中国科学院
CHINESE ACADEMY OF SCIENCES

State of Genetically Modified Pigs for Agriculture in China

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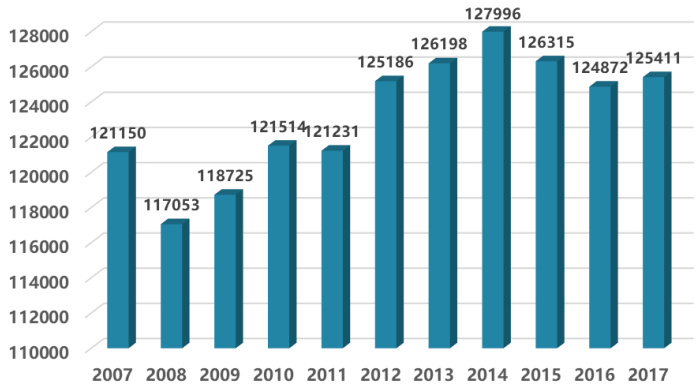
2021-08-31

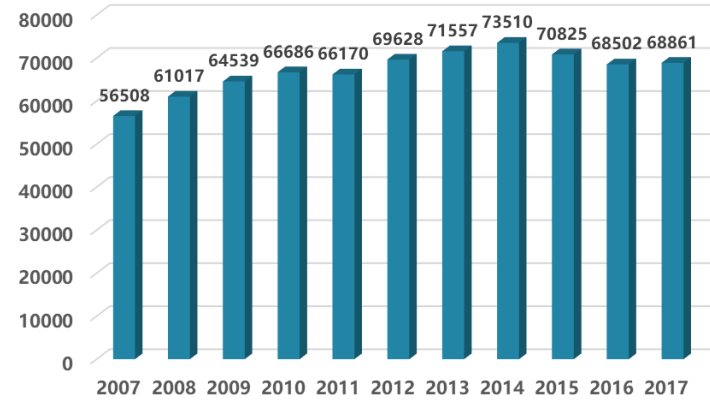
Pork is the main meat food in China (65%)

China consumes more than 50% pork of the World

World: about 1.4 billion pigs; China: about 0.7 billion pigs



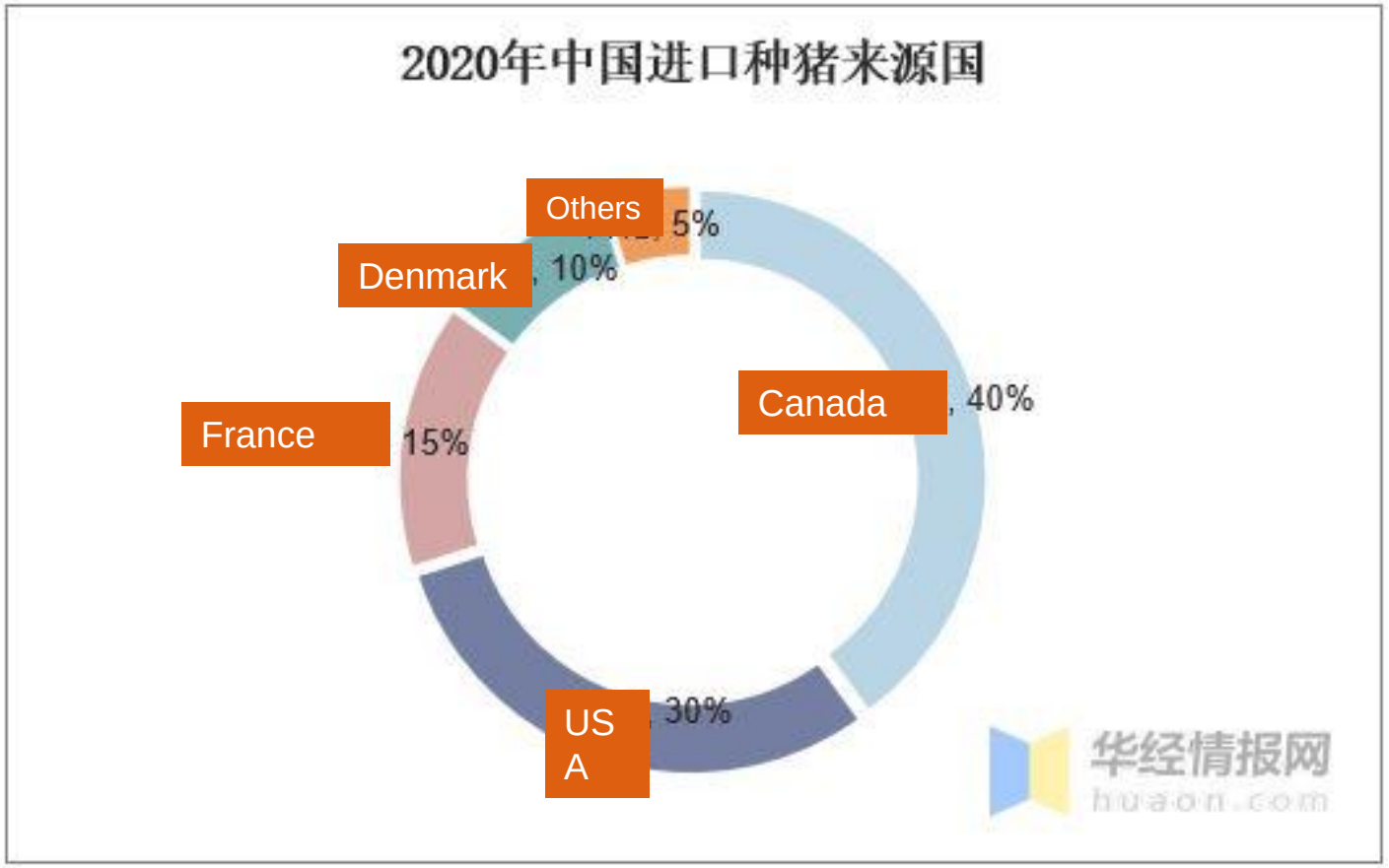
The number of slaughtered pig in the world

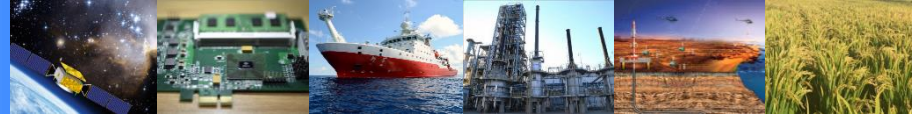


The number of slaughtered pig in China

China has no native breeds of pigs with high productivity

Import 20 thousand elite boars from other countries each year



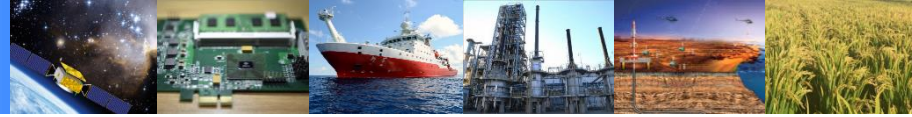


The Chinese government attaches importance to generation of genetically modified pig strains with favorable traits for agriculture

1. Setting up National special funding for transgenic livestock

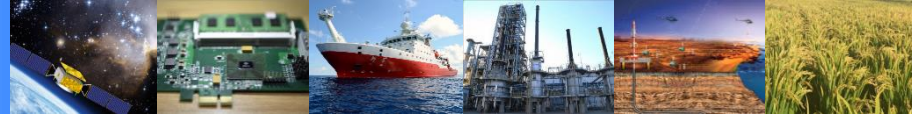
2. Setting up big pig facilities for large scale characterization of GM pigs

3. More than 15 research teams working on GM pigs



The genetical modification of pigs can be made to:

- ✓ Promote growth: GH, IGF
- ✓ Improve meat quality: FAT1, FAT2, MSTN
- ✓ Reduce environmental emissions: NSP-degrading and phytatea-degrading enzymes
- ✓ Gain resistance to pathogens or other environmental stress



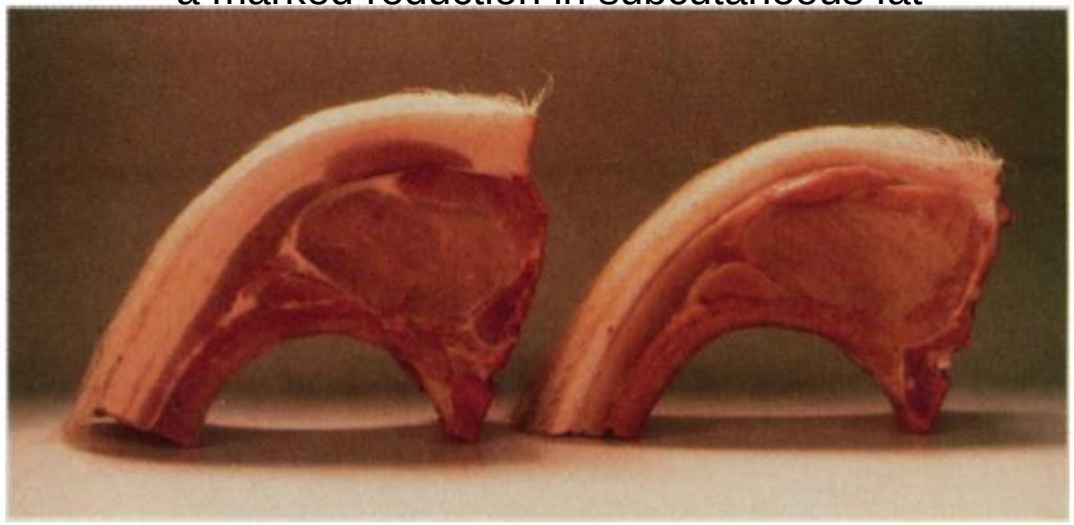
1. Genetical Modification of pigs for promoting growth

Over expression of growth hormone

Significant improvements in
both daily weight gain and feed efficiency

Group	Average daily weight gain (g)	Feed efficiency (kg feed/ kg gain)
Founder animals*		
Control	743 ± 32 (6)	3.12 ± 0.15
Transgenic	690 ± 65 (6)	2.62 ± 0.12
	$P = 0.480$	$P = 0.026$
37-06 G2 progeny†		
Control	760 ± 24 (8)	2.99 ± 0.12 (8)
Transgenic	874 ± 30 (5)	2.46 ± 0.16 (5)
	$P = 0.016$	$P = 0.026$
37-06 G3 progeny‡		
Control	867 ± 21 (15)	ND
Transgenic	933 ± 31 (8)	
	$P = 0.098$	
31-04 G2 progeny§		
Control	869 ± 44 (7)	ND
Transgenic	988 ± 62 (7)	
	$P = 0.15$	
Combined progeny		
Control	815 ± 17 (30)	ND
Transgenic	905 ± 21 (20)	
	$P = 0.001$	

