

# Supplementary Material

## Changes in 24(S)-Hydroxycholesterol Are Associated with Cognitive Performance in Early Huntington's Disease: Data from the TRACK and ENROLL HD Cohorts

**Supplementary Table 1.** Pearson correlation of oxysterol concentration and ratios in TRACK-HD across years 1, 3, and 4.

Oxysterol	Test	Year 1		Year 3		Year 4	
		<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
24(S)-OHC	Emotion Recognition	<b>0.25</b>	<b>0.004</b>	<b>0.26</b>	<b>0.002</b>	0.15	0.072
24(S)-OHC	Smell Identification Test	0.12	0.157	0.11	0.216	---	---
24(S)-OHC	Spot the change-5k	0.16	0.064	0.20	<b>0.019</b>	0.14	0.121
24(S)-OHC	Spot the change-7k	---	---	0.11	0.204	0.16	0.077
24(S)-OHC	Stroop Test - Word Reading	<b>0.27</b>	<b>0.001</b>	0.13	0.122	0.09	0.292
24(S)-OHC	Symbol Digit Modality	<b>0.17</b>	<b>0.049</b>	<b>0.23</b>	<b>0.006</b>	0.13	0.122
24(S)-OHC	Trail Making A	-0.15	0.073	-0.14	0.103	---	---
24(S)-OHC	Trail Making B	<b>-0.17</b>	<b>0.041</b>	<b>-0.17</b>	<b>0.041</b>	---	---
24(S)/25-OHC	Emotion Recognition	<b>0.22</b>	<b>0.016</b>	<b>0.24</b>	<b>0.005</b>	<b>0.30</b>	<b>&lt;0.001</b>
24(S)/25-OHC	Smell Identification Test	0.16	0.074	0.19	<b>0.032</b>	---	---
24(S)/25-OHC	Spot the change-5k	<b>0.18</b>	<b>0.041</b>	<b>0.24</b>	<b>0.005</b>	<b>0.34</b>	<b>&lt;0.001</b>
24(S)/25-OHC	Spot the change-7k	---	---	0.16	0.067	0.10	0.284
24(S)/25-OHC	Stroop Test - Word Reading	<b>0.23</b>	<b>0.009</b>	<b>0.25</b>	<b>0.004</b>	<b>0.34</b>	<b>&lt;0.001</b>
24(S)/25-OHC	Symbol Digit Modality	<b>0.24</b>	<b>0.007</b>	<b>0.18</b>	<b>0.040</b>	<b>0.20</b>	<b>0.023</b>
24(S)/25-OHC	Trail Making A	<b>-0.22</b>	<b>0.012</b>	-0.11	0.218	---	---
24(S)/25-OHC	Trail Making B	<b>-0.19</b>	<b>0.028</b>	-0.16	0.065	---	---
24(S)/27-OHC	Emotion Recognition	<b>0.17</b>	<b>0.053</b>	<b>0.28</b>	<b>0.001</b>	<b>0.23</b>	<b>0.008</b>
24(S)/27-OHC	Smell Identification Test	0.13	0.112	0.14	0.112	---	---
24(S)/27-OHC	Spot the change-5k	<b>0.19</b>	<b>0.026</b>	<b>0.29</b>	<b>&lt;0.001</b>	<b>0.32</b>	<b>&lt;0.001</b>
24(S)/27-OHC	Spot the change-7k	---	---	<b>0.25</b>	<b>0.003</b>	0.10	0.285
24(S)/27-OHC	Stroop Test - Word Reading	<b>0.21</b>	<b>0.013</b>	<b>0.24</b>	<b>0.004</b>	<b>0.24</b>	<b>0.004</b>
24(S)/27-OHC	Symbol Digit Modality	<b>0.22</b>	<b>0.009</b>	<b>0.21</b>	<b>0.013</b>	0.16	0.063
24(S)/27-OHC	Trail Making A	-0.16	0.060	-0.10	0.247	---	---
24(S)/27-OHC	Trail Making B	-0.16	0.056	-0.13	0.126	---	---
25-OHC	Emotion Recognition	0.03	0.751	-0.12	0.181	-0.15	0.087
25-OHC	Smell Identification Test	-0.01	0.901	<b>-0.20</b>	<b>0.024</b>	---	---
25-OHC	Spot the change-5k	0.01	0.911	-0.11	0.213	-0.16	0.075
25-OHC	Spot the change-7k	---	---	-0.10	0.241	-0.02	0.865
25-OHC	Stroop Test - Word Reading	0.00	0.961	-0.15	0.078	<b>-0.21</b>	<b>0.014</b>
25-OHC	Symbol Digit Modality	-0.08	0.339	-0.06	0.512	-0.08	0.354
25-OHC	Trail Making A	0.05	0.552	0.09	0.299	---	---
25-OHC	Trail Making B	0.03	0.718	0.09	0.294	---	---

