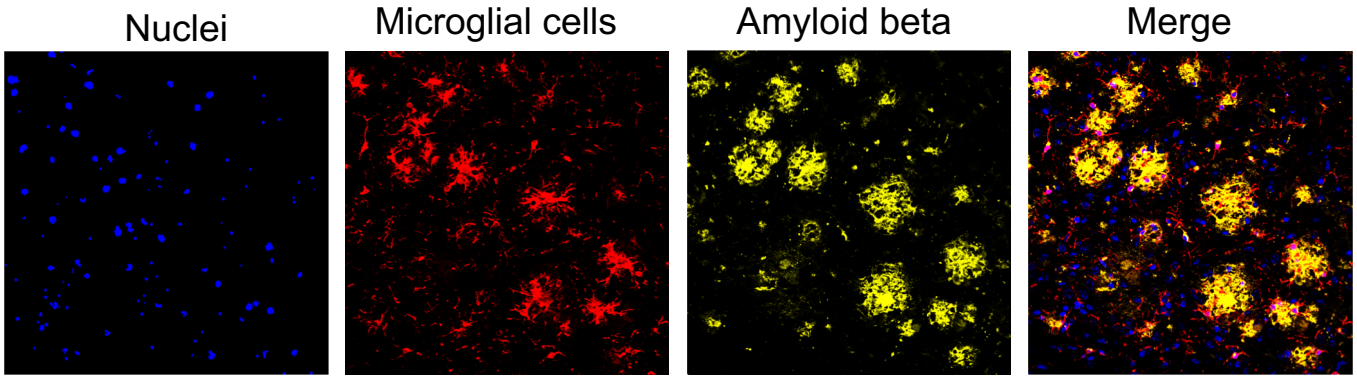
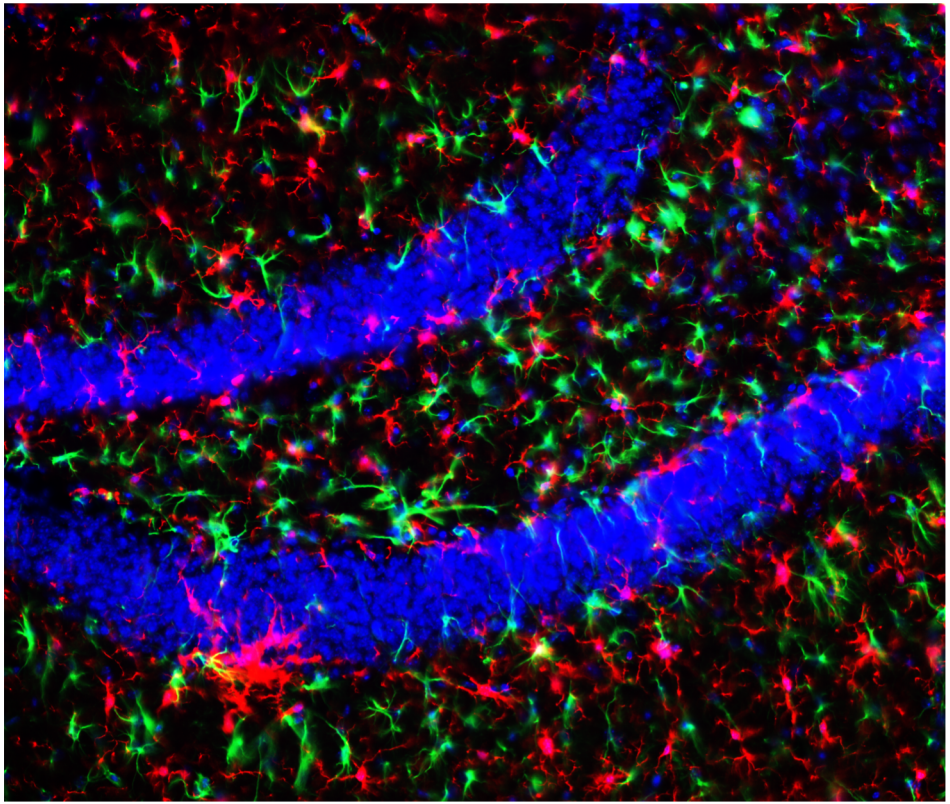
Analysis of cellular networks on tissue samples

Immunofluorescence staining



Analysis of cellular networks on tissue samples



Immunofluorescence staining

5xFAD - 2 months of age

Blu → DAPI
Rosso → Microglia
Verde → Astrocytes

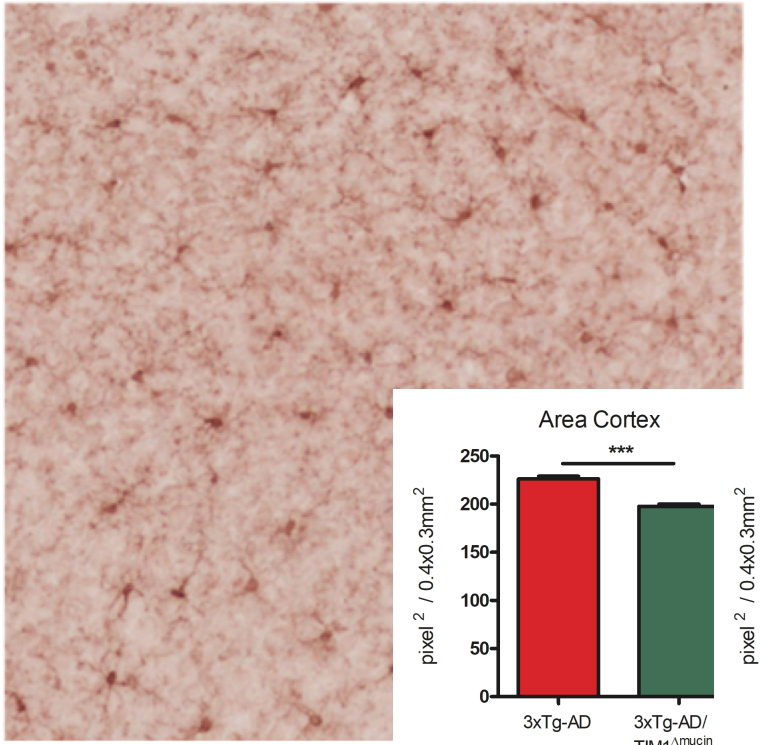
Hippocampus

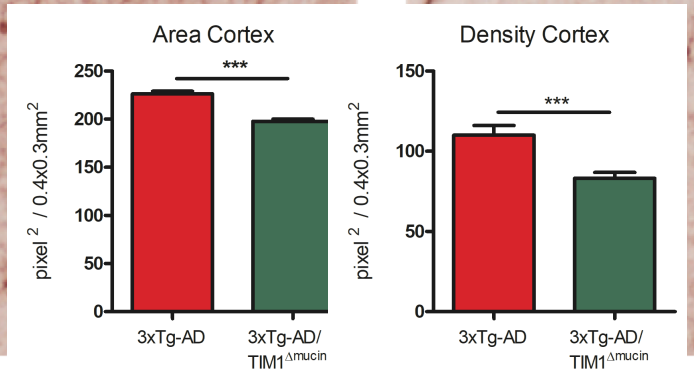
TIM-1 deficiency reduces microglia activation in 3xTg-AD/TIM-1$^{\Delta mucin}$ - IMMUNOHISTOCHEMISTRY STUDY

