

## Supplementary Information for

### MRBLE-pep measurements reveal accurate binding affinities for B56, a PP2A regulatory subunit.

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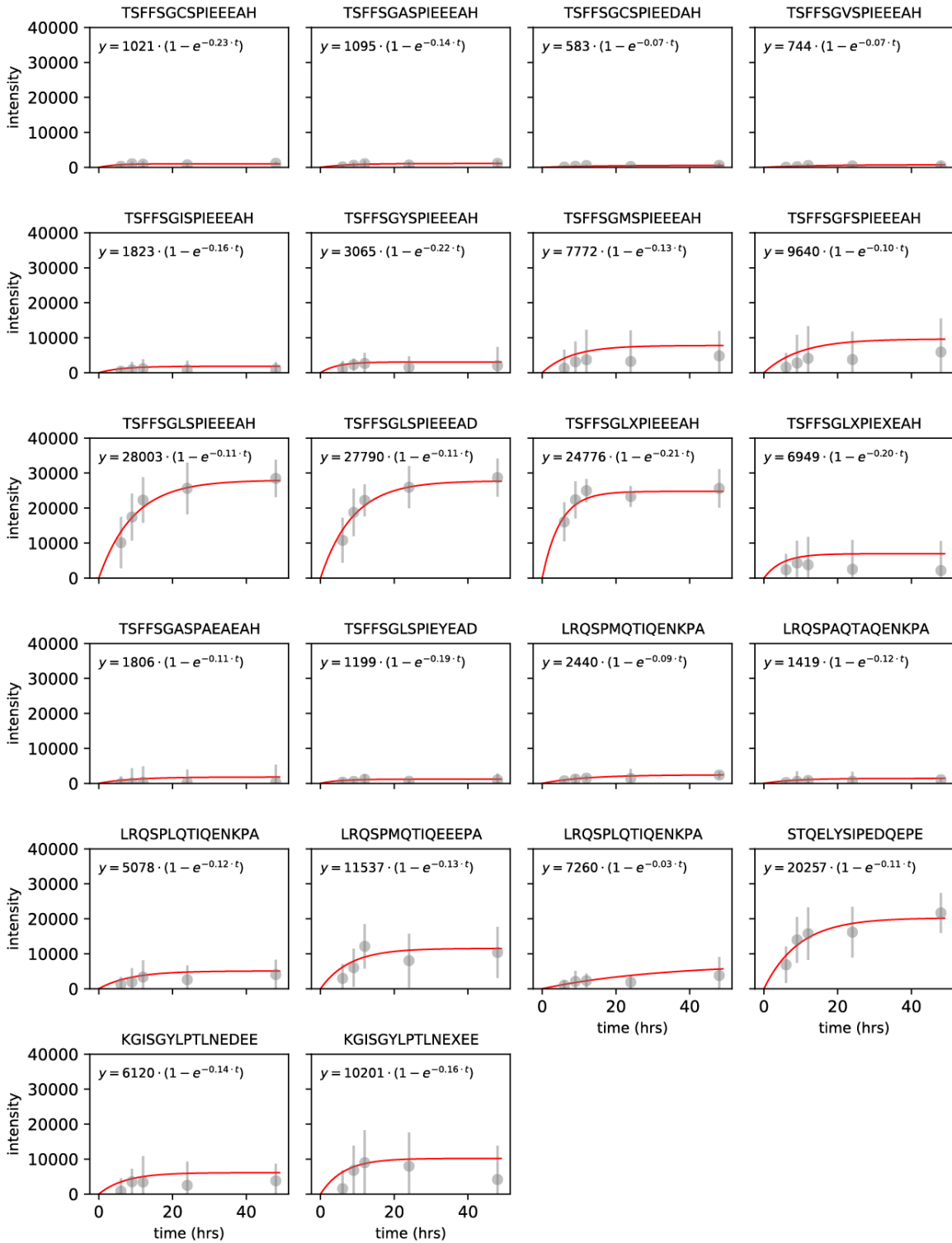
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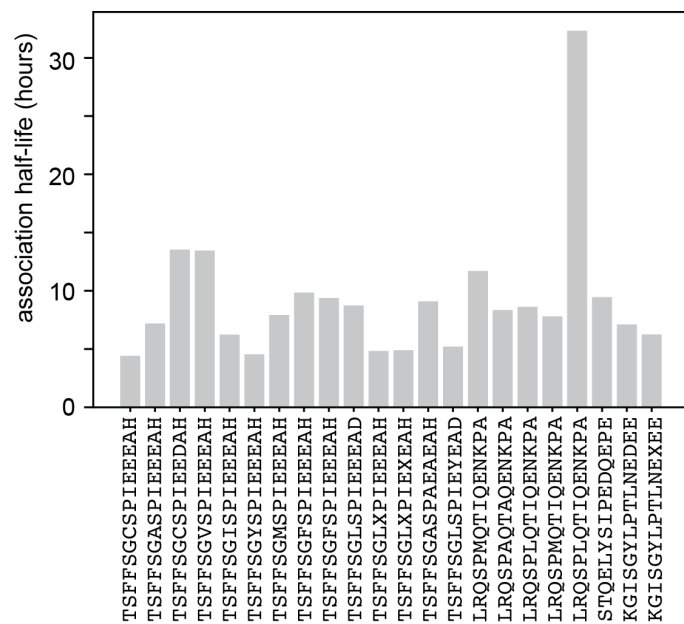
<sup>6</sup> Chan Zuckerberg Biohub, San Francisco, CA 94110

**Table S1:** Mean  $K_d$  (nM) and mean  $\Delta\Delta G$  (kcal/mol) for peptides calculated from triplicate measurements for both libraries.

