Supplemental Methods.

Animal Infection Protocol

Six juvenile pigtailed macaques (*Macaca nemestrina*) were infected with SIV by intravenous inoculation with both an immunosuppressive swarm (SIV/DeltaB670) at a dose of 10,000 times 50% animal infectious dose and a neurovirulent clone (SIV/17E-Fr) at a dose of 50 times 50% animal infectious dose, as described previously.[10] Sacrifice and necropsy were at 7 days post-infection. Negative controls were six juvenile pigtailed macaques, mock inoculated with PBS and then sacrificed. Spleen tissue samples were collected from all 12 macaques and fresh frozen. Animal protocols were approved by the Johns Hopkins University Institutional Animal Care and Use Committee in accordance with Animal Welfare Act regulations and the USPHS Policy on Humane Care and Use of Laboratory Animals.

Animal Studies Ethics Statement

"Animal studies were approved by the Johns Hopkins Animal Care and Use Committee (IACUC protocol #PR12M310); animals were humanely treated in accordance with federal guidelines and institutional policies. Pigtailed macaques (Macaca nemestrina) used in this study were obtained from nonhuman primate breeding facilities within the United States, and were housed in Johns Hopkins University facilities that are fully accredited by the association for the Assessment and Accreditation of Laboratory Animal Care, International (AALAC). All housing met or exceeded guidelines of the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" and the United States Department of Agriculture Animal Welfare Act. Macaques were fed a balanced, commercial macaque chow (Purina Mills, Gray Summit, MO, USA) once a day supplemented with a variety of food enrichment."

Tissue Sample Collection and Preparation

Miltex 3 mm biopsy punches of fresh-frozen spleens were resuspended in 500 µL 0.1% ascorbic acid in a 1 mL Kontes Duall Tissue Grinder, and homogenized by hand for 10 minutes. Metabolites were extracted by a modified[6] Bligh-Dyer protocol: 150 µL homogenate was added to 600 µL methanol at -20°C. 450 µL room-temperature chloroform was then added, and the solution vortexed and returned to an ice bath. The solution was then vortexed every 5 minutes for 30 minutes, returning to the ice bath each time. At the penultimate step, 150 µL ice cold MilliQ water was added, and samples were maintained at -20°C for 2 hours to create phase separation. The upper (aqueous) phase was then collected.

Next, in order to maximize metabolite yield, the above extraction procedure was repeated with 2% ammonium hydroxide at pH 9, and with 1% formic acid at pH 2. Specifically, the organic phase from the first extraction was dried and then resuspended in water. The methanol and chloroform steps were repeated as above, and then the pH 9 buffer was added to create the phase. The aqueous phase following this step was collected, and the procedure was repeated with a buffer at pH 2. All three extractions were filtered with a 50,000 MWCO to remove large particulates, and flowthroughs were combined, dried, and resuspended in 2% ACN/0.1% FA for HPLC-MS analysis.

Protein Normalization for Quantitative Profiling

For quantitative metabolite profiling, protein amounts in each homogenate were estimated by a modified Lowry method after a deoxycholate precipitation (2D Quant Kit, GE Healthcare). Protein estimations were confirmed by 1D gel electrophoresis and SYPRO Ruby (Life Technologies) staining. For gels, 2 µg sample was combined with 2.5 µL SDS sample buffer, and 1 µL 100mM DTT, and the final solution diluted up to 10 µL with MilliQ water. Running buffer was prepared by diluting MES SDS running buffer (Life Technologies) 1:20 in MilliQ water with 20% methanol. Samples were denatured for 10 minutes at 70°C, and run for ~40 minutes at 200 V constant voltage.