

# Allen Brain Observatory

## TECHNICAL WHITE PAPER: PHENOTYPIC CHARACTERIZATION OF TRANSGENIC MOUSE LINES

### OVERVIEW

The Allen Brain Observatory contains data collected via two-photon calcium imaging to quantify neuron activity in the mouse neocortex in response to visual stimuli. A Cre/Tet-dependent fluorescent calcium indicator, GCaMP6f, was used to register neural activity in the visual cortex of mice exposed to various visual stimuli. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6. These transgenic strategies drive GCaMP6f expression based on specificity conferred by a Cre driver alone (for example, reporter lines Ai148 and Ai162), or based on the cell-type specific expression of Cre recombinase and tetracycline-controlled transactivator protein (tTA) under the control of the *Camk2a* promoter with the Ai93 and Ai94 reporters. Overall animal health and breeding observations are described in this document, including:

- A summary of transgenic line creation strategy
- Images of GCaMP6 expression based on 2-photon serial tomography, to detect the native baseline presence of cells harboring GCaMP6
- Phenotypic characteristics of transgenic animals, including summaries of growth
- Technical notes and considerations for animal breeding and health

The following transgenic mouse lines are included:

Transgenic Line	Presence in cortical layers	Target cell class	Details on page
<b>Cux2-CreERT2; Camk2a-tTA; Ai93</b>	2/3, 4	Excitatory	2
<b>Emx1-IRES-Cre; Camk2a-tTA; Ai93</b>	2/3, 4, 5	Excitatory	4
<b>Fezf2-CreER; Ai148</b>	5	Excitatory	6
<b>Nr5a1-Cre; Camk2a-tTA; Ai93</b>	4	Excitatory	8
<b>Ntsr1-Cre_GN220; Ai148</b>	6	Excitatory	10
<b>Pvalb-IRES-Cre; Ai162</b>	2/3, 4, 5	Inhibitory	12
<b>Rbp4-Cre_KL100; Camk2a-tTA; Ai93</b>	5	Excitatory	14
<b>Rorb-IRES2-Cre; Camk2a-tTA; Ai93</b>	4	Excitatory	16
<b>Scnn1a-Tg3-Cre; Camk2a-tTA; Ai93</b>	4	Excitatory	18
<b>Slc17a7-IRES2-Cre; Camk2a-tTA; Ai93</b>	2/3, 4, 5	Excitatory	20
<b>Slc17a7-IRES2-Cre; Camk2a-tTA; Ai94</b>	2/3, 4, 5	Excitatory	22
<b>Sst-IRES-Cre; Ai148</b>	4, 5	Inhibitory	24
<b>Tlx3-Cre_PL56; Ai148</b>	5	Excitatory	26
<b>Vip-IRES-Cre; Ai148</b>	2/3, 4	Inhibitory	28

Under the *Transgenic Characterization* tab of the Allen Brain Observatory, image data is available to view native GCaMP6 fluorescence for each line using 2-photon serial tomography, as performed on animals that have not undergone surgery (described in the “Visual Coding Overview” whitepaper in [Documentation](#)).

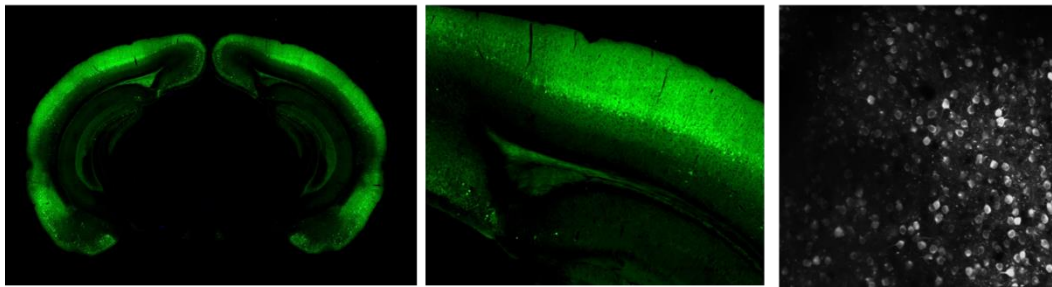
## Cux2-CreERT2; Camk2a-tTA; Ai93 (TITL-GCaMP6f)

### Overview

Cux2-CreERT2;Camk2a-tTA;Ai93(TITL-GCaMP6f) transgenic mice express a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons under the Camk2a promoter. Further specificity is regulated by the tamoxifen-inducible Cux2 promoter, induction of which results in Cre-mediated expression of GCaMP6f in excitatory neurons. GCaMP6f expression is enriched in cortical layers 2, 3 and 4 and in thalamus, midbrain, pons, medulla, and cerebellum.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



*Native gene expression:*

*in situ* hybridization of *Cux2* (NCBI Accession: NM\_007804.2) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/72128748>

*in situ* hybridization of *Camk2a* (NCBI Accession: NM\_009792.3) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/79490122>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

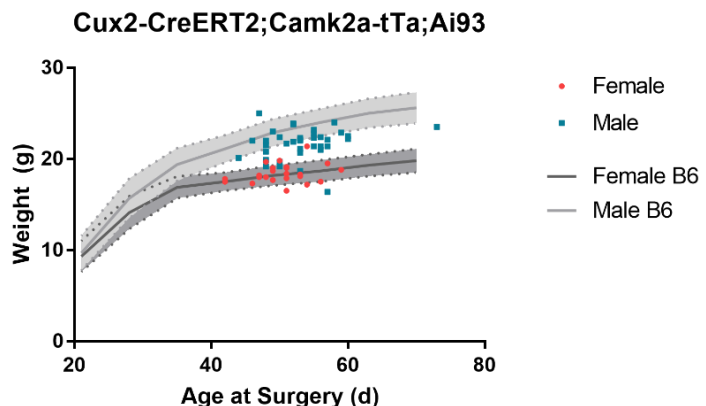
Male: 53.24  $\pm$  5.24

Female: 50  $\pm$  4.46

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male: 21.68  $\pm$  1.67

Female: 18.37  $\pm$  1.05



## Technical

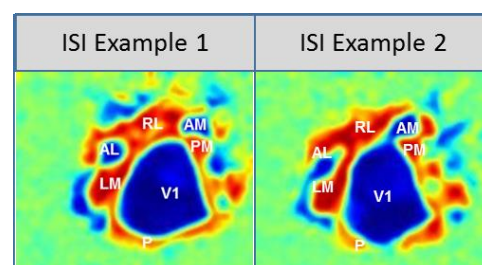
**Breeding Considerations:** Cux2-CreERT2 is a knock-in allele that replaces the first coding ATG of Cux2 and abolishes the gene function. There is no Cre recombinase prior to tamoxifen exposure. Crosses generating homozygous tTA animals were avoided due to tTA toxicity causing prenatal lethality. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for Cre and/or GCaMP6f were included. Despite the fact that Cux2-CreERT2 is a “knock-in” allele that abolished Cux2 gene function, heterozygotes and homozygotes have been used for breeding with no reported gross defects, although sustained inbreeding of homozygous Cux2-CreERT2 may be contraindicated (Gil-Sanz C, 2015). Germline deletion of the Ai93(TITL-GCaMP6f) LoxP-STOP-LoxP cassette is possible when crossing a Cux2-CreERT2;Ai93 mouse with a mouse heterozygous for Camk2a-tTA. To avoid germline deletion of the stop cassette, breeding sets (pairs and trios) were comprised of either a heterozygous Cux2-CreERT2 mouse crossed with a heterozygous Camk2a-tTA;homozygous Ai93 mouse or a heterozygous Cux2-CreERT2; heterozygous Camk2a-tTA mouse crossed with a homozygous Ai93 mouse.

**Inducible transgene induction procedure:** Adult mice (p35) received 5 daily doses of tamoxifen (200mg/kg, oral gavage) followed by a week of recovery prior to surgery.

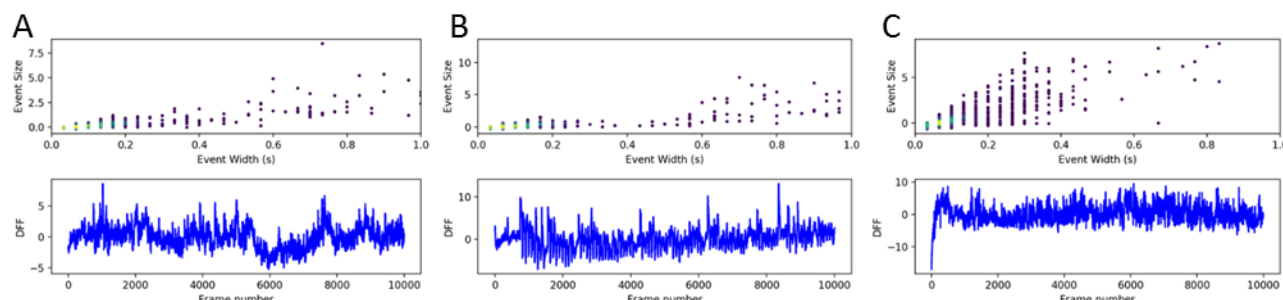
**General observations:** No difference was observed between heterozygous and homozygous animals for GCaMP6 expression pattern or physiology. 23.5% of 132 mice needed to be re-weighed to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=39, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Cux2-CreERT2; Camk2a-tTA; Ai93 are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **Interictal events were observed in a small number of pipeline animals (5.1% of animals).** Separate testing identified a single animal exhibiting interictal events (Steinmetz et al., 2017).



## Summary

No significant issues were observed with these examples except for an infrequent occurrence of interictal events.

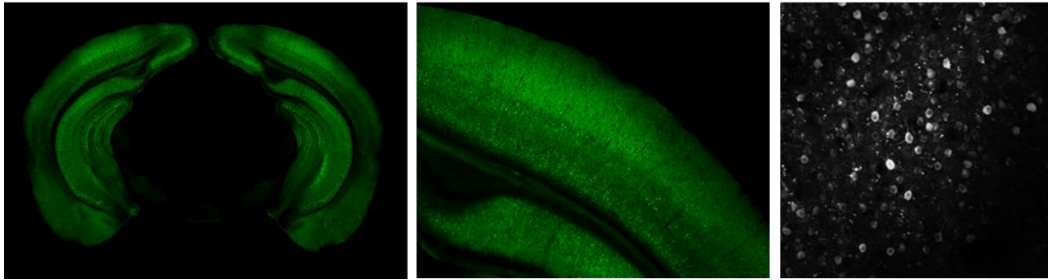
## Emx1-IRES-Cre; Camk2a-tTA; Ai93 (TITL-GCaMP6f)

### Overview

Emx1-IRES-Cre;Camk2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Emx1* promoter, resulting in mice that exhibit GCaMP6f in excitatory neurons across layers of cortex as well as in hippocampus.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



#### Native reporter expression:

*in situ* hybridization of *Emx1* (NCBI Accession: XM\_132640.3) in C57BL/6J

<http://mouse.brain-map.org/experiment/show/100145374>

*in situ* hybridization of *Camk2a* (NCBI Accession: NM\_009792.3) in C57BL/6J

<http://mouse.brain-map.org/experiment/show/79490122>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