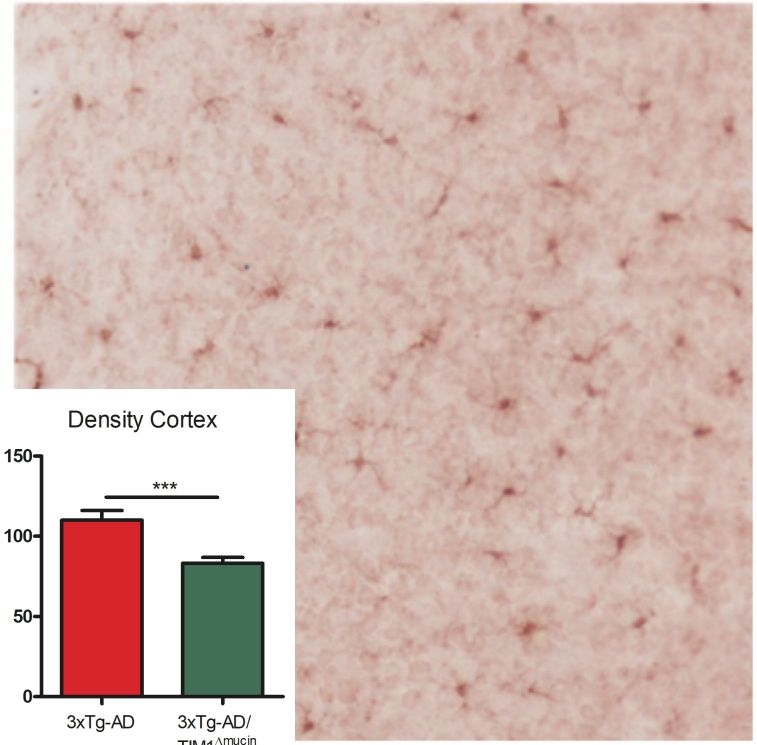
3xTg-AD

3xTg-AD/TIM1$^{\Delta mucin}$

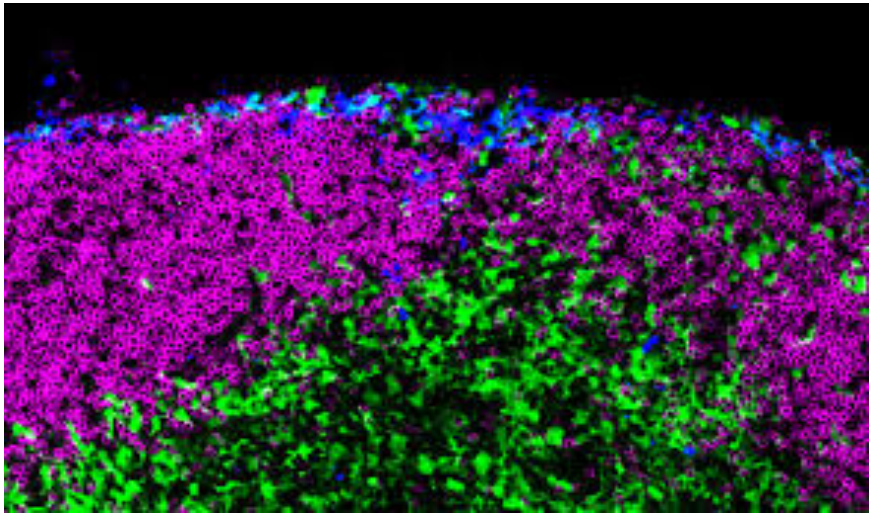
Cortex

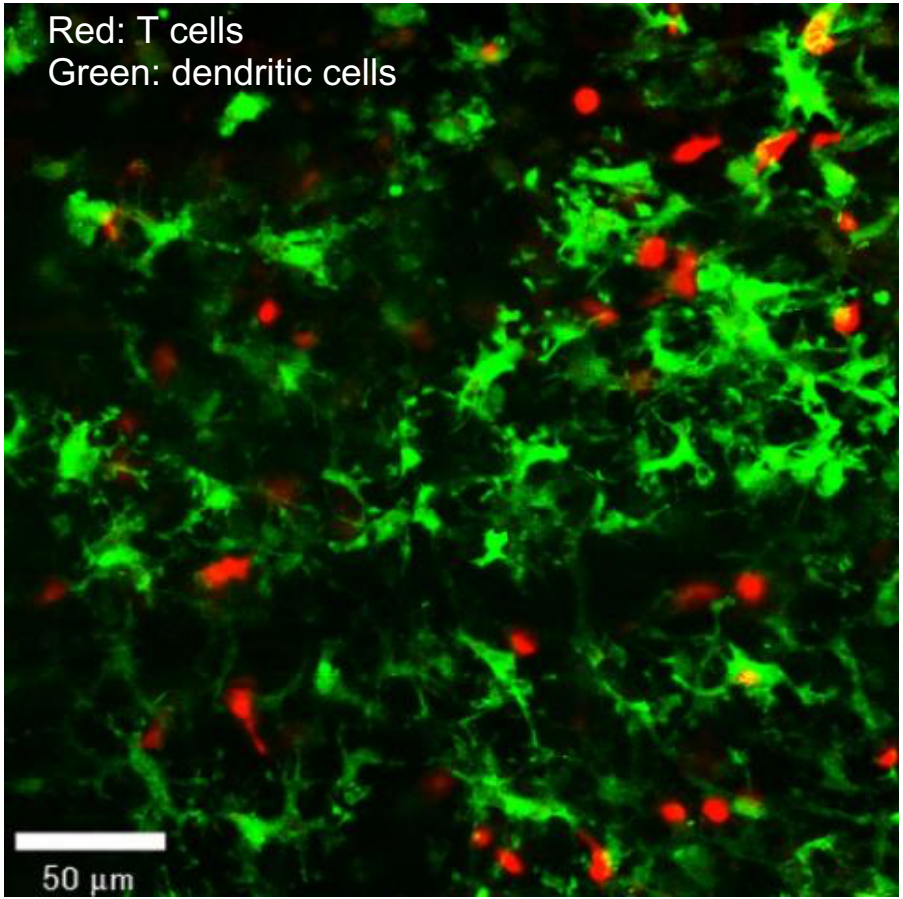






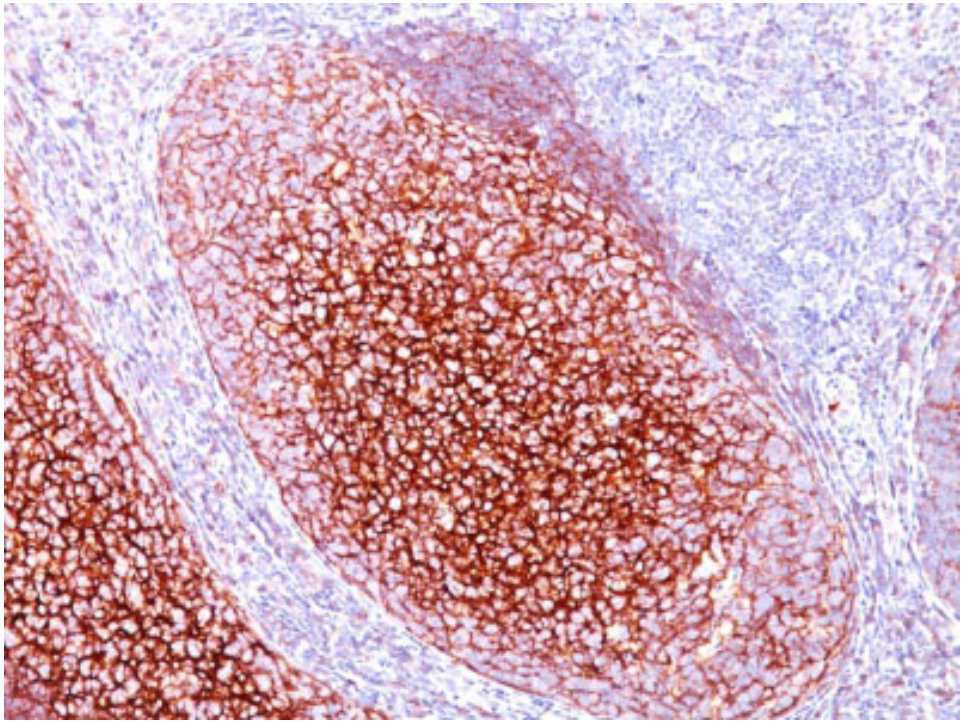
Lymphocytes and dendritic cells in the lymph nodes - immunofluorescence



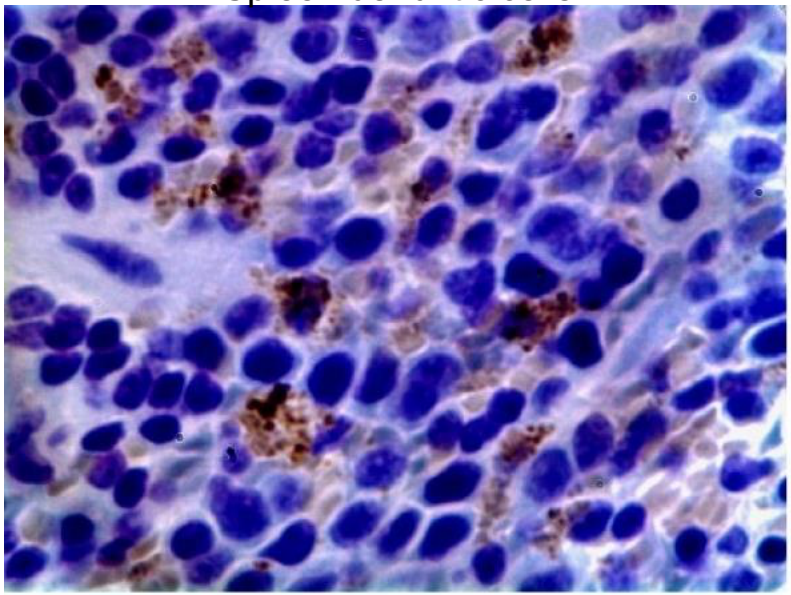


Immunohistochemistry for the study of tissue morphology

Follicular dendritic cells

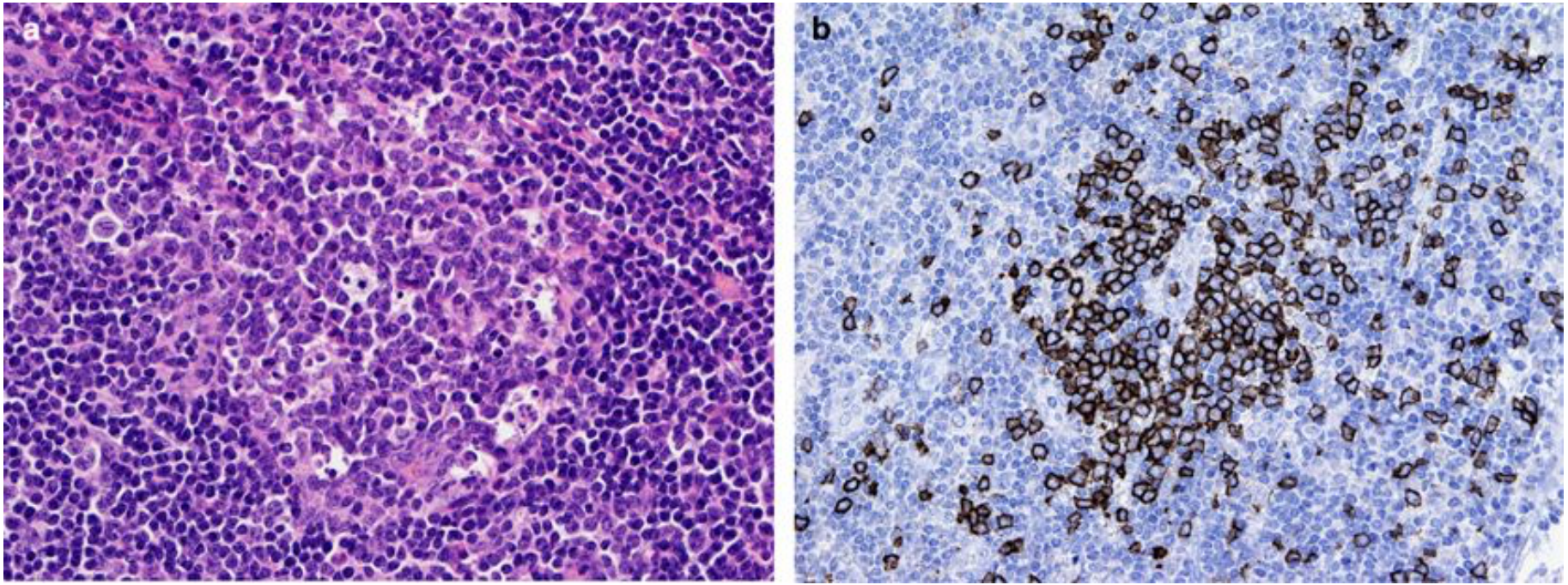


Spleen dendritic cells



Immunohistochemistry-Paraffin: Dendritic Cell Marker Antibody (AP-MAB0801) [NB110-85474] - Formalin fixed and paraffin embedded mouse spleen tissue section was subjected to IHC staining of mouse DCs using NB110-85474 (x 40).

