



## REVIEW ARTICLE OPEN



# Orchestration of synaptic functions by WAVE regulatory complex-mediated actin reorganization

Kyung Ah Han<sup>1,2</sup> and Jaewon Ko<sup>1,2</sup>  

© The Author(s) 2023

The WAVE regulatory complex (WRC), composed of five components—Cyfip1/Sra1, WAVE/Scar, Abi, Nap1/Nckap1, and Brk1/HSPC300—is essential for proper actin cytoskeletal dynamics and remodeling in eukaryotic cells, likely by matching various patterned signals to Arp2/3-mediated actin nucleation. Accumulating evidence from recent studies has revealed diverse functions of the WRC in neurons, demonstrating its crucial role in dictating the assembly of molecular complexes for the patterning of various *trans*-synaptic signals. In this review, we discuss recent exciting findings on the physiological role of the WRC in regulating synaptic properties and highlight the involvement of WRC dysfunction in various brain disorders.

*Experimental & Molecular Medicine* (2023) 55:1065–1075; <https://doi.org/10.1038/s12276-023-01004-1>

## INTRODUCTION

Actin, a major cytoskeletal element, is essential for a range of fundamental cellular processes in eukaryotes. Actin plays a key role in neuronal morphogenesis and synapse formation during nervous system development<sup>1</sup>. In mature neurons, actin is the most prominent cytoskeletal component in both presynaptic and postsynaptic compartments, including dendritic spines, which mediate most excitatory synaptic transmission in the brain<sup>2–4</sup>. In addition, actin cytoskeletal remodeling has been implicated in structural synaptic plasticity and is intimately linked to the proper operation of molecular machineries critical for synaptic functions involving a cohort of various actin-binding proteins that regulate the assembly and disassembly of actin filaments<sup>5–7</sup>. Notably, it has been established that the Arp2/3 (actin-related proteins-2 and -3) complex, an evolutionarily conserved actin nucleating hub, acts in conjunction with dozens of other nucleation-promoting factors to drive polymerization, organization, and recycling of the actin filament network<sup>8</sup>.

Among nucleation-promoting factors, members of the WASP (Wiskott-Aldrich syndrome protein), neuronal WASP, and WAVE (WASP family verprolin homologous protein; also known as SCAR [suppressor of cyclic AMP [cAMP] receptor] family have been highlighted as ubiquitous regulators of actin cytoskeletal remodeling<sup>9–11</sup>. These proteins exist in a heteropentameric macromolecular complex (~400 kDa) known as the WRC (WAVE regulatory complex)<sup>11,12</sup>. The WRC is assembled from five different proteins—CYFIP (cytoplasmic FMR1-interacting protein; also known as Sra1), NAP (NCK-associated protein; also known as Nckap1), ABI (Abelson-interacting protein), HSPC300 (hematopoietic stem progenitor cell 300; also known as Brk1), and WAVE—that are all essential for WRC functions<sup>11</sup> (Fig. 1a). Remarkably, for each component protein, there are homologous proteins that likely exhibit tissue-specific and/or cell-type-specific expression, employ distinct regulatory mechanism(s) and/or differentially activate

Arp2/3-mediated actin polymerization, as elaborated below. The same is true for orthologous subunits in different organisms. The WRC is intrinsically inactive but is activated upon interaction with numerous cytosolic proteins, small GTPases, and transmembrane receptors, causing its translocation to the plasma membrane, where it activates the Arp2/3 complex<sup>11</sup>. Notable regulators include the Rho-family GTPase Rac1 and Arf GTPase Arf1, which allosterically relieve autoinhibition of the WRC by releasing the WCA (WASP homology 2-central-acidic) domain<sup>13,14</sup> (Fig. 1b). Moreover, PIP<sub>3</sub> [phosphatidylinositol-(3,4,5)-triphosphate] further enhances Rac1-mediated WRC activation<sup>15,16</sup>. Although our understanding is still incomplete, extensive research on the basic biology and regulatory mechanisms of the WRC has significantly contributed to our view of how actin networks are organized in eukaryotic cells.

In the current review, we describe the known functions of WRC components at neuronal synapses. We then discuss the role of the WRC in orchestrating key aspects of synapse development. Finally, we highlight the association of WRC dysfunctions with certain brain disorders and consider their ramifications. Owing to space constraints, we limit our presentation of background information on the WRC to neuroscience-related topics; recent review articles are available for those interested in greater detail<sup>11,17</sup>.

## THE ROLE OF WRC COMPONENTS DURING NERVOUS SYSTEM DEVELOPMENT

Although the significance of the WRC in regulating actin dynamics and remodeling has gradually come to be appreciated, the roles of individual WRC components in various processes during nervous system development have not yet been convincingly defined. Because each of the five components—Cyfip1/Sra1, WAVE/Scar, Abi, Nap1/Nckap1, and HSPC300/Brk1—also mediates complex formation with other distinct proteins, it is possible to speculate

<sup>1</sup>Department of Brain Sciences, Daegu Gyeongbuk Institute of Science and Technology (DGIST), 333 Techno Jungangdae-Ro, Hyeonpoong-Eup, Dalseong-Gun, Daegu 42988, Korea. <sup>2</sup>Center for Synapse Diversity and Specificity, DGIST, Daegu 42988, Korea. ✉email: jaewonko@dgist.ac.kr

Received: 7 February 2023 Revised: 13 March 2023 Accepted: 13 March 2023  
Published online: 1 June 2023



haploinsufficiency of either result in abnormal dendritic spines<sup>26,27</sup>. Another study showed that both *Cyfp1* and *Cyfp2* are also localized at GABAergic synapses<sup>28</sup>. Strikingly, overexpression of *Cyfp1* or *Cyfp2* decreases inhibitory synapse structure and transmission, disrupting the excitatory-to-inhibitory (E/I) synaptic balance<sup>28</sup>. Similarly, postsynaptic loss of *Cyfp1* increases inhibitory synapse size and strength, concomitant with the upregulation of GABA<sub>A</sub> receptor  $\beta$ 2/3 subunits and the synaptic adhesion protein Nlgn3 (neuroigin-3)<sup>28</sup>. Studies on *Cyfp1* heterozygous (*Cyfp1*<sup>+/-</sup>) mice illustrate the postsynaptic role of *Cyfp1*, demonstrating altered synapse composition in the hippocampus of these mice, with decreased levels of SynGAP1 (a synaptic Ras GTPase activating protein 1) and GluA1 (an AMPAR [ $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor] subunit) and increased levels of mGluR1/5 [metabotropic glutamate receptor 1 and 5], GluN2B (an NMDAR [N-methyl-D-aspartate receptor] subunit), and F-actin<sup>29,30</sup>. *Cyfp1* also participates in the regulation of presynaptic nerve terminal size and vesicle release probability through its actions on the WRC downstream of Rac1<sup>31</sup>, whereas *Cyfp2* regulates presynaptic short-term plasticity through its actions on presynaptic mitochondria in medial prefrontal cortical neurons<sup>32</sup>. However, it remains unclear how *Cyfp* proteins, which are present at both excitatory and inhibitory synapses, form complexes with Rac1, WAVE1, FMRP, and other proteins to bidirectionally regulate the synaptic E/I ratio. In addition, it is unknown whether the identified presynaptic roles of *Cyfp* proteins are universal across diverse brain regions.

Each *Cyfp* paralog also performs distinct functions. Analyses of *Cyfp1/2*-haploinsufficient mice showed that *Cyfp2*<sup>+/-</sup>, but not *Cyfp1*<sup>+/-</sup>, mice exhibit cell-type-specific defects in spine morphogenesis<sup>27</sup>. In line with this observation, *Cyfp1* and *Cyfp2* distinctively regulate retinal ganglion cell axon growth and guidance in zebrafish<sup>33</sup>. In particular, upon axon-axon contact, *Cyfp2* is cotransported with RNPs (ribonucleoprotein particles) to the growth cone periphery, where it switches its association, dissociating from RNPs and associating with the WRC to regulate actin polymerization and filopodial dynamics<sup>33</sup>. Expression of *Cyfp1* fails to rescue the axon-sorting defect phenotype associated with *Cyfp2* deletion<sup>33</sup>, indicating the nonredundant function of *Cyfp* paralogs during retinal development. This axon-segregating function of *Cyfp2* likely relies on its binding to a subset of transmembrane receptors involved in processes that govern proper axon sorting<sup>34</sup>. It is also possible that *Cyfp2* functions are distinct across diverse animal species (e.g., see ref. <sup>35</sup>). Intriguingly, single-cell RNA sequencing analyses revealed that *Cyfp1* is expressed in both neurons and astrocytes, whereas *Cyfp2* is predominantly expressed in neurons<sup>36</sup>, a finding that warrants future studies using conditional KO (knockout) lines to systematically analyze the effects of neuron-specific or astrocyte-specific deletion. It is also important to consider differences in the post-transcriptional regulatory activities of *Cyfp* paralogs in analyses of phenotypes arising from cell-type-specific deletion<sup>37</sup>.

