

# FoxP2 is a Parvocellular-Specific Transcription Factor in the Visual Thalamus of Monkeys and Ferrets

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**Although the parallel visual pathways are a fundamental basis of visual processing, our knowledge of their molecular properties is still limited. Here, we uncovered a parvocellular-specific molecule in the dorsal lateral geniculate nucleus (dLGN) of higher mammals. We found that FoxP2 transcription factor was specifically expressed in X cells of the adult ferret dLGN. Interestingly, FoxP2 was also specifically expressed in parvocellular layers 3–6 of the dLGN of adult old world monkeys, providing new evidence for a homology between X cells in the ferret dLGN and parvocellular cells in the monkey dLGN. Furthermore, this expression pattern was established as early as gestation day 140 in the embryonic monkey dLGN, suggesting that parvocellular specification has already occurred when the cytoarchitectonic dLGN layers are formed. Our results should help in gaining a fundamental understanding of the development, evolution, and function of the parallel visual pathways, which are especially prominent in higher mammals.**

**Keywords:** ferret, FoxP2, monkey, parvocellular, X cells

## Introduction

A fundamental basis of vision is parallel processing of sensory information, which is especially prominent in higher mammals such as primates and carnivores. Visual information is conveyed to the cerebral cortex via the dorsal lateral geniculate nucleus (dLGN) along parallel visual pathways. The parallel visual pathways are composed of 3 pathways with distinct anatomical and physiological characteristics. The 3 pathways are known as the X, Y, and W pathways in carnivores and are also known as the parvocellular (P), magnocellular (M), and koniocellular (K) pathways in primates (Sherman and Spear 1982; Livingstone and Hubel 1987; DeYoe and Van Essen 1988; Felleman and Van Essen 1991; Maunsell 1992; Hendry and Reid 2000; Sherman and Guillery 2004; Wassle 2004; Jones 2007; Nassi and Callaway 2009). It has been suggested that X and Y cells in carnivores are homologous to M and P cells in primates, respectively, while the alternative view is that X and Y cells are homologous to linear and non-linear M cells, respectively, and that P cells are unique to primates (Kaplan 2004). A deeper understanding of the parallel visual pathways will provide not only information about visual recognition, but will also contribute to our understanding of the evolution of the visual system and the general mechanisms by which the brain integrates information from multiple sources to create a unified, coherent percept of the external world.

Although considerable progress has been made in our knowledge of the anatomical circuitry and physiological

properties that distinguish these 3 pathways, our knowledge of their molecular properties is still limited. To obtain insights into the molecular properties of the parallel pathways, earlier studies have searched for molecules specifically expressed in 1 of the 3 pathways by using carnivores and primates (Hendry et al. 1984; Hockfield and Sur 1990; Bickford et al. 1998; Tochitani et al. 2001; Prasad et al. 2002; Kawasaki et al. 2004; Murray et al. 2008); however, the identification of molecules specifically expressed in X and/or P cells has not yet been achieved. Furthermore, none of the molecules selectively expressed in one of Y, W, M, or K pathways appear to mediate the development and function specific to that pathway; the previously identified molecules are primarily expressed postnatally and are mostly related to calcium signaling or to the cytoskeleton.

As the first step toward understanding the molecular properties of the parallel visual pathways, we searched for parvocellular-specific transcription factors in the visual system. We previously identified molecules expressed in the dLGN of ferrets, which are carnivores (Kawasaki et al. 2004), and further examined detailed expression patterns of these molecules in the ferret dLGN. We searched for molecules that were selectively expressed in the inner dLGN because X cells are located in the inner dLGN of adult ferrets. Here, we report a parvocellular-specific transcription factor in the dLGN of both ferrets and old world monkeys. FoxP2 is a member of the forkhead transcription factor family, and pioneering studies have shown that human FOXP2 is responsible for human developmental speech-language abnormalities (Lai et al. 2001; Fisher and Scharff 2009). The forkhead transcription factor family consists of >30 transcription factors, many of which play crucial roles in developmental processes and in the pathophysiology of disease (Hannenhalli and Kaestner 2009; Jackson et al. 2010). Interestingly, we further show that FoxP2 is expressed at the earliest time point during development among molecules specifically expressed in 1 of the 3 pathways. Our findings should help shed light not only on the molecular properties but also on the evolution of the parallel visual pathways and the pathophysiology of diseases that might be related to the parallel visual pathways such as developmental dyslexia (Livingstone et al. 1991; Farrag et al. 2002; Schulte-Korne and Bruder 2010).

## Materials and Methods

### Animals

Pigmented sable ferrets (*Mustela putorius furo*) and macaque monkeys (*Macaca fuscata* and *Macaca mulatta*) of either sex were

used. Adult animals were deeply anesthetized with pentobarbital and transcardially perfused with 4% paraformaldehyde (PFA). Brains were post-fixed in 4% PFA, cryoprotected in 30% sucrose, and embedded in optimal cutting temperature (OCT) compound. In some experiments, fresh-frozen tissues were used. Timed-pregnant monkeys were anesthetized with ketamine and isoflurane, and the fetuses were delivered by cesarean section. Brains were swiftly removed and immersion fixed for 5 days in 4% PFA. The area of interest was cut from the brain in the coronal plane, and cryoprotected in 25% sucrose before embedding in OCT compound. All experiments were performed in accordance with the National Institutes of Health and institutional guidelines for the care and use of laboratory animals and were approved by the Animal Care and Use Committees of the University of Tokyo and the University of California, Davis.

### Tracer Injection

Labeling of retinal ganglion cell (RGC) axons was performed as described previously (Kawasaki et al. 2004). After ferrets were anesthetized, 0.5% Alexa-conjugated cholera toxin B subunits (CTBs; Molecular Probes) solution (2–5  $\mu$ l) was injected into the vitreous humor.

