Supplementary information

Methods

Purification of HTT protein

HTT-GST fusion protein coding sequences were cloned into pGEX-6P vectors. Recombinant plasmids were transformed into E. coli BL-21 cells by heat shock and production of HTT protein fragments was induced with 100 μM IPTG at 16°C and 200 rpm overnight. After lysis of bacteria by sonication, GST fusion proteins were captured with Glutathione Sepharose 4B beads (GE Healthcare). HTT protein fragments were cleaved from GST tags by incubating beads overnight at 4°C with PreScission Protease enzyme (GE Healthcare). Purified fusion proteins were then dialyzed against PBS with 1% Tween20 and stored at -80°C until further use. HTT proteins were produced containing the N-terminal sequence of HTT protein with 548 amino acids and a polyglutamine repeat expansion of the indicated length.

	Disease stage	Number of samples analysed	Mean CAG repeat length	Standard deviation
Monocytes	Pre	8	43.00	1.927
	Early	10	45.20	5.453
	Moderate	7	43.29	2.752
T cells	Pre	7	42.57	1.618
	Early	10	45.20	5.453
	Moderate	7	43.29	2.752
B cells	Pre	7	42.57	1.618
	Early	9	45.78	5.449
	Moderate	7	43.29	2.752
Buccal cells	Pre	15	43.53	1.767
	Early	31	43.45	3.520
	Moderate	5	44.60	1.949

Supplementary Table 1. HTT CAG repeat length for HD patients participating in the study. The CAG repeat length for patients in the cohort was 40-47 CAG repeats, with the exception of one early stage patient with a larger repeat size of 59.

		Caudate atrophy (%/year)			Ventricular expansion (ml/year)			Whole brain atrophy (%/year)		
	n[A]	Crude	Adjusted [B]	Burden adjusted [C]	Crude	Adjusted [B]	Burden adjusted [C]	Crude	Adjusted [B]	Burden adjusted [C]
Monocytes	11	0.031	0.026	0.007	0.013	0.016	0.093	0.005	0.010	0.138
T cells	11	0.047	0.086	0.099	0.090	0.121	0.685	0.052	0.049	0.518
B cells	10	0.215	0.289	0.474	0.410	0.487	0.864	0.289	0.360	0.883
Buccal cells	18	0.786	0.621	0.392	0.560	0.475	0.475	0.545	0.484	0.806

Supplementary Table 2 P-values to assess the level of association between mHTT levels in peripheral immune cells and brain atrophy/expansion rates.

[A] One mHTT observation (two for buccal epithelial cells) is based on the mean of two measurements taken for that subject

