

Electronic supplementary information (ESI)

Nonspecific nuclear uptake of anti-MUC1 aptamers by dead cells: The role of cell viability monitoring in aptamer targeting of membrane-bound protein cancer biomarkers

Shane Patrick Flanagan^a, Ronen Fogel^a, Adrienne Lesley Edkins^b, Lance St. John Ho^a and Janice Limson^{a*}

^a Biotechnology Innovation Centre, Rhodes University, Makhanda, South Africa

^b Department of Biochemistry and Microbiology, Rhodes University, Makhanda, South Africa

* Corresponding author: E-mail: j.limson@ru.ac.za (JL)

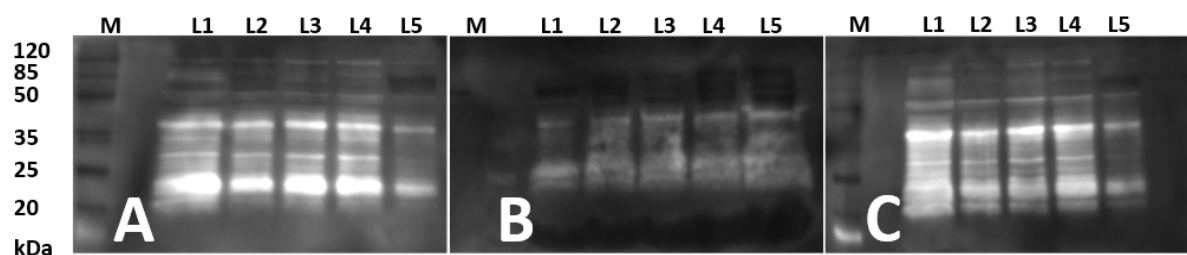


Fig S1. Southwestern blot of biotin-labelled (A) 5TR4 aptamer, (B) 5TRG2 aptamer, and (C) S22 aptamer binding to cell lysates of the tested cancer cell lines. Lane annotations: Protein molecular weight marker (M), MCF-7 (L1), Hs578T (L2), A549 (L3), SW480 (L4), and SW620 (L5).

