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Tracking mechanisms of viral dissemination *in vivo*

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Abstract

Dissemination and replication of viruses into hosts is a multi-step process where viral particles infect, navigate and indoctrinate various cell types. Doing so, viruses can reach tissues that are distant from their infection site by building subcellular mechanisms which are, sometimes, disruptive. Modeling these steps, at appropriate resolution and within animal models, is cumbersome. Yet, mimicking these strategies *in vitro* fails to recapitulate the complexity of the cellular ecosystem. Here, we will discuss how the cell biology community established relevant *in vivo* platforms to dissect the cellular and molecular programs governing viral dissemination. We will focus on the zebrafish model and will briefly describe how it provides a transparent window to unravel new cellular mechanisms of viral dissemination *in vivo*.

Highlights

- The zebrafish model allows *in vivo* investigations of virus-induced molecular processes at subcellular resolution
- Viruses have evolved multiple strategies for disseminating over long distance, including by indoctrinating host cell types with high migration potential
- Organoids derived from stem cells emerge as powerful alternatives to unravel new molecular mechanisms of viral dissemination

Main text

Introduction

To disseminate into hosts, pathogens had to evolve strategies to navigate and indoctrinate complex three-dimensional environments that cannot be fully recapitulated in two-dimensional monolayer cell culture. They deploy molecular stratagems to hijack resident stromal cells of multiple tissues and favor their dissemination and replication. While mimicking these strategies *in vitro* can be useful, established animal models are powerful systems for unraveling cellular and molecular programs favoring viral dissemination. Cell biologists have adapted relevant *in vivo* platforms, such as zebrafish embryos and mice, to develop exciting approaches

allowing to dissect *in vivo* subcellular processes that foster viral spreading. More recently, *ex vivo* tissue culture and organoids derived from human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) have been developed to study viral dissemination strategies in complex multidimensional settings. While bacterial dissemination *in vivo* was recently reviewed [1], we aim here at discussing recent work related to the cell biology of virus dissemination *in vivo*, list the current technical limitations and propose future developments.

Command and conquer

Viral dissemination into host refers to the ability of viral particles to reach tissular compartments remotely located from their initial infection site. This includes fluid-to-tissue, tissue-to-fluid and tissue-to-tissue dissemination, in addition to cell-to-cell virus transmission (see for instance [2, 3]). Such large-scale transitional stages require that the virus builds well-orchestrated subversion molecular mechanisms, which can be either disruptive or *a contrario* quiet. In any case, it is critical to decipher the subcellular processes involved in viral dissemination at the multi-organ scale, and to this end, relevant *in vivo* models represent instrumental approaches.

Tracking viral dissemination from the bloodstream using zebrafish embryos

A common way for viruses to disseminate is to use afferent fluid canals that naturally irrigate the organism. The bloodstream is the perfect culprit as it provides a fast track for distant viral dissemination, which may occur through various molecular mechanism [4]. The zebrafish embryo and larvae (*danio rerio*) represent a very attractive model to study virus dissemination from the bloodstream toward various organs [5]. Indeed, the genetic manipulation of zebrafish is straightforward and fluorescent fish lines allowing to tracking multiple cell types (endothelial, immune cells, etc..) are readily available [6]. Zebrafish display both innate and adaptive vertebrate-type immunity and recent developments now offer genetically immuno-compromised strains ideal for xenotransplantation of human patient samples and for unraveling the contribution of key immune elements to pathophysiology [7]. Moreover, microscopic studies are eased by the transparency of the embryo and its small size, avoiding body hindrance under the microscope. The model has been widely used in developmental and cell

biology, as well as in cancer biology [8, 9] and recently emerged as a powerful system to track viral dissemination *in vivo* [10, 11].

Studies investigating the cell biology of fish viruses have obviously used the zebrafish model extensively [12]. However, in the case of mammalian-tropic viruses, zebrafish embryos are mostly employed to study immune responses as well as pathogenesis, antiviral agents potency and toxicity (see for instance [13-19]). This model is also powerful to identify viral tropism, as it has recently been exemplified for the human norovirus (HuNoV), which can be detected in cells of the hematopoietic lineage and the intestine [14]. Hence, this powerful *in vivo* model offers exciting opportunities to decipher molecular mechanistic of pathogen dissemination.

The bloodstream carries a number of viruses and bacteria, but this environment is very hostile, as it contains a large number of host defenses, including immune cells, antibodies, proteins of the complement, and DNA traps (for review see [20]). To quickly escape the bloodstream, the easiest strategy is probably to infect endothelial cells lining blood vessels. Although relatively efficient at first, endothelial leakage or dysfunction is permanently under tight surveillance, and thus, this strategy does not preserve the virus from the host's immune defenses. The *Tg(fli1:GFP)* zebrafish line exhibiting fluorescent endothelial cells is an ideal model to study such process, as exemplified with the Infectious Hematopoietic Necrosis Virus (IHNV), which strongly disrupt the blood vessels for dissemination [21]. To a lesser extent, Chikungunya virus (CHKV) was also shown to infect endothelial cells of the brain vasculature of zebrafish larvae, leading to neuroinvasion [10]. In contrast, the authors of this latter study found that Sindbis virus (SINV) was infecting the central nervous system (CNS) independently of endothelial infection. This represents one of the first study to our knowledge investigating various routes of neuroinvasion, which was made possible thanks to advanced zebrafish embryo imaging. In contrast, the invasion of the CNS by Zika virus (ZIKV) was recently investigated in IFNAR-deficient mice, but the outcome was less evident [22]. Indeed, the authors nicely showed that the blood-brain barrier (BBB) was not disrupted, but it remains to be determined whether the virus reach the CNS through basolateral release, transcytosis or transinfection processes. Such information requires high resolution intravital imaging, which are technically difficult to implement in mice (see Text Box 1).

Viruses also evolved a subtler method to invade remote organs, which consists of hiding into blood cell components, such as platelets or monocytes for instance [11, 23], conferring a protective shell to them. When hiding, the virus may either replicate into the host cell, or just be carried in an intracellular compartment of the circulating host cell. This latter strategy however is also hazardous for the virus, as the particles will need to escape the cell later on to disseminate, while avoiding being directed toward degradative endolysosomes [24]. Nevertheless, an associated benefit from subverting a blood cell is the possibility to hitch a ride *incognito* over long distances, opening the door to long-range dissemination. It also allows the virus to sneakily cross tight endothelial barriers usually impermeable to microbes without disrupting it and thus, without inducing a strong immune response. This phenomenon is referred to as the *Trojan Horse hypothesis* [4, 25] and was described more than 35 years ago [26]. Recent work highlighted that ZIKV could be carried by human circulating monocytes. Tracking the behavior of these monocytes into the bloodstream of zebrafish embryos revealed key cellular strategies used by ZIKV to favor distant dissemination [11] (See Fig.1). Indeed, molecular mechanistic insights revealed that intravascular arrest of ZIKV-exposed monocytes was increased when compared to their non-exposed counterparts. These findings correlated with the increased expression of adhesion molecules at the surface of ZIKV-exposed monocytes, suggesting that the virus promotes cell attachment to the endothelial cells. Interestingly, ZIKV not only favors the intravascular arrest of infected monocytes, it further stimulates their extravasation which is a step that is instrumental for viral dissemination. This *in vivo* experimental approach was originally developed for tumor metastasis [27], highlighting the successful adaptation of powerful tools designed by cell biologists to unravel key strategies of viral dissemination.

