# Rhodopsin and melanopsin coexist in mammalian sperm cells and activate different signaling pathways for thermotaxis

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# Supplementary Information

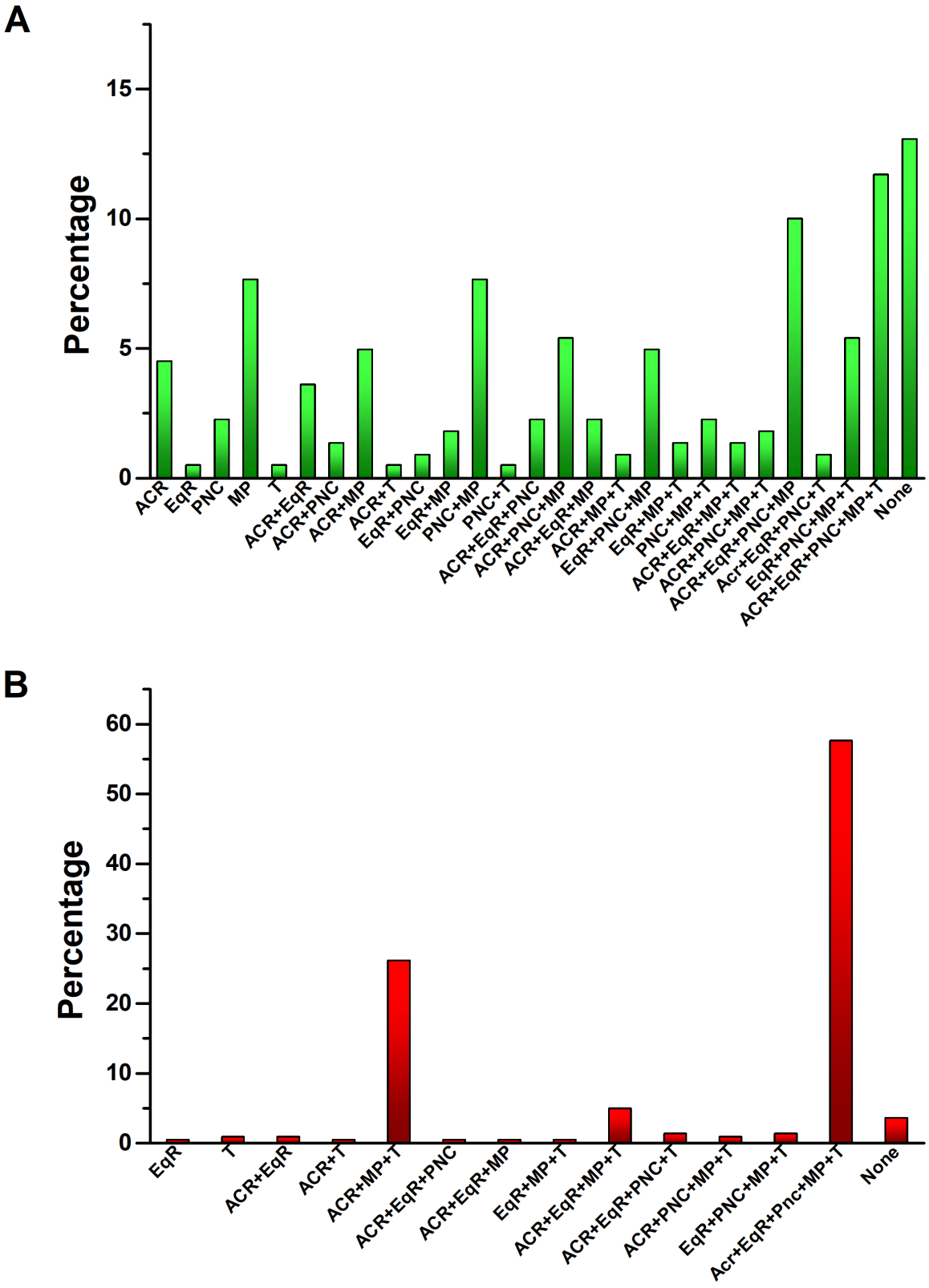


**Figure S1.** Distribution of rhodopsin and melanopsin in human sperm cells. The cells are those shown in Figure 2. The figure was drawn from the data of individual cells shown in Table S1. N is the number of cells analyzed. Abbreviations: Acr, acrosome; EqR, equatorial ring; PNC, postnuclear cap; MP, midpiece; T, tail. See Table S1 and Methods for more details.

