

Intestinal activating transcription factor 4 regulates stress-related behavioral alterations via paraventricular thalamus in male mice

Authors

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Running title:

Enteric ATF4 regulates stress-related behaviors

Supplementary figures, figure legends and tables

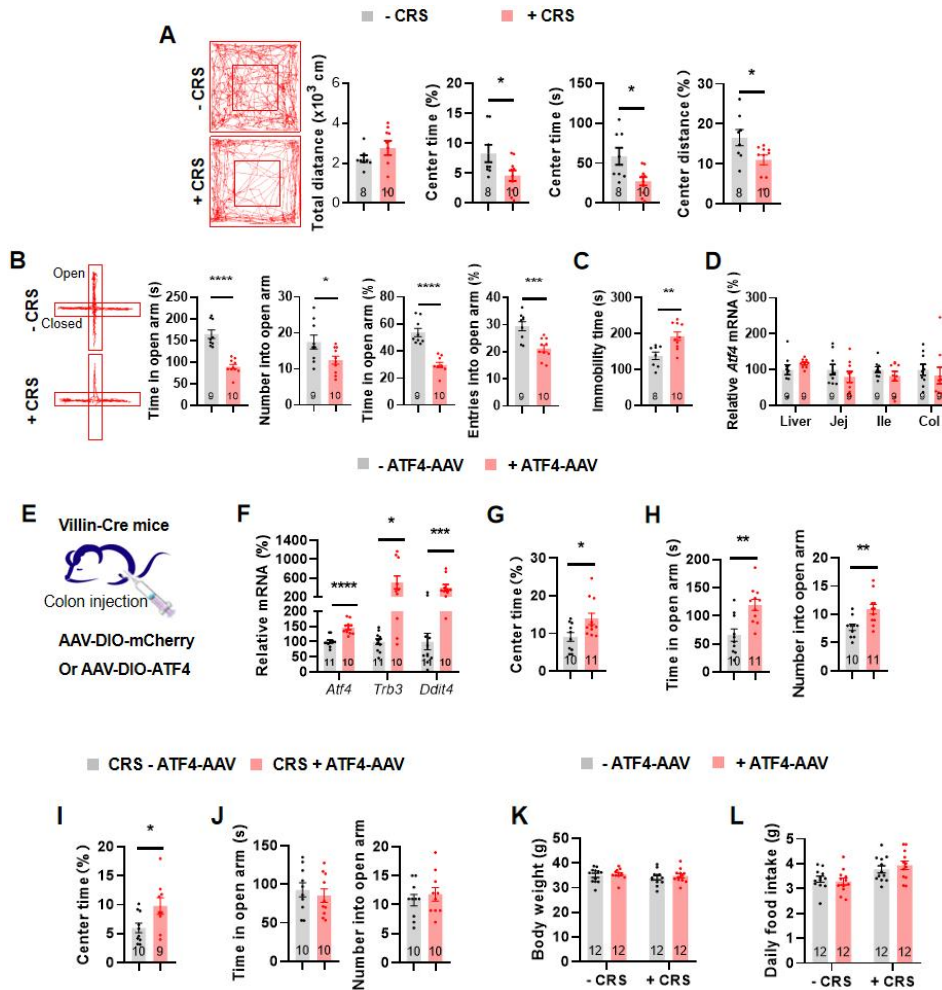


Fig. S1. Behavioral effects of mice subjected to chronic restraint stress (CRS) or AAVs.

A. Representative tracks of mice in open field test (OFT), travel distance (Unpaired t test with Welch's correction, $t_{12.67} = 1.368$, $P = 0.1952$), percentage of time spent in center ($t_{16} = 2.287$, $P = 0.0361$), time spent in center ($t_{16} = 2.806$, $P = 0.0127$), and percentage of distance in center area ($t_{16} = 2.438$, $P = 0.0268$).

B. Representative tracks of mice in elevated plus maze test (EPM), the time spent in the open arms ($t_{17} = 6.769$, $P < 0.0001$), the number of entries into the open arms ($t_{17} = 2.320$, $P = 0.0330$), percentage of time spent in the open arms ($t_{17} = 6.960$, $P < 0.0001$), and percentage of entries into the open arms ($t_{17} = 4.078$, $P = 0.0008$).

C. Immobility time of tail suspension test (TST) ($t_{16} = 3.405$, $P = 0.0036$).

D. Gene expression of *Atf4* in liver (Unpaired *t* test with Welch's correction, $t_{9,422} = 1.178$, $P = 0.2677$), jejunal (Jej) ($t_{16} = 0.9478$, $P = 0.3573$), ileal (Ile) ($t_{16} = 1.157$, $P = 0.2641$) and colonic (Col) (Mann Whitney test, $P = 0.2224$) lamina propria by RT-PCR.

E. Schematic of Colonic injection of AAV in Villin-Cre mice.

F. Gene expression of *Atf4* ($t_{19} = 5.319$, $P < 0.0001$), *Trb3* (Unpaired *t* test with Welch's correction, $t_{9,086} = 3.043$, $P = 0.0138$) and *Ddit* (Mann Whitney test, $P = 0.0001$) in colon epithelial cells by RT-PCR.

G. Percentage of time spent in center in OFT ($t_{19} = 2.426$, $P = 0.0254$).

H. The time spent in the open arms ($t_{19} = 3.636$, $P = 0.0018$), the number of entries into the open arms ($t_{19} = 3.095$, $P = 0.0060$) in EPM.

I. Percentage of time spent in center in OFT ($t_{17} = 2.363$, $P = 0.0303$).

J. The time spent in the open arms ($t_{18} = 0.5953$, $P = 0.5591$), the number of entries into the open arms ($t_{18} = 0.6591$, $P = 0.5182$) in EPM.

K. Body weight (Adenovirus Factor: $F_{1,44} = 0.6048$, $P = 0.4409$; CRS factor: $F_{1,44} = 1.052$, $P = 0.3107$; Adenovirus \times CRS: $F_{1,44} = 0.02925$, $P = 0.8650$).

L. Average of daily food intake (Adenovirus Factor: $F_{1,44} = 0.06367$, $P = 0.8020$; CRS factor: $F_{1,44} = 13.44$, $P = 0.0007$; Adenovirus \times CRS: $F_{1,44} = 0.7795$, $P = 0.3821$).

Studies for A-D were conducted using 8–9-week-old male wild-type mice with or without CRS; studies for F-L were conducted using 8–9-week-old male Villin-Cre mice receiving colonic injection of AAVs expressing mCherry (- AAV-ATF4) or ATF4 (+ AAV-ATF4) under normal states (- CRS) or CRS (+ CRS). Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's *t* test or specific test marked in the data. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

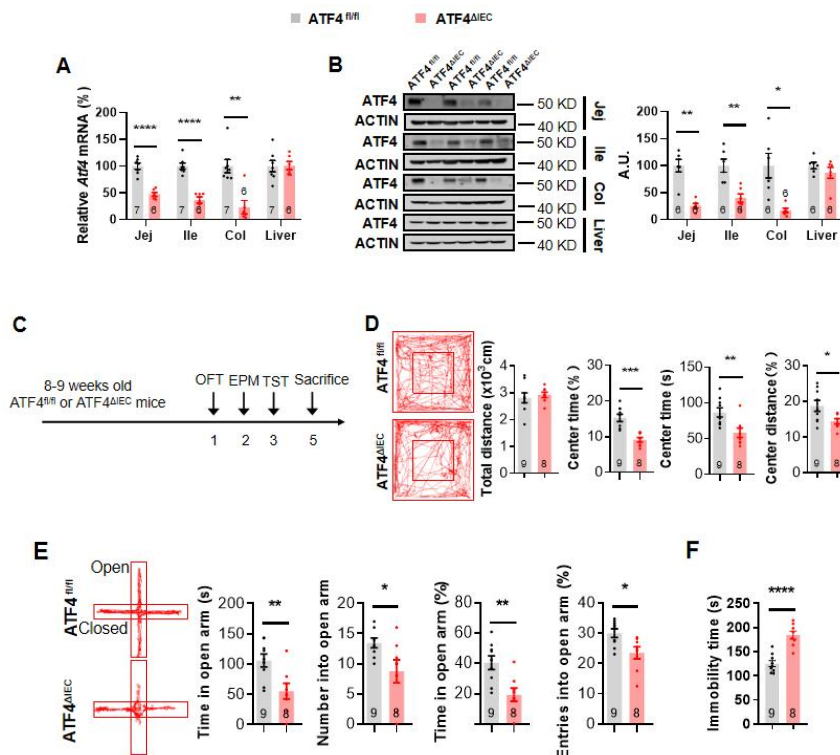


Fig. S2 Embryonic intestinal ATF4 deletion exhibits stress-related behaviors.

A. Gene expression of *Atf4* in jejunal (Jej) ($t_{11} = 7.463$, $P < 0.0001$), ileal (Ile) ($t_{11} = 7.625$, $P < 0.0001$) and colonic (Col) (Mann Whitney test, $P = 0.0082$) epithelial cells and liver ($t_{11} = 0.08589$, $P = 0.9331$) by RT-PCR.

B. ATF4 protein in jejunal ($t_{6.270} = 5.960$, $P = 0.0009$), ileal ($t_{10} = 4.152$, $P = 0.0020$) and colonic ($t_{5.322} = 3.605$, $P = 0.0139$) epithelial cells and liver ($t_{10} = 1.132$, $P = 0.2840$) by western blotting (left) and quantified by densitometric analysis (right), A.U.: arbitrary units.

C. Timeline of the behavioral tests. OFT, open field test; EPM, elevated plus maze test; TST, tail suspension test.

D. Representative tracks of mice in OFT, travel distance ($t_{15} = 0.4957$, $P = 0.6273$), percentage of time spent in center ($t_{15} = 4.857$, $P = 0.0002$), time spent in center ($t_{15} = 3.077$, $P = 0.0077$), and percentage of distance in center area ($t_{15} = 2.440$, $P = 0.0276$).

E. Representative tracks of mice in EPM, the time spent in the open arms ($t_{15} = 3.038$, $P = 0.0083$), the number of entries into the open arms (Unpaired t test with Welch's correction, $t_{9.559} = 2.272$, $P = 0.0475$), percentage of time spent in the open arms ($t_{15} = 3.335$, $P = 0.0045$), and percentage of entries into the open arms ($t_{15} = 2.699$, $P = 0.0165$).

F. Immobility time of tail suspension test (TST) ($t_{15} = 5.780$, $P < 0.0001$).

Studies were conducted using 8–9-week-old male $ATF4^{fl/fl}$ or $ATF4^{\Delta IEC}$ mice. Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test or specific test marked in the data. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

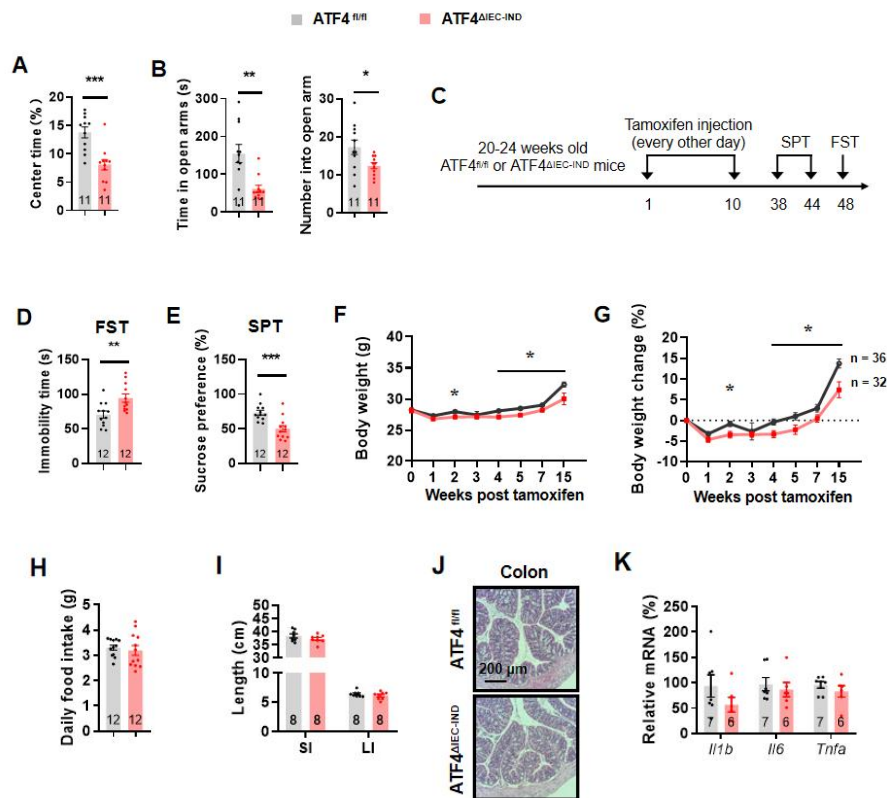


Fig. S3 Effects of inducible intestinal ATF4 knockout mice.

