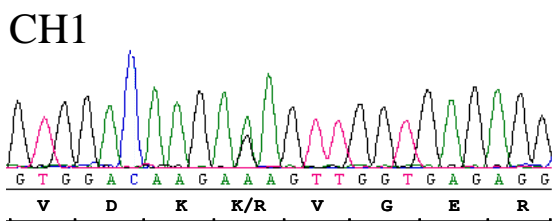


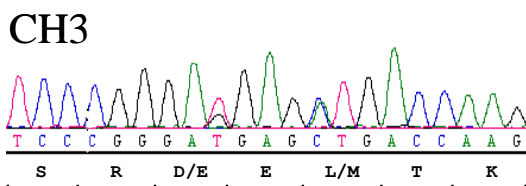
Supplementary Materials

Sequencing of CH1 and CH3 amplification products coupled to visual analysis of the returned chromatographs revealed the heterozygous allotype manifested itself as overlapping peaks corresponding to two different nucleotides each encoded by alternate G1m alleles (Supplementary Figure 1A). In contrast the homozygous allotype manifested itself as a single chromatograph peak, corresponding to two identical, overlapping G1m alleles (Supplementary Figure 1B).

A: Example Heterozygous Chromatogram

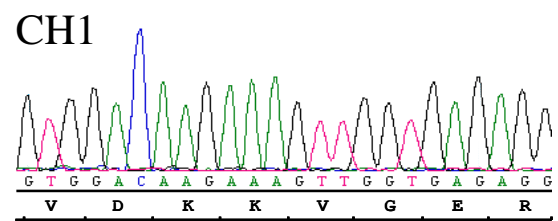


G1m(17)/G1m(3)

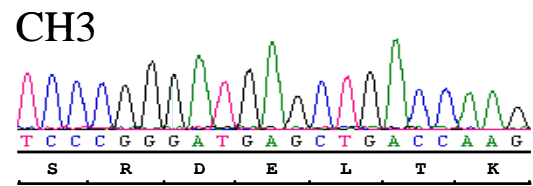


G1m(1)/nG1m(1)

B: Example Homozygous Chromatogram



G1m(17)/G1m(17)

