HDAC2 regulates atypical antipsychotic responses through the modulation of *mGlu2* promoter activity

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1

Animals were chronically (21 days) injected with vehicle (black), 10 mg/kg clozapine (red), 4 mg/kg risperidone (green), or 1 mg/kg haloperidol (blue), and sacrificed one day after the last injection.

(**a-c**) [^{3}H]Ketanserin binding in *5HT2A*$^{+/+}$ mouse frontal cortex after vehicle, or chronic clozapine (**a**), risperidone (**b**) or haloperidol (**c**). Vehicle compared to clozapine, $F[2,77] = 180.4$, $P < 0.001$; vehicle compared to risperidone, $F[2,95] = 93.95$, $P < 0.001$; vehicle compared to haloperidol, $F[2,104] = 2.17$, $P > 0.05$. Maximum number of binding sites (B_{max}) for [^{3}H]LY341495 obtained from individual saturation curves are decreased by chronic clozapine and risperidone (n = 4-8 independent saturation curves per group). $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant; Student's *t*-test.

(**d**) [^{3}H]Raclopride binding in *5HT2A*$^{+/+}$ mouse striatum after vehicle or chronic haloperidol. Vehicle compared to haloperidol, $F[2,56] = 29.27$, $P < 0.001$. Maximum number of binding sites (B_{max}) for [^{3}H]raclopride obtained from individual saturation curves are increased by chronic haloperidol (n = 4 independent saturation curves per group). $^{*}P < 0.05$; Student's *t*-test.

(**e-f**) [^{3}H]LY341495 binding in *5HT2A*$^{-/-}$ mouse frontal cortex after vehicle, or chronic clozapine (**e**) or risperidone (**f**). Vehicle compared to clozapine, $F[2,128] = 2.39$, $P > 0.05$; vehicle compared to risperidone, $F[2,93] = 93.95$, $P > 0.05$; vehicle compared to haloperidol, $F[2,104] = 2.17$, $P > 0.05$. Maximum number of binding sites (B_{max}) for [^{3}H]LY341495 obtained from individual saturation curves are not affected by chronic

clozapine and risperidone (n = 4-8 independent saturation curves per group). n.s., not significant; Student's *t*-test.

(**g**) Expression of *5HT2C*, *dopamine D2*, *mGlu2*, and *mGlu3* mRNA in *5HT2A*$^{-/-}$ mouse frontal cortex assayed by qRT-PCR. Experiments were performed after vehicle, or chronic clozapine, risperidone or haloperidol (n = 6 mice per group). Expression of *5HT2C,* $F[3,20] = 0.50$, $P > 0.05$; Expression of *D2,* $F[3,20] = 0.02$, $P > 0.05$; Expression of *mGlu2,* $F[3,20] = 0.09$, $P > 0.05$; Expression of *mGlu3,* $F[3,20] = 1.35$, $P > 0.05$; one-way ANOVA. $P > 0.05$; Bonferroni's *post hoc* test of one-way ANOVA.

(h) Expression of *5HT2A*, *5HT2C*, *dopamine D2*, *mGlu2*, and *mGlu3* mRNA in mouse thalamus and striatum assayed by qRT-PCR. Experiments were performed after vehicle, or chronic clozapine (n = 6 mice per group). The α value was corrected for multiple independent null hypotheses by using the Holm's sequentially rejective Bonferroni method. $^{*}P < 0.011$; Student's *t*-test. Error bars represent s.e.m.

Supplementary Figure 2

(**a**) Mice were chronically (21 days) injected with vehicle (black), 10 mg/kg clozapine (red), 4 mg/kg risperidone (green), or 1 mg/kg haloperidol (blue), and sacrificed one day after the last injection. Fragmented chromatin was immunoprecipitated with antibody recognizing acetyl-histone H4 (H4ac), methyl-histone H3 at lysine 4 (H3K4me1/2/3), tri-methyl-histone H3 at lysine 27 (H3K27me3), or tri-methyl-histone H3 lysine 9 (H3K9me3), and the level of association of the *5HT2A, 5HT2C, dopamine D2, mGlu2, or mGlu3* promoters was measured by qPCR.

Decreased H4ac at the *mGlu2* promoter by chronic clozapine and risperidone, but not haloperidol, in mouse frontal cortex. Note also that H4ac was decreased at the *5HT2A* promoter by chronic clozapine, but not risperidone or haloperidol (n = 8-12 mice per

group). The α value was corrected for multiple independent null hypotheses by using the Holm's sequentially rejective Bonferroni method. *$P < 0.011$; Student's *t*-test.

H3K4me1/2/3 was not altered (n = 8-14 mice per group). The α value was corrected for multiple independent null hypotheses by using the Holm's sequentially rejective Bonferroni method. $P > 0.011$; Student's *t*-test.

Increased H3K27me3 at the *mGlu2* promoter by chronic clozapine and risperidone, but not haloperidol, in mouse frontal cortex (n = 8-14 mice per group). The α value was corrected for multiple independent null hypotheses by using the Holm's sequentially rejective Bonferroni method. *$P < 0.011$; Student's *t*-test.

H3K9me3 was not altered (n = 8-14 mice per group). The α value was corrected for multiple independent null hypotheses by using the Holm's sequentially rejective Bonferroni method. $P > 0.011$; Student's *t*-test. Error bars represent s.e.m.

(**b**) Subchronic treatment with antipsychotics does not affect the histone modification status at the *mGlu2* promoter. Mice were sub-chronically (2 days) injected with vehicle (black), 10 mg/kg clozapine (red), 4 mg/kg risperidone (green), or 1 mg/kg haloperidol (blue), and sacrificed one day after the last injection. Fragmented chromatin was immunoprecipitated with antibody recognizing acetyl-histone H3 (H3ac) or tri-methyl-histone H3 at lysine 27 (H3K27me3), and the level of association of the *5HT2A, dopamine D2, mGlu2,* or *mGlu3* promoters was measured by qPCR (n = 8 mice per group). Binding of H3ac at the *5HT2A* promoter, $F[3,28] = 0.32$, $P > 0.05$; binding of H3ac at the *D2* promoter, $F[3,28] = 0.62$, $P > 0.05$; binding of H3ac at the *mGlu2* promoter, $F[3,28] = 1.88$, $P > 0.05$; binding of H3ac at the *mGlu3* promoter, $F[3,28] = 0.66$, $P > 0.05$. $P > 0.05$; Bonferroni's *post hoc* test of one-way ANOVA. Binding of H3K27me3 at the *5HT2A* promoter, $F[3,28] = 0.20$, $P > 0.05$; binding of H3K27me3 at

the *D2* promoter, F[3,28] = 2.20, $P > 0.05$; binding of H3K27me3 at the *mGlu2* promoter, F[3,28] = 0.73, $P > 0.05$; binding of H3K27me3 at the *mGlu3* promoter, F[3,28] = 0.58, $P > 0.05$; one-way ANOVA. $P > 0.05$; Bonferroni's *post hoc* test of one-way ANOVA.

(**c**) Chronic treatment with the antidepressant fluoxetine does not affect the histone modification status at the *mGlu2* promoter. Mice were chronically (21 days) injected with fluoxetine (20 mg/kg), or vehicle, and sacrificed one day after the last injection. Fragmented chromatin was immunoprecipitated with antibody recognizing H3K27me3, and the level of association of the *5HT2A, dopamine D2, mGlu2,* or *mGlu3* promoters was measured by qPCR (n = 6 mice per group). The α value was corrected for multiple independent null hypotheses by using the Holm's sequentially rejective Bonferroni method. $P > 0.011$; Student's *t*-test

(**d**) In *5HT2A*$^{-/-}$ mice, chronic treatment with atypical antipsychotics does not affect the histone modification status at the *mGlu2* promoter. Mice were chronically (21 days) injected with vehicle (black), 10 mg/kg clozapine (red), or 4 mg/kg risperidone (green), and sacrificed one day after the last injection. Fragmented chromatin was immunoprecipitated with antibody recognizing H3ac or H3K27me3, and the level of association of the *dopamine D2, mGlu2,* or *mGlu3* promoters was measured by qPCR (n = 8 mice per group). Binding of H3ac at the *D2* promoter, F[2,21] = 0.91, $P > 0.05$; binding of H3ac at the *mGlu2* promoter, F[2,21] = 0.20, $P > 0.05$; binding of H3ac at the *mGlu3* promoter, F[2,21] = 0.10, $P > 0.05$; one-way ANOVA. $P > 0.05$; Bonferroni's *post hoc* test of one-way ANOVA. Binding of H3K27me3 at the *D2* promoter, F[2,21] = 2.44, $P > 0.05$; binding of H3K27me3 at the *mGlu2* promoter, F[2,21] = 0.42, $P > 0.05$; binding of H3K27me3 at the *mGlu3* promoter, F[2,21] = 1.66, $P > 0.05$; one-way ANOVA. $P > 0.05$; Bonferroni's *post hoc* test of one-way ANOVA. Error bars represent s.e.m.

