

+ CXCR5 R180 test

PPD & Icos vs. Golgi Addition

January 21st, 2025

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY41	Old Adult		25E6	2-15-12	Nitrogen Inside	2	4.9E6	9.8	↑1.3 (1.6)
cvd 6022	PPD+ Adult		15E6	3-9-23		2	8.0E6	16.0	↑3.3

1:41 pm Thaw Start

NY041 used LN2

1:51 pm spin

2:05 pm DNase

2:15 pm stain Ctrl

Incubation @ 2:45 pm

8:59 PPD AND CD107a added
(4 μ L) (0.96 μ L)

780 μ L volume = 2.34 M cells

cvd 6022 + 80 μ L unstained ~240K cells

One NY041 and 1 cvd 6022 still pink

↑ Making it a ctrl

↑ And making it the golgi immediate

9 AM start in the AM ✓

- Golgi 0 @ 8:59 pm ✓
- Golgi 1 @ < 9:59 pm > ✓
- Golgi 2 ^{surf} @ < 10:59 pm > ✓
2^{intra} @ < 10:59 pm > ✓
- Golgi 3 @ < 11:59 pm > ✓
- Golgi 4 @ < 12:59 pm > ✓

Ab prep @ 8:45 AM

9:13 AM spin

9:30 AM zombie spin 9:45 AM

9:36 Hot spin 1400 B

9:46 cold spin 1400 B @ 10:20

10:06 samples Hot

↑ NO Icos @ this point

ctrl SEB

15
x 5
75 μ L

Extra
0 1 2 3 4
3M 3M 3M 3M 3M

+ CD107a

300
30 μ L

Intra
2M

5 μ L
x .66
3.30 μ L PPD
x 6
19.8 μ L PPD

An unstained is critical!

1.2
x .66
720
72
0.792 μ L

0.800
x 5
4 μ L

(PPD stim)
(PPD retention)
(Icos surface)

1.2
x .8
0.96

50 μ L Volume PFA-PBS 0.4% to all sds

10:36 AM wash

membrane

10:49 AM

Samples cold → 11:14 AM spin @ 11:22 AM

10:54 AM

Fix → 04 → 14 → 24

11:30 AM

strep → 11:45 AM

11:58 AM

spin @ 11:50

12:01 PM

sds Intra → 12:28

golgi @ 12:28 pm ✓

2nd wash @ 12:36 pm ✓

samples Intra @ 12:52 pm → 1:32 pm

3d @ 1:34 pm
4th @ 1:44 pm
Done 1:53

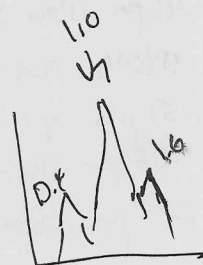
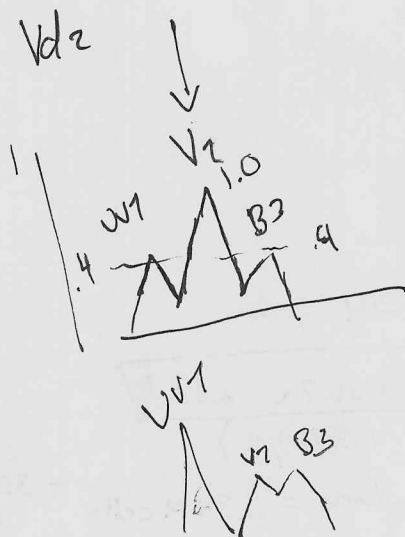
$$\begin{array}{r}
 300 \mu\text{L scb Fix} \\
 \times 36 \\
 \hline
 10,800
 \end{array}
 \qquad
 \begin{array}{r}
 600 \mu\text{L for samples} \\
 \times 6 \\
 \hline
 3.6 \text{ mL}
 \end{array}$$

$$\begin{array}{r}
 10.8 \\
 3.6 \\
 \hline
 15.4
 \end{array}
 \leftarrow
 \begin{array}{c}
 \text{Fix} \quad 25-75 \\
 \hline
 3.75 \text{ Fix} \\
 11.25 \text{ Diluent}
 \end{array}$$