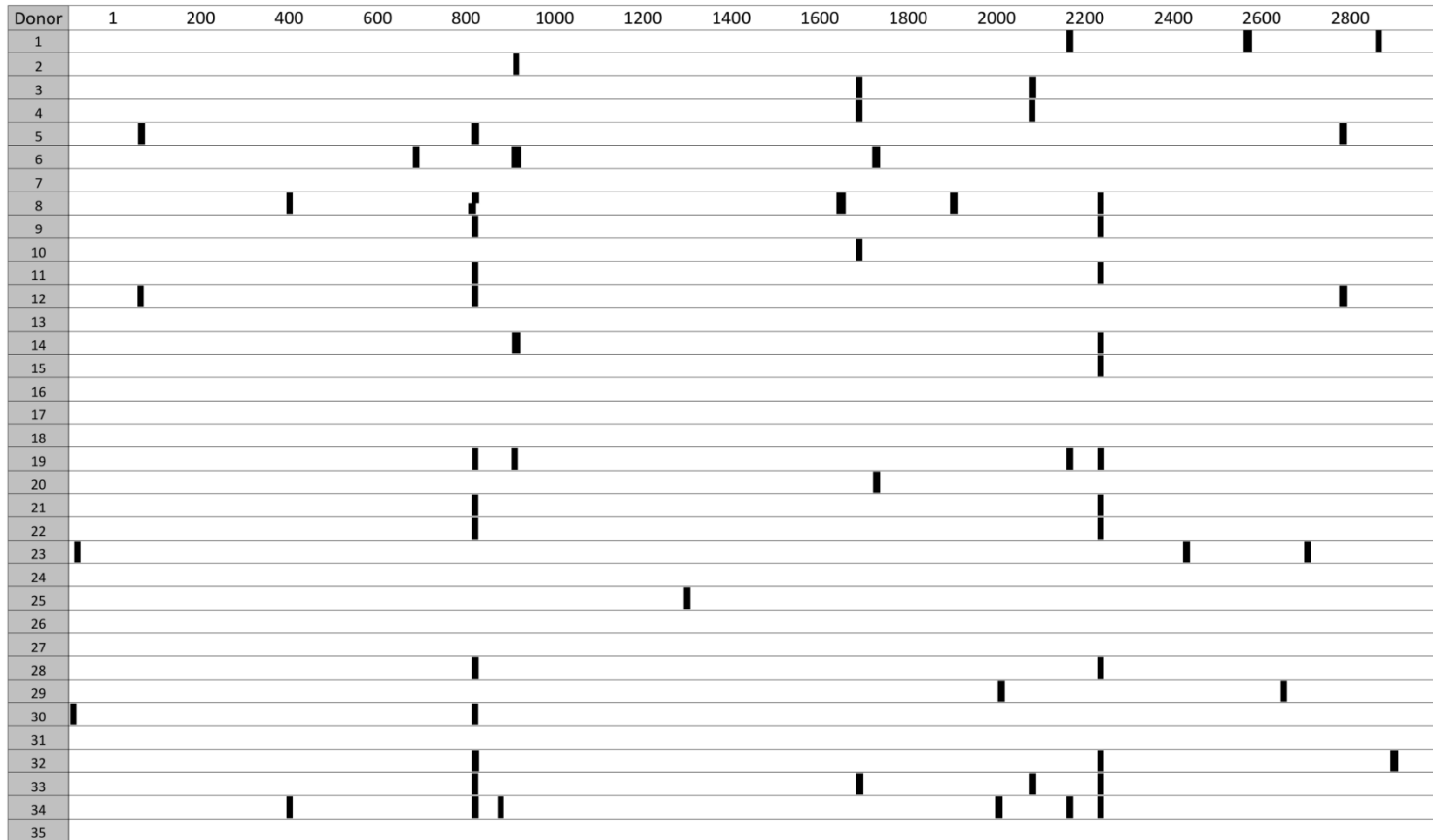


