



Research Institute for
Farm Animal Biology

Current and future directions in livestock genetic improvement: advancing resilience and sustainability through genotype-to-phenotype research

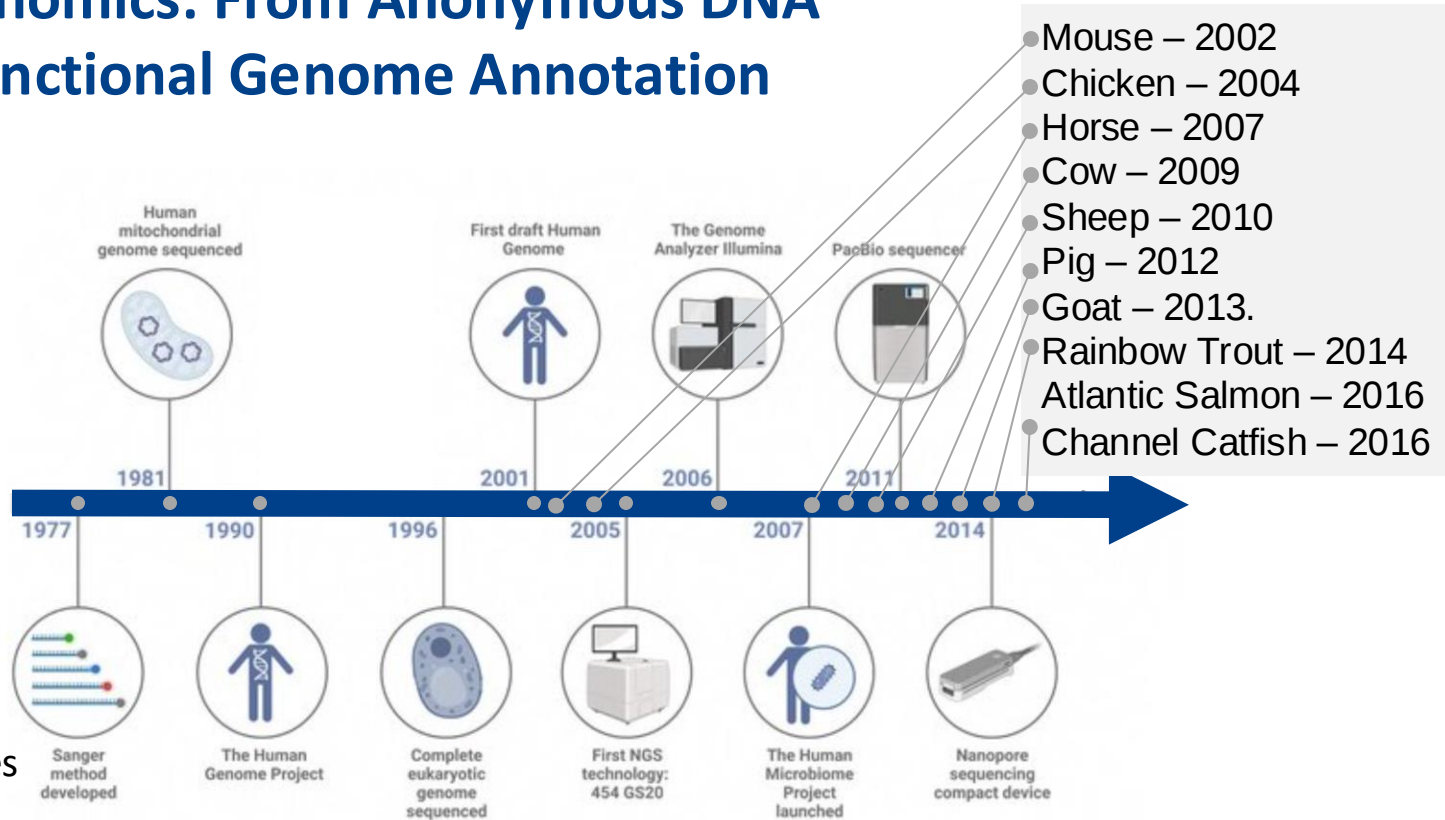
Klaus Wimmers

3rd EAAP Regional Meeting 2025, Krakow
10.04.2025



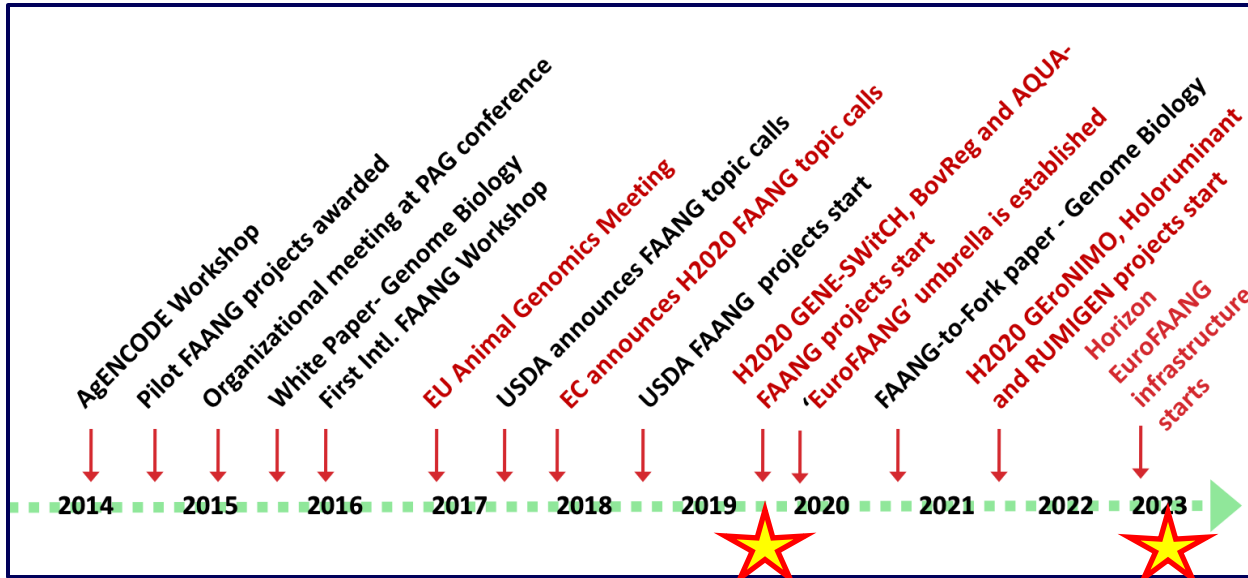
Advancing Genomics: From Anonymous DNA Markers to Functional Genome Annotation

- Microsatellites
- Linkage maps
- QTL
- ESTS
- SNPs
- SNP chips
- GWAS
- Genomic selection
- Reference genomes
- Pan-genomes



Functional annotation of animal genomes (FAANG)

Background: history about “EuroFAANG” initiatives



- COST action FAANG-Europe

- EuroFAANG cluster from H2020 funded projects

- the new EuroFAANG RI project



➔ promoting genotype-to-phenotype research (G2P)

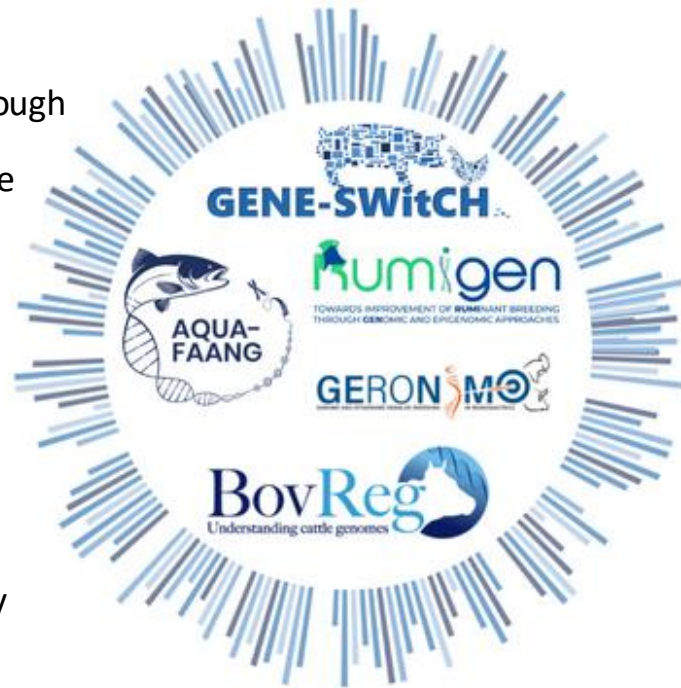
Sustainability

Research Aims

- ✓ Increase efficiency through precision breeding
- ✓ Improve animal welfare and health
- ✓ Minimise environment impact

Joint Strategies

- ✓ Communication & Dissemination
- ✓ Training
- ✓ Research Methodology



Three Pillars of Sustainability in Animal Husbandry



Environmental Sustainability

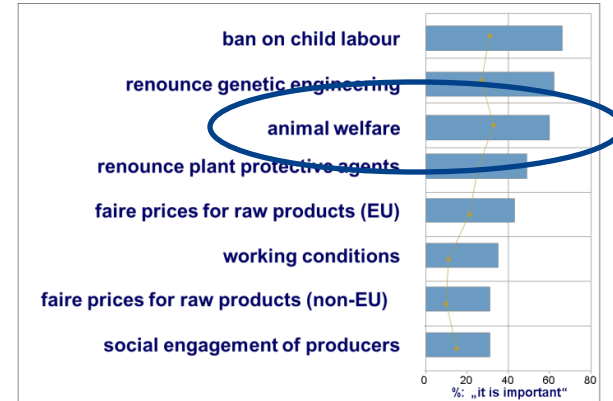
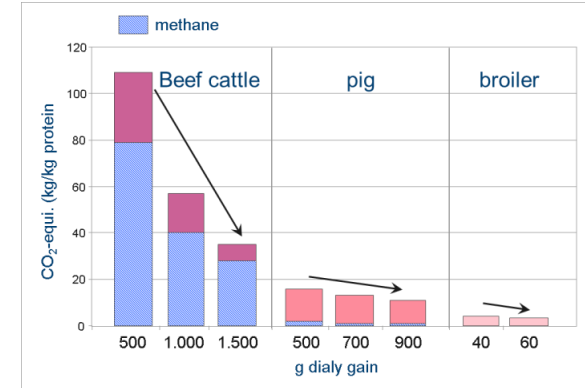
- Reducing emissions
- Improving resource efficiency
- Promoting biodiversity
- ...

Economic Sustainability

- Ensuring farm profitability
- Optimizing resource and land use
- Strengthening animal health
- ...

Social Sustainability

- Safeguarding animal welfare and health
- Encouraging socially accepted farming practices
- Securing product quality and food safety
- ...

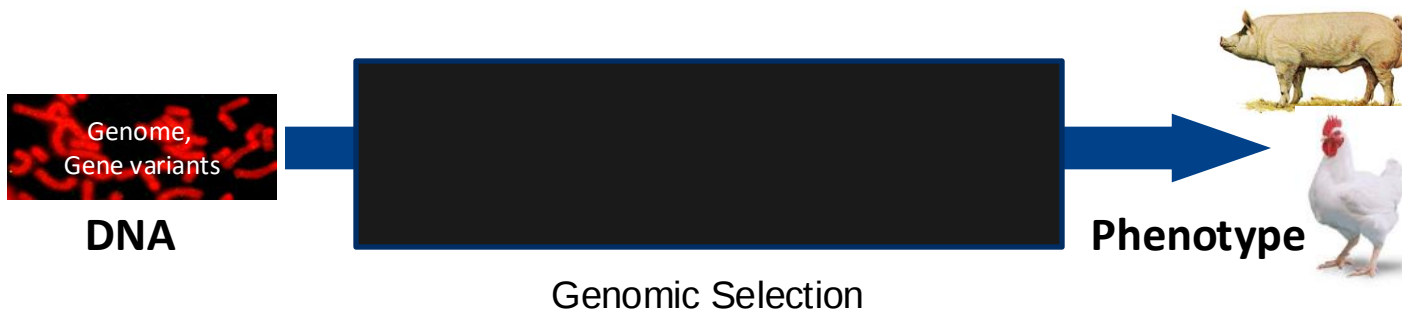


➔ Important issues: resource efficiency, resilience

Need to elucidate molecular phenotypes



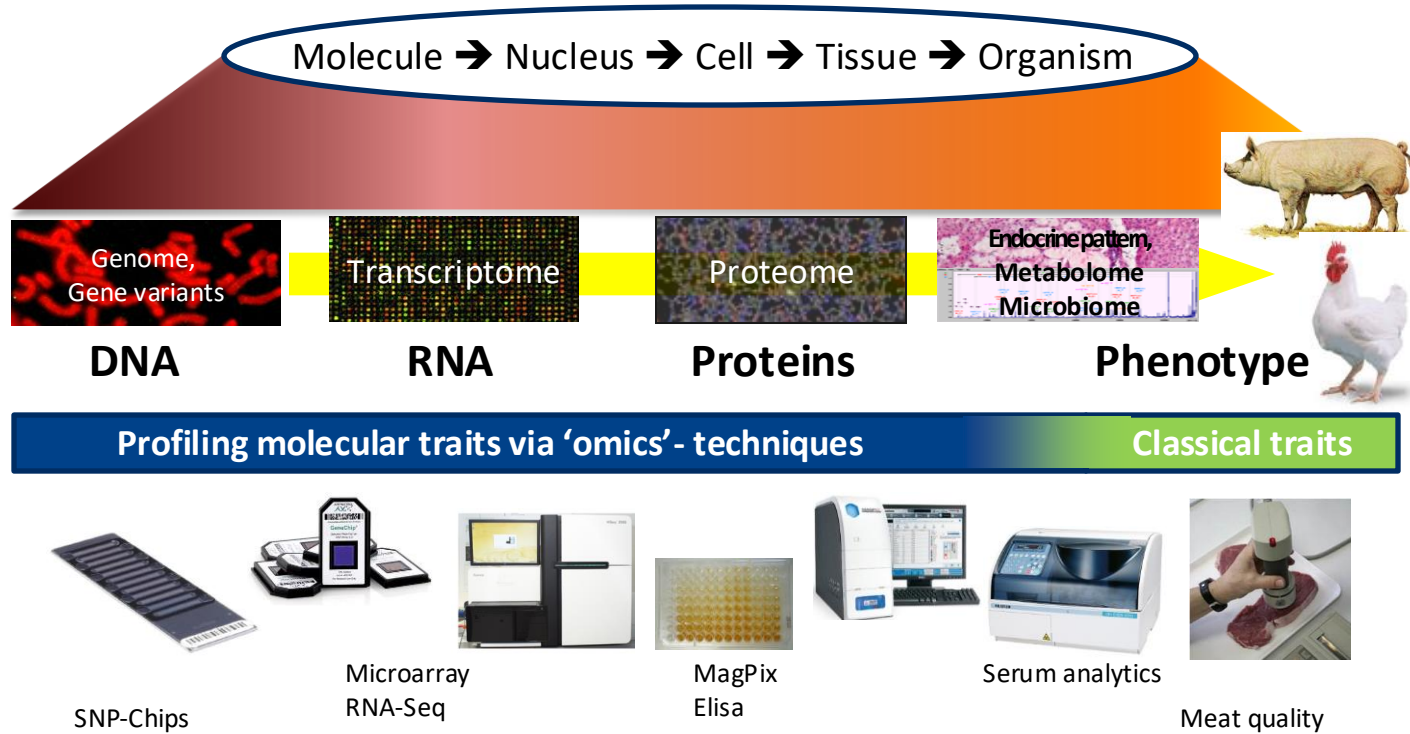
Environment + **Genotype** = **Phenotype**



