Treadmill workout activates PPARα in the hippocampus to upregulate ADAM10, decrease plaques and improve cognitive functions in 5XFAD mouse model of Alzheimer's disease

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Running title: Treadmill protects the hippocampus via PPARα

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Supplemental results

Figure S1. Upregulation of ADAM10 in the DG of Tg mice by treadmill exercise. Six-month-old Tg mice (n=6/group) were allowed to gently run on treadmill. After 2 months of treadmill exercise, DG sections were DAB-immunostained for ADAM10 (A). The density of cells expressing ADAM10 was quantified in dorsal (B), ventral (C) and DG (D) by using the Image J. A total of 24 cells (4 cells per section; one section per mouse; six mice per group) were used for Image J quantification. Statistical analyzes was conducted by using One-way ANOVA followed by Tukey's multiple comparison tests. ***$p<0.001$.

Figure S2. Comparison of hippocampal volumes from the brain of non-Tg mice, Tg mice and Tg mice with treadmill running exercise. A) Photograph shows the coronal section containing stereology module grid to measure the volume of hippocampus using the Stereo Investigator software (MicroBrightfield) driving a motorized stage microscope. B) Hippocampal volumes (mm^3) of different groups of mice (n=6 per group). Results were statistically analyzed by One-way ANOVA followed by Tukey's multiple comparison tests. ***$p<0.001$.

Figure S3. Treadmill exercise attenuates the burden of Aβ in the hippocampus of Tg-mice. Six to seven-month old Tg-mice (n=6/group) were allowed to perform the treadmill exercise. Using the 6E10 monoclonal antibody, the levels of Aβ proteins were examined in hippocampal homogenates of mice by the Western Blot (A). Actin was used as the loading control. All the protein bands were scanned and densitometric analysis representing mean±SD for Aβ levels are relative to non-Tg controls. (B, C) Quantification of relative Aβ level and CTF-β level in protein bands indicates - ***$p<0.001(=2.3700x10^{-5})$ vs non-Tg mice; ***$p<0.001(=1.4300x10^{-5})$ vs Tg-mice with treadmill exercise and ***$p<0.001(=0.0070)$ vs non-Tg mice; ***$p<0.001(=0.0032x10^{-5})$ vs Tg-mice with treadmill exercise. (D, E) Hippocampal sections were double labeled using

Thio-S and Aβ 6E10 antibody for demonstrating the Aβ pathology in cortex and hippocampus region of mice with and without treadmill exercise. Results are mean ± SD of six per group. All the quantification of Aβ plaques was performed using the Image J. Statistical analysis were conducted by using One-way ANOVA followed by Tukey's multiple comparison tests. Thio-S positive plaque in hippocampus and cortex region were further characterized for (F) the total area fraction (Thio-S area as a percentage of total hippocampal area) - ***p<0.001(=1.8100x10^{-18}) vs non-Tg mice and ***p<0.001(=8.9453x10^{-9}) vs Tg-mice with treadmill exercise; (G) the plaque count - ***p<0.001 (=1.9361x10^{-26}) vs non-Tg mice and ***p<0.001 (=2.0106x10^{-11}) vs Tg-mice with treadmill exercise; (H) the average plaque size – p***0.001 (=4.6257 x10^{-14}) vs non-Tg mice and ***p<0.001 (=9.9493x10^{-7}) vs Tg-mice with treadmill exercise. ns – Non-significant.

Figure S4. Treadmill exercise upregulates the expression of PPARα in cortex and hippocampus of Tg-mice. Tg-mice (6-7 month old), were initially trained to perform the treadmill exercise. Representative images of immunofluorescence staining showing NeuN (green) and PPARα (red) in (A) cortex and (B) hippocampal CA1 region of mice. DAPI (blue) was used to stain nuclei. (C) Higher magnification of images shows significant expression of PPARα (yellow) in center of NeuN+ cells and (D) Mean fluorescence intensity (MFI) analysis of PPARα using the Image J indicates - ***p<0.001 (=3.4162x10^{-7}) vs non-Tg mice; p<0.001 (=01.1103x10^{-5}) vs Tg-mice after treadmill exercise. (E) Using the PPARα monoclonal antibody, relative level of PPARα were also examined in hippocampal homogenates of mice by Western Blot. Statistical analysis was conducted by using One-way ANOVA followed by Tukey's multiple comparison tests. (F) Quantification of relative PPARα level in protein bands indicates - ***p<0.001 (=5.1400x10^{-5}) vs non-Tg mice; ***p<0.001(=0.00015) vs Tg-mice after treadmill exercise. ns – Non-significant.

Figure S5. Treadmill exercise attenuates Aβ plaque pathology in Tg-mice via the PPARα pathway. Six to seven month old Tg-mice and $Tg^{\Delta PPAR\alpha}$ mice (n=6/group) were allowed to perform the treadmill exercise. Campbell-Switzer Silver staining of hippocampal sections performed for demonstrating the Aβ pathology in cortex and hippocampal region of Tg-mice and $Tg^{\Delta PPAR\alpha}$ mice with and without treadmill exercise (A). Aβ plaque pathology characterized for number of plaques (B), density of plaques (C) and average size of plaques (D). Results are mean±SD of six per group. All the quantification of Aβ plaques performed using the Image J. Statistical analysis were conducted by using One-way ANOVA followed by Tukey's multiple comparison tests. The number of plaques in Cortex - ***p<0.001 (=$1.4908x10^{-5}$) vs Non-Tg mice; ***p<0.001 (=0.0002) vs Tg-mice after treadmill exercise; ns (=0.8144) vs $Tg^{\Delta PPAR\alpha}$ mice after treadmill exercise and in hippocampus - ***p<0.001(=$5.4492x10^{-5}$) vs Non-Tg mice; ***p<0.001 (=0.0018) vs Tg-mice after treadmill exercise; ns (=0.9954) vs $Tg^{\Delta PPAR\alpha}$ mice after treadmill exercise. The size of plaques - ***p<0.001 (=$3.7900x10^{-39}$) vs non-Tg mice; ***p<0.001 (=$1.0900 x10^{-23}$) vs Tg-mice after treadmill exercise; ns (=0.2829) vs $Tg^{\Delta PPAR\alpha}$ mice after treadmill exercise and the density of plaques - ***p<0.001(=$1.6789x10^{-15}$) vs non-Tg mice; ***p<0.001 (=$1.3098x10^{-11}$) vs Tg-mice after treadmill exercise; ns (=0.7856) vs $Tg^{\Delta PPAR\alpha}$ mice after treadmill exercise. ns – Non-significant.

Figure S6. Treadmill exercise attenuates the memory deficits in Tg-mice via the PPARα pathway. Six to seven-month old Tg-mice and $Tg^{\Delta PPAR\alpha}$ mice (n=6/group) were allowed to perform running exercise on the rotating treadmill. Following the treadmill exercise, behavioral tests such as Barnes maze, Novel-object recognition test and T-maze performed for assessing the memory functions of Tg-mice and $Tg^{\Delta PPAR\alpha}$ mice. Barnes maze test showing (A) representative heat maps, (B) latency to the goal box - ***p<0.001 (=$1.050x10^{-6}$) vs Non-Tg mice; ***p<0.001

(=4.1200X 10^{-7}) vs Tg-mice with treadmill exercise; ns (=0.4404) vs $Tg^{\Delta PPAR\alpha}$ mice with treadmill exercise, (C) Number of errors made - ***p<0.001 (=4.2660x10^{-6}) vs Non-Tg mice; ***p<0.001 (=7.8300 X10^{-5}) vs Tg-mice with treadmill exercise; ns (=0.2986) vs $Tg^{\Delta PPAR\alpha}$ mice with treadmill exercise. Context-dependent hippocampal behavior was analyzed using the T-maze test showing (D) positive turns - ***p<0.001(=1.233x10^{-5}) vs non-Tg mice; ***p<0.001 (=1.0081X10^{-7}) vs Tg-mice with treadmill exercise; ns (=0.6189) vs $Tg^{\Delta PPAR\alpha}$ mice with treadmill exercise and (E) negative turns -***p<0.001(=1.0021x10^{-7}) vs non-Tg mice; ***p<0.001 (=1.2165X10^{-3}) vs Tg-mice with treadmill exercise; ns (=0.6019) vs $Tg^{\Delta PPAR\alpha}$ mice with treadmill exercise. Novel-object recognition test showing (F) representative heat maps, (G) object exploration time - ***p<0.001 (=1.2128x10^{-9}) vs non-Tg mice; ***p<0.001 (=5.8675X10^{-7}) vs Tg-mice with treadmill exercise; ns (=0.8433) vs $Tg^{\Delta PPAR\alpha}$ mice with treadmill exercise. (H) Open field test demonstrating the velocity behavior in general locomotor activity of mice indicates - ns (=0.1333) vs non-Tg mice; ns (=0.3011) vs Tg-mice with treadmill exercise; ns (=0.9398) vs $Tg^{\Delta PPAR\alpha}$ mice with treadmill exercise. Results are mean ± SD of six per group. Statistical analyzes was conducted by using one-way ANOVA followed by Tukey's multiple comparison tests. ns – Non-significant.