### Abi

Members of the Abi protein family, which includes Abi1, Abi2, and Abi3/NESH, were initially identified as substrates of c-Abl tyrosine kinase (with the exception of Abi3)<sup>38–40</sup>. Abi proteins form complexes with various proteins (e.g., Eps8, Sos1, or WAVE2) linked to the Rac1-orchestrated actin remodeling pathway that leads to enhanced Arp2/3-mediated actin nucleation<sup>40–42</sup>. Abi1 promotes tyrosine phosphorylation of several proteins, including Mena (mammalian enabled), BCAP (B-cell adaptor for PI3-kinase), Cdc3 and WAVE2, promoting localization of these proteins to specific subcellular compartments<sup>43–46</sup>. Intriguingly, Abi1 itself is phosphorylated at serine 88 by CaMKII $\alpha$  (Ca<sup>2+</sup>/calmodulin-dependent kinase II $\alpha$ ), which is essential for Abi1-dependent modulation of spine morphogenesis<sup>47</sup>. Abi1, initially enriched in growth cones, is relocated to filopodia and dendritic spines and

becomes restricted to the postsynaptic density matrix through interactions with ProSAP2/Shank3 (SH3 and multiple ankyrin repeat domains 3). Upon synaptic activation, it translocates to the nucleus through interactions with heterogeneous nuclear ribonucleoprotein K or is retransported to excitatory synaptic sites through interaction with the motor protein Kif26B<sup>48–50</sup>. In the nucleus, Abi1 forms a trimeric complex with Myc and Max transcription factors that facilitates the transcription of E-box-regulated genes, including epidermal growth factor receptors<sup>48</sup>. All three Abi proteins are characterized by their involvement in dynamic actin cytoskeleton remodeling, as demonstrated in various model organisms<sup>42,48,51–56</sup>, but whether and how each Abi paralog exerts distinct actions in the regulation of actin dynamics at synaptic sites remain unexplored.

### WAVE/SCAR

Three members of the WAVE protein family (WAVE1–3), also known as the SCAR family, possess modular domains that interact with the small GTPases Cdc42 or Rac1 or the adapter protein IRSp53, leading to activation of the Arp2/3 complex and subsequent actin remodeling and branching at the leading edges of cells<sup>57</sup>. WAVE1 is highly expressed in the brain, and its deletion abnormally alters the size of both presynaptic terminals and postsynaptic spines<sup>58</sup>. Moreover, *Wave1*-KO mice exhibit deficits in sensorimotor function, including impaired motor coordination and balance, reduced anxiety levels, and deficits in hippocampus-dependent learning and memory<sup>59</sup>. Because removal of WAVE1 impedes assembly of the WRC, it is likely that WAVE1 and its binding proteins, such as WRP (WAVE1-associated RacGAP protein) and profilin, form a localized hub that serves to properly regulate Rac signaling. Notably, as a kinase-anchoring protein, WAVE1 is linked to various protein kinases, including cAMP-dependent PKA (protein kinase A) and Cdk5 (cyclin-dependent kinase 5)<sup>60,61</sup>. Cdk5 phosphorylates three residues in WAVE1, an action that is reversed by stimulation of D1-type dopamine receptors and NMDARs<sup>62,63</sup>. An acute challenge of mice with cocaine following a 2-week course of cocaine administration and subsequent 2-week withdrawal period similarly dephosphorylates residues phosphorylated by Cdk5<sup>60</sup>. Moreover, WAVE1 is expressed in MSNs (medium spiny projection neurons) in the striatum; further analyses using D1-MSN-expressing, neuron-specific *Wave1*-KO mice showed that WAVE1 is essential for activity-dependent regulation of dendritic spine density and excitatory synaptic transmission selectively in D1-MSN neurons<sup>60</sup>.

Similar to WAVE1, WAVE2 is involved in maintaining dendritic spine density and size through a mechanism that involves an IRSp53-dependent pathway<sup>64</sup>; it also controls dysbindin-1-mediated dendritic morphogenesis<sup>65</sup>. Moreover, WAVE2 interacts with c-Abl tyrosine kinase, which is modulated by Abi1<sup>66</sup>. Notably, c-Abl-mediated tyrosine phosphorylation of WAVE2 is required for the actin remodeling activity of WAVE2<sup>66</sup>. WAVE3 is also phosphorylated by c-Abl, and this phosphorylated form of WAVE3 regulates lamellipodia formation and cell migration<sup>67</sup>. Puzzlingly, Abi1 is crucial for c-Abl-mediated tyrosine phosphorylation of WAVE2 but not WAVE3<sup>66,67</sup>, implying the presence of additional intermediate(s) that could be uniquely associated with WAVE3-containing WRCs. Because each WAVE paralog exhibits differential localization in growth cones<sup>68</sup>, it is plausible that complexes containing distinct WAVE orthologs have different roles during synapse development. Future analyses of the interactomes of each WAVE paralog in vivo will be required to better understand the conserved and divergent functions of the WRC.

### Nap1/NCKAP1

Nap1, also termed NCKAP1 (Nck-associated protein 1), was demonstrated to interact directly with Rac1 and the other WRC components, *Cyfp1* and Abi1, to regulate WAVE1 activity, which is required for Arp2/3-mediated actin polymerization and branching

at protrusive membrane edges and subsequent lamellipodial extension<sup>54,69,70</sup>. Nap1 is localized along lamellipodia and mediates cell migration and laminar-specific neuronal differentiation in the developing neocortex<sup>55,71–73</sup>. It is also responsible for remodeling the motility and adhesion machinery by forming complexes with OL-protocadherin<sup>72</sup>. Moreover, Nap1 expression in cortical neurons is upregulated by BDNF<sup>73</sup>, and its stability is modulated by interaction with HSP90 (heat shock protein 90)<sup>74</sup>. These studies clearly establish a role for Nap1 in developing neurons, but the function of Nap1 at mature stages of synapse development and details of the underlying mechanism remains unclear.

### BRK1/HSPC300

Brk1 (BRICK1)/HSPC300, the smallest component (~8 kDa) of the WRC, has been most extensively studied in the context of cytoskeletal remodeling in *Arabidopsis*<sup>75,76</sup>, with these studies suggesting a crucial role of HSPC300 in promoting Arp2/3 activity<sup>77</sup>. However, a study employing RNAi (RNA interference) showed that the effects of HSPC300 knockdown in cultured *Drosophila* S2 cells are modest relative to those observed with RNAi-mediated ablation of other WRC components<sup>54</sup>. Moreover, other studies have shown that HSPC300 is not required for the assembly of the WAVE complex in vitro or Arp2/3-mediated actin polymerization<sup>42,78</sup>. In the only currently available study, *Drosophila* HSPC300 was shown to regulate synaptic morphology at NMJs (neuromuscular junctions) by forming a complex with Rac1-WAVE proteins<sup>79</sup>. However, the lack of studies targeting vertebrate HSPC300 orthologs has hindered the establishment of the critical roles of these proteins in the WRC.

## SYNAPTIC FUNCTIONS OF THE WRC

### Postsynaptic spine morphogenesis

Dendritic spines are morphologically diverse, protrusive structures studding from dendritic shafts that receive the most excitatory synaptic inputs<sup>2,80,81</sup>. They are almost exclusively enriched with F-actin, a polymerized form of actin filaments; notably, remodeling of F-actin governs excitatory synapse physiology. Numerous actin regulators that mediate the tight control of assembly and signaling in dendritic spines have been identified and shown to collectively orchestrate actin remodeling dynamics in conjunction with a host of spine-enriched activators and/or inhibitors that tune the activity of various small GTPases<sup>82–84</sup>. Arp2/3 is the most notable central regulator, and together with other F-actin regulators, such as formins and profilins, it drives actin filament assembly, dendritic spine maturation, and dendrite branching<sup>81,85,86</sup>. A subset of protein kinases provides phosphorylation-based regulation of the WRC<sup>87</sup>, altering its conformation in a manner that facilitates interactions with Arp2/3. The WRC is activated and relocated to membranes by the concerted actions of upstream factors, including Rac1 and other proteins, as well as by PIP<sub>3</sub><sup>11,88</sup>. Rac1-mediated promotion of spine growth involves NMDA receptor activation and direct interaction with the WRC component Cyfip1<sup>25</sup>. In addition, PIP<sub>3</sub> binds to the WRC component WAVE2 to regulate the formation of filopodia-like protrusion structures that project from spines during structural plasticity in hippocampal CA1 pyramidal neurons<sup>89,90</sup>. Furthermore, the WRC physically interacts with diverse synaptic receptors that likely transduce patterned extracellular signals into the intracellular compartment<sup>34</sup> (Table 1; see also below for details). Whether the WRC-Arp2/3 axis operates similarly across diverse cell types and brain regions needs to be systematically investigated in future studies.

### Axon guidance

Accurate formation of synaptic connections during nervous system development requires that axonal growth cones detect a

vast array of guidance cues that direct them to their appropriate synaptic targets. These extracellular cues lead to coordinated regulation of actin and microtubule networks. The Arp2/3 complex negatively regulates growth cone translocation and pathfinding but not growth cone morphology<sup>91,92</sup>. In addition, the Arp2/3 complex is required for axon guidance and initiation of growth cone filopodia in *Caenorhabditis elegans* (*C. elegans*) through multiple actin modulatory pathways, including the WRC<sup>93–98</sup>. A number of transmembrane molecules are also linked to the Arp2/3-mediated polymerization of branched actin needed for proper axon guidance in *Drosophila* embryos. Robo1 (roundabout guidance receptor 1) directly interacts with the WRC, which is further modulated by the presence of Slit (a repulsive ligand for the Robo family of receptors in *Drosophila*) secreted from midline glia<sup>99</sup>. The Robo-WRC interaction is essential for Robo1-mediated growth cone repulsion at the midline<sup>99</sup>. In addition, neogenin, a well-known axon guidance regulator, recruits the WRC-Arp2/3 complex to promote actin nucleation and thereby maintain adherens junction dynamics and tension<sup>100</sup> (Fig. 2). Overall, these studies suggest that by regulating actin cytoskeletal dynamics, the WRC-Arp2/3 axis could act as a central hub for axon guidance. However, whether other axon guidance regulators are also coupled to the WRC-Arp2/3 complex and how they functionally interact in a combinatorial manner in vertebrate neurons remains to be determined.

