Identification of genes regulating GABAergic interneuron maturation

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Highlights

We identify 247 genes expressed in cortical GABAergic interneurons by FACS-array.

Dgkg is dominantly expressed in somatostatin -expressing GABAergic interneurons.

Dgkg alters neurite outgrowth of GABAergic neurons.

Abbreviations

GABA: gamma-aminobutyric acid, GE: ganglionic eminence, Gad67: Glutamic acid decarboxylase-67, FACS: fluorescence activated cell sorting, Dgkg: Diacylglycerol kinase gamma, SST: somatostatin, PV: parvalbumin, Vstm2a: V-set and transmembrane domain containing 2A, Dlx: Distal less homeobox, DIV: day in vitro, PA: phosphatidic acid, DGK: Diacylglycerol kinase, GEO: gene expression omnibus, RPKM: Reads per kilobase per million

Database: The gene expression data are available in the GEO database under the accession number GSE93295.

Keywords: GABAergic interneuron, FACS-array, neurite outgrowth, Dgkg, Diacylglycerol kinase

ABSTRACT

During embryonic development, GABAergic interneurons, a main inhibitory component in the cerebral cortex, migrate tangentially from the ganglionic eminence (GE) to cerebral cortex. After reaching the cerebral cortex, they start to extend their neurites for constructing local neuronal circuits around the neonatal stage. Aberrations in migration or neurite outgrowth are implicated in neurological and psychiatric disorders such as epilepsy, schizophrenia and autism. Previous studies revealed that in the early phase of cortical development the neural population migrates tangentially from the GE in the telencephalon and several genes have been characterized as regulators of migration and specification of GABAergic interneurons. However, much less is known about the molecular mechanisms of GABAergic interneurons-specific maturation at later stages of development. Here, we performed genome-wide screening to identify genes related to the later stage by flow cytometry based-microarray (FACS-array) and identified 247 genes expressed in cortical GABAergic interneurons. Among them, *Dgkg*, a member of diacylglycerol kinase family, was further analyzed. Correlational analysis revealed that *Dgkg* is dominantly expressed in somatostatin (SST)-expressing GABAergic interneurons. The functional study of *Dgkg* using GE neurons indicated alteration in neurite outgrowth of GABAergic neurons. This study shows a new

functional role for *Dgkg* in GABAergic interneurons as well as the identification of other candidate genes for their maturation.

INTRODUCTION

In the cerebral cortex, there are two major types of neurons divided by function and the migration mode during brain development (Lodato and Arlotta, 2015). Glutamatergic pyramidal neurons in the cerebral cortex are produced in the ventricular zone and radially migrate on the radial glial processes. On the other hand, progenitors of GABAergic interneurons arise from the embryonic subcortical progenitor zone, called the ganglionic eminence (GE), and migrate to the cortex tangentially in streams located above and below the cortical plate (CP) during embryonic development in mice (Marin and Rubenstein, 2001). After termination of tangential migration at the postnatal stage, GABAergic interneurons switch to a mode of radial migration, invade the CP, and extend their neurites to construct local neural circuits (Hatanaka et al., 2016; Marin, 2013; Marin and Rubenstein, 2001). Within the cortex, the GE gives rise to several types of interneurons, including the parvalbumin (PV)-expressing fast-spiking interneurons, the somatostatin (SST)-expressing interneurons and the vasointestinal peptide (VIP)-expressing multipolar interneurons (Bandler et al., 2017; Kepecs and Fishell, 2014; Kessaris et al., 2014; Tremblay et al., 2016). GABAergic interneurons are an extremely heterogeneous group of neurons (Lodato and Arlotta, 2015). Previous studies suggest that disruption of GABAergic neuronal function leads to a variety of

neurological and psychiatric disorders such as epilepsy, schizophrenia and autism spectrum disorders (Chu and Anderson, 2015; Le Magueresse and Monyer, 2013; Marin, 2012). Therefore, proper migration and neurite outgrowth are critical for normal brain function and behavior.

During embryonic development, neurogenesis, migration and specification of cortical GABAergic interneurons are regulated by a number of transcription factors and their targets. For example, distal-less homeobox (*Dlx*) transcription factors, *Dlx1* and *Dlx2*, function at multiple stages of GABAergic maturation, NK2 homeobox 1, *Nkx2-1* acts as master regulator in promoting GE-derived interneuron fates, and combinatorial binding of NKX2-1 and LIM homeobox protein (*Lhx*), Lhx6, activates genes expressed in cortical migrating interneurons (Bandler et al., 2017; Kepecs and Fishell, 2014; Kessaris et al., 2014; Lodato and Arlotta, 2015; Marin, 2012). After reaching their proper position at the postnatal stage, GABAergic interneurons begin to mature and extend their neurites for generating axon and dendrites (Hatanaka et al., 2016; Marin, 2013; Yamasaki et al., 2010). However, much less is known about the signaling molecules involved in the postnatal migration and maturation of GABAergic interneurons.

To identify the genes involved in maturation of GABAergic interneurons, we

performed fluorescence activated cell sorting (FACS)-array analysis at postnatal day 0 (P0) by using GAD67-GFP knock-in mice (Tamamaki et al., 2003), in which a cDNA-encoding enhanced GFP (EGFP) is targeted to the locus encoding glutamic acid carboxylase 67 (GAD67). We identified 247 genes (132 up-regulated and 115 down-regulated) expressed in cortical GABAergic interneurons. Further analyses of candidate genes revealed that *Dgkg*, a member of diacylglycerol kinase family, regulates neurite outgrowth of GABAergic interneurons.

MATERIALS AND METHODS

Animals

Female mice for primary culture were purchased (ICR; CLEA Japan, Inc., Tokyo, Japan). The vaginal plug day was assigned as day 0.5 (E0.5) for mouse. For FACS-array analysis, neonatal pups were obtained by crossing GAD67-GFP$^{+/GFP}$ (Tamamaki et al., 2003) and WT mice (C57BL/6J). Mice were housed in a room with a 12 hour light / dark cycle (light on at 8:00 a.m. and off at 8:00 p.m.) and provided with *ad libitum* access to water and food. All of the protocols for animal experiments were approved by the Animal Research Committee of Osaka Bioscience Institute, Hiroshima University RIKEN Brain Science Institute, and performed in accordance with

institutional guidelines and regulations.

DNA constructs

For overexpression of *Dgkg*, *Vstm2a* and *AW551984*, PCR products containing each coding region were subcloned into a pcDNA3-3xFLAG vector. For subcellular localization analysis of *Dgkg*, a coding sequence of *Dgkg* was subcloned into C-terminus of pβActin-EGFP (gifted from S. Okabe, The University of Tokyo). To silence the expression of *Dgkg* (GenBank Accession No. NM_138650.2), 4 types of shRNA were designed by BLOCK-iT RNAi Designer (Thermo Fisher Scientific, Waltham, USA). Each sequence is shown in Supplementary Table 1. The annealed oligonucleotide was subcloned into pENTR-U6 vector (Thermo Fisher Scientific). All plasmids were purified by NucleoBond Xtra Midi kit (Takara Bio Inc., Shiga, Japan) and verified with DNA sequencing (3130 Genetic Analyzer; Thermo Fisher Scientific, Waltham, USA).

Primary neuronal culture

E14.5 pups were transferred from pregnant ICR into ice-cold Hank's balanced salt solution (HBSS) and the GE was dissected out following previously published procedures (Olsson et al., 1995). Neurons were dissociated using SUMITOMO Nerve-Cell Culture System (SUMITOMO BAKELITE CO., LTD., Tokyo, Japan) and plated

on a poly-L-lysine coated glass bottom dish (MATSUNAMI GLASS IND., LTD., Osaka, Japan) at 1 x 10^6 cells / 35 mm dish for imaging and 3 x 10^6 cells / 35 mm dish (IWAKI, Chiba, Japan) for RNA extraction. Cells were treated with 10 μM Ara C (Sigma-Aldrich, Missouri, USA) to inhibit the growth of non-neuronal cells. Neurons were cultured in B27 supplement minus (Thermo Fisher Scientific) / minimum essential medium (MEM) (Thermo Fisher Scientific) with 5% fetal bovine serum (FBS) (Biowest, Nuaille, France), 0.5 mM L-glutamine (Thermo Fisher Scientific), 100 U/ml penicillin and 100 μg/ml streptomycin (Nacalai tesque, Kyoto, Japan) at 37°C, 5% CO_2.

Cell line culture

HEK293T (Fig. 4B) and NIH3T3 (Fig. 5B) cells were cultured in Dulbecco's modified eagle medium (DMEM) (Thermo Fisher Scientific) supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C, 5% CO_2.

