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Final report of the research group of

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The study of the initial cellular phase of Alzheimer's Disease

1. Scientific report

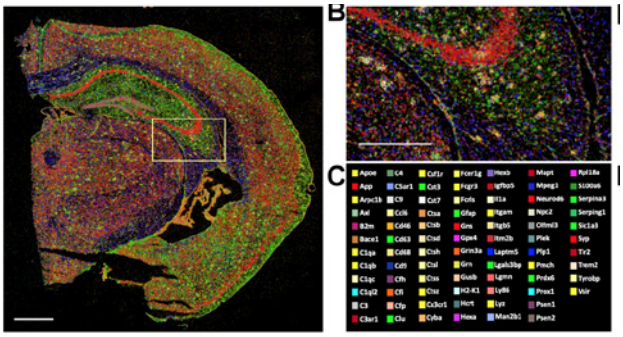
Alzheimer disease (AD) affects roughly 10% of the population aged 65 and up to 50% of people aged 85, while the worldwide cost was estimated € 703 billion (World Alzheimer Report 2016). Although the need for novel therapies is great, a deeper understanding of molecular and cellular mechanisms is first required to make this possible. The last few years have provided a significant paradigm shift in that regard. Failing trials in the clinic[1–4]Alois Alzheimer described the neuropathology of the disease that was to bear his name. 1 Subsequently, our understanding of Alzheimer disease (AD show that the postulated linear relationship between amyloid peptide (Aβ) accumulation, neuronal degeneration and dementia is no longer tenable[4,5]. On the other hand, we see a shift from the classical neurocentric view[6] towards a more integrated view[4] incorporating astroglia and microglia, mainly because the majority of newly identified AD risk genes[7–12] are expressed in these cells. Gene expression profiles show that microglial[13,14] and astroglial[15–17] cells adopt many different states in AD, which might explain their disparate roles in the development and progression of the pathology[18].

We have made enormous progress over the last three years, also thanks to the support from the GSKE to map what we called in 2016 "the cellular phase of Alzheimer's Disease"[19]. We have implemented over the three years three novel technologies that allow asking now questions on the pathogenic cellular mechanisms in a complete novel and exciting way. These are single cell sequencing[20], spatial transcriptomics analysis (Chen et al., submitted) and finally creation of human-mouse chimeric mice[21] allowing to study human microglia in the context of the brain.

We have published a series of high impact papers that describe the first results of these approaches and which have provided a strong foundation for functional studies on a treasure of novel pathways and drug targets relevant to inflammation and neurodegeneration in Alzheimer's disease. All the findings have been highlighted and discussed on Alz Forum. We summarize below the different publications that acknowledge the support of GSKE, and highlight the major finding in each work from a basic science and/or translational point of view.

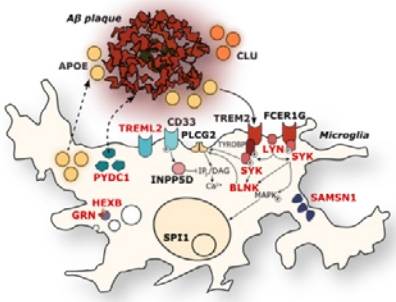
1.1. Spatial transcriptomics reveal microglia-astroglia crosstalk in the amyloid-β plaque cell niche of Alzheimer's disease

The linear cause-consequence relationship linking amyloid-β peptide (Aβ) accumulation to neuronal dysfunction in Alzheimer disease (AD) is gradually replaced by the concept that Aβ initiates complex inflammatory-like cellular alterations that progressively become Aβ independent and lead to brain dyshomeostasis. Little is known about the pathophysiology of this cellular phase of AD. We use here two orthogonal technologies, Spatial Transcriptomics and *in situ* sequencing (figure), to analyse the transcriptome changes in cells in the amyloid-β plaque niche in a knock-in mouse model for AD. We identify a multicellular co-expressed gene network of 57 Plaque-Induced Genes (PIGs) that define a series of co-ordinated and spatially restricted microglia, astroglia and oligodendrocyte responses to progressing amyloid plaques encompassing complement, oxidative stress and inflammation. A separate oligodendrocyte network suggests abnormal myelination. Spatial Transcriptomics provides an unprecedented approach to untangle the dysregulated cellular network in the vicinity of pathogenic hallmarks of AD and other brain diseases. The work demonstrates the increasing interaction of the PiGs when exposed more strongly to amyloid plaques and provide several