We acknowledge that the zebrafish model has limitations to study pathogenesis and long-term dissemination in the case where the zebrafish cells are not permissive to the infection. Although there is no evidence to our knowledge that ZIKV can efficiently infect zebrafish, this latter study took advantage of the relative permissiveness of this *in vivo* model for xenografts to investigate virus-induced cell extravasation across the vascular wall [11]. Similarly, the human Cytomegalovirus (HCMV) was shown to induce monocyte adhesion to endothelial cells and transmigration [28], and while the zebrafish is not permissive to HCMV infection [29], one could for instance propose of

injecting HCMV-infected human cells in zebrafish to monitor virus-induced cell migration in an *in vivo* context. Transplanting cells into adult zebrafish has recently been made possible thanks to the development of immuno-compromised models (i.e. rag2 mutant zebrafish [30]) where subcellular imaging can be envisioned upon further generation of optically-cleared zebrafish that lack T, B, and natural killer cells (i.e. the prkdc^{-/-}, il2rga^{-/-} zebrafish [31]). Nevertheless, such advantage comes with the drawback that it does not allow to understand the immune response to viral infection, which shapes viral illness allowing the development of antiviral medicine [32].

Concluding remarks

The benefits of detailed *in vivo* cell biology analyses that the transparent zebrafish embryo has to offer should not be overlooked. Indeed, zebrafish allows *in vivo* investigations of virus-induced molecular processes at subcellular resolution, including studies addressing the mechanism of attachment, rolling and extravasation through the endothelial cell wall, the adhesion molecules involved and drastic actin rearrangements and the influence of a natural sheer stress in these processes. Yet, this model has limitations and future development are still needed (see Outstanding Questions box). In addition, the zebrafish embryo represents a powerful platform to develop correlative imaging approaches designed to track viral dissemination at nanoscale and thereby identify mechanisms of viral subversion leading to dramatic intracellular membranous rearrangement [33]. Correlative Light and Electron microscopy has recently been used in this system for tracking the dissemination and uptake of extracellular vesicles [34] that are similar in many aspects (size, morphology, content, etc...) to viral particles. Alternatively, it would be exciting to apply such CLEM strategies to mouse models of viral infection [35]. The zebrafish embryo not only allows to capture cellular behavior of viruses, it also allows to discover new viruses. A recent study reported the creation of a strain that expresses green fluorescent protein (GFP) under an interferon-stimulated gene promoter. Here, GFP expression can be used to track immune antiviral response and thereby provides an ideal platform to develop new strategies for discovering viruses while testing their impact on vertebrate models [36].

Text Box 1. Beyond the zebrafish: the mouse model to study viral dissemination.

The mouse represents an attractive model to study virus-host interactions [37]. However, they are more difficult to image than zebrafish embryos and because viruses can target various tissues, intravital microscopy (IVM) requires specific settings according to the organ to be imaged. A detailed protocol for IVM of the lung to study influenza virus infection was recently released [38], and while this protocol could be adapted to study SARS-CoV-2 infection for instance, it is less relevant to viral infections targeting other organs. Moreover, a large number of mouse models of viral infection requires immunocompromised or humanized mice, which makes this approach non-trivial and not fully relevant to human disease. Yet, the mouse model has the advantage to have an immune system more closely related to humans than zebrafish and to better recapitulate pathogenesis of viral infections. A pioneering study developed IVM in mouse lymph nodes to monitor the dissemination of the human immunodeficiency virus type 1 (HIV-1) through virus-induced migration of T cells [39], after a CD169-dependent virus transfer from sinus-lining macrophages [40]. Visualization of fluorescently-tagged vesicular stomatitis virus (VSV) particles in live mice showed circulating leukocytes transporting the oncolytic virus toward tumors [41]. These studies could track the migratory properties of infected cells, but underlying molecular mechanistic was not proposed. Murooka & colleagues intriguingly showed that the HIV-infected T cells exhibited unusually elongated, thin and branched trailing edges [39], which must result from dramatic cytoskeletal rearrangements, which remain to be fully characterized *in vivo*. Using IVM and electron tomography in the bone-marrow of HIV-infected mice, the description of several scenario leading to HIV cell-cell transfer was further depicted (Figure 1, [42]), and here again, host proteins involved in these complex processes remain to be identified. Recently, the vaccinia virus (VACV) was shown to enhance rapid and directed cell motility through the subversion of EGFR signaling [43]. The authors could highlight *in vitro* that inhibitors of the host factors ADAM10, EGFR, MAPK and FAK reduced the radial velocity and directional migration of infected cells and that depletion of the viral-encoded protein VGF also decreases virus-induced cell migration. Mouse IVM revealed that VGF was responsible for virus spread and lesion formation in mice ear pinnae. Because FAK is involved in focal adhesion formation, one may envision that VACV controls the dynamics of focal adhesion of infected cells to promote cell migration. Finally,

Drosophila melanogaster has been used to track and understand viral dissemination of the Nora virus, notably through the creation of transgenic reporter lines that emit fluorescence upon infection [44]. However, flies have not been used to study mammalian viruses as relevance would rightfully be questionable. In contrast, investigating the cell biology of viral dissemination in mosquitoes represent a highly relevant *in vivo* model for arboviruses, but we could not identify such studies.

Text Box 2. Alternatives to animal models.

With current ethical concerns regarding animal research, non-animal models mimicking complex 3D environments have been developed. Human explants probably represent the most relevant alternative to measure physiological viral infection *ex vivo* (see for instance [45-47]), but the administrative and technical difficulties to obtain human-derived samples, associated to the complicated methods required to image and genetically manipulate it, makes such approach cumbersome to implement in cell biology studies. In contrast, stem cell derived organoids represent attractive alternatives to study physiological viral dissemination *in-a-dish* [48, 49]. Nature, size, genetics, imaging and reproducibility are properties justifying why organoids are acclaimed. Recently for instance, intestinal organoids were used to study SARS-CoV-2 intracellular replication [50]. They observed viral particles in double membrane vesicles (DMV), Golgi apparatus and inside the endomembrane system, while virus secretion was occurring both from the apical and basolateral side (see Figure 1), which is consistent with the observation that virus particles shed in feces from infected patients [51]. Organoids can also be adequately used to study apoptosis or cell division, as exemplified with ZIKV-infected cerebral organoids [52, 53], but should prove useful in a variety of other subcellular studies. As opposed to animal models, organoids offer a unique human system, while working in tissue-like conditions. Although it is not fully appropriate to study adaptive immune responses or inter-organ physiopathology, they should be considered as relevant animal-free models to address the cell biology of viral dissemination in greater details.

Figure Legend: Strategies to track viral dissemination *in vivo*

Viruses employ several strategies to disseminate and reach adjacent tissues (Central scheme). They either freely diffuse through the vascular wall when its integrity is

altered; they may infect endothelial cells and are released in the extravascular space; they can be transported across the vascular wall without infection or they can be transported upon infection of circulating immune cells that can stop and cross the endothelium layer. Animal models such as the zebrafish embryo, the mouse model and the versatile organoids provide very useful imaging and analysis platforms for tracking, at high spatio-temporal resolution, the dynamics and cellular strategies of viral dissemination. For example, intravascular behavior of ZIKV-infected monocytes can be tracked in real time in zebrafish embryos (see Ayala-Nunez et al. 2019). Budding of HIV-1 from a T-cell can be tracked with electron tomography in mice (See Ladinsky et al.) profile emanating from the surface of a little cytoplasm between the nuclear envelope (NE) and the budding plasma membrane. Dissemination of SARS-CoV-2 can be tracked in enterocytes of intestinal organoids (see [50]).