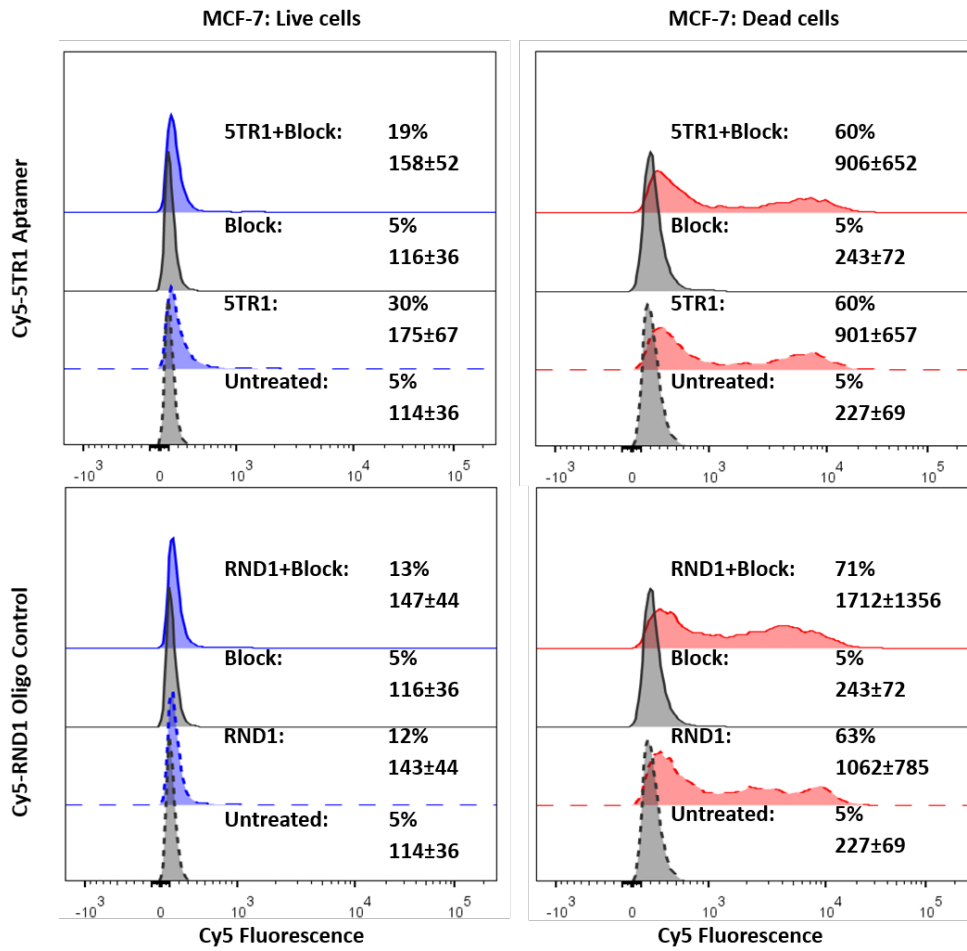


Fig S2. Blocking efficacy of salmon sperm dsDNA on 5TR1 aptamer binding to MCF-7 cells. Representative histogram of live and dead MCF-7 cell population stained with 25 pmol Cy5- labelled 5TR1 or 25 pmol Cy5-labelled RND1. Insert indicates the cell population percentage stained positive for Cy5 fluorescence, and the median Cy5 fluorescence intensity of the total live or dead cell population. The number of live cell events counted: 10 000.