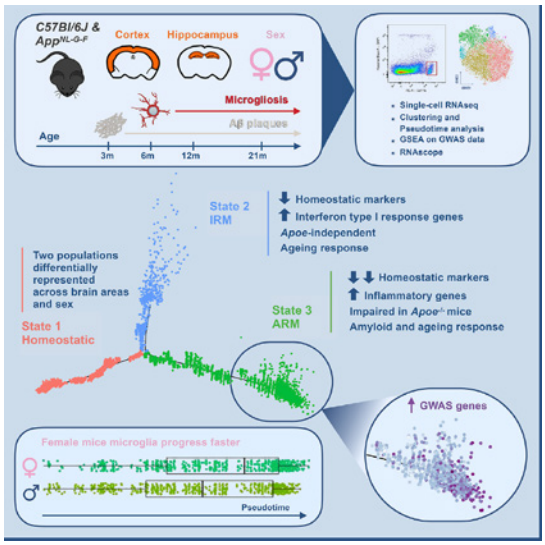
novel drug targets that should allow to modulate the astroglia/microglia response to amyloid plaques. This is a very active area of research in the field.

1.2. Novel Alzheimer risk genes determine the microglia response to amyloid-β but not to TAU pathology



Genetic variants even when not reaching genome-wide significance still add to the genetic risk of developing Alzheimer's disease (AD) as assessed by polygenic risk scores. Whether and how such subthreshold risk loci translate into relevant disease pathways, is unknown. We investigate here the involvement of AD risk variants in the transcriptional responses of two AD mouse models: APPswe/PS1^{L166P} (APPtg) and Thy-TAU22 (TAUtg). A unique gene expression module, highly enriched for AD risk genes, is specifically responsive to Aβ but not to TAU pathology. We identify in this module 7 established AD (*APOE*, *CLU*, *INPP5D* aka SHIP1, *CD33* (Siglech in mice), *PLCG2*, *SPI1* and *FCER1G*) and 11 risk genes implicated in AD in GWAS without reaching genome wide significance (*GPC2, TREML2, SYK, GRN, SLC2A5, SAMSN1, PYDC1, HEXB, RRBP1, LYN* and BLNK), that become significantly upregulated when exposed to Aβ (see figure). Single microglia sequencing confirms that Aβ, not TAU, pathology induces a marked transcriptional change in microglia, resulting in increased proportions of activated response microglia. Moreover, 15 of these 18 AD risk genes are expressed in microglia, with 7 significantly higher expressed in homeostatic microglia. We conclude that genetic risk of AD functionally translates into different microglia pathway responses to Aβ pathology. This insight puts an important part of the AD genetic risk downstream of the amyloid pathway but upstream of TAU pathology.

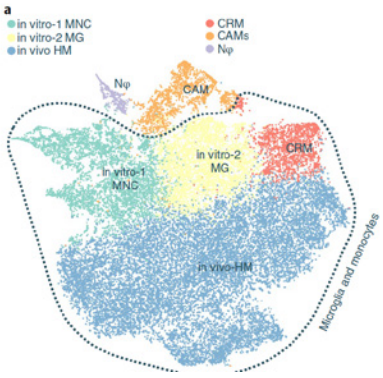
1.3. The major risk factors for Alzheimer's disease: Age, Sex and Genes, modulate the microglia response to Aβ plaques



Microglia are involved in Alzheimer's disease (AD) by adopting activated phenotypes. How ageing in the absence or presence of β-amyloid (Aβ) deposition in different brain areas affects these phenotypes and whether sex and AD risk genes are involved, remains largely unknown. Here we analyzed the gene expression profiles of more than 10,000 individual microglia cells isolated from cortex and hippocampus of male and female *App*$^{NL-G-F}$ at 4 different stages of Aβ deposition and in age-matched control mice. Microglia adopt two major activated states (figure), both during normal aging and during exposure to Aβ. The activated response microglia (ARM) is enhanced by amyloid plaques, is composed of specialized subgroups overexpressing MHC type II and tissue repair genes (*Dkk2*, *Gpnmb*, *Spp1*), induced upon prolonged Aβ exposure, and is strongly enriched with AD risk genes. Microglia in female mice advance faster in the activation trajectories. Similar activated states were also found in a second AD model and in human brain. Abolishing the expression of *Apoe*, the major genetic risk factor for AD, impairs the ARM response, while the second response type, enriched for interferon response genes, remains unaffected. Our data indicate that ARMs are the converging point of multiple AD risk factors.