Figure S7. Treadmill exercise did not alter the PPARβ expression in cortex and hippocampus region of Tg- mice. Six- to seven-month-old Tg-mice (n=6/group) were allowed to perform exercise on rotating treadmill. Representative images of immunofluorescence staining showing NeuN (green) and PPARβ (red) in (A) cortex and (B) hippocampal CA1 region of mice with and without treadmill exercise. DAPI (blue) was used to stain nuclei. Using the PPARβ monoclonal antibody, PPARβ levels was also examined in hippocampal homogenates of mice by the Western Blot (C). Actin was used as the loading control. All the protein bands were scanned and densitometric analysis representing mean ± SD for PPARβ levels relative to non-Tg controls. (D)

MFI analysis of PPARβ was performed using the Image J shows – ns (=0.2789) vs non-Tg mice; ns (=0.9739) vs Tg-mice after treadmill exercise. (E) Quantification of relative PPARβ level in protein bands indicates – ns (=0.3287) vs non-Tg mice; ns (0.9440) vs Tg-mice after treadmill exercise. Results are mean ± SD of six per group. Statistical analysis was conducted by using One-way ANOVA followed by Tukey's multiple comparison tests. ns – Non-significant.

Figure S8. Generation of 5XFAD mice lacking PPARβ ($Tg^{\Delta PPAR\beta}$). Representative PCR of PPARβ, PSEN1 and APP transgene DNA expression in 5XFAD (Tg), $PPAR\beta^{-/-}$ and $Tg^{\Delta PPAR\beta}$ mice.

Figure S9. Treadmill exercise results in upregulation of ADAM10 in Tg-mice independent of PPARβ. After the treadmill exercise, Tg and $Tg^{\Delta PPAR\beta}$ mice sacrificed for monitoring the level of *p*ADAM10 and mADAM10 in hippocampal homogenates by Western Blot using the monoclonal ADAM10 antibody (A). Actin was used as the loading control. Bands were scanned and densitometric analysis for *p*ADAM10 and *m*ADAM10 levels relative to Non-Tg controls performed using the NIH Image J Software. Results are mean ± SD of six per group. Statistical analysis was conducted by using One-way ANOVA followed by Tukey's multiple comparison tests. (B, C) Quantification of relative *p*ADAM10 and *m*ADAM10 level in protein bands are mean±SD of six per group indicates - for *p*ADAM10 – $p<0.001$ (=0.0014) vs Non-Tg mice; $p<0.001$ (=0.0003) vs Tg-mice after treadmill exercise; ***p (=0.0004) vs $Tg^{\Delta PPAR\beta}$ mice after treadmill exercise and *m*ADAM10 - ***$p<0.001$ (=0.0008) vs non-Tg mice; ***$p<0.001$ (=0.0016) vs Tg-mice after treadmill exercise; ***p (=0.0007) vs $Tg^{\Delta PPAR\beta}$ mice after treadmill exercise. Abbreviations: *p*ADAM10 - proADAM10; *m*ADAM10 – mature ADAM10; ns – Non-significant.

Figure S10. Treadmill exercise attenuates the burden of Aβ in Tg-mice and $Tg^{\Delta PPAR\beta}$ mice. Six to seven-month old Tg-mice and $Tg^{\Delta PPAR\beta}$ mice (n=6/group) were allowed to perform running exercise on rotating treadmill. After treadmill exercise, mice were sacrificed and Diaminobenzidine staining of hippocampal brain sections were performed using the monoclonal 82E1 antibody for demonstrating the Aβ pathology in cortex and hippocampus region of mice with and without treadmill exercise (A). The Aβ plaque pathology was characterized for number of plaques (B), average size of plaques (C) and density of plaques (D). Results are mean ± SD of six per group. All the quantification of Aβ plaques was performed using the Image J. Statistical analysis were conducted by using One-way ANOVA followed by Tukey's multiple comparison tests. The number of plaques in hippocampus region - ***p<0.001 (=$6.2981x10^{-5}$) vs non-Tg mice; ***p<0.001(=0.0008) vs Tg-mice, ***p<0.001 (0.0062) vs $Tg^{\Delta PPAR\beta}$ mice after treadmill exercise. The size of plaques - ***p<0.001 (=$2.574x10^{-4}$) vs Non-Tg mice; ***p<0.001 (=$6.8685x10^{-13}$) vs Tg-mice after treadmill exercise; ***p<0.001 (=$1.1085x10^{-13}$) vs $Tg^{\Delta PPAR\beta}$ mice and density of plaques - ***p<0.001(=$3.9723x10^{-22}$) vs Non-Tg mice; ***p<0.001(=$9.5411x10^{-14}$) vs Tg-mice after treadmill exercise; ***p0.001 (=$9.2455x10^{-23}$) vs $Tg^{\Delta PPAR\beta}$ mice after treadmill exercise. ns – Non-significant.

Figure S11. Treadmill exercise reduces the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in serum and hippocampus of Tg-mice via PPARα, but not PPARβ. After treadmill exercise, ELISA quantification of Aβ1-40 (A, C, E) and Aβ1-42 (B, D, F) was performed in serum (A, B), TBS (C, D) and TBS+Triton X-100 (E, F) extracted hippocampal tissues. Six to seven-month old mice (n=6 per group) were used in two independent experiments. **p<0.01 and ***p<0.001 versus Non-Tg mice; ***p<0.01 and p<0.001 versus Tg-mice with treadmill exercise and **p<0.01 versus $Tg^{\Delta PPAR\beta}$ mice with treadmill exercise by sample t-tests. ns – Non-significant.

Figure S12. Treadmill exercise attenuates the memory deficits in $Tg^{\Delta PPAR\beta}$ mice. Tg-mice and $Tg^{\Delta PPAR\beta}$ mice (6-7-month-old; n=6/group) were allowed to perform running exercise on rotating treadmill. After treadmill exercise, behavioral tests such as Barnes maze, Novel-object recognition test, and T-maze were conducted for assessing the memory functions of Tg-mice and $Tg^{\Delta PPAR\beta}$ mice. (A) Barnes maze test showing representative heat maps, (B) latency to the goal box - ***p<0.001 (=1.051x10^{-6}) vs non-Tg mice; ***p<0.001 (=4.1200X10^{-7}) vs Tg-mice after treadmill exercise; p<0.001 (=1.4812x10^{-5}) vs $Tg^{\Delta PPAR\beta}$ mice after treadmill exercise, (C) Number of errors made - ***p<0.001 (=7.0594x10^{-6}) vs non-Tg mice; ***p<0.001 (=7.8300 X10^{-5}) vs Tg-mice after treadmill exercise; ***p<0.001 (=0.0003) vs $Tg^{\Delta PPAR\beta}$ mice after treadmill exercise. Analysis of context-dependent hippocampal behavior using the T-maze test shows (D) positive turns - ***p<0.001 (=1.5100x10^{-7}) vs non-Tg mice; ***p<0.001 (=1.1800X10^{-6}) vs Tg-mice after treadmill exercise; p<0.001 (=0.0001) vs $Tg^{\Delta PPAR\beta}$ mice with treadmill exercise and (E) negative turns -***p<0.001 (=1.5100x10^{-7}) vs non-Tg mice; ***p<0.001 (=1.0080x10^{-6}) vs Tg-mice with treadmill exercise; p<0.001 (=0.0013) vs $Tg^{\Delta PPAR\beta}$ mice with treadmill exercise. (F) Novel-object recognition test showing representative heat maps, (G) object exploration time - ***p<0.001 (=0.0002) vs non-Tg mice; ***p<0.001 (=0.0016) vs Tg-mice with treadmill exercise; p<0.001 (=0.0012) vs $Tg^{\Delta PPAR\beta}$ mice with treadmill exercise. (H) Open field test demonstrating the velocity in general locomotor behavior of mice indicates - ns (=0.1029) vs non-Tg mice; ns (=0.2982) vs Tg-mice with treadmill exercise; ns (=0.3826) vs $Tg^{\Delta PPAR\beta}$ mice with treadmill exercise. Results are mean ± SD of six per group. Statistical analyzes were performed by using one-way ANOVA followed by Tukey's multiple comparison tests. ns – Non-significant.

