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MAZ mediates the cross-talk between CT-1 and NOTCH1 signaling during gliogenesis

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Neurons and glia cells are differentiated from neural stem/progenitor cells (NSCs/NPCs) during brain development. Concomitant activation of JAK/STAT and NOTCH1 signaling is required for gliogenesis, a process to generate glia cells to ensure proper brain functions. NOTCH1 signaling is down-regulated during neurogenesis and up-regulated during gliogenesis. However, the underlying mechanism remains elusive. We report here that cardiotrophin-1 (CT-1) activates NOTCH1 signaling through the up-regulation of ADAM10, a rate-limiting factor of NOTCH1 signaling activation. We found that a transcriptional factor, Myc-associated zinc finger protein (MAZ), plays an important role in ADAM10 transcription in response to CT-1 in NPCs. MAZ knockdown inhibits CT-1 stimulated gliogenesis and it can be rescued by over-expressing human NICD. Our results provide a link between NOTCH1 activation and neuronal secreted CT-1, suggesting that CT-1 plays an important role in ensuring the coordinated activation of NOTCH1 signaling during gliogenesis.

Glia cells which account for more than 90% of cells in the brain, are essential for neuronal functions¹. They not only provide nutrients but also regulate the activity of neurons and mediate synapse transmission efficiency^{2,3}. Both neurons and glia cells are differentiated from neural stem/progenitor cells (NSCs/NPCs) during brain development, which processes are generally termed as neurogenesis and gliogenesis, respectively^{1,4}. Gliogenesis starts later than neurogenesis during cortical development and it is stimulated by the newborn neurons⁴. However, the molecular mechanisms controlling the neurogenesis to gliogenesis switch are still not very clear; and deregulated or defective gliogenesis has been indicated in many neural developmental diseases such as autism and schizophrenia¹. Several important signal pathways such as Notch and CT-1/JAK/STAT signaling pathways have been shown to be involved in gliogenesis^{5,6}.

Notch signaling acts as an important and delicate molecular switch for NSCs cell fate during mammalian cortical development^{5,6}. Four Notch receptors, NOTCH1-4, have been identified so far⁷. NOTCH1, the most studied one, is required for multiple cell fate determination processes, such as maintaining of NSCs pluripotency^{1,8}, promoting astrocytes differentiation⁹⁻¹¹ and neuronal maturation¹²⁻¹⁴. Interestingly, activated NOTCH1 signaling is essential for both NSC self-renewing and gliogenesis while down-regulation of NOTCH1 signaling is required for neurogenesis^{5,15-17}. It is not clear how NOTCH1 signaling is switched between those processes, especially how it is up-regulated during gliogenesis⁴.

NOTCH1 pathway activation involves three proteolytic cleavage steps. First, NOTCH1 is processed by a Furin-like enzyme in the Golgi apparatus and the self-heterodimer NOTCH1 receptor is transported to cell membrane. After binding to the NOTCH1 ligands on neighboring cells, such as Jagged 1 (JAG-1) or Delta-like 1 (DLL-1), NOTCH1 is cleaved by the site 2 (S2) enzymes, a disintegrin and metalloprotease 10 and/or 17 (ADAM10 & ADAM17), at outside juxtamembrane region¹⁸. Subsequently the transmembrane stub is cleaved by γ -secretase at site 3 (S3) to generate NOTCH1 intracellular domain (NICD). Within those proteolytic steps, the S2 cleavage is the rate-limiting step for NOTCH1 activation¹⁹. NICD translocates to the nucleus and interacts with transcription factor CSL (C_{BF}1, S_u(H), L_{ag}1) to activate downstream targets such as Hes1, Hes5, and nuclear factor I/A

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(NFIA)^{16,19}. It has been shown that NOTCH1 activates NFIA to release DNA (cytosine-5)-methyltransferase 1 (DNMT1) from the promoter of Glial fibrillary acidic protein (GFAP, a glia marker protein) during gliogenesis^{5,9,20,21}. Interrupting NOTCH1 signaling pathway by deletion of *CBF-1* can cause severe defects in gliogenesis²².

In addition to Notch, cardiotrophin-1 (CT-1), an interleukin-6 (IL-6) family cytokine secreted by newborn neurons, is also involved in gliogenesis^{1,23–26}. CT-1 stimulates gliogenesis by activating the JAK/STAT pathway and knockout of *CT-1* in mouse brain causes the reduction of glia cells^{24,25}. Previous studies also showed that early activation of JAK/STAT pathway in NSCs leads to the premature generation of glial cells in embryonic mouse brain, while inhibition of the JAK/STAT pathway prevents the production of glia cells²⁷. In addition, the expression of GFAP requires simultaneous activation of both NOTCH1 and JAK/STAT pathways as both CBF-1 and STAT3 bind to the promoter region of GFAP⁹. Therefore, both NOTCH1 and CT-1/JAK/STAT signals are essential for gliogenesis.

To ensure a synchronized activation of both NOTCH1 and CT-1/JAK/STAT pathways during gliogenesis, signal crosstalk between the two pathways has been proposed¹⁶. It has been shown that NOTCH1 activation leads to STAT3 activation²⁸. On the other hand, STAT3 can induce NOTCH1 ligands expression²¹. To further understand the relationship between the NOTCH1 and CT-1 pathways during gliogenesis, we investigated the role of CT-1 on NOTCH1 activation. Here we show that CT-1 can activate NOTCH1 signaling through the induction of ADAM10 expression. Myc-associated zinc finger protein (MAZ) plays an essential role in CT-1 stimulated ADAM10 expression and gliogenesis. Our results revealed a novel mechanism for the regulation of NOTCH1 signaling by CT-1 which is essential for gliogenesis.

Results

CT-1 induces the levels of ADAM10 and NICD in NPCs. Since both Notch and CT-1 signal pathways are involved in gliogenesis, we firstly investigated whether CT-1 has any effect on NOTCH1 pathway. Cultured NPCs isolated from embryonic day 11.5–13.5 (E11.5–13.5) mouse frontal cortex were treated with CT-1 for 72 hrs. The levels of several important components in the NOTCH1 pathway including NOTCH1 ligands (JAG-1 and DLL-1), S2 enzymes (ADAM10 and ADAM17), full length NOTCH1 receptor and NICD were inspected. As shown in Fig. 1A, the level of NICD was increased ~158% after CT-1 stimulation, while the full length NOTCH1 receptor was moderately enhanced ~39% (Fig. 1B). On the other hand, JAG-1 and DLL-1 levels were apparently not affected (Fig. S1A,B). Interestingly, the level of ADAM10 was induced ~112% by CT-1, which is comparable to that of NICD (Fig. 1C). However, the level of ADAM17 was decreased by ~55% (Fig. 1D). Similar effects were also observed in the mouse embryonic fibroblast cell line NIH3T3 cells with 24 hrs CT-1 stimulation (Fig. 1A–D).

