Table 2B: sequence and stability variability across nanobodies following stage 1 mutagenesis

unstable

partially stable

stable

|  |  |
| --- | --- |
|   |  IMGT# 1111111111111111 |
|   |  11111111122222223444444444455555566667777777777888888888899999999990000011222222222 |
|   |  12345678912345678901234569012345678901234567890123456789012345678901234567890123489012345678 |
| **MUT#** |  **MAQVQLQESGGGLVQAGGSLRLSCAASMGWFRQAPGKEREFVAATYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCWGQGTQVTVSS** |
| 7 |  **1KXQ** V V ***G*** I  |
| 6 |  **1KXT** V Y C L RAN  |
| 11 |  **1KXV** V S Y G G T V M  |
| 4 |  **2X6M** V R ***G*** R A I  |
| 8 |  **3CFI** P S G W G S T  |
| 9 |  **3G9A** A C L N T R  |
| 4 |  **3K1K** V P R Y W GSS E  |
| 9 |  **3K74** P Y G W M K  |
| 13 |  **3V0A** V P S G W SAW G  |
| 6 |  **3ZKQ** P T G W T T  |
| 4 |  **4GRFf** V P I ***G*** C  |
| 8 |  **4HEP** V P E I ***G*** Y  |
| 9 |  **4I13** I V G N  |
| 2 |  **4KML** P G SSD T T  |
| 7 |  **4KRM** G G I  |
| 3 |  **4LGS** V A S L A  |
| 2 |  **4LHJ** V Y L TSN  |
| 7 |  **4MQS** I ***G*** C I  |
| 4 |  **4OCL** V A T  |
| 5 |  **4QGY** V I ***G*** C P M  |
| 2 |  **4QKX** Y Q L N  |
| 7 |  **4S10** S R  |
| 8 |  **4WEM** E Q YLN G  |
| 5 |  **4WEN** P T Y K H E  |
| 4 |  **4WEU** K Y  |
| 4 |  **4X7C** V P Y Q L S N  |
| 3 |  **1G6V** V ***G*** T G I  |
| 9 |  **1RJC** ***G*** V A M  |
| 5 |  **1ZV5** V I D ***G*** F S  |
| 4 |  **3J6A** ***G*** GA I  |
| 3 |  **4I0C** E A ***G*** P R M  |
| 6 |  **4W6W** ***G*** CVN ***E*** L  |
| 5 |  **1JTP** ***G*** ***L*** I  |
| 3 |  **2P42** V ***G*** L T  |
| 4 |  **3K7U** LF  |
| 9 |  **4C58** G ***G*** CS S ***L*** I  |
| 4 |  **4HEM** V T R  |
| 3 |  **4LAJ** V P I ***G*** C  |
| 10 |  **4LGP** V P I W C V V I  |
| 4 |  **4W6X** T ***G*** CS ***R*** I  |
| 2 |  **4W6Y** A V ***G*** S T  |
| 6 |  **5IVN** V P T I ***G*** C I  |

\*52Gly and 90X are bolded and italicized. The number of mutations made to each nanobody is reported in the left-most column