Loss of the golgin GM130 causes Golgi disruption, Purkinje neuron loss, and ataxia in mice

Chunyi Liu 1,2, Mei Mei 1,*, Qiuling Li 1,*, Peristera Roboti 4, Qianqian Pang 1,2, Zhengzhou Ying 1,2, Fei Gao 3, Martin Lowe 4,5, and Shilai Bao 1,2,6

1State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China 2The University of Chinese Academy of Sciences, Beijing, China 3State Key Laboratory of Stem cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China 4Faculty of Life Sciences, University of Manchester, The Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK. *These authors contributed equally to this work §Corresponding authors. Email: sbaao@genetics.ac.cn and martin.lowe@manchester.ac.uk

Submitted to Proceedings of the National Academy of Sciences of the United States of America

The Golgi apparatus lies at the heart of the secretory pathway where it is required for secretory trafficking and cargo modification. Disruption of Golgi architecture and function has been widely observed in neurodegenerative disease, but whether Golgi dysfunction is causal with regard to the neurodegenerative process, or is simply a manifestation of neuronal death, remains unclear. Here we report that targeted loss of the golgin GM130 leads to a profound neurological phenotype in mice. Global knockout of mouse GM130 results in developmental delay, severe ataxia, and postnatal death. We further show that selective deletion of GM130 in neurons causes fragmentation and defective positioning of the Golgi apparatus, impaired secretory trafficking, and dendritic atrophy in Purkinje cells. These cellular defects manifest as reduced cerebellar size and Purkinje cell number, leading to ataxia. Purkinje cell loss and ataxia first appear during postnatal development but progressively worsen with age. Our data therefore indicate that targeted disruption of the mammalian Golgi apparatus and secretory traffic results in neuronal degeneration in vivo, supporting the view that Golgi dysfunction can play a causative role in neurodegeneration.

GM130 | Golgi apparatus | polarized secretion | Purkinje cell | ataxia

Introduction

As an important compartment of the endomembrane system, the Golgi apparatus is present in all eukaryotic cells. The Golgi apparatus lies at the heart of the secretory pathway and plays a critical role in the post-translational modification and trafficking of secretory cargo proteins and lipids (1). In addition to these core functions, the Golgi apparatus also contributes to cell cycle regulation and cytoskeletal dynamics (2-4). The Golgi apparatus has a characteristic architecture, comprising one or more stacks of cisternae that in vertebrate cells are laterally connected to form the Golgi ribbon (5, 6). The vertebrate Golgi is typically positioned adjacent to the centrosome, a localization that is dependent upon interactions with microtubules and the microtubule motor protein dynein (7). In migrating cells or in polarized cells such as neurons, the Golgi is positioned towards the leading edge or apical dendrite respectively, allowing both polarized delivery of secretory cargo to these plasma membrane domains (8-10). In developing neurons, the Golgi can also exist as non-centrosomally associated outposts, thought to be important for localized delivery of cargo direct to the newly forming dendritic plasma membrane as well as local microtubule nucleation to support dendrite morphogenesis (11-14).

Although the Golgi apparatus is well characterized at the molecular level, its roles in development and in tissue homeostasis, and how its dysfunction contributes to disease, remain relatively poorly characterized. For example, we know that the Golgi apparatus undergoes fragmentation in many neurodegenerative diseases, including Alzheimer’s disease, Parkinson’s disease, Amyotrophic Lateral Sclerosis (ALS) and spinocerebellar ataxia type 2 (SCA2) (15-19). However, whether Golgi fragmentation or impairment of secretory traffic in neurons can cause neurodegeneration, or simply reflects a consequence of cell death, remains unclear (20). Several studies have shown that polarized membrane delivery via the Golgi apparatus is important for neuronal morphogenesis during brain development (8-10, 21), but whether impairment of this process can cause neuronal death with consequent neurological impairment in vivo is currently unknown.

Members of the golgin family of coiled-coil proteins are required for maintenance of Golgi organization and are important for the specificity and efficiency of membrane traffic at the Golgi apparatus (22, 23). One of the best-studied golgins, GM130 (also known as GOLGA2), contributes to Golgi ribbon morphology and can tether transport vesicles to facilitate endoplasmic reticulum (ER) to Golgi traffic (24-27). It has also been implicated in Golgi positioning and cytoskeletal regulation (4, 28), and can contribute to the organization of neuronal Golgi outposts, at least in Drosophila (13). However, the physiological importance and in vivo functions of GM130 have yet to be explored in a mammal.

