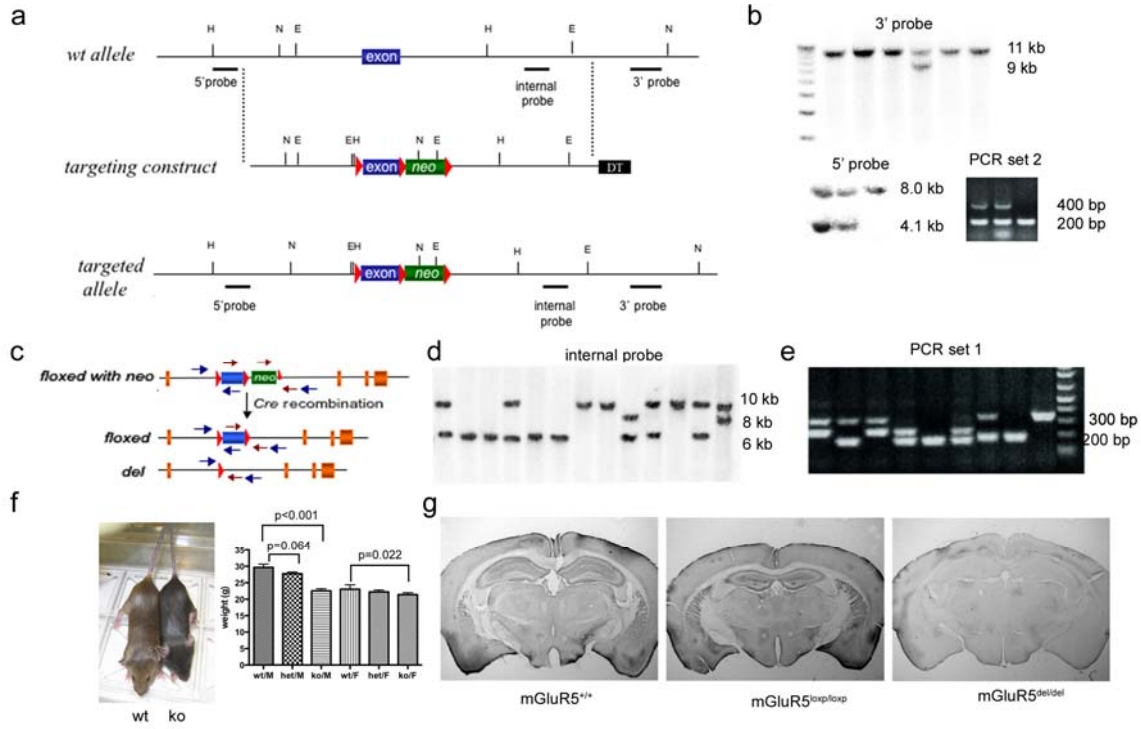
Supplementary Figure 1



S. Fig.1. Generating mGluR5$^{loxP/loxP}$ and mGluR5$^{del/del}$ mice.

a, Structure of the Grm5 gene locus (top), the targeting vector (middle), and mutate locus (bottom). Blue boxes, exon 7; green box, loxP-flanked PGK-*neo* cassette inserted into the intron 470 bp downstream of exon 7; black box, DT (diptheria toxin-A) cassette. The 5' and 3' arms of the targeting construct were 6.8 kb and 6.3 kb, respectively. N, Nco1 sites; H, HindIII sites; E, EcoRV sites.

b, Southern blot with DNA prepared from injected ES clones. With 3' probe, WT and targeted loci generated 11.0 kb and 9.0 kb NcoI fragments, respectively. For the 5' probe, the 8.0 kb and the 4.1 kb HindIII segment corresponded to the WT allele and mutant allele, respectively. Genotypes werw also confirmed by PCR (primer set 2)

c, Schemes for creating mGluR5$^{loxP/loxP}$ and mGluR5$^{del/del}$ allele using cre recombination. Locations of primers for two sets of PCR genotyping were indicated.

