The stromal interaction molecule 1 (STIM1) is an ER-Ca\(^{2+}\) sensor and an essential component of ER-Ca\(^{2+}\) store operated Ca\(^{2+}\) entry. Loss of STIM1 affects metabotropic glutamate receptor 1 (mGluR1)-mediated synaptic transmission, neuronal Ca\(^{2+}\) homeostasis, and intrinsic plasticity in Purkinje neurons (PNs). Long-term changes of intracellular Ca\(^{2+}\) signaling in PNs led to neurodegenerative conditions, as evident in individuals with mutations of the ER-Ca\(^{2+}\) channel, the inositol 1,4,5-triphosphate receptor. Here, we asked whether changes in such intrinsic neuronal properties, because of loss of STIM1, have an age-dependent impact on PNs. Consequently, we analyzed mRNA expression profiles and cerebellar morphology in PN-specific STIM1 KO mice (STIM1\(^{-/-}\)) of both sexes across ages. Our study identified a requirement for STIM1-mediated Ca\(^{2+}\) signaling in maintaining the expression of genes belonging to key biological networks of synaptic function and neurite development among others. Gene expression changes correlated with altered patterns of dendritic morphology and greater inner-dendritic spine density in aging STIM1\(^{-/-}\) mice. Together, our data identify STIM1 as an important regulator of Ca\(^{2+}\) homeostasis and neuronal excitability in turn required for maintaining the optimal transcriptional profile of PNs with age. Our findings are significant in the context of understanding how dysregulated calcium signals impact cellular mechanisms in multiple neurodegenerative disorders.

**Key words:** calcium signaling; climbing fibers; excitability; mGluR1; motor coordination; RNA-Seq

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**Significance Statement**

In Purkinje neurons (PNs), the stromal interaction molecule 1 (STIM1) is required for mGluR1-dependent synaptic transmission, refilling of ER Ca\(^{2+}\) stores, regulation of spike frequency, and cerebellar memory consolidation. Here, we provide evidence for a novel role of STIM1 in maintaining the gene expression profile and optimal synaptic connectivity of PNs. Expression of genes related to neurite development and synaptic organization networks is altered in PNs with persistent loss of STIM1. In agreement with these findings the dendritic morphology of PNs and climbing fiber innervations on PNs also undergo significant changes with age. These findings identify a new role for dysregulated intracellular calcium signaling in neurodegenerative disorders and provide novel therapeutic insights.

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**Introduction**

Cerebellar Purkinje neurons (PNs) play a key role in coordination of vertebrate movements. They are GABAergic neurons that integrate synaptic inputs from other regions of the brain and cerebellum and generate the sole output of the cerebellar cortex to the deep cerebellar nuclei (Albus, 1971; Ito, 2006; Dean et al., 2010). In several vertebrates studied, such as zebrafish (Namikawa et al., 2019), mouse (Burright et al., 1995; Clark et al., 1997; Watase et al., 2002; Liu et al., 2009; Prestori et al., 2019), and humans (Koeppen, 2005; Koeppen et al., 2013; Rossi et al., 2014; Hekman and Gomez, 2015), PN malfunction leads to deficits in motor function. In humans, the deficits form a spectrum of neurodegenerative disorders called ataxias, characterized by postural instability, gait disturbances, and motor incoordination (Perkins et al.,...
Materials and Methods

**Animals.** All experimental procedures were performed in accordance with Institutional Animal Ethics Committee approved by the Control and Supervision of Experiments on Animals (New Delhi, India). All transgenic mice were bred and maintained in the NCBS Animal Facility (Bangalore, India). Conditional KO using cre-lox system was adopted to generate STIM1 KO exclusively in the PNs. Homozygous Stim1 flox mice, in which exon 2 of the Stim1 gene was flanked by loxP sites (Oh-Hora et al., 2008), were bred with mice that express the Cre gene under the control of the PCP2 promoter (B6.129-Tg(Pcp2-cre)2Mpin/J, The Jackson Laboratory, RRID:MSR_JAX:001460 (Barski et al., 2000) and with a Cre reporter mouse strain Ai14Tdtomato (B6.Cg-Gt(ROSA) 26Sor tm95.1(CAG-GCaMP6f)Hze/J, RRID:MSR_JAX:007914, The Jackson Laboratory). A transgenic mouse strain Stim1 flox/flox; STIM1 flox/flox; PCP2Cre/+ was considered to be PN type-specific STIM1 KO (STIM1 PKO) mice, and a double transgenic mouse STIM1 flox/flox; STIM1 PKO mice was taken as control. The offspring obtained were genotyped using PCR of genomic DNA extracted from the tail clippings. The WT Stim1 gene and the floxed Stim1 gene were detected by the following primer pairs: Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’. Stim1-forward, 5’-CTGACACCTGCAATGAGATC-3’ and Stim1 reverse, 5’-GCCGCCTAGCACTGACCTAC-3’.

**Confocal imaging and image analysis.** Confocal images were obtained using a confocal microscope (Olympus FV1000 or FV3000 laser scanning confocal microscope) with a 20x objective (PlanApo, NA 0.75) and a 40x objective (PlanApo, NA 1.30; Olympus oil immersion), or a 60x objective (PlanApo, NA 1.42; Olympus oil immersion). For estimation of STIM1 levels in the PNs, images were acquired at 1.0-μm-thickness intervals with frame size of 512 × 512 pixels. Fluorescence intensity analysis was performed by marking the outlines of PN soma as ROIs using Imaris software. The mean intensity of STIM1 and tdTomato fluorescence was calculated from a minimum of 50-60 PNs in sections 3 animals of each genotype. For estimation of VGLUT2 puncta along PC dendrites at both proximal and distal ends, ImageJ software (Biplane, version 9.1.2) was used (Kaneko et al., 2011). The Filament tracer software (Auto Depth) in ImageJ was used to track each dendritic filament keeping the largest diameter threshold as 3 μm and thinnest diameter as 1.86 μm. Spot detection in ImageJ software was used to quantify VGLUT2 puncta by setting spot diameter threshold as 2 μm and the total distance close to the filament as 3 μm in case of proximal dendrites and 2 μm in case of distal dendrites. For quantifying the dendritic arborization, confocal images were captured at 1 μm intervals from 100-μm-thickness sections. Images of primary and secondary dendrites of PNs were analyzed with Filament tracer (Auto Depth) to measure the total dendritic length, dendritic area, dendritic volume, and number of intersections (Kaneko et al., 2011). Confocal sections with frame size of 1024 × 1024 pixels were captured at 0.5 μm intervals using 60x objective for quantifying distal spine density. Filament tracer (Auto Depth) was used to trace the dendrites by setting the largest diameter threshold as 2 μm and minimum end point diameter as 0.31 μm, and spine density was quantified setting the maximum spine length threshold to 1.5 μm (De Bartolo et al., 2015).
Rotarod test. STIM1PKO, STIM1PHet, and control mice were habituated to the rotarod (IITC, model 775, Series 8 Software) by providing a short training session where they are subjected to a constant speed of 5 rpm for 400 s. After habituating the mice to the rotarod, mice were tested for four trials a day for 5 consecutive days. In each session, the velocity of the rotation was increased with a constant acceleration of 9 rpm/min starting from 5 rpm and finally reaching to 45 rpm with a ramp speed of 240 s (Hartmann et al., 2014). The time at which the mouse fell off the rotarod was recorded, and the mean latency on the rod was calculated for the four trials for each mouse across 5 d of sessions. The same set of mice were aged and used for the rotarod assay across different ages.

Isolation and culturing of mouse PNs. Cerebellar PNs were isolated and cultured from postnatal P1 mice (Ai14Tdtomato+/−; PCP2Cre+; STIM1loxPlox, Ai14Tdtomato+/−; PCP2Cre+; STIM1loxPlox) as described previously (Tabata et al., 2000). Mice were killed by decapitation, and their cerebella were dissected and washed in Ca2+− and Mg2+-free HBSS containing gentamicin (10 µg/ml Invitrogen, 15750045). The tissue was dissected in 2.5 ml of HBSS containing trypsin (0.1% w/v; Sigma Millipore, T1426) at 33°C for 13−15 min. The cerebella were gently triturated in 2 ml of HBSS supplemented with MgSO4−7H2O (12 µM) and DNase I (1 U/ml Sigma Millipore, 11284392001) into small aggregates; ~5 ml of HBBS was added to the cell suspension and centrifuged at 1200 rpm for 30 min at 4°C. Supernatant was removed, and the density of dissociated cells was adjusted to 5 × 106 cells/ml with DMEM: Nutrient Mixture F-12 (DMEM/F12 Invitrogen, 10565018) supplemented with N2 supplement (Invitrogen, 17502048) and FBS (10% v/v; Invitrogen, 10270106); ~100 µl of the suspension was seeded onto the poly-D-lysine-coated coverslip sealed to the plastic culture dish (diameter, 35 mm; Nunc, 153066). After 3 h of incubation in the CO2 incubator, 2 ml of culture medium was added to each dish. Culture medium used was 1:1 mixture of DMEM/F12 and Neurobasal medium (Invitrogen, 21103-049) supplemented with N2-B, B-27 nutrients, and gentamicin (10 µg/ml Invitrogen, 15750045). The cells were maintained in the CO2 incubator, and half of the old medium was replaced after 3 d with a fresh one supplemented additionally with BSA (100 µg/ml; A3156, Sigma Millipore) and a glial proliferation inhibitor, cytosine arabinoside (4 mM; Sigma Millipore, C1768).

