Of Mice and Men and Wildlings

*Illustrations by NIH Medical Arts and M. Kranz AGD Design

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Department of Medicine II, Gastroenterology, Hepatology, Endocrinology,
and Infectious Diseases

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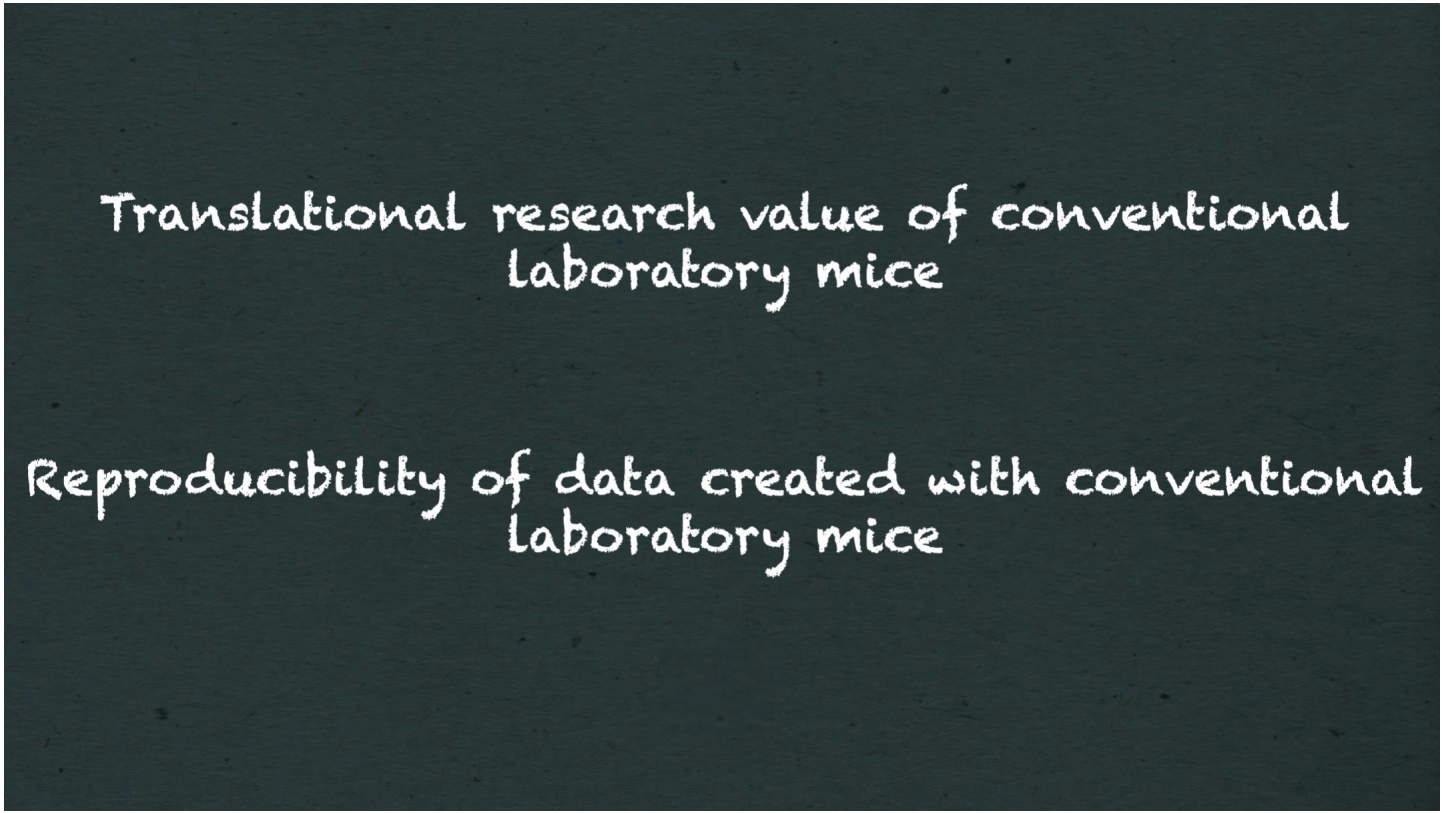
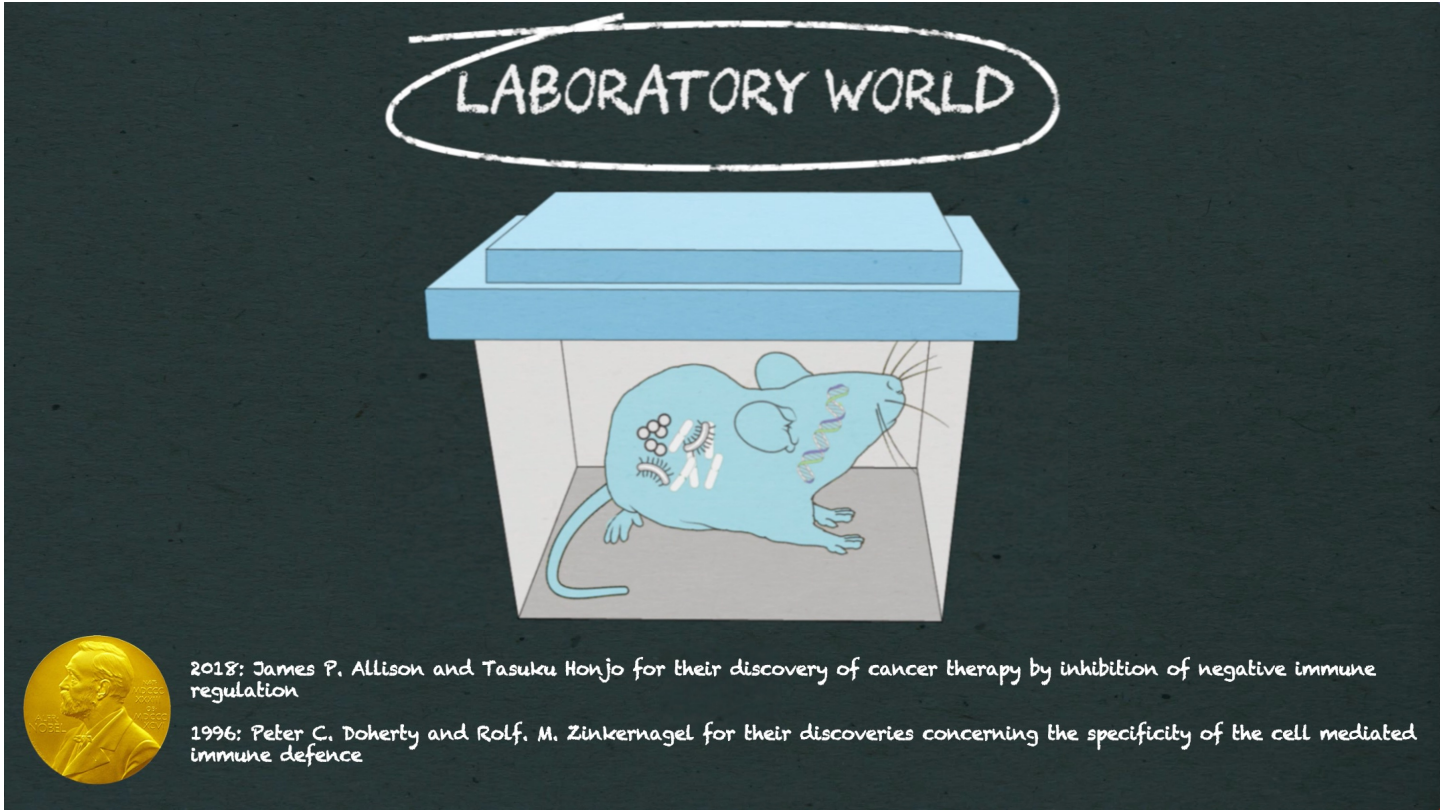
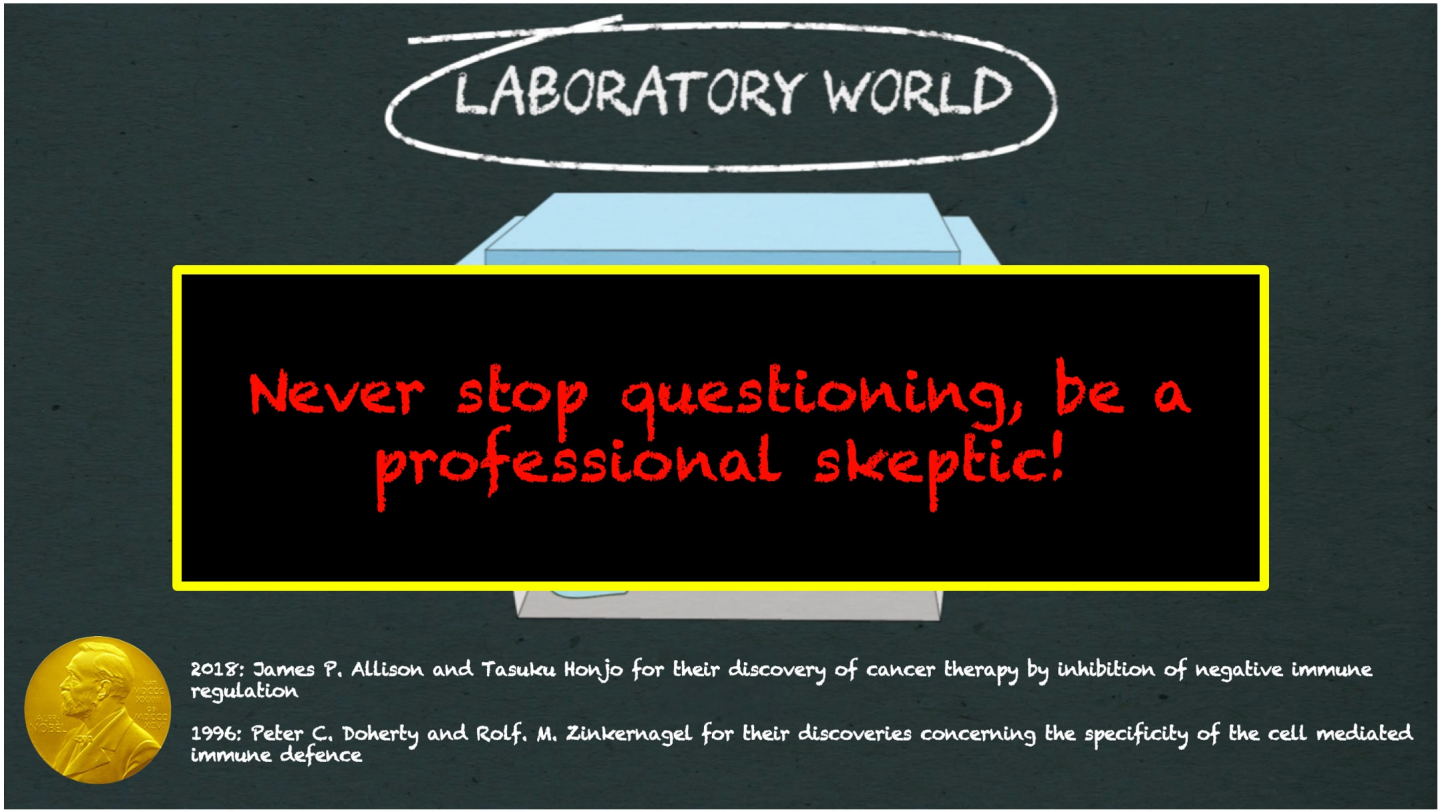
Topics of this presentation



Mouse models in translational research: Two examples of success stories in which mouse models revolutionized medicine and the treatment of patients



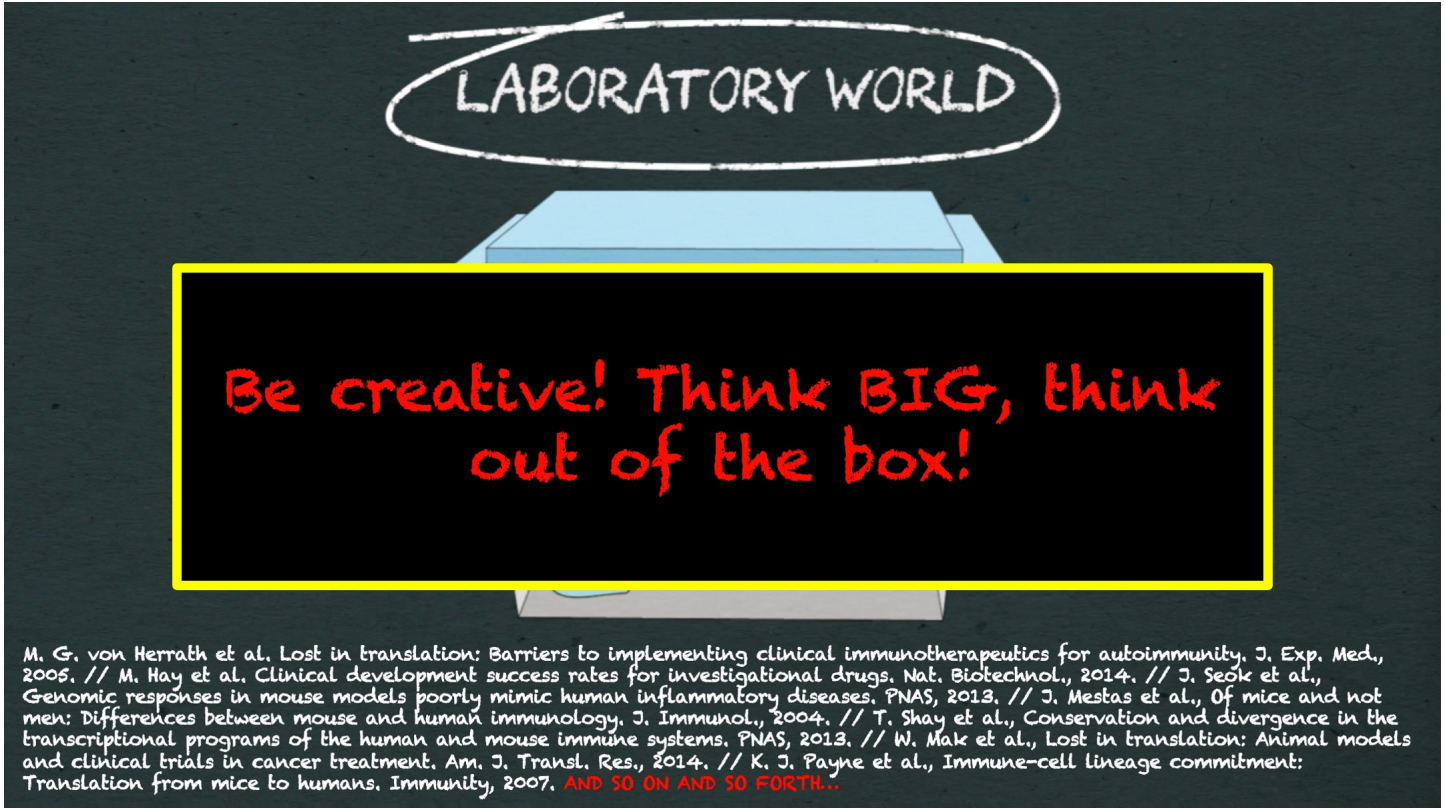
However, never stop questioning your models!



