# Non-coding RNA *TERC* functions as an ageing-related mitochondrial retrograde signal

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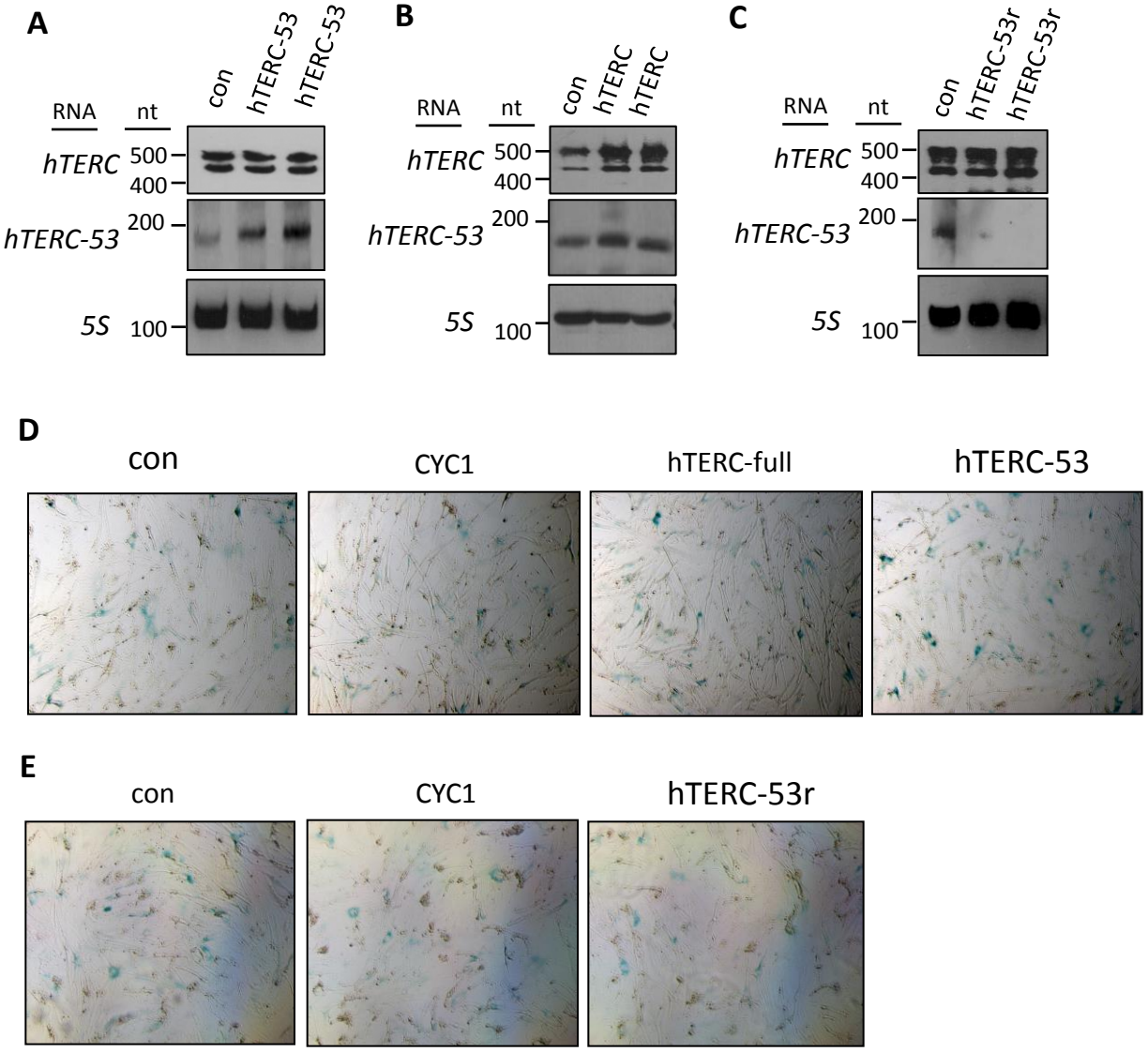



**Figure S1. SA-β-gal staining of 2BS cell lines, related to Figure 1.**
(A) Northern blots of total *hTERC*, *hTERC-53* and *5S* rRNA in HEK cells (con), or HEK cells overexpressing *hTERC-53* (hTERC-53). (B) Northern blots of total *hTERC-53* and *5S* rRNA in HEK cells (con), or HEK cells overexpressing *hTERC* (hTERC). (C) Northern blots of total *hTERC-53* and *5S* rRNA in HEK cells (con), or HEK cells overexpressing *hTERC-53r* (hTERC-53r). (D) 2BS cell lines generated with the empty vector (con), or the vector expressing yeast *CYC1* RNA (CYC1), full length *hTERC* (hTERC-full) or *hTERC-53* (hTERC-53) were grown to 37 PDs, and then stained for SA-β-gal. (E) 2BS cell lines generated with the empty vector (con), or the vector expressing yeast *CYC1* RNA (CYC1) or anti-sense *hTERC-53* (hTERC-53r) were grown to 43 PDs and stained for SA-β-gal.