### Synaptic vesicle clustering and presynaptic assembly

F-actin is highly and preferentially localized around presynaptic vesicle clusters and enriched at the presynaptic active zone, providing a structural substrate for synaptic vesicle clustering, nerve terminal arborization, and axon guidance<sup>101–103</sup>. Intriguingly, synaptic activity promotes presynaptic F-actin assembly, which is essential for proper development of the presynaptic active zone and synaptic vesicle pools<sup>103</sup>. Arp2/3-dependent F-actin networks coordinate the concomitant activation of diverse pathways involving synaptic vesicle proteins, active zone proteins, and/or adaptors that dictate synaptic vesicle localization and clustering<sup>102</sup> (Fig. 2). This WRC component also appears to be involved in synaptic vesicle clustering at nascent synapses through netrin- and Rac1-dependent pathways<sup>51,104</sup> and synaptic cell-adhesion signaling pathways<sup>105</sup> in *C. elegans*. Moreover, orthologs of WRC components strongly accumulate in axons, and functional studies using fly lines harboring a null mutation of each component have delineated common abnormalities in axonal and synaptic morphology—specifically, reduced synaptic terminal length at the NMJ—in *Drosophila*<sup>20,70,106–108</sup>. These studies reinforce the idea that the cooperative operation of WRC components in recruiting functional presynaptic machinery involving Arp2/3-mediated actin remodeling is evolutionarily conserved. However, understanding whether and how the WRC contributes to presynaptic assembly, particularly in vertebrate presynaptic neurons, downstream of presynaptic F-actin organization, will require further investigation.

### Transduction of extracellular synaptic adhesion signals to intracellular machinery

Actin dynamics are intimately involved in the regulation of cell surface expression and trafficking of synaptic receptors<sup>109</sup>. The WRC interacts with a host of transmembrane receptors that likely orchestrate *trans*-synaptic adhesion pathways<sup>11,34</sup>. Intriguingly, a highly conserved consensus peptide motif composed of six residues (Φ-x-T/S-F-X-X; Φ = hydrophobic amino acid and X = any amino acid), termed the WIRS (WRC interacting receptor sequence), is present in the cytoplasmic regions of ~115 transmembrane proteins<sup>34</sup>. The WIRS binding surface is occupied by both the Cyfip and Abi subunits of the WRC and is present only in the fully assembled complex<sup>34</sup>. Further analyses have also established that WIRS motifs are prevalent in other potential

**Table 1.** WIRS-containing transmembrane proteins.

Gene name	Protein name	Sequences and positions of WIRS
PTPRS	Receptor-type tyrosine-protein phosphatase S	LATFCV (aa 1526–1531) LGSFDH (aa 1940–1945)
PTPRD	Receptor-type tyrosine-protein phosphatase delta	LGSFDH (aa 1904–1909)
PTPRF	Receptor-type tyrosine-protein phosphatase F	LGSFDH (aa 1899–1904)
EPHA3	Ephrin type-A receptor 3	LDSFLR (aa 707–712) ITTFRT (aa 910–915)
EPHA5	Ephrin type-A receptor 5	LDTFLK (aa 761–766)
EPHA6	Ephrin type-A receptor 6	LDSFLR (aa 759–764) IVSFLD (aa 926–931) FTTFDL (aa 987–992)
EPHA7	Ephrin type-A receptor 7	FTTFCS (aa 922–927)
EPHA8	Ephrin type-A receptor 8	LDTFLR (aa 721–726) FRTFSS (aa 812–817)
EPHA10	Ephrin type-A receptor 10	FSTFPS (aa 929–934) FPSFGS (aa 932–937)
EPHB1	Ephrin type-B receptor 1	LDSFLR (aa 705–710)
EPHB2	Ephrin type-B receptor 2	LDSFLR (aa 707–712) YTSFNT (aa 912–917) FTSFVD (aa 939–944)
EPHB3	Ephrin type-B receptor 3	LDSFLR (aa 719–724) YTTFIT (aa 924–929) FASF DL (aa 951–956)
EPHB4	Ephrin type-B receptor 4	LDSFLR (aa 701–706) FGSFEL (aa 933–938)
EPHB6	Ephrin type-B receptor 6	LDSFLR (aa 756–761) LSSF AF (aa 786–791) LCTFSD (aa 974–979)
BAI2	Adhesion G protein-coupled receptor B2	YPSFLS (aa 1395–1400) FHFTDR (aa 1492–1497) WSTFKS (aa 1544–1549)
BAI3	Adhesion G protein-coupled receptor B3	WDTFKN (aa 1470–1475)
NLGN1	Neuroigin-1	LHTFNT (aa 834–839) FNTFTG (aa 837–842)
NLGN3	Neuroigin-3	YNTFAA (aa 827–832)
NLGN4X	Neuroigin-4	LHTFNT (aa 792–797) FNTFSG (aa 795–800)
LPHN1	Latrophilin-1	ISTFCF (aa 879–884)
UNC5A	Netrin receptor UNC5A	YGTFNF (aa 444–449)
UNC5C	Netrin receptor UNC5C	FGSFNS (aa 533–538)
UNC5D	Netrin receptor UNC5D	FQTFNF (aa 424–429) LDSFGT (aa 670–675)
SLITRK6	SLIT and NTRK-like protein 6	FLSFQD (aa 746–751)
DSC2	Desmocollin-2	WHSFTQ(aa 826–831)
DSC3	Desmocollin-3	WHSFTQ(aa 821–826)
PCDHA1	Protocadherin alpha-1	FITFGK (aa 913–918)
PCDHA2	Protocadherin alpha-2	FITFGK (aa 911–916)
PCDHA5	Protocadherin alpha-5	FITFGK (aa 899–904)
PCDHA6	Protocadherin alpha-6	FITFGK (aa 913–918)
PCDHA7	Protocadherin alpha-7	FITFGK (aa 900–905)
PCDHA8	Protocadherin alpha-8	FITFGK (aa 913–918)
PCDHA9	Protocadherin alpha-9	FITFGK (aa 913–918)
PCDHA10	Protocadherin alpha-10	FITFGK (aa 911–916)
PCDHA11	Protocadherin alpha-11	FITFGK (aa 912–917)
PCDHA12	Protocadherin alpha-12	FITFGK (aa 904–909)
PCDHA13	Protocadherin alpha-13	FITFGK (aa 913–918)
PCDHAC1	Protocadherin alpha-C1	FITFGK (aa 926–931)

**Table 1.** continued

Gene name	Protein name	Sequences and positions of WIRS
PCDHAC2	Protocadherin alpha-C2	FITFGK (aa 970–975)
PCDH9	Protocadherin-9	LSTFAP (aa 1158–1163)
PCDH11X	Protocadherin-11X	LTTFTP (aa 1323–1328)
PCDH11Y	Protocadherin-11Y	LTTFAP (aa 1316–1321)
PCDH8	Protocadherin-8	MSTFCK (aa 984–989)
PCDH10	Protocadherin-10	MPSFVP (aa 955–960) FSTFGK (aa 1000–1005)
PCDH17	Protocadherin-17	FCTFGK (aa 998–1003)
PCDH18	Protocadherin-18	FSTFGK (aa 983–988)
PCDH19	Protocadherin-19	FATFGK (aa 1015–1020)
PCDH12	Protocadherin-12	FQTFGK (aa 1087–1092)
FAT2	Protocadherin Fat 2	LVTFGP (aa 4128–4133)
FAT3	Protocadherin Fat 3	MTTFHP (aa 4263–4268) LSSFQS (aa 4339–4344) FSTFAV (aa 4482–4487)
CELSR3	Flamingo homolog 1	LASFNS (aa 3250–3255)
TMEM132A	Transmembrane protein 132A	FVTFAP (aa 965–970)
TMEM132C	Transmembrane protein 132C	FTTFTT (aa 1056–1061)
TMEM132D	Transmembrane protein 132D	FTTFTA (aa 1047–1052)
TMEM132E	Transmembrane protein 132E	FTTFTT (aa 1015–1020)
ROBO1	Roundabout homolog 1	MKTFNS (aa 1050–1055)
ROBO3	Roundabout homolog 3	LQTFHG (aa 1029–1034)
KIRREL1	Kin of IRRE-like protein 1	YSSFKD (aa 572–577)
NEO1	Neogenin	LKSFAV (aa 1359–1364)

aa amino acid.

The indicated residue numbers are based on human protein sequences.

candidate WRC ligands (Table 1). Because many WIRS-containing proteins are also considered synaptic cell-adhesion molecules (CAMs), these studies suggest the tantalizing concept that various synaptic adhesion pathways, in collaboration with other types of WRC ligands, are involved in the recruitment of the WRC to cellular membranes. Indeed, the WRC forms complexes with the cell-adhesion protein SYG-1 at presynaptic sites and regulates both synaptogenesis and axonal branching in egg-laying motor neurons of *C. elegans*<sup>105</sup>.

