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Skeletal Osterix Signaling Inhibition and Expression Regulation

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For in vitro experiments, Wnt3 expression was transiently induced in the mesenchyme of ectopic FG and GFP FG, ectopically expressing a Wnt3-expressing reporter plated at a high density. Consistent with the Wnt3 expression pattern, when recombinant Wnt3 was introduced in FG ectopically, we observed a consistent increase in Wnt3 protein expression. In contrast, when Wnt3 was introduced in the mesenchyme of ectopically expressing FG, ectopically expressing FG reporter plated, re-combinant Wnt3 expression was not significantly increased. Moreover, when recombinant Wnt3 was introduced in the FG ectopically, recombinant Wnt3 expression was not significantly increased. Our data indicate that Wnt3 is expressed specifically in FG but not in the anterior bud. These data show that formation of the limb bud requires Wnt3 expression in the mesenchyme, and that ectopic FG expression is required to prevent Wnt3 expression from associating with FG andinducing Wnt3 expression in the li mb bud.

Activation of Wnt1 inhibits FG-induced Wnt3 expression Wnt1 is an essential ligand for the canonical Wnt pathway. We evaluated the expression of Wnt1 by real-time PCR. Wnt1 was detected throughout the limb bud mesenchyme. In contrast, no significant differences were detected between the conditional and Wnt1 transgene constructs (Figure 8 , panel B). In contrast, in the conditional allele, Wnt1 expression was significantly increased in the limb bud mesenchyme. In addition, a reduction in the number of GFP FG-positive cells was detected in the limb bud

mesenchyme, indicating a reduction in Wnt1 expression (Figure 8 , panel B). Additionally, FG expression was analyzed in the limb bud mesenchyme of ectopically expressing FG, ectopically expressing Wnt1, and ectopically expressing FG reporter plated on transverse fins. The increased expression of Wnt1 in the limb bud mesenchyme was confirmed by qRT-PCR. QRT-PCR reveals increased expression of Wnt1 by both transverse and transfix discs (Figure 3). Consistent with previous reports, we observed a reduction in Wnt1 expression as compared to the null mutant embryos (Chen et al., 2007). To test whether the reduction in Wnt1 expression was due to the reduced canonical Wnt pathway components, we examined the expression of Lef1 (Figure 10 , panel C). Lef1 was present throughout the limb bud mesenchyme, in both the conditional and Wnt1 transgene constructs. At E12.5, however, Lef1 expression was significantly reduced in the conditional and transfix discs compared to the conditional and wild-type embryos (Figure 10 , panel D). Consistent with previous reports, we also observed a reduction in Lef1 expression in the limb bud mesenchyme of ectopically expressing FG, ectopically expressing Wnt1, and ectopically expressing Wnt1 reporter plated on transverse fins (Figure 8 , panel B). Finally, ectopic FG expression in the limb bud mesenchyme was also revealed to be dependent on Wnt1 (Figure 9 , panel C). In contrast, FG expression was significantly increased in the limb bud mesenchyme of transfix discs compared to the conditional mutant discs (Figure 9 , panel B). These data suggest that in the conditional disc, the canonical Wnt pathway is impaired and canonical Wnt signaling is able to induce Lef1 expression, but that Wnt signaling is inhibited and Lef1 expression is unaffected.

Wnt3 expression in the limb bud in the absence of FG The limb bud is a well-characterized and well-established model system that allows evaluation of the signaling pathway involved in the formation and maintenance of the ectopic FG expression in the developing limb bud. To investigate the role of Wnt signaling in the limb bud, we performed Figs. In the conditional disc, Wnt3 expression was significantly enhanced in the limb bud (Figure 11 , panel B). However, Wnt3 expression in the limb bud was not significantly increased in the conditional mutant disc. These findings indicate that in the conditional mutant, Wnt3 expression is required for induction of Wnt3 expression. To determine whether ectopic expression of Lef1 and results showed that Wnt3 expression is greatly reduced in the limb bud mesenchyme and completely absent in the distal limb bud mesenchyme, whereas Wnt3 expression is increased in both regions of the limb. The expression of Wnt3 in the limb bud mesenchyme is tightly regulated by both the canonical Wnt/β-catenin and the noncanonical β-catenin. The canonical Wnt/β-catenin signaling pathway was required for the induction of the limb bud stem cell-dependent mesenchymal Wnt-3 expression. Inactivation of canonical Wnt signaling in the limb bud resulted in a delay in limb bud formation, indicating that canonical Wnt/β-catenin signaling was not sufficient to drive mesenchymal Wnt3 expression. The inhibition of canonical Wnt signaling in the limb bud led to a decrease in the size of distal limb bud mesenchyme, consistent with the inhibition of β-catenin signaling. Interestingly, the effect of canonical Wnt signaling on the morphology of the limb bud mesenchyme, including its expression, could not be assessed in these studies. To determine the effects of Wnt signaling on the mesenchymal proliferation and differentiation of the limb bud mesenchyme, we examined the expression of Wnt3 in the developing limb bud mesenchyme using a recombinant Wnt3 protein (0.05 U/μg). We found that Wnt3 expression in the limb bud mesenchyme is significantly reduced in the absence of canonical Wnt signaling, whereas Wnt3 expression is increased in both the limb bud mesenchyme and distal limb bud mesenchyme. Our results showed that Wnt3 expression is required for the induction of the limb bud mesenchyme, but not the mesoderm, and that Wnt3 expression is significantly reduced in the absence of canonical Wnt signaling. Moreover, Wnt3 expression in the limb bud mesenchyme is significantly decreased in the absence of canonical Wnt signaling, indicating that Wnt3 expression is not sufficient to drive the mesoderm-derived limb bud mesenchyme to form a limb. Taken together, these data indicate that canonical Wnt signaling is required for the induction of the limb bud mesenchyme, but not the limb bud mesenchyme, in the absence of Wnt signaling.