Supplementary Figure 3

Methylation status of the *mGlu2* (*grm2*) gene CpG island is not affected by chronic clozapine in mouse frontal cortex.

(**a**) Schematic view of amplified region of the *mGlu2* gene.

(**b,c**) Methylation status of 39 CpG sites close to the *mGlu2* promoter region obtained from bisulfite sequencing in mouse liver and frontal cortex. Open and filled circles represent unmethylated and methylated cytosines, respectively. Representative example is shown (**b**). Ten to twelve clones were sequenced for each mouse (n = 3 mice per group). Percentage of methylated clones is shown (**c**).

(**d,e**) Effect of chronic clozapine on the methylation status of 39 CpG sites close to the *mGlu2* promoter region obtained from bisulfite sequencing in mouse frontal cortex. Open and filled circles represent unmethylated and methylated cytosines, respectively. Representative example is shown (**d**). Ten to twelve clones were sequenced for each mouse (n = 6 mice per group). Percentage of methylated clones is shown (e). $*P < 0.05$; n.s., not significant; Student's *t*-test. Error bars represent s.e.m.

Supplementary Figure 4

(**a**) ChIP in postmortem human frontal cortex. Digested chromatin was immunoprecipitated with antibody recognizing H3ac, or control IgG, and the level of association of the *5HT2A, 5HT2C, mGlu2, mGlu3*, or *B2M* promoters was measured by qPCR (n = 6-9 postmortem frontal cortex samples per group). Results are shown as enrichment values (bound/input).

(**b,c**) The HDAC inhibitors SAHA and MS-275 affect the histone code of the *mGlu2* and *mGlu3* promoters when infused into the frontal cortex. Experiments were performed 24 h after a single infusion (0.5 μl) of vehicle, SAHA (10 μM) or MS-275 (10 μM) into the

frontal cortex. (**b**) Expression of *5HT2A*, *5HT2C*, *mGlu2*, and *mGlu3* mRNA in mouse frontal cortex assayed by qRT-PCR (n = 6 mice per group). Expression of *5HT2A,* $F[2,15] = 1.91$, $P > 0.05$; expression of *5HT2C,* $F[2,15] = 2.25$, $P > 0.05$; expression of *mGlu2,* $F[2,15] = 5.02$, $P < 0.05$; expression of *mGlu3,* $F[2,15] = 6.66$, $P < 0.01$; one-way ANOVA. $P < 0.05$; $^{**}P < 0.01$; Bonferroni's *post hoc* test of one-way ANOVA. (**c**) Fragmented chromatin was immunoprecipitated with antibody recognizing acetyl-histone H3 (H3ac), and the level of association of the *5HT2A, 5HT2C, mGlu2, or mGlu3* promoters was measured by qPCR (n = 6 mice per group). Binding of H3ac at the *5HT2A* promoter, $F[2,15] = 3.51$, $P > 0.05$; binding of H3ac at the *5HT2C* promoter, $F[2,15] = 2.23$, $P > 0.05$; binding of H3ac at the *mGlu2* promoter, $F[2,15] = 15.72$, $P < 0.001$; binding of H3ac at the *mGlu3* promoter, $F[2,15] = 56.03$, $P < 0.001$; one-way ANOVA. $^{**}P < 0.01$; $^{***}P < 0.001$; Bonferroni's *post hoc* test of one-way ANOVA.

(**d**) Luciferase reporter assay: the HDAC inhibitors TSA, SAHA and MS-275 activate the *mGlu2* promoter activity in HEK293 cells. HEK293 cells were transfected with mouse *mGlu2* promoter-luciferase plasmid, 5 h later, treated with vehicle, TSA (10 μM), SAHA (10 μM) or MS-275 (10 μM) for 20 h, and then analyzed for luciferase activity (n = 3 independent experiments in triplicate). Effect of TSA, $F[3,8] = 13.72$, $P < 0.01$; effect of SAHA, $F[3,8] = 7.05$, $P < 0.01$; effect of MS-275, $F[3,8] = 17.79$, $P < 0.001$; one-way ANOVA. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; Bonferroni's *post hoc* test of one-way ANOVA.

(**e**) ChIP assay. HEK293 cells were transfected with mouse *mGlu2* promoter-luciferase plasmid, 5 h later, treated with vehicle, SAHA (10 μM) or MS-275 (10 μM) for 20 h, and then ChIP assays with anti-H3ac antibody were performed (n = 3 independent experiments in triplicate). Effect of SAHA or MS-275, $F[2,6] = 16.09$, $P < 0.01$; one-way ANOVA. $^{*}P < 0.05$; $^{**}P < 0.01$; Bonferroni's *post hoc* test of one-way ANOVA.

(**f,g**) Chronic SAHA is associated with increased levels of H3ac in mouse frontal cortex. Mice were treated chronically (21 days) with vehicle, SAHA (20 mg/kg) or clozapine (10 mg/kg), and sacrificed one day after the last injection. Chronic SAHA, and not chronic clozapine, up-regulates protein levels of H3ac in nuclear fractions (n = 6 mice per group). $^{**}P < 0.01$; n.s., not significant; Student's *t*-test (**f**). Western blot in nuclear and cytoplasmic fractions (**g**). Representative immunoblots are shown.

(**h**) Effect of chronic SAHA on genes previously implicated in cognitive function and synaptic plasticity[33,34]. Mice were chronically (21 days) treated with SAHA (20 mg/kg) or vehicle, and sacrificed one day after the last injection (n = 6 mice per group). The α value was corrected for multiple independent null hypotheses by using the Holm's sequentially rejective Bonferroni method. $^{*}P < 0.012$; Student's *t*-test.

Effect of HDAC2 over-expression on genes that display increased mRNA levels in frontal cortex following chronic SAHA. Experiments were performed in frontal cortex of mice that received intra-frontal cortex administration of HSV-GFP or HSV-HDAC2 (n = 6 mice per group). The α value was corrected for multiple independent null hypotheses by using the Holm's sequentially rejective Bonferroni method. $^{*}P < 0.012$; Student's *t*-test. Error bars show s.e.m.

Supplementary Figure 5

(**a**) Western blot analysis with anti-FLAG antibody. Cell lysates were prepared from HEK293 cells transfected with pcDNA3.1 (mock), pcDNA3.1-FLAG-HDAC2 or pcDNA3.1-FLAG-HDAC2-H141A.

(**b**) HSV-mediated transgene expression in frontal cortex. HSV-GFP and HSV-FLAG-HDAC2 were injected into frontal cortex, and FLAG-HDAC2 expression was measured by Western blotting.

(**c**) Whole-cell recordings of GFP-positive and GFP-negative neurons in acute brain slice, showing the inhibitory effects of LY379268 (1 μM) on the sEPSC amplitude evoked by the hallucinogenic 5HT2A agonist DOI (5 μM) in cortical pyramidal neurons (n = 2-3 mice per group, 10 neurons per mice). Effect of HSV-GFP or HSV-HDAC2, F[3,51] = 4.07; $P < 0.05$; effect of DOI and/or LY379, F[2,51] = 21.58, $P < 0.001$; two-way ANOVA. *$P < 0.05$; ***$P < 0.001$; Bonferroni's *post hoc* test of one-way ANOVA.