To confirm that the induction of ADAM10 and NICD by CT-1 leads to the NOTCH1 pathway activation, we examined two NOTCH1 down-stream targets, *Hes1* and *Hes5*. The mRNA levels of both *Hes1* and *Hes5* were significantly induced by CT-1 stimulation in both NPCs (Fig. 1E) and NIH3T3 cells (Fig. 1F). Thus, our results indicate that CT-1 stimulation can elevate both ADAM10 and NICD levels and activate NOTCH1 signaling.

ADAM10 is essential for CT-1 induced NOTCH1 cleavage. As CT-1 could induce ADAM10 but not ADAM17 and activate NOTCH1 signaling, we postulated that CT-1 might activate NOTCH1 signaling through ADAM10. To test this hypothesis, both ADAM10 and ADAM17 knockout cell lines were generated with TALEN method as described in *materials and methods*. The knockout of ADAM10 or ADAM17 was confirmed by Western blot analyses (Fig. 2A). Similar to NIH3T3 cells, the levels of NICD and ADAM10 were up-regulated after CT-1 stimulation for 24 hrs in WT cells (Fig. 2B–E). CT-1 was still able to up-regulate both ADAM10 and NICD levels in ADAM17 KO cells (Fig. 2C–E). However, the elevation of NICD level was not observed in ADAM10 KO cells (Fig. 2B,D), indicating that ADAM10 but not ADAM17 is essential for CT-1 stimulated NICD elevation.

CT-1 induces the transcription of ADAM10 through MAZ. We went on to explore how CT-1 induces the expression of ADAM10 and found that *Adam10* mRNA level was induced at 6 hrs after CT-1 stimulation, with a peak at 8 hrs in NIH3T3 cells (Fig. 3A), suggesting that CT-1 regulates the transcription of ADAM10. We therefore tried to find the potential important element(s) in the promoter of *ADAM10*. Luciferase reporter constructs with different regions of mouse *Adam10* promoter were generated and used for transcriptional analysis. As shown in Fig. 3B, *Adam10* promoter regions from –1060 to –107 could significantly up-regulate the luciferase activity in the presence of CT-1, comparable to the induction of endogenous ADAM10 mRNA level (Fig. 3A). This suggests that a response element is located within this region.

Although STAT3 is the downstream transcription factor of CT-1 in the up-regulation of GFAP expression during gliogenesis²⁵, there is no STAT3 binding motif located within the ADAM10 promoter region excluding STAT3 as the responsible transcriptional factor²⁹. Therefore, we characterized *Adam10* promoter furthermore and found that the –615 to –107 and –1060 to –289 could also respond to CT-1 stimulation (Fig. 3C). These suggest that the promoter region around –615 to –289 is potentially important. Using two online bioinformatics tools (www.cbil.upenn.edu/tess and www.tools.genome.duke.edu/genereregulation/mcpromoter), we searched for potential protein binding motif in that region and found a Myc-associated zinc finger protein (MAZ) binding motif CCCTCCC located at –458 to –452. Therefore, two deletion mutants (–1060 ~ –289ΔMAZ & –615 ~ –107ΔMAZ) were generated and tested. The deletion of MAZ binding motif completely abolished their response to CT-1 stimulation (Fig. 3B). CHIP assays confirmed that MAZ could indeed bind to the *Adam10* promoter as the anti-MAZ antibody could pull down the fragment of *Adam10* promoter which contained the MAZ binding motif but not the *Adam10* intron1 (Fig. 3C). Furthermore, CHIP assay also showed that CT-1 up-regulated the binding of MAZ to *Adam10* promoter (Fig. 3D). Taken together, our results indicate that the transcription factor, MAZ, plays a role in CT-1 stimulated transcriptional regulation on *ADAM10*.