Calcium imaging in PNs. Dissociated PNs were cultured for 14 DIV on poly-D-lysine-coated coverslips. For calcium measurements, the cells were loaded with Fluo-4 acetoxymethyl ester (Fluo-4 AM; Invitrogen) containing 0.002% Phloric F-127 (Sigma Millipore) in culture medium for 30 min in the dark (Deb et al., 2016). After incubation, cells were washed with calcium containing HBSS (contains 2 mM Ca2+) and placed in 0 Ca2+ HBBS (20 mM HEPES, 137 mM NaCl, 5 mM KCl, 2 mM MgCl2, pH 7.3) containing 0.5 mM EGTA and 10 µM glucose and imaged for calcium channel currents. And 15−20 frames for each condition were acquired at ~512 pixels using Scan Image 3.8 and further analyzed by ScanImage software. For quantifying the changes in fluorescence, ROI was drawn around the cell of interest during KCl stimulation was calculated by computing the average rate of change in fluorescence intensity (ΔF) between the time at which Fmax (ΔF/F) occurs and 11 second time points and expressed as ΔF/F.

Western blots. Microdissected PNL and ML with white matter using Trizol according to the manufacturer’s protocol. Tissue was homogenized in 500 µl TRIzol (Invitrogen, catalog #1596026) using micropestle homogenizer followed by vortexing the sample before proceeding with RNA isolation protocol. Purity of the isolated RNA was checked by a NanoDrop spectrophotometer (Thermo Fisher Scientific), and its integrity was detected by running it on a 1% Tris-EDTA agarose gel; ~500 ng of total RNA isolated was used per sample for cDNA synthesis. For the DNase treatment, a reaction volume of 22.1 µl containing 500 ng of isolated RNA, 0.5 U of DNase 1 (amplification grade), 1 µl DTT, and 20 U of RNase inhibitor (RNase OUT) was incubated at 37°C for 30 min followed by heat inactivation at 70°C for 10 min. cDNA synthesis was initiated for the DNase-treated sample to which is added 200 U of Moloney murine leukemia virus reverse transcriptase, 50 µM random primer, and 1 X Transcriptor first-strand synthesis buffer in a final volume of 20 µl. The reaction mixture was subjected to 25°C for 10 min followed by treatment at 42°C for 60 min, and finally heat inactivation at 70°C for 10 min. All reagents used were purchased from Invitrogen.

Real-time qPCRs were performed using the KAPA SYBR FAST qPCR kit (Sigma Millipore, catalog #KK4601) on an ABI 7500 Fast machine (Applied Biosystems) operated with ABI 7500 software version 2 in a total volume of 10 µl. Primers were designed by using Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/). Sequences of primers for all set of genes are listed in Table 1. The fold change of gene expression of STIM1PKO relative to WT was normalized according to the 2−ΔΔCt method where ΔCt = [Ct (target gene) − Ct (GAPDH)] STIM1PKO − [Ct (target gene) − Ct (GAPDH)] STIM1WT.

Western blots. Microdissected PNL and ML were homogenized in 1× cell lysis buffer (RIPA buffer, Sigma Millipore, catalog #R0278) containing protease inhibitor cocktail (Sigma Millipore, catalog #P8340) and phosphatase inhibitors (Cell Signaling Technologies, catalog #58705) using micropestle homogenizer. The homogenized tissues were centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatant was collected as the total cell lysate. Protein quantity of the cell lysate was estimated by the Bradford assay, and an equal amount of protein was loaded onto SDS-PAGE 8%-12% acrylamide gels. Proteins were transferred onto nitrocellulose membrane and blocked using 5% skim milk in TBS-T...
(TBS with 0.1% Tween 20) for an hour at 37°C. Blots were then incubated overnight with primary antibodies at 4°C. The primary antibodies used were rabbit anti-STIM1 (1:1000; CST, catalog #D88E10) and mouse anti-β-actin (loading control) at 1:3000 (BD Biosciences, catalog #612656). After washing with PBS-T, blots were then incubated for 1 h with secondary antibodies. The secondary antibodies were anti-

### Table 1. Primers used for real-time qPCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5'-3')</th>
<th>Reverse (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pp8</td>
<td>CACGCGAACGACCTGAAAGG</td>
<td>CACGCGAACGACCTGAAAGG</td>
</tr>
<tr>
<td>Stim1</td>
<td>ACAATCGACTGCTGATGAGG</td>
<td>ACAATCGACTGCTGATGAGG</td>
</tr>
<tr>
<td>Gabra6</td>
<td>TGCTGAGAAGCTGACATGGC</td>
<td>TGCTGAGAAGCTGACATGGC</td>
</tr>
<tr>
<td>Itpr1</td>
<td>TGAAAGAGCAAACGAGGAGC</td>
<td>TGAAAGAGCAAACGAGGAGC</td>
</tr>
<tr>
<td>Pvalb</td>
<td>ATGGGGAGGGCCAGATTGTC</td>
<td>ATGGGGAGGGCCAGATTGTC</td>
</tr>
<tr>
<td>Oral3</td>
<td>CTTCTGCGCTGTTTGTTCAGT</td>
<td>CTTCTGCGCTGTTTGTTCAGT</td>
</tr>
<tr>
<td>Casq2</td>
<td>AGCCGGCACTGCTCATTACAGT</td>
<td>AGCCGGCACTGCTCATTACAGT</td>
</tr>
<tr>
<td>Kctd17</td>
<td>GGCT GCCCTACCATCTCCTGTTG</td>
<td>GGCT GCCCTACCATCTCCTGTTG</td>
</tr>
<tr>
<td>Syt1l</td>
<td>CAAGAGGAACTCAGAAGTTG</td>
<td>CAAGAGGAACTCAGAAGTTG</td>
</tr>
<tr>
<td>Vamp3</td>
<td>CCGTGCTGCTGTTTGTTCAGT</td>
<td>CCGTGCTGCTGTTTGTTCAGT</td>
</tr>
<tr>
<td>Dlg4</td>
<td>ACCAGGTGAGAACAGGCG</td>
<td>ACCAGGTGAGAACAGGCG</td>
</tr>
<tr>
<td>Gluag2</td>
<td>TGGAGGGATTTGCTGATCCGG</td>
<td>TGGAGGGATTTGCTGATCCGG</td>
</tr>
<tr>
<td>Robo2</td>
<td>CTGTGCTGCCATTCTTCTCCG</td>
<td>CTGTGCTGCCATTCTTCTCCG</td>
</tr>
<tr>
<td>Setd6</td>
<td>TGGCTGCTGCCATTCTTCTCCG</td>
<td>TGGCTGCTGCCATTCTTCTCCG</td>
</tr>
<tr>
<td>Bod</td>
<td>GGTGATGTCTTCCACCAGTACTCCG</td>
<td>GGTGATGTCTTCCACCAGTACTCCG</td>
</tr>
<tr>
<td>Insr</td>
<td>TTTCAATGATGAGCAGGCT</td>
<td>TTTCAATGATGAGCAGGCT</td>
</tr>
<tr>
<td>Gapdh</td>
<td>CTTTG6GATCAGAAGAGAG</td>
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</table>

