UniversityHospital Zurich
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Cell-type specific proteomics in the brain

Technical Journal Club

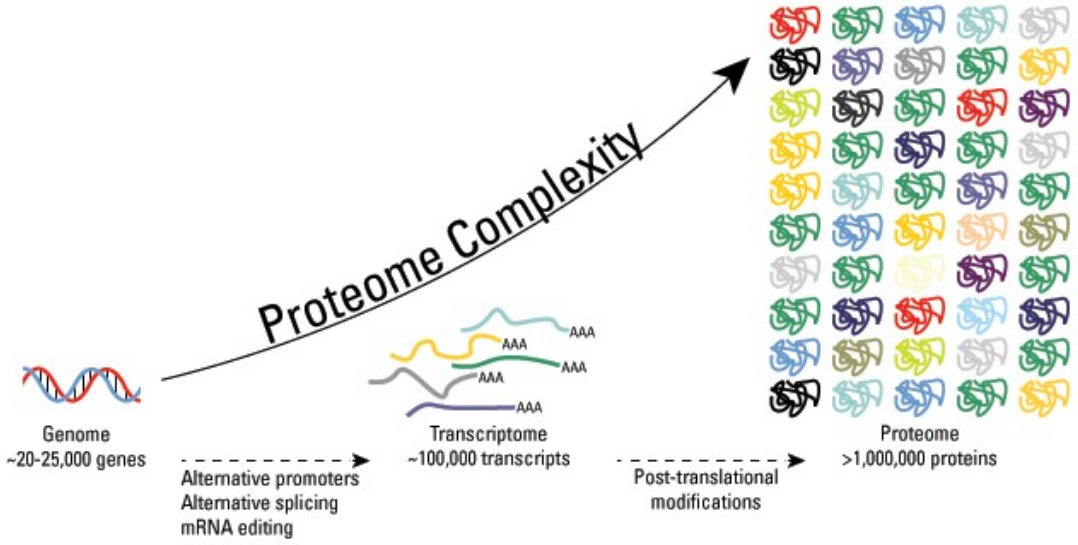
13.02.2018

Daniel Kirschenbaum

Introduction:

- the complexity of the phenotype -

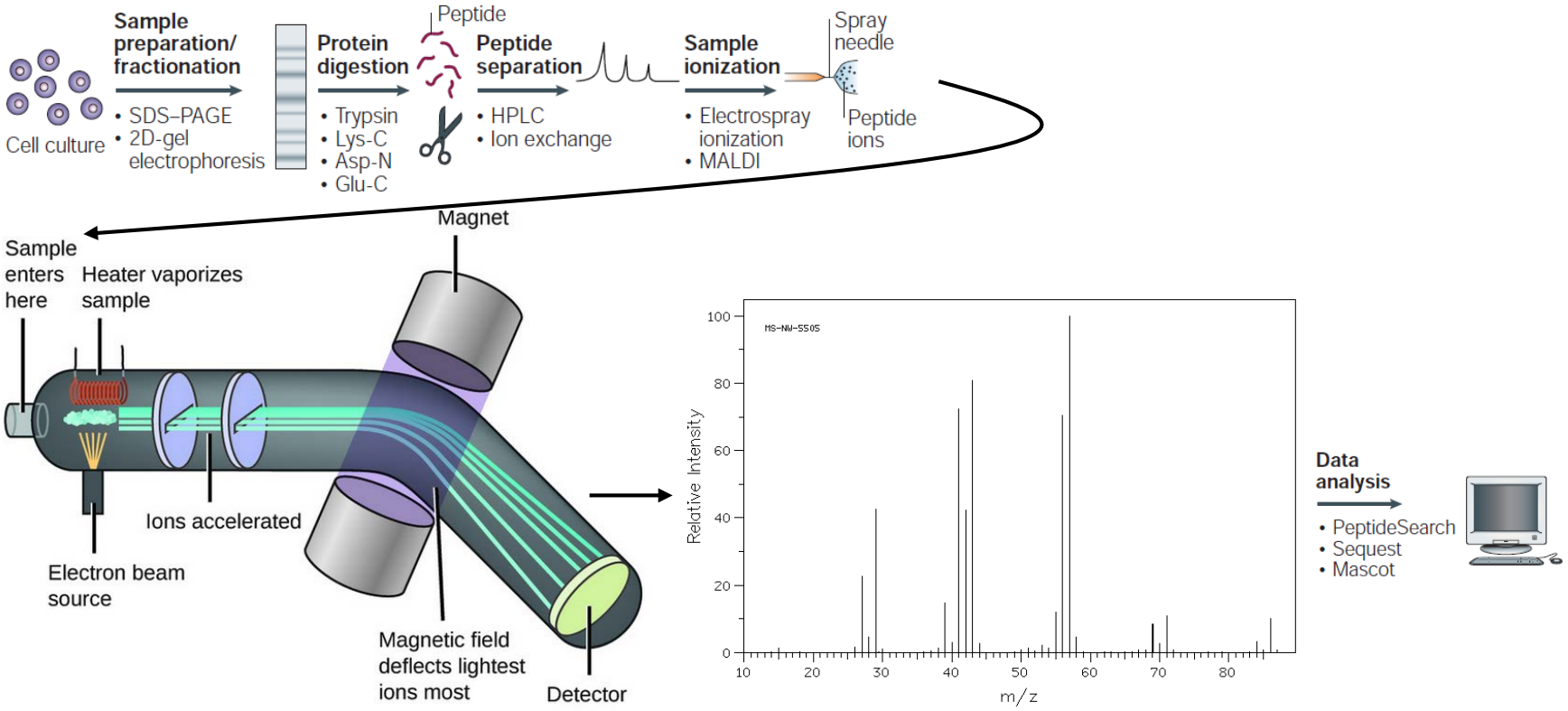
- There is a pool of ~20.000 protein coding genes in human.
- There are epigenetically controlled proteom-phenotypes resulting in cell types and tissues.
- Almost all steps of signal processing and cellular reactions manifest in proteomic changes.
- Proteomic changes can involve the abundance of proteins and protein isoforms, their interactions or their chemical modifications (e.g. phosphorylation).
- In order to understand the phenotype of cells and organisms, looking at their proteome is essential.



Introduction:

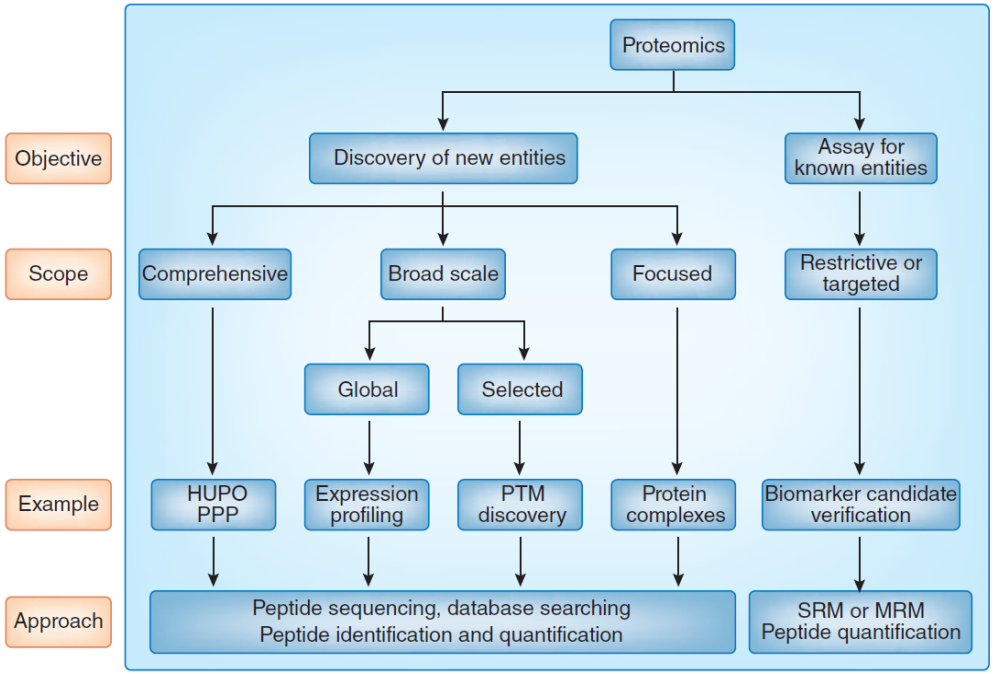
- current approaches in proteomics -

- Mass spectrometry of bulk tissues has been used widely.
- Proteomic scale mass spectrometry has developed substantially in the recent years.
- Proteome, Glycoproteome, Interactome, Cell-surface-proteome etc.

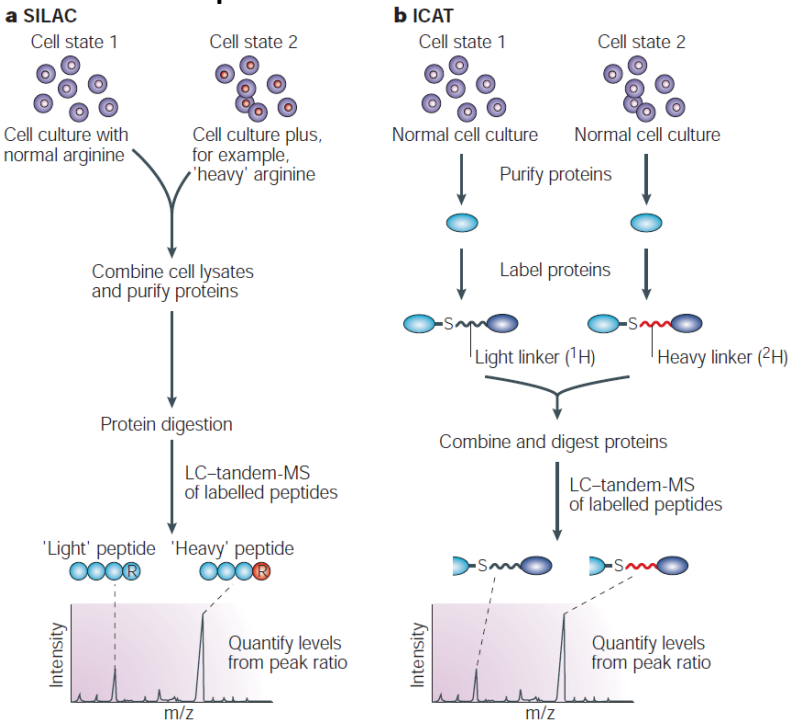


Introduction:

- the field of mass spectrometry-



Relative-quantitative



...absolute-quantitative

Mallick & Kuster, 2010, Nat. Biotech
Steen & Mann, 2004, Nat. Rew.

