







# Supplementary Materials: Drivers and Distribution of Henipavirus-Induced Syncytia: What Do We Know?

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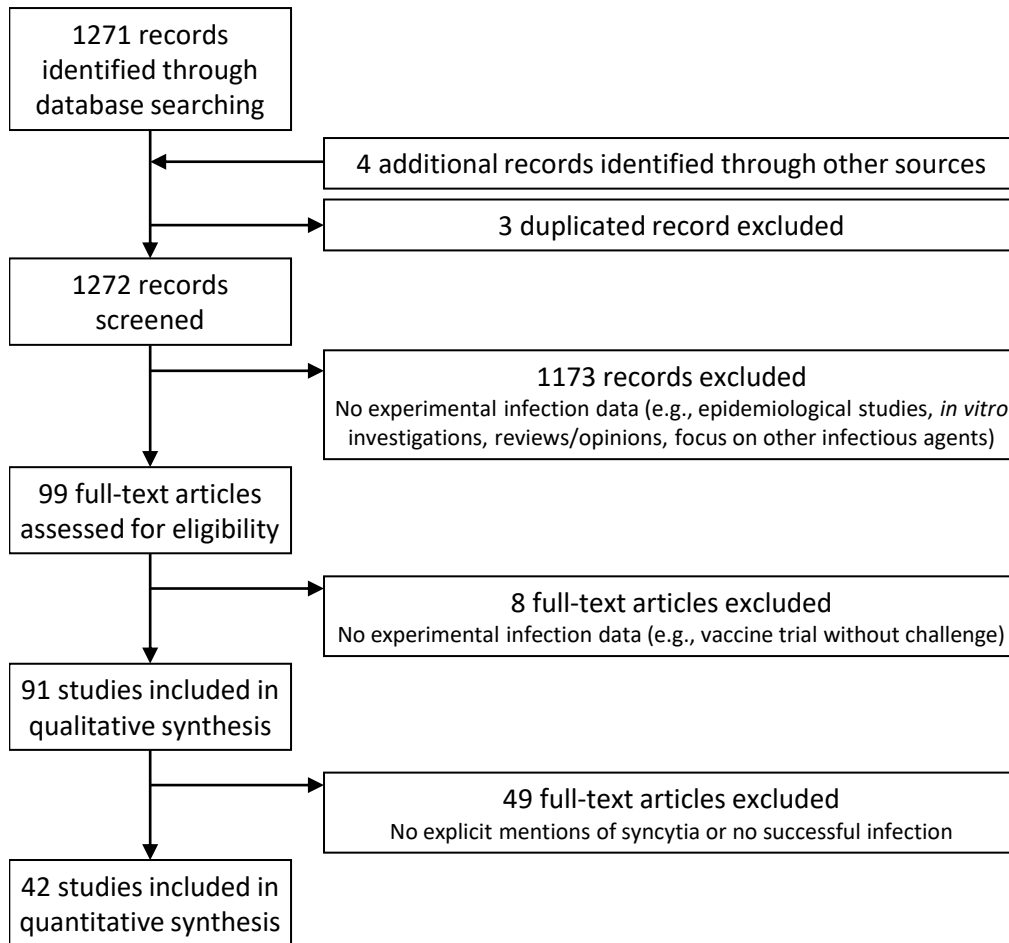
## 1. Meta-analysis methodology

We screened the Web of Science Core Collection database on October 29, 2020 using the following keywords: (“in vivo” OR infect\* OR histo\*) AND (henipavir\* OR hendra OR nipah OR “equine morbilli\*”) (1 271 records). We also considered studies referenced in full-texts assessed for eligibility and potentially reporting datasets of interest (4 records). We then selected publications reporting data from in vivo experimental infections using HeV, NiV or CedV. To ensure that we included in our meta-analysis only studies that actively screened for syncytia, we excluded the publications that never mentioned syncytia, multinucleated cells or cell fusion in the method or result sections. Data collected from individuals who received a treatment (passive or active immunization or antiviral) or were inoculated deficient virus (e.g., knockout of non-structural proteins) were also excluded from the meta-analysis. The complete selection procedure is described in Figure S1 following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [1]. Studies included in the quantitative analyses are listed in Table S1.

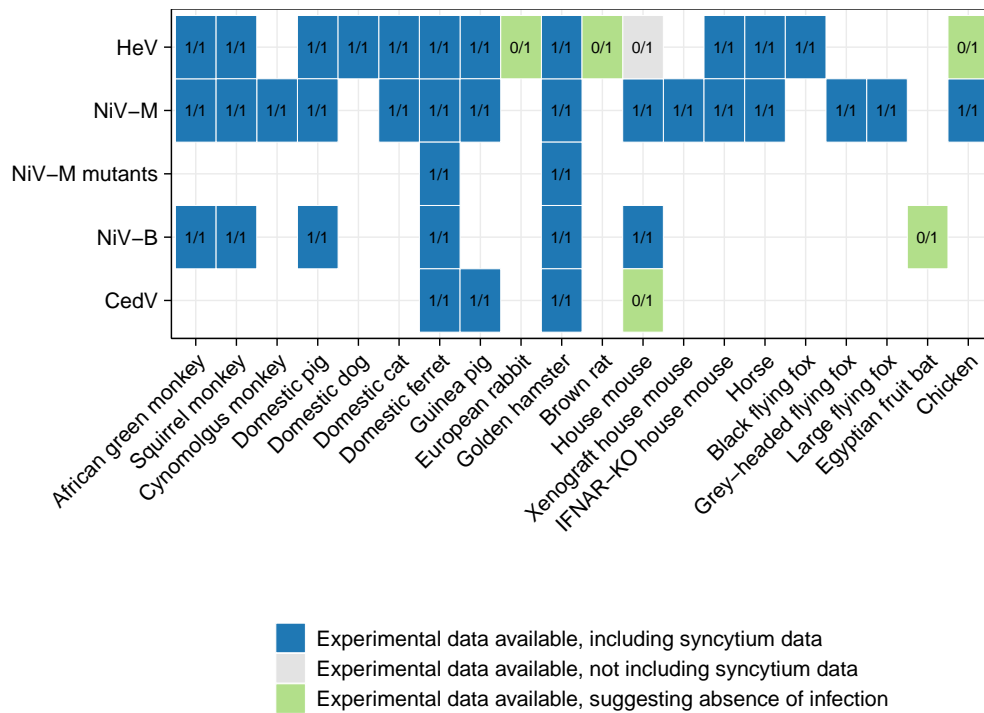
For each study, data on syncytium occurrence in tissue samples (assessed by histological analyses) were manually extracted from the publication text and tables. Data on virus culture, viral RNA detection (detected by reverse transcription polymerase chain reaction) or viral antigen detection (detected by immunohistochemistry) were also included in the data set when available. Data were compiled as detection/non-detection of syncytia and viral material in individual samples or as proportion of positive samples depending on the resolution at which data were reported. Syncytia were considered as detected if said so explicitly, and non-detected if reported that no syncytia were detected, no lesion were detected, or detected lesions were limited to X and Y (not including syncytia). When such explicit information was not available, syncytium data were recorded as not available (in particular, non-detection was not inferred from the absence of report of detection). Individual metadata were also included in the data set, in particular host species, viral strain, route and dose of inoculation and time between inoculation and euthanasia. Data availability is summarized in Figure S2.

