

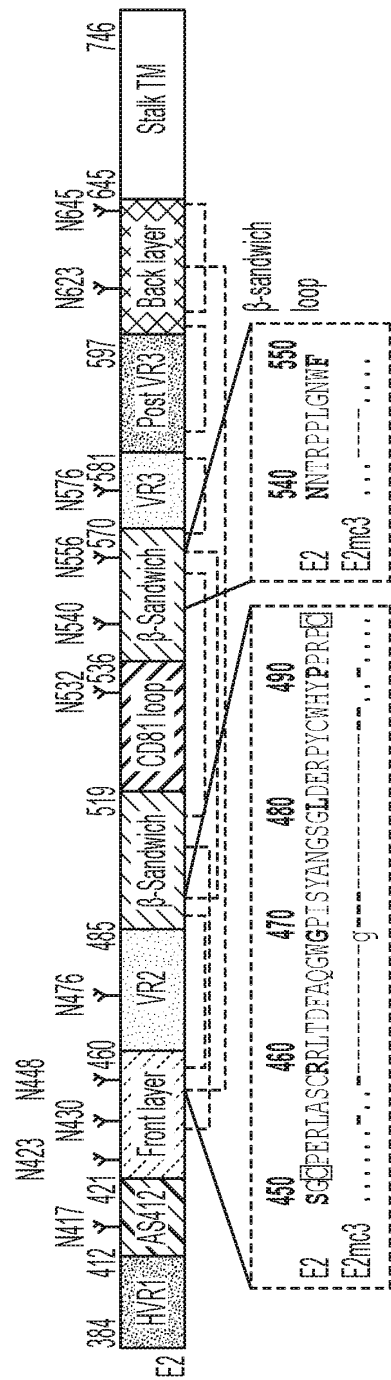


FIG. 1A

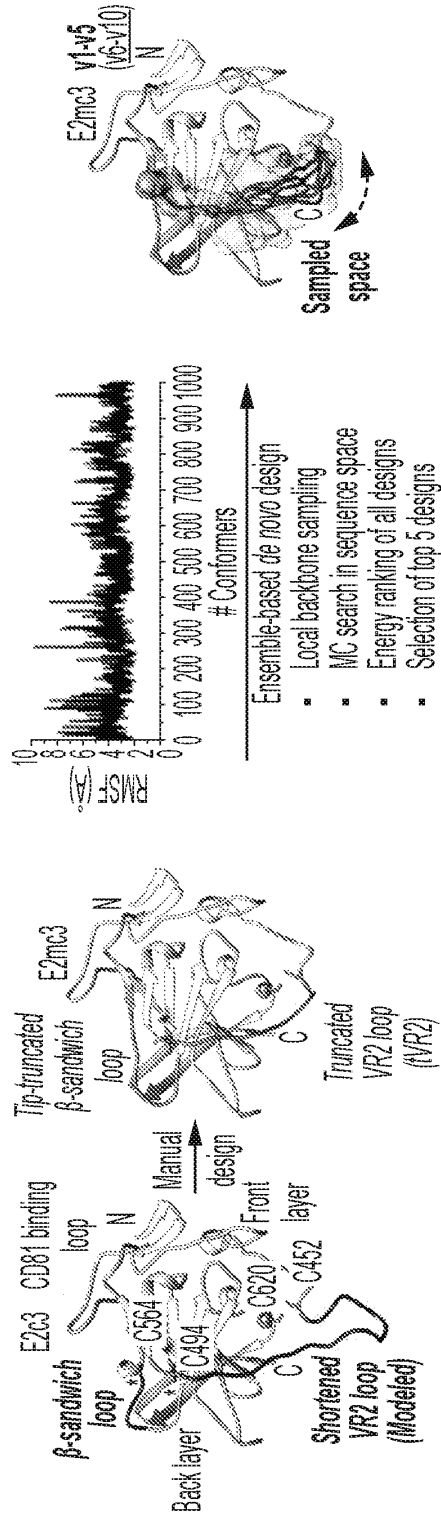


FIG. 1B

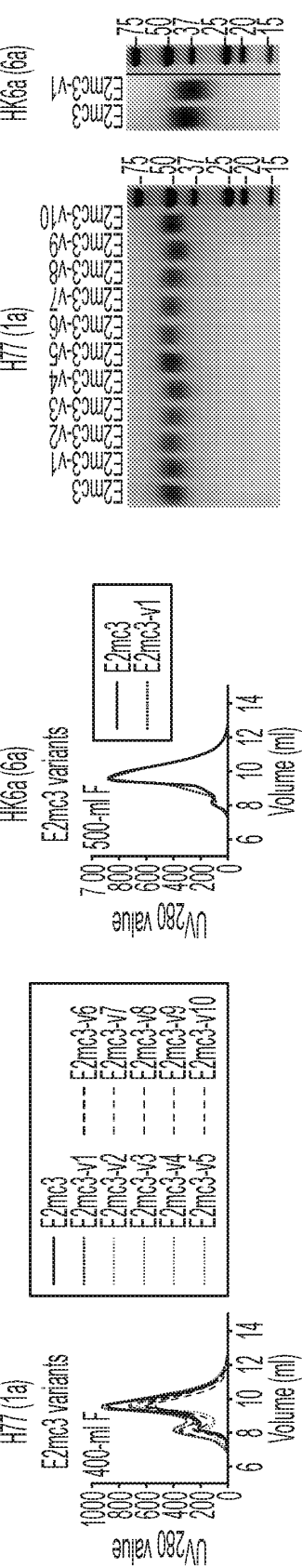


FIG. 1C

FIG. 1D

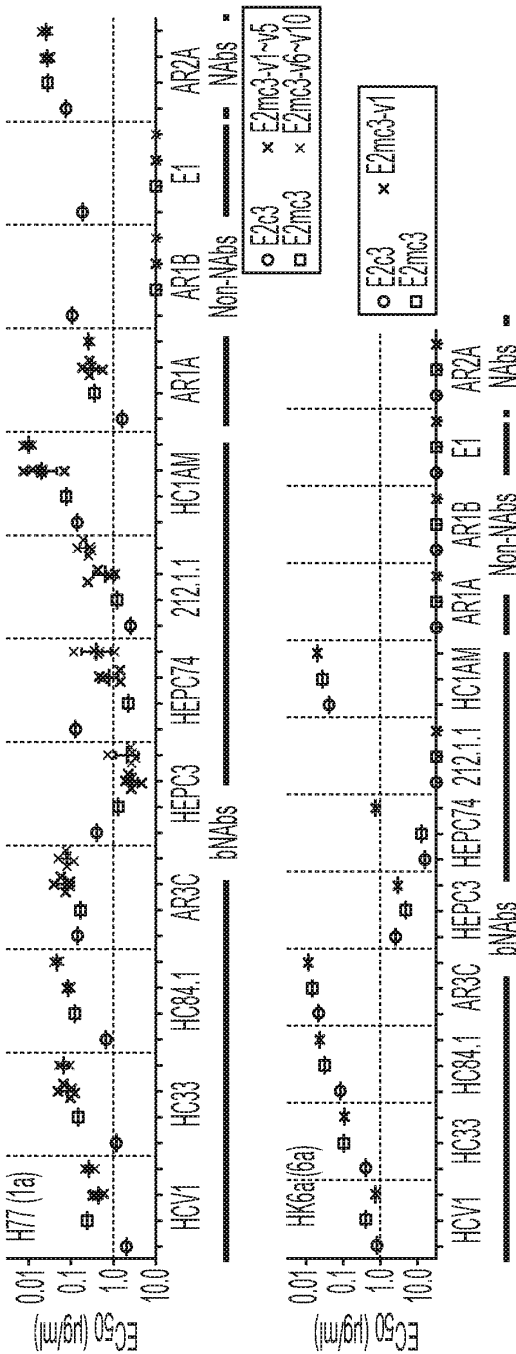


FIG. 1E

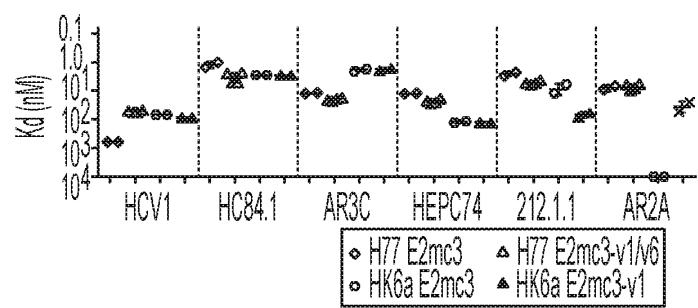


FIG. 1F

| Thermostability of E2 cores derived from HCV genotypes 1 and 6 <sup>a</sup> |          |       |          |          |           |       |          |
|-----------------------------------------------------------------------------|----------|-------|----------|----------|-----------|-------|----------|
|                                                                             | H77 (1a) |       |          |          | HK6a (6a) |       |          |
|                                                                             | E2c3     | E2mc3 | E2mc3-v1 | E2mc3-v6 | E2c3      | E2mc3 | E2mc3-v1 |
| T <sub>m</sub> (°C)                                                         | 75.7     | 79.9  | 80.9     | 80.1     | 78.0      | 77.9  | 79.4     |
| ΔT <sub>1/2</sub> (°C)                                                      | 10.8     | 9.5   | 9.5      | 9.3      | 8.5       | 8.9   | 10.0     |

<sup>a</sup>T<sub>m</sub> and ΔT<sub>1/2</sub> are derived from differential scanning calorimetry (DSC) analysis.

FIG. 1G

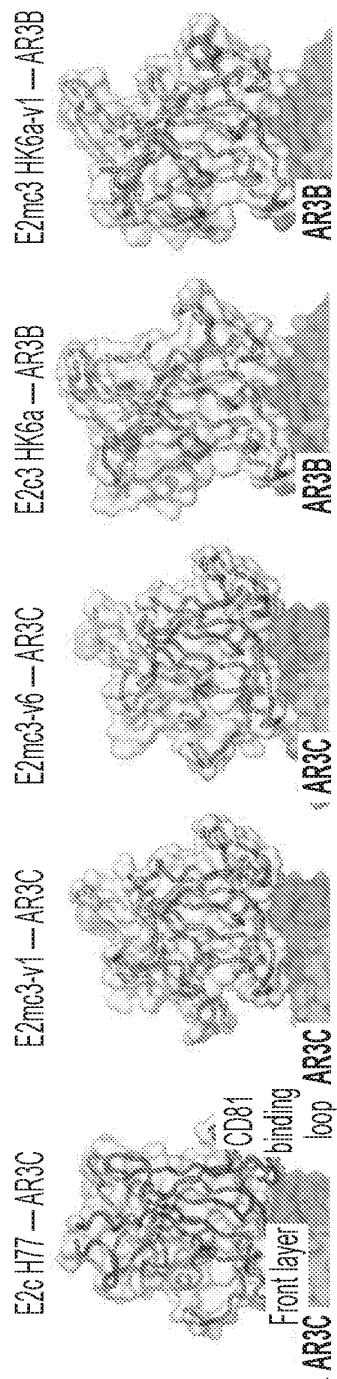


FIG. 2A

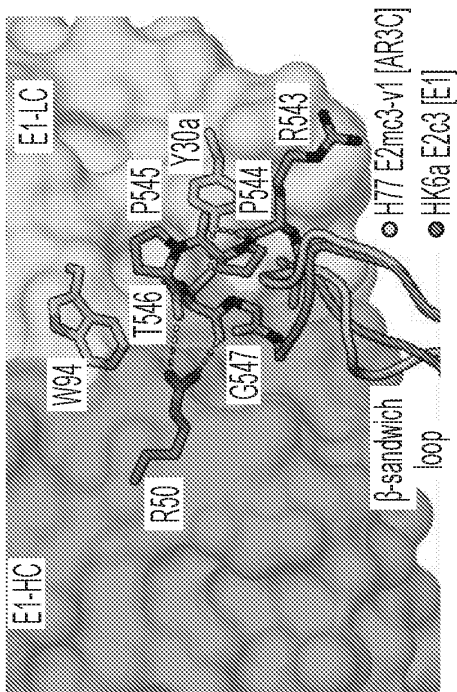


FIG. 2B

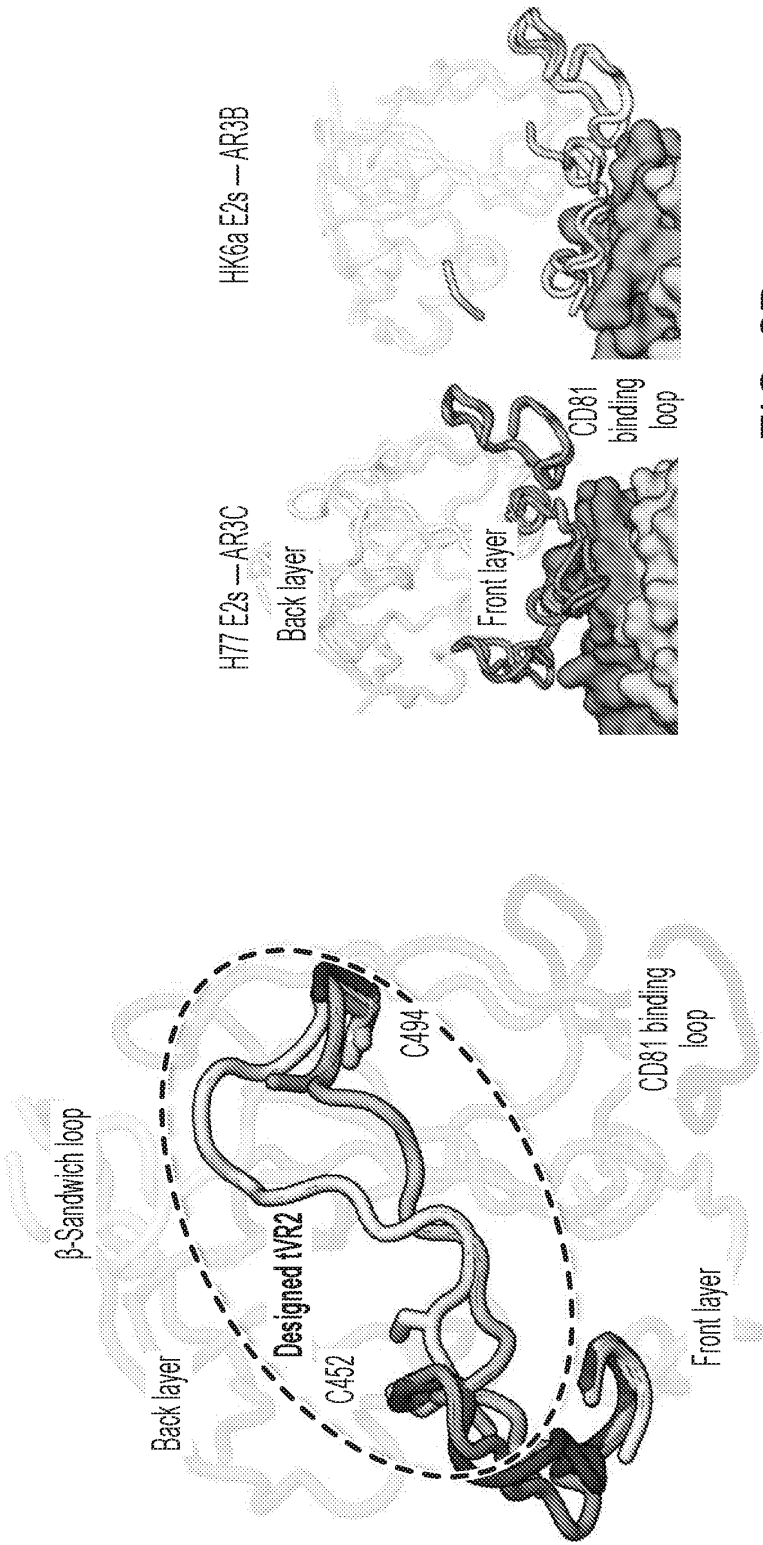


FIG. 2D

- H77 E2c
- H77 E2mc3-v1
- H77 E2mc3-v6
- HK6aE2mc3
- HK6a E2mc3-v1

FIG. 2C

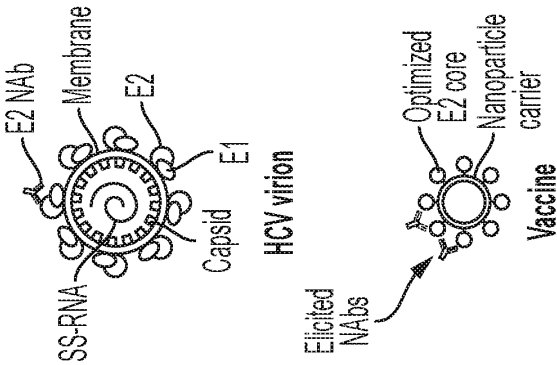


FIG. 3A

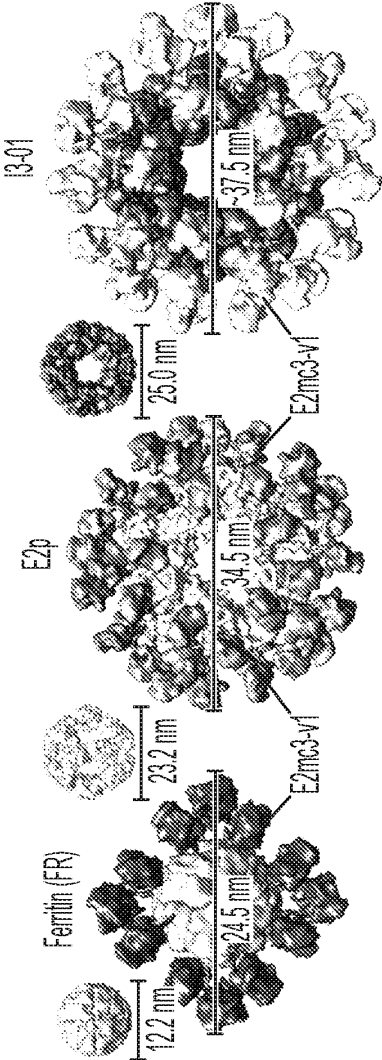


FIG. 3B

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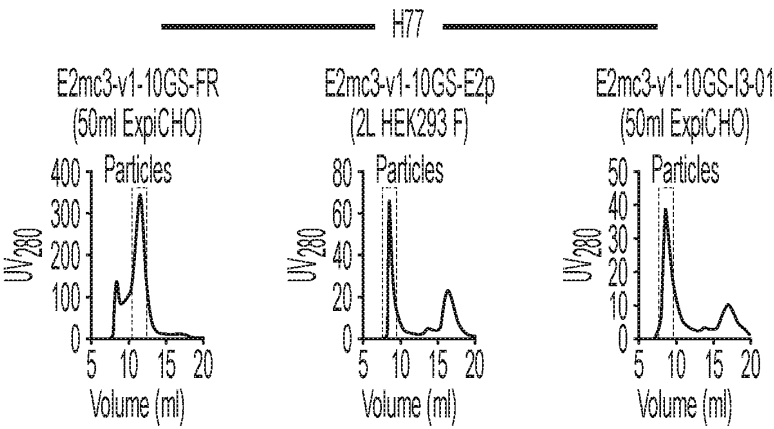


FIG. 3C

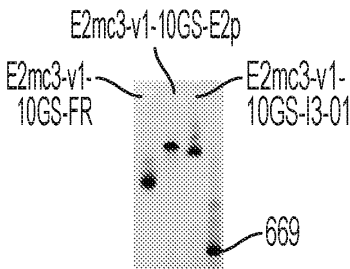


FIG. 3D

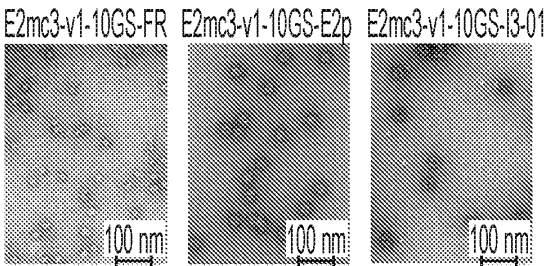
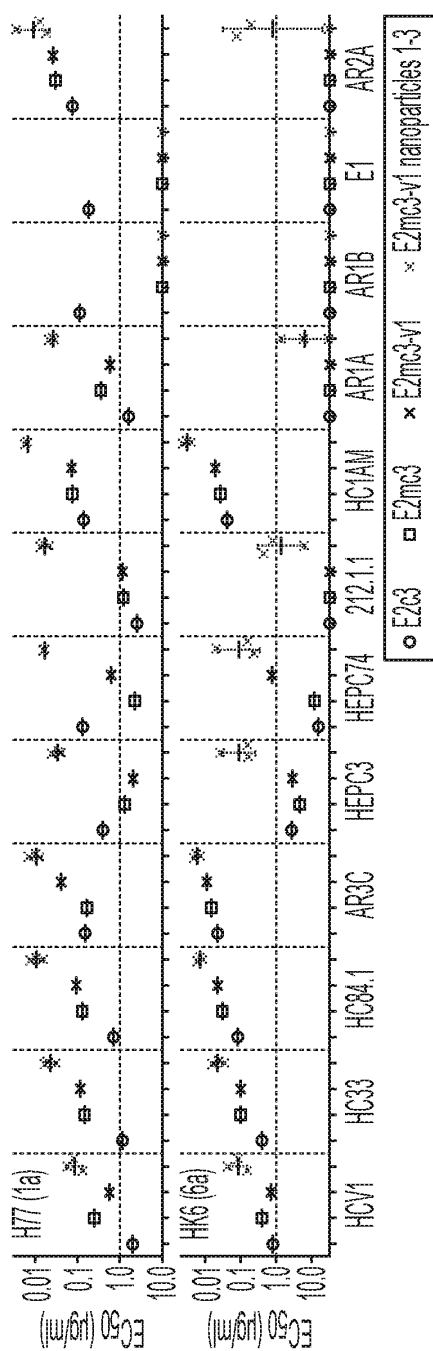


FIG. 3E



ॐ

| E2 core immunogens | E2mc3-v1 |        |      |        |       |      | E2mc3-v1-10GS-FR |        |      |        |       |      | E2mc3-v1-10GS-E2p |        |      |        |       |      | E2mc3-v1-10GS-I3-01 |        |      |        |       |      |
|--------------------|----------|--------|------|--------|-------|------|------------------|--------|------|--------|-------|------|-------------------|--------|------|--------|-------|------|---------------------|--------|------|--------|-------|------|
|                    | HCv1     | HC84.1 | AR3C | HEPC74 | 212.1 | AR2A | HCv1             | HC84.1 | AR3C | HEPC74 | 212.1 | AR2A | HCv1              | HC84.1 | AR3C | HEPC74 | 212.1 | AR2A | HCv1                | HC84.1 | AR3C | HEPC74 | 212.1 | AR2A |
|                    | 0.23     | 0.28   | 0.25 | 0.35   | 0.25  | 0.10 | 0.19             | 0.27   | 0.21 | 0.19   | 0.08  | 0.04 | 0.51              | 1.00   | 0.93 | 0.77   | 0.45  | 0.80 | 0.61                | 1.53   | 1.37 | 1.27   | 0.68  | 0.19 |
|                    | 0.53     | 1.14   | 1.21 | 0.97   | 0.56  | 1.25 | 1.12             | 0.92   | 1.04 | 1.60   | 1.14  | 0.42 | 0.53              | 1.41   | 1.21 | 0.97   | 0.66  | 1.25 | 1.12                | 0.92   | 1.04 | 1.60   | 1.14  | 0.42 |
|                    | 0.89     | 1.81   | 1.72 | 1.44   | 0.86  | 1.33 | 1.15             | 1.91   | 1.27 | 1.77   | 1.28  | 0.41 | 0.89              | 1.81   | 1.72 | 1.44   | 0.86  | 1.33 | 1.15                | 1.91   | 1.27 | 1.77   | 1.28  | 0.41 |

