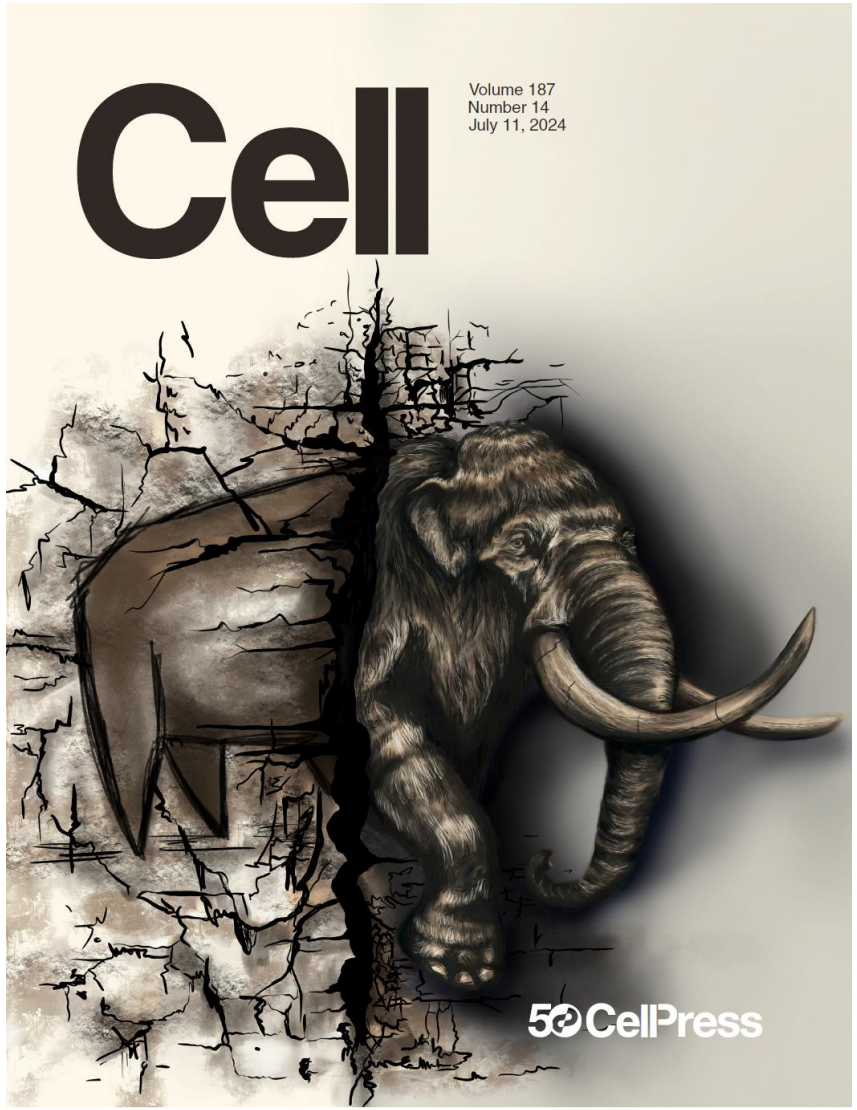
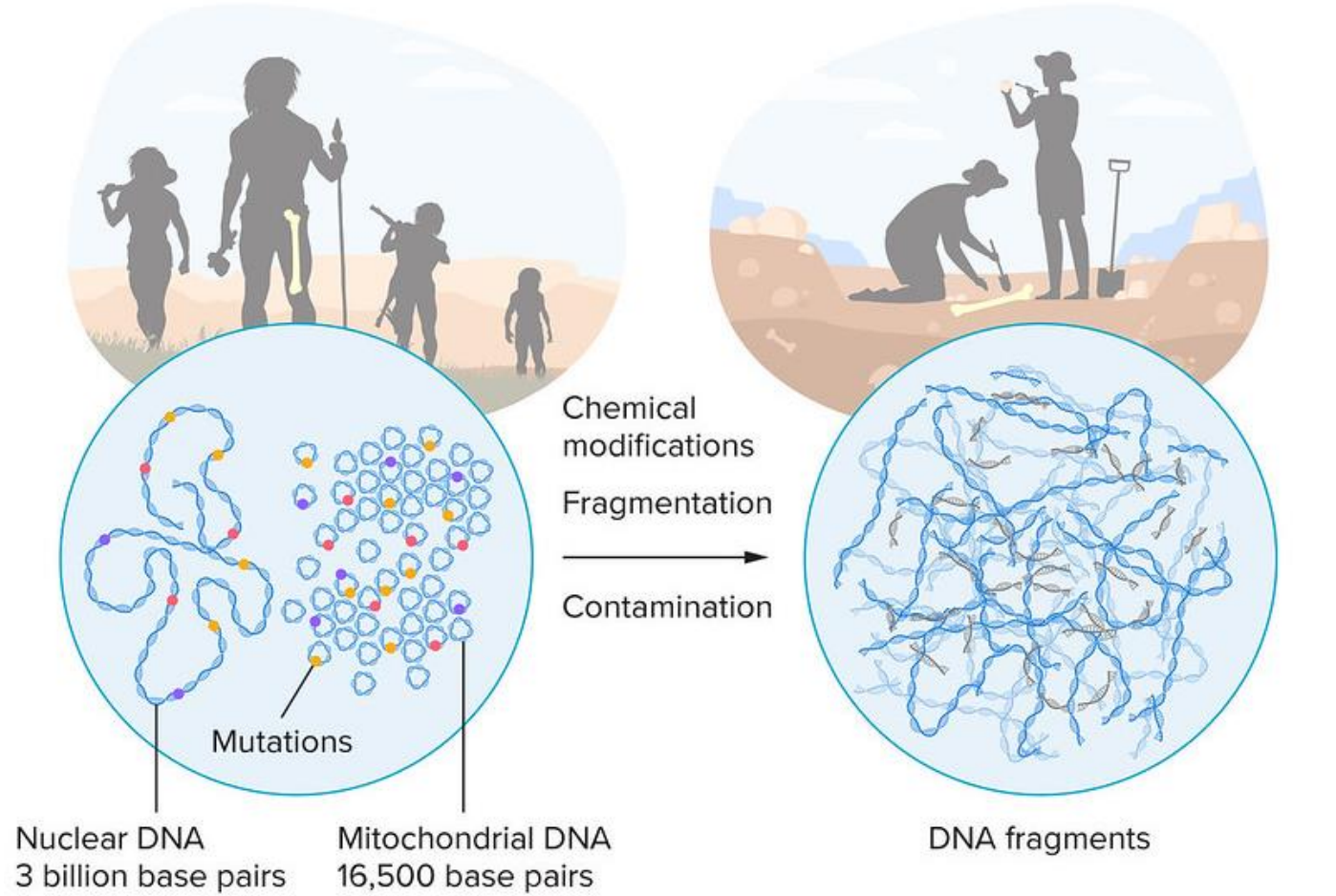
THREE-DIMENSIONAL GENOME ARCHITECTURE PERSISTS IN A 52,000-YEAR-OLD WOOLLY MAMMOTH SKIN SAMPLE



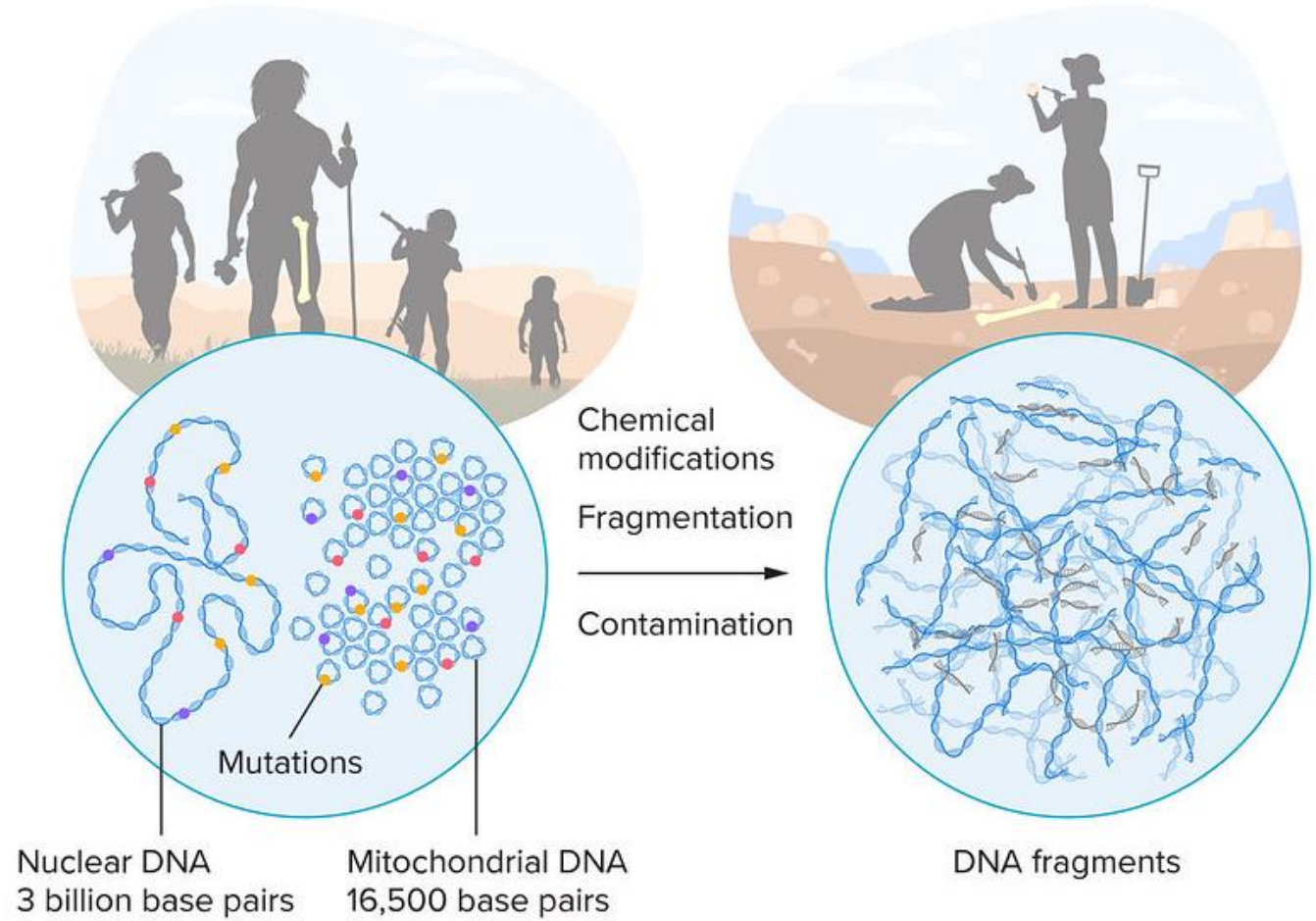
Marcela Sandoval-Velasco*, Olga Dudchenko*#, Juan Antonio Rodríguez*, Cynthia Pérez Estrada*, Marianne Dehasque, Claudia Fontsere, Sarah S.T. Mak, Ruqayya Khan, Vinícius G. Contessoto, Antonio B. Oliveira Junior, Achyuth Kalluchi, Bernardo J. Zubillaga Herrera, Jiyun Jeong, Renata P. Roy, Ishawnia Christopher, David Weisz, Arina D. Omer, Sanjit S. Batra, Muhammad S. Shamim, Neva C. Durand, Brendan O'Connell, Alfred L. Roca, Maksim V. Plikus, Mariya A. Kusliy, Svetlana A. Romanenko, Natalya A. Lemskaya, Natalya A. Serdyukova, Svetlana A. Modina, Polina L. Perelman, Elena A. Kizilova, Sergei I. Baiborodin, Nikolai B. Rubtsov, Gur Machol, Krisha Rath, Ragini Mahajan, Parwinder Kaur, Andreas Gnirke, Isabel Garcia-Treviño, Rob Coke, Joseph P. Flanagan, Kelcie Pletch, Aurora Ruiz-Herrera, Valerii Plotnikov, Innokentiy S. Pavlov, Naryya I. Pavlova, Albert V. Protopopov, Michele Di Pierro, Alexander S. Graphodatsky, Eric S. Lander, M. Jordan Rowley, Peter G. Wolynes, José N. Onuchic, Love Dalén, Marc A. Marti-Renom#, M. Thomas P. Gilbert#, Erez Lieberman Aiden#

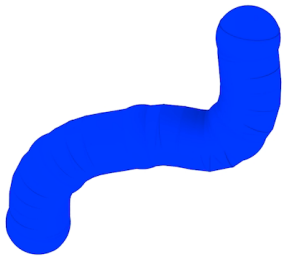
*first authors #corresponding authors

DNA FRAGMENTS SURVIVE IN ANCIENT SAMPLES…



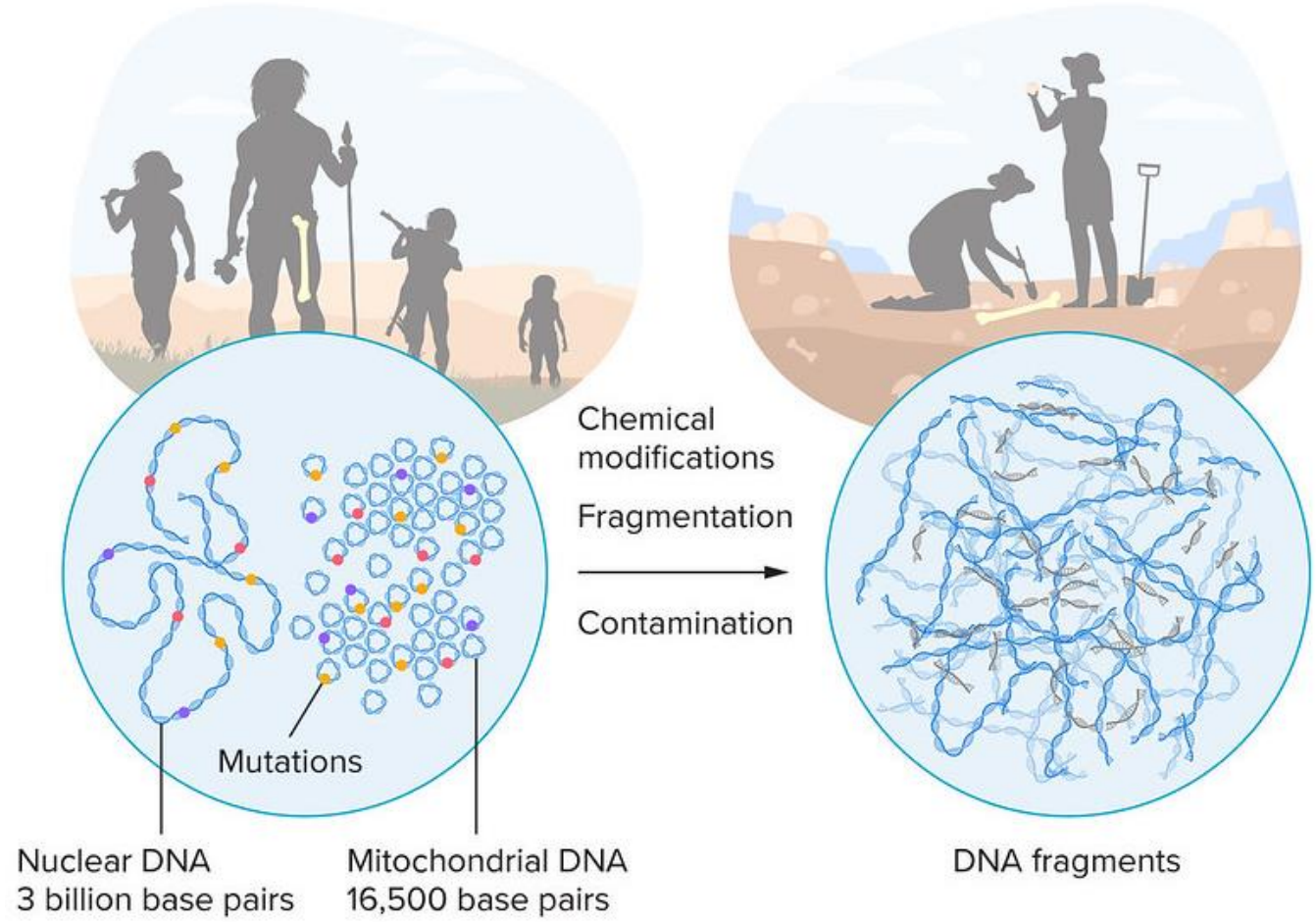
...BUT ONLY FRAGMENTS

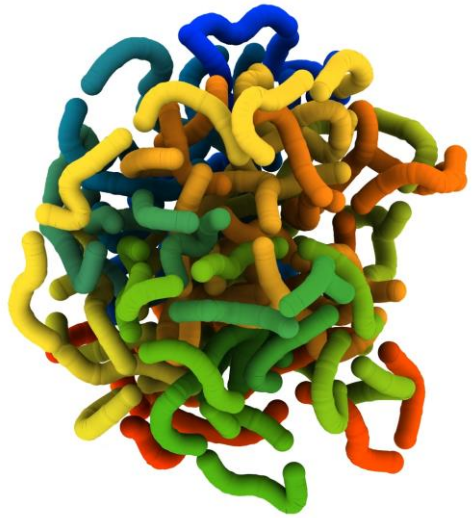




Vinícius Contessoto
Antonio Oliveira Jr.
José Onuchic [CTBP]

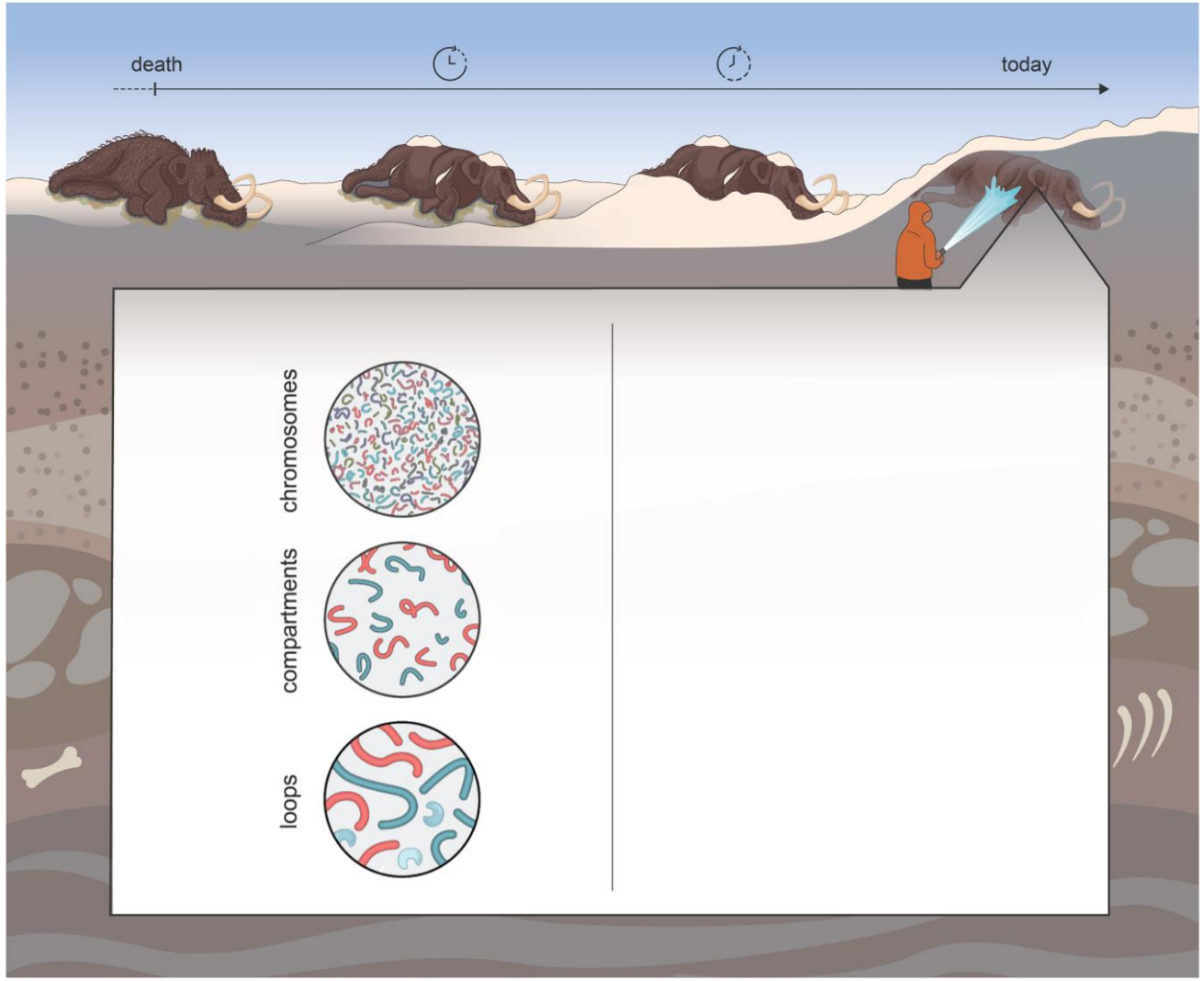
...BUT ONLY FRAGMENTS



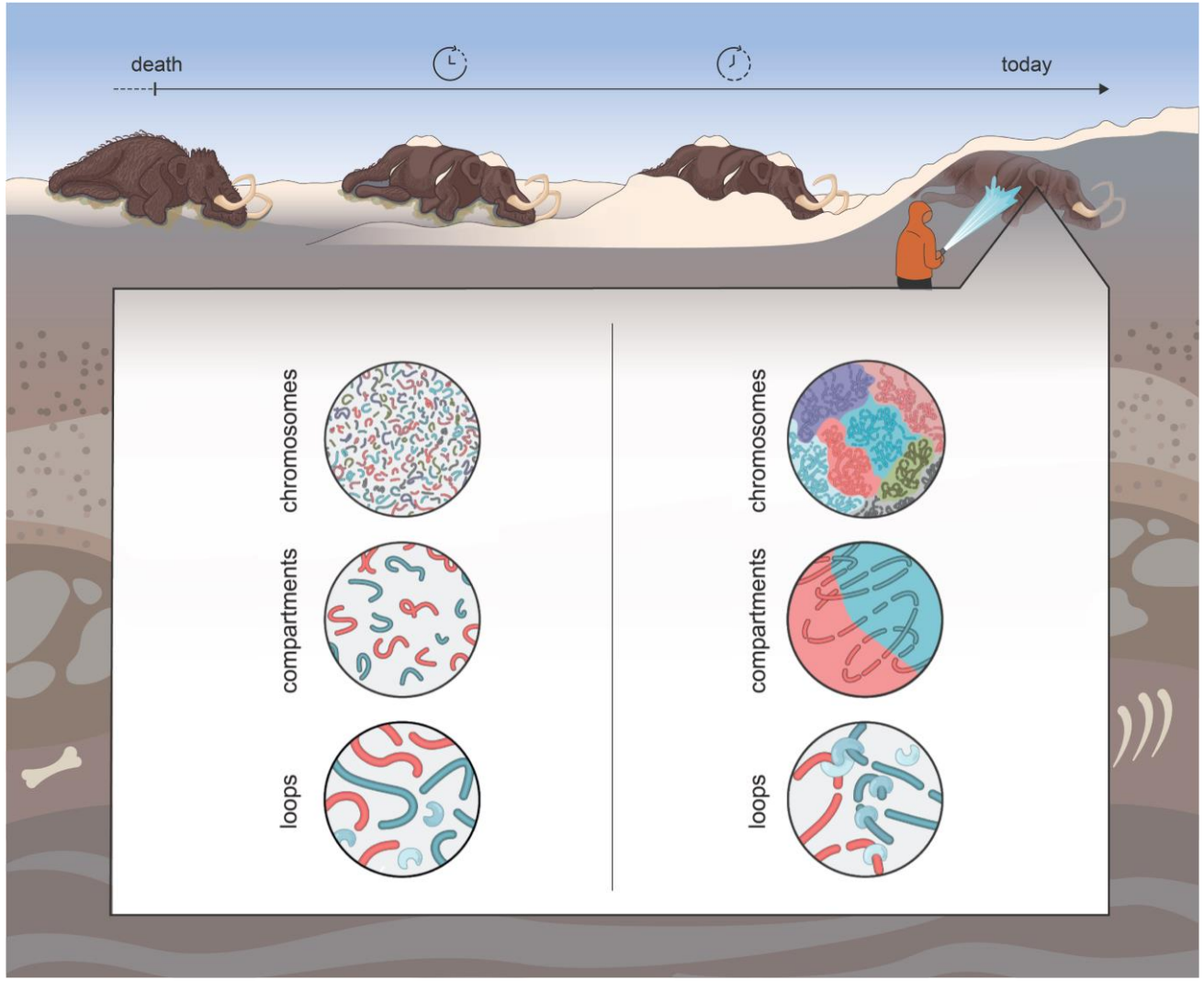


Vinícius Contessoto
Antonio Oliveira Jr.
José Onuchic [CTBP]

COULD THE THREE-DIMENSIONAL ARRANGEMENT OF DNA FRAGMENTS SURVIVE?



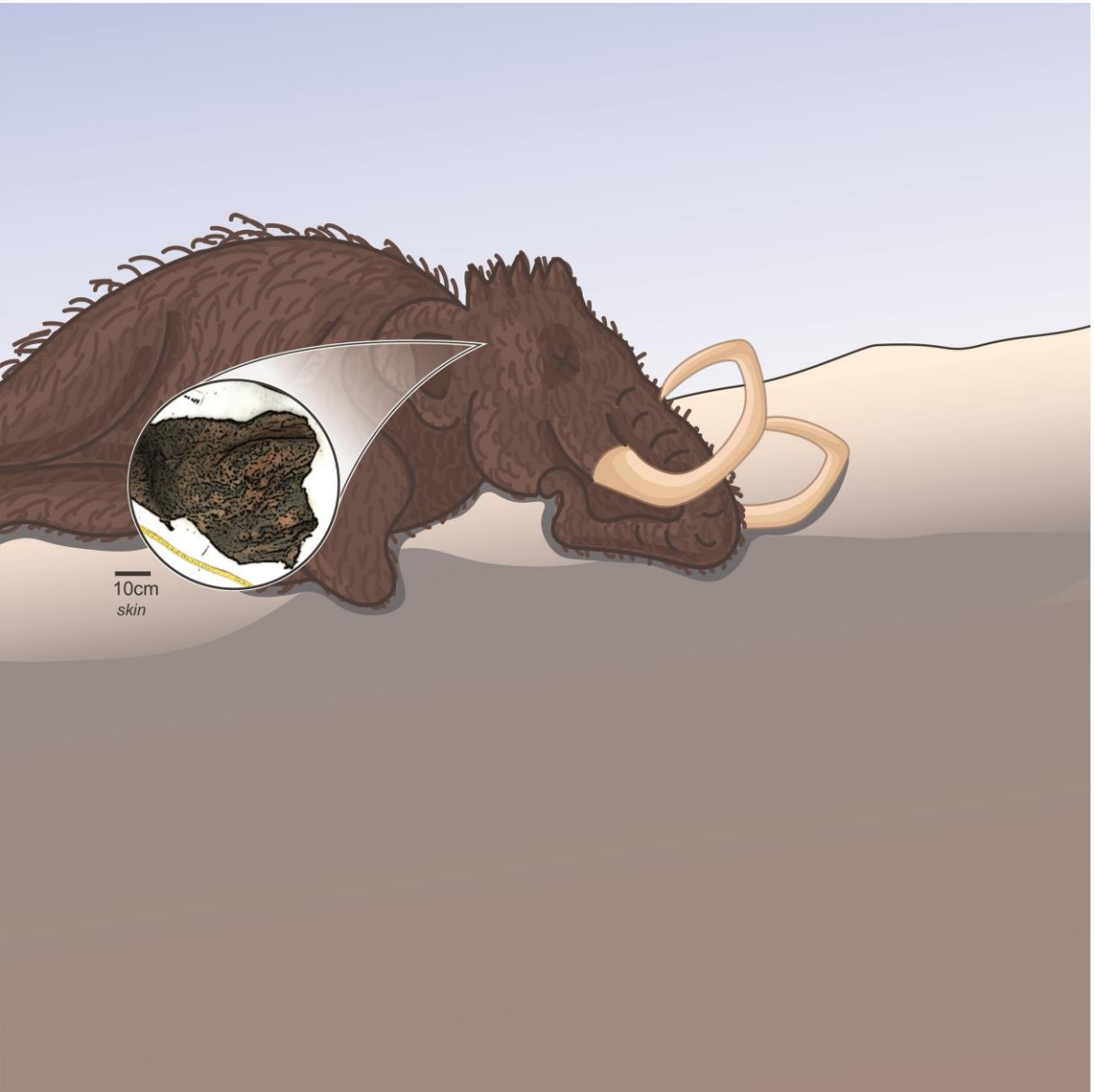
COULD THE THREE-DIMENSIONAL ARRANGEMENT OF DNA FRAGMENTS SURVIVE?



