**Supplemental Material for:**

**Title:** Blood handling and leukocyte isolation methods impact the global transcriptome of immune cells

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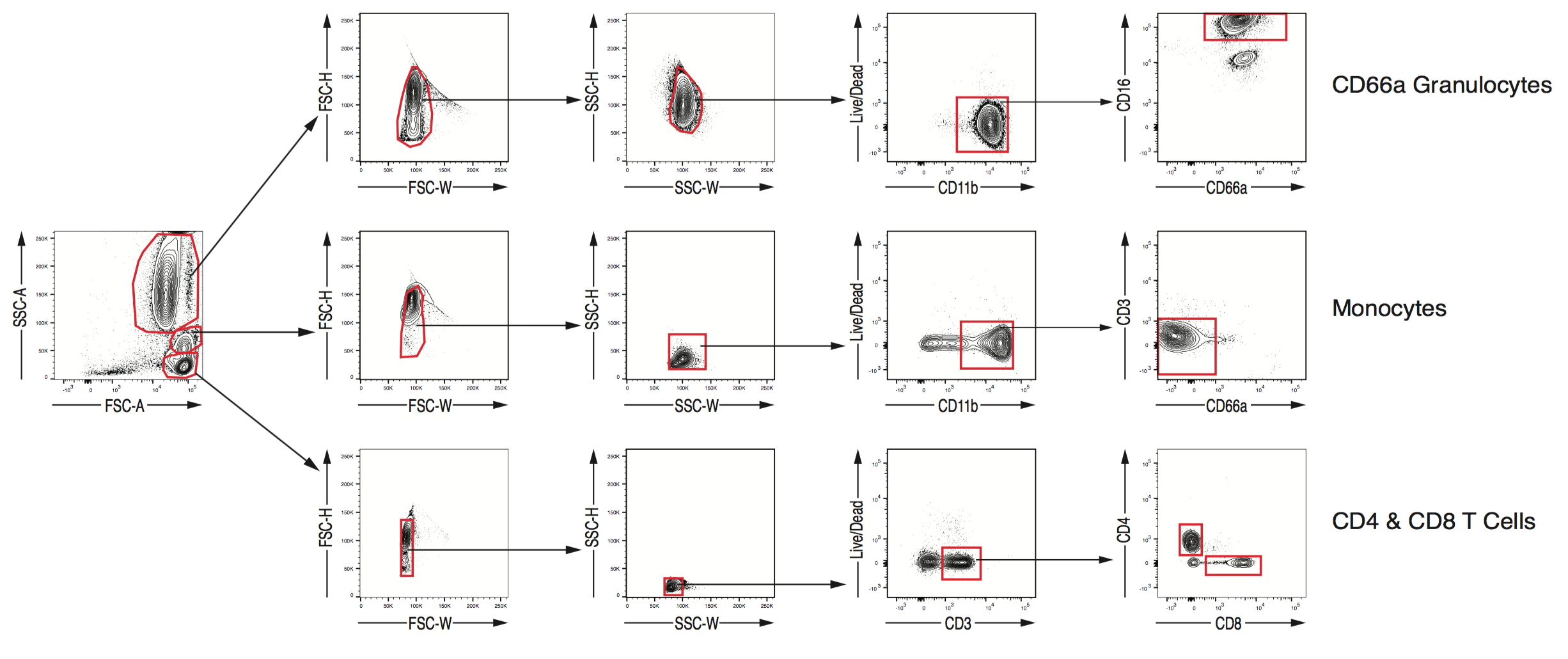