a marked reduction in subcutaneous fat



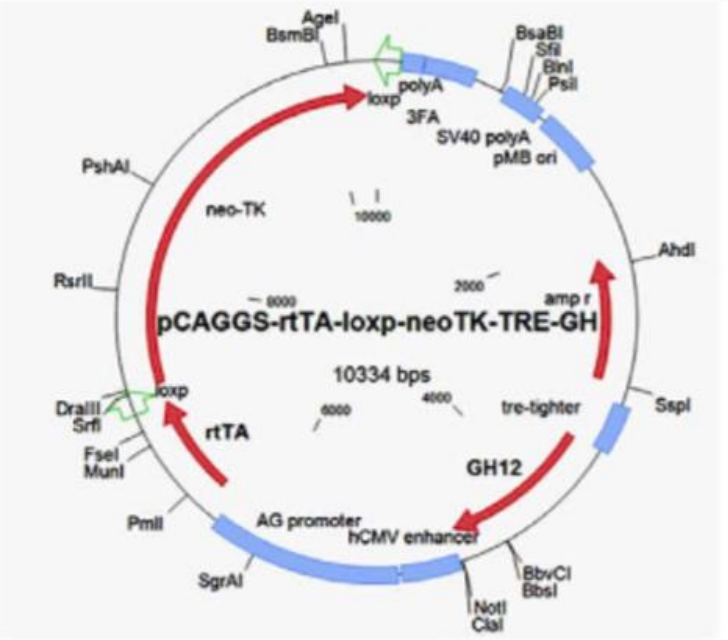
pathological changes

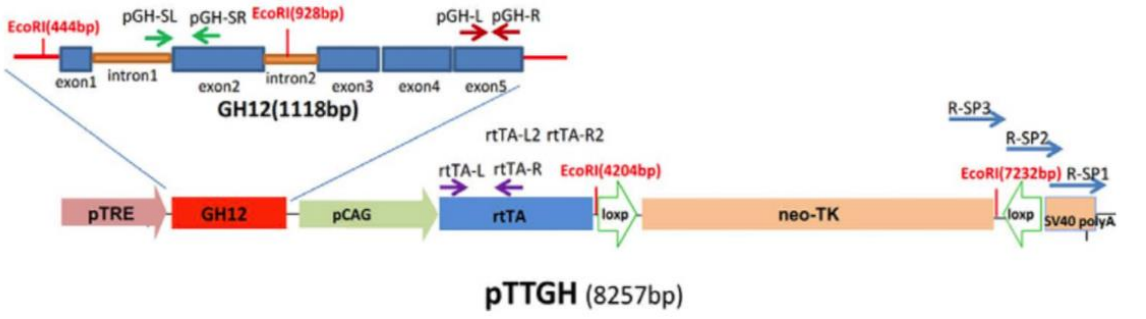
Diagnosis*	Number of animals	
	Transgenic	Control
Gastric ulcers	5/5	0/3
Synovitis	4/5	0/3
Cardiac myocyte nuclear hypertrophy	4/5	0/3
Dermatitis	4/5	1/3
Nephritis	3/5	0/3
Pneumonia	3/5	1/3

Science,1989

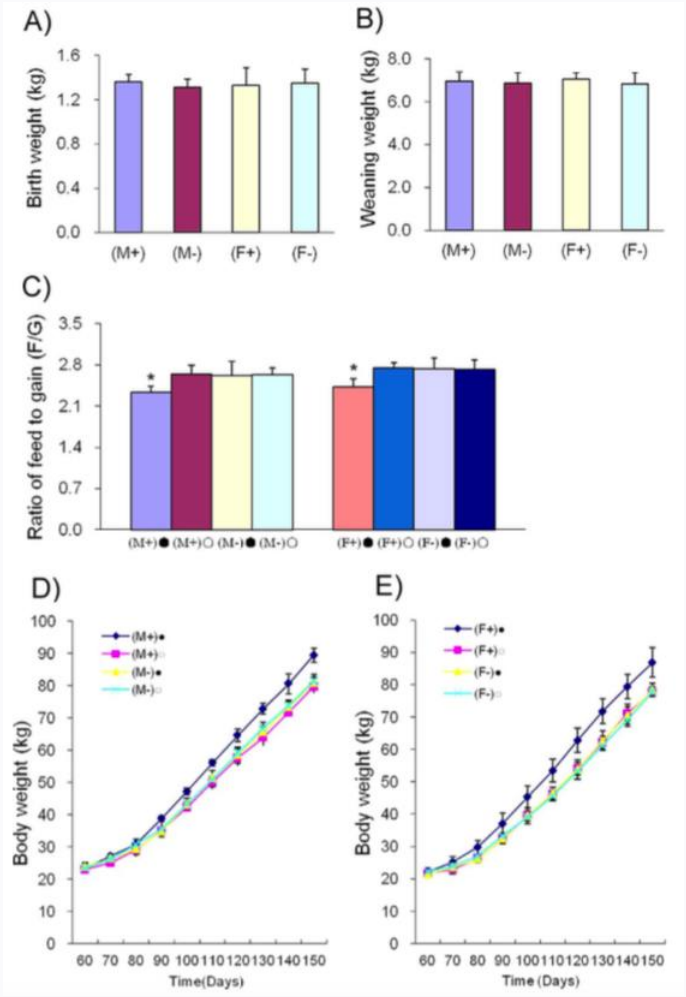
The transgenic pig with controllable expression of growth hormone

the pCAGGS-rtTA-TRE-GH12 (pTTGH) vector





Growth rates and feed efficiencies



Scientific Reports,2015

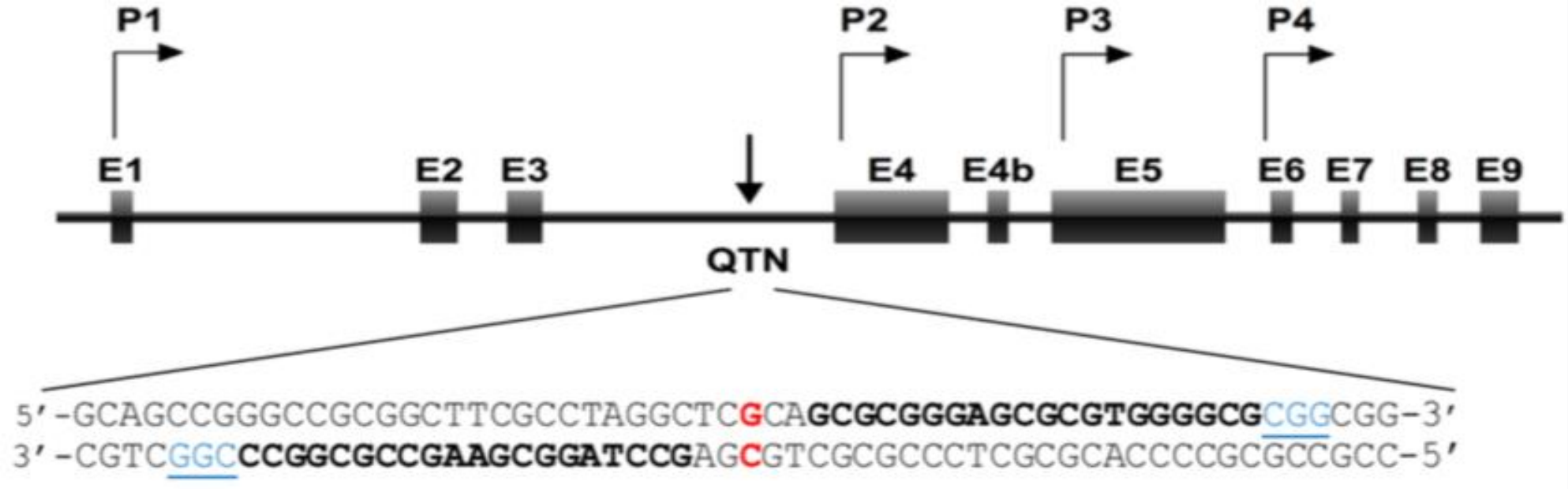
Editing porcine *IGF2* regulatory element to improve meat production in Chinese Bama pigs

IGF2, an important growth factor which affects skeletal muscle and fat deposition

IGF2-intron 3–nucleotide 3072:

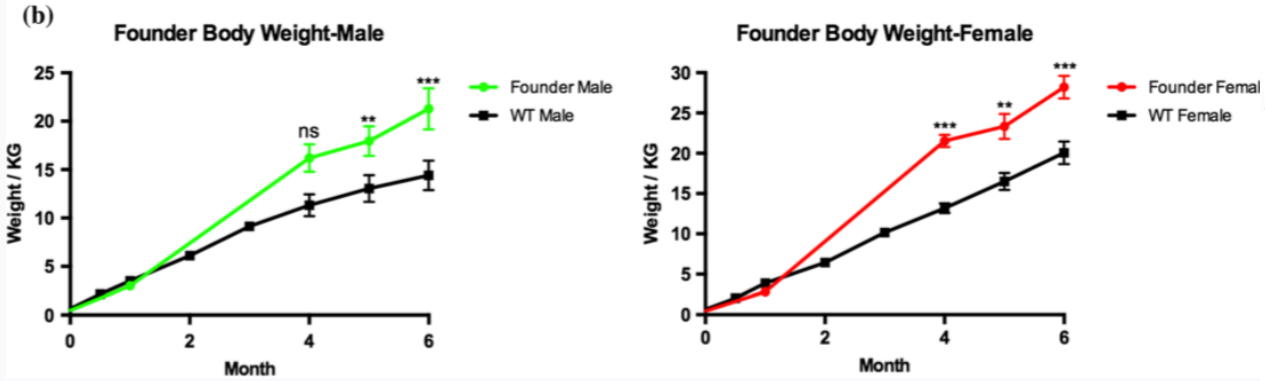
5′-GCTC**G**C-3′, the G allele, recognized by repressor ZBED6, negatively regulates *IGF2* expression

