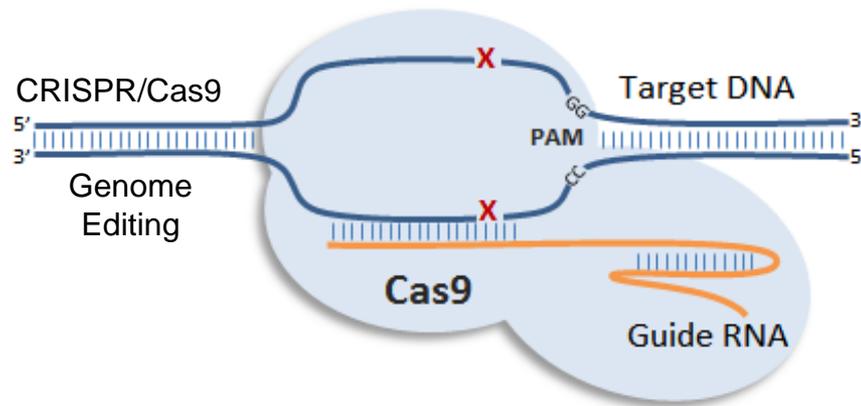# Genetic engineering in non-human primates for brain disorder research

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The need for additional genetic models in neuroscience research

Genetic engineering in mice has revolutionized biomedical research. However, its impact on developing treatments for brain disorders has been limited.

■**Limitations of rodent models for brain disorders：**

1. Huge differences in both structure and function between rodent and human brain.

2. Evolutional divergence in behaviors and underlying circuits.

3. Difficulty in studying higher brain function, e.g. cognition, executive function and emotion.

4. Numerous failures in translating preclinical success in rodents to clinical trials in humans
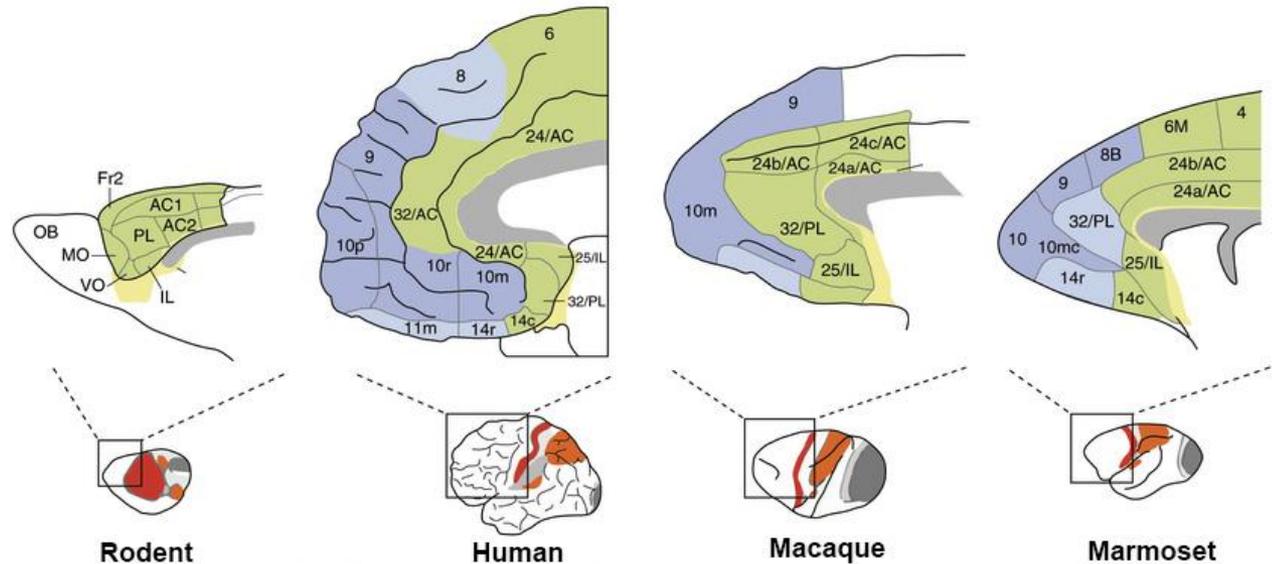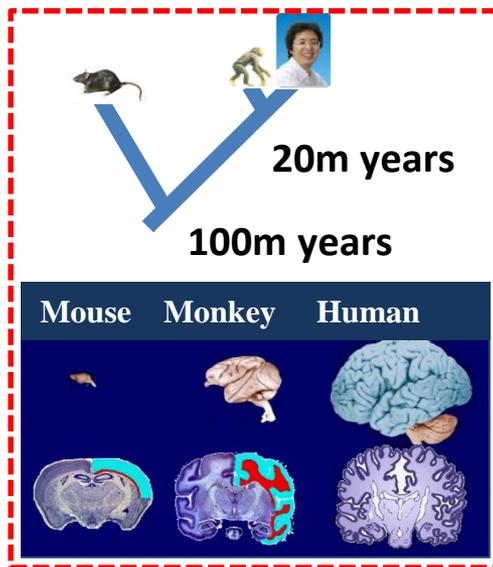
The need for additional models in neuroscience research

The lack of predictive animal models is considered one of bottlenecks to the development of new drugs for brain disorders

mGluR5 antagonists and Fragile X syndrome

Neuron
Report

Correction of Fragile X Syndrome in Mice

Neuron
Report

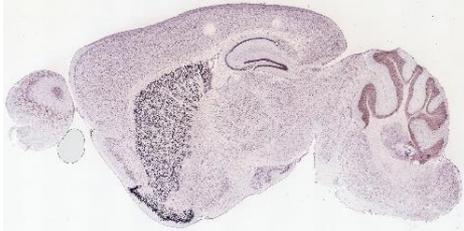**Chronic Pharmacological mGlu5 Inhibition Corrects Fragile X in Adult Mice**

R&D

Roche abandons another Fragile X R&D program after PhII trials flunk out
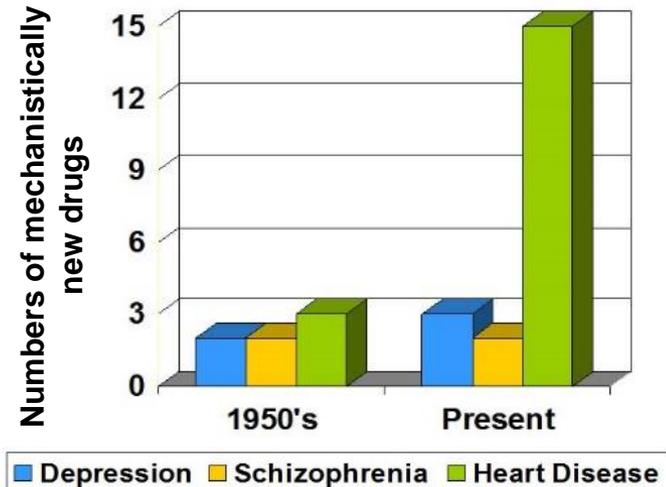
PDE10 inhibitor and Huntington disease