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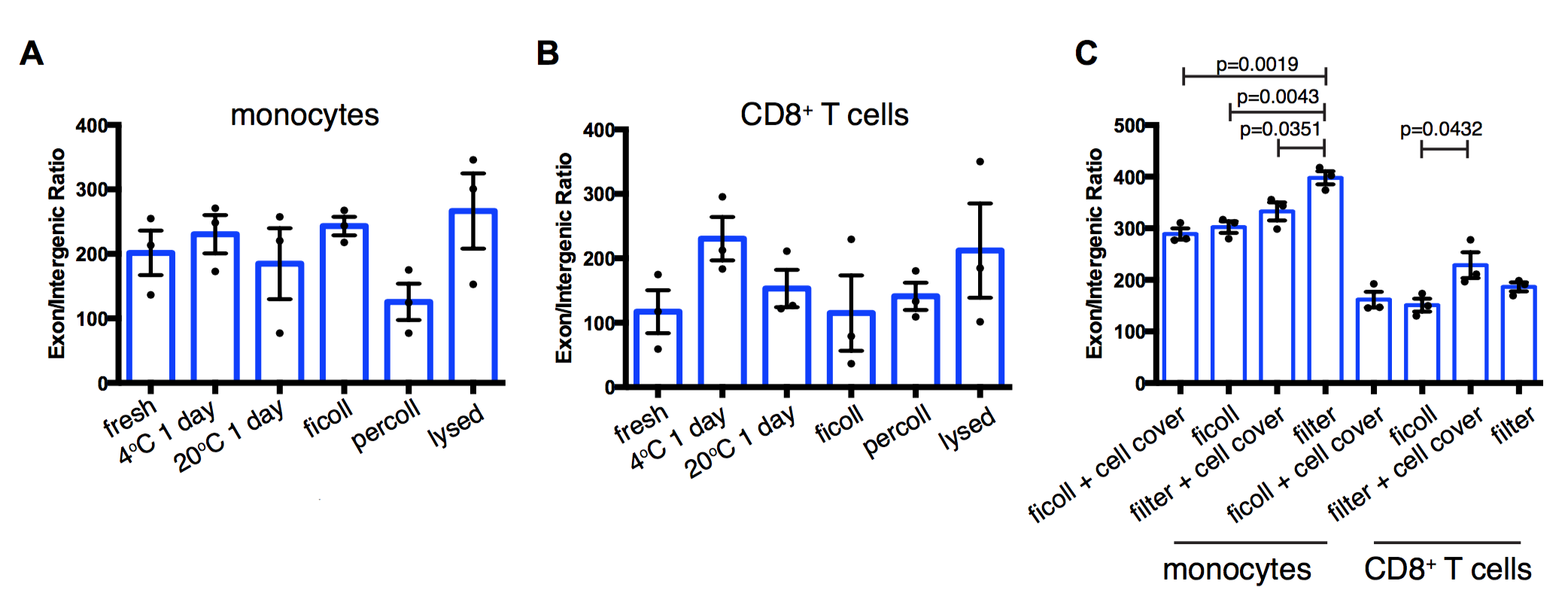
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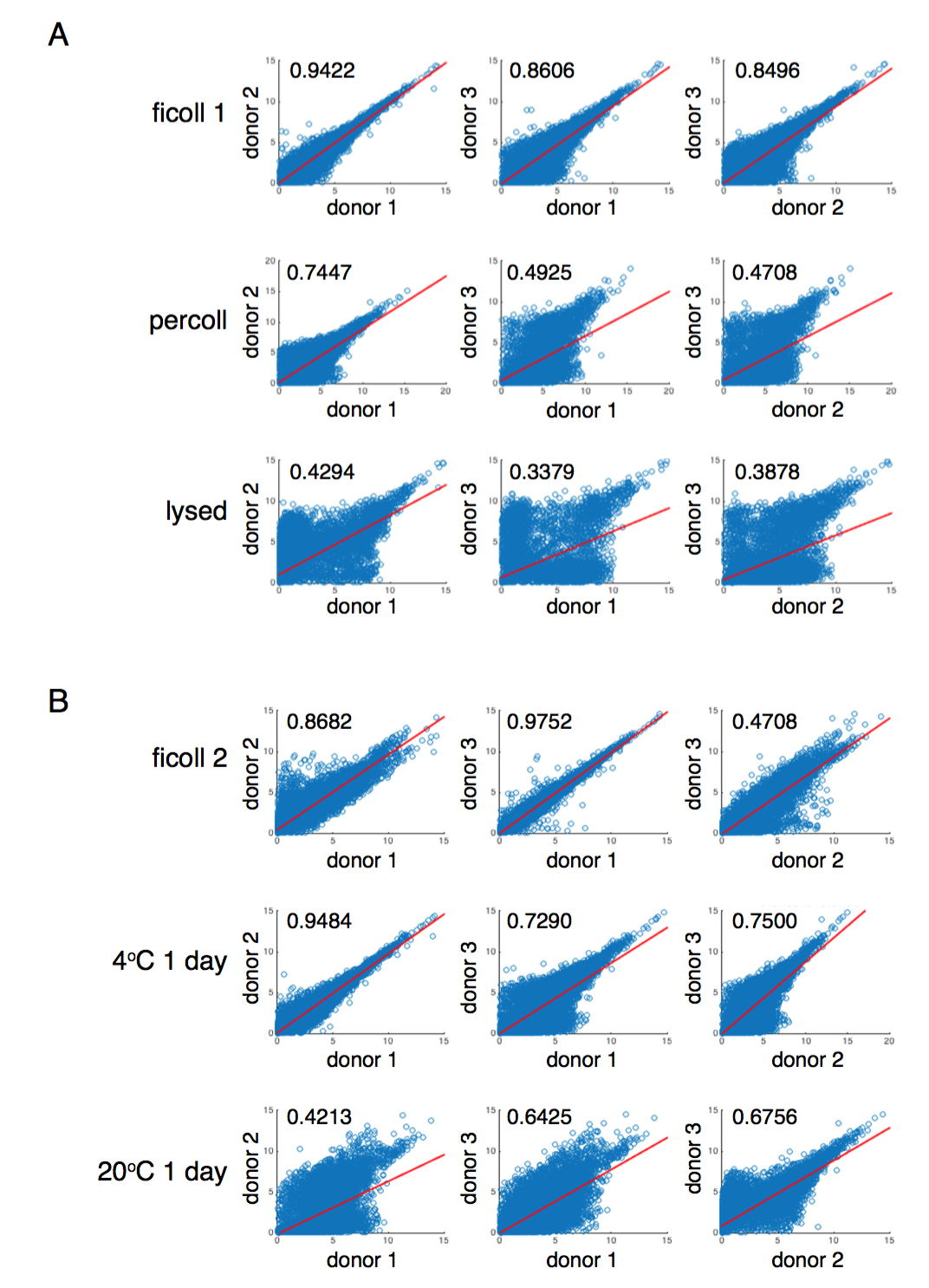
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