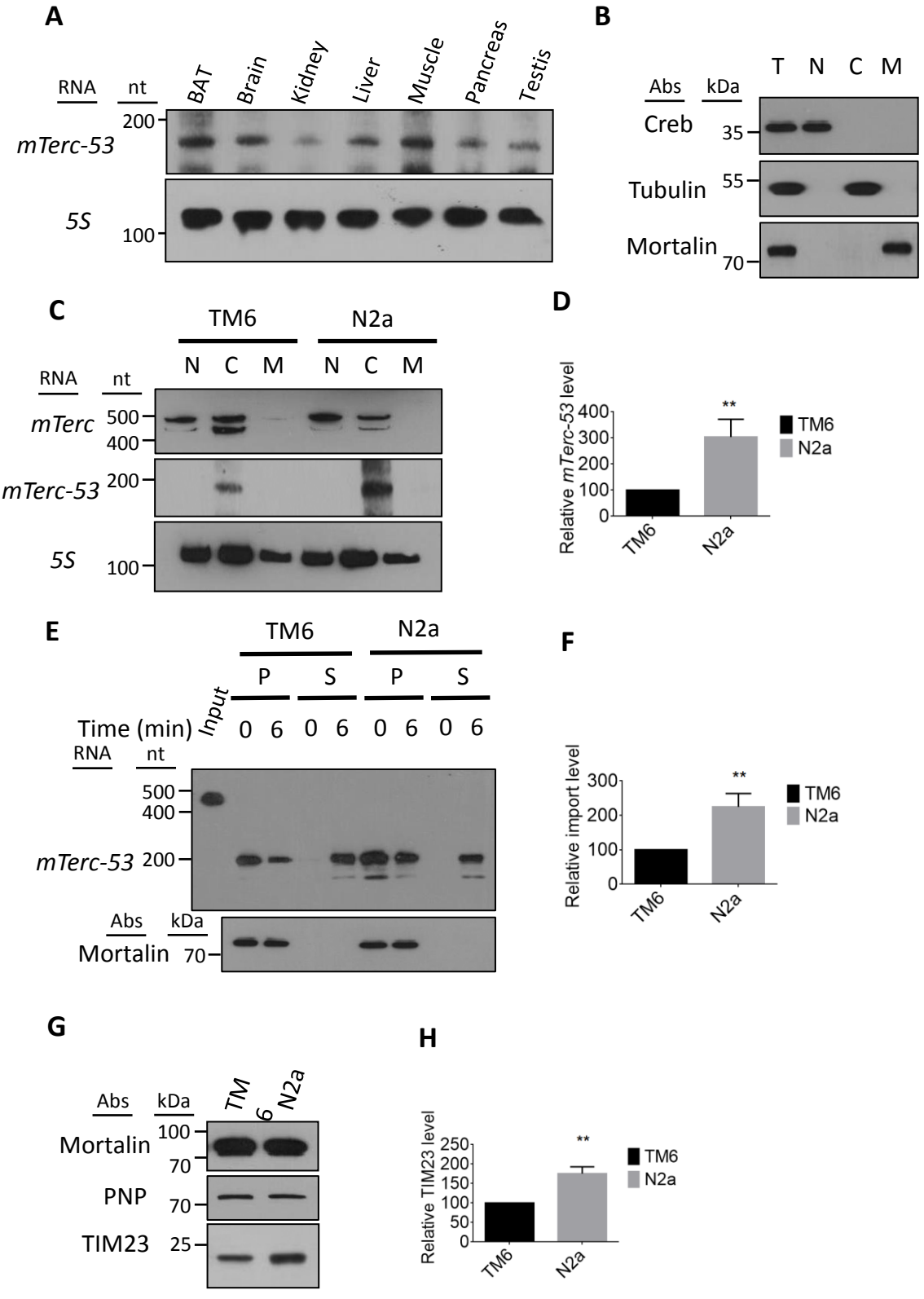

A
RNA
nt
BAT
Brain
Kidney
Liver
Muscle
Pancreas
Testis
200
mTerc-53
5S
100
B
T N C M
Abs
kDa
Creb
35
Tubulin
55
Mortalin
70
C
TM6
N2a
N C M N C M
RNA
nt
mTerc
500
400
mTerc-53
200
5S
100
D
Relative mTerc-53 level
400
300
200
100
0
**
TM6
N2a
E
TM6
N2a
P S P S
Input
Time (min)
0 6 0 6 0 6 0 6
RNA
nt
500
400
mTerc-53
200
Abs
kDa
Mortalin
70
F
Relative import level
300
200
100
0
**
TM6
N2a
G
Abs
kDa
TM6
N2a
Mortalin
100
70
PNP
70
TIM23
25
H
Relative TIM23 level
250
200
150
100
50
0
**
TM6
N2a

