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# Supplementary Materials for

## Ubiquitin-proteasomal regulation of chromatin remodeler INO80 in the nucleus accumbens mediates persistent cocaine craving

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**

(available at advances.sciencemag.org/cgi/content/full/5/10/eaay0351/DC1)

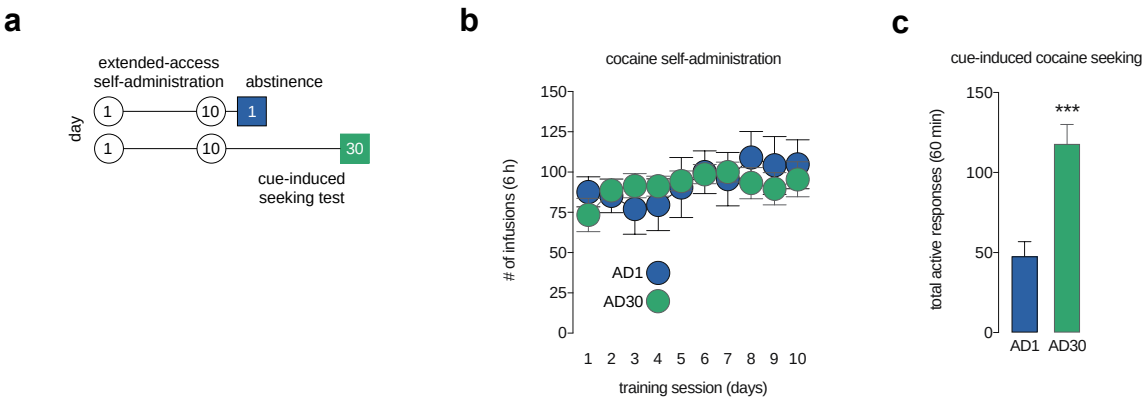


**Fig. S1. Incubated cocaine craving during prolonged abstinence following extended-access cocaine self-administration.** (**A**) Schematic of experimental timeline for extended-access self-administration and cue-induced seeking test. (**B**) Extended-access cocaine self-administration training behavior (two-way repeated measures ANOVA, treatment × session: $F_{(9,170)} = 0.445$, $p = 0.909$, $n$ = 9-10 rats/group). (**C**) Total active responses during a cue-induced seeking test (*t*-test, $t_{(17)} = 4.496$, $p = 0.0003$; $n$ = 9-10 rats/group). Data are mean ± sem. ***$p < 0.001$. AD: abstinence day.