A FIVE-YEAR FOSSIL HUNT BROUGHT US TO A PERMAFROST CAVE…

Video credit: Love Dalén

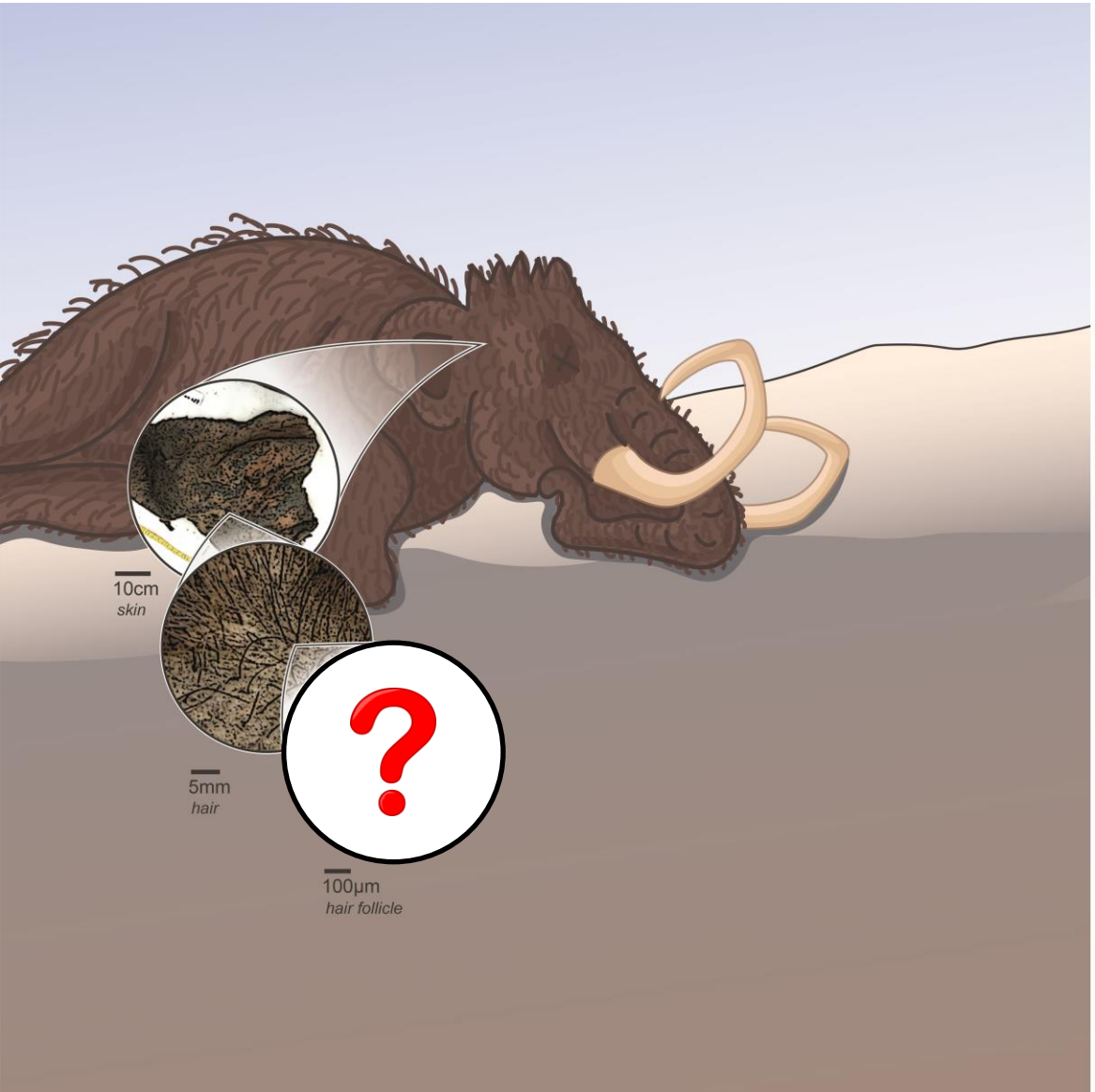
...WHERE WE FOUND A SKIN FROM A 52,000-YEAR-OLD WOOLLY MAMMOTH



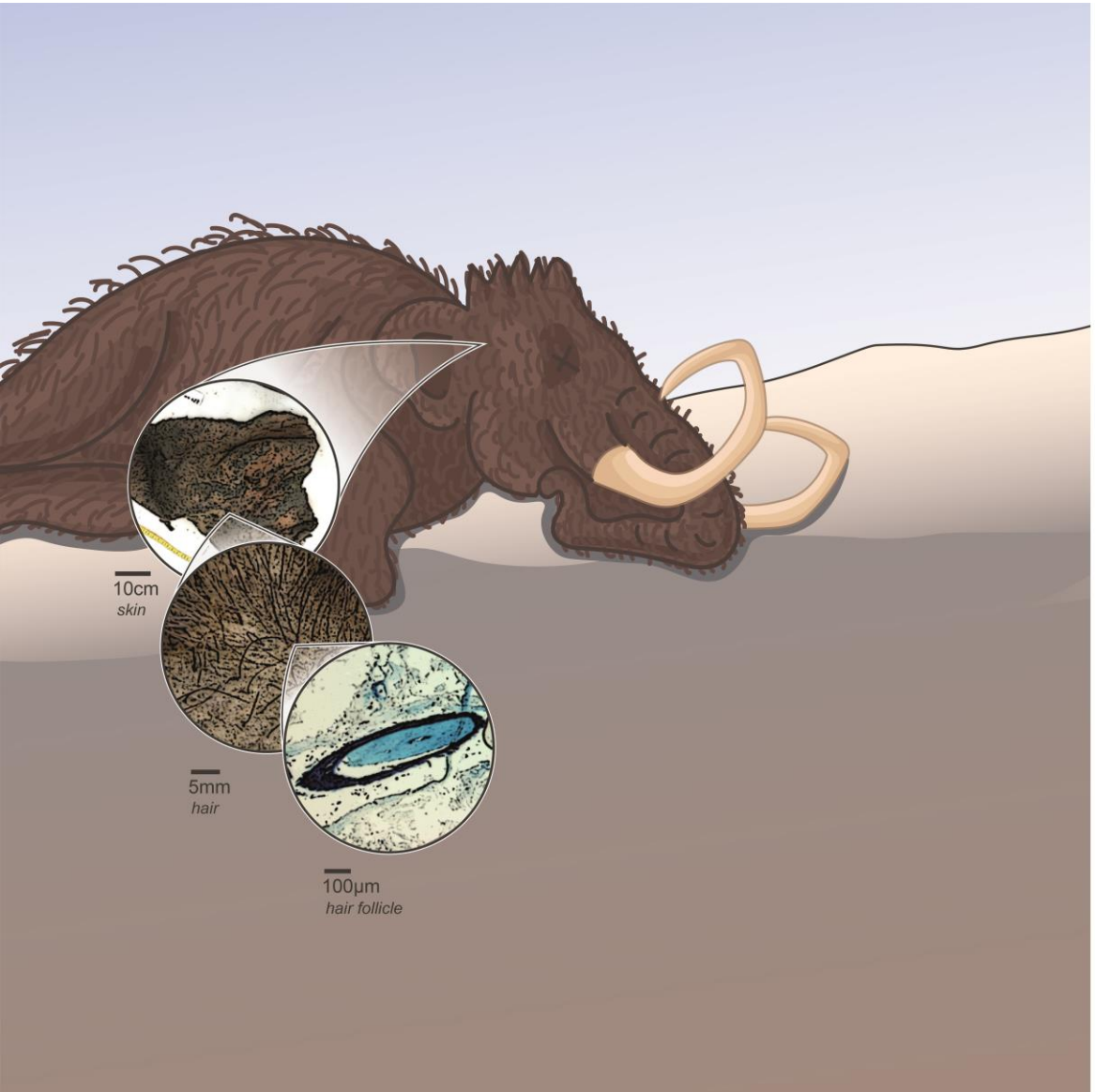
THE HAIRS WERE STILL THERE

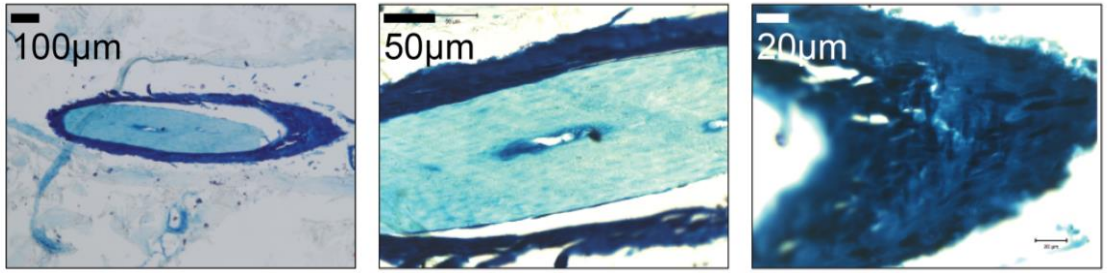
Valeri Plotnikov
Dan Fisher
Photo: Love Dalen

WE KEPT ZOOMING IN USING A MICROSCOPE

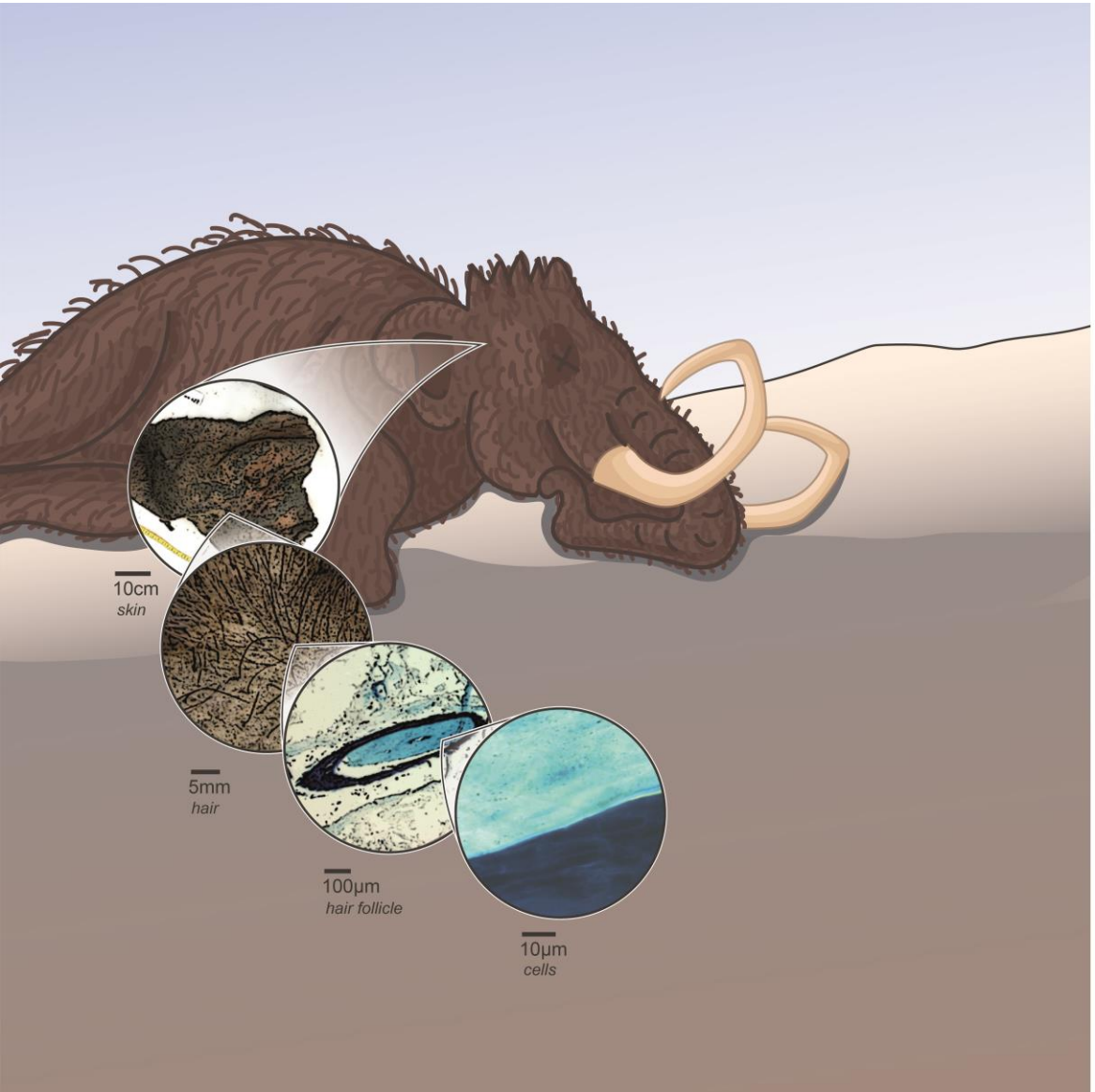


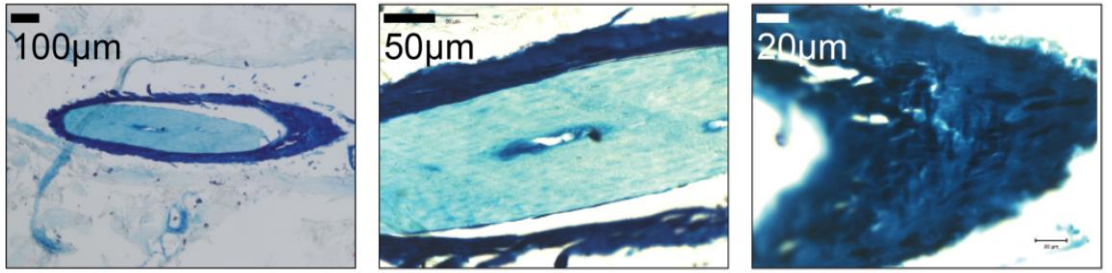
NOW WE COULD SEE THE HAIR FOLLICLES...



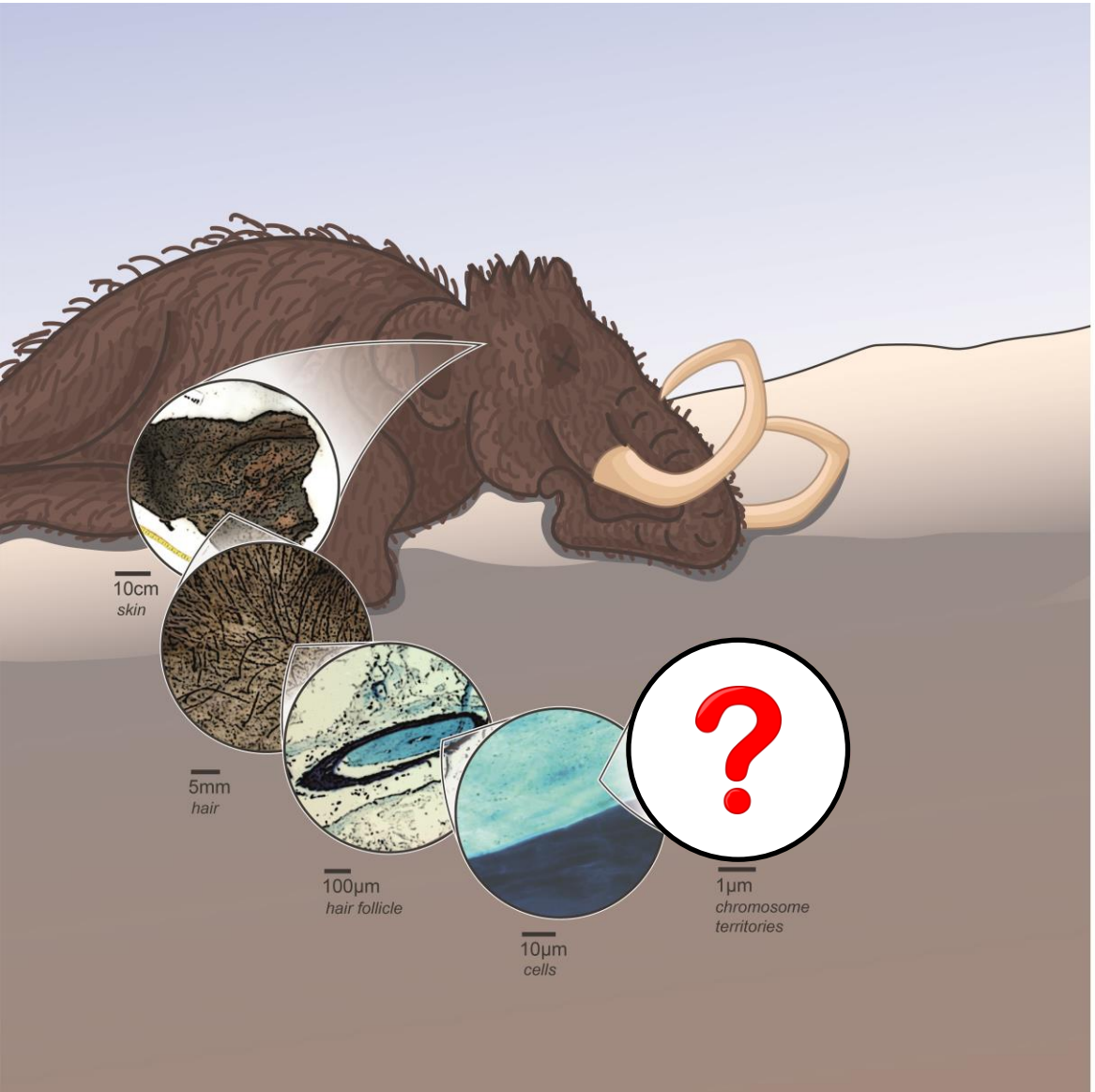


...AND EVEN INDIVIDUAL CELLS

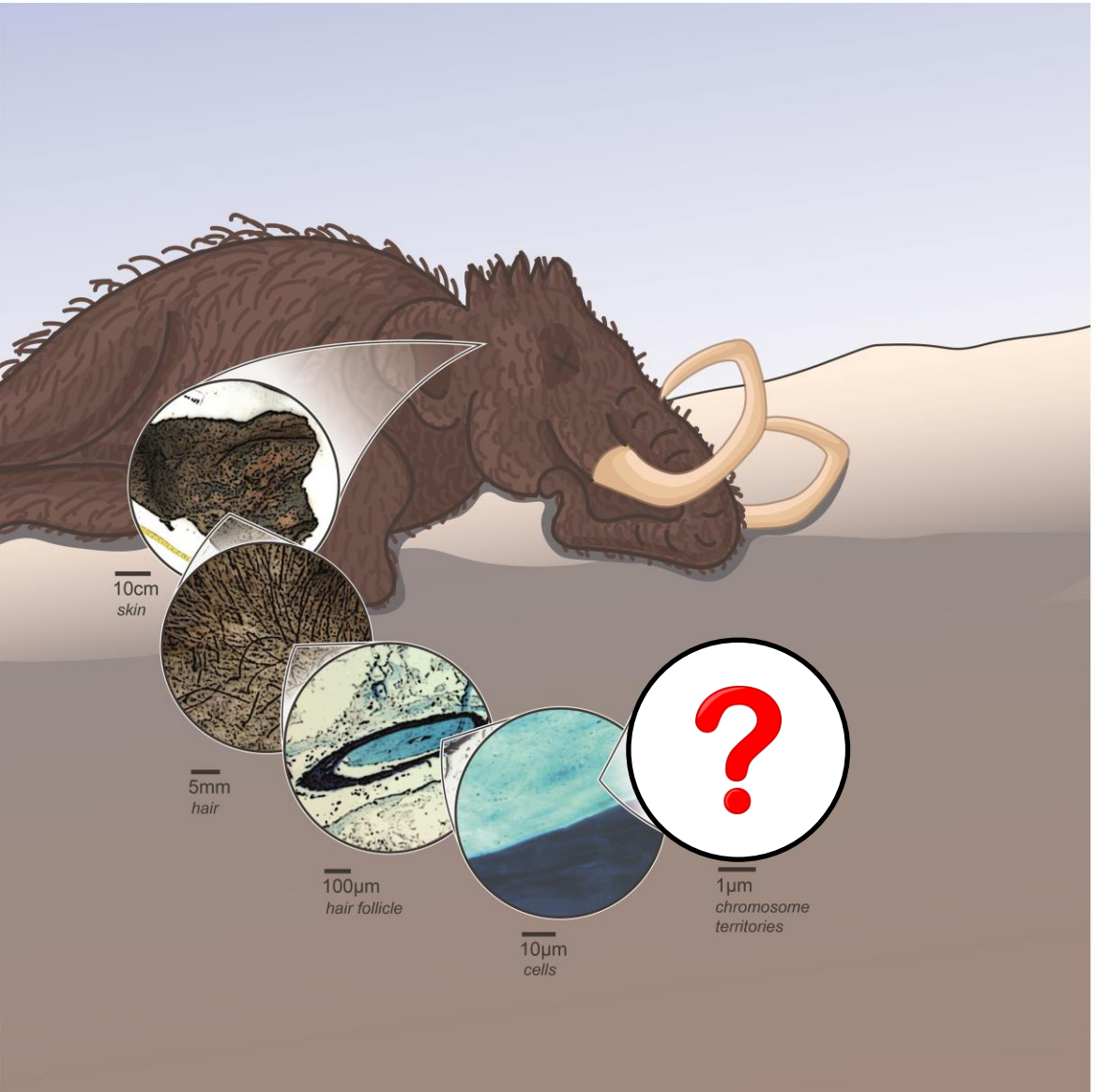


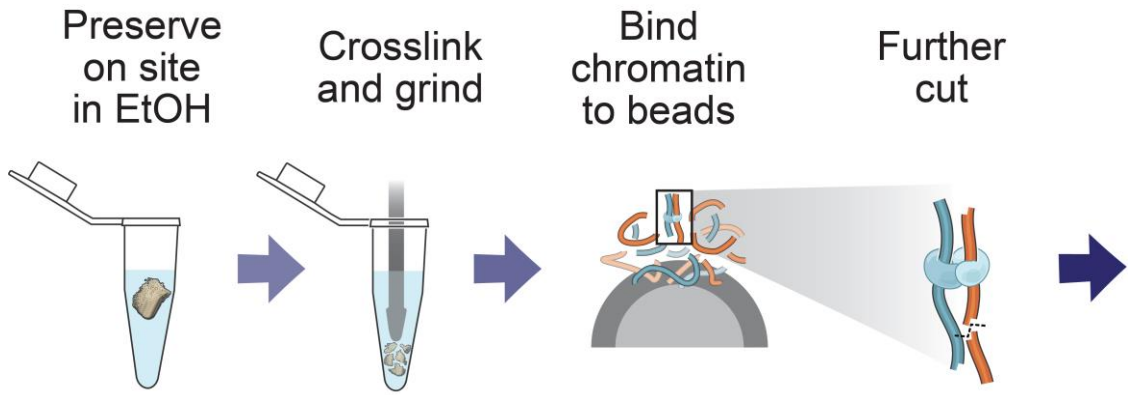


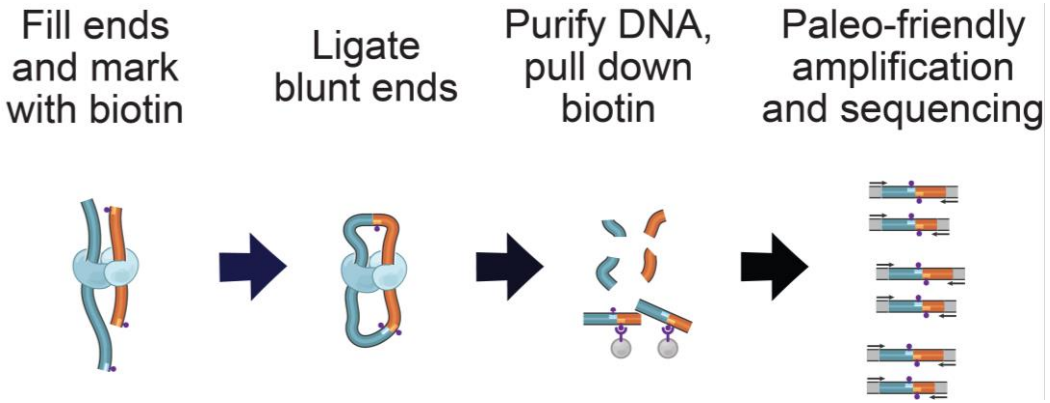
FINALLY WE ARRIVED AT NUCLEAR SCALE,



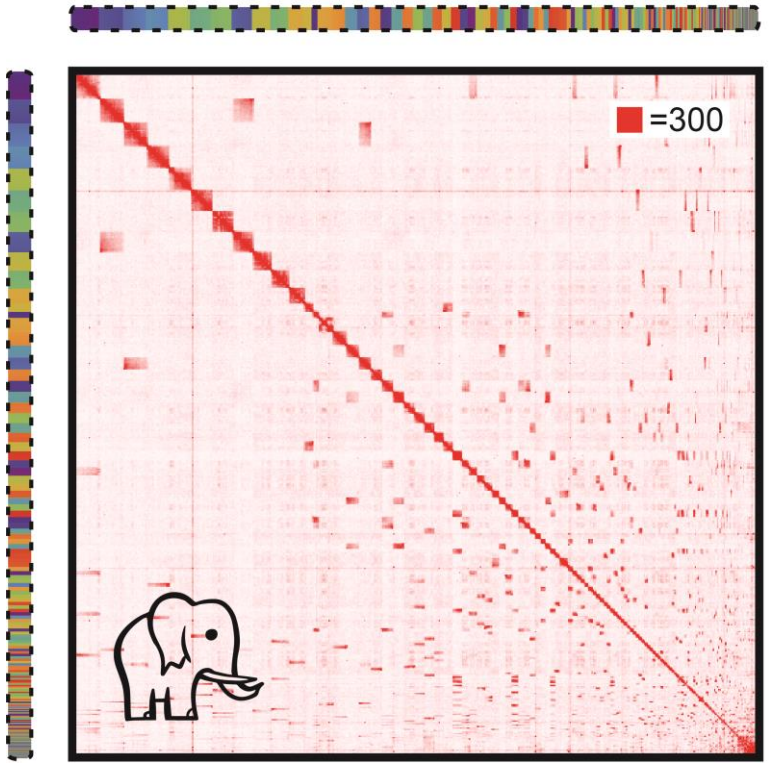
FINALLY WE ARRIVED AT NUCLEAR SCALE, BUT ZOOMING REQUIRED A NEW METHOD: PALEOHI-C



