Herinneringen
(Memories)

Catherine Verfaillie
KU Leuven

23-11-2018

“Herinneringen” is gefinancierd binnen het Interreg V programma Vlaanderen-Nederland, het grensoverschrijdend samenwerkingsprogramma met financiële steun van het Europees Fonds voor Regionale Ontwikkeling. Meer info: www.grensregio.eu

Aims for KU Leuven team

- To obtain iPSC from patients with genetic and sporadic forms of AD, as well as normal controls
- To generate cortical neurons to test the effect of toxins on the transcriptome of iPSC neurons
- If time permits, to test role of identified aberrant transcripts / miRNAs on cortical neuronal function, as it relates to AD phenotypes

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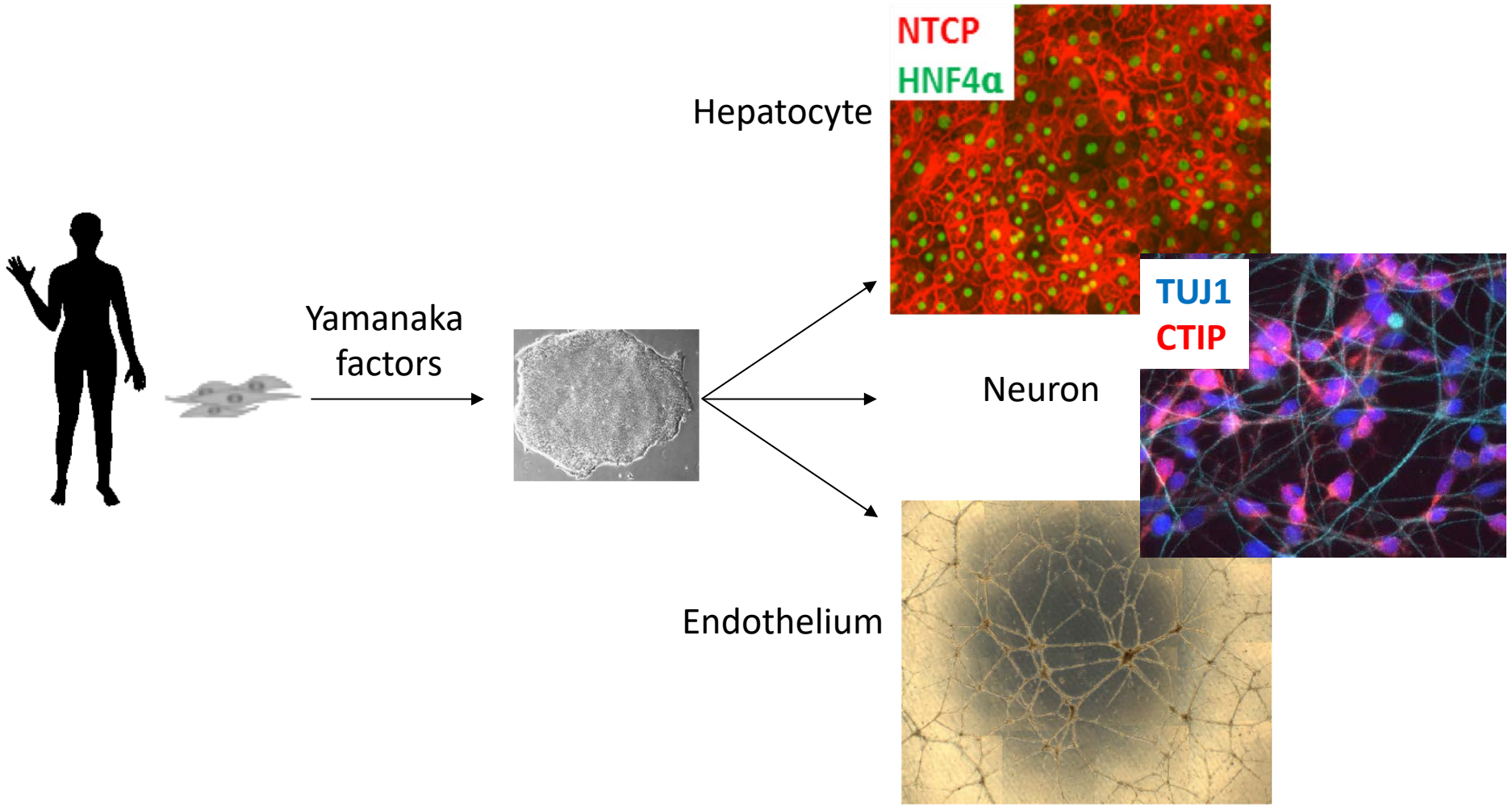
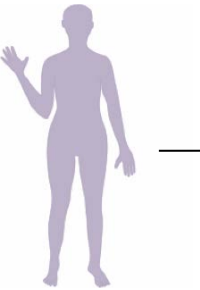
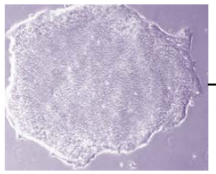
Induced pluripotent stem cells