A. Percentage of time spent in center in open field test (OFT) ($t_{20} = 4.269$, $P = 0.0004$).

B. The time spent in the open arms (Mann Whitney test, $P = 0.0032$), the number of entries into the open arms (Unpaired t test with Welch's correction, $t_{13.30} = 2.368$, $P = 0.0337$) in elevated plus maze test (EPM).

C. Timeline of the tamoxifen injection and behavioral tests of D and E. FST, forced swim test; SPT, sucrose preference test.

- D. Immobility time of forced swim test (FST) ($t_{22} = 3.121, P = 0.0050$).
- E. Sucrose preference in sucrose preference test (SPT) ($t_{22} = 4.026, P = 0.0006$).
- F. Body weight curve after tamoxifen administration.
- G. Relative body weight change curve after tamoxifen administration.
- H. Average of daily food intake (Unpaired *t test* with Welch's correction, $t_{16.27} = 0.5048, P = 0.6204$).
- I. Length of small intestine (SI) ($t_{14} = 1.177, P = 0.2590$) and large intestine (LI) ($t_{14} = 1.005, P = 0.3317$).
- J. Hematoxylin and eosin (H&E) of colon.
- K. Gene expression of *Il1b* ($t_{11} = 1.344, P = 0.2059$), *Il6* (Mann Whitney test, $P = 0.6282$) and *Tnfa* (Mann Whitney test, $P = 0.4452$) in colonic epithelial cells by RT-PCR.

Studies were conducted using 14–16-week-old male ATF4^{fl/fl} or ATF4^{ΔIEC-IND} mice. Data are expressed as the mean ± SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's *t test* or specific test marked in the data. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

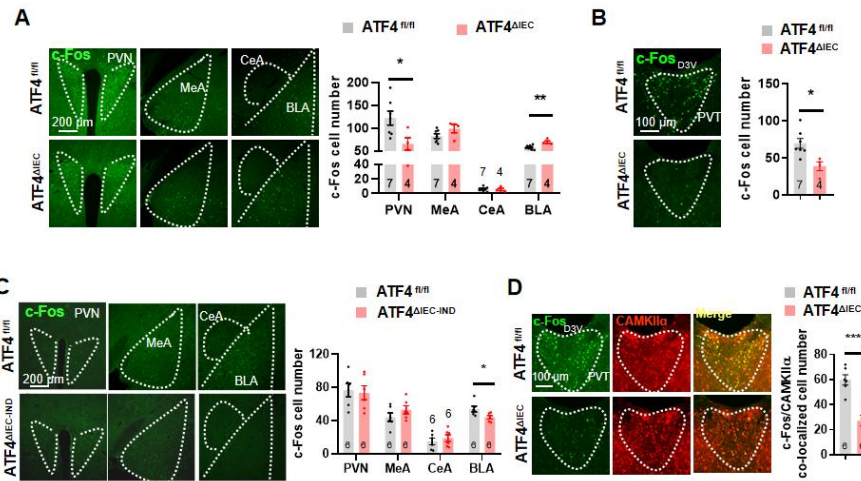


Fig. S4 Immunofluorescence (IF) staining in the brain of intestinal ATF4 knockout mice.

A. IF staining for c-Fos (green) in the paraventricular nucleus of hypothalamus (PVN) ($t_9 = 2.479$, $P = 0.0351$), the medial amygdala (MeA) (Mann Whitney test, $P = 0.0788$), the central amygdala (CeA) ($t_9 = 0.3443$, $P = 0.7385$) and the basolateral amygdala (BLA) ($t_9 = 3.686$, $P = 0.0050$), and quantification of c-Fos cell numbers (right).

B. IF staining for c-Fos (green) in the paraventricular thalamus (PVT) (left) and quantification of c-Fos cell numbers (right) ($t_9 = 3.092$, $P = 0.0129$); D3V, dorsal 3rd ventricle.

C. IF staining for c-Fos (green) in the paraventricular nucleus of hypothalamus (PVN) ($t_{10} = 0.2690$, $P = 0.7934$), the medial amygdala (MeA) ($t_{10} = 1.252$, $P = 0.2392$), the central amygdala (CeA) ($t_{10} = 0.6847$, $P = 0.5091$) and the basolateral amygdala (BLA) ($t_{10} = 2.416$, $P = 0.0363$), and quantification of c-Fos cell numbers (right).

D. IF staining for c-Fos (green), CAMKII α (red) or merge (yellow) in PVT (left), and quantification of c-Fos and CAMKII α colocalized cell numbers (right) ($t_{10} = 5.472$, $P = 0.0003$).

Studies for A, B, D were conducted using 8–9-week-old male ATF4^{fl/fl} or ATF4^{ΔIEC} mice; studies for C were conducted using 14–16-week-old male ATF4^{fl/fl} or

ATF4^{ΔIEC-IND} mice. Data are expressed as the mean ± SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test or specific test marked in the data. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

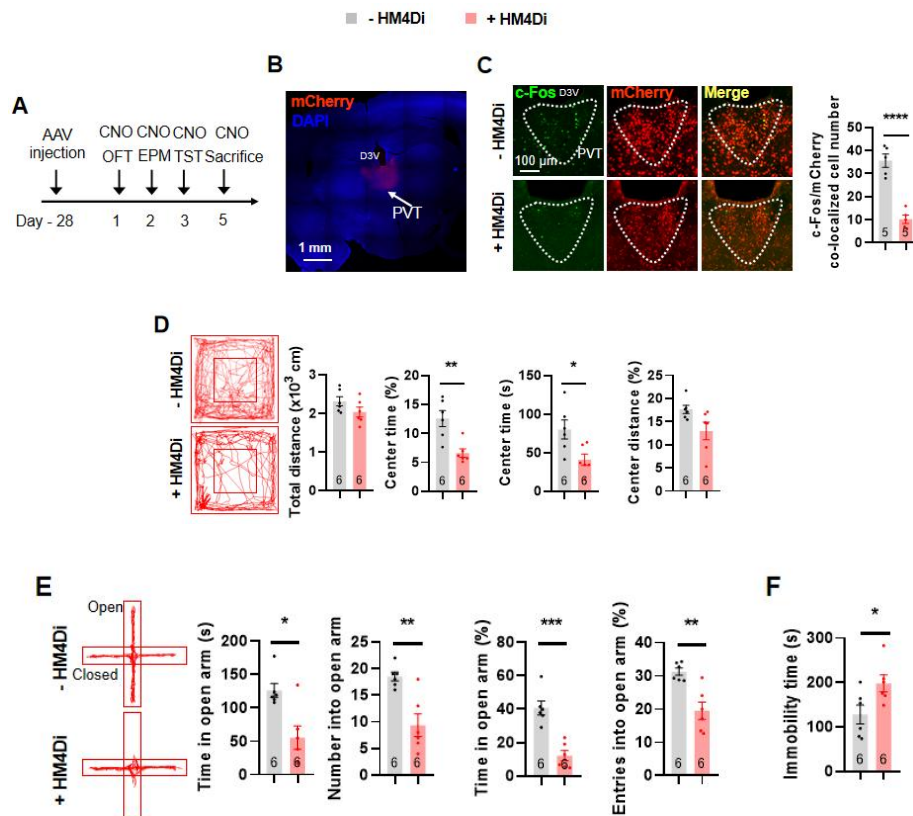


Fig. S5 Inhibition of paraventricular thalamus (PVT) glutamatergic neurons exhibits stress-related behavioral alterations.

A. Timeline of experiment procedure and behavioral tests. OFT, open field test; EPM, elevated plus maze test; TST, tail suspension test.

B. Post hoc visualization of mCherry (red) and DAPI (blue) in PVT of wild-type (WT) mice receiving AAV-CAMKII α -HM4Di-mCherry stereotaxic injections; D3V, dorsal 3rd ventricle.

C. Immunofluorescence (IF) staining for c-Fos (green), mCherry (red) or merge (yellow) in PVT (left), and quantification of c-Fos and mCherry colocalized cell numbers (right) ($t_8 = 7.556$, $P < 0.0001$).

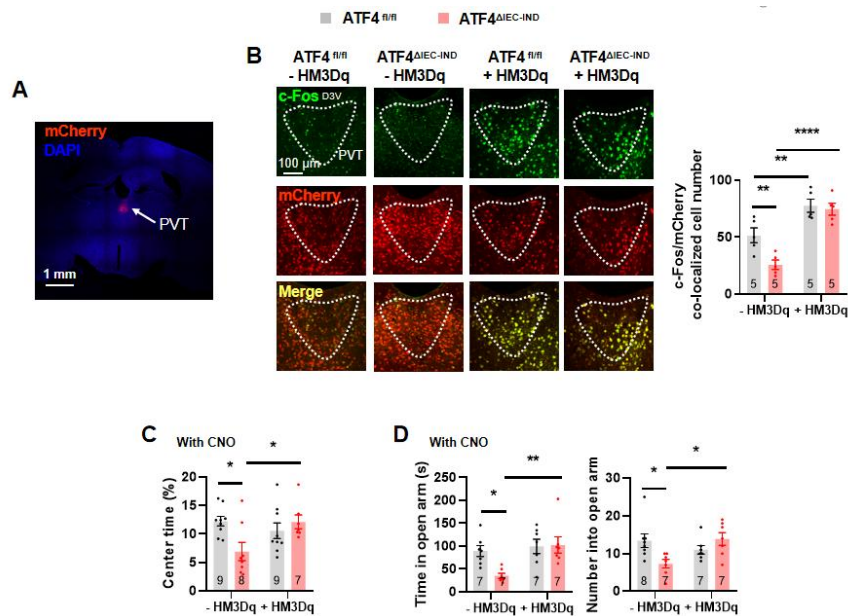
D. Representative tracks of mice in OFT, travel distance ($t_{10} = 1.581$, $P = 0.1450$),

percentage of time spent in center ($t_{10} = 3.807$, $P = 0.0034$), time spent in center ($t_{10} = 2.719$, $P = 0.0216$), and percentage of distance in center area ($t_{10} = 2.214$, $P = 0.0512$).

E. Representative tracks of mice in EPM, the time spent in the open arms (Mann Whitney test, $P = 0.0411$), the number of entries into the open arms ($t_{10} = 4.009$, $P = 0.0025$), percentage of time spent in the open arms ($t_{10} = 5.536$, $P = 0.0002$), and percentage of entries into the open arms ($t_{10} = 4.129$, $P = 0.0020$).

F. Immobility time of tail suspension test (TST) ($t_{10} = 2.449$, $P = 0.0343$).

Studies were conducted using 7–8-week-old male WT mice receiving AAVs expressing mCherry (- HM4Di) or HM4Di (+ HM4Di), followed by CNO injection. Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test or specific test marked in the data. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.



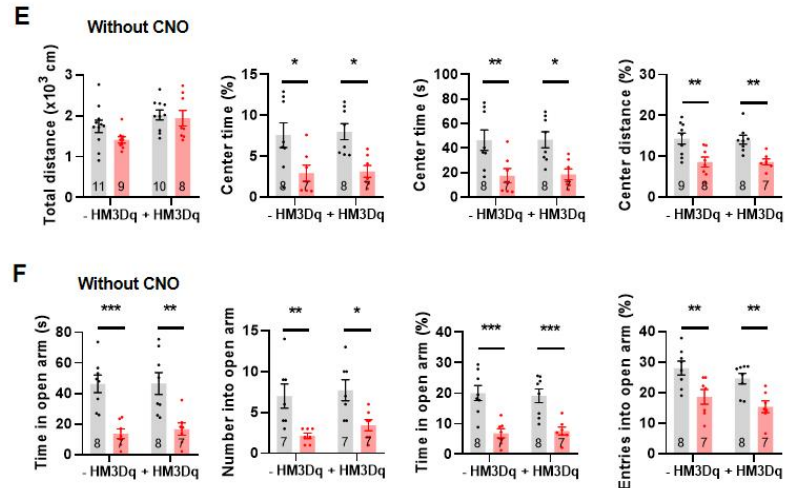


Fig. S6 Behavioral effects of inducible intestinal ATF4 deletion mice with paraventricular thalamus (PVT) glutamatergic neurons activation.

A. Post hoc visualization of mCherry (red) and DAPI (blue) in PVT of ATF4^{ΔIEC-IND} mice receiving AAV-CAMKII α -HM3Dq-mCherry stereotaxic injections; D3V, dorsal 3rd ventricle.