| | Rhodopsin | | | | | Melanopsin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cell** | **Acr** | **EqR** | **PNC** | **MP** | **T** | **Acr** | **EqR** | **PNC** | **MP** | **T** |
| 1 | 123 | 349 | 1630 | 1018 | 196 | 582 | 1684 | 2018 | 1745 | 243 |
| 2 | 164 | 452 | 1294 | 1310 | 143 | 581 | 2434 | 2647 | 1663 | 229 |
| 3 | 181 | 243 | 226 | 250 | 44 | 185 | 279 | 479 | 293 | 78 |
| 4 | 151 | 180 | 343 | 215 | 51 | 170 | 413 | 429 | 424 | 87 |
| 5 | 415 | 215 | 284 | 400 | 63 | 169 | 300 | 459 | 466 | 131 |
| 6 | 263 | 787 | 508 | 624 | 69 | 197 | 357 | 314 | 328 | 116 |
| 7 | 699 | 300 | 171 | 312 | 60 | 182 | 373 | 285 | 295 | 143 |
| 8 | 186 | 356 | 333 | 250 | 111 | 613 | 1983 | 1816 | 214 | 110 |
| 9 | 286 | 361 | 335 | 174 | 32 | 1079 | 1168 | 723 | 435 | 156 |
| 10 | 126 | 154 | 138 | 324 | 84 | 430 | 785 | 750 | 447 | 151 |
| 11 | 175 | 90 | 131 | 317 | 48 | 151 | 308 | 423 | 200 | 95 |
| 12 | 488 | 417 | 426 | 644 | 182 | 529 | 1271 | 1798 | 1302 | 327 |
| 13 | 762 | 661 | 3202 | 1629 | 189 | 967 | 1948 | 2951 | 1745 | 278 |
| 14 | 498 | 385 | 208 | 202 | 53 | 176 | 374 | 339 | 301 | 94 |
| 15 | 314 | 392 | 122 | 240 | 55 | 212 | 353 | 302 | 329 | 100 |
| 16 | 105 | 594 | 2954 | 1302 | 155 | 349 | 1913 | 2957 | 1735 | 282 |
| 17 | 940 | 882 | 457 | 758 | 102 | 340 | 434 | 314 | 296 | 72 |
| 18 | 1093 | 591 | 601 | 1126 | 63 | 505 | 446 | 427 | 468 | 83 |
| 19 | 222 | 374 | 276 | 917 | 167 | 201 | 381 | 319 | 258 | 121 |
| 20 | 160 | 296 | 229 | 206 | 112 | 401 | 1591 | 1156 | 184 | 105 |
| 21 | 239 | 308 | 379 | 200 | 50 | 206 | 246 | 330 | 236 | 69 |
| 22 | 78 | 233 | 174 | 214 | 57 | 454 | 1491 | 930 | 368 | 139 |
| 23 | 296 | 232 | 314 | 185 | 78 | 220 | 244 | 320 | 290 | 76 |
| 24 | 378 | 675 | 811 | 343 | 124 | 158 | 227 | 433 | 268 | 63 |
| 25 | 275 | 321 | 195 | 178 | 53 | 477 | 717 | 559 | 180 | 94 |
| 26 | 446 | 239 | 121 | 243 | 113 | 297 | 188 | 154 | 190 | 68 |
| 27 | 423 | 344 | 205 | 218 | 84 | 280 | 425 | 388 | 267 | 112 |
| 28 | 443 | 215 | 223 | 256 | 72 | 138 | 310 | 457 | 689 | 167 |
| 29 | 248 | 310 | 830 | 879 | 142 | 600 | 1554 | 2008 | 1418 | 208 |
| 30 | 379 | 213 | 166 | 255 | 70 | 203 | 209 | 207 | 229 | 57 |
| 31 | 86 | 141 | 120 | 236 | 44 | 191 | 304 | 369 | 499 | 86 |
| 32 | 199 | 210 | 116 | 256 | 55 | 238 | 356 | 403 | 472 | 84 |
| 33 | 124 | 224 | 171 | 334 | 39 | 139 | 310 | 426 | 372 | 82 |
| 34 | 216 | 246 | 395 | 356 | 66 | 388 | 1264 | 1319 | 667 | 146 |
| 35 | 59 | 76 | 83 | 136 | 40 | 200 | 419 | 432 | 138 | 82 |
| 36 | 330 | 315 | 391 | 628 | 251 | 306 | 302 | 300 | 314 | 122 |
| 37 | 732 | 3438 | 1188 | 192 | 59 | 238 | 720 | 394 | 163 | 97 |
| 38 | 235 | 255 | 264 | 403 | 63 | 176 | 550 | 504 | 535 | 111 |
| 39 | 402 | 243 | 130 | 359 | 106 | 550 | 1083 | 964 | 416 | 202 |
| 40 | 165 | 500 | 454 | 379 | 98 | 708 | 1294 | 1400 | 546 | 188 |
| 41 | 205 | 225 | 573 | 390 | 81 | 651 | 1299 | 1030 | 506 | 169 |