Mouse models: lost in translation(al research). Beside these rare success stories, the overwhelming majority of data does not successfully translate from the mouse to humans!

M. G. von Herrath et al. Lost in translation: Barriers to implementing clinical immunotherapeutics for autoimmunity. J. Exp. Med., 2005. // M. Hay et al. Clinical development success rates for investigational drugs. Nat. Biotechnol., 2014. // J. Seok et al., Genomic responses in mouse models poorly mimic human inflammatory diseases. PNAS, 2013. // J. Mestas et al., Of mice and not men: Differences between mouse and human immunology. J. Immunol., 2004. // T. Shay et al., Conservation and divergence in the transcriptional programs of the human and mouse immune systems. PNAS, 2013. // W. Mak et al., Lost in translation: Animal models and clinical trials in cancer treatment. Am. J. Transl. Res., 2014. // K. J. Payne et al., Immune-cell lineage commitment: Translation from mice to humans. Immunity, 2007. AND SO ON AND SO FORTH...

Mouse models: lost in translation(al research). What is the reason for failure in translation, how can we create better translational research models?



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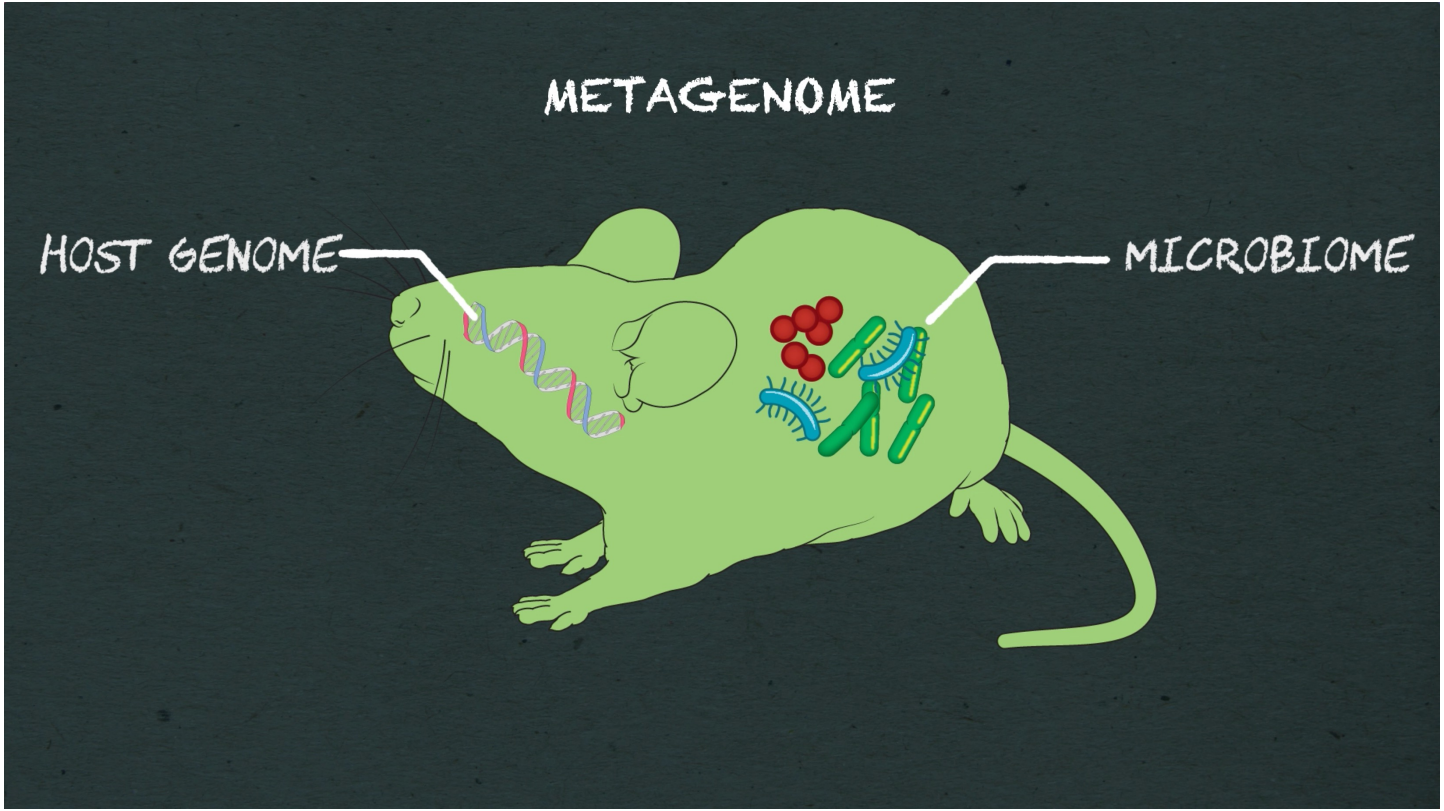
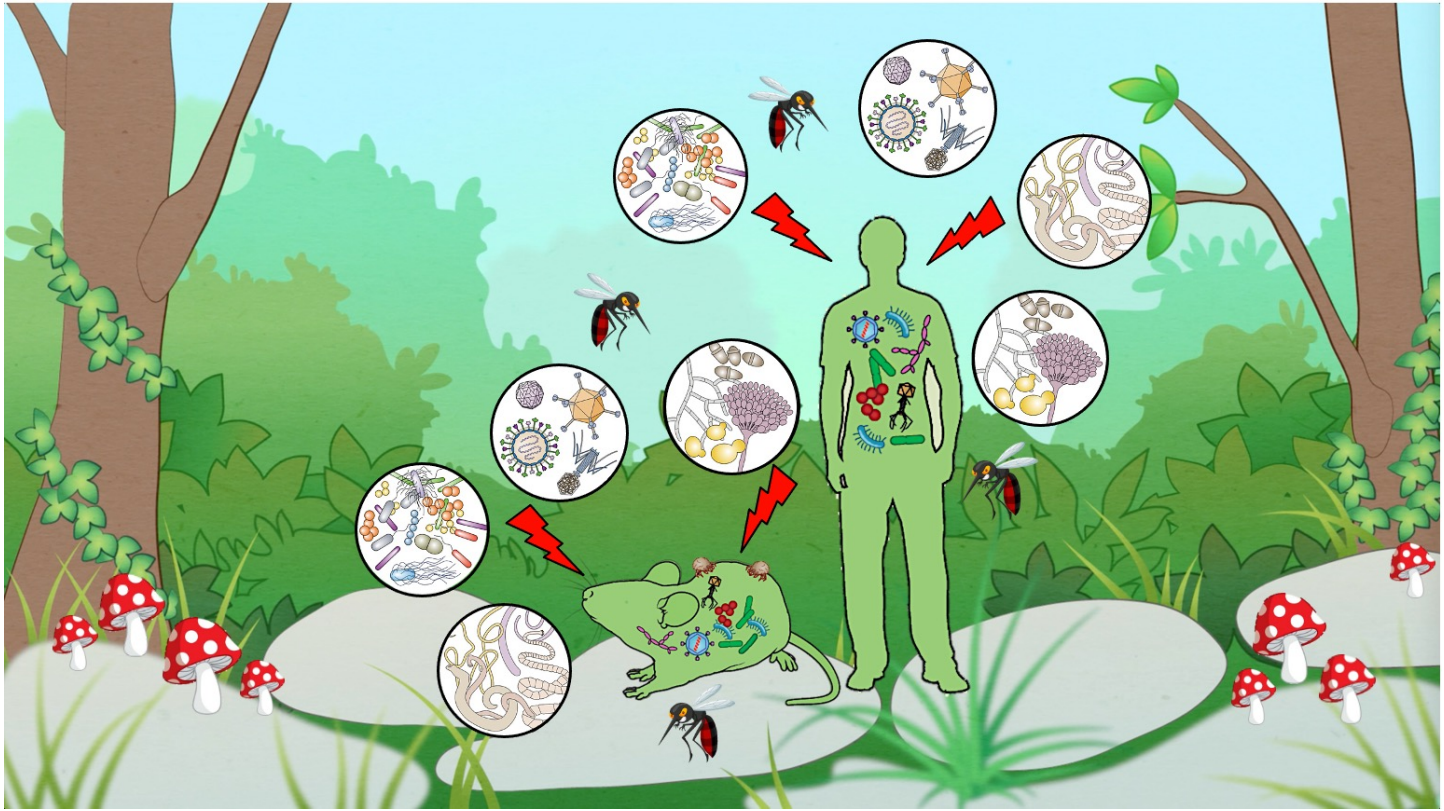
All mammals are metagenomic organisms

All mammals are metagenomic organisms and the metagenome, the combination of the host genome and the microbiome, is the driver of the mammalian phenotype

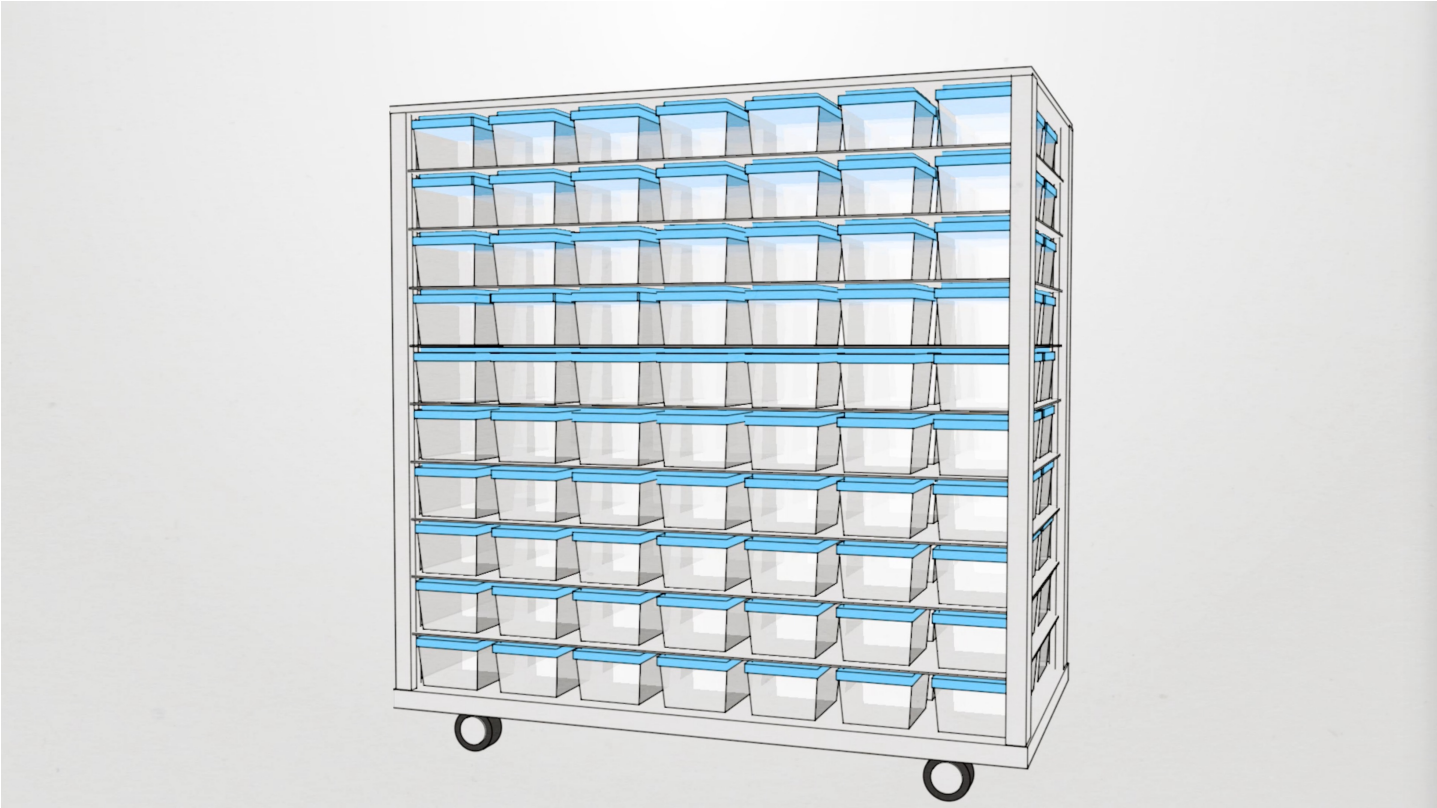


Of mice and men, and naturally co-evolved microbiota

We are Born to be Wild – this is the common link among mammals



Lab mice have a very special physiology, something that only exists in the lab and that we have created, they have lost the common link among mammals