### Table 2. Fold changes in mRNA on STIM1 KO in PNs

<table>
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<th>Gene</th>
<th>STIM1 K0</th>
<th>STIM1 K0</th>
<th>p</th>
<th>STIM1 K0</th>
<th>STIM1 K0</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itp1</td>
<td>1.02 ± 0.09</td>
<td>0.92 ± 0.05</td>
<td>0.38765</td>
<td>1.01 ± 0.08</td>
<td>0.71 ± 0.09*</td>
<td>0.03393</td>
</tr>
<tr>
<td>Pvalb</td>
<td>1.00 ± 0.06</td>
<td>0.91 ± 0.05</td>
<td>0.22470</td>
<td>1.01 ± 0.05</td>
<td>0.68 ± 0.05**</td>
<td>0.00098</td>
</tr>
<tr>
<td>Oral3</td>
<td>1.04 ± 0.14</td>
<td>1.03 ± 0.14</td>
<td>0.98871</td>
<td>1.02 ± 0.08</td>
<td>0.64 ± 0.14*</td>
<td>0.04566</td>
</tr>
<tr>
<td>Casq2</td>
<td>1.03 ± 0.11</td>
<td>0.81 ± 0.11</td>
<td>1.99599</td>
<td>1.02 ± 0.02</td>
<td>0.80 ± 0.04**</td>
<td>0.00596</td>
</tr>
<tr>
<td>Syt1l</td>
<td>1.02 ± 0.09</td>
<td>0.78 ± 0.10</td>
<td>0.10524</td>
<td>1.01 ± 0.06</td>
<td>0.70 ± 0.11*</td>
<td>0.04867</td>
</tr>
<tr>
<td>Vamp3</td>
<td>1.04 ± 0.12</td>
<td>0.15 ± 0.16</td>
<td>0.54134</td>
<td>1.01 ± 0.06</td>
<td>0.81 ± 0.05*</td>
<td>0.03345</td>
</tr>
<tr>
<td>Dlg4</td>
<td>1.01 ± 0.04</td>
<td>0.92 ± 0.06</td>
<td>0.03635</td>
<td>1.00 ± 0.04</td>
<td>0.77 ± 0.09*</td>
<td>0.04507</td>
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<td>Kctd17</td>
<td>1.04 ± 0.13</td>
<td>0.15 ± 0.12</td>
<td>0.56564</td>
<td>1.01 ± 0.09</td>
<td>0.79 ± 0.05*</td>
<td>0.03983</td>
</tr>
<tr>
<td>Syt1l</td>
<td>1.02 ± 0.09</td>
<td>0.89 ± 0.06</td>
<td>0.26332</td>
<td>1.01 ± 0.08</td>
<td>0.80 ± 0.05*</td>
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<tr>
<td>Vamp3</td>
<td>1.06 ± 0.19</td>
<td>0.87 ± 0.09</td>
<td>0.37641</td>
<td>1.02 ± 0.06</td>
<td>0.65 ± 0.13</td>
<td>0.04647</td>
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<tr>
<td>Dlg4</td>
<td>1.04 ± 0.12</td>
<td>0.79 ± 0.07</td>
<td>0.12287</td>
<td>1.01 ± 0.08</td>
<td>0.77 ± 0.06*</td>
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<tr>
<td>Robo2</td>
<td>1.02 ± 0.04</td>
<td>0.79 ± 0.15</td>
<td>0.24809</td>
<td>1.01 ± 0.08</td>
<td>0.75 ± 0.08*</td>
<td>0.04005</td>
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<td>Setd6</td>
<td>1.02 ± 0.07</td>
<td>0.12 ± 0.07</td>
<td>0.04939</td>
<td>1.00 ± 0.06</td>
<td>0.83 ± 0.05*</td>
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<td>Bad</td>
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<td>0.16 ± 0.15</td>
<td>0.94048</td>
<td>1.01 ± 0.07</td>
<td>0.83 ± 0.03*</td>
<td>0.04470</td>
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<td>Vamp3</td>
<td>1.02 ± 0.09</td>
<td>0.07 ± 0.09</td>
<td>0.32820</td>
<td>1.01 ± 0.09</td>
<td>0.59 ± 0.08*</td>
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<td>Dlx17</td>
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<td>0.19 ± 0.06</td>
<td>0.22536</td>
<td>1.00 ± 0.15</td>
<td>0.11 ± 0.06*</td>
<td>0.03543</td>
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</table>

*The fold changes of the indicated genes from biologically significant pathways in WT and STIM1 KO conditions of 14-week- and 1-year-old mice. All measurements are done by qRT-PCR. Fold changes are normalized to WT levels (reference gene, Gapdh: n = 6). Data are mean ± SEM. **p < 0.01; ***p < 0.001; ****p < 0.0001: two-tailed Student’s t test.

**STIM1**WT, **STIM1**KO, and **STIM1**Pro mice, and Sidak’s multiple comparison test was used for comparison between **STIM1**WT and **STIM1**Pro mice.

### Data availability.

The RNA-Seq data associated with this manuscript has been submitted to GEO with accession number GSE158513.
Results

Characterization of STIM1 protein in cerebellar PNs across ages

STIM1 is a key component of SOCE (Liou et al., 2005; Roos et al., 2005), and previous studies have shown that loss of STIM1 in PNs leads to changes in intracellular calcium homeostasis and reduced neuronal excitability. At the organismal level, loss of STIM1 in PNs affects motor learning and memory consolidation (Hartmann et al., 2014; Ryu et al., 2017). To understand the molecular basis of these deficits, we generated PN-specific STIM1 KO (STIM1^PKO) mice using the conditional Cre-lox system (see Materials and Methods). Exon 2, which encodes the EF hand of STIM1 (Oh-Hora et al., 2008), was deleted using a PCP2 (L7)-Cre transgenic mouse strain that exhibits near-specific expression in PNs postembryonic day 19 (Barski et al., 2000). Therefore, we tested for loss of STIM1 from PNs at 6

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Figure 1. Genotyping of control and STIM1 KO transgenic mice. A, Top, Schematic diagram showing exon 2 of STIM1 gene flanked by loxP recombination sites (yellow triangles). Deletion of this exon following Cre-mediated recombination is expected to result in a frame shift that generates a premature stop codon in the next exon. Middle, Schematic diagram showing Cre reporter cassette inserted into the intron between endogenous exons 1 and 2 of the Rosa26 locus. Bottom, Schematic diagram showing insertion of Cre-recombinase cDNA into the exon 4 of PCP2 gene. Primers are indicated by arrows (FP, forward primer; RP, reverse primer). B, Top, Reporter mouse line with tdTomato-expressing Purkinje cells was generated by cross breeding floxed stop tdTomato mice (Ai14-tdTomato) and PCP2-Cre. Bottom, STIM1^KO mouse line of tdTomato-expressing Purkinje cells was generated by cross breeding homozygous double transgenic STIM1^flox/flox; Ai14-tdTomato with STIM1^flox/flox; PCP2- Cre/cre. C, Agarose gel with genotyping of STIM1 KO mice. PCRs of genomic DNA from Stim1^flox/flox; Ai14td/1; PCP2- Cre/1 (STIM1PKO) mice are given on lanes 1-3 and 4-6. A single band at 399 bp is for homozygous STIM1^flox (lanes 1, 4), two bands at 297 bp (RosaWT) and 196 bp (Ai14td insert in the Rosa locus) (lanes 2, 5), and 421 bp for PCP2Cre (lanes 3, 6). PCRs with genomic DNA from Stim1^flox/1; Ai14td/1; PCP2- Cre/1 (STIM1PHet) mice are shown in lanes 7-9. D, Genotyping of control mice. Lanes 1-4 with Ai14^WT; PCP2- Cre/1; Cre/1 (STIM1^WT); sizes of DNA bands are as described above for C, L, DNA ladder for both C and D.
weeks by immunohistochemistry. Despite expression of tdTomato in PNs at 6 weeks, indicating PCP2-Cre activity, a substantial level of STIM1 remained in the PNs (Fig. 2A, white arrowheads). Expression of STIM1 in the soma of PNs was not detected at 12 weeks, as evident by loss of colocalization between anti-STIM1 antibody and tdTomato (red; Fig. 2B, white arrowheads). In general, we observed that STIM1 expression was low in axonal processes (Fig. 2, white asterisks) compared with PN soma and dendrites (Fig. 2, black arrows on PN dendrites). STIM1 expression in the granule layer (GL) of both control and STIM1PKO appears unaltered in the immunostained sections (GL, red asterisks). Quantification of STIM1 in PN soma, obtained by fluorescence intensity measurements, also identified residual STIM1 in STIM1PKO PNs from mice 6 weeks of age, that was significantly reduced by 12 weeks (Fig. 3A,
B). Loss of STIM1 did not affect the viability of PNs until 1 and 1.5 years, as evident in Figures 2C and 3C. The time lag between Cre expression (3 weeks) and loss of STIM1 protein (12 weeks) might be because of slow protein turnover of STIM1. Alternately, there might be a difference between recombination efficiency of the STIM1 flox allele and the tdTomato flox allele. A few interneurons in the ML also express tdTomato indicating PCP2-Cre expression in cells other than PNs. Subsequent studies for understanding STIM1 function in PNs were performed in mice 12 weeks of age or older.

