Molecular characterization of activated neurons by ribosome profiling

Caihong Zhu
Institute of Neuropathology, Aguzzi group
01.12.2015

Journal Club

Outline

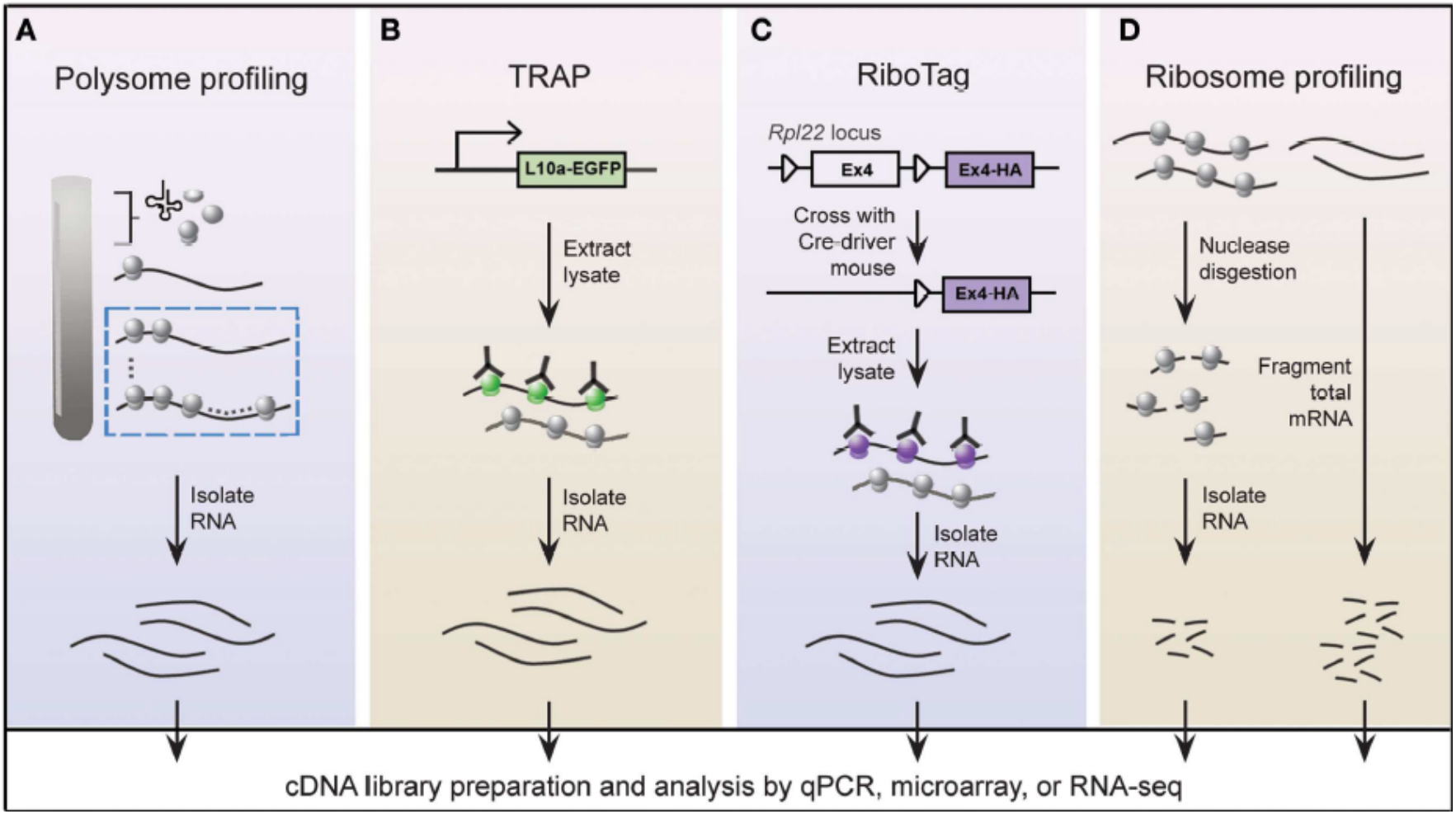
- Genome wide methods to study protein translation
- Molecular profiling of activated neurons by phosphorylated ribosome capture
- Molecular profiling of activated olfactory neurons identifies odorant receptors for odors *in vivo*

Genome wide methods to study translation

- Globally monitor gene expression: mRNA levels by microarray or RNA-seq

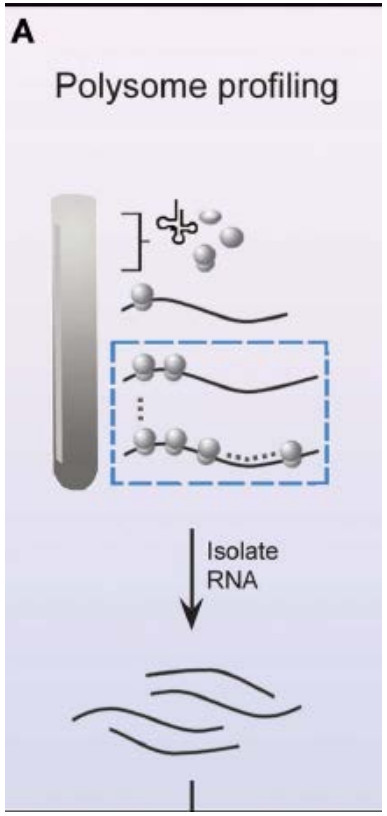
- Protein level not always correlates with mRNA level
  - mRNA regulation
  - Translation from non-AUG codons, nonsense reading-through
  - Programmed ribosome pausing

Genome wide methods to study translation



Kapeli & Yeo, Frontiers in Neuroscience, 2012

Genome wide methods to study translation

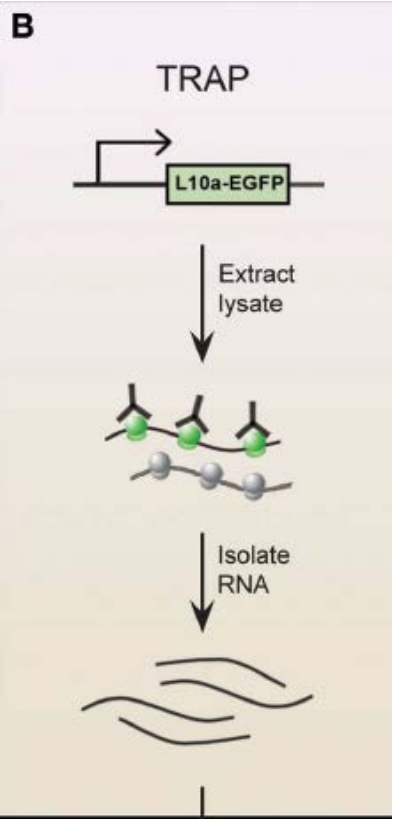


Polysome profiling: purification of polysome associated mRNA by centrifugation through sucrose gradient. Well-translated mRNA vs. Poor-translated mRNA

- **Pros:** Original method to examine translation status of transcriptome.
- **Cons:** Labor intensive; scaling issues; does not differentiate between active and stalled ribosomes; mostly used in in vitro cell culture.

Zong et al, PNAS, 1999

Genome wide methods to study translation

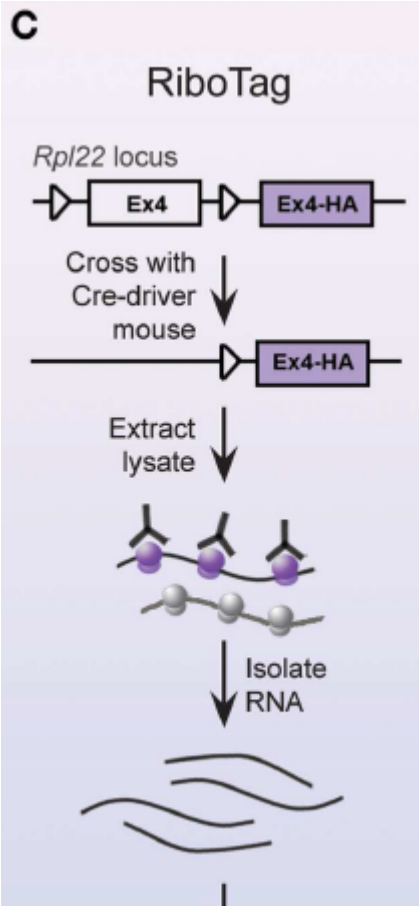


TRAP (translating ribosome affinity purification): BAC transgenic mice expressing EGFP fused with ribosome protein L10 under various promoters, IP of EGFP-L10a associated mRNA.

- **Pros:** Examines ribosome-associated mRNAs within a specific cell type in vivo
- **Cons:** Each BAC TRAP mouse line is limited to studying one cell type; transgenic overexpression of EGFP-L10a; EGFP antibody; does not differentiate between active and stalled ribosomes.

Heiman et al, Cell, 2008; Doyle et al, Cell, 2008

Genome wide methods to study translation



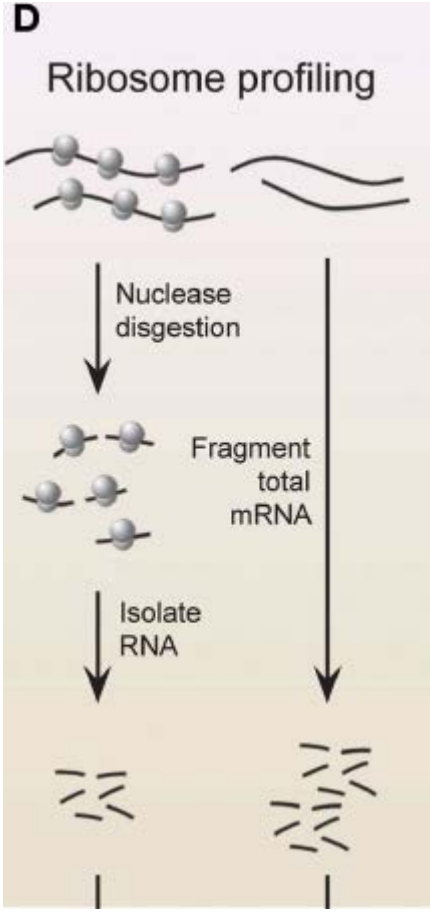
Improved TRAP methods

- Cre-dependent expression of an EGFPtagged ribosomal protein construct (EEF1A1-LSL.EGFPL10)
- RiboTag: Knock-in a Cre-dependent expression cassette of fused Rpl22-HA protein into Rpl22 locus
- Retro-TRAP: aGFP nanobody-Rpl10 + GFP expression

- **Pros:** Expand the range of cell type to be investigated in vivo
- **Cons:** Do not differentiate between active and stalled ribosomes

Sanz et al, PNAS, 2008; Stanley et al, Cell, 2013; Ekstrand et al, Cell, 2014

Genome wide methods to study translation



Ribosome profiling: nuclease digestion of ribosome-mRNA complex, followed by centrifugation through sucrose gradient or cushion to purify ribosome protected mRNA

- **Pros:** Determine ribosome position and translation efficiency for individual mRNA; reveal novel translational regulatory features (e.g uORF, start and termination sites, ribosome stall)
- **Cons:** Is challenging to apply to in vivo

Ingolia et al, Science, 2009; Guo et al, Nature, 2010; Ingolia et al, Cell, 2011

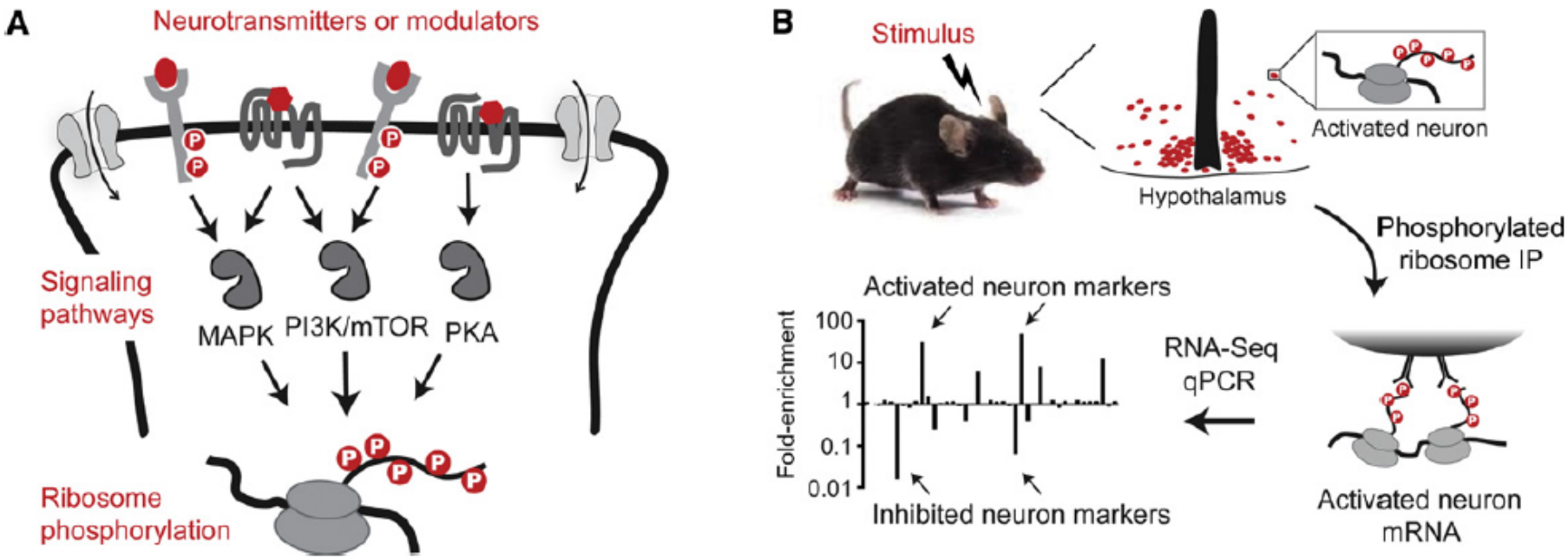
Molecular Profiling of Activated Neurons by Phosphorylated Ribosome Capture

