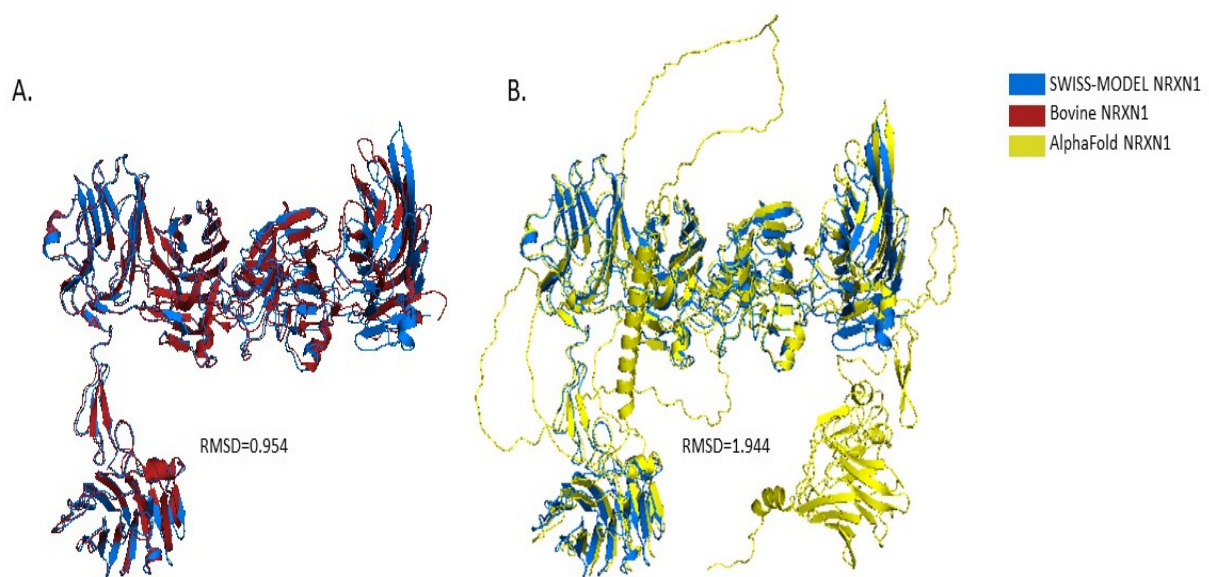
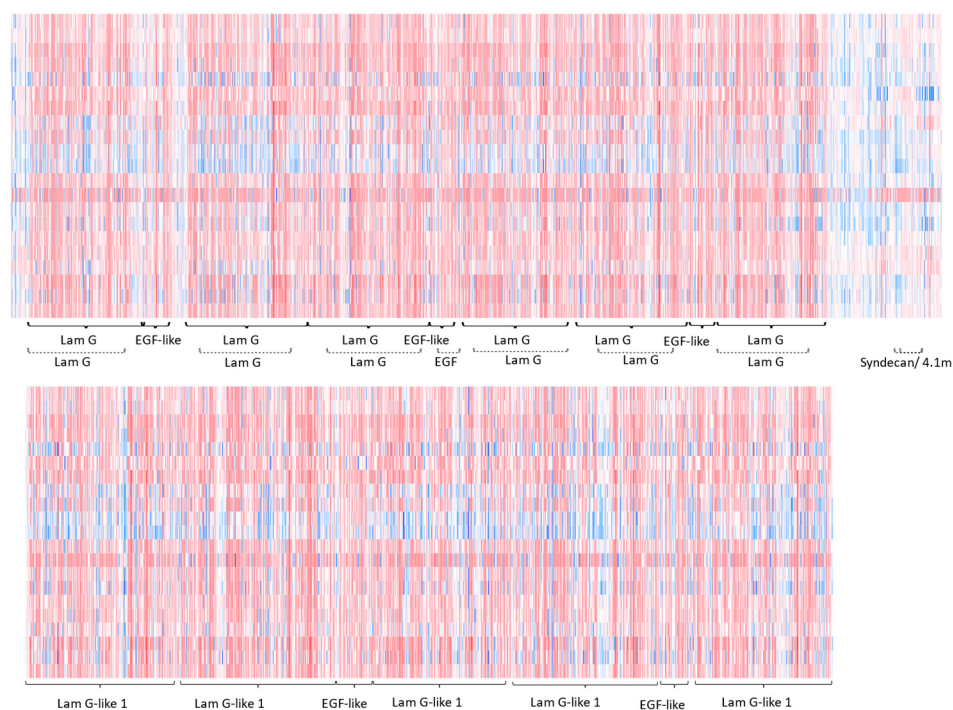