Loss of STIM1 in PNs leads to deficits in motor learning and coordination

Previous studies have reported the importance of STIM1 in regulating cerebellar motor behavior (Hartmann et al., 2014) and memory consolidation (Ryu et al., 2017). To identify the exact stage at which the motor learning phenotype develops and to investigate its progression with age, control and STIM1PKO mice were subjected to a standard rotarod assay over 5 d (Hartmann et al., 2014) (see Materials and Methods). During each test session, animals were placed on a rotating rod that was accelerated continuously from 5 to 45 rpm, and the time at which the animal fell off the accelerating rotarod was noted. The ability to stay longer on the rotarod indicates better motor learning and coordination. Whereas both control and STIM1PKO mice performed comparably at either 9 or 14 weeks (Fig. 4A, B), a significant decrease in time spent by STIM1PKO mice on the accelerating rotarod was evident from 17 weeks onward, particularly after 4 d of training (Fig. 4C). Comparable rotarod performance at 9 and 14 weeks is not surprising given that STIM1 immunoreactivity from STIM1PKO PNs is lost at 12 weeks. In control mice, average latency to fall improved from 152.4 ± 7.2 s (day 1) to 232 ± 12.7 s over 5 d (Fig. 4C) in case of mice 17 weeks of age. In contrast, STIM1PKO mice fell off the rotarod with a similar latency as controls on day 1 (146.4 ± 10.1 s) but over 5 d did not exhibit improvement to the same extent as controls (day 5, 163.1 s; Fig. 4C). The same trend was observed at 29 weeks (Fig. 4D) and at 1 year of age (Fig. 4E). To our surprise, we also observed a decrease in the latency on the rotarod in case of STIM1PH mice compared with that of control mice at all ages tested, indicating an effect of the loss of one copy of STIM1 in PNs on motor learning. Progression of the deficit in motor learning with age appeared similar in control, STIM1PH, and STIM1PKO mice, as evident by comparing motor performance across ages on the fifth day of training (Fig. 4F).
Reduced membrane depolarization and loss of mGluR1 stimulated Ca^{2+} signals in STIM1 KO PNs

It is likely that altered calcium homeostasis in STIM1^{PRO} PNs underlies the motor learning deficits observed in STIM1^{PRO} mice. Because STIM1 is an integral component of SOCE in both nonexcitable (Liou et al., 2005; Roos et al., 2005; Oh-Hora et al., 2008; H. Cheng et al., 2016) and excitable cells (Venkiteswaran and Hasan, 2009; Gruszczynska-Biegala et al., 2011; Hartmann et al., 2014; Ryu et al., 2017), as a first step we investigated SOCE in cultured PNs obtained from postnatal day 1 mice and cultured for 14 d (Fig. 5). In comparison with control PNs (Aii14Tdtomato^{cre/loxp}; PCP2-Cre^{cre/loxp}), SOCE from STIM1^{PRO} PNs was muted but not abolished (Fig. 5A,B). The continued presence of reduced levels of STIM1 protein even after gene KO, as evident in Figures 2 and 3A, B, probably contributes to the SOCE in STIM1^{PRO} PNs. Our efforts to culture PNs either beyond 14 d or from cerebella of mice older than postnatal day 7 were not successful, hindering attempts to measure SOCE from PNs in which the STIM1 protein was abolished. Instead, calcium transients were measured in acute cerebellar slices from PNs expressing the genetically encoded calcium sensor, GCaMP6f using two-photon calcium imaging (Fig. 6). Changes in excitability between control PNs and STIM1^{PRO} PNs were measured from cerebellar slices of mice 17 weeks of age and stimulated with 75 mM KCl. WT PNs show robust cytosolic calcium elevations on addition of KCl (Fig. 6A–E, blue trace), whereas calcium responses from STIM1^{PRO} PNs were significantly reduced compared with that of controls (Fig. 6A–E, red trace). The significant reduction in calcium responses of STIM1^{PRO} PNs, on depolarization with KCl, agrees with the motor deficit observed in mice 17 weeks of age (Fig. 4C). These data are also in agreement with a range of electrophysiological measurements, including current clamps undertaken in-depth in a previous study (Ryu et al., 2017). The authors found reduced firing frequency and reduced excitability in STIM1^{PRO} PNs, which they attribute to the dysregulation of ionic currents through multiple membrane channels by changes in intracellular calcium dynamics (Ryu et al., 2017).

The mGluR1 is abundantly expressed in PNs (Lein et al., 2007; Ohtani et al., 2014). Mice with genomic deletion of mGluR1 exhibit severe ataxic symptoms (Alba et al., 1994) that can be rescued by expression of mGluR1 exclusively in PNs (Ichise et al., 2000). Moreover, autoantibodies against mGluR1 in humans with paraneoplastic cerebellar ataxia (Smitt et al., 2000; Coesmans et al., 2003) impair cerebellar motor coordination. Thus, mGluR1 signaling in PNs is required for cerebellar function, including motor learning and coordination. We assessed mGluR1 activation in the PNs of both control and STIM1^{PRO} mice by stimulating cerebellar slices with the mGluR1 agonist DHPG (200 μM, Fig. 6F–H). In control PNs, DHPG stimulation evoked large calcium transients in most PNs (Fig. 6F,H), although initiation of the calcium responses by DHPG occurred at varying time points in different PNs (Fig. 6F). This variation in the response time could be because of differences in the time taken for DHPG to reach the area focused under the microscope. Interestingly, in STIM1^{PRO} PNs, DHPG application failed to evoke measurable calcium transients (Fig. 6G,H). The specificity of mGluR1 activation was tested by addition of CPCCOEt, an mGluR1 antagonist (Hartmann et al., 2008). Application of CPCCOEt (200 μM) abolished the DHPG-induced calcium responses otherwise observed in control PNs (Fig. 6I). Notably, loss of mGluR1 activation observed in STIM1^{PRO} PNs from mice 17 weeks of age (Fig. 6G,H) occurs within a few weeks of loss of STIM1 protein (12 weeks, Fig. 2B) and also correlates with the onset of motor dysfunction noticed in STIM1^{PRO} mice (17 weeks, Fig. 4C). These data demonstrate that STIM1 is required for a significant proportion of mGluR1-activated calcium transients that appear in the soma and dendrites of PNs. A previous study demonstrated significant reduction in DHPG-evoked

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Motor coordination deficits in STIM1 KO mice. A, Mean latency on the rotarod for STIM1^{WT} and STIM1^{KO} mice at 9 weeks (STIM1^{WT}; n = 5) and (B) at 14 weeks (STIM1^{WT}, n = 7 and STIM1^{KO}, n = 5). Two-way ANOVA, post hoc test, and Sidak’s multiple comparison test were used for comparisons. C–E, Mean latency on the rotarod for STIM1^{WT} (n = 23), STIM1^{PHet} (n = 13), and STIM1^{KO} (n = 24) mice. Significant changes in latencies were estimated using two-way ANOVA, post hoc test, followed by Tukey’s multiple comparisons test. **p < 0.05; ***p < 0.001; ****p < 0.0001; comparison of STIM1^{WT} and STIM1^{KO} mice. F, Latencies to fall from the accelerated rotarod on day 5 for STIM1^{WT}, STIM1^{PHet}, and STIM1^{KO} mice across different ages, shown as box plots. Horizontal line indicates the median. Black solid diamond represents the mean. Colored diamonds represent individual data points. In every box plot, the limits extend from 25th to 75th percentile.
inward currents and local Ca\(^{2+}\) transients in the dendrites of STIM1\(^{PKO}\) PNs, which attribute to reduced levels of Ca\(^{2+}\) in dendritic ER stores that in turn are insufficient to stimulate DHPG-evoked slow EPSC Ca\(^{2+}\) currents (sEPSCs) (Hartmann et al., 2014). Because DHPG-evoked ER-Ca\(^{2+}\) release followed by Ca\(^{2+}\) entry resulting from the overlap in time of sEPSCs (Hartmann et al., 2014), these cannot be easily distinguished from each other, but it is likely that the larger amplitude and longer time scale of the sEPSCs contribute primarily to the Ca\(^{2+}\) signals seen in Figure 6F. Thus, our findings further augment the role of STIM1 in regulating neuronal excitability and synaptic transmission.