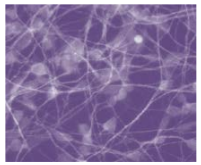


Disease modeling using iPSC

Normal

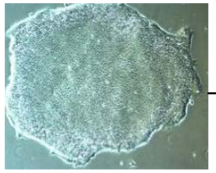


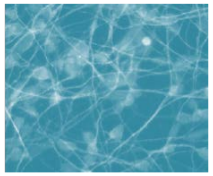


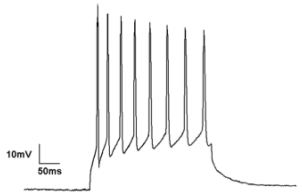


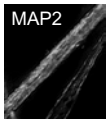
CRISPR/Cas

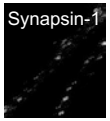
Diseased

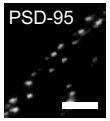


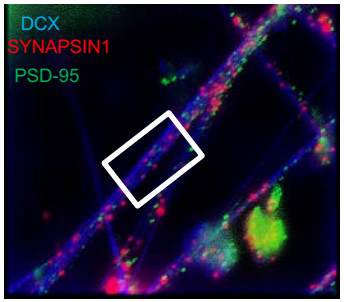


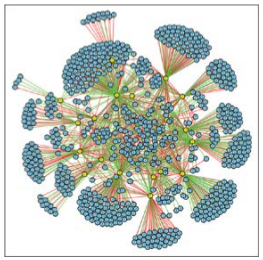












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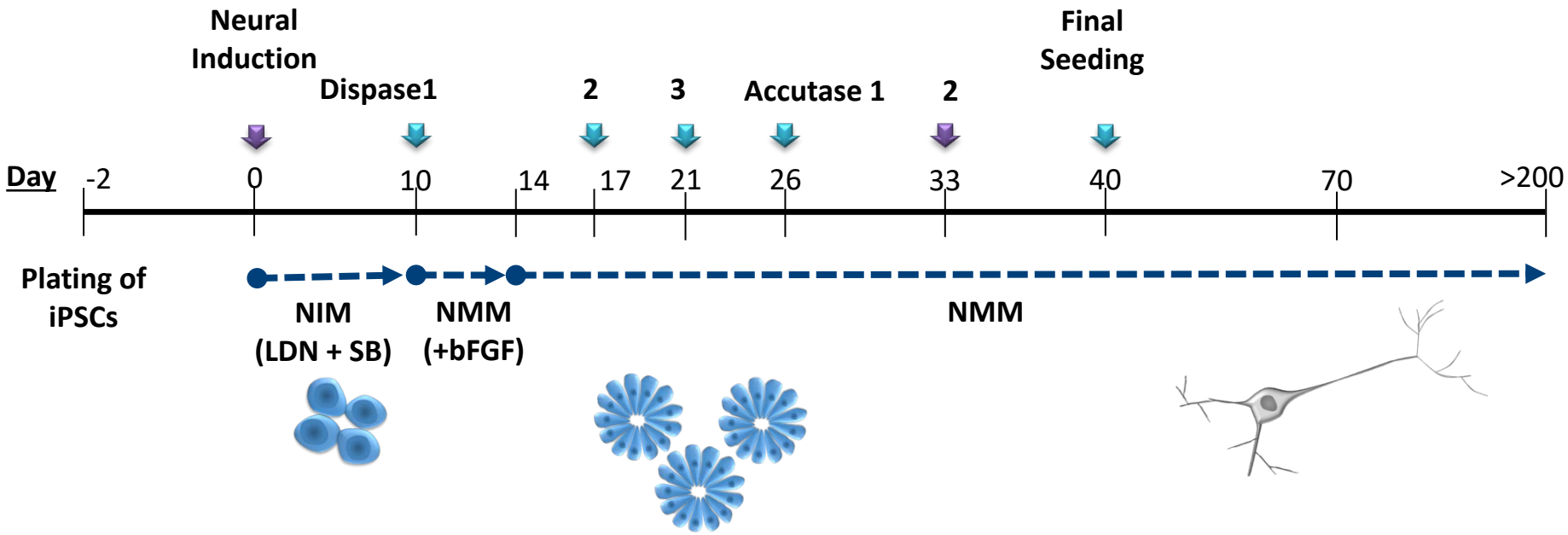
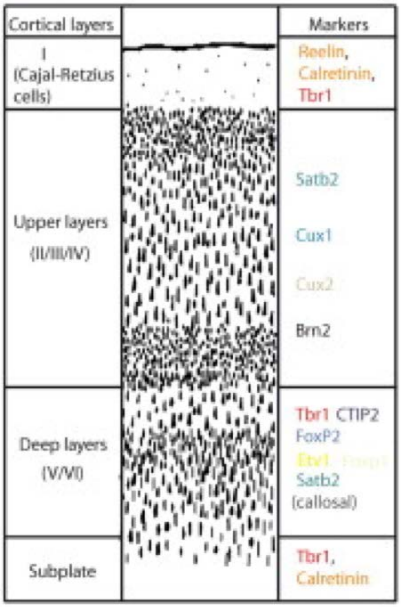
Generation of cortical glutamatergic projection neurons