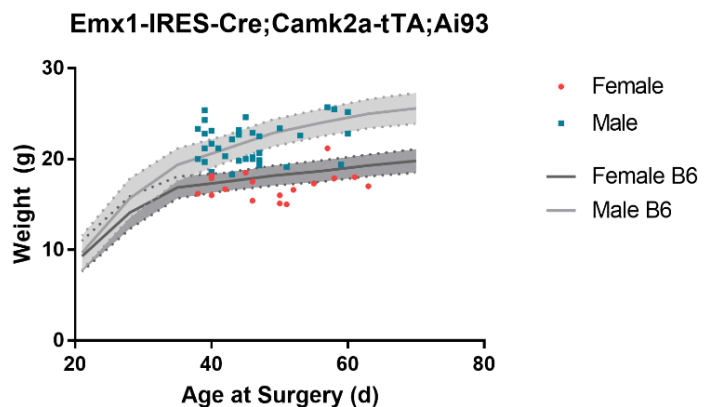
Male:  $45.76 \pm 6.67$

Female:  $49.55 \pm 7.89$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $21.81 \pm 2.14$

Female:  $17.13 \pm 1.49$



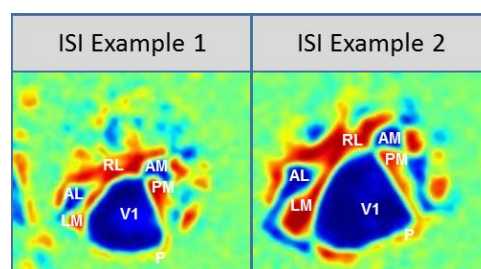
## Technical

**Breeding Considerations:** Breeding sets (pairs and trios) included only tTA heterozygous animals as animals homozygous for the tTA transgenes caused undesirable and/or lethal phenotypes. It is important to note that Emx1-IRES-Cre is expressed in mouse germline. Therefore, the mating scheme should avoid combining this Cre transgene and the Cre-dependent reporter (containing LoxP-STOP-LoxP cassette) until the last cross, which creates the experimental animal. That scheme prevents the exposure of the Cre-dependent reporter to Cre recombinase activity in the mouse germline. The exposure of the Cre-dependent reporter to Cre recombinase in the germline results in STOP deletion from the LoxP-STOP-LoxP cassette, and subsequent expression of the reporter that is not Cre-specific.

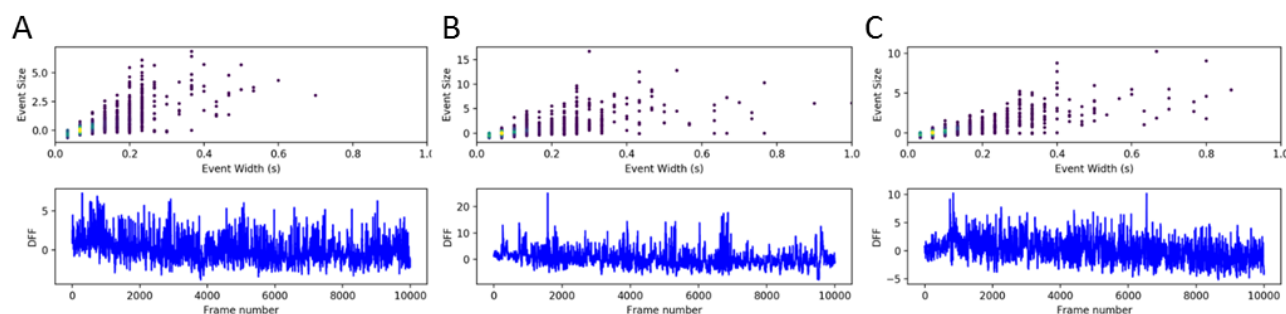
**General observations:** 39 of 149 mice (26.2%) needed to be re-weighted to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32. Five mice were excluded due to insufficient surgical weight. Five mice were excluded due to eye anomalies (e.g., anophthalmia, microphthalmia, or cloudy eyes). Interictal events were observed in ~42% of mice as measured at the Allen Institute (Steinmetz et al., 2017).

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n= 19 higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Emx1-IRES-Cre;Camk2a-tTA;Ai93 mice are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). Mouse A exhibited these abnormal events which are absent from mouse B and C. **Animals of this line exhibited a high prevalence (42%) of interictal events.**



## Summary

An abnormality observed in this line is the high occurrence of interictal events (42%); all mice are screened for interictal events, and excluded from subsequent testing if activity is detected.



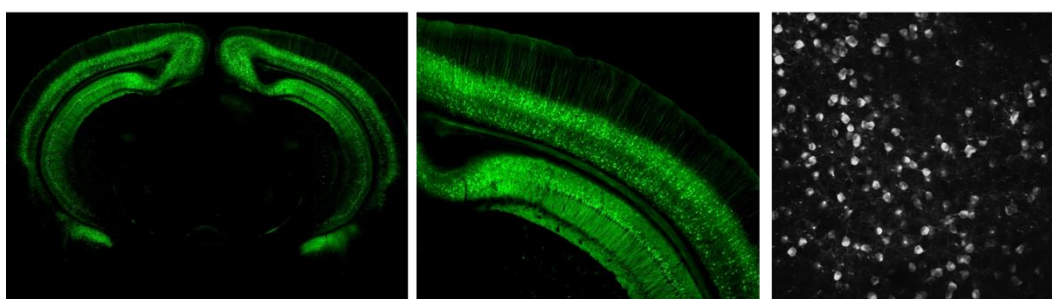
## Fezf2-CreER; Ai148 (TIT2L-GCaMP6f-ICL-tTA2)

### Overview

Fezf2-CreER;Ai148 (TIT2L-GCaMP6f-ICL-tTA2) transgenic mice express a tamoxifen-inducible Cre recombinase under the control of the Fezf2 promoter, resulting in expression of Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f in layer 5 (and to a lesser extent in layer 6) as well as specific populations in the amygdala and hippocampus.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



Native gene expression:

*in situ* hybridization of *Fezf2* (NCBI Accession: NM\_080433.1) in C57BL/6J

<http://mouse.brain-map.org/experiment/show/75651163>

### Growth and Development

Mouse weights from this transgenic line are collected just prior to surgery (p37-p63), compared to standard C57BL/6J growth curves obtained from The Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

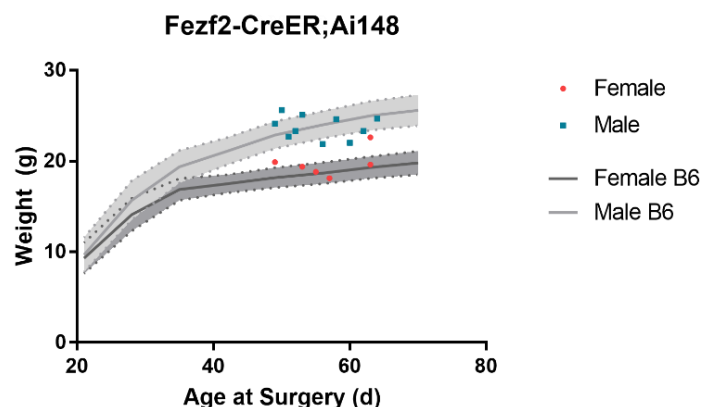
Male: 55.5  $\pm$  5.29

Female: 56.66  $\pm$  5.57

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male: 23.73  $\pm$  1.29

Female: 19.73  $\pm$  1.54



### Technical

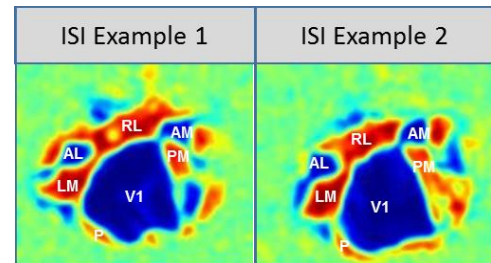
**Breeding Considerations:** Breeding sets (pairs and trios) consisted of crossing a heterozygous Fezf2-CreER mouse to either a heterozygous or homozygous Ai148 (Ai148(TIT2L-GCaMP6f-ICL-tTA2) mouse.

*Inducible transgene induction procedure:* Adult mice (p35) received 5 daily doses of tamoxifen (200mg/kg, oral gavage) followed by a week of recovery prior to surgery.

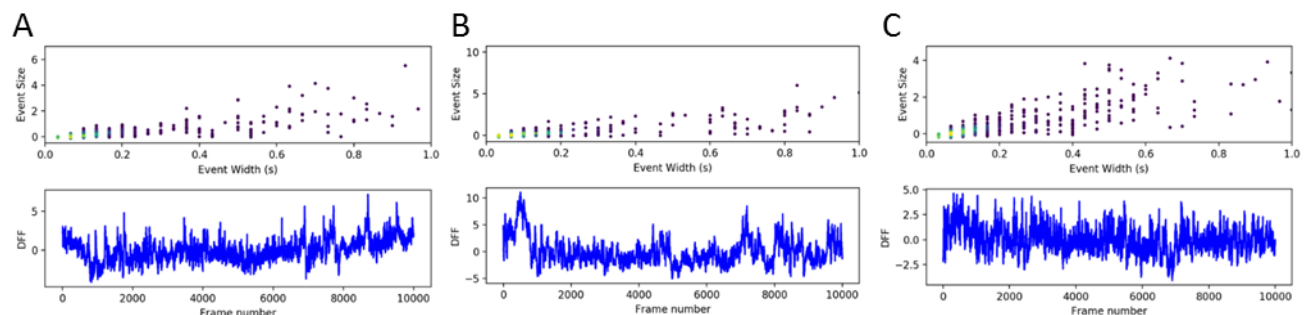
*General observations:* 15 of 61 mice (24.6%%) needed to be re-weighted to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32. Three mice were excluded due to insufficient surgical weight. Nine mice did not survive Tamoxifen induction. Two mice were excluded due to eye anomalies.

## Other Characterization

*Intrinsic signal imaging.* Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=12, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two *Fzef2-CreER;Ai148* mice are shown.



