Neurochemical Research (2019) 44:312–322
https://doi.org/10.1007/s11064-018-2676-7

ORIGINAL PAPER

MHC Class I Molecules and PirB Shape Neuronal Morphology by Affecting the Dendritic Arborization of Cortical Neurons

Yuqing Shen[1] · Huanhuan Zhao[1] · Ping Li[1] · Yaqin Peng[1] · Pengfei Cui[1] · Fengqin Miao[1] · Ying Zhang[1] · Aifeng Zhang[2] · Jianqiong Zhang[1,3]

Received: 8 June 2018 / Revised: 29 October 2018 / Accepted: 30 October 2018 / Published online: 8 November 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Neuronal MHC class I proteins have been previously reported to regulate synaptic plasticity. Several reports indicate MHC class I proteins are expressed early during development of the nervous system, suggesting they may also play a role in neuronal development. Using cultured cortical neurons, we show MHC class I proteins aggregate at specific sites in neuronal cell bodies, which overlap with the actin cytoskeleton. Knockout of MHC class I in cultured neurons increases total dendritic length and the number of branch points. These effects are abolished by reintroducing MHC class I expression. Similarly, blocking of MHC class I proteins or PirB by an MHCI antibody or a soluble PirB ectodomain respectively, mimics the knock out phenotype of increased dendritic branching. This effect is correlated with decreased phosphorylation of both LIMK and cofilin, suggesting it may be mediated by an induction of cofilin activity. Finally, layer II and III cortical neurons in the sensorimotor region of an MHC class I deficiency mouse model show increased dendritic growth and branching. Altogether, our results suggest MHC class I plays a role in inhibiting or limiting the degree of dendrite arborization during the development of cortical neurons.

Keywords MHC class I molecules · Dendrite arborization · Cortical neurons · Cofilin phosphorylation

Abbreviations

MHC Major histocompatibility complex
PirB Paired immunoglobulin-like receptor B

Introduction

During development neurons need to differentiate, extend and branch dendritic arbors to form a seamless synaptic integration within the nascent neuronal network. The final morphology of each neuron not only physically determines its field of reception, the unique shapes and patterning of neuronal arbors, but also physiologically and anatomically classifies individual neuron subtypes, ultimately defining the very nature of their function [1]. Both extrinsic and intrinsic mechanisms contribute to determining the unique organization of an individual neuron's dendritic arbor. A group of well-established extrinsic signals are known to specifically regulate the development of cortical pyramidal cell dendrites including Sema3 [2], Notch [3] and brain derived nerve growth factors (BDNF) [4].

After the first report that neuronal MHC class I proteins regulate synaptic plasticity in the lateral geniculate nucleus (LGN) during development [5], additional efforts have further validated the role of these proteins in activity-dependent refinement of synaptic plasticity [6–8]. Based on these findings, published studies indicate that blocking signaling pathways regulated by MHC class I proteins and their partners can reduce neurodegeneration caused by ischemic stroke [9–11] and Alzheimer's disease [12]. In previous work, we have found that expression of MHC class I in cultured hippocampal neurons appeared as early as 3 days in vitro (DIV) [13], a time when most neurons are

✉ Jianqiong Zhang
Zhjq@seu.edu.cn

1 Department of Microbiology and Immunology, Key Laboratory of Developmental Genes and Human Disease, Ministry of Education, Medical School, Southeast University, Nanjing, China

2 Department of Pathology, Medical School, Southeast University, Nanjing, China

3 Jiangsu Key Laboratory of Molecular and Functional Imaging, Medical School, Zhongda Hospital, Southeast University, Nanjing, China

in the process of extending neurites, far before the time of developing synapsis between neurons. We also found that in vivo expression of MHC class I was elevated as early as embryonic day E16.5 in cerebral cortex [14]. Taken together, these results suggest that in addition to their role in regulating synaptic plasticity, MHC class I proteins may participate in additional pathways including the regulation of early neuronal development.

Using a co-culture system of mouse embryonic retina explants with thalamic explants, shedding of soluble MHC class I (sMHC I) was demonstrated to inhibit the outgrowth of retinal neurites [15, 16]. A follow up study from the same group demonstrated sMHC I can also inhibit neurite outgrowth from embryonic dorsal root ganglia (DRG) in culture [17]. Additionally, others report that basal dendrites of L2/3 pyramidal cells exhibited more branching in H2-Db and Kb knockout (H2-Db/Kb KO) mice, with the functional outcome of increased connectivity with other neuron terminals [18]. However, the mechanism(s) underpinning how MHC class I dictates neuron morphologies or how MHC class I molecules affect neurite outgrowth in cultured cortical neurons has not been clearly described. In this study, we find that: (i) knockout of MHC class I can increase dendrite number and complexity in vivo and in vitro, and (ii) Blocking of MHC class I or PirB can increase cofilin activity by reducing the phosphorylation on ser 3. Our data suggest a partial molecular signaling cascade responsible for MHC class I regulated dendritic branching during cortical neuron development.

Materials and Methods

Animals

All animals used in this study were C57BL/6J mice and maintained under pathogen-free conditions in our animal facility. All animal experiments were carried out in accordance with the protocols evaluated and approved by Institutional Animal Care and Use Committee (IACUC) of the Medical School of Southeast University (approval ID: SYXK-2010.4987). Constitutive H2-Db/Kb KO mice were purchased from Taconic Bioscience (USA) and flow cytometry was used to confirm the Knockout of H-2K/D gene.

Lentivirus Preparation

The 1080 base pairs that were used for expressing H2-Db were subcloned into AgeI and EcoRI (NEB, USA) sites of FUGW-GFP vector (Clontech, USA) according to the manufacturer protocol. PCMV-dR8.9 and VSV-G (Clontech, USA) were used as helper plasmids. The lentivirus was generated by transfection of HEK 293T cells with FUGW or FUGW-H2-Db plasmid with the helper plasmid using Lipofectamine™ 2000 (Invitrogen, USA). 6–14 h after transfection, the culture medium was replaced with fresh DMEM (Gibco, USA), supplemented with 10% FBS (Gibco, USA) and 1/500 volume of ViralBoost™ (ALSTEM, LLC, USA). After 48 h, the supernatant was collected and filtered through 0.45 μm cellulose acetate filters. 1 volume of cold Lentivirus Precipitation Solution was added to the filtered supernatant (ALSTEM, LLC, USA) and refrigerated overnight. Higher titer stocks were obtained by discarding supernatant and resuspending the viral pellets with DMEM (Gibco, USA).

Primary Neuron Culture

Cortical neuronal cells were isolated from E16.5–18.5 fetus as previously described [19]. Primary Cortical neurons were grown in 6-well plates or on glass coverslips (coated with 100 μg/ml poly-D-lysine, Sigma-Aldrich, USA) in 24-well plates in Neurobasal media (Gibco, USA), supplemented with B27 (Invitrogen, USA), GlutaMAX (Invitrogen, USA) and D -glucose (Sigma-Aldrich, USA). Half volume of the media was changed after 3 DIV. For re-expression of H2-Db in H2-Db/Kb KO mice, neuronal cells were transfected with lentivirus at 1 DIV. For blocking of MHC class I molecules, neuronal cells were treated with anti-MHC I antibody (OX18, Abcam, USA) or isotype-matched mouse IgG control (Invitrogen, USA) at 3 DIV. For blocking of the ligand bound by PirB, neuronal cells were treated with soluble PirB ectodomains (sPirB, R&D systems, USA) and bovine serum albumin (BSA, AMRESCO, USA) at 3 DIV.

