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NOTE

Down-Regulation of Robust Acute Type I IFN Responses Distinguishes Non-Pathogenic SIV Infection of Natural Hosts from Pathogenic SIV Infection of Rhesus Macaques

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Abstract

The mechanisms underlying the AIDS-resistance of natural hosts for SIV remain unknown. Recently, it was proposed that natural SIV hosts avoid disease because their plasmacytoid dendritic cells (pDCs) are intrinsically unable to produce IFN α in response to SIV RNA stimulation. However, here we show that: i) acute SIV infections of natural hosts are associated with a rapid and robust type-I IFN response *in vivo*; ii) pDCs are the principal *in vivo* producers of IFN α/β at peak acute infection in lymphatic tissues; and iii) natural SIV hosts down-regulate these responses in the early chronic infection. In contrast, persistently high type-I IFN responses are observed during pathogenic SIV infection of rhesus macaques.

A hallmark of pathogenic HIV/SIV infections is persistent chronic activation of the immune system that is associated with i) dysfunctions in cellular and humoral immunity; ii) elevated levels of activation-induced cell death in all lymphocyte subsets; and iii) failure to effectively control virus replication (reviewed in **7, 26**). The chronic inflammatory environment in pathogenic HIV/SIV infection disrupts T cell homeostasis through i) suppression of regenerative bone marrow functions; ii) reduction of thymic output; and iii) structural and functional damage of peripheral lymphoid tissues (**12**). Indeed, it is now widely accepted that persistent chronic immune activation is a major driving force for CD4+ T cell depletion and AIDS progression (**8, 12**).

SIV infection of natural hosts such as sooty mangabeys (SMs), African green monkeys (AGM) and mandrills is typically non-progressive even though these “natural” infections share key features with pathogenic HIV infection of humans and SIV infection of rhesus macaques (RM) including: i) chronic high levels of viremia; ii) viral tropism for CD4+ T cells; iii) short half-lives of virus-infected cells; and iv) severe acute depletion of mucosal CD4+ T cells (**10, 11, 22, 25, 28**). A striking difference between nonpathogenic SIV infection in natural hosts and pathogenic HIV and SIV infections in humans and RMs respectively, is the lower levels of immune activation during the chronic phase of infection **observed** in natural hosts (**16, 25**). While several groups have documented **T cell proliferation and** immune activation during the acute phase of SIV infection in natural hosts (**5, 11, 17, 20-22**), it has recently been proposed that a key

biologically distinguishing feature of SMs (and potentially other natural hosts of SIV) is a host species specific attenuated innate immune response to infection that includes the lack of plasmacytoid dendritic cell (pDC) activation and type I IFN production in response to SIV infection (**19**). This hypothesis is largely based on *in vitro* experiments, in which isolated peripheral blood pDCs from SMs were reported to show diminished TLR 7/9 mediated type I IFN responses to SIV RNA as compared to pDCs from RMs, and limited *in vivo* studies including longitudinal flow cytometric comparisons confined to the acute phase of infection, along with cross-sectional gene expression comparisons from a single time point during chronic infection. To further study these questions in a biologically relevant *in vivo* setting, we sought to 1) determine if pDCs in lymphatic tissues of natural hosts produce type I IFN during acute infection and 2) determine if type I IFN responses are attenuated following SIV infection in SM compared to RM. In addition, we extended our analysis to a second widely studied SIV natural host, the African green monkey (AGM; Table 1). Here we provide direct *in vivo* evidence that during acute SIV infection RMs, SMs and AGMs all mount a similarly robust type I IFN response, and that a key distinguishing feature between non-pathogenic SIV infection of SMs and AGMs and pathogenic SIVmac infection of RMs is the timely resolution of this vigorous *in vivo* type I IFN response in natural hosts but not RMs, resulting in limited chronic activation of type I IFN responsive pathways.