*Screening for interictal events.* Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **No interictal events were observed.**



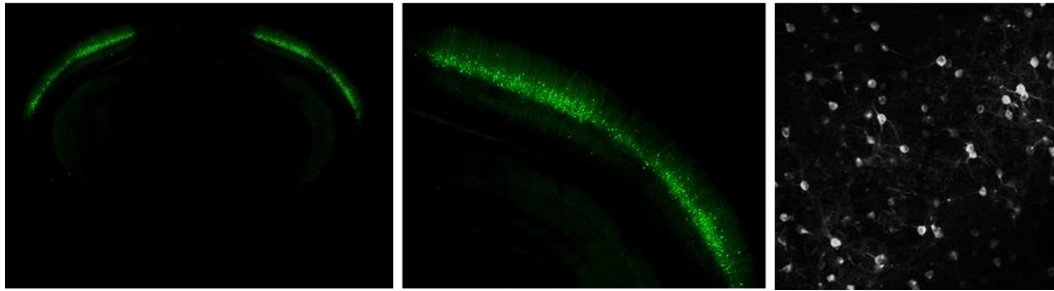
## Nr5a1-Cre; CaMK2a-tTA; Ai93 (TITL-GCaMP6f)

### Overview

Nr5a1-Cre;CaMK2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Nr5a1* promoter in a modified BAC, resulting in mice that exhibit GCaMP6f in excitatory neurons in cortical layer 4 as well as in the ventromedial hypothalamus.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



*Native reporter expression:*

*in situ* hybridization of *Nr5a1* (NCBI Accession: NM\_139051.2) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/734>

*in situ* hybridization of *Camk2a* (NCBI Accession: NM\_009792.3) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/79490122>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

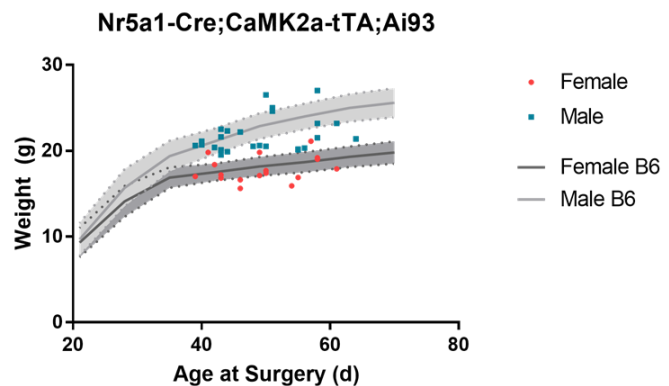
Male:  $49 \pm 7.19$

Female:  $49.47 \pm 6.76$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $21.75 \pm 2.04$

Female:  $17.84 \pm 1.49$





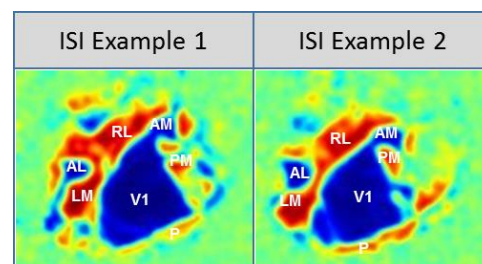
## Technical

**Breeding Considerations:** Breeding sets (pairs and trios) were comprised of two breeding schemes. The first was crossing a heterozygous Nr5a1-Cre mouse to a Camk2a-tTA;Ai93(TITL-GCaMP6f) mouse that was heterozygous for tTA and either heterozygous or homozygous for GCaMP6f. The second option was crossing a heterozygous Nr5a1-Cre; heterozygous Camk2a-tTA mouse with a homozygous Ai93(TITL-GCaMP6f) mouse. In some cases, a heterozygous GCaMP6f mouse was used when homozygous GCaMP6f mice were unavailable. Crosses generating homozygous Cre and/or tTA were avoided as homozygous Cre and/or tTA causes undesirable phenotypes and/or prenatal lethality. To avoid germline deletion of the stop cassette, the breeding scheme avoided crossing a mouse consisting of Cre and GCaMP6f (Cre-dependent reporter) to a tTA mouse. This scheme would have resulted in the deletion of the LoxP-STOP-LoxP cassette causing expression in all cells, independent of Nr5a1-Cre activity. Nr5a1-Cre is an endogenous bacterial artificial chromosome (BAC) transgene.

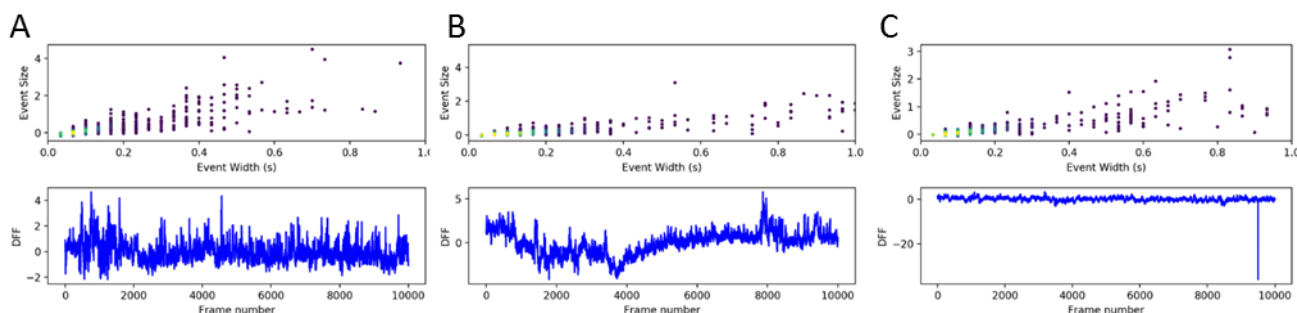
**General observations:** 15 of 51 mice (29.4%) needed to be re-weighted to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32. One mouse was excluded due to insufficient surgical weight. Two mice were excluded due to eye anomalies.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=24, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Nr5a1-Cre;CaMK2a-tTA;Ai93 mice are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **No interictal events were observed.**



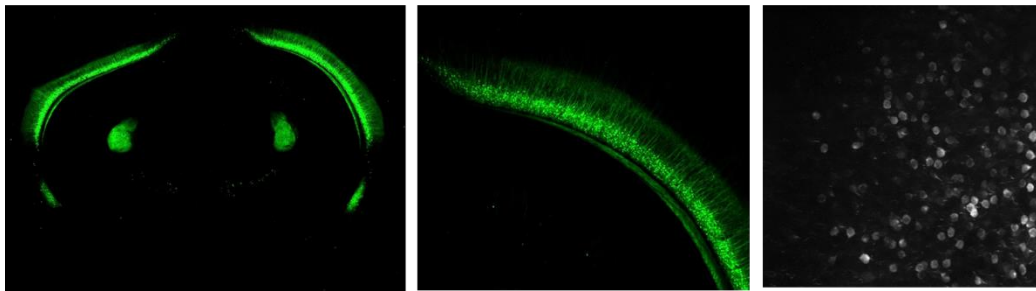
## Ntsr1-Cre\_GN220; Ai148 (TIT2L-GCaMP6f-ICL-tTA2)

### Overview

Ntsr1-Cre-GN220;Ai148 (TIT2L-GCaMP6f-ICL-tTA2) transgenic mice express a tamoxifen-inducible Cre recombinase under the control of the portion of the Ntsr1 promoter, resulting in expression of Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f in layer 6.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



*Native gene expression:*

*in situ* hybridization of *Ntsr1* (NCBI Accession: NM\_018766.1) in C57BL/6J

<http://mouse.brain-map.org/experiment/show/73519704>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

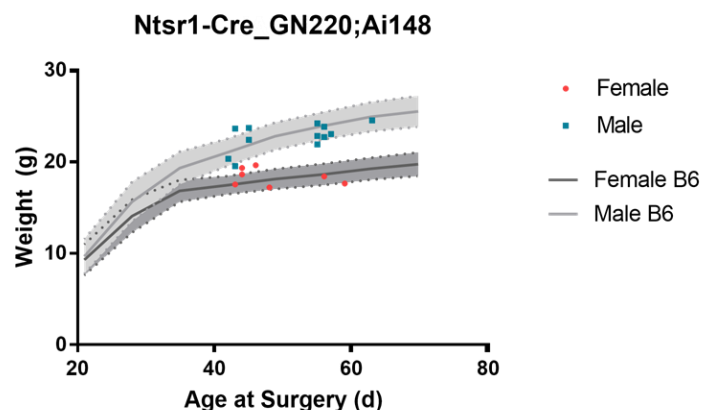
Male:  $51.25 \pm 7.12$

Female:  $48.57 \pm 6.37$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $22.7 \pm 1.51$

Female:  $18.31 \pm 0.92$



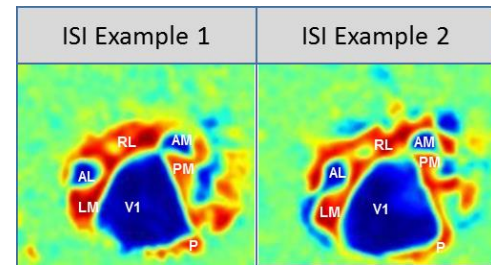
## Technical

**Breeding Considerations:** Ntsr1-Cre\_GN220 is a BAC transgenic and expressed in the germline. Breeding sets included (pairs and trios), crossing heterozygous Ntsr1-Cre\_GN220 and either heterozygous or homozygous Ai148(TIT2L-GCaMP6f-ICL-tTA2).

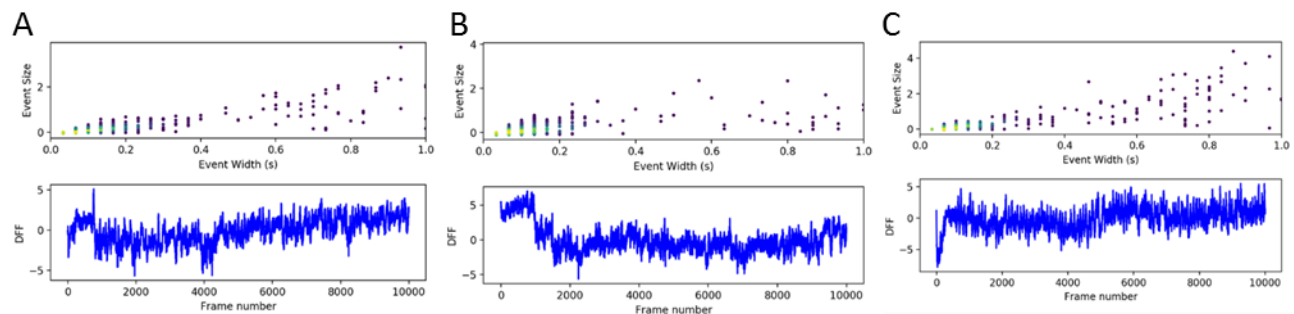
**General observations:** 7 of 43 mice needed to be re-weighted to meet the eligible surgical weight (18.3g Male and 15g Female) criteria at p32. Two mice were excluded due to insufficient weight, and four mice were excluded due to eye anomalies.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on  $n=10$ , retinotopic metrics for V1 for this line fell within the acceptable range for our current pipeline quality standards. Higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Ntsr1-Cre\_GN220;Ai148 mice are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **No interictal events were observed.**



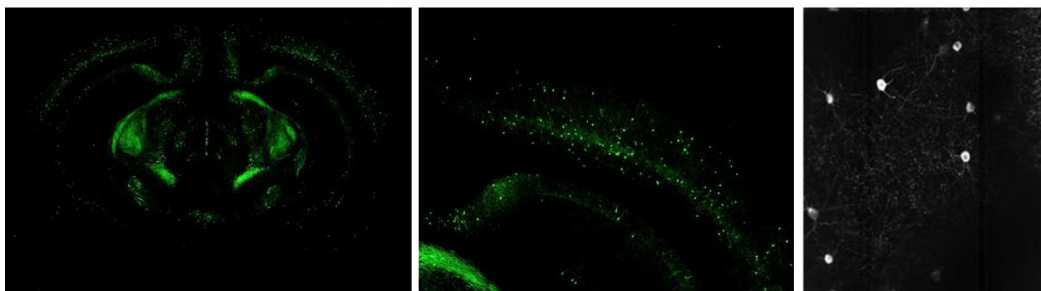
## Pvalb-IRES-Cre; Ai162 (TIT2L-GCaMP6s-ICL-tTA2)

### Overview

Pvalb-IRES-Cre; Ai162 (TIT2L-GCaMP6s-ICL-tTA2) transgenic mice express a Cre/Tet-dependent fluorescent calcium indicator GCaMP6s, with specificity provided by Cre expression under control of the parvalbumin promoter to achieve Cre-mediated expression of GCaMP6s in inhibitory neurons. The reporter is expressed throughout cortex and hippocampus. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6s.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



Native gene expression:

*in situ* hybridization of *Pvalb* (NCBI Accession: x) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/79556738>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

Male:  $62.6 \pm 5.17$

Female:  $49 \pm 8.09$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $23.18 \pm 1.19$

Female:  $17.18 \pm 1.39$



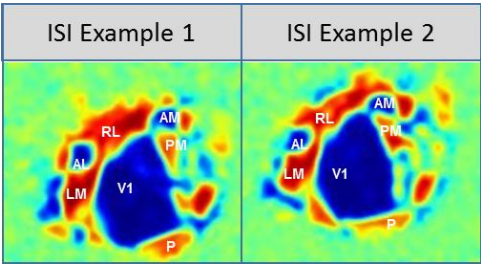
Technical

*Breeding Considerations:* Breeding pairs were comprised of crossing a heterozygous Pvalb-IRES-Cre mouse to either a heterozygous or homozygous Ai162(TIT2L-GCaMP6s-ICL-tTA2) mouse. Parvalbumin is expressed in male germline and is a knock-in allele.

