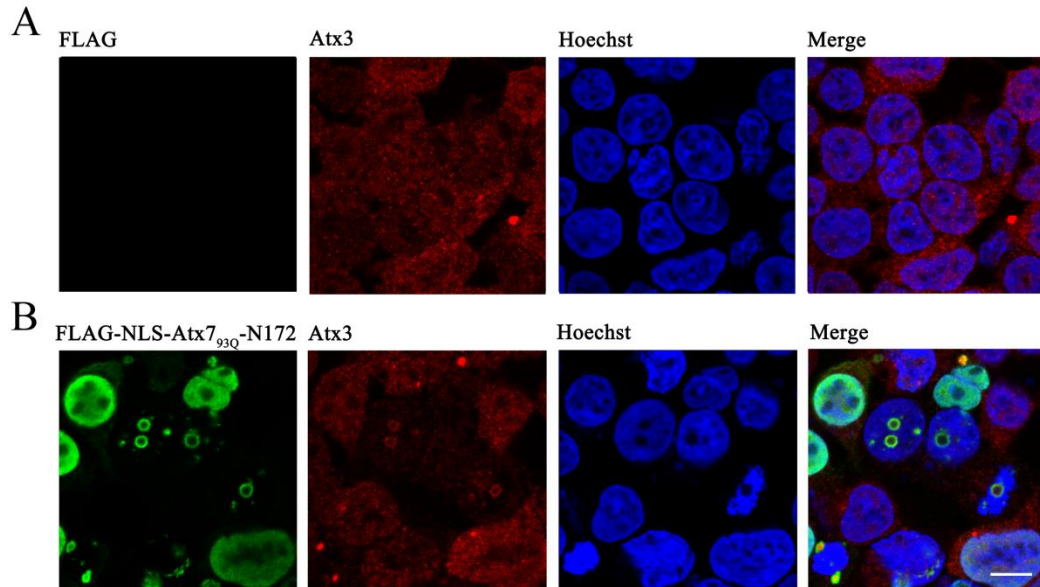


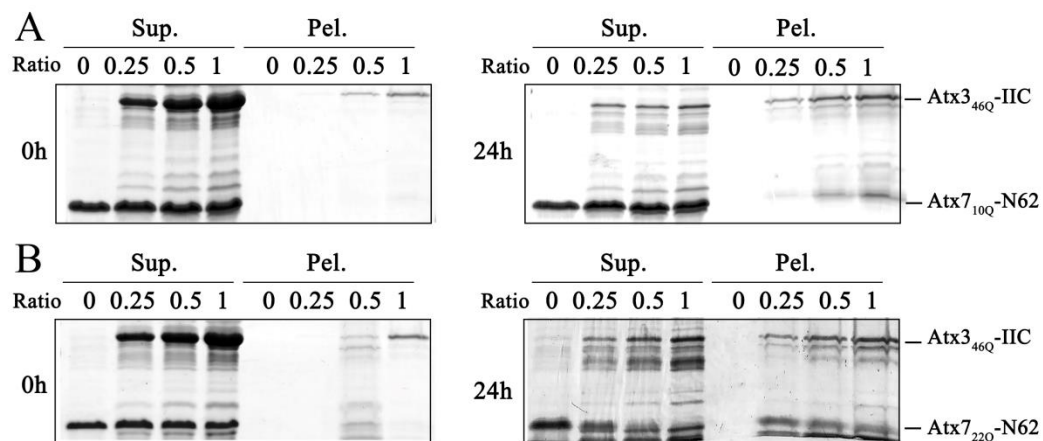
Supplementary Figure S1. The PQE Htt fragment sequesters endogenous Atx3 into insoluble aggregates

Supernatant/pellet fractionation combined with western blot analysis was carried out to detect the sequestration of endogenous Atx3 by WT Htt_{100Q}-N90 and its 3KR mutant. HEK 293T cells were transfected with empty vector, Htt_{100Q}-N90 or its 3KR mutant. About 48 h after transfection, the cell lysates were subject to supernatant/pellet fractionation and then western blot analysis with an anti-FLAG or anti-Atx3 antibody. WT, Htt_{100Q}-N90; 3KR, the 3KR mutant of Htt_{100Q}-N90; Vec., vector; Sup., supernatant; Pel., pellet.