27-OHC	Emotion Recognition	0.10	0.274	0.00	0.985	-0.07	0.424
27-OHC	Smell Identification Test	0.00	0.975	-0.01	0.953	---	---
27-OHC	Spot the change-5k	-0.02	0.799	-0.06	0.469	-0.16	0.066
27-OHC	Spot the change-7k	---	---	-0.10	0.242	0.01	0.887
27-OHC	Stroop Test - Word Reading	0.08	0.349	-0.03	0.687	-0.11	0.207
27-OHC	Symbol Digit Modality	-0.01	0.895	0.06	0.448	-0.03	0.719
27-OHC	Trail Making A	-0.03	0.725	-0.04	0.665	---	---
27-OHC	Trail Making B	-0.04	0.642	-0.06	0.469	---	---

Pearson correlation analyses were performed across control, pre-manifest HD, and manifest HD participants to assess the relationship between 24(S)-OHC concentration, 24(S)/25-OHC ratio, 24(S)/27-OHC ratio, 25-OHC concentration, and 27-OHC concentration to performance on cognitive tests. Correlation  $r$  and  $p$  values are displayed by year and those that reached statistical significance of  $p < 0.05$  are bolded.

**Supplementary Table 2.** Analyses of TFC and UHDRS motor score with oxysterol concentration and ratios in TRACK-HD across years 1, 3, and 4.

		<b>Year 1</b>	<b>Year 3</b>	<b>Year 4</b>
<b>Oxysterol</b>	<b>Test</b>	<i>p</i>	<i>p</i>	<i>p</i>
24(S)-OHC	TFC Score	0.286	0.203	<b>&lt;0.001</b>
24(S)/25-OHC	TFC Score	0.052	0.090	<b>&lt;0.001</b>
24(S)/27-OHC	TFC Score	<b>0.011</b>	<b>0.002</b>	<b>0.010</b>
25-OHC	TFC Score	0.320	0.924	0.268
27-OHC	TFC Score	0.542	0.209	0.683
24(S)-OHC	Motor Score	0.277	0.652	0.515
24(S)/25-OHC	Motor Score	0.577	0.230	0.685
24(S)/27-OHC	Motor Score	<b>0.007</b>	0.141	0.806
25-OHC	Motor Score	0.428	0.703	0.825
27-OHC	Motor Score	0.557	0.102	0.600

Binomial regression (TFC) and zero-inflated Poisson model (Motor score) were performed across pre-manifest HD and manifest HD participants to assess their relationship with 24(S)-OHC concentration, 24(S)/25-OHC ratio, 24(S)/27-OHC ratio, 25-OHC concentration, 27-OHC concentration over years 1, 3, and 4. Statistically significant ( $p < 0.05$ ) relationships are bolded.

**Supplementary Table 3.** Pearson correlation values and false-discovery rate (FDR)-adjusted  $p$  values from analyses of cognitive test performance and oxysterol concentrations and ratios in ENROLL-HD.