*General observations:* Out of 21 pipeline mice, four had to be re-weighted to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32.

Other Characterization

*Intrinsic signal imaging.* Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=6, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Pvalb-IRES-Cre; Ai162 mice are shown.



*Screening for interictal events.* Labelling in inhibitory lines was too sparse to assess with confidence the presence of interictal events through calcium imaging. Therefore we did not exclude animals based on properties of calcium events.



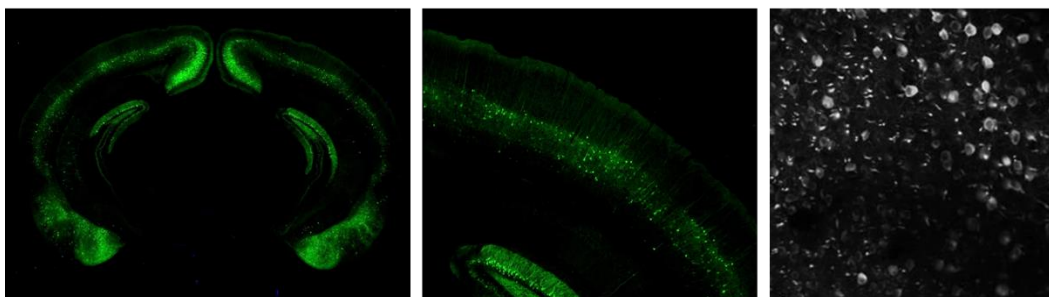
## Rbp4-Cre\_KL100; Camk2a-tTA; Ai93 (TITL-GCaMP6f)

### Overview

Rbp4-Cre\_KL100;CaMK2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Rbp4* promotor that results in Cre-mediated expression of GCaMP6f in excitatory neurons in cortical layer 5 as well as dentate gyrus.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



Native gene expression:

*in situ* hybridization of *Rbp4* (NCBI Accession: NM\_011255.1) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/71016583>

*in situ* hybridization of *Camk2a* (NCBI Accession: NM\_009792.3) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/79490122>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

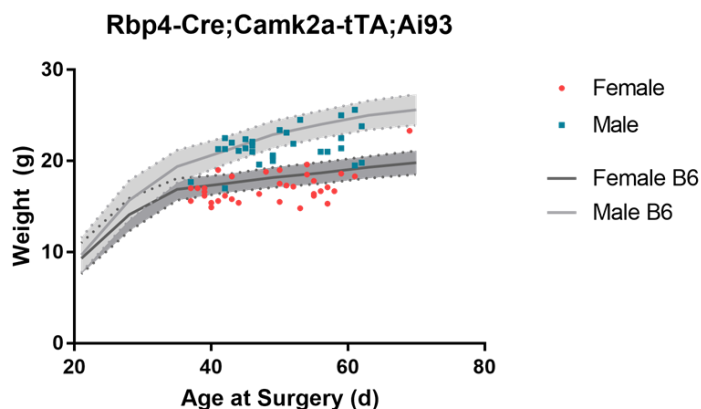
Male:  $50.17 \pm 7.33$

Female:  $48.48 \pm 8.16$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $21.54 \pm 1.92$

Female:  $17.12 \pm 1.67$



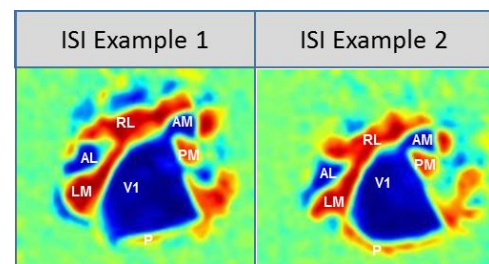
## Technical

**Breeding Considerations:** Breeding sets (pairs and trios) included heterozygotes of Rbp4-Cre\_KL100 and Camk2a-tTA with no gross abnormalities reported in offspring. Crosses generating homozygous CRE were avoided due to low survivability past p40 and reported eye issues of anophthalmia and microphthalmia. Crosses producing homozygous tTA animals were avoided due to tTA toxicity causing prenatal lethality. Rbp4-Cre\_KL100 is endogenous and expresses in the germline. Therefore, germline deletion of the Ai93(TITL-GCaMP6f) LoxP-STOP-LoxP cassette is possible when crossing a Rbp4-Cre\_KL100;Ai93(TITL-GCaMP6f) mouse with a mouse heterozygous for Camk2a-tTA. To avoid germline deletion of the stop cassette, breeding sets (pairs and trios) were comprised of either a heterozygous Rbp4-Cre\_KL100 mouse crossed with a heterozygous Camk2a-tTA;homozygous Ai93(TITL-GCaMP6f) mouse or a heterozygous Rbp4-Cre\_KL100;heterozygous Camk2a-tTA mouse crossed with a homozygous Ai93(TITL-GCaMP6f) mouse.

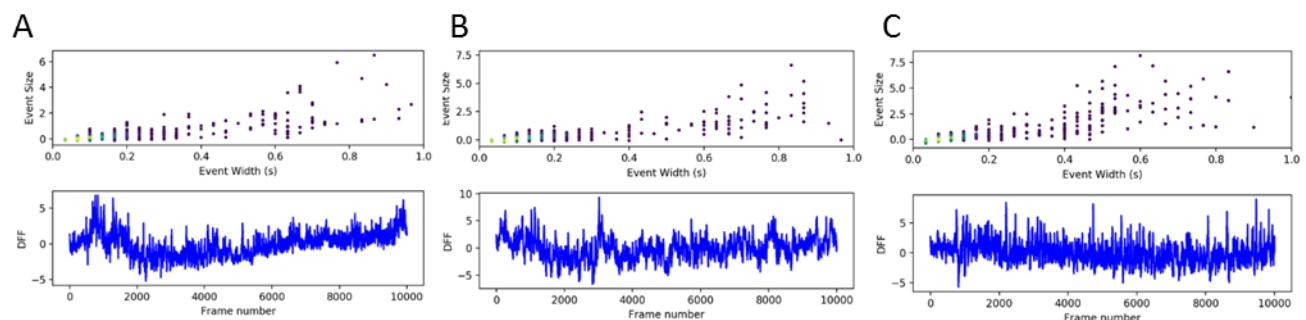
**General observations:** A small fraction (7.2%) of Rbp4-Cre;CaMK2a-tTA;Ai93 mice exhibited a tremulous phenotype and were therefore excluded from the experimental pipeline. 24.5% of 61 mice needed to be reweighed to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32. One mouse was excluded due to insufficient weight. 3% of mice exhibited eye anomalies and were thus excluded.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=24, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Rbp4-Cre\_KL100;CaMK2a-tTA;Ai93 mice are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **No interictal events were observed.**



## Summary

A small subset of animals display tremulous phenotype.

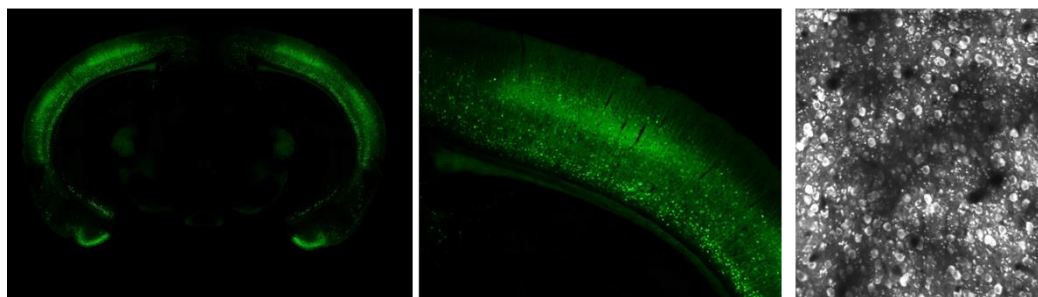
## Rorb-IRES2-Cre; Camk2a-tTA; Ai93 (TITL-GCaMP6f)

### Overview

Rorb-IRES2-Cre;CaMK2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Rorb* promotor, which results in Cre-mediated expression of GCaMP6f in excitatory neurons in cortical layer 4 (dense patches) and layers 5,6 (sparse) as well as in superior colliculus and thalamus.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



*Native gene expression:*

*in situ* hybridization of *Rorb* (NCBI Accession: N M\_146095.1) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/79556597>

*in situ* hybridization of *Camk2a* (NCBI Accession: NM\_009792.3) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/79490122>

### Growth and Development

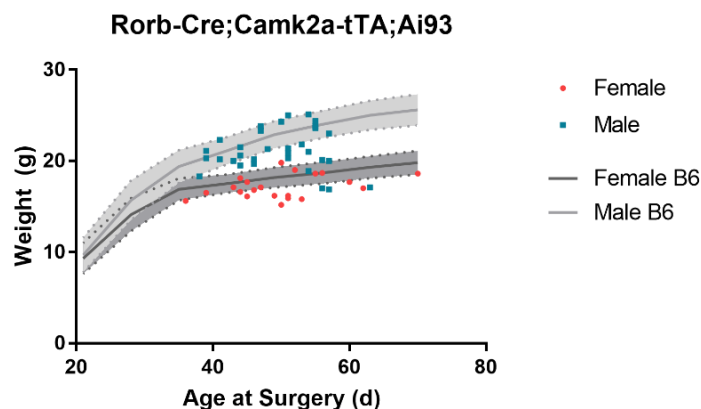
Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

Male:  $49.59 \pm 6.42$   
 Female:  $49.90 \pm 7.85$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $21.14 \pm 2.25$   
 Female:  $17.15 \pm 1.26$



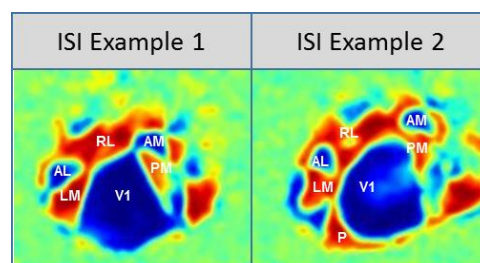
## Technical

**Breeding Considerations:** Originally both Rorb-IRES2-Cre heterozygotes and homozygotes were used for breeding. Although the desirable genotype for experimental animals was heterozygosity for all three transgenes, in some instances animals homozygous for GCaMP6f were included. Animals found to be homozygous for Cre were eliminated from the pipeline as these animals exhibited an unusual locomotor phenotype of "high stepping". The breeding scheme was changed to only generate heterozygous Cre animals. Crosses generating homozygous tTA animals were avoided due to tTA toxicity causing prenatal lethality. Germline deletion of the Ai93(TITL-GCaMP6f) LoxP-STOP-LoxP cassette is possible when crossing a Rorb-IRES2-Cre;Ai93(TITL-GCaMP6f) mouse with a mouse heterozygous for Camk2a-tTA. To avoid germline deletion of the stop cassette, breeding sets (pairs and trios) were comprised of either a heterozygous Rorb-IRES2-Cre mouse crossed with a heterozygous Camk2a-tTA;homozygous Ai93(TITL-GCaMP6f) mouse or a heterozygous Rorb-IRES2-Cre;heterozygous Camk2a-tTA mouse crossed with a homozygous Ai93(TITL-GCaMP6f) mouse. Rorb-IRES2-Cre is a knock-in allele at the stop codon of the *Rorb* gene.