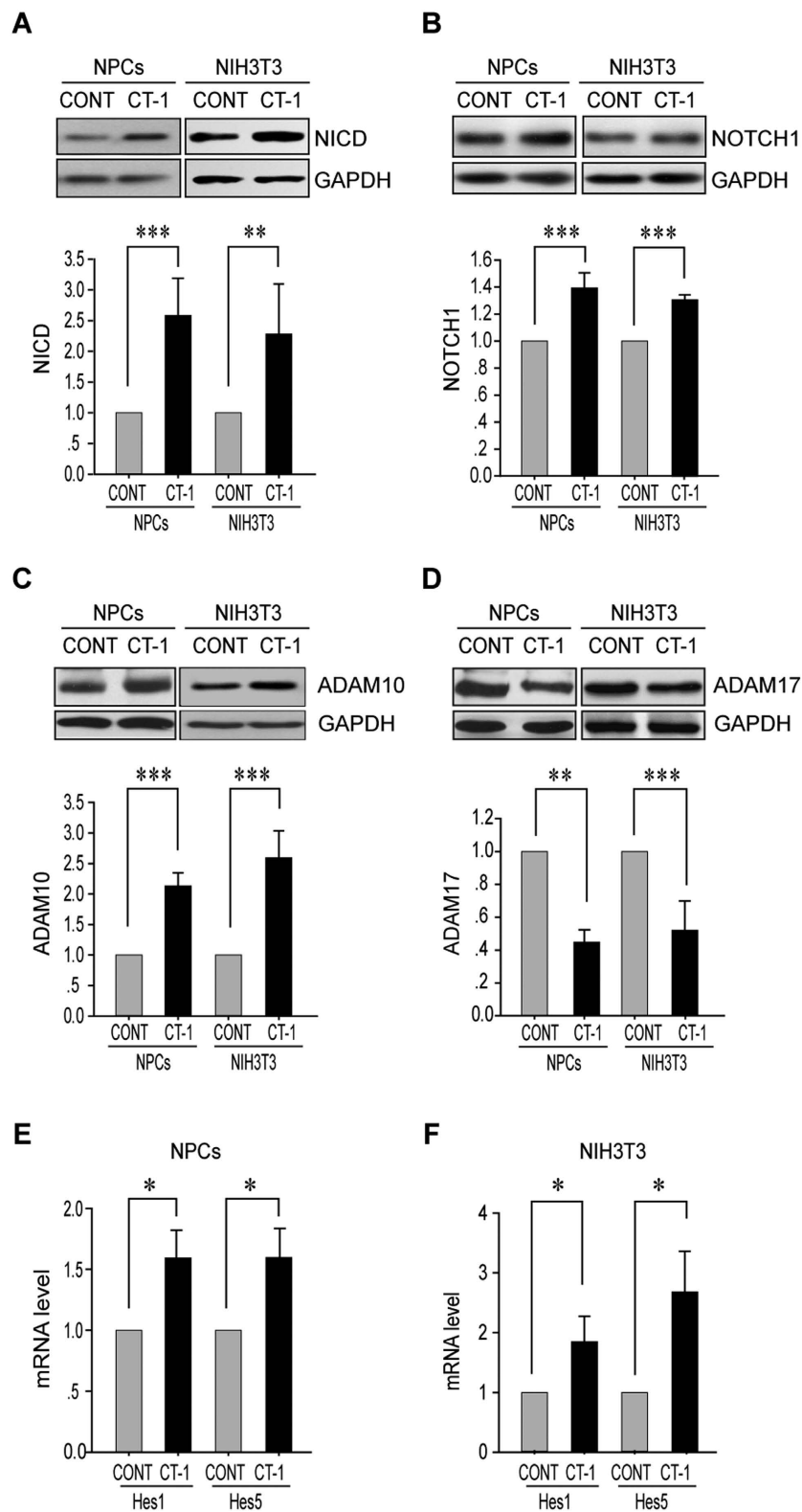


Figure 1. CT-1 up-regulates ADAM10 and NICD levels in NPCs and NIH3T3 cells. NPCs isolated from E11.5–13.5 mouse embryonic cortex were stimulated with CT-1 (100 ng/ml) or buffer (control, Cont) for 72 hrs, and NIH3T3 cells were stimulated with CT-1 (100 ng/ml) or buffer (control, Cont) for 24 hrs. Cell lysates were subjected to Western Blot analysis for both NICD (A) and full length NOTCH1 receptor (B) and two S2 enzymes ADAM10 (C) and ADAM17 (D). GAPDH was used as loading control. Representative blots and statistical analysis are shown in the upper and lower panels respectively. Two downstream effectors of NOTCH1 pathway, *Hes1* & *Hes5* were examined by qRT-PCR after CT-1 stimulation for 24 hrs in NIH3T3 cells (E) or 48 hrs in NPCs (F). The blots were cropped to improve the clarity and conciseness and full-length blots are presented in Supplementary Fig. S3. All data represent means \pm SEM (one-way ANOVA). $N \geq 5$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

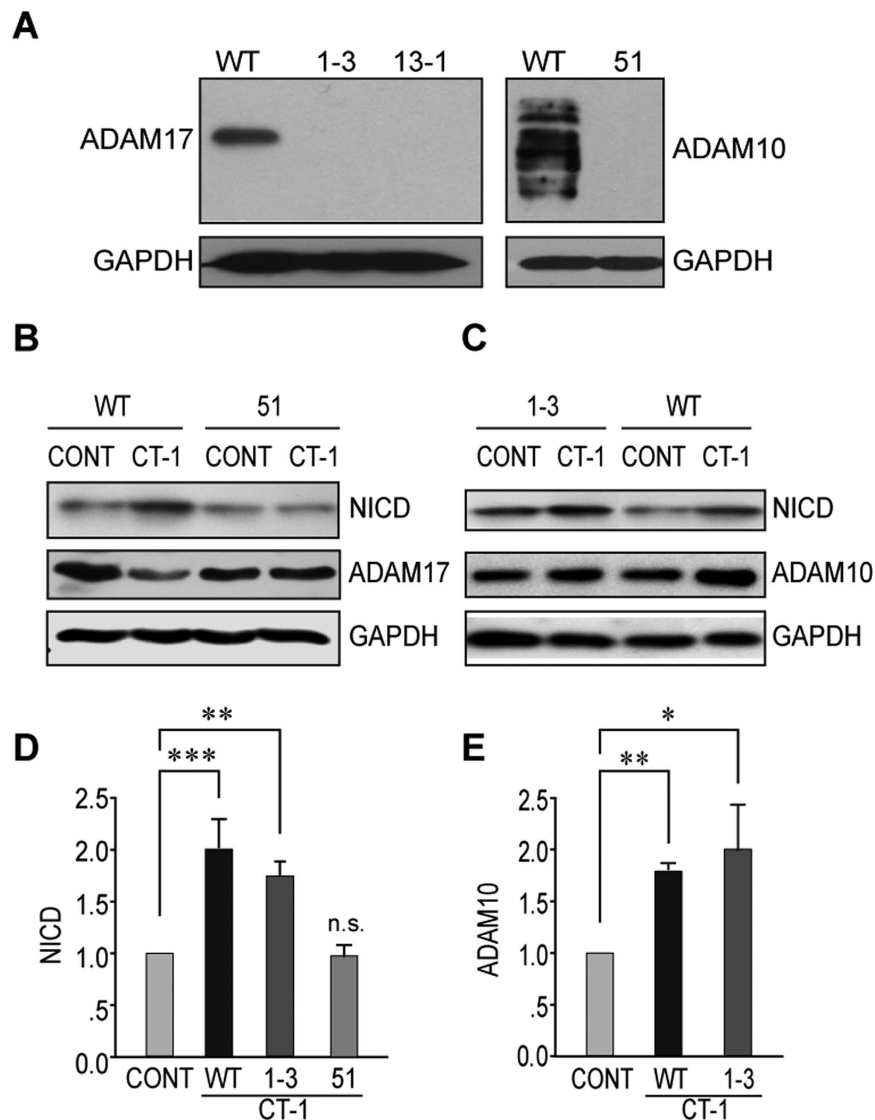


Figure 2. ADAM10 is essential for CT-1 induced NICD increase. (A) Confirmation of ADAM17 KO 293T cell lines (1-3 and 13-1) and ADAM10 KO 293T cell line (51) by Western blot analysis. (B,C) Different cell lines were stimulated with 100 ng/ml CT-1 for 24 hrs. Cell lysates were analyzed for NICD, ADAM10, or ADAM17 levels with GAPDH as loading control. Representative blots are shown in (B,C). (D,E) Statistical analysis for NICD and ADAM10 levels from (B,C). The blots were cropped to improve the clarity and conciseness and full-length blots are presented in Supplementary Fig. S3. All data represent means \pm SEM (one-way ANOVA). $N = 4$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

