Huntingtin-mediated axonal transport requires arginine methylation by PRMT6

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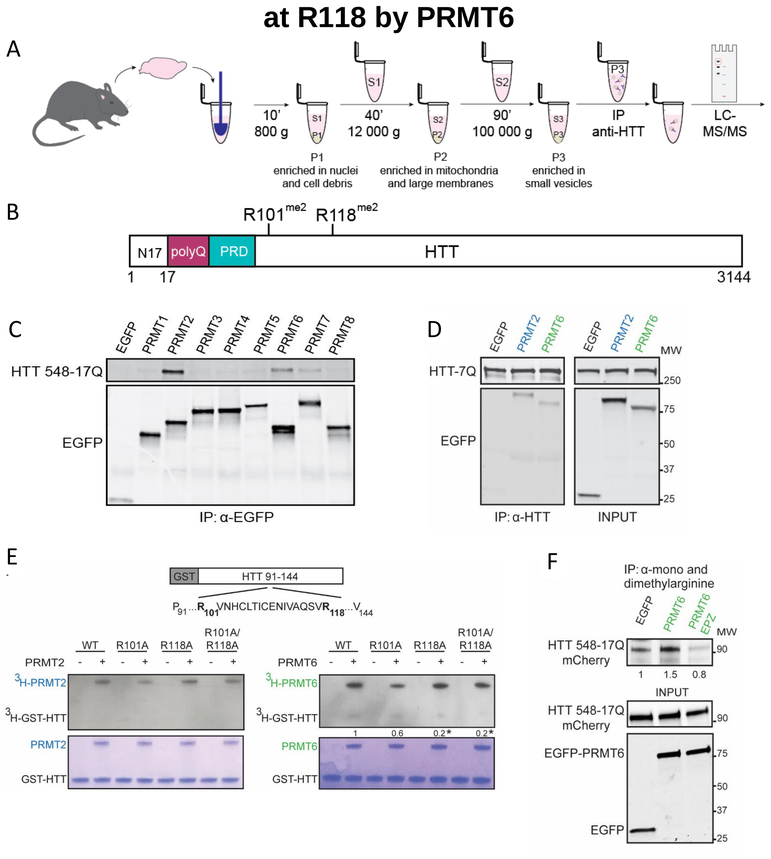
Abstract

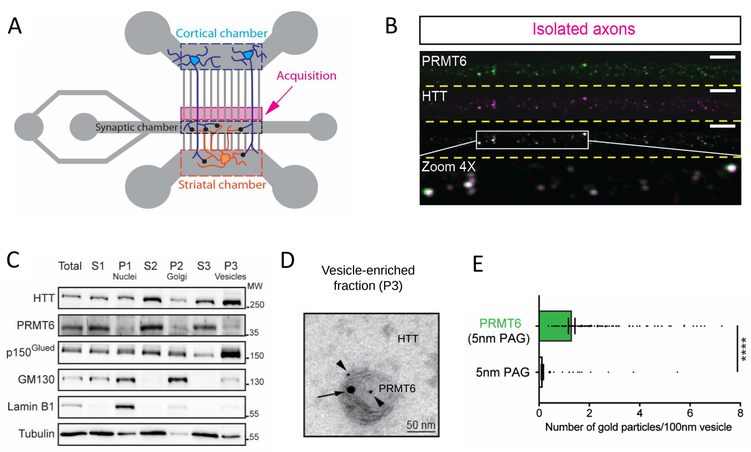
The huntingtin (HTT) protein transports various organelles, including vesicles containing neurotrophic factors, from embryonic development throughout life. To better understand how HTT mediates axonal transport and why this function is disrupted in Huntington's disease (HD), we study vesicle-associated HTT and find that it is dimethylated at a highly conserved arginine residue (R118) by the protein arginine methyltransferase 6 (PRMT6). Without R118 methylation, HTT associates less with vesicles, anterograde trafficking is diminished, and neuronal death ensues—very similar to what occurs in HD. Inhibiting PRMT6 in HD cells and neurons exacerbates mutant HTT (mHTT) toxicity and impairs axonal trafficking, whereas overexpressing PRMT6 restores axonal transport and neuronal viability, except in the presence of a methylation-defective variant of mHTT. In HD flies, overexpressing PRMT6 rescues axonal defects and eclosion. Arginine methylation thus regulates HTT-mediated vesicular transport along the axon, and increasing HTT methylation could be of therapeutic interest for HD.