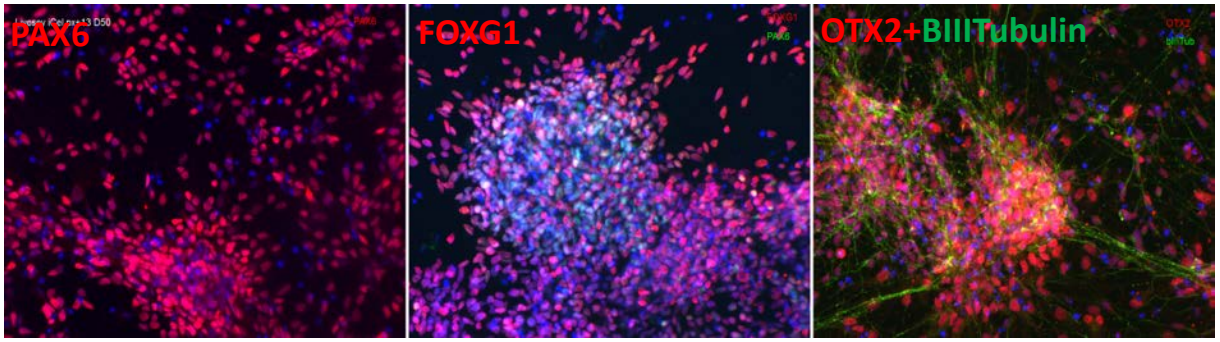


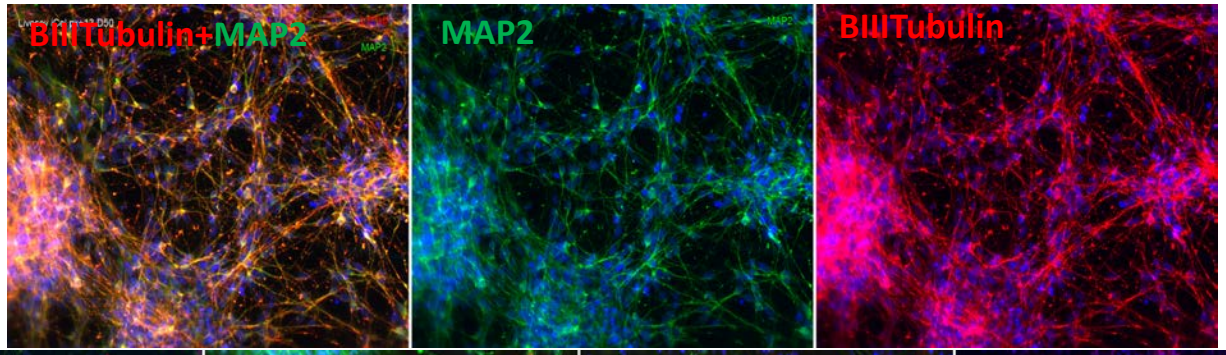
NIM : Neural induction medium,
NMM : Neural maintenance medium, bFGF : basic Fibroblast Growth Factor