[B] Adjusted for age and gender

[C] Additionally adjusted for disease burden

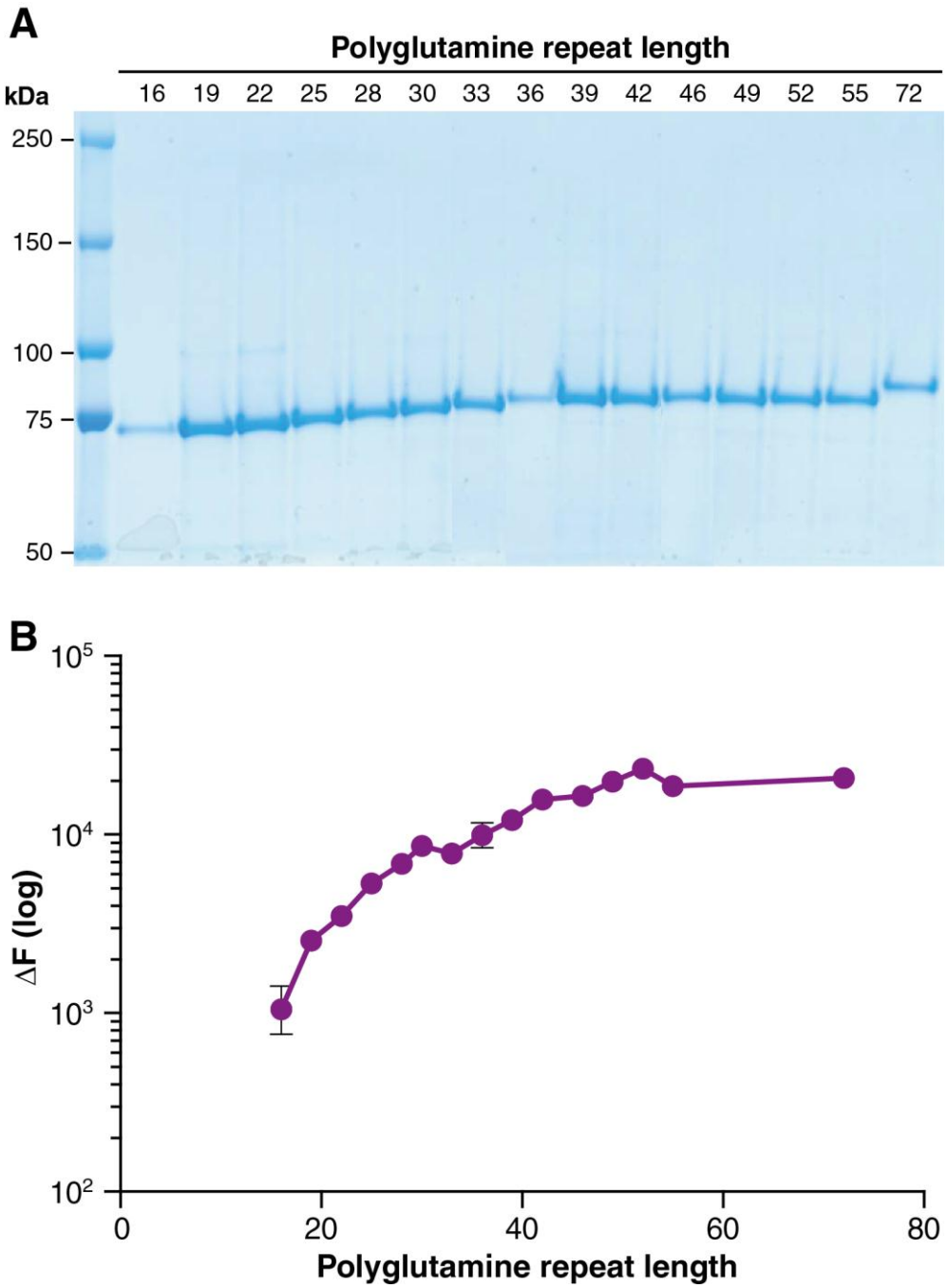
Antibody	Epitope	TR-FRET label	Description	Source
IgG	–	-	IgG	Alpha Diagnostic (#20008-250)
2B7	HTT aa 7-13	Terbium cryptate donor fluorophore (CisBio)	Mouse monoclonal IgG	(1)
4C9	HTT aa 51-71	-	Mouse monoclonal IgG	Novartis Pharma AG
3B5H10	N171 (65Q)	-	Mouse monoclonal IgG	Sigma Aldrich (P1874)
MW1	Polyglutamine	D2 acceptor fluorophore (CisBio)	Mouse monoclonal IgG	(2) Provided by the Developmental Studies Hybridoma Bank (developed under the auspices of the NICHD, maintained by the University of Iowa).
1H6	HTT aa 116-129	-	Mouse monoclonal IgG	(3)
2166	HTT aa 443-457	AlexaFluor (A)488 acceptor fluorophore (Invitrogen)	Mouse monoclonal IgG	Millipore (MAB2166)

Supplementary Table 3 Table of primary antibodies used in the study.