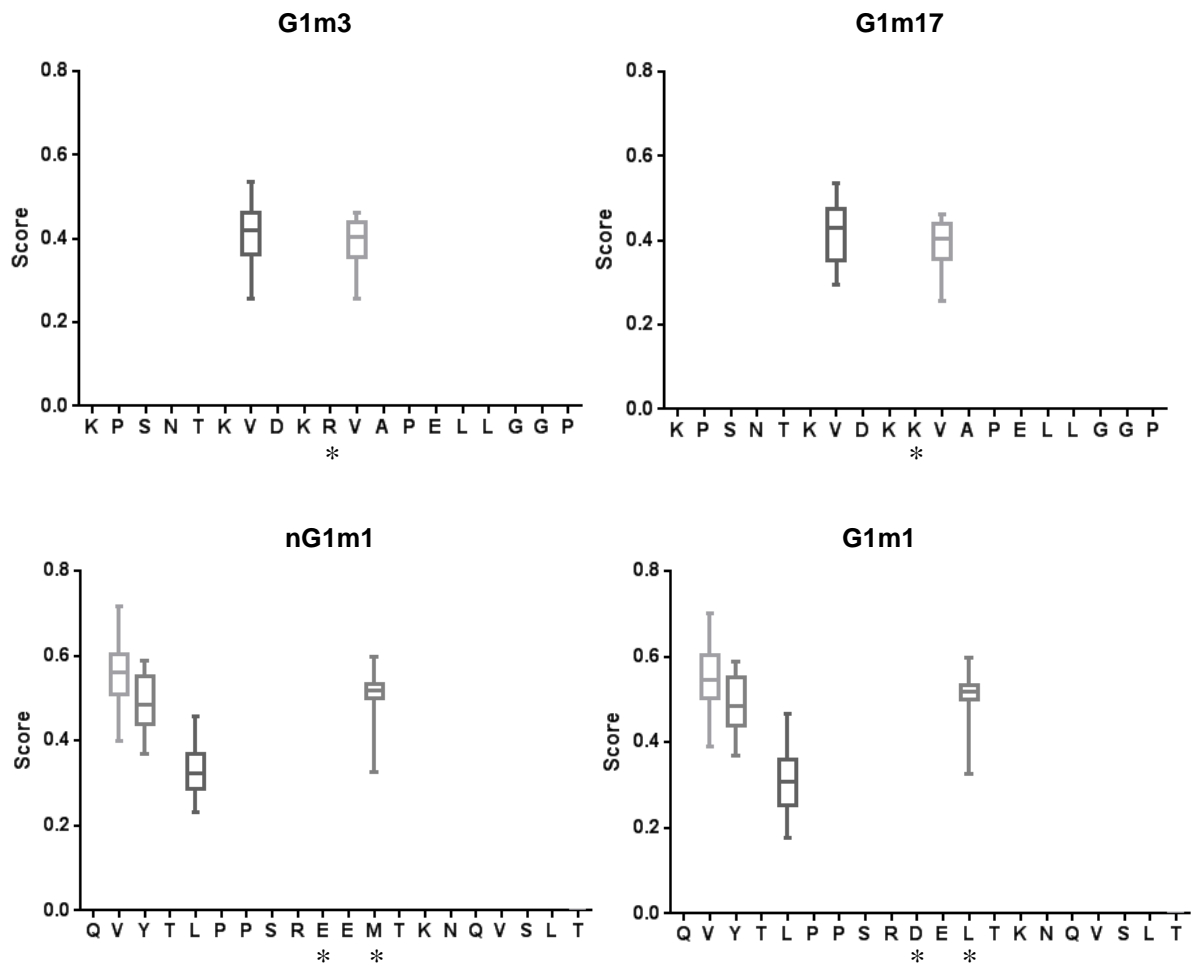
G1m(1)/G1m(1)

