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Supplemental Information

Ten-Eleven Translocation Proteins Modulate the Response to Environmental Stress in Mice

Ying Cheng, Miao Sun, Li Chen, Yujing Li, Li Lin, Bing Yao, Ziyi Li, Zhiqin Wang, Jack Chen, Zhigang Miao, Ning Xin, Luoxiu Huang, Emily G. Allen, Hao Wu, Xingshun Xu, and Peng Jin

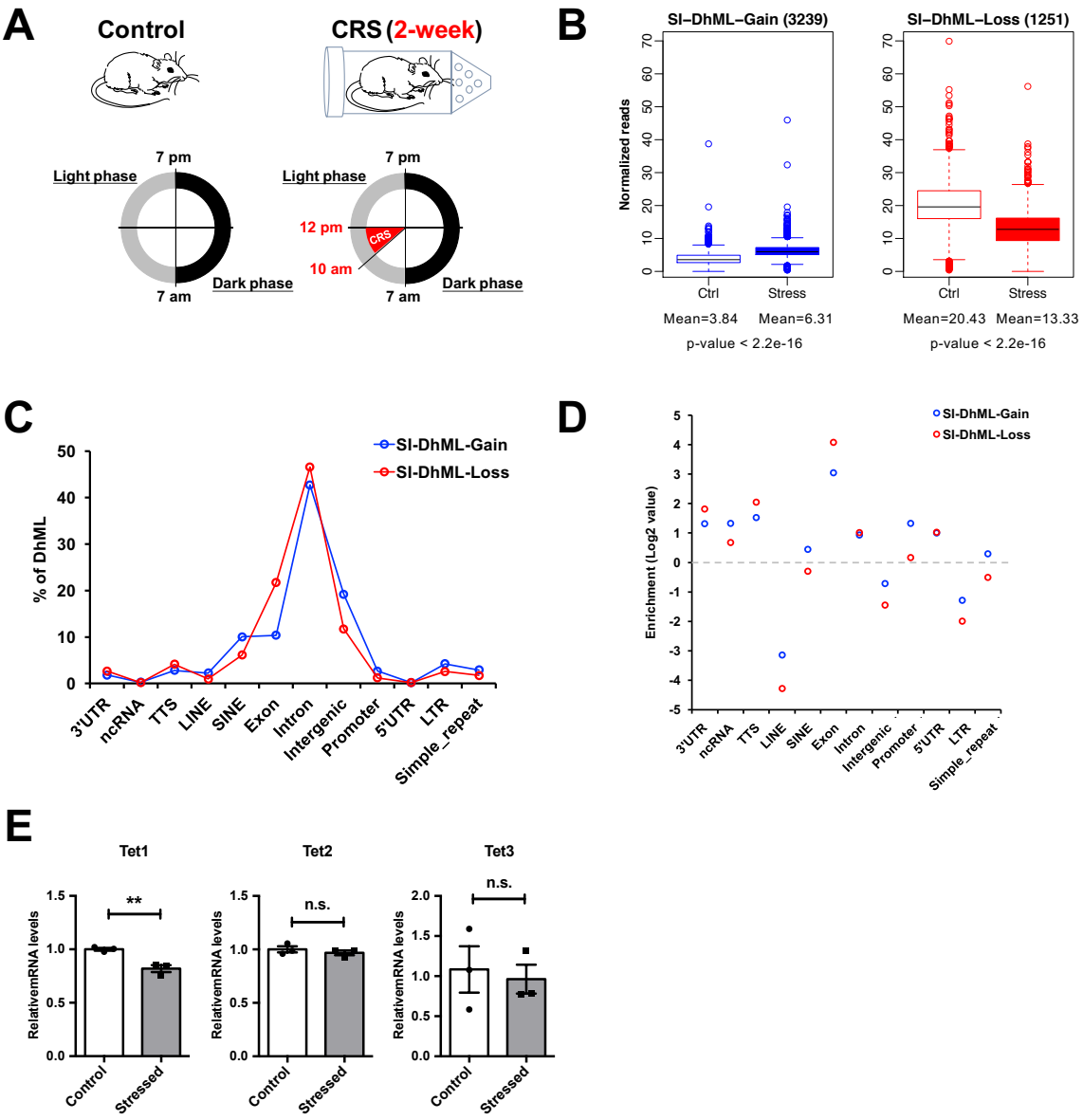

Figure S1. Chronic restraint stress results in decreased 5hmC and Tet1 mRNA levels in PFC, Related to Figure 1.