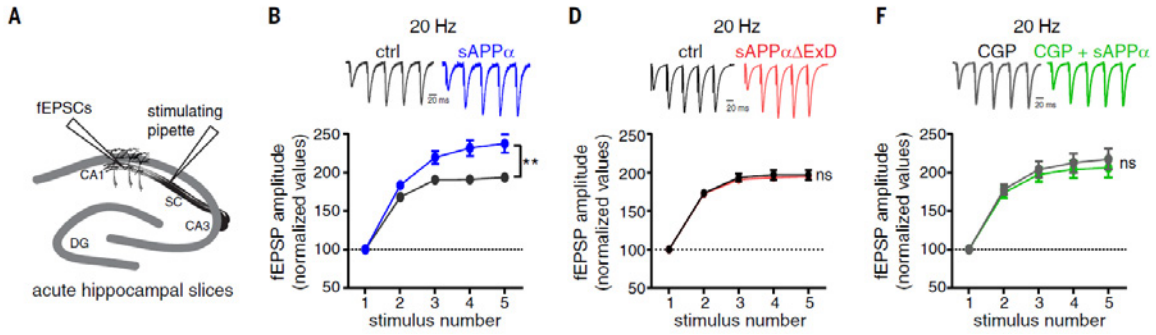
1.4. Stem cell derived human microglia transplanted in mouse brain to study human disease

While genetics highlight the role of microglia in Alzheimer's disease (AD), one third of putative AD-risk genes lack adequate mouse orthologs. Here, we successfully engraft human microglia derived from embryonic stem cells in the mouse brain. The cells recapitulate transcriptionally human primary microglia *ex vivo (figure)* and show expression of human specific AD-risk genes. Oligomeric Amyloid-β induces a divergent response in human vs. mouse microglia. This model can be used to study the role of microglia in neurological diseases. ESC-derived human microglia transplanted into mouse brain represents clearly a step forward to model part of the GWAS defined risk of AD. Despite certain limitations that should be considered (e.g. lack of adaptive immune system, variability in the grafting efficiency of different pluripotent stem cells, iPSC), we anticipate that our approach will be widely applicable to study other neurological diseases. The use of human H9 cells in combination with CRISPR/Cas9 technology opens unanticipated possibilities to model human specific genetic aspects of brain disease.

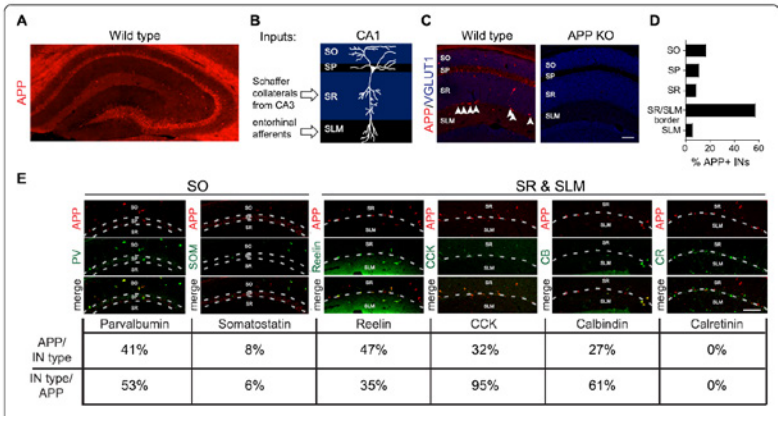


1.5. Secreted Amyloid-β Precursor Protein Functions as a $GABA_BR1a$ Ligand to Modulate Synaptic Transmission

The Amyloid-β Precursor Protein (APP) is central to the pathogenesis of Alzheimer's disease, yet its physiological function remains unresolved. Increasing evidence suggests that APP has a synaptic function mediated by an unidentified receptor for the shed APP ectodomain (sAPP). Here we show that sAPP binds the gamma-aminobutyric acid type B receptor subunit 1a (GABABR1a), which predominantly localizes to synaptic boutons. This interaction is direct and mediated by a conserved peptide stretch in the extension domain of APP and the sushi 1 domain specific to the GABABR1a subunit. sAPP-GABABR1a binding suppresses basal synaptic transmission and augments short-term facilitation in hippocampal synapses via inhibition of synaptic vesicle release (figure). Further, a 17 amino acid peptide corresponding to the GABABR1a binding region within APP reversibly suppresses spontaneous neuronal activity in vivo. Our findings identify GABABR1a as a synaptic receptor for sAPP and reveal a physiological role for sAPP in regulating GABABR1a function to modulate synaptic transmission.