Sample size was approximated by the minimum plausible sample size based on the available data if no exact sample size was available. Time post-infection was approximated by the rounded averaged value when the range of plausible value was of three days or less; if a range of more than three days was plausible, the data were excluded from any analysis or plot considering time post-infection. Syncytium prevalence was then calculated as the ratio of individuals in which syncytia were detected / individuals for which syncytia detection or non-detection is reported at least once for a given tissue, physiologic system, and/or time-point depending on the analysis.

We used the software R (version 3.6.2) [2] and packages `tydir` (version 1.1.2) [3], `ggplot2` (version 3.3.2) [4] and `cowplot` (version 1.0.0) [5] for data curation, analysis and visualization. The complete data set and the codes used to reproduce the figures and analyses are available at <https://doi.org/10.5281/zenodo.5338802> [? ].



**Figure S1.** Selection process of the studies included in the meta-analysis of syncytium formation following experimental henipavirus infections *in vivo*.



**Figure S2.** Map of syncytium data availability across host and virus species following experimental henipavirus infection in vivo. A publication was counted as reporting syncytium data if it either explicitly reported the observation or non-observation of syncytia, or if it reported the absence of any lesion from all the explored tissues. Numbers indicate the number of publications reported syncytium data / number of publications reporting experimental infection data. Blue cells indicate host-virus combinations for which at least one publication reported syncytium data; grey cells indicate combinations for which no syncytium data are available; and green cells indicate combinations for which experimental inoculation did not induce any host response (immune or pathological); blank spaces indicate that no data are available.

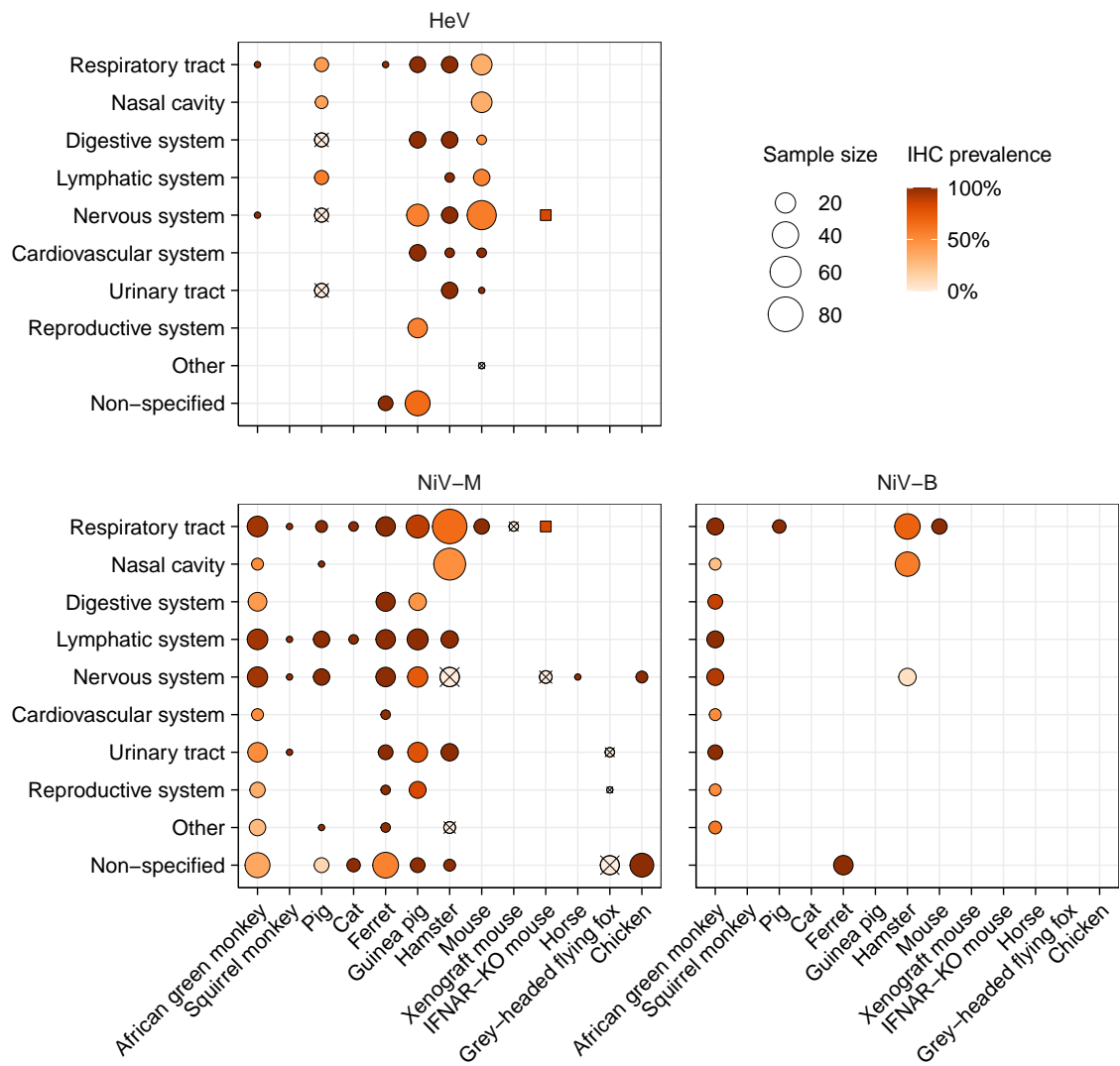
**Table S1. 1/2.** Studies included in the meta-analysis of syncytium formation following experimental henipavirus infections *in vivo*. TCID<sub>50</sub>: median tissue-culture infectious dose; LD<sub>50</sub>: median lethal dose; PFU: plaque forming units.

