

Supplementary Information

Dynamics-aware Evolutionary Profiling Uncouples Structural Rigidity from Functional Motion to Enable Enhanced Variant Interpretation

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Table S1. List of analyzed *Homo sapiens* and *cross-species* proteins. More detailed information is available in the data repository. (<https://github.com/karagol-taner/Dynamics-aware-Evolutionary-Profiling>)

ID ¹	PDB ID ²	UniProt ID ³
1	2xolB	O30077
2	3fhfA	Q58134
3	3bjdB	Q9HY91
4	3letB	Q87P32
5	2pxfA	Q1GDR9
6	4xb4A	P74102
7	3oyvA	A7M120
8	2o70F	A1L259
9	3nr1A	Q8N4P3
10	2imsA	Q16611
11	3rznA	Q9NZD2
12	3fcnA	Q2RPE2
13	2o74F	A1L259
14	2zcaB	Q53VY0
15	5w4aB	-
16	2p54A	Q07869
17	3jz9A	Q5ZSQ3
18	1j77A	Q9RGD9
19	4hfva	Q5ZUE7
20	5w4dB	-
21	1r8mE	Q99418
22	5cofA	Q1R1X2
23	2foza	Q9NX46
24	1c1kA	P13342
25	1ah7A	P09598
26	2bbrA	Q98325
27	3nkeB	Q46896
28	5fiaB	Q5ZXU7
29	2incA	Q87798
30	4qn8A	Q5ZRR7
31	3p5pA	Q41594
32	1z1lA	O00408
33	3jzaB	Q9H0U4
34	3m7dA	Q977W1
35	2fefB	Q9I1R6
36	2osaA	P15882
37	1fczA	P13631
38	2x96A	Q10714
39	4uc8A	P03418
40	2fefA	Q9I1R6
41	4qn8B	Q5ZRR7
42	3hfwA	P54922
43	2gnoA	Q9WZM9
44	2y1qA	P37571
45	1wbeA	P68265
46	5fbfA	P24021
47	3lyfD	D3K5I7
48	1vdkB	P84127
49	7bqiA	Q9BQS8
50	3eqxA	Q8E9K5
51	2ovjA	Q9H0H5
52	2ixmA	Q15257

53	1iomA	Q5SIM6
54	1w2wA	Q06489
55	1d2tA	Q9S1A6
56	4z9pA	P18272
57	3owtA	P11938
58	2zd2B	A1B060
59	3lgbB	P20457
60	4ny3A	Q15257
61	5b1qB	Q9WT50
62	2dwkA	Q9D394
63	3m6zA	Q977W1
64	5x1uB	Q5WZ95
65	6exaB	Q5ZYC7
66	5b1qA	Q9WT50
67	3owtB	P11938
68	1w2wE	Q06489
69	5zmoA	Q9L0M9
70	4n67A	A0A0H3LV04
71	1qgiA	P33673
72	7e2sA	P72583
73	5lc8A	Q8RNT4
74	1y6iA	P72583
75	4ervA	Q15413
76	2abkA	P0AB83
77	5muxB	P45859
78	1s9uA	Q8ZPK0
79	5j47A	P03072
80	5muxC	P45859
81	1tezB	P05327
82	1hbnE	P11558
83	7aqxA	P26332
84	1e6yD	P07962
85	8b3wA	M4T0T6
86	3p3oA	Q70KH6
87	4djaA	A9CH39
88	6mm7C	P05132
89	3cmlA	Q6UDW7
90	5imuA	O05446
91	3bqkA	Q6UDW7
92	8cx1A	A7KH32
93	2ws1A	P38913
h1	1by2A	Q08380
h2	1dtdB	P48052
h3	1ef1C	P26038
h4	1ef1D	P26038
h5	1egwB	Q02078
h6	1egwC	Q02078
h7	1ewfA	P17213
h8	1f5nA	P32455
h9	1fczA	P13631
h10	1fs1A	Q13309
h11	1fs1C	Q13309
h12	1lj5A	P05121
h13	1m9zA	P37173
h14	1mlwA	P17752
h15	1nkpD	P01106

h16	1o4kA	P12931
h17	1wojA	P09543
h18	2cb5A	Q13867
h19	2f60K	O00330
h20	2osaA	P15882
h21	2p54A	Q07869
h22	2pq8A	Q9H7Z6
h23	2vkpB	Q96KE9
h24	2wyaA	P54868
h25	2wzbA	P00558
h26	2wzoA	Q3YBR2
h27	2xdgA	Q02643
h28	2xvsA	Q8N0Z6
h29	2znrA	Q96FJ0
h30	3bqpB	P07602
h31	3lblA	Q00987
h32	3luiB	Q15036
h33	3m66A	Q96E29
h34	4bt9A	P13674
h35	4bt9B	P13674
h36	4ddpA	Q14457
h37	4fqnB	Q9BSQ5
h38	4gv2A	Q9Y6F1
h39	4hkdB	Q13188
h40	4jnuA	Q7Z3B4
h41	4jnuB	Q7Z3B4
h42	4jo7A	Q9BVL2
h43	4jo7C	Q9BVL2
h44	4xglA	O15091
h45	4ykdA	Q9BSQ5
h46	4zyaA	O43776
h47	4zyaB	O43776
h48	5opmA	P49902
h49	5v03B	Q9H2S1
h50	5w2fA	P41214
h51	5wbxB	Q9H2S1
h52	6mdwA	Q9H040
h53	6mdxA	Q9H040
h54	6mi3B	Q9Y6K9
h55	6uxEB	Q9Y697
h56	6y43A	Q8NI99
h57	7jfIC	Q14653
h58	7jfID	Q14653

¹Protein IDs 1–93 are cross-species alpha-helical proteins; IDs h1–h58 are Homo sapiens medium-sized proteins. Proteins were selected from available molecular dynamics trajectories within the ATLAS database (<https://www.dsimb.inserm.fr/ATLAS>) (methods).

²PDB ID from the RCSB PDB (<https://www.rcsb.org/>)

³UniProt ID of the protein.