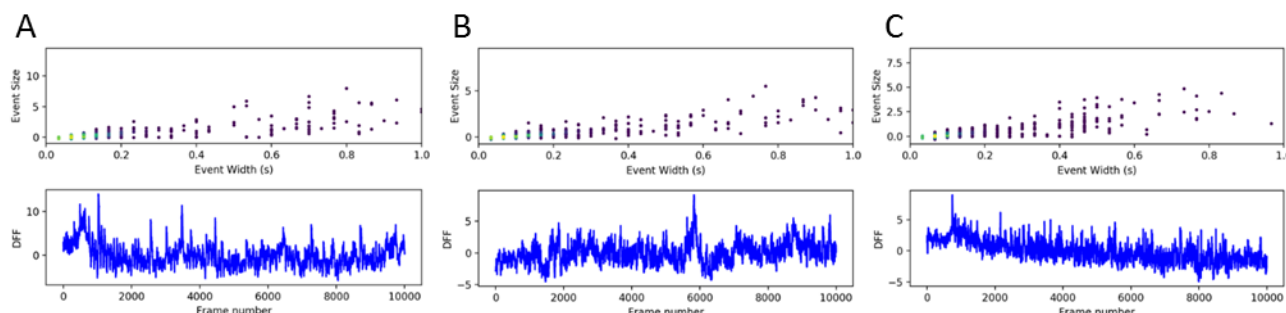
**General observations:** 2.96 % of 148 mice needed to be re-weighed to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32. Mice homozygous for Rorb-IRES2-Cre exhibited "high stepping". No abnormal phenotypes were observed for heterozygous mice.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=25, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Rorb-IRES2-Cre;CaMK2a-tTA;Ai93 mice are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **Interictal events were observed in experimental animals (2.2 % frequency). Data from these animals were excluded.**



## Summary

Homozygous Cre animals for this line exhibit "high stepping". Heterozygous Cre animals were used for breeding and experimental data collection.

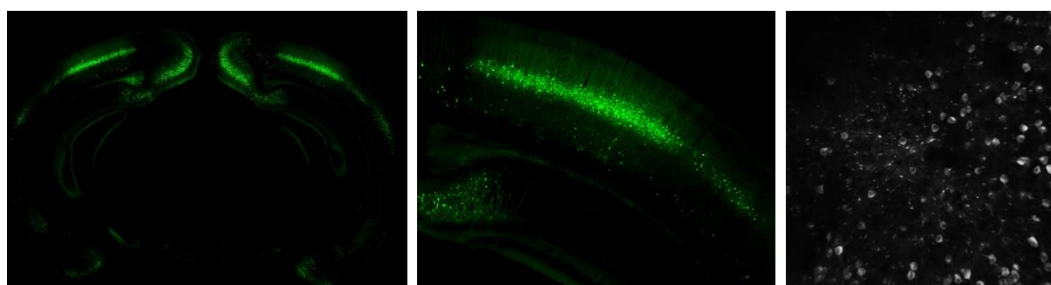
## Scnn1a-Tg3-Cre; Camk2a-tTA; Ai93 (TITL-GCaMP6f)

### Overview

Scnn1a-Tg3-Cre;Camk2a-tTA; Ai93(TITL-GCaMP6f) mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, and a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. The *Scnn1a* promoter confers further specificity, resulting in mice that exhibit GCaMP6f in excitatory neurons in cortical layer 4 and in restricted populations within the cortex, thalamus, and in cerebellum.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



*Native gene expression:*

*in situ* hybridization of *Scnn1a* (NCBI Accession: NM\_011324.1) in C57BL/6J; a subset of Scnn1a-positive neurons are represented in Tg3 strain.

<http://mouse.brain-map.org/experiment/show/70562125>

*in situ* hybridization of *Camk2a* (NCBI Accession: NM\_009792.3) in C57BL/6J

<http://mouse.brain-map.org/experiment/show/79490122>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

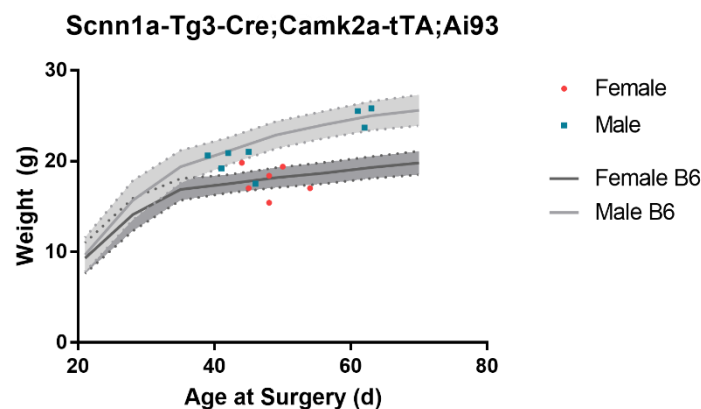
Male:  $49.87 \pm 10.2$

Female:  $48.16 \pm 3.60$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $21.77 \pm 2.96$

Female:  $17.83 \pm 1.67$





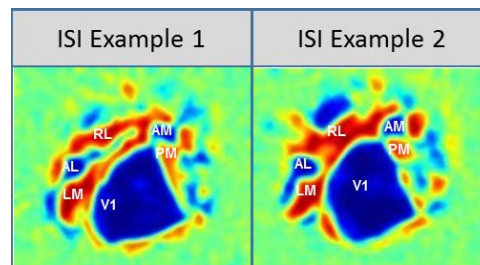
## Technical

**Breeding Considerations:** Breeding sets (pairs and trios) were comprised of two breeding schemes. The first was crossing a heterozygous Scnn1a-Tg3-Cre mouse to a Camk2a-tTA;Ai93(TITL-GCaMP6f) mouse that was heterozygous for tTA and either heterozygous or homozygous for GCaMP6f. The second scheme was crossing a heterozygous Scnn1a-Tg3-Cre; heterozygous Camk2a-tTA mouse crossed with a homozygous Ai93(TITL-GCaMP6f) mouse. In some cases, a heterozygous GCaMP6f mouse was used when homozygous GCaMP6f mice were unavailable. Crosses generating homozygous Cre and/or tTA were avoided as homozygous Cre and/or tTA causes undesirable phenotypes and/or prenatal lethality. To avoid germline deletion of the stop cassette, the breeding scheme avoided crossing a mouse consisting of Cre and GCaMP6f (Cre-dependent reporter) to a tTA mouse. This scheme could have resulted in the deletion of the LoxP-STOP-LoxP cassette causing expression in all cells specific and not specific to Cre. Scnn1a-Tg3-Cre is an endogenous bacterial artificial chromosome (BAC) transgene.

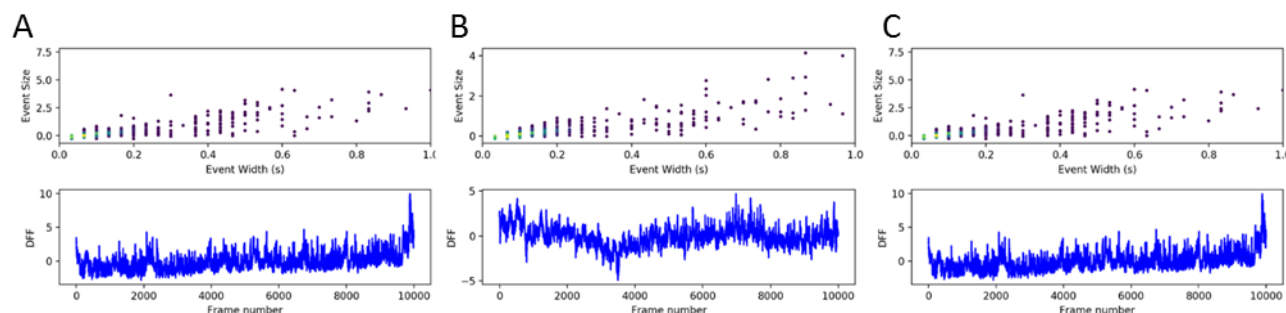
**General observations:** 25 of 85 mice (29.4%) needed to be re-weighed to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32. Fourteen mice were excluded due to insufficient surgical weight.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on  $n=7$ , higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93 mice are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **No interictal events were observed.**



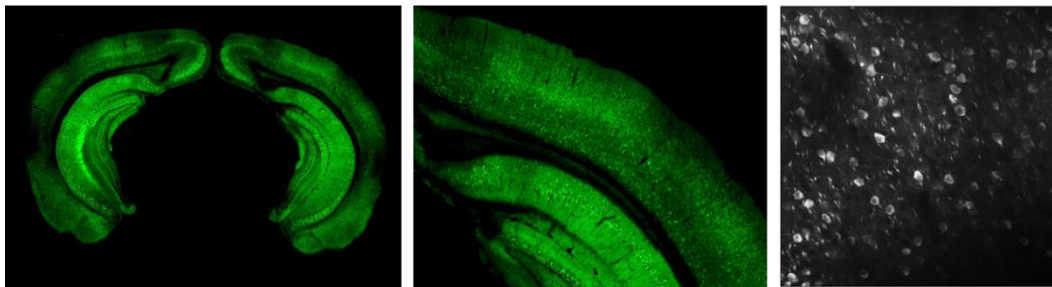
## Slc17a7-IRES2-Cre; Camk2-tTa; Ai93 (TITL-GCaMP6f)

### Overview

Slc17a7-IRES2-Cre; Camk2a-tTA; Ai93 (TITL-GCaMP6f) transgenic mice express a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons under control of the *Camk2a* promoter. Further specificity is achieved by the *Slc17a7* promoter, resulting in mice expressing GCaMP6f in excitatory neurons across all cortical layers.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



Native gene expression:

*in situ* hybridization of *Slc17a7* (NCBI Accession: x) in C57BL/6J

<http://mouse.brain-map.org/experiment/show/70436317>

*in situ* hybridization of *Camk2a* (NCBI Accession: NM\_009792.3) in C57BL/6J

<http://mouse.brain-map.org/experiment/show/79490122>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