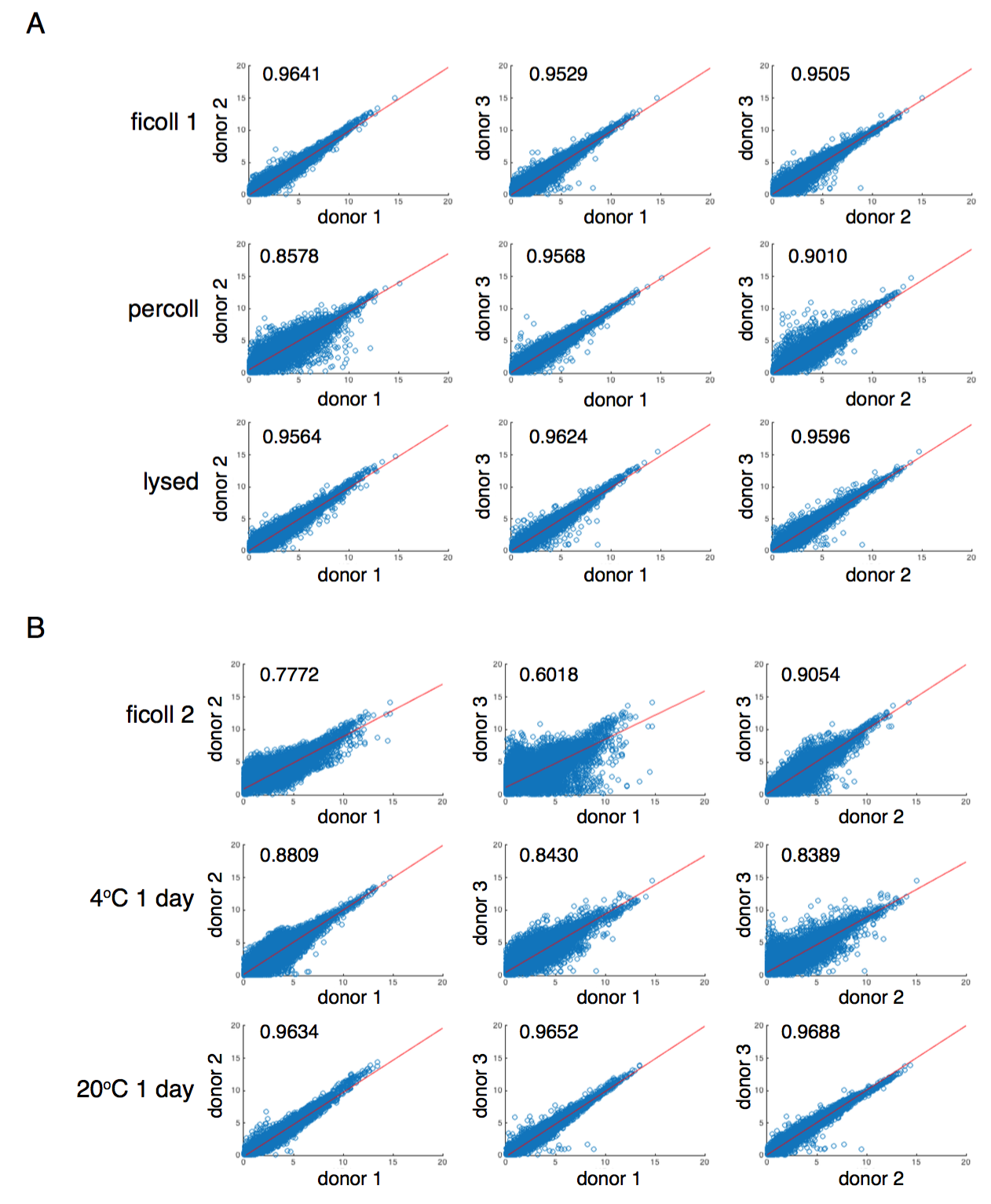
**Supplemental Figure 1**. Sorting strategy used to profile peripheral blood mononuclear cells (PBMCs) from blood of healthy donors across all conditions tested. Antibodies are listed in Table 1.