33  
E

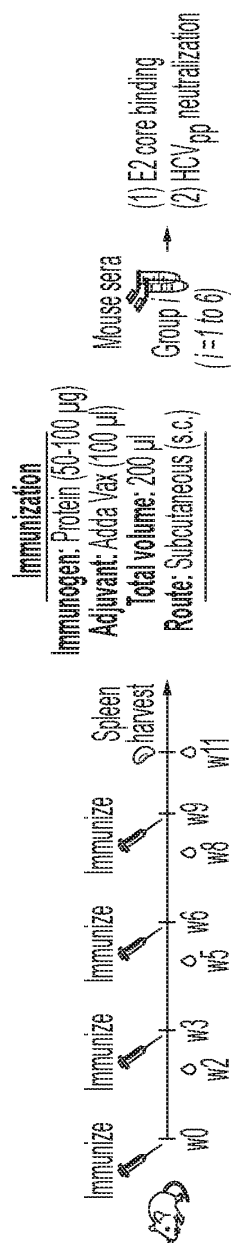


FIG. 4A

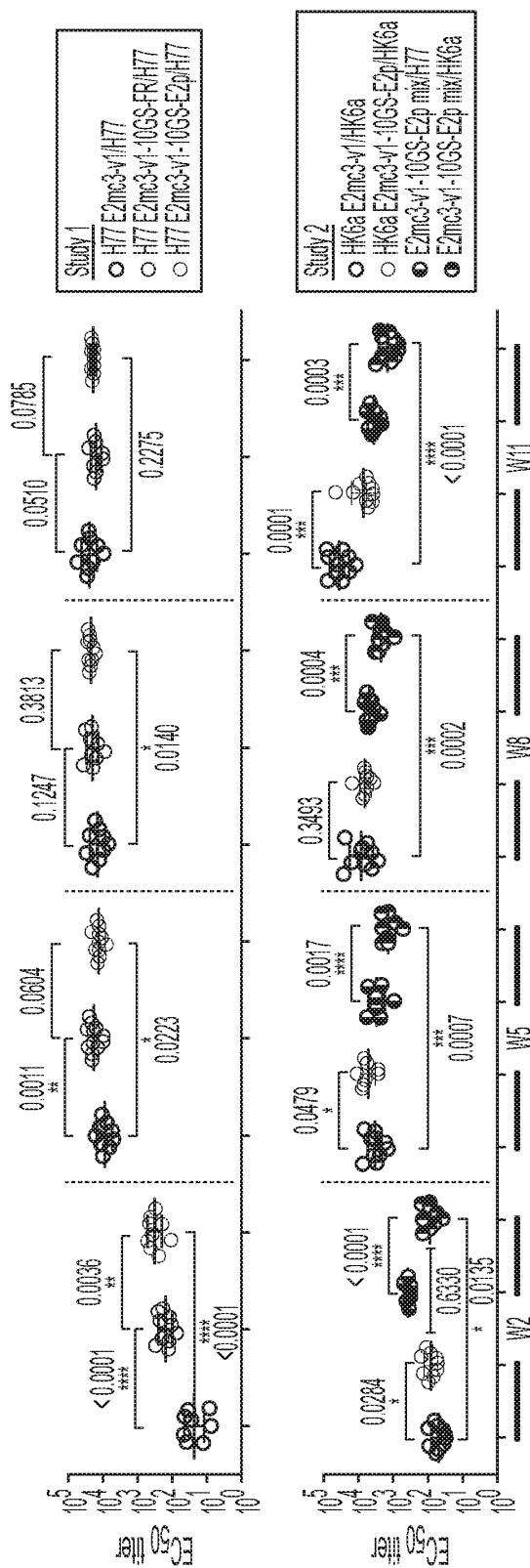


FIG. 4B

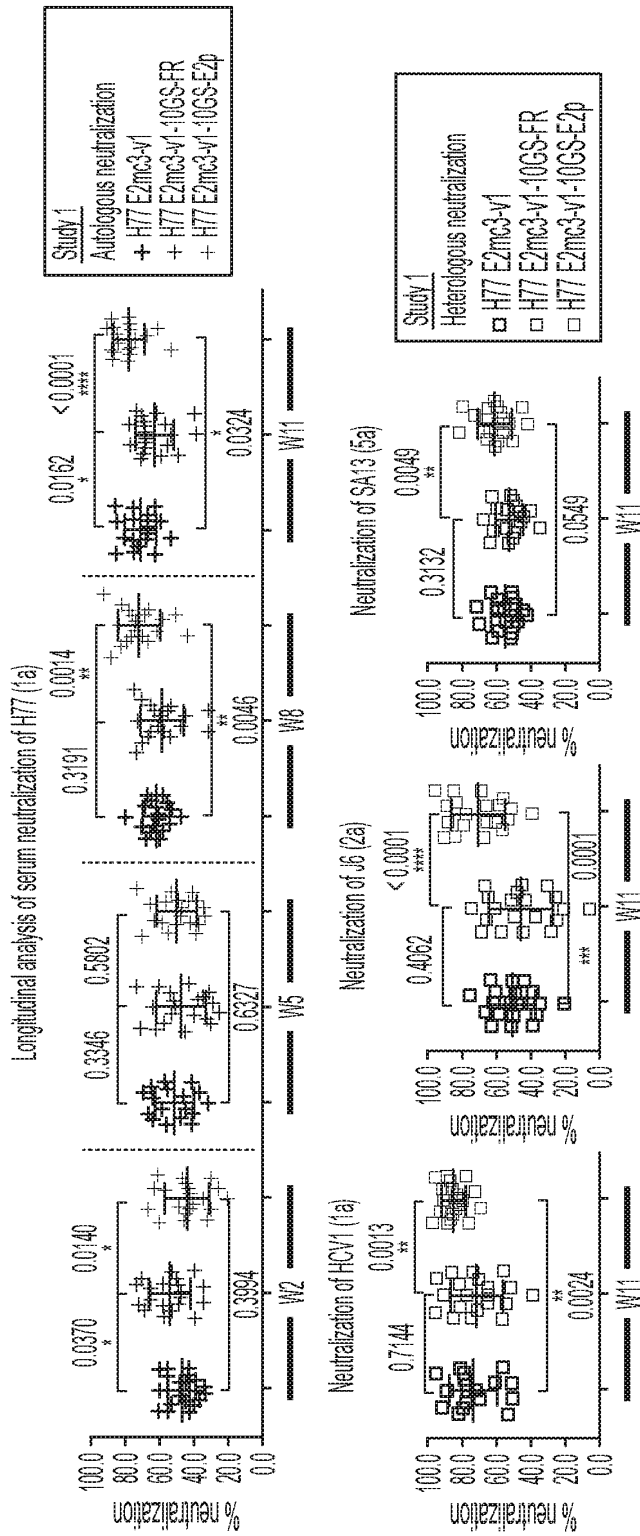


FIG. 4C

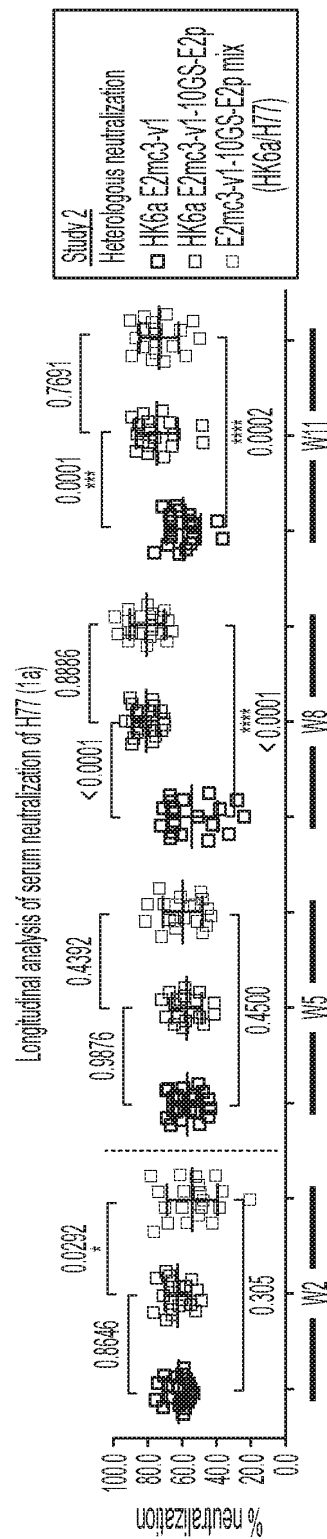


FIG. 4D

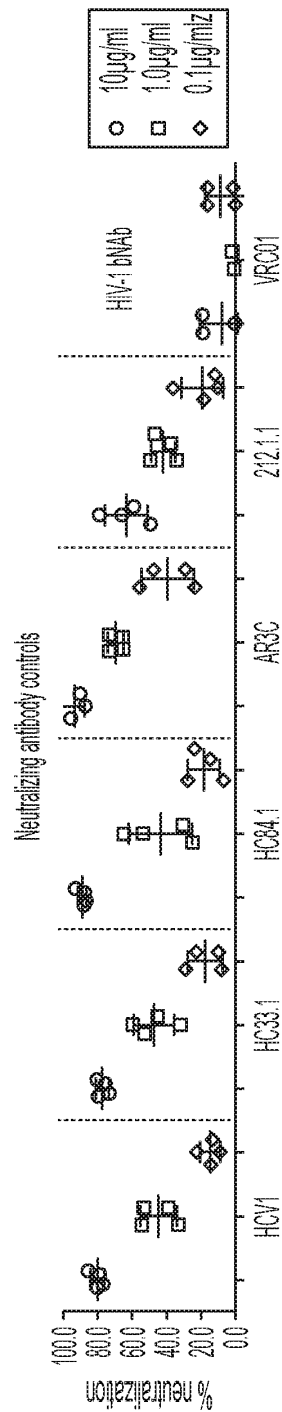


FIG. 4E

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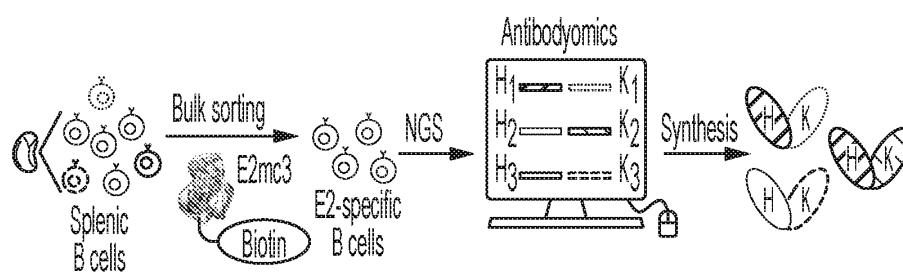


FIG. 5A

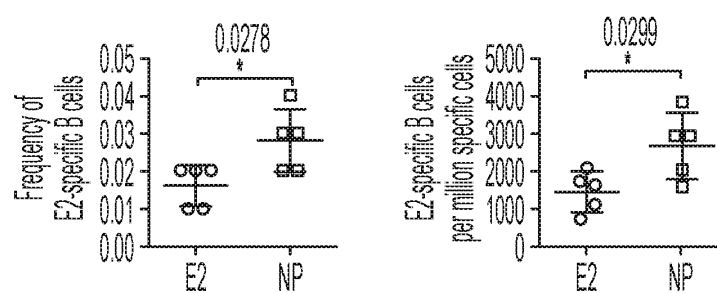


FIG. 5B

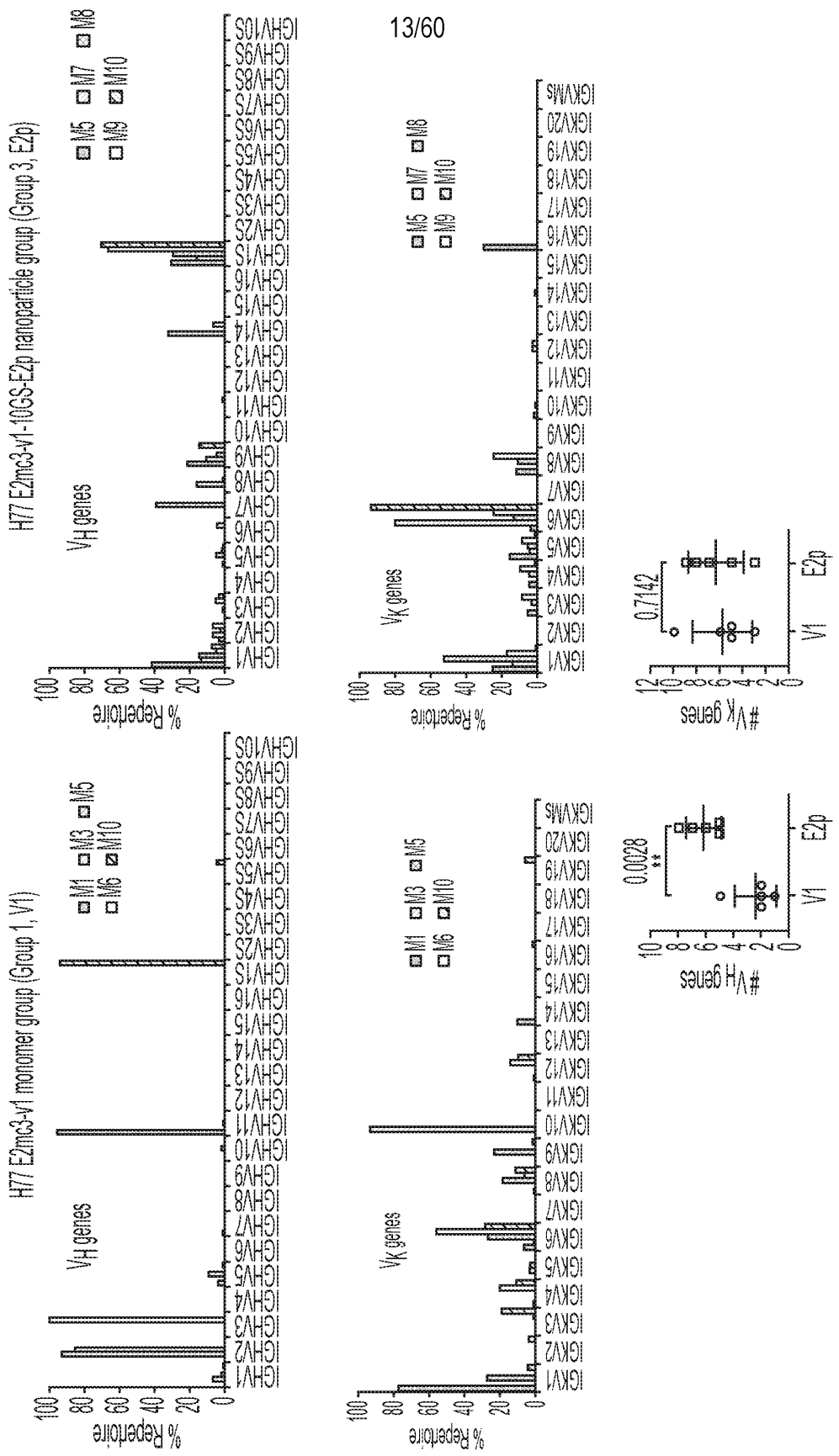


FIG. 5C

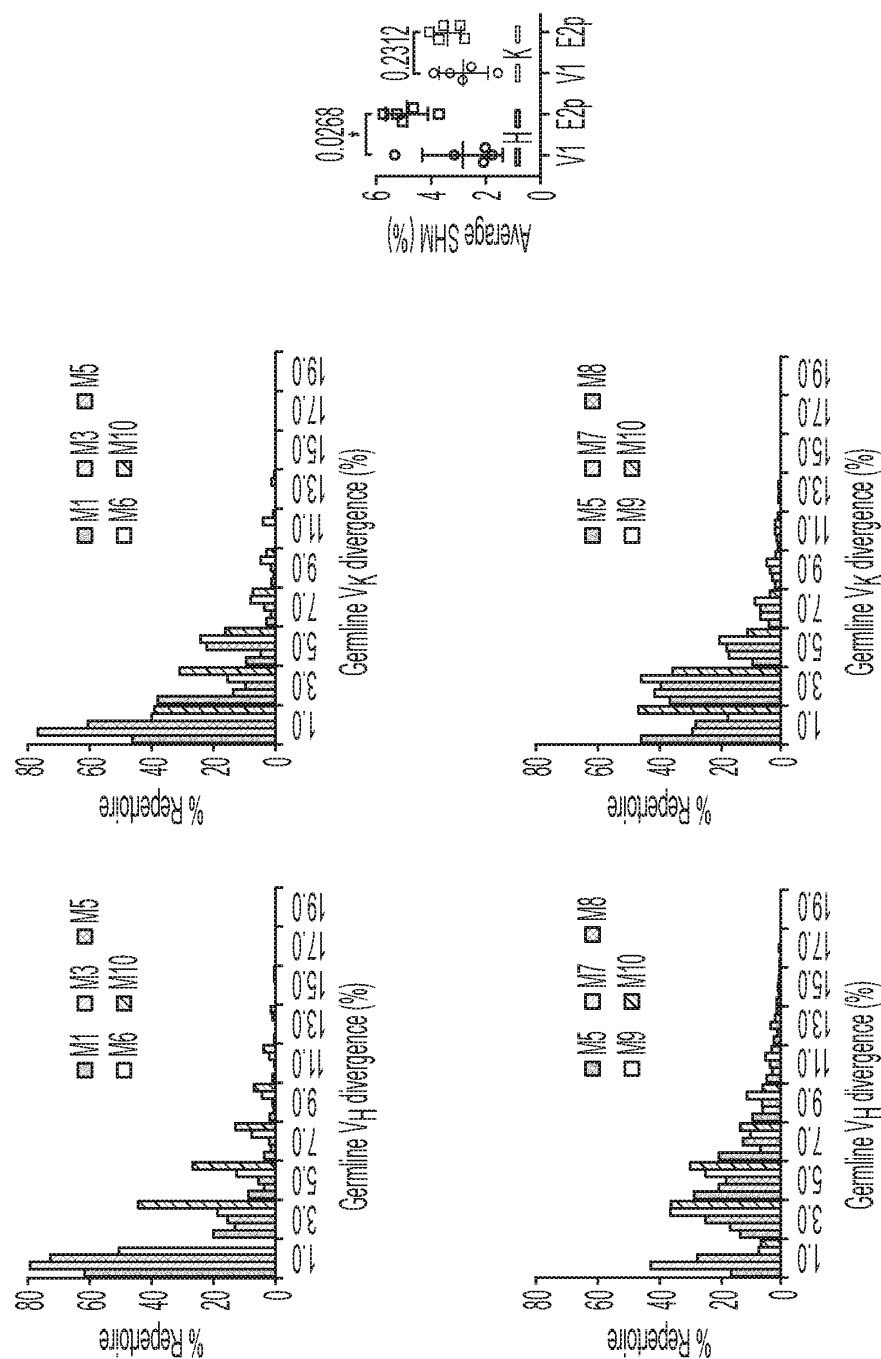


FIG. 5D

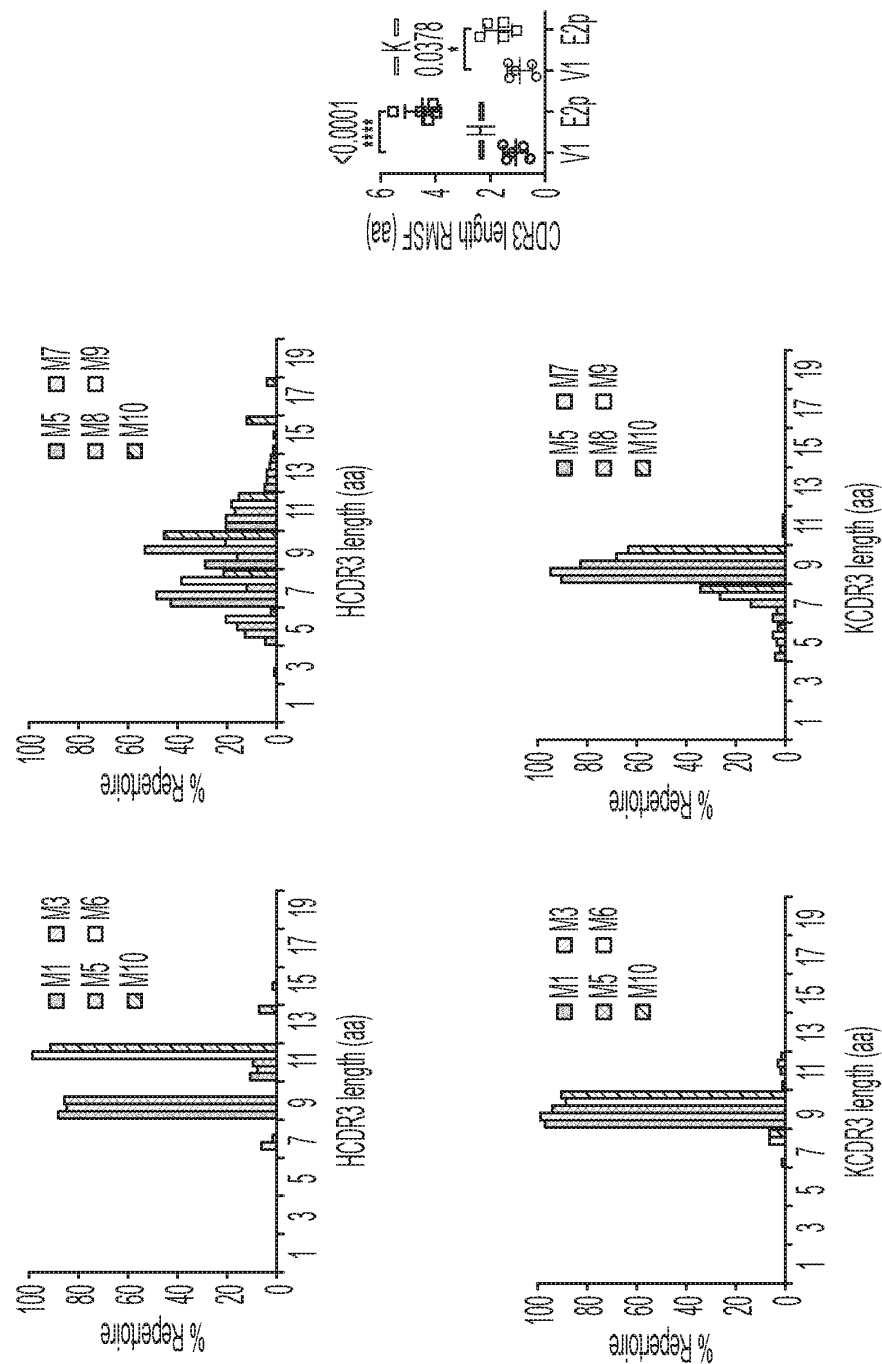
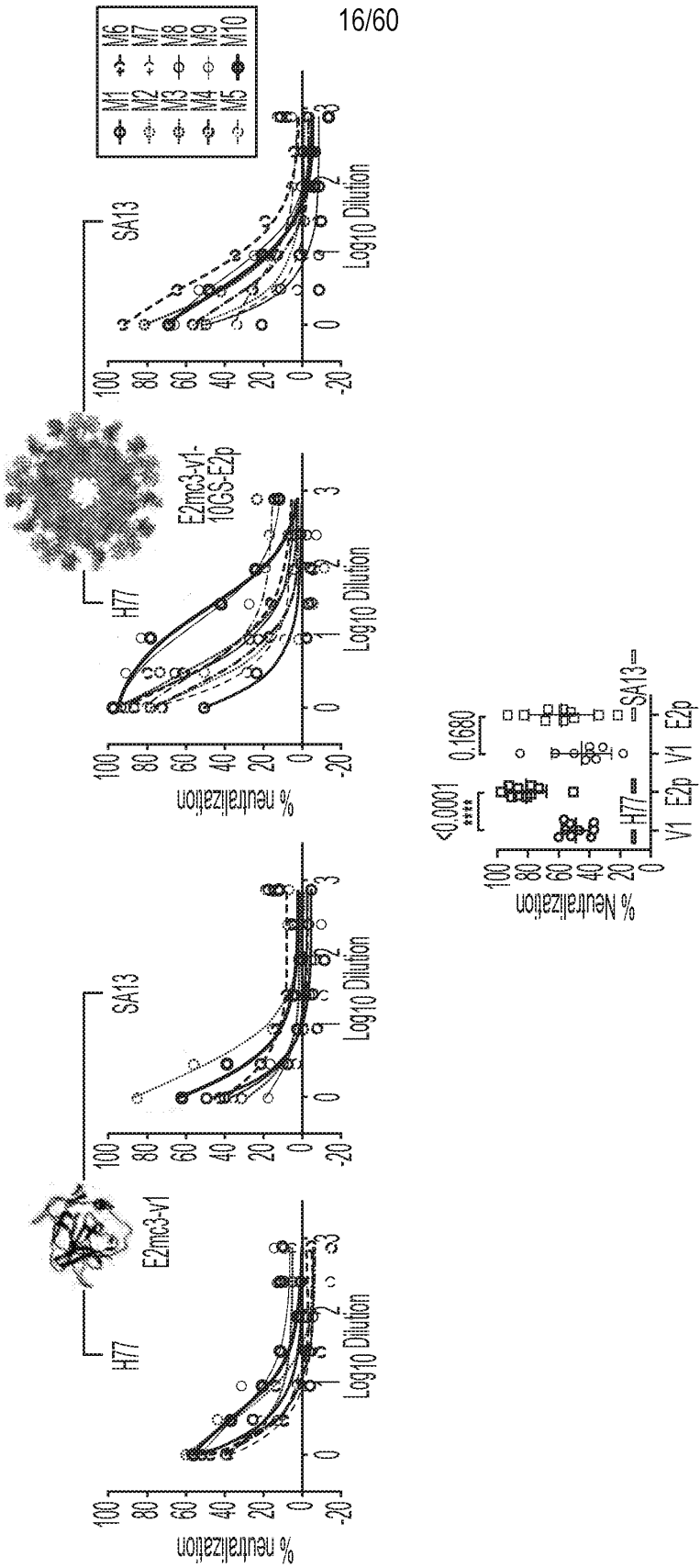