Introduction:

- the problem of abbundance, representativeness and specificity -

- Analyzing the proteom of the brain in a cell-type specific manner used to be challenging.
- Approaches included:
  - Laser capture microdisseciton
  - FACS sorting
  - Ex vivo methods: cell culture preparations or rapid isolation of primary cells

Limited protein yield and throughput. Cell surface marker bias.

- Cell culture is not necessarily representative of the brain.
- Isolation of cells may perturbe the proteome and samples may not be pure enough.

Labeling and identifying cell-type-specific proteomes in the mouse brain

Toke P Krogager[1,3], Russell J Ernst[1,3], Thomas S Elliott[1,3], Laura Calo[2], Václav Beránek[1], Ernesto Ciabatti[1], Maria Grazia Spillantini[2], Marco Tripodi[1], Michael H Hastings[1] & Jason W Chin[1]

February 2018, Nat. Biotechnology

Cell-type-specific metabolic labeling of nascent proteomes *in vivo*

Beatriz Alvarez-Castelao[1], Christoph T Schanzenbächer[1,2,6], Cyril Hanus[1,5,6], Caspar Glock[1], Susanne tom Dieck[1], Aline R Dörrbaum[1,2], Ina Bartnik[1], Belquis Nassim-Assir[1], Elena Ciirdaeva[1], Anke Mueller[3], Daniela C Dieterich[3], David A Tirrell[4], Julian D Langer[1,2] & Erin M Schuman[1]

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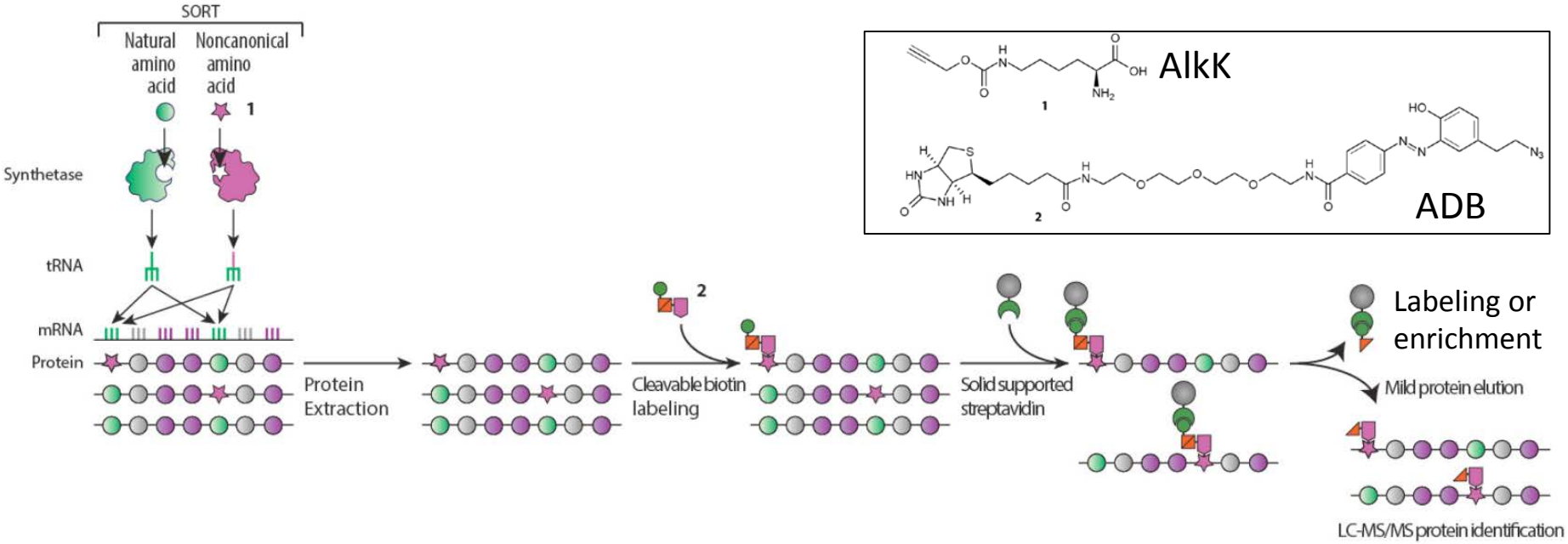
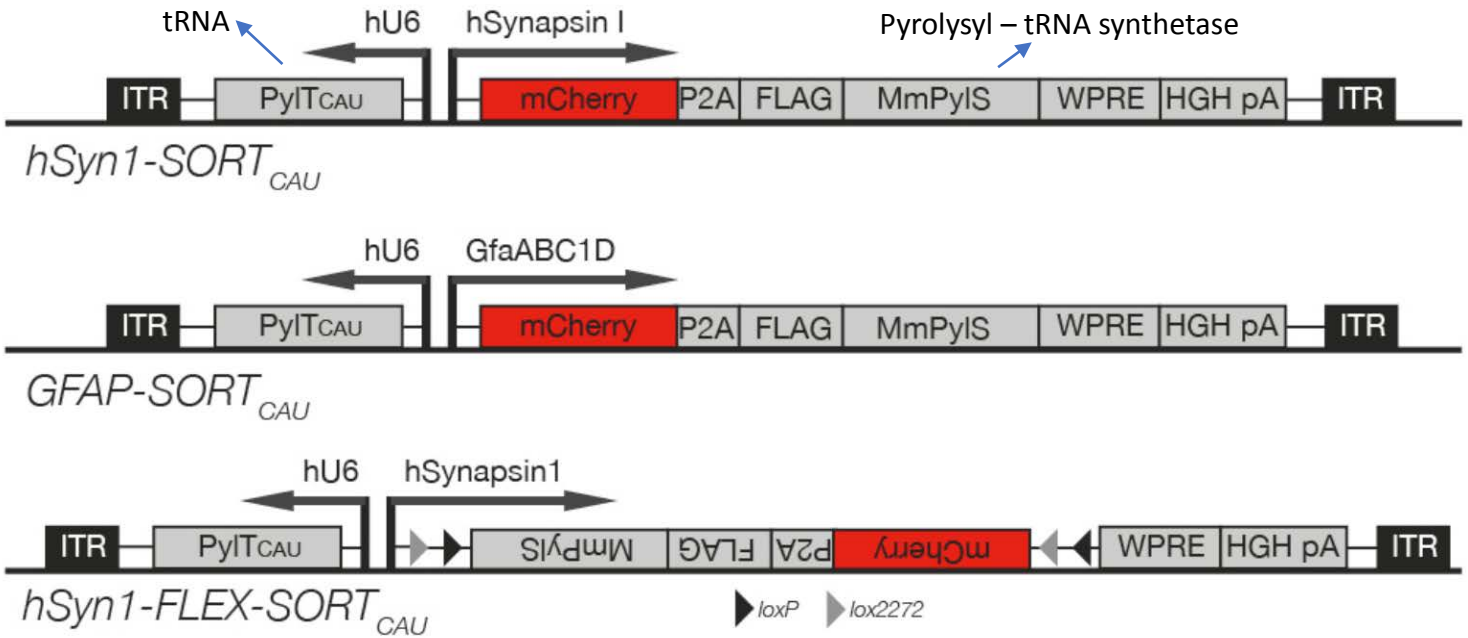
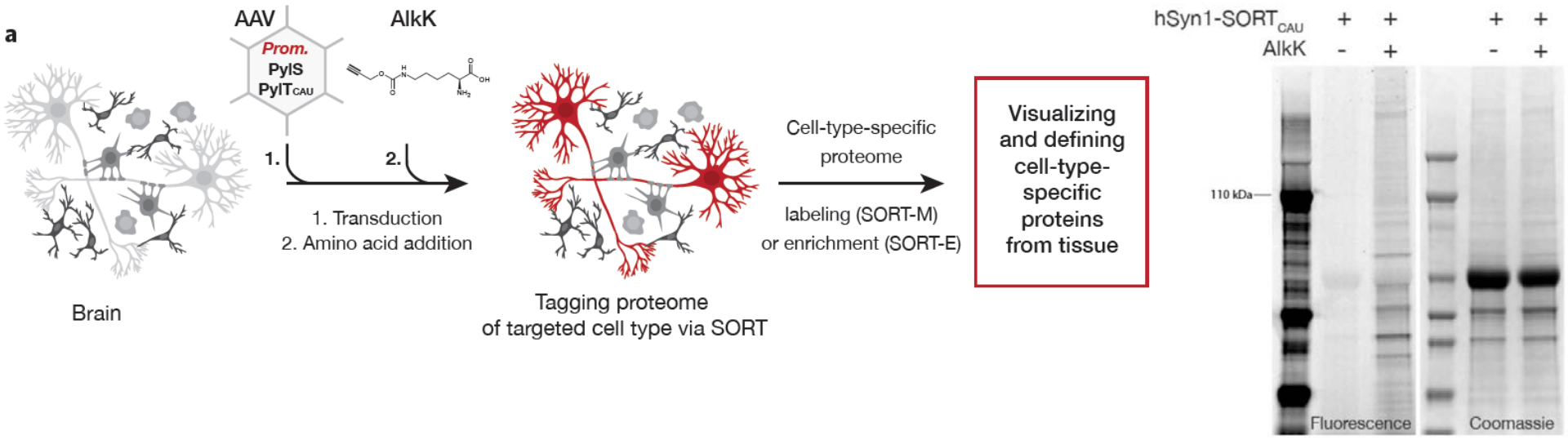