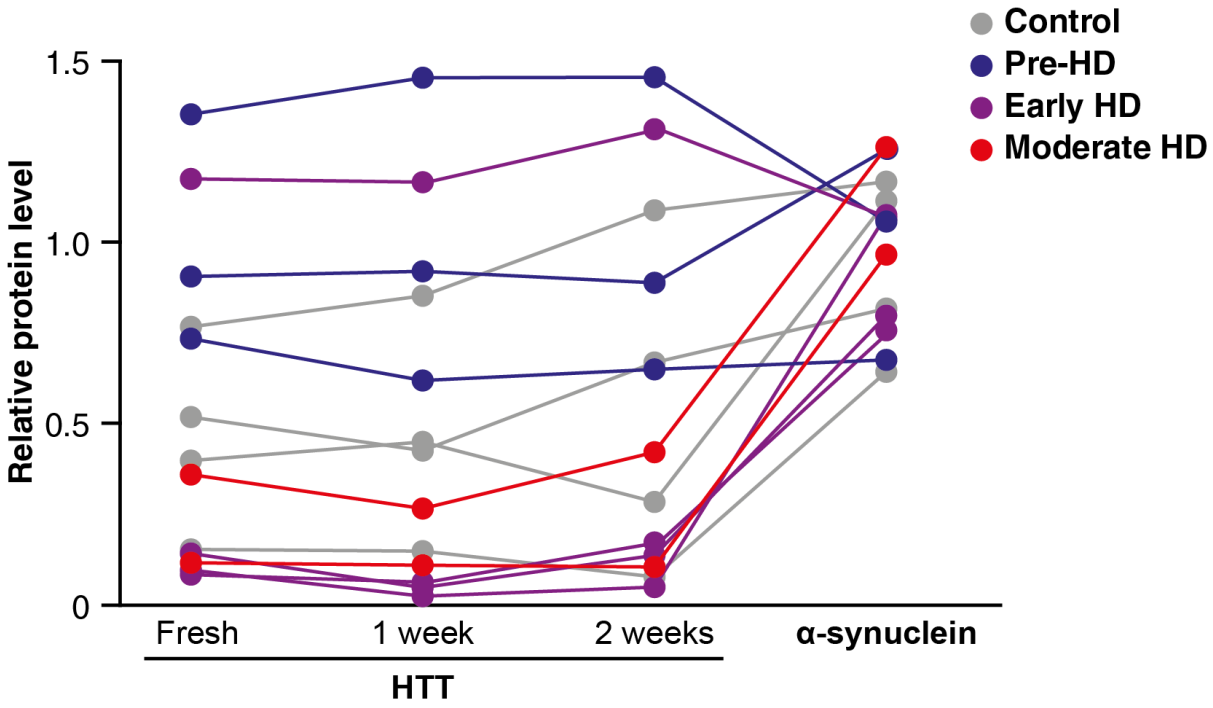
(1) Weiss A, et al. *Anal Biochem.* 2011;410(2):304-6.

(2) Ko J, Ou S, Patterson PH. *Brain Res Bull.* 2001;56(3-4):319-29.

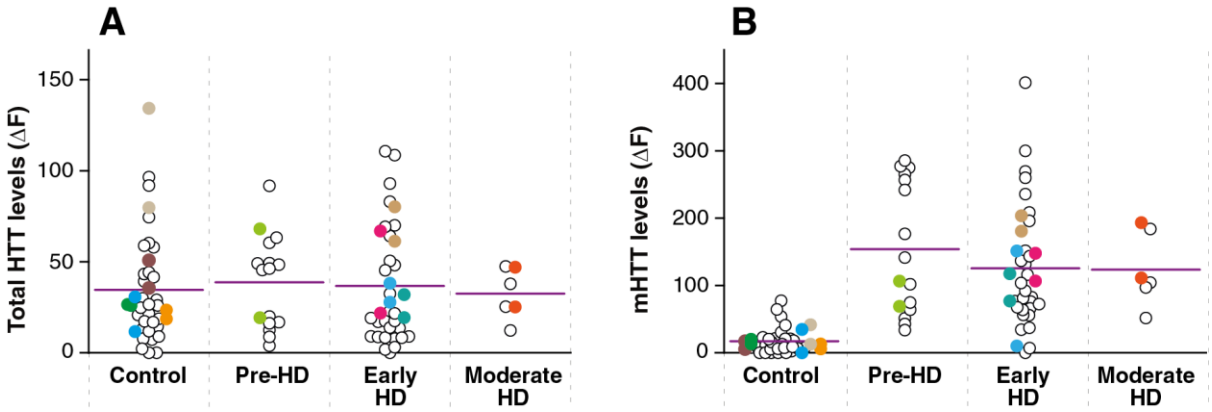
(3) Lunkes A, et al. *Mol Cell*. 2002;10(2):259-69.