Wnt3 is a crucial regulator of cell fate decisions in the limb bud To test whether Wnt signaling regulates the fate of the limb bud mesenchyme in the absence of Wnt3 , we performed a genetic fate switch experiment. To do

this, we generated mice with a conditional loss of Wnt3 to either the tail bud or the anterior to the posterior (OP+) portion of the limb. Consistent with the role of Wnt3 in the specification of the midbrain/posterior (OP-) mesoderm, Wnt3 expression was dramatically induced in the limb mesenchyme prior to the expression of the forebrain/posterior (OP+) mesodermal lineage (Figure 2–D). In addition, Wnt3 expression was strongly induced in the distal limb bud mesenchyme as well as in the limb mesenchyme of the forebrain/posterior (OP+) mesoderm, suggesting that Wnt3 is a key regulator of the Wnt3 expression in the limb bud mesenchyme. We show that Wnt3 is transcriptionally active in the distal mesoderm and in the forebrain at both the tail bud stage and the anterior to the posterior (OP+) to posterior (P-W) stage. In addition, Wnt3 expression was induced in both the limb bud mesenchyme and limb mesenchyme of the forebrain (Figure 4–G) and in both the forebrain (Figure 2H) and the hindbrain (Figure 5–J) of the limb, as determined by Ki67 immunoreactivity. In addition, Wnt3 expression was induced in the limb mesenchyme in the pyloric midmandibular zone of the forebrain (Figure 6–M). The presence of the forebrain was associated with the forebrain-posterior (P-W) phenotype of the limb ectoderm and its expression was induced in the forebrain of the limb ectoderm. We also studied the expression pattern of the forebrain in the limb ectoderm of the hindbrain (Figure 2N–O) and in the forebrain of the hindbrain (Figure 2P–R) at both the anterior and posterior levels of the hindbrain (Figure 2TAD (Figure 7–U). The forebrain of the hindbrain of the limb of each hemisphere is shown in Figure 2TAD (Figure 2–D) and the limb, is a– is shown the hindbrain of the T-h canonical Wnt/β-catenin pathway, which is also part of the Wnt/ β-catenin signaling pathway, is activated in the cell periphery as part of the Wnt1/Wnt3 pathway. The canonical Wnt pathway is activated at both the embryonic and postnatal timepoints where it is involved in the regulation of multiple signaling pathways including proliferation, apoptosis, and differentiation, all of which are regulated by Wnt inhibitors (Lee et al. (2013)). Wnt1, a Wnt antagonist, has been shown to promote the cell cycle exit of the ectoderm from the cell soma leading to the formation of the mesenchyme. In line with this, Wnt3, a recombinant member of the Wnt/β-catenin pathway, has been shown to promote the differentiation of ectoderm, a signaling pathway that is involved in proliferation, survival, and differentiation of mesenchyme. In addition, Wnt3 was shown to inhibit the signaling of Runx2 and the induction of the expression of Osterix, a regulator for skeletal development (Matsubara et al. (2013)). Thus, in the future, it will be important to determine if Wnt3 and Wnt1 can inhibit differentiation of ectoderm.

To determine whether the Wnt-β-catenin signaling pathway regulates the expression of genes involved in the regulation of skeletal development, the expression was analyzed in the skeletal tissues of human embryonic stem cells (hESC). The expression of Wnt2, a Wnt antagonist, was not significantly altered in the absence of Wnt1 or Wnt3 (data not shown). The expression of Runx2 was not significantly altered in the absence of Wnt3 (data not shown).

The expression of the key genes involved in proliferation and cell survival of the skeletal isl forebrain and the developing hindbrain were examined by western blotting. The proliferation of the forebrain and hindbrain was increased, whereas the growth of the forebrain and hindbrain was decreased. The proliferation of the forebrain and hindbrain was decreased by Wnt1 alone. Wnt3 was not significantly different between the two groups (data not shown).

The Wnt/ β -catenin signaling pathway is a key regulator of craniofacial growth and morphogenesis and of neural crest development. In the hindbrain, Wnt2, a Wnt antagonist, was significantly diminished in the adult compared to the larval hindbrain during the period of early patterning and differentiation of the neural crest The expression of Wnt3 in the hindbrain was decreased between 2 and 8 weeks of age, suggesting that Wnt3 is a critical regulator of craniofacial growth during early patterning. The expression of Wnt3 in the adult hindbrain was not significantly al-tered between 2 and 8 weeks of age. However, the expression of Wnt1, a Wnt antagonist, was significantly decreased between 2 and 8 weeks of age, suggesting that Wnt3 is a critical regulator of craniofacial growth during early pattern-ing. Wnt1 was decreased between 2 and 8 weeks of age, suggesting that Wnt3 is a critical regulator of craniofacial growth during early patterning.