Zachary A. Knight,[1,*] Keith Tan,[1] Kivanc Birsoy,[1] Sarah Schmidt,[1] Jennifer L. Garrison,[1] Robert W. Wysocki,[1] Ana Emiliano,[1] Mats I. Ekstrand,[1] and Jeffrey M. Friedman[1,*]
[1]Laboratory of Molecular Genetics, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

- Goal: To systemically identify functional populations of neurons that control behavior (eg. cellular components of neural circuit that controls feeding in the hypothalamus)

- Method: phosphorylated ribosome capture and profiling

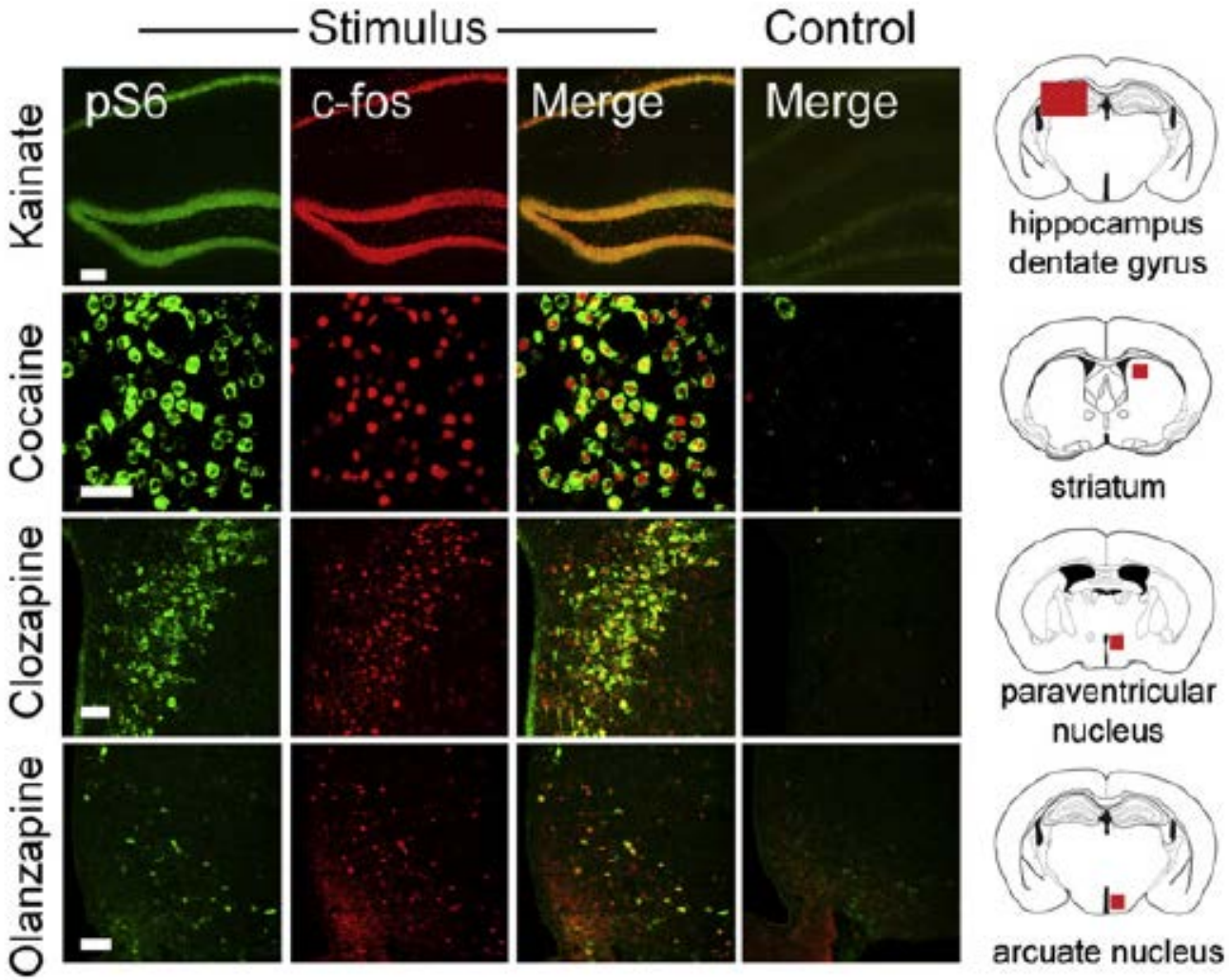
Phosphorylated ribosome profiling



A: Ribosome protein S6 is a common target of a set of core pathways induced by neurotransmitters or modulators and is phosphorylated upon these stimulation

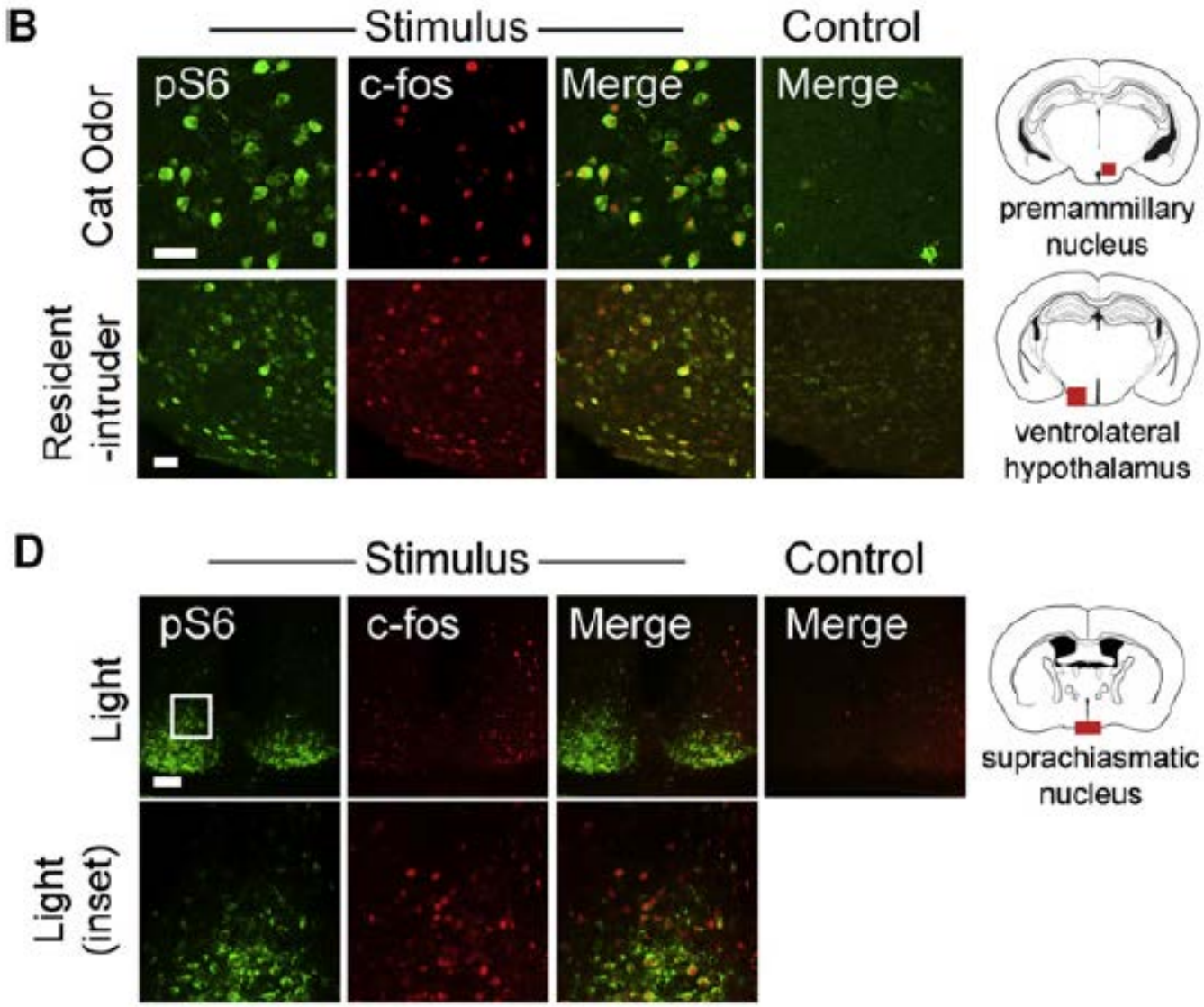
B: Strategy of phosphorylated ribosome profiling in vivo

Colocalization of pS6 and c-fos following diverse stimuli

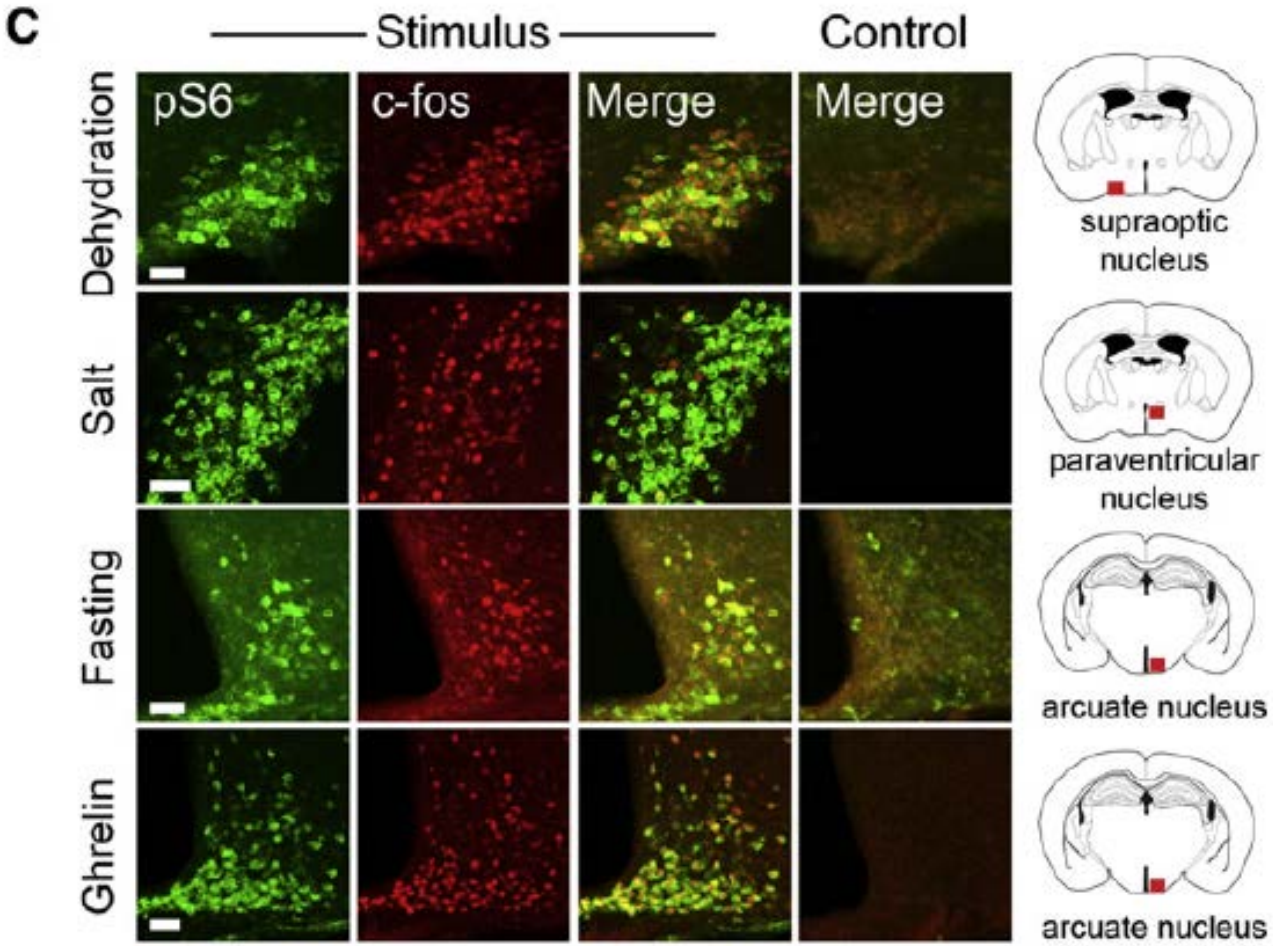


C-fos is a immediate early gene widely used to visualize the neurons that respond to numerous stimuli

