

1/4

FIG. 1

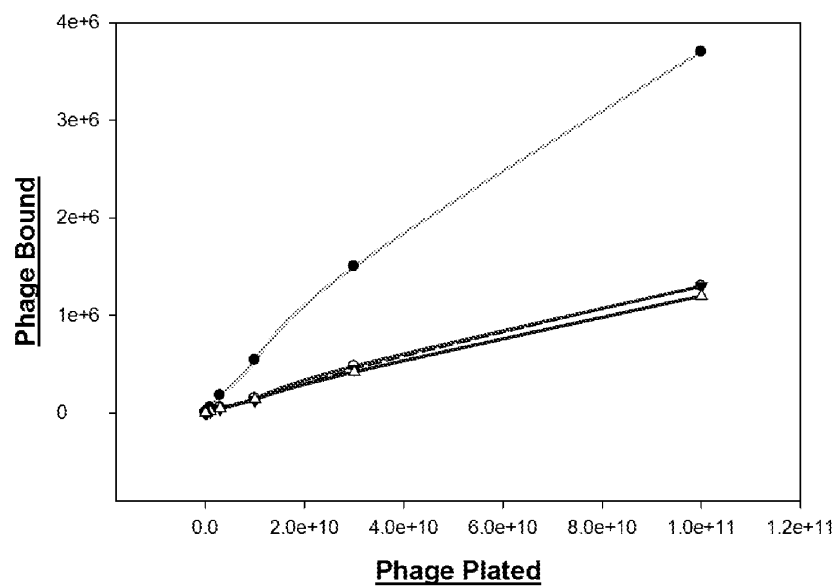


FIG. 2

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNW  
VRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTIS  
RDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYIS  
YWAYWGQGTLLTVSSGGGGSGGGGGSGGGGGSQTVVTQ  
EPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWWQQKPG  
QAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQ  
PEDEAEYYCVLWYSNRWVFGGGTKLTVL

3/4

FIG. 3

| anti-CD3e scFv | KD (nM)<br>hum CD3e | kon(1/Ms) | kdis(1/s) | KD (nM)<br>cyno CD3e | kon(1/Ms) | kdis(1/s) | cyno/hum<br>ratio |
|----------------|---------------------|-----------|-----------|----------------------|-----------|-----------|-------------------|
| wt             | 4.4                 | 4.71E+05  | 2.07E-03  | 3.9                  | 4.63E+05  | 1.83E-03  | 0.9               |
| 2B2            | 3.8                 | 6.08E+05  | 2.32E-03  | 3.5                  | 5.57E+05  | 1.93E-03  | 0.9               |
| 9F2            | 4.1                 | 3.61E+05  | 1.33E-03  | 3.4                  | 3.38E+05  | 1.05E-03  | 0.8               |
| 5A2            | 4.3                 | 5.66E+05  | 2.36E-03  | 4.2                  | 4.75E+05  | 1.93E-03  | 1.0               |
| 6A2            | 4.7                 | 5.22E+05  | 2.48E-03  | 4.9                  | 4.56E+05  | 2.22E-03  | 1.0               |
| 2D2            | 6.4                 | 5.27E+05  | 3.38E-03  | 6.6                  | 4.71E+05  | 3.09E-03  | 1.0               |
| 3F2            | 8.0                 | 7.04E+05  | 5.02E-03  | 6.6                  | 7.12E+05  | 4.38E-03  | 0.8               |
| 2E4            | 14.4                | 4.16E+05  | 5.99E-03  | 13.2                 | 4.04E+05  | 5.32E-03  | 0.9               |
| 2H2            | 16.0                | 5.87E+05  | 9.06E-03  | 16.0                 | 5.25E+05  | 8.37E-03  | 1.0               |
| 10B2           | 17.9                | 4.90E+05  | 8.74E-03  | 16.6                 | 4.93E+05  | 8.15E-03  | 0.9               |
| 1A2            | 19.9                | 5.99E+05  | 1.19E-02  | 17                   | 5.31E+05  | 9.03E-03  | 0.9               |
| 1C2            | 36.8                | 6.63E+05  | 2.44E-02  | 30                   | 6.69E+05  | 1.97E-02  | 0.8               |
| 2A4            | 46.3                | 3.64E+05  | 1.66E-02  | 43.4                 | 3.53E+05  | 1.53E-02  | 0.9               |
| 10E4           | 49.8                | 5.22E+05  | 2.60E-02  | 46.8                 | 5.08E+05  | 2.38E-02  | 0.9               |
| 8A5            | 109                 | 7.46E+05  | 8.10E-02  | 103                  | 7.23E+05  | 7.44E-02  | 0.9               |
| 2G5            | 117                 | 9.94E+05  | 1.15E-01  | 115                  | 9.64E+05  | 1.11E-01  | 1.0               |
| 1G4            | 132.9               | 1.67E+05  | 2.20E-02  | 133.7                | 1.64E+05  | 2.19E-02  | 1.0               |

FIG. 4

| Anti-huCD3ε<br>variants | T <sub>h</sub> (°C) |
|-------------------------|---------------------|
| WT                      | 59.2                |
| 2B2                     | 57.4                |
| 3F2                     | 52.2                |
| 2E4                     | 55                  |
| 2H2                     | 53                  |
| 10B2                    | 51.5                |
| 2A4                     | 56.2                |
| 2G5                     | 58                  |
| 1G4                     | 60.3                |