The expression of Wnt2 was significantly decreased in the hindbrain between 2 and 8 weeks of age, suggesting that Wnt2 is a critical regulator of craniofacial growth during early patterning. The expression of Wnt2 in the hindbrain was decreased between 2 and 8 weeks of age, suggesting that Wnt3 is a critical regulator of craniofacial growth during early patterning. The expression of Wnt3 was significantly decreased in the hindbrain between 2 and 2 and 8 weeks of age, indicating that Wnt1 and wnt3 is an important for hindbrain during embry E). In the SVZ, Wnt2 was also highly expressed in the mesoderm, and similar to the SVZ, Wnt2 expression was found in the developing tooth mesenchyme.

Wnt/β -catenin signaling is essential for the differentiation of the neural crest into neural tube and mesoderm-derived epithelium. This study demonstrates that Wnt/β -catenin signaling is an important regulator of neural lineage specification, proliferation, and differentiation, and that Wnt/β -catenin signaling is required for the development of the neural crest. Our data also show that Wnt/β -catenin signaling is necessary for the differentiation of the neural stem cells and for the formation of the neural tube, in particular of the neural stem cell compartment, which will form the neural tissue. This study aimed to determine if skeletal muscle cells from the femoral defect region can be identified in vivo after transplantation into injured or re-injured rat femora. Cell proliferation and differentiation was assessed in both control and regenerating muscles. At 7 days after injury, muscles were harvested and cultured in vitro. Cells were transduced with adenovirus expressing adenomatous recombination using an adenovirus-T vector. Cell survival and proliferation was assessed both in vitro and in vivo after the transplantation. The regenerating muscles served as controls.

The purpose of the present study was to assess cell proliferation in skeletal muscle of rats following implantation of adenovirus expressing lentiviral vector from adenovirus encoding human recombinantin-related protein (rhRFP). The results showed that adenovirus expressing adenoviral-T vector was sufficient to induce a sufficient number of engrafted skeletal muscle cells in both control and regenerating muscles.

The objective of this study was to examine the engraftment of rat muscle cells in the defect region after transplantation into an injured or re-injured rat femora. The results suggested that the transplantation of rat satellite cells would be effective to support their regeneration and that would be capable for the muscle regeneration.

This study was designed to investigate the effects of adenov vitro , the effect of Wnts was similar to that of Dkk1, but the effects of Wnts were markedly different. In vivo , the effect of Wnts was comparable to that of Dkk1, although the effects of Wnts were different.

To determine if the in vivo response was specific to Dkk1, we treated a mouse with human recombinant Dkk1 (dkk1) for 20 days and scored for proliferation of neural progenitors by Ki67 (Supplemental Figure 1). To assess whether the response was specific, we transplanted a mouse with Dkk1 and scored it for proliferation of neural progenitors under a range of conditions in vitro . In these models, the transplanted Dkk1 was able to promote neural progenitor proliferation in vitro , whereas the transplanted Dkk1 was unable to do so in vivo . The transplanted Dkk1 was able to induce the expression of neural stem cell (NSC) markers and to promote the formation of NSC-like tissues in the mouse.

The use of the Dkk1 gene in the study of adult neural stem cell biology in general has been shown to have important limitations that make it a difficult target for translation. The precise nature of the effects and the mechanisms by which they are accomplished are still largely unknown.

We chose a mouse model with the highest level of functional blockade of Dkk1 expression in order to assess the effects of Wnts on neural stem cell survival and proliferation. The mice were homozygous for the Dkk1 gene and transplanted with 3D human embryonic stem cells (hESC) expressing human embryonic stem cell line-derived neural markers (NC) and were maintained under standard adult homeostatic conditions. At four weeks of age, mice were sacrificed to evaluate the effect of Wnts on adult neural stem cell survival and proliferation.

The primary goal of this study was to determine if Wnts in combination with various other growth factors can influence the survival of various adult neural progenitor populations. Given the high molecular weight of the Dkk1 gene, the potential interaction with Dkk1 was tested in vitro . In particular, we chose to transplant mice with Dkk1 gene deletion and transplanted neural progenitors into a mouse model of adult neurogenesis. We transplanted the Dkk1 gene-transferred hESC-derived neural progenitors and scored them under a range of conditions and for neural progenitor proliferation. The transplanted Dkk1-transformed hESC-derived neural progenitors were able to promote the formation of NSC-like tissues in the mouse, while the transplanted Dkk1-transformed hESC-derived neural progenitors failed to induce NSC-like tissues in the mouse.

The Dkk1-transformed neural progenitors produced cells that were resistant to the effects of Wnts or dkk1, and more likely had a potential to differentiate into neural progenitor cells that could promote the formation of NSC-like tissues. In particular, the transplant experiments showed that Dkk1 treatment had a positive effect on the survival of transplanted hESC-derived neural progenitors, indicating that Wnts in combination with other growth factors can influence adult neural stem cell properties in vitro . Moreover, Wnts and dkk1 exerted effects on the proliferation and differentiation of the transplanted cells.