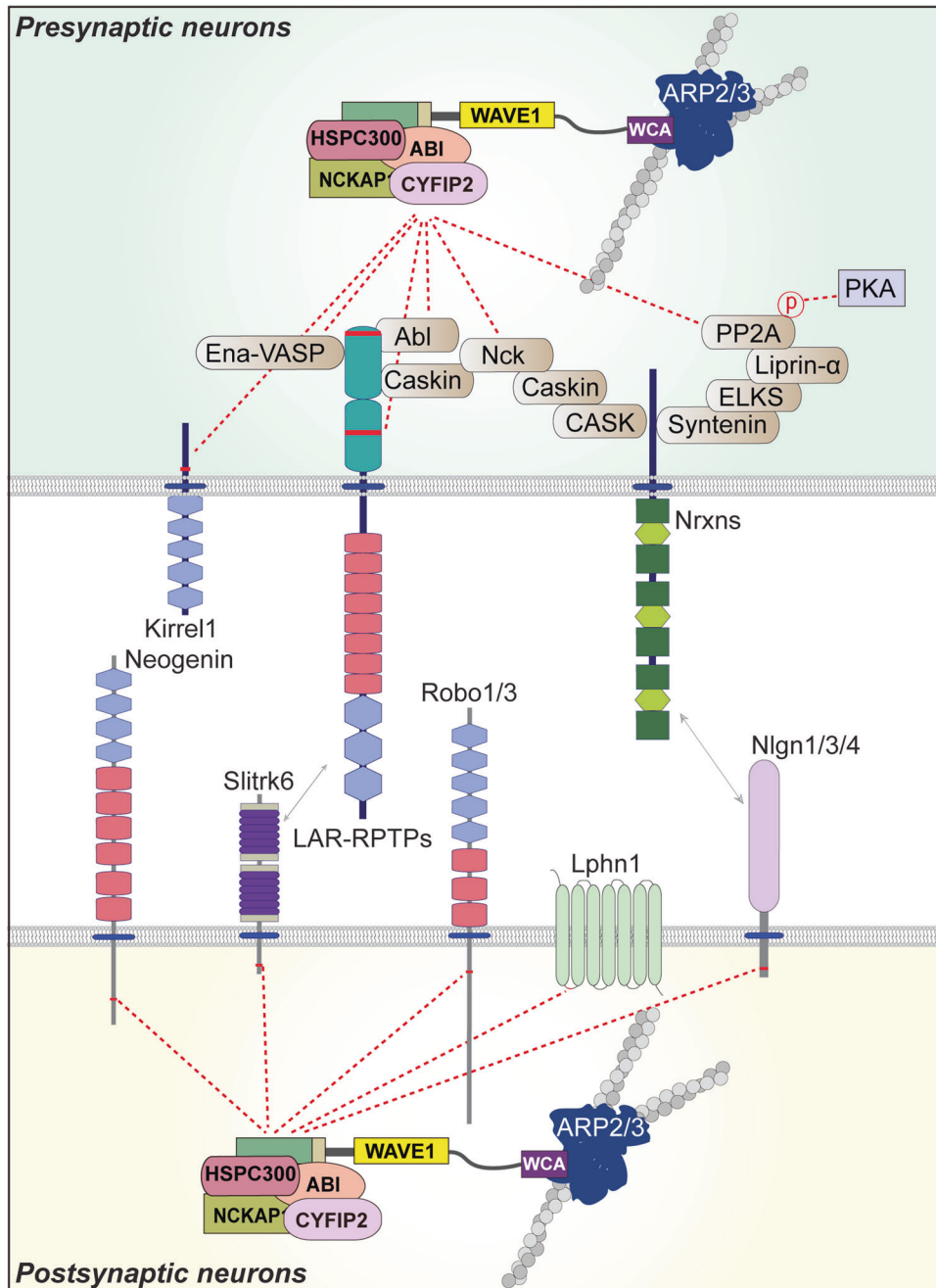
Another study reported that *Drosophila* Nlgn1 directly interacts with the WRC through its WIRS motif and regulates NMJ growth and synaptic transmission through its WRC-binding activity<sup>110</sup>. Because vertebrate Nlgn1 also possess the WIRS motif in their cytoplasmic regions<sup>110</sup> (Table 1), the regulation of F-actin assembly through the Nlgn-mediated synaptic adhesion pathway is likely an evolutionarily conserved mechanism. Intriguingly, the WRC regulatory pathway appears to integrate cues from other signaling pathways encompassing PKA<sup>62,63,111</sup>. Moreover, a subset of protein kinase pathways, including PKA and post-synaptic cAMP signaling, is essential for compartmentalized signaling at excitatory synapses<sup>112–114</sup>, suggesting the fascinating hypothesis that differentially activated WRC pathways determine the strength of intracellular signals in postsynaptic neurons (Fig. 2). This hypothesis is particularly compelling because synaptic CAMs are thought to mediate the specificity of neural circuit architecture<sup>113,115,116</sup>. Another avenue for future research would be to investigate whether specific paralogs of WRC components align with specific synaptic adhesion pathways. To this end, it would be invaluable to determine the expression patterns of each WRC component in distinct cell types, ideally at single-cell resolution.

## THE ASSOCIATION OF WRC DYSFUNCTION WITH BRAIN DISORDERS

Considering the broad ramifications of the WRC and its interacting networks in governing actin-regulatory machinery in cells, it should come as no surprise that dysfunction in the WRC is associated with a variety of genetic disorders. Because of the universal significance of actin dynamics and remodeling and the fact that mutations affecting the expression and/or other biochemical properties of a single WRC component also influence those of other components, dysregulation of WRC components could manifest as neurological disorders, immune deficiencies, or cancer<sup>11,117</sup>. Here, in keeping with the focus of this review, we primarily discuss links between the dysfunction of WRC components and brain disorders (Fig. 3).

### Disturbance of *Cyfp1* functions linked to neurodevelopmental and neuropsychiatric diseases

Deletions in the 15q11.2 region of the human genome (15q11.2 microdeletion), also called Burnside Butler syndrome, are a rare chromosomal anomaly clinically associated with developmental delay, mental retardation, epilepsy, autism spectrum disorder (ASD), schizophrenia, and congenital heart defects<sup>118</sup>. One of the genes within this chromosomal locus is *CYFIP1*<sup>119,120</sup>. Because homozygous deletion of *Cyfp1* in mice is embryonic lethal, most animal studies on this syndrome have primarily been performed using *Cyfp1*<sup>+/-</sup> mice. These mice exhibit impairments in motor learning and coordination, altered sensory motor gating, aberrant sensory perception/novelty seeking, and enhanced extinction of inhibitory avoidance—phenotypes that are frequently found in individuals with ASD and schizophrenia<sup>26,121–123</sup>. Conversely, transgenic mice overexpressing *Cyfp1* exhibit a series of distinct



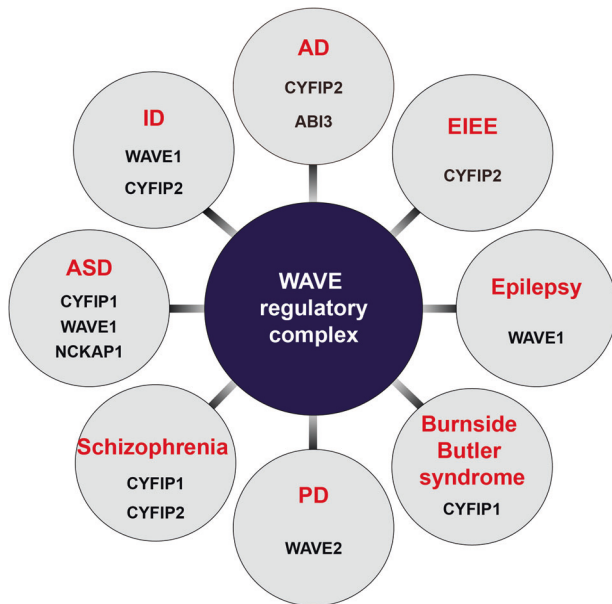
**Fig. 2 Molecular model of WRC recruitment at neuronal synapses.** WRC recruitment is regulated by cell-adhesion proteins and their interacting scaffold proteins at synapses. Various scaffold proteins present in the presynaptic active zone play important roles in WRC recruitment and function. Prominent among them are LAR-RPTPs and Kirrel1. Neurexins (Nrnxns) can also bind to the WRC but do not do so directly; instead, they bind indirectly through presynaptic scaffolding proteins, including syntenin, ELKS, liprin- $\alpha$ , PP2A, CASK, Caskins and Nck. In addition to interacting directly with the WRC through their WIRS motif, LAR-RPTPs can also indirectly bind the WRC through interactions with Ena, Abl, and Caskins. Robo receptors, Nlgn, neogenin, Slitrks, and latrophilins are also located at the postsynapse and might recruit the WRC through their WIRS motif.

behavioral phenotypes, including increased fear with mild learning and memory deficits but no ASD-like behavioral abnormalities<sup>124</sup>. These studies suggest that altered *CYFIP1* dosage contributes to divergent endophenotypes associated with diverse neurodevelopmental and neuropsychiatric disorders. Although incompletely understood, abnormalities in NMDAR or mTOR (mammalian target of rapamycin) signaling and altered white matter changes (e.g., thinning of myelin sheath in the corpus callosum) have been proposed as possible pathophysiological mechanisms underlying *Cyfp1* dysfunction-associated brain

diseases<sup>26,30,122,125</sup>. However, it remains to be determined how *Cyfp1* dysfunction-related mechanisms cause alterations in specific neural circuits responsible for specific behavioral domains associated with the indicated neurological disorders.

