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Supplemental information

An adult-stage transcriptional program for survival of serotonergic connectivity

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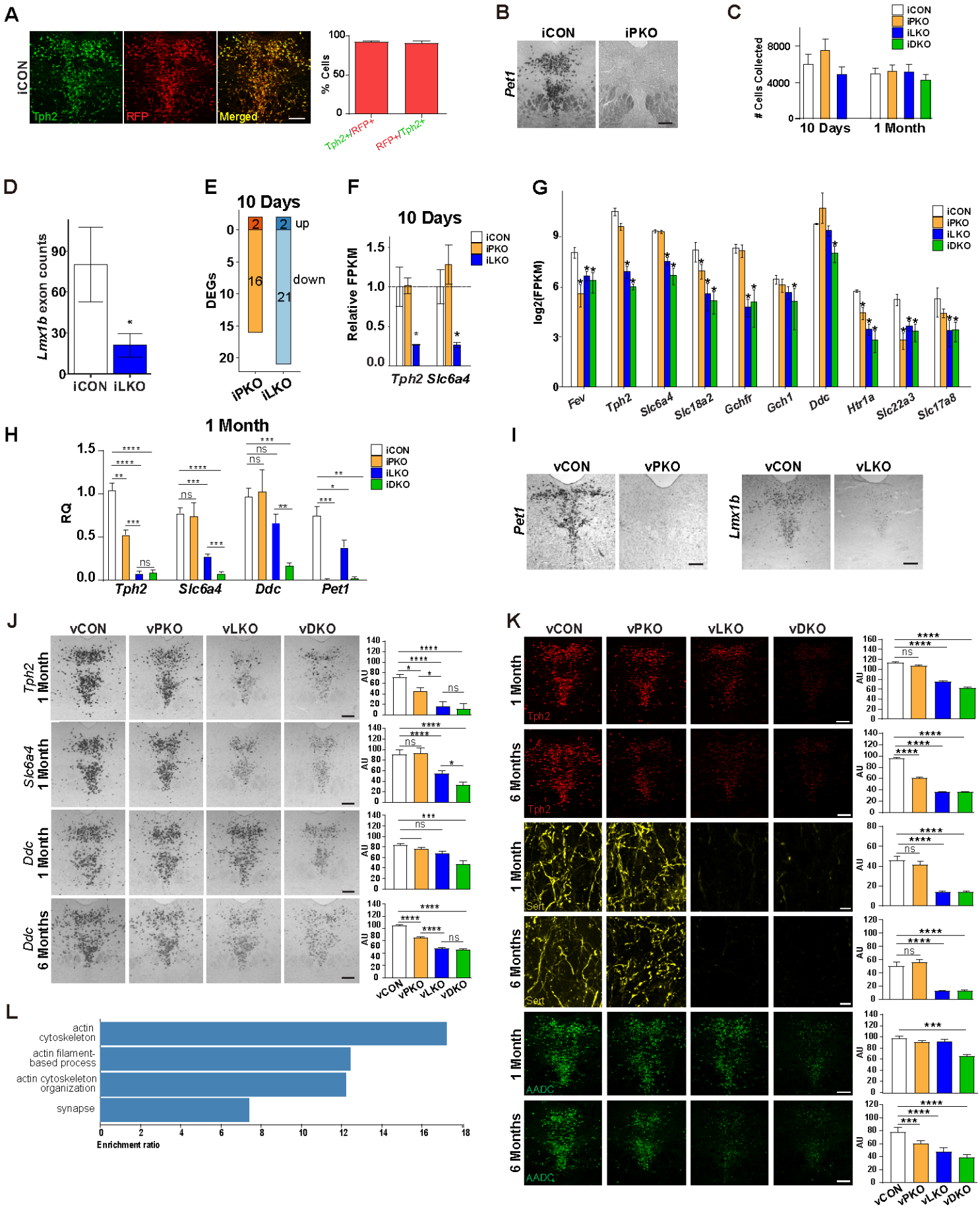

A
iCON
Tph2
RFP
Merged
% Cells
Tph2+/RFP+
RFP+/Tph2+
B
iCON
iPKO
Pet1
C
iCON
iPKO
iLKO
iDKO
Cells Collected
10 Days
1 Month
D
Lmx1b exon counts
iCON iLKO
E
10 Days
DEGs
up
down
iPKO
iLKO
F
10 Days
Relative FPKM
Tph2 Slc6a4
G
log2(FPKM)
Fev
Tph2
Slc6a4
Slc18a2
Gchfr
Gch1
Ddc
Htr1a
Slc22a3
Slc17a8
H
1 Month
RQ
Tph2
Slc6a4
Ddc
Pet1
I
vCON
vPKO
vLKO
Pet1
Lmx1b
J
vCON
vPKO
vLKO
vDKO
Tph2 1 Month
Slc6a4 1 Month
Ddc 1 Month
Ddc 6 Months
AU
K
vCON
vPKO
vLKO
vDKO
1 Month
6 Months
Tph2
Sert
AADC
L
actin cytoskeleton
actin filament-based process
actin cytoskeleton organization
synapse
Enrichment ratio

Supplemental Fig 1. Adult stage targeting of 5-HT neuron gene expression, Related to Figure 1.

A. Tph2-CreER targeting efficiency (Tph2+/RFP+) and specificity (RFP+/Tph2+) assessed at 1 month post-TAM. n=2 iCON mice.

B. *In situ* hybridization analysis of adult stage Pet1 targeting at 1 month post-TAM; n= 3 mice. Scale bar: 100µm.

C. Number of neurons flow sorted for RNA-seq at 10 days and 1 month post-TAM.

D. Reduced *Lmx1b* exon 4 reads demonstrates effective *Lmx1b* targeting.

E. iPKO and iLKO DEGs at 10 days post-TAM; FDR≤0.05.

F. Relative expression of *Tph2* and *Slc6a4* in iCON, iPKO and iLKO neurons 10 days post-TAM.

G. Relative expression of 5-HT neurotransmission genes and *Slc17a8* at 1 month post-TAM.

H. qPCR analysis of 5-HT gene expression 1 month post-TAM; 2 mice were dissected per replicate (n), n=3-5 mice per genotype; ± SEM; one-way ANOVA.