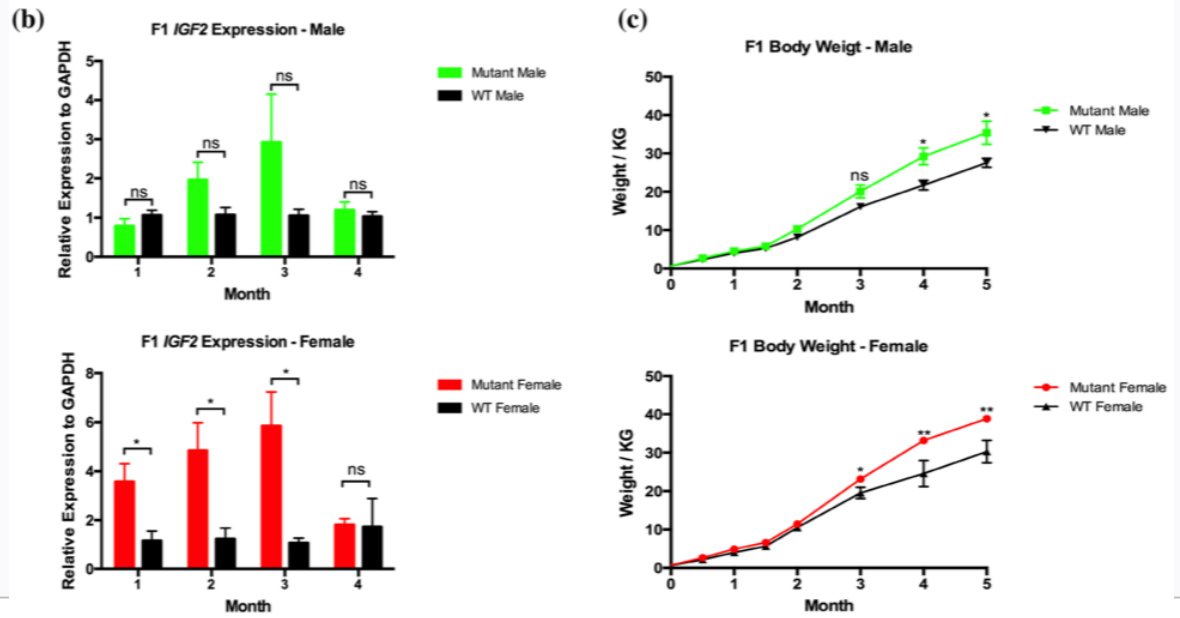
Experiment Design



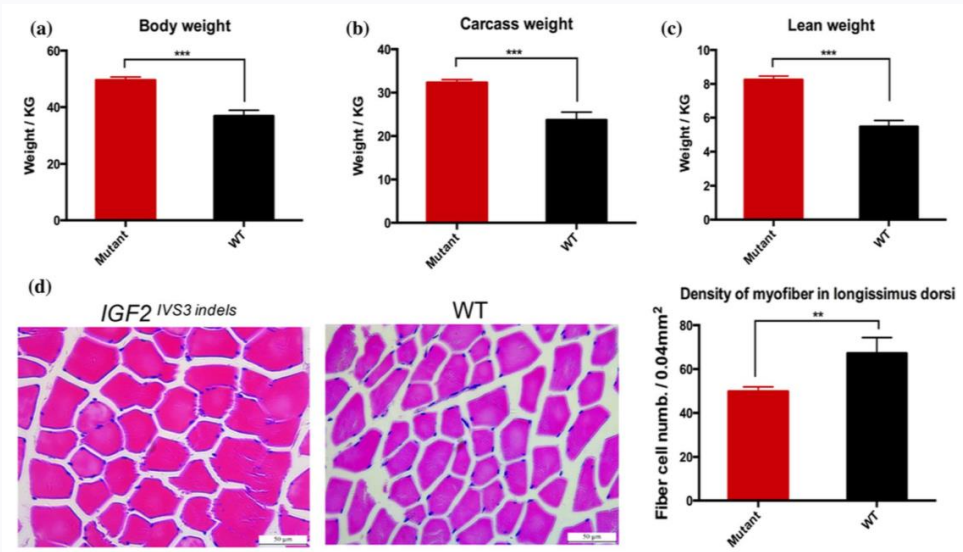
Cellular and Molecular Life Sciences,2018

Growth performance of founder and F1 gene-edited pigs

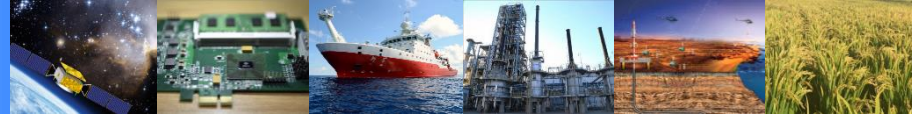




Meat quality and other phenotypes



Cellular and Molecular Life Sciences,2018



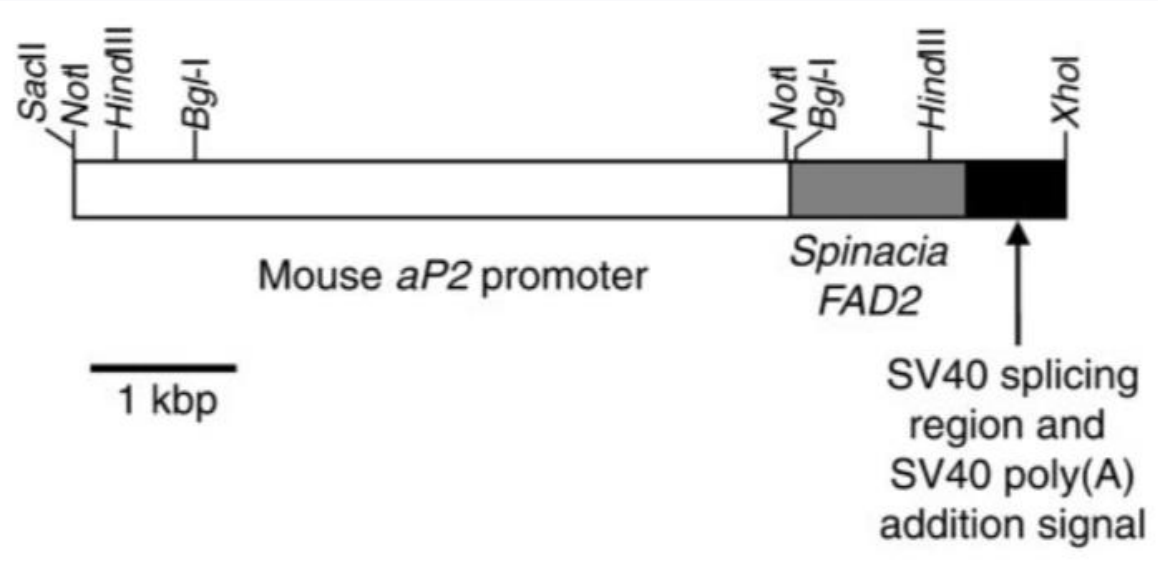
2. Improvement of the pig meat quality

Expression of FAD2 in transgenic pigs promotes synthesis of polyunsaturated fatty acids

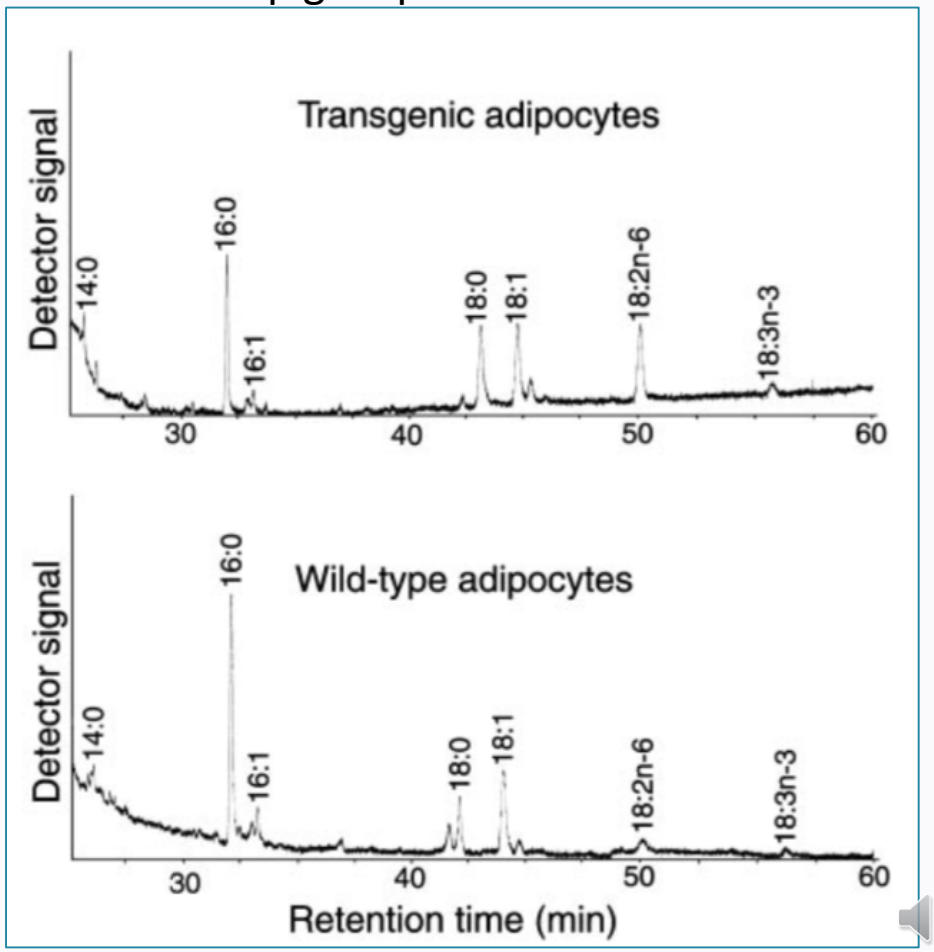
Mammalians lack the desaturases required for synthesis of polyunsaturated fatty acids

FAD2: a delta12 fatty acid desaturase from spinach

The coding region of cDNA for a 12 desaturase (FAD2) from *Arabidopsis thaliana*



fatty acid composition of accumulated lipids in pig adipose cells.



PNAS,2004

The delta-15 desaturase (fat1) gene pigs synthesize n-3 PUFAs from n-6 PUFAs

Transgenic pigs with hfat-1, humanized Caenorhabditis elegans gene, encoding an n-3 fatty acid desaturase

