**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.

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| **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.*