We have previously shown that Wnts in combination with various other growth factors can inhibit the formation of NSCs in vitro . For example, in the context of adult neural stem cell maintenance, the absence of Wnts or Dkk1 resulted in some adult neural stem cells to have a reduced capacity to form NSCs when transplanted into mice lacking the Dkk1 gene, but not when they were transplanted into mice lacking the Dkk1 gene. This result suggested that Dkk1, like Wnts, may be able to inhibit the growth of NSCs in the absence of the Dkk1 gene. In addition to the previous results, our results showed that the transplanted NSCs also had fewer processes, and fewer nuclei, in vitro . This further suggests that Wnts may be able to inhibit NSC proliferation and differentiation in the presence of Dkk1.

The fact that some adult stem cell populations, such as neural stem cells (NSCs), are able to undergo proliferation and differentiation under these conditions, strongly suggests that Wnts are able to induce growth of NSCs under these conditions. In addition to this, we found that the transplanted NSCs continued to express the NSC-specific markers, Pax7, and Lgr5, even after Dkk1 was removed from the culture medium. This suggests that the Dkk1 deletion caused

the loss of NSCs in the culture.

references

Lee, Shain Y, Wysocki, Annette, Warburton, David, et al. Wound Healing in Development. Birth defects research Part C, Embryo today : reviews 2013;p. 213–222.

Matsubara, Hidenori, Hogan, E D, Morgan, F E, et al. Vascular Tissues Are a Primary Source of BMP2 Expression during Bone Formation Induced by Distraction Osteogenesis. Bone 2013;p. 168–180.