the polyunsaturated fatty acid profiles of total lipids

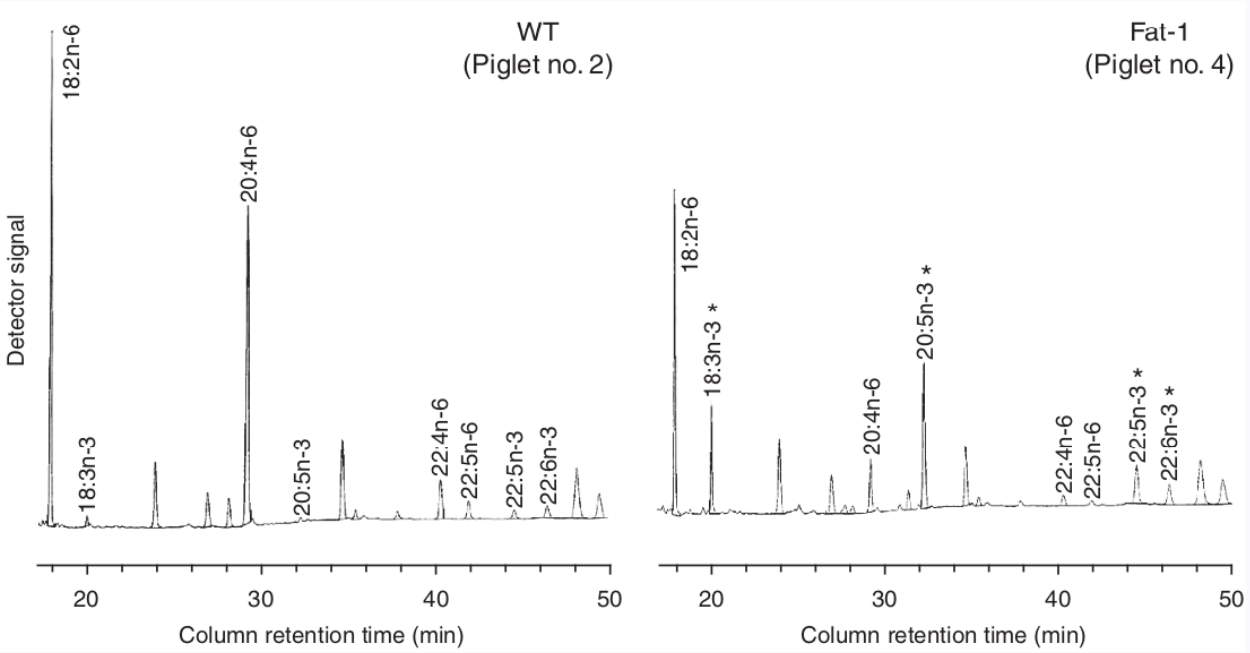


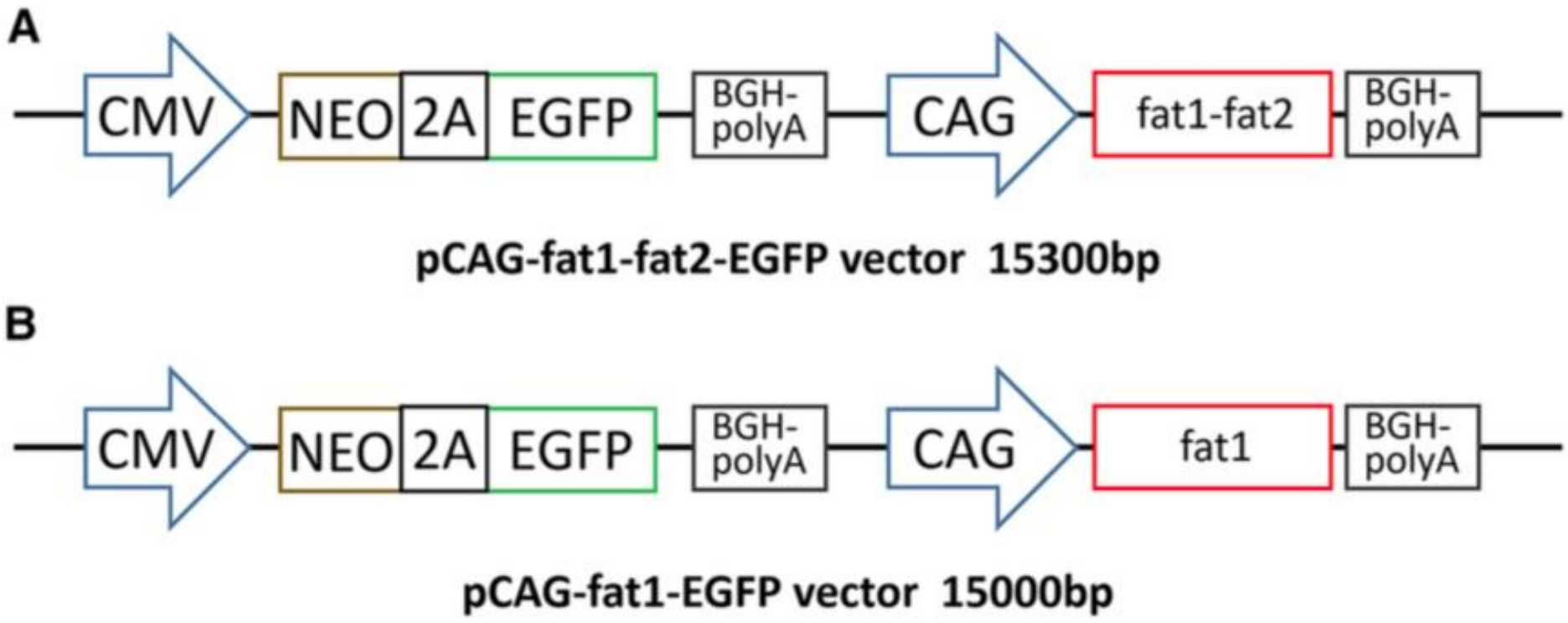
Table 1 *n*-3 and *n*-6 fatty acids concentration and *n*-6/*n*-3 ratios in tail samples from h*fat-1* transgenic and wild-type piglets

Fatty acids in tails[a]	Transgenic piglets ($n = 8$)	Wild-type piglets ($n = 8$)
ALA (18:3 *n*-3, %)	0.94 ± 0.10	0.63 ± 0.04
EPA (20:5 *n*-3, %)	4.21 ± 0.60	0.26 ± 0.07
DPA (22:5 *n*-3, %)	1.69 ± 0.19	0.35 ± 0.05
DHA (22:6 *n*-3, %)	1.75 ± 0.23	0.95 ± 0.21
Total *n*-3 FA (%)	8.59 ± 0.84	2.18 ± 0.25
Total *n*-6 FA (%)	14.28 ± 1.31	18.46 ± 1.41
n-6/*n*-3 ratio	1.69 ± 0.30	8.52 ± 0.62

Nature Biotechnology,2006

Co-expression of fat1 and FAD2 in transgenic pigs

ndogenously produced n-6 PUFAs was used as substrates to synthesize n-3 PUFAs

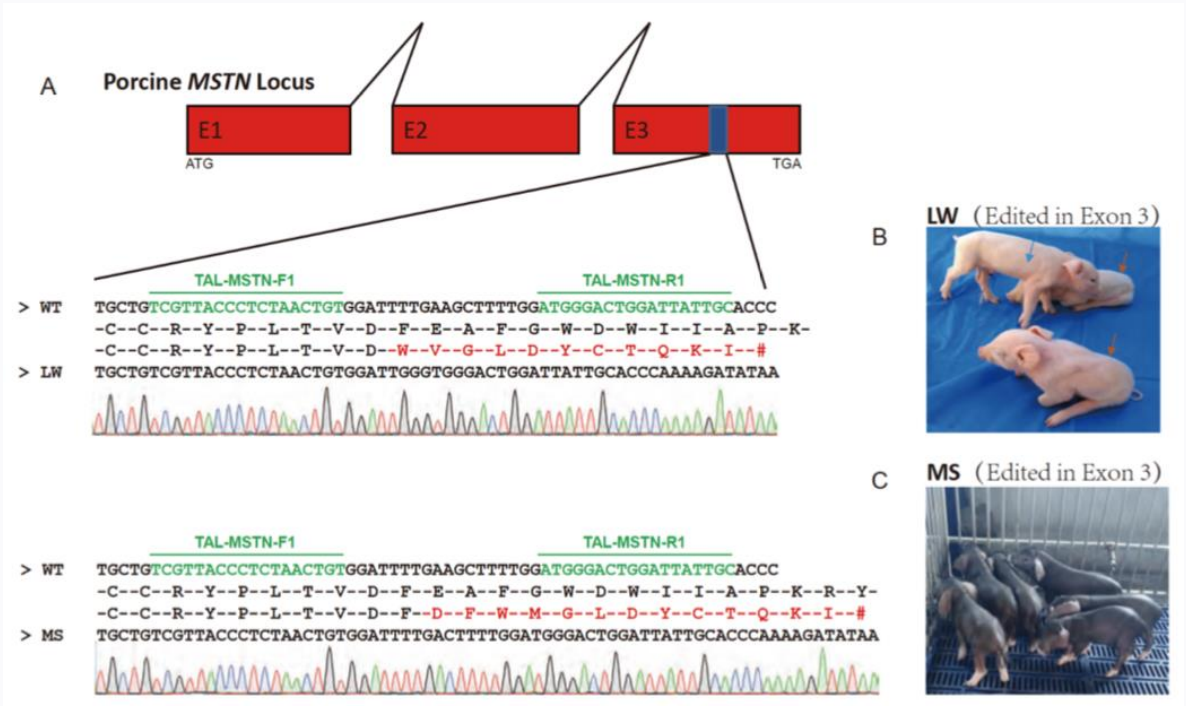


Transgenic Res,2019

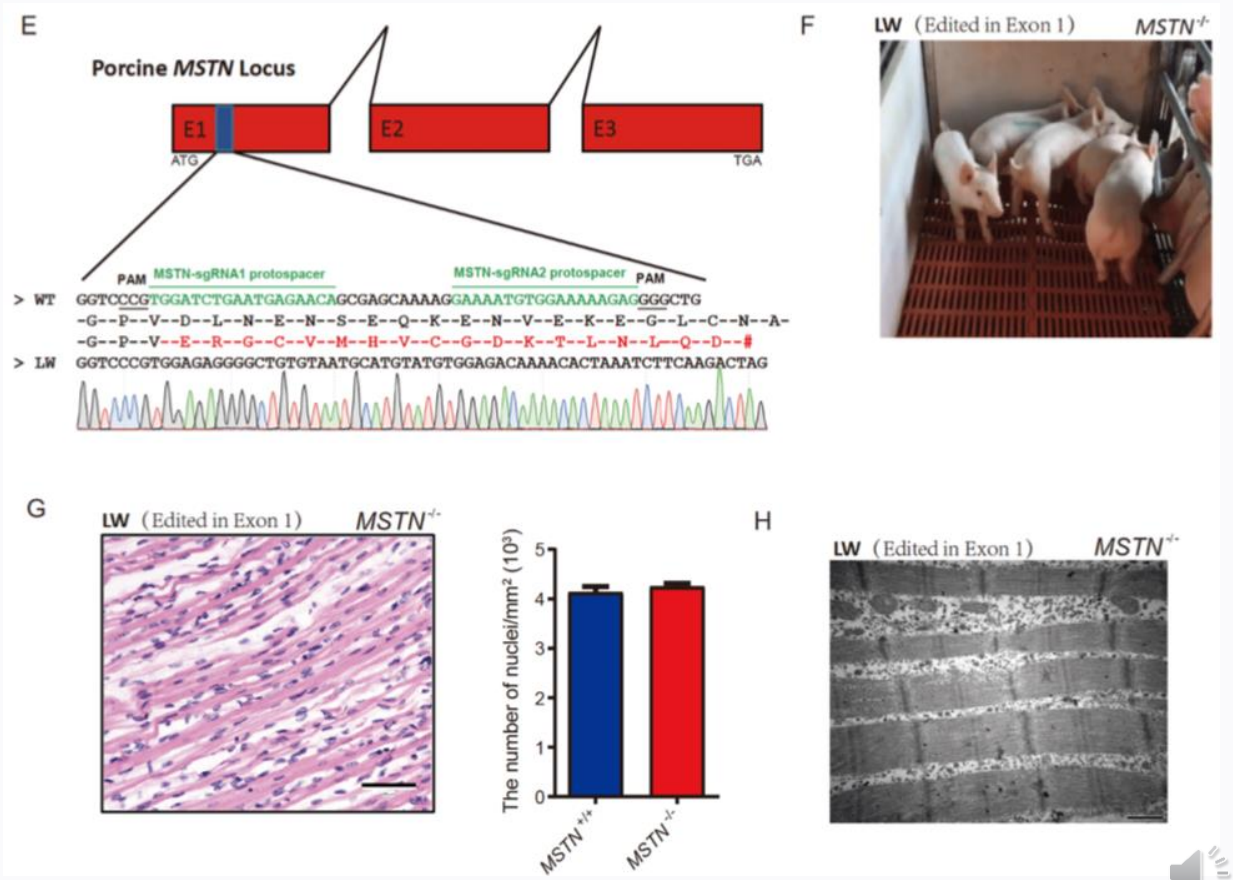
MSTN-edited pigs to overcome lameness and sustainably improve nutritional meat production

Genes related to muscle mass: **myostatin (*MSTN*)**, negatively regulates skeletal muscle cell proliferation

hindlimb weakness

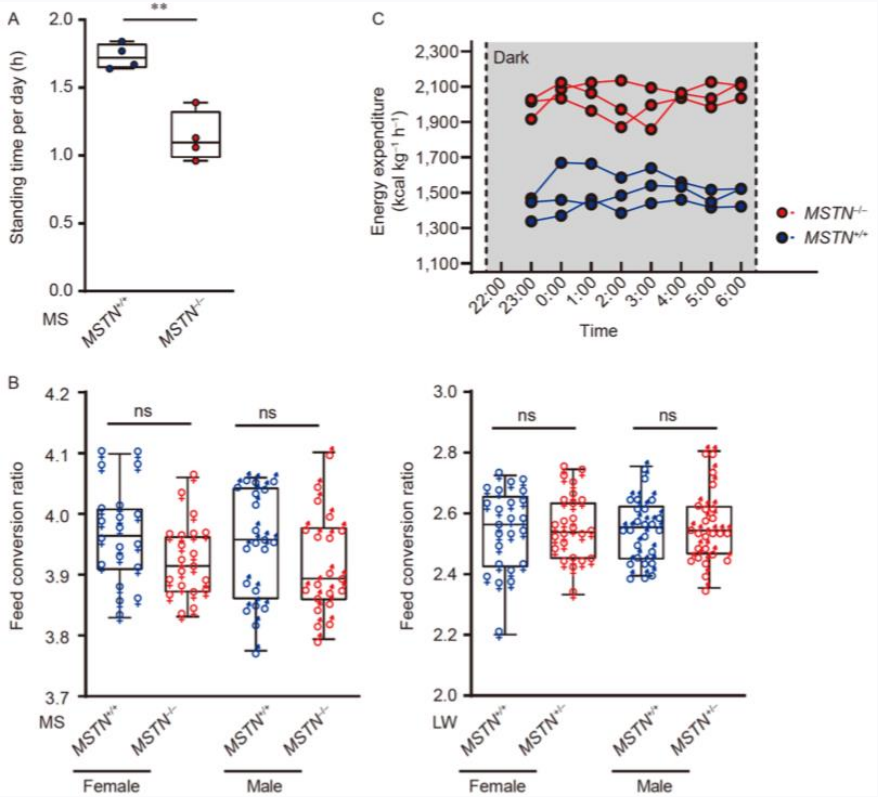


Alternative edit-site-based solution
avoids ER stress and overcomes the hindlimb weakness

