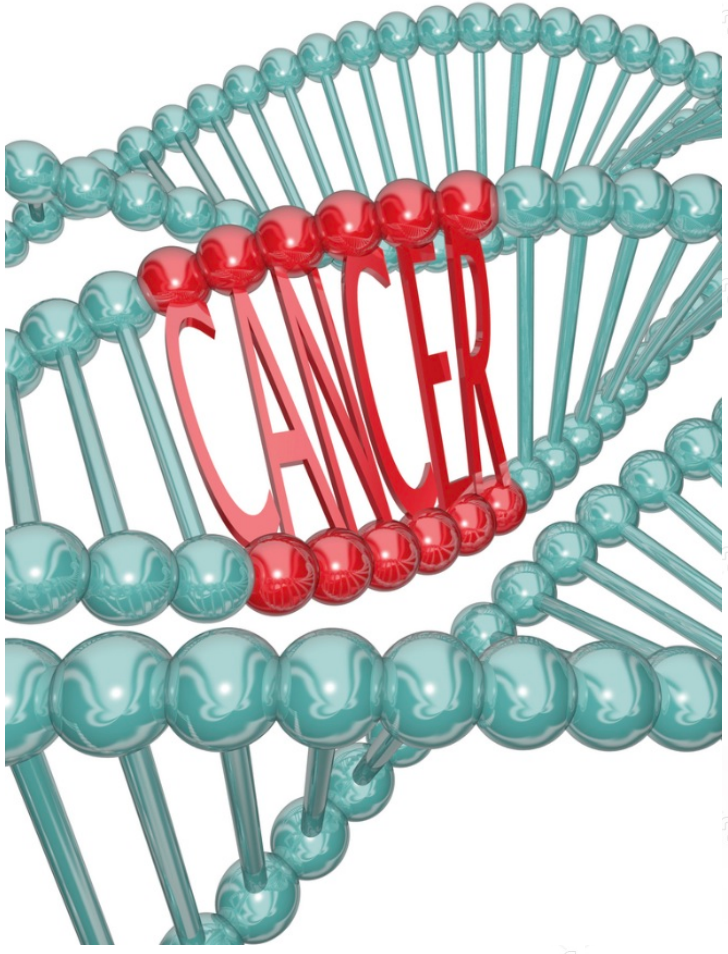


Case studies in comparative cancer genomics

Will Meyerson

Image credit:
https://www.shutterstock.com/image-photo/basset-hound-dog-dressed-veterinarian-wearing-63823888
https://vector.childrenshospital.org/2014/04/the-challenge-of-cancer-genomics-embarking-on-clarity-2/

Cancer afflicts all multicellular organisms … to varying extents

Image from http://hikersnotebook.net/Burls

- Domazet-Lošo, T. *et al.* Naturally occurring tumours in the basal metazoan *Hydra*. *Nat. Commun.* 5:4222 doi: 10.1038/ncomms5222 (2014).
- Doonan, J. and Sablowski, R. Walls around tumours – why plants do not develop cancer. *Nat. Reviews Cancer*. 10:794-802 doi:10.1038/nrc2942 (2010).

How can we learn about biomedicine from animals?

- Animals as experimental subjects – fewer legal, practical, and (possibly) moral barriers to experimentation in animals
- Animals as creatures with extreme physiology – animals have a wider range of phenotypes and genotype than humans, making some generalizable trends more obvious
  - e.g. polar bears when they evolutionarily diverged from brown bears, took on a diet of seal blubber, and subsequently underwent strongly selected mutations in cholesterol processing genes
- Animals as controls for some aspects of human culture
  - e.g. let's say brain cancer has increased in humans in recent decades in tandem with increased cell-phone use. If brain cancer similarly increased in (non-cell-phone-using) animals over the same interval, then we need to look for other explanatory variables

Outline: Three Papers

1. Elephants and TP53 [animals as extreme physiology]
2. Dogs and an ancient tumor [animals as extreme physiology]
3. Veterinary health expenditures in USA [animals as counter-cultural controls] (not actually about cancer genomics, just for fun)

TP53 copy number expansion is associated with the evolution of increased body size and an enhanced DNA damage response in elephants

Michael Sulak, Lindsey Fong, Katelyn Mika, Sravanthi Chigurupati, Lisa Yon, Nigel P Mongan, Richard D Emes, Vincent J Lynch

The University of Chicago, United States; University of Nottingham, United Kingdom; Weill Cornell Medical College, United States; University of Nottingham UK, United Kingdom

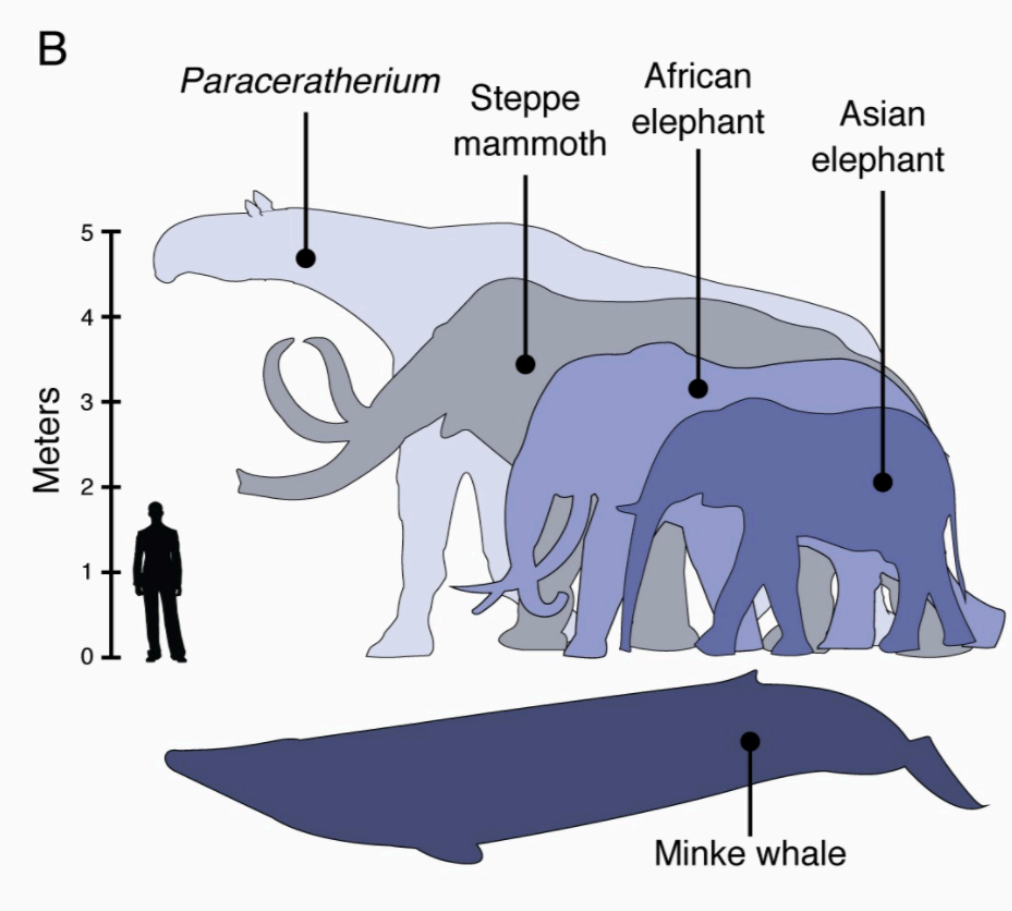
DOI: http://dx.doi.org/10.7554/eLife.11994