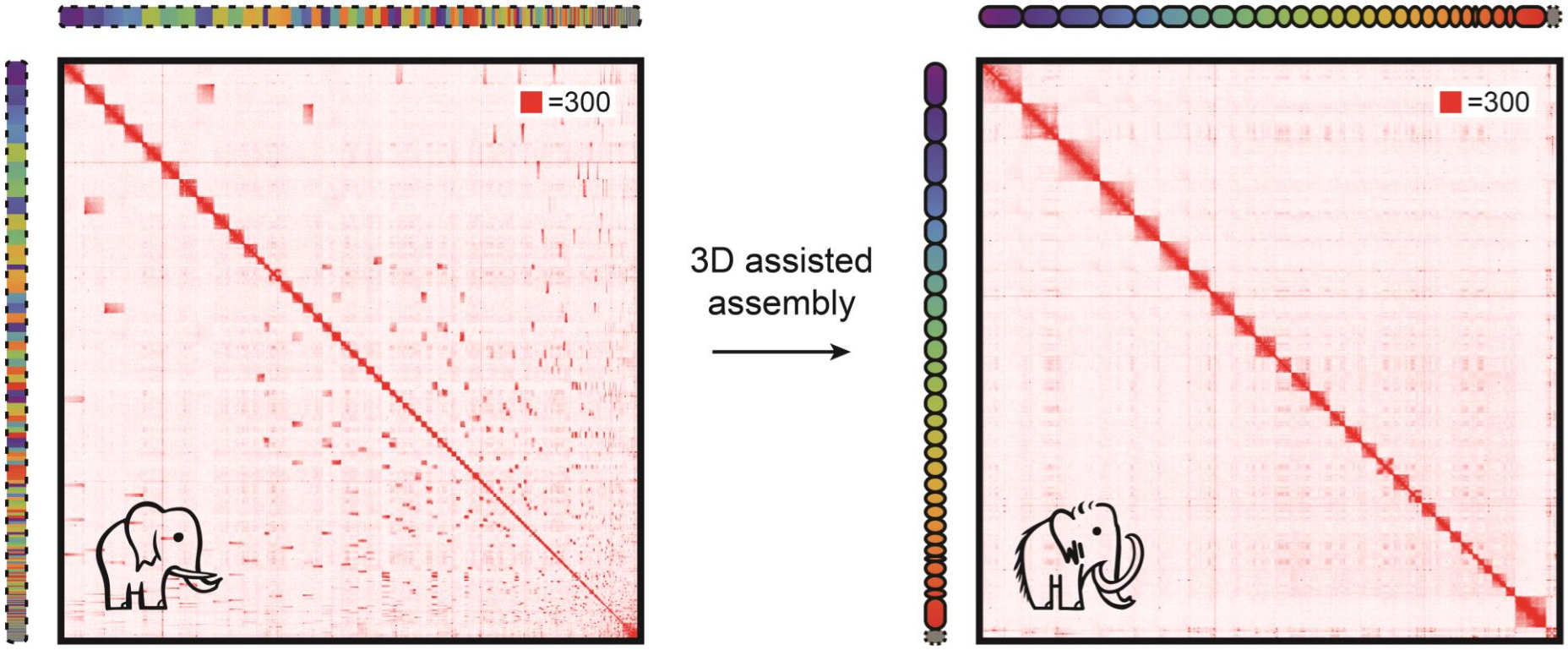




HERE IS WHAT THE PALEOHI-C DATA LOOKED LIKE

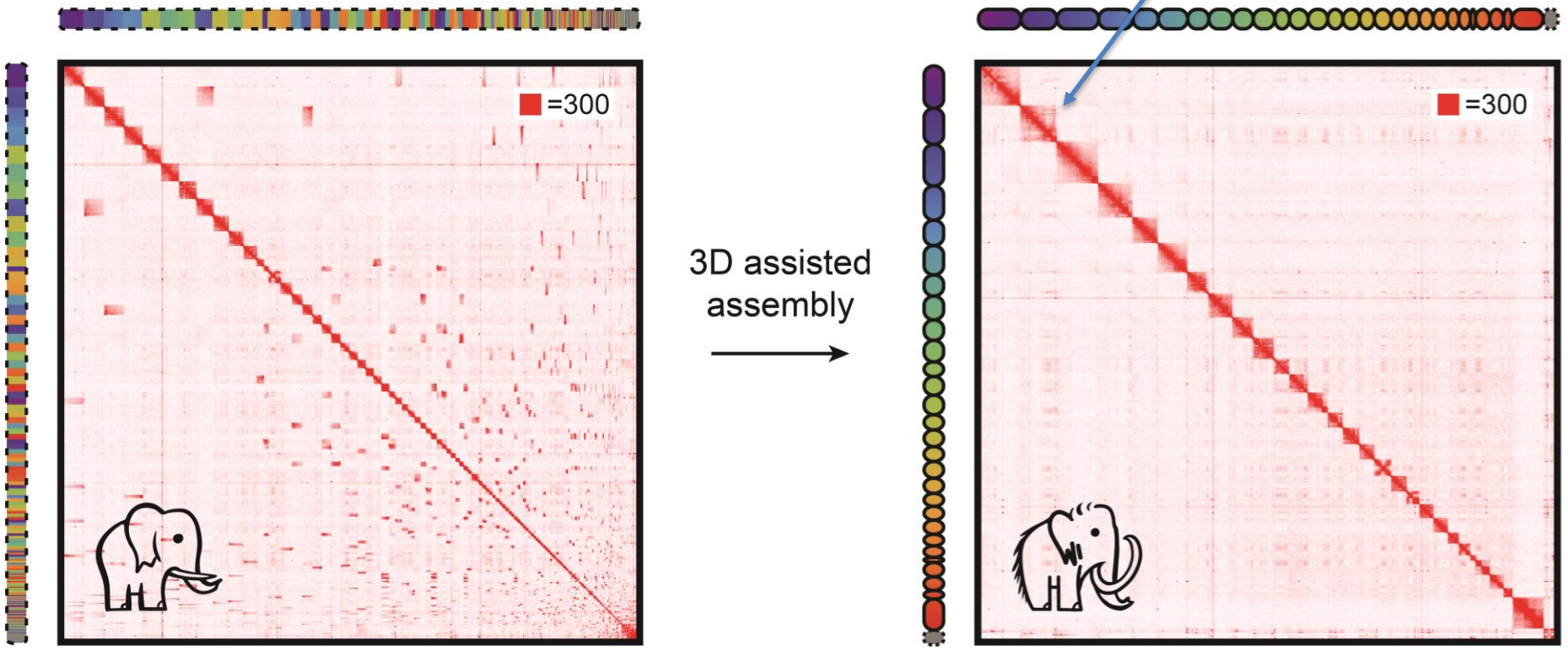


THIS IS WHAT THE ASSEMBLY LOOKED LIKE

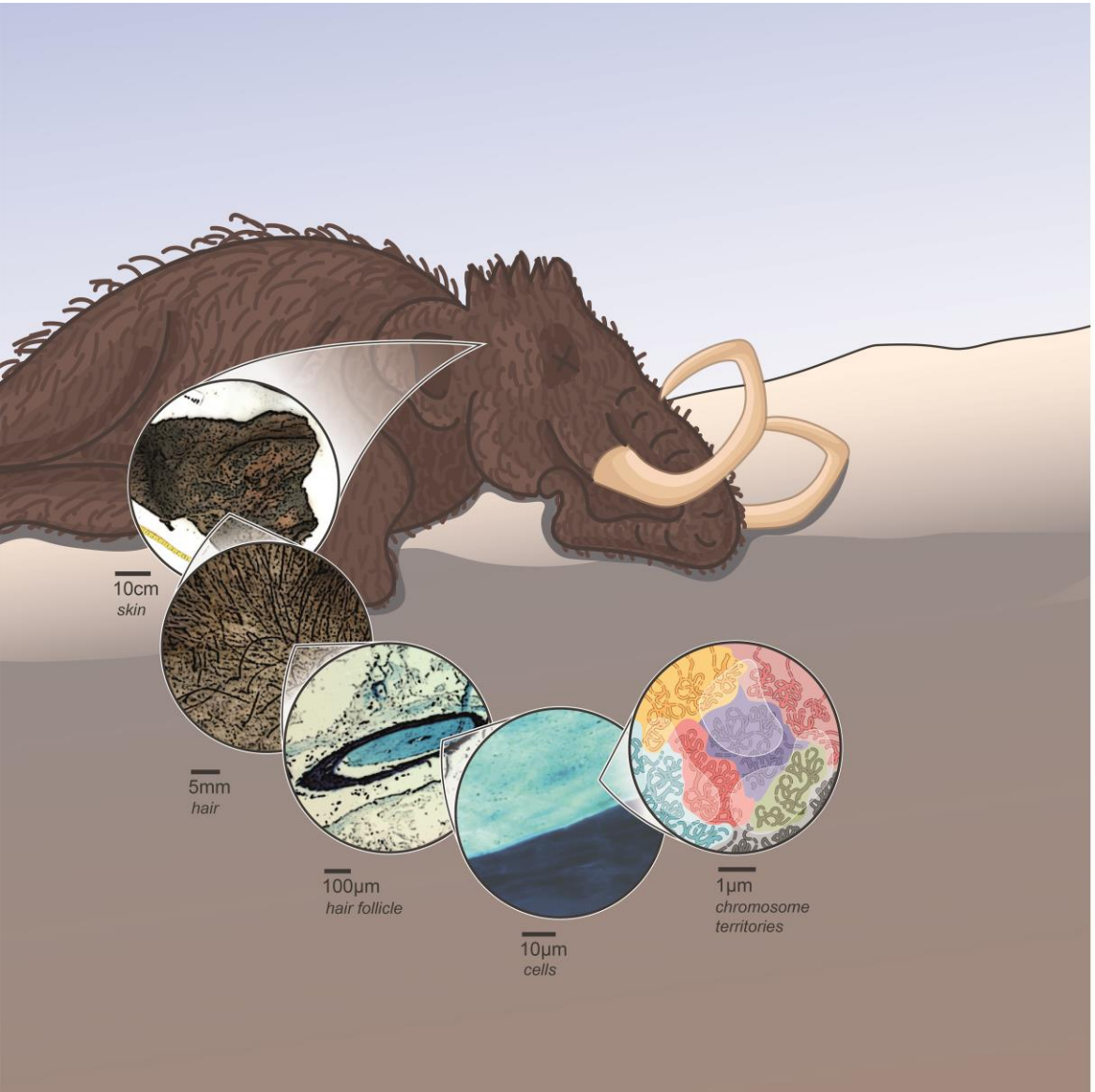


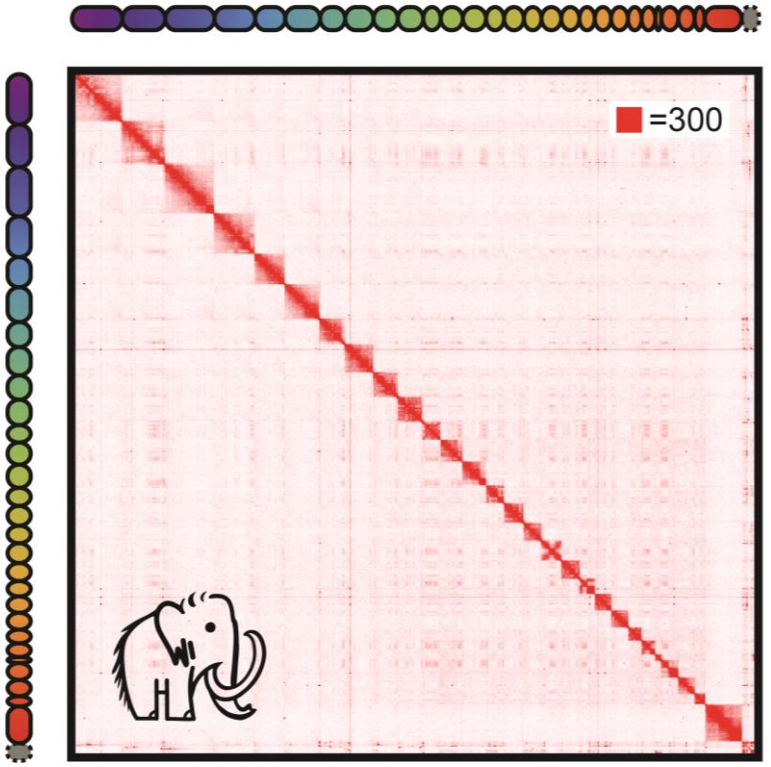
THIS IS WHAT THE ASSEMBLY LOOKED LIKE

By the way this is
a mammoth chromosome territory

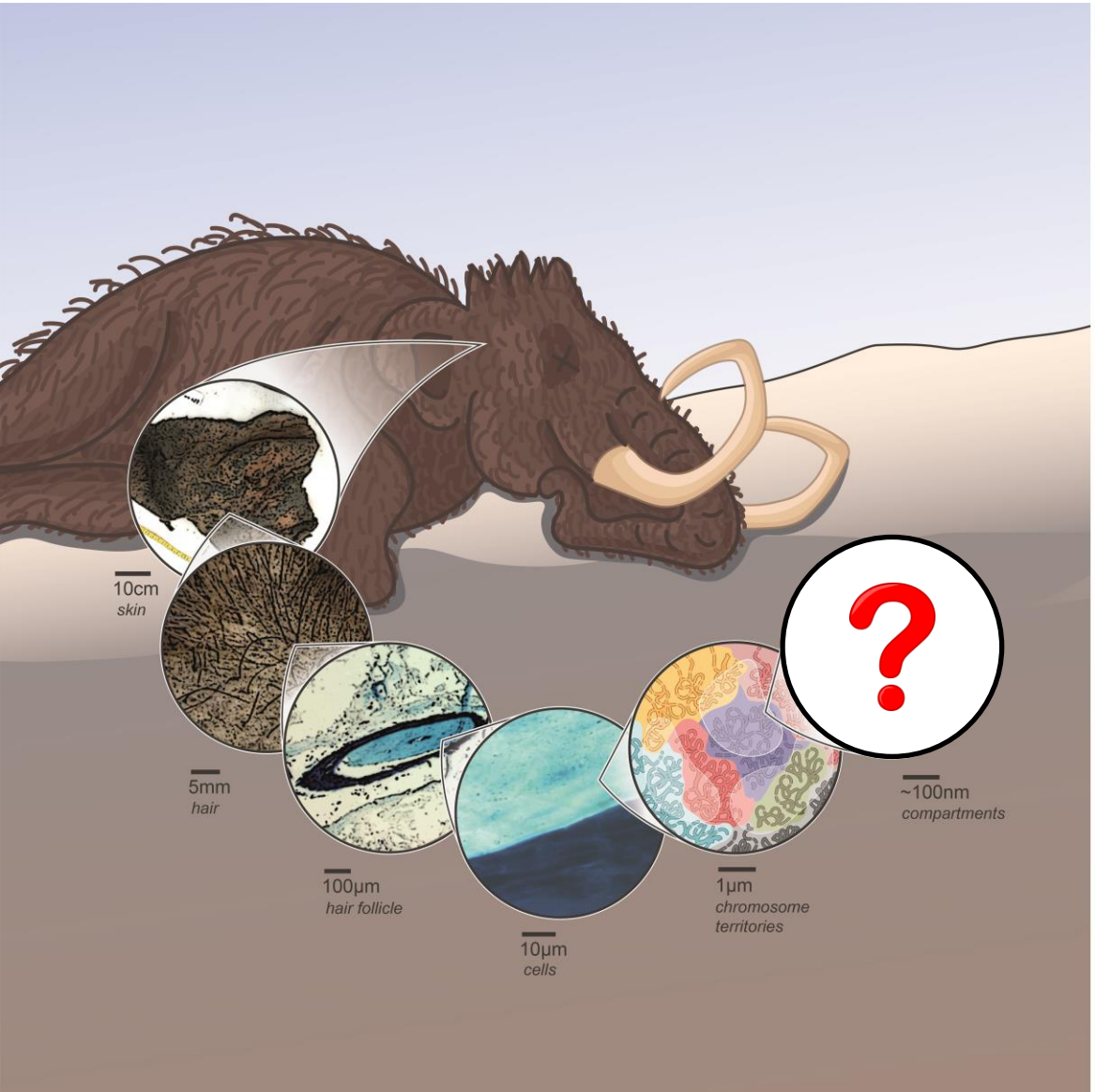


SO YES, WE CAN PEEK INSIDE THE NUCLEUS OF A WOOLLY MAMMOTH

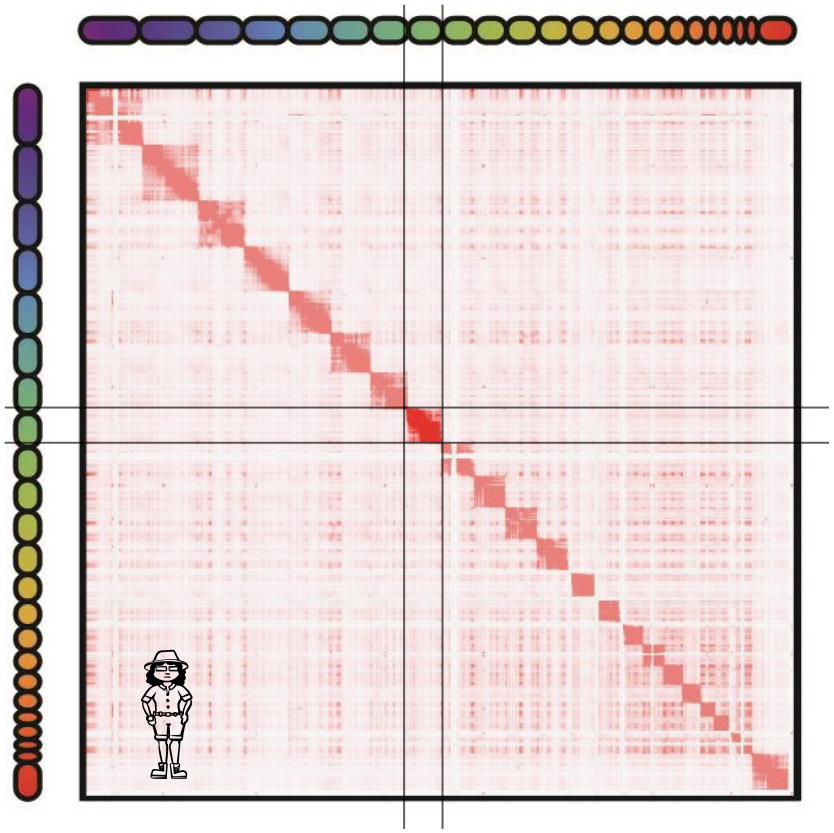


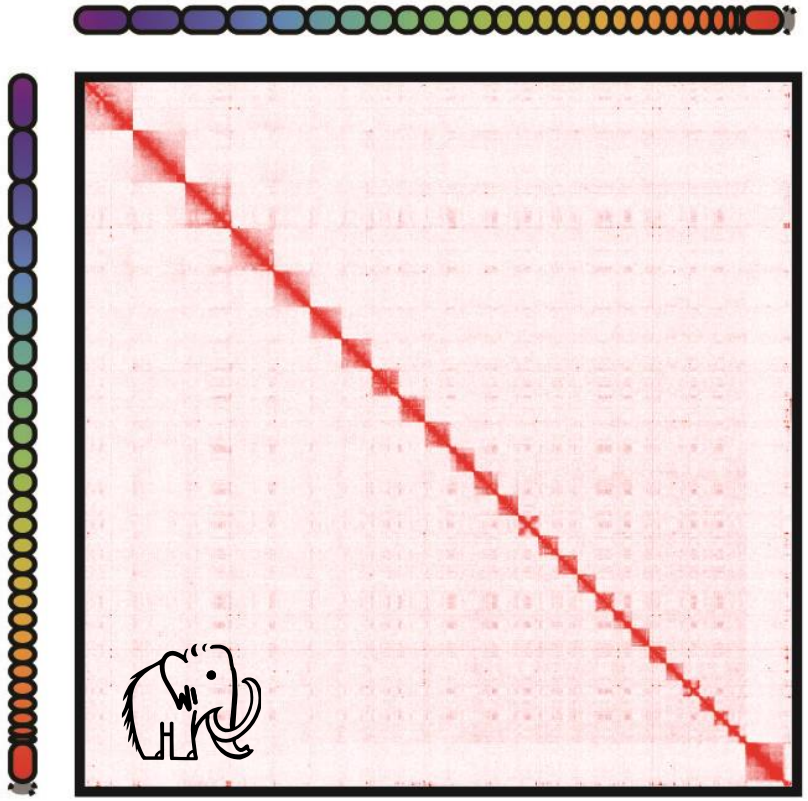


CAN WE KEEP GOING?

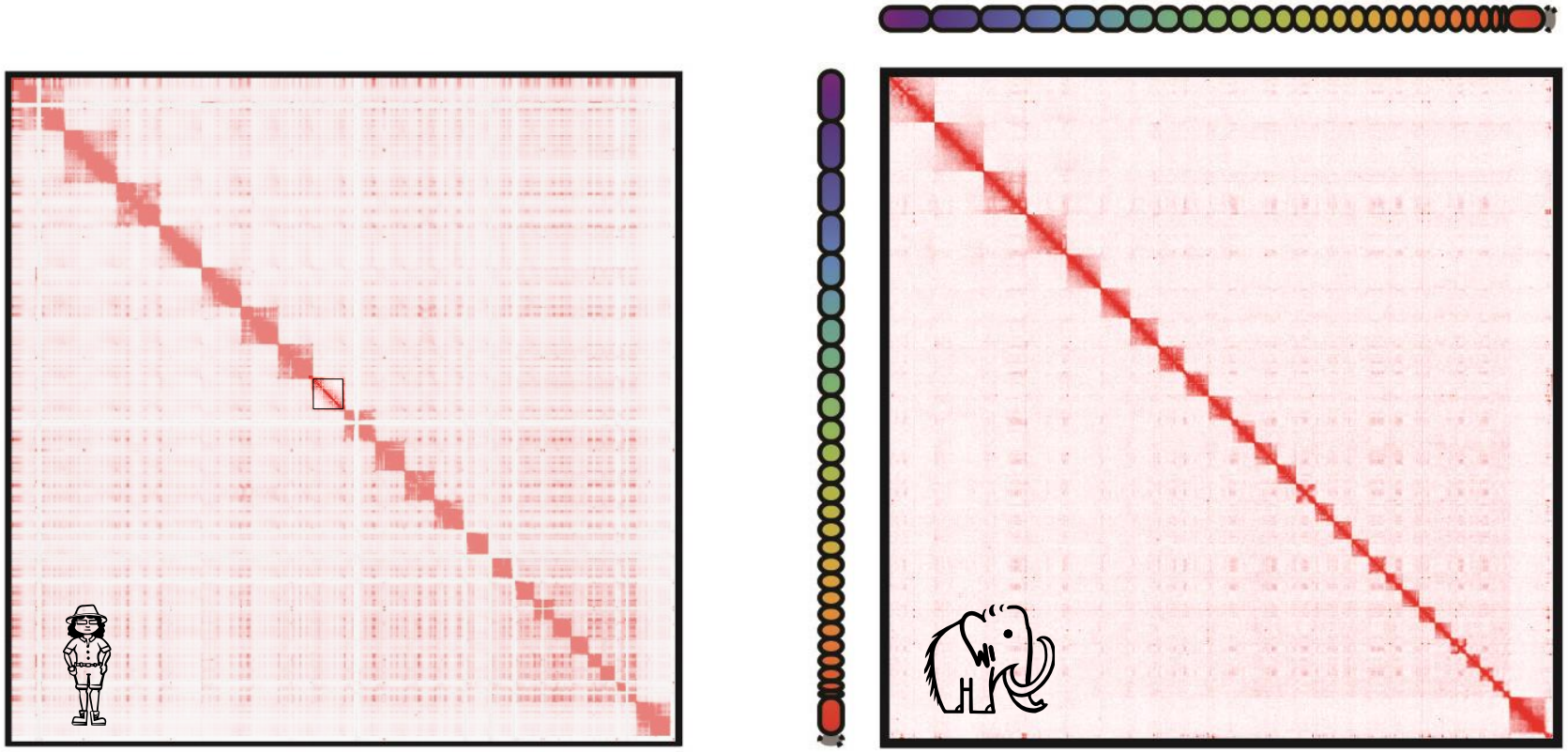


WHAT ABOUT COMPARTMENTS?





DO COMPARTMENTS PERSIST?



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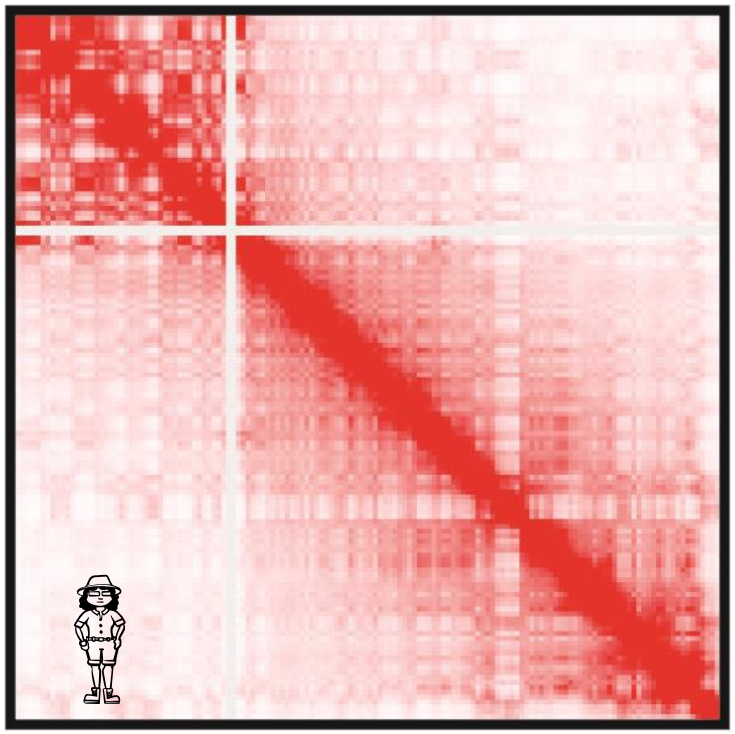
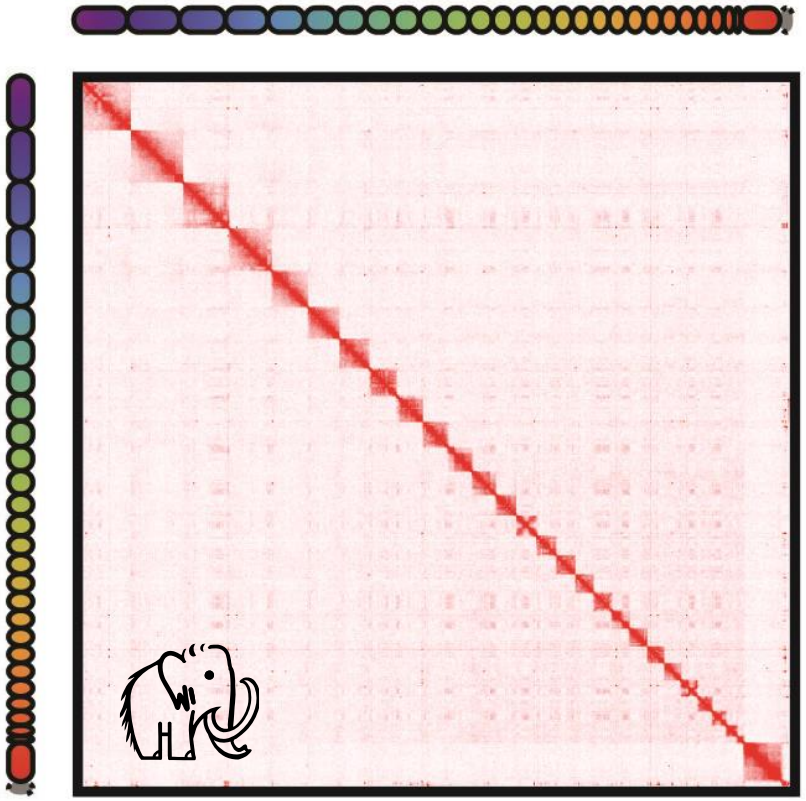
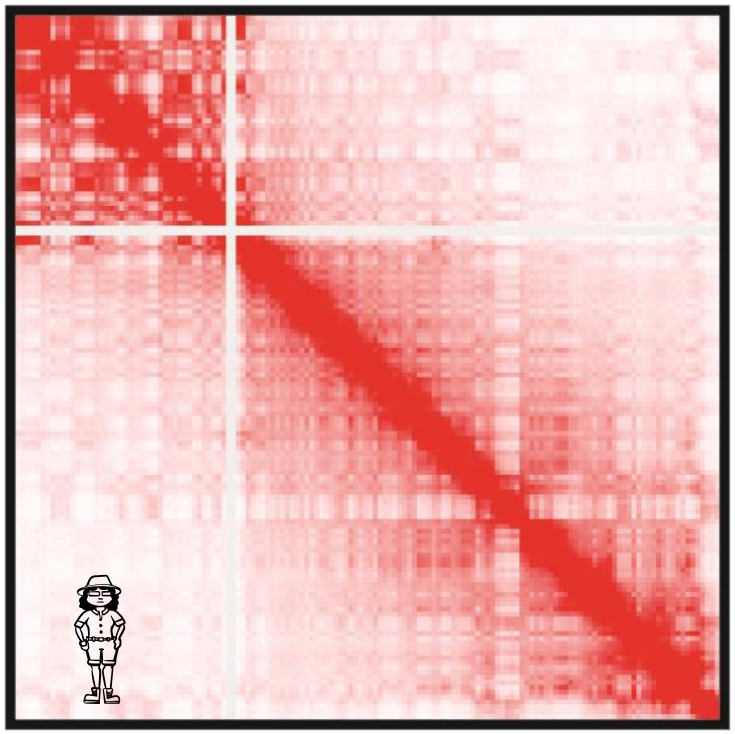
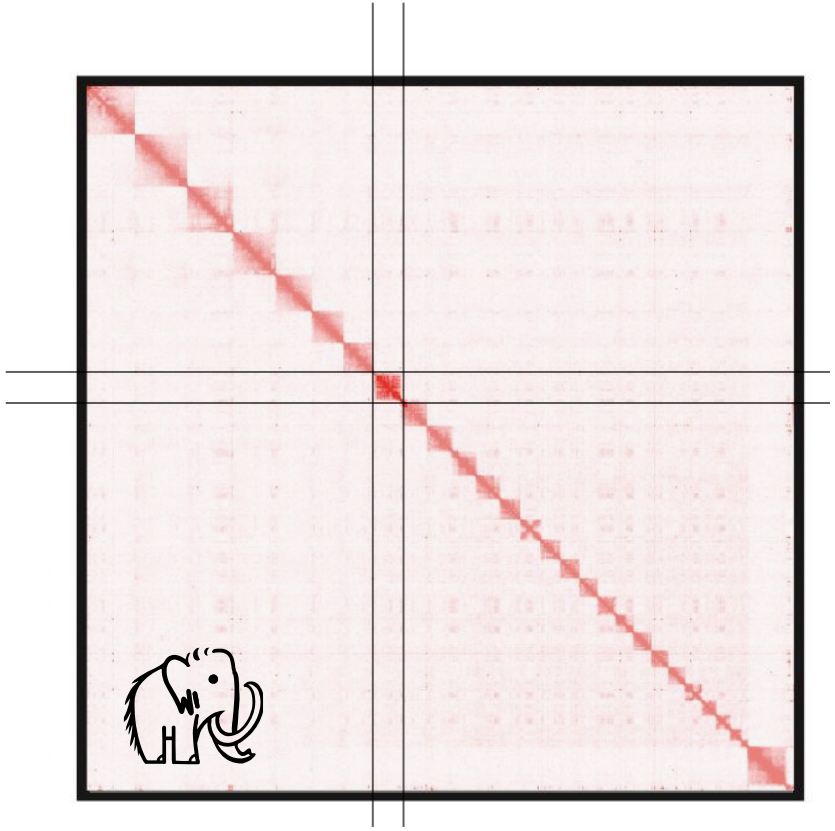


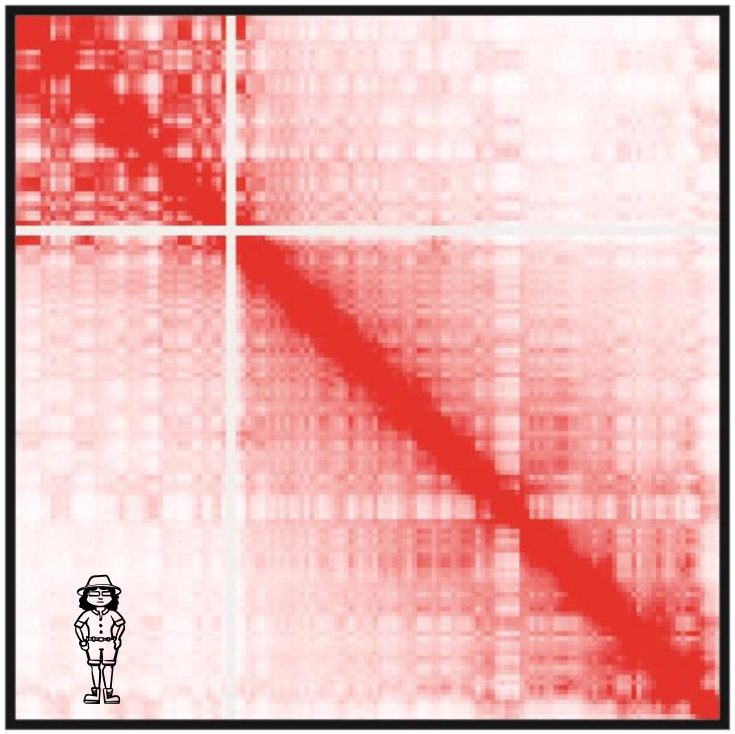


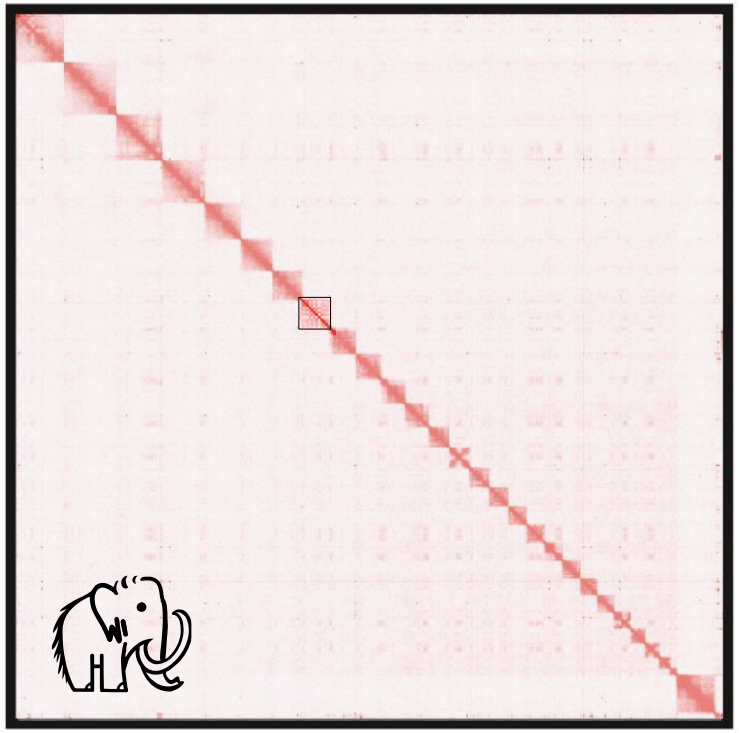
DO COMPARTMENTS PERSIST?





