

In the format provided by the authors and unedited.

# Integrated single-cell analysis of multicellular immune dynamics during hyperacute HIV-1 infection

Samuel W. Kazer<sup>1,2,3,4</sup>, Toby P. Aicher<sup>1,2,4</sup>, Daniel M. Muema<sup>5,6</sup>, Shaina L. Carroll<sup>7</sup>, Jose Ordoñas-Montanes<sup>1,2,3,4,8,9</sup>, Vincent N. Miao<sup>1,2,4,10</sup>, Ang A. Tu<sup>4,11,12</sup>, Carly G. K. Ziegler<sup>1,2,4,10</sup>, Sarah K. Nyquist<sup>1,2,4,13,14</sup>, Emily B. Wong<sup>5,15,16,17</sup>, Nasreen Ismail<sup>6</sup>, Mary Dong<sup>1</sup>, Amber Moodley<sup>6</sup>, Bonnie Berger<sup>14,18</sup>, J. Christopher Love<sup>1,11,12</sup>, Krista L. Dong<sup>1</sup>, Alasdair Leslie<sup>5,16</sup>, Zaza M. Ndhlovu<sup>1,5,6,19</sup>, Thumbi Ndung'u<sup>1,5,6,16,20</sup>, Bruce D. Walker<sup>1,6,19,21</sup> ✉ and Alex K. Shalek<sup>1,2,3,4,10,11,13,21</sup> ✉

<sup>1</sup>Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA. <sup>2</sup>Institute for Medical Engineering and Science (IMES), Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>3</sup>Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>4</sup>Broad Institute of MIT and Harvard, Cambridge, MA, USA. <sup>5</sup>African Health Research Institute, Durban, South Africa. <sup>6</sup>HIV Pathogenesis Programme, Nelson R. Mandela School of Medicine, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban, South Africa. <sup>7</sup>Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA. <sup>8</sup>Division of Gastroenterology, Boston Children's Hospital, Boston, MA, USA. <sup>9</sup>Harvard Stem Cell Institute, Cambridge, MA, USA. <sup>10</sup>Program in Health Sciences and Technology, Harvard Medical School & Massachusetts Institute of Technology, Boston, MA, USA. <sup>11</sup>Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>12</sup>Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>13</sup>Program in Computational and Systems Biology, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>14</sup>Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>15</sup>Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA. <sup>16</sup>Division of Infection and Immunity, University College London, London, UK. <sup>17</sup>Harvard Medical School, Boston, MA, USA. <sup>18</sup>Department of Mathematics, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>19</sup>Howard Hughes Medical Institute, Chevy Chase, MD, USA. <sup>20</sup>Max Planck Institute for Infection Biology, Berlin, Germany. <sup>21</sup>These authors contributed equally: Bruce D. Walker, Alex K. Shalek. ✉e-mail: [BWALKER@mgm.harvard.edu](mailto:BWALKER@mgm.harvard.edu); [shalek@mit.edu](mailto:shalek@mit.edu)

## **Supplementary Information for:**

# **Integrated Single-Cell Analysis of Multicellular Immune Dynamics during Hyper-Acute HIV-1 Infection**

Samuel W. Kazer<sup>1,2,3,4</sup>, Toby P. Aicher<sup>1,2,4</sup>, Daniel M. Muema<sup>5,6</sup>, Shaina L. Carroll<sup>7</sup>, Jose Ordovas-Montanes<sup>1,2,3,4,8,9</sup>, Vincent N. Miao<sup>1,2,4,10</sup>, Ang A. Tu<sup>4,11,12</sup>, Carly G. K. Ziegler<sup>1,2,4,10</sup>, Sarah K. Nyquist<sup>1,2,4,13,14</sup>, Emily B. Wong<sup>5,15,16</sup>, Nasreen Ismail<sup>6</sup>, Mary Dong<sup>1</sup>, Amber Moodley<sup>6</sup>, Bonnie Berger<sup>14,17</sup>, J. Christopher Love<sup>1,11,12</sup>, Krista L. Dong<sup>1</sup>, Alasdair Leslie<sup>5,16</sup>, Zaza M. Ndhlovu<sup>1,5,6,18</sup>, Thumbi Ndung'u<sup>1,5,6,16,19</sup>, Bruce D. Walker<sup>1,6,18,†,\*</sup>, and Alex K. Shalek<sup>1,2,3,4,10,11,13,†,\*</sup>

