mitt}$). Astonishingly, we found some intriguing differences between mr-$Lrp4^{LacZ}$ mice and mr-$Lrp4^{mitt}$ mice, which were ignored or not paid special attention in previous research. LRP4 LDLα domain not only affects the survival rate and body weight of mice. Especially, LRP4 LDLα domain manipulates dendritic arborization of neurons *in vivo* and *in vitro*. These results indicate LRP4 LDLα domain plays a crucial role in the development of the central nervous system during dendritic branching.

Materials and methods

Animal

All transgenic or wild type male mice were housed in ventilated cages. 5 or fewer adult mice were feed in each cage. Sufficient water and food were free intake to mice, with a 12-h light/dark cycle, room temperature at 22 to 25°C and humidity is 50-60%. All experiments involving animals were conducted according to the "guidelines for the care and use of experimental animals" issued by

Nanchang University. The Committee on the Ethics of Animal Experiments of the University of Nanchang approved the protocol (Permit Number: 2016–0002). For *in vivo* experiment, surgery was performed under sodium pentobarbital anesthesia (50 mg/kg, ip injection), and all efforts were made to minimize suffering. After terminal experiments, mice were euthanized by carbon dioxide inhalation followed by cervical dislocation.

Nissl's staining

Brain slides were cut in 40 μm, washing with distilled water for 3 min. Dyeing in the staining buffer (0.2% Cresyl Violet solution) for 5 min in 60°C. Then dehydrating slides with 50%, 75%, 90% ethanol (Sangon Biotech,China,A500737) for 20 sec. Putting the slides into 100% ethyl alcohol 3 times, each time for 20 sec， transferring slides into xylene (Sangon Biotech, China, A530011) 3 times, each time for 10 min. Samples were mounted in Hydromount (National Diagnostics, USA, HD-106).

Golgi staining

Golgi staining was performed by using FD Rapid Golgi Stain™ Kit (FD NeuroTechnologies, USA, PK-401). Staining solution D, solution E and ultra-pure water were mixed in a ratio of 1:1:2. Slides were incubated with staining solution at room temperature for 10 min and washed twice with ultra-pure water, each time for 4 min. Transferring brain slides to the plate hole containing 50%, 75% and 90% ethanol for 4 min each time, then the brain slides were put into the holes containing 10 ml 90% ethanol or 100% ethanol for 3 times, 4 min for each time. Followed the samples were put into the xylene for 1 h. Images were randomly taken. Neurons with clear dendritic branches were

subjected to Sholl Analysis by using image J. The investigator who performed analysis was blinded to genotypes.

Cell culture and plasmid transfection

HEK293T (RRID:CVCL_0063) cells were cultured with media (DMEM + 10% Fetal bovine serum +1% Pens/Strep) in a cell culture incubator at 37°C and 5% CO_2, and changed the medium once every 3 days. After the cell density reached about 60%, added the plasmids (pFLAG-CMV1 vector, pFLAG-CMV1-Lrp4-LDLα, or pFLAG-CMV1-Reelin) and polyethyleneimine (Ploysciences, USA, 24314) to 50 μl of serum-free DMEM culture medium according to the ratio of 3 μg of plasmid to 15 μl of PEI, then mixed together at room temperature. After 20 min, the mixture was added to HEK293T cells. After cultured in an incubator at 37°C and 5% CO_2 for 4-8 h, the culture medium was replaced with new HEK293T culture medium. After 24 h, conditioned medium collected from the supernatant was added into the primary neurons (DIV4). Primary neurons were changed half-medium every other day, and after 6 days (DIV10), the neurons were preformed to immunofluorescent staining.

Astrocyte and neuron co-culture

Primary cell separation and co-culture were performed as described previously [11] with minor modifications. The isolated hippocampi of E18 mice were cut for small pieces, and digested in 0.25% trypsin at 37°C for 10-15 min. For primary neuron culture, dissociated cells were resuspended in primary culture medium (Neurobasal+1% GlutaMax^{TM}+5%FBS+1% Pens/Strep) and plated onto poly-L-lysine–coated coverslips in 12 well-plates for 4 h. And then replacing medium with serum-

111 free medium (Neurobasal + 1% GlutaMAX^{TM} + 2% B27 +1% Pens/Strep) and cytosine arabinoside
112 (Med ChemExpress, China, HY-13605) (10 μM) to inhibit glia proliferation. Half of the medium
113 was changed every other day. For astrocyte culture, dissociated cells were resuspended in the plating
114 medium (DMEM + 10% Fetal bovine serum +1% GlutaMAX^{TM} +1% Pens/Strep) and plated into
115 culture flasks for 3 d. The flasks were shaken at 250 rpm for 24 h to remove microglia and
116 oligodendrocytes. Astrocytes were passaged every 3 d at a ratio 1:3 and seeded onto coverslips
117 before co-culture. After neuron were cultured 8 d (DIV8), three coverslips seeded astrocytes were
118 placed with one neuron coverslip in one 35-mm dish containing serum-free medium for incubating
119 7 d (DIV15) until immunofluorescent staining.
120
121 *Immunofluorescent staining*
122 The coverslips of astrocyte and neuron co-culture were fixed at room temperature for 20 min in 4%
123 paraformaldehyde. After rinsing 10 min with phosphate buffered saline(PBS) (0.01 M, pH 7.4) at
124 room temperature, the coverslips were immersed in antibody blocking solution (10% donkey serum,
125 1% calf serum albumin, 0.5% Triton X-100 in PBS) at room temperature for 2 h. After this, the
126 coverslips were rinsed with 0.01 M PBS at room temperature for 3 times, the primary antibody (anti-
127 β3-Tubuiln antibody, Thermo Fisher Scientific, PA5-95875) was diluted 1:1000 with antibody
128 blocking solution, and was added into the coverslips for 4°C overnight. The coverslips were washed
129 with 0.01 M PBS at room temperature for 3 times, each time for 10 min. The secondary antibody
130 (anti-IgG，Invritrogen, 1832035) was diluted 1:1000 with antibody blocking solution, and then the
131 coverslips were incubated at room temperature for 2 h in dark. After washing 3 times for 10 min
132 with PBST, samples were mounted in Hydromount (National Diagnostics). Z serial images were