| | Rhodopsin | | | | | Melanopsin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cell** | **Acr** | **EqR** | **PNC** | **MP** | **T** | **Acr** | **EqR** | **PNC** | **MP** | **T** |
| 42 | 331 | 222 | 193 | 300 | 69 | 376 | 1207 | 1323 | 570 | 159 |
| 43 | 635 | 620 | 1017 | 1214 | 150 | 571 | 1666 | 1795 | 1737 | 254 |
| 44 | 199 | 278 | 261 | 352 | 97 | 220 | 445 | 469 | 423 | 140 |
| 45 | 174 | 415 | 250 | 430 | 65 | 150 | 449 | 572 | 434 | 102 |
| 46 | 417 | 346 | 484 | 572 | 63 | 236 | 622 | 753 | 1068 | 101 |
| 47 | 191 | 780 | 344 | 215 | 53 | 637 | 1421 | 1185 | 367 | 151 |
| 48 | 249 | 252 | 185 | 386 | 66 | 181 | 443 | 452 | 344 | 101 |
| 49 | 89 | 274 | 167 | 278 | 38 | 196 | 394 | 414 | 233 | 76 |
| 50 | 335 | 338 | 241 | 413 | 72 | 431 | 1238 | 1550 | 682 | 200 |
| 51 | 197 | 301 | 276 | 250 | 112 | 538 | 2011 | 1547 | 207 | 103 |
| 52 | 472 | 246 | 152 | 239 | 50 | 154 | 381 | 337 | 261 | 114 |
| 53 | 166 | 227 | 307 | 260 | 71 | 437 | 1019 | 953 | 436 | 170 |
| 54 | 89 | 142 | 129 | 233 | 36 | 189 | 499 | 419 | 168 | 77 |
| 55 | 406 | 269 | 182 | 296 | 63 | 518 | 1161 | 1023 | 543 | 164 |
| 56 | 116 | 97 | 246 | 260 | 33 | 134 | 268 | 491 | 279 | 54 |
| 57 | 118 | 810 | 615 | 899 | 79 | 105 | 242 | 364 | 624 | 66 |
| 58 | 937 | 405 | 199 | 267 | 70 | 329 | 245 | 175 | 197 | 63 |
| 59 | 337 | 530 | 1385 | 566 | 194 | 471 | 580 | 752 | 930 | 387 |
| 60 | 204 | 438 | 132 | 397 | 131 | 140 | 990 | 359 | 764 | 92 |
| 61 | 632 | 334 | 245 | 387 | 114 | 619 | 543 | 476 | 519 | 276 |
| 62 | 685 | 1084 | 1230 | 113 | 13 | 284 | 296 | 296 | 25 | 6 |
| 63 | 1392 | 2122 | 2102 | 1679 | 768 | 438 | 1765 | 2097 | 1898 | 413 |
| 64 | 872 | 744 | 473 | 790 | 153 | 220 | 846 | 993 | 783 | 114 |
| 65 | 677 | 801 | 3116 | 782 | 165 | 514 | 532 | 1775 | 932 | 137 |
| 66 | 124 | 538 | 559 | 456 | 243 | 73 | 415 | 627 | 480 | 91 |
| 67 | 474 | 552 | 470 | 1085 | 187 | 506 | 603 | 1234 | 1323 | 444 |
| 68 | 146 | 343 | 335 | 300 | 60 | 167 | 745 | 377 | 291 | 67 |
| 69 | 73 | 137 | 207 | 261 | 73 | 70 | 397 | 511 | 528 | 67 |
| 70 | 249 | 304 | 118 | 240 | 59 | 137 | 354 | 283 | 259 | 91 |
| 71 | 229 | 941 | 590 | 850 | 167 | 115 | 975 | 994 | 1686 | 161 |
| 72 | 442 | 1113 | 2978 | 1073 | 244 | 157 | 396 | 790 | 475 | 201 |
| 73 | 239 | 376 | 148 | 173 | 47 | 69 | 198 | 128 | 135 | 38 |
| 74 | 68 | 248 | 369 | 177 | 44 | 79 | 498 | 272 | 370 | 62 |
| 75 | 168 | 177 | 159 | 211 | 76 | 320 | 252 | 227 | 220 | 115 |
| 76 | 397 | 570 | 318 | 528 | 172 | 299 | 764 | 1543 | 990 | 120 |
| 77 | 325 | 199 | 456 | 703 | 140 | 645 | 458 | 871 | 660 | 306 |
| 78 | 353 | 74 | 97 | 242 | 92 | 256 | 169 | 302 | 364 | 234 |
| 79 | 474 | 2843 | 1049 | 1918 | 86 | 220 | 738 | 409 | 470 | 68 |
| 80 | 164 | 727 | 1630 | 188 | 74 | 148 | 946 | 447 | 248 | 97 |
| 81 | 607 | 319 | 629 | 810 | 232 | 412 | 559 | 1460 | 830 | 319 |
| 82 | 170 | 171 | 454 | 548 | 148 | 368 | 522 | 782 | 629 | 266 |