**Protein Landscape: Which Proteins are "Soft Machines"?
(Ranked by Mean DCS)**

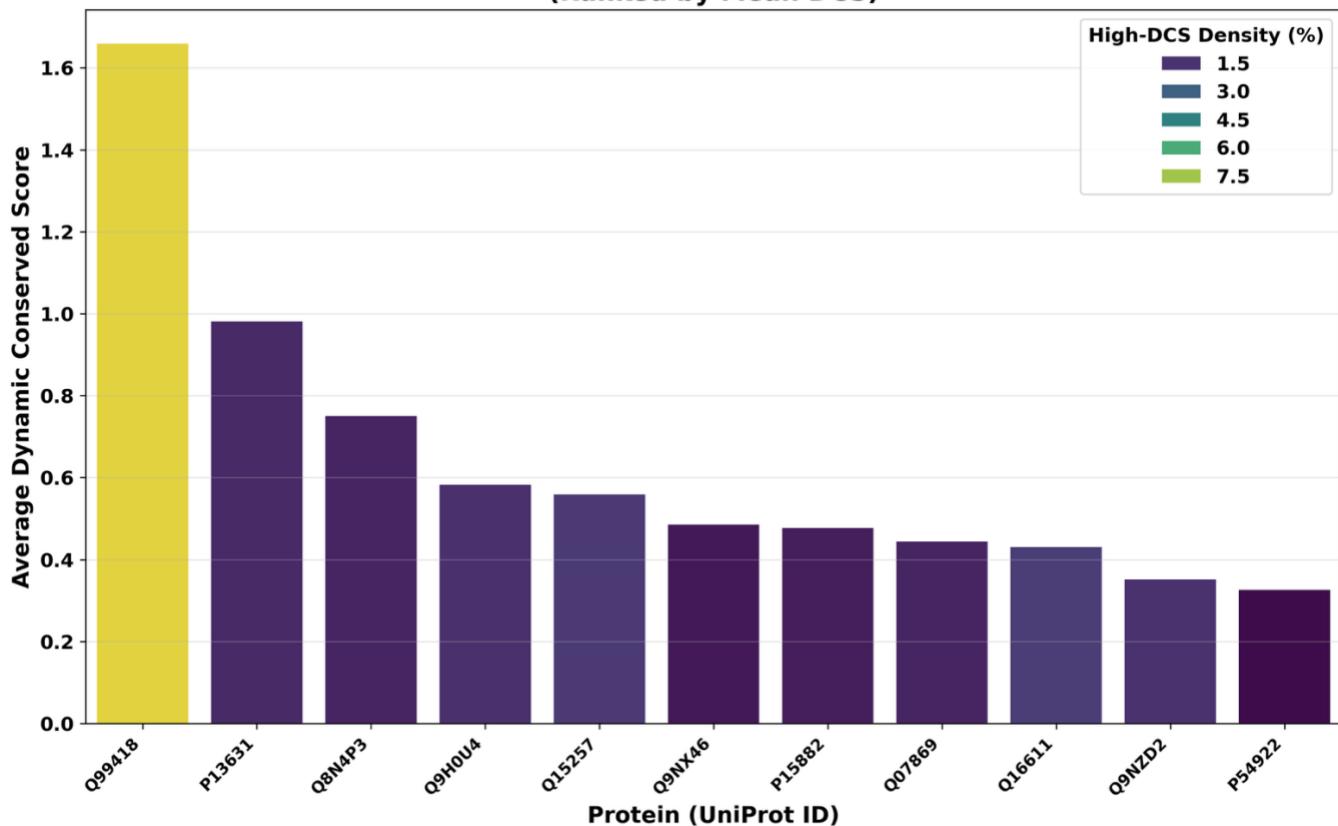


Figure S1. Top-ranked cross-species alpha-helical proteins based on highest average DCS, derived from available molecular dynamics data and structural analysis (see Methods).

Protein Landscape: Which Proteins are "Structural Rocks"?
(Ranked by Mean RCS)

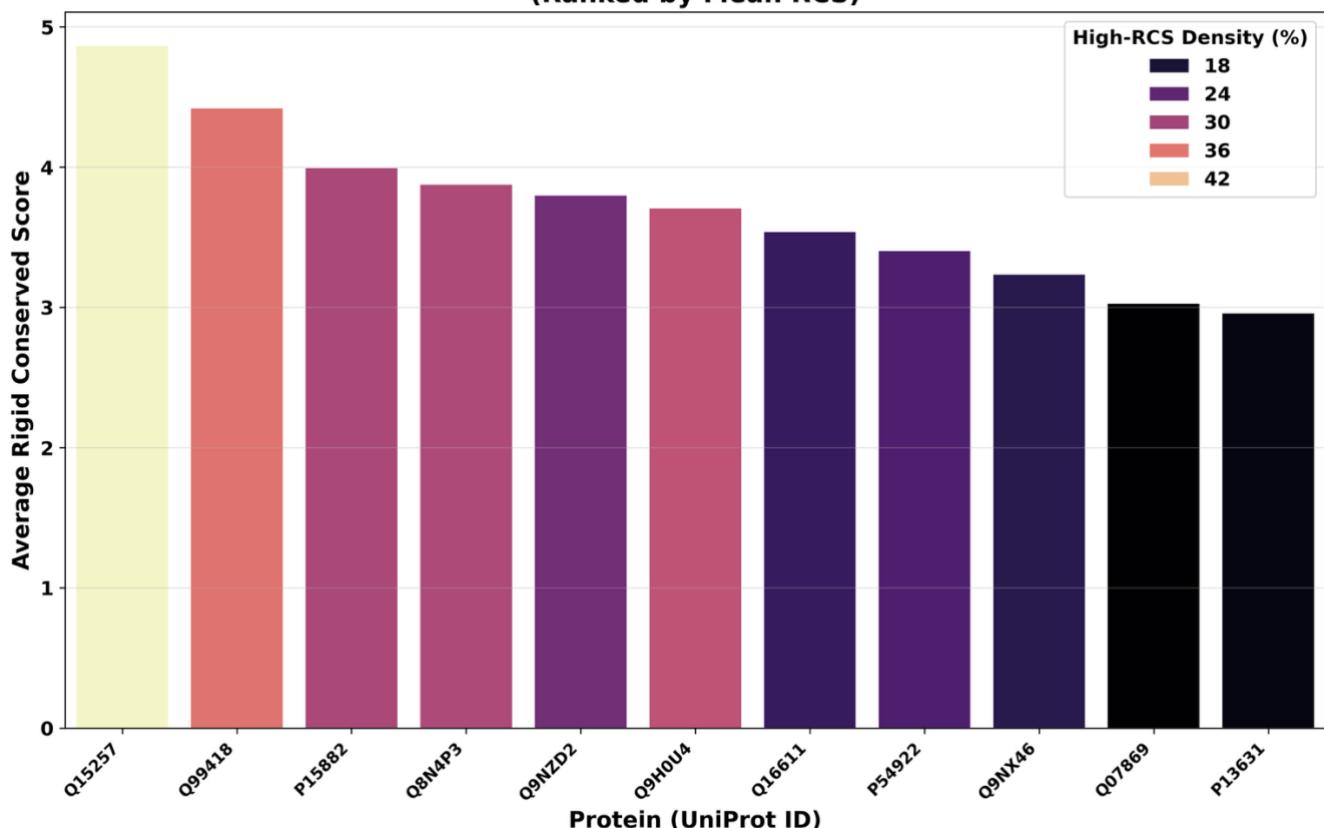


Figure S2. Top-ranked cross-species alpha-helical proteins based on highest average RCS, derived from available molecular dynamics data and structural analysis (see Methods).

