Apoptosis reveals human evolutionary heritage sequestered in non-coding DNA

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Abstract

Cell-free chromatin particles (cfChPs), primarily derived from non-coding DNA (ncDNA) and released from apoptotic cells into the bloodstream, can be horizontally transferred into living cells[1]. However, their potential role in generating ncDNA within recipient cells remains unknown. We previously reported that cfChP-sized fragments generated upon sonication of high-molecular-weight DNA can indiscriminately transmit themselves into foreign cells across species and kingdom boundaries[2]**.** We hypothesised that cfChP-like DNA–protein complexes generated following organismal death are exchanged among species and progressively accumulate to form a dense patchwork that constitutes ncDNA, and that apoptosis may dissociate these complexes and render them amenable to detection. To test this hypothesis, we used species-specific DNA fluorescent in situ hybridisation probes and antibodies against archaea, eubacteria, plants, algae, fungi, protozoa, Drosophila, fish, chicken, mouse, rat, pig, dog and monkey on intact and apoptotic human cells. We observed no reactivity with intact cells but strong reactivity with the ncDNA component of apoptotic cells. Our findings indicate that ncDNA represents a dense conglomeration of interspecies DNA–protein complexes that become detectable following apoptosis. These results suggest that human evolutionary heritage resides within ncDNA, transmitted horizontally through apoptotic processes.

Main

The genomes of complex multicellular eukaryotes are largely composed of non-protein-coding DNA (ncDNA). Although a fraction of these genomes has important regulatory functions[3], the reason why 99% of the human genome should comprise ncDNA remains unresolved.

We previously reported that circulating cell-free chromatin particles (cfChPs), primarily composed of ncDNA fragments released from apoptotic cells, undergo horizontal transfer into living cells, where they become detectable using fluorescent in situ hybridisation (FISH)[1]. These findings suggested that DNA sequences, normally undetectable within the ncDNA of intact cells, can be visualised following apoptosis, albeit within foreign host cells. We also demonstrated that ncDNA contained within cfChPs harbours an intrinsic protein synthesis machinery, including RNA polymerase, ribosomal RNA, and ribosomal proteins, enabling them to autonomously synthesise various proteins that are typically produced by genomic DNA[1]. These proteins could be detected using immunofluorescence (IF) within the host cells using appropriate antibodies.

In a related study, we reported that mechanical shearing of high-molecular-weight DNA via sonication results in the generation of cfChP-like small DNA–protein complexes, which can readily enter into foreign cells without being restricted to species and kingdom boundaries[2]. Based on this finding, we hypothesised that

cfChP-like DNA–protein complexes generated upon organismal death, especially during evolutionary periods of severe environmental stress[4–6], were exchanged among species and progressively accumulated to form the dense patchwork that now constitutes the ncDNA. We further hypothesised that apoptosis may dissociate the constituents of the compacted ncDNA and make them amenable to detection by FISH and IF[1].

To test this hypothesis, we used species-specific FISH probes to detect ncDNA sequences unique to various taxa, from archaea to monkeys, within apoptotic human cells, using intact cells as negative controls. Since cfChPs like DNA-protein complexes can autonomously synthesise proteins[1], we also tested whether multiple species-specific antibodies react with intact and apoptotic cells. Before we began our experiments, we confirmed that our FISH probes and antibodies were strictly species-specific and exhibited no cross-reactivity (Supplementary Fig. 1).

We believe that our findings would provide insights into the evolutionary origin and composition of ncDNA and clarify whether interspecies exchange of DNA–protein complexes has contributed to the progressive expansion of the non-coding genome across evolution. The latter would be consistent with emerging reports that evolutionarily advanced species possess progressively higher fractions of ncDNA[7–9].

Hypoxia-induced apoptosis

We first investigated whether apoptosis induced by hypoxia or occurring naturally in human cells and tissues reacts with species-specific DNA FISH probes and antibodies, whereas intact living cells do not. Accordingly, we subjected A375 human melanoma cells to hypoxia (1% oxygen) and exposed the flow-sorted Annexin V-positive apoptotic cells and the Annexin V-negative non-apoptotic (living) cells to nine different species-specific FISH probes and antibodies. Cells or tissues from each of these species acted as positive controls (Figs. 1a and 1b). We observed that the FISH probes and antibodies did not react with intact A375 cells but reacted strongly with apoptotic A375 cells and positive-control cells/tissues. We validated these findings in A375 cells by performing similar experiments using four additional human cell lines, HEK-293 (embryonic kidney), HeLa (cervical cancer), HEKa (epidermal keratinocytes) and HADF (adult dermal fibroblasts), with results identical to those obtained in A375 cells (Supplementary Fig. 2).

Fig. 1

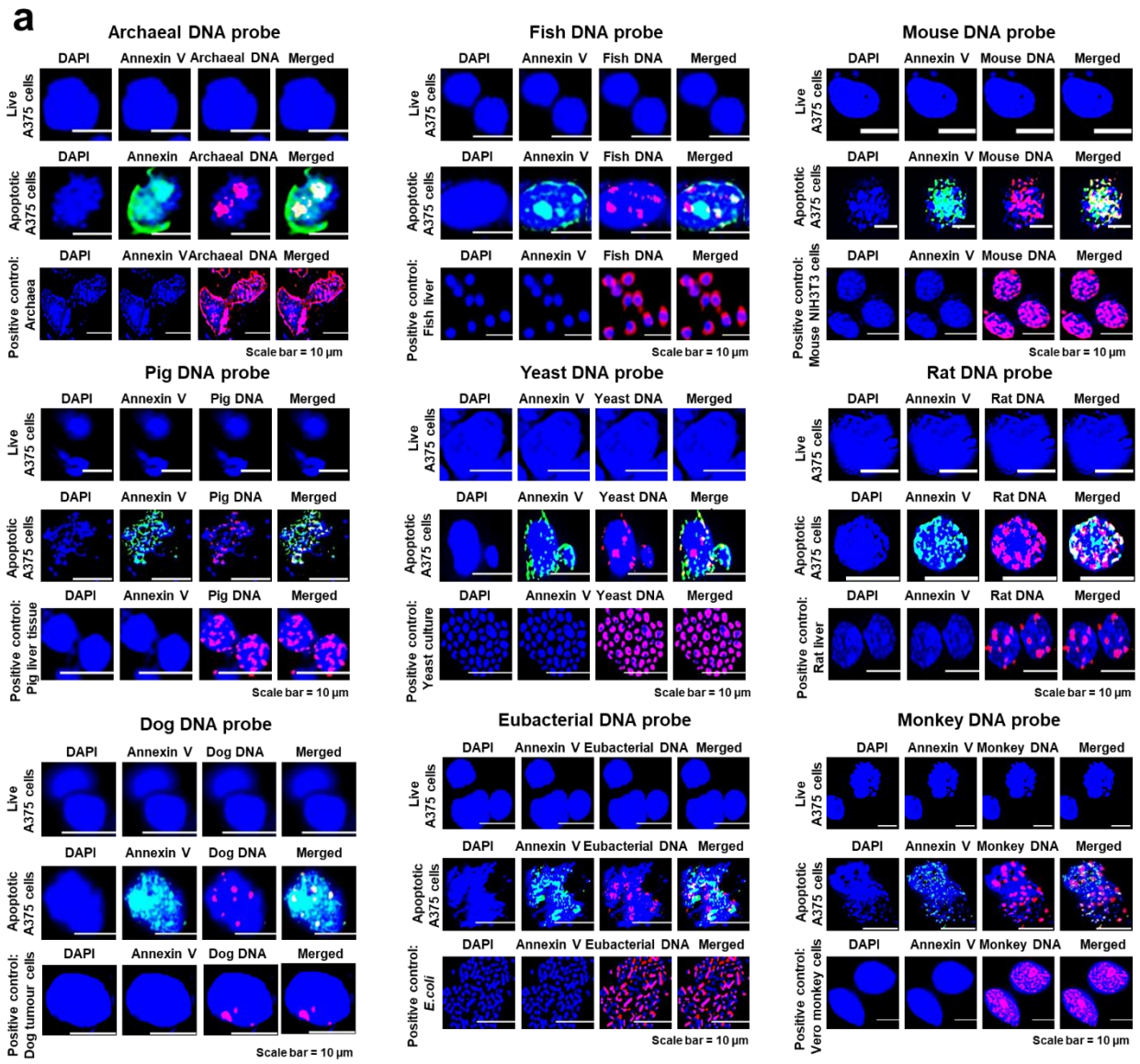
a
Archaeal DNA probe
DAPI
Annexin V
Archaeal DNA
Merged
Live A375 cells
Apoptotic A375 cells
Positive control: Archaea
Scale bar = 10 µm
Fish DNA probe
Fish DNA
Positive control: Fish liver
Mouse DNA probe
Mouse DNA
Positive control: Mouse NIH3T3 cells
Pig DNA probe
Pig DNA
Positive control: Pig liver tissue
Yeast DNA probe
Yeast DNA
Merge
Positive control: Yeast culture
Rat DNA probe
Rat DNA
Positive control: Rat liver
Dog DNA probe
Dog DNA
Positive control: Dog tumour cells
Eubacterial DNA probe
Eubacterial DNA
Positive control: E.coli
Monkey DNA probe
Monkey DNA
Positive control: Vero monkey cells