| | Rhodopsin | | | | | Melanopsin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cell** | **Acr** | **EqR** | **PNC** | **MP** | **T** | **Acr** | **EqR** | **PNC** | **MP** | **T** |
| 83 | 146 | 300 | 472 | 1021 | 72 | 133 | 526 | 793 | 1551 | 109 |
| 84 | 107 | 143 | 119 | 129 | 69 | 387 | 393 | 411 | 283 | 151 |
| 85 | 163 | 144 | 152 | 364 | 97 | 480 | 367 | 404 | 344 | 224 |
| 86 | 558 | 1468 | 870 | 884 | 368 | 222 | 934 | 787 | 369 | 197 |
| 87 | 280 | 286 | 450 | 727 | 112 | 330 | 690 | 1121 | 1781 | 132 |
| 88 | 399 | 885 | 720 | 1221 | 392 | 199 | 623 | 988 | 1340 | 241 |
| 89 | 246 | 167 | 160 | 235 | 94 | 289 | 272 | 378 | 388 | 207 |
| 90 | 215 | 188 | 133 | 352 | 68 | 307 | 464 | 451 | 337 | 152 |
| 91 | 1616 | 1282 | 1681 | 1125 | 104 | 709 | 1609 | 1848 | 1656 | 93 |
| 92 | 361 | 1228 | 730 | 1167 | 363 | 175 | 978 | 910 | 1250 | 209 |
| 93 | 578 | 1016 | 952 | 1145 | 759 | 264 | 1465 | 1169 | 1463 | 291 |
| 94 | 408 | 731 | 378 | 309 | 79 | 211 | 831 | 830 | 847 | 103 |
| 95 | 2808 | 2806 | 2210 | 720 | 105 | 821 | 1203 | 746 | 245 | 66 |
| 96 | 1117 | 404 | 75 | 109 | 64 | 294 | 385 | 248 | 132 | 43 |
| 97 | 836 | 567 | 735 | 740 | 148 | 380 | 524 | 1046 | 930 | 121 |
| 98 | 667 | 493 | 377 | 577 | 61 | 441 | 767 | 1139 | 1283 | 100 |
| 99 | 634 | 459 | 247 | 275 | 100 | 163 | 540 | 269 | 525 | 82 |
| 100 | 2503 | 1384 | 985 | 1584 | 216 | 527 | 798 | 566 | 1808 | 266 |
| 101 | 79 | 214 | 150 | 347 | 65 | 129 | 521 | 686 | 936 | 120 |
| 102 | 608 | 354 | 551 | 424 | 118 | 289 | 460 | 736 | 581 | 69 |
| 103 | 95 | 223 | 582 | 118 | 47 | 194 | 331 | 246 | 105 | 48 |
| 104 | 363 | 548 | 201 | 409 | 78 | 362 | 981 | 1070 | 1053 | 181 |
| 105 | 1328 | 599 | 1038 | 2045 | 64 | 413 | 843 | 803 | 1276 | 91 |
| 106 | 401 | 679 | 810 | 465 | 43 | 340 | 901 | 965 | 1085 | 104 |
| 107 | 602 | 858 | 277 | 539 | 59 | 325 | 498 | 545 | 719 | 59 |
| 108 | 152 | 352 | 376 | 248 | 47 | 140 | 441 | 210 | 266 | 41 |
| 109 | 575 | 341 | 674 | 273 | 46 | 298 | 526 | 1000 | 873 | 78 |
| 110 | 578 | 362 | 387 | 341 | 62 | 207 | 904 | 702 | 480 | 111 |
| 111 | 416 | 582 | 571 | 527 | 92 | 171 | 404 | 391 | 507 | 145 |
| 112 | 139 | 225 | 350 | 258 | 130 | 385 | 868 | 998 | 235 | 181 |
| 113 | 278 | 370 | 1221 | 732 | 100 | 220 | 412 | 1837 | 1205 | 232 |
| 114 | 223 | 300 | 455 | 498 | 145 | 485 | 902 | 821 | 538 | 356 |
| 115 | 455 | 250 | 560 | 434 | 70 | 281 | 564 | 579 | 461 | 125 |
| 116 | 233 | 312 | 401 | 317 | 79 | 180 | 657 | 651 | 519 | 128 |
| 117 | 34 | 82 | 41 | 115 | 25 | 32 | 42 | 39 | 40 | 44 |
| 118 | 200 | 337 | 789 | 930 | 190 | 549 | 1820 | 1797 | 1134 | 206 |
| 119 | 267 | 383 | 358 | 230 | 70 | 209 | 565 | 772 | 399 | 97 |
| 120 | 277 | 261 | 424 | 438 | 86 | 171 | 457 | 620 | 451 | 139 |
| 121 | 217 | 338 | 752 | 545 | 90 | 197 | 504 | 476 | 346 | 116 |
| 122 | 197 | 392 | 470 | 1131 | 141 | 571 | 1771 | 1228 | 1486 | 193 |
| 123 | 255 | 433 | 1257 | 1607 | 172 | 504 | 1542 | 3129 | 2105 | 234 |

