

Supplemental Information

Title

Analysis of mutant and total huntingtin expression in Huntington's disease murine models.

Authors

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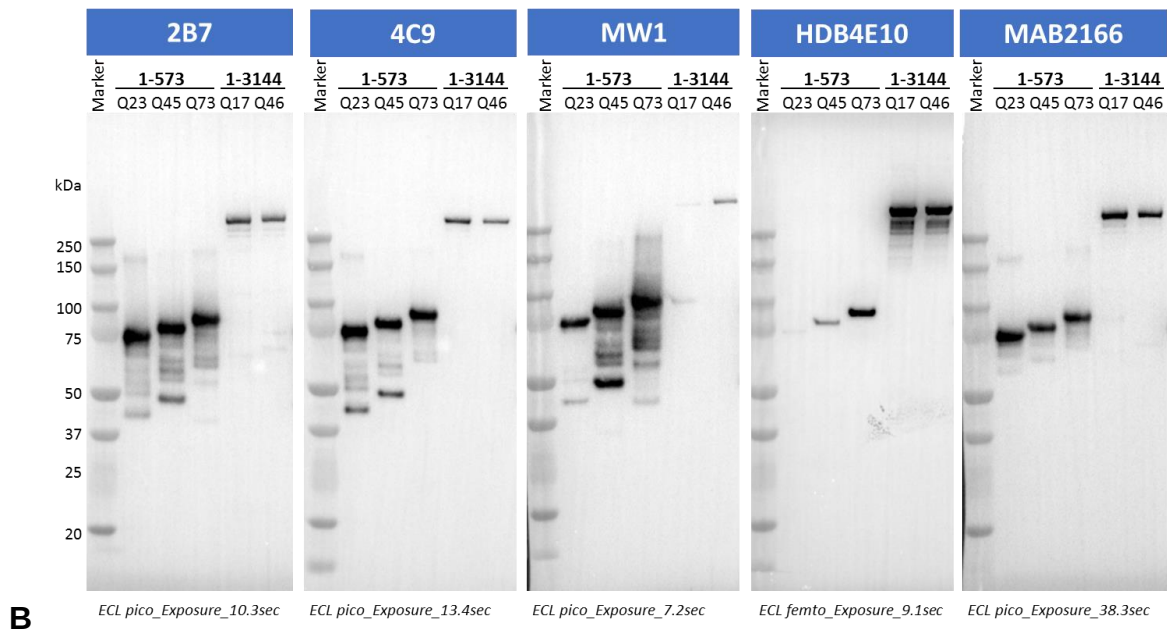
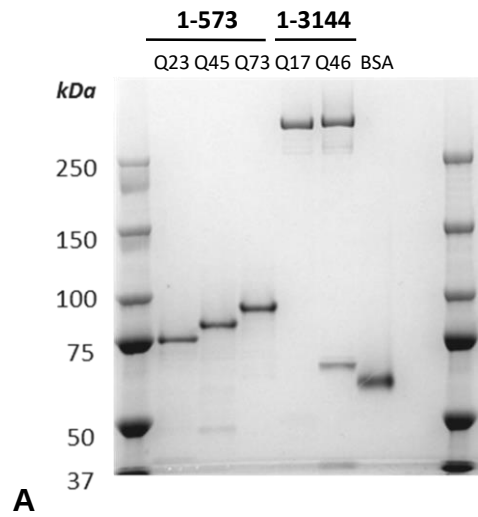
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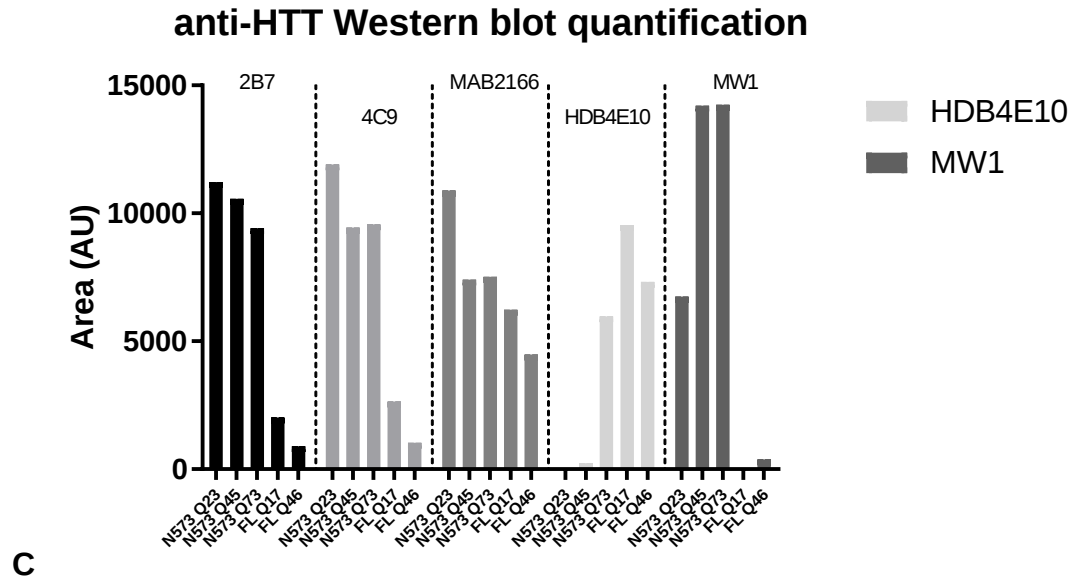
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S-1 Full-length Western blot of HTT recombinant proteins