H&E and Immunohistochemistry for the study of tissue morphology



(**a**) Plasmacytoid dendritic cells nodule in a reactive lymph node, closely associated with high endothelial venules and containing macrophages with tingible bodies (hematoxylin and eosin stain). (**b**) Anti-CD303, the most specific marker for plasmacytoid dendritic cells, stains a cluster and surrounding scattered plasmacytoid dendritic cells.

Fibroblastic reticular cell networks and their topological properties

PLOS | BIOLOGY

RESEARCH ARTICLE

Topological Small-World Organization of the Fibroblastic Reticular Cell Network Determines Lymph Node Functionality

Mario Novkovic[1☯], Lucas Onder[1☯], Jovana Cupovic[1☯], Jun Abe[2], David Bomze[1], Viviana Cremasco[3], Elke Scandella[1], Jens V. Stein[2], Gennady Bocharov[4], Shannon J. Turley[5], Burkhard Ludewig[1]*

1 Institute of Immunobiology, Kantonsspital St. Gallen, St. Gallen, Switzerland, **2** Theodor Kocher Institute, University of Bern, Bern, Switzerland, **3** Novartis Institutes for Biomedical Research, Cambridge, Massachusetts, United States of America, **4** Institute of Numerical Mathematics, Russian Academy of Sciences, Moscow, Russia, **5** Department of Cancer Immunology, Genentech, South San Francisco, California, United States of America

☯ These authors contributed equally to this work.
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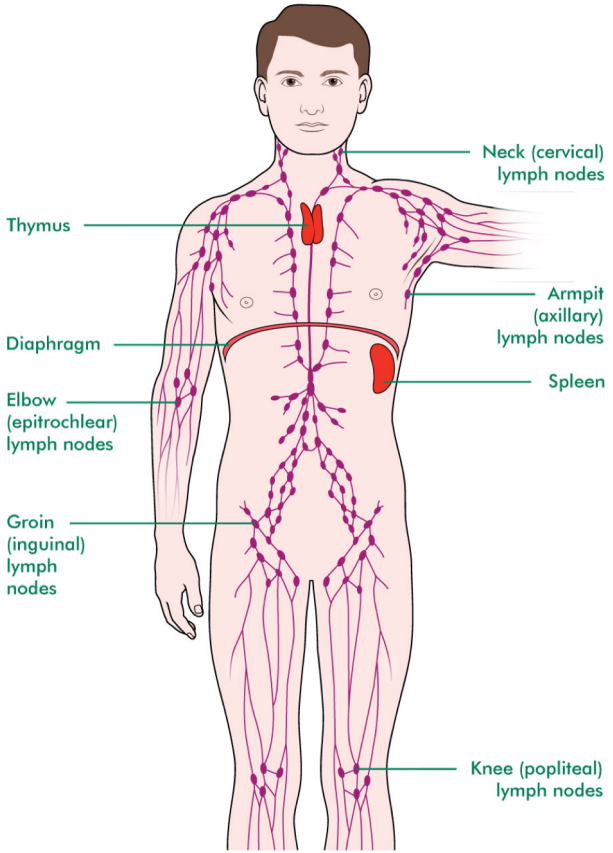
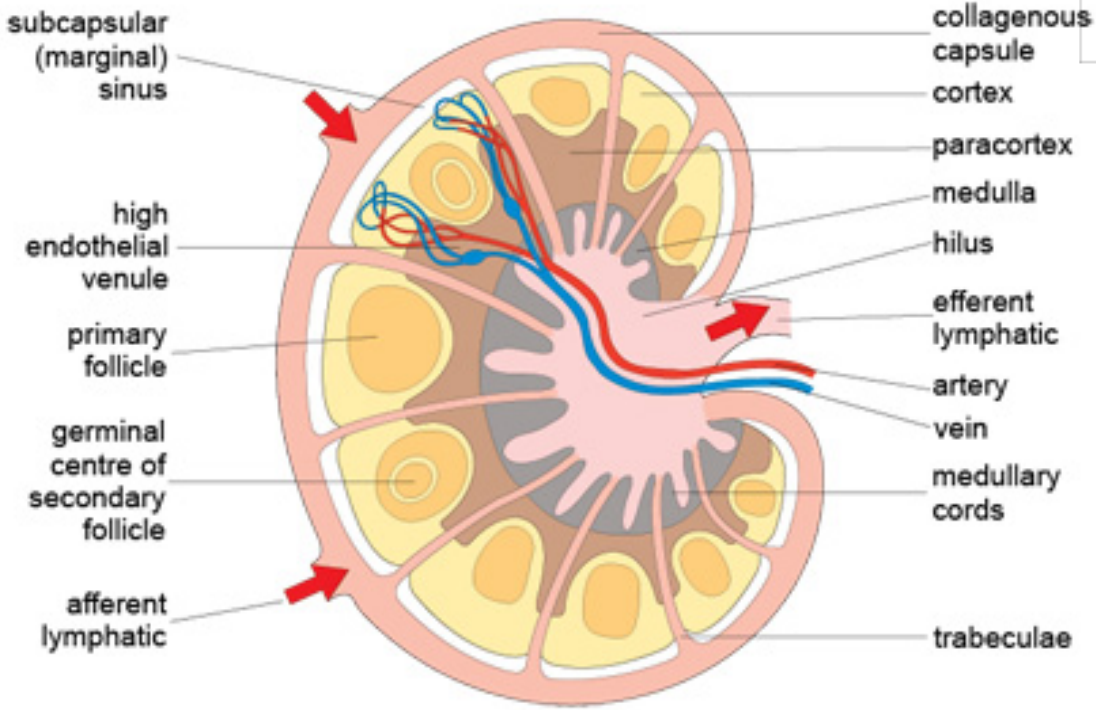
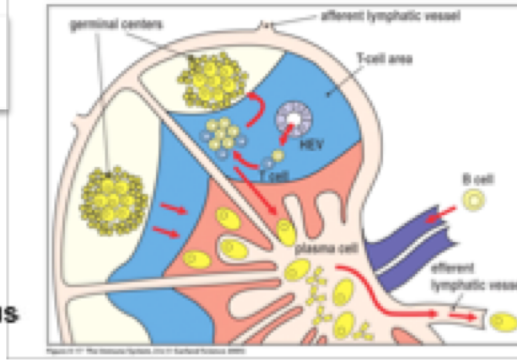
Lymph nodes

- Placed at convergence points of larger lymphoid vessels
- Detection of invading pathogens is favored
- Place for the establishment of efficient interactions between the immune system and microbial antigens
- Are fundamental for the initiation of immune responses