| | Rhodopsin | | | | | Melanopsin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cell** | **Acr** | **EqR** | **PNC** | **MP** | **T** | **Acr** | **EqR** | **PNC** | **MP** | **T** |
| 124 | 321 | 272 | 329 | 408 | 87 | 193 | 568 | 596 | 606 | 191 |
| 125 | 194 | 400 | 1457 | 1477 | 255 | 548 | 1487 | 1749 | 1244 | 256 |
| 126 | 99 | 286 | 325 | 299 | 60 | 142 | 392 | 573 | 486 | 101 |
| 127 | 149 | 147 | 155 | 279 | 50 | 116 | 244 | 469 | 354 | 114 |
| 128 | 230 | 356 | 384 | 334 | 30 | 309 | 426 | 454 | 431 | 82 |
| 129 | 187 | 216 | 150 | 330 | 41 | 215 | 547 | 408 | 289 | 88 |
| 130 | 340 | 331 | 554 | 387 | 76 | 164 | 448 | 579 | 450 | 121 |
| 131 | 179 | 220 | 556 | 1496 | 159 | 432 | 1813 | 1734 | 1570 | 216 |
| 132 | 475 | 302 | 504 | 493 | 68 | 230 | 530 | 604 | 521 | 125 |
| 133 | 255 | 416 | 323 | 307 | 77 | 197 | 829 | 667 | 501 | 130 |
| 134 | 117 | 151 | 298 | 657 | 59 | 80 | 111 | 171 | 154 | 55 |
| 135 | 166 | 292 | 351 | 626 | 162 | 329 | 742 | 1126 | 493 | 325 |
| 136 | 138 | 255 | 259 | 341 | 127 | 312 | 799 | 1171 | 455 | 313 |
| 137 | 260 | 183 | 318 | 668 | 118 | 265 | 743 | 769 | 310 | 255 |
| 138 | 145 | 317 | 541 | 350 | 76 | 155 | 590 | 648 | 473 | 121 |
| 139 | 341 | 178 | 249 | 539 | 79 | 368 | 230 | 229 | 248 | 88 |
| 140 | 103 | 133 | 120 | 77 | 33 | 51 | 93 | 82 | 66 | 46 |
| 141 | 149 | 446 | 477 | 574 | 86 | 145 | 585 | 1020 | 1090 | 120 |
| 142 | 179 | 253 | 281 | 617 | 126 | 165 | 543 | 504 | 542 | 147 |
| 143 | 295 | 271 | 383 | 715 | 94 | 134 | 703 | 709 | 713 | 139 |
| 144 | 260 | 460 | 521 | 480 | 133 | 266 | 949 | 1402 | 1045 | 193 |
| 145 | 171 | 187 | 168 | 734 | 134 | 104 | 127 | 118 | 278 | 73 |
| 146 | 415 | 290 | 332 | 241 | 78 | 138 | 296 | 860 | 570 | 114 |
| 147 | 324 | 292 | 979 | 381 | 55 | 197 | 1135 | 875 | 1017 | 95 |
| 148 | 40 | 183 | 30 | 61 | 28 | 30 | 49 | 44 | 33 | 26 |
| 149 | 78 | 236 | 753 | 312 | 33 | 66 | 362 | 203 | 124 | 20 |
| 150 | 198 | 246 | 498 | 380 | 112 | 311 | 835 | 905 | 283 | 161 |
| 151 | 388 | 270 | 517 | 635 | 74 | 125 | 409 | 504 | 505 | 128 |
| 152 | 431 | 783 | 228 | 289 | 116 | 212 | 1130 | 453 | 629 | 119 |
| 153 | 235 | 293 | 436 | 427 | 93 | 148 | 483 | 513 | 471 | 199 |
| 154 | 136 | 165 | 234 | 616 | 92 | 150 | 169 | 198 | 278 | 95 |
| 155 | 78 | 343 | 448 | 507 | 91 | 129 | 723 | 1123 | 1035 | 141 |
| 156 | 143 | 230 | 196 | 213 | 65 | 144 | 350 | 291 | 210 | 85 |
| 157 | 605 | 520 | 277 | 360 | 56 | 241 | 588 | 521 | 400 | 86 |
| 158 | 250 | 266 | 399 | 787 | 70 | 164 | 458 | 592 | 794 | 117 |
| 159 | 54 | 125 | 122 | 311 | 89 | 43 | 64 | 67 | 66 | 66 |
| 160 | 121 | 379 | 824 | 493 | 73 | 160 | 535 | 1002 | 985 | 104 |
| 161 | 64 | 82 | 40 | 39 | 34 | 27 | 36 | 23 | 24 | 26 |
| 162 | 136 | 113 | 155 | 132 | 158 | 79 | 80 | 90 | 67 | 48 |
| 163 | 153 | 358 | 369 | 665 | 61 | 174 | 447 | 744 | 390 | 106 |
| 164 | 316 | 484 | 659 | 387 | 118 | 187 | 678 | 1237 | 569 | 143 |