Transfection

HEK293T and NIH3T3 cells were plated on 6 well plates (Becton, Dickinson and Company, New Jersey, USA) at 1 x 10^6 and 2 x 10^5 cells/well 1 day before transfection, respectively. HEK293T cells and GE primary neurons were transfected by the calcium phosphate method (Sambrook and Russell, 2006). At DIV1, 3 μg of each 3 x FLAG tagged plasmid and 1 μg of pβActin-DsRed (gifted from S. Okabe) were used in this

experiment. Following 4 hours of incubation, the medium was changed to fresh one. For GE primary culture in Fig. 5A and C, 0.5 μg of pβActin-Dgkg-EGFP and pβActin-EGFP were transfected by Lipofectamine 2000, according to manufacturer's protocols (Thermo Fisher Scientific). Similarly, 3 μg of shRNA for *LacZ* and *Dgkg* were transfected by Lipofectamine 2000 in Fig. 5C. In the case of shDgkgALL, 0.75 μg of 4 types shDgkg was used. In Fig. 5B, each shRNA was transfected to NIH3T3 cells by Lipofectamine LTX according to manufacturer's protocols (Thermo Fisher Scientific).

Western blotting

Two days after transfection, cells were washed with phosphate buffered saline (PBS) and lysed in radio-immunoprecipitation assay (RIPA) buffer contained 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 1% Na-deoxycholate, 0.1% SDS, 50 mM NaF, 1 mM Na_3VO_4, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 1 mM phenylmethylsulfonyl fluoride. After sonication and centrifugation at 17,000 x g for 30 min, the supernatant was used for SDS-PAGE. Cell lysates (20 μg) were separated by using 10% polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Merck Millipore, Massachusetts, USA). The membranes were blocked with 5% skim milk (Thermo Fisher Scientific) and incubated with primary antibody in 2% skim milk for overnight at 4°C. Secondary antibodies were applied the next day for 1 hr at room

temperature. The antibodies used in this study were as follows: monoclonal anti-FLAG M2 antibody (F3165, 1:1,000; Sigma-Aldrich) and IRDye800CW Goat anti-mouse IgG (H+L) (926-32210, 1:10,000; LI-COR Biosciences, Nebraska, USA). Finally, membranes were washed by tris-buffered saline-tween (TBST) and bands were detected by LI-COR Odyssey Infrared Imaging System (LI-COR Biosciences).

Imaging and neurite-outgrowth analysis

For checking GFP signals in sorted cells, images were obtained using the Eclipse TE300 inverted microscope (Nikon, Tokyo, Japan). To measure neurite length, images were obtained using the FV300 confocal laser-scanning microscope (Olympus, Tokyo, Japan) in Fig. 4C-D, FV3000 confocal microscope (Olympus) in Fig. 5A and BZ-9000 (Keyence, Osaka, Japan) in Fig. 5C. The length of neurites was measured by using the NeuronJ plugin in ImageJ software, which allows rapid tracing and quantification of neurite length (Meijering et al., 2004).

Immunocytochemistry in primary cultured neurons from the GE

In Fig. 5A and C, immunocytochemistry by using anti-GFP antibody was performed to enhance the exogenous GFP signals. First, GE primary neurons were washed with PBS twice, followed by fixation with 2% paraformaldehyde (PFA) / 4% sucrose in 0.1 M PBS for 20 min. Then, cells were washed with PBS, permeabilized with PBS-T (0.2%

Triton X) for 5 min, incubated with a blocking solution (PBS-T containing 5% normal goat serum) for 60 min, with anti-GFP polyclonal antibody (A-11122, 1:500, Thermo Fisher Scientific) for overnight at 4 °C. Cells were washed with PBS-T, incubated with anti-rabbit IgG - Alexa 488 (A-11034, 1:1000, Thermo Fisher Scientific) for 2 hours, washed with PBS-T and mounted with VECTASHIELD (Vector Laboratories, Burlingame, CA, USA).

Tissue dissociation and flow cytometric purification of cortical GABAergic neurons

Preparation and purification of cortical GABAergic neurons were performed by modifying a previously published protocol (Lobo et al., 2006). Cerebral cortices from P0 GAD67-GFP$^{+/GFP}$ neonates were dissected in ice-cold HBSS. The cortices were minced, then, dissociated with 0.25% Trypsin, 2,250 U DNaseI in Hanks' balanced salt solution (HBSS) at 37°C for 20 min. The enzymatic reaction was stopped by adding DMEM /10% newborn bovine serum (NBS) and the tissue was triturated to prepare single cell suspension. After centrifugation, cells were resuspended in media and filtered through a 35 μm cell strainer tube (CORNING, New York, USA). Cells were sorted on a FACS-Aria (Becton, Dickinson and Company) for fluorescein-5-isothiocyanate (FITC) signals (GFP). As a negative control, cells of WT mice were used

to calibrate FITC signals. Cells with high FITC signals (>10 fold to WT) were considered GFP positive cells. In total, 1 x 10^6 cells were collected in media (DMEM/10% NBS). The cells were then lysed in 100 μl XB buffer (PicoPure RNA Isolation Kit, Thermo Fisher Scientific) and incubated at 42°C for 30 min for complete lysis. Cell lysates were stored at – 80°C until use.

Total RNA extraction, qRT-PCR and microarray analysis with sorted cortical cells

RNA extraction was performed using the PicoPure RNA Isolation Kit according to the manufacturer's instructions (Thermo Fisher Scientific). Quantity and quality of total RNA were verified with the NanoDrop spectrometer (Thermo Fisher Scientific) and Agilent 2100 Bioanalyzer (Nanochip, Agilent Technologies, California, USA). For the microarray experiment, a Chemiluminescent microarray (Applied Biosystems, CA, USA) was used as previously described (Watanabe et al., 2009). Four independent biological samples were used for this analysis. Five hundred nanograms of total RNA was used for cRNA labeling with the NanoAmp RT-IVT Labeling Kit and hybridized onto ABI Mouse Genome Survey arrays according to manufacturer's protocol (Thermo Fisher Scientific). For reliable detection, only probes with signal / noise ratio > 3 and quality flags < 5,000 were used. Normalized values, obtained by global median normalization, were used for comparison of each group. Statistical analysis was

performed with R (http://www.r-project.org). Probes expressed dominantly in GFP positive cells were extracted according to 2 criteria, 1. q-value < 0.05 (Benjamini-Hocheberg), 2. Mean fold change (GFP (+) / GFP (-)) > 3 or < 0.33. For the quantitative reverse transcription- polymerase chain reaction (qRT-PCR) experiment, 1 μg of total RNA was reverse transcribed with Super ScriptII (Thermo Fisher Scientific). Quantitative PCR was performed using SYBR Green real-time PCR system as described previously (7900HT, Thermo Fisher Scientific) (Akashi and Takumi, 2005). *Gapdh* gene was used as an internal control. Primer sequences are shown in Supplementary Table 2. Microarray data have been deposited to the GEO under the accession number GSE93295.

Bioinformatic analysis

The expression of target genes in various cortical cell types was investigated using a dataset from a previous study (GSE71585), which was obtained from the gene expression omnibus (GEO) (Tasic et al., 2016). Briefly, the authors performed single cell RNA sequencing in primary visual cortex of adult mice and identified 49 transcriptomic cell types by clustering 1,424 core cells. Reads per kilobase per million (RPKM) in Refseq were used for comparing the expressions of target genes. In this step, we omitted LOC102632463 because it did not contain Refseq. The ggplot2

package for R was used to generate violin plots (Wickham, 2009). The corrplot package for R was used to make the correlational plot. The gene lists obtained by FACS-array analysis were submitted to the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 to analyze component enrichments (Huang da et al., 2009). Three annotation categories in Gene_Ontology were selected (GOTERM_BP_DIRECT, GOTERM_CC_DIRECT, GOTERM_MF_DIRECT). Statistical analysis was done by using modified Fisher's exact test.

qRT-PCR in primary cultured neurons and NIH3T3 cells

Total RNA was extracted by using TRI Reagent (Molecular Research Center, Ohio, USA) from NIH3T3 cells and GABAergic neurons at DIV1 and 6. Reverse transcription and real time PCR were performed as described above. *Gapdh* gene was used as an internal control. Primer sequences are described in Supplementary Table 2.

Statistics

Statistical analysis was done by StatView 5.0 (SAS Institute, Cary, NC, USA) or R. Data were analyzed by Student's *t*-test, Benjamini and Hocheberg procedure and Dunnett's test. The significant criteria were set at $p < 0.05$.

