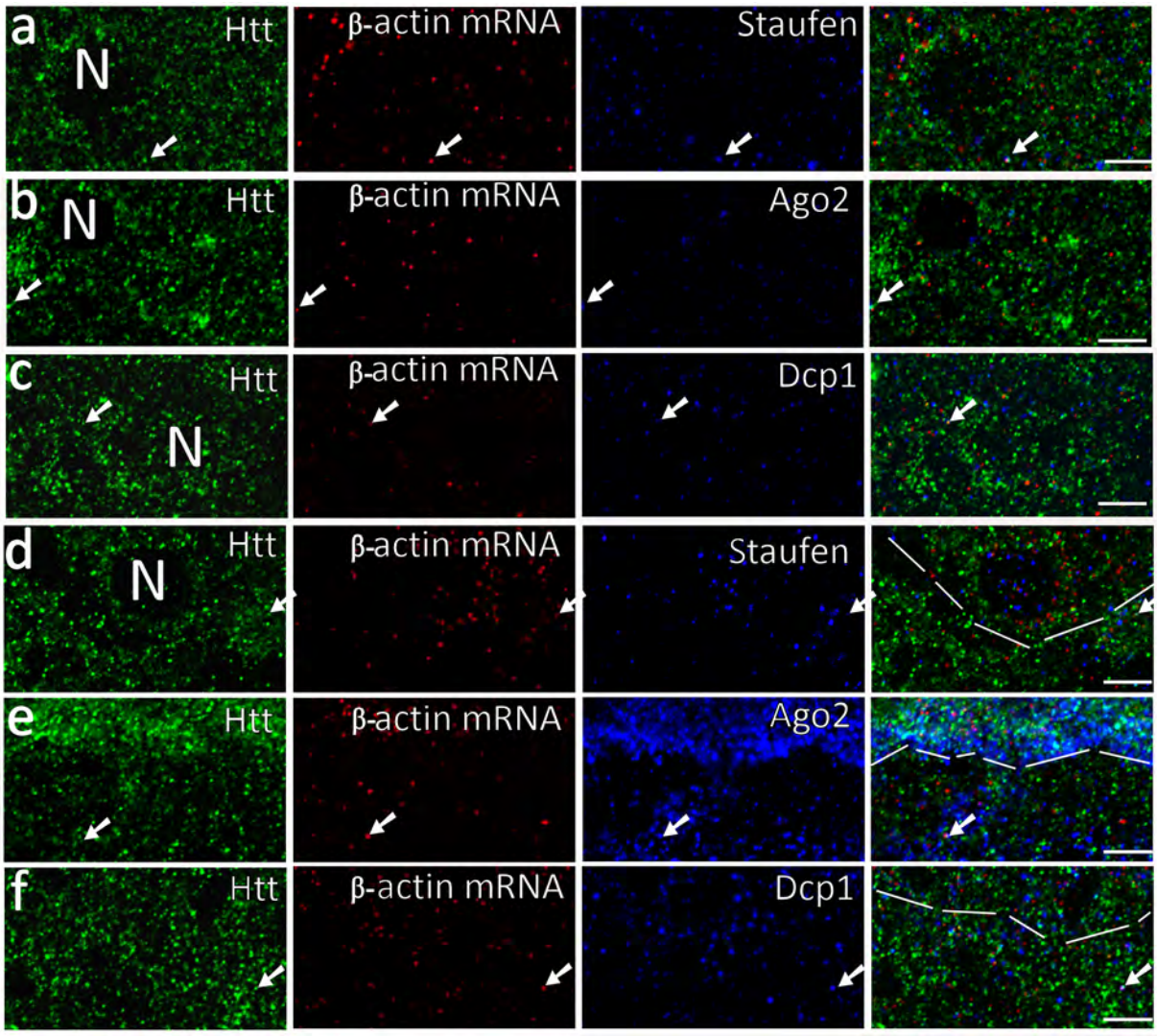
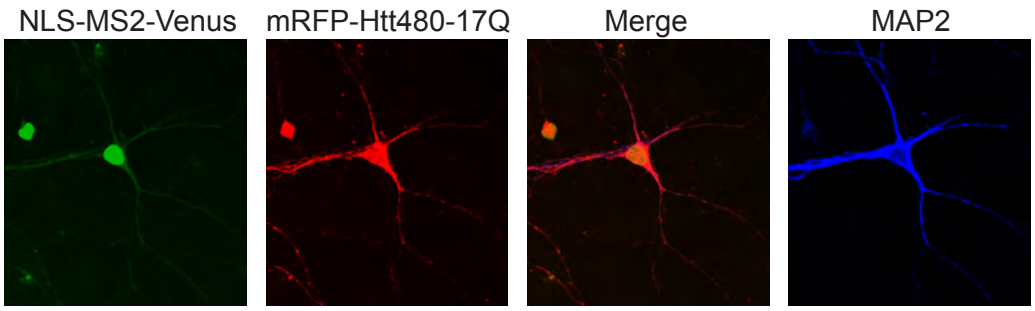
SUPPLEMENTARY INFORMATION