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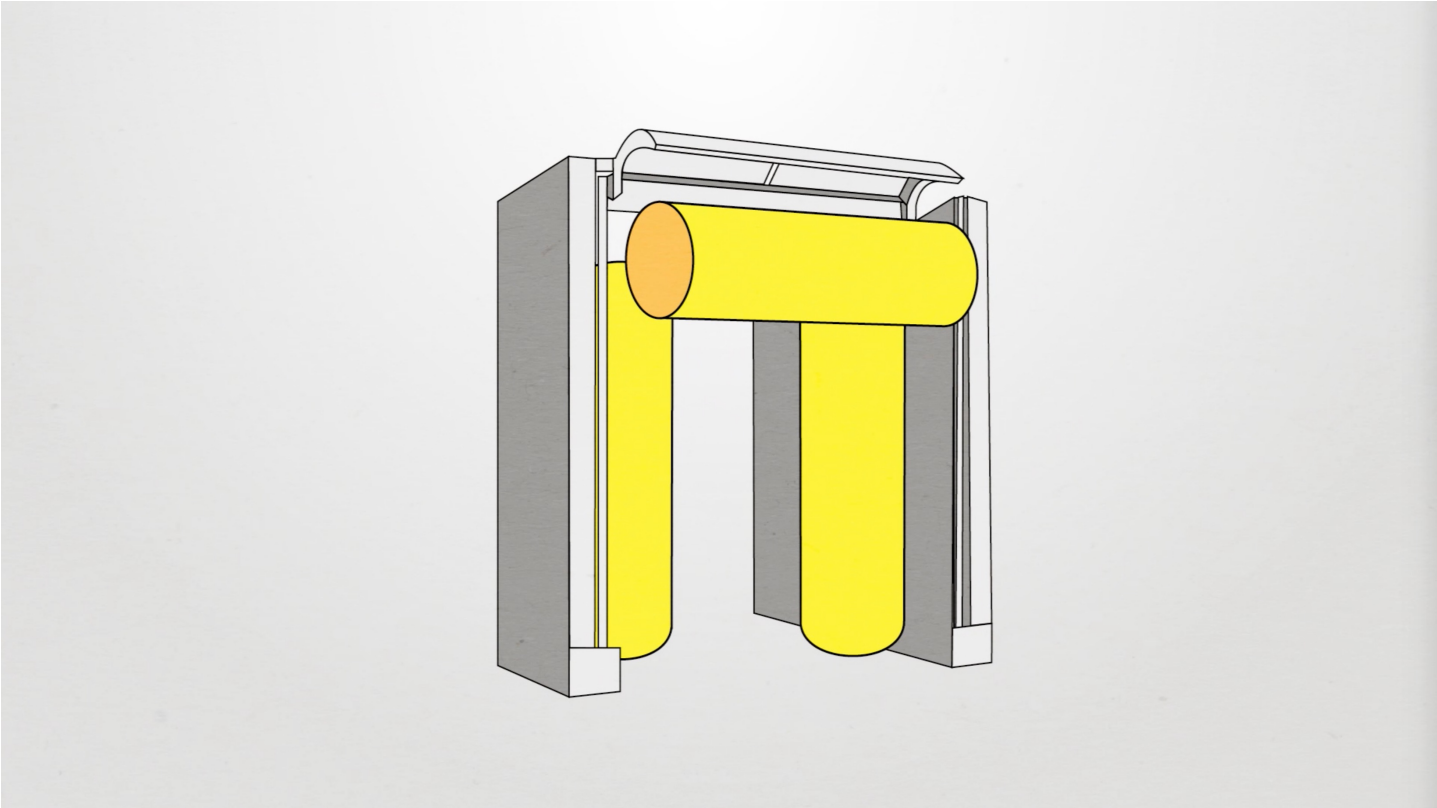

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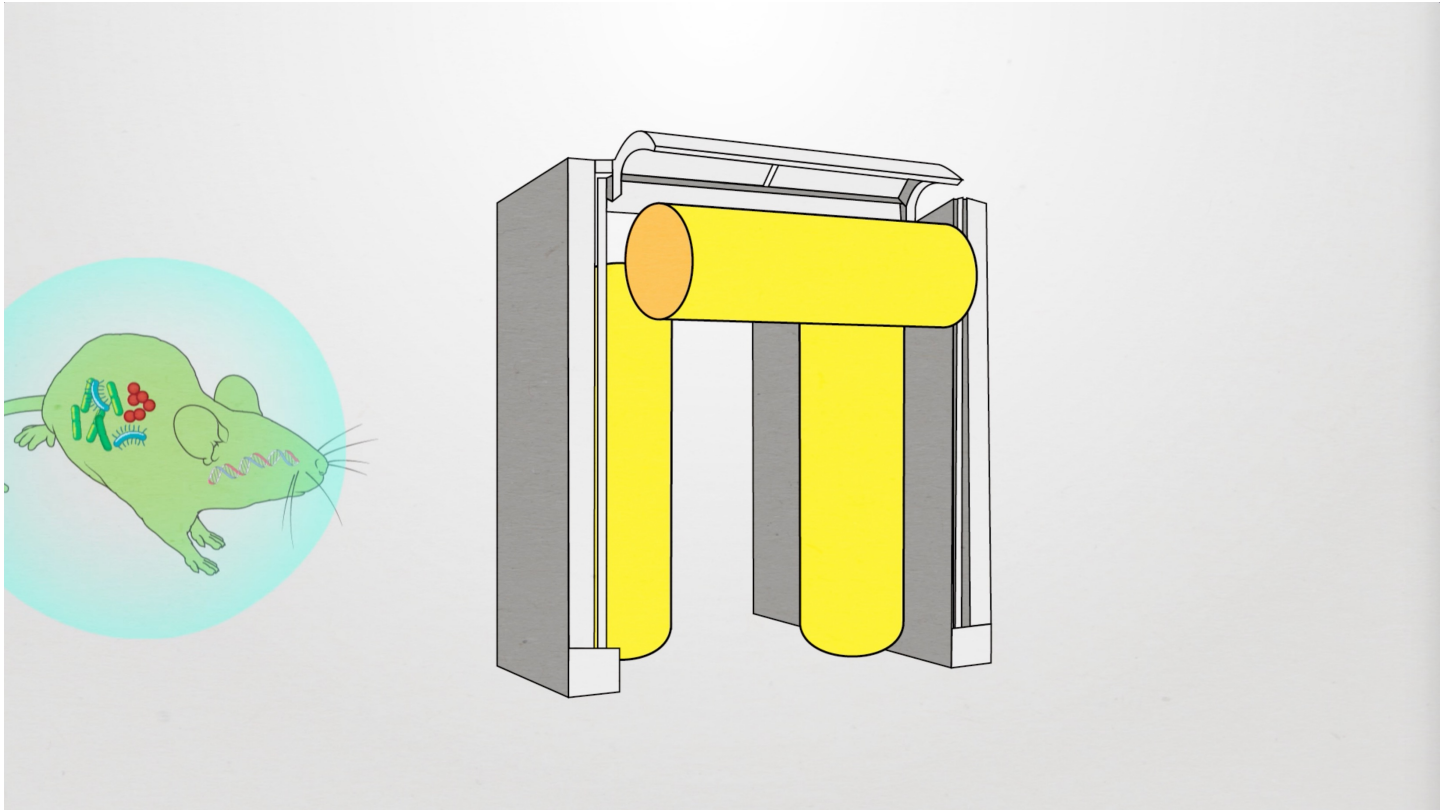

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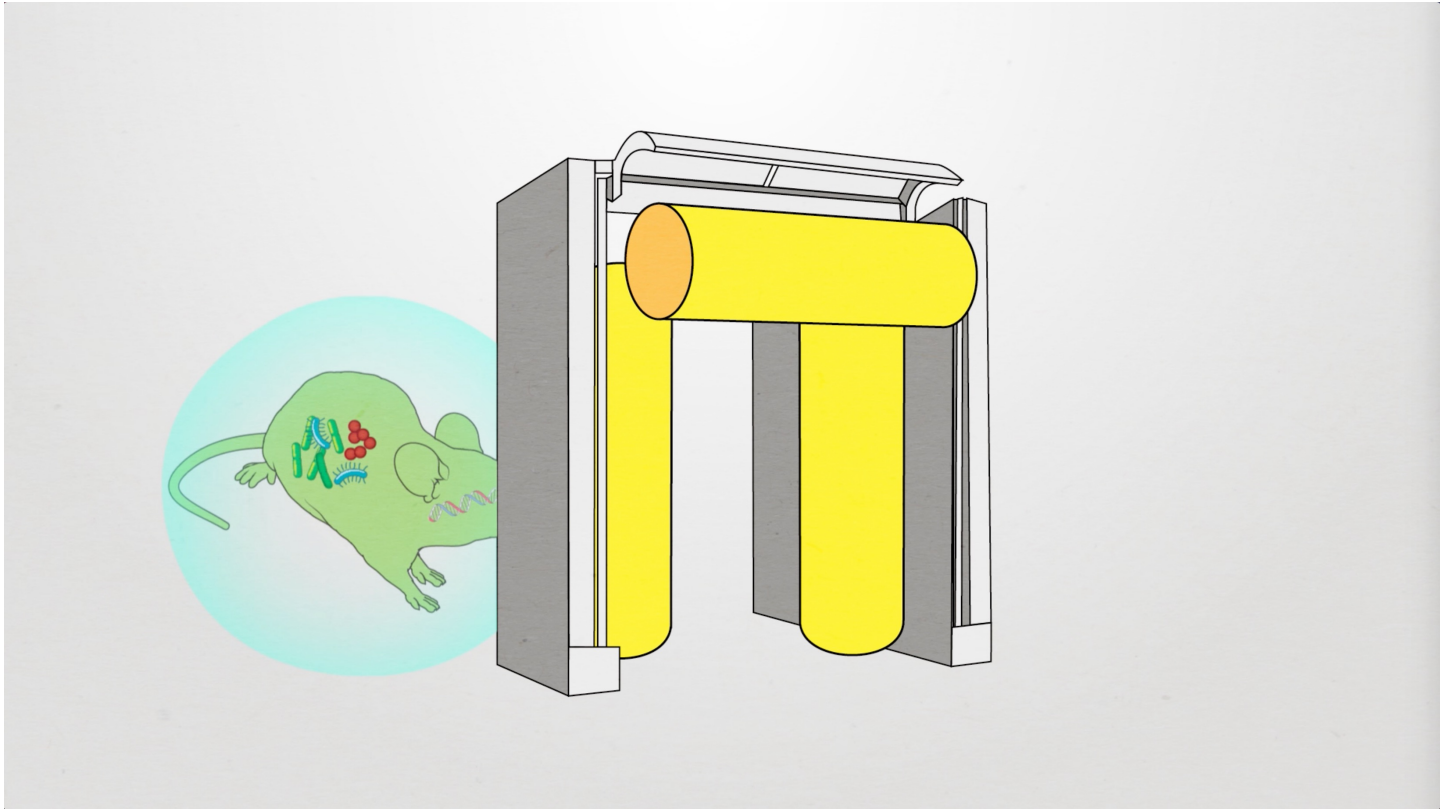

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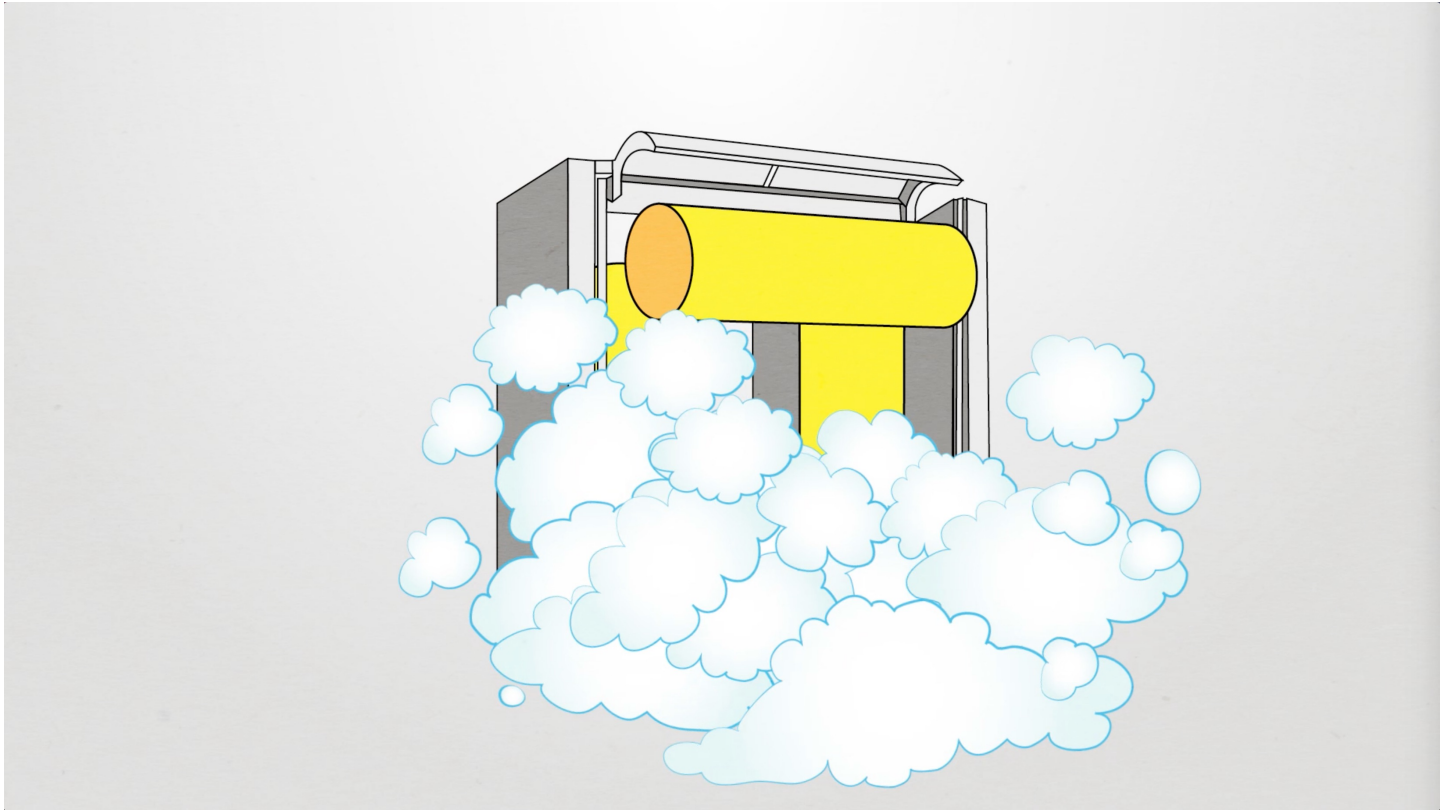