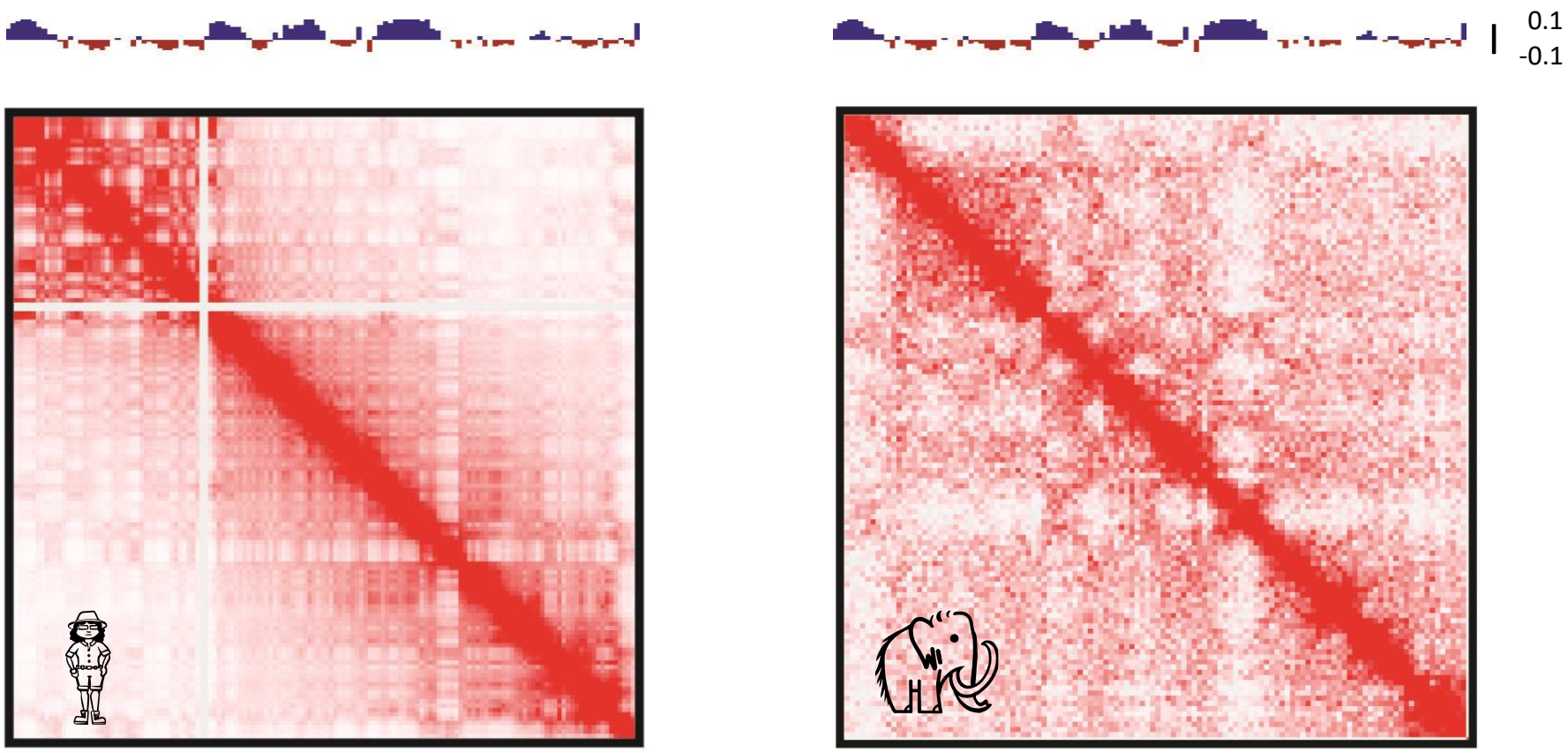
DO COMPARTMENTS PERSIST?



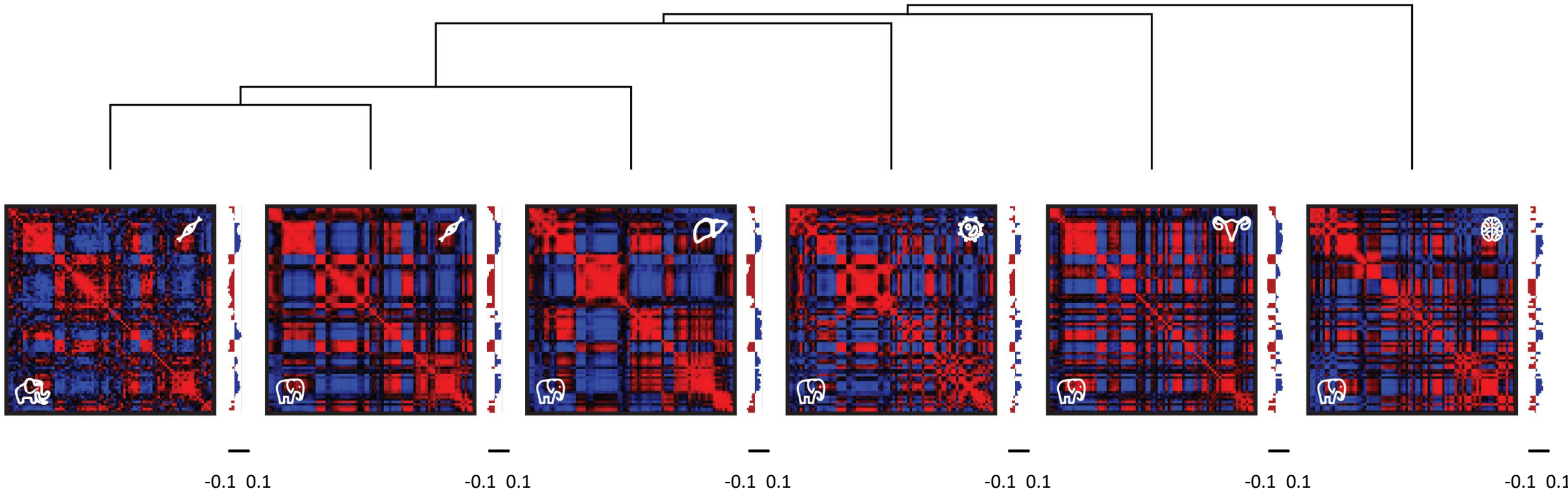


COMPARTMENTS PERSIST!

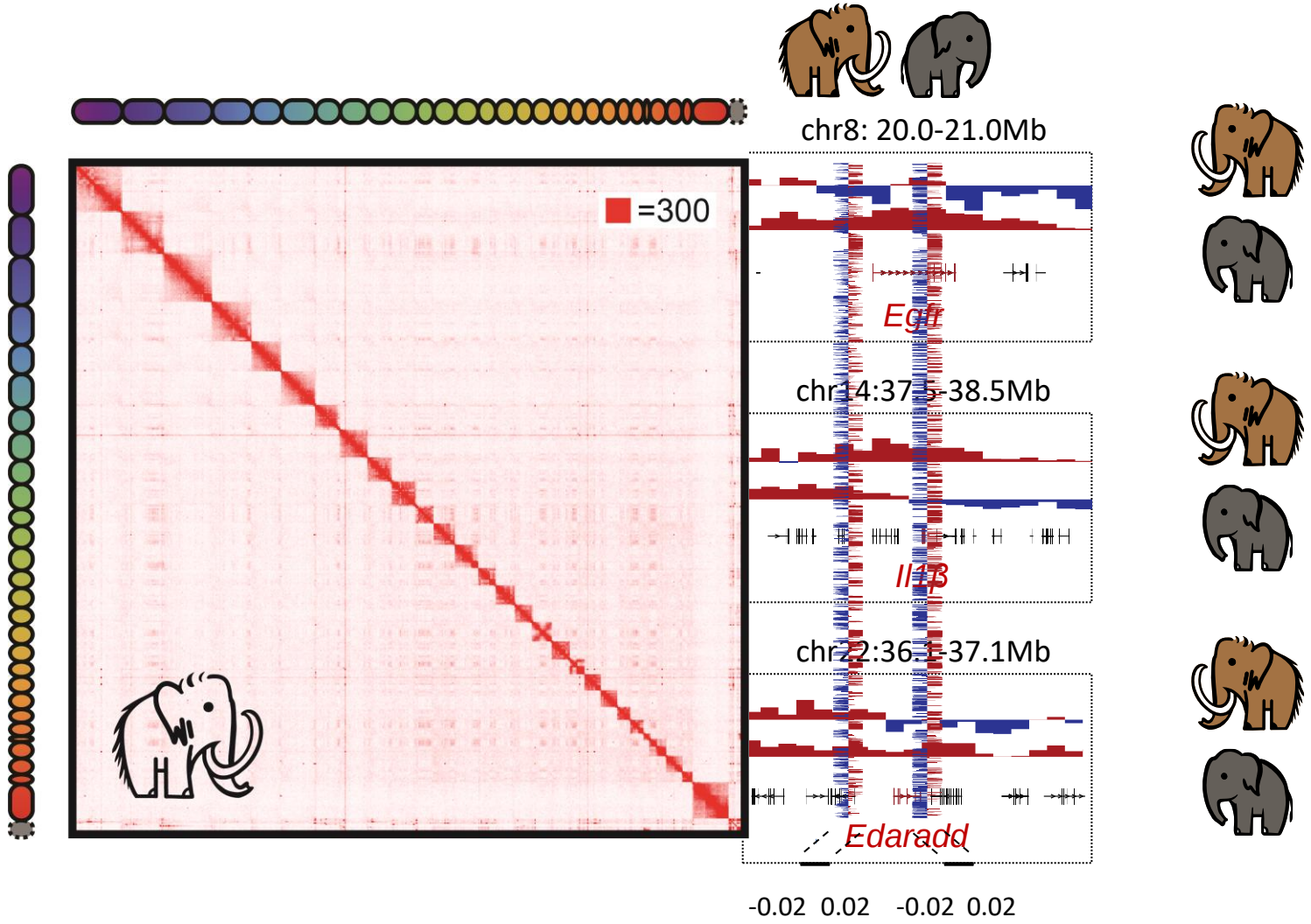
Active
Inactive



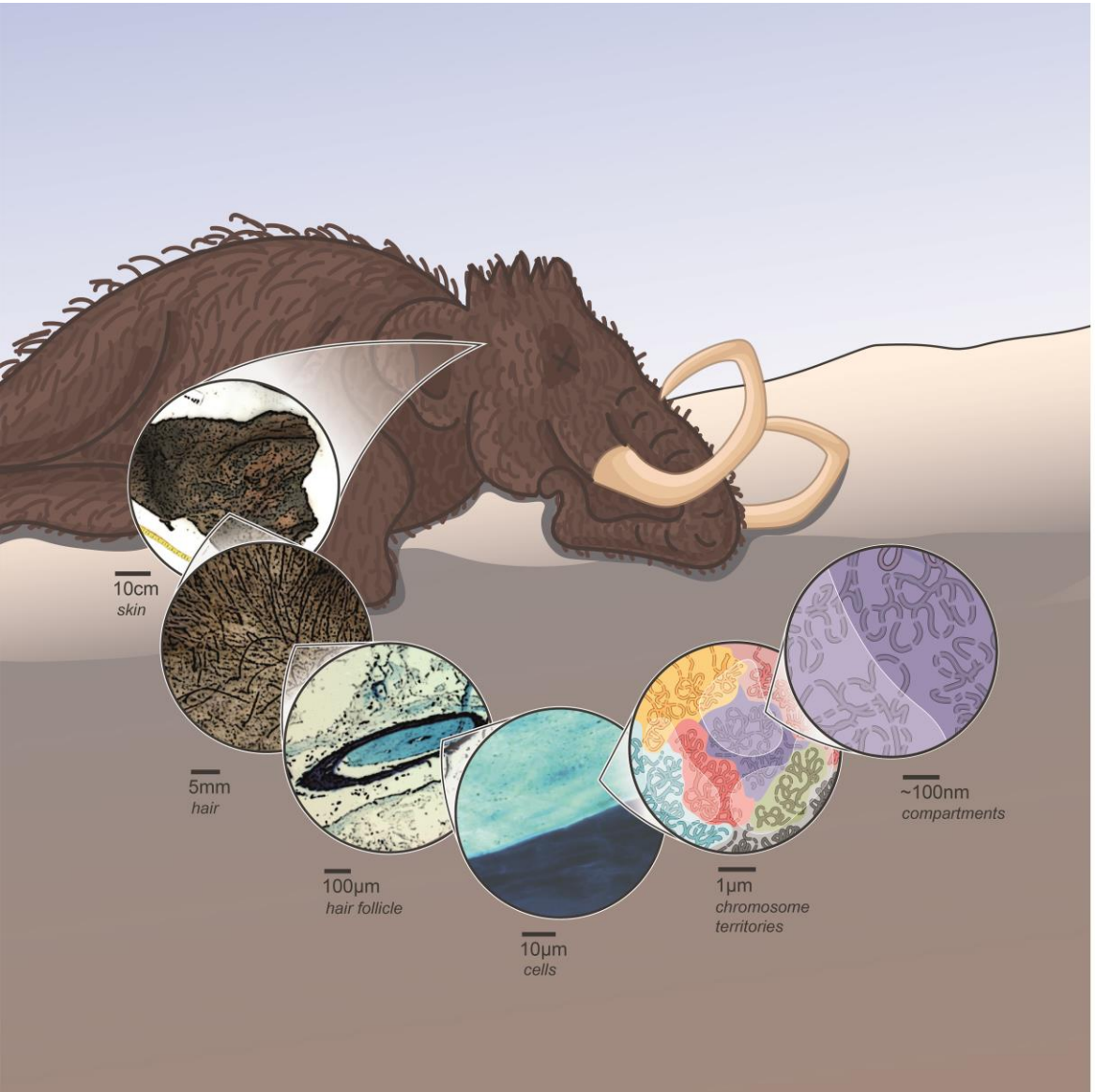
THE FIDELITY OF PRESERVATION IS HIGH ENOUGH TO PICK UP CELL-SPECIFIC PATTERNS…

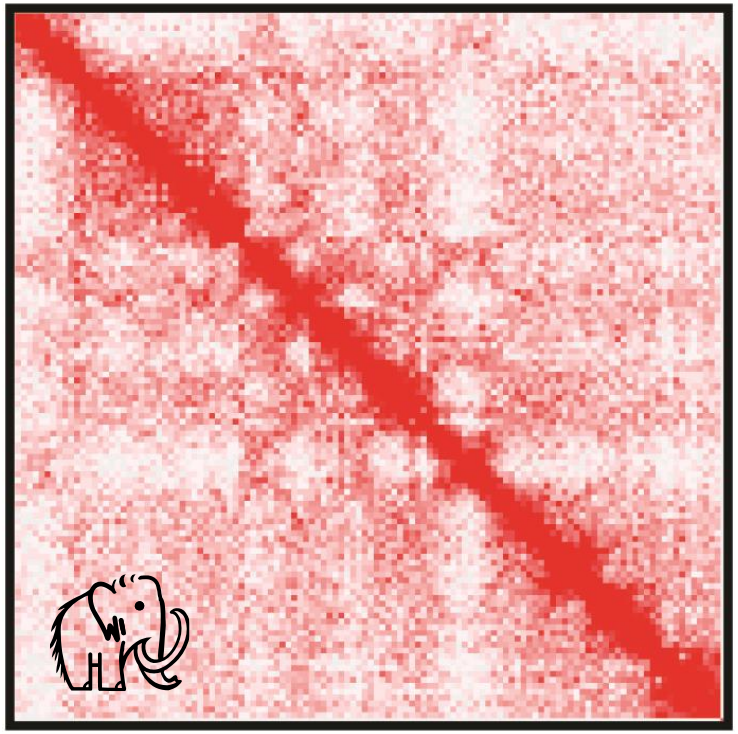


…AND WE CAN USE THE SIGNAL TO FIGURE OUT WHICH GENES WERE ‘ON’ IN THE SKIN 52,000 YEARS AGO

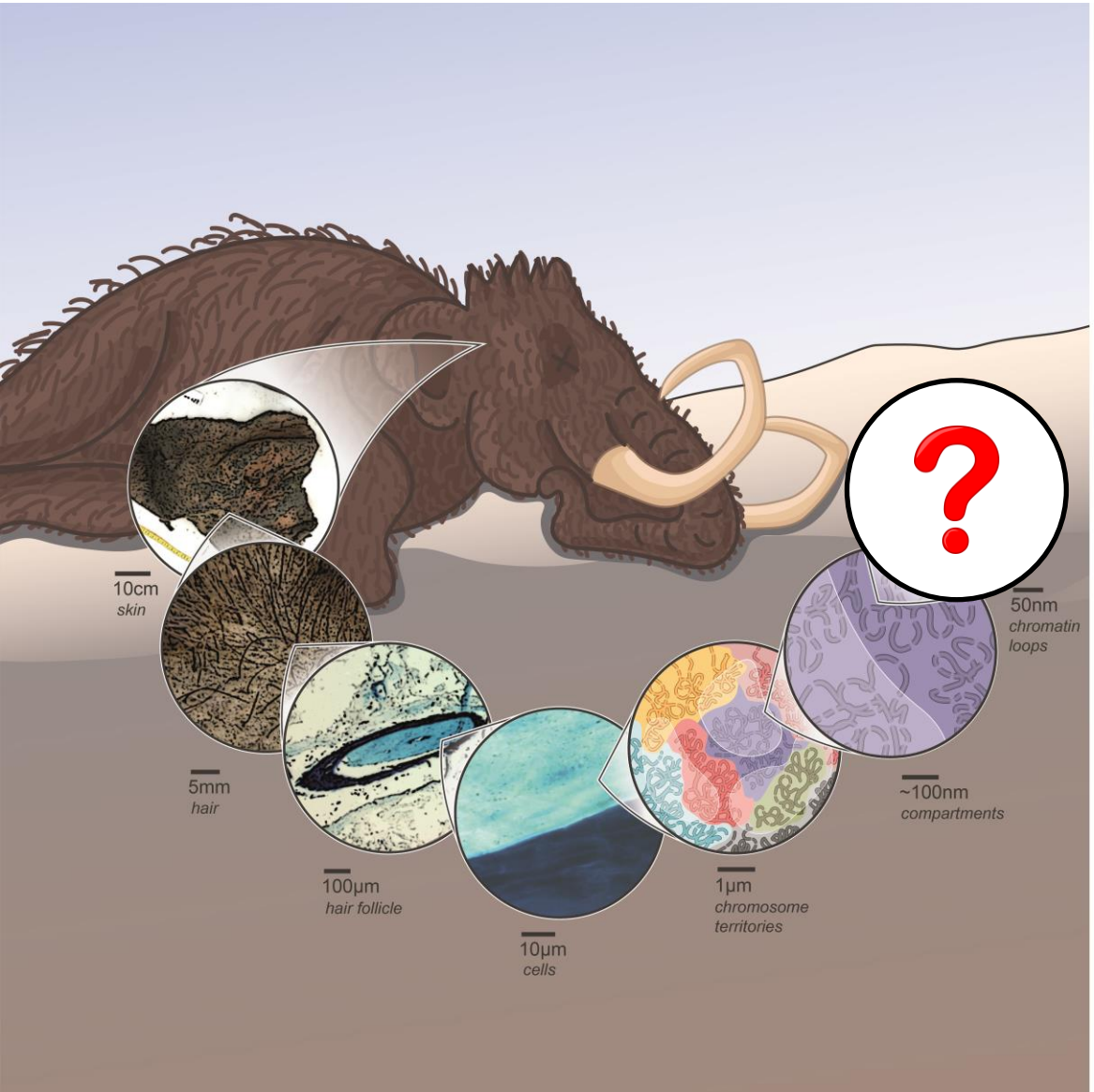


YES, WE CAN SEE COMPARTMENTS!

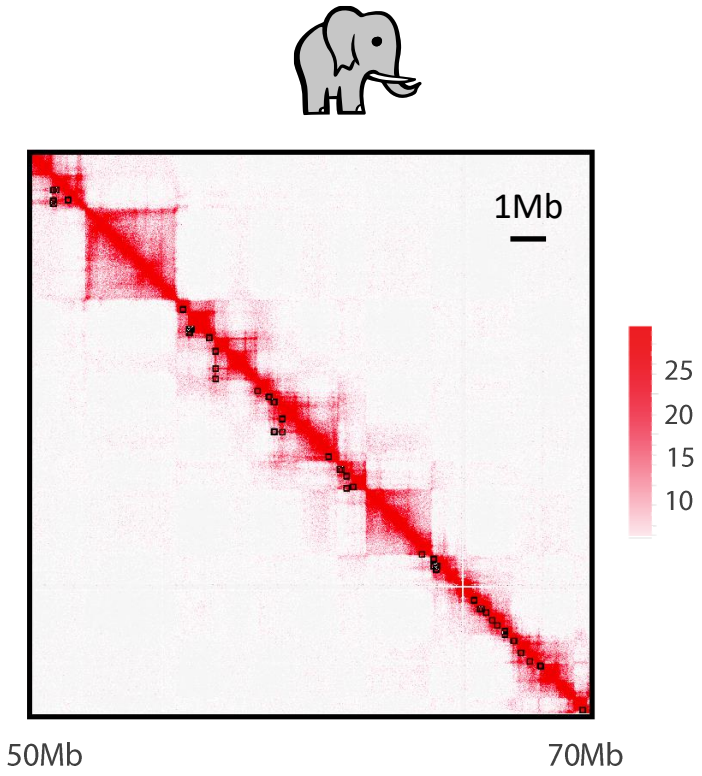


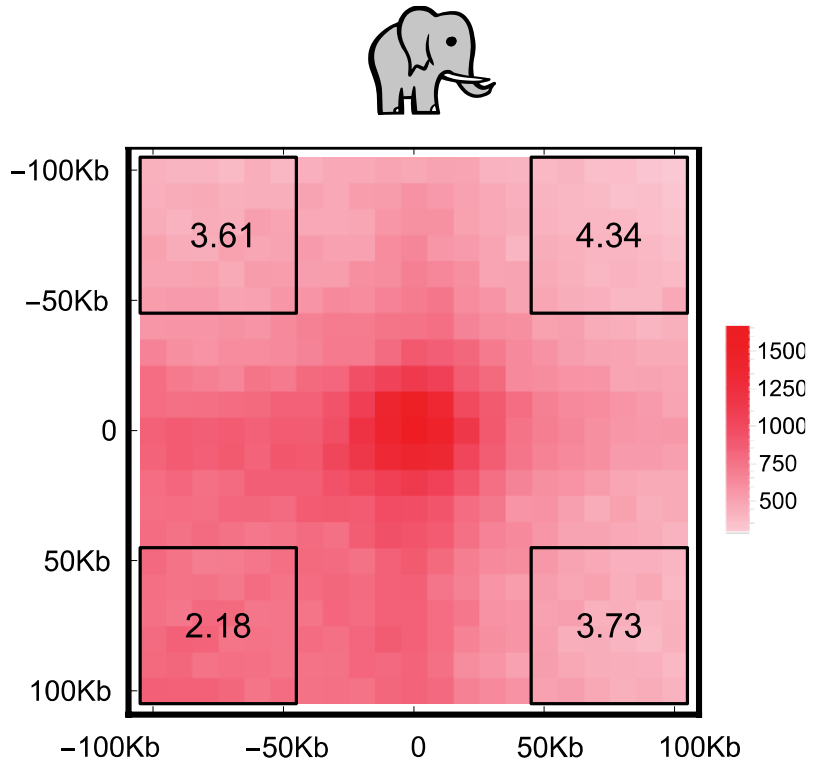


CAN WE ZOOM IN EVEN FURTHER?



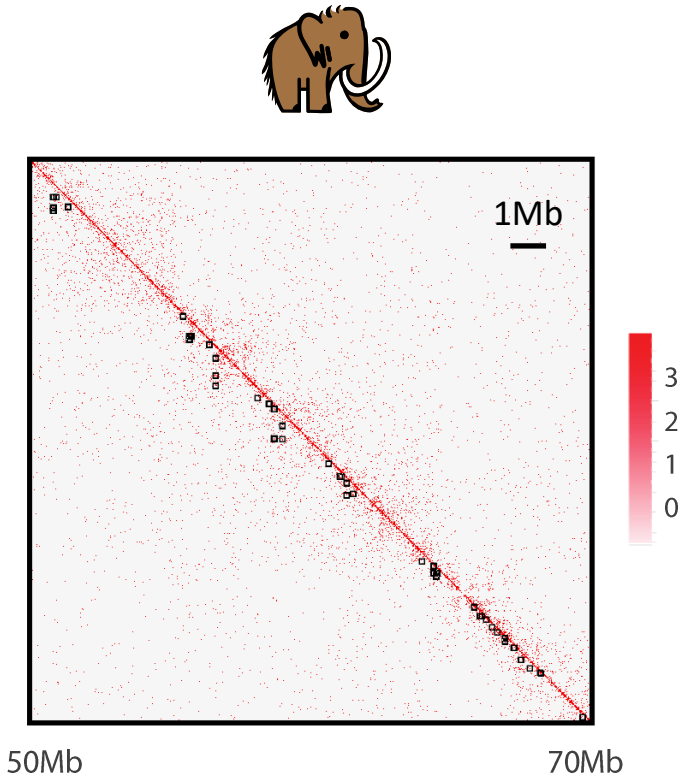
DO LOOPS PERSIST?