(A) Design of chronic restraint stress (CRS) experiment. Mice subjected to CRS were individually placed into a customized well-ventilated 50-ml conical tube, in which the mice could change from the supine to prone position but were unable to move forward or backward. The non-stressed littermate controls remained undisturbed in their home cages. The experiments occurred daily from 10 am to 12 pm for 2 hours over 2 weeks in the original home cages, and the mice were released from the tube afterwards. (B) Comparison of normalized reads in WT-SI-DhML-Gain (3239) and WT-SI-DhML-Loss (1251) exhibited significant differences between Control and Stressed samples (two-tailed unpaired *t*-test; p-value < 2.2e–16). (C) Identified WT-SI-DhML-Gain and WT-SI-DhML-Loss were annotated to various genomic regions, revealing the majority of WT-SI-DhML were found in exons, introns and intergenic regions. (D) Enrichment (Log_2 value) of WT-SI-DhML in each genomic region revealed an increase of WT-SI-DhML in gene body, including exon, intron and TSS, etc. (E) Quantitative reverse-transcription PCR determined Tet1, Tet2, and Tet3 expression in the PFC of control and stressed WT mice. Tet1 exhibited a significant decrease upon CRS, whereas Tet2 and Tet3 showed little changes. n = 3, error bars indicate mean ± SEM; two-tailed unpaired *t*-test; n.s., not significant; **, P < 0.01.

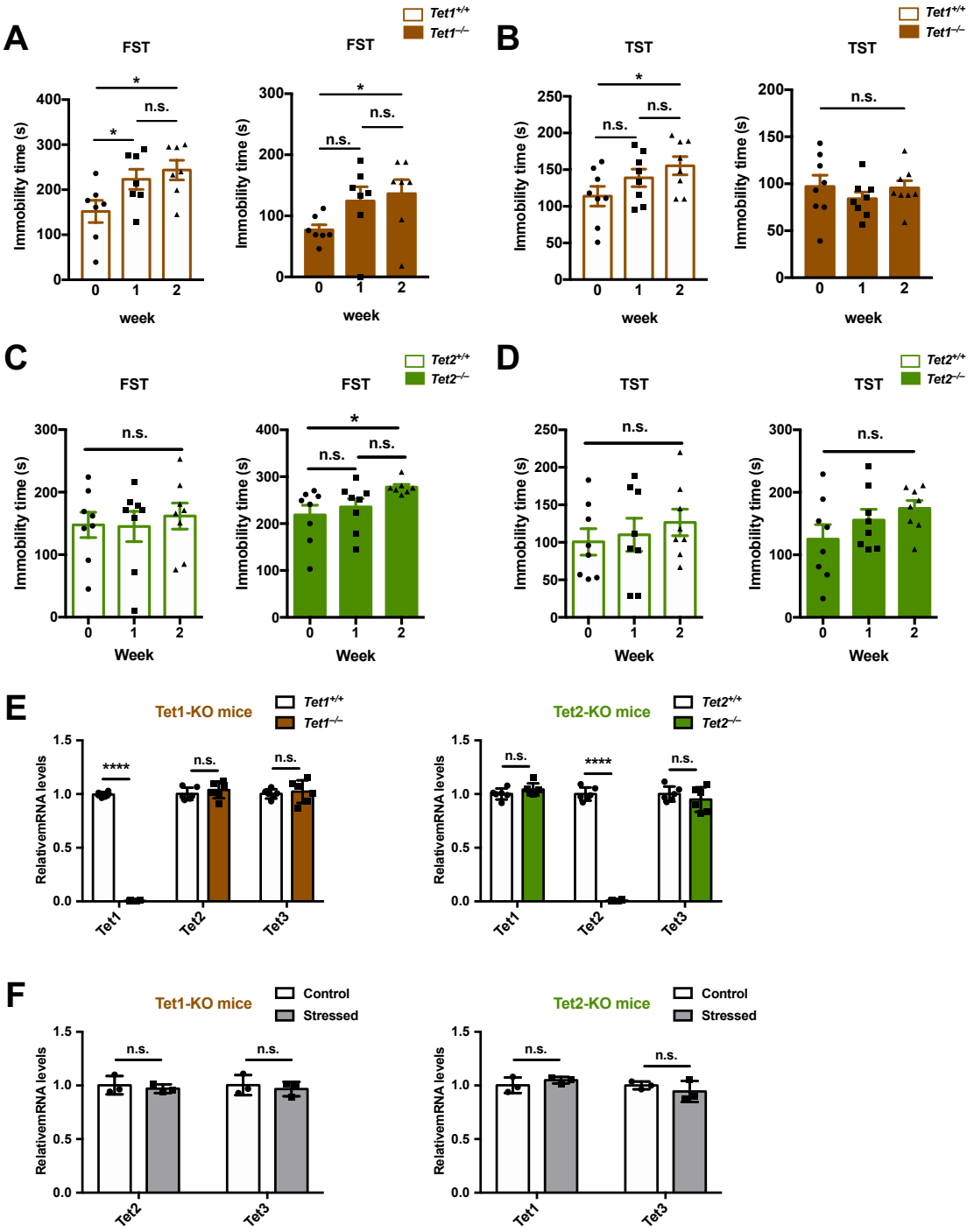

Figure S2. Forced swimming and tail suspension tests of Tet1 KO and Tet2 KO mice, Related to Figure 2.

(A-B) Comparison of the immobility time of *Tet1*$^{+/+}$ and *Tet1*$^{-/-}$ mice across weeks in FST (A) and TST (B). (C-D) Comparison of the immobility time of *Tet2*$^{+/+}$ and *Tet2*$^{-/-}$ mice across weeks in FST (C) and TST (D). (E-F) Quantitative reverse-transcription PCR determined Tet1, Tet2, and Tet3 expression in the PFC of Tet1 KO and Tet2 KO mice. When compared with littermate control, there is no significant change of Tet2/3 in Tet1 KO mice nor Tet1/3 in Tet2 KO mice (E). Under stress condition, no significant change of Tet2/3 in Tet1 KO mice nor Tet1/3 in Tet2 KO mice was observed (F). n = 3, error bars indicate mean ± SEM; two-tailed unpaired *t*-test; n.s., not significant; ****, P < 0.0001.

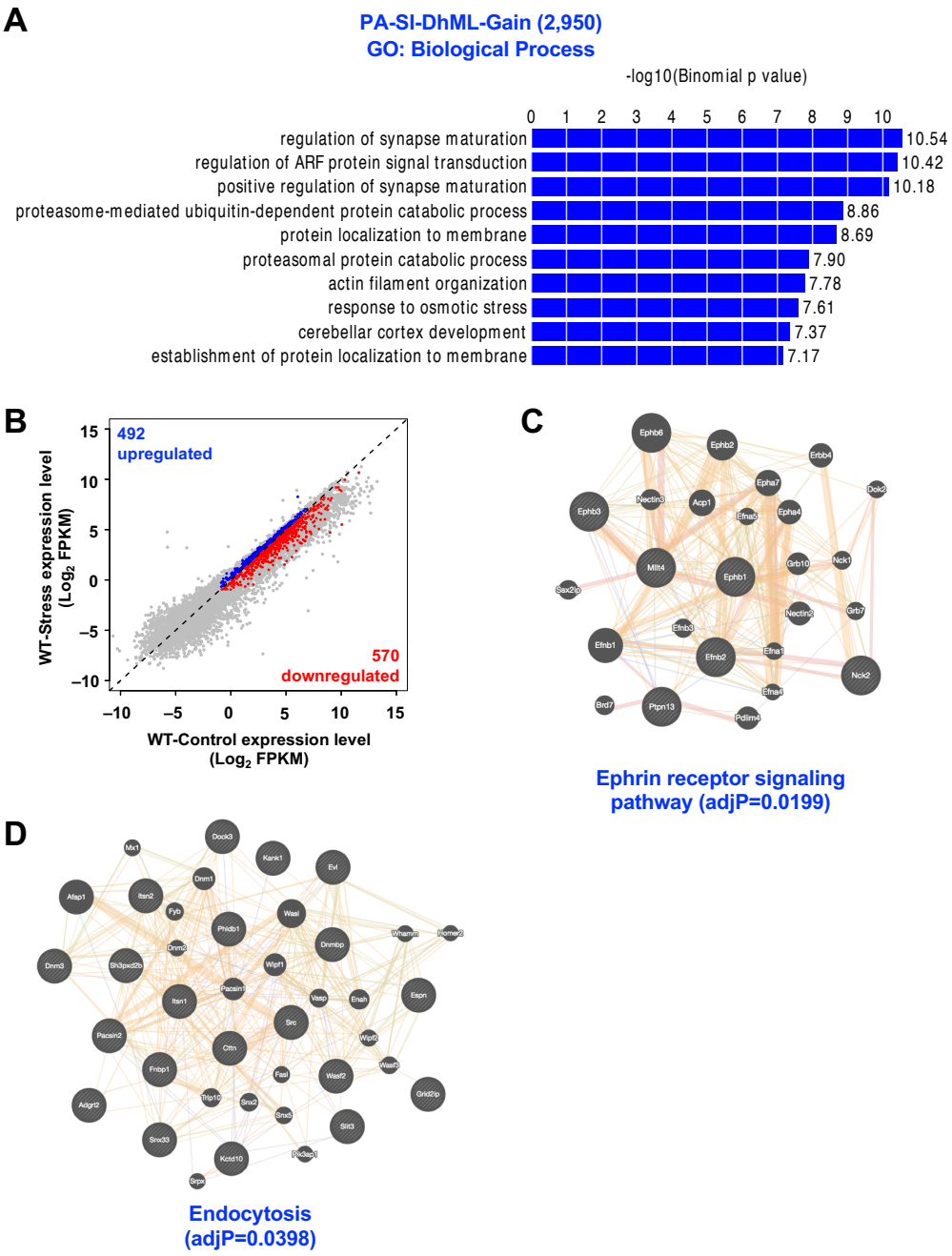

Figure S3. PA-SI-DhML involves in different pathway, Related to Figure 3.
(A) Gene Ontology (GO)- 'biological process' analysis on 2950 PA-SI-DhML-Gain. (B) RNA-seq experiments were performed to evaluate the global gene expression change stressed versus control WT mice (n = 3). 1608 genes that associated with 2950 PA-SI-DhML-Gain were used to investigate whether they were differentially expressed. Genes with log2FC > 0.1 or < –0.1 and FPKM > 0.5 are defined as significantly upregulated and downregulated genes. Logarithmic scale (Log) of FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values were plotted, with significantly upregulated and downregulated genes highlighted in blue and red, respectively. (C-D) Genes associated with PA-SI-DhML-Gain were further subjected to protein interaction network module analysis by the WebGestalt, which has mouse protein-protein interaction modules and finds hierarchical modules. The genes associated with PA-SI-DhML-Gain showed significant correlation with the genes involving in ephrin receptor signaling pathway (C) and endocytosis (D).

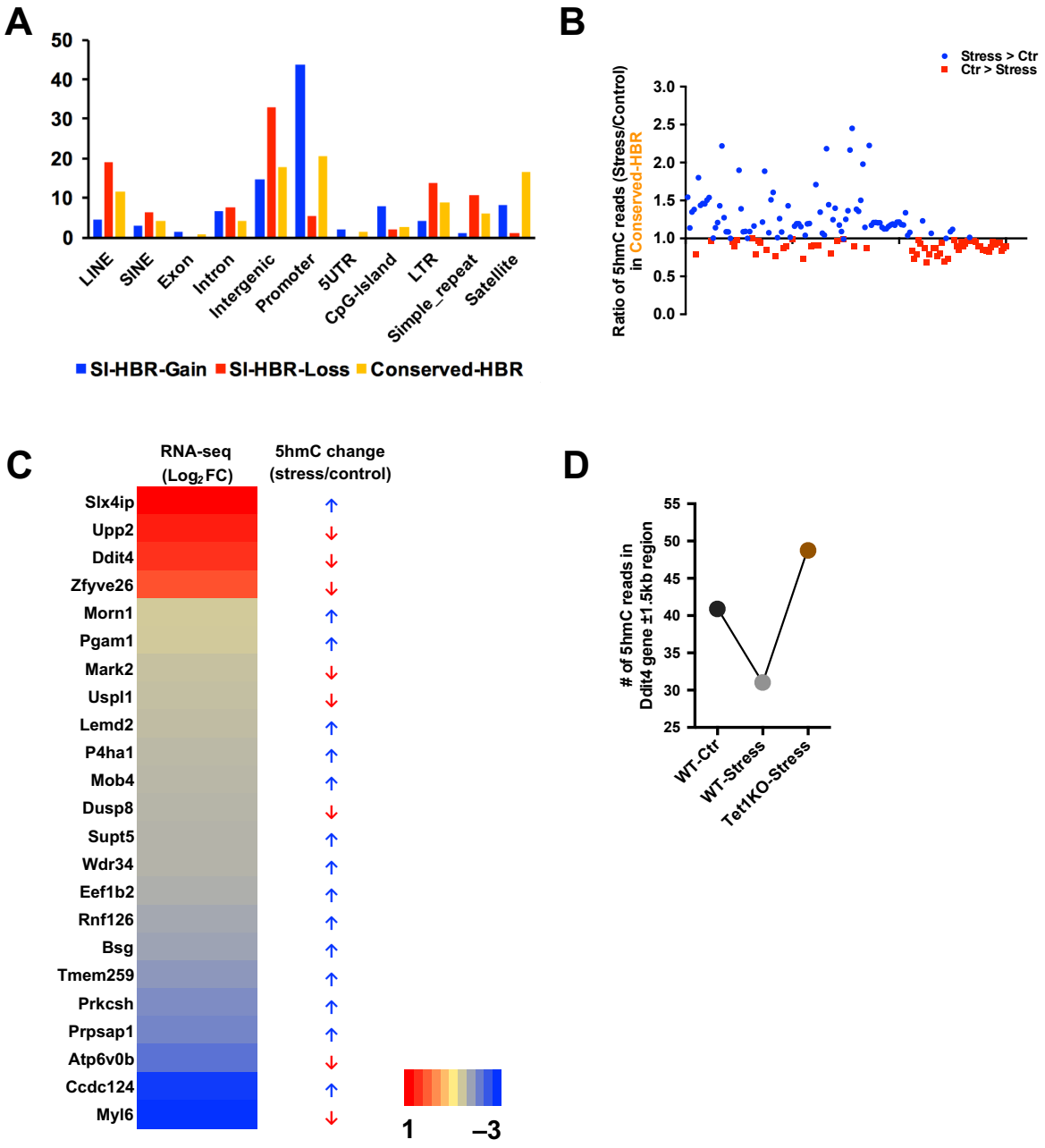

Figure S4. CRS leads to an enriched HIF1α binding in specific regions, Related to Figure 4.
(A) Genomic distribution of SI-HBR-Gain, Conserved-HBR and SI-HBR-Loss. Among SI-HBR-Gain, 136 out of 315 (43%) HBR were annotated to gene promoter regions (promoter-associated SI-HBR-Gain). (B) Normalized 5hmC reads were counted from each Conserved-HBR. The ratio of 5hmC reads was calculated by normalizing the 5hmC reads in stressed WT PFC to those in control WT PFC. Blue dots indicate the HBR with more 5hmC in stressed WT PFC, and red squares indicate the HBR with more 5hmC in control WT PFC. (C) CRS resulted in SI-HBR-Gain in the promoter of a set of genes, among which 4 upregulated ($Log_2FC > 0.5$) and 19 downregulated ($Log_2FC < –0.5$) genes contain the dynamical change of 5hmC (> 10% change of reads) in their promoter regions (±1-kb) (Table S3). Heat map indicates those upregulated and downregulated genes in the order of Log_2FC values. (D) In whole gene body ± 1.5-kb region of Ddit4 gene, 5hmC reads were counted in control WT and stressed WT/Tet1KO mice. Normalized 5hmC reads reduced in WT mice upon stress, while increased 5hmC reads were found in stressed Tet1-KO mice.

SUPPLEMENTA TABLES:

Table S1. Summary of RNA-seq analyses. (See Excel file) **(Related to Figure 3)**

Table S2. Summary of SI-HBR-Gain associated genes expression and 5hmC changes. (See Excel file) **(Related to Figure 4)**

Table S3. Summary of primers used in the experiments. (See Excel file) **(Related to Figure 2, Figure 4 and Figure S1)**