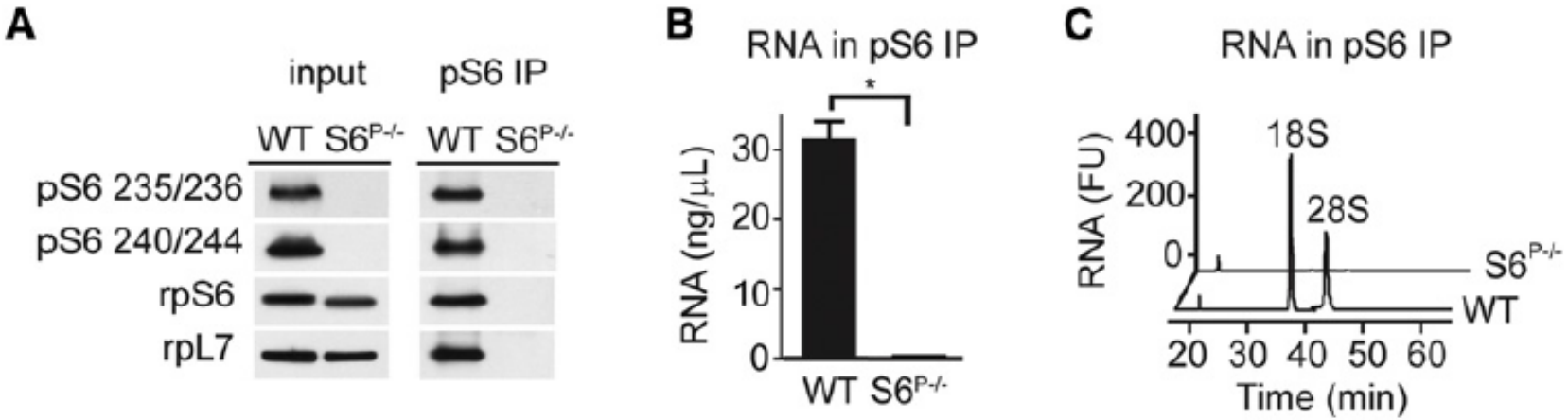
Colocalization of pS6 and c-fos following diverse stimuli



Colocalization of pS6 and c-fos following diverse stimuli

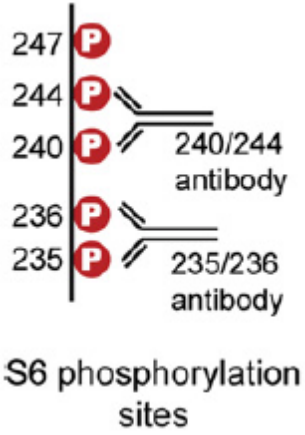


Selective capture of phosphorylated ribosome *in vitro*

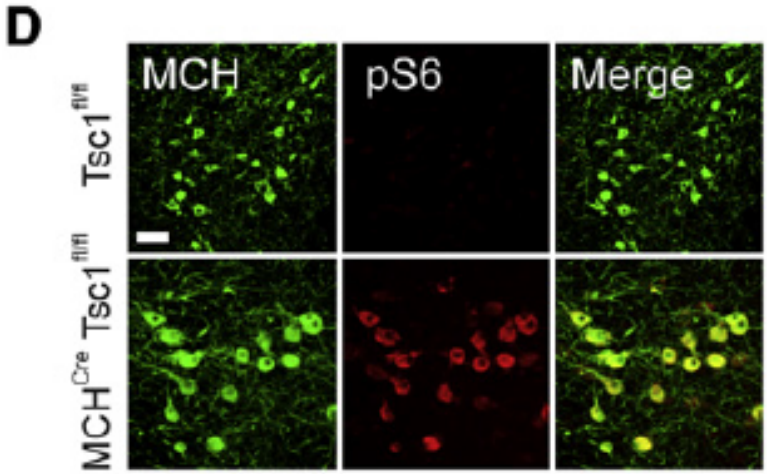


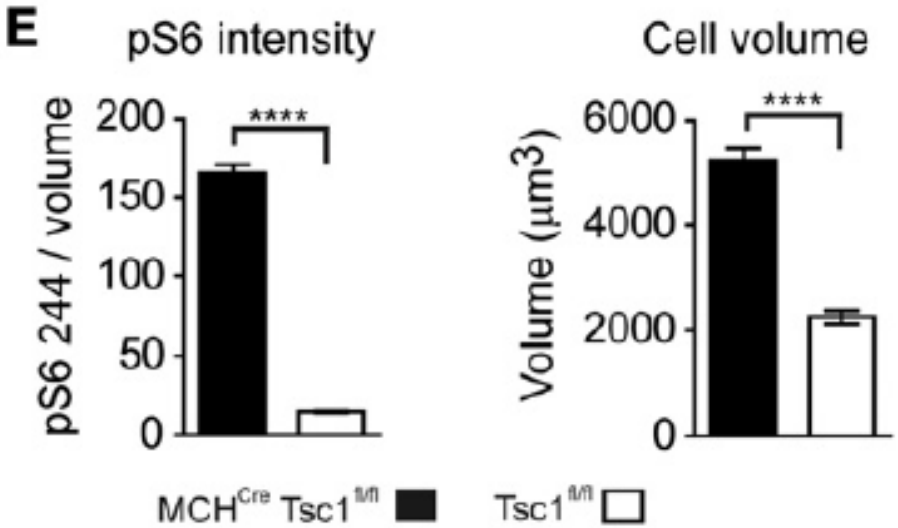
ip by pS6 240/244 antibody

- Mouse embryonic fibroblasts (MEF)
- 5 phosphorylation sites on S6: Ser 235, 236, 240, 244, 247
- $S6^{P-/-}$: 5 serine sites are mutated to alanine
- 100-fold more RNA was isolated in pS6 ip from WT MEF compared to $S6^{P-/-}$ controls.



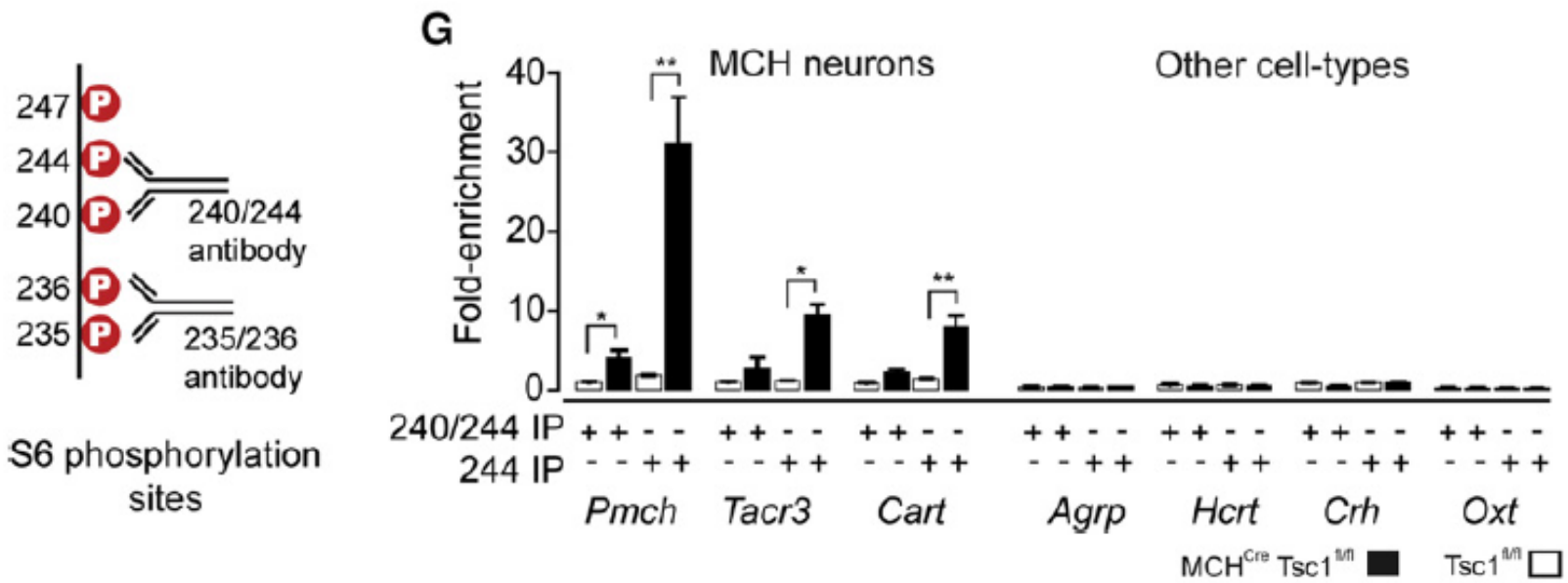
Selective capture of phosphorylated ribosome *in vivo*





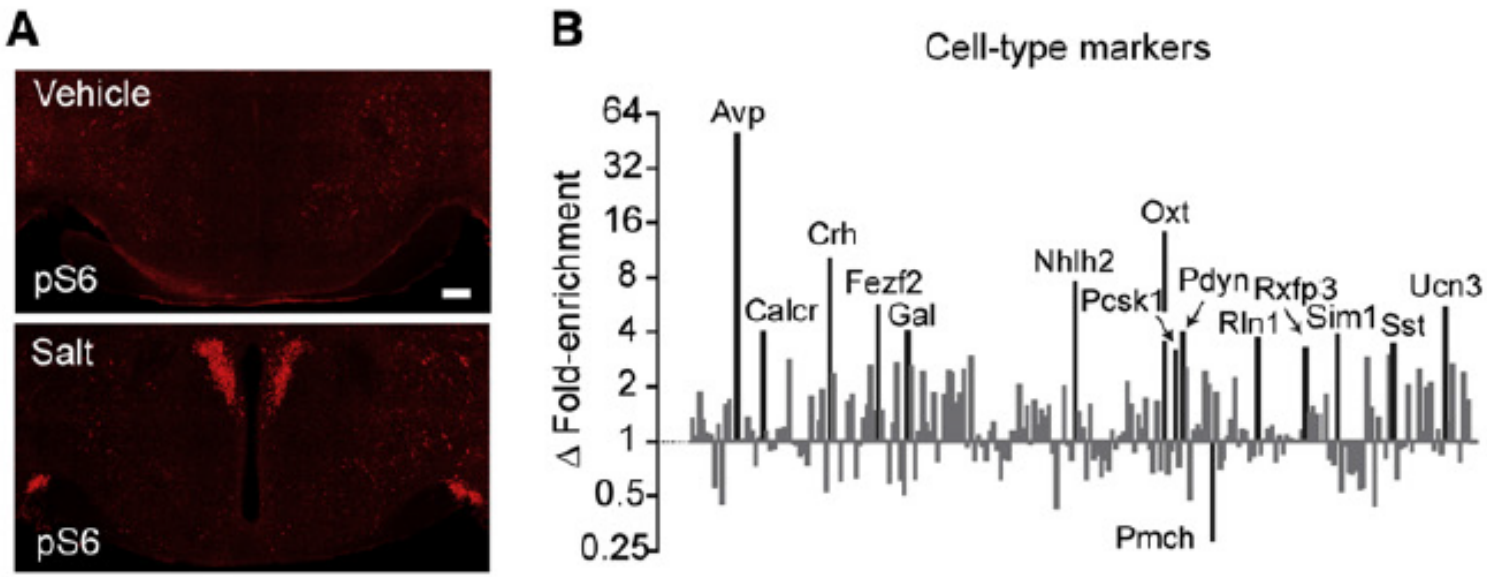
- Melanin-concentrating hormone (MCH) neurons
- Deletion of Tsc1 (tuberous sclerosis 1) activates the mTORC1 pathway, resulting in constitutive S6 phosphorylation and increased cell size
- Specifically deletion of Tsc1 in MCH neurons