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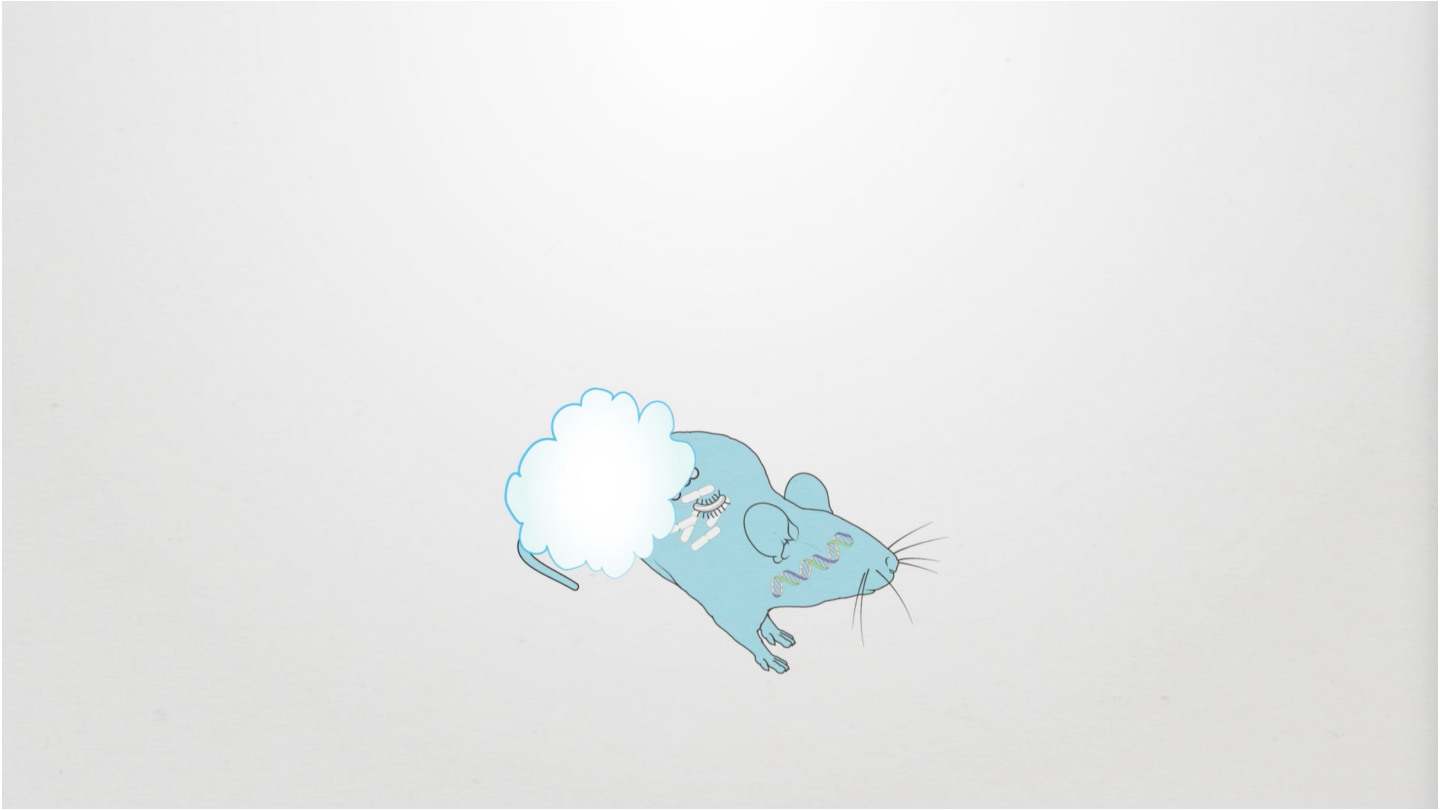

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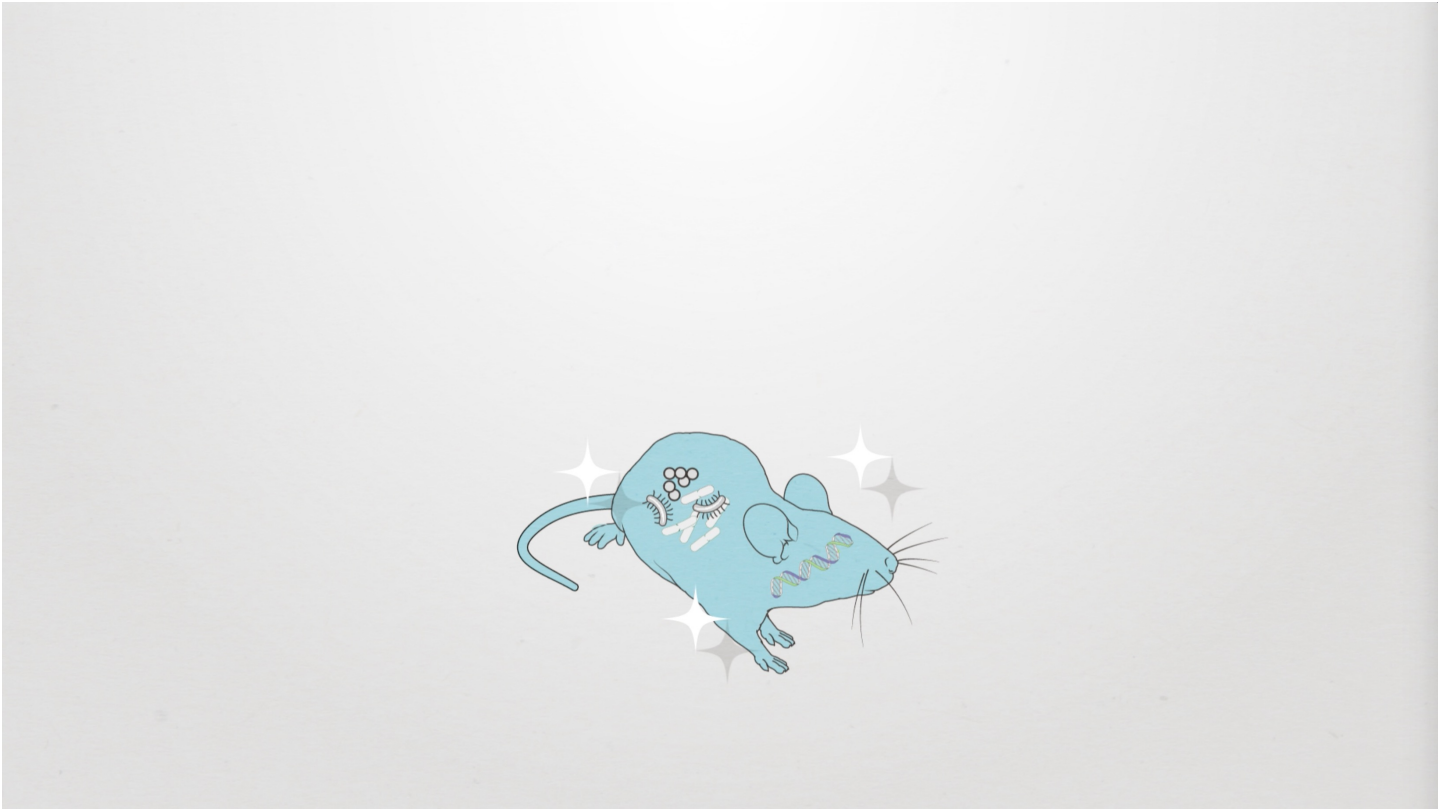


Humans are not like lab mice, humans are pretty dirty, especially during the critical time when their microbiome and immune system evolves!

Conclusion: Restore the common link “Born to be Wild”

All mammals are „Born to be Wild“, thus:

We could make the microbiome of lab mice wild and dirty again – like ours – by giving them the microbiome of actual wild mice,

this approach should mature their immune system, make them more like us humans and increase their translational research value!

We are not alone with our opinion!

L. K. Beura *et al.*, Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature*, 2016.

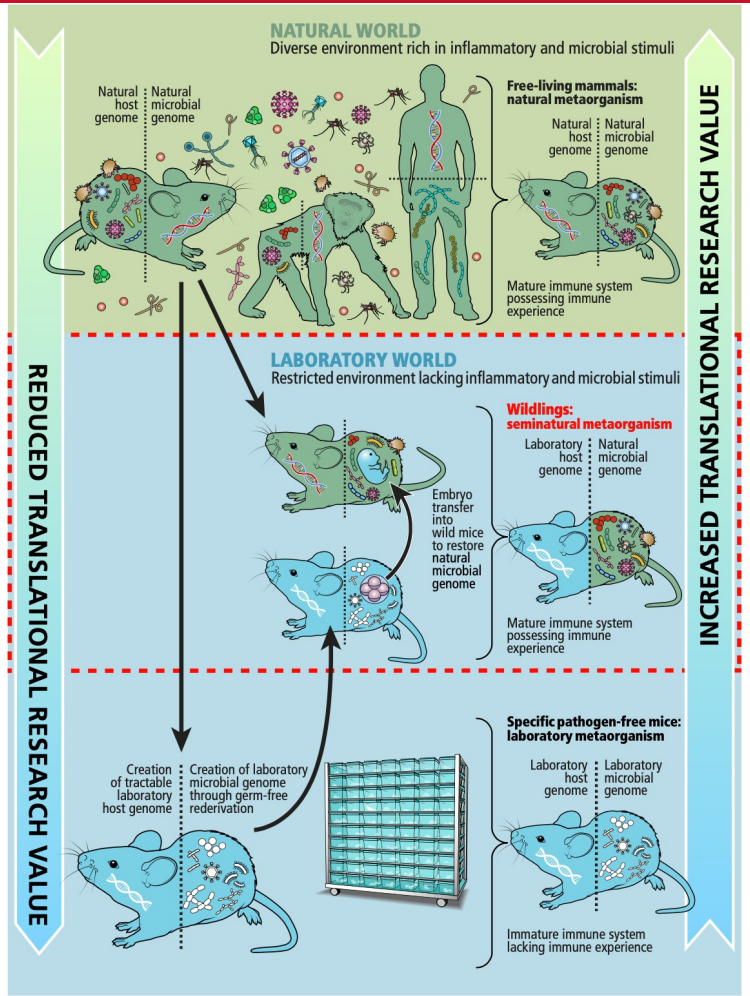
T. A. Reese *et al.*, Sequential infection with common pathogens promotes human-like immune gene expression and altered vaccine response. *Cell Host Microbe*, 2016.

S. P. Rosshart *et al.*, Wild mouse gut microbiota promotes host fitness and improves disease resistance. *Cell*, 2017.

S. Abolins *et al.*, The comparative immunology of wild and laboratory mice, Mus musculus domesticus. *Nat Commun*, 2017.