collected with an Olympus fluorescence microscope (FSX100) and collapsed into a single image.

Experimental design and Statistical analysis

The study was not pre-registered. For assignment of experimental groups, no special randomization methods were employed. Sample sizes and the number of cells were determined by past experience and not by a statistical sample size calculation. The experiments reported in this work did not require institutional approval. Exclusion criteria were not pre-determined in this study. Data were statistically analyzed using GraphPad Prism 5.0 (RRID: SCR_002798, GraphPad Software, CA, USA) and the results were expressed as mean ± standard error (Mean ± SEM). The analysis and experimental group assignments were performed by a different person than the experimenter. The measurement data were analyzed by one-way ANOVA; the comparison between groups was analyzed by independent sample T-test. The mice numbers of independent experiments were noted in the figure legend. The difference was considered statistically significant, and $P < 0.05$ was considered to be statistically significant，$P > 0.05$ (#) was considered no significance.

Results

LRP4 LDLα domain was necessary to keep body weight and survival rate

Body weight and survival rate of mr-Lrp4^{LacZ} mice, mr-Lrp4^{mitt} mice and the control mice were monitored for more than 60 d. Compared with the control mice, the survival rate was significantly lower in the mr-Lrp4^{LacZ} group (Fig1 B). The mr-Lrp4^{LacZ} mice gained significantly less body weight and brain weight (Fig1 C-E). However, mr-Lrp4^{mitt} mice appeared healthy and were indistinguishable from the control mice. There was no significantly difference between mr-Lrp4^{mitt}

mice and the control mice in body weight, survival rate and brain weight (Fig 1B-1E). mr-Lrp4^{mitt} mice and mr-Lrp4^{LacZ} mice were all Lrp4-null mice, but there were serious differences in phenotype between mr-Lrp4^{LacZ} and mr-Lrp4^{mitt} mice. The mr-Lrp4^{mitt} mice remained LDLα domain of LRP4 theoretically, as shown in Fig 1A. Therefore, we speculate whether the LDLα domain played a certain function to keep mice alive and body weight, which lead to phenotypic differences between the two types of mice.

The thickness of cerebral cortex reduced in mr-Lrp4^{LacZ} mice

Nissl's staining was carried out to observe the cerebral cortex region of mr-Lrp4^{LacZ} mice, mr-Lrp4^{mitt} mice and the control mice. The cerebral cortex of mr-Lrp4^{LacZ} mice was markedly thinner in layer I, II/III and IV than those of the control group, while the cerebral cortex of mr-Lrp4^{mitt} mice showed no difference from those of the control mice (Fig2 A, B). There was a remarkable difference in thickness of mr-Lrp4^{LacZ} mice compared with mr-Lrp4^{mitt} and the control mice. We speculated that the LDLα domain of LRP4 maintained the normal structure of the cerebral cortex.

The dendritic branches of neurons increased in mr-Lrp4^{mitt} mice

Golgi staining was performed to identify morphological structure of prefrontal cortex neurons in mr-Lrp4^{LacZ}, mr-Lrp4^{mitt} mice and the control mice. The complexity of neurons was analyzed by Sholl analysis. Sholl analysis centered on neuronal cell body, a series of concentric circles were drawn, and obtained the number of intersections of neuronal processes varying with the distance from the cell body. Compared with the control mice, there was no difference in total dendrites length presented in mr-Lrp4^{LacZ} mice, as shown in figure 3, but the total dendrite length in mr-Lrp4^{mitt} mice

177 was longer (Fig3 B). In mr-Lrp4^{LacZ} mice, we observed no significant difference in total dendrites
178 branches number of the prefrontal cortex neurons compared with the control group (Fig 3 C, D).
179 Besides, more dendrite branches were showed in mr-Lrp4^{mitt} mice than the control mice (Fig3 C, D).
180 It indicated that the LRP4 LDLα domain may play a role of promoting dendritic arborization.
181
182 *LRP4 LDLα domain increased dendrite arborization in vitro*
183 In mr-Lrp4^{mitt} mice, the LDLα domain was secreted because of LRP4 lack of transmembrane
184 domain. To assess whether LRP4 LDLα domain played a role in neuronal dendritic arborization, we
185 transfected pFLAG-CMV1-Lrp4 LDLα into primary neurons of wild type mice, pFLAG-CMV1-
186 Vector was negative control and pFlag-CMV1-Reelin plasmid was positive control. The number of
187 dendrites branches increased in the LRP4 LDLα group compared with the negative samples (Fig4
188 B, C).
189 Studies showed that LRP4 was expressed in neuron and astrocyte, and LRP4 knockout in
190 astrocytes suppressed glutamatergic release by increasing ATP release [11]. To explore whether the
191 LRP4 LDLα promoting dendritic arborization were from neuron or astrocyte, we co-cultured the
192 astrocytes with the neurons from wild type mice and/or mr-Lrp4^{mitt} mice. Neurons of wild type mice
193 showed more branches being co-cultured with astrocytes from mr-Lrp4^{mitt} mice than astrocytes from
194 wild type mice. Without controversy, the Lrp4^{mitt} mice neurons boosted more dendritic arborization
195 being co-cultured with astrocytes from Lrp4^{mitt} mice than astrocytes from wild type mice (Fig5 B,
196 C). The data further confirmed that free LRP4 LDLα domain in astrocytes promoted dendrite
197 arborization of neurons.
198