Published September 19, 2016

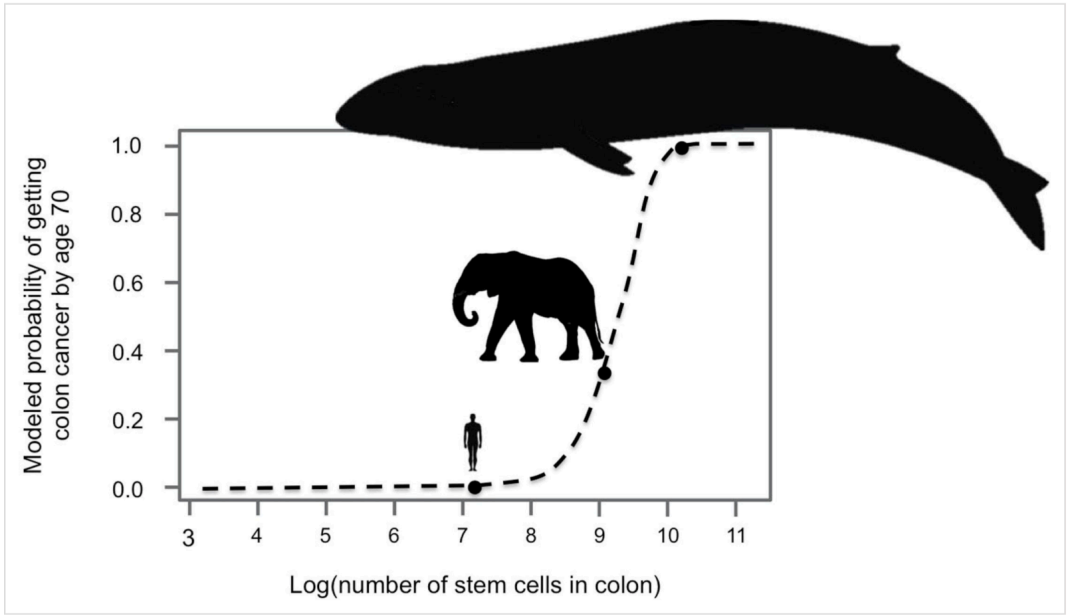
Cite as eLife 2016;5:e11994

This article has been corre

Some animals are really big



We would expect big animals to get cancer much earlier, but they don't (Peto's paradox)



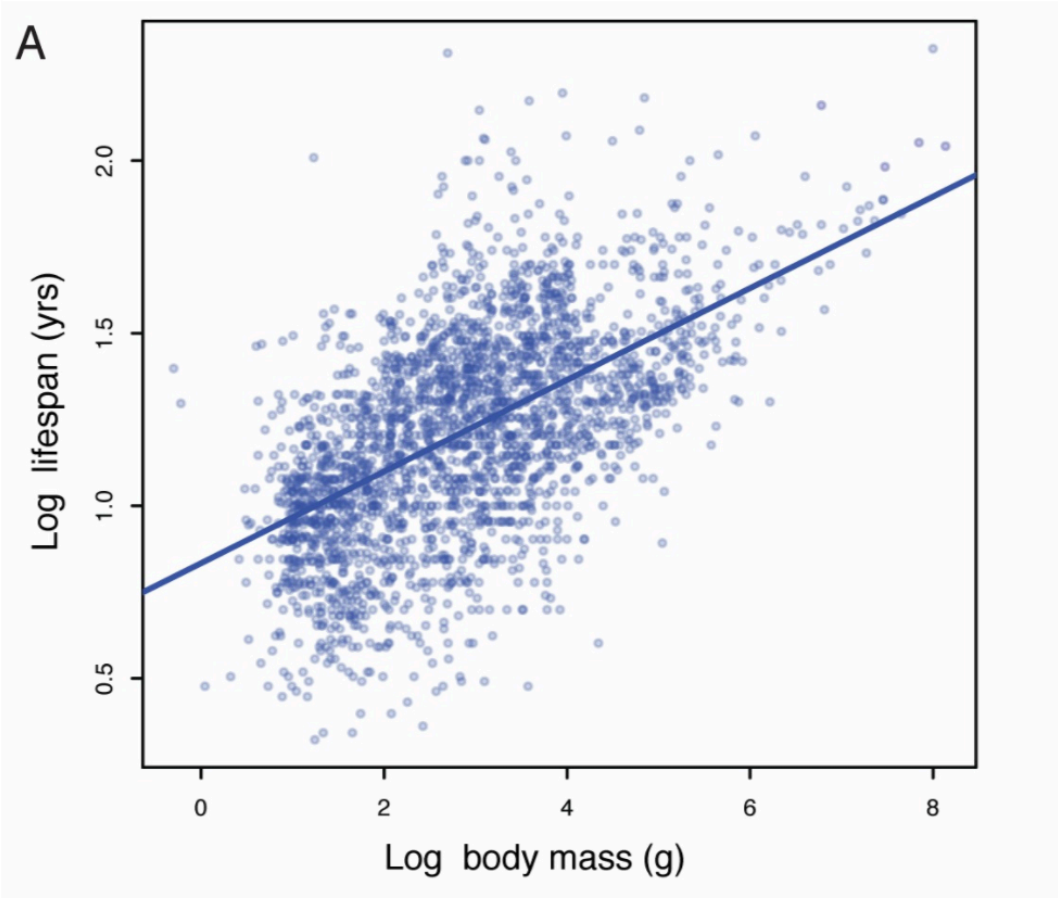
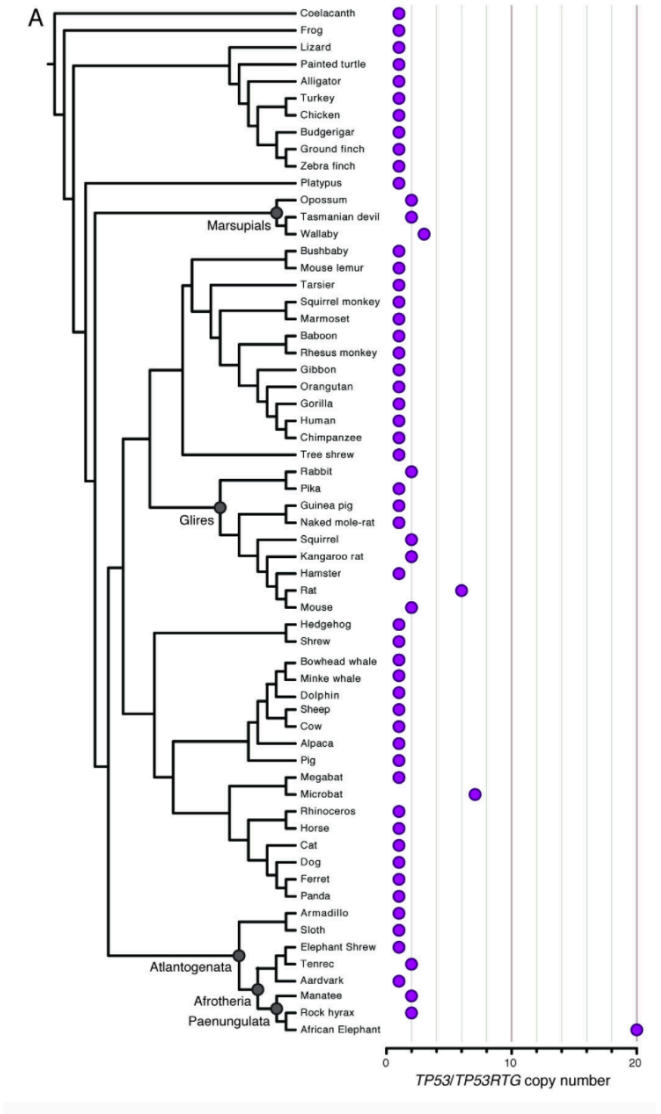