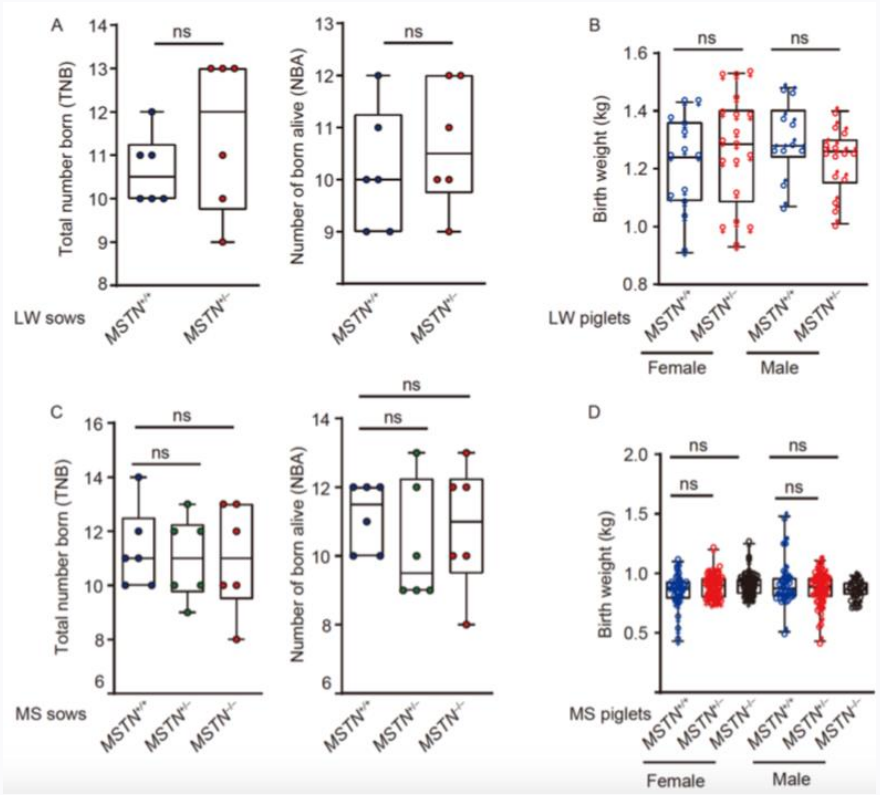


Sci China Life Sci,2021

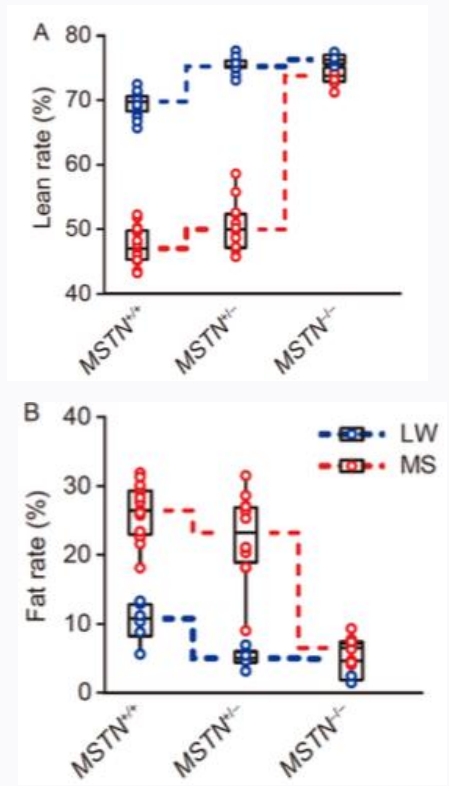
Similar feed-conversion ratio



No dystocia or significant effects on the maternal reproductive traits



different trends of change for lean and fat rates



Sci China Life Sci,2021

3. Reduction of environmental impact

Only 1/3 of feed nitrogen and phosphorus were utilized from feedstuff diets in pig production. Inefficient feed digestion can cause serious nutrient emissions to the environment.

- Phytates, negatively charged saturated cyclic acids, bind to positively charged molecules in the diet such as minerals and protein, thereby reducing nutrient digestibility and increasing discharge of the unabsorbed nutrients to the environment.

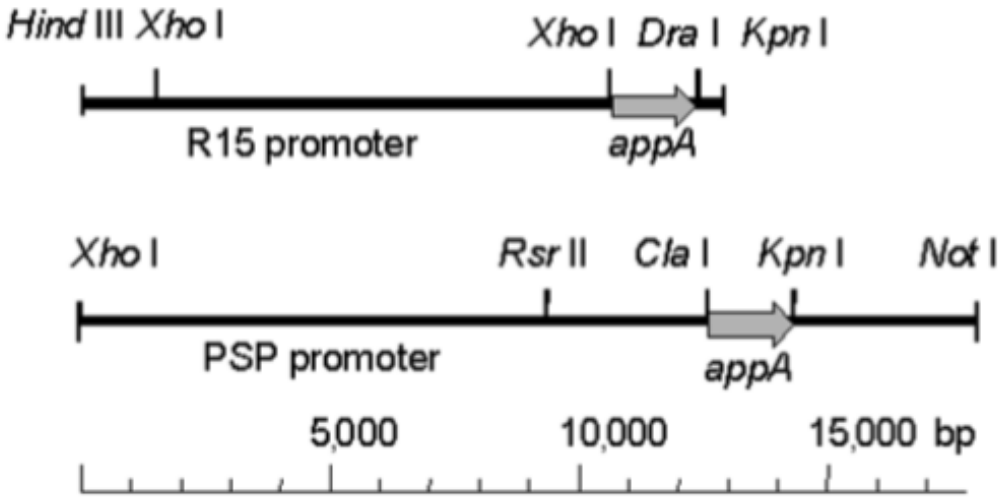
- Pigs are inherently incapable of digesting Non-starch polysaccharides that are primarily present in plant cell walls.

Salivary gland-specific expression of phytase

Phytate phosphorus passes undigested, most important manure pollutant

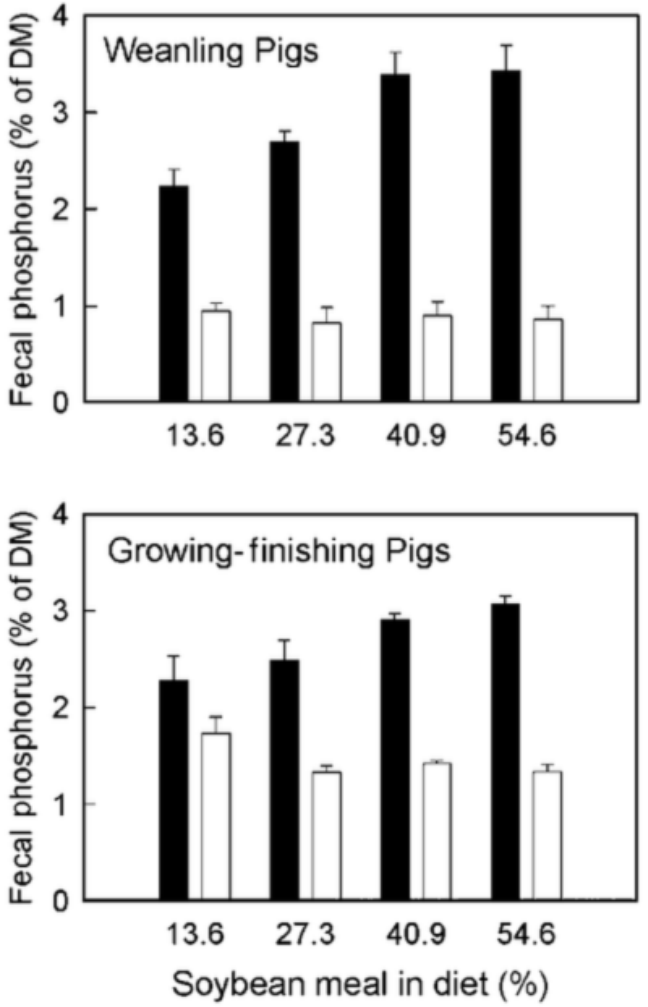
Phytase: allows the pigs to digest the phosphorus in phytate

Phytase transgene constructs



◆ salivary gland-specific expression promoter: PSP

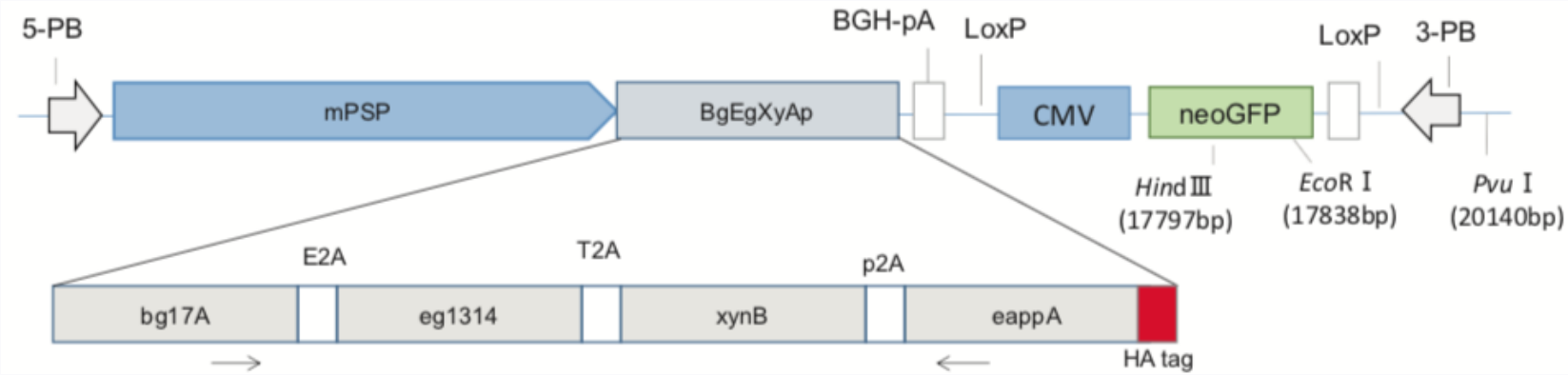
Total phosphorus content of fecal matter



Nature Biotechnology,2001

Expression of three microbial enzymes, b-glucanase, xylanase, and phytase in the salivary glands