Discussion

Here we demonstrated that the survival rate and body weight of mr-Lrp4^{LacZ} mice were lower than mr-Lrp4^{mitt} mice and the control mice. The brain tissue also was smaller. Second, the cerebral cortex was thinner in layer I, II/III and IV of mr-Lrp4^{LacZ} mice than those of the control group. In mr-Lrp4^{mitt} mice, LRP4 has the LDLα domain，but this domain was missing in mr-Lrp4^{LacZ} mice. We speculated that these changes may be related to the function of the LDLα domain. Therefore, in order to clarify the role of the LRP4 LDLα domain, we performed Golgi staining to observe the branches of neuronal cells. The results showed that there were more dendritic branches in neurons of mr-Lrp4^{mitt} mice than in the control group. On the contrary, there was no difference between mr-Lrp4^{LacZ} mice and the control group. Third, neuronal cells transfected with LRP4 LDLα plasmid have more dendritic branches than the control group. When neurons were co-cultured with astrocytes of mr-Lrp4^{mitt} mice, the number of dendritic branches increased. These results indicated that LDLα domain of LRP4 promoted more dendritic arborization in neurons.

LRP4, as a member of the low-density lipoprotein receptor family, contains a large extracellular N-terminal region, a transmembrane domain and a short C-terminal region [16]. The extracellular region has eight LDLa domains (class A repeats), four β-propeller domains (class B repeats). LRP4 has a fundamental role during formation, maintenance and regeneration of the NMJ [17]. LRP4 β1 domain binds with agrin to form the agrin-LRP4 binary complex to activate acetylcholine receptor (AChR) clustering in NMJ [5, 6]. Study results showed that treatment ecto-LRP4 (Lrp4 ECD only) into myotubes, ecto-LRP4 increased the number of agrin-induced AChR clusters. This indicates that soluble ecto-LRP4 is sufficient to serve as a receptor for agrin to initiate pathways for AChR clustering. Ecto-LRP4 acts via a similar mechanism of full-length LRP4 in

muscles to stimulate AChR clustering [1]. In this experiment, the release of LDLα domain in mr-Lrp4^{mitt} mice may promote the increase of neuronal branches.

LRP4 plays a crucial role in CNS, including maintaining synapses, especially in synaptic transmission [18, 19]. Studies demonstrated that in the brain glutamate release was reduced in lacking LRP4 mice. LRP4 knockout astrocytes suppressed presynaptic glutamatergic release by increasing ATP release. ATP released from astrocytes was converted to adenosine that activates adenosine A1 receptors in glutamatergic pre-synapses. Synaptic plasticity was affected [11]. Besides, LRP4 played a role in dendritic development and synaptogenesis in the CNS. Knockdown of LRP4 in embryonic cortical and hippocampal neurons causes a reduction in density of primary dendrites, overexpression of LRP4 in these cultured neurons had the opposite effect inducing more but shorter primary dendrites (Karakatsani et al., 2017). Neuron-specific knockdown of LRP4 by in utero electroporation of LRP4 miRNA also resulted in neurons with fewer primary dendrites in the developing cortex and hippocampus in vivo (Karakatsani et al., 2017). Embryonic cortical neurons from Lrp4^{mitt} mice had fewer but longer primary dendrites and transfection of agrin compensated the dendritic branching deficits in LRP4-deficient neurons (Handara et al., 2019).

Overall, our data point to functional links between LRP4 LDLα domain in modulating dendritic branching in developing CNS neurons. LRP4 LDLα domain promoted more dendritic branches formation. LRP4 LDLα domain binds with DKK1, Sclerostin, ApoE, Gremlin1, Wise. DKK1, Sclerostin and Wise are factors that inhibit Wnt signaling by binding LRP5/6 [20-22]. DKK1 mutation causes the phenotypes of a double ridge and polysyndactyly [23]. Mutation of Sclerostin results in sclerosteosis [24]. Moreover, LRP4 ECD enhances sclerostin-mediated inhibition of Wnt/β-catenin signaling [25]. LRP4 mutation increases serum sclerostin level in

osteocytes [26]. However, the significance of the LRP4 interaction with the Wnt signaling pathway in brain remains unclear [27]. LRP4 and Wise interaction is revealed to regulate the patterning and formation of gland development [28]. ApoE, as one of LRP4 ligands, is essential for the development of the nervous system, the regulation of synaptic plasticity, neuroprotection, and the innervation of the muscle [8]. LRP4 interaction with ApoE promotes Aβ uptake[29]. ApoE also interacts with Reelin [8] and inhibits Reelin boost dendritic arborization [30-33]. Therefore, we speculated that free LRP4 LDLα in mr-Lrp4^{mitt} mice may promote dendritic arborization by relieving the inhibition of ApoE on the Reelin.