Huntingtin mediates dendritic transport of β-actin mRNA in rat neurons

Bin Ma, Jeffrey N Savas, Man-Shan Yu, Brady P Culver, Moses V Chao, and Naoko Tanese

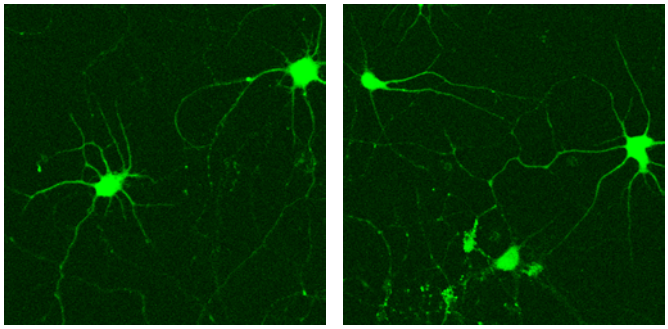


Supplementary Figure S1 Co-localization of Htt and β-actin mRNA in rat (P25) cortex (a-c), and hippocampus (d-f). Htt, β-actin mRNA, and RNA-binding proteins are shown in green, red, and blue, respectively. Co-localization of Htt/β-actin mRNA, Htt/RNA-binding proteins and β-actin mRNA/RNA-binding proteins are shown in yellow, cyan and magenta in merged images, respectively. The arrows indicate the co-localization of Htt, β-actin mRNA, and RNA-binding proteins. N: Nucleus. Scale bar: 10.0 μm. (a) Htt co-localizes with transport RNPs detected with staufen in the cortex. (b) Htt co-localizes with Ago2-containing RNPs in the cortex. (c) Htt co-localizes with a P-body marker DCP1 in the brain cortex. (d) Htt co-localizes with transport RNPs detected with staufen in the hippocampus. (e) Htt co-localizes with Ago2-containing RNPs in the hippocampus. (f) Htt co-localizes with a P-body marker DCP1 in the hippocampus. The white line in (d-f) outlines the border between the soma (upper part) and dendrites (lower part) in the hippocampus.

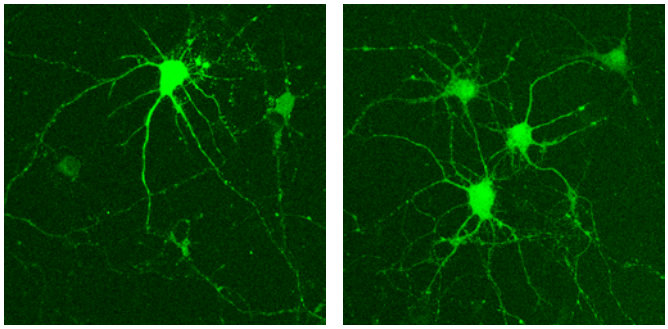


Supplementary Figure S2 Primary rat cortical neurons were transfected with plasmids encoding NLS-MS2-Venus and mRFP-Htt480-17Q but without an MS2-mRNA reporter. The MS2-Venus protein is confined to the nucleus via NLS in the absence of a target mRNA containing MS2 binding sites.