#### **Cyfp2 mutations in early infantile epileptic encephalopathy**

As described in the previous section (*Role of WRC components during nervous system development*), *Cyfp1* and *Cyfp2* perform different neuronal functions, as supported by distinct interactome profiles and spatiotemporal expression patterns in the brain<sup>36,126</sup>.



**Fig. 3 Implication of WRC components in various neurological disorders.** Schematic of the neurological disorders discussed in the current paper that are related to dysfunctions of each WRC component. Dysfunction of WAVE1/2, NAP1, ABI3 and CYFIP1/2 might cause neurodevelopmental, neuropsychiatric, and neurodegenerative disorders. ASD autism spectrum disorder, AD Alzheimer's disease, EIEE early infantile epileptic encephalopathy, ID intellectual disability, PD Parkinson's disease.

Consistent with this, deletions in the chromosomal region (5q33.3–5q35.1) harboring *CYFIP2* have been observed in patients with symptoms distinct from those with *CYFIP1* mutations<sup>117</sup>. In addition, reduced *CYFIP2* levels were reported in patients with schizophrenia and AD (Alzheimer's disease)<sup>127,128</sup>. Intriguingly, de novo Arg87-residue *CYFIP2* variants are associated with various facets of neurodevelopmental disorders, including EIEE (early infantile epileptic encephalopathy)<sup>129–131</sup>. Structural studies have demonstrated that Arg87 *CYFIP2* variants likely disrupt hydrogen bonding between *CYFIP2* and *WAVE1* or *Nap1*, leading to structural instability of the WRC and dysregulation of Rac1-mediated WRC activity<sup>132</sup>. Intriguingly, *Cyfp2*<sup>+R87C</sup> knock-in mice recapitulate a variety of neurological phenotypes that resemble symptoms of patients with West syndrome<sup>133</sup>. Because West syndrome and Ohtahara syndrome are categorized as subtypes of EIEE<sup>134</sup>—both of which are known to involve presynaptic defects<sup>135</sup>—it will be interesting to examine how the reported functions of *CYFIP2* are linked to their pathogenesis mechanisms. Other *CYFIP2* missense variants, notably including presumably pathogenic variants at the Asp724 residue, exhibit variable clinical phenotypes<sup>130</sup>. Moreover, mice with a *Cyfp2* deficiency display visual impairments that are frequently observed in individuals with intellectual disability<sup>136</sup>. Determining how these phenotypes are caused by *Cyfp2* dysfunction will require future studies that integrate the various cellular and clinical phenotypes observed in *Cyfp2* KO and/or missense variants.

#### Dysfunctions of WAVE are linked to neurodevelopmental and neurodegenerative disorders

Exome sequencing and whole-genome sequencing have also identified a number of de novo truncating or missense variants of *WAVE1* in patients with various neurodevelopmental symptoms<sup>137–140</sup>. Certain *WAVE1* variants were predicted to disrupt the WCA domain<sup>11</sup>. However, systematic analyses to determine whether the reported *WAVE1* variants are pathogenic in the regulation of actin polymerization processes in the nervous system

are currently lacking. *WAVE2* is also suggested to be involved in PD (Parkinson's disease)<sup>141</sup>. *WAVE2* interacts with *LRRK2* (leucine-rich repeat kinase 2), a key culprit involved in the pathogenesis of PD<sup>141</sup>. *LRRK2* phosphorylates *WAVE2*, stabilizes its levels and modulates the dynamics of *WAVE2*-mediated phagocytic activity of macrophage cells<sup>141</sup>. Several subsequent reports appear to support the association of *WAVE2* with PD<sup>142,143</sup>, although the mechanistic basis of *WAVE2* action in the pathogenesis of PD is still unclear.

#### Association of *Abi3* with Alzheimer's disease

Rare coding variants of *ABI3* were initially shown to be linked to AD<sup>144</sup>, a relationship that appears to find consistent support in follow-up studies<sup>145,146</sup>. Moreover, deletion of *Abi3* exacerbates various pathophysiological features in a mouse model of AD<sup>147</sup>. *ABI3* was also recently proposed as an early biomarker for AD<sup>148</sup>. Overall, a series of these studies have strongly implicated *ABI3* in AD pathogenesis, likely through its involvement in microglial motility and/or phagocytosis, in relation to microglial migration into amyloid plaques<sup>149,150</sup>. Whether *ABI3* is also involved in other neurodegenerative disorders remains to be determined.

#### Implication of *NAP1/NCKAP1* in neurodevelopmental disorders

Several heterozygous de novo and ultrarare deleterious variants of *NAP1* have been reported in individuals with various symptoms found in neurodevelopmental disorders<sup>151</sup>. In support of this observation, *NAP1* loss of function induces defective neuronal differentiation and abnormal neuronal migration in mice<sup>73,151</sup>. Although these observations provide compelling evidence that *Nap1* could be a contributing factor to neurodevelopmental disorders with an ASD core, it is unclear whether *NAP1* variants indeed lead to dysregulation of the WRC. Intriguingly, *NAP1* appears to exhibit cell-type-specific expression in the developing human brain<sup>151</sup>. Given the low correlation between mRNA and protein levels in the brain, future work is warranted to determine whether cell-type-specific expression patterns of *Nap1* mRNA are recapitulated at the protein level.

#### OUTLOOK AND FUTURE AVENUES

The significance of the WRC in regulating various structural and functional aspects of nervous system development and synapse formation has been clearly recognized. However, to fully understand WRC mechanisms, researchers will need to address a number of questions. For example, how is the WRC activated and/or clustered in response to different patterns of extracellular signals in neurons? In particular, do interactions of the WRC with various synaptic adhesion proteins produce distinct *trans*-synaptic signals that converge on a downstream pathway? Does the WRC act similarly in both pre- and postsynaptic neurons? What determines the composition of the WRC in different types of neurons? Does the distinct composition of the presynaptic WRC relate to distinct modes of synaptic transmission by influencing release probability? Do other actin-regulatory pathways (e.g., formins) universally crosstalk with the WRC-mediated Arp2/3 pathway across diverse cell types in various brain areas? Answering these questions will enable researchers to develop various tools for controlling the localization and/or strength of WRC signaling activities, providing an unprecedented opportunity to elucidate the role of the WRC in mediating the specificity and diversity of neural circuit architectures. Given that the Arp2/3 complex is also expressed in nonneuronal cells<sup>152</sup>, it will be illuminating to investigate the nonneuronal roles of each WRC component, ideally using conditional KO mouse lines.

#### REFERENCES

- Cingolani, L. A. & Goda, Y. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. *Nat. Rev. Neurosci.* **9**, 344–356 (2008).