Study	Species	Inoculation route	Dose	Virus
Baseler et al. 2014 [6]	Hamster	Oronasal	1×10 <sup>7</sup> TCID <sub>50</sub>	NiV-M
Baseler et al. 2014 [6]	Hamster	Oronasal	1×10 <sup>7</sup> TCID <sub>50</sub>	NiV-B
Bossart et al. 2009 [7]	Ferret	Oronasal	500 TCID <sub>50</sub>	NiV-M
Bossart et al. 2009 [7]	Ferret	Oronasal	5×10 <sup>4</sup> TCID <sub>50</sub>	NiV-M
Clayton et al. 2016 [8]	Ferret	Oronasal	5000 TCID <sub>50</sub>	NiV-B
Clayton et al. 2016 [8]	Ferret	Oronasal	5000 TCID <sub>50</sub>	NiV-M
Cong et al. 2017 [9]	African green monkey	Intratracheal	13000 PFU	NiV-M
Cong et al. 2017 [9]	African green monkey	Aerosol	40300 PFU	NiV-M
de Wit et al. 2014 [10]	Hamster	drinking	5×10 <sup>8</sup> TCID <sub>50</sub>	NiV-B
Escaffre et al. 2018 [11]	Hamster	Aerosol	85000 PFU	NiV-M
Escaffre et al. 2018 [11]	Hamster	Aerosol	2×10 <sup>4</sup> PFU	NiV-M
Escaffre et al. 2018 [11]	Hamster	Aerosol	1×10 <sup>5</sup> PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal/oral	13×10 <sup>5</sup> PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal	23000 PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal	2500 - 65000 PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal/oral	700 - 13×10 <sup>5</sup> PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal	59000 PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal/oral	81000 PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal	65000 PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal	2500 PFU	NiV-M
Geisbert et al. 2010 [12]	African green monkey	Intratracheal	7000 PFU	NiV-M
Geisbert et al. 2014 [13]	African green monkey	Intravenous	5×10 <sup>5</sup> PFU	NiV-M
Geisbert et al. 2019 [14]	African green monkey	Intranasal	2×10 <sup>4</sup> PFU	NiV-B
Geisbert et al. 2019 [14]	African green monkey	Intranasal	2000 PFU	NiV-B
Guillaume et al. 2009 [15]	Hamster	Intraperitoneal	1000 PFU	HeV
Guillaume-Vasselin et al. 2016 [16]	Hamster	Intraperitoneal	1×10 <sup>4</sup> LD <sub>50</sub>	HeV
Hammoud et al. 2018 [17]	African green monkey	Aerosol	100 PFU	NiV-M
Hammoud et al. 2018 [17]	African green monkey	Aerosol	1000 PFU	NiV-M
Hooper et al. 1997a [18]	Horse	Intranasal	Unknown	HeV
Hooper et al. 1997a [18]	Horse	Intravenous/aerosol	2×10 <sup>7</sup> TCID <sub>50</sub>	HeV
Hooper et al. 1997b [19]	Cat	Intranasal	5000 TCID <sub>50</sub>	HeV
Hooper et al. 1997b [19]	Cat	Oral	5000 TCID <sub>50</sub>	HeV
Hooper et al. 1997b [19]	Cat	Direct contact	5000 TCID <sub>50</sub>	HeV
Hooper et al. 1997b [19]	Cat	Subcutaneous	5000 TCID <sub>50</sub>	HeV
Hooper et al. 1997b [19]	Guinea pig	Subcutaneous	5000 TCID <sub>50</sub>	HeV
Johnston et al. 2015 [20]	African green monkey	Intratracheal	25000 PFU	NiV-M
Li et al. 2010 [21]	Pig	Intranasal	2×10 <sup>7</sup> PFU	HeV
Li et al. 2010 [21]	Pig	Oronasal	66×10 <sup>6</sup> PFU	HeV

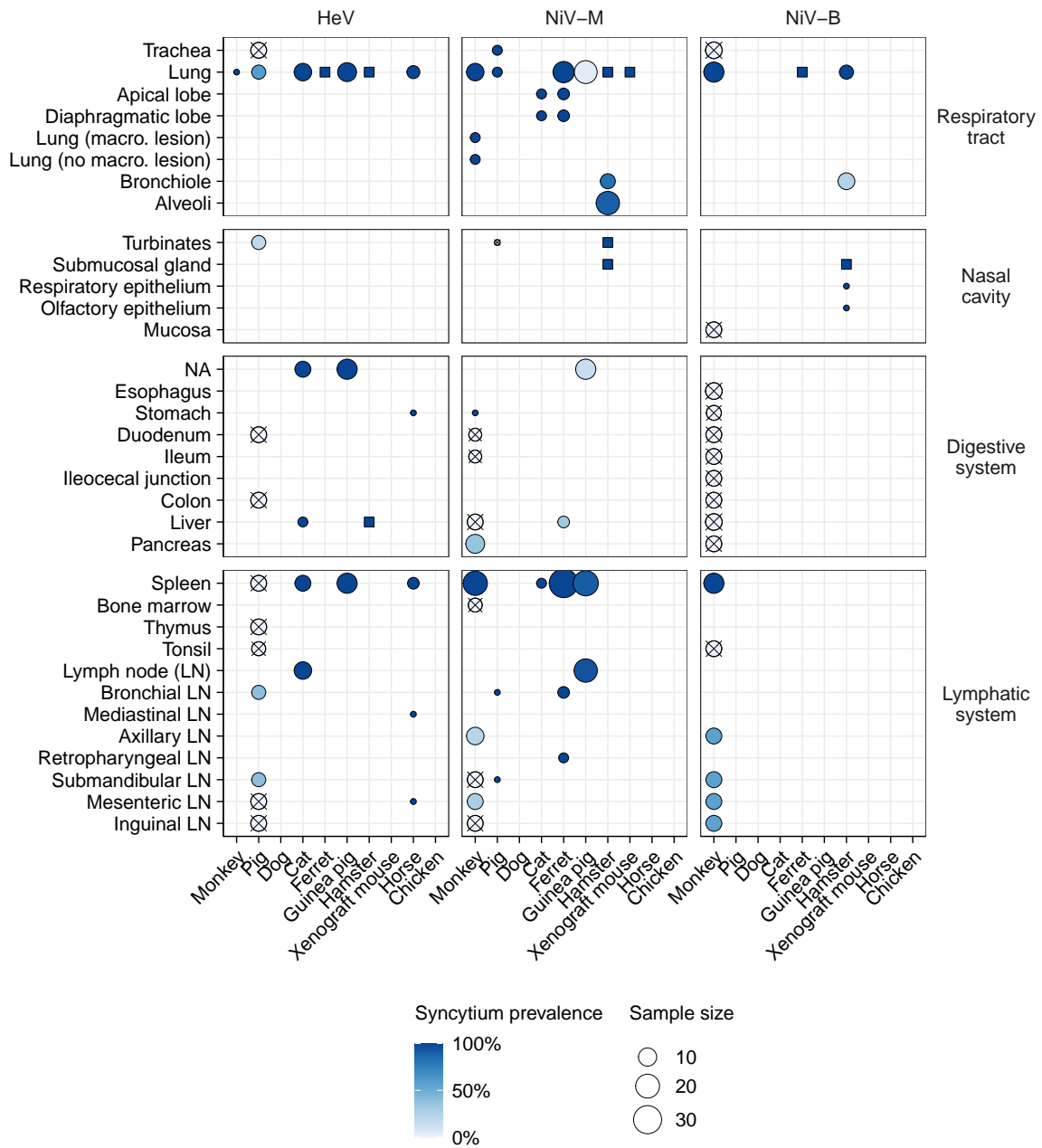
**Table S1. 2/2.** Studies included in the meta-analysis of syncytium formation following experimental henipavirus infections *in vivo*. TCID<sub>50</sub>: median tissue-culture infectious dose; PFU: plaque forming units.