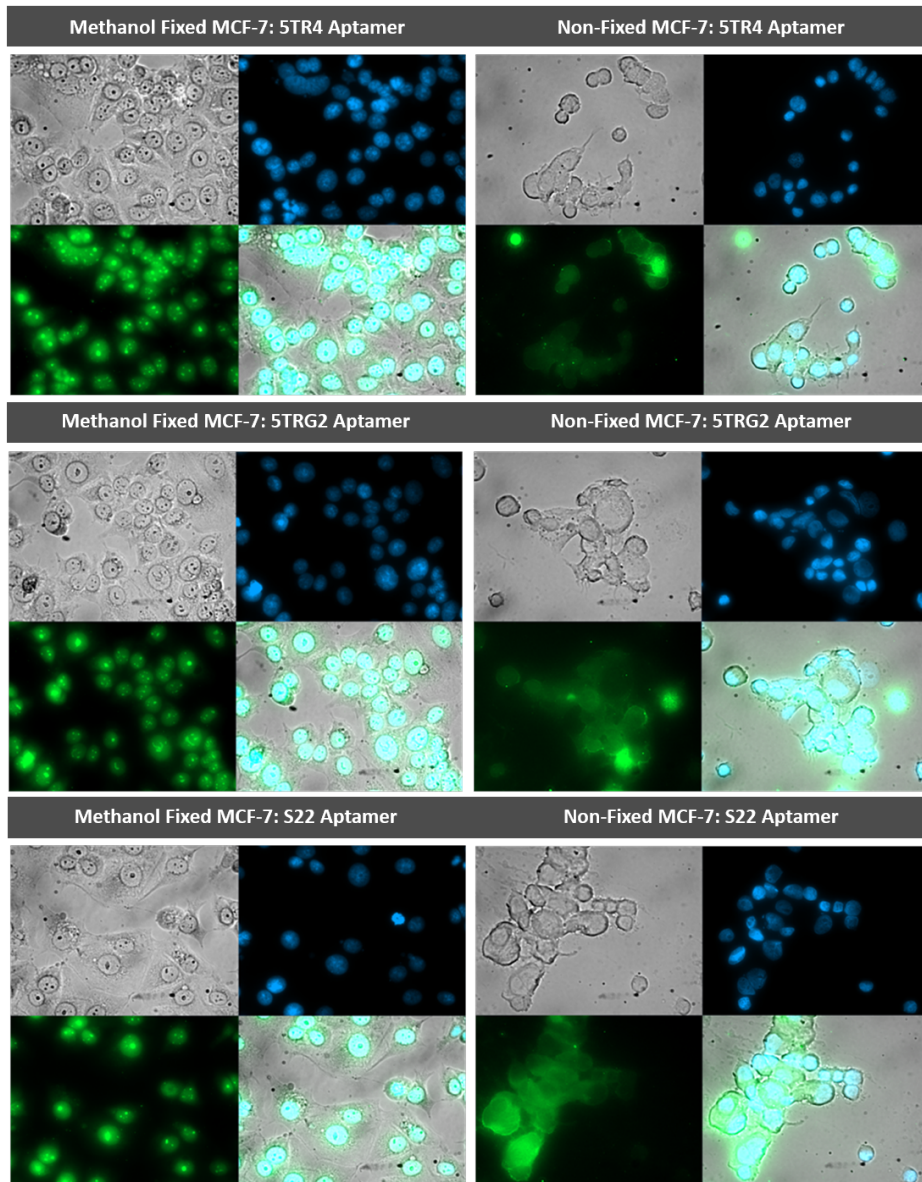


Fig S3A. Fluorescence microscopy imaging of methanol- and non-fixed MCF-7 cells stained with DAPI (blue) and FITC-labelled (green) aptamers 5TR4, 5TRG2 and S22. Images were captured using the Zeiss Axiovert epi-fluorescent microscope at a magnification of 400X.