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Fig. 1

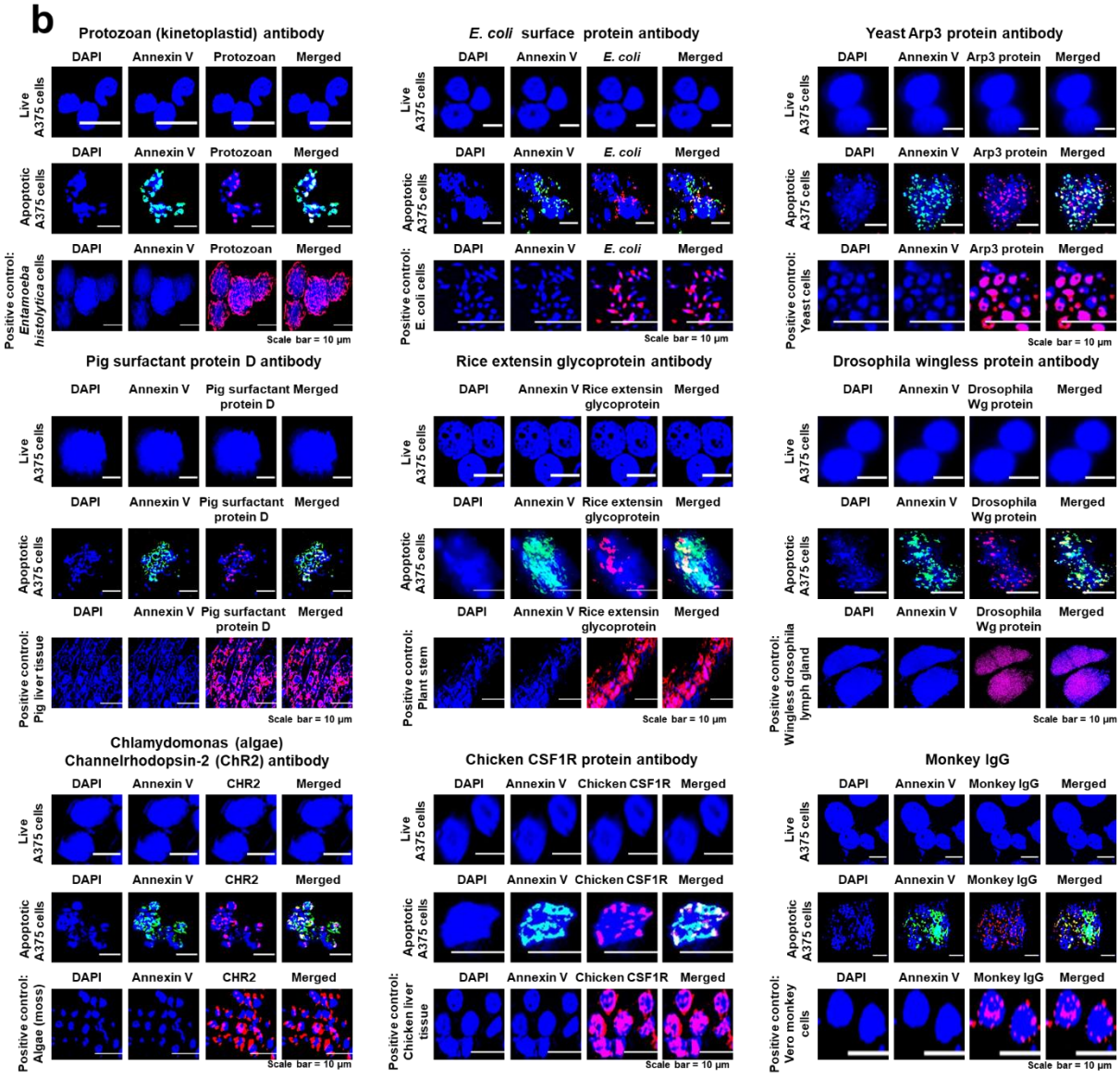

Fig. 1: Detection of species-specific nucleic acid and protein markers in apoptotic human melanoma cells. **a.** Live A375 cells and the corresponding flow-sorted Annexin V-positive apoptotic cells were simultaneously probed with species-specific DNA FISH probes. Apoptotic cells (middle row in each panel) but not live cells (top row in each panel) reacted with the FISH probes. The bottom row in each panel represents positive-control cells or tissues of the respective species. The dog FISH probe used was specific for the X (magenta) and Y (green) chromosomes. As A375 cells originated from a female patient with malignant

melanoma, only the magenta X chromosome is visible in the apoptotic A375 cells. Similarly, green signals (Y chromosome) are not seen in the positive-control cells since they were derived from a female dog tumour. **b.** Live A375 cells and the corresponding flow-sorted Annexin V-positive apoptotic cells were simultaneously probed with species-specific antibodies. Apoptotic cells (middle row in each panel) but not live cells (top row in each panel) reacted with the antibodies. The bottom row in each panel represents positive-control cells/tissues of the respective species. All experiments presented in Fig. 1 were performed twice by two different investigators.

Spontaneously occurring apoptosis

We next investigated the reactivity of cells that had spontaneously undergone apoptosis under normal culture conditions or in normal human tissues. We examined three human cell lines, namely, MDA-MB-231 (breast cancer), MRC-5 (embryonic lung fibroblast) and A375 (malignant melanoma), as well as three normal human tissues (brain, liver and kidney). We identified spontaneously occurring apoptotic cells among the viable cells via immunostaining with an antibody against Annexin V. We observed that FISH probes against rat, archaea, fungal, fish, bacterial and mouse DNA reacted strongly with Annexin V-positive apoptotic cells but not with Annexin V-negative viable cells (Fig. 2a, top row in each panel). Annexin V-positive apoptotic cells also reacted with antibodies against protozoan kinetoplastid, rat RTA1 protein, rice extensin glycoprotein, mouse MHC-II protein, *E. coli* O15 protein and chicken CSF1R protein, whereas Annexin V-negative viable cells were non-reactive (Fig. 2a, bottom row in each panel).

In normal human tissues, formalin-fixed, paraffin-embedded sections of brain, liver and kidney were stained with Annexin V antibody to identify the spontaneously occurring apoptotic cells among the viable cells. We found that in each case, only Annexin V-positive apoptotic cells reacted with FISH probes against pig, mouse, fungal, monkey, archaea and fish DNA, whereas Annexin V-negative live cells were non-reactive (Fig. 2b, top row in each panel). Likewise, the Annexin V-positive apoptotic cells within these organs reacted with antibodies against the *Drosophila* Wg protein, rat RTA1 protein, rice extensin glycoprotein, mouse MHC-II protein, algae CHR2 protein, and pig surfactant protein D, whereas the Annexin V-negative cells were non-reactive (Fig. 2b, bottom row in each panel).