|                            |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|----------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 001 Gallus_gallus          | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 002 Xiphophorus_maculatus  | E | V | F | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 003 Ficedula_albicollis    | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 004 Macropus_eugenii       | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 005 Ochotona_princeps      | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 006 Taeniopygia_guttata    | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 007 Homo_sapiens           | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 008 Anas_platyrhynchos     | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 009 Gasterosteus_aculeatus | E | V | F | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 010 Pelodiscus_sinensis    | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 011 Dipodomys_ordii        | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 012 Gadus_morhua           | E | V | F | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 013 Tursiops_truncatus     | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 014 Lepisosteus_oculatus   | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 015 Astyanax_mexicanus     | E | V | V | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 016 Pteropus_vampyrus      | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 017 Meleagris_gallopavo    | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 018 Oreochromis_niloticus  | E | V | F | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 019 Procavia_capensis      | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 020 Tetraodon_nigroviridis | E | L | F | P | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 021 Choloepus_hoffmanni    | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 022 Mus_musculus           | E | V | I | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |
| 023 Poecilia_formosa       | E | V | F | R | E | S | S | T | T | G | M | V | V | G | I | V | A | A | A | A | L | C | I | L | I | L | L | Y | A | M | Y | K | Y | R | N | R | D | E | G | S | Y | H | V | D | E | S | R | N | Y |

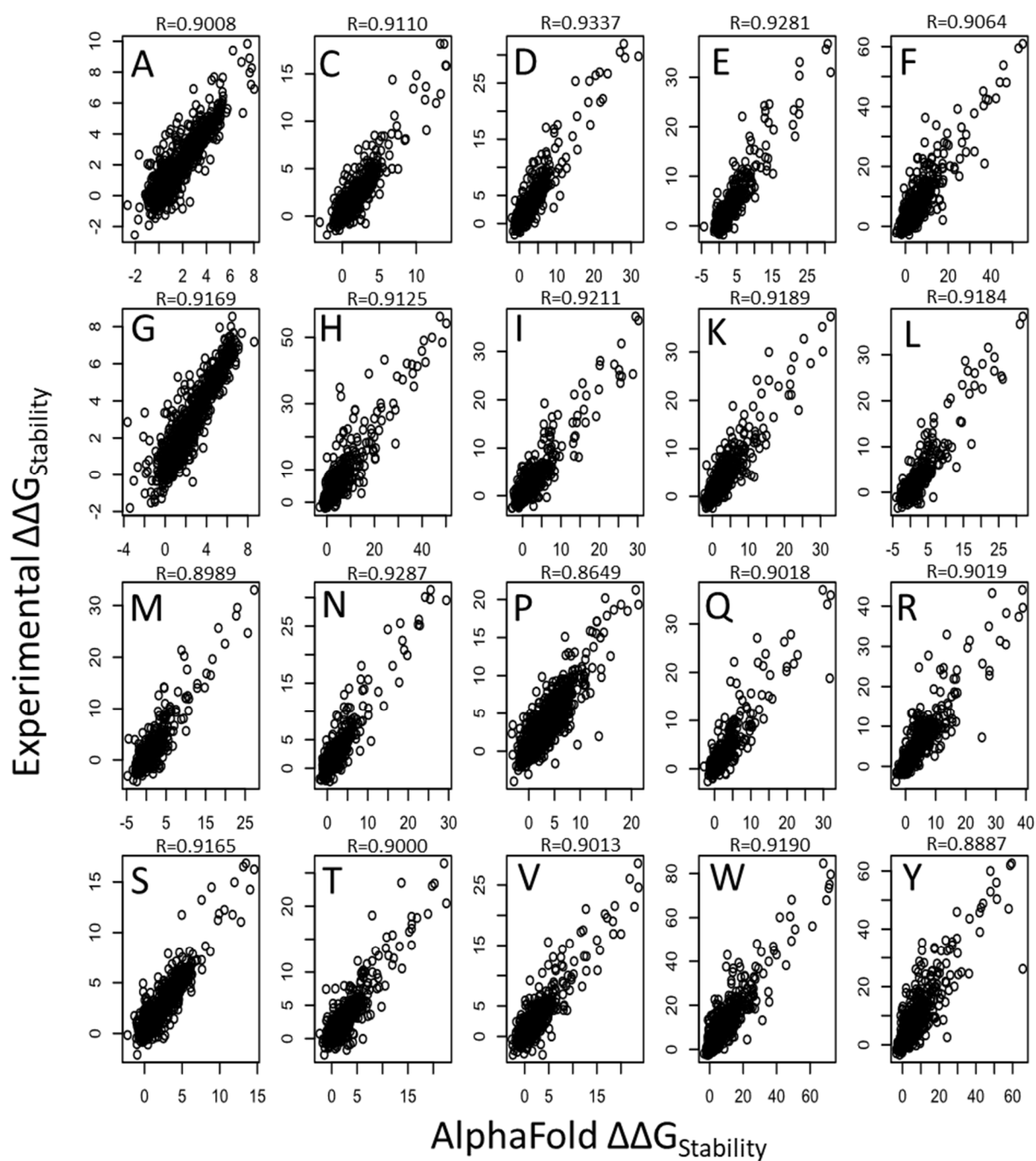
**Supplementary Figure S1.** Multiple Sequence alignment of NRXN1a protein sequences from 23 species. The yellow box highlights the region corresponding to the transmembrane region.