As pDCs are the major source of type I interferon (IFN- α/β) produced in response to viral infections (**18**) and as pDCs migrate to inflamed lymphatic

tissues (LT) through high endothelium venules during viral and bacterial infections (**3, 29**), we studied pDC localization and IFN- α/β expression in peripheral lymph nodes (LN) early after SIV infection in RMs, SMs and AGMs, analyzing IFN- α/β at the protein level by immunohistochemistry on lymphatic tissue sections. Although the limited availability of tissues from our Sooty mangabey cohort limited our ability to perform detailed kinetic assessments of type I IFNs, each nonhuman primate species clearly showed an up-regulation of IFN- α/β at 14 dpi of the acute phase of infection with cells showing distinctively plasmacytoid morphology (putative pDCs) being the principal cell population producing these type I IFN proteins (**Fig. 1A**). Furthermore, type I IFN α production co-localized with expression of the pDC marker CD123 (**Fig. 1B**). Although these data demonstrate that at a time point around peak viral replication during the acute stage of SIV infection that pDCs of all three species are fully capable of producing type I IFNs, these data do not rule out the potential important contribution of other cell types as a source of type I IFNs in SIV infection.

Despite the clear demonstration of IFN expression by pDCs at the protein level following SIV infection in RMs, SMs, and AGMs, the presence of numerous variants of type I interferons and possible species-specific differences in immunoreactivity with available reagents rendered interpretation of quantitative analysis of type I interferon protein by immunohistochemistry potentially problematic. However, the biological activity of type I interferons can be assessed by measuring IFN-stimulated genes (ISGs), an approach which has

been reported to be a more sensitive measure of type I IFN induction *in vivo* than either IFN mRNA, and protein levels (23). Therefore we focused our efforts and available tissues to study the kinetics and magnitude of interferon dependent responses in lymph nodes by measuring two highly specific IFN- α/β -inducible effector molecules, myxovirus resistance protein A (MxA or Mx1) and interferon stimulated gene 15 (ISG15), proteins with highly conserved sequences across species which showed excellent, consistent reactivity in preliminary experiments using our immunohistochemistry methods (4, 13, 24, 27) (Fig. 2A). Of note, to compare IFN-specific responses by SIV-infected SM and RM, and HIV-infected humans, in their study, Mandl et al measured expression of mRNA for MX1 (and other markers) in PBMC by quantitative real-time RT PCR, but only at a single time point in chronic infection (19). Using quantitative image analysis tools to measure the protein expression levels for both MxA and ISG15 in high resolution whole lymph node Aperio tissue scans, we found vigorous type I IFN responses to SIV infection during the acute stage of infection with similar kinetics and peaks in RMs and in SMs and AGMs, with these IFN- α/β -inducible responses mirroring viral replication patterns (Fig. 2B-C) (9, 11 and data not shown).

Importantly, at 7 dpi all non-human primate species showed equivalent levels of MxA responses (Fig. 2C). It is not entirely clear why SMs showed statistically lower expression in MxA at 14 dpi and similar levels at 28 dpi compared to RMs, however this may be due to the low numbers of animals that were available for this study. Furthermore, these data show that AGMs begin to down-regulate MxA responses by 28 dpi, whereas only two SMs had attenuated

this response at this time point (**Fig. 2C**). However, by the chronic stage of infection (ranging from 4 – 16 months post infection) both AGMs and SMs showed completely attenuated type I IFN MxA responses, in stark contrast to RMs that maintained high levels of MxA at all time points (**Fig. 2**). The striking difference between non-pathogenic SIV infection of SMs and AGMs and pathogenic infection of RMs, and likely human HIV infection, was not a relative or absolute inability of natural hosts to mount a type I IFN response or a functional block of pDC production of IFN- α/β proteins in response to SIV *in vivo* (**Fig. 1-2**), but the relatively rapid down-regulation of these responses during the transition to chronic infection, despite ongoing viral replication (**Fig. 2**). Given the quantifiably lower ISG15 protein expression levels during acute infection in AGMs and SMs, in contrast to MxA (**Fig. 2C**), it is still possible that natural hosts respond in a qualitatively distinct way to type I IFNs produced rapidly following SIV infection compared to RMs or alternatively resident lymph node cell populations have divergent sensitivities to type I IFNs. However, the presented data are fully consistent with the observation of a strong up-regulation of type-I interferon stimulated genes (ISGs) during acute SIV infection of AGMs and SMs as measured by microarray analysis (1, 14, 17).