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Abbreviations

LRP4: Low-density lipoprotein receptor-related protein 4; CNS: Central Nervous System; AChR: Acetylcholine receptor; NMJ: neuromuscular junction; ECD: extracellular domain; ICD：intracellular domain; ATP: Adenosine Triphosphate; DIV: Days in vitro; DMEM: dulbecco's modified eagle medium; PBS: phosphate buffered saline

Acknowledgements

We thank Dr. Baoming Li (Hangzhou Normal University), Dr. Bingxing Pan (Nanchang University) and Dr. Shangdong Liang (School of Basic Medical Sciences in Nanchang University) for assistance in this project. Thanks to Dr. Shiwen Luo for providing HEK293T cells line. Thanks to Dr. Haitao Wu for providing the LRP4 plasmids.

Authors' contributions

YM and WSQ initiated and designed the study. GAM and CP performed the Nissl's staining and Golgi staining. YM and JHY performed Cell culture and plasmid transfection. RDY, ZYZ, WYQ, FEK, ZSQ and LXS analyzed data. YM and WSQ wrote the manuscript with input from all coauthors. All authors read and approved the final manuscript.

Funding

This work was supported by grants from National Natural Science Foundation of China (81860242, 31460260, 31400936), Natural Science Foundation of Jiangxi Province (20171BAB205036, 160102).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experiments involving animals were conducted according to the "guidelines for the care and use of experimental animals" issued by Nanchang University. The Committee on the Ethics of Animal Experiments of the University of Nanchang approved the protocol.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Figure legend

Figure 1. LRP4 LDLα domain is necessary to keep body weight and survival rate

(A) Schematic diagram of Lrp4-LacZ and Lrp4-mitt; (B-C) Comparing with mr-Lrp4^{mitt} and the control mice, reduced survival rate in mr-Lrp4^{LacZ} mice (B), and reduced body weight of mr-Lrp4^{LacZ} mice(C); (D) Representative brain of the control，mr-Lrp4^{LacZ} and mr-Lrp4^{mitt} mice; (E) Brain weights of the control，mr-Lrp4^{LacZ} and mr-Lrp4^{mitt} mice. Values are means ± SEM. Mice number per group no less than 10. * $P < 0.05$, ** $P < 0.01$.

Figure 2. LRP4 LDLα domain affects the thickness of mouse cerebral cortex

(A) Representative images of Nissl's staining the cerebral cortex of the control，mr-Lrp4^{LacZ} and mr-Lrp4^{mitt} mice; (B) The thickness of the cerebral cortex in layer Ⅰ, Ⅱ/Ⅲ and Ⅳ decreased in mr-Lrp4^{LacZ} mice. Values are means ± SEM. Mice number per group no less than 5. * $P < 0.05$, ** $P < 0.01$.

Figure 3. LRP4 LDLα domain enhances the dendrite arborization of neurons in mr-Lrp4^{mitt} mice

(A) Representative images of Golgi staining the neurons in cerebral cortex of the control，mr-Lrp4^{LacZ} and mr-Lrp4^{mitt} mice; (B)The total dendrite length of neurons increased in mr-Lrp4^{mitt} mice; (C-D) The total dendrite branch number of neurons increased in mr-Lrp4^{mitt}

mice. Values are means ± SEM. Mice number per group no less than 3 and neuron number per group no less than 30. * $P < 0.05$, ** $P < 0.01$, # $P > 0.05$.

Figure 4. LRP4 LDLα domain increases the dendrites arborization in primary neurons

(A-B) pFLAG-CMV1-Lrp4-LDLα, pFLAG-CMV1-Reelin and pFLAG-CMV1 plasmid were transfected into HEK293T cells, then the conditioned medium was collected to add into primary cultured wild type mice neurons; (C) LRP4 LDLα domain improved the number of dendrites branches in primary neurons. Intersections of neuron dendrites branches were scored using Image J sholl analysis. Mice number per group no less than 6 and neuron number per group no less than 30. ** $P < 0.01$, ***$P < 0.001$.

Figure 5. LRP4 LDLα domain in astrocytes promotes dendrite arborization in primary co-cultured neurons

(A-B) Primary neurons of mr-Lrp4^{mitt} mice or wild type mice were co-cultured with astrocytes of mr-Lrp4^{mitt} mice or wild type mice; (C) Co-cultured with astrocytes from mr-Lrp4^{mitt} mice, neuronal dendrite branch number of wild type mice was more than co-cultured with astrocytes from wild type mice; Similar difference are present in the co-cultured neurons of mr-Lrp4^{mitt} mice with astrocytes from mr-Lrp4^{mitt} mice or from wild type mice. Intersections of neuron dendrites branches were scored using Image J sholl analysis. Mice number per group no less than 6 and neuron number per group no less than 30. ** $P < 0.01$.