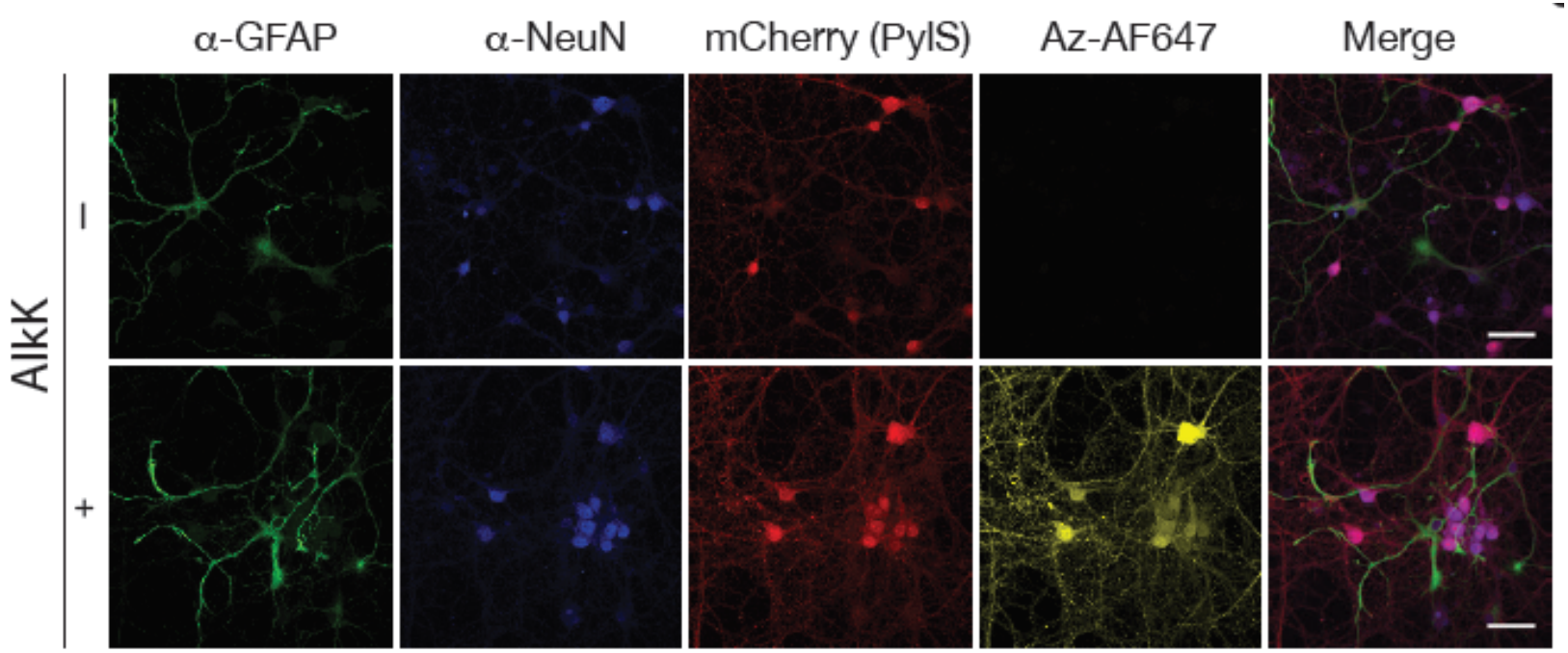
SORT – stochastic orthogonal recording of translation
AlkK - Nε- (propargyloxycarbonyl)-L-lysine
ADB - azide diazobenzene biotin

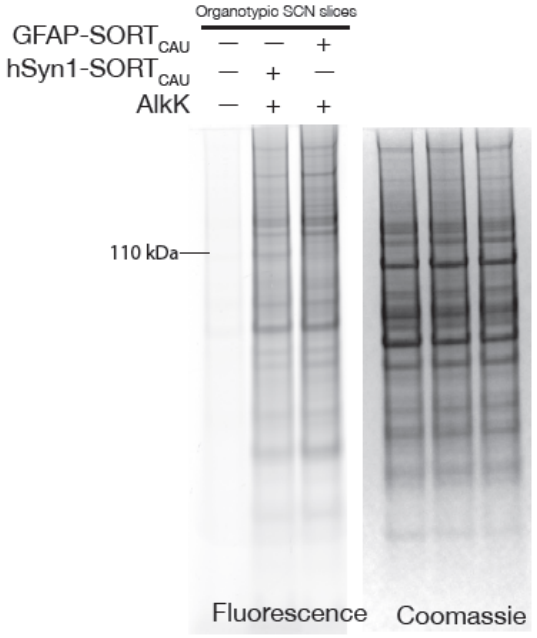

tRNA
hU6
hSynapsin I
Pyrolysyl – tRNA synthetase
ITR
PylTCAU
mCherry
P2A
FLAG
MmPylS
WPRE
HGH pA
ITR
hSyn1-SORTCAU
hU6
GfaABC1D
ITR
PylTCAU
mCherry
P2A
FLAG
MmPylS
WPRE
HGH pA
ITR
GFAP-SORTCAU
hU6
hSynapsin1
ITR
PylTCAU
MmPylS
FLAG
P2A
mCherry
WPRE
HGH pA
ITR
hSyn1-FLEX-SORTCAU
loxP
lox2272

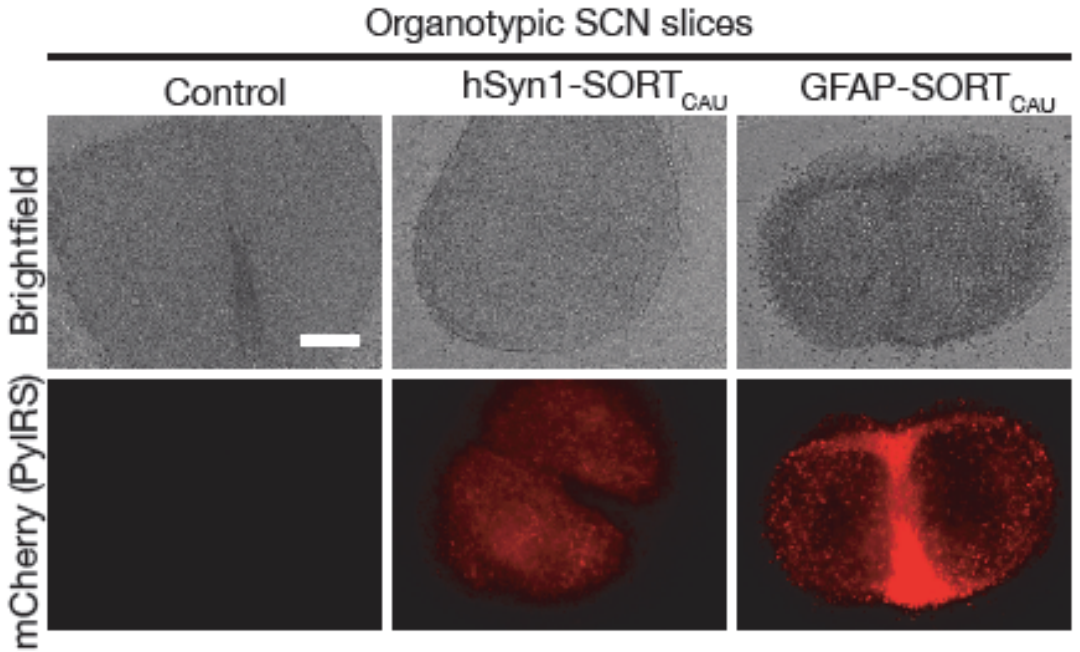

a
AAV
Prom.
PylS
PylTCAU
AlkK
1.
2.
1. Transduction
2. Amino acid addition
Brain
Tagging proteome
of targeted cell type via SORT
Cell-type-specific
proteome
labeling (SORT-M)
or enrichment (SORT-E)
Visualizing
and defining
cell-type-
specific
proteins
from tissue
hSyn1-SORTCAU + + + +
AlkK - + - +
110 kDa
Fluorescence
Coomassie