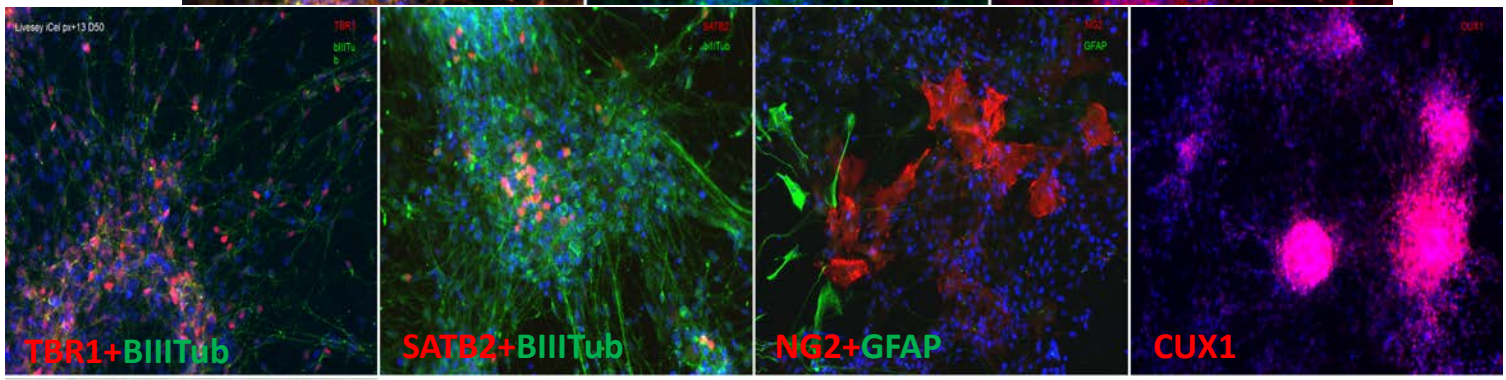
Shi et al Nature Protocols 2012

Cortical glutamatergic projection neurons



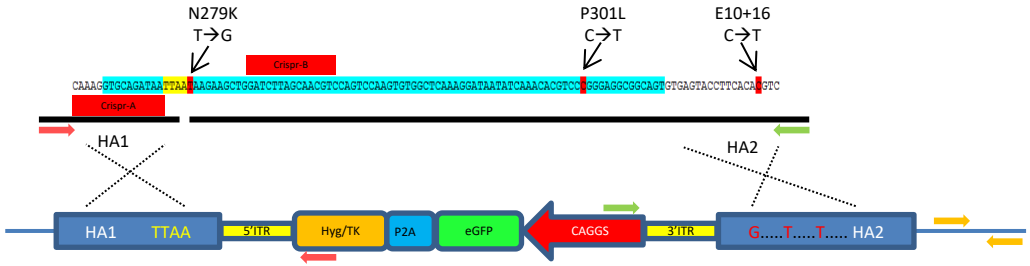


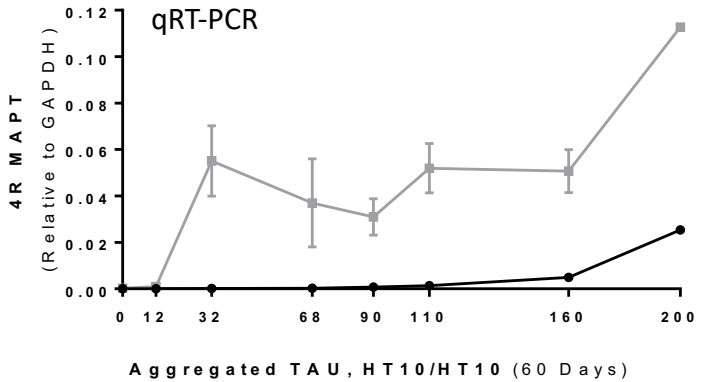


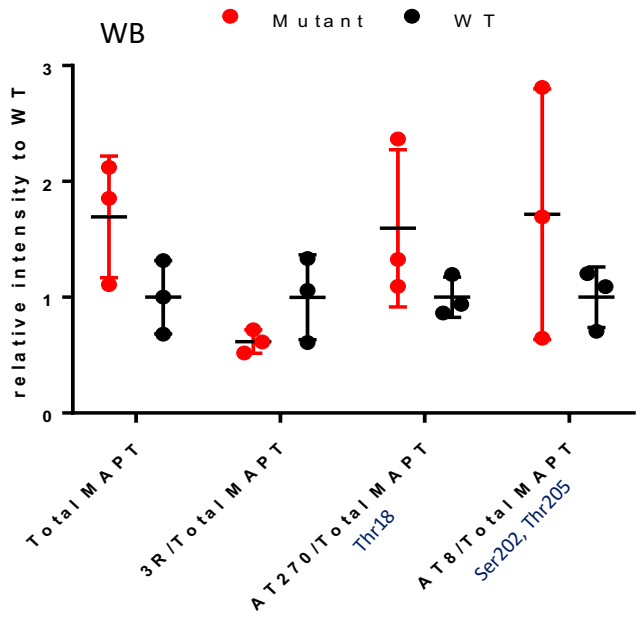


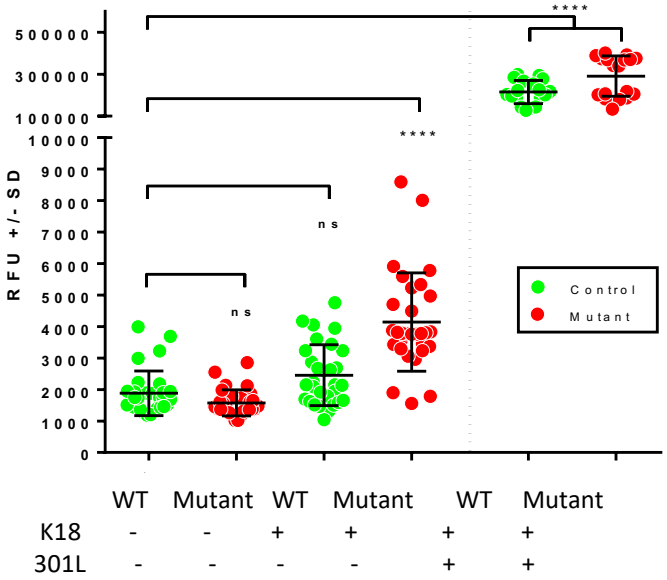
Tau mutant cortical neurons

increased 4R-TAU, P-TAU and aggregated TAU









Garcia Leon et al, Alzheimer's and dementia 2018

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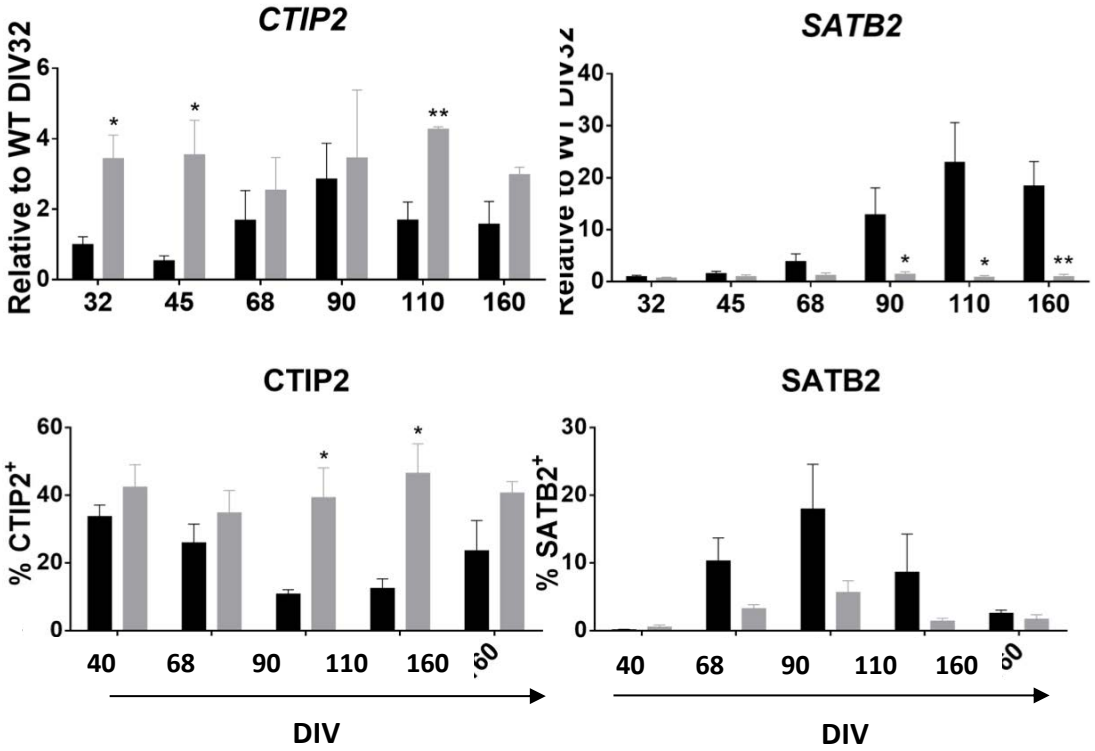
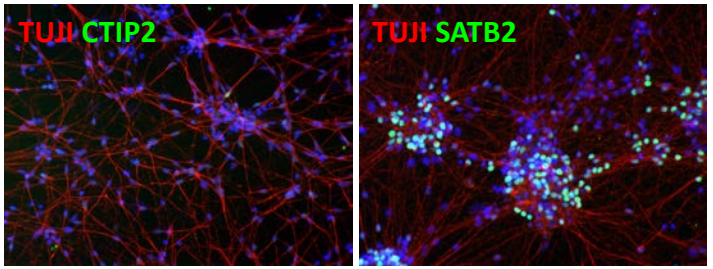
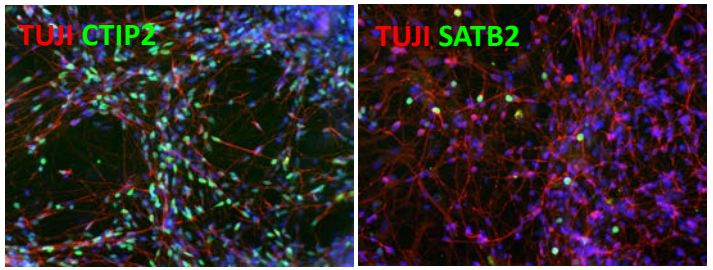
Tau mutant cortical neurons