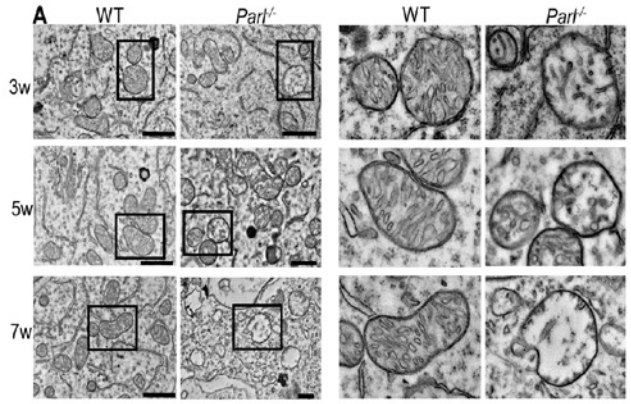
1.6. Contribution of GABAergic interneurons to amyloid-β plaque pathology in an APP knock-in mouse model



	Parvalbumin	Somatostatin	Reelin	CCK	Calbindin	Calretinin
APP/ IN type	41%	8%	47%	32%	27%	0%
IN type/ APP	53%	6%	35%	95%	61%	0%

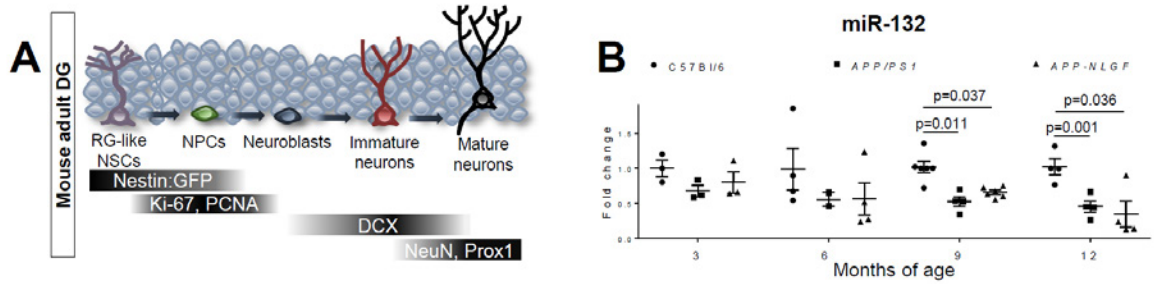
The amyloid-β (Aβ) peptide, the primary constituent of amyloid plaques found in Alzheimer's disease (AD) brains, is derived from sequential proteolytic processing of the Amyloid Precursor Protein (APP). However, the contribution of different cell types to Aβ deposition has not yet been examined in an in vivo, non-overexpression system. Here, we show that endogenous APP is highly expressed in a heterogeneous subset of GABAergic interneurons throughout various laminae of the hippocampus (figure), suggesting that these cells may have a profound contribution to AD plaque pathology. We then characterized the laminar distribution of amyloid burden in the hippocampus of an APP knock-in mouse model of AD. To examine the contribution of GABAergic interneurons to plaque pathology, we blocked Aβ production specifically in these cells using a cell type-specific knock-out of BACE1. We found that during early stages of plaque deposition, interneurons contribute to approximately 30% of the total plaque load in the hippocampus. The greatest contribution to plaque load (75%) occurs in the stratum pyramidale of CA1, where plaques in human AD cases are most prevalent and where pyramidal cell bodies and synaptic boutons from perisomatic-targeting interneurons are located. These findings reveal a crucial role of GABAergic interneurons in the pathology of AD. Our study also highlights the necessity of using APP knock-in models to correctly evaluate the cellular contribution to amyloid burden since APP overexpressing transgenic models drive expression in cell types according to the promoter and integration site and not according to physiologically relevant expression mechanisms.