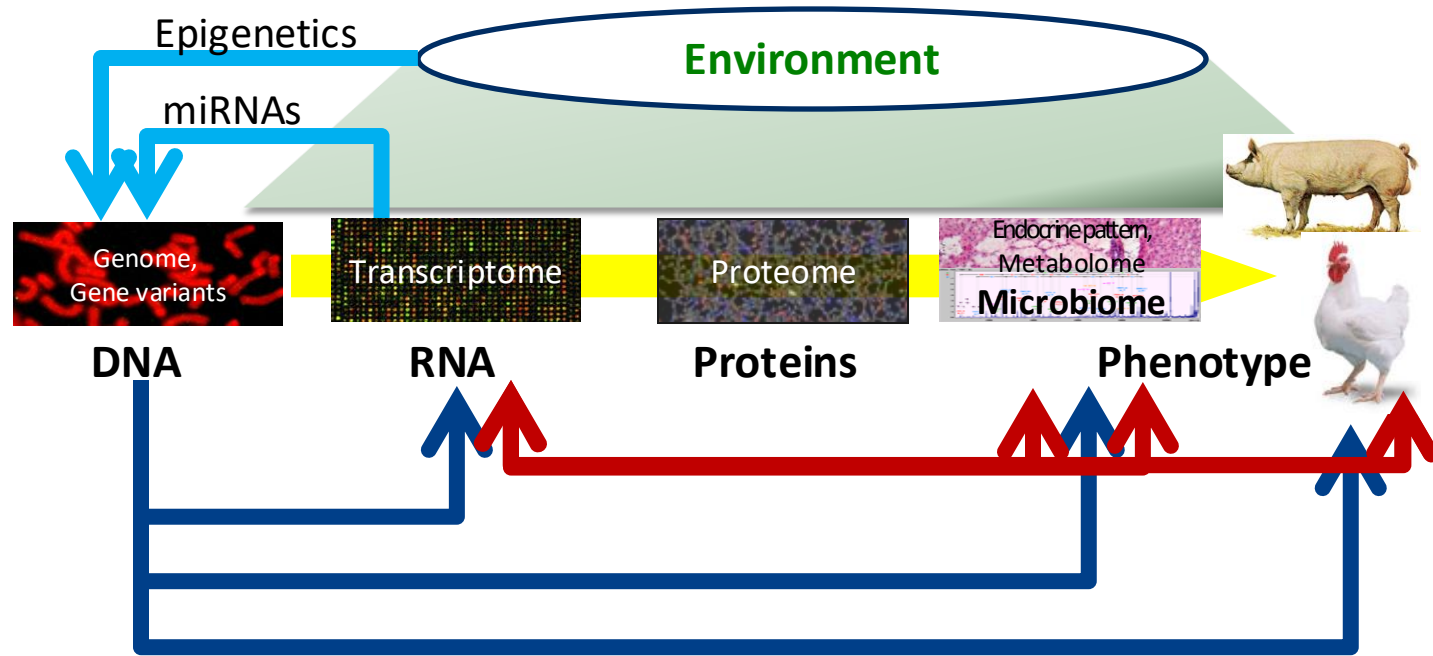
Genotype to phenotype (G2P) research explores how an organism's genetic makeup (genotype) determines its observable traits or characteristics (phenotype).

➔ **precise prediction of individual phenotypes and breeding values, as well as the identification of causal genetic variants underlying complex traits**

Genotype-Phenotype Map



Approaches along the Genotype–Phenotype Map



- Dissecting complex (quantitative) traits
- Generating hypotheses
- Identifying molecular variation underlying trait expression and inheritance
- Discovering diagnostic biomarkers and biosignatures

...growing importance of resilience in a changing world

Transformation of farm animal husbandry:

...need for resilience in the face of increasing disturbances or disruptions due to climate change, changing farming and management conditions...

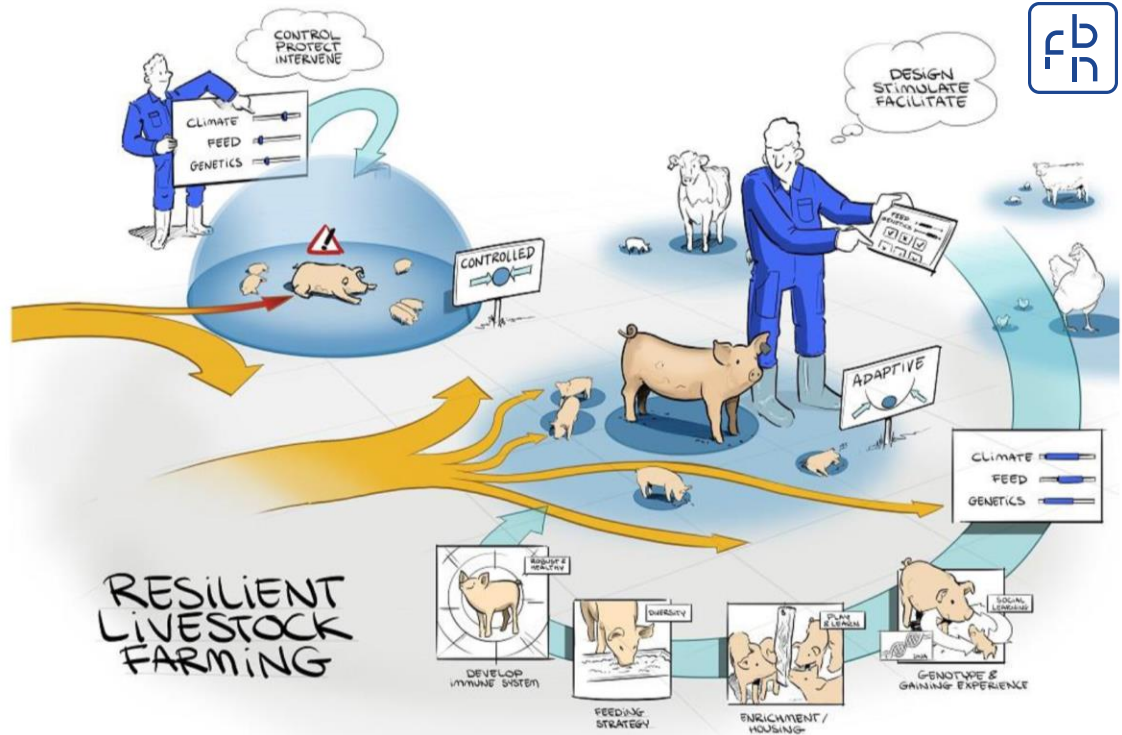
- conventional farming:

high level of environmental control(feeding, climate, hygiene, daily rhythm)

- organic farming: closer to natural conditions, environmental stimuli

 - ➔ improved welfare

 - ➔ higher resilience/higher robustness

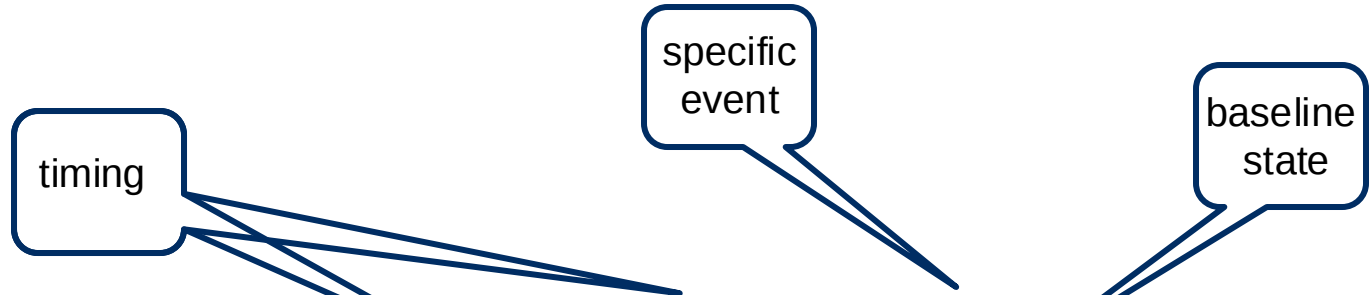


<https://www.wur.nl/en/research-results/research-institutes/livestock-research/themes/resilience.htm>

Resilience, Robustness, and Resistance: Core Traits for Ensuring Animal Health and Welfare



European Partnership on
Animal Health and Welfare



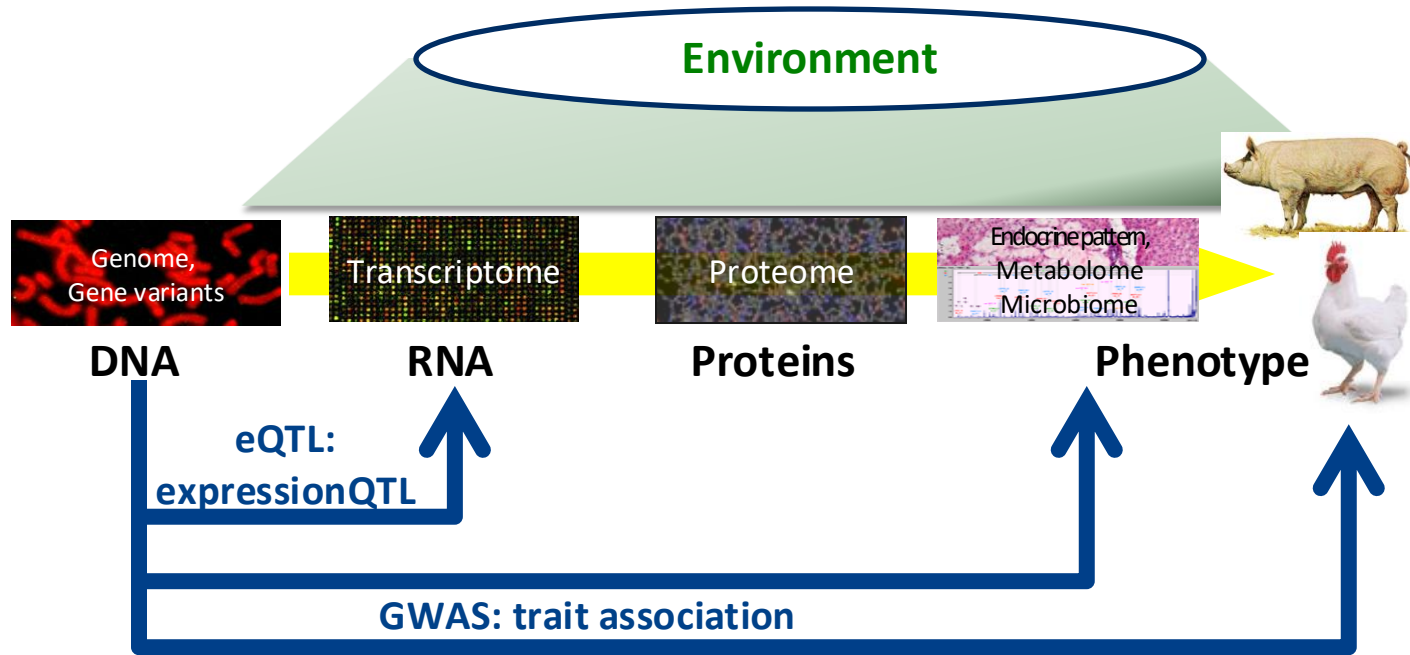
The **resilience** of an animal is the ability to cope with short-term disturbances, including those caused by pathogens, through response and rapid recovery to its original state. (*sponge*)

The **robustness** of an animal in production is the ability to maintain its functionality under a wide range of conditions and stresses without substantial change. (*stone*)

The **resistance** of an animal is its capability to avoid or mitigate the negative effects that any type of challenge might cause to it. (*shield*)

➔ linked to behavioral, immune, stress, and metabolic responses

Approaches along the Genotype–Phenotype Map



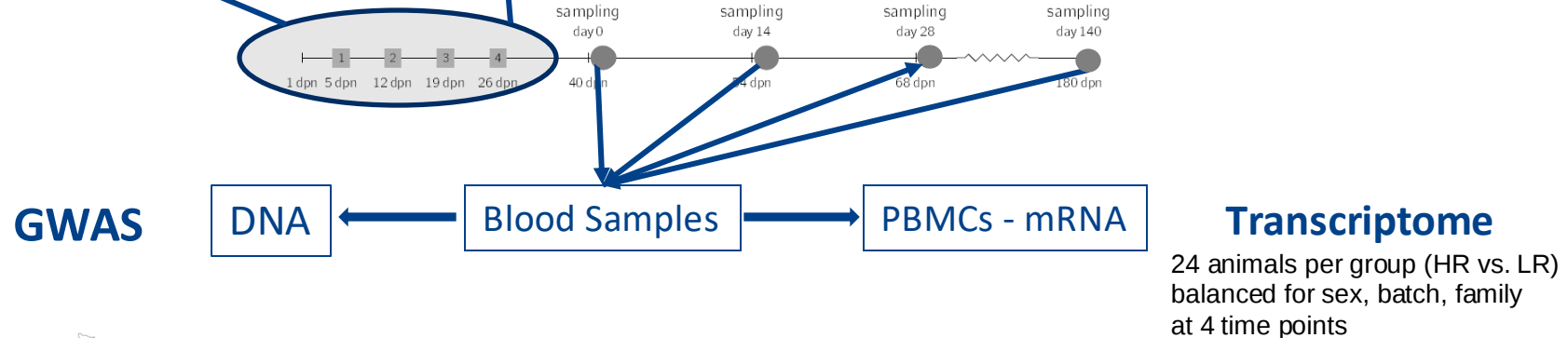
Connecting genetic variation to phenotypic outcomes

— including RNA expression patterns, endogenous molecular traits, and organism-level traits



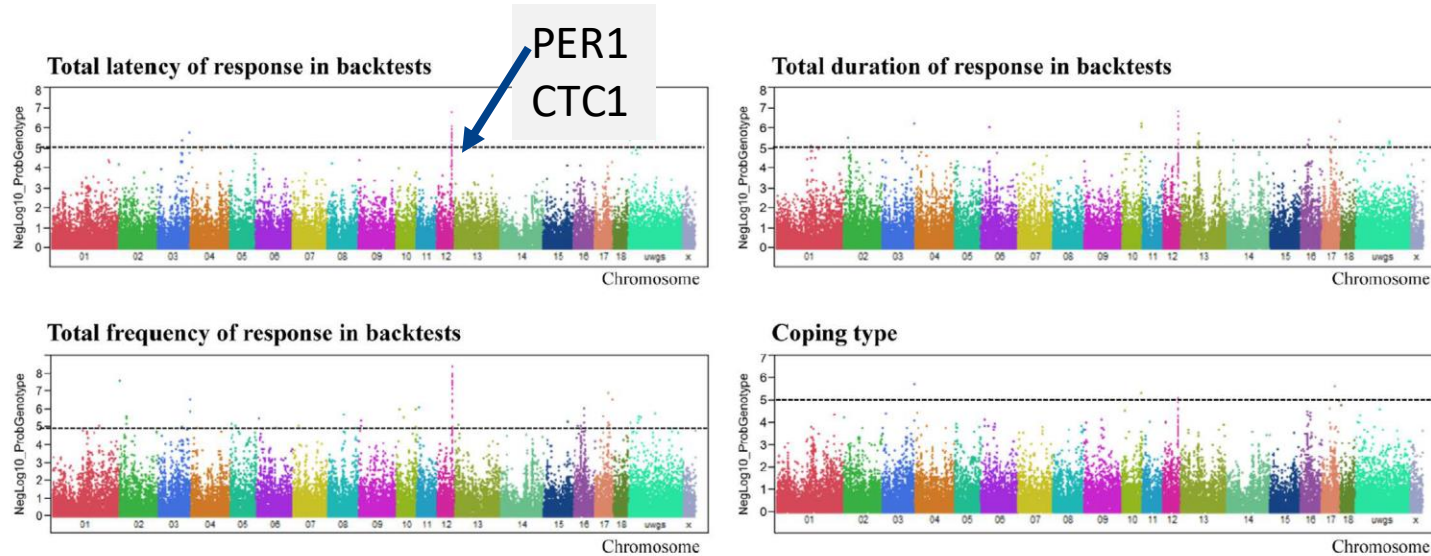
Animal personality and coping styles are basic concepts for evaluating animal welfare. Struggling response of piglets in so-called backtests early in life reflects their coping strategy.

latency, duration and frequency of struggling attempts
 → animals assigned to groups according to their coping behaviour: **high reactive vs. low reactive** (pro-active vs. re-active)