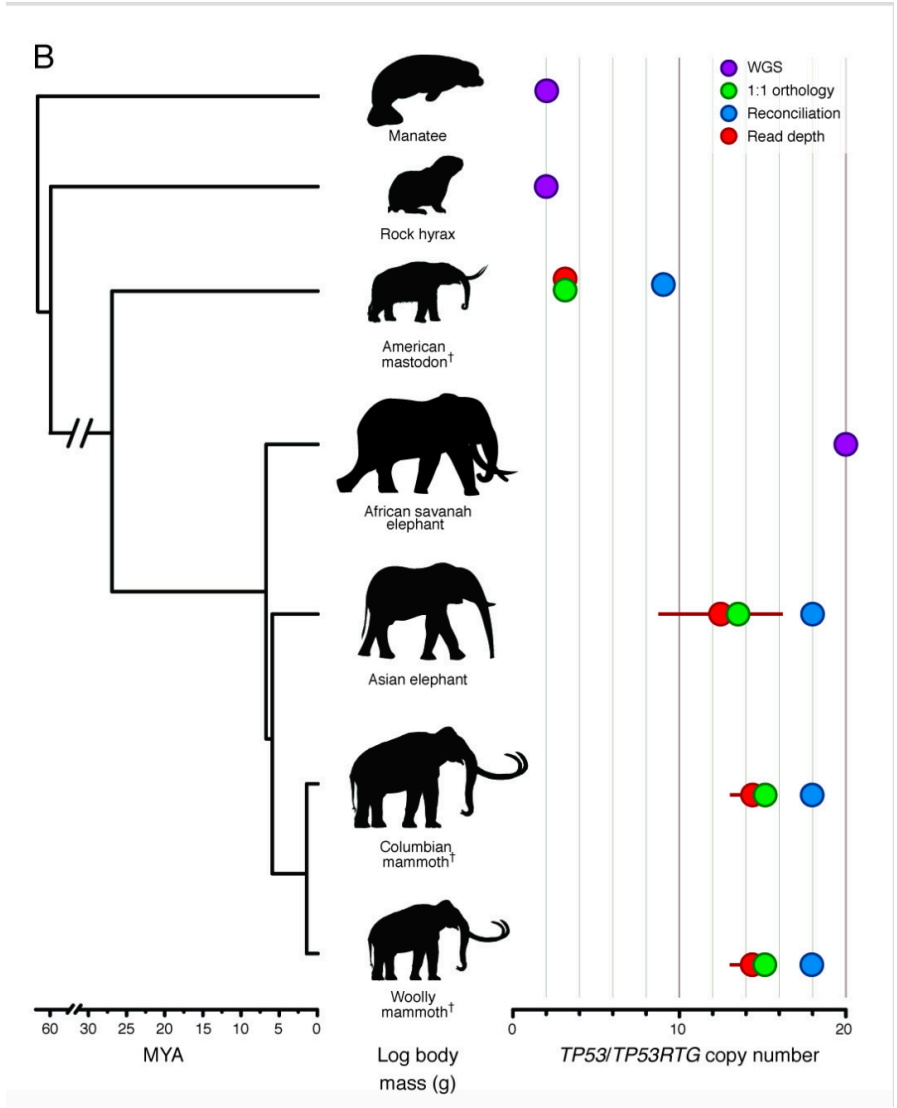
Figure credit: Gaugrhan S. *et al*., Evolutionary biology: How elephants beat cancer. eLife 2016;5:e21864.

Sulak et al. followed up with hints that elephants have more copies of TP53 “The Guardian of the Genome”

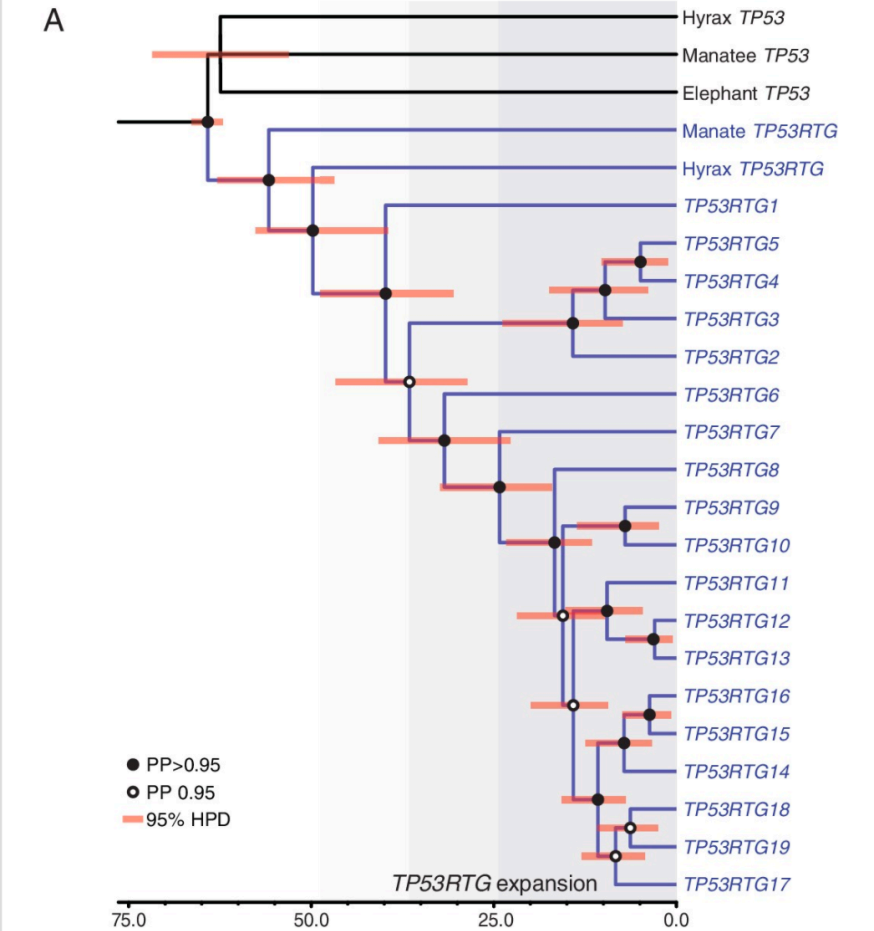
- Used BLAT on Human TP53 sequence against 61 vertebrate genomes including the American mastodon, woolly mammoth, and Columbian mammoth to find canonical TP53 in each species
- From CDS of the canonical TP53 in each vertebrate, used BLAT on the rest of that mammal’s genome to find TP53 orthologues

