Secretagogin-dependent matrix metalloprotease-2 release from neurons regulates neuroblast migration

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PNAS. February, 2017 ($IF_{(5years)}$: 10.3)

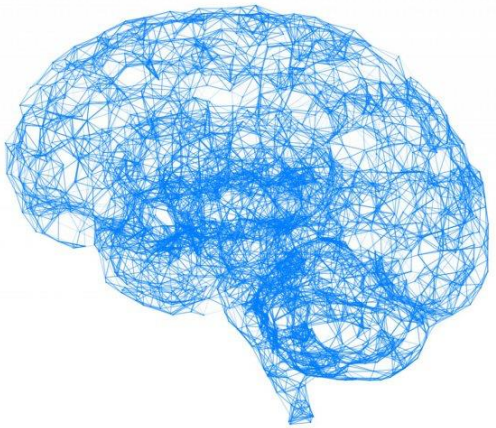
Thomas Haider

JC/TS Current Topics in Applied Immunology SS2017

March 6th, 2017

Background

Central Nervous System



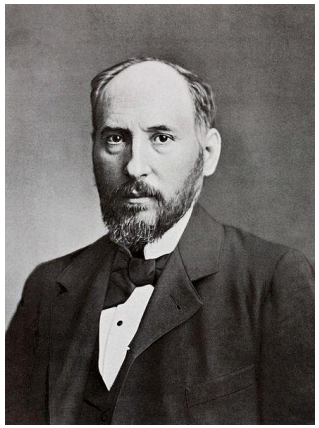
- Complex network

- Brain: 100 billion neurons (100 000 000 000)
  - Each neuron – connections (synapses) to up to 10,000 neurons
  - Estimated 1,000 trillion synapses! (1 000 000 000 000 000)

- During development high plasticity necessary
  - Learning
  - Memories

Front Neurosci. November 2009, Volume 3, Article 31.
Front Neurosci. June 2015, Volume 8, Article 23.

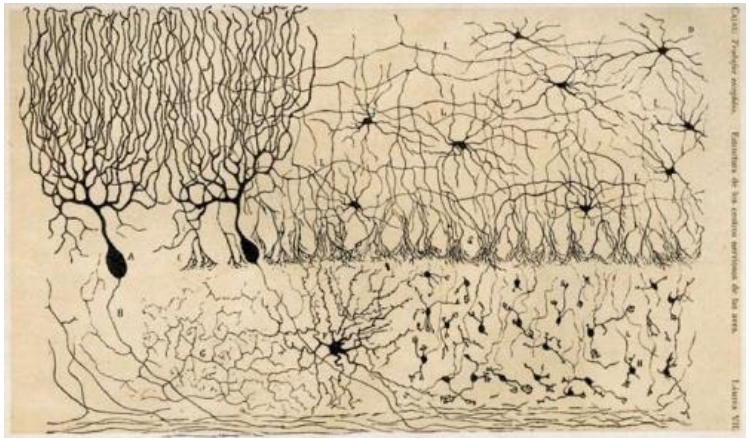
Central Nervous System



Plasticity in adult brains?

"Once development was ended, the fonts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult center the nerve paths are something fixed and immutable, nothing may be regenerated."

(S. Ramon y Cajal, 1928)



Dialogues Clin Neurosci. 2004 Jun; 6(2): 135–141.

Central Nervous System

Plasticity in adult brains!

- 1960s and 1970s: damaged axons can grow
- 1990s: “adult neurogenesis” in primates and humans

- Regions of adult neurogenesis
  - Hippocampus (dentate gyrus) – Memories
  - Other areas controversially discussed

Dialogues Clin Neurosci. 2004 Jun; 6(2): 135–141.
Nature. 2011, Vol 478.

Central Nervous System

- In adults reduction of plasticity important
  - To protect developed circuits
  - To keep learned information
  - Avoid malignant formation

- Remaining plasticity tightly regulated
  - Deficits cause disease (Schizophrenia, Autism, Malignancies,…)

Front Neurosci. November 2009, Volume 3, Article 31.
Front Neurosci. June 2015, Volume 8, Article 23.

Damage to the Central Nervous System

Caused by different events

- Ischemic (e.g. Stroke)
- Traumatic (e.g. Spinal cord/traumatic brain injury)
- Inflammatory (e.g. Multiple sclerosis)
- Haemorrhagic (e.g. aneurysma bleeding)
- Degenerative (e.g. Alzheimer disease, ALS)
- Neoplasms (e.g. Glioblastoma)
- Genetic (e.g. trisomy 21)
- Infectious (e.g. encephalitis spongiforme, Creutzfeldt-Jakob)
- Toxic-nutritive (e.g. ethanol abuse)

-> **Problem: Limited regenerative capacity**

Front Neurosci. November 2009, Volume 3, Article 31.
Front Neurosci. June 2015, Volume 8, Article 23.

Central Nervous System

Regulation of plasticity

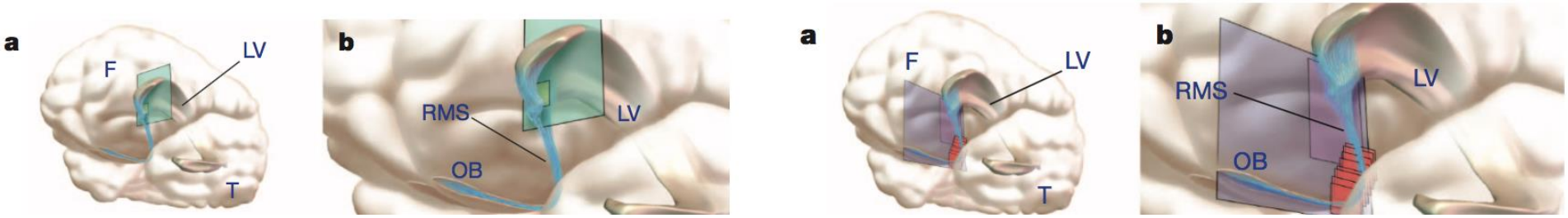
- Regulation on multiple cellular levels

- Hormones (sexual hormones, stress hormones…)

- Inhibitors
  - MAG – myelin-neuron interaction, oligodendrocyte differentiation
  - NoGoA – inhibition of neurite growth
  - OMgp, CSPG,…

Rostral Migratory Stream

- Glia-enriched conduit of forward-migrating neuroblasts
- Target: olfactory bulb

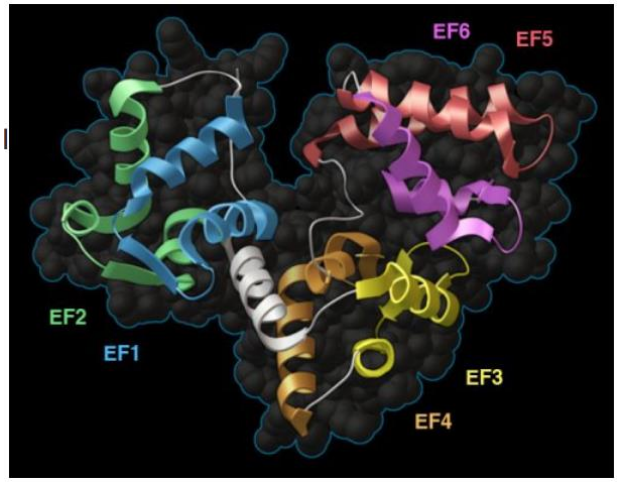


- Regulation: Growth factors, ephrins, neuron-glia cell interactions
- Astroglial “tunnel” directs outgrowth
- Extensive neuroblast-glial interaction to modulate tunnel

Dialogues Clin Neurosci. 2004.
Nature. 2011, Vol 478.