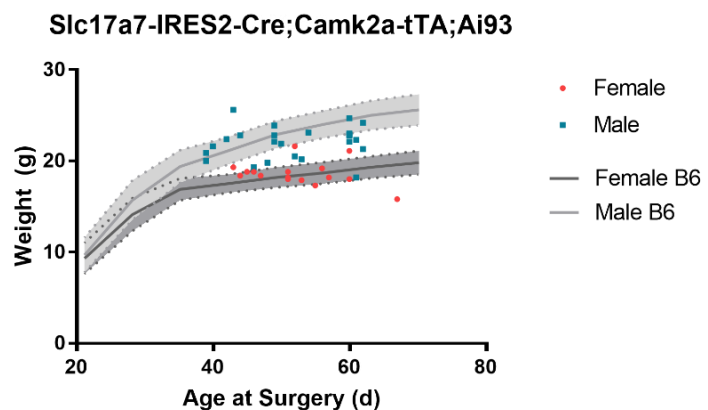
Male: 50.76  $\pm$  10.4

Female: 52.37  $\pm$  6.63

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male: 21.99  $\pm$  1.72

Female: 18.61  $\pm$  1.35



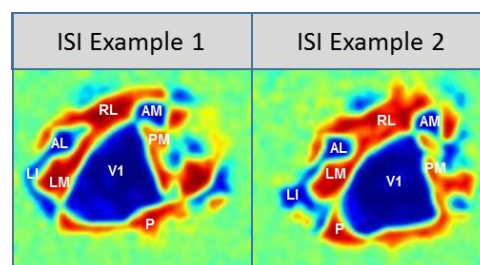
## Technical

**Breeding Considerations:** Breeding sets (pairs and trios) were comprised of crossing a heterozygous *Slc17a7-IRES2-Cre* mouse to a *Camk2a-tTA;Ai93(TITL-GCaMP6f)* mouse that is heterozygous for tTA and homozygous for GCaMP6f. Crosses generating homozygous tTA were avoided as tTA toxicity causes embryonic lethality. *Slc17a7-IRES2-Cre* (*Vglut1-IRES2-Cre-D*) is a knock-in allele at the stop codon of the *Slc17a7* gene and expresses in the mouse germline. Therefore, the breeding scheme should avoid crossing a mouse consisting of Cre and GCaMP6f (Cre-dependent reporter) to a tTA mouse. This scheme would result in the deletion of the LoxP-STOP-LoxP cassette causing expression in all cells specific and not specific to Cre.

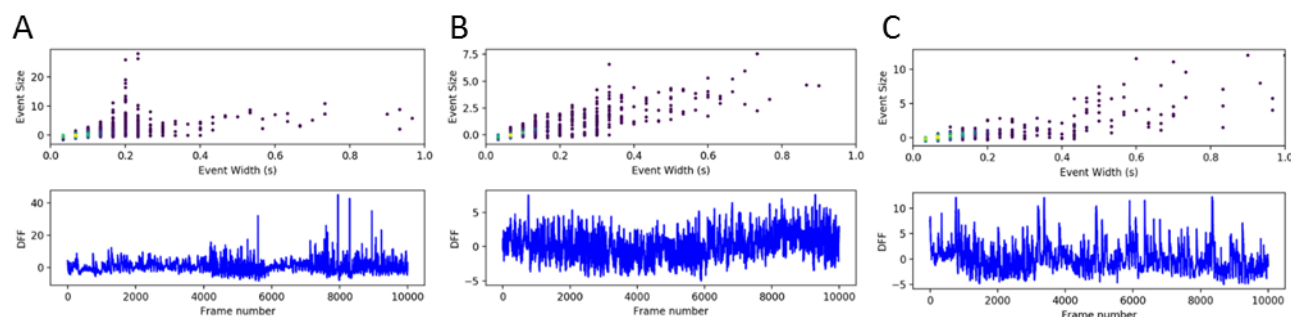
**General observations:** 26.67% of 147 mice needed to be re-weighed to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32. 2% of the mice were excluded from experiments due to eye anomalies.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on  $n=27$ , higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two *Slc17a7-IRES2-Cre;Camk2a-tTA;Ai93* mice are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **Interictal events were observed in pipeline animals (8.3 % of animals, example in panel A below). Data from these animals was excluded.**



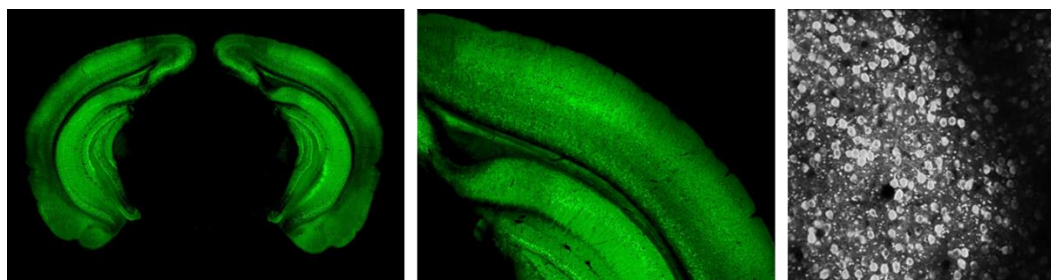
## Slc17a7-IRES2-Cre; Camk2a-tTa; Ai94 (TITL-GCaMP6s)

### Overview

Slc17a7-IRES2-Cre; Camk2a-tTA; Ai94(TITL-GCaMP6s) transgenic mice express a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6s, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Slc17a7* promoter, resulting in mice that express GCaMP6s in excitatory neurons across all layers of cortex.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



Native gene expression:

*in situ* hybridization of *Slc17a7* (NCBI Accession: x) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/70436317>

*in situ* hybridization of *Camk2a* (NCBI Accession: NM\_009792.3) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/79490122>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

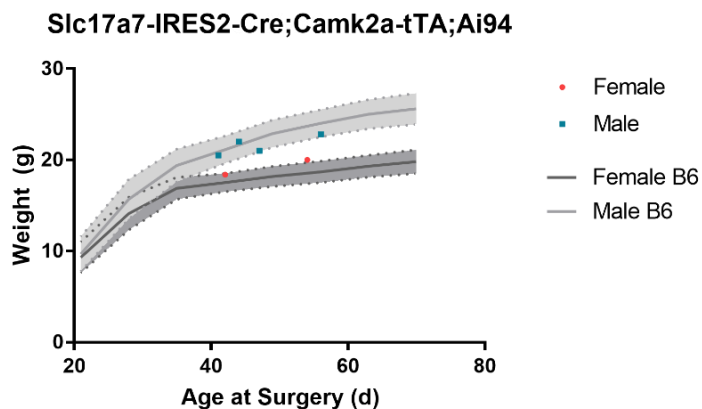
Male:  $47 \pm 6.48$

Female:  $48 \pm 8.48$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $21.57 \pm 1.02$

Female:  $19.2 \pm 1.13$



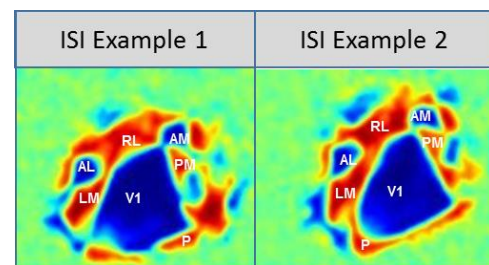
## Technical

**Breeding Considerations:** Breeding sets (pairs and trios) were comprised of crossing a heterozygous *Slc17a7-IRES2-Cre* mouse to a *Camk2a-tTA;Ai94(TITL-GCaMP6s)* mouse that is heterozygous for tTA and homozygous for GCaMP6f. Crosses generating homozygous tTA were avoided as tTA toxicity causes embryonic lethality. *Slc17a7-IRES2-Cre* (*Vglut1-IRES2-Cre-D*) is a knock-in allele at the stop codon of the *Slc17a7* gene and expresses in the mouse germline. Therefore, the breeding scheme should avoid crossing a mouse consisting of Cre and GCaMP6s (Cre-dependent reporter) to a tTA mouse. This scheme would result in the deletion of the LoxP-STOP-LoxP cassette causing expression in all independent of Cre expression.

**General observations:** 21.4% of 14 mice needed to be re-weighted to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=4, higher visual areas (HVA) being used for imaging in the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two *Slc17a7-IRES2-Cre;Camk2a-tTA;Ai94* mice are shown.



**Screening for interictal events.** Based on experiments and simulation of calcium response profile of GCaMP6s, we could not distinguish normal spontaneous activity from abnormal interictal events for the slow GCaMP6. We therefore did not exclude animals based on calcium event properties.



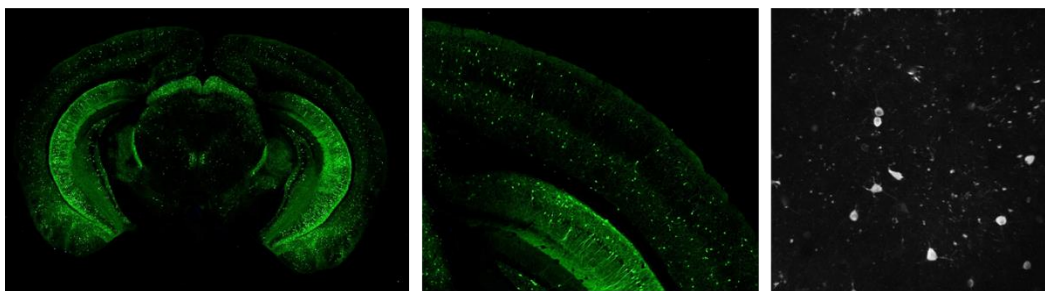
## Sst-IRES-Cre; Ai148 (TIT2L-GCaMP6f-ICL-tTA2)

### Overview

Sst-IRES-CRE; Ai148 (TIT2L-GCaMP6f-ICL-tTA2) transgenic mice express GCaMP6f in cells expressing Cre under control of the Sst promoter, resulting in GCaMP6f expression in scattered cells throughout the brain. Localized areas of enrichment include restricted populations in thalamus, amygdala, midbrain, hindbrain, cortical subplate, and Purkinje cell layer.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



*Native gene expression:*

*in situ* hybridization of Sst (NCBI Accession: x) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/1001>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

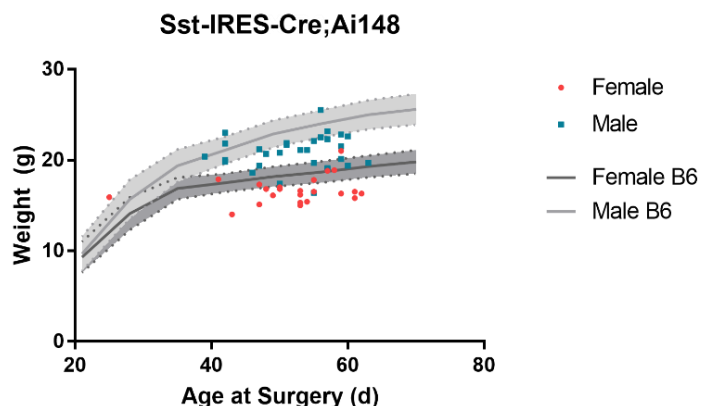
Male:  $52.48 \pm 6.40$

Female:  $51.86 \pm 8.14$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $20.85 \pm 1.80$

Female:  $16.66 \pm 1.50$



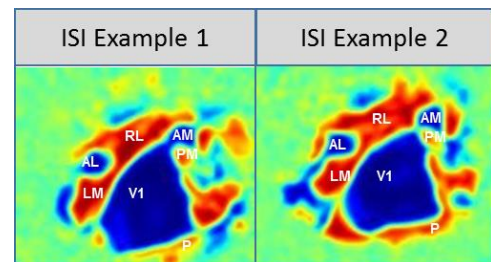
## Technical

**Breeding Considerations:** Sst-IRES-Cre is a knock-in allele. Breeding sets (pairs and trios) consisted of crossing a heterozygous Sst-IRES-Cre mouse to either a heterozygous or homozygous Ai148(TIT2L-GCaMP6f-ICL-tTA2).

