Glossary

Epistasis - in the most general sense, epistasis is interactions leading to non-independence of effects. For proteins, this means that the effect of a mutation in a protein sequence on fitness can vary based on co-occurring mutations.

Fitness - ability of a protein sequence to perform a specific, desired function.

Fitness landscape - both (1) a dataset mapping many protein sequences to fitness within a defined region of sequence space and (2) a conceptual framework for thinking about the mapping of protein sequence to fitness.

Function - a task performed by a protein sequence, typically referring to either a native task or a task desired by a protein engineer.

Homology - sharing a common origin at all levels (organism, population and species), which often results in similarity. For proteins both sequences and structures can be considered homologous due to common origin. [1]

Multiple sequence alignment - an arrangement of three or more sequences such that similar regions are aligned. Gaps can be inserted within some sequences at a penalty such that as much of the similar regions of the sequences are aligned as possible.

Mutagenesis - introduction of genetic mutations. In protein engineering, mutagenesis is typically performed on a single DNA sequence encoding a protein.

Mutant - a resulting DNA (and, equivalently, protein) sequence from mutagenesis on an initial starting sequence. Mutagenesis for protein engineering can either result in a single mutant or a library (pool) of mutants. Parent sequence - another word for the initial starting sequence prior to mutagenesis. This is not to be conflated with “wild type sequence”.

Sequence identity - similarity between two (typically aligned) sequences

Thermostability - ability of a protein to preserve its structure and function under extremes of temperature conditions. [2]

Tree of life - referring to the phylogenetic tree of life, which depicts the relationships of biological species based on their last common ancestors.

Variant - within this text we define variant the same way as mutant (see previous).

Wild type sequence - a protein sequence that arises in nature and predominates within a natural population. While a wild type sequence can function as a parent sequence, these two terms have distinct meanings and should not be conflated.

References

[1] Helga Ochoterena, Alexander Vrijdaghs, Erik Smets, and Regine Claßen-Bockhoff. The Search for Common Origin: Homology Revisited. *Systematic Biology*, 68(5):767–780, 02 2019. ISSN 1063-5157. doi: 10.1093/sysbio/syz013. URL `https://doi.org/10.1093/sysbio/syz013`. (document)

[2] X-X Zhou, Y-B Wang, Y-J Pan, and W-F Li. Differences in amino acids composition and coupling patterns between mesophilic and thermophilic proteins. *Amino acids*, 34(1):25–33, 2008. (document)

GB1 Additional Results [*]