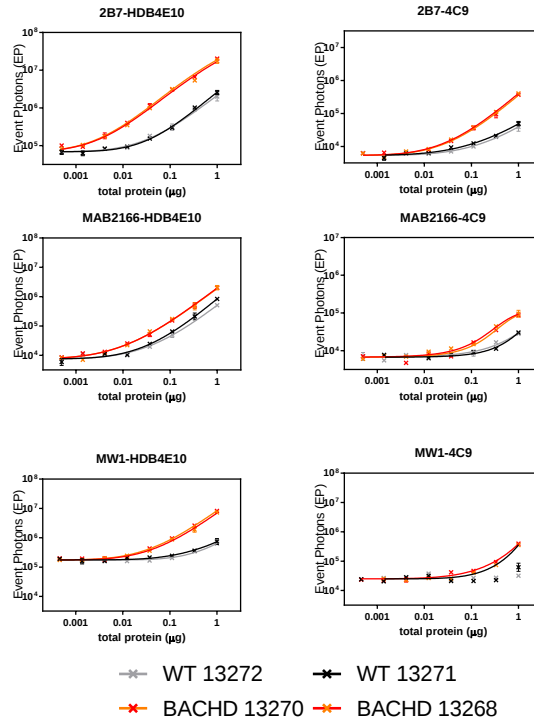


(A) Coomassie staining of the five standard protein used in the present work (left to right for each panel: N573 Q22, N573 Q45, N573 Q72, FL Q17, FL Q46, BSA 1 ug). (B) high exposure Western blots of the five standard proteins (left to right for each panel: N573 Q22, N573 Q45, N573 Q72, FL Q17, FL Q46) stained with the five antibodies used in the present work. (C) densitometry of the Western blot HTT bands in panel (B)

S-2 Fibroblast cell information

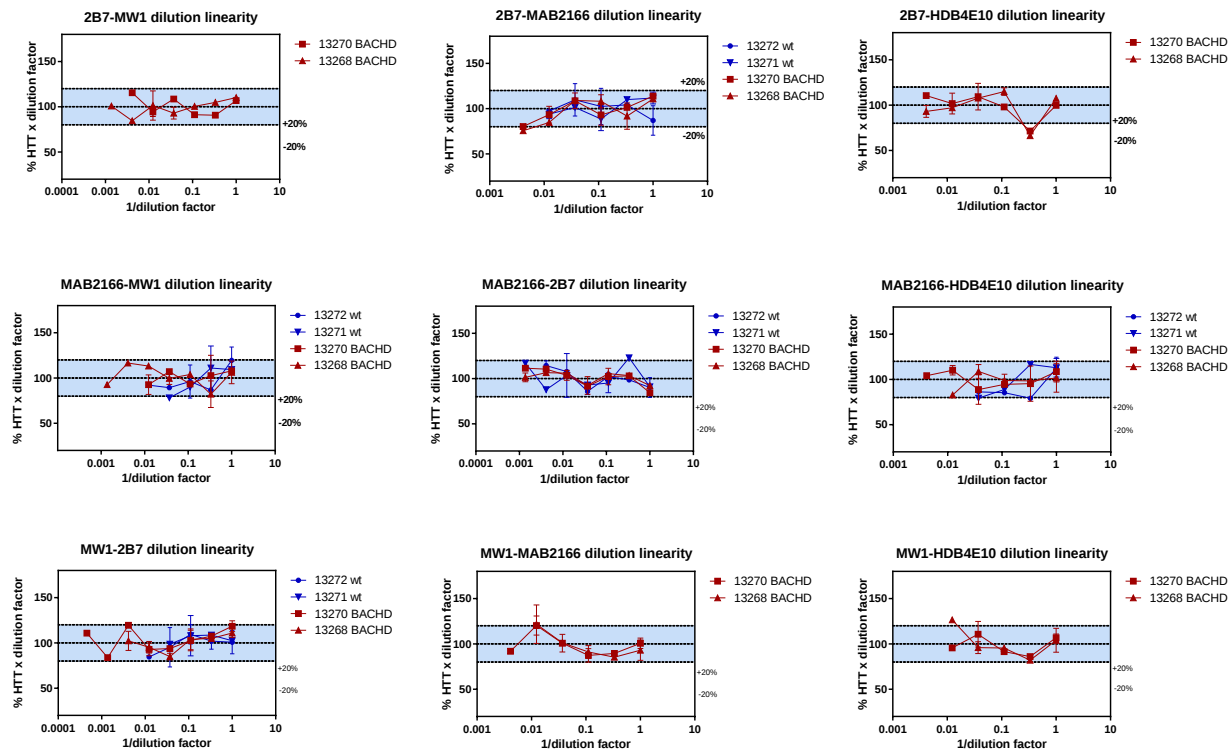
Catalog_ID	Age of onset	HD	CAG repeats Laragen	Age	Gender	Notes
GM21756	unknown	JHD	66/18		Female	Clinically affected;
GM04723	14	JHD	70/20	19	Female	clinically affected; neuro exam 3/82 shows bradykinesia, juvenile features of HD with markedly reduced saccadic velocity, Parkinsonian features, and marginal dystonia;
GM07492	unknown	normal	21/18	17	Male	
GM07532	unknown	normal	23/19	16	Female	Caucasian mother and Japanese father;
GM04857	unknown		50/40	23	Female	clinically affected; onset at age 28 years; 9 affected sibs and 2 with probable HD; neurological exam 3/82 shows bradykinesia;
GM04721	35	HD	49/36	37	Female	clinically affected; neuro exam 3/82 shows chorea & facial twitches; 1st cousin to proband;
GM01085	35	HD	45/23	44	Male	moderate ataxia, chorea, dysarthria, and dysphagia;
GM04693	41	HD	47/23	33	Male	clinically affected;
GM04775	unknown	normal	24/17	46	Female	