Here, we generated GM130 knockout mice and investigated the consequences of GM130 loss upon Golgi architecture and function within the nervous system. We find that loss of GM130 leads to disrupted organization and altered positioning of the Golgi apparatus in cerebellar Purkinje cells, which is accompanied by impaired polarized trafficking to the apical dendrite. Importantly, we find that these cellular defects manifest as a loss of...
GM130 protein in GM130−/− mice. B, Representative image of wild type and GM130−/− littermates. C, Body weight histogram of GM130fl/fl (n=20), GM130−/− (n=41) and GM130−/− (n=21) mice. **P < 0.01. D, Survival curve of GM130+/+, GM130−/− and GM130−/− mice. E, Expression pattern of GM130 in P3 and P14 mice. F, Body weight of pancreas, lung and neural specific knockout mice compared with wild type littermates at 8-9 weeks of age. **P < 0.01.

Fig. 3. Age dependent cerebellar atrophy in GM130-nKO mice. A, Gross morphology of brains from adult control and GM130-nKO mice. B, C, Nissl staining of sagittal sections of brain (B) and cerebellum (C) from 7 month old control and GM130-nKO mice. Black arrows indicate the position of the Purkinje cell layer (GL) and molecular layer (ML) are indicated. Scale bar in C, 50 µm. D, Purkinje cells (labeled with calbindin-D28K, green) in lobule X of the cerebellum of GM130-nKO and littermate control mice at age 2 weeks (upper) and 4 weeks (lower). Nuclei are stained with DAPI (blue). Scale bar, 500 µm. E, F, Quantification of the Purkinje cell density and molecular layer thickness in lobules X and IX of GM130-nKO and littermate control mice at age 2 weeks (E) and 4 weeks (F). n = 3; *P < 0.05, **P < 0.01. Data are presented as the mean ± SD. G, Immunohistochemical staining of the astrocyte marker glial fibrillary acidic protein (GFAP, red) and calbindin-D28K (green) in the cerebellum of control and GM130-nKO mice. Scale bar, 20 µm.

Golgi apparatus and impairment of secretory trafficking result in neuronal loss in vivo, and thus may contribute to the phenotypes observed in neurodevelopmental and neurodegenerative disease.

Results
Generation of GM130 knockout mice. To determine the physiological importance of GM130 in vivo, we generated a global knockout mouse (GM130−/−) by homologous recombination (Fig. S1). The GM130−/− mice, which lacked detectable GM130 (Fig. 1A), were born at a normal Mendelian ratio, indicating GM130 is not essential for embryonic development. However, deletion of GM130 resulted in reduced growth (Fig. 1B, C), and post-natal death before age post-natal day 35 (P35) (Fig. 1D).

To explore how loss of GM130 causes growth retardation and death, the temporal expression of GM130 in different mouse organs was examined. We found that GM130 is highly expressed in the brain of newborn mice (P3), and although widely expressed it is particularly abundant in liver, pancreas, and lung during post-natal development (P14) (Fig. 1E). To determine how organ-specific loss of GM130 affects post-natal development, we generated conditional knockout mice in which GM130 was deleted in the brain by crossing GM130fl/fl with mice lacking GM130 in either the pancreas, or lung (Fig. 1F), although GM130 was highly expressed in these organs. However, when GM130 was deleted in the brain by crossing GM130fl/fl with mice bearing a Nestin-Cre transgene, which is expressed throughout
the nervous system (29), the neuron-specific knockout offspring (referred to as GM130-nKO mice), showed significant growth retardation (Fig. 1F). This result indicates that GM130 expression in the brain is required for normal growth in mice. The GM130-nKO mice were able to breed and did not show reduced survival relative to GM130 wild-type (referred as Ctrl) littermates up to 1.5 years of age. The growth retardation observed in GM130-nKO mice was less than that in GM130-/- mice, suggesting that GM130 has functions in cell types beyond those in which Nestin-Cre is active.