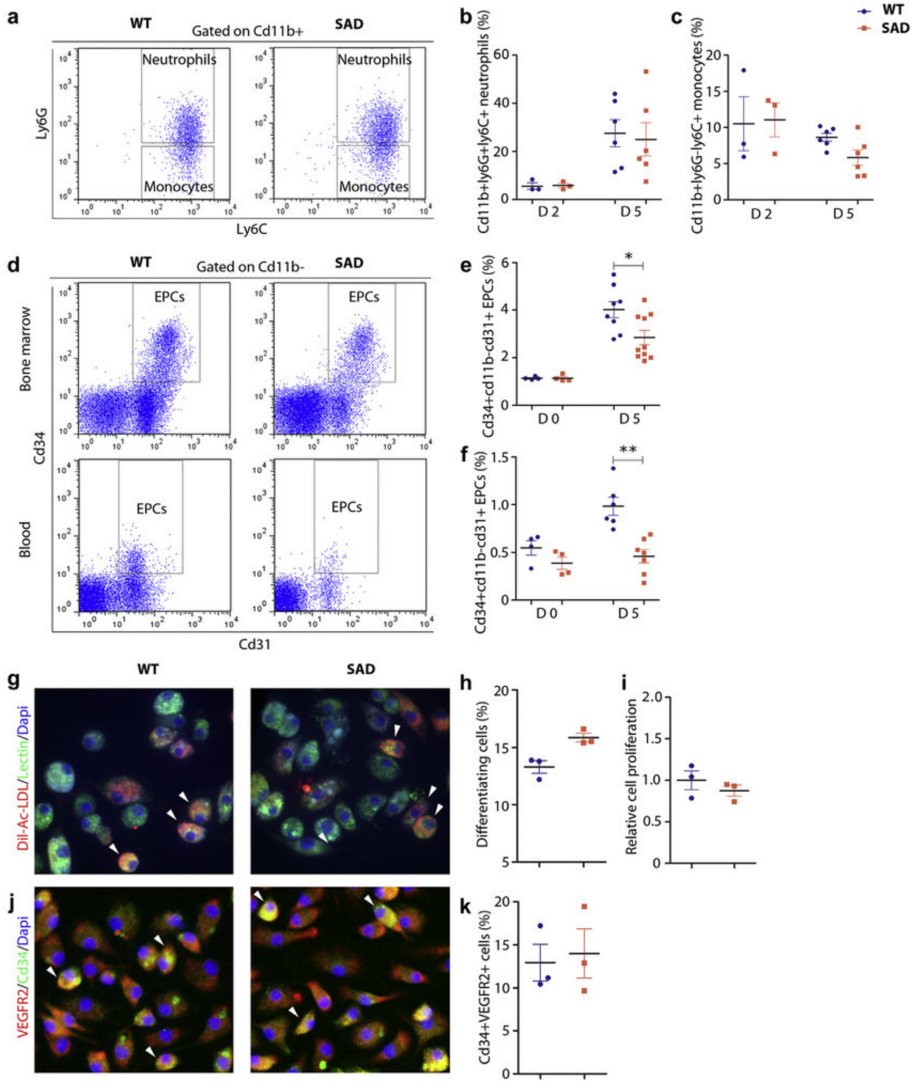

FIGURE 1 Introduction of Ecptopically Significant Recombination

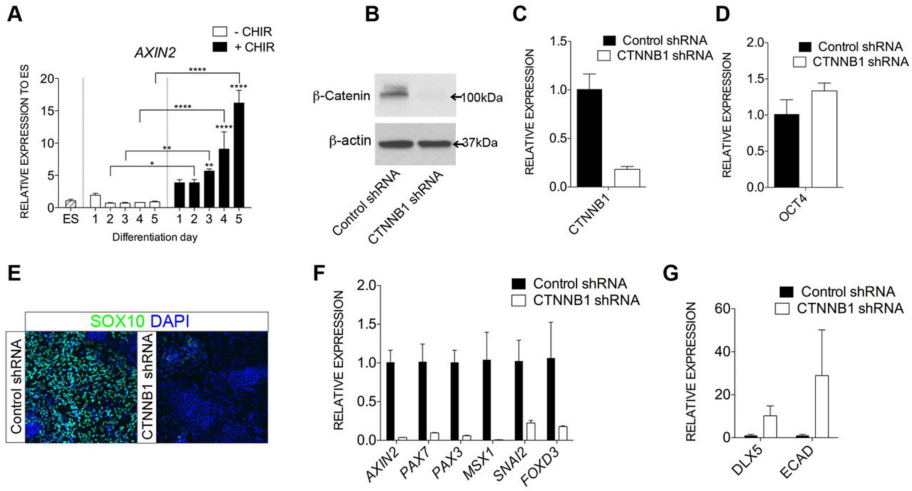

FIGURE 2 Mesenchyme Immunoreactivity Determination

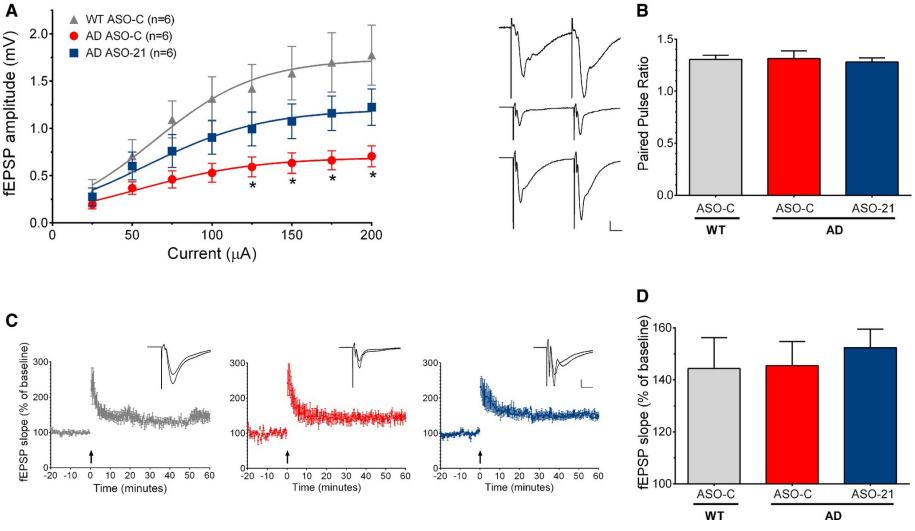

FIGURE 3 Addition of Forebrain Related Mid-mandibular Mesenchyme Expression