Elephants have the most retrogenes of “The Guardian of the Genome” (TP53)

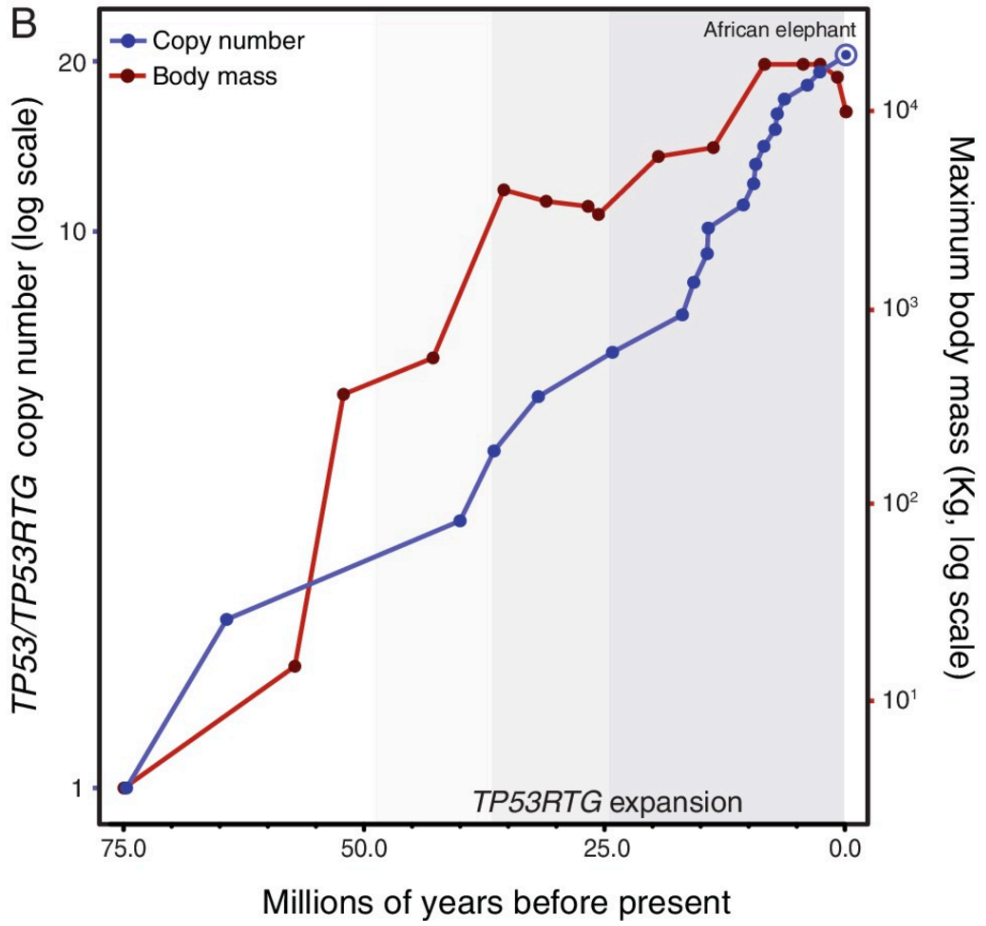




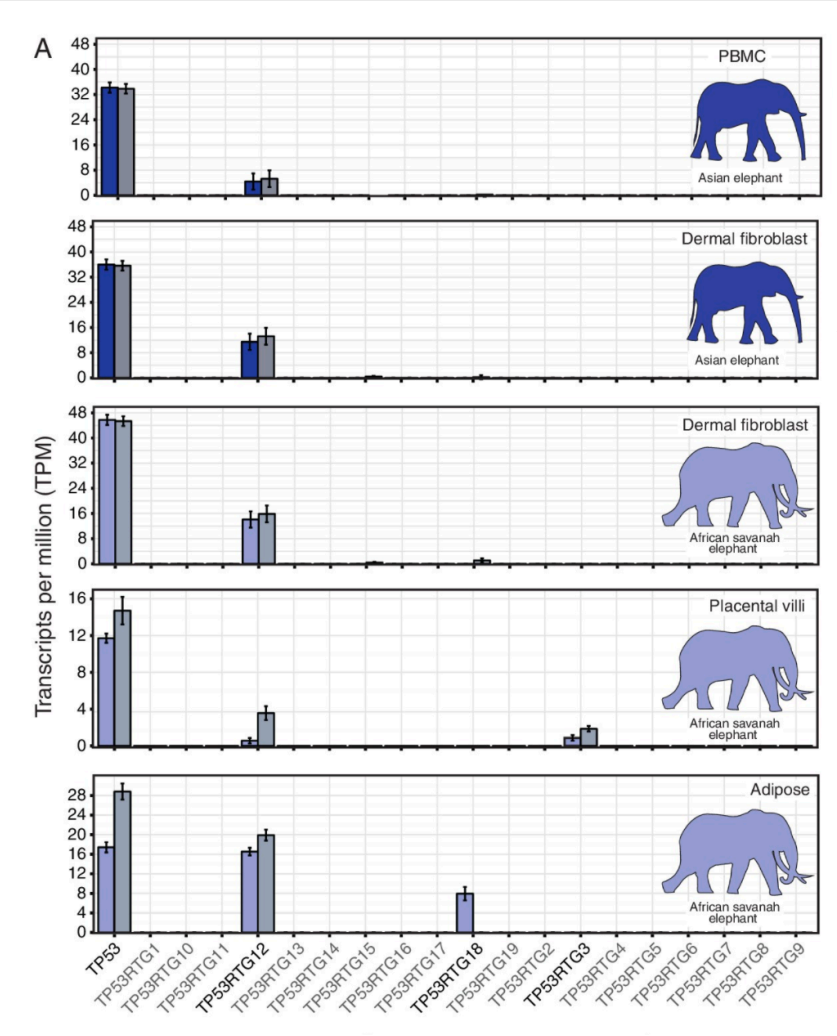
Throughout history, elephant size has increased in tandem with TP53 retrogene copy number



Time calibrated Bayesian phylogeny of *TP53/TP53RTG* genes



At least some of these TP53 retrogenes are transcribed in some profiled cell types