Together, these data unequivocally demonstrate that SMs and AGMs mount robust type I IFN responses to acute SIV infection and have functional IFN- α/β producing pDCs *in vivo*. However, in contrast to pathogenic infection of RMs, SIV-infected SMs and AGMs resolve these innate immune responses in the transition to chronic infection. While these data do not address whether or not

the observed robust type I IFN response and up-regulation of ISGs in SMs and AGMs occurs via a TLR-7/9-dependent or -independent mechanism, we conclude that a muted IFN α response by pDCs is neither a feature of nor a requirement for non-pathogenic SIV infection in SMs or AGMs.

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Table 1

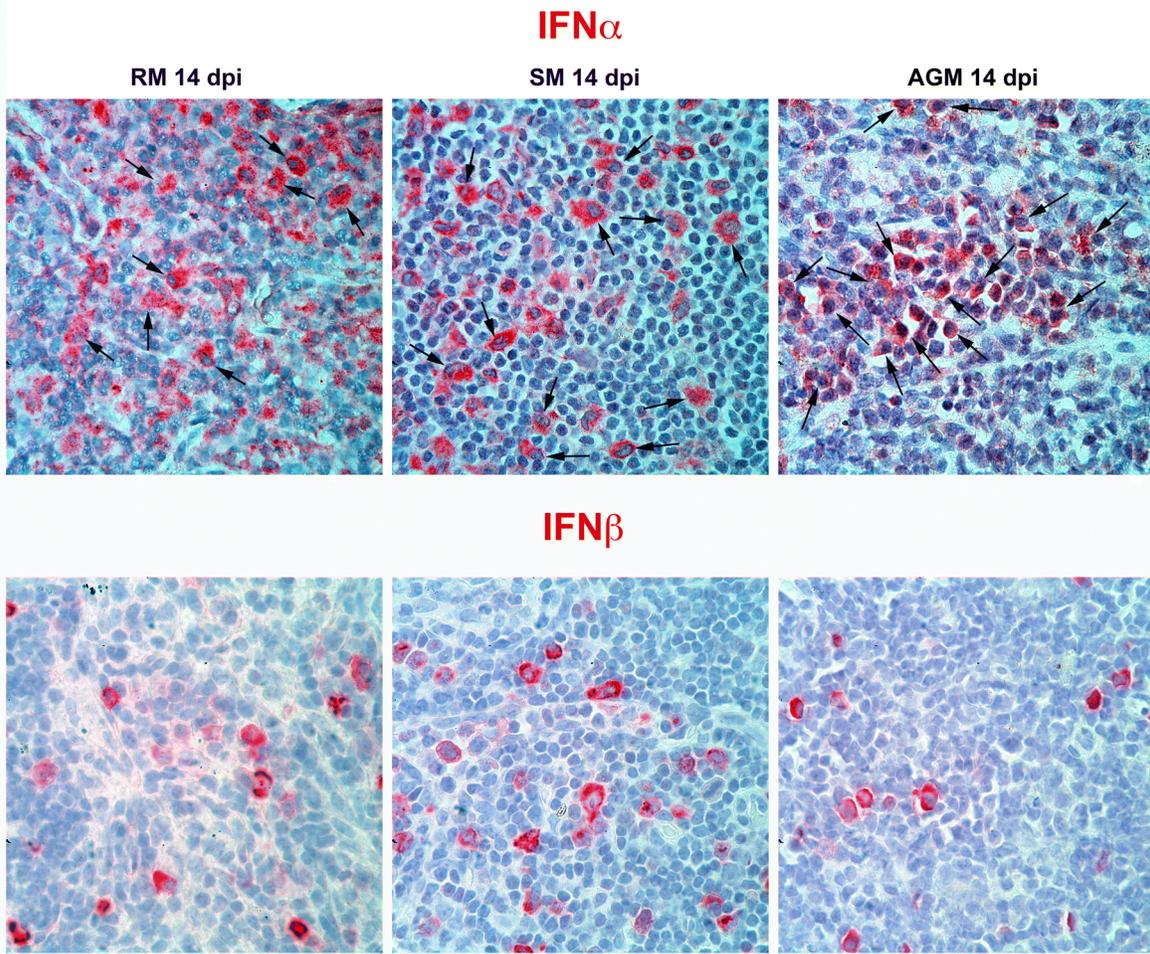
Animal ID	Species	Virus	LN samples (dpi)	Source (References)
AG-2	AGM	SIVagm1	Pre, +7, +14, +28	NIH/LMM (9)
AG-3	AGM	SIVagmVer	Chronic	NIH/LMM (9)
AG-4	AGM	SIVagmVer	Chronic	NIH/LMM (9)
AG-8	AGM	SIVagm1	Pre, +7, +14, +28	NIH/LMM (9)
AG-10	AGM	SIVagmVer	Chronic	NIH/LMM (9)
AG-14	AGM	SIVagm1	Pre, +7, +14, +28	NIH/LMM (9)
AG-17	AGM	SIVagmVer	Chronic	NIH/LMM (9)
AG-19	AGM	SIVagm1	Pre, +7, +14, +28	NIH/LMM (9)
AG-21	AGM	SIVagmVer90	Pre, +7, +14, +28	NIH/LMM (9)
AG-22	AGM	SIVagmVer90	Pre, +7, +14, +28	NIH/LMM (9)