RESULTS and DISCUSSION

Identification of GABAergic neuron-specific genes by FACS-array at the neonatal stage

To isolate GABAergic neurons, we utilized cerebral cortices of heterozygous GAD67-GFP knock-in mice that faithfully label GABAergic neurons (Tamamaki et al., 2003). Dissociated cerebral cortices were then separated into GFP (+) and GFP (-) cell fractions by FACS. The fluorescent signals derived from GAD67-GFP knock-in mice exhibited a bimodal distribution with higher GFP signal intensities, whereas those from wild-type (WT) mice contained only a fraction with lower GFP signal intensities (Fig. 1A). After sorting of the GFP (+) fraction, almost all of GFP (+) cells had GFP signals. On the other hand, GFP (-) cells rarely had GFP signals (Fig. 1B). We next performed qRT-PCR with neuronal marker genes. Fez family zinc finger 2 (*Fezf2*) (Inoue et al., 2004), a glutamatergic marker, and glutamic acid decarboxylase 1 (*Gad1*), a GABAergic marker, showed decreased and increased expression, respectively, in GFP (+) cells, whereas no change was observed in the expression of actin beta (*Actb*), a housekeeping gene, between GFP (+) and GFP (-) cells (Fig. 1C). These results suggest GABAergic interneurons were correctly sorted from a mixed cortical cell population by FACS. Using this system, we performed FACS-array, microarray analysis to identify genes differentially expressed in GFP (+) and GFP (-) cells (Lobo et al., 2006). We found that several known markers specific for GABAergic interneurons such as *Gad1/2*

and *Dlx* families were exclusively expressed in the GFP (+) fraction (Fig. 1D; GABAergic). Importantly, marker genes for other neuron types, glutamatergic neurons, astrocytes and oligodendrocytes, showed a drastic reduction in the GFP (+) fraction (Fig. 1D; Glutamatergic, Astrocyte, Oligodendrocyte). Comparing GFP (+) and GFP (-) cells, 132 up-regulated and 115 down-regulated genes were identified by relatively strict filtering ($q < 0.05$, fold change > 3 and < 0.33) (Table 1 and 2). Because the list of up-regulated genes contained many genes specific for GABAergic interneurons that were already published (Aoto et al., 2015; Batista-Brito et al., 2008; Blasco-Ibanez et al., 1998; Cobos et al., 2005; Esumi et al., 2008; Faux et al., 2010; Hardt et al., 2008; Hurtado-Chong et al., 2009; Li et al., 2012; Lin et al., 2015; Miyoshi et al., 2015; Murthy et al., 2014; Sugino et al., 2006; Wang et al., 2011), we concluded that the remaining genes on the list are strong candidates for GABAergic neuron-specific genes.

The top 30 of up-regulated genes in GFP (+) included *Htr3a, Gad1*, *Sst*, *Slc32a1* and *Dlx5*. These five genes are well-known markers, subtype specific peptides or transcription factors in cortical GABAergic interneurons (Table 3). The functional roles in GABAergic interneurons of eight genes, *Nxph1*, *Nrxn3*, *Penk*, *Erbb4*, *Igf1*, *Ackr3*, *Prox1* and *Asic4*, have been investigated in previous studies (Aoto et al., 2015; Batista-Brito et al., 2008; Blasco-Ibanez et al., 1998; Hardt et al., 2008; Hurtado-Chong

et al., 2009; Li et al., 2012; Lin et al., 2015; Miyoshi et al., 2015; Murthy et al., 2014; Sugino et al., 2006; Wang et al., 2011). Among the remaining 17 genes (Fig. 2A), *Maf*, *Rpp25*, *Drd2*, *Adarb2*, *Tac1*, *Dgkg*, *Synpr*, *Npy2r* and *Rxrg* were independently reported to be expressed in GABAergic neurons, but their function at the postnatal stage in GABAergic interneurons was still unknown (Batista-Brito et al., 2008; Dolle et al., 1994; Hardt et al., 2008; McKinsey et al., 2013; Miyoshi et al., 2015; Perreault et al., 2012; Sugino et al., 2006). We performed gene ontology (GO) analysis of differentially expressed genes to characterize differentially expressed genes in GABAergic interneurons, by using Database for Annotation, Visualization and Integrated Discovery (DAVID) (Fig. 2B, C). The terms for synapse development (synapse, postsynaptic membrane and cell junction) were enriched in up-regulated genes and proliferation (cell cycle and cell division) in down-regulated genes. These data suggest that GABAergic interneurons at P0 stop proliferation and start maturation for constructing the local network.

To obtain further insights, we studied the subtype-specificity of target genes. From neuropeptide expression data, cortical interneurons can be largely classified into 3 groups: parvalbumin (*Pv*), somatostatin (*Sst*) and serotonin receptor 3a (*Htr3a*) expressing interneurons, and these 3 groups account for nearly 100% of cortical

GABAergic interneurons (Tremblay et al., 2016). Furthermore, a recent elegant study reported cortical GABAergic cells can be divided into more detailed components by using single cell RNA sequencing (Tasic et al., 2016). Therefore, we applied a bioinformatic approach to identify the subclass of target genes. First, we analyzed the expression of our 16 target genes (LOC102632463 was omitted from 17 target genes because it did not contain Refseq) in 49 transcriptomic cell types found in the previous study (obtained from GEO, GSE71585) (Tasic et al., 2016). Although several target genes such as *Neb* or *Drd2* showed low expression levels in adult cortical neurons, most of the target genes still exhibited GABAergic neuron specificities (Fig. 3A: *Smad3* to *Igtp* [from left to right]: GABAergic neurons, L2 *Ngb* to L6b *Rgs12*: glutamatergic neurons, *Astro Aqp4*: astrocytes, *OPC Pdgfra*: oligodendrocyte precursor cells, *Oligo 96*Rik* and *Opalin*: oligodendrocytes, Micro *Ctss*: microglia, Endo *Xdh*: endothelial cells, SMC *Myl9*: smooth muscle cells). In this study, we focused on 3 genes, *Dgkg*, *Vstm2a* and *AW551984,* because the function of *AW551984* and *Vstm2a* is unknown and *Dgkg* seems to be a kinase unique to GABAergic neurons without a known function in neurons. These three genes showed high expression, especially in SST-positive GABAergic interneurons (Fig. 3A). Therefore, we further performed correlational analysis of these three genes in the dataset (Fig. 3B). *Dgkg* and *AW551984* showed a

relatively strong correlation with *Sst* expression and *Vstm2a* expression was correlated with *Vip* while there were no positive correlation between these 3 genes and other neuronal or glial marker genes (Fig. 3B).

The expression of three target genes is increased during maturation of GABAergic neurons.

The expression level of *Gad1* is known to be developmentally regulated (Kim et al., 2015). Therefore, the above target genes may be related to maturation of GABAergic interneurons. We thus checked whether the regulation of their expression is dependent on maturation of GABAergic interneurons by using *in vitro* primary culture. Like the expression of *Gad1*, mRNA expression levels of three genes (*Dgkg*, *Vstm2a* and *AW551984*) in cultured GABAergic neurons at DIV6 were increased compared to those at DIV1, although the difference for *AW551984* was not statistically significant (Fig. 4A). Thus, these three genes may regulate maturation of cortical GABAergic interneurons.

Dgkg promotes neurite outgrowth of GABAergic neurons

To identify the function of the three genes, we overexpressed them in GABAergic neurons and investigated neurite outgrowth. We first constructed FLAG-tagged target proteins and validated them by Western blot in HEK293T cells (Fig. 4B). Each

construct showed a band with the expected size. For overexpression in GABAergic neurons, we prepared primary cultured neurons derived from the GE and introduced these plasmids (Fig. 4C and D). All samples extended their neurite in a time dependent manner from DIV2 to DIV8. At DIV2, the neurite length of neurons expressing each of the three genes was not different from that of control. However, from DIV4 to DIV8, *Dgkg* consistently promoted neurite outgrowth at each time point. *AW551984* showed a trend of increasing neurite outgrowth, particularly at DIV6. *Vstm2a* did not influence neurite outgrowth at any time point (Fig. 4C and D).

Next, we checked subcellular localization of Dgkg protein in GABAergic primary neurons. In cell lines, Dgkg is reported to be localized in plasma membrane and cytoplasmic space (Tanino et al., 2013). Similar to this report, Dgkg-EGFP was predominantly localized in cytoplasm and in neurites, suggesting Dgkg plays a role in maturation of GABAergic neurons. To support of this idea, we further examined whether knockdown of *Dgkg* expression causes delayed maturation. First, we designed 4 types of short hairpin RNA (shRNA) for *Dgkg* (shDgkg) and validated them in NIH3T3 cells by qRT-PCR. Compared to control (shLacZ), mRNA expression of *Dgkg* was decreased in all of shDgkg treated samples (Fig. 5B). We then introduced these shRNA into GE primary neurons and measured the length of neurite. At both time

points of DIV4 and DIV7, the neurite length of shDgkg treated neurons was significantly decreased compared to that of shLacZ control (Fig. 5C).