**KO of STIM1 alters the profile of gene expression in PNs**

A key aim of this study was to understand long-term molecular and cellular changes that occur in fully differentiated PNs because of dysregulated intracellular calcium signaling and SOCE by loss of STIM1. Changes in gene expression have been reported on reduced SOCE in nonexcitable immune cells (Feske, 2007), the developing mouse brain (Somasundaram et al., 2014), *Drosophila* pupal neurons (Richhariya et al., 2017), and human neural precursor cells (Gopurappilly et al., 2018). Gene expression profiles of mature differentiated neurons with loss of STIM1/nSOCE have not been published to date. To test for gene expression changes in STIM1\(^{PKO}\) PNs, we micro-dissected PNs from 1-year-old STIM1\(^{WT}\) and control animals. RNA was isolated from the PNL+ML (Fig. 7A) and from the GL. Specificity of tissue dissection was tested by measuring levels of RNA of a Purkinje neuronal marker, the Purkinje cell protein-2 (PCP2), and a granule cell marker, the GABA(A) receptor \(\alpha_6\) subunit (GABRA6) (Boyden et al., 2006). Micro-dissected PNL and ML from control and STIM1\(^{PKO}\) cerebellum showed high expression of PCP2 compared with the granular layer (Fig. 7B, left) and a low level of the granule cell marker GABRA6 (Fig. 7B, right), suggesting minimal contamination from granule neurons in the PN-ML sample. Importantly, a significant reduction of STIM1 RNA was observed in PN-ML isolated from the STIM1 KO mice compared with control mice (Fig. 7B, middle). STIM1 expression levels in the GL were not significantly altered (Fig. 7B, middle) as observed in the immunostained sections in Figure 2. Western blot data also showed that the STIM1 protein was significantly reduced in STIM1\(^{PKO}\) PN-ML (Fig. 7C).

RNA expression studies were performed with RNA isolated from the PN-ML of STIM1\(^{PKO}\) and control animals. Analysis of the RNA sequencing data demonstrated significant changes in gene expression in micro-dissected PN-ML from STIM1\(^{PKO}\) cerebellum compared with controls. Differentially expressed genes are shown as a volcano plot where red dots and green dots represent sets of transcripts expressed at significantly lower and higher levels than control and STIM1\(^{PKO}\) PN-ML, respectively (Fig. 7D; EdgeR analysis). The expression of 285 genes was significantly different between control and STIM1\(^{PKO}\) PN-ML based on differential analysis by both EdgeR and DeSeq and \(p < 0.05\). Of these, 168 genes were downregulated and 117 were upregulated (Fig. 7E).

Interestingly, calcium signaling genes, such as \(IP_{3}R1, IP_{3}R2,\) and Orai3, and genes encoding calcium-binding proteins, such as parvalbumin (\(Pv\)), calmodulin1 (\(Calm1\)), calsequestrin 2 (\(Casq2\)), and S100B (\(S100\beta\)), were significantly downregulated in STIM1\(^{PKO}\) PN-ML (Fig. 7F). Expression of Orai1 appears very low in PNs of both STIM1\(^{WT}\) (biological replicate read counts: 0, 0, 3) and STIM1\(^{PKO}\) (biological replicate read counts: 0, 2, 0) mice. Expression of Orai2 is slightly higher than Orai1 in both genotypes (STIM1\(^{WT}\) biological replicate read counts: 7, 11, and 12 and STIM1\(^{PKO}\) biological replicate read counts: 11, 8, and 7; from data in GEO: GSE158513). Neither Orai1 nor Orai2 expression appears affected in PN+ML from STIM1\(^{PKO}\) mice. Change in S100\(\beta\) levels were intriguing because this protein is expressed in Bergmann glial cells (Fig. 3D, marked with white arrowheads) that are unipolar astrocytes located around PNs (Fig. 3D, marked with white asterisks) and Bergmann fibers enwrap synapses on Purkinje cell dendrites (Landry et al., 1989; Yamada and Watanabe, 2002; Hachem et al., 2007). Altered expression of S100\(\beta\) in STIM1\(^{PKO}\) suggests that loss of mGluR1 signals and reduced excitability in PNs also affect gene expression of closely associated glial cells (see Discussion).

Abolishing STIM1 protein in PNs also reduced the expression of various ion channels and pumps, such as calcium voltage-gated channel auxiliary subunit \(\gamma 5\) (\(Caeng5\)), ATPase Na\(^+\)/K\(^+\) transporting subunit \(\alpha\ 3\) (\(Atp1A3\)), and synaptic signaling genes, such as Synaptotagmin 11 (\(Syt11\)) and vesicle-associated membrane protein 1 (\(Vamp1\)) (Fig. 7F). Thus, loss of STIM1 affects the expression of key components of intracellular Ca\(^{2+}\) signaling and homeostasis as well as some components of membrane excitability. Genes involved in regulation of cell cycle process, such as \(Pik4\) (Polo-like kinase 4), \(Ovol\) (Ovo-like transcriptional...
repressor 1), and Map6d1 (MAP6 domain containing 1), and those involved in regulation of cellular carbohydrate metabolic process, such as Bad (BclIIAssociated Agonist of Cell Death), Insr (Insulin Receptor), and Kat2b (Lysine Acetyltransferase 2B), are significantly upregulated (Fig. 7G). Together, these data suggest that signaling through STIM1 has an important role in regulating gene expression in mature differentiated PNs.

Biological pathways affected by absence of STIM1 in PNs
To further understand the nature of signaling mechanisms regulated by STIM1 in murine PNs, we used Metascape to predict the GO of differentially regulated genes (Figs. 8, 9). Endocytic recycling, neuron projection development, protein transport, ER calcium ion homeostasis, postsynapse organization, and glutamate receptor signaling pathway were among significantly downregulated GO biological processes (Fig. 8A). A network plot of GO biological processes for the downregulated gene set was constructed using Metascape (Fig. 8B) to help understand intercluster gene similarities and intrACLuster gene redundancies among the enriched GO terms. Regulation of neuron projection development (blue) and postsynapse organization (gray) share several genes, and the interconnecting edges indicate the relative

Figure 6. Reduced membrane depolarization and mGluR1 activation in STIM1 KO PNs. A, Line plot of the mean traces (± SEM) of normalized GCaMP6f fluorescence responses (ΔF/F) in PN soma on 75 mM KCl stimulation (STIM1WT, 84 PNs, 8 mice; STIM1KO, 44 PNs, 5 mice). B, Representative images of changes in GCaMP6f fluorescence from PN soma at indicated time points following KCl stimulation. Red arrowheads indicate PN soma. Scale bars, 20 μm. C-E, Bar graphs with peak ΔF/F (STIM1WT, 12.41 ± 0.57; STIM1KO, 7.08 ± 0.40; p = 4.10 × 10^{-12}), area under the curve (STIM1WT, 360.21 ± 27.82; STIM1KO, 273.59 ± 31.92, p = 0.04338), and rate of calcium entry (STIM1WT, 2.54 ± 0.27; STIM1KO, 0.56 ± 0.04, p = 2.11 × 10^{-11}) quantified from A, respectively. Each bar is compared with control shown in blue. *p < 0.05; **p < 0.0001; two-tailed Student’s t test. Line plots of GCaMP6f fluorescence responses (ΔF/F) in PN soma of STIM1WT (30 PNs, 4 mice) and STIM1KO (55 PNs, 4 mice) on addition of the mGluR1 agonist, 200 μM DHPG. H, Snapshots of GCaMP6f responses from PN soma at indicated time points on DHPG stimulation. Red arrowheads indicate the PN soma. Scale bars, 20 μm. I, Mean traces (±SEM) of normalized GCaMP6f fluorescence responses (ΔF/F) in PN soma on DHPG stimulation in slices incubated with 200 μM CPCCOEt, an mGluR1 antagonist (STIM1WT, 39 PNs, 4 mice; STIM1KO, 43 PNs, 4 mice).
Figure 7. RNA Seq reveals gene expression changes in PNs from STIM1PKO mice. A, Images of a micro-dissected PNL+ML and a schematic representation for extracting RNA and protein. Scale bar, 1 mm. B, Bar graphs represent comparative expression levels of a PN marker, PCP2 (Purkinje cell protein 2), STIM1 (Stromal Interaction Molecule 1), and a GL marker GABRA6 (GABA type A receptor subunit alpha6) normalized to GAPDH (glyceraldehyde 3-phosphate dehydrogenase) in samples of PNL+ML and GL. RNA expression was measured by qRT-PCR from the micro-dissected samples. Fold changes of STIM1 and GABRA6 are relative to the expression levels of Control PCP2 (n = 6 mice, 1 year). Relative fold change of PCP2 in STIM1WT (PNL+ML), 1.02 ± 0.10; STIM1PKO (PNL+ML), 1.03 ± 0.09 versus PCP2 levels in STIM1WT (GL), 0.22 ± 0.02; STIM1PKO (GL), 0.33 ± 0.08; p = 0.00038 for STIM1WT (PNL+ML) versus STIM1WT (GL) and p = 0.00031 for STIM1PKO (PNL+ML) versus STIM1PKO (GL). Relative fold change of STIM1 in STIM1WT (PNL+ML), 3.43 ± 0.21 × 10⁻¹; STIM1PKO (PNL+ML), 0.969 ± 0.23 × 10⁻¹; p = 1.5 × 10⁻⁵. Relative fold change of GABRA6 in STIM1WT (PNL+ML), 0.01 ± 0.002 versus STIM1WT (GL), 0.17 ± 0.06; p = 0.03931 and STIM1PKO (PNL+ML), 0.01 ± 0.003 versus STIM1PKO (GL), 0.12 ± 0.02, p = 0.00195. Data are mean ± SEM. * p < 0.05; ** p < 0.01; *** p < 0.001; two-tailed Student’s t test. C, Western blot showing STIM1 levels in control and STIM1 KO micro-dissected samples. D, Volcano plot of gene expression changes. Red dots and green dots represent sets of transcripts expressed at significantly lower and higher levels in STIM1PKO PNs, respectively.
relatedness of these two processes. As might be expected, endo-
cytic recycling (red), receptor-mediated endocytosis (green),
lysosomal transport (pink), and membrane organization (light
blue) form a tight network. Interestingly, genes in the ER calcium
ion homeostasis cluster (brown) form a distinct cluster that is
interlinked through the glutamate receptor-signaling pathway
(light blue) with postsynapse organization and receptor-medi-
ated endocytosis (Fig. 8B; Extended Data Fig. 8-1). This analysis
suggests that loss of STIM1-mediated Ca\(^{2+}\) signaling in PNs fur-
ther affects the expression of genes that influence ER calcium
ion homeostasis, glutamate receptor signaling, neuron excitability,
and synaptic transmission.