**Motor defects in GM130 knockout mice.** The GM130-/- mice displayed a striking ataxia phenotype (Movie S1 and Fig. S3A). The mice staggered and could not stand steady on their hind legs, indicating GM130 is required for proper motor control during post-natal development. Consistent with the ataxia phenotype seen in GM130-/- mice, GM130-nKO mice also displayed motor coordination defects (Movie S2). These were mild in young animals but progressively worsened with age (Movie S3), and tremor was obvious. The GM130-nKO mice also displayed a limp reflex when lifted by their tail (Fig. 2A), consistent with a neurodegenerative defect (30). A footprint assay revealed an ataxic walking gait and diminished stride (Fig. 2B, C). In contrast, both Nestin-Cre transgenic mice and GM130-/- mice did not display any motor abnormalities. To assess motor coordination quantitatively, the GM130-nKO mice and control littermates were subjected to rotarod testing. In multiple trials, GM130-nKO mice showed a mild reduction in time spent on the rotarod at 3 weeks of age (Fig. 2D), which was much more profound at 8 and 12 weeks of age. GM130-/- years of age. The growth retardation observed in GM130-nKO mice, suggesting that GM130 is required for proper motor control during post-natal development. Consistent with the ataxia phenotype seen in GM130-/- mice, GM130-nKO mice also displayed motor coordination defects (Movie S2). These were mild in young animals but progressively worsened with age (Movie S3), and tremor was obvious. The GM130-nKO mice also displayed a limp reflex when lifted by their tail (Fig. 2A), consistent with a neurodegenerative defect (30). A footprint assay revealed an ataxic walking gait and diminished stride (Fig. 2B, C). In contrast, both Nestin-Cre transgenic mice and GM130-/- mice did not display any motor abnormalities. To assess motor coordination quantitatively, the GM130-nKO mice and control littermates were subjected to rotarod testing. In multiple trials, GM130-nKO mice showed a mild reduction in time spent on the rotarod at 3 weeks of age (Fig. 2D), which was much more profound at 8 and 12 weeks of age.
age (Fig. 2E and Fig. S3B-D). GM130-nKO mice also needed a longer time to cross balance beams of multiple sizes (Fig. 2F, G).
Together, these results indicate that deletion of GM130 in the central nervous system leads to severe neurological dysfunction.

**Progressive cerebellar atrophy and Purkinje cell loss in GM130-nKO mice.** To reveal the basis of the motor phenotype of GM130-nKO mice, adult brains from GM130\textsuperscript{fl/fl} and GM130-nKO mice were analyzed. No gross changes in brain architecture were observed in the forebrain or midbrain of GM130-nKO mice; however, the cerebellar size was dramatically reduced (Fig. 3A, B).

Therefore, we focused our attention on the cerebellum. Histological analysis by Nissl staining revealed a dramatic loss of Purkinje cells in adult GM130-nKO cerebellum (Fig. 3C). By comparing the cerebellum at different ages, we found that loss of Purkinje cells, marked by antibodies to calbindin-D28K, began in the third post-natal week, most noticeably from lobules X and IX (Fig. 3D-F), with Purkinje cell degeneration occurring in other regions as the mice became older (Fig. S4A-C). In contrast to Purkinje cells, there was no degeneration of neurons within the molecular or granule layers of the cerebellum (Fig. S4D). The results indicate a progressive cerebellar atrophy and degeneration of Purkinje cells in the disruption of Golgi architecture was seen in granule cells occurring early in development. The progressive degeneration of Purkinje cells correlated with impaired motor function, which was also progressive in nature (Fig. 2E and Fig. S3B-D). The loss of Purkinje cells in the GM130-nKO mice also correlated with a significant decrease in the thickness of the molecular layer (Fig. 3F) and increased staining of the astrocyte marker GFAP, consistent with neuronal damage, in cerebellar regions where GM130 is important for maintaining Golgi organization in many cell types (25, 31). GM130 also participates in ER to Golgi traffic (8-10, 21). GM130 participates in ER to Golgi traffic (8-10, 21). GM130 participates in ER to Golgi traffic (8-10, 21). GM130 participates in ER to Golgi traffic (8-10, 21). GM130 participates in ER to Golgi traffic (8-10, 21). GM130 participates in ER to Golgi traffic (8-10, 21).