As a possible mechanism, the regulation of neurite length by Dgkg may be due to phosphatidic acid (PA). A diacylglycerol kinase (DGK) family including *Dgkg* is known to phosphorylate diacylglycerol to produce PA (Wang et al., 2006). PA is a pleiotropic lipid and plays various roles as a second messenger, such as cell proliferation, vesicle membrane trafficking and cytoskeletal organization (Wang et al., 2006). In neurons, PA is reported to promote neurite outgrowth, dendritic extension / branching and growth corn motility by modulating cytoskeletal organization via regulation of key proteins such as protein kinase A or RA-RhoGAP (Rap-activated Rho GTPase-activating protein) (Ammar et al., 2014; Kurooka et al., 2011; Song and Poo, 1999; Zhu et al., 2016). We hypothesize that *Dgkg* regulates neurite outgrowth via PA in GABAergic interneurons. Alternatively, the kinase activity independent pathway may be related to neurite extension in GABAergic interneurons. Tanino *et al.* reported that filopodia-like protrusions are induced by *Dgkg* in N1E-115 neuroblastoma and its effect is independent of its kinase activity (Tanino et al., 2013), although this molecular signal is still unknown. It will be important to investigate GABAergic interneurons in *Dgkg* KO mice. Although no study on *Dgkg* KO mice has been reported so far, the KO mice

of *Dgkb,* a subfamily member, show decreased neurite length (Shirai et al., 2010). Because *Dgkg* is included in a type I DGK family with *Dgka* and *Dgkb*, they may share their function to regulate neurite outgrowth using the same signaling pathway (Merida et al., 2008).

The long-term imaging using organ culture preparations of perinatal stages demonstrates that at the earlier stage, cortical interneurons migrate, as development proceeds, exhibit sea urchin-like morphology, alternating between extended and retracted short processes, and cease the migration, and then suddenly extend a long axon-like process at the around P0 stage (Hatanaka et al., 2016; Yamasaki et al., 2010), which is equivalent to the time when samples were collected for FACS-array in this study. We hypothesize that *Dgkg* may play a role in this maturation step of GABAergic interneurons.

We revealed that *Dgkg* is dominantly expressed in SST-expressing GABAergic neurons (Fig. 3A and B). SST interneurons, one of the major class of inhibitory neurons in the cerebral cortex, are often characterized by highly dense wiring into the local network such as Martinotti cells (Tremblay et al., 2016; Urban-Ciecko and Barth, 2016). This dense wiring projects not only intra but also inter cortical layers, providing strong inhibition to many cell types across the cortical layers. From this morphological

view, proper maturation by *Dgkg* may be an important process for the intact morphology or function of SST-expressing GABAergic interneurons.

In summary, we identified 247 genes expressed in cortical GABAergic interneurons including *Dgkg* by FACS-array. Bioinformatics revealed *Dgkg* may play a role in SST-expressing GABAergic interneurons. We also found gain/loss of function of *Dgkg* alters neurite outgrowth in GABAergic neurons. These results suggest that *Dgkg* has a vital regulatory role in the construction of cortical interneuron networks.

ACKNOWLEDGEMENTS

We thank S. Okabe for providing the plasmid and the technical staff of the Takumi Lab for technical assistance. This work was supported in part by KAKENHI (16H06316, 16H06463, 16K13110, 16K19066), Japan Society of Promotion of Science and Ministry of Education, Culture, Sports, Science, and Technology, CREST, Japan Science and Technology Agency, Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP, and the Takeda Science Foundation. KF was Japan Society for the Promotion of Science (JSPS) Research Fellow.

Author contributions

KF and KT conducted the experiments KF, KT, TN and Tsuyoshi Toya performed data analysis. YY provided materials. KF, KT and Toru Takumi wrote the paper. Toru Takumi supervised the project.

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Table 1. Up-regulated genes in GABAergic neurons (132 genes)

Gene symbol	Mean ratio	q-value	Gene symbol	Mean ratio	q-value
Htr3a	65.35	0.008	Mkx	5.88	0.011
Gad1	59.81	0.010	Ngef	5.72	0.038
Maf	59.49	0.009	A830011K09Rik	5.67	0.045
Neb	58.88	0.031	Rgs2	5.58	0.029
Sst	46.11	0.007	Cxcr4	5.49	0.005
Gm14204	43.57	0.038	Akap5	5.38	0.008
Slc32a1	39.36	0.032	4933416M06Rik	5.33	0.028
Rpp25	39.02	0.007	Fgd5	5.24	0.043
Drd2	33.11	0.038	Kcnip2	5.23	0.017
Adarb2	32.30	0.003	1810011O10Rik	5.20	0.006
Tac1	30.51	0.029	C230057M02Rik	5.18	0.038
Nxph1	28.28	0.015	Plcxd3	5.17	0.040
Dlgap2	27.54	0.004	Slc10a4	5.10	0.030
Nrxn3	26.29	0.030	Kirrel3	5.06	0.036
Penk	23.12	0.047	Vax1	5.05	0.042
Erbb4	21.10	0.019	Dpp10	5.03	0.023
Igf1	18.66	0.008	Camk1g	5.02	0.030
Oprm1	18.59	0.018	Rara	5.01	0.048
Dgkg	18.01	0.038	Gpr176	4.96	0.025
Ackr3	17.06	0.014	Bcl11b	4.95	0.010
Prox1	14.69	0.031	Rnf128	4.91	0.014

Dlx5	14.29	0.033	Cntnap2	4.91	0.034
Synpr	14.21	0.019	Foxp1	4.78	0.019
LOC102632463	14.09	0.028	Kcnt1	4.67	0.045
Asic4	14.06	0.029	Dclk3	4.63	0.012
Vstm2a	13.42	0.014	Lgi1	4.53	0.002
AW551984	12.99	0.006	4931423N10Rik	4.44	0.029
Npy2r	12.98	0.003	E230016M11Rik	4.43	0.030
Sntg2	12.75	0.033	Meg3	4.34	0.015
Rxrg	12.46	0.012	Ebf1	4.31	0.028
Chrm3	12.24	0.048	Zfp618	4.29	0.038
Fam65b	11.90	0.005	Sowaha	4.29	0.038
Foxp2	11.84	0.009	Cxcl14	4.25	0.038
Kcns3	11.79	0.026	Gm15345	4.21	0.042
Tcerg1l	11.67	0.038	Slitrk3	4.21	0.045
Zfp503	11.57	0.021	Fxyd7	4.12	0.048
Gria1	11.11	0.038	Ap1s2	4.10	0.036
Spock3	10.95	0.021	Tnfrsf13b	4.00	0.043
Isl1	10.64	0.016	Mirg	3.99	0.020
Ptprm	10.53	0.029	Fut9	3.79	<0.001
Zfhx3	10.32	0.040	Unc13c	3.71	0.008
Cacng3	10.30	0.034	Meis1	3.69	0.048
Kcnc2	10.25	0.005	Gng7	3.67	0.038
Grik1	10.16	0.003	Et14	3.67	0.042
Pde4dip	9.86	0.002	Zfp641	3.67	0.021

Dlx2	9.76	0.048	Vstm2l	3.61	0.015
Ptchd4	9.50	0.023	Lqi1	3.61	0.012
Grik3	8.94	0.010	Bend4	3.49	0.029
Cntnap3	8.89	0.034	AI593442	3.49	0.022
Sox2ot	8.22	0.028	Commd8	3.48	0.047
Sp8	8.12	0.041	Drp2	3.39	0.023
Pnoc	8.00	0.023	Kirrel	3.37	0.045
Tshz1	7.95	0.043	Fgf12	3.32	0.028
Arl4d	7.71	0.044	Epb41l4b	3.32	0.024
P2ry1	7.44	0.036	Kctd12	3.31	0.037
Zfp536	6.94	0.005	Peg10	3.30	0.018
Sorcs3	6.92	0.038	Cacna2d2	3.30	0.032
Cacna2d3	6.85	0.021	Cxxc4	3.29	0.021
Olfm3	6.78	0.029	Trerf1	3.25	0.018
Gng4	6.76	0.009	Rasl10b	3.22	0.014
Slc27a2	6.28	0.048	Pacsin1	3.19	0.029
Gm20754	6.27	0.006	Cdc42ep5	3.19	0.045
Elfn1	6.27	0.029	Dlgap1	3.18	0.005
Syt13	6.05	0.041	Trpc7	3.11	0.029
Gabrg3	6.02	0.009	E130308A19Rik	3.09	0.050
Npy	5.97	0.014	Gm25145	3.01	0.032

Table 2. Down-regulated genes in GABAergic neurons (115 genes)

Gene symbol	Mean ratio	q-value	Gene symbol	Mean ratio	q-value
Satb2	0.02	0.003	Spc25	0.20	0.042
Neurod6	0.02	0.016	Ptprz1	0.21	0.029
Bhlhe22	0.03	0.002	Lcorl	0.21	0.025
Tbr1	0.03	0.019	Kif26b	0.21	0.019
Celsr1	0.03	0.037	Sybu	0.21	0.016
Tnc	0.03	0.010	Rgs5	0.22	0.019
Zfp36l1	0.04	0.013	Myo6	0.22	0.009
Lhx2	0.04	0.016	Echdc2	0.22	0.037
Fabp7	0.04	0.047	Fam210b	0.22	0.026
Hbb-bt	0.05	0.004	2810417H13Rik	0.23	0.021
Hba-a1	0.05	0.014	Slc12a4	0.23	0.041
Cd9	0.05	0.044	Fat1	0.23	0.012
Hbb-bs	0.06	0.004	Pcdh20	0.23	0.011
Slco1c1	0.06	0.008	Plat	0.23	0.029
Nrarp	0.07	0.048	Ncaph	0.24	0.005
Smpdl3a	0.07	0.043	Rrm2	0.24	0.025
Mmd2	0.07	0.031	Ttyh1	0.24	0.034
4933405E24Rik	0.07	0.036	Tpx2	0.24	0.023
Qk	0.08	0.010	Cck	0.24	0.017
Sox21	0.08	0.018	Nckap5	0.25	0.031
Gas1	0.08	0.041	Magt1	0.25	0.048