<b>Oxysterol</b>	<b>Test</b>	<b><math>r</math></b>	<b><math>p</math> (FDR-adjusted)</b>
24(S)/25-OHC	Stroop Interference	0.22	<b>0.0006</b>
24(S)/25-OHC	Stroop Color Naming	0.21	<b>0.0008</b>
24(S)/25-OHC	Symbol Digit Modality	0.19	<b>0.0029</b>
24(S)/25-OHC	Stroop Word Reading	0.19	<b>0.0029</b>
24(S)/27-OHC	Stroop Interference	0.16	<b>0.0156</b>
24(S)-OHC	MMSE	n/a	<b>0.0192</b>
24(S)/25-OHC	Verbal-Categorical	0.15	<b>0.0218</b>
24(S)/27-OHC	Symbol Digit Modality	0.14	<b>0.0347</b>
24(S)/25-OHC	MMSE	n/a	<b>0.0379</b>
24(S)/27-OHC	Stroop Color Naming	0.13	<b>0.0379</b>
24(S)-OHC	Symbol Digit Modality	0.13	<b>0.0398</b>
24(S)/25-OHC	Verbal-Letters	0.13	<b>0.0425</b>
24(S)/27-OHC	MMSE	n/a	<b>0.0445</b>
24(S)-OHC	Stroop Word Reading	0.12	<b>0.0471</b>
24(S)/25-OHC	Trail Making B	-0.12	0.0525
24(S)/27-OHC	Stroop Word Reading	0.12	0.0525
24(S)-OHC	Verbal-Categorical	0.12	0.0541
24(S)/25-OHC	Trail Making A	-0.12	0.0570
24(S)-OHC	Stroop Interference	0.11	0.0570
24(S)-OHC	Trail Making A	-0.11	0.0570
24(S)/27-OHC	Trail Making B	-0.11	0.0762
24(S)-OHC	Verbal-Letters	0.10	0.0928
24(S)/27-OHC	Verbal-Categorical	0.09	0.1006
24(S)/27-OHC	Verbal-Letters	0.10	0.1006
24(S)/27-OHC	Trail Making A	-0.09	0.1006
24(S)-OHC	Stroop Color Naming	0.09	0.1006
24(S)-OHC	Trail Making B	-0.10	0.1006
25-OHC	Stroop Color Naming	-0.10	0.1006
25-OHC	Stroop Interference	-0.10	0.1006
25-OHC	MMSE	n/a	0.3276
25-OHC	Stroop Word Reading	-0.05	0.4307
27-OHC	Stroop Interference	-0.05	0.4753
27-OHC	Trail Making A	-0.05	0.4753
25-OHC	Symbol Digit Modality	-0.04	0.5061
25-OHC	Verbal-Categorical	-0.04	0.5061
25-OHC	Verbal-Letters	-0.04	0.5631
27-OHC	MMSE	n/a	0.5800
27-OHC	Stroop Color Naming	-0.03	0.6467
25-OHC	Trail Making B	0.03	0.7031
27-OHC	Verbal-Categorical	0.02	0.7454
27-OHC	Verbal-Letters	0.02	0.7454

27-OHC	Stroop Word Reading	0.02	0.7493
25-OHC	Trail Making A	0.01	0.9370
27-OHC	Trail Making B	-0.01	0.9370
27-OHC	Symbol Digit Modality	0.00	0.9408

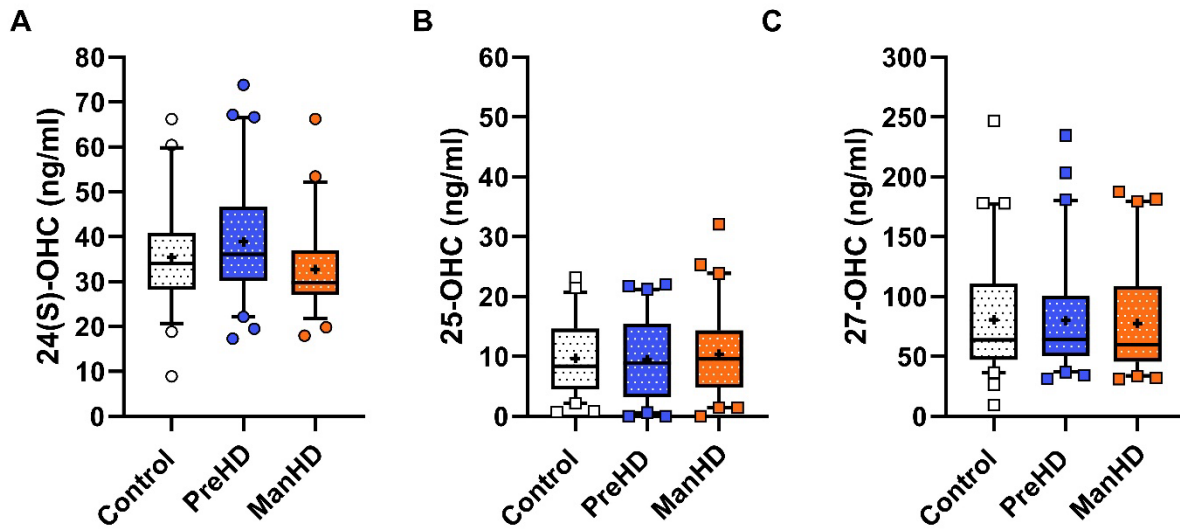
Binomial regression was performed for MMSE and therefore does not have  $r$  values available. Pearson correlation analyses for all other tests were performed. In all comparisons, analyses were conducted across control, pre-manifest HD, and manifest HD participants to assess the relationship between 24(S)-OHC concentration, 24(S)/25-OHC ratio, 24(S)/27-OHC ratio, 25-OHC concentration, 27-OHC concentration to performance on cognitive tests. FDR-adjusted  $p$  values are displayed by rank and statistically significant (FDR-adjusted  $p < 0.05$ ) relationships are bolded.