Biological pathways associated with regulation of cell cycle
process, mitotic cell cycle, signal release, cell development, cellu-
lar carbohydrate metabolic process, and transmembrane trans-
port are significantly upregulated in the STIM1\(^{PRO}\) PNs (Fig.
8C). Clustering of upregulated genes from “GO biological pro-
cess” shows that regulation of cell cycle processes is the most
enriched pathway with gene sets being shared among its various
subclusters; no intercluster connections with other GO terms
were observed (Fig. 8D; Extended Data Fig. 8-2).

A similar GO analysis of cellular components identified three
interrelated clusters of “neuron to neuron synapse, “axon,” and
“presynapse” among the downregulated genes in STIM1\(^{PRO}\), sup-
porting an effect of STIM1-mediated Ca\(^{2+}\) signaling on multiple
synaptic components, including postsynaptic densities, synaptic
membranes, and glutamatergic synapses (Fig. 9A,B; Extended
Data Fig. 9-1). A second set of interrelated clusters, “early endo-
some,” “cytoplasmic vesicle part,” and “membrane coat,” suggest
an effect on vesicular transport. Interestingly, the KEGG pathway
and Reactome analysis identified an interconnected network that
links to Alzheimer’s disease and includes several nodes belonging
to ion homeostasis (Fig. 9C,D, Extended Data Fig. 9-2). The func-
tional relevance of single nodes in the network plots is hard
to assess from this analysis. Together, these findings indicate that
regulation of gene expression by STIM1 impacts various biologi-
cal, cellular, and molecular processes that could in turn alter syn-
aptic morphology and function as well as neuronal excitability.

Gene expression changes because of loss of STIM1 are age-
dependent

Next, we investigated the age at which gene expression changes
occur after STIM1 KO in PNs. Accordingly, candidate genes from
various GO-enriched categories (Fig. 8) were chosen, includ-
ing calcium signaling, voltage-gated ion channels, synaptic
signaling, neuron projection development, transcription factors,
and carbohydrate metabolic process, and their expression was
measured by qRT-PCR in RNA isolated from the dissected cere-
bella of control and STIM1\(^{PRO}\) mice at 14 weeks and 1 year.
Interestingly, expression level of genes encoding IP\(_1\), R1, Orai3,
and calcium-binding proteins, such as Pvalb, Calm1, Casq2, and
S100B, were significantly downregulated at 1 year (Fig. 10A, red
vs blue bars) but not at 14 weeks of age (Fig. 10A, dark pink vs
light purple bars). A similar pattern of age-dependent changes in
gene expression was observed for all other gene classes tested by
qRT-PCR (Fig. 10B–F). Results of the qRT-PCR experiments from
STIM1\(^{PRO}\) animals 1 year of age helped to validate the change in
gene expression of various ion channels and pumps, such as Ca<sup>c5</sup>, Atp1a3, and Kctd17 (Fig. 10B), and synaptic sig-
naling genes, such as Syt11 and Vamp1 (Fig. 10C). In addition,
we confirmed the downregulation of genes involved in neuron
projection development, such as Dlg4 (discs large homolog 4),
Robo2 (Roundabout guidance receptor 2), Gigg2 (GRB10
Interacting GYF Protein 2), and Map4 (Microtubule-associated
protein 4) (Fig. 10D) and those involved in regulating gene
expression, such as Tjp2b (Transcription factor AP-2 β) and
Setd6 (SET domain containing 6) (Fig. 10E) in STIM1\(^{PRO}\) PNs.
Two upregulated genes, Bad and Insr, both related to carbohy-
drate metabolic process, were also validated (Fig. 10F).

Possibly the altered gene expression observed in 1-year-old
STIM1\(^{PRO}\) PNs arises from a combinational effect of loss of
mGlur1 signals through STIM1 (Fig. 6G,H), and other mecha-
nisms that are dysregulated because of prolonged loss of STIM1-
dependent signaling in PNs, such as reduced excitability (Fig.
6A–E). Alternatively, in the initial phase of STIM1 KO, certain
compensatory mechanisms might exist that are abrogated with
age. These findings suggest that changes in neuronal activity
(Fig. 6) and onset of motor dysfunction (Fig. 4), because of
loss of STIM1 in PNs, precede changes in gene expression
(Figs. 7–10).

Dendritic morphology of PNs undergoes subtle changes in
STIM1 KO animals

PNs integrate afferent inputs received on an elaborate dendritic
tree and their function is largely influenced by development and
maintenance of dendritic morphology and spine density (K. J. Lee
et al., 2005; McKay and Turner, 2005). Based on our findings that
genes for neuron projection development, such as Dlg4, Robo2,
Gigg2, and Map4 (Figs. 8, 10), were significantly downregulated
in PNs of STIM1\(^{PRO}\) mice, and that changes in the dendritic pat-
ttern might also occur because of altered regulation of genes for
aspects of metabolic regulation (Figs. 8, 9) (Bauernafield et al.,
2014; Steiner, 2019; Maiezzini et al., 2020); we investigated the
dendritic morphology of PNs. Dendritic arborization of the pri-
mary and secondary dendritic branches of PNs were traced using
the Filament Tracer (Auto Depth) tool of Imaris software (Fig.
11A; see Materials and Methods). The number of intersections
observed at specific distances from the soma were quantified to
obtain a measure of the dendritic branch complexity (Fig. 11B).
A significant reduction in the number of intersections was observed
at a distance of 120-140 μm from the Purkinje soma (p < 0.001
at 120 μm, p < 0.05 at 130 μm, and p < 0.01 at 140 μm, two-way
ANOVA, Sidak’s multiple comparisons test; Fig. 11B) as indicated
in Figure 11A (white arrowheads, at 120 μm), suggesting reduced
inputs from parallel fibers (Ichikawa et al., 2002, 2016) and
climbing fibers (CFs) (Sugawara et al., 2013). Interestingly, overall
dendritic volume of STIM1\(^{PRO}\) PNs was reduced to a significant
extent from 2322.23 ± 69.48 μm\(^3\) (WT) to 1840.56 ± 71.48 μm\(^3\)
(STIM1\(^{PRO}\); p < 0.00005; Fig. 11C). A modest effect was also
observed on the dendritic area (Fig. 11D). Total dendritic length
remained similar between WT and STIM1\(^{PRO}\) PNs (Fig. 11E).
Because this method does not allow reliable tracing of finer
dendritic branches, it is possible that changes, beyond those seen
in the primary and secondary dendrites, have not been detected
in our study. Previous studies have demonstrated the importance
of STIM2-mediated neuronal SOCE in maintenance of mushroom
spines in hippocampal neurons (Sun et al., 2014). However, spine
density at the distal end of the STIM1PKO PNs appeared no different from WT (Fig. 11F,G). An estimation of different subtypes of spines in STIM1PKO PNs would have been informative. However, the resolution of confocal images obtained was insufficient to identify spine subtypes. Moreover, the spine density observed appeared higher than from published data with Golgi staining (Sugawara et al., 2013; Xiao et al., 2020). This discrepancy may be either because of the different methods used or the fact that we

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**Figure 8.** Biological processes affected by the absence of STIM1 in the PNs. **A**, Biological processes enriched among genes that are downregulated in STIM1 KO PNs. **B**, Network analysis of significant biological processes from **A**. **C**, Biological processes enriched among genes that are upregulated in STIM1 KO PNs. **D**, Network analysis of significant biological processes of upregulated genes. In the network plots, each circle node represents GO terms where its size is proportional to the number of input genes included in the term, and its color represents its cluster identity. Nodes that share common genes are connected by edges. Edge width corresponds to the number of genes that are shared between nodes, and edge length represents the similarity coefficient between nodes. GO analysis was performed using Metascape and parameters specific for Mus musculus with p value cutoff as 0.01, count threshold at 3, and minimum enrichment as 1.5. Details of GO terms are provided in Extended Data Figures 8-1 and 8-2.
have used older mice for our studies. Our data demonstrate that signaling through STIM1 helps maintain the dendritic arborization and dendritic morphology of PNs, and suggest that altered expression of genes because of loss of STIM1 could be responsible in part for the changes in dendritic morphology.