Figure S13. Treadmill exercise upregulates the PPARγ expression in cortex and hippocampus region of Tg-mice. Six to seven-month old mice (n=6/group) were allowed to

perform running exercise on rotating treadmill. Representative images of immunofluorescence staining showing NeuN (green) and PPARγ (red) in (A) cortex and (B) hippocampal CA1 region of Tg-mice with and without treadmill exercise. DAPI (blue) was used to stain nuclei. (C) MFI analysis of PPARγ using the Image J was performed in mice after treadmill exercise and compared to non-exercise group of mice – ***p<0.001 (=1.5370x10x10^{-7}) vs Non-Tg mice and ***p<0.001 (=1.4200x10^{-6}) vs Tg-mice with treadmill exercise. (D) Using the PPARγ monoclonal antibody, we examined the level of PPARγ in hippocampal homogenates of mice by the Western Blot. Actin was used as the loading control. Bands were scanned and densitometric analysis representing mean ± SD for PPARγ levels was studied relative to non-Tg controls. Results are mean ± SD of six per group. Statistical analyzes was conducted by using one-way ANOVA followed by Tukey's multiple comparison tests. (E) Quantification of relative PPARα level in protein bands indicates - ***p<0.001 (=5.1400x10^{-5}) vs non-Tg mice; ***p<0.001(=0.0015) vs Tg-mice with treadmill exercise. ns – Non-significant**.**

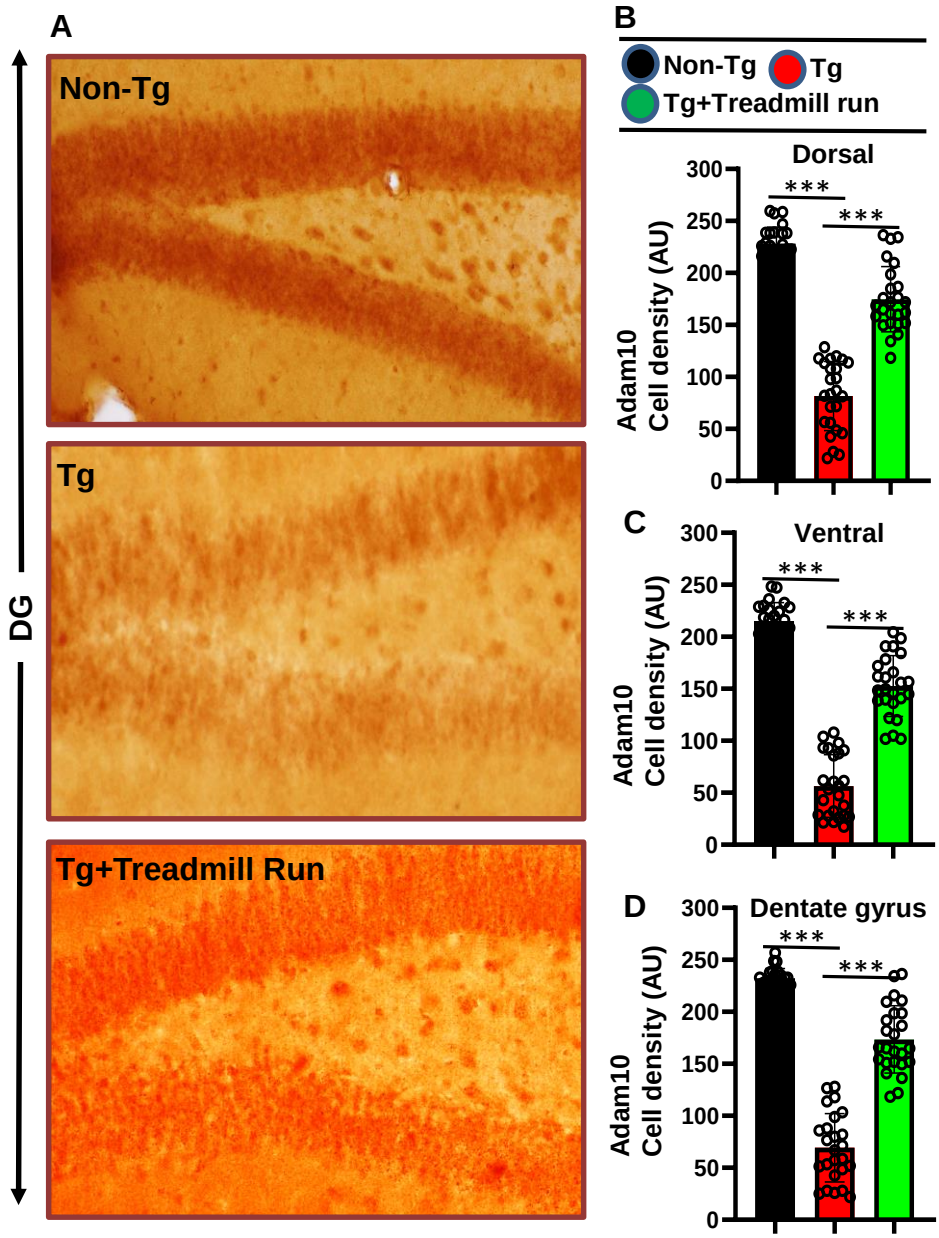
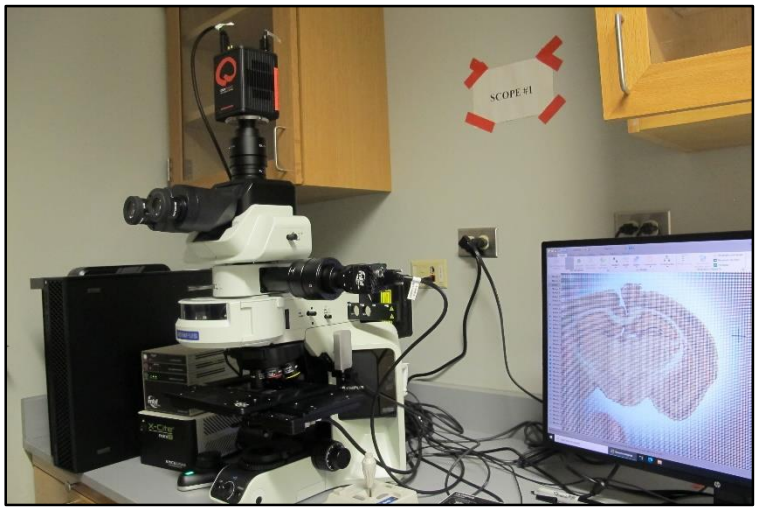

A
Non-Tg
Tg
Tg+Treadmill Run
DG
B
Non-Tg
Tg
Tg+Treadmill run
Dorsal
Adam10 Cell density (AU)
300
250
200
150
100
50
0
***
***
C
Ventral
Adam10 Cell density (AU)
***
***
D
Dentate gyrus
Adam10 Cell density (AU)
***
***

Figure S1

A



B

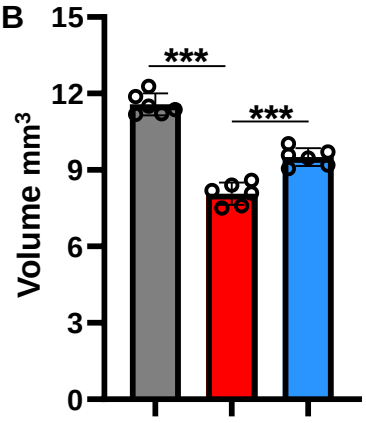


Figure S2

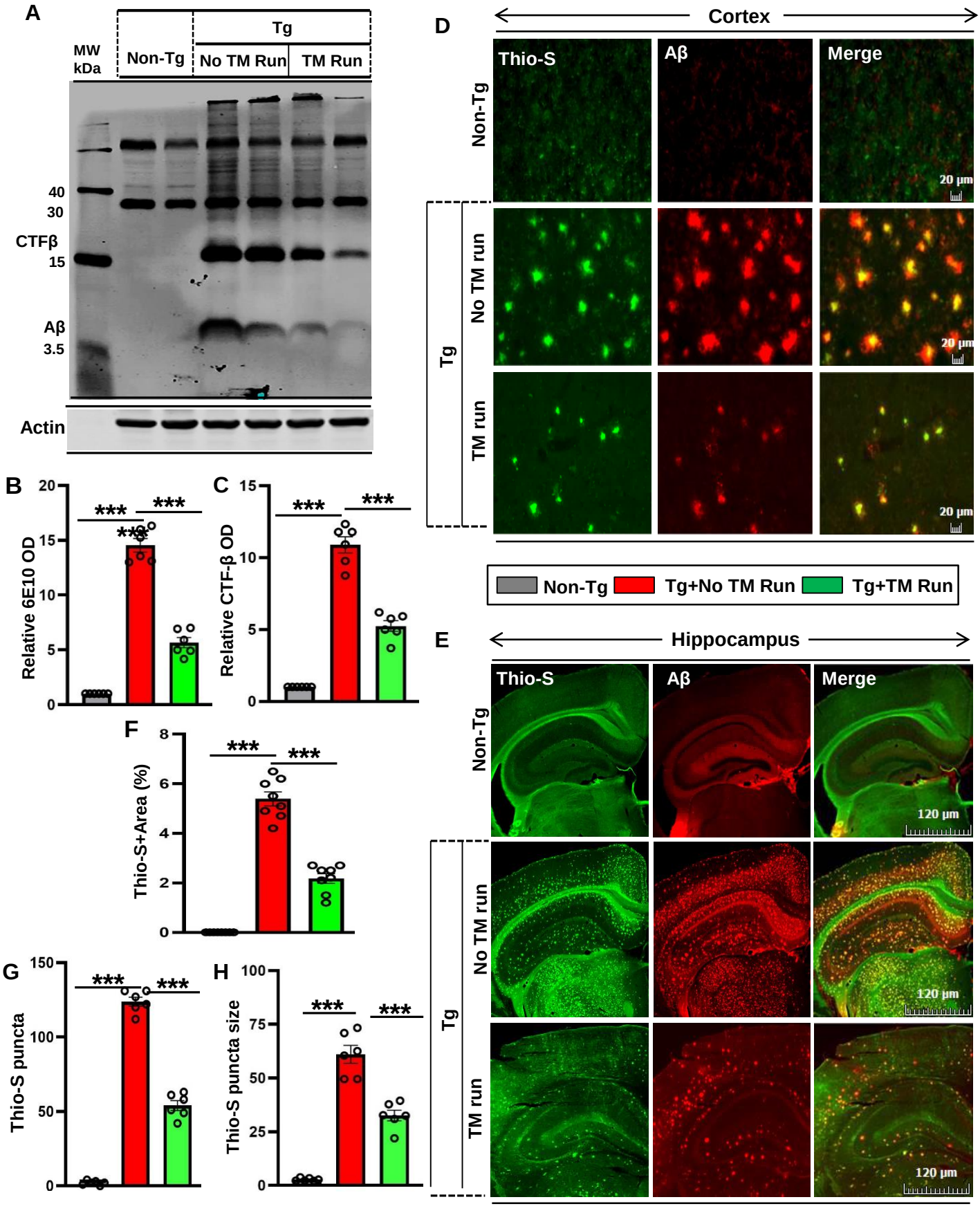

A
MW kDa
Non-Tg
Tg
No TM Run
TM Run
40
30
CTFβ
15
Aβ
3.5
Actin
B
Relative 6E10 OD
C
Relative CTF-β OD
D
Cortex
Thio-S
Aβ
Merge
Non-Tg
Tg
No TM run
TM run
20 μm
Non-Tg
Tg+No TM Run
Tg+TM Run
E
Hippocampus
120 μm
F
Thio-S+Area (%)
G
Thio-S puncta
H
Thio-S puncta size
***