**Figure S2. *mTerc-53* levels in different mouse tissues, *mTerc-53* localization in neuronal N2a cells, in vitro import of *mTerc* into N2a mitochondria and export of *mTerc-53*, and N2a mitochondrial protein levels, related to Figure 4.**

(A) Northern blots of cytosolic *mTerc-53* and *5S* rRNA in different mouse tissues. BAT (Brown Adipose Tissue). (B) Immunoblots of different cellular fractions of mouse neuronal cells N2a: total cell lysate (T), the nucleus (N), the cytosol (C) and mitochondria (M). Creb, β-tubulin, and mortalin were used as markers for the nucleus, the cytosol and mitochondria respectively. (C) Northern blots of *mTerc*, *mTerc-53* and *5S* in equal cellular volume of nuclear, cytosolic and mitochondrial fractions in mouse TM6 cells and mouse neuronal N2a cells. (D) Quantification of the relative cytosolic *mTerc-53* level in panel (C) (n = 3). (E) In vitro import of *mTerc* into TM6 or N2a mitochondria and export of *mTerc-53* from the mitochondria; P (Pellet), S (Supernatant). (F) Quantification of the relative import efficiency in panel (E) (n=3). (G) Immunoblot of TM6 and N2a mitochondria. (H) Quantification of the relative TIM23 level in panel (G) (n = 3). Statistical comparisons are performed using unpaired *t*-tests; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Data are presented as mean ± standard error of the mean (s.e.m.).

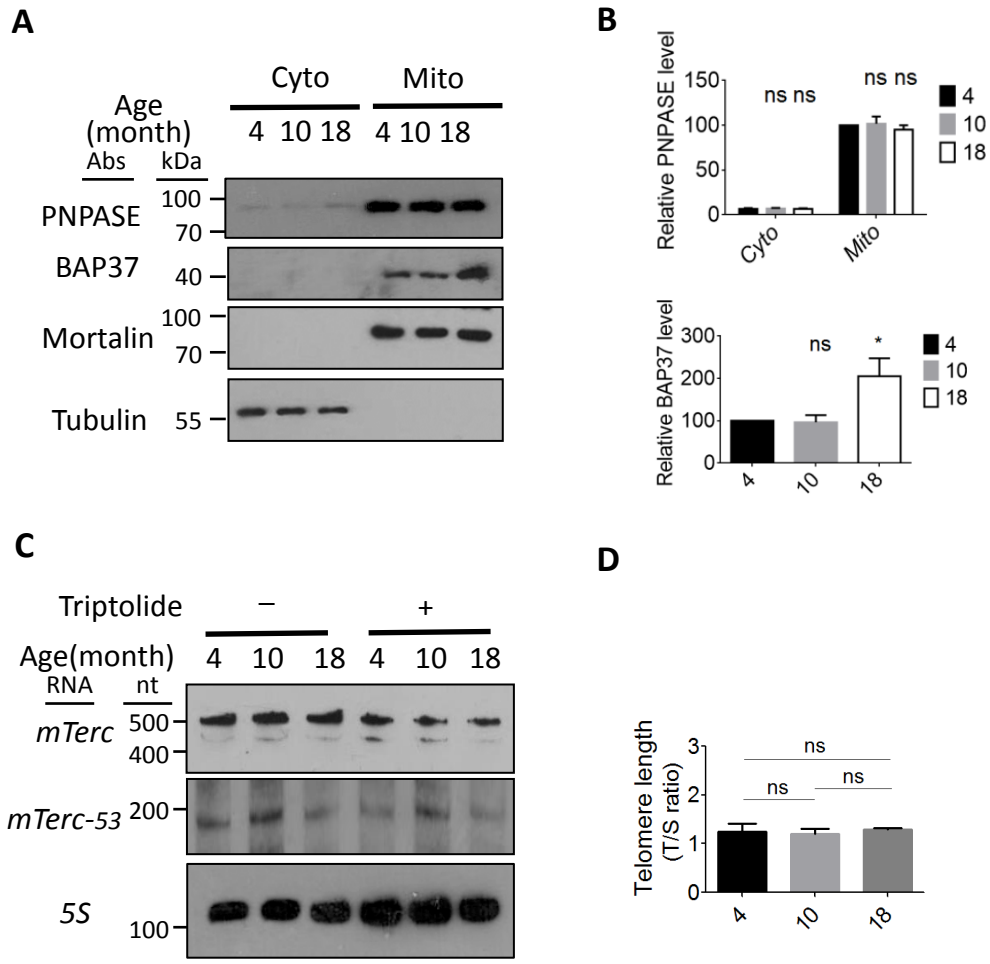



**Figure S3. Protein level changes in the brains of 4 months, 10 months and 18 months old mice, and the effect of triptolide treatment on cytosolic *mTerc* and *mTerc-53* levels, related to Figure 4.**

(A) Immunonlots of the cytosol and mitochondria isolated from the brains of 4 months, 10 months and 18 months old mice. (B) Quantification of the relative PNPASE levels and the mitochondrial BAP37 levels in panel (A) (n = 6). (C) Northern blots of cytosolic *mTerc*, *mTerc-53* and *5S* rRNA in the brain cells of 4 months, 10 months and 18 months old mice with or without triptolide treatment (2 μM for 3 hs). (D) Comparison of the telomere length in the brains of 4 months, 10 months and 18 months old mice. Statistical comparisons are performed using unpaired *t*-tests; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Data are presented as mean ± standard error of the mean (s.e.m.).