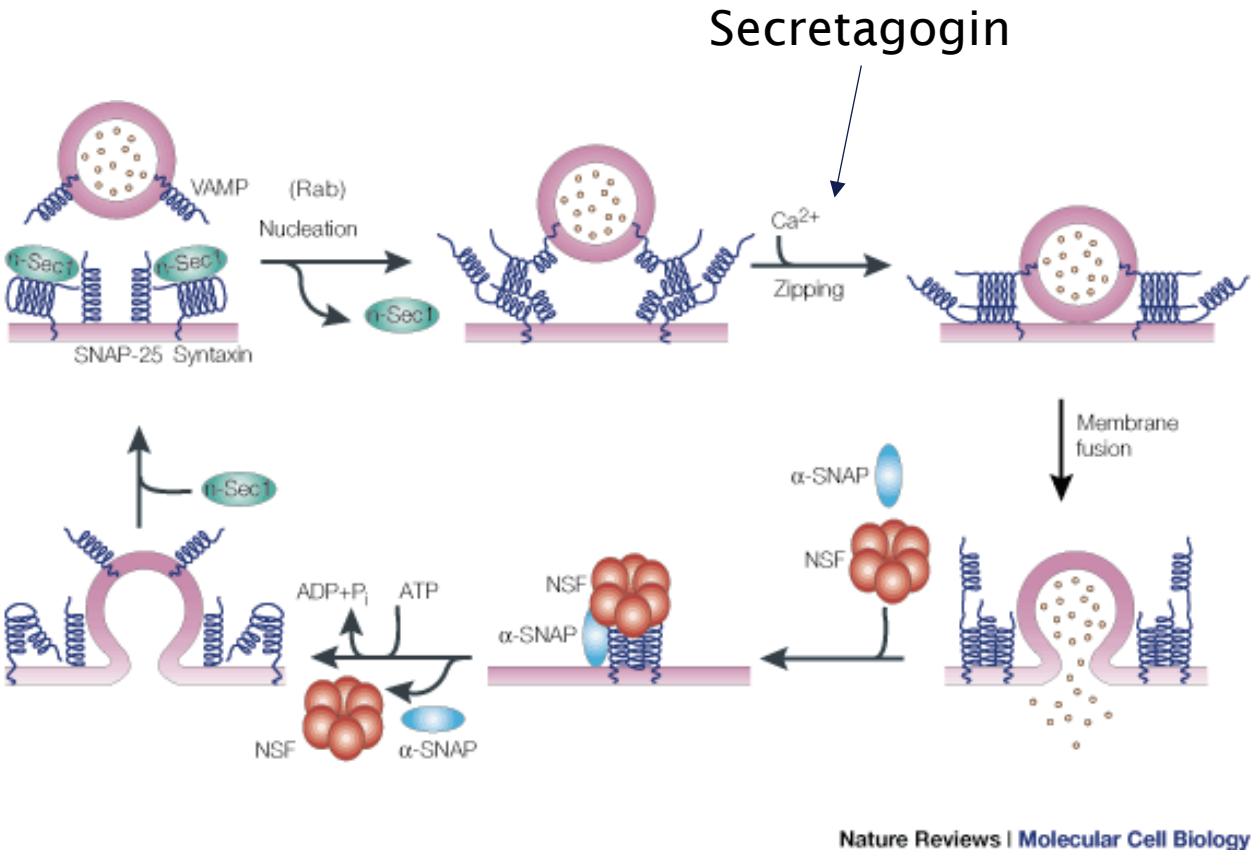
Secretagogin

- First described in pancreatic beta-cells (2000)
- Calcium-binding protein
- Upon Ca-binding -> conformational change
- Interaction with SNARE among others
- Neuropeptide release from hypothalamic neurons



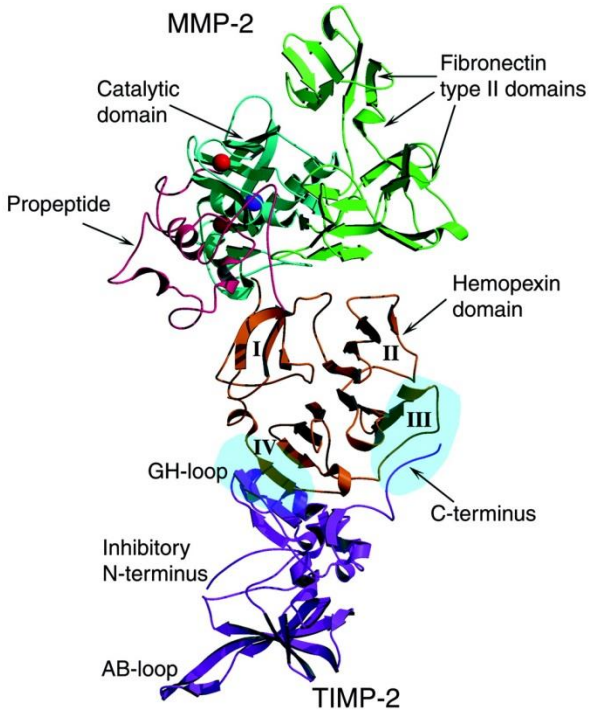
J Biol Chem 275(32):24740-24751
http://sbkb.org/fs/secretagogin

SNARE Protein complex



Matrix Metalloproteinases

- Endoproteinases
- Degrade proteins of the extracellular matrix
- Regulation with specific inhibitors (TIMPs)
- Association with various diseases
- Lung – ventilation during CABG reduces MMP release



Yong VW (2005). Nat Rev Neurosci 6(12):931-944.
Proc Natl Acad Sci U S A. 2002 May 28;99(11):7414-9.
Beer et al., journal of surgical research 195 (2015)

Matrix Metalloproteinases in the CNS

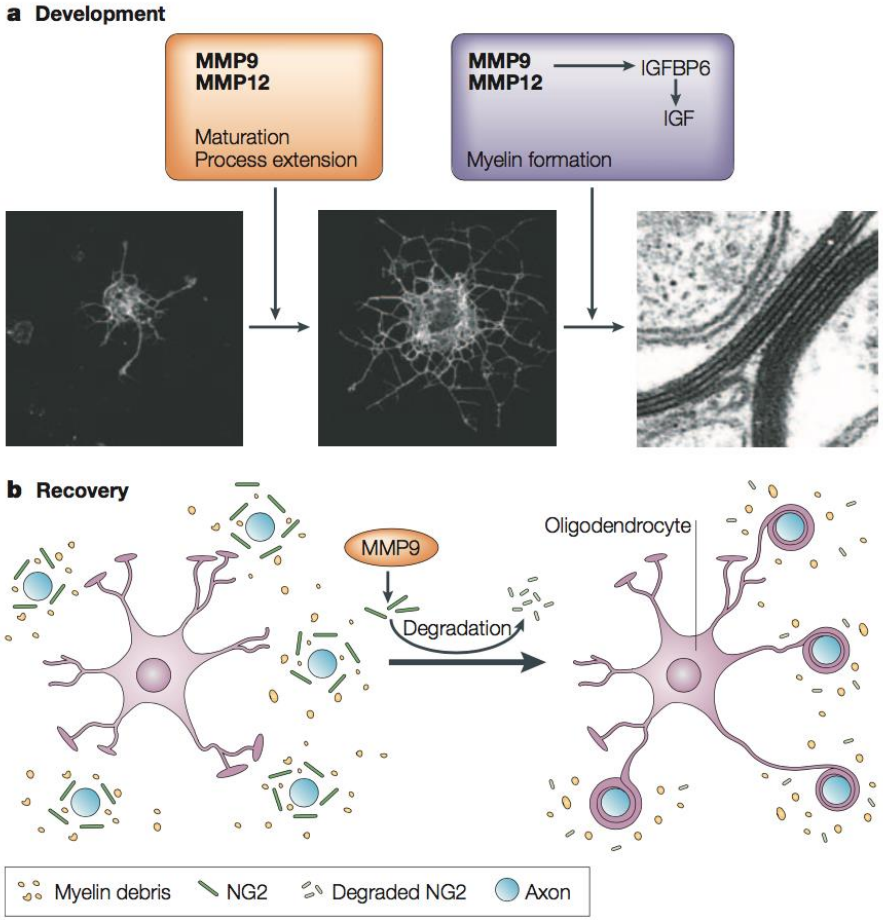


Table 1 | **Outcomes of adult MMP-null mice in CNS insults**

Genotype	Reported outcome	Reference
MMP2$^{-/-}$	Earlier onset and more severe EAE due to a compensatory increase in MMP9	27
	No difference from wild type after focal ischaemia	116
	Reduced glioma growth	117
MMP9$^{-/-}$	Better recovery from spinal cord injury	25
	Reduced apoptosis of retinal ganglion neurons after optic nerve ligation	49
	Less severe EAE disease course	118
	Improved histological and motor outcome in brain trauma	119
	Better histological outcome from ischaemic stroke	120
	Increased haemorrhage, neurological deficits and lethality after intracerebral haemorrhage	121
	Impaired remyelination after a spinal cord lesion	26
MMP12$^{-/-}$	Worse disease course in EAE	14
	Better recovery from spinal cord injury	23
	Better functional recovery from intracerebral haemorrhage	122

The varied outcomes highlight the influence of both the beneficial and detrimental properties of matrix metalloproteinases (MMPs) in the CNS. EAE, experimental autoimmune encephalomyelitis.

Yong VW (2005). Nat Rev Neurosci 6(12):931–944.

Purpose of the Study

Elucidate mechanisms of communication between newborn neurons and a contingent of nonastroglial cells resident in the rostral migratory stream (RMS).

Identify and describe a **novel subsets of neurons** involved in migration of neuroblasts in the RMS.