Study	Species	Inoculation route	Dose	Virus
McEachern et al. 2008 [22]	Cat	Oronasal	$5 \times 10^4$ TCID <sub>50</sub>	NiV-M
Middleton et al. 2007 [23]	Guinea pig	Intraperitoneal	$5 \times 10^4$ TCID <sub>50</sub>	NiV-M
Middleton et al. 2017 [24]	Dog	Oronasal	$2 \times 10^6$ TCID <sub>50</sub>	HeV
Mire et al. 2013 [25]	Ferret	Intranasal	5000 PFU	NiV-M
Mire et al. 2015 [26]	African green monkey	Intratracheal/intranasal	$5 \times 10^5$ PFU	NiV-B
Mire et al. 2019 [27]	African green monkey	Intratracheal/intranasal	$5 \times 10^5$ PFU	NiV-B
Mungall et al. 2006 [28]	Cat	Subcutaneous	5000 TCID <sub>50</sub>	NiV-M
Mungall et al. 2006 [28]	Cat	Subcutaneous	500 TCID <sub>50</sub>	NiV-M
Mungall et al. 2007 [29]	Cat	Subcutaneous	500 TCID <sub>50</sub>	NiV-M
Munster et al. 2012 [30]	Hamster	Intranasal	$1 \times 10^5$ TCID <sub>50</sub>	NiV-M
Pallister et al. 2009 [31]	Ferret	Oronasal	5000 TCID <sub>50</sub>	NiV-M
Prasad et al. 2019 [32]	African green monkey	Aerosol	$1 \times 10^4$ PFU	NiV-B
Prasad et al. 2019 [32]	African green monkey	Aerosol	1000 PFU	NiV-B
Rockx et al. 2010 [33]	African green monkey	Intratracheal	$4 \times 10^5$ TCID <sub>50</sub>	HeV
Satterfield et al. 2015 [34]	Ferret	Intranasal	5000 PFU	NiV-M
Satterfield et al. 2016 [35]	Ferret	Intranasal	5000 PFU	NiV-M
Satterfield et al. 2019 [36]	Ferret	Intranasal	5000 PFU	NiV-M
Tanimura et al. 2006 [37]	Chicken	Allantoic	158489 PFU	NiV-M
Tanimura et al. 2006 [37]	Chicken	Yolk	7943 PFU	NiV-M
Torres-Velez et al. 2008 [38]	Guinea pig	Intraperitoneal	$6 \times 10^4$ PFU	NiV-M
Valbuena et al. 2014 [39]	Xenograft mouse	Xenograft injection	Unknown	NiV-M
Weingartl et al. 2005 [40]	Pig	Intranasal/oral/ocular	$25 \times 10^4$ PFU	NiV-M
Weingartl et al. 2006 [41]	Pig	Intranasal	$25 \times 10^4$ PFU	NiV-M
Westbury et al. 1995 [42]	Dog	Subcutaneous	5000 TCID <sub>50</sub>	HeV
Westbury et al. 1995 [42]	rat	Subcutaneous	5000 TCID <sub>50</sub>	HeV
Westbury et al. 1995 [42]	rabbit	Subcutaneous	5000 TCID <sub>50</sub>	HeV
Westbury et al. 1995 [42]	house_mouse	Subcutaneous	5000 TCID <sub>50</sub>	HeV
Westbury et al. 1995 [42]	Chicken	Subcutaneous	5000 TCID <sub>50</sub>	HeV
Westbury et al. 1996 [43]	Cat	Oral/intranasal/subcutaneous	3981 TCID <sub>50</sub>	HeV
Williamson et al. 2000 [44]	Guinea pig	Subcutaneous	$5 \times 10^4$ TCID <sub>50</sub>	HeV
Williamson et al. 2001 [45]	Guinea pig	Subcutaneous	$5 \times 10^4$ TCID <sub>50</sub>	HeV
Williamson et al. 2001 [45]	Guinea pig	Subcutaneous	$3 \times 10^4$ TCID <sub>50</sub>	HeV
Wong et al. 2003 [46]	Hamster	Intraperitoneal	270 PFU	NiV-M
Wong et al. 2003 [46]	Hamster	Unknown	Unknown	NiV-M
Woon et al. 2020 [47]	Ferret	Oral/intranasal	$3 \times 10^4$ TCID <sub>50</sub>	HeV
Woon et al. 2020 [47]	Black flying fox	Oral/intranasal	$3 \times 10^4$ TCID <sub>50</sub>	HeV
Yoneda et al. 2013 [48]	African green monkey	Intraperitoneal	$1 \times 10^6$ TCID <sub>50</sub>	NiV-M

