**Appendix**

1. Measurements of Fura-2 AM fluorescence emission at 510 nm of Ca2+-bound (340 nm excitation) and Ca2+-free fura-2 AM (380 nm excitation) on 1321N1 WT (C1-C3) and hHA-P2Y2R transfected 1321N1 cells (C4-C6). Cells were treated with 100 μM ATP at cycle number 10 using Tecan Infinite M200**®** injection system.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Fura-2 AM 380 nm excitation** | | | | | | | |  | | |  | | |  | |  | | |  | |  | | | |  | | |  | | |  | | |  | | |  | | |  | | |  | | |  |  | |  | |  |  | |
| Cycle Nr. | 1 | 2 | 3 | 4 | | 5 | | | 6 | | | 7 | | | 8 | | | 9 | | | | 10 | | 11 | | | 12 | | | 13 | | | 14 | | | 15 | | | 16 | | | 17 | | | 18 | | | 19 | | 20 | | |
| Time [s] | 0 | 3 | 6 | 9 | | 12 | | | 15 | | | 18 | | | 21 | | | 24 | | | | 27.6 | | 30 | | | 33 | | | 36 | | | 39 | | | 42 | | | 45 | | | 48 | | | 51 | | | 54 | | 57 | | |
| C1 | 1457 | 1468 | 1462 | 1450 | | 1501 | | | 1464 | | | 1398 | | | 1488 | | | 1471 | | | | 1396 | | 1347 | | | 1367 | | | 1326 | | | 1418 | | | 1344 | | | 1338 | | | 1373 | | | 1349 | | | 1348 | | 1327 | | |
| C2 | 1584 | 1581 | 1580 | 1613 | | 1594 | | | 1565 | | | 1670 | | | 1629 | | | 1653 | | | | 1412 | | 1380 | | | 1386 | | | 1392 | | | 1378 | | | 1352 | | | 1378 | | | 1389 | | | 1386 | | | 1393 | | 1374 | | |
| C3 | 1317 | 1288 | 1309 | 1347 | | 1329 | | | 1328 | | | 1333 | | | 1297 | | | 1327 | | | | 1281 | | 1290 | | | 1315 | | | 1322 | | | 1333 | | | 1343 | | | 1287 | | | 1269 | | | 1305 | | | 1306 | | 1284 | | |
| C4 | 1440 | 1420 | 1394 | 1447 | | 1431 | | | 1405 | | | 1431 | | | 1408 | | | 1460 | | | | 1261 | | 823 | | | 803 | | | 832 | | | 784 | | | 829 | | | 848 | | | 831 | | | 845 | | | 816 | | 825 | | |
| C5 | 1462 | 1466 | 1452 | 1452 | | 1432 | | | 1464 | | | 1468 | | | 1453 | | | 1454 | | | | 1231 | | 855 | | | 851 | | | 829 | | | 861 | | | 850 | | | 804 | | | 813 | | | 859 | | | 868 | | 842 | | |
| C6 | 1377 | 1412 | 1410 | 1416 | | 1414 | | | 1387 | | | 1432 | | | 1438 | | | 1448 | | | | 1301 | | 910 | | | 865 | | | 912 | | | 890 | | | 888 | | | 925 | | | 911 | | | 896 | | | 910 | | 923 | | |
| **Fura-2 AM 340 nm excitation** | | | | |  | |  | | |  | | |  | | | |  | | |  | | |  | | |  | | |  | | |  | | |  | | |  | | |  | | |  | | |
| Cycle Nr. | 1 | 2 | 3 | 4 | | 5 | | | 6 | | | 7 | | | 8 | | | 9 | | | | 10 | | 11 | | | 12 | | | 13 | | | 14 | | | 15 | | | 16 | | | 17 | | | 18 | | | 19 | | 20 | | |
| Time [s] | 0.7 | 3.7 | 6.7 | 9.7 | | 12.7 | | | 15.7 | | | 18.7 | | | 21.7 | | | 24.7 | | | | 28.3 | | 30.7 | | | 33.7 | | | 36.7 | | | 39.7 | | | 42.7 | | | 45.7 | | | 48.7 | | | 51.7 | | | 54.7 | | 57.7 | | |
| C1 | 1784 | 1760 | 1701 | 1730 | | 1807 | | | 1777 | | | 1713 | | | 1789 | | | 1825 | | | | 1760 | | 1717 | | | 1718 | | | 1789 | | | 1776 | | | 1819 | | | 1861 | | | 1814 | | | 1809 | | | 1784 | | 1730 | | |
| C2 | 1791 | 1757 | 1746 | 1778 | | 1781 | | | 1773 | | | 1799 | | | 1756 | | | 1851 | | | | 1544 | | 1548 | | | 1571 | | | 1624 | | | 1559 | | | 1620 | | | 1598 | | | 1588 | | | 1556 | | | 1617 | | 1542 | | |
| C3 | 1372 | 1397 | 1425 | 1461 | | 1443 | | | 1391 | | | 1362 | | | 1437 | | | 1445 | | | | 1406 | | 1422 | | | 1498 | | | 1437 | | | 1454 | | | 1471 | | | 1435 | | | 1448 | | | 1417 | | | 1449 | | 1431 | | |
| C4 | 1592 | 1592 | 1619 | 1623 | | 1653 | | | 1609 | | | 1642 | | | 1629 | | | 1696 | | | | 1927 | | 1937 | | | 2071 | | | 2083 | | | 2085 | | | 2049 | | | 2056 | | | 2062 | | | 2061 | | | 2040 | | 2017 | | |
| C5 | 1716 | 1689 | 1700 | 1653 | | 1672 | | | 1636 | | | 1714 | | | 1677 | | | 1713 | | | | 1827 | | 1884 | | | 1954 | | | 1993 | | | 2019 | | | 1947 | | | 1984 | | | 1947 | | | 1935 | | | 1939 | | 1933 | | |
| C6 | 1564 | 1546 | 1530 | 1505 | | 1548 | | | 1583 | | | 1552 | | | 1616 | | | 1580 | | | | 1936 | | 2091 | | | 2097 | | | 2145 | | | 2098 | | | 2155 | | | 2029 | | | 2032 | | | 2076 | | | 2055 | | 2044 | | |