### Immunohistochemistry

Immunohistochemistry was performed as described previously with slight modifications (Kawasaki et al. 2000). Sections (thickness 14–40  $\mu$ m) were made using a cryostat, permeabilized with 0.1–0.5% Triton X-100/phosphate-buffered saline, and incubated overnight with primary antibodies, which included rabbit anti-FOXP2, goat anti-FOXP2 (Abcam), mouse anti-NeuN, rabbit anti-calbindin-D28, mouse Cat-301 (Chemicon), mouse anti-adenomatous polyposis coli (APC) (Calbiochem), mouse anti-HuC/D (Molecular Probes), mouse anti-glial fibrillary acidic protein (GFAP) (Sigma), and mouse SMI-32 (Sternberger Monoclonals) antibodies. Rabbit and goat anti-FOXP2 antibodies recognize amino acids within residues 700 to the C-terminus and amino acids 703–715 of human FOXP2, respectively. After incubation with secondary antibodies and Hoechst 33342 (or DAPI), the sections were washed. For immunoenzyme staining, after incubation with primary antibodies, sections were incubated with an alkaline phosphatase-conjugated secondary antibody (Biosource) and visualized using nitro-blue tetrazolium chloride/5-bromo-4-chloro-3'-indolylphosphate p-toluidine salt (NBT/BCIP) as substrates. In some experiments, the sections were then subjected to fluorescent Nissl (Molecular Probes) and Hoechst 33342 staining. Experiments were repeated at least 3 times in different animals and gave consistent results.

### In Situ Hybridization

*In situ* hybridization was performed as described previously (Iwai and Kawasaki 2009). The DNA fragment corresponding to the 3' UTR of *Foxp2* mRNA (GeneBank Accession No. NM\_053242, nucleotide 4318–6760) was used to generate the *Foxp2* RNA probe. Sections prepared from fresh-frozen tissues were treated with 4% PFA and 0.25% acetic anhydride. The sections were incubated overnight with digoxigenin-labeled RNA probes in hybridization buffer (50% formamide, 5 $\times$  saline-sodium citrate buffer, 5 $\times$  Denhardt's solution, 0.3 mg/mL yeast RNA, 0.1 mg/mL herring sperm DNA, and 1 mM dithiothreitol). The sections were then incubated with an alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche) and were visualized using NBT/BCIP as substrates. In some experiments, the sections were subjected to Hoechst 33342 staining and immunohistochemistry. Experiments were repeated at least 3 times in different animals and gave consistent results.

### Quantification

Quantification of soma area sizes was performed as described previously (Hockfield and Sur 1990). To measure the soma areas of Cat-301-positive and FoxP2-positive neurons, horizontal sections (thickness 14  $\mu$ m) from the adult ferret dLGN were subjected to triple fluorescent labeling with an antibody (for Cat-301 or FoxP2),

fluorescent Nissl, and Hoechst 33342. After subtraction of tissue background fluorescent intensities, images of fluorescent Nissl staining were thresholded at mean + standard deviation (SD) of all pixel intensities. Neurons which had well-defined Hoechst 33342-positive nuclei were selected, and their soma area sizes were quantified using the particle analysis tool of ImageJ. Statistical significance was evaluated using Mann–Whitney's *U* test.

To quantify the percentages of FoxP2-positive cells that were also *VGLUT2*-positive, horizontal sections (thickness 14  $\mu$ m) were subjected to *VGLUT2 in situ* hybridization, FoxP2 immunohistochemistry, and Hoechst 33342 staining. The numbers of *VGLUT2*-positive cells and FoxP2-positive cells were counted. To quantify the percentages of dLGN neurons that were also FoxP2-positive, horizontal sections (thickness 14  $\mu$ m) were stained using anti-FOXP2 and anti-HuC/D antibodies and Hoechst 33342. Neurons which had well-defined Hoechst 33342-positive nuclei were selected, and the numbers of FoxP2-positive and HuC/D-positive cells were counted. Statistical significance was analyzed using Student's *t*-test.

## Results

### Expression of FoxP2 in the Adult Ferret dLGN

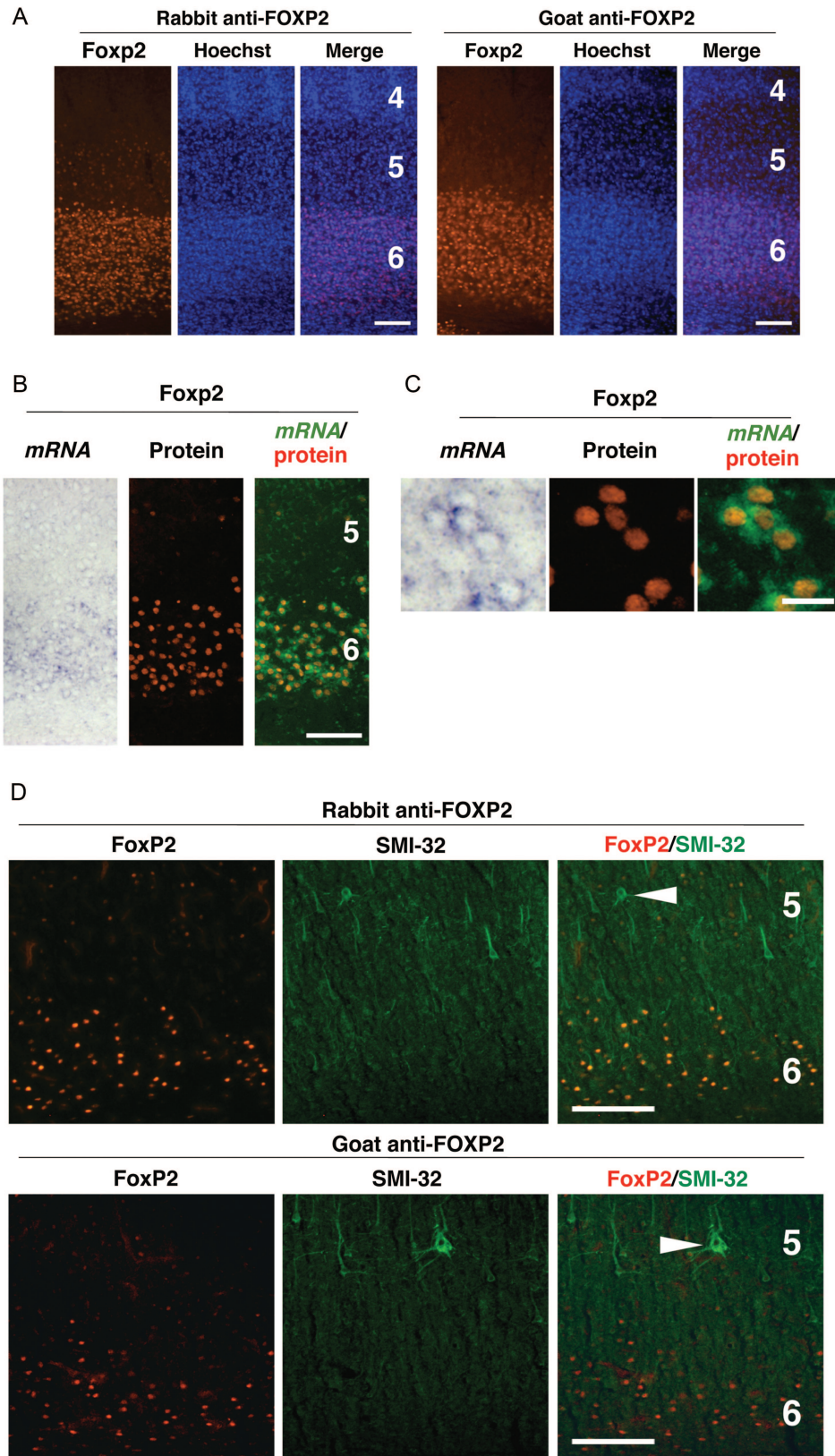
We first confirmed the specificities of 2 anti-FOXP2 antibodies. Consistent with previous reports (Ferland et al. 2003), these antibodies recognized layer 6 strongly and layer 5 weakly in the cerebral cortex of mice and ferrets (Fig. 1A, D). Furthermore, the distribution patterns of Foxp2 immunoreactivities were consistent with those of *Foxp2* mRNA (Fig. 1B, C). These results clearly indicate that anti-FOXP2 antibodies used here specifically recognize Foxp2 protein.