Real-Time Quantitive Polymerase Chain Reaction

Total RNA from cultured neurons was extracted using the Trizol protocol (Invitrogen, USA). One microgram of total RNA was reverse transcribed using the PrimeScript RT reagent kit with gDNA Eraser (Takara, Japan). Expression of mouse βIII-tubulin and PirB genes were analyzed using Power SYBR Green PCR Master Mix (Roche, Germany), with βIII-tubulin used as an internal control. The primers were described as before [10].

Golgi Impregnation

P30 female mice were euthanized with sodium pentobarbital and the brains were quickly removed. Golgi impregnation of 200 μm sagittal sections was stained by using the FD Rapid GolgiStain kit (FD Neuro Technologies, USA) according to the manufacturer protocol. Briefly, the brain slices were immersed in the initial impregnation solution in darkness at

room temperature. After 2 weeks of incubation, the slices were transferred into solution C and incubated for 5 days at 4 °C. Slices were then washed in ddH_2O and transferred into a freshly prepared mixture of solutions D and E. After dehydration through graded ethanol, slices were cleared in xylene solution and mounted with Permount Mounting Medium (Fisher Scientific, USA).

Immunofluorescence Staining

Reagent used in immunofluorescence experiments were: mouse anti-MHC I (OX18, 1:100, Abcam, USA), mouse anti-microtubule-associated protein2 (MAP2, 1:1000, Abcam, USA), TRITC-Phalloidin (1:1000, Sigma-Aldrich, USA), DAPI (1:1000, Sigma-Aldrich, USA) and secondary antibodies conjugated with Alexa Fluor 488 (1:400, Invitrogen, USA). Coverslips were fixed by 4% paraformaldehyde (Sigma Aldrich, USA) in 0.01 M PBS with 4% sucrose for 20 min. Cells were rinsed in PBS, permeabilized with 0.25% Triton X-100 (Sigma-Aldrich, USA) for 15 min and washed three times with PBS. Cells were then blocked for 11 h with 10% BSA in PBS and incubated with primary antibodies overnight at 4 °C in a humid chamber. After rinsing three times in PBS, cells were incubated with a secondary antibody for 1 h in a humid chamber at room temperature. Cells were then rinsed with PBS and mounted on slides in Prolong Gold Anti-fade Reagent (Invitrogen, USA). The coverslips were observed with a fluorescence microscope (Olympus Fluoview FV 1000, Japan).

Sholl Analysis

For the Sholl analysis, concentric circles with an increasing radius (10 μ increments) were placed around the cell body. The number of intersections of the dendrites and the concentric circles per radial distance from the soma were quantified. Briefly, 8-bit images of cultured neurons were traced using the NeuronJ software (USA) and tracing files were generated. The data were organized and analyzed.

Western Blotting Analysis

The whole-cell lysates were lysed by protein lysis buffer (50 mM Tris–Cl PH7.4, 150 mM NaCl, 1% NP-40, 0.5% Sodium deoxycholate, 0.1% SDS) with protease inhibitor and phosphatase inhibitor (Roche, USA). The concentration of protein was quantified by the BCA protein assay kit (Pierce, USA). Equal amounts of protein (30–40 μg) were denatured at 100 °C for 5 min in a protein sample buffer, separated by SDS-PAGE and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). Membranes were incubated in 5% BSA (AMRESCO, USA) in Tris-buffered saline with 0.05% Tween 20 (Sigma-Aldrich, USA) (TBST buffer) at room temperature for 2 h to block nonspecific binding, and then probed with primary antibodies overnight at 4 °C. The primary antibodies and concentrations used were described as follows: anti-cofilin and phospho-Ser3 cofilin (1:1000, Cell Signaling, USA), anti-LIMK1 and phospho-LIMK1 (1:1000, Bioworld, USA), anti-GFP (1:2500, Santa Cruz Biotechnology, USA). The membrane was then washed with TBST and incubated with appropriate peroxidase-conjugated secondary antibody for 1 h at room temperature. Immunodetection was performed with an enhanced chemilumi-nescent (ECL) substrate (Pierce, USA). The signals were imaged with a luminoimage analyzer (Tanon, China) and quantification was performed by densitometry using Image J software (USA).

Statistics

For each treatment, around 10–25 cells were randomly selected, measured, and the number was averaged. Three or more independent experiments were performed. Data are presented as mean ± standard error of the mean (SEM). Statistical significance was determined using ANOVA or two-tailed *t*-tests as indicated. Probability outcomes are summarized in graphs as follows: $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Results

MHC Class I Co-localizes with F-Actin in Neuronal Growth Cones

First, we studied the cellular distribution of MHC class I molecules during the development of cultured neurons. MHC class I molecules were observed to co-localize with sites enriched for F-actin at 2, 4 and 7 DIV. During the earliest stage (2 DIV), MHC class I immunoreactivity was distributed in patches along the cell periphery and along the initial budding and protrusions of neurites (Fig. 1a). During neurite growth, MHC class I immunoreactivity was observed in the soma and also concentrated at the neurite tip (4 DIV, Fig. 1b). As long as the neurites continue to extend, growth cones were highly labeled with MHC class I molecules (7 DIV, Fig. 1c). The accumulation of MHC class I overlapped with the F-actin-rich regions as indicated by Phalloidin labeling. The pattern of MHC class I expression in cultured neurons suggest their potential involvement in regulating neurite outgrowth and pathfinding.