Selective capture of phosphorylated ribosome *in vivo*



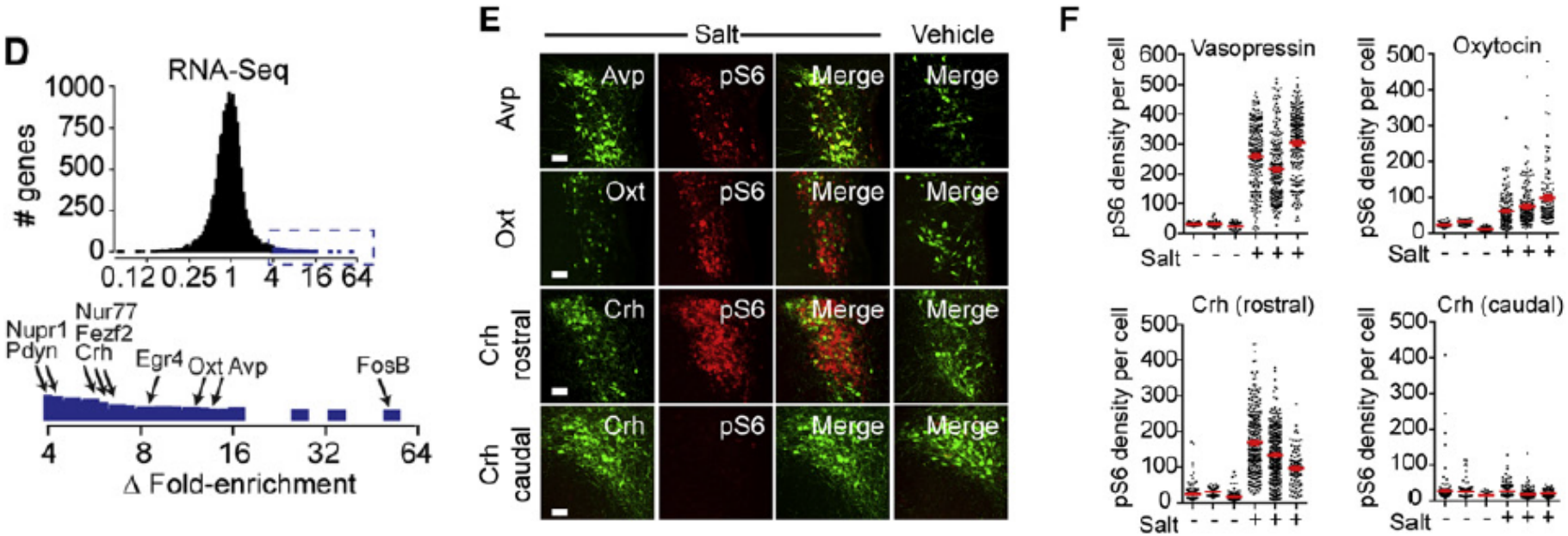
- Polyclonal Ab $pS6^{240/244}$ enriched Pmch mRNA by 4-fold (ip/input)
- Phosphorylation of S6 occurs sequentially in an order 236-235-240-244-247. Ab against C-terminal may exhibit wider dynamic range in response to stimuli and enable greater enrichment
- Preincubation of $pS6^{240/244}$ Ab with a phosphopeptide containing $pS6^{240}$ phosphorylation site, yielding Ab that recognize only $pS6^{244}$ and resulting >30-fold enrichment for Pmch

Molecular identification of hypothalamic neurons activated by salt



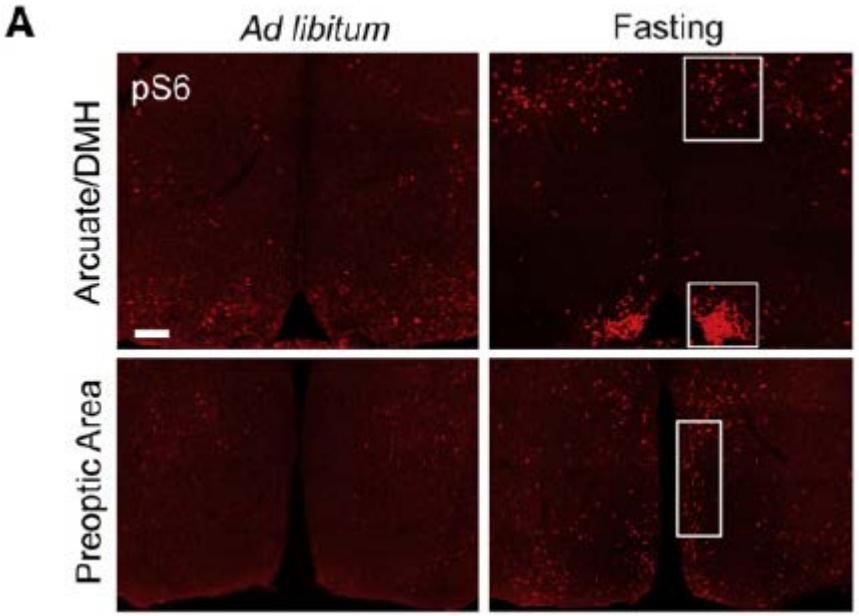
- Salt challenge induced a dramatic increase of pS6 in regions of the hypothalamus that mediate osmoregulation
- Custom array of 225 Taqman probe: fold change(ip/input) of salt challenged animals /fold change(ip/iput) of control animals
- Known markers of neurons respond to salt challenge were highly enriched: vasopressin (Avp), oxytocin (Oxt), corticotrophin-releasing hormone (Crh).
- Novel markers: relaxin-1 (Rln1), Urocortin 3(Ucn3), somatostatin (Sst)

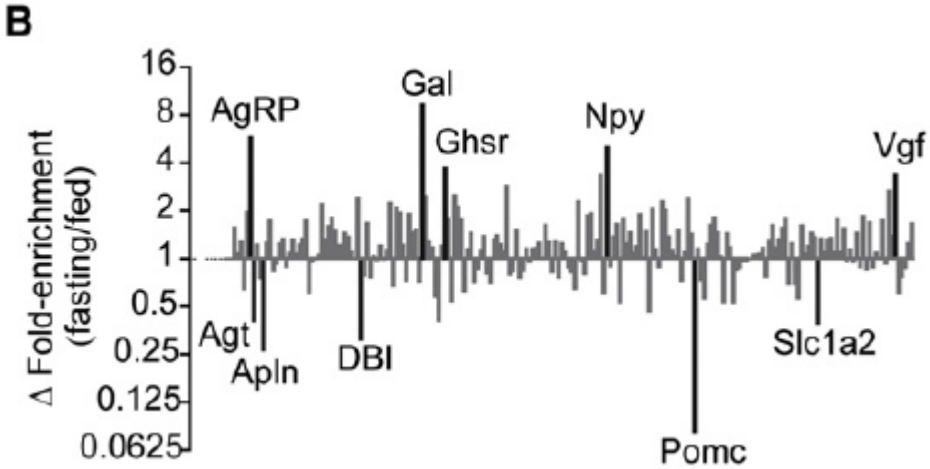
Molecular identification of hypothalamic neurons activated by salt



- RNA-seq of differential enrichment of cell-type-specific genes in pS6 ip, but not after total ribosome ip
- Colocalization between Avp, Oxt and Crh with pS6 in salt-treated and control animals

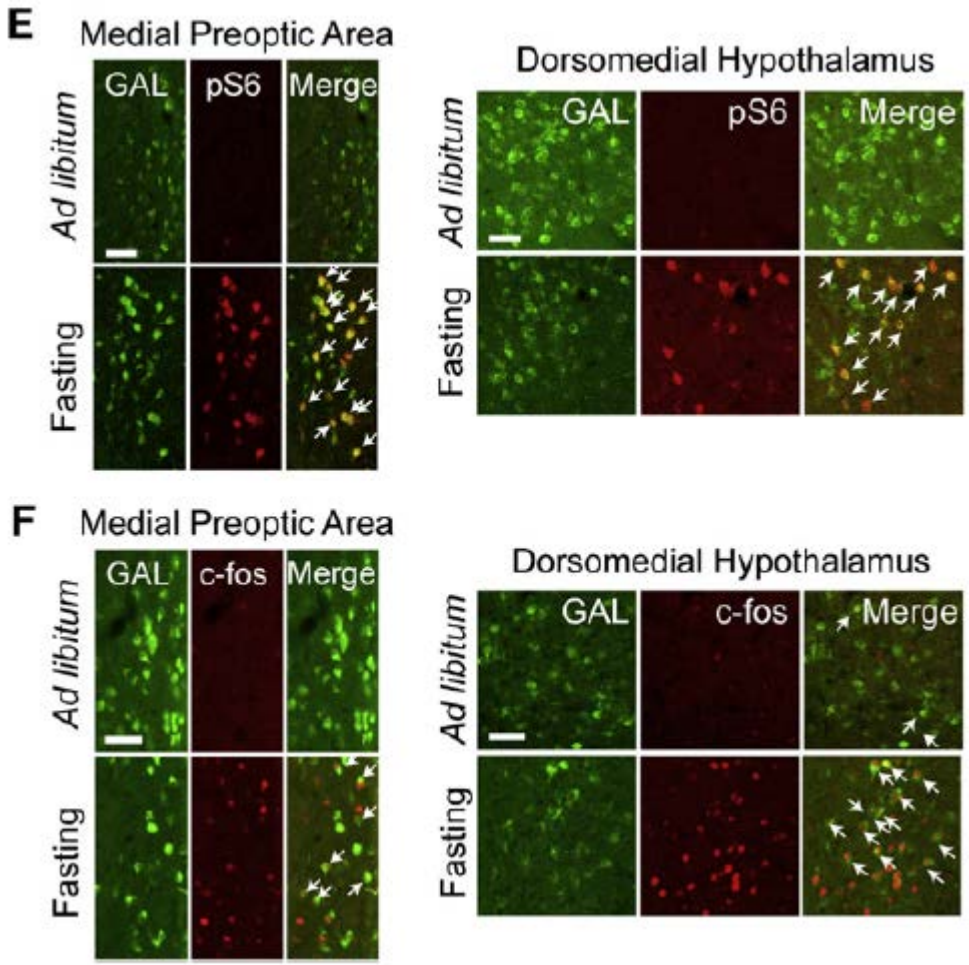
Molecular identification of hypothalamic response to fasting





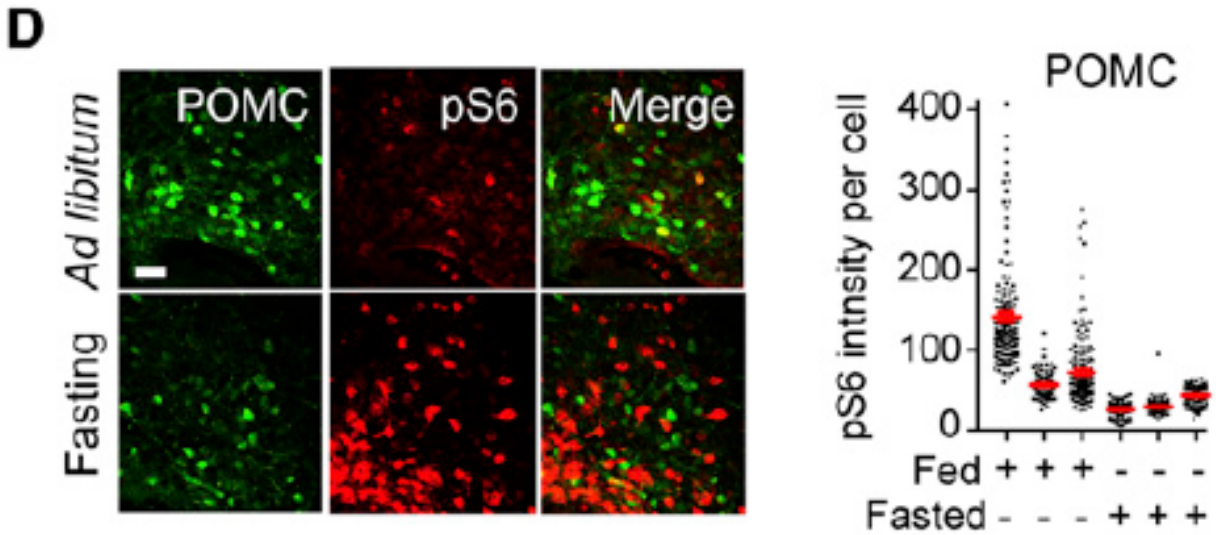
- Fasting (o.n) induced a strong increase of pS6 in arcuate nucleis and dorsomedial hypothalamus (DMH), as well as in preoptic area
- Fold change(ip/input) of salt challenged animals /fold change(ip/iput) of control animals
- Markers of neurons respond to fasting were highly enriched: Agrp, Npy, ghrelin receptor (Ghsr) and Vgf. These neuropeptides are known to promoter food uptake.

