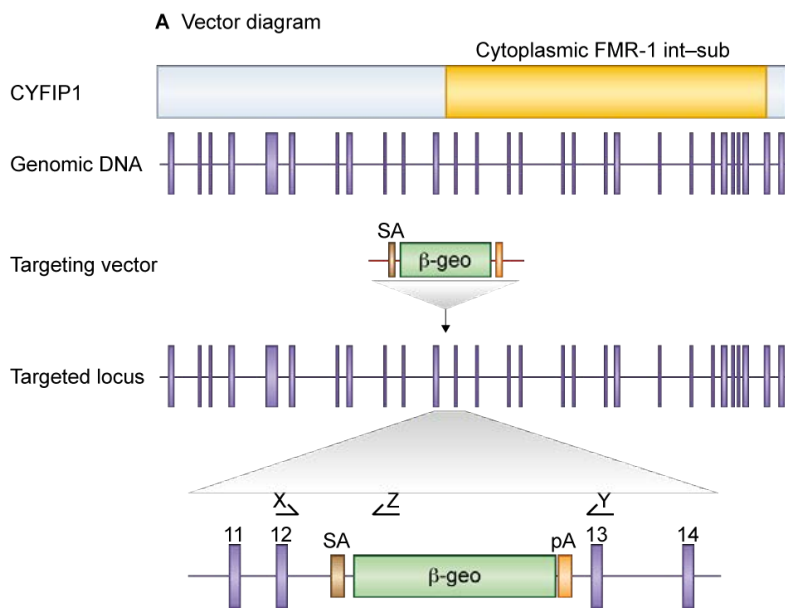


# *Cyfp1* (CYFIP1)

## 1 Mouse generation

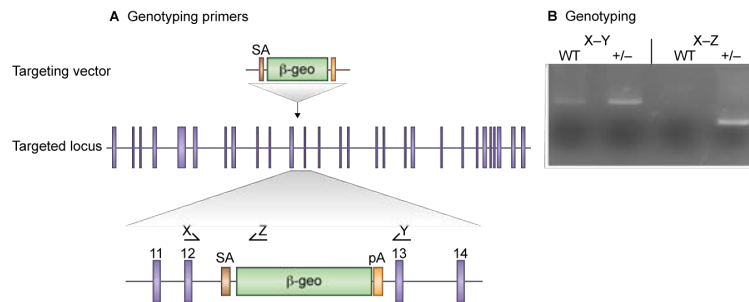
### 1.1 Mutation



**Figure 1:** *Details of the mutation*

A mouse embryonic stem (ES) cell line (XG769, strain 129/Ola) with an insertional mutation in *Cyfp1* was obtained from BayGenomics ([baygenomics.ucsf.edu/](http://baygenomics.ucsf.edu/)). The insertional mutation in XG769, by the gene-trapping vector, pGT1lxf, that was designed to create an in-frame fusion between the 5' exons of the trapped gene and a reporter,  $\beta$ -geo (a fusion of  $\beta$ -galactosidase and neomycin phosphotransferase II), occurred in intron 12-13. Thus, the gene-trapped locus is predicted to yield a fusion transcript containing exons 1-12 of *Cyfp1* and beta-geo. The ES cells were injected into C57BL/6 blastocysts to create chimeric mice, which were bred with 129S5 mice to generate heterozygous *Cyfp1* mutant mice.

## 1.2 Genotyping



**Figure 2:** *Genotyping details*

Genomic DNA was isolated from ES cells or mouse tissues by Wizard SV 96 Genomic DNA purification system (Promega Cat A2371). Genotyping PCR consisted of a 1.2kb product amplified from the wild-type (wt) allele using a forward primer X and a reverse primer Y as above. A 1kb product was amplified from the targeted allele using primer X with reverse primer Z (5'- GGTACGTTGGTGTAGATGG -3'), within the  $\beta$ -geo cassette. After enzymatic amplification for 35 cycles (45 seconds at 94 degC, 45 seconds at 55 degC, and 1 minute at 72 degC), the PCR products were size-fractionated on a 2% agarose gel in 1x Tris borate-EDTA buffer. Primers used for genotyping (X,Y, Z). PCR genotyping of gene trap Zm mice using a common forward primer, X, and reverse primers Y and Z to amplify the wt and mutant alleles respectively.

