Supplemental Materials for:

Concordance of immunological events between intrarectal and intravenous $SHIV_{AD8\text{-}EO}$ infection when assessed by Fiebig-equivalent staging

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SUPPLEMENTAL FIGURES

A

Timepoint (pc)	D0	D3	D7	D10	D14	D17	wk3	wk4	wk5	wk6	wk7	wk8	wk12	wk16	wk20
Intrarectal challenge															
HH7	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
DGEM	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
HZ2	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
DGAM	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
HR8	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
DG81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Intravenous challenge															
DG63	-	+	+	+	+	+	+	+	+	+	+	+	+	+	N.D.
DGBH	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
DGGL	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
HEM	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
HPZ	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
HVL	-	+	+	+	+	+	+	+	+	+	+	+	+	+	

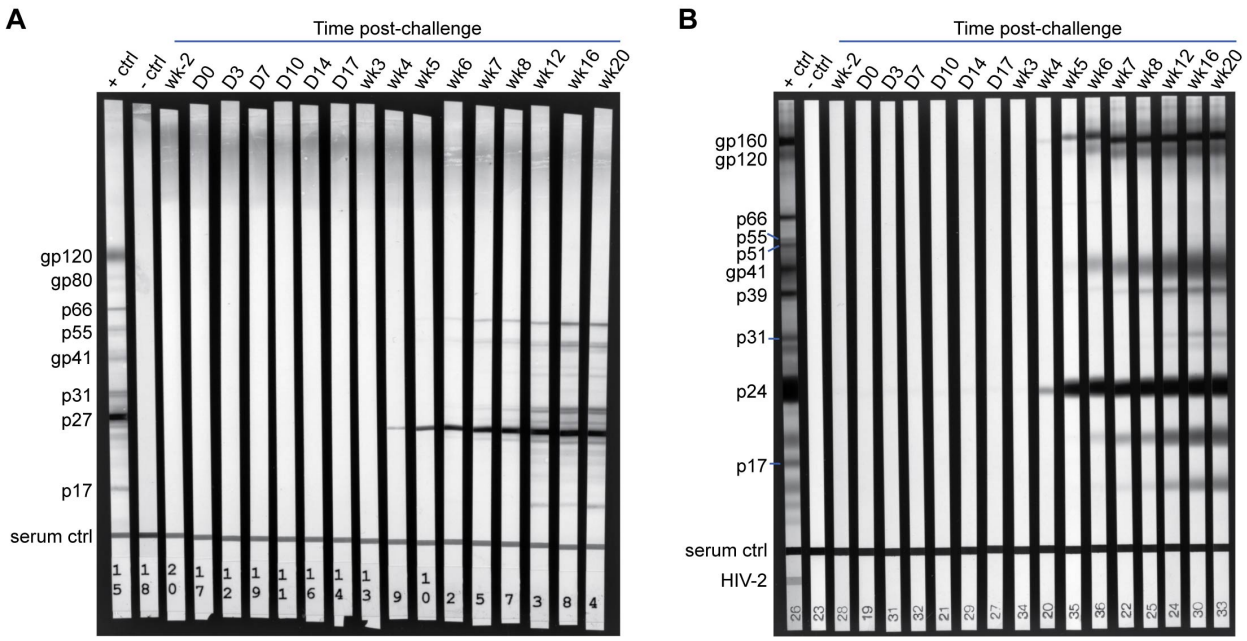
B

Timepoint (pc)	D0	D3	D7	D10	D14	D17	wk3	wk4	wk5	wk6	wk7	wk8	wk12	wk16	wk20
Intrarectal challenge															
HH7	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
DGEM	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
HZ2	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
DGAM	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
HR8	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
DG81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Intravenous challenge															
DG63	-	-	-	+	+	+	-	-	-	-	-	-	-	-	N.D.
DGBH	-	-	-	+	+	+	-	-	-	-	-	-	-	-	
DGGL	-	-	+	+	+	+	+	+	+	+	+	+	-	+	
HEM	-	-	-	+	+	+	+	-	-	-	-	-	-	-	
HPZ	-	-	-	+	+	+	+	-	-	-	+	+	-	-	
HVL	-	-	-	+	+	+	+	-	-	-	-	-	-	-	