Figure S3

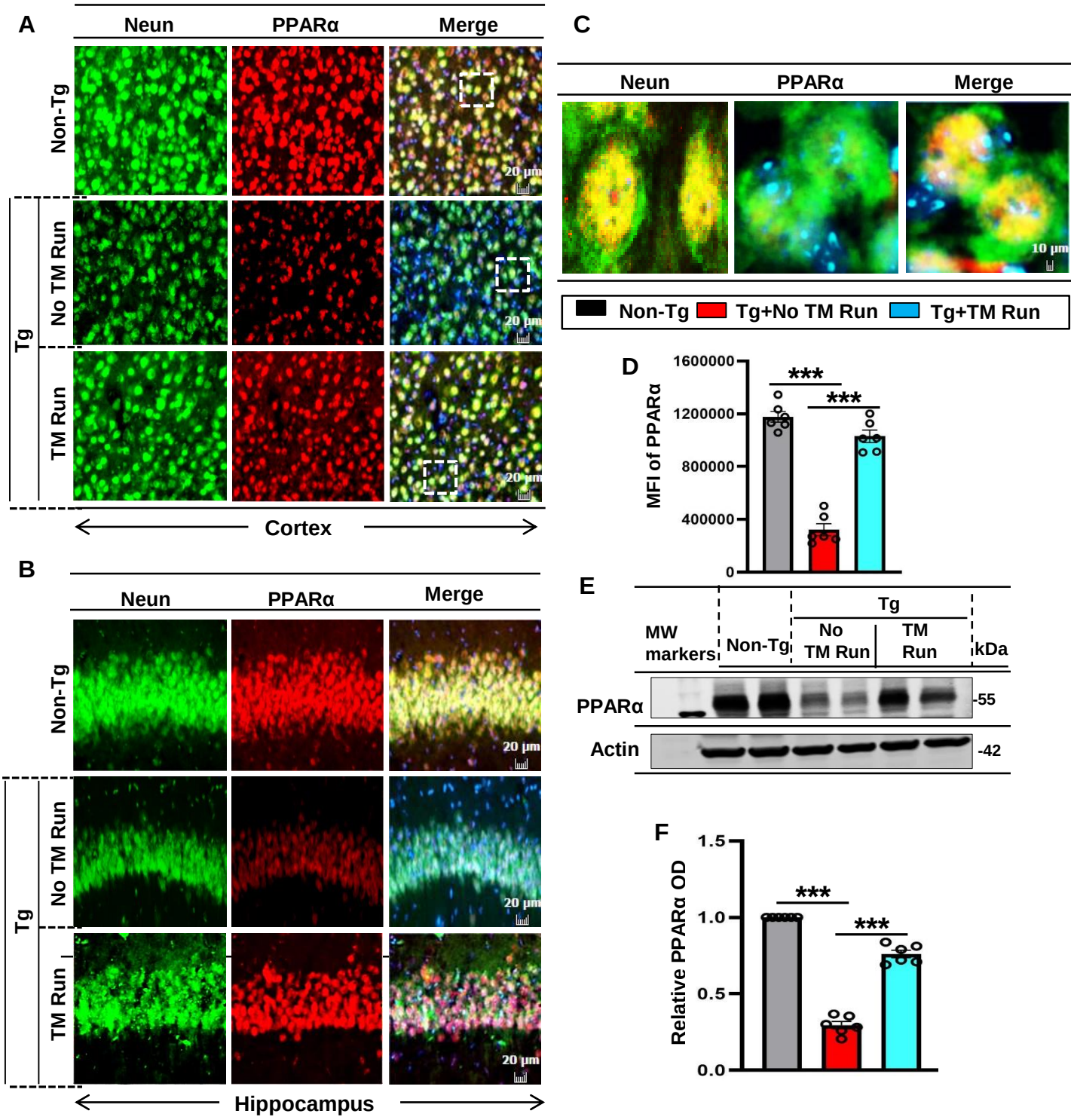

A
Neun
PPARα
Merge
Non-Tg
Tg
No TM Run
TM Run
20 μm
Cortex
B
Hippocampus
C
10 μm
Non-Tg
Tg+No TM Run
Tg+TM Run
D
MFI of PPARα
1600000
1200000
800000
400000
0
***
E
MW markers
kDa
-55
-42
F
Relative PPARα OD
1.5
1.0
0.5
0.0

Figure S4

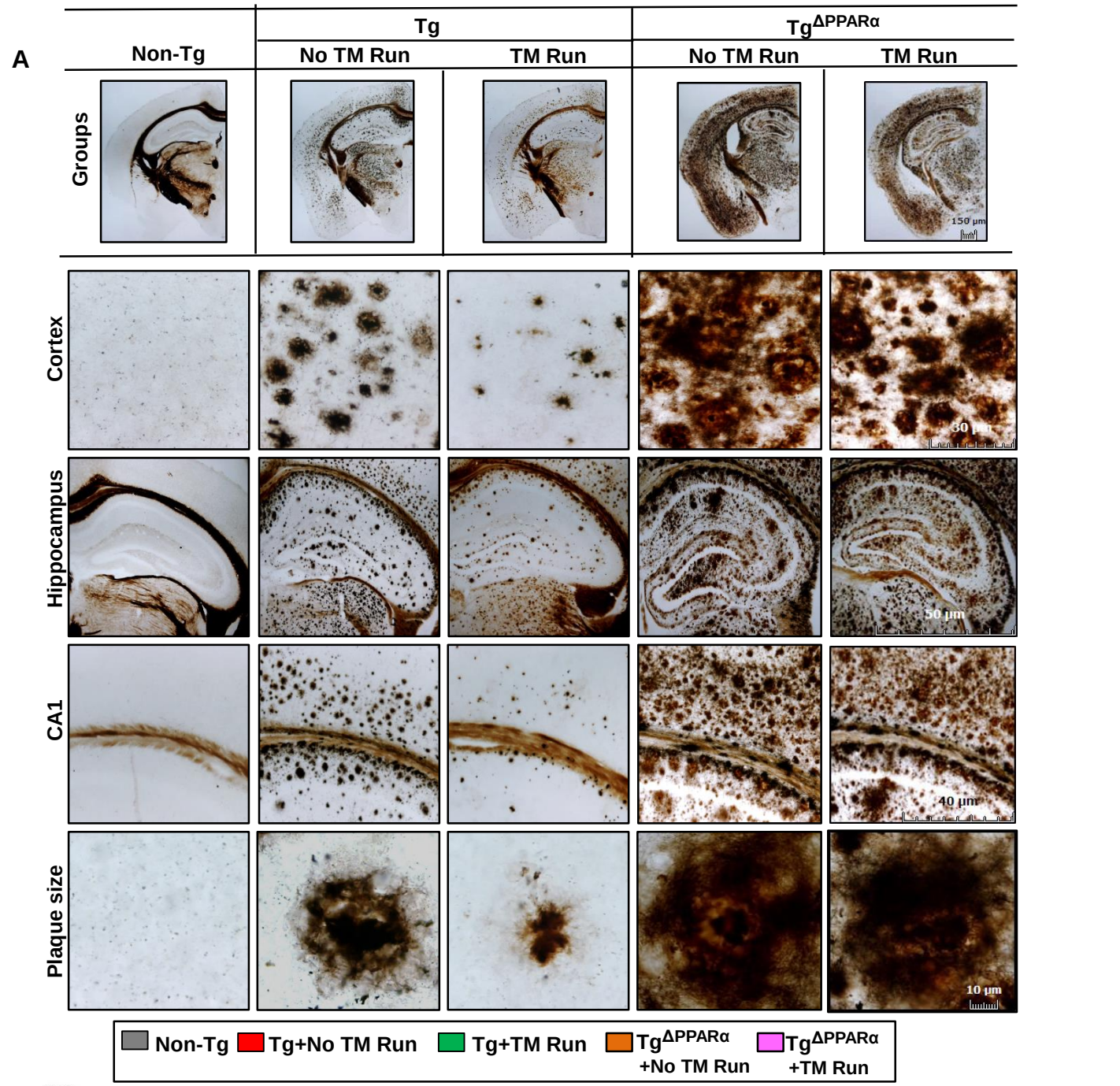

A
Non-Tg
Tg
No TM Run
TM Run
Tg^ΔPPARα
No TM Run
TM Run
Groups
Cortex
Hippocampus
CA1
Plaque size
150 µm
30 µm
50 µm
40 µm
10 µm
Non-Tg
Tg+No TM Run
Tg+TM Run
Tg^ΔPPARα +No TM Run
Tg^ΔPPARα +TM Run
B
Cortex
Hippocampus
Number of plaques/mm²
***
***
ns
C
plaques density (AU)
***
***
ns
D
plaques area /mm²
***
***
ns

