**Supplemental Digital Content,
Table S1: Individual macaque study treatment and sample collection parameters.** Breakdown of individual macaques included in retrospective analysis. Monkeys were included from multiple different studies as noted in the table. The social housing status, antiretroviral therapy, and instruments and reagents used for each group are also listed.

**Table S2. Macaque demographics by housing condition.** Summary of the demographic characteristics of each experimental group including uncontrolled variables such as animal source and machine used for analyses.

**Table S3: Flow cytometry panels.**  Specific reagents used for each flow cytometry panel included in this analysis. Panels used for each macaque can be seen in Table S1.

**Table S4: Sample size for each study component by timepoint.**  Due to the retrospective nature of this study, sample size was limited by available data. The number of samples available at each timepoint is outlined.

**Table S5. Variables in singly and socially housed macaque groups.** Controlled and uncontrolled variables with the potential to differ between the macaque housing groups analyzed in this retrospective study. Post hoc analyses were performed to assess the impact of animal sedation order, age, or machine used on group outcomes; data from a second FACS machine was subsequently excluded. \*This statement indicates there may be unidentified variables that changed between these time periods, but is not intended to indicate that any specific change occurred in 2013.

**Figure S1: Day 10 change from baseline platelet count and activation markers in singly housed macaques compared to socially housed macaques.** Baseline values (2 timepoints) were averaged and subtracted from post-inoculation day 10 (for *n* for each timepoint see Table S4). Housing groups were compared using a Mann-Whitney test for non-parametric data. Reduction in platelet counts (A) in whole blood CBCs were not different between housing groups at day 10.Change in P-selectin expression (B) at day 10 was different between housing groups (\*\*P = 0.004). Singly housed macaques showed no change from baseline post-inoculation.Change in MHC-I expression (C) was not different between housing groups at day 10 post-inoculation.Change in CD40L expression (D) at day 10 was different between housing groups (\*\*P = 0.006). Singly housed macaques showed no change from baseline post-inoculation.

**Figure S2: Changes in monocyte subset percentages in singly compared to socially housed macaques.** Housing groups were compared from baseline (3 timepoints) through 7, 10, and 14 days after inoculation with SIV (for *n* for each timepoint see Table S4). CD14 and CD16 monocyte expression profiles were compared using flow cytometry (see Figure S3 for gating strategy), and used to determine percentage of each monocyte subtype (A-C). Error bars display the standard error of the mean, comparisons between housing groups over time were performed with a mixed effects model using the Šidák correction for multiple comparisons. \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001.

**Figure S3: Flow cytometry gating strategy.** Example gating strategy for monocyte (A) and platelet (B and C) whole blood flow cytometry panels.