MAZ is important for CT-1 induced gliogenesis. We showed above that MAZ is responsible for CT-1 induced ADAM10 up-regulation, we then examined the role of MAZ in NPCs. NPCs were transfected with either MAZ-shRNAs or human MAZ (hMAZ) expressing construct and incubated with either CT-1 or control media. MAZ knockdown blocked CT-1 stimulated up-regulation of ADAM10 and NICD in NPCs (Figs 4A and S2). On the other hand, both ADAM10 and NICD levels were enhanced by hMAZ in the absence of CT-1 stimulation (Figs 4B and S2). When NPCs over-expressing hMAZ were stimulated with CT-1, the levels of NICD and ADAM10 were not further induced but down-regulated relatively (Figs 4C and S2).

We then went on to investigate whether MAZ is involved in gliogenesis. Specific markers were adopted for the detection of cell lineages, with GFAP for astrocytes, Nestin for NPCs, and Map2 or Tuj1 for neurons. Similar to previous report²⁵, CT-1 stimulation of NPCs substantially induced the expression of GFAP as well as the percentage of GFAP+ cells (Figs 4C and S2, Fig. 5A,B). Such effect could be significantly blocked by MAZ knock-down (Figs 4C and S2, Fig. 5A,B). Meanwhile, the levels of Nestin or Map2 or the ratio of Nestin+ or Tuj1+ cells were affected but not very significantly by MAZ knock-down (Figs 4C and 5C,D). We also inspected whether the MAZ expression could enhance the levels of GFAP. No apparent increase of GFAP expression was observed (Figs 4D and S2). Those results indicate that MAZ may play a necessary but not sufficient role in gliogenesis.

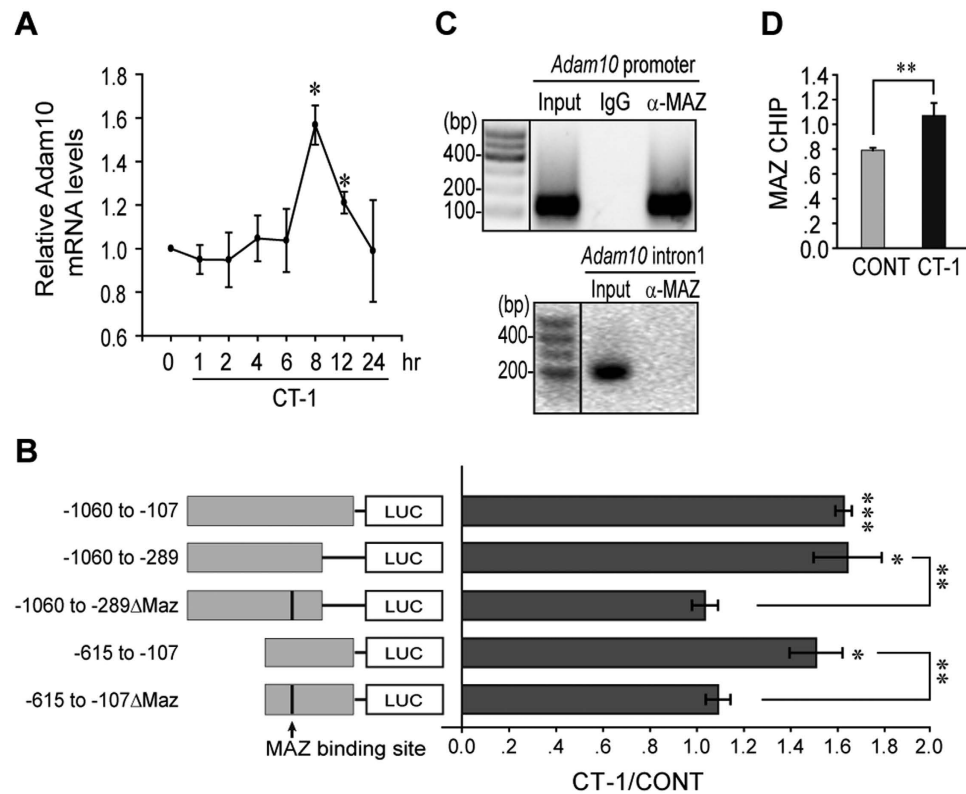


Figure 3. CT-1 induces ADAM10 transcription through MAZ. (A) NIH3T3 cells were treated with CT-1 at different time points. *Adam10* mRNA levels were determined by qRT-PCR. (B) Luciferase reporter constructs with different regions of *Adam10* promoter were transfected into NIH3T3 cells. Relative luciferase activities were determined after stimulation with CT-1 or control buffer for 12 hrs. (C) After incubation with CT-1 for 24 hrs, nuclei of NIH3T3 cells were extracted and ChIP were performed with anti-MAZ antibody or IgG control followed by amplification with primers targeting the -526 to -410 bp of *Adam10* promoter region. An adjacent fragment of *Adam10* intron1 was included as a negative control. ‘Input’ indicates PCR amplification of total DNA. (D) The relative amount of *Adam10* promoter fragments by CHIP assay in (D) were determined by qRT-PCR. All data represent means \pm SEM (one-way ANOVA). $N = 4$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Since MAZ regulates the level of ADAM10 as well as NICD, we hypothesized that MAZ might act upstream of NOTCH1 cleavage in the NOTCH1 signaling pathway. To test this speculation, we co-expressed either mouse ADAM10 or human NICD fragment together with MAZ shRNAs in NPCs and inspected different cellular markers 72 hrs after CT-1 stimulation. The up-regulation of GFAP level under CT-1 stimulation was repressed by MAZ knockdown, and the down-regulation could be rescued by over-expression of either ADAM10 (Fig. S2) or hNICD (Fig. 4E). Similarly, the increased percentage of GFAP⁺ cells under CT-1 stimulation on NPCs was also repressed by MAZ knockdown and the suppression could be rescued by over-expression of hNICD (Fig. 5A,B). This indicates that the activation of NOTCH cleavage could rescue the inhibitory effects of MAZ knockdown on CT-1 induced gliogenesis. Taken together, our results strongly suggested that MAZ plays an important role in CT-1 induced gliogenesis of NPCs through the up-regulation of ADAM10 and NOTCH1 cleavage.