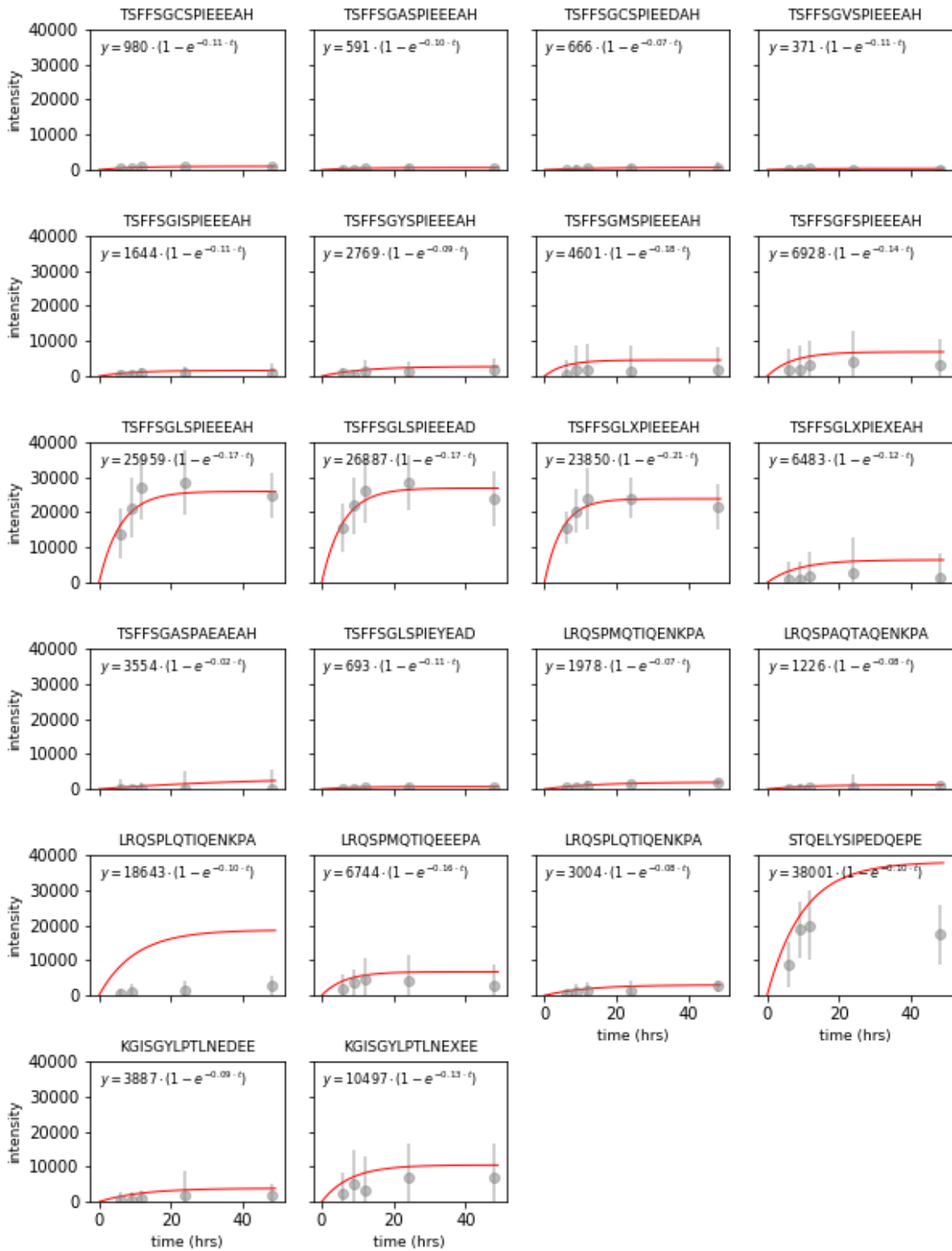
| Code | Sequence        | Mean (Kd) | SE (Kd)  | Mean ( $\Delta\Delta G$ ) | SE ( $\Delta\Delta G$ ) | Library |
|------|-----------------|-----------|----------|---------------------------|-------------------------|---------|
| 1    | TSFFSGCSPIEEEAH | 71073.44  | 10306.54 | 2.82                      | 0.23                    | 1       |
| 2    | TSFFSGASPIEEEAH | 104654.44 | 19522.14 | 3.04                      | 0.19                    | 1       |
| 3    | TSFFSGCSPIEEDA  | 158673.79 | 20642.92 | 3.30                      | 0.28                    | 1       |
| 4    | TSFFSGVSPIEEEAH | 344022.43 | 57937.01 | 3.76                      | 0.28                    | 1       |
| 5    | TSFFSGISPIEEEAH | 77163.64  | 3446.67  | 2.88                      | 0.31                    | 1       |
| 6    | TSFFSGYSPIEEEAH | 40576.78  | 6039.53  | 2.49                      | 0.19                    | 1       |
| 7    | TSFFSGMSPIEEEAH | 39828.93  | 15393.48 | 2.39                      | 0.12                    | 1       |
| 8    | TSFFSGFSPIEEEAH | 21125.87  | 3881.26  | 2.10                      | 0.19                    | 1       |
| 9    | TSFFSGLSPIEEEAH | 824.49    | 299.18   | 0.07                      | 0.02                    | 1       |
| 10   | TSFFSGLSPIEEAD  | 719.33    | 248.05   | 0.00                      | 0.00                    | 1       |
| 11   | TSFFSGLXPIEEEAH | 430.93    | 157.18   | -0.32                     | 0.03                    | 1       |
| 12   | TSFFSGLXPIEXEAH | 25598.31  | 4459.53  | 2.21                      | 0.17                    | 1       |
| 13   | TSFFSGASPAEAEAH | 283395.36 | 38820.16 | 3.64                      | 0.35                    | 1       |
| 14   | TSFFSGLSPIEYEAD | 92865.40  | 8706.23  | 2.99                      | 0.25                    | 1       |
| 15   | LRQSPMQTIQENKPA | 44031.44  | 11871.24 | 2.51                      | 0.29                    | 1       |
| 16   | LRQSPAQTAQENKPA | 77891.82  | 18278.86 | 2.86                      | 0.28                    | 1       |
| 17   | LRQSPLQTIQENKPA | 27692.73  | 3245.71  | 2.27                      | 0.29                    | 1       |
| 18   | LRQSPMQTIEEEPA  | 12766.19  | 4588.22  | 1.73                      | 0.12                    | 1       |
| 19   | LRQSPLQTIQENKPA | 27809.86  | 4479.58  | 2.27                      | 0.29                    | 1       |
| 20   | STQELYSIPEDQEPE | 1893.03   | 632.36   | 0.59                      | 0.06                    | 1       |
| 21   | KGISGYLPTLNDEE  | 43087.35  | 10136.94 | 2.50                      | 0.16                    | 1       |
| 22   | KGISGYLPTLNEXEE | 13148.79  | 4776.67  | 1.71                      | 0.05                    | 1       |
| 1    | DFTRLQDIPEETESR | 755.78    | 313.65   | 0.43                      | 0.04                    | 2       |
| 2    | DFTRMQDIPEETEXR | 11812.68  | 2771.72  | 2.14                      | 0.09                    | 2       |
| 3    | DFTRAQDAPAETESR | 39734.00  | 12185.37 | 2.83                      | 0.21                    | 2       |
| 4    | DFTRMQDIPEETESR | 10706.87  | 2184.70  | 2.09                      | 0.12                    | 2       |
| 5    | NKRLSTIDEXGSILS | 311.00    | 76.28    | 0.18                      | 0.06                    | 2       |
| 6    | NKRLSTIDEEGSILS | 161.85    | 47.09    | -0.43                     | 0.04                    | 2       |
| 7    | NKRASTADASGSILS | 25288.43  | 6720.82  | 2.58                      | 0.12                    | 2       |
| 8    | NKRLSTIDESGSILS | 3947.35   | 139.40   | 1.52                      | 0.20                    | 2       |
| 9    | LRQSPLQTIQEEPA  | 1216.59   | 635.59   | 0.67                      | 0.12                    | 2       |
| 10   | LRQXPLQTIQEEPA  | 346.25    | 119.03   | 0.00                      | 0.00                    | 2       |
| 11   | LRQSPLKTIKEEPA  | 54.63     | 18.22    | -1.08                     | 0.08                    | 2       |
| 12   | LRQSPLQTIQEEPA  | 124.51    | 35.18    | -0.58                     | 0.06                    | 2       |
| 13   | LRQSPMQTIEEEPA  | 6576.38   | 773.96   | 1.82                      | 0.15                    | 2       |
| 14   | LRQSPLQTIQENKPA | 2501.93   | 753.05   | 1.20                      | 0.08                    | 2       |
| 15   | LRQSPAQTAQENKPA | 32042.00  | 9690.55  | 2.71                      | 0.16                    | 2       |
| 16   | LRQSPMQTIQENKPA | 9325.89   | 886.41   | 2.03                      | 0.18                    | 2       |
| 17   | TSFFSGASPAEAEAH | 31616.29  | 8755.70  | 2.71                      | 0.18                    | 2       |
| 18   | TSFFSGLXPIEEEAH | 119.18    | 30.79    | -0.60                     | 0.07                    | 2       |
| 19   | TSFFSGLSPIEEEAH | 128.32    | 41.73    | -0.58                     | 0.02                    | 2       |
| 20   | TSFFSGFSPIEEEAH | 581.48    | 224.80   | 0.30                      | 0.06                    | 2       |
| 21   | TSFFSGISPIEEEAH | 12282.41  | 2181.89  | 2.18                      | 0.15                    | 2       |
| 22   | TSFFSGVSPIEEEAH | 21912.73  | 5620.81  | 2.50                      | 0.11                    | 2       |
| 23   | TSFFSGCSPIEEDA  | 21088.21  | 5071.72  | 2.48                      | 0.12                    | 2       |
| 24   | TSFFSGCSPIEEEAH | 9551.44   | 1878.54  | 2.02                      | 0.12                    | 2       |