(**d**) Viral-mediated over-expression of HDAC2, but not HDAC1 or HDAC4, in frontal cortex attenuates the inhibition of MK801-stimulated locomotor response by LY379268. Mice were administered LY379268 (8 mg/kg) or vehicle, followed by MK801 (0.5 mg/kg). Left panels depict the time courses of MK801-induced locomotion measured in 5 min blocks. Time of injection is indicated by arrow (n = 6-14 mice per group) (see also Fig. 5f).

(**e**) Viral-mediated over-expression of HDAC2 in frontal cortex does not affect the exploratory activity in the open field test. Mice were placed in the locomotor chamber and allowed to habituate for 5 min. After habituation, horizontal activity (left panel) and exploration time of the center portion of the environment (right panel) was measured for 60 min. The effect of viral-mediated over-expression of HDAC1, HDAC2, HDAC4, and control GFP, is shown (n = 8-20 mice per group). Effect of HSV-mediated over-expression on horizontal activity, F[3,48] = 0.32, $P > 0.05$, effect of HSV-mediated over-expression on center time, F[3,52] = 0.04, $P > 0.05$; two-way ANOVA. $P > 0.05$; Bonferroni's *post hoc* test of one-way ANOVA.

(**f**) Working memory paradigm. Mice performance (percentage of correct trials) in a continuous delayed alternation test using 10 s or 40 s as the delay time (left panel). Note that, in trained animals, the performance was stabilized at 10 s versus 40 s delay times

for at least five consecutive days (n = 12-14 mice per group). Animals treated with MK801 (0.5 mg/kg) made significantly fewer correct choices after the 10 s delay (right panel). Pretreatment with LY379268 reversed the MK801-impaired delayed alternation memory performance (n = 11-20 per group). Effect of delay time, $F[1,79] = 7.44$, $P < 0.01$; effect of MK801 and/or LY379, $F[2,79] = 5.32$, $P < 0.01$; two-way ANOVA. $^{**}P < 0.01$; n.s., not significant; Bonferroni's *post hoc* test of two-way ANOVA. The dashed line indicates chance performance.

(**g**) Effect of viral-mediated over-expression of HDAC2 on the reversal of MK801-impaired delayed alternation memory performance (percentage of correct trials at 40 s delay) by LY379268. Mice (HSV-GFP and HSV-HDAC2) made significantly fewer correct choices after the 40 s delay relative to the 10 s delay (see also Fig. 5h). Mice were administered LY379268 (5 mg/kg) or vehicle, followed by MK801 (0.5 mg/kg; n = 10-16 mice per group). The dashed line indicates chance performance. Effect of HSV-HDAC2, $F[1,67] = 0.26$, $P > 0.05$; Effect of LY379, $F[2,67] = 1.67$, $P > 0.05$; two-way ANOVA. $P > 0.05$; Bonferroni's *post hoc* test of two-way ANOVA. Error bars represent s.e.m.

Supplementary Figure 6

(**a,b**) Head-twitch response induced by the hallucinogenic drug DOI (2 mg/kg) one day after chronic (21 days, **a**) or subchronic (2 days, **b**) treatment with clozapine (10 mg/kg), risperidone (4 mg/kg), haloperidol (1 mg/kg), or vehicle (n = 6-23 mice per group). Effect of chronic treatment, $F[3,51] = 26.22$, $P < 0.001$; effect of subchronic treatment, $F[2,19] = 2.85$, $P > 0.05$; one-way ANOVA. $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant; Bonferroni's *post hoc* test of one-way ANOVA.

(**c**) Chronic SAHA decreases the head-twitch behavioral response, and potentiates the antipsychotic-like behavioral effect of clozapine. Animals were chronically (21 days)

treated with clozapine (10 mg/kg) and/or SAHA (20 mg/kg), or vehicle, and the head-twitch response induced by the hallucinogen DOI (2 mg/kg) was determined five days after the last injection (n = 5-6 mice per group). DOI-dependent head-twitch response, $F[3,15] = 40.31$, $P < 0.001$; one-way ANOVA. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant; Bonferroni's *post hoc* test of one-way ANOVA.

(**d**) Dose response effect of LY379268 on the locomotor behavior induced by MK801. The left panel shows representative time courses of MK801-induced locomotion measured in 5 min blocks. Mice were administered LY379268 (3 or 12 mg/kg) or vehicle, followed by MK801 (0.5 mg/kg). Time of injection is indicated by arrow. The left panel shows bar graph summaries of the total MK801-induced locomotion as a summation of horizontal activity from t = 15 to t = 120 min. Mice were administered LY379268 (at the indicated dose) or vehicle, followed by MK801 (0.5 mg/kg). Time of injection is indicated by arrow (n = 6-14 mice per group). Effect of MK801 and/or LY379, $F[5,56] = 10.39$, $P < 0.001$; one-way-ANOVA. **$P < 0.01$; ***$P < 0.001$; n.s., not significant; Bonferroni's *post hoc* test of one-way ANOVA.

(**e**) Co-administration of chronic SAHA (20 mg/kg) and acute LY379268 (1.5 mg/kg) inhibits the MK801-stimulated locomotor activity, an effect not seen with administration of either compound alone. Note also that basal locomotor activity before MK801 injection was not affected by chronic SAHA. Time courses of MK801-induced locomotion measured in 5 min blocks. Mice were administered LY379268 (1.5 mg/kg) or vehicle, followed by MK801 (0.5 mg/kg). Time of injection is indicated by arrow (n = 8-12 mice per group). Error bars show s.e.m.

Supplementary Figure 7

(**a**) Full gel size corresponding to Fig. 3e (V, vehicle; C, clozapine).

(**b**) Full gel size corresponding to Fig. 3h (C, control; Sz, schizophrenic; U, untreated; T, treated).

(**c**) Full gel size corresponding to Supplementary Fig. 5a.

(**d**) Full gel size corresponding to Supplementary Fig. 5b (1, HSV-GFP; 2, HSV-FLAG-HDAC2).

(**e**) Full gel size corresponding to Supplementary Fig. 6e (1, nuclear; 2, cytoplasmic).