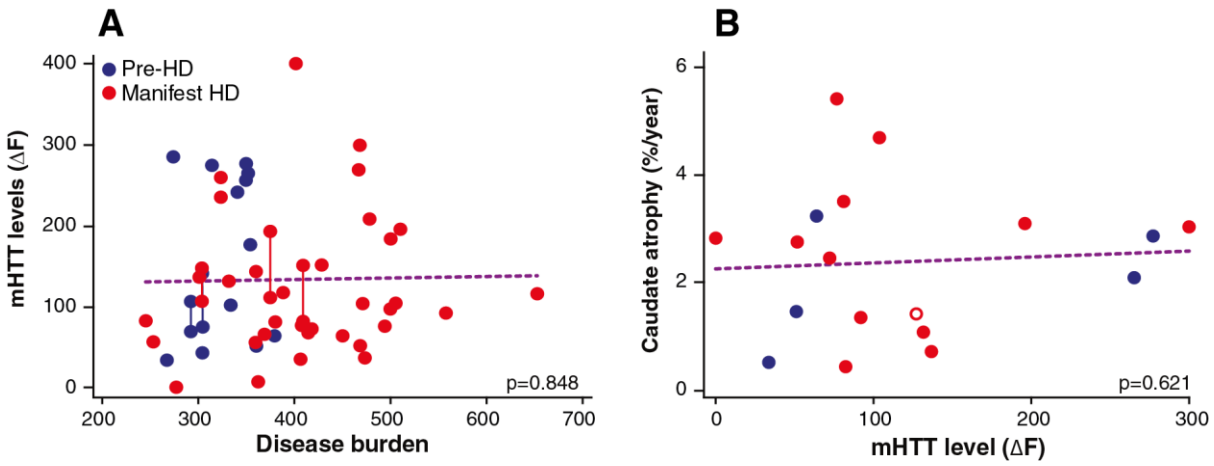
Supplementary Figure 1 TR-FRET using 2B7-MW1 detects mHTT protein with a 10-20 fold higher sensitivity than wild-type HTT protein (**A**) Coomassie-stained gel for lengths and purity verification of N-terminal HTT protein fragments (548aa) with increasing polyglutamine repeat lengths. GST-tagged HTT proteins were expressed in E. coli and isolated via PreScission column purification followed by GST-cleavage. (**B**) 2B7-MW1 TR-FRET signal intensities for detection of 11 fmol purified HTT protein with increasing polyglutamine repeat lengths.