Model	Split	Train rho	Train MSE	Test rho	Test MSE	Avg Epochs Trained
ESM-1b (per AA)	low_vs_high	0.83	0.03	0.53	3.26	60.00
	one_vs_rest	0.58	0.79	0.29	1.83	22.73
	sampled	0.92	0.24	0.91	0.28	69.27
	three_vs_rest	0.76	0.58	0.79	0.86	36.45
	two_vs_rest	0.59	0.60	0.47	1.41	56.64
ESM-1b (mean)	low_vs_high	0.75	0.09	0.13	4.33	41.00
	one_vs_rest	0.56	0.84	0.31	1.88	22.00
	sampled	0.71	1.20	0.68	1.18	31.27
	three_vs_rest	0.36	1.12	0.54	1.59	22.64
	two_vs_rest	0.38	0.99	0.35	1.58	24.27
ESM-1b (mut mean)	low_vs_high	0.83	0.03	0.48	3.39	48.45
	one_vs_rest	0.68	0.54	0.27	1.67	26.00
	sampled	0.92	0.31	0.89	0.40	61.27
	three_vs_rest	0.81	0.50	0.80	0.88	37.36
	two_vs_rest	0.69	0.50	0.55	1.28	65.00
ESM-1v (per AA)	low_vs_high	0.82	0.03	0.53	3.26	54.73
	one_vs_rest	0.65	0.70	0.09	1.92	25.00
	sampled	0.94	0.17	0.92	0.22	70.91
	three_vs_rest	0.87	0.32	0.83	0.71	54.64
	two_vs_rest	0.65	0.48	0.37	1.48	55.36
ESM-1v (mean)	low_vs_high	0.74	0.09	0.11	4.54	42.55
	one_vs_rest	0.65	0.79	-0.08	1.88	24.73
	sampled	0.73	1.12	0.69	1.13	34.27
	three_vs_rest	0.42	1.07	0.49	1.53	23.27
	two_vs_rest	0.40	0.92	0.18	1.54	29.36
ESM-1v (mut mean)	low_vs_high	0.82	0.03	0.47	3.50	54.36
	one_vs_rest	0.68	0.48	0.19	2.13	24.55
	sampled	0.93	0.27	0.90	0.35	66.36
	three_vs_rest	0.80	0.53	0.80	0.95	35.36
	two_vs_rest	0.68	0.51	0.50	1.34	54.18
ESM-untr (per AA)	low_vs_high	0.79	0.04	0.32	3.78	77.91
	one_vs_rest	-0.08	0.84	0.12	1.88	24.18
	sampled	0.85	0.54	0.81	0.57	119.27
	three_vs_rest	0.43	1.11	0.52	1.55	23.36
	two_vs_rest	0.00	1.00	0.22	1.58	29.18
ESM-untr (mean)	low_vs_high	0.61	0.10	0.10	4.71	54.18
	one_vs_rest	-0.17	0.84	0.05	1.82	25.64
	sampled	0.55	1.45	0.55	1.40	30.27
	three_vs_rest	0.33	1.19	0.46	1.69	22.64
	two_vs_rest	-0.10	1.00	0.09	1.57	30.00
ESM-untr (mut mean)	low_vs_high	0.73	0.06	0.16	4.14	63.55
	one_vs_rest	0.44	0.95	0.21	1.62	22.00
	sampled	0.73	0.82	0.70	0.85	72.27
	three_vs_rest	0.48	0.93	0.57	1.26	25.00
	two_vs_rest	0.51	0.70	0.40	1.63	142.18
CNN	low_vs_high	0.84	0.03	0.47	3.40	99.00
	one_vs_rest	0.48	2.52	0.15	2.11	22.73
	sampled	0.91	0.24	0.90	0.30	99.00
	three_vs_rest	0.83	0.40	0.81	0.75	99.00
	two_vs_rest	0.58	1.05	0.39	1.64	28.09

[*] All additional result tables are averages of 10 separate runs with different seeds; graphs show mean and standard dev