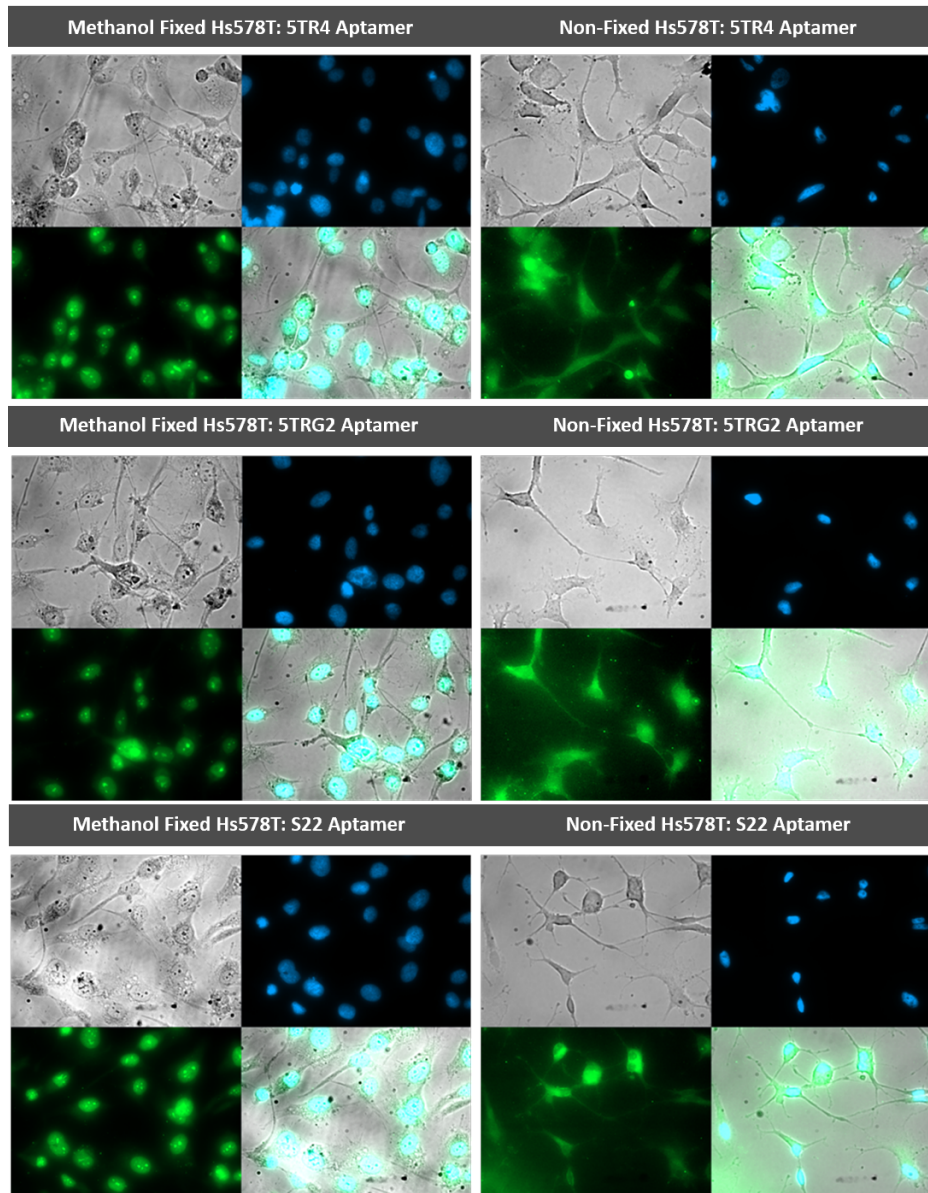


Fig S3B. Fluorescence microscopy imaging of methanol- and non-fixed Hs578T cells stained with DAPI (blue) and FITC-labelled (green) aptamers 5TR4, 5TRG2 and S22. Images were captured using the Zeiss Axiovert epi-fluorescent microscope at a magnification of 400X.