### 1.3 Breeding

No  $Zm^{-/-}$  mice were produced from  $Zm^{+/-}$  intercrosses. Male and female  $Zm^{+/-}$  mice developed normally to adulthood, were fertile, exhibited normal body size and no gross abnormalities. Genotypes of 3-week-old pups from  $Zm^{+/-}$  intercrosses identified 8 wt and 18  $Zm^{+/-}$  progeny (Chi-squared  $p=0.0125$ ). Backcrosses onto the 129S5/SvEvBrd background were used to maintain the colony and to generate heterozygous and wildtype mice to study.

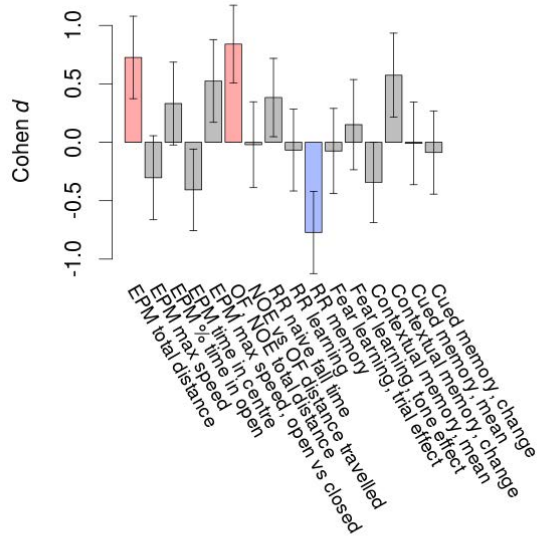
## 2 Behaviour

### 2.1 Definitions

**Table 1:** *Definitions of Behaviour Variables*

| Experiment              | Variable                      | Units            | Description                                                                                                                                                                   |
|-------------------------|-------------------------------|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Elevated Plus Maze      | EPM total distance            | cm               | Total distance (cm) travelled in any arm or central zone of the EPM                                                                                                           |
| Elevated Plus Maze      | EPM max speed                 | cm/s             | Maximum speed (cm/s) travelled in any arm or central zone of the EPM                                                                                                          |
| Elevated Plus Maze      | EPM % time in open            | percent          | Percentage of time in the open or closed arms of the EPM spent in open arms                                                                                                   |
| Elevated Plus Maze      | EPM time in centre            | s                | Total time (s) spent in the central zone of the EPM                                                                                                                           |
| Elevated Plus Maze      | EPM max speed, open vs closed | cm/s             | Difference between the maximum speed (cm/s) observed in the open arms and the closed arms of the EPM                                                                          |
| Open Field/Novel Object | OF, NOE total distance        | log10 cm         | Total distance travelled (log10 cm) during initial exposure to the open field and in presence of the novel object                                                             |
| Open Field/Novel Object | NOE vs OF distance travelled  | cm               | Difference in distance travelled (cm) in presence of the novel object and during initial exposure to open field                                                               |
| Rotarod                 | RR naive fall time            | log10 s          | Fall time on accelerating rotarod (log10 s), naive performance in session 1                                                                                                   |
| Rotarod                 | RR learning                   | s/trial          | Learning on rotarod, measured as increase in fall time per trial (s/trial) in session 1                                                                                       |
| Rotarod                 | RR memory                     | s                | Memory on rotarod, measured as excess fall time at middle of session 2 relative to middle of session 1                                                                        |
| Fear Training           | Fear learning, trial effect   | percent freezing | Fear learning, measured as extra % time freezing before third trial compared to % time freezing before first trial                                                            |
| Fear Training           | Fear learning, tone effect    | percent freezing | Fear learning, measured as increase in % time freezing due to third tone compared to increase in % time freezing due to first tone                                            |
| Contextual Memory       | Contextual memory, mean       | percent freezing | Contextual memory, measured as difference in % time freezing during first 120 s re-exposure to the box compared to first 120 s in the box on previous day                     |
| Contextual Memory       | Contextual memory, change     | percent freezing | Contextual memory, measured as increase in % time spent freezing from first time bin of 30 s to fourth bin of 30 s during 120 s re-exposure to the box                        |
| Cued Memory             | Cued memory, mean             | percent freezing | Cued memory, measured as increase in % time spent freezing during 120 s of tone re-exposure compared to increase in % time spent freezing during initial tone on previous day |
| Cued Memory             | Cued memory, change           | percent freezing | Cued memory, measured as increase in % time spent freezing from first time bin of 30 s to fourth bin of 30 s during 120 s re-exposure to the tone                             |