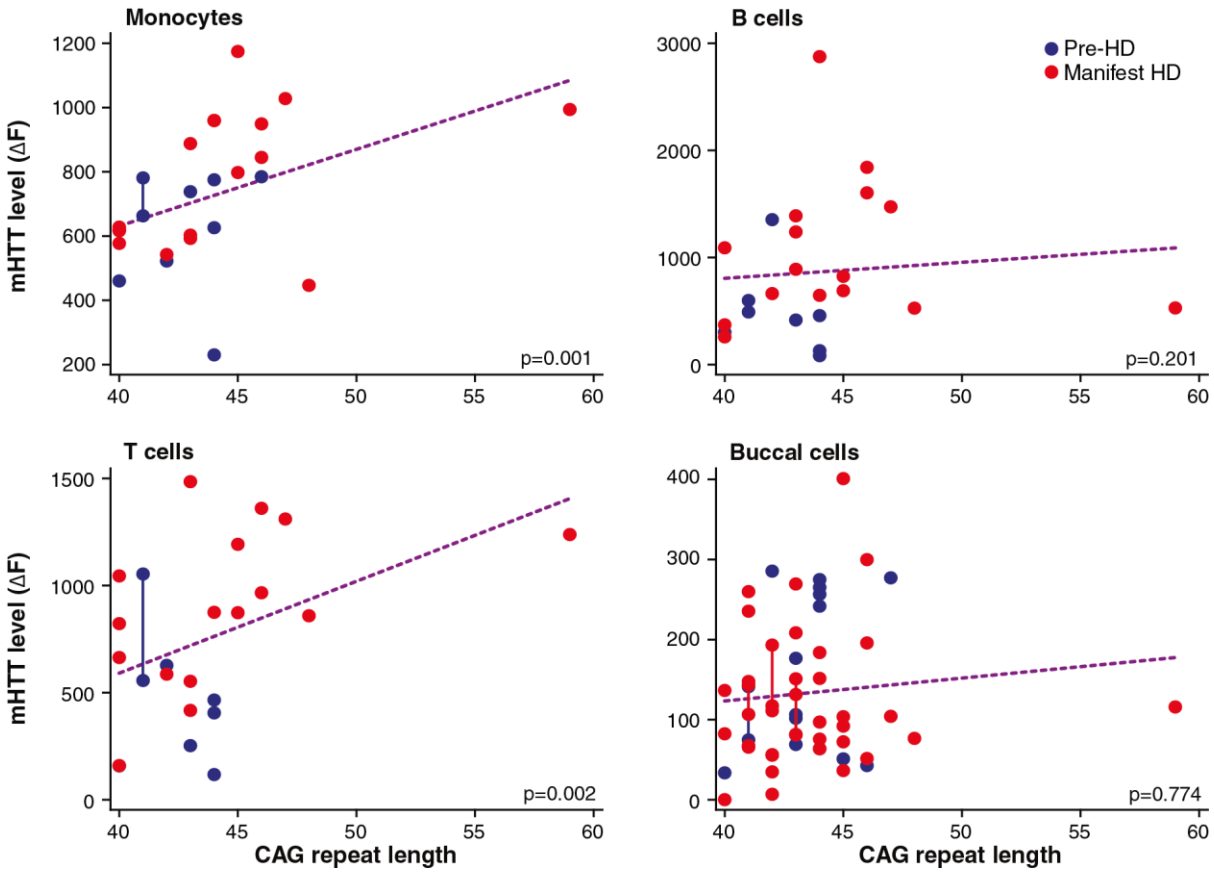
Supplementary Figure 2 TR-FRET quantification of HTT protein is robust and reproducible. HTT protein levels were quantified directly after lysis (fresh), after one week storage at -80°C and a single freeze/thaw cycle (1 week), and after two weeks storage at -80°C and two freeze/thaw cycles with detection by an independent HTT antibody and fluorophore pair (2 weeks). HTT protein level quantification was reproducible for the three independent measurements, verifying the reliability and robustness of the assay. Specificity of the signal for HTT protein was verified by analysis of the same samples with TR-FRET assays for an unrelated protein, α-synuclein (example data for total HTT in primary monocytes).