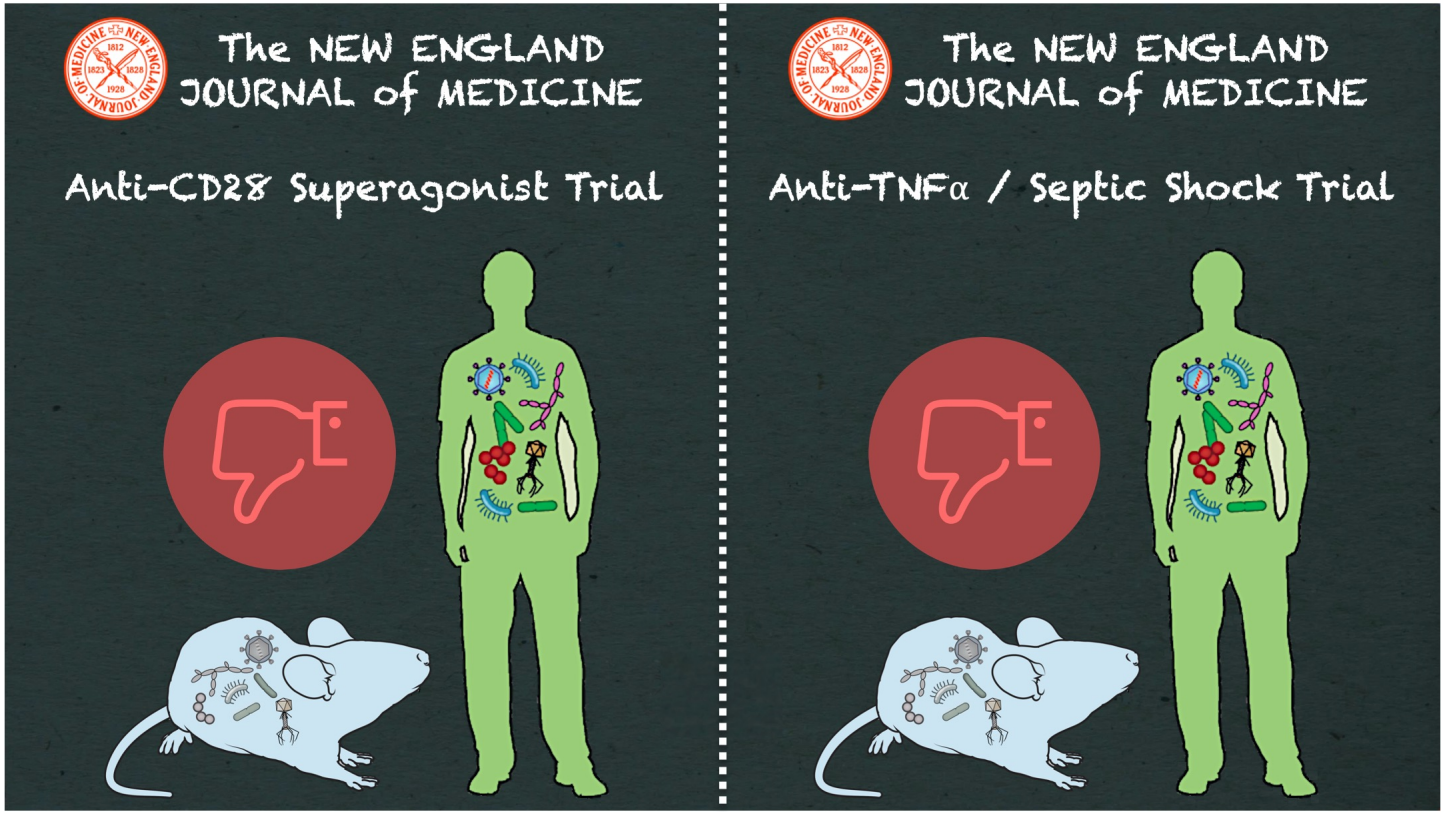
J. M. Leung *et al.*, Rapid environmental effects on gut nematode susceptibility in rewilded mice. *PLoS Biol*, 2018.

Let us make lab mice, their immune system and microbiome wild (dirty) again



Rosshart et al., Cell, 2017.
Rosshart et al., Science, 2019.

Could wildlings have prevented failed clinical trials?



Could wildlings have prevented the failed human trial of CD28SA?

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE BRIEF REPORT

Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412

Ganesh Suntharalingam, F.R.C.A., Meghan R. Perry, M.R.C.P., Stephen Ward, F.R.C.A., Stephen J. Brett, M.D., Andrew Castello-Cortes, F.R.C.A., Michael D. Brunner, F.R.C.A., and Nicki Panoskaltsis, M.D., Ph.D.

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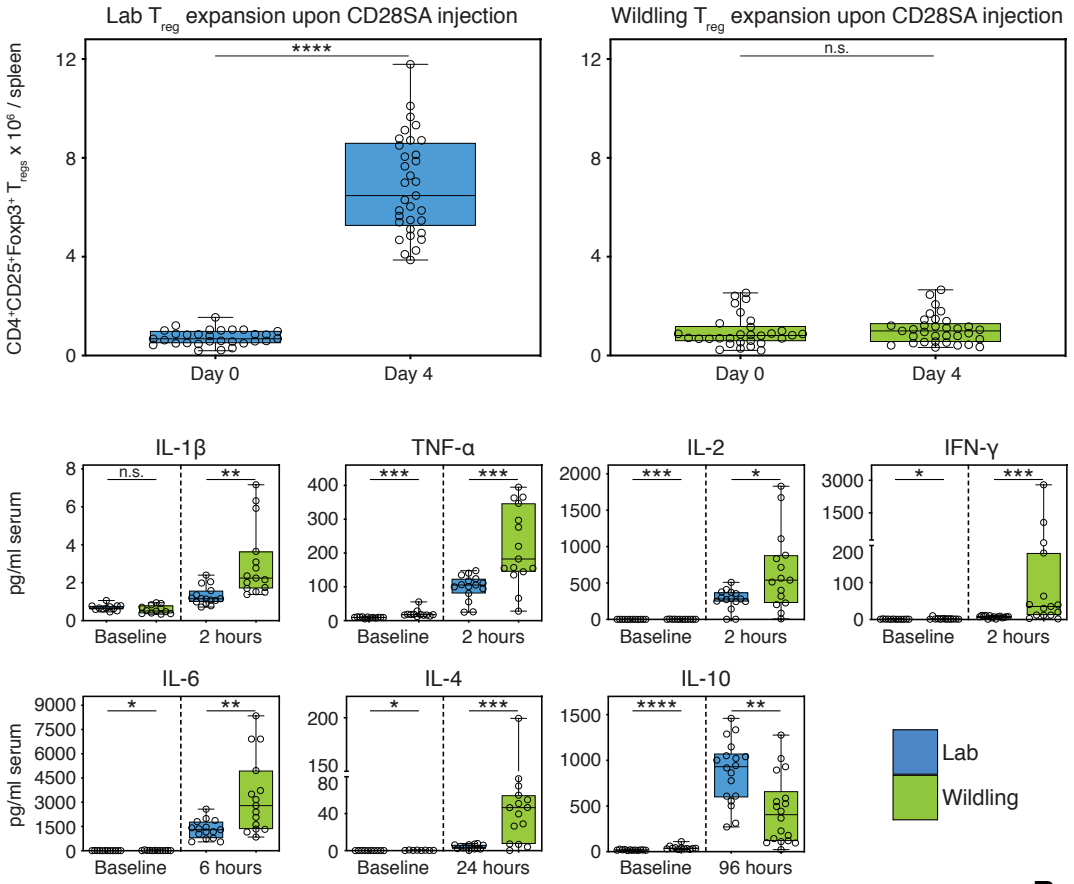
Rosshart et al., Science, 2019.

Could wildlings have prevented the failed human trial of CD28SA?



Rosshart et al., Science, 2019.

Could wildlings have prevented the failed human trial of TNFα?

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Treatment of Septic Shock with the Tumor Necrosis Factor Receptor:Fc Fusion Protein

Charles J. Fisher, Jr., M.D., Jan M. Agosti, M.D., Steven M. Opal, M.D., Stephen F. Lowry, M.D., Robert A. Balk, M.D., Jerald C. Sadoff, M.D., Edward Abraham, M.D., Roland M.H. Schein, M.D., and Ernest Benjamin, M.D. for the Soluble TNF Receptor Sepsis Study Group*

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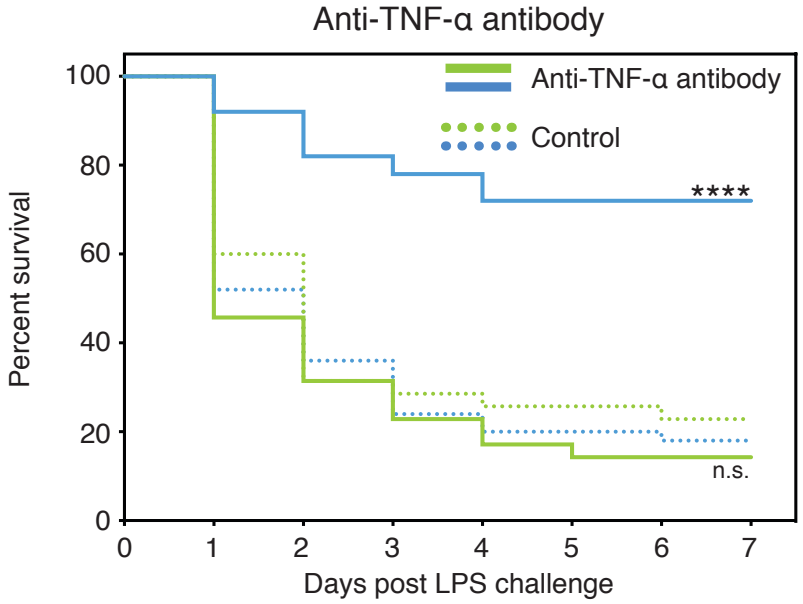
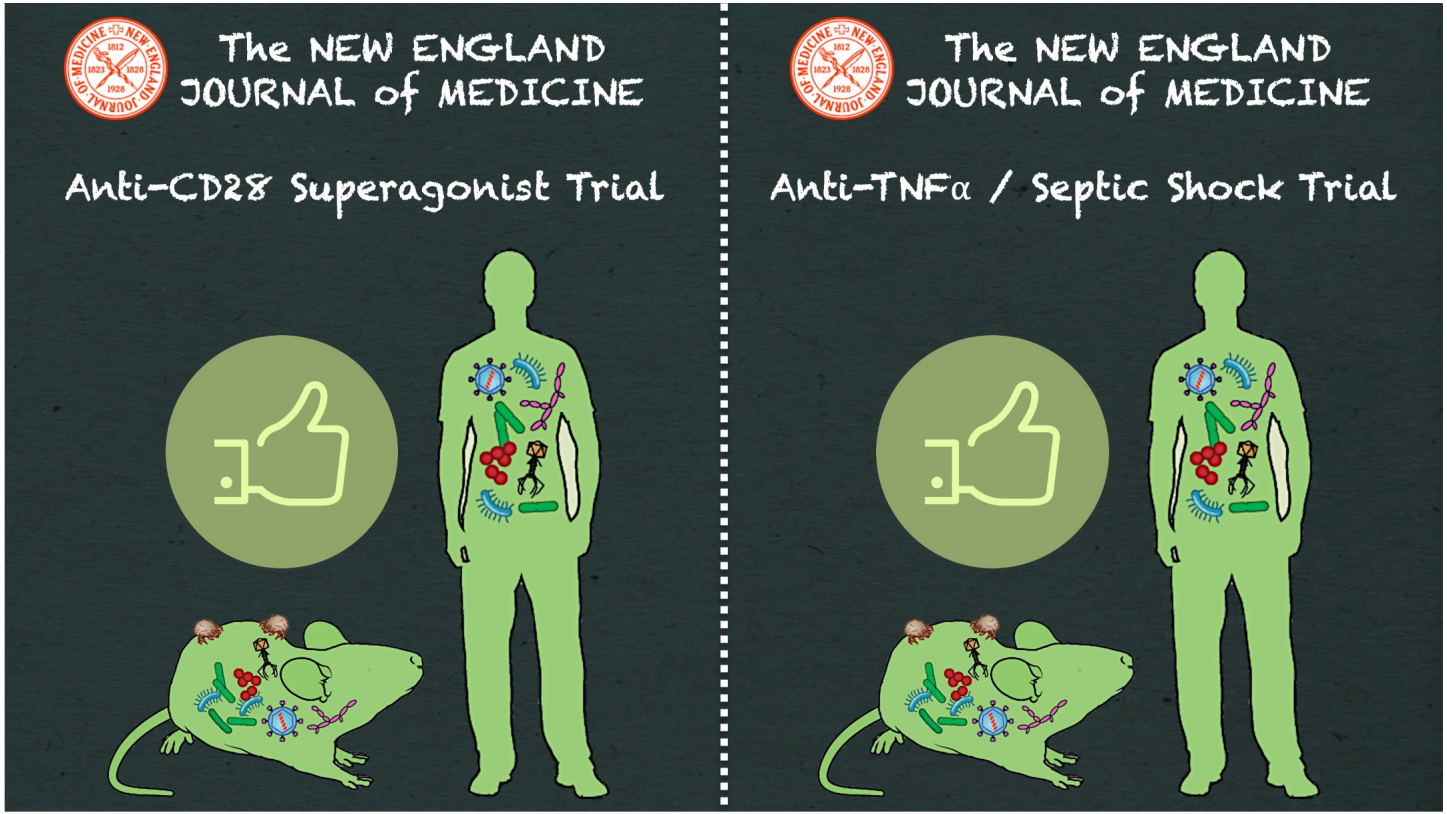
Rosshart et al., Science, 2019.

Could wildlings have prevented the failed human trial of TNFα?