**SDC, Table S1.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Housing Type | Database Monkey Group ID | Monkey Database ID | Age at SIV Inoculation (Years) | Weight at Inoculation (kg) | Treatment  | dPI Treatment | Source | Hematologic Analyzer | Platelet FACS Machine | Monocyte FACS Machine | Flow panels |
| SOCIAL | 76 | 422 | 2.6 | 4.06 | 2.5mg/kg Dolutegravir, 20mg/kg TFV, 40mg/kg FTC SID SQ | 12 | JHU | Procyte | Calibur | Fortessa | C, 1, 2 |
| SOCIAL | 423 | 2.5 | 3.84 |
| SOCIAL | 424 | 2.3 | 3.60 |
| SOCIAL | 425 | 2.3 | 3.14 |
| SOCIAL | 426 | 2.2 | 3.03 |
| SOCIAL | 427 | 2.2 | 3.08 |
| SOCIAL | 067/073 | 367 | 4.0 | 7.49 | 30mg/kg TFV SID SQ for first two weeks then 10mg/kg, 480mg/kg Atazanavir, 10mg/kg Integrase Inhibitor L000870812 BID PO, 24mg/kg Ritonavir BID PO | 12 | JHU | Procyte | Calibur | Fortessa | C |
| SOCIAL | 377 | 3.8 | 6.19 |
| SOCIAL | 378 | 3.6 | 8.09 |
| SOCIAL | 386 | 2.8 | 4.69 | 30mg/kg TFV SID SQ for first two weeks then 10mg/kg, 480mg/kg Atazanavir, 10mg/kg Integrase Inhibitor L000870812 BID PO, 24mg/kg Ritonavir BID PO | 12 | JHU | Procyte | Calibur | Fortessa | C, 1, 2 |
| SOCIAL | 387 | 2.7 | 3.69 |
| SOCIAL | 388 | 2.4 | 3.79 |
| SOCIAL | 406 | 5.2 | 7.89 | 30mg/kg TFV SID SQ for first two weeks then 20mg/kg, 270mg/kg Atazanavir, 10mg/kg Integrase Inhibitor L000870812 BID PO, 24mg/kg Ritonavir BID PO | 12 | New Iberia | Procyte | Calibur | Fortessa | C |
| SOCIAL | 407 | 3.7 | 4.89 |
| SOCIAL | 408 | 3.5 | 5.19 |
| SOCIAL | 71 | 390 | 3.6 | 4.39 | 2.5mg/kg Dolutegravir, 20mg/kg TFV, 40mg/kg FTC SID SQ, 200mg Maraviroc BID PO | 12 | JHU | Procyte | Calibur | Fortessa | C |
| SOCIAL | 394 | 3.3 | 4.59 |
| SOCIAL | 400 | 3.8 | 4.89 | New Iberia |
| SOCIAL | 401 | 3.1 | 4.99 |
| SOCIAL | 74 | 409 | 3.1 | 4.19 | 2.5mg/kg Dolutegravir, 20mg/kg TFV, 40mg/kg FTC SID SQ, 200mg Maraviroc BID PO | 12 | JHU | Procyte | Calibur | Fortessa | C |
| SOCIAL | 410 | 2.7 | 3.49 |
| SOCIAL | 411 | 2.8 | 4.29 |
| SOCIAL | 412 | 2.7 | 3.49 |
| SOCIAL | 413 | 2.6 | 3.89 |
| SOCIAL | 414 | 2.4 | 3.79 |
| SOCIAL | 65 | 368 | 3.9 | 5.39 | 480mg/kg Darunavir BID PO, 10mg/kg Integrase Inhibitor L000870812 BID PO, 24mg/kg Ritonavir BID PO, 25/50mg/kg Abacavir BID PO | 12 | JHU | Procyte | Calibur | Fortessa | C |
| SOCIAL | 369 | 3.8 | 4.89 |
| SOCIAL | 370 | 3.1 | 5.39 |
| SOCIAL | 371 | 3.4 | 3.89 |
| SOCIAL | 372 | 3.0 | 3.49 |
| SOCIAL | 385 | 3.0 | 4.39 |
| SOCIAL | 64 | 361 | 3.9 | 5.49 | 30mg/kg TFV SID SQ for first two weeks then 10mg/kg, 270mg/kg Atazanavir, 10mg/kg Integrase Inhibitor L000870812 BID PO, 24mg/kg Ritonavir BID PO | 12 | JHU | Hemavet | Calibur | Fortessa | C |