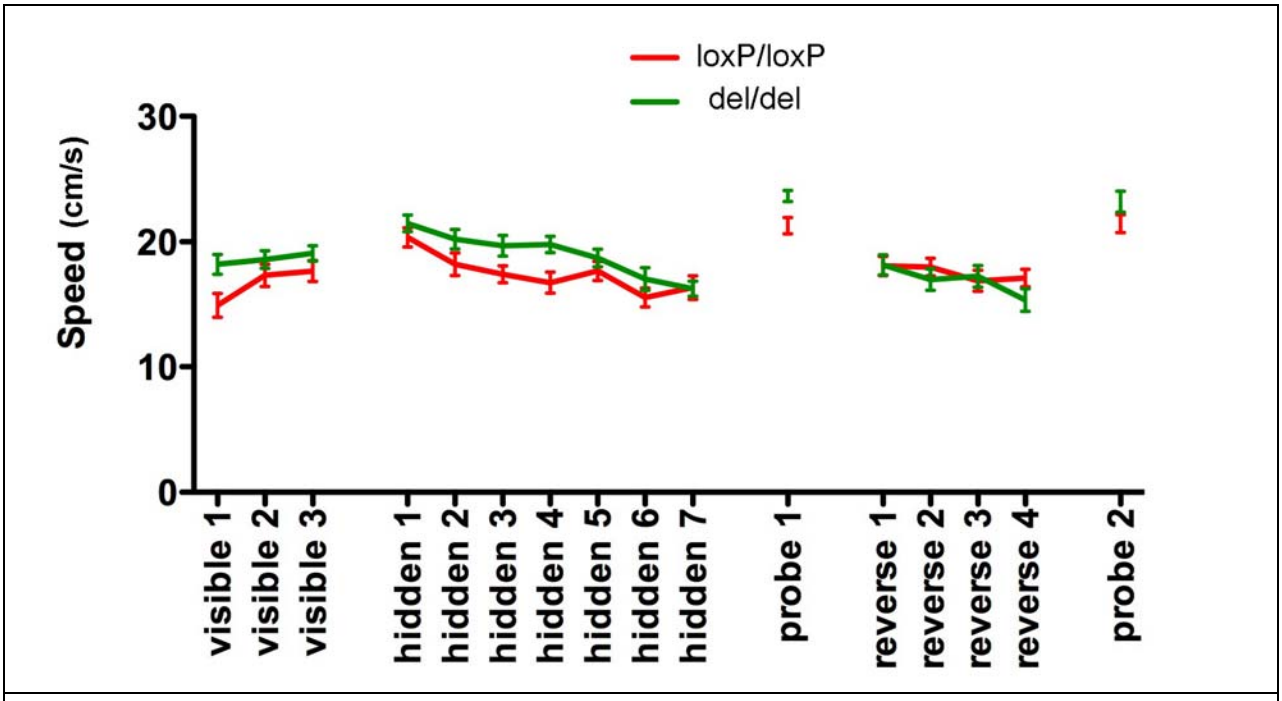
d, Southern blot analysis of EcoRV-digested tail DNA confirmed the germline cre recombination of loxP sites, which further led to mGluR5$^{loxP/loxP}$ and mGluR5$^{del/del}$ allele. (WT, floxed and deletion loci gave rise to 10.0 kb, 8.0 kb and 6.6 kb fragments, respectively).

e, cre recombination was confirmed by PCR products (primer set 2) of expected size (*wt*, 200bp; *loxP*, 270 bp; *del*, 320 bp).

f, ko mice weigh less then their wild-type littermates, confirmed the report(Bradbury et al., 2005) on a previously generated line of mGluR5 knockout mice(Lu et al., 1997). Comparisons were made between mGluR5$^{del/del}$ mice and mGluR5$^{+/+}$ mice produced by mGluR5$^{del/+}$ het x het breeding. Male and female mGluR5$^{del/del}$ mice weigh 20% and 9% less, respectively, than their wild-type littermates of the same gender. We found no difference in body weight between mGluR5$^{+/+}$ and mGluR5$^{loxP/loxP}$ mice produced by mGluR5$^{+/loxP}$ het x het breeding.

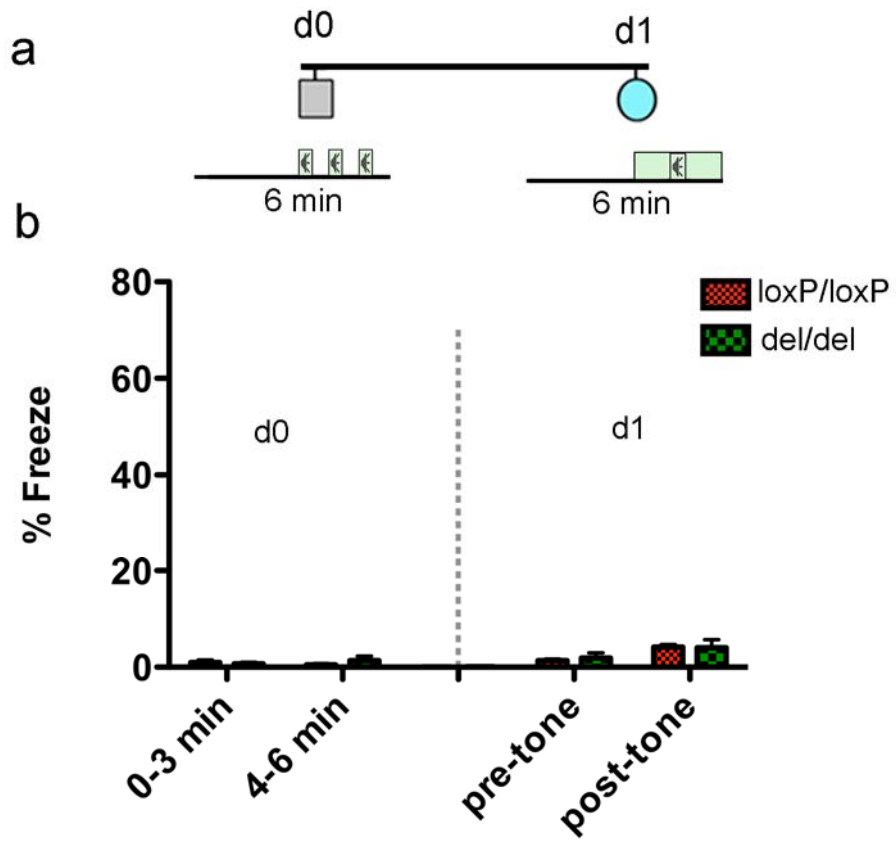
g, Immunohistochemistry confirmed the deletion of the mGluR5 products in mGluR5$^{del/del}$ mice. mGluR5 was stained by using polyclonal rabbit anti-mGluR5 antibody that recognizes 21 residues corresponding to the C-terminal of mGluR5 (Milipore, 06-451;1:500 dilution)(Kirschstein et al., 2007).