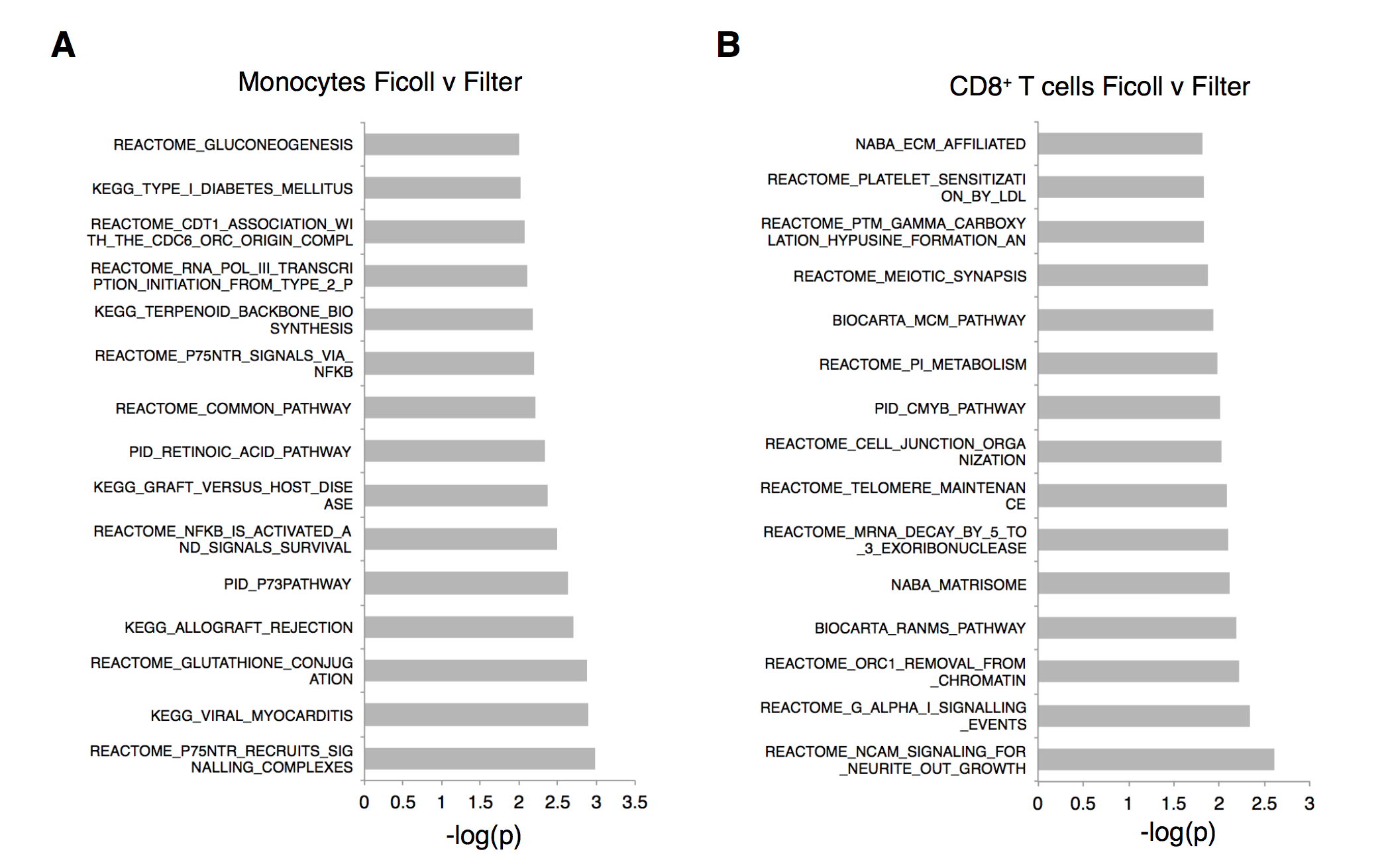
**Supplemental Figure 2**. Exon/intergenic ratios are plotted for each indicated condition for (**A**) monocytes, (**B**) T cells and (**C**) for filtration as compared to ficoll. Statistically significant comparisons are indicated and were calculated by one-way ANOVA with Tukey’s multiple comparisons test.