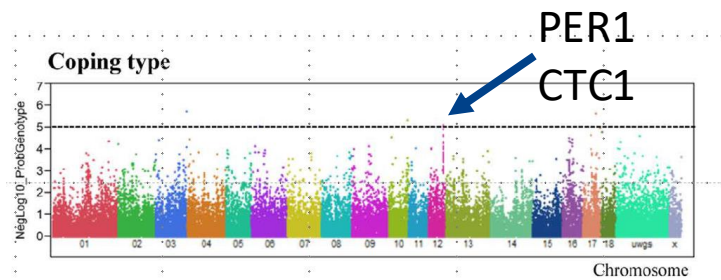


GWAS for backtest traits

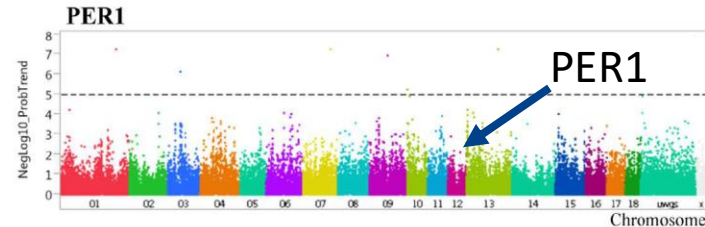
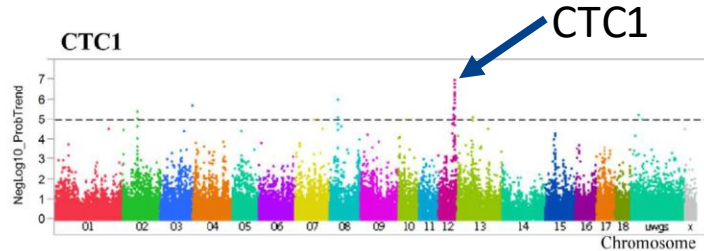
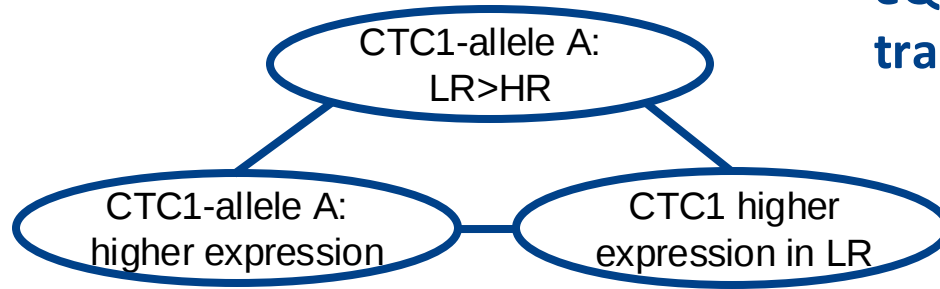
Significant SNPs for latency, duration and frequency of struggling attempts at 5, 12, 19 and 26 days of age and across all replications



association with
coping traits



eQTL: GWAS for transcript abundance of trait associated genes in hypothalamus



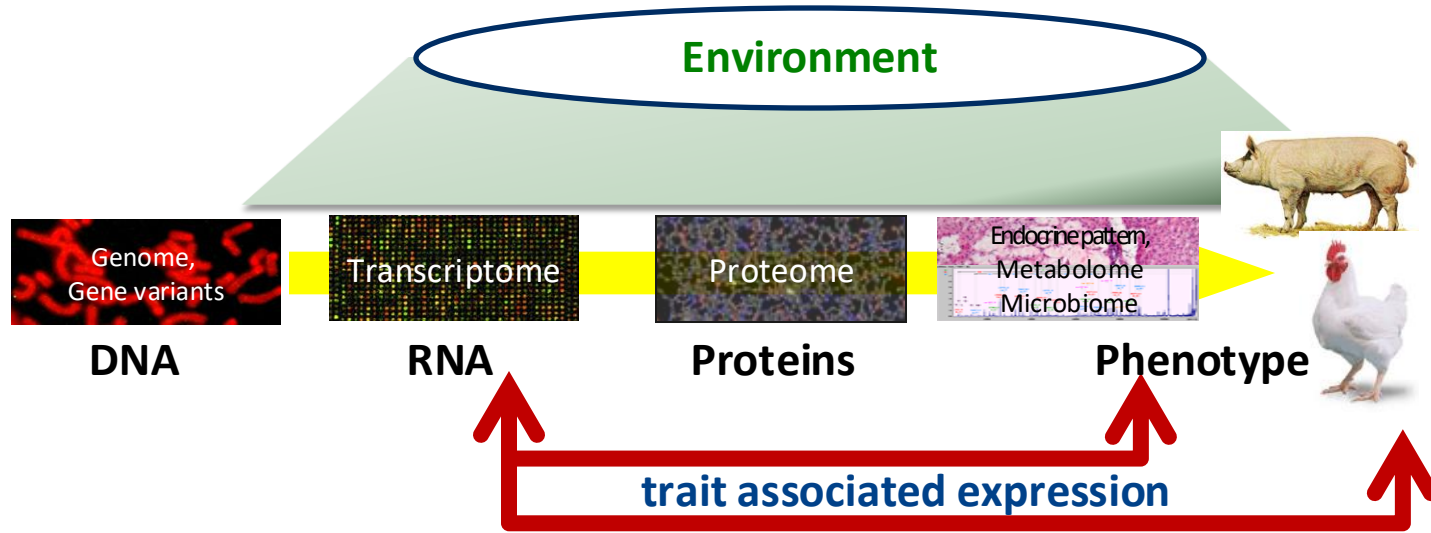
cis- and trans eQTL of trait-associated genes

Ponsuksili et al., 2015
²Beckstein et al., 2014
¹Zhang et al., 2020

Coping behaviour links to circadian rhythm:

- telomere maintenance complex component 1 (CTC1): circadian patterns of activity and food intake²
- Period circadian clock 1 (PER1) glucocorticoid rhythmicity, innate routine & feeding behavior¹

Approaches along the Genotype–Phenotype Map



Connecting RNA expression variation to phenotypic outcomes

Transcriptional response to stress - triggered by aggressive behaviour



- High reactive animals are in a more alert physiological state, primed for defense and recovery, compared to low reactive animals.
- Aggression and stress have negative effect on production, meat quality and animal welfare



Aggressiveness examined at the age of ~10 weeks by mixing and counting skin lesions (lesion score, LS):

High aggressiveness (**H**), high lesion score; Low aggressiveness (**L**), low lesion score

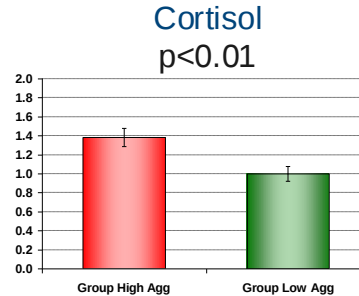
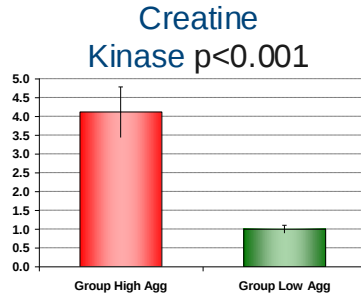
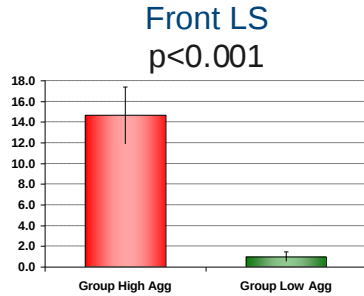
At slaughter age pigs (n=271) with different aggressiveness were mixed [**HH**, **HL**, **LL**, **U** (unmixed)] prior to transport and slaughter to induce different levels of aggressive behavior

Phenotypes measured at slaughter:

- **Stress response**: cortisol, creatine kinase, lactate, glucose
- **Aggressive behavior**: carcass lesions at front, middle, rear & total
- **Meat quality**: pH at 3hr, 6hr, 24hr; driploss; Minolta L,a,b
- **transcriptome**



Transcriptional response to stress - triggered by aggressive behaviour



high vs low stress group
n=8 n=8

PBMC
M. longissimus dorsi
liver
adrenal gland



mRNA

➔ identification of genes and pathways associated with stress responsiveness and aggressiveness

Transcriptional response to stress – PBMC



1038 differentially expressed genes are related to:

Canonical pathway	diff	#	pVal BH
Dendritic cell maturation	HF > LF	12	6.20E-04
IL-6 signaling	HF > LF	8	7.69E-03
IL-8 signaling	HF > LF	8	4.67E-02
Toll-like receptor signaling	HF > LF	5	1.83E-02
TREM 1 signaling	HF > LF	9	3.22E-05
Glucocorticoid receptor signaling	HF > LF	10	4.27E-02
NF- κ B Signaling	HF > LF	11	1.58E-03
Acute Phase Response Signaling	HF > LF	10	4.68E-03
IL-10 Signaling	HF > LF	5	3.47E-02
Complement System	HF > LF	3	8.32E-02

H showed shifts in pathways of innate and acquired immunity, with functional interconnection to the HPA axis.

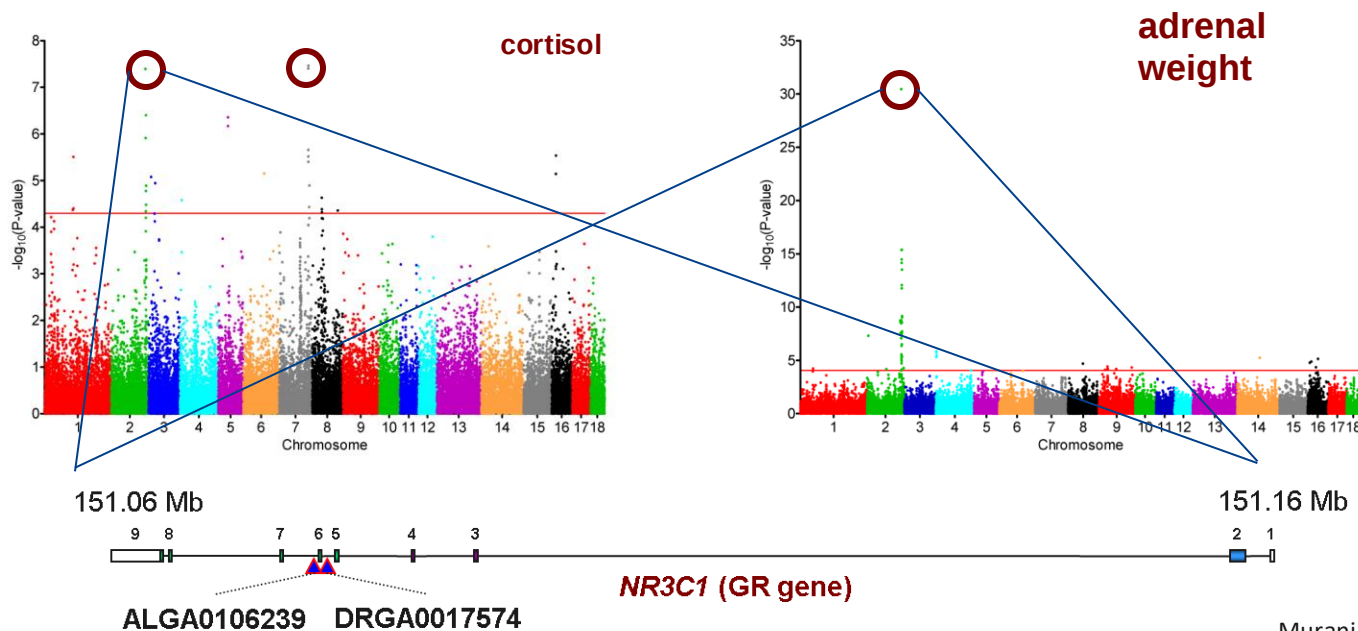
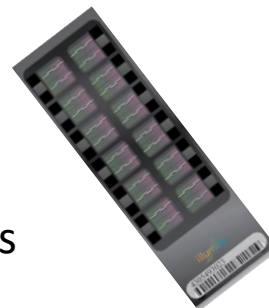
- ➔ Changes in immune effectors and cell surface receptors indicate alerting in terms of response and responsiveness
- ➔ Relates to behavioral, immune, stress, and metabolic responses

Data collection:

animals & samples: German Landrace (LR; n=834)

plasma cortisol level (ELISA); weight of adrenal gland

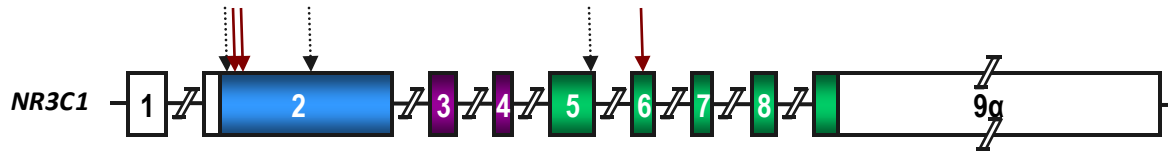
genotypes: Illumina PorcineSNP60 BeadChip (n=564); 42,359 SNPs



Detection of coding SNP in *NR3C1*



6 SNP in the coding region identified: 3 missense, 3 silent

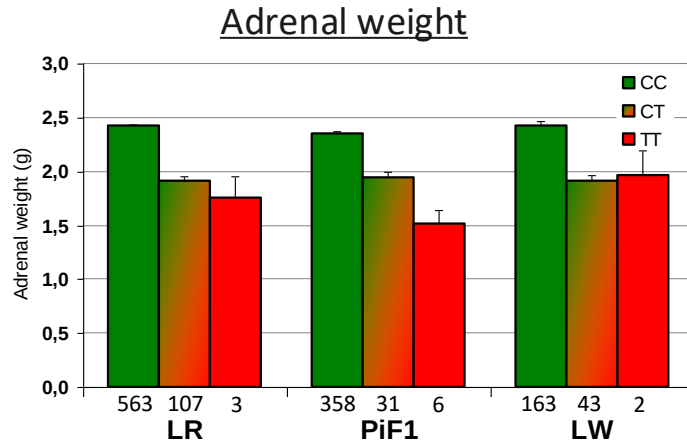


SNP	Position	Substitution	LR (n=834)	LW (n=274)	PiF1 (n=537)
c.39A>C	Exon 2	p.Glu13Asp	0.013	0.035	0.029
c.55G>C	Exon 2	p.Val19Leu	0.000	0.000	0.186
c.1829C>T	Exon 6	p.Ala610Val	0.082	0.104	0.060

Association of the cSNPs with cort and adrw

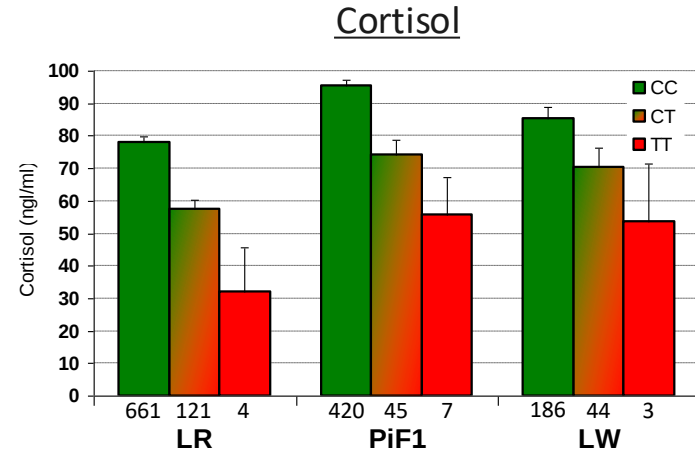


No, or weak and inconsistent effects of SNPs c.39A>C and c.55G>C
SNP c.1829C>T (exon 6; Ala/Val) showed large and consistent effects:



$p=2.0E-39$ $p=4.3E-17$ $p=1.6E-12$

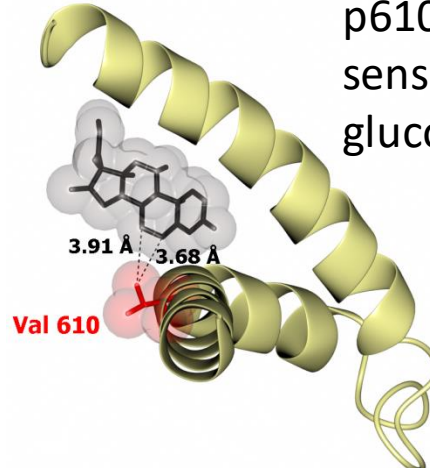
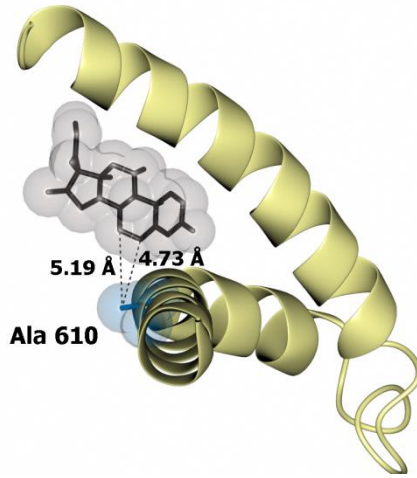
Substitution effect $\cong -1.20$ SD