Supplementary Figure 1. Sequence Chromatographs Corresponding to (A) Heterozygote and (B) Homozygote Samples.

Overlapping peaks corresponding to alternately encoded alleles at either the CH1 or CH3 loci allow differentiation of heterozygote and homozygote donors. Polymorphic nucleotides specified by arrows. Single letter amino acid code is shown below the corresponding nucleotide triplet.

Supplementary Figure 2. HLA-DR-associated peptides produced by 5×10^6 dendritic cells from 35 different donors exposed to KLH. HLA-DR-associated peptides can originate from various regions of a protein and typically occur as multiple length variants which share the same HLA-DR binding core and form a “cluster”. Clusters are indicated as black boxes along the protein sequence.

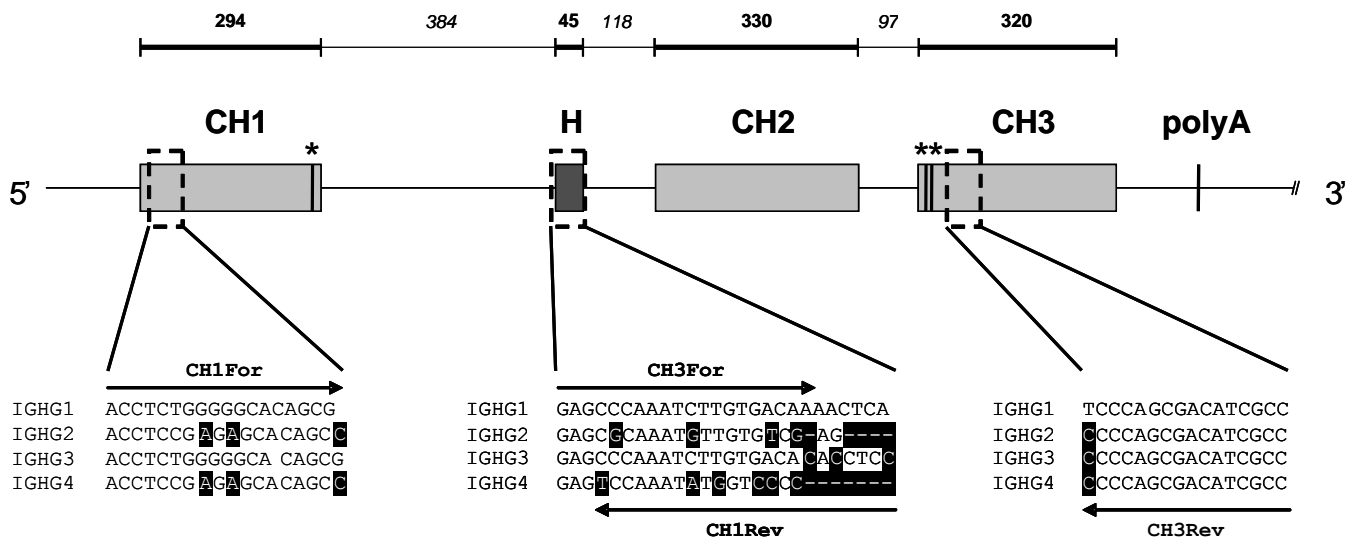




Supplementary Figure 3. *In silico* HLA-DR binding analysis of peptides spanning IgG1 allotypic variations (asterisked). Peptides spanning the selected sequence were tested as 9mer peptides in one amino acid increments against a panel of 34 HLA-DR alleles using iTope™ (Perry LC, Jones TD, Baker MP. New approaches to prediction of immune responses to therapeutic proteins during preclinical development. *Drugs in R & D* 2008; 9:385-96). The binding scores for each 9mer peptide are indicated above the the first amino acid of the peptide. The maximum and minimum scores are indicated, the boxed region represents the interquartile range and the median score is indicated by the line. Equivalent peptides that gave binding scores with each allotype were tested for significant differences using an unpaired, two-tailed Student's T test. No significant differences were observed ($p < 0.05$).