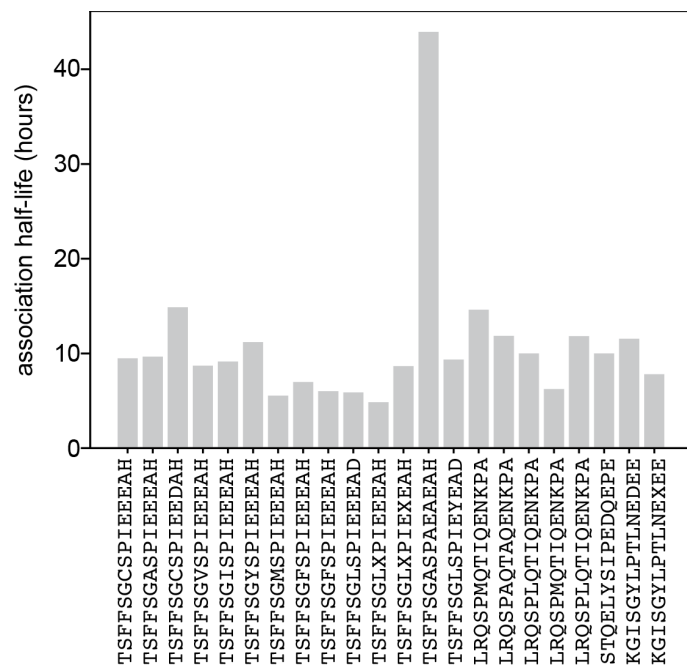
**Fig. S1.** Measurement of time required to reach binding equilibrium for all peptides in MRBLE-pep library 1. Grey markers indicate median bead intensity at a given time point, error bars indicate the standard deviation, and the red line indicates a fit to a kinetic binding curve (equation and fit parameters given at top).



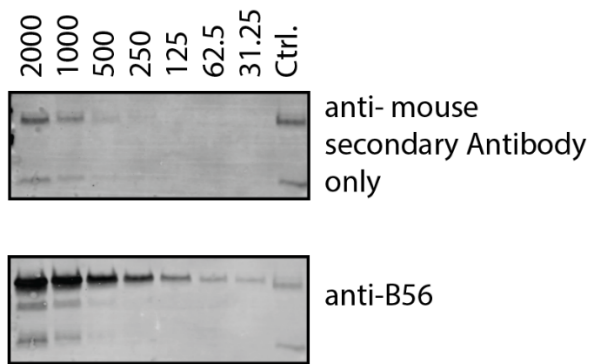
**Fig. S2.** Association half-life for all peptides from MRBLE-pep library 1 (determined from kinetic binding fit parameters).