B. Immunofluorescence (IF) staining for c-Fos (green), mCherry (red) or merge (yellow) in PVT (left), and quantification of c-Fos and mCherry colocalized cell numbers (right) (Genotype Factor: $F_{1,16} = 6.957$, $P = 0.0179$; HM3Dq factor: $F_{1,16} = 46.93$, $P < 0.0001$; Genotype \times HM3Dq: $F_{1,16} = 4.360$, $P = 0.0531$).

C. Percentage of time spent in center in open field test (OFT) (Genotype Factor: $F_{1,29} = 2.145$, $P = 0.1538$; HM3Dq factor: $F_{1,29} = 1.841$, $P = 0.1853$; Genotype \times HM3Dq: $F_{1,29} = 7.147$, $P = 0.0122$).

D. The time spent in the open arms (Genotype Factor: $F_{1,24} = 3.561$, $P = 0.0713$; HM3Dq factor: $F_{1,24} = 8.223$, $P = 0.0085$; Genotype \times HM3Dq: $F_{1,24} = 4.445$, $P = 0.0456$), the number of entries into the open arms (Genotype Factor: $F_{1,25} = 1.162$, $P = 0.2914$; HM3Dq factor: $F_{1,25} = 1.958$, $P = 0.1740$; Genotype \times HM3Dq: $F_{1,25} = 8.900$, $P = 0.0063$) in elevated plus maze test (EPM).

E. Representative tracks of mice in OFT, travel distance (Genotype Factor: $F_{1,34} = 2.112$, $P = 0.1553$; HM3Dq factor: $F_{1,34} = 8.248$, $P = 0.0070$; Genotype \times HM3Dq: $F_{1,34} = 0.8104$, $P = 0.3743$), percentage of time spent in center (Genotype Factor: $F_{1,26} = 17.81$, $P = 0.0003$; HM3Dq factor: $F_{1,26} = 0.07846$, $P = 0.7816$; Genotype \times

HM3Dq: $F_{1,26} = 0.009319$, $P = 0.9238$), time spent in center (Genotype Factor: $F_{1,26} = 18.54$, $P = 0.0002$; HM3Dq factor: $F_{1,26} = 0.01205$, $P = 0.9134$; Genotype \times HM3Dq: $F_{1,26} = 0.003922$, $P = 0.9505$), and percentage of distance in center area (Genotype Factor: $F_{1,28} = 22.61$, $P < 0.0001$; HM3Dq factor: $F_{1,28} = 0.01271$, $P = 0.9110$; Genotype \times HM3Dq: $F_{1,26} = 0.01376$, $P = 0.9075$).

F. Representative tracks of mice in EPM, the time spent in the open arms (Genotype Factor: $F_{1,26} = 32.63$, $P < 0.0001$; HM3Dq factor: $F_{1,26} = 0.08644$, $P = 0.7711$; Genotype \times HM3Dq: $F_{1,26} = 0.06746$, $P = 0.7971$), the number of entries into the open arms (Genotype Factor: $F_{1,24} = 18.88$, $P = 0.0002$; HM3Dq factor: $F_{1,24} = 0.9032$, $P = 0.3514$; Genotype \times HM3Dq: $F_{1,24} = 0.07373$, $P = 0.7883$), percentage of time spent in the open arms (Genotype Factor: $F_{1,26} = 37.18$, $P < 0.0001$; HM3Dq factor: $F_{1,26} = 0.002266$, $P = 0.9624$; Genotype \times HM3Dq: $F_{1,26} = 0.1678$, $P = 0.6854$), and percentage of entries into the open arms (Genotype Factor: $F_{1,26} = 37.18$, $P = 0.0001$; HM3Dq factor: $F_{1,26} = 2.548$, $P = 0.1225$; Genotype \times HM3Dq: $F_{1,26} = 0.002497$, $P = 0.9605$).

Studies were conducted using 10–12-week-old male ATF4^{fl/fl} or ATF4 ^{Δ IEC-IND} mice receiving AAVs expressing mCherry (- HM3Dq) or HM3Dq (+ HM3Dq), with CNO injection (A-D), or without CNO injection (E and F). Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

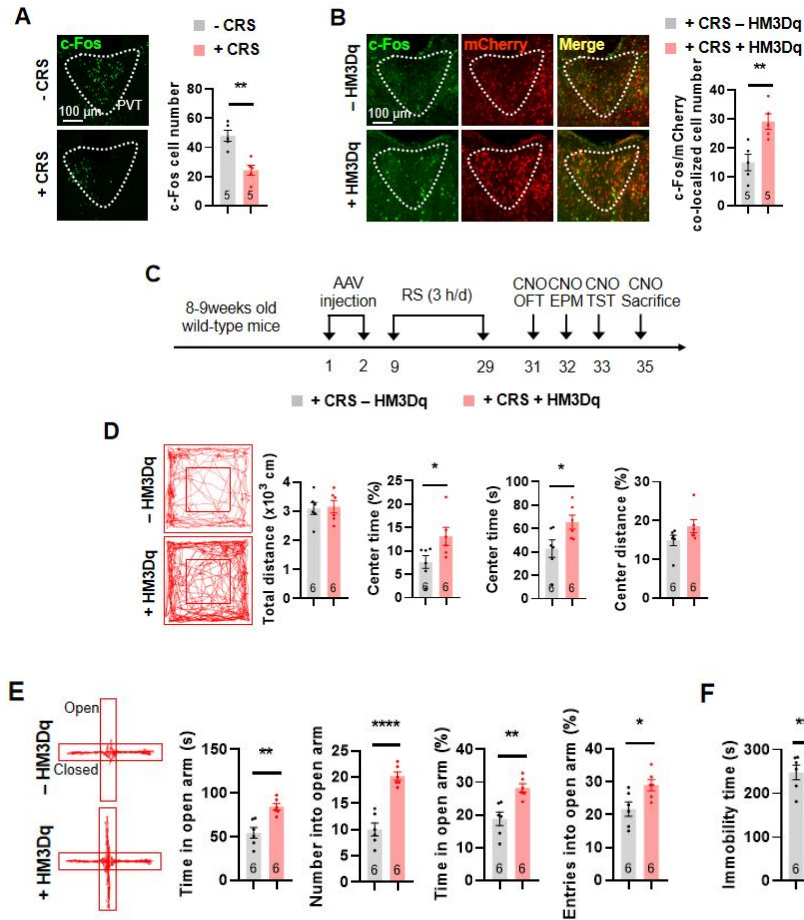


Fig. S7 Activation of paraventricular thalamus (PVT) glutamatergic neurons resists to chronic restraint stress (CRS) induced stress-related behaviors.

A. Immunofluorescence (IF) staining for c-Fos (green) in PVT (left) and quantification of c-Fos cell numbers (right) ($t_8 = 4.597$, $P = 0.0018$); D3V, dorsal 3rd ventricle.

B. IF staining for c-Fos (green), mCherry (red) or merge (yellow) in PVT (left), and quantification of c-Fos and mCherry colocalized cell numbers (right) ($t_8 = 3.649$, $P = 0.0065$).

C. Timeline of the behavioral tests. OFT, open field test; EPM, elevated plus maze test; TST, tail suspension test.

D. Representative tracks of mice in OFT, travel distance ($t_{10} = 0.1943$, $P = 0.8499$), percentage of time spent in center ($t_{10} = 2.334$, $P = 0.0417$), time spent in center ($t_{10} = 2.362$, $P = 0.0398$), and percentage of distance in center area (Mann Whitney test, $P =$

0.0931).

E. Representative tracks of mice in EPM, the time spent in the open arms ($t_{10} = 4.149$, $P = 0.0020$), the number of entries into the open arms ($t_{10} = 6.916$, $P < 0.0001$), percentage of time spent in the open arms ($t_{10} = 3.896$, $P = 0.0030$), and percentage of entries into the open arms ($t_{10} = 2.649$, $P = 0.0244$).

F. Immobility time of tail suspension test (TST) ($t_{10} = 5.232$, $P = 0.0004$).

Studies for A were conducted using 8–9-week-old male wild-type (WT) mice with or without CRS; studies for B-F were conducted using 8–9-week-old male WT mice receiving AAVs expressing mCherry (- HM3Dq) or HM3Dq (+ HM3Dq), followed by CRS and CNO injection. Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test or specific test marked in the data. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

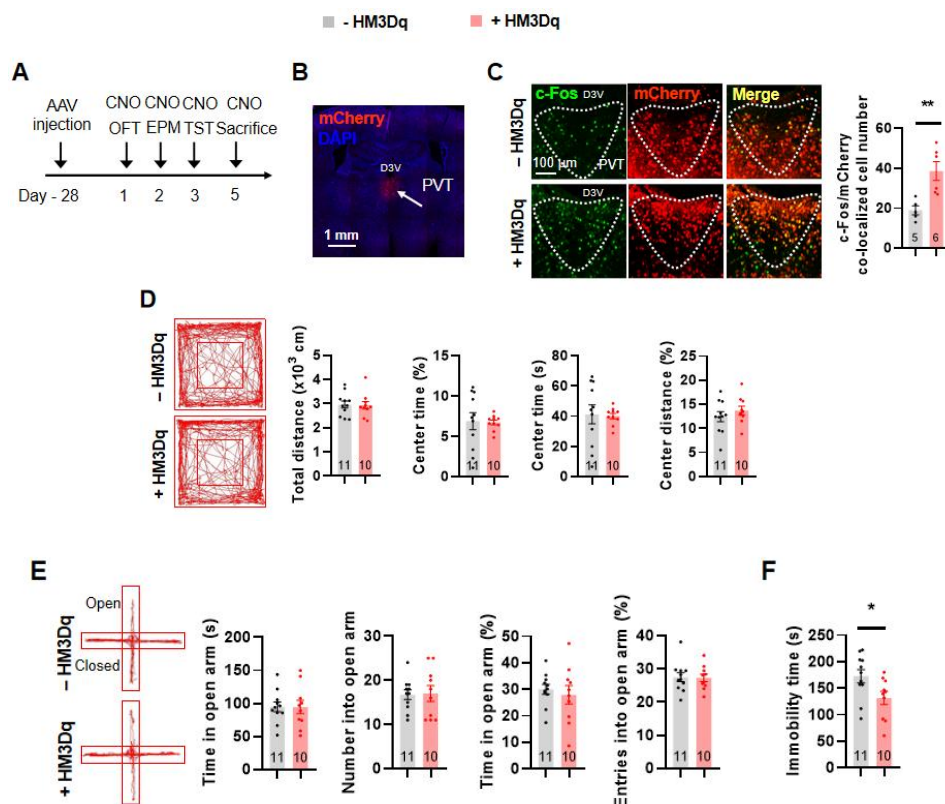


Fig. S8 Chemogenetic activation of PVT in non-stressed animals has no effects with stress-related behavioral alterations.

- A. Timeline of experiment procedure and the behavioral tests. OFT, open field test; EPM, elevated plus maze test; TST, tail suspension test.
- B. Post hoc visualization of mCherry (red) and DAPI (blue) in PVT of wild-type mice receiving AAV-CAMKII α -HM3Dq-mCherry stereotaxic injections; D3V, dorsal 3rd ventricle.
- C. Immunofluorescence (IF) staining for c-Fos (green), mCherry (red) or merge (yellow) in PVT (left), and quantification of c-Fos and mCherry colocalized cell numbers (right) ($t_{19} = 3.499$, $P = 0.0067$).
- D. Representative tracks of mice in OFT, travel distance ($t_{19} = 0.1451$, $P = 0.8861$), percentage of time spent in center (Unpaired t test with Welch's correction, $t_{11.58} = 0.1473$, $P = 0.8855$), time spent in center (Unpaired t test with Welch's correction, $t_{11.62} = 0.1631$, $P = 0.8733$), and percentage of distance in center area ($t_{19} = 0.9327$, $P = 0.3627$).
- E. Representative tracks of mice in EPM, the time spent in the open arms ($t_{19} = 0.01298$, $P = 0.9898$), the number of entries into the open arms ($t_{19} = 0.1327$, $P = 0.8958$), percentage of time spent in the open arms ($t_{19} = 0.5384$, $P = 0.5965$), and percentage of entries into the open arms ($t_{19} = 0.1099$, $P = 0.9136$).
- F. Immobility time of TST ($t_{19} = 2.311$, $P = 0.0322$).

Studies were conducted using 8–9-week-old male WT mice receiving AAVs expressing mCherry (- HM3Dq) or HM3Dq (+ HM3Dq), followed by CNO injection. Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test or specific test marked in the data. $**P < 0.01$.