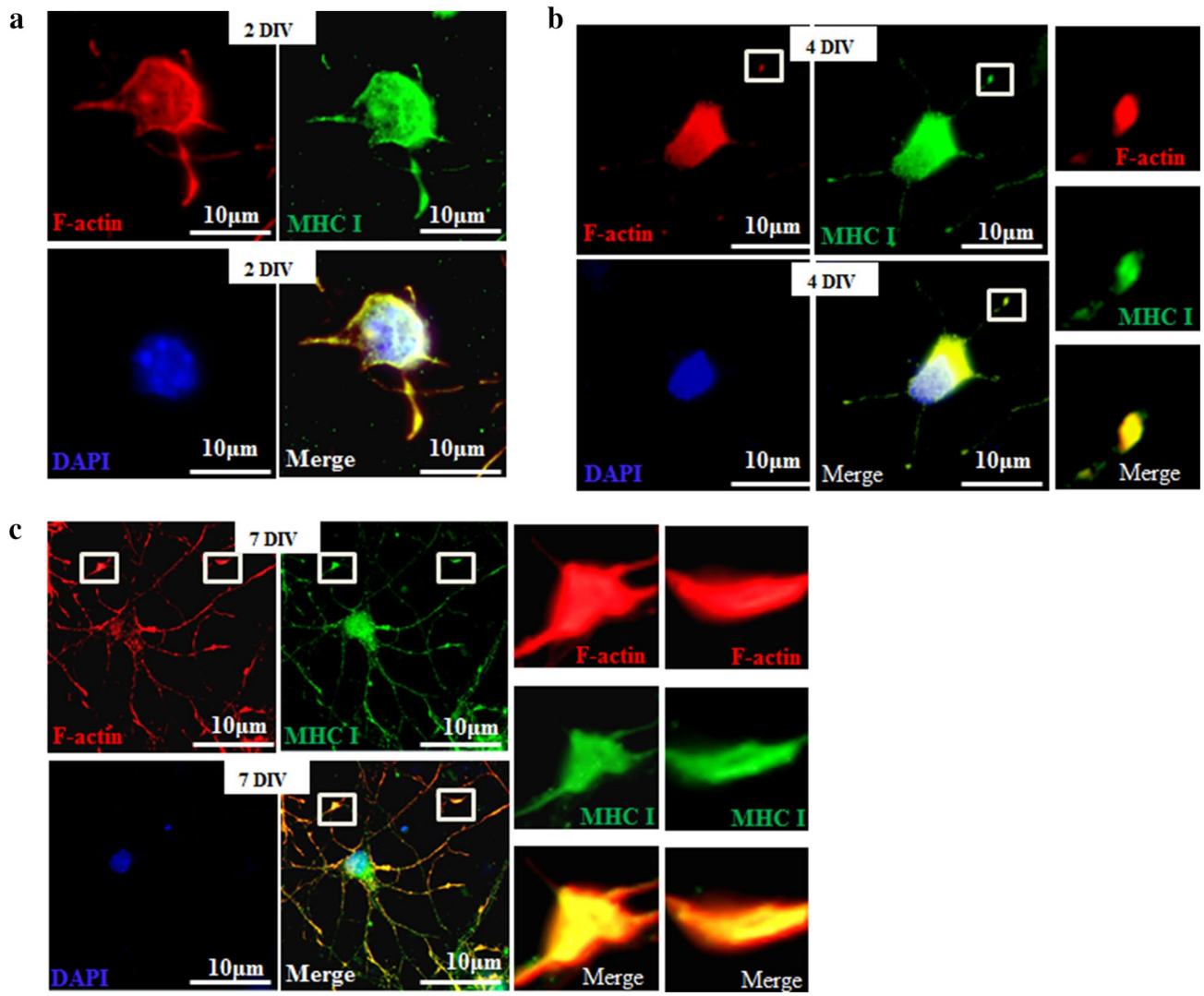

Fig. 1 MHC class I molecules colocalized with filopodia and neurite tip in cultured cortical neurons. Double immunolabeling of MHC (green) and F-actin (red) at 2 DIV (days in vitro, **a**), 4 DIV (**b**) and 7 DIV (**c**) were detected in cultured cortical neurons. MHC class I immunoreactivity is distributed in patches at the cell periphery and protrusions (2 DIV). During neurite growth (4 DIV), MHC class I immunoreactivity was shown in cell body and at the neurite tip (shown by magnification of the boxed region). At 7 DIV, MHC class I immunoreactivity was shown in cell body and along the process, where it is concentrated at or close to the neurite tips (shown by magnification of the boxed region). Bars: 10 μm. (Color figure online)

Neurons Cultured from H2-Db/Kb KO Cortices Exhibit Increased Dendritic Complexity

Based on the observation MHC class I was targeted to discrete actin-rich sites, we investigated whether knockdown of MHC class I would affect neurite outgrowth. Neuronal morphology was examined in cultured cortical neurons of WT and H2-Db/Kb KO mice. As compared to WT neurons, KO neurons were observed to have both increased total dendritic length and increased number of branch points as measured by Sholl analysis (Fig. 2).

To study the gain-of-function phenotype of MHC class I molecules on dendritic formation, H2-Db was overexpressed in KO neurons by lentivirus starting at 1 DIV. The exogenous expression of H2-Db-GFP fusion protein was monitored by western blot (Fig. 3a). Compared with empty vector control, H2-Db overexpression decreased total dendritic length as well as the number of branch points (Fig. 3b–d). Collectively, these observations suggest that MHC class I plays a role in restricting dendrite formation.

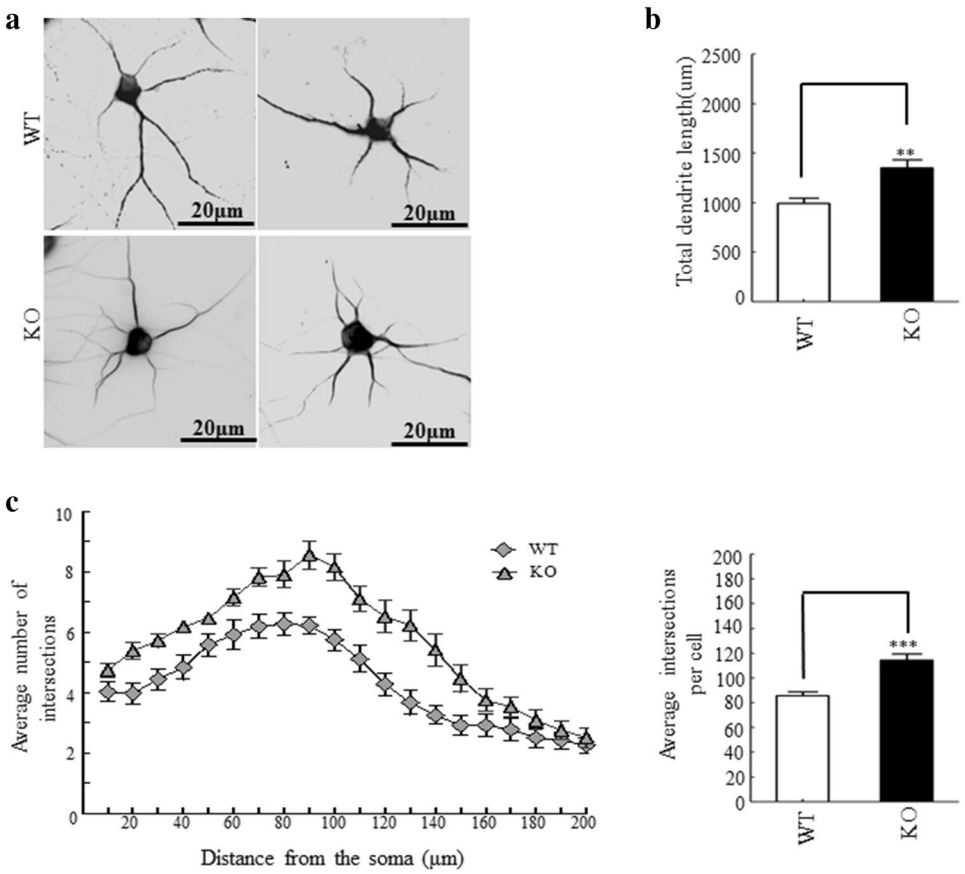

Fig. 2 Loss of MHC class I molecules led to enhanced neurite growth and branching. **a** Cultured cortical neurons from WT or H2-Kb/Db knockout (KO) mouse brain were fixed and immunostained for MAP2. KO neurons showed a higher number of neurites than WT neurons. Two representative figures are shown in each condition. Bars: 20 μm. **b** Quantification of neurite length ($p = 0.007$). **c** Sholl analysis was performed on these neurons. Sholl profile and the average total numbers of intersections per cell were shown ($n = 20$ WT and 20 KO cells from three independent animals, respectively; $p = 0.0002$)

Neurite Branching is Promoted with Acute Functional Blockade of MHCI or PirB

PirB was discovered in a search for receptors that are known to bind MHCI proteins in the immune system. It was also determined to be a receptor for neuronal MHCI. In order to make sure PirB is expressed in cultured neurons at 3 DIV when we applied sPirB, we performed real-time PCR to compare the expression level at different cultured days. As shown in Fig. 4a, the expression of PirB decreased as the culture time extended. Although it is low, it is still detectable at 3 DIV and this expression level is higher than that of 7 DIV. To study the role of MHC class I and PirB in the contest of neurite outgrowth, either a polyclonal MHC class I antibody (OX18, 1 μg/ml) or a soluble PirB ectodomain (0.5 μg/ml) were added to the culture medium at an early stage of neuron development (3 DIV). As shown in Figs. 5 and 4b–d, neurons treated with either MHC class I antibody or PirB ectodomain exhibited a dramatic increase in dendritic arborization. We detected a marked increase in dendrite length and also a substantial enhancement in the number of dendritic intersections, as revealed by Sholl analysis.