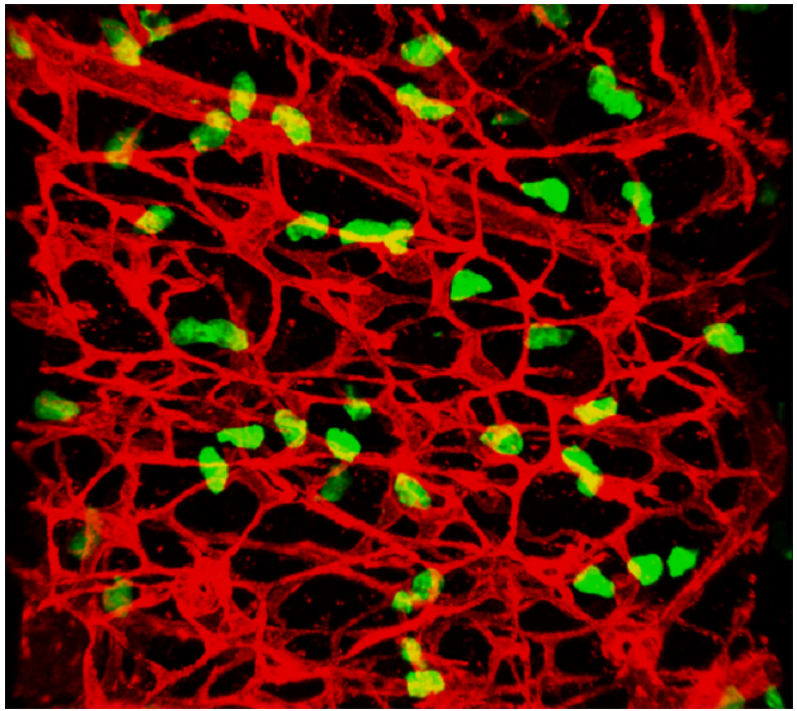
The structure of lymph nodes





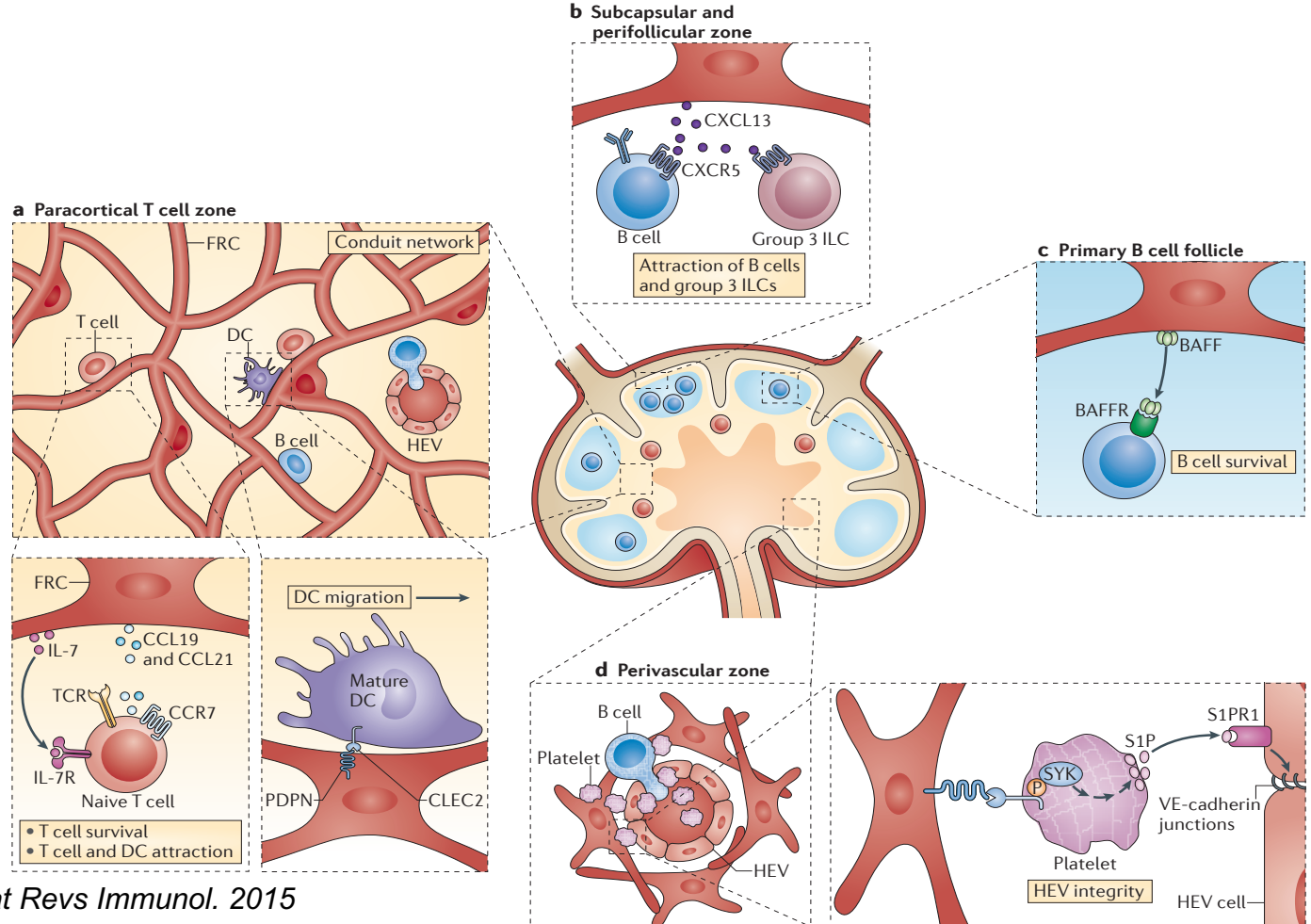
Fibroblastic Reticular Cells (FRCs)

- **Present in:**
  - **Lymph nodes**
  - **Spleen**
  - **Thymus**
  - **Other lymphoid tissues**
- **Immunological specialized myofibroblasts**
- **Form stellate cell-cell contacts creating a three dimensional open network in which leukocytes migrate**



Gp38+ stromal cells (red) form a dense sponge-like network of fibroblastic cells throughout the T zone of lymph nodes and thereby physically guide migrating T cells (green) (3D reconstruction of a labeled vibratome section).

FRCs organize the lymph node microarchitecture

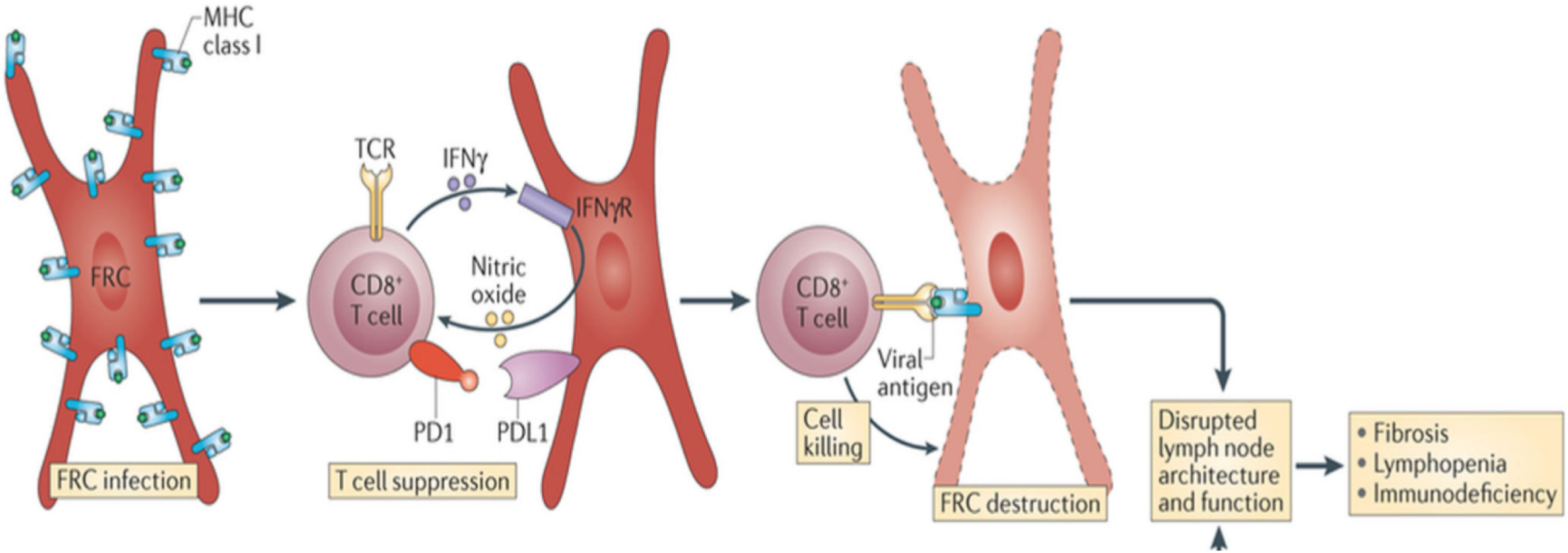


Fletcher et al., Nat Revs Immunol. 2015

Subsets of FRCs in the lymph nodes

Name	Defining features	Defining functions	Refs
T cell zone reticular cells	• PDPN^{+}desmin^{+} MADCAM1^{-} • CCL19, CCL21 and IL-7 secretion	• Maintaining the T cell zone • Constructing the conduit network	1–5,9
Marginal reticular cells	• Subcapsular location • PDPN^{+}desmin^{+}MADCAM1^{+}IL-7^{hi} CXCL13^{+}RANKL^{hi} • Not found in tertiary lymphoid organs	• Rich source of IL-7 • Differentiation into FDCs	8,13,31
B cell zone reticular cells	• Resident cells: PDPN^{+}CCL19^{+}BAFF^{+} and negative for FDC markers • Inducible cells: PDPN^{+} subset of CD21^{-} FRCs with a history of CD21 expression; convert into CXCL13^{+} cells during the B cell response	• Maintaining B cell survival and follicle boundaries	30,36,78
FDCs	CD21^{+}CD35^{+}MFGE8^{+}CXCL13^{+}ICAM1^{+} VCAM1^{+}BAFF^{+}	• Maintaining germinal centres • Facilitating the production of high-affinity antibodies	6,13,32
Pericytic FRCs	• PDPN^{+} • Located around HEVs • PDPN signals to CLEC2 on platelets	Preventing bleeding from HEVs into lymph nodes	40

Fibroblastic Reticular Cells (FRCs): Network damage during viral infection



Main aims of the application

Study the properties of biological and simulated FRC networks

Find a connection between FRC networks and lymph node functionality

Steps of the application

1. Reconstruction of FRC biological networks
2. Study of the FRC Network Structural Integrity
3. In Silico Prediction of the FRC Network Topological Robustness
4. Evaluation of LN functionality after FRC ablation
5. Conclusions

Step 1: Reconstruction of FRC biological networks

- **Biological FRC networks were reconstructed using *Imaris* and data from 6 mice, and their properties analysed with *R*.**
- **Topological analysis of the network was performed using the *igraph* package in R and RStudio.**
- **In-silico network simulation, using an in-house *R* algorithm and the *IGraph* library.**

Information regarding the previous slide

R is a statistical programming language used for analysis of data and mathematical model reconstruction

IGraph is a library for R (available for python, too). A library is a sort of plugin that is used to carry out very specific tasks

Python is a multipurpose programming language that can be used to develop softwares for companies, videogames, and data analysis

Step 1: Reconstruction of FRC biological networks

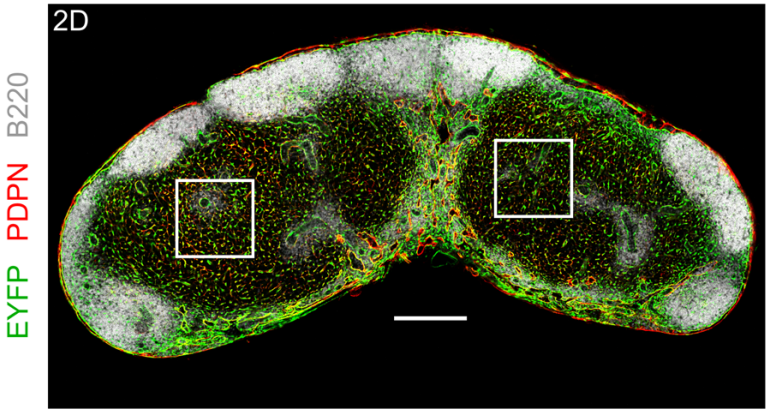
Three-dimensional reconstructions of the T cell zone FRC network were performed using *Imaris* (Bitplane).