Number upper cortical neurons decreased



Wild-type (DIV90)





Mutant (DIV90)

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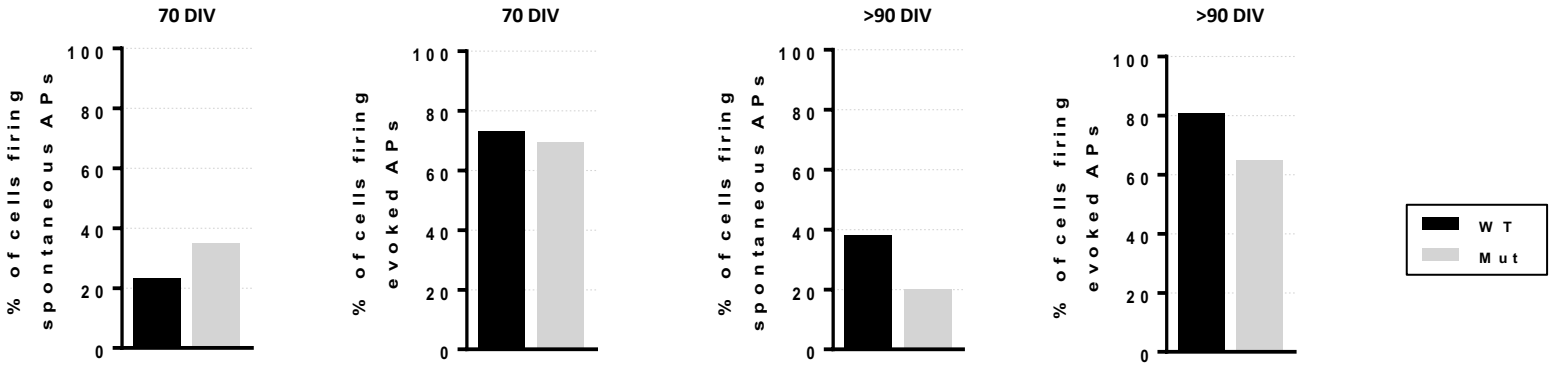
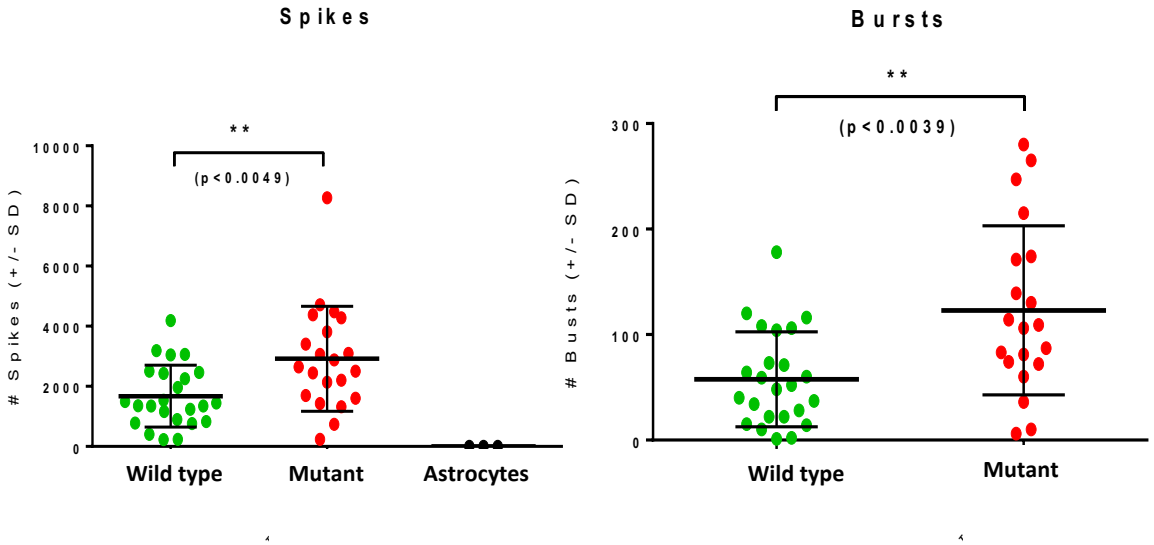
Tau mutant cortical neurons

hyper-excitability in mutant neurons (MEA)