1.Vesicle-associated HTT is methylated at R118 by PRMT6



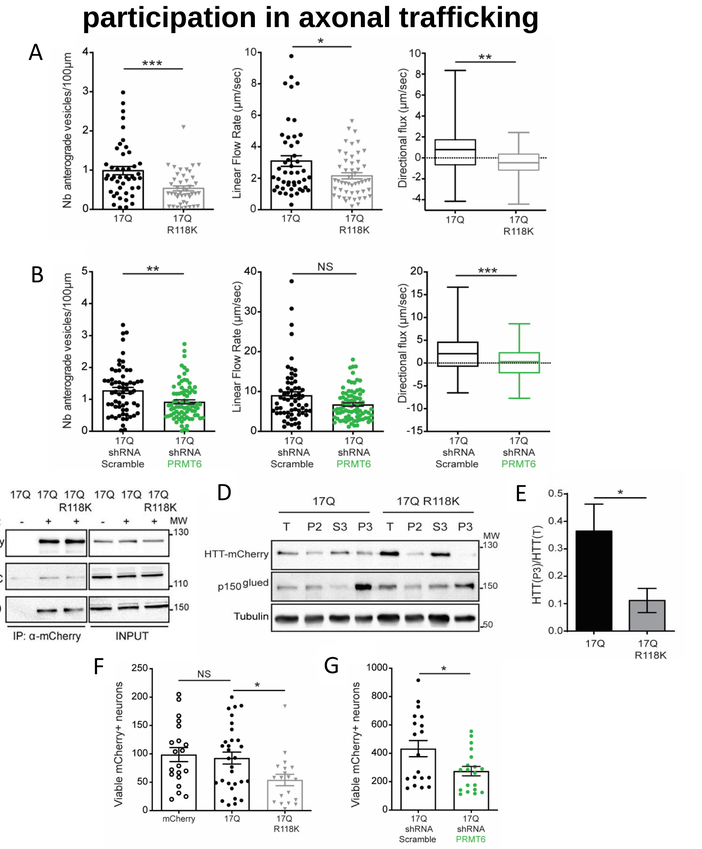
(A and B) Schematic of vesicle-associated HTT purification from mouse brain for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis and identification of methylated arginine residues (R101 and R118). N17, N-terminal 17 amino acids; PRD, proline-rich domain; polyQ, polyglutamine tract. **(C)** Immunoprecipitation (IP) assay in HEK293T cells overexpressing HTT 548-17Q together with either soluble EGFP or EGFP-tagged PRMT1–PRMT8. **(D)** IP assay in STHdh$^{Q7/Q7}$ cells overexpressing EGFP-PRMT2 or EGFP-PRMT6 **(E)** In vitro methylation assay performed by incubating wild-type or mutant GST-HTT fusion proteins and recombinant PRMT2 (left panel) or PRMT6 (right panel) in the presence of [3H]-SAM. Top: fluorography. Bottom: Coomassie Brilliant blue staining. Student's t test, ⁎p < 0.05. Quantification (average) of [3H]-HTT/HTT ratio is shown at the bottom of the fluorography panel. **(F)** IP assay with an anti-mono- and di-methylarginine antibody in mouse cortical neurons overexpressing HTT 548-17Q-mCherry together with soluble EGFP or EGFP-PRMT6 treated or not with EPZ (10 μM). Quantification (average) of HTT IP/HTT input ratio is shown at the bottom of the IP panel.

2. PRMT6 localizes to axonal vesicles

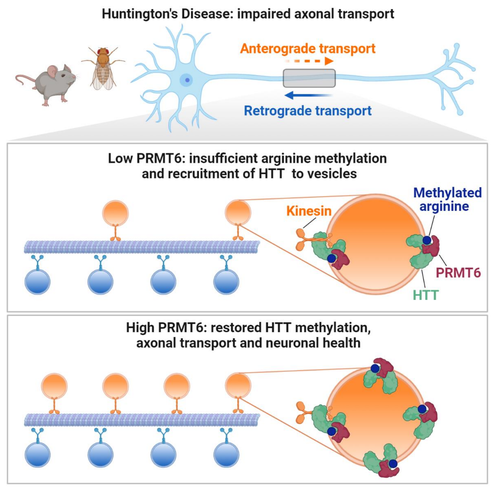


(A) Schematic depicting the microfluidic chamber reconstituting the corticostriatal network. **(B)** Immunofluorescence analysis of PRMT6 and HTT in DIV14 mouse primary neurons plated in microfluidic chambers as in (A). Bar, 2 μm. **(C)** Subcellular fractionation of mouse brain extracts. Purity of fractions was verified by immunoblotting for the presence of laminB1 (nuclear marker), GM130 (Golgi marker), and p150Glued (enriched in the P3 fraction). S1, S2, and S3 represent the corresponding cytosolic fractions. **(D)** Immunogold transmission electron microscopy (TEM) analysis of the P3 fraction from (E). PRMT6 is indicated with an arrowhead (5 nm gold nanoparticles); HTT is indicated with an arrow (15 nm gold nanoparticles). Bar, 50 nm. **(E)** Quantification of immunogold-labeled vesicles isolated from mouse brain from (D). PRMT6, 5nm gold nanoparticles with anti-PRMT6 primary antibody; 5 nm PAG, negative control without anti-PRMT6 primary antibody. Student's t test, ⁎⁎⁎⁎p < 0.0001.