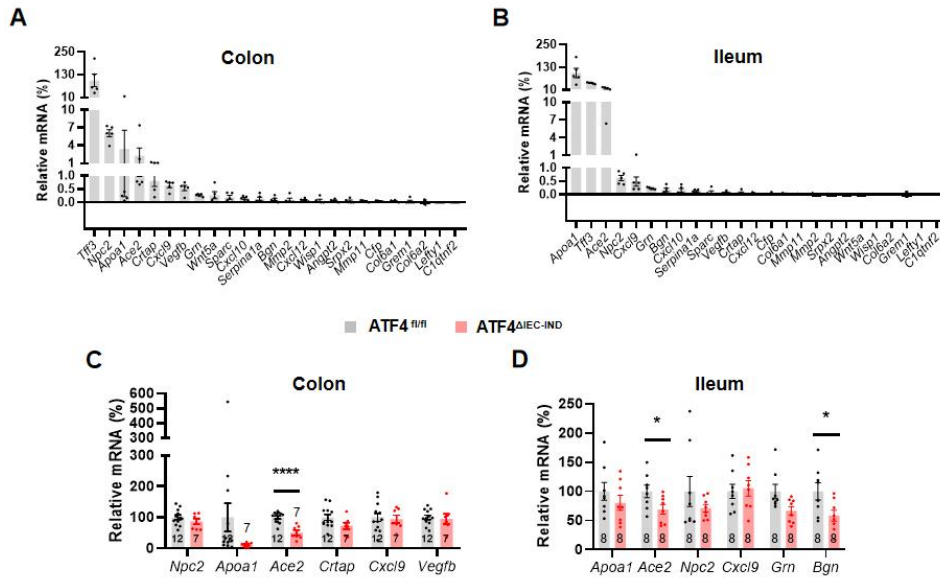


Fig. S9 Gene expression of candidate genes in intestinal ATF4 knockout mice.

A. Gene expression of 25 candidates in colonic epithelial cells by RT-PCR.

B. Gene expression of 25 candidates in ileal epithelial cells by RT-PCR.

C. Gene expression of *Npc2* ($t_{17} = 1.078$, $P = 0.2961$), *Apoa1* (Mann Whitney test, $P = 0.0221$), *Ace2* ($t_{17} = 5.439$, $P < 0.0001$), *Crtap* ($t_{17} = 1.790$, $P = 0.0912$), *Cxcl9* ($t_{17} = 0.2802$, $P = 0.7827$) and *Vegfb* ($t_{17} = 0.3359$, $P = 0.7411$) in colonic epithelial cells by RT-PCR.

D. Gene expression of *Apoa1* ($t_{14} = 0.9845$, $P = 0.3416$), *Ace2* ($t_{14} = 2.279$, $P = 0.0389$), *Npc2* (Unpaired t test with Welch's correction, $t_{7.865} = 1.089$, $P = 0.3084$), *Cxcl9* ($t_{14} = 0.2923$, $P = 0.7743$), *Grn* (Mann Whitney test, $P = 0.0650$) and *Bgn* ($t_{14} = 2.393$, $P = 0.0313$) in ileal epithelial cells by RT-PCR.

Studies for A and B were conducted using 8–9-week-old male wild-type mice; studies for C and D were conducted using 14–16-week-old male $ATF4^{fl/fl}$ or $ATF4^{\Delta IEC-IND}$ mice. Data are expressed as the mean \pm SEM ($n = 5$ for A and B, the number of samples are indicated in the bar graph for C and D), with individual data points. Data were analyzed via two-tailed unpaired Student's t test or specific test marked in the data. $*P < 0.05$, $****P < 0.0001$.

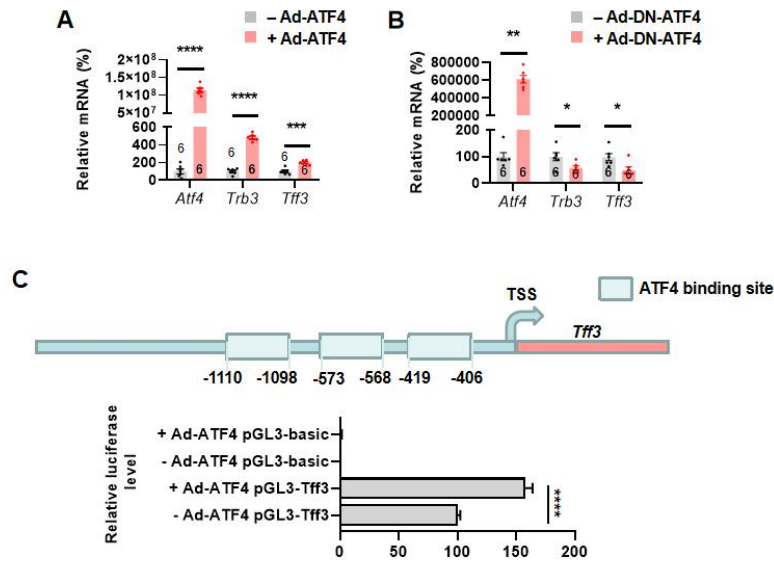


Fig. S10 ATF4 regulates TFF3 expression directly in vitro.

A. Gene expression of *Atf4* (Unpaired t test with Welch's correction, $t_5 = 19.92$, $P < 0.0001$), *Trb3* ($t_{10} = 17.49$, $P < 0.0001$) and *Tff3* ($t_{10} = 5.717$, $P = 0.0002$) by RT-PCR in CCD-841 cells infected with Ad-GFP (- Ad-ATF4) or Ad-ATF4 (+ Ad-ATF4).

B. Gene expression of *Atf4* (Mann Whitney test, $P = 0.0022$), *Trb3* ($t_{10} = 2.491$, $P = 0.0319$) and *Tff3* ($t_{10} = 2.662$, $P = 0.0238$) by RT-PCR in CCD-841 cells infected with Ad-GFP (- Ad-DN-ATF4) or Ad-DN-ATF4 (+ Ad-DN-ATF4).

C. The ATF4 binding sites in the *Tff3* promoter (top) and luciferase activity assessed in CCD-841 cells expressing the *Tff3* promoter plasmid (pGL3-Tff3) or blank pGL3-basic plasmid (pGL3-basic), with Ad-GFP (- Ad-ATF4) or Ad-ATF4 (+ Ad-ATF4) infected (bottom).

Studies were conducted in CCD-841 cells with at least two independent experiments. Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test or specific test marked in the data. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

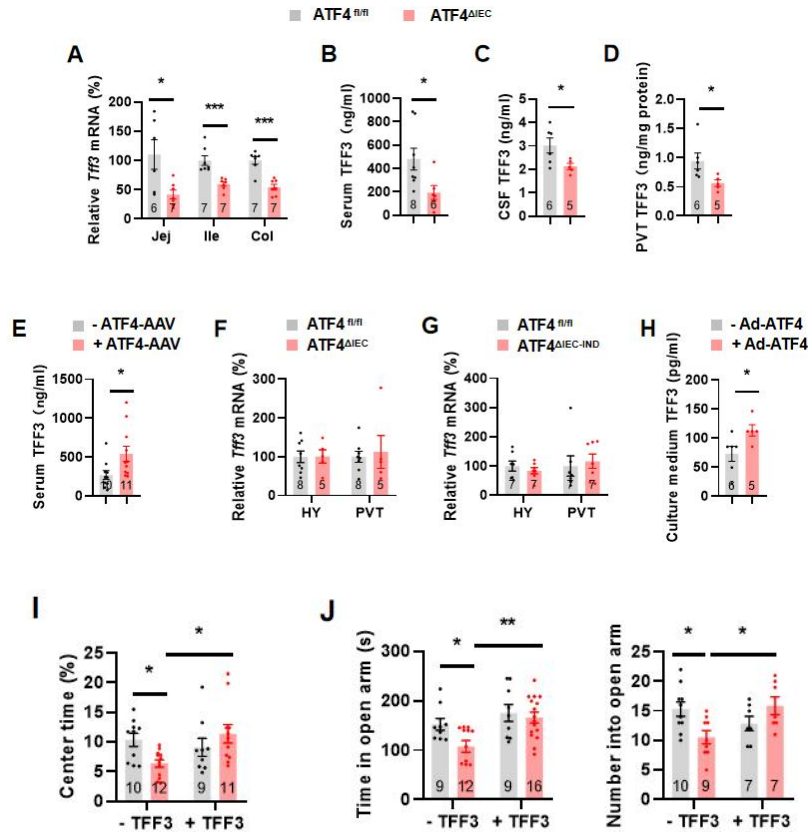


Fig. S11 TFF3 levels in different tissues and different transgenic mice.

A. Gene expression of *Tff3* in jejunal (Jej) (Unpaired t test with Welch's correction, $t_{5.905} = 2.584$, $P = 0.0422$), ileal (Ile) ($t_{12} = 4.486$, $P = 0.0007$) and colonic (Col) ($t_{12} = 5.592$, $P = 0.0001$) epithelial cells by RT-PCR.

B. TFF3 levels in serum by ELISA ($t_{12} = 2.394$, $P = 0.0339$, CV of ATF4^{fl/fl}: 54.55%, CV of ATF4^{ΔIEC}: 74.54%).

C. TFF3 levels in cerebrospinal fluid (CSF) by ELISA ($t_9 = 2.394$, $P = 0.0403$, CV of ATF4^{fl/fl}: 26.05%, CV of ATF4^{ΔIEC}: 13.43%).

D. TFF3 levels in the paraventricular thalamus (PVT) by ELISA ($t_9 = 2.396$, $P = 0.0401$, CV of ATF4^{fl/fl}: 35.39%, CV of ATF4^{ΔIEC}: 21.70%).

E. TFF3 levels in serum by ELISA (Mann Whitney test, $P = 0.0107$, CV of -ATF4-AAV: 65.18%, CV of +ATF4-AAV: 59.68%).

F. Gene expression of *tff3* in hypothalamus (HY) ($t_{11} = 0.03445$, $P = 0.9731$) and PVT (Mann Whitney test, $P = 0.5237$) by RT-PCR.

G. Gene expression of *tff3* in hypothalamus (HY) ($t_{12} = 0.7755$, $P = 0.4531$) and PVT (Mann Whitney test, $P = 0.7095$) by RT-PCR.

H. TFF3 levels in culture medium by ELISA ($t_9 = 2.383$, $P = 0.0410$, CV of - Ad-ATF4: 43.73%, CV of + Ad-ATF4: 19.26%).

I. Percentage of time spent in center in open field test (OFT) (Genotype Factor: $F_{1,38} = 0.4814$, $P = 0.4920$; TFF3 factor: $F_{1,38} = 2.343$, $P = 0.1341$; Genotype \times TFF3: $F_{1,38} = 6.522$, $P = 0.0148$).

J. The time spent in the open arms (Genotype Factor: $F_{1,42} = 4.387$, $P = 0.0423$; TFF3 factor: $F_{1,42} = 9.758$, $P = 0.0032$; Genotype \times TFF3: $F_{1,42} = 1.857$, $P = 0.1803$), the number of entries into the open arms (Genotype Factor: $F_{1,29} = 0.4649$, $P = 0.5007$; TFF3 factor: $F_{1,29} = 1.249$, $P = 0.2730$; Genotype \times TFF3: $F_{1,29} = 9.164$, $P = 0.0051$) in elevated plus maze test (EPM).

Studies for A-D, F were conducted using 8–9-week-old male ATF4^{fl/fl} or ATF4 ^{Δ IEC} mice; studies for E were conducted using 8–9-week-old male Villin-Cre mice receiving colonic injection of AAVs expressing mCherry (- AAV-ATF4) or ATF4 (+ AAV-ATF4); studies for G were conducted using 14–16-week-old male ATF4^{fl/fl} or ATF4 ^{Δ IEC-IND} mice; studies for H were conducted in CCD-841 cells with Ad-GFP (- Ad-ATF4) or Ad-ATF4 (+ Ad-ATF4) infected; studies for I and J were conducted using 14–16-week-old male ATF4^{fl/fl} or ATF4 ^{Δ IEC-IND} mice receiving i.p. injection of saline (- TFF3) or TFF3 (+ TFF3). Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test for A-H, or via two-way ANOVA for I and J, or specific test marked in the data. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

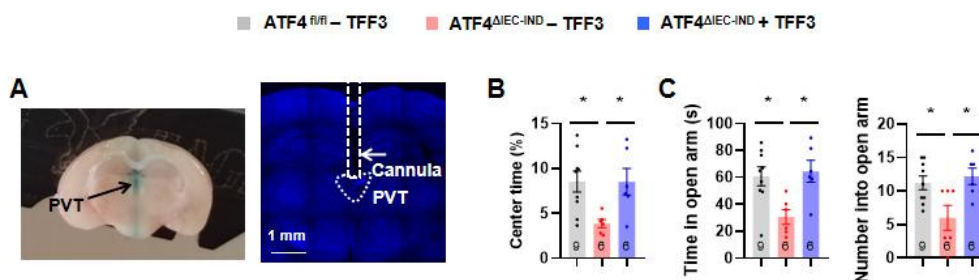


Fig. S12 Behavioral alterations of paraventricular thalamus (PVT) injection of

TFF3 intestinal ATF4 deletion mice.