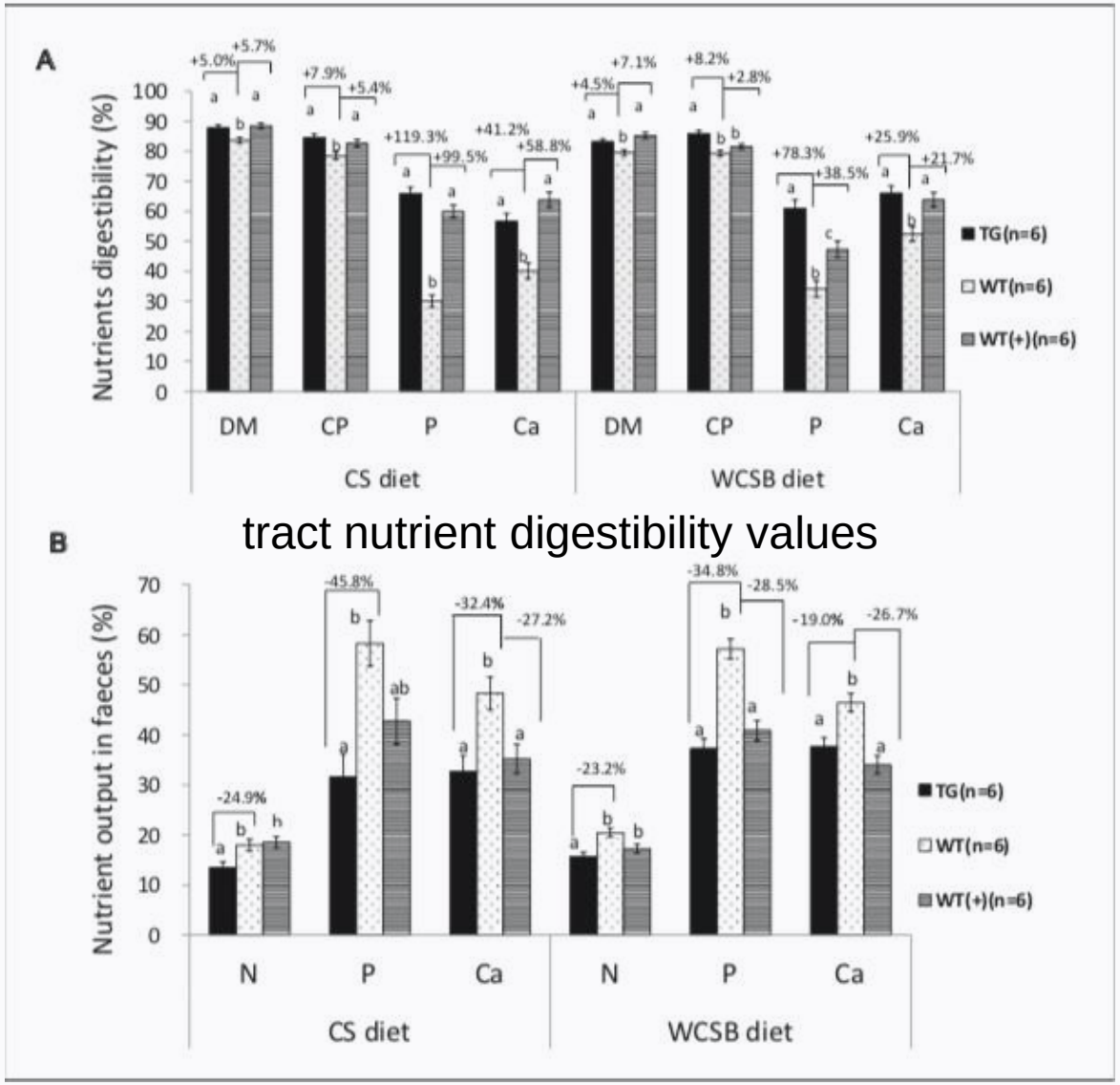
Experiment Design



- salivary gland-specific expression promoter: mPSP
- **NSP-degrading enzymes**: two β-glucanases genes (bg17A and eg1314), a xylanase gene (xynB)
- **Phytate-degrading enzyme**: phytase gene (eappA)

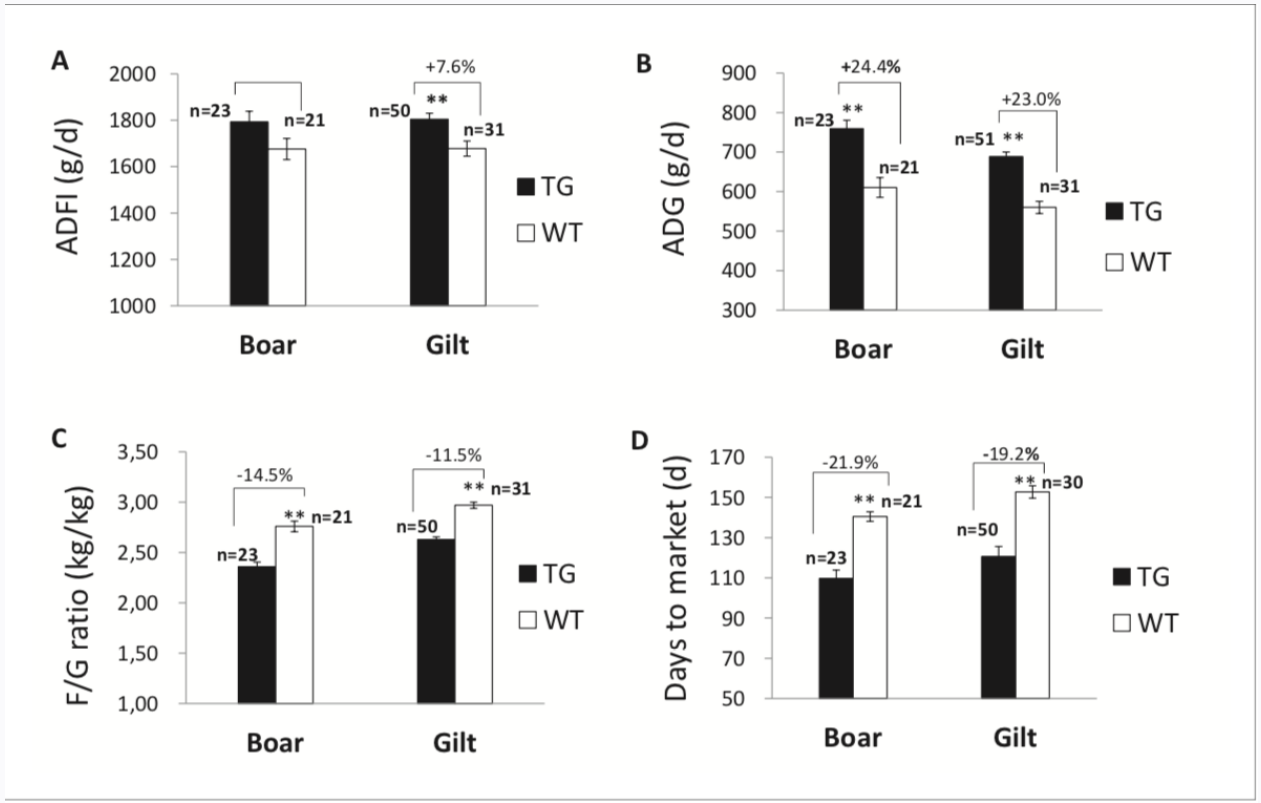
eLife,2018

fecal nutrient output



tract nutrient digestibility values

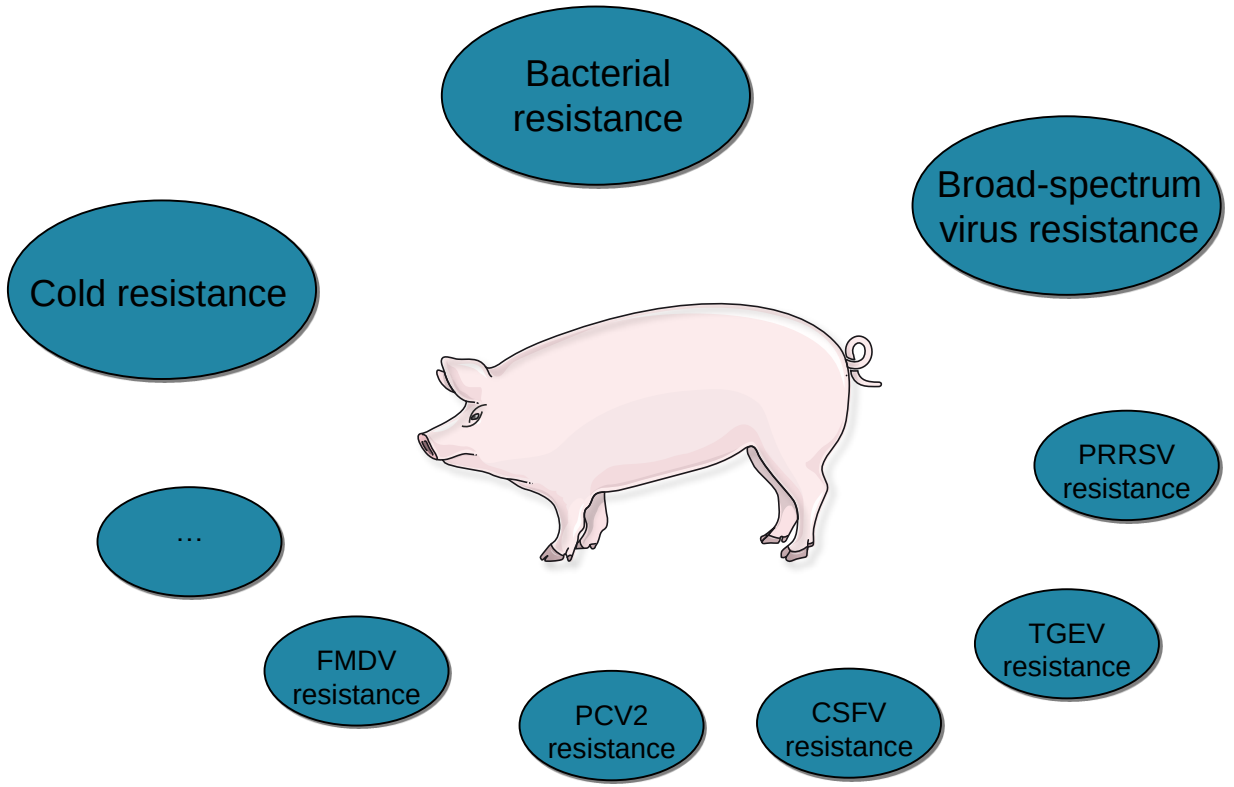
Growth performance



eLife,2018

4. Stress resistance pigs

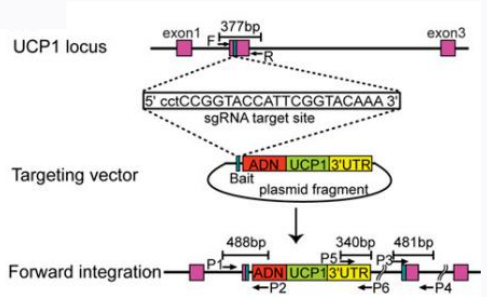
- Overexpressing endogenous resistance genes
- Introducing exogenous resistance genes
- Editing pathogens targeting receptor genes
- Introducing synthetical virus-killing genes



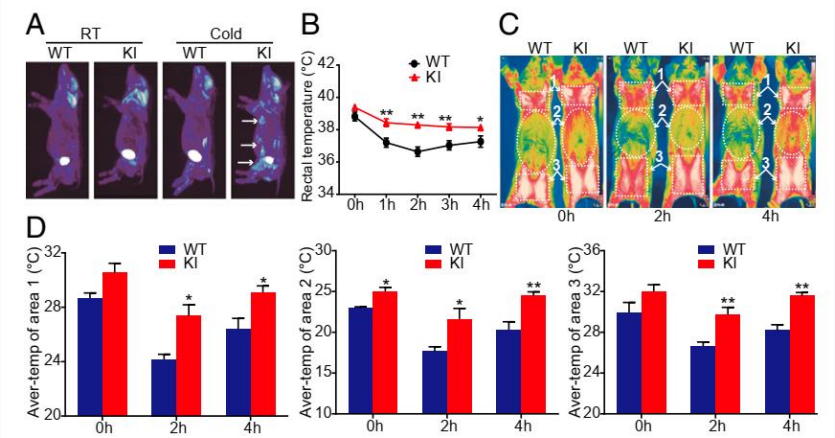
Generation of adiponectin-UCP1 KI transgenic pigs: Cold resistance transgene pigs

Pigs lack a functional UCP1 gene, resulting in poor thermoregulation and susceptibility to cold.