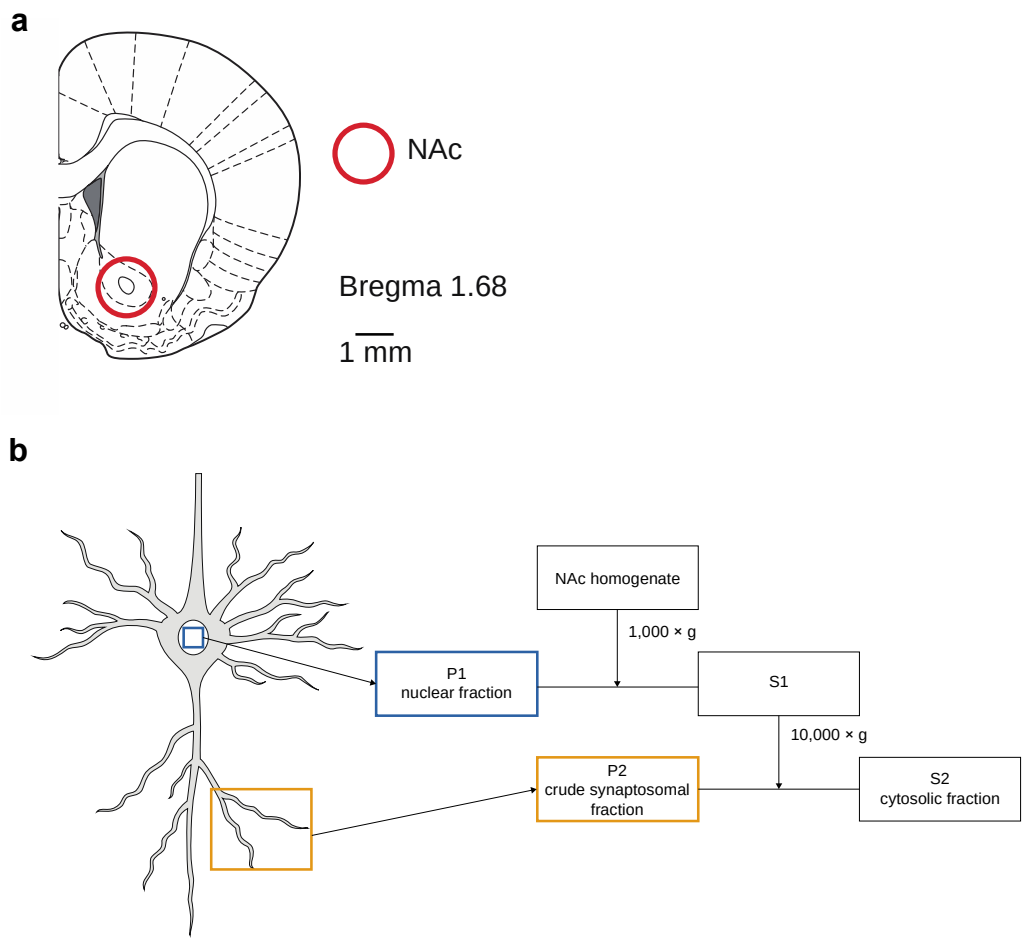


**Fig. S2. Experimental timeline and tissue collection.** (**A**) Schematic of dissected 2-mm-diameter nucleus accumbens (NAc) punches (red) from 1 mm coronal slice prepared with a brain matrix for immunoblotting, ChIP-seq, qPCR and qChIP studies. (**B**) schematic of NAc tissue processing to obtain nuclear, cytosolic and synaptosomal fractions.

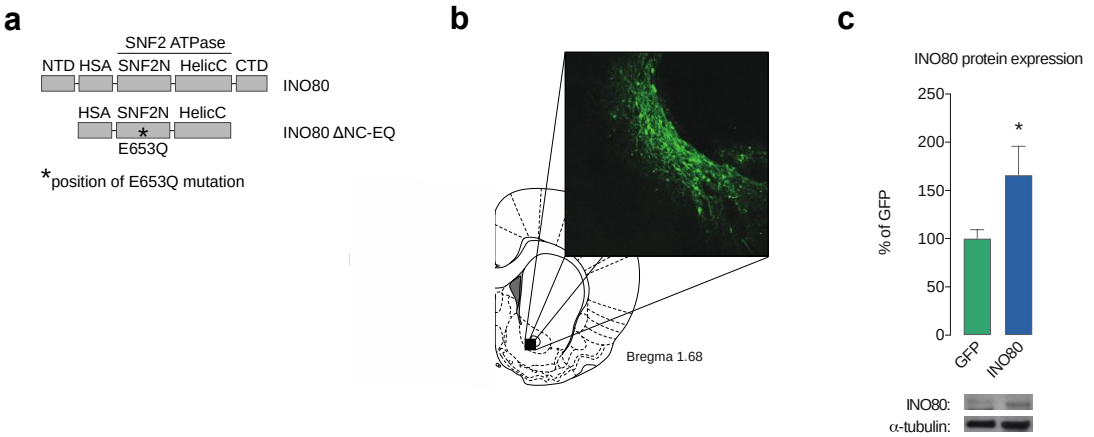


**Fig. S3. Custom HSV vectors made to manipulate INO80.** (**A**) Schematics depicting custom HSV vectors for INO80 and INO80 ΔNC-EQ. (**B**) Anatomical placement of viral infection and representative image of HSV-infected area in the NAc adjacent to the anterior commissure. (**C**) INO80 protein expression in NAc tissue infected with HSV-INO80 ($t_{(9)}$ = 2.255, $p$ = 0.025; $n$ = 5-6 rats/group). Data are mean ± sem. *$p$ < 0.05. HSV: herpes-simplex virus.