Functional Blockade of Either MHCI or PirB Results in Decreased Phosphorylation of Cofilin

The ADF/cofilin family of actin binding proteins is an important regulator of dendritic complexity. Therefore, we investigated whether increased arborization of neurons by inhibition of MHC class I or PirB was accompanied by a change in cofilin activity. Phosphorylation at Ser 3, which causes reduced cofilin activity, was decreased either in the neurons treated with the MHC class I antibody or PirB ectodomain (Fig. 6). At the same time, the protein kinase LIMK that phosphorylates cofilin at ser 3, also showed reduced phosphorylation. These results imply MHC class I or PirB mediated effects on branching may be mediated by an reduction of cofilin activity.

MHC Class I Deficiency in Cortical and Hippocampal Neurons Increases Dendritic Growth and Branching in the Postnatal Cerebral Cortex

We investigated if MHC class I can also affect the development of neurites in vivo. Dendritic branching of layer II and III pyramid neurons in the sensorimotor cortex and neurons

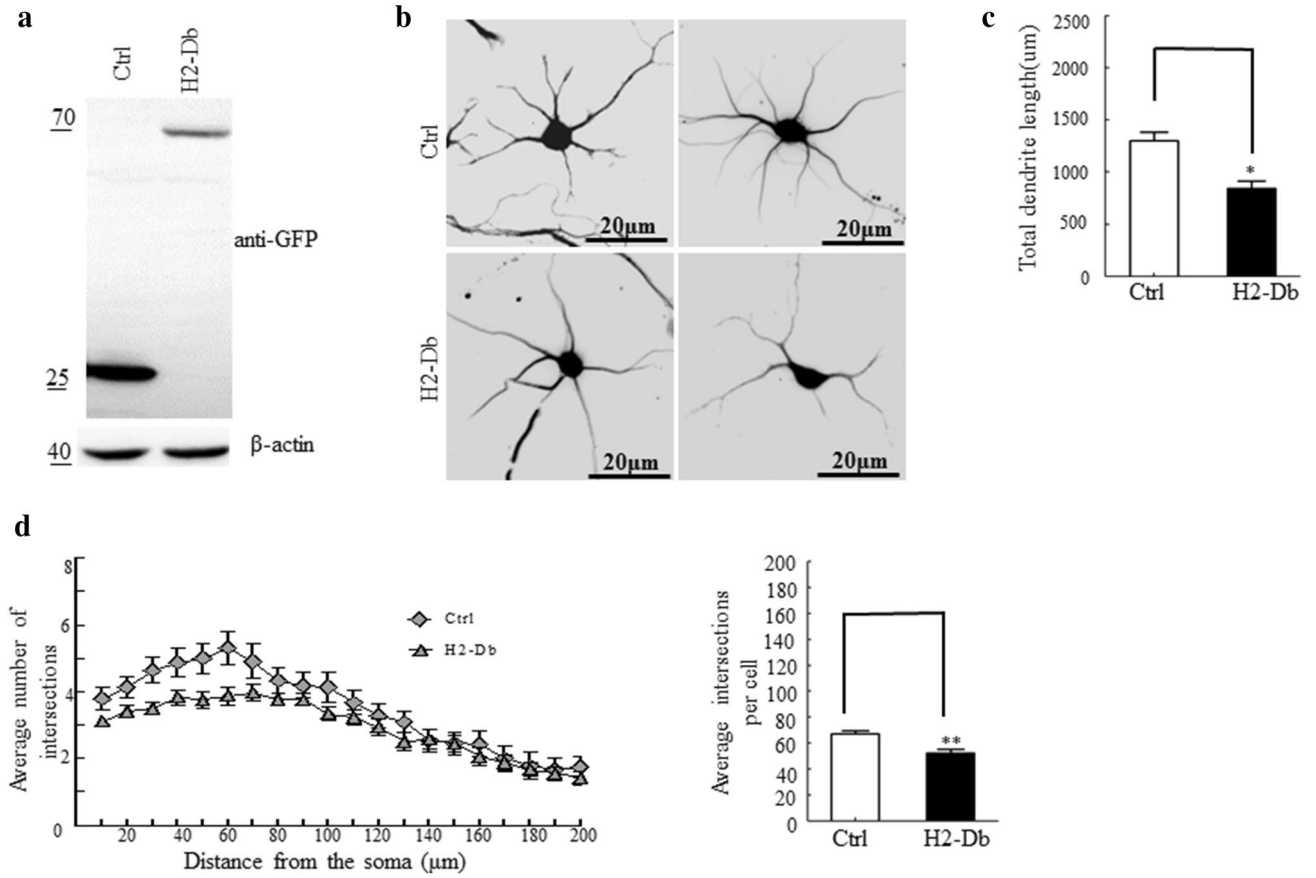

Fig. 3 Enhanced expression of MHC class I molecules decreased neurite growth and branching. **a** Overexpression of H2-Db in neurons was achieved using lentivirus delivery. Cell lysates were immunoblotted with GFP and actin antibodies to confirm the expression of H2-Db. Bars: 20 μm. **b** Cortical neurons were fixed and immunostained for MAP2. Two representative figures are shown in control and H2-Db overexpression neurons. **c** Quantification of neurite length ($p = 0.0121$). **d** Sholl analysis was performed on these neurons. Sholl profile and the average total numbers of intersections per cell were shown ($n = 20$ Ctrl and 25 H2-Db overexpression cells from three independent experiments, respectively; $p = 0.0051$)

in hippocampus of P30 mice was analyzed using Golgi–Cox staining. Dendritic branching complexity was assessed by reconstructing individual pyramidal cells for Sholl analysis. As shown in Figs. 7 and 8, both dendrite length and branch complexity are increased in H2-Db/Kb KO neurons ($p < 0.0001$).

Discussion

The function of MHC class I molecules in synaptic remodeling and plasticity have been described in many papers. It was reported that MHC I are required and sufficient for proper formation of precise connections between retina and LGN [20]. In this study, we investigated the effect of MHC class I molecules on dendrite formation in cultured cortical neurons. Based on our previous observation, the presence of MHC class I molecules appeared early in cultured hippocampal neurons [13]. We found that cultured cortical neurons showed early MHC class I expression at 2 DIV, well before synaptogenesis. In addition, MHC class I was observed to co-localize with F-actin at dendritic growth cones, suggesting a role in regulating neurite outgrowth. Neurons from H2-Kb/Db KO mice showed increased dendrite formation while re-expression of H2-Db reversed this phenotype. Taken together, these results detail that in addition to their roles in the regression of synapses, MHC class I can regulate early neuronal morphogenesis by inhibition of the dendrite formation.