**General observations:** 27 of 96 mice needed to be re-weighted to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32; however no mice were excluded due to insufficient weight. Dermatitis appeared in some animals around p100 or earlier in the following pattern: on the back of neck, under chin and at base of and/or on tail. 15.63% of mice developed either or both dermatitis and rectal prolapse and were excluded. 4% of the mice were excluded due to eye anomalies. These health observations should be considered in the breeding strategy of this line.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=34, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Sst-IRES-CRE;Ai148 mice are shown.



**Screening for interictal events.** Labelling in inhibitory lines was too sparse to assess with confidence the presence of interictal events through calcium imaging. We therefore did not exclude animals based on calcium events properties.

## Summary

This line is prone to dermatitis and rectal prolapse; animals require careful monitoring for these health issues.

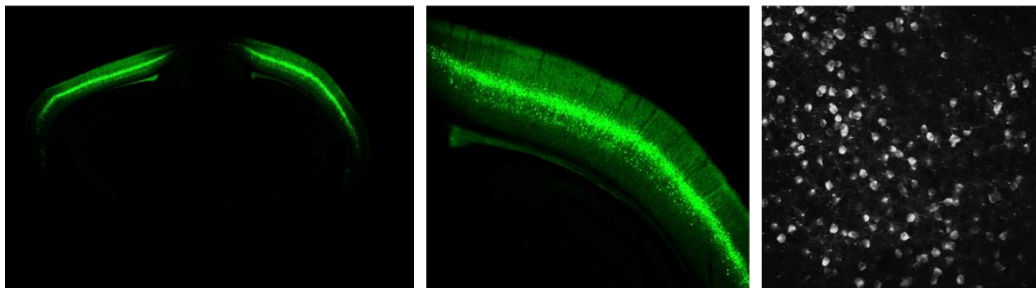
## Tlx3-Cre\_PL56; Ai148 (TIT2L-GCaMP6f-ICL-tTA2)

### Overview

Tlx3-Cre\_PL56; Ai148 (TIT2L-GCaMP6f-ICL-tTA2) transgenic mice express GCaMP6f in cells expressing Cre under control of the Tlx3 promoter, resulting in GCaMP6f expression in scattered cells throughout the brain. GCaMP6f expression is enriched in layer 5a of cortex and in restricted populations of pons and medulla.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



Native gene expression:

*in situ* hybridization of *Tlx3* (NCBI Accession: x) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/71609033>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from the Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

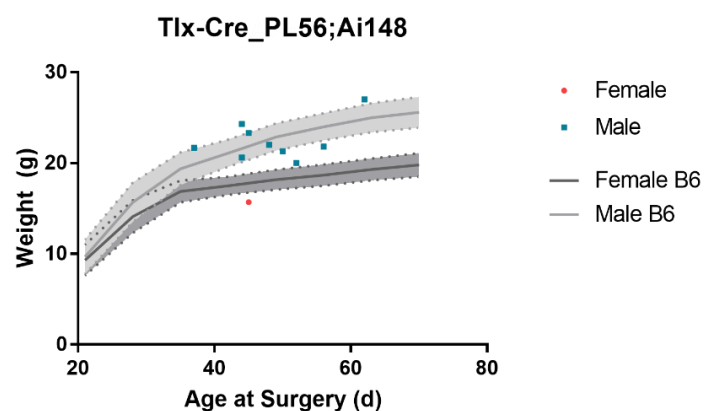
Male:  $48.67 \pm 7.4$

Female: 45

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $22.44 \pm 2.14$

Female: 15.7



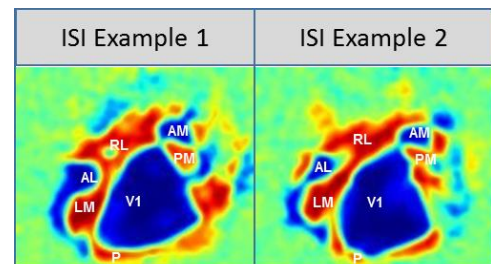
## Technical

**Breeding Considerations:** Tlx3-Cre\_PL56 is a Bacterial Artificial Chromosome (BAC) transgene and is expressed in the female germline. Breeding sets included (pairs and trios) of crossing a heterozygous Tlx3-Cre\_PL56 mouse to either a heterozygous or homozygous Ai148 (TIT2L-GCaMP6f-ICL-tTA2) mouse.

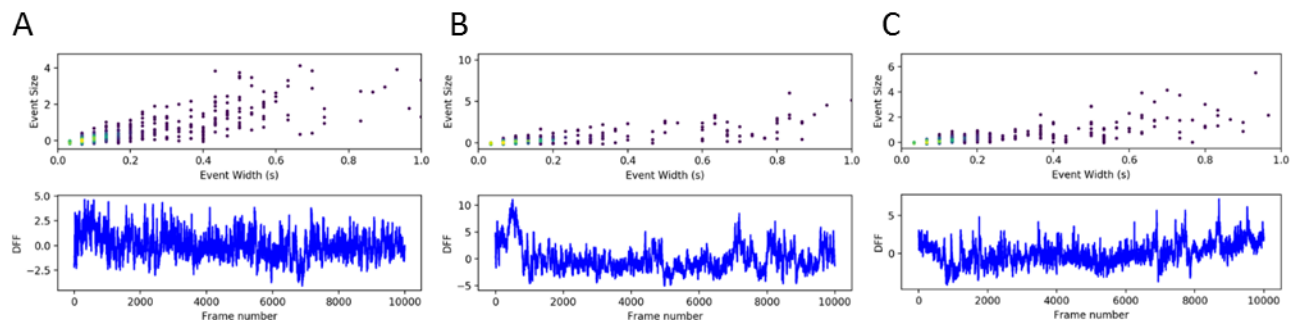
**General observations:** 20% of 49 mice needed to be re-weighted to meet the eligible surgical weight (18.3g Male and 15g Female) criteria at p32. No mice were excluded due to insufficient weight. Three mice were excluded due to microphthalmia, malocclusion, and hydrocephalus.

## Other Characterization

**Intrinsic signal imaging.** Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=10, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Tlx3-Cre\_PL56; Ai148 mice are shown.



**Screening for interictal events.** Representative animals from each line were screened for abnormal calcium activity such as epileptiform interictal events that may be a consequence of GCaMP6 expression (see Visual Coding Overview). The quantification of full FOV calcium events amplitude and width (top) and prominence and width of all detected calcium events in a 5 min recording session (bottom; shows  $\Delta F/F$  traces for the entire field of view) using two photon calcium imaging in 3 animals. Interictal events are typically large and short events that showcase whole field large fluctuation ( $>10\%$   $\Delta F/F$ ) in calcium associated with very short transients ( $<300$  ms prominence). **No evidence of interictal events were observed.**



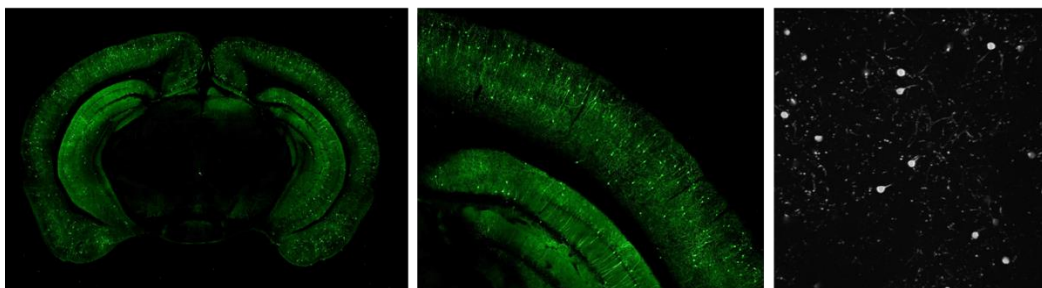
## Vip-IRES-Cre; Ai148 (TIT2L-GCaMP6f-ICL-tTA2)

### Overview

Vip-IRES-Cre; Ai148 (TIT2L-GCaMP6f-ICL-tTA2) transgenic mice express GCaMP6f in cells expressing Cre under control of the Vip promoter, resulting in GCaMP6f expression in scattered cells throughout the brain. GCaMP6f is expressed in scattered cells throughout the brain, with enriched expression in superficial cortical layers and restricted populations in hindbrain and midbrain.

### Transgene Expression

Native GCaMP6 expression (green) was imaged using 2P tomography from perfused brains (*left, full coronal plane; middle, visual cortex*). An example field-of-view obtained during calcium imaging is also shown (*right*).



*Native gene expression:*

*in situ* hybridization of *Vip* (NCBI Accession: x) in C57BL/6J  
<http://mouse.brain-map.org/experiment/show/77371835>

### Growth and Development

Mouse weights from this transgenic line collected just prior to surgery (p37-p63) compared to standard C57BL/6J growth curves obtained from Jackson Laboratory.

#### Mean ( $\pm$ SD) Age at surgery (days):

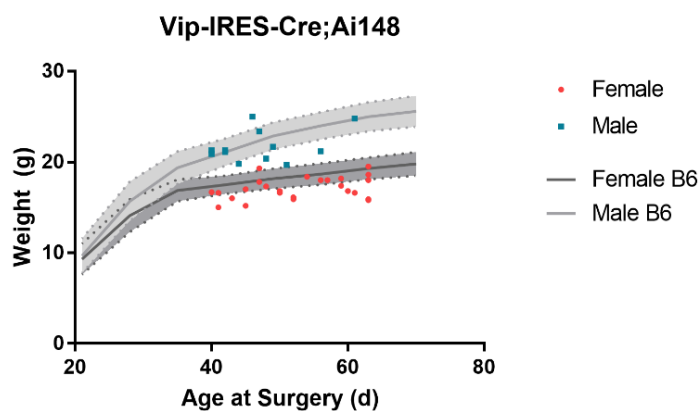
Male:  $47.16 \pm 6.43$

Female:  $52.88 \pm 7.93$

#### Mean ( $\pm$ SD) Weight at surgery (g):

Male:  $21.71 \pm 1.76$

Female:  $17.1 \pm 1.20$





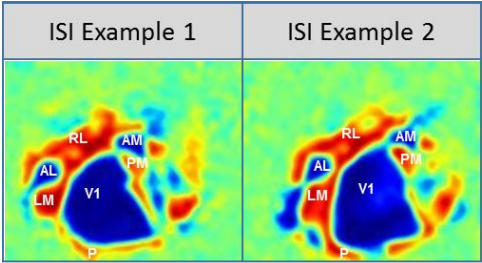
Technical

*Breeding Considerations:* Vip-IRES-Cre is a bacterial artificial chromosome (BAC) transgene. Breeding sets (pairs and trios) consisted of crossing a heterozygous Vip-IRES-Cre mouse to either a heterozygous or homozygous Ai148(TIT2L-GCaMP6f-ICL-tTA2).