Figure S5

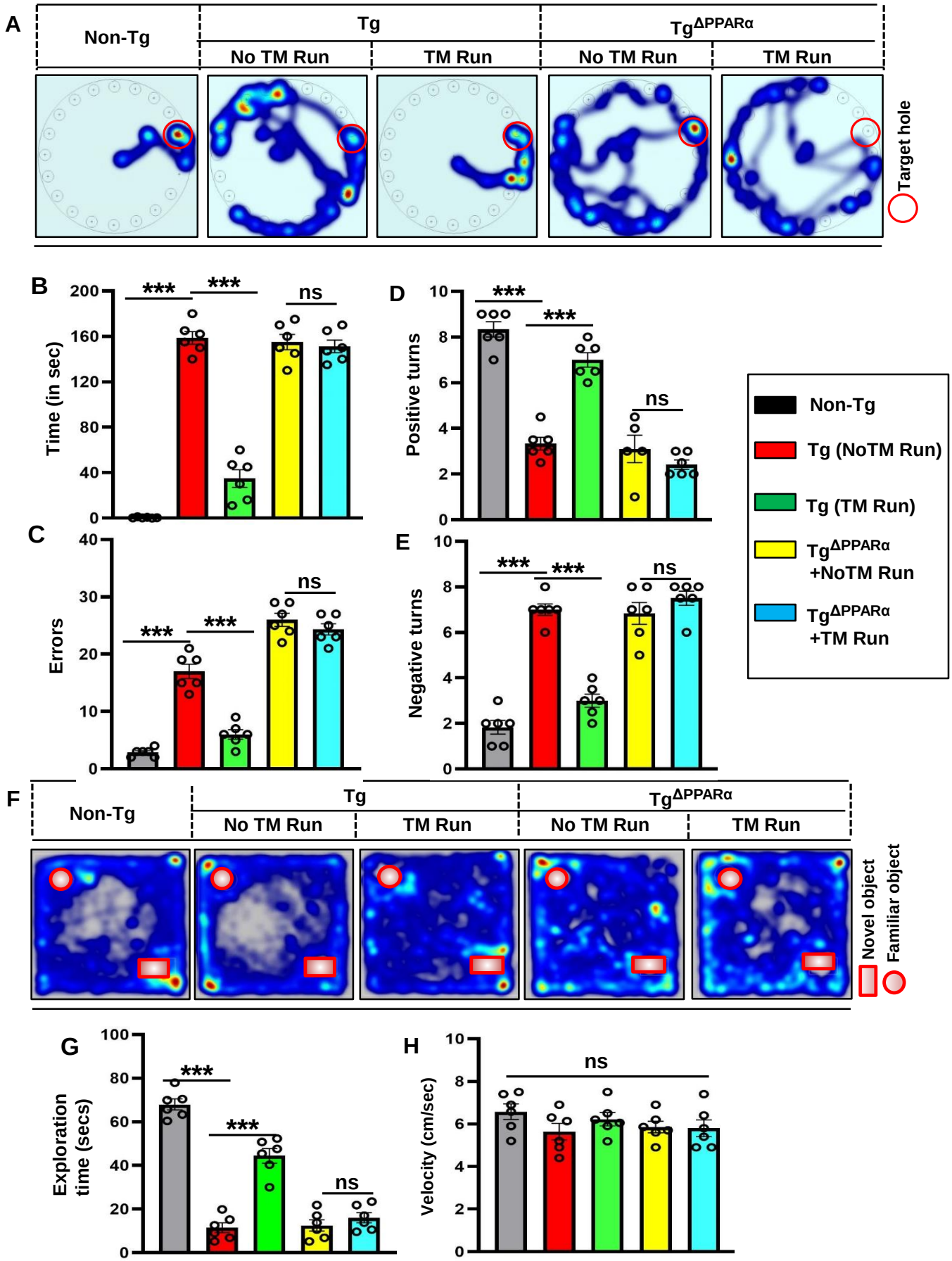

A
Non-Tg
Tg
No TM Run
TM Run
TgΔPPARα
No TM Run
TM Run
Target hole
B
Time (in sec)
***
***
ns
D
Positive turns
***
***
ns
C
Errors
***
***
ns
E
Negative turns
***
***
ns
Non-Tg
Tg (NoTM Run)
Tg (TM Run)
TgΔPPARα +NoTM Run
TgΔPPARα +TM Run
F
Non-Tg
Tg
No TM Run
TM Run
TgΔPPARα
No TM Run
TM Run
Novel object
Familiar object
G
Exploration time (secs)
***
***
ns
H
Velocity (cm/sec)
ns

Figure S6

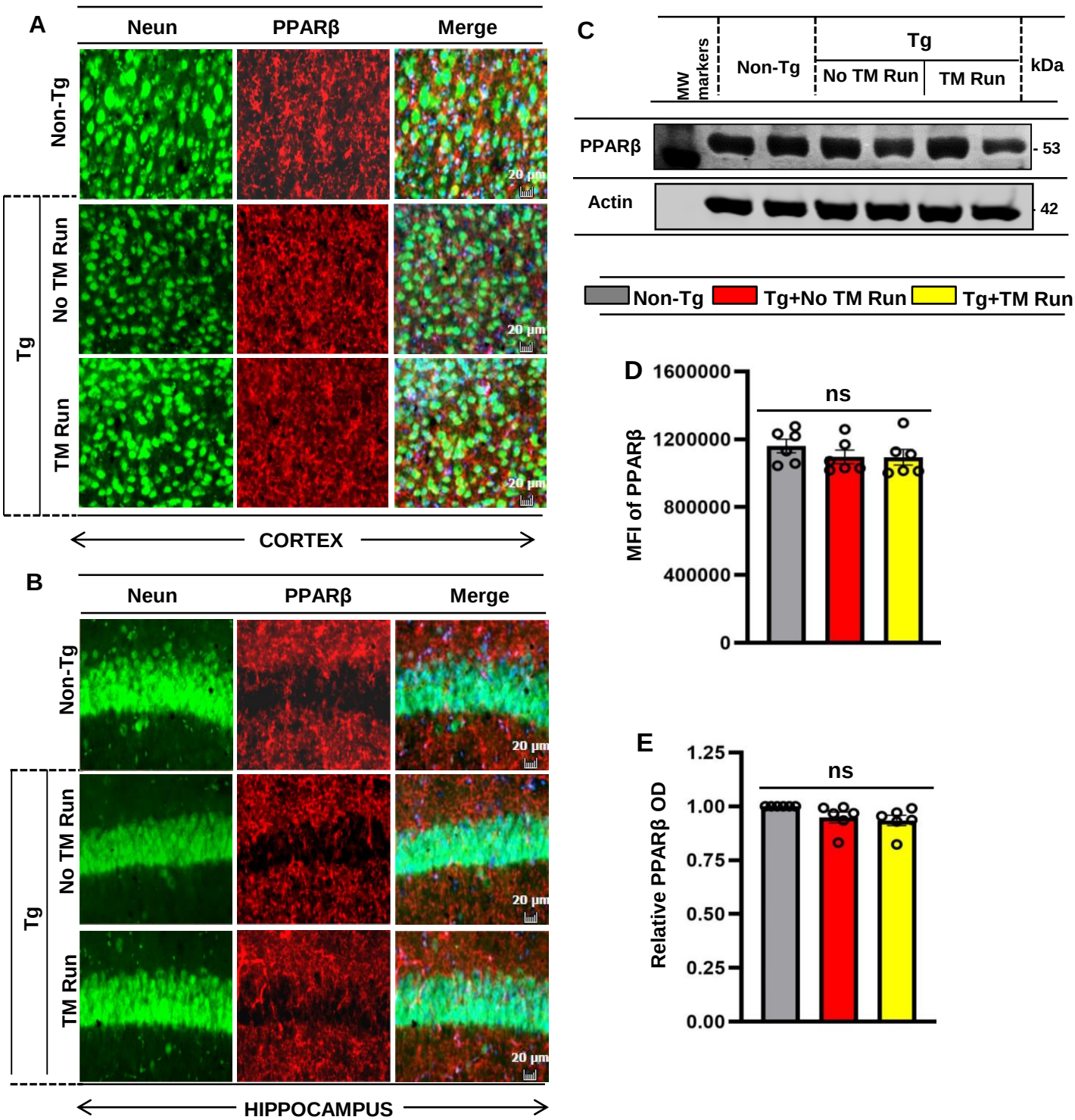

A
Neun
PPARβ
Merge
Non-Tg
Tg
No TM Run
TM Run
20 μm
CORTEX
B
HIPPOCAMPUS
C
MW markers
Non-Tg
Tg
No TM Run
TM Run
kDa
PPARβ
53
Actin
42
Non-Tg
Tg+No TM Run
Tg+TM Run
D
MFI of PPARβ
1600000
1200000
800000
400000
0
ns
E
Relative PPARβ OD
1.25
1.00
0.75
0.50
0.25
0.00
ns