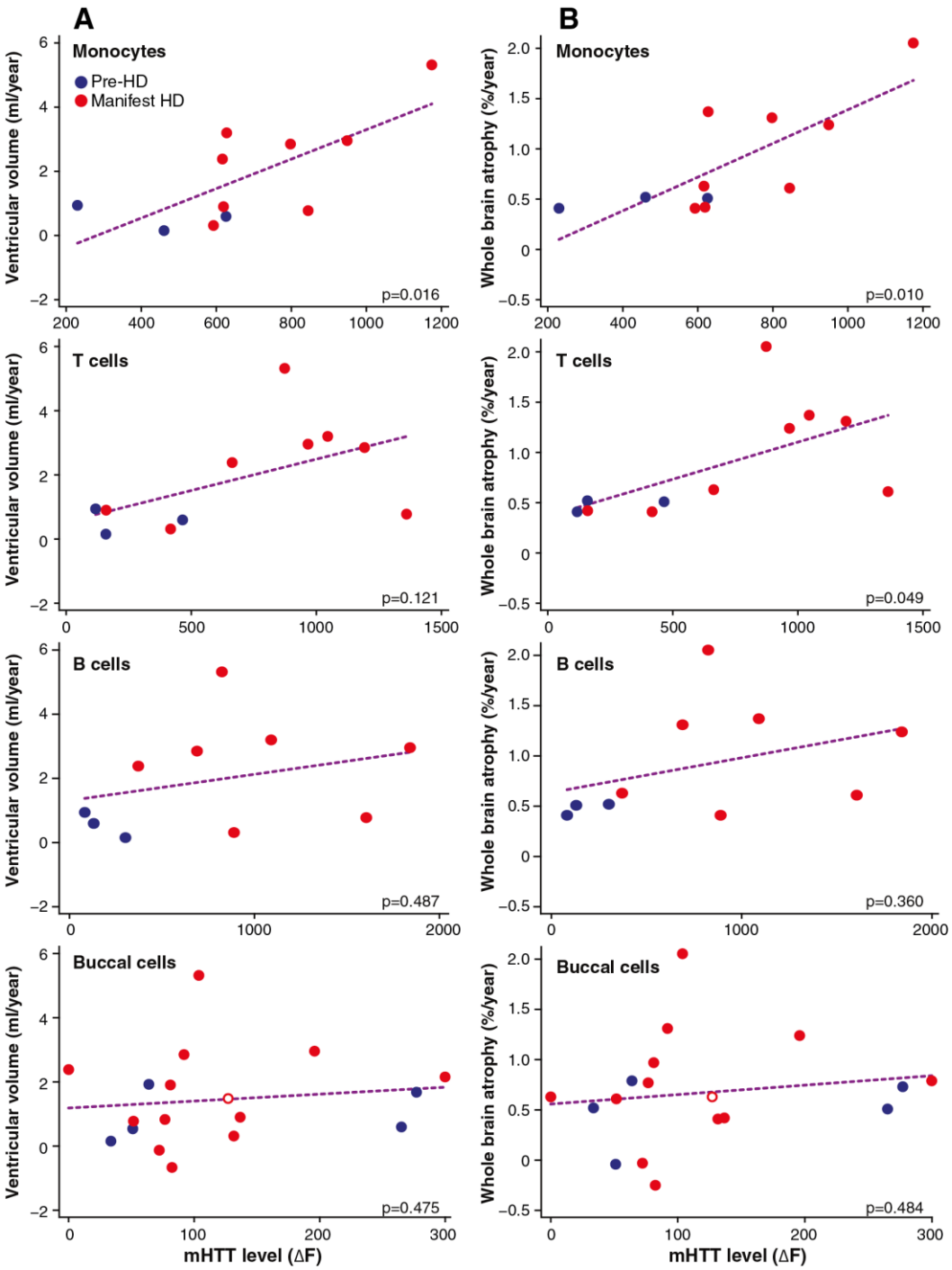
Supplementary Figure 3 Relationship between HTT levels in buccal epithelial cells and disease stage. Total and mutant HTT protein levels were quantified by TR-FRET in buccal epithelial cells. (**A**) Total HTT levels in buccal epithelial cells showed no significant differences between HD patients and control subjects, or between HD gene carriers at different disease stages. (**B**) Mutant HTT protein was detected in samples from HD patients and pre-manifest HD mutation carriers, as compared to controls. No significant differences in mean mHTT levels between different disease stages were observed. Colored circles indicate multiple samples from a single subject.



