

# Xenogene Zell-, Gewebe- und Organtransplantation – von der Grundlagenforschung zur klinischen Anwendung

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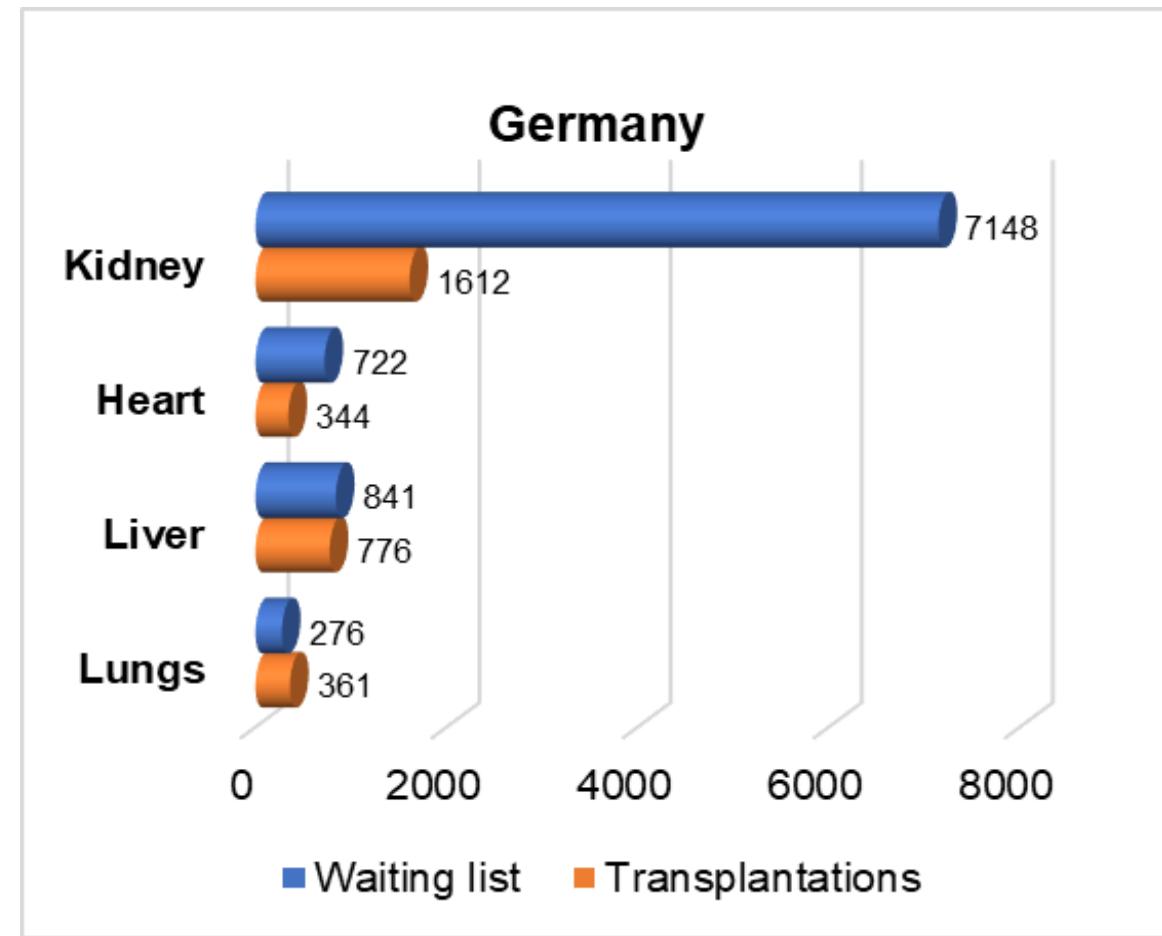
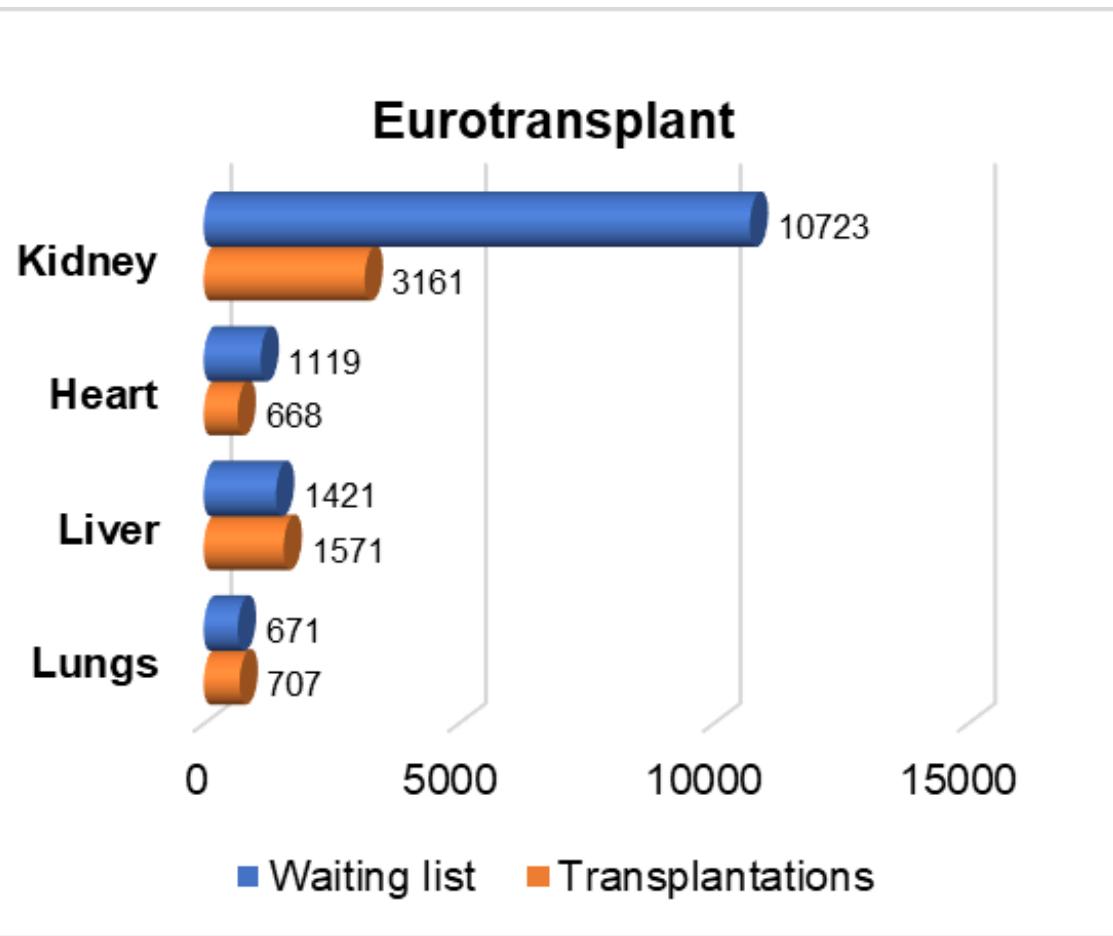
# Darlegung potentieller Interessenskonflikte

Der Inhalt des folgenden Vortrages ist Ergebnis des Bemühens um größtmögliche Objektivität und Unabhängigkeit.

Als Referent weise ich darauf hin, dass es **persönliche Verbindungen** zu Unternehmen gibt, deren Produkte im Kontext des folgenden Vortrages von Interesse sind. Dabei handelt es sich um die folgenden Unternehmen und Verbindungen:

Unternehmen	Verbindungen
Mitgründer der XTransplant GmbH	(Honorar für Vortrags-, Autoren-, Gutachter- oder Beratungstätigkeiten; Honorar für Vorbereitung von Fortbildungen; Erstattung von reise- oder Übernachtungskosten; Erstattung von Teilnahmegebühren an Fortbildungen; Patente; Geld aus Lizenzen/Tantiemen; Honorar für Durchführung von Auftragsstudien; Erhalt von Forschungsgeldern; andere)
Mitgründer der MWM Biomodels GmbH	Sprecher des DFG SFB/TR 127

# Lack of human organs for transplantation



Eurotransplant Annual Report 2019

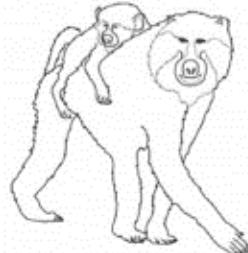
# The changing view on xenotransplantation

Concordant

## Advantages:

- anatomical similarities
- physiological compatibility
- rejection resembles allotransplants (?)

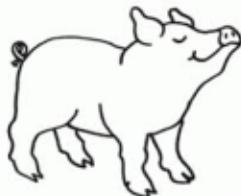
## Disadvantages:



- long gestation period
- single offspring
- organ size unsuitable for human adults
- transmission of infections
- high costs
- ethical considerations

Discordant

## Advantages:



- anatomical and physiological similarities
- short gestation period (114 days)
- large litters, rapid growth of offspring
- organ size suitable for human adults
- designated pathogen-free breeding (DPF)
- genetic modification well established**

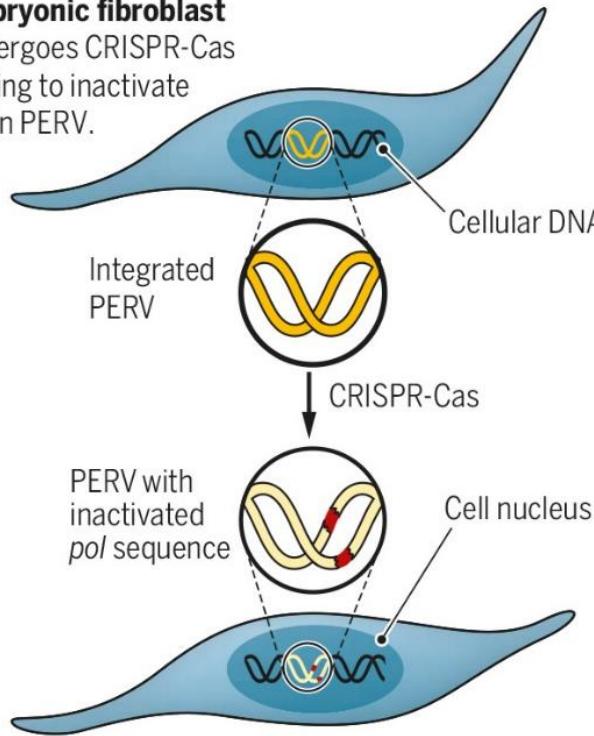
## Disadvantages:

- hyperacute / humoral rejection
- physiological incompatibilities
- porcine endogenous retroviruses (PERV)

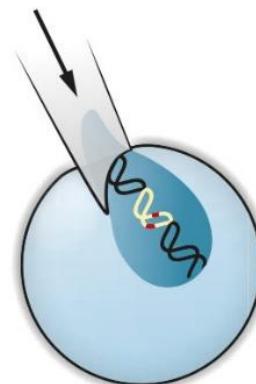


# Pigs with inactivated PERV integrants

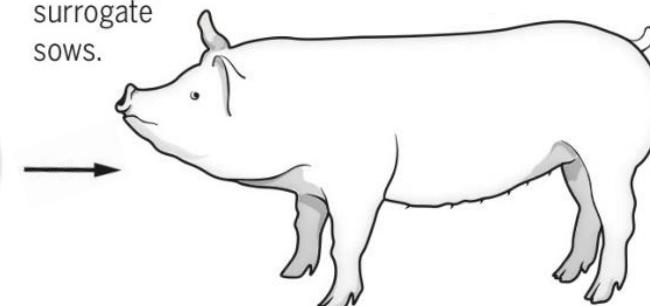
**Embryonic fibroblast**  
undergoes CRISPR-Cas  
editing to inactivate  
*pol* in PERV.