Figure S7

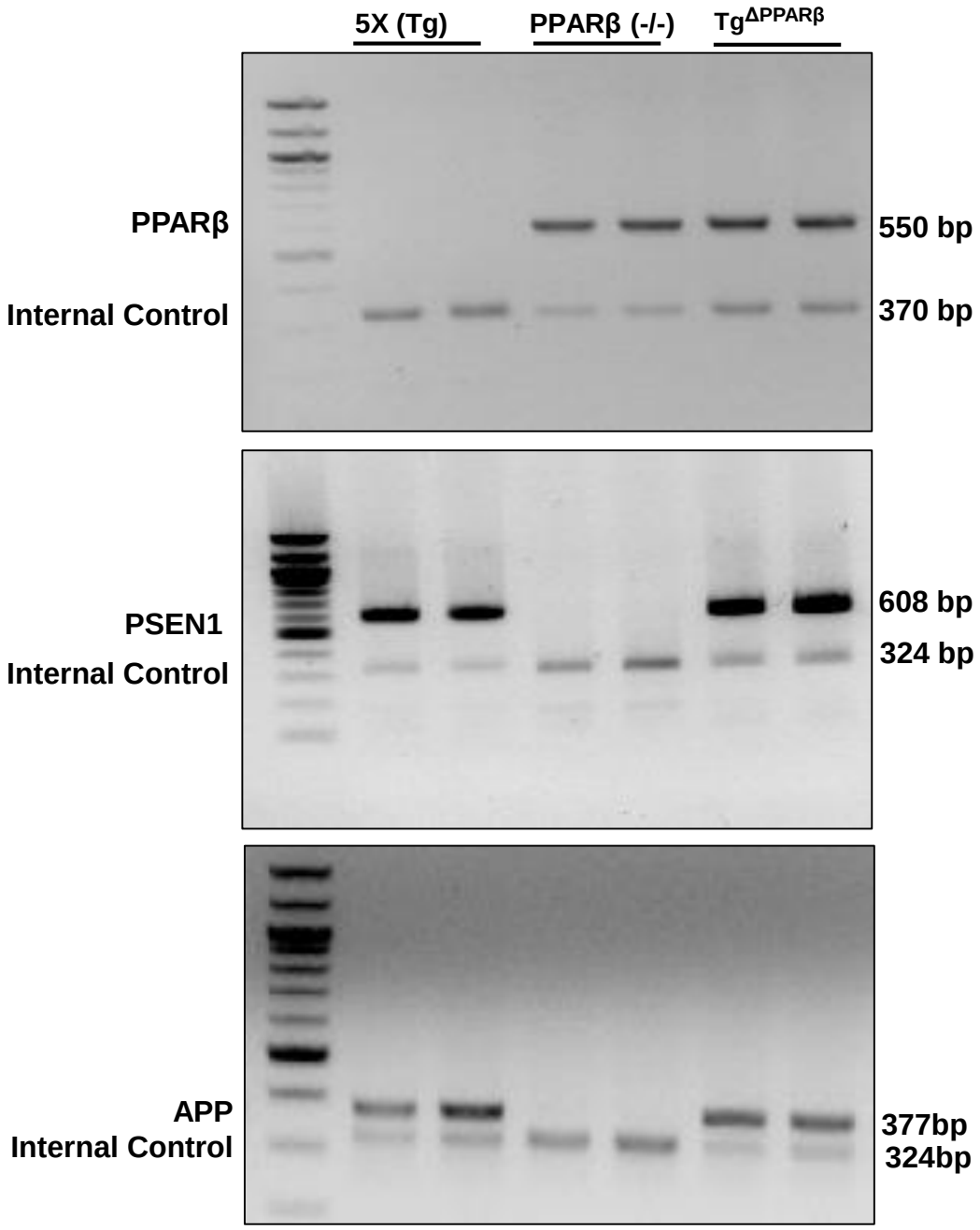

5X (Tg)
PPARβ (-/-)
TgΔPPARβ
PPARβ
Internal Control
550 bp
370 bp
PSEN1
Internal Control
608 bp
324 bp
APP
Internal Control
377bp
324bp

Figure S8

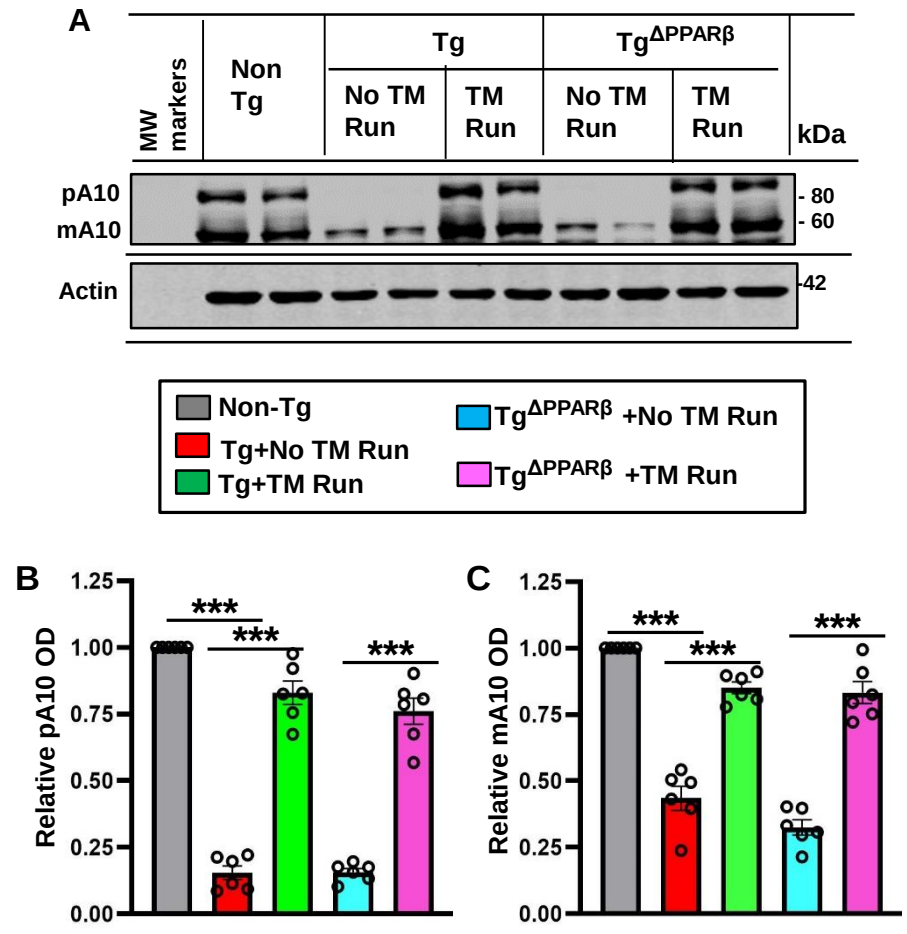

A
MW markers
Non Tg
Tg
No TM Run
TM Run
TgΔPPARβ
No TM Run
TM Run
kDa
pA10
mA10
- 80
- 60
Actin
-42
Non-Tg
Tg+No TM Run
Tg+TM Run
TgΔPPARβ +No TM Run
TgΔPPARβ +TM Run
B
Relative pA10 OD
1.25
1.00
0.75
0.50
0.25
0.00
***
***
***
C
Relative mA10 OD
1.25
1.00
0.75
0.50
0.25
0.00
***
***
***