Discussion

Signal crosstalk between the NOTCH1 and CT-1/JAK/STAT pathways has been proposed to ensure a synchronized activation of both pathways for proper gliogenesis¹⁶. In this study, we demonstrate that CT-1 induces NOTCH1 signaling through the regulation of the speed-limiting S2 enzyme ADAM10. CT-1 can induce ADAM10 expression via transcription to ensure NOTCH1 activation and proper gliogenesis. MAZ is an essential transcriptional factor for CT-1 induced ADAM10 up-regulation and gliogenesis (Fig. 6). Our results revealed an interesting and important crosstalk mechanism between NOTCH1 and CT-1 signaling pathways for gliogenesis.

It has been shown previously that ciliary neurotrophic factor (CNTF) stimulation of embryonic stem cells⁸ or NSCs³⁰ induces Notch activation as well as gliogenesis. Both CNTF and CT-1 belong to the IL-6 family cytokine, but CNTF is not expressed in embryonic brain while CT-1 is secreted by newborn neurons to stimulate gliogenesis of NSCs in the brain²⁵. Unlike CNTF which promotes NOTCH1 receptor expression dramatically³¹, we showed that CT-1 only moderately enhances the level of NOTCH1 receptor. Instead, CT-1 induces ADAM10 level significantly.

ADAM10, the critical S2 enzyme of NOTCH1, has been considered as the physiological enzyme for NOTCH1 due to the similar phenotypes, including undeveloped heart and neural tube which lead to early embryonic lethality at E9.5, in *Adam10* and *Notch1* knockout mice^{19,32–34}. Conditional ADAM10 knockout in mouse brain leads

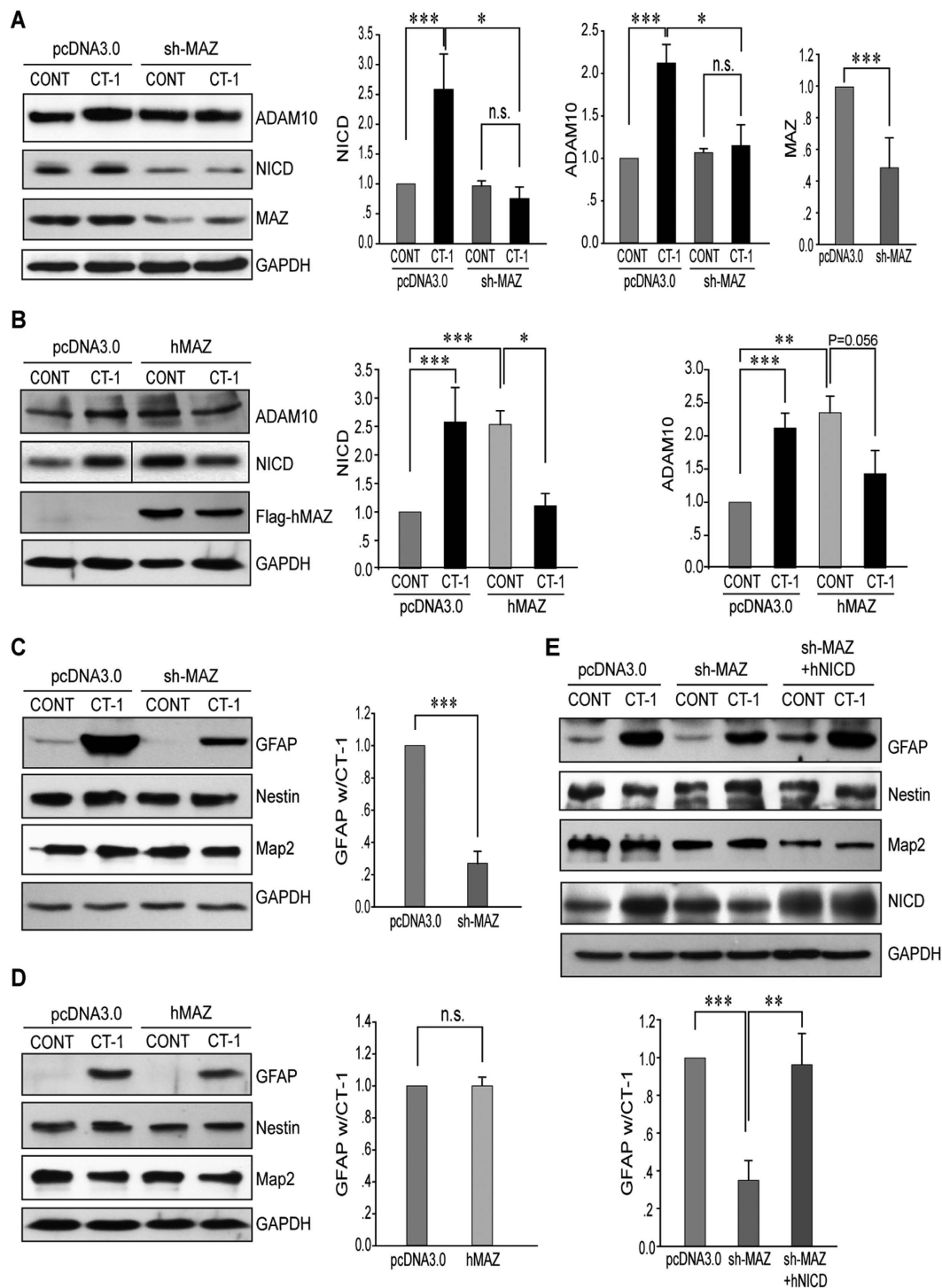


Figure 4. MAZ regulates CT-1 induced expression of NICD, ADAM10 and GFAP in NPCs. NPCs isolated from E11.5–13.5 mouse embryonic cortex were transfected with MAZ, sh-MAZ, hNICD or control constructs as indicated before seeding onto culture dishes. Cell lysates were prepared 72 hrs after CT-1 stimulation and NICD, ADAM10, MAZ, GFAP, Nestin or Map2 levels were analyzed by Western blot analysis. The blots were cropped to improve the clarity and conciseness and full-length blots are presented in Supplementary Fig. S3. All data represent means \pm SEM (one-way ANOVA). $N \geq 4$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

to disturbed cerebral cortex layout and less NSCs in cortex³⁵. However, the role of ADAM10 in gliogenesis was not clear as the conditional knockout of ADAM10 was driven by Nestin, which starts to express early during NSCs proliferation stage. Recently we reported that ADAM10 co-localizes with NICD and partially with glial cell marker S100 β in the intermediate zone (IZ) and cortical plate (CP) in E18.5 mouse cortex and ADAM10