Neuron

Phosphodiesterase 10A Inhibition Improves Cortico-Basal Ganglia Function in Huntington's Disease Models

Pfizer Amaryllis trial ends in disappointment: no improvement in Huntington's disease symptoms

Genome-editing in primates to generate genetic models and cell type-specific tools for brain disorder research

Common marmoset

Small (~350g), **~14 years of life span**, New World monkey

140 days gestation

Mature in 1-1.5 years

Continuous (non-seasonal) breeders

Give birth twice a year and produce twins/triplets each birth

Very social and communicative

Cynomolgus macaque

Large (7-20lb), **~30 years of life span**, native to Southeast Asia
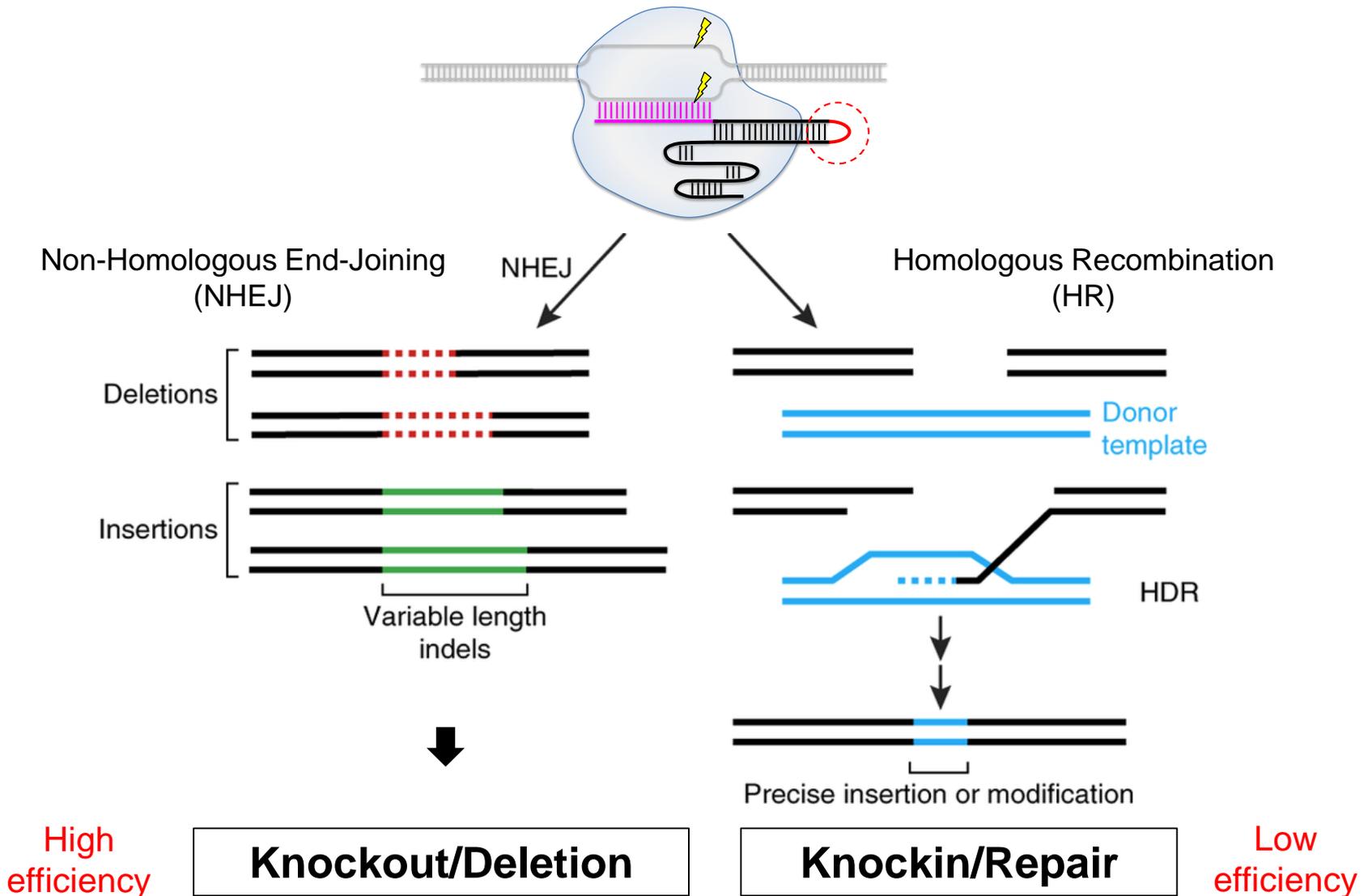
160 days gestation

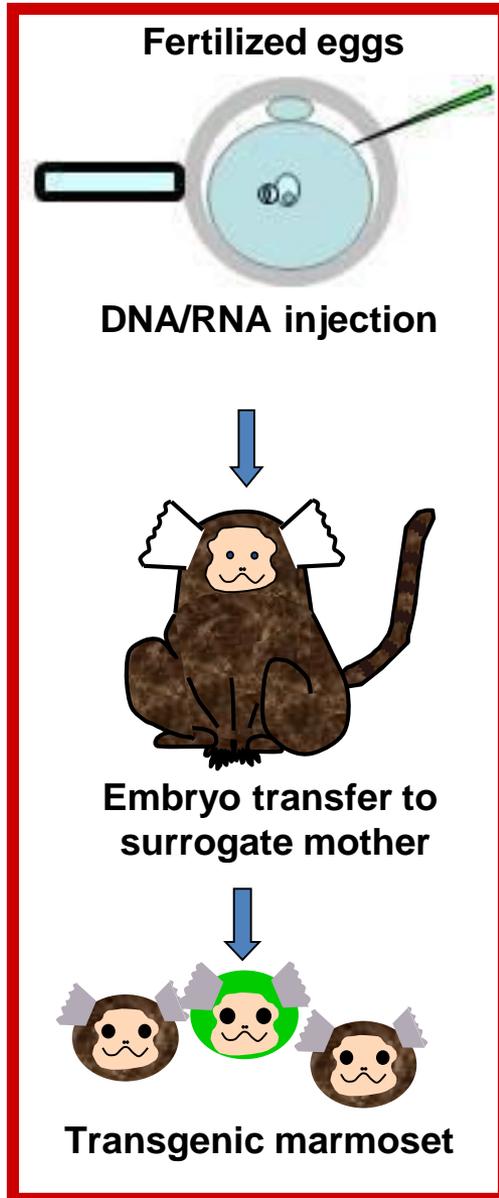**Mature in 3.5 years**

Very similar to rhesus monkey

Continuous (non-seasonal) breeders

Similar brain structure to humans

Great for study of higher brain function

Genome-editing in embryos by CRISPR

Sander & Joung. *Nat Biotechnol* (2014)