The model of the FRC network was created as an undirected, unweighted graph with no isolate components by defining nodes as the FRC centers of mass and edges as physical connections between neighboring FRCs.

Step 1: Reconstruction of FRC biological networks

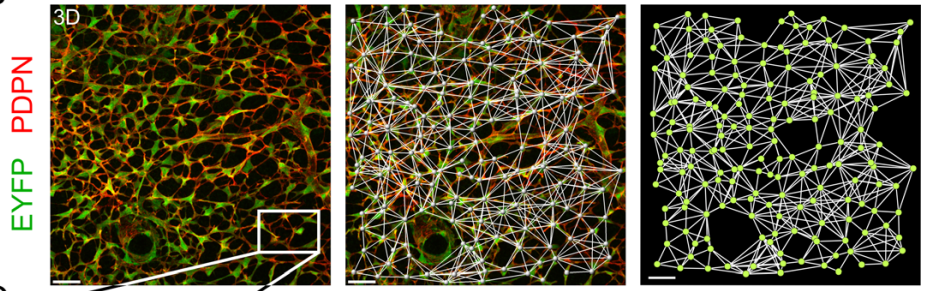
classical podoplanin (PDPN)-expressing T cell zone FRCs

A

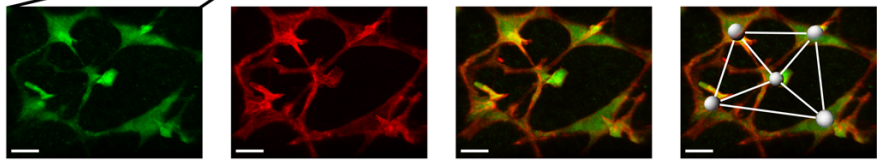


Confocal microscopy
Z stacks covering a volume of 304 x 304 x 32 µm

B

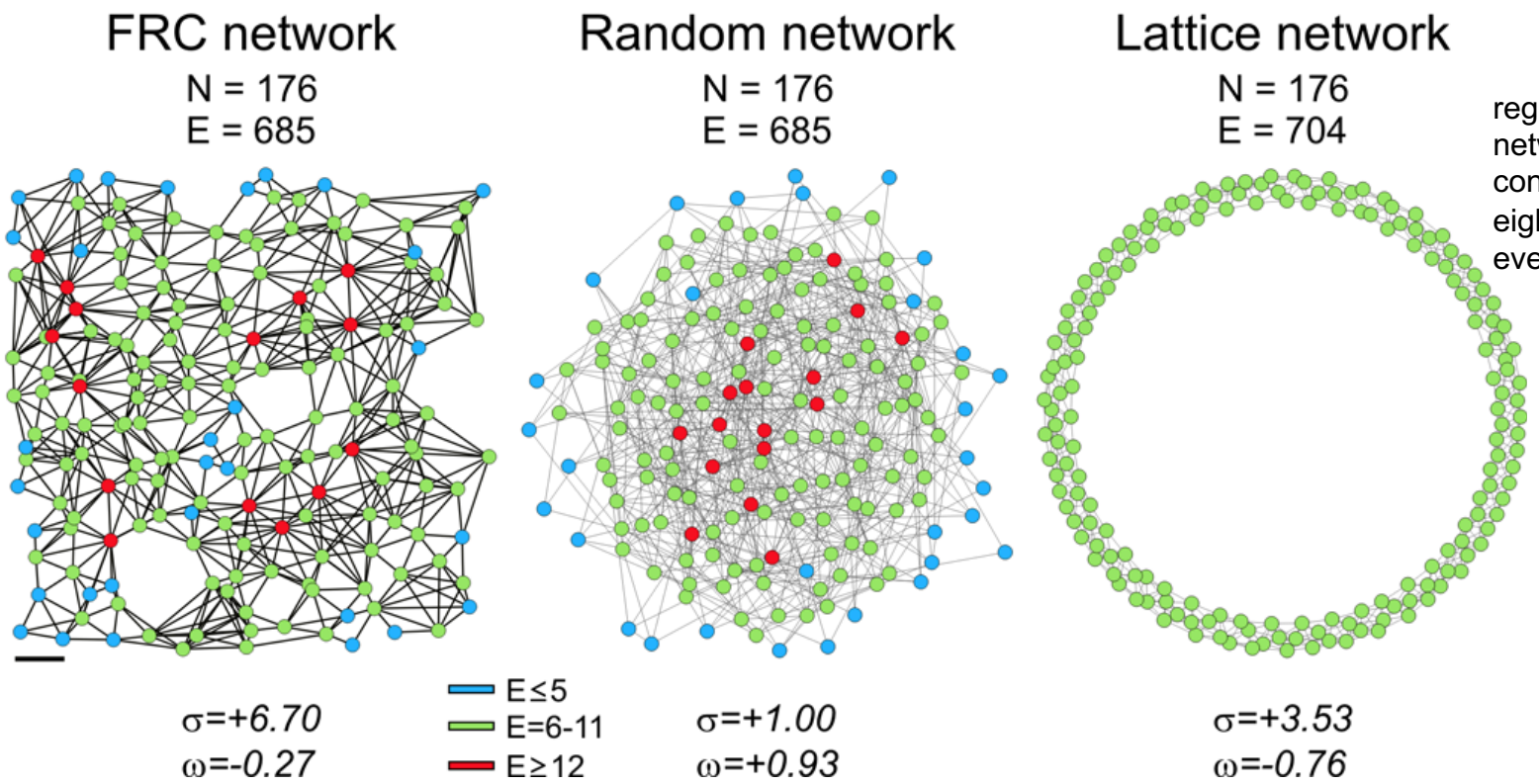


C



Step 1: Reconstruction of FRC biological networks

Topology of the T cell zone FRC network



regular ring lattice network was constructed with eight edges for every node

N, the number of nodes; E, the number of edges

Small world networks

- most nodes can be reached from every other node by a small number of steps, even though most nodes are not direct neighbors.
- fast and efficient information transfer.
- characterized by small shortest path lengths (node-to-node distances).
- high capacity for clustering (i.e., connectivity between neighboring nodes), which is strikingly different from random networks in which all nodes have the same probability of containing an edge.
- can be classified as a small-world network by comparing network-level statistics to equivalent random and lattice networks.
- Robustness to perturbation

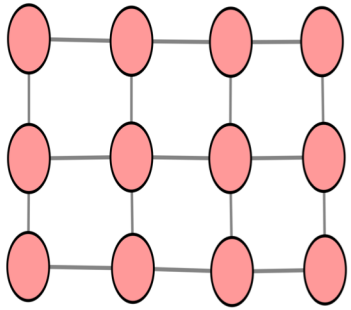
Small world networks

- **can be described by the σ and ω parameters, which classify a network as small world if σ > 1 and ω % 0 (range −0.5 to +0.5)**

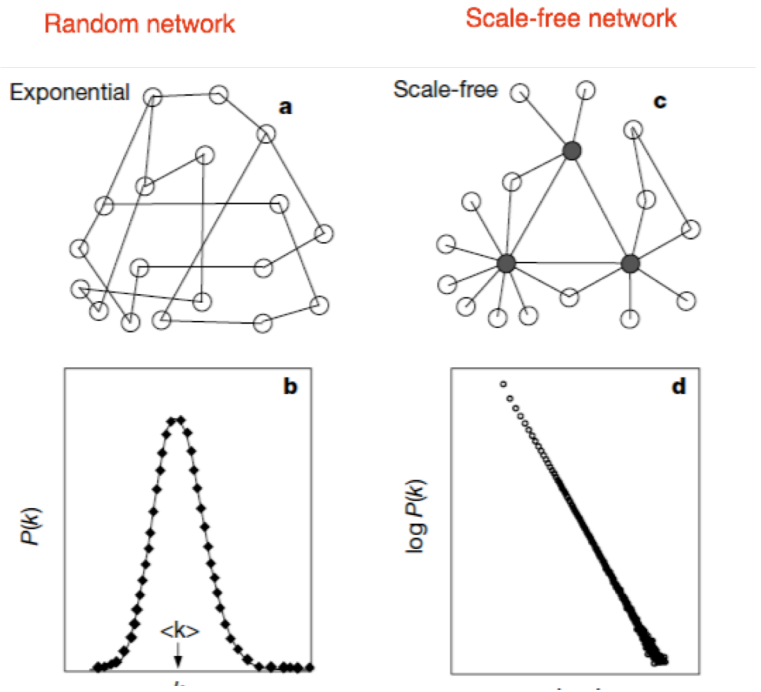
- Random networks will show σ approx. 1 and positive $0 < \omega < 1$, while lattice networks will have $\sigma > 1$ like small-world networks but negative $-1 < \omega < 0$.

Lattice networks

Lattice-like network is a network in which nodes are connected to their immediate neighbours and forms a regular grid. Usually man-made. Lowest heterogeneity and lowest randomness.