We then examined the distribution pattern of FoxP2-positive cells in the adult ferret dLGN. We found that FoxP2 was predominantly expressed in the inner dLGN, while *Zic2*-positive cells were found throughout the dLGN (Fig. 2A). The ferret dLGN consists of the A/A1 and C layers, which occupy the inner and outer dLGN, respectively (Linden et al. 1981). To visualize the dLGN layers, we labeled RGC axons by injecting Alexa 488- and Alexa 555-conjugated CTBs into the ipsilateral and contralateral eyes, respectively. We found that FoxP2-positive cells were almost exclusively distributed in the A and A1 layers (Fig. 2B, C). Two independent anti-FOXP2 antibodies recognized the same cells (Fig. 2D). FoxP2-positive cells were consistently distributed in the A/A1 layers throughout the entire dLGN (Fig. 2E).

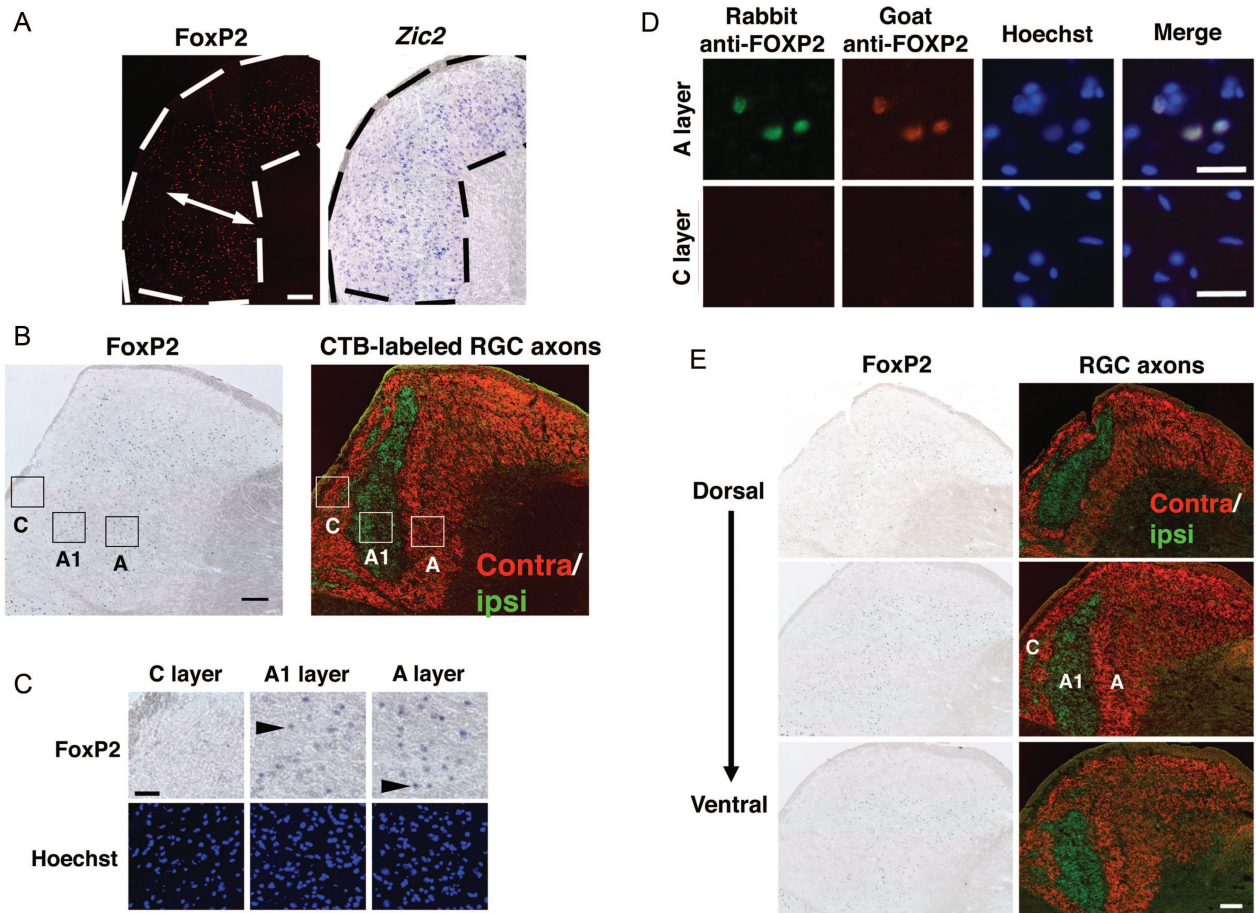
Next, we examined FoxP2 expression at the cellular level. We found that all FoxP2-positive cells expressed the neuronal markers NeuN and HuC/D (Fig. 3A, arrows), whereas they did not express the astrocyte marker GFAP or the oligodendrocyte marker APC (Fig. 3B). FoxP2-positive neurons also expressed *VGLUT2* mRNA ( $93.9 \pm 2.2\%$ ,  $n = 153$ ; 3 dLGNs from 3 animals; Fig. 3C, arrows), while they did not express *GAD1* mRNA (Fig. 3C). These results suggest that FoxP2-positive cells in the adult ferret dLGN are glutamatergic relay neurons.