**Supplementary Figure S2.** (A) Structural alignment of SWISS-MODEL structure 3qcw.1.A with the bovine NRXN1 structure (PDB ID: 3poy). (B) Structural alignment of SWISS-MODEL structure 3qcw.1.A with the AlphaFold NRXN1 structure (Uniprot ID: Q9ULB1).



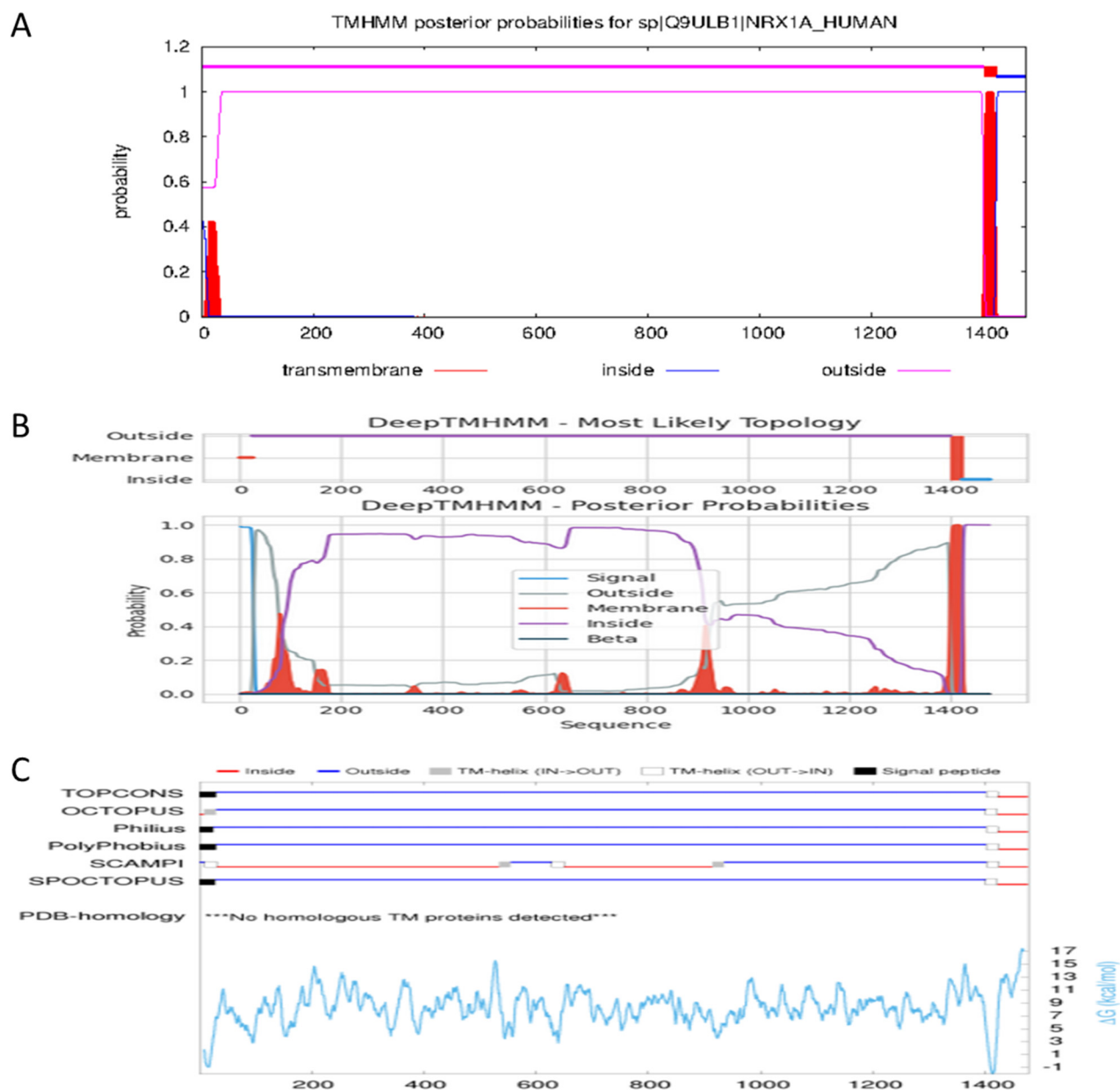
**Supplementary Figure S3.** Heatmap displaying the effects of saturation mutagenesis for the full-length human NRXN1 AlphaFold model (Top) and experimental bovine NRXN1 model (Bottom). The color gradient depicts the change in stability following