Dok5	0.08	0.010	Lingo1	0.25	0.036
Abhd4	0.09	0.016	Lpp	0.25	0.039
Zbtb18	0.09	0.002	Nfia	0.26	0.028
Nr4a3	0.09	0.010	Gldc	0.26	0.037
Dmrta2	0.10	0.009	Phgdh	0.26	0.030
Clstn2	0.10	0.029	Insm1	0.26	0.048
Fkbp9	0.10	0.038	Adamts3	0.27	0.011
Ndrg1	0.10	0.006	Dusp16	0.27	0.033
Palmd	0.10	0.038	Csmd2	0.27	0.014
Vim	0.11	0.018	Prc1	0.28	0.009
Acot1	0.12	0.042	Fam49a	0.28	0.030
Luzp2	0.12	0.009	Cacna2d1	0.28	0.023
Prex1	0.12	0.005	Hmgn5	0.28	0.021
Fn1	0.13	0.045	Tgfb	0.29	0.029
Ptn	0.13	0.004	Crot	0.29	0.040
Tjp2	0.13	0.002	Dusp6	0.29	0.011
Wwc1	0.14	0.002	Fgd4	0.29	0.040
Wwtr1	0.14	0.046	Nlgn1	0.29	0.002
Fgfr3	0.15	<0.001	Pygb	0.29	0.033
Pou3f2	0.15	0.018	Cks2	0.29	0.041
Dab1	0.15	0.041	Slc27a1	0.30	0.014
Nrp1	0.15	0.020	Cyp20a1	0.30	0.011
Mmp14	0.16	0.032	Kif20a	0.31	0.002
Sertm1	0.17	0.002	Ckap2	0.31	0.019

Megf10	0.17	0.039	Ttc28	0.31	0.042
Inhba	0.18	0.031	Maml2	0.31	0.037
Hs3st1	0.18	0.018	Pou3f1	0.32	0.017
Nrg1	0.18	0.034	Cdc25c	0.32	0.023
Ccna2	0.18	0.003	Rbm24	0.32	0.034
Tns3	0.18	0.023	Nusap1	0.32	0.038
Klf3	0.18	0.039	Psat1	0.32	0.038
Dync1i1	0.18	0.029	Cenpa	0.32	0.044
Cyp7b1	0.19	0.040	Aspm	0.32	0.032
Hey1	0.19	0.012	Lrig1	0.33	0.048
Ltbp1	0.20	0.038	Ak3	0.33	0.045
Mfap4	0.20	0.038	Mir99ahg	0.33	0.012
Nos1	0.20	0.002			

Table 3. Top 30 genes differentially expressed in GFP-positive and -negative cells analyzed by FACS-array

Up-regulated			Down-regulated		
Gene symbol	**Mean ratio**	**q-value**	**Gene symbol**	**Mean ratio**	**q-value**
Htr3a	65.35	0.008	Satb2	0.02	0.003
Gad1	59.81	0.010	Neurod6	0.02	0.016
Maf	59.49	0.009	Bhlhe22	0.03	0.002
Neb	58.88	0.031	Tbr1	0.03	0.019
Sst	46.11	0.007	Celsr1	0.03	0.037
Gm14204	43.57	0.038	Tnc	0.03	0.010
Slc32a1	39.36	0.032	Zfp36l1	0.04	0.013
Rpp25	39.02	0.007	Lhx2	0.04	0.016
Drd2	33.11	0.038	Fabp7	0.04	0.047
Adarb2	32.30	0.003	Hbb-bt	0.05	0.004
Tac1	30.51	0.029	Hba-a1	0.05	0.014
Nxph1	28.28	0.015	Cd9	0.05	0.044
Dlgap2	27.54	0.004	Hbb-bs	0.06	0.004
Nrxn3	26.29	0.030	Slco1c1	0.06	0.008
Penk	23.12	0.047	Nrarp	0.07	0.048
Erbb4	21.10	0.019	Smpdl3a	0.07	0.043
Igf1	18.66	0.008	Mmd2	0.07	0.031
Oprm1	18.59	0.018	4933405E24Rik	0.07	0.036
Dgkg	18.01	0.038	Qk	0.08	0.010

Ackr3	17.06	0.014	Sox21	0.08	0.018
Prox1	14.69	0.031	Gas1	0.08	0.041
Dlx5	14.29	0.033	Dok5	0.08	0.010
Synpr	14.21	0.019	Abhd4	0.09	0.016
LOC102632463	14.09	0.028	Zbtb18	0.09	0.002
Asic4	14.06	0.029	Nr4a3	0.09	0.010
Vstm2a	13.42	0.014	Dmrta2	0.10	0.009
AW551984	12.99	0.006	Clstn2	0.10	0.029
Npy2r	12.98	0.003	Fkbp9	0.10	0.038
Sntg2	12.75	0.033	Ndrg1	0.10	0.006
Rxrg	12.46	0.012	Palmd	0.10	0.038

Supplementary Table 1. Target sequences of shRNAs for Dgkg knockdown

shRNA name	Target sequence (5'-3')
shDgkg#1	GCGACCTCAGGATAAGCTAGA
shDgkg#2	GGATCAGATTGTGAGCCAAAT
shDgkg#3	GCTGCAAGGCATGGACTATGA
shDgkg#4	GGATGACGTTTCACCGCAAAT

Supplementary Table 2. Primer sets for qRT-PCR

Primer name	Sequence (5'-3')
Actb Fw	CGTGCGTGACATCAAAGAGAA
Actb Rv	TGGATGCCACAGGATTCCAT
AW551984 Fw	TCTATGGATTTGGAGCCACCC
AW551984 Rv	CCGCCAACAAACGCTTTACTT
Dgkg Fw	AACTTCACAAAGCACCCACCG
Dgkg Rv	AGGATTTTGGTCAAGCTGCCC
Gad1 Fw	TGGAGCAGATCCTGGTTGACT
Gad1 Rv	GCCATTCACCAGCTAAACCAA
Gapdh Fw	ACGGGAAGCTCACTGGCATGGCCTT
Gapdh Rv	CATGAGGTCCACCACCCTGTTGCTG
Fezf2 Fw	ACCCAAAAACTTCACCTGCGA
Fezf2 Rv	ACACAAACGGTCTAGCTCCGGT
Vstm2a Fw	ACAACTCTGCCAACCAACGAA
Vstm2a Rv	TGGCTTGTAGCTATCCTCGCA

FIGURE LEGENDS

Figure 1. Purification and expression analysis of cortical GABAergic interneurons by FACS-array

(A) Fluorescence activated cell sorting (FACS) analysis of cortical cells in WT and GAD67-GFP$^{+/GFP}$ neonatal pups. Histograms show cell counts (vertical axis) and GFP signal intensity (horizontal axis). **(B)** Representative images of cortical cells before sorting, and GFP (-) and GFP (+) fractions after sorting. Scale bar indicates 500 μm. **(C)** Relative mRNA expression of *Fezf2* (Glutamatergic neuronal marker), *Actb* (housekeeping gene) and *Gad1* (GABAergic neuronal marker) determined by qRT-PCR. Vertical axis indicates relative fold change (log base 10) of each gene calculated by the ratio of post-sorted GFP (+) to GFP (-). Each bar indicates independent biological replicates. *Gapdh* gene was used as an internal control. **(D)** Gene expression level of cell type specific markers by FACS-array. Vertical axis indicates relative fold change (log base 10) of each gene calculated by GFP (+) / GFP (-) ratio. Error bars are means ± s.e.m. N = 4 for each cell type (GFP (+) and GFP (-)).

Figure 2. mRNA expression of target genes and ontology analysis

(A) Relative mRNA expression levels of 17 candidate genes determined by FACS-array. N = 4 for each cell type. Error bars are means ± s.e.m. **(B, C)** Gene ontology

analysis of differentially expressed genes ($q < 0.05$, fold change > 3 or < 0.33). Enriched ontology terms among differentially expressed genes are listed in order larger significance ($p < 0.05$, modified Fisher's exact test). Red, green and black bars indicate cellular component, biological process and molecular function, respectively. The blue line represents $p = 0.05$.