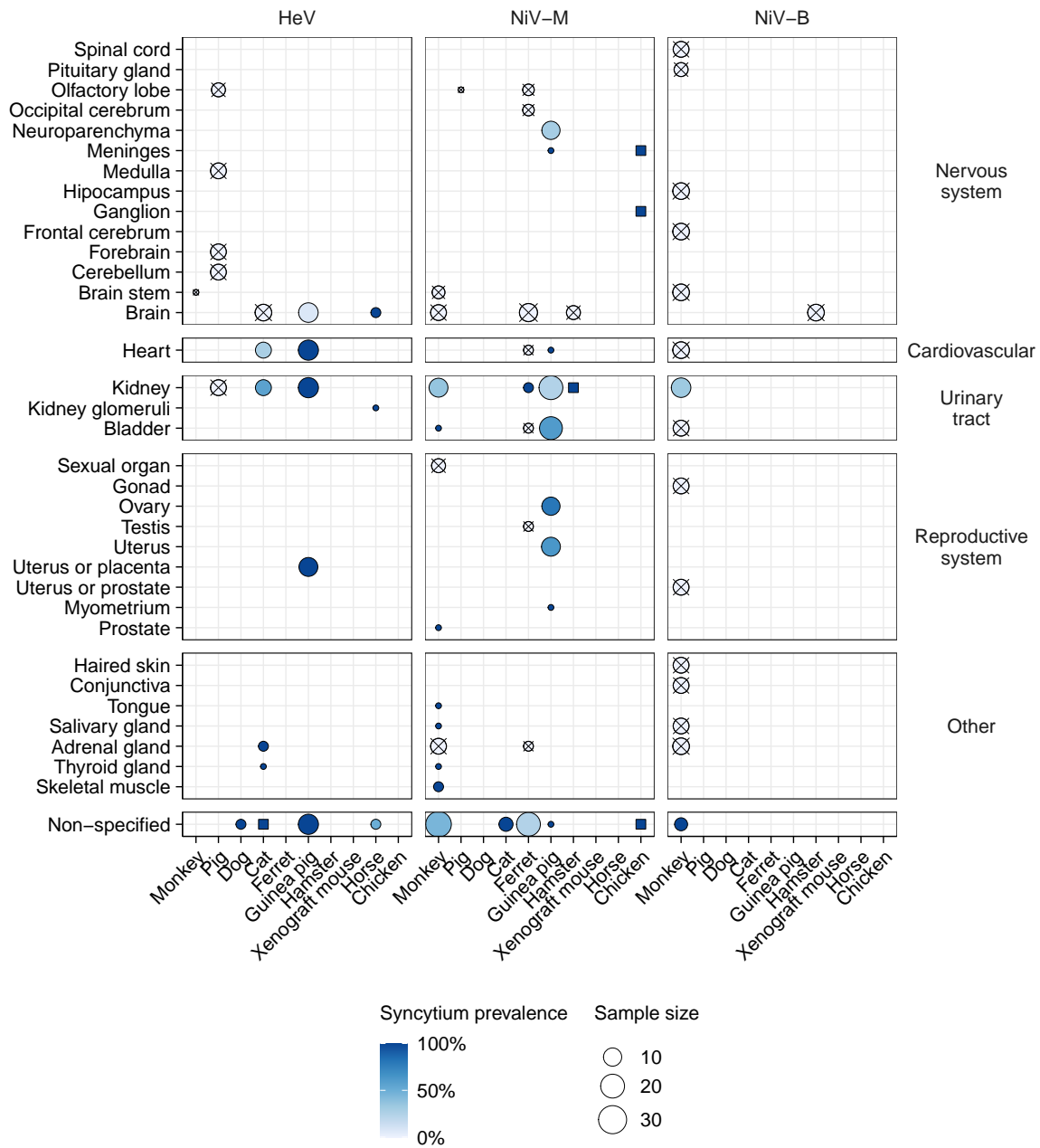
## 2. Additional Results



**Figure S3.** Proportion of individuals presenting viral antigens detected by immunohistochemistry (IHC) following experimental henipavirus infection in vivo. Dark orange squares indicate that viral antigens were detected but the exact proportion of individuals is unknown; blank spaces indicate that no data are available. Data obtained using different inoculation routes or doses and collected at different time post-infection were merged together. This figure represents a realistic map of potential syncytium data, as syncytia are detected by histology, which uses similar sample collection and preparation procedures that IHC (minus the staining of viral proteins via immunolabeling). Note that the scale for sample size is different from the figures reporting syncytium data due to larger sample sizes available for IHC. Data were extracted from the studies listed in [S1](#).



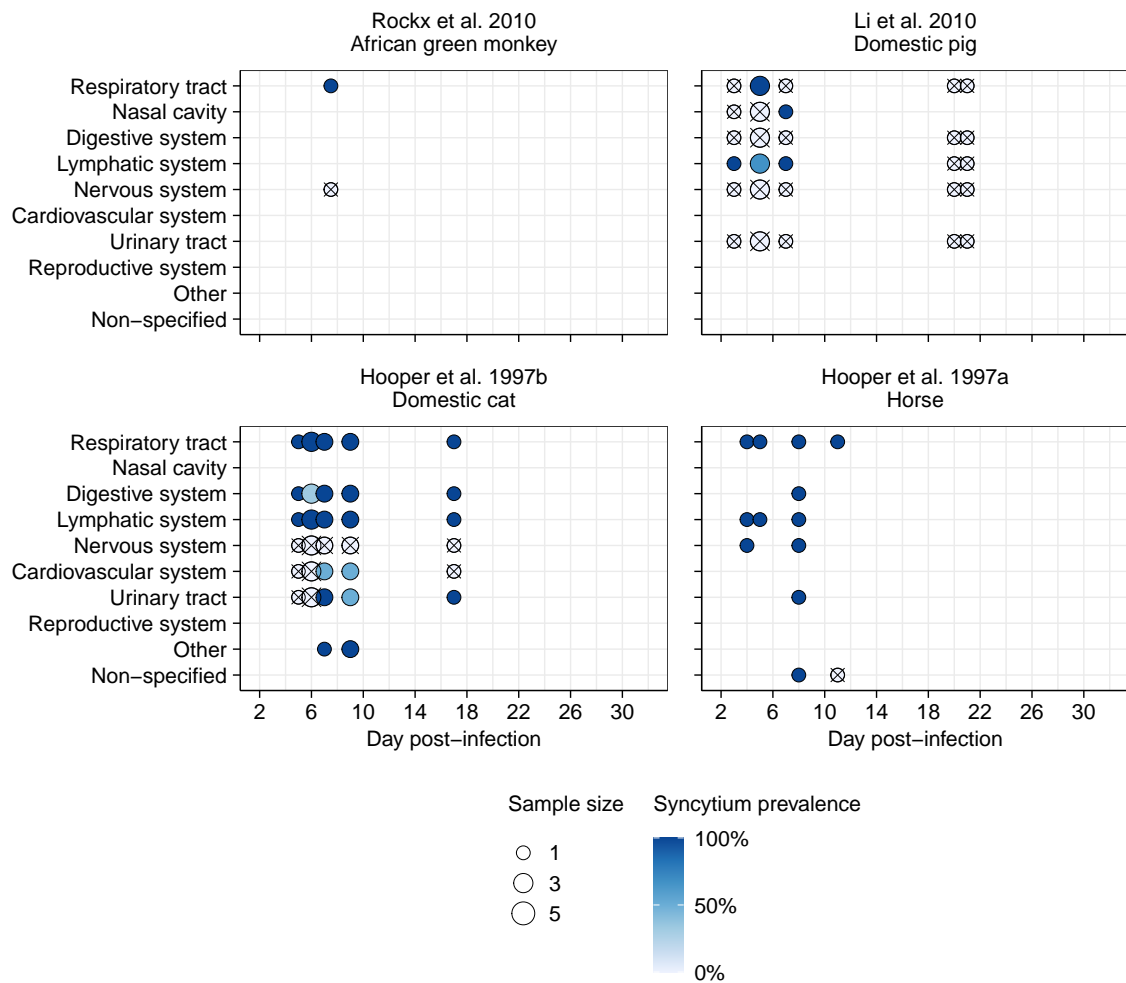
**Figure S4. 1/2.** Proportion of individuals presenting syncytia in different tissues following experimental henipavirus infection in vivo. Tissues are broken down at with varying levels of resolution (e.g., brain versus hippocampus, despite the hippocampus being part of the brain) corresponding to the diversity of resolution levels used across studies. Dot size indicates the number of individuals for which information regarding the detection or non-detection of syncytia was available, and dot color indicates the proportion of individuals presenting syncytia among those. Dark blue squares indicate that syncytia have been reported but that the exact proportion of individuals was unknown; blank spaces indicate that no data are available. Macro.: macroscopic; GI tract: gastro-intestinal tract. Data were extracted from the studies listed in S1.