Materials and Methods

Materials and Methods

- Fetal Tissue – Two male fetal brains with normal development (between gestational weeks 31–33) from Vienna brain bank
- Stereotactic removal of unilateral olfactory bulb
  - Wistar rats
  - Secretagogin$^{-/-}$ conditional knockout mice
- Immunohistochemistry
- Immunoprecipitation and Shotgun Proteomic Analysis
- qPCR
- Western blotting
- Secretagogin Silencing using siRNA
- In-vivo inhibition of MMP activity with Marimastat

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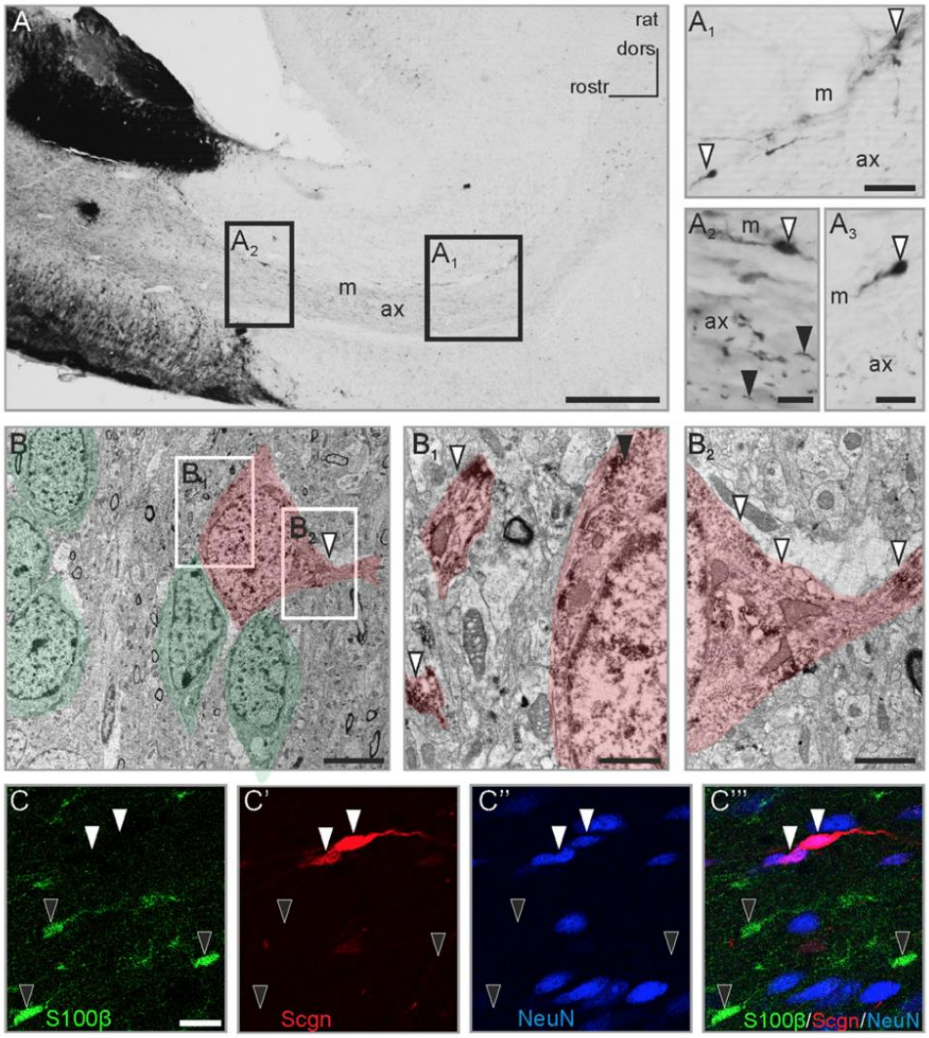
Results

Secretagogin labels a distinct cell subset



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Results I

- Secretagogin positive cells are present in the RMS
- Secretagogin positive cells are in close contact to chain-migrating neuroblasts
- Secretagogin positive cells are differentiated neurons!

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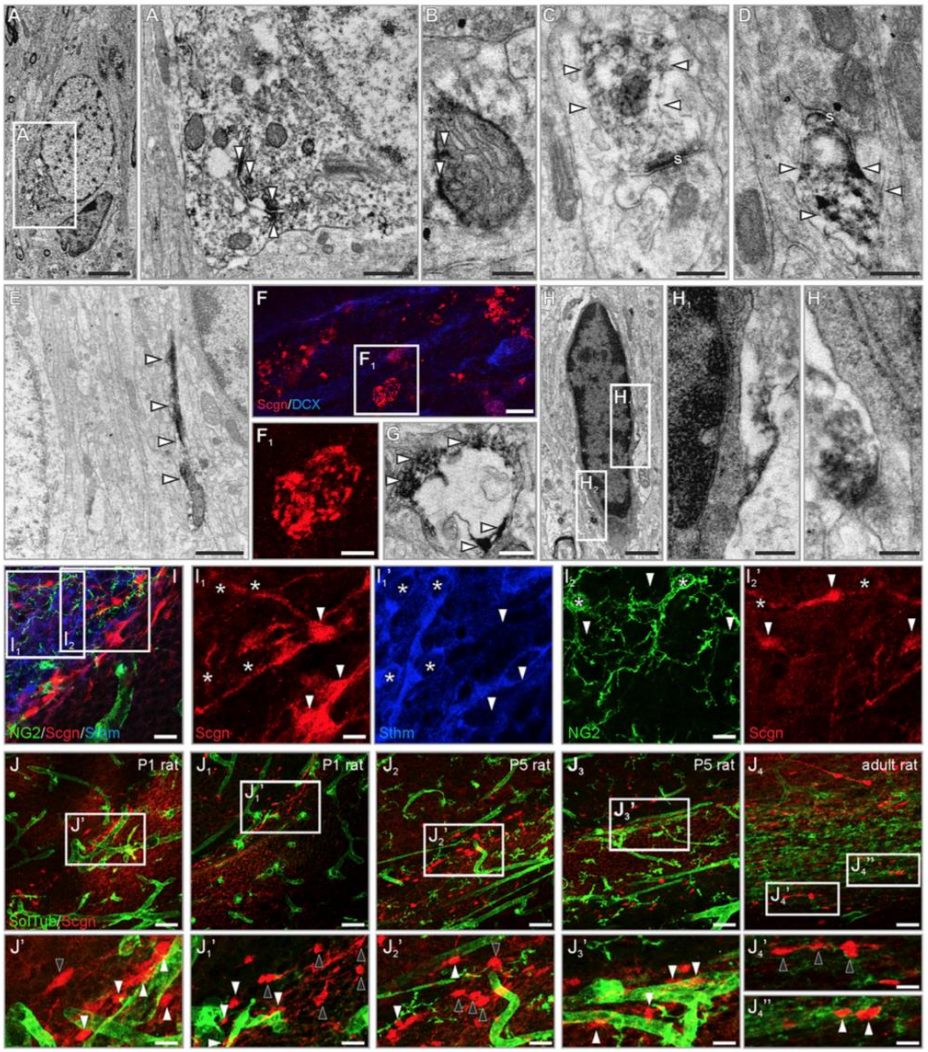
Secretagogin compartmentalization

- Secretagogin compartmentalization associated with ER and mitochondria



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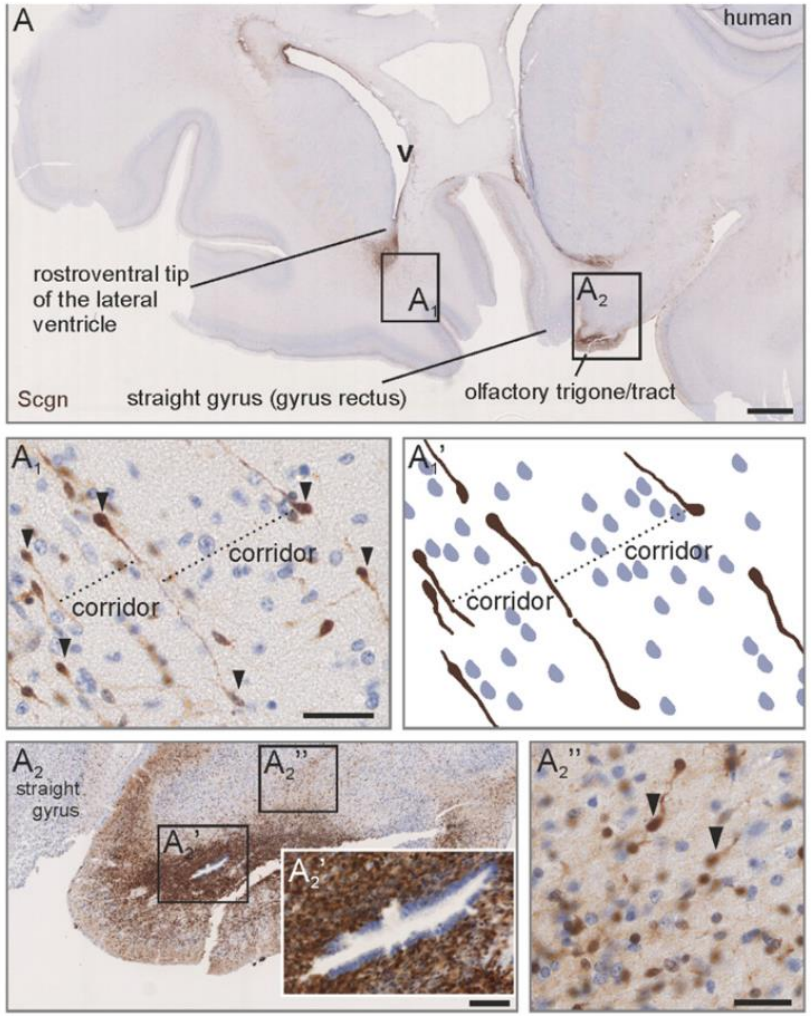
Secretagogin staining in human fetal tissue



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Are these secretagogin^{+} cells resident in the RMS or specialized neuroblasts?