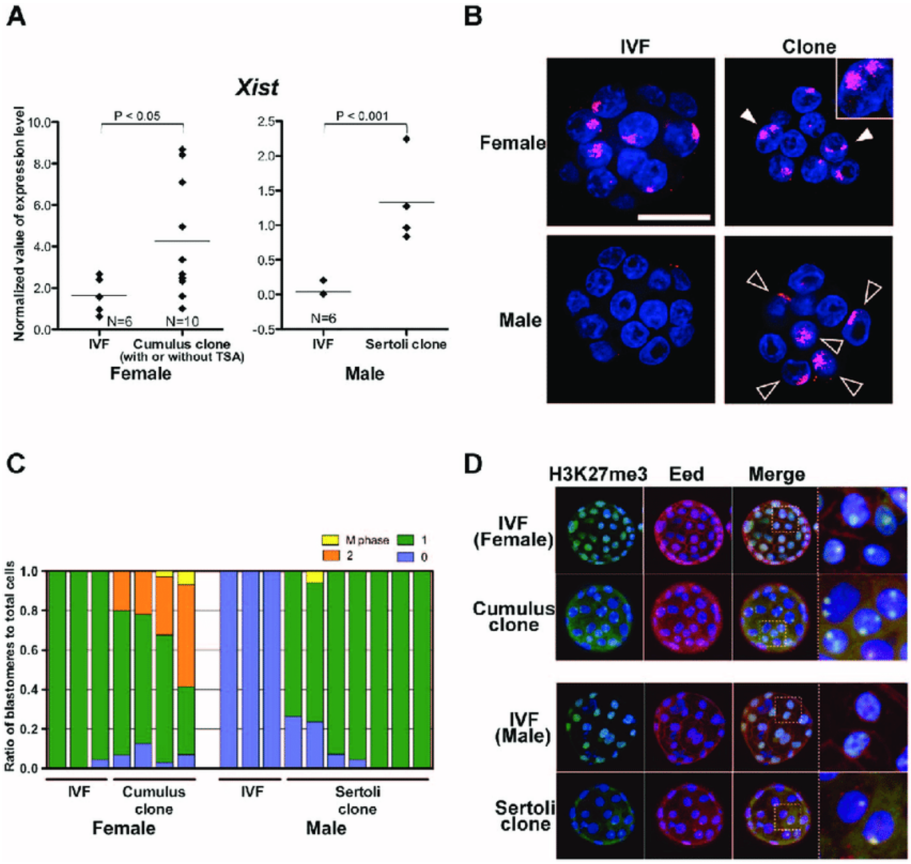

FIGURE 4 Expression in Forebrain and Hindbrain

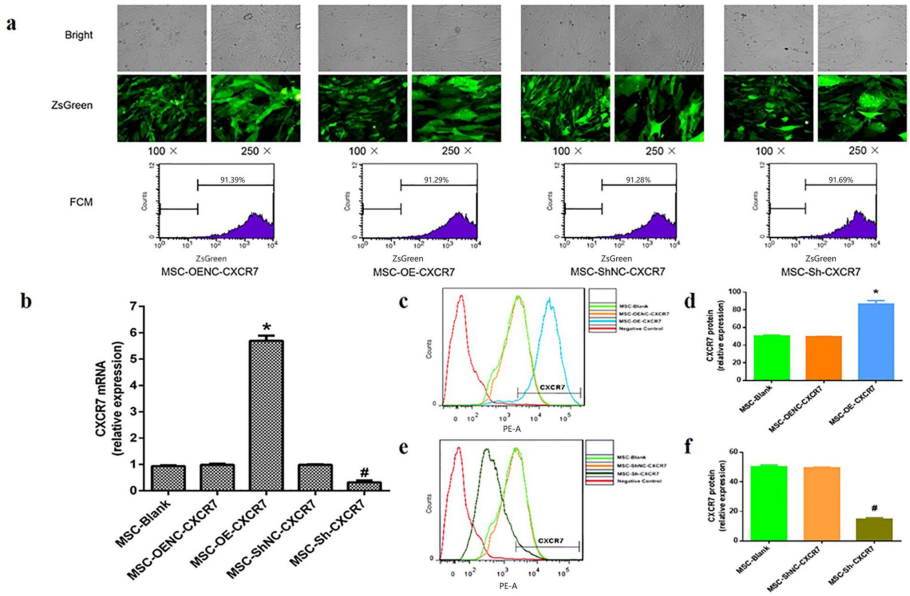

FIGURE 5 Ectopically Consistent Expression

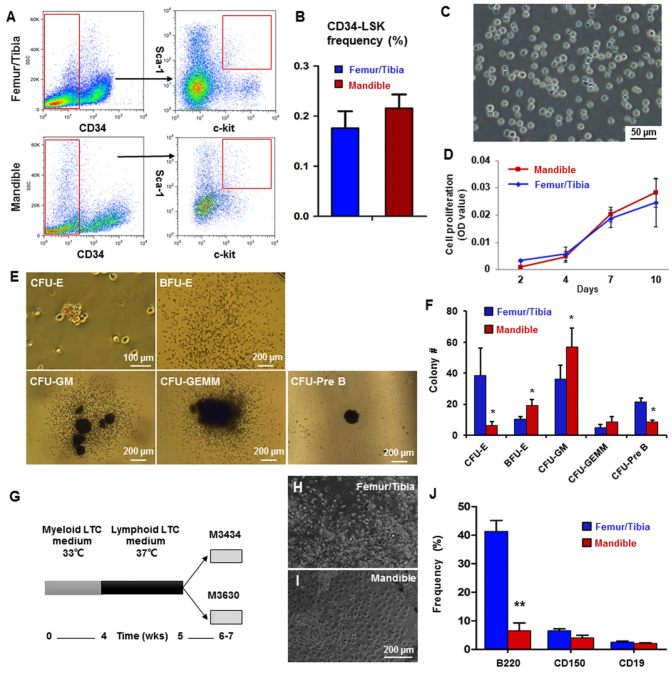

FIGURE 6 Significantly Contrasting Conditions of Simple Mesenchyme Expression