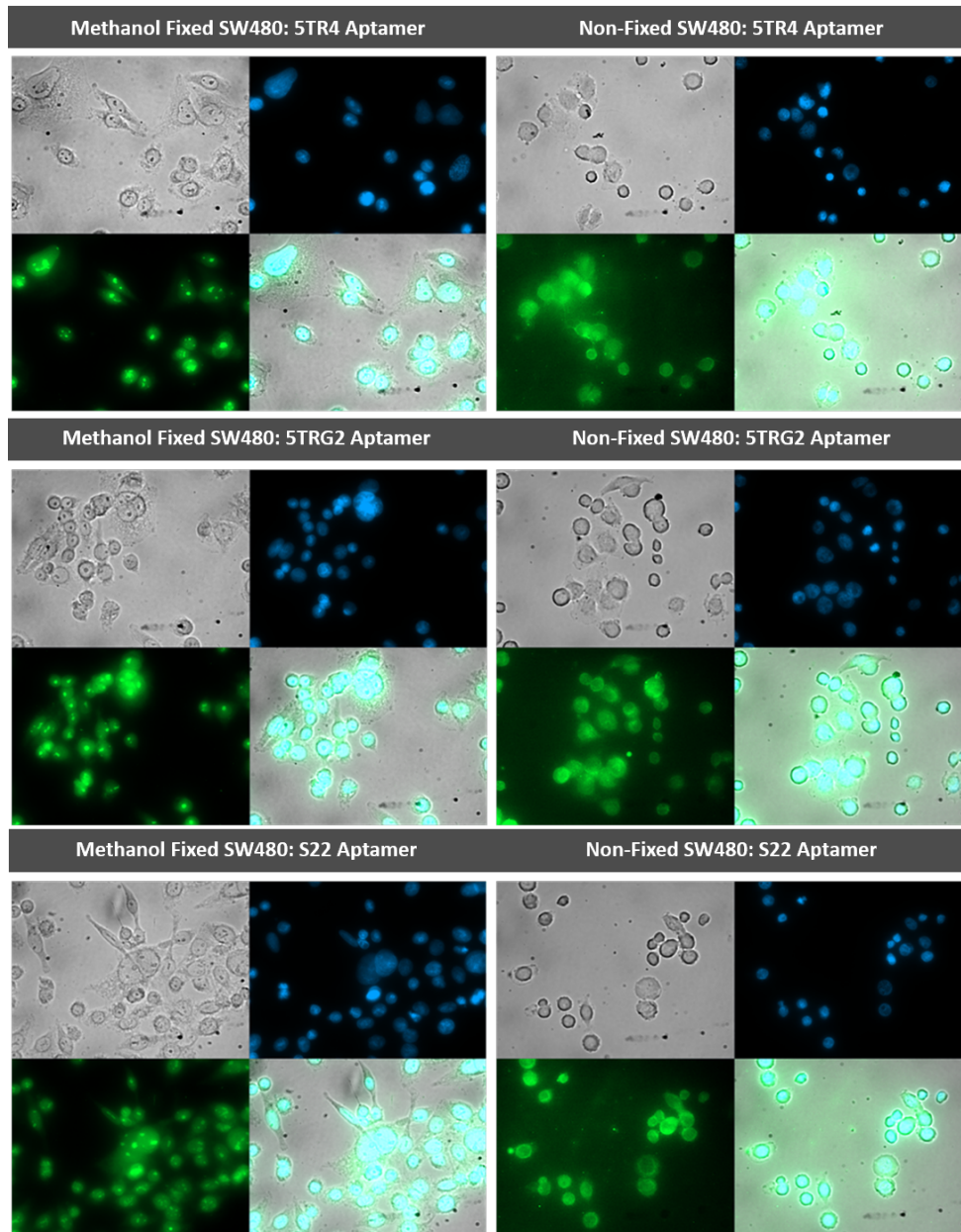


Fig S3C. Fluorescence microscopy imaging of methanol- and non-fixed SW480 cells stained with DAPI (blue) and FITC-labelled (green) aptamers 5TR4, 5TRG2 and S22. Images were captured using the Zeiss Axiovert epi-fluorescent microscope at a magnification of 400X.