FIG. 5E



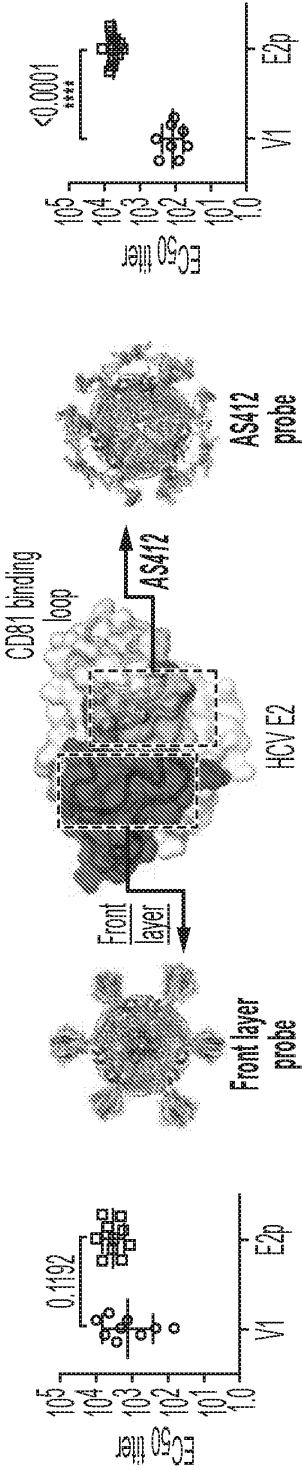


FIG. 5G

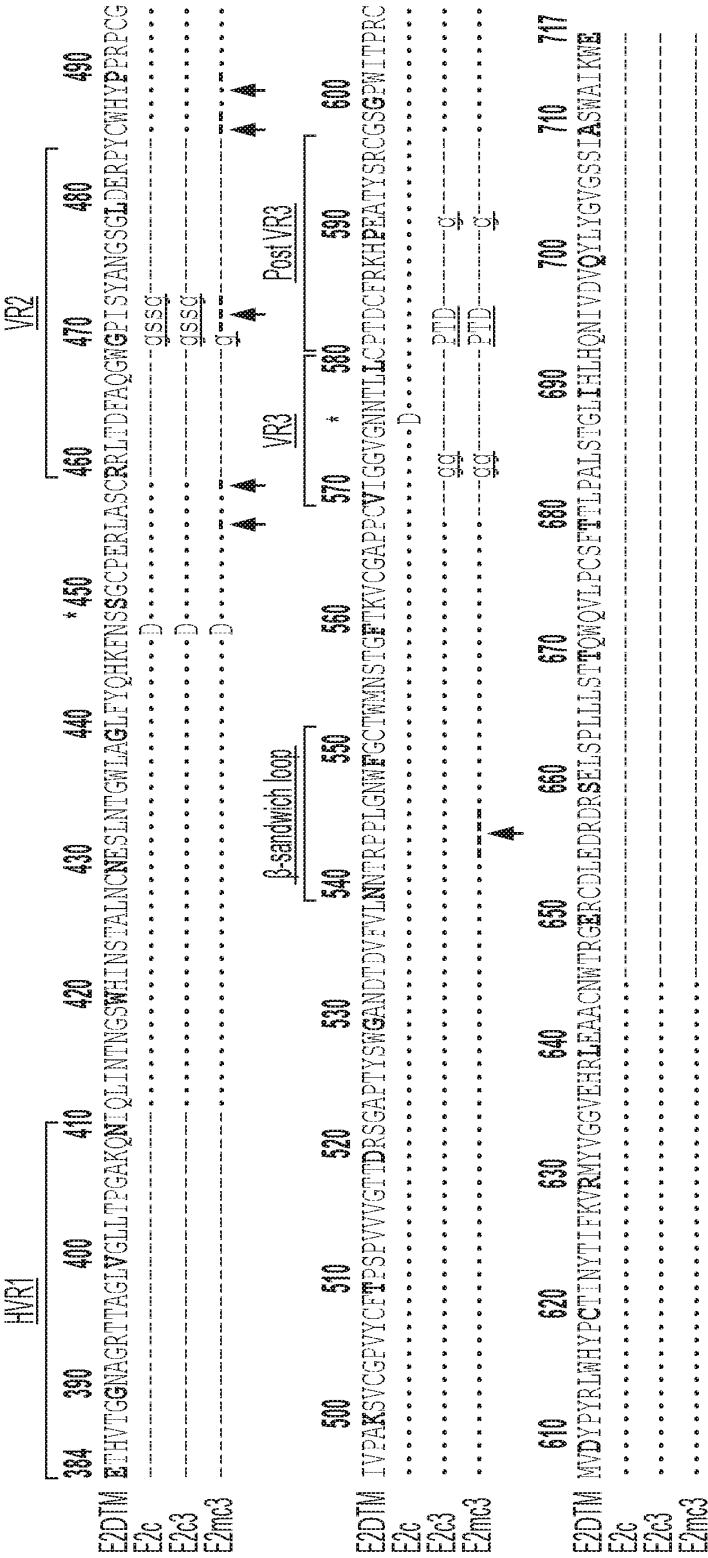
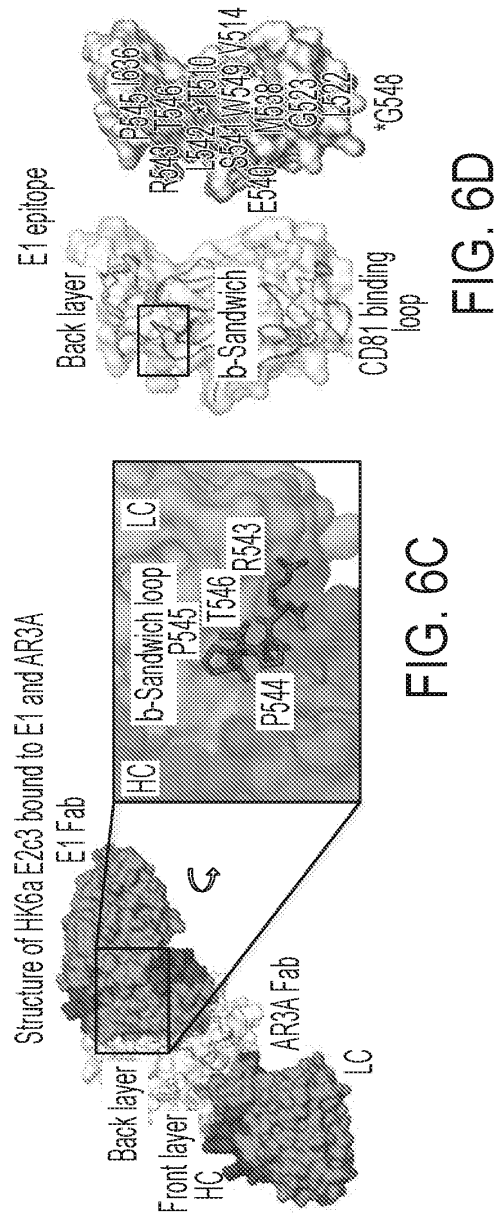
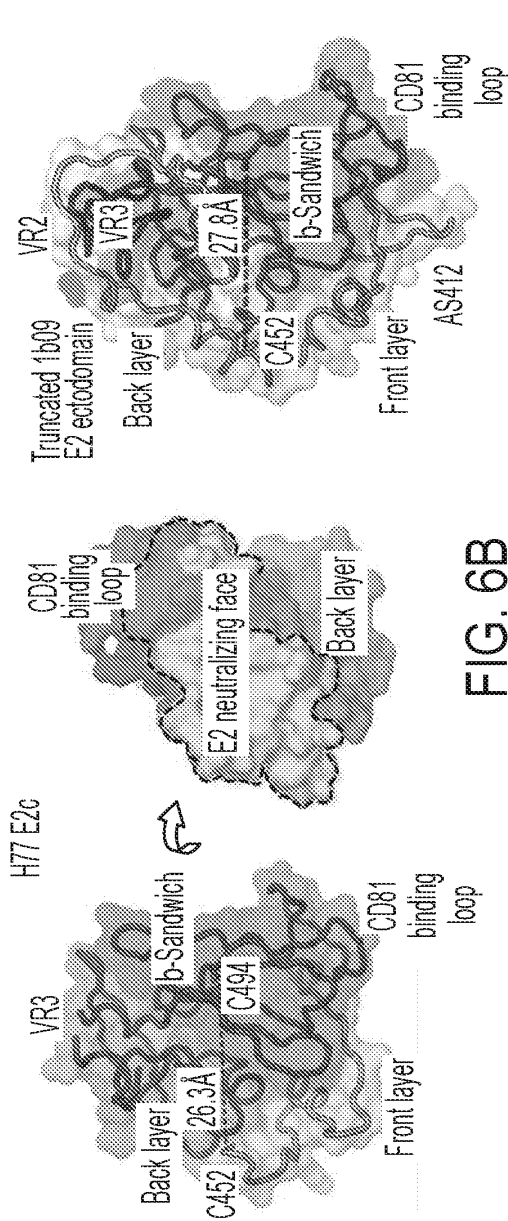


FIG. 6A



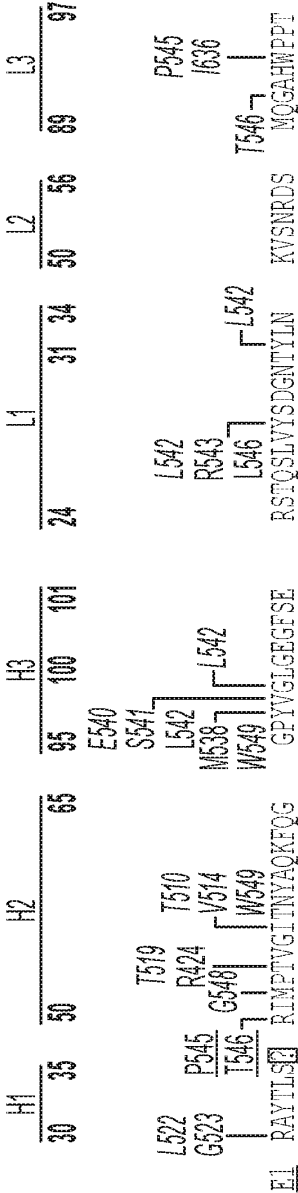


FIG. 6E

| Redesigned E2mc3 tVR2 ensembles |                     |
|---------------------------------|---------------------|
| Loop length #1                  | Loop length #2      |
| C452GGGGGGHYPRPC494             | C452GGGGGGHYPRPC494 |
| 6 a.a.                          | 5 a.a.              |

| Replace PERASG with |                 |
|---------------------|-----------------|
| Name                | Energy Sequence |
| v1                  | -139.205 QNWDEP |
| v2                  | -138.005 KVNIDP |
| v3                  | -137.772 EKVEEL |
| v4                  | -137.611 PDENMK |
| v5                  | -137.544 KRECKM |
| v6                  | -136.423 PKTEV- |
| v7                  | -136.358 KRVDI- |
| v8                  | -136.207 PSDMV- |
| v9                  | -136.163 PNEEE- |
| v10                 | -136.086 KKEIR- |

FIG. 6F

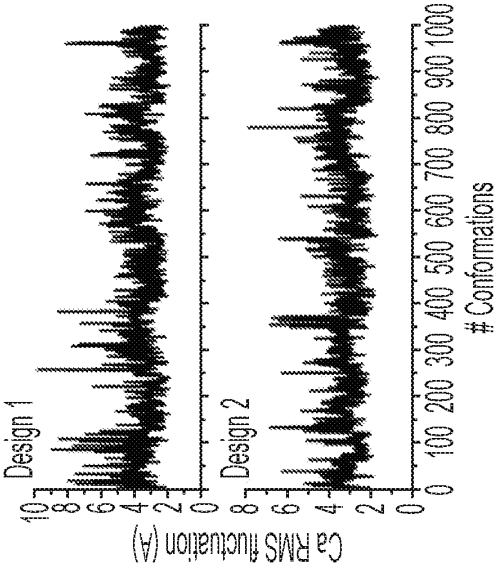


FIG. 6G

FIG. 6H

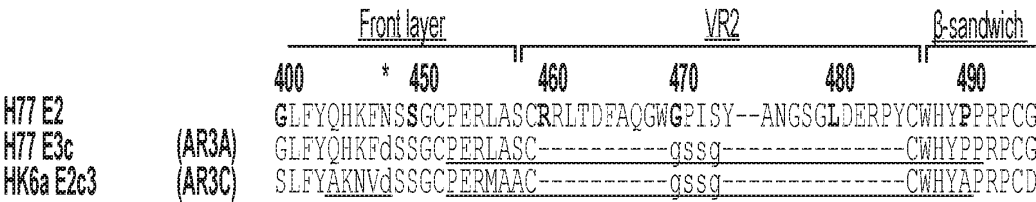


FIG. 6I

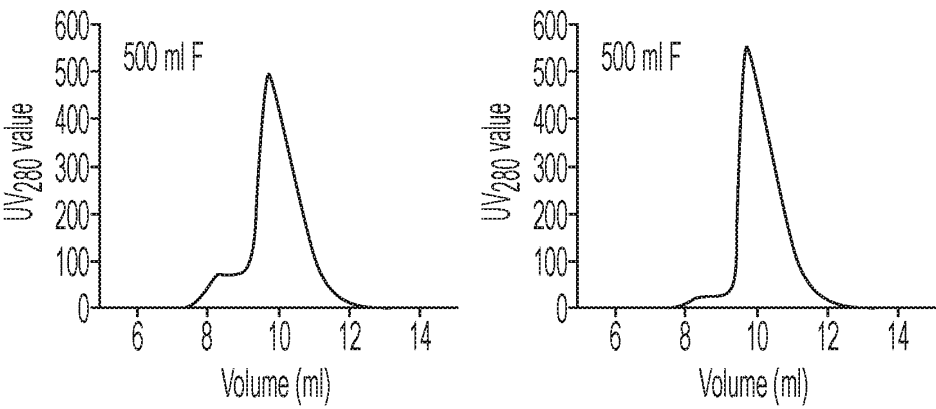


FIG. 7A

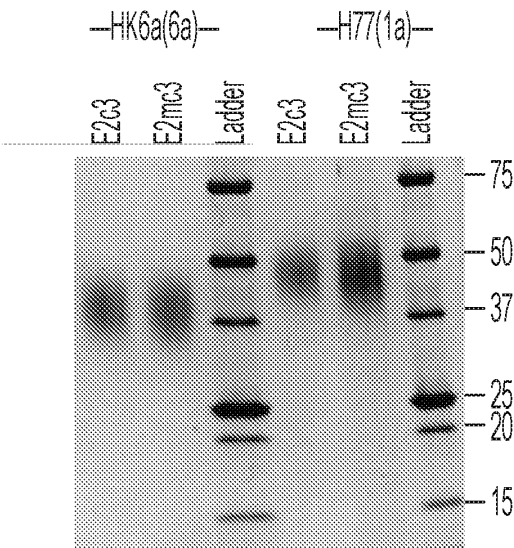
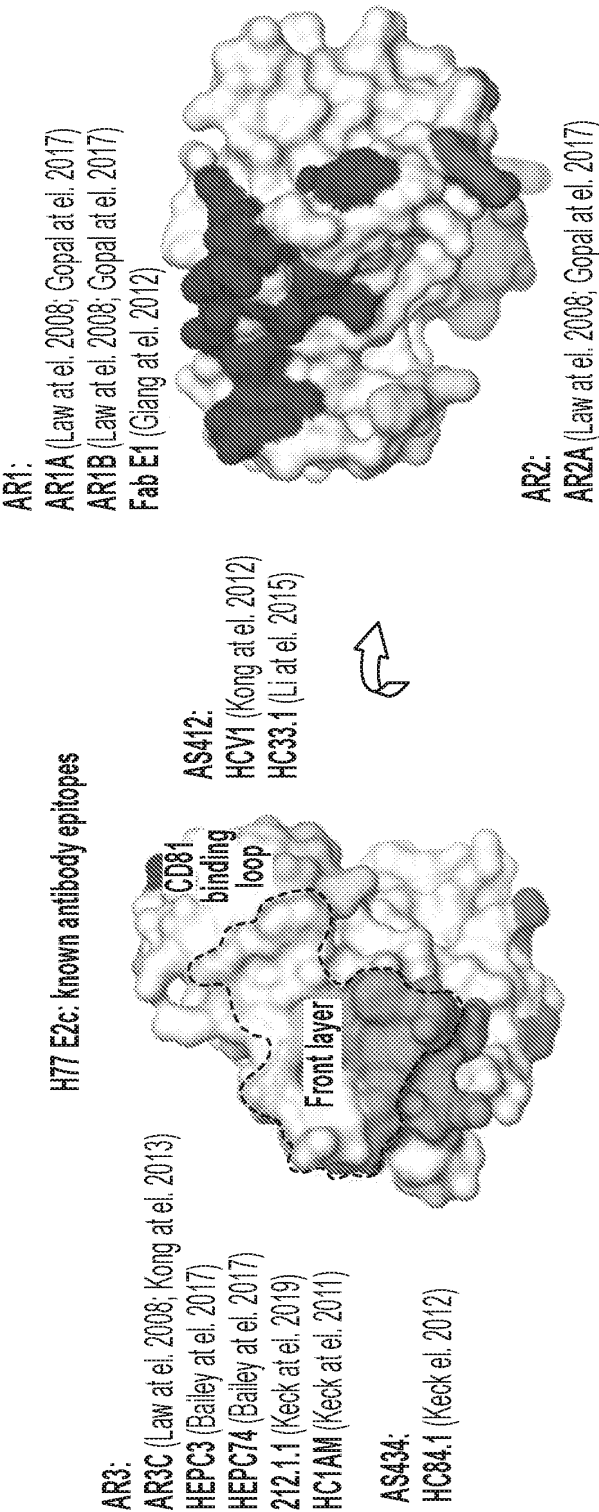


FIG. 7B



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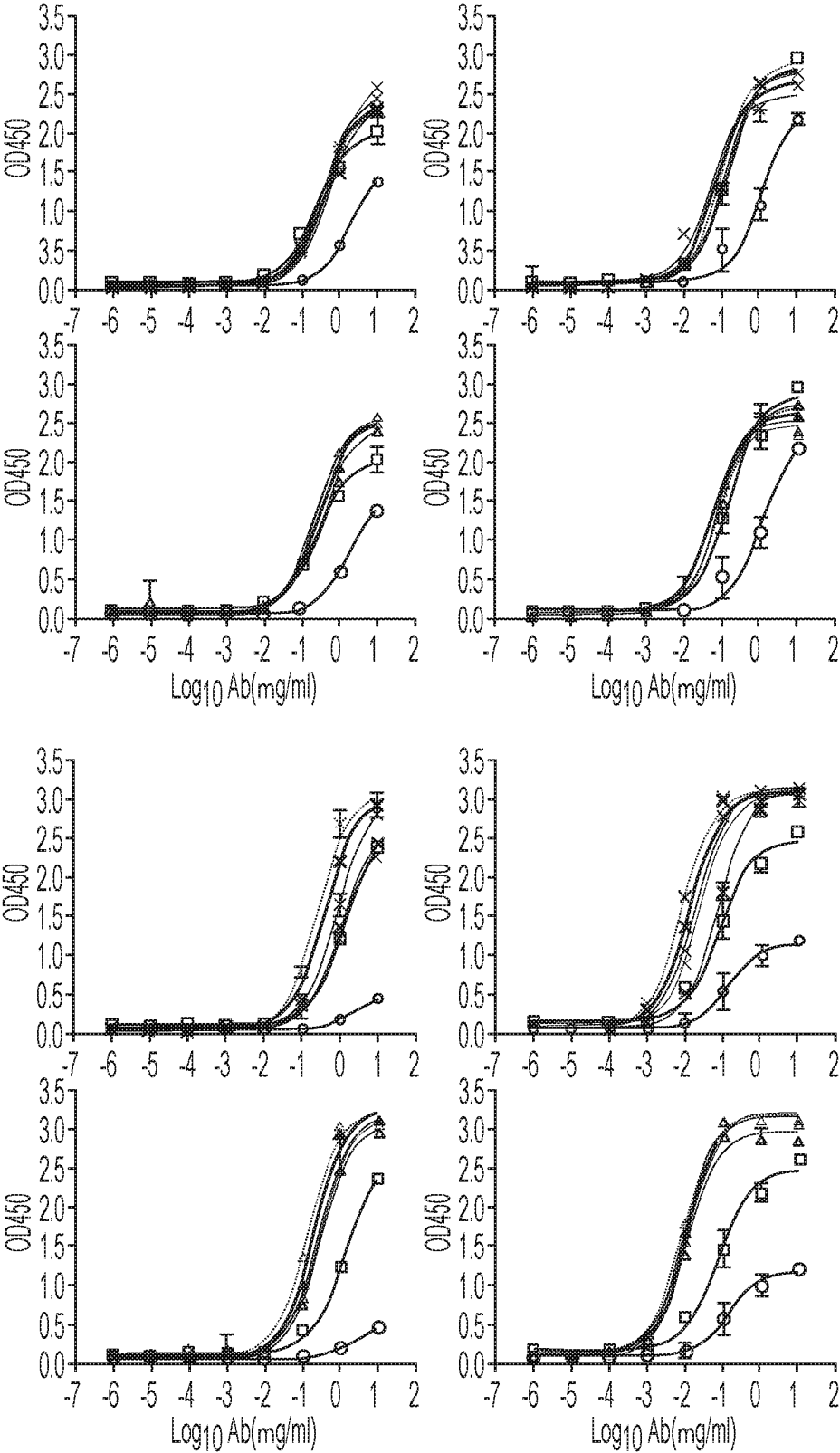