Figures

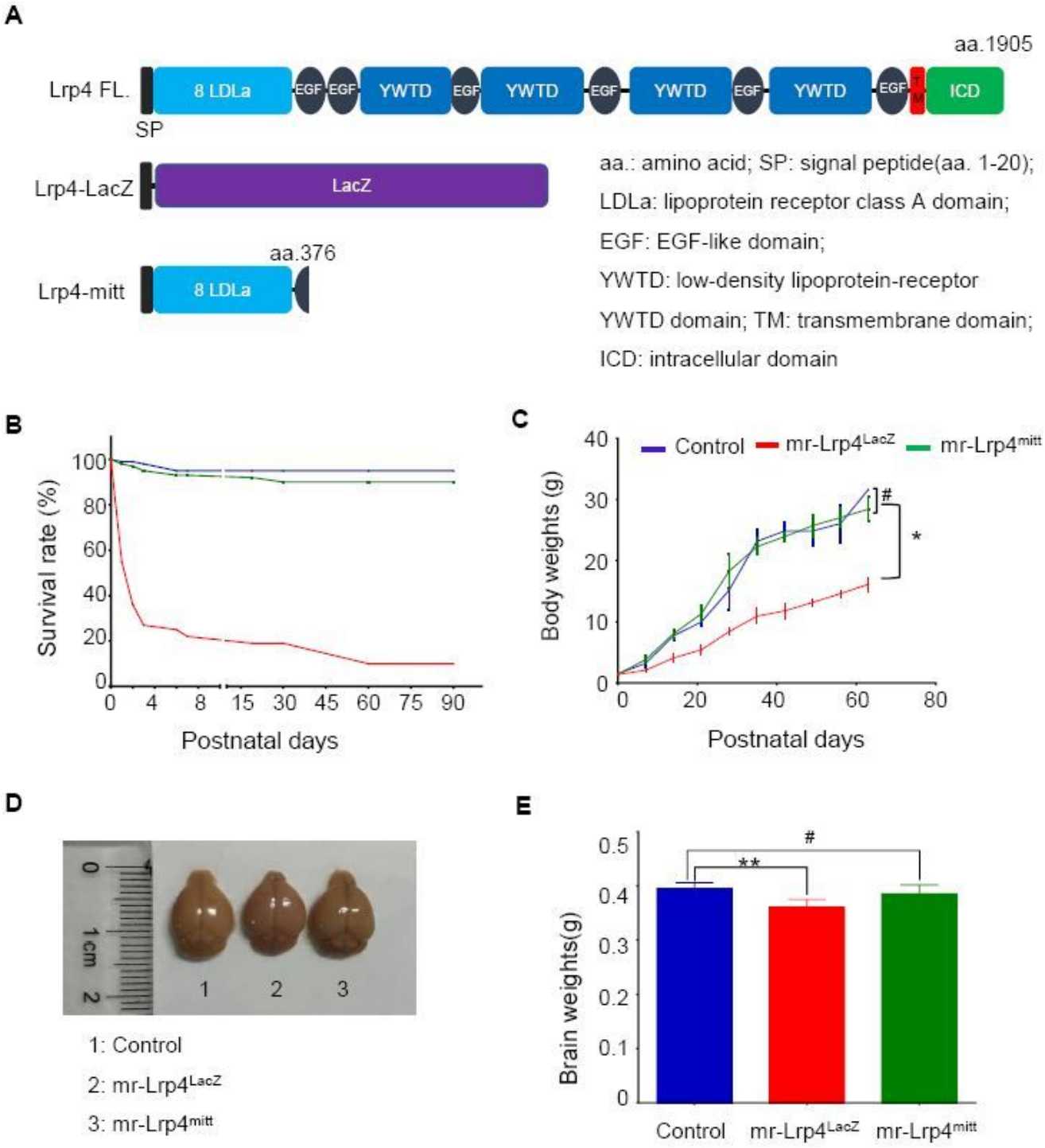

Figure 1

Figure 1

LRP4 LDLα domain is necessary to keep body weight and survival rate (A) Schematic diagram of Lrp4-LacZ and Lrp4-mitt; (B-C) Comparing with mr-Lrp4mitt and the control mice, reduced survival rate in mr-Lrp4LacZ mice (B), and reduced body weight of mr-Lrp4LacZ mice(C); (D) Representative brain of the

control□mr-Lrp4LacZ and mr-Lrp4mitt mice; (E) Brain weights of the control□mr-Lrp4LacZ and mr-Lrp4mitt mice. Values are means ± SEM. Mice number per group no less than 10. * P < 0.05, ** P < 0.01.