Molecular identification of hypothalamic response to fasting



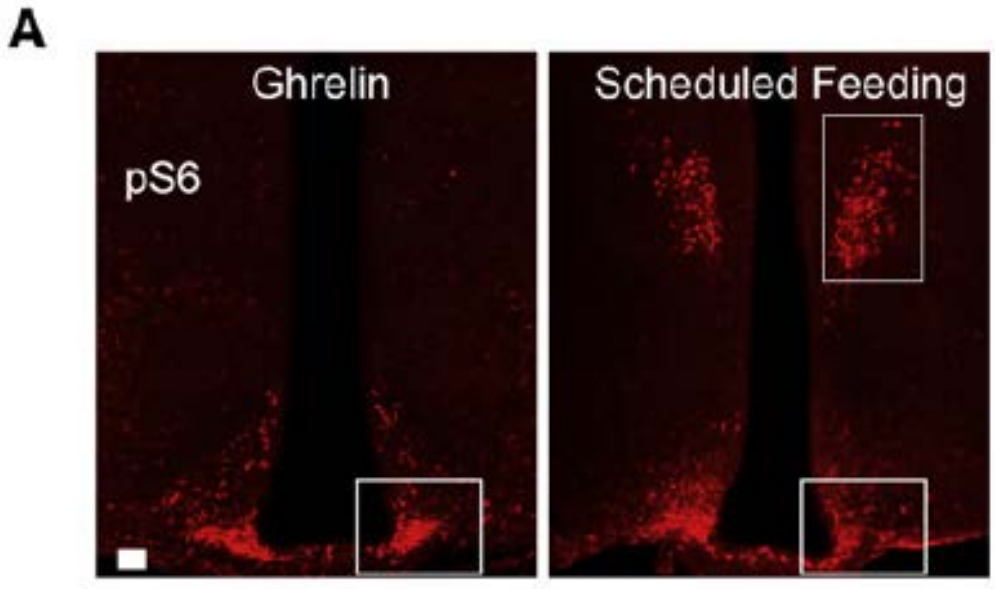
- Galanin (Gal) is also enriched in medial preoptic area (MPA) and dorsomedial hypothalamus (DMH) in pS6 ip after fasting
- I.c injection of galanin has been shown to stimulate feeding.
- New: Regulation of Gal neurons by changes in nutritional state

Molecular identification of hypothalamic response to fasting



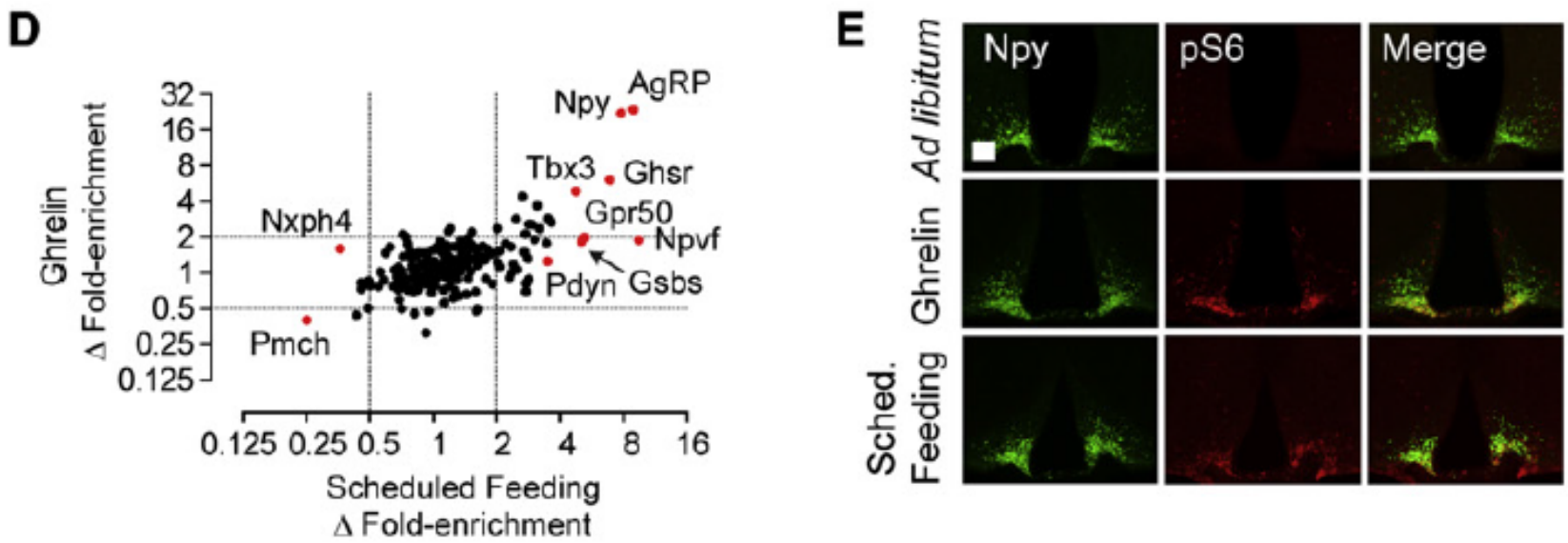
- POMC neuropeptide has been shown to inhibit food take, Pomc expression in downregulated during food deprivation
- POMC neurons were inhibited by fasting

Molecular identification of hypothalamic response to scheduled feeding/ghrelin treatment



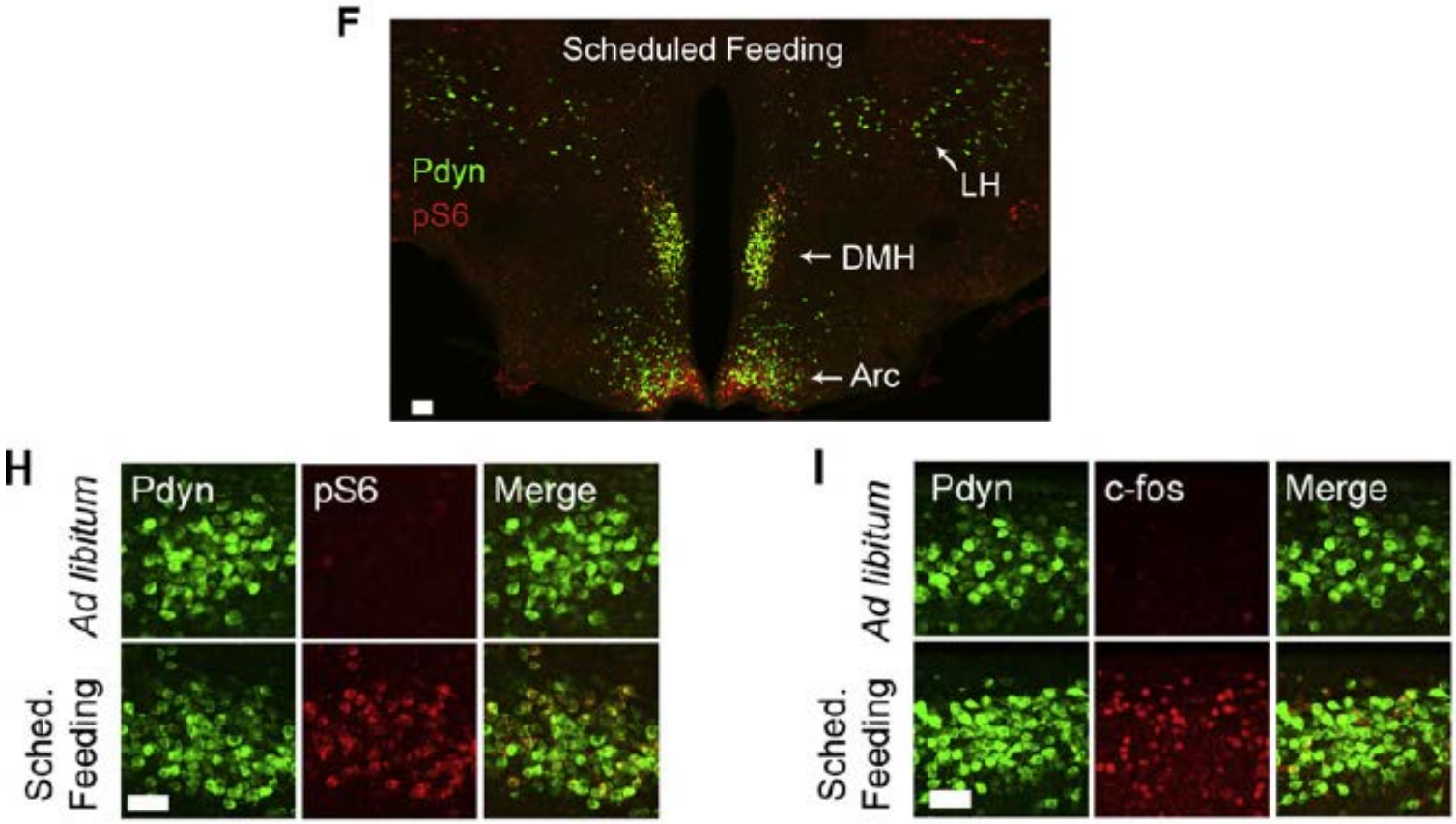
- Food access within 3-hour window (CT4-CT7) for 10 days
- Brain collected at CT6, after food presentation at CT4
- Ghrelin: increases prior meal, i.p injection, analyse 1 hour after injection
- Scheduled feeding induced intense pS6 in dorsomedial hypothalamus (DMH) and Arcuate nucleis, peaked within the meal window and decline thereafter
- Ghrelin injection induced pS6 in Arcuate nucleis, but not in DMH

Molecular identification of hypothalamic response to scheduled feeding/ghrelin treatment



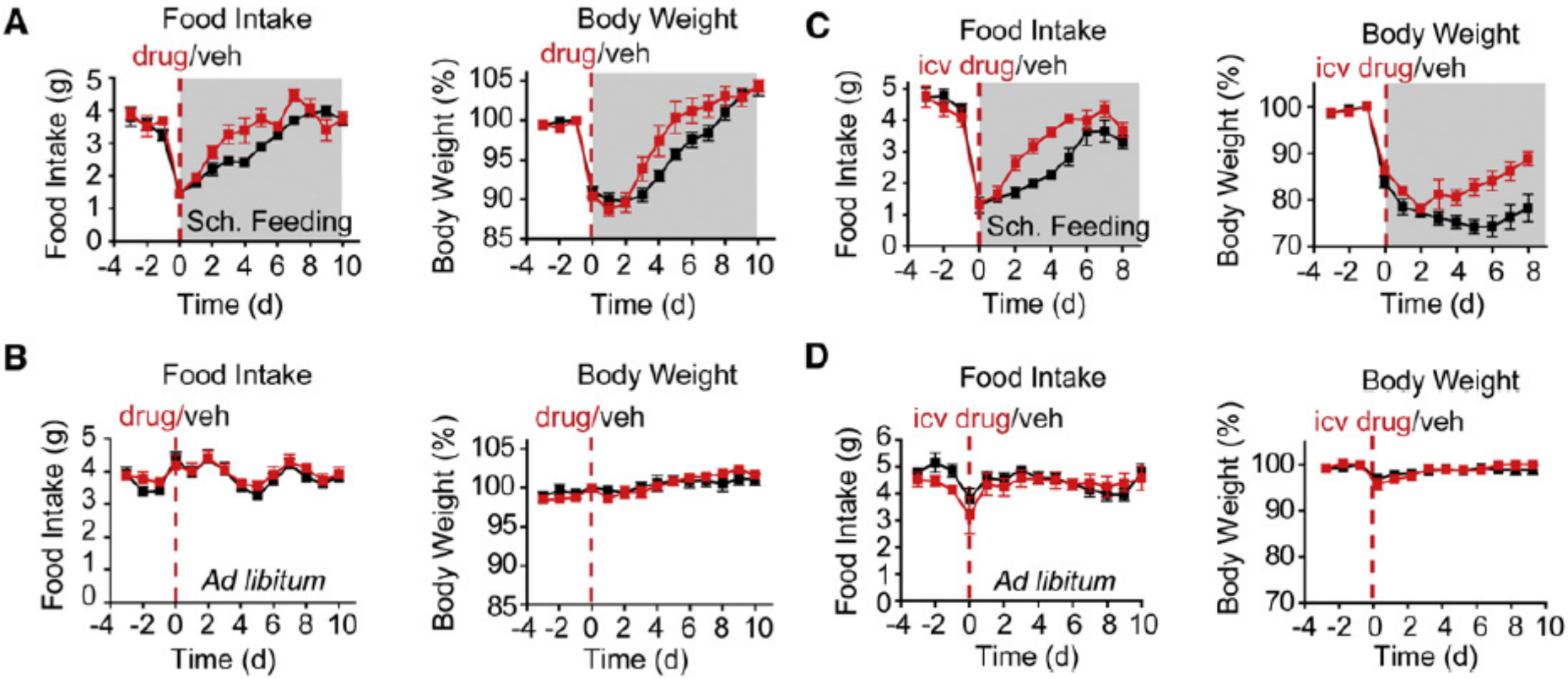
- Fold change(ip/input) of SF or ghrelin treated animals /fold change(ip/iput) of control animals
- SF and ghrelin both activated AgRP, Npy, Ghsr neurons
- Specific markers of neurons respond to SF, but not to ghrelin, highly enriched: prodynorphin(Pdyn), Gpr50, Npvf and Gsbs

Molecular identification of hypothalamic response to scheduled feeding/ghrelin treatment



- Specific markers of neurons respond to SF highly enriched: prodynorphin(Pdyn), Gpr50, Npvf and Gsbs
- Pdyn signals by activating k-opioid receptor (KOR)

KOR signaling restrains food intake during scheduled feeding



- Antagonizing KOR signaling by i.p JDTic or i.cv norbinaltorphomine impaired meal termination in scheduled feeding animals

Conclusion

- S6 phosphorylation can be used as a tag to capture ribosome in activated neurons
- pS6 tagged ribosome can be used to capture activated neurons by various stimuli, identified Gal neurons in fasting, Pdyn neurons in scheduled feeding
- pS6 in other cells: immune system, lung, intestine or kidney
- !! c-fos or pS6 do not retrieve markers for all activated neurons!