mutagenesis: red indicates a decrease in protein stability, white represents a neutral change, and blue represents increased stability. The solid brackets show the relative locations of recognized functional domains. The dotted brackets indicate conserved domains as reported from the Conserved Domain Database.



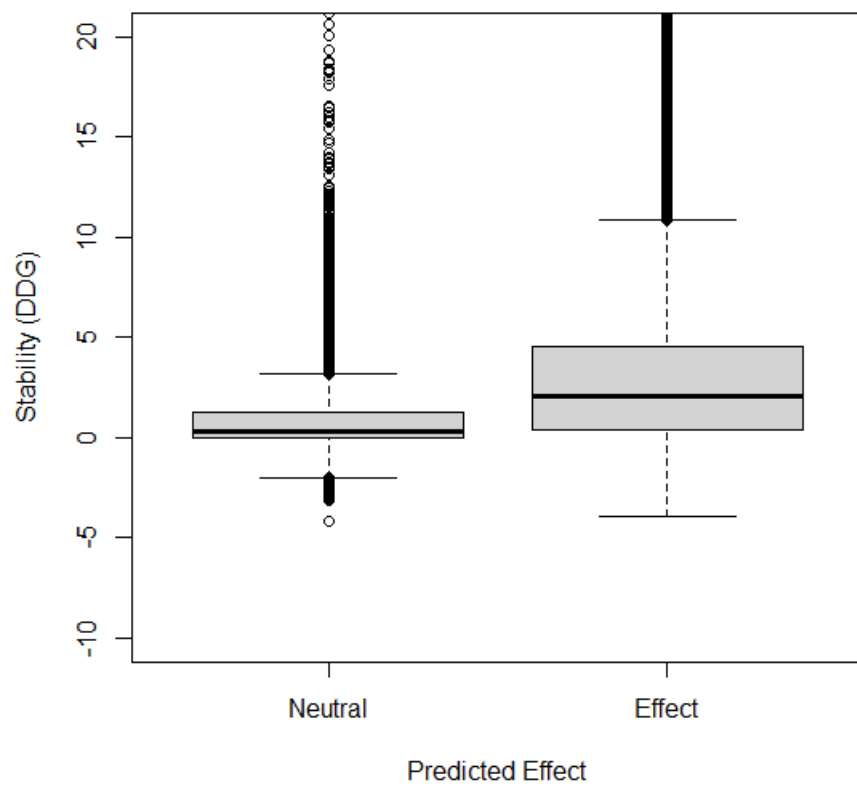
**Supplementary Figure S4.** Scatterplots depicting the corrections of  $\Delta\Delta G$  of substitutions to 20 different residues between the AlphaFold and experimental structures.