**Disruption of Golgi architecture and positioning upon GM130 knockout.** Studies in cultured cells have revealed a role for GM130 in maintaining mammalian Golgi ribbon organization and pericentrosomal positioning (25, 31). GM130 also participates in vesicle tethering during ER to Golgi trafficking (24, 26, 27), and can function as a scaffold for activation of Cdc42 or Stk25 that is relevant for cell migration (32-34). To elucidate the cellular basis of the ataxic phenotype and Purkinje cell degeneration of GM130-nKO mice, we used antibodies to golgin-84 and TGN38, markers of the cis and trans-Golgi respectively, to analyze Golgi structure in Purkinje cells from mice at ages of P8, P14 and P28 by immunostaining. Purkinje cells from control mice at all ages had an elaborate Golgi ribbon, with Golgi elements extending around the nucleus of the cell soma, whereas loss of GM130 resulted in a compaction of the Golgi apparatus at P14 and P28 (Fig. 4A, B; see also Fig. S8). Changes in Golgi ultrastructure in the Purkinje cells were clearly observed using transmission electron microscopy with a loss of cisternal stacking and cisternal length and an accumulation of vesicular profiles localized to the perinuclear region (Fig. 4C, D). These results are consistent with our observations in GM130\textsuperscript{-/-} MEFS cells (Fig. S6). A similar phenotype in GM130\textsuperscript{-/-} cells (Fig. 3F) shows that the Golgi apparatus undergoes dynamic changes in its dendritic localization during neuronal development.

Importantly, we found that loss of GM130 altered the position of the Golgi apparatus in the soma of Purkinje cells. In control mice, the Golgi ribbon extended around the nucleus, it was enriched at the apical pole and extended to the initial segment of the primary dendrite, close to the molecular layer (Fig. 4A, B, E). In contrast, the Golgi was predominantly found at the opposite side of the soma to the primary dendrite in GM130-nKO mice at P14 and P28, indicating a loss of apical polarity of the Golgi apparatus (Fig. 4A, B, E). This finding is consistent with the loss of Golgi polarity seen upon shRNA-mediated depletion of GM130 from hippocampal granule cells (10). The pericentrosomal positioning of the mammalian Golgi apparatus helps determine its polarized distribution in various cell types (7). To determine whether loss of GM130 results in altered association of the Golgi with the centrosome in Purkinje cells, Golgi and centrosome positioning were analyzed in parallel. As shown in Fig. 4F, in control mice the Golgi apparatus was closely associated with the Purkinje cell centrosome, labeled with γ-tubulin, which was located apically near the base of the primary dendrite. In GM130-nKO mice, the centrosome retained its apical polarity, but the Golgi apparatus was completely dissociated from it (Fig. 4F). A likely explanation for this effect is the loss of GM130 binding protein AKAP450, which helps link the Golgi apparatus to the centrosome (35), from the Golgi in the GM130-nKO Purkinje neurons (Fig. S5A). In contrast, the Golgi association of Stk25, which has been implicated in Golgi polarity (32), was retained in the absence of GM130 (Fig. S5B). Together these results indicate that although GM130 is not required for initial Golgi polarization, it is essential to maintain the polarized distribution of the Golgi apparatus in Purkinje cells, most likely through its association with AKAP450 and the centrosome.

**Deficient secretory cargo trafficking upon GM130 knockout.** The polarized distribution of the Golgi apparatus in neuronal cells is required for directed trafficking of secretory cargos into the dendrite, which is important for dendritic growth during development (8-10, 21). GM130 participates in ER to Golgi traffic (24, 26), functioning as a tether for ER-derived transport vesicles (27). Secretory trafficking was therefore analyzed in GM130 knockout cells, using the model cargo vesicular stomatitis virus G protein (referred as VSVG) fused to GFP. In both cultured primary MEFS and cerebellar neurons, deletion of GM130 led to a reduced rate of trafficking of VSVG-GFP from the ER to the Golgi apparatus (Fig. 5A, B and Fig. S9), indicating a role for GM130 in this trafficking step in these cells. We then analyzed the morphology of dendrites in Purkinje cells of the knockout mice. In GM130-nKO mice, the dendrites were smaller than those seen at P9, indicating not only a failure to expand but also significant amount of dendritic atrophy. In wild-type mice, an elaborate dendritic tree was obvious at P9 and by P30 there was a dramatic expansion of dendritic arbors, as expected (Fig. 5C). Dendritic morphology was relatively normal at P9 in GM130-nKO mice (Fig. 5C), indicating that GM130 is dispensable for initiation of dendrite formation and initial growth of the dendritic tree. However, strikingly, there was a dramatic reduction both in dendritic size and arborization in GM130-nKO Purkinje cells at P30 compared to wild-type (Fig. 5C). Indeed the dendrite was smaller than that seen at P9, indicating not only a failure to expand but also significant amount of dendritic atrophy. Together these results indicate impaired secretion and defective dendritic maintenance upon loss of GM130.