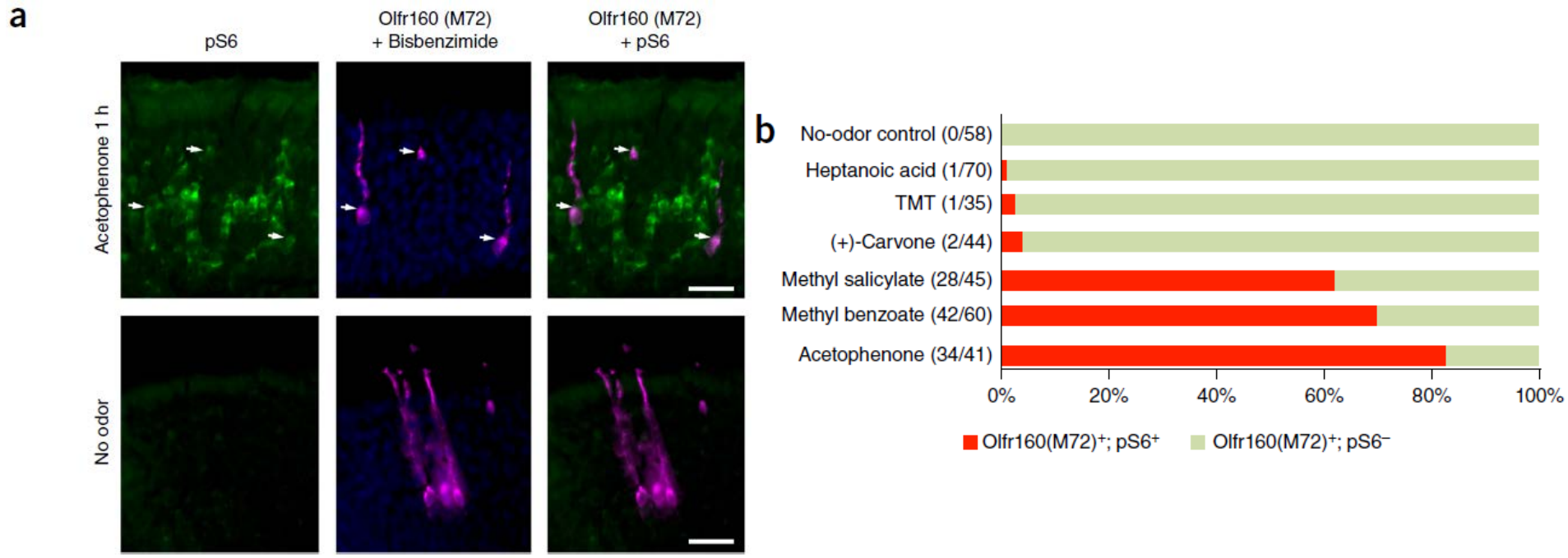
Molecular profiling of activated olfactory neurons identifies odorant receptors for odors *in vivo*

Yue Jiang[1–3], Naihua Natalie Gong[1], Xiaoyang Serene Hu[1], Mengjue Jessica Ni[1], Radhika Pasi[1] & Hiroaki Matsunami[1,4]

- Goal: Comprehensive mapping odorant receptors (ORs) and corresponding odors

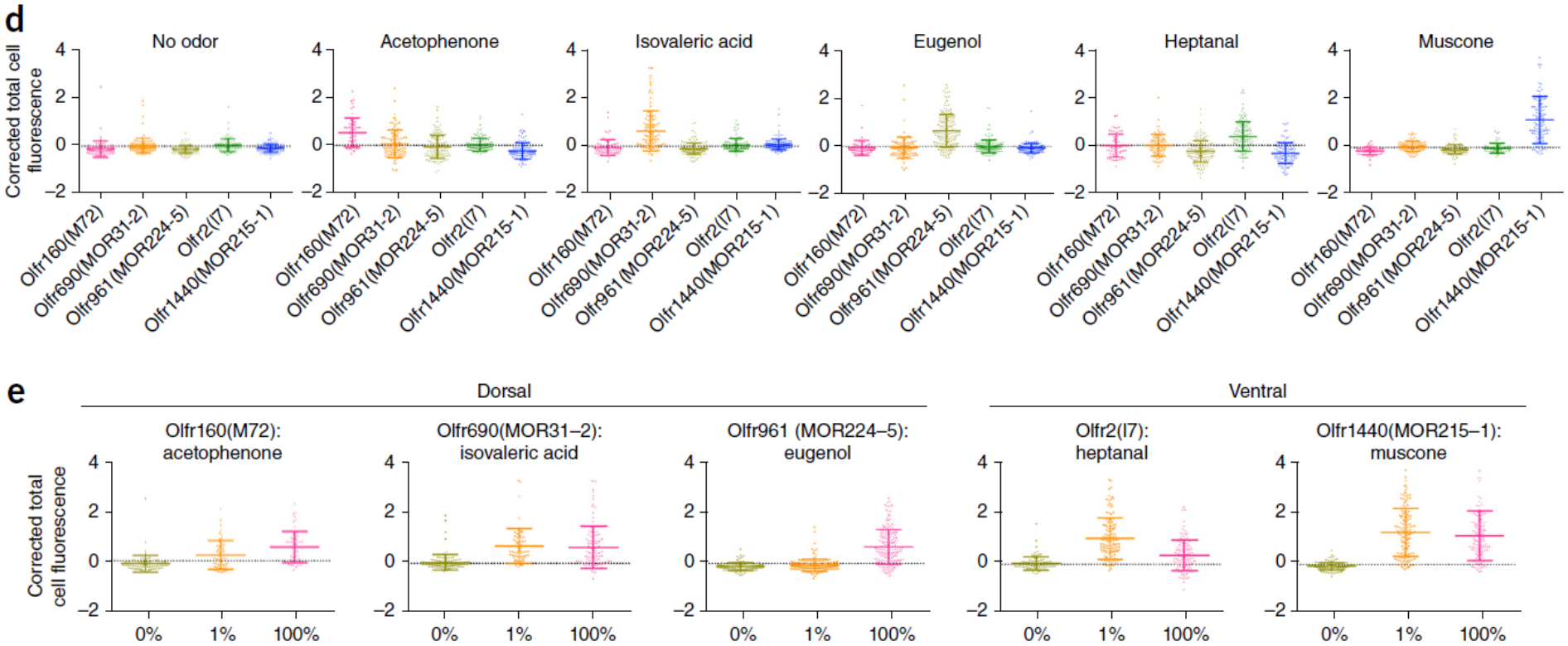
- Method: phosphorylated ribosome immunoprecipitation and profiling

Odor exposure leads to S6 phosphorylation in olfactory sensory neurons (OSNs) in olfactory epithelium (OE)



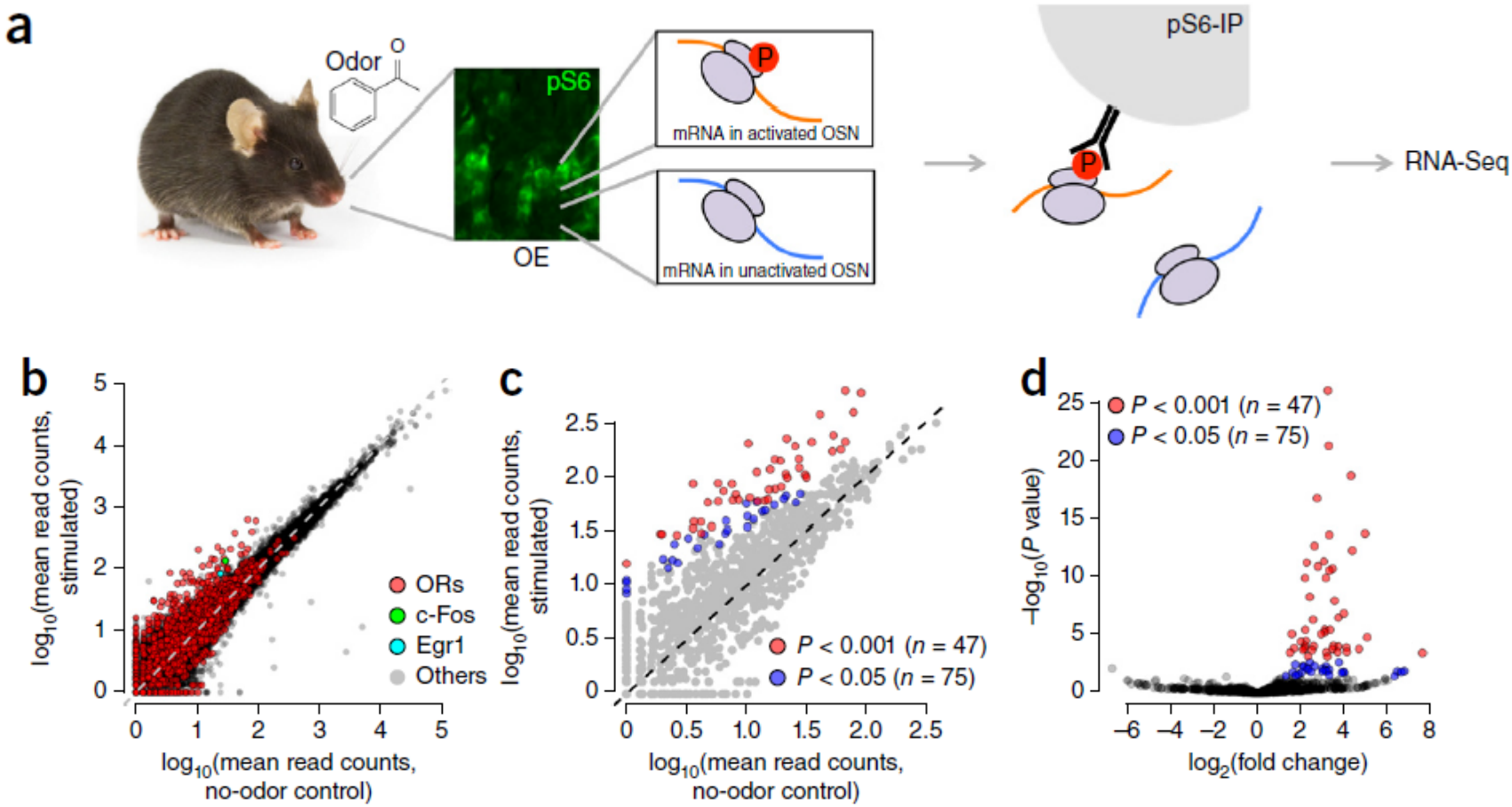
- Olfr160: known receptor for acetophenone
- Olfr160 agonist: methyl salicylate, methyl benzoate, acetophenone

Odor exposure leads to S6 phosphorylation in olfactory sensory neurons (OSNs) in olfactory epithelium (OE)



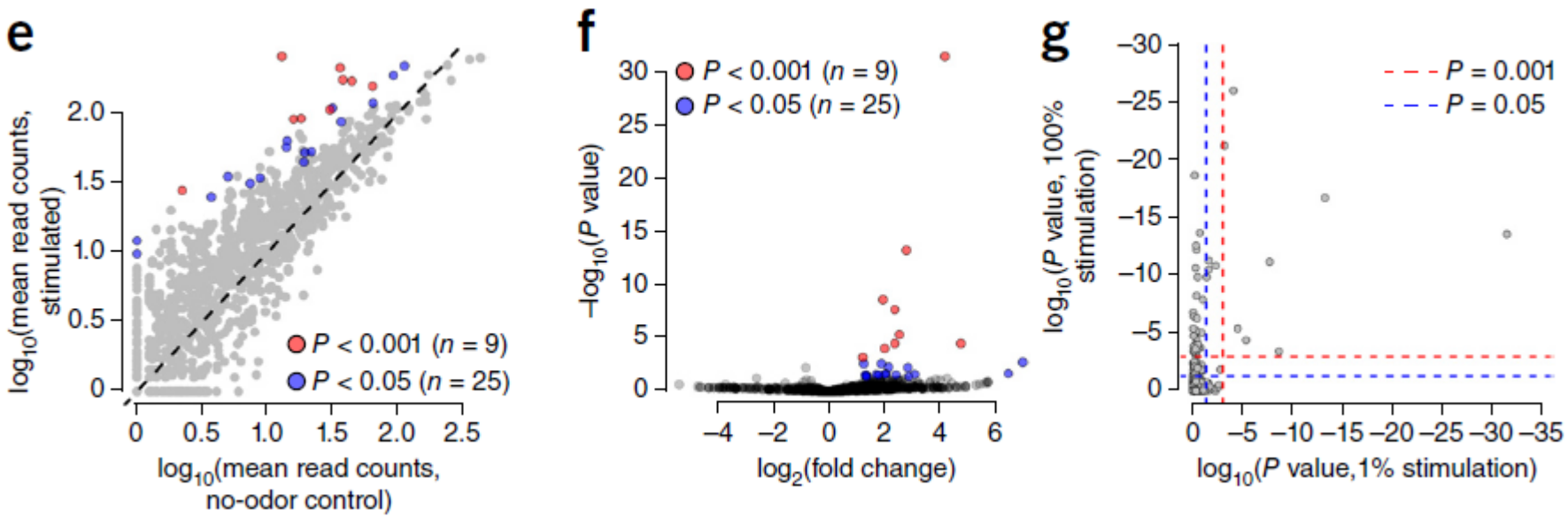
- Different odorant-odorant receptor pairs induced pS6
- Induction of pS6 is dose-dependent