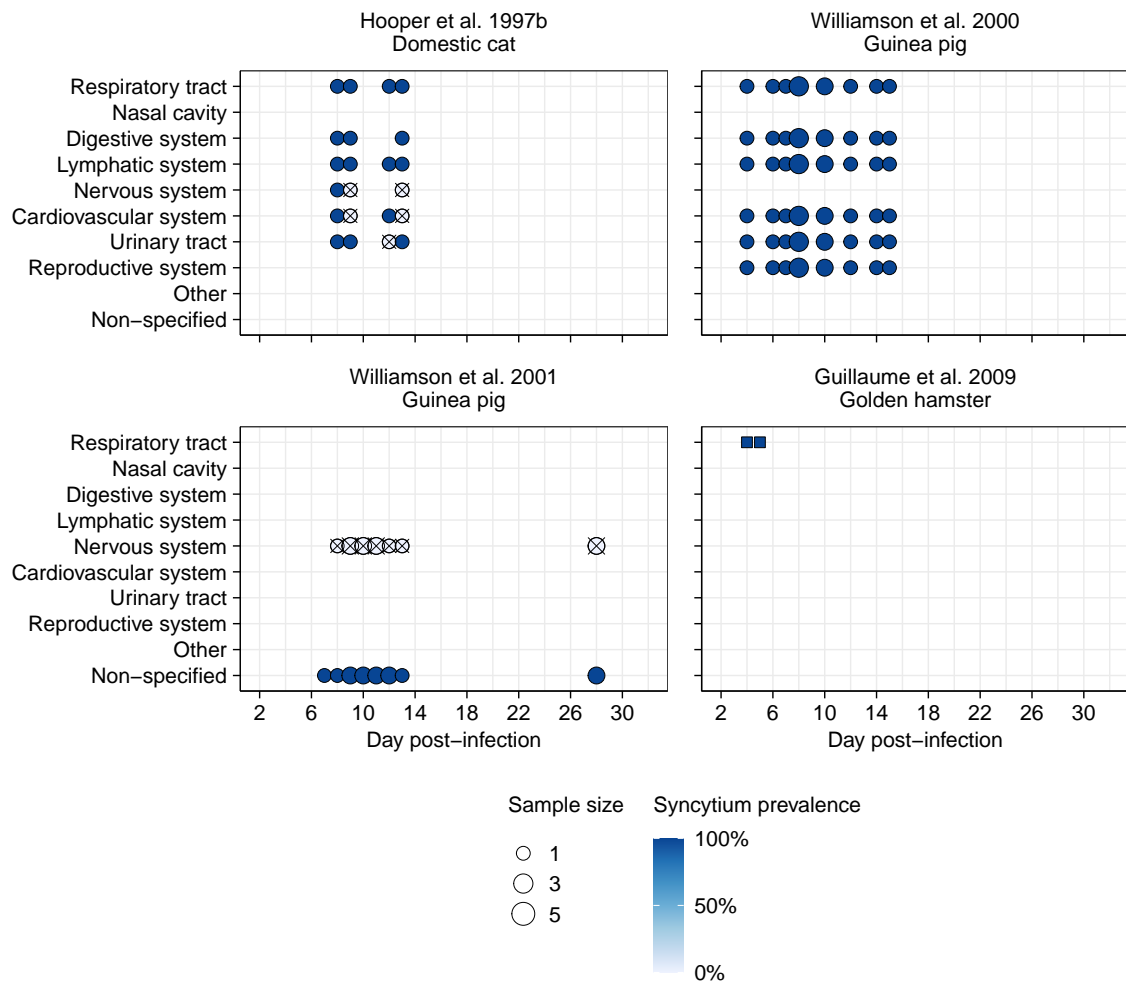
**Figure S4. 2/2.** Proportion of individuals presenting syncytia in different tissues following experimental henipavirus infection in vivo. Tissues are broken down at with varying levels of resolution (e.g., brain versus hippocampus, despite the hippocampus being part of the brain) corresponding to the diversity of resolution levels used across studies. Dot size indicates the number of individuals for which information regarding the detection or non-detection of syncytia was available, and dot color indicates the proportion of individuals presenting syncytia among those. Dark blue squares indicate that syncytia have been reported but that the exact proportion of individuals was unknown; blank spaces indicate that no data are available. Macro.: macroscopic.



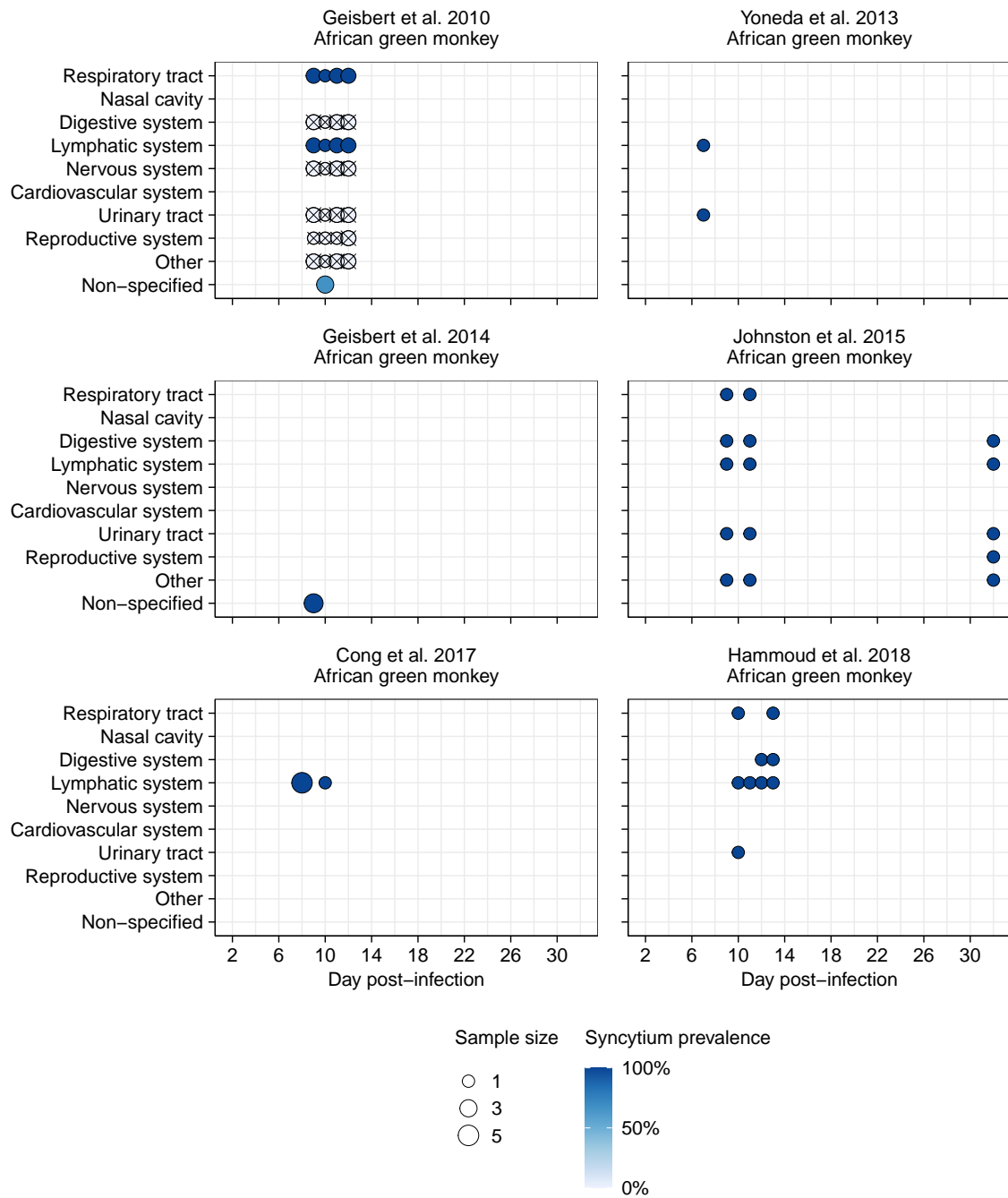
The proportion of individuals presenting syncytia over time post-infection broken down by study is represented in Figures S5–S7. The proportion of individuals presenting syncytia was calculated by dividing the number of individuals for which syncytia were detected by the number of individuals for which information regarding the detection or non-detection of syncytia was available, for each given tissue and at each given time-point. If several samples were collected from a given individual, the individual was considered as presenting syncytia if syncytia were detected in at least one sample (e.g., if several lymph nodes were collected from an individual and syncytia were detected in at least one, this individual was considered as presenting syncytia in lymphatic tissues). “Cardiovascular system” corresponds to heart and major blood vessels. “Other” regroups conjunctiva, skin, lip, pharynx, salivary gland and thyroid samples. “Organ mix” corresponds to unspecified tissues.