FIG. 7D

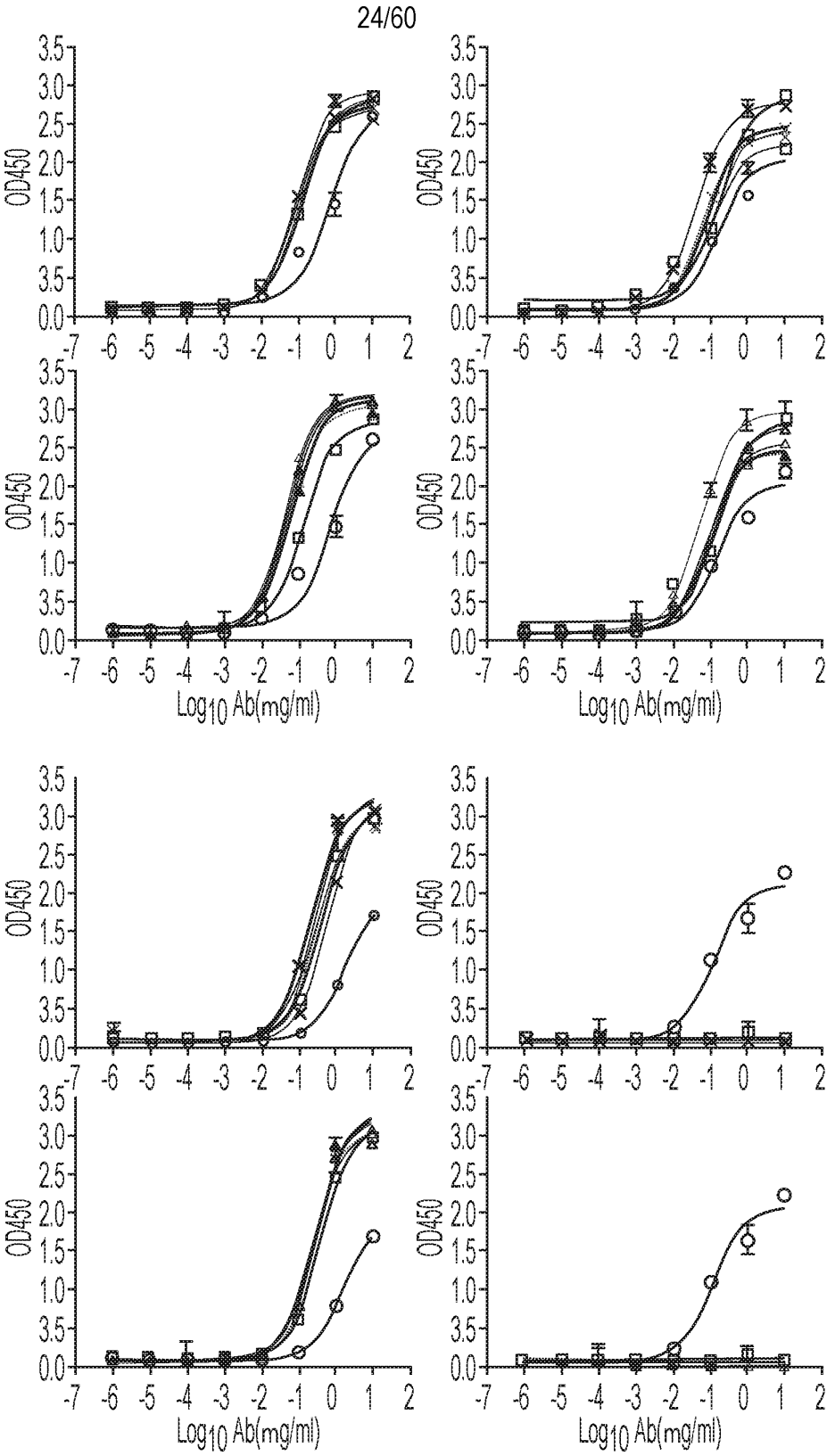


FIG. 7D (Continued)

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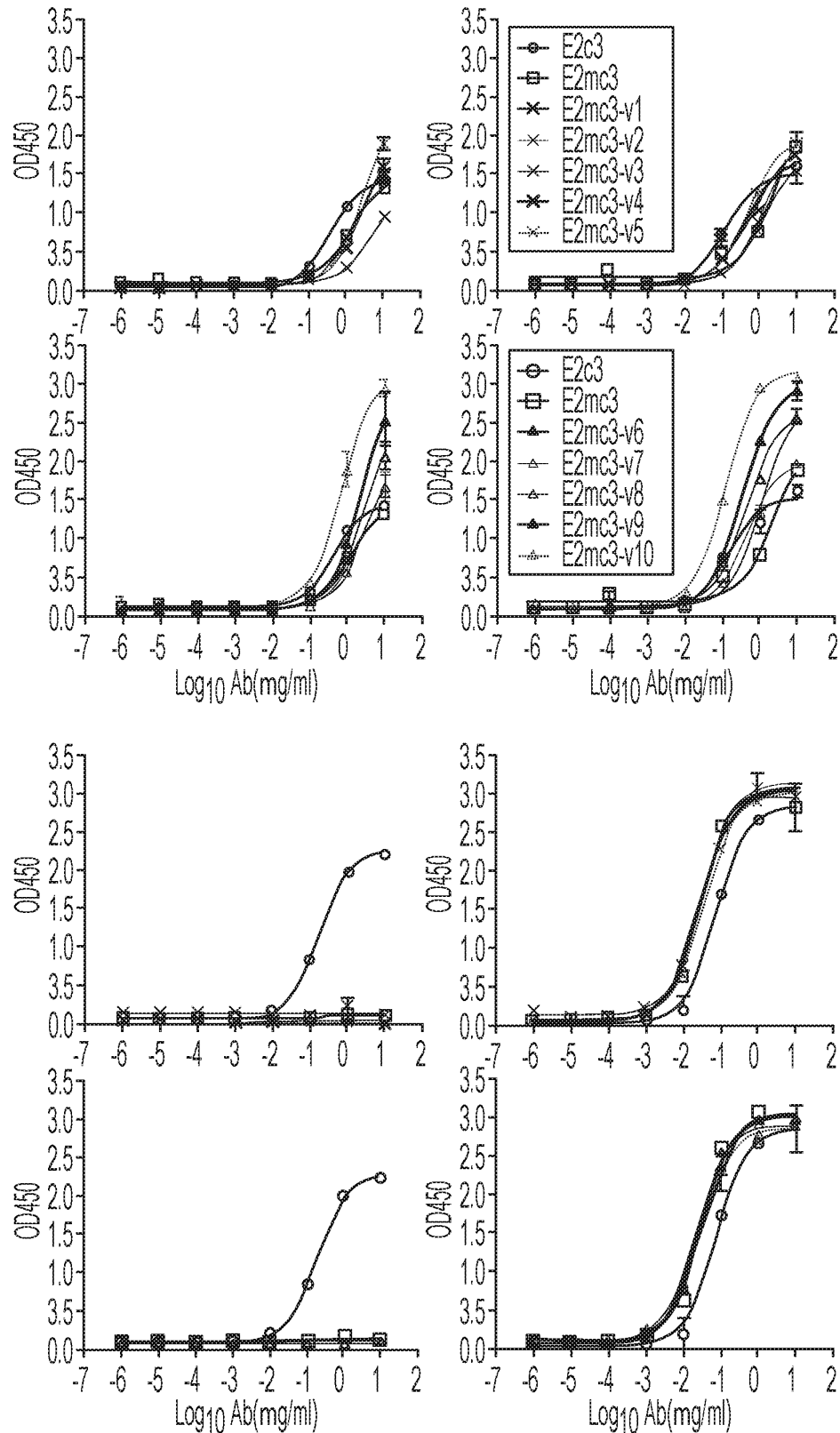


FIG. 7D (Continued)

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EC<sub>50</sub> values of H77 E2 core constructs binding to 12 HCV-specific antibodies<sup>a</sup>.

|           | HCV1   | HC33.1  | HC84.1  | AR3C    | HEPC3  | HEPC74 | 212.1.1 | HC1AM    | AR1A   | AR1B   | E1     | AR2A    |
|-----------|--------|---------|---------|---------|--------|--------|---------|----------|--------|--------|--------|---------|
| E2c3      | 2.022  | 1.153   | 0.6954  | 0.1458  | 0.3926 | 0.1335 | 2.479   | 0.1379   | 1.56   | 0.1084 | 0.1799 | 0.07568 |
| E2mc3     | 0.2485 | 0.1481  | 0.1269  | 0.17    | 1.286  | 2.198  | 1.215   | 0.07587  | 0.3604 | -      | -      | 0.02879 |
| E2mc3-v1  | 0.5451 | 0.1114  | 0.08747 | 0.04025 | 1.938  | 0.6034 | 1.065   | 0.07059  | 0.5547 | -      | -      | 0.02548 |
| E2mc3-v2  | 0.5896 | 0.1219  | 0.09616 | 0.09921 | 2.296  | 1.395  | 1.045   | 0.02245  | 0.2802 | -      | -      | 0.02889 |
| E2mc3-v3  | 0.3661 | 0.04667 | 0.08207 | 0.09939 | 4.551  | 1.427  | 0.8706  | 0.02003  | 0.2646 | -      | -      | 0.02851 |
| E2mc3-v4  | 0.3184 | 0.06764 | 0.07956 | 0.07655 | 2.637  | 0.4576 | 0.4189  | 0.01254  | 0.1926 | -      | -      | 0.02373 |
| E2mc3-v5  | 0.4349 | 0.09598 | 0.08494 | 0.05845 | 2.634  | 0.5341 | 0.2482  | 0.007939 | 0.3064 | -      | -      | 0.03428 |
| E2mc3-v6  | 0.3588 | 0.08911 | 0.04386 | 0.119   | 2.864  | 0.4247 | 0.2895  | 0.01092  | 0.2432 | -      | -      | 0.02549 |
| E2mc3-v7  | 0.2567 | 0.05072 | 0.04014 | 0.05046 | 2.719  | 0.4663 | 0.313   | 0.01209  | 0.2532 | -      | -      | 0.02785 |
| E2mc3-v8  | 0.2086 | 0.05031 | 0.04738 | 0.08043 | 3.186  | 1.098  | 0.2663  | 0.009017 | 0.2487 | -      | -      | 0.0201  |
| E2mc3-v9  | 0.3242 | 0.05871 | 0.05566 | 0.08429 | 2.568  | 0.3426 | 0.1992  | 0.01026  | 0.2615 | -      | -      | 0.02962 |
| E2mc3-v10 | 0.2225 | 0.09384 | 0.04887 | 0.08056 | 0.7269 | 0.1174 | 0.1355  | 0.007812 | 0.2874 | -      | -      | 0.0284  |

<sup>a</sup> EC<sub>50</sub> values were calculated from the besting fitting in GraphPad Prism 6.

FIG. 7E

Antigenetic profiling of HK6a E2mc3 designs against E2c3 by ELISA

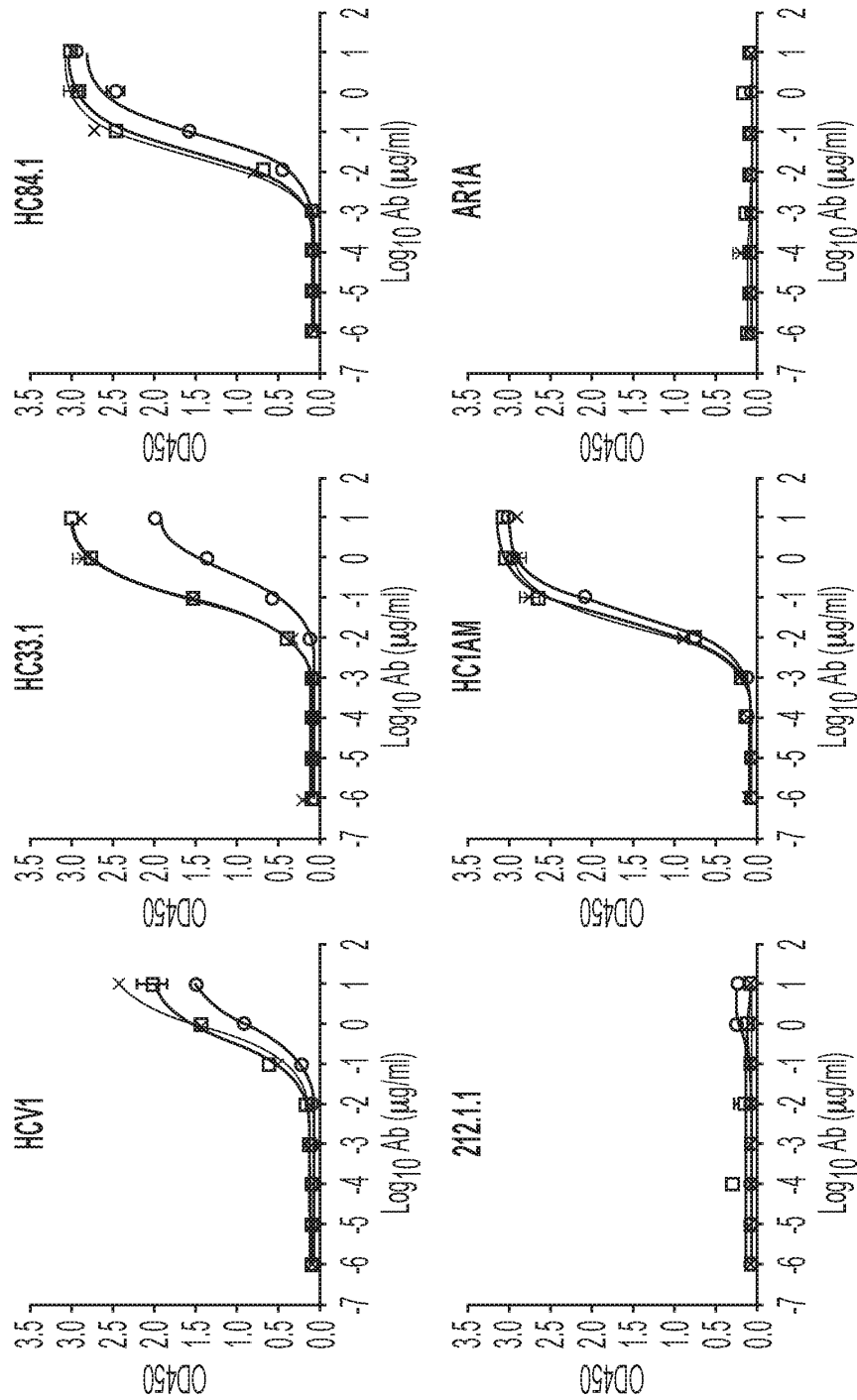


FIG. 7F

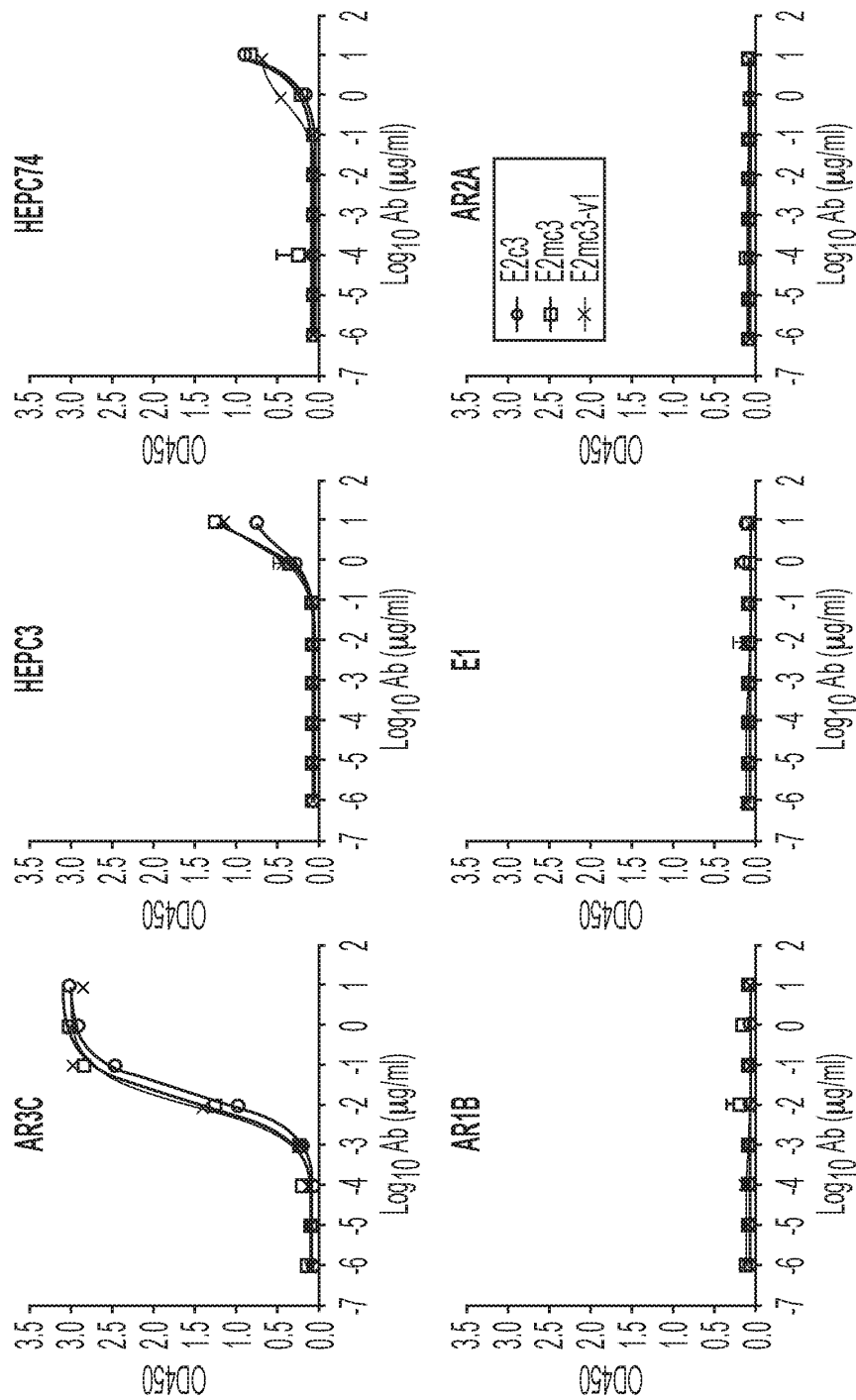


FIG. 7F (Continued)

EC<sub>50</sub> values of HK6a E2 core constructs binding to 12 HCV-specific antibodies<sup>a</sup>.

|          | HCV1   | HC33.1 | HC84.1  | AR3C    | HEPC3 | HEPC74 | 212.1.1 | HC1AM   | AR1A | AR1B | E1 | AR2A |
|----------|--------|--------|---------|---------|-------|--------|---------|---------|------|------|----|------|
| E2c3     | 0.8293 | 0.4099 | 0.08365 | 0.02219 | 2.637 | 15.88  | -       | 0.0418  | -    | -    | -  | -    |
| E2mc3    | 0.3883 | 0.1034 | 0.03069 | 0.01498 | 4.767 | 12.29  | -       | 0.02714 | -    | -    | -  | -    |
| E2mc3-y1 | 0.7244 | 0.1054 | 0.02383 | 0.01109 | 2.938 | 0.771  | -       | 0.02011 | -    | -    | -  | -    |

<sup>a</sup> EC<sub>50</sub> values were calculated from the besting fitting in Prism.

FIG. 7G

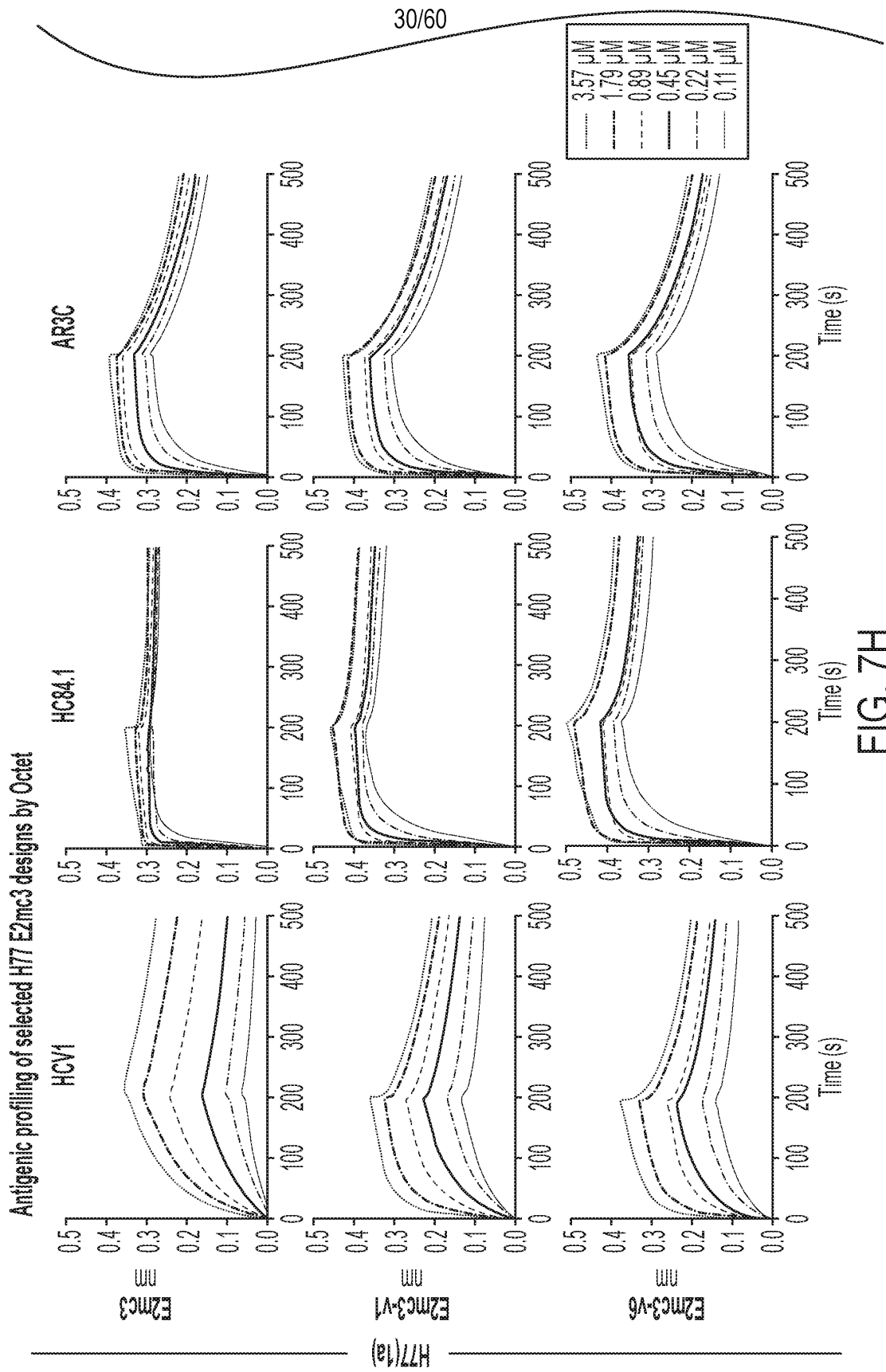


FIG. 7H

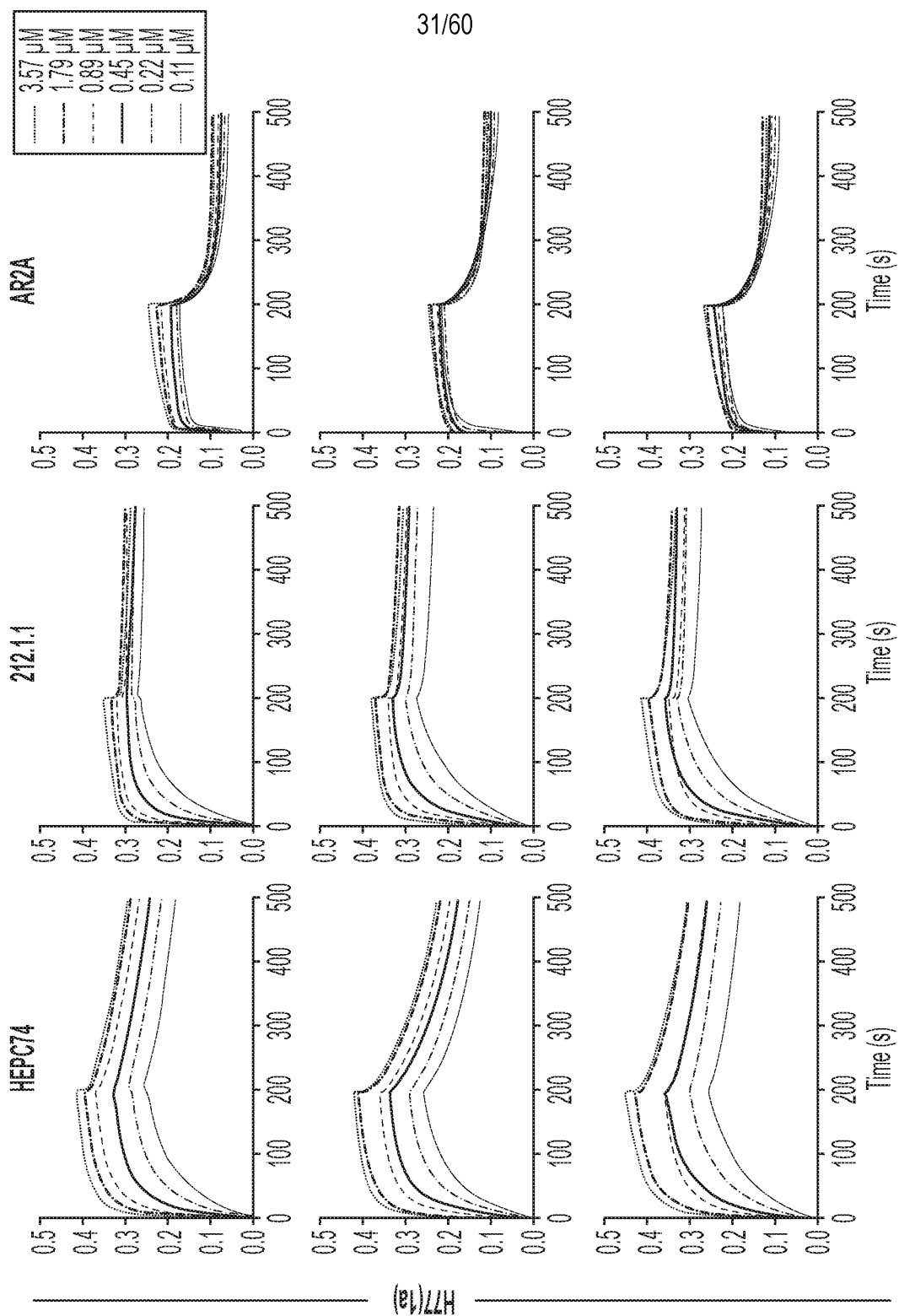


FIG. 7H (Continued)