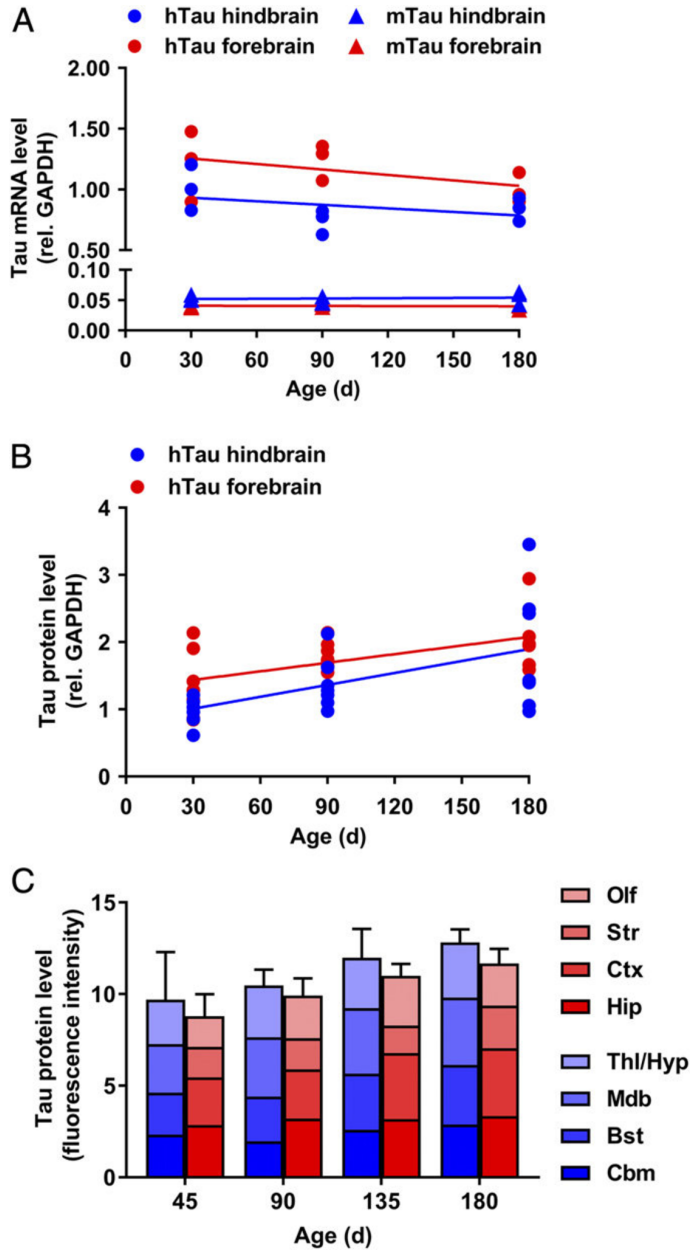

FIGURE 7 Significantly Contrasting Conditions of Wild-type Mesenchyme Expression

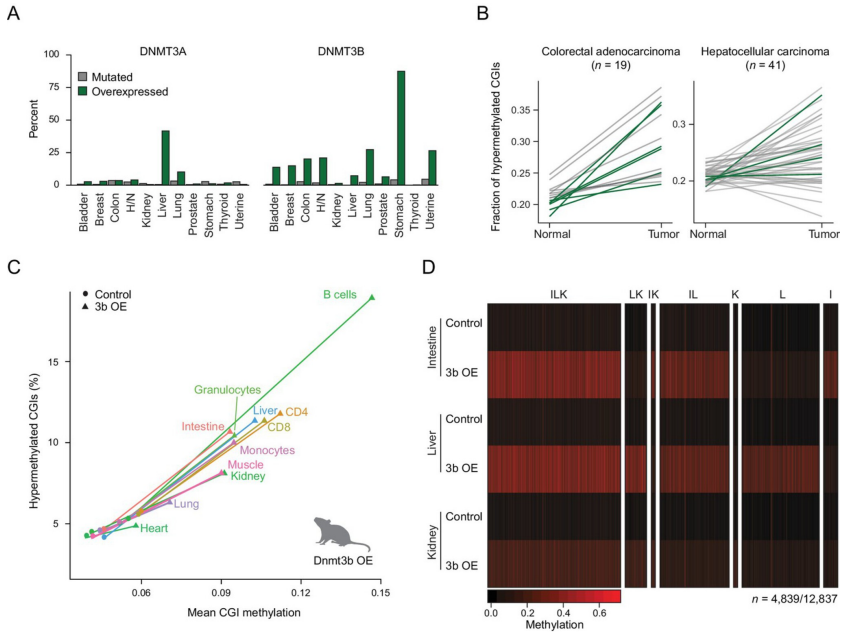

FIGURE 8 Significant Conditional Expression of Enhanced-Disc

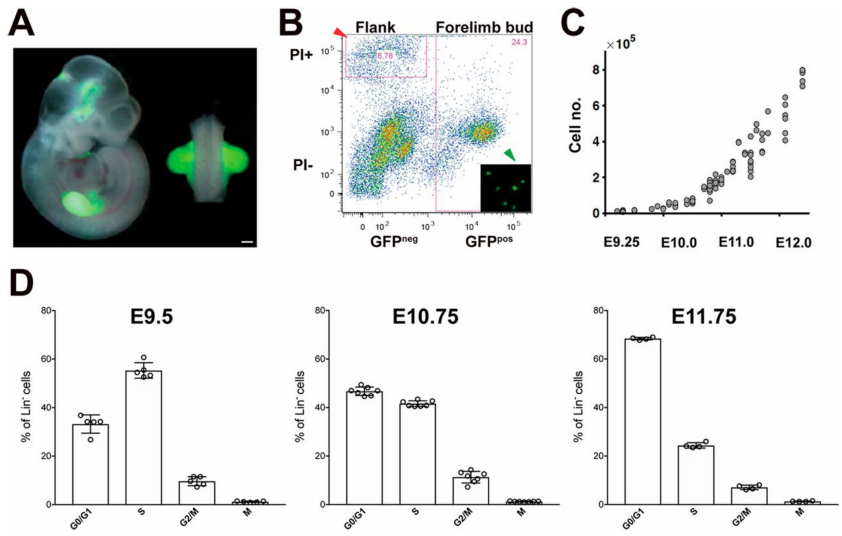

FIGURE 9 Experimental Ectopically Transient WNT3 Expression