**Somatic cell nuclear transfer**  
is performed whereby  
the fibroblast nucleus  
that contains the  
inactivated *pol* gene  
is transferred into  
a denucleated oocyte.



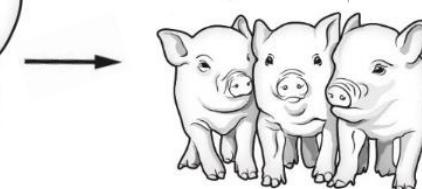
**The edited oocyte**  
is transplanted into  
surrogate sows.



- A primary porcine fetal fibroblast cell line (FFF3) with about 25 copies of functional PERVs was used
- Use of p53 inhibitor during genetic modification was necessary to grow up 100% PERV-inactivated FFF3 cell clones
- Five out of eight PERV-inactivated FFF3 cell clones carried chromosomal abnormalities
- No difference in SCNT efficiency between PERV-inactivated (0.9%) and WT cells (0.8%)
- The physiological functions of endogenous retroviruses, which exists in all mammalian species, remain largely unknown

Niu et al., *Science* 357, 1303-1307 (2017)

**PERV-inactivated piglets** are produced,  
from which organs  
could be used for  
human transplant.



New York Times, 8-10-2017

# The changing view on xenotransplantation



## nature biotechnology EDITORIAL

In the 1990s, concerns about potential transfer of porcine retroviruses to xenotransplant recipients shut down commercial programs, even though **transmission had not (and still has not) been demonstrated *in vivo***. Given the continuing shortage of human organs for transplant, **return of commercial funding to xenotransplantation is encouraging**.

**Government funders should take note.** Yes, stem cell-derived therapies offer great long-term promise for degenerative diseases. But **xenotransplants represent an additional intriguing option - one with potentially shorter horizons to the clinic.**

**NATURE BIOTECHNOLOGY**  
VOLUME 34 NUMBER 1 JANUARY 2016

## Science AAAS

The science stories likely to make headlines in 2020

<https://www.sciencemag.org/news/2020/01/science-stories-likely-make-headlines-2020>

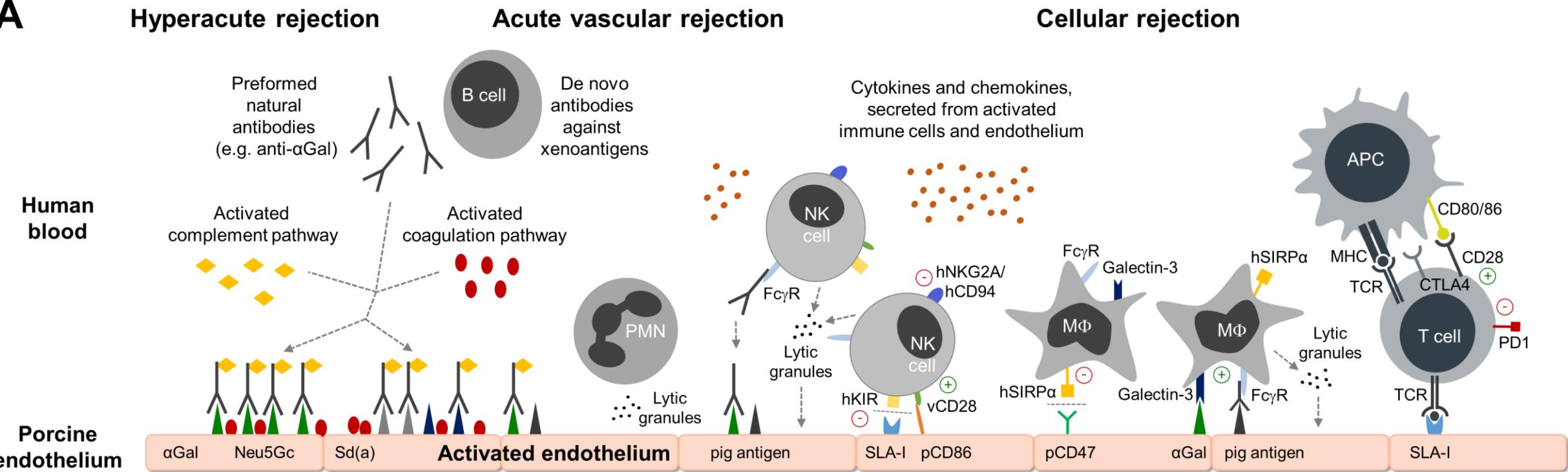
By Science News Staff | Jan. 2, 2020, 10:50 AM

### Making xenotransplants survive

The genome editor CRISPR is reinvigorating the beleaguered field of xenotransplantation, which aims to surgically replace human organs or tissues with ones harvested from animals such as pigs. Novel clinical trials of the strategy could launch this year. Xenotransplantation has long promised to alleviate a chronic shortage of human livers, hearts, and other organs. It could also provide corneas to cure blindness and insulin-producing islet cells to replace those destroyed by diabetes. Recent CRISPR experiments have modified genes in pigs to prevent or dampen human immune responses to their tissue and have removed DNA from the porcine genome that could spawn potentially dangerous viruses in a person. Transplants from these edited pigs to monkeys, a key test of safety and efficacy before human trials, have demonstrated long-term viability in their new hosts.

# Strategies to overcome pig-to-human xenotransplant rejection

A



B

## Deletion of carbohydrate antigens:

Knockout of GGT1, CMAH, B4GALNT2

## Inhibition of complement activation:

Expression of hCD46, hCD55, hCD59

## Prevention of coagulation disorder:

Expression of hTHBD, hEPCR, hTFPI, hCD39

## Inhibition of NK-cell activation:

Expression of HLA-E/hB2M

## Inhibition of MΦ activation:

Expression of hCD47

## Suppression of inflammation:

Expression of hA20, hHO-1

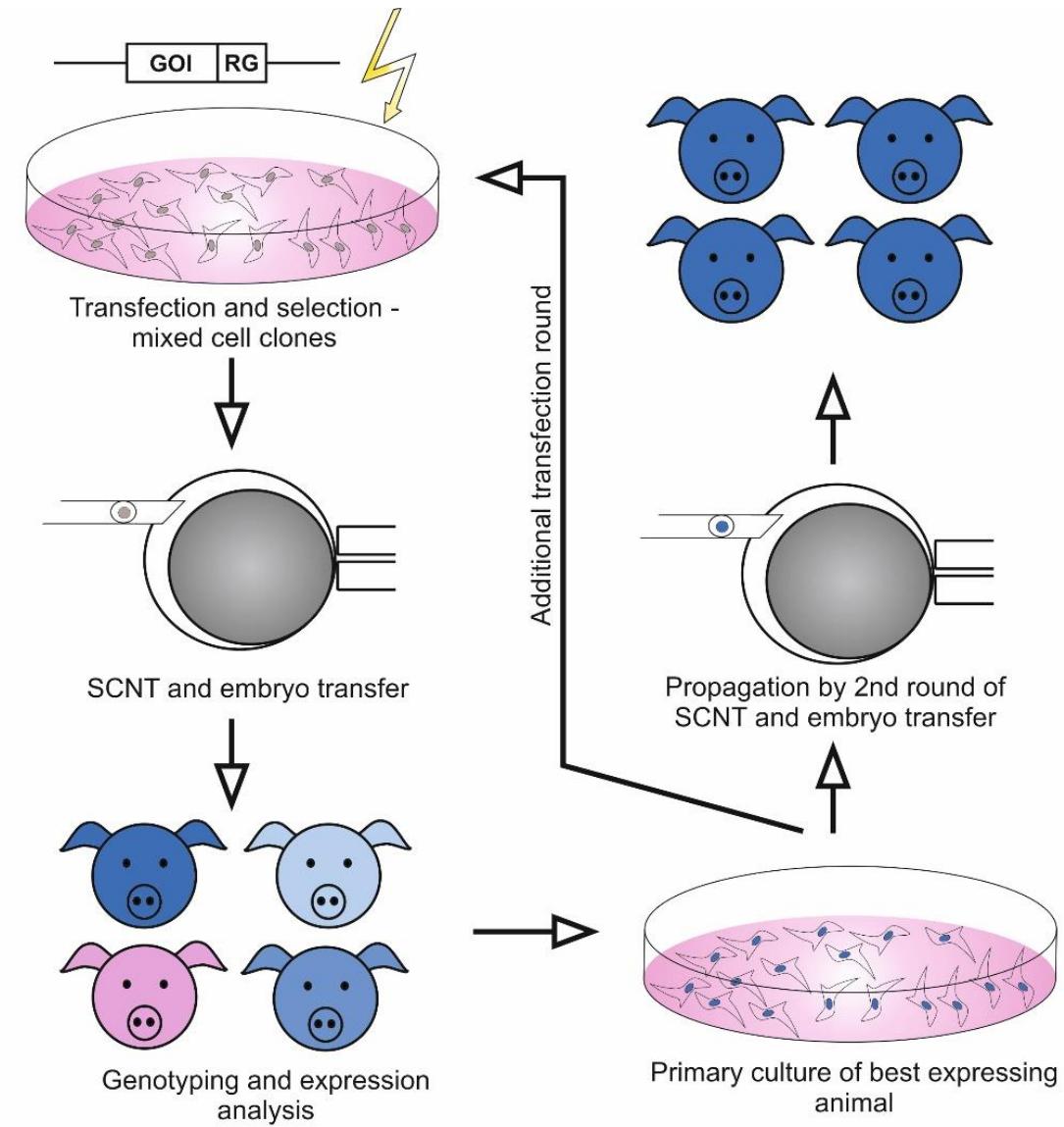
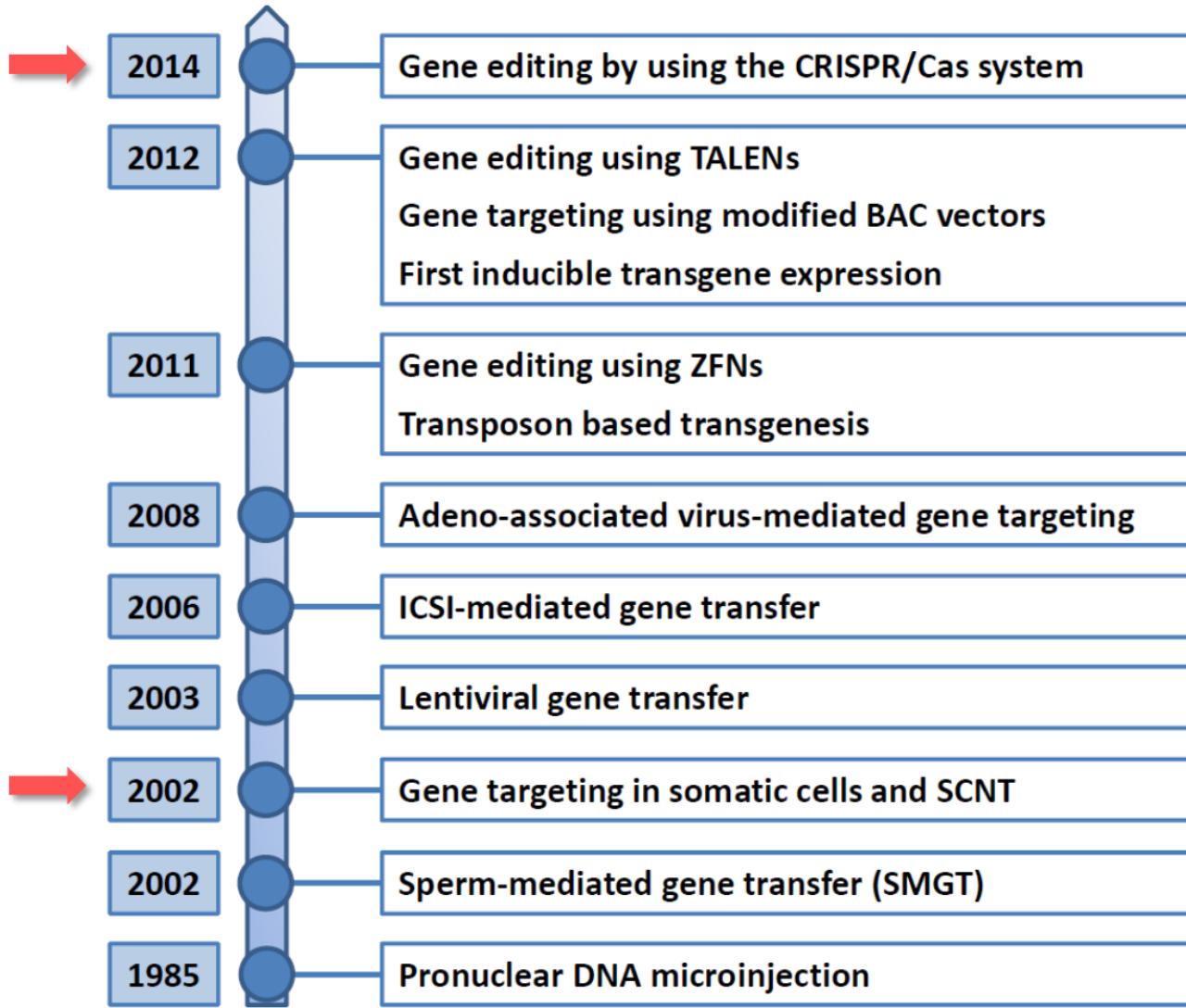
## Inhibition of SLA antigen presentation:

Deletion of SLA-I, pB2M, pCIITA

## Inhibition of T-cell activation:

Expression of CTLA4-Ig, LEA29Y, hPD-L1, anti-hCD2-mAb

# Toolbox for the genetic modification of pigs



# Structure the TRR 127 Xenotransplantation

## Biology of xenogeneic cell, tissue and organ transplantation

### Project Group A

Immunity, tolerance

A1 Schwinzer,  
de Figueiredo

A2 Bähr, Michel,  
Murray, Kupatt

A3 Chavakis

A4 Jaeckel,  
Noyan

A5 Waskow, Ludwig,  
Linkermann

### Project Group B

Novel transgenic strategies

B1&2 Schnieke, Lucas-Hahn,  
Petersen, Fischer

B3 Kemter, Böttcher,  
Klymiuk, Wolf

### Project Group Z

Central support projects

Z1 Marckmann,  
Sautermeister,  
Hoppe

Z2 Tönjes, Godehardt,  
Denner, Kaufer

Z3 Wolf, Kessler,  
Schnieke, Petersen

Z4 Hinkel,  
Schönmann,  
Lampe

### Project Group C

Preclinical and clinical XT

C1 Speier, Cohrs,  
Kemter

C3 Wolf-van Buerck,  
Seissler

C4 Ludwig, Sancho Zapatero,  
Welzel, Bornstein

C7 Hilfiker, Cebotari,  
Büttner, Niemann

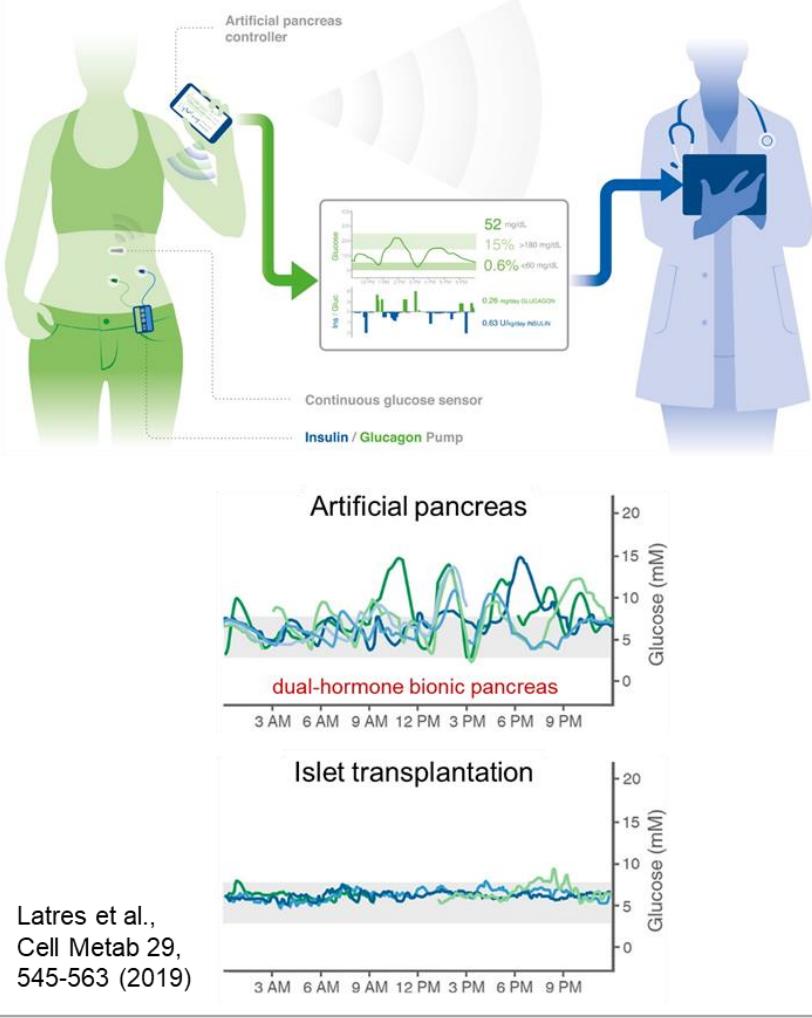
C8 Längin, Abicht,  
Brenner, Reichart

from bench

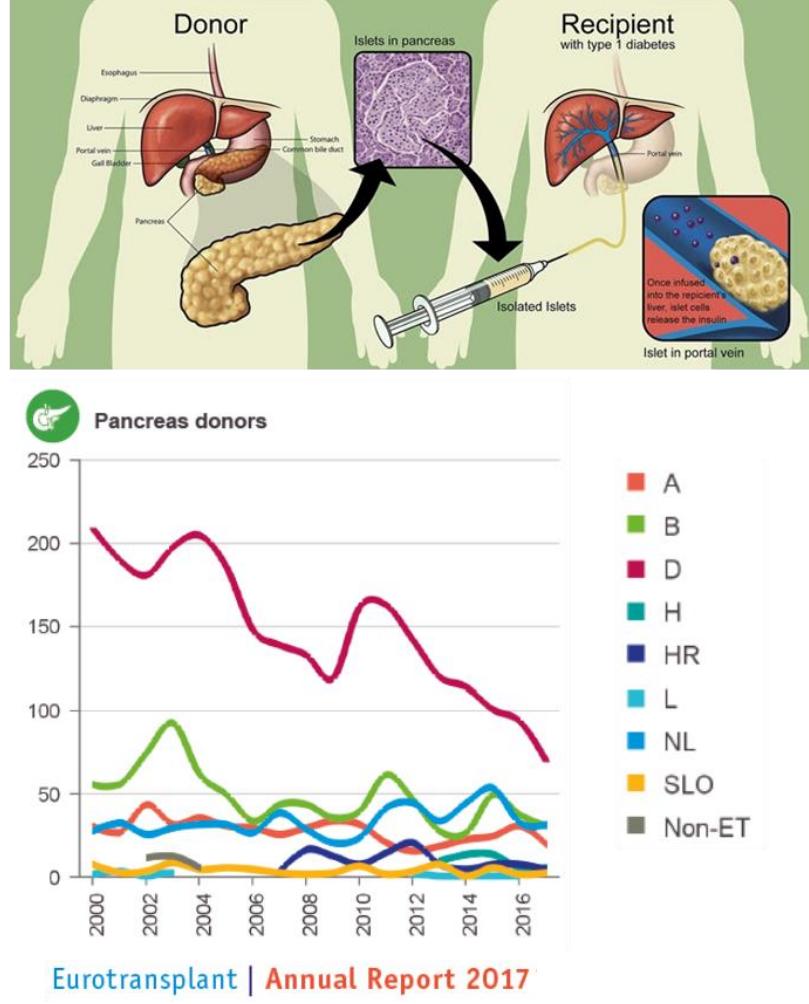
to bedside

# The need for alternative sources of pancreatic islets

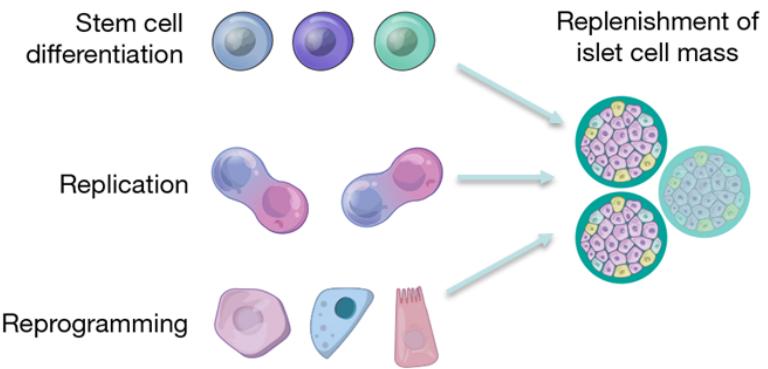
## Artificial pancreas



## Allotransplantation of islets



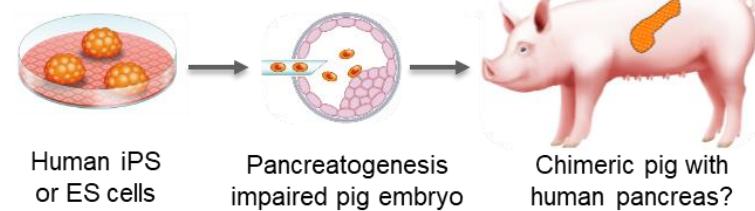
## Differentiation of stem cells and reprogramming of endogenous cells



Zhou & Melton, Nature 557, 351-358 (2018)