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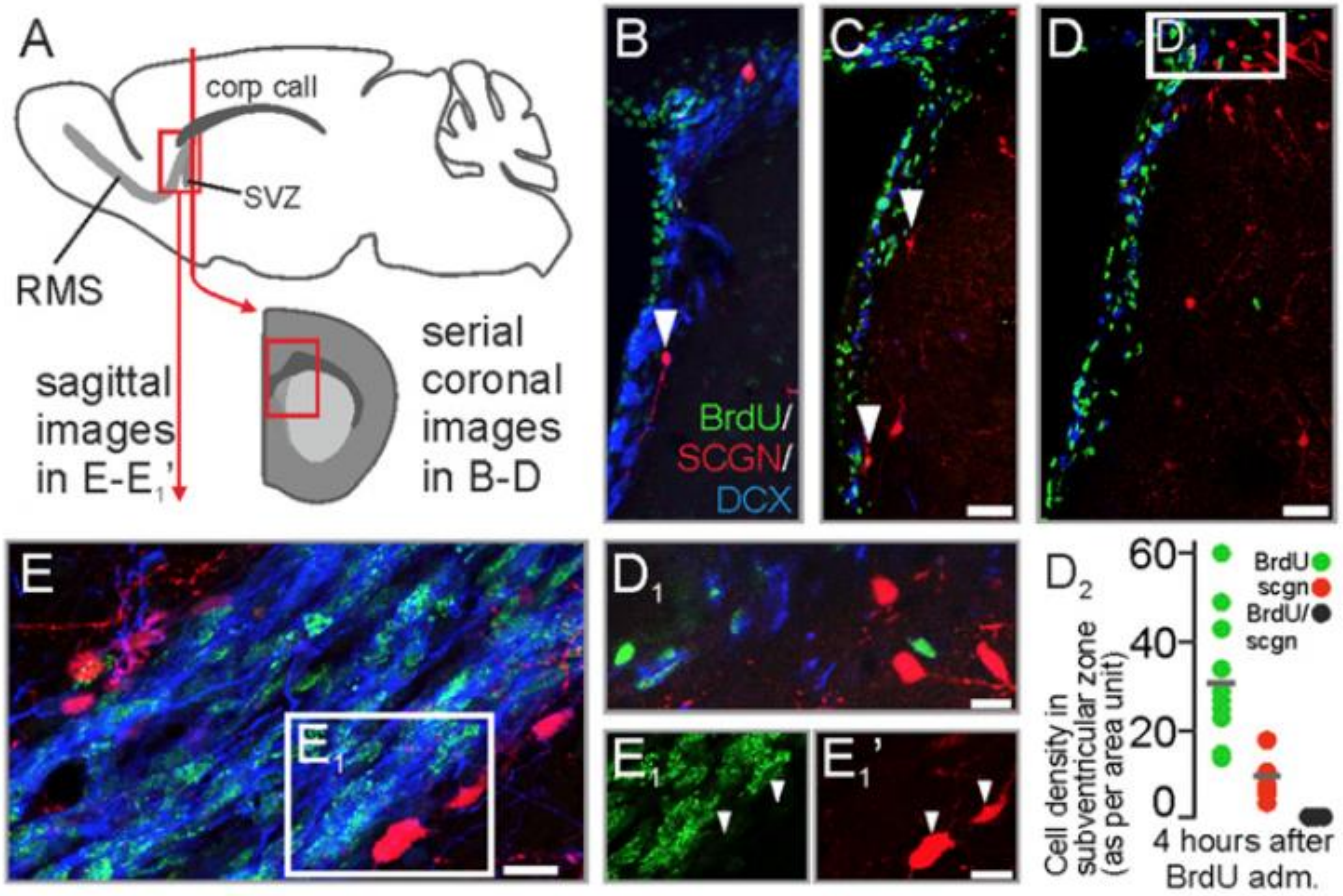
Turnover analysis



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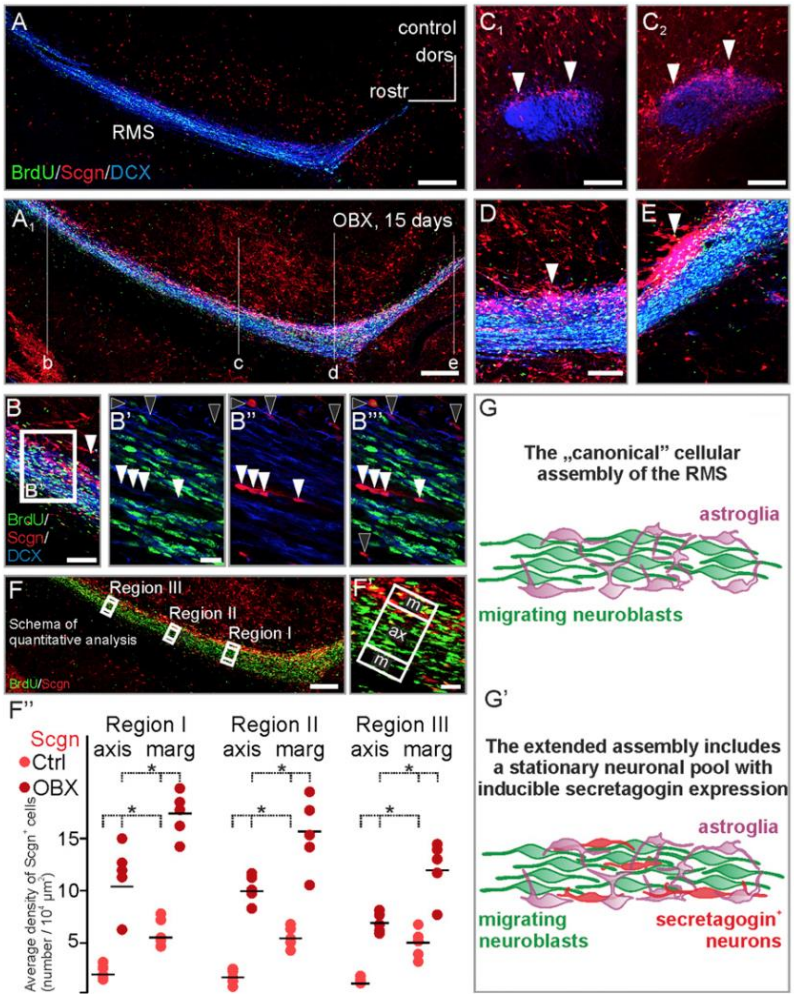
Secretagogin^{+} cells following bulbectomy

- Previous studies show RMS enlarges after bulbectomy
- 15 days after injury SCGN expression significantly increased



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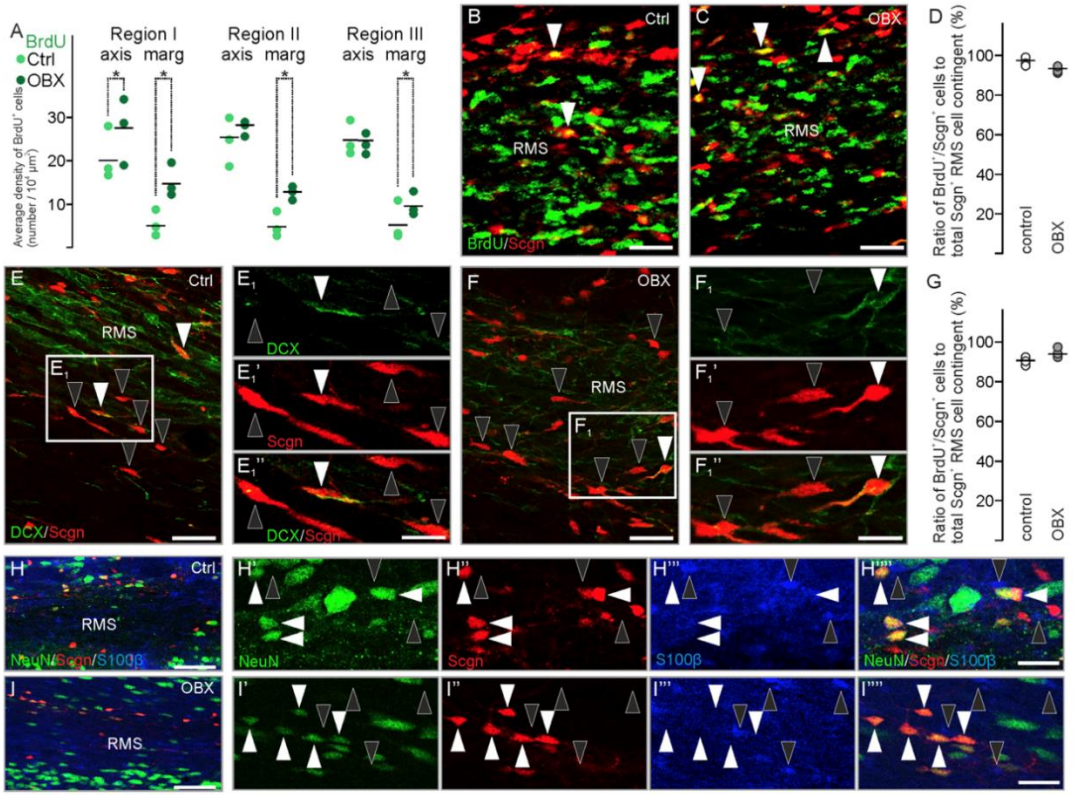
Secretagogin^{+} cells following bulbectomy