## 2.2 Behaviour Overview



**Figure 3:** *Behaviour Overview. Alteration in a repertoire of eight innate and eight learned behaviour variables is shown. Cohen d effect sizes of the mutation are presented  $\pm$  SE.*

Mutant mice showed little overall behavioural difference from wildtypes, with three of 16 behaviour variables significantly impacted in these mutant mice. In the elevated plus maze task, one behavioural variable, EPM total distance, was significantly increased in mutants. In the open field/novel object exploration task, one behavioural variable, OF NOE total distance, was significantly increased in mutants. In the rotarod task, one behavioural variable, RR memory, was significantly decreased in mutants. Fear learning, contextual memory and cued memory tasks were unaffected. Definitions of variables may be found below.

## 2.3 Elevated Plus Maze - Innate/Instinctive Behaviour

**Table 2:** *Analysis of Innate or Instinctive Variables: mean(SEM) of behaviour scores for wildtype and mutant males and females, with p-values of differences*

| Variable                      | Units | Wildtype M (n=9) | Wildtype F (n=8) | Mutant M (n=10) | Mutant F (n=8) | P(sex x mutation) | P(mutation) |
|-------------------------------|-------|------------------|------------------|-----------------|----------------|-------------------|-------------|
| EPM total distance            | cm    | 670 (26)         | 762 (53)         | 833 (63)        | 788 (31)       | 0.16              | 0.044 *     |
| EPM max speed                 | cm/s  | 17.5 (1.3)       | 19.2 (1.4)       | 17.5 (0.6)      | 17.2 (0.7)     | 0.35              | 0.4         |
| EPM percent time in open      | %     | 19.3 (5.3)       | 10.8 (6.6)       | 25 (8.1)        | 17 (4.5)       | 0.96              | 0.37        |
| EPM time in centre            | s     | 161 (12)         | 113 (18)         | 129 (10)        | 109 (20)       | 0.35              | 0.2         |
| EPM max speed, open vs closed | cm/s  | -7.7 (1.4)       | -10.9 (2.4)      | -7.6 (1.1)      | -6 (1.3)       | 0.13              | 0.14        |

EPM total distance: Total distance (cm) travelled in any arm or central zone of the EPM

EPM max speed: Maximum speed (cm/s) travelled in any arm or central zone of the EPM

EPM percent time in open: Percentage of time in the open or closed arms of the EPM spent in open arms

EPM time in centre: Total time (s) spent in the central zone of the EPM

EPM max speed, open vs closed: Difference between the maximum speed (cm/s) observed in the open arms and the closed arms of the EPM