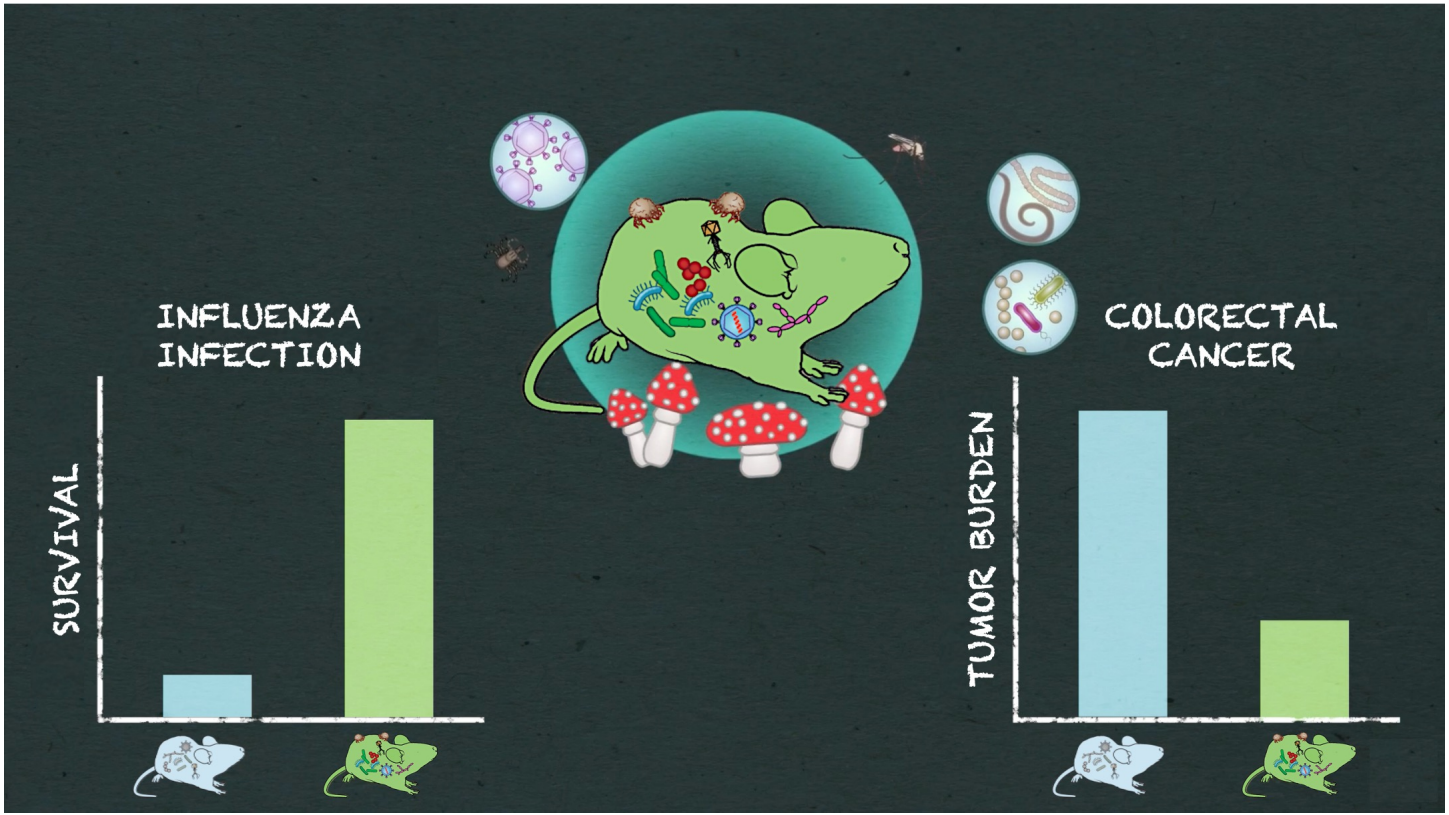
Rosshart et al., Science, 2019.

In preclinical trials, where rodent and non-human primate models failed to predict the human response to harmful drug treatments, wildlings accurately phenocopied human immune responses and could have prevented these tragically failed human trials



Rosshart et al., Science, 2019.

Natural microbiota-based models may enable the discovery of microbiota-related novel disease treatments that may be translatable into the human system



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Rosshart et al., Cell, 2017.

Concluding remarks

Wildlings may enable the discovery of novel disease treatments that cannot be studied in conventional laboratory mice

Further, they may increase the safety and success rate of transitioning bench results to bedside practice

3R: Moreover, using such models may be the more ethical approach (non-human primates)

Reproducibility of data created with conventional lab mice

Irreproducible and confliciting data due to divergent microbiota among commercial vendors and research institutes

„Embrace the diversity and document everything"

or

„use standardized microbiota"

Reproducibility of data created with conventional lab mice

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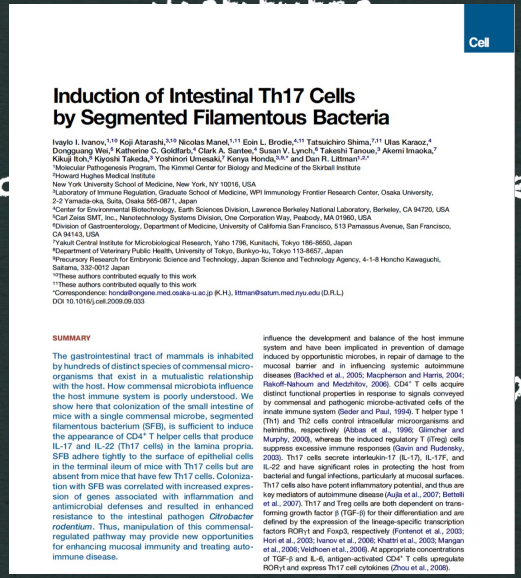

Cell

Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria

Ivaylo I. Ivanov,[1,10] Koji Atarashi,[5,10] Nicolas Manel,[1,11] Eoin L. Brodie,[4,11] Tatsuichiro Shima,[7,11] Ulas Karaoz,[4] Dongguang Wei,[4] Katherine C. Goldfarb,[4] Clark A. Santee,[4] Susan V. Lynch,[8] Takeshi Tanoue,[9] Akemi Imaoka,[7] Kikuji Itoh,[5] Kiyoshi Takeda,[3] Yoshinori Umesaki,[7] Kenya Honda,[5,6,*] and Dan R. Littman[1,2,*]
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[2]Howard Hughes Medical Institute
New York University School of Medicine, New York, NY 10016, USA
[3]Laboratory of Immune Regulation, Graduate School of Medicine, WPI Immunology Frontier Research Center, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan
[4]Center for Environmental Biotechnology, Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[5]Carl Zeiss SMT, Inc., Nanotechnology Systems Division, One Corporation Way, Peabody, MA 01960, USA
[6]Division of Gastroenterology, Department of Medicine, University of California San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143, USA
[7]Yakult Central Institute for Microbiological Research, Yaho 1796, Kunitachi, Tokyo 186-8650, Japan
[8]Department of Veterinary Public Health, University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan
[9]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama, 332-0012 Japan
[10]These authors contributed equally to this work
[11]These authors contributed equally to this work
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DOI 10.1016/j.cell.2009.09.033

SUMMARY

The gastrointestinal tract of mammals is inhabited by hundreds of distinct species of commensal microorganisms that exist in a mutualistic relationship with the host. How commensal microbiota influence the host immune system is poorly understood. We show here that colonization of the small intestine of mice with a single commensal microbe, segmented filamentous bacterium (SFB), is sufficient to induce the appearance of $CD4^+$ T helper cells that produce IL-17 and IL-22 (Th17 cells) in the lamina propria. SFB adhere tightly to the surface of epithelial cells in the terminal ileum of mice with Th17 cells but are absent from mice that have few Th17 cells. Colonization with SFB was correlated with increased expression of genes associated with inflammation and antimicrobial defenses and resulted in enhanced resistance to the intestinal pathogen *Citrobacter rodentium*. Thus, manipulation of this commensal-regulated pathway may provide new opportunities for enhancing mucosal immunity and treating autoimmune disease.

influence the development and balance of the host immune system and have been implicated in prevention of damage induced by opportunistic microbes, in repair of damage to the mucosal barrier and in influencing systemic autoimmune diseases (Backhed et al., 2005; Macpherson and Harris, 2004; Rakoff-Nahoum and Medzhitov, 2006). $CD4^+$ T cells acquire distinct functional properties in response to signals conveyed by commensal and pathogenic microbe-activated cells of the innate immune system (Seder and Paul, 1994). T helper type 1 (Th1) and Th2 cells control intracellular microorganisms and helminths, respectively (Abbas et al., 1996; Glimcher and Murphy, 2000), whereas the induced regulatory T (iTreg) cells suppress excessive immune responses (Gavin and Rudensky, 2003). Th17 cells secrete interleukin-17 (IL-17), IL-17F, and IL-22 and have significant roles in protecting the host from bacterial and fungal infections, particularly at mucosal surfaces. Th17 cells also have potent inflammatory potential, and thus are key mediators of autoimmune disease (Aujla et al., 2007; Bettelli et al., 2007). Th17 and Treg cells are both dependent on transforming growth factor β (TGF-β) for their differentiation and are defined by the expression of the lineage-specific transcription factors $ROR\gamma t$ and Foxp3, respectively (Fontenot et al., 2003; Hori et al., 2003; Ivanov et al., 2006; Khattri et al., 2003; Mangan et al., 2006; Veldhoen et al., 2006). At appropriate concentrations of TGF-β and IL-6, antigen-activated $CD4^+$ T cells upregulate $ROR\gamma t$ and express Th17 cell cytokines (Zhou et al., 2008).

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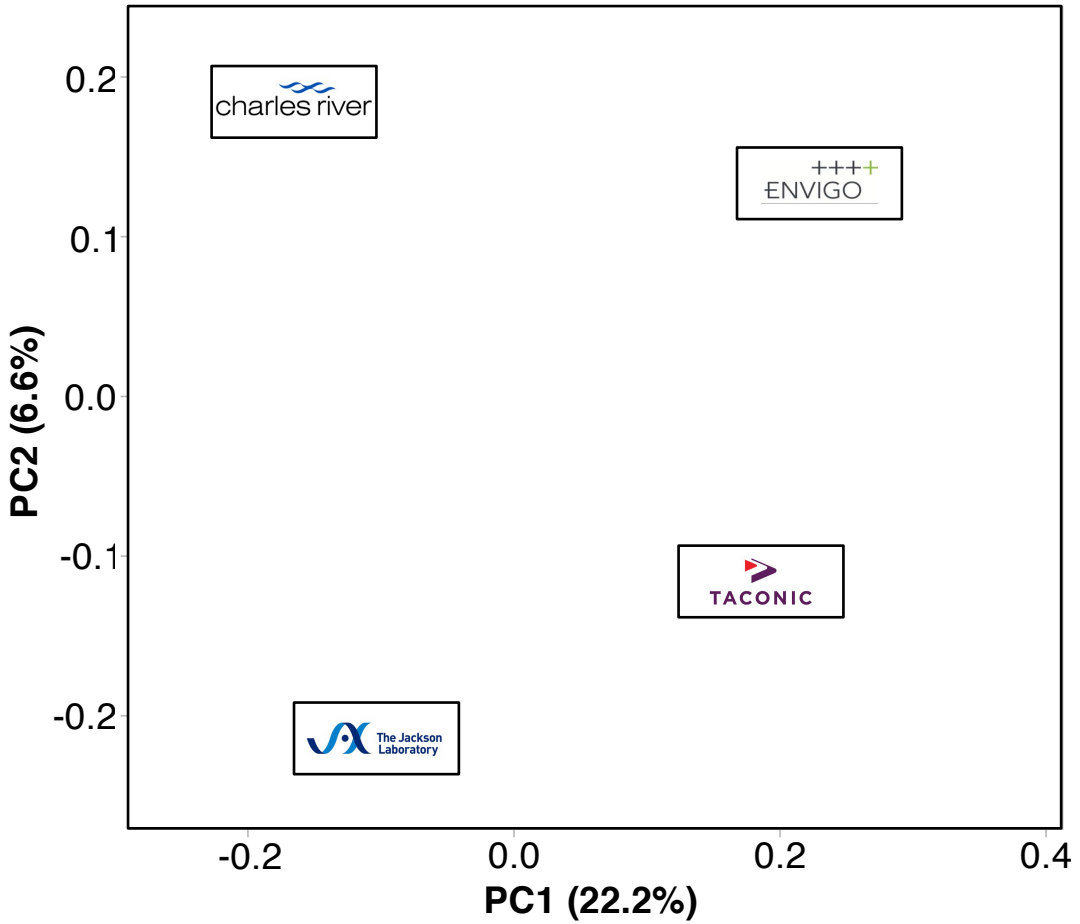
Thought experiment: Should we embrace diversity?