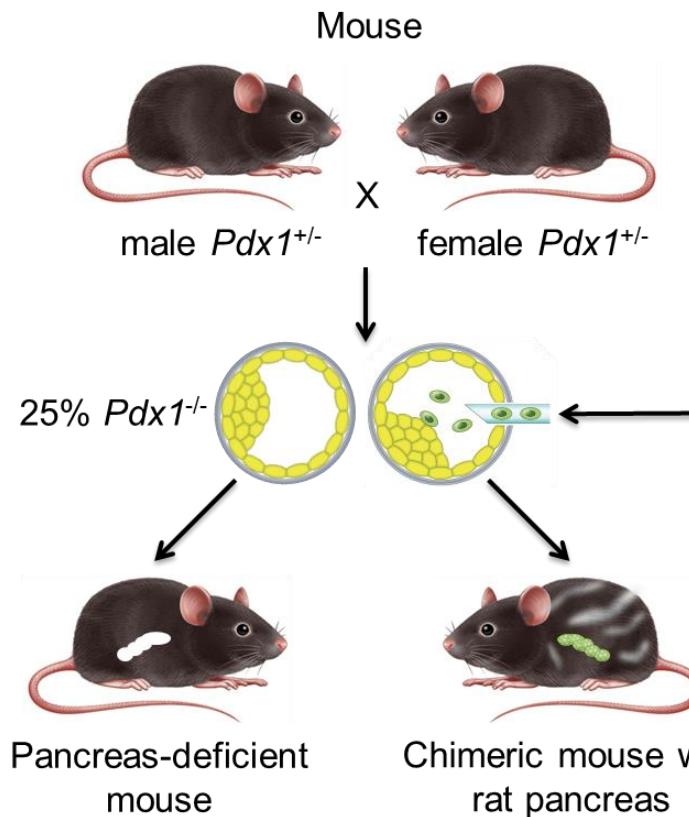
## Human pancreas from animal hosts

Kobayashi et al., Cell 142, 787-799 (2010)

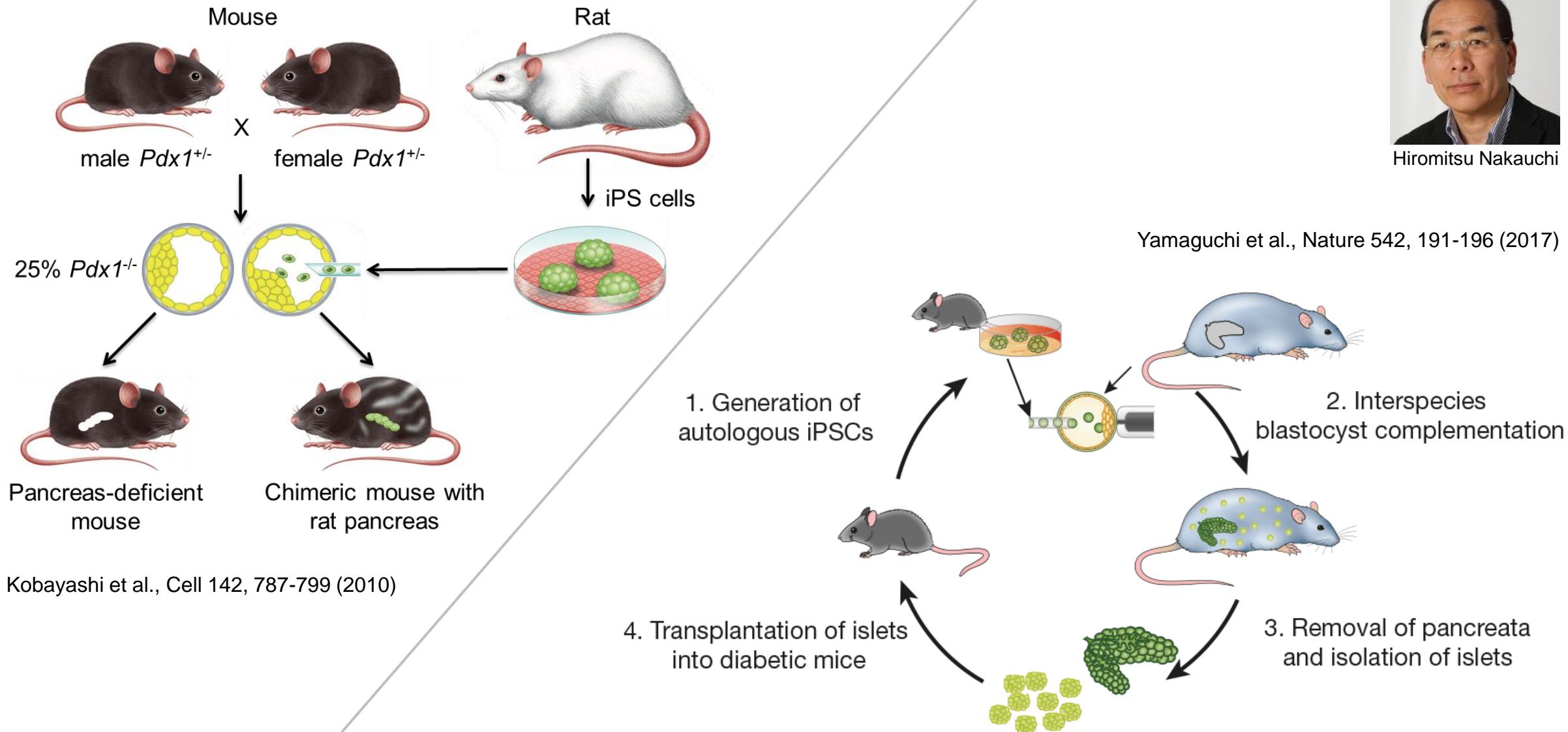


Matsunari et al., PNAS 110, 4557-4562 (2013)

# Rat pancreas in mouse and vice versa

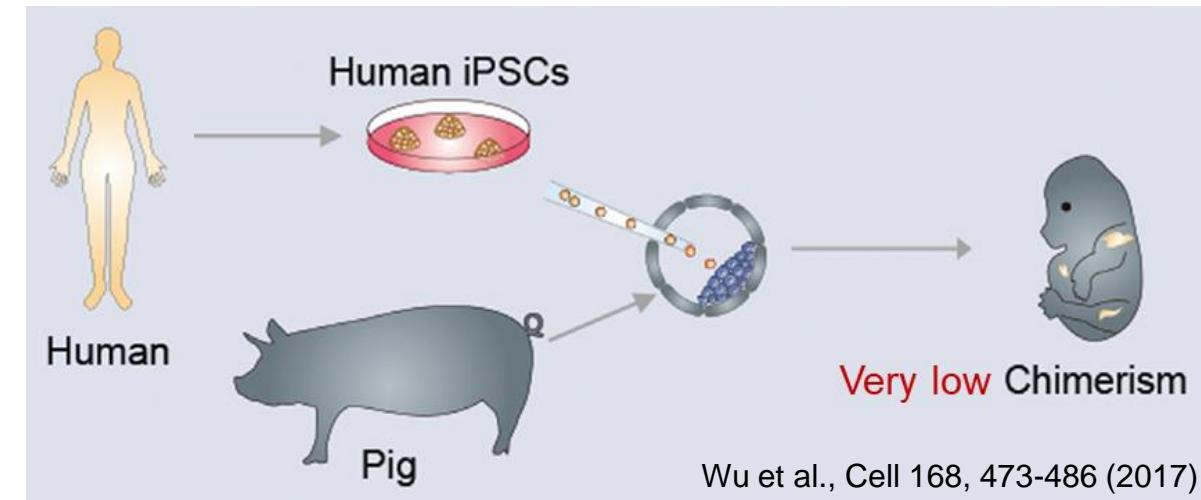
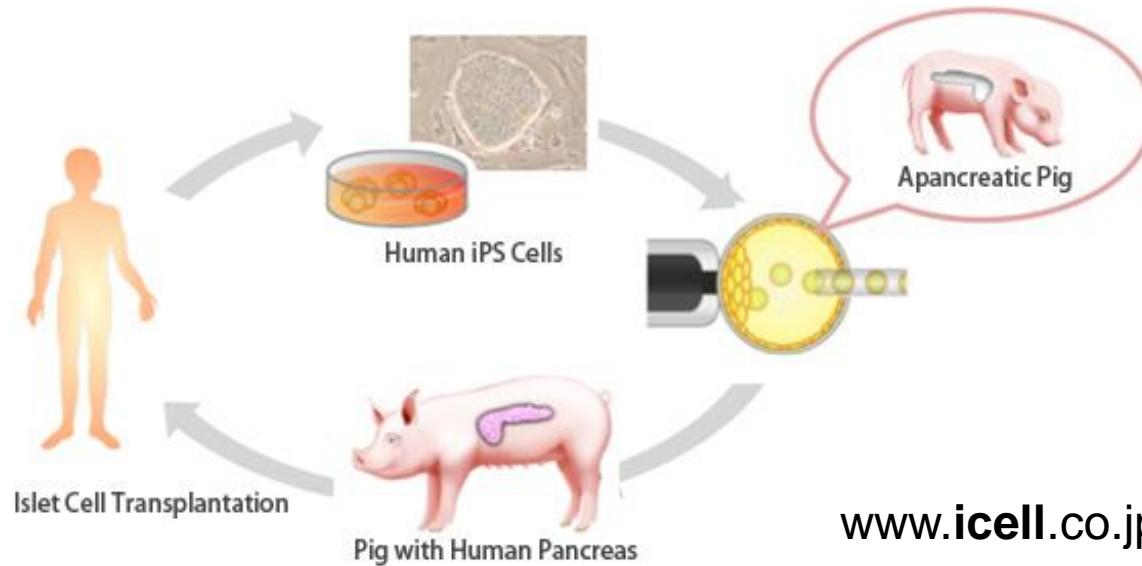


Kobayashi et al., Cell 142, 787-799 (2010)



Hiromitsu Nakauchi

# Human organs from animal hosts?



Divergence times (M years) between human and various animal species

Calculated with TIMETREE (<http://www.timetree.org/>). Above trajectory: median divergence times; below trajectory: estimated divergence time (confidence interval).

	Human	Mouse	Rat	Rabbit	Pig	Dog	Macaque
Human		89	89	89	94	94	28.8
Mouse	90 (85 - 97)		15.9	80	94	94	89
Rat	90 (85 - 97)	20.9 (18.4 - 23.3)		80	94	94	89
Rabbit	90 (85 - 97)	82 (77 - 88)	82 (77 - 88)		94	94	89
Pig	96 (91 - 101)	96 (91 - 101)	96 (91 - 101)	96 (91 - 101)		81	94
Dog	96 (91 - 101)	96 (91 - 101)	96 (91 - 101)	96 (91 - 101)	78 (76 - 82)		94
Macaque	29.4 (28.0 - 31.4)	90 (85 - 97)	90 (85 - 97)	90 (85 - 97)	96 (91 - 101)	96 (91 - 101)	

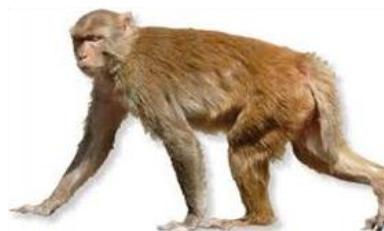
# Porcine islet xenotransplantation is a realistic option

Long-term control of diabetes in immunosuppressed non-human primates (NHP) by the transplantation of adult porcine islets