$$\begin{array}{r}
 600 \\
 \times 4 \\
 \hline
 2.4 \text{ mL} \\
 \times 4 \\
 \hline
 9.6 \text{ mL}
 \end{array}
 \qquad
 \begin{array}{r}
 600 \mu\text{L / wash} \\
 \times 2 \\
 \hline
 1.2 \text{ mL} \\
 32 \\
 \hline
 2.4 \\
 36.0 \\
 38.4 \text{ mL} \\
 28.8 \text{ mL} \\
 9.6 \text{ mL} \\
 \hline
 76.8 \text{ mL wash}
 \end{array}
 \qquad
 \begin{array}{r}
 1.2 \text{ mL wash} \\
 \times 4 \\
 \hline
 4.8 \text{ mL / sample} \\
 \times 6 \\
 \hline
 28.8 \text{ mLs}
 \end{array}$$

$$\underline{8 \text{ mL}} + \underline{72 \text{ mL H}_2\text{O}}$$

Time 0	0.69	0.03	
Time 1	0.89	0.02	
Time 2	1.27	0.03	← Surface
	~ 1.17		← 1.51 intrac
Time 3	1.60	0.02	
Time 4	1.81	<u>0.02</u>	



January 21st, 2025

Intra Ice

January 21st, 2025

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!	6	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	6	Cold Stain 30min @4C	6	4°C for 15 min	RBC Lysis then Fix/Perm	Intranuclear Stain 40 min @RT	6
1	UV1	BUV395	CD62L	(DREG-56)	1							1.2	7.2				
2	UV2	BUV563	CD69	(FNSO)	2					1.8	10.8	0.5	3			0.8	4.8
3	UV3	BUV615	CD4	(1G1)	2							0.5	3			0.1	0.6
4	UV4	BUV737	CD38	(86)	2					1.5	9	0.5	3			0.2	1.2
5	UV5	BUV805	CD4	(SK3)	1												
6	UV6	BUV805	CD4	(SK3)	1												
7	UV7	BUV805	CD4	(SK3)	1												
8	UV8	BUV805	CD4	(SK3)	1												
9	UV9	BUV805	CD4	(SK3)	1												
10	UV10	BUV805	CD4	(SK3)	1												
11	UV11	BUV805	CD4	(SK3)	1												
12	UV12	BUV805	CD4	(SK3)	1												
13	UV13	BUV805	CD4	(SK3)	1												
14	UV14	BUV805	CD4	(SK3)	1												
15	UV15	BUV805	CD4	(SK3)	1												
16	UV16	BUV805	CD4	(SK3)	1												
17	UV17	BUV805	CD4	(SK3)	1												
18	UV18	BUV805	CD4	(SK3)	1												
19	UV19	BUV805	CD4	(SK3)	1												
20	UV20	BUV805	CD4	(SK3)	1												
21	UV21	BUV805	CD4	(SK3)	1												
22	UV22	BUV805	CD4	(SK3)	1												
23	UV23	BUV805	CD4	(SK3)	1												
24	UV24	BUV805	CD4	(SK3)	1												
25	UV25	BUV805	CD4	(SK3)	1												
26	UV26	BUV805	CD4	(SK3)	1												
27	UV27	BUV805	CD4	(SK3)	1												
28	UV28	BUV805	CD4	(SK3)	1												
29	UV29	BUV805	CD4	(SK3)	1												
30	UV30	BUV805	CD4	(SK3)	1												
31	UV31	BUV805	CD4	(SK3)	1												
32	UV32	BUV805	CD4	(SK3)	1												
33	UV33	BUV805	CD4	(SK3)	1												
34	UV34	BUV805	CD4	(SK3)	1												
35	UV35	BUV805	CD4	(SK3)	1												
36	UV36	BUV805	CD4	(SK3)	1												
37	UV37	BUV805	CD4	(SK3)	1												
38	UV38	BUV805	CD4	(SK3)	1												
39	UV39	BUV805	CD4	(SK3)	1												
40	UV40	BUV805	CD4	(SK3)	1												
41	UV41	BUV805	CD4	(SK3)	1												
42	UV42	BUV805	CD4	(SK3)	1												
43	UV43	BUV805	CD4	(SK3)	1												
44	UV44	BUV805	CD4	(SK3)	1												
45	UV45	BUV805	CD4	(SK3)	1												
46	UV46	BUV805	CD4	(SK3)	1												
47	UV47	BUV805	CD4	(SK3)	1												
48	UV48	BUV805	CD4	(SK3)	1												
49	UV49	BUV805	CD4	(SK3)	1												
50	UV50	BUV805	CD4	(SK3)	1												
51	UV51	BUV805	CD4	(SK3)	1												
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53	UV53	BUV805	CD4	(SK3)	1												
54	UV54	BUV805	CD4	(SK3)	1												
55	UV55	BUV805	CD4	(SK3)	1												
56	UV56	BUV805	CD4	(SK3)	1												
57	UV57	BUV805	CD4	(SK3)	1												
58	UV58	BUV805	CD4	(SK3)	1												
59	UV59	BUV805	CD4	(SK3)	1												
60	UV60	BUV805	CD4	(SK3)	1												
61	UV61	BUV805	CD4	(SK3)	1												
62	UV62	BUV805	CD4	(SK3)	1												
63	UV63	BUV805	CD4	(SK3)	1												
64	UV64	BUV805	CD4	(SK3)	1												
65	UV65	BUV805	CD4	(SK3)	1												
66	UV66	BUV805	CD4	(SK3)	1												
67	UV67	BUV805	CD4	(SK3)	1												
68	UV68	BUV805	CD4	(SK3)	1												
69	UV69	BUV805	CD4	(SK3)	1												
70	UV70	BUV805	CD4	(SK3)	1												
71	UV71	BUV805	CD4	(SK3)	1												
72	UV72	BUV805	CD4	(SK3)	1												
73	UV73	BUV805	CD4	(SK3)	1												
74	UV74	BUV805	CD4	(SK3)	1												
75	UV75	BUV805	CD4	(SK3)	1												
76	UV76	BUV805	CD4	(SK3)	1												
77	UV77	BUV805	CD4	(SK3)	1												
78	UV78	BUV805	CD4	(SK3)	1												
79	UV79	BUV805	CD4	(SK3)	1												
80	UV80	BUV805	CD4	(SK3)	1												
81	UV81	BUV805	CD4	(SK3)	1												
82	UV82	BUV805	CD4	(SK3)	1												
83	UV83	BUV805	CD4	(SK3)	1												
84	UV84	BUV805	CD4	(SK3)	1												
85	UV85	BUV805	CD4	(SK3)	1												
86	UV86	BUV805	CD4	(SK3)	1												
87	UV87	BUV805	CD4	(SK3)	1												
88	UV88	BUV805	CD4	(SK3)	1												
89	UV89	BUV805	CD4	(SK3)	1												
90	UV90	BUV805	CD4	(SK3)	1												
91	UV91	BUV805	CD4	(SK3)	1												
92	UV92	BUV805	CD4	(SK3)	1												
93	UV93	BUV805	CD4	(SK3)	1												
94	UV94	BUV805	CD4	(SK3)	1												
95	UV95	BUV805	CD4	(SK3)	1												
96	UV96	BUV805	CD4	(SK3)	1												
97	UV97	BUV805	CD4	(SK3)	1												
98	UV98	BUV805	CD4	(SK3)	1												
99	UV99	BUV805	CD4	(SK3)	1												
100	UV100	BUV805	CD4	(SK3)	1												