Cell culture & slice culture

Dissociated rat cortex

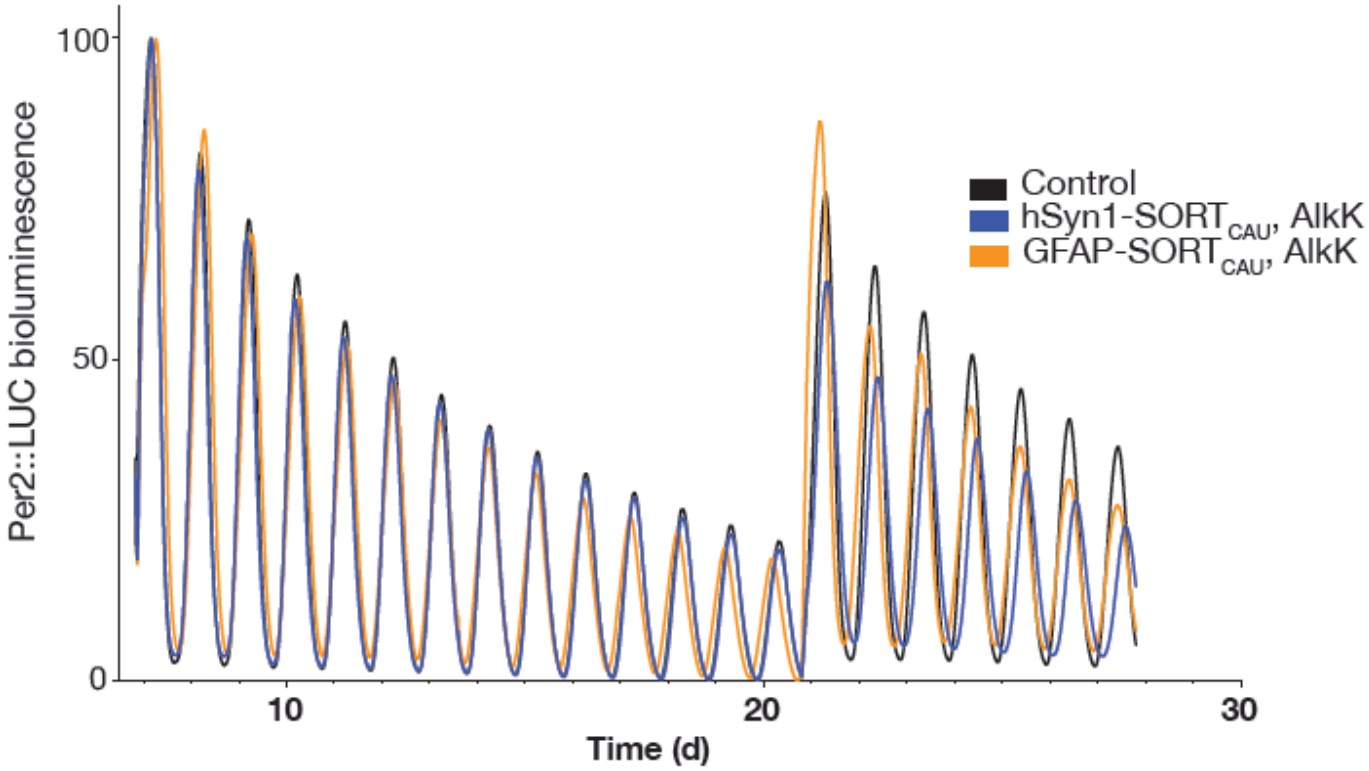




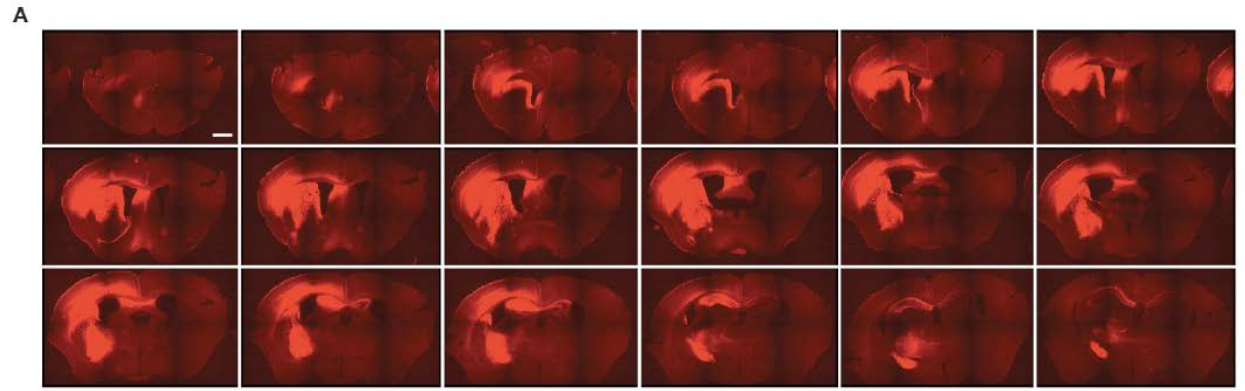


SORT – tagging does not influence signalling

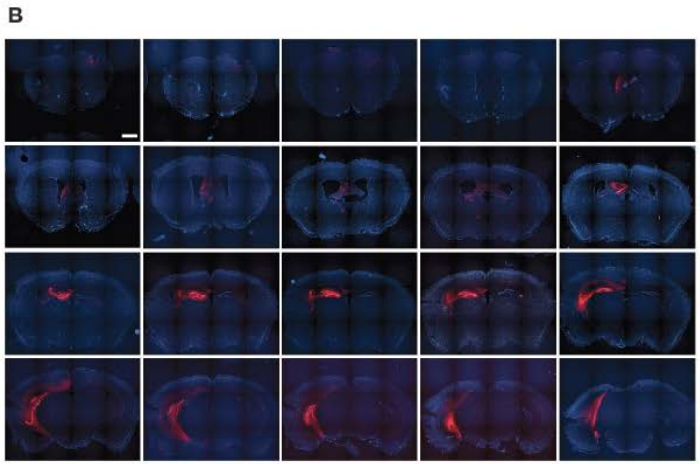
- SCN shows strong circadian oscillations detectable by the Period2::Luciferase system. It is a system very sensitive to perturbartions.
- SORT – does not alter the the circadian pattern.



In vivo



hSyn1-SORT$_{CAU}$



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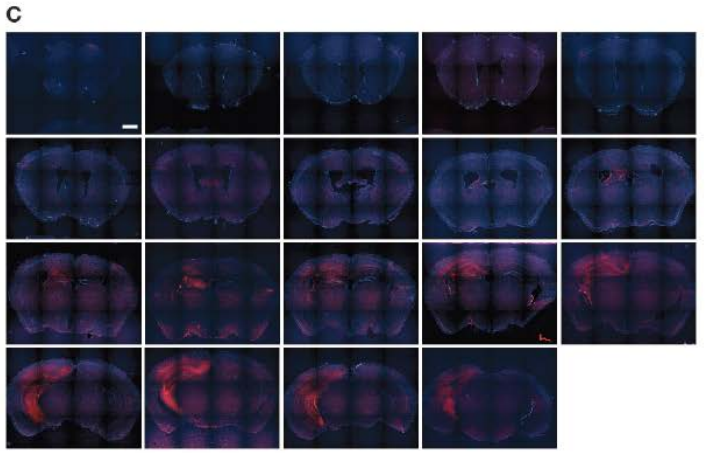
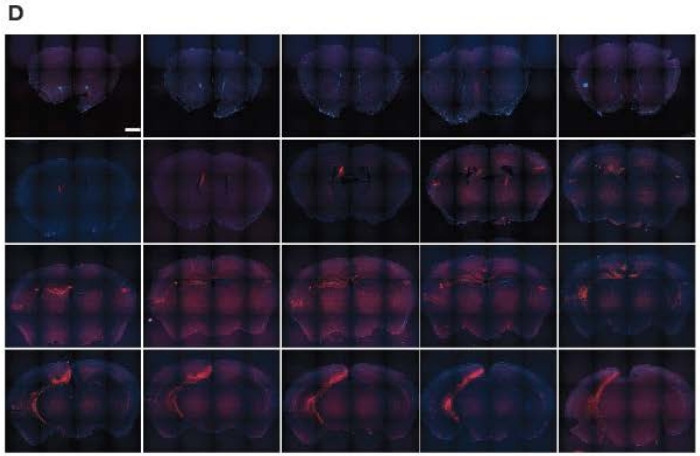
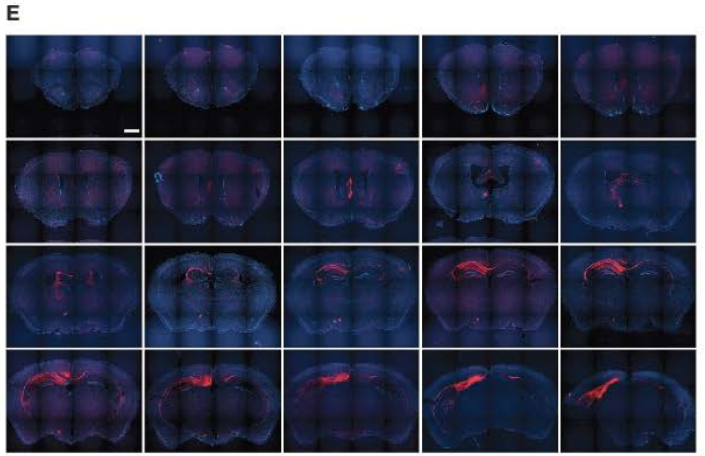