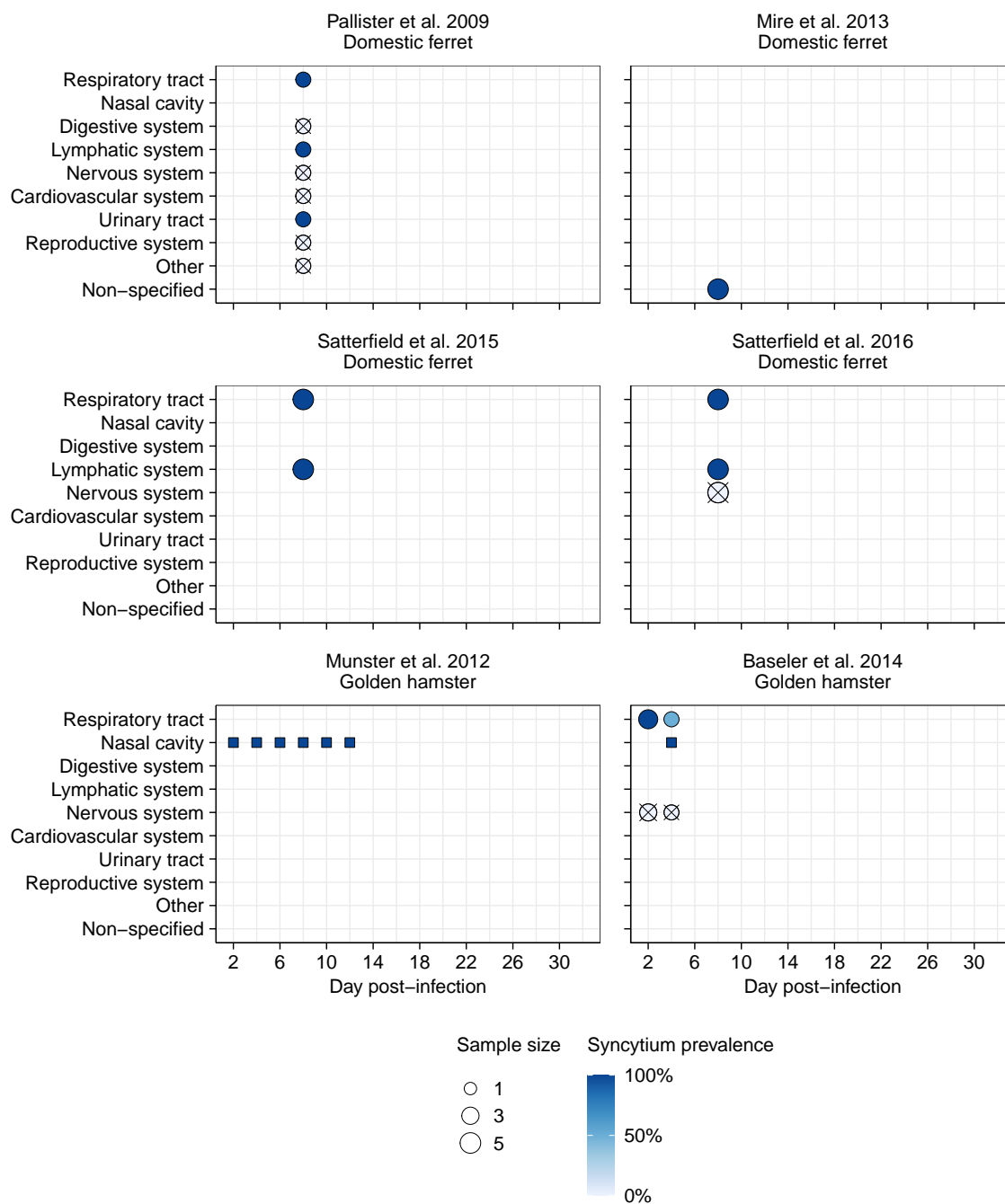
**Figure S5. 1/2.** Proportion of individuals presenting syncytia over time post-infection following experimental Hendra virus infection in vivo. Dot size indicates the number of individuals for which information regarding the detection or non-detection of syncytia was available, and dot color indicates the proportion of individuals presenting syncytia among those. Crossed circles indicate that no syncytia were observed; dark blue squares indicate that syncytia have been reported but that the exact proportion of individuals was unknown; blank spaces indicate that no data are available.



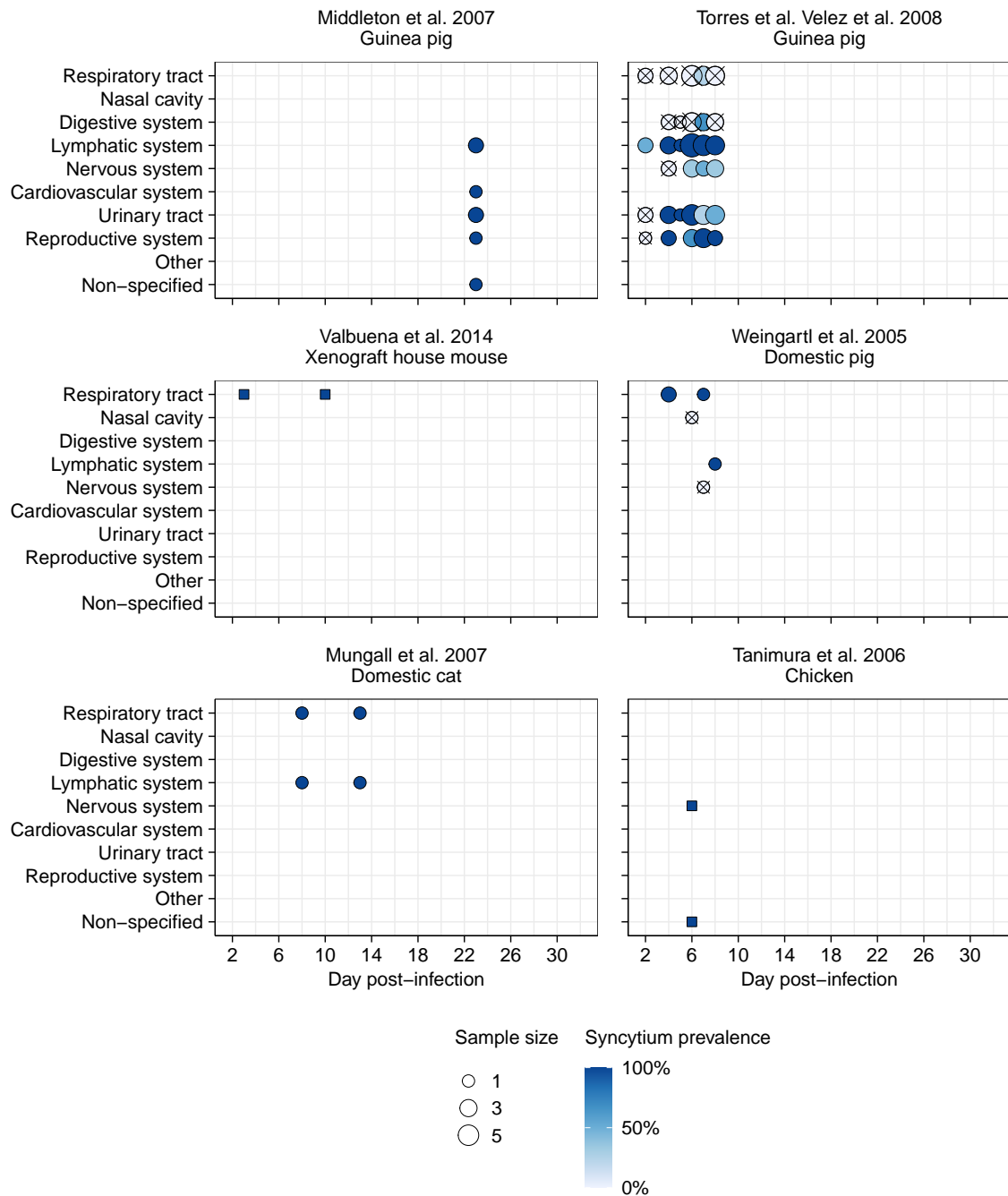
**Figure S5. 2/2.** Proportion of individuals presenting syncytia over time post-infection following experimental Hendra virus infection in vivo. Dot size indicates the number of individuals for which information regarding the detection or non-detection of syncytia was available, and dot color indicates the proportion of individuals presenting syncytia among those. Crossed circles indicate that no syncytia were observed; dark blue squares indicate that syncytia have been reported but that the exact proportion of individuals was unknown; blank spaces indicate that no data are available.