- Induction of expression in resident cells rather than introduction of new contigent of cells



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Secretagogin$^{-/-}$ mice

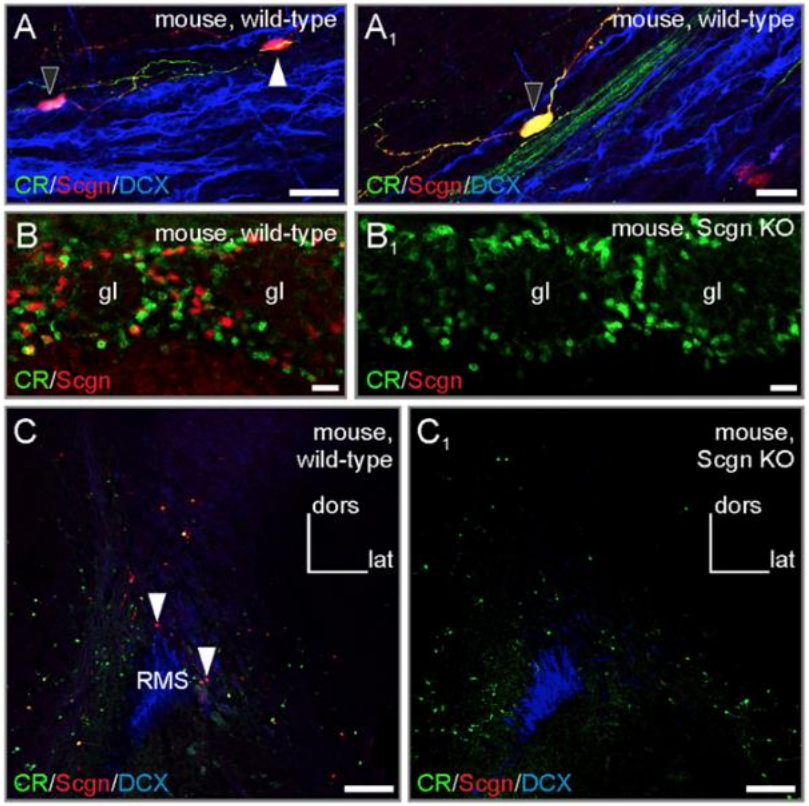

Fig. S3. Secretagogin$^{-/-}$ mice demonstrate successful loss of function. (*A* and A_1) Secretagogin^{+} neurons in wild-type RMSs typically expressed calretinin (black arrowheads). The white arrowhead points to a secretagogin^{+}/calretinin^{-} neuron). (*B*–C_1) Secretagogin$^{-/-}$ mice lacked secretagogin expression in both the olfactory bulb (*B* and B_1) and RMS (*C* and C_1). (Scale bars: 100 μm in *C* and C_1; 20 μm in *B* and B_1; 10 μm in *A* and A_1.)

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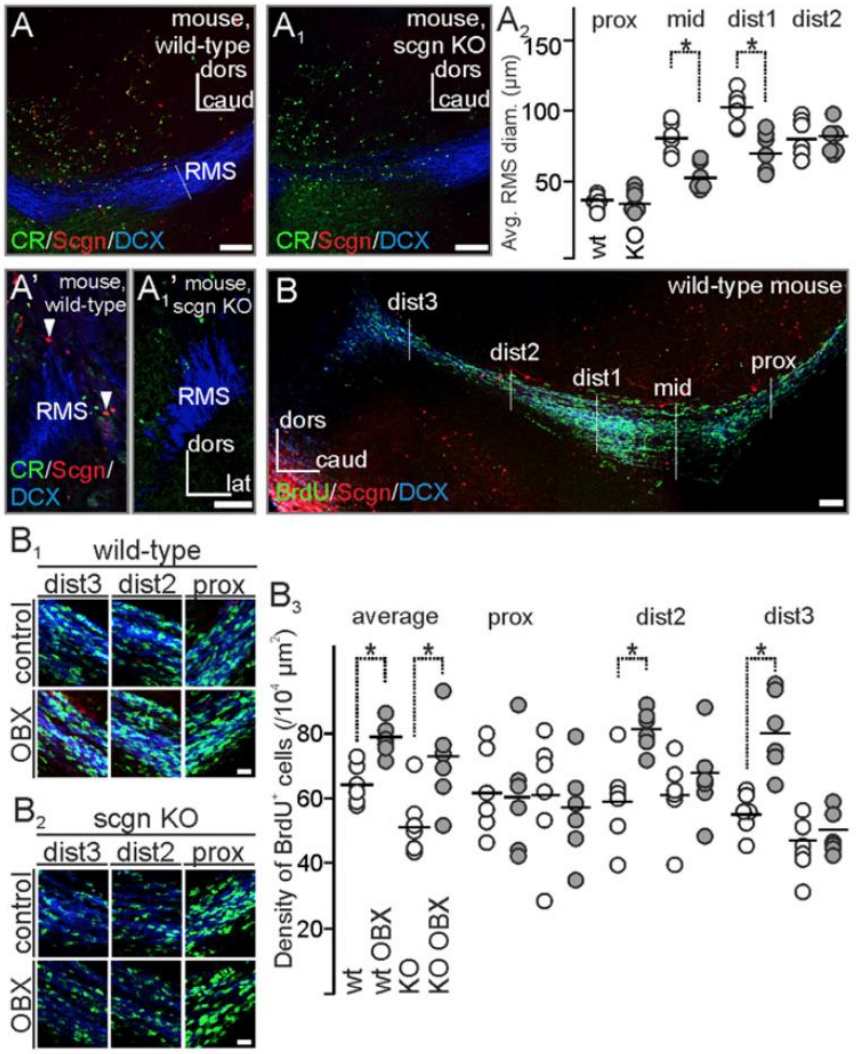
Secretagogin$^{-/-}$ mice

- Altered RMS
- Bulbectomy (OBX) – no increase of BrdU+ cells in KO mice

➢ *bulbectomy-induced mobilization of neuro-blasts toward the injury site is slowed in the absence of secretagogin*