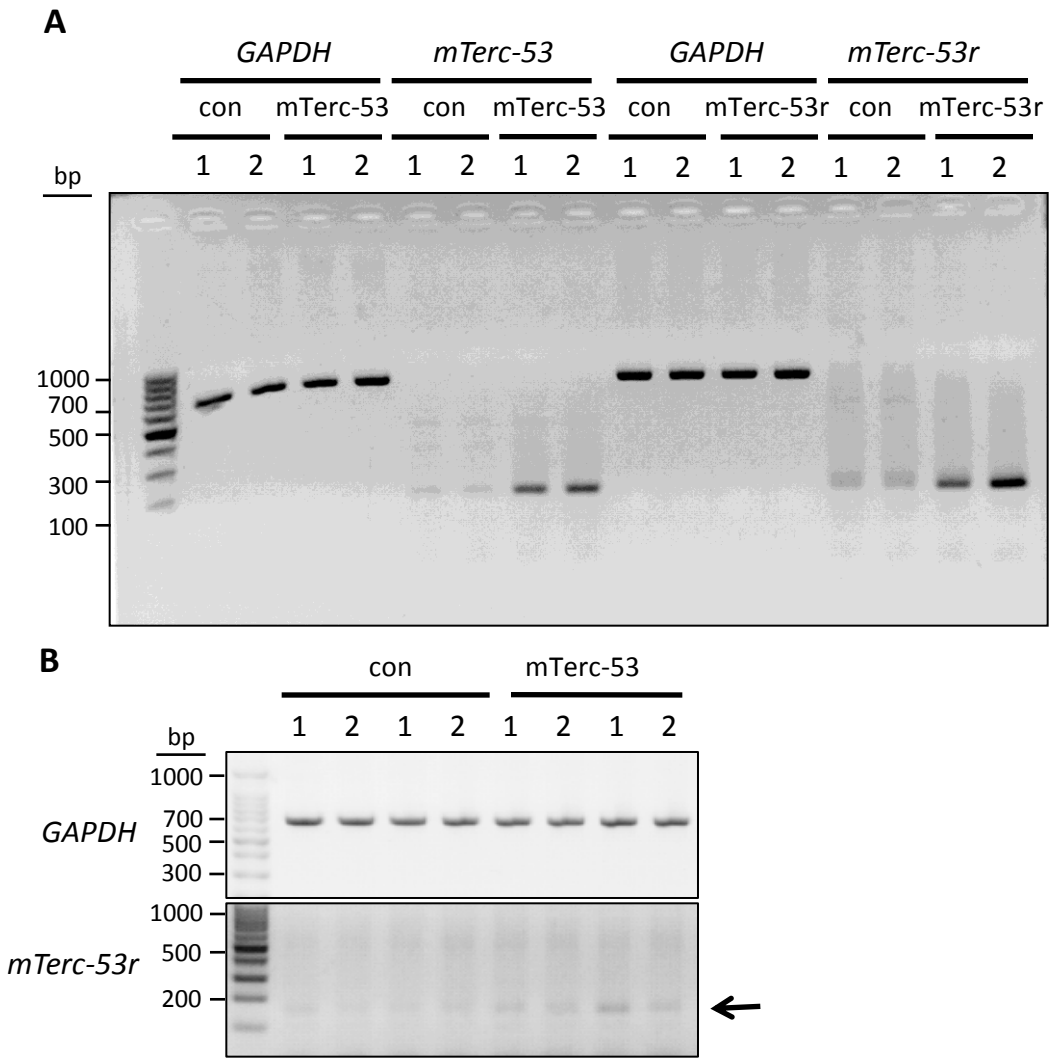
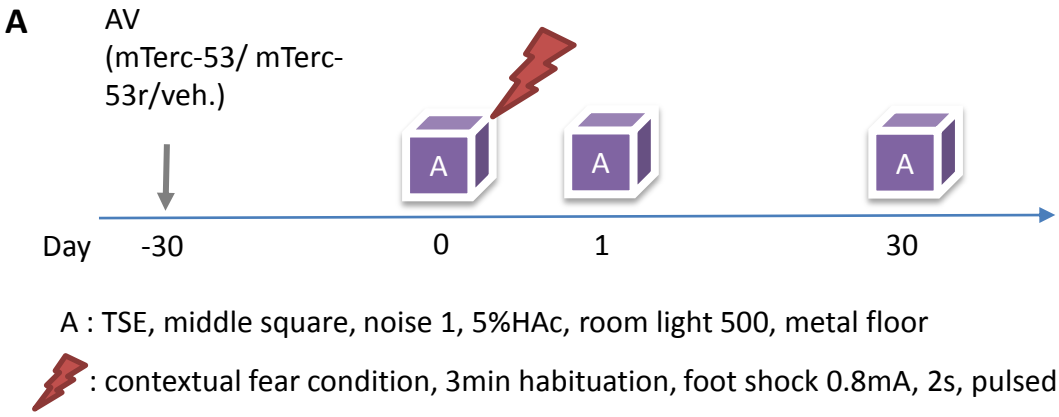
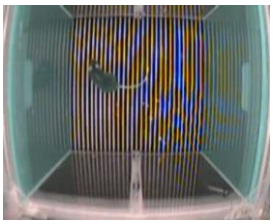