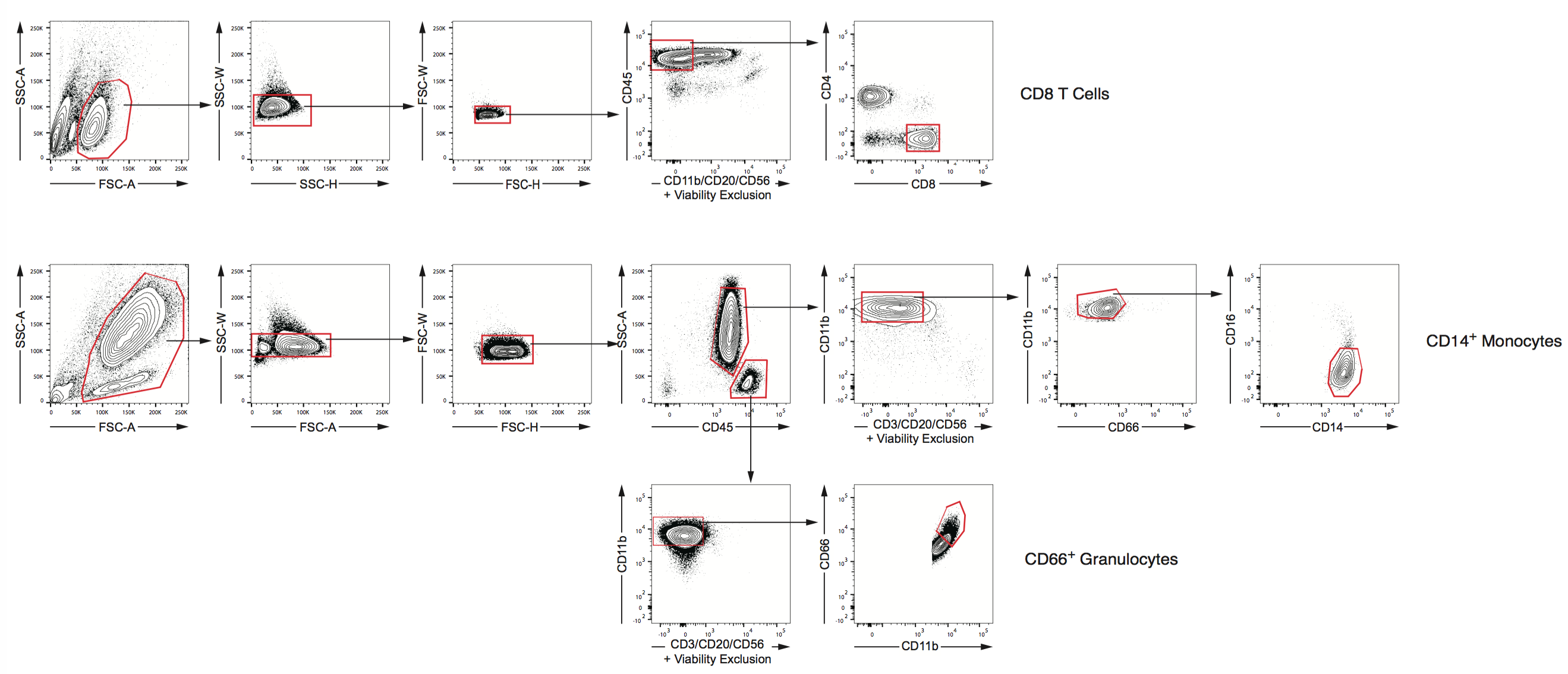
**Supplemental Figure 3. Pairwise scatter plots of coding transcriptomes generated from monocytes for each indicated comparison.** Regression lines and R2 values are shown on each plot for **(A**) ficoll, percoll and lysis processing conditions, and (**B**) ficoll, 4°C for 1 day or 20°C for 1 day conditions.



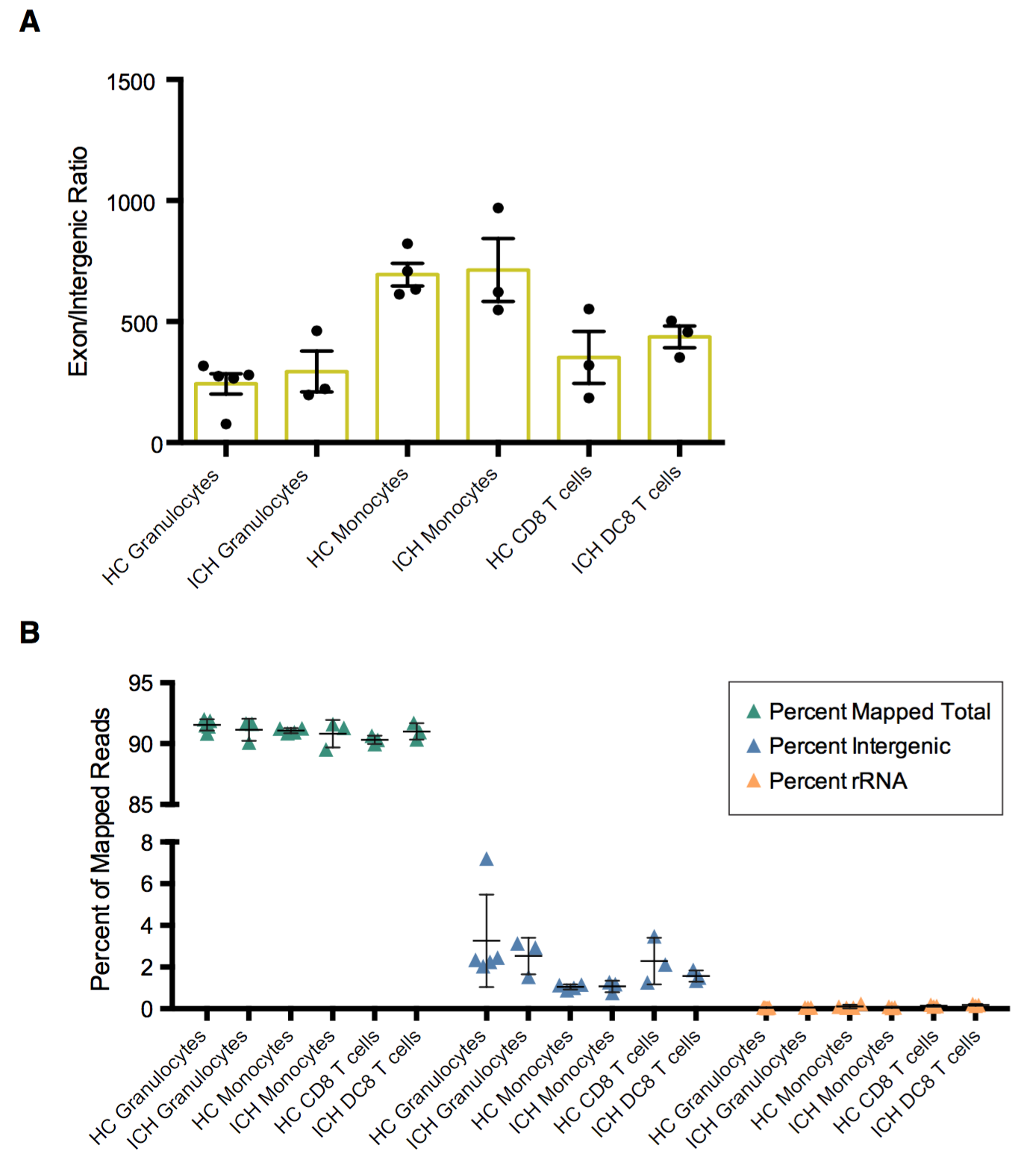
**Supplemental Fig 4. Pairwise scatter plots of coding transcriptomes generated from CD8+ T cells for each indicated comparison.** Regression lines and R2 values are shown on each plot for **(A**) ficoll, percoll and lysis processing conditions, and (**B**) ficoll, 4°C for 1 day or 20°C for 1 day conditions.



**Supplemental Figure 5.** **ssGSEA results for ficoll and filter methods for isolation of PBMCs.** Forest plots of top 15 significantly altered gene sets when PBMCs are isolated using filters for monocytes (**A**) and CD8+ T cells (**B**).



**Supplemental Figure 6**. Flow cytometry isolation scheme for sequencing data generated from cells isolated from intracerebral hemorrhage (ICH) and matched healthy donors (HD).



**Supplemental Figure 7**. Quality control metrics for sequencing data generated from cells isolated from intracerebral hemorrhage (ICH) and matched healthy donors (HD). (**A**) Exon/intergenic ratio for each indicated condition. No statistically significant differences were found when comparing healthy to ICH within each cell type by students t test. (**B**) Percent mapped reads for each indicated condition. No statistically significant differences were found when comparing healthy to ICH within each cell type by students t test for each percent metric plotted.