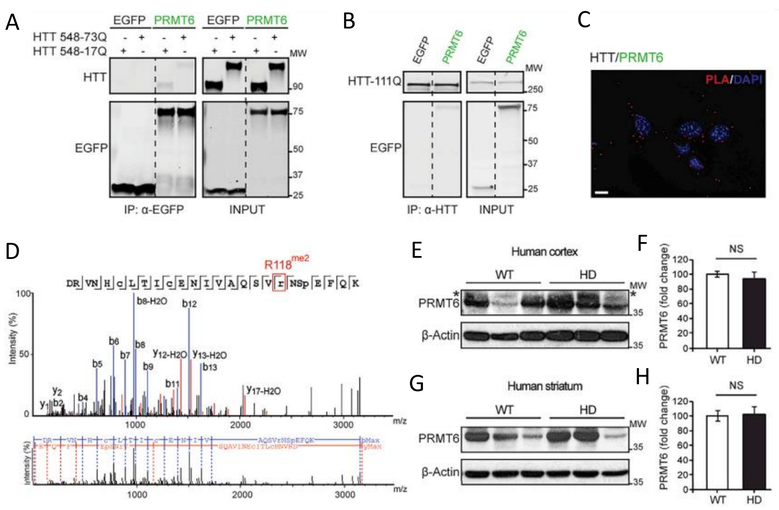
3. Methylation of HTT at R118 regulates its participation in axonal trafficking



A) Analysis of the trafficking kinetics in DIV14 cortical neurons transduced with lentiviral vectors expressing either wild-type HTT 548-17Q or HTT 548-17Q R118K fused to mCherry. Two-tailed non-parametric Mann-Whitney test, ⁎p < 0.05, ⁎⁎p < 0.01, and ⁎⁎⁎p < 0.001. **(B)** Analysis of trafficking kinetics in DIV14 cortical neurons transduced with lentiviral vectors expressing wild-type HTT 548-17Q fused to mCherry together with scrambled or PRMT6 shRNA. Two-tailed non-parametric Mann-Whitney test, ⁎⁎p < 0.01 and ⁎⁎⁎p < 0.001. **(C)** Immunoprecipitation assay in primary rat neurons transduced with mCherry-tagged HTT 548-17Q or HTT 548-17Q R118K. **(D)** Immunoblotting analysis of subcellular fractionation of primary rat cortical neurons transduced as in (A). **(E)** Quantification of HTT 548-mCherry signal in the P3 fraction over total fraction from (C). Student's t test, ⁎p < 0.05. **(F)** Analysis of cell viability in DIV11 mouse primary cortical neurons expressing either mCherry or HTT 548-17Q-mCherry or HTT 548-17Q R118K-mCherry. One-way ANOVA, Tukey's post hoc test, ⁎p < 0.05. **(G)** Analysis of cell viability in DIV11 mouse primary cortical neurons transduced with lentiviral vectors expressing HTT 548-17Q fused to mCherry together with scrambled or PRMT6 shRNA. One-way ANOVA, Tukey's post hoc test, ⁎p < 0.05.