A. The photo of the green dye trace in the brain via PVT cannula injection (left) and post hoc visualization of DAPI (blue) in the brain of PVT cannula implantation (right).

B. Percentage of time spent in center in open field test (OFT) ($F_{2,18} = 4.976$; $P = 0.0190$).

C. The time spent in the open arms ($F_{2,18} = 6.131$; $P = 0.0093$), the number of entries into the open arms ($F_{2,18} = 5.519$; $P = 0.0135$) in elevated plus maze test (EPM).

Studies for A were conducted using 10-14-week-old male wild-type (WT) mice receiving PVT injection of green dye; studies for B and C were conducted using 12-14-week-old male $ATF4^{fl/fl}$ or $ATF4^{\Delta IEC-IND}$ mice receiving PVT injection of saline (- TFF3) or TFF3 (+ TFF3). Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via one-way ANOVA. $*P < 0.05$.

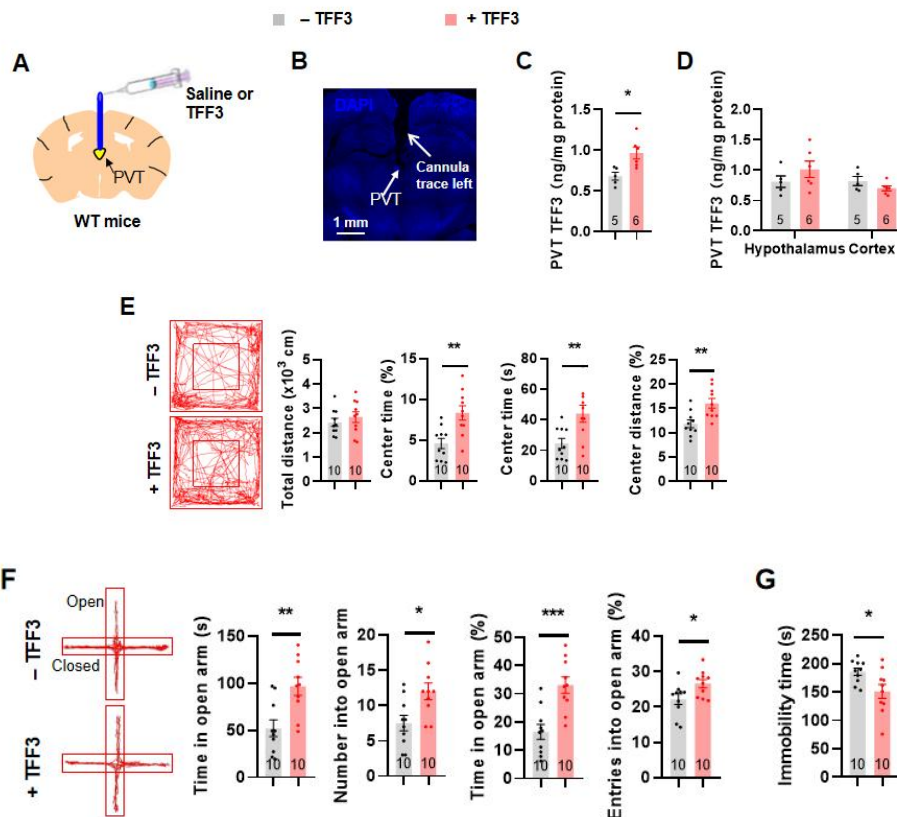


Fig. S13 The behavioral changes of wild-type mice with paraventricular thalamus (PVT) injection of TFF3.

- A. Schematic of PVT injection of TFF3 in wild-type (WT) mice.
- B. Post hoc visualization of DAPI (blue) in the brain of PVT cannula implantation.
- C. TFF3 levels in the PVT by ELISA ($t_9 = 3.058$, $P = 0.0136$, CV of - TFF3: 16.07%, CV of + TFF3: 18.93%).
- D. TFF3 levels in hypothalamus ($t_9 = 1.183$, $P = 0.2672$, CV of - TFF3: 25.86%, CV of + TFF3: 32.85%) and cortex ($t_9 = 1.468$, $P = 0.1761$, CV of - TFF3: 20.23%, CV of + TFF3: 14.48%) by ELISA.
- E. Representative tracks of mice in OFT, travel distance ($t_{18} = 0.7819$, $P = 0.4445$), percentage of time spent in center ($t_{18} = 3.479$, $P = 0.0027$), time spent in center ($t_{18} = 3.042$, $P = 0.0070$), and percentage of distance in center area ($t_{18} = 3.310$, $P = 0.0039$).
- F. Representative tracks of mice in EPM, the time spent in the open arms ($t_{18} = 3.401$, $P = 0.0032$), the number of entries into the open arms ($t_{18} = 2.812$, $P = 0.0115$), percentage of time spent in the open arms ($t_{18} = 4.204$, $P = 0.0005$), and percentage of entries into the open arms ($t_{18} = 2.336$, $P = 0.0313$).
- G. Immobility time of TST ($t_{18} = 2.506$, $P = 0.0220$).

Studies were conducted using 8–9-week-old male WT mice receiving PVT injection of saline (- TFF3) or TFF3 (+ TFF3). Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

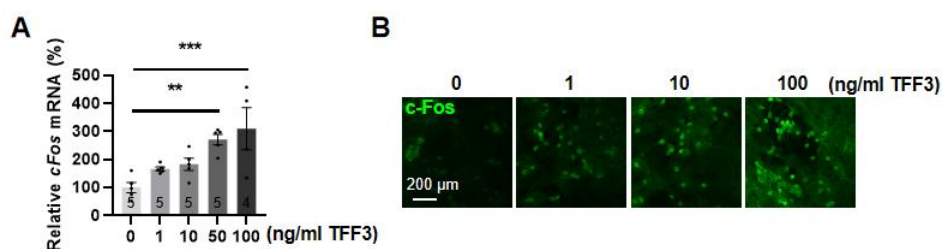


Fig. S14 TFF3 treatment regulates c-Fos expression in vitro thalamic primary neurons.

A. Gene expression of *c-Fos* in thalamic primary neurons by RT-PCR (Brown-Forsythe ANOVA test, $F_{4,4.635} = 5.555$; $P = 0.0498$).

B. Immunofluorescence (IF) staining for c-Fos (green) in thalamic primary neurons. Studies were conducted in thalamic primary neurons with TFF3 concentration gradient treatment. Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via Brown-Forsythe ANOVA test. $**P < 0.01$, $***P < 0.001$.

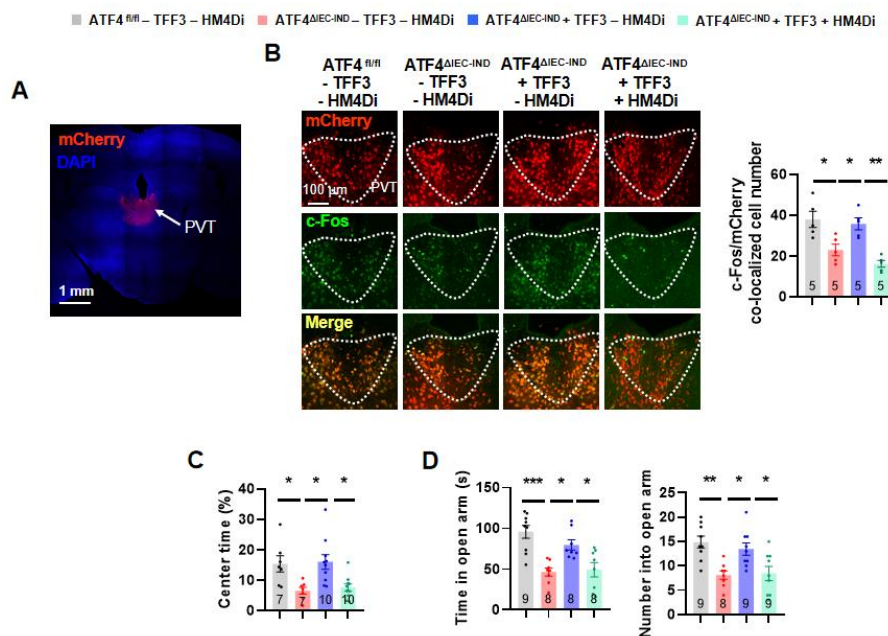


Fig. S15 Immunofluorescence (IF) staining in the brain and behavioral effects of intestinal ATF4 knockout mice with HM4Di and TFF3 treatments.

A. Post hoc visualization of mCherry (red) and DAPI (blue) in PVT of ATF4^{ΔIEC-IND} mice receiving AAV-CAMKII α -HM4Di-mCherry stereotaxic injections; D3V, dorsal 3rd ventricle.

B. Immunofluorescence (IF) staining for c-Fos (green), mCherry (red) or merge (yellow) in PVT (left), and quantification of c-Fos and mCherry colocalized cell numbers (right) ($F_{3,16} = 12.21$; $P = 0.0002$).

C. Percentage of time spent in center in open field test (OFT) ($F_{3,30} = 6.334$; $P =$

0.0019).

D. The time spent in the open arms ($F_{3,29} = 11.34$; $P < 0.0001$), the number of entries into the open arms ($F_{3,31} = 7.805$; $P = 0.0005$) in elevated plus maze test (EPM).

Studies were conducted using 12–14-week-old male $ATF4^{fl/fl}$ or $ATF4^{\Delta IEC-IND}$ mice receiving AAVs expressing mCherry (- HM4Di) or HM4Di (+ HM4Di), followed by CNO and TFF3 i.p. injection. Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

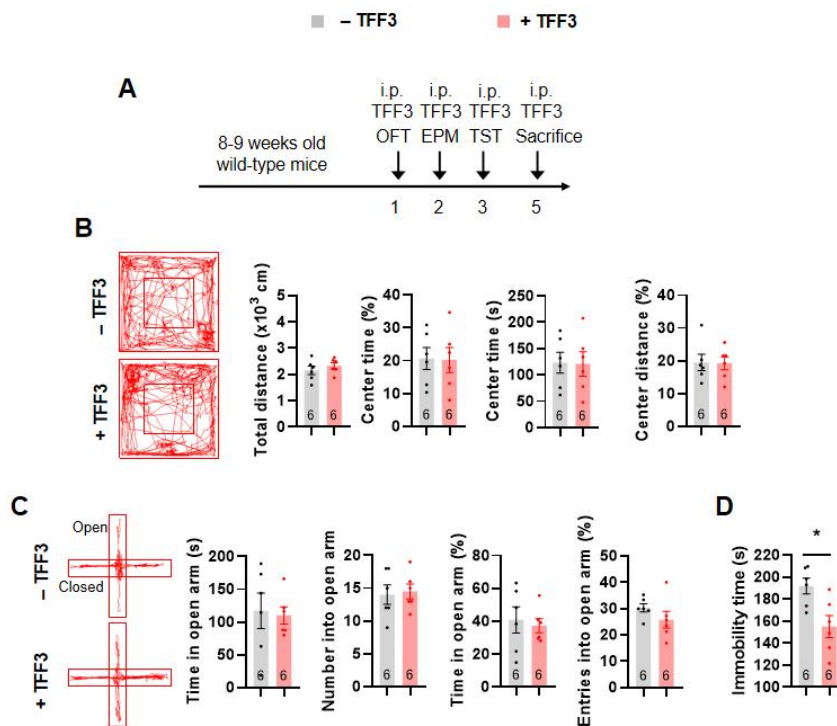


Fig. S16 The behavioral changes of TFF3 i.p. injection of wild-type mice.

A. Timeline of experiment procedure and the behavioral tests. OFT, open field test; EPM, elevated plus maze test; TST, tail suspension test.

B. Representative tracks of mice in OFT, travel distance ($t_{10} = 0.8924$, $P = 0.3931$), percentage of time spent in center ($t_{10} = 0.08366$, $P = 0.9350$), time spent in center ($t_{10} = 0.07995$, $P = 0.9379$), and percentage of distance in center area ($t_{10} = 0.08124$, $P = 0.9369$).

C. Representative tracks of mice in EPM, the time spent in the open arms ($t_{10} = 0.2379$, $P = 0.8167$), the number of entries into the open arms ($t_{10} = 0.2666$, $P = 0.7952$), percentage of time spent in the open arms ($t_{10} = 0.3909$, $P = 0.7041$), and percentage of entries into the open arms ($t_{10} = 1.277$, $P = 0.2305$).

D. Immobility time of TST ($t_{10} = 2.966$, $P = 0.0141$).

Studies were conducted using 8–9-week-old male wild-type mice receiving TFF3 i.p. injection. Data are expressed as the mean \pm SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-tailed unpaired Student's t test. $*P < 0.05$.