**Supplemental Table 1. Antibodies used for cell sorting in this study.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Supplemental Table 1: Antibodies used for the cell sorting** | | | | |
| 1. **Sort panel I** | | | | |
| **Marker** | **Label** | **Clone** | **Supplier** | **Catalog #** |
| CD3 | APC/H7 | SK7 | BD | 560176 |
| CD4 | V450 | RPA-T4 | BD | 560345 |
| CD8 | V500 | RPA-T8 | BD | 560774 |
| CD11b | FITC | ICRF44 | eBioscience | 11-0118-42 |
| CD16 | PE/Cy7 | 3G8 | BD | 557744 |
| CD25 | PE | M-A251 | BD | 555432 |
| CD45 | PerCP/Cy5.5 | H130 | Tonbo | 65-0459-T100 |
| CD66a | PE | B1.1 | BD | 564105 |
| CD127 | AF 647 | HIL-7R-M21 | BD | 558598 |
| Live/Dead | Fixable red dye |  | Life Technologies | L34972 |
| 1. **Sort panel II – T cells** | | | | |
| **Marker** | **Label** | **Clone** | **Supplier** | **Catalog #** |
| CD2 | APC/H7 | RPA-2.10 | BD | 562638 |
| CD4 | violetFluor 450 | RPA-T4 | Tonbo | 75-0049-T100 |
| CD8 | PE/Cy7 | RPA-T8 | Tonbo | 60-0088-T100 |
| CD11b | Biotin | M1/70 | Tonbo | 30-0112-U500 |
| CD20 | Biotin | 2H7 | Biolegend | 302350 |
| CD25 | PE | M-A251 | BD | 555432 |
| CD45 | FITC | HI30 | Tonbo | 35-0459-T100 |
| CD56 | Biotin | HCD56 | Biolegend | 318320 |
| CD127 | APC | R34-34 | Tonbo | 20-1278-T100 |
| Streptavidin | PE-CF594 |  | BD | 562318 |
| Live/Dead | Fixable red dye |  | Life Technologies | L34972 |
| 1. **Sort panel II – Innate cells** | | | | |
| **Marker** | **Label** | **Clone** | **Supplier** | **Catalog #** |
| CD2 | Biotin | RPA-2.10 | Biolegend | 300204 |
| CD11b | FITC | ICRF44 | Tonbo | 35-0118-T100 |
| CD14 | APC/Cy7 | M5E2 | Biolegend | 301820 |
| CD16 | v450 | 3G8 | BD | 560474 |
| CD20 | Biotin | 2H7 | Biolegend | 302350 |
| CD45 | PE | HI30 | Tonbo | 50-0459-T100 |
| CD56 | Biotin | HCD56 | Biolegend | 318320 |
| CD66a/c/e | PE/Cy7 | ASL-32 | Biolegend | 342310 |
| Streptavidin | BV510 |  | Biolegend | 405234 |
| Live/Dead | Fixable red dye |  | Life Technologies | L34972 |

**Supplemental Table 2**. Summary statistics performed by one-way ANOVA with Tukey’s multiple comparisons test for data shown in Figure 2.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Figure** | **Cell Type** | **Tukey's multiple comparisons test** | **Mean Diff.** | **95.00% CI of diff.** | **Significant?** | **Summary** | **Adjusted P Value** |
| **2A** | CD4 T cells | Freshly Isolated vs. 1 Day 20C | 14.31 | -1.799 to 30.42 | No | ns | 0.0767 |
| CD4 T cells | Freshly Isolated vs. 1 Day 4C | 20.44 | 4.336 to 36.55 | Yes | \* | 0.0188 |
| CD4 T cells | 1 Day 20C vs. 1 Day 4C | 6.134 | -9.974 to 22.24 | No | ns | 0.5118 |
| CD8 T cells | Freshly Isolated vs. 1 Day 20C | 6.237 | -3.844 to 16.32 | No | ns | 0.2193 |
| CD8 T cells | Freshly Isolated vs. 1 Day 4C | 7.647 | -2.434 to 17.73 | No | ns | 0.1272 |
| CD8 T cells | 1 Day 20C vs. 1 Day 4C | 1.41 | -8.671 to 11.49 | No | ns | 0.905 |
| Monocytes | Freshly Isolated vs. 1 Day 20C | 2.31 | -5.792 to 10.41 | No | ns | 0.6743 |
| Monocytes | Freshly Isolated vs. 1 Day 4C | 3.249 | -4.852 to 11.35 | No | ns | 0.4798 |
| Monocytes | 1 Day 20C vs. 1 Day 4C | 0.9396 | -7.162 to 9.041 | No | ns | 0.9333 |
| Granulocytes | Freshly Isolated vs. 1 Day 20C | -3.483 | -9.523 to 2.556 | No | ns | 0.2573 |
| Granulocytes | Freshly Isolated vs. 1 Day 4C | -14.86 | -20.89 to -8.816 | Yes | \*\*\* | 0.0007 |
| Granulocytes | 1 Day 20C vs. 1 Day 4C | -11.37 | -17.41 to -5.333 | Yes | \*\* | 0.0028 |
| **2B** | CD4 T cells | Ficoll Gradient vs. Whole Blood Lysis | 21.83 | 8.283 to 35.37 | Yes | \*\* | 0.0062 |
| CD4 T cells | Ficoll Gradient vs. Collagenase + Percoll Gradient | 26.84 | 13.3 to 40.39 | Yes | \*\* | 0.0022 |