*Note: Only 20 cycles of the 40 collected are shown.*

1. To express in intracellular calcium concentration in cells, calculate the ratio of Fura-2 AM fluorescence emission in response to 340 nm and 380 nm excitation (340/380).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 340/380 | | | | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Cycle Nr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Time [s] | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27.6 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 |
| C1 | 1.224 | 1.199 | 1.163 | 1.193 | 1.204 | 1.214 | 1.225 | 1.202 | 1.241 | 1.261 | 1.275 | 1.257 | 1.349 | 1.252 | 1.353 | 1.391 | 1.321 | 1.341 | 1.323 | 1.304 |
| C2 | 1.131 | 1.111 | 1.105 | 1.102 | 1.117 | 1.133 | 1.077 | 1.078 | 1.120 | 1.093 | 1.122 | 1.133 | 1.167 | 1.131 | 1.198 | 1.160 | 1.143 | 1.123 | 1.161 | 1.122 |
| C3 | 1.042 | 1.085 | 1.089 | 1.085 | 1.086 | 1.047 | 1.022 | 1.108 | 1.089 | 1.098 | 1.102 | 1.139 | 1.087 | 1.091 | 1.095 | 1.115 | 1.141 | 1.086 | 1.109 | 1.114 |
| C4 | 1.106 | 1.121 | 1.161 | 1.122 | 1.155 | 1.145 | 1.147 | 1.157 | 1.162 | 1.528 | 2.354 | 2.579 | 2.504 | 2.659 | 2.472 | 2.425 | 2.481 | 2.439 | 2.500 | 2.445 |
| C5 | 1.174 | 1.152 | 1.171 | 1.138 | 1.168 | 1.117 | 1.168 | 1.154 | 1.178 | 1.484 | 2.204 | 2.296 | 2.404 | 2.345 | 2.291 | 2.468 | 2.395 | 2.253 | 2.234 | 2.296 |
| C6 | 1.136 | 1.095 | 1.085 | 1.063 | 1.095 | 1.141 | 1.084 | 1.124 | 1.091 | 1.488 | 2.298 | 2.424 | 2.352 | 2.357 | 2.427 | 2.194 | 2.231 | 2.317 | 2.258 | 2.215 |

*Note: Only 20 cycles of the 40 collected are shown.*

1. To normalize Ca2+ mobilization, use the average of the first 9 cycles (unstimulated) as baseline and divide each cycle recording by the baseline.

*E.g.*, C1 baseline calculation: (1.224 + 1.199 + 1.163 + 1.193 + 1.204 + 1.214 +1.225 +1.202 + 1.241)/9 = **1.207**

C1 normalized data: 1.224/1.207 = **1.014**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cycle Nr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Time [s] | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27.6 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 |
| C1 | 1.014 | 0.993 | 0.964 | 0.988 | 0.997 | 1.005 | 1.015 | 0.996 | 1.028 | 1.044 | 1.056 | 1.041 | 1.117 | 1.037 | 1.121 | 1.152 | 1.094 | 1.111 | 1.096 | 1.080 |
| C2 | 1.020 | 1.003 | 0.997 | 0.995 | 1.008 | 1.022 | 0.972 | 0.973 | 1.010 | 0.987 | 1.012 | 1.023 | 1.053 | 1.021 | 1.081 | 1.046 | 1.032 | 1.013 | 1.047 | 1.013 |
| C3 | 0.971 | 1.011 | 1.015 | 1.011 | 1.012 | 0.977 | 0.953 | 1.033 | 1.015 | 1.023 | 1.028 | 1.062 | 1.014 | 1.017 | 1.021 | 1.040 | 1.064 | 1.013 | 1.035 | 1.039 |
| C4 | 0.968 | 0.982 | 1.017 | 0.982 | 1.012 | 1.003 | 1.005 | 1.013 | 1.017 | 1.338 | 2.061 | 2.259 | 2.193 | 2.329 | 2.165 | 2.123 | 2.173 | 2.136 | 2.190 | 2.141 |
| C5 | 1.014 | 0.995 | 1.011 | 0.983 | 1.008 | 0.965 | 1.008 | 0.997 | 1.018 | 1.282 | 1.903 | 1.983 | 2.076 | 2.025 | 1.978 | 2.131 | 2.068 | 1.946 | 1.929 | 1.983 |
| C6 | 1.031 | 0.994 | 0.985 | 0.965 | 0.994 | 1.036 | 0.984 | 1.020 | 0.991 | 1.351 | 2.086 | 2.201 | 2.135 | 2.140 | 2.203 | 1.991 | 2.025 | 2.103 | 2.050 | 2.010 |

*Note: Only 20 cycles of the 40 collected are shown.*