Because glutamatergic neurons in the A/A1 layers consist of X and Y cells, these results raised the possibility that FoxP2 is selectively expressed in X and/or Y cells. We found that FoxP2 expression was exclusive to cells which did not express either Cat-301 or SMI-32, both Y-cell markers (Fig. 3D), suggesting that FoxP2 is expressed by X cells, but not Y cells (Hockfield and Sur 1990; Bickford et al. 1998). Consistently, the soma sizes of FoxP2-positive neurons were indeed significantly smaller than those of Cat-301-positive neurons ( $P < 0.01$ , Mann–Whitney's *U* test, 4 dLGNs from 3





**Figure 1.** Characterization of anti-FOXP2 antibodies. (A) Immunohistochemical localization of Foxp2 protein in the mouse cerebral cortex at P13. Two independent anti-FOXP2 antibodies showed Foxp2 expression primarily in layer 6 and weakly in layer 5. Cortical layers are indicated with numbers. (B) Localization of *Foxp2* mRNA and Foxp2 protein in the mouse cerebral cortex at P10. The merged image shows that *Foxp2* mRNA-positive cells (pseudo-colored in green) and Foxp2 protein-positive cells (orange) are completely overlap. Cortical layers are indicated with numbers. (C) High magnification images of B. (D) Foxp2 immunoreactivities in the adult ferret visual cortex. Consistent with the results obtained using mice, 2 independent anti-FOXP2 antibodies predominantly recognized layer 6 and weakly recognized layer 5 in the ferret primary visual cortex at P101. SMI-32-positive cells (arrowheads) indicate the location of layer 5. Cortical layers are indicated with numbers in merged images. Coronal sections. Experiments were repeated at least 3 times in different animals and gave consistent results. Scale bars: 100  $\mu$ m (A, B, D) and 25  $\mu$ m (C).



**Figure 2.** FoxP2 expression in the adult ferret dLGN. (A) Double staining with FoxP2 immunohistochemistry and *Zic2* *in situ* hybridization using horizontal sections of the adult ferret dLGN. While *Zic2*-positive cells were detected throughout the entire dLGN (dashed line), FoxP2-positive cells were mostly restricted to the inner dLGN (double arrow). (B) FoxP2-positive cells were restricted to the A/A1 layers of the ferret dLGN. LGN layers were labeled by injecting CTBs into the eyes. Horizontal sections were stained with anti-FOXP2 antibody. (C) High magnification images of areas in B (boxes). (D) Double immunostaining with 2 independent anti-FOXP2 antibodies. Both antibodies resulted in the same expression patterns. (E) Distribution patterns of FoxP2-positive cells across the adult ferret dLGN. The serial horizontal sections from CTB-injected animals were stained with anti-FOXP2 antibody. Experiments were repeated at least 3 times in different animals and gave consistent results. Scale bars: 200  $\mu\text{m}$  (A, B, E), 50  $\mu\text{m}$  (C), and 25  $\mu\text{m}$  (D).

animals; Fig. 3E, F). The averaged cross-sectional areas of FoxP2- and Cat-301-positive somata were  $172.3 \pm 58.9 \mu\text{m}^2$  ( $n = 104$ ) and  $497.2 \pm 146 \mu\text{m}^2$  ( $n = 75$ ), respectively. These values are consistent with previous reports of the sizes of X and Y cells (Friedlander et al. 1981; Hockfield and Sur 1990). Thus, these results indicate that FoxP2-expressing neurons in the adult ferret dLGN are X cells.

#### Expression of FoxP2 in the Adult Monkey dLGN

To test if FoxP2 expression is also functional cell-class-specific in another mammalian species, we examined the distribution pattern of FoxP2 in the dLGN of adult old world monkeys. We found that FoxP2 was selectively expressed in parvocellular layers 3–6 (Fig. 4A–C). In contrast, Cat-301 was found in magnocellular layers 1–2, and calbindin-D28-positive cells were located in the koniocellular layers (interlaminar zones and layer S) as previously reported (Fig. 4D; Hendry et al. 1984; Jones and Hendry 1989). These results clearly indicate that FoxP2 is selectively expressed in the parvocellular layers of the adult monkey dLGN. Taken together, FoxP2 is the first known parvocellular-specific molecule in the visual system.