GFAP-SORT$_{CAU}$

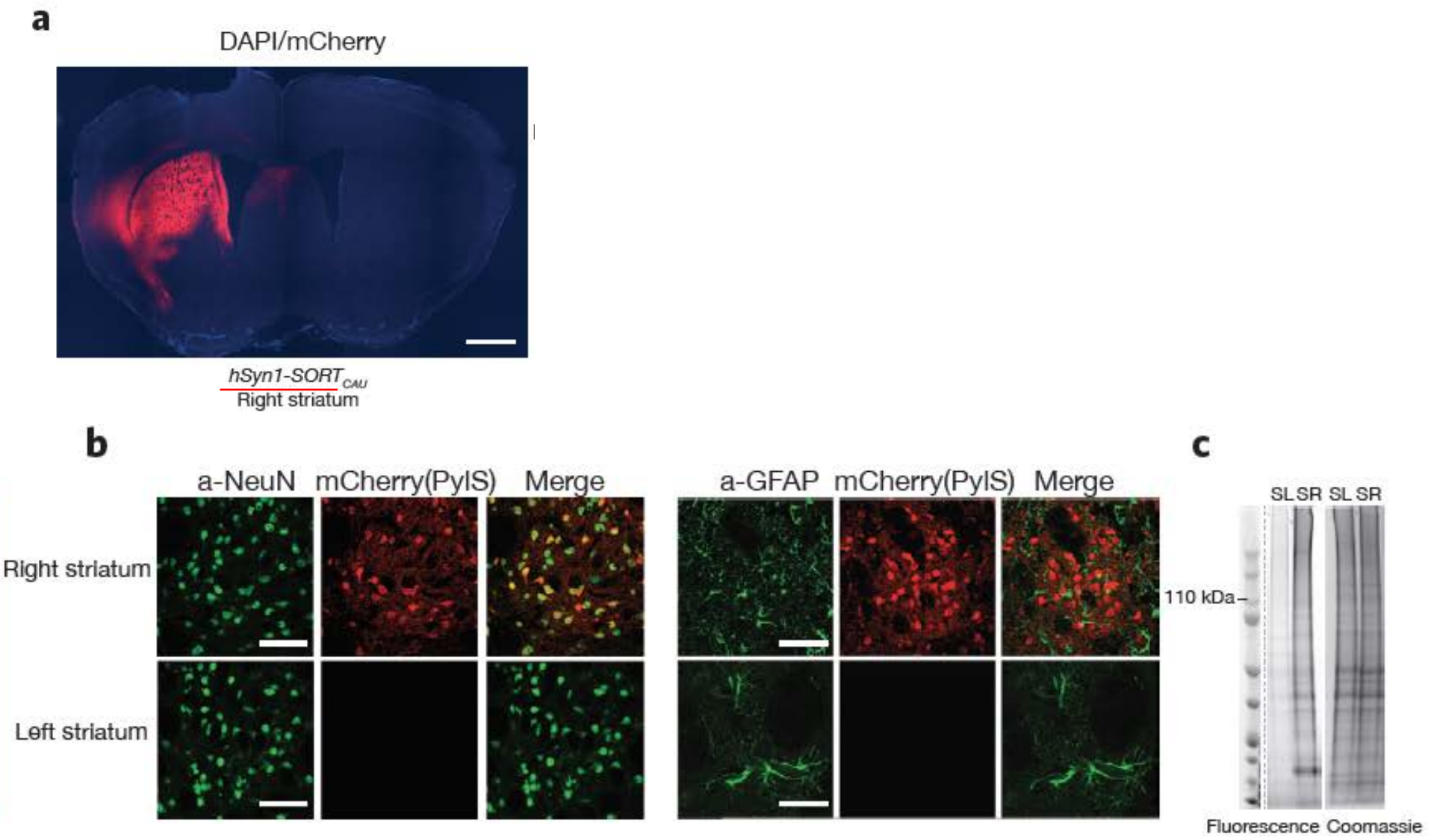


Vgat-Cre
hSyn1-SORT$_{CAU}$

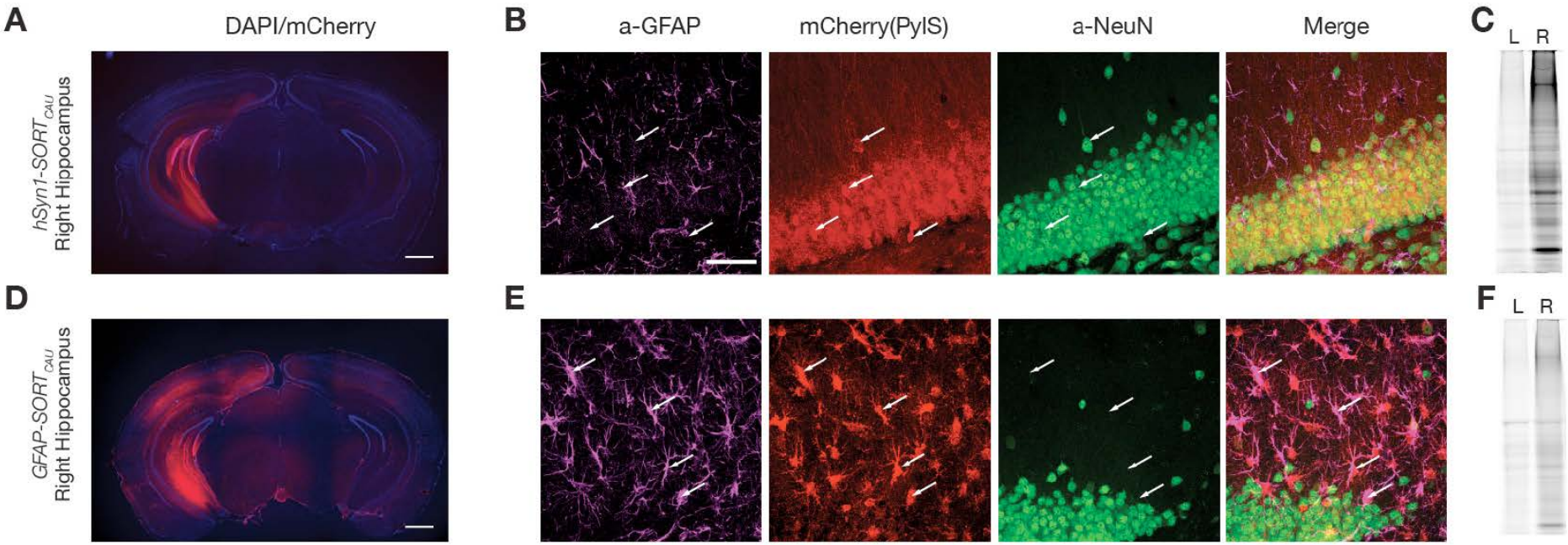


Vglut-Cre
hSyn1-SORT$_{CAU}$

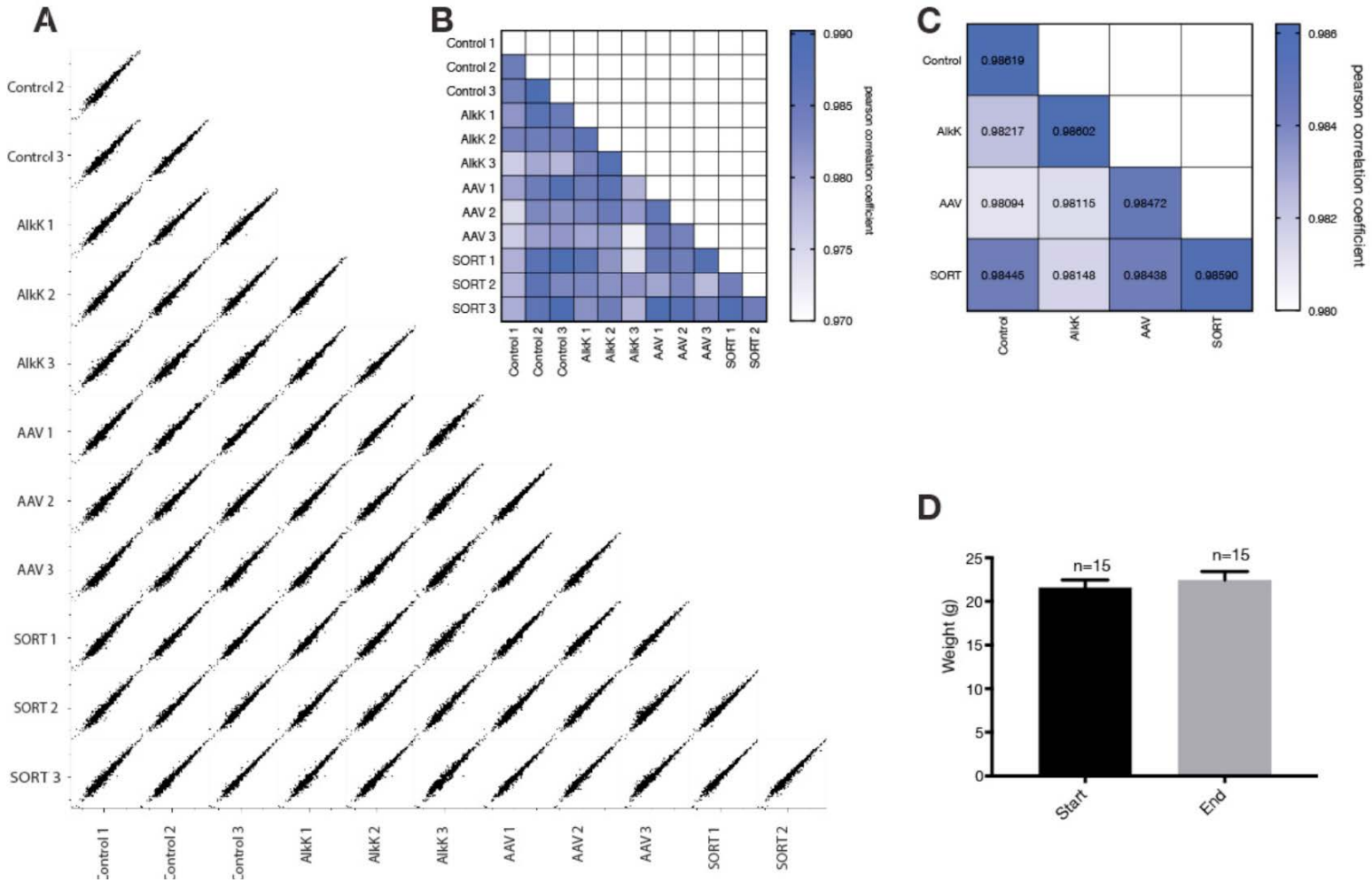
In vivo


a
DAPI/mCherry
hSyn1-SORT CAU
Right striatum
b
a-NeuN
mCherry(PylS)
Merge
a-GFAP
mCherry(PylS)
Merge
Right striatum
Left striatum
c
SL SR SL SR
110 kDa
Fluorescence
Coomassie

In vivo

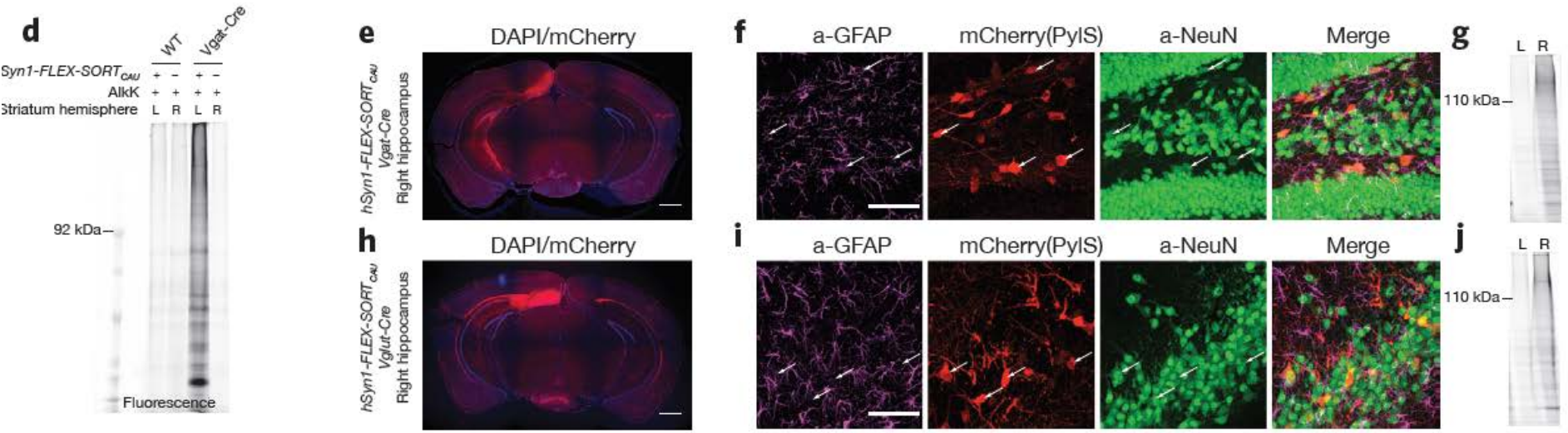

A
DAPI/mCherry
hSyn1-SORT CAU
Right Hippocampus
B
a-GFAP
mCherry(PylS)
a-NeuN
Merge
C
L
R
D
GFAP-SORT CAU
Right Hippocampus
E
F
L
R