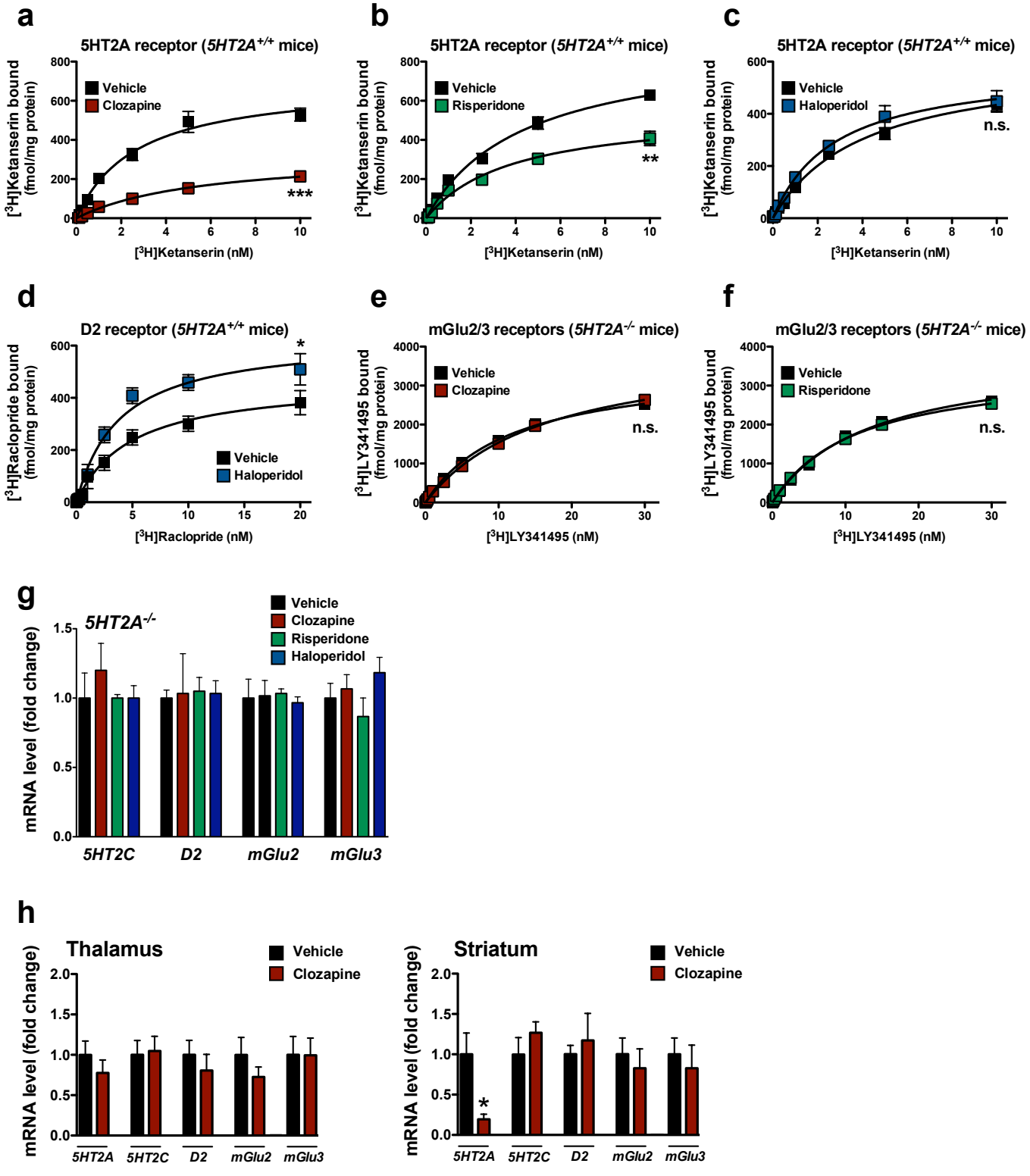
(**f**) Full gel size corresponding to Supplementary Fig. 6f (V, vehicle; S, SAHA; C, clozapine).

*, Unspecific bands of unknown nature.

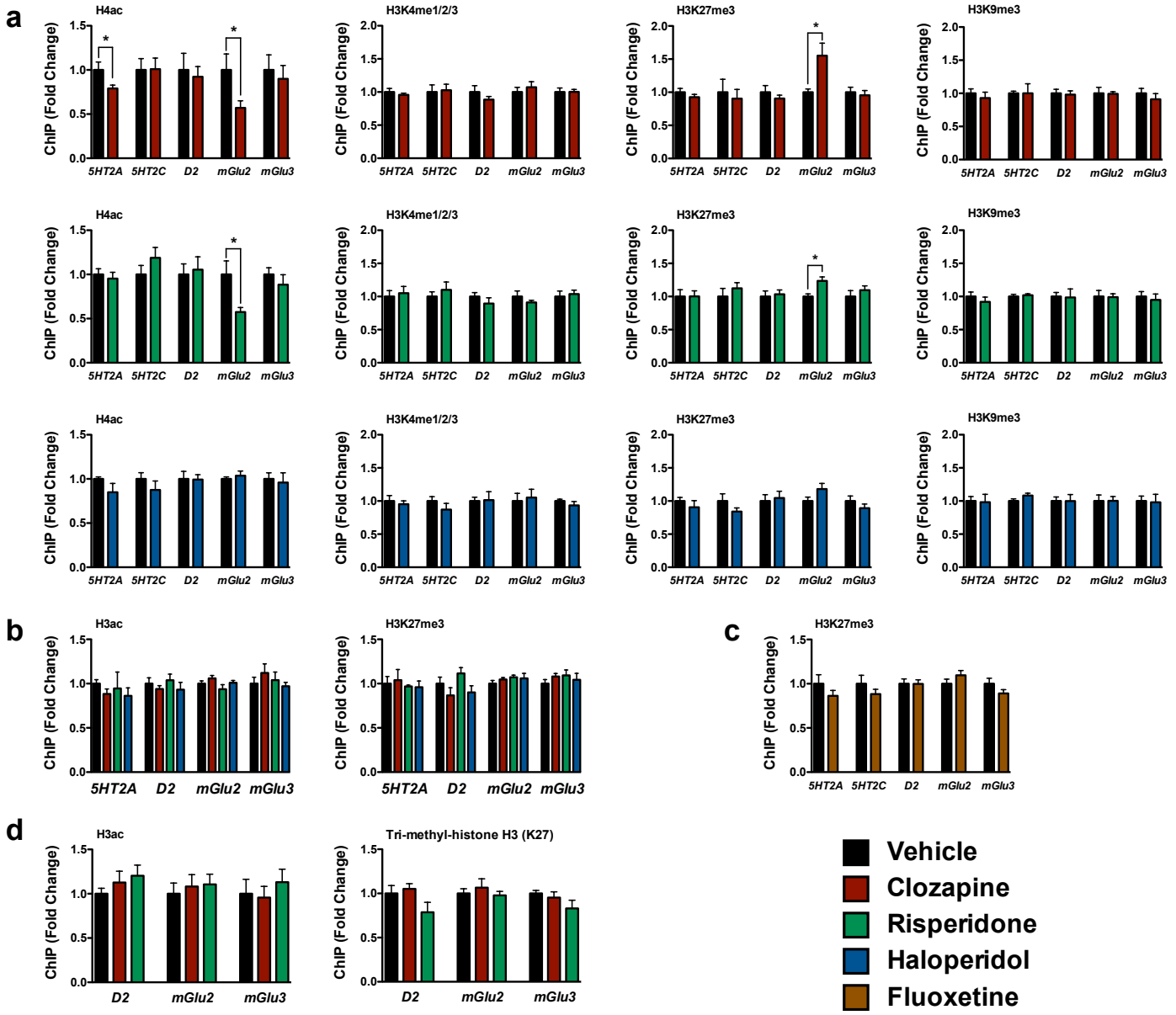
Supplementary Figure 8.

Schematic model of improved efficacy of antipsychotic drug action by HDAC2 inhibition

Activation of the 5HT2A by the endogenous neurotransmitter serotonin represses the promoter activity of *HDAC2*. Atypical antipsychotic drugs reverse the 5HT2A-dependent repression of *HDAC2*, an effect that is associated with increased HDAC2 binding and repressive histone modifications at the *mGlu2* promoter. Inhibition of HDAC2 by SAHA prevents these atypical antipsychotic drug-dependent repressive histone modifications at the *mGlu2* promoter, which improves their therapeutic efficacy.