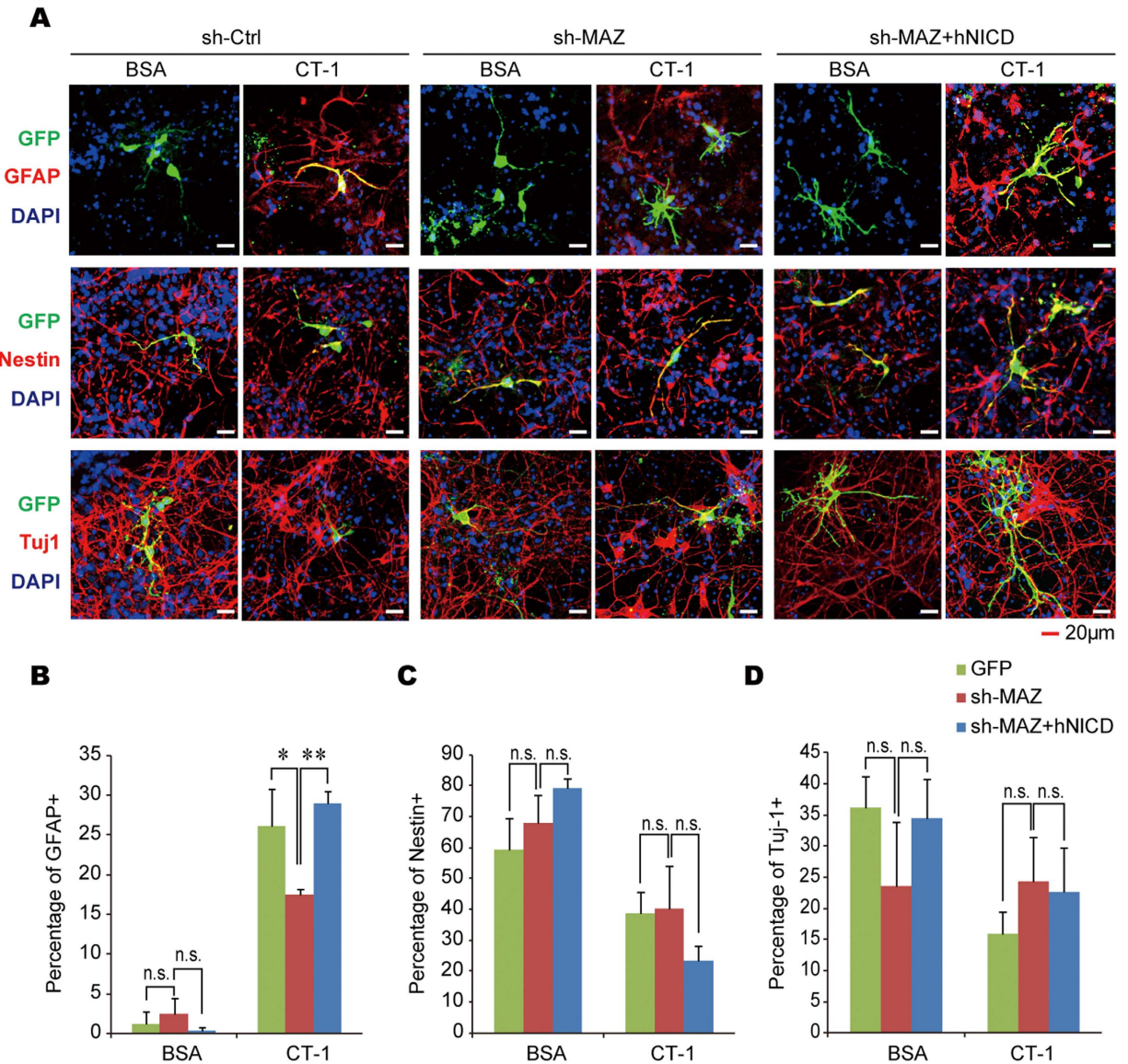


Figure 5. MAZ is required for CT-1 induced gliogenesis of NPCs. NPCs were isolated from E13.5–14.5 mouse embryonic cortex and transfected with GFP, sh-MAZ, hNICD or control constructs as indicated. Cells were treated with or without CT-1 for 72 hrs and subjected to immunofluorescent analysis. Nestin, Tuj1, and GFAP positive (+) cells were counted in those GFP positive cells. **(A)** The representative images. **(B–D)** Statistical analyses of the percentage of GFAP+, Nestin+, and Tuj1+ cells in GFP+ cells. All data represent means \pm SEM (one-way ANOVA). $N \geq 4$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

expression in the IZ and CP is much higher than that in stem layer VZ and SVZ³⁶. Considering that both NICD and ADAM10 were stimulated by CT-1, it is possible that higher levels of ADAM10 and NICD in the IZ and CP are likely to be stimulated by CT-1 secreted by surrounding newborn neurons.

In this study, we find that MAZ acts as an important transcription factor for the up-regulation of ADAM10 and NICD in response to CT-1 stimulation. In NPCs, CT-1 stimulation induced gliogenesis can be blocked by MAZ knockdown and this inhibitory effect can be rescued by both ADAM10 and NICD over-expression. Previous studies on MAZ, also named SAF-1 or Pur-1, were focused on its function as a downstream transcription factor of IL-1 or IL-6 signal during inflammation³⁷. Very little is known for its function during development. Interestingly, it is reported recently that when the binding of MAZ was blocked with a *cis* double-stranded oligodeoxynucleotides in a neural stem cell line C17.2 cells, the cells tend to differentiate into neurons³⁸. This is consistent with our results that MAZ knockdown leads to suppression of NOTCH signaling. Because down-regulation of NOTCH signaling is required for neurogenesis^{5,15–17}, inhibition of MAZ function in C17.2 cells may attenuate NOTCH signaling and promotes neurogenesis. Further understanding will be needed in conditional knockout mice model on MAZ and/or CT-1.