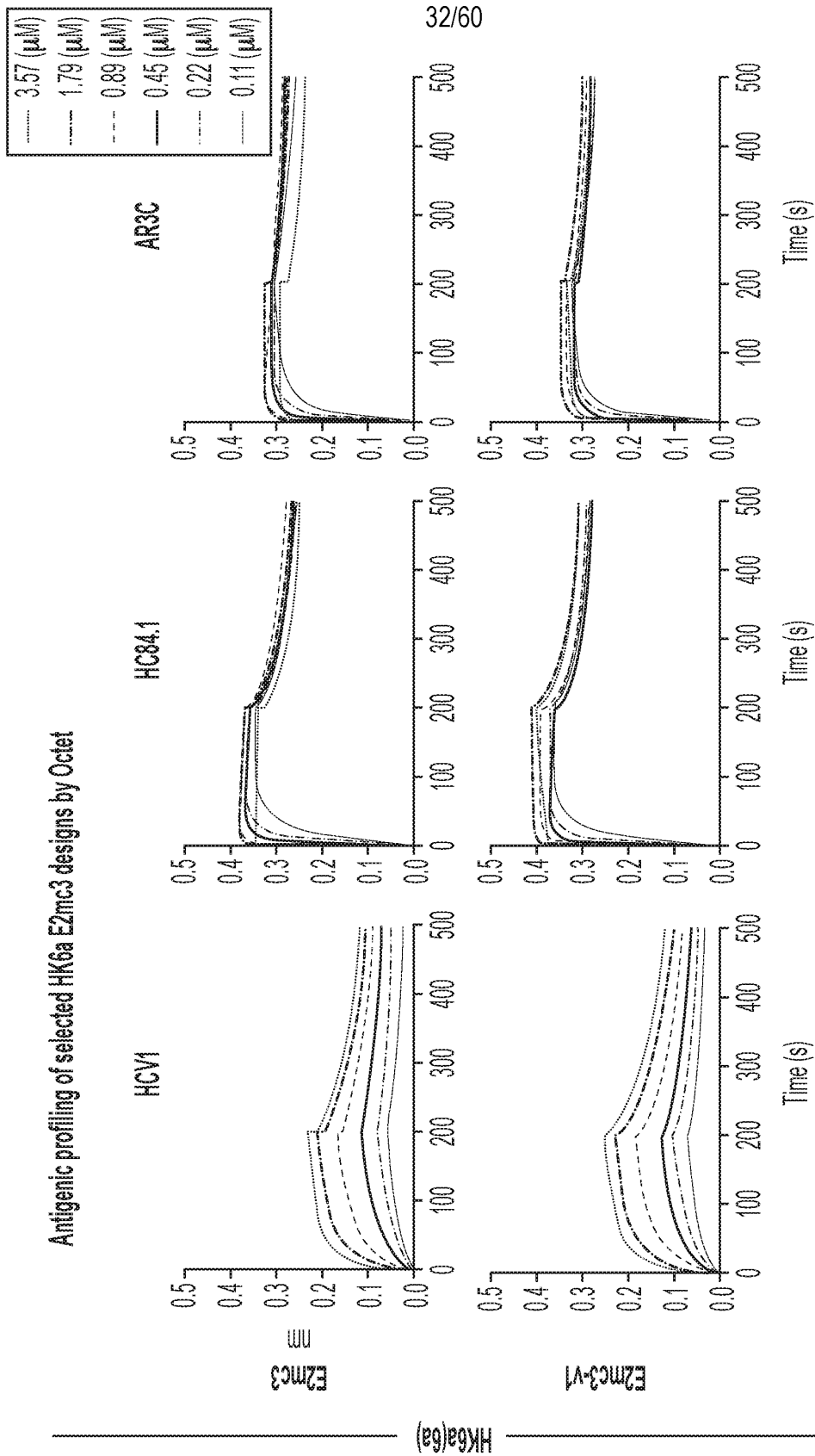


FIG. 71

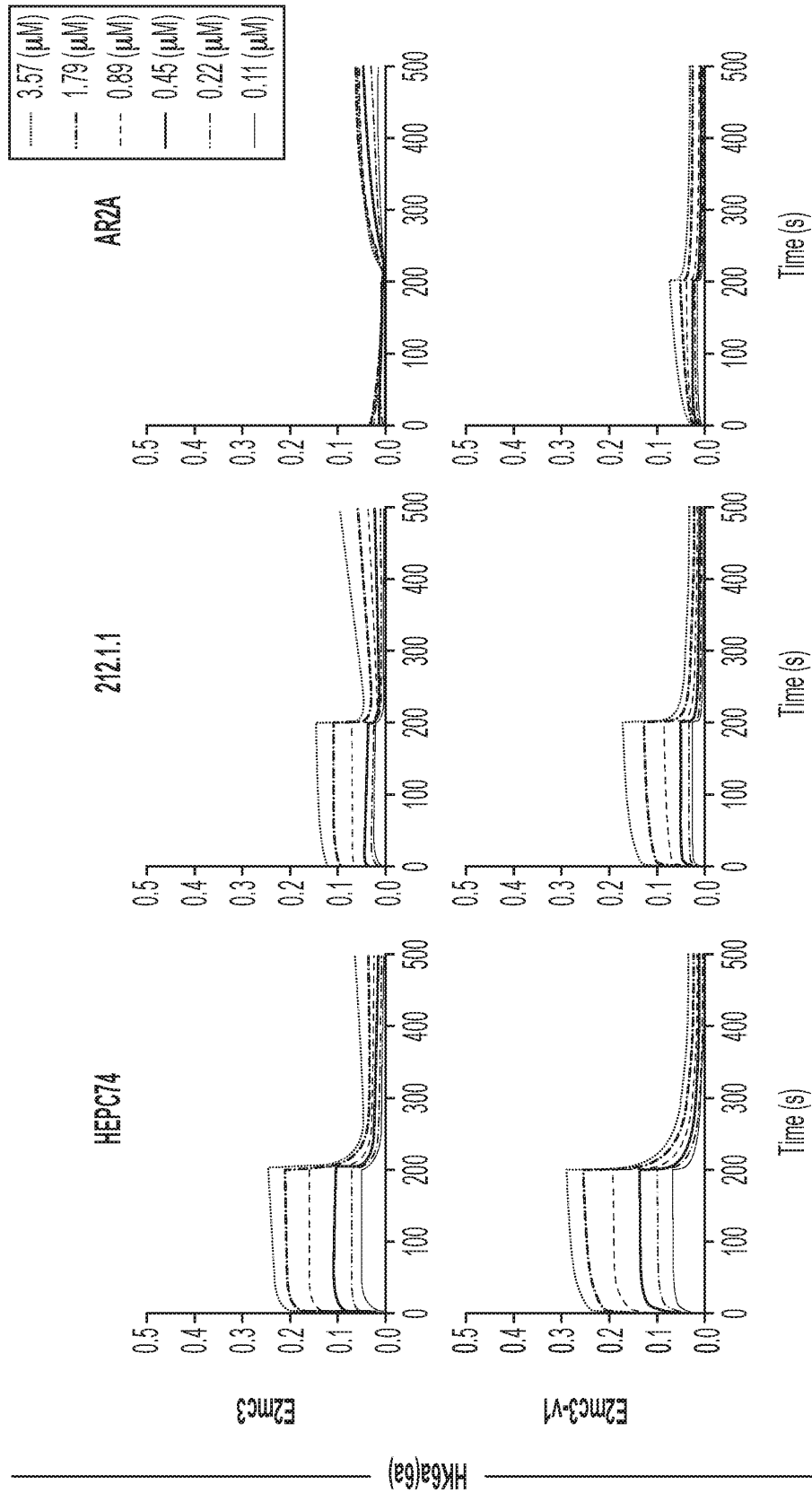


FIG. 71 (Continued)

K<sub>D</sub> values of E2mc3 variants binding to six representative HCV-specific antibodies measured by Octeta<sup>®</sup>.

|      | HCV1     |          |          | HC84.1   |          |          | AR3C     |          |          | HEPC74   |          |          | 212.1.1  |          |          | AR2A   |        |        |
|------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|--------|--------|--------|
|      | Ave[6]   | Ave[3]   | Ave[6]   | Ave[6]   | Ave[3]   | Ave[6]   | Ave[6]   | Ave[3]   | Ave[6]   | Ave[6]   | Ave[3]   | Ave[6]   | Ave[6]   | Ave[3]   | Ave[6]   | Ave[6] | Ave[3] | Ave[6] |
| H77  | E2mc3    | 5.96E-07 | 6.21E-07 | 1.00E-09 | 1.45E-09 | 1.20E-08 | 1.26E-08 | 1.26E-08 | 1.26E-08 | 1.20E-08 | 1.20E-08 | 2.27E-09 | 3.04E-09 | 9.74E-09 | 6.61E-09 |        |        |        |
|      | E2mc3-v1 | 5.19E-08 | 5.16E-08 | 2.24E-09 | 2.37E-09 | 1.73E-08 | 1.93E-08 | 2.55E-08 | 2.55E-08 | 2.72E-08 | 5.28E-09 | 6.06E-09 | 8.76E-09 | 6.17E-09 |          |        |        |        |
|      | E2mc3-v6 | 4.60E-08 | 4.83E-08 | 4.97E-09 | 4.91E-09 | 1.95E-08 | 2.23E-08 | 1.84E-08 | 2.21E-08 | 4.27E-09 | 5.24E-09 | 9.47E-09 | 5.92E-09 |          |          |        |        |        |
| HK6a | E2mc3    | 6.99E-08 | 7.10E-08 | 2.85E-09 | 2.98E-09 | 1.67E-09 | 2.06E-09 | 1.19E-07 | 1.29E-07 | 1.16E-08 | 6.11E-09 |          |          |          |          |        |        |        |
|      | E2mc3-v1 | 8.75E-08 | 8.38E-08 | 2.68E-09 | 2.61E-09 | 1.60E-09 | 1.98E-09 | 1.20E-07 | 1.26E-07 | 5.78E-08 | 8.28E-08 | 5.18E-08 | 2.62E-08 |          |          |        |        |        |

FIG. 7J

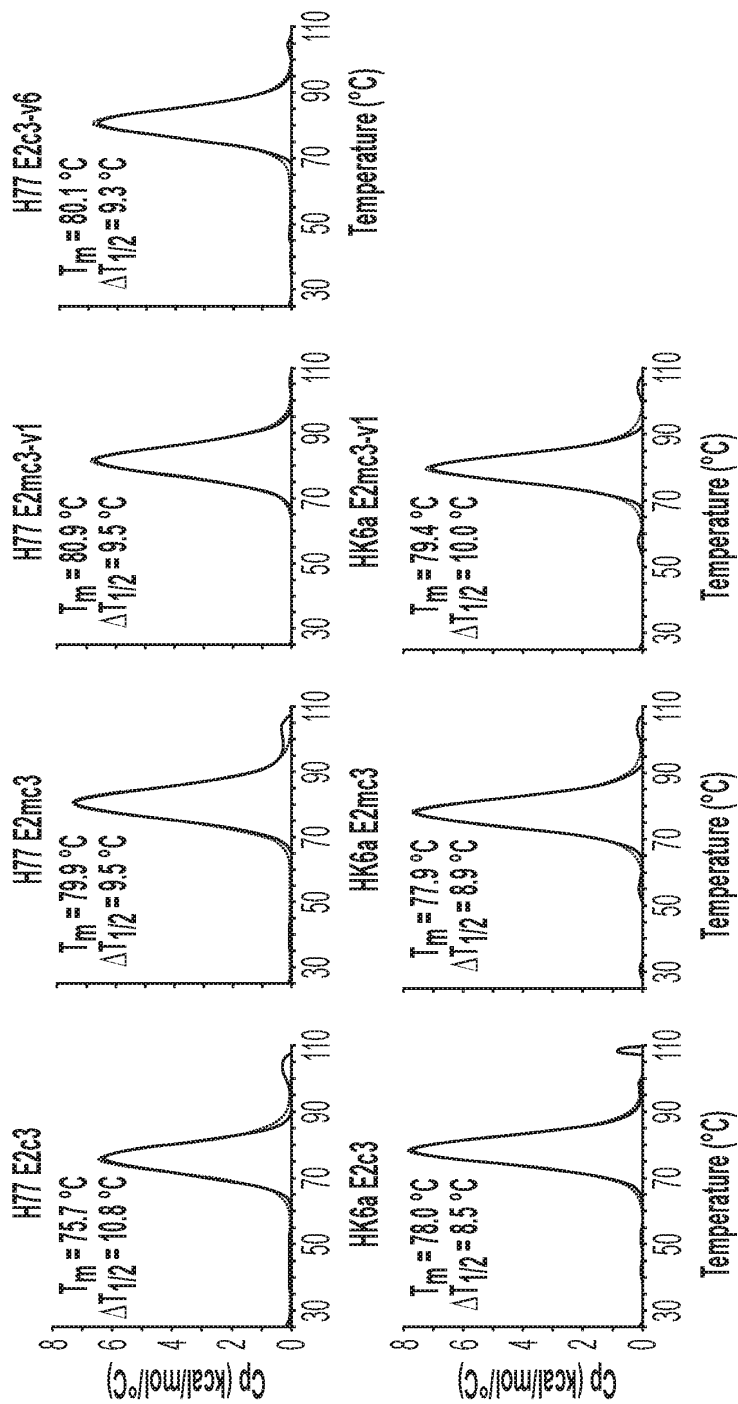
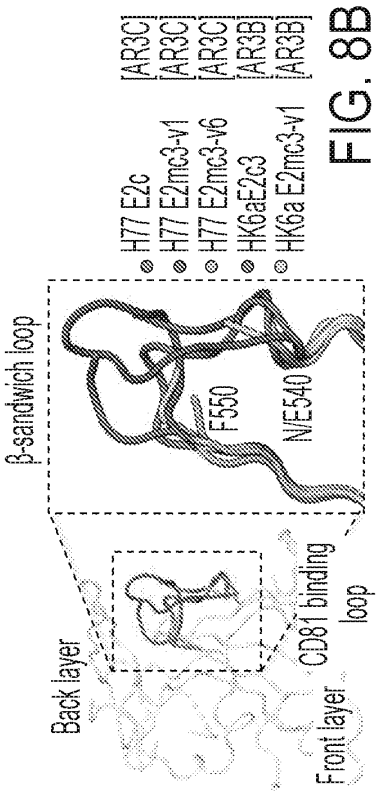
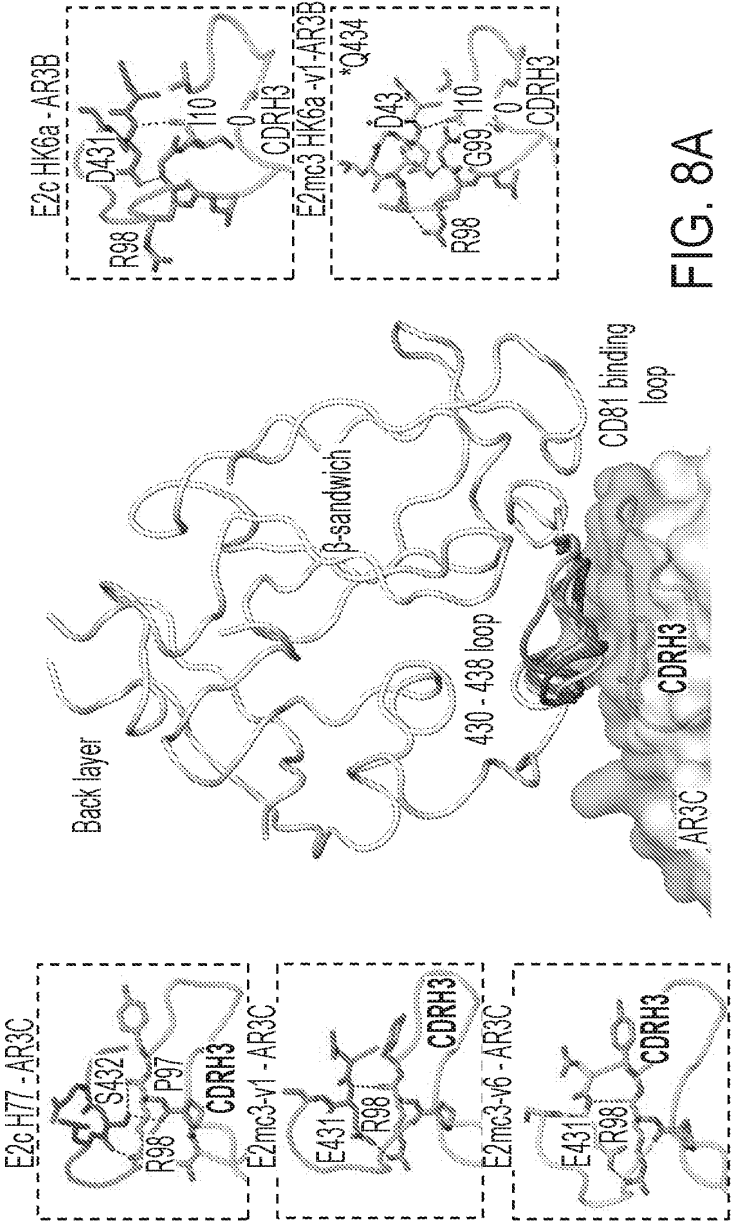