Fig. 2

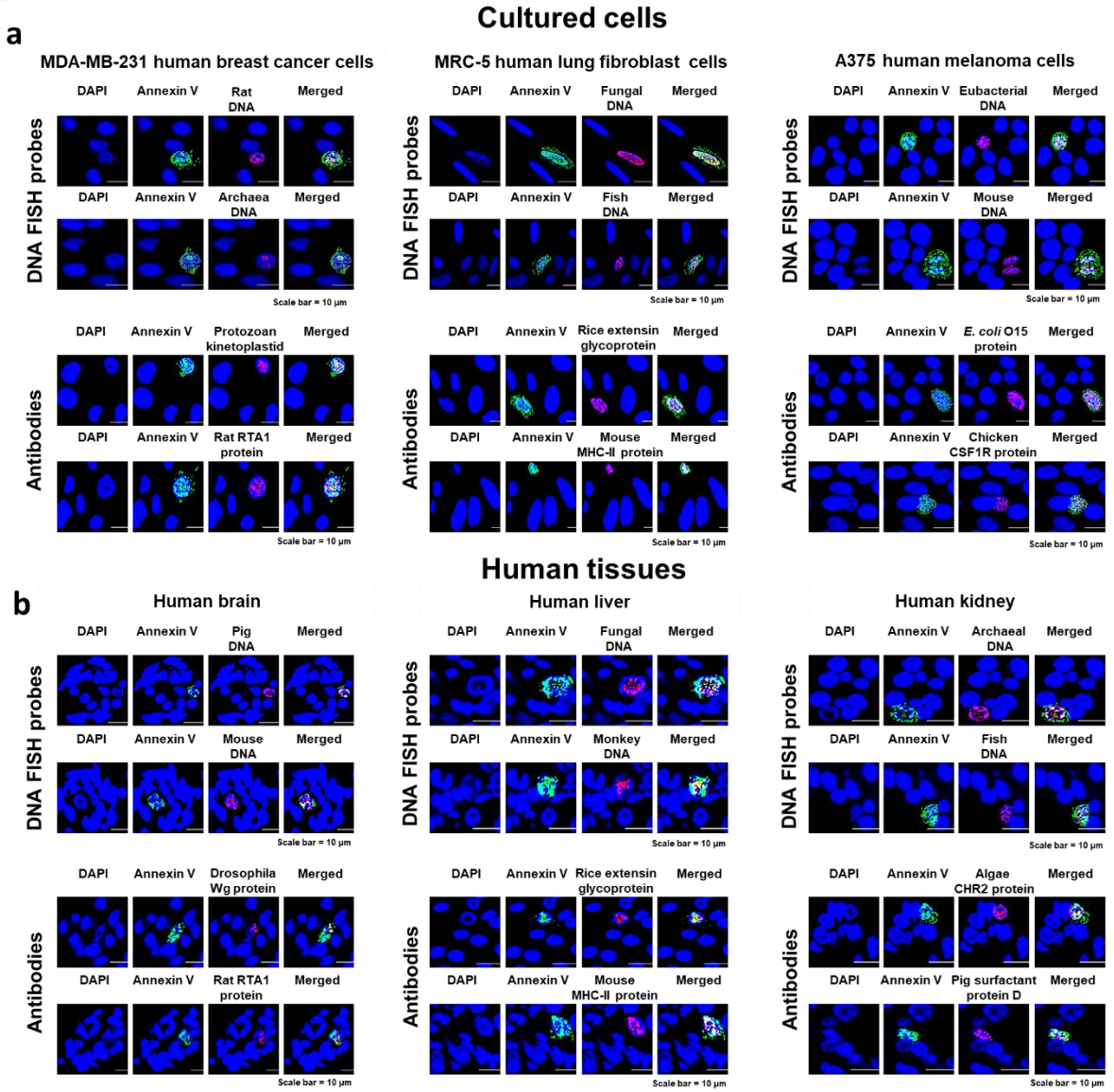

Fig. 2: Spontaneously occurring apoptotic cells, but not viable cells, reveal phylogenetic diversity. **a.** MDA-MB-231 human breast cancer, MRC-5 human embryonic lung, and A375 human melanoma were simultaneously stained with an antibody against Annexin V and a panel of species-specific DNA FISH probes and antibodies. Annexin V-positive apoptotic cells, but not Annexin V-negative viable cells, exhibited reactivity with species-specific DNA FISH probes and antibodies. **b.** Formalin-fixed, paraffin-embedded sections of human brain, liver and kidney tissues were simultaneously stained with an antibody against Annexin V and a panel of species-specific DNA FISH probes and antibodies. Annexin V-positive

apoptotic cells, but not Annexin V-negative viable cells, react with species-specific DNA FISH probes and antibodies. All experiments presented in Fig. 2 were performed twice by two different investigators.

Phylogenetic diversity of ncDNA

We next tested the hypothesis that ncDNA represents a conglomeration of DNA–protein complexes from different species that become detectable following apoptosis. We had previously used a human long non-coding RNA probe comprising a set of Stellaris RNA FISH probes containing up to 48 unique sequences, each labelled with a fluorophore, and reported that the probe collectively bound to the non-coding RNA target transcript[1]. We found that, when tested against human DNA, the RNA probe aligned with the entire length of DNA with almost 100% coverage[1]. Since the non-coding RNA probe could also detect ncDNA, we used it to identify ncDNA in apoptotic bodies of hypoxia-induced apoptotic A375 cells. To generate apoptotic bodies, 1×10^6 A375 cells were subjected to hypoxia-induced apoptosis, and 100 µL of the cell suspension containing approximately 1.5×10^5 apoptotic cells was dropped onto glass slides from a height of 2 ft. This manoeuvre allowed the apoptotic cells to disperse into apoptotic bodies, which spread evenly on the glass slides. The slides were simultaneously probed with an antibody against Annexin V, a human whole-genome probe and the non-coding RNA probe. The Annexin V positive apoptotic bodies were found to colocalise with both the whole genomic and the non-coding RNA probes. Upon merging the images of whole genomic and non-coding RNA

probe, 96.3% of the signals were found to colocalise, confirming that the non-coding RNA probe reliably detected ncDNA in virtually all apoptotic bodies (Fig. 3a). Consequently, all subsequent images obtained using the non-coding RNA probe were considered to represent ncDNA.

We examined the apoptotic bodies of A375 cells using dual FISH probes against ncDNA and those against archaea, fish, mouse, rat and monkey DNA (Fig. 3b). We found a high degree of co-localisation in each case (89.2–95.6%) between ncDNA and species-specific probes, as indicated in the adjacent histograms. We then performed immunoFISH using a FISH probe against ncDNA and antibodies against various species-specific proteins. We observed a similarly high degree of co-localisation of ncDNA and various proteins (range between 93.2% and 99.6%), as indicated in the adjacent histograms (Fig. 3c). These findings were further validated in human embryonic kidney (HEK-293) and human cervical cancer (HeLa) cells (Supplementary Fig. 3). Collectively, these results indicated that almost all apoptotic bodies derived from human cells consist of ncDNA and contain sequences from various species, capable of synthesising their respective species-specific proteins that become detectable following apoptosis.