## 2.4 Open Field & Novel Object Exploration - Innate/Instinctive Behaviour

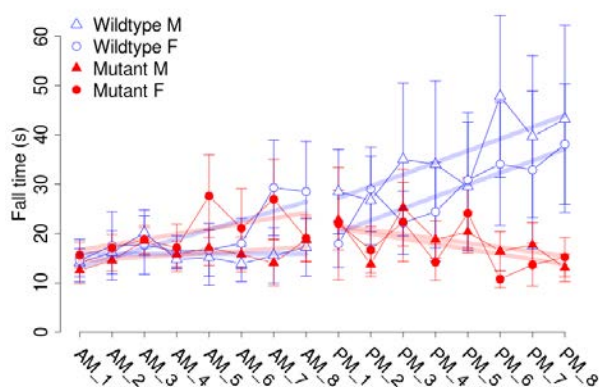
**Table 3:** Analysis of Innate or Instinctive Variables: mean(SEM) of behaviour scores for wildtype and mutant males and females, with p-values of differences

| Variable                     | Units    | Wildtype M (n=9) | Wildtype F (n=8) | Mutant M (n=10) | Mutant F (n=8) | P(sex x mutation) | P(mutation) |
|------------------------------|----------|------------------|------------------|-----------------|----------------|-------------------|-------------|
| OF, NOE total distance       | log10 cm | 2.88 (0.04)      | 3.03 (0.08)      | 3.15 (0.09)     | 3.16 (0.12)    | 0.41              | 0.022 *     |
| NOE vs OF distance travelled | cm       | -101 (44)        | 246 (220)        | -118 (245)      | 260 (207)      | 0.94              | 0.99        |

OF, NOE total distance: Total distance travelled (log10 cm) during initial exposure to the open field and in presence of the novel object

NOE vs OF distance travelled: Difference in distance travelled (cm) in presence of the novel object and during initial exposure to open field

## 2.5 Motor Behaviour - Innate/Instinctive Ability, Learning & Memory



**Figure 4:** RR histories. Mutant and wildtype (WT) males and females as indicated. Data represent mean  $\pm$  SEM.

**Table 4:** RR analysis: mean(SEM) of behaviour scores for wildtype and mutant males and females, with p-values of differences

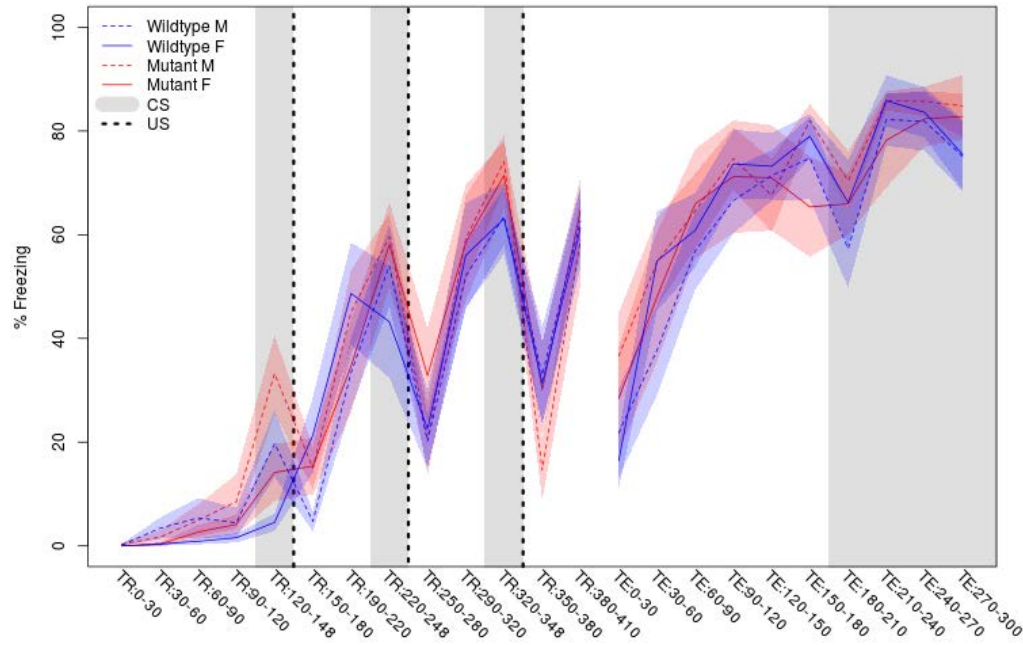
| Variable           | Units   | Wildtype M (n=9) | Wildtype F (n=8) | Mutant M (n=10) | Mutant F (n=8) | P(sex x mutation) | P(mutation) |
|--------------------|---------|------------------|------------------|-----------------|----------------|-------------------|-------------|
| RR naive fall time | log10 s | 1.15 (0.08)      | 1.01 (0.18)      | 1.15 (0.04)     | 1.23 (0.05)    | 0.29              | 0.29        |
| RR learning        | s/trial | 0 (0.7)          | 1.9 (1.6)        | 0.4 (0.6)       | 1.1 (1)        | 0.54              | 0.88        |
| RR memory          | s       | 19.7 (11)        | 8.8 (6.2)        | 2.6 (2.4)       | -3 (2)         | 0.7               | 0.032 *     |