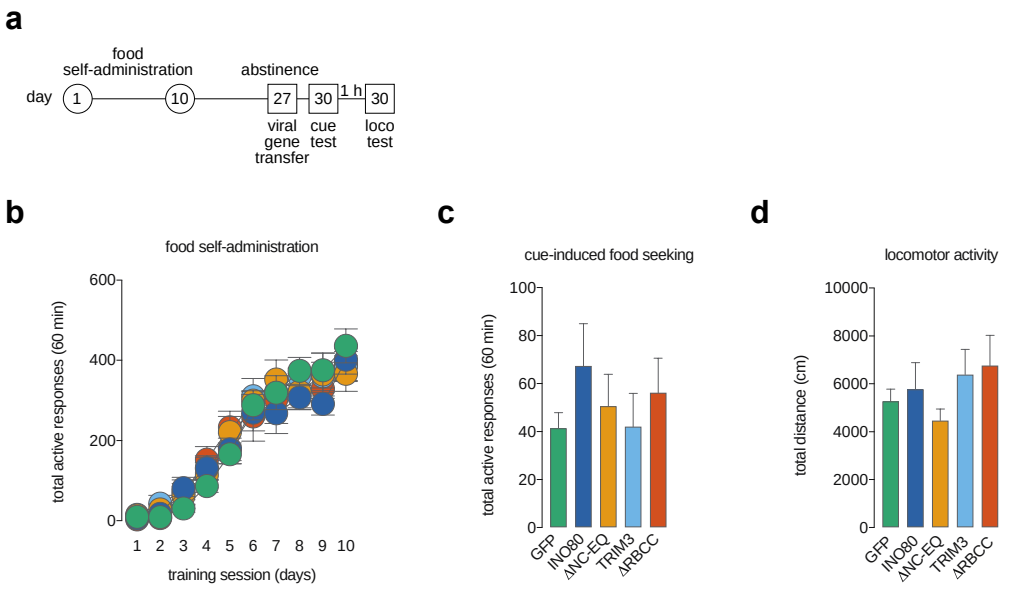


**Fig. S4. Cue-induced food seeking and locomotor activity following food self-administration.** (**A**) Schematic of experimental timeline for food self-administration, viral-mediated gene transfer and cue-induced seeking test. (**B**) Self-administration training behavior prior to viral-mediated gene transfer (two-way repeated measures ANOVA, treatment group $F_{(4,410)} = 0.566$, $p = 0.688$; $n = 7$-16 rats/group). (**C**) Total active responses during a 1 h cue-induced seeking test on "AD30" (one-way ANOVA, treatment group $F_{(4,41)} = 0.816$, $p = 0.522$; $n = 7$-16 rats/group). (**D**) Total distance traveled in a locomotor test (one-way ANOVA, treatment group $F_{(4,41)} = 0.95$, $p = 0.445$; $n = 7$-16 rats/group). Data are mean ± sem. ΔNC-EQ: catalytically inactive mutant INO80; ΔRBCC: catalytically inactive mutant TRIM3.