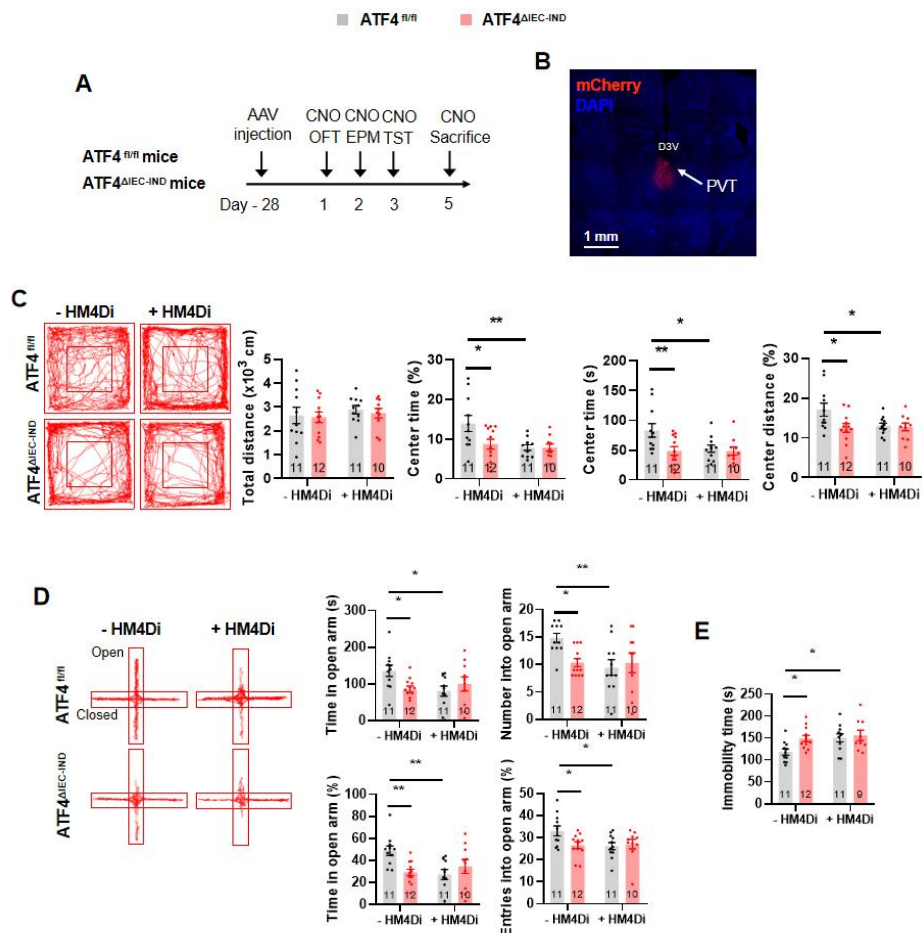


Fig. S17 The behavioral changes of inducible intestinal ATF4 deletion mice with paraventricular thalamus (PVT) glutamatergic neurons inhibition.

A. Timeline of experiment procedure and the behavioral tests. OFT, open field test; EPM, elevated plus maze test; TST, tail suspension test.

B. Post hoc visualization of mCherry (red) and DAPI (blue) in PVT of ATF4^{ΔIEC-IND} mice receiving AAV-CAMKIIα-HM4Di-mCherry stereotaxic injections; D3V, dorsal 3rd ventricle.

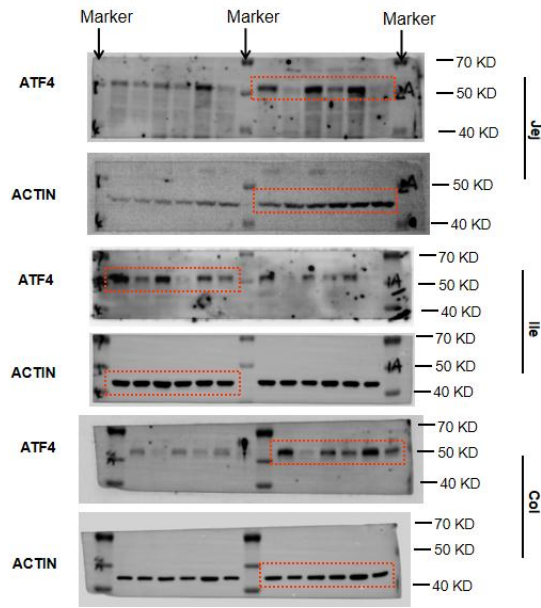
C. Representative tracks of mice in OFT, travel distance (Genotype Factor: $F_{1,40} = 0.2109$, $P = 0.6485$; HM4Di factor: $F_{1,40} = 0.7010$, $P = 0.4074$; Genotype × HM4Di: $F_{1,40} = 0.01985$, $P = 0.8887$), percentage of time spent in center (Genotype Factor: $F_{1,40} = 3.740$, $P = 0.0602$; HM4Di factor: $F_{1,40} = 6.629$, $P = 0.0138$; Genotype × HM4Di: $F_{1,40} = 3.736$, $P = 0.0604$), time spent in center (Genotype Factor: $F_{1,40} = 5.489$, $P = 0.0242$; HM4Di factor: $F_{1,40} = 3.498$, $P = 0.0688$; Genotype × HM4Di: $F_{1,40} = 3.518$, $P = 0.0680$), and percentage of distance in center area (Genotype Factor: $F_{1,40} = 4.380$, $P = 0.0428$; HM4Di factor: $F_{1,40} = 2.710$, $P = 0.1076$; Genotype × HM4Di: $F_{1,40} = 3.554$, $P = 0.0667$).

D. Representative tracks of mice in EPM, the time spent in the open arms (Genotype Factor: $F_{1,40} = 1.120$, $P = 0.2963$; HM4Di factor: $F_{1,40} = 2.024$, $P = 0.1626$; Genotype × HM4Di: $F_{1,40} = 5.824$, $P = 0.0205$), the number of entries into the open arms (Genotype Factor: $F_{1,40} = 2.195$, $P = 0.1463$; HM4Di factor: $F_{1,40} = 4.826$, $P = 0.0339$; Genotype × HM4Di: $F_{1,40} = 4.708$, $P = 0.0360$), percentage of time spent in the open arms (Genotype Factor: $F_{1,40} = 1.850$, $P = 0.1814$; HM4Di factor: $F_{1,40} = 3.102$, $P = 0.0858$; Genotype × HM4Di: $F_{1,40} = 8.893$, $P = 0.0049$), and percentage of entries into the open arms (Genotype Factor: $F_{1,40} = 2.186$, $P = 0.1471$; HM4Di factor: $F_{1,40} = 2.903$, $P = 0.0962$; Genotype × HM4Di: $F_{1,40} = 3.797$, $P = 0.0584$).

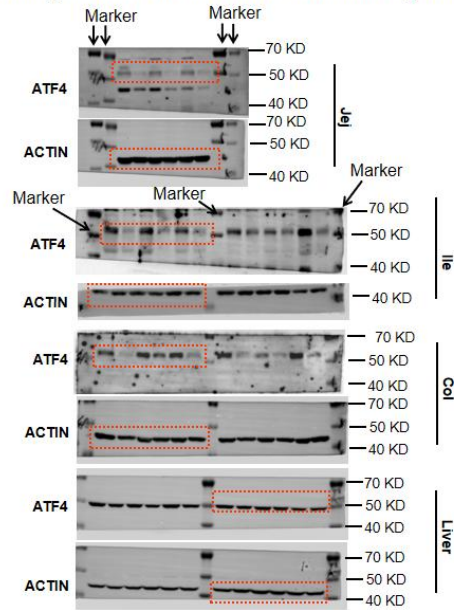
E. Immobility time of TST (Genotype Factor: $F_{1,39} = 4.227$, $P = 0.0465$; HM4Di factor: $F_{1,39} = 4.856$, $P = 0.0335$; Genotype × HM4Di: $F_{1,39} = 2.028$, $P = 0.1624$).

Studies were conducted using 24–28-week-old male ATF4^{fl/fl} or ATF4^{ΔIEC-IND} mice receiving AAVs expressing mCherry (- HM4Di) or HM4Di (+ HM4Di), with CNO injection. Data are expressed as the mean ± SEM (the number of samples are indicated in the bar graph), with individual data points. Data were analyzed via two-way ANOVA. * $P < 0.05$, ** $P < 0.01$.

A The full gels of the western bolts about the Fig. 1C



B The full gels of the western bolts about the Fig. 2B



C The full gels of the western bolts about the Fig. S2B

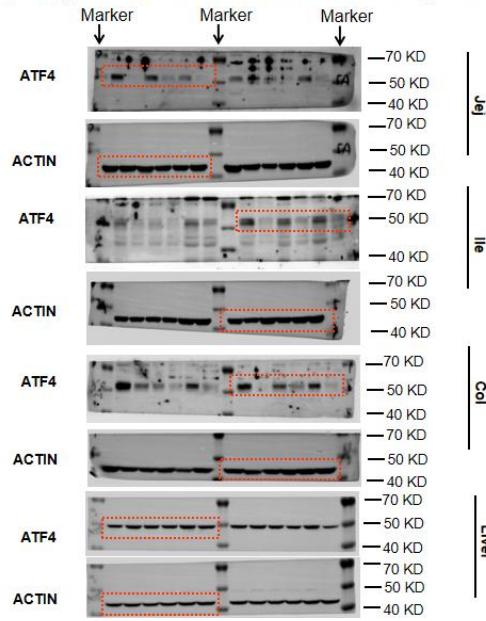


Fig. S18 The full gels of the western bolts in the related Figures.

- A. The full gel of the western blots with molecular weight markers in Fig. 1C.
- B. The full gel of the western blots with molecular weight markers in Fig. 2B.
- C. The full gel of the western blots with molecular weight markers in Fig. S2B.

Supplementary Table 1. Primers used for gene amplification.

Gene	Direction	Primer sequence 5'→3'
<i>Atf4</i>	F	ATCTGGAGGTGGCCAAGCAC
	R	TGGTTTCCAGGTCATCCATT
<i>Atf4-2</i>	F	ATCTGGAGGTGGCCAAGCAC
	R	TGGTTTCCAGGTCATCCATT
<i>Fos</i>	F	CCAGTCCTCACCTCTTCCAG
	R	TCCAGCACCAGGTTAATTCC
<i>Trb3</i>	F	TGTCTTGCGCACCTCAA
	R	CCAGCTTCGTCCTCTCACAGT
<i>Tff3</i>	F	GCCCTCTGGCTAATGCTGTT

	R	TTGGGATACTGGAGTCAAAGC
<i>Gapdh</i>	F	TGTGTCCGTCGTGGATCTGA
	R	CCTGCTTCACCACCTTCTTGAT
<i>Srpx2</i>	F	ATGGTACGCAGGCTCAGGTTA
	R	TGAGTAGCATGTGGCTTCTCC
<i>Clqmf2</i>	F	CTGACCCAATGCTTGGTGC
	R	GCCCTTTGGTCCTTGTTTTCC
<i>Col6a2</i>	F	GCTCCTGATTGGGGACTCT
	R	CCAACACGAAATACACGTTGAC
<i>Crtap</i>	F	TCACACCTATCTCCTGAAGCA
	R	TGTCGGAAATGGACGTTCTCC
<i>Mmp2</i>	F	CCCATGAAGCCTTGTTTACCA
	R	TGGAAGCGGAACGGAAACT
<i>Col6a1</i>	F	CTGCTGCTACAAGCCTGCT
	R	CCCATAAGGTTTCAGCCTCA
<i>Angpt2</i>	F	CCTCGACTACGACGACTCAGT
	R	TCTGCACCACATTCTGTTGGA
<i>Wnt5a</i>	F	CAACTGGCAGGACTTTCTCAA
	R	CATCTCCGATGCCGGAACT
<i>Apoa1</i>	F	GGCACGTATGGCAGCAAGAT
	R	CCAAGGAGGAGGATTCAAACCTG
<i>Grem1</i>	F	CTGGGGACCCTACTGCCAA
	R	TTTGCACCAATCTCGCTTCAG
<i>Serpina1a</i>	F	TAGGGAGCAAGGGTGACACTC
	R	ACTGTCTGGTCTGTTGAGGGT
<i>Sparc</i>	F	GTGGAAATGGGAGAATTTGAGGA
	R	CTCACACACCTTGCCATGTTT
<i>Mmp11</i>	F	CCGGAGAGTCACCGTCATC
	R	GCAGGACTAGGGACCCAATG

<i>Wisp1</i>	F	CAGCACCCTAGAGGAAACGA
	R	CTGGGCACATATCTTACAGCATT
<i>Cxcl9</i>	F	GGAGTTCGAGGAACCCTAGTG
	R	GGGATTTGTAGTGGATCGTGC
<i>Cxcl12</i>	F	TGCATCAGTGACGGTAAACCA
	R	TTCTTCAGCCGTGCAACAATC
<i>Lefty1</i>	F	CCAACCGCACTGCCCTTAT
	R	CGCGAAACGAACCAACTTGT
<i>Npc2</i>	F	AGGACTGCGGCTCTAAGGT
	R	AGGCTCAGGAATAGGGAAGGG
<i>Cxcl10</i>	F	CCAAGTGCTGCCGTCATTTTC
	R	GGCTCGCAGGGATGATTTCAA
<i>Vegfb</i>	F	GCCAGACAGGGTTGCCATAC
	R	GGAGTGGGATGGATGATGTCAG
<i>Bgn</i>	F	TGCCATGTGTCCTTTCGGTT
	R	CAGGTCTAGCAGTGTGGTGTC
<i>Ace2</i>	F	TCCAGACTCCGATCATCAAGC
	R	GCTCATGGTGTTTCAGAATTGTGT
<i>Grn</i>	F	ATGTGGGTCCTGATGAGCTG
	R	GCTCGTTATTCTAGGCCATGTG
<i>Cfp</i>	F	TTCACCCAGTATGAGGAGTCC
	R	GCTGACCATTGTGGAGACCT

Supplementary Table 2. Normality of distribution, equal variances, t/F values and degree of freedom of the data.