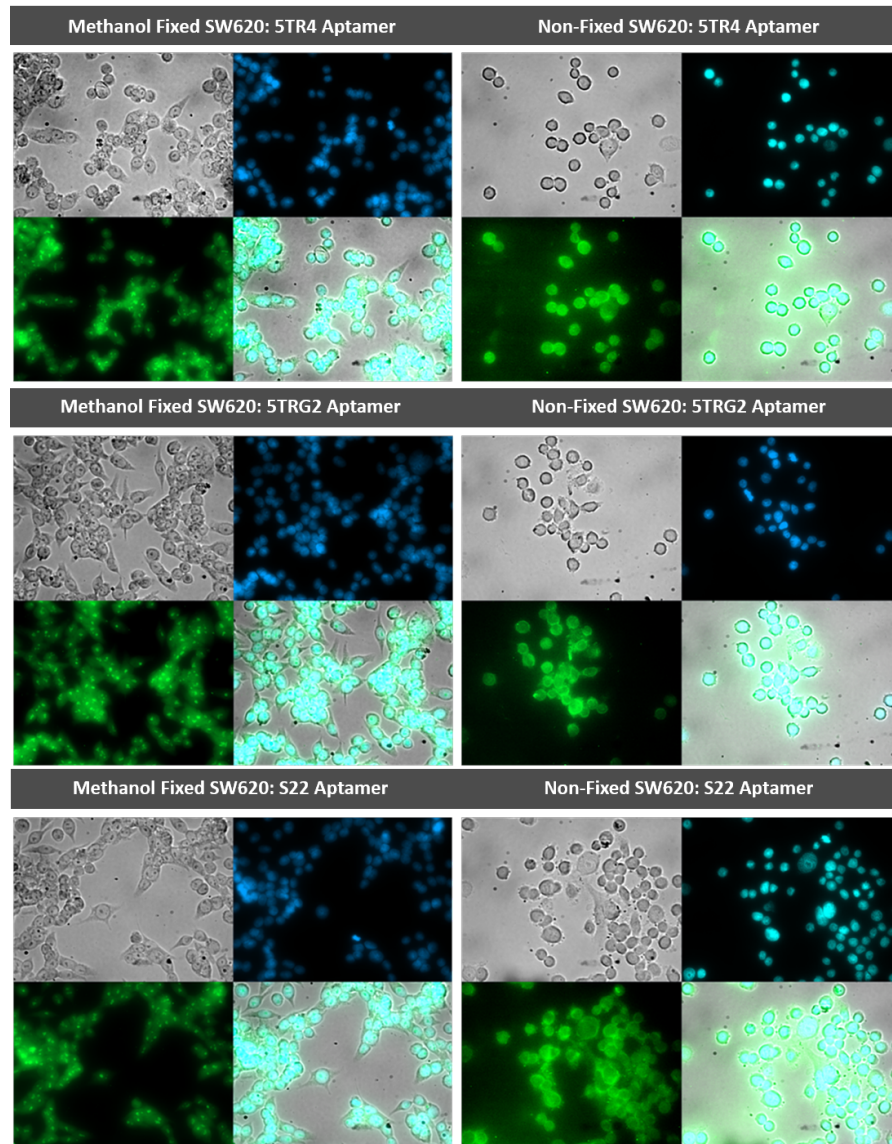


Fig S3D. Fluorescence microscopy imaging of methanol- and non-fixed SW620 cells stained with DAPI (blue) and FITC-labelled (green) aptamers 5TR4, 5TRG2 and S22. Images were captured using the Zeiss Axiovert epi-fluorescent microscope at a magnification of 400X.