Figure S9

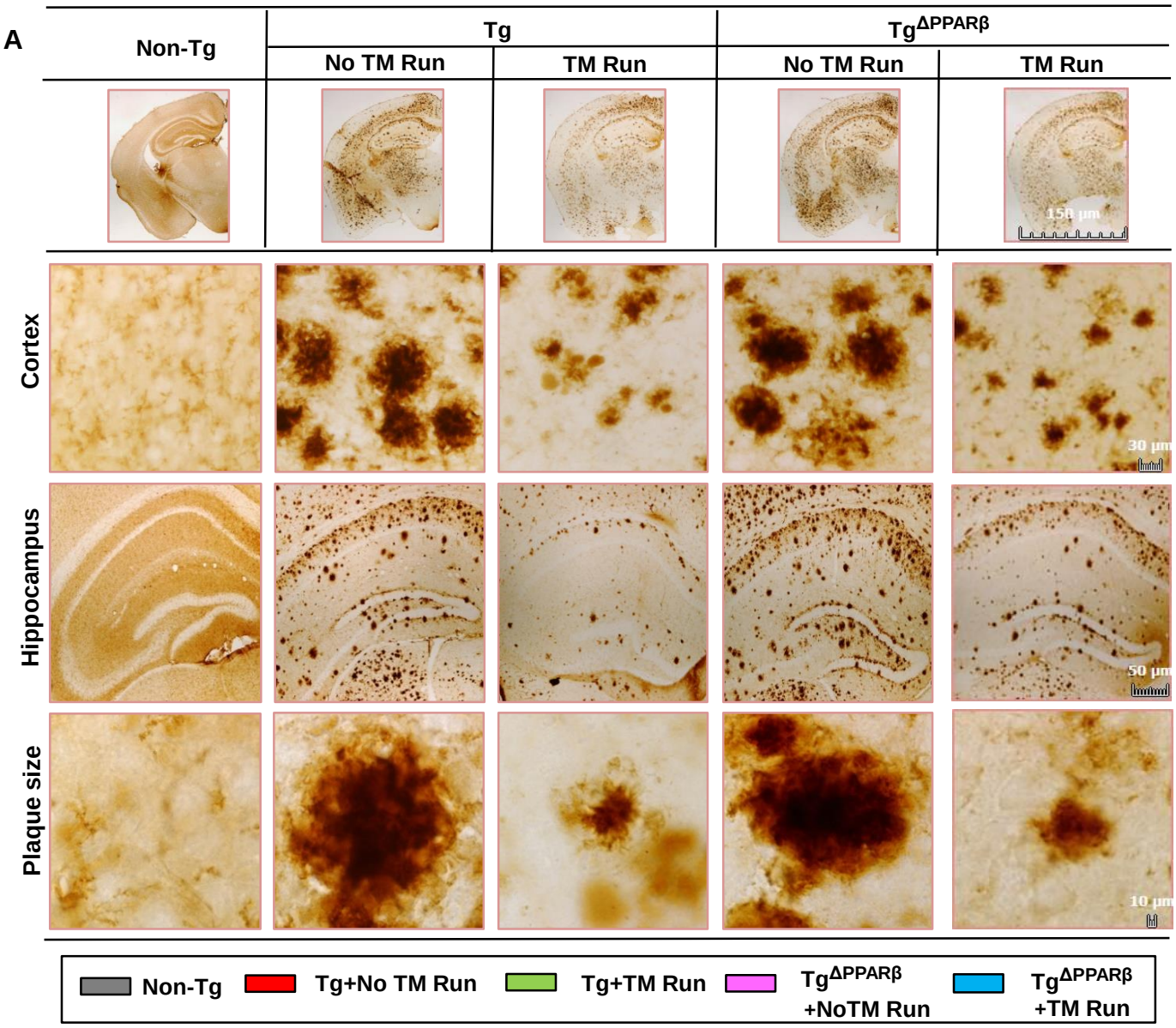

A
Non-Tg
Tg
No TM Run
TM Run
Tg^ΔPPARβ
No TM Run
TM Run
Cortex
Hippocampus
Plaque size
150 µm
30 µm
50 µm
10 µm
Non-Tg
Tg+No TM Run
Tg+TM Run
Tg^ΔPPARβ +NoTM Run
Tg^ΔPPARβ +TM Run
B
No. of plaques/mm²
C
plaques area/mm²
D
plaques density (AU)
***

Figure S10

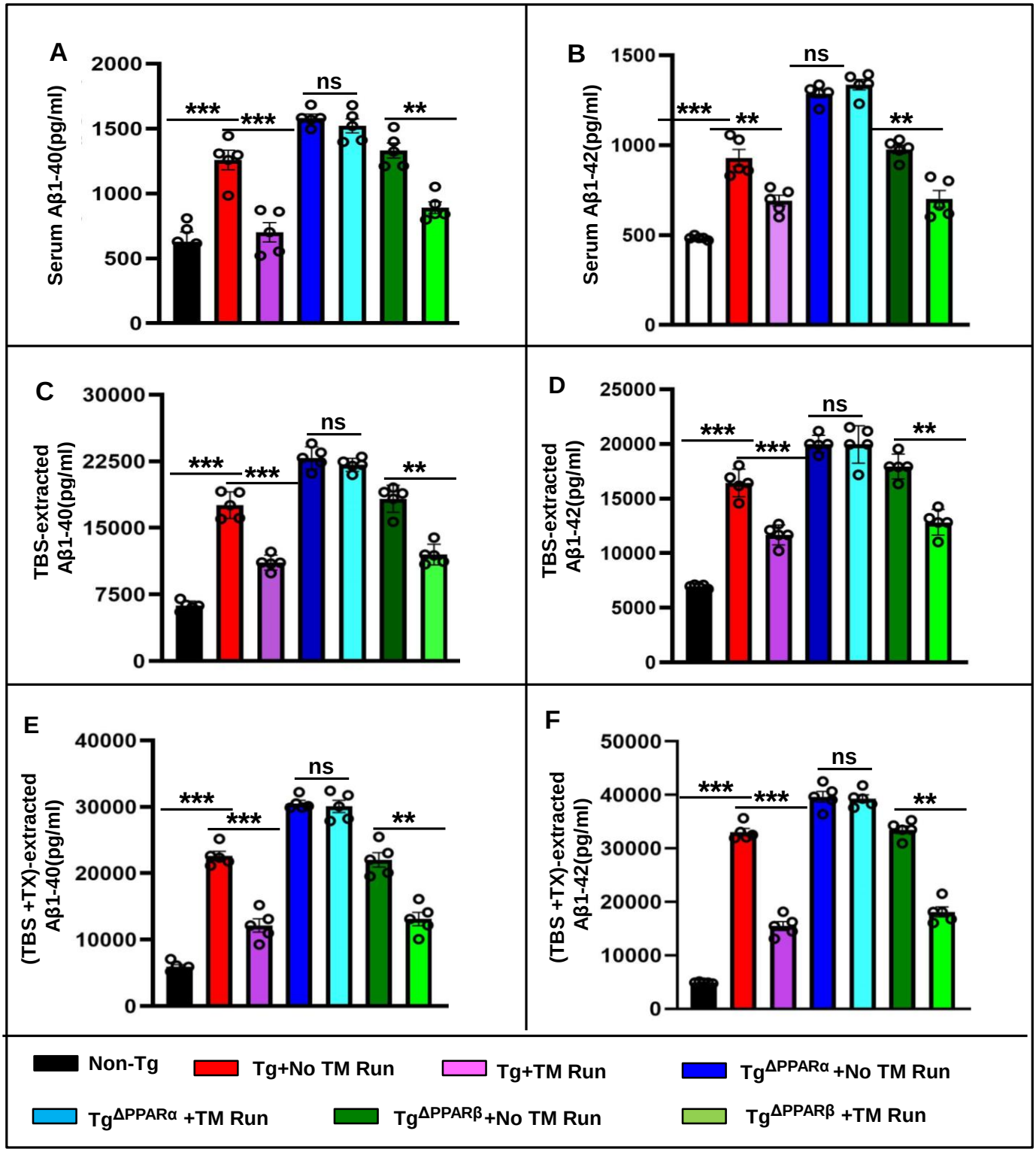

A
Serum Aβ1-40(pg/ml)
2000
1500
1000
500
0
***
***
ns
**
B
Serum Aβ1-42(pg/ml)
1500
1000
500
0
***
**
ns
**
C
TBS-extracted Aβ1-40(pg/ml)
30000
22500
15000
7500
0
***
***
ns
**
D
TBS-extracted Aβ1-42(pg/ml)
25000
20000
15000
10000
5000
0
***
***
ns
**
E
(TBS +TX)-extracted Aβ1-40(pg/ml)
40000
30000
20000
10000
0
***
***
ns
**
F
(TBS +TX)-extracted Aβ1-42(pg/ml)
50000
40000
30000
20000
10000
0
***
***
ns
**
Non-Tg
Tg+No TM Run
Tg+TM Run
Tg^ΔPPARα +No TM Run
Tg^ΔPPARα +TM Run
Tg^ΔPPARβ +No TM Run
Tg^ΔPPARβ +TM Run