SORT – tagging does not influence protein expression *in vivo*

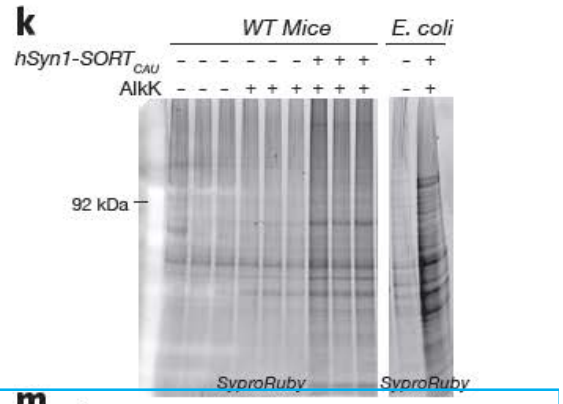


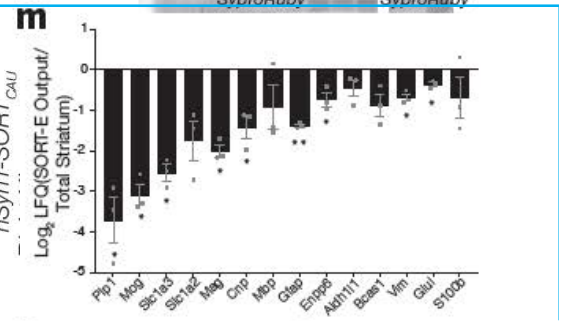
In vivo

Cell (promoter) specific protein tagging



In vivo – mass spectrometry





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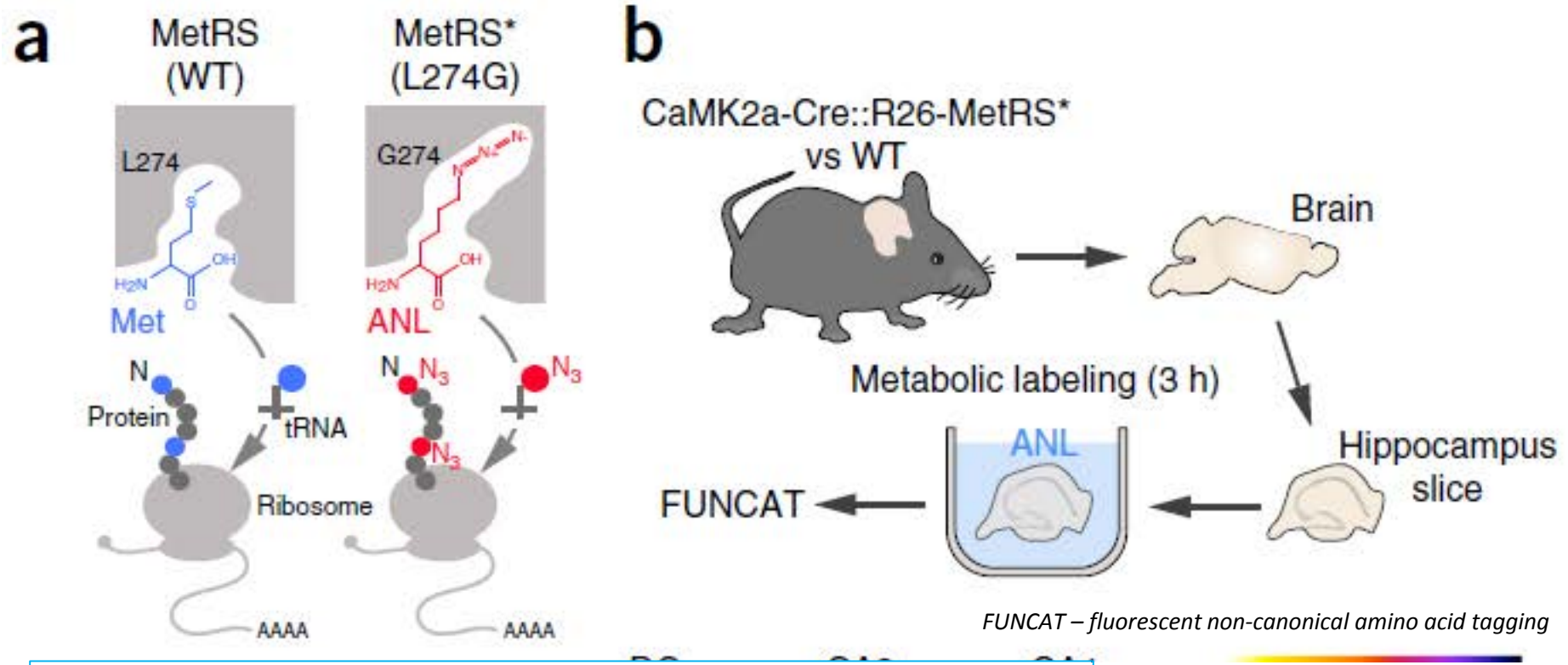
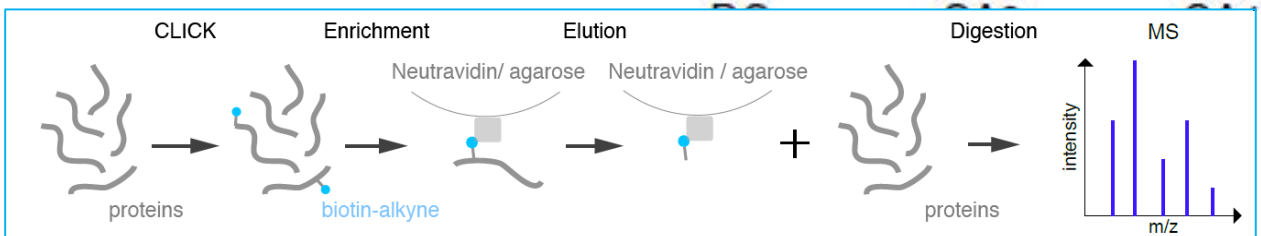


FUNCAT – fluorescent non-canonical amino acid tagging



BONCAT – bio-orthogonal non-canonical amino acid tagging

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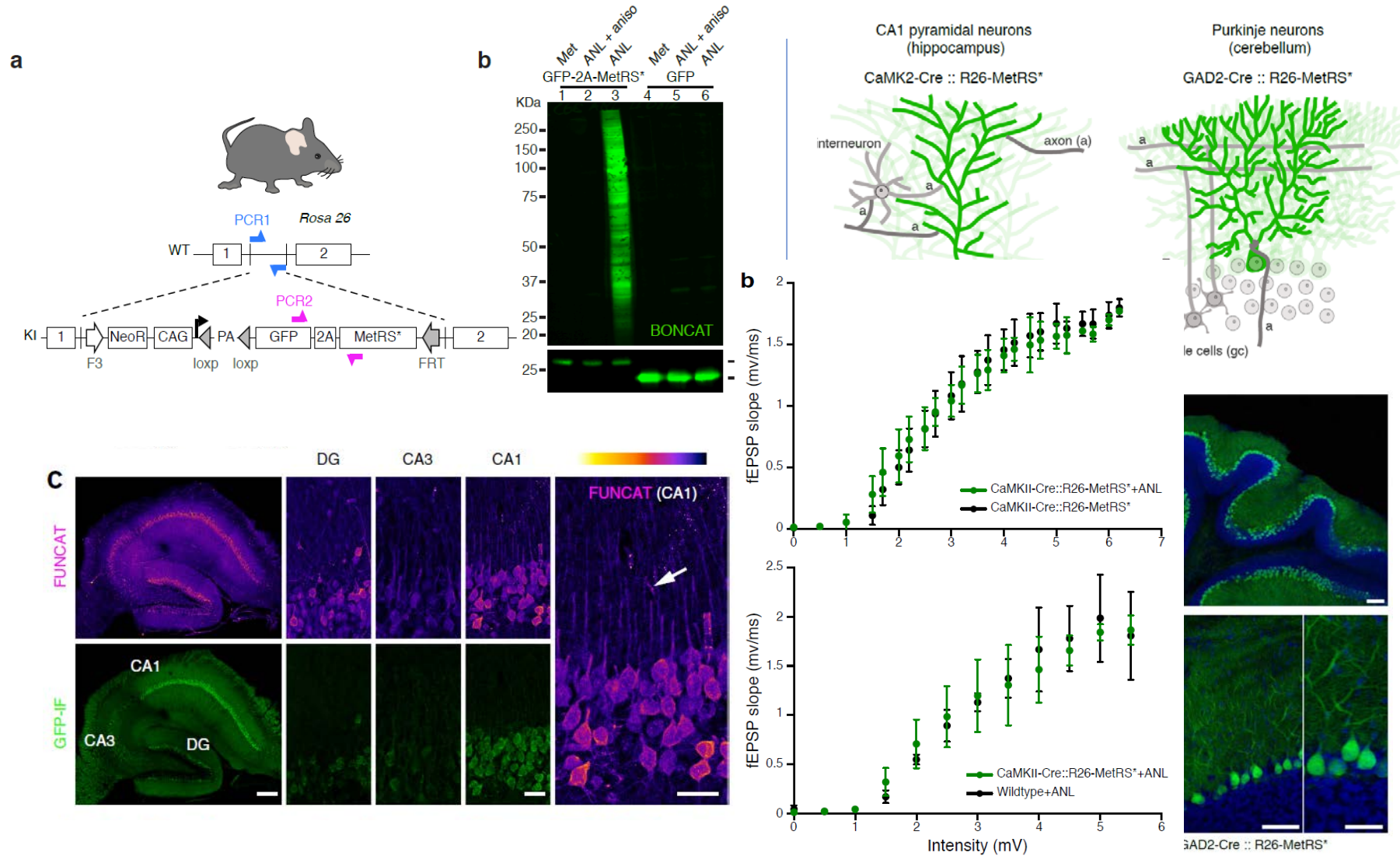
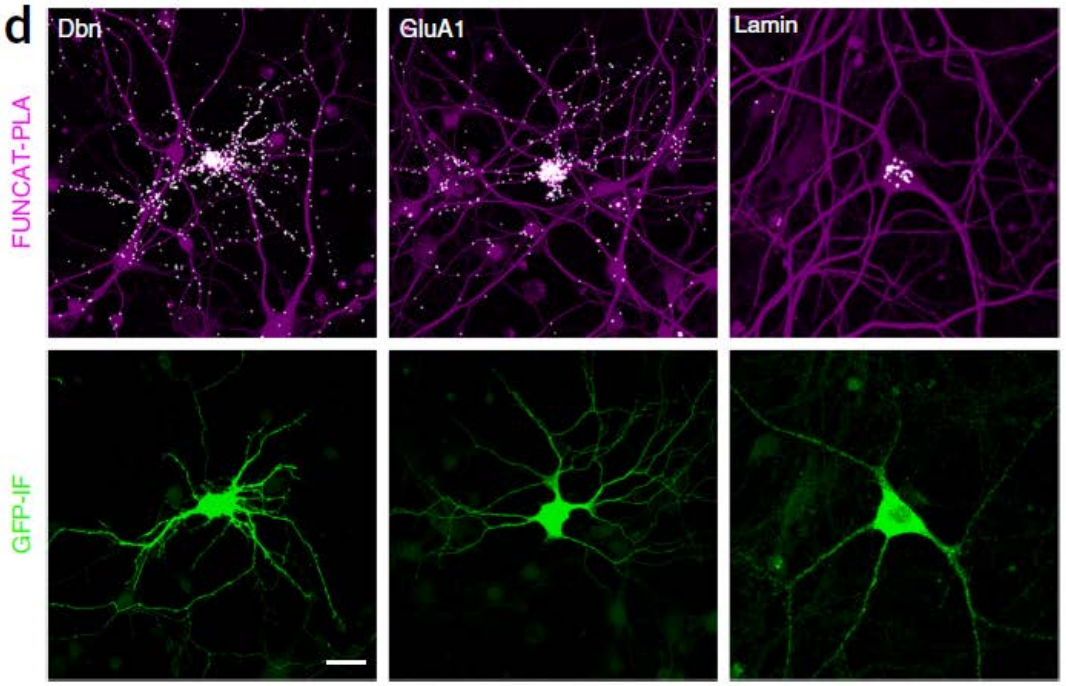
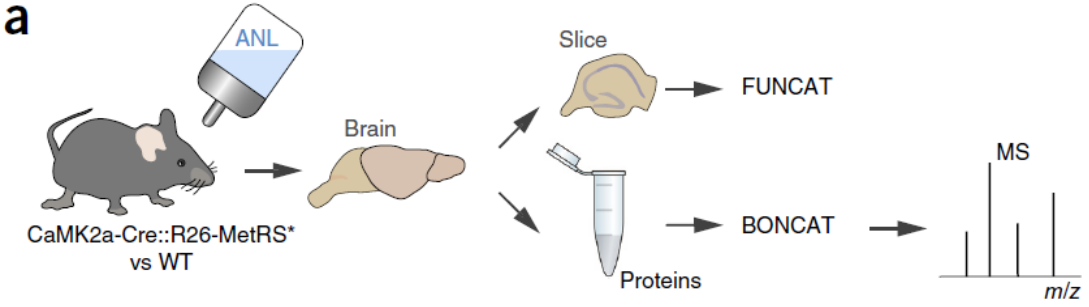
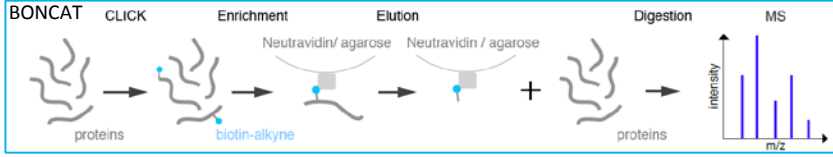