Development of a G1m-specific CH1 and CH3 PCR-based Allotype Screen

A high throughput PCR-based allotype screen was developed that allowed discrimination of heterozygote and homozygote G1m(17)/G1m(3) and G1m(1)/nG1m(1) donor samples. Selective amplification of G1m CH1 and CH3 loci was achieved by designing PCR primers to regions of sequence diversity identified between the different human IgG isotypes (Supplementary Figure 3).



Supplementary Figure 4. G1m Constant Region Exon/Intron Organisation.

The length of exons (bold) and introns (italics) is given in base pairs. Dashed boxes define those regions of sequence to which the G1m specific primers anneal. Residues that differ from G1m nucleotide sequence at these positions are highlighted in black. Alignment generated using the ClustalW method. Primers are depicted as arrows with the tail and head of the arrow representing the 5' and 3' termini, respectively. H, hinge region. Asterisks (*) indicate position of allotype specific polymorphisms. Not drawn to scale. (Modified from IMGT Repertoire, <http://www.imgt.org>).

| Donor | Day 5 | | | | Day 6 | | | | Day 7 | | | | Day 8 | | | |
|-------|-------|-------------|----------------|-------|-------|-------------|----------------|-------|-------|-------------|----------------|-------|-------|-------------|----------------|-------|
| | Media | Trast. G1m3 | Trast. G1m1,17 | KLH | Media | Trast. G1m3 | Trast. G1m1,17 | KLH | Media | Trast. G1m3 | Trast. G1m1,17 | KLH | Media | Trast. G1m3 | Trast. G1m1,17 | KLH |
| 2 | 2909 | 2943 | 1619 | 4831 | 2209 | 2233 | 2088 | 4051 | 1669 | 1249 | 1478 | 1708 | 1669 | 1249 | 1478 | 1708 |
| 3 | 2840 | 2495 | 1666 | 3657 | 443 | 515 | 533 | 1892 | 1912 | 2019 | 2007 | 2426 | 1912 | 2019 | 2007 | 2426 |
| 4 | 1099 | 1511 | 1230 | 3350 | 269 | 346 | 402 | 3445 | 2118 | 2236 | 1494 | 2144 | 2118 | 2236 | 1494 | 2144 |
| 5 | 958 | 1093 | 1003 | 1629 | 848 | 908 | 914 | 3488 | 1478 | 1264 | 1028 | 3658 | 1636 | 1534 | 1821 | 6468 |
| 7 | 1211 | 1588 | 1769 | 8999 | 630 | 768 | 1044 | 11101 | 2970 | 3271 | 5166 | 20113 | 2963 | 4854 | 5372 | 14131 |
| 9 | 830 | 706 | 833 | 3274 | 790 | 722 | 774 | 7946 | 810 | 657 | 575 | 8304 | 593 | 410 | 664 | 10139 |
| 10 | 918 | 814 | 730 | 5036 | 610 | 614 | 806 | 6660 | 1196 | 807 | 744 | 7548 | 804 | 565 | 776 | 12929 |
| 15 | 3769 | 4026 | 4016 | 7730 | 2674 | 3253 | 1872 | 4596 | 2643 | 2573 | 1938 | 4925 | 2054 | 2306 | 1847 | 10731 |
| 16 | 2100 | 2630 | 1992 | 5222 | 1376 | 1567 | 552 | 1877 | 3602 | 5270 | 2713 | 10170 | 6629 | 7693 | 4991 | 15998 |
| 17 | 746 | 906 | 686 | 4035 | 1365 | 2116 | 1971 | 5182 | 1923 | 1600 | 1358 | 5460 | 1795 | 2057 | 1593 | 10008 |
| 23 | 842 | 932 | 1287 | 3576 | 540 | 641 | 989 | 2595 | 611 | 551 | 516 | 1498 | 1093 | 1366 | 1405 | 3955 |
| 24 | 867 | 1746 | 2025 | 6326 | 355 | 604 | 690 | 2743 | 934 | 994 | 1194 | 4225 | 1260 | 2165 | 2328 | 8350 |
| 25 | 383 | 703 | 649 | 2262 | 509 | 809 | 677 | 1761 | 469 | 456 | 385 | 3303 | 325 | 649 | 667 | 5502 |