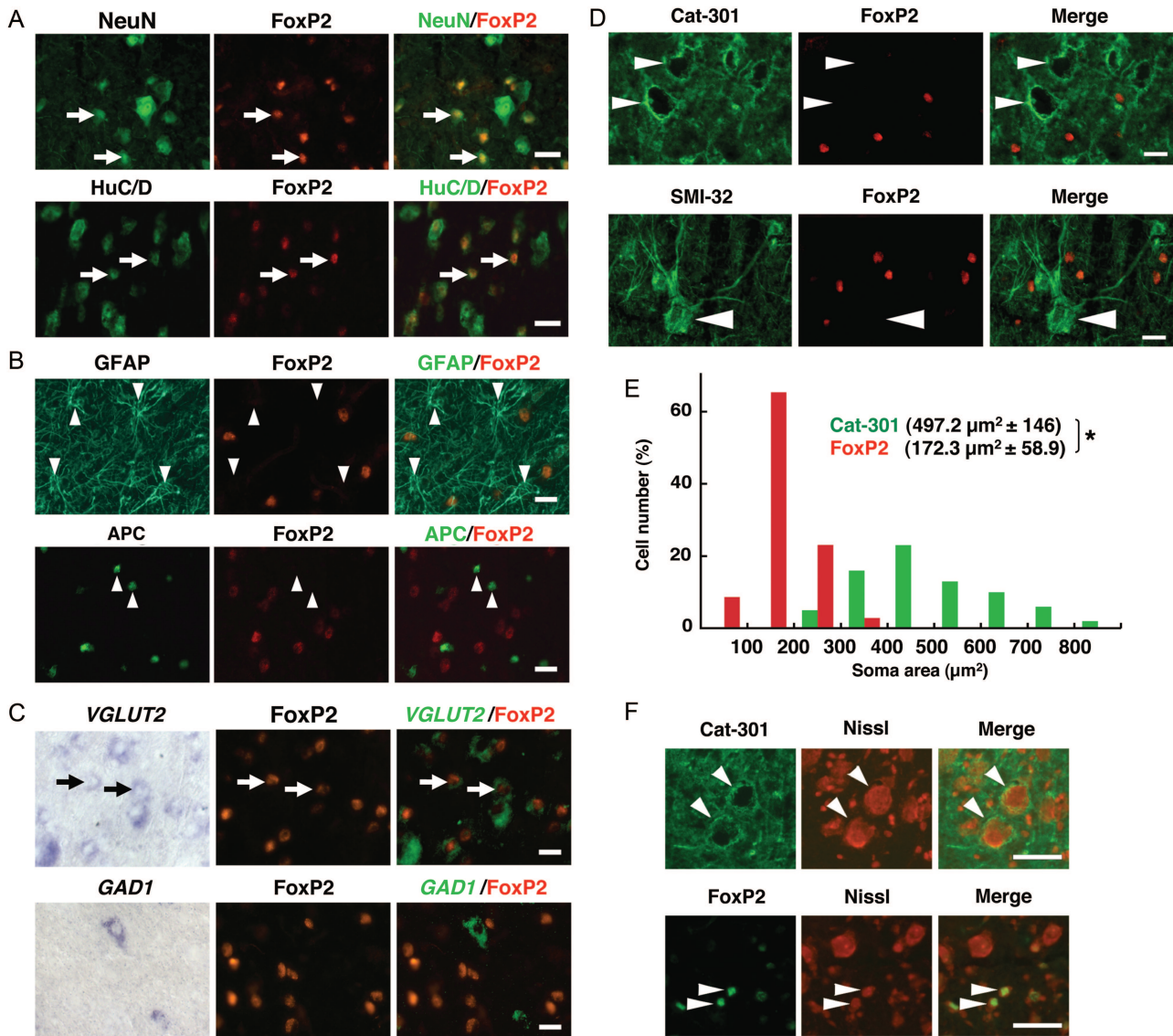
There is a longstanding question about the relationship between M and P cells in primates and Y and X cells in

carnivores. It has been suggested that X and Y cells in carnivores are homologous to M and P cells in primates, respectively, while the alternative view is that X cells are homologous to linear M cells, Y cells to non-linear M cells, and P cells are unique to primates (Kaplan 2004). Our results show that FoxP2 is selectively expressed in X cells in ferrets and P cells in primates. The expression patterns conserved in these 2 species strongly suggest a homology between X and P cells.

#### FoxP2 Expression in the dLGN during Development

Although the mechanisms underlying the development of the parallel visual pathways have been of great interest, the Y/M-specific molecules found so far are expressed postnatally. In contrast, FoxP2 is expressed in the parvocellular layers in the embryonic monkey dLGN on gestation day (GD) 140 (Fig. 5A), suggesting that M and P properties are already specified on GD140. Intriguingly, a small number of FoxP2-positive cells were also found in the magnocellular layers on GD140 (Fig. 5A). They could be eliminated during development. Interestingly, on GD85, when cytoarchitectonic layers are about to be formed (Yan et al. 1996), FoxP2 immunoreactivity was stronger in the dorsal portion of the dLGN, although not as clearly so as on GD140 (Fig. 5B). This suggests that the specific