| SOCIAL | 362 | 3.9 | 4.99 |
| SOCIAL | 363 | 3.8 | 6.09 |
| SOCIAL | 364 | 3.6 | 6.49 |
| SOCIAL | 365 | 2.6 | 4.49 |
| SOCIAL | 366 | 2.4 | 4.49 |
| SOCIAL | 69 | 389 | 2.9 | 4.10 | 2.5mg/kg Dolutegravir, 20mg/kg TFV, 40mg/kg FTC SID SQ | 12 | JHU | Procyte | Calibur | Fortessa | C |
| SOCIAL | 391 | 2.8 | 4.00 |
| SOCIAL | 392 | 2.7 | 4.10 |
| SOCIAL | 393 | 2.6 | 4.10 |
| SINGLE | 058 | 340 | 4.4 | 6.49 | 25mg/kg Fisetin SID PO | 12 | SNBL | Hemavet | Calibur | Fortessa | B, 1, 2 |
| SINGLE | 341 | 4.4 | 8.78 |
| SINGLE | 342 | 4.4 | 6.49 |
| SINGLE | 060 | 345 | 5.9 | 10.58 | 30mg/kg TFV SID SQ for first 14 days then 10mg/kg, 270mg/kg Atazanavir BID PO, 10mg/kg Integrase Inhibitor L000870812 BID PO, 24mg/kg Ritonavir BID PO | 12 | SNBL | Hemavet | Calibur | Fortessa | C |
| SINGLE | 347 | 5.7 | 8.48 |
| SINGLE | 050 | 266 | 4.5 | 3.29 | 12.5mcg Flucanazole SID PO, 5mg Paroxitine SID PO | 12 | New Iberia | Hemavet | Calibur | Calibur | A |
| SINGLE | 308 | 3.3 | 4.59 |
| SINGLE | 309 | 3.2 | 3.99 |
| SINGLE | 311 | 3.1 | 6.99 |
| SINGLE | 313 | 3.4 | 6.79 | JHU |
| SINGLE | 321 | 4.1 | 4.49 |
| SINGLE | 049 | 307 | 3.9 | 6.09 | 30mg/kg TFV SID SQ, 270mg/kg Atazanavir BID PO, 10mg/kg Integrase Inhibitor L000870812 BID PO | 42 | Iberia | Hemavet | Calibur | Calibur | A |
| SINGLE | 310 | 3.1 | 4.39 |
| SINGLE | 312 | 4.1 | 4.99 |
| SINGLE | 052 | 203 | 9.3 | 7.59 | Untreated | N/a | Yerkes | Hemavet | Calibur | Calibur | A |
| SINGLE | 268 | 6.1 | 6.89 |
| SINGLE | 272 | 4.5 | 12.18 |
| SINGLE | 274 | 6.1 | 3.29 |
| SINGLE | 320 | 3.5 | 7.79 |
| SINGLE | 265 | 4.6 | 18.37 | JHU |
| SINGLE | 044 | 291 | 3.0 | 3.72 | Untreated | N/a | Yerkes | Hemavet | Calibur | Calibur | A |
| SINGLE | 292 | 3.0 | 3.99 |
| SINGLE | 294 | 3.0 | 2.36 |
| SINGLE | 054 | 329 | 2.7 | 4.59 | 30mg/kg TFV SID SQ, 270mg/kg Atazanavir BID PO, 10mg/kg Integrase Inhibitor L000870812 BID PO | 12 | JHU | Hemavet | Calibur | Fortessa | B |
| SINGLE | 330 | 2.5 | 3.59 |
| SINGLE | 331 | 2.4 | 3.19 |
| SINGLE | 061 | 348 | 5.5 | 10.48 | Untreated | N/a | SNBL | Hemavet | Calibur | Fortessa | B, 1, 2 |
| SINGLE | 349 | 5.2 | 9.88 |
| SINGLE | 350 | 5.3 | 6.69 |
| SINGLE | 351 | 5.2 | 9.48 | B |
| SINGLE | 352 | 5.0 | 6.39 |
| SINGLE | 353 | 5.0 | 7.69 |
| SINGLE | 057 | 337 | 5.6 | 9.78 | 30mg/kg TFV SID SQ for first two weeks then 10mg/kg, 480mg/kg Darunavir BID PO, 10mg/kg Integrase Inhibitor L000870812 BID PO, 24mg/kg Ritonavir BID PO | 12 | SNBL | Hemavet | Calibur | Fortessa | B, 1, 2 |
| SINGLE | 338 | 5.5 | 7.69 |
| SINGLE | 339 | 5.5 | 7.99 |