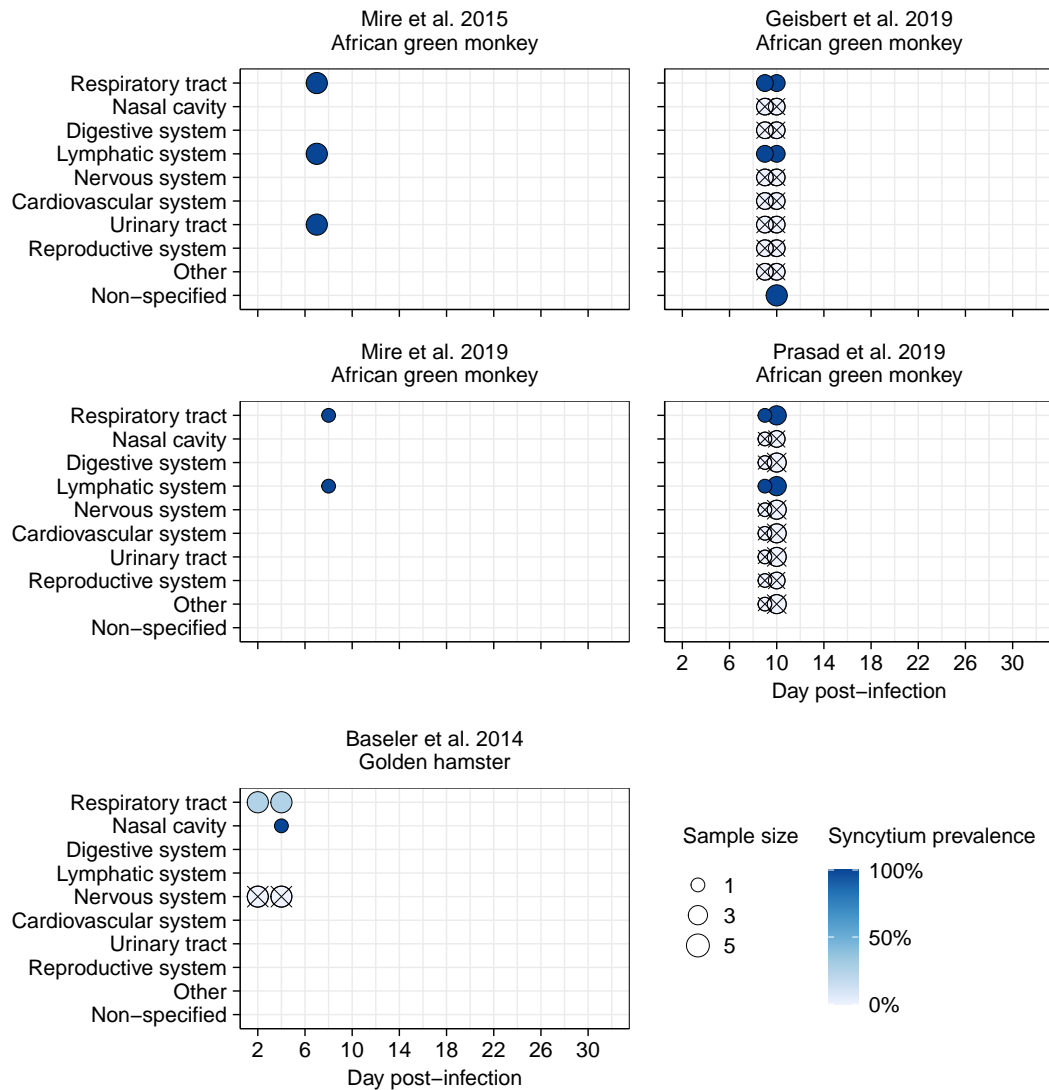
**Figure S6. 1/3.** Proportion of individuals presenting syncytia over time post-infection following experimental Nipah Malaysia virus infection in vivo. Dot size indicates the number of individuals for which information regarding the detection or non-detection of syncytia was available, and dot color indicates the proportion of individuals presenting syncytia among those. Crossed circles indicate that no syncytia were observed; dark blue squares indicate that syncytia have been reported but that the exact proportion of individuals was unknown; blank spaces indicate that no data are available.



**Figure S6. 2/3.** Proportion of individuals presenting syncytia over time post-infection following experimental Nipah Malaysia virus infection in vivo. Dot size indicates the number of individuals for which information regarding the detection or non-detection of syncytia was available, and dot color indicates the proportion of individuals presenting syncytia among those. Crossed circles indicate that no syncytia were observed; dark blue squares indicate that syncytia have been reported but that the exact proportion of individuals was unknown; blank spaces indicate that no data are available.



**Figure S6. 3/3.** Proportion of individuals presenting syncytia over time post-infection following experimental Nipah Malaysia virus infection in vivo. Dot size indicates the number of individuals for which information regarding the detection or non-detection of syncytia was available, and dot color indicates the proportion of individuals presenting syncytia among those. Crossed circles indicate that no syncytia were observed; dark blue squares indicate that syncytia have been reported but that the exact proportion of individuals was unknown; blank spaces indicate that no data are available.



**Figure S7.** Proportion of individuals presenting syncytia over time post-infection following experimental Nipah Bangladesh virus infection in vivo. Dot size indicates the number of individuals for which information regarding the detection or non-detection of syncytia was available, and dot color indicates the proportion of individuals presenting syncytia among those. Crossed circles indicate that no syncytia were observed; dark blue squares indicate that syncytia have been reported but that the exact proportion of individuals was unknown; blank spaces indicate that no data are available.

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