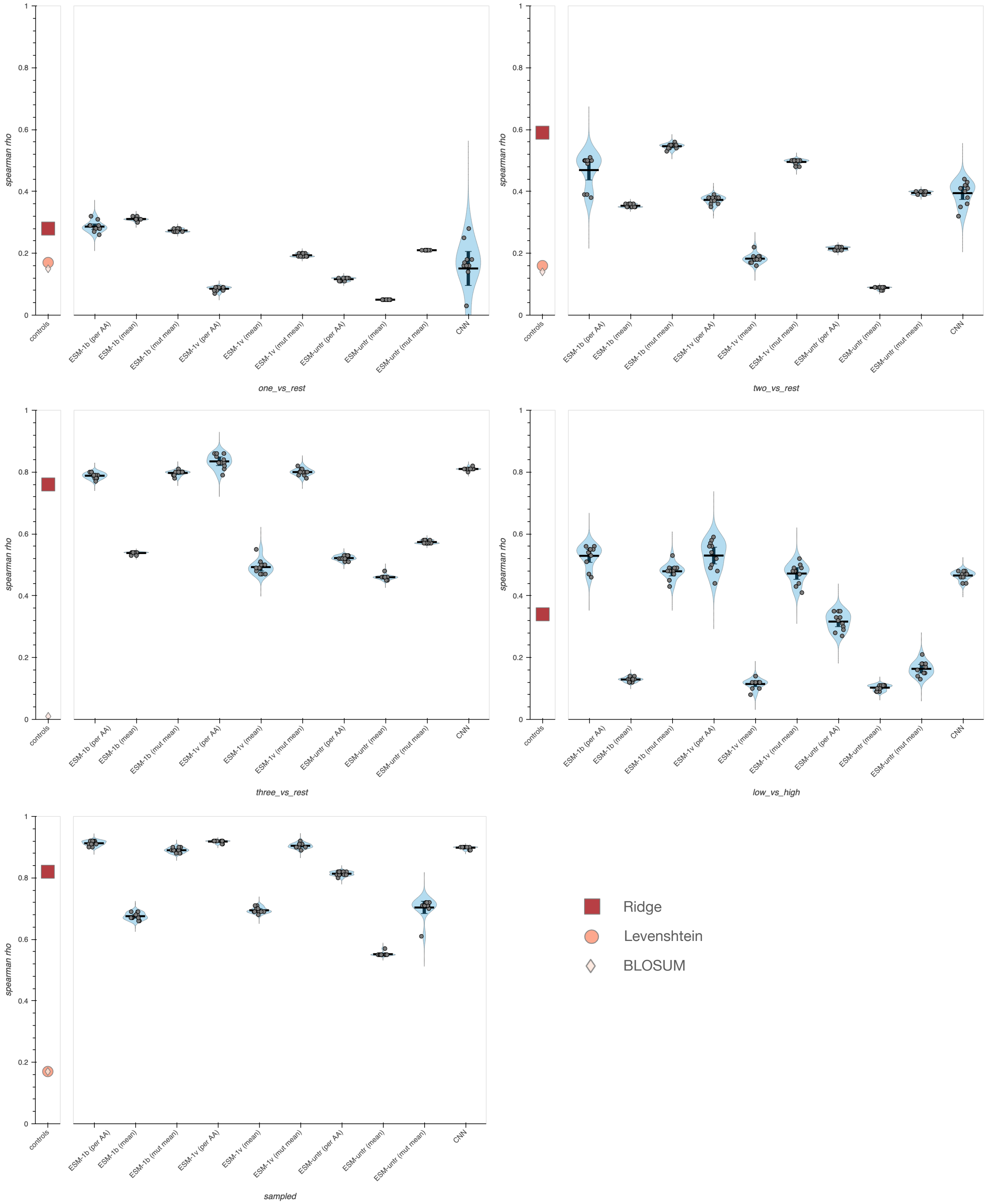
AAV Additional Results

Model	Split	Train rho	Train MSE	Test rho	Test MSE	Avg Epochs Trained
	low_vs_high	0.78	1.15	0.38	14.52	49.67
	one_vs_many	0.38	5.73	0.03	9.85	29.73
ESM-1b (per AA)	sampled	0.90	2.07	0.90	2.12	44.27
	seven_vs_many	0.89	2.56	0.65	2.70	43.82
	two_vs_many	0.84	2.42	0.61	6.79	67.45
	low_vs_high	0.59	2.03	0.15	19.69	38.73
	one_vs_many	0.38	5.70	0.04	9.78	31.09
ESM-1b (mean)	sampled	0.78	5.16	0.78	5.19	35.09
	seven_vs_many	0.76	6.24	0.46	5.96	26.91
	two_vs_many	0.73	5.07	0.22	9.74	42.82
	low_vs_high	0.74	1.49	0.32	15.54	32.64
	one_vs_many	0.66	3.96	0.40	13.86	49.09
ESM-1b (mut mean)	sampled	0.89	2.62	0.88	2.70	33.45
	seven_vs_many	0.88	3.40	0.60	4.51	27.64
	two_vs_many	0.82	3.12	0.59	7.41	34.73
	low_vs_high	0.78	1.11	0.35	10.83	39.91
	one_vs_many	0.30	5.77	0.12	9.55	22.82
ESM-1v (per AA)	sampled	0.91	1.88	0.91	1.94	37.64
	seven_vs_many	0.91	2.22	0.70	2.58	33.55
	two_vs_many	0.87	1.82	0.70	6.00	50.82
	low_vs_high	0.58	2.05	0.23	22.12	38.45
	one_vs_many	0.34	5.77	0.15	9.56	23.36
ESM-1v (mean)	sampled	0.78	5.30	0.77	5.29	30.91
	seven_vs_many	0.76	6.03	0.45	8.85	26.73
	two_vs_many	0.71	4.98	0.14	12.14	37.27
	low_vs_high	0.75	1.50	0.29	16.14	29.27
	one_vs_many	0.67	4.40	0.43	18.06	39.82
ESM-1v (mut mean)	sampled	0.89	2.94	0.89	3.03	30.27
	seven_vs_many	0.89	3.42	0.63	4.35	25.00
	two_vs_many	0.84	2.54	0.61	8.19	40.64
	low_vs_high	0.56	1.78	0.06	23.25	62.73
	one_vs_many	0.40	5.79	0.18	9.54	25.91
	sampled	0.78	4.15	0.77	4.25	100.36
	seven_vs_many	0.74	4.70	0.38	15.27	56.18
	two_vs_many	0.73	4.32	0.20	11.02	118.27
ESM-untr (mean)	low_vs_high	0.42	2.22	0.22	25.52	70.18
	one_vs_many	0.32	5.80	0.01	9.54	25.73
ESM-untr (per AA)	sampled	0.62	6.55	0.62	6.58	69.73
	seven_vs_many	0.62	6.33	0.23	18.30	44.36
	two_vs_many	0.57	6.15	0.14	9.15	90.27
	low_vs_high	0.63	1.64	0.24	19.26	47.91
	one_vs_many	0.52	4.54	0.41	8.66	112.73
ESM-untr (mut mean)	sampled	0.81	3.70	0.81	3.79	68.55
	seven_vs_many	0.80	3.80	0.56	7.40	60.27
	two_vs_many	0.75	4.25	0.18	8.92	49.36
	low_vs_high	0.80	1.03	0.28	15.41	99.00
	one_vs_many	0.65	5.78	0.35	9.53	37.18
CNN	sampled	0.92	1.51	0.91	1.70	99.00
	seven_vs_many	0.92	1.44	0.73	5.00	99.00
	two_vs_many	0.86	1.74	0.58	10.39	99.00