**Figure S4. exogenous expression of *mTerc-53* and *mTerc-53r* in mouse hippocampi, related to Figure 4 and 5.**

(A) Total RNA was isolated from *mTerc-53*, or *mTerc-53r* overexpressing mouse hippocampi or control hippocampi (con) and RT-PCR was performed with primers for *GAPDH*, *mTerc-53* or *mTerc-53r*. Two mouse hippocampi were examined (1 and 2). (B) *mTerc-53r* level was examined in mouse hippocampi with exogenous expression of *mTerc-53* or the control hippocampi.



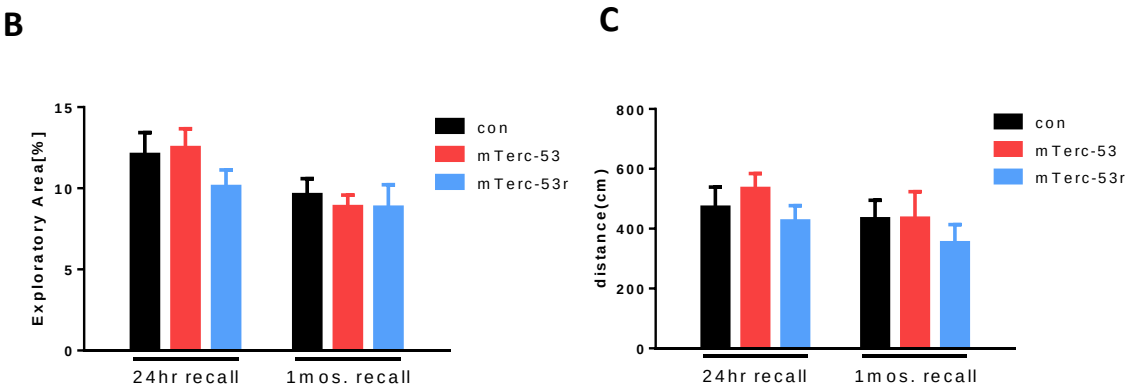
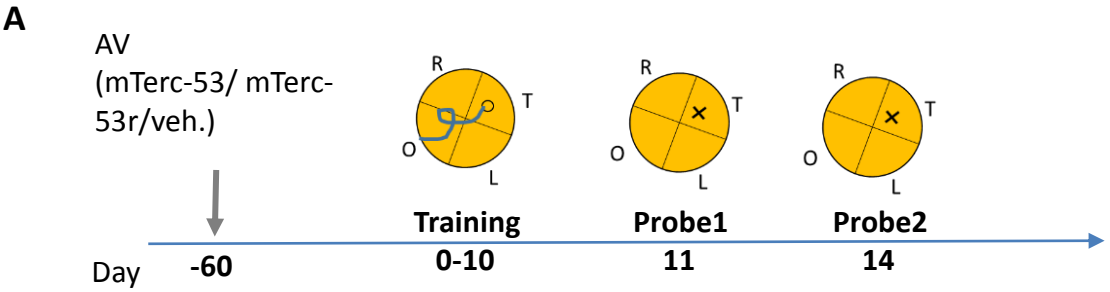
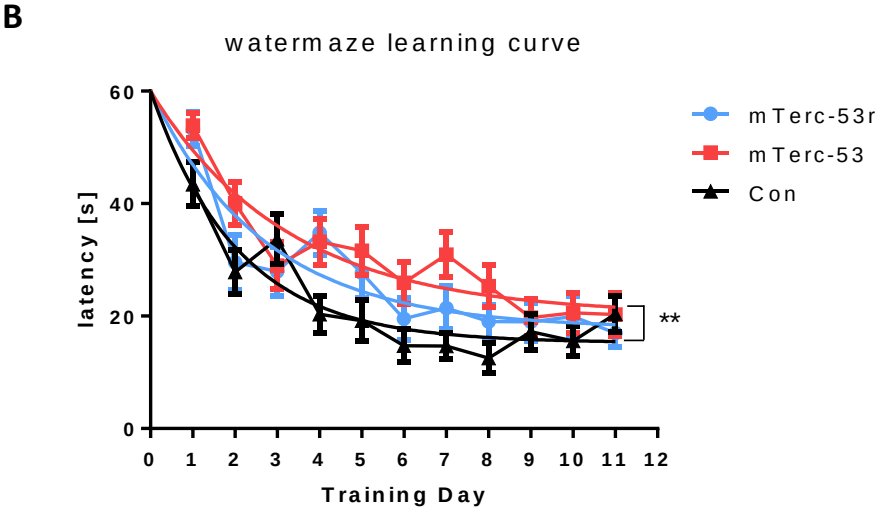
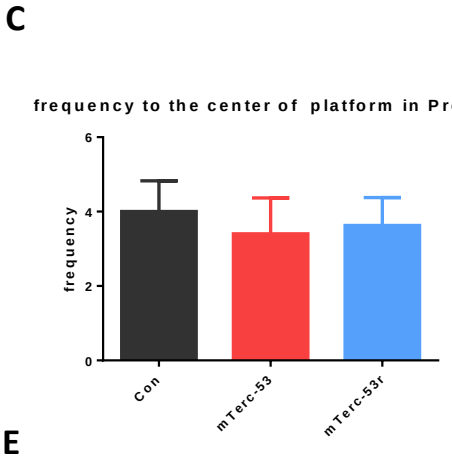
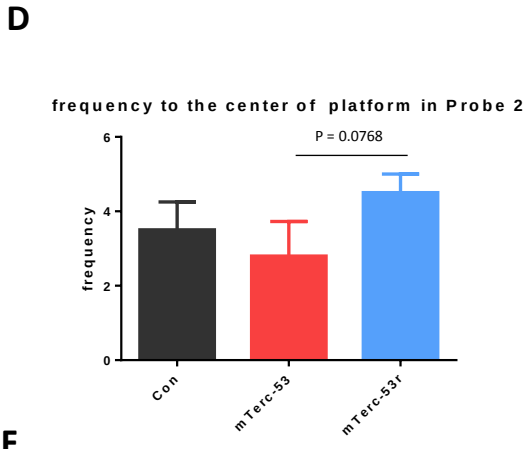