RR naive fall time: Fall time on accelerating rotarod (log10 s), naive performance in session 1

RR learning: Learning on rotarod, measured as increase in fall time per trial (s/trial) in session 1

RR memory: Memory on rotarod, measured as excess fall time at middle of session 2 relative to middle of session 1

## 2.6 Fear Conditioning - Learning & Memory



**Figure 5:** FC histories. Percent freezing presented as mean  $\pm$  SEM. TR:N-n, percent time spent freezing during time interval  $N < t \leq n$  seconds on training day. TE:N-n, same on testing day.

**Table 5:** FC analysis: mean(SEM) of behaviour scores for wildtype and mutant males and females, with p-values of differences

| Variable                    | Units      | Wildtype M (n=9) | Wildtype F (n=8) | Mutant M (n=10) | Mutant F (n=8) | P(sex x mutation) | P(mutation) |
|-----------------------------|------------|------------------|------------------|-----------------|----------------|-------------------|-------------|
| Fear learning, trial effect | % freezing | 50.7 (7.6)       | 54.4 (10.7)      | 47.6 (6)        | 54.5 (9.7)     | 0.85              | 0.85        |
| Fear learning, tone effect  | % freezing | -7.6 (5)         | 7.1 (5.8)        | -3.4 (6.6)      | 8.4 (3.8)      | 0.79              | 0.61        |
| Contextual memory, mean     | % freezing | 53.8 (5.5)       | 51.6 (9.5)       | 42.4 (6)        | 50.8 (6.5)     | 0.45              | 0.35        |
| Contextual memory, change   | % freezing | 29.8 (7)         | 38.7 (9.8)       | 40.5 (9.2)      | 55.7 (3.8)     | 0.7               | 0.1         |
| Cued memory, mean           | % freezing | -13.3 (7)        | 3.7 (6.9)        | -12.6 (5.5)     | 3.3 (8.9)      | 0.94              | 0.97        |
| Cued memory, change         | % freezing | 4.4 (6.8)        | 7.3 (4.6)        | 8 (5.6)         | -0.3 (5.7)     | 0.35              | 0.8         |

Fear learning, trial effect: Fear learning, measured as extra percent time freezing before third trial compared to percent time freezing before first trial

Fear learning, tone effect: Fear learning, measured as increase in percent time freezing due to third tone compared to increase in percent time freezing due to first tone

Contextual memory, mean: Contextual memory, measured as difference in percent time freezing during first 120 s re-exposure to the box compared to first 120 s in the box on previous day

Contextual memory, change: Contextual memory, measured as increase in percent time spent freezing from first time bin of 30 s to fourth bin of 30 s during 120 s re-exposure to the box

Cued memory, mean: Cued memory, measured as increase in percent time spent freezing during 120 s of tone re-exposure compared to increase in percent time spent freezing during initial tone on previous day

Cued memory, change: Cued memory, measured as increase in percent time spent freezing from first time bin of 30 s to fourth bin of 30 s during 120 s re-exposure to the tone