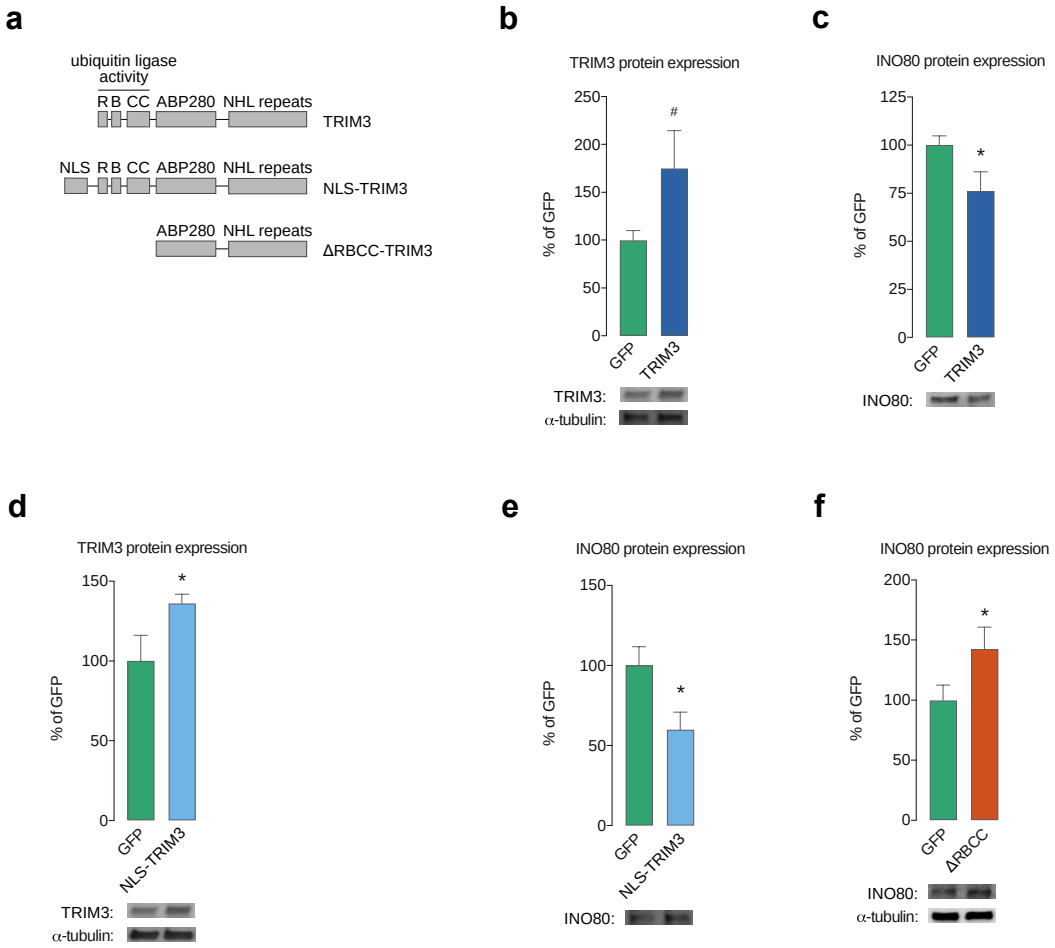


**Fig. S5. Custom HSV vectors made to manipulate TRIM3.** (**A**) Schematics depicting custom HSV vectors for TRIM3, NLS-TRIM3 and ΔRBCC-TRIM3. (**B**, **C**) TRIM and INO80 protein expression in NAc tissue infected with HSV-TRIM3 (TRIM3: *t*-test, $t_{(8)} = 1.815$, $p = 0.054$; INO80: *t*-test, $t_{(7)} = 2.289$, $p = 0.028$; $n = 4$-5 rats/group). (**D**, **E**) TRIM and INO80 protein expression in NAc tissue infected with HSV-NLS-TRIM3 (TRIM3: *t*-test, $t_{(7)} = 1.904$, $p = 0.049$; INO80: *t*-test, $t_{(9)} = 2.497$, $p = 0.017$; $n = 4$-6 rats/group). (**F**) INO80 protein expression in NAc tissue infected with HSV-ΔRBCC-TRIM3 ($t_{(8)} = 1.905$, $p = 0.047$; $n = 5$ rats/group). Data are mean ± sem. *$p < 0.05$, #$p = 0.05$. HSV: herpes-simplex virus; ΔRBCC: ring-binding coiled coil; NLS: nuclear localizing signal.

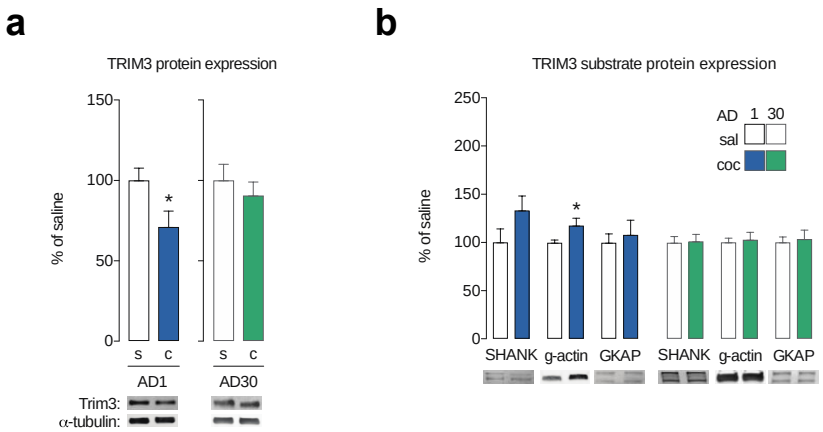


**Fig. S6. Expression of TRIM3 and synaptic substrates during abstinence following extended-access self-administration.** (**A**) TRIM3 protein expression in NAc P2 fractions (AD1: *t*-test, $t_{(14)} = 2.35$, $p = 0.034$; AD30: *t*-test, $t_{(18)} = 0.706$, $p = 0.489$; $n = 7$-10 rats/group). (**B**) SHANK, γ-actin and GKAP protein expression in NAc P2 fractions on AD1 and AD30 (SHANK AD1: *t*-test, $t_{(15)} = 1.592$, $p = 0.066$; γ-actin AD1: *t*-test, $t_{(13)} = 2.288$, $p = 0.02$; GKAP AD1: *t*-test, $t_{(16)} = 0.225$, $p = 0.825$; SHANK AD30: *t*-test, $t_{(17)} = 0.111$, $p = 0.913$; $n = 9$-10; γ-actin AD30: *t*-test, $t_{(18)} = 0.321$, $p = 0.752$; GKAP AD30: *t*-test, $t_{(18)} = 0.317$, $p = 0.755$; $n = 7$-10 rats/group). Data are mean ± sem. *$p < 0.05$. AD: abstinence day; P2: crude synaptosomal fraction.