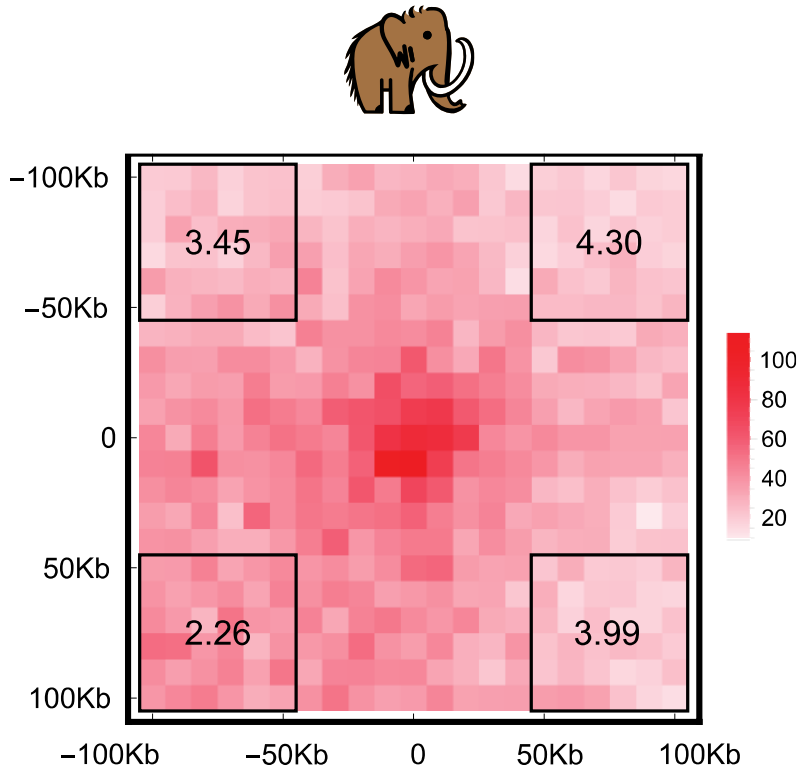




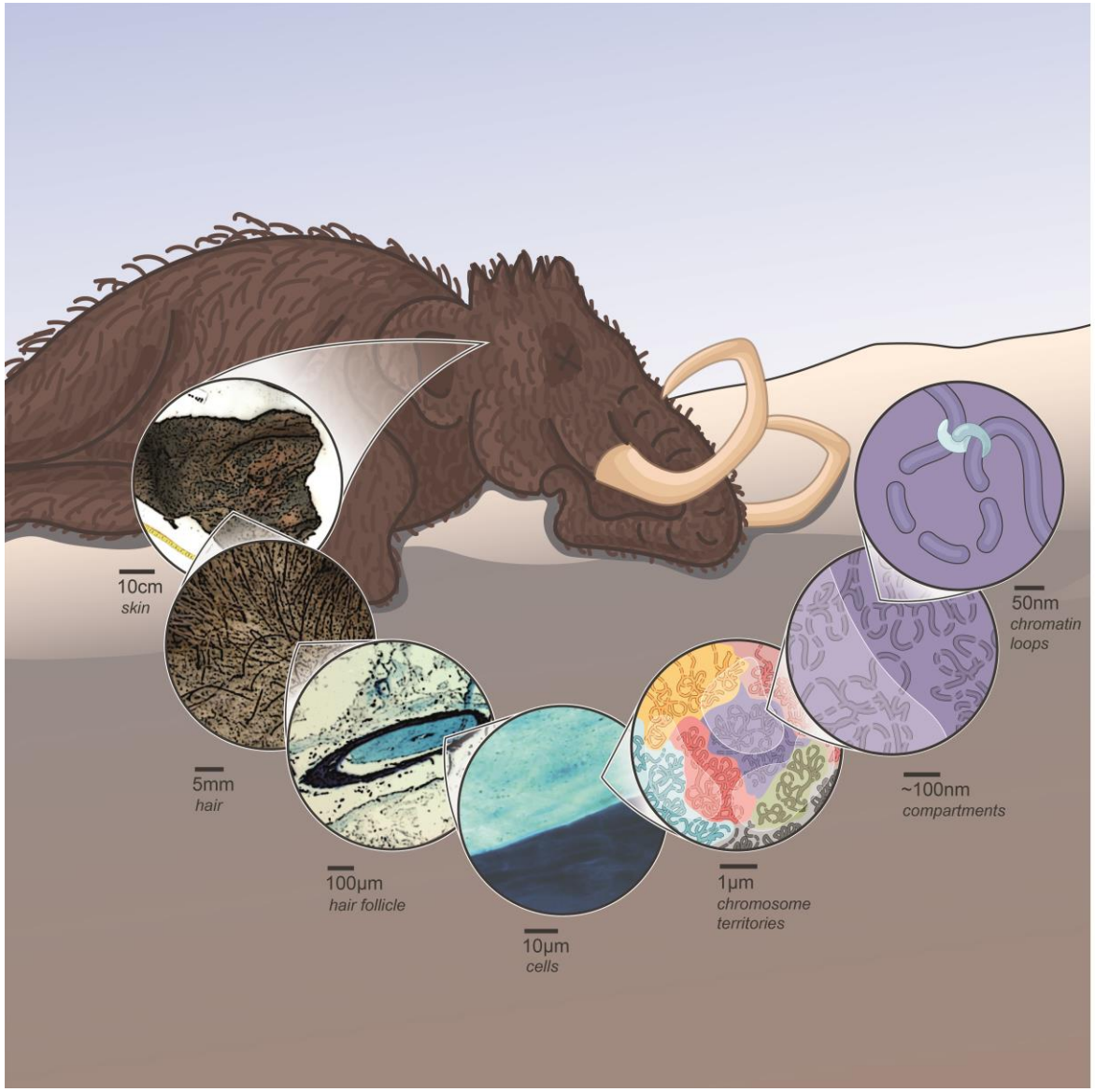
Rao, Huntley et al., Cell 2014: Aggregate Peak Analysis (APA)

LOOPS PERSIST FOR 52,000 YEARS!





WE CAN SEE FOSSILS OF ANCIENT CHROMOSOMES!



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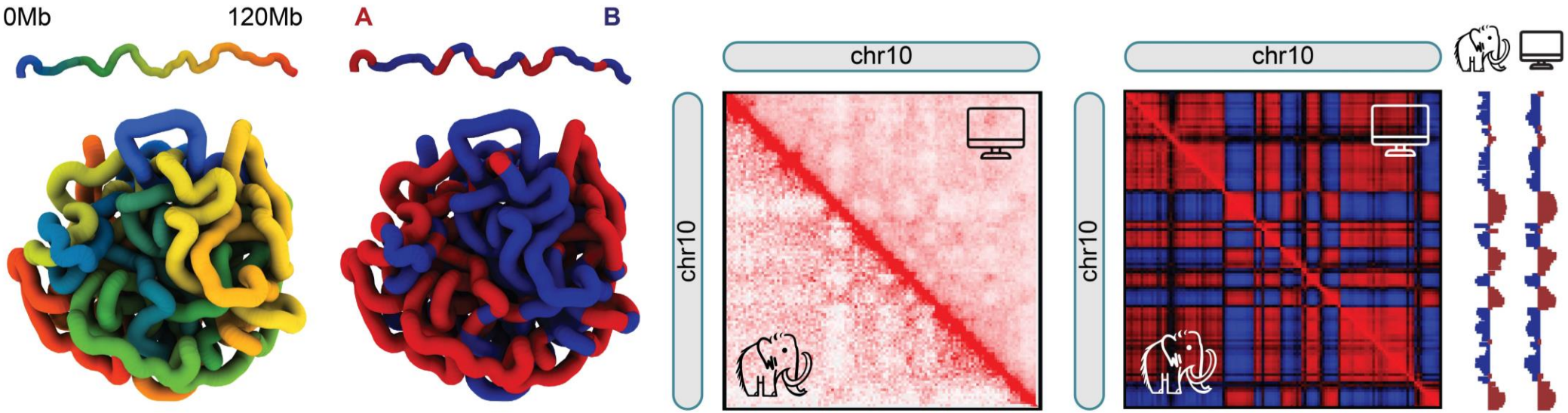


Simulations: Vinícius Contessoto, Antonio Oliveira Jr., José Onuchic [CTBP]

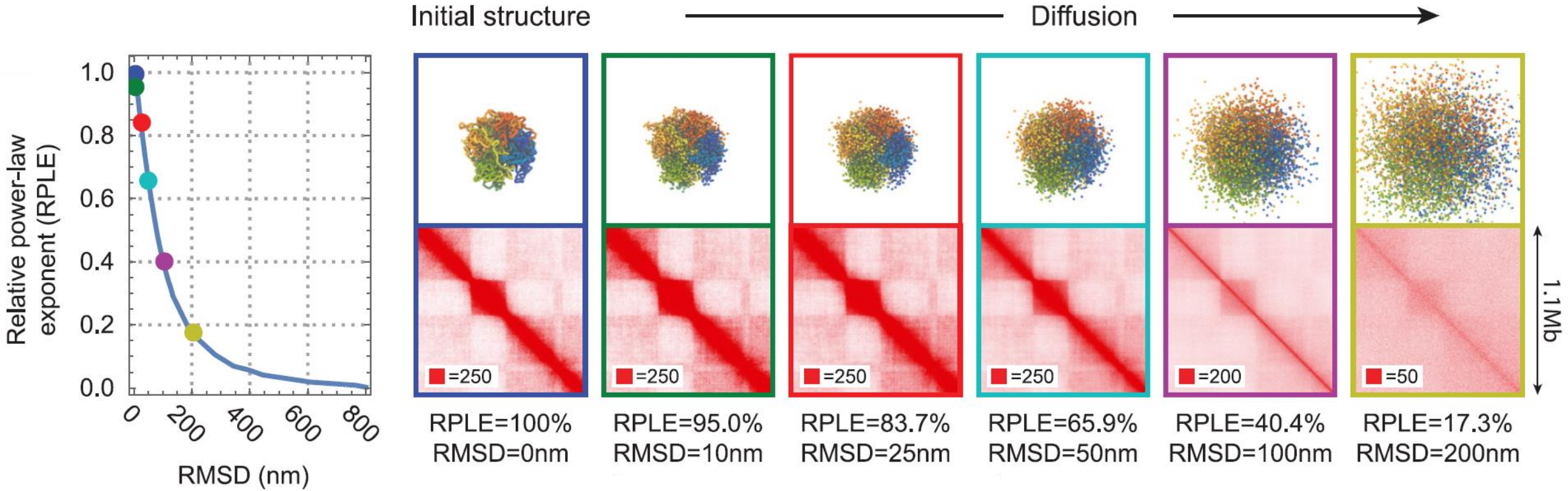
ANCIENT DNA FRAGMENTS IN OUR SAMPLES APPEAR NOT TO HAVE DIFFUSED MUCH

SURVIVAL OF ARCHITECTURAL FEATURES RULES OUT RMSD>50NM

Diffusivity and viscosity estimates for woolly mammoth chromatin based on the preservation of various architectural features

Feature	RMSD (nm)	Diffusivity (m^2/s)	Viscosity ($kg/m \cdot s$)
Nucleus	≤ 5000	$\leq 2.5 \times 10^{-24}$	$\geq 4.6 \times 10^{9}$
Chromosome territories	≤ 2000	$\leq 4.1 \times 10^{-25}$	$\geq 2.9 \times 10^{10}$
Barr body	≤ 1000	$\leq 1.0 \times 10^{-25}$	$\geq 1.2 \times 10^{11}$
Point-to-point loops	≤ 50	$\leq 2.5 \times 10^{-28}$	$\geq 4.6 \times 10^{13}$

SO DOES THE MODELING



Simulations: Bernardo Zubillaga Herrera, Michele Di Pierro [CTBP]

TAKEN TOGETHER WE ARE CONFIDENT THAT RMSD IS <50NM

Diffusivity and viscosity estimates for woolly mammoth chromatin based on the preservation of various architectural features

Feature	RMSD (nm)	Diffusivity (m^2/s)	Viscosity (kg/m·s)
Nucleus	$\leq$5000	$\leq 2.5 \times 10^{-24}$	$\geq 4.6 \times 10^{9}$
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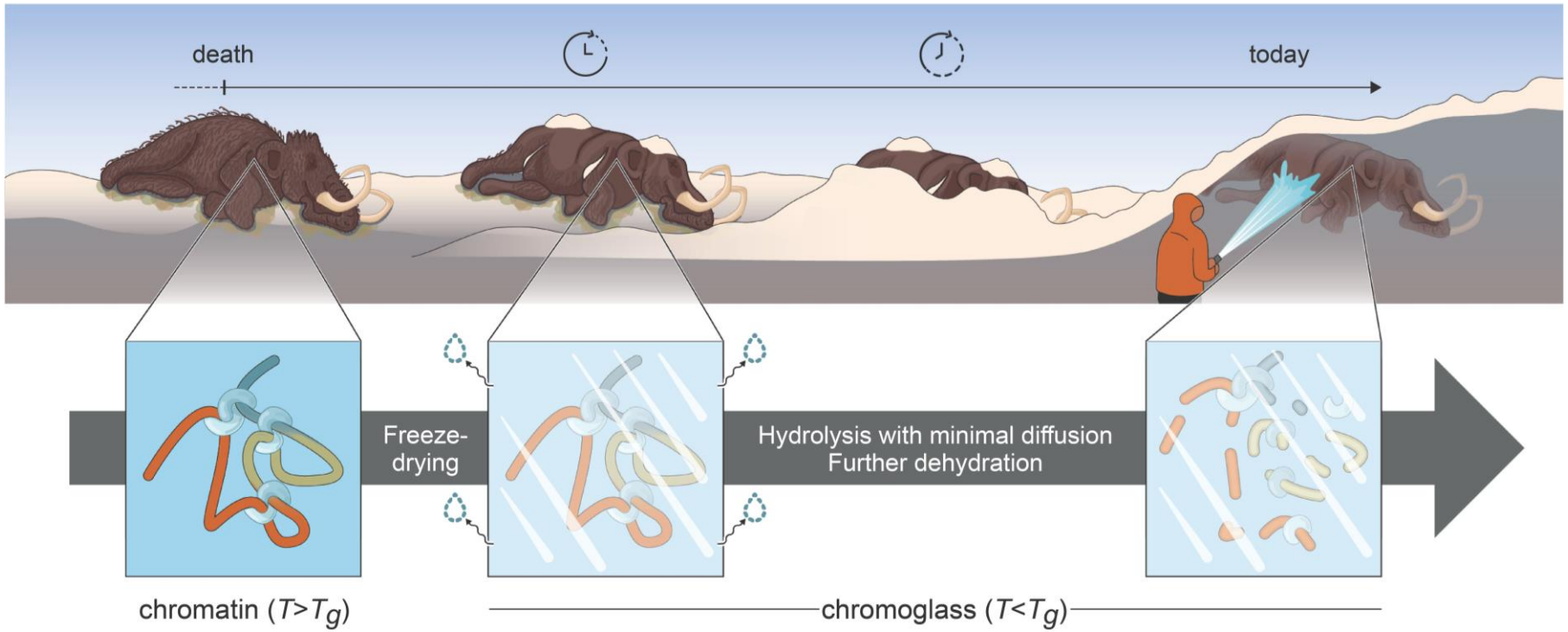
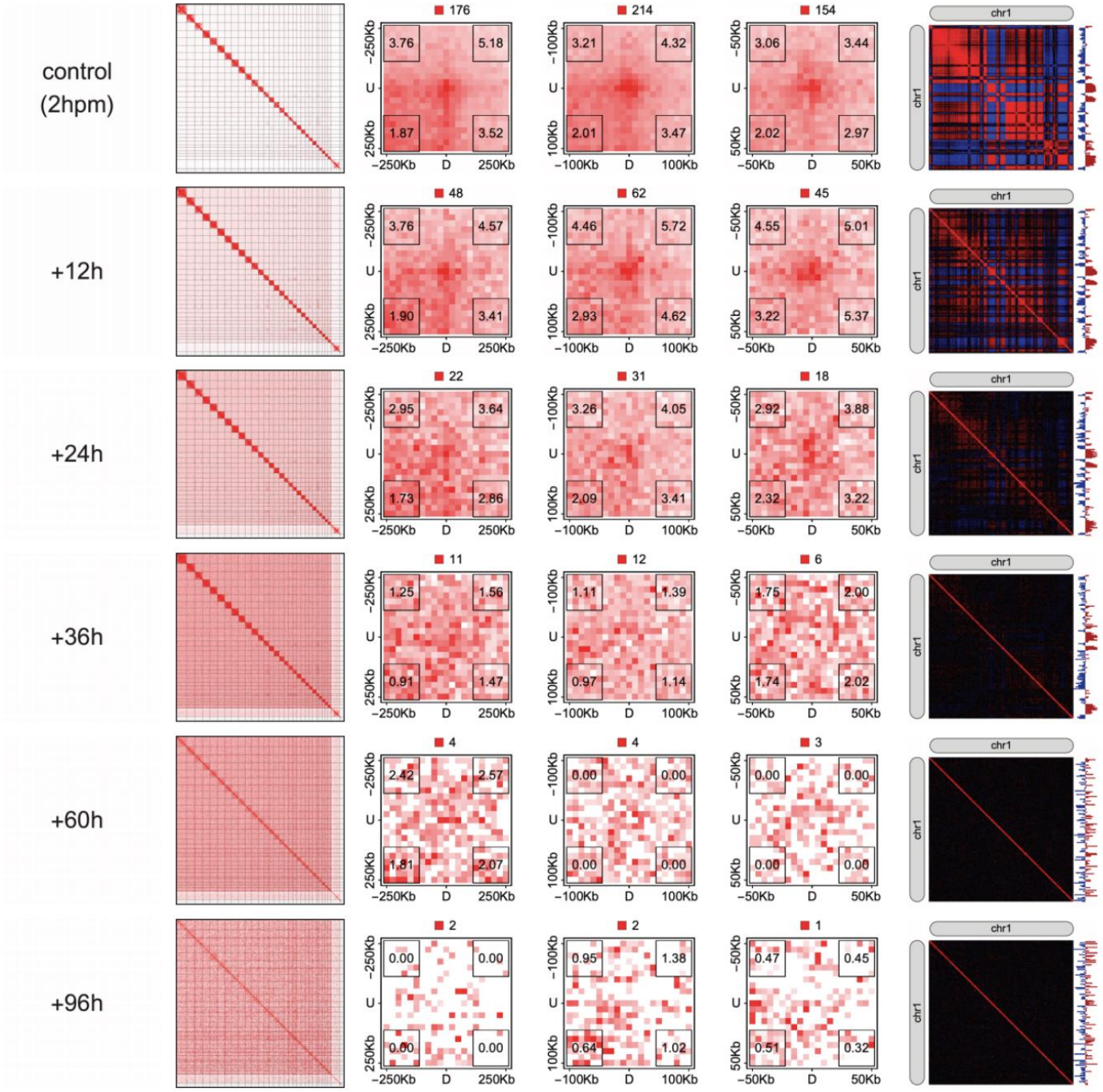
Barr body	$\leq$1000	$\leq 1.0 \times 10^{-25}$	$\geq 1.2 \times 10^{11}$
Point-to-point loops	$\leq$50	$\leq 2.5 \times 10^{-28}$	$\geq 4.6 \times 10^{13}$
Contact probability …			
… using simple diffusion model	$\leq$50	$\leq 2.5 \times 10^{-28}$	$\geq 4.6 \times 10^{13}$
… using excluded volume model	$\leq$20	$\leq 4.1 \times 10^{-29}$	$\geq 2.9 \times 10^{14}$

RMSD IS CONSISTENT WITH GLASSY STATE

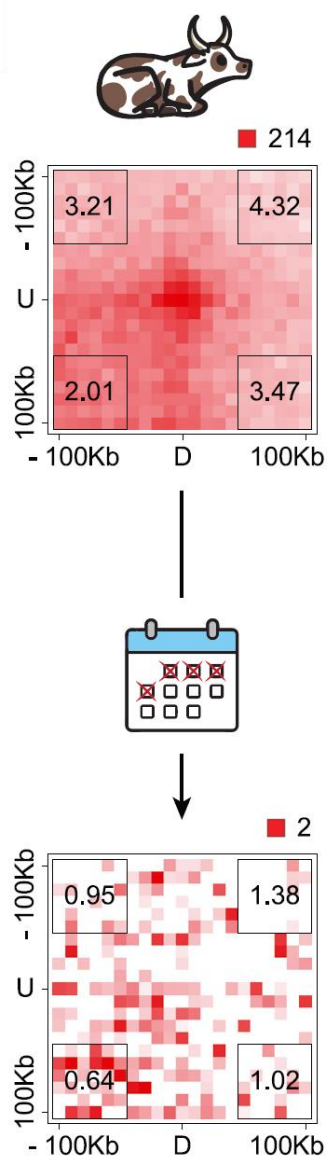


WE PERFORMED SOME EXPERIMENTS IN MODERN SAMPLES TO TEST THE HYPOTHESIS

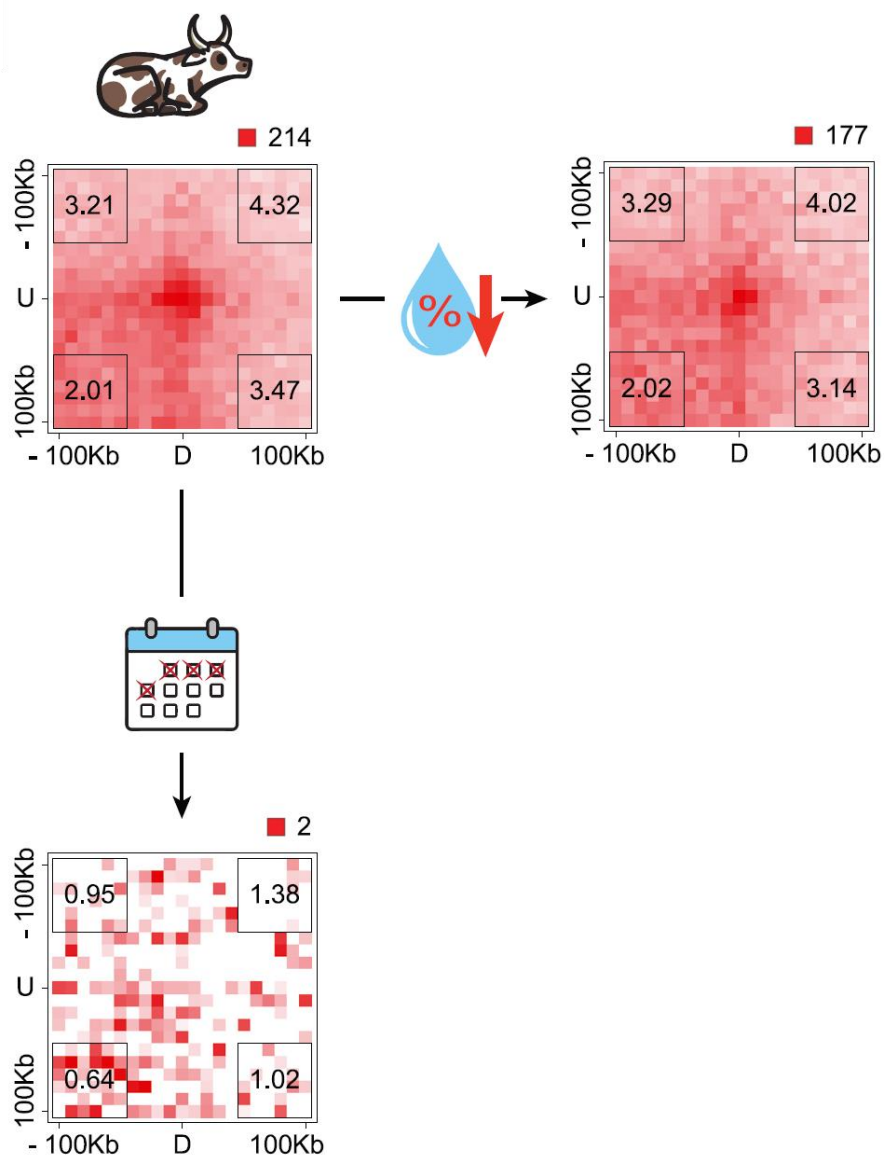
W/O INTERVENTION ARCHITECTURE DEGRADES IN 4 DAYS



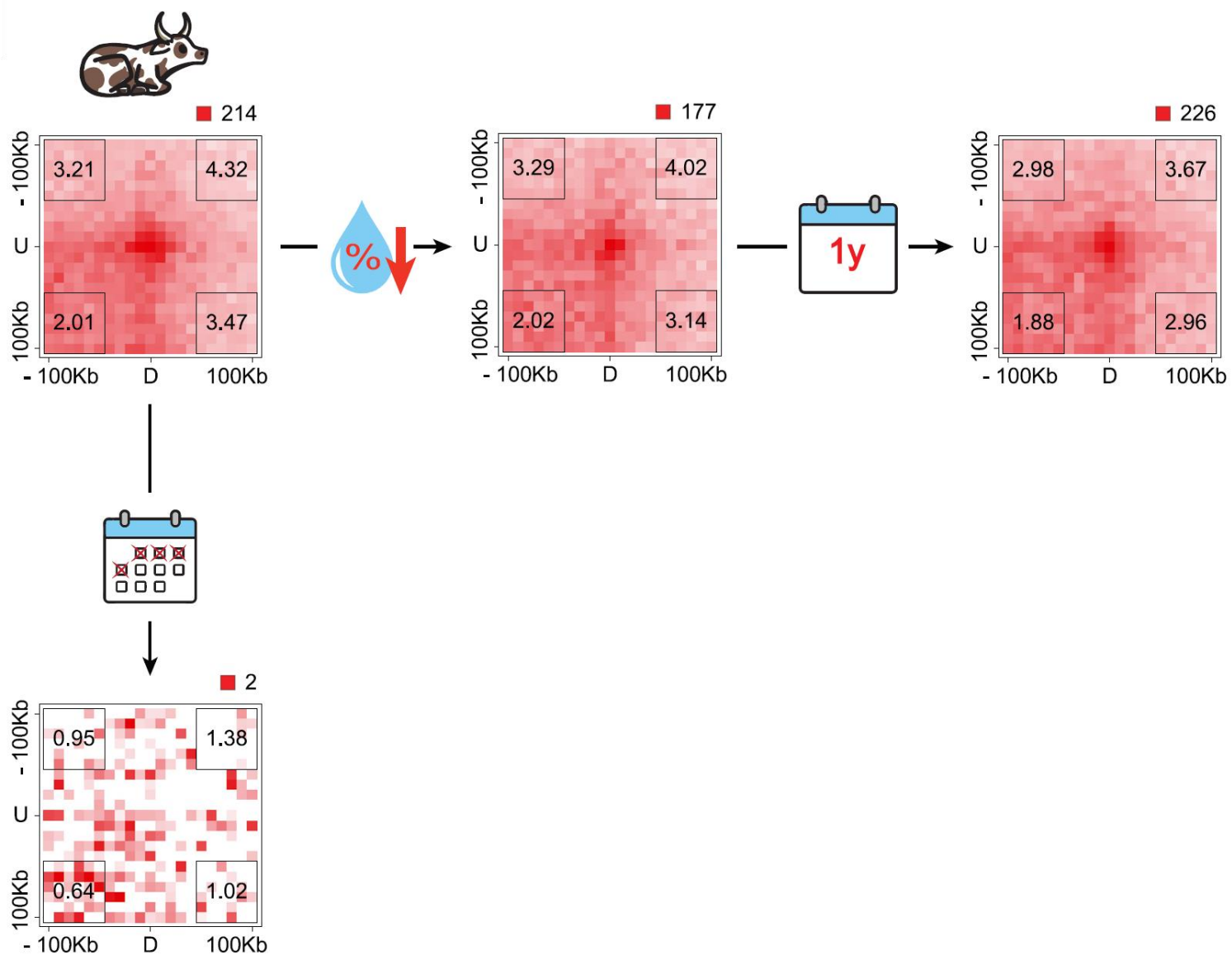
W/O INTERVENTION ARCHITECTURE DEGRADES IN 4 DAYS



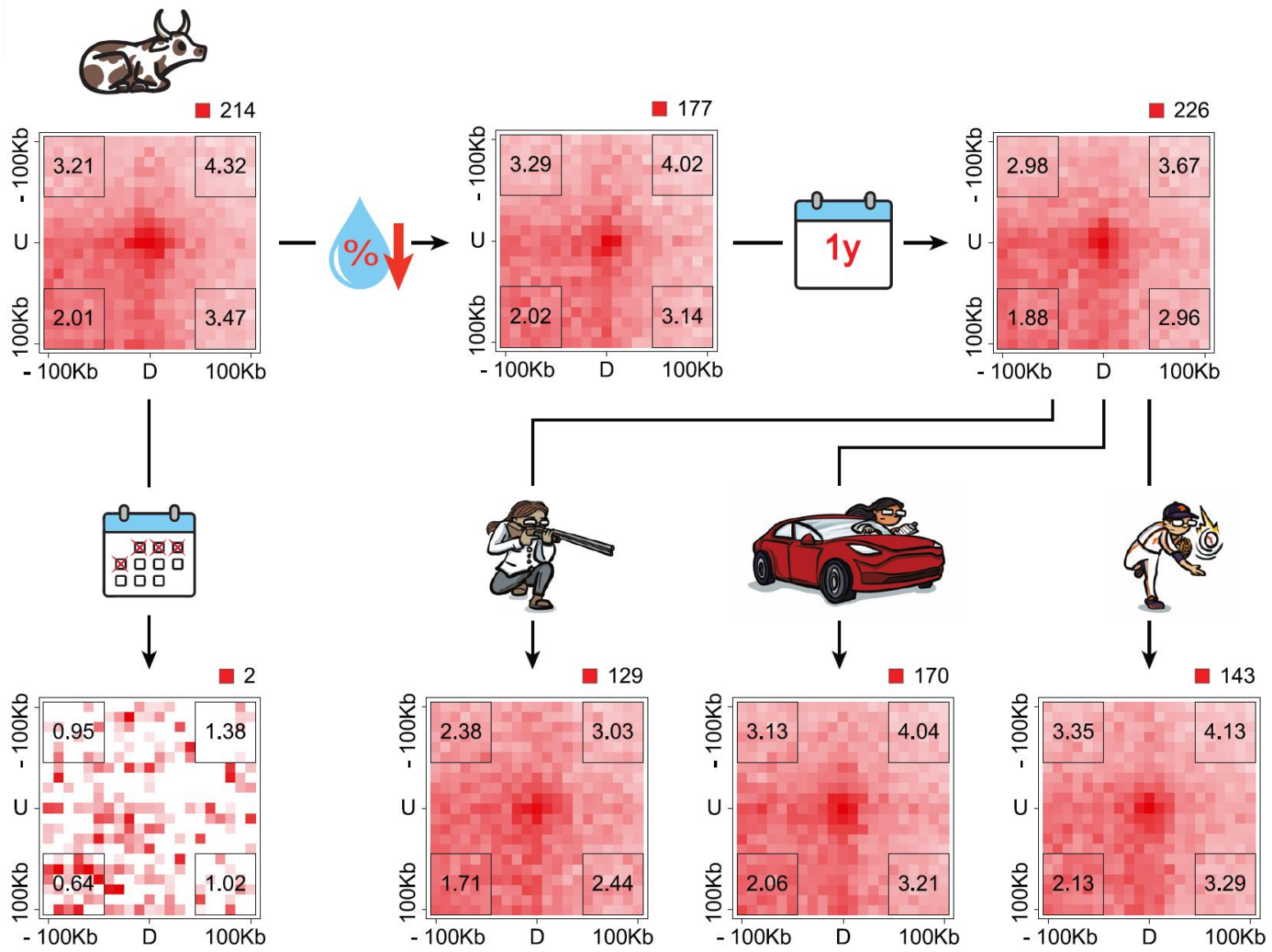
DEHYDRATION PRESERVES 3D ARCHITECTURE ...