**Supplementary Table 4.** False-discovery rate (FDR)-adjusted  $p$  values from analyses of UHDRS and CAP scores with oxysterol concentrations and ratios in ENROLL-HD.

<b>Oxysterol</b>	<b>Test</b>	<b><math>p</math> (FDR-adjusted)</b>
24(S)/25-OHC	UHDRS TFC Score	<b>1.85E-09</b>
24(S)/25-OHC	UHDRS FA Score	<b>1.85E-09</b>
24(S)/27-OHC	UHDRS TFC Score	<b>7.45E-07</b>
24(S)/27-OHC	UHDRS FA Score	<b>1.51E-06</b>
24(S)-OHC	UHDRS TFC Score	<b>4.80E-05</b>
25-OHC	CAG-Age-Product	<b>5.08E-05</b>
25-OHC	UHDRS FA Score	<b>0.0002</b>
24(S)/27-OHC	UHDRS TMS	<b>0.0013</b>
24(S)-OHC	UHDRS FA Score	<b>0.0020</b>
24(S)/25-OHC	UHDRS TMS	<b>0.0050</b>
24(S)-OHC	CAG-Age-Product	<b>0.0061</b>
25-OHC	UHDRS TFC Score	0.0590
27-OHC	UHDRS TMS	0.0590
24(S)-OHC	UHDRS TMS	0.0590
27-OHC	CAG-Age-Product	0.0590
27-OHC	UHDRS FA Score	0.0863
24(S)/27-OHC	CAG-Age-Product	0.0946
24(S)/25-OHC	CAG-Age-Product	0.3130
25-OHC	UHDRS TMS	0.4113
27-OHC	UHDRS TFC Score	0.5589

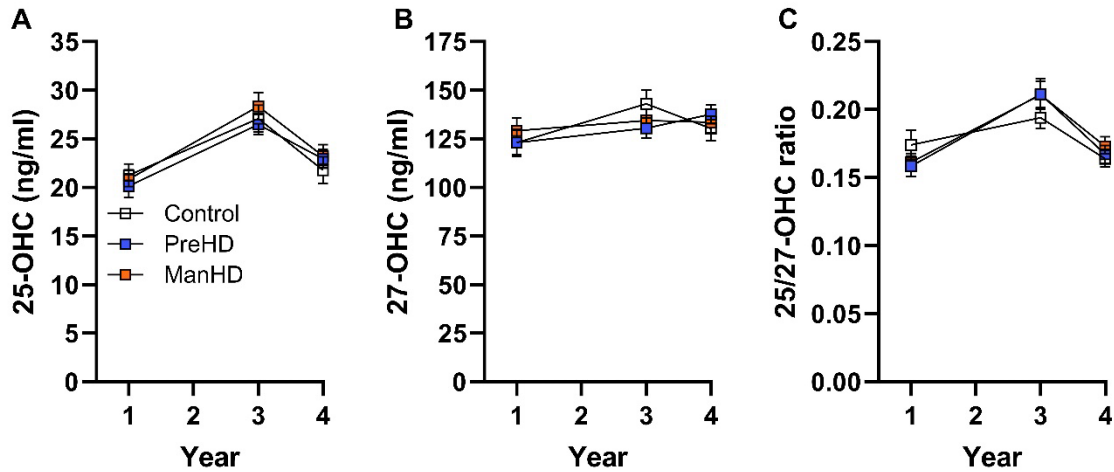
Binomial regression (UHDRS TFC score, UHDRS FA score), Pearson correlation (CAP), zero-inflated Poisson model (UHDRS TMS), were performed across pre-manifest HD and manifest HD participants to assess their relationship with 24(S)-OHC concentration, ratio of 24(S)-OHC to 25-OHC, 24(S)/25-OHC ratio, 24(S)/27-OHC ratio, 25-OHC concentration, 27-OHC concentration. FDR-adjusted  $p$  values are displayed by rank and statistically significant (FDR-adjusted  $p < 0.05$ ) relationships are bolded.