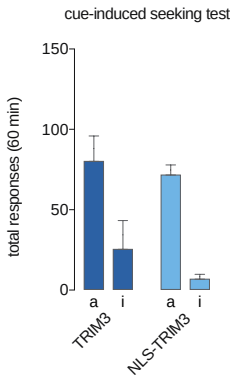


**Fig. S7. Similar cocaine-seeking behavior in rats that received intra-NAc injections of HSV-TRIM3 or HSV-NLS-TRIM3.** Total active and inactive responses during a 60-min cue-induced seeking test on AD30 for rats that self-administered cocaine (one-way ANOVA, treatment $F_{(1,18)}$ = 0.245; Fisher PLSD test for total active lever: TRIM3 vs NLS-TRIM3 $p$ = 0.597; $n$ = 5-6 rats/group)HSV: herpes-simplex virus; NLS: nuclear localizing signal.

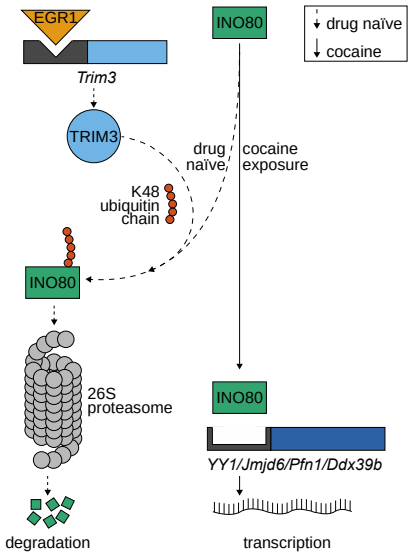


**Fig. S8. Schematic of INO80 signaling in the NAc.** Under basal conditions (drug naïve; dotted lines), Egr1 transcribes *Trim3* and TRIM3 mediates the polyubiquitination of INO80 for degradation by the 26S proteasome complex. During prolonged abstinence following cocaine exposure (solid line), there are reductions in Egr1 binding along the *Trim3* promoter, TRIM3 protein expression and TRIM3-mediated proteasomal degradation of INO80. This leads to an increase in INO80 expression that mediates cue-induced cocaine seeking and changes in INO80 binding patterns along the rat genome.

**Table S1. Primer list for qPCR.**

| Gene symbol | Gene name | Ensembl accession no. | Primer sequence, 5'−3' | |
|---|---|---|---|---|
| *Trim3* | Tripartite Motif-Containing protein 3 | ENSRNOG00000018356 | F | GAGCCAGCATCTTCCAACTGTA |
| | | | R | AACCCAACAAGCCCTAACAC |
| *Yy1* | YY1 Transcription Factor | ENSG00000100811 | F | TTTGCCAGAATGAAGCCAAGAA |
| | | | R | GCTCTCAACGAACGCTTTGC |
| *Jmjd6* | Arginine Demethylase and Lysine Hydroxylase | ENSG00000070495 | F | AACTGTCTTTGTACCAGGGGG |
| | | | R | CGCCCTCTTACCGTCTTG |
| *Ddx39b* | DExD-Box Helicase 39B | ENSG00000198563 | F | AGTGCTTAGCTCTTCTGTCGG |
| | | | R | GTCCACATCGTTCTCTGCCA |

**Table S2. Primer list for ChIP.**

| Gene symbol | Gene name | Ensembl accession no. | Primer sequence, 5'−3' | |
|---|---|---|---|---|
| *Trim3* | Tripartite Motif-Containing 3 | ENSRNOT00000024850.5 | F | gggtctgagagatgtgggcg |

**Data S1. INO80 ChiP-seq data tables.** INO80 peaks (cocaine, saline); INO80 diffReps (cocaine vs. saline); Kegg INO80 (cocaine vs. saline)