| 26 | 2844 | 3378 | 2873 | 11810 | 3814 | 4601 | 4077 | 13221 | 3111 | 3363 | 2962 | 10595 | 2859 | 3279 | 3016 | 13131 |
| 27 | 3287 | 3402 | 3267 | 11271 | 2670 | 2920 | 1955 | 9689 | 5286 | 3612 | 4094 | 19576 | 6713 | 4515 | 4838 | 28535 |
| 28 | 750 | 1036 | 1035 | 1599 | 262 | 382 | 287 | 483 | 972 | 1339 | 898 | 2941 | 905 | 1401 | 1180 | 2955 |
| 29 | 693 | 1044 | 1227 | 7503 | 1435 | 1985 | 1879 | 13748 | 1119 | 1212 | 1106 | 14362 | 1169 | 1193 | 1818 | 22793 |
| 33 | 1520 | 1319 | 875 | 5012 | 2172 | 2088 | 1427 | 7394 | 2063 | 2461 | 3030 | 5615 | 1770 | 1842 | 1345 | 1454 |
| 37 | 1075 | 1466 | 1564 | 2188 | 1220 | 920 | 806 | 1431 | 740 | 1071 | 2429 | 1276 | 1172 | 1174 | 816 | 376 |
| 39 | 1161 | 1139 | 1057 | 3799 | 756 | 1148 | 1094 | 6263 | 1299 | 431 | 463 | 6319 | 1608 | 1588 | 1962 | 5519 |
| 40 | 1809 | 1518 | 1425 | 5176 | 1897 | 1396 | 1222 | 10915 | 855 | 803 | 643 | 5714 | 1641 | 2099 | 1887 | 4194 |
| 41 | 1752 | 1887 | 2344 | 2931 | 634 | 1041 | 972 | 3120 | 2591 | 2503 | 4059 | 3906 | 1683 | 2648 | 2168 | 3152 |
| 42 | 1376 | 1827 | 1689 | 3775 | 1328 | 1424 | 1234 | 7080 | 1258 | 1224 | 1290 | 2835 | 5410 | 4153 | 3018 | 1037 |
| 43 | 1509 | 2173 | 2028 | 4310 | 1090 | 1166 | 1030 | 5029 | 588 | 794 | 639 | 4768 | 1465 | 2407 | 2322 | 5674 |
| 44 | 1250 | 1744 | 1973 | 3685 | 1049 | 1574 | 1848 | 6676 | 1010 | 962 | 1306 | 6666 | 2189 | 2833 | 3443 | 4022 |
| 45 | 2578 | 4052 | 4441 | 4112 | 1388 | 1949 | 2044 | 5427 | 4464 | 2541 | 2708 | 3106 | 2239 | 2548 | 2490 | 4386 |
| 48 | 356 | 535 | 284 | 371 | 306 | 298 | 309 | 897 | 384 | 128 | 77 | 499 | 342 | 323 | 260 | 257 |
| 49 | 317 | 520 | 436 | 250 | 556 | 581 | 589 | 399 | 5420 | 4471 | 6165 | 6794 | 349 | 571 | 523 | 315 |
| 52 | 3229 | 3550 | 2934 | 4492 | 4121 | 2513 | 2051 | 8916 | 3665 | 1400 | 1578 | 5669 | 6184 | 2775 | 3985 | 2190 |
| 54 | 2290 | 3558 | 3667 | 5222 | 2043 | 1622 | 1562 | 7178 | 2602 | 1562 | 1303 | 3515 | 2137 | 2195 | 3468 | 2063 |
| 55 | 4779 | 3067 | 3318 | 6727 | 4362 | 2374 | 2004 | 5885 | 5280 | 1818 | 1142 | 2513 | 6764 | 4294 | 4122 | 3243 |
| 56 | 7646 | 8492 | 6515 | 7668 | 8393 | 6541 | 5329 | 9821 | 5093 | 3683 | 2689 | 3457 | 10941 | 6704 | 9108 | 2837 |
| 57 | 1865 | 2676 | 2385 | 4047 | 881 | 1084 | 1196 | 5635 | 4864 | 3296 | 7042 | 4443 | 939 | 868 | 983 | 6849 |
| 58 | 3735 | 5748 | 5688 | 5714 | 1782 | 1688 | 1667 | 5419 | 3265 | 3542 | 1623 | 3144 | 1193 | 1483 | 1935 | 6081 |
| 59 | 1983 | 2858 | 3249 | 4849 | 1475 | 1289 | 1313 | 8376 | 3188 | 2304 | 770 | 5151 | 2148 | 1407 | 1562 | 10156 |
| 60 | 3120 | 3883 | 4392 | 5406 | 796 | 1030 | 1376 | 6288 | 1765 | 391 | 486 | 3452 | 1401 | 1598 | 2000 | 12567 |
| 61 | 805 | 1243 | 1407 | 2291 | 873 | 1249 | 1279 | 4949 | 7390 | 1789 | 3618 | 4539 | 1541 | 1177 | 1141 | 4356 |
| 66 | 966 | 1234 | 1685 | 3573 | 801 | 1031 | 907 | 3077 | 323 | 342 | 500 | 1925 | 1459 | 1023 | 1274 | 5019 |