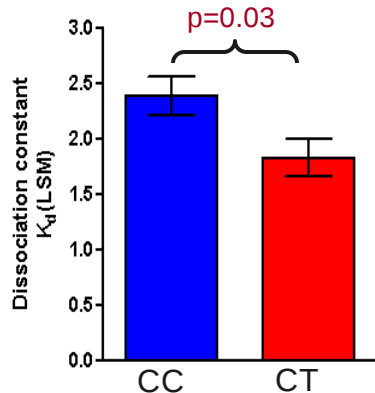
$p=4.2E-14$ $p=1.30E-06$ $p=0.018$

Substitution effect $\cong -0.65$ SD

Effects of SNP c.1829C>T

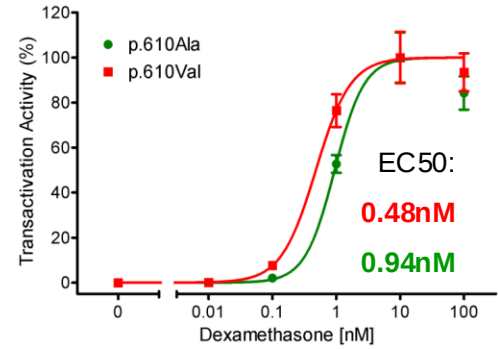


p610Val increases sensitivity of GR to glucocorticoids

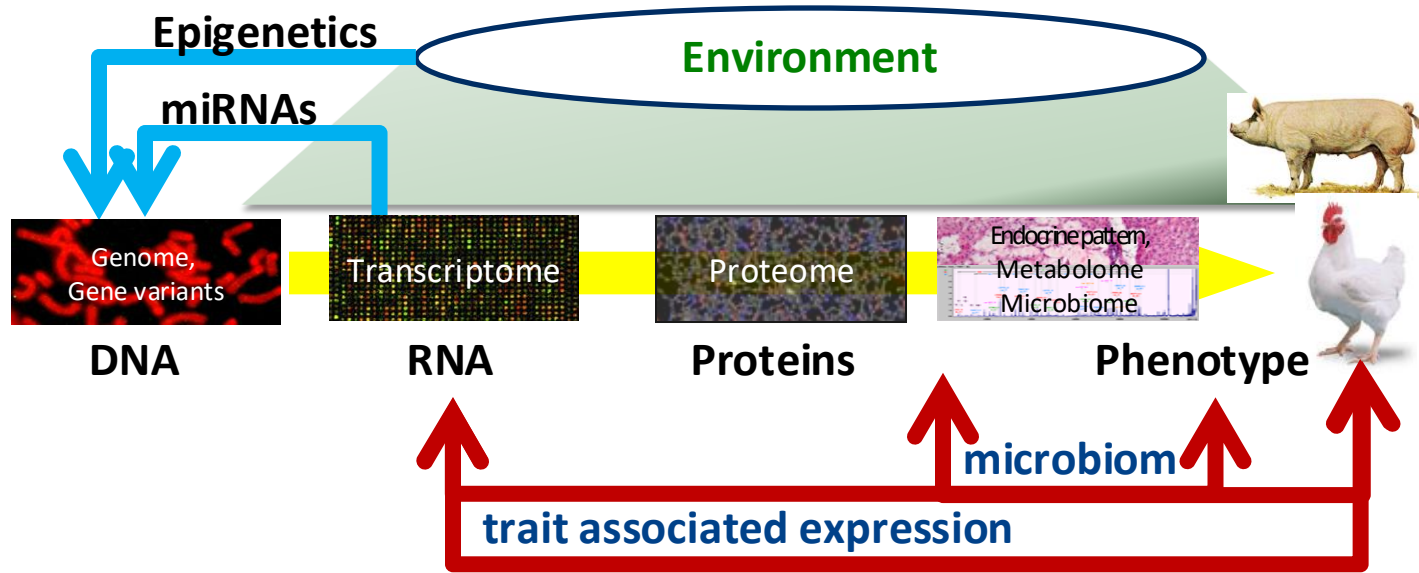


p.610Val with reduced K_d
→ higher binding affinity

→ causal polymorphism affecting cortisol receptor sensitivity, with implications for behavioral, immune, stress, and metabolic responses, and for the use of cortisol as a biomarker of stress



Approaches along the Genotype–Phenotype Map



Connecting Multi-Omics variation to phenotypic outcomes

Sustainability and Phosphorus

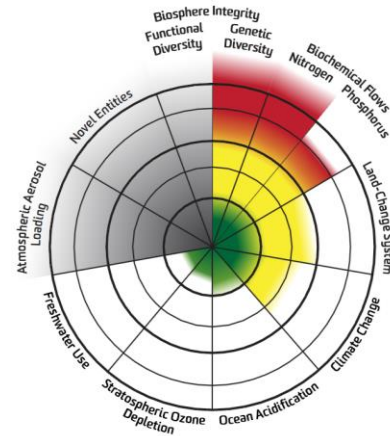


Surface mining in Togo
(A. Pugachevsky)

Eutrophication in marine and freshwater systems (IOW)

Challenges:

- Essential for all living beings
- Finite resource
- Environmental issues
- Dependency on imports

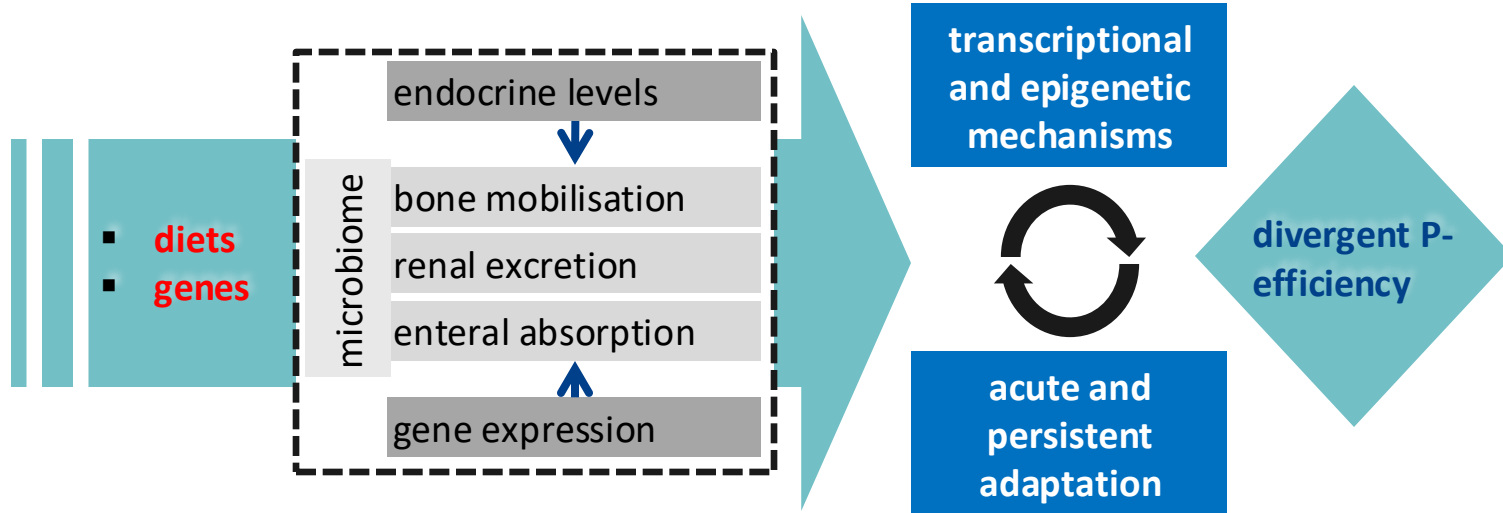


P use exceeds planetary boundaries

Sustainable Phosphorus utilisation



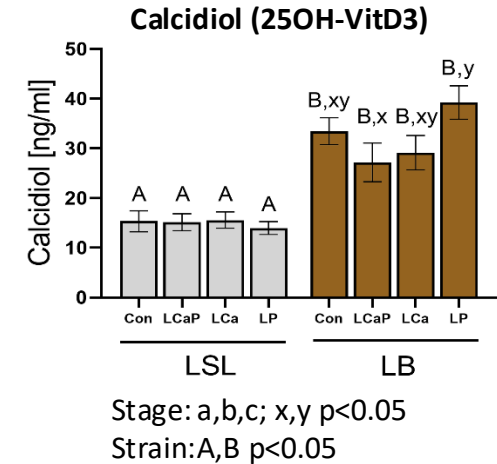
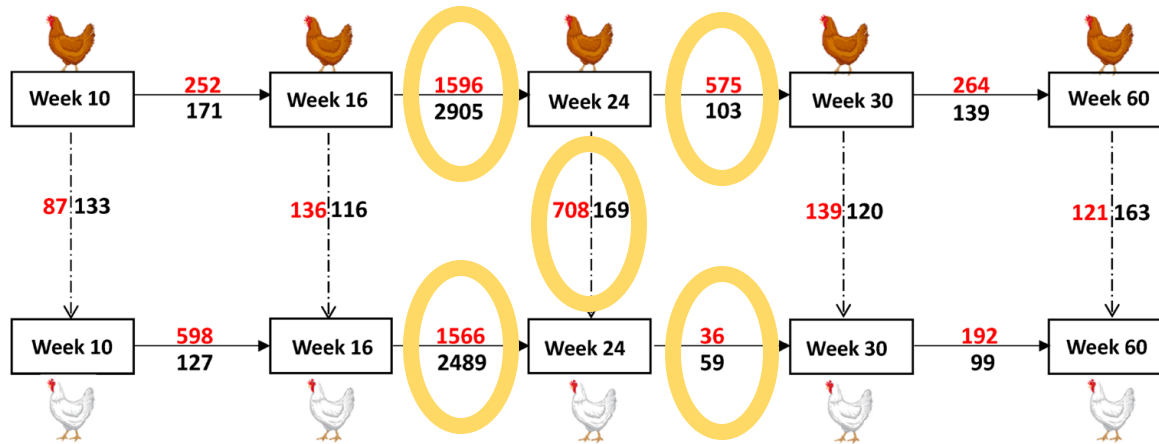
P homeostasis is maintained by tight regulation



P supplementation according to physiological and genetic requirements without excess:

- adequate diets
- genetic selection
- modulation of microbiota

Differences between LSL vs. LB hens based on jejunal transcriptomic profiling and the endocrine system (e.g., vitamin D): effects by age, breed, and diet

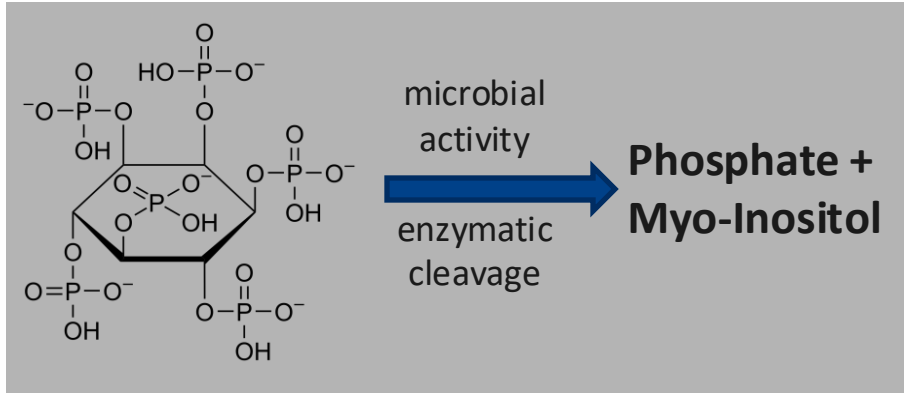


P-Fowl datasets:

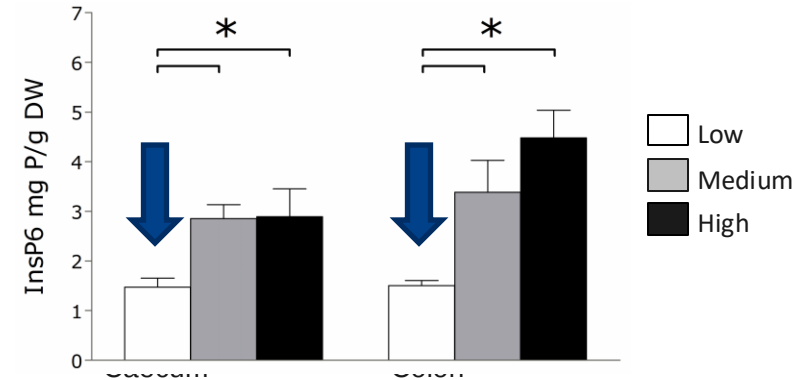
- 11 zoo-technical or physiological characteristics of phenotype (project 1)
- 58 immune traits (project 7)
- 183 metabolites, enzymes or hormones (projects 1, 3 & 5)
- 209 microbial taxa from duodenum digesta & mucosa (project 4)
- 291 miRNA (project 9)
- 13689 mRNA (project 3)

Faktors: Phytases, Microbiota

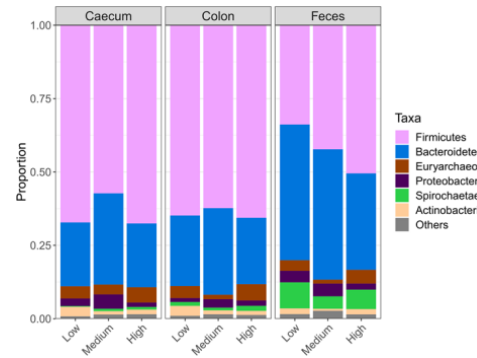
➤ **Phytate** (Inositolphosphat; **InsP6**)



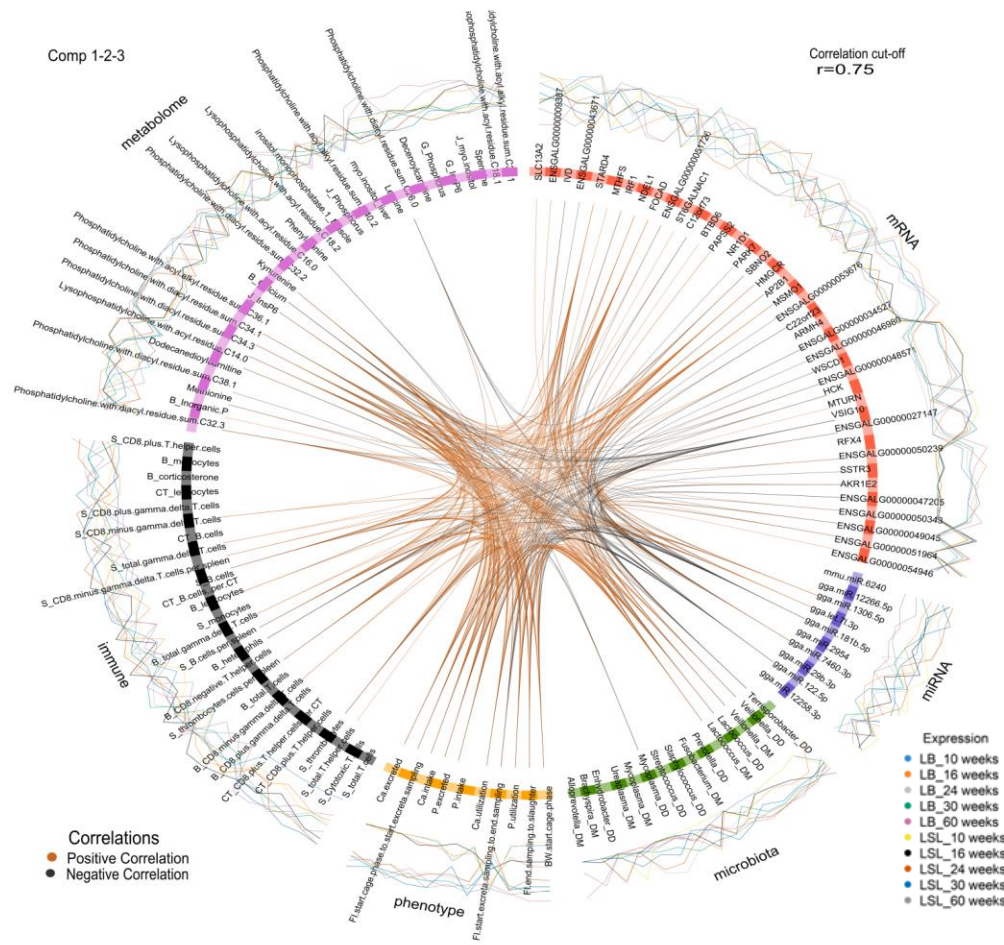
Reduced levels of mineral P lead to increased mobilization of phytate-bound P in the gut...