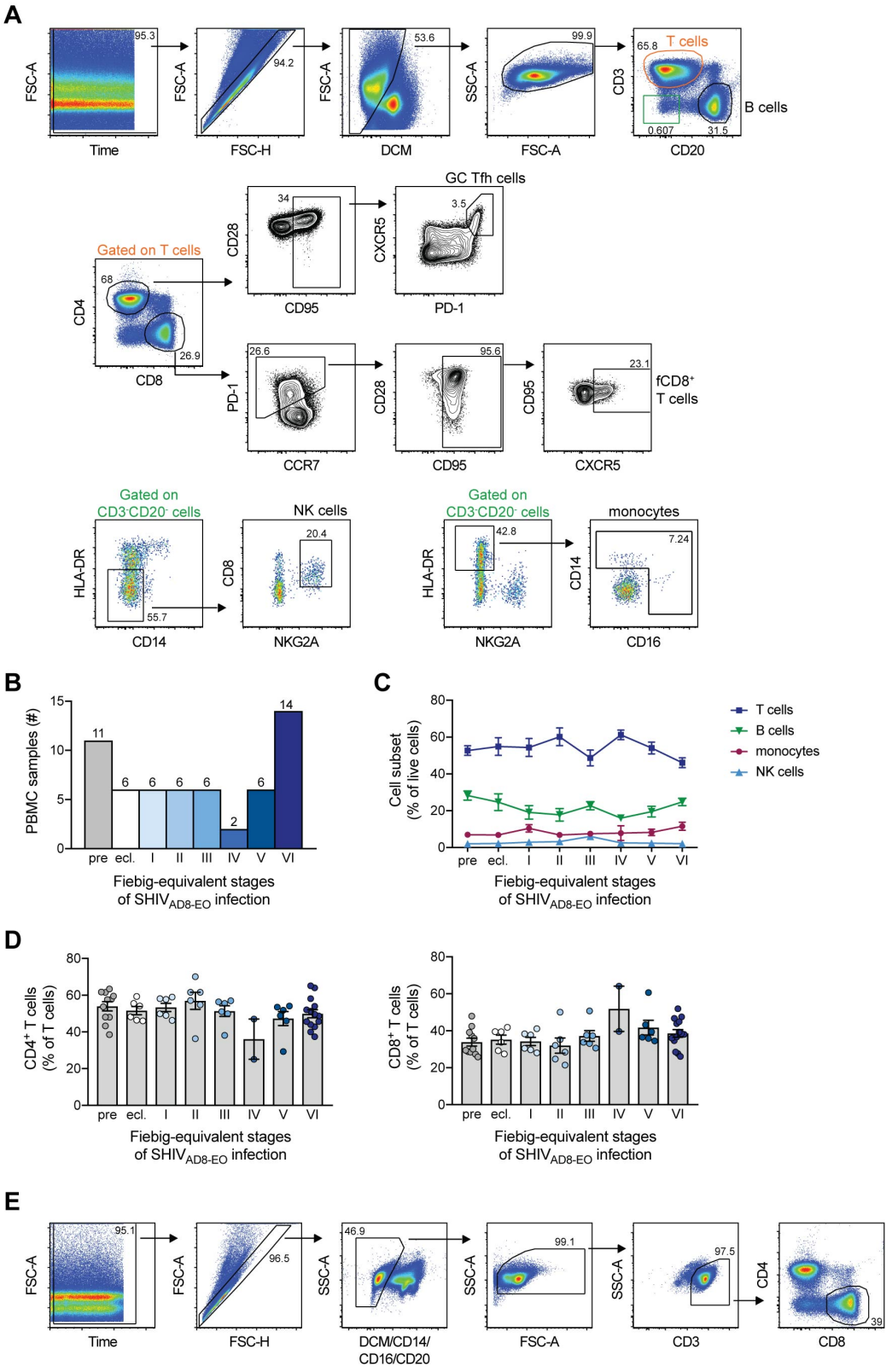
C

Timepoint (pc)	D0	D3	D7	D10	D14	D17	wk3	wk4	wk5	wk6	wk7	wk8	wk12	wk16	wk20
Intrarectal challenge															
HH7	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
DGEM	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
HZ2	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
DGAM	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
HR8	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
DG81	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Intravenous challenge															
DG63	-	-	-	-	-	+	+	+	+	+	+	+	+	+	N.D.
DGBH	-	-	-	-	-	+	+	+	+	+	+	+	+	+	
DGGL	-	-	-	-	-	+	+	+	+	+	+	+	+	+	
HEM	-	-	-	-	-	+	+	+	+	+	+	+	+	+	
HPZ	-	-	-	-	-	+	+	+	+	+	+	+	+	+	
HVL	-	-	-	-	+	+	+	+	+	+	+	+	+	+	

Supplemental Figure 1. Patterns of viral marker detection throughout $SHIV_{AD8\text{-}EO}$ infection. Detection of plasma (**A**) SIV Gag RNA, (**B**) SIV p27 antigen, and (**C**) HIV-1/HIV-2 antibodies during the course of $SHIV_{AD8\text{-}EO}$ infection. The symbols "+" and "-" denote detection and absence of detection, respectively, of the corresponding viral markers. D, day: N.D., not determined; pc, post-challenge: wk, week.