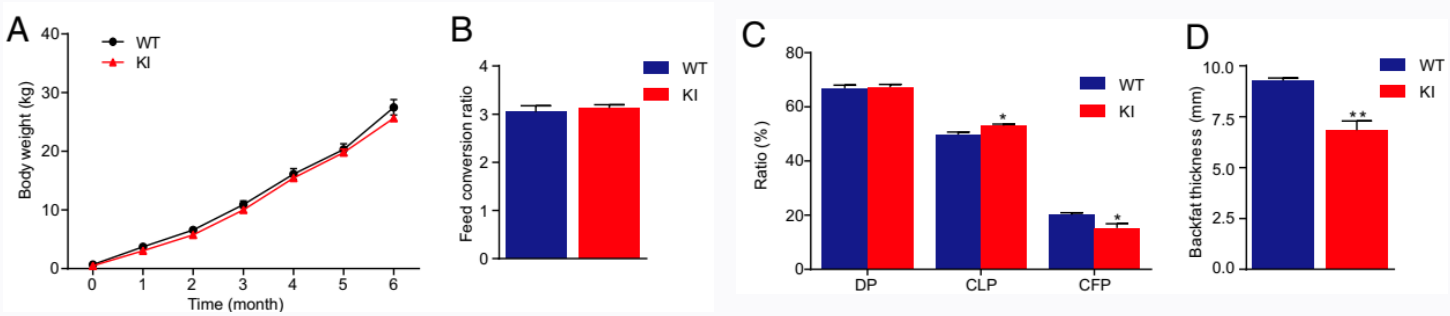
Generation of adiponectin-UCP1 KI transgenic pigs



UCP1 KI pigs exhibited improved thermoregulation



Decreased Fat Deposition and Increased Adipose Lipolysis in UCP1 KI Pigs



PNAS, 2017

Anti-bacterial transgenic pigs

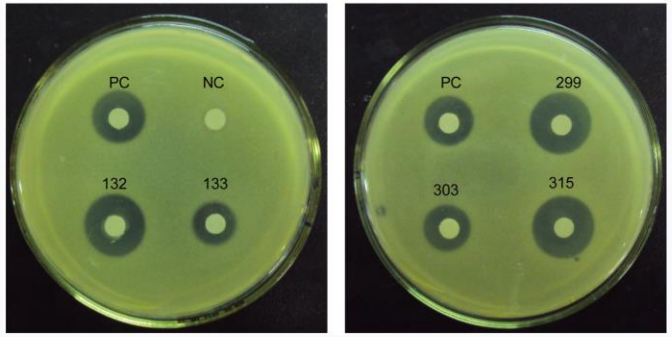
Lysozyme has Broad-spectrum antibacterial activities

lysozyme →

Bacillus subtilis
Bacillus cereus,
Staphylococcus aureus,
Escherichia coli,
Klebsiella pneumoniae,
Pseudomonas aeruginosa,
Streptococcus agalactiae,
Salmonella typhimurium,
…

Human lysozyme gene transgenic pigs

Transgenic pigs milk can inhibit growth of *E.coli*



Transgenic pigs milk can inhibit the growth of *E.coli* in the duodenum of sucking pigs

Item	Non-transgenic	Transgenic	*p*-value
Escherichia coli			
Duodenum	7.62±0.24	6.56±0.17	<0.001
Jejunum	7.00±0.39	6.85±0.66	0.707
Ileum	7.70±0.39	7.58±0.43	0.691
Colon	7.09±0.35	6.77±0.34	0.236

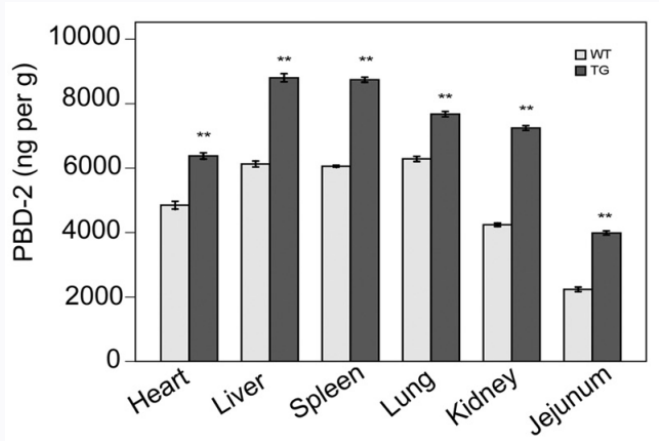
PLOS ONE, 2014; PLOS ONE, 2015

Anti-bacterial transgenic pigs

Porcine beta-defensin 2 (PBD-2) can against *Actinobacillus pleuropneumoniae*

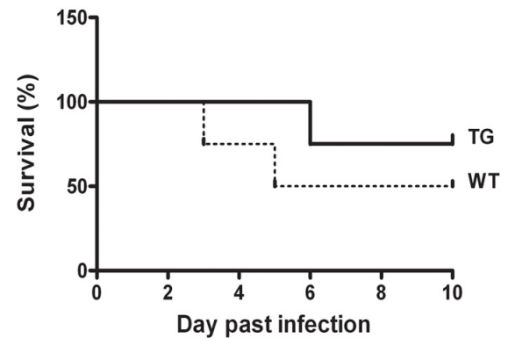
Actinobacillus pleuropneumoniae is an important respiratory pathogen causing porcine contagious pleuropneumonia

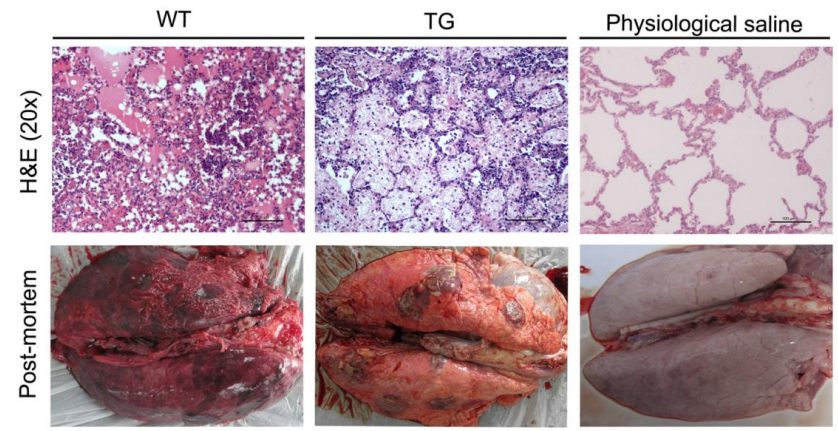
Construct of transgenic pigs overexpressing PBD-2



PBD-2 gene is driven by the CAG promoter

Overexpression of porcine beta-defensin 2 (PBD-2) enhanced resistance to cohabitation infection by A. pleuropneumoniae.

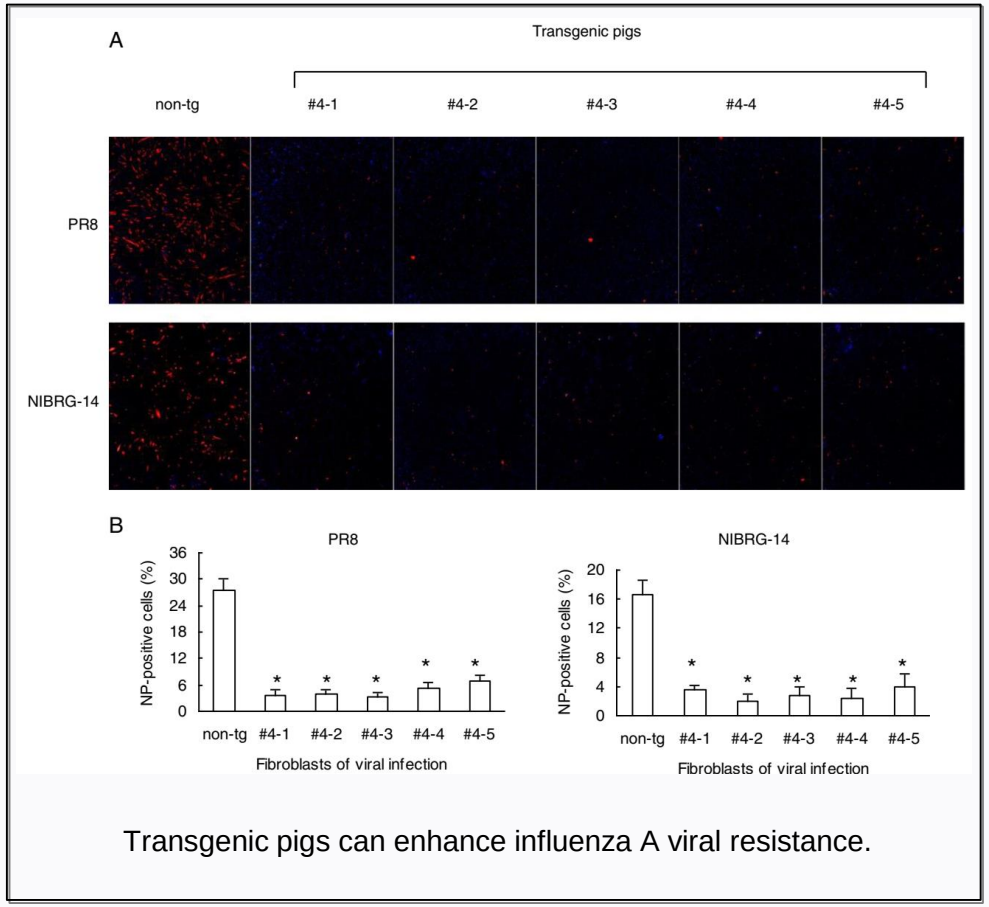




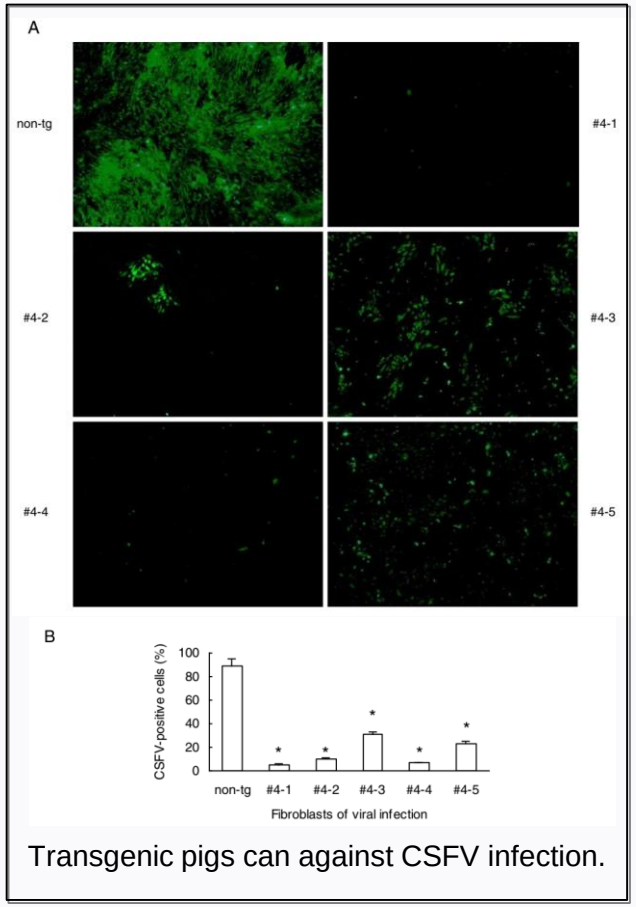
Infection and Immunity, 2015

Broad-spectrum antiviral transgenic pigs

1. The transgenic pigs over-expressing myxovirus resistance gene have broad-spectrum antiviral activities



Transgenic pigs can enhance influenza A viral resistance.