Supplementary Figure S2. Immunofluorescence images showing sequestration of endogenous Atx3 into inclusions by a nuclear form of the PQE Atx7 fragment in cells

(A) Vector control. (B) NLS-Atx7_{93Q}-N172. HEK 293T cells were transfected with the FLAG-NLS-Atx7_{93Q}-N172 plasmid or control vector and then subjected to confocal microscopic imaging. Atx7_{93Q}-N172 and endogenous Atx3 were stained with an anti-FLAG (green) or anti-Atx3 antibody (red), and nuclei were stained with Hoechst (blue). Scale bar: 10 μ m. NLS, nuclear localization signal.



Supplementary Figure S3. Co-precipitation of Atx3_{46Q}-IIC with Atx7-N62 *in vitro*

(A) Atx_{346Q}-IIC with Atx_{710Q}-N62. (B) Atx_{346Q}-IIC with Atx_{722Q}-N62. The protein mixtures were incubated for 24 h and then subject to supernatant/pellet fractionation and SDS-PAGE (15% gel). Atx_{346Q}-IIC was mixed with Atx_{710Q}-N62 or Atx_{722Q}-N62 (50 μM) at an increasing molar ratio of Atx_{346Q}-IIC/Atx7-N62 (0:1, 0.25:1, 0.5:1 and 1:1). Atx3-IIC, C-terminal fragment (residues 221–373) of isoform-II Atx3; Atx7-N62, N-terminal fragment (residues 1–62) of Atx7. Sup., supernatant; Pel., pellet.

Supplementary Table S1. List of the constructs applied in this study

Constructs	Vectors	Restriction enzyme sites	Additional
Trx-Atx _{710Q} -N62	pET-32M	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-62, N-Trx tag
Trx-Atx _{722Q} -N62	pET-32M	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-62, N-Trx tag
Trx-Atx _{733Q} -N62	pET-32M	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-62, N-Trx tag
Trx-Atx _{722Q} -N62(T3N9)	pET-32M	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-62, Q32T/Q38N
Atx _{322Q} -IIC	pET-22b(+)	<i>Bam</i> HI/ <i>Xho</i> I	Residues 221-373, isoform II
Atx _{36Q} -IIC	pET-22b(+)	<i>Bam</i> HI/ <i>Xho</i> I	Residues 221-373, isoform II
GB1-Atx _{346Q} -IIC	pGBTNH	<i>Nde</i> I/ <i>Xho</i> I	Residues 221-373, isoform II, GB1 tag
HA-Atx _{322Q}	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Full length, isoform I, WT
HA-Atx _{310Q}	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Full length, isoform I
HA-Atx _{33Q}	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Full length, isoform I
HA-Atx _{223Q} -N317	pcDNA3.0	<i>Bam</i> HI/ <i>Not</i> I	Residues 1-317
HA-Atx _{29Q} -N317	pcDNA3.0	<i>Bam</i> HI/ <i>Not</i> I	Residues 1-317
HA-Atx _{23Q} -N317	pcDNA3.0	<i>Bam</i> HI/ <i>Not</i> I	Residues 1-317
HA-AR _{23Q}	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Full length, WT
HA-AR _{10Q}	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Full length
HA-AR _{3Q}	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Full length
FLAG-AR	pcDNA3.0	Seamless cloning kit	3x FLAG, Full length
pGL3-ARE	pGL3	<i>Sac</i> I/ <i>Xho</i> I	3x ARE
FLAG-Htt _{18Q} -N552	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-552
FLAG-Htt _{100Q} -N552	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-552

FLAG-Htt _{100Q} -N90	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-90
FLAG-Htt _{100Q} -N90-3KR	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-90, K6R/K9R/K15R
FLAG-Atx _{710Q} -N172	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-172
FLAG-Atx _{793Q} -N172	pcDNA3.0	<i>Bam</i> HI/ <i>Xho</i> I	Residues 1-172

Supplementary Table S2. List of the antibodies used in this study

Antibody	Source	Catalog number
Anti-FLAG (mouse)	Sigma-Aldrich	F1804
Anti-FLAG (rabbit)	Proteintech	20543-1-AP
Anti-HA (mouse)	Sigma-Aldrich	H9658
Anti-AR (mouse)	Santa Cruz	sc-7305
Anti-Atx3 (rabbit)	Abclonal	A1243
GAPDH (mouse)	Proteintech	60004-1-Ig