1.7. PARL deficiency in mouse causes Complex III defects, coenzyme Q depletion, and Leigh-like syndrome



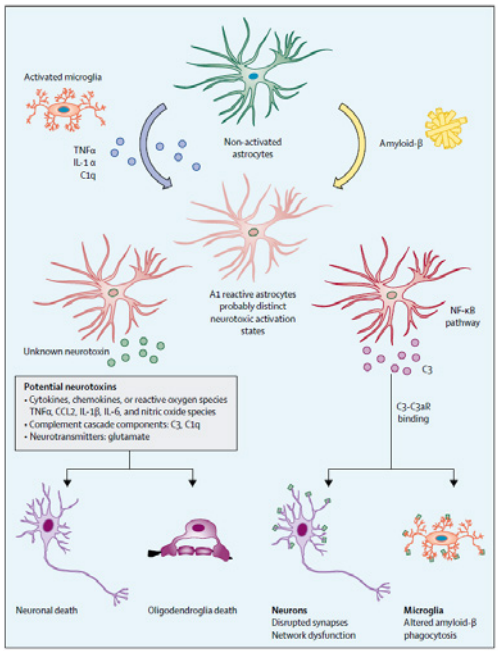
The mitochondrial intramembrane rhomboid protease Parl has been implicated in diverse functions *in vitro*, but its physiological role in vivo remains unclear. Here we show that Parl ablation in mouse causes a striking necrotizing encephalomyelopathy similar to Leigh syndrome, a mitochondrial disease characterized by disrupted energy production. Mice with conditional Parl deficiency in the nervous system, but not in muscle, develop a similar phenotype as germline Parl knockouts demonstrating the vital role of Parl in neurological homeostasis. Genetic modification of two major Parl substrates, Pink1 and Pgam5, do not modify this severe neurological phenotype. Parl-/- brain mitochondria are affected by severe ultrastructural changes (figure), and defects in Complex III activity, coenzyme Q biosynthesis, and mitochondrial calcium metabolism. Parl is necessary for the stable expression of Ttc19, required for Complex III activity, and of Coq4, essential in coenzyme Q biosynthesis. Thus, Parl plays a previously overseen constitutive role in the maintenance of the respiratory chain in the nervous system, and its deficiency causes progressive mitochondrial dysfunction, structural abnormalities and Leigh-like syndrome

1.8. microRNA-132 restores adult hippocampal neurogenesis and memory deficits in Alzheimer's disease



Adult hippocampal neurogenesis (AHN) is functionally linked to mnemonic and cognitive plasticity in humans and rodents. In Alzheimer's disease (AD), the process of generating new neurons at the hippocampal neurogenic niche is impeded, but the mechanisms involved are unknown. Here we identify miR-132, one of the most consistently downregulated microRNAs in AD, as a potent regulator of AHN, exerting cell-autonomous proneurogenic effects in the adult neurogenic niche. Using distinct AD mouse models (figure), cultured human primary and established neural stem cells, and human patient material, we demonstrate that AHN is directly impacted by AD pathology. miR-132 replacement in adult mouse AD hippocampus restores AHN and relevant memory deficits. Our findings corroborate the significance of AHN in AD and reveal the possible therapeutic significance of targeting miR-132 in neurodegeneration.

1.9. The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. (review)



Astrocytes, also called astroglia, maintain homoeostasis of the brain by providing trophic and metabolic support to neurons. They recycle neurotransmitters, stimulate synaptogenesis and synaptic neurotransmission, form part of the blood–brain barrier, and regulate regional blood flow. Although astrocytes have been known to display morphological alterations in Alzheimer's disease for more than a century, research has remained neurocentric. Emerging evidence suggests that these morphological changes reflect functional alterations that affect disease. Genetic studies indicate that most of the risk of developing late onset Alzheimer's disease, the most common form of the disease, affecting patients aged 65 years and older, is associated with genes (ie, APOE, APOJ, and SORL) that are mainly expressed by glial cells (ie, astrocytes, microglia, and oligodendrocytes). This insight has moved the focus of research away from neurons and towards glial cells and neuroinflammation. Molecular studies in rodent models suggest a direct contribution of astrocytes to neuroinflammatory and neurodegenerative processes causing Alzheimer's disease; however, these models might insufficiently mimic the human disease, because rodent astrocytes differ considerably in morphology, functionality, and gene expression. In-vivo studies using stem-cell derived human astrocytes are allowing exploration of the human disease and providing insights into the neurotoxic or protective contributions of these cells to the pathogenesis of disease. The first attempts to develop astrocytic biomarkers and targeted therapies are emerging.