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References

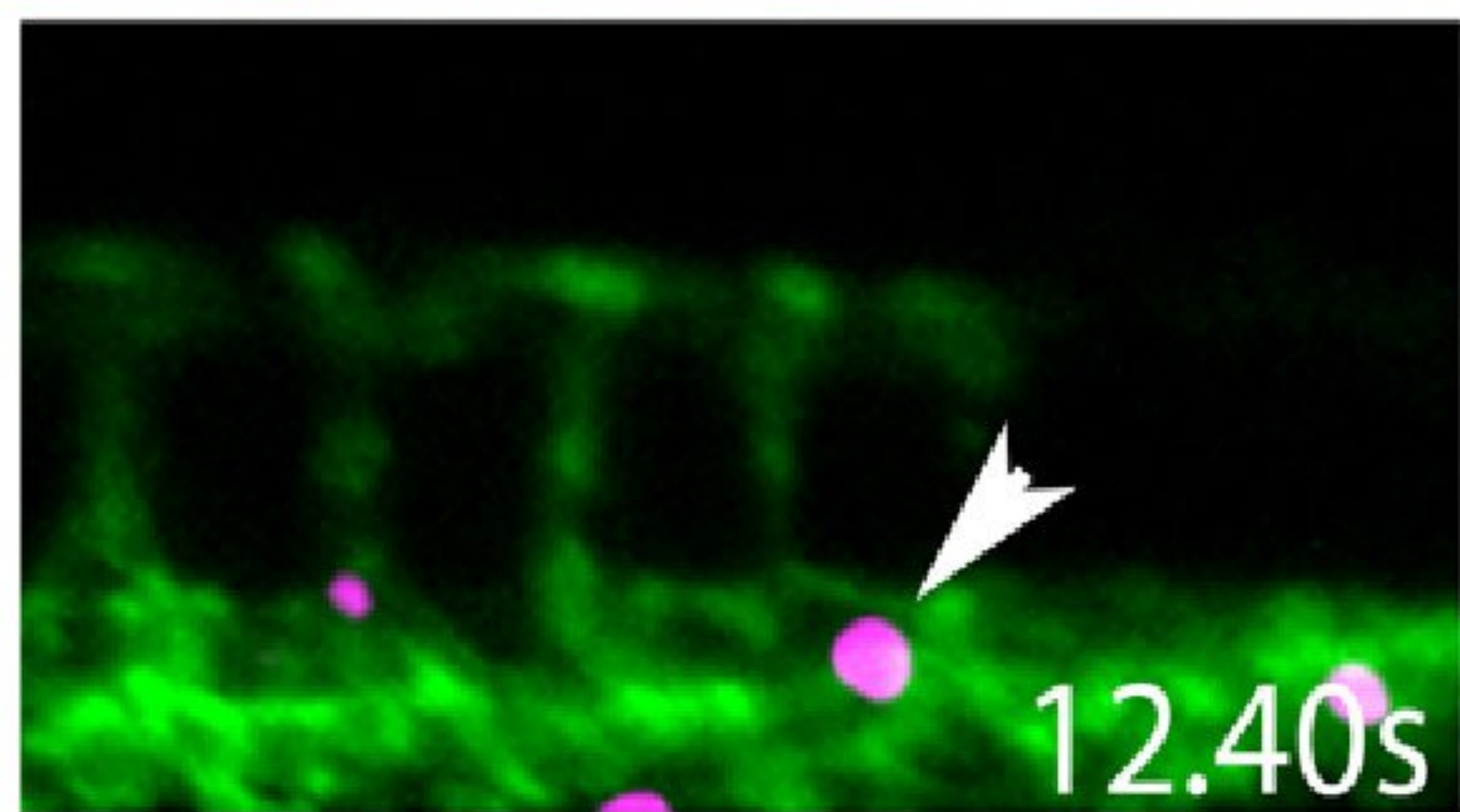
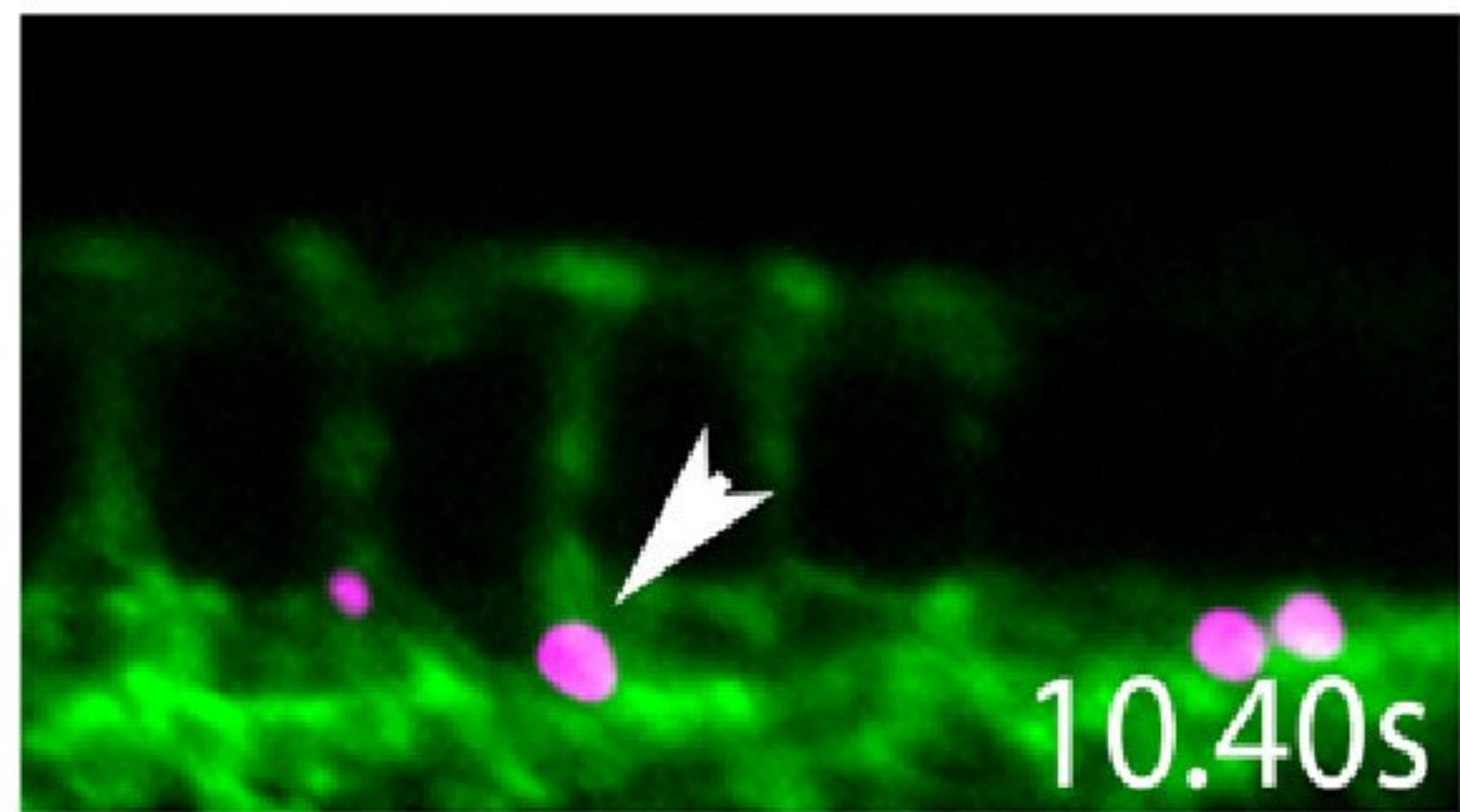
1. Torraca, V. and Mostowy, S. (2018) Zebrafish Infection: From Pathogenesis to Cell Biology. *Trends Cell Biol* 28 (2), 143-156.
2. Cifuentes-Munoz, N. et al. (2018) Direct cell-to-cell transmission of respiratory viruses: The fast lanes. *PLoS Pathog* 14 (6), e1007015.
3. Graw, F. and Perelson, A.S. (2016) Modeling Viral Spread. *Annu Rev Virol* 3 (1), 555-572.
4. Ayala-Nunez, N.V. and Gaudin, R. (2020) A viral journey to the brain: Current considerations and future developments. *PLoS Pathog* 16 (5), e1008434.
5. Levraud, J.P. et al. (2014) Through the looking glass: witnessing host-virus interplay in zebrafish. *Trends Microbiol* 22 (9), 490-7.
6. Ruzicka, L. et al. (2019) The Zebrafish Information Network: new support for non-coding genes, richer Gene Ontology annotations and the Alliance of Genome Resources. *Nucleic Acids Res* 47 (D1), D867-D873.
7. Fazio, M. et al. (2020) Zebrafish patient avatars in cancer biology and precision cancer therapy. *Nat Rev Cancer* 20 (5), 263-273.
8. Follain, G. et al. (2018) Hemodynamic Forces Tune the Arrest, Adhesion, and Extravasation of Circulating Tumor Cells. *Dev Cell* 45 (1), 33-52 e12.