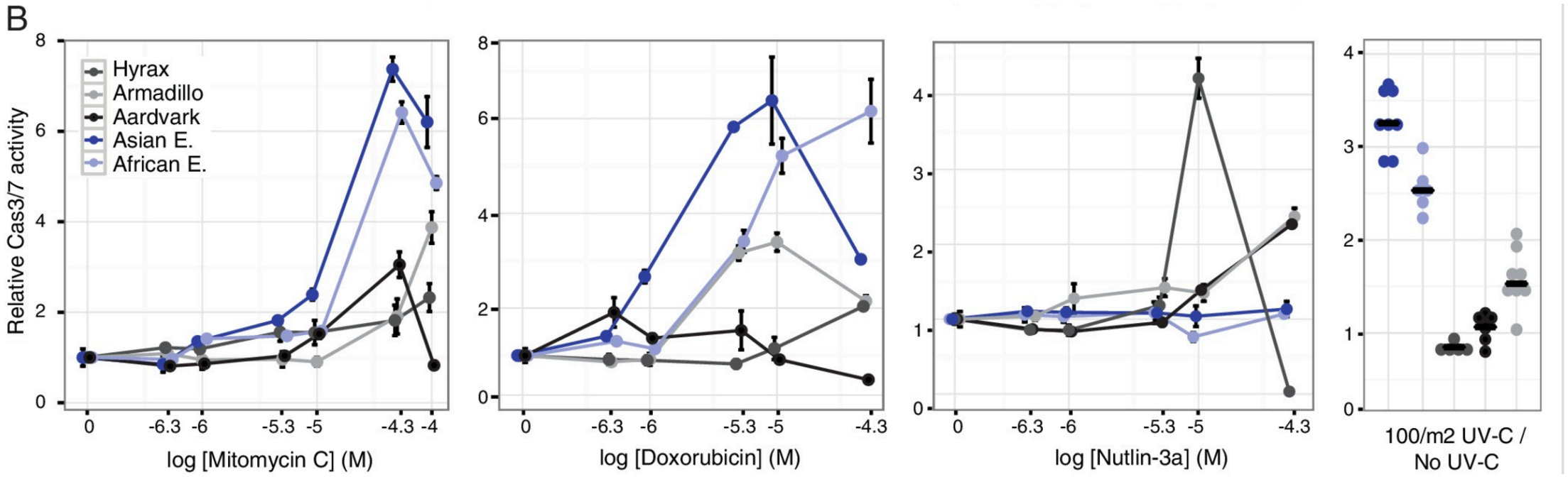


It is not obvious to me from this graph, but the authors claim evidence for five different TP53 retrogenes being transcribed

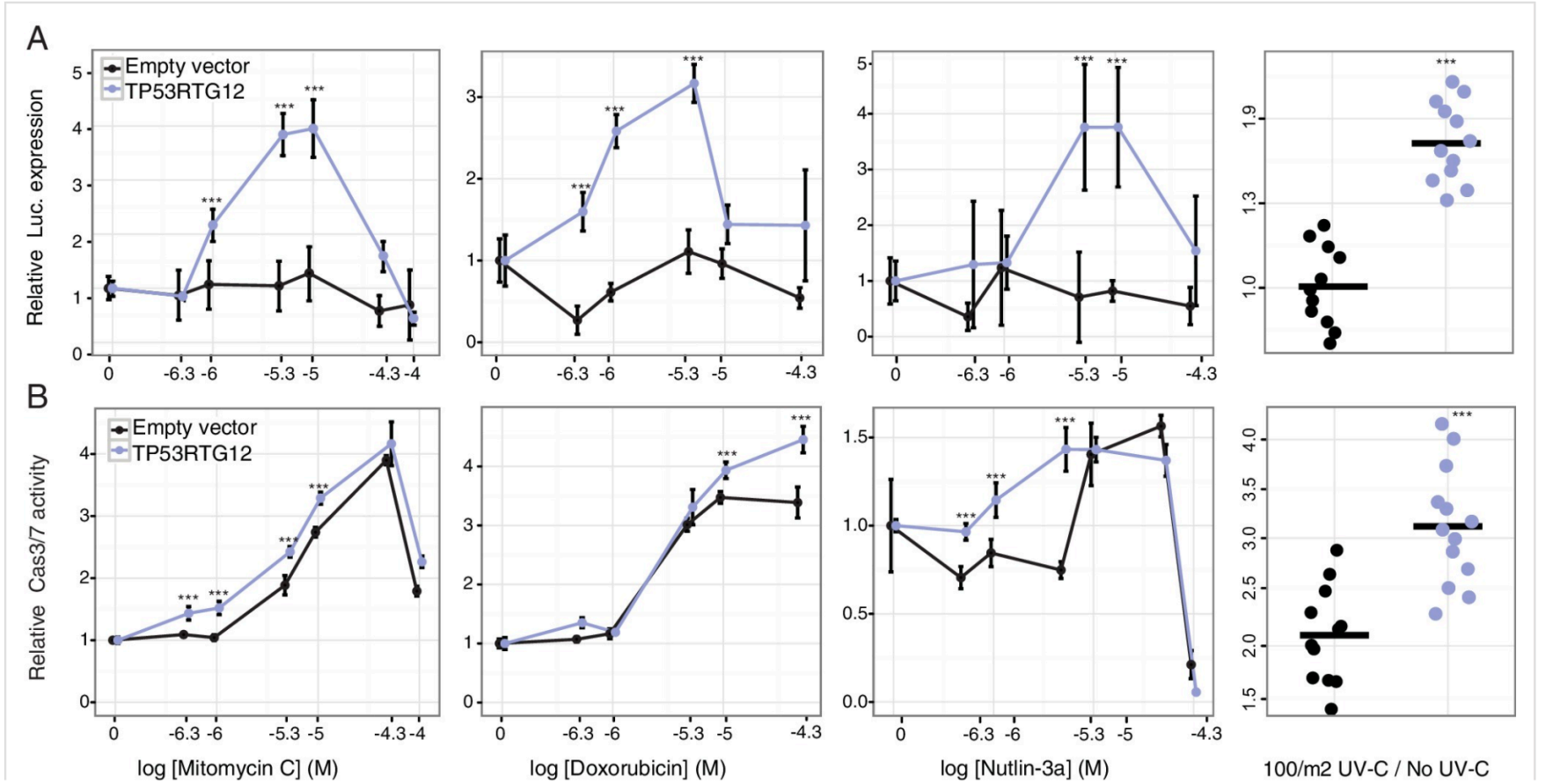
A lot less impressive when you see how few retrogenes are actually transcribed

Maybe there are yet-unprofiled tissues that express other retrogenes?