An interesting observation is that over-expression of hMAZ alone without CT-1 stimulation did not induce the level of GFAP, even though the level of NICD was induced in NPCs. This suggests that MAZ plays a necessary

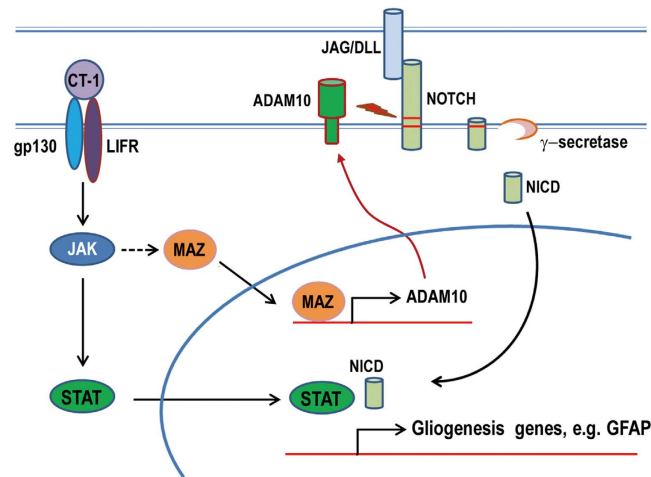


Figure 6. A schematic model for the crosstalk between CT-1 and NOTCH signalings during gliogenesis. CT-1 plays an important role in ensuring co-activation of JAK/STAT and Notch1 signaling during gliogenesis. CT-1 regulates the rate-limiting S2 enzymes of NOTCH, ADAM10 via transcription factor, Maz. Maz is essential for CT-1 induced up-regulation of ADAM10, NICD and GFAP, as well as gliogenesis.

but not sufficient role in gliogenesis. MAZ may have to coordinate with other components in CT-1 signaling pathways, e.g. JAK/STAT, to induce gliogenesis.

Another interesting observation is that the level of total MAZ was decreased about 43% after CT-1 stimulation for 72 hrs in NPCs (Figs S2A and E), even though CT-1 could up-regulate the binding of MAZ to ADAM10 promoter (Fig. 3D). This suggests that a negative feedback signal may exist here in the regulation of MAZ expression. However, the levels of ADAM10 and NICD are down-regulated instead of further induced by CT-1 in MAZ over-expressing NPCs cells (Figs 4B and S2A,C,D). Therefore, it is very likely that CT1 stimulation in the presence of high level of MAZ may also activate negative feedback signal to prevent the further elevation of ADAM10 and NICD levels. Our results suggest that the components involved in gliogenesis are well coordinated. How and why such sophisticated mechanism is required for gliogenesis will be an interesting subject for future study.

In summary, the gliogenesis of NSCs/NPCs requires both CT-1 and NOTCH1 signal pathways. In addition to activation of the JAK/STAT pathway, CT-1 also up-regulates Notch signaling through the regulation of S2 enzyme ADAM10. Such collaborative and synergic crosstalk between the two essential pathways is important to ensure the proper gliogenesis during brain development.

Material and Methods

Antibodies and inhibitors. The following antibodies were purchased from Abcam: ADAM10 (ab1997), NICD (ab8925), GFAP (ab7260), NOTCH1 (ab27526), DLL-1 (ab76655), JAG-1 (ab7771); and Nestin (ab6142), MAP2 (ab11268). Goat anti-ADAM17 (sc6416) and rabbit anti-MAZ (sc-28745) antibodies were purchased from Santa Cruz Biotech. Mouse anti-GAPDH, anti-alpha Tubulin, anti-beta Actin, and goat anti-rabbit and anti-mouse secondary antibodies were all purchased from Kangchen Bio-tech. Goat anti-Rat secondary antibody was purchased from Beyotime. CT-1 peptide (300–32) was purchased from Peptide.

Plasmids. pCMV6-flag-hMAZ construct was purchased from Origene. Human NICD construct was a gift from Dr. Hongyan Wang. Mouse MAZ shRNA constructs were purchased from Genechem Inc. Three different hairpins were cloned into pGV102/GFP to generate MAZ-shRNA constructs: 5'-gaTGCTGAGCTCGGCTTATATctcgagATATAA GCCGAGCTCAGCATC-3', 5'-ggCCCTTCA AATGTGAGAAATctcgagATTTCTCACATTT GAAGGGCC-3', 5'-gaGTAAGGTTGGGTGGTTAAActcgagTT TAACCACCCAACCTTA CTC-3', named as MAZ-shRNA1, 2, 3, respectively. Scramble sequence for control shRNA was: 5'-CACCGTTCTCCGAACGTGTCACGTCAAGAGATTACGTGACAC GTTCG GAGAATTTTTTG-3'.

To generate ADAM10 luciferase reporter constructs, different regions of the mouse *Adam10* promoter (NM_11487) were generated by PCR and cloned into NheI/HindIII sites of pGL3. The primers used are listed below. -1060 F: 5'-ctagctagcggccgctctctctc-3'; -615 F: 5'-ctagctagcttttga ggccaagaagc-3'; -451 F: 5'-aggccaatcctgctctcgc-3'; -459 R: 5'-cggggccgctggagactcgc-3'; -289 R: 5'-ccaagcttcgagc ggccaataac-3'; -107 R: 5'-ccaagcttgaagcgcctctcgc-3'; -106 R: 5'-ccaagcttgaagcgcctctcgc-3'. The -615 to -107 Δ MAZ (deleted -458 to -452) construct was generated by two fragments and vector ligation directly. The -1060 to -289 Δ MAZ (deleted -458 to -452) construct was generated with NovoRec[®] PCR one-step cloning kit (Sino Biotech) using NovoRec recombinase. Two circles of PCR and recombination reactions were used to generate the deletion construct. The primers used were -1060 rec-F1: 5'-agaacattctctatcgataGCCCCGCTCCTCTCCTCCCA-3' and -459 rec-R1: 5'-gatctcgagccgggctagcCGGGCCGCTGGAGACTCCG-3'; -451 rec-F2: 5'-cggagtctcca gcgcccccAGGCCAATCCCTGCTCTCCG-3' and -289 rec-R2: 5'-accaacagtaccggaatgccCGCGACGG CACCCAATAC-3'. Small letters indicate the recombinant arms used in the reactions.