**Protein Landscape: Which Proteins are "Soft Machines"?
(Ranked by Mean DCS)**

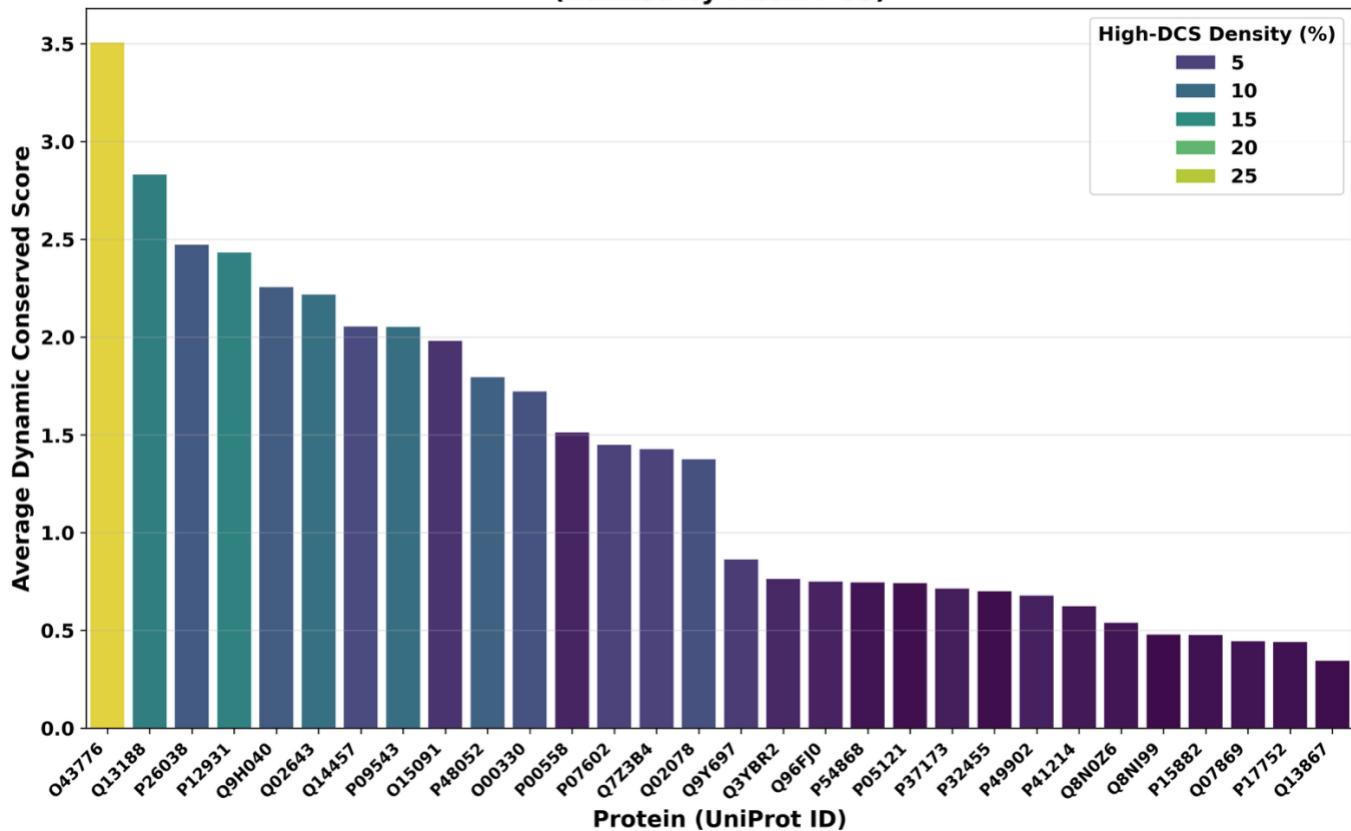


Figure S3. Top-ranked human medium-size proteins based on highest average DCS, derived from available molecular dynamics data and structural analysis (see Methods).

Protein Landscape: Which Proteins are "Structural Rocks"?
(Ranked by Mean RCS)

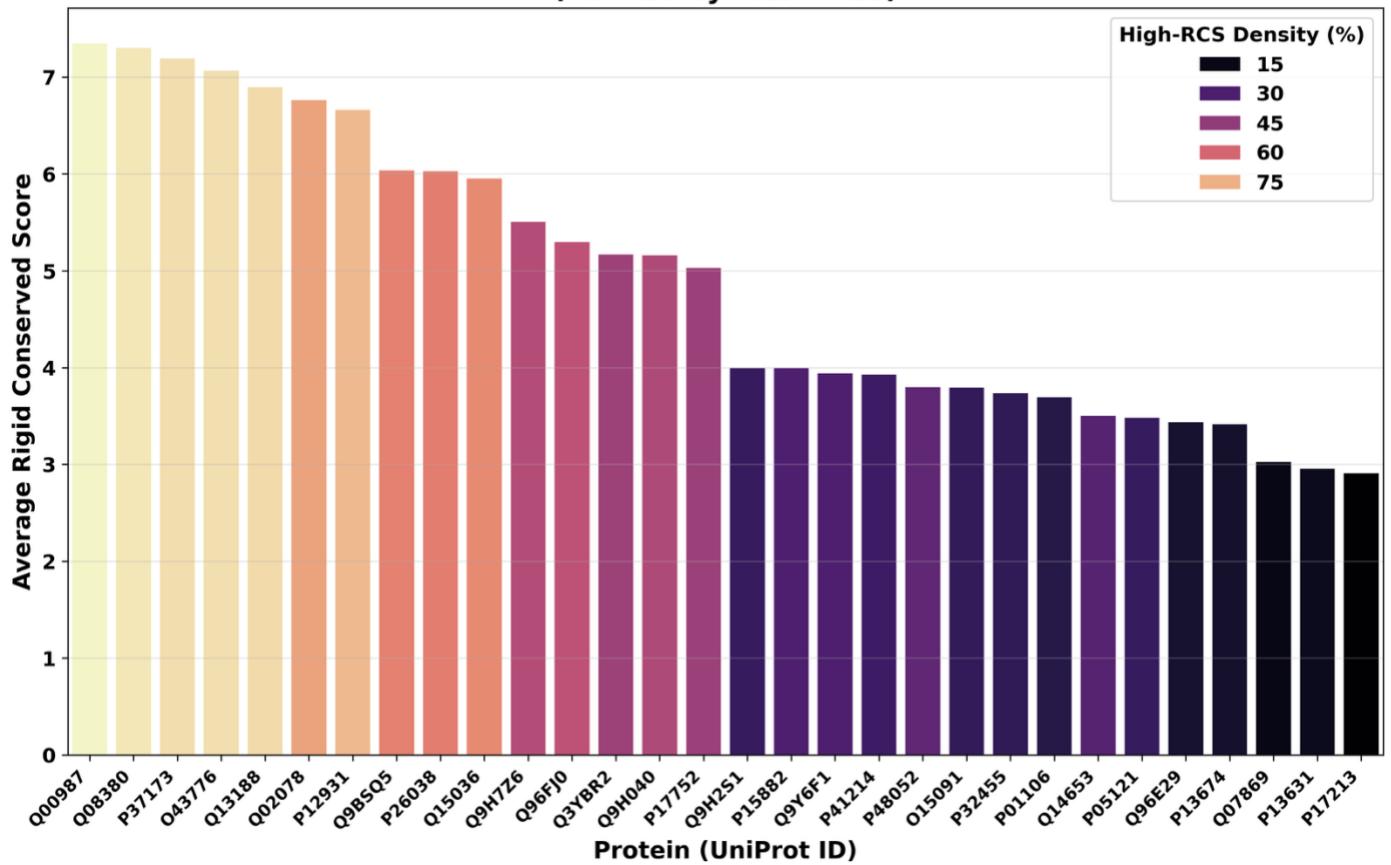


Figure S4. Top-ranked human medium-size proteins based on highest average RCS, derived from available molecular dynamics data and structural analysis (see Methods).