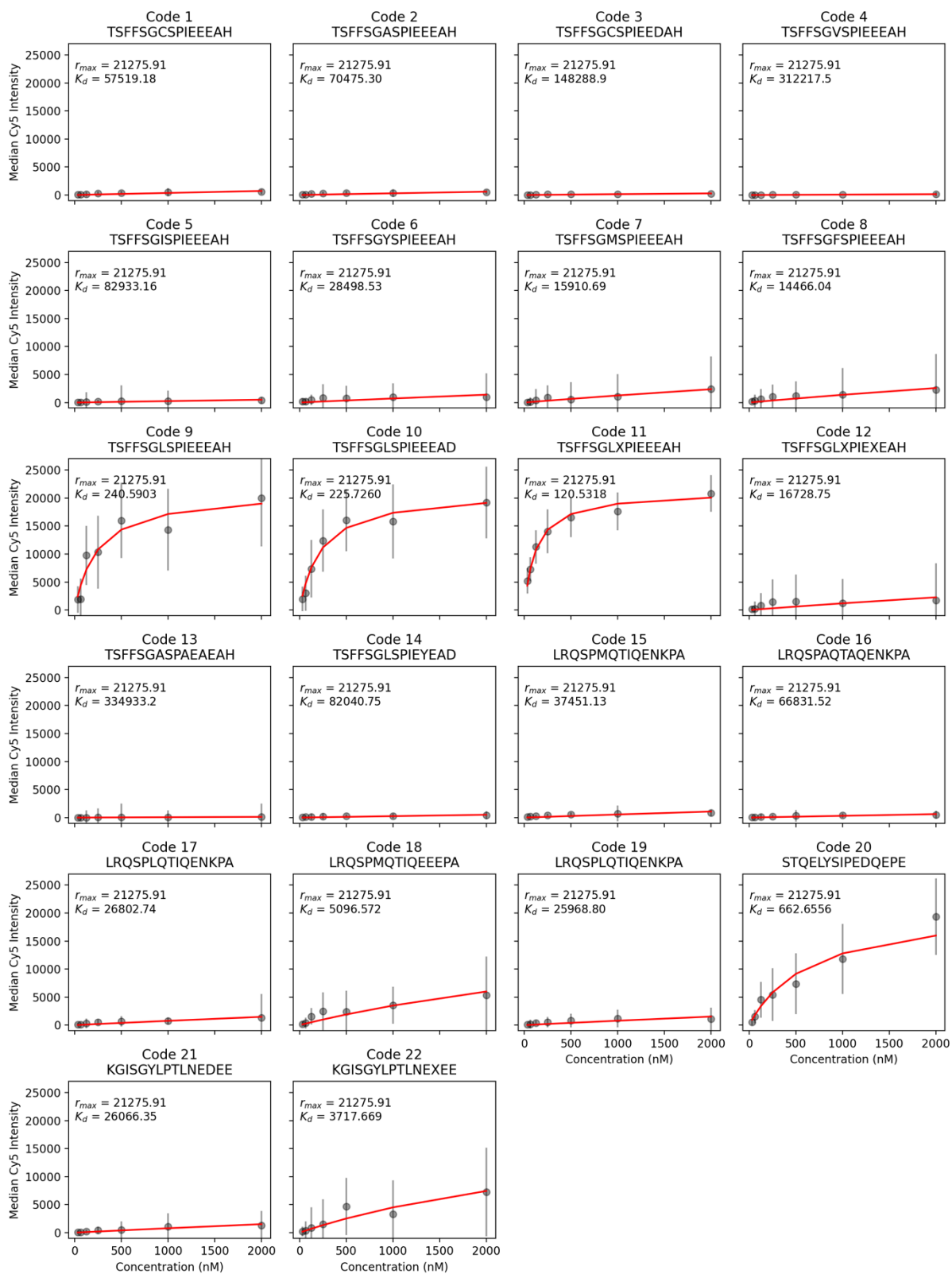
**Fig. S3.** Measurement of time required to reach binding equilibrium for all peptides in MRBLE-pep library 1. Grey markers indicate median bead intensity at a given time point, error bars indicate the standard deviation, and the red line indicates a fit to a kinetic binding curve (equation and fit parameters given at top).



**Fig. S4.** Association half-life for all peptides from MRBLE-pep library 1 (determined from kinetic binding fit parameters).

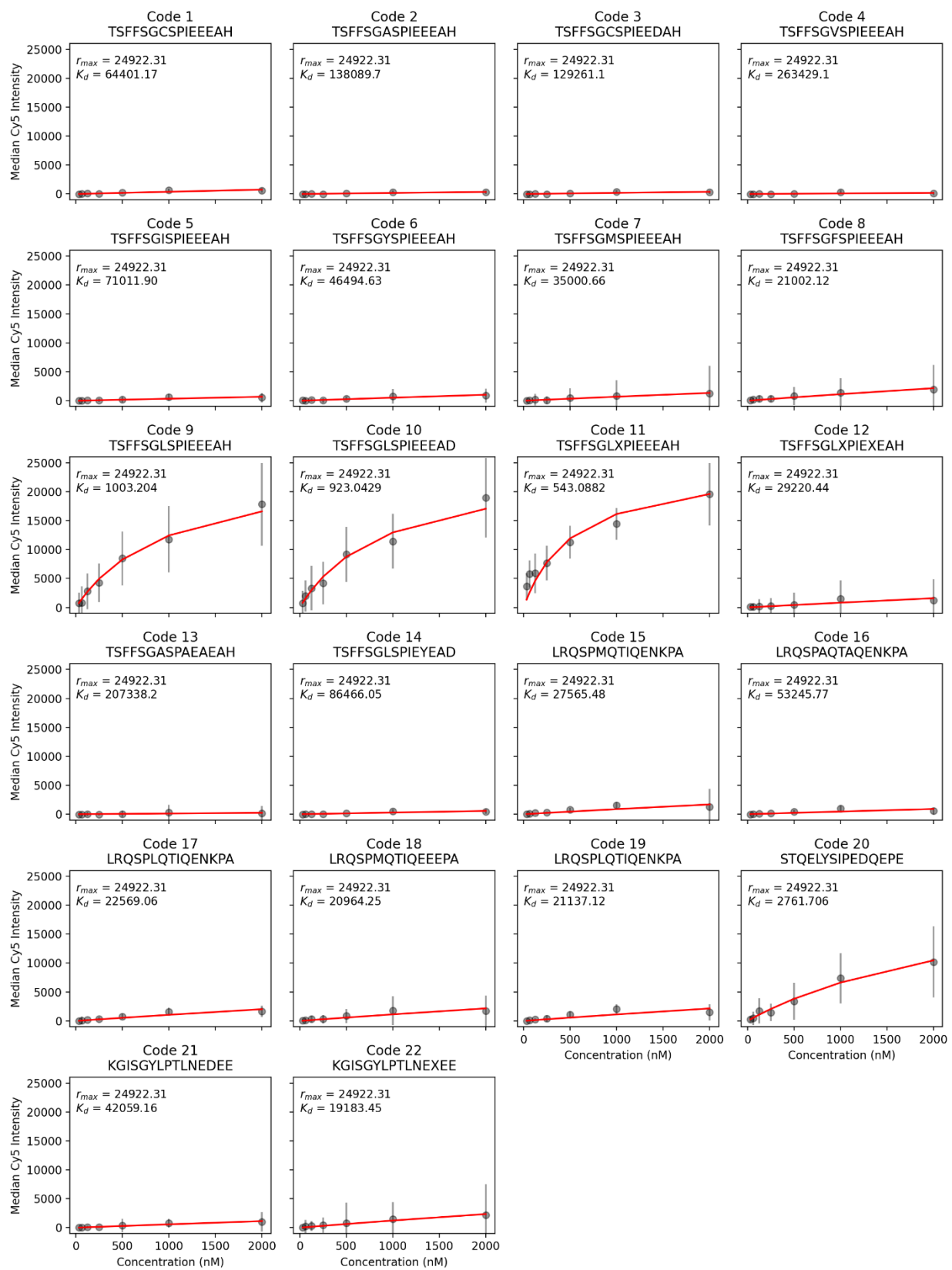


**Fig. S5.** Western Blot with the unbound fractions of a MRBLE-pep concentration experiment. The unbound fraction from each concentration of a MRBLE-pep concentration experiment was probed for unbound B56 protein.