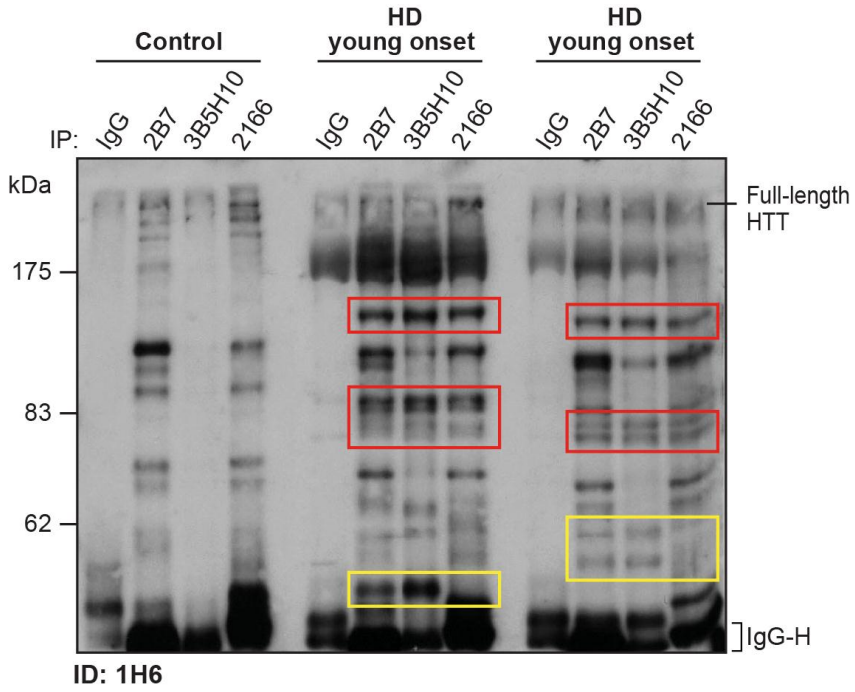
Supplementary Figure 4 Associations between mHTT levels in buccal epithelial cells and disease burden score and caudate atrophy rate. (**A**) Mutant HTT protein levels in buccal epithelial cells do not show an association with HD disease burden score. Repeated measurements for a single subject are joined by a line. (**B**) Mutant HTT levels in buccal epithelial cells are not associated with caudate atrophy rates measured by serial volumetric MRI. Hollow points indicate an average of two mHTT readings for a single subject.



Supplementary Figure 5 Associations between mHTT levels in peripheral immune cells and CAG repeat length. Mutant HTT protein levels in monocytes and T cells, but not B cells and buccal epithelial cells, show a statistically significant positive association with CAG repeat length. Repeated measurements for a single subject are joined by a line. Consistent with other analyses, p-values are adjusted for age and gender. The analysis performed to obtain these p-values is analogous to that for the analysis of the association between mHTT and disease burden score, as described in the statistical methods.



Supplementary Figure 6 Associations between mHTT levels in peripheral immune cells and ventricular expansion and whole brain atrophy rates. Mutant HTT levels in monocytes are significantly associated with (**A**) ventricular expansion and (**B**) whole brain atrophy rates measured by serial volumetric MRI. A weak association was also observed between mHTT levels in T cells and whole brain atrophy. Hollow points indicate an average of two mHTT readings for a single subject.



Supplementary Figure 7 Mutant HTT protein fragments are present in HD PBMCs. HTT protein in PBMCs from two young-onset HD patients (*HTT* CAG repeat lengths of 76 and 59) and an age-matched control was immunoprecipitated with 2B7, 3B5H10 or 2166 anti-HTT antibodies, as compared to an IgG control. Immunoprecipitates were blotted with the 1H6 anti-HTT antibody. Several mHTT-specific bands were found in the lysates from the young-onset HD patient PBMCs (colored boxes), including N-terminal HTT fragments (yellow boxes) immunoprecipitated with the 2B7 and 3B5H10 antibodies, but not with the more C-terminal epitope-binding 2166. IP = immunoprecipitation; ID = immunodetection; IgG-H = antibody heavy chain.