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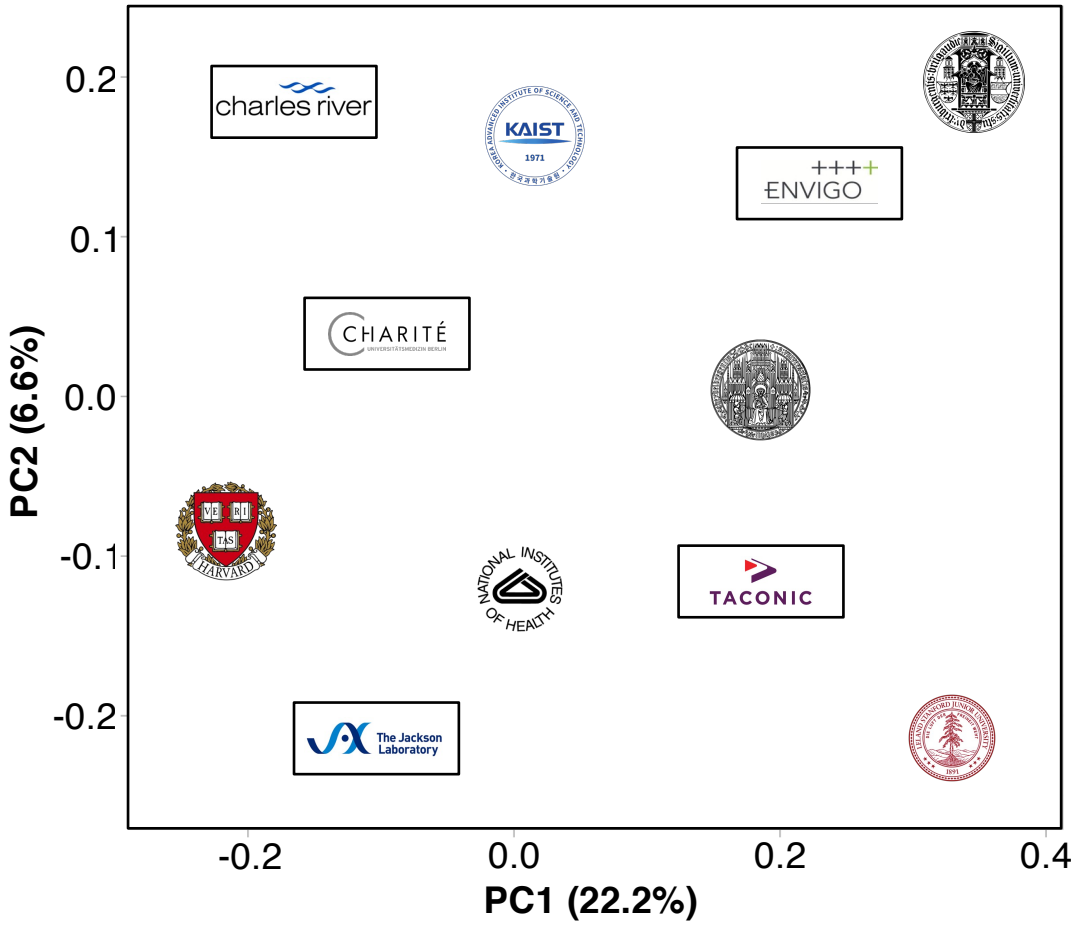
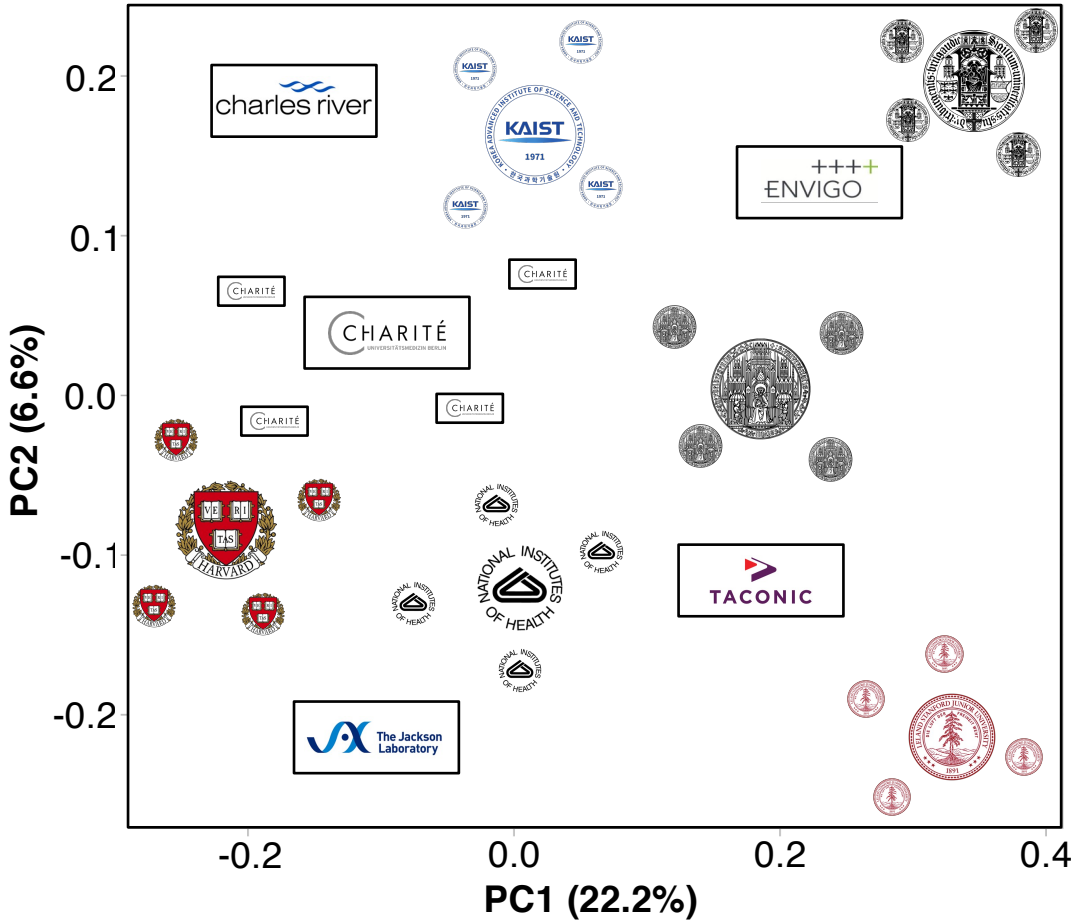
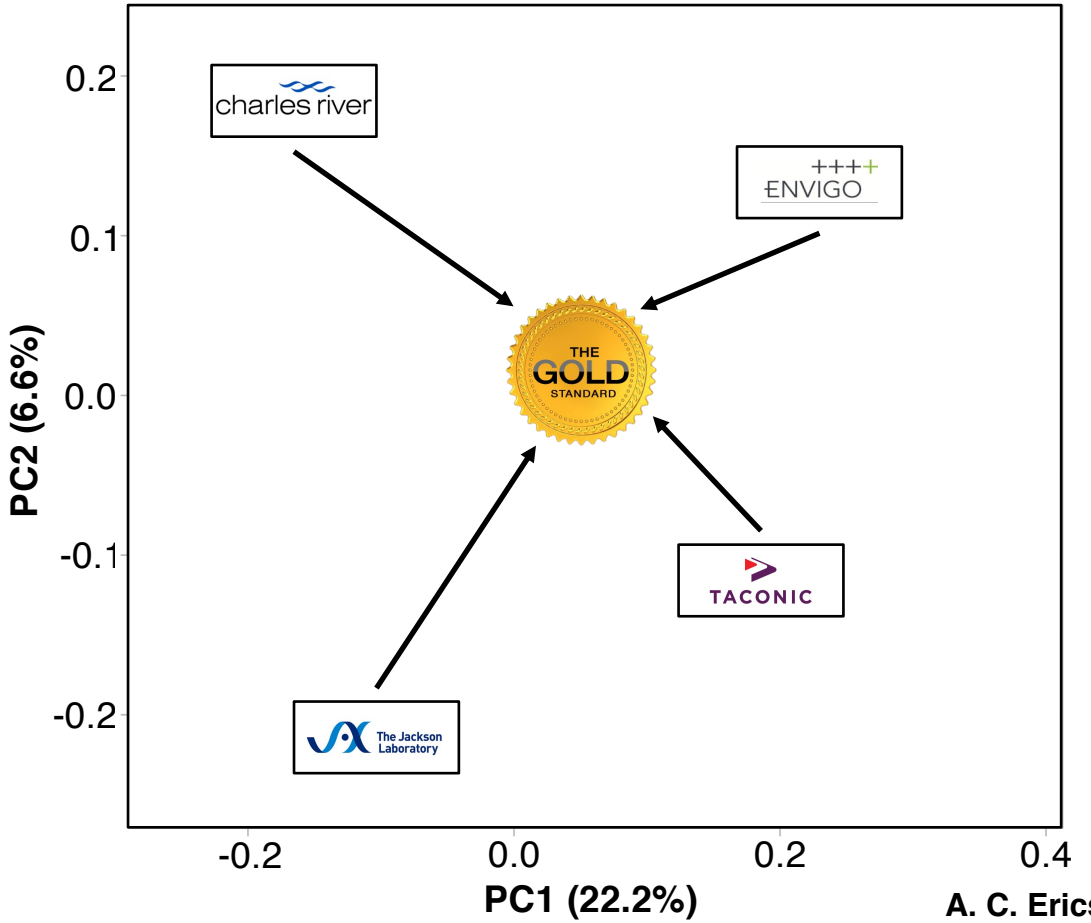


Thought experiment: Should we embrace diversity? This approach does not solve the underlying problem of divergent microbiota and will fail due to the complexity of the system!

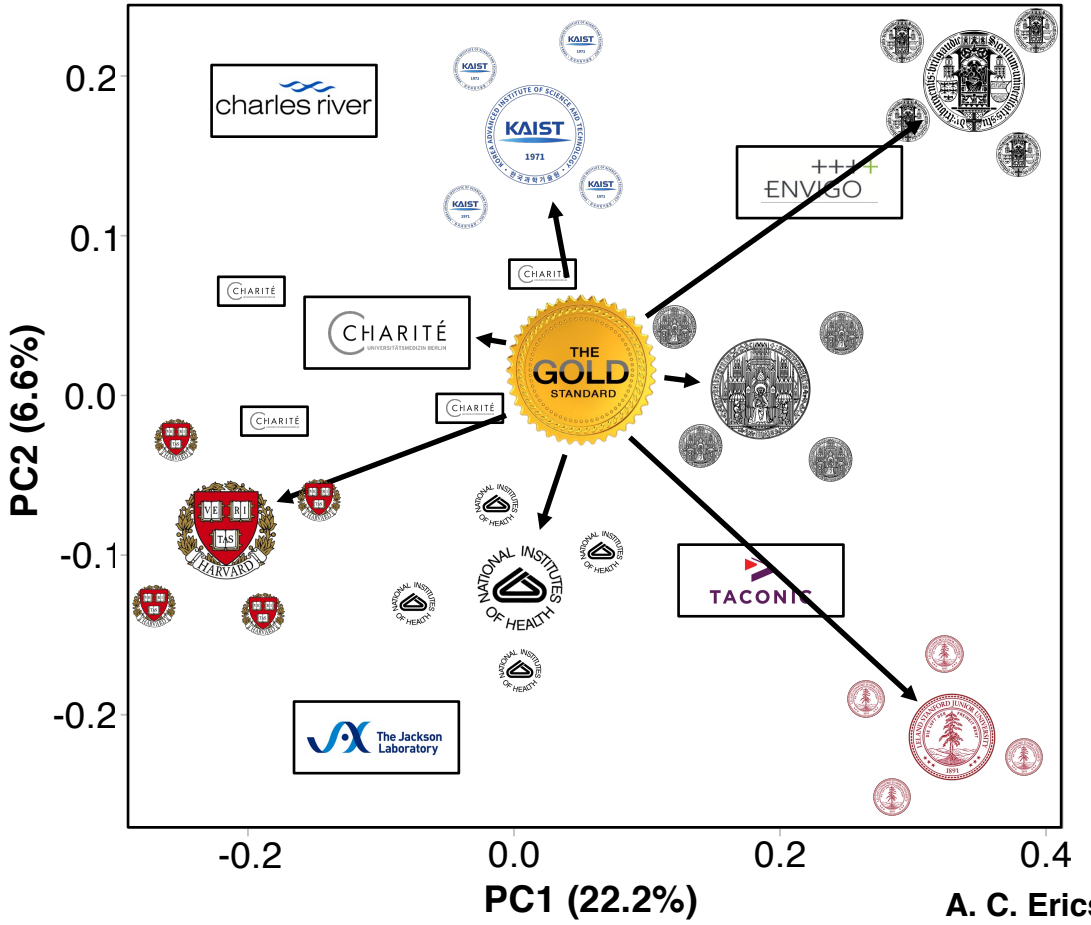


Thought experiment: Should we standardize?



A. C. Ericsson et al., Sci. Rep., 2019.

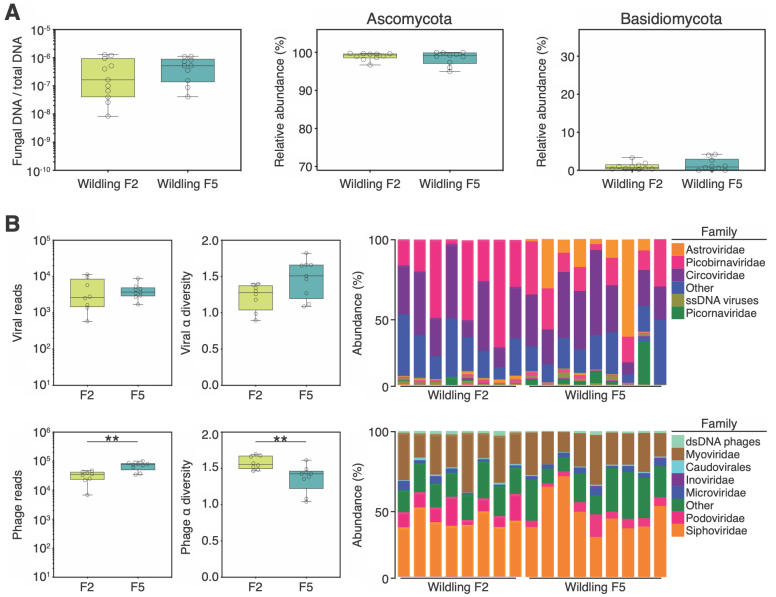
Thought experiment: Should we standardize? Standardization does make sense. However, it will not work with conventional laboratory microbiota, they are not stable and not resilient!



A. C. Ericsson et al., Sci. Rep., 2019.

Characteristics of microbiota for standardization? Can natural microbiota-based models help with this issue?

Wildling microbiota possess multigenerational stability and are their composition is fully characterized



Rosshart et al., Science, 2019.

Wildlings carry typical naturally occurring “pathogens” of wild mice and this composition is fully characterized. However, wildlings are healthy animals, they show no signs of spontaneous disease, carry no human pathogens and are BSL-1.