S-3 Performance of HDB4E10 and 4C9 containing assays against rat brain homogenates



Two wild type rats (13272 and 13271) and two BACHD rats (13270 and 103266) brain homogenates were diluted starting 1 from 6.7 μg/ml of total protein for 7 dilution points. Each dilution curve was tested via SMC using the antibody pair reported in the title of the graph.

S-4 Dilution linearity in wild type and BACHD rat brain homogenates

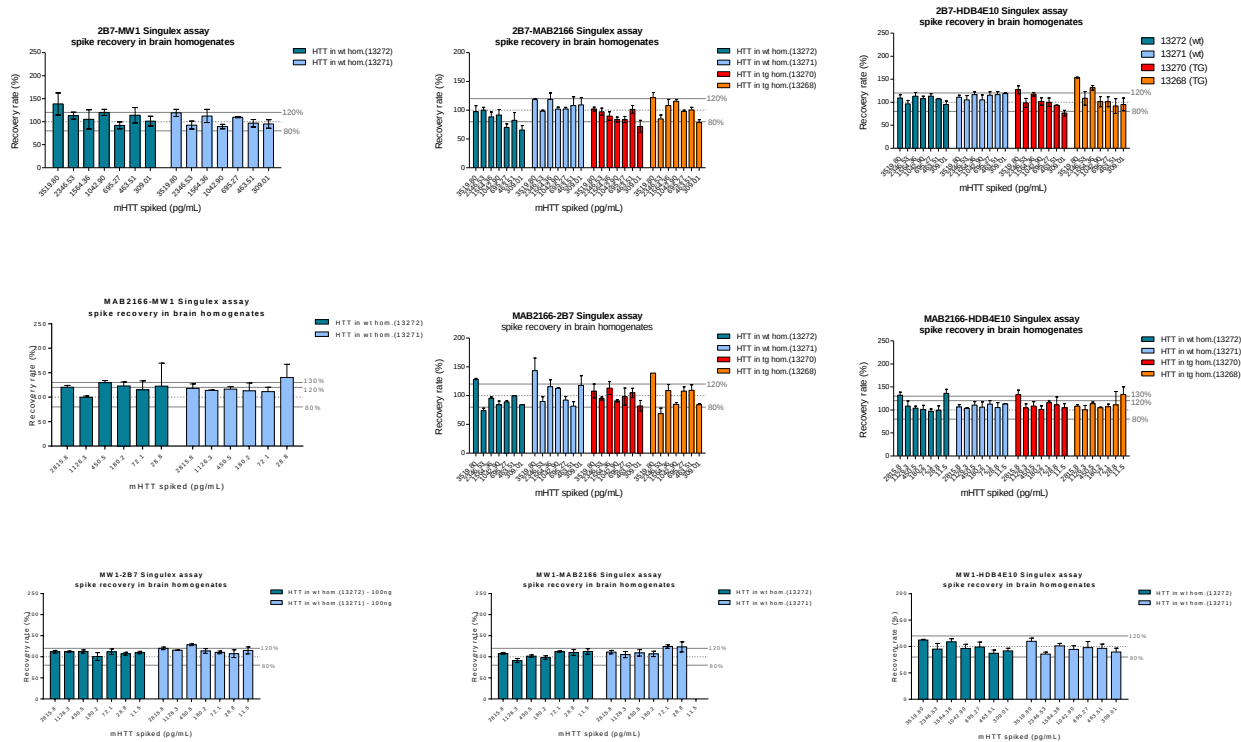


Two wild type rats (13272 and 13271) and two BACHD rats (13270 and 103266) brain homogenates were diluted starting 1.3 $\mu\text{g/ml}$ of total protein for the 2B7-MW1 assay and from 6.7 $\mu\text{g/ml}$ of total protein for all other antibody pairs for 7 dilution points. Each dilution curve was tested via SMC using the antibody pair reported in the title of the graph. The graphs show the percentage recovery (x-axis) with respect to the average concentration of each dilution multiplied for the dilution factor (y-axis). The light blue band represent the acceptable $\pm 20\%$ RE% range. Each point is represented as the average and standard deviation of three independent replicates.