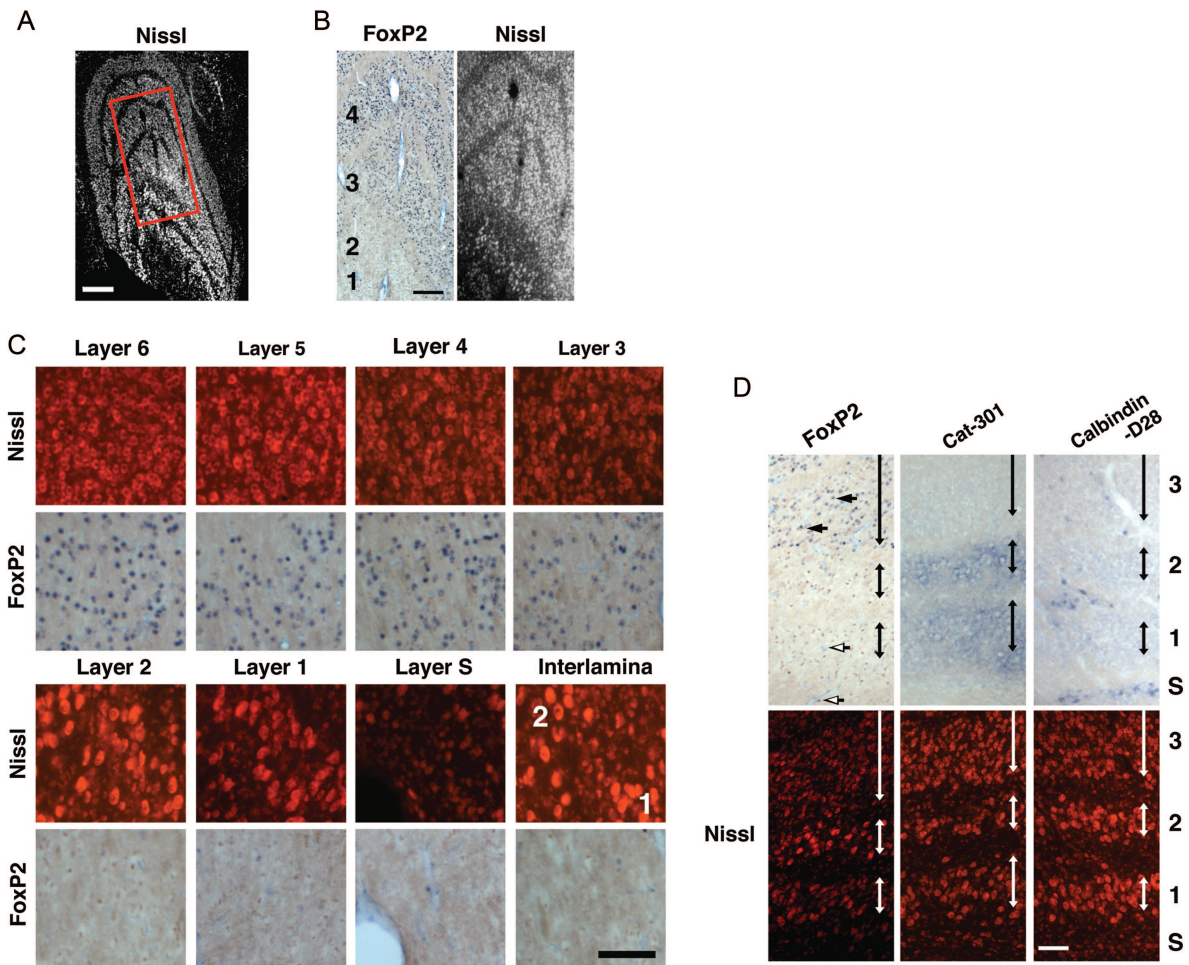


**Figure 3.** Characterization of FoxP2-positive cells in the A/A1 layer at the cellular level. (A) Double immunostaining with anti-FOXP2 antibody plus either anti-NeuN or anti-HuC/D antibody. FoxP2 was expressed in a subset of NeuN- or HuC/D-positive neurons (arrows). (B) Double immunostaining with anti-FOXP2 antibody plus either anti-GFAP or anti-APC antibody. GFAP-positive astrocytes (arrowheads) and APC-positive oligodendrocytes (arrowheads) did not express FoxP2. (C) Double staining with *in situ* hybridization and FoxP2 immunohistochemistry. FoxP2 expression was almost entirely limited to *VGLUT2*-positive cells (arrows). (D) Double immunostaining for FoxP2 plus either Cat-301 or SMI-32. Cat-301-positive or SMI-32-positive Y cells did not express FoxP2 (arrowheads). (E) Histogram of soma area sizes of Cat-301-positive cells and FoxP2-positive cells. FoxP2-positive cells were significantly smaller than Cat-301-positive cells (mean  $\pm$  SD;  $*P < 0.01$ , Mann-Whitney's *U* test). (F) Double staining for fluorescent Nissl plus either Cat-301 or FoxP2. Soma area sizes were measured using Nissl staining patterns. Cat-301-positive cells were larger than FoxP2-positive cells (arrowheads). Horizontal sections. Experiments were repeated at least 3 times in different animals and gave consistent results. Scale bars: 20  $\mu\text{m}$  (A–D) and 50  $\mu\text{m}$  (F).

expression of FoxP2 begins when the cytoarchitectonic layers are formed during development. Taken together, our results indicate that pattern formation within the developing monkey dLGN may involve 2 distinct steps: The initial gradual differentiation along the inner–outer axis, followed by the formation of sharp boundaries between the M and P layers. In summary, FoxP2 begins to be expressed in the monkey dLGN at the earliest time point during development among molecules that are known to be specifically expressed in 1 of the 3 pathways. FoxP2 could mediate the formation of the parallel visual pathways during development.

We also examined FoxP2 expression in the developing ferret dLGN. Similar to the results obtained using adult ferrets, FoxP2 was preferentially expressed in the inner dLGN of newborn ferrets at P1, which roughly corresponds to

GD60–80 in monkey (Fig. 5C; Issa et al. 1999; Huberman et al. 2005). A subset of HuC/D-positive cells in the inner dLGN were FoxP2-positive ( $85.4 \pm 11.2\%$ ,  $n = 155$ ; 6 dLGNs from 3 animals; Fig. 5D), suggesting that FoxP2 is expressed in a subset of neurons in the inner dLGN. Interestingly, the percentage of the neurons in which FoxP2 was expressed in the newborn dLGN was almost the same as that in the adult dLGN from our data ( $81.7 \pm 5.6\%$ ,  $n = 178$ ; 3 dLGNs from 3 animals) and the percentage of X cells, which were physiologically identified, in the adult dLGN (Price and Morgan 1987). It seems reasonable to speculate that FoxP2-positive cells in the newborn ferret dLGN correspond to future X cells. Because Cat-301 and SMI-32 are not expressed soon after birth in the dLGN, the detailed identity of FoxP2-positive neurons in the neonatal ferret dLGN remains to be elucidated.