...EVEN AFTER A YEAR AT ROOM TEMPERATURE



DEHYDRATED CHROMATIN IS VERY ROBUST



THREE-DIMENSIONAL GENOME ARCHITECTURE PERSISTS IN A 52,000-YEAR-OLD WOOLLY MAMMOTH SKIN SAMPLE

Marcela Sandoval-Velasco[#], Olga Dudchenko[#,†], Juan Antonio Rodríguez[#], Cynthia Pérez Estrada[#], Marianne Dehasque, Claudia Fontsere, Sarah S.T. Mak, Ruqayya Khan, Vinícius G. Contessoto, Antonio B. Oliveira Junior, Achyuth Kalluchi, Bernardo J. Zubillaga Herrera, Jiyun Jeong, Renata P. Roy, Ishawnia Christopher, David Weisz, Arina D. Omer, Sanjit S. Batra, Muhammad S. Shamim, Neva C. Durand, Brendan O'Connell, Alfred L. Roca, Maksim V. Plikus, Mariya A. Kusliy, Svetlana A. Romanenko, Natalya A. Lemskaya, Natalya A. Serdyukova, Svetlana A. Modina, Polina L. Perelman, Elena A. Kizilova, Sergei I. Baiborodin, Nikolai B. Rubtsov, Gur Machol, Krisha Rath, Ragini Mahajan, Parwinder Kaur, Andreas Gnirke, Isabel Garcia-Treviño, Rob Coke, Joseph P. Flanagan, Kelcie Pletch, Aurora Ruiz-Herrera, Valerii Plotnikov, Innokentiy S. Pavlov, Naryya I. Pavlova, Albert V. Protopopov, Michele Di Pierro, Alexander S. Graphodatsky, Eric S. Lander, M. Jordan Rowley, Peter G. Wolynes, José N. Onuchic, Love Dalén, Marc A. Marti-Renom[†], M. Thomas P. Gilbert[†], Erez Lieberman Aiden[†]

Cell 2024

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RICE | CTBP
Center for Theoretical Biological Physics

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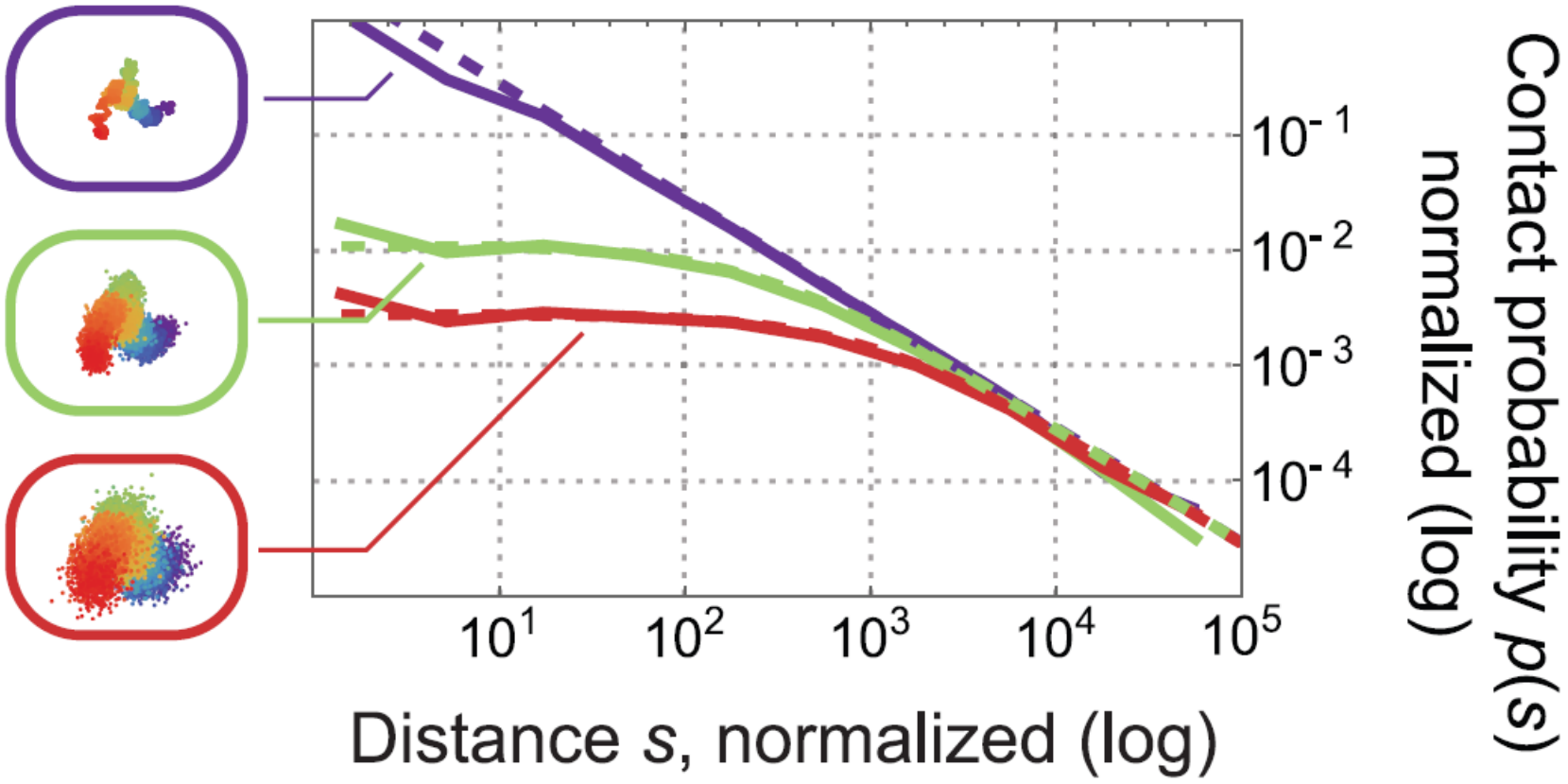
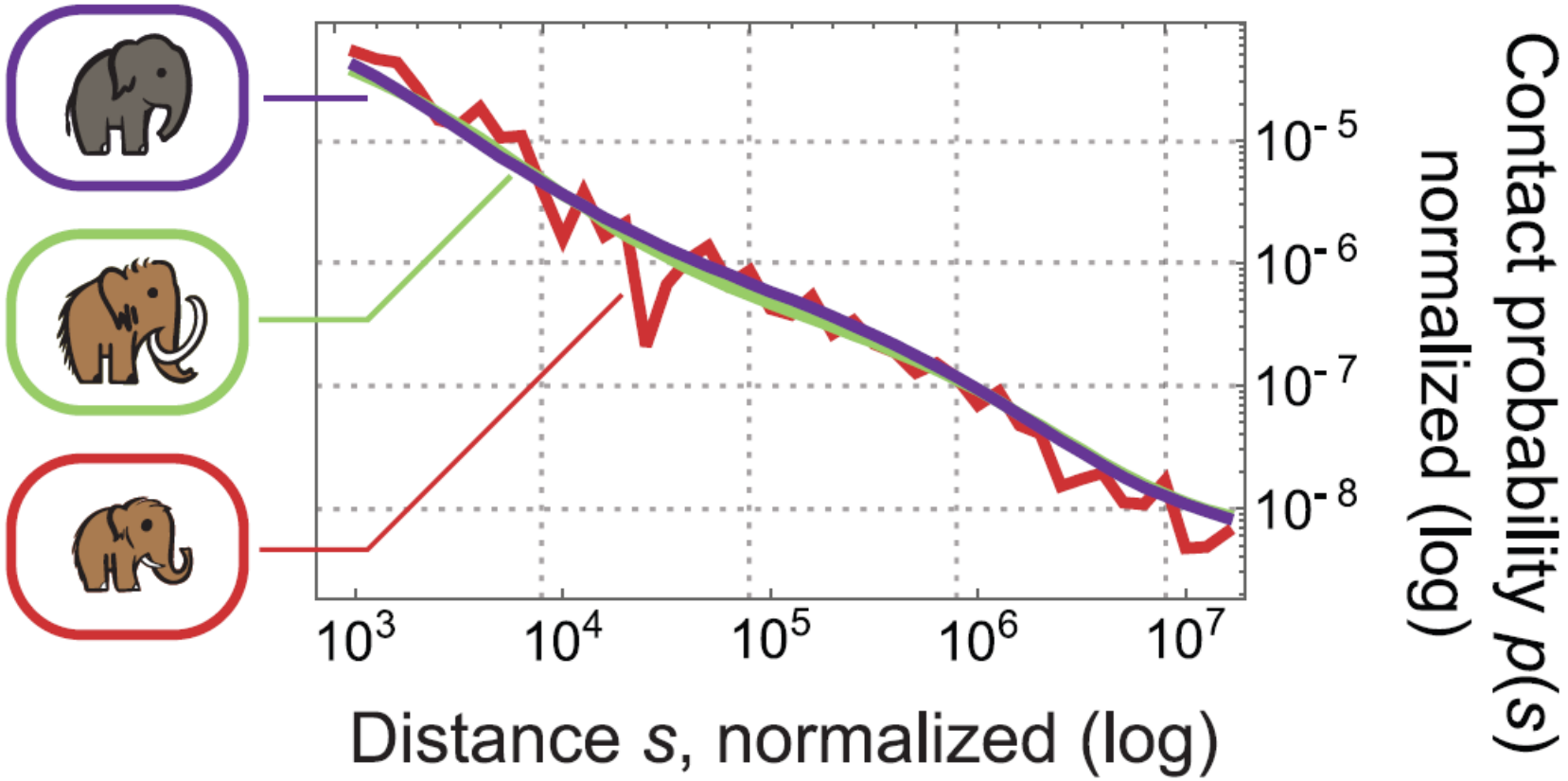
LEO Foundation
The Welch Foundation Advancing Chemistry. Improving Life.
The Robert and Janice McNair Foundation
FULBRIGHT
IBM
Pawsey supercomputing centre
AMD

AS PARTICLES DIFFUSE FINE STRUCTURE IS DISTURBED



BUT IN MAMMOTH FINE STRUCTURE IS PRESERVED AT ALL ASSAYABLE SCALES



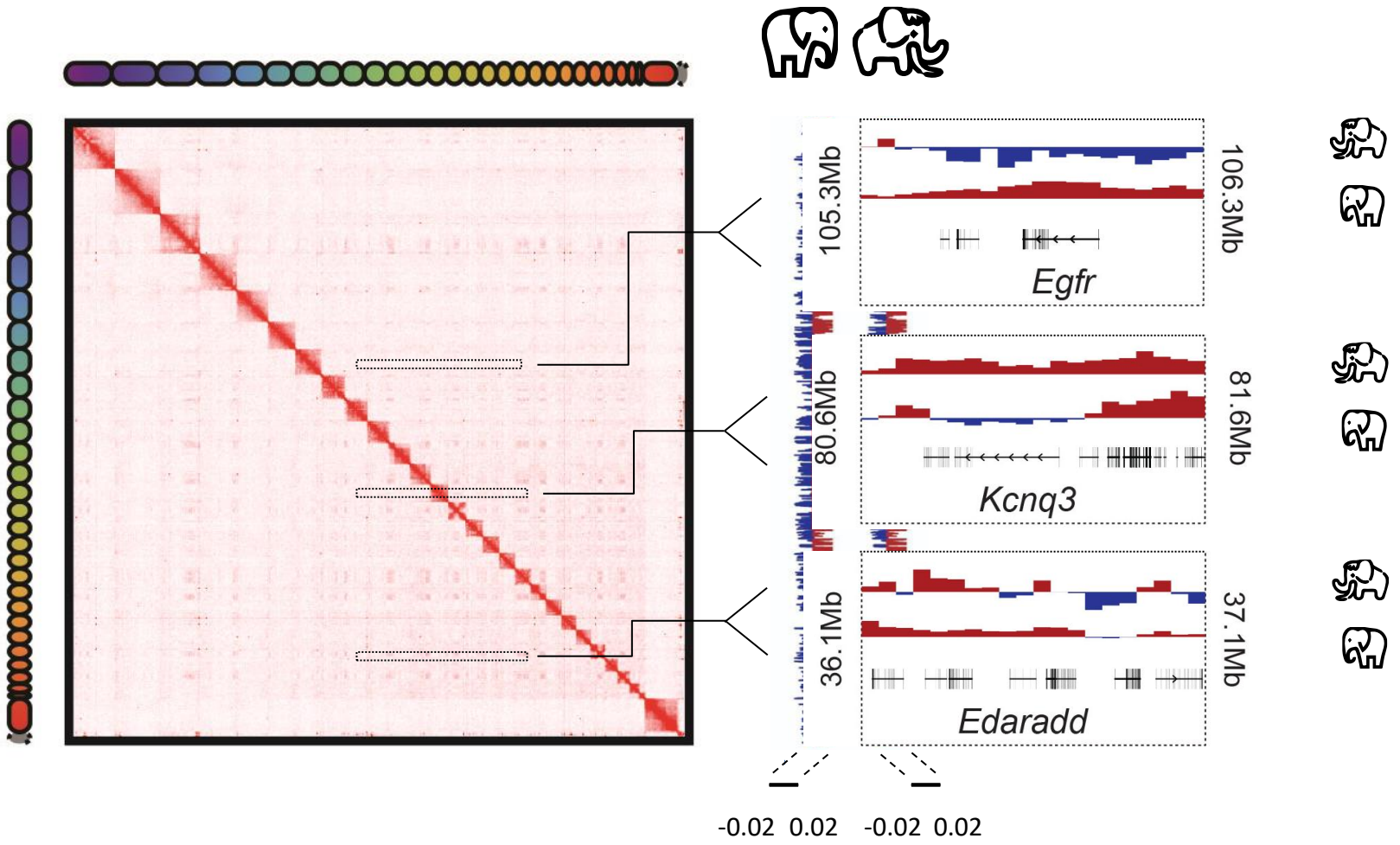
A BIT OF CONTEXT

Alan Taylor Siberian mammoth pirates, The Atlantic, 2016

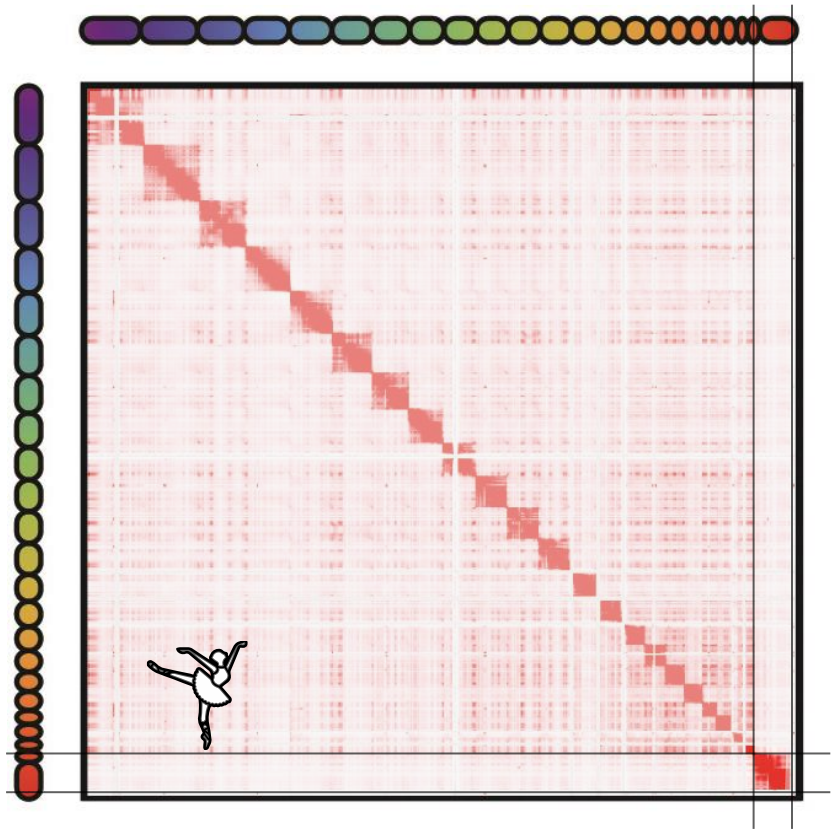
SOME GENES IN MAMMOTH ARE DIFFERENT…

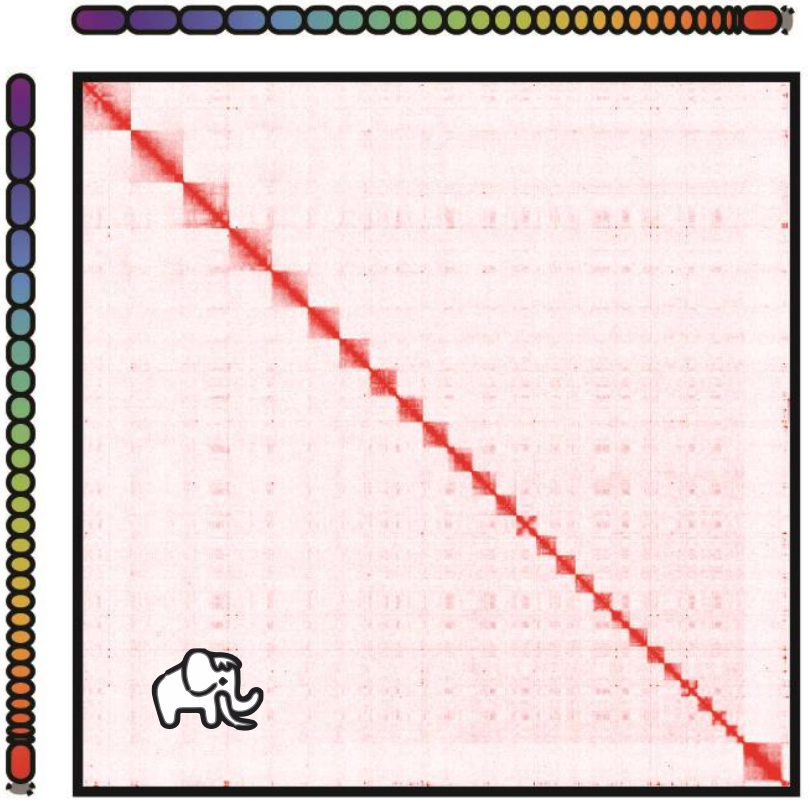
Achyuth Kalluchi

Jordan Rowley

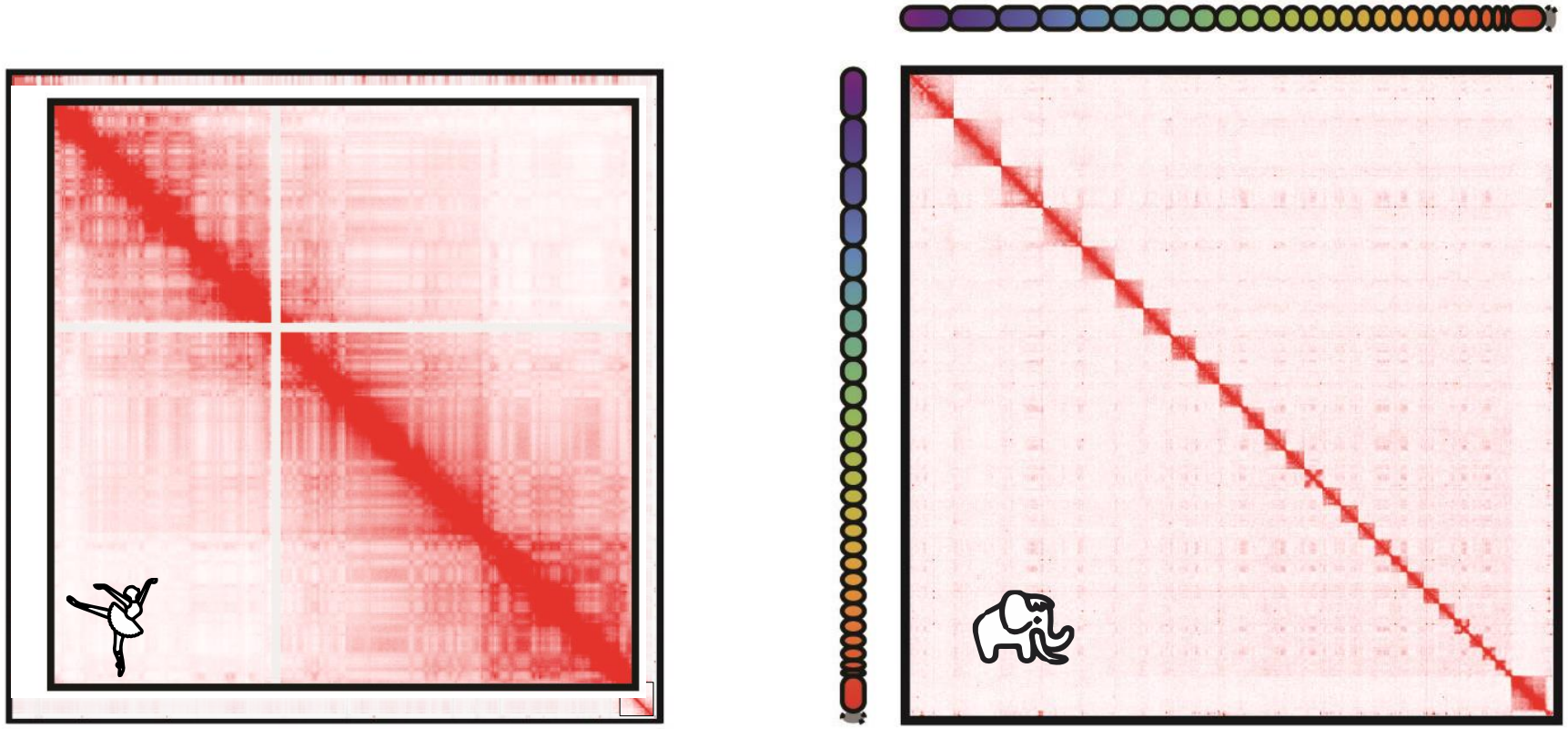


...AND SO IS CHR X





...AND SO IS CHR X



Darrow, Huntley et al., PNAS 2016

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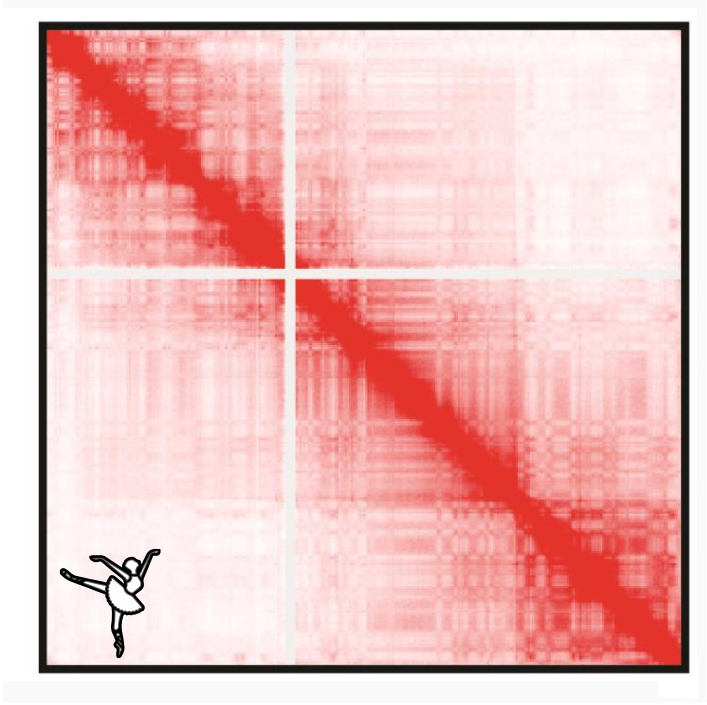