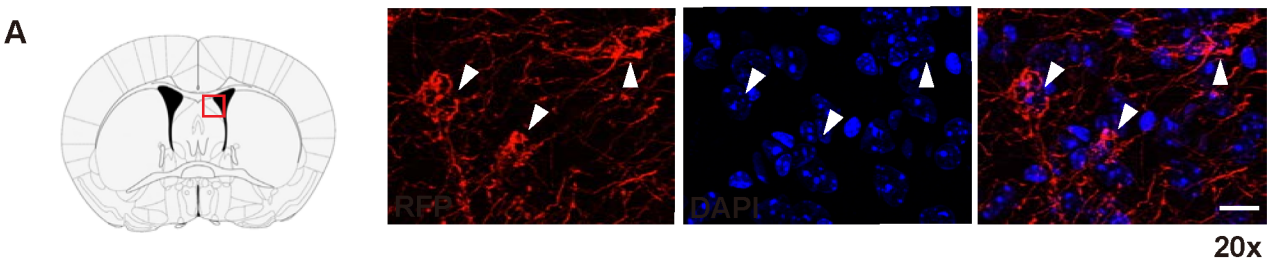
I. ISH analysis of *Pet1* (left) or *Lmx1b* (right) 1 month after AAV-Cre injection into the DRN of vCON and vPKO or vLKO mice. Scale bar: 100µm.

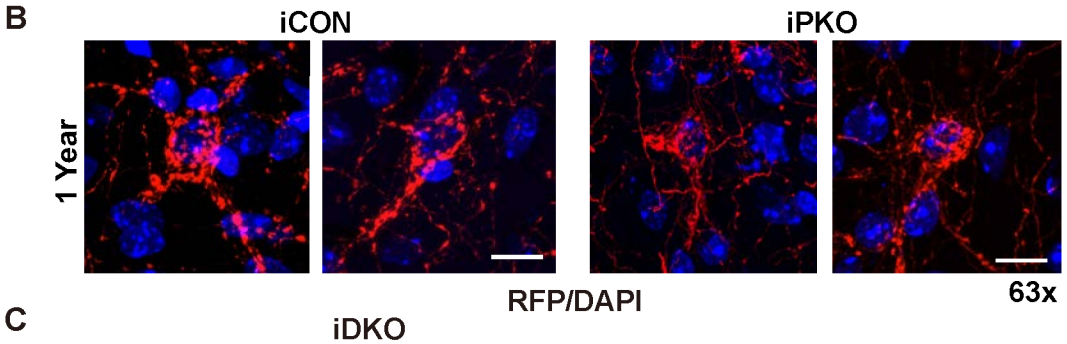
J. ISH of *Tph2, Slc6a4, and Ddc* in vCON, vPKO, vLKO and vDKO mice at 1 or 6 months post AAV-Cre injection; ± SEM; n= 1,200 -1,500 cells from 3 sections obtained from 3 mice per genotype; 1-way ANOVA. Scale bar: 100µm.

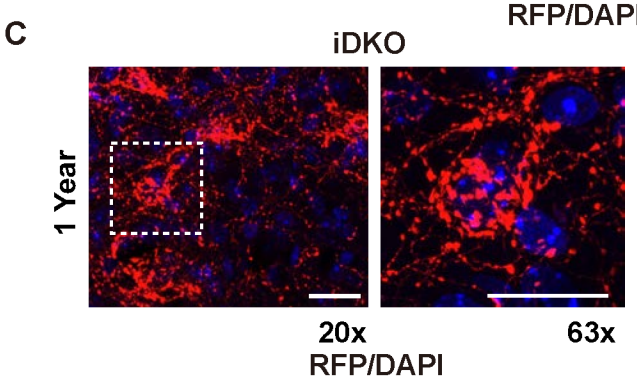
K. Immunofluorescence staining 1 month and 6 months post AAV-Cre injections; ± SEM; n= 1,200 -1,500 cells from 3 sections obtained from each mouse per genotype; 1-way ANOVA. Scale bar, 100µm, Tph2 panels; 5µm, Sert panels; 100µm, AADC panels.

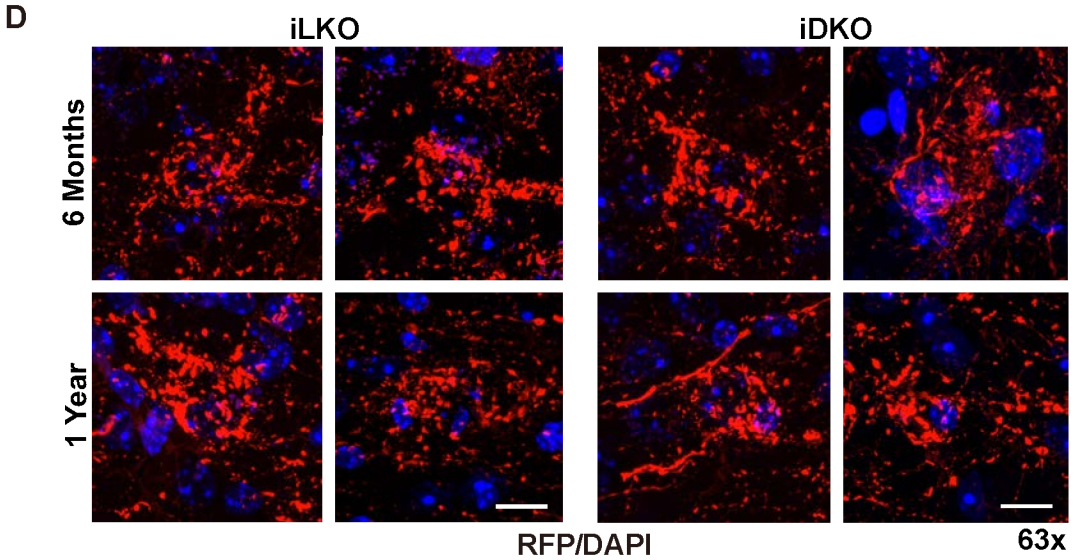
L. Gene Ontology (Biological Process) term enrichment for iLKO DEGs at 10 days post-TAM.

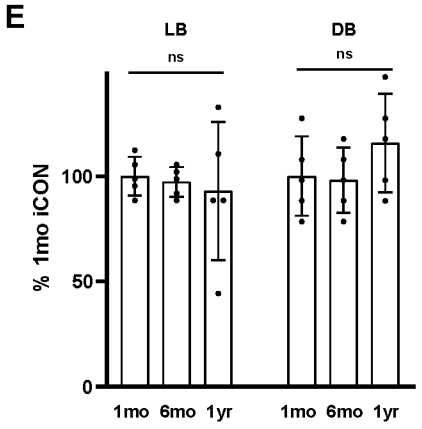
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns, not significant in G-J.