Both extracellular and intracellular signals can regulate dendritic growth, such as neuotrophins, semaphorins, Notch and some cell adhesion molecules. sMHC I

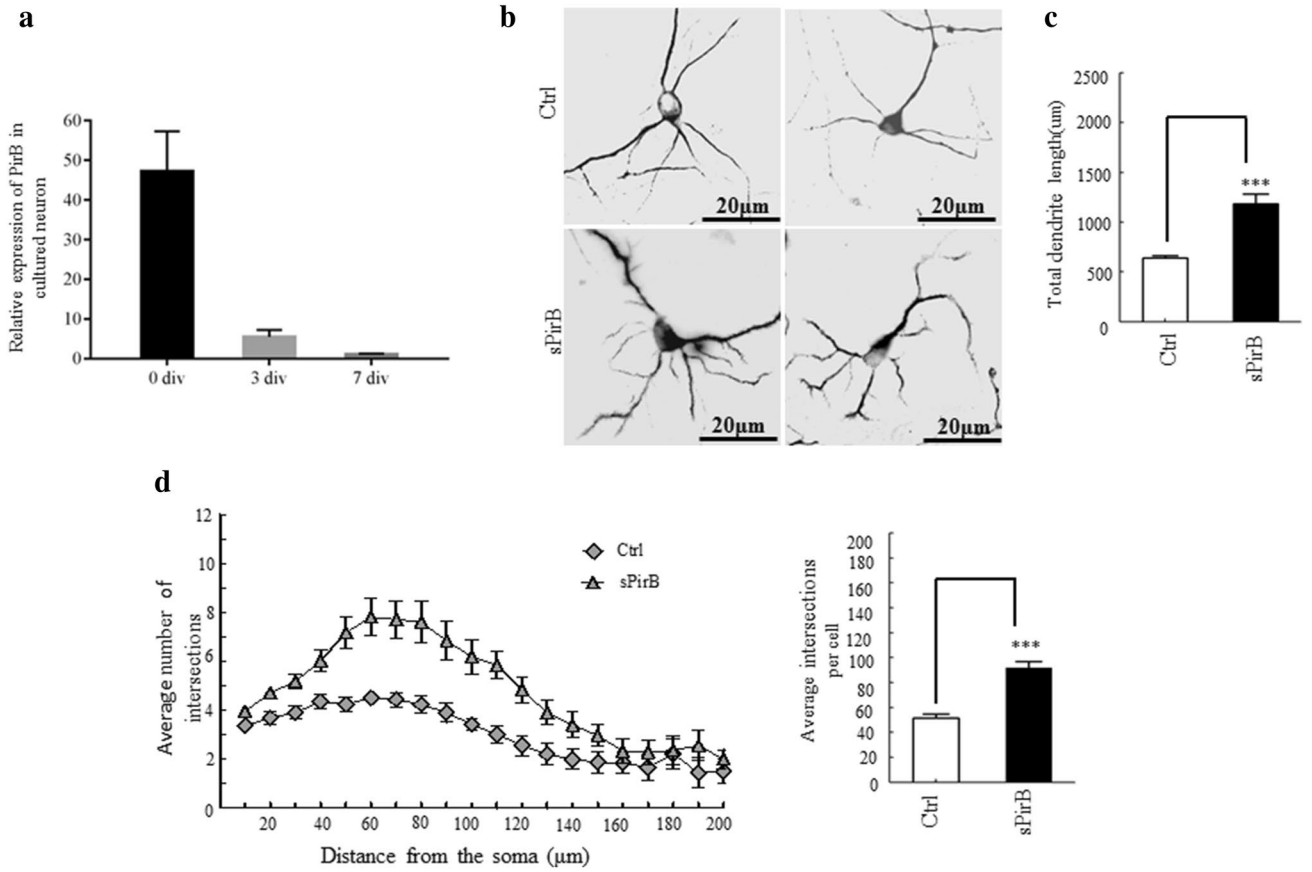

Fig. 4 Blocking PirB signaling by PirB ectodomain increased neurite growth and branching. **a** Differences in mRNA expression levels of PirB were determined by real-time PCR. The expression levels were normalized to that of 7 DIV neurons. **b** A soluble PirB ectodomain was added into the culture medium at 3 DIV. Neuron morphology was monitored by MAP2 signals at 5 DIV. Two representative figures are shown in each condition. Bars: 20 μm. **c** Quantification of neurite length ($p = 0.0002$). **d** Sholl analysis was performed on these neurons. Sholl profile and the average total numbers of intersections per cell were shown ($n = 17$ vehicles and 20 sPirB treated cells from three independent experiments, respectively, $p < 0.0001$)

molecules have been reported to be another extracellular signals that can decrease neurite outgrowth from retina and thalamic tissue culture. sMHC I are produced either by shedding from intact MHC I molecules or by alternative splicing of its heavy chain. However, in our cultured medium, we were unable to detect sMHC I (unpublished data), which suggests the inhibitory effect was driven by membrane bound MHC I and not by sMHC I. The binding partner of membrane bound MHC I in the central nervous system has not been completely defined. PirB was known to bind MHC I in forebrain neurons and PirB KO mice had the same phenotype as MHC I KO in regulating visual cortical plasticity [21], therefore PirB may well be one of the receptors of neuronal MHC class I molecules [22]. Acute blockade of PirB was shown to trigger the formation of new functional synapses of layer V pyramidal neurons [23] and PirB can negatively regulate spine density, stability and the threshold for adult ocular dominance plasticity [24], suggesting that PirB is involved in the pathway of MHC I regulated synaptic plasticity. In this study, we did not detect PirB expression by immunofluorescent staining, either due to the low level of PirB expression or due to antibody we used not recognizing the epitope displayed by neurons. However,