pS6 ip enriches OR mRNAs from odor stimulated OE



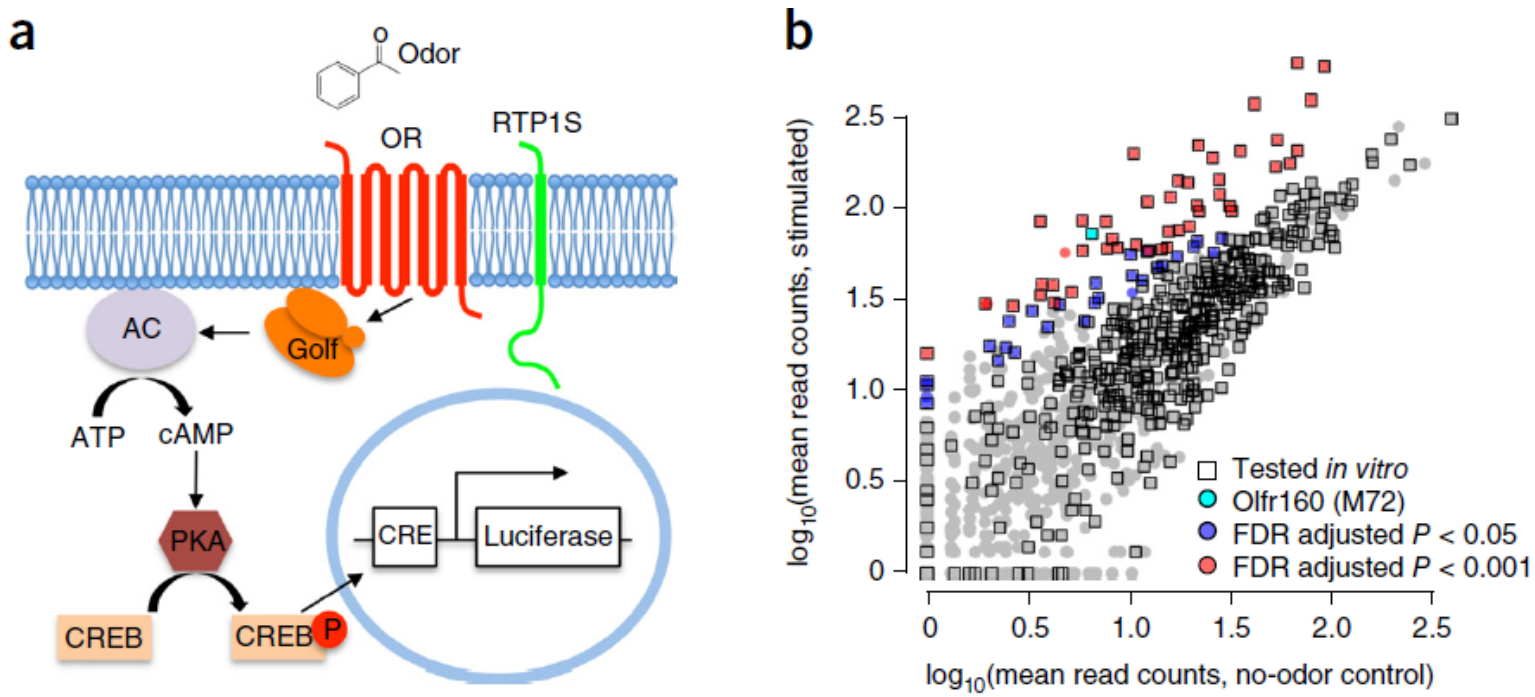
- RNA-seq read counts odor stimulated samples/unstimulated samples
- 75 ORs were enriched ($p<0.05$) in stimulated group (100% acetophenone)

pS6 ip enriches OR mRNAs from odor stimulated OE



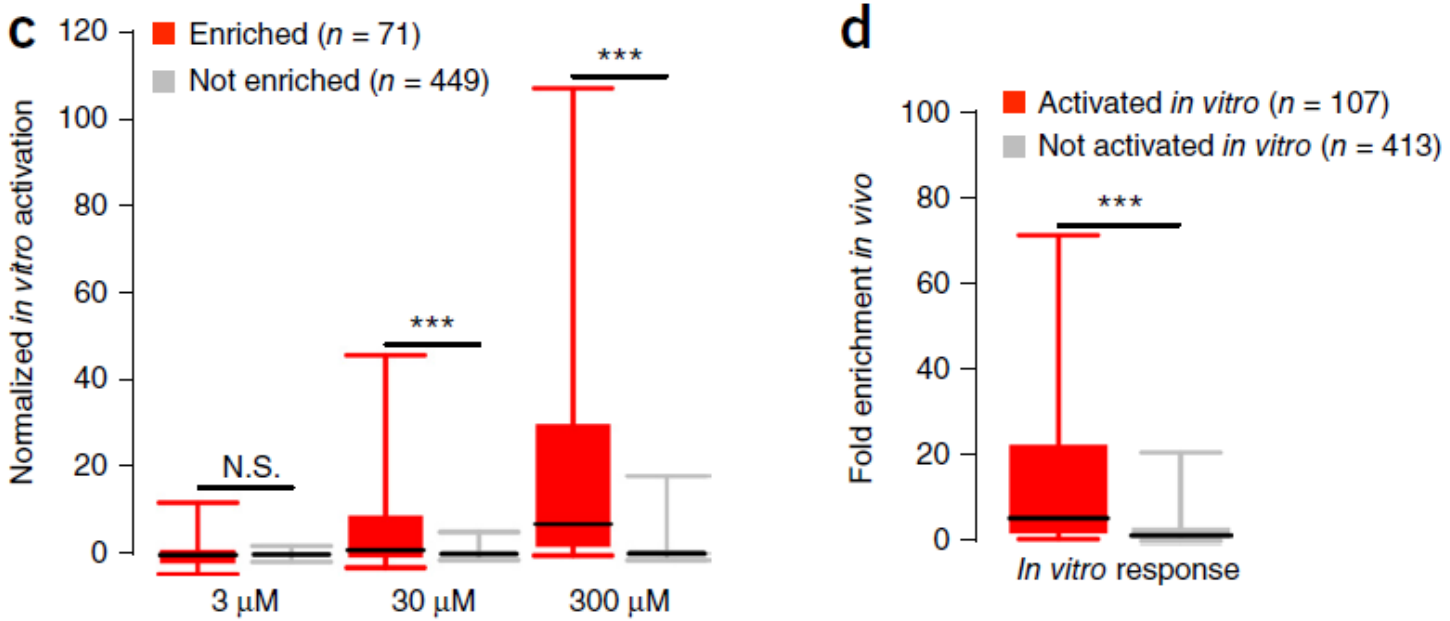
- RNA-seq read counts odor stimulated samples/unstimulated samples
- 25 ORs were enriched (p<0.05) in stimulated group (1% acetophenone)
- 9/9 of ORs (p<0.001) were in 100% acetophenone stimulated ORs; 14/25 of ORs (p<0.05) were in 100% acetophenone stimulated ORs

Enriched ORs tent to respond to acetophenone in vitro



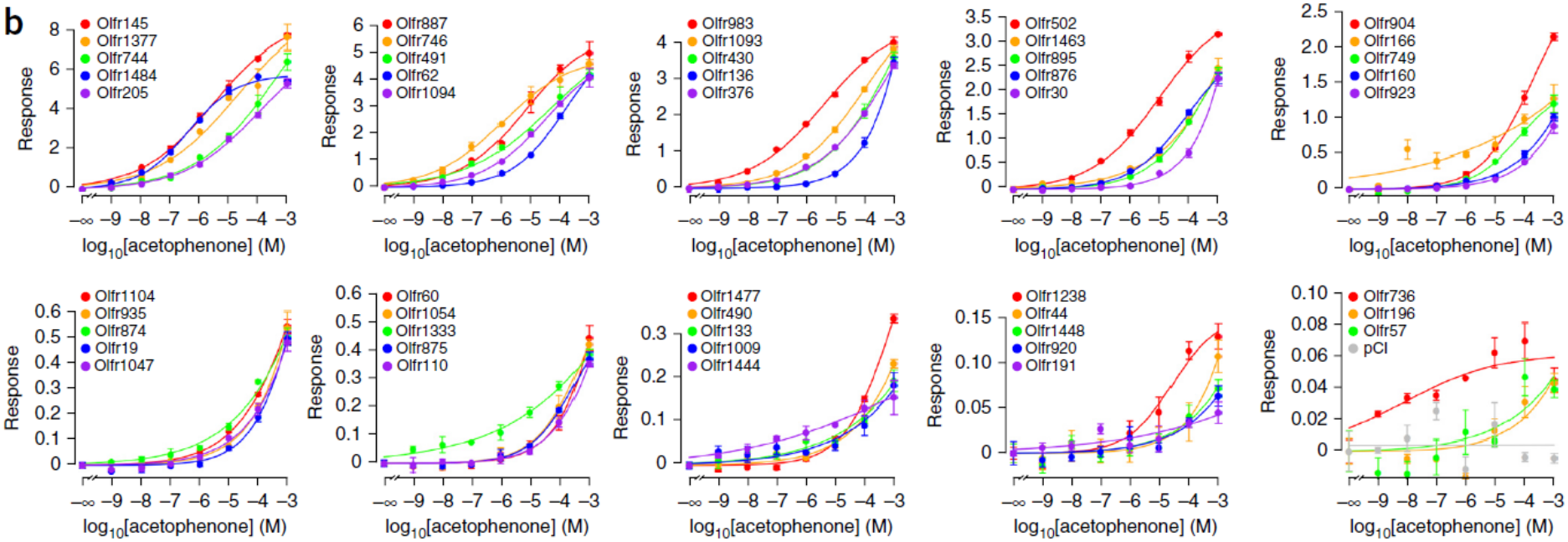
- AC, adenylyl cyclase; CRE, cAMP response element; CREB, cAMP response element-binding protein; PKA, protein kinase A; RTP1S, receptor transporting protein 1 (short)
- 71/75 of ORs enriched by 100% acetophenone stimulated OSNs in vivo and 449 unenriched ORs were tested in vitro

Enriched ORs tent to respond to acetophenone in vitro



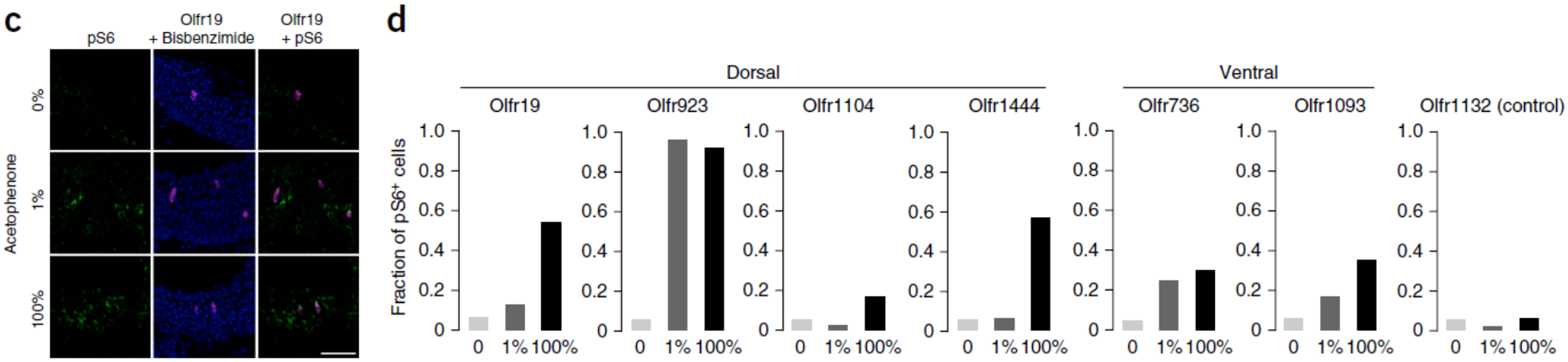
- 71 enriched ORs and 449 unenriched ORs were transiently expressed Hana 3A cells, and then tested for response to acetophenone
- Enriched ORs showed significantly higher response to 30uM or higher acetophenone stimulation than unenriched ORs
- 49/71 (69%) in vivo enriched ORs responded; 58/449 (13%) in vivo unenriched ORs responded in vitro, 43/58 showed a trend in vivo
- ORs enriched in vitro were also enriched in pS6 ip analysis in vivo