Figure 3. Expression profiling in 49 clustered cortical cells and correlation patterns of target genes

(A) Expression profiles of 16 candidate genes in various cortical cells defined in a previous study (Tasic et al., 2016). The violin plots represent distribution of each mRNA expression. Each cortical cell type (49 types), listed in the bottom panel, was clustered by using single cell RNA sequencing with 1,424 cells in the primary visual cortex. Dots in violin plots represent median of RPKM. **(B)** Correlational analysis by using a previous dataset (Tasic et al., 2016). Correlations between 3 target genes (*Dgkg*, *Vstm2a* and *AW551984*) and each marker gene are shown. Warmer and colder colored circles indicate positive and negative correlation, respectively. The areas of circles show the absolute value of its corresponding correlation coefficients. *Snap25* (pan-neuronal); *Gad1* (pan-GABAergic); *Vip*, *Sst* and *Pvalb* (GABAergic subtype); *Slc17a7* (pan-glutamatergic); *Rorb* (mostly layer 4 and 5a); *Foxp2* (layer 6); *Aqp4* (astrocytes);

Pdgfra (oligodendrocyte precursor cells, OPCs); *Mog* (oligodendrocytes); *Itgam* (microglia); *Flt1* (endothelial cells); and *Bgn* (smooth muscle cells, SMC).

Figure 4. Functional evaluation of target genes, *Dgkg*, *Vstm2a* and *AW551984*

(A) Relative mRNA expression levels in cultured GABAergic neurons were determined by qRT-PCR. *Gapdh* gene was used as an internal control. Mean expression of DIV1 was set to 1. N = 3 (DIV1) and 5 (DIV6). **p < 0.01 (Student's *t*-test). Error bars are means ± s.e.m. **(B)** Immunoblot showing detection of exogenous proteins by using anti-FLAG antibody. Arrowheads on the left side indicate the predicted molecular mass of AW551984, DGKG and VSTM2A from top to bottom. **(C)** Representative images of cultured GE neurons overexpressing each plasmid containing the coding region at DIV2-8. Each neuron was visualized with cotransfection of pβActin-DsRed. Scale bar, 100 μm. **(D)** Quantitative analysis of neurite length induced by target genes. GE primary cultured neurons were transfected with target genes and pβActin-DsRed on DIV1. Total neurite length in a cell was measured by NeuronJ, a plugin of ImageJ. Each neurite length was measured at DIV2-8. N = 123, 102, 109, and 82 (Control), 74, 90, 84, and 80 (*Dgkg*), 66, 54, 39, and 35 (*Vstm2a*) and 38, 40, 33, and 28 (*AW551984*) at DIV2, 4, 6, and 8, respectively. **p < 0.01 (Dunnett's test). Error bars are means ± s.e.m.

Figure 5. Effect of Dgkg knockdown in GABAergic neurons

(A) Subcellular localization of Dgkg proteins in primary cultured neurons from the GE. Each plasmid including pβActin-EGFP (left panel) and pβActin-Dgkg fused to EGFP (right panel) was transfected to GE neurons (DIV1). Immunocytochemistry by using anti-EGFP antibody was performed to enhance the signal intensity of EGFP (DIV4). Scale bars indicate 20 μm. **(B)** NIH3T3 cells were transfected with 4 types of shRNA targeting Dgkg. After 48 hours of transfection, cells were used for qRT-PCR to validate knockdown effect. *Gapdh* gene was used as an internal control. "DgkgALL" means cells treated with all 4 type shRNAs for *Dgkg*. The *Dgkg* expression level treated with shRNA for *LacZ* gene was set to 1. **(C)** Quantitative analysis of neurite length induced with shRNAs for *Dgkg*. GE primary cultured neurons were transfected with each shRNA and pβActin-EGFP at DIV1. Immunocytochemistry by using anti-EGFP antibody was performed to enhance the signal intensity of EGFP at DIV4 and 7. Total neurite length in a cell was measured by NeuronJ, a plugin of ImageJ. At DIV4, 123 (Control; shLacZ), 108 (shDgkg#1), 113 (shDgkg#3) and 105 (shDgkgALL) and at DIV7, 69 (Control; shLacZ), 103 (shDgkg#1), 104 (shDgkg#3) and 100 (shDgkgALL) cells were used for this analysis. Multiple comparison test on each time point was performed (**$p < 0.01$, Dunnett's test). Error bars are means ± s.e.m.

Table 1. Upregulated genes in GABAergic neurons

List of genes that meet 2 criteria including $q < 0.05$ and mean ratio GFP (+) / GFP (-) > 3.

Table 2. Down-regulated genes in GABAergic neurons

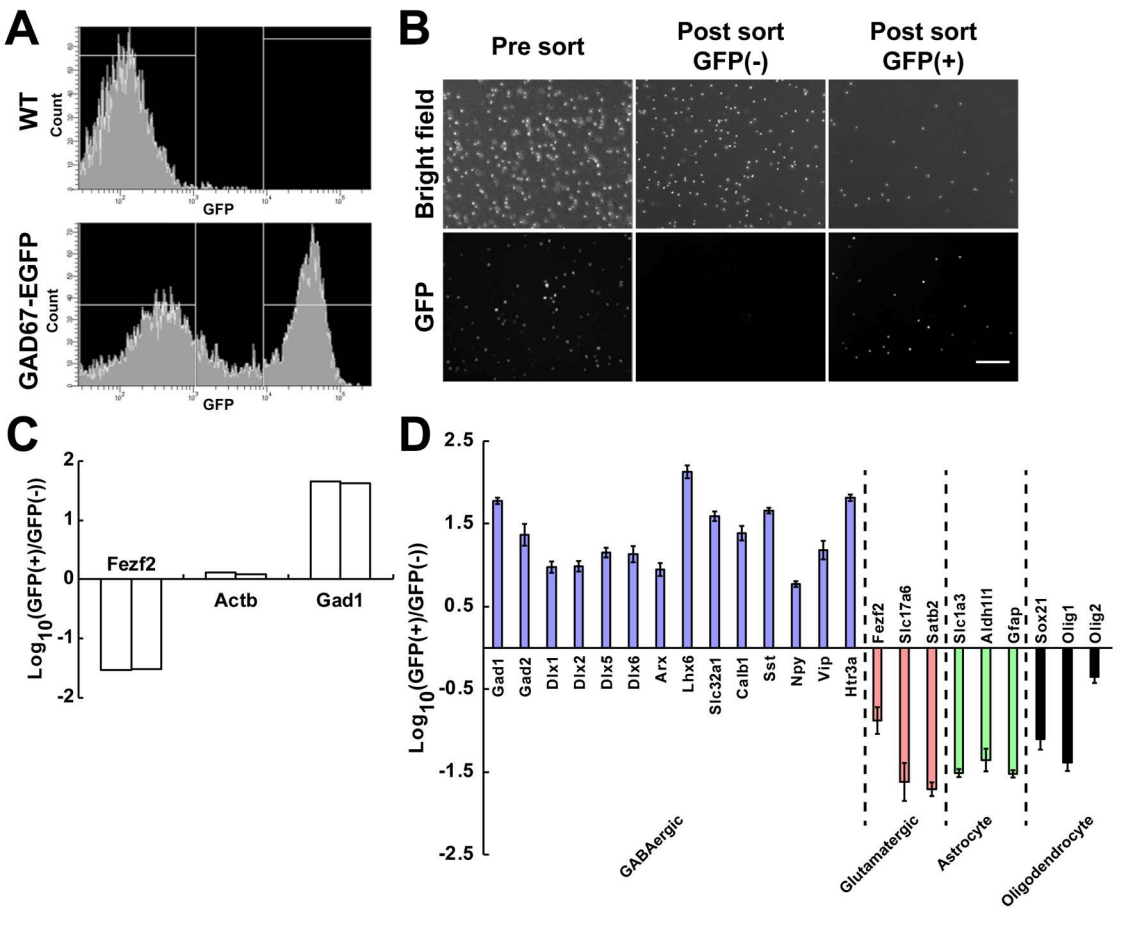
List of genes that meet 2 criteria including $q < 0.05$ and mean ratio GFP (+) / GFP (-) < 0.33.