Loss of STIM1 in PNs enhances climbing fiber innervation of PN dendrites
Appropriate CF-PN synaptic wiring influences information processing and integration essential for motor learning and coordination (Ichikawa et al., 2016). Multiple CFs innervate PNs at birth and subsequently undergo pruning so that a single CF innervates a PN in mature adults. This extensive pruning of CF-PN synapses continues until postnatal week 3 (Crepel, 1982; Hashimoto et al., 2009; Watanabe and Kano, 2011) and depends on mGluR1 activation during the late phases (Kano et al., 1995, 1997, 1998; Offermanns et al., 1997; Hashimoto et al., 2001). Attenuation of mGluR1 signaling and reduced excitability were observed in PNs of STIM1KO animals (Fig. 6). Consequently, we examined the density and distribution of CF-PN synapses by counting VGLUT2 (vesicular glutamate transporter type 2) puncta along the dendrites of PNs from animals 14 weeks and 1 year of age (Fig. 12). A significant increase in VGLUT2 puncta was observed along the proximal dendrites of STIM1KO PNs in mice 14 weeks of age (Fig. 12A,B), whereas the density of VGLUT2 puncta at the distal dendrites appeared comparable between control and STIM1KO cerebellum (Fig. 12C,D). Interestingly, in animals 1 year of age, the density of VGLUT2 puncta along both the proximal (Fig. 12E,F) and distal dendrites of STIM1KO PNs (Fig. 12G,H) increased significantly, suggesting that loss of STIM1 leads to long-term changes in synapse formation extending from 14 weeks to 1 year. Transcriptional profiling data from animals 1 year of age show that genes for endocytic recycling, postsynapse organization, and glutamate receptor signaling, all of which are likely to impact synapse function, were significantly downregulated in STIM1KO PNs (Fig. 8A,B). Reduced expression of synaptic components in STIM1KO PNs may thus be a compensatory mechanism for appropriate loss of synapse elimination, as suggested by excess VGLUT2 puncta in 1-year-old animals.
Figure 10. Age-dependent gene expression changes in STIM1PKO PNs. Bar graphs of fold changes in expression levels of the indicated genes from biologically significant pathways, such as (A) calcium signaling, (B) voltage-gated ion channels, (C) synaptic signaling, (D) neuron projection development, (E) transcription factor, and (F) carbohydrate metabolic process. Fold changes were normalized to GAPDH. All measurements are by qRT-PCR of cDNA prepared from RNA isolated from micro-dissected PNL 1 ML (n = 6). Data are mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; two-tailed Student's t test. Pcp2, Purkinje cell protein 2; Stim1, stromal interaction molecule 1; Gabra6, GABA type A receptor subunit alpha6; Itpr1, inositol 1,4,5-trisphosphate receptor 1; Pvalb, parvalbumin; Calm1, calmodulin1; Casq2, calsequestrin 2; Cacng5, calcium voltage-gated channel auxiliary subunit g5; Atp1a3, ATPaseNa+/K+ transporting subunit a3; Kctd17, potassium channel tetramerization domain containing 17; Syt11, synaptotagmin 11; Vamp1, vesicle-associated membrane protein 1; Dlg4, discs large homolog 4; Robo2, roundabout guidance receptor 2; Gigyl2, GRB10-interacting GYF protein 2; Map4, microtubule-associated protein 4; Tlap2b, transcription factor AP-2β; Setd6, SET domain containing 6; Bad, Bcl-2-associated agonist of cell death; Insr, insulin receptor; Gapdh, glyceraldehyde 3-phosphate dehydrogenase. Details of fold change and exact p value are provided in Table 2.
Discussion

In this study, we have identified molecular and cellular changes that occur in murine PNs with cell-specific loss of STIM1. Excitability and Ca\(^{2+}\) signaling in response to mGluR1 stimulation are affected early and are concomitant with loss of STIM1 protein (after 12 weeks), whereas changes in gene expression appear to be age-dependent and are evident in older animals.

Older STIM\(^{PKO}\) mice also exhibit an age-dependent increase in VGLUT2 puncta, which correspond to CF-PN synapses (Fremeau et al., 2001) accompanied by subtle changes in dendritic morphology (Fig. 13). Altered dendritic morphology and excessive CF-PN inputs imply that error encoding during motor learning tasks is likely to be impaired in aged STIM\(^{PKO}\) mice (Nguyen-Vu et al., 2013). This idea requires further testing. The

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Figure 11. Alteration in dendritic morphology observed in STIM1 KO PNs. A, Confocal analysis of dendritic arborization in PNs of control and STIM1 KO mice using Filament Tracer (Auto Depth) in Imaris software. Confocal images of PN dendrites (left) and their projection image, traced using Imaris (right) are shown. White arrowheads indicate 120 \(\mu\)m. Scale bar, 20 \(\mu\)m. B, Sholl analysis with number of intersections (y axis) and distance from the soma (in \(\mu\)m; x axis) for PN dendrites from control and STIM1\(^{PKO}\) animals. *p < 0.05; **p < 0.01; ***p < 0.001; two-way ANOVA with Sidak’s multiple comparison test. C, Bar graph with quantification of the total dendritic volume (STIM1\(^{WT}\), 2322.24 \(\pm\) 69.48 \(\mu\)m\(^3\); STIM1\(^{PKO}\), 1840.56 \(\pm\) 71.49 \(\mu\)m\(^3\); p = 5.82 \times 10^{-4}\) (D) dendritic area (STIM1\(^{WT}\), 2979.99 \(\pm\) 90.96 \(\mu\)m\(^2\); STIM1\(^{PKO}\), 2658.20 \(\pm\) 100.96 \(\mu\)m\(^2\); p = 0.0202) and (E) dendritic length (STIM1\(^{WT}\), 361.74 \(\pm\) 12.36 \(\mu\)m; STIM1\(^{PKO}\), 351.35 \(\pm\) 14.18 \(\mu\)m; p = 0.5821) of control and STIM1\(^{PKO}\) dendrites (STIM1\(^{WT}\), n = 54; STIM1\(^{PKO}\), n = 38 PNs). F, Spine morphology on distal dendrites of PNs expressing tdTomato (from left; scale bar, 4 \(\mu\)m) followed by enlarged images of the marked regions (scale bar, 2 \(\mu\)m). The enlarged confocal image is followed by an overlay with the corresponding projection image traced using Imaris and the projection image from Imaris analysis (extreme right). White arrowheads indicate some of the spines present on a distal dendrite. G, Bar graph represents the quantitative analysis of spine density (spines/10 \(\mu\)m) along the distal dendrites of PN for the indicated genotype (STIM1\(^{WT}\), 31.28 \(\pm\) 0.38 (n = 16 PNs); STIM1\(^{PKO}\), 31.08 \(\pm\) 0.43 (n = 14 PNs); p = 0.7324). Three mice (age 1 year) were analyzed per group. Data are mean \(\pm\) SEM. *p < 0.05; **p < 0.01; two-tailed Student’s t test; n.s., not significant.
loss of mGluR1 Ca\textsuperscript{2+} signals in STIM1\textsuperscript{PKO} PNs through adult life might result in the increase of CF-PN puncta with age. Based on the observation that parallel changes in gene expression occur and affect multiple synapse components, it is likely that reduced mGluR1 signaling in the PN dendrites sets up a negative feedback loop between reduced synaptic activity, ER-

Ca\textsuperscript{2+} homeostasis, and changes in gene expression, thus further affecting PN synaptic function and CF-PN synapses with age.