9. Osmani, N. and Goetz, J.G. (2019) Multiscale Imaging of Metastasis in Zebrafish. *Trends Cancer* 5 (12), 766-778.
10. Passoni, G. et al. (2017) Imaging of viral neuroinvasion in the zebrafish reveals that Sindbis and chikungunya viruses favour different entry routes. *Dis Model Mech* 10 (7), 847-857.
11. Ayala-Nunez, N.V. et al. (2019) Zika virus enhances monocyte adhesion and transmigration favoring viral dissemination to neural cells. *Nat Commun* 10 (1), 4430.
12. Zou, P.F. and Nie, P. (2017) Zebrafish as a Model for the Study of Host-Virus Interactions. *Methods Mol Biol* 1656, 57-78.
13. Langevin, C. et al. (2019) IFN Signaling in Inflammation and Viral Infections: New Insights from Fish Models. *Viruses* 11 (3).
14. Van Dycke, J. et al. (2019) A robust human norovirus replication model in zebrafish larvae. *PLoS Pathog* 15 (9), e1008009.
15. Haddad, J.G. et al. (2020) The Geraniin-Rich Extract from Reunion Island Endemic Medicinal Plant *Phyllanthus phillyreifolius* Inhibits Zika and Dengue Virus Infection at Non-Toxic Effect Doses in Zebrafish. *Molecules* 25 (10).
16. Burgos, J.S. et al. (2008) Zebrafish as a new model for herpes simplex virus type 1 infection. *Zebrafish* 5 (4), 323-33.
17. Selakovic, Z. et al. (2019) Second generation of diazachrysenes: Protection of Ebola virus infected mice and mechanism of action. *Eur J Med Chem* 162, 32-50.
18. Palha, N. et al. (2013) Real-time whole-body visualization of Chikungunya Virus infection and host interferon response in zebrafish. *PLoS Pathog* 9 (9), e1003619.
19. Gabor, K.A. et al. (2014) Influenza A virus infection in zebrafish recapitulates mammalian infection and sensitivity to anti-influenza drug treatment. *Dis Model Mech* 7 (11), 1227-37.
20. Hickey, M.J. and Kubes, P. (2009) Intravascular immunity: the host-pathogen encounter in blood vessels. *Nat Rev Immunol* 9 (5), 364-75.
21. Ludwig, M. et al. (2011) Whole-body analysis of a viral infection: vascular endothelium is a primary target of infectious hematopoietic necrosis virus in zebrafish larvae. *PLoS Pathog* 7 (2), e1001269.
22. Papa, M.P. et al. (2017) Zika Virus Infects, Activates, and Crosses Brain Microvascular Endothelial Cells, without Barrier Disruption. *Front Microbiol* 8, 2557.
23. Simon, A.Y. et al. (2015) Dengue virus binding and replication by platelets. *Blood* 126 (3), 378-85.
24. Robinson, M. et al. (2018) Viral journeys on the intracellular highways. *Cell Mol Life Sci* 75 (20), 3693-3714.
25. Santiago-Tirado, F.H. and Doering, T.L. (2017) False friends: Phagocytes as Trojan horses in microbial brain infections. *PLoS Pathog* 13 (12), e1006680.
26. Peluso, R. et al. (1985) A Trojan Horse mechanism for the spread of visna virus in monocytes. *Virology* 147 (1), 231-6.
27. Follain, G. et al. (2020) Fluids and their mechanics in tumour transit: shaping metastasis. *Nat Rev Cancer* 20 (2), 107-124.
28. Smith, M.S. et al. (2004) Human cytomegalovirus induces monocyte differentiation and migration as a strategy for dissemination and persistence. *J Virol* 78 (9), 4444-53.
29. Cazorla-Vazquez, S. et al. (2019) Human cytomegaloviral multifunctional protein kinase pUL97 impairs zebrafish embryonic development and increases mortality. *Sci Rep* 9 (1), 7219.