RCS (Global) vs AlphaMissense

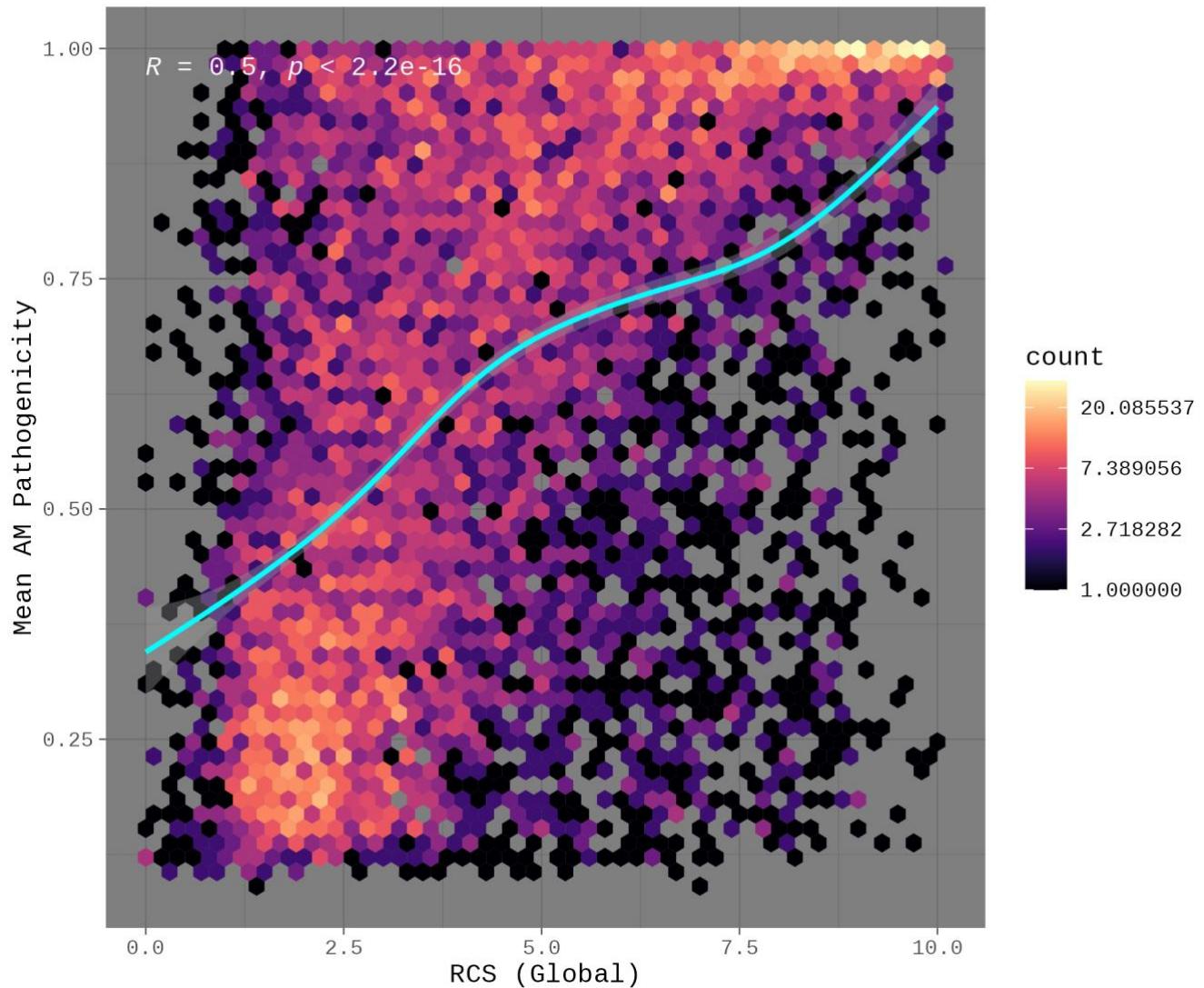


Figure S5. Comparison between Rigid Conserved Score (RCS) and AlphaMissense scores.