Panel of Data	Normality of distribution?	Equal variances?	t values (t test) or F values (ANOVA)	Total Degree of freedom
Fig.1B Jej	yes;yes	yes	3.531	8
Fig. 1B Ile	yes;yes	yes	2.926	8
Fig. 1B Col	no;yes	no	—	—

Fig.1C Jej	yes;yes	yes	2.687	11
Fig. 1C Ile	yes;yes	yes	3.323	11
Fig. 1C Col	yes;yes	yes	2.729	11
Fig.1E Total distance	yes;yes	yes	0.0771	19
Fig.1E Center time (s)	yes;yes	yes	2.425	19
Fig. 1E Center distance (%)	yes;yes	yes	2.251	19
Fig. 1F Time in open arm (%)	yes;yes	yes	3.596	19
Fig. 1F Entries into open arm (%)	yes;yes	yes	2.529	19
Fig. 1G	yes;yes	yes	2.684	19
Fig.1H Total distance	yes;yes	yes	1.488	17
Fig.1H Center time (s)	yes;yes	yes	2.367	17
Fig. 1H Center distance (%)	yes;yes	yes	2.4	17
Fig. 1I Time in open arm (%)	yes;yes	yes	0.6447	18
Fig. 1I Entries into open arm (%)	yes;yes	yes	0.02775	18
Fig. 1J	yes;yes	yes	4.204	17
Fig. 2A Jej	yes;yes	no	11.04	9.285
Fig. 2A Ile	yes;yes	no	12.24	9.174
Fig. 2A Col	yes;yes	yes	5.288	12
Fig. 2A Liver	yes;yes	yes	0.3067	13
Fig. 2B Jej	yes;yes	yes	8.774	10
Fig. 2B Ile	yes;yes	yes	8.615	10
Fig. 2B Col	yes;yes	no	9.894	5.713
Fig. 2B Liver	yes;yes	yes	0.4219	10
Fig.2D Total distance	yes;yes	yes	0.4083	20
Fig.2D Center time (s)	yes;yes	yes	4.647	20
Fig. 2D Center distance (%)	yes;yes	yes	3.867	20
Fig. 2E Time in open arm (%)	yes;yes	no	3.519	13.28
Fig. 2E Entries into open arm (%)	yes;yes	yes	3.617	20
Fig. 2F	yes;yes	yes	2.409	10
Fig. 3A	yes;yes	yes	3.213	8
Fig. 3B	yes;yes	yes	2.748	10
Fig. 3D Total distance	all-yes	yes	Genotype Factor: $F_{1,35} = 0.01699$; HM3Dq factor: $F_{1,35} = 0.5218$; Genotype \times HM3Dq: $F_{1,35} = 0.4704$	total=38
Fig. 3D Center time (s)	yes,yes,no,yes	yes	Genotype Factor: $F_{1,31} = 0.885$; HM3Dq factor: $F_{1,31} = 3.067$; Genotype \times HM3Dq: $F_{1,31} = 5.286$	total=34
Fig. 3D Center distance (%)	all-yes	yes	Genotype Factor: $F_{1,30} = 3.866$; HM3Dq factor: $F_{1,30} = 4.824$; Genotype \times HM3Dq: $F_{1,30} = 6.279$	total=33

Fig. 3E Time in open arm (%)	all-yes	yes	Genotype Factor: $F_{1,24} = 4.114$; HM3Dq factor: $F_{1,24} = 6.458$; Genotype \times HM3Dq: $F_{1,24} = 2.875$	total=27
Fig. 3E Entries into open arm (%)	all-yes	yes	Genotype Factor: $F_{1,24} = 4.435$; HM3Dq factor: $F_{1,24} = 5.363$; Genotype \times HM3Dq: $F_{1,24} = 3.373$	total=27
Fig. 3F	all-yes	yes	Genotype Factor: $F_{1,24} = 5.006$; HM3Dq factor: $F_{1,24} = 4.436$; Genotype \times HM3Dq: $F_{1,24} = 7.477$	total=27
Fig. 4B Jej	yes,yes	yes	2.968	10
Fig. 4B Ile	yes,yes	yes	3.591	10
Fig. 4B Col	no,yes	no	—	—
Fig. 4C	yes,yes	yes	2.692	12
Fig. 4D	yes,yes	yes	3.941	9
Fig. 4E	yes,yes	yes	2.945	9
Fig. 4F	all-yes	yes	Genotype Factor: $F_{1,16} = 3.068$; TFF3 factor: $F_{1,16} = 8.243$; Genotype \times TFF3: $F_{1,16} = 7.311$	total=19
Fig. 4H Total distance	all-yes	yes	Genotype Factor: $F_{1,38} = 0.2085$; TFF3 factor: $F_{1,38} = 0.05511$; Genotype \times TFF3: $F_{1,38} = 6.033e-005$	total=41
Fig. 4H Center time (s)	all-yes	yes	Genotype Factor: $F_{1,38} = 1.595$; TFF3 factor: $F_{1,38} = 3.336$; Genotype \times TFF3: $F_{1,38} = 4.781$	total=41
Fig. 3H Center distance (%)	all-yes	no	Genotype Factor: $F_{1,38} = 1.044$; TFF3 factor: $F_{1,38} = 5.912$; Genotype \times TFF3: $F_{1,38} = 6.511$	total=41
Fig.4I Time in open arm (%)	all-yes	yes	Genotype Factor: $F_{1,42} = 4.330$; TFF3 factor: $F_{1,42} = 9.751$; Genotype \times TFF3: $F_{1,42} = 1.801$	total=45
Fig.4I Entries into open arm (%)	yes,yes,no,yes	yes	Genotype Factor: $F_{1,29} = 4.784$; TFF3 factor: $F_{1,29} = 2.426$; Genotype \times TFF3: $F_{1,29} = 3.737$	total=32
Fig.4J	all-yes	yes	Genotype Factor: $F_{1,42} = 10.73$; TFF3 factor: $F_{1,42} = 13.03$; Genotype \times TFF3: $F_{1,42} = 1.091$	total=45
Fig.5C	all-yes	yes	$F_{2,23} = 3.886$	total=25
Fig.5D Total distance	all-yes	yes	$F_{2,18} = 1.182$	total=20
Fig. 5D Center time (s)	all-yes	yes	$F_{2,18} = 5.074$	total=20
Fig. 5D Center distance (%)	no, yes, yes	yes		

Fig.5E Time in open arm (%)	all-yes	yes	$F_{2,18} = 14.37$	total=20
Fig.5E Entries into open arm (%)	all-yes	yes	$F_{2,18} = 12.32$	total=20
Fig.5F	all-yes	yes	$F_{2,18} = 6.345$	total=20
Fig.6A	all-yes	yes	$F_{2,12} = 15.17$	total=14
Fig.6C Total distance	all-yes	no	$F_{3,000,21.48}^* = 1.289$	total=24.48
Fig. 6C Center time (s)	all-yes	yes	$F_{3,31} = 7.131$	total=34
Fig. 6C Center distance (%)	all-yes	yes	$F_{3,32} = 7.854$	total=35
Fig.6D Time in open arm (%)	all-yes	yes	$F_{3,31} = 7.916$	total=34
Fig.6D Entries into open arm (%)	all-yes	yes	$F_{3,31} = 7.815$	total=34
Fig.6E	all-yes	yes	$F_{3,36} = 9.359$	total=39

Panel of Data	Normality of distribution?	Equal variances?	t values (t test) or F values (ANOVA)	Total Degree of freedom
Fig. S1A Total distance	yes, yes	no	1.368	12.67
Fig. S1A Center time (%)	yes, yes	yes	2.287	16
Fig. S1A Center time (s)	yes, yes	yes	2.287	16
Fig. S1A Center distance (%)	yes, yes	yes	2.806	16
Fig. S1B Time in open arm (s)	yes, yes	yes	6.769	17
Fig. S1B Number into open arm	yes, yes	yes	2.32	17
Fig. S1B Time in open arm (%)	yes, yes	yes	6.96	17
Fig. S1B Entries into open arm (%)	yes, yes	yes	4.078	17
Fig. S1C	yes, yes	yes	3.405	16
Fig. S1D Liver	yes, yes	no	1.178	9.422
Fig. S1D Jej	yes, yes	yes	0.9478	16
Fig. S1D Ile	yes, yes	yes	1.157	16
Fig. S1D Col	yes, no	yes	—	—
Fig. S1F Aft4	yes, yes	yes	5.319	19
Fig. S1F Trb3	yes, yes	no	3.043	9.086
Fig. S1F Ddit4	no, no	no	—	—
Fig. S1G Center time (%)	yes, yes	yes	2.426	19
Fig. S1H Time in open arm (s)	yes, yes	yes	3.636	19
Fig. S1H Number into open arm	yes, yes	yes	3.095	19
Fig. S1I Center time (%)	yes, yes	yes	2.363	17
Fig. S1J Time in open arm (s)	yes, yes	yes	0.5953	18
Fig. S1J Number into open arm	yes, yes	yes	0.6591	18
Fig. S1K	all-yes	yes	Adenovirus Factor: $F_{1,44} = 0.6048$; CRS factor: $F_{1,44} = 1.052$;	total=47

			Adenovirus × CRS: $F_{i,44} = 0.02925$	
Fig. S1L	all-yes	yes	Adenovirus Factor: $F_{i,44} = 0.06367$; CRS factor: $F_{i,44} = 13.44$; Adenovirus × CRS: $F_{i,44} = 0.7795$	total=47
Fig. S2A Jej	yes, yes	yes	7.463	11
Fig. S2A Ile	yes, yes	yes	7.625	11
Fig. S2A Col	no, no	yes	—	—
Fig. S2A Liver	yes, yes	yes	0.08589	11
Fig. S2B Jej	yes, yes	yes	3.887	10
Fig. S2B Ile	yes, yes	yes	3.971	10
Fig. S2B Col	yes, no	yes	—	—
Fig. S2B Liver	yes, yes	yes	0.2664	10
Fig. S2D Total distance	yes, yes	yes	0.4957	15
Fig. S2D Center time (%)	yes, yes	yes	4.857	15
Fig. S2D Center time (s)	yes, yes	yes	3.077	15
Fig. S2D Center distance (%)	yes, yes	yes	2.44	15
Fig. S2E Time in open arm (s)	yes, yes	yes	3.038	15
Fig. S2E Number into open arm	yes, yes	no	2.272	9.559
Fig. S2E Time in open arm (%)	yes, yes	yes	3.335	15
Fig. S2E Entries into open arm (%)	yes, yes	yes	2.699	15
Fig. S2F	yes, yes	yes	5.78	15
Fig. S3A Center time (%)	yes, yes	yes	4.269	20
Fig. S3B Time in open arm (s)	yes, no	no	—	—
Fig. S3B Number into open arm	yes, yes	no	2.368	13.3
Fig. S3D	yes, yes	yes	3.121	22
Fig. S3E	yes, yes	yes	4.026	22
Fig. S3H	yes, yes	no	0.5048	16.27
Fig. S3I SI	yes, yes	yes	1.177	14
Fig. S3I LI	yes, yes	yes	1.005	14
Fig. S3K II1b	yes, yes	yes	1.344	11
Fig. S3K II6	no, yes	yes	—	—
Fig. S3K Tnfa	no, yes	yes	—	—
Fig. S4A PVN	yes, yes	yes	2.479	9
Fig. S4A MeA	yes, no	yes	—	—
Fig. S4A CeA	yes, yes	yes	0.3443	9
Fig. S4A BLA	yes, yes	yes	3.686	9
Fig. S4B	yes, yes	yes	3.092	9
Fig. S4C PVN	yes, yes	yes	0.269	10
Fig. S4C MeA	yes, yes	yes	1.252	10
Fig. S4C CeA	yes, yes	yes	0.6847	10
Fig. S4C BLA	yes, yes	yes	2.416	10