**STIM1 and Ca\textsuperscript{2+} signaling in Purkinje neurons**

Interestingly, within a few weeks of loss of STIM1 from PNs, mGluR1 responses reduce significantly as also the ability to learn
and perform a motor coordination task (Figs. 4, 6). An important physiological output of mGluR1/IP3R-mediated Ca2+ signaling is LTD of PF-PN synapses. Loss of either mGluR1 or the IP3R1 abrogates LTD (Aiba et al., 1994; Inoue et al., 1998; Ichise et al., 2000). LTD requires that the PNs receive within a 100–500 ms window both repetitive PF inputs for stimulating mGluR1/IP3R-mediated Ca2+ release and CF-activated action potentials (Sarkisov and Wang, 2008). Attenuated Ca2+ entry in STIM1PKO mice affects refilling of ER stores in PNs, which in turn suppresses mGluR1-stimulated Ca2+ signals and therefore the ability to respond to repetitive PF inputs (Hartmann et al., 2014). Based on the reduced KCl responses observed in PNs of STIM1PKO mice, it is likely that PN response to CF inputs is also attenuated. The status of LTD in STIM1PKO thus needs further investigation.

Unlike IP3R1 (Sugawara et al., 2013) or mGluR1 mutant mice (Aiba et al., 1994), loss of STIM1 in PNs does not lead to either neurodegeneration or ataxia. STIM1PKO mice appeared normal under standard cage conditions, and motor learning deficits appear only when they are challenged with a more complex motor task, such as walking on an accelerated rotarod. With age, their ability to perform the motor coordination task deteriorates, but no more than the deterioration found in aging WT mice (Fig. 4F). We did not observe loss of PNs at either 1 year (Fig. 2C) or 1.5 years (Fig. 3C) of age. Thus, cellular and physiological phenotypes of STIM1PKO mice appear in a milder version of phenotypes in mGluR1 mutant mice and mice with PN specific KO of the IP3R1 (Sugawara et al., 2013).

Both neuronal and glial calcium homeostasis may be affected in the STIM1PKO cerebellum

Transcriptomic analysis of PNs from STIM1PKO mice identified STIM1-dependent mechanisms as essential for maintaining the expression of multiple genes that impact Ca2+ signaling and Ca2+ homeostasis with age (Figs. 7F,G, 8-10). PNs express a range of Ca2+ channels, Ca2+ -binding proteins, Ca2+ -dependent kinases, and phosphatases that together presumably help maintain cellular Ca2+ homeostasis, regulate various Ca2+ -dependent processes, and modulate the multiple inputs received by PNs (Preztori et al., 2019). Among such downregulated genes, the IP3R1 is significant because previous studies have reported downregulation of IP3R1 expression in mouse models for SCAs (Lin et al., 2000; Serra et al., 2004; Crespo-Barreto et al., 2010; Ingram et al., 2016; Stoyas et al., 2020). STIM1PKO PNs also show reduced expression of a range of Ca2+ -binding proteins, including parvalbumin, which is expressed abundantly in PNs (Caillard et al., 2000; Schwaller et al., 2002). PNs from SCA1 patients (Vig et al., 1996) and transgenic mice expressing the human SCA1 causing gene (Vig et al., 1998) also show significant reduction in parvalbumin expression. Also downregulated in STIM1PKO PNs is calmodulin 1, another the EF hand-containing calcium-binding protein that regulates activity of several Ca2+ -regulated enzymes, such as αCaMKII and βCaMKII required for cerebellar LTD and motor learning (Hansel et al., 2006; Van Woerden et al., 2009). Calmodulin levels are also reduced in the cerebellar vermis of a spontaneously ataxic mouse (Pogo) (K. Y. Lee et al., 2011).

Downregulated genes for Ca2+ -binding proteins include S100β, which is not expressed to a detectable extent in PNs but is expressed abundantly in the Bergmann glia (Fig. 3D), whose processes enwrap synapses of PN dendrites (Yamada and Watanabe, 2002). Our data demonstrate that changes in Ca2+ homeostasis because of loss of STIM1 in PNs can impact glial gene expression by mechanism(s) that need identification. In the hippocampus, loss of S100B from glial cells affects neuronal plasticity, indicating the significance of reduced S100B on neuronal function and the importance of glia-neuron interactions (Nishiyama et al., 2002).

Gene expression changes observed in PNs from STIM1PKO mice suggest the existence of specific transcription factors whose activity is affected by altered cellular Ca2+ homeostasis. Our study does not identify such factors; but interestingly, we observed that expression of Setd6 was significantly downregulated in STIM1PKO PNs from mice 1 year of age (Figs. 7F, 10E). SETD6 is an N-lysine methyltransferase that directly methylates RelA (Chang et al., 2011; Levy et al., 2011) and PAK4 (p21-activated kinase 4) (Vershinin et al., 2016). A recent study demonstrated that knockdown of Setd6 interferes with memory consolidation, gene expression patterns, and spine morphology in the hippocampus of rat (Webb et al., 2020). Reduced expression of Setd6 and another transcription factor Tafap2b (Transcription factor AP-2 β) in PNs from STIM1PKO mice (Figs. 7F, 10E) might thus be responsible for some of the altered gene expression patterns observed in STIM1PKO PNs.
**STIM1 in Purkinje neurons impacts expression of multiple genes in common with cerebella from ataxic individuals**

A common feature of a number of downregulated genes, corresponding to different biological processes, is their known effect on maintenance of normal gait and motor coordination. These include *Itpr1* (Lin et al., 2000; Serra et al., 2004; Crespo-Barreto et al., 2010; Ingram et al., 2016; Stoyas et al., 2020), *Dlg4* (Feyder et al., 2010), *Robo2* (Gibson et al., 2014), and *Gigy2* (Giovannone et al., 2009). *IP3R1* mutant mice exhibit ataxia and dystonia (Matsumoto et al., 1996; Hisatsune et al., 2013), and human patients with loss or mutation of one copy of the *IP3R1* exhibit SCAI5, SCA29, or Gillespie’s syndrome (Hasan and Sharma, 2020). *Dlg4* encodes PSD-95 (postsynaptic density protein 95), a membrane-associated guanylate kinase and a major scaffolding protein in the excitatory postsynaptic density that regulates synaptic strength (Kim and Sheng, 2004; Funke et al., 2005; D. Cheng et al., 2006). *Dlg4* KO mice show impaired motor coordination (Feyder et al., 2010). *Robo2* is a transmembrane receptor for the secreted molecule Slit2 (Slit homolog 2 protein) and functions during axon guidance and cell migration (Ma and Tessier-Lavigne, 2007; Giovannone et al., 2012). PN-specific *Robo2*-deficient mice exhibit gait alterations (Gibson et al., 2014). Mice heterozygous for *Gigy2* exhibit motor dysfunction (Giovannone et al., 2009). GO analysis indicates that these genes belong to independent biological processes (Fig. 8A; Extended Data Fig. 8-1). Network analysis (Fig. 8B) suggests that genes, such as *Itpr1*, *Dlg4*, *Robo2*, and *Gigy2*, impact postsynaptic organization of PNs and could thus affect synaptic plasticity required for motor learning. In this context, the reduction in dendritic volume in PNs of STIM1KO mice (Fig. 11C) is also significant because reduced dendritic volume is observed in mice with KO of the SCAS causing gene β-III spectrin (Gao et al., 2011). Reduced expression of ion channels and ion pumps (*Cacng5*, *Atp1a3*, *Kcnd1l7*) as well as synaptic transmission-related genes, such as *Sytl1* and *Vamp1* in STIM1KO mice (Fig. 10B,C), indicates an effect of STIM1 KO on the density and composition of membrane channels and pumps in PNs.

Disruption of intracellular Ca^{2+} signaling in PNs has been proposed as a key mechanism in the pathogenesis of SCAs (Liu et al., 2009; Schorge et al., 2010; Kasumu and Bezprozvanny, 2012; Shimobayashi and Kapfhammer, 2017). Previous studies on SCA models identified several major biological pathways in common with STIM1KO analysis, such as calcium signaling, glutamate receptor signaling, and synaptic LTD (Serra et al., 2004; Gatchel et al., 2008; Crespo-Barreto et al., 2010; Ingram et al., 2016). Defective intracellular Ca^{2+} signaling is also associated with several other neurodegenerative disorders that affect motor coordination, such as Parkinson’s disease (Zhou et al., 2016) and Huntington’s disease (Wu et al., 2011, 2016; Nekrasov et al., 2016). Pharmaceutical agents that modulate STIM1 function and potentially restore Ca^{2+} homeostasis could thus function as therapeutics for multiple neurodegenerative disorders associated with dysregulated intracellular Ca^{2+} signaling.

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