| | Rhodopsin | | | | | Melanopsin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cell** | **Acr** | **EqR** | **PNC** | **MP** | **T** | **Acr** | **EqR** | **PNC** | **MP** | **T** |
| 165 | 298 | 226 | 247 | 286 | 188 | 303 | 376 | 304 | 200 | 101 |
| 166 | 351 | 255 | 503 | 890 | 108 | 265 | 276 | 330 | 337 | 91 |
| 167 | 113 | 145 | 128 | 813 | 73 | 94 | 87 | 84 | 201 | 59 |
| 168 | 634 | 285 | 283 | 442 | 106 | 144 | 146 | 134 | 191 | 70 |
| 169 | 173 | 223 | 740 | 464 | 125 | 512 | 1346 | 1621 | 1030 | 231 |
| 170 | 232 | 296 | 160 | 728 | 37 | 134 | 400 | 509 | 682 | 76 |
| 171 | 255 | 266 | 249 | 401 | 49 | 213 | 314 | 798 | 689 | 116 |
| 172 | 55 | 117 | 35 | 63 | 32 | 61 | 69 | 44 | 40 | 36 |
| 173 | 180 | 203 | 560 | 579 | 83 | 190 | 440 | 859 | 993 | 117 |
| 174 | 201 | 194 | 183 | 346 | 94 | 225 | 331 | 392 | 528 | 140 |
| 175 | 168 | 395 | 244 | 358 | 159 | 331 | 873 | 790 | 418 | 218 |
| 176 | 99 | 390 | 366 | 544 | 121 | 149 | 850 | 801 | 1385 | 175 |
| 177 | 848 | 428 | 409 | 1265 | 240 | 363 | 1006 | 1234 | 627 | 357 |
| 178 | 157 | 350 | 477 | 375 | 89 | 224 | 1112 | 1668 | 652 | 142 |
| 179 | 428 | 190 | 235 | 678 | 111 | 258 | 231 | 238 | 307 | 110 |
| 180 | 210 | 629 | 699 | 624 | 126 | 200 | 1077 | 1450 | 1199 | 192 |
| 181 | 101 | 319 | 565 | 427 | 60 | 146 | 679 | 760 | 786 | 102 |
| 182 | 453 | 314 | 575 | 358 | 135 | 217 | 583 | 1483 | 730 | 230 |
| 183 | 224 | 212 | 352 | 452 | 55 | 161 | 312 | 499 | 615 | 118 |
| 184 | 162 | 226 | 541 | 394 | 66 | 234 | 324 | 531 | 578 | 139 |
| 185 | 190 | 483 | 635 | 1334 | 178 | 620 | 1877 | 1856 | 1477 | 274 |
| 186 | 80 | 397 | 244 | 485 | 40 | 83 | 747 | 620 | 738 | 87 |
| 187 | 197 | 182 | 171 | 160 | 88 | 204 | 321 | 281 | 216 | 79 |
| 188 | 189 | 213 | 214 | 189 | 80 | 230 | 323 | 375 | 236 | 83 |
| 189 | 452 | 381 | 1287 | 1094 | 264 | 629 | 1685 | 1848 | 1477 | 359 |
| 190 | 270 | 444 | 550 | 416 | 87 | 469 | 1437 | 1297 | 877 | 196 |
| 191 | 12 | 9 | 7 | 8 | 13 | 11 | 11 | 11 | 9 | 11 |
| 192 | 288 | 384 | 260 | 338 | 140 | 443 | 1141 | 813 | 384 | 287 |
| 193 | 677 | 503 | 561 | 377 | 136 | 339 | 960 | 1066 | 439 | 395 |
| 194 | 359 | 213 | 201 | 628 | 51 | 140 | 262 | 581 | 644 | 102 |
| 195 | 162 | 322 | 478 | 569 | 73 | 161 | 397 | 534 | 709 | 120 |
| 196 | 280 | 189 | 496 | 623 | 94 | 148 | 399 | 1158 | 965 | 157 |
| 197 | 381 | 312 | 326 | 561 | 59 | 175 | 411 | 658 | 502 | 110 |
| 198 | 225 | 384 | 1334 | 1438 | 303 | 666 | 1566 | 1894 | 2019 | 297 |
| 199 | 391 | 395 | 601 | 535 | 94 | 243 | 1275 | 1175 | 863 | 254 |
| 200 | 140 | 157 | 136 | 243 | 78 | 147 | 183 | 162 | 186 | 83 |
| 201 | 98 | 110 | 126 | 306 | 53 | 126 | 334 | 422 | 491 | 98 |
| 202 | 131 | 118 | 505 | 360 | 61 | 210 | 475 | 827 | 531 | 106 |
| 203 | 79 | 1148 | 218 | 319 | 74 | 146 | 486 | 441 | 427 | 89 |
| 204 | 333 | 483 | 290 | 229 | 60 | 319 | 570 | 699 | 280 | 94 |
| 205 | 142 | 187 | 99 | 262 | 58 | 123 | 417 | 491 | 329 | 94 |