Supplementary Figure 2



S. Fig.2. Genotype comparisons of swimming speed during MWM test. Data was analyzed by training block (3 trials per day) except probe tests. mGluR5$^{del/del}$ mice swam slightly faster than mGluR5$^{loxP/loxP}$ during the visible platform test ($F_{1,72}$ = 4.61, p = 0.039) and during the hidden platform test ($F_{1,216}$ = 3.05, p =0.089). Speeds during both probe tests were similar between the two groups (t-test, p > 0.05). An overall slightly faster speed seen in mGluR5$^{del/del}$ mice may be also related to lighter weight (Bradbury et al., 2005) and less immobility(Li et al., 2006) and in these animals.

Supplementary Figure 3



S. Fig. 3. 85 db tone (2900Hz) alone induced little freezing in mice.
a, The experimental paradigm. Mice were trained at day 0 (d0) with 3 tones (85 db 2900Hz) each lasted 20-s starting at 3 minute with 1-min interval in context 1 (marked by grey box). On the next day, mice were put into different context (marked by light blue circle) for 6 minutes, with a tone presentation lasting for 3 minutes.
b, Freezing in d0 and d1 was scored at 3-min intervals ($mGluR5^{del/del}$, n=5; $mGluR5^{loxP/loxP}$, n=7). In d0, both groups displayed less than 1.5% pre-tone freezing (0-3 min) or post-tone freezing (4-6 min). In d1, pre-tone freezing (contextual control) were also less than 1.5% for both groups. 3-min tone induced little freezing in mice (<3 % in both groups, as compared to pre-tone freezing).

Supplementary Table 1. Primer sequences and PCR genotyping conditions.

PCR set	Primer name	Primer sequences
Set 1	Top	5'-GATGTCCCACTTACCTGATGT-3'
	Bottom-1	5'-AGTTCCGTGTCTTTATTCTTAGC-3'
	Bottom-2	5'-AGGCGCTTCCAAAATAGAGG-3'
Set 2	*neo* primer:	5'-GGCTCTTTACTATTGCTTTATGATAATG-3'
	Top	5'-TTGCTAGCTGAAAAGGACGAAACA-3'
	Bottom	5'-TCGTTTTGAATCTTGGGGACAGTTAC-3'

We used two sets of primers for PCR genotyping:

For the PCR set 1, annealing temperature was set at 61 ^{o}C. The top primer hybridizes to sequences 50 bp upstream of first loxP site, and the bottom-1 primer is located 300 bp upstream of exon5. Bottom-2 primer is 240 bp downstream of last loxP site. Primer set 1 yields 200 bp, 270 bp and 320 bp amplicons from the *wt*, *floxed* (with *neo* deletion) and *del* allele respectively.

For the PCR set 2, annealing temperature was set at 52 ^{o}C. The top primer hybridizes to sequences 314 bp downstream of exon7. The bottom primer is located 100 bp downstream of the last loxP site. Neo primer is 300 bp upstream to last loxP site. DNA from *wt*, *floxed* (with *neo*), *floxed* (without *neo*) yields 217 bp. 463 bp and 323 bp PCR products respectively.

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Kirschstein T, Bauer M, Muller L, Ruschenschmidt C, Reitze M, Becker AJ, Schoch S, Beck H (2007) Loss of metabotropic glutamate receptor-dependent long-term depression via downregulation of mGluR5 after status epilepticus. J Neurosci 27:7696-7704.

Li X, Need AB, Baez M, Witkin JM (2006) Metabotropic glutamate 5 receptor antagonism is associated with antidepressant-like effects in mice. J Pharmacol Exp Ther 319:254-259.

Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J Neurosci 17:5196-5205.