**Figure 4.** Parvocellular-specific expression of FoxP2 in the adult monkey dLGN. (A) dLGN layers visualized with Nissl staining. (B) Immunostaining for FoxP2 within the red box in A. FoxP2 is specifically expressed in the parvocellular layers. LGN layers are indicated with numbers. (C) High magnification images. FoxP2 was strongly expressed in parvocellular layers 3–6, but was absent in magnocellular layers 1–2 and in the koniocellular layers (layer S and interlaminar zones). (D) Distinct distribution patterns of FoxP2-, Cat-301-, and calbindin-D28-positive cells. Sections were stained with fluorescent Nissl plus either anti-FoxP2, Cat-301, or anti-calbindin-D28 antibody. FoxP2-positive cells were specifically located in the parvocellular layers (arrowheads) and were rarely found in the magnocellular and koniocellular layers. Faint FoxP2 signals were observed around small vessels (open arrowheads). Cat-301-immunoreactivity was detected in layers 1 and 2, and calbindin-D28-immunoreactivity was found in the layer S and interlaminar zones. LGN layers are indicated with numbers and arrows. Experiments were repeated at least 3 times in different animals and gave consistent results. Scale bars: 500  $\mu$ m (A), 250  $\mu$ m (B), and 100  $\mu$ m (C, D).

## Discussion

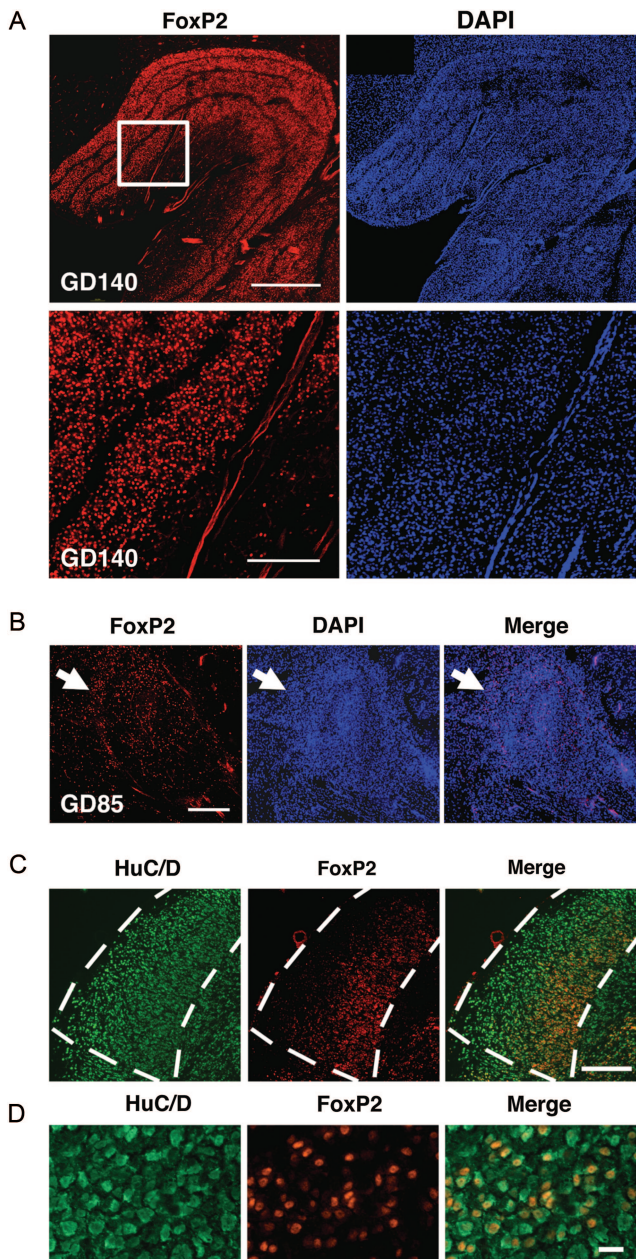
We have shown that FoxP2 is selectively expressed in X cells of the ferret dLGN and in the parvocellular layers of the monkey dLGN. There is a longstanding question about the relationship between M and P cells in primates and Y and X cells in carnivores. It has been suggested that X and Y cells in carnivores are comparable with M and P cells in primates, respectively, while the alternative view is that X and Y cells are homologous to linear and non-linear M cells, respectively, and that P cells are unique to primates (Kaplan 2004). Because our results demonstrated that FoxP2 was selectively expressed in X cells in ferrets and P cells in primates, our findings provide new evidence for a homology between X and P cells.

Earlier studies have investigated molecules specifically expressed in 1 of the 3 pathways (Hendry et al. 1984; Hockfield and Sur 1990; Bickford et al. 1998; Tochitani et al. 2001; Prasad et al. 2002; Kawasaki et al. 2004; Murray et al. 2008). Among these molecules whose expression patterns

were confirmed using *in situ* hybridization or immunohistochemistry, none are expressed specifically in X/P cells. TCF7L2 is more highly expressed in the P layers than in the M layers, but is also expressed in the K layers (Murray et al. 2008). FoxP2 is the first molecule found to be parvocellular-specific in the visual system. Furthermore, FoxP2 is the first transcription factor found to be selectively expressed in one of the M, P, and K pathways in the visual system. In addition, FoxP2 is expressed at the earliest time point during development among molecules specifically expressed in 1 of the 3 pathways. Thus, both the time course and specificity of FoxP2 suggest that FoxP2 is involved in the development and/or function of the parallel visual pathways.

Selective expression of transgenes in either M or P cells would be extremely beneficial for exploring the anatomical, functional, and developmental properties of the parallel pathways. Useful transgenes would include optogenetic molecules (e.g. channelrhodopsin and halorhodopsin), trans-synaptic tracers (e.g. wheat germ agglutinin (WGA) and WGA-Cre),





**Figure 5.** FoxP2 expression in the monkey and ferret dLGN during development. (A) FoxP2 expression in the monkey dLGN on GD140. FoxP2 was specifically expressed in the parvocellular layers. High magnification images in the white box of the upper panel are shown in the lower panels. (B) FoxP2 expression in the dLGN (arrows) on GD85. FoxP2 immunoreactivity was stronger in the dorsal portion of the dLGN, although not as clearly so as on GD140. Frontal sections. (C) Double immunostaining for FoxP2 and HuC/D using the P1 ferret dLGN. The area within the dashed lines indicates the dLGN. Whereas HuC/D-positive cells are distributed throughout the dLGN, FoxP2-positive cells are prominent in the inner dLGN. (D) High magnification images of the inner dLGN at P14. FoxP2-positive cells were a subset of HuC/D-positive neurons. Horizontal sections. Scale bars: 1 mm (A, upper), 250  $\mu$ m (A, lower), 500  $\mu$ m (B), 200  $\mu$ m (C), and 20  $\mu$ m (D).