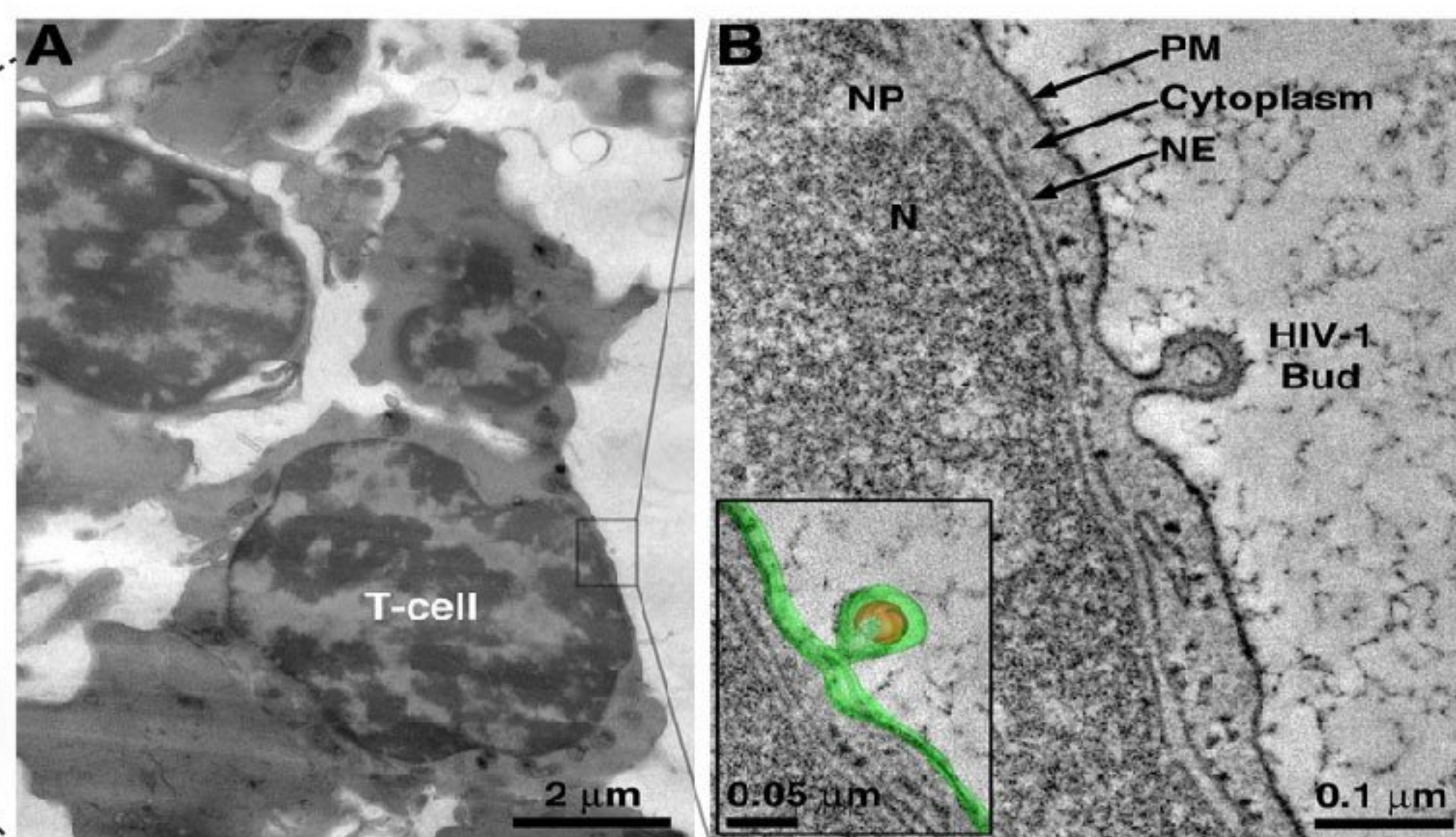
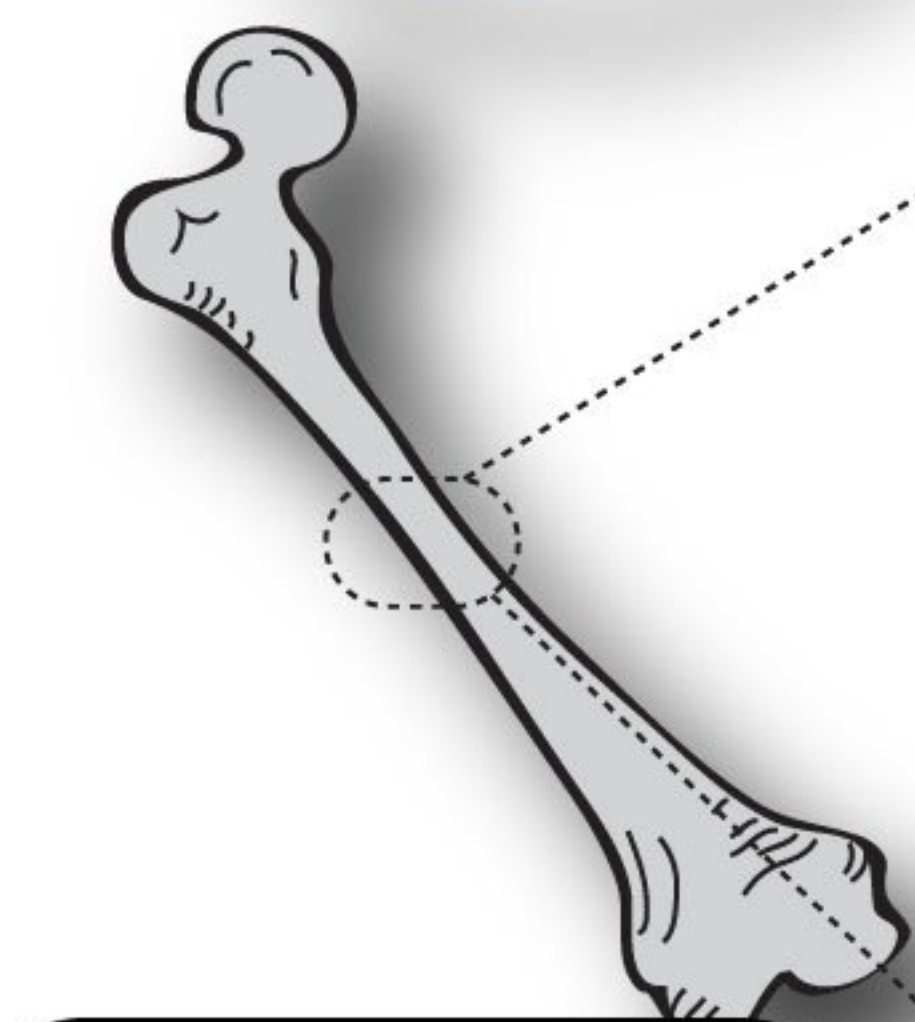
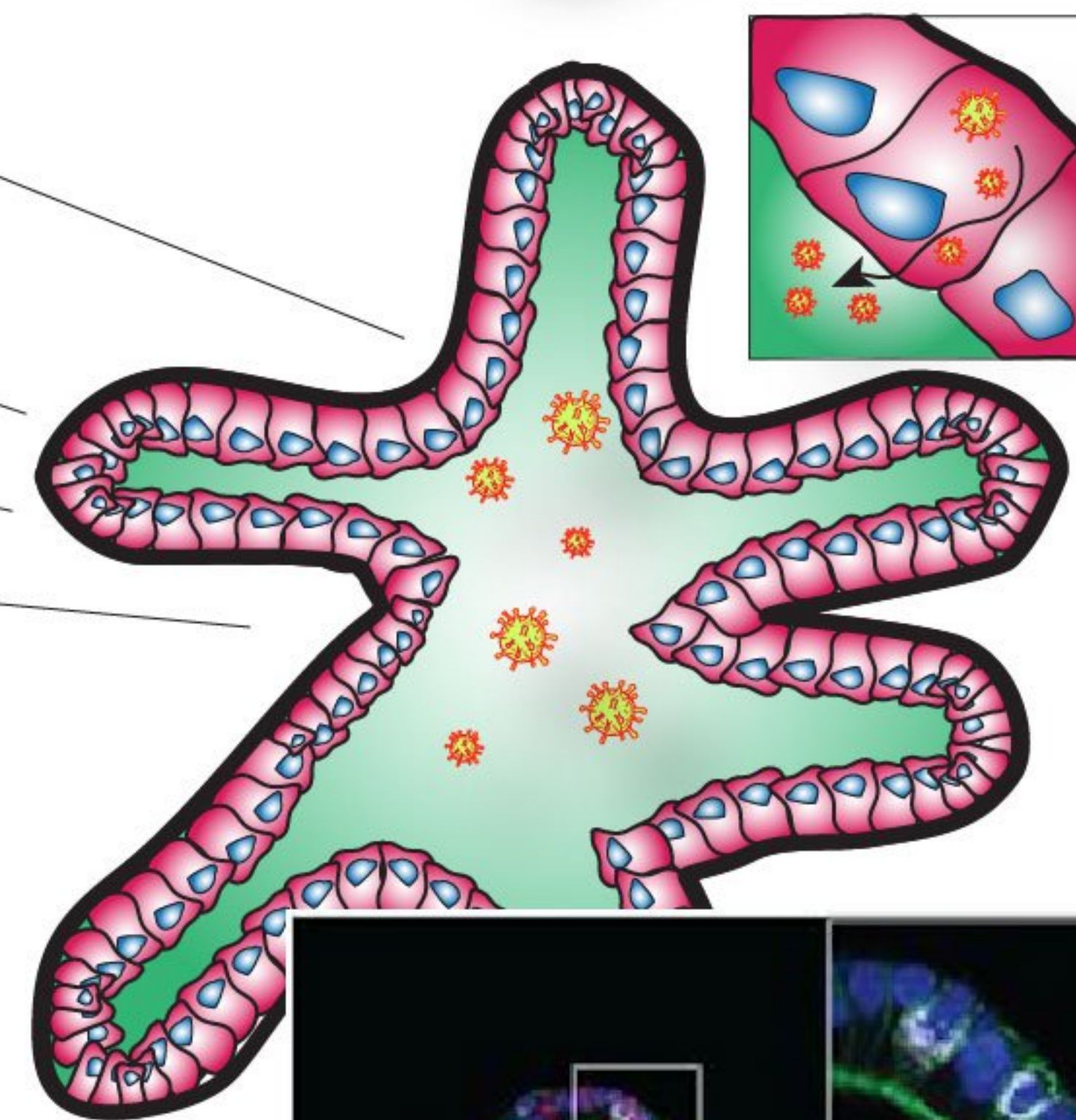
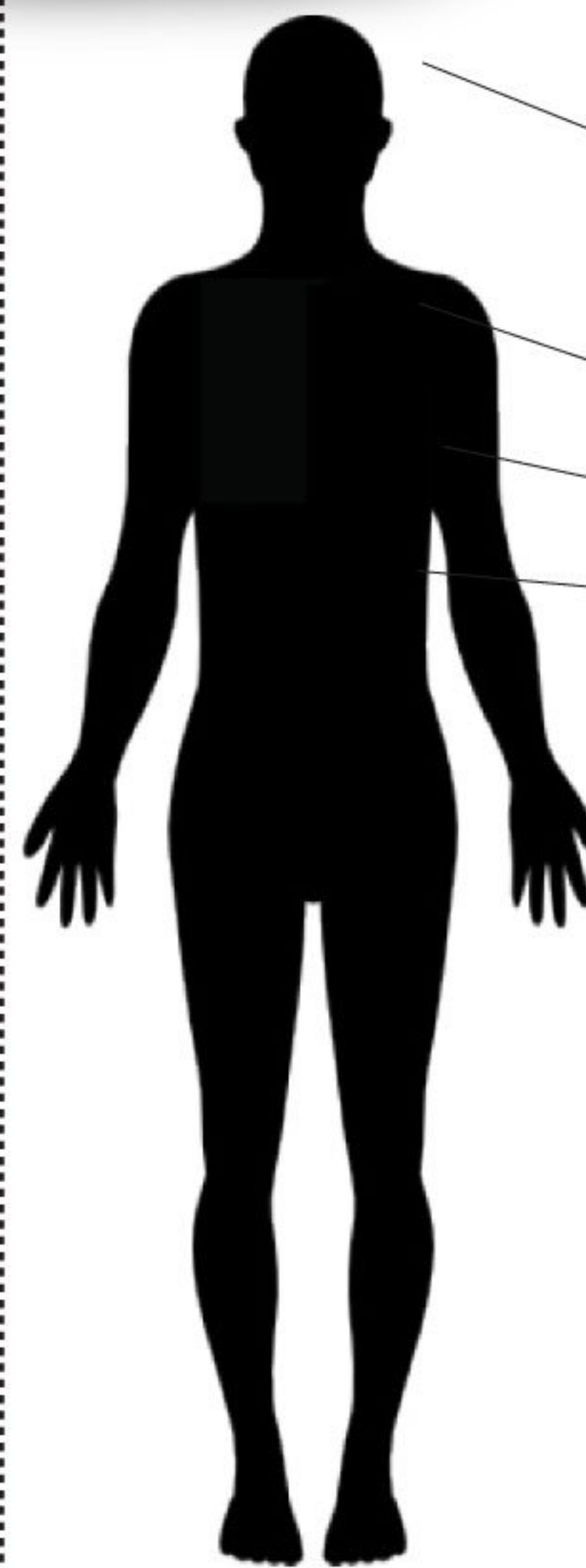
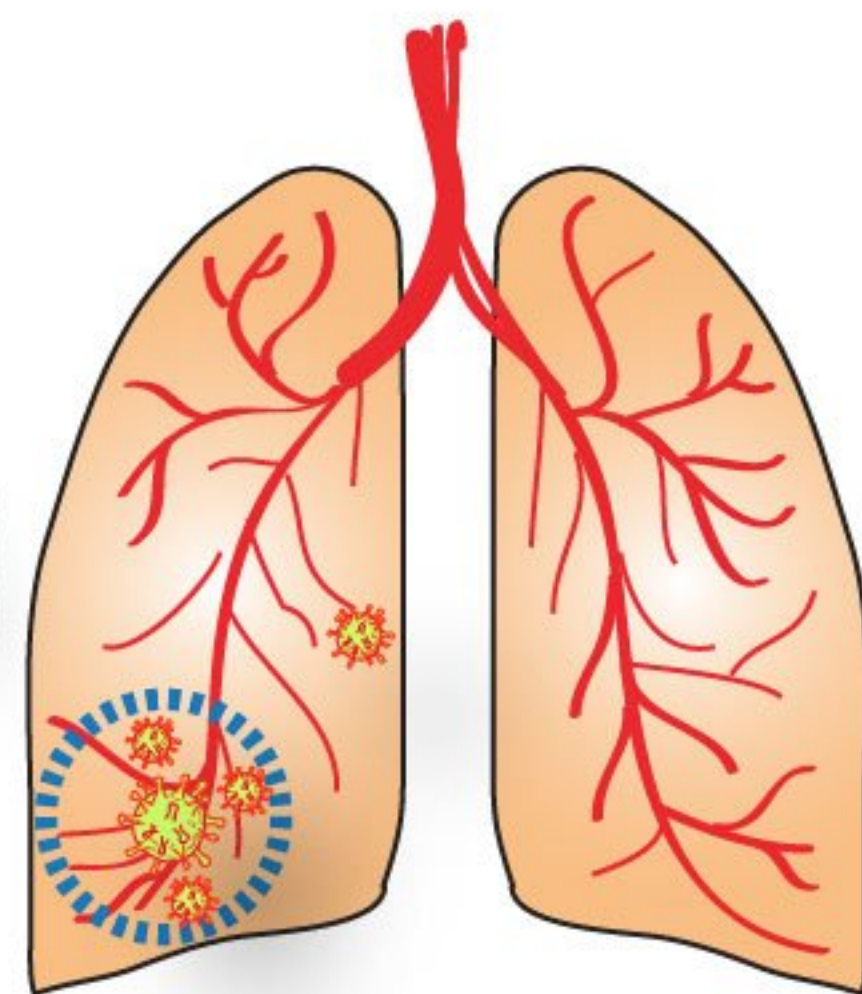
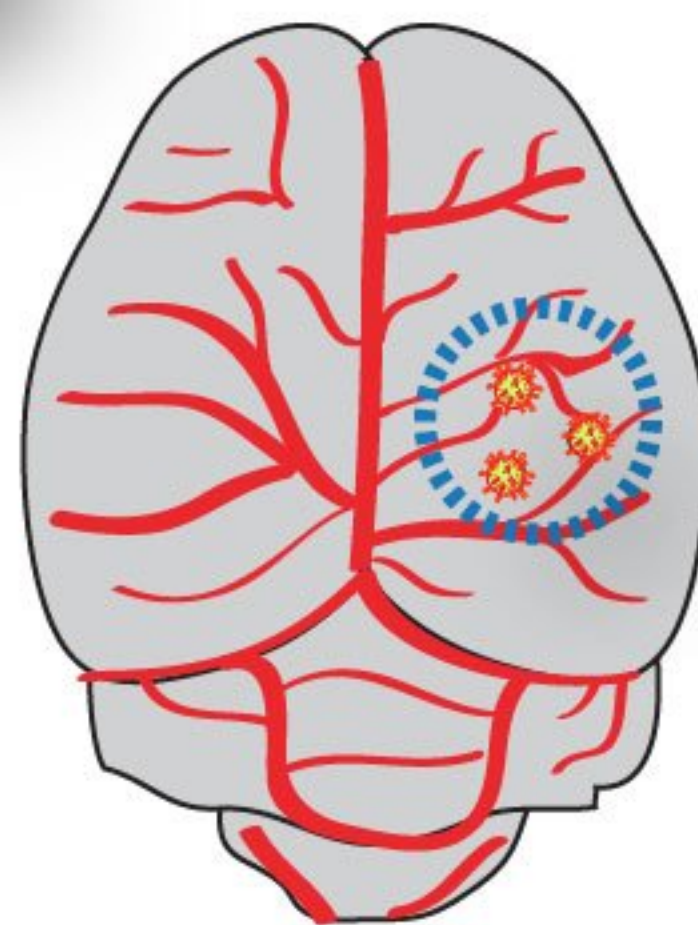
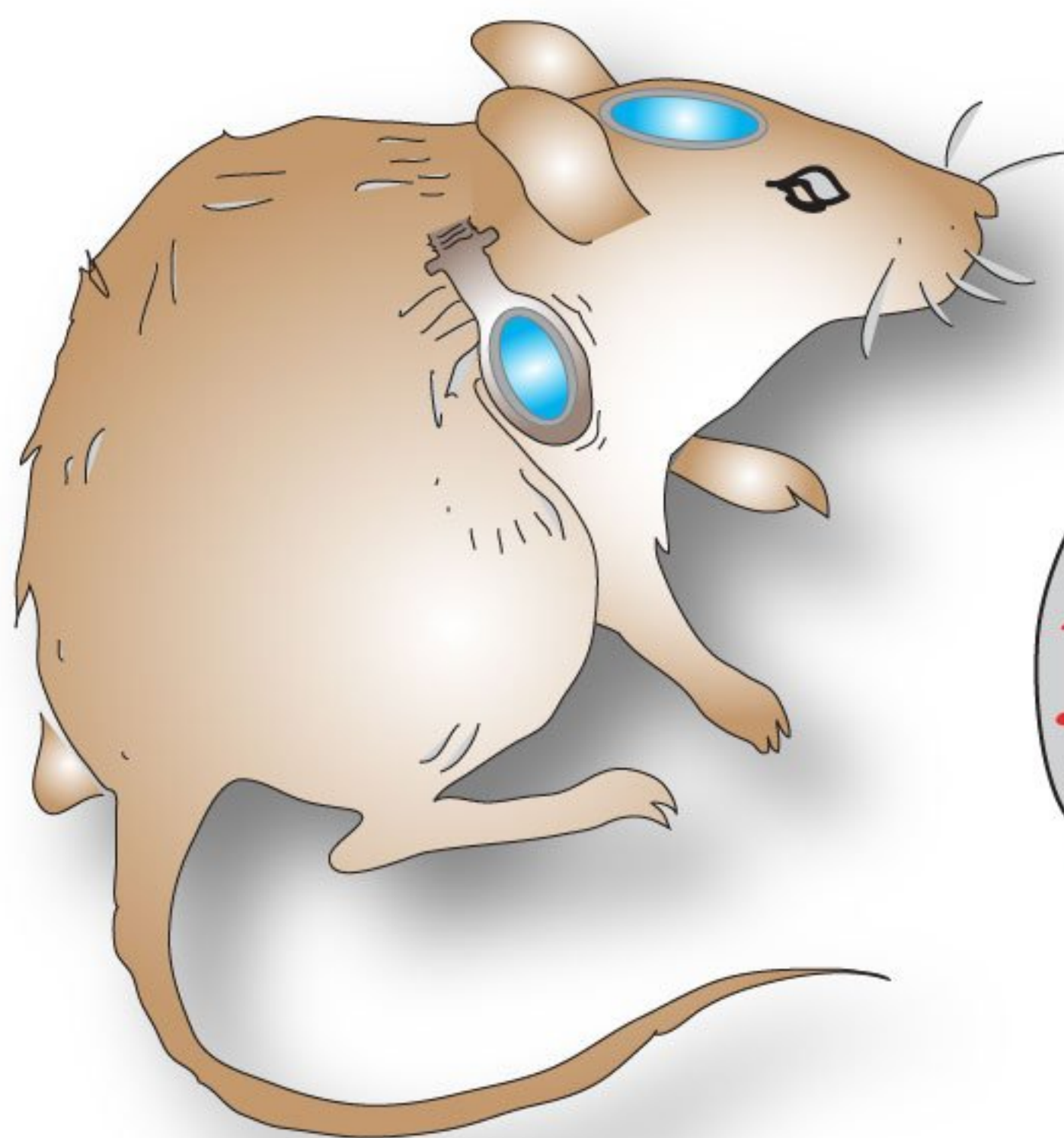
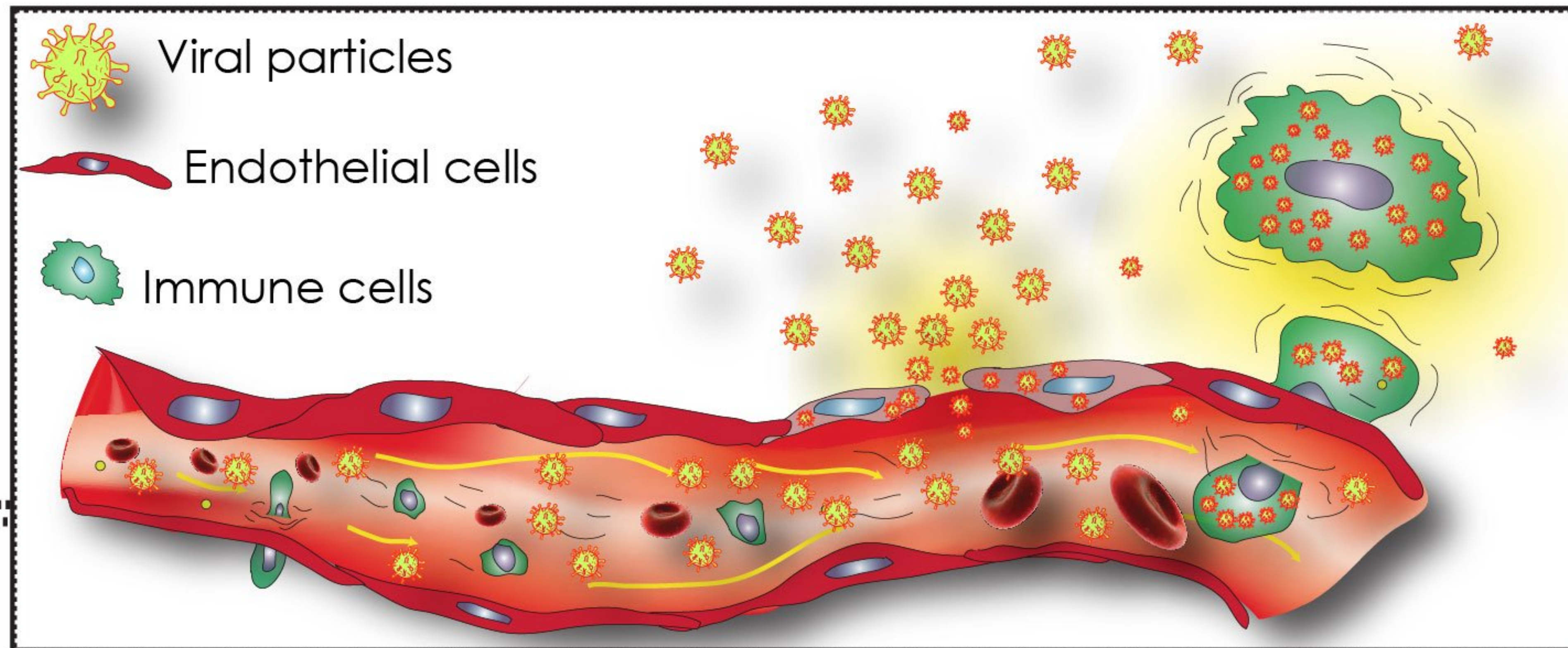
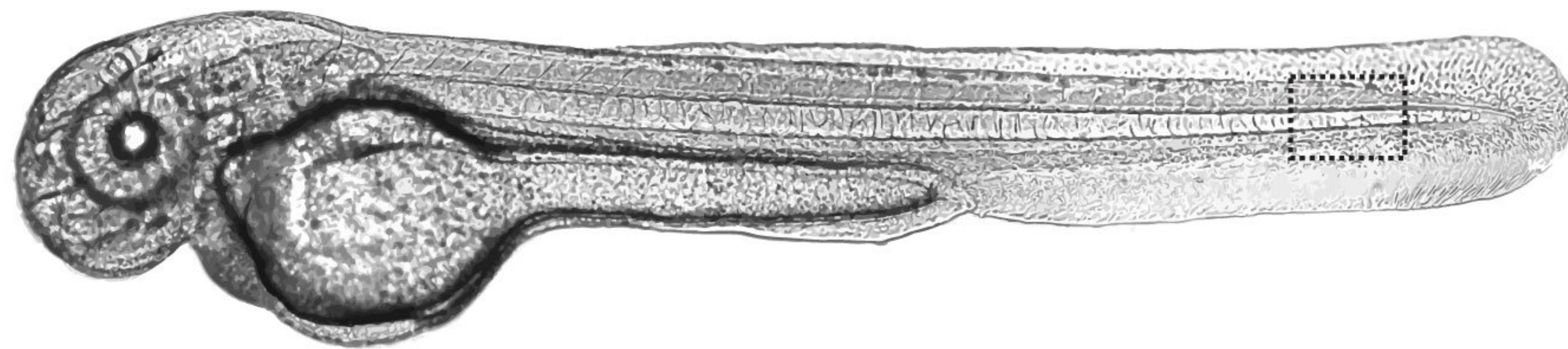
30. Tang, Q. et al. (2014) Optimized cell transplantation using adult rag2 mutant zebrafish. *Nat Methods* 11 (8), 821-4.
31. Yan, C. et al. (2019) Visualizing Engrafted Human Cancer and Therapy Responses in Immunodeficient Zebrafish. *Cell* 177 (7), 1903-1914 e14.
32. Goody, M.F. et al. (2014) Studying the immune response to human viral infections using zebrafish. *Dev Comp Immunol* 46 (1), 84-95.
33. Wolff, G. et al. (2020) Double-Membrane Vesicles as Platforms for Viral Replication. *Trends Microbiol.*
34. Hyenne, V. et al. (2019) Studying the Fate of Tumor Extracellular Vesicles at High Spatiotemporal Resolution Using the Zebrafish Embryo. *Dev Cell* 48 (4), 554-572 e7.
35. Karreman, M.A. et al. (2016) Intravital Correlative Microscopy: Imaging Life at the Nanoscale. *Trends Cell Biol* 26 (11), 848-863.