αβ T cell SFC panel

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of Live/Dead mix (1:2500) @RT for 15min
Wash 1.5 ml 5% PBS-FBS, spin 1300 rpm, 8min
Add CD161 bcrn antibody for 10 minutes at RT
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min
Add Hoechst mix, incubate @ 37C for 30min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min
Add ColdStain mix, incubate @ 4C for 30min
Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mix, incubate @ 4C for 15min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min
0.8 ml Nuclear FixPerm, incubate @ 4C for 30min
(vortex every 10 minutes)

First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min
Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min
Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min
Resuspend in 80 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

32 5 doses

RF

5.1411 CD5c
B-A19
CL405-652c4
Coralite

AlexaFluor 405

Dylight 405

Span Violet 423

Super Bright 438

Viobright 423

32 5 doses

4-on-off

48 31.2 46.8 93.6 187.2 280.8

12.6 37.8 41.2 75.6 151.2 43.8 15.6 31.2 46.8 93.6 187.2 280.8

12.6 37.8 41.2 75.6 151.2 43.8 15.6 31.2 46.8 93.6 187.2 280.8

... 2000 2 0 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1

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Inf 914-4 HU ♂

41415

2022-02-10

2022-06-16

↑ 3 up 2024

12A Row G, Col 2

(12B Row A Col 2)