The following examples show the process of determining the dilution linearity of the 2B7-MW1 for the 13270 BACHD brain.

Conc (pg mHTT/ug total protein)	Dilution factor		Conc. X Dil. factor	RE% w.r.t. average
5717.27	1.00	==>	5717.27	5.61
1617.40	3.00	==>	4852.20	-10.37
542.56	9.00	==>	4883.05	-9.80
215.05	27.00	==>	5806.33	7.26
62.25	81.00	==>	5041.90	-6.86
25.43	243.01	==>	6180.03	14.16
		Average	5413.46	

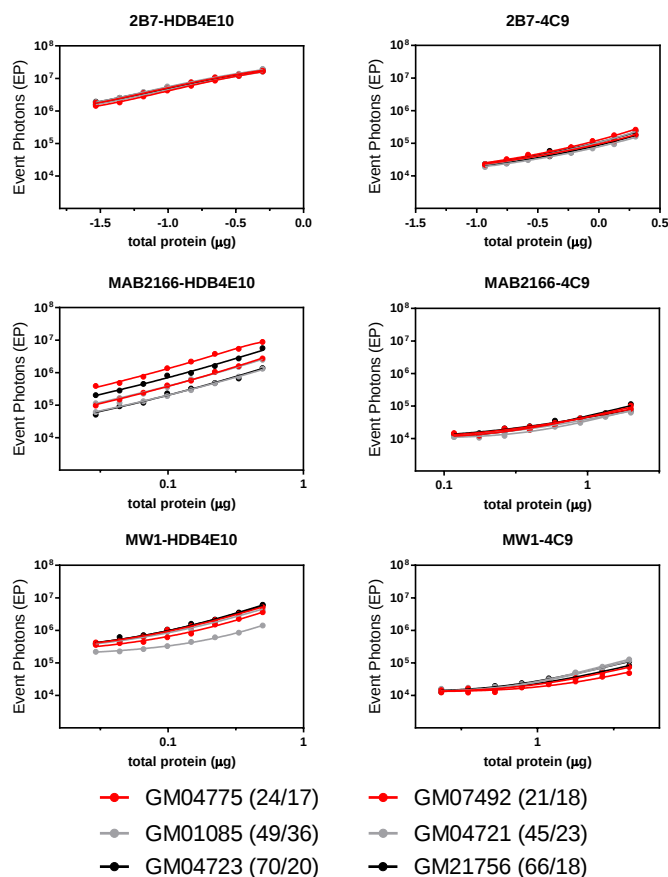
S-5 Spike recovery in wild type and BACHD rat brain homogenates.



Two wild type (13272 and 13271) and two BACHD (13270 and 103266) brain samples were spiked with a serial dilution of the recombinant FL HTT Q46 and assayed via SMC using the antibody pairs reported in the title of each graph. The amount of brain homogenate used for each assay is reported in the table below. The percentage of the recovered spikes for each spike dilution is reported in the x-axis, while the nominal spike concentration is reported on the y-axis. Each point is represented as the average and standard deviation of three independent replicates.

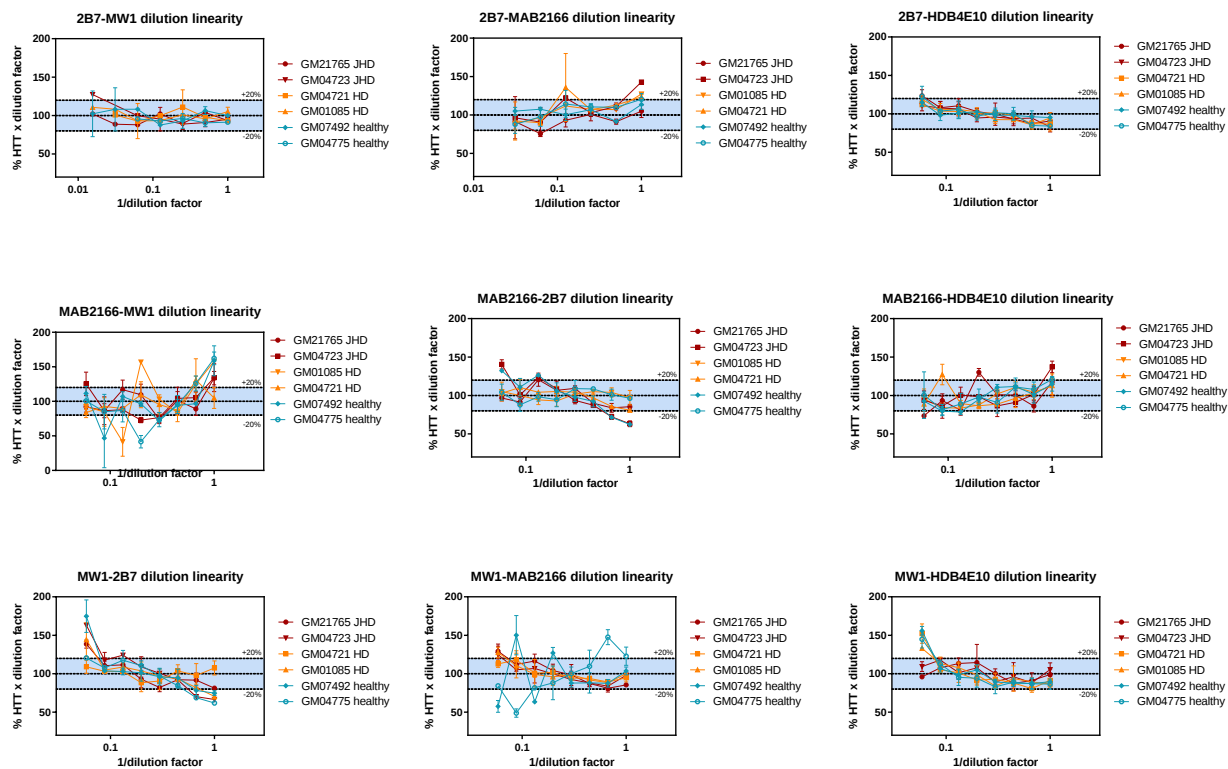
	Starting total protein concentration	
assays	ug/mL wt	ug/mL HD
2B7-MW1	0.13	X
2B7-MAB2166	0.33	0.33
2B7-HDB4E10	0.67	0.67
MAB2166-MW1	0.67	0.20
MAB2166-2B7	0.33	0.33
MAB2166-HDB4E10	0.67	0.67
MW1-2B7	0.67	0.67
MW1-MAB2166	0.67	0.67
MW1-HDB4E10	0.67	0.20