FIG. 7K



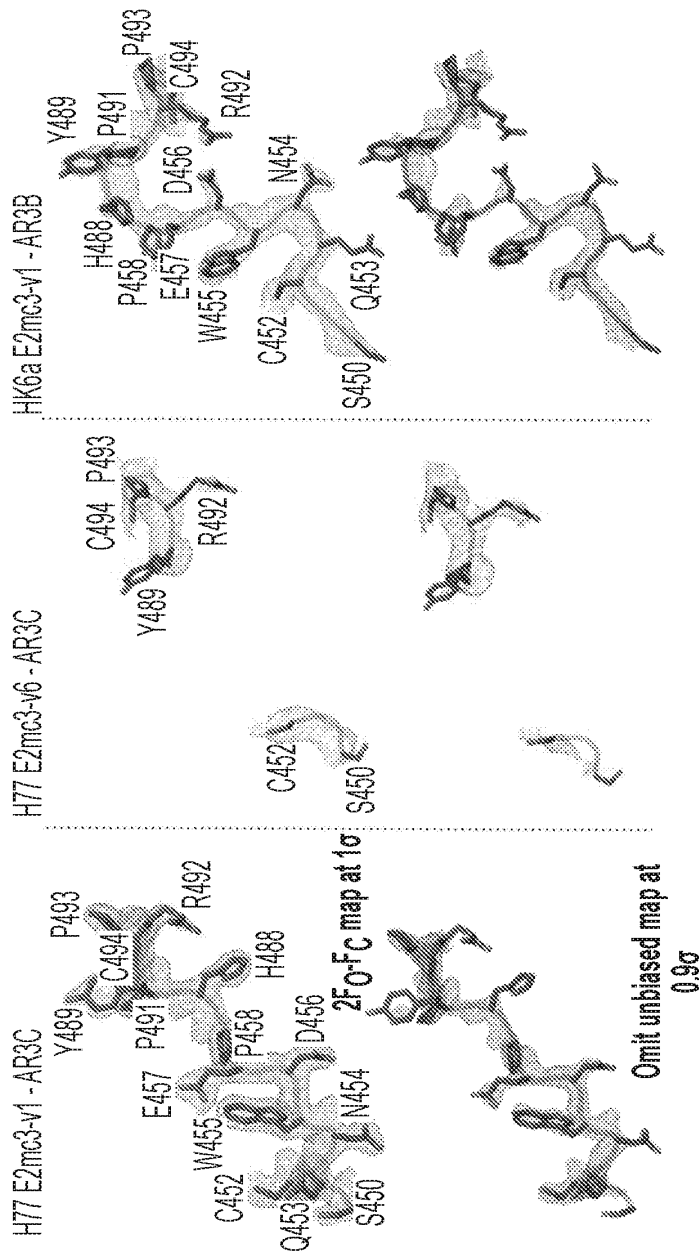


FIG. 9A

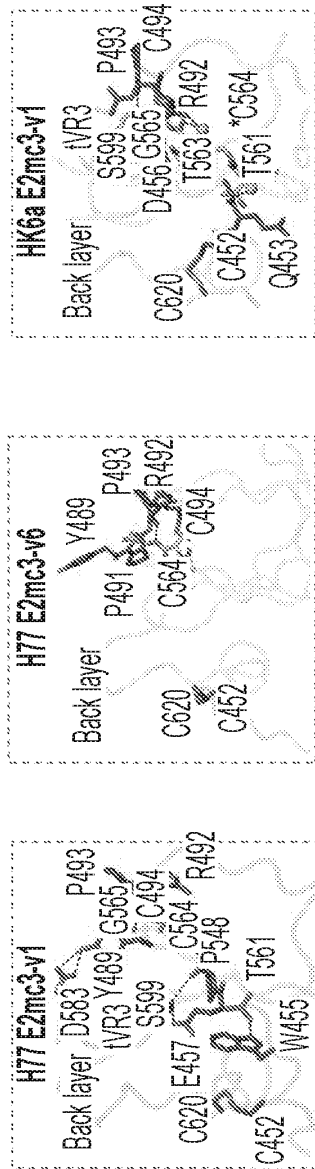


FIG. 9B

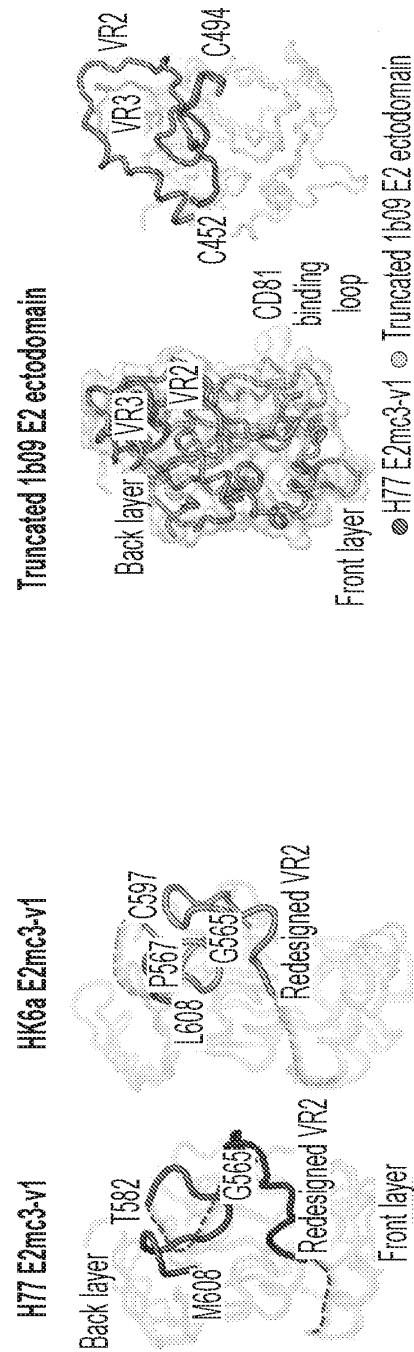
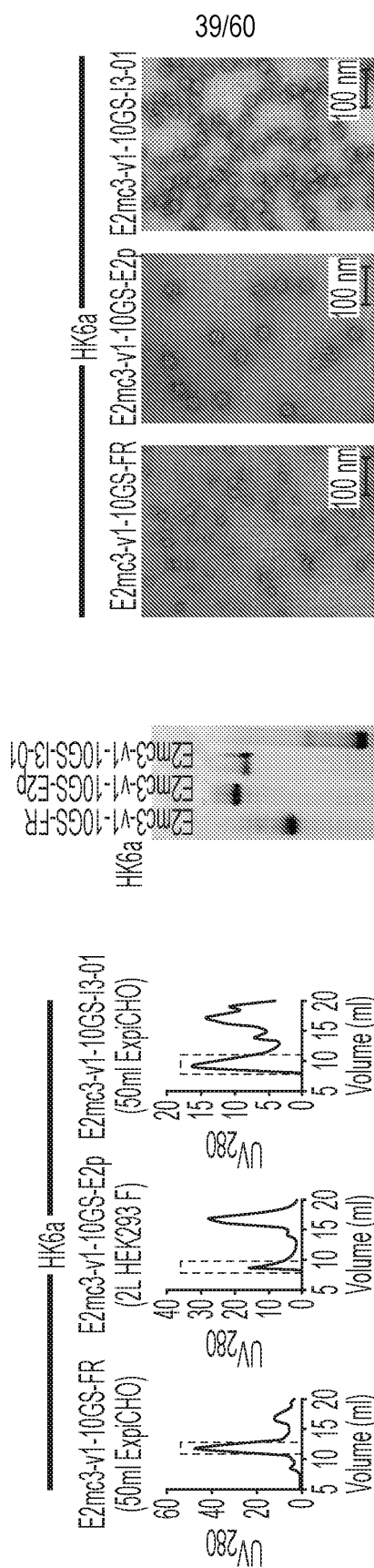


FIG. 9C

FIG. 9D



109.54

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FIG. 10A

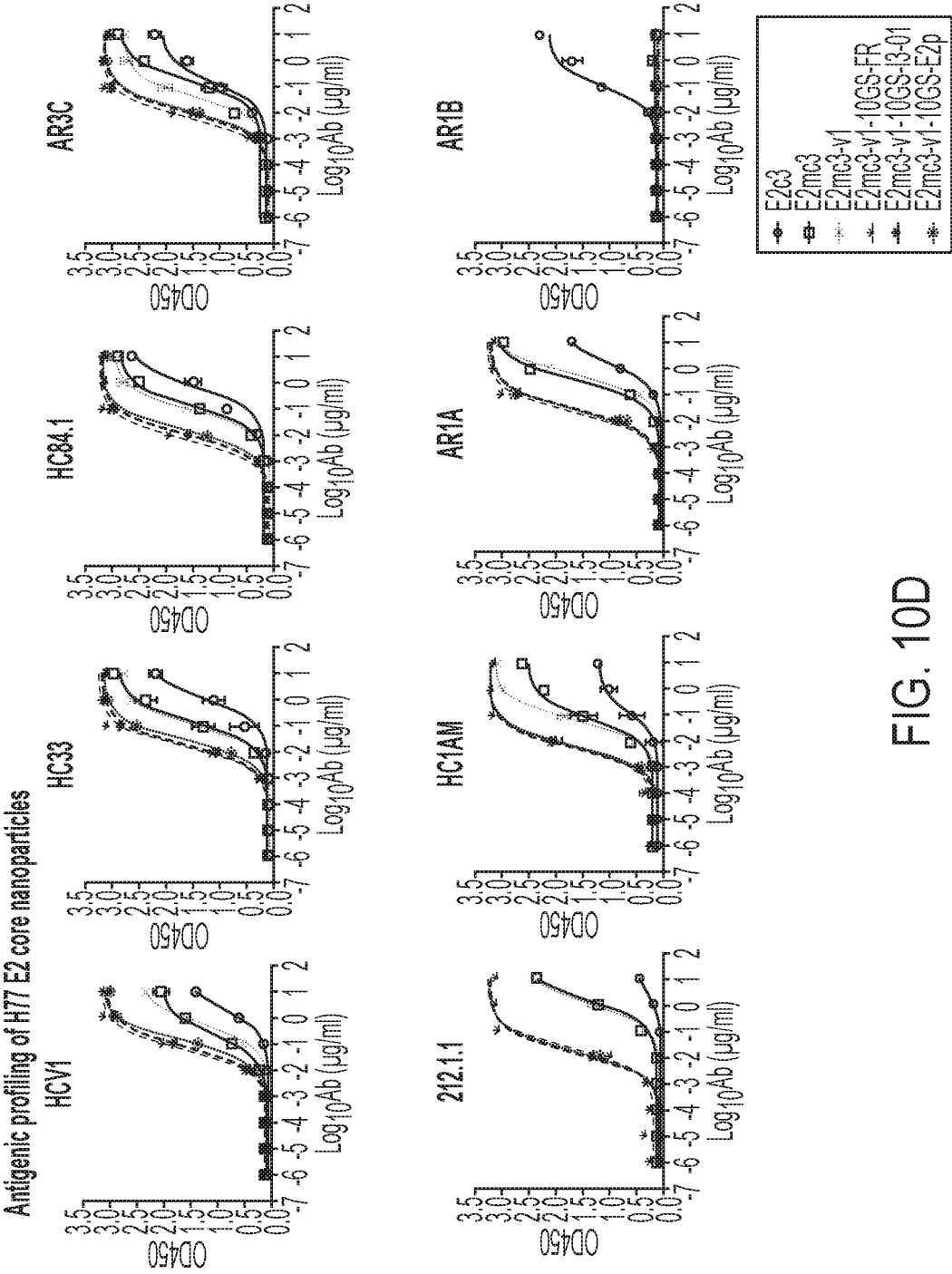


FIG. 10D

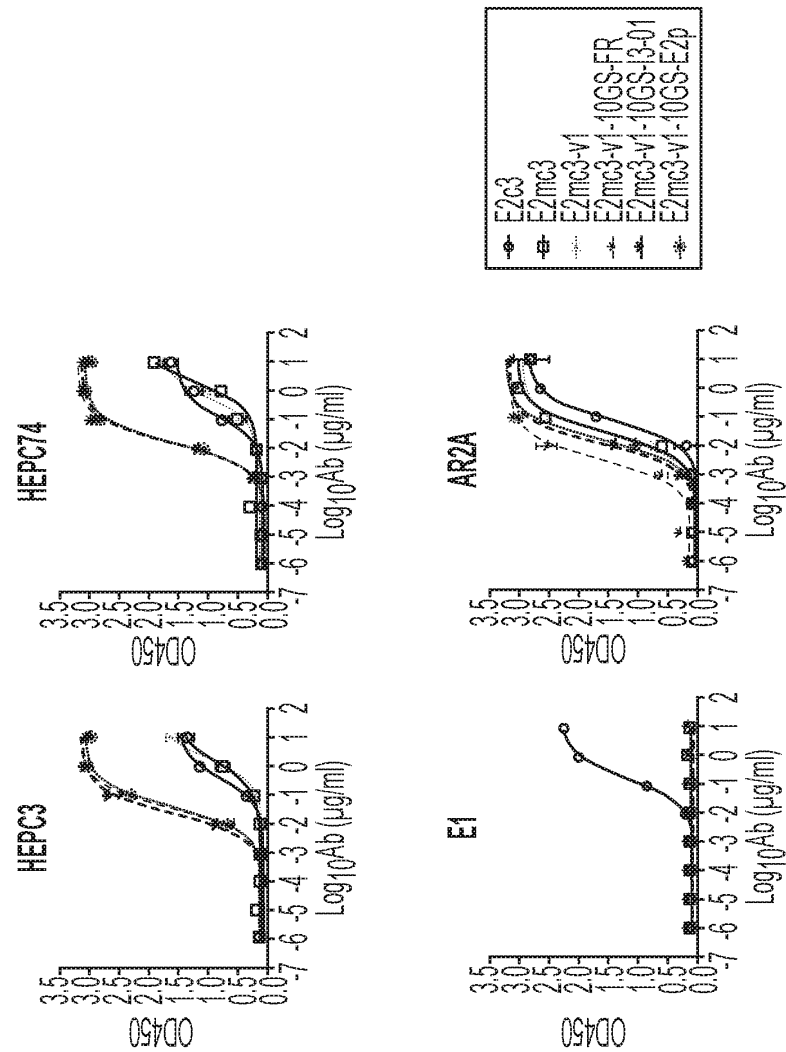


FIG. 10D (Continued)

EC<sub>50</sub> values of various H77 E2 core nanoparticles binding to 12 HCV-specific antibodies <sup>a</sup>.

|               | HCV1    | HC33    | HC84.1   | AR3C     | HEPC3   | HEPC74  | 212.1.1 | HC1AM    | AR1A    | AR1B   | E1     | AR2A     |
|---------------|---------|---------|----------|----------|---------|---------|---------|----------|---------|--------|--------|----------|
| E2c3          | 2.022   | 1.153   | 0.6954   | 0.1458   | 0.3926  | 0.1335  | 2.479   | 0.1379   | 1.56    | 0.1084 | 0.1799 | 0.07568  |
| E2mc3         | 0.2485  | 0.1481  | 0.1269   | 0.17     | 1.286   | 2.198   | 1.215   | 0.07587  | 0.3604  | -      | -      | 0.02879  |
| E2mc3-v1      | 0.5451  | 0.1114  | 0.08747  | 0.04025  | 1.938   | 0.6034  | 1.065   | 0.07059  | 0.5547  | -      | -      | 0.02548  |
| v1-10GS-FR    | 0.05775 | 0.01682 | 0.007175 | 0.007275 | 0.03598 | 0.01634 | 0.02125 | 0.007    | 0.0214  | -      | -      | 0.003544 |
| v1-10GS-E2p   | 0.1301  | 0.0311  | 0.01563  | 0.01266  | 0.03929 | 0.01786 | 0.01591 | 0.006578 | 0.02986 | -      | -      | 0.01755  |
| v1-10GS-I3-01 | 0.07697 | 0.01976 | 0.01088  | 0.01108  | 0.02433 | 0.01551 | 0.01348 | 0.005776 | 0.02758 | -      | -      | 0.01209  |

<sup>a</sup>EC<sub>50</sub> values were calculated from the besting fitting in Prism.

FIG. 10E

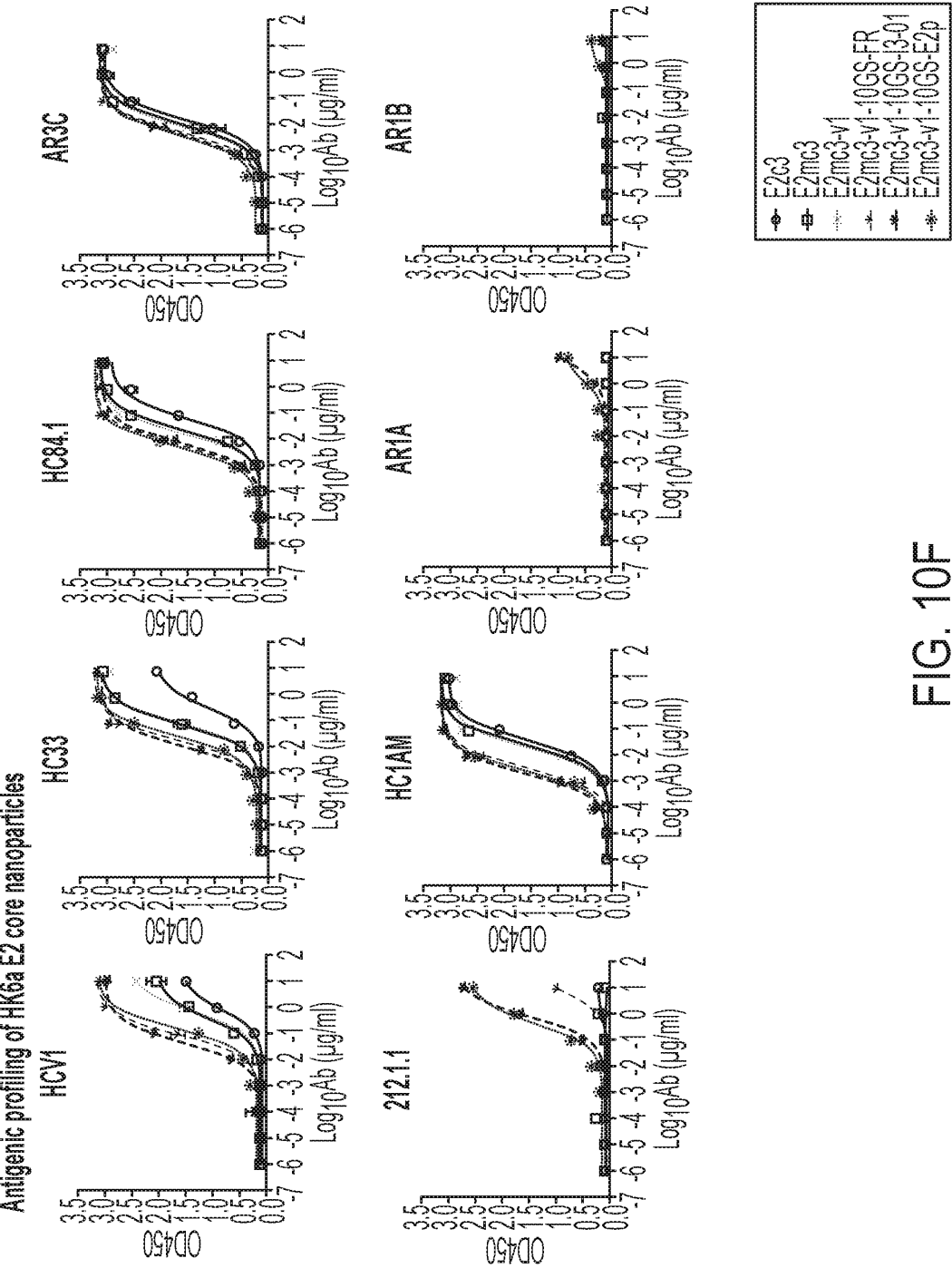


FIG. 10F

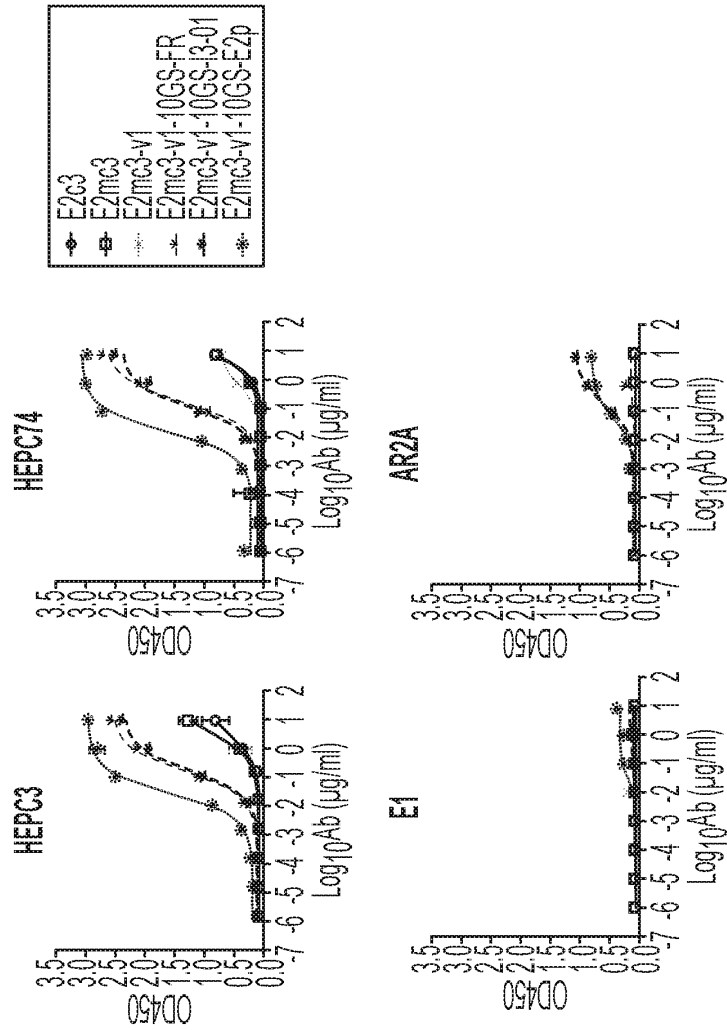


FIG. 10F (Continued)

EC<sub>50</sub> values of various HK6a E2 core nanoparticles binding to 12 HCV-specific antibodies <sup>a</sup>.

|               | HCV1    | HC33    | HC84.1   | AR3C     | HEPC3   | HEPC74 | 212.1.1 | HC1AM    | AR1A  | AR1B | E1 | AR2A    |
|---------------|---------|---------|----------|----------|---------|--------|---------|----------|-------|------|----|---------|
| E2c3          | 0.8293  | 0.4099  | 0.08365  | 0.02219  | 2.637   | 15.88  | -       | 0.0418   | -     | -    | -  | -       |
| E2mc3         | 0.3883  | 0.1034  | 0.03069  | 0.01498  | 4.767   | 12.29  | -       | 0.02714  | -     | -    | -  | -       |
| E2mc3-v1      | 0.7244  | 0.1054  | 0.02383  | 0.01109  | 2.938   | 0.771  | -       | 0.02011  | -     | -    | -  | -       |
| v1-10GS-FR    | 0.0923  | 0.02377 | 0.007617 | 0.007139 | 0.1703  | 0.2297 | 6.501   | 0.003927 | -     | -    | -  | -       |
| v1-10GS-E2p   | 0.1586  | 0.03368 | 0.006394 | 0.005718 | 0.02632 | 0.0206 | 0.4494  | 0.003214 | 1.405 | -    | -  | 0.07849 |
| v1-10GS-I3-01 | 0.04669 | 0.01564 | 0.008569 | 0.005155 | 0.1617  | 0.1534 | 0.7927  | 0.002361 | 6.271 | -    | -  | 0.1968  |

<sup>a</sup>EC<sub>50</sub> values were calculated from the besting fitting in Prism.