➔ Application of phytases in animal feed to enhance the bioavailability of phytate phosphorus



...as a result of microbiome shifts



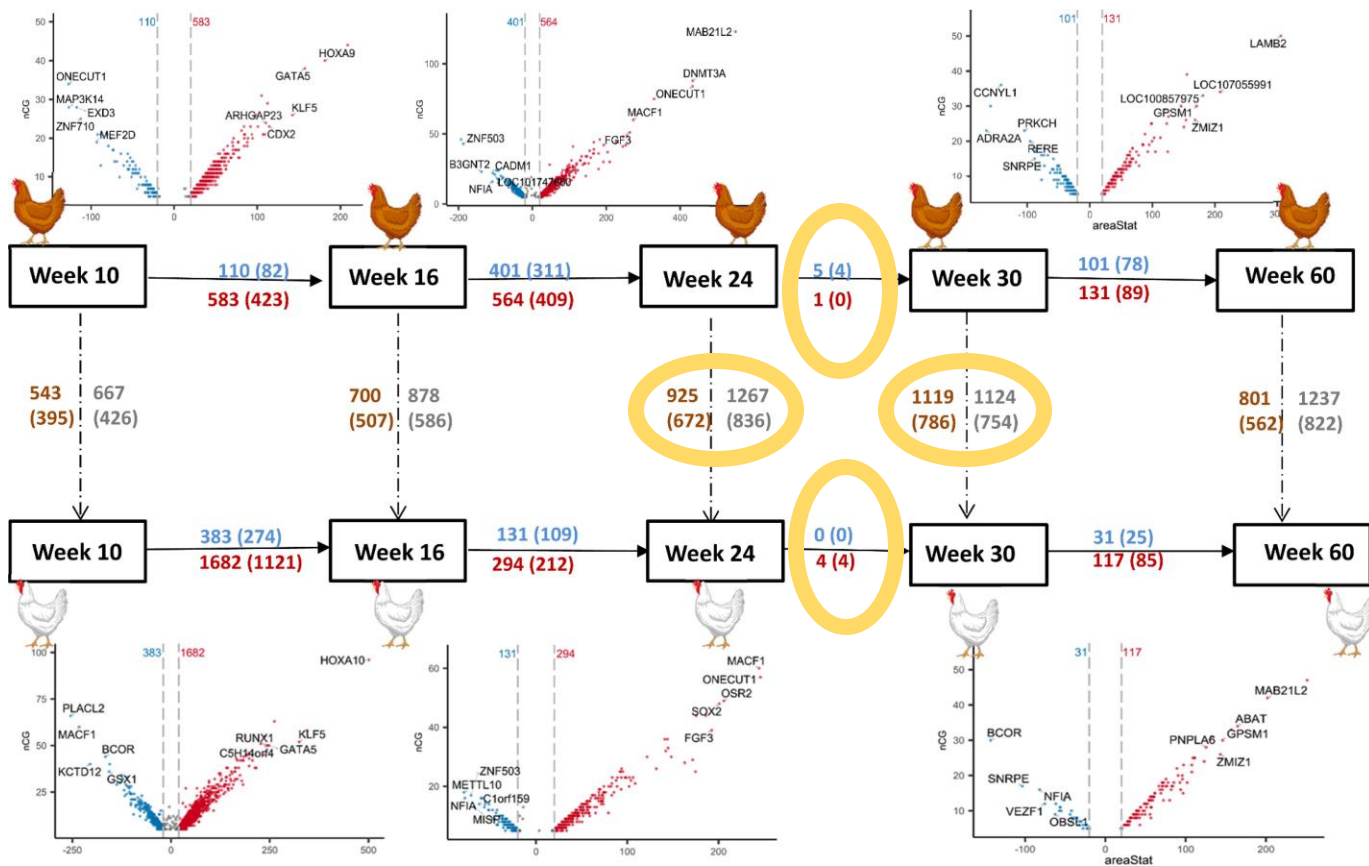
multi-omics-biosignature:

- 40 mRNA,
- 10 miRNA,
- 15 microbial taxa,
- 29 metabolites,
- 30 immune traits
- 10 phenotype traits

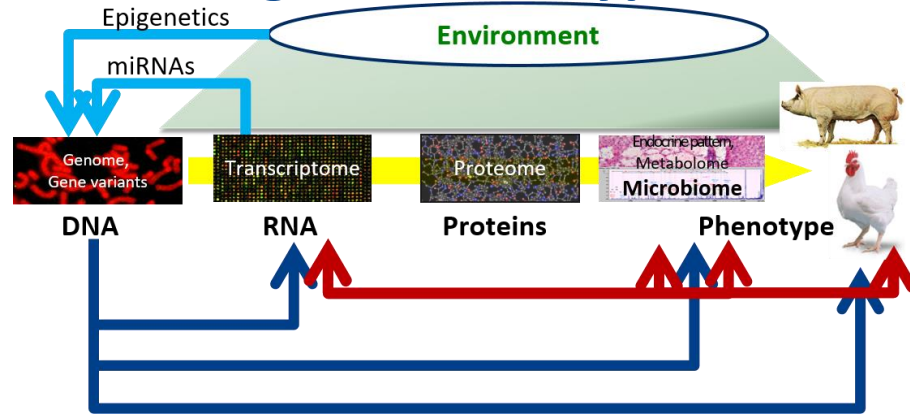
interestingly DNA-methyltransferases are among the differentially expressed genes



Differential DNA-methylation



Approaches along the Genotype–Phenotype Map



Connecting DNA Variants, RNA Expression, and Multi-Omics to Phenotypic Outcomes

- Identified candidate and causal genes, as well as key pathways involved in immune response, coping behavior, and aggression
→ supporting improved animal welfare
- Discovered a multi-omics biosignature for phosphorus efficiency
→ advancing nutritional sustainability and precision breeding

➔ DNA markers, biomarkers, and biosignatures provide a foundation for tools enabling precise prediction of individual phenotypes and breeding values

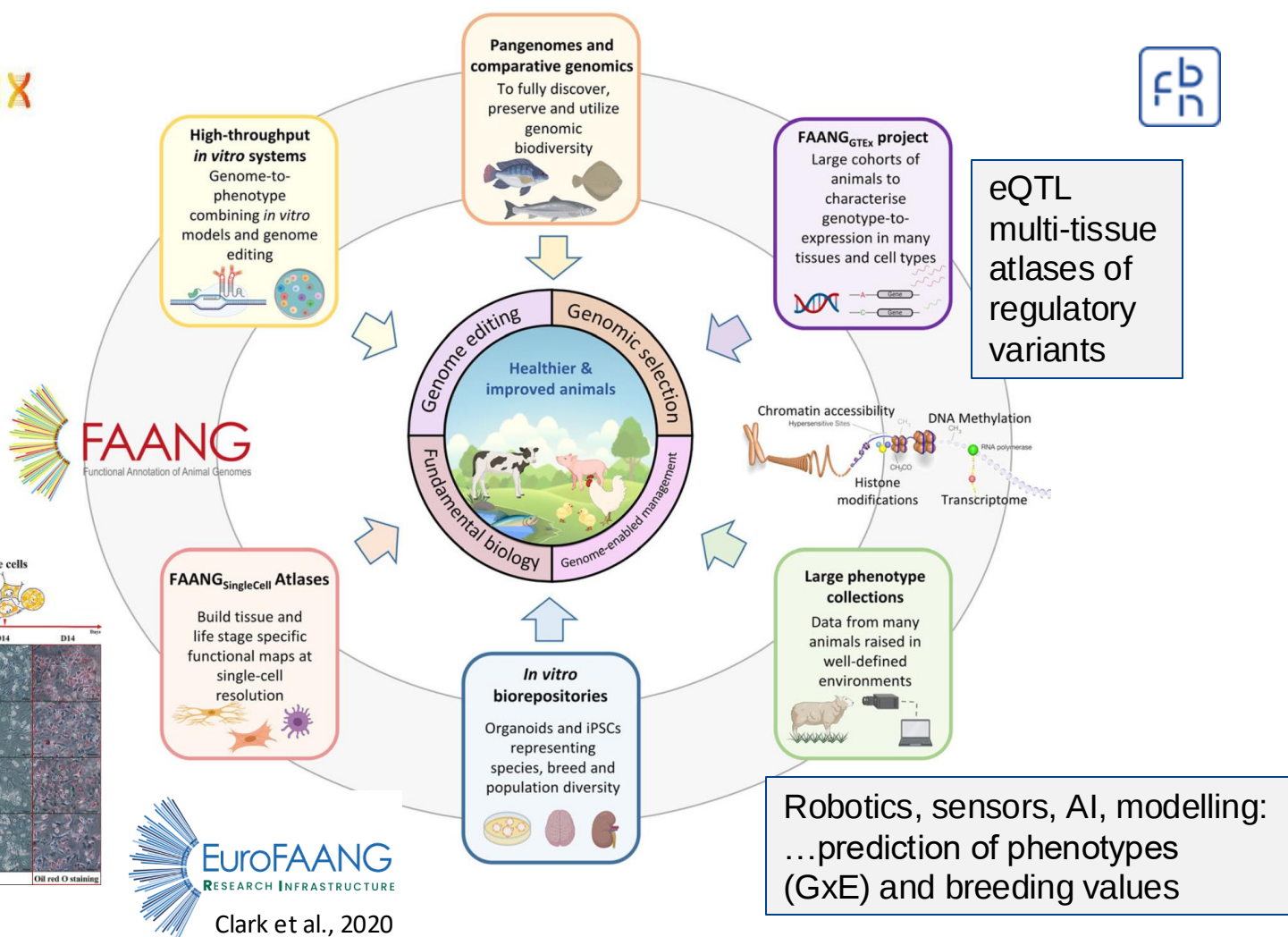


Adipogenic differentiated live cells

Diagram illustrating the timeline of adipogenic differentiation. The timeline starts at D-1 (Confluent cells) and proceeds through D0, D1, D4 (Adipogenic differentiation starts), D7, D14, and D21 (Differentiated cells). Below the timeline, a grid of microscopy images shows the progression of adipogenic differentiation for four conditions: AS-FP, AS-FS, DL-FP, and DL-FS. The images are organized into two columns: 'Live cells' (left) and 'Oil red O staining' (right). The 'Live cells' column shows the morphology of the cells at each time point, while the 'Oil red O staining' column shows the accumulation of lipid droplets (stained red) in the cells. The images are arranged in a 4x6 grid, with rows corresponding to the conditions and columns corresponding to the time points and staining methods.



EuroFAANG
RESEARCH INFRASTRUCTURE
Clark et al., 2020





GATTACA: comprehensive genetic profiling, manipulation, and selection before birth, combined with on-the-spot genetic diagnosis, enable societal stratification based on genetics



...accurate prediction of phenotypic outcomes under varying conditions to enhance genetic selection and align animal abilities with their environment

Thank you for your kind attention



Research Institute for Farm Animal Biology
Wilhelm-Stahl-Allee 2
18196 Dummerstorf, Germany

www.fbn-dummerstorf.de

K.Wimmers
wimmers@fbn-dummerstorf.de

Background: FAANG and G2P-Research



The FAANG initiative, which stands for **Functional Annotation of Animal Genomes**, aims to enhance our understanding of how genetic information translates into phenotypes in animals. Specifically, the goals of the FAANG initiative include:

- **Characterizing Functional Elements:** Identifying and annotating functional elements within animal genomes, such as promoters, enhancers, and non-coding RNAs, to understand their roles in gene regulation and expression.
- **Understanding Gene Regulation:** Investigating the regulatory mechanisms that control gene expression, including the dynamics of transcription, chromatin structure, and epigenetic modifications.
- **Linking Genotype to Phenotype:** Establishing connections between genetic variations (genotype) and observable traits (phenotype) in animals, which can have implications for breeding programs, animal health, and production efficiency.
- **Developing Genomic Resources:** Generating comprehensive datasets and resources, such as reference genomes, transcriptomes, and epigenomes, to facilitate research and breeding efforts in diverse animal species.
- **Enhancing Animal Welfare and Productivity:** Contributing to improvements in animal welfare, health, and productivity by providing insights into the genetic basis of desirable traits and diseases.

GenoPHEnix objectives:

- **1. Building shared capacity for deep phenotyping of farmed animals, including identification of biomarkers for breeding and management.**
- **2. Creation of a common data structure and access to genome and phenome data in farmed animals.**
- **3. Expanded Biobanking Capabilities and Access to Cellular Models for Farmed Animal Research**

basic

applied

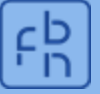


Funded by
the European Union



625





Titel of the chapter

Our four focus topics



01

Individualising
farm animal
husbandry



02

Farming animals
in sustainable
resource cycles



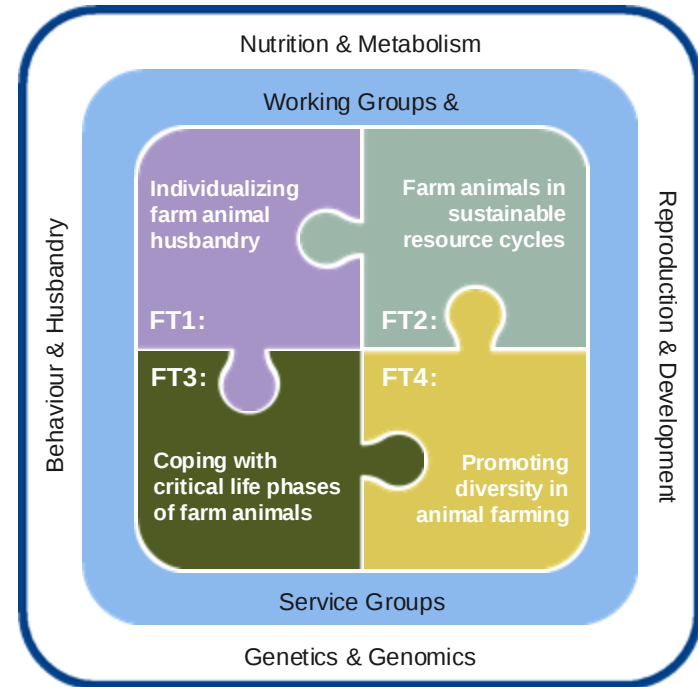
03

Coping with
critical life
phases of farm
animals



04

Promoting
diversity in
animal farming



● immune response and performance

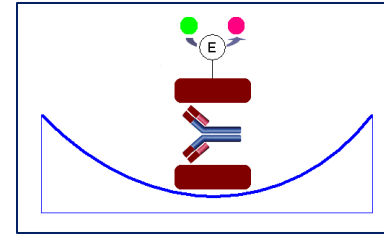
Animals with favourable productivity and immune response exhibit lifelong characteristic expression pattern → biomarkers

● immune response and coping behavior

Proactive animals show a increase preparedness to cope with biotic and abiotic stressors → biomarkers

● aggressive behavior and stress response

Focussed analysis of key genes within functional pathway can provide causal variation → breeding tools