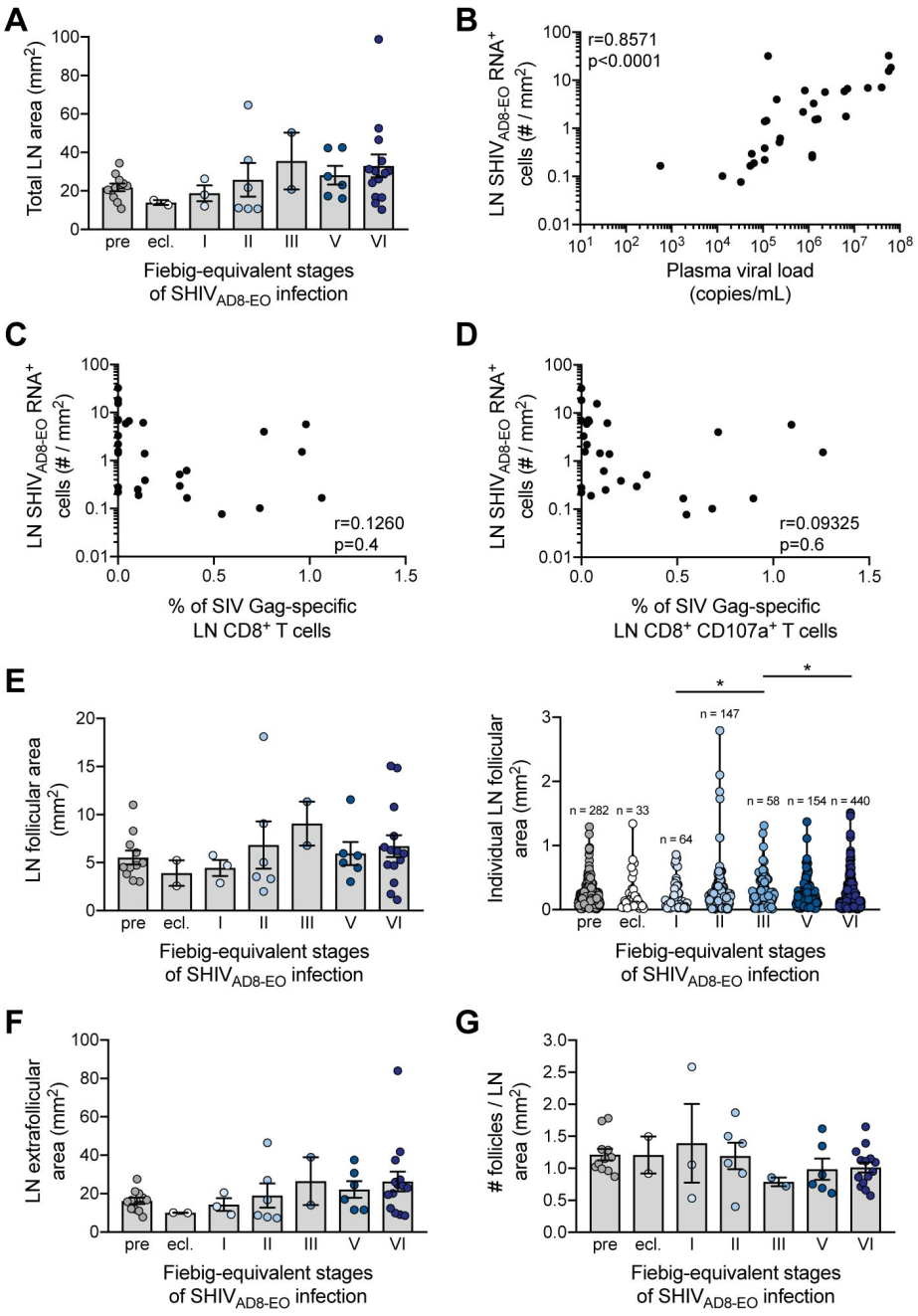


Supplemental Figure 2. Western blot assays for detection of SIV and HIV antibodies. Representative example of the western blots for detection of antibodies against (**A**) SIV antigens and (**B**) HIV antigens in the plasma of $SHIV_{AD8\text{-}EO}$-challenged monkeys. The SIV western blot was used to detect antibodies against the Gag and Pol antigens (p17, p27, p31, p55, and p66), whereas the HIV western blot was used to detect antibodies against the Env antigens (gp41, gp120, and gp160). Positive and negative control samples provided by each of the western blot kits are also illustrated. ctrl, control; D, day; wk, week.