36. Balla, K.M. et al. (2020) Linking Virus Discovery to Immune Responses Visualized during Zebrafish Infections. *Curr Biol* 30 (11), 2092-2103 e5.
37. Sewald, X. (2018) Visualizing Viral Infection In Vivo by Multi-Photon Intravital Microscopy. *Viruses* 10 (6).
38. Ueki, H. et al. (2020) Multicolor two-photon imaging of in vivo cellular pathophysiology upon influenza virus infection using the two-photon IMPRESS. *Nat Protoc* 15 (3), 1041-1065.
39. Murooka, T.T. et al. (2012) HIV-infected T cells are migratory vehicles for viral dissemination. *Nature* 490 (7419), 283-7.
40. Sewald, X. et al. (2015) Retroviruses use CD169-mediated trans-infection of permissive lymphocytes to establish infection. *Science* 350 (6260), 563-567.
41. Naumenko, V. et al. (2018) Visualizing Oncolytic Virus-Host Interactions in Live Mice Using Intravital Microscopy. *Mol Ther Oncolytics* 10, 14-27.
42. Ladinsky, M.S. et al. (2019) Mechanisms of virus dissemination in bone marrow of HIV-1-infected humanized BLT mice. *Elife* 8.
43. Beerli, C. et al. (2018) Vaccinia virus hijacks EGFR signalling to enhance virus spread through rapid and directed infected cell motility. *Nat Microbiol.*
44. Ekstrom, J.O. and Hultmark, D. (2016) A Novel Strategy for Live Detection of Viral Infection in *Drosophila melanogaster*. *Sci Rep* 6, 26250.
45. Tabata, T. et al. (2018) Zika Virus Replicates in Proliferating Cells in Explants From First-Trimester Human Placentas, Potential Sites for Dissemination of Infection. *J Infect Dis* 217 (8), 1202-1213.
46. Ganor, Y. et al. (2019) HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy. *Nat Microbiol* 4 (4), 633-644.
47. Tajpara, P. et al. (2019) A Preclinical Model for Studying Herpes Simplex Virus Infection. *J Invest Dermatol* 139 (3), 673-682.
48. Lancaster, M.A. and Knoblich, J.A. (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345 (6194), 1247125.
49. Marton, R.M. and Pasca, S.P. (2020) Organoid and Assembloid Technologies for Investigating Cellular Crosstalk in Human Brain Development and Disease. *Trends Cell Biol* 30 (2), 133-143.
50. Lamers, M.M. et al. (2020) SARS-CoV-2 productively infects human gut enterocytes. *Science*.