Regular graphs tend to have long average paths and high clustering (as the nodes tend to be densely connected in groups).



$$P(k) \sim k^{-\gamma}$$

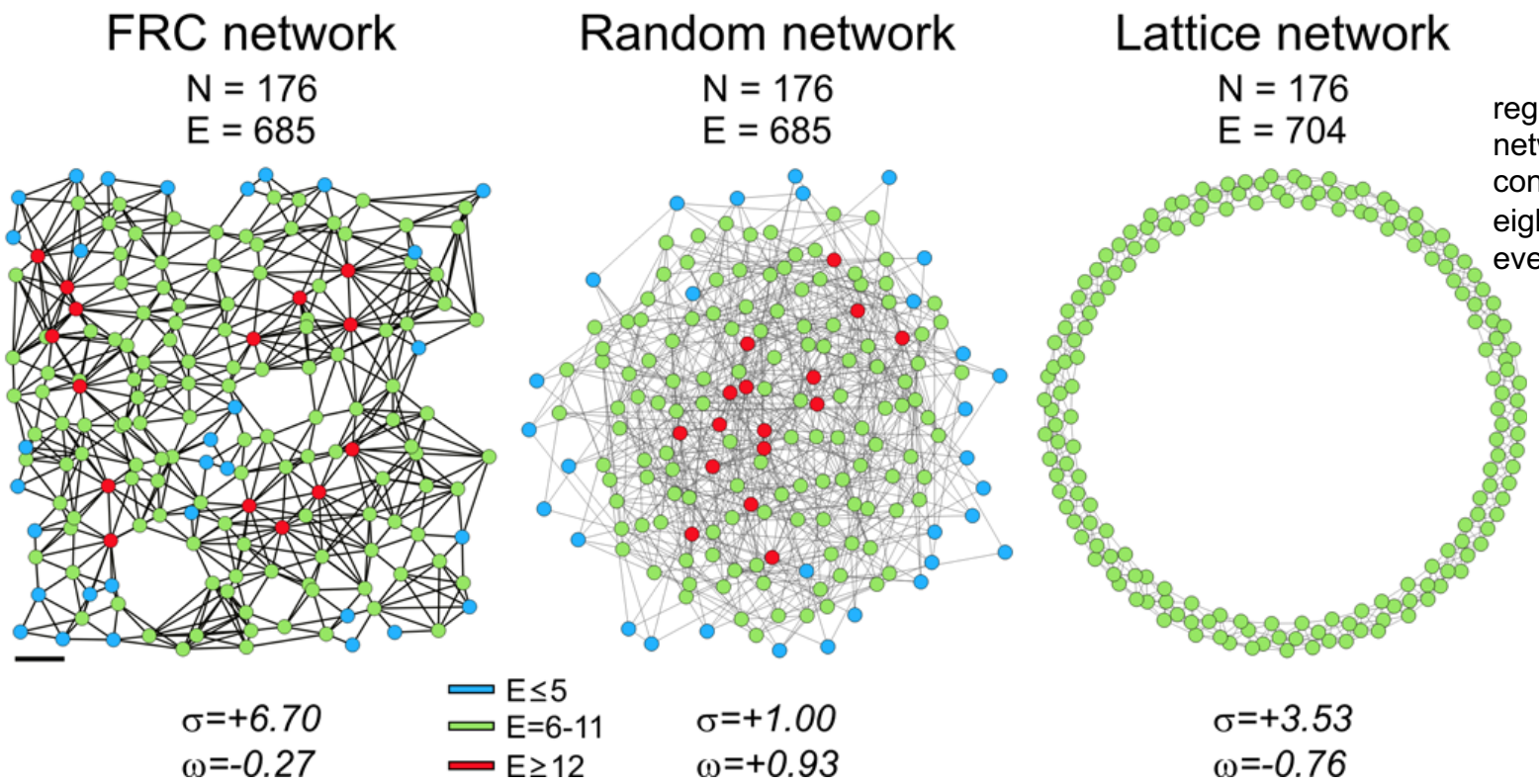
y= degree exponent

Random networks are described by the Erdos-Renyi model in which objects (nodes) form random connections (edges) between each other with the same probability. Most nodes will have approximately the same number of connections, centered on the network average with a Poisson degree distribution. Low heterogeneity

Scale-free networks are characterized by a power-law degree distribution with most nodes possessing few connections and very few nodes showing large numbers of connections. Highly connected nodes are called hubs, and they maintain the whole network structure

Step 1: Reconstruction of FRC biological networks

Topology of the T cell zone FRC network



regular ring lattice network was constructed with eight edges for every node

N, the number of nodes; E, the number of edges

Step 1: Reconstruction of FRC biological networks

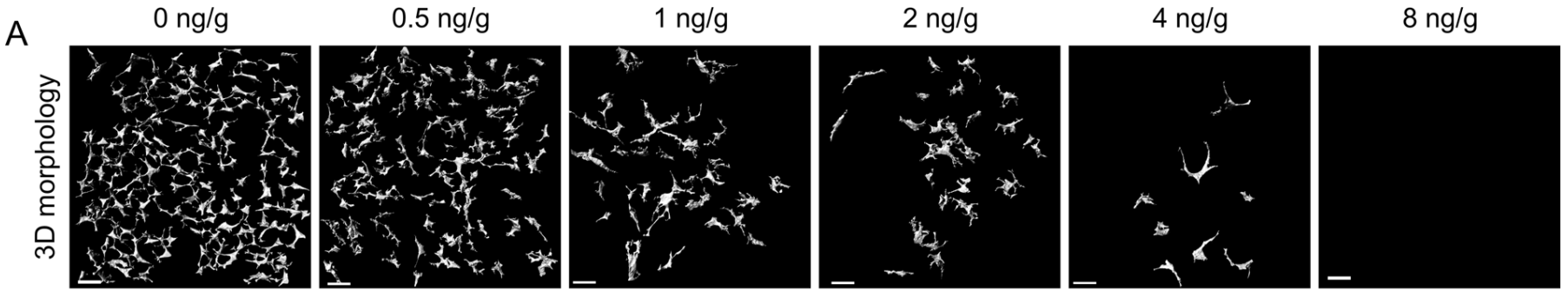
The initial network analysis with $\sigma = 6.128 \pm 0.659$ and $\omega = -0.308 \pm 0.069$ ($n = 6$ mice) indicates that FRCs of the T cell zone form a small-world network with lattice-like properties.

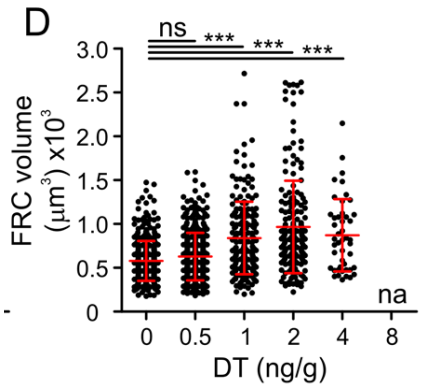
Step 2: Study of the FRC Network Structural Integrity

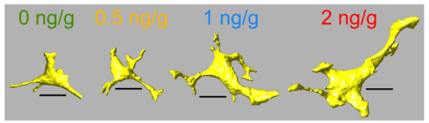
- **Usage of mice that express both the diphtheria toxin receptor (DTR) and EYFP under the control of the Ccl19 promoter (Ccl19eyfp/idtr).**
- **Partial removal of nodes/FRCs**
- **Graded doses of DT were applied**

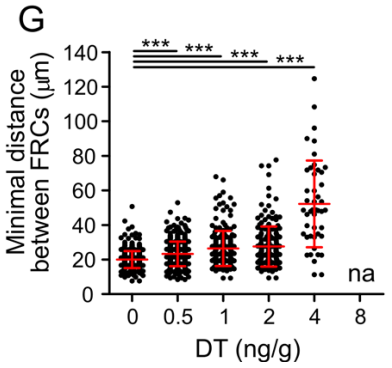
Step 2: Study of the FRC Network Structural Integrity

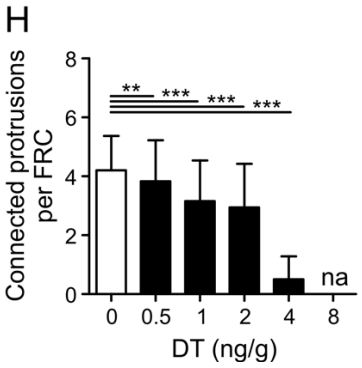
Alterations in FRC morphology following partial FRC ablation











Explanation of the previous slide

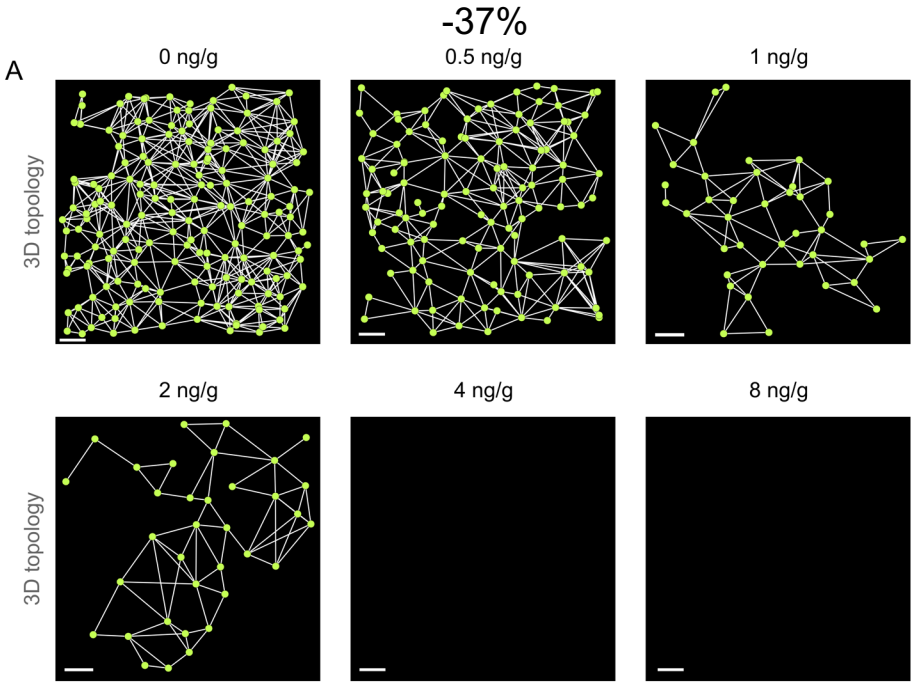
Injection of increasing doses of DT toxin, in mice, to study the FRC network response.