Fig. 3

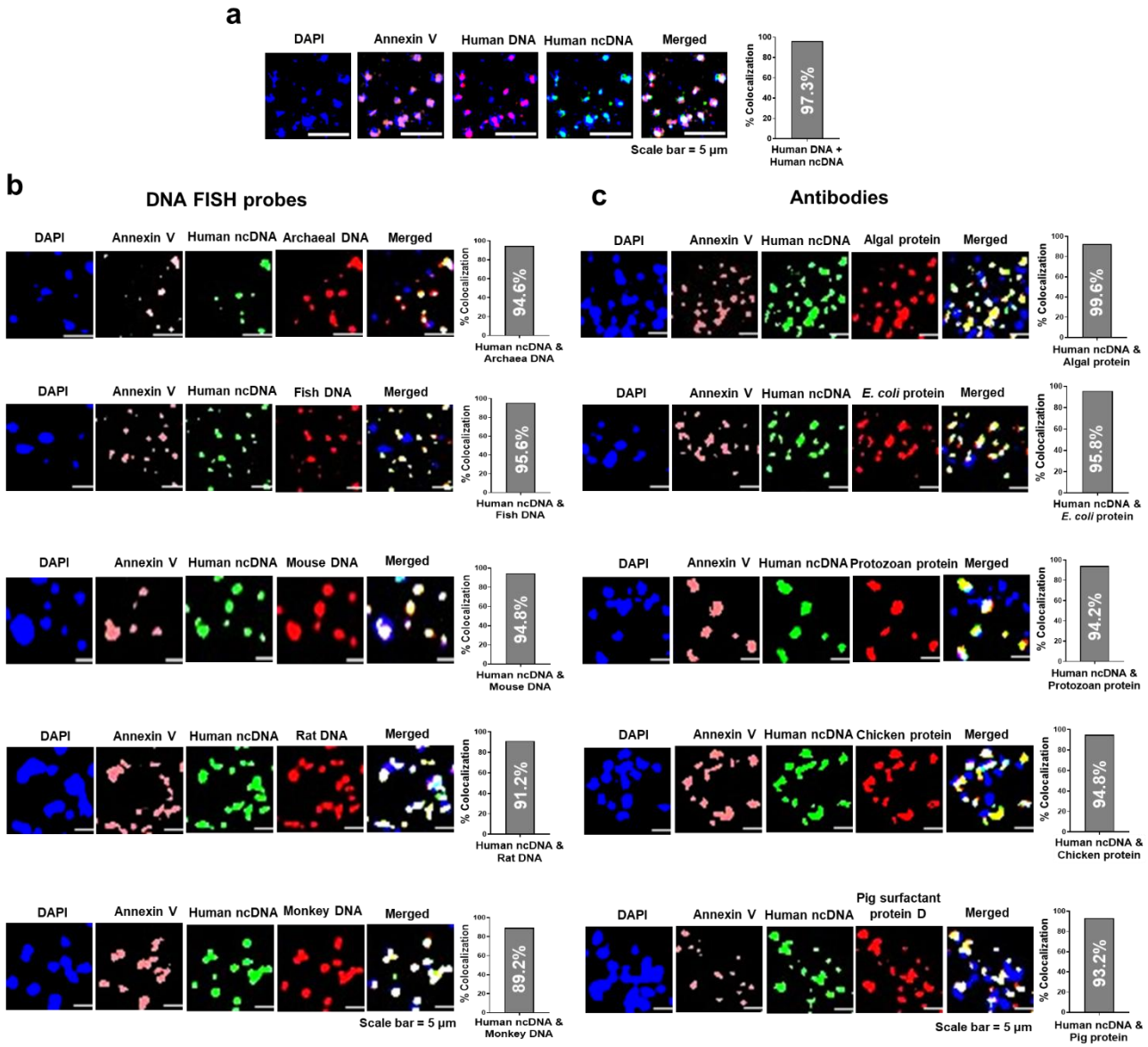

Fig. 3: ncDNA in apoptotic bodies of A375 human melanoma cells reveals extensive phylogenetic diversity. a. Apoptotic bodies were simultaneously probed with an antibody against Annexin V (pink), a whole-genome DNA FISH probe (red) and a long non-coding RNA probe (green) to detect ncDNA. One thousand apoptotic bodies positive for human whole-genome DNA were analysed to determine ncDNA positivity the percentage of apoptotic bodies which were also positive for ncDNA (histogram). A high degree of co-localisation of whole human genomic DNA and ncDNA (96.3%) is observed, indicating that nearly all apoptotic

bodies were composed of ncDNA. The merged images are exclusive of Annexin V signals. **b.** Apoptotic bodies were simultaneously probed with an antibody against Annexin V (pink), a long non-coding RNA probe (green), and a variety of species-specific DNA FISH probes (red). One thousand ncDNA-positive apoptotic bodies were analysed to determine the percentage of apoptotic bodies positive for species-specific DNA (histogram). A high degree of co-localisation of ncDNA and species-specific DNA is observed. For fluorescence colour consistency, FISH probes against pig, eubacteria and fungi were pseudo-coloured as red, and the corresponding human long non-coding RNA probe was pseudo-coloured as green. Not all DAPI-stained apoptotic particles reacted with Annexin V antibody, likely owing to uneven staining. The merged images are exclusive of Annexin V signals. **c.** Apoptotic bodies were simultaneously probed with an antibody against Annexin V (pink), a long non-coding RNA probe (green) and various species-specific antibodies (red). One thousand apoptotic bodies positive for ncDNA were analysed to determine the percentage of apoptotic bodies positive for species-specific antibodies (histogram). A high degree of co-localisation of ncDNA and species-specific antibodies is observed. Not all DAPI-stained apoptotic particles reacted with Annexin V, likely owing to uneven staining of the apoptotic cells from which they were derived. The merged images are exclusive of Annexin V signals. All experiments presented in Fig. 3 were performed twice by two different investigators.

Discussion

The present report is founded on two of our earlier studies[1,2]. The first study revealed that circulating cfChPs, largely composed of ncDNA, originate from apoptotic cells generated during normal cellular turnover and that they can horizontally transfer to healthy cells. Once inside their new hosts, they randomly assemble into complex concatemers, some of which are ostensibly multi-megabase pairs in size, to become detectable by specific FISH probes and antibodies[1]. The second indicated that high-molecular-weight DNA, when

sonicated to generate 500–3000-bp fragments, similar in size to circulating cfChPs, can undergo unrestricted horizontal transfer into heterologous cells without being restricted to inter-specific and inter-kingdom boundaries[2]. Thus, sonicated particles from humans, bacteria and plants could readily enter into nuclei of mouse cells, whereas those from humans, bacteria and plants could spontaneously enter into bacteria. This observation suggests that, over the course of evolution, DNA–protein complexes from dying organisms, especially during periods of severe environmental stress[3–5], may have exchanged themselves between species and across kingdom boundaries, accumulated in recipient genomes and formed the dense patchwork of sequences that now constitute ncDNA. Findings from our first study suggested that components of these patchwork sequences become detectable using species-specific FISH probes and antibodies following cellular apoptosis. Our current results support this concept by showing that human cell apoptosis exposes the origin and nature of DNA–protein complexes accumulated over evolutionary time and sequestered within human ncDNA. This implies that the phylogenetic diversity of ncDNA in every human cell represents a record of evolutionary history, reflecting a persistent species-spanning legacy. These findings lead us to hypothesise that the “silent” ncDNA within human cells represents the remnants of an ancient genome once functional within primitive organisms, and that the 1% of protein-coding DNA gradually emerged to serve the complex functional requirements of higher-order organisms. Our earlier work[1] had suggested that the “ancient genome” retains the potential to

perform the various functions attributed to the “modern genome”, including DNA, RNA and protein synthesis, guided by distinct biological rules that remain to be elucidated.

We were unable to confirm the phylogenetic diversity that we detected in the apoptotic cells using current DNA sequencing technologies, as the extensively fragmented apoptotic DNA failed quality check analysis, making it unfit for DNA sequencing.

Collectively, we propose that evolution may not have proceeded through random mutations in a hypothetical primordial genome, but rather through the progressive accumulation of DNA–protein complexes derived from diverse species to create an ever-expanding genome that is now represented by ncDNA. In this framework, an appropriate combination of DNA–protein sequences occurring by chance could have favoured the emergence of new species during the long course of evolution.

Methods

Ethics

This study was approved by the Institutional Ethics Committee (IEC) of Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre (TMC) for using archival samples of normal human brain, liver and kidney tissues (approval no. 900927).

FISH probes and antibodies

A list of the FISH probes and antibodies used in this study, their species origin, and procurement sourcea is provided in Supplementary Table 1 a–c. A list of the apoptosis detection reagents is provided in Supplementary Table 1d. FISH probes specific for human, mouse, pig, rat and monkey were custom-synthesised, and their species specificity was certified by the respective companies (company names and their locations are provided in Supplementary table 1a). DNA probes for eubacteria, fish, archaea, dogs and fungi were procured commercially, and species specificity was certified by the respective manufacturers (the names of the companies and their country locations are listed in Supplementary table 1a). Nonetheless, we independently tested each DNA FISH probe and antibody against cells and tissues of the various species examined to confirm species specificity and exclude cross-reactivity (Supplementary Fig. 1).

The human long non-coding RNA probe, composed of a set of Stellaris RNA FISH probes containing up to 48 unique sequences, each labelled with a fluorophore that collectively binds along an RNA target transcript, was procured commercially as indicated. For fluorescent colour compatibility, two differently labelled long non-coding RNA probes (red and green) were used as required.

Cell lines and culture

A list of cell lines used in this study, their tissue origins, culture media used and procurement sources is provided in Supplementary Table 2. The cells were cultured in 35-mm dishes at 37 °C, 95% air, 5% CO_2, and 95% humidity. For the experiments, 6×10^4 cells were plated per dish and used the following day when the cell density was approximately 1×10^5.

Flow cytometry

Cells were incubated in a hypoxic chamber (1% O_2) for 72 h, harvested by gentle scraping, pelleted, and resuspended in Annexin binding buffer. To identify apoptotic cells, cells were stained with FITC-labelled Annexin V for 20 min in the dark and sorted using a FACS Aria III (BD Biosciences, San Jose, CA, USA) with FACS Diva software (version 4.0.1.2; Becton, Dickinson and Company). Cells were gated based on forward- and side-scatter characteristics, and Annexin V-positive cells were sorted, collected in PBS, and processed for IF and FISH.

IF and FISH

<u>IF and FISH on cultured cells</u>

The various cell lines were cultured in 35-mm dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS) in a

humidified incubator at 37 ºC with 5% CO_2. Cells (1×10^5) were harvested, cytospun onto positively charged slides, and fixed in 4% paraformaldehyde (PFA). For IF analysis, the unbound PFA was neutralised with 0.3 M glycine, treated with saponin buffer containing 5% normal goat serum (1 h), and immunostained with appropriate primary and secondary antibodies. The slides were mounted using VECTASHIELD DAPI (Vector Laboratories, Newark, CA, USA, cat. no. H1200). For the FISH analysis, the cells were dehydrated in a molecular-grade ethanol series (70%, 80% and 100%) and hybridised with the appropriate FISH probes diluted in hybridisation buffer (10% dextran sulfate, 10% deionised formamide and 2X SSC dissolved in nuclease-free water) in a humidified chamber at 37 ºC overnight (16 h). Slides were washed in 2×SSC for 15 min at 37 ºC and 1×SSC at room temperature (RT) for 10 min. The slides were mounted using VECTASHIELD DAPI. For both IF and FISH, the images were acquired using an Applied Spectral Bio-Imaging System (Yokne'am Illit, Israel).