Fertilized eggs

DNA/RNA injection

Embryo transfer to surrogate mother

Transgenic marmoset

Transgenic primates

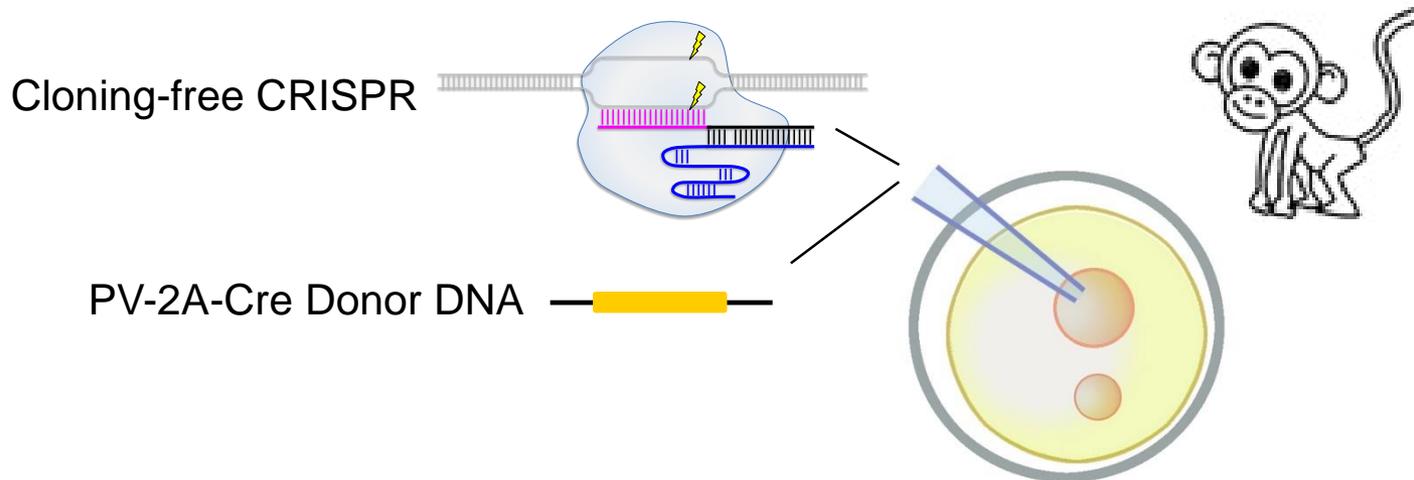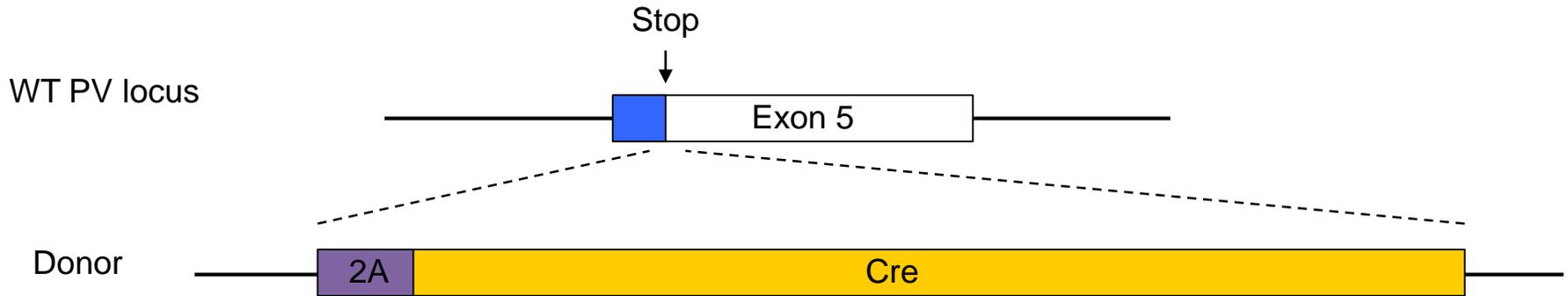**Disease models (knockouts and knockins):**

- **Gene deletions**
- **Gene insertions**
- **Point mutations**
- **Conditional mutations**

Genetic tools (knockins and transgenes):

- **Cell type-specific Cre lines**
- **Cell type-specific indicators**
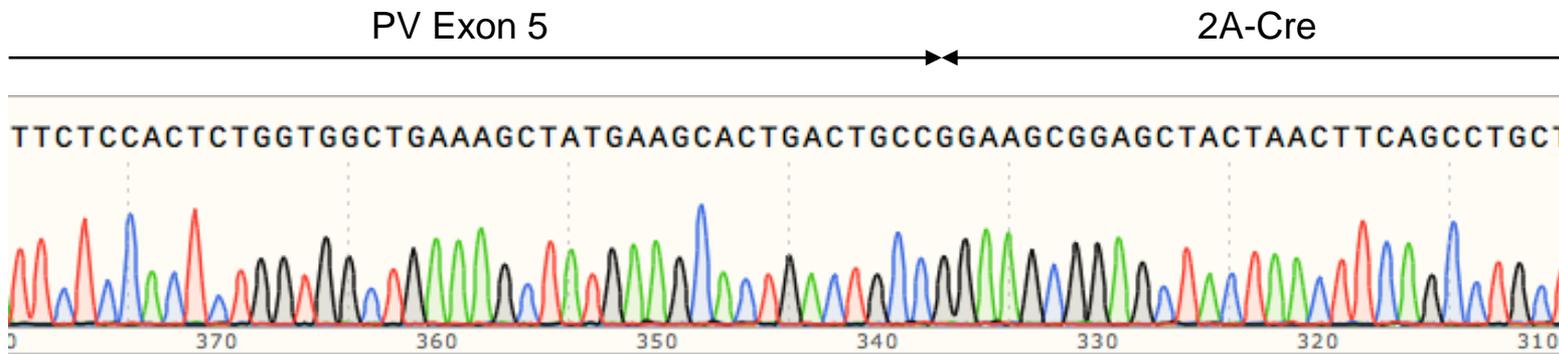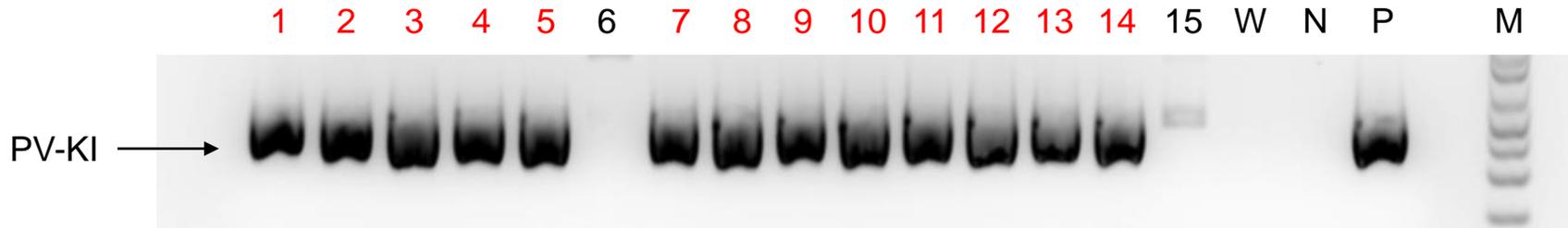- **Cell type-specific actuators**