Kurita el al. Supplementary Fig 1



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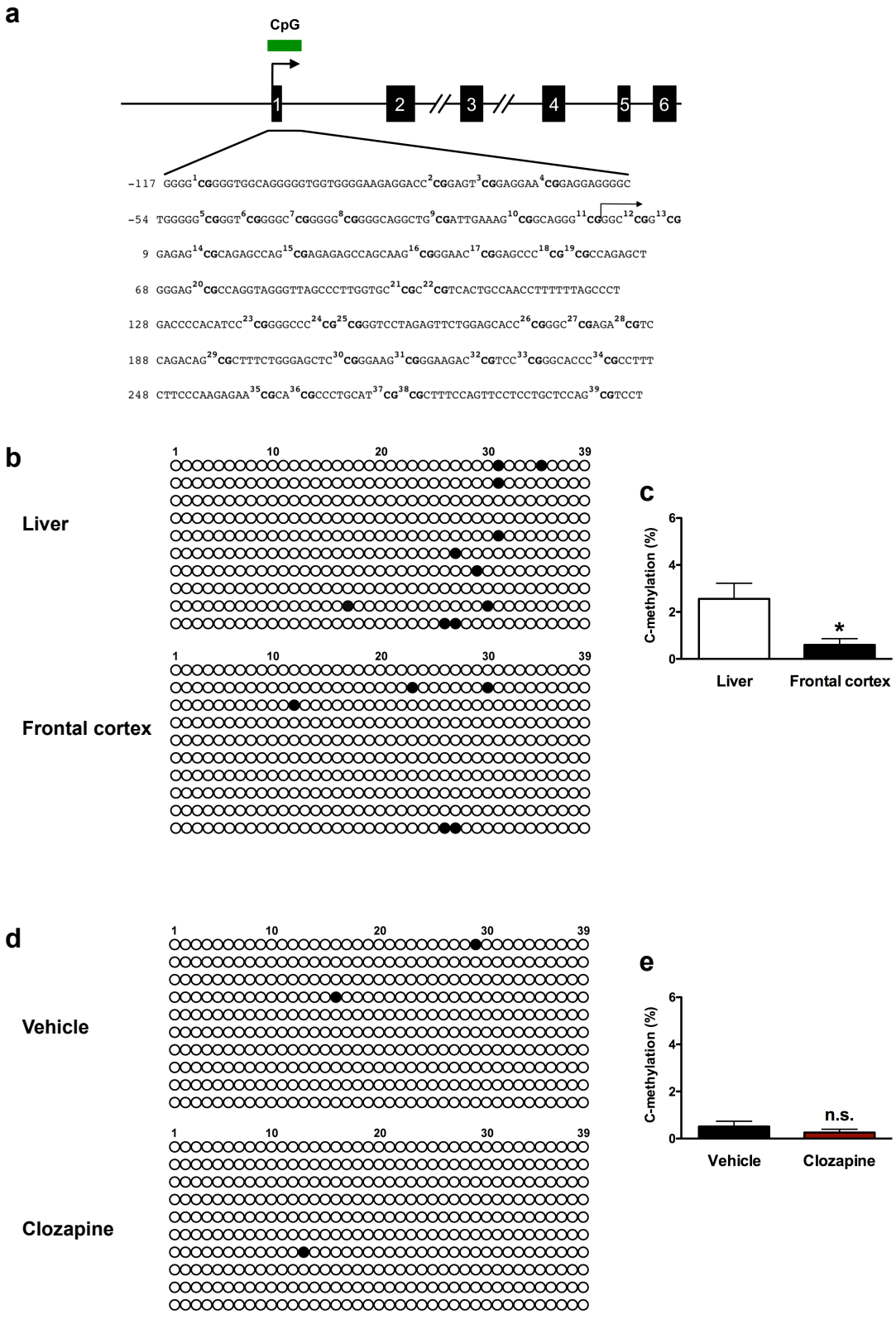

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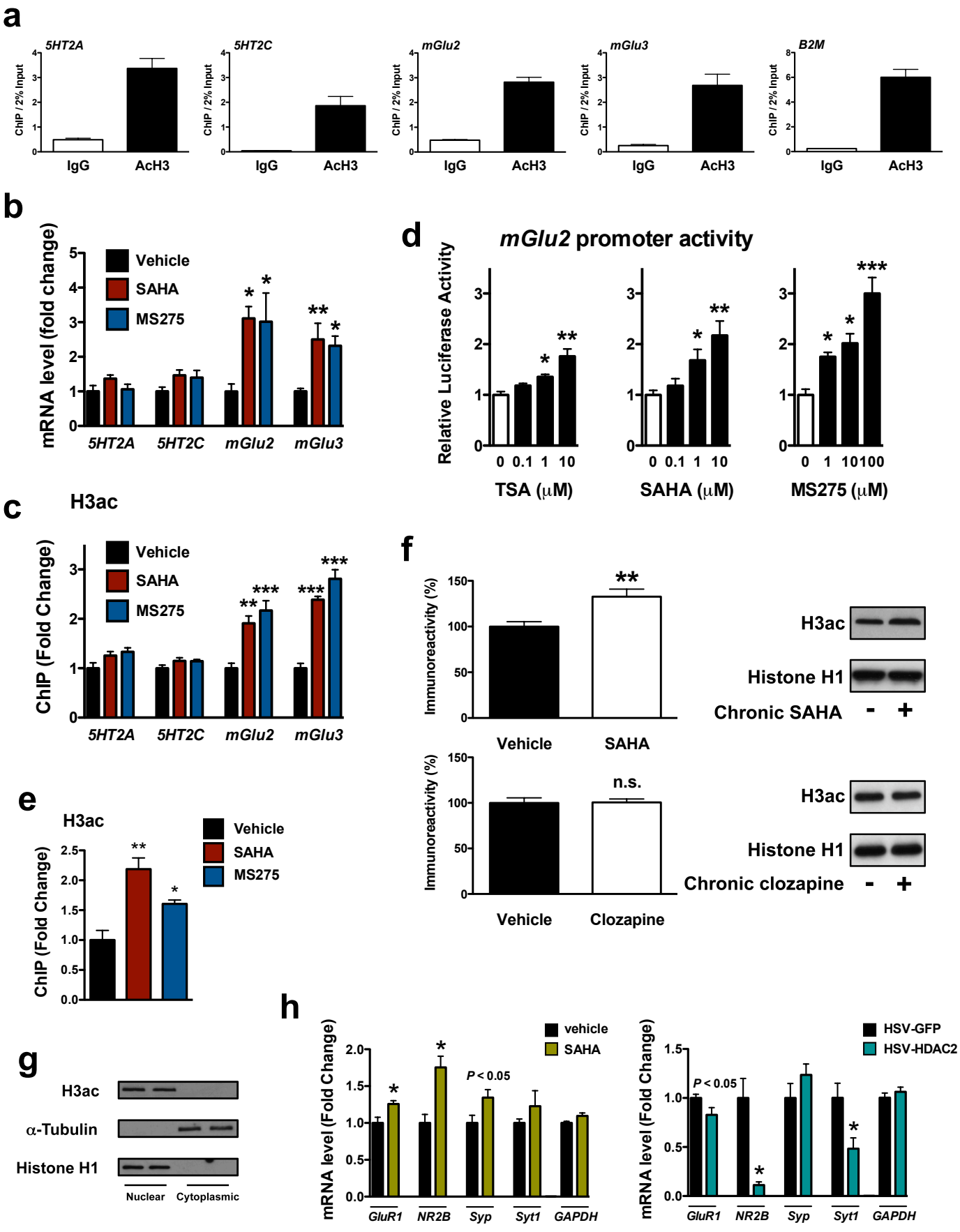