| CD4 T cells | Whole Blood Lysis vs. Collagenase + Percoll Gradient | 5.019 | -8.523 to 18.56 | No | ns | 0.5283 |
| CD8 T cells | Ficoll Gradient vs. Whole Blood Lysis | 10.18 | 1.301 to 19.06 | Yes | \* | 0.0291 |
| CD8 T cells | Ficoll Gradient vs. Collagenase + Percoll Gradient | 10.79 | 1.914 to 19.68 | Yes | \* | 0.0227 |
| CD8 T cells | Whole Blood Lysis vs. Collagenase + Percoll Gradient | 0.6132 | -8.268 to 9.494 | No | ns | 0.9757 |
| Monocytes | Ficoll Gradient vs. Whole Blood Lysis | 9.179 | -4.263 to 22.62 | No | ns | 0.1711 |
| Monocytes | Ficoll Gradient vs. Collagenase + Percoll Gradient | -11.97 | -25.41 to 1.471 | No | ns | 0.076 |
| Monocytes | Whole Blood Lysis vs. Collagenase + Percoll Gradient | -21.15 | -34.59 to -7.708 | Yes | \*\* | 0.007 |
| Granulocytes | Ficoll Gradient vs. Whole Blood Lysis | -38.12 | -54.85 to -21.39 | Yes | \*\* | 0.001 |
| Granulocytes | Ficoll Gradient vs. Collagenase + Percoll Gradient | -17.54 | -34.27 to -0.8039 | Yes | \* | 0.0418 |
| Granulocytes | Whole Blood Lysis vs. Collagenase + Percoll Gradient | 20.58 | 3.853 to 37.32 | Yes | \* | 0.0216 |
| **2C** | CD4 T cells | Ficoll vs. Ficoll + Cellcover | -2.658 | -14.36 to 9.04 | No | ns | 0.8834 |
| CD4 T cells | Ficoll vs. Whole Blood Filtration | 10.22 | -1.474 to 21.92 | No | ns | 0.0884 |
| CD4 T cells | Ficoll vs. Whole Blood Filtration + Cellcover | 10.08 | -1.622 to 21.77 | No | ns | 0.0936 |
| CD4 T cells | Ficoll + Cellcover vs. Whole Blood Filtration | 12.88 | 1.184 to 24.58 | Yes | \* | 0.0318 |
| CD4 T cells | Ficoll + Cellcover vs. Whole Blood Filtration + Cellcover | 12.73 | 1.036 to 24.43 | Yes | \* | 0.0336 |
| CD4 T cells | Whole Blood Filtration vs. Whole Blood Filtration + Cellcover | -0.1481 | -11.85 to 11.55 | No | ns | >0.9999 |
| CD8 T cells | Ficoll vs. Ficoll + Cellcover | -0.7315 | -7.549 to 6.086 | No | ns | 0.985 |
| CD8 T cells | Ficoll vs. Whole Blood Filtration | -0.4832 | -7.301 to 6.334 | No | ns | 0.9955 |
| CD8 T cells | Ficoll vs. Whole Blood Filtration + Cellcover | -0.3196 | -7.137 to 6.498 | No | ns | 0.9987 |
| CD8 T cells | Ficoll + Cellcover vs. Whole Blood Filtration | 0.2484 | -6.569 to 7.066 | No | ns | 0.9994 |
| CD8 T cells | Ficoll + Cellcover vs. Whole Blood Filtration + Cellcover | 0.412 | -6.406 to 7.229 | No | ns | 0.9972 |
| CD8 T cells | Whole Blood Filtration vs. Whole Blood Filtration + Cellcover | 0.1636 | -6.654 to 6.981 | No | ns | 0.9998 |
| Monocytes | Ficoll vs. Ficoll + Cellcover | 4.604 | -3.839 to 13.05 | No | ns | 0.3625 |
| Monocytes | Ficoll vs. Whole Blood Filtration | 12.23 | 3.782 to 20.67 | Yes | \*\* | 0.0073 |
| Monocytes | Ficoll vs. Whole Blood Filtration + Cellcover | 13.91 | 5.47 to 22.36 | Yes | \*\* | 0.0033 |
| Monocytes | Ficoll + Cellcover vs. Whole Blood Filtration | 7.621 | -0.8225 to 16.06 | No | ns | 0.0777 |
| Monocytes | Ficoll + Cellcover vs. Whole Blood Filtration + Cellcover | 9.309 | 0.8657 to 17.75 | Yes | \* | 0.0316 |
| Monocytes | Whole Blood Filtration vs. Whole Blood Filtration + Cellcover | 1.688 | -6.755 to 10.13 | No | ns | 0.916 |
| Granulocytes | Ficoll vs. Ficoll + Cellcover | 0.1653 | -15.45 to 15.78 | No | ns | >0.9999 |
| Granulocytes | Ficoll vs. Whole Blood Filtration | -39.91 | -55.52 to -24.29 | Yes | \*\*\* | 0.0002 |
| Granulocytes | Ficoll vs. Whole Blood Filtration + Cellcover | -30.85 | -46.46 to -15.23 | Yes | \*\* | 0.001 |
| Granulocytes | Ficoll + Cellcover vs. Whole Blood Filtration | -40.07 | -55.68 to -24.46 | Yes | \*\*\* | 0.0002 |
| Granulocytes | Ficoll + Cellcover vs. Whole Blood Filtration + Cellcover | -31.01 | -46.63 to -15.4 | Yes | \*\*\* | 0.001 |
| Granulocytes | Whole Blood Filtration vs. Whole Blood Filtration + Cellcover | 9.059 | -6.554 to 24.67 | No | ns | 0.3162 |