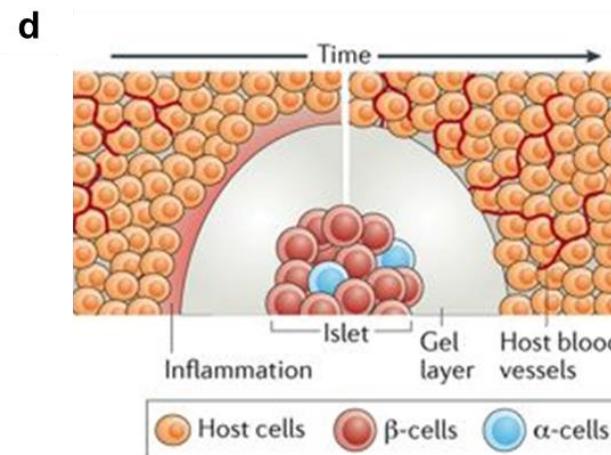
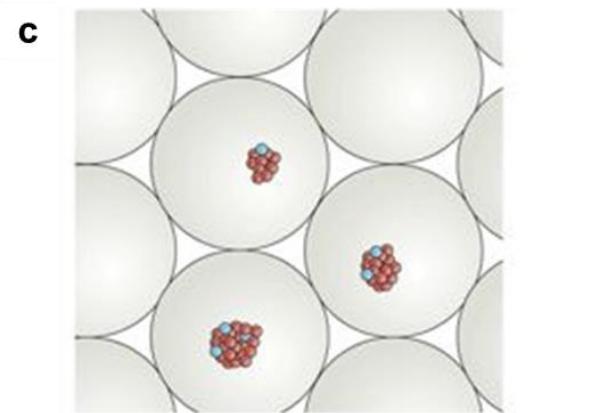
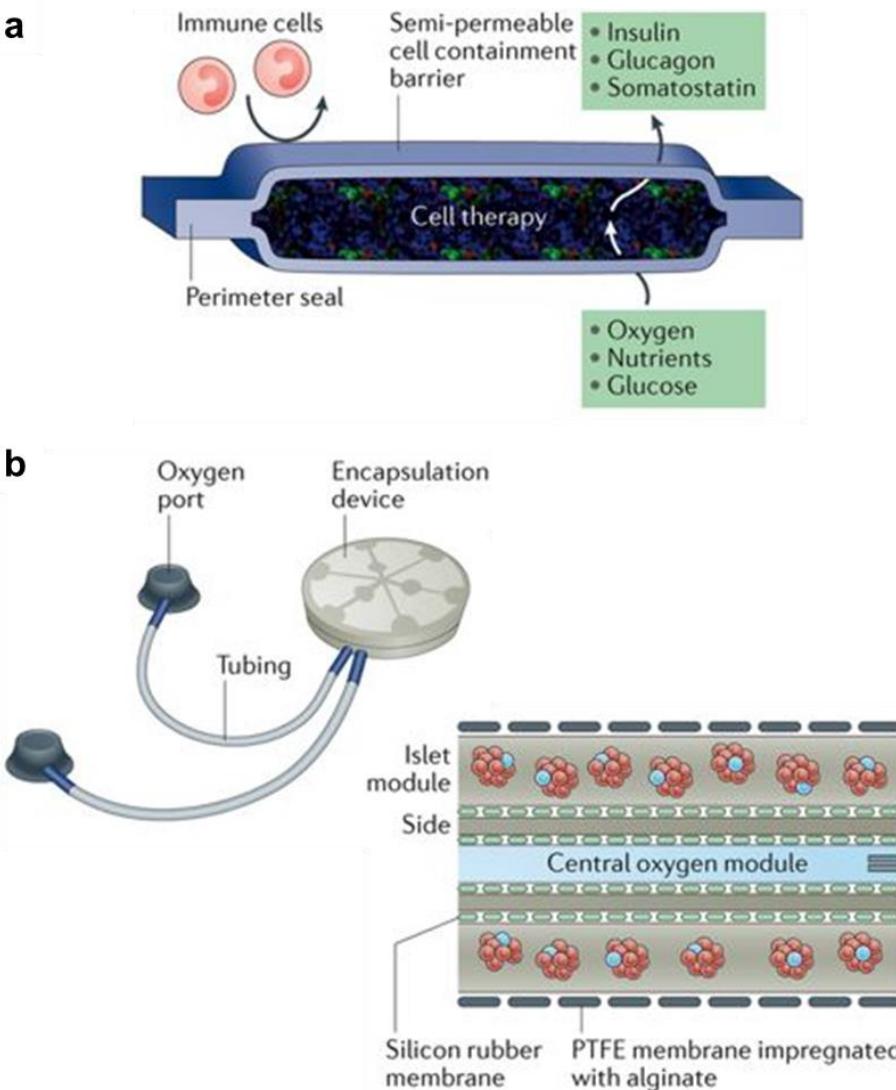
Recipient			Donor pigs		Islet infusion		Insulin independence
ID	Sex	Bwt (kg)	No. of pigs used	Bwt (kg)	Total islet (IEQ)	Day	
R051	M	7	3 (2M/1F)	71, 68, 84	530 000	>603	
R080	F	4.3	3 (2M/1F)	86, 78, 75	430 000	167	
R082	F	5.1	2 (2M)	62, 82	510 000	512	
R084	F	5.3	2 (2F)	98, 95	530 000	303	
R089	F	5.4	2 (2M)	72, 63	540 000	180	
Mean $\pm$ SD			2.4 $\pm$ 0.5	77.8 $\pm$ 11.6	508 000 $\pm$ 45 000		

## Immunosuppression:

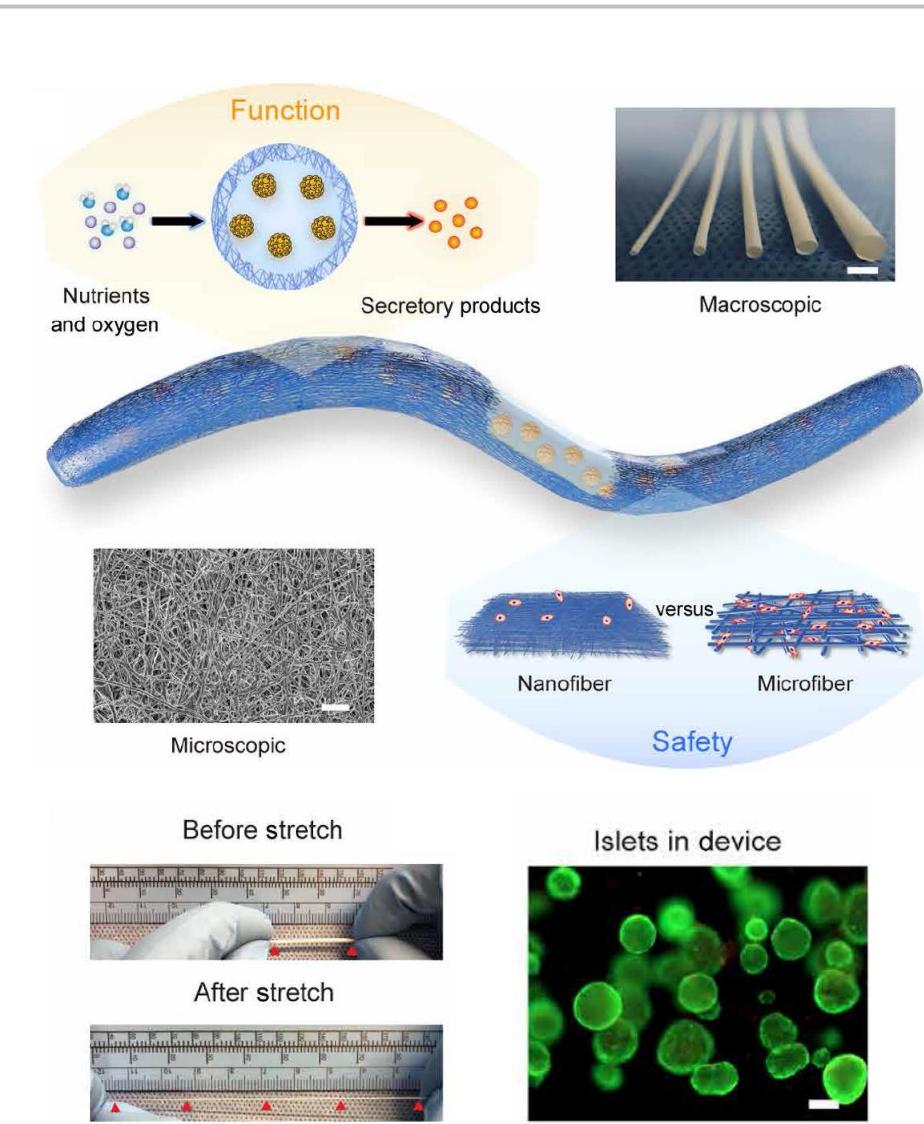
- Anti-CD154 mAb
- Sirolimus
- Anti-thymocyte globulin (ATG, Thymoglobulin)
- Cobra venom factor (CVF)
- TNF- $\alpha$  neutralizing mAb (Humira)