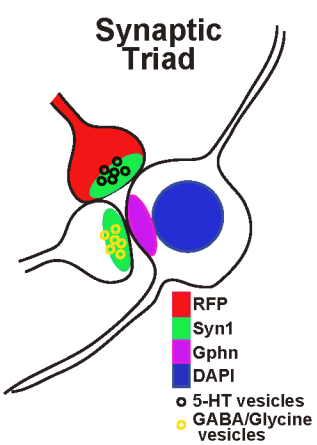

A
RFP
DAPI
20x

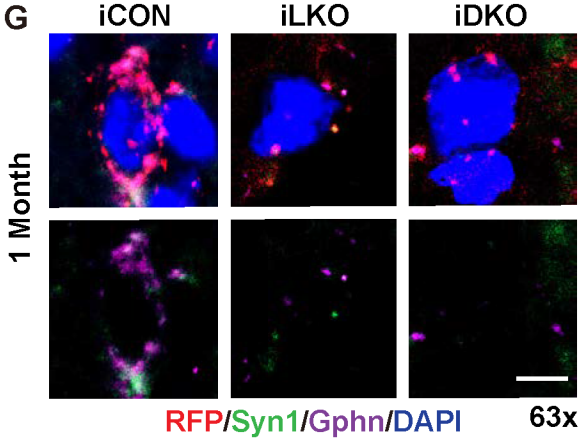

B
iCON
iPKO
1 Year
RFP/DAPI
63x


C
iDKO
1 Year
20x
63x
RFP/DAPI

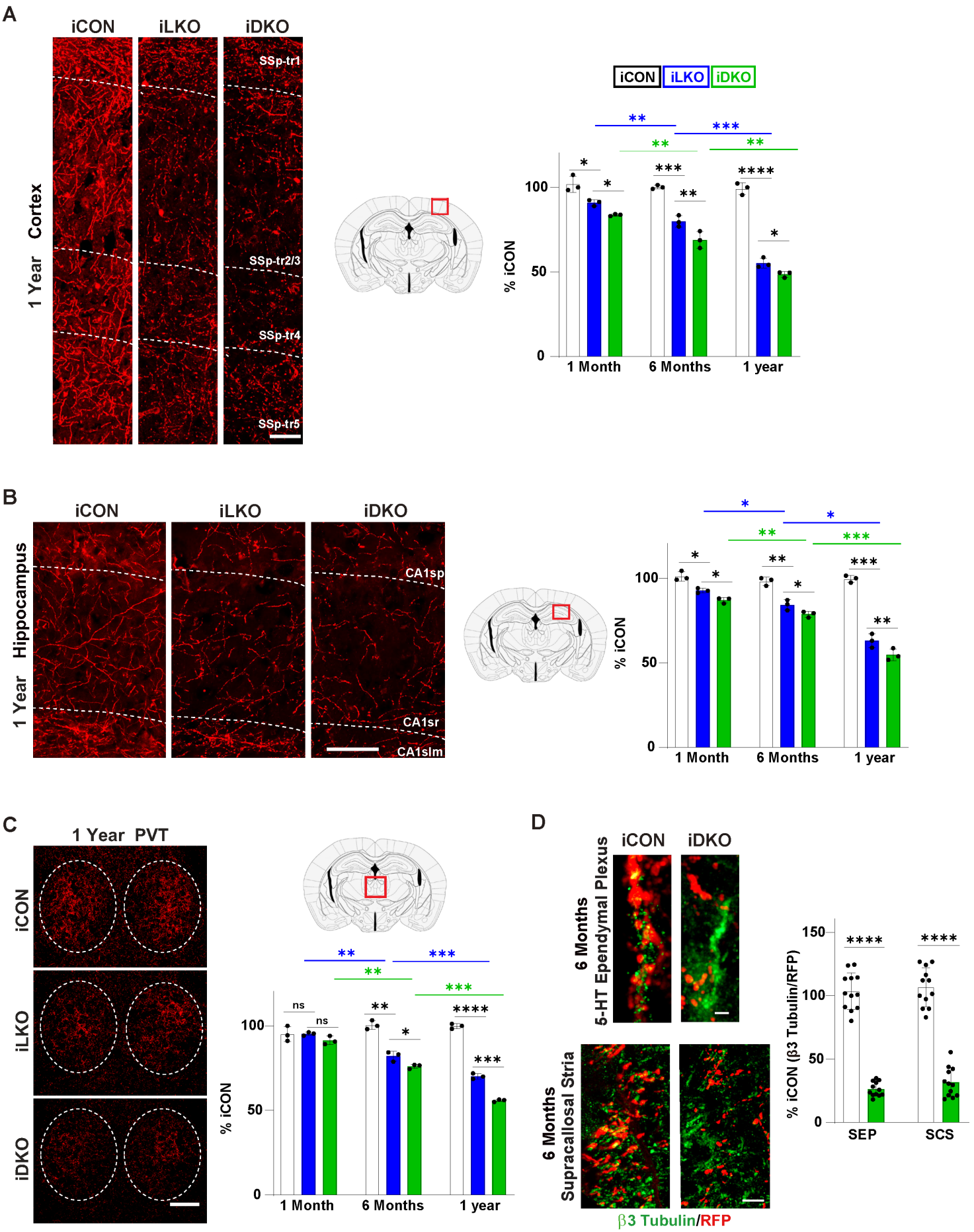

D
iLKO
iDKO
6 Months
1 Year
RFP/DAPI
63x


E
LB
DB
ns
ns
% 1mo iCON
100
50
0
1mo 6mo 1yr
1mo 6mo 1yr

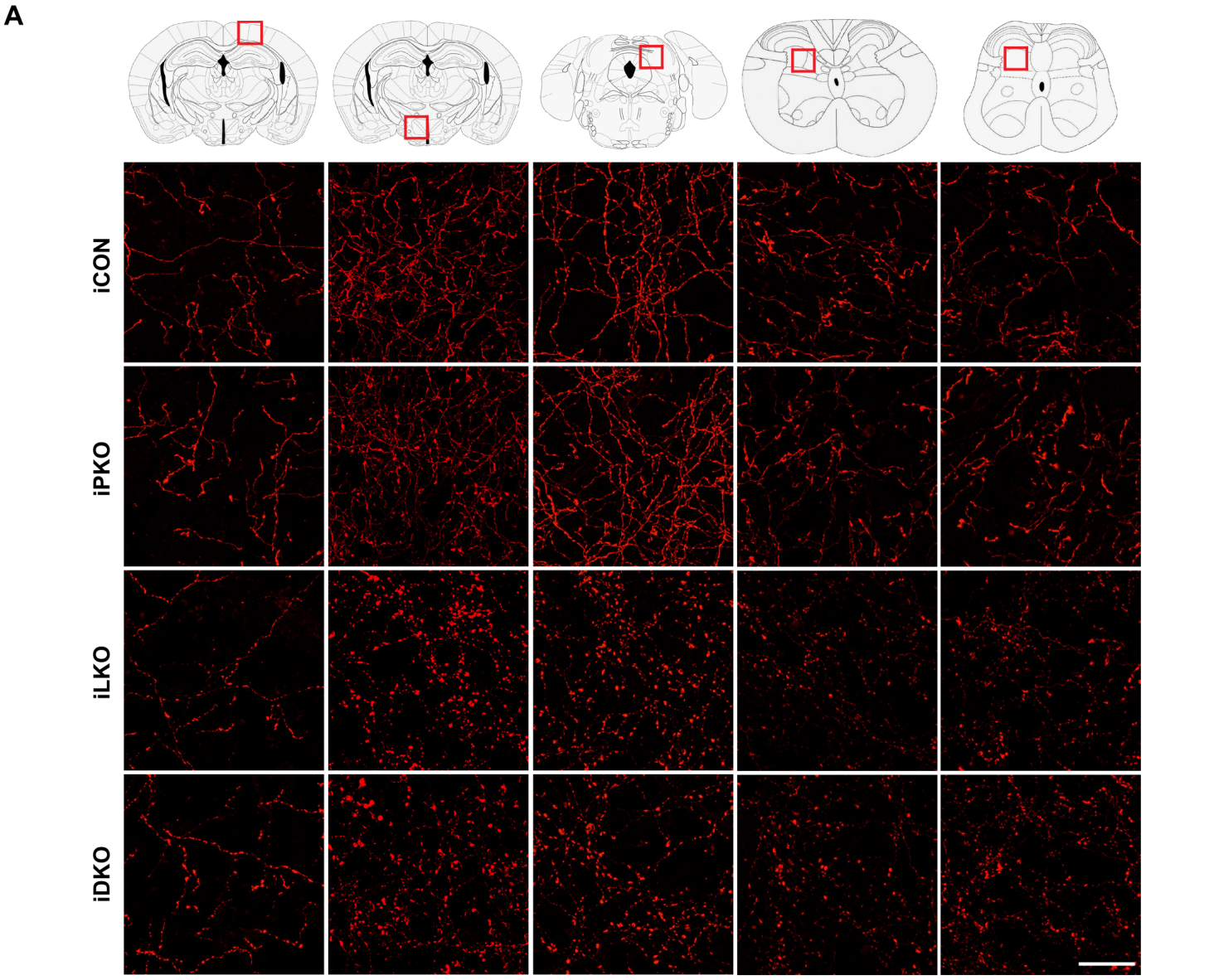

F
Synaptic Triad
RFP
Syn1
Gphn
DAPI
5-HT vesicles
GABA/Glycine vesicles


G
iCON
iLKO
iDKO
1 Month
RFP/Syn1/Gphn/DAPI
63x

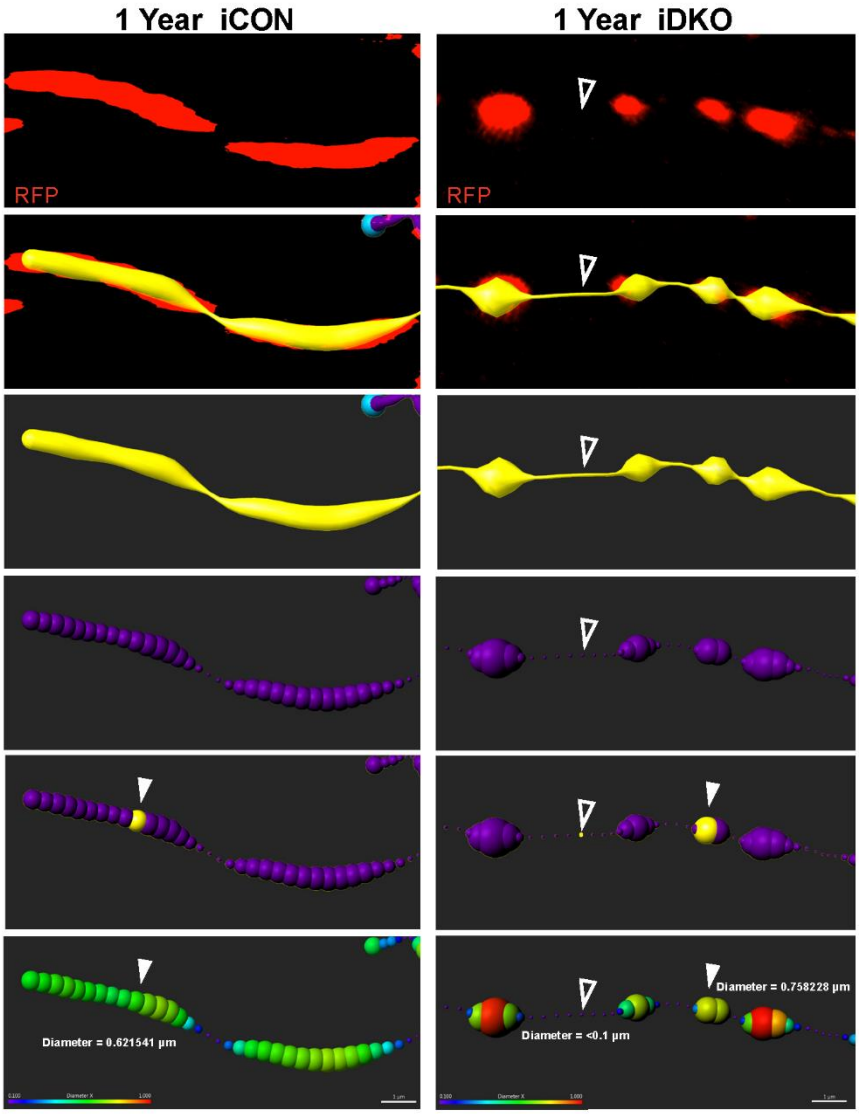
Supplemental Figure 2. Degeneration of pericellular baskets, Related to Figure 2.
A. Coronal schematic highlighting location of dLS where pericellular baskets can be readily imaged. Arrowheads indicate representative dense baskets (DBs) identified at low magnification.
B. Representative images of DBs in iCON (left) and iPKO (right) mice 1 year post-TAM. Scale bar: 10µm.
C. Confocal images of readily identifiable DBRs found in iDKO mice 1 year post-TAM. Low magnification (left) indicates that DBRs are easy to identify while higher magnification (right) reveals abnormal morphology. Scale bars: 10µm.
D. Representative images of DBRs at 6 months and 1 year post-TAM. Scale bar: 10µm.
E. iCON LB and DBs numbers do not change across time points. Data expressed % of iCON numbers at 1 month post-TAM. 1-way ANOVA. n=3 mice per genotype, 2-3 sections per animal were used for counting.
F. Schematic of 5-HT synaptic triad.
G. Representative images of Synapsin1, Gephyrin, and RFP colocalization in iCON, iLKO and iDKO mice at 1 month post-TAM. Scale bar: 5µm.