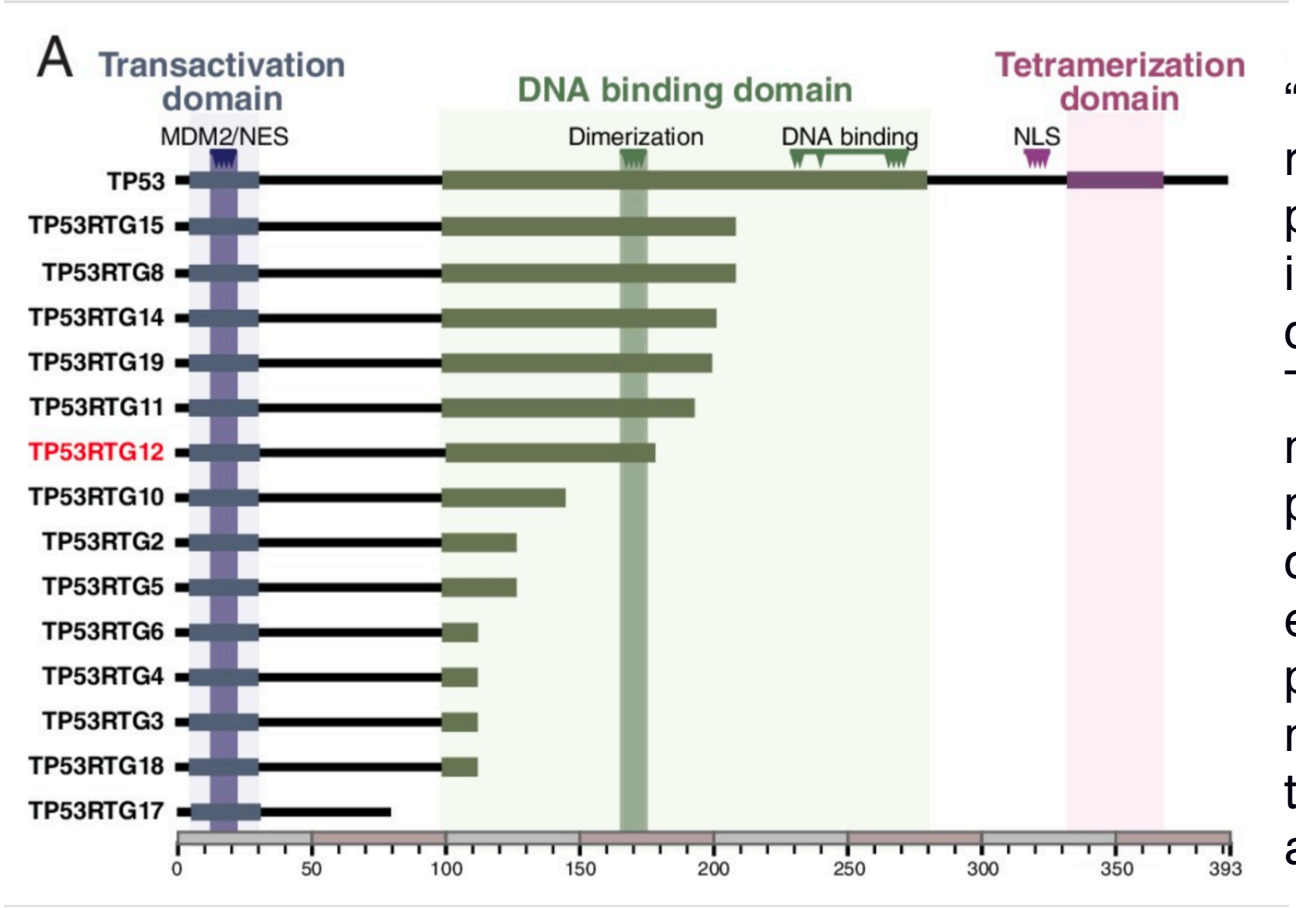
Elephant cells undergo apoptosis more easily than do the cells of related species



Transfecting mouse cells with elephant retro-TP53 makes mouse cells undergo apoptosis more readily



TP53RTG proteins are unlikely to directly regulate TP53 target genes because they lack critical residues required for nuclear localization, tetramerization, and DNA-binding



"While TP53RTG12 does not appear to directly regulate gene expression, many of the TP53RTG proteins (including TP53RTG12) retain the MDM2 interaction motif in the transactivation domain and dimerization sites in the DNA binding domain. These data suggest at least two non-exclusive models of TP53RTG action: (1) TP53RTG proteins may act as 'decoys' for the MDM2 complex allowing the canonical TP53 protein to escape negative regulation and (2) TP53RTG proteins may protect canonical TP53 from MDM2 mediated ubiqutination, which requires tetramerization, by dimerizing with canonical TP53 and thereby preventing the formation of tetramers"

The cost of TP53

- Few animals have extra TP53
- Overexpression of (canonical) TP53 in mice leads to earlier death and reduced fertility (Maier et al., 2004)
- Why can elephants get away with it?
  - Their answer: “It is possible that the costs were minimized because functional *TP53RTG* genes evolved through non-functional intermediates, which accumulated loss of function mutations that minimized redundancy with *TP53*.”

Assessment/Reflections

- What if elephants just have more copy number of genes generally? Would be nice to see some controls of other genes
- I wasn't that impressed with the effect size of the retrogenes' impact on apoptosis in mice
- Can we design a drug that has TP53-like effects for cancer primary prevention of cancer?
- Can we just someday add new copies of modified TP53 through gene-editing to new human embryos for life-long cancer protection?
- In the end, elephant cancer prevention is not That impressive:
  - Sure they're 60x bigger. But they don't smoke. They have slower metabolism. Lifespan is up to 50-70 years, while humans live a bit longer, and cancer risk is exponential with age, and only linear (on theoretical expectation) with body size. Would like more detailed epidemiology of elephant risk for cancer and human risk for cancer at same ages.
  - More impressive is humans' cancer prevention vs little animals (mice that get cancer after 4 years etc)
  - Other species, other strategies are potentially more impressive, but not necessarily imitable in humans (naked mole rats have increased contact inhibition among cells)

Transmissible Dog Cancer Genome Reveals the Origin and History of an Ancient Cell Lineage

Elizabeth P. Murchison[1,2,*,†], David C. Wedge[1,*], Ludmil B. Alexandrov[1], Beiyuan Fu[1], Inigo Martincorena[1], Zemin Ning[1], Jose M. C. Tubio[1], Emma I. Werner[1], Jan Allen[3], Andrigo Barboza De Nardi[4], Edward M. Donelan[3], Gabriele Marino[5], Ariberto Fassati[6], Peter J. Campbell[1], Fengtang Yang[1], Austin Burt[7], Robin A. Weiss[6], Michael R. Stratton[1,†]

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Science 24 Jan 2014:
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Cancer's evolution is cut short

- Typically, a cancer dies with its host
- We might wonder: what would happen to a tumor if it had time to keep evolving?
  - This could exaggerate trends that would make their early signs on human-relevant time-scales more obvious in retrospect
- With the advent of modern cell culture, we've been able to observe cancer progress for longer, but our observations here have two important limitations:
  1. Cell culture does not recapitulate the immune, nutrient, spatial, and metastatic complexity of living hosts
  2. Cell culture has existed thus far on a time-scale of decades, which is not dramatically longer than tumors in living hosts

Canine transmissible venereal tumor (CTVT) is the oldest tumor in the world

- Canine transmissible venereal tumor (CTVT) is a tumor whose cells are passed from dog to dog during mating, allowing it to survive the death of its host
- Normally, host immune systems prevent cancerous cells from one organism from growing in another, but CTVT has powerful immune-evasion mechanisms
- CTVT has endured in this way for millennia and spread to every inhabited continent

Analyzed the genomes of CTVT specimens from two dogs, continents apart

- Two primary analysis dogs: an Australian Aboriginal camp dog and and American cocker spaniel in Brazil
- WGS @ 60 - 100X, 100 bp paired ends reads from tumor + normal (liver)

We don’t have the founder dog’s normal. So how do they call *somatic* SNVs?