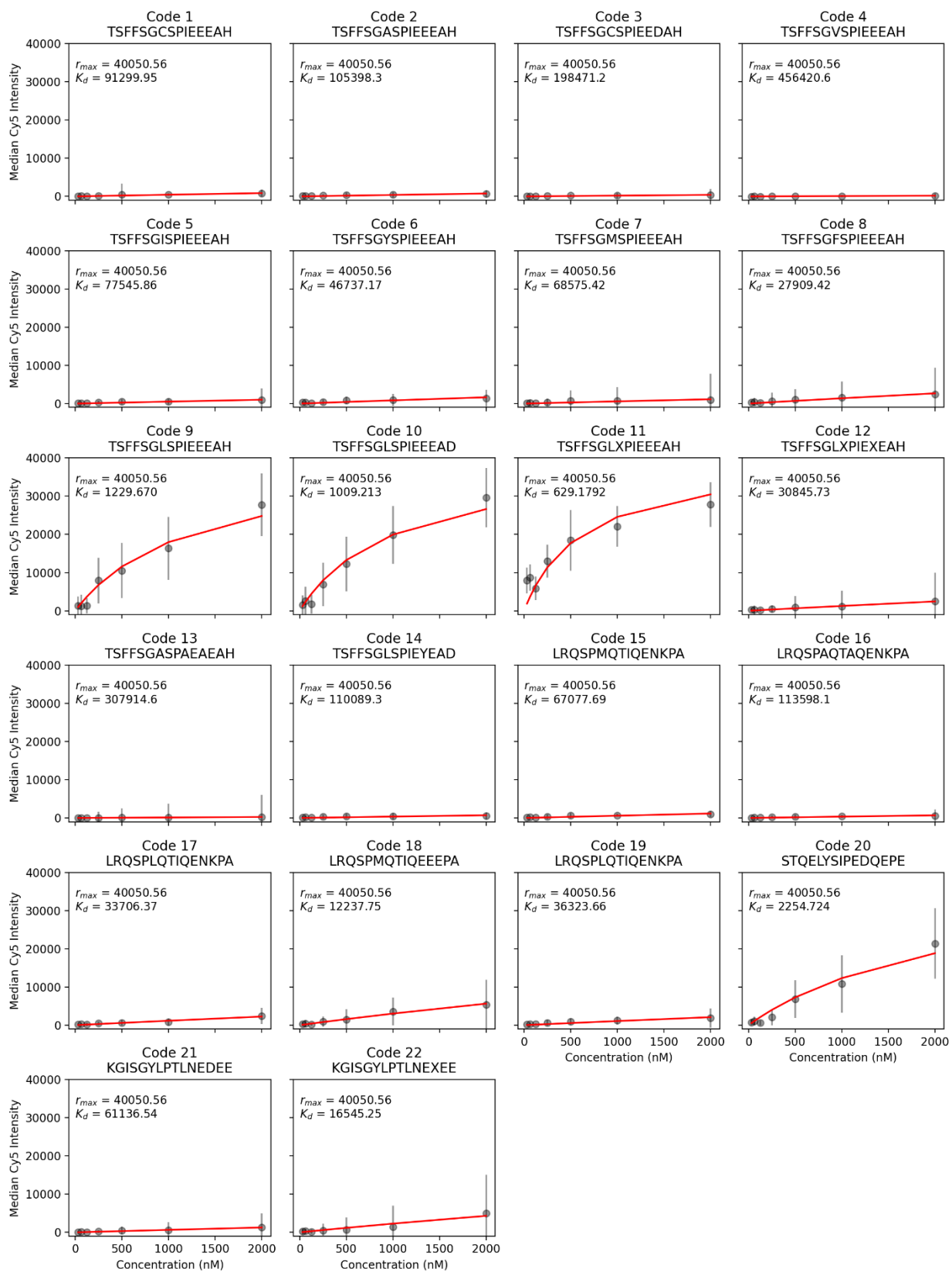


**Fig. S6.** Concentration-dependent binding data (grey markers, median intensity and standard deviation over all beads) and associated Langmuir isotherm fits (red lines) for MRBLE-pep library 1, replicate #1.