2. Matus, A. Actin-based plasticity in dendritic spines. *Science* **290**, 754–758 (2000).
3. Matus, A., Brinkhaus, H. & Wagner, U. Actin dynamics in dendritic spines: a form of regulated plasticity at excitatory synapses. *Hippocampus* **10**, 555–560 (2000).
4. Yuste, R. & Bonhoeffer, T. Genesis of dendritic spines: insights from ultrastructural and imaging studies. *Nat. Rev. Neurosci.* **5**, 24–34 (2004).
5. Dillon, C. & Goda, Y. The actin cytoskeleton: integrating form and function at the synapse. *Annu. Rev. Neurosci.* **28**, 25–55 (2005).
6. Kennedy, M. B., Beale, H. C., Carlisle, H. J. & Washburn, L. R. Integration of biochemical signalling in spines. *Nat. Rev. Neurosci.* **6**, 423–434 (2005).
7. Pollard, T. D. Regulation of actin filament assembly by Arp2/3 complex and formins. *Annu. Rev. Biophys. Biomol. Struct.* **36**, 451–477 (2007).
8. Goley, E. D. & Welch, M. D. The ARP2/3 complex: an actin nucleator comes of age. *Nat. Rev. Mol. Cell Biol.* **7**, 713–726 (2006).
9. Takenawa, T. & Suetsugu, S. The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. *Nat. Rev. Mol. Cell Biol.* **8**, 37–48 (2007).
10. Campellone, K. G. & Welch, M. D. A nucleator arms race: cellular control of actin assembly. *Nat. Rev. Mol. Cell Biol.* **11**, 237–251 (2010).
11. Rottner, K., Stradal, T. E. B. & Chen, B. WAVE regulatory complex. *Curr. Biol.* **31**, R512–R517 (2021).
12. Chen, Z. et al. Structure and control of the actin regulatory WAVE complex. *Nature* **468**, 533–538 (2010).
13. Yang, S. et al. Arf GTPase activates the WAVE regulatory complex through a distinct binding site. *Sci. Adv.* **8**, eadd1412 (2022).
14. Ding, B. et al. Structures reveal a key mechanism of WAVE regulatory complex activation by Rac1 GTPase. *Nat. Commun.* **13**, 5444 (2022).
15. Oikawa, T. et al. PtdIns(3,4,5)P<sub>3</sub> binding is necessary for WAVE2-induced formation of lamellipodia. *Nat. Cell Biol.* **6**, 420–426 (2004).
16. Yang, Y. et al. Leep1 interacts with PIP3 and the Scar/WAVE complex to regulate cell migration and macropinocytosis. *J. Cell Biol.* **220**, e202010096 (2021).
17. Schaks, M., Giannone, G. & Rottner, K. Actin dynamics in cell migration. *Essays Biochem.* **63**, 483–495 (2019).
18. Schenck, A., Bardoni, B., Moro, A., Bagni, C. & Mandel, J. L. A highly conserved protein family interacting with the fragile X mental retardation protein (FMRP) and displaying selective interactions with FMRP-related proteins FXR1P and FXR2P. *Proc. Natl Acad. Sci. USA* **98**, 8844–8849 (2001).
19. Cory, G. O. & Ridley, A. J. Cell motility: braking WAVES. *Nature* **418**, 732–733 (2002).
20. Schenck, A. et al. CYFIP/Sra-1 controls neuronal connectivity in *Drosophila* and links the Rac1 GTPase pathway to the fragile X protein. *Neuron* **38**, 887–898 (2003).
21. Derivery, E. et al. The Arp2/3 activator WASH controls the fission of endosomes through a large multiprotein complex. *Dev. Cell* **17**, 712–723 (2009).
22. Napoli, I. et al. The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell* **134**, 1042–1054 (2008).
23. Pathania, M. et al. The autism and schizophrenia associated gene CYFIP1 is critical for the maintenance of dendritic complexity and the stabilization of mature spines. *Transl. Psychiatry* **4**, e374 (2014).
24. Abekhouk, S. et al. New insights into the regulatory function of CYFIP1 in the context of WAVE- and FMRP-containing complexes. *Dis. Model Mech.* **10**, 463–474 (2017).
25. De Rubéis, S. et al. CYFIP1 coordinates mRNA translation and cytoskeleton remodeling to ensure proper dendritic spine formation. *Neuron* **79**, 1169–1182 (2013).
26. Bozdagi, O. et al. Haploinsufficiency of Cyfip1 produces fragile X-like phenotypes in mice. *PLoS ONE* **7**, e42422 (2012).
27. Han, K. et al. Fragile X-like behaviors and abnormal cortical dendritic spines in cytoplasmic FMR1-interacting protein 2-mutant mice. *Hum. Mol. Genet.* **24**, 1813–1823 (2015).
28. Davenport, E. C. et al. Autism and schizophrenia-associated CYFIP1 regulates the balance of synaptic excitation and inhibition. *Cell Rep.* **26**, 2037–2051 (2019).
29. Sahasrabudhe, A. et al. Cyfip1 regulates SynGAP1 at hippocampal synapses. *Front. Synaptic Neurosci.* **12**, 581714 (2020).
30. Kim, N. S. et al. CYFIP1 dosages exhibit divergent behavioral impact via diametric regulation of NMDA receptor complex translation in mouse models of psychiatric disorders. *Biol. Psychiatry* **92**, 815–826 (2022).
31. Hsiao, K., Harony-Nicolas, H., Buxbaum, J. D., Bozdagi-Gunal, O. & Benson, D. L. Cyfip1 regulates presynaptic activity during development. *J. Neurosci.* **36**, 1564–1576 (2016).
32. Kim, G. H. et al. Altered presynaptic function and number of mitochondria in the medial prefrontal cortex of adult Cyfip2 heterozygous mice. *Mol. Brain* **13**, 123 (2020).
33. Cioni, J. M. et al. Axon-axon interactions regulate topographic optic tract sorting via CYFIP2-dependent WAVE complex function. *Neuron* **97**, 1078–1093 (2018).
34. Chen, B. et al. The WAVE regulatory complex links diverse receptors to the actin cytoskeleton. *Cell* **156**, 195–207 (2014).
35. Marsden, K. C. et al. A Cyfip2-dependent excitatory interneuron pathway establishes the innate startle threshold. *Cell Rep.* **23**, 878–887 (2018).
36. Zhang, Y., Kang, H. R. & Han, K. Differential cell-type-expression of CYFIP1 and CYFIP2 in the adult mouse hippocampus. *Anim. Cells Syst.* **23**, 380–383 (2019).
37. Biembengut, I. V., Silva, I. L. Z., Souza, T. & Shigunov, P. Cytoplasmic FMR1 interacting protein (CYFIP) family members and their function in neural development and disorders. *Mol. Biol. Rep.* **48**, 6131–6143 (2021).
38. Dai, Z. & Pendergast, A. M. Abi-2, a novel SH3-containing protein interacts with the c-Abl tyrosine kinase and modulates c-Abl transforming activity. *Genes Dev.* **9**, 2569–2582 (1995).
39. Shi, Y., Alin, K. & Goff, S. P. Abl-interactor-1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, suppresses v-abl transforming activity. *Genes Dev.* **9**, 2583–2597 (1995).
40. Ichigotani, Y., Fujii, K., Hamaguchi, M. & Matsuda, S. In search of a function for the E3B1/Abi2/Argbp1/NESH family (Review). *Int. J. Mol. Med* **9**, 591–595 (2002).
41. Innocenti, M. et al. Phosphoinositide 3-kinase activates Rac by entering in a complex with Eps8, Abi1, and Sos-1. *J. Cell Biol.* **160**, 17–23 (2003).
42. Innocenti, M. et al. Abi1 is essential for the formation and activation of a WAVE2 signalling complex. *Nat. Cell Biol.* **6**, 319–327 (2004).
43. Tani, K. et al. Abl interactor 1 promotes tyrosine 296 phosphorylation of mammalian enabled (Mena) by c-Abl kinase. *J. Biol. Chem.* **278**, 21685–21692 (2003).
44. Leng, Y. et al. Abelson-interactor-1 promotes WAVE2 membrane translocation and Abelson-mediated tyrosine phosphorylation required for WAVE2 activation. *Proc. Natl Acad. Sci. USA* **102**, 1098–1103 (2005).
45. Maruoka, M. et al. Identification of B cell adaptor for PI3-kinase (BCAP) as an Abl interactor 1-regulated substrate of Abl kinases. *FEBS Lett.* **579**, 2986–2990 (2005).
46. Lin, T. Y., Huang, C. H., Chou, W. G. & Juang, J. L. Abi enhances Abl-mediated CDC2 phosphorylation and inactivation. *J. Biomed. Sci.* **11**, 902–910 (2004).
47. Park, E., Chi, S. & Park, D. Activity-dependent modulation of the interaction between CaMKII $\alpha$  and Abi1 and its involvement in spine maturation. *J. Neurosci.* **32**, 13177–13188 (2012).
48. Proepper, C. et al. Abelson interacting protein 1 (Abi-1) is essential for dendrite morphogenesis and synapse formation. *EMBO J.* **26**, 1397–1409 (2007).
49. Proepper, C. et al. Heterogeneous nuclear ribonucleoprotein k interacts with Abi-1 at postsynaptic sites and modulates dendritic spine morphology. *PLoS ONE* **6**, e27045 (2011).
50. Heinrich, J. et al. The postsynaptic density protein Abelson interactor protein 1 interacts with the motor protein Kinesin family member 26B in hippocampal neurons. *Neuroscience* **221**, 86–95 (2012).
51. Stavoe, A. K. et al. Synaptic vesicle clustering requires a distinct MIG-10/Lamellipodin isoform and ABI-1 downstream from Netrin. *Genes Dev.* **26**, 2206–2221 (2012).
52. Rogers, S. L., Wiedemann, U., Stuurman, N. & Vale, R. D. Molecular requirements for actin-based lamella formation in *Drosophila* S2 cells. *J. Cell Biol.* **162**, 1079–1088 (2003).
53. McShea, M. A. et al. Abelson interactor-1 (ABI-1) interacts with MRL adaptor protein MIG-10 and is required in guided cell migrations and process outgrowth in *C. elegans*. *Dev. Biol.* **373**, 1–13 (2013).
54. Kunda, P., Craig, G., Dominguez, V. & Baum, B. Abi, Sra1, and Kette control the stability and localization of SCAR/WAVE to regulate the formation of actin-based protrusions. *Curr. Biol.* **13**, 1867–1875 (2003).
55. Steffen, A. et al. Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation. *EMBO J.* **23**, 749–759 (2004).
56. Innocenti, M. et al. Abi1 regulates the activity of N-WASP and WAVE in distinct actin-based processes. *Nat. Cell Biol.* **7**, 969–976 (2005).
57. Pollitt, A. Y. & Insall, R. H. WASP and SCAR/WAVE proteins: the drivers of actin assembly. *J. Cell Sci.* **122**, 2575–2578 (2009).
58. Hazai, D. et al. Ultrastructural abnormalities in CA1 hippocampus caused by deletion of the actin regulator WAVE-1. *PLoS ONE* **8**, e75248 (2013).
59. Soderling, S. H. et al. Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. *Proc. Natl Acad. Sci. USA* **100**, 1723–1728 (2003).
60. Ceglia, I. et al. WAVE1 in neurons expressing the D1 dopamine receptor regulates cellular and behavioral actions of cocaine. *Proc. Natl Acad. Sci. USA* **114**, 1395–1400 (2017).
61. Westphal, R. S., Soderling, S. H., Alto, N. M., Langeberg, L. K. & Scott, J. D. Scar/WAVE-1, a Wiskott-Aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. *EMBO J.* **19**, 4589–4600 (2000).
62. Kim, Y. et al. Phosphorylation of WAVE1 regulates actin polymerization and dendritic spine morphology. *Nature* **442**, 814–817 (2006).
63. Ceglia, I., Kim, Y., Nairn, A. C. & Greengard, P. Signaling pathways controlling the phosphorylation state of WAVE1, a regulator of actin polymerization. *J. Neurochem.* **114**, 182–190 (2010).
64. Choi, J. et al. Regulation of dendritic spine morphogenesis by insulin receptor substrate 53, a downstream effector of Rac1 and Cdc42 small GTPases. *J. Neurosci.* **25**, 869–879 (2005).