- Some common germline dog SNPs are known from dog population genetics
- Find the ratio of known homozygous germline SNPs to known heterozygous germline SNPs in each diploid segment of this dog cancer genome
- Assume that this same ratio approximately holds for unknown germline SNPs in the same diploid segments [Assumption X]
- In non-rearranged portions of dog cancer genome, assume all homozygous variants are germline SNPs (infinite sites assumption)
- Estimate number of heterozygous unknown germline SNPs by [Assumption X]
- (Not sure what they do in non-diploid regions)

Results of Relevance to this Particular Tumor

- Founder dog lived 11,000 years ago
- Last common ancestor of a CTVT sample from Brazil and a sample from Australia was about 460 years ago
  - Dates back to age of exploration
- High rate of inbreeding among founder-dog's ancestors
  - Tasmanian devils live on an island and are only other example of transmissible tumor. Low genetic diversity may make it easier for tumor transmission

Results of Relevance to Cancer Genomics

- CTVT harbors nearly 2 million SNVs (~95% shared between two dogs continents apart)
  - 100-1000X as many SNVs in typical tumor, 10X as much as most-mutant PCAWG lung cancer, but actually less than the most mutant PCAWG colon cancer (2.4M)
- > 2000 candidate SVs (~~90% shared), yet mostly diploid
- No evidence for subclones
  - Any present variants under positive selection in CTVT have completed their selective sweep
  - “This suggests that CTVT is not undergoing positive selection at high frequency, possibly indicating that it is well adapted to its niche”
- Largest mutational signature is UV signature (42%)
  - It is the cancer cells on the surface that are most easily transmitted between dogs
- (Only) four identified driver mutations (SETD2, CDKN2A, MYC, ERG)
  - Surely there are more, but we presumably know little about dog cancer drivers
- 646 genes completely knocked out by homozygous deletion or LOF + deletion
  - Apparently these genes are truly non-essential for cellular survival

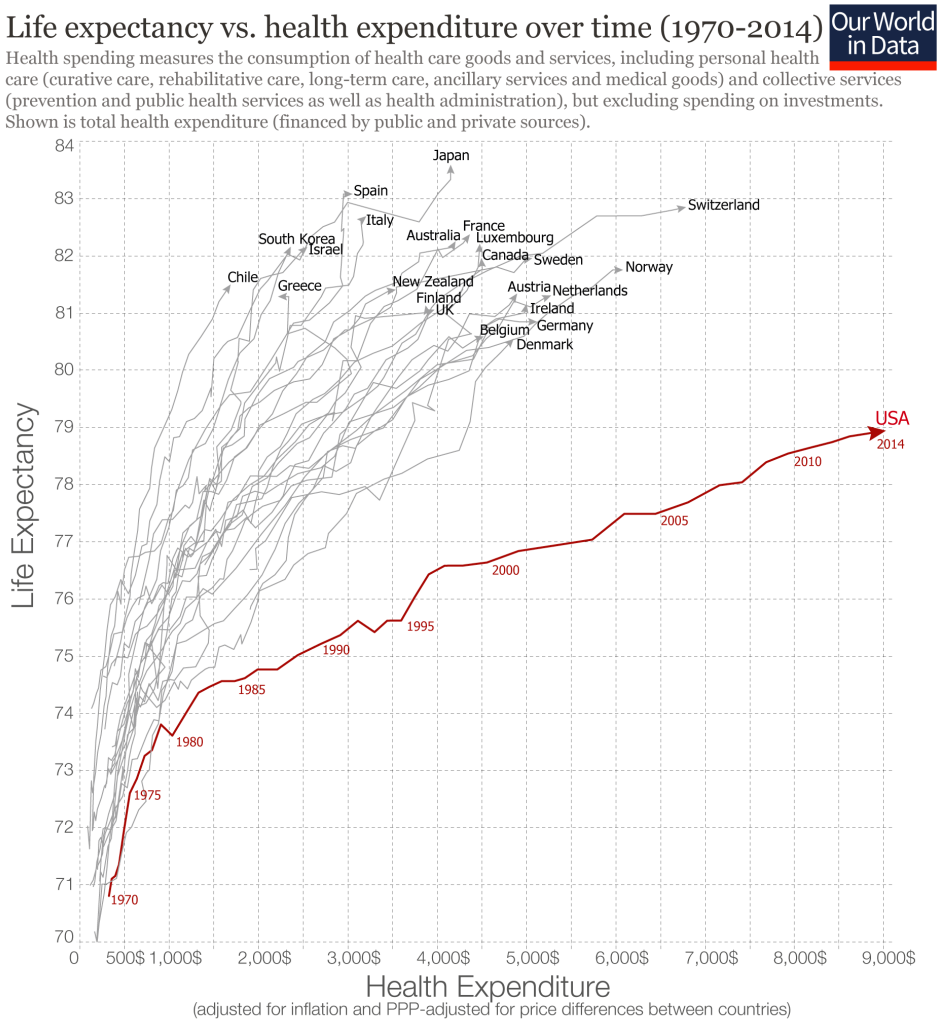
IS AMERICAN PET HEALTH CARE (ALSO) UNIQUELY INEFFICIENT?

Liran Einav
Amy Finkelstein
Atul Gupta

Working Paper 22669
http://www.nber.org/papers/w22669

NATIONAL BUREAU OF ECONOMIC RESEARCH
1050 Massachusetts Avenue
Cambridge, MA 02138
September 2016

Among developed countries, the US is an outlier in terms of spending more on health care for smaller life expectancy gains



From ourWorldindata.org

Why does US health care cost so much?

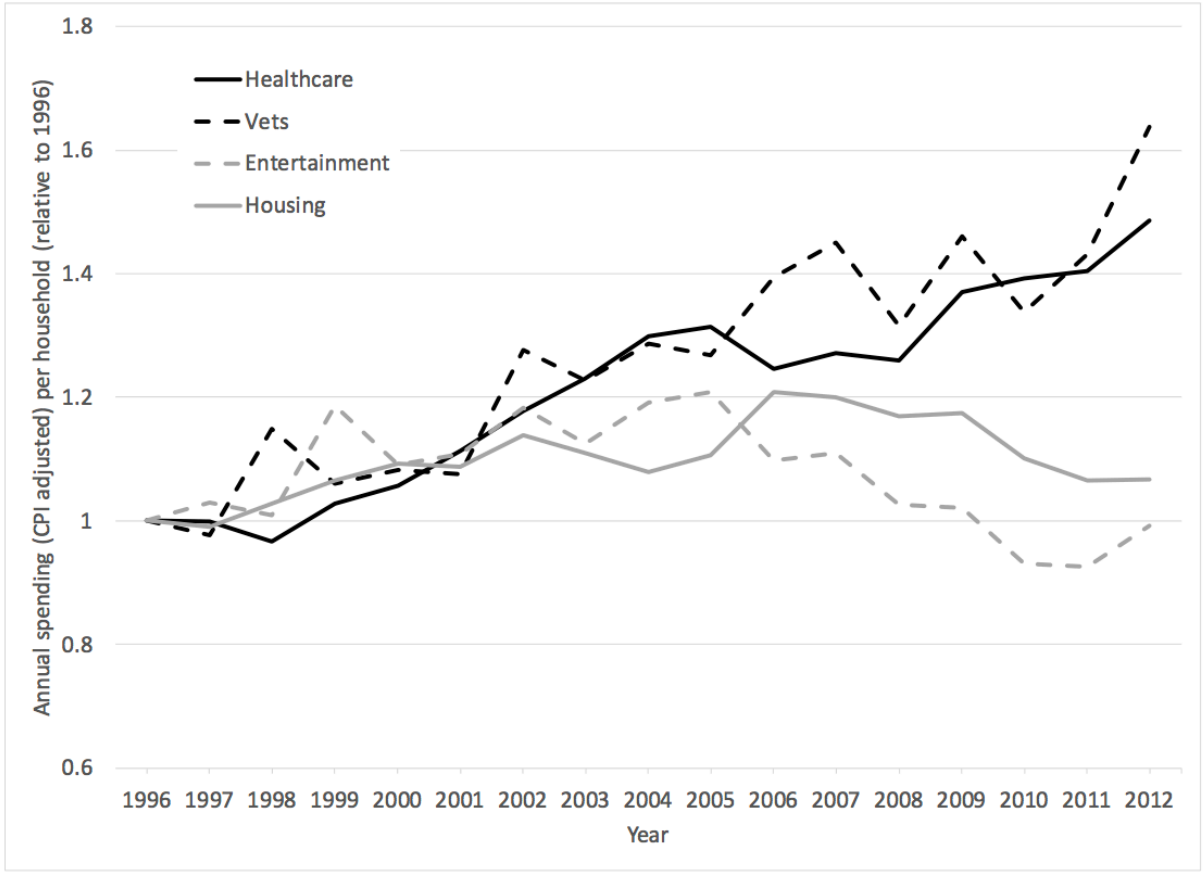
- US malpractice law incentivizing defensive medicine?
- De-coupling of cost Insurance?
- Regulations?
- American lifestyle (e.g. lack of exercise)
- …