S-6 Performance of HDB4E10 and 4C9 containing assays human fibroblast lysates



Two control GM04775 (24/17) and GM07492 (21/18), two HD GM04721 (49/36) and GM01085 (45/23) and two JHD GM04723 (70/20) and GM21756 (66/18) fibroblast cell lysates were diluted starting from the total protein concentrations reported in the table in S-7. Each dilution curve was tested via SMC using the antibody pair reported in the title of the graph

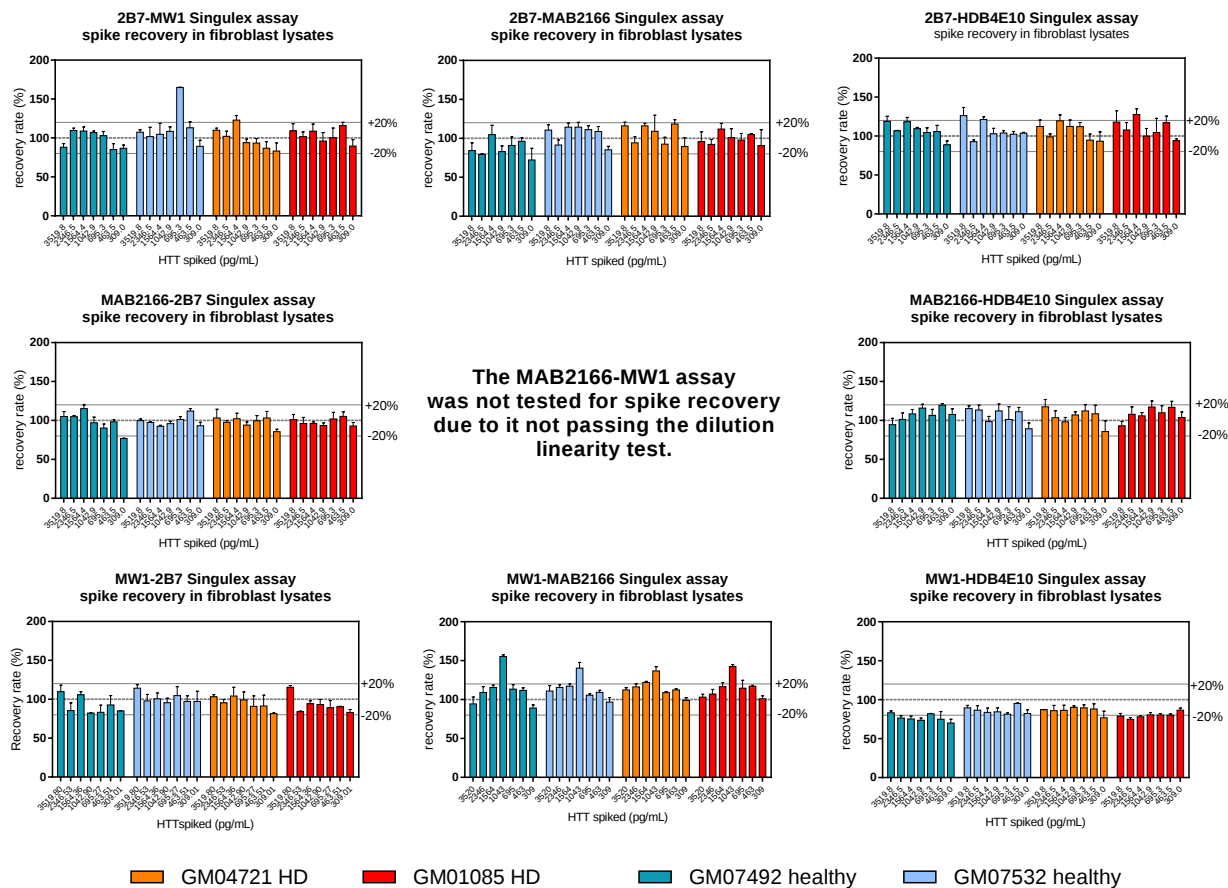
S-7 Dilution linearity in HD and JHD human fibroblast lysates