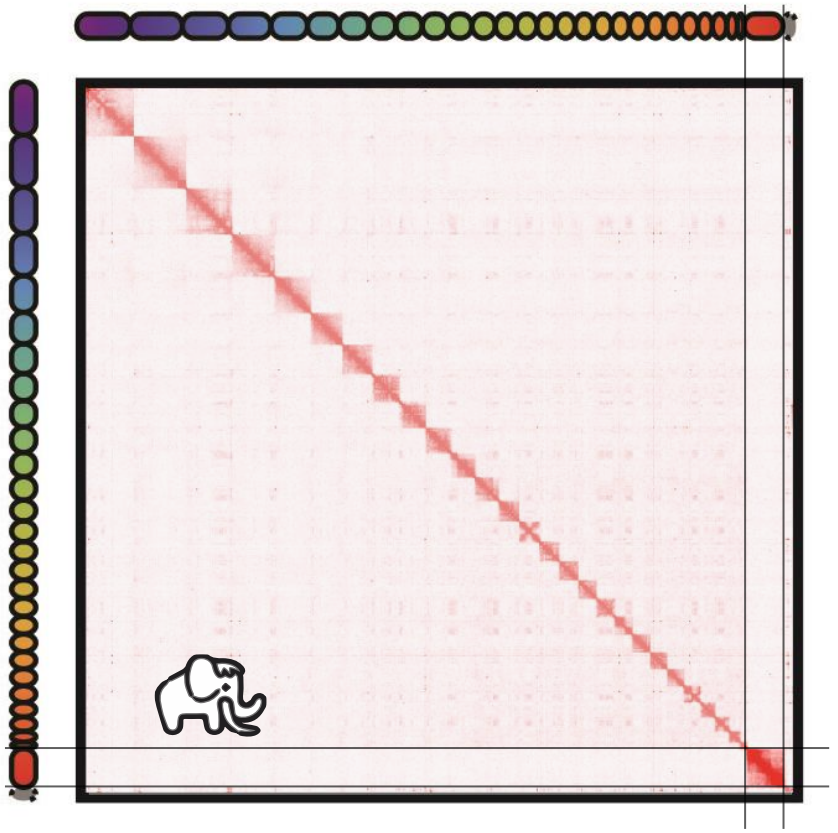

Darrow, Huntley et al., PNAS 2016

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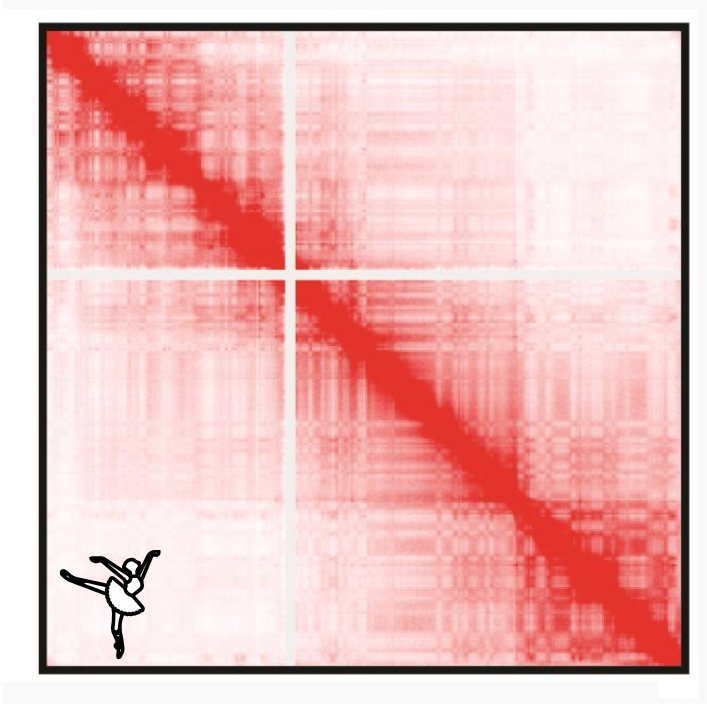
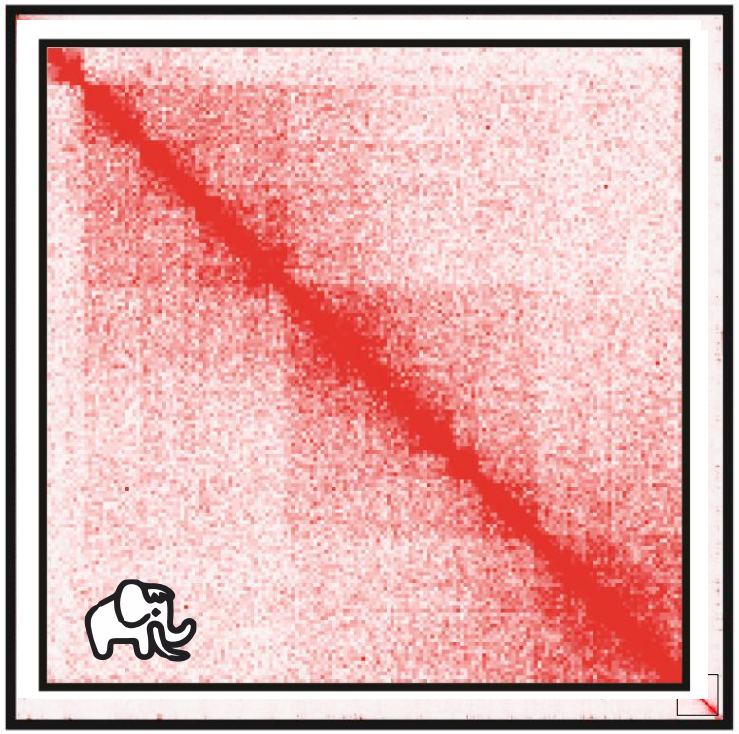
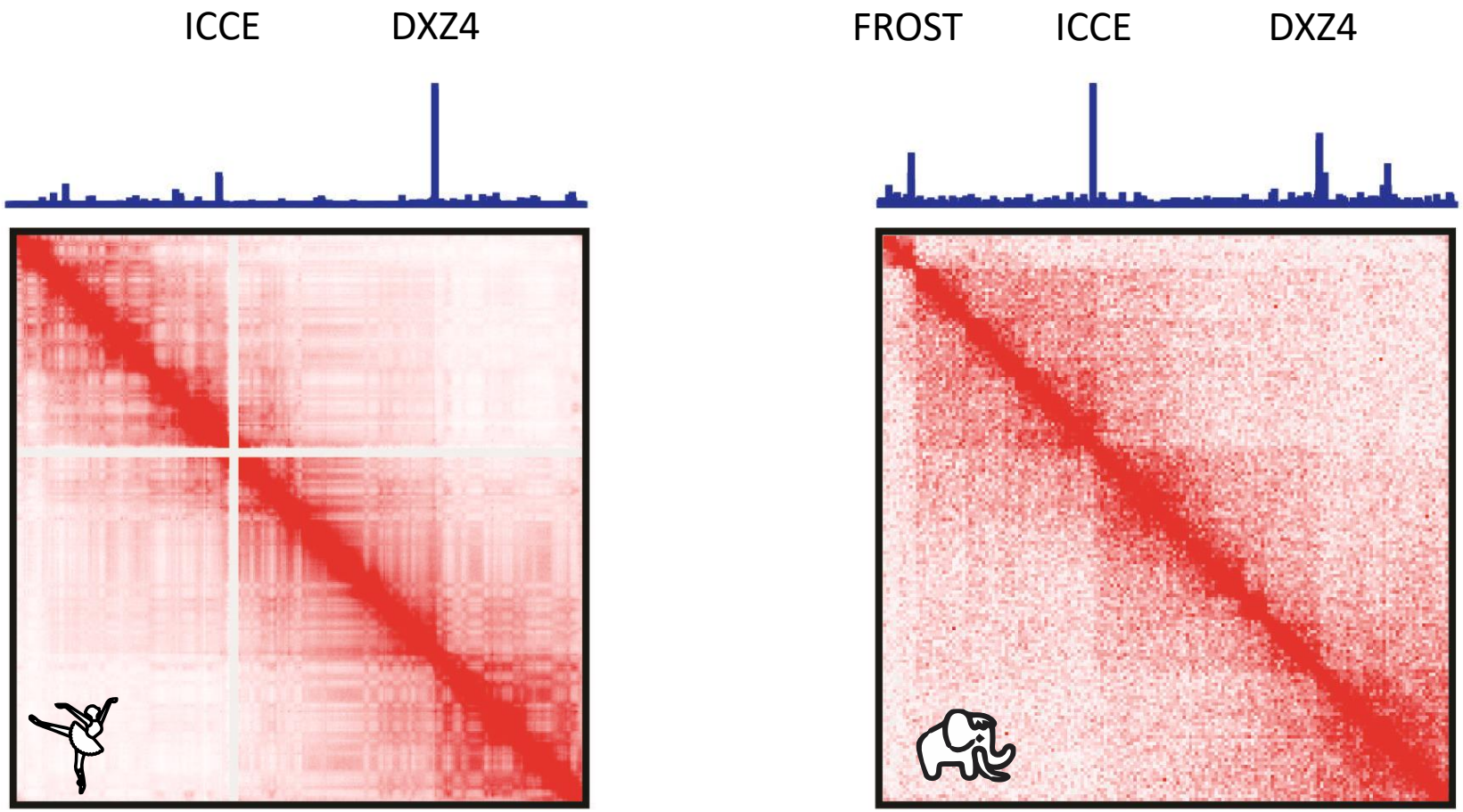




Darrow, Huntley et al., PNAS 2016

IT'S BEEN PUSHING ITS BOUNDARIES

CTCF alignments



Darrow, Huntley et al., PNAS 2016

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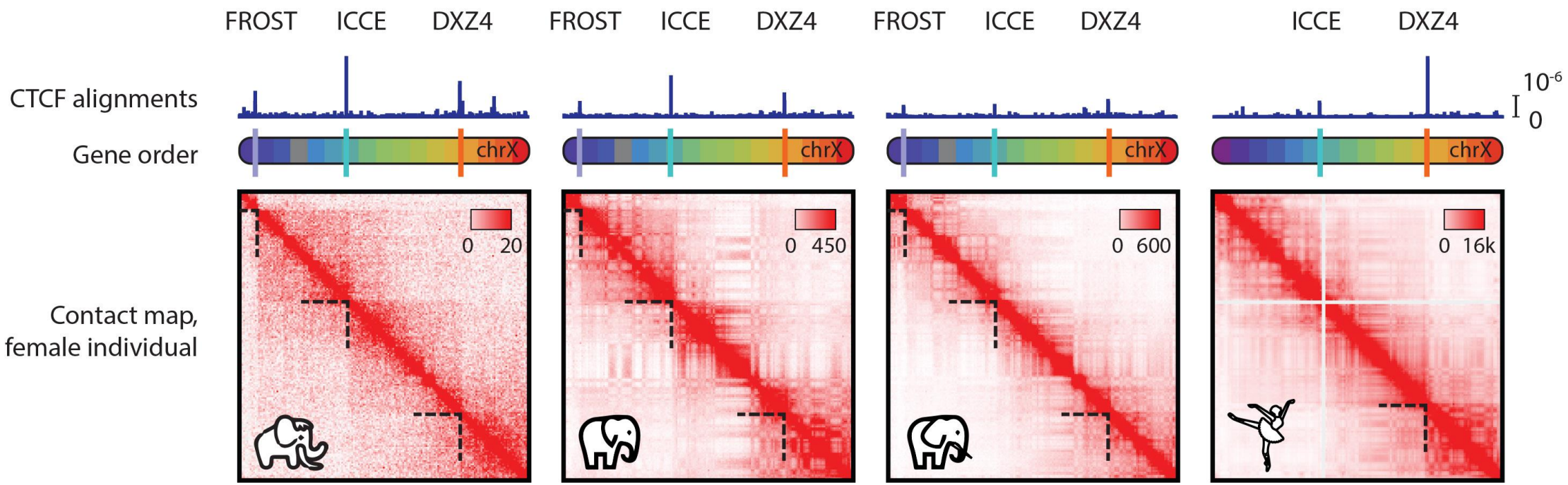

THANK YOU!


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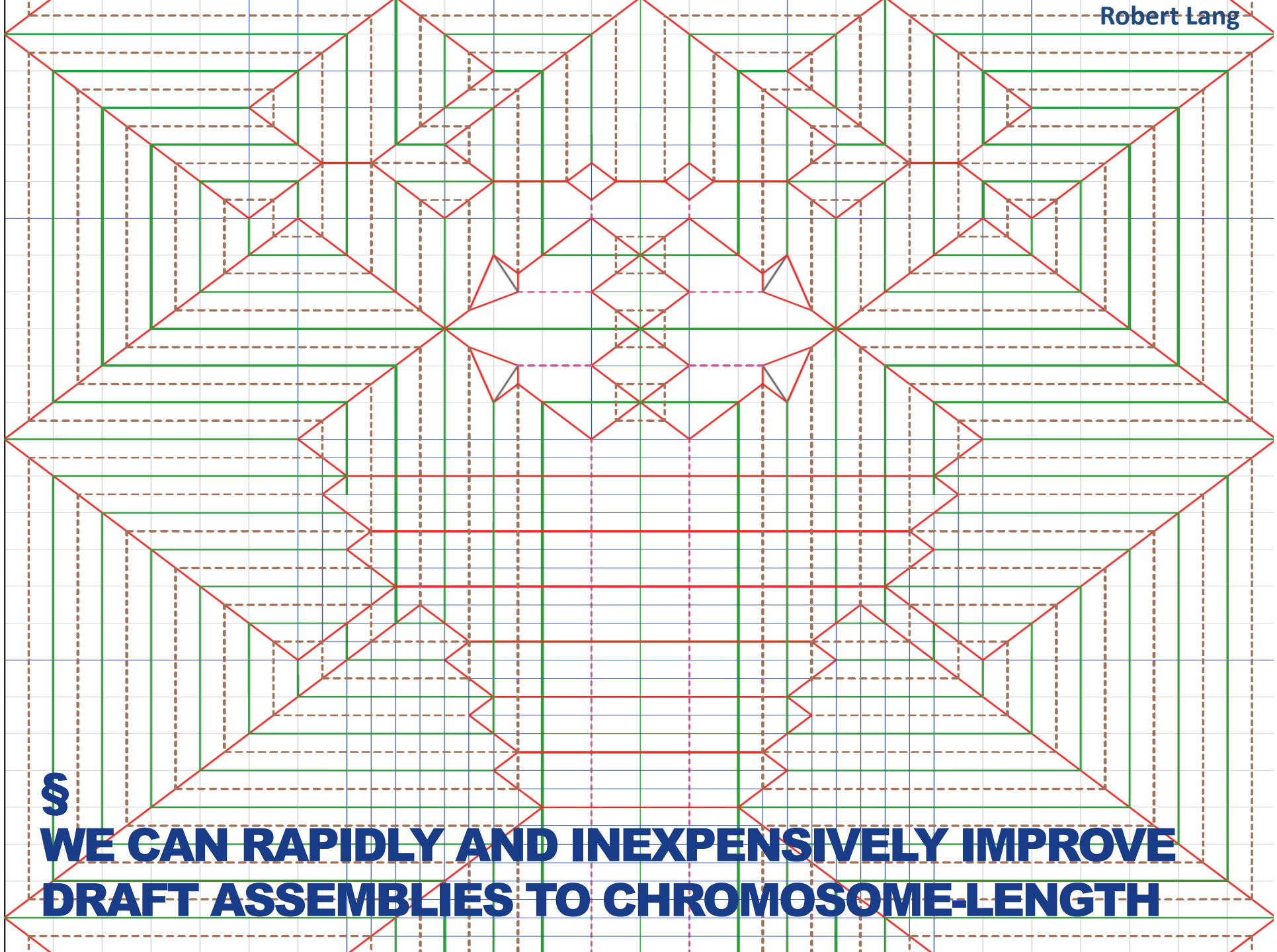
3 Laboratory of P.A. Lazarev Mammoth Museum of the Research Institute of Applied Ecology of the North, North-Eastern Federal University Named after M. K. Ammosov, Russian Federation

§

HI-C IS A POWERFUL SOURCE OF LINKING DATA

Folded by Jason Ku

Photo: Erik Demaine



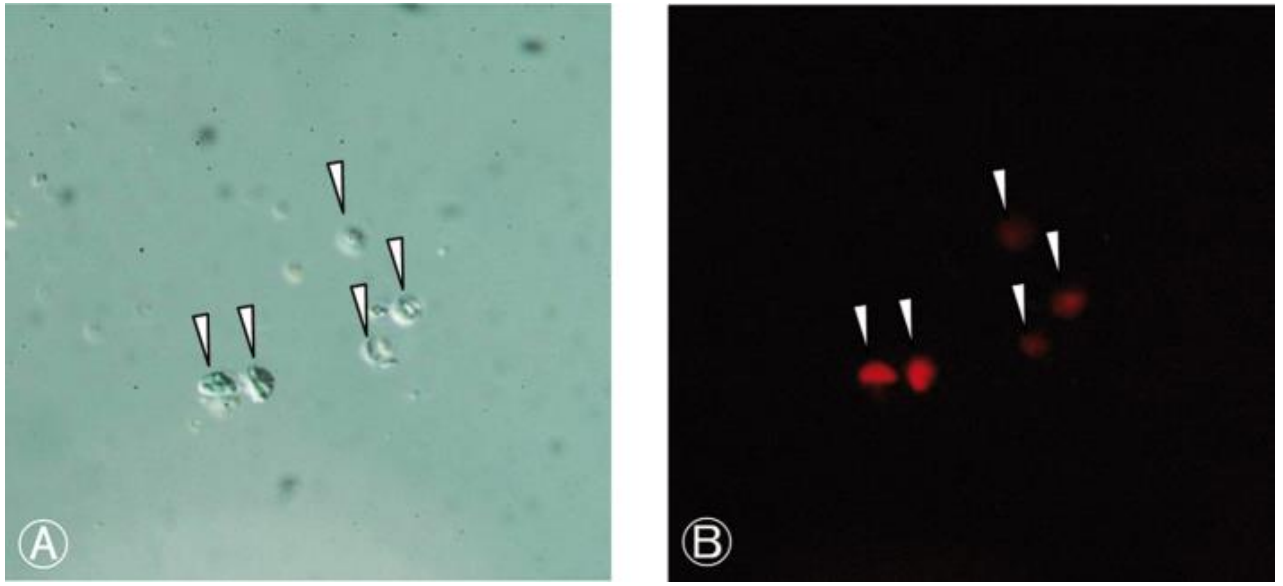
§

WE CAN RAPIDLY AND INEXPENSIVELY IMPROVE DRAFT ASSEMBLIES TO CHROMOSOME-LENGTH

BREAK SLIDE

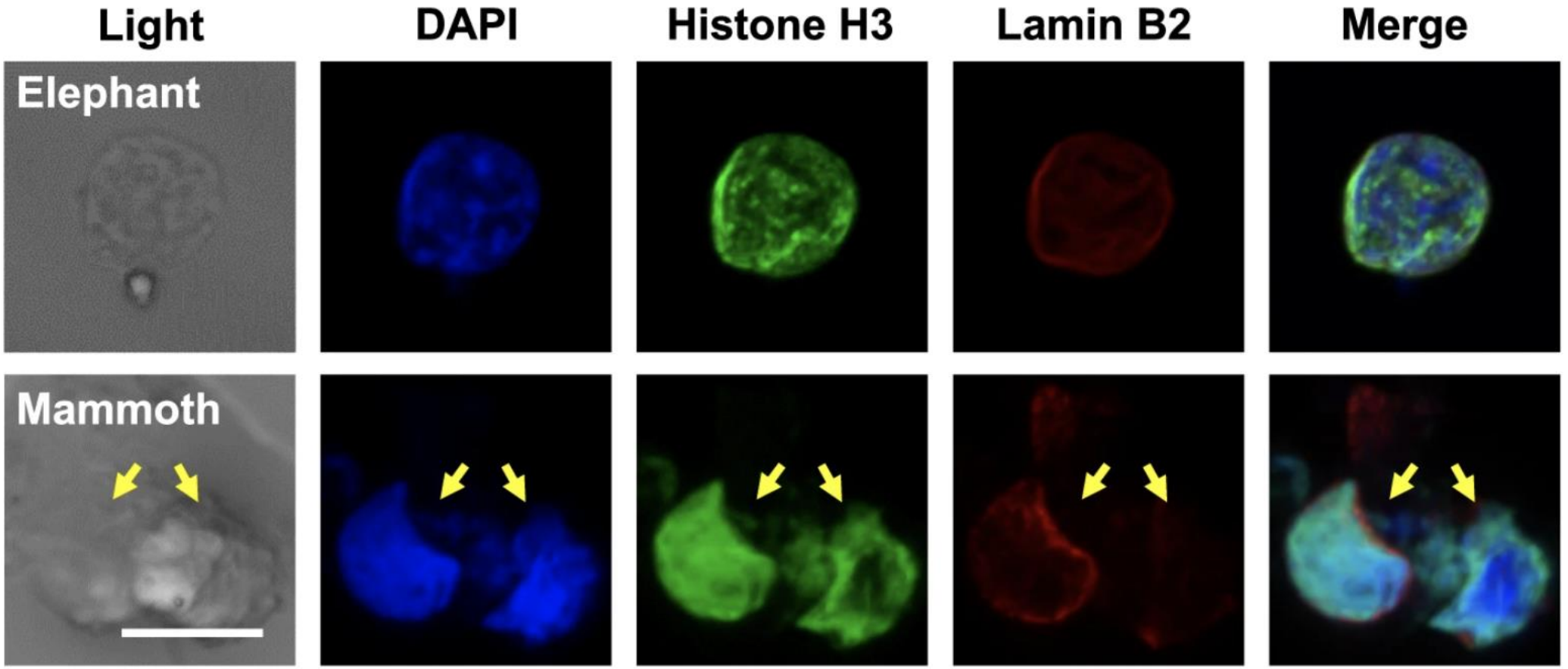
Video credits: Aleksandr Grafodatsky, Albert Protopopov

PREVIOUSLY, PROMISING RESULTS WERE REPORTED IN A 15,000 Y.O. MAMMOTH…



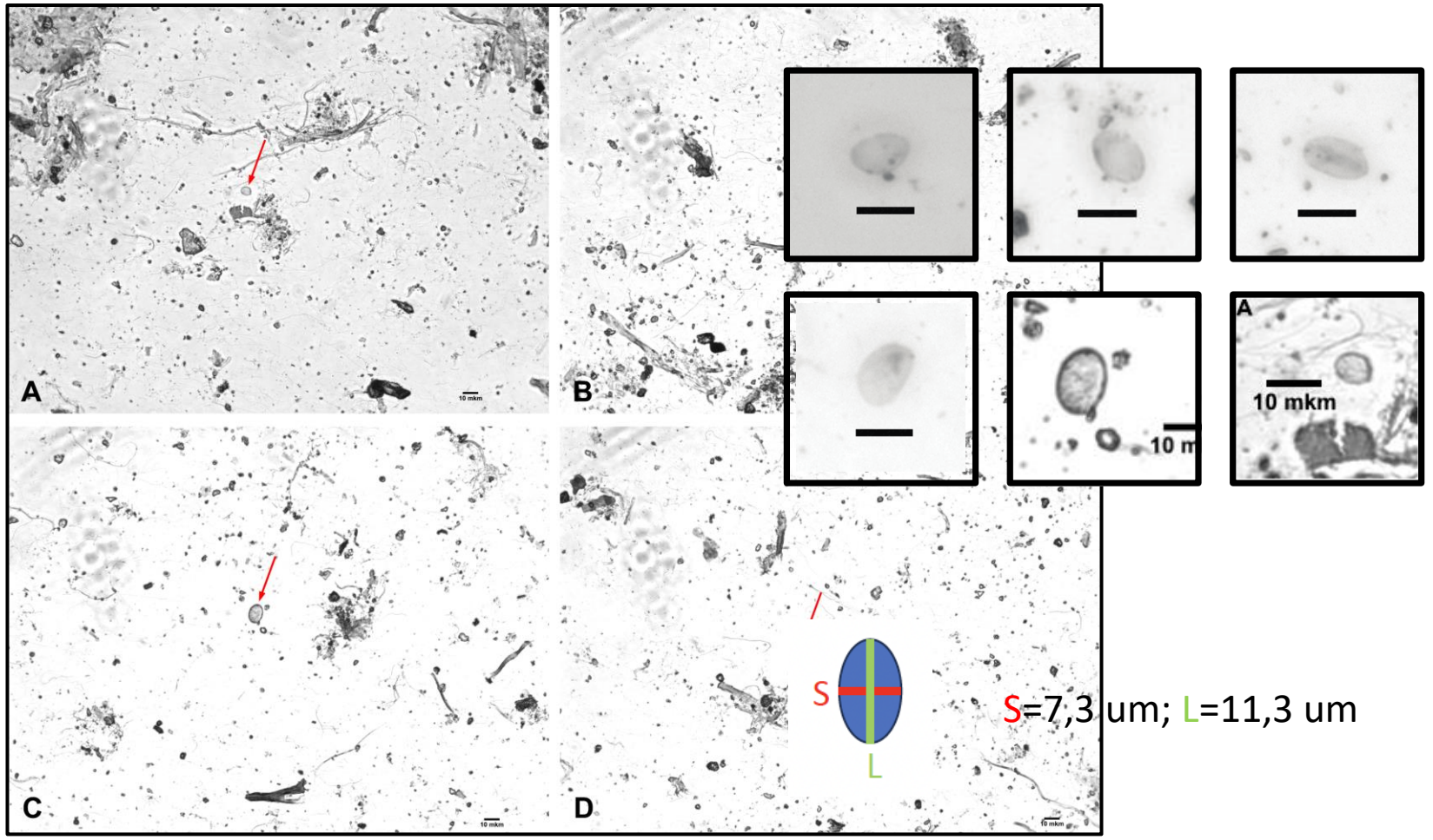
Dark field and PI from Kato et al., Proc Jpn Acad Ser B Phys Biol Sci. 2009

…AND A 28,000 Y.O. ‘YUKA’ MAMMOTH



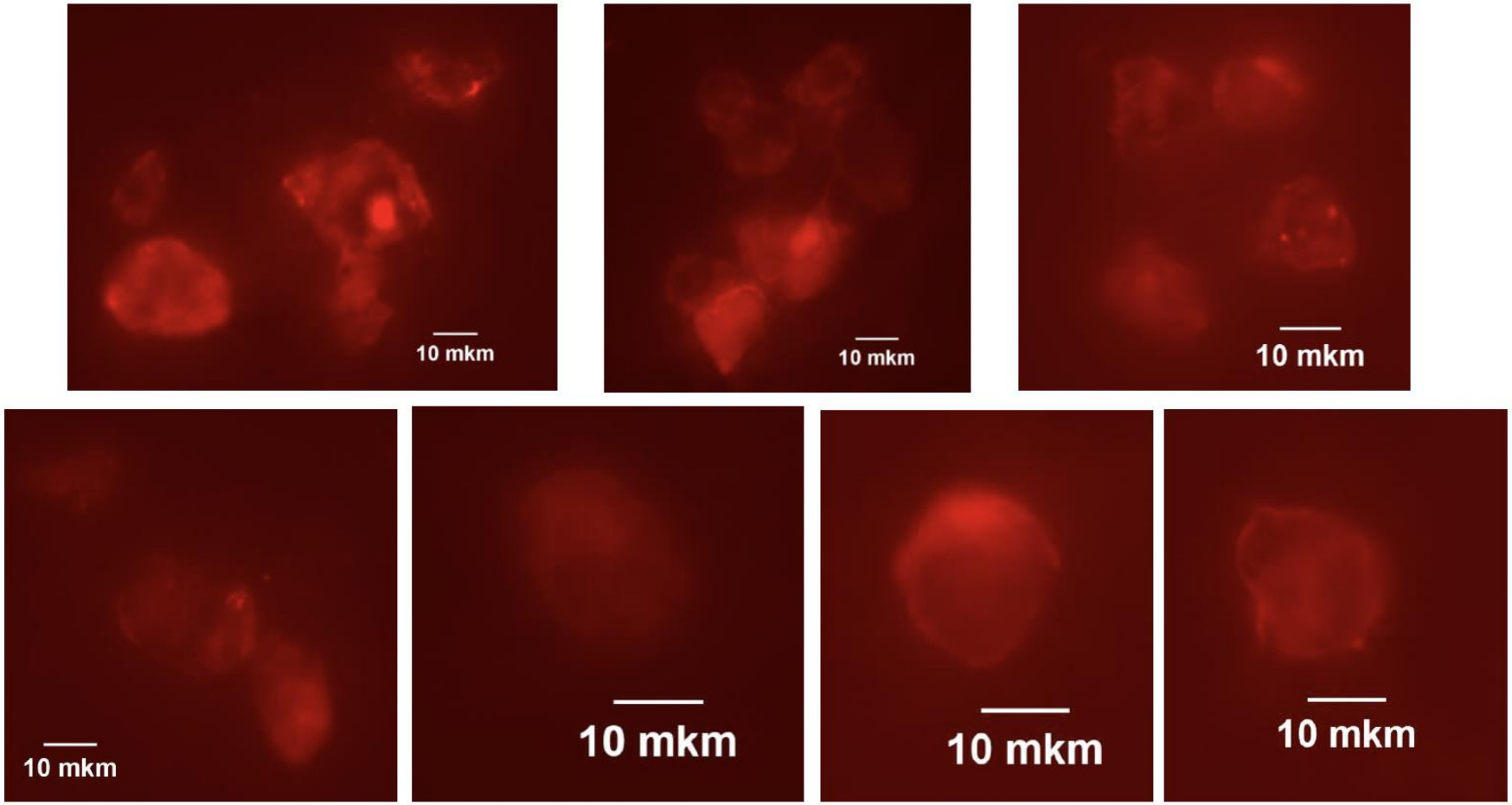
Immunostaining from Yamagata et al., *Sci Rep*. 2019

WE SEE ROUNDISH THINGS ON GIEMSA



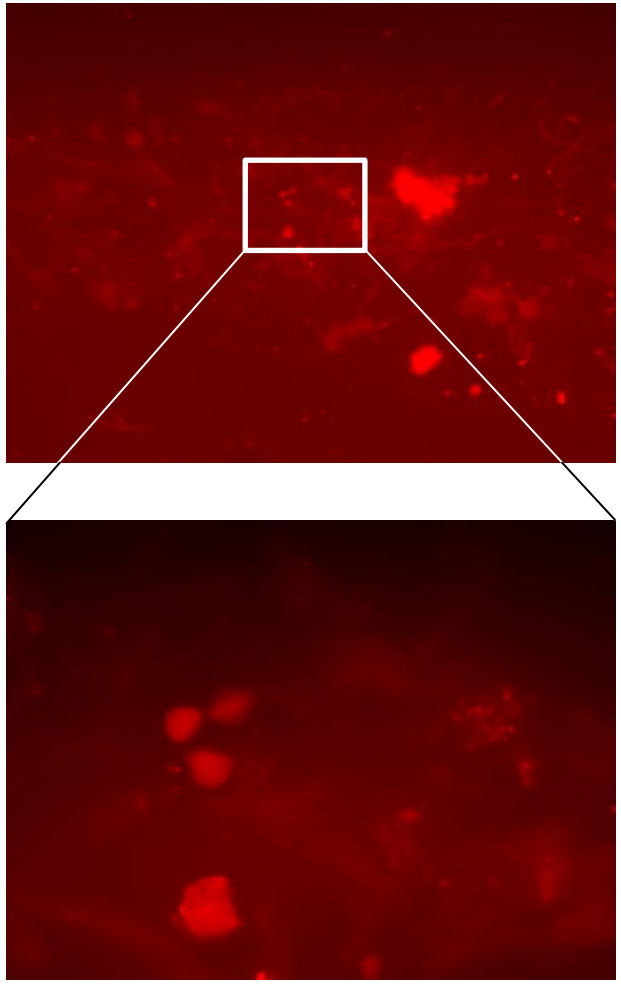
FA fixed CW nuclei, Giemsa in Buffer A, 10um scale bar

WE SEE SIMILAR SHAPES WITH PI STAINING…



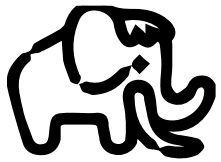
FA fixed CW nuclei, PI in Hi-C lysis buffer, 10um scale bar. ! Likely non-specific !