The injection, in mice, of 0.5 ng/g of DT resulted in moderate FCR ablation, while dose above 2 ng/g resulted in substantial damage to the FCR network.

FRC numbers decreased by 37%, 67%, 70%, 91% and 100% in mice treated with 0.5, 1, 2, 4 and 8 ng/g DT, respectively. Interestingly, high doses of DT induce and enlargement in FCR cell body, probably because these morphological changes are a consequence of FRC relaxation by which the cells compensate for the loss of neighboring cells or the need to cover more space.

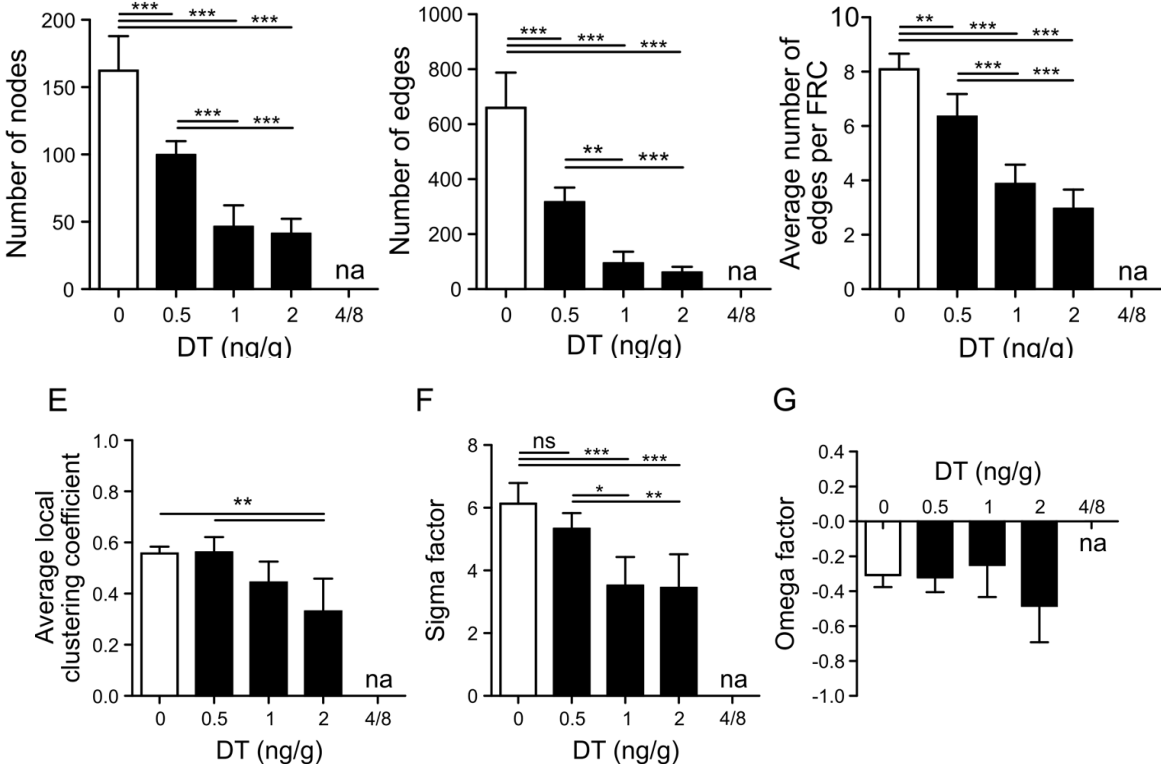
Step 2: Study of the FRC Network Structural Integrity

Gradual FRC ablation reveals thresholds for FRC network integrity



Step 2: Study of the FRC Network Structural Integrity

Gradual FRC ablation reveals thresholds for FRC network integrity



Step 2: Study of the FRC Network Structural Integrity

Conclusions:

- **the number of nodes and edges dropped substantially when only 37% of the FRCs were ablated (0.5ng DT)**

- **the network structure was destroyed when more than 70% of the cells were ablated**

- **The number of edges per FRC and the local clustering coefficient were not profoundly altered at the DT dose of 0.5 ng/g.**

- **small-worldness as determined by the σ factor was not significantly affected when the FRC network was mildly perturbed by the low- dose DT injection**

- **the ω factor is not sensitive to strong alterations in the FRC network introduced by partial node removal (Fig G), suggesting that the FRC network remains preferentially latticed**

Step 2: Study of the FRC Network Structural Integrity

Main Conclusion

essential FRC network features remain stable when <40% of the cells are removed, while an ablation of >70% of FRCs results in complete network failure

Step 3: In Silico Prediction of the FRC Network Topological Robustness

Assumption: the rapid loss of LN-FRCs during viral infection occurs randomly.

Hence, though a computer simulation, the authors performed a series of simulations removing randomly chosen nodes from the representative FRC network, for statistical significance, with the mentioned characteristics (lattice-like and small-worldness).

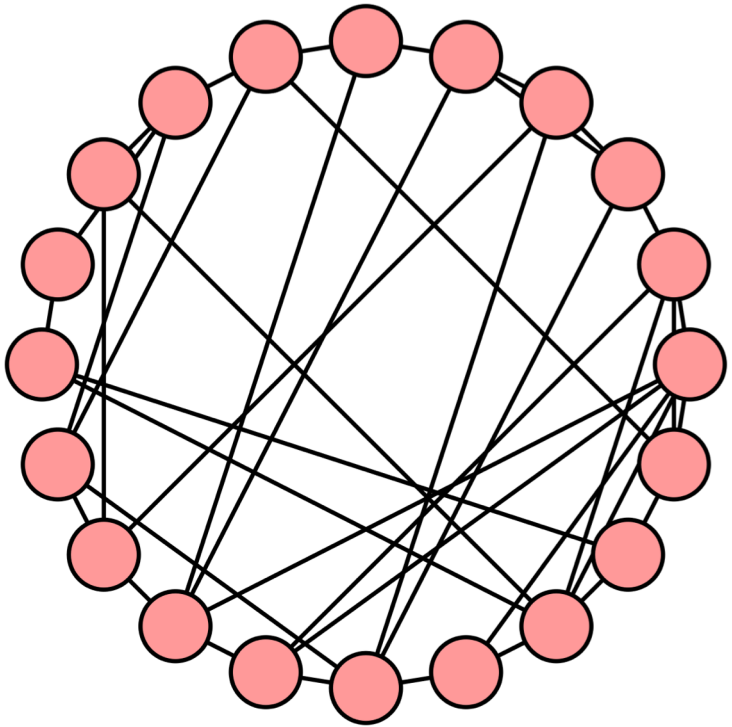
Using computational methods allows for evaluating the model using very large numbers of simulations, 1000 rounds starting from the representative FRC network.

Step 3: In Silico Prediction of the FRC Network Topological Robustness

FRC Network Model

The network model used in this work refers to a small world lattice-like networks

This means that the nodes are highly connected (lattice-like) with each other and the average short path of this network is small (small world)



Step 3: In Silico Prediction of the FRC Network Topological Robustness

Disrupting the network

Using the in-house *R* algorithm "generate network & remove node", it was possible to study the network fragmentation kinetic.

Step 3: In Silico Prediction of the FRC Network Topological Robustness

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At each step the robustness of the residual network was evaluated and a fragmentation curve was generated.

Step 3: In Silico Prediction of the FRC Network Topological Robustness

Disrupting the network

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Robustness is the "ability of the network to withstand failures and perturbations" (from https://en.wikipedia.org/wiki/Robustness_of_complex_networks)

Step 3: In Silico Prediction of the FRC Network Topological Robustness

Disrupting the network

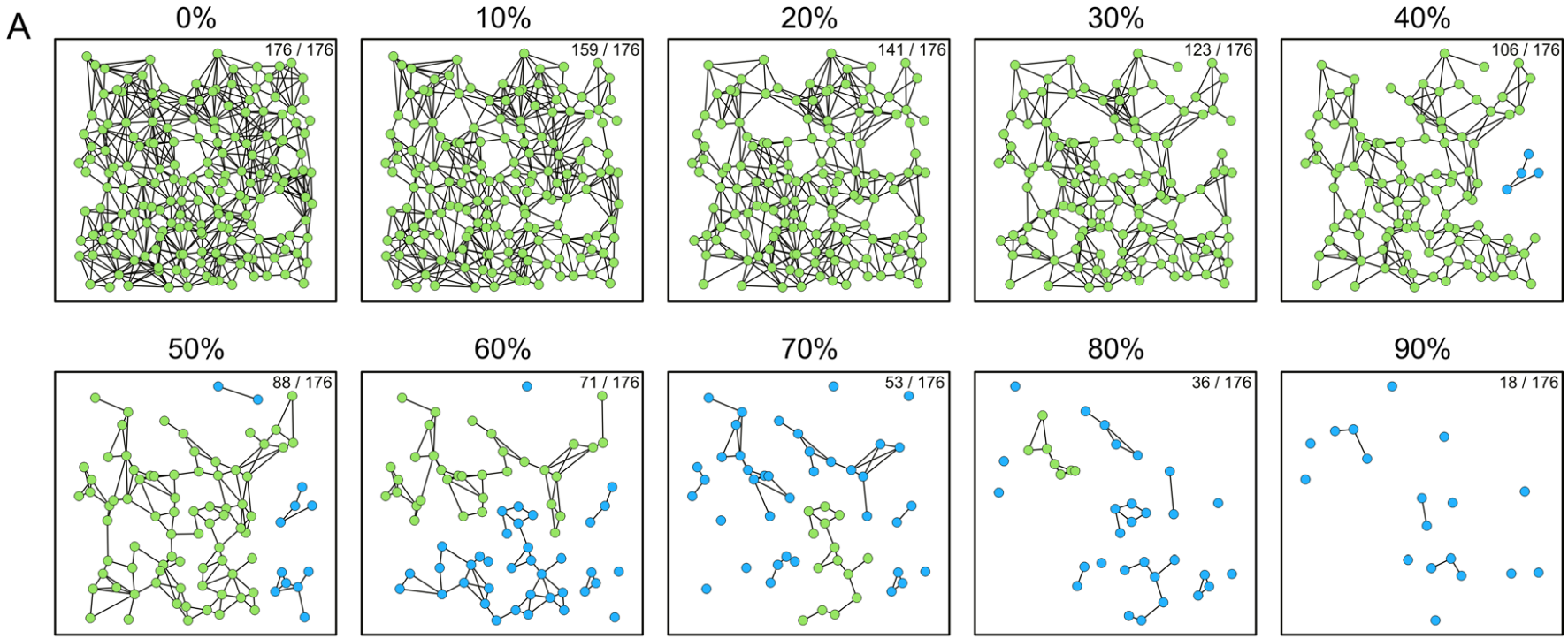
Using the in-house *R* algorithm "generate network & remove node", it was possible to study the network fragmentation kinetic.