65. Ito, H. et al. Dysbindin-1, WAVE2 and Abi-1 form a complex that regulates dendritic spine formation. *Mol. Psychiatry* **15**, 976–986 (2010).
66. Stuart, J. R., Gonzalez, F. H., Kawai, H. & Yuan, Z. M. c-Abl interacts with the WAVE2 signaling complex to induce membrane ruffling and cell spreading. *J. Biol. Chem.* **281**, 31290–31297 (2006).
67. Sossey-Alaoui, K., Li, X. & Cowell, J. K. c-Abl-mediated phosphorylation of WAVE3 is required for lamellipodia formation and cell migration. *J. Biol. Chem.* **282**, 26257–26265 (2007).
68. Nozumi, M., Nakagawa, H., Miki, H., Takenawa, T. & Miyamoto, S. Differential localization of WAVE isoforms in filopodia and lamellipodia of the neuronal growth cone. *J. Cell Sci.* **116**, 239–246 (2003).
69. Eden, S., Rohatgi, R., Podtelejnikov, A. V., Mann, M. & Kirschner, M. W. Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature* **418**, 790–793 (2002).
70. Bogdan, S. & Klambt, C. Kette regulates actin dynamics and genetically interacts with Wave and Wasp. *Development* **130**, 4427–4437 (2003).
71. Ibarra, N., Pollitt, A. & Insall, R. H. Regulation of actin assembly by SCAR/WAVE proteins. *Biochem Soc. Trans.* **33**, 1243–1246 (2005).
72. Nakao, S., Platek, A., Hirano, S. & Takeichi, M. Contact-dependent promotion of cell migration by the OL-protocadherin-Nap1 interaction. *J. Cell Biol.* **182**, 395–410 (2008).
73. Yokota, Y., Ring, C., Cheung, R., Pevny, L. & Anton, E. S. Nap1-regulated neuronal cytoskeletal dynamics is essential for the final differentiation of neurons in cerebral cortex. *Neuron* **54**, 429–445 (2007).
74. Xiong, Y. et al. Nck-associated protein 1 associates with HSP90 to drive metastasis in human non-small-cell lung cancer. *J. Exp. Clin. Cancer Res.* **38**, 122 (2019).
75. Vadde, B. V. L. Tip growth: SPIRRIG and BRICK1 regulate root hair development by modulating the spatiotemporal dynamics of actin. *Plant Cell* **33**, 2106–2107 (2021).
76. Le, J., Mallery, E. L., Zhang, C., Brankle, S. & Szymanski, D. B. Arabidopsis BRICK1/HSPC300 is an essential WAVE-complex subunit that selectively stabilizes the Arp2/3 activator SCAR2. *Curr. Biol.* **16**, 895–901 (2006).
77. Djakovic, S., Dyachok, J., Burke, M., Frank, M. J. & Smith, L. G. BRICK1/HSPC300 functions with SCAR and the Arp2/3 complex to regulate epidermal cell shape in Arabidopsis. *Development* **133**, 1091–1100 (2006).
78. Gautreau, A. et al. Purification and architecture of the ubiquitous Wave complex. *Proc. Natl Acad. Sci. USA* **101**, 4379–4383 (2004).
79. Qurashi, A. et al. HSPC300 and its role in neuronal connectivity. *Neural Dev.* **2**, 18 (2007).
80. Yuste, R. Dendritic spines and distributed circuits. *Neuron* **71**, 772–781 (2011).
81. Spence, E. F. & Soderling, S. H. Actin out: regulation of the synaptic cytoskeleton. *J. Biol. Chem.* **290**, 28613–28622 (2015).
82. Tolias, K. F., Duman, J. G. & Um, K. Control of synapse development and plasticity by Rho GTPase regulatory proteins. *Prog. Neurobiol.* **94**, 133–148 (2011).
83. Hedrick, N. G. & Yasuda, R. Regulation of Rho GTPase proteins during spine structural plasticity for the control of local dendritic plasticity. *Curr. Opin. Neurobiol.* **45**, 193–201 (2017).
84. Penzes, P. & Rafalovich, I. Regulation of the actin cytoskeleton in dendritic spines. *Adv. Exp. Med. Biol.* **970**, 81–95 (2012).
85. Spence, E. F., Kanak, D. J., Carlson, B. R. & Soderling, S. H. The Arp2/3 complex is essential for distinct stages of spine synapse maturation, including synapse unsilencing. *J. Neurosci.* **36**, 9696–9709 (2016).
86. Sturmer, T. et al. Transient localization of the Arp2/3 complex initiates neuronal dendrite branching in vivo. *Development* **146**, dev113397 (2019).
87. Mendoza, M. C. Phosphoregulation of the WAVE regulatory complex and signal integration. *Semin Cell Dev. Biol.* **24**, 272–279 (2013).
88. Hume, P. J., Humphreys, D. & Koronakis, V. WAVE regulatory complex activation. *Methods Enzymol.* **540**, 363–379 (2014).
89. Ueda, Y. & Hayashi, Y. PIP(3) regulates spinule formation in dendritic spines during structural long-term potentiation. *J. Neurosci.* **33**, 11040–11047 (2013).
90. Basu, S. & Lamprecht, R. The role of actin cytoskeleton in dendritic spines in the maintenance of long-term memory. *Front. Mol. Neurosci.* **11**, 143 (2018).
91. Strasser, G. A., Rahim, N. A., VanderWaal, K. E., Gertler, F. B. & Lanier, L. M. Arp2/3 is a negative regulator of growth cone translocation. *Neuron* **43**, 81–94 (2004).
92. San Miguel-Ruiz, J. E. & Letourneau, P. C. The role of Arp2/3 in growth cone actin dynamics and guidance is substrate dependent. *J. Neurosci.* **34**, 5895–5908 (2014).
93. Norris, A. D., Dyer, J. O. & Lundquist, E. A. The Arp2/3 complex, UNC-115/abLIM, and UNC-34/Enabled regulate axon guidance and growth cone filopodia formation in *Caenorhabditis elegans*. *Neural Dev.* **4**, 38 (2009).
94. Shakir, M. A. et al. The Arp2/3 activators WAVE and WASP have distinct genetic interactions with Rac GTPases in *Caenorhabditis elegans* axon guidance. *Genetics* **179**, 1957–1971 (2008).
95. Demarco, R. S. & Lundquist, E. A. RACK-1 acts with Rac GTPase signaling and UNC-115/abLIM in *Caenorhabditis elegans* axon pathfinding and cell migration. *PLoS Genet.* **6**, e1001215 (2010).
96. Alan, J. K., Struckhoff, E. C. & Lundquist, E. A. Multiple cytoskeletal pathways and PI3K signaling mediate CDC-42-induced neuronal protrusion in *C. elegans*. *Small GTPases* **4**, 208–220 (2013).
97. Wallace, A. G., Raduwan, H., Carlet, J. & Soto, M. C. The RhoGAP HUM-7/Myo9 integrates signals to modulate RHO-1/RhoA during embryonic morphogenesis in *Caenorhabditis elegans*. *Development* **145**, dev168724 (2018).
98. Mohamed, A. M., Boudreau, J. R., Yu, F. P., Liu, J. & Chhin-Sang, I. D. The *Caenorhabditis elegans* Eph receptor activates NCK and N-WASP, and inhibits Ena/VASP to regulate growth cone dynamics during axon guidance. *PLoS Genet.* **8**, e1002513 (2012).
99. Chaudhari, K., Gorla, M., Chang, C., Kania, A. & Bashaw, G. J. Robo recruitment of the Wave regulatory complex plays an essential and conserved role in midline repulsion. *eLife* **10**, e64474 (2021).
100. Lee, N. K. et al. Neogenin recruitment of the WAVE regulatory complex maintains adherens junction stability and tension. *Nat. Commun.* **7**, 11082 (2016).
101. Murthy, V. N. & De Camilli, P. Cell biology of the presynaptic terminal. *Annu. Rev. Neurosci.* **26**, 701–728 (2003).
102. Nelson, J. C., Stavoe, A. K. & Colon-Ramos, D. A. The actin cytoskeleton in pre-synaptic assembly. *Cell Adhes. Migr.* **7**, 379–387 (2013).
103. Rust, M. B. & Maritzen, T. Relevance of presynaptic actin dynamics for synapse function and mouse behavior. *Exp. Cell Res.* **335**, 165–171 (2015).
104. Stavoe, A. K. & Colon-Ramos, D. A. Netrin instructs synaptic vesicle clustering through Rac GTPase, MIG-10, and the actin cytoskeleton. *J. Cell Biol.* **197**, 75–88 (2012).
105. Chia, P. H., Chen, B., Li, P., Rosen, M. K. & Shen, K. Local F-actin network links synapse formation and axon branching. *Cell* **156**, 208–220 (2014).
106. Schenck, A. et al. WAVE/SCAR, a multifunctional complex coordinating different aspects of neuronal connectivity. *Dev. Biol.* **274**, 260–270 (2004).
107. Hummel, T., Leifker, K. & Klambt, C. The Drosophila HEM-2/NAP1 homolog KETTE controls axonal pathfinding and cytoskeletal organization. *Genes Dev.* **14**, 863–873 (2000).
108. Zallen, J. A. et al. SCAR is a primary regulator of Arp2/3-dependent morphological events in Drosophila. *J. Cell Biol.* **156**, 689–701 (2002).
109. Hanley, J. G. Actin-dependent mechanisms in AMPA receptor trafficking. *Front. Cell Neurosci.* **8**, 381 (2014).
110. Xing, G. et al. Neurexin-Neurologin 1 regulates synaptic morphology and functions via the WAVE regulatory complex in Drosophila neuromuscular junction. *eLife* **7**, e30457 (2018).
111. Yamashita, H., Ueda, K. & Kioka, N. WAVE2 forms a complex with PKA and is involved in PKA enhancement of membrane protrusions. *J. Biol. Chem.* **286**, 3907–3914 (2011).
112. Liang, M. et al. Oligomerized liprin-alpha promotes phase separation of ELKS for compartmentalization of presynaptic active zone proteins. *Cell Rep.* **34**, 108901 (2021).
113. Lim, D., Kim, D., Um, J. W. & Ko, J. Reassessing synaptic adhesion pathways. *Trends Neurosci.* **45**, 517–528 (2022).
114. Sando, R., Ho, M. L., Liu, X. & Südhof, T. C. Engineered synaptic tools reveal localized cAMP signaling in synapse assembly. *J. Cell Biol.* **221**, e202109111 (2022).
115. Kim, H. Y., Um, J. W. & Ko, J. Proper synaptic adhesion signaling in the control of neural circuit architecture and brain function. *Prog. Neurobiol.* **200**, 101983 (2021).
116. Südhof, T. C. The cell biology of synapse formation. *J. Cell Biol.* **220**, e202103052 (2021).
117. Zhang, Y., Lee, Y. & Han, K. Neuronal function and dysfunction of CYFIP2: from actin dynamics to early infantile epileptic encephalopathy. *BMB Rep.* **52**, 304–311 (2019).
118. Cox, D. M. & Butler, M. G. Distal partial trisomy 15q26 and partial monosomy 16p13.3 in a 36-year-old male with clinical features of both chromosomal abnormalities. *Cytogenet Genome Res.* **145**, 29–34 (2015).
119. Clifton, N. E., Thomas, K. L., Wilkinson, L. S., Hall, J. & Trent, S. FMRP and CYFIP1 at the synapse and their role in psychiatric vulnerability. *Complex Psychiatry* **6**, 5–19 (2020).
120. Hosak, L. New findings in the genetics of schizophrenia. *World J. Psychiatry* **3**, 57–61 (2013).
121. Bachmann, S. O. et al. Behavioral training rescues motor deficits in Cyfip1 haploinsufficiency mouse model of autism spectrum disorders. *Transl. Psychiatry* **9**, 29 (2019).
122. Oguro-Ando, A. et al. Increased CYFIP1 dosage alters cellular and dendritic morphology and dysregulates mTOR. *Mol. Psychiatry* **20**, 1069–1078 (2015).
123. Dominguez-Iturza, N. et al. The autism- and schizophrenia-associated protein CYFIP1 regulates bilateral brain connectivity and behaviour. *Nat. Commun.* **10**, 3454 (2019).
124. Fricano-Kugler, C. et al. CYFIP1 overexpression increases fear response in mice but does not affect social or repetitive behavioral phenotypes. *Mol. Autism* **10**, 25 (2019).