DCS (Global) vs AlphaMissense

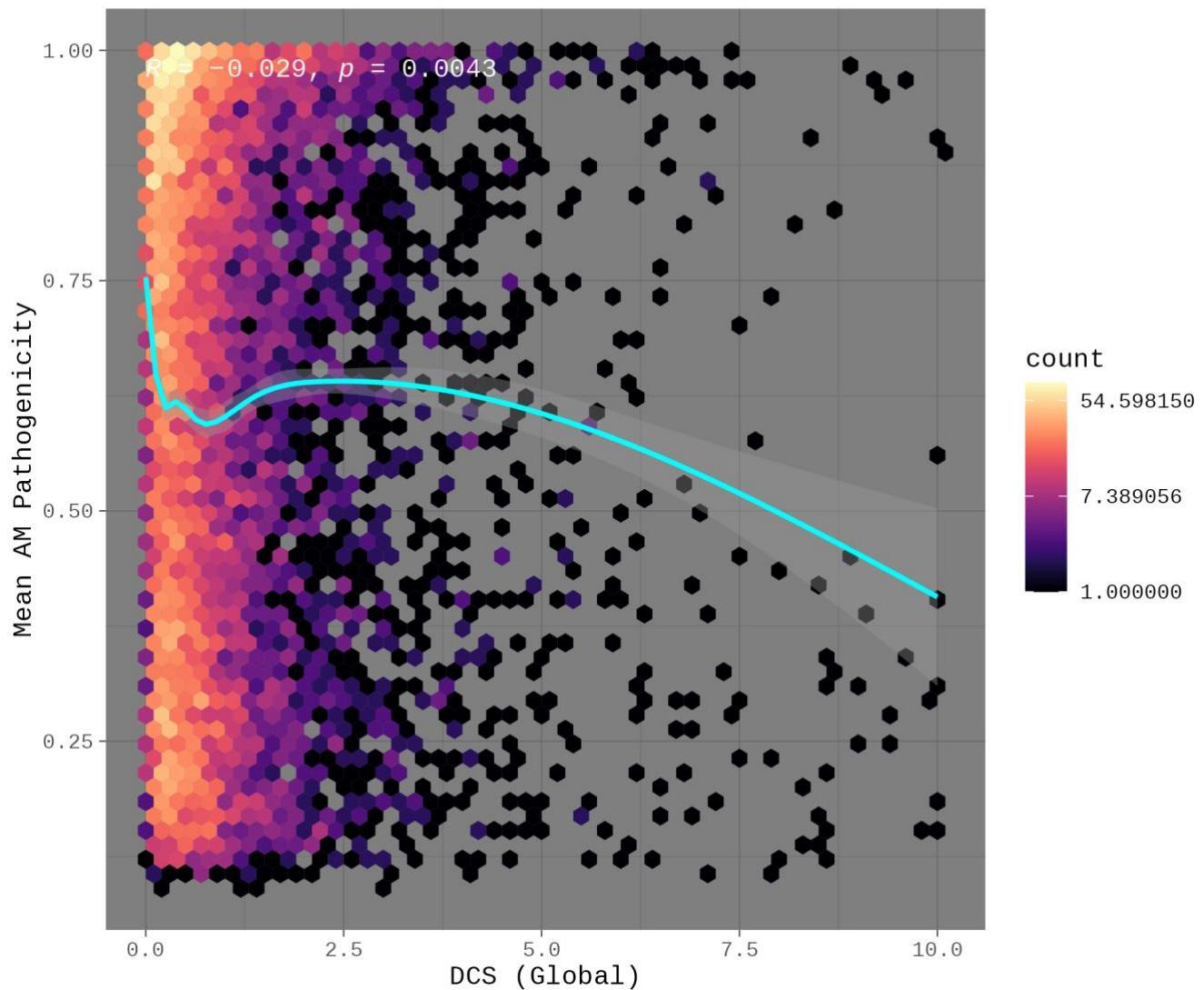


Figure S6. Comparison between Dynamic Conserved Score (DCS) and AlphaMissense scores.

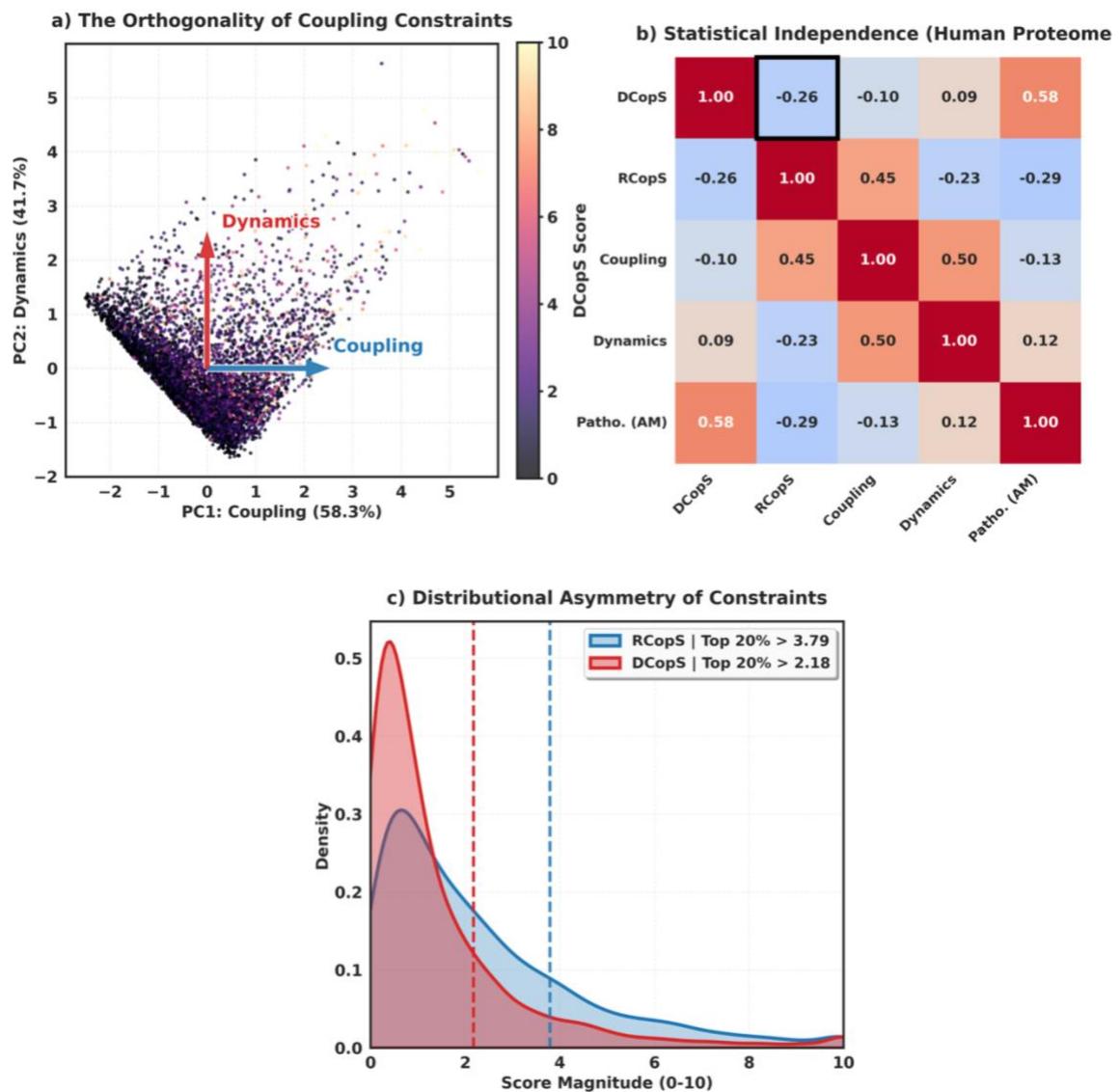


Figure S7. Descriptive Statistics of DCopS and RCopS.

(a) Principal Component Analysis (PCA) biplot of the pan-proteome residue landscape (93 alpha-helical proteins), illustrating the relationship between Evolutionary Coupling and Structural Dynamics. b) Spearman correlation matrix quantifying the relationships between derived metrics. (c) Density plots of DCopS and RCopS scores.