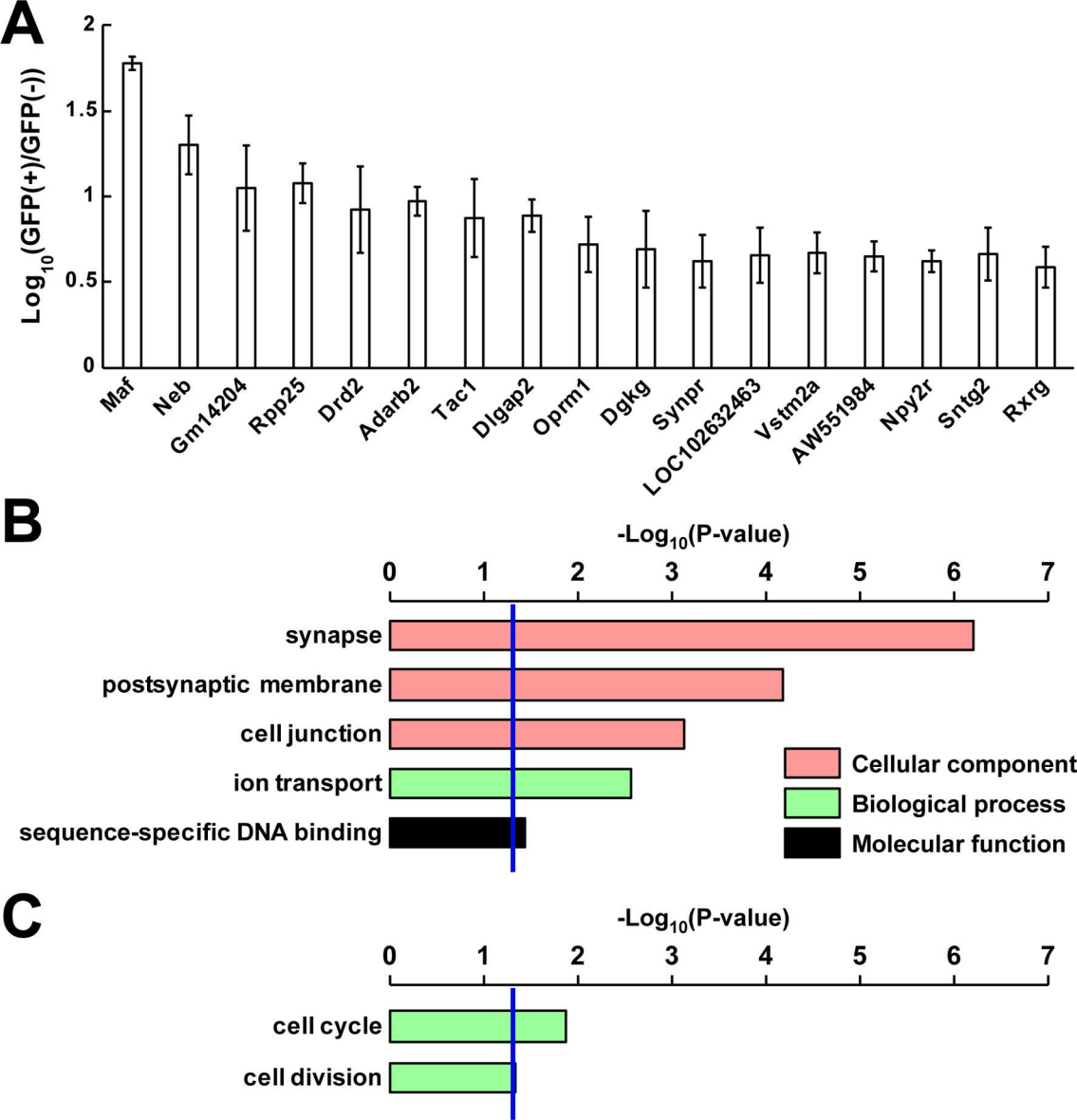
Table 3. Top 30 genes differentially expressed in GFP-positive and -negative cells analyzed by FACS-array

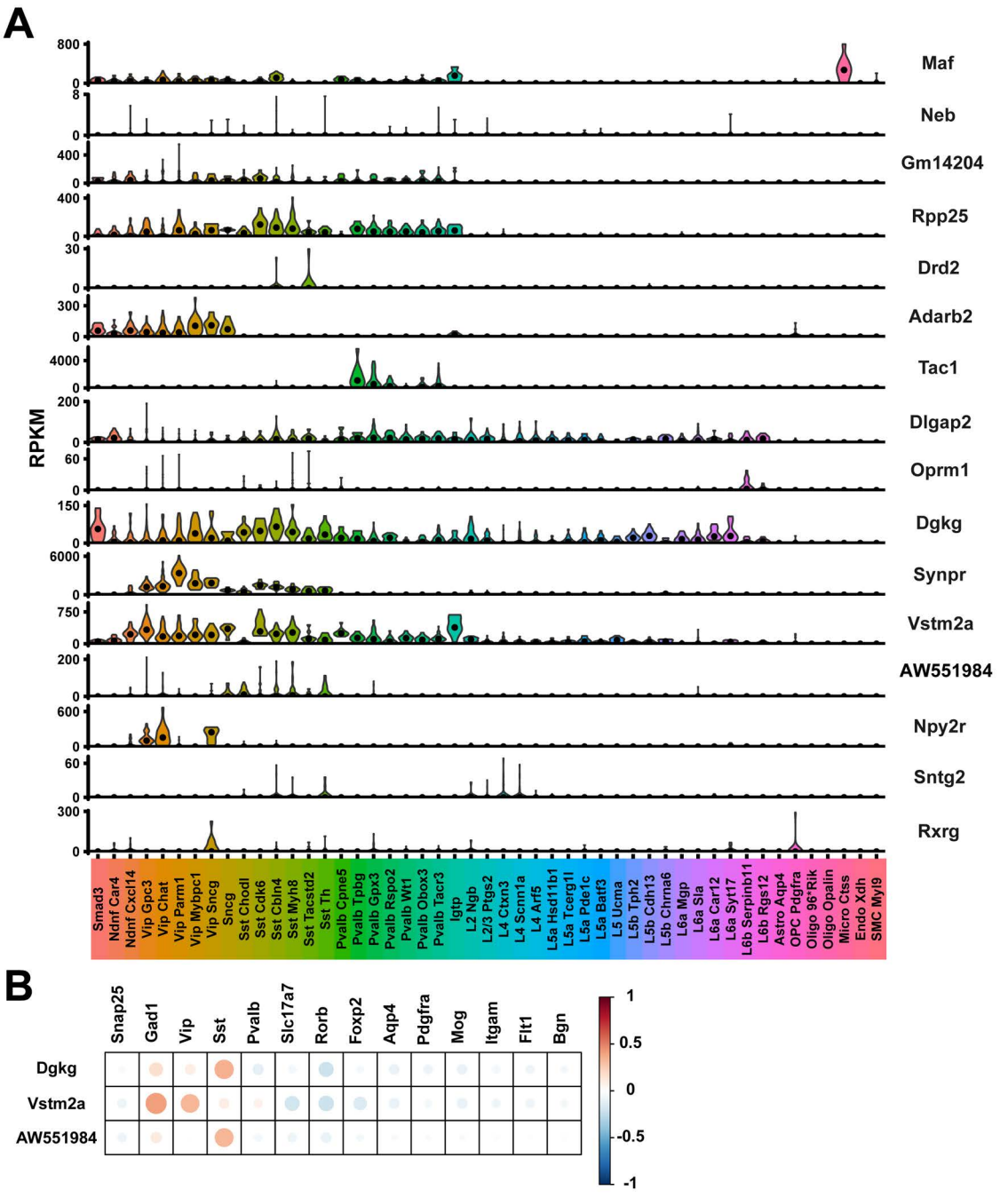
Four independent samples were used in this experiment. The p value was corrected using the Benjamini-Hochberg procedure with FDR (q value) < 0.05.