Generation of PV-Cre knock-in monkeys

Macaque Parvalbumin (PV) locus

Generation of PV-Cre knock-in monkeys
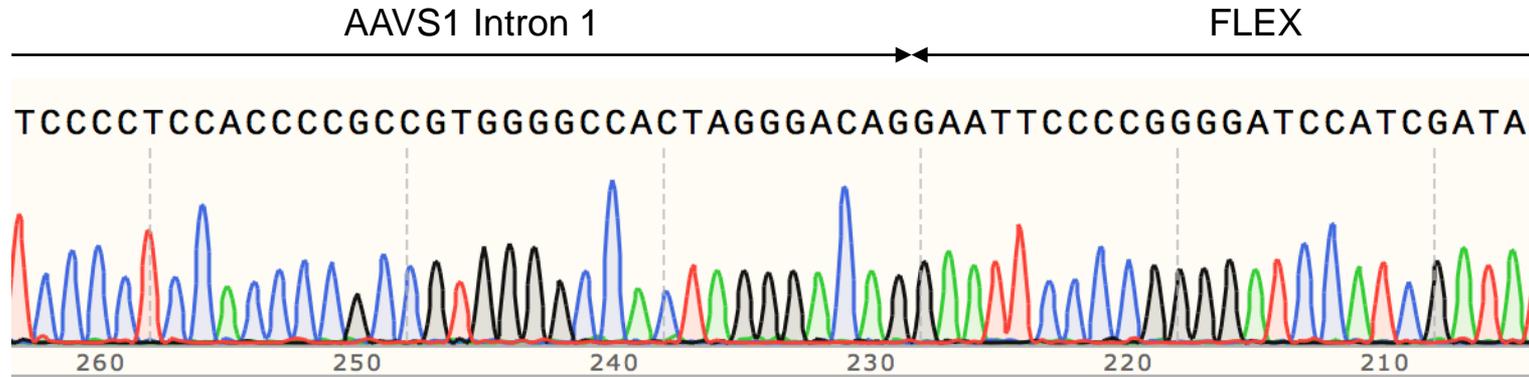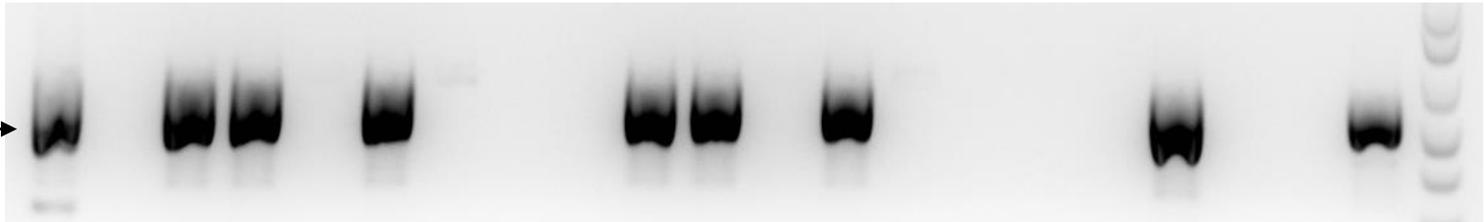
PV-Cre knock-in monkey