<u>IF and FISH on formalin-fixed paraffin-embedded tissue sections</u>

Formalin-fixed paraffin-embedded sections (5 µm thickness) of human brain, liver and kidney were deparaffinised thrice in xylene (100%), re-hydrated through an alcohol series (100% thrice, 80%, 70% and 50%) and distilled water and subjected to antigen retrieval at 90 ºC using sodium citrate buffer (pH 6) for 20 min. Slides were cooled to RT (approximately 25–30 ºC), treated with saponin buffer (1 h) and

stained with Annexin V. Slides were then fixed in 2% PFA for 10 min and subjected to FISH as described in the previous section.

IF and FISH on apoptotic bodies

To generate apoptotic bodies, 1×10^6 A375 melanoma cells were subjected to hypoxia-induced apoptosis. The apoptotic cell suspension containing approximately 1.5×10^5 apoptotic bodies were diluted to 100 µL phenol red-free DMEM and dropped onto glass slides from a height of 2 ft to evenly disperse the apoptotic bodies. Slides were then processed for IF and FISH (described in the subsequent section).

Attempts to sequence apoptotic DNA

We extracted DNA from apoptotic A375 human melanoma cells to confirm our FISH findings of phylogenetic diversity using whole-genome sequencing. However, DNA from two biological replicates failed quality assessment, which precluded genomic sequencing. One of the two agarose gel images is provided in Supplementary Figure 4.

Image acquisition and processing

For fluorescence microscopy, we used the Spectral Bio-Imaging System installed on a BX63 Olympus microscope. Images were captured under a 40× air objective lens using FISH View Software 8.0. All images were captured at identical optical settings for comparison and to prevent saturation and photo bleaching. All images were processed using FISH View Software 8.0.

Use of a long non-coding RNA probe to detect ncDNA

We previously used a human long non-coding RNA probe comprising a set of Stellaris RNA FISH probes containing a pool of up to 48 unique sequences, each of which was labelled with a fluorophore[1]. The probe collectively binds to the non-coding RNA target transcript[1]. We reported that, when tested against human DNA, the RNA probe aligned with the full DNA length with almost 100% coverage[1]. Leveraging the ability of the non-coding RNA probe to detect ncDNA, we used it to identify ncDNA. Apoptotic bodies were simultaneously probed with antibodies against Annexin V, the non-coding RNA probe, and various species-specific FISH probes.

Procurement and processing of positive-control cells and tissues

Sources of cells and tissues used as positive controls are provided in Supplementary Table 3. Cells and tissues were processed as follows: archaebacteria, eubacteria (*E. coli)*, fungus (yeast) and protozoa (*Entamoeba*

histolytica) cells were fixed in 4% PFA, smeared onto slides, air-dried and processed for FISH and IF. Algal samples (moss tissue) were washed with PBS, fixed in 4% PFA, smeared on slides, air-dried and processed for FISH and IF. NIH3T3 mouse fibroblasts, dog cells and monkey cells were cytospun onto slides, fixed in 4% PFA and processed for FISH and IF. Drosophila lymph gland, chicken liver, rat liver and pig liver tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin blocks and sectioned for FISH and IF.

Attempts to sequence apoptotic DNA

We extracted DNA from apoptotic A-375 human melanoma cells to confirm our FISH findings of phylogenetic diversity of apoptotic cells using whole genome sequencing. However, the DNA from two biological replicates failed the quality check, which precluded any genomic sequencing.

Statistical analyses

Our study did not involve any formal statistical analysis.

Data Availability

All the data are included in the manuscript. Additional data will be provided upon reasonable request to the corresponding author.

References

1. Banerjee, S. *et al.* Horizontally transferred cell-free chromatin particles function as autonomous ‘satellite genomes’ and vehicles for transposable elements within host cells. *eLife* **13**, RP103771 (2025).

2. Raghuram, G. V. *et al*. Physical shearing imparts biological activity to DNA and ability to transmit itself horizontally across species and kingdom boundaries. *BMC Mol. Biol.* **18**, 21 (2017).

3. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74 (2012).

4. Bijlsma, R. & Loeschcke, V. Environmental stress, adaptation and evolution: an overview. *J. Evol. Biol.* **18**, 744-749 (2005).

5. Hoffmann, A. A. & Parsons, P. A. *Extreme Environmental Change and Evolution*, (1997).

6. Nevo, E. Evolution under environmental stress at macro- and microscales. *Genome Biol. Evol.* **3**, 1039-1052 (2011).

7. Mattick, J. S. RNA regulation: a new genetics? *Nat. Rev. Genet.* **5**, 316-323 (2004).

8. Eddy, S. R. The C-value paradox, junk DNA and ENCODE. *Curr. Biol.* **22**, R898-R899 (2012).

9. Taft, R. J., Pheasant, M. & Mattick, J. S. The relationship between non-protein-coding DNA and eukaryotic complexity. *BioEssays* **29**, 288-299 (2007).

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Author Contributions

R.J., A.R., L.K., R.S., M.M. and S.B. performed the immunofluorescence and FISH experiments. R.L. was responsible for cell culture and provided the live and hypoxia-induced apoptotic cells. G.V.R. supervised the immunofluorescence and FISH experiments and wrote the manuscript. S.S. performed flow-cytometry sorting experiments, supervised the IF and FISH analyses and wrote the manuscript. I.M. conceptualised and was responsible for overall supervision of the work, procured funding, wrote the manuscript and finalised and approved the final version.

Competing Interests

The authors declare no conflict of interest.

Materials & Correspondence

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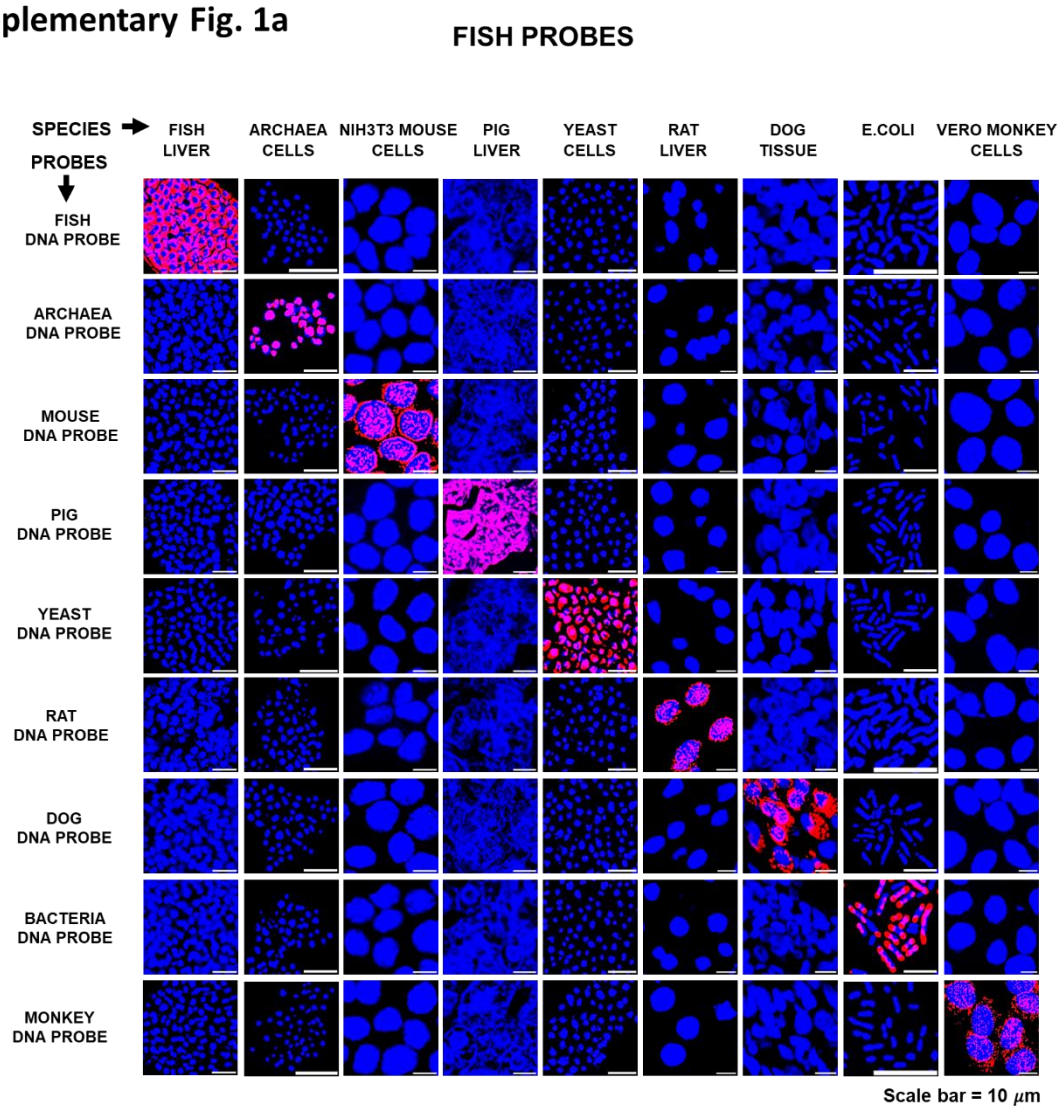
Additional Information