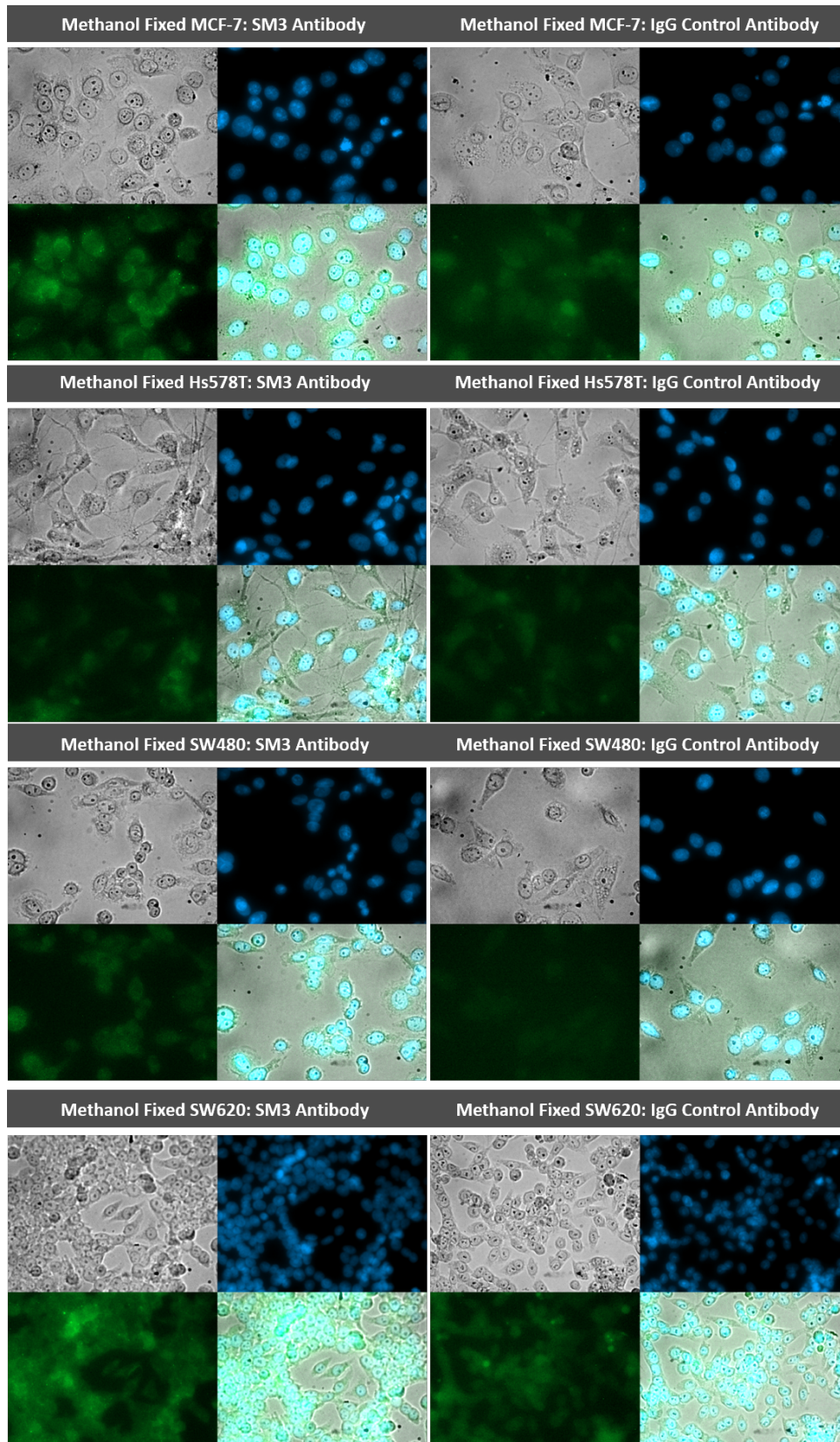


Fig S3E. Fluorescence microscopy imaging of methanol-fixed MCF-7, Hs578T, SW480, and SW620 cells stained with DAPI (blue) and FITC-labelled (green) antibodies SM3 and the IgG isotype control. Images were captured using the Zeiss Axiovert epi-fluorescent microscope at a magnification of 400X.

Table S1. Median, median absolute deviation and mean rank scores of Fig 2 and Fig 4 datasets.

| | Fig 2C Cy5 Fluorescence | | | |
|--------------------|--|-------------------|--|-------------------|
| | MCF-7: Live cells | | MCF-7: Dead cells | |
| | Median \pm median absolute deviation | Mean ranks | Median \pm median absolute deviation | Mean ranks |
| Untreated cells | 77 \pm 24 | 46360 | 171 \pm 51 | 9357 |
| Cy5-5TR1 1 pmol | 77 \pm 24 | 46579 | 218 \pm 69 | 12808 |
| Cy5-5TR1 10 pmol | 81 \pm 25 | 50798 | 436 \pm 172 | 20604 |
| Cy5-5TR1 25 pmol | 104 \pm 32 | 66599 | 5040 \pm 3696 | 37151 |
| Cy5-5TR1 50 pmol | 121 \pm 37 | 77979 | 10605 \pm 9630 | 41048 |
| Cy5-5TR1 100 pmol | 148 \pm 48 | 89173 | 18876 \pm 15740 | 45948 |
| Cy5-RND1 1 pmol | 76 \pm 24 | 45297 | 205 \pm 67 | 11622 |
| Cy5-RND1 10 pmol | 79 \pm 25 | 48146 | 702 \pm 402 | 23763 |
| Cy5-RND1 2 5pmol | 83 \pm 26 | 51413 | 1570 \pm 830 | 30189 |
| Cy5-RND1 50 pmol | 93 \pm 28 | 59881 | 2772 \pm 1899 | 33543 |
| Cy5-RND1 100 pmol | 98 \pm 28 | 63312 | 5831 \pm 3586 | 39452 |
| Dyl650-SM3 | 781 \pm 553 | 123591 | 9139 \pm 7518 | 43531 |
| Dyl650-IgG Isotype | 248 \pm 89 | 110482 | 5445 \pm 4656 | 38365 |

| Fig 4A Cy5 Fluorescence: Median ± median absolute deviation | | | | |
|--|--------------------------|---------------------------|--------------------------|--------------------------|
| | MCF-7: Live cells | Hs578T: Live cells | SW480: Live cells | SW620: Live cells |
| Untreated cells | 127 ± 43 | 166 ± 56 | 97 ± 31 | 90 ± 29 |
| Cy5-5TR1 | 562 ± 227 | 626 ± 237 | 476 ± 210 | 124 ± 54 |
| Cy5-RND1 | 291 ± 109 | 302 ± 110 | 175 ± 68 | 140 ± 50 |