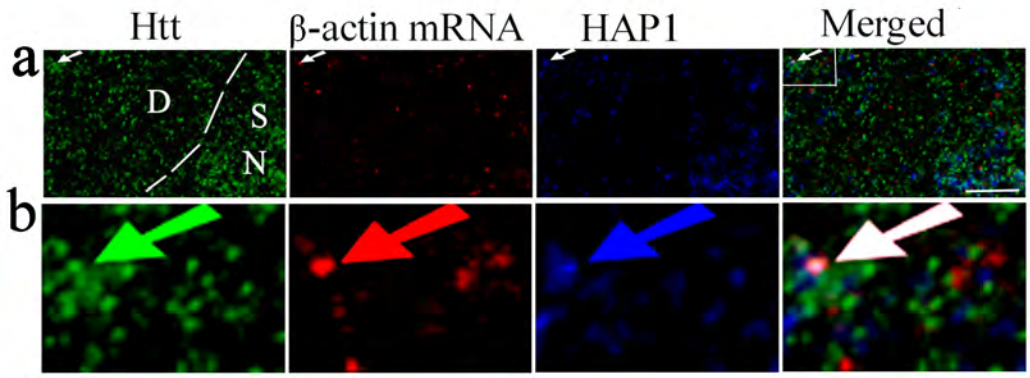
shRNA-scrambled-GFP



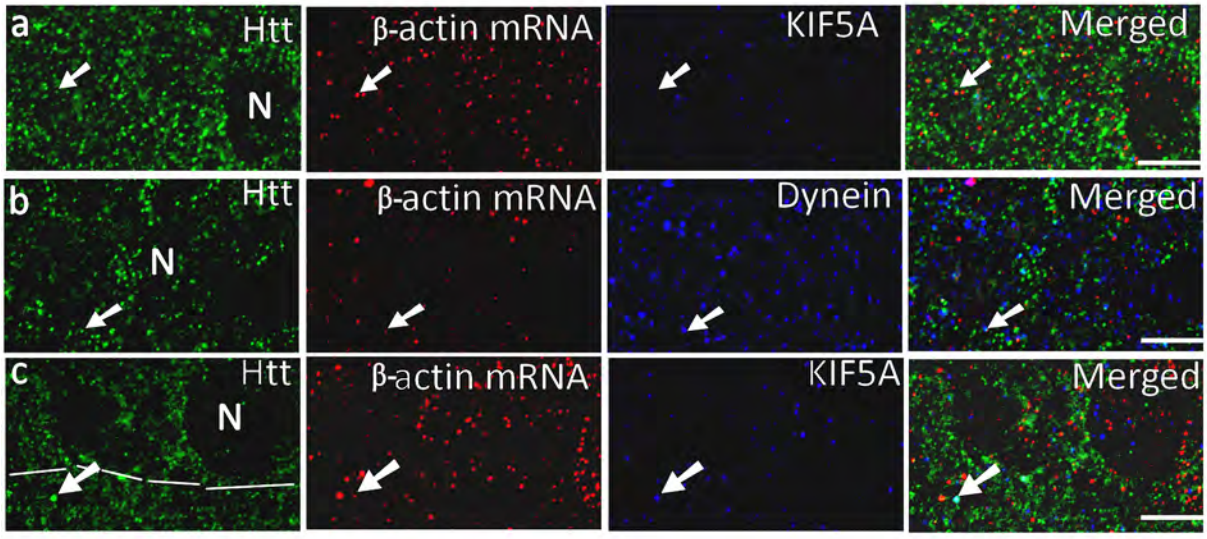
shRNA-Htt-1-GFP



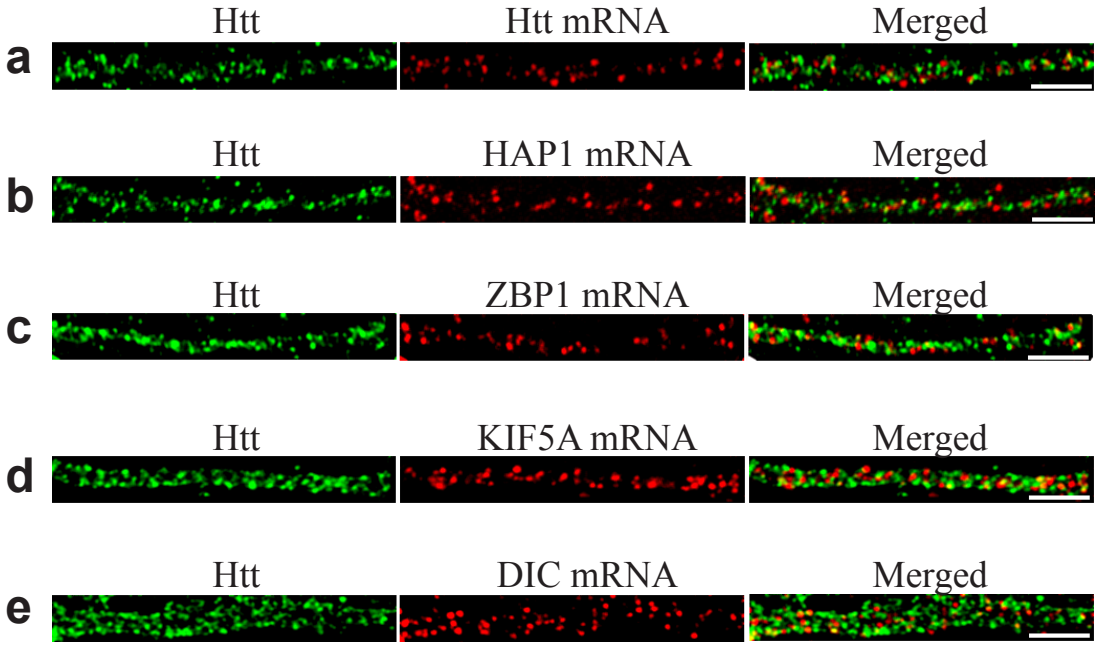
Supplementary Figure S3 Primary rat cortical neurons at DIV4 were infected with lentivirus expressing scrambled shRNA (top) or shRNA-Htt-1 (bottom) for 96 hrs. The neurons were fixed and GFP expression examined under confocal microscope.



Supplementary Figure S4 Co-localization of Htt, β-actin mRNA, and HAP1 and in the rat (P25) hippocampus. Htt, β-actin mRNA, and HAP1 are shown in green, red, and blue, respectively. Co-localization of Htt/β-actin mRNA, Htt/HAP1, and β-actin mRNA/HAP1 are shown in yellow, cyan, and magenta in the merged images, respectively. The arrows indicate the co-localization of Htt, β-actin mRNA, and HAP1. (a) The white line marks the border between the soma (right) and dendrites (left) in the hippocampus. N: nucleus; S: soma; D: dendrite. Scale bar: 10.0 μm. (b) High-magnification views of the boxed region in the merged image in (a).



Supplementary Figure S5 Co-localization of Htt, β-actin mRNA, and motor proteins in rat (P25) cortex (a-b), and hippocampus (c). Scale bar: 10.0 μm. Htt, β-actin mRNA, and motor proteins are shown in green, red, and blue, respectively. Co-localization of Htt/β-actin mRNA, Htt/motor proteins, and β-actin mRNA/motor proteins is shown in yellow, cyan, and magenta in the merged images, respectively. The arrows indicate the co-localization of Htt, β-actin mRNA, and motor proteins. N: nucleus. (a) Htt co-localizes with β-actin mRNA and KIF5A in the cortex. (b) Htt co-localizes with β-actin mRNA and dynein in the cortex. (c) Htt co-localizes with β-actin mRNA and KIF5A in the hippocampus. The white dashed line in (c) marks the border of the soma (upper part) and dendrites (lower part) in the hippocampus.