VS: We have the money and this is how we want to spend it.

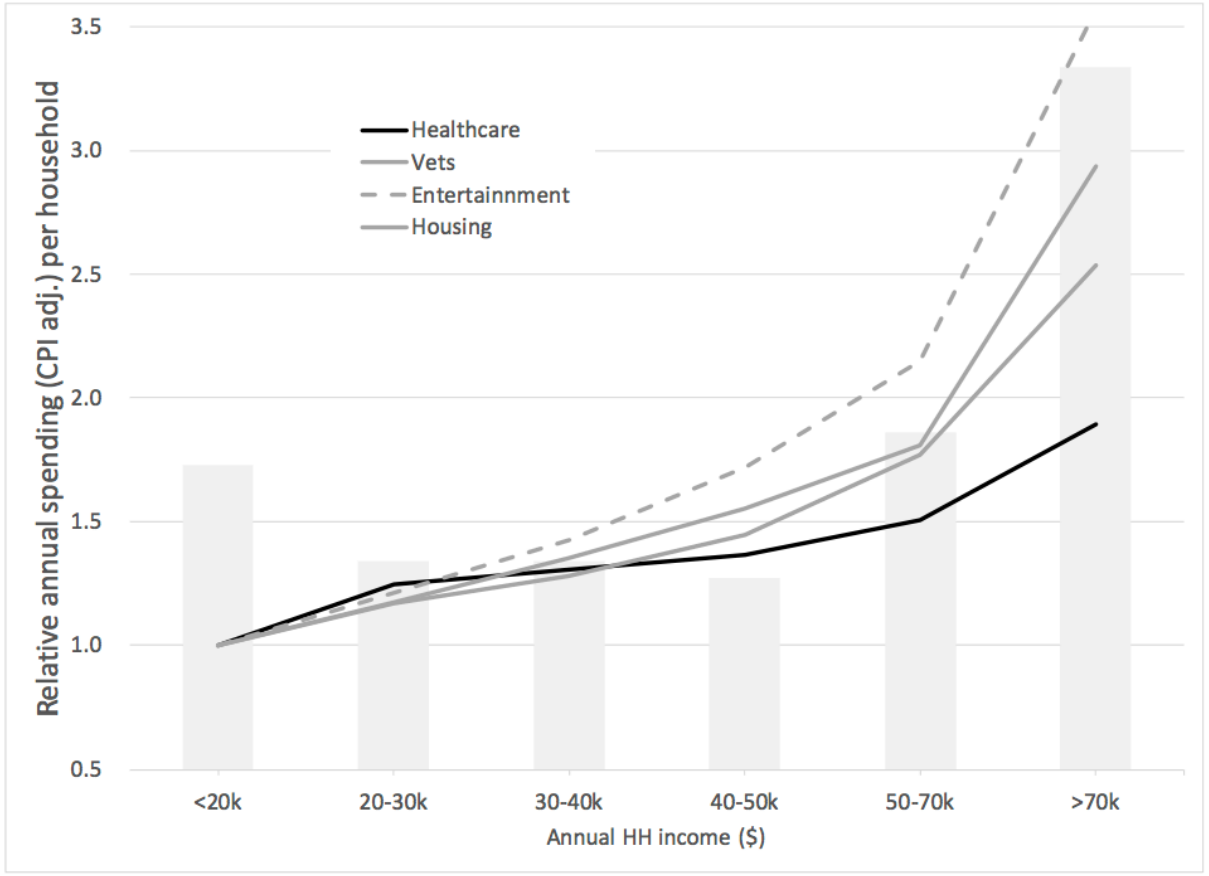
This paper tried to address this question by looking at pet healthcare costs

- The cost of healthcare is an extremely complicated question, and is not settled by any one study, including this one
- I remain mostly undecided on why US healthcare is so expensive and how to fix it, this is just an interesting paper

US pet healthcare cost increases have tracked US human healthcare costs



Richer Americans spend more (in general) including on human and pet healthcare



Paper's (sometimes implicit) Argument:

- Unlike human healthcare, pet healthcare is not dominated by insurance markets, regulations, and malpractice suits
- Therefore, pet healthcare costs are a truer expression of what people would prefer to spend on healthcare
- Yet we observe the same spending trends in pet healthcare as in human healthcare
- If the same things are driving increased spending on pet and human healthcare, it is not insurance markets, regulations, and malpractice suits driving that cost
- Instead the high cost of US healthcare is because American people are willing and able to spend a lot on healthcare
- Elegant logic (although of course, the same things might not be driving the increased spending on pet and human healthcare, and just because people are willing to spend a lot on healthcare, doesn't mean that they *should be* willing)

My reflections: Would be interesting to see pet healthcare costs in other countries

- USHH = US Human Healthcare costs
- USPH = US Pet Healthcare costs
- ODHH = Other developed country human healthcare costs
- ODPH = Other developed country pet healthcare costs
- We know USHH > ODHH
- We know USPH are proportional to USHH
- What if ODPH are proportional to ODHH?
  - Reinforce this paper's argument that Americans are willing and able to spend more on healthcare (for people and pets) than are people in other developed countries
- What if ODPH are instead proportional to USPH?
  - Perhaps market structures/regulations in other developed countries cause less spending less on health care than their people would prefer to spend
- What if the trend of ODPH varies from country to country, with no particularly close relationship to that country's ODPH?
  - Then cast doubt on premise that the same things are driving increased costs in pet and human healthcare

Conclusions

- Varying risk among animals to cancer can in principle inspire us to new cancer prevention strategies
- An ancient transmissible tumor in dogs offers a window into the long-run behavior of tumors
- Understanding pet healthcare costs can helps us deconvolute the driving forces of human healthcare costs