| Fig 4C Dyl650 Fluorescence: Median ± median absolute deviation | | | | |
|---|--------------------------|---------------------------|--------------------------|--------------------------|
| | MCF-7: Live cells | Hs578T: Live cells | SW480: Live cells | SW620: Live cells |
| Untreated cells | 127 ± 43 | 166 ± 56 | 97 ± 31 | 90 ± 29 |
| Dyl650-SM3 | 499 ± 271 | 293 ± 201 | 227 ± 96 | 256 ± 96 |
| Dyl650-IgG Isotype | 336 ± 118 | 294 ± 174 | 242 ± 93 | 189 ± 64 |

| Fig 4B FITC Fluorescence: Median ± median absolute deviation | | | | |
|---|--------------------------|---------------------------|--------------------------|--------------------------|
| | MCF-7: Live cells | Hs578T: Live cells | SW480: Live cells | SW620: Live cells |
| Untreated cells | 375 ± 109 | 750 ± 217 | 267 ± 75 | 175 ± 36 |
| FITC-5TR1 | 1327 ± 601 | 1624 ± 736 | 810 ± 382 | 550 ± 225 |
| FITC-5TR4 | 1591 ± 705 | 1998 ± 892 | 1123 ± 519 | 676 ± 286 |
| FITC-5TRG2 | 1212 ± 510 | 1498 ± 632 | 771 ± 333 | 483 ± 188 |
| FITC-S22 | 1123 ± 504 | 1111 ± 412 | 667 ± 295 | 430 ± 144 |

| Fig 4D FITC Fluorescence: Median ± median absolute deviation | | | | |
|---|--------------------------|---------------------------|--------------------------|--------------------------|
| | MCF-7: Dead cells | Hs578T: Dead cells | SW480: Dead cells | SW620: Dead cells |
| Perm cells | 351 ± 108 | 710 ± 204 | 339 ± 92 | 310 ± 110 |
| FITC-MH1 | 55361 ± 25824 | 23870 ± 9699 | 15435 ± 7105 | 13483 ± 6934 |
| FITC-SecAb | 15904 ± 6513 | 6526 ± 2325 | 3263 ± 1564 | 2607 ± 1275 |

| Fig 4A Cy5 Fluorescence: Mean ranks | | | | |
|---|--------------------------|---------------------------|--------------------------|--------------------------|
| | MCF-7: Live cells | Hs578T: Live cells | SW480: Live cells | SW620: Live cells |
| Untreated cells | 6625 | 7618 | 7348 | 10664 |
| Cy5-5TR1 | 22198 | 22467 | 22924 | 16368 |
| Cy5-RND1 | 16179 | 14916 | 14729 | 17969 |
| Fig 4C Dyl650 Fluorescence: Mean ranks | | | | |
| | MCF-7: Live cells | Hs578T: Live cells | SW480: Live cells | SW620: Live cells |
| Untreated cells | 2659 | 4614 | 3045 | 2834 |
| Dyl650-SM3 | 8409 | 6563 | 7230 | 8217 |
| Dyl650-IgG Isotype | 6934 | 6824 | 7726 | 6950 |
| Fig 4B FITC Fluorescence: Mean ranks | | | | |
| | MCF-7: Live cells | Hs578T: Live cells | SW480: Live cells | SW620: Live cells |
| Untreated cells | 7413 | 12442 | 8557 | 6547 |
| FITC-5TR1 | 29780 | 29335 | 28881 | 30650 |
| FITC-5TR4 | 32625 | 32963 | 33647 | 33638 |
| FITC-5TRG2 | 28253 | 28113 | 28100 | 28145 |
| FITC-S22 | 26932 | 22148 | 25817 | 26023 |
| Fig 4D FITC Fluorescence Mean ranks | | | | |
| | MCF-7: Dead cells | Hs578T: Dead cells | SW480: Dead cells | SW620: Dead cells |
| Perm cells | 6160 | 5818 | 6072 | 6780 |
| FITC-MH1 | 22162 | 22797 | 22155 | 21496 |
| FITC-SecAb | 16680 | 16386 | 16775 | 16725 |