### TRACK-HD, Year 2 method development

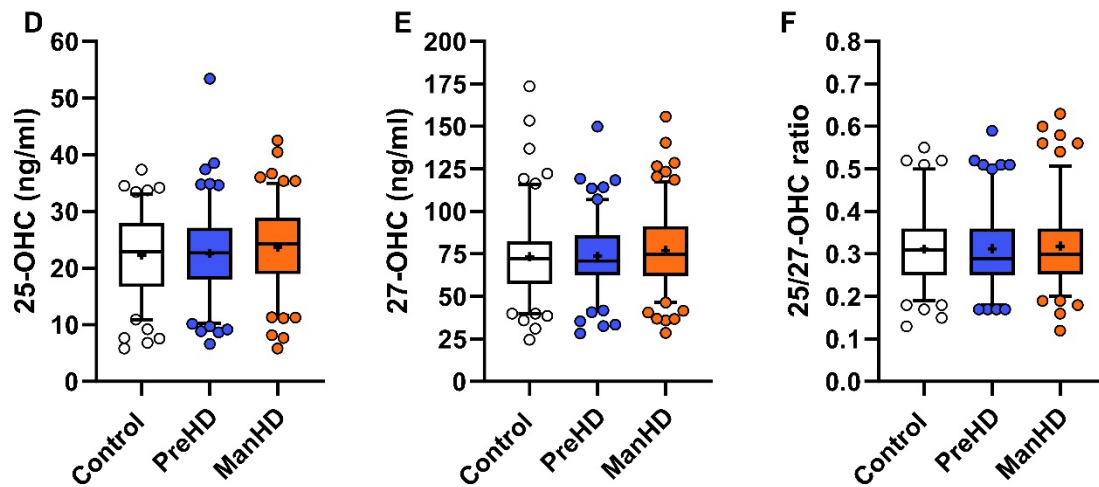


**Supplementary Figure 1. Oxysterols were detectable in plasma from TRACK-HD.** Samples from TRACK-HD, year 2, were used for assay development and quantification of A) 24(S)-OHC, B) 25-OHC, and C) 27-OHC. TRACK-HD had samples from controls, pre-manifest HD (PreHD), and manifest HD (ManHD). Box and whiskers span 5-95% of the data, with line at median and cross at the mean, and individual circles are shown for participants outside the 5-95% span.