A
iCON
iLKO
iDKO
1 Year Cortex
SSp-tr1
SSp-tr2/3
SSp-tr4
SSp-tr5
iCON iLKO iDKO
% iCON
1 Month
6 Months
1 year
B
iCON
iLKO
iDKO
1 Year Hippocampus
CA1sp
CA1sr
CA1slm
% iCON
1 Month
6 Months
1 year
C
1 Year PVT
iCON
iLKO
iDKO
% iCON
1 Month
6 Months
1 year
D
iCON
iDKO
6 Months 5-HT Ependymal Plexus
6 Months Supracallosal Stria
β3 Tubulin/RFP
% iCON (β3 Tubulin/RFP)
SEP
SCS

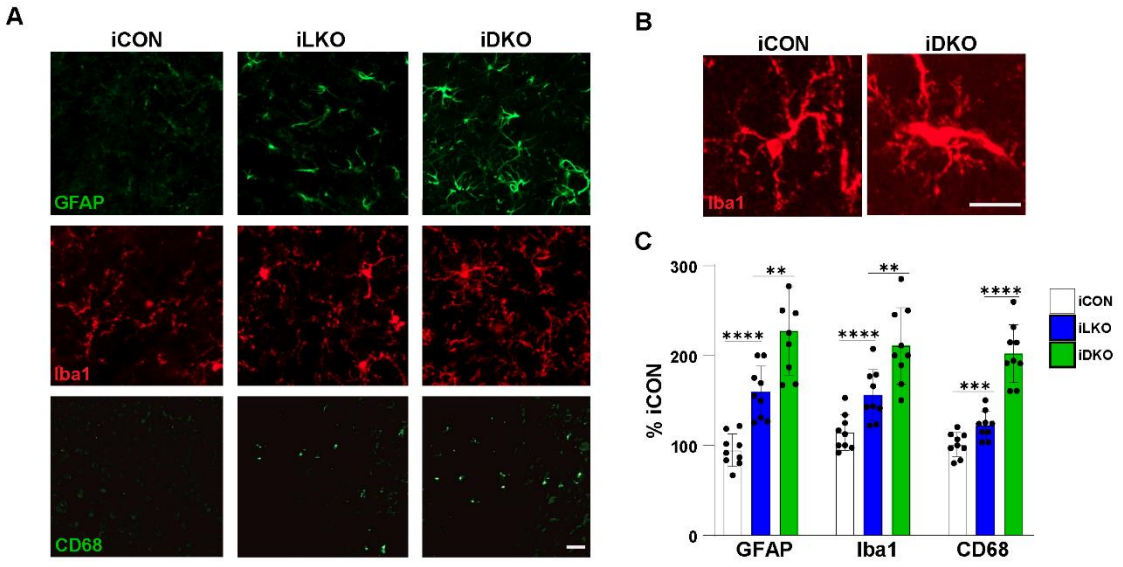
Supplemental Figure 3. Degeneration of serotonergic axons, Related to Figure 3.
A-C, Left, representative images of TdTomato+ (anti-RFP) axons in somatosensory cortex (A), hippocampus (B) and paraventricular nucleus (C) at 1 year post-TAM. Right, quantification of TdTomato+ axon densities; ±SEM; n=3 mice per genotype; 2-way ANOVA. Scale bars: A, 50μm; B, C, 100μm.
D. Left, representative images of β3 Tubulin co-immunostaining with RFP at 6 months post-TAM. Scale bars, 2μm, 10μm. Right, relative ratio of β3 Tubulin co-immunostaining with RFP at 6 months post-TAM; ±SEM; n=3 mice per genotype; 1-way ANOVA.
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; ns, not significant.


A
iCON
iPKO
iLKO
iDKO
B
Developmental Axons
P0 Control
Regenerating Axons
iCON 1mo PCA
Tandem Varicosities & Spheroids
iCON
iDKO
iCON PCA
P0 CON
****
#/10^4 µm^2
15
10
5
0
C
Syn1 / RFP
iCON
iDKO
1mo PCA
6 Months
Syn1
****
% RFP
80
60
40
20
0
iCON (PCA)
iDKO