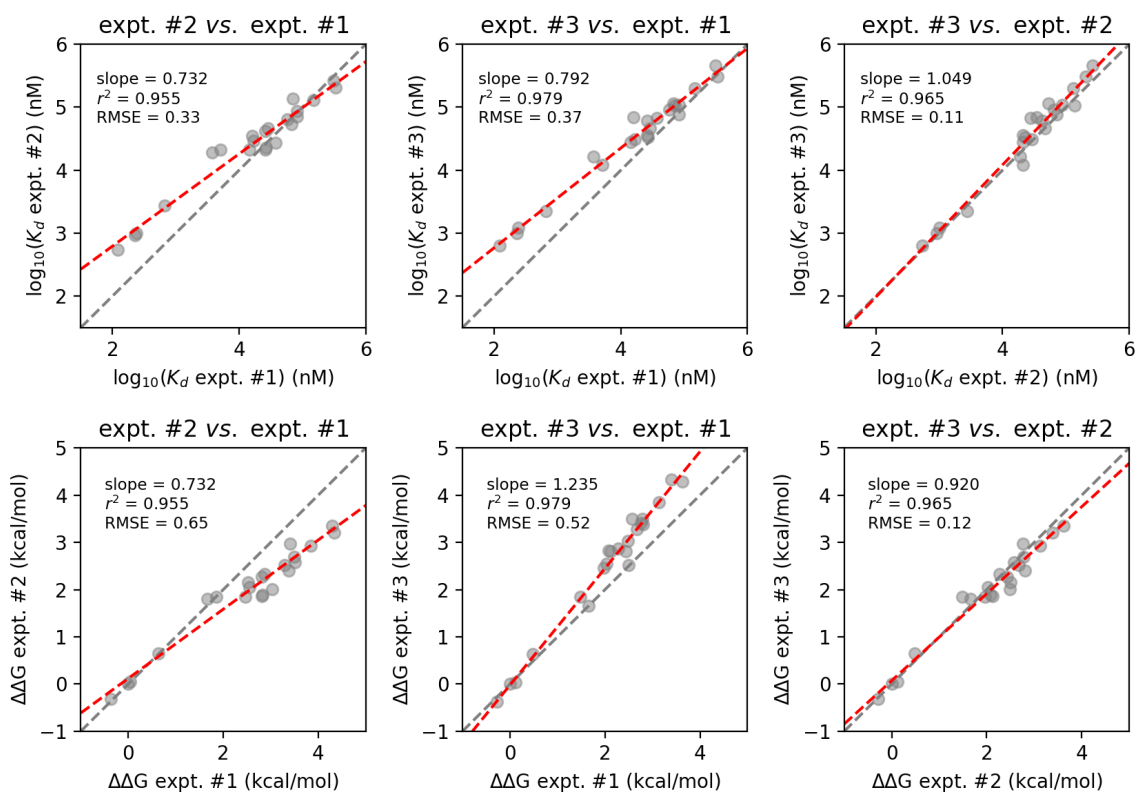




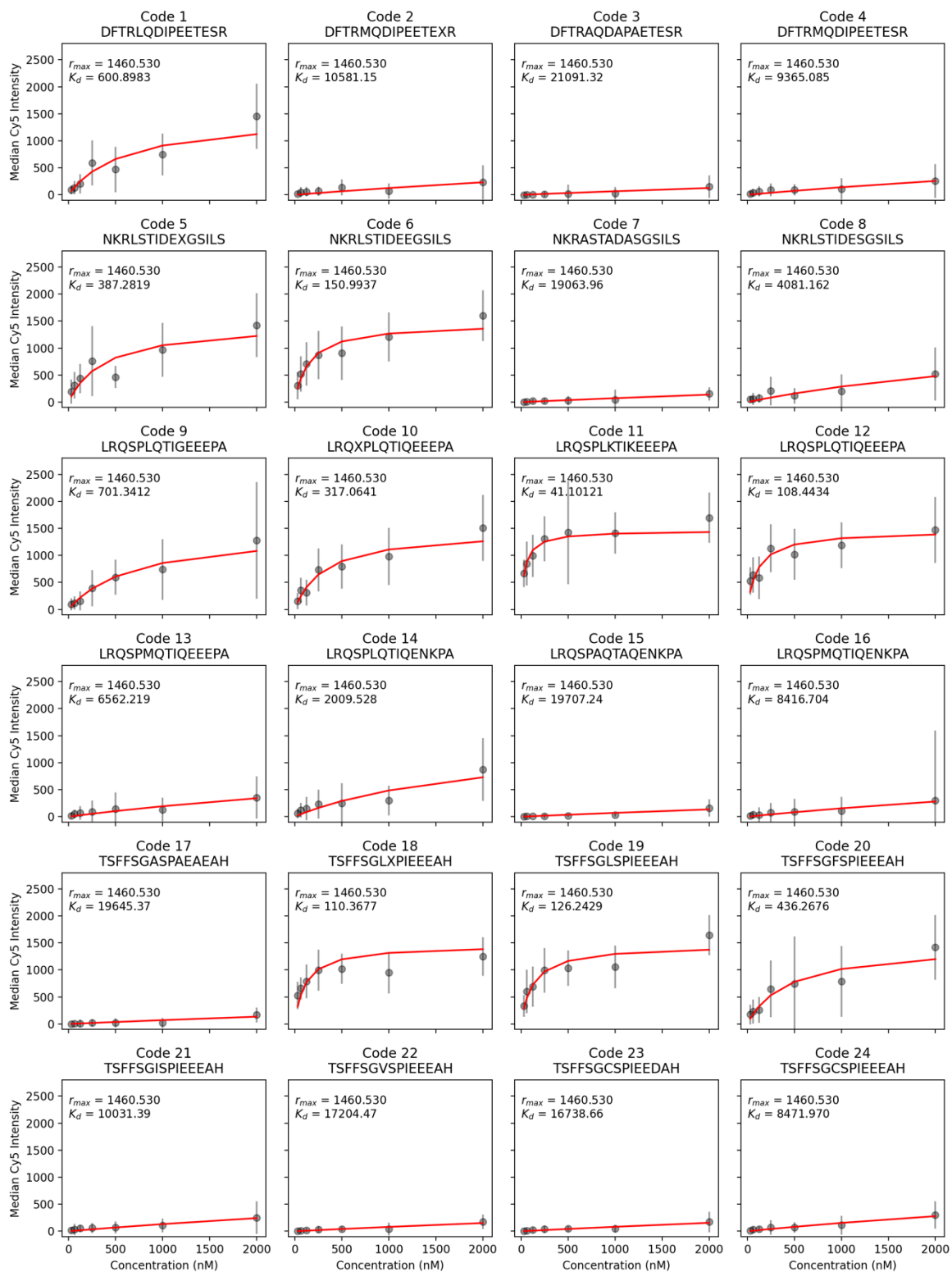
**Fig. S7.** Concentration-dependent binding data (grey markers, median intensity and standard deviation over all beads) and associated Langmuir isotherm fits (red lines) for MRBLE-pep library 1, replicate #2.



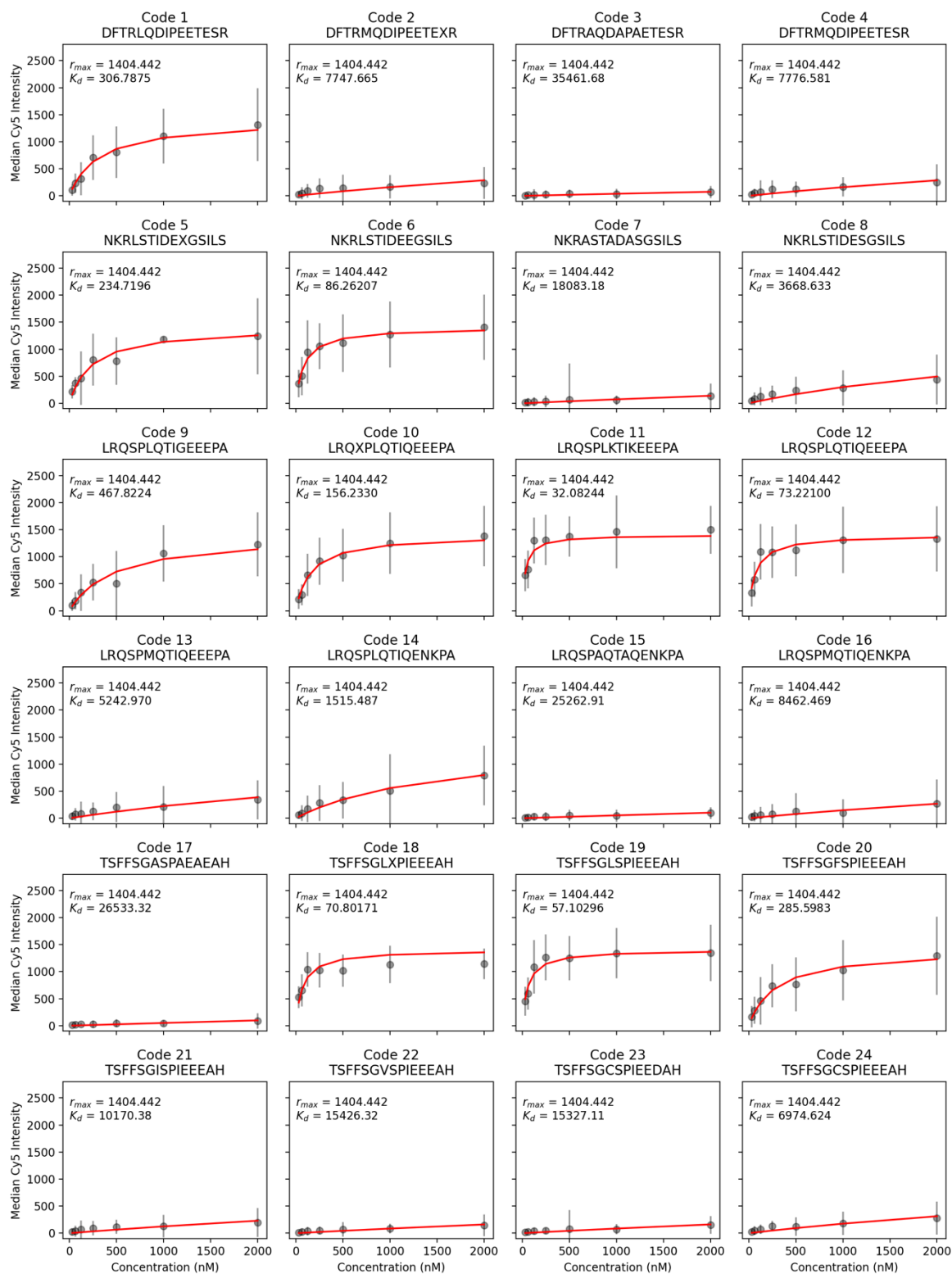
**Fig. S8.** Concentration-dependent binding data (grey markers, median intensity and standard deviation over all beads) and associated Langmuir isotherm fits (red lines) for MRBLE-pep library 1, replicate #3.