Two control GM04775 (24/17) and GM07492 (21/18), two HD GM04721 (49/36) and GM01085 (45/23) and two JHD GM04723 (70/20) and GM21756 (66/18) fibroblast cell lysates were diluted starting from the total protein concentrations reported in the table below. Each dilution curve was tested via SMC using the antibody pair reported in the title of the graph. The graphs show the percentage recovery (x-axis) with respect to the average concentration of each dilution multiplied for the dilution factor (y-axis). The light blue band represent the acceptable $\pm 20\%$ RE% range. Each point is represented as the average and standard deviation of three independent replicates.

	Starting total protein concentration
assays	ug/mL
2B7-MW1	3.33
2B7-MAB2166	13.33
2B7-HDB4E10	3.33
MAB2166-MW1	13.33
MAB2166-2B7	1.67
MAB2166-HDB4E10	3.33
MW1-2B7	3.33
MW1-MAB2166	26.67
MW1-HDB4E10	3.33

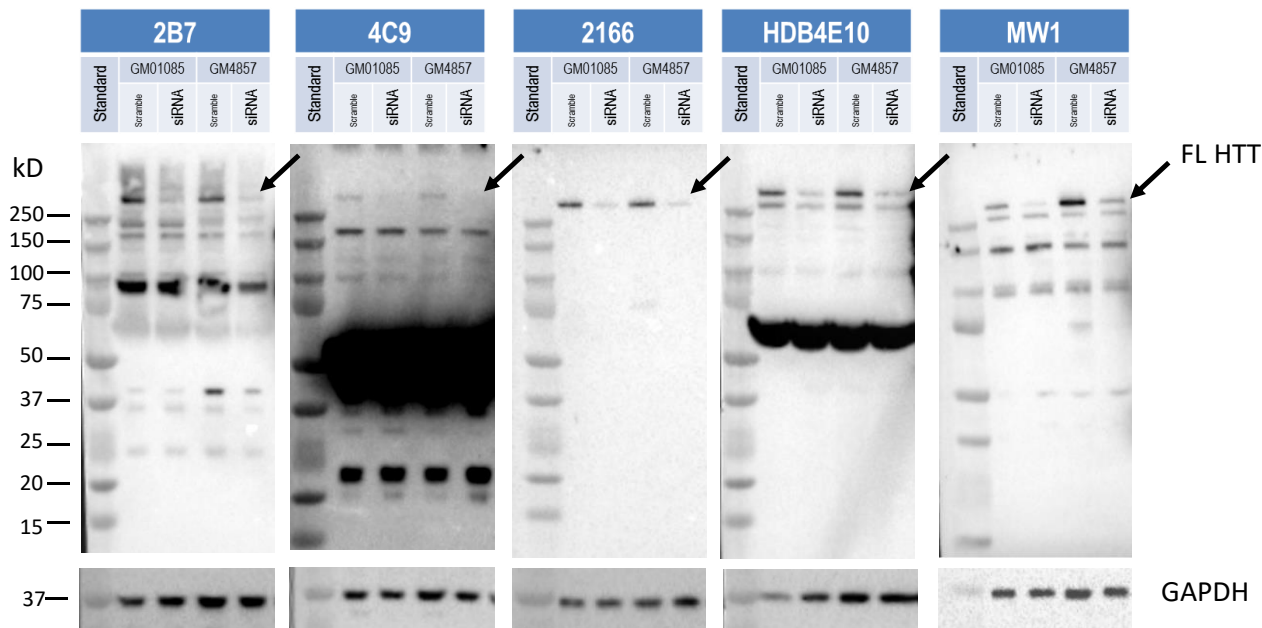
S-8 Spike recovery in wild type, HD and JHD human fibroblast lysates



Two control GM04775 (24/17) and GM07492 (21/18), two HD GM04721 (49/36) and GM01085 (45/23) and two JHD GM04723 (70/20) and GM21756 (66/18) fibroblast cell lysates were spiked with a serial dilution of the recombinant FL HTT Q46 and assayed via SMC using the antibody pairs reported in the title of each graph. The amount of brain homogenate used for each assay is reported in the table below. The percentage of the recovered spikes for each spike dilution is reported in the x-axis, while the nominal spike concentration is reported on the y-axis. Each point is represented as the average and standard deviation of three independent replicates.