**TRACK-HD, Year 3**  
hypothesis-generating cohort

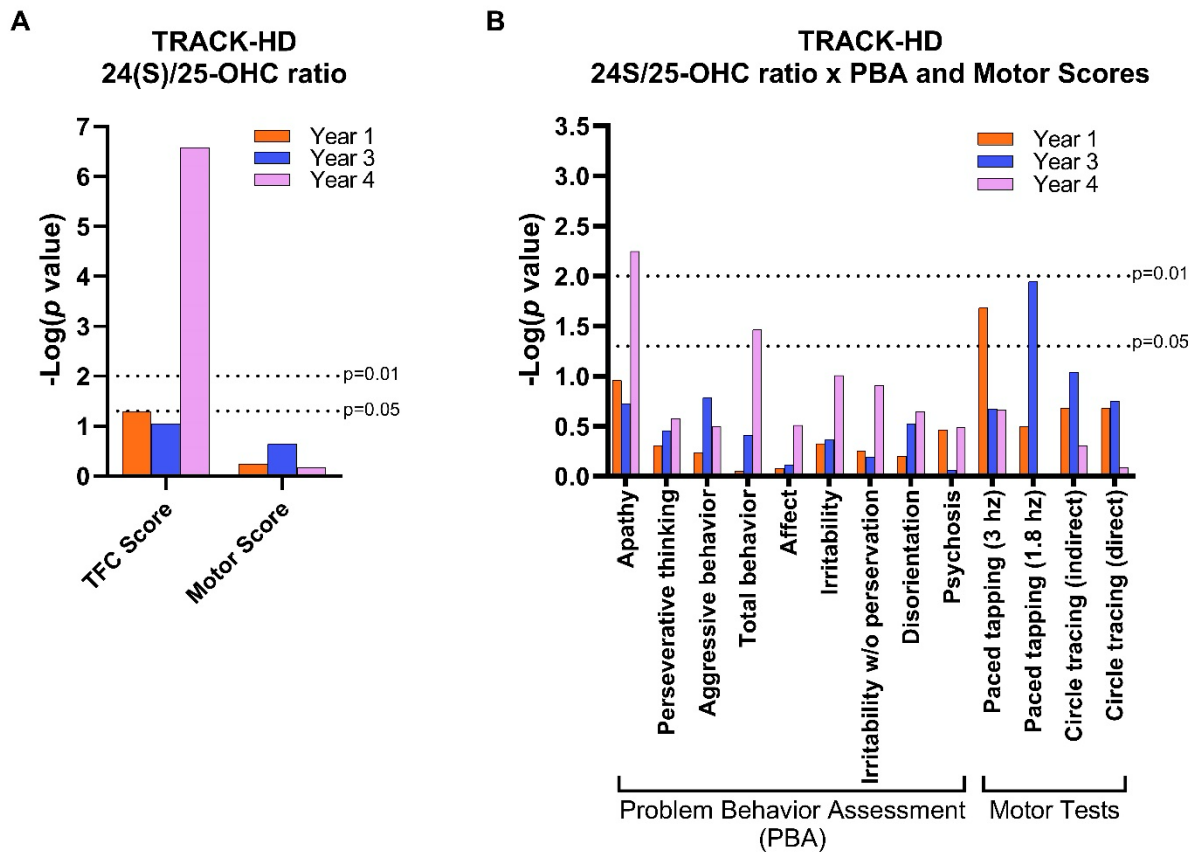


**ENROLL-HD**  
hypothesis-testing cohort



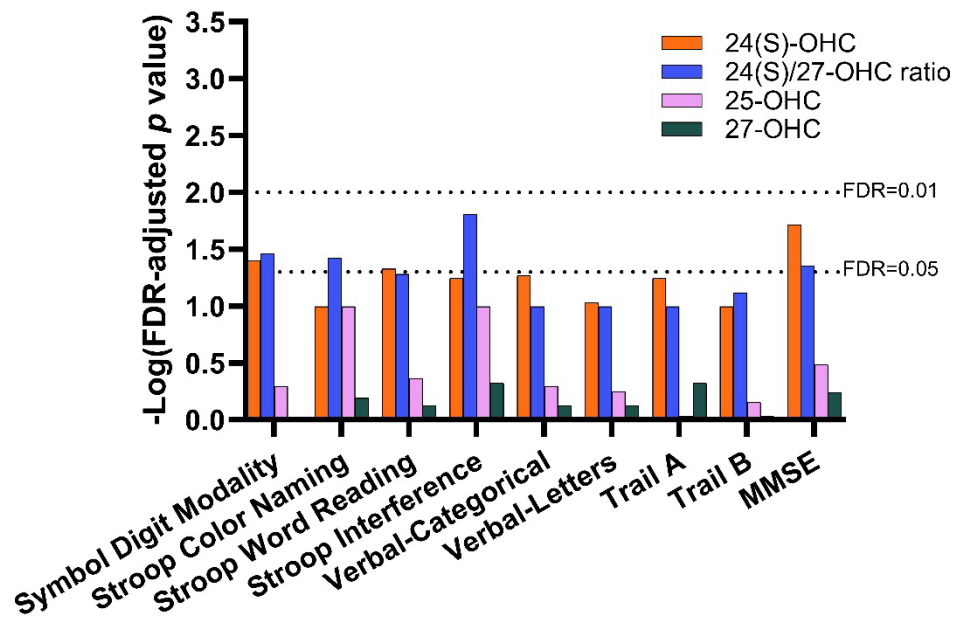
**Supplementary Figure 2. 25-OHC, 27-OHC, and their ratio was not altered during manifest Huntington’s disease in independent registry cohorts.** A-C) TRACK-HD registry cohort, which was used as a hypothesis-generating cohort; D-F) ENROLL-HD registry cohort, which was used as a hypothesis-testing cohort. Groups in both registries consisted of controls, pre-manifest HD (PreHD), and manifest HD (ManHD). A and D) 25-OHC concentrations. B and E) 27-OHC concentrations C and F) 25/27-OHC ratio. TRACK-HD data were compared by one-way ANOVA with repeated measure (year) with Tukey post-hoc test and shown as mean  $\pm$  standard error. ENROLL-HD data were compared by 1-way ANOVA with Tukey post hoc between all groups and shown as box and whiskers that span 5-95% of the data, with line at median and cross at the mean, and individual circles are shown for participants outside the 5-95% span.