| | Rhodopsin | | | | | Melanopsin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cell** | **Acr** | **EqR** | **PNC** | **MP** | **T** | **Acr** | **EqR** | **PNC** | **MP** | **T** |
| 206 | 112 | 129 | 528 | 534 | 52 | 140 | 396 | 902 | 749 | 77 |
| 207 | 55 | 166 | 359 | 440 | 82 | 97 | 350 | 451 | 603 | 98 |
| 208 | 155 | 225 | 371 | 471 | 80 | 168 | 429 | 832 | 696 | 98 |
| 209 | 175 | 208 | 190 | 227 | 92 | 163 | 407 | 466 | 498 | 182 |
| 210 | 257 | 294 | 450 | 539 | 53 | 161 | 516 | 807 | 1250 | 90 |
| 211 | 2246 | 2333 | 1450 | 651 | 180 | 520 | 650 | 702 | 382 | 73 |
| 212 | 580 | 684 | 1197 | 614 | 246 | 328 | 330 | 402 | 183 | 109 |
| 213 | 353 | 757 | 887 | 368 | 131 | 144 | 256 | 474 | 283 | 62 |
| 214 | 1824 | 1085 | 379 | 1005 | 291 | 485 | 360 | 242 | 502 | 95 |
| 215 | 970 | 894 | 497 | 764 | 124 | 342 | 436 | 334 | 309 | 80 |
| 216 | 1074 | 598 | 611 | 1058 | 82 | 485 | 433 | 446 | 466 | 92 |
| 217 | 896 | 442 | 212 | 247 | 75 | 322 | 261 | 172 | 189 | 60 |
| 218 | 467 | 591 | 463 | 288 | 233 | 218 | 290 | 259 | 264 | 111 |
| 219 | 656 | 171 | 247 | 351 | 77 | 242 | 150 | 236 | 198 | 75 |
| 220 | 394 | 206 | 156 | 242 | 80 | 205 | 208 | 212 | 232 | 56 |
| 221 | 478 | 229 | 131 | 261 | 101 | 324 | 185 | 150 | 204 | 68 |
| 222 | 119 | 808 | 672 | 960 | 69 | 102 | 243 | 372 | 661 | 61 |

**Table S1.** Intensity of rhodopsin and melanopsin staining in different regions of individual human sperm cells. Each row represents an individual sperm cell. The values shown are the relative fluorescens intensities in the acrosome (Acr), equatorial ring (EqR), postnuclear cap (PNC), midpiece (MP) and tail (T). Fluorescens intensities of rhodopsin and melanopsin higher than the threshold values (staining per unit area larger than the sum mean+SD of the negative control and larger than 150% of the mean of the negative control) are highlighted in green and red, respectively. Cells were considered as stained when the measured intensity of their staining per unit area was both larger than the sum mean+SD of the negative control (staining with secondary antibody only) and larger than 150% of the mean of the negative control. Only highlighted values were considered as true staining. The values shown are mean of 6 sperm samples from different donors.