Fig. S4D	yes, yes	yes	5.472	10
Fig. S5C	yes, yes	yes	7.556	8
Fig. S5D Total distance	yes, yes	yes	1.581	10
Fig. S5D Center time (%)	yes, yes	yes	3.807	10
Fig. S5D Center time (s)	yes, yes	yes	2.719	10
Fig. S5D Center distance (%)	yes, yes	yes	2.214	10
Fig. S5E Time in open arm (s)	no, yes	yes	—	—
Fig. S5E Number into open arm	yes, yes	yes	4.009	10
Fig. S5E Time in open arm (%)	yes, yes	yes	5.536	10
Fig. S5E Entries into open arm (%)	yes, yes	yes	4.129	10
Fig. S5F	yes, yes	yes	2.449	10
Fig. S6B	all-yes	yes	Genotype Factor: $F_{1,16} = 6.957$; HM3Dq factor: $F_{1,16} = 46.93$; Genotype \times HM3Dq: $F_{1,16} = 4.360$	total=19
Fig. S6C Center time (%)	yes, yes, no, yes	yes	Genotype Factor: $F_{1,29} = 2.145$; HM3Dq factor: $F_{1,29} = 1.841$; Genotype \times HM3Dq: $F_{1,29} = 7.147$	total=32
Fig. S6D Time in open arm (s)	yes, yes, yes, no	yes	Genotype Factor: $F_{1,24} = 3.561$; HM3Dq factor: $F_{1,24} = 8.223$; Genotype \times HM3Dq: $F_{1,24} = 4.445$	total=27
Fig. S6D Number into open arm	no, yes, yes, yes	yes	Genotype Factor: $F_{1,25} = 1.162$; HM3Dq factor: $F_{1,25} = 1.958$; Genotype \times HM3Dq: $F_{1,25} = 8.900$	total=28
Fig. S6E Total distance	all-yes	yes	Genotype Factor: $F_{1,34} = 2.112$; HM3Dq factor: $F_{1,34} = 8.248$; Genotype \times HM3Dq: $F_{1,34} = 0.8104$	total=37
Fig. S6E Center time (%)	all-yes	yes	Genotype Factor: $F_{1,26} = 17.81$; HM3Dq factor: $F_{1,26} = 0.07846$; Genotype \times HM3Dq: $F_{1,26} = 0.009319$	total=29
Fig. S6E Center time (s)	all-yes	yes	Genotype Factor: $F_{1,26} = 18.54$; HM3Dq factor: $F_{1,26} = 0.01205$; Genotype \times HM3Dq: $F_{1,26} = 0.003922$	total=29
Fig. S6E Center distance (%)	all-yes	yes	Genotype Factor: $F_{1,28} = 22.61$; HM3Dq factor: $F_{1,28} = 0.01271$; Genotype \times HM3Dq: $F_{1,28} = 0.01376$	total=31
Fig. S6F Time in open arm (s)	all-yes	yes	Genotype Factor: $F_{1,26} = 32.63$; HM3Dq factor: $F_{1,26} = 0.08644$; Genotype \times HM3Dq: $F_{1,26} = 0.06746$	total=29
Fig. S6F Number into open arm	all-yes	no	Genotype Factor: $F_{1,24} = 18.88$; HM3Dq factor: $F_{1,24} = 0.9032$; Genotype \times HM3Dq: $F_{1,24} = 0.07373$	total=27
Fig. S6F Time in open arm (%)	all-yes	yes	Genotype Factor: $F_{1,26} = 37.18$; HM3Dq factor: $F_{1,26} = 0.002266$;	total=29

			Genotype × HM3Dq: $F_{1,26} = 0.1678$	
Fig. S6F Entries into open arm (%)	all-yes	yes	Genotype Factor: $F_{1,26} = 37.18$; HM3Dq factor: $F_{1,26} = 2.548$; Genotype × HM3Dq: $F_{1,26} = 0.002497$	total=29
Fig. S7A	yes, yes	yes	4.597	8
Fig. S7B	yes, yes	yes	3.649	8
Fig. S7D Total distance	yes, yes	yes	0.1943	10
Fig. S7D Center time (%)	yes, yes	yes	2.334	10
Fig. S7D Center time (s)	yes, yes	yes	2.362	10
Fig. S7D Center distance (%)	no, no	yes	—	—
Fig. S7E Time in open arm (s)	yes, yes	yes	4.149	10
Fig. S7E Number into open arm	yes, yes	yes	6,916	10
Fig. S7E Time in open arm (%)	yes, yes	yes	3.896	10
Fig. S7E Entries into open arm (%)	yes, yes	yes	2.649	10
Fig. S7F	yes, yes	yes	5.232	10
Fig. S8C	yes, yes	yes	3.499	9
Fig. S8D Total distance	yes, yes	yes	0.1451	19
Fig. S8D Center time (%)	yes, yes	no	0.1473	11.58
Fig. S8D Center time (s)	yes, yes	no	0.1631	11.62
Fig. S8D Center distance (%)	yes, yes	yes	0.9327	19
Fig. S8E Time in open arm (s)	yes, yes	yes	0.01298	19
Fig. S8E Number into open arm	yes, yes	yes	0.1327	19
Fig. S8E Time in open arm (%)	yes, yes	yes	0.5384	19
Fig. S8E Entries into open arm (%)	yes, yes	yes	0.1099	19
Fig. S8F	yes, yes	yes	2.311	19
Fig. S9C Npc	yes, yes	yes	1.078	17
Fig. S9C Apoa1	no, yes	no	—	—
Fig. S9C Ace2	yes, yes	yes	5.439	17
Fig. S9C Crtap	yes, yes	yes	1.79	17
Fig. S9C Cxcl9	yes, yes	yes	0.2802	17
Fig. S9C Vegfb	yes, yes	yes	0.3359	17
Fig. S9D Apoa1	yes, yes	yes	0.9845	14
Fig. S9D Ace2	yes, yes	yes	2.279	14
Fig. S9D Npc2	yes, yes	no	1.089	7.865
Fig. S9D Cxcl9	yes, yes	yes	0.2923	14
Fig. S9D Grn	no, yes	yes	—	—
Fig. S9D Bgn	yes, yes	yes	2.393	14
Fig. S10A Atf4	yes, yes	no	19.92	5
Fig. S10A Trb3	yes, yes	yes	17.49	10
Fig. S10A Tff3	yes, yes	yes	5.717	10
Fig. S10B Atf4	no, yes	no	—	—

Fig. S10B Trb3	yes, yes	yes	2.491	10
Fig. S10B Tff3	yes, yes	yes	2.662	10
Fig. S11A Jej	yes, yes	no	2.584	5.905
Fig. S11A Ile	yes, yes	yes	4.486	12
Fig. S11A Col	yes, yes	yes	5.592	12
Fig. S11B	yes, yes	yes	2.394	12
Fig. S11C	yes, yes	yes	2.394	9
Fig. S11D	yes, yes	yes	2.396	9
Fig. S11E	yes, no	yes	—	—
Fig. S11F HY	yes, yes	yes	0.03445	11
Fig. S11F PVT	yes, no	no	—	—
Fig. S11G HY	yes, yes	yes	0.7755	12
Fig. S11G PVT	no, yes	yes	—	—
Fig. S11H	yes, yes	yes	2.383	9
Fig. S11I Center time (%)	yes, no, yes, yes	yes	Genotype Factor: $F_{1,38} = 0.4814$; TFF3 factor: $F_{1,38} = 2.343$; Genotype \times TFF3: $F_{1,38} = 6.522$	total=41
Fig. S11J Time in open arm (s)	all-yes	yes	Genotype Factor: $F_{1,42} = 4.387$; TFF3 factor: $F_{1,42} = 9.758$; Genotype \times TFF3: $F_{1,42} = 1.857$	total=45
Fig. S11J Number into open arm	all-yes	yes	Genotype Factor: $F_{1,29} = 0.4649$; TFF3 factor: $F_{1,29} = 1.249$; Genotype \times TFF3: $F_{1,29} = 9.164$	total=32
Fig. S12B Center time (%)	all-yes	yes	$F_{2,18} = 4.976$	total=20
Fig. S12C Time in open arm (s)	all-yes	yes	$F_{2,18} = 6.131$	total=20
Fig. S12C Number into open arm	all-yes	yes	$F_{2,18} = 5.519$	total=20
Fig. S13C	yes, yes	yes	3.058	9
Fig. S13D Hypothalamus	yes, yes	yes	1.183	9
Fig. S13D Cortex	yes, yes	yes	1.468	9
Fig. S13E Total distance	yes, yes	yes	0.7819	18
Fig. S13E Center time (%)	yes, yes	yes	3.479	18
Fig. S13E Center time (s)	yes, yes	yes	3.042	18
Fig. S13E Center distance (%)	yes, yes	yes	3.31	18
Fig. S13F Time in open arm (s)	yes, yes	yes	3.401	18
Fig. S13F Number into open arm	yes, yes	yes	2.812	18
Fig. S13F Time in open arm (%)	yes, yes	yes	4.204	18
Fig. S13F Entries into open arm (%)	yes, yes	yes	2.336	18
Fig. S13G	yes, yes	yes	2.506	18
Fig. S14A	all-yes	no	$F_{4,4,635} = 5.555$	total=8.635
Fig. S15B	all-yes	yes	$F_{3,16} = 12.21$	total=19

Fig. S15C Center time (%)	all-yes	yes	$F_{3,30} = 6.334$	total=33
Fig. S15D Time in open arm (s)	all-yes	yes	$F_{3,29} = 11.34$	total=32
Fig. S15D Number into open arm	all-yes	yes	$F_{3,31} = 7.805$	total=34
Fig. S16B Total distance	yes, yes	yes	0.8924	10
Fig. S16B Center time (%)	yes, yes	yes	0.08366	10
Fig. S16B Center time (s)	yes, yes	yes	0.07995	10
Fig. S16B Center distance (%)	yes, yes	yes	0.08124	10
Fig. S16C Time in open arm (s)	yes, yes	yes	0.2379	10
Fig. S16C Number into open arm	yes, yes	yes	0.2666	10
Fig. S16C Time in open arm (%)	yes, yes	yes	0.3909	10
Fig. S16C Entries into open arm (%)	yes, yes	yes	1.277	10
Fig. S16D	yes, yes	yes	2.966	10
Fig. S17C Total distance	all-yes	yes	Genotype Factor: $F_{1,40} = 0.2109$; HM4Di factor: $F_{1,40} = 0.7010$; Genotype \times HM4Di: $F_{1,40} = 0.01985$	total=43
Fig. S17C Center time (%)	all-yes	no	Genotype Factor: $F_{1,40} = 3.740$; HM4Di factor: $F_{1,40} = 6.629$; Genotype \times HM4Di: $F_{1,40} = 3.736$	total=43
Fig. S17C Center time (s)	all-yes	yes	Genotype Factor: $F_{1,40} = 5.489$; HM4Di factor: $F_{1,40} = 3.498$; Genotype \times HM4Di: $F_{1,40} = 3.518$	total=43
Fig. S17C Center distance (%)	all-yes	yes	Genotype Factor: $F_{1,40} = 4.380$; HM4Di factor: $F_{1,40} = 2.710$; Genotype \times HM4Di: $F_{1,40} = 3.554$	total=43
Fig. S17D Time in open arm (s)	all-yes	yes	Genotype Factor: $F_{1,40} = 1.120$; HM4Di factor: $F_{1,40} = 2.024$; Genotype \times HM4Di: $F_{1,40} = 5.824$	total=43
Fig. S17D Number into open arm	yes, yes, no, yes	yes	Genotype Factor: $F_{1,40} = 2.195$; HM4Di factor: $F_{1,40} = 4.826$; Genotype \times HM4Di: $F_{1,40} = 4.708$	total=43
Fig. S17D Time in open arm (%)	all-yes	yes	Genotype Factor: $F_{1,40} = 1.850$; HM4Di factor: $F_{1,40} = 3.102$; Genotype \times HM4Di: $F_{1,40} = 8.893$	total=43
Fig. S17D Entries into open arm (%)	yes, yes, yes, no	yes	Genotype Factor: $F_{1,40} = 2.186$; HM4Di factor: $F_{1,40} = 2.903$; Genotype \times HM4Di: $F_{1,40} = 3.797$	total=43
Fig. S17E	all-yes	yes	Genotype Factor: $F_{1,39} = 4.227$; HM4Di factor: $F_{1,39} = 4.856$; Genotype \times HM4Di: $F_{1,39} = 2.028$	total=42