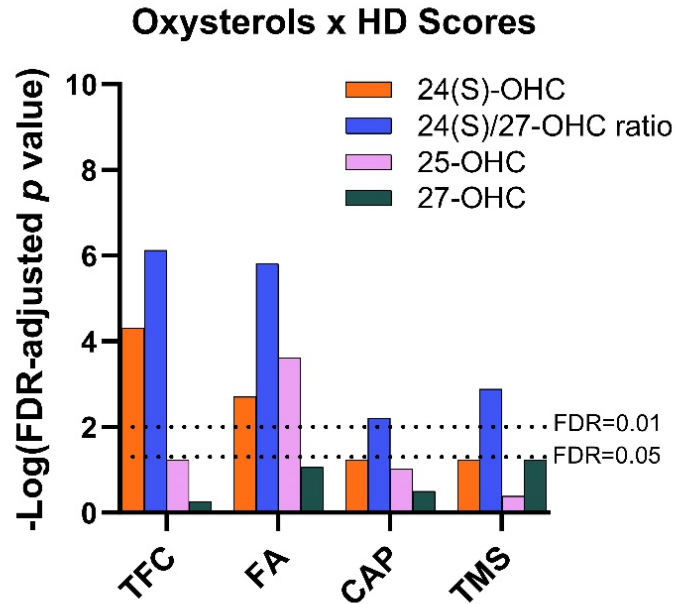


**Supplementary Figure 3. 24(S)/25-OHC ratio correlated with TFC score but did not meaningfully correlate with motor score or performance on problem behavior assessment and motor tests in TRACK-HD.** A) *p* values from analyses of plasma 24(S)/25-OHC ratios with TFC (binomial regression) and motor score (zero-inflated Poisson) B) Log-transformed *p* values from correlation analysis of tests and 24(S)/25-OHC ratio over years 1, 3, and 4 across all TRACK-HD participants.