AG-24	AGM	SIVagmVer90	Pre, +7, +14, +28	NIH/LMM (9)
FBn	SM	SIVsmm	Chronic	Yerkes (2)
FBq	SM	SIVsmm	Chronic	Yerkes (2)
FFq	SM	SIVsmm	Chronic	Yerkes (2)
FFs	SM	SIVsmm	Pre, +7, +14, +30	Yerkes (5, 10)
FLh	SM	-	SIV-	Yerkes (2)
FOz	SM	-	SIV-	Yerkes (2)
FRs	SM	SIVsmm	Pre, +7, +14, +30	Yerkes (5, 10)
FSs	SM	SIVsmm	Pre, +7, +14, +30	Yerkes (5, 10)
FUv	SM	SIVsmm	Pre, +7, +14, +30	Yerkes (5, 10)
FWv	SM	SIVsmm	Pre, +7, +14, +30	Yerkes (5, 10)
FXn	SM	SIVsmm	Chronic	Yerkes (2)
FZr	SM	-	SIV-	Yerkes (2)
04D169	RM	-	SIV-	NIH/VRC
95084	RM	SIVmac239	Chronic	WNPRC (5, 6)
R268	RM	SIVmacE660	Chronic	UPitt
R437	RM	SIVmacE660	Chronic	UPitt
R443	RM	SIVmac239	Chronic	UPitt
R451	RM	SIVmac239	Chronic	UPitt
R453	RM	SIVmac239	Chronic	UPitt
R455	RM	SIVmac239	Chronic	UPitt
R457	RM	SIVmac239	Chronic	UPitt
R462	RM	SIVmac239	Chronic	UPitt
R472	RM	SIVmac239	Chronic	UPitt
R475	RM	SIVmac239	Chronic	UPitt
RCo8	RM	SIVmac239	Pre, +7, +14, +30	Yerkes (5)
RJi8	RM	SIVmac239	Chronic	Yerkes (5)
RJi9	RM	SIVmac239	Pre, +7, +14, +30, Chronic	Yerkes (5)
RKb9	RM	SIVmac239	Pre, +7, +14, +30, Chronic	Yerkes (5)
RM340	RM	SIVmac239	Pre, +7, +28	NCI-Frederick (5, 6)
RSq8	RM	SIVmac239	+14	Yerkes (15)
RWf7	RM	SIVmac239	Pre, +14	Yerkes (15)
RWu8	RM	SIVmac239	Chronic	Yerkes (5)