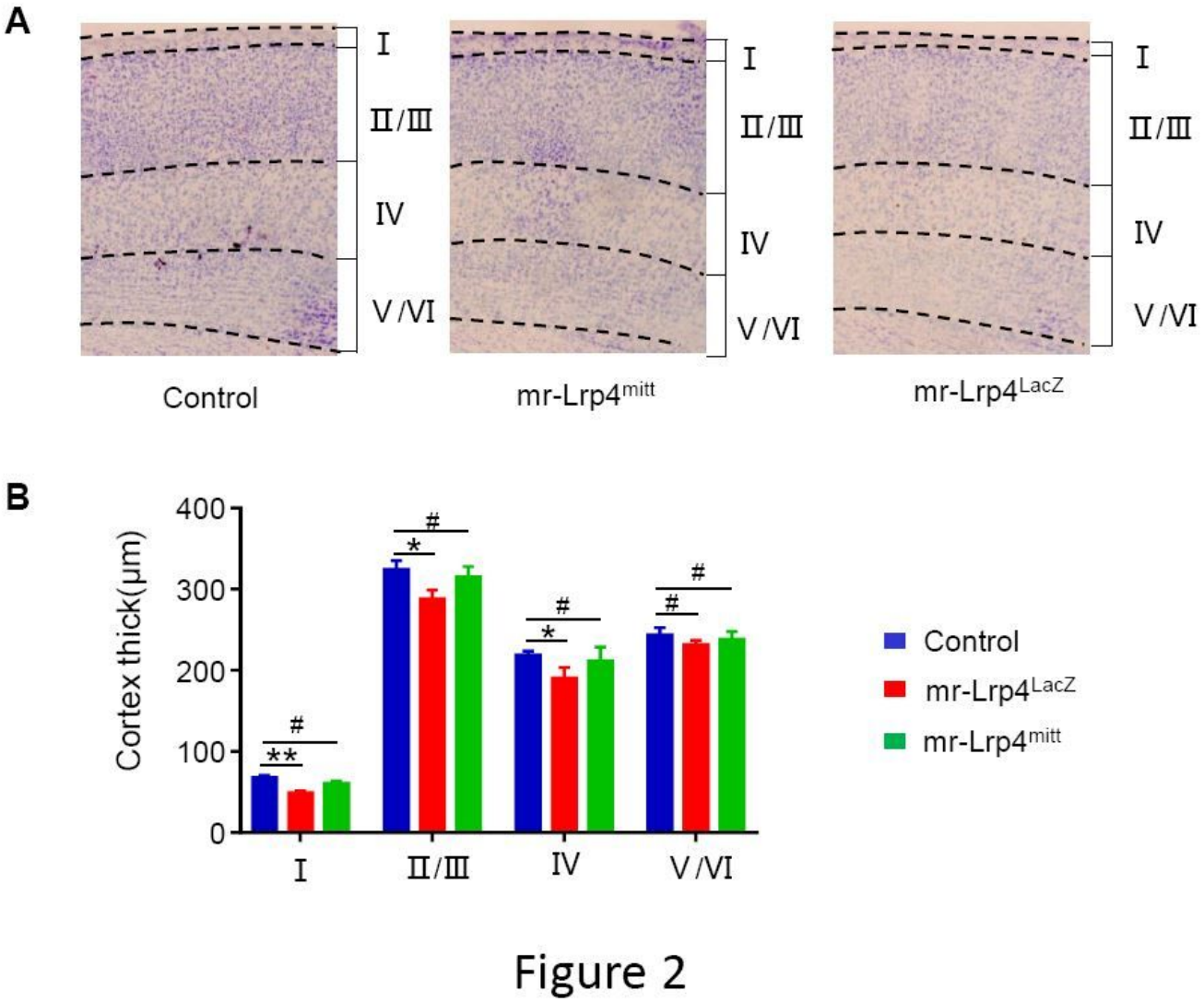

Figure 2

Figure 2

LRP4 LDLα domain affects the thickness of mouse cerebral cortex (A) Representative images of Nissl's staining the cerebral cortex of the control□mr-Lrp4LacZ and mr-Lrp4mitt mice; (B) The thickness of the cerebral cortex in layer □, □/□ and □ decreased in mr-Lrp4LacZ mice. Values are means ± SEM. Mice number per group no less than 5. * P < 0.05, ** P < 0.01.