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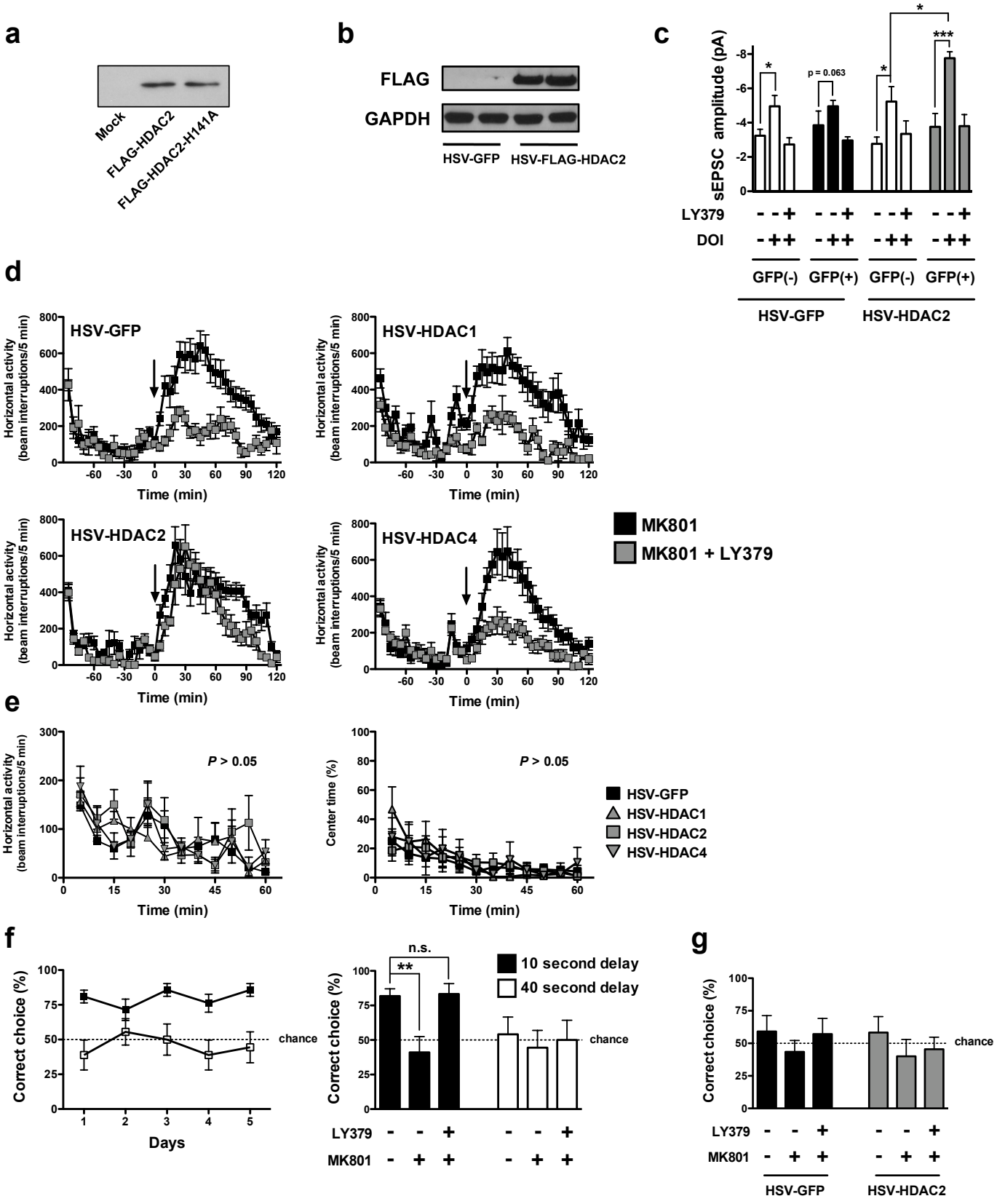

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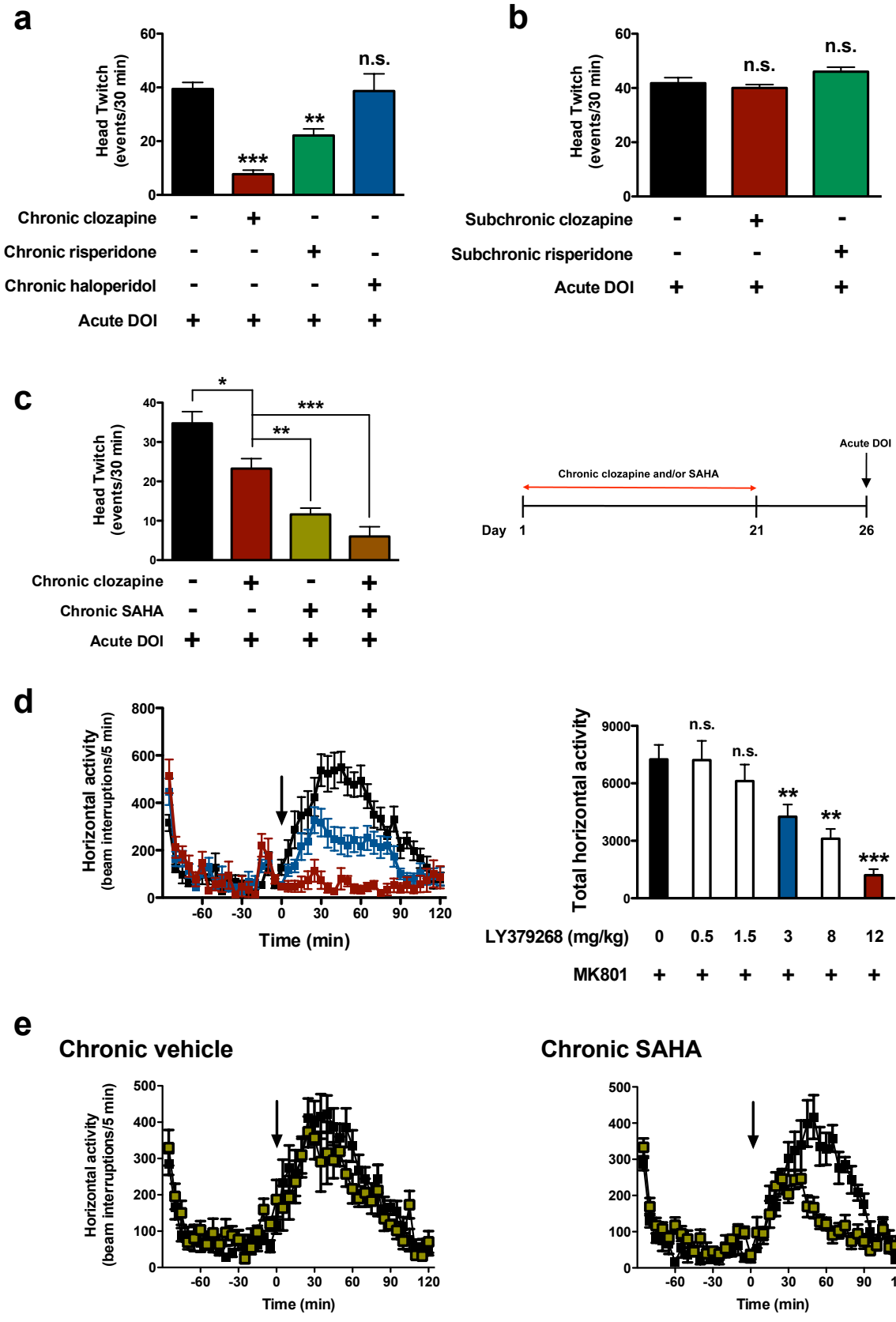