Figure Legend

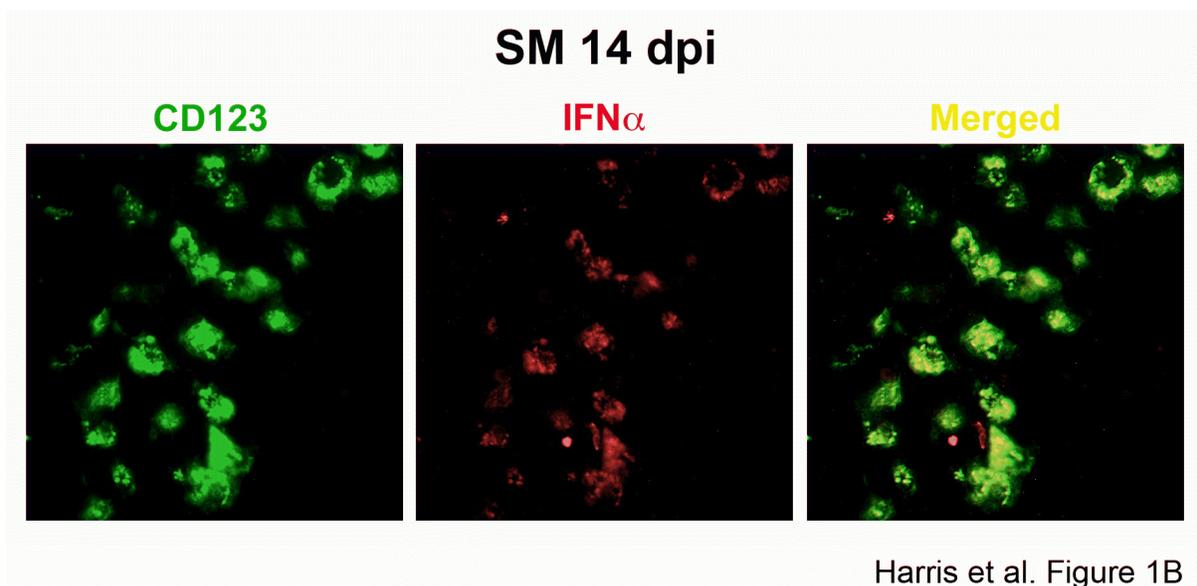
Figure 1. *In vivo* IFN α/β protein expression in pDCs during acute SIV infection of RMs, SMs and AGMs. *A.* Immunohistochemical staining (1000x) of IFN α (top panels) and IFN β (bottom panels) in lymph node sections from animals 14 days p.i. Arrows identify examples of IFN α/β expression in cells with a clear plasmacytoid morphology, putative pDCs. Tissues were stained with antibodies to IFN α (clones MMHA-2 and MMHA-3) or IFN β (MMHB-16) (all from PBL InterferonSource) using the biotin-free MACH-3 polymer-ALP system (Biocare Medical), developed with Vulcan Fast Red (Biocare Medical) and counterstained with hematoxylin. *B.* Immunofluorescent staining (400x) shows that CD123+ pDCs (green) in SMs at 14 days p.i. do in fact express IFN α protein (red), as the merged images clearly demonstrate colocalization (yellow).

Figure 2. Rapid and robust LN type I IFN responses in SIV-infected RMs, SMs and AGMs that is promptly resolved in SMs and AGMs but not RMs. *A.* Representative high magnification (400x) images from the T cell zone immunohistochemically stained for MxA, a specific type I IFN responsive gene in RM, AGM and SM prior to and during the acute and chronic stage of SIV infection (scale bar 25 μ m). *B-C.* Quantitative image analysis of high-resolution whole lymph node scans immunohistochemically stained for MxA (left panels) and ISG15 (right panels) from SIV-infected RMs (black symbols), SMs (blue symbols) and AGMs (red symbols). Archived tissue sections from three previous