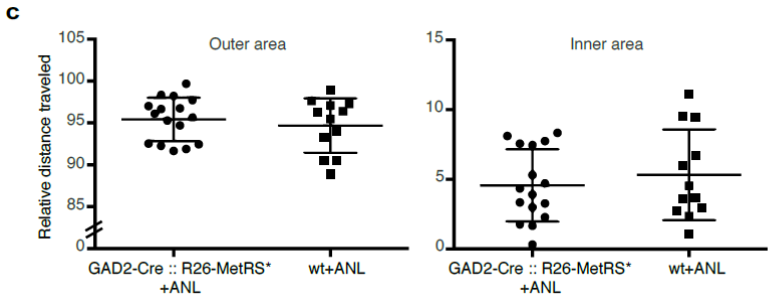
Hippocampal cultures. Protein localisation is unaltered.

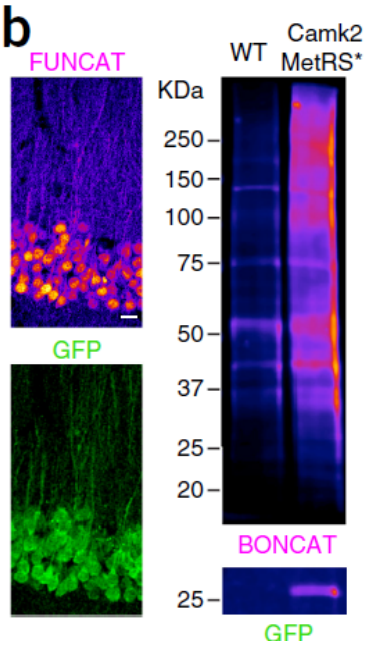


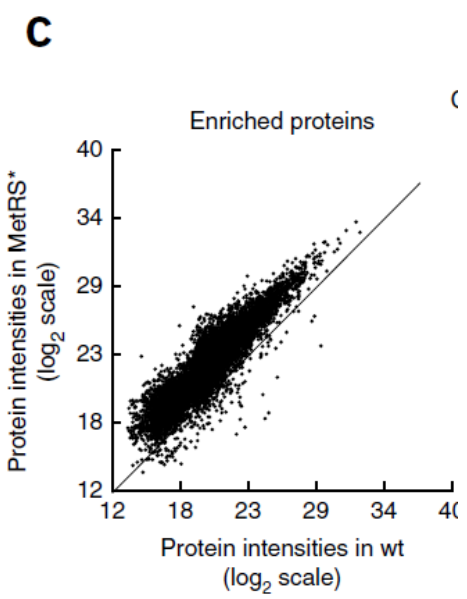




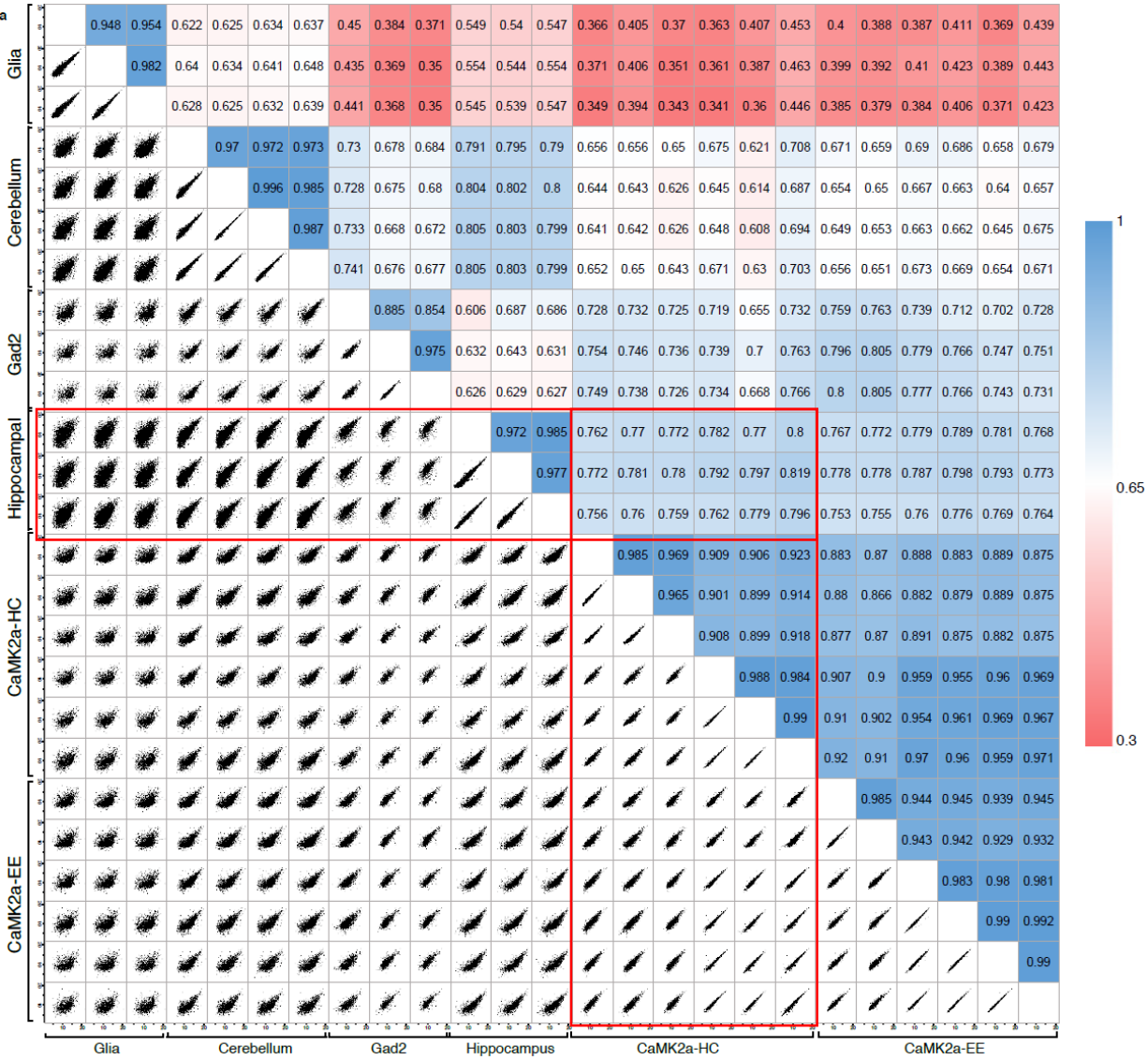
Behaviour – no difference in open field test