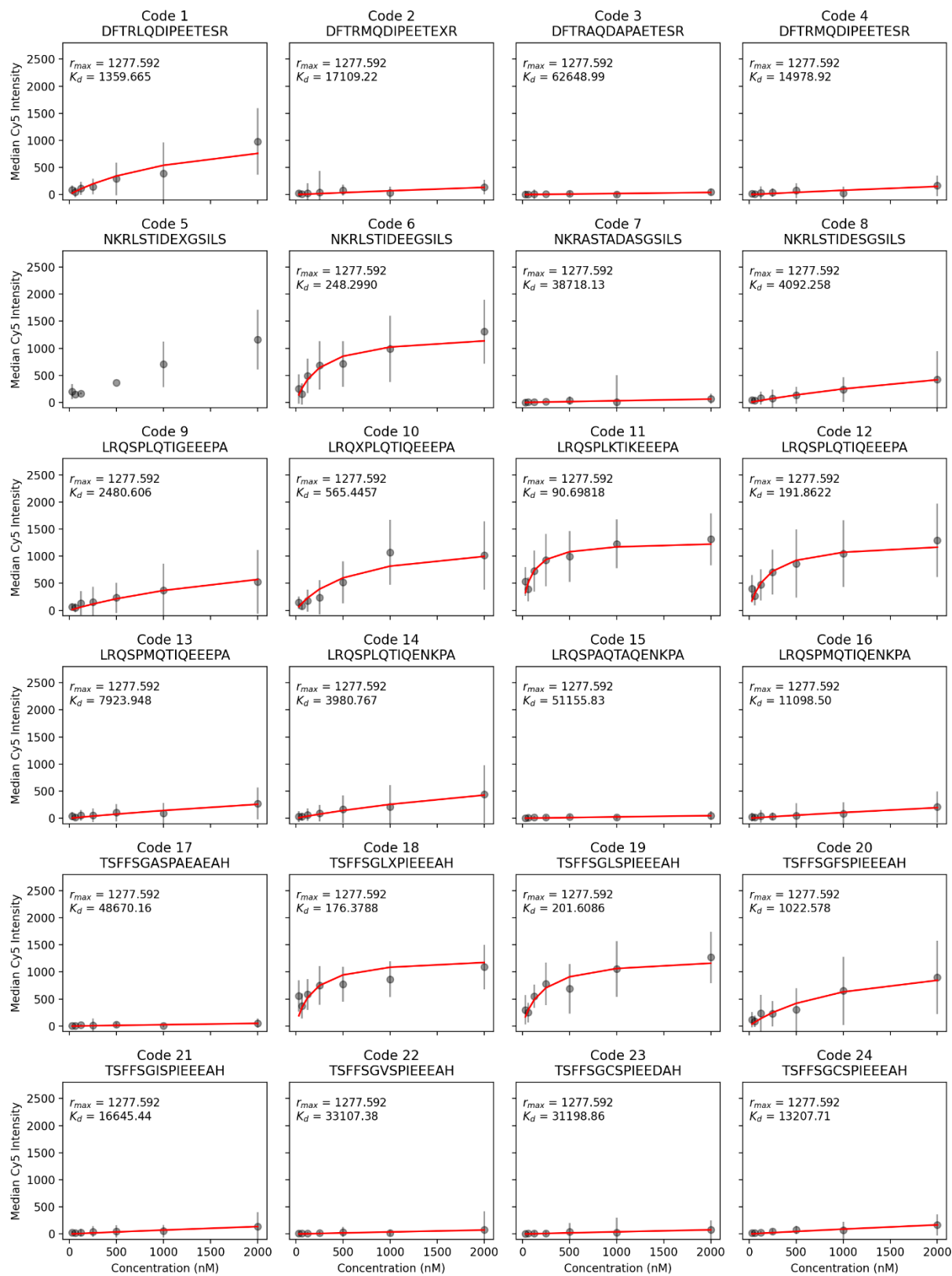
**Fig. S9.** Comparison of measured affinities between technical replicates for MRBLE-pep library 1. **(A)** Comparison of  $K_d$ s for each B56-peptide interaction (returned values from global Langmuir isotherm fits). Black dashed line indicates the 1:1 identity line; red dashed line indicates a linear regression to  $\log_{10}$ -transformed  $K_d$  values. **(B)** Comparison of  $\Delta\Delta G$ s for each B56-peptide interactions (calculated relative to a Kif4A ‘reference’ peptide sequence of TSFFSGLSPIIEEAD). Black dashed line indicates the 1:1 identity line; red dashed line indicates a linear regression.