Supplementary Figure S6 Htt protein co-localizes with mRNAs encoding components of the dendritic transport machinery. Htt protein and indicated mRNAs are shown in green and red, respectively. Co-localization of Htt and mRNA is seen in yellow in merged images. Htt mRNA, HAP1 mRNA, ZBP1 mRNA, kinesin-1 mRNA, and DIC mRNA are shown in (a), (b), (c), (d), and (e), respectively. The left part of each image is the proximal part of the dendrite. Scale bar: 5.0 μm.

Supplementary Table S1

Dynamics of β-actin mRNA trafficking

Total # structures		130	100%
Oscillating/Stationary		109	83.8%
Anterograde		8	6.2%
Retrograde		13	10.0%
Velocity		0.0026-0.078 μm/s	
Anterograde (μm/s)		Retrograde (μm/s)	
1	0.068	9	0.078
2	0.041	10	0.064
3	0.0026	11	0.018
4	0.019	12	0.018
5	0.011	13	0.029
6	0.014	14	0.011
7	0.021	15	0.023
8	0.021	16	0.057
		17	0.018
		18	0.031
		19	0.012
		20	0.048
		21	0.012
Average	0.0207	Average	0.0322

SD	0.0247	SD	0.0222
Average total	0.0294		
SD total	0.0215		
p value	0.449; No significant difference between anterograde and retrograde		

Supplementary Table S2

Parameters and settings used for confocal microscopy

Fluorescent dye	**Laser**	**Excitation wave length (nm)**	**Emission filter (nm)**	**Detector**
Alexa 488	Argon	488	BP 505–530	Normal
DyLight 549	HeNe1	543	BP 560–615	Normal
DyLight 649	HeNe2	633	649–756	META

SUPPLEMENTARY VIDEO LEGENDS

Supplementary Video 1 Co-trafficking of Htt with β-actin mRNA in rat cortical neurons was imaged live over 652.5 seconds. β-actin mRNA (detected by NLS-MS2-Venus) is in green and mRFP-Htt480-17Q in red. Co-localization of Htt with β-actin mRNA appears in yellow in this merged image. The left part of the image is the proximal part of the dendrite.

Supplementary Video 2 Co-trafficking of HAP1 with β-actin mRNA in rat cortical neurons was imaged live over 1656.5 seconds. β-actin mRNA (detected by NLS-MS2-Venus) is in green and HAP1 in red. Co-localization of HAP1 with β-actin mRNA appears in yellow in the merged image. The left part of each image is the proximal part of the dendrite.

Supplementary Video 3 Trafficking of β-actin mRNA in rat cortical neurons was imaged live over 1016.0 seconds. β-actin mRNA is detected by NLS-MS2-Venus.

Supplementary Video 4 Trafficking of β-actin mRNA in rat cortical neurons after a 30-minute treatment with 2 μg/ml nocodazole was imaged live over 1016.0 seconds. β-actin mRNA is detected by NLS-MS2-Venus.

Supplementary Video 5 Co-trafficking of KIF5A with β-actin mRNA in rat cortical neurons was imaged live over 107.0 seconds. β-actin mRNA (detected by NLS-MS2-

RFP) is in red and KIF5A (GFP) in green. Co-localization of KIF5A with β-actin mRNA is shown in yellow in the merged image. The second granule from the left shows anterograde movement along the dendrites. The left part of each image is the proximal part of the dendrites. The distance that the granule traveled is 1.39 μm. The velocity of the granule is 0.013 μm/s.

Supplementary Video 6 Co-trafficking of DIC with β-actin mRNA in rat cortical neurons was imaged live over 1040.4 seconds. β-actin mRNA (detected by NLS-MS2-RFP) is in red and DIC (EGFP) in green. Co-localization of DIC with β-actin mRNA is shown in yellow in the merged image. The left part of each image is the proximal part of the dendrites. The distance that the granule traveled is 1.57 μm.