To further assess secretory trafficking in the cerebellum of GM130-nKO mice, we focused on synaptic receptors that have to transit the Golgi apparatus on their way to the neuronal plasma membrane, where they function in neurotransmission (36, 37). Levels of plasma membrane AMPA-type glutamate receptor subunits were assessed by blotting the post-synaptic density (PSD) fraction isolated from the cerebellum of GM130-nKO mice. There were decreased amounts of both GluR1 and
GluR2 in the PSD fraction of the GM130-nKO mice compared to control, even though total abundance was not affected (Fig 5D, E), consistent with impaired secretory traffic to the synaptic membrane. In support of this conclusion, we found soma to dendritic trafficking of GluR1 was reduced in GM130-nKO Purkinje neurons (Fig. 5F). It has been reported that decreased abundance of the GluR2 subunit can lead to high Ca2+ influx (38), which can result in Purkinje cell death. There was also reduced PSD95, which scaffolds several types of neurotransmitter receptors including NMDA and AMPA-type glutamate receptors at the post-synaptic membrane (39), in the PSD fraction (Fig. 5D, E). Reduced PSD95 can cause reduced AMPA receptor abundance at the synapse and longer NMDA mediated LTP (40). Thus, defective neurotransmission as a consequence of reduced neurotransmitter receptor abundance and stability at the synapse is likely to contribute to impaired functionality and long-term survival of Purkinje cells in the cerebellum of GM130-nKO mice.

**Analysis of golgins and GRASP65 in the cerebellum.**

Given the particular sensitivity of Purkinje neurons to loss of GM130, it was of interest to assess relative levels of GM130 and related golgins, in different neuronal types, both in control and GM130-nKO mice. As shown in Fig. S8C, in the cerebellum of GM130-knockout mice, there is a shift in abundance of GM130 in Purkinje cells and in neurons within the granule and molecular layers of control cells. The staining for GM130 was stronger in Purkinje cells, reflecting the increased abundance of Golgi membranes in this cell type. The same was true for two other golgins, golgin-84 and TMF1, which are localized to the cis-Golgi and trans-Golgi respectively (Fig. 4A and SS1). Similarly, staining for the cis-Golgi golgin GMAP-210, which, like GM130, also functions in ER to Golgi transport (27, 41), was strong in Purkinje neurons, although harder to detect in other neuronal types present within the cerebellum (Fig. S8E). In GM130-nKO mice, the levels of the golgins studied were largely unaffected, although there was a slight reduction in abundance of GMAP-210 (Fig. 4A and SSS1). GRASP65, which is anchored to the Golgi membrane via association with GM130 (25, 42), was present in Purkinje cells and other neuronal types within the cerebellum of control mice. In GM130-nKO mice, it was lost from the Golgi, as expected (Fig. S8F). Together these results suggest that the particular sensitivity of Purkinje cells to loss of GM130 is not due to a deficit in expression of other golgins or GRASP65 in this cell type.

**Discussion**

In this study we report that targeted knockout of the golgin GM130 in mice leads to degeneration of Purkinje neurons within the cerebellum. Within Purkinje neurons, GM130 is required for Golgi positioning via association with the centrosome, and for efficient ER to Golgi trafficking (Fig. S510). Both processes are required for polarized delivery of secretory cargo to the dendrite, which is required for growth and maintenance of the dendritic tree. Loss of GM130 leads to dendrite atrophy, Purkinje cell degeneration and generation of an ataxic phenotype in mice. Previous work has shown that the polarized distribution of the Golgi apparatus is required for dendritic initiation from the soma and subsequent growth, a process that requires the delivery of large amounts of newly synthesized plasma membrane components via the secretory pathway (8, 21). Knockout of GM130 did not affect initiation or early growth of the Purkinje cell dendrite, but was required for maintenance of the dendritic tree. This finding suggests there is a higher requirement for directed secretory traffic for the expansion and maintenance of the dendritic tree, as opposed to its initial formation, at least in Purkinje cells. GM130 impairs membrane delivery into the apical dendrite in two ways: loss of Golgi positioning and lower rates of ER to Golgi traffic, most likely due to defects in vesicle tethering.