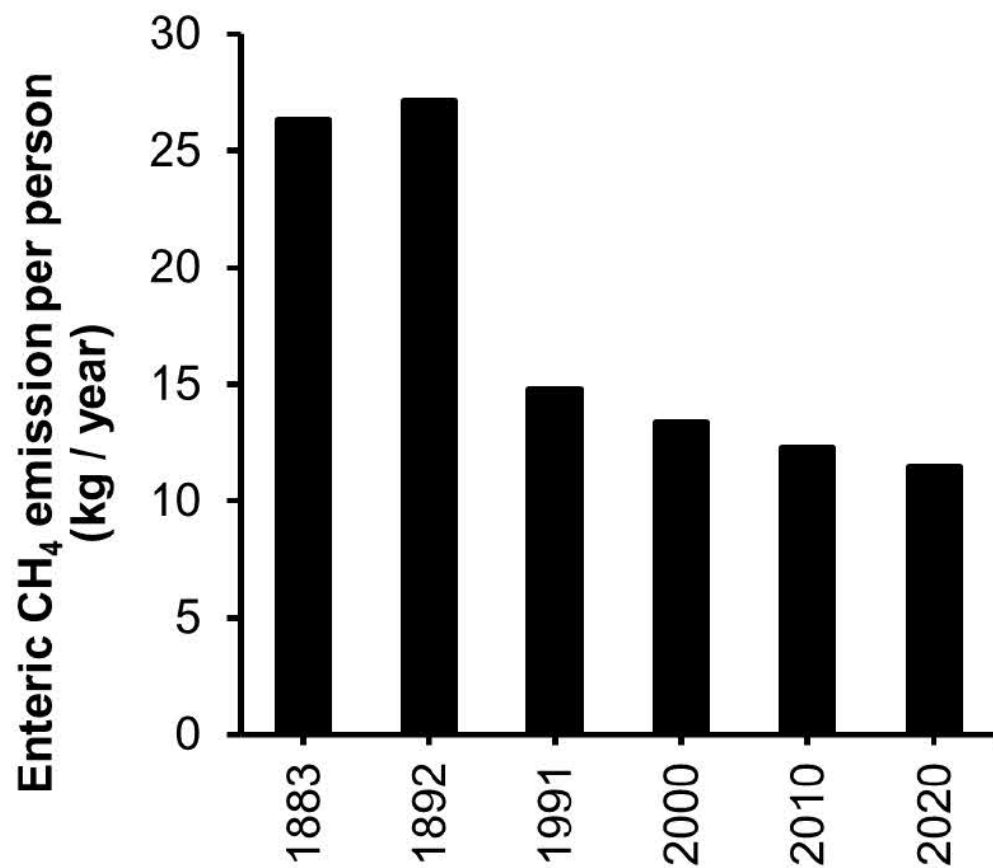
ELISA



backtest



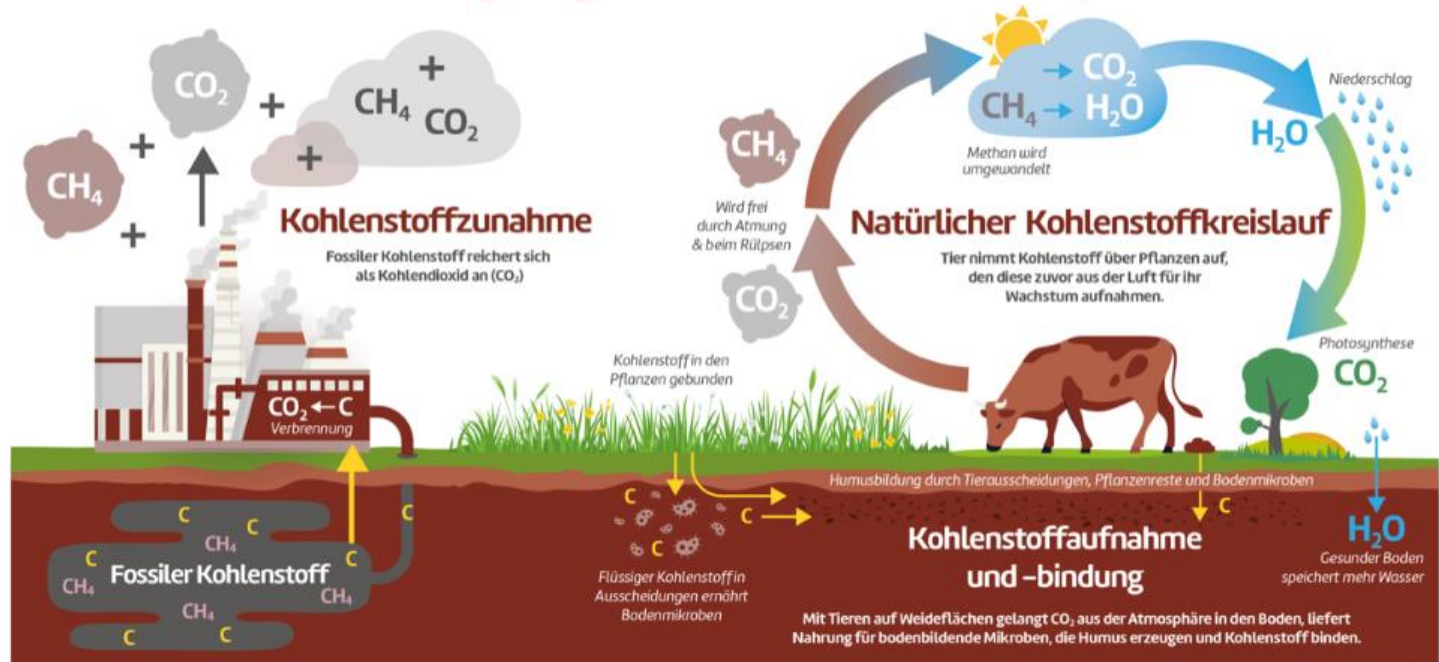
cortisol level



Kuhla, Viereck, 2022

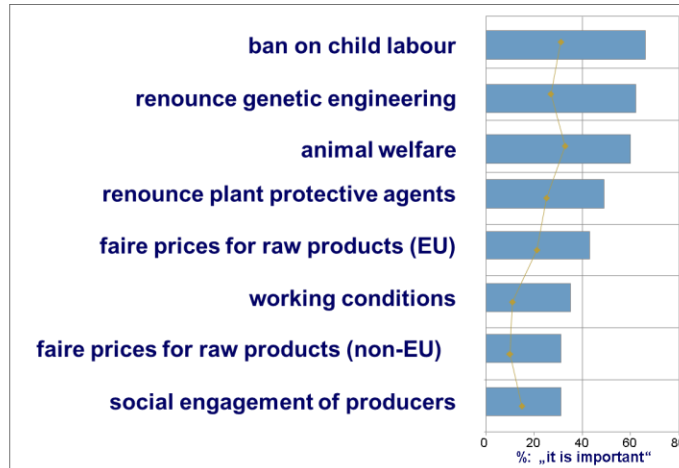
Emissionen aus der Nutztierhaltung sind Teil eines natürlichen Kreislaufs, Emissionen aus fossilen Energieträgern reichern sich in der Atmosphäre an.

fokusfleisch

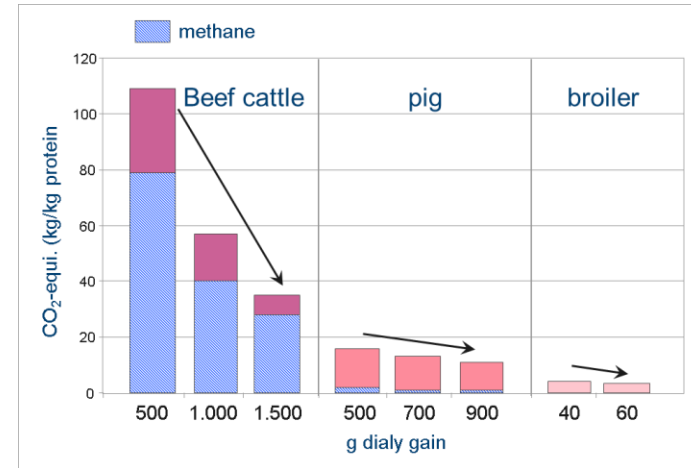


Quellen: globalfoodjustice.org

welfare + efficiency = sustainability

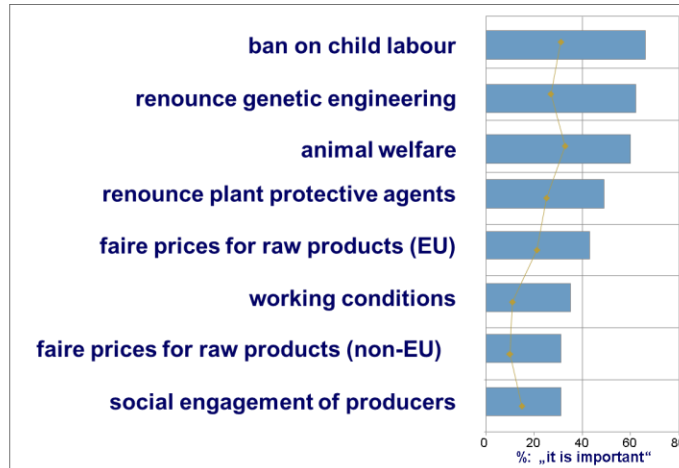


Nestle Ernährungsstudie 2011

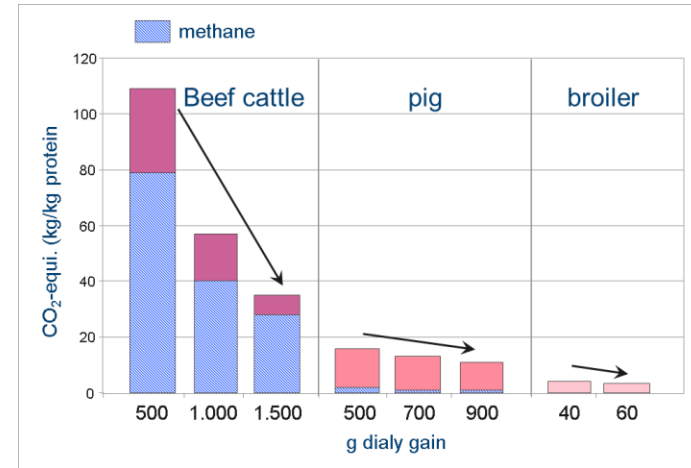


Flachowsky et al. 2011

welfare + efficiency = sustainability



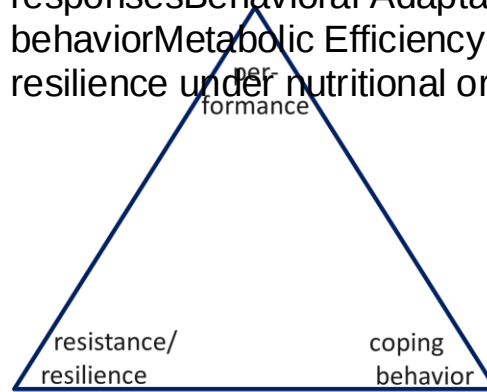
Nestle Ernährungsstudie 2011



Flachowsky et al. 2011

Linked to behavioral, immune, stress, and metabolic responses

Physiological Regulation – covering immune and stress responses
Behavioral Adaptability – covering coping strategies and social behavior
Metabolic Efficiency – covering energy balance, nutrient use, and resilience under nutritional or environmental stress



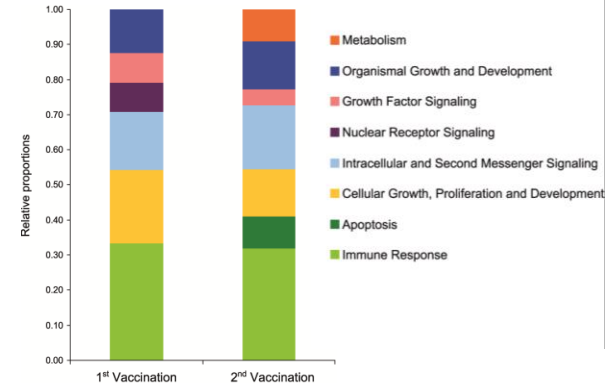
Resilience: coping and aggressive behaviour; immune response; interactions with performance

PBMC transcriptional response to TT



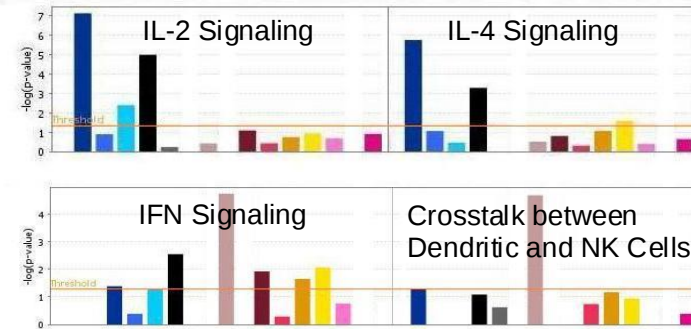
after 1 st vacc.	2 h	4 h	8 h	30 h	75 h	d 14
# DE genes	679	1196	1104	773	549	642

after 2 nd vacc.	2 h	4 h	8 h	30 h	75 h	d 14
# DE genes	485	358	824	942	1121	527

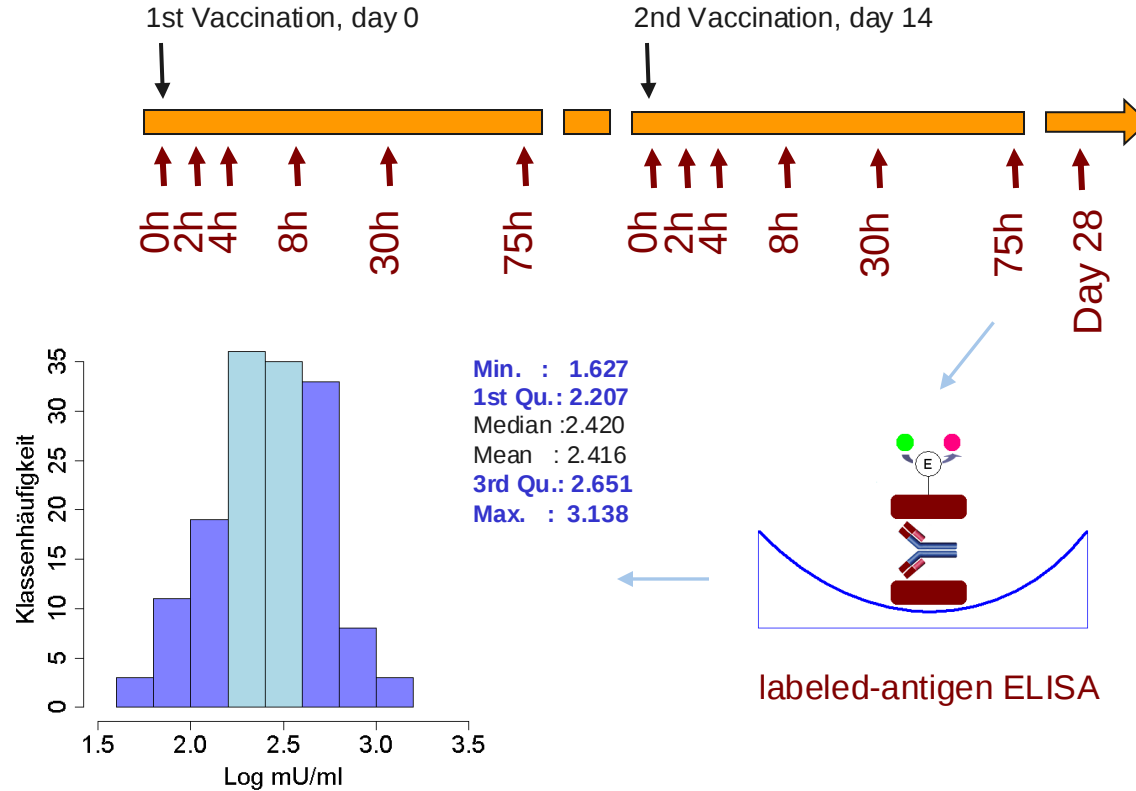


→ immune challenge provokes an immediate transcriptional response of immune and metabolic pathways

→ TT provokes a Th1 and Th2 response



Immune response phenotype



Performance phenotypes



> 30 single traits

- Backfat
- Fat area
- Loin eye area
- Lean meat content
- Feed intake/conversion
- Final weight
- ...