	Starting total protein concentration	
assays	ug/mL wt	ug/mL HD
2B7-MW1	0.13	0.13
2B7-MAB2166	1.67	1.67
2B7-HDB4E10	0.07	0.07
MAB2166-MW1	X	X
MAB2166-2B7	0.02	0.02
MAB2166-HDB4E10	0.20	0.20
MW1-2B7	0.07	0.07
MW1-MAB2166	8.00	0.67
MW1-HDB4E10	0.07	0.07

S-9 HTT silencing in human fibroblasts



Two fibroblasts cell lines GM01085 (45/23) and GM04857 (50/40) were subjected to HTT silencing via siRNA. Cell lysates from both donors were used to determine the silencing efficiency via western blot using the antibodies under investigation. The present Western blot does not have a sufficient resolution to allow the detection of the mHTT band at higher molecular weight than the wild type one. The following table reports all the numeric % decrease of HTT detected via SMC with respect to the scramble treated cells.

Antibody pair	GM01085 (45/23)		GM04857 (50/40)	
	% decrease w.r.t scramble	StDev	% decrease w.r.t scramble	StDev
2B7-2166	-66.43	3.93	-72.71	3.43
2B7-MW1	-25.8557	15.76097	-49.8191	7.831169
2B7-HDB4E10	-46.2	6.8	-53.4	5.5
2166-2B7	-26.96	10.1	-9.74	23.87
2166-HDB4E10	-15.7868	17.9155	-13.3887	20.17093
2166-MW1	6.7	19.46	4.45	12.87
MW1-2166	-58.47	1.56	-71.31	2.17
MW1-2B7	-19.68	7.6	-66.45	2.53
MW1-HDB4E10	-48.09	8.11	-67.53	4.87

S-10 CSF and brain HTT and mHTT levels in a BACHD life-span study

Month	BRAIN			CSF			BRAIN		
	mHTT (2B7-MW1)			mHTT (2B7-MW1)			total HTT (2B7-MAB2166)		
	Avg	SD	N	Avg	SD	N	Avg	SD	N
7	9296.72	2932.29	18	23.65	14.20	9	34.75	14.57	20
8	5408.79	2011.28	19	19.54	5.50	15	36.08	10.64	19
9	7876.58	1521.02	10	19.17	3.54	10	30.39	11.61	10
10	5224.18	2107.52	9	16.47	8.46	6	32.29	13.63	5
11	2930.50	959.90	10	9.47	4.87	10	28.73	9.75	10
12	4690.37	1761.62	6	9.35	4.04	6	20.07	6.37	8
13	4812.71	1216.79	9	10.21	4.10	9	24.74	10.39	11
14	7213.60	3292.98	8	14.16	6.52	8	29.58	5.46	9
17	7809.51	3141.59	10	25.20	13.13	9	31.97	14.58	6
19	6305.68	2094.90	10	29.64	15.82	8	33.53	7.82	11
20	12127.24	4098.56	11	26.35	11.28	7	26.06	12.32	12

Numerical values of the CSF and brain HTT and mHTT levels in a BACHD life-span study