125. Silva, A. I. et al. Cyfp1 haploinsufficient rats show white matter changes, myelin thinning, abnormal oligodendrocytes and behavioural inflexibility. *Nat. Commun.* **10**, 3455 (2019).
126. Bonaccorso, C. M. et al. Fragile X mental retardation protein (FMRP) interacting proteins exhibit different expression patterns during development. *Int. J. Dev. Neurosci.* **42**, 15–23 (2015).
127. Focking, M. et al. Proteomic and genomic evidence implicates the postsynaptic density in schizophrenia. *Mol. Psychiatry* **20**, 424–432 (2015).
128. Tiwari, S. S. et al. Alzheimer-related decrease in CYFIP2 links amyloid production to tau hyperphosphorylation and memory loss. *Brain* **139**, 2751–2765 (2016).
129. Zweier, M. et al. Spatially clustering de novo variants in CYFIP2, encoding the cytoplasmic FMRP interacting protein 2, cause intellectual disability and seizures. *Eur. J. Hum. Genet.* **27**, 747–759 (2019).
130. Begemann, A. et al. New insights into the clinical and molecular spectrum of the novel CYFIP2-related neurodevelopmental disorder and impairment of the WRC-mediated actin dynamics. *Genet. Med.* **23**, 543–554 (2021).
131. Nakashima, M. et al. De novo hotspot variants in CYFIP2 cause early-onset epileptic encephalopathy. *Ann. Neurol.* **83**, 794–806 (2018).
132. Schaks, M., Reinke, M., Witke, W. & Rottner, K. Molecular dissection of neurodevelopmental disorder-causing mutations in CYFIP2. *Cells* **9**, 1355 (2020).
133. Kang, M. et al. CYFIP2 p.Arg87Cys causes neurological defects and degradation of CYFIP2. *Ann. Neurol.* **93**, 155–163 (2023).
134. Pavone, P. et al. West syndrome: a comprehensive review. *Neurol. Sci.* **41**, 3547–3562 (2020).
135. Patzke, C. et al. Analysis of conditional heterozygous STXB1 mutations in human neurons. *J. Clin. Invest.* **125**, 3560–3571 (2015).
136. Chaya, T. et al. Deficiency of the neurodevelopmental disorder-associated gene Cyfp2 alters the retinal ganglion cell properties and visual acuity. *Hum. Mol. Genet.* **31**, 535–547 (2022).
137. Srivastava, S. et al. Expansion of the genotypic and phenotypic spectrum of WASF1-related neurodevelopmental disorder. *Brain Sci.* **11**, 931 (2021).
138. Zhao, A. et al. Trio exome sequencing identified a novel de novo WASF1 missense variant leading to recurrent site substitution in a Chinese patient with developmental delay, microcephaly, and early-onset seizures: a mutational hotspot p.Trp161 and literature review. *Clin. Chim. Acta* **523**, 10–18 (2021).
139. Ito, Y. et al. De novo truncating mutations in WASF1 cause intellectual disability with seizures. *Am. J. Hum. Genet.* **103**, 144–153 (2018).
140. Shimojima Yamamoto, K. et al. Recurrent de novo pathogenic variant of WASF1 in a Japanese patient with neurodevelopmental disorder with absent language and variable seizures. *Hum. Genome Var.* **8**, 43 (2021).
141. Kim, K. S. et al. Regulation of myeloid cell phagocytosis by LRRK2 via WAVE2 complex stabilization is altered in Parkinson's disease. *Proc. Natl Acad. Sci. USA* **115**, E5164–E5173 (2018).
142. Dwyer, Z. et al. Leucine-rich repeat kinase-2 (LRRK2) modulates microglial phenotype and dopaminergic neurodegeneration. *Neurobiol. Aging* **91**, 45–55 (2020).
143. Fenner, B. M., Fenner, M. E., Prowse, N. & Hayley, S. P. LRRK2 and WAVE2 regulate microglial-transition through distinct morphological phenotypes to induce neurotoxicity in a novel two-hit in vitro model of neurodegeneration. *J. Cell Physiol.* **237**, 1013–1032 (2022).
144. Sims, R. et al. Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. *Nat. Genet.* **49**, 1373–1384 (2017).
145. Conway, O. J. et al. ABI3 and PLCG2 missense variants as risk factors for neurodegenerative diseases in Caucasians and African Americans. *Mol. Neurodegener.* **13**, 53 (2018).
146. Castillo, E. et al. Comparative profiling of cortical gene expression in Alzheimer's disease patients and mouse models demonstrates a link between amyloidosis and neuroinflammation. *Sci. Rep.* **7**, 17762 (2017).
147. Karahan, H. et al. Deletion of *Abi3* gene locus exacerbates neuropathological features of Alzheimer's disease in a mouse model of Abeta amyloidosis. *Sci. Adv.* **7**, eabe3954 (2021).
148. Cao, M. et al. ABI3 is a novel early biomarker of Alzheimer's disease. *J. Alzheimers Dis.* **87**, 335–344 (2022).
149. Turner, A. K., Shaw, B. C., Simpson, J. F. & Estus, S. Identification and quantitation of novel ABI3 isoforms relative to Alzheimer's disease genetics and neuropathology. *Genes* **13**, 1607 (2022).
150. Satoh, J. I. et al. Microglia express ABI3 in the brains of Alzheimer's disease and Nasu-Hakola disease. *Intractable Rare Dis. Res.* **6**, 262–268 (2017).
151. Guo, H. et al. NCKAP1 disruptive variants lead to a neurodevelopmental disorder with core features of autism. *Am. J. Hum. Genet.* **107**, 963–976 (2020).
152. Murk, K., Blanco Suarez, E. M., Cockbill, L. M., Banks, P. & Hanley, J. G. The antagonistic modulation of Arp2/3 activity by N-WASP, WAVE2 and PICK1 defines dynamic changes in astrocyte morphology. *J. Cell Sci.* **126**, 3873–3883 (2013).

## ACKNOWLEDGEMENTS

This study was supported by the National Creative Research Initiative Program (NRF-2022R1A3B1077206 to J.K.) and the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MSIT) (2021R1C1C2010767 to K.A.H.).

## AUTHOR CONTRIBUTIONS

J.K. conceptualized the review; K.A.H. created the figures, and J.K. edited the figures; J.K. wrote an initial version of the manuscript; and K.A.H. edited, revised, and reviewed the manuscript. Both authors have read and agreed to the final version of the manuscript.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

**Correspondence** and requests for materials should be addressed to Jaewon Ko.

**Reprints and permission information** is available at <http://www.nature.com/reprints>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2023