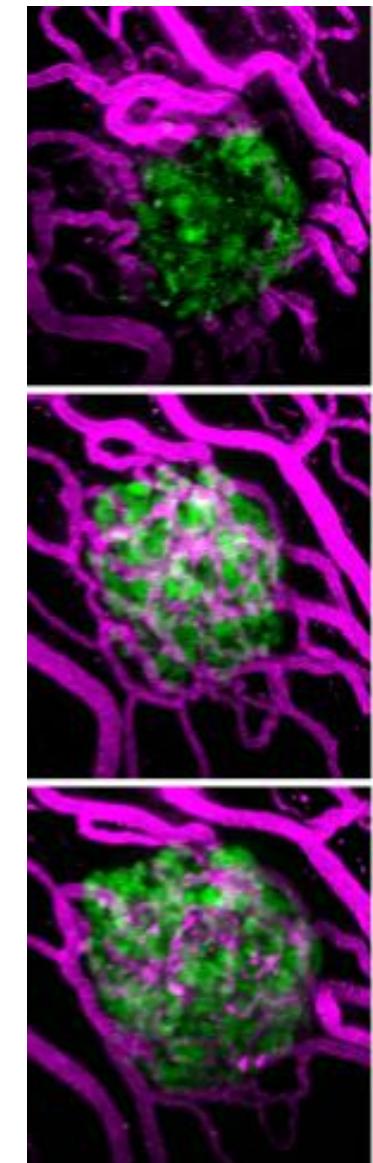
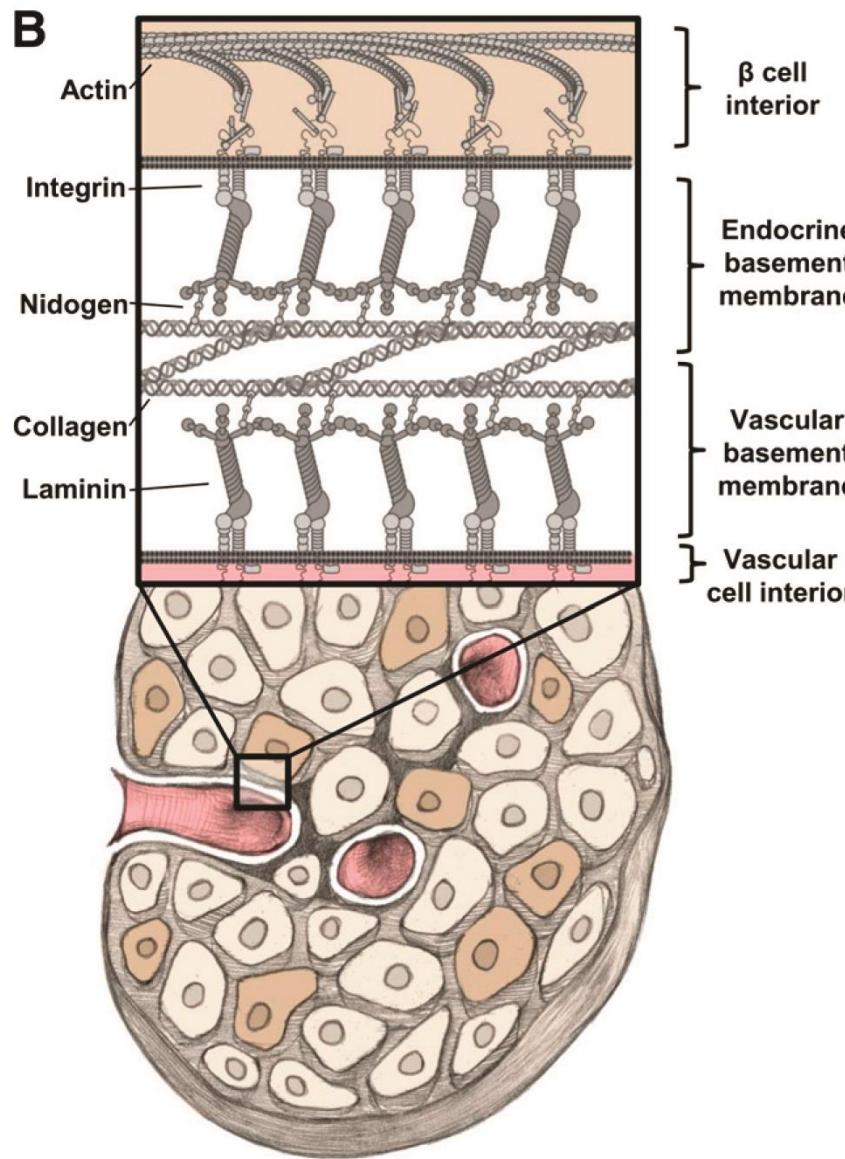
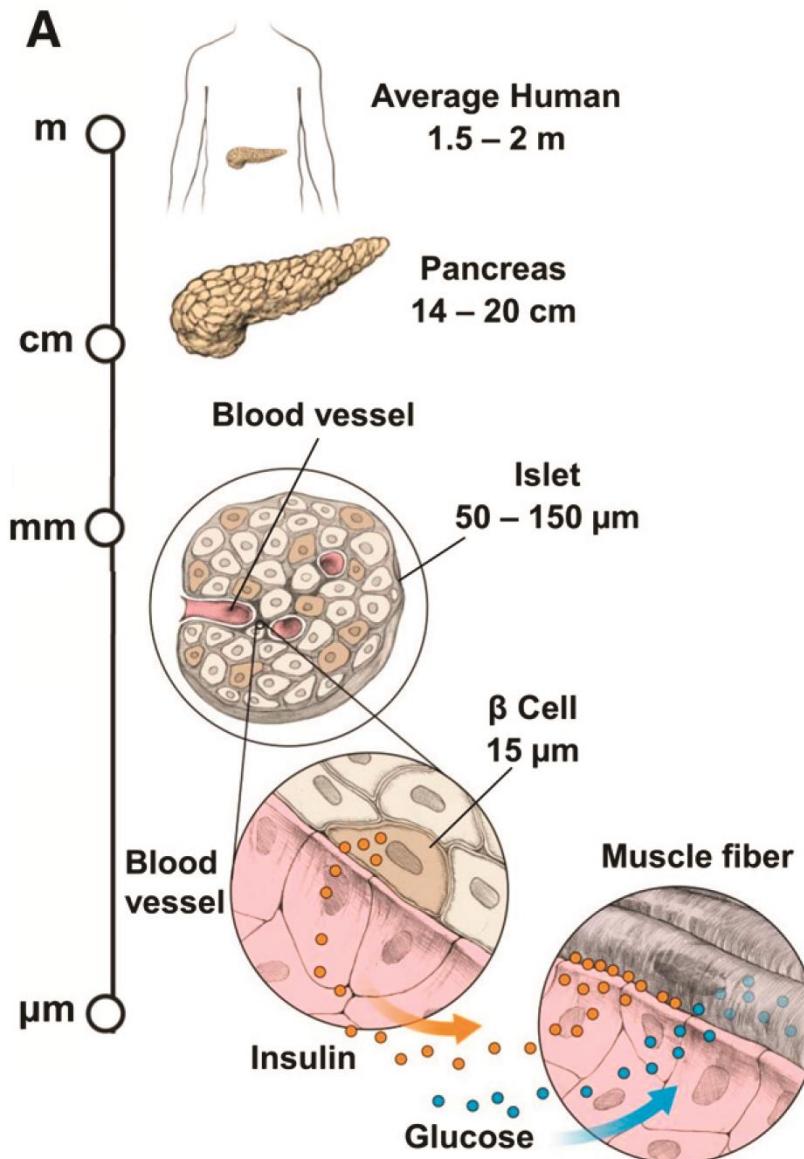
# Islet encapsulation strategies



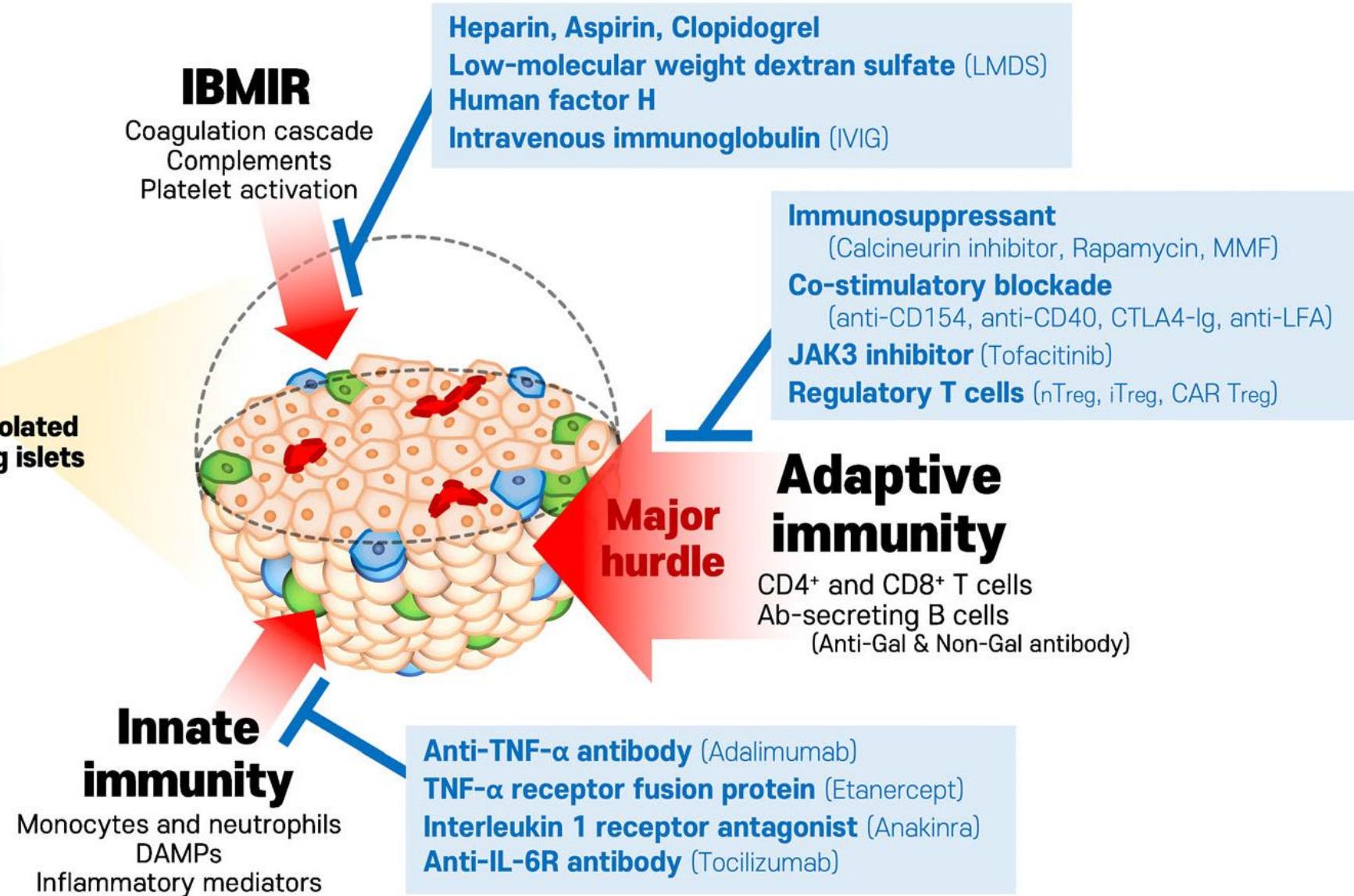
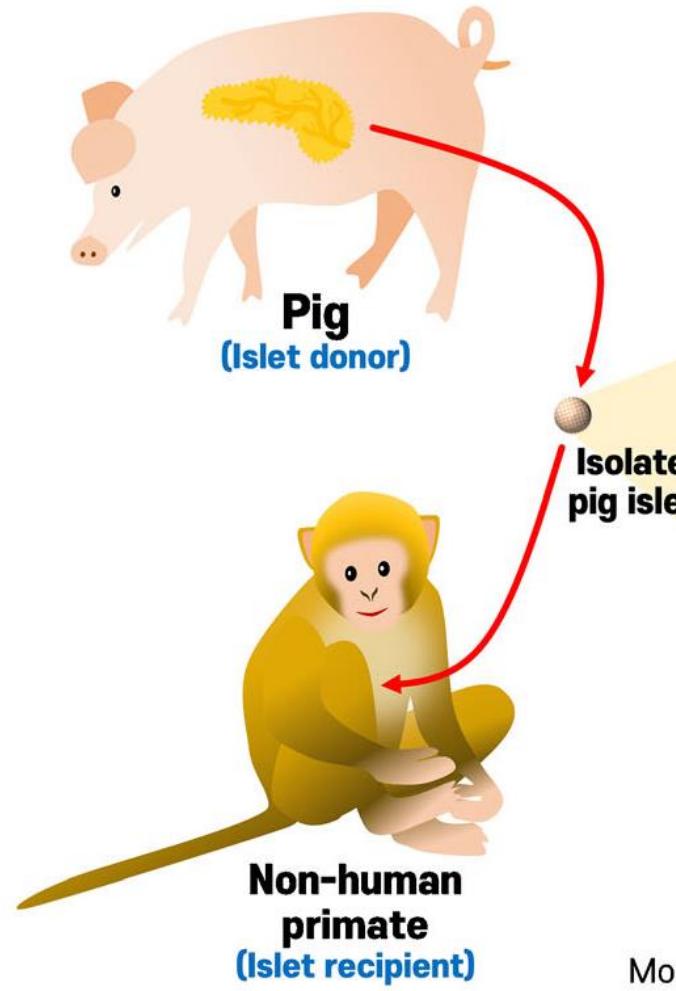
Nature Reviews | Drug Discovery