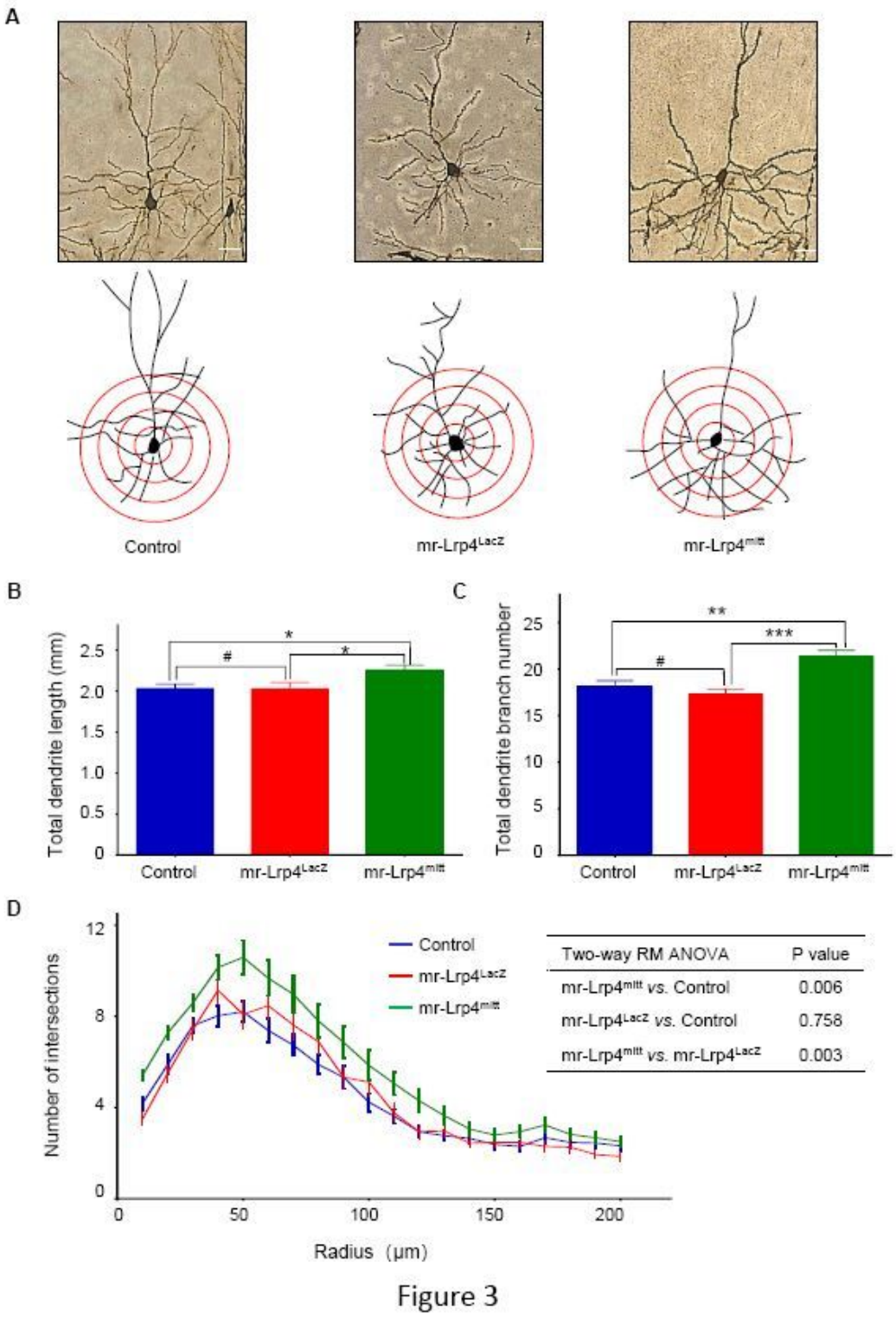

Figure 3

Figure 3

LRP4 LDLα domain enhances the dendrite arborization of neurons in mr-Lrp4mitt mice (A) Representative images of Golgi staining the neurons in cerebral cortex of the control mr-Lrp4LacZ and mr-Lrp4mitt mice; (B)The total dendrite length of neurons increased in mr-Lrp4mitt mice; (C-D) The total dendrite branch number of neurons increased in mr-Lrp4mitt mice. Values are means ± SEM. Mice number per group no less than 3 and neuron number per group no less than 30. * $P < 0.05$, ** $P < 0.01$, # $P > 0.05$.

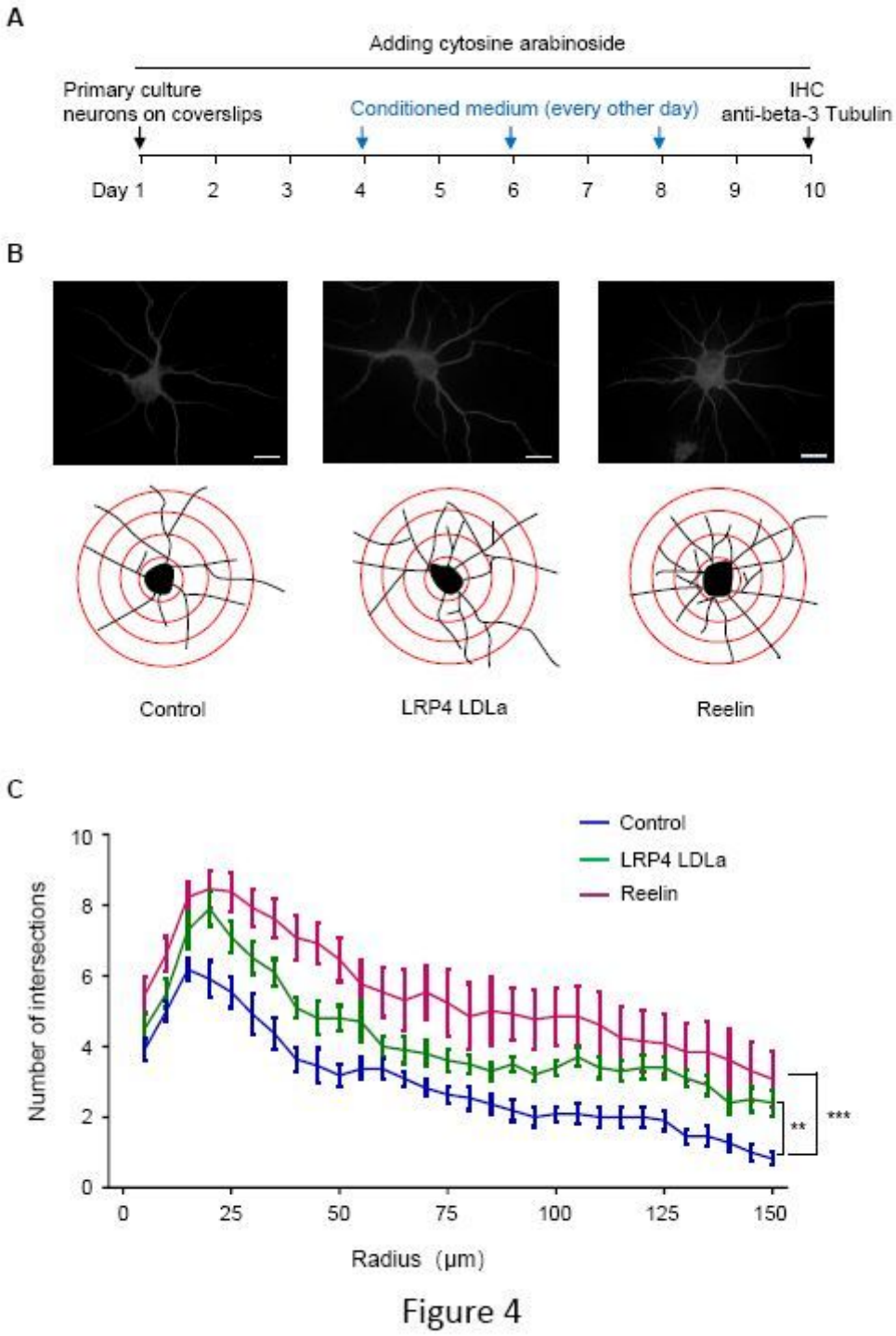

Figure 4

Figure 4

LRP4 LDLα domain increases the dendrites arborization in primary neurons (A-B) pFLAG-CMV1-Lrp4-LDLα, pFLAG-CMV1-Reelin and pFLAG-CMV1 plasmid were transfected into HEK293T cells, then the conditioned medium was collected to add into primary cultured wild type mice neurons; (C) LRP4 LDLα domain improved the number of dendrites branches in primary neurons. Intersections of neuron dendrites