| Animal | Treatment | VCL (μm/s) | VSL (μm/s) | VAP (μm/s) | LIN | WOB | Motility (%) |
|---|---|---|---|---|---|---|---|
| 1 | IBMX | 219±15 | 57±10 | 86±8 | 0.68±0.09 | 0.41±0.05 | 46 |
| | 2-APB | 247±26 | 41±8 | 70±8 | 0.57±0.08 | 0.29±0.02 | 39 |
| | Control | 233±22 | 50±9 | 71±5 | 0.71±0.11 | 0.32±0.04 | 36 |
| 2 | IBMX | 267±20 | 56±7 | 102±9 | 0.61±0.05 | 0.39±0.02 | 33 |
| | 2-APB | 230±22 | 48±5 | 76±6 | 0.66±0.04 | 0.36±0.02 | 44 |
| | Control | 206±14 | 47±5 | 71±5 | 0.65±0.04 | 0.35±0.01 | 36 |
| 3 | IBMX | 217±18 | 56±7 | 86±7 | 0.63±0.05 | 0.44±0.03 | 38 |
| | 2-APB | 271±12 | 46±4 | 94±4 | 0.51±0.03 | 0.36±0.01 | 54 |
| | Control | 187±14 | 52±5 | 71±5 | 0.72±0.03 | 0.40±0.01 | 24 |
| 4 | IBMX | 267±16 | 61±4 | 94±5 | 0.65±0.03 | 0.39±0.01 | 49 |
| | 2-APB | 250±10 | 64±4 | 93±4 | 0.70±0.02 | 0.38±0.01 | 38 |
| | Control | 269±13 | 60±4 | 92±4 | 0.64±0.02 | 0.37±0.01 | 37 |
| 5 | IBMX | 284±11 | 71±4 | 110±5 | 0.65±0.02 | 0.40±0.01 | 42 |
| | 2-APB | 269±9 | 59±3 | 106±4 | 0.59±0.02 | 0.41±0.01 | 50 |
| | Control | 250±14 | 66±7 | 107±7 | 0.60±0.04 | 0.45±0.02 | 42 |
| 6 | IBMX | 258±19 | 65±9 | 94±9 | 0.67±0.05 | 0.40±0.02 | 60 |
| | 2-APB | 256±33 | 51±7 | 100±14 | 0.54±0.05 | 0.42±0.04 | 31 |
| | Control | 254±20 | 64±7 | 91±9 | 0.70±0.04 | 0.36±0.02 | 31 |

**Table S2.** Effects of inhibitors of the transducin/cyclic nucleotide pathway and of the PLC pathway on the motility parameters of sperm cells of mice that do not express melanopsin. Sperm samples from *Opn4*$^{-/-}$*; Opn4:tdTomato*$^{+}$ mice were subjected to IBMX (1 mM), 2APB (0.1 mM), or, as a control, the solvent DMSO, and then the motility was analyzed (37°C). Abbreviations: VSL, straight-line velocity (the time-average velocity of the sperm head along a straight line from its first position to its last position, expressed in μm/s); VCL, curvilinear velocity (time-averaged velocity of a sperm head along its actual curvilinear path, expressed in μm/s); VAP, average path velocity (velocity over an average path generated by a roaming average, expressed in μm/s), LIN, linearity [defined as (VSL/VCL)×100][1]; MOT (%), percentage of motile spermatozoa. The values are the mean ± SEM of the indicated 6 mice (~300 cells analyzed for each mouse). None of the parameters in the presence of an inhibitor was significantly different from the no-inhibitor control.

| Animal | VCL (μm/s) | VSL (μm/s) | VAP (μm/s) | LIN | WOB | Motility (%) |
|---|---|---|---|---|---|---|
| Rho$^{-}$ Mel$^{-}$ (n=7) | 269±9 | 56±2 | 92±4* | 0.63±0.02 | 0.36±0.02* | 46 |
| Rho$^{+}$ Mel$^{+}$ (n=8) | 266±9 | 73±7 | 120±3 | 0.62±0.04 | 0.45±0.01 | 33 |

**Table S3.** Motility parameters of sperm cells of mice that do not express both melanopsin and rhodopsin (*Opn2*$^{-/-}$*; Opn4*$^{-/-}$*; Opn4:tdTomato*$^{+}$) and of wild-type mice. The results are expressed as mean ± SEM. *$P$<0.001 according to unpaired $t$ test. See Table S2 for details.

## References


1. Davis, R. O. & Siemers, R. J. Derivation and reliability of kinematic measures of sperm motion. *Reprod. Fertil. Dev.* **7,** 857–869 (1995).