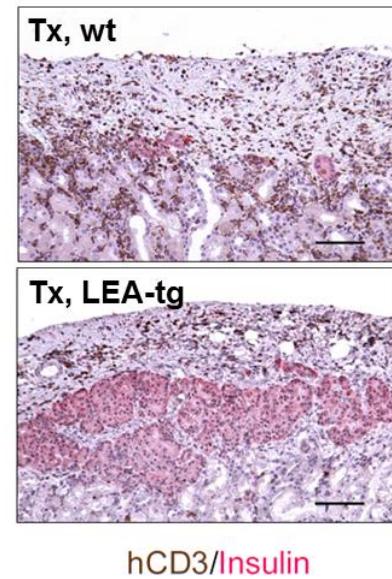
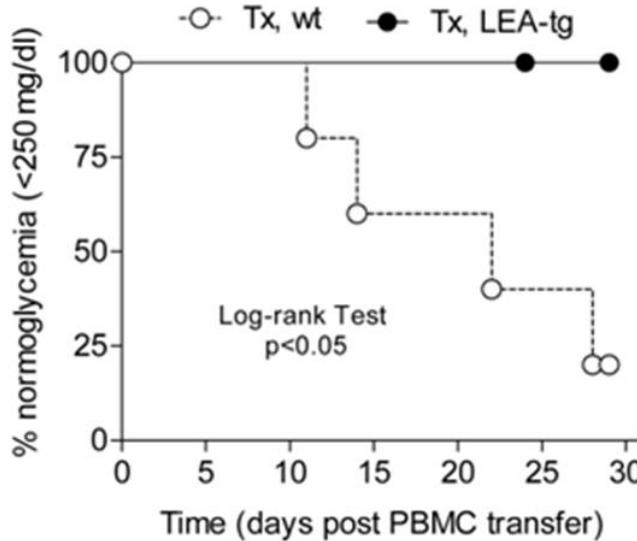
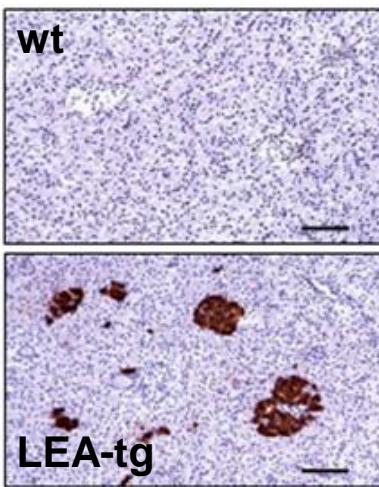
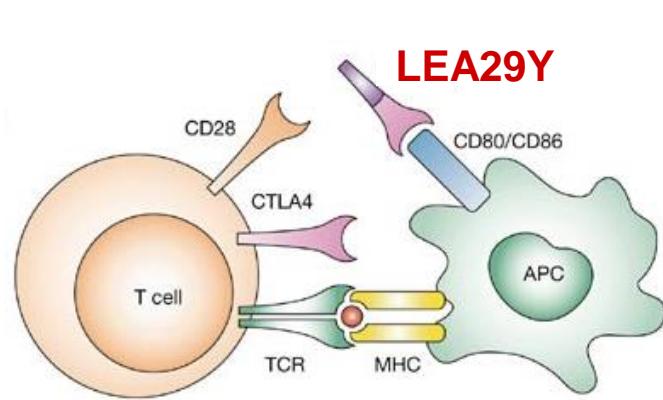
# Beta cells are in intimate contact with capillaries



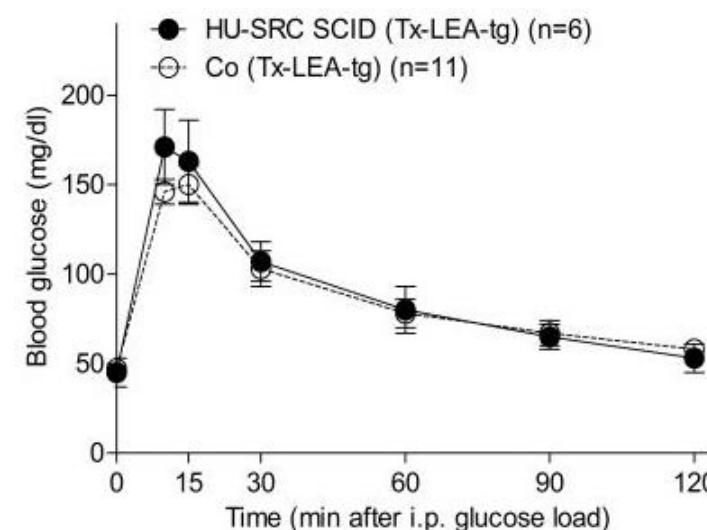
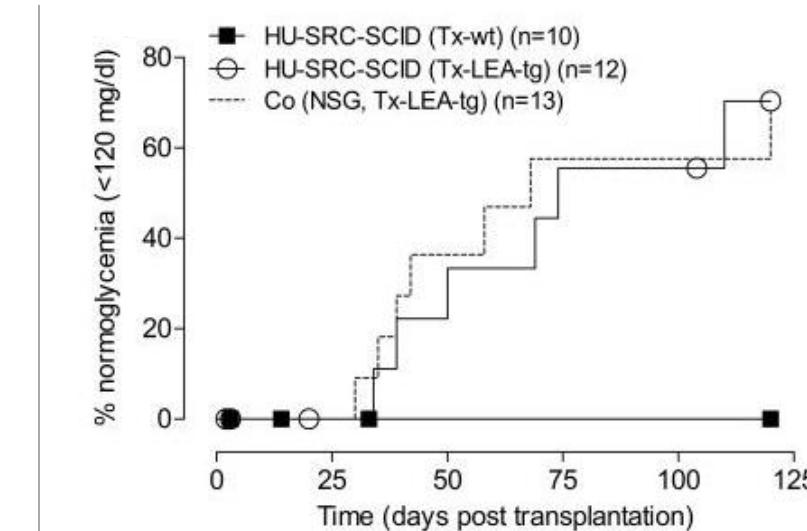
# Immunomodulatory approaches for porcine islet xenotransplantation



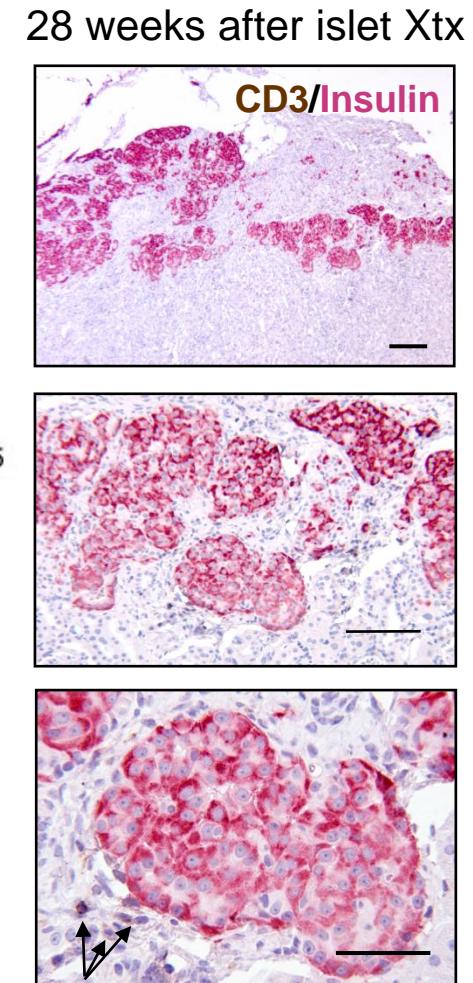
# Protection of porcine xeno-islets by expression of LEA29Y



Klymiuk et al., Diabetes 61, 1527-1532 (2012)



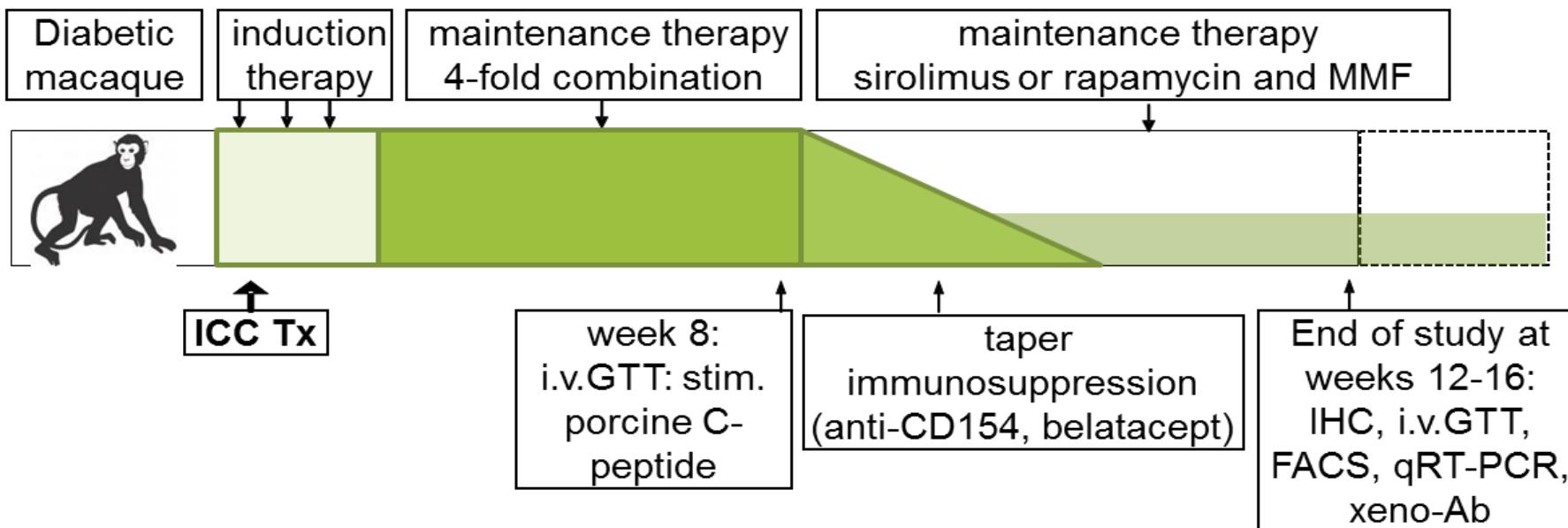
Wolf-van Bürck et al., Sci Rep 7, 3572 (2017)



# Testing of LEA29Y porcine islets in diabetic nonhuman primates

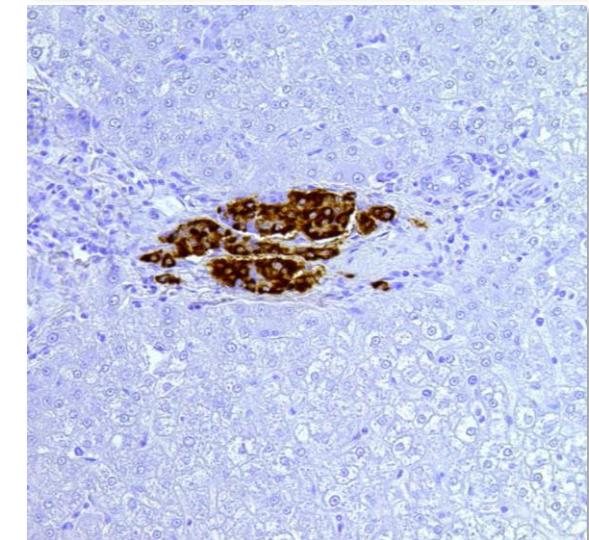
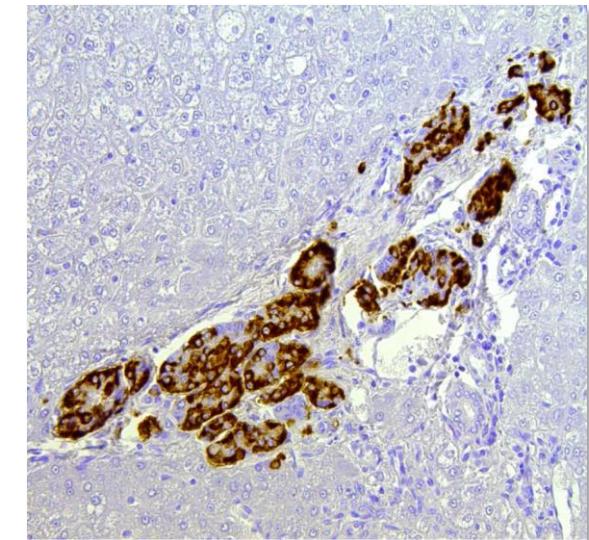
## Methodology:

XTx of LEA29Y-GTKO ICCs in diabetic rhesus macaques with low-dose systemic immunosuppression



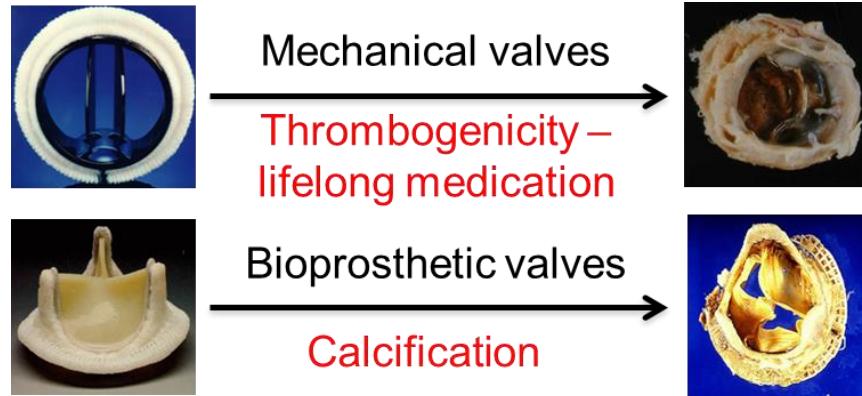
## Output:

Determination of the minimal systemic immunosuppression required for /NSLEA29Y-GTKO islet survival in NHP



# The need for alternative sources of heart valves and hearts

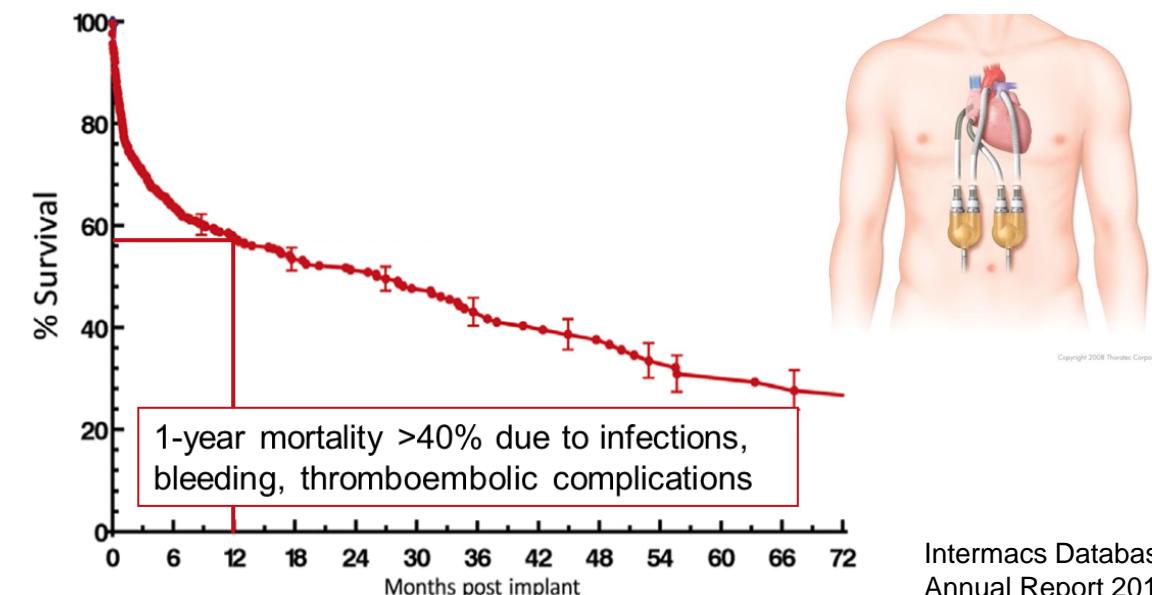
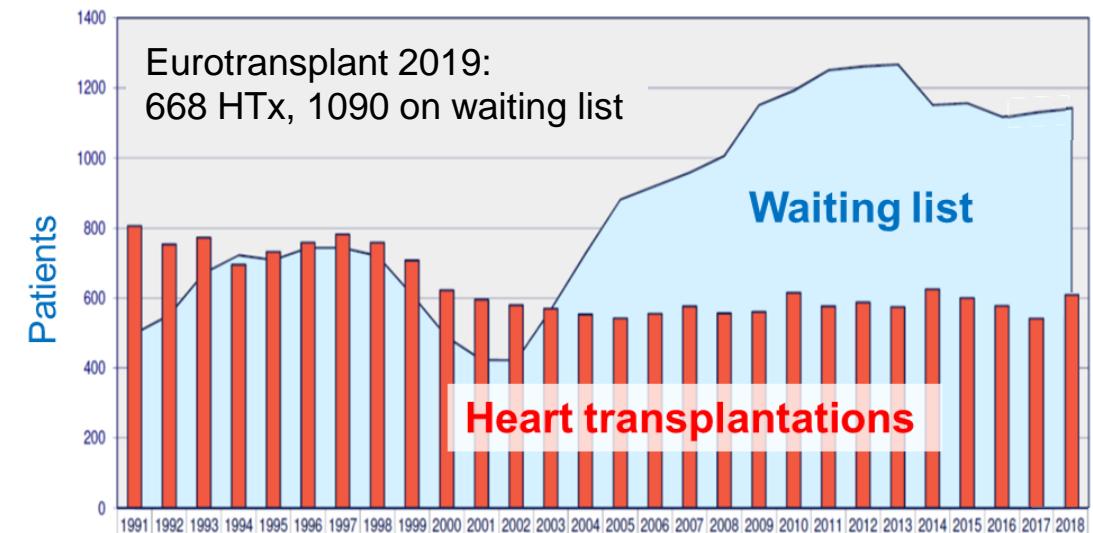
- 290'000 heart valve replacements worldwide (2003)
- 850'000 heart valve replacements worldwide (2050)
- 60% develop complications within 10 years post-op



Decellularized allogeneic valves  
Remodeling and growth potential



Problem: Limited availability in number and size



# Mechanisms of porcine xenograft rejection & strategies to overcome them

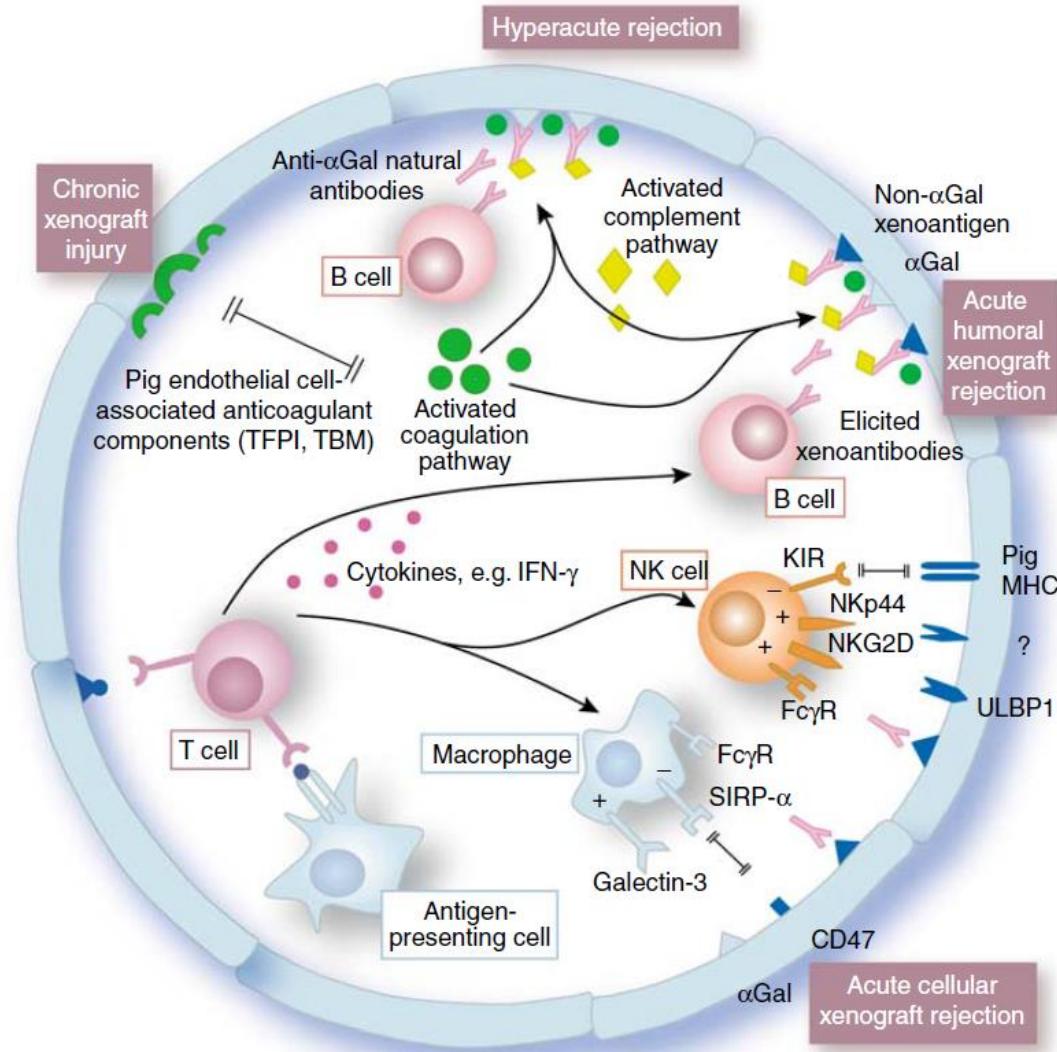
**Expression of hTBM, hEPCR, hCD39, hTFPI, hCD73**

**Expression of hHO-1, A20**

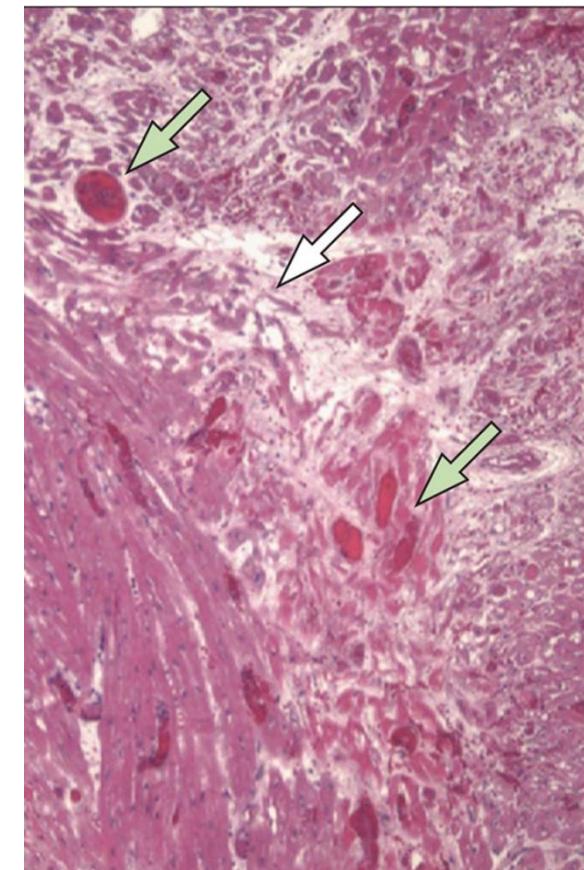