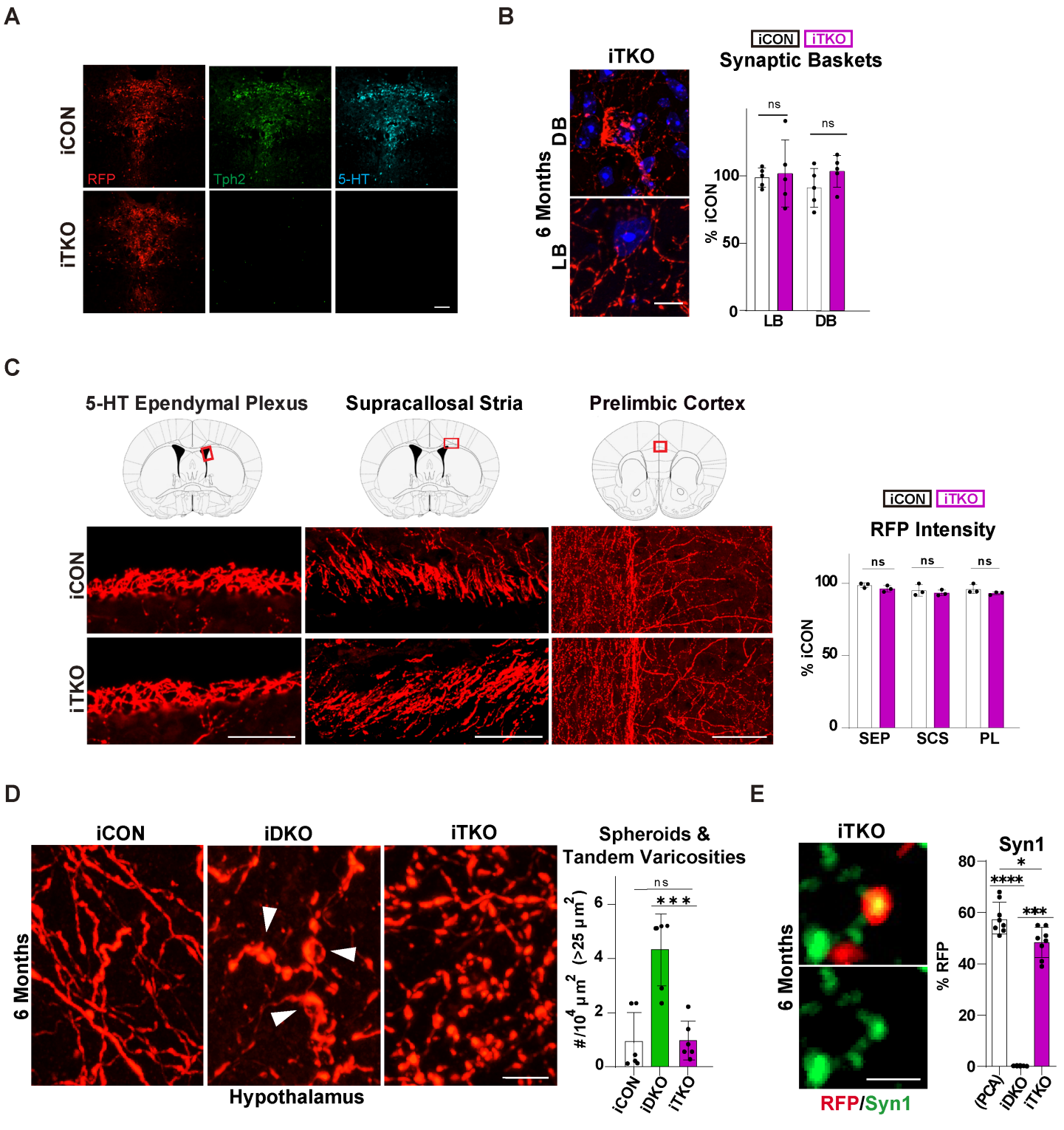
Supplemental Figure 4. Progressive dystrophic morphology, Related to Figure 4.
A. Progressive morphological alterations of TdTomato+ iLKO and iDKO axons at 1 year post-TAM. Left to right: cortex, hypothalamus, inferior colliculus, cervical spinal cord and sacral spinal cord (n=3-5 mice analyzed per genotype).
B. Representative images of control 5-HT axon morphology at birth (upper panel) and adult regenerating axons at 1 month post-PCA administration (lower panel). Right, quantification of tandem varicosities and spheroids; ±SEM; n=3 mice per genotype.
C. Regenerating iCON axons at 1 month post-PCA accumulate Synapsin 1 in varicosities but iDKO fibers do not at 6 month post-TAM. Right, quantification; mean ± SEM; n=3 mice per genotype.
One-way ANOVA in B, C; ****$p<0.0001$. Scale bars: 20µm in A, B; 2µm in C.



Supplemental Figure 5. Axon fragmentation analysis, Related to Figure 4.
Imaris 3D reconstruction modeling and axon segment diameter analysis at 1 year post-TAM. Modeled axon segments representing areas below the detection threshold of 0.1µm (open arrowheads) were counted. Fragmentation index was calculated by the number of modeled <0.1µm assignments divided by the sum of <0.1µm and >0.1µm assignments (filled arrowheads).



Supplemental Figure 6. Microglia activation and astrogliosis, Related to Figure 4.
A. GFAP, Iba1, and CD68 immunoreactivities are significantly increased in iLKO and iDKO brains compared to iCON at 6 months post-TAM. Scale bar: 20μm for each panel.
B. High magnification image of Iba1+ cells in iCON brains exhibiting small cell bodies and ramified processes compared to those in iDKO brains exhibiting an activated morphology. Scale bar: 20μm.
C. Quantification of GFAP, Iba1, and CD68 immunoflourescence in iCON, iLKO and iDKO cortex; ±SEM; n=3 mice using 3 sections per animal per genotype; 1-way ANOVA; **p<0.01, ***p<0.001, ****p<0.0001.


A
iCON
iTKO
RFP
Tph2
5-HT
B
iTKO
6 Months
DB
LB
iCON
iTKO
Synaptic Baskets
ns
ns
% iCON
100
50
0
LB
DB
C
5-HT Ependymal Plexus
Supracallosal Stria
Prelimbic Cortex
iCON
iTKO
iCON
iTKO
RFP Intensity
ns
ns
ns
% iCON
100
50
0
SEP
SCS
PL
D
iCON
iDKO
iTKO
6 Months
Hypothalamus
Spheroids &
Tandem Varicosities
ns
***
#/10^4 µm^2 (>25 µm^2)
6
4
2
0
iCON
iDKO
iTKO
E
iTKO
6 Months
RFP/Syn1
Syn1
*
****
***
% RFP
80
60
40
20
0
(PCA)
iDKO
iTKO

Supplemental Figure 7. Loss of adult brain 5-HT does not lead to loss of serotonergic connectivity or dystrophic morphology, Related to Figure 4.