Cell culture, transfection & Western Blot. NIH3T3 and 293T cells were grown in DMEM supplemented with 10% fetal bovine serum, 2 mmol/L glutamine, and 1% penicillin/streptomycin. Cells were transfected using Fugene (Roche 14738300) or X-tremeGENE 9 (Roche 06365787001). CT-1 was dissolved in DMEM containing 0.1% BSA. The following stimulator and inhibitors were used: 100 ng/ml CT-1, and 10 μ g/ml Cycloheximide. After proper incubation time, the cells were washed with PBS, and then lysed in cell lysis buffer (PBS, pH 7.4, with 1% (v/v) TritonX-100, 1 \times protease inhibitors, and 5 mmol/L 1,10-phenanthroline). Lysates were subjected to Western blot. In brief, samples were loaded and separated on SDS-PAGE and then electrically blotted to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories). After incubation in blocking buffer for 1 h, the membrane was incubated with the primary antibody at 4 °C overnight and then with the HRP-conjugated secondary antibody for 1 h, ECL was used to visualizing the protein bands. All films were scanned and analyzed with Quantity ONE based on intensity and normalized to GAPDH.

TALEN knockout 293T cell line. ADAM17 and ADAM10 knockout 293T cell line was made with TALEN technique by Viewsolid Biotech. In brief, TALEN primers were designed to target exon 1 of *ADAM17* (NM_003183.4): 5'-CTCAGACTACGATATTCTctcttctatctaatATCCAGCAGCATTCGGT-3' or the exon 8 of *ADAM10* (NM_001110.2): 5'-TTTTGATGATGGCGTACTtggtctggcttgggTTGGAGCACCTTCAGGTA-3'. Capital letters are the reading frame for TALEN arms. Two *ADAM17* KO cell lines, 1-3 and 13-1 and one *ADAM10* KO cell line 51 were successfully generated. A frame shift was generated after 138bp of human *ADAM17* CDS and a stop codon was generated at 168bp or 159bp for 1-3 or 13-1, respectively. For *ADAM10* knockout cell line 51, a frame shift after 990bp of *ADAM10* CDS and an early stop was generated at 1026bp.

NPCs isolation and differentiation assay. NPCs was isolated from C57BL/6 or CD-1(ICR) mice purchased from Shanghai SLAC laboratory animal Inc or Institute of Genetics and Developmental Biology (IGDB), Chinese Academy of Sciences (CAS). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Fudan University and IGDB, CAS. The protocol was approved by the Committee on the Ethics of Animal Experiments of Fudan University, Shanghai, and IGDB, CAS, Beijing. NPCs isolation and culture were based on previous report³⁹. In brief, E11.5-14.5 embryonic frontal cortex were dissected in cold PBS and NPCs were cultured in Neurobasal medium (Invitrogen) containing 500 μ M glutamine, 2% B27 supplement (Sigma), 1% penicillin and streptomycin (Invitrogen) and 40 ng/ml FGF2 (Peprotech). Transfections on NPCs were performed with X-tremeGENE HP DNA transfection reagent (Roche, 06366236001) before seeding the isolated NPCs on culture dishes. For CT-1 stimulated differentiation, NPCs were treated with CT-1 (100 ng/ml) for indicated time.

qRT-PCR. NPCs and NIH3T3 cells were lysed and total RNA was extracted with RNAeasy kit (QIAGEN). The levels of *ADAM10* mRNA were determined with qRT-PCR kit (Takara RR036A and RR820A). *GAPDH* gene was used as internal control. The primers used were: *Adam10*: 5'-cctgccatttcactctgtcattta-3' & 5'-gtgccccggctcctctctact-3'; *GAPDH*: 5'-acagcaactccactcttccact-3' and 5'-ttgctcagtgtccttctgtggg-3'. *Hes1*, *Hes5* and β -*actin* primers were adopted from a previous report⁴⁰.

Luciferase reporter assay. NIH3T3 cells were transfected with 1 μ g pGL3 with *ADAM10* promoter controlled luciferase gene and 1 μ g pSV- β -galactosidase using 4 μ l Fugene or X-tremeGENE 9 in Opti-MEM (Invitrogen). After 12 hrs CT-1 stimulation, the media were changed and cells were lysed for measurement of luciferase activity using a luciferase assay system (Promega E1483) and galactosidase activity with β -galactosidase Reporter gene assay kit (Beyotime RG0036). Luciferase activity normalized to β -galactosidase activity represents the promoter activity.

Chromatin Immunoprecipitation (CHIP) assay. CHIP with MAZ antibody was performed with Cell signaling SimpleCHIP[®] kit according to manufactory's protocol. The enrichment of specific DNA sequences was determined by PCR with the following primers: 5'-aggtagcactttcacaggga-3' and 5'-gccgctctctctgtccgct-3'. The PCR product is 117bp fragment correspondent to the -526 to -410 region of *Adam10* promoter. An irrelevant 204bp fragment located on *Adam10* intron1 +1398 to +1601 was amplified with primers 5'-gtcttgctgctgtttcacttt-3' and 5'-ctttcaccacaatgcttatgct-3'.

Immunofluorescent experiments. Cultured NPCs were washed with PBS and fixed in 4%PFA in PBS for 30 min at 4 °C. Then cells were treated with blocking buffer at room temperature (RT) for 1 hr. Primary antibody were added on cells and incubated at 4 °C overnight. Cells were washed with PBS 3 times next day before adding secondary antibody and incubated at RT for 1 hr. Cells were washed again with PBS and sealed for microscope imaging with Zeiss LSM700.

Statistic analysis. All WB bands were quantified by using Quantity one software. The results were normalized to its corresponding loading control GAPDH. Then the CT-1 stimulated condition was compared to BSA treated control condition in each set of experiments. All experiments were repeated at least three times and the statistical significance was evaluated by one-way ANOVA test. Differences were taken as statistically significant at $p < 0.05$.

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Author Contributions

Y.Z. designed the study and Y.Z. and Z.X. wrote the main manuscript text and prepared Fig. 6. B.L., A.M., Y.W., Z.L., and Q.L. prepared Figs 1–4. A.M. and F.Z. prepared Fig. 5. All authors reviewed the manuscript.

Additional Information

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