PCA - Principal Component Analysis:

Variance Explained by Each Factor

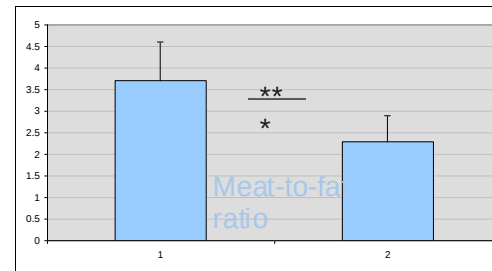
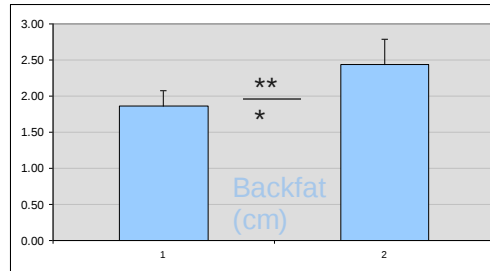
Factor1	Factor2	Factor3	Factor4
2.536402	3.302546	2.507998	1.964000

39%

10%

7.8%

6.1%



Performance phenotypes



> 30 single traits

- Backfat
- Fat area
- Loin eye area
- Lean meat content
- Feed intake/conversion
- Final weight
- ...

PCA - Principal Component Analysis:

Variance Explained by Each Factor

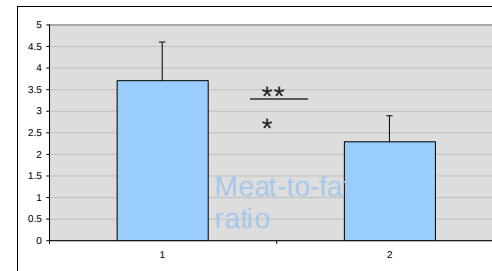
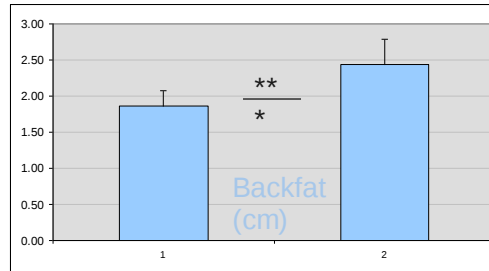
Factor1	Factor2	Factor3	Factor4
2.536402	3.302546	2.507998	1.964000

39%

10%

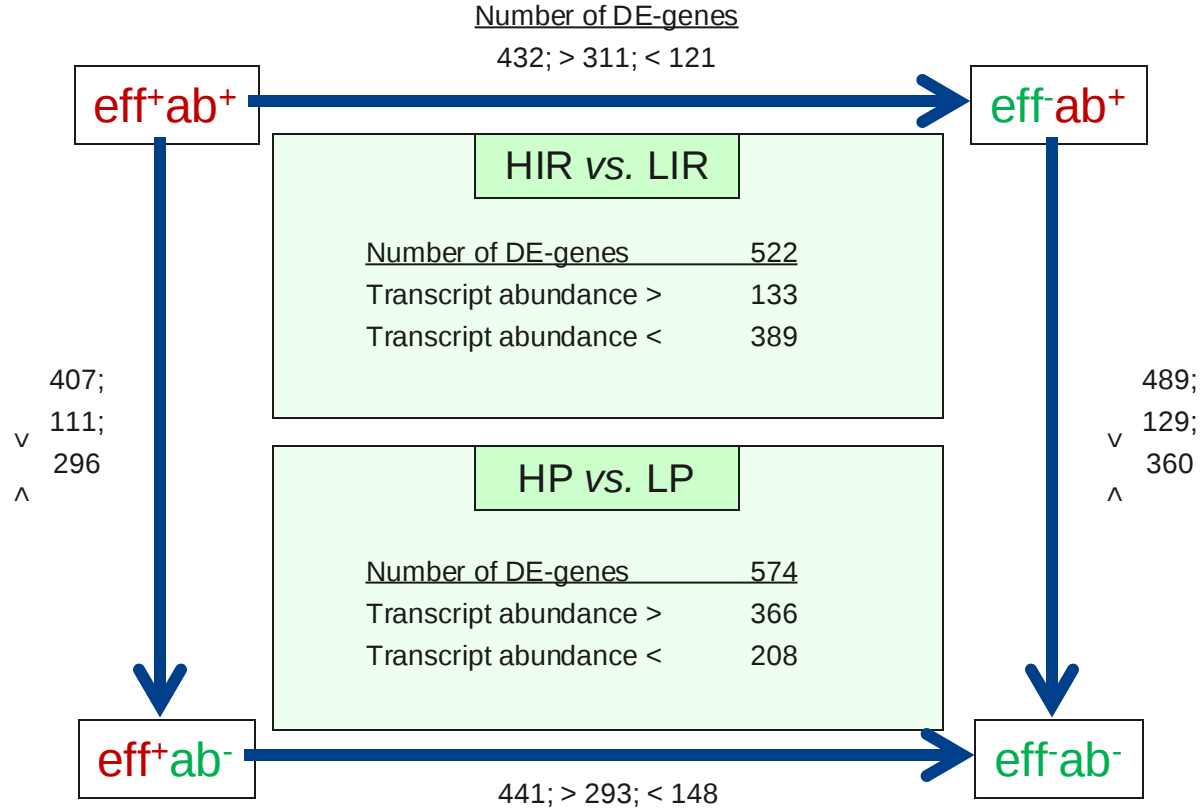
7.8%

6.1%



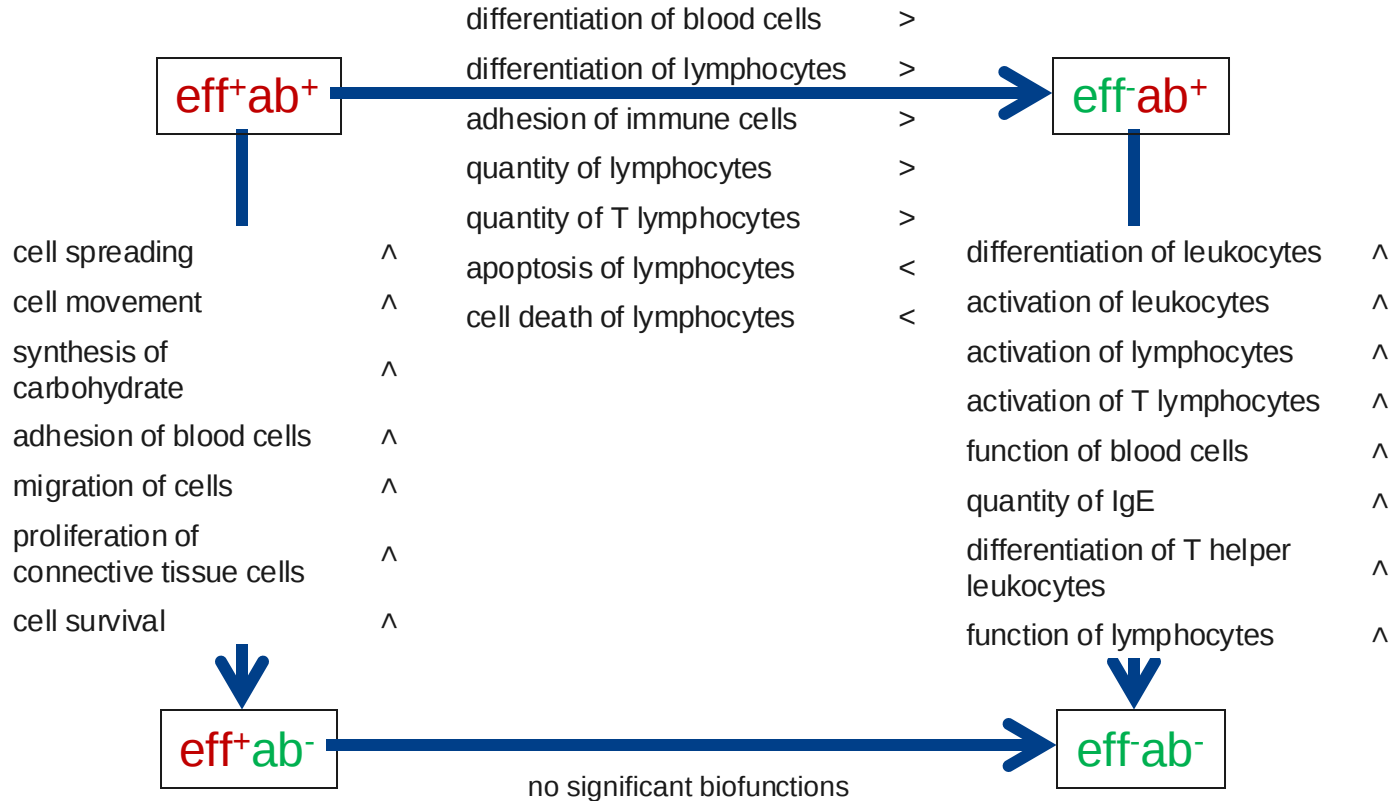
PBMC transcriptional response to TT

...against the background of divergent immunity and performance



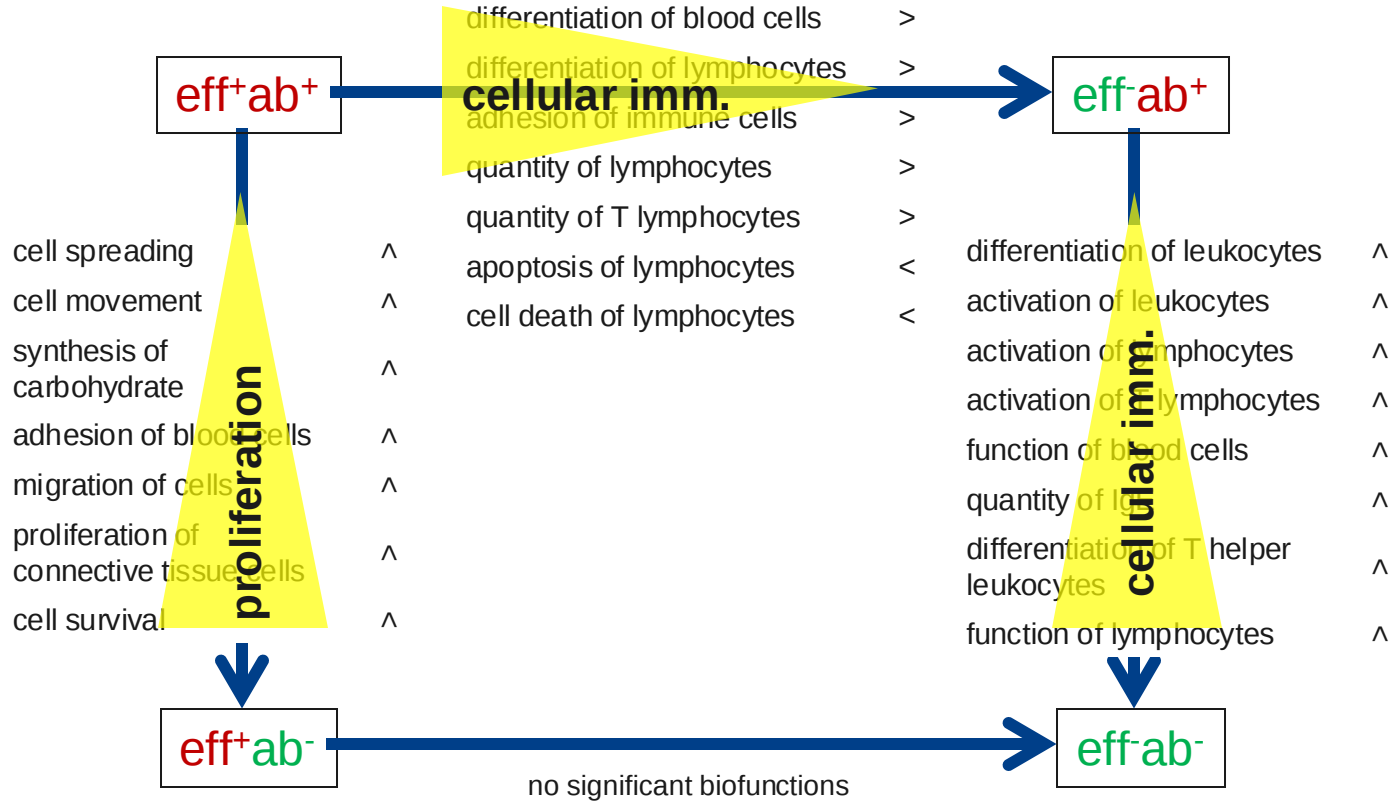
PBMC transcriptional response to TT

...against the background of divergent immunity and performance



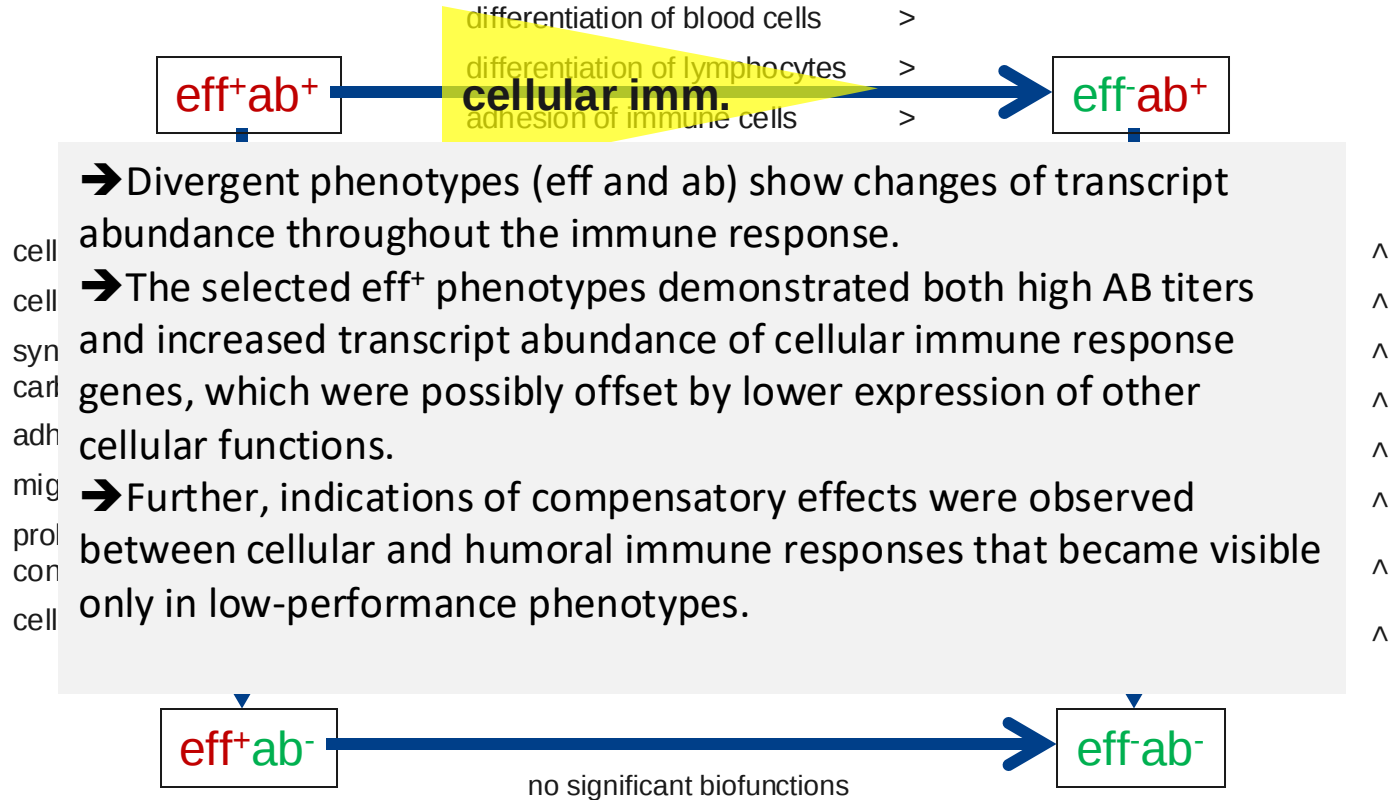
PBMC transcriptional response to TT

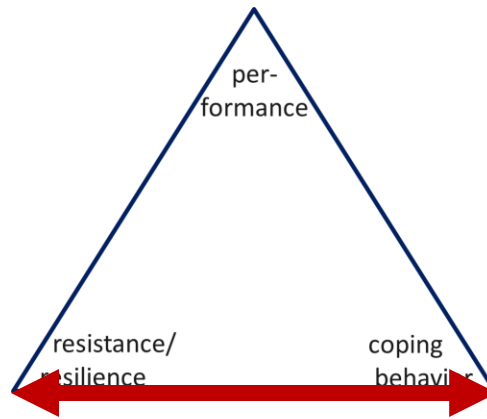
...against the background of divergent immunity and performance