Supplementary Table 1. Average cpm values (to the nearest whole number) for G1m3 donors tested against both G1m3 and G1m1,17 trastuzumab (Trast.) for the time-course T cell assay (days 5 to 8).

| Donor | Day 5 | | | | Day 6 | | | | Day 7 | | | | Day 8 | | | |
|-------|-------|-------------|----------------|-------|-------|-------------|----------------|-------|-------|-------------|----------------|-------|-------|-------------|----------------|-------|
| | Media | Trast. G1m3 | Trast. G1m1,17 | KLH | Media | Trast. G1m3 | Trast. G1m1,17 | KLH | Media | Trast. G1m3 | Trast. G1m1,17 | KLH | Media | Trast. G1m3 | Trast. G1m1,17 | KLH |
| 1 | 1827 | 2591 | 2255 | 10352 | 1670 | 1914 | 2079 | 11504 | 1856 | 1498 | 1697 | 1374 | 1856 | 1498 | 1697 | 1374 |
| 6 | 1432 | 1540 | 1276 | 9149 | 1039 | 1339 | 1239 | 14570 | 2211 | 2333 | 1226 | 29792 | 2011 | 3043 | 1740 | 25014 |
| 8 | 3568 | 3661 | 3713 | 2693 | 522 | 590 | 753 | 433 | 6099 | 5388 | 5132 | 1188 | 6571 | 6473 | 6092 | 564 |
| 11 | 1708 | 1898 | 1529 | 6924 | 664 | 798 | 735 | 6213 | 2295 | 1781 | 1281 | 9819 | 2208 | 1827 | 1603 | 11686 |
| 12 | 1200 | 1384 | 1214 | 3189 | 134 | 193 | 228 | 826 | 1230 | 1139 | 996 | 2958 | 1385 | 1166 | 1037 | 5007 |
| 13 | 1947 | 2344 | 2134 | 6095 | 1277 | 1537 | 1630 | 6225 | 2025 | 2217 | 1497 | 7273 | 1971 | 1863 | 2149 | 7127 |
| 14 | 1139 | 957 | 1972 | 3773 | 1132 | 1466 | 2074 | 6900 | 3332 | 1724 | 5200 | 5849 | 2944 | 1365 | 4550 | 4480 |
| 18 | 530 | 408 | 616 | 1695 | 2537 | 2118 | 2819 | 7134 | 3019 | 1856 | 1940 | 4927 | 2476 | 2387 | 2301 | 8498 |
| 19 | 858 | 1476 | 1499 | 12593 | 876 | 1129 | 1416 | 10896 | 1398 | 1987 | 2325 | 24340 | 1305 | 1990 | 2980 | 27407 |
| 20 | 1562 | 2077 | 2700 | 7766 | 346 | 423 | 391 | 2671 | 1608 | 1518 | 1315 | 20386 | 1700 | 1494 | 1763 | 45674 |
| 21 | 1132 | 2068 | 1679 | 4196 | 1442 | 5071 | 1917 | 7009 | 1060 | 1467 | 1013 | 2275 | 1312 | 1433 | 2037 | 4189 |
| 22 | 2925 | 2996 | 1784 | 3970 | 3727 | 3937 | 2586 | 3496 | 2327 | 1271 | 676 | 875 | 5930 | 4341 | 2703 | 1273 |
| 28 | 750 | 1036 | 1035 | 1599 | 262 | 382 | 287 | 483 | 972 | 1339 | 898 | 2941 | 905 | 1401 | 1180 | 2955 |
| 30 | 675 | 699 | 582 | 3028 | 1031 | 956 | 753 | 1666 | 1088 | 712 | 640 | 1747 | 1062 | 629 | 803 | 2593 |
| 31 | 939 | 1655 | 1478 | 8775 | 1480 | 1535 | 1024 | 6159 | 1522 | 2617 | 1644 | 9851 | 1396 | 2303 | 1984 | 5220 |
| 32 | 312 | 466 | 424 | 1525 | 99 | 113 | 146 | 350 | 450 | 571 | 346 | 669 | 433 | 284 | 137 | 356 |
| 34 | 294 | 284 | 131 | 82 | 514 | 327 | 163 | 142 | 86 | 78 | 507 | 87 | 455 | 431 | 478 | 63 |
| 35 | 738 | 896 | 736 | 7555 | 1170 | 1473 | 926 | 14167 | 894 | 1252 | 653 | 9081 | 1354 | 2307 | 1501 | 3259 |
| 36 | 1282 | 1784 | 1217 | 4769 | 2035 | 3100 | 1633 | 7320 | 1616 | 2753 | 1328 | 5865 | 2651 | 4065 | 2129 | 2210 |
| 38 | 2557 | 1511 | 1375 | 5525 | 2478 | 1005 | 999 | 5807 | 2925 | 506 | 699 | 1331 | 3087 | 1863 | 2000 | 785 |
| 45 | 2578 | 4052 | 4441 | 4112 | 1388 | 1949 | 2044 | 5427 | 4464 | 2541 | 2708 | 3106 | 2239 | 2548 | 2490 | 4386 |
| 47 | 1160 | 1906 | 1368 | 2566 | 1084 | 1266 | 806 | 3098 | 1508 | 1091 | 547 | 2456 | 1467 | 2572 | 1475 | 3936 |
| 50 | 467 | 346 | 355 | 388 | 491 | 288 | 410 | 315 | 419 | 501 | 461 | 200 | 648 | 391 | 389 | 202 |
| 51 | 1964 | 3662 | 3355 | 4043 | 1652 | 2080 | 2096 | 6306 | 2071 | 1425 | 1158 | 5401 | 1823 | 3476 | 3279 | 3732 |
| 53 | 1755 | 1674 | 2447 | 1894 | 1492 | 771 | 905 | 1364 | 4484 | 2441 | 4064 | 8716 | 1493 | 1148 | 836 | 666 |
| 62 | 2530 | 2043 | 1882 | 4189 | 4339 | 2612 | 1953 | 7103 | 3014 | 2228 | 1640 | 2885 | 2271 | 3368 | 2335 | 1720 |
| 63 | 1349 | 1653 | 1839 | 2317 | 1505 | 1593 | 1517 | 3269 | 1255 | 851 | 730 | 2463 | 1734 | 1838 | 2231 | 5175 |
| 64 | 1427 | 1199 | 746 | 5990 | 1464 | 1645 | 1129 | 9515 | 958 | 1111 | 706 | 4606 | 1210 | 1572 | 1436 | 2149 |
| 65 | 1505 | 1676 | 1168 | 5999 | 1977 | 1940 | 1524 | 17909 | 1532 | 2125 | 8479 | 16059 | 1710 | 1828 | 1487 | 14384 |
| 67 | 1013 | 1065 | 1068 | 3626 | 799 | 1041 | 829 | 5054 | 663 | 565 | 508 | 4471 | 788 | 1579 | 1608 | 4733 |
| 68 | 1937 | 2221 | 2067 | 4407 | 2080 | 2021 | 1961 | 5649 | 1099 | 863 | 768 | 3997 | 2057 | 2423 | 2903 | 5552 |