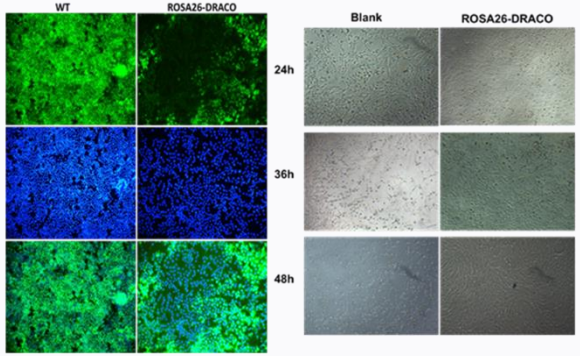


Transgenic pigs can against CSFV infection.

Cell Regeneration, 2014

Broad-spectrum antiviral transgenic pigs

2. The transgenic pigs express DRACO gene have broad-spectrum antiviral activities



Transgenic pigs can enhance CSFV and PRV resistance

3. Gene editing pigs enhance NLRP3 expression have broad-spectrum antiviral activities

Transgenic pigs can enhance NLRP3 expression during the stimulation

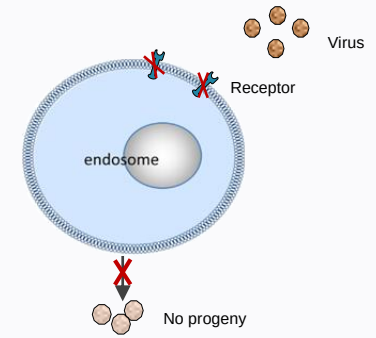
The Journal of Immunology, 2020

Specific viral resistance transgenic pigs

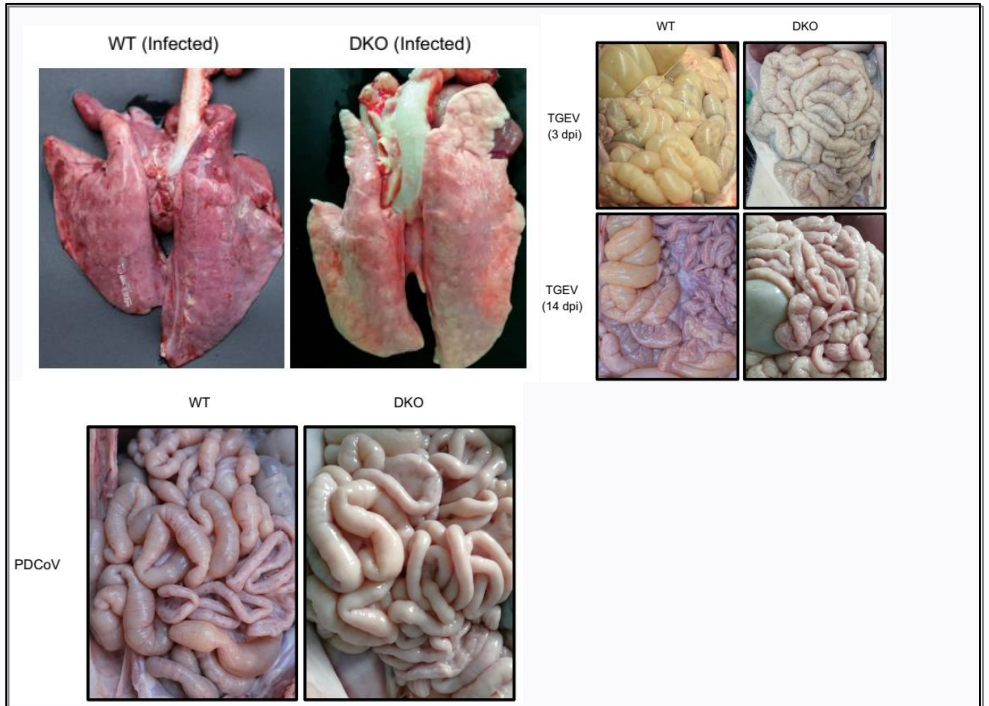
1. Knockout virus entry receptors CD163 and porcine aminopeptidase N (pAPN) Gene

pigs are resistant to PRRSV and porcine transmissible gastroenteritis virus (TGEV) and decreased susceptibility to deltacoronavirus (PDCoV

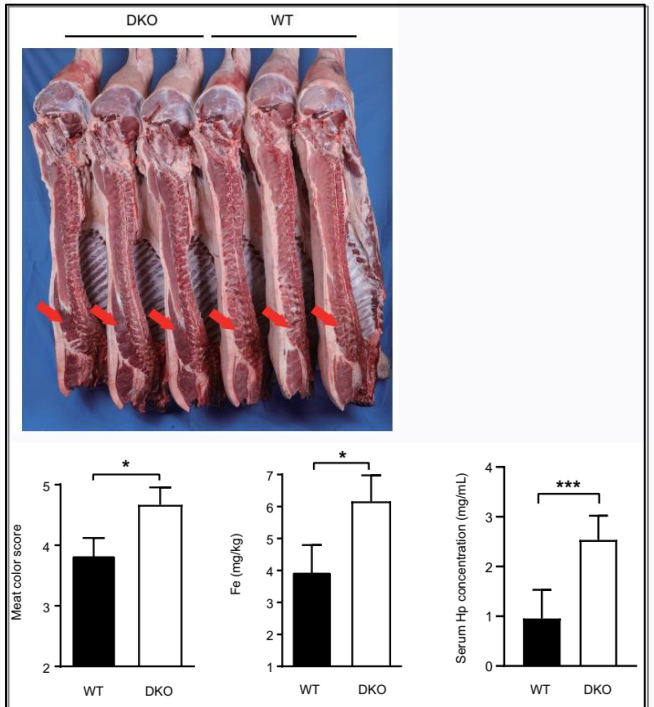
The strategy of antivirus



CD163&pAPN DKO pigs



CD163 and pAPN DKO pigs are completely resistant to genotype 2 PRRSV and TGEV, and also decreased susceptibility to PDCoV.

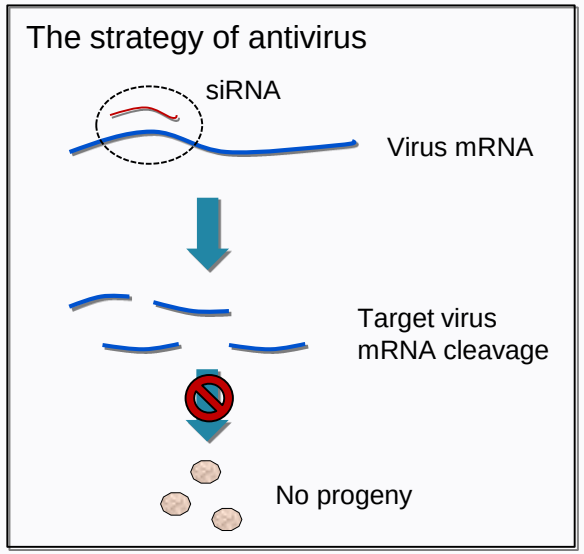


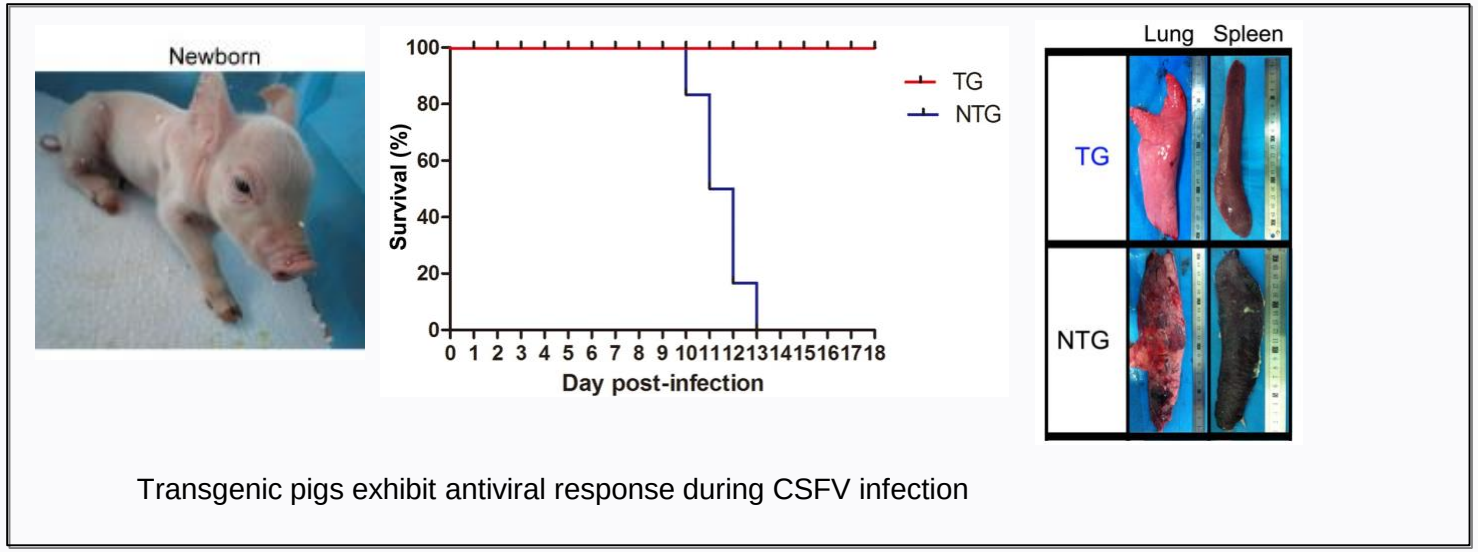
DKO pigs maintain normal production performance.

eLife, 2020

Specific viral resistance transgenic pigs

2. Constructing CSFV resistance pigs through knockin the shRNAs of targeting virus RNA





Transgenic pigs exhibit antiviral response during CSFV infection

Others anti-viral transgenic pigs through introducing RNA interference

Type of virus	Journal	Organization
Foot-and-mouth disease virus (FMDV)	eLife，2015	Shihezi University
Porcine circovirus type 2 (PCV2)	Chinese Journal of Veterinary Science，2017	South China Agricultural University

PLOS Pathogens, 2020

Thanks