Garcia Leon et al, Alzheimer's and dementia 2018

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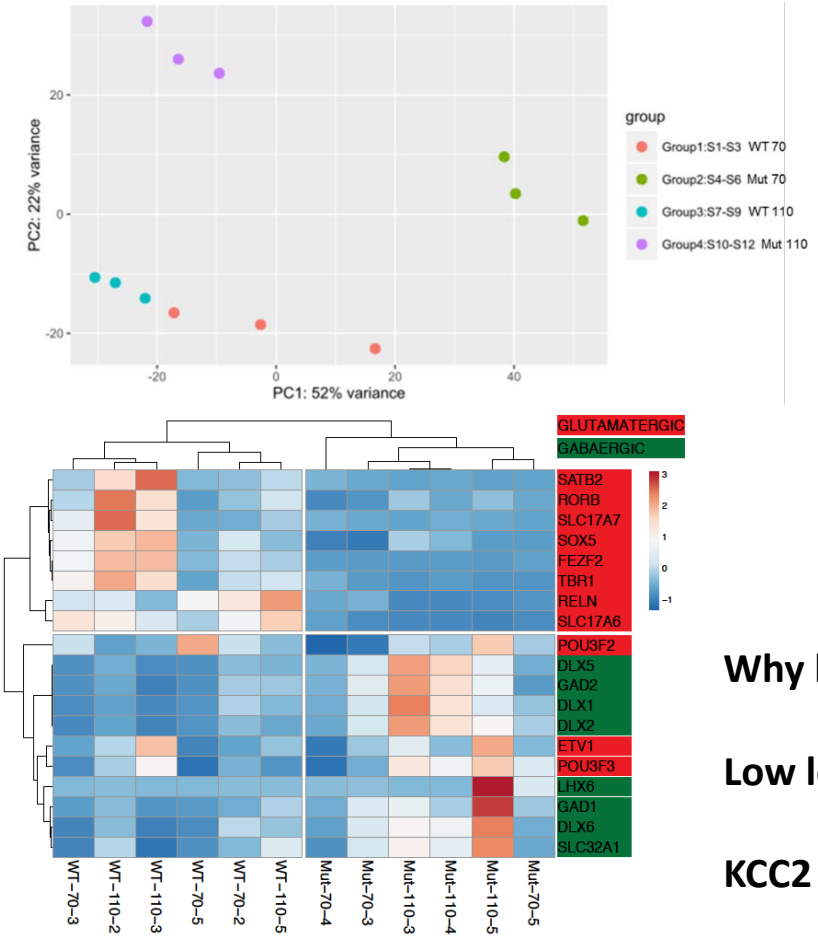
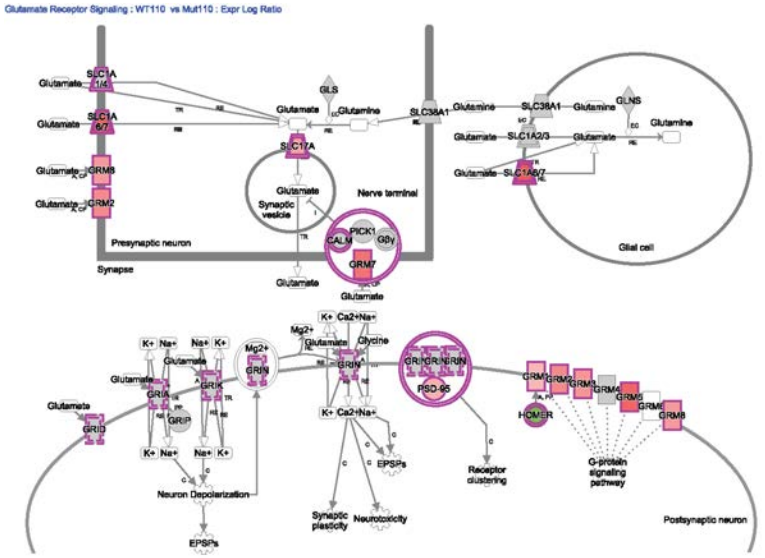
Tau mutant cortical neurons

Shift to gabaergic in stead of glutamatergic neurons





Why hyper-excitability if gabaergic?

Low levels of KCC2 in TAU mutant cells, hence maybe excitatory

KCC2 decreased in APP deficiency, maybe also because of TAU?

Garcia Leon et al, Alzheimer's and dementia 2018

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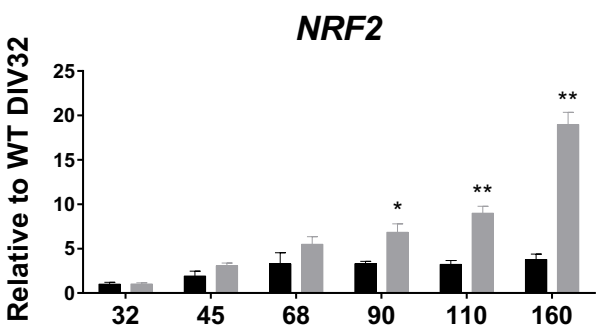
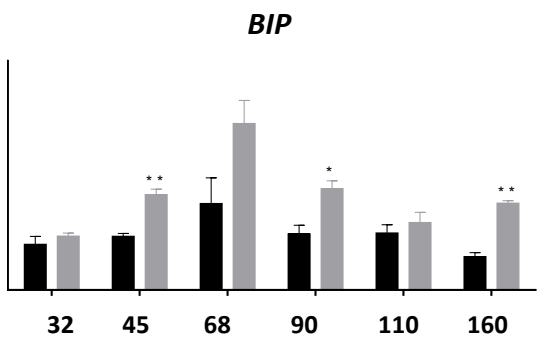
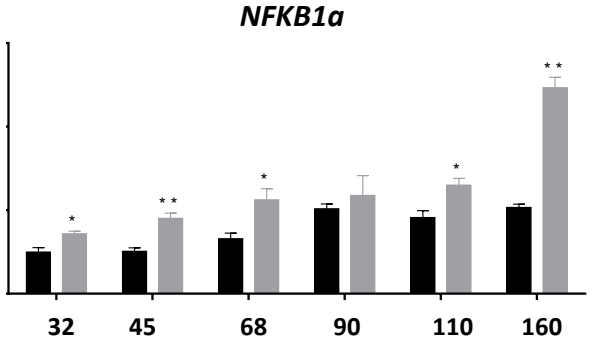
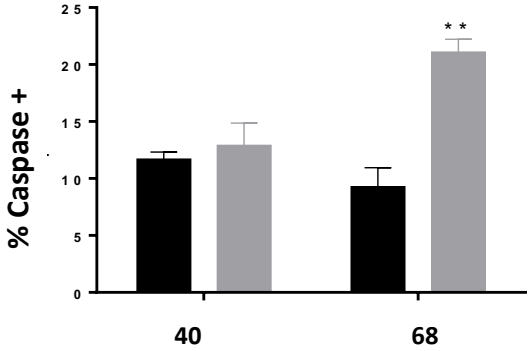
Tau mutant cortical neurons