Thermostability Additional Results

Model	Split	Train rho	Train MSE	Test rho	Test MSE	Avg Epochs Trained
ESM-1b (per AA)	human	0.76	9.54	0.71	11.98	33.9
	human_cell	0.76	13.60	0.68	18.01	38.2
	mixed_split	0.74	25.72	0.67	39.86	35.2
ESM-1b (mean)	human	0.75	9.97	0.70	11.98	36.0
	human_cell	0.74	14.43	0.67	17.94	40.8
	mixed_split	0.72	27.38	0.67	39.87	44.0
ESM-1v (per AA)	human	0.77	8.96	0.69	12.59	32.8
	human_cell	0.78	12.37	0.67	18.16	36.5
	mixed_split	0.76	22.16	0.65	41.12	33.2
ESM-1v (mean)	human	0.75	9.61	0.69	12.59	36.6
	human_cell	0.76	13.44	0.67	18.09	43.0
	mixed_split	0.73	25.55	0.66	40.28	37.2
ESM-untr (per AA)	human	0.45	18.33	0.48	18.85	51.2
	human_cell	0.47	25.01	0.48	25.23	59.4
	mixed_split	0.41	54.94	0.45	61.79	131.5
ESM-untr (mean)	human	0.48	17.64	0.52	17.84	52.2
	human_cell	0.49	24.31	0.52	24.30	60.6
	mixed_split	0.34	62.60	0.37	71.80	137.0
CNN	human	0.68	12.41	0.51	18.02	77.5
	human_cell	0.71	16.73	0.49	25.36	83.4
	mixed_split	0.73	27.89	0.34	84.49	99.0