branches were scored using Image J sholl analysis. Mice number per group no less than 6 and neuron number per group no less than 30. ** P < 0.01, ***P < 0.001.

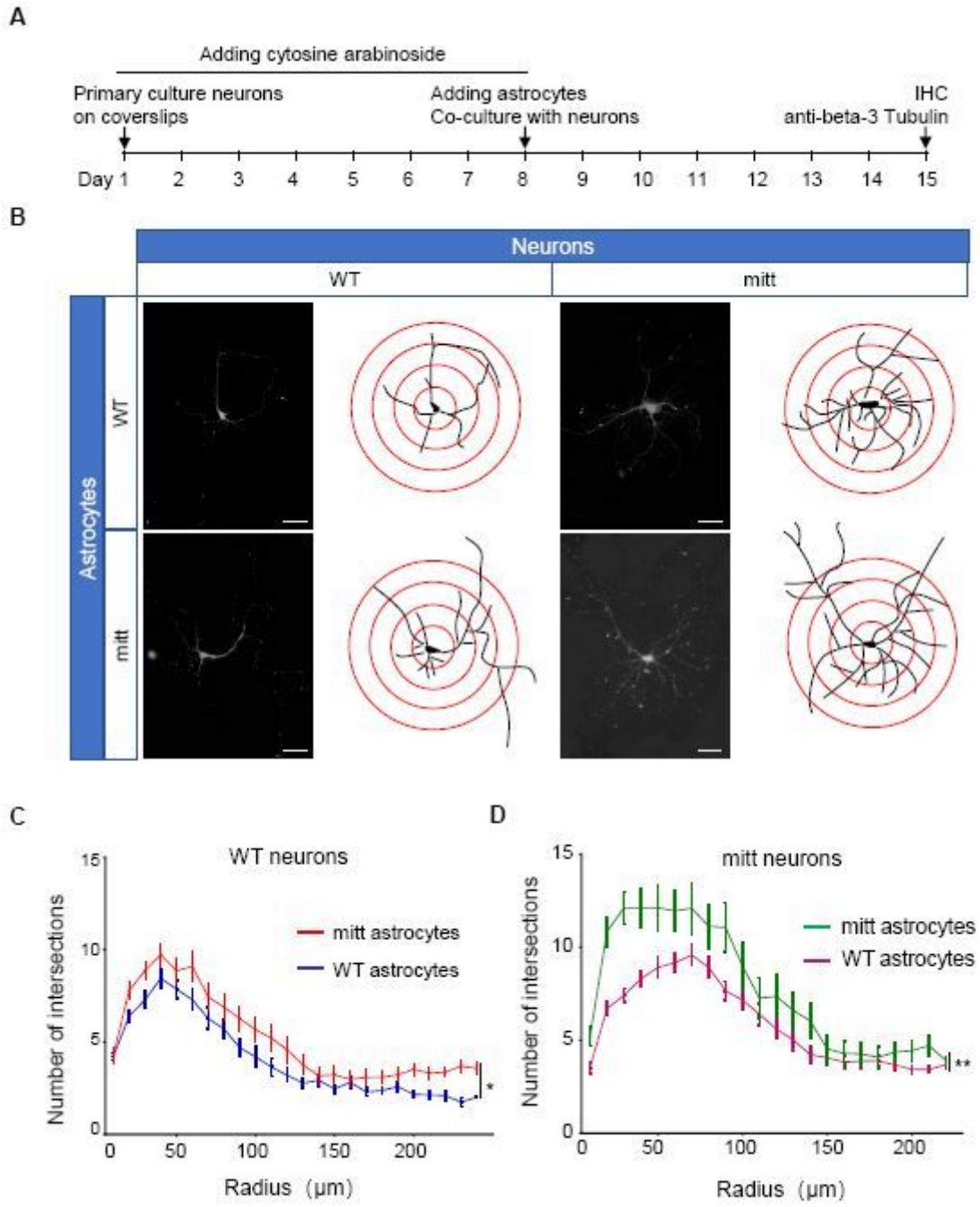

Figure 5

Figure 5

LRP4 LDLα domain in astrocytes promotes dendrite arborization in primary co-cultured neurons (A-B) Primary neurons of mr-Lrp4mitt mice or wild type mice were co-cultured with astrocytes of mr-Lrp4mitt mice or wild type mice; (C) Co-cultured with astrocytes from mr-Lrp4mitt mice, neuronal dendrite branch number of wild type mice was more than co-cultured with astrocytes from wild type mice; Similar

difference are present in the co-cultured neurons of mr-Lrp4mitt mice with astrocytes from mr-Lrp4mitt mice or from wild type mice. Intersections of neuron dendrites branches were scored using Image J sholl analysis. Mice number per group no less than 6 and neuron number per group no less than 30. ** $P < 0.01$.