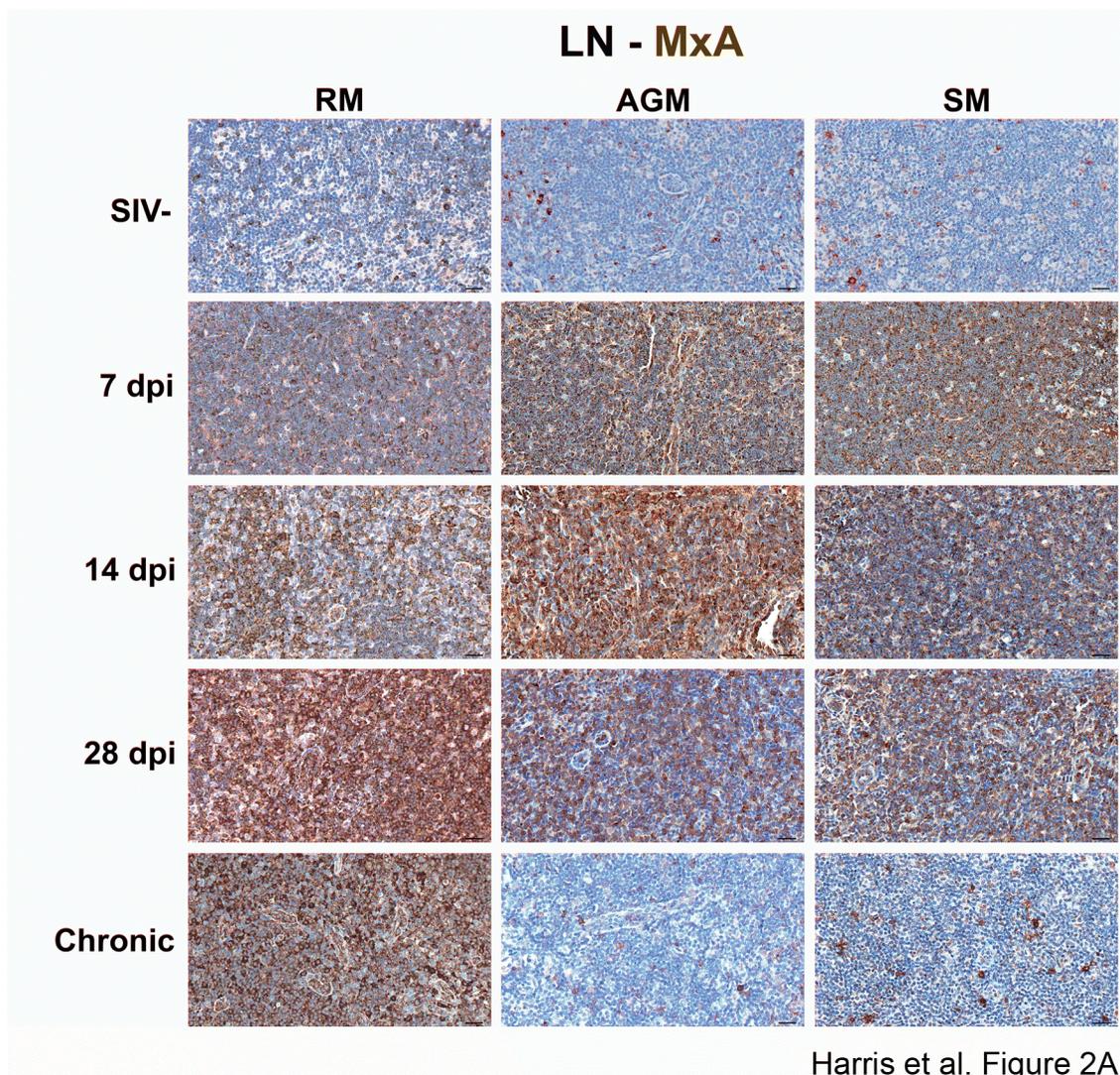
acute infection studies, in which we had collected lymph node biopsies that spanned from pre-infection time points through 30 days post infection and into the chronic stage longitudinally (Table 1) (5, 9, 11, 15). In addition, we used cross-sectional tissue samples collected at various times during the chronic stage and from SIV- animals to add to our original data set (Table 1). Quantitative image analysis was performed on immunohistochemically stained, high-resolution whole lymph node tissue scans obtained from an Aperio ScanScope CS (Aperio Technologies, Inc.) to measure the protein expression levels of both MxA (anti-MxA antibody M143 generously provided by Dr. Georg Kochs, Universitätsklinikum Freiburg) and ISG15 (Sigma Prestige Antibodies® Powered by Atlas Antibodies) and shown kinetically for each non-human primate species (B) or at each time point showing comparative expression levels for all the species (C). Each circle represents a quantified lymph node section. For some animals there were two independent lymph nodes collected and measured and are thus represented as separate independent data points. Second lymph nodes from individual animals are indicated by unfilled plot symbols. Mann-Whitney non-parametric U tests were performed using Prism 4.0 software (Prism, San Diego, CA) with resulting p-values shown.