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Silencing of Secretagogin

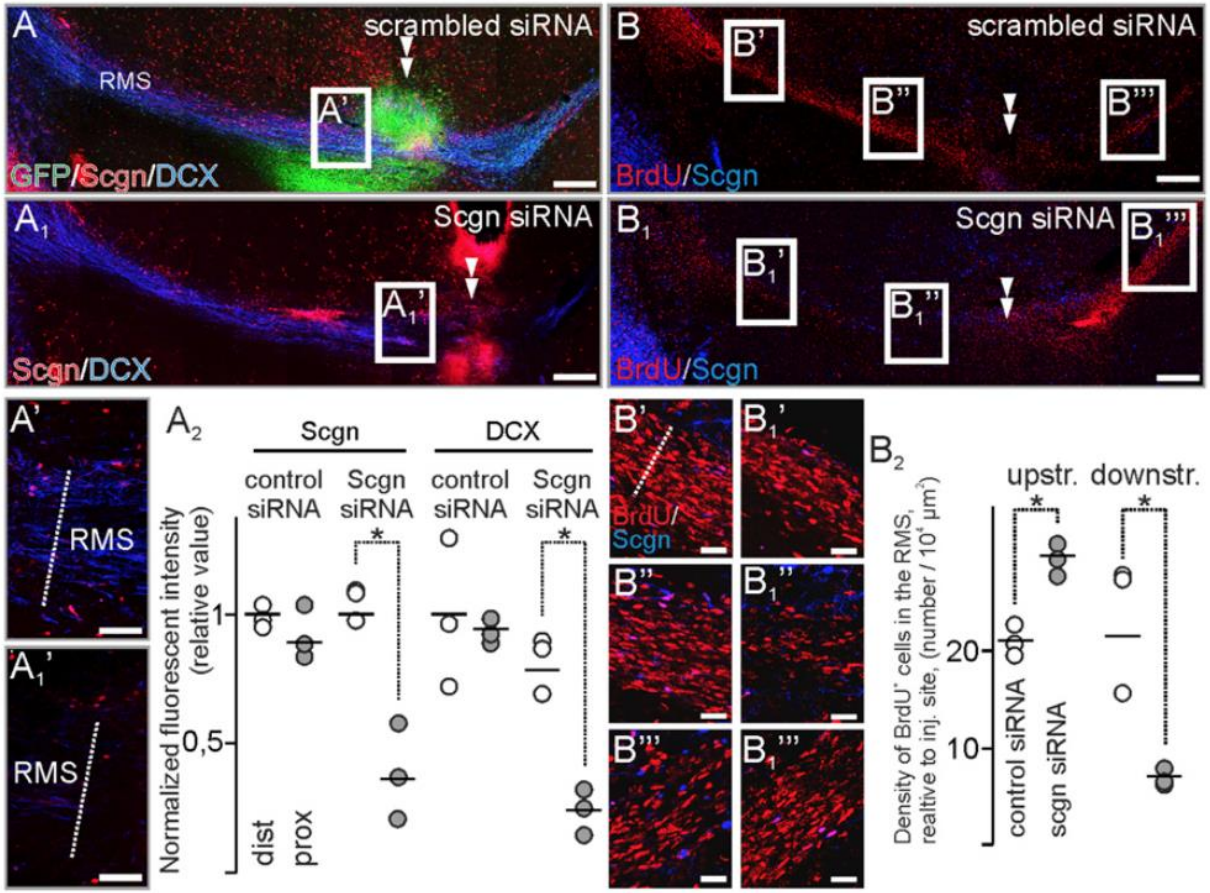

Fig. 6. In vivo secretagogin silencing decreases DCX expression and slows neuroblast migration in the RMS. (*A*–*A*$_2$) Secretagogin (*A*$_1$ and *A*$_{1'}$) but not scrambled siRNA (*A* and *A*′) reduced DCX expression locally (arrowheads in *A* and *A*$_1$). This effect is independent of olfactory bulbectomy (Fig. S4). Note the reduced secretagogin expression upon using specific (*A*$_{1'}$) but not scrambled (*A*′) siRNA and compared with distal RMS free of silencing effect (*A*$_2$). (*B*–*B*$_2$) The density of BrdU$^+$ cells decreased rostral but increased caudal to the silencing site (*B*$_2$). $P < 0.05$, Student's *t* test. Images in *A*, *A*$_1$, *B*, and *B*$_1$ were acquired using the tile-and-stitch function. (Scale bars: 200 µm in *A*, *A*$_1$, *B*, and *B*$_1$; 25 µm in *A*′ and *A*$_{1'}$; 15 µm in *B*′–*B*$_{1'''}$.)

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Silencing of Secretagogin in OBX rats

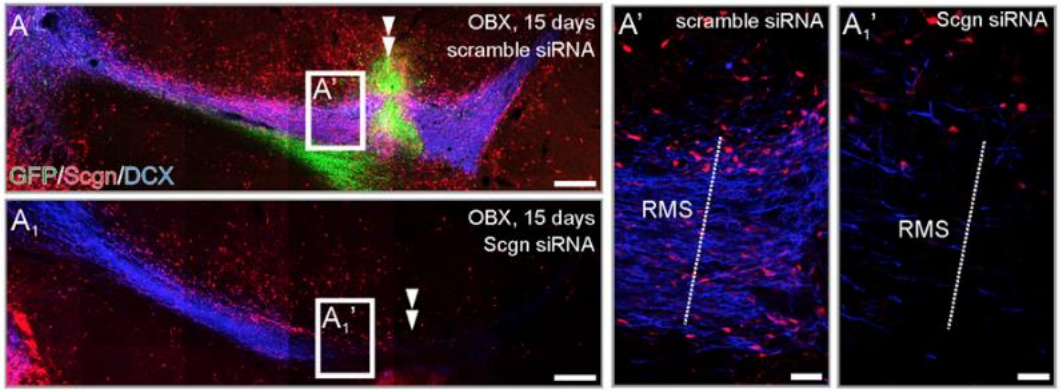

Fig. S4. Secretagogin expression in bulbectomized rats following in vivo gene silencing. Secretagogin (A_1 and $A_{1'}$) but not scrambled siRNA (A and A') reduced DCX expression locally (double arrowheads in A and A') in bulbectomized rats. Images in A and A' were acquired using the tile-and-stitch function. (Scale bars: 200 μm in A and A_1; 25 μm in A' and $A_{1'}$.)

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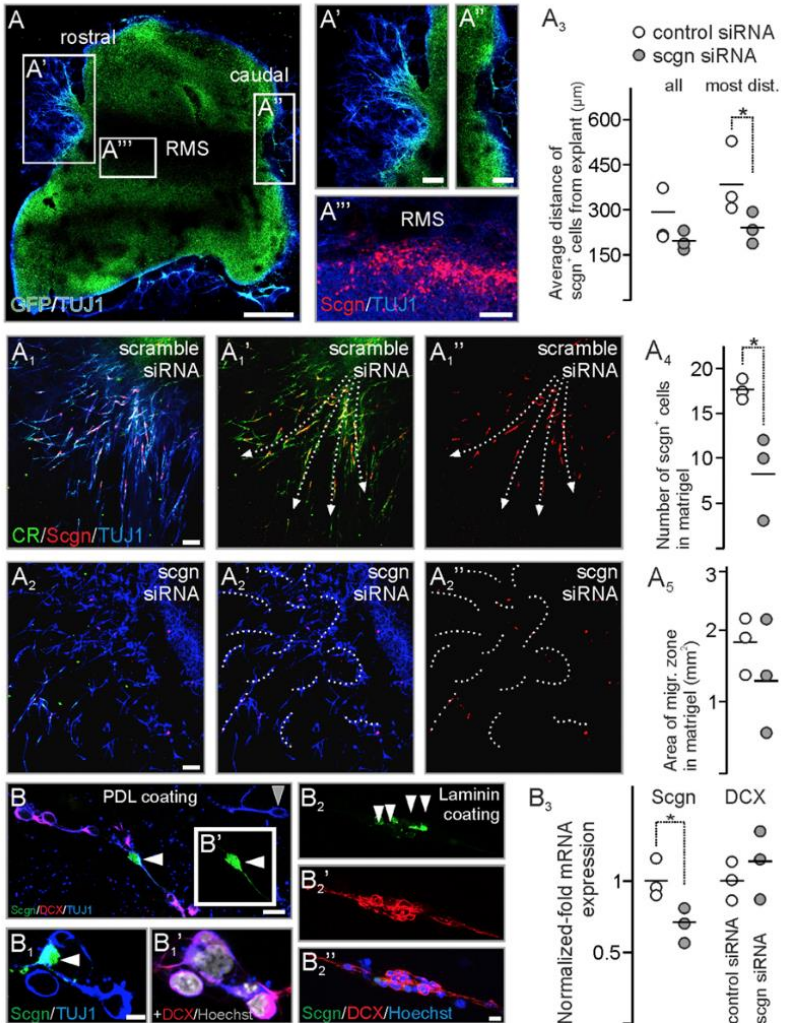
RMS explants



DCX expression is not directly regulated by secretagogin but instead that secretagogin expression regulates cell motility through an extracellular mechanism.

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Functional aspects of Secretagogin?

- Micropunches of RMS -> Immunoprecipitation with Secretagogin then mass spectrometry