|        |        |        |        |        |        |        |        |        |        |        |       |      |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|------|
| 2.903  | 1.953  | 1.342  | 1.798  | 3.251  | 2.1    | 1.751  | 1.023  | 1.507  | 4.065  | 3.027  | 2.919 | A    |
| -0.013 | 1.961  | 0.836  | 5.591  | 2.916  | 1.472  | 1.242  | 0.891  | 1.029  | 0      | 2.766  | 0.023 | C    |
| 4.573  | 2.203  | 0      | 4.077  | 3.198  | 2.506  | 2.673  | 1.951  | 0.981  | 10.925 | -0.012 | 4.793 | D    |
| 3.586  | 1.647  | 0.204  | 6.448  | 3.902  | 2.884  | 2.323  | 1.21   | 0.697  | 9.325  | 2.42   | 4.179 | E    |
| 2.657  | 0.618  | 0.434  | 3.913  | 0.169  | 2.334  | 1.4    | -0.58  | 0.314  | 15.659 | 1.518  | 1.819 | F    |
| 4.619  | 2.55   | 2.43   | 0      | 4.585  | 2.013  | 2.181  | 2.391  | 1.91   | 4.578  | 3.509  | 3.367 | G    |
| 3.747  | 1.323  | 2.231  | 34.835 | 3.223  | 2.714  | 1.93   | 1.335  | 0.114  | 13.957 | 2.91   | 2.784 | H    |
| 4.098  | 0.561  | -0.146 | 6.048  | 4.114  | 3.953  | 1.671  | 2.827  | -0.616 | 6.594  | 2.774  | 5.323 | I    |
| 1.609  | 1.119  | 0.637  | 6.616  | 3.037  | 1.488  | 0.607  | 0.068  | 0.373  | 10.869 | 2.572  | 3.542 | K    |
| 3.003  | 0.746  | 0.379  | 5.451  | 1.257  | 1.395  | 1.229  | 3.726  | -0.212 | 6.526  | 2.15   | 5.767 | L    |
| 2.229  | 0.465  | -0.129 | 4.316  | 0.829  | 1.638  | -0.225 | 0.018  | 0.282  | 6.565  | 2.385  | 2.801 | M    |
| 3.871  | 1.971  | 0.77   | 3.545  | 3.105  | -0.002 | 1.392  | 1.376  | 0.885  | 9.049  | 1.525  | 3.012 | N    |
| 2.156  | 5.428  | -0.956 | 7.132  | 3.204  | 6.338  | 7.619  | -0.5   | 4.226  | 3.688  | 3.483  | 1.961 | P    |
| 3.253  | 1.363  | 0.964  | 5.99   | 3.394  | 1.741  | 0.9    | 0.784  | 0.776  | 8.092  | 2.898  | 4.021 | Q    |
| 2.645  | -0.015 | 1.751  | 9.118  | 3.624  | 1.902  | -0.23  | 0.668  | 0.841  | 9.482  | 2.731  | 3.495 | R    |
| 4.152  | 2.211  | 1.494  | 2.11   | 2.452  | 1.719  | 1.54   | 2.145  | 2.082  | 5.141  | 3.483  | 3.275 | S    |
| 4.313  | 1.863  | 1.255  | 6.476  | 1.4    | 1.871  | 1.386  | 2.542  | 1.326  | 7.923  | 3.39   | 7.503 | T    |
| 4.852  | 1.544  | 0.327  | 10.448 | 3.387  | 4.627  | 1.968  | 1.952  | 0      | 5.339  | 2.745  | 5.896 | V    |
| 3.201  | 1.293  | 0.751  | 5.485  | -0.021 | 2.174  | 1.266  | -0.201 | -0.116 | 26.032 | 2.69   | 1.677 | W    |
| 2.736  | 0.637  | 0.78   | 6.169  | 1.304  | 2.387  | 1.634  | -0.014 | 0.413  | 19.998 | 1.925  | 2.113 | Y    |
| 3.21   | 1.572  | 0.768  | 6.778  | 2.617  | 2.363  | 1.713  | 1.181  | 0.841  | 9.19   | 2.544  | 3.514 | Mean |
| C691   | R692   | D693   | G694   | W695   | N696   | R697   | Y698   | V699   | C700   | D701   | C702  |      |