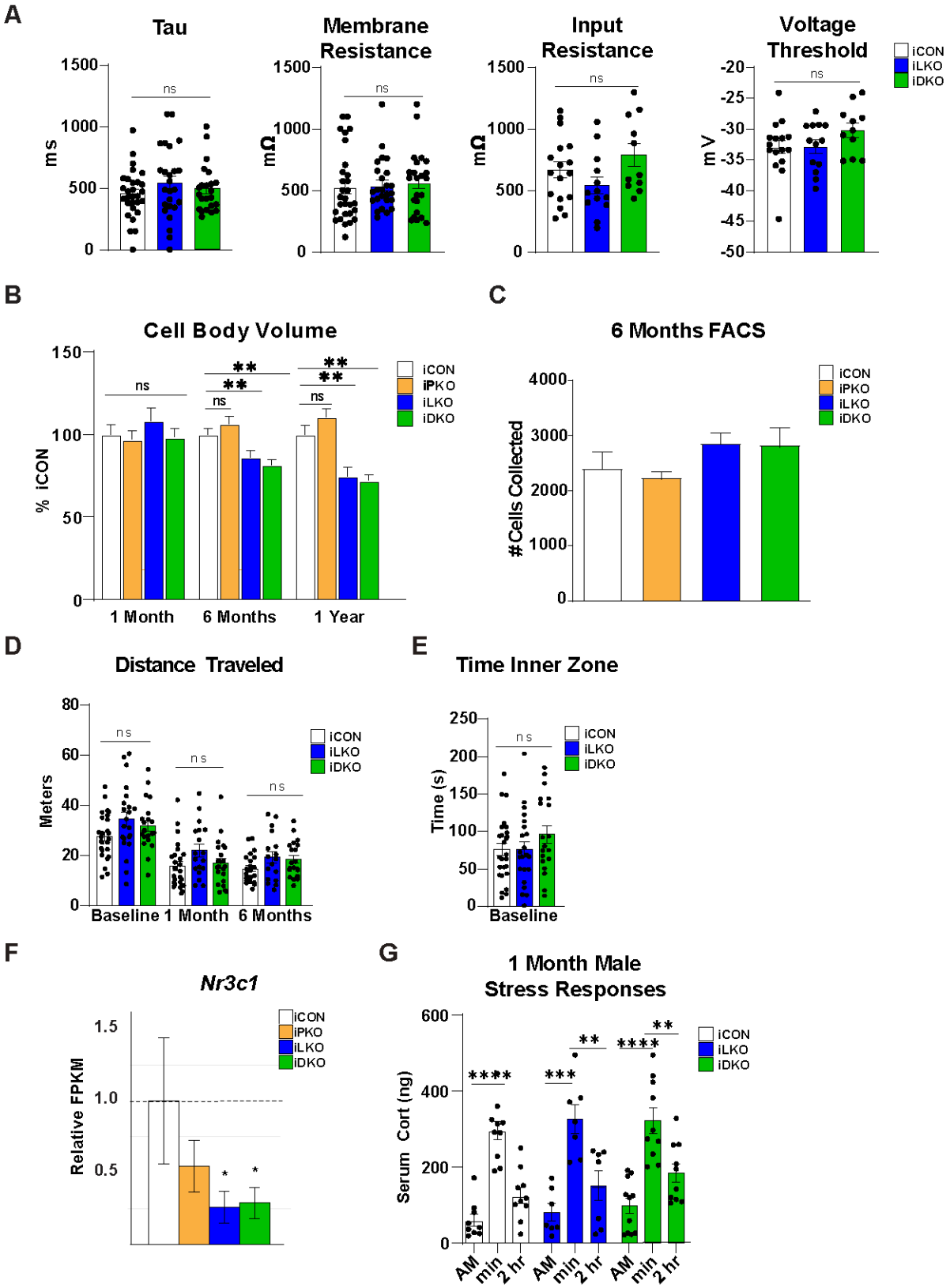
A. Adult stage tamoxifen-inducible *Tph2* targeting.

B. Left, representative images of DBs and LBs in iTKO mice versus iCON at 6 months post-TAM. Right, quantification of pericellular baskets numbers: ±SEM; n=3 animals per genotype; unpaired t-test with Welch's correction; ns, not significant. Scale bar: 10µm.

C. Left, representative images of TdTomato+ (anti-RFP) axons in indicated regions. Right, quantification of RFP fluorescence at 6 months post-TAM: ±SEM; n=3 mice per genotype; unpaired t-test with Welch's correction; ns, not significant. Scale bars: 50µm for SEP and SCS; 100µm for PL.

D. Left, TdTomato+ fibers and spheroids (arrowheads) at 6 months post-TAM. Right, quantification of tandem varicosities and spheroids; ±SEM, n=3 mice per genotype; 1-way ANOVA; ***p<0.001, ns, not significant. Scale bar: 5µm.

E. Accumulation of Synapsin1 in iTKO varicosities at 6 months post-TAM; ±SEM; n=3 mice per genotype; 1-way ANOVA; *p<0.05, ***p<0.001, ****p<0.0001. Scale bar:5µm.


A
Tau
ms
1500
1000
500
0
ns
Membrane Resistance
mΩ
1500
1000
500
0
ns
Input Resistance
mΩ
1500
1000
500
0
ns
Voltage Threshold
mV
-20
-25
-30
-35
-40
-45
-50
ns
iCON
iLKO
iDKO
B
Cell Body Volume
% iCON
150
100
50
0
ns
**
**
ns
**
**
ns
1 Month
6 Months
1 Year
iCON
iPKO
iLKO
iDKO
C
6 Months FACS
#Cells Collected
4000
3000
2000
1000
0
iCON
iPKO
iLKO
iDKO
D
Distance Traveled
Meters
80
60
40
20
0
ns
ns
ns
Baseline
1 Month
6 Months
iCON
iLKO
iDKO
E
Time Inner Zone
Time (s)
250
200
150
100
50
0
ns
Baseline
iCON
iLKO
iDKO
F
Nr3c1
Relative FPKM
1.5
1.0
0.5
*
*
iCON
iPKO
iLKO
iDKO
G
1 Month Male Stress Responses
Serum Cort (ng)
600
400
200
0
****
***
**
****
**
AM
min
2 hr
AM
min
2 hr
AM
min
2 hr
iCON
iLKO
iDKO

Supplemental Figure 8. Passive membrane properties, cell body volumes and behavior assays, Related to Figure 6.

A. Tau, membrane resistance, input resistance, and voltage threshold at 1 month post-TAM; ±SEM; n=14-18 neurons per genotype; 1-way ANOVA.

B. Cell body volumes at 6 months and 1 year post-TAM; ±SEM; 150-200 cells were analyzed per mouse, n=2-3 mice per genotype; 1-way ANOVA.