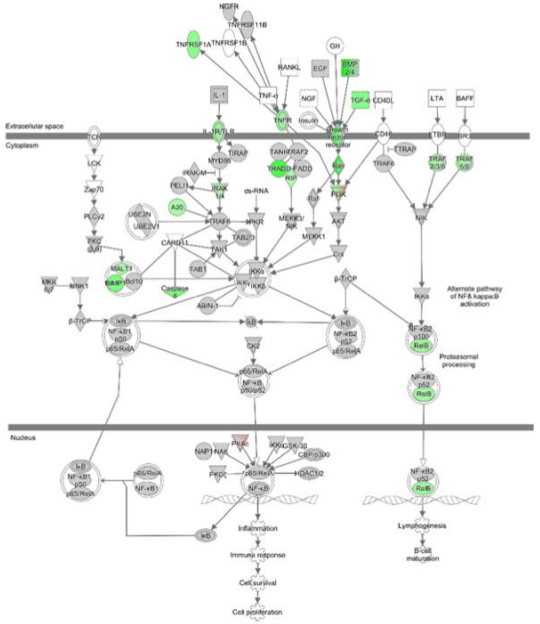
Increase in multiple stress pathway transcripts, and apoptosis











Garcia Leon et al, Alzheimer's and dementia 2018

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Tau mutant cortical neurons

- Triple mutant iPSC neurons express high levels 4R-TAU, P-TAU and form aggregated Tau without overexpression of 301L-TAU

- Multiple functional differences between triple mutant and wild type PSC-neurons:
  - Hyperexcitability
  - Decreased upper layer neurons and increased gabaergic neurons
  - Increase in stress pathway activation and cell apoptosis
  - Decreased neurite outgrowth (not shown)

Garcia Leon et al, Alzheimer's and dementia 2018

Status Verfaillie lab

- Cell lines identified and banked
- Creation neuroprogenitors from iPSC
- Initial testing (by ReMynd) of effect external ‘toxins’ on Ca-flues and secreted TAU and Aβ42

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Herinneringen iPSC cells

Herinneringen code	Line name	Age of donor	Sex of donor
Group 1: aged individuals with no AD (between 70 and 90 years of age)			
HER-01	HPSI0115i-zihe_1	75-79	female
HER-02	HPSI0214i-rayr_1	75-79	male
HER-03	UCSD223i-NDC1-1	86	male
HER-04	SCRP6007i	88	female

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Group 2: patient with sporadic AD (between 70 and 90 years of age)			
HER-06	UCSD234i-SAD2-3	83	male
HER-07	AD8K213 (RIKEN HPS0258)	76	male (not yet available)
HER-08	AD-iPS5	82	female (not yet available)
HER-12	CW50119	84	male (ordered, MTA OK)
HER-13	CW50120	82	male (ordered, MTA OK)
HER-14	CW50133	87	female (ordered, MTA OK)
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Group 4: individual with protective (Islandic) APP mutation (A673T, c.2017G>A)			
HER-05	01279.A27 knock-in	unknown	male (not available as iPSC)

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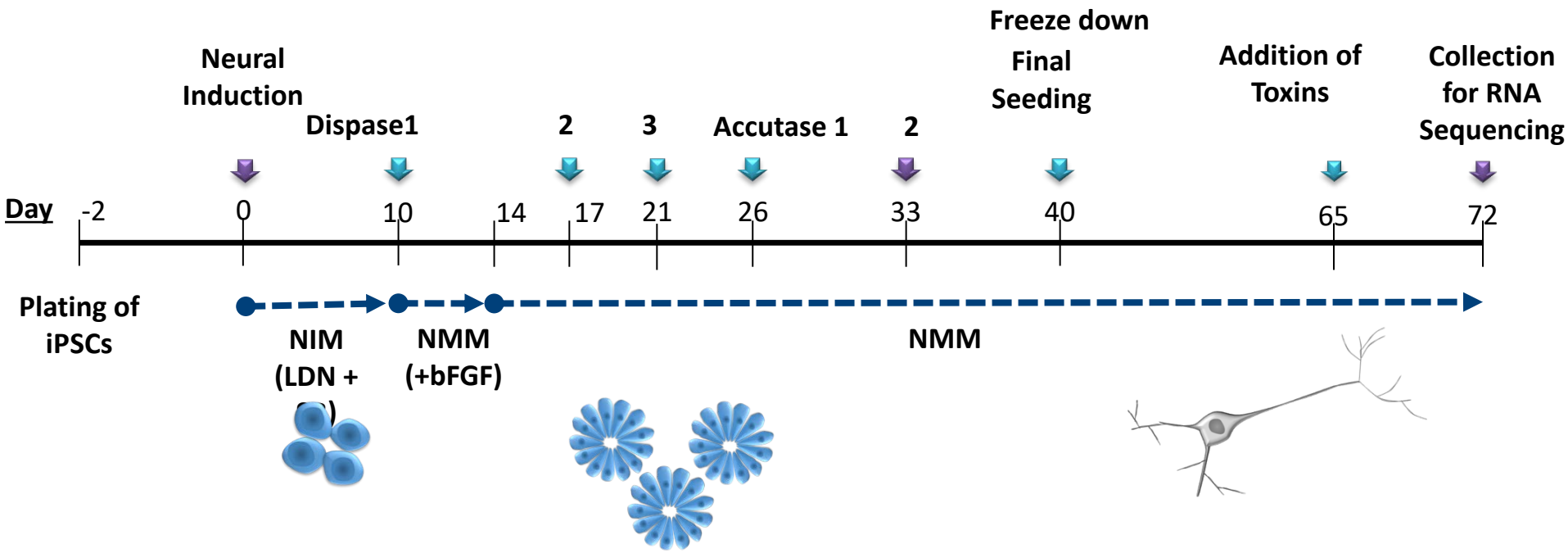
Generation of cortical glutamatergic projection neurons