Figure S11

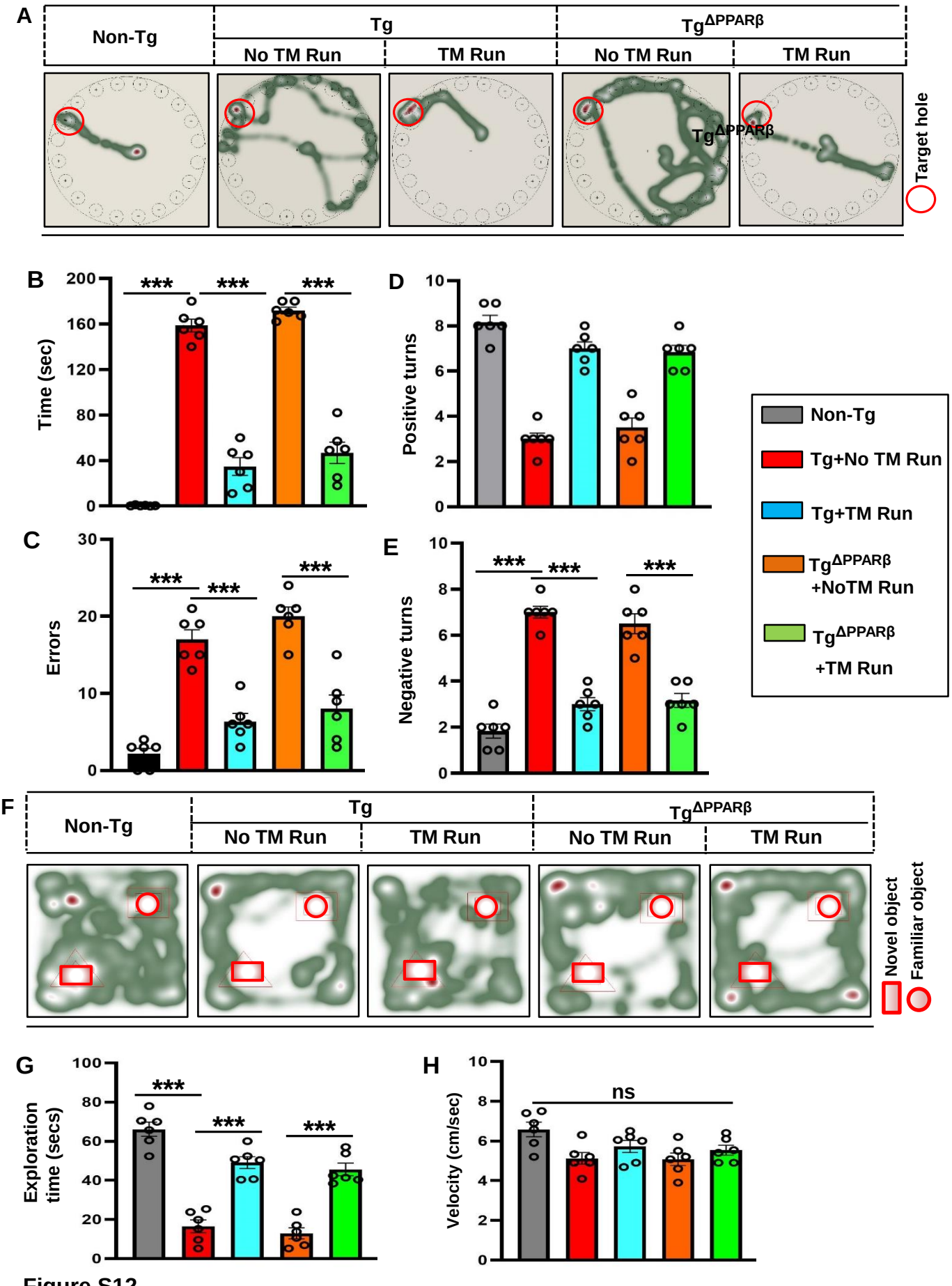

A
Non-Tg
Tg
No TM Run
TM Run
TgΔPPARβ
No TM Run
TM Run
TgΔPPARβ
Target hole
B
Time (sec)
***
***
***
D
Positive turns
C
Errors
E
Negative turns
Non-Tg
Tg+No TM Run
Tg+TM Run
TgΔPPARβ +NoTM Run
TgΔPPARβ +TM Run
F
Non-Tg
Tg
No TM Run
TM Run
TgΔPPARβ
No TM Run
TM Run
Novel object
Familiar object
G
Exploration time (secs)
H
Velocity (cm/sec)
ns

Figure S12

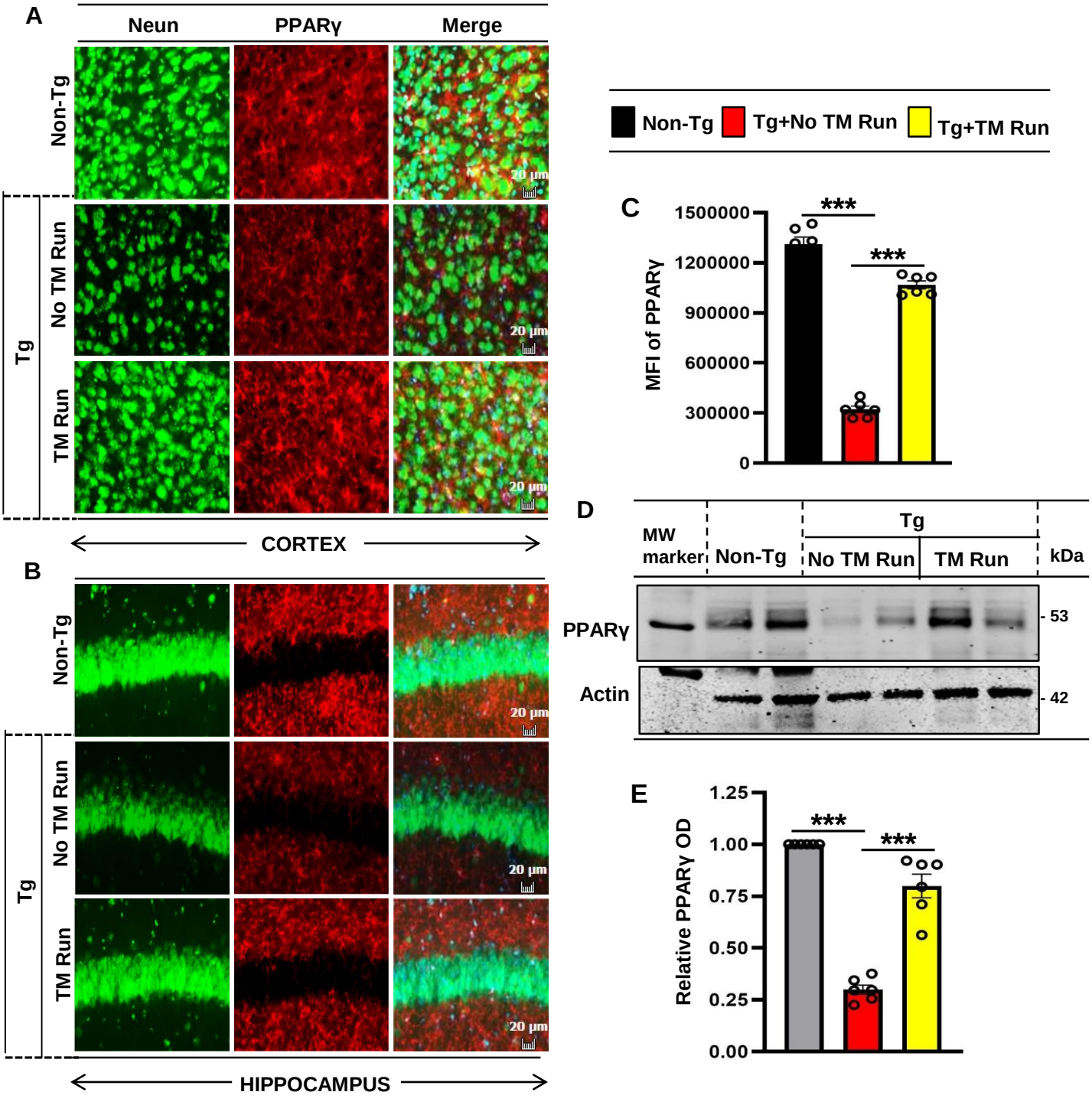

A
Neun
PPARγ
Merge
Non-Tg
Tg
No TM Run
TM Run
20 μm
CORTEX
B
HIPPOCAMPUS
Non-Tg
Tg+No TM Run
Tg+TM Run
C
MFI of PPARγ
1500000
1200000
900000
600000
300000
0
***
D
MW marker
Non-Tg
Tg
No TM Run
TM Run
kDa
PPARγ
Actin
53
42
E
Relative PPARγ OD
1.25
1.00
0.75
0.50
0.25
0.00

Figure S13

Table S1. List of antibodies used

Target and Antibody	Source	Catalog No. and Host	Application/Dilution
Adam10	Millipore	AB19026 (Rabbit)	IHC/1:500; WB/1:1000
Adam 17	Abcam	AB 2051 (Rabbit)	WB/1:1000
Bace1	Invitrogen	PA5-14878 (Rabbit)	WB/1:1000
Psen1	Millipore	AB5308 (Rabbit)	WB/1:1000
Neun	Millipore Invitrogen	NAB377 (Mouse) 702022 (Rabbit)	IHC/1:500 WB/1:500
PPARα	Santa Cruz	sc398394 (Mouse)	IF/1:250; WB/1:250
PPARβ	Santa Cruz	sc74517 (Mouse)	IF/1:200; WB/1:250
PPARγ	Santa Cruz	sc7273 (Mouse)	IF/1:200; WB/1:200
β Amyloid (6E10)	Biolegend	803001 (Mouse)	IF/1:400; WB/1:1000
β Amyloid (82e1)	IBL	103230 (Mouse)	IHC/1:500; WB/1:1000
Campbell Switzer staining	NSA	Stains – NSA	NSA procedure
β Actin	Abcam	ab6276 (Mouse)	WB/1:1000

WB, Western blot; ICC, immunocytochemistry; IHC, immunohistochemistry

Table S2. Details of primers used for genotyping 5XFAD mice lacking PPARβ

PPARβ null:	Forward:	CAGGATGTCCTTCCACAGAGACAG
	Reverse:	TTAGCCACTGCATCATCTGGG
	Neo Primer:	GCAATCCATCTTGTTCAATGGC
5XFAD:	Mutant reverse:	CGGGCCTCTTCGCTATTAC
	Wild type reverse:	TATACAACCTTGGGGGATGG
	Common:	ACCCCCATGTCAGAGTTCCT