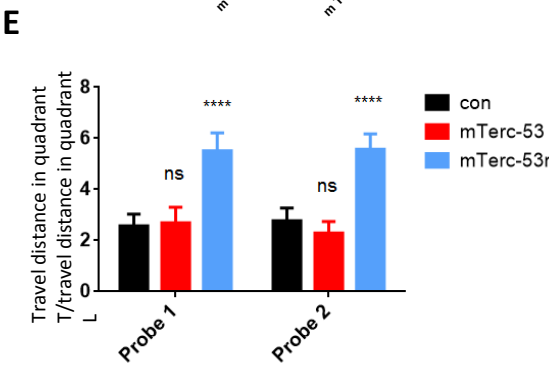

**Figure S5. Contextual fear conditioning, related to Figure 4.**
(A) Schematic of the contextual fear conditioning paradigm with a photo of a mouse in context A. (B) Exploratory area of control mice (n = 20) or mice overexpressing *mTerc-53* (n = 20) or *mTerc-53r* (n = 20) in the hippocampus 24 hours (24hr recall) or 1 month (1mos. recall) after training. (C) Travel distance of the mice.


A
AV
(mTerc-53/ mTerc-53r/veh.)
R
T
O
O
L
Training
0-10
Probe1
11
Probe2
14
Day
-60


B
watermaze learning curve
latency [s]
Training Day
mTerc-53r
mTerc-53
Con
**


C
frequency to the center of platform in Probe 1
frequency
Con
mTerc-53
mTerc-53r


D
frequency to the center of platform in Probe 2
P = 0.0768
frequency
Con
mTerc-53
mTerc-53r


E
Travel distance in quadrant T/travel distance in quadrant L
ns
****
ns
****
Probe 1
Probe 2
con
mTerc-53
mTerc-53r