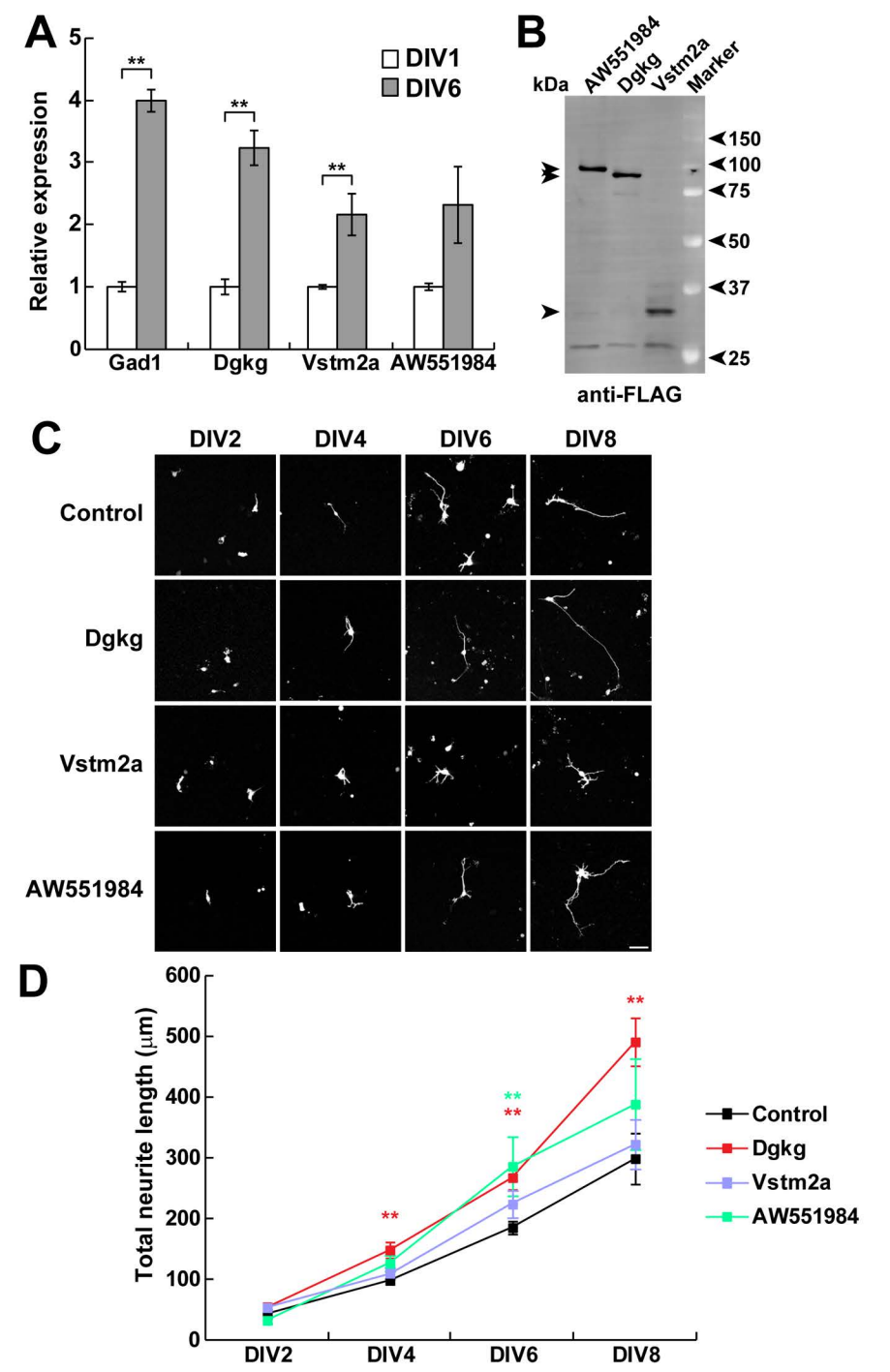
Table S1. Target sequences of shRNAs for Dgkg knockdown

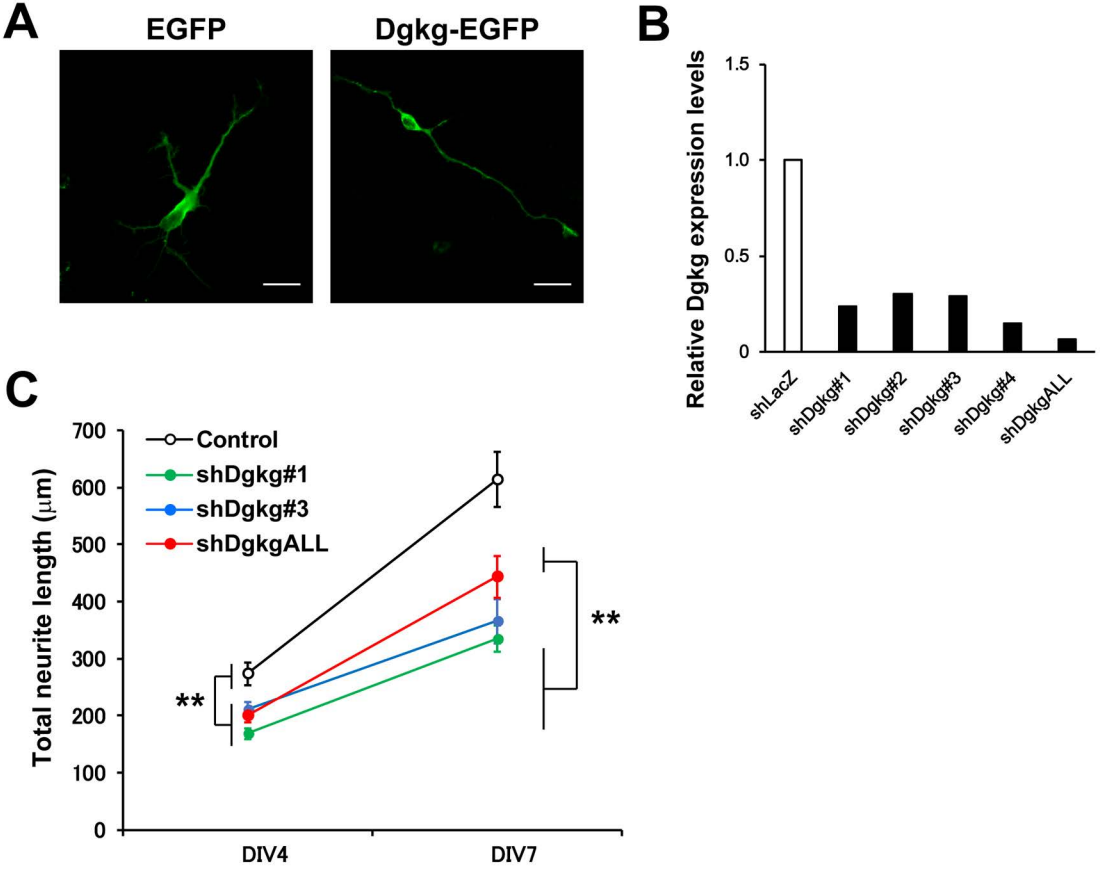
Table S2. Primer sets for qRT-PCR


A
WT
Count
GFP
GAD67-EGFP
Count
GFP
B
Pre sort
Post sort GFP(-)
Post sort GFP(+)
Bright field
GFP
C
Log10(GFP(+)/GFP(-))
Fezf2
Actb
Gad1
D
Log10(GFP(+)/GFP(-))
Gad1
Gad2
Dlx1
Dlx2
Dlx5
Dlx6
Arx
Lhx6
Slc32a1
Calb1
Sst
Npy
Vip
Htr3a
Fezf2
Slc17a6
Satb2
Slc1a3
Aldh1l1
Gfap
Sox21
Olig1
Olig2
GABAergic
Glutamatergic
Astrocyte
Oligodendrocyte


A
Log10(GFP(+)/GFP(-))
2
1.5
1
0.5
0
Maf
Neb
Gm14204
Rpp25
Drd2
Adarb2
Tac1
Dlgap2
Oprm1
Dgkg
Synpr
LOC102632463
Vstm2a
AW551984
Npy2r
Sntg2
Rxrg
B
-Log10(P-value)
0
1
2
3
4
5
6
7
synapse
postsynaptic membrane
cell junction
ion transport
sequence-specific DNA binding
Cellular component
Biological process
Molecular function
C
-Log10(P-value)
0
1
2
3
4
5
6
7
cell cycle
cell division


A
RPKM
Maf
Neb
Gm14204
Rpp25
Drd2
Adarb2
Tac1
Dlgap2
Oprm1
Dgkg
Synpr
Vstm2a
AW551984
Npy2r
Sntg2
Rxrg
Smad3
Ndnf Car4
Ndnf Cxcl14
Vip Gpc3
Vip Chat
Vip Parm1
Vip Mybpc1
Vip Sncg
Sncg
Sst Chodl
Sst Cdk6
Sst Cbln4
Sst Myh8
Sst Tacstd2
Sst Th
Pvalb Cpne5
Pvalb Tpbg
Pvalb Gpx3
Pvalb Rspo2
Pvalb Wt1
Pvalb Obox3
Pvalb Tacr3
Igtp
L2 Ngb
L2/3 Ptgs2
L4 Ctxn3
L4 Scnn1a
L4 Arf5
L5a Hsd11b1
L5a Tcerg1l
L5a Pde1c
L5a Batf3
L5 Ucma
L5b Tph2
L5b Cdh13
L5b Chrna6
L6a Mgp
L6a Sla
L6a Car12
L6a Syt17
L6b Serpinb11
L6b Rgs12
Astro Aqp4
OPC Pdgfra
Oligo 96*Rik
Oligo Opalin
Micro Ctss
Endo Xdh
SMC Myl9
B
Snap25
Gad1
Vip
Sst
Pvalb
Slc17a7
Rorb
Foxp2
Aqp4
Pdgfra
Mog
Itgam
Flt1
Bgn
Dgkg
Vstm2a
AW551984
1
0.5
0
-0.5
-1


A
Relative expression
DIV1
DIV6
**
Gad1
Dgkg
Vstm2a
AW551984
B
kDa
AW551984
Dgkg
Vstm2a
Marker
150
100
75
50
37
25
anti-FLAG
C
DIV2
DIV4
DIV6
DIV8
Control
Dgkg
Vstm2a
AW551984
D
Total neurite length (μm)
Control
Dgkg
Vstm2a
AW551984


A
EGFP
Dgkg-EGFP
B
Relative Dgkg expression levels
1.5
1.0
0.5
0
shLacZ
shDgkg#1
shDgkg#2
shDgkg#3
shDgkg#4
shDgkgALL
C
Total neurite length (μm)
700
600
500
400
300
200
100
0
Control
shDgkg#1
shDgkg#3
shDgkgALL
**
**
DIV4
DIV7