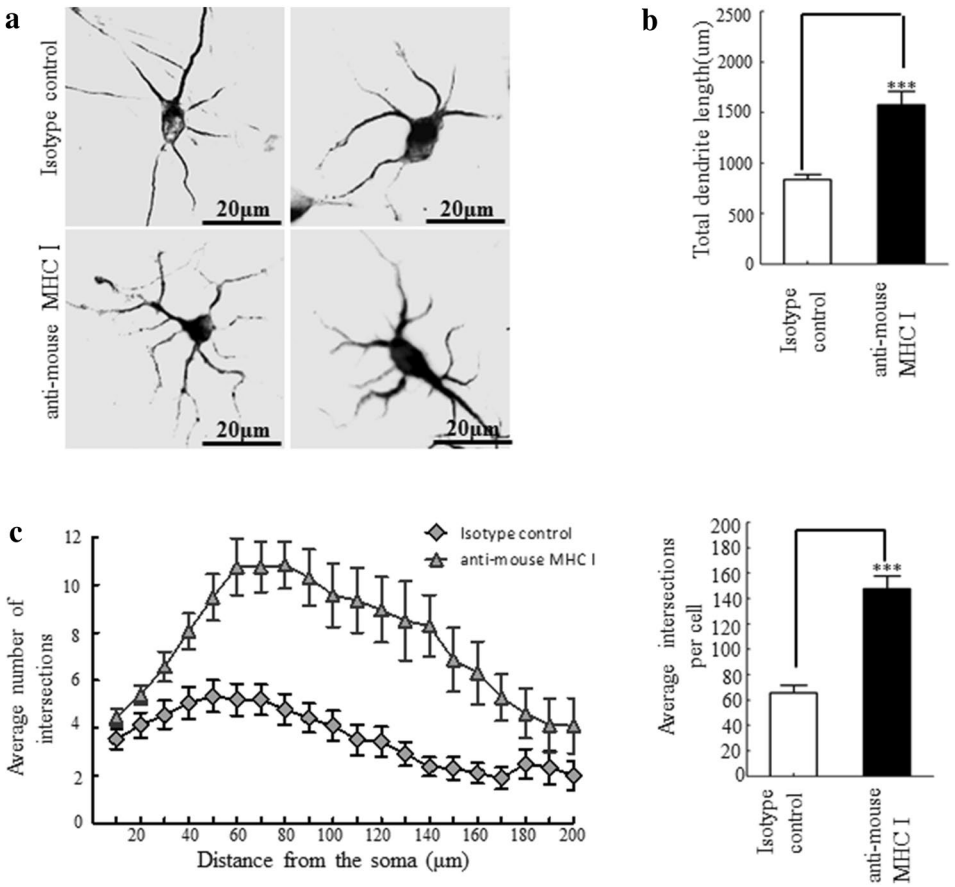

Fig. 5 Blocking MHC class I molecules signaling by anti-mouse MHC class I antibody increased neurite growth and branching. **a** A polyclonal MHC class I antibody was added into the culture medium at 3 DIV. Neuron morphology was monitored by MAP2 signals at 5 DIV. Two representative figures are shown in each condition. Bars: 20 μm. **b** Quantification of neurite length ($p < 0.0001$). **c** Sholl analysis was performed on these neurons. Sholl profile and the average total numbers of intersections per cell were shown ($n = 20$ Isotype control and 22 anti-mouse MHC class I antibody treated cells from three independent experiments, respectively; $p < 0.0001$)

when using anti-MHC class I antibodies or a PirB extracellular peptide to block MHC class I or ligand bound by PirB, neurons showed increased dendrite formation and arborization, suggesting the possible role of MHC class I and PirB signaling on inhibition of neurite growth. Based on this result, we are unable to confirm whether PirB is the partner of MHC class I on the neuron or exclude any other molecules that might play a role in this interaction. Another candidate molecule that can transfer the signal from MHC class I to intracellular molecules is CD3ζ, which has been reported to regulate dendrite formation in cultured neurons [25]. Nevertheless, no membrane receptors containing CD3ζ have yet been reported in neurons. Future studies will need to investigate the binding partners of MHC class I molecules that is required for regulating neurite outgrowth.

Dendrite branching is a dynamic process beginning with the extension and contraction of filopodia. Dendrites form when actin filaments are destabilized and microtubules invade the filopodia [26]. This process is regulated by a host of cellular factors, including microtubule regulatory proteins. Cofilin is a regulator of actin polymeration, which has been reported to promote dendritic development in cortical neurons [27]. The function of cofilin is negatively regulated by phosphorylation of Ser-3 which is mediated by LIMK1/2 [28]. In this study, we show cofilin activity is decreased by MHC I or PirB in cultured cortical neurons. Blocking MHC I or PirB by anti-MHC class I antibody or PirB ectodomain resulted in activation of cofilin through decreased Ser-3 phosphorylation by LIMK1. At this point it remains unclear whether members of the RhoGTPase family, described as the upstream regulator of LIMK1/2, are also under the control of MHCI or PirB.

MHC class I molecules not only regulate dendrite formation in cultured neurons, but also affect neuron morphology in vivo. As shown by our group and by others,

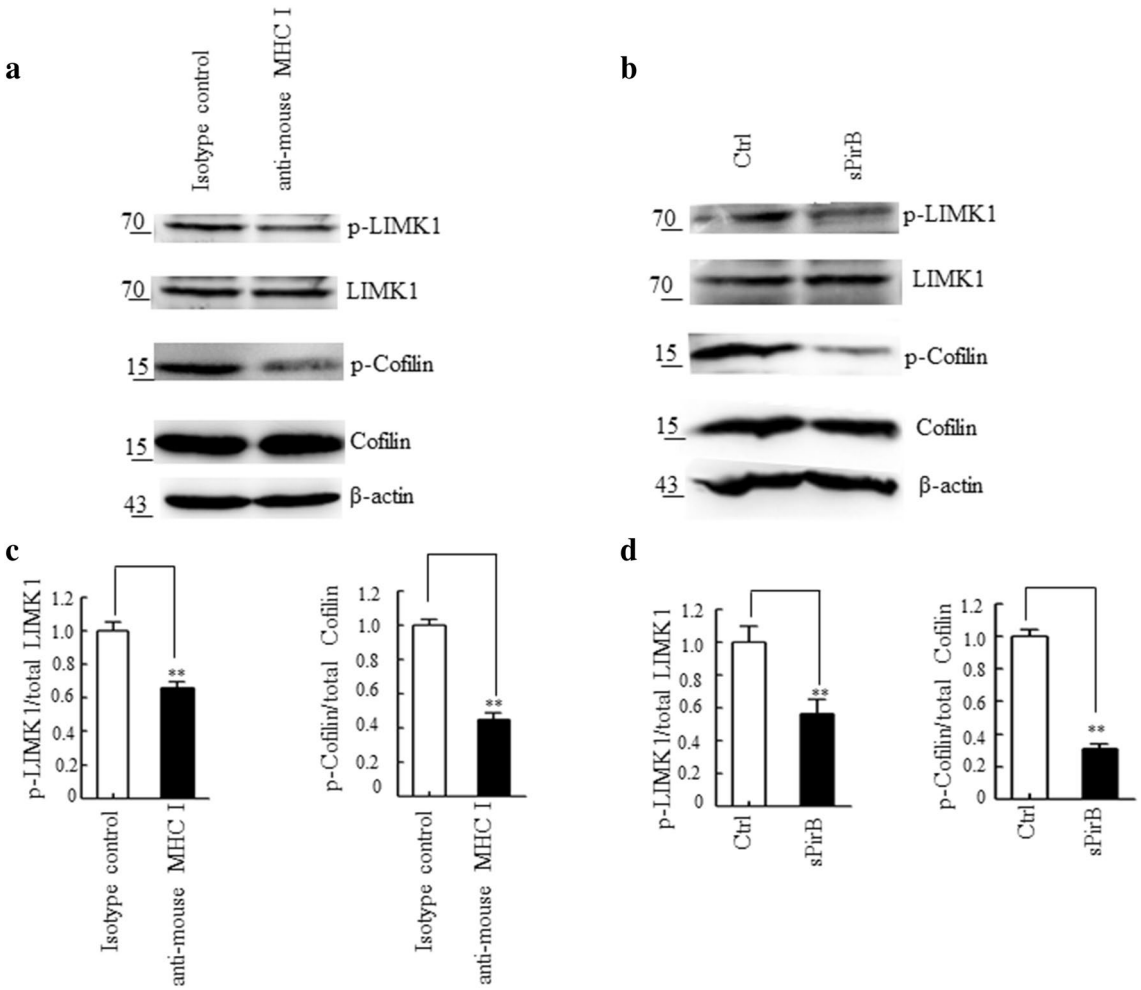

Fig. 6 Functional blockade of either MHC I or PirB resulted in decreased phosphorylation of cofilin. **a** A polyclonal MHC class I antibody and **c** a soluble PirB ectodomain was added into the culture medium at 3 DIV and cell lysate was collected at 48 h later. The level of indicated proteins in neuron cells was determined by immunoblot. **b** and **d** Quantification of specific phospho-cofilin and phosphor-LIMK levels from Western blot analysis. Phospho-cofilin/total cofilin values and Phospho-LIMK1/total LIMK1 values were calculated relative to the average value for controls [data from three independent experiments, $p = 0.0073$ and $p = 0.0042$ (**b**), $p = 0.0097$ and $p = 0.0054$ (**d**)]

pyramidal cells in both cortex and hippocampus have more complex dendrites in H2-Kb/Db KO mice. Dendrites are at the receive endo of the synaptic flow of information, and dendritic development is determined in part by the amount of information receiving by each neuron. Consequently, defects in dendritic development often accompany neurodevelopmental disorders such as autism and mental retardation [29], to name a few. Other neuro-physiological conditions, such as Schizophrenia are genetically linked to genes in the MHC class I region [30]. Whether changes of MHC class I can be involved in those diseases by changing of neuron connectivity through shaping of dendritic morphology requires further investigation.