- 64 Secretagogin-specific proteins

- **AnnexinV** – known to be associated with MMP-2 release

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Annexin V and MMP-2 in RMS

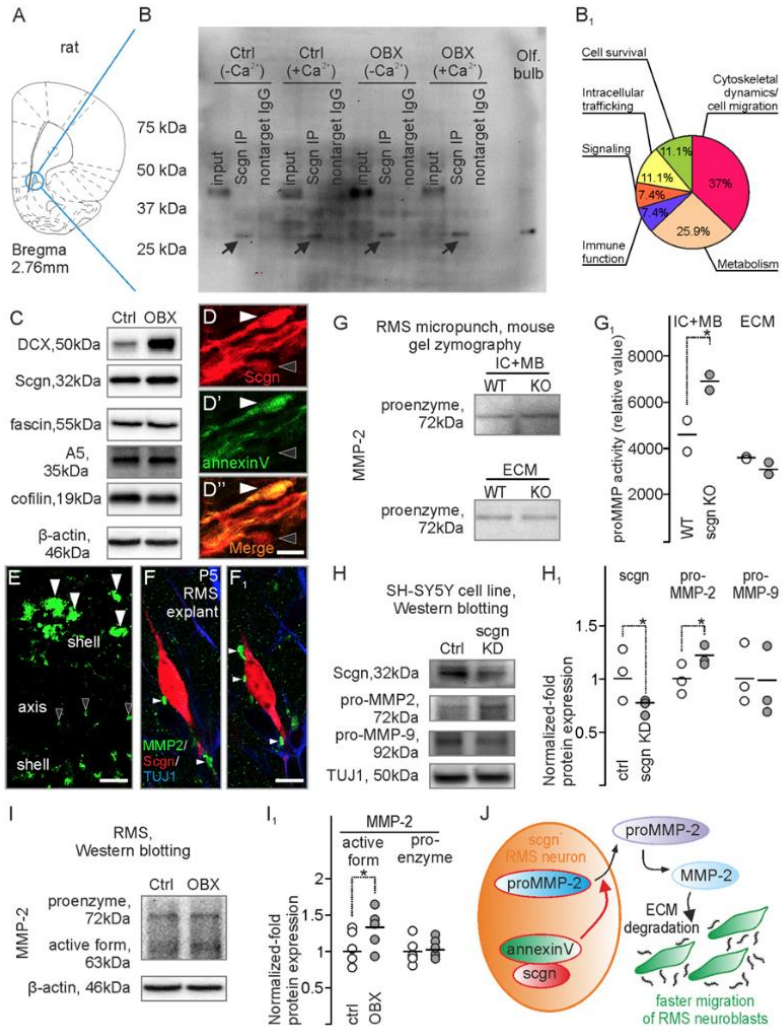

Fig. 8. Secretagogin regulates MMP-2 release. (*A* and *B*) RMS micropunch samples from bulbectomized and control rats were immunoprecipitated with secretagogin in Ca^{2+}-containing or Ca^{2+}-free isolation buffers and were subjected to mass spectrometry. (B_1) Functional distribution of secretagogin-interacting proteins recruited from samples of bulbectomized animals and homogenized in Ca^{2+}-containing isolation buffer. (*C*) Western blotting verified DCX, secretagogin, fascin, annexin V, and cofilin expression in the RMS. (*D–D''*) Post hoc immunohistochemistry resolved annexin V immunoreactivity in secretagogin$^+$ neurons in the RMS (white arrowheads). The black arrowhead points to an annexin V$^-$/secretagogin$^+$ neuron. (*E*) In situ zymography revealed gelatinase activity in the RMS that was reminiscent of the distribution of secretagogin$^+$ neurons. White and black arrowheads indicate profiles in the shell and axial domains of the RMS, respectively. (*F* and F_1) MMP2$^+$ profiles on the extracellular surface of secretagogin$^+$ neurons in RMS explants (serial reconstruction, 700-nm thin optical slices, consecutive z-stack images). (*G* and G_1) Gel zymography from RMS micropunches showed increased proMMP-2 levels in both intracellular and membrane-bound fractions of secretagogin$^{-/-}$ mice, but proMMP-2 levels decreased in the extracellular fraction. (*H* and H_1) Secretagogin silencing increased the expression of the proenzyme form of MMP-2 but not of MMP-9 in SH-SY5Y neuroblastoma cells in vitro. (*I* and I_1) Olfactory bulbectomy increased the level of the active but not of the proenzyme form of MMP-2 in rat RMS. (*J*) Schematic overview of the secretagogin-regulated mechanism in the RMS. Secretagogin regulates the externalization of proMMP-2, thereby limiting the amount of the active form of MMP-2. MMP-2 degrades the extracellular matrix to promote neuroblast migration. $P < 0.05$, Student's *t* test. A5, annexin V; Ctrl, control; OBX, bulbectomized. (Scale bars; 40 μm in *D*; 10 μm in *D''* and *E*; and 5 μm in F_1.)

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Inhibition of MMP-2

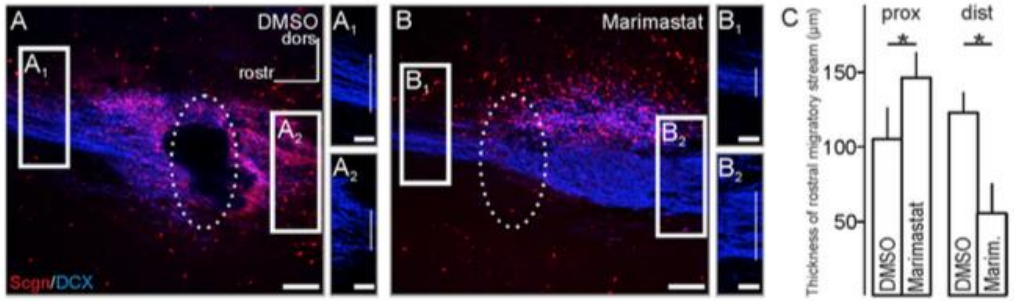

Fig. S7. Marimastat blocks forward neuroblast migration. (*A–C*) Focal administration of the dual MMP-2/MMP-9 inhibitor Marimastat (1 µ M/0.1 µL) (Tocris) increased the diameter of the proximal RMS (B_2) but decreased the diameter of the distal RMS (B_1), compared with control (DMSO) application (A_2 and A_1, respectively) with secretagogin$^+$ cells accumulating upstream the application site. The effect of Marimmastat is quantified in *C*. (Scale bars: 100 µm in *A* and *B*; 20 µm in A_1, A_2, B_1, and B_2.) $^*P < 0.05$.

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Conclusion

Conclusion

Identification of **differentiated neurons** in the mammalian RMS that express **secretagogin** to initiate a molecular cascade through **annexin V** to increase **MMP-2 release**, thus remodelling the extra-cellular matrix to aid neuroblast migration.

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Discussion

Discussion

- Resident cells in the RMS
- Enzyme externalization mechanism hitherto only known in tumor biology
- Up-regulation of Secretagogin after injury
- Depletion of this cell-type irreversible?
- Molecular mechanism / interaction of Secretagogin and Annexin V ?

Discussion

- Feedback mechanism?
- Different regions of the CNS?
- Involved in diseases?
- Previous work shows that secretagogin^{+} cells are still present in olfactory tract in aged brains
- Potential therapeutic target after CNS injuries?

Thank you for your attention!

Results

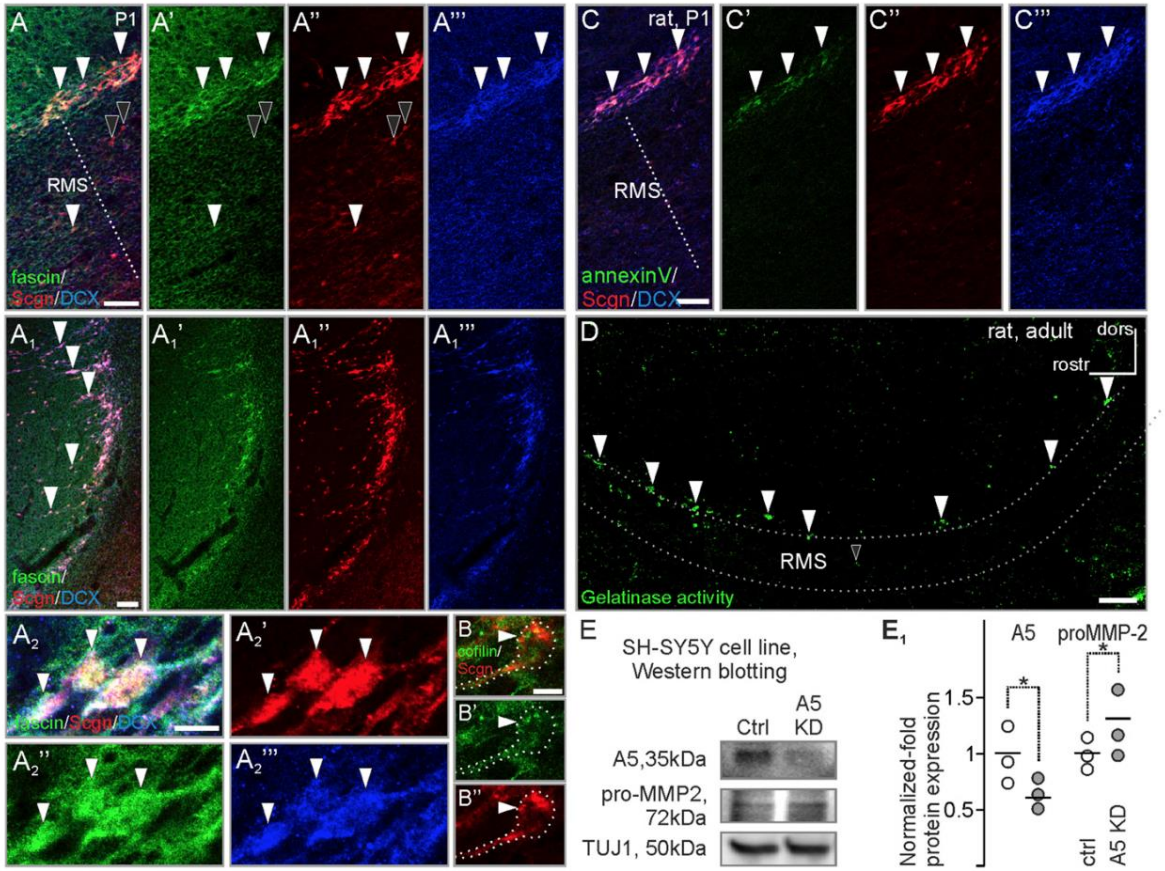

Fig. S5. Secretagogin is coexpressed with the motility proteins fascin and cofilin. (*A*–*A*$_{2'''}$) Fascin^{+}/secretagogin^{+} neurons were identified not only in the marginal region of the RMS in P1 rats (white arrowheads in *A*–*A*‴; black arrowheads point to fascin^{-}/secretagogin^{+} neurons) but also in groups leaving the RMS from its proximal limb transversally (white arrowheads in *A*$_1$–*A*$_1$‴). (*B*–*B*″) Cofilin colocalized with secretagogin. The dotted line surrounds a cofilin^{+}/secretagogin^{+} cell. (*C*–*C*‴) Secretagogin^{+} cells coexpress annexin V in the dorsal margin of the RMS. (*D*) In situ zymography revealed gelatinase activity in the RMS, which was reminiscent of the distribution of secretagogin^{+} neurons. (*E* and *E*$_1$) Knockdown of annexin V expression increased proMMP-2 expression. (Scale bars: 40 μm in *A*, *A*$_1$, *B*, and *C*; 200 μm in *D*; and; 10 μm in *A*$_2$.)