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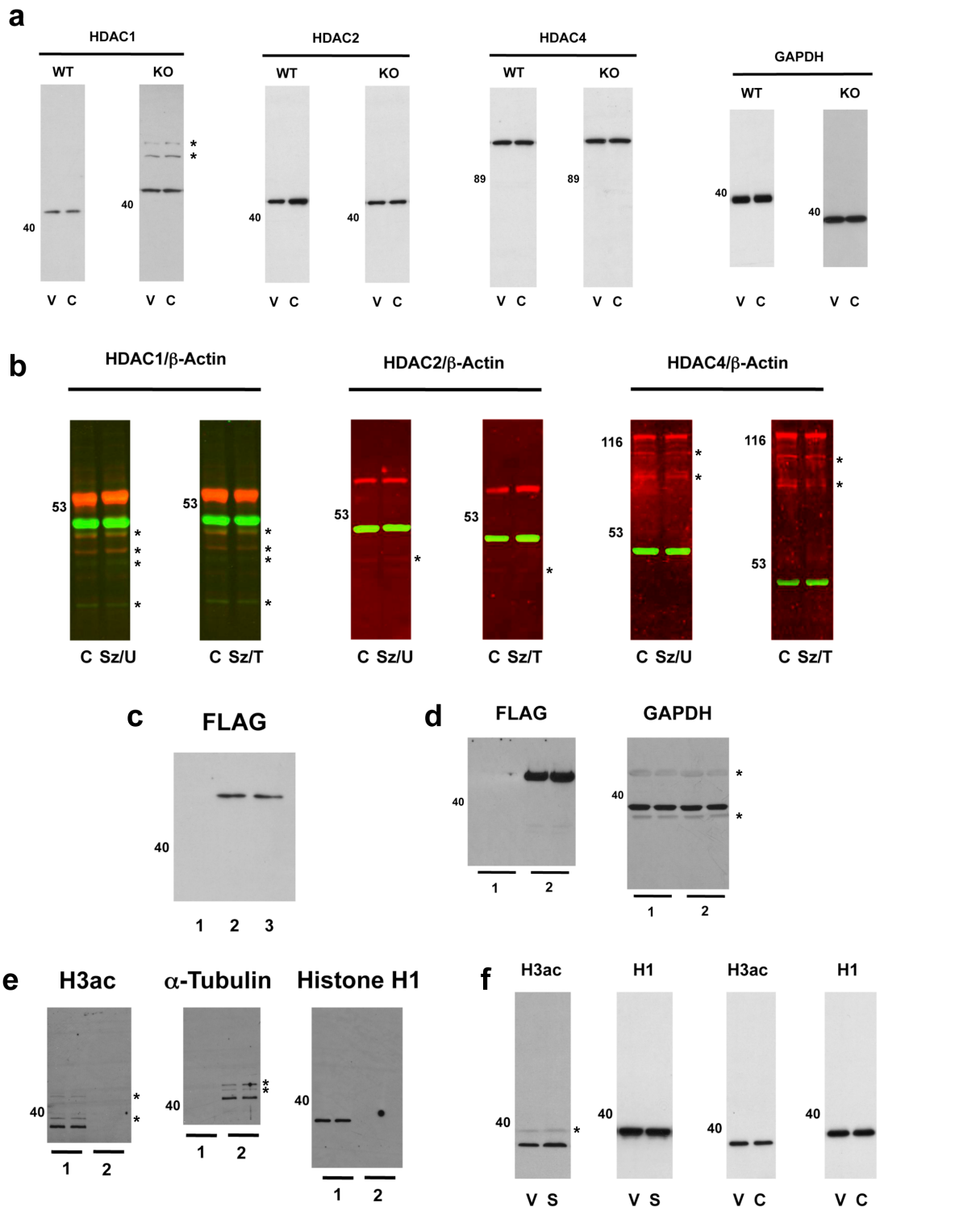

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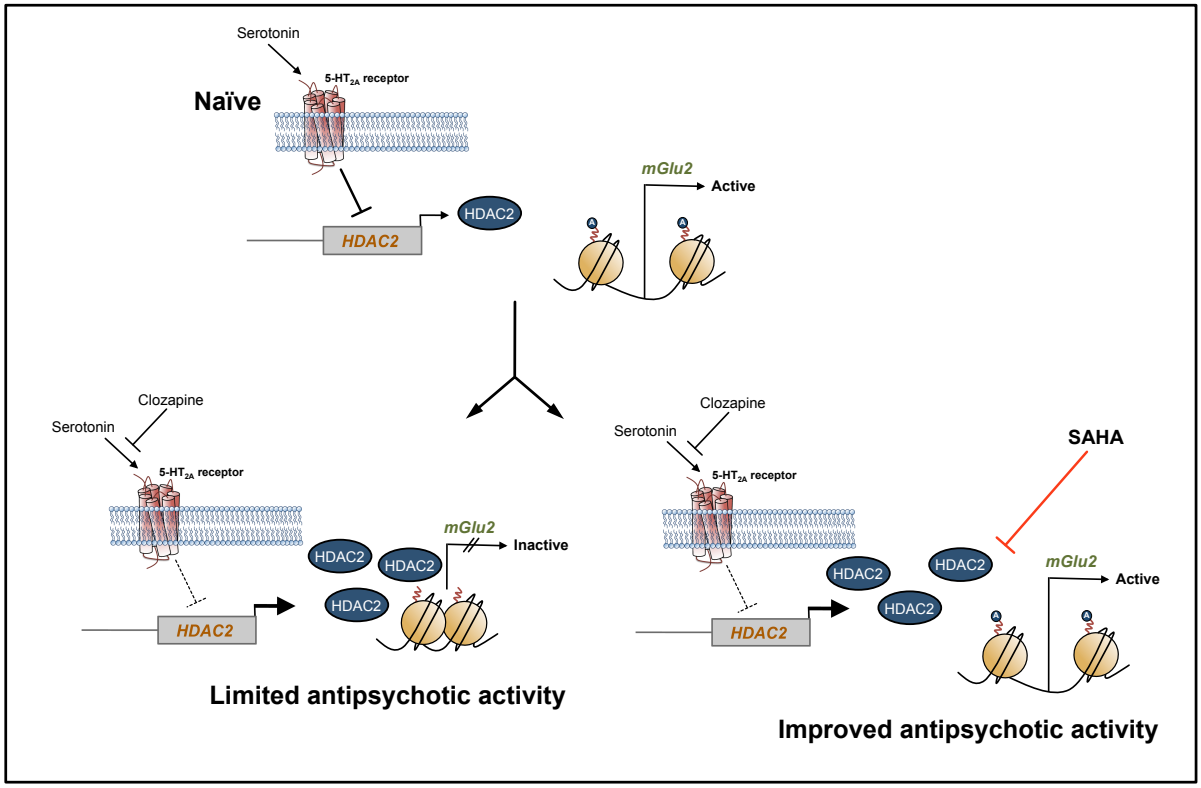

Kurita el al. Supplementary Fig 8

Supplementary Table 1. Demographic characteristics and *antemortem* diagnoses of untreated schizophrenic subjects, treated schizophrenic subjects, and their respective control subjects.

	Gender (F/M)	*Age at death (years)*	*Postmortem delay (h)*	*RIN**	*Storage time (months)*	*Antipsychotic treatment*	*Additional drugs*
Schizophrenic 1 [a,b]	M	30	51	7.1	152	Untreated	
Control 1 [a,b]	M	29	18	5.3	149		
Schizophrenic 2 [a,b]	M	48	20	8.3	83	Untreated	
Control 2 [a,b]	M	47	18	7.8	103		BDZ
Schizophrenic 3 [a,b]	M	31	11	9.2	74	Untreated	
Control 3 [a,b]	M	31	13	8.2	60		ETH (0.96 g/l)
Schizophrenic 4 [a,b]	M	45	3	9.3	67	Untreated	BDZ
Control 4 [a,b]	M	44	21	8.5	53		
Schizophrenic 5 [a,b]	M	27	24	3.8	65	Untreated	
Control 5 [a,b]	M	28	30	8.3	139		
Schizophrenic 6 [a,b]	M	46	22	8.2	42	Untreated	BIP
Control 6 [a,b]	M	46	24	8.5	28		
Schizophrenic 7 [a,b]	M	48	11	8.0	11	Untreated	
Control 7 [a,b]	M	49	8	8.8	4		
Schizophrenic 8 [a,b]	M	45	18	5.4	20	Untreated	
Control 8 [a,b]	M	47	15	7.4	5		
Schizophrenic 9 [a,b]	M	34	15	8.9	23	Untreated	
Control 9 [a,b]	M	36	48	7.7	138		
Schizophrenic 10 [a]	M	52	7	9.1	27	Untreated	BDZ
Control 10 [a]	M	51	13	8.1	7		
Schizophrenic 11 [a]	F	59	9	8.7	18	Untreated	
Control 11 [a]	F	58	20	7.2	137		
Schizophrenic 12 [a,b]	M	30	18	4.9	90	OLA	
Control 12 [a,b]	M	30	11	8	91		THC
Schizophrenic 13 [a,b]	M	32	8	7.4	86	QUE	BDZ, PAR
Control 13 [a,b]	M	32	20	6.7	144		ETH (2.37 g/l)
Schizophrenic 14 [a,b]	M	56	12	4	20	OLA, CLT	
Control 14 [a,b]	M	54	16	8.2	11		ETH (0.58 g/l)
Schizophrenic 15 [a,b]	M	35	3	9.2	81	QUE	BDZ
Control 15 [a,b]	M	36	23	8.2	12		
Schizophrenic 16 [a,b]	M	35	11	8.6	31	CLZ	BDZ, CLM, PAR
Control 16 [a,b]	M	36	18	9.2	101		BZG, ETH (1.69 g/l)
Schizophrenic 17 [a,b]	M	37	8	7.1	36	OLA	BDZ, DPH, ETH (0.9 g/l)
Control 17 [a,b]	M	38	33	7.6	166		ETH (1.62 g/l)
Schizophrenic 18 [a]	M	44	6	8.2	92	CLT, LEV	BIP, BDZ
Control 18 [a]	M	44	23	7.9	11		
Schizophrenic 19 [a]	M	23	16	9.1	81	SUL	
Control 19 [a]	M	23	17	7.1	54		
Schizophrenic 20 [b]	F	42	38	4	78	CLZ, QUE, SUL	BDZ
Control 20 [b]	F	38	22	7.6	7		
Schizophrenic 21 [b]	M	33	23	6.2	68	CLZ	
Control 21 [b]	M	33	4	9.1	60		
Schizophrenia group	2F/19M	39.6 ± 2.1	15.9 ± 2.5	7.3 ± 0.4	59.3 ± 7.6		
Control group	2F/19M	39.5 ± 2.0	19.7 ± 2.0	7.8 ± 0.1	70.48 ± 12.6		