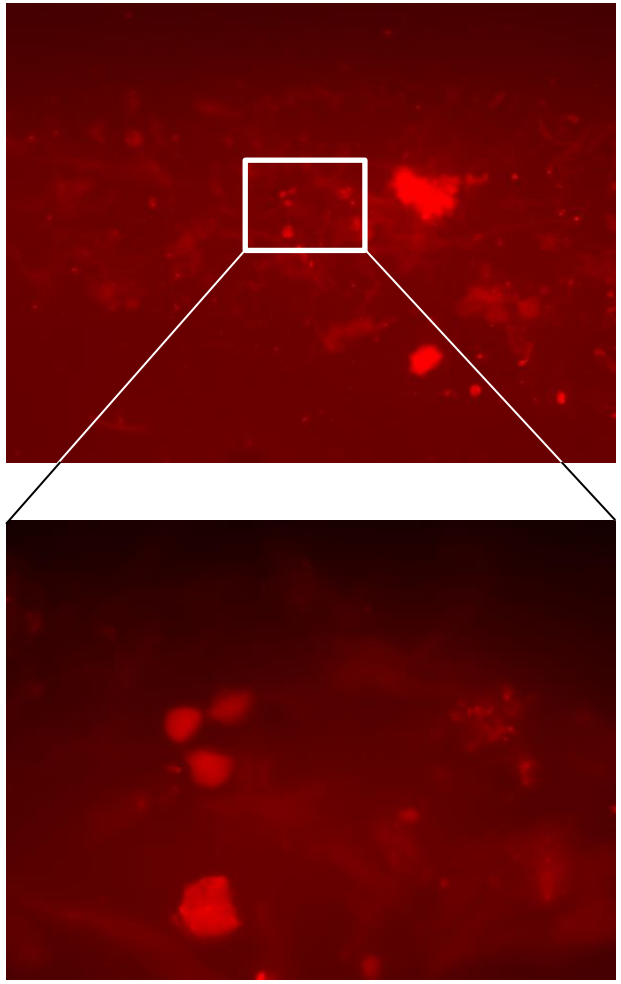
…AND EB

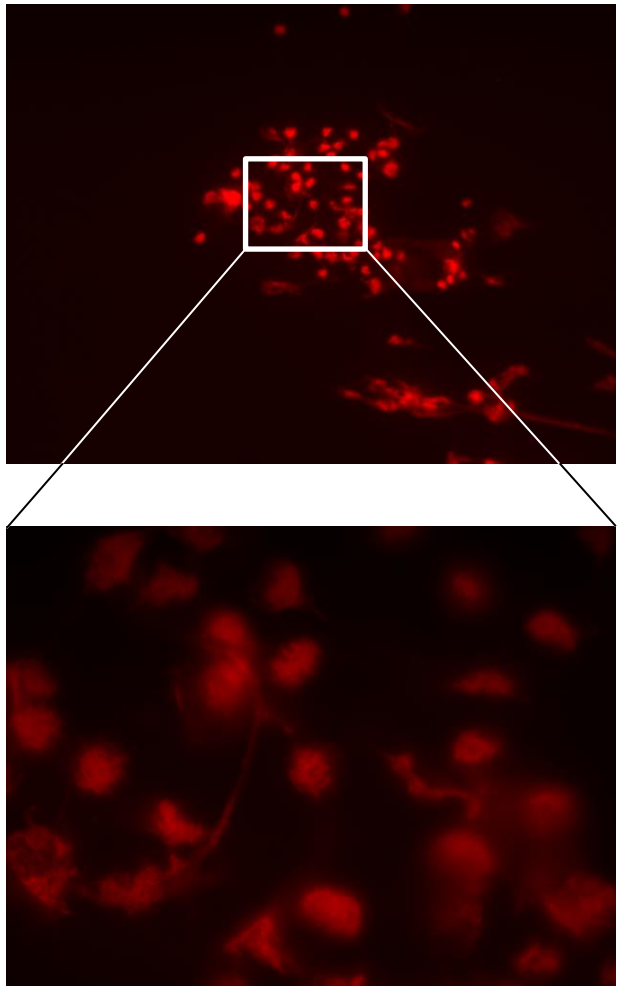


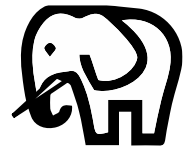
No-FA CW nuclei, ethidium bromide in Hi-C lysis buffer, 100x

THEY ARE SIMILAR IN SIZE AND SHAPE TO ELEPHANT NUCLEI



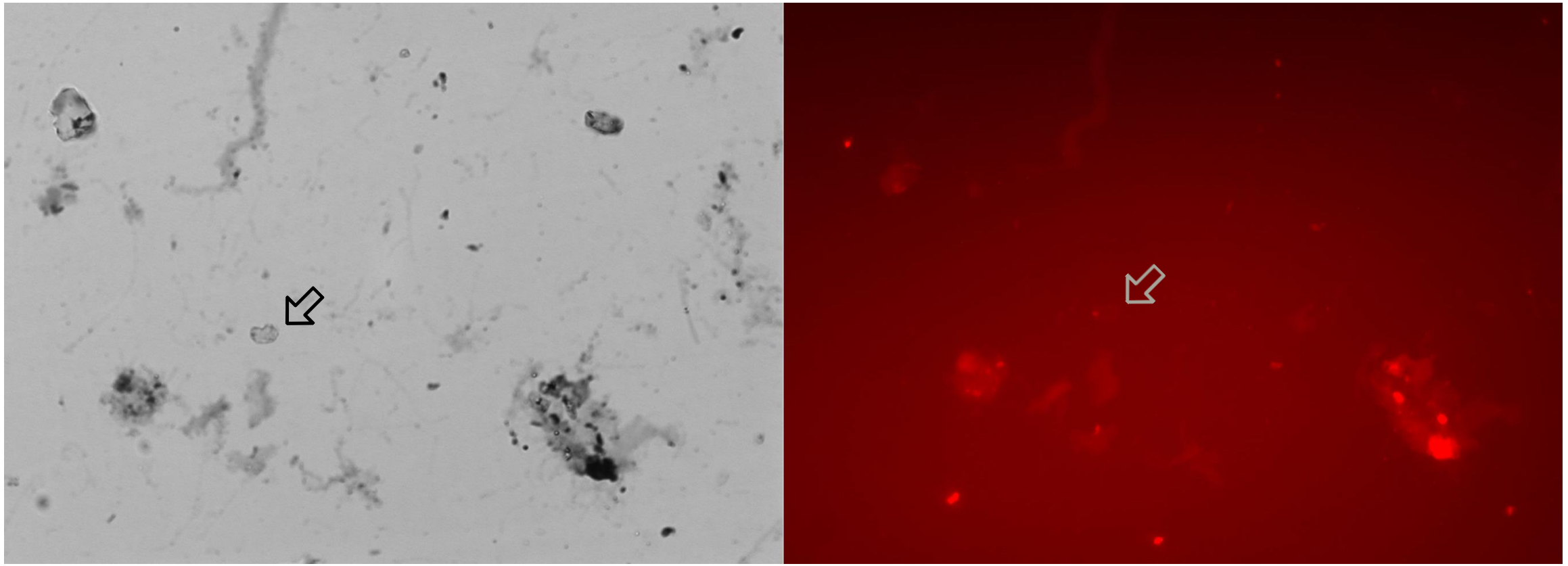






No-FA CW nuclei, ethidium bromide in Hi-C lysis buffer, 100x

[[WE ARE HAVING SOME TROUBLE CONNECTING THE TWO]]



Consecutive Giemsa and PI staining, no FA crosslinking

WE TRIED DOING FISH ON THESE STRUCTURES, BUT THE RESULTS ARE INCONCLUSIVE

- African elephant gDNA
- African elephant chromosome-specific probes
- rDNA
- Telomeric DNA

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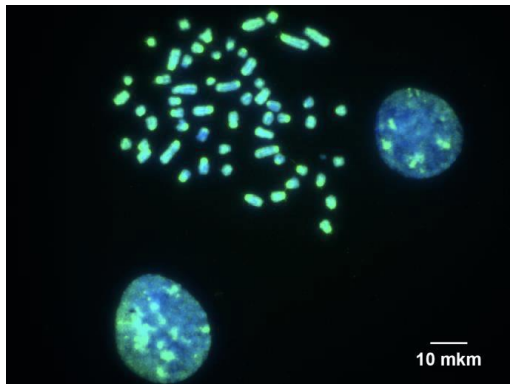

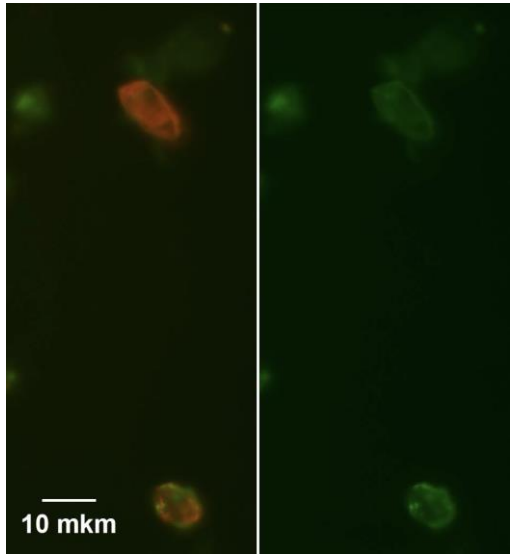

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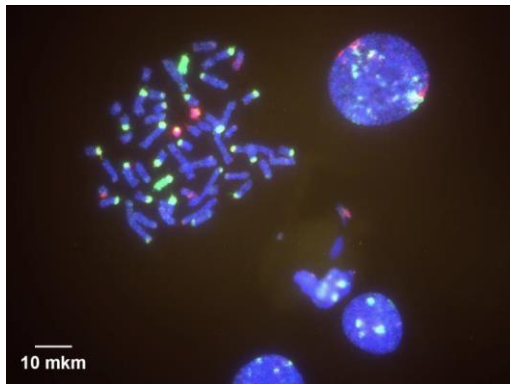

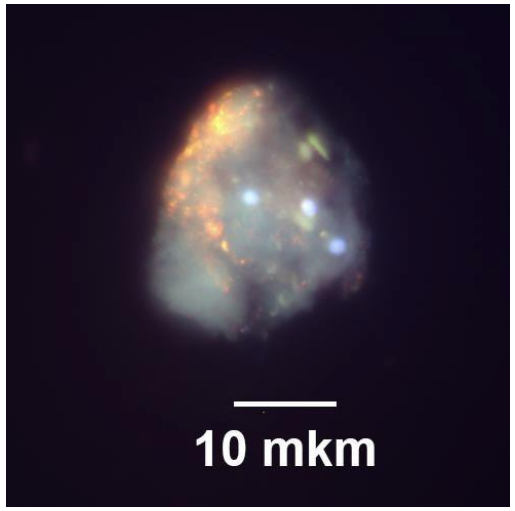

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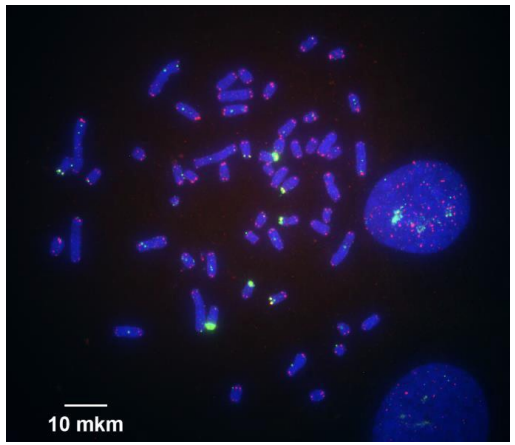
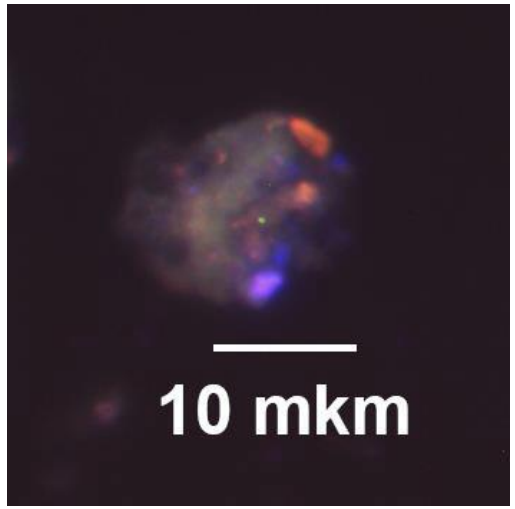
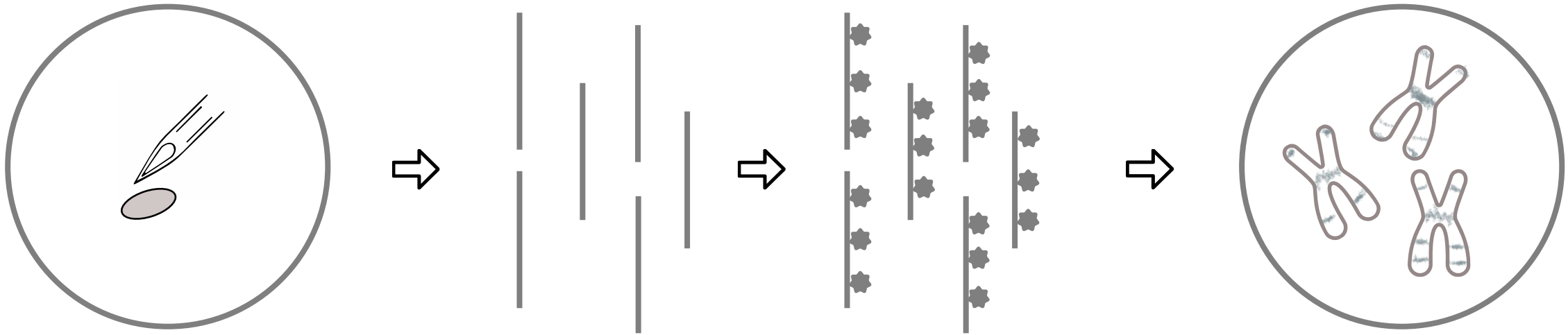




WE TRIED TURNING THE TABLES ON THE MAMMOTH



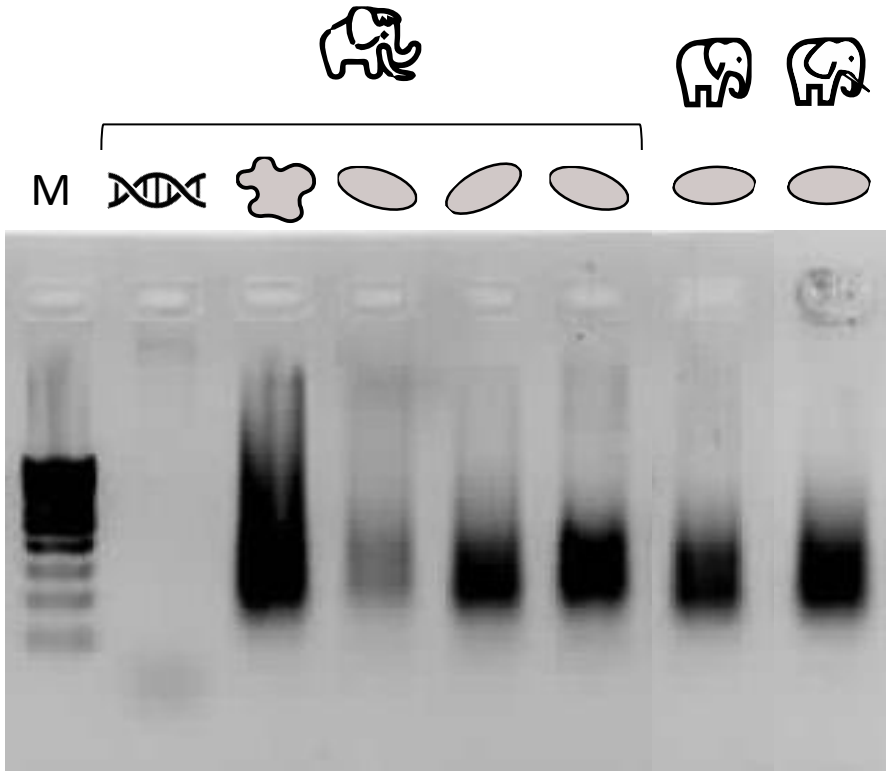
Micro-dissect the **mammoth**

Amplify
(& sequence, in progress)

Label

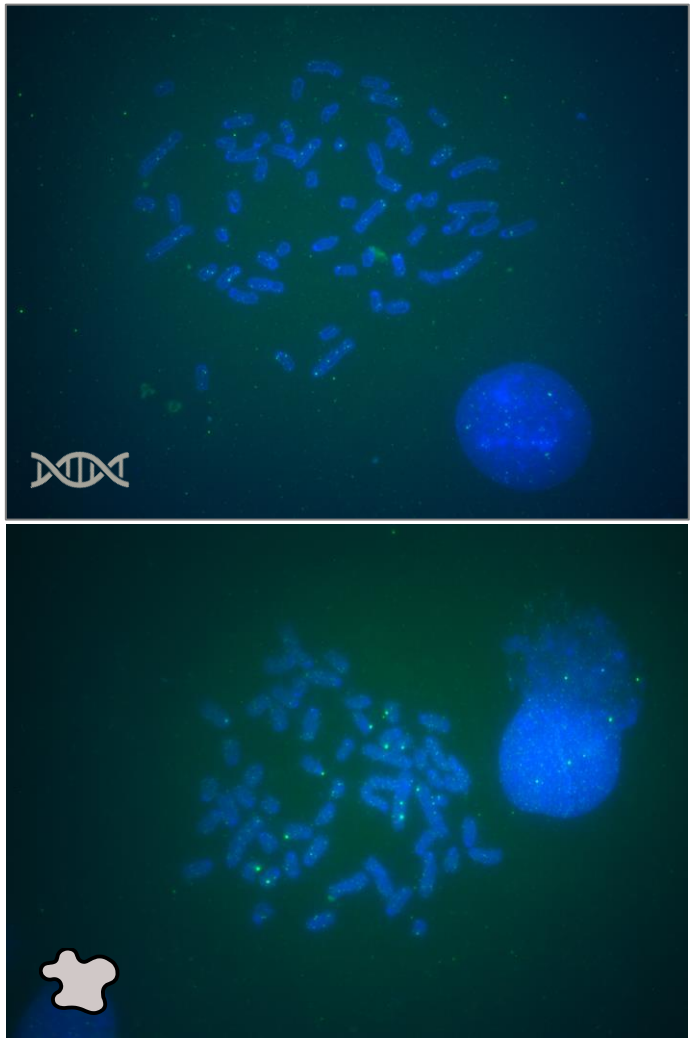
Stain the **elephant**

MICRODISSECTION PROBES CONTAIN DNA…

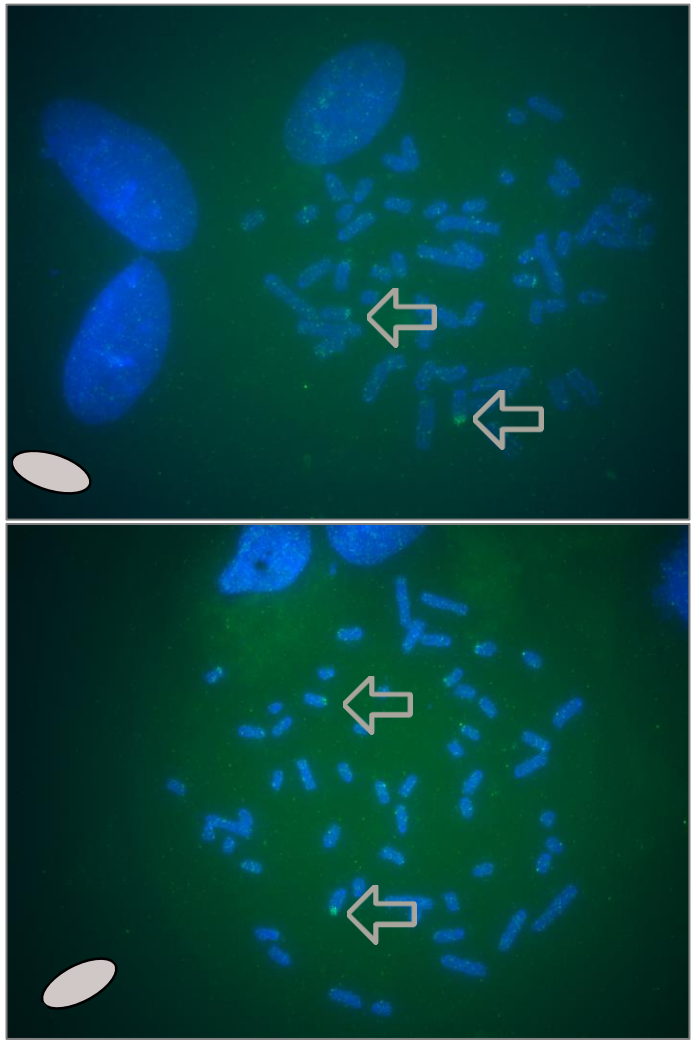


...THAT LABELS SOMETHING IN ELEPHANT!

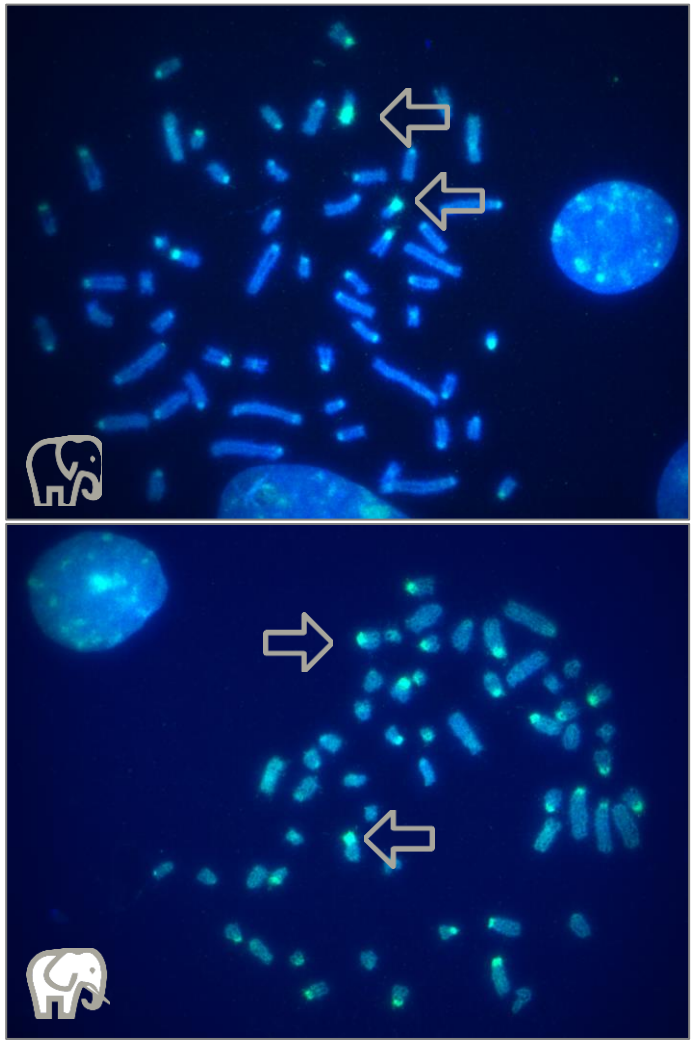
Negative control (debris and WGS)



Mammoth microdissections

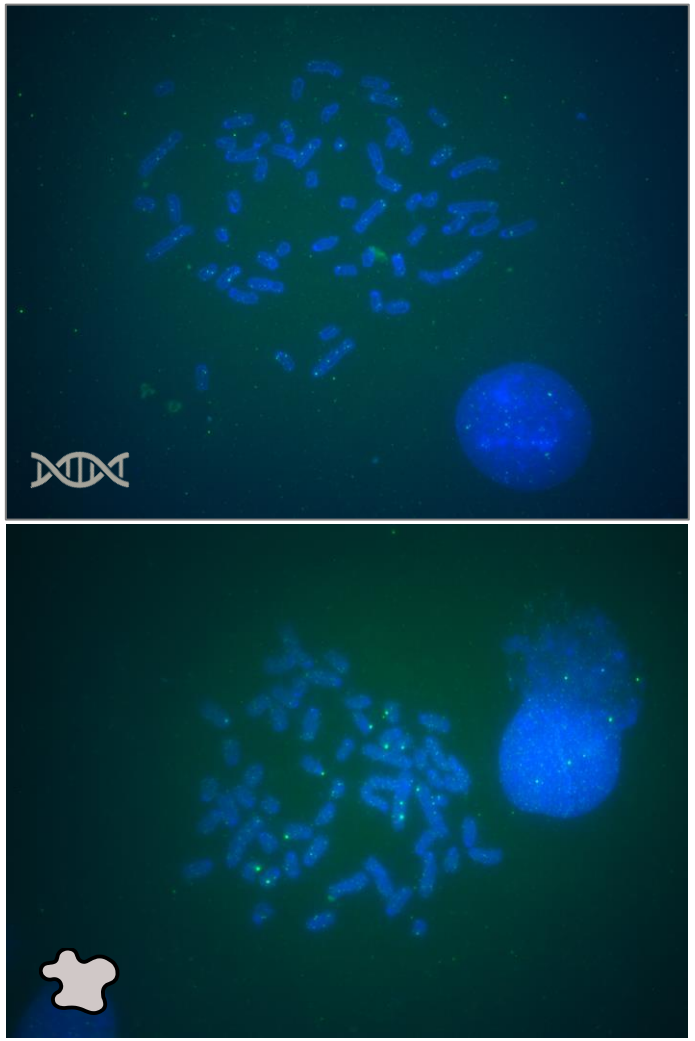


Positive control (modern elephants)



LOOKS LIKE RDNA?

Negative control (debris and WGS)



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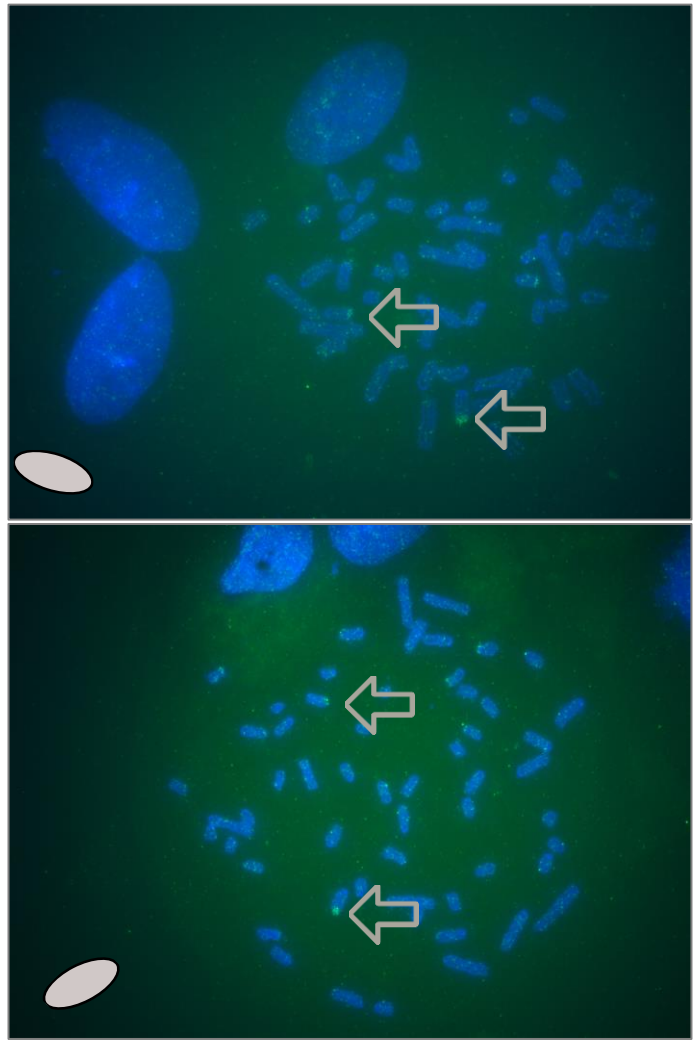

Positive control (modern elephants)