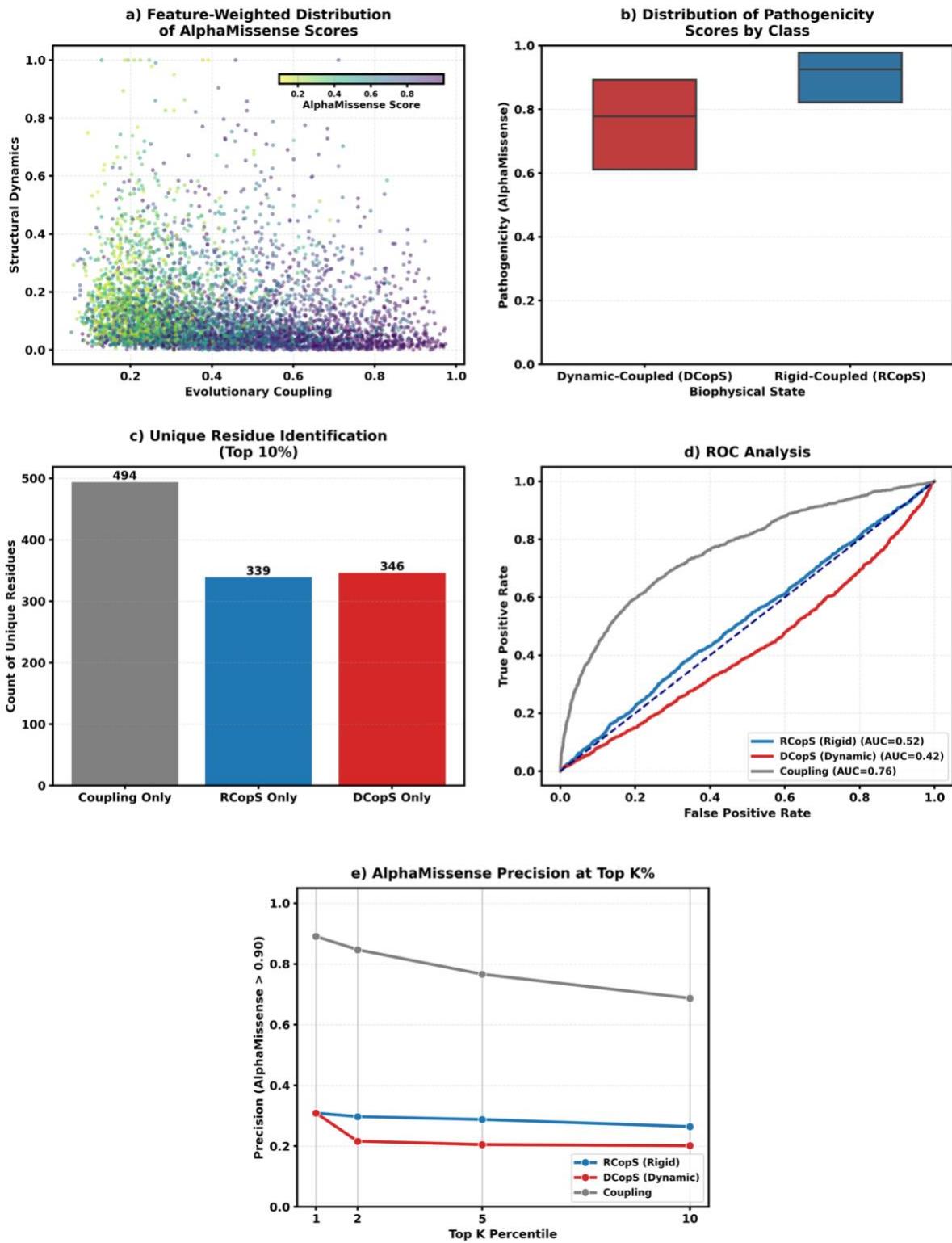


Figure S8. Statistical Analysis of Dynamics-aware Evolutionary Coupling Profiling Metrics.
 (a) Feature-weighted landscape of AlphaMissense scores plotted against evolutionary cooupling and structural dynamics. (b) Distribution of AlphaMissense pathogenicity scores across biophysical classes. (c) Unique residue identification within the top 10% of scores. (d) Receiver Operating Characteristic (ROC) analysis (e) Precision at Top-K% analysis.

RCopS (Global) vs AlphaMissense

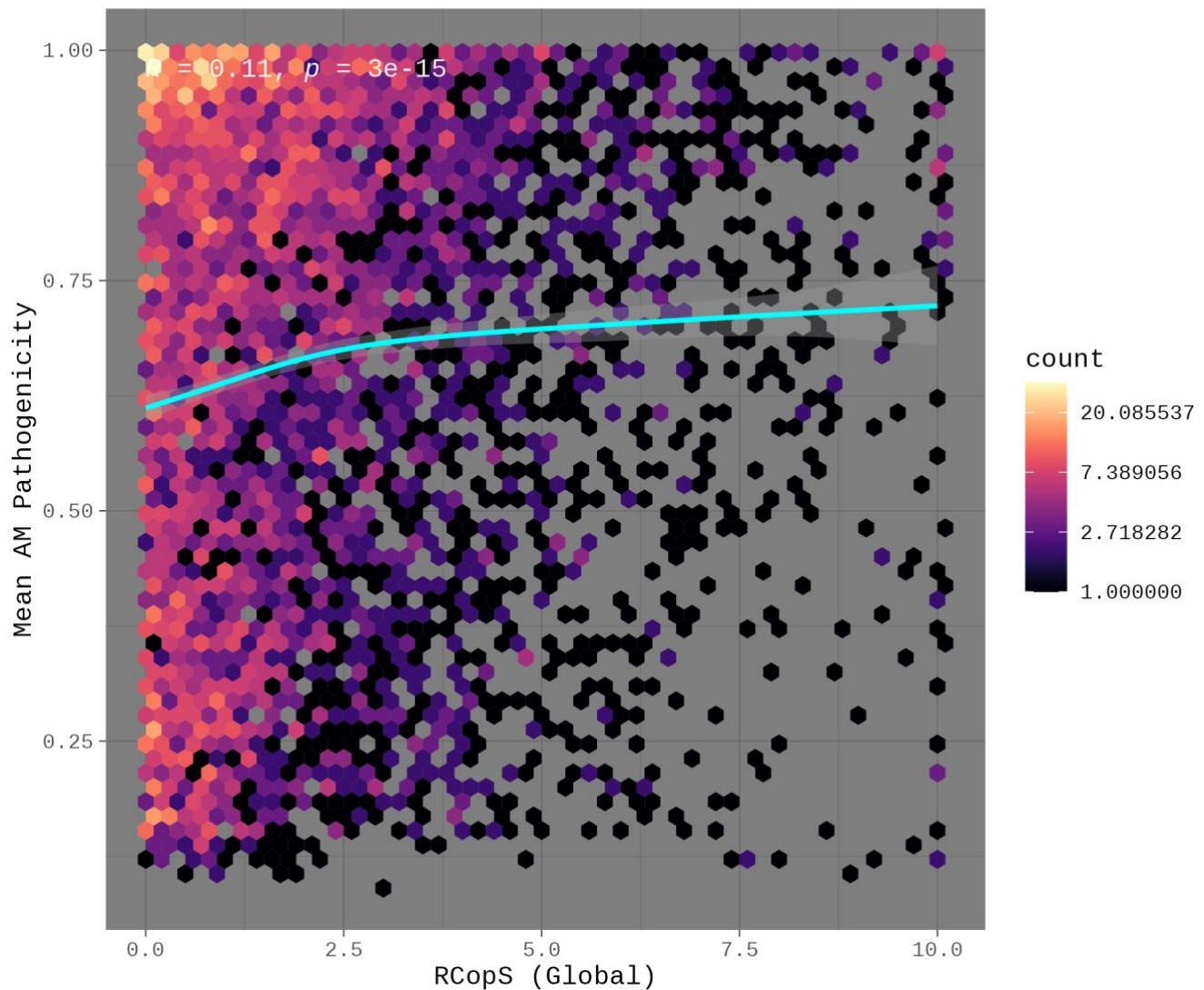


Figure S9. Comparison between Rigid Co-evolutionary Coupling Score (RCopS) and AlphaMissense scores.