FIG. 10G

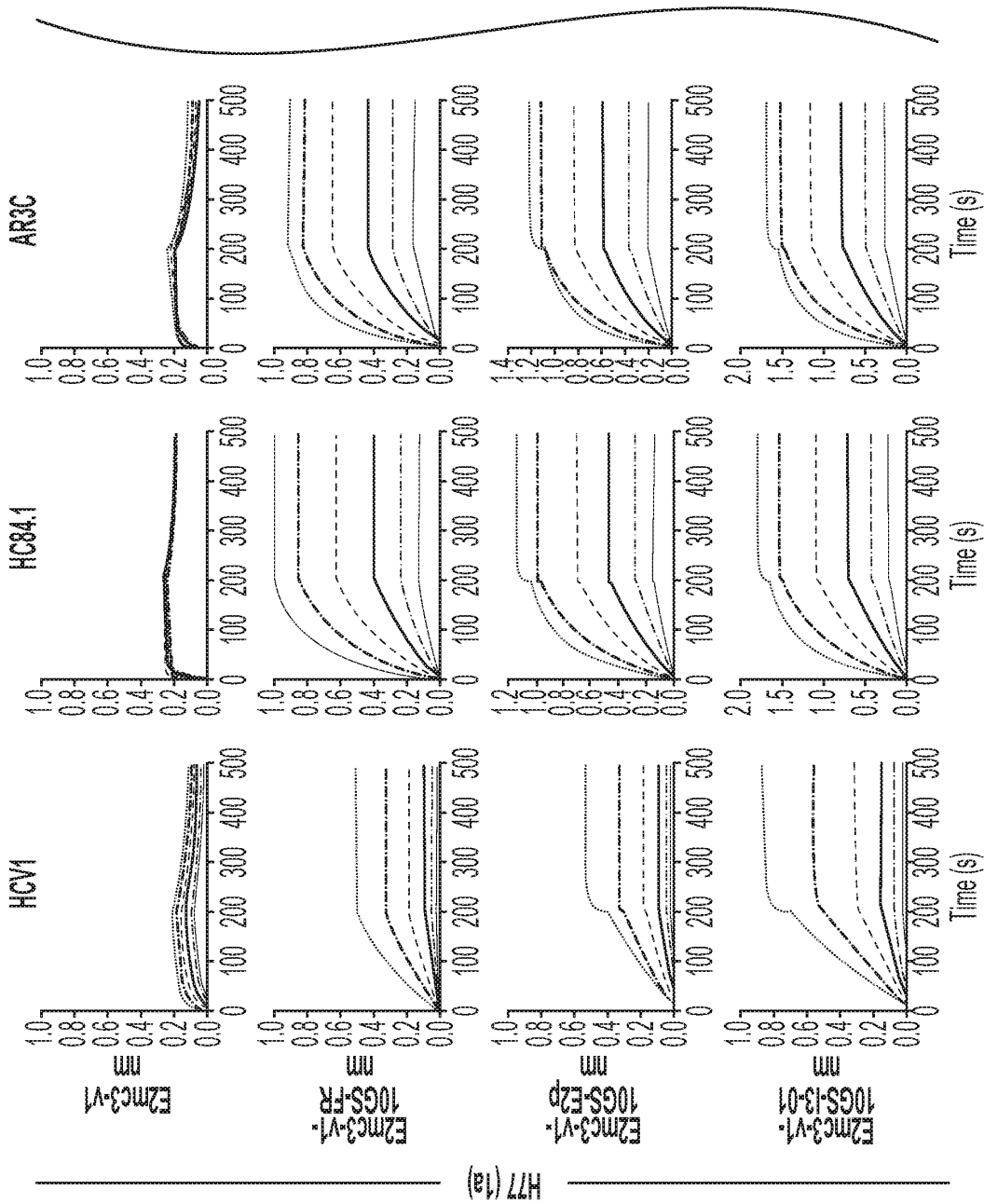


FIG. 10H

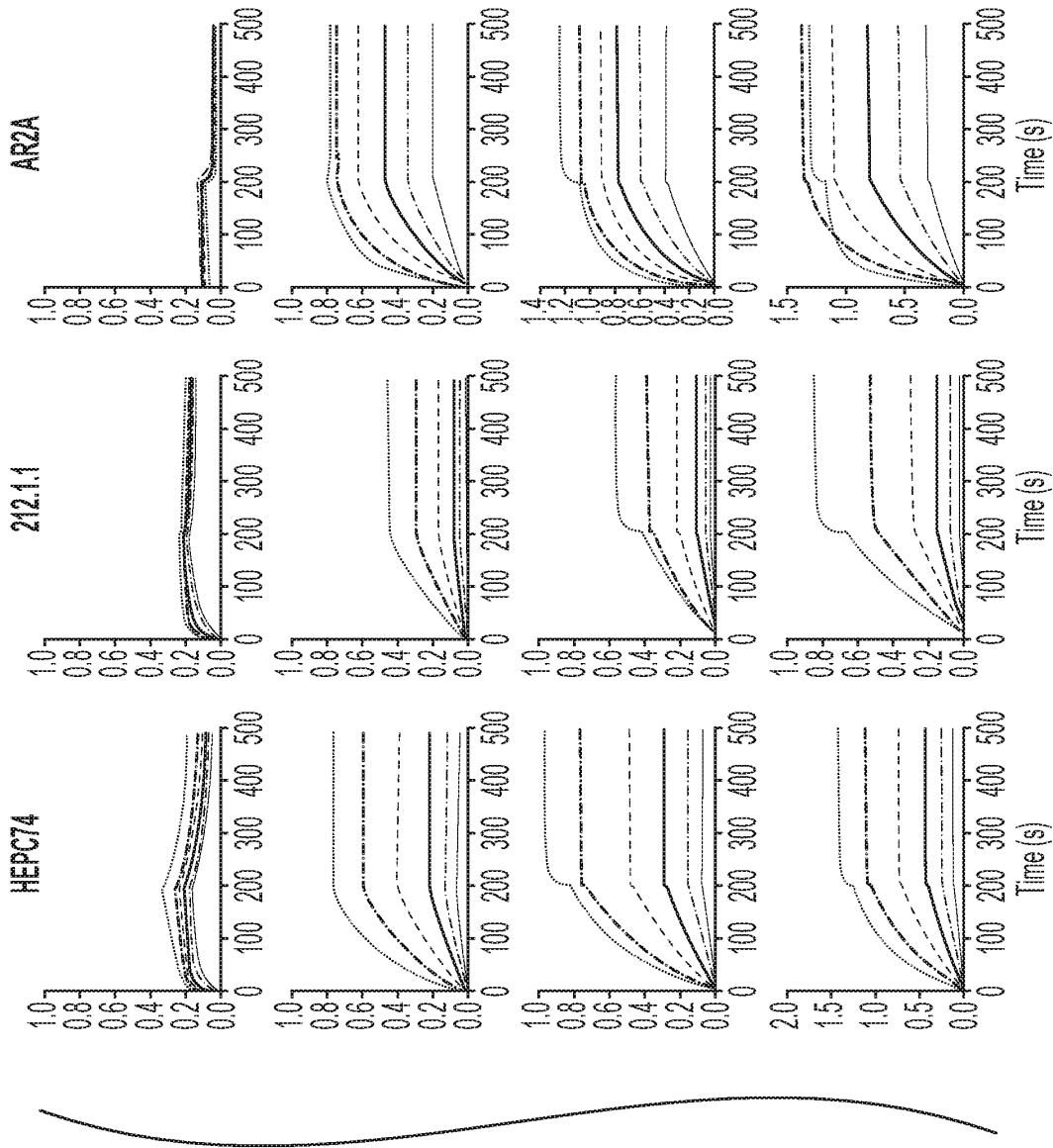


FIG. 10H (Continued)

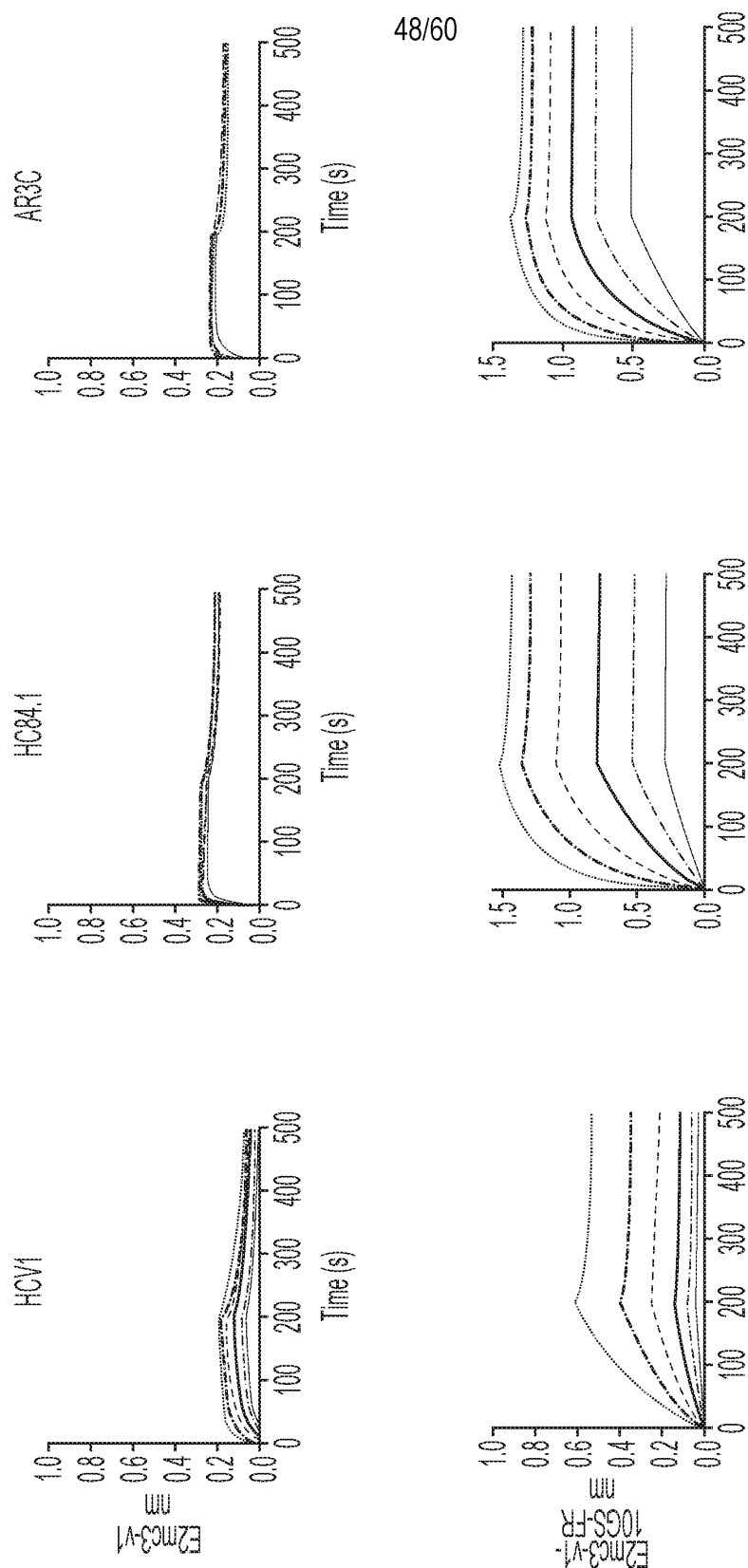


FIG. 10

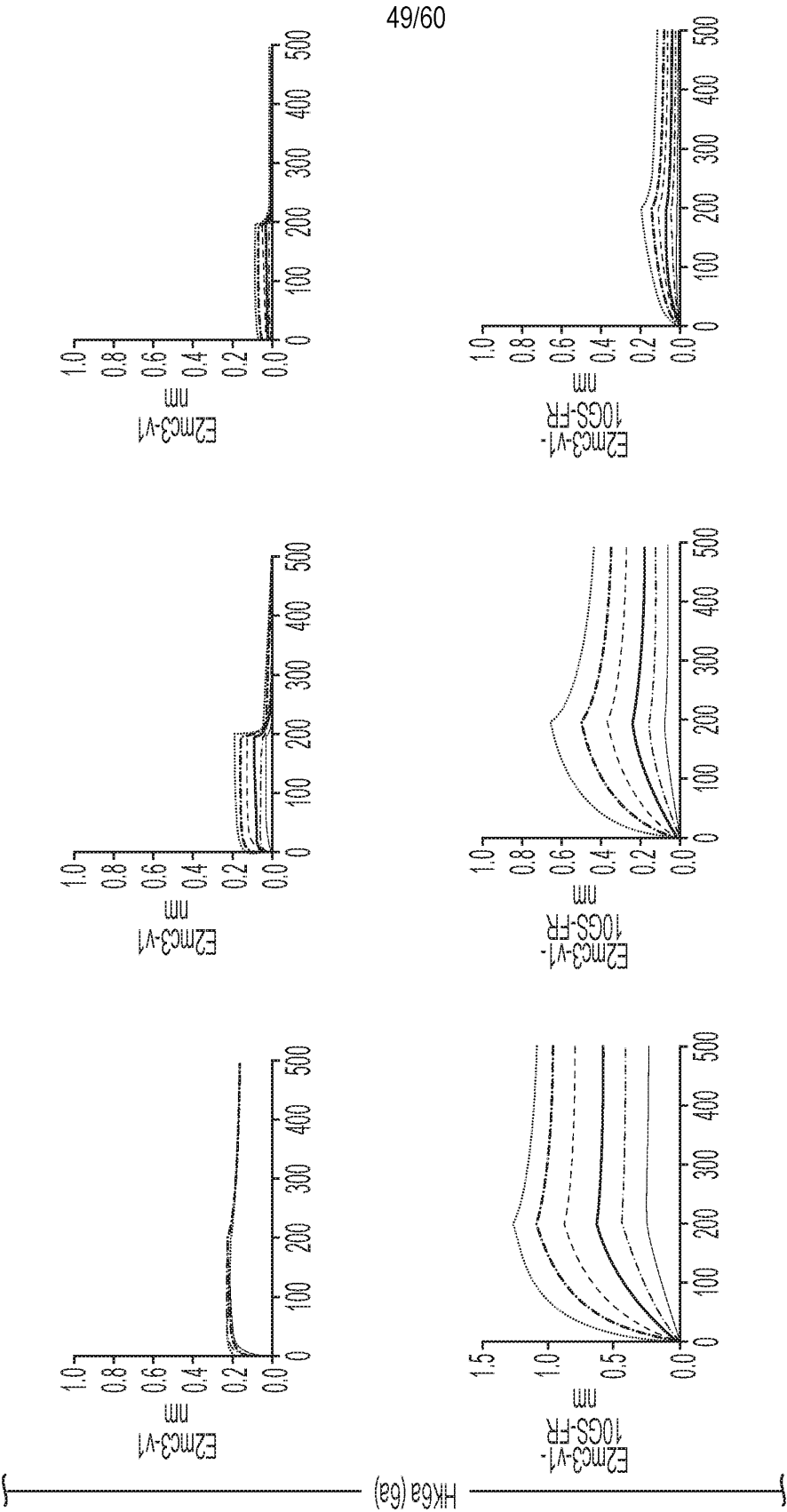


FIG. 10I (Continued)

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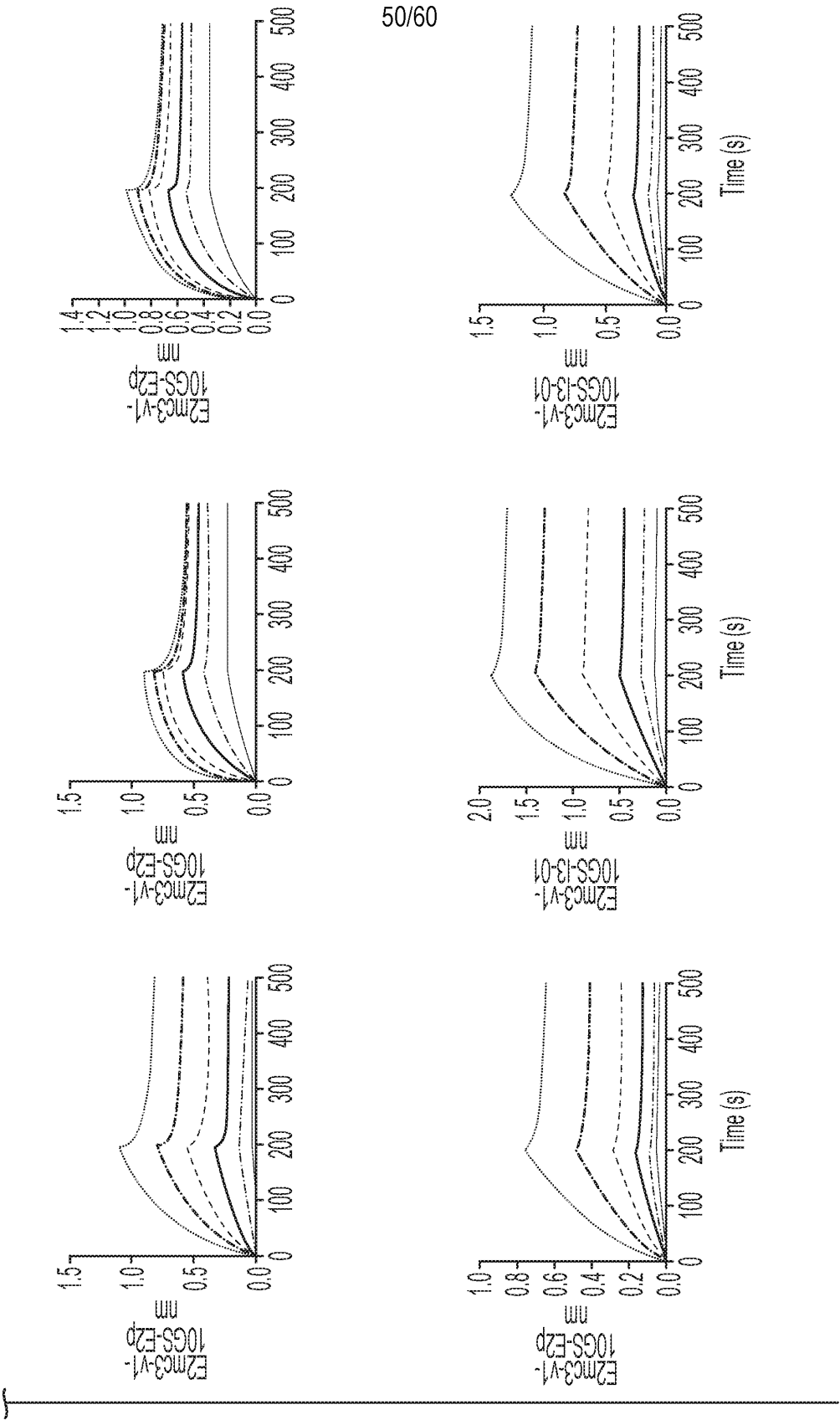
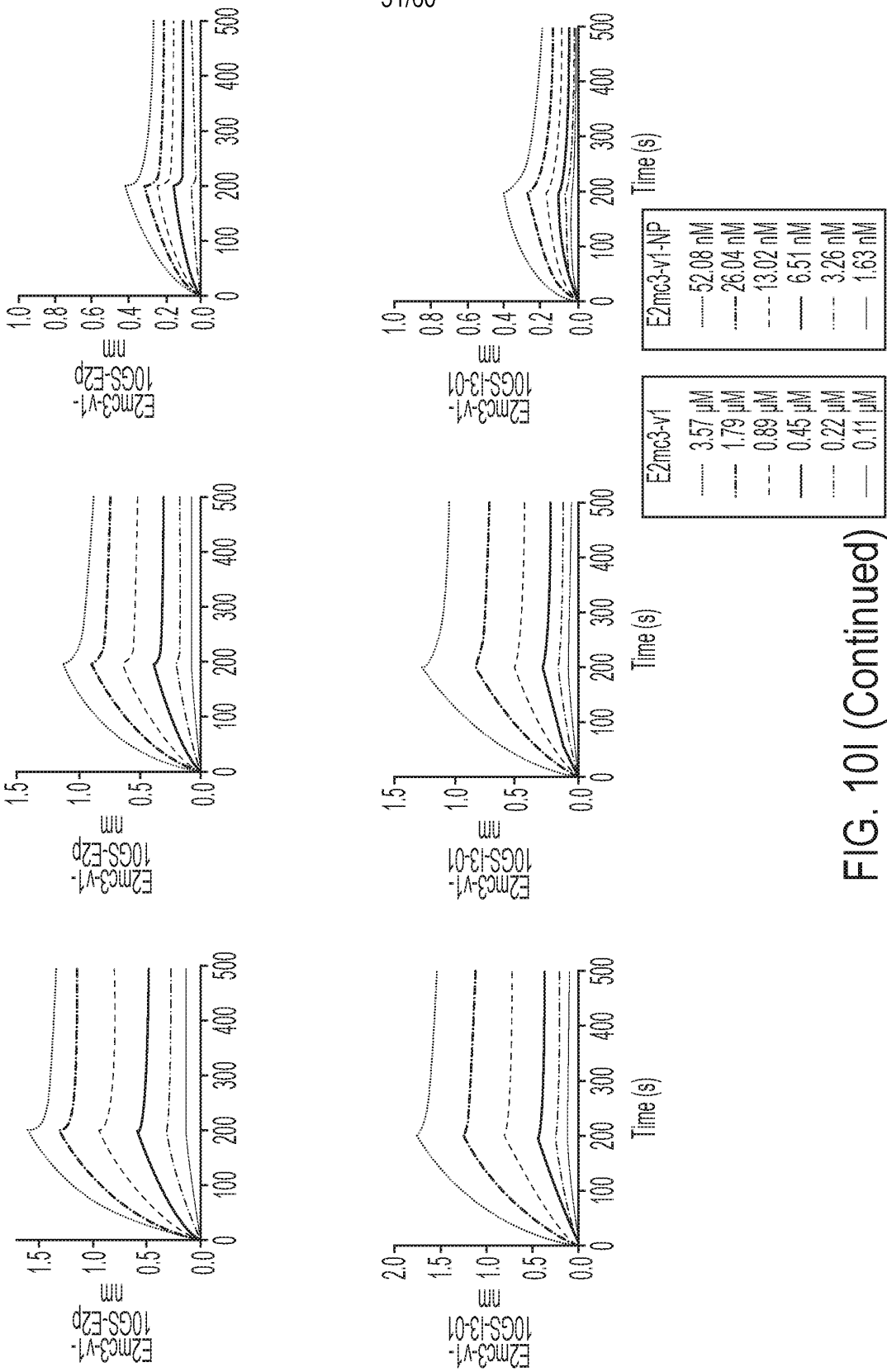


FIG. 10I (Continued)



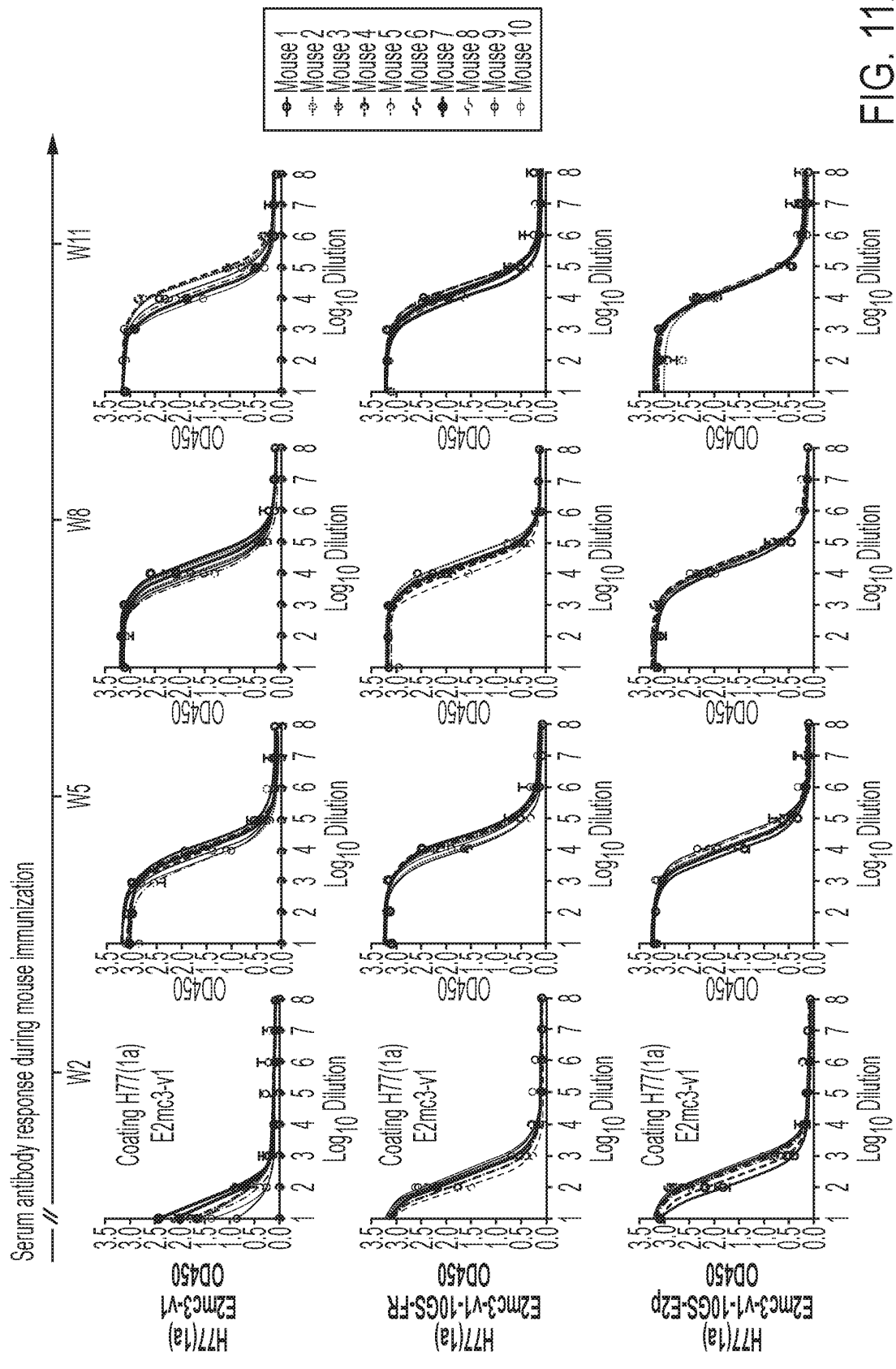


FIG. 11A

EC<sub>50</sub> values of mouse sera from study #1 binding o H77 E2mc3-v1<sup>a</sup>

|                 | W2       |                 |                  | W5       |                 |                  | W8       |                 |                  | W11      |                 |                  |
|-----------------|----------|-----------------|------------------|----------|-----------------|------------------|----------|-----------------|------------------|----------|-----------------|------------------|
|                 | E2mc3-v1 | E2mc3-v1-<br>FR | E2mc3-v1-<br>E2p | E2mc3-v1 | E2mc3-v1-<br>FR | E2mc3-v1-<br>E2p | E2mc3-v1 | E2mc3-v1-<br>FR | E2mc3-v1-<br>E2p | E2mc3-v1 | E2mc3-v1-<br>FR | E2mc3-v1-<br>E2p |
| M1              | 45.65    | 187.5           | 111.4            | 16664    | 26151           | 7611             | 16656    | 20693           | 15734            | 26922    | 17649           | 17777            |
| M2              | 35.33    | 120.5           | 253.5            | 11702    | 13274           | 10329            | 24286    | 19668           | 25039            | 41201    | 14666           | 17494            |
| M3              | 27.42    | 228.7           | 421.2            | 7427     | 22099           | 14695            | 12729    | 33686           | 24650            | 25983    | 19679           | 17012            |
| M4 <sup>b</sup> | -        | 133.5           | 379.6            | -        | 25538           | 11473            | -        | 17101           | 21928            | -        | 27042           | 16985            |
| M5              | 13.41    | 124.7           | 298.8            | 5242     | 20640           | 18250            | 6957     | 35842           | 26595            | 20309    | 16396           | 23639            |
| M6              | 38.96    | 196.4           | 199              | 12123    | 29183           | 12959            | 19740    | 22697           | 27719            | 54269    | 18537           | 23137            |
| M7              | 32.78    | 121.4           | 438.6            | 10134    | 20558           | 14652            | 13000    | 14444           | 23248            | 13421    | 10252           | 22005            |
| M8              | 48.96    | 74.5            | 403.9            | 10186    | 9582            | 12977            | 31134    | 9176            | 26215            | 28858    | 10330           | 20674            |
| M9              | 8.99     | 258.1           | 477.4            | 6559     | 10058           | 22251            | 9314     | 18901           | 19345            | 24221    | 15651           | 16983            |
| M10             | 8.097    | 315.6           | 515.7            | 4895     | 16103           | 12300            | 10764    | 21685           | 18924            | 9493     | 17209           | 20705            |

<sup>a</sup> In study #1, mice were injected with H77 E2mc3-v1 and three nanoparticles. The EC<sub>50</sub> values were calculated from the besting fitting in GraphPad Prism 6.