## **Affiliations**

<sup>1</sup>Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, USA

<sup>2</sup>Institute for Medical Engineering and Science (IMES), Massachusetts Institute of Technology, Cambridge, MA, USA

<sup>3</sup>Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, USA

<sup>4</sup>Broad Institute of MIT and Harvard, Cambridge, MA, USA

<sup>5</sup>African Health Research Institute, Durban, South Africa

<sup>6</sup>HIV Pathogenesis Programme, Nelson R. Mandela School of Medicine, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban, South Africa

<sup>7</sup>Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA

<sup>8</sup>Division of Gastroenterology, Boston Children's Hospital, Boston, MA, USA

<sup>9</sup>Harvard Stem Cell Institute, Cambridge, MA, USA

<sup>10</sup>Program in Health Sciences and Technology, Harvard Medical School and Massachusetts Institute of Technology, Boston, MA, USA

<sup>11</sup>Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology,  
Cambridge, MA, USA

<sup>12</sup>Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA,  
USA

<sup>13</sup>Program in Computational and Systems Biology, Massachusetts Institute of Technology,  
Cambridge, MA, USA

<sup>14</sup>Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of  
Technology, Cambridge, MA, USA

<sup>15</sup>Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA; Harvard  
Medical School, Boston, MA, USA

<sup>16</sup>Division of Infection and Immunity, University College London, London, UK

<sup>17</sup>Department of Mathematics, Massachusetts Institute of Technology, Cambridge, MA, USA

<sup>18</sup>Howard Hughes Medical Institute, Chevy Chase, MD, USA

<sup>19</sup>Max Planck Institute for Infection Biology, Berlin, Germany

<sup>†</sup>These authors contributed equally to this work.

\*Corresponding author emails: [shalek@mit.edu](mailto:shalek@mit.edu) (A.K.S.); [BWALKER@mgh.harvard.edu](mailto:BWALKER@mgh.harvard.edu)  
(B.D.W.)

## SUPPLEMENTARY TABLES

| Individual | Time Point (days relative to first positive viral RNA test) |                |               |                |                |                |                 |               |
|------------|-------------------------------------------------------------|----------------|---------------|----------------|----------------|----------------|-----------------|---------------|
|            | <i>Pre-Infection</i>                                        | <i>0 Weeks</i> | <i>1 Week</i> | <i>2 Weeks</i> | <i>3 Weeks</i> | <i>4 Weeks</i> | <i>6 Months</i> | <i>1 Year</i> |
| P1         | -70                                                         | 3              | 10            | 17             | 24             | 31             | 168             | 330           |
| P2         | -42                                                         | 3              | N.C.          | 17             | 24             | 31             | 161             | 329           |
| P3         | -35                                                         | 1              | 7             | 14             | 21             | 28             | 162             | 329           |
| P4         | -95                                                         | 1              | 7             | 14             | 24             | N.C.           | 164             | 413           |

  

| Individual | Age at Detection | STI at HIV Detection | Fiebig Stage at Detection | HLA           |               |               |
|------------|------------------|----------------------|---------------------------|---------------|---------------|---------------|
|            |                  |                      |                           | HLA-A         | HLA-B         | HLA-C         |
| P1         | 24               | Yes                  | I                         | 24:02 / 29:02 | 07:02 / 44:03 | 07:01 / 07:02 |
| P2         | 21               | N/A                  | I                         | 68:01 / 68:02 | 57:02 / 58:02 | 06:02 / 18    |
| P3         | 24               | Yes                  | I                         | 02:05 / 66:01 | 14:01 / 39:10 | 8:04 / 12:03  |
| P4         | 21               | Yes                  | I                         | 01:01 / 66:01 | 39:10 / 81:01 | 12:03 / 18    |