51. Wu, Y. et al. (2020) Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 5 (5), 434-435.
52. Watanabe, M. et al. (2017) Self-Organized Cerebral Organoids with Human-Specific Features Predict Effective Drugs to Combat Zika Virus Infection. *Cell Rep* 21 (2), 517-532.
53. Tang, H. et al. (2016) Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. *Cell Stem Cell* 18 (5), 587-90.

Zebrafish

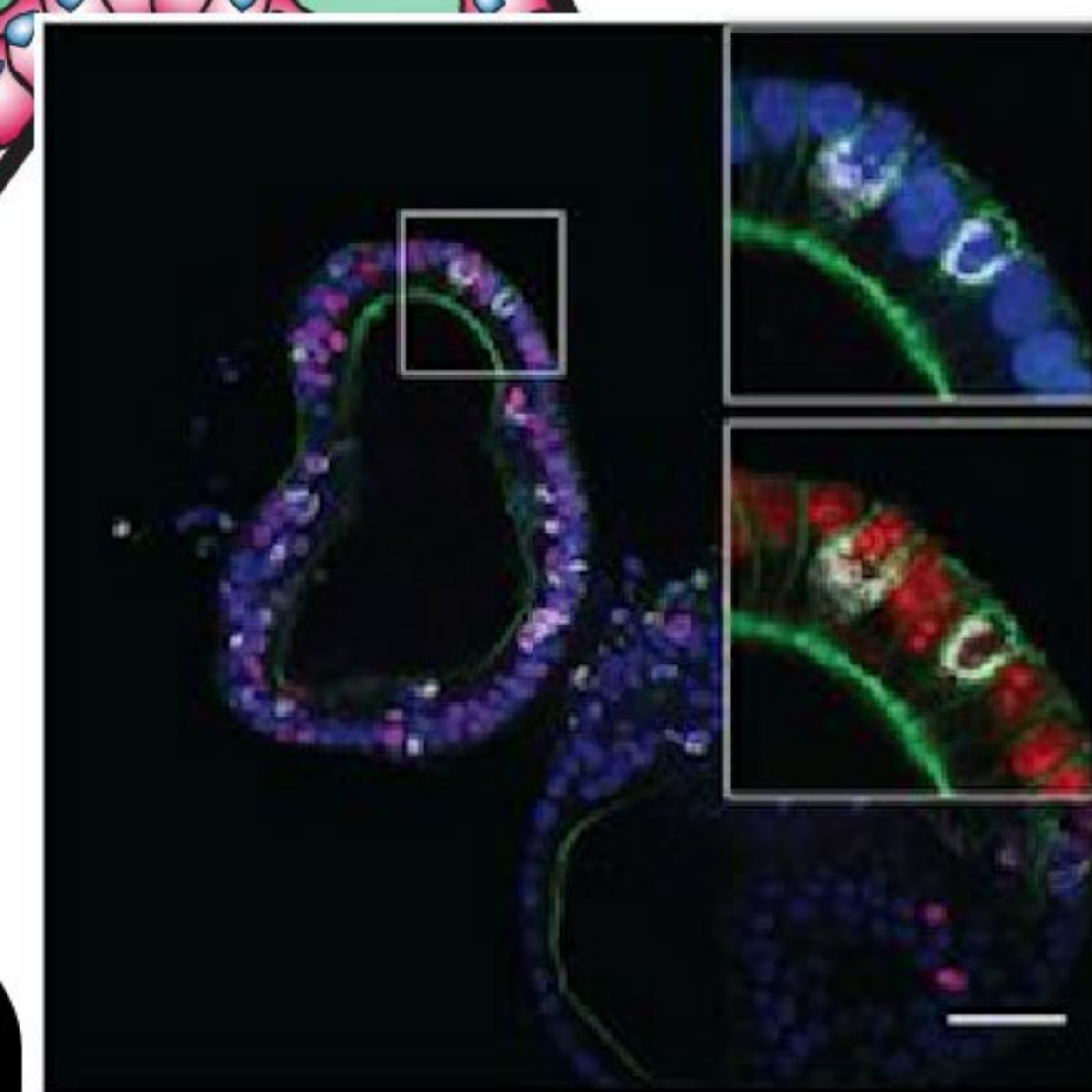


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(Ladinsky et al.)

Organoids



(Lamers et al.)

Mouse