DCopS (Global) vs AlphaMissense

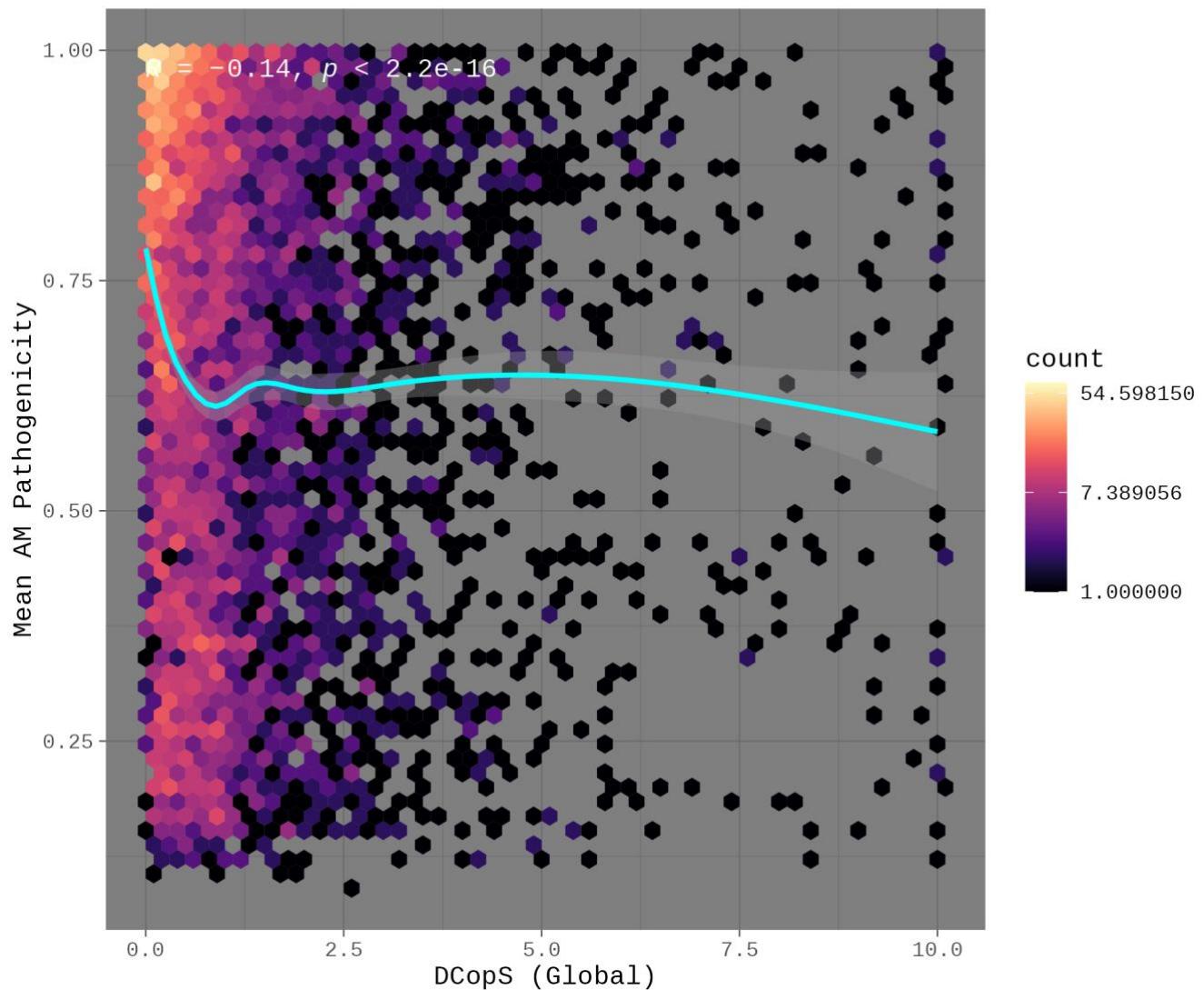


Figure S10. Comparison between Dynamics Co-evolutionary Coupling Score (DCopS) and AlphaMissense scores.

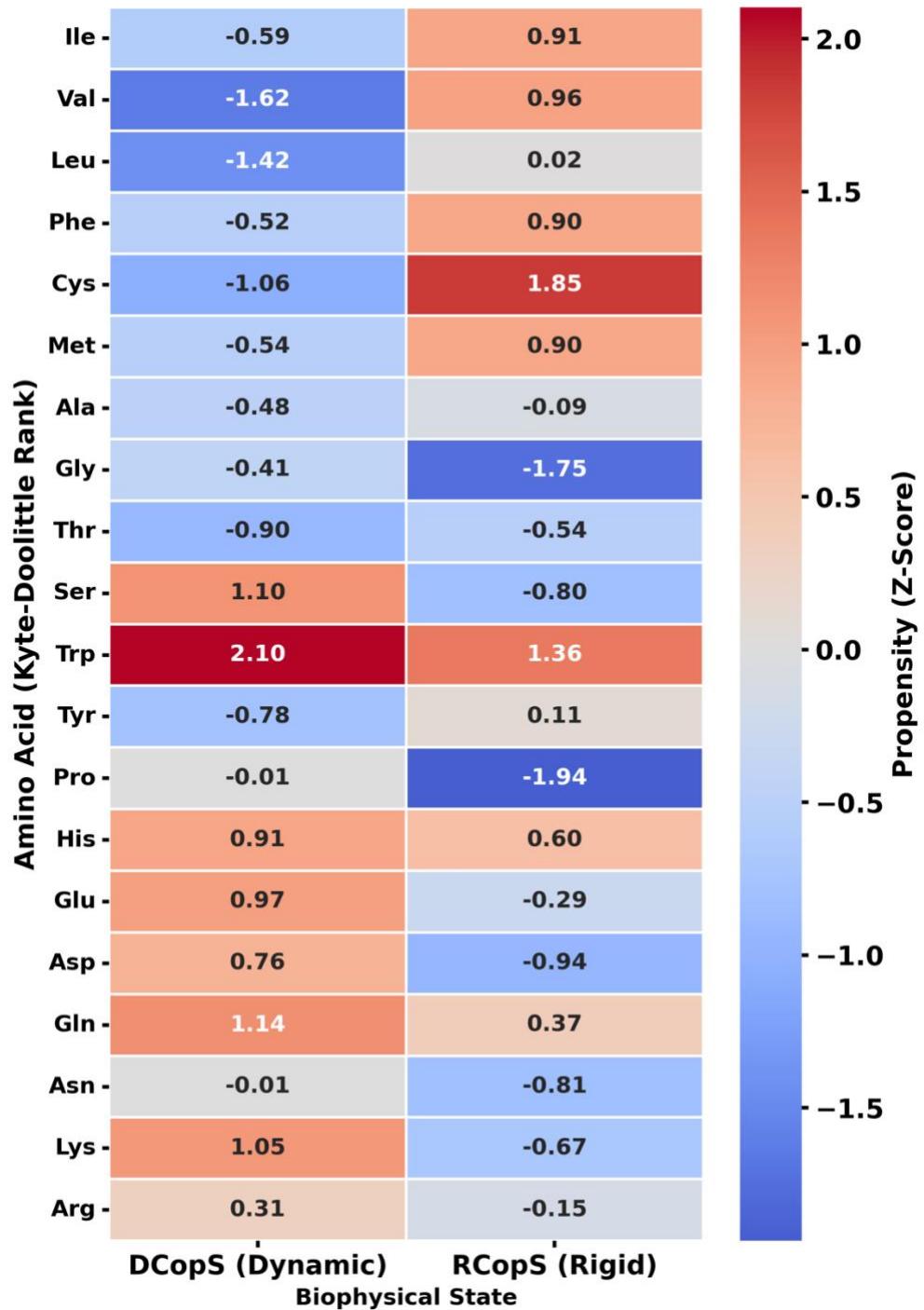


Figure S11. Amino Acid Propensity Heatmap of DCopS and RCopS metrics.