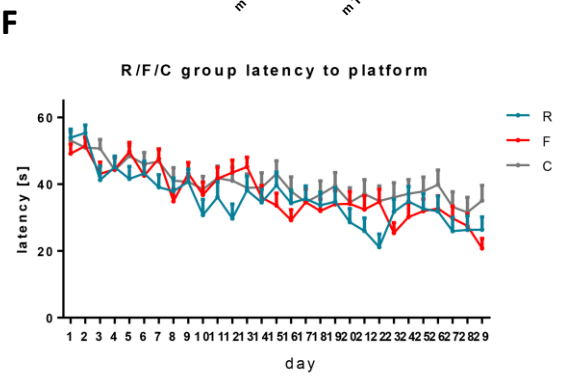

F
R/F/C group latency to platform
latency [s]
day
R
F
C

**Figure S6. Morris Water Maze (MWM) test, related to Figure 4.**
(A) Schematic of the MWM paradigm. (B) Latency learning curve of control mice (n = 12) or mice overexpressing *mTerc-53* (n = 10) or *mTerc-53r* (n = 10) in the hippocampus to locate the hidden platform. Two-way repeated measures ANOVA was used for comparison. (C, D) Frequency of locating the hidden platform in probe 1 (C) and 2 (D). Statistical comparisons are performed using unpaired *t*-tests; (E) Ratio of travel distance in quadrant T to that in quadrant L in probe 1 and 2. (F) Latency learning curve of 18 months old control mice (C) or mice overexpressing *mTerc-53* (F) or *mTerc-53r* (R) in the hippocampus to locate the hidden platform (n = 8). Two-way repeated measures ANOVA was used for comparison.*$P$<0.05, **$P$<0.01, ***$P$<0.001, ****$P$<0.0001. Data are presented as mean ± standard error of the mean (s.e.m.).

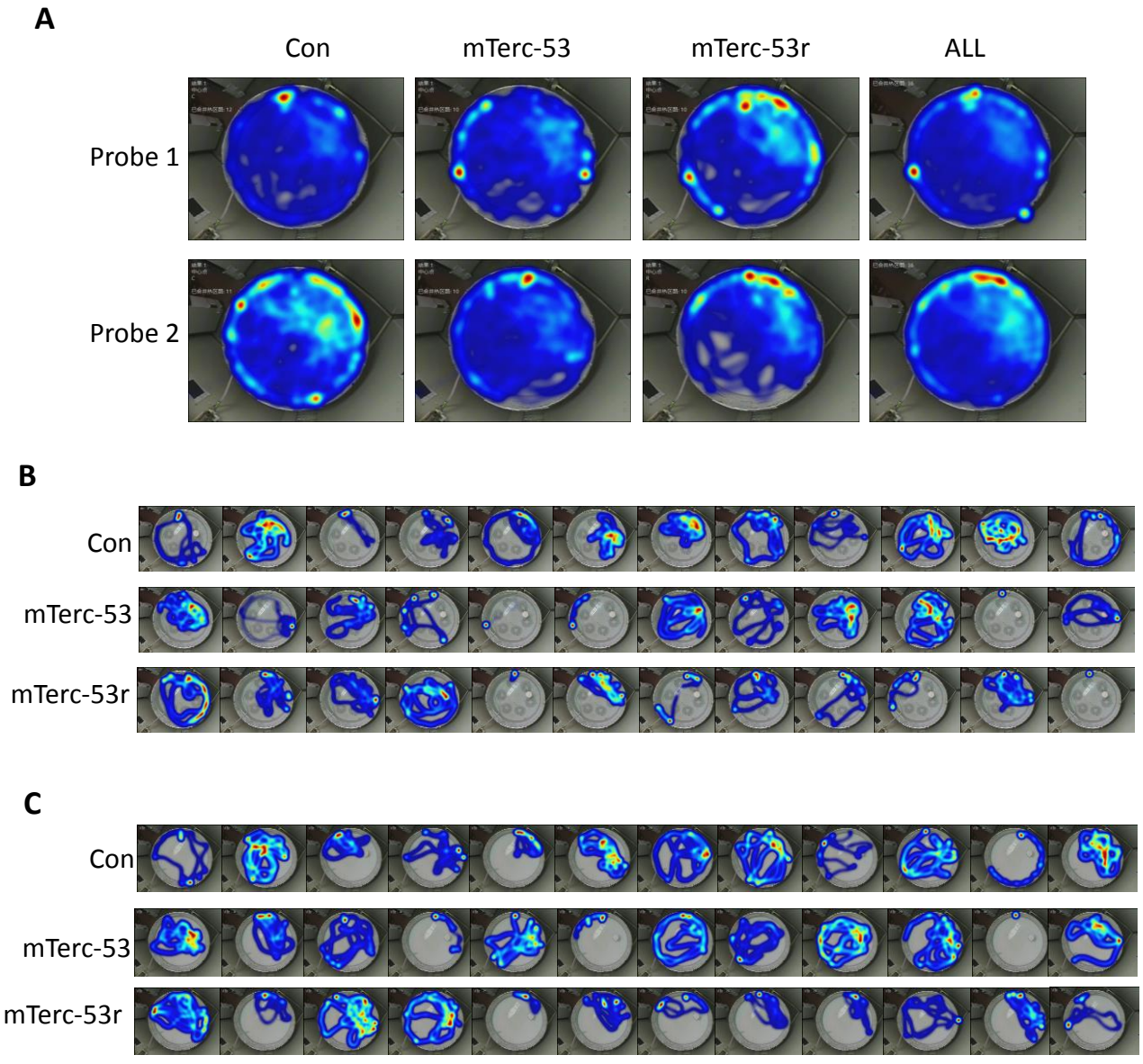



**Figure S7. Morris Water Maze (MWM) test, related to Figure 4.**
(A) Average traveling heat maps in three groups and in total for two probes. (B) Individual traveling heat maps during probe 1. (C) Individual traveling heat maps during probe 2.