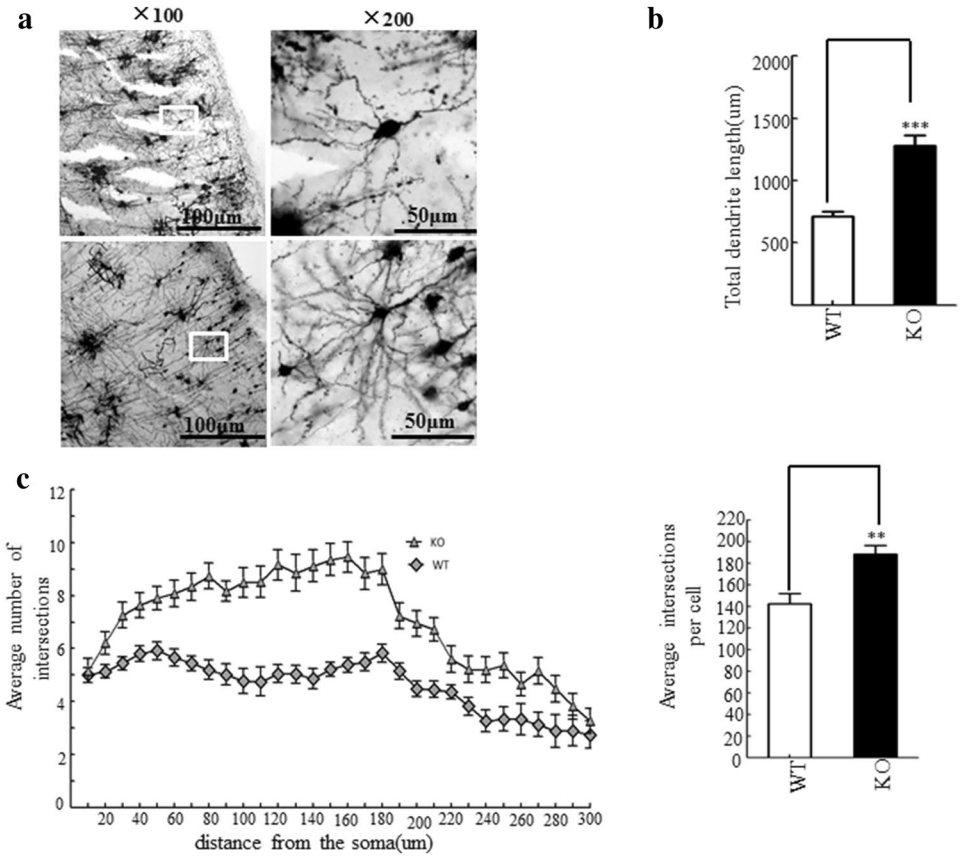

Fig. 7 Loss of MHC class I molecules increased dendritic growth and branching in upper layer pyramid neurons in P30 sensorimotor cortex. **a** Cell morphology of layer II and III pyramid neurons in the sensorimotor cortex of P30 WT or H2-Kb/Db knockout (KO) mouse were checked by Golgi–Cox staining. Bars: 100 μm. (Neuron in the boxed region were shown at higher magnification, bars: 50 μm). **b** Total length of basal and apical dendrites of pyramid neurons was quantified ($p < 0.0001$). **c** Sholl analysis was performed on these neurons. Sholl profile and the average total numbers of intersections per cell were shown. ($n = 18$ WT and 20 KO cells from three and four independent animals, respectively; $p = 0.0011$)

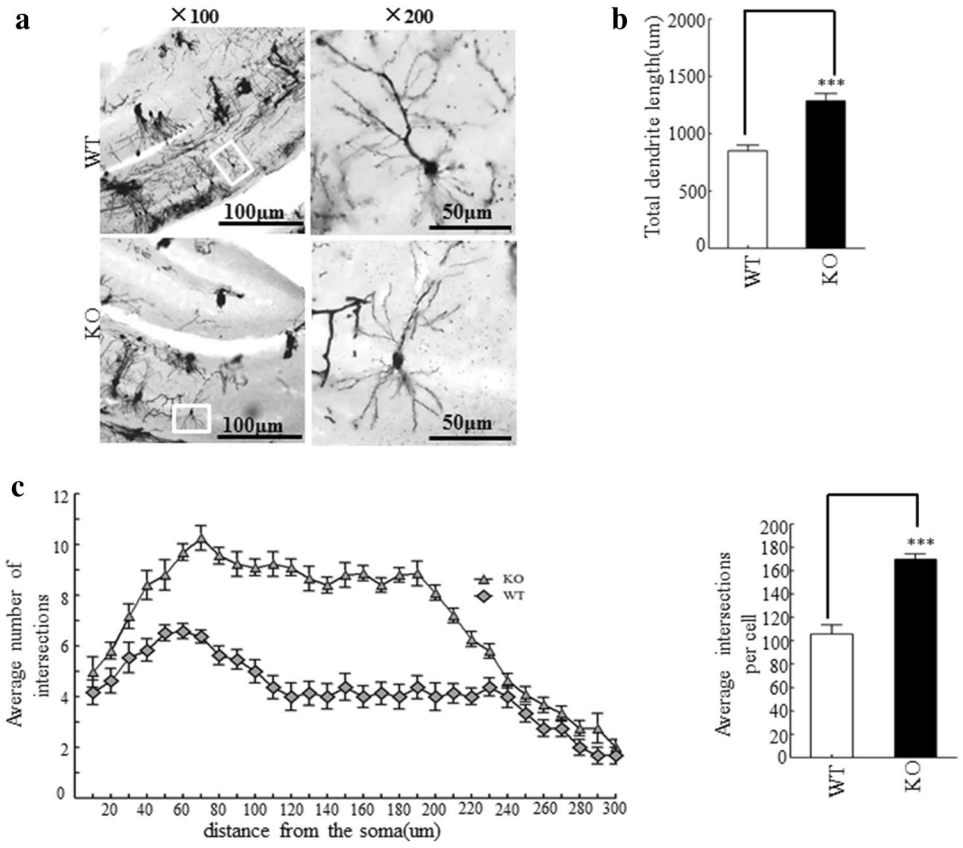

Fig. 8 Loss of MHC class I molecules increased dendritic growth and branching in P30 hippocampal area CA1. **a** Cell morphology of hippocampus neurons of P30 WT or H2-Kb/Db knockout (KO) mouse were checked by Golgi–Cox staining. Bars: 100 μm. (Neurons in the boxed region were shown at higher magnification, bars: 50 μm). **b** Total length of basal and apical dendrites of pyramid neurons was quantified ($p = 0.0008$). **c** Sholl analysis was performed on these neurons. Sholl profile and the average total numbers of intersections per cell were shown. ($n = 20$ WT and 20 KO cells from three and three independent animals, respectively; $p = 0.0001$)

Acknowledgements This work was supported by National Nature Science Foundation of China (Grant No. 81571615), the Fundamental Research Funds for the Central Universities (Grant No. 2242018k30004) and The Natural Science Foundation of Jiangsu Province of China (Grant No. BK20151421).

Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. McAllister AK (2000) Cellular and molecular mechanisms of dendrite growth. Cerebral cortex 10(10):963–973
2. Polleux F, Morrow T, Ghosh A (2000) Semaphorin 3A is a chemoattractant for cortical apical dendrites. Nature 404(6778):567–573
3. Redmond L, Oh SR, Hicks C, Weinmaster G, Ghosh A (2000) Nuclear Notch1 signaling and the regulation of dendritic development. Nat Neurosci 3(1):30–40
4. Lom B, Cohen-Cory S (1999) Brain-derived neurotrophic factor differentially regulates retinal ganglion cell dendritic and axonal arborization in vivo. J Neurosci 19(22):9928–9938
5. Corriveau RA, Huh GS, Shatz CJ (1998) Regulation of class I MHC gene expression in the developing and mature CNS by neural activity. Neuron 21(3):505–520
6. Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ (2000) Functional requirement for class I MHC in CNS development and plasticity. Science 290(5499):2155–2159
7. Letellier M, Willson ML, Gautheron V, Mariani J, Lohof AM (2008) Normal adult climbing fiber monoinnervation of cerebellar Purkinje cells in mice lacking MHC class I molecules. Dev Neurobiol 68(8):997–1006
8. Wu ZP, Washburn L, Bilousova TV, Boudzinskaia M, Escande-Beillard N, Querubin J, Dang H, Xie CW, Tian J, Kaufman DL (2011) Enhanced neuronal expression of major histocompatibility complex class I leads to aberrations in neurodevelopment and neurorepair. J Neuroimmunol 232(1–2):8–16
9. Adelson JD, Barreto GE, Xu L, Kim T, Brott BK, Ouyang YB, Naserke T, Djurisic M, Xiong X, Shatz CJ, Giffard RG (2012) Neuroprotection from stroke in the absence of MHCI or PirB. Neuron 73(6):1100–1107
10. Wang J, Zhang Y, Xia J, Cai T, Du J, Chen J, Li P, Shen Y, Zhang A, Fu B, Gao X, Miao F, Zhang J, Teng G (2018) Neuronal PirB upregulated in cerebral ischemia acts as an attractive theranostic target for ischemic stroke. J Am Heart Assoc 7(3):e007197
11. Xia J, Zhang Y, Zhao H, Wang J, Gao X, Chen J, Fu B, Shen Y, Miao F, Zhang J, Teng G (2017) Non-invasive monitoring of CNS MHC-I molecules in ischemic stroke mice. Theranostics 7(11):2837–2848
12. Kim T, Vidal GS, Djurisic M, William CM, Birnbaum ME, Garcia KC, Hyman BT, Shatz CJ (2013) Human LilrB2 is a beta-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. Science 341(6152):1399–1404
13. Lv D, Shen Y, Peng Y, Liu J, Miao F, Zhang J (2015) Neuronal MHC class I expression is regulated by activity driven calcium signaling. PloS ONE 10(8):e0135223
14. Liu J, Shen Y, Li M, Shi Q, Zhang A, Miao F, Liu J, Wu X, He Y, Zhang J (2013) The expression pattern of classical MHC class I molecules in the development of mouse central nervous system. Neurochem Res 38(2):290–299
15. Escande-Beillard N, Washburn L, Zekzer D, Wu ZP, Eitan S, Ivkovic S, Lu Y, Dang H, Middleton B, Bilousova TV, Yoshimura Y, Evans CJ, Joyce S, Tian J, Kaufman DL (2010) Neurons preferentially respond to self-MHC class I allele products regardless of peptide presented. J Immunol 184(2):816–823
16. Washburn LR, Zekzer D, Eitan S, Lu Y, Dang H, Middleton B, Evans CJ, Tian J, Kaufman DL (2011) A potential role for shed soluble major histocompatibility class I molecules as modulators of neurite outgrowth. PloS ONE 6(3):e18439
17. Wu ZP, Bilousova T, Escande-Beillard N, Dang H, Hsieh T, Tian J, Kaufman DL (2011) Major histocompatibility complex class I-mediated inhibition of neurite outgrowth from peripheral nerves. Immunol Lett 135(1–2):118–123
18. Adelson JD, Sapp RW, Brott BK, Lee H, Miyamichi K, Luo L, Cheng S, Djurisic M, Shatz CJ (2016) Developmental sculpting of intracortical circuits by MHC class I H2-Db and H2-Kb. Cereb Cortex 26(4):1453–1463
19. Kaech S, Banker G (2006) Culturing hippocampal neurons. Nat Protoc 1(5):2406–2415
20. Lee H, Brott BK, Kirkby LA, Adelson JD, Cheng S, Feller MB, Datwani A, Shatz CJ (2014) Synapse elimination and learning rules co-regulated by MHC class I H2-Db. Nature 509(7499):195–200
21. Syken J, Grandpre T, Kanold PO, Shatz CJ (2006) PirB restricts ocular-dominance plasticity in visual cortex. Science 313(5794):1795–1800
22. Shatz CJ (2009) MHC class I: an unexpected role in neuronal plasticity. Neuron 64(1):40–45
23. Bochner DN, Sapp RW, Adelson JD, Zhang S, Lee H, Djurisic M, Syken J, Dan Y, Shatz CJ (2014) Blocking PirB up-regulates spines and functional synapses to unlock visual cortical plasticity and facilitate recovery from amblyopia. Sci Transl Med 6(258):258ra140
24. Djurisic M, Vidal GS, Mann M, Aharon A, Kim T, Ferrao Santos A, Zuo Y, Hubener M, Shatz CJ (2013) PirB regulates a structural substrate for cortical plasticity. Proc Natl Acad Sci USA 110(51):20771–20776
25. Angibaud J, Louveau A, Baudouin SJ, Nerriere-Daguin V, Evain S, Bonnamain V, Hulin P, Csaba Z, Dournaud P, Thinard R, Naveilhan P, Noraz N, Pellier-Monnin V, Boudin H (2011) The immune molecule CD3zeta and its downstream effectors ZAP-70/Syk mediate ephrin signaling in neurons to regulate early neuritogenesis. J Neurochem 119(4):708–722
26. Sainath R, Gallo G (2015) Cytoskeletal and signaling mechanisms of neurite formation. Cell Tissue Res 359(1):267–278
27. Rosario M, Schuster S, Juttner R, Parthasarathy S, Tarabykin V, Birchmeier W (2012) Neocortical dendritic complexity is controlled during development by NOMA-GAP-dependent inhibition of Cdc42 and activation of cofilin. Genes Dev 26(15):1743–1757
28. Simhadri PK, Malwade R, Vanka R, Nakka VP, Kuppusamy G, Babu PP (2017) Dysregulation of LIMK-1/cofilin-1 pathway: a possible basis for alteration of neuronal morphology in experimental cerebral malaria. Ann Neurol 82(3):429–443
29. Forrest MP, Parnell E, Penzes P (2018) Dendritic structural plasticity and neuropsychiatric disease. Nat Rev Neurosci 19(4):215–234
30. Jia P, Wang L, Fanous AH, Chen X, Kendler KS, Zhao Z (2012) A bias-reducing pathway enrichment analysis of genome-wide association data confirmed association of the MHC region with schizophrenia. J Med Genet 49(2):96–103