<sup>b</sup> M4 in the E2mc3-v1 group died shortly after the first injection, as indicated by "-".

FIG. 11B

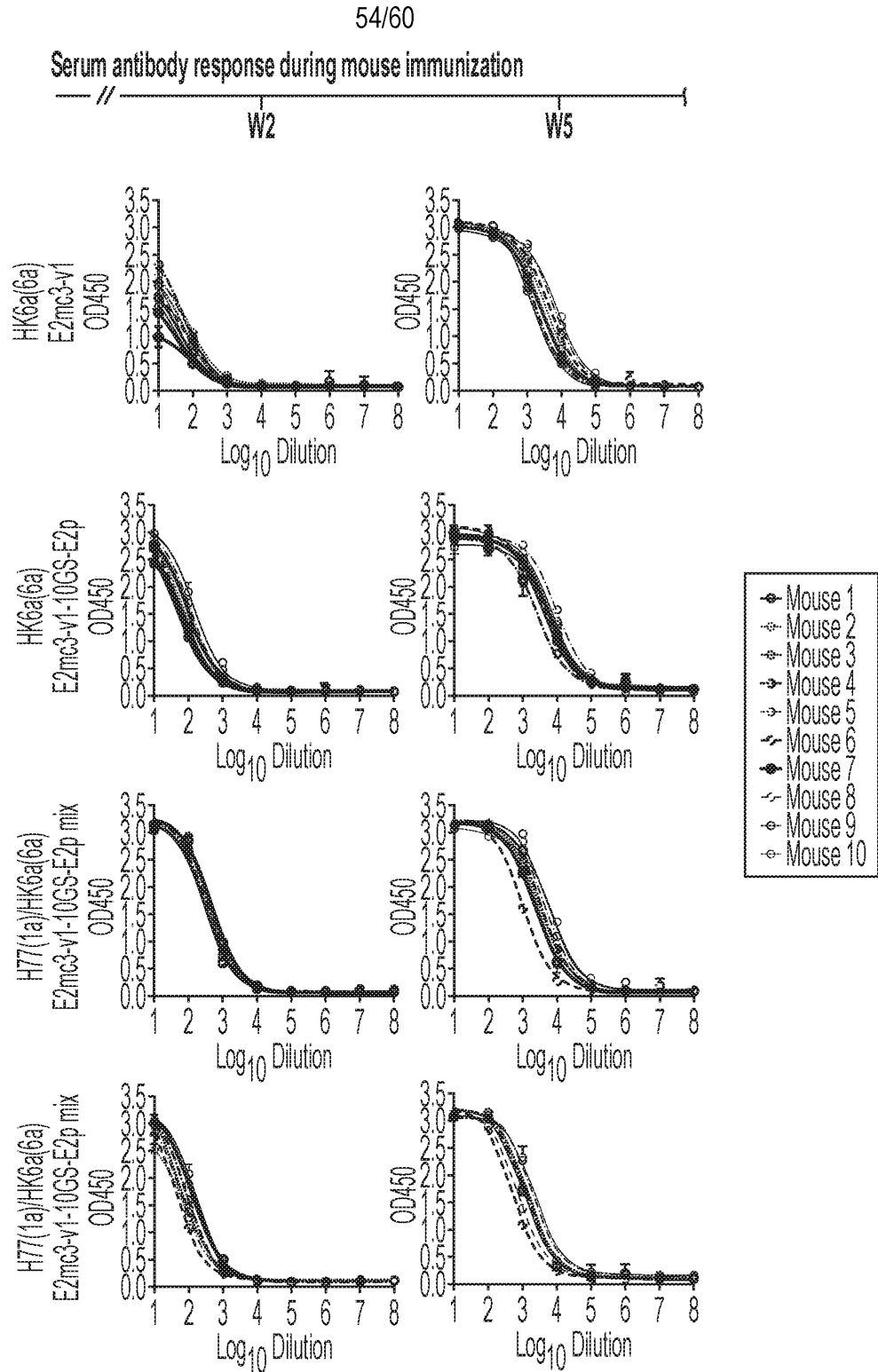


FIG. 11C

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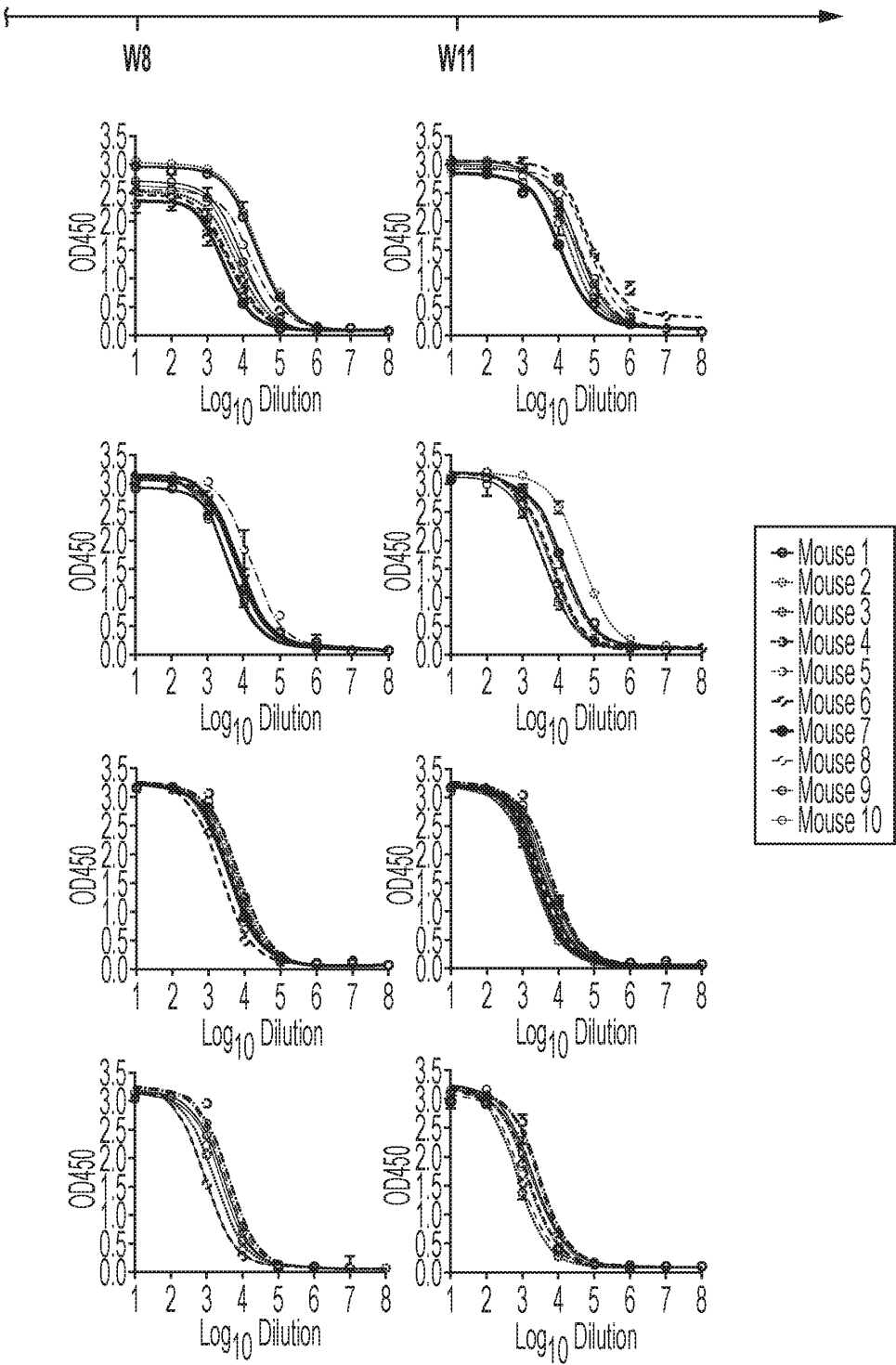


FIG. 11C (Continued)

EC<sub>50</sub> values of mouse sera from study #2 binding o H77 or HK6a E2mc3-v1<sup>a</sup>

|                 | W2            |               |                 |                  | W5            |               |                 |                  | W8            |               |                 |                  | W11           |               |                 |                  |
|-----------------|---------------|---------------|-----------------|------------------|---------------|---------------|-----------------|------------------|---------------|---------------|-----------------|------------------|---------------|---------------|-----------------|------------------|
|                 | E2mc3-v1/HK6a | E2p-<br>/HK6a | E2p-<br>mix/H77 | E2p-<br>mix/HK6a | E2mc3-v1/HK6a | E2p-<br>/HK6a | E2p-<br>mix/H77 | E2p-<br>mix/HK6a | E2mc3-v1/HK6a | E2p-<br>/HK6a | E2p-<br>mix/H77 | E2p-<br>mix/HK6a | E2mc3-v1/HK6a | E2p-<br>/HK6a | E2p-<br>mix/H77 | E2p-<br>mix/HK6a |
| M1              | 36.44         | 57.23         | 306.2           | 87.92            | 2536          | 4152          | 3007            | 1177             | 23668         | 5399          | 4670            | 2171             | 27580         | 13634         | 3734            | 1375             |
| M2              | 91.76         | 85.23         | 332.7           | 78.19            | 3859          | 7285          | 3007            | 1483             | 26324         | 6025          | 4616            | 2034             | 77268         | 44791         | 4154            | 1108             |
| M3              | 51.32         | 51.48         | 399.2           | 62.26            | 2864          | 6936          | 2352            | 1128             | 5734          | 6315          | 3498            | 1668             | 18754         | 5244          | 2068            | 719.9            |
| M4 <sup>b</sup> | 53.05         | 65.53         | 359.2           | 81.48            | 1675          | 2658          | 3169            | 1508             | 4243          | 6359          | 6933            | 4249             | 19549         | 3916          | 6201            | 3204             |
| M5              | 60.35         | 116.9         | 419.5           | 53.01            | 6542          | 10938         | 4165            | 2108             | 15942         | 14805         | 6033            | 3367             | 40012         | 9942          | 3898            | 2609             |
| M6              | 67.18         | 52.19         | 340.6           | 38.93            | 3359          | 2649          | 924             | 514.4            | 4006          | 7421          | 2276            | 886              | 73413         | 6369          | 2238            | 743              |
| M7              | 96.09         | 52.14         | 358.9           | 145.9            | 3193          | 4062          | 2111            | 767.9            | 2660          | 6536          | 4169            | 2153             | 11387         | 5148          | 2616            | 1280             |
| M8              | 31.99         | 99.93         | 482.1           | 150.3            | 4293          | 6946          | 3802            | 1447             | 6461          | 6138          | 4654            | 2050             | 74357         | 5673          | 3055            | 881.3            |
| M9              | 35.05         | 156.6         | 318.9           | 160.7            | 1430          | 5808          | 5347            | 2225             | 8582          | 3526          | 6081            | 2866             | 39734         | 3810          | 5125            | 2255             |
| M10             | 31.36         | 137.7         | 304.2           | 120.1            | 7300          | 5819          | 5406            | 2204             | 7968          | 4166          | 5594            | 2682             | 27205         | 3709          | 5003            | 2141             |

<sup>a</sup> In study #2, three groups of mice were injected with HK6a E2mc3-v1, HK6a E2mc3-v1-10GS-E2p, and HK6a/H77 E2mc3-v1-10GS-E2p mix. In serum analysis, the coating antigen was HK6a E2mc3-v1 for group 1 and group 2, while both H77 E2mc3-v1 and HK6a E2mc3-v1 were used as coating antigen for group 3 and analyzed in two separate experiments. In this table, the coating antigen is shown after “”. The EC<sub>50</sub> values were calculated from the besting fitting in GraphPad Prism 6.

FIG. 11D

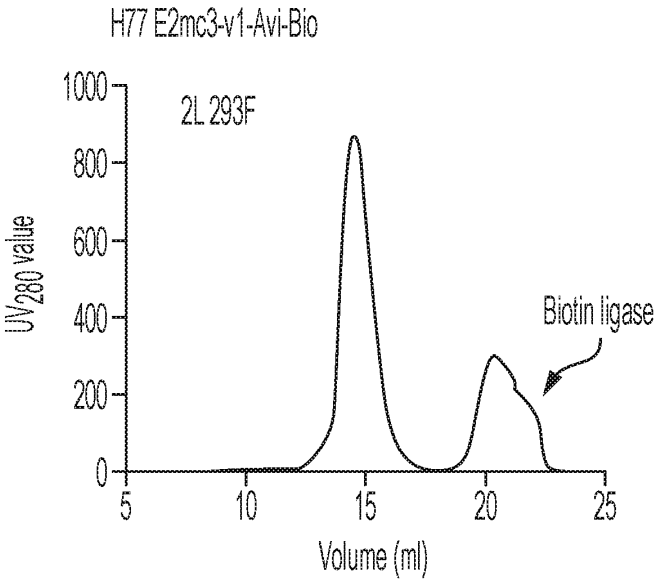


FIG. 12A

H77 E2mc3-v1-specific bulk sorting of mouse splenic B cells from study #1

| Mouse sample | Sorted cells | Frequency of E2mc3-v1-specific B cells | E2mc3-v1-specific B cells per 10 million splenic cells |
|--------------|--------------|----------------------------------------|--------------------------------------------------------|
| G1-1         | 269          | 0.02%                                  | 1771                                                   |
| G1-3         | 294          | 0.01%                                  | 769                                                    |
| G1-5         | 70           | 0.02%                                  | 2107                                                   |
| G1-6         | 383          | 0.02%                                  | 1655                                                   |
| G1-10        | 42           | 0.01%                                  | 1151                                                   |
| G3-5         | 639          | 0.02%                                  | 2065                                                   |
| G3-7         | 519          | 0.04%                                  | 3851                                                   |
| G3-8         | 296          | 0.03%                                  | 2968                                                   |
| G3-9         | 145          | 0.02%                                  | 1618                                                   |
| G3-10        | 565          | 0.03%                                  | 2962                                                   |

FIG. 12B

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Antibodyomics analysis of next-generation sequencing (NGS) data obtained for E2mc3-v1-sorted mouse splenic B cells<sup>a</sup>

| Mouse sample | N <sub>Raw</sub> | N <sub>Align</sub> | Chain | N <sub>Chain</sub> | <Length> | N <sub>Usable</sub> | Perc <sub>Usable</sub> |
|--------------|------------------|--------------------|-------|--------------------|----------|---------------------|------------------------|
| G1-1         | 754,261          | 448,586            | H     | 292,627            | 574.1    | 286,978             | 98.1%                  |
|              |                  |                    | K     | 155,959            | 515.9    | 154,780             | 99.2%                  |
| G1-3         | 786,781          | 509,031            | H     | 351,785            | 543.4    | 350,416             | 99.6%                  |
|              |                  |                    | K     | 157,246            | 518.2    | 156,928             | 99.8%                  |
| G1-5         | 995,967          | 250,954            | H     | 13,791             | 552.7    | 13,629              | 98.8%                  |
|              |                  |                    | K     | 237,163            | 508.5    | 235,538             | 99.3%                  |
| G1-6         | 830,128          | 96,295             | H     | 57,820             | 571.1    | 55,754              | 96.4%                  |
|              |                  |                    | K     | 38,475             | 555.8    | 37,189              | 96.7%                  |
| G1-10        | 826,370          | 358,931            | H     | 180,016            | 657.9    | 170,128             | 94.5%                  |
|              |                  |                    | K     | 178,915            | 505.7    | 177,145             | 99.0%                  |
| G3-5         | 2,370,390        | 757,676            | H     | 471,407            | 636.6    | 469,623             | 99.6%                  |
|              |                  |                    | K     | 286,269            | 509.2    | 285,645             | 99.8%                  |
| G3-7         | 2,310,407        | 378,836            | H     | 194,360            | 571.7    | 193,698             | 99.7%                  |
|              |                  |                    | K     | 184,476            | 498.1    | 180,878             | 98.0%                  |
| G3-8         | 1,215,653        | 414,054            | H     | 180,968            | 625.0    | 180,367             | 99.7%                  |
|              |                  |                    | K     | 233,086            | 509.9    | 232,190             | 99.6%                  |
| G3-9         | 1,788,762        | 348,596            | H     | 191,028            | 631.3    | 188,161             | 98.5%                  |
|              |                  |                    | K     | 157,568            | 506.9    | 157,069             | 99.7%                  |
| G3-10        | 971,175          | 471,436            | H     | 262,416            | 646.6    | 260,219             | 99.2%                  |
|              |                  |                    | K     | 209,020            | 524.2    | 207,506             | 99.3%                  |

<sup>a</sup> Listed items include the mouse sample ID, number of raw reads (N<sub>Raw</sub>), number of sequences after V<sub>H</sub>/V<sub>K</sub> gene assignment and removing fragments with a V-gene alignment of 250bp or shorter (N<sub>Align</sub>), Chain type (H or K), number of V<sub>H</sub>/V<sub>K</sub> chains, average read length of specific chain type, number of usable full-length antibody chains after the Antibodyomics pipeline processing (N<sub>Usable</sub>), and percentage of usable chains (Perc<sub>Usable</sub> = N<sub>Usable</sub>/N<sub>Chain</sub> × 100%). NGS was performed on Ion S5 using an Ion 530 chip.

FIG. 12C

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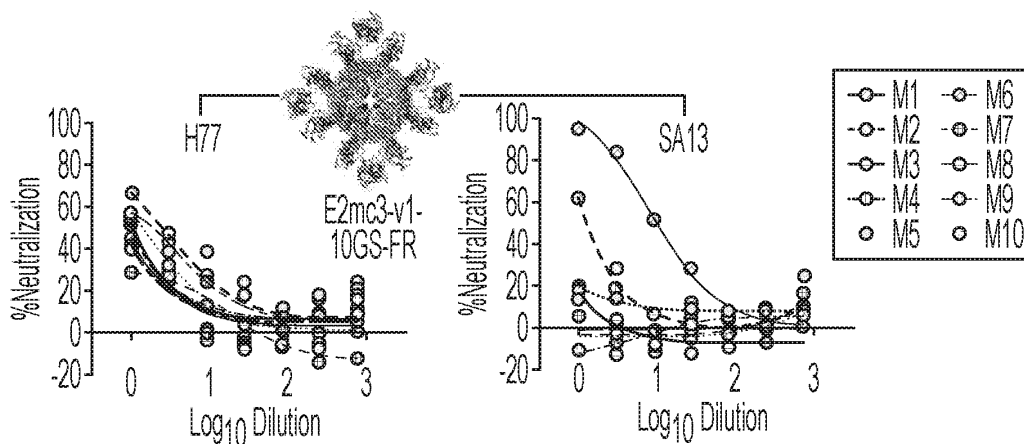
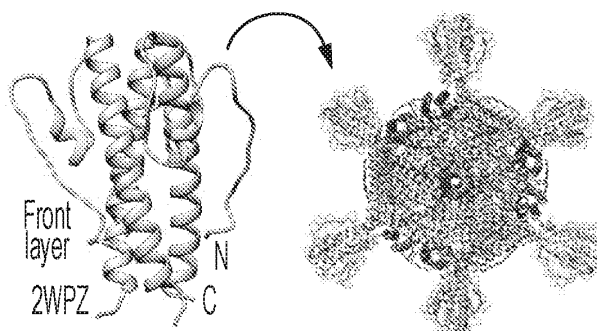


FIG. 13A

## Front layer epitope-scaffold design

|              |                                                          |
|--------------|----------------------------------------------------------|
| Front layer  | HINSTALNCNESLNTGWLGLFYQH KFDSSG                          |
| 2WPZ_A_FL    | HINSTALNCNESLNTGWLGLFYQH KFDSSGRMKQLEDKVETNLSKVYHNENVARL |
| 2WPZ_A_(PDB) | -----RMKQLEDKVEENLSKVYHNENEVARL                          |
|              | *****                                                    |
| 2WPZ_A_FL    | KKLVGER                                                  |
| 2WPZ_A_(PDB) | KKLVGER                                                  |
|              | *****                                                    |



## AS412 epitope-scaffold design

|              |                                                           |
|--------------|-----------------------------------------------------------|
| AS412        | ----QLINTNG--SWHIN                                        |
| 1T07_A_AS412 | ----RLVNCNG--SWLIGLDRPPYPGAKGEDIYNNVSRKAWDEWQKHQTMLINERRL |
| 1T07_A_(PDB) | GHMSRTVMCRKYHEELPGLDRPPYPGAKGEDIYNNVSRKAWDEWQKHQTMLINERRL |
|              | * * * . * *                                               |
| 1T07_A_AS412 | NMMNAEDRKFLQQEMDKFLSGEDY                                  |
| 1T07_A_      | NMMNAEDRKFLQQEMDKFLSGEDY                                  |
|              | *****                                                     |

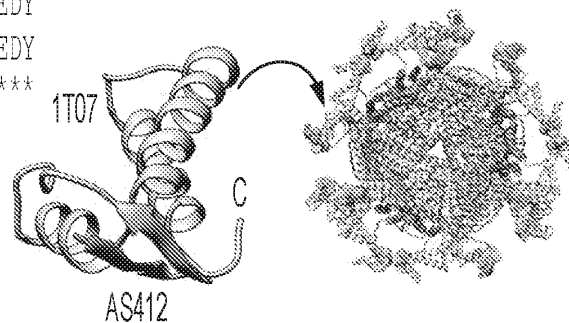


FIG. 13B