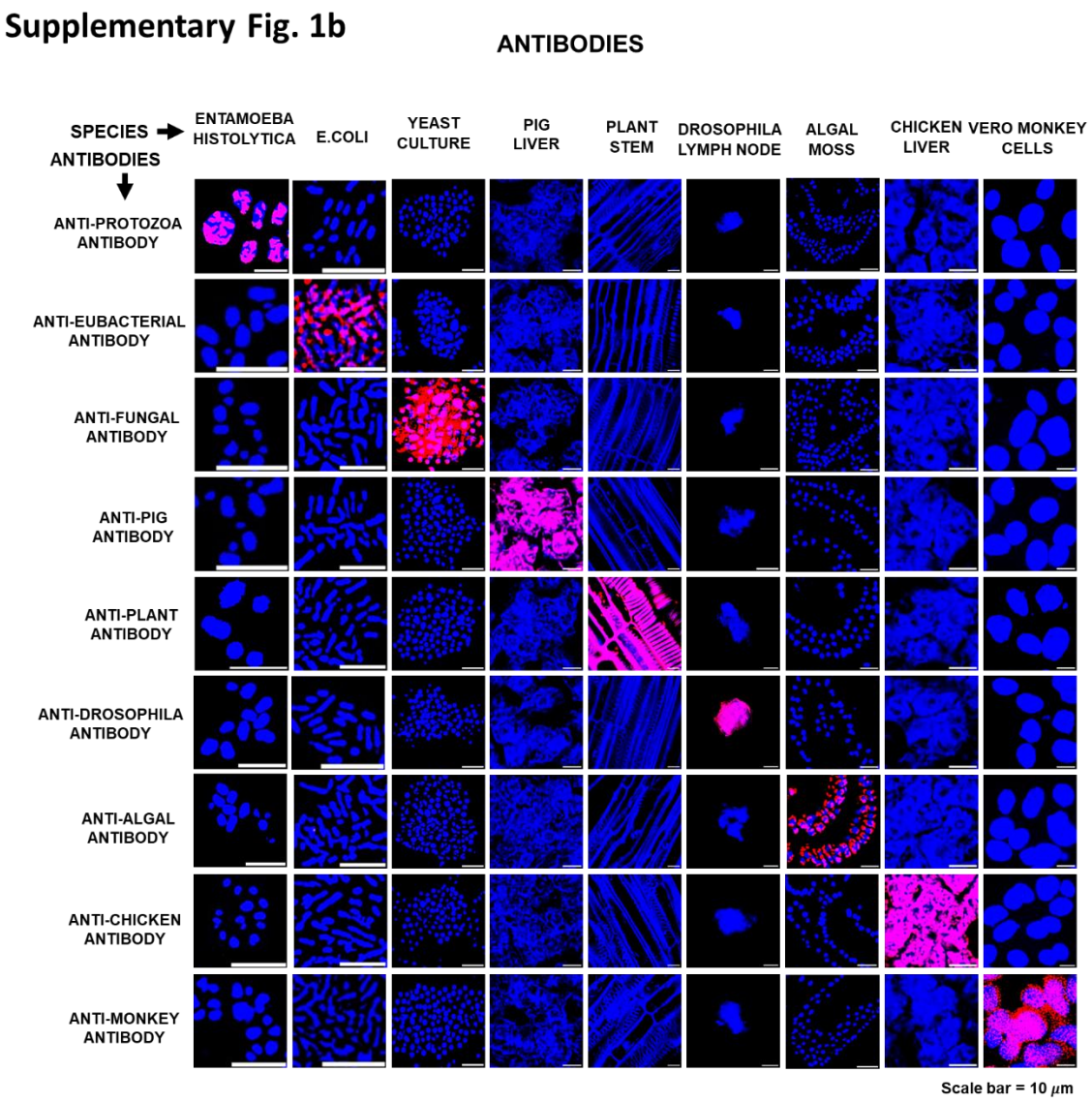
Supplementary Information is available for this paper.

Supplementary Information

Supplementary figures

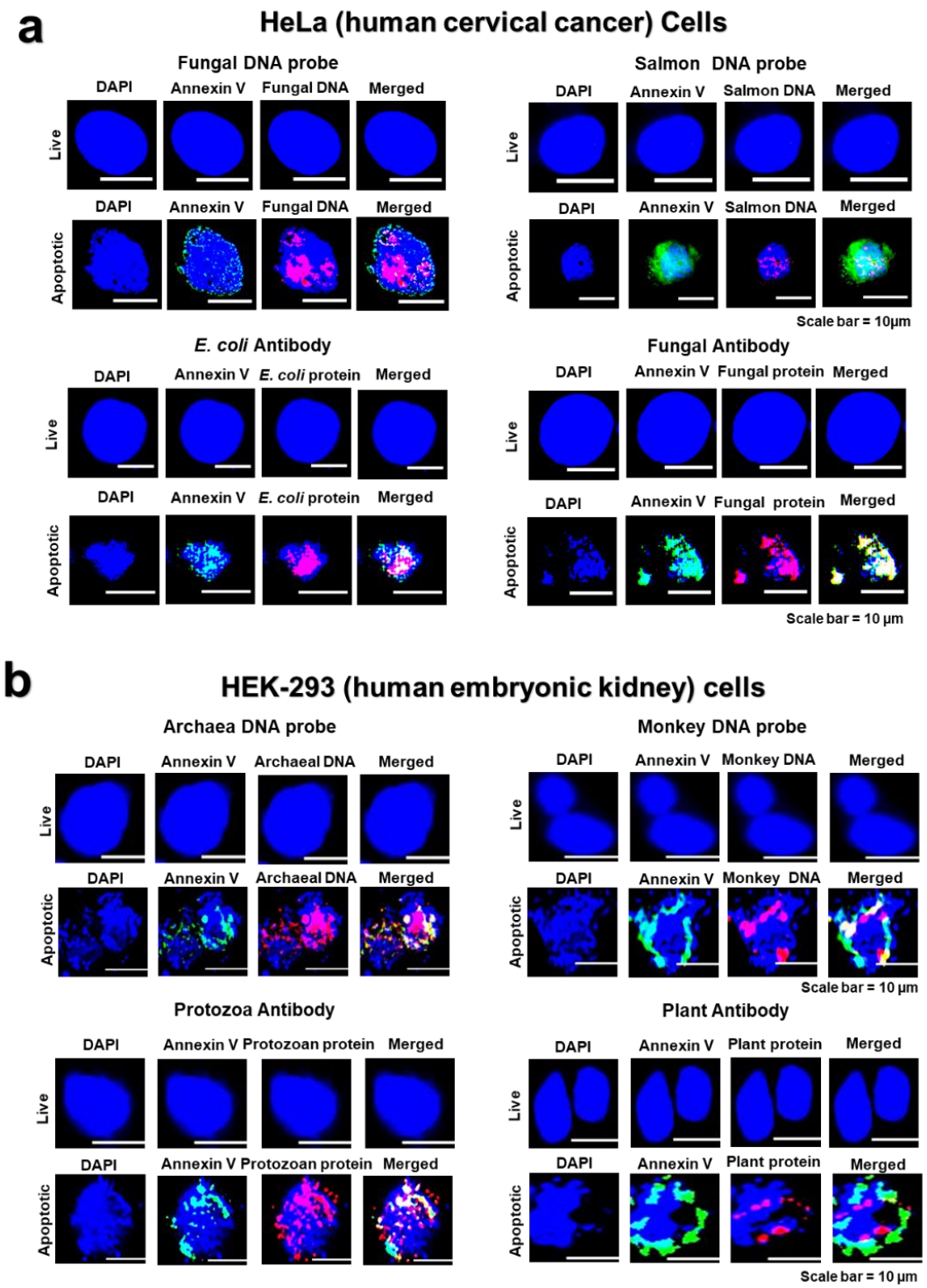
Supplementary Fig. 1a





Supplementary Fig. 1. Experiments to confirm species-specificity of FISH probes and antibodies. **a.** Cells were fixed with paraformaldehyde for 10 min, whereas formalin-fixed, paraffin-embedded sections were used in the case of tissues. Cells and tissue sections were probed with species-specific probes as indicated. **b.** Same as in (a), except cells and tissue sections were probed with appropriate species-specific antibodies as indicated.