Knock-in efficiency: 60-70%

Aida *et al. Unpublished*

Generation of Opsin knock-in monkeys

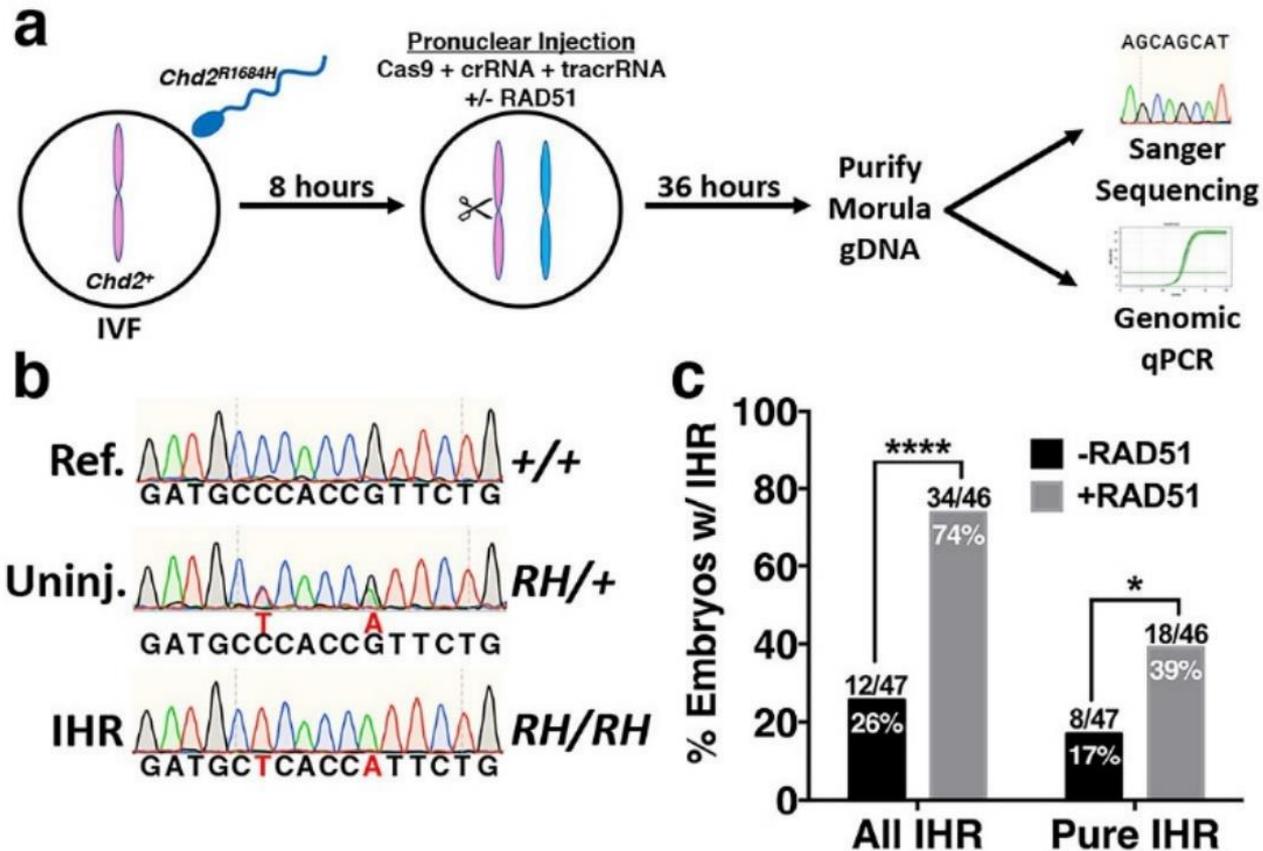## AAVS1-FLEX-ChrimsonR knock-in monkey

AAVS1-KI

Live monkey

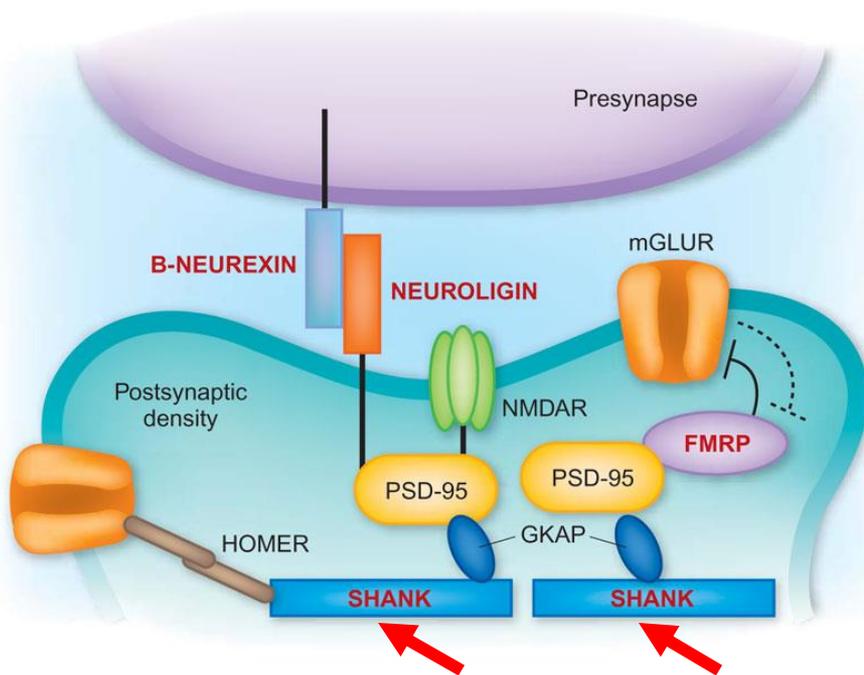# RAD51 significantly increases homozygous knockin rates
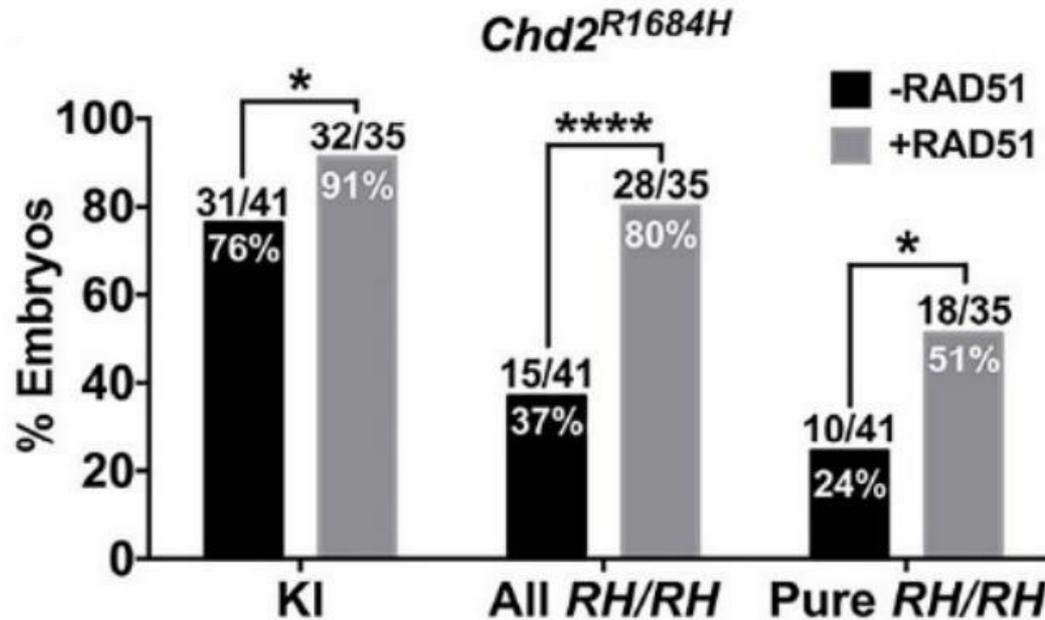
Direct testing of interhomolog repair

Dramatically shorten the time to obtain homozygous animals

Shank3 mutations cause autism spectrum disorder

Identification of a recurrent breakpoint within the *SHANK3* gene in the 22q13.3 deletion syndrome

M C Bonaglia, R Giorda, E Mani, G Aceti, B-M Anderlid, A Baroncini, T Pramparo, and O Zuffardi

Peça et al., *Nature*, 2011
Mei et al., *Nature*, 2016
Zhou et al., *Neuron*, 2016
Wang et al., *J. Clin. Invest.* 2017

Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders

Christelle M Durand[1], Catalina Betancur[2], Tobias M Boeckers[3], Juergen Bockmann[3], Pauline Chaste[1], Fabien Fauchereau[1,4], Gudrun Nygren[5], Maria Rastam[5], I Carina Gillberg[5], Henrik Anckarsäter[5], Eili Sponheim[6], Hany Goubran-Botros[1], Richard Delorme[1], Nadia Chabane[7], Marie-Christine Mouren-Simeoni[7], Philippe de Mas[8], Eric Bieth[8], Bernadette Rogé[9], Delphine Héron[10], Lydie Burglen[11], Christopher Gillberg[5,12], Marion Leboyer[2,13] & Thomas Bourgeron[1,4]
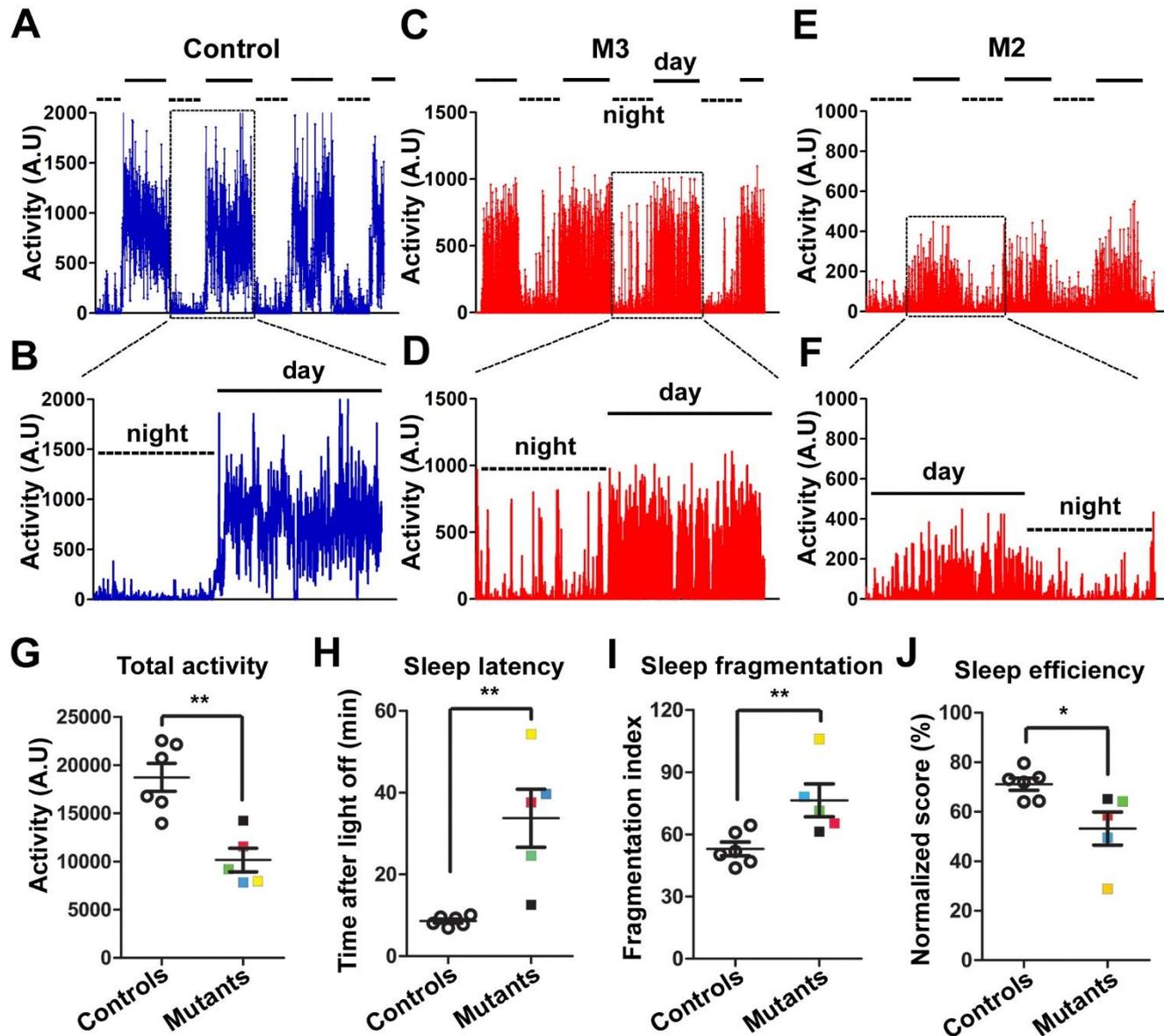
Joe Buxbaum, Yonghui Jiang, Craig Powell, Tom Sudhof, etc

Cynomolgus macaque monkeys with Shank3 mutations

A Heterozygous Shank3 deletion

B Homozygous Shank3 deletion
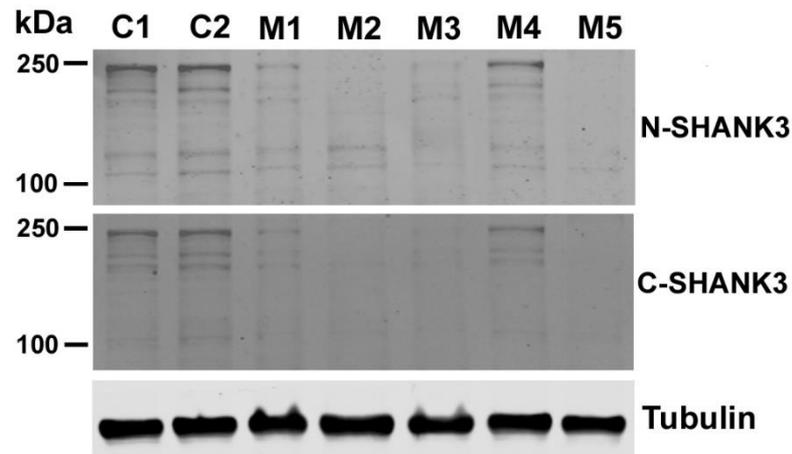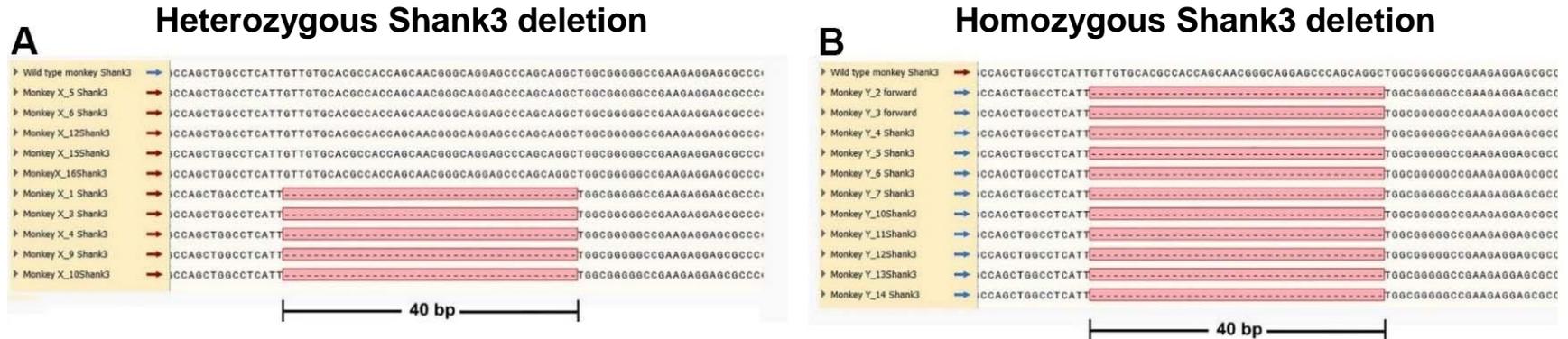
Monkey ID / Antibody	M1	M2	M3	M4	M5
N-Shank3	47%	32%	28%	83%	21%
C-Shank3	23%	11%	20%	72%	10%

Shank3 mutant macaques show lower motor activity and sleep disruption

Jitendra Sharma

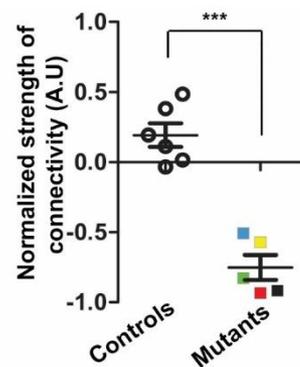# Shank3 mutant macaques show impaired social interaction