Dynamics-Aware Evolutionary Profiling

ADEPT Automated Dynamics-aware Evolutionary Profiling Tool
Karagöl et al. 2025-2026 (version 0.9.6-web)

Methodology & Protocol

The screenshot displays the ADEPT web interface with four main sections:

- DATA CONFIGURATION**: Includes tabs for Conservation Mode (selected) and Coupling Mode. It shows a PROJECT NAME input field containing "MyProtein" and an INPUT MODE section with Separate Files (selected) and Combined CSV options.
- Biophysical Landscape**: Shows a status message "Awaiting Analysis". It includes sections for MOLECULAR DYNAMICS (RMSF), EVOLUTIONARY CONSERVATION / COUPLING, and PROTEIN STRUCTURE (PDB/CIF), each with a "Select [Type] Data" button.
- 3D Structure Mapping**: Shows a "Upload PDB Structure" button.
- PARAMETERS**: Allows adjustment of CONSERVATION WEIGHT (blue slider at 1.0) and DYNAMICS (RMSF) WEIGHT (orange slider at 1.0). A "Run Analysis" button is present.
- Sequence Heatmap**: Shows a placeholder area with a "Run analysis to generate map" button.

Figure S12. Screenshot of *ADEPT* (Automated Dynamics-aware Evolutionary Profiling Tool) web page. (<https://www.karagolresearch.com/adept>)

Table S2. List of available variants from our analysis that are also available in the ClinVar database (likely pathogenic and/or pathogenic variants only).

Protein_ID	UniProt_ID	Gene_Name	Protein_Change	DCS_Score	RCS_Score	AM_Score	ClinVar_Significance	ClinVar_Accession
proteinh46	O43776	NARS1	p.Thr17Met	5.77521546	7.95821059	0.0926	Likely pathogenic	VCV000986308
proteinh46	O43776	NARS1	p.Lys60Thr	4.00639923	7.81799684	0.5261	Likely pathogenic	VCV001700235
proteinh25	P00558	PGK1	p.Lys30Thr	3.25673338	0.62755102	0.4744	Likely pathogenic	VCV000625219
proteinh25	P00558	PGK1	p.Asp315Asn	2.67962719	7.80165816	0.9902	Likely pathogenic	VCV001506855
proteinh3	P26038	MSN	p.Asp534Ala	2.4318675	5.62064272	0.7441	Likely pathogenic	VCV000804017
proteinh25	P00558	PGK1	p.Leu88Pro	2.11855213	2.75985128	0.9854	Pathogenic	VCV000009946
proteinh25	P00558	PGK1	p.Thr378Pro	1.94739253	4.21664677	0.9588	Pathogenic	VCV000009956
proteinh25	P00558	PGK1	p.Asp285Val	1.92554557	8.03571429	0.988	Pathogenic	VCV000009951
proteinh25	P00558	PGK1	p.Val266Met	1.57763655	5.38551617	0.951	Pathogenic	VCV000009944
proteinh22	Q9H7Z6	KAT8	p.Lys175Glu	1.40072916	1.26938159	0.9993	Pathogenic	VCV000976462
proteinh22	Q9H7Z6	KAT8	p.Lys175Thr	1.40072916	1.26938159	0.9962	Likely pathogenic	VCV001329928
proteinh25	P00558	PGK1	p.Cys316Arg	1.3832589	5.31089883	0.9905	Pathogenic	VCV000009948
proteinh25	P00558	PGK1	p.Arg206Pro	1.24916379	5.25797872	0.8115	Pathogenic	VCV000009943
proteinh25	P00558	PGK1	p.Ile47Asn	1.24334798	6.28362462	0.9973	Pathogenic	VCV000009952
proteinh25	P00558	PGK1	p.Ser320Asn	1.08210237	6.24430091	0.9549	Pathogenic	VCV000009953
proteinh25	P00558	PGK1	p.Cys50Trp	0.99629656	4.84524262	0.9987	Likely pathogenic	VCV001687119
proteinh25	P00558	PGK1	p.Ser62Asn	0.99281612	6.71550695	0.9942	Likely pathogenic	VCV000391568
proteinh25	P00558	PGK1	p.Asp164Val	0.98935074	9.00911854	0.9987	Pathogenic	VCV000009954
proteinh25	P00558	PGK1	p.Ala354Pro	0.89768392	2.78468302	0.9487	Likely pathogenic	VCV001506865
proteinh25	P00558	PGK1	p.Asp268Asn	0.87869967	1.81393834	0.1682	Pathogenic	VCV000009942
proteinh26	Q3YBR2	TBRG1	p.Ala210Thr	0.78132944	5.20144657	0.0969	Likely pathogenic	VCV000996584
proteinh25	P00558	PGK1	p.Gly158Val	0.67394095	4.78818389	0.9952	Pathogenic	VCV000009947
proteinh52	Q9H040	SPRTN	p.Tyr117Cys	0.53461618	6.18179543	0.4674	Pathogenic	VCV000143916
proteinh22	Q9H7Z6	KAT8	p.Met217Leu	0.50597802	4.19456031	0.794	Likely pathogenic	VCV002683826
proteinh24	P54868	HMGCS2	p.Asp136Gly	0.48261225	0.99257963	0.9681	Likely pathogenic	VCV001327468

proteinh24	P54868	HMGCS2	p.Val141Asp	0.4373275	2.39868044	0.9622	Likely pathogenic	VCV001327463
proteinh22	Q9H7Z6	KAT8	p.Lys181Asn	0.24001222	1.71821534	0.9818	Pathogenic	VCV000976463
proteinh24	P54868	HMGCS2	p.Glu74Lys	0.22019061	2.26038743	0.9304	Likely pathogenic	VCV001327462
proteinh24	P54868	HMGCS2	p.Gly212Arg	0.2128617	8.40290199	0.9716	Pathogenic/Likely pathogenic	VCV000009259
proteinh24	P54868	HMGCS2	p.Gly219Glu	0.12474414	3.39906778	0.9653	Likely pathogenic	VCV003896593
proteinh27	Q02643	GHRHR	p.Arg94Trp	0.08629653	2.71380786	0.6164	Likely pathogenic	VCV001449976
proteinh27	Q02643	GHRHR	p.Arg94Gln	0.08629653	2.71380786	0.2761	Pathogenic/Likely pathogenic	VCV000161436
proteinh24	P54868	HMGCS2	p.Tyr167Cys	0.0810042	3.31068489	0.7319	Pathogenic	VCV000009262
proteinh24	P54868	HMGCS2	p.Ala171Val	0.07501354	2.45557137	0.4387	Likely pathogenic	VCV000870423
proteinh24	P54868	HMGCS2	p.Gly55Asp	0.06409203	2.36807632	0.9764	Likely pathogenic	VCV002628076
proteinh24	P54868	HMGCS2	p.Phe174Leu	0.05413154	1.28795812	0.7558	Pathogenic/Likely pathogenic	VCV000009257