Supplemental Figure 3. Flow cytometry identification of LN cell populations and immunophenotyping of PBMCs. (**A**) Gating strategy to identify T cells, B cells, NK cells, and

monocytes, as well as $CD4^+$ and $CD8^+$ T cell subsets, by flow cytometry in the LNs of $SHIV_{AD8\text{-}EO}$-challenged monkeys. (**B**) Distribution of PBMC samples for flow cytometry immunophenotyping analysis across the Fiebig-equivalent stages of $SHIV_{AD8\text{-}EO}$ infection. Frequency of (**C**) monocytes, NK cells, B cells, and T cells, and of (**D**) $CD4^+$ (*left*) and $CD8^+$ (*right*) T cells at the different stages of $SHIV_{AD8\text{-}EO}$ infection. (**E**) Gating strategy to identify LN $CD8^+$ T cells from $SHIV_{AD8\text{-}EO}$-challenged monkeys for flow cytometry analysis of $CD8^+$ T cell responses to 6 h stimulation with SIV Gag peptide pool. Graphs show the absolute number (**B**), the mean ± SEM (**C**), and the mean ± SEM and individual datapoints (**D**). The Kruskal-Wallis test followed by Dunn's multiple comparisons test was used to detect significant differences between all stages except for Fiebig-equivalent stage IV (low *n*) (**C-D**). DCM, dead cell marker; ecl., eclipse; $fCD8^+$, follicular $CD8^+$; GC, germinal center; LN, lymph node; NK, natural killer; Tfh, T follicular helper.



Supplemental Figure 4. Size of LN sections as determined by RNAscope *in situ* hybridization. (**A**) Size of whole LN sections in each stage of $SHIV_{AD8\text{-}EO}$ infection. Correlations between the number of $SHIV_{AD8\text{-}EO}$ RNA^+ cells per mm^2 of LN and either (**B**) plasma viral load, (**C**) frequency of SIV Gag-specific LN $CD8^+$ T cells co-expressing CD69 and IFNγ, MIP-1β, or TNF, or expressing CD107a, or (**D**) frequency of SIV Gag-specific LN $CD8^+$ T cells expressing CD107a. (**E**) Size of combined follicles per LN section (*left*) or of individual LN follicles (*right*) in each stage of $SHIV_{AD8\text{-}EO}$ infection. (**F**) Size of the extrafollicular area in each stage of $SHIV_{AD8\text{-}EO}$ infection. (**G**) Number of follicles per mm^2 of LN in each stage of $SHIV_{AD8\text{-}EO}$ infection. Bar graphs show the mean ± SEM and individual datapoints (**A, E *left*, F, and G**). The Kruskal-Wallis

test followed by Dunn's multiple comparisons test was used to detect significant differences between the pre-challenge phase, Fiebig-equivalent stage II, V, and VI of $SHIV_{AD8\text{-}EO}$ infection (**A, E *left*, F, and G**), or between all stages (**E *right***). The Spearman's rank correlation test was used for correlation analyses (**B-D**). ecl., eclipse; LN, lymph node.

SUPPLEMENTAL TABLES

Supplemental Table 1. Laboratory assays to identify the Fiebig-equivalent stages of $SHIV_{AD8\text{-}EO}$ infection

Fiebig stages of HIV-1 infection[A]	Fiebig-equivalent stages of $SHIV_{AD8\text{-}EO}$ infection
Quantitative HIV-1 RNA PCR	Quantitative SIV Gag RNA RT-PCR
HIV-1 p24 ELISA	SIV p27 ELISA
HIV-1/HIV-2 antibody ELISA (IgM-sensitive)	HIV-1/HIV-2 antibody ELISA (IgM-sensitive)
HIV western blot	SIV western blot & HIV western blot

[A]E. W. Fiebig, D. J. Wright, B. D. Rawal, P. E. Garrett, R. T. Schumacher, L. Peddada, C. Heldebrant, R. Smith, A. Conrad, S. H. Kleinman, M. P. Busch, Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* **17**, 1871-1879 (2003).

Supplemental Table 2. Antibodies used in flow cytometry

Marker	Fluorochrome	Clone	Supplier
CCR7	AF700	150503	BD Biosciences
CD3	APC-Cy7	SP34-2	BD Biosciences
CD4	BUV661	SK3	BD Biosciences
CD8	BV570	RPA-T8	Biolegend
CD14	BV510	M5E2	Biolegend
CD16	BUV496	3G8	BD Biosciences
CD16	BV510	3G8	Biolegend
CD20	BUV737	2H7	BD Biosciences
CD20	BV510	2H7	Biolegend
CD28	ECD	CD28.2	Beckman Coulter
CD69	BV605	FN50	Biolegend
CD95	PECy5	DX2	BD Biosciences
CD107a	BV650	H4A3	Biolegend
CXCR5	PECy7	MU5UBEE	eBioscience
DCM	Zombie UV	-	Biolegend
DCM	Aqua	-	Invitrogen
HLA-DR	PECy5.5	TU36	Life Technologies
IFNγ	FITC	B27	BD Biosciences
MIP-1β	PE	D21-1351	BD Biosciences
NKG2A	APC	REA110	MACS Miltenyi Biotec
PD-1	BV711	EH12.2H7	Biolegend
TNF	BV785	Mab11	Biolegend