A recent study reported that RNA interference-mediated depletion of GM130 in hippocampal neurons results in mild impairment of dendritic initiation (10). In contrast, we find that in Purkinje neurons, dendritic initiation still occurs in the absence of GM130. Notably, we find that Purkinje neurons are particularly susceptible to loss of GM130 in vivo. The reason for this is currently unclear. A possible explanation is redundancy in golgin function, which may vary between different types of neurons (22, 23). However, the abundant Purkinje cell expression of other cis-Golgi golgins that could in theory compensate for loss of GM130, argues against this possibility. Rather, we favor the hypothesis that Purkinje cells are particularly susceptible to perturbations of secretory traffic due to their extremely large dendritic tree, which requires a significant input of material for both its growth and its maintenance. The relatively large amounts of Golgi in Purkinje cells would be consistent with this idea. Interestingly, knockout of the GM130 binding partner GRASP65 in mice fails to elicit a phenotype (43). This has been attributed to compensation by the related protein GRASP55 (43), which does not interact with GM130 in vivo (44). Thus, even though loss of GM130 resulted in a failure to recruit GRASP65 to the Golgi, the phenotypes we observe are likely independent of GRASP65.

Cell-culture based studies have implicated GM130 in a number of cellular processes in addition to secretory trafficking, including cytokoskeletal regulation (22, 32, 33), synaptic function, and cell division (3, 31, 33, 34). It was therefore surprising that the GM130 knockout mice did not display any overt developmental phenotype; pups were born at normal weight and looked morphologically normal. These findings would appear inconsistent with a major role for GM130 in cell migration or cell division in vivo, processes that are particularly important during embryonic development. However, an alternative explanation is that GM130 function in these processes is redundant, possibly with another golgin, or that the developing animal can compensate for loss of GM130 in a way not possible in cultured cells. Further studies will be required to discriminate between these possibilities. Interestingly, a human patient with a loss-of-function GM130 mutation has recently been described (45). This patient lacked any neonatal phenotype, but developed neuromuscular defects in the first year of life. Hence, in humans, it would seem GM130 is also dispensable during embryonic development. It would also appear that an important role for GM130 in the nervous system is conserved between mice and humans.

How similar the neuronal degeneration we observe upon GM130 knockout is to that observed in progressive neurodegenerative disease is currently unclear. The loss of Purkinje cells in the GM130 knockout mice starts around 3 weeks into postnatal development and progressively worsens as the mice age. The most common neurodegenerative diseases typically manifest only later in life, although the spinocerebellar ataxias, in which Purkinje cell death is currently observed, can appear much earlier in life (46). The cellular phenotypes we observe in the GM130 knockout mice could be considered neurodevelopmental. Although Purkinje neurons are born and specified prior to the time when phenotypes start to manifest, in mice they continue to develop their dendritic tree for up to 3 weeks following birth (47, 48). Hence, a failure of Purkinje cells to properly grow or maintain the dendritic tree during the first weeks of post-natal development could explain the neurological defects we observe. This is different from an inability to maintain a fully formed dendritic network, as occurs when mature neurons undergo degeneration in later onset disease. Nevertheless, the demonstration that Golgi dysfunction causes neuronal loss in vivo, combined with the observation that neuronal loss and ataxia worsen with age upon loss of GM130, indicates that this process could, in principle, result in or at least contribute to the neurodegeneration that occurs in human disease. In support of this possibility, it has been shown that α-synuclein can perturb ER to Golgi traffic in Parkinson’s disease models (49), that the observations that the

Footnote Author
Aβ fragment of APP that causes Alzheimer’s disease, and the pathogenic form of ataxin-2 that causes spinocerebellar ataxia type 2, both disrupt Golgi organization (19, 50). Hence, Golgi dysfunction and defective secretory trafficking, which could be attributable to a number of primary causes, may represent a significant pathogenic mechanism of neurodegenerative disease in humans.

Materials and Methods

Detailed methods for knockout mouse generation and phenotypic analysis, immunohistochemistry and electron microscopy, and biochemical frac-

tion and trafficking experiments can be found in the Supplementary Information Material and Methods. This also contains details of relevant antibodies used in this study and the methods used for quantitative analysis of data.

Acknowledgements. We appreciate the input of Peizhun Zhang and Yaqing Wang in these studies. We are grateful to Philip Woodman, Viki Allan, Stephen High and Hugh Piggins for their comments on the manuscript. We also thank Samantha Forbes of the University of Manchester Faculty of Life Sciences EM core facility. This work was supported by the National Natural Sciences Foundation of China (grants 31571379 and 31371378) and the BBSRC (grant BB/007711/1 and partnering award BB/H516001/R).