Antipsychotics were not detected in blood samples of untreated schizophrenics. Therapeutic levels of clotiapine (CLT), levomepromazine (LEV), olanzapine (OLA), quetiapine (QUE), sulpiride (SUL), and clozapine (CLZ) were detected in blood samples of treated schizophrenics. All schizophrenic subjects included, except schizophrenic 9, schizophrenic 11, schizophrenic 14, schizophrenic 16, and schizophrenic 18, committed suicide. Abbreviations: benzodiazepines (BDZ), and biperiden (BIP), benzoylecgonine (BZG), clomipramine (CLM), diphenhydramine (DPH), paracetamol (PAR), and tetrahydrocannabinol (THC). Ethanol in blood is coded as ETH. [a]Postmortem human brain samples assayed by Western blot. [b]Postmortem human brain samples assayed by ChIP. *RIN, RNA integrity number.

Supplementary Table 2

Mouse qRT-PCR primer pairs

Gene Name	GenBank	Primer pairs	
		Forward	Reverse
Hdac1	**BC092070**	TGCGTGGAAAGAAAACAACC	ACCCAGACCCCTCCTAAATG
Hdac2	**NM_008229**	GGGACAGGCTTGGTTGTTTC	GAGCATCAGCAATGGCAAGT
Hdac3	**NM_010411**	GCCAAGACCGTGGCGTATT	GTCCAGCTCCATAGTGGAAGT
Hdac4	**NM_207225**	CAATCCCACAGTCTCCGTGT	CAGCACCCCACTAAGGTTCA
Hdac5	**NM_001077696**	TGTCACCGCCAGATGTTTTG	TGAGCAGAGCCGAGACACAG
Hdac6	**NM_010413**	GTGGAAAGAAGTGTGCTGTACC	CCCACAACTAGGTCCTCTTCAG
Hdac7	**NM_019572**	GGTGGACCCCCTTTCAGAAG	TGGGTAGCCAGGAGTCTGGA
Hdac8	**NM_027382**	CATCGAAGGTTATGACTGTGTCC	GTTCTGGTGAAACAGGCTCTT
Hdac9	**NM_024124**	GCGAGACACAGATGCTCAGAC	TGGGTTTTCCTTCCATTGCT
Htr2a	**NM_172812**	GCAGTCCATCAGCAATGAGC	GCAGTGGCTTTCTGTTCTCC
Htr2c	**NM_008312**	GTATTCCCTCCCTTCCTTGC	CGTGTGTGAATGAGCAGAGC
Drd2	**NM_010077**	CTCTACCCTCCAATCCACTC	CATCCACAGCCTCCTCTAAG
Grm2	**NM_001160353**	CCATCTTCTACGTCACCTCC	AGGAACAAGCTGGGATCCAG
Grm3	**NM_181850**	TGACTACAGAGTGCAGACGAC	TCGCAGTTCCACTGACACTG
GluR1	**NM_001113325**	TGTGTTTGTGAGGACTACGGCA	GGATTCTTTGCCACCTTCATT
NR2B	**NM_008171.3**	CCCAGATCCTCGATTTCATT	GCCAAACTGGAAGAACATGG
Syp	**NM_009305.2**	GCCACGGACCCAGAGAACAT	GGAAGCCAAACACCACTGAG
Syt1	**NM_001252341**	CATCGACCAGATCCACTTGT	TCGTTTCCTACTTGGCACAC
I -actin	**NM_007393**	AGGTGACAGCATTGCTTCTG	GCTGCCTCAACACCTCAAC
GAPDH	**NM_008084**	TGCGACTTCAACAGCAACTC	CTTGCTCAGTGTCCTTGCTG
mapkapk5	**NM_010765**	CATTGCCCAGTGTATCCTCC	ACCTGCTTTACCACCTCTGC
rpS3	**NM_012052**	AGGTTGTGGTGTCTGGGAAG	GAGGCTTCTTGGGACCAATC

PCR primer pairs for ChIP assay in mouse samples

Gene name	GenBank	Primer pairs		Location	Nucleotide location from TSS[1]
		Forward	Reverse		
Htr2a	**NT_039606**	CCTGGACACATCATCACTGG	AGACAGCTTAGGGGACAGCA	Promoter	-295 to -138
Htr2c	**NT_039718**	GCCATGGATGACCTCAGTTT	TACACTGCTCAGGGCCTGTT	Promoter	-238 to -94
Drd2	**NT_039472**	GCCCTATGGCTTGAAGGTAA	GACAGGCGGCGCTAGAGT	Promoter	+48 to +178
Grm2	**NT_039477**	ATCCTGCTGCTACCACGTCT	TGGACACAGAAACTGGATGC	-1.4 kb upstream promoter	-1419 to -1299
Grm2	**NT_039477**	GCCACTGTCTCATCTGTTCC	ATCCCGCTCTTGACAGGT	Promoter	-340 to -188
Grm2	**NT_039477**	TTAATGAGCACCGTGGCATA	CGTGTGTATCCTTGGAGCAG	Exon 2	+1979 to +2113
Grm3	**NT_039299**	TTCACTCGCTCACACTGCTC	AAGCTCTGCTAAGGCTCACG	Promoter	-694 to -555
Actb	**NT_081055**	GAGACATTGAATGGGGCAGT	ATGAAGAGTTTTGGCGATGG	Promoter	-321 to -230

[1]TSS: transcriptional start site

PCR primer pairs for ChIP assay HEK293 cell cultures

Promoter	Assay	Primer pairs	
		Forward	Reverse
Grm2	Agarose gel	CACACTGTGGACAGCTCCAG	CCGTCTTCGAGTGGGTAGAA
Grm2	qPCR	TAGGGTTAGCCCTTGGTGAA	CCGTCTTCGAGTGGGTAGAA
Luciferase	Agarose gel	GAGCGGCTACGTTAACAACC	GTGAGAATTCACGGCGATCT

PCR primer pairs for ChIP assay in postmortem human brain samples

Gene name	GenBank	Primer pairs		Location	Nucleotide location from TSS[1]
		Forward	Reverse		
Htr2a	**NG_013011**	CTCCTGGCTGTTGCTACCTT	TTTCCACGGGAATGGAGTAG	Promoter	-92 to +7
Htr2c	**NG_012082**	AGTGCTGATTGGCTGCTCTT	CACCAGAGCGCCTACCTC	Promoter	-68 to +26
Grm2	**NT_022517**	GGGATTCAGCACCACGAG	CTCCTCCGTTCCTCCAGAC	Promoter	-204 to -76
Grm3	**NT_007933**	GCGAGGTGGTAGCAGAAAAG	CTTTTTCGCATTCTCCCAAA	Promoter	-258 to -165
B2M	**NT_010194**	GGGCACCATTAGCAAGTCAC	GGCGCTCATTCTAGGACTTC	Promoter	! 318 to ! 220

[1]TSS: transcriptional start site