Supplementary Table 2. Average cpm values (to the nearest whole number) for G1m1,17 donors tested against both G1m3 and G1m1,17 trastuzumab for the time-course T cell assay (days 5 to 8).

| Donors: G1m3 | | | | | Donors: G1m1,17 | | | | |
|--------------|--------|-------------|----------------|---------|-----------------|--------|-------------|----------------|--------|
| Donor | Media | Trast. G1m3 | Trast. G1m1,17 | KLH | Donor | Media | Trast. G1m3 | Trast. G1m1,17 | KLH |
| 2 | 4.33 | 4.50 | 4.83 | 57.50 | 1 | 2.67 | 3.67 | 4.00 | 14.00 |
| 3 | 2.50 | 3.00 | 3.00 | 18.25 | 6 | 1.00 | 9.50 | 10.50 | 43.00 |
| 4 | 4.50 | 4.67 | 4.67 | 25.00 | 8 | 8.17 | 12.33 | 11.00 | 39.50 |
| 5 | 0.17 | 2.17 | 5.67 | 10.25 | 11 | 18.00 | 16.67 | 27.33 | 49.25 |
| 7 | 15.50 | 20.00 | 20.83 | 50.50 | 12 | 17.50 | 21.17 | 17.67 | 22.50 |
| 9 | 6.17 | 5.50 | 6.17 | 43.25 | 13 | 41.17 | 18.50 | 21.83 | 102.25 |
| 10 | 5.83 | 6.33 | 7.00 | 20.75 | 14 | 53.50 | 58.33 | 59.00 | 180.75 |
| 15 | 33.67 | 37.67 | 31.50 | 12.75 | 18 | 2.83 | 1.50 | 3.33 | 17.50 |
| 16 | 18.50 | 13.17 | 2.33 | 39.25 | 19 | 3.17 | 4.67 | 7.50 | 13.25 |
| 17 | 9.00 | 6.17 | 5.83 | 31.25 | 20 | 2.00 | 1.17 | 1.17 | 25.00 |
| 23 | 43.67 | 51.33 | 36.50 | 41.25 | 21 | 8.17 | 12.33 | 10.50 | 20.75 |
| 24 | 9.67 | 19.17 | 21.33 | 89.75 | 22 | 7.00 | 12.17 | 11.83 | 45.25 |
| 25 | 9.17 | 20.40 | 24.83 | 32.50 | 28 | 8.83 | 11.67 | 9.33 | 33.75 |
| 26 | 16.40 | 13.33 | 11.50 | 36.50 | 30 | 4.00 | 1.33 | 4.83 | 25.25 |
| 27 | 41.33 | 37.67 | 52.00 | 119.50 | 31 | 33.00 | 25.67 | 15.83 | 68.25 |
| 28 | 8.83 | 11.67 | 9.33 | 33.75 | 32 | ND | ND | ND | ND |
| 29 | 4.00 | 1.83 | 1.83 | 24.75 | 34 | 0.33 | 0.17 | 0.83 | 0.25 |
| 33 | 9.83 | 11.67 | 14.17 | 86.00 | 35 | 21.83 | 39.67 | 15.17 | 42.67 |
| 37 | 16.67 | 13.17 | 16.83 | 40.25 | 36 | 17.40 | 19.33 | 15.33 | 33.33 |
| 39 | 4.00 | 1.83 | 3.50 | 21.50 | 38 | 7.50 | 7.83 | 11.33 | 37.00 |
| 40 | 27.00 | 17.17 | 19.83 | 123.50 | 45 | 59.50 | 86.00 | 88.83 | 115.00 |
| 41 | 87.17 | 92.75 | 144.75 | 140.75 | 47 | 82.83 | 55.17 | 52.50 | 56.00 |
| 42 | 231.67 | 263.50 | 260.50 | 443.50 | 50 | 0.33 | 0.17 | 0.17 | 2.00 |
| 43 | 20.80 | 19.20 | 3.83 | 121.50 | 51 | 209.67 | 487.50 | 301.50 | 450.50 |
| 44 | 57.00 | 65.50 | 82.83 | 151.75 | 53 | 91.67 | 90.67 | 61.50 | 94.75 |
| 45 | 59.50 | 86.00 | 88.83 | 115.00 | 62 | 130.80 | 98.17 | 141.33 | 444.75 |
| 48 | 0.33 | 0.00 | 0.17 | 0.75 | 63 | 265.17 | 374.83 | 392.00 | 603.25 |
| 49 | ND | ND | ND | ND | 64 | 272.40 | 205.33 | 271.50 | 575.25 |
| 52 | 210.33 | 265.00 | 297.33 | 461.25 | 65 | 94.33 | 77.17 | 118.50 | 199.00 |
| 54 | 174.60 | 322.83 | 295.17 | 505.25 | 67 | 152.50 | 354.83 | 260.25 | 107.75 |
| 55 | 70.50 | 31.67 | 20.50 | 78.25 | 68 | 430.50 | 503.00 | 460.83 | 379.00 |
| 56 | 181.33 | 136.50 | 143.50 | 278.50 | | | | | |
| 57 | 483.67 | 437.33 | 598.83 | 1092.75 | | | | | |
| 58 | 410.50 | 454.67 | 516.83 | 800.25 | | | | | |
| 59 | 385.00 | 514.50 | 304.17 | 440.75 | | | | | |
| 60 | 501.67 | 586.33 | 648.33 | 617.00 | | | | | |
| 61 | 130.67 | 148.67 | 207.17 | 727.25 | | | | | |
| 66 | 75.50 | 121.00 | 72.40 | 241.75 | | | | | |

Supplementary Table 3. Average spw values for homozygous donors (either G1m3 or G1m1,17) donors tested against both G1m3 and G1m1,17 trastuzumab (Trast.) for IL-2 ELISpot assay. ND = no data.