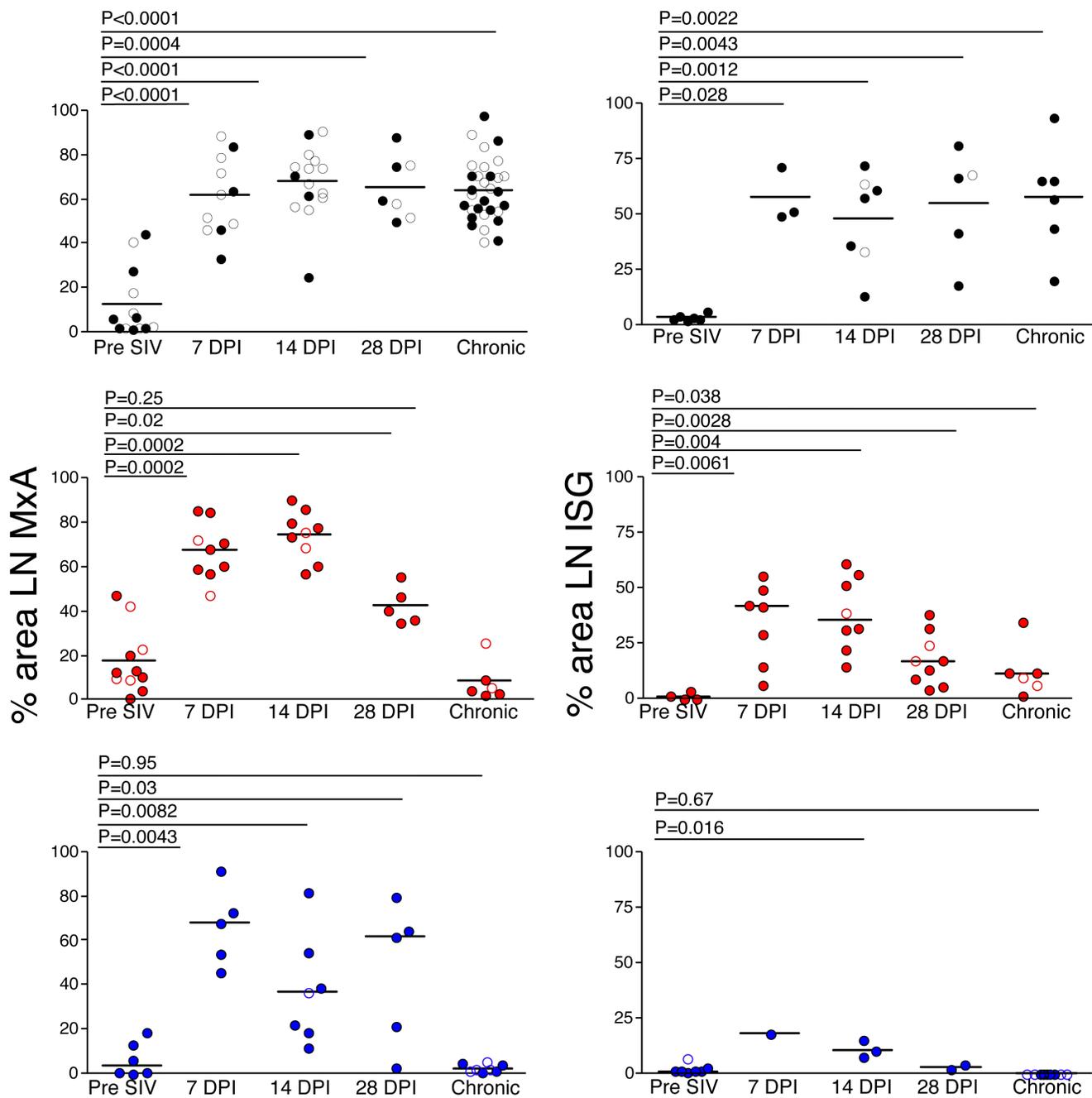
Harris et al. Figure 1A



Harris et al. Figure 1B



Harris et al. Figure 2A



Harris et al. Figure 2B