At each step the robustness of the residual network was evaluated and a fragmentation curve was generated.

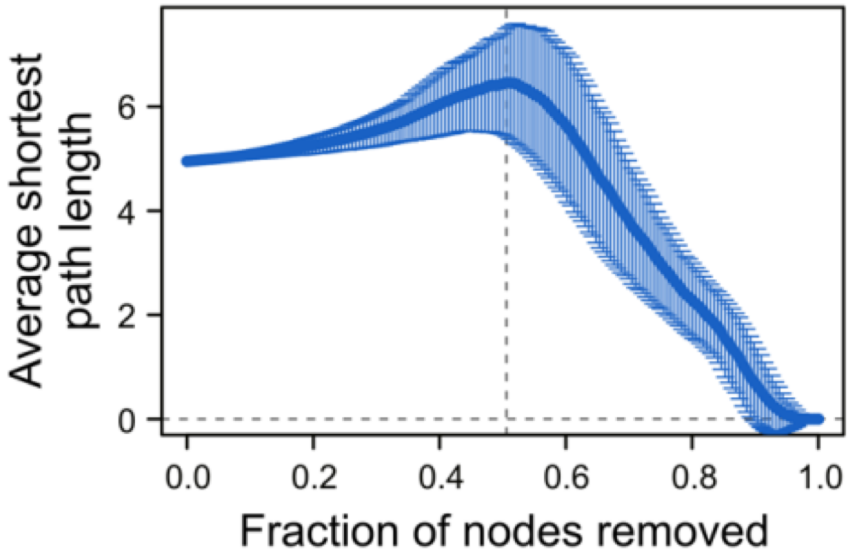
Robustness is the "ability of the network to withstand failures and perturbations" (from https://en.wikipedia.org/wiki/Robustness_of_complex_networks)

A numerical value ranging in between [1/N - 0.5] defines the robustness of a network with N nodes.

Step 3: In Silico Prediction of the FRC Network Topological Robustness



Step 3: In Silico Prediction of the FRC Network Topological Robustness

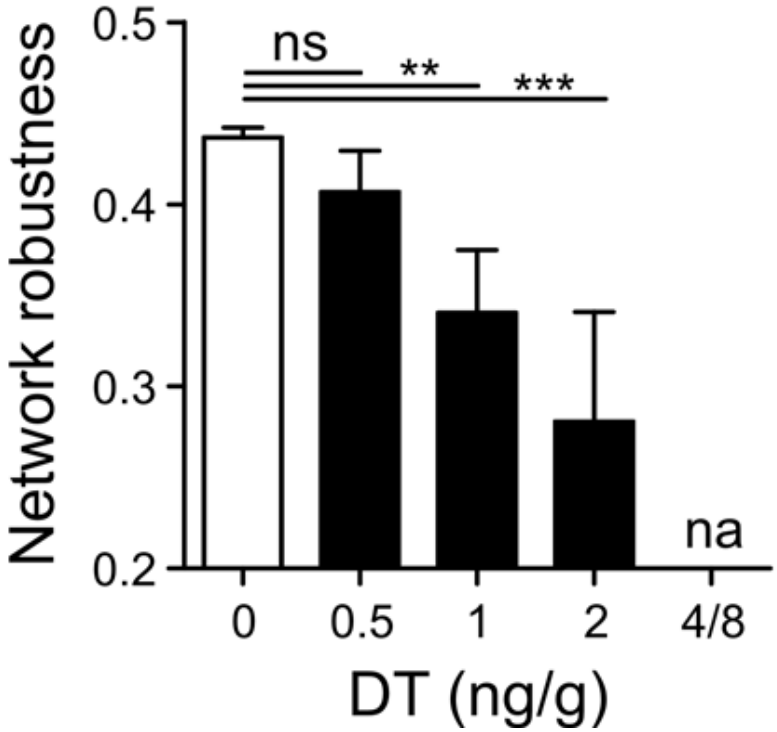


The curve shows how the average shortest path length does not significantly change until 50% of the nodes are removed from the network.

Step 3: In Silico Prediction of the FRC Network Topological Robustness

Biological meaning of in silico predictions

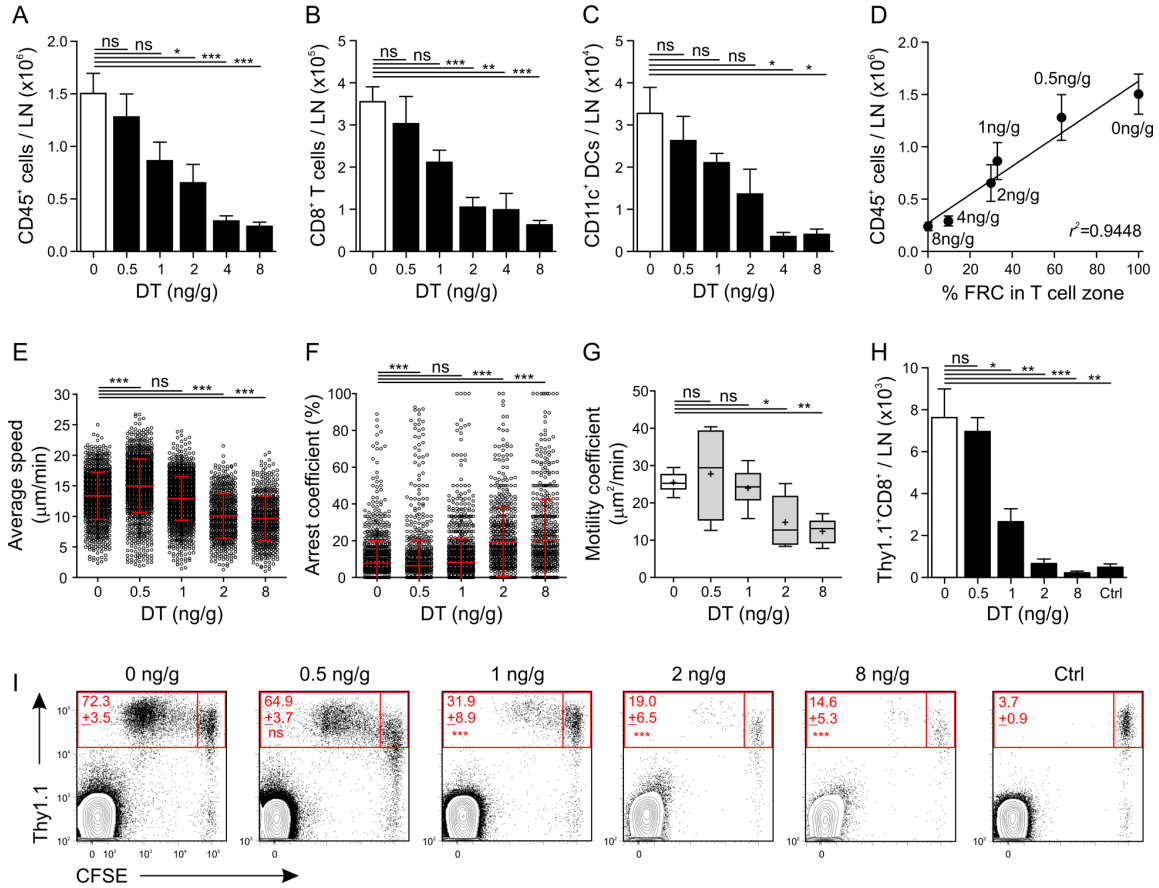
The in silico model predicts that the FRC network displays significant topological robustness against random node removal and is able to tolerate up to half of the network being destroyed.



Network robustness R values for FRC networks at indicated doses of DT. Data represent mean ± SD for 3–6 mice per group from two independent experiments.

0.5ng/g DT: 37% FRC removal
1ng/g DT: 67% FRC removal

Step 4: Evaluation of LN functionality after FRC ablation



Step 4: Evaluation of LN functionality after FRC ablation

- Substantial changes in LN cellularity and T cell migration occur when >70% of FRCs are removed
- Failure of T cell expansion at 50% removal of FRCs
- High correlation between topology and biological function as shown in Fig D
- The analysis demonstrates the connection between LN functionality and FRC topology

Step 5: Conclusions

The methodology is suitable to assess FRC network topology

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LN FRCs form a small-world network with lattice-like properties.

The analysis demonstrates the connection between LN functionality and FRC topology

Graph theory-based analysis is useful for the understanding of immune processes.

R index of the FRC network can be considered as a biologically relevant and consistent measure of robustness with global functional implications in the immune system.