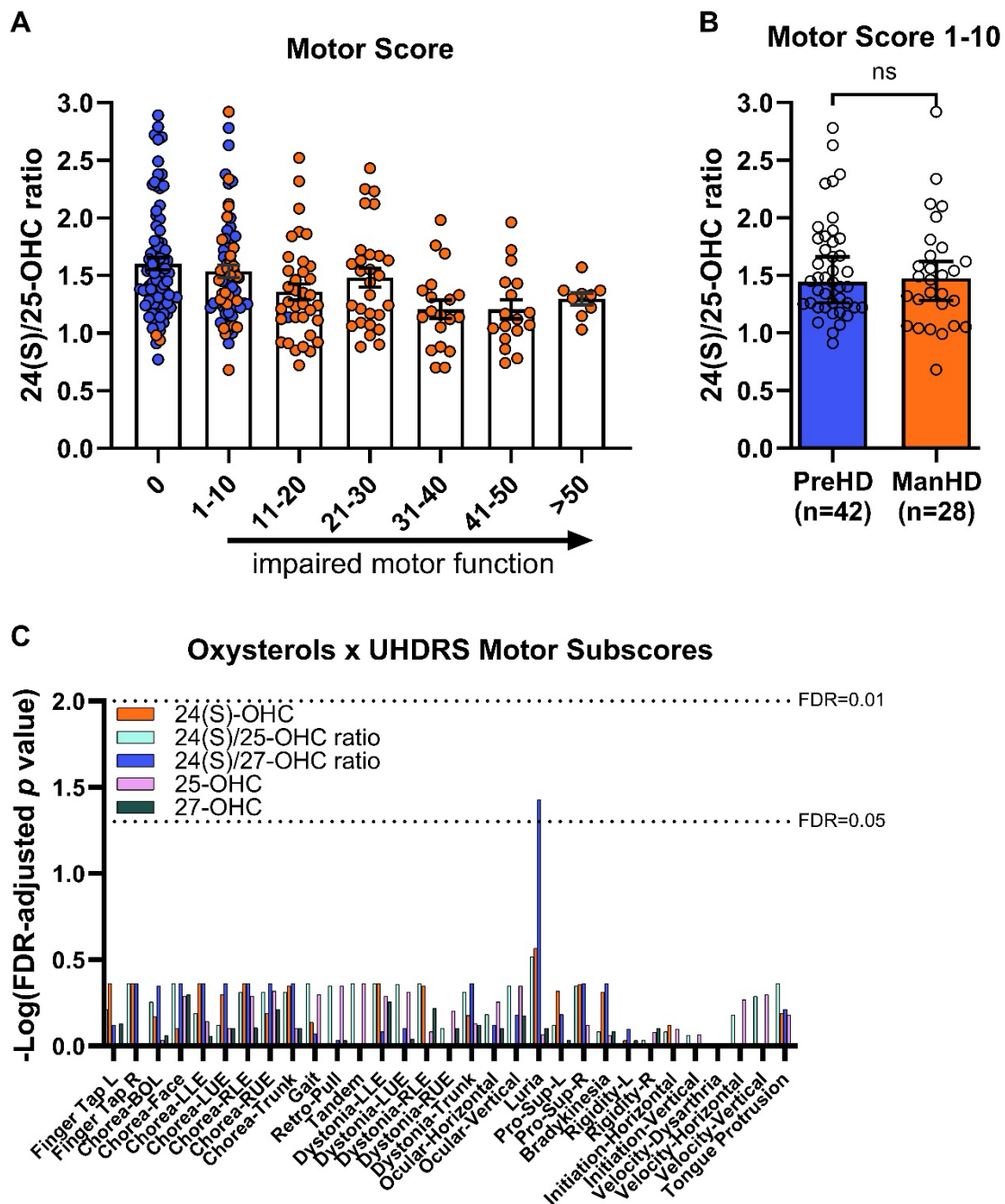
### Oxysterols x Cognitive Scores



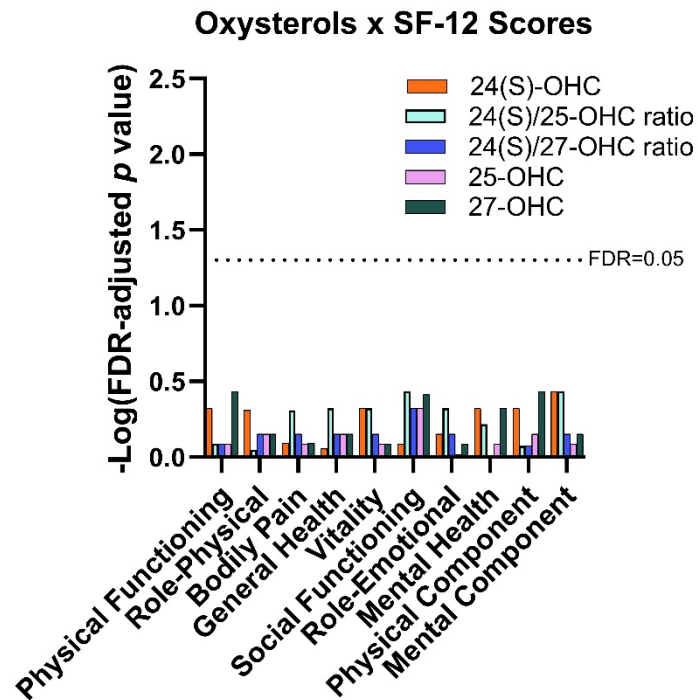
**Supplementary Figure 4. 24(S)-OHC, 25-OHC, 27-OHC, and 24(S)/27-OHC and cognitive tests in the ENROLL-HD.** Log-transformed  $p$  values from cognitive tests and plasma oxysterol concentrations and ratios; binomial regression (MMSE) and Pearson correlations (all other tests).  $P$  values were adjusted for false-discovery rate (FDR).



**Supplementary Figure 5. 24(S)/25-OHC ratio correlated with UHDRS and CAP scores in ENROLL-HD.** Log-transformed  $p$  values from analyses of UHDRS and CAP scores with plasma hydroxycholesterols and ratios; binomial regression (TFC, Functional), Pearson correlation (CAP), zero-inflated Poisson model (Motor score), with  $p$  values were adjusted for false-discovery rate (FDR).



**Supplementary Figure 6. 24(S)/25-OHC ratio is not strongly related to motor score or motor sub scores in ENROLL-HD.** A) Total motor score and 24(S)/25-OHC ratio across pre-manifest HD (blue) and manifest HD (orange) participants. B) 24(S)/25-OHC ratio in pre-manifest (PreHD) and manifest HD (ManHD) at Motor Score of 1-10, t-test. C) Oxysterols and ratios of oxysterols are not correlated with UHDRS motor sub scores. Log-transformed *p* values from analyses of UHDRS motor examination sub scores and plasma oxysterol and oxysterol ratios with zero-inflated Poisson model, with *p* values were adjusted for false-discovery rate (FDR).



**Supplementary Figure 7. Oxysterols and oxysterol ratios did not correlate significantly with SF-12 sub scores and component scores in ENROLL-HD.** Log-transformations of  $p$  values from Pearson correlations.  $P$  values were adjusted for false-discovery rate (FDR).