Yang Zhou, Rogier Landman et al.
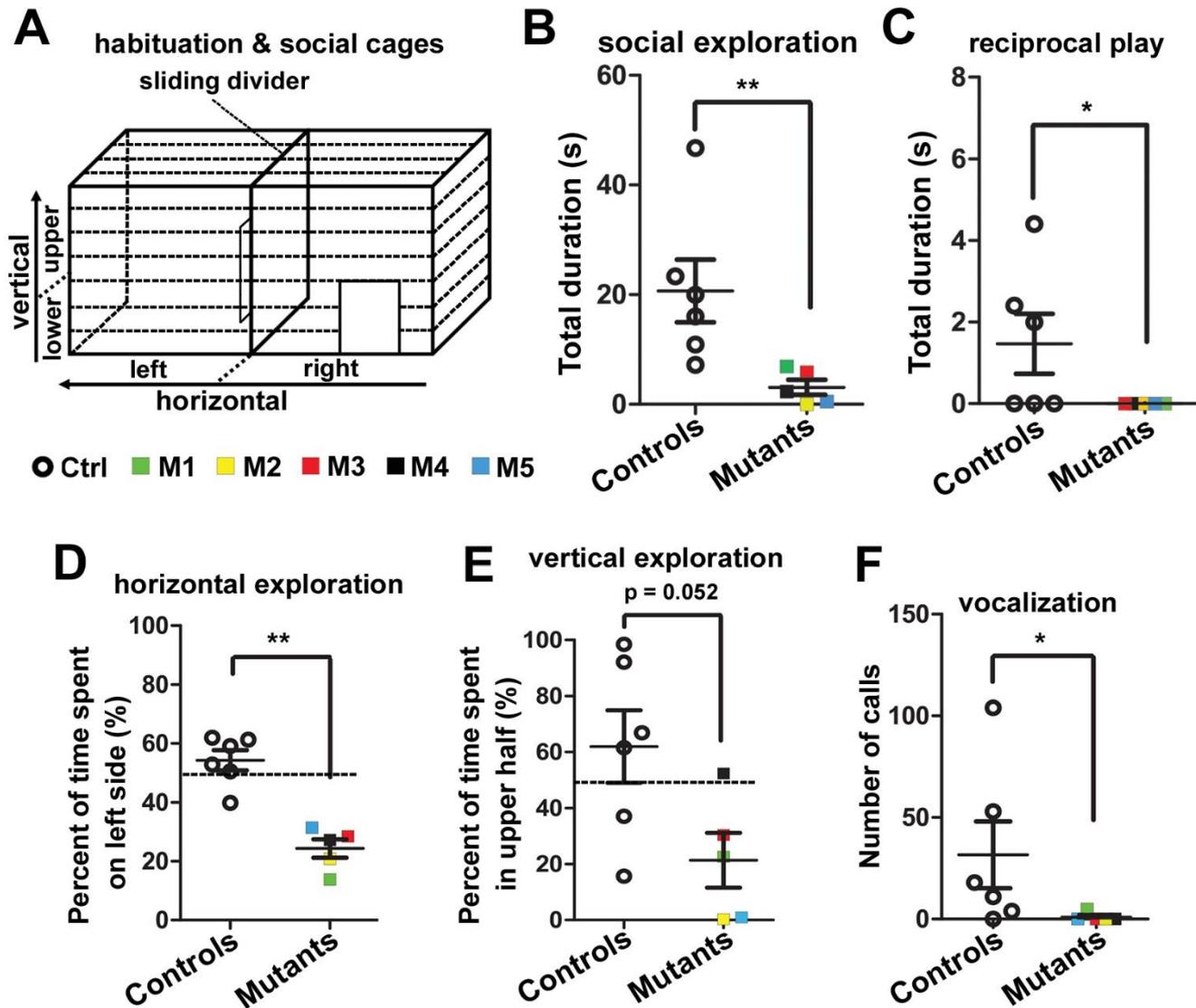
Altered resting state local connectivity in Shank3 mutant macaques
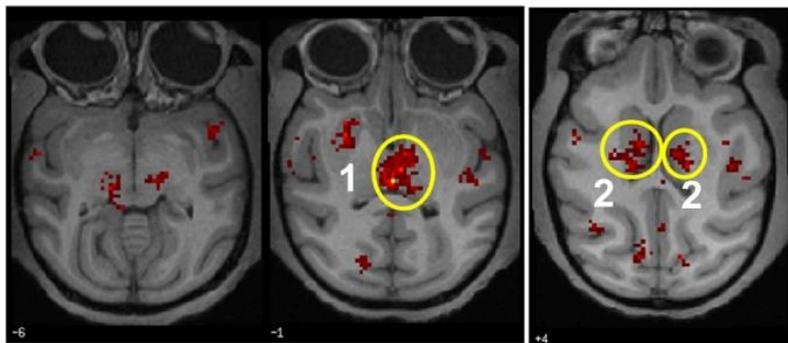
Jitendra Sharma et al.,

Generating Shank3 mutant marmosets with CRISPR/Cas9

Live birth of two Shank3 mutant marmosets

Heterozygous 18-base pair Shank3 deletion in exon 21, resulting in a frameshift and premature stop codon—mimic a human ASD mutation.
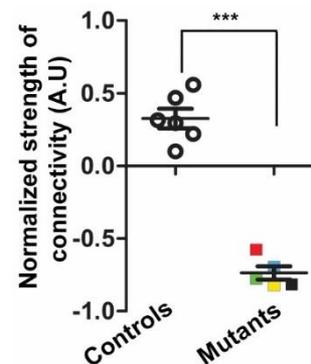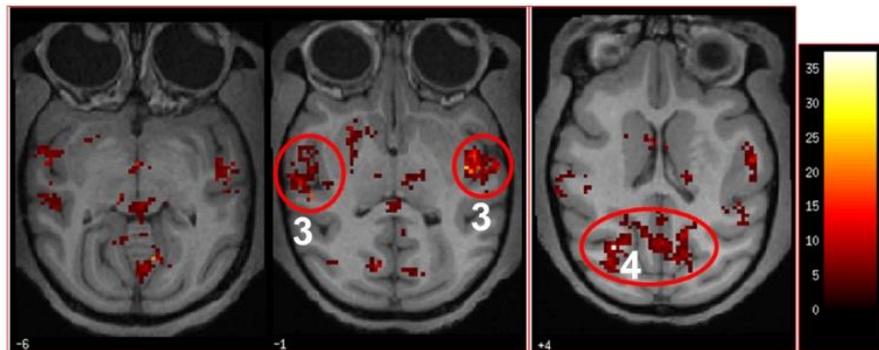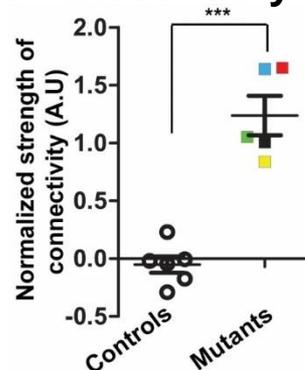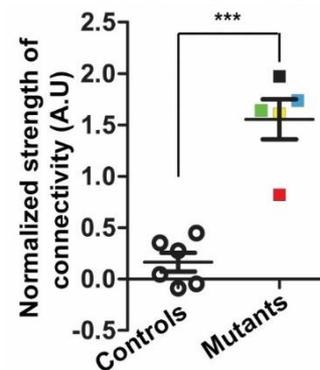
Automated 3D behavioral tracking