2. Publications mentioning support from GSKE:

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   Secreted amyloid-β precursor protein functions as a GABA(B)R1a ligand to modulate synaptic transmission. **Science**. 2019 Jan 11;363(6423).
2. Spinazzi M, Radaelli E, Horré K, Arranz AM, Gounko NV, Agostinis P, Maia TM, Impens F, Morais VA, Lopez-Lluch G, Serneels L, Navas P, **De Strooper B**.
   PARL deficiency in mouse causes Complex III defects, coenzyme Q depletion, and Leigh-like syndrome. **Proc Natl Acad Sci U S A**. **2019** Jan 2;116(1):277-286.
3. Renzo Mancuso, Johanna Van Den Daele, Nicola Fattorelli, Leen Wolfs, Sriram Balusu, Oliver Burton, Annerieke Sierksma, Yannick Fourne, Suresh Poovathingal, Amaia Arranz-Mendiguren, Carlo Sala Frigerio, Christel Claes, Lutgarde Serneels, Tom Theys, V. Hugh Perry, Catherine Verfaillie, Mark Fiers, **Bart De Strooper**.
   Stem cell derived human microglia transplanted in mouse brain to study human disease. **Nature Neuroscience 2019** pages2111–2116
4. Amaia M Arranz, **Bart De Strooper**
   The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. **Lancet Neurology 2019**: 406-414
5. Carlo Sala Frigerio, Leen Wolfs, Nicola Fattorelli, Nicola Thrupp, Iryna Voytyuk, Inga Schmidt, Renzo Mancuso, Wei-Ting Chen, Maya Woodbury, Gyan Srivastava, Thomas Möller, Eloise Hudry, Sudeshna Das, Takaomi Saido, Eric Karran, Bradley Hyman, V. Hugh Perry, Mark Fiers, **Bart De Strooper**.
   The major risk factors for Alzheimer's disease: Age, Sex and Genes, modulate the microglia response to Aβ plaques. **Cell Reports 2019**, pp 1293–1306
6. Rice HC, Marcassa G, Chrysidou I, Horré K, Young-Pearse TL, Müller UC, Saito T, Saido TC, Vassar R, de Wit J, **De Strooper B**.
   Contribution of GABAergic interneurons to amyloid-β plaque pathology in an APP knock-in mouse model. **Mol Neurodegener. 2020** Jan 8;15(1):3. doi: 10.1186/s13024-019-0356-y

In press:

7. Annerieke Sierksma, Ashley Lu,, Evgenia Salta, Renzo Mancuso, Jesus Zoco, David Blum, Luc Buée, **Bart De Strooper***, Mark Fiers*, Novel Alzheimer risk genes determine the microglia response to amyloid-β but not to TAU pathology. **Embo Mol.Med., in press**

Resubmitted to Cell

8. Wei-Ting Chen, Ashley Lu, Katleen Craessaerts, Benjamin Pavie, Carlo Sala Frigerio, Renzo Mancuso, Xiaoyan Qian, Jana Lalakova, Malte Kühnemund, Iryna Voytyuk, Leen Wolfs, An Snellinx, Sebastian Munck, Aleksandra Jurek, Jose Fernandez Navarro, Takaomi C Saido, Joakim Lundeberg, Mark Fiers, **Bart De Strooper**. Spatial and temporal transcriptomics reveal microglia-astroglia crosstalk in the amyloid-β plaque cell niche of Alzheimer's disease. https://www.biorxiv.org/content/10.1101/719930v1

Resubmitted to Cell Stem Cell

9. Evgenia Salta, Hannah Walgrave, Sriram Balusu, Elke Vanden Eynden, Sarah Snoeck, Katleen Craessaerts, Nicky Thrupp, Leen Wolfs, Katrien Horré, Yannick Fourne, Alicja Ronisz, Edina Silajdžić, Zsuzsanna Callaerts-Vegh, Rudi D'Hooge, Dietmar Rudolf Thal, Henrik Zetterberg, Sandrine Thuret, Mark Fiers, Carlo Sala Frigerio, **Bart De Strooper** microRNA-132 restores adult hippocampal neurogenesis and memory deficits in Alzheimer's disease

3. Honors over the last three years

2018 European Grand Prix for Research (France, awarded by Fondation pour la Recherche sur Alzheimer)
2018 Commander in the Order of Leopold (Belgian national honorary order)
2018 Brain Prize, awarded by the Lundbeck Foundation (Denmark)
2018 Recognized as Highly cited researcher 2018 (Clarivate, Web of Science Group)
2019 Recognized as Highly cited researcher 2019 (Clarivate, Web of Science Group)
2019 Recognized as an Expertscape World Expert in Alzheimer's disease (nº 8 worldwide), presenilins (nº1 worldwide) and Amyloid beta-Peptides (nº 4 worldwide)