Wildlings carry pathogens that originate from wild mice

	Wildlings (n = 24)	Wild mice (n = 20)
Viruses	**Percent positive mice***	
Adenovirus type 1 & 2 (MAV-1 & MAV2)	0	0
Ectromelia virus (Mousepox)	0	0
Hantaan (HTNV/HANT)	0	0
Lactate dehydrogenase-elevating virus (LDV/LDH)	0	0
Minute virus of mice (MVM)	0	0
Mouse coronavirus (MHV)	83	90
Mouse cytomegalovirus (MCMV)	50	85
Mouse parvoviruses (MPV)	46	75
MPV 1	42	55
MPV 2	4	20
MPV 5	0	0
Generic parvovirus NS-1 (NS-1)	0	0
Mouse pneumonitis virus (K)	0	0
Mouse rotavirus (MRV/EDIM/ROTA-A)	13	20
Murine norovirus (MNV)	67	50
Mouse theilovirus (TMEV/GDVII)	0	0
Mouse thymic virus (MTLV)	21	40
Pneumonia virus of mice (PVM)	0	0
Polyoma virus (POLY)	50	70
Prospect Hill virus (PHV)	0	0
Reovirus tupe 1, 2, 3, 4 (REO)	0	0
Sendai virus (SEND)	0	0
Lymphocytic choriomeningitis virus (LCMV)	71	60
Encephalitozoon cuniculi (ECUN)	0	0
Bacteria	**Percent positive mice***	
Beta hemolytic Streptococcus Group A	0	0
Beta hemolytic Streptococcus Group B	0	5
Beta hemolytic Streptococcus Group C	0	0
Beta hemolytic Streptococcus Group G	0	0
Bordetella bronchiseptica	0	0
Campylobacter	0	0
Cilia-associated respiratory bacillus (CARB)	0	0
Citrobacter rodentium	0	0
Bordetella hinzii	0	0
Clostridium piliforme	0	0
Corynebacterium bovis	0	0
Corynebacterium kutscheri	0	0
Encephalitozoon cuniculi (ECUN)	0	0
Helicobacter species	100	100
Helicobacter bilis	0	0
Helicobacter ganmani	100	100
Helicobacter hepaticus	13	10
Helicobacter mastomyrinus	4	5
Helicobacter rodentium	0	0
Helicobacter typhlonius	100	100
Klebsiella oxytoca	0	5
Klebsiella pneumoniae	0	0
Mycoplasma pulmonis (MPUL)	0	0
Pasteurella pneumotropica (Heyl)	75	50
Pasteurella pneumotropica (Jawetz)	100	80
Pseudomonas aeruginosa	8	5
Salmonella Genus	0	0
Staphylococcus aureus	0	0
Proteus mirabilis	0	5
Streptobacillus moniliformis	0	0
Streptococcus pneumoniae	0	0
Parasites/Protozoa/Fungi	**Percent positive mice***	
Pneumocystis	0	0
Crytosporidium	0	0
Demodex	63	70
Entamoeba	33	10
Giardia	29	20
Spironucleus muris	83	65
Tritrichomonas	67	70
Mite species	100	100
Myobia musculi	100	95
Myocoptes musculinus	96	100
Radfordia affinis	83	90
Radfordia ensifera	0	0
Pinworm species	96	85
Aspiculuris tetraptera	58	25
Syphacia muris	0	5
Syphacia obvelata	42	50

* The pathogen profile was determined with the PRIA™ (PCR Rodent Infectious Agent) Panel Surveillance Plus and the Serology Profile Assessment Plus by Charles River infectious agent testing (Charles River Laboratories). A mouse was considered pathogen-exposed if it tested positive in at least one of these independent assays.

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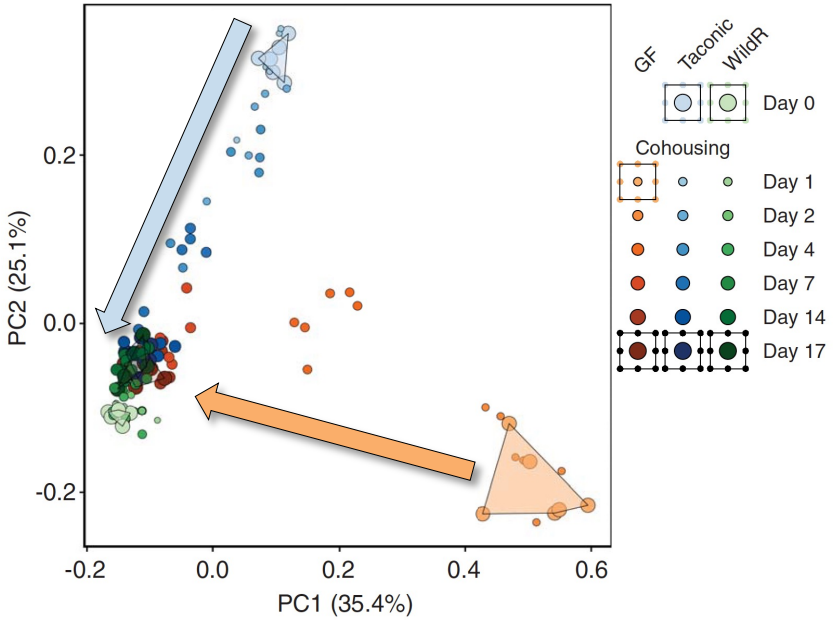
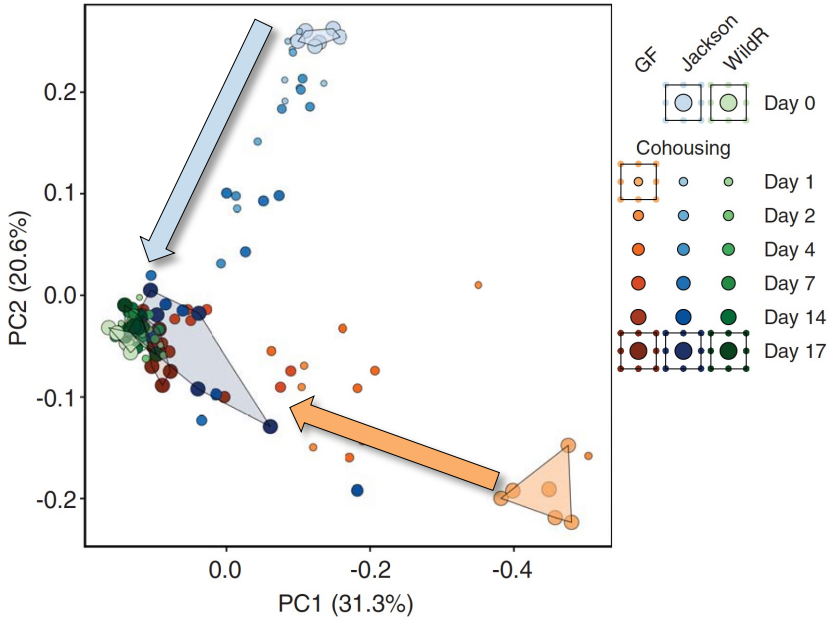
Rosshart et al., Science, 2019.

Can natural microbiota-based models help with irreproducibility of biomedical data? Natural microbiota evolved under evolutionary pressure in the natural world. Thus, they may be stable and resilient and may therefore make a standardization possible!

Microbial challenge through co-housing





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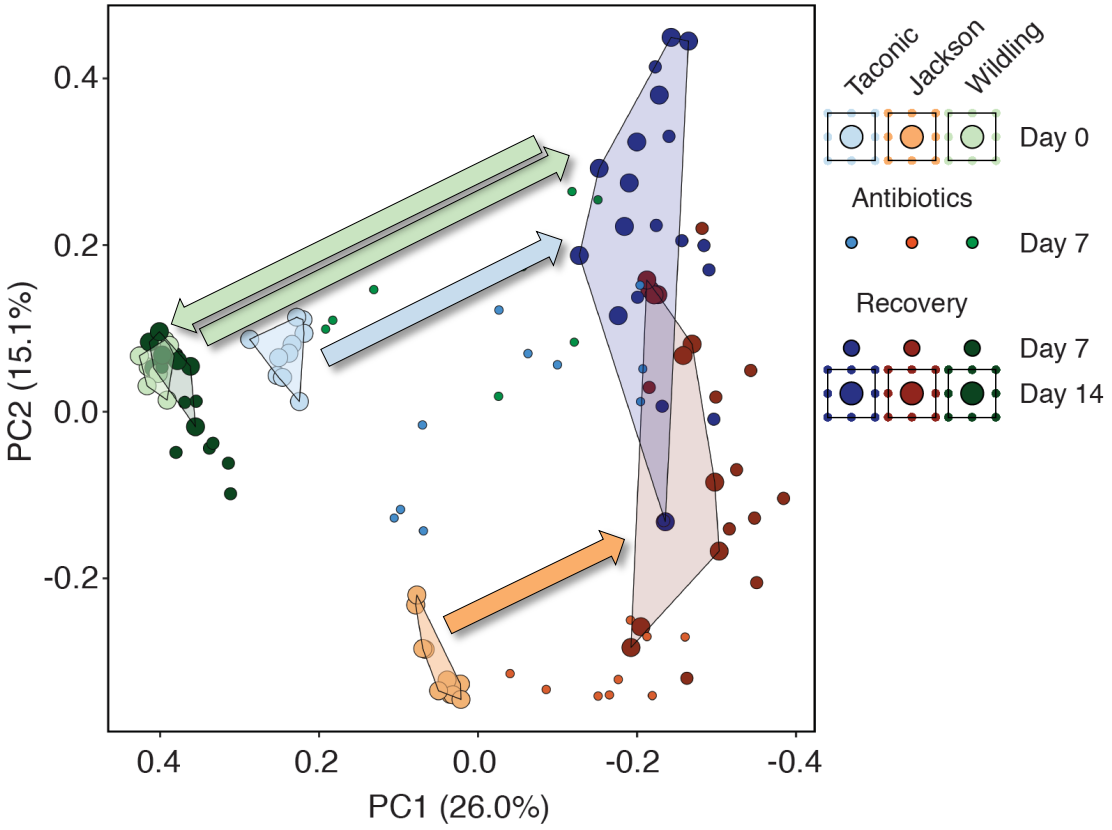
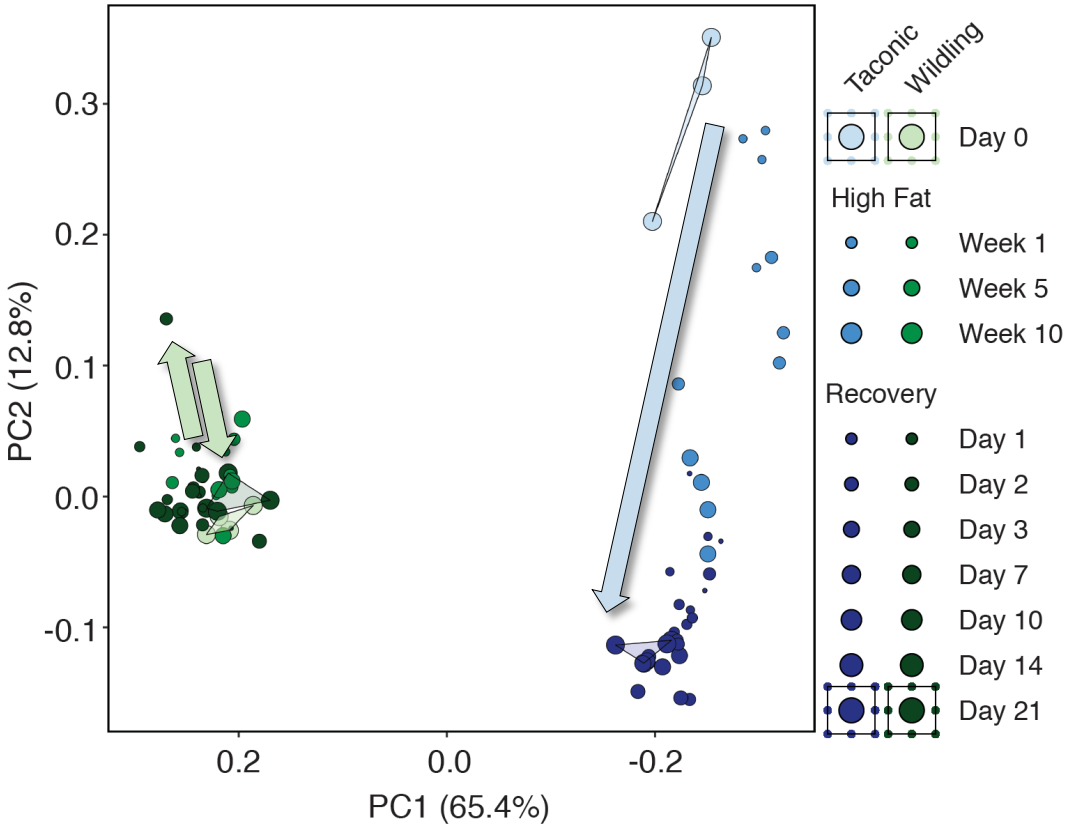
Rosshart et al., Science, 2019.

Antibiotic challenge with amoxicillin / clavulanate



Rosshart et al., Science, 2019.

Dietary challenge with high-fat diet



Rosshart et al., Science, 2019.

Concluding remarks I

The microbiota composition of the wildling model is fully characterized: "We do know exactly what we are dealing with"

Wild mouse microbiota possess multigenerational stability in lab mice under conventional SPF conditions, wildlings have no "special needs" as regards to housing and they are perfectly healthy (BSL-1)

Wild mouse microbiota possess extraordinary resilience against various environmental challenges
and could be utilized in a feasible fashion

Indispensable characteristics for highly-controlled, long-term work and reproducible experimentation

Increased translational research value, a better model for human diseases

Concluding remarks II

Thus, natural gut microbiota have characteristics that are important for standardization and that would make it possible, if you want to standardize. Moreover, this standard would increase the translational research value of the model system, a better model for human diseases

Natural microbiota- and pathogen-based models may help to discover novel disease treatments that cannot be studied in conventional laboratory mice

And all of this together may be the more ethical approach, may enhance the reproducibility of biomedical studies and increase the safety and success of translating immunological results from animal models to humans alongside a reduction in costs

What are the next steps?

"If you wish to be out front, act as if you were behind"

– Lao Tzu, ancient chinese poet and philosopher –

❀ Our mouse model from 2017 is already commercially available through Taconic Biosciences and we continue to optimize our models, e.g. we work on a goldstandard microbiome with potential commercial applications

❀ We use these novel mouse models in basic and preclinical research studies to:

I) discover novel treatments for a wide range of human diseases of global relevance including transplant rejection, GvHD, cancer, infectious diseases, allergies, autoimmune and inflammatory diseases, neurological disorders as well as cardiovascular diseases

II) support drug development in collaboration with the pharmaceutical industry

❀ We are aiming to establish new clinical microbiome studies and a SFB/TRR initiative