**SDC, Table S2.**

|  |  |  |
| --- | --- | --- |
|  | **Socially housed (*n* = 41)** | **Singly housed (*n* = 35)** |
| Sex | All male | All male |
| Avg. age at inoculation | 3.1 years (Range: 2.2 – 5.2) | 4.5 years (Range: 2.4 – 9.3) |
| Avg. weight at inoculation | 4.64 kg (Range: 3.03 – 8.09) | 6.86 kg (Range: 2.36 – 18.37) |
| Source | From 2 different institutions | From 4 different institutions |
| Hematology  | Both machines | Hemavet only |
| Monocyte FACS machine  | Fortessa only | Fortessa only (Calibur excluded) |

**SDC, Table S3.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Monocyte panel A** |  |  |  |  |
| *Antibody* | *Clone* | *Supplier* | *Catalogue #* | *RRID* | *Fluorochrome* |
| CD14 | M5E2 | BD Biosciences | 557153 | AB\_396589 | FITC |
| CD16 | 3G8 | BD Biosciences | 555408 | AB\_395808 | PE-Cy5 |
| **Monocyte panel B** |  |  |  |  |
| *Antibody* | *Clone* | *Supplier* | *Catalogue #* | *RRID* | *Fluorochrome* |
| CD14 | M5E2 | BD Biosciences | 563420 | AB\_2744286 | Brilliant Violet 650 |
| CD16 | 3G8 | BioLegend | 302026 | AB\_2278418 | AlexaFluor 700 |
| **Monocyte panel C** |  |  |  |  |
| *Antibody* | *Clone* | *Supplier* | *Catalogue #* | *RRID* | *Fluorochrome* |
| TLR2 | 11G7 | BD Biosciences | 558318 | AB\_647100 | AlexaFluor 488 |
| CD14 | M5E2 | BD Biosciences | 563420 | AB\_2744286 | Brilliant Violet 650 |
| CD16 | 3G8 | BioLegend | 302026 | AB\_2278418 | AlexaFluor 700 |
| **Platelet panel 1**  |  |  |  |  |
| *Antibody* | *Clone* | *Supplier* | *Catalogue #* | *RRID* | *Fluorochrome* |
| CD42a | ALMA.16 | BD Biosciences | 558818 | AB\_397129 | FITC |
| P-selectin | AC1.2 | BD Biosciences | 550561 | AB\_393753 | PE |
| CD40L | 24-31 | BioLegend | 310808 | AB\_314831 | PE-Cy5 |
| **Platelet panel 2** |  |  |  |  |
| *Antibody* | *Clone* | *Supplier* | *Catalogue #* | *RRID* | *Fluorochrome* |
| CD42a | ALMA.16 | BD Biosciences | 558819 | AB\_397130 | PE |
| HLA-ABC | G46-2.6 | BD Biosciences | 555552 | AB\_395935 | FITC |

**SDC, Table S4.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Time point | Social (*n*) | Single (*n*) |
| Monocytes | PB1 | 41 | 34 |
| PB2 | 41 | 34 |
| PB3 | 40 | 34 |
| 7 | 41 | 35 |
| 10 | 41 | 33 |
| 14 | 41 | 17 |
| CD14 | PB1 | 41 | 17 |
| PB2 | 41 | 17 |
| PB3 | 34 | 17 |
| 7 | 41 | 17 |
| 10 | 41 | 16 |
| 14 | 41 | 11 |
| CD16 | PB1 | 41 | 17 |
| PB2 | 41 | 17 |
| PB3 | 34 | 17 |
| 7 | 41 | 17 |
| 10 | 41 | 16 |
| 14 | 41 | 11 |
| Platelets | PB1 | 9 | 6 |
| PB2 | 9 | 9 |
| 7 | 9 | 6 |
| 10 | 9 | 8 |
| 14 | 9 | 3 |
| P-selectin | PB1 | 9 | 9 |
| PB2 | 9 | 6 |
| 10 | 8 | 9 |
| CD40L | PB1 | 9 | 9 |
| PB2 | 9 | 6 |
| 10 | 8 | 9 |
| MHC-I | PB1 | 9 | 9 |
| PB2 | 9 | 6 |
| 10 | 8 | 7 |

**SDC, Table S5.**

|  |  |
| --- | --- |
| **Controlled Variables** | **Uncontrolled Variables (detailed in Table S1)** |
| Pigtailed macaques | Variety of origins for macaques |
| All males, 2-6 years old | Total amount of accessible enclosure space |
| MANE-A1\*084:01 negative | CBC machine changed in 2015 |
| Same room in a single facility | Changes between studies in FACS panel |
| Same food, water, and enrichment program | Different ART/interventions at d12 PI |
| Common SIV inoculum stocks | Unidentified variables associated with pre- & post-2013\* |
| Intravenous route of inoculation  |  |
| Sampling by same 2 DVMs |  |
| Same animal caretaker |  |
| Same PCR assay performed by same technician |  |
| FACS staining and run by same technician |  |
| FACS re-analyzed by single researcher  |  |
| Data stored on electronic database |  |



**SDC Figure S1.**

 

**SDC, Figure S2.**

**SDC, Figure S3.**

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