**Supplementary Figure S5.** Heatmap of residues 691-702, the equivalent location of a consensus sequence for a hydroxylation site in homologous proteins. The color gradient corresponds to the degree of stability change: dark red to light red indicates highly to moderately destabilizing mutations, white indicates neutral, and dark blue to light blue indicates highly to moderately stabilizing mutations. The  $\Delta\Delta G$  values are displayed in each cell.



**Supplementary Figure S6.** Transmembrane prediction from THMM (A), DeepTMHMM (B), and TOPCONS (C). The sequence position is on the x axis. The vertical axes depict either probability or free folding energy



**Supplementary Figure S7.** Boxplot of SNAP2 predictions vs. stability prediction from FoldX. There was a significant difference between stability scores of mutations that are predicted to have an effect by SNAP2 and those predicted to be neutral.

**Supplementary Table S1.** Pairwise Results of Tukey Test comparing the DDG associated with mutations generated in residues in conserved domains and non-conserved regions of the NRXN1 AlphaFold structure.

[illegible]



**Supplementary Table S2.** Disease-causing NRXN1 mutations with DDG, Mean DDG, and SNAP2 scores from HGMD

| Accession | DNA Mutation | Protein Variant                | Phenotype                             | Reference                           | Source  | DDG   | Mean DDG | SNAP2   |
|-----------|--------------|--------------------------------|---------------------------------------|-------------------------------------|---|-------|----------|---------|
| CM081373  | c.53T>A      | p.p.L18Q                       | Autism spectrum disorder              | Kim (2008) Am J Hum Genet 82:199    | PubMed 18179900 (NM_001135659.3)                | 0.24  | 0.45     | neutral |
| CM186462  | c.64G>C      | p.p.G22R                       | Autism spectrum disorder              | Takata (2018) Cell Rep 22:734       | PubMed 29346770 (Transcript: ENST00000404971.5) | 0.03  | 0.78     | effect  |
| CM1313262 | c.455G>A     | p.p.G152D                      | Autism spectrum disorder              | Koshimiz u (2013) PLoS One 8:e74167 | PubMed 24066114 (NM_001135659)                  | 1.53  | 2.23     | neutral |
| CM1617422 | c.587C>A     | p.p.P196H                      | Autism spectrum disorder              | Wang (2016) Nat Commun 7:13316      | PubMed 27824329 (NM_001135659.1)                | 1.96  | 1.74     | neutral |
| CM122179  | c.943T>C     | p.p.Y315H (Y282H) <sup>#</sup> | Autism                                | Liu (2012) J Psychiatr Res 46:630   | PubMed 22405623 (Table 1)                       | 1.62  | 2.16     | neutral |
| CM1617273 | c.1174A>C    | p.p.N392H (N359H) <sup>#</sup> | Autism spectrum disorder              | Wang (2016) Nat Commun 7:13316      | PubMed 27824329 (NM_001135659.1)                | 0.38  | 0.1      | neutral |
| CM081374  | c.2242C>A    | p.p.L748I (L708I) <sup>#</sup> | Autism spectrum disorder              | Kim (2008) Am J Hum Genet 82:199    | PubMed 18179900 (NM_001135659.3)                | 0.73  | 0.82     | neutral |
| CM1819545 | c.2354G>A    | p.p.R785Q (R745Q) <sup>#</sup> | Autism spectrum disorder              | Guo (2018) Mol Autism 9:64          | PubMed 30564305 (NM_001135659)                  | 1.58  | 3.29     | effect  |
| CM118317  | c.2557C>T    | p.p.R853C (R813C) <sup>#</sup> | Intellectual disability, nonsyndromic | Gauthier (2011) Hum Genet 130:563   | PubMed 21424692 (Supplementary Table 1)         | 2.48  | 2.2      | effect  |
| CM137480  | c.2653C>T    | p.p.H885Y (H845Y)              | Autism spectrum disorder              | Jiang (2013) Am J Hum               | PubMed 23849776 (NM_001135659.3)                | -1.01 | 0.62     | neutral |