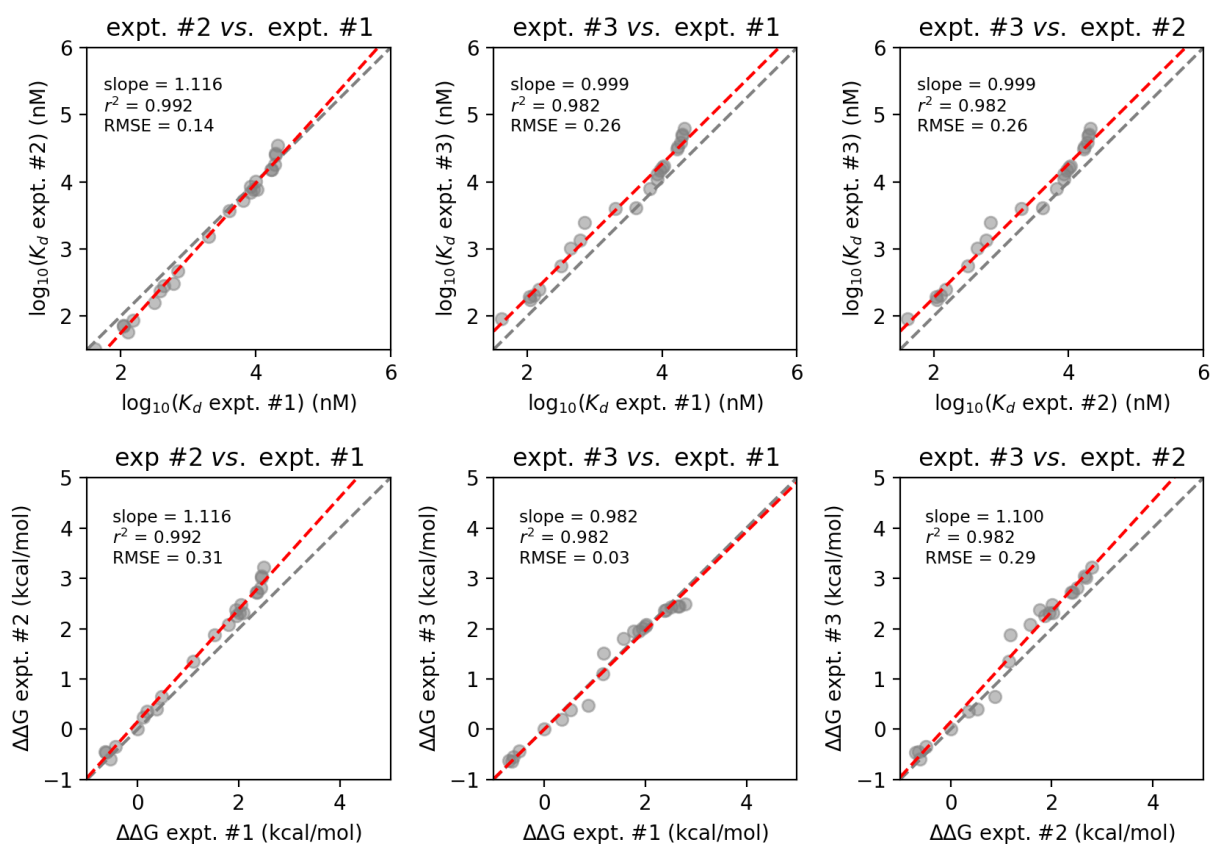
**Fig. S10.** Concentration-dependent binding data (grey markers, median intensity and standard deviation over all beads) and associated Langmuir isotherm fits (red lines) for MRBLE-pep library 2, replicate #1.



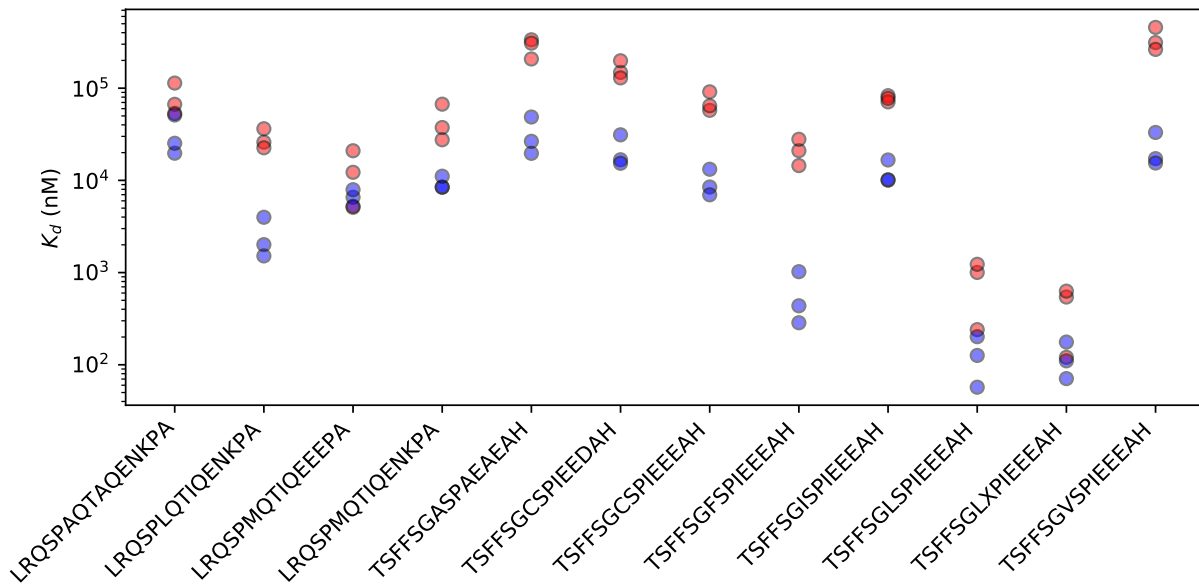
**Fig. S11.** Concentration-dependent binding data (grey markers, median intensity and standard deviation over all beads) and associated Langmuir isotherm fits (red lines) for MRBLE-pep library 2, replicate #2.



**Fig. S12.** Concentration-dependent binding data (grey markers, median intensity and standard deviation over all beads) and associated Langmuir isotherm fits (red lines) for MRBLE-pep library 1, replicate #3.



**Fig. S13.** Comparison of measured affinities between technical replicates for MRBLE-pep library 2. **(A)** Comparison of  $K_d$ s for each B56-peptide interaction (returned values from global Langmuir isotherm fits). Black dashed line indicates the 1:1 identity line; red dashed line indicates a linear regression to  $\log_{10}$ -transformed  $K_d$  values. **(B)** Comparison of  $\Delta\Delta G$ s for each B56-peptide interactions (calculated relative to a Kif4A 'reference' peptide sequence of TSFFSGLSPIIEEAD). Black dashed line indicates the 1:1 identity line; red dashed line indicates a linear regression.



**Fig. S14.** Measured  $K_d$ s for 12 peptides containing Kif4A-like or FoxO3-like motifs across 3 technical replicates each of MRBLE-pep library 1 (red markers) and library 2 (blue markers).