S-11 Time-course HTT and mHTT levels in wild type and Q175 tissues

Total HTT wt			pg HTT/ug total protein)																													
Days	0			32			61			87			106			149																
	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N														
Heart	2.428	0.549	8	2.391	0.801	7	2.004	0.665	7	1.257	0.545	6	1.010	0.393	7	1.893	0.448	6														
Liver	12.580	3.601	5	4.473	2.89	6	1.710	1.631	5	2.642	1.025	6	3.394	0.708	5	1.237	0.484	3														
Lung	6.383	0.945	7	10.473	1.297	6	9.408	1.266	5	7.240	1.044	6	7.637	1.789	6	5.370	0.892	5														
Spleen	5.770	2.045	8	7.242	1.035	5	9.325	1.364	6	8.032	2.023	5	6.300	3.466	6	7.210	1.408	4														
Muscle	11.984	2.678	7	3.585	0.539	4	1.951	1.601	7	1.005	0.992	6	1.240	1.123	6	1.512	0.596	5														
Kidney	6.550	1.136	6	8.008	1.366	6	10.412	2.261	5	7.890	1.674	7	6.482	2.395	5	8.282	0.952	6														
Spinal cord	7.881	3.848	7	28.422	5.308	6	28.363	5.351	3	25.368	1.965	4	22.510	5.338	3	18.953	3.318	4														
Cerebellum	5.343	1.224	8	14.460	5.344	5	11.980	6.725	6	17.592	5.055	6	16.297	4.730	7	25.048	5.366	4														
Cortex	3.659	0.353	8	15.588	3.729	6	17.537	2.443	6	17.593	3.111	6	20.923	2.885	6	19.565	2.836	4														
Striatum	8.889	1.253	8	11.815	2.270	6	12.915	3.113	5	12.924	2.157	7	16.315	4.864	6	19.702	2.664	5														
Hippocampus				15.315	1.351	2	16.387	3.202	3	22.273	3.260	3	22.220	3.528	3	20.923	1.839	3														
Olfactory bulb	4.373	0.524	7	11.584	5.893	7	10.875	4.258	6	16.512	4.482	6	11.945	2.015	6	16.618	6.487	5														
Total HTT Q175			pg HTT/ug total protein)																													
Days	0			32			61			87			106			149																
	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N														
Heart	2.963	0.292	8	1.456	1.023	5	0.778	0.611	5	1.330	0.478	5	1.060	0.271	5	0.782	0.299	6														
Liver	3.325	1.590	8	2.580	1.005	5	1.586	0.362	5	2.698	2.132	5	2.996	1.176	5	1.960	0.929	6														
Lung	4.326	1.342	6	5.188	3.103	5	4.868	1.384	5	4.904	1.189	5	5.624	1.177	5	3.755	2.335	6														
Spleen	3.925	1.458	8	3.466	0.835	5	3.228	1.280	5	5.000	1.892	5	4.418	1.650	5	4.312	1.092	6														
Muscle	7.340	3.676	8	1.126	1.510	5	1.030	1.263	5	0.502	0.502	5	0.750	0.900	5	0.920	0.510	6														
Kidney	4.740	0.884	8	4.624	1.679	5	4.058	2.153	5	4.336	1.053	5	4.446	0.672	5	3.990	1.724	6														
Spinal cord	9.790	4.309	8	15.458	3.367	5	15.445	3.571	2	18.742	7.669	5	15.352	5.200	6	19.955	3.490	6														
Cerebellum	2.785	0.943	8	11.480	5.099	5	11.030	5.293	5	16.680	3.990	4	16.947	5.519	3	11.210	5.787	4														
Cortex	3.700	0.516	8	9.466	1.616	5	11.370	1.047	5	20.670	5.211	5	21.884	8.116	5	14.487	3.478	6														
Striatum	7.600	1.539	8	8.892	2.691	5	9.266	2.491	5	9.700	1.818	5	14.662	9.695	5	14.480	4.522	6														
Hippocampus				11.120	2.414	3	11.967	3.961	3	14.287	0.526	3	20.423	0.465	3	14.853	2.857	3														
Olfactory bulb	4.873	1.662	8	10.358	7.085	5	9.442	4.249	5	7.856	2.945	5	8.644	0.691	5	10.912	5.004	6														
mHTT Q175			pg mHTT/ug total protein)																													
Days	0			32			61			87			106			149																
	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N	Avg	SD	N														
Heart	26.048	2.328	8	20.400	1.148	5	14.156	2.811	5	20.542	3.418	5	20.078	1.842	5	11.218	2.254	6														
Liver	16.961	23.389	8	16.272	14.954	5	11.420	9.397	5	10.924	9.276	5	20.862	18.328	5	8.817	4.225	6														
Lung	9.865	3.562	8	29.780	26.698	5	11.522	2.745	5	15.442	6.241	5	15.722	6.643	5	10.097	6.238	6														
Spleen	11.574	6.742	8	14.392	9.533	5	20.306	5.041	5	20.362	5.307	5	23.880	7.647	5	20.885	11.590	6														
Muscle	30.075	8.348	8	9.874	1.991	5	5.508	1.759	5	6.036	2.388	5	5.256	1.962	5	3.923	1.800	6														
Kidney	2.151	3.452	8	3.276	1.655	5	4.110	3.186	5	2.592	1.116	5	3.492	1.095	5	5.255	1.807	6														
Spinal cord	48.702	8.410	6	78.228	33.84	5	77.440	11.484	3	78.320	35.574	5	70.668	34.840	5	56.597	34.600	6														
Cerebellum	156.695	10.039	8	170.076	44.537	5	159.700	32.697	5	116.694	71.964	5	152.414	29.512	5	112.585	24.281	6														
Cortex	40.229	5.961	8	122.430	33.961	5	119.180	41.411	5	145.114	65.921	5	147.866	79.006	5	72.618	29.507	6														
Striatum	42.718	4.722	8	123.700	23.378	5	98.236	21.746	5	94.594	31.123	5	105.628	50.086	5	77.543	26.573	6														
Hippocampus	27.150	15.752	8	170.696	29.983	5	116.664	50.343	5	112.378	68.726	5	177.556	63.563	5	112.111	47.751	6														
Olfactory bulb	37.566	12.659	8	88.098	20.344	5	91.468	11.524	5	78.128	3.397	5	76.292	11.552	5	75.685	30.294	6														
mHTT/HTT ratio Q175																																
Days	0		32		61		87		106		149																					
	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD																				
Heart	8.792	0.411	14.011	4.415	11.195	6.592	15.445	2.737	11.942	2.300	14.352	2.534																				
Liver	11.828	3.378	6.307	2.815	7.201	2.750	4.049	2.100	6.963	2.997	4.498	1.238																				
Lung	2.280	0.384	5.729	2.760	2.778	0.434	3.149	0.664	2.796	0.590	2.689	0.962																				
Spleen	4.732	0.869	4.152	1.309	6.291	1.116	4.032	0.831	5.405	1.189	4.844	1.206																				
Muscle	4.097	0.825	8.769	5.111	5.348	3.031	12.024	5.711	7.008	3.939	4.264	1.252																				
Kidney	4.539	0.395	0.708	0.197	1.018	0.426	0.598	0.132	0.785	0.122	1.117	0.297																				
Spinal cord	4.975	0.850	5.061	1.079	5.014	0.925	4.179	1.143	4.603	1.211	5.166	1.454																				
Cerebellum	56.264	6.935	14.815	3.411	12.256	2.493	9.694	2.193	8.994	1.862	11.806	3.167																				

Numerical values of the time-course HTT and mHTT levels in wild type and Q175 tissues