**Supplementary Table 1: Time point, clinical information, and HLA genotype for participants studied.**

| <b>P1</b>          | <b>Pre-Infection</b> | <b>0 Weeks</b> | <b>1 Week</b> | <b>2 Weeks</b> | <b>3 Weeks</b> | <b>4 Weeks</b> | <b>6 Months</b> | <b>1 Year</b> |
|--------------------|----------------------|----------------|---------------|----------------|----------------|----------------|-----------------|---------------|
| B cells            | 133                  | 249            | 22            | 194            | 134            | 315            | 242             | 241           |
| CD4+ T cells       | 899                  | 606            | 157           | 389            | 294            | 669            | 718             | 751           |
| CTLs               | 144                  | 138            | 50            | 277            | 247            | 487            | 404             | 396           |
| DCs                | 72                   | 14             | 2             | 19             | 23             | 42             | 37              | 79            |
| Monocytes          | 547                  | 622            | 162           | 571            | 450            | 692            | 409             | 876           |
| NK cells           | 175                  | 143            | 92            | 422            | 308            | 422            | 341             | 512           |
| Plasmablasts       | 6                    | 9              | 4             | 23             | 4              | 16             | 9               | 0             |
| <b>Total Cells</b> | 1976                 | 1781           | 489           | 1895           | 1460           | 2643           | 2160            | 2855          |

  

| <b>P2</b>          | <b>Pre-Infection</b> | <b>0 Weeks</b> | <b>1 Week</b> | <b>2 Weeks</b> | <b>3 Weeks</b> | <b>4 Weeks</b> | <b>6 Months</b> | <b>1 Year</b> |
|--------------------|----------------------|----------------|---------------|----------------|----------------|----------------|-----------------|---------------|
| B cells            | 171                  | 58             | N/A           | 23             | 169            | 54             | 109             | 157           |
| CD4+ T cells       | 869                  | 231            | N/A           | 63             | 37             | 144            | 83              | 567           |
| CTLs               | 170                  | 120            | N/A           | 178            | 249            | 103            | 160             | 259           |
| DCs                | 93                   | 121            | N/A           | 20             | 44             | 48             | 85              | 42            |
| Monocytes          | 1324                 | 1745           | N/A           | 587            | 691            | 538            | 952             | 1664          |
| NK cells           | 85                   | 64             | N/A           | 136            | 215            | 70             | 79              | 152           |
| Plasmablasts       | 8                    | 11             | N/A           | 268            | 41             | 29             | 31              | 11            |
| <b>Total Cells</b> | 2720                 | 2350           | 0             | 1275           | 1446           | 986            | 1499            | 2852          |

  

| <b>P3</b>          | <b>Pre-Infection</b> | <b>0 Weeks</b> | <b>1 Week</b> | <b>2 Weeks</b> | <b>3 Weeks</b> | <b>4 Weeks</b> | <b>6 Months</b> | <b>1 Year</b> |
|--------------------|----------------------|----------------|---------------|----------------|----------------|----------------|-----------------|---------------|
| B cells            | 154                  | 95             | 53            | 88             | 138            | 138            | 53              | 76            |
| CD4+ T cells       | 967                  | 245            | 272           | 305            | 612            | 501            | 160             | 341           |
| CTLs               | 278                  | 236            | 311           | 520            | 608            | 474            | 100             | 131           |
| DCs                | 29                   | 5              | 37            | 34             | 47             | 29             | 5               | 13            |
| Monocytes          | 920                  | 1017           | 1122          | 1214           | 577            | 1361           | 146             | 893           |
| NK cells           | 151                  | 166            | 270           | 343            | 119            | 171            | 70              | 62            |
| Plasmablasts       | 57                   | 18             | 41            | 72             | 37             | 30             | 7               | 8             |
| <b>Total Cells</b> | 2556                 | 1782           | 2106          | 2576           | 2138           | 2704           | 541             | 1524          |