*General observations:* 33 of 85 mice needed to be re-weighed to meet eligible surgical weight (18.3g Male and 15g Female) criteria at p32. One mouse was excluded due to insufficient weight (surgical age cut-off: P63). Seven mice were excluded due to eye anomalies (microphthalmia, anophthalmia and/or cloudy eyes).

Other Characterization

*Intrinsic signal imaging.* Intrinsic signal imaging provides a map of retinotopic space that may identify abnormalities in the visual pathway. Based on n=25, higher visual areas (HVA) being used for imaging for the Allen Brain Observatory were all present and largely targetable based on ability to identify the appropriate retinotopic location. Example images of ISI-based functional area maps from two Vip-IRES-Cre; Ai148 mice are shown.



*Screening for interictal events.* Labelling in inhibitory lines was too sparse to assess with confidence the presence of interictal events through calcium imaging. We therefore did not exclude animals based on calcium events properties.

## REFERENCES

**Ai93(TITL-GCaMP6f)**Source: <https://www.jax.org/strain/024103>**Originating Lab:** Allen Institute for Brain Science

Madisen L, Garner AR, Shimaoka D, Chuong AS, Klapoetke NC, Lu L, van der Bourg A, Niino Y, Egolf L, Monetti C, Gu H, Mills M, Cheng A, Tasic B, Nguyen TN, Sunkin SM, Benucci A, Nagy A, Miyawaki A, Helmchen F, Empson RM, Knoepfel T, Boyden ES, Reid RC, Carandini M, Zeng H. (2015) Transgenic mice for intersectional targeting of neural sensors and effectors with high specificity and performance. *Neuron* 85(5):942-958.

**Ai94(TITL-GCaMP6s)**Source: <https://www.jax.org/strain/024104>**Originating Lab:** Allen Institute for Brain Science

Madisen L, Garner AR, Shimaoka D, Chuong AS, Klapoetke NC, Lu L, van der Bourg A, Niino Y, Egolf L, Monetti C, Gu H, Mills M, Cheng A, Tasic B, Nguyen TN, Sunkin SM, Benucci A, Nagy A, Miyawaki A, Helmchen F, Empson RM, Knoepfel T, Boyden ES, Reid RC, Carandini M, Zeng H. (2015) Transgenic mice for intersectional targeting of neural sensors and effectors with high specificity and performance. *Neuron* 85(5):942-958.

**Ai148(TIT2L-GCaMP6f-ICL-tTA2)**Source: <https://www.jax.org/strain/030328>**Originating Lab:** Allen Institute for Brain Science

A suite of transgenic driver and reporter mouse lines with enhanced brain cell type targeting and functionality. Daigle TL, Madisen L, Hage TA, Valley MT, Knoblich U, Larsen RS, Takeno MM, Huang L, Gu H, Larsen R, Mills M, Bosma-Moody A, Siverts L, Walker M, Graybuck LT, Yao Z, Fong O, Garren E, Lenz G, Chavarha M, Pendergraft J, Harrington J, Hirokawa KE, Harris JA, McGraw M, Ollerenshaw DR, Smith K, Baker CA, Ting JT, Sunkin SM, Lecoq J, Lin MZ, Boyden ES, Murphy GJ, da Costa N, Waters J, Li L, Tasic B, Zeng H. bioRxiv 224881; doi: <https://doi.org/10.1101/224881>

**Ai162(TIT2L-GCaMP6s-ICL-tTA2)**Source: <https://www.jax.org/strain/031562>**Originating Lab:** Allen Institute for Brain Science

Daigle TL; Madisen L; Hage TA; Valley MT; Knoblich U; Larsen RS; Takeno MM; Huang L; Gu H; Larsen R; Mills M; Bosma-Moody A; Siverts L; Walker M; Graybuck LT; Yao Z; Fong O; Garren E; Lenz G; Chavarha M; Pendergraft J; Harrington J; Hirokawa KE; Harris JA; McGraw M; Ollerenshaw DR; Smith K; Baker CA; Ting JT; Sunkin SM; Lecoq J; Lin MZ; Boyden ES; Murphy GJ; da Costa N; Waters J; Li L; Tasic B; Zeng H. 2018. A suite of transgenic driver and reporter mouse lines with enhanced brain cell type targeting and functionality *Cell* 174(2):465-480.e22. PubMed: 30007418MGI: J:260362

**Camk2a-tTA**Source: <https://www.jax.org/strain/007004>**Originating Lab:** Mark Mayford

Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER. (1996) Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83. PubMed: 8939850 MGI: J:37107.

**Cux2-CreERT2**Source: [https://www.mmrrc.org/catalog/sds.php?mmrrc\\_id=32779](https://www.mmrrc.org/catalog/sds.php?mmrrc_id=32779)**Originating Lab:** Ulrich Mueller

Franco SJ, Gil-Sanz C, Martinez-Garay I, Espinosa A, Harkins-Perry SR, Ramos C, Müller U. (2012) Fate-restricted neural progenitors in the mammalian cerebral cortex. *Science* 337(6095):746-9. Gil-Sanz C, Espinosa A, Fregoso SP, Bluske KK, Cunningham CL, Martinez-Garay I, Zeng H, Franco SJ, Müller U. (2015) Matters Arising: Lineage tracing using Cux2-Cre and Cux2-CreERT2 mice. *Neuron* 86 (4): 1091-1099.

**Emx1-IRES-Cre**Source: <https://www.jax.org/strain/005628>**Originating Lab:** Kevin Jones

Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR. (2002) Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J Neurosci* 22(15):6309-14.

**Fezf2-CreER**

**Source:** Cold Spring Harbor Laboratories

**Originating Lab:** Z. Josh Huang

*Unpublished*

**Nr5a1-Cre**

**Source:** <https://www.jax.org/strain/006364>

**Originating Lab:** Bradford B. Lowell

Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, Kenny CD, Christiansen LM, White RD, Edelstein EA, Coppari R, Balthasar N, Cowley MA, Chua S Jr, Elmquist JK, Lowell BB. (2006) Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 49(2):191-203.

**Ntsr1-Cre\_GN220**

**Source:** [https://www.mmrrc.org/catalog/sds.php?mmrrc\\_id=30648](https://www.mmrrc.org/catalog/sds.php?mmrrc_id=30648)

**Originating Labs:** Nathaniel Heintz & Charles Gerfen

Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci*. 2007 Sep 12;27(37):9817-23.

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature*. 2003 Oct 30;425(6961):917-25.

**Pvalb-IRES-Cre**

**Source:** <https://www.jax.org/strain/008069>

**Originating Lab:** Silvia Arber, Friedrich Miescher Institute

Hippenmeyer S; Vrieseling E; Sigrist M; Portmann T; Laengle C; Ladle DR; Arber S. 2005. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLoS Biol* 3(5):e159

**Rbp4-Cre\_KL100**

**Source:** [https://www.mmrrc.org/catalog/sds.php?mmrrc\\_id=31125](https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31125)

**Originating Labs:** Nathaniel Heintz & Charles Gerfen

Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR. (2007) Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* 27(37):9817-23.

Gerfen CR, Paletzki R, Heintz N. (2013) GENSAT BAC cre-recombinase driver lines to study the functional organization of cerebral cortical and basal ganglia circuits. *Neuron* Dec 18;80(6):1368-83.

**Rorb-IRES2-Cre**

**Source:** <https://www.jax.org/strain/023526>

**Originating Lab:** Allen Institute for Brain Science

Harris JA, Hirokawa KE, Sorensen SA, Gu H, Mills M, Ng LL, Bohn P, Mortrud M, Ouellette B, Kidney J, Smith KA, Dang C, Sunkin S, Bernard A, Oh SW, Madisen L, Zeng H. (2014) Anatomical characterization of Cre driver mice for neural circuit mapping and manipulation. *Front Neural Circuits* 8:76.

**Scnn1a-Tg3-Cre**

**Source:** <https://www.jax.org/strain/009613>

**Originating Lab:** Allen Institute for Brain Science

Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

**Slc17a7-IRES2-Cre**

**Source:** <https://www.jax.org/strain/023527>

**Originating Lab:** Allen Institute for Brain Science

Harris JA, Hirokawa KE, Sorensen SA, Gu H, Mills M, Ng LL, Bohn P, Mortrud M, Ouellette B, Kidney J, Smith KA, Dang C, Sunkin S, Bernard A, Oh SW, Madisen L, Zeng H. 2014. Anatomical characterization of Cre driver

mice for neural circuit mapping and manipulation. *Front Neural Circuits* 8:76. PubMed: 25071457 MGI: J:220523.

#### **Sst-IRES-Cre**

**Source:** <https://www.jax.org/strain/013044>

**Originating Lab:** Z. Josh Huang

NIH Neuroscience Blueprint Cre Driver Network. 2009. Cre recombinase-expressing mice generated for the NIH Neuroscience Blueprint Cre Driver Network MGI Direct Data Submission: MGI: J:151755

Taniguchi H; He M; Wu P; Kim S; Paik R; Sugino K; Kvitsani D; Fu Y; Lu J; Lin Y; Miyoshi G; Shima Y; Fishell G; Nelson SB; Huang ZJ. 2011. A resource of cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron* 71(6):995-1013. PubMed: 21943598 MGI: J:177261

#### **Tlx3-Cre\_PL56**

**Source:** [https://www.mmrrc.org/catalog/sds.php?mmrrc\\_id=41158](https://www.mmrrc.org/catalog/sds.php?mmrrc_id=41158)

**Originating Labs:** Nathaniel Heintz and Charles Gerfen

Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci*. 2007 Sep 12;27(37):9817-23. (Full Text Journal Article).

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature*. 2003 Oct 30;425(6961):917-25. (Medline PMID: 14586460).

#### **Vip-IRES-Cre**

**Source:** <https://www.jax.org/strain/010908>

**Originating Lab:** Z. Josh Huang

NIH Neuroscience Blueprint Cre Driver Network. 2009. Cre recombinase-expressing mice generated for the NIH Neuroscience Blueprint Cre Driver Network MGI Direct Data Submission :. MGI: J:151755

Taniguchi H; He M; Wu P; Kim S; Paik R; Sugino K; Kvitsani D; Fu Y; Lu J; Lin Y; Miyoshi G; Shima Y; Fishell G; Nelson SB; Huang ZJ. 2011. A resource of cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron* 71(6):995-1013. PubMed: 21943598 MGI: J:177261

Steinmetz NA, Buettnering C, Lecoq J, Lee CR, Peters AJ, et al. 2017. Aberrant cortical activity in multiple GCaMP6-expressing transgenic mouse lines. *bioRxiv* 138511; doi: <https://doi.org/10.1101/138511>