Tracking 2 marmosets

Collaboration with David Hayden and John Fisher II (CSAIL MIT)

Testing learning and cognition in homecage box

Elias Issa and Jim DiCarlo

Our Primate Genetic Engineering Team

Guoping Feng, (MIT and Stanley Center for Psychiatric Research at Broad Institute)

Feng Zhang, (MIT and Broad Institute)

Robert Desimone and McGovern Institute for Brain Research
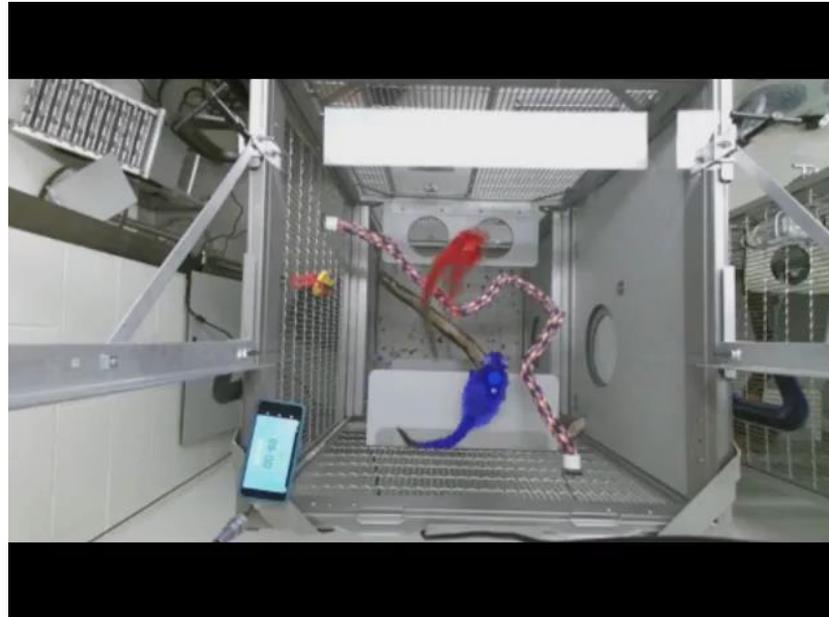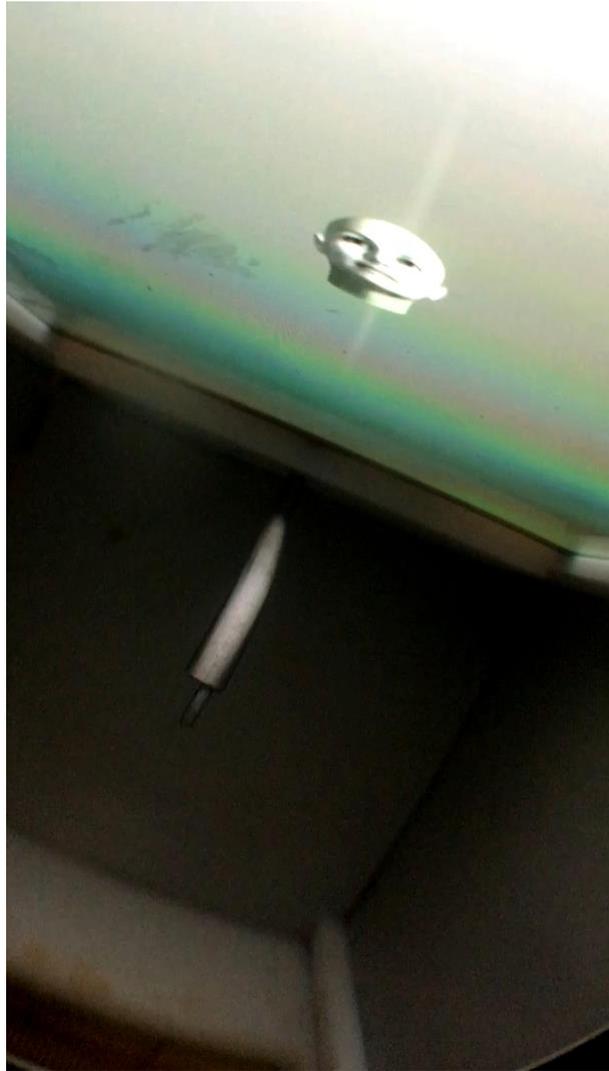
Steve Hyman and the Stanley Center for Psychiatric Research

Steve McCarroll, (Harvard Medical School and Stanley Center for Psychiatric Research)

Laura Brattain, Thomas Quatieri, Edward Wack and MIT Lincoln Lab

Yang Zhou, Qiangge Zhang, Rogier Landman, Julia Hyman, Jitendra Sharma, Charles Jennings, Martin Wienisch, tomomi Aida, Olivia Meisner, Shivangi Parmar Alan Wisler, Karthik Srinivasan, Ricardo del Rosario (MIT and Broad)

James Fox, *Robert Marini and Monika Burns,* (Division of Comparative Medicine, MIT)

Many others at MIT and Stanley Center/Broad Institute (Jacquelyn Stathopoulos*)*

Key Collaborators:

Jim Pickel, (Director of NIMH Transgenic Core)

Afonso Silva, (Investigator, NIMH)

Hideyuki Okano and Erika Sasaki (Professors at Keio University and CIEA.

Shihua Yang (Professor, South China Agricultural University)

Peng Xiang (Professor, Sun Yat-sen University)

Jon Hennebold (Director of Reproduction lab, Oregon Primate Center)

Liping Wang (Professor, Shenzhen Institute of Advanced Technology, CAS)

Huihui Zhou (Professor, Shenzhen Institute of Advanced Technology, CAS)

SIAT Peacock Team: Xingtian Wu, Fuqiang Xu, Yu Chen, Yang Zhan

Support

**A close collaboration between Stanley Center at Broad Institute
and
McGovern Institute at MIT**

Involving faculty members from Broad, Harvard, McGovern Institute, Picower Institute, Dept of Brain and Cognitive Science, Dept of Comparative Medicine

- **McGovern Institute for Brain Research at MIT**
- **Stanley Center for Psychiatric Research at Broad**
- **Poitras Center for Affective Disorder Research at MIT**
- **Tan-Yang Center for Autism Research at MIT**
- **NIH BRAIN Initiative**
- **Broad *Next10* Initiative**
- **Massachusetts Life Science Center**
- **Brain Research Foundation**
- **Cure Huntington Disease Initiative (CHDI)**
- **Roche**
- **Friends of McGovern Institute (Ted Poitras, Silas Chou)**