C. Numbers of TdTomato+ neurons flow sorted at 6 months post-TAM.

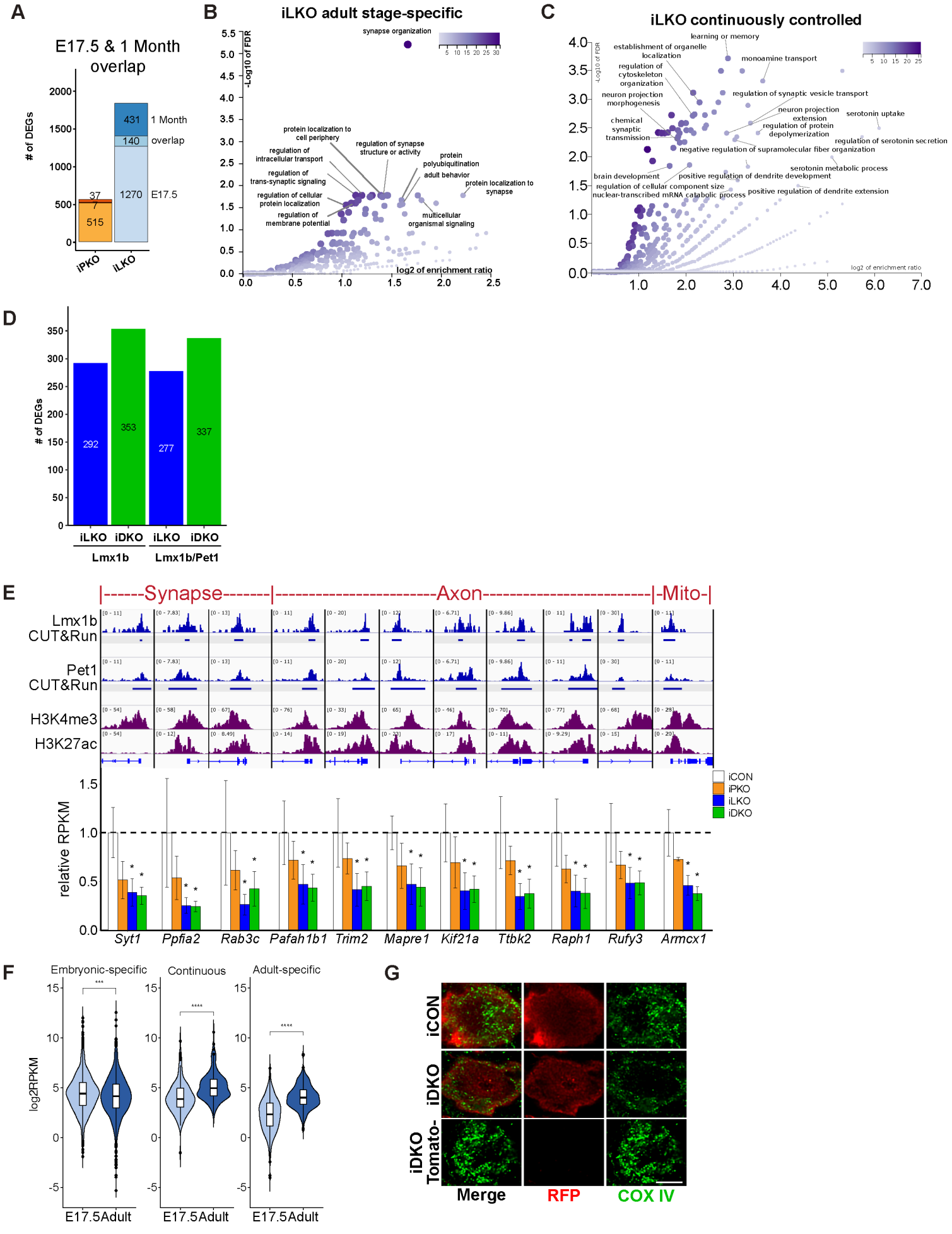
D. Total distance traveled pre-TAM and at 1 and 6 months post-TAM; ±SEM; n=20-28 mice per genotype; 2-way repeated measures ANOVA.

E. Baseline testing of time in the inner zone of the open field pre-TAM; n=20-28 mice per genotype; 2-way ANOVA and unpaired t-test with Welch's correction.

F. Relative expression (FPKM) of *Nr3c1*. *FDR≤0.05.

G. Serum CORT levels after restraint stress in male mice at 1 month post-TAM; ± SEM; n=8-10 mice per genotype; 2-way repeated measures ANOVA;

**p<0.01, ***p<0.001, ****p<0.0001, ns, not significant.


A
E17.5 & 1 Month overlap
# of DEGs
2000
1500
1000
500
0
37
7
515
431
140
1270
1 Month
overlap
E17.5
iPKO
iLKO
B
iLKO adult stage-specific
-Log10 of FDR
synapse organization
protein localization to cell periphery
regulation of intracellular transport
regulation of trans-synaptic signaling
regulation of cellular protein localization
regulation of membrane potential
regulation of synapse structure or activity
protein polyubiquitination
adult behavior
protein localization to synapse
multicellular organismal signaling
log2 of enrichment ratio
C
iLKO continuously controlled
-Log10 of FDR
learning or memory
establishment of organelle localization
monoamine transport
regulation of cytoskeleton organization
neuron projection morphogenesis
regulation of synaptic vesicle transport
chemical synaptic transmission
neuron projection extension
serotonin uptake
regulation of protein depolymerization
regulation of serotonin secretion
negative regulation of supramolecular fiber organization
serotonin metabolic process
brain development
positive regulation of dendrite development
regulation of cellular component size
nuclear-transcribed mRNA catabolic process
positive regulation of dendrite extension
log2 of enrichment ratio
D
# of DEGs
292
353
277
337
iLKO
iDKO
iLKO
iDKO
Lmx1b
Lmx1b/Pet1
E
|------Synapse------|----------Axon----------|-Mito-|
Lmx1b CUT&Run
Pet1 CUT&Run
H3K4me3
H3K27ac
relative RPKM
iCON
iPKO
iLKO
iDKO
Syt1
Ppfia2
Rab3c
Pafah1b1
Trim2
Mapre1
Kif21a
Ttbk2
Raph1
Rufy3
Armcx1
F
Embryonic-specific
Continuous
Adult-specific
log2RPKM
E17.5
Adult
G
iCON
iDKO
iDKO Tomato-
Merge
RFP
COX IV

Supplemental Figure 9. Adult-stage control of synapse, axon, and mitochondrial genes, Related to Figure 7.

A. Adult stage-specific DEGs determined by comparison of E17.5 Pet1 and Lmx1b DEGs to 1 month post-TAM Pet1 and Lmx1b DEGs.

B. Biological Process GO term enrichment of 1 month post-TAM adult stage-specific iLKO down DEGs.

C. Biological Process GO term enrichment of 1 month post-TAM continuously controlled iLKO down DEGs.

D. Number of iLKO (blue) or iDKO (green) down DEGs bound by Lmx1b and co-bound by Lmx1b and Pet1 within ± 5kb of TSSs.

E. Upper, Genome browser views of Lmx1b and Pet1 co-occupancy (reads in blue) at continuously controlled iLKO and iDKO DEG promoters, defined by enrichment of H3K4me3 and H3K27ac (reads in purple). Lower, bar plots of continuously controlled DEGs; *FDR≤0.05.

F. Violin plots of showing upregulation of adult stage-specific genes. (Wilcoxon rank sum test, ***p≤0.001, ****p≤0.0001).

G. Images show mitochondrial fragmentation (COX IV) only in TdTomato+ iDKO cell bodies. Scale bar: 1µm.