neuronal activity reporters [e.g. green fluorescent protein (GFP)-based  $Ca^{2+}$  sensors], and synaptic marker proteins (e.g. synaptophysin-GFP and PSD-95-GFP) (Yoshihara et al. 1999; Boyden et al. 2005; Deisseroth et al. 2006; Arenkiel et al. 2007; Gradinaru et al. 2010; Sehara et al. 2010; Ako et al. 2011). Our results showed that FoxP2 is selectively expressed

in X/P cells in the dLGN, and even low expression of FoxP2 was undetectable in Y/M cells, indicating that the promoter region of the monkey FoxP2 gene is an ideal candidate for targeting P cells for transgene expression. Our results provide a potential means for targeting X/P cells.

Because techniques for genetic manipulation are commonly available in mice, it would be beneficial if mice could be used for investigating the function and development of the parallel visual pathways. Recent pioneering studies suggested that mice, like higher mammals, also have X-, Y-, and W-like cells (Grubb and Thompson 2004; Huberman et al. 2008; Huberman et al. 2009; Krahe et al. 2011). Therefore, it would be intriguing to compare these cell types and the expression patterns of Foxp2.

#### **FoxP2 Expression during Development**

We found that the selective expression of FoxP2 was less obvious in the monkey dLGN on GD85, and that the P layer-specific expression pattern became prominent by GD140. These results raised the possibility that pattern formation within the monkey dLGN involves 2 distinct steps: The initial gradual differentiation along the inner–outer axis, followed by the formation of sharp boundaries between the M and P layers. Similar 2-step development has been reported in the specification of the spinal cord. Early in development, sonic hedgehog makes gradual gene expression patterns in the spinal cord (Jessell 2000). Then reciprocal suppression of transcription factors produces sharp boundaries between domains. Because of this similarity, it seems reasonable to speculate that pattern formation in the dLGN also involves morphogens and reciprocal suppression of transcription factors. Interestingly, FoxP2 was reported to act as a transcriptional suppressor (Shu et al. 2001).

Interestingly, a recent study reported that FoxP2 showed a graded expression pattern, with higher expression in the P layers than in the M layers, in the neonatal marmoset dLGN (Mashiko et al. 2012). Therefore, the graded expression of FoxP2 in the immature dLGN might be conserved between old world monkeys and marmosets early in development.

#### **Functional Implications of FoxP2 in Normal and Pathophysiological Conditions**

Because Foxp2 plays crucial roles in the development of other organs such as the lungs (Shu et al. 2007), it seems reasonable to speculate that FoxP2 is also involved in the formation of the parallel visual pathways in higher mammals during development. Although FoxP2 function in the parvocellular layers is currently unknown, affected KE family members, who have mutated *FOXP2* gene and speech disabilities, showed suggestive phenotypes. They showed not only speech impairment but also mild impairment on picture arrangement tests (Watkins et al. 2002). Therefore, in addition to speech disabilities, visual impairments could also underlie the behavioral phenotype of the KE family (Vargha-Khadem et al. 2005), and FoxP2 expression in the parvocellular layers could potentially account for this phenotype. It would be intriguing to examine parvocellular-specific visual function in KE family members. Furthermore, our findings also suggest that *FOXP2* might be clinically relevant to the pathophysiology of developmental dyslexia. Developmental dyslexia is a common learning disability involving a specific impairment in

reading ability, but its pathophysiology is largely unknown. Previously, the involvement of the parvocellular pathways in developmental dyslexia has been proposed (Farrag et al. 2002). Because a recent study showed that one single nucleotide polymorphism in *FOXP2* was significantly associated with dyslexia (Wilcke et al. 2012), *FOXP2* is an important candidate for further research into the pathophysiology of developmental dyslexia.

### Future Perspectives

Higher mammals such as ferrets and monkeys have well-developed visual systems and have been used for electrophysiological and morphological experiments. Thus, molecular analyses using ferrets and monkeys would be an important future direction for understanding well-developed visual systems in humans. Recently, transgenic techniques using virus vectors became available in higher mammals (Chan et al. 2001; Lois et al. 2002; Sasaki et al. 2009). Combining with these techniques, the information about the molecular organization of the dLGN may open the door for the exploration of the evolution, development, physiological functions, and pathophysiology of the visual system in higher mammals.

### Funding

This work was supported by the 21st COE Program; the Global COE Program; Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology-JAPAN; PRESTO from Japan Science and Technology Agency; Human Frontier Science Program; Takeda Science Foundation; Takeda Medical Research Foundation; Astellas Foundation for Research on Metabolic Disorders; the Danone Institute of Japan; the Life Science Foundation of Japan; the Kurata Memorial Hitachi Science and Technology Foundation; the Novartis Foundation for the Promotion of Science; Mitsubishi Foundation; Fukuda Foundation for Medical Technology; Yamada Science Foundation; Hokuto Foundation; Daiichi-Sankyo Foundation of Life Sciences; Research Foundation for Opto-Science and Technology; Santen Pharmaceutical and the National Institutes of Health (EY016182, United States of America); and Research Fellowship for Young Scientists from Japan Society for the Promotion of Science (to L.I.).

### Notes

We are especially thankful to the late Dr Lawrence C. Katz (Duke University/HHMI) for his advice at the initial phase of this project. We are grateful to Drs C.A. Mason and T. Kuwajima (Columbia University) for critical reading of this manuscript. We thank Drs S. Tsuji, H. Bito, T. Kadowaki, M. Araie (The University of Tokyo), E. Nishida (Kyoto University), Y. Sasai (RIKEN-CDB), and S. Nakanishi (Osaka Bioscience Institute) for their warm and continuous support. We also thank K. Tanno for her technical assistance, and Z. Blalock and Kawasaki lab members for their support. *Conflict of Interest:* None declared.

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