GB1 Dataset



spearman rho
0
0.2
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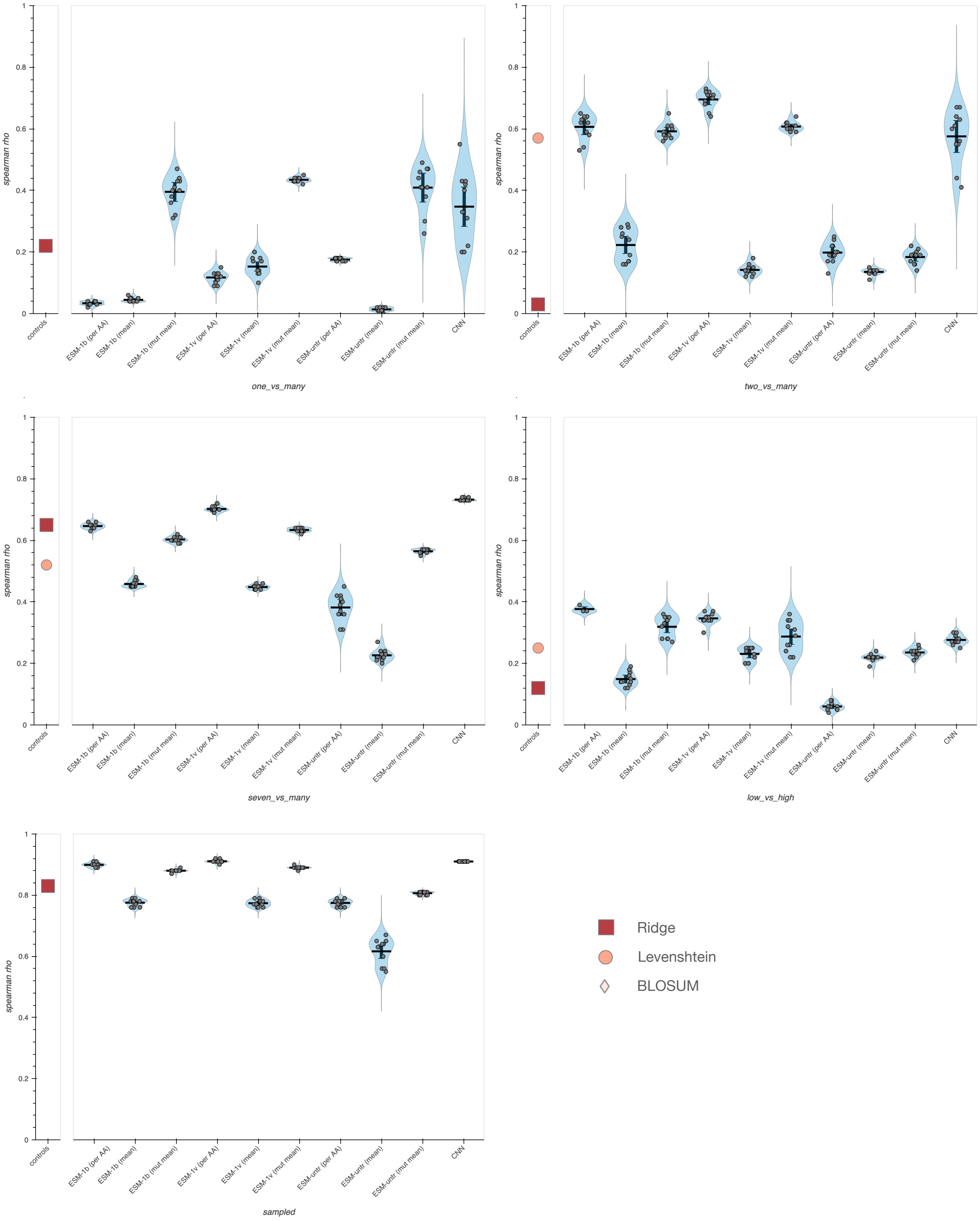
1
controls
ESM-1b (per AA)
ESM-1b (mean)
ESM-1b (mut mean)
ESM-1v (per AA)
ESM-1v (mean)
ESM-1v (mut mean)
ESM-untr (per AA)
ESM-untr (mean)
ESM-untr (mut mean)
CNN
one_vs_rest
two_vs_rest
three_vs_rest
low_vs_high
sampled
Ridge
Levenshtein
BLOSUM

AAV Dataset



spearman rho
1
0.8
0.6
0.4
0.2
0
controls
ESM-1b (per AA)
ESM-1b (mean)
ESM-1b (mut mean)
ESM-1v (per AA)
ESM-1v (mean)
ESM-1v (mut mean)
ESM-untr (per AA)
ESM-untr (mean)
ESM-untr (mut mean)
CNN
one_vs_many
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BLOSUM

Thermostability Dataset