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Results

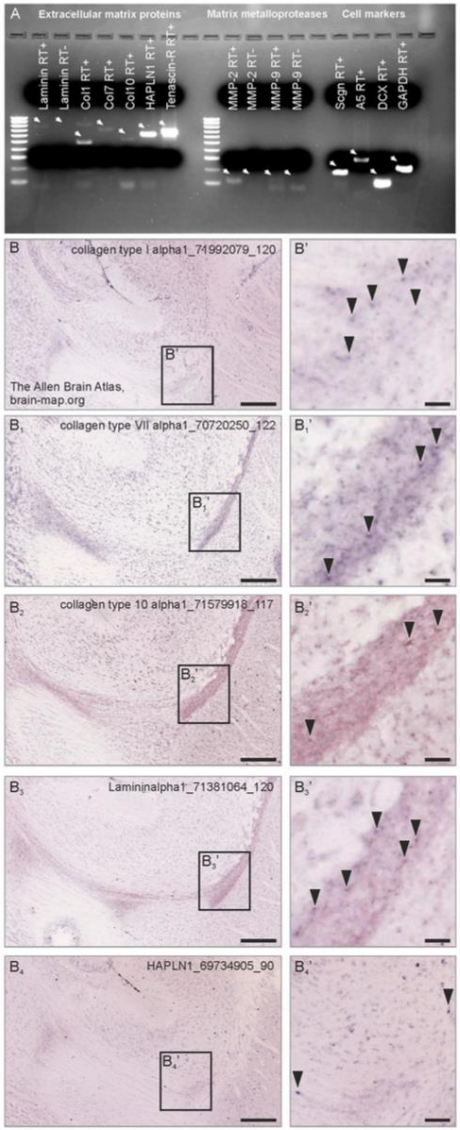

Fig. S6. The presence of extracellular matrix components and matrix metalloproteases in the RMS. (*A*) PCR amplicons from RMS micropunches indicate the presence of laminin, collagen types 1, 7, and 10, the link protein HAPLN-1, and tenascin-R. In contrast to MMP-2, MMP-9 mRNA expression was at the detection limit. White arrows indicate cDNA bands (or the lack of, in case of the RT lanes). (*B*–*B*$_4$′) Corresponding in situ hybridization images from the open source database of the Allen Brain Atlas (experiment numbers are indicated in the figure images) demonstrate the presence of collagen types 1, 7, and 10, laminin, and and HAPLN-1 (black arrowheads) in the adult mouse RMS. Col, collagen; HPLN-1, hyaluronan and proteoglycan link protein 1. (Scale bars: 300 μm for *B*, *B*$_1$, *B*$_2$, *B*$_3$, and *B*$_4$; 20 μm for *B*′, *B*$_1$′, *B*$_2$′, *B*$_3$′, and *B*$_4$′.)

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Results

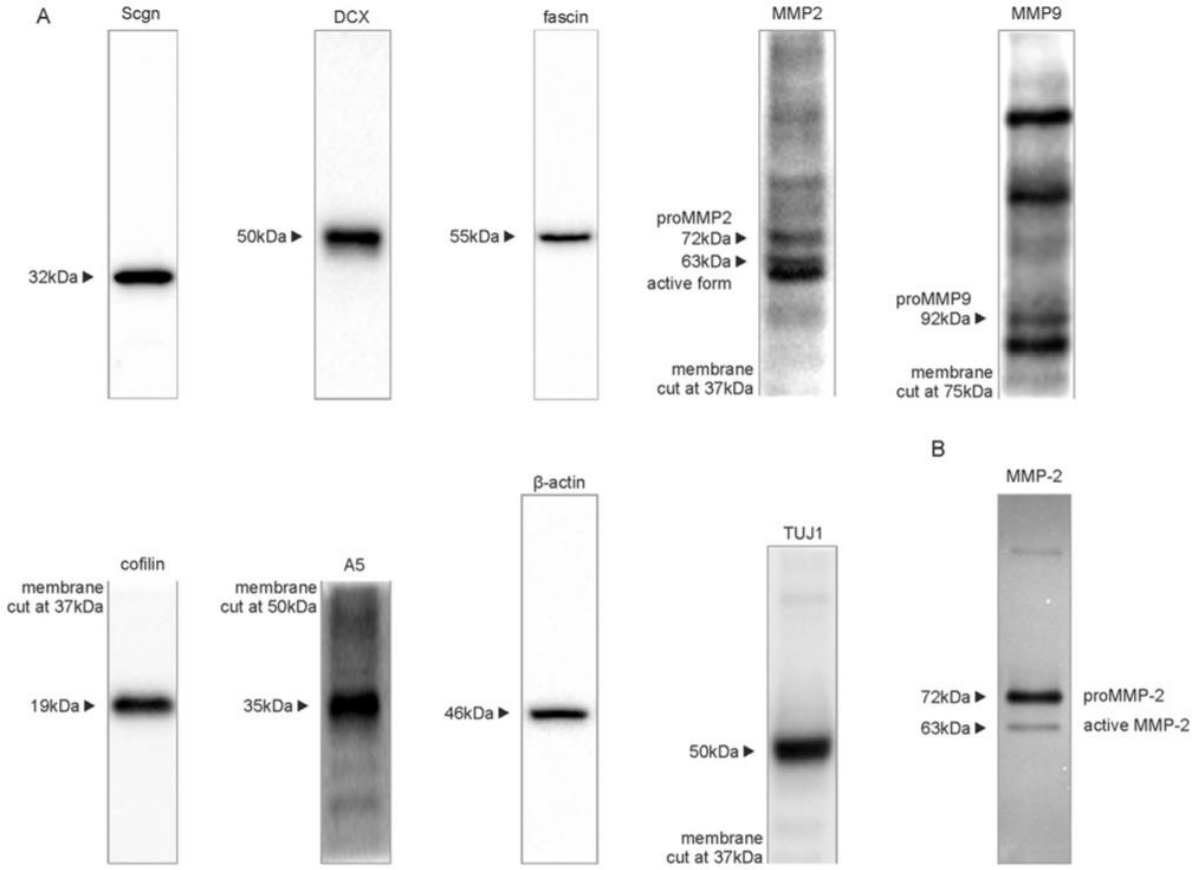

Fig. S8. Full lanes of Western blots and zymography gels. (*A*) Western blotting. (*B*) Gel zymography.

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Results

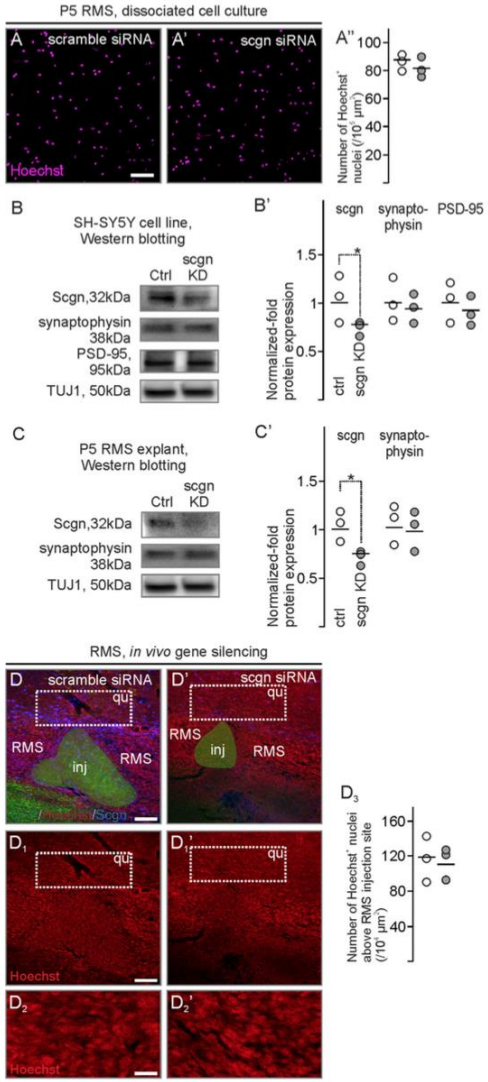

Fig. S9. In vitro, ex vivo, and in vivo secretagogin silencing (siRNA) does not affect cell survival or the expression of synaptic markers. (*A*–*A*″) Control experiments for secretagogin siRNA selectivity. The density of live cells as determined by counting Hoechst$^+$ nuclei was identical after the application of scrambled or secretagogin-specific siRNA in P5 dissociated RMS cultures. (*B* and *B*′) Secretagogin silencing in SH-SY5Y neuroblastoma cells left the expression of the pre- and postsynaptic markers synaptophysin and PSD-95, respectively, unaltered. (*C* and *C*′) Synaptophysin expression did change after secretagogin silencing in P5 RMS explants. (*D*–*D*$_3$) In vivo gene silencing did not affect the density of Hoechst$^+$ cells at the application site immediately dorsal to the RMS (areas within the dashed boxes). High-power images are shown in *D*$_2$ and *D*$_2$′. inj, injection site; qu, quantification. (Scale bars: 20 µm in *A*, *D*, and *D*$_1$; 8 µm in *D*$_2$.)

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