  

| <b>P4</b>          | <b>Pre-Infection</b> | <b>0 Weeks</b> | <b>1 Week</b> | <b>2 Weeks</b> | <b>3 Weeks</b> | <b>4 Weeks</b> | <b>6 Months</b> | <b>1 Year</b> |
|--------------------|----------------------|----------------|---------------|----------------|----------------|----------------|-----------------|---------------|
| B cells            | 60                   | 61             | 22            | 42             | 58             | N/A            | 174             | 166           |
| CD4+ T cells       | 100                  | 155            | 124           | 391            | 87             | N/A            | 232             | 1041          |
| CTLs               | 58                   | 71             | 92            | 537            | 267            | N/A            | 120             | 155           |
| DCs                | 94                   | 93             | 33            | 29             | 70             | N/A            | 30              | 50            |
| Monocytes          | 1277                 | 1788           | 2014          | 618            | 2286           | N/A            | 1199            | 869           |
| NK cells           | 18                   | 35             | 59            | 214            | 24             | N/A            | 61              | 88            |
| Plasmablasts       | 274                  | 9              | 43            | 97             | 27             | N/A            | 9               | 4             |
| <b>Total Cells</b> | 1881                 | 2212           | 2387          | 1928           | 2819           | 0              | 1825            | 2373          |

**Supplementary Table 2: Cell frequencies by time point and participant.**

| <b>B</b>      | <b>Plasmablast</b> | <b>CD4</b>    | <b>NK</b>    | <b>Monocyte</b> | <b>CD1C+ DC</b> | <b>CTL</b>   | <b>Prolif. T</b> |
|---------------|--------------------|---------------|--------------|-----------------|-----------------|--------------|------------------|
| <i>LYZ</i>    | <i>LYZ</i>         | <i>LYZ</i>    | <i>LYZ</i>   | <i>CD3E</i>     | <i>CD3E</i>     | <i>LYZ</i>   | <i>LYZ</i>       |
| <i>CD14</i>   | <i>CD14</i>        | <i>CD14</i>   | <i>CD14</i>  | <i>CD3G</i>     | <i>CD3G</i>     | <i>CD14</i>  | <i>CD14</i>      |
| <i>TRBC2</i>  | <i>TRBC2</i>       | <i>IGJ</i>    | <i>IGJ</i>   | <i>CD3D</i>     | <i>CD3D</i>     | <i>IGJ</i>   | <i>IGJ</i>       |
| <i>TRDC</i>   | <i>TRDC</i>        | <i>IGHM</i>   | <i>IGHM</i>  | <i>IGJ</i>      | <i>IGJ</i>      | <i>IGHM</i>  | <i>IGHM</i>      |
| <i>CD3E</i>   | <i>CD3E</i>        | <i>IGHG1</i>  | <i>IGHG1</i> | <i>IGHM</i>     | <i>IGHM</i>     | <i>IGHG1</i> | <i>IGHG1</i>     |
| <i>CD3G</i>   | <i>CD3G</i>        | <i>IGHG2</i>  | <i>IGHG2</i> | <i>IGHG1</i>    | <i>IGHG1</i>    | <i>IGHG2</i> | <i>IGHG2</i>     |
| <i>CD3D</i>   | <i>CD3D</i>        | <i>IGHG3</i>  | <i>IGHG3</i> | <i>IGHG2</i>    | <i>IGHG2</i>    | <i>IGHG3</i> | <i>IGHG3</i>     |
| <i>CCL5</i>   | <i>CCL5</i>        | <i>IGHG4</i>  | <i>IGHG4</i> | <i>IGHG3</i>    | <i>IGHG3</i>    | <i>IGHG4</i> | <i>IGHG4</i>     |
| <i>MNDA</i>   |                    | <i>IGHD</i>   | <i>IGHD</i>  | <i>IGHG4</i>    | <i>IGHG4</i>    | <i>IGHD</i>  | <i>IGHD</i>      |
| <i>VCAN</i>   |                    | <i>IGHA1</i>  | <i>IGHA1</i> | <i>IGHD</i>     | <i>IGHD</i>     | <i>IGHA1</i> | <i>IGHA1</i>     |
| <i>FCGR3A</i> |                    | <i>FCGR3A</i> | <i>CD3E</i>  | <i>IGHA1</i>    | <i>IGHA1</i>    |              |                  |
| <i>NKG7</i>   |                    | <i>VCAN</i>   | <i>CD3D</i>  | <i>TRBC2</i>    | <i>TRBC2</i>    |              |                  |
|               |                    |               | <i>CD3G</i>  | <i>TRDC</i>     | <i>TRDC</i>     |              |                  |
|               |                    |               | <i>TRAC</i>  |                 |                 |              |                  |

**Supplementary Table 11: Genes used to determine residual RNA contamination in each cell type.**