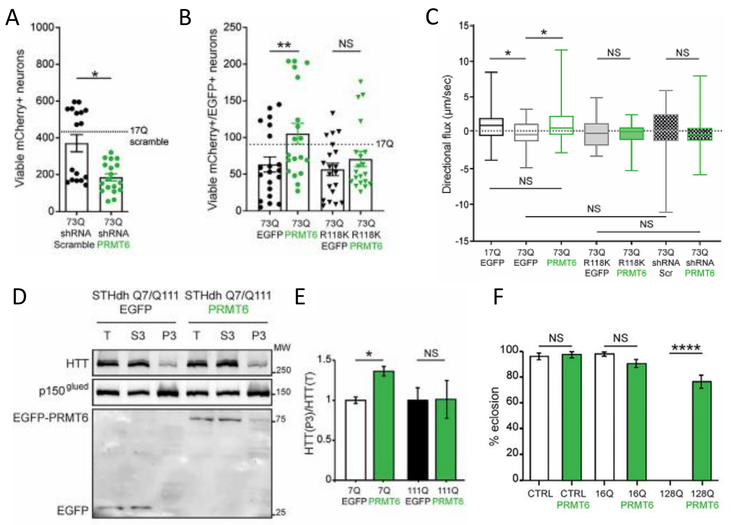


4. Arginine methylation of HTT regulates axonal trafficking



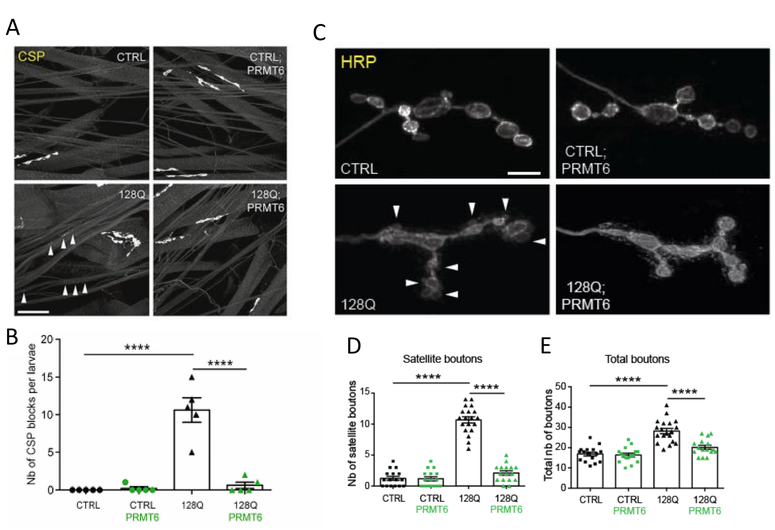
(A) CoIP assay in HEK293T cells overexpressing wild-type (HTT 548-17Q) or mutant N-terminal HTT (HTT 548-73Q) together with soluble EGFP or EGFP-tagged PRMT6. **(B)** CoIP assay in STHdh$^{Q111/Q111}$ cells overexpressing EGFP or EGFP-PRMT6. **(C)** PLA in STHdh$^{Q111/Q111}$ cells. Nuclei were revealed with DAPI. Shown are representative images from three independent experiments. Bar, 10 μm. **(D)** Electrospray ionization (ESI)-MS/MS analysis of human full-length mHTT (HTT-82Q) purified from transfected HEK293 cells. **(E and G)** Immunoblotting analysis of PRMT6 expression in the cortex (E) and striatum (G) of HD patients or healthy individuals. **(F and H)** Quantification of (E) and (G), respectively. Graph shows mean ± SEM; n = 3. Student's t test. NS, not significant.

5. PRMT6 modifies mHTT-induced toxicity



(A) Analysis of cell viability in DIV11 mouse primary cortical neurons transduced with lentiviral vectors expressing HTT 548-73Q fused to mCherry together with scrambled or PRMT6 shRNA. One-way ANOVA, Tukey's post hoc test, ⁎p < 0.05. **(B)** Analysis of cell viability in mouse primary cortical neurons expressing mCherry-tagged HTT 548-73Q or HTT 548-73Q R118K together with EGFP or EGFP-PRMT6. Two-way ANOVA, Tukey's post hoc test, ⁎⁎p < 0.01. **(C)** Analysis of the trafficking kinetics in DIV12–DIV14 neurons transduced with lentiviral vectors expressing mCherry-tagged HTT 548-17Q, HTT 548-73Q, or HTT 548-73Q R118K together with EGFP, EGFP-PRMT6, scrambled shRNA, and PRMT6 shRNA. Kruskal-Wallis test, Dunn's post hoc, ⁎p < 0.05. **(D)** Immunoblotting analysis of subcellular fractionation of STHdhQ7/Q111 transduced with EGFP or EGFP-PRMT6 lentivirus. **(F)** Quantification of HTT and mHTT signals in the P3 fraction over total fraction from (E). Student's t test, ⁎p < 0.05. **(G)** Eclosion assays in control flies or flies expressing full-length HTT 16Q or HTT 128Q. Two-way ANOVA, Tukey's post hoc test, ⁎⁎⁎⁎p < 0.0001.

6. PRMT6 expression rescues HTT-mediated axonal and neuromuscular junction defects in Drosophila



(A) Representative larval segmental nerves from ELAV-GeneSwitch (ElavGS); w1118 (CTRL), PRMT6 (PRMT6; w1118), HTT 128Q (128Q), or PRMT6; HTT 128Q (PRMT6; 128Q) larvae (CSP, cysteine string protein [arrowhead]). Bar, 15 μM. **(B)** Quantification of CSP blocks in (A) (⁎⁎⁎⁎p < 0.0001, n = 5). **(C)** Immunofluorescence images of neuromuscular junctions at muscle 4 segment A2-A3 stained with the presynaptic marker horseradish peroxidase (HRP; arrowhead). Bar, 10 μM. **(D and E)** Quantification of the average number of satellite boutons (D) and the total number of boutons (E) (n = 16–19). Graphs in (B) and (D)–(E) represent mean ± SEM. Statistical comparison in (B) and (D)–(E) were determined using one-way ANOVA with Tukey's multiple comparisons test (⁎p < 0.05 and ⁎⁎⁎⁎p < 0.0001; NS, not significant).

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