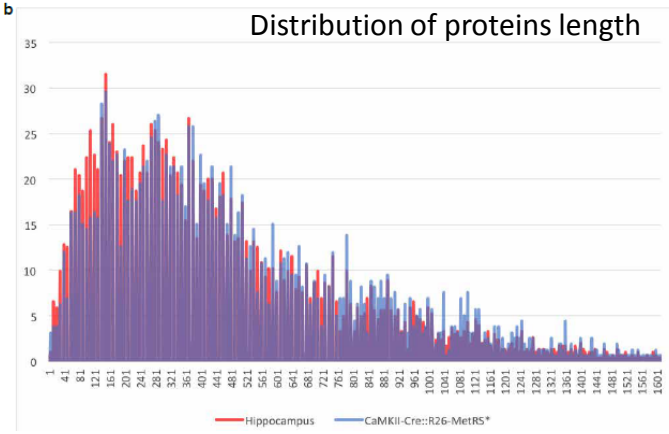


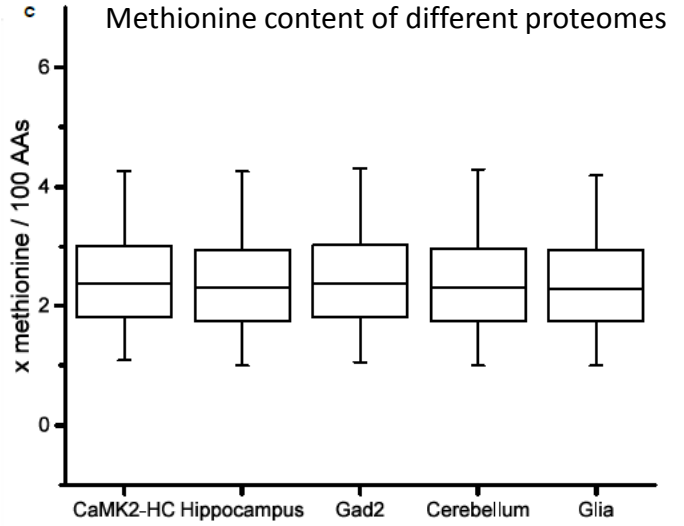




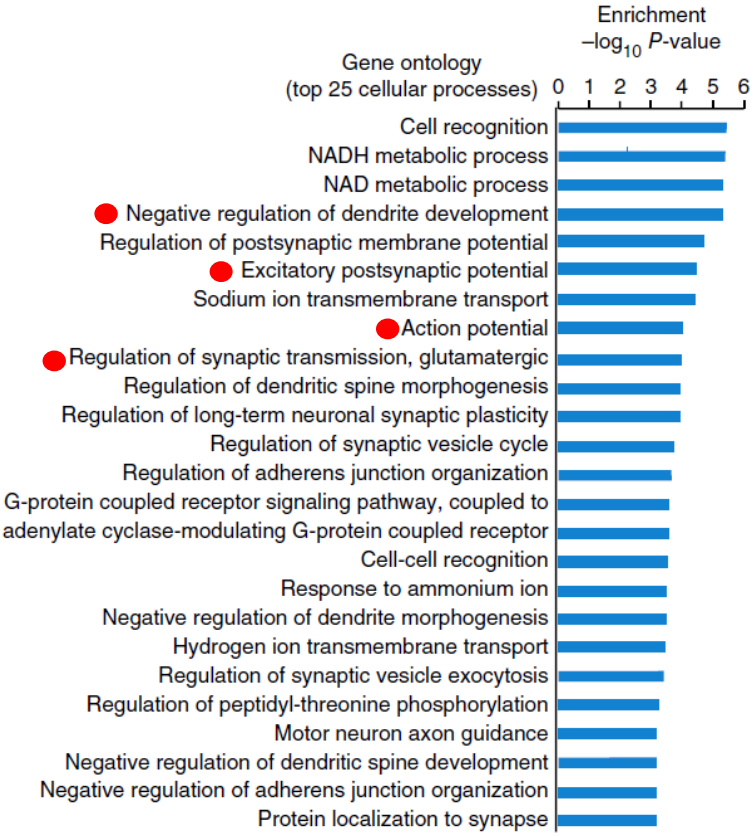
No major differences in protein expression, protein length and methionine content.

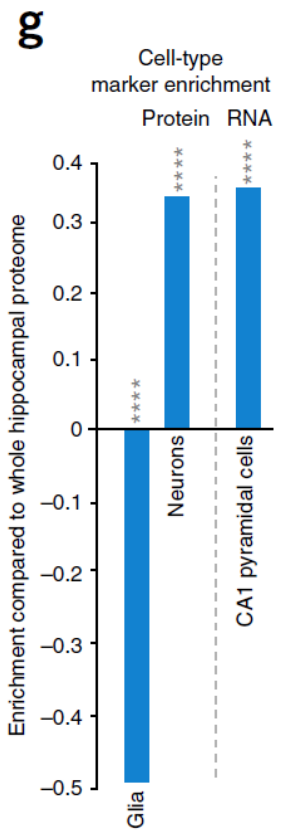


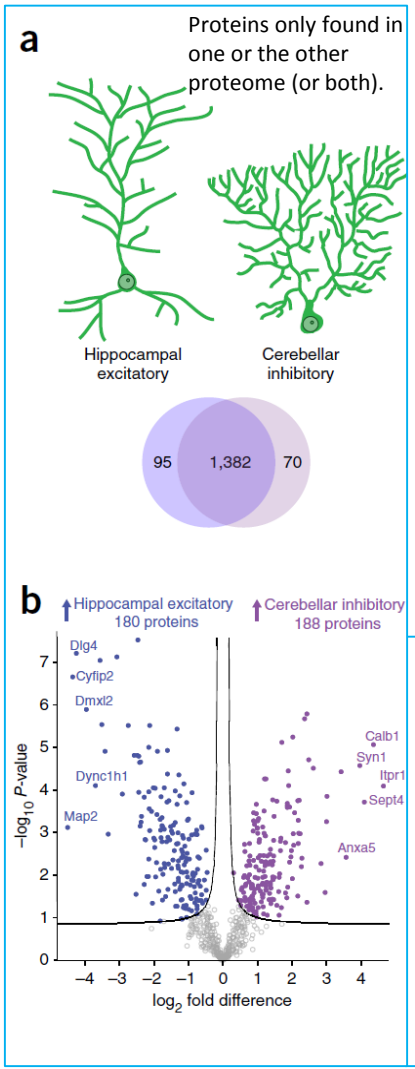


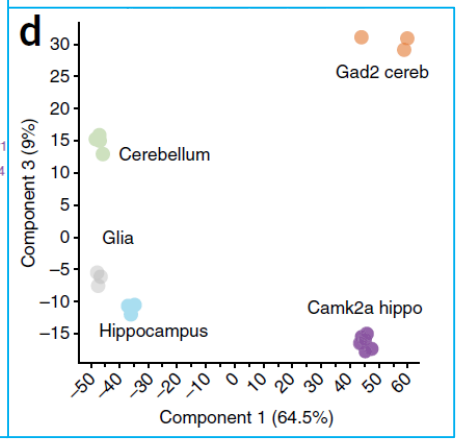


Enrichment of proteins and protein networks in excitatory hippocampal neurons compared to CTRL proteome.

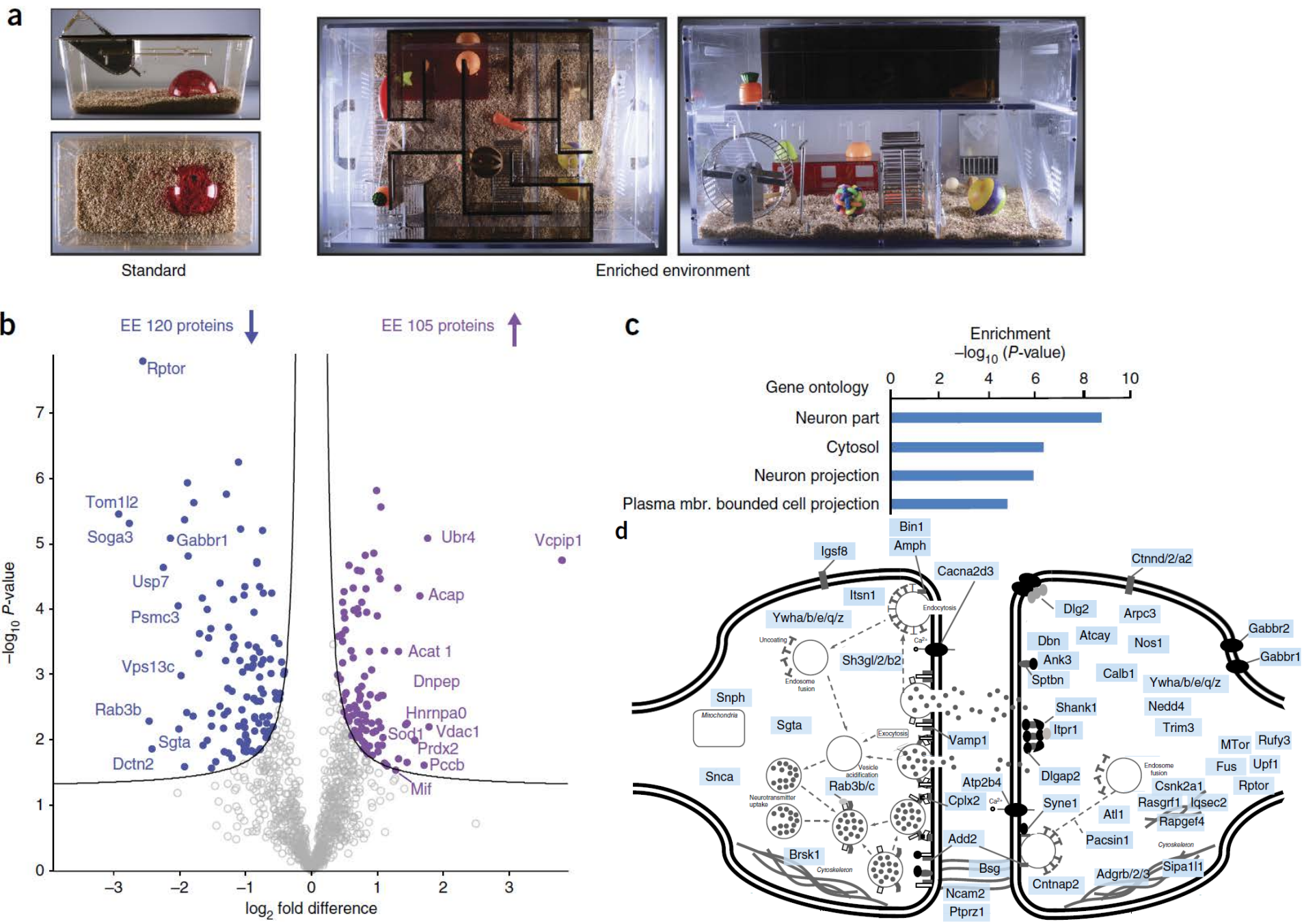








Effects of enriched environment on the proteome.



Summary

Both methods allow us to do targeted, cell-typre specific proteomics in the brain.

SORT

- Viral transduction
- Flexible in terms of disease models.
- Variability due to injections and infection rate.
- Easier to generate desired vectors.

FUNCAT/BONCAT

- Genetic labeling
- May require complex breeding.
- Less inherent variability.
- Limited to the available mice.

Shortcomings of both methods

- Samples are pooled from multiple mice.
- Both studies yield ~2000 proteins whereas the proteom contains more than that (~12.000/cell).
- The stoichiometry of labeling is not established.
  - How does variable AA intake influence ncAA incorporation and proteom labeling?
  - Optimum between labeling and proteom malfunction?

Thank you for your attention!