|               |           |   |  |  |  |       |       |         |
|---------------|-----------|---|--|--|--|-------|-------|---------|
|               |           |   |  | Genet<br>93:249  |  |       |       |         |
| CM142515      | c.2663A>G | p.p.E888<br>G<br>(E848G) <sup>#</sup>   | Schizophreni<br>a  | Fromer<br>(2014)<br>Nature<br>506:179                            | PubMed 24463507<br>(Supplementary<br>Table 1)                | 4.13  | 4.74  | effect  |
| CM148697      | c.2713T>A | p.p.F905I<br>(F865I) <sup>#</sup>       | Autism<br>spectrum<br>disorder &<br>intellectual<br>disability | Yangngam<br>(2014)<br>Genet Test<br>Mol<br>Biomarker<br>s 18:510 | PubMed 24832020<br>(Figure 1)                                | 2.89  | 3.27  | effect  |
| CM122180      | c.2797C>G | p.p.L933<br>V<br>(L893V) <sup>#</sup>   | Autism   | Liu (2012)<br>J Psychiatr<br>Res 46:630                          | PubMed 22405623<br>(Table 1)                                 | 4.14  | 2.01  | neutral |
| CM122181      | c.3523A>G | p.p.I1175<br>V<br>(I1135V) <sup>#</sup> | Autism   | Liu (2012)<br>J Psychiatr<br>Res 46:630                          | PubMed 22405623<br>(Table 1)                                 | 1.25  | 5.59  | neutral |
| CM141413<br>2 | c.3932G>T | p.p.R206<br>L(R1241L<br>) <sup>#</sup>  | Nicotine<br>dependence<br>risk,<br>association<br>with         | Yang<br>(2015) Mol<br>Psychiatry<br>20:1467                      | PubMed 25450229<br>(Table 2.)                                | -0.02 | -0.06 | effect  |
| CM141413<br>1 | c.4135A>C | p.p.T274(<br>T1309P) <sup>#</sup>       | Nicotine<br>dependence<br>risk,<br>association<br>with         | Yang<br>(2015) Mol<br>Psychiatry<br>20:1467                      | PubMed 25450229<br>(Table 3.,<br>rs77665267,<br>NM_138735.5) | -0.15 | -0.09 | effect  |
| CM118318      | c.4511A>G | p.p.H150<br>4R(H143<br>4R) <sup>#</sup> | Schizophreni<br>a  | Gauthier<br>(2011)<br>Hum<br>Genet<br>130:563                    | PubMed 21424692<br>(Figure 1)                                | -0.5  | -0.18 | neutral |

<sup>#</sup>The mutation position in parentheses is aligned based on the canonical sequence of human NRXN1a (UniProt ID: QU9LB1).