Supplementary Fig. 2



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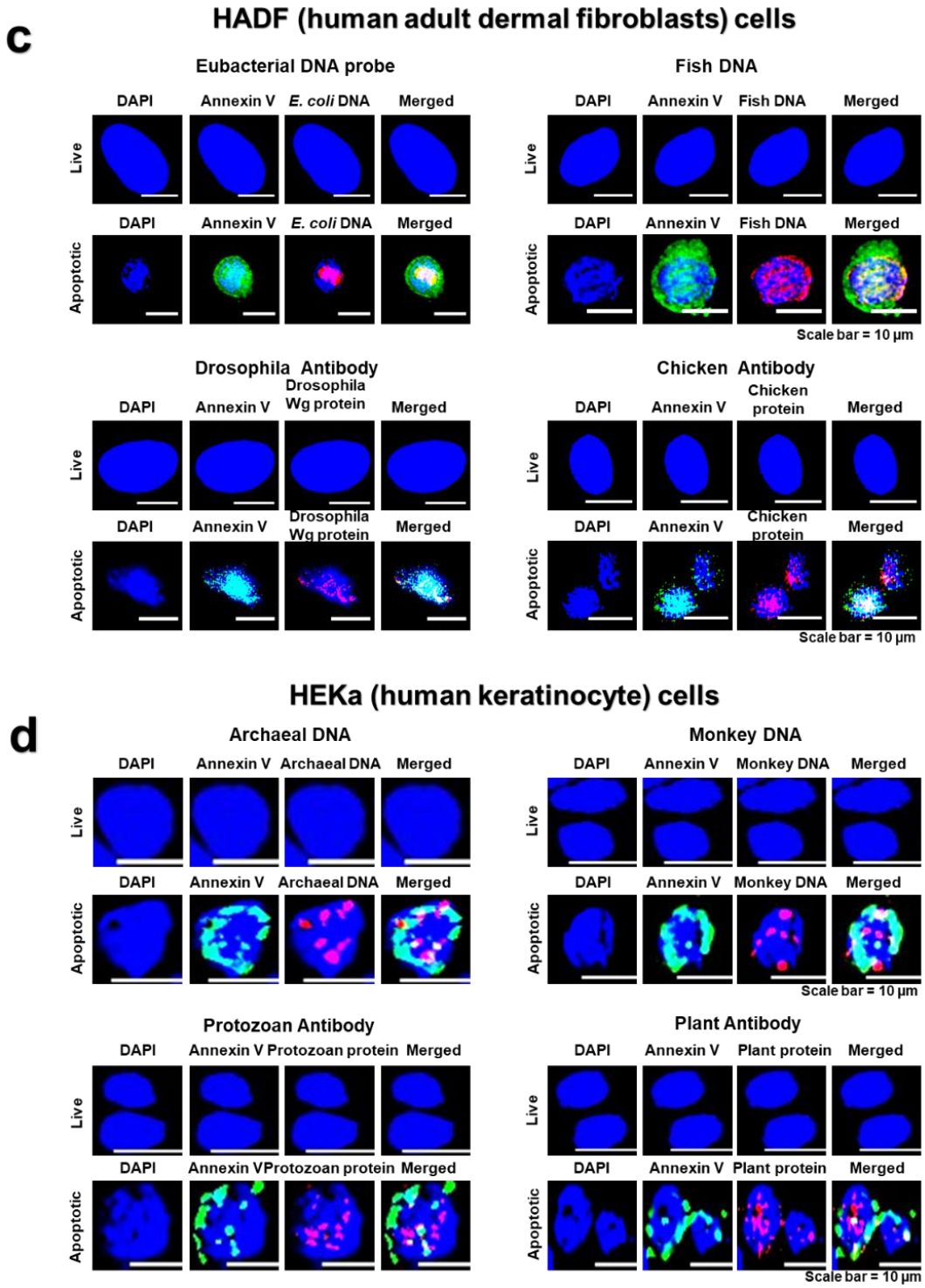
Supplementary Fig. 2 (Cont….)



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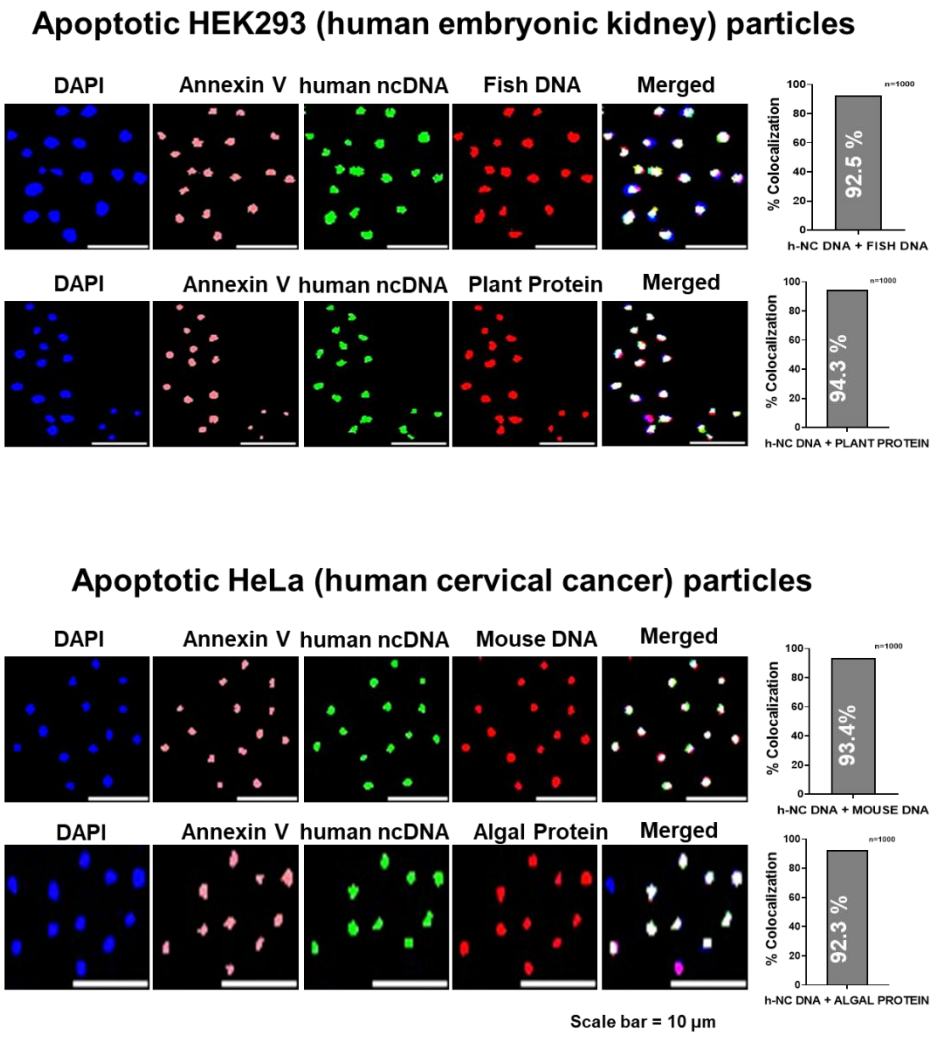
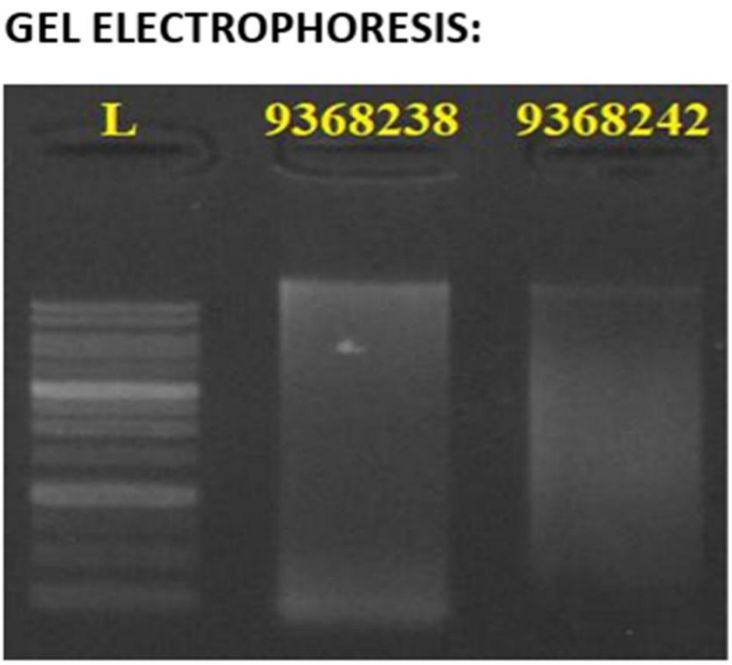
Supplementary Fig. 2. Apoptosis of diverse human cell types reveals their phylogenetic diversity. **a–d.** HeLa (human cervical cancer), HEK-293 (human embryonic kidney), human dermal fibroblast cells and human keratinocytes. Methodological details are provided in Fig. 1.