PBMC transcriptional response to TT

...against the background of divergent immunity and performance





transcriptional response to TT vaccination of piglets classified as either **high or low-reactive** according to latency, total duration, and frequency of struggling bouts in a backtest

Top 5 Biofunctions, which DEGs were mapped to (day 0)



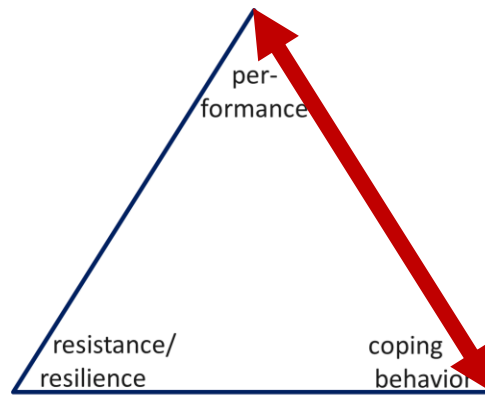
Biofunction	Diff.	p-value	# of genes
adhesion of endothelial cells	HR > LR	1.29E-02	5
quantity of blood platelets	HR > LR	9.55E-04	9
vasculogenesis	HR > LR	9.77E-03	27
quantity of megakaryocytes	HR < LR	2.30E-03	5
senescence of			

Top 5 Biofunctions, which DEGs were mapped to (day 28)



Biofunction	Diff.	p-value	# of genes
adhesion of blood cells	HR > LR	1.30E-04	15
adhesion of granulocytes	HR > LR	5.77E-03	5
attachment of cells	HR > LR	3.82E-04	7
binding of blood cells	HR > LR	2.77E-03	9
binding of blood platelets	HR > LR	1.88E-05	6



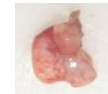


Analysis of adrenal transcriptome response to
psychosocial stress of **aggressive behaviour**
&
identification of genes and pathways associated
with stress responsiveness and aggressiveness

aggressive behaviour and stress



- aggression commonly occurs when **mixing** unfamiliar pigs which disturbs the social dominance order¹
- aggression: powerful psychosocial stressor activating **hypothalamic-pituitary-adrenocortical (HPA) axis** & **sympatho-adrenomedullary (SAM) system**²
- aggression and stress have **negative effect** on production, meat quality and animal welfare³



Summary of findings



- the coping groups HR and LR were discriminated according to molecular features related to

cell communication, vasculogenesis, inflammation, wound healing, immune response (and nervous systems functions)

- the tetanus toxoid vaccination blurred transcriptional differences between coping groups temporarily (day 14)
- no clear inherited shift toward Th1 or Th2 immunity was observed between coping styles
- at the juvenile stage, pigs with proactive coping (HR) may favor molecular pathways that support effective defense and recovery mechanisms.

➔ **HR are in a more alert physiological state, primed for defense and recovery**

Stage (weeks)	Strain	Gene	FoldChange	p value	FDR
10 vs16	LB	DNMT3A	0.54	8.36E-06	9.84E-05
	LB	DNMT3B	1.96	7.46E-05	6.21E-04
	LSL	DNMT3A	0.55	2.46E-06	3.17E-05
	LSL	DNMT3B	1.94	4.04E-05	3.26E-04
16 vs 24	LB	DNMT1	3.15	8.02E-16	6.08E-15
	LB	DNMT3A	2.87	5.18E-15	3.43E-14
	LB	DNMT3B	2.25	1.86E-06	3.77E-06
	LSL	DNMT1	2.39	6.10E-12	2.65E-11
	LSL	DNMT3A	2.42	1.33E-12	6.35E-12
	LSL	DNMT3B	1.80	2.87E-04	4.88E-04
24	LB vs LSL	DNMT1	1.43	6.32E-03	3.34E-02

immune response and performance

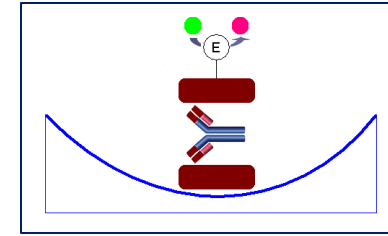
Animals with favourable productivity and immune response exhibit lifelong characteristic expression pattern → biomarkers

immune response and coping behavior

Proactive animals show an increase in preparedness to cope with biotic and abiotic stressors → biomarkers

aggressive behavior and stress response

Focussed analysis of key genes within functional pathway can provide causal variation → breeding tools



ELISA

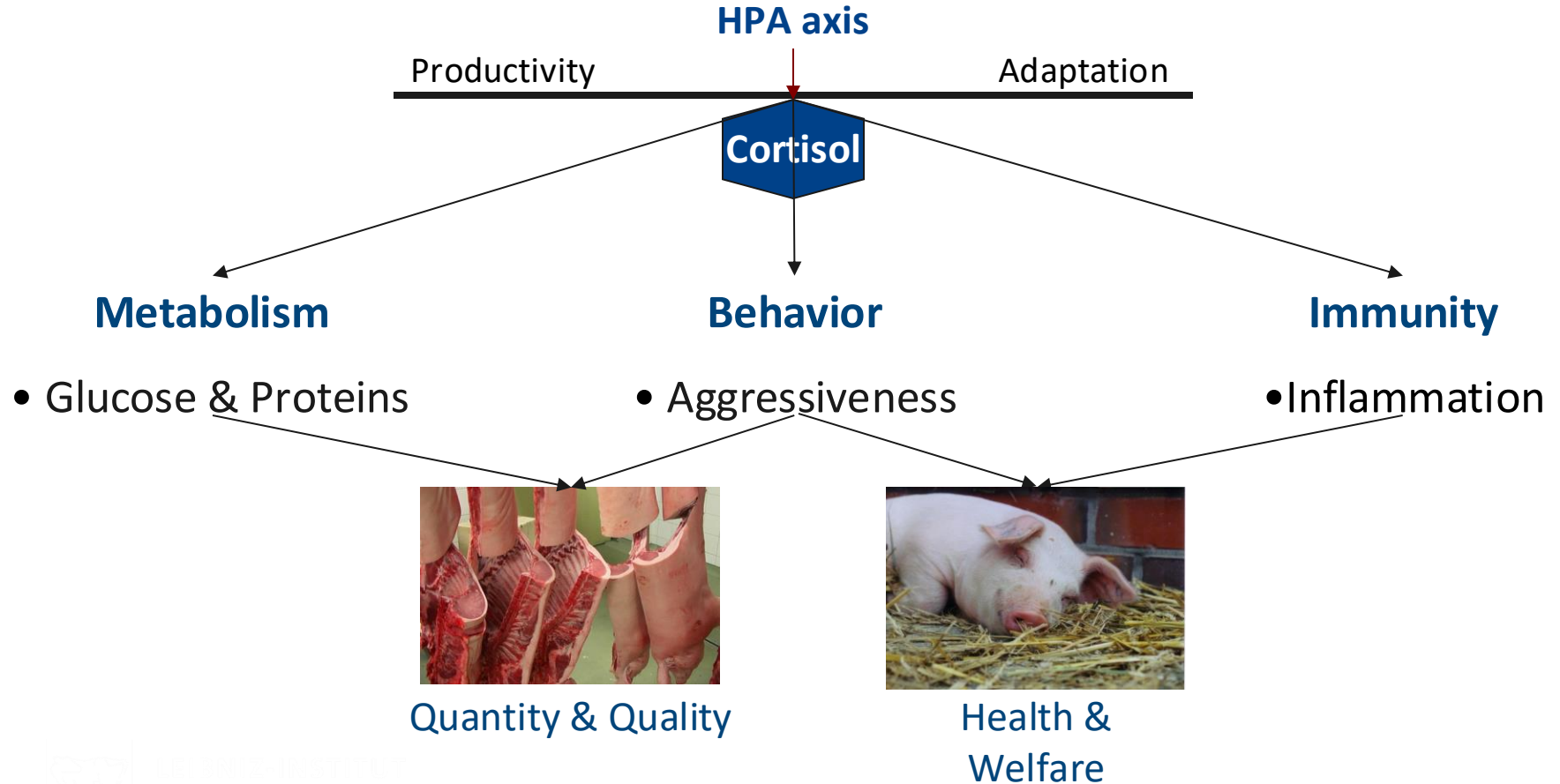


backtest



cortisol level

Cortisol and pig production



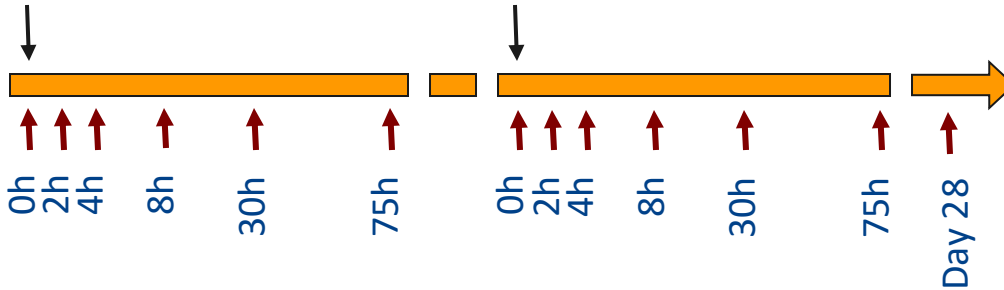
PBMC transcriptional response to TT



Pilot study: subset n = 3 x 6 piglets vaccinated

1st Vaccination, day 0

2nd Vaccination, day 14



after 1 st vacc.	2 h	4 h	8 h	30 h	75 h	d 14
# DE genes	679	1196	1104	773	549	642
after 2 nd vacc.	2 h	4 h	8 h	30 h	75 h	d 14
# DE genes	485	358	824	942	1121	527

Blood Samples



PBMCs - mRNA

transcriptome



→ immune challenge provokes an immediate transcriptional response of immune and metabolic pathways

→ TT provokes a Th1 and Th2 response

Does fight-or-flight behaviour shape the immune response to vaccination?

coping behaviour

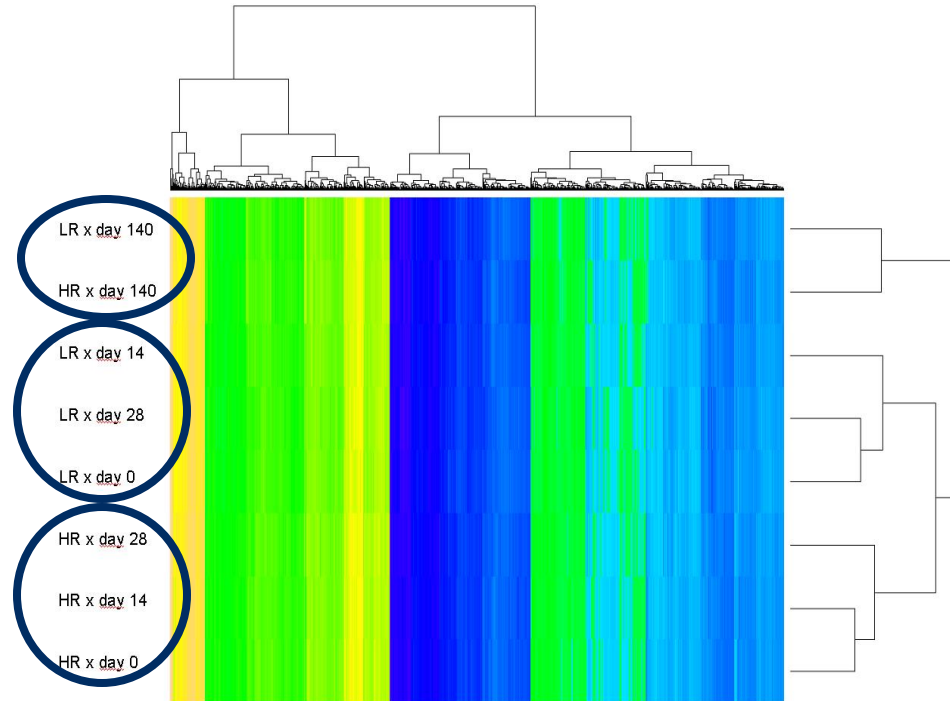
aggressive behaviour

PBMC transcriptional response to TT

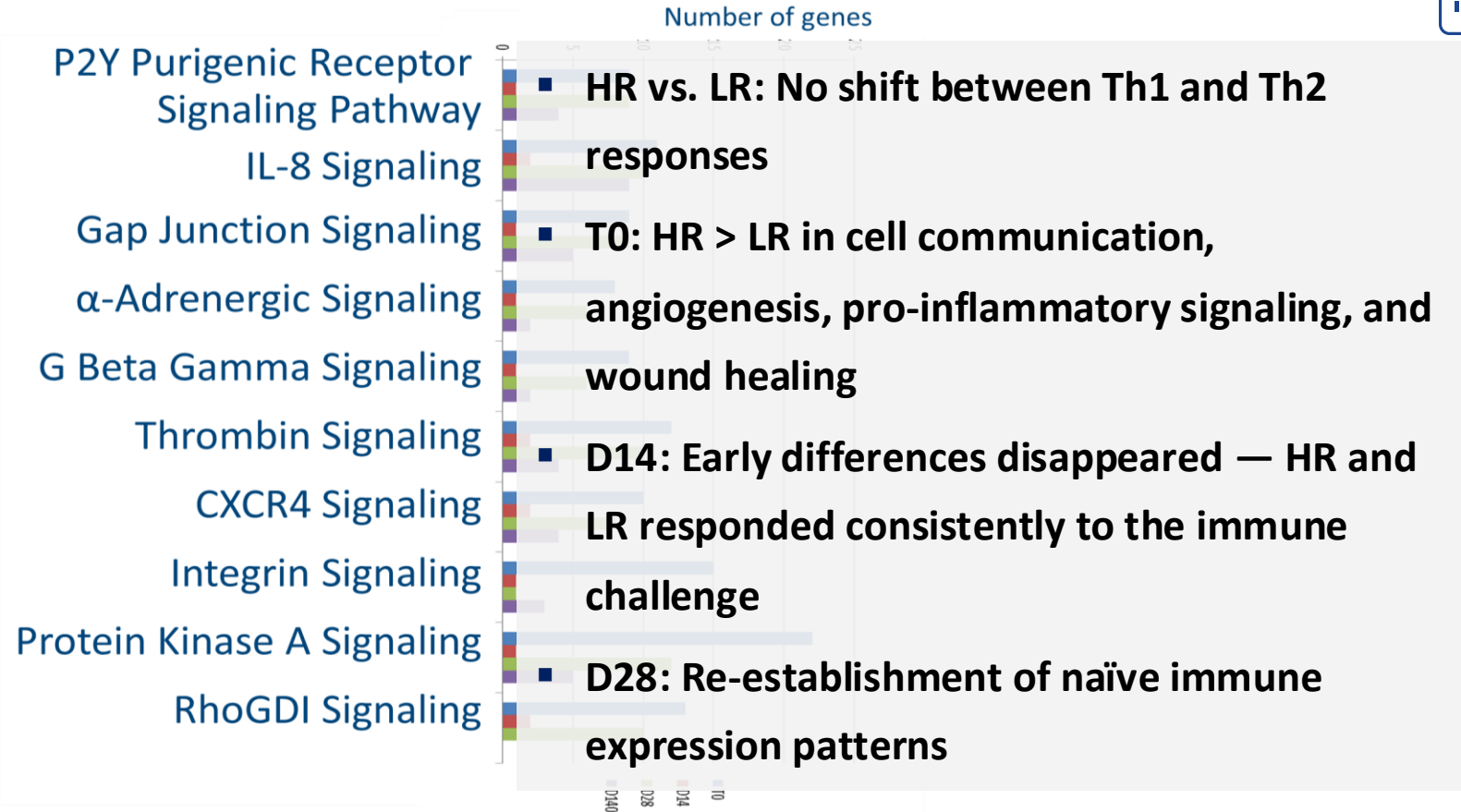
...against the background of divergent behaviour



- Transcriptional responses differ between proactive and reactive animals across time points
- Heatmap patterns group by coping style at early stages (d0, d14, d28)
- Age-specific effects dominate at later stage (d140)



Top 10 Canonical Pathways Linked to DEGs



➔ HR are in a more alert physiological state, primed for defense and recovery