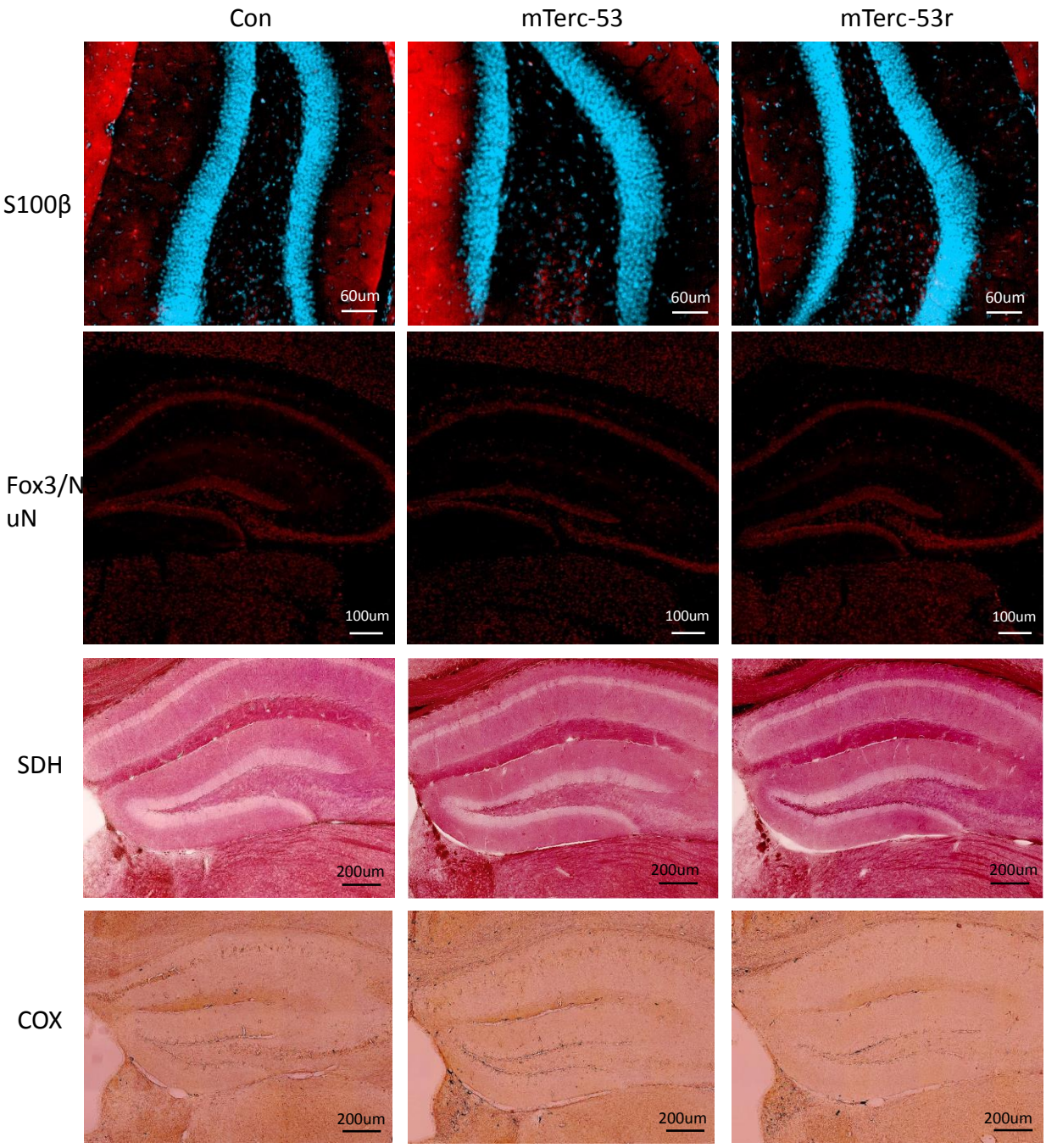



**Figure S8. Histological examination of the hippocampi of control mice or mice overexpressing *mTerc-53* or *mTerc-53r,* related to Figure 5.** S100β (red) and Fox3/NeuN immunostaining, and COX/SDH histochemistry of the hippocampi.

# Table legends

**Table S1. Differentially expressed genes between Terc-53 (53) and control cells (Con).**
**Table S2. Differentially expressed genes between Terc-53r (53r) and control cells (Con).**
**Table S3. Gene ontology of differentially expressed genes between Terc-53 (53) and control cells (Con).**
**Table S4. Gene ontology of differentially expressed genes between Terc-53r (53r) and control cells (Con).**