Identification of acetophenone ORs



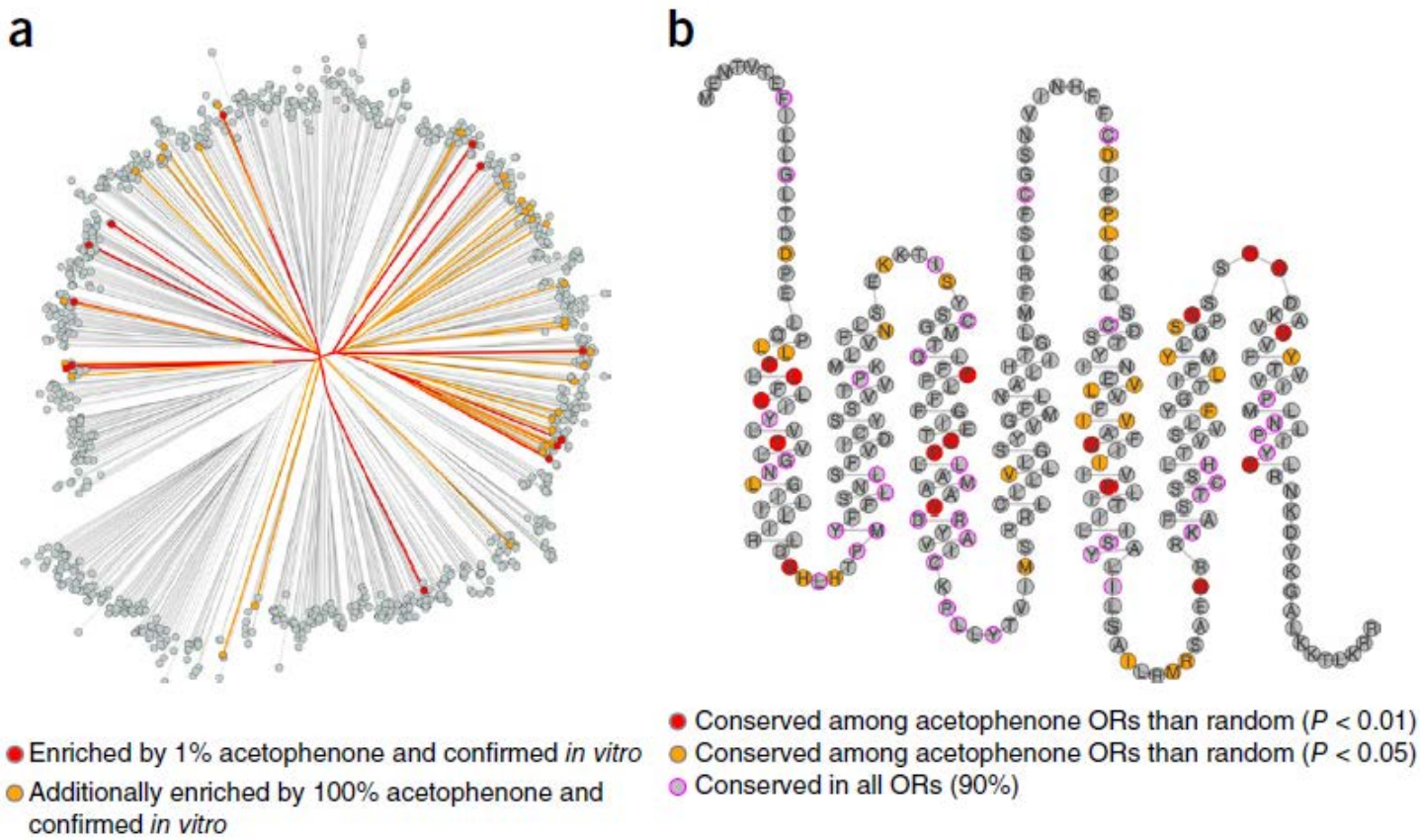
- 48 of ORs that enriched in pS6 ip showed dose-dependent luciferase response to acetophenone

Identification of acetophenone ORs



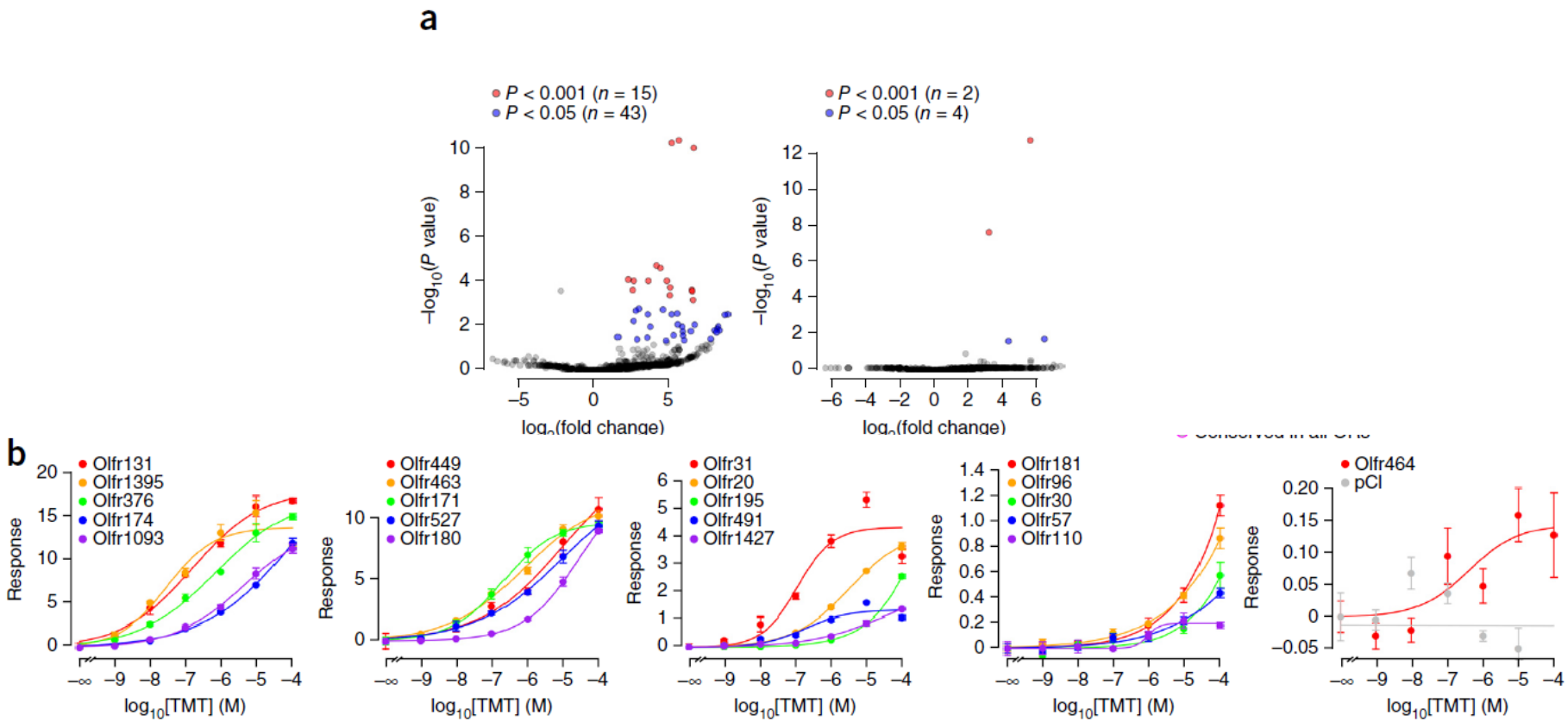
- 6 of the newly identified acetophenone ORs
- Fluorescent RNA in situ hybridization for OR mRNA
- These ORs were phosphorylated upon acetophenone stimulation

Sequence-function relationshop of acetophenone ORs



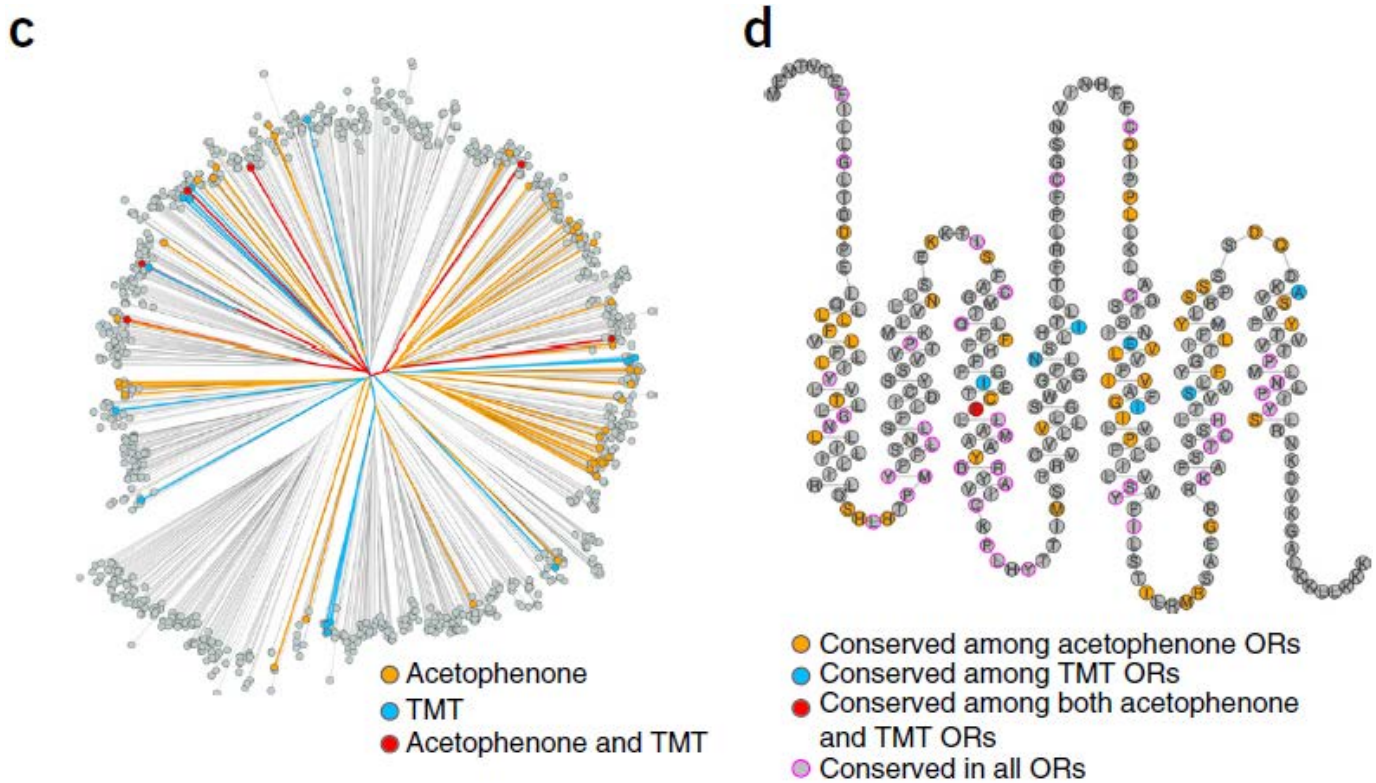
- Phylogenetic tree of OR protein sequences, acetophenone ORs are not clustered in one or a few subfamilies
- Grantham distance: conserved AA sites in transmembrane domain, where odorants bind to ORs

Identification of TMT ORs



- TMT: 2,3,5-Trimethyl-3-thiazoline. A fox feces component induced fear-related response in mice
- 43 ORs were enriched ($p<0.05$) in 100% TMT stimulated group, 4 ORs were enriched ($p<0.05$) in 1% TMT stimulated group
- 21/42 of ORs that enriched in pS6 ip showed dose-dependent luciferase response to TMT

Sequence-function relationshop of TMT ORs



- Phylogenetic tree of OR protein sequences, TMT ORs are not clustered in one or a few subfamilies
- Grantham distance:: conserved AA sites in transmembrane domain, where odorants bind to ORs

Conclusion

- pS6 ip screen is high throughput, can screen most, if not all, ORs in one experiment
- pS6 is does not require transgenic animals
- By pS6, new acetophenone and TMT ORs were identified
- High correlation between in vivo and in vitro mapping

Ribosome profiling in neurodegenerative diseases

- Infect or cross disease models to BacTRAP mice to study roles of neuron subtypes in neurodegeneration

NATURE NEUROSCIENCE VOLUME 18 | NUMBER 9 | SEPTEMBER 2015

Identification of neurodegenerative factors using translatome–regulatory network analysis

Lars Brichta[1], William Shin[2,3], Vernice Jackson-Lewis[4–7], Javier Blesa[4–7], Ee-Lynn Yap[1], Zachary Walker[1], Jack Zhang[1], Jean-Pierre Roussarie[1], Mariano J Alvarez[2], Andrea Califano[2,8], Serge Przedborski[4–8] & Paul Greengard[1,8]

- Ribosome profiling in infected animals or disease models (in vivo)

Science **350**, 82 (2015)

Multiple repressive mechanisms in the hippocampus during memory formation

Jun Cho,[1,2]* Nam-Kyung Yu,[2]* Jun-Hyeok Choi,[2] Su-Eon Sim,[2] SukJae Joshua Kang,[2] Chuljung Kwak,[2] Seung-Woo Lee,[2] Ji-il Kim,[2] Dong Il Choi,[2] V. Narry Kim,[1,2]† Bong-Kiun Kaang[2]†

- Tags of ribosome in infected animals or disease models, ip ribosome-mRNA complex and profiling

Thank you for your attention!