NIM : Neural induction medium,
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Shi et al Nature Protocols 2012

Status differentiations

	Celline	Renamed	IPSC	Day -2	Neural induction	Dispase 1	Dispase 2	Dispase 3	Dispase 4	Accutase 1	Freeze down DIV33
1	ApoE4 Batch A1	HER-09 A1	p27	04/07/2018	06/07/2018	16/07/2018	23/07/2018	27/07/2018	31/07/2018	31/07/2018	08/08/2018
2	ApoE4 Batch A2	HER-09 A2	p31	22/07/2018	24/07/2018	03/08/2018	10/08/2018	14/08/2018	20/08/2018	20/08/2018	24/08/2018
3	Tau Batch B1	HER-10 B1	px+16	14/08/2018	16/08/2018	26/08/2018	02/09/2018	05/09/2018	10/09/2018	12/09/2018	17/09/2018
4	Tau Batch B2	HER-10 B2	px+16	26/08/2018	28/08/2018	07/09/2018	14/09/2018	20/09/2018	26/09/2018	26/09/2018	01/10/2018
5	Cellectis C1	HER-11 C1	px+11	04/09/2018	06/09/2018	16/09/2018	23/09/2018	27/09/2018		02/10/2018	09/10/2018
6	Cellectis C2	HER-11 C2	px+13	12/09/2018	14/09/2018	24/09/2018	01/10/2018	05/10/2018		10/10/2018	17/10/2018
7	Herinneringen 02 D1	HER-02 D1	p31	14/09/2018	16/09/2018	26/09/2018	03/10/2018	08/10/2018	11/10/2018	15/10/2018	19/10/2018
8	Herinneringen 01 E1	HER-01 E1	p30	16/09/2018	18/09/2018	28/09/2018	05/10/2018	10/10/2018		15/10/2018	22/10/2018
9	Herinneringen 02 D2	HER-02 D2	p35	21/09/2018	23/09/2018	03/10/2018	10/10/2018	15/10/2018	19/10/2018	22/10/2018	26/10/2018
10	Herinneringen 01 E2	HER-01 E2	p31	21/09/2018	23/09/2018	03/10/2018	10/10/2018	15/10/2018	19/10/2018	19/10/2018	26/10/2018
11	Herinneringen 03 F1	HER-03 F1	p15	21/09/2018	23/09/2018	03/10/2018	10/10/2018	15/10/2018	19/10/2018	19/10/2018	26/10/2018
12	Herinneringen 03 F2	HER-03 F2	p16	26/09/2018	28/09/2018	08/10/2018	15/10/2018	19/10/2018	24/10/2018	24/10/2018	31/10/2018
13	Herinneringen 10 B3	HER-10 B3	px+15	08/10/2018	10/10/2018	20/10/2018	27/10/2018	31/10/2018		05/11/2018	12/11/2018
14	Herinneringen 06 G1	HER-06 G1	p43	08/10/2018	10/10/2018	20/10/2018	27/10/2018	31/10/2018		05/11/2018	12/11/2018
15	Herinneringen 06 G2	HER-06 G2	p45	12/10/2018	14/10/2018	24/10/2018	31/10/2018	05/11/2018	09/11/2018	12/11/2018	16/11/2018
16	Herinneringen 04 H1	HER-04 H1	p15	25/10/2018	27/10/2018	06/11/2018	13/11/2018	16/11/2018	20/11/2018	23/11/2018	29/11/2018
17	Herinneringen 04 H2	HER-04 H2		31/10/2018	02/11/2018	12/11/2018	19/10/2018	23/11/2018		28/11/2018	05/12/2018

QC done/ongoing

- iPSC lines:
  - Pluripotency assay
  - SNP assay
- Cortical neuron progenitors
  - qRT-PCR

Cells provided to ReMynd

- Wild type Cellectis ChiPSC6B
- Triple Tau mutant Cellectis ChiPSC6B

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Timing going forward

- On already established NPCs
  - Terminal plating till DIV65 (Mo1&2/2019)
  - Exposure to toxins for 7 days (Mo2/2019)
  - Collection samples RNAseq, TAU and Aβ42 (Mo2/2019)

- On sporadic AD sample still be obtained
  - iPSC bank (Mo2/2019)
  - NPC bank (Mo3&4/2019)
  - Terminal plating, exposure, collection (Mo4&5/2019)

Who did the work!!!

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