Supplementary Fig. 3



Supplementary Fig. 3: Non-coding DNA in apoptotic bodies of cell types other than A375 human melanoma cells reveals their phylogenetic diversity. Methodological details are provided in Fig. 3.

Supplementary Fig. 4



Supplementary Fig. 4: Agarose gel electrophoresis of hypoxia-induced apoptotic cells showing a smear pattern, which failed the quality-control test for whole genome sequencing.

Supplementary tables

Supplementary Table 1a. DNA FISH probes.

FISH probe	Cat. No.	Company	FISH probe dilution
Human genomic DNA (Cy3) Red	Custom-synthesised	Applied Spectral Imaging, Yokne'am Illit, Israel	1:5
Mouse genomic DNA (Cy3) Red	Custom-synthesised	Chromosome Science Labo Inc., Sapporo, Japan	1:10
Pig genomic DNA **(**FITC**)**	Custom-synthesised	Chromosome Science Labo Inc., Sapporo, Japan	1:10
Rat genomic DNA **(**Cy3**)** Red	Custom-synthesised	Chromosome Science Labo Inc., Sapporo, Japan	1:10
Monkey genomic DNA (Cy3) Red	Custom-synthesised	Chromosome Science Labo Inc., Sapporo, Japan	1:10
Eubacteria genomic DNA Probe (FITC)	EUB338	Chromosome Science Labo Inc., Sapporo, Japan	1:10
Salmon **fish** genomic DNA (Cy3) Red	Salmon cy3	Chromosome Science Labo Inc., Sapporo, Japan	1:10
Archaea-Bacterial DNA, (Cy3) Red	ARC915	Chromosome Science Labo Inc., Sapporo, Japan	1:10
Dog XY chromosome FISH probe;X (Cy3) and Y (FITC)	DXY-15	Chromosome Science Labo Inc., Sapporo, Japan	1:10
Pan-**fungal** genomic DNA (FITC)	Pan-fungal	Chromosome Science Labo Inc.,	1:10

		Sapporo, Japan	

Supplementary Table 1b. Long non-coding RNA probes.

Probe description	**Company**	**Cat. no.**	**FISH probe concentration**
Stellaris® FISH Probes, Human LSINCT5 with FAM	LGC-Biosearch Technologies, Petaluma, CA, USA	VSMF2034 3-5	125 nM
Human 18S ribosomal RNA probe 5′ 6-FAM (5′-UGAGACAAGCAUAUGCUACC UGGC-3′)	Integrated DNA Technologies, San Diego, CA, USA	Custom-synthesised	500 nM

Supplementary Table 1c. Antibodies.

Antigen	**Company**	**Cat. no.**	**Antibody dilution**
Monkey Mouse monoclonal anti-monkey IgG	Life Diagnostic Inc., West Chester, PA, USA	10C2-9	1:250
Mouse Rat monoclonal anti-mouse MHC-II	Abcam, Cambridge, UK	ab25333	1:700
Rat Mouse monoclonal anti-rat RT1A	Bio-legend, San Diego, CA, USA	205201	1:500
Pig Mouse monoclonal anti-pig surfactant Protein D	Bio-Rad, Hercules, CA, USA	MCA2725	1:200
Drosophila Mouse monoclonal anti-*Drosophila* Wg (Wingless; the Drosophila Wnt ortholog)	Developmental Studies Hybridoma Bank, Iowa City, USA	4D4	1:20

Bacteria Mouse monoclonal anti-*Escherichia coli* O15 antigen	Bio-Rad, Hercules, CA, USA	4330-9508	1:200
Algae Mouse monoclonal anti-*Chlamydomonas* Channel rhodopsin 2	Progen GmbH, Heidelberg, Germany	651180	1:200
Fungi Mouse monoclonal anti-*Saccharomyces cerevisiae* Arp3	Santa Cruz Biotechnology, Dallas, TX, USA	Sc-376625	1:500
Protozoa Mouse monoclonal anti-*Leishmania donovani* Kinetoplastid membrane Protein 11	Ray Biotech, Peachtree Corners, GA, USA	DS-MB-01745	1:500
Chicken Mouse monoclonal anti-chicken CSF1R antibody	My-BioSource, San Diego, CA, USA	MBS224833	1:200
Plant Rat monoclonal anti-rice Extensin Glycoprotein	Agrisera AB, Vännäs, Sweden	AS18 4210	1:10
DNA Mouse monoclonal anti-DNA antibody	Novus Biologicals, Centennial, CO, USA	NB110-89473	1:10
Human Rabbit polyclonal anti-human RNA Polymerase III A	GeneTex, Irvine, CA, USA	GTX106241	1:200
Human Mouse monoclonal anti-Human Ribosomal protein (RPLP0)	Sigma-Merck Millipore, Burlington, MA, USA	SAB1402899	1:100

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Supplementary Table 1d. Apoptosis detection reagents.

Reagent name	Company	Cat. no.	Reagent dilution
BD Pharmingen™ FITC Annexin V Apoptosis Detection Kit	BD Biosciences, San Jose, CA, USA	556547	1:20
BD Pharmingen™ PE Annexin V Apoptosis Detection Kit	BD Biosciences, San Jose, CA, USA	559763	1:20
Rabbit polyclonal anti-Annexin V antibody	Antibodies.com, Cambridge, UK	A13619	1:100
Goat polyclonal anti-rabbit IgG (H+L) secondary antibody, DyLight-633	ThermoFisher Scientific, Waltham, MA, USA	35562	1:500

Supplementary Table 2. List of cell lines.

Cell line	Tissue Origin	Source	Culture medium used
A375	Human melanoma	ATCC (CRL-1619)	DMEM + 10% FBS
MRC-5	Human lung fibroblast	ATCC (CCL-212)	EMEM + 10% FBS
MDA-MB-231	Human breast cancer	ATCC (HTB-26)	DMEM + 10% FBS
HEK-293	Human embryonic kidney	ATCC (CRL-1573™)	DMEM + 10% FBS
HeLa	Human cervical cancer	ATCC (CRM-CCL-2™)	DMEM + 10% FBS
HADF	Human dermal fibroblast	HiMedia (CL005)	DMEM + 10% FBS
HEKa	Human keratinocyte	ThermoFisher Scientific (C0055C)	DMEM + 10% FBS

DMEM, Dulbecco's Modified Eagle Medium; EMEM, Eagle's Minimum Essential Medium; FBS, Foetal bovine serum

Supplementary Table 3. Positive-control cells and tissues.

Cells/tissue	**Procurement source**
Archaebacteria cells	National Centre for Cell Science (NCCS), Pune, India
Eubacteria (*E. coli* cells)	From Dr. Vivek Bhat, Department of Microbiology, ACTREC, India
Plant stem	Garden in ACTREC, India
Algae (moss)	Garden in ACTREC, India
Fungus (yeast cells)	From Dr. Sunil Shetty, PI, ACTREC
Protozoa (*Entamoeba histolytica)*	From Professor Samudrala Gourinath, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India
Drosophila lymph gland	From Dr. Rohan Khadilkar, ACTREC, India
Fish liver tissue	From fishmonger, Navi Mumbai
Chicken liver tissue	From a poultry shop
Mouse cells (NIH3T3 embryonic fibroblast)	From ATCC (CRL-1658)
Rat liver tissue	From the animal house, ACTREC, India
Pig liver tissue	From a butcher shop
Female dog cells	Spontaneous malignant round cell tumour in a female dog, developed at the animal facility in ACTREC, India
Monkey cells (Vero)	From ATCC (CCL-81)