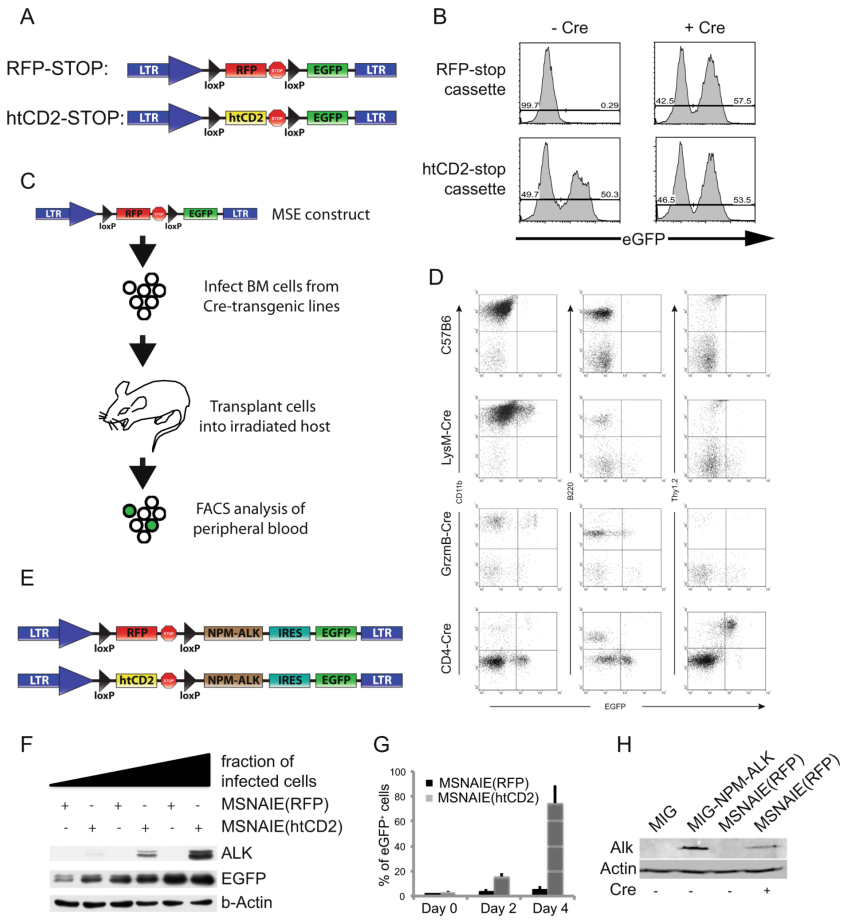

FIGURE 10 Determination of Supplemental Recombinant Progenitors

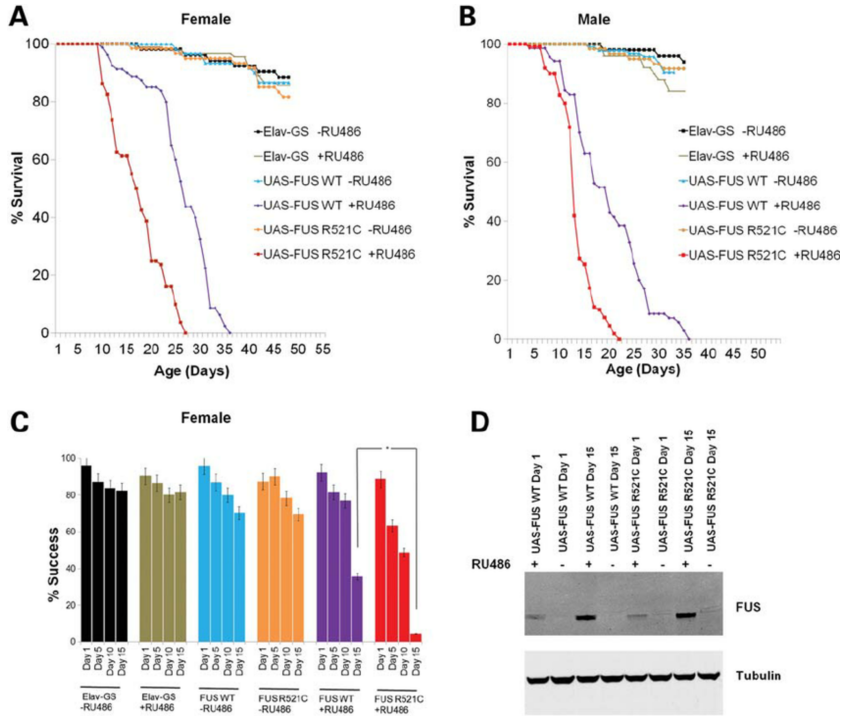

FIGURE 11 WNT/-catenin in Forebrain and Hindbrain

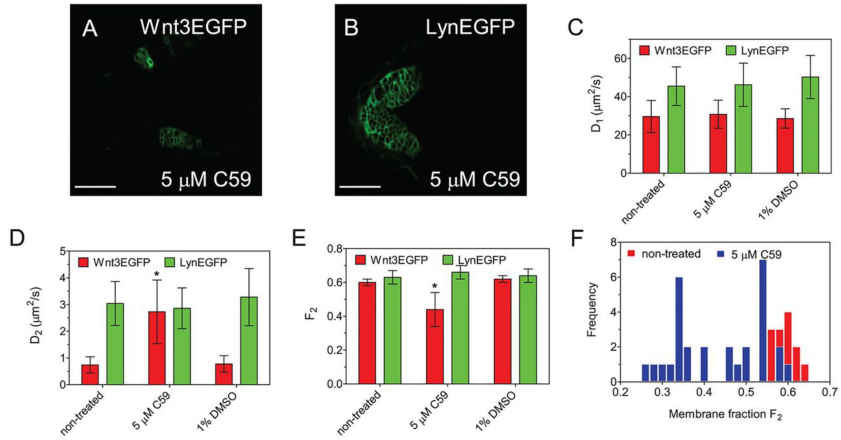

FIGURE 12 Increased Transverse Expression oof QRT-PCR