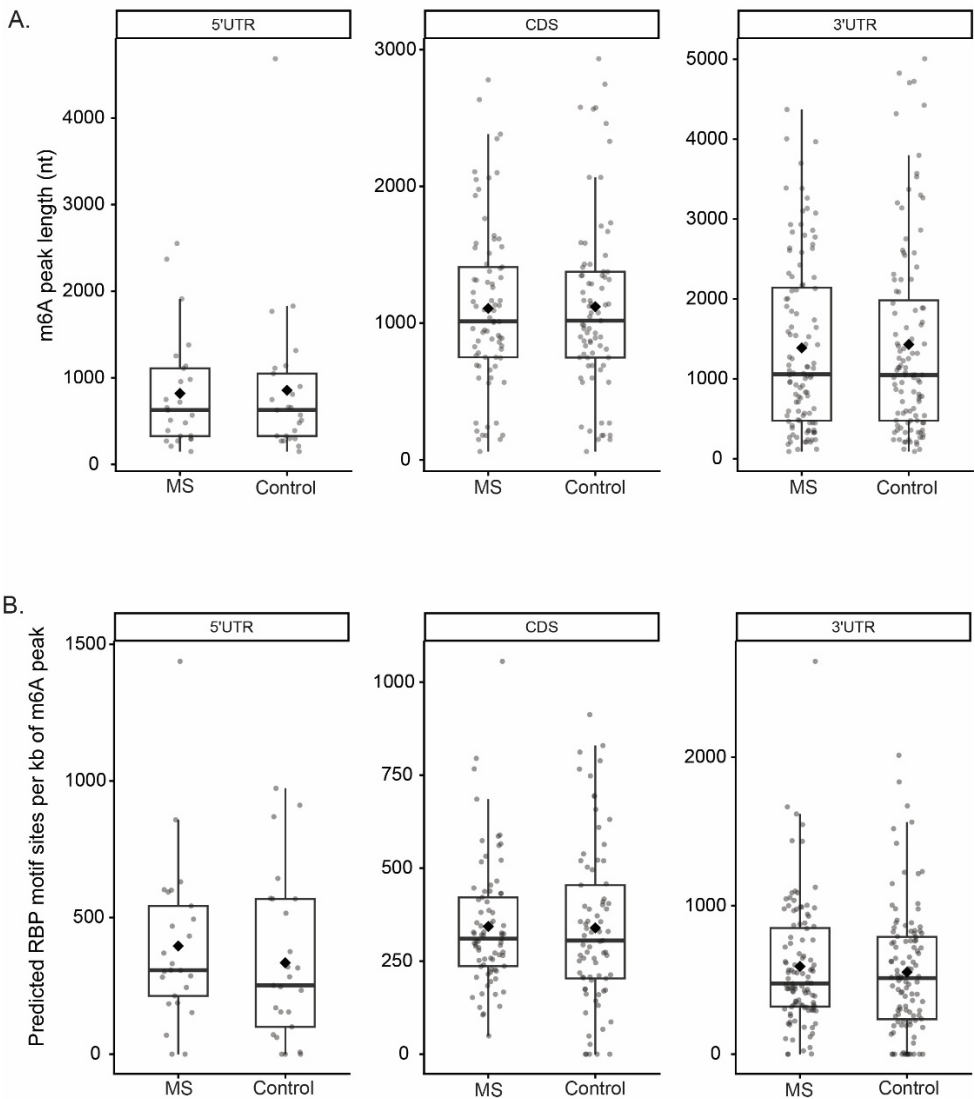
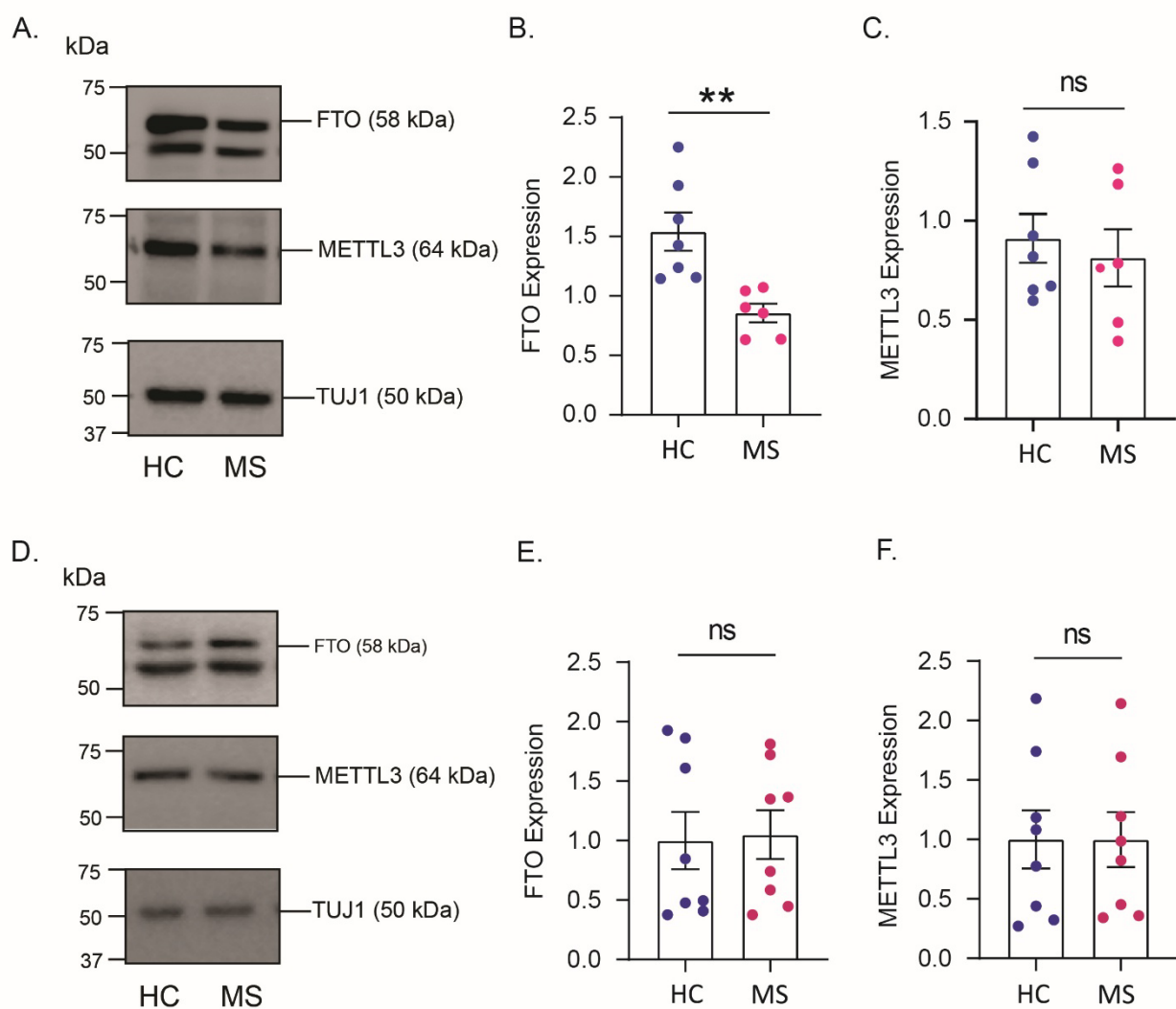


SUPPLEMENTARY DATA



Supplementary Figure 1. Comparison of m⁶A peak length and predicted RBP motif density between MS-hypermethylated and matched control peaks. (A) Distribution of m⁶A peak lengths for MS-hypermethylated and matched control peaks in the 5'UTR, CDS, and 3'UTR. **(B)** Density of predicted RBP motif sites overlapping m6A peaks, expressed as motif sites per kilobase of m6A peak, for MS-hypermethylated and matched control peaks in the 5'UTR, CDS, and 3'UTR. RBP motif densities were compared between MS-hypermethylated and matched control peaks using Wilcoxon rank-sum tests separately for the 5'UTR, CDS, and 3'UTR. The data underlying this figure are available at <https://doi.org/10.6084/m9.figshare.3336832>.

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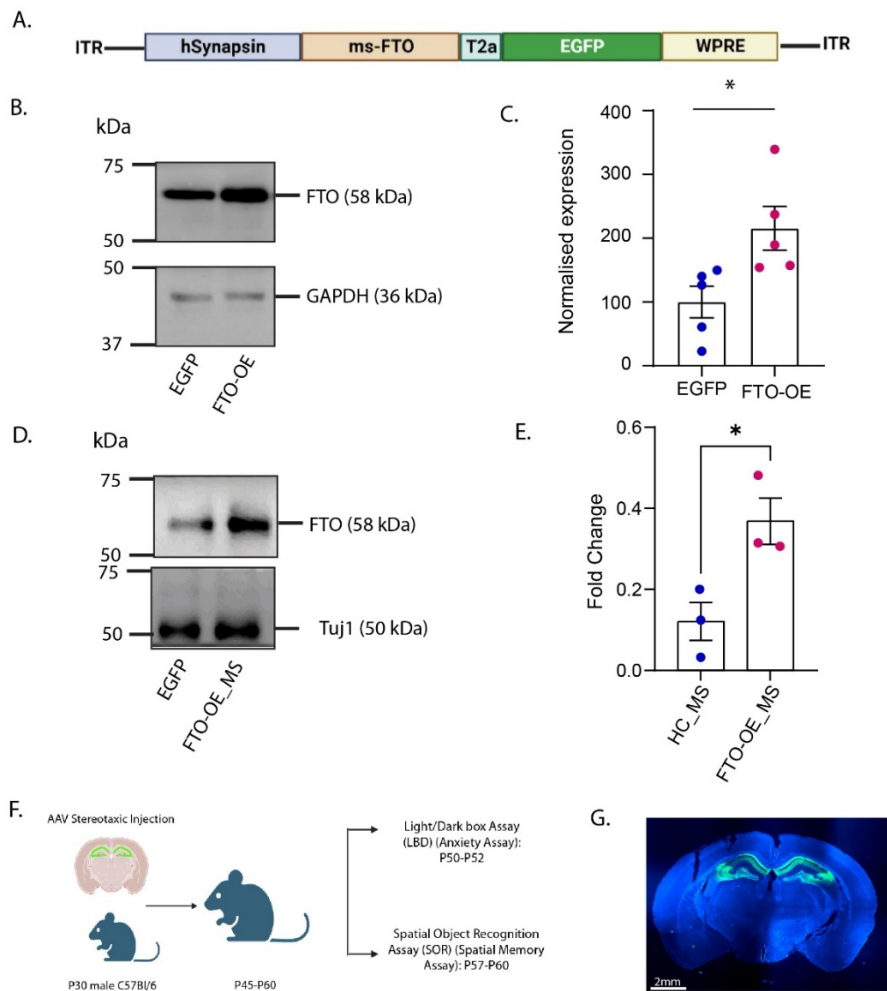
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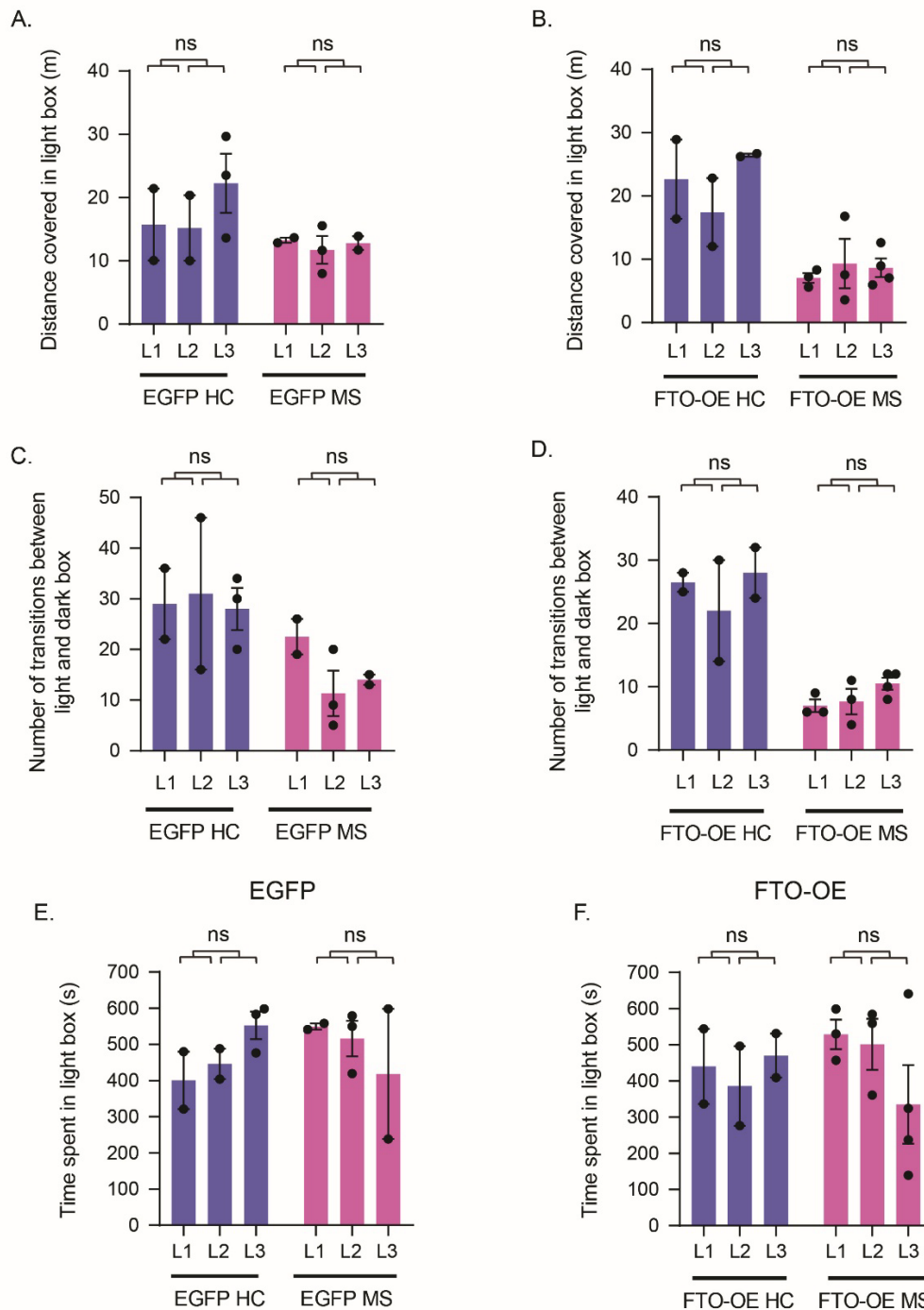
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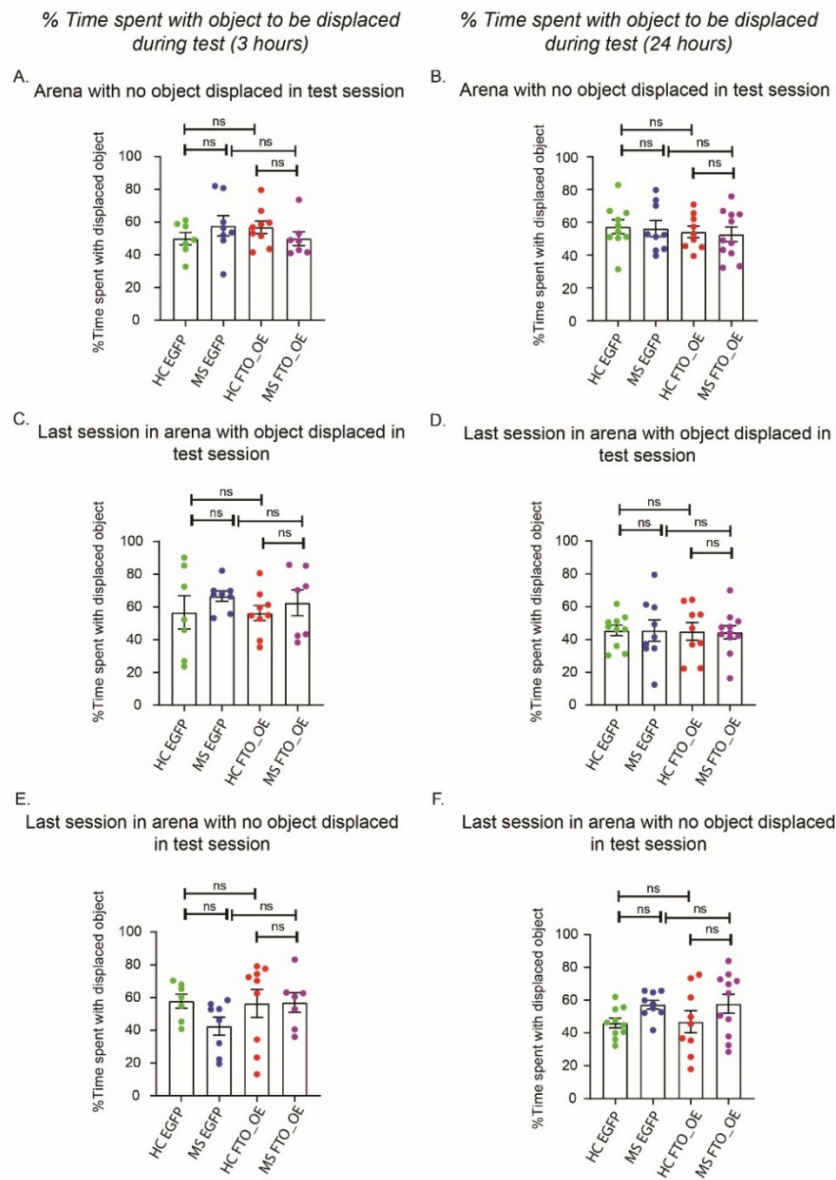
Supplementary Figure 2. Expression of writers and erasers in the hippocampus upon maternal separation in females. (A) Immunoblot showing expression of FTO and METTL3 in P21 (females). Quantification of **(B)** FTO and **(C)** METTL3 expression at P21 (females); N=3-4 pregnant dams, n = 6-7 pups. **(D)** Immunoblot showing expression of FTO and METTL3 at P28 (females). Quantification of **(E)** FTO and **(F)** METTL3 expression in P28 (females); N=3-4 pregnant dams, n=8 pups, *p<0.05, **p<0.01, ns=not significant. Data represents Mean ± SEM. Unpaired Student's t-test with Welch's correction. The data underlying this figure are available at <https://doi.org/10.6084/m9.figshare.3336832>.



Supplementary Figure 3. Experimental design and validation of FTO overexpression. (A) Schematic representation of the AAV vector used for the overexpression of FTO. **(B)** Immunoblot showing FTO expression. **(C)** quantification for FTO overexpression in HEK293T. Data shown as Mean±SEM, N = 5 independent cultures, *p<0.05. Student's paired t-test. **(D)** Immunoblot showing FTO expression in mouse **(E)** quantification of FTO overexpression in P60 C57BL/6 male mice. Data shown as Mean ± SEM, N = 3 animals, *p<0.05. Student's unpaired t-test with Welch's correction. **(F)** Experimental timeline and behavioural assays **(G)** Representative image of coronal brain section showing expression of EGFP, confirming successful injection in hippocampus (P60). The data underlying this figure are available at <https://doi.org/10.6084/m9.figshare.3336832>.



Supplementary Figure 4. Litter effect amongst animals in anxiety assay. (A–F) Litter effect amongst EGFP and FTO-OE injected HC and MS animals, with respect to **(A–B)** distance and **(C–D)** transitions, and **(E–F)** time. Data shown as Mean±SEM. Two-way ANOVA with Fisher's LSD, N=3 pregnant dams, n=7–10 pups, ns = not significant. The data underlying this figure are available at <https://doi.org/10.6084/m9.figshare.3336832>.

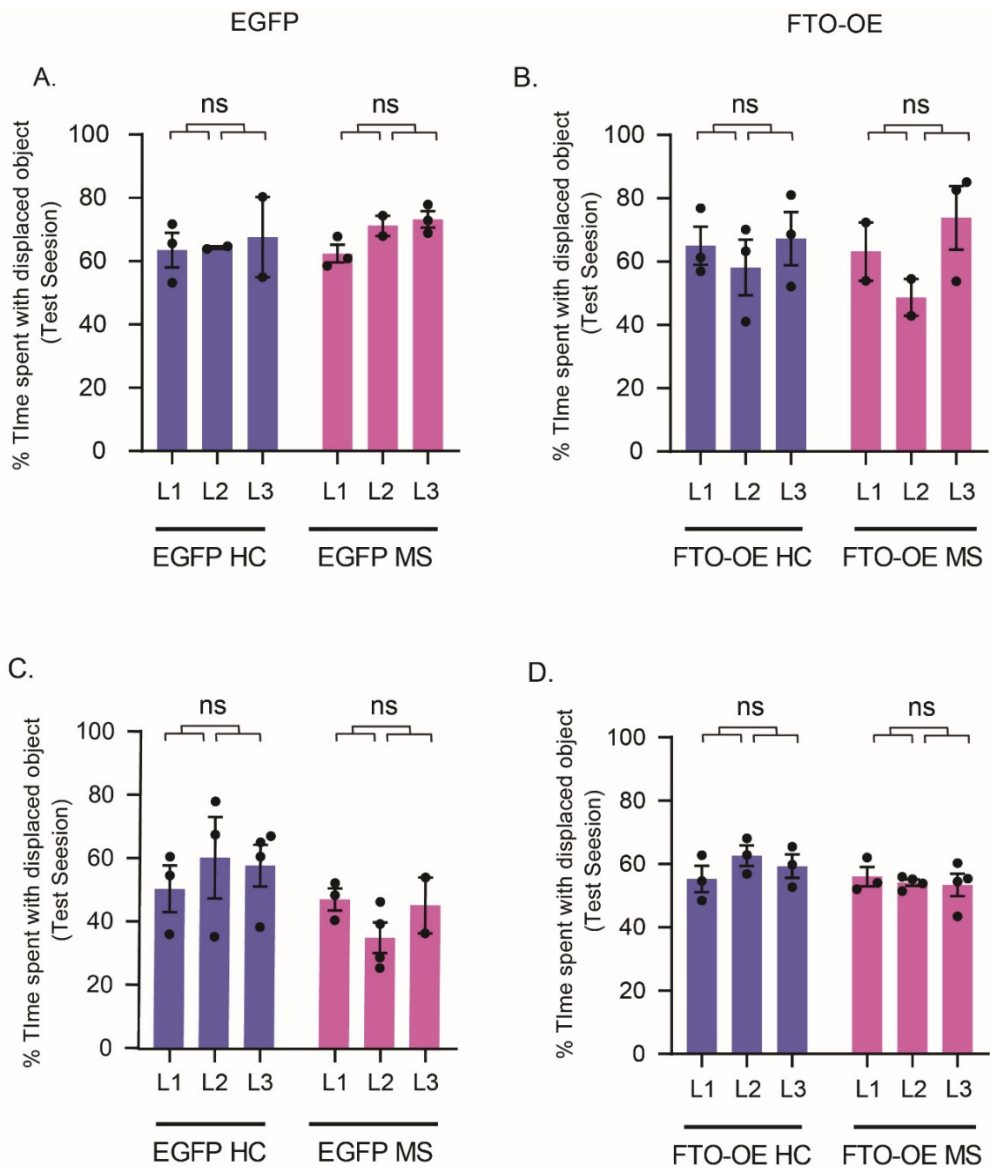


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Supplementary Figure 5. Time spent with object used for SOR shows no object bias. (A, B)

Percentage time spent with objects in the arena with no object displaced during test session; **(A)** 3hrs, N=3 pregnant dams, n=7–9 pups, and **(B)** 24hrs, N=3 pregnant dams, n=9–11 pups, post training. **(C, D)** Percentage time spent in the last training session with objects in the arena with object displaced during test session; **(C)** 3hrs, N=3 pregnant dams, n=7–9 pups, and **(D)** 24hrs, N=3 pregnant dams, n=9–11 pups, post training. **(E, F)** Percentage time spent in the last training session with objects in the arena with object displaced during test session; **(E)** 3hrs, N=3 pregnant dams, n=7–9 pups and **(F)** 24hrs, N=3 pregnant dams, n=9–11 pups, post training. Data shows

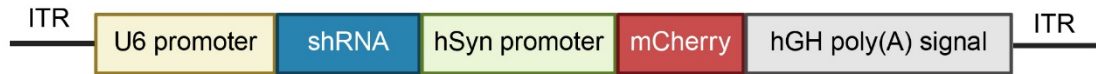
Mean±SEM. Two-way ANOVA with Bonferroni's correction, ns = not significant. The data underlying this figure are available at <https://doi.org/10.6084/m9.figshare.3336832>.



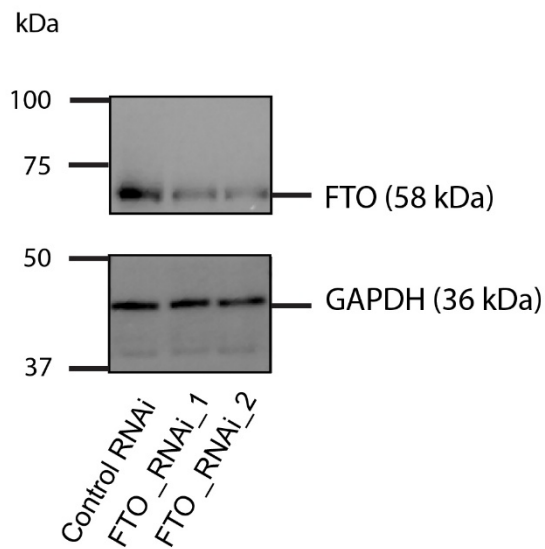
Supplementary Figure 6. Litter effect of animals used for spatial object recognition (SOR) assay. (A–D) Litter effect amongst EGFP and FTO-OE injected HC and MS animals, used for SOR **(A–B)** 3hrs post-training, N=3 pregnant dams, n=7–9 pups; **(C–D)** 24hrs post-training, N=3 pregnant dams, n=9–11 pups. Data shown as Mean±SEM. Two-way ANOVA with Bonferroni's correction, ns = not significant. The data underlying this figure are available at <https://doi.org/10.6084/m9.figshare.3336832>.

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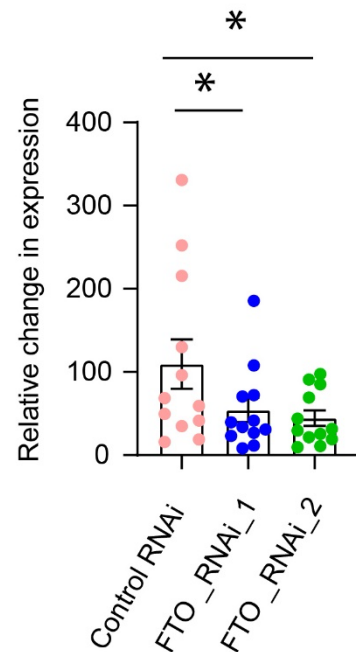
A.



B.



C.



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67 **Supplementary Figure 7. Experimental design and validation of FTO knockdown. (A)**

68 Schematic of AAV vector used for the knockdown of FTO. **(B)** Immunoblot and **(C)** Quantification

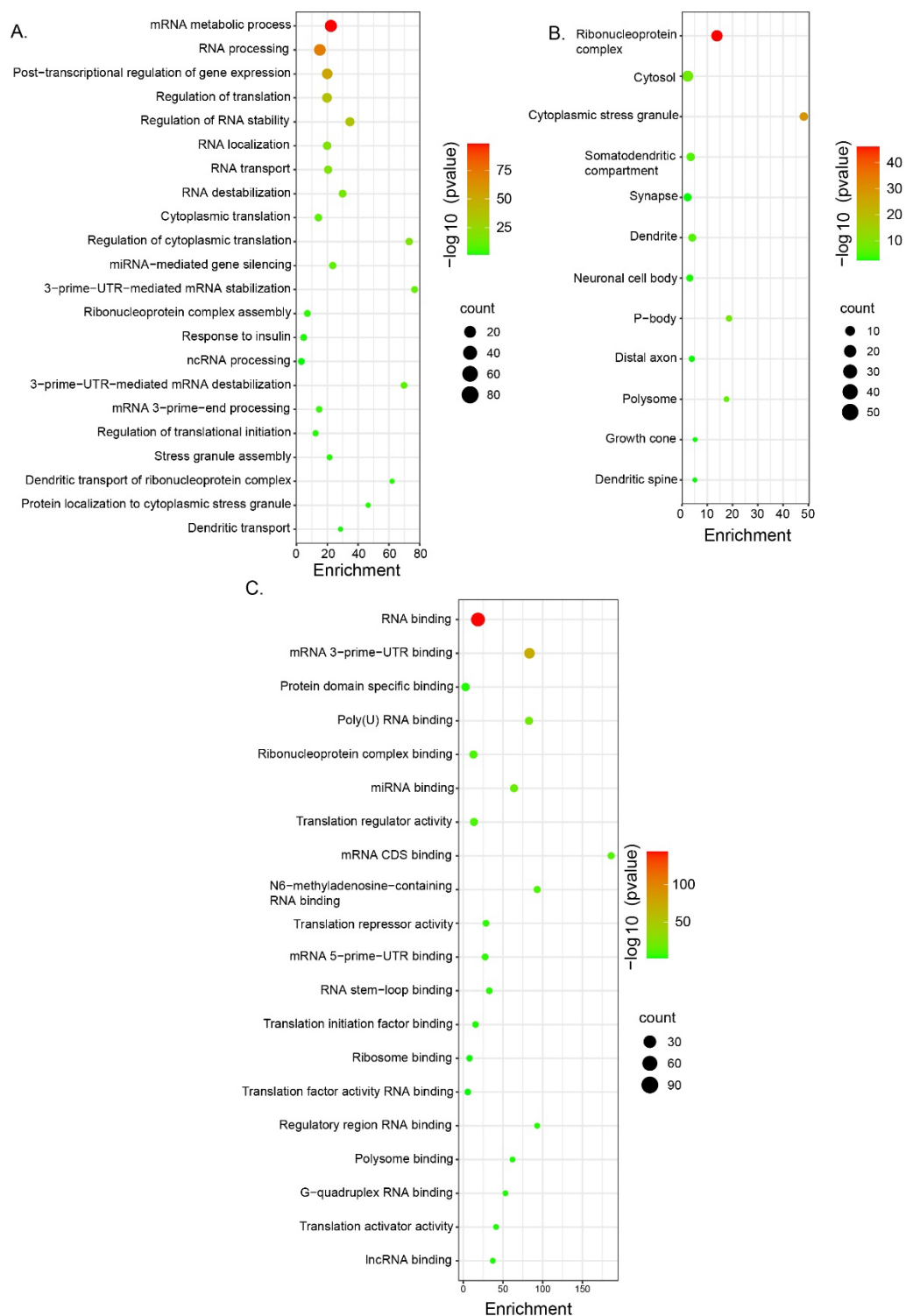
69 for FTO knockdown in HEK293T cells. Data shown as Mean $\pm$ SEM, n=12 independent cultures,

70 *p<0.05. Student's paired t-test. The data underlying this figure are available at

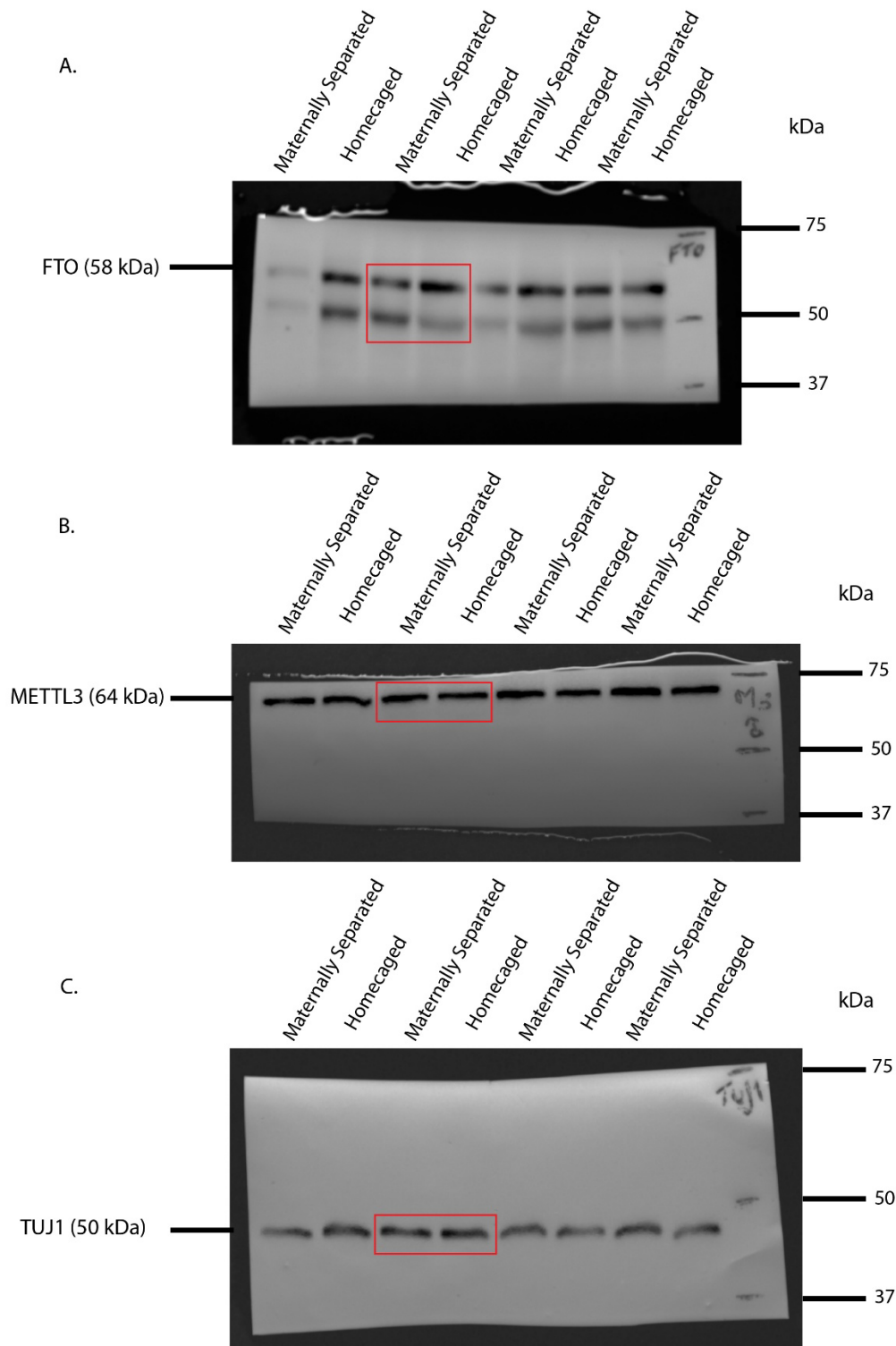
71 <https://doi.org/10.6084/m9.figshare.3336832>.

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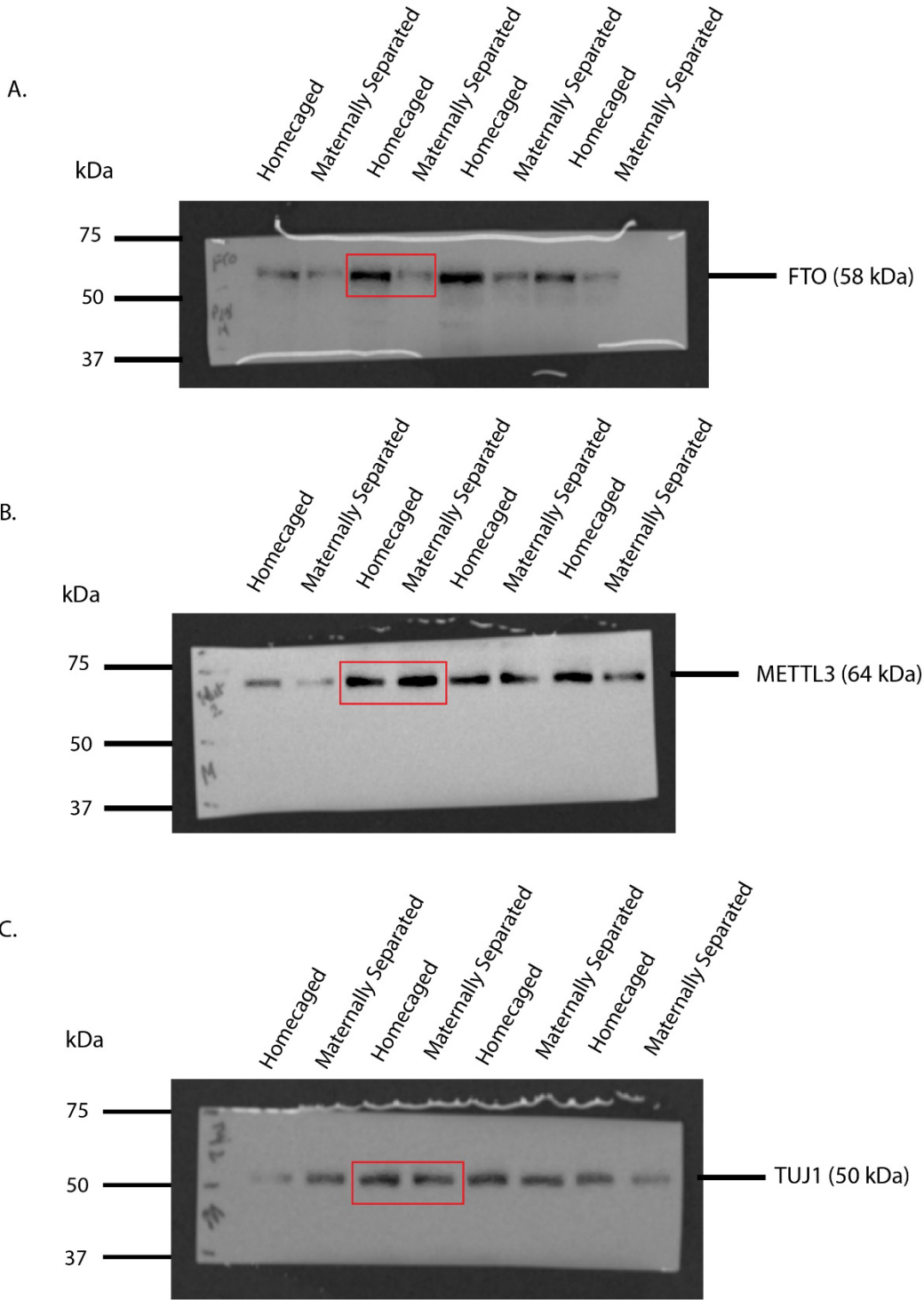
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Supplementary Figure 8. Gene Ontology enrichment of RNA-binding proteins represented by enriched predicted motifs in MS-hypermethylated m⁶A peaks. (A) Biological process, (B) Cellular components, and (C) Molecular function enriched terms, FDR<0.05. The data underlying this figure are available at <https://doi.org/10.6084/m9.figshare.3336832>.



Supplementary Figure 9. Full immunoblots showing expression of (A) FTO, (B) METTL3 and (C) Tuj1 in males, in P21 males. (See Figure 2H).

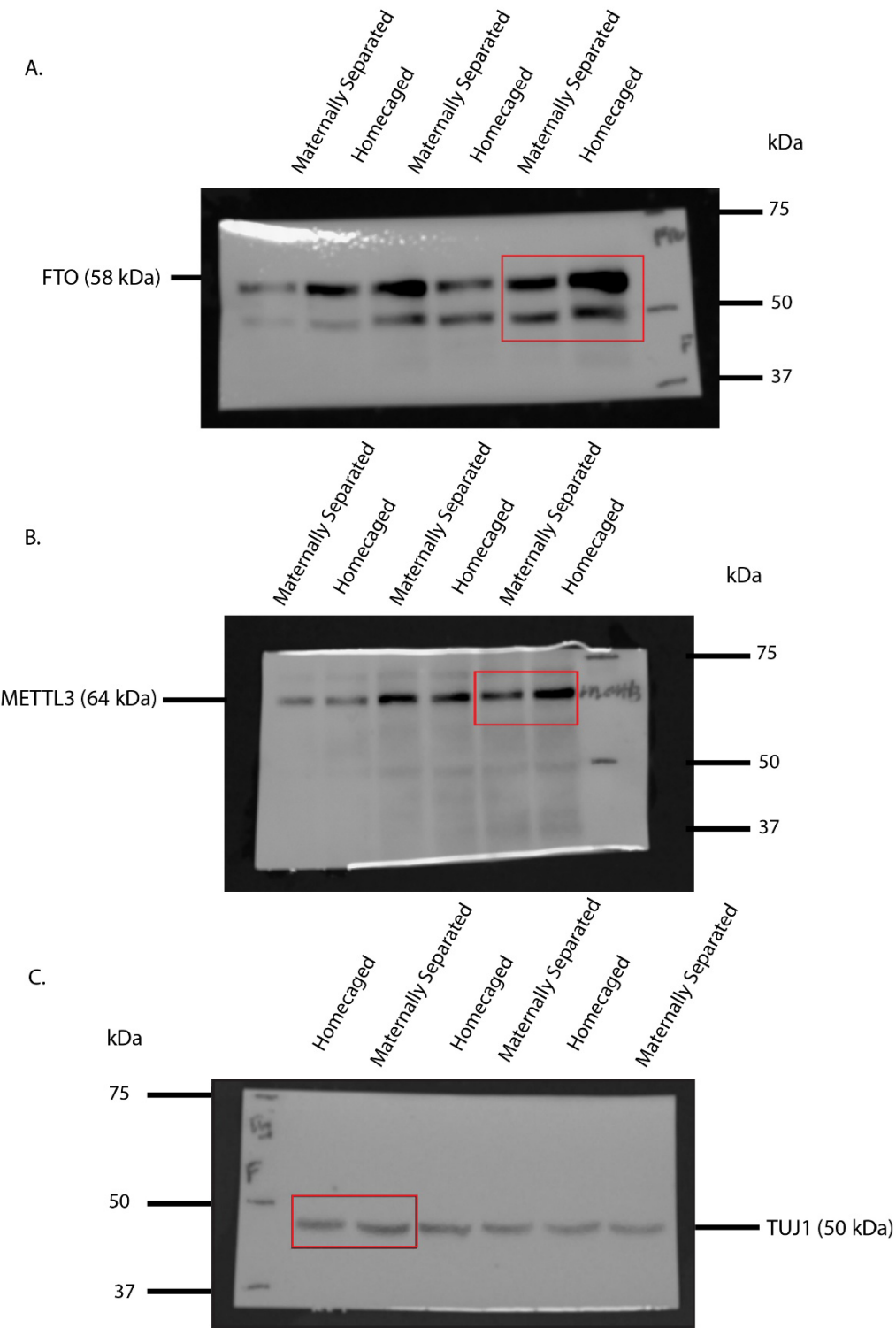


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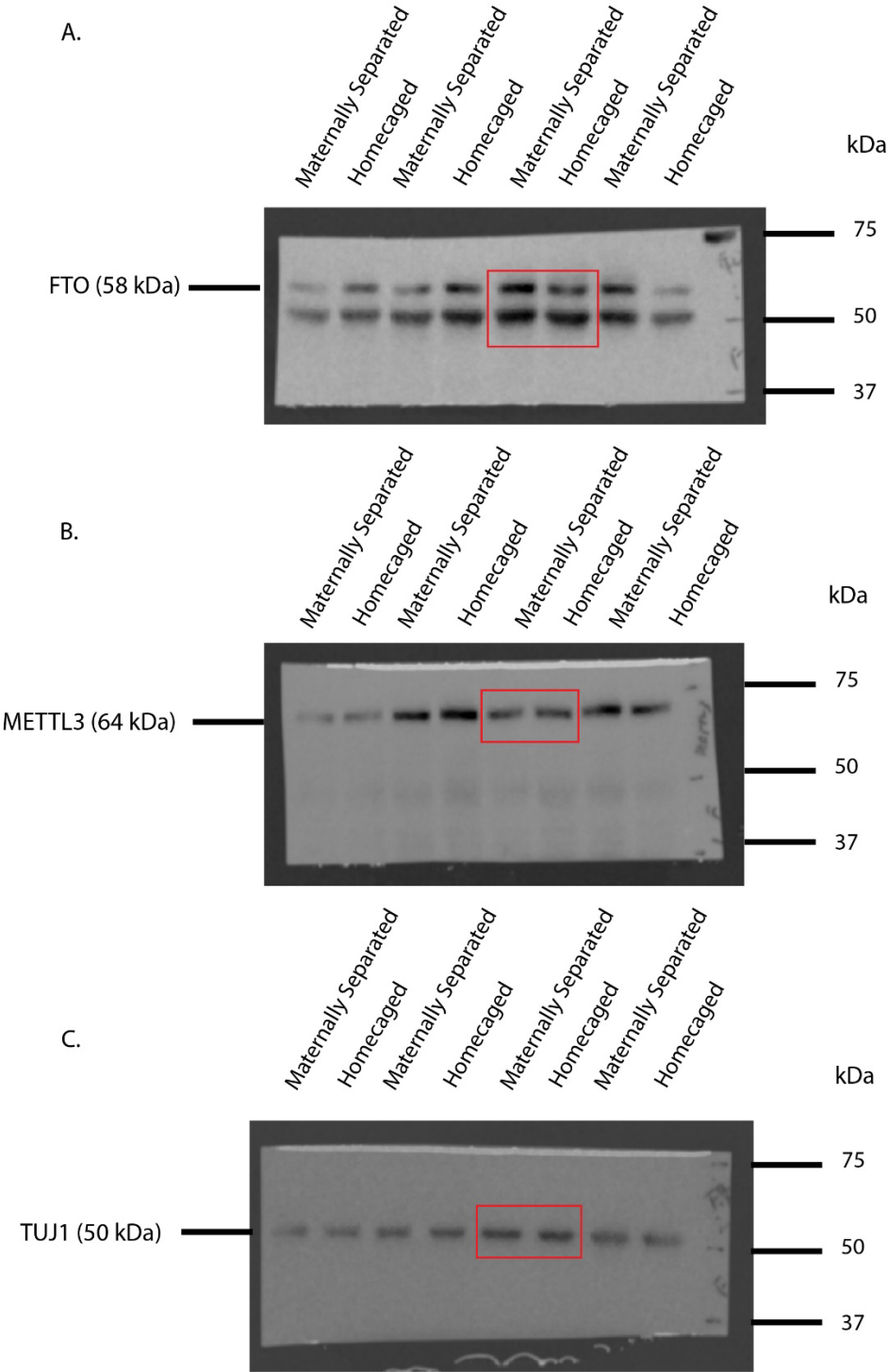
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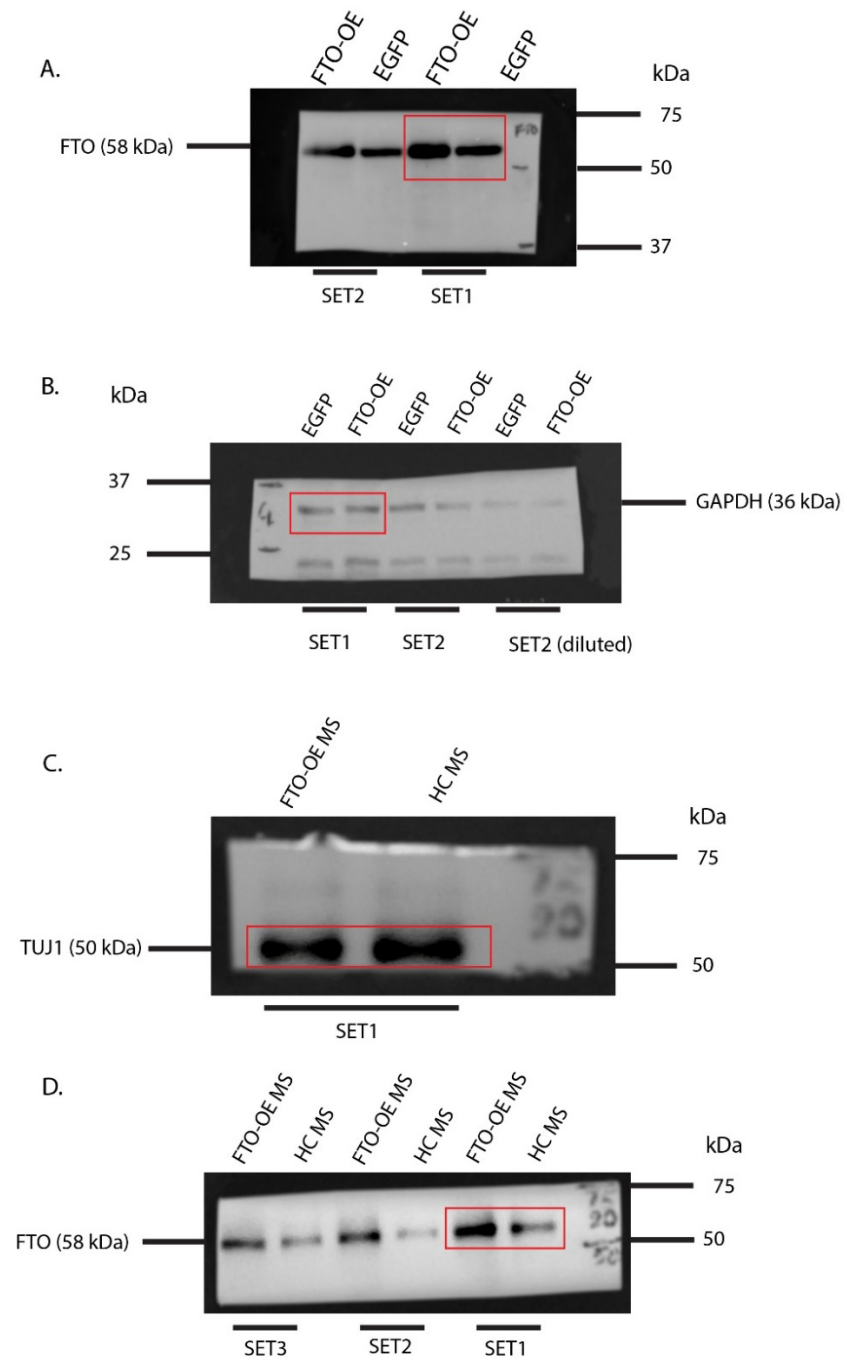
Supplementary Figure 10. Full immunoblots showing expression of **(A)** FTO, **(B)** METTL3 and **(C)** Tuj1 in males, in P28 males. (See Figure 2K).



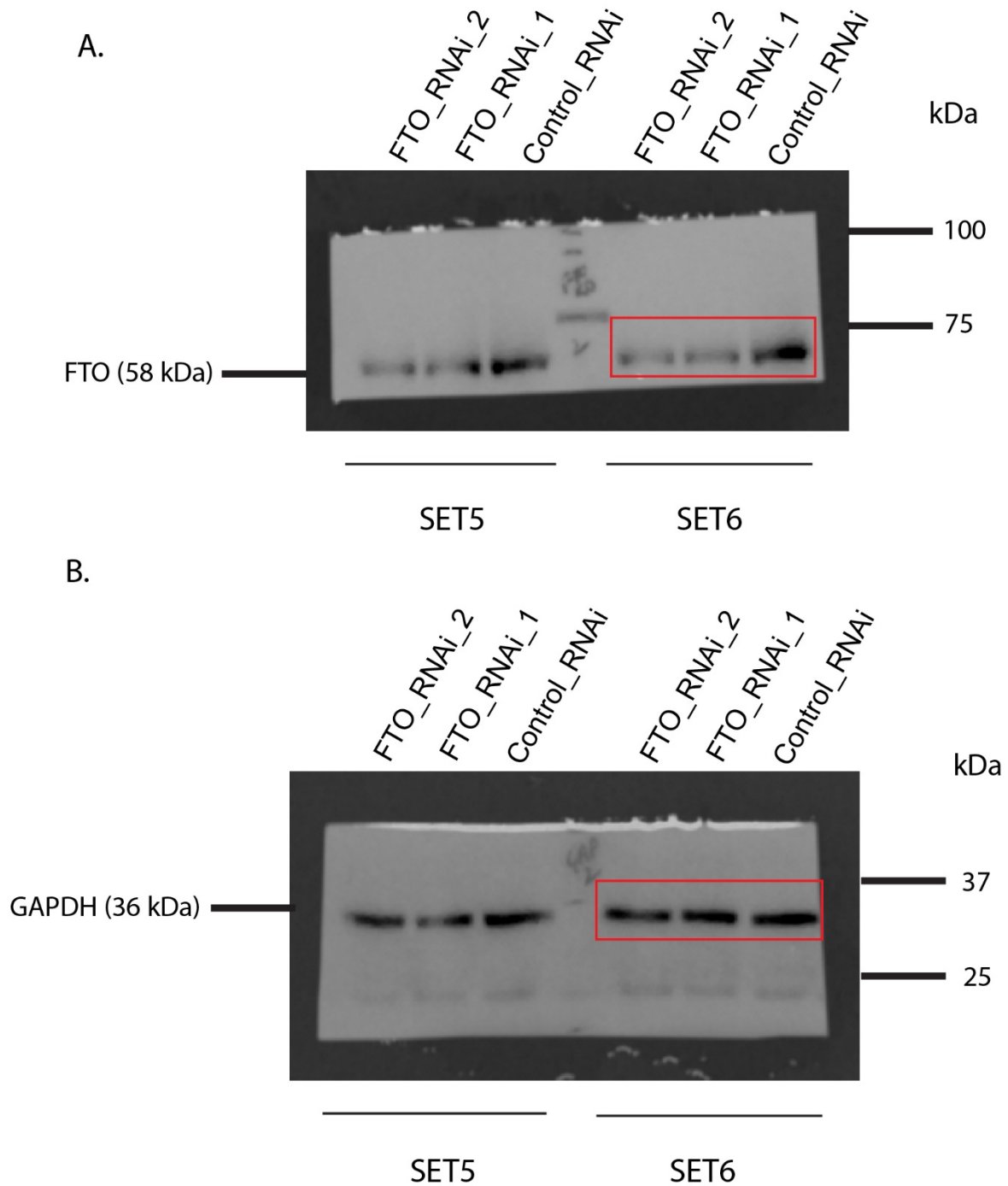
Supplementary Figure 11. Full immunoblots showing expression of **(A)** FTO, **(B)** METTL3 and **(C)** Tuj1, in P21 females. (See Supplementary Figure 2A).



Supplementary Figure 12. Full immunoblots showing expression of **(A)** FTO, **(B)** METTL3 and **(C)** Tubulin β 1, in P28 females. (See Supplementary Figure 2D).



Supplementary Figure 13. Full immunoblots for FTO overexpression verification showing expression of **(A)** FTO and **(B)** GAPDH in HEK293T cells. (See Supplementary Figure 3B). Full immunoblot showing expression of **(A)** FTO and **(B)** GAPDH **(C)** Tuj1 **(D)** FTO in C57BL/6 mice. (See Supplementary Figure 3D).



Supplementary Figure 14. Full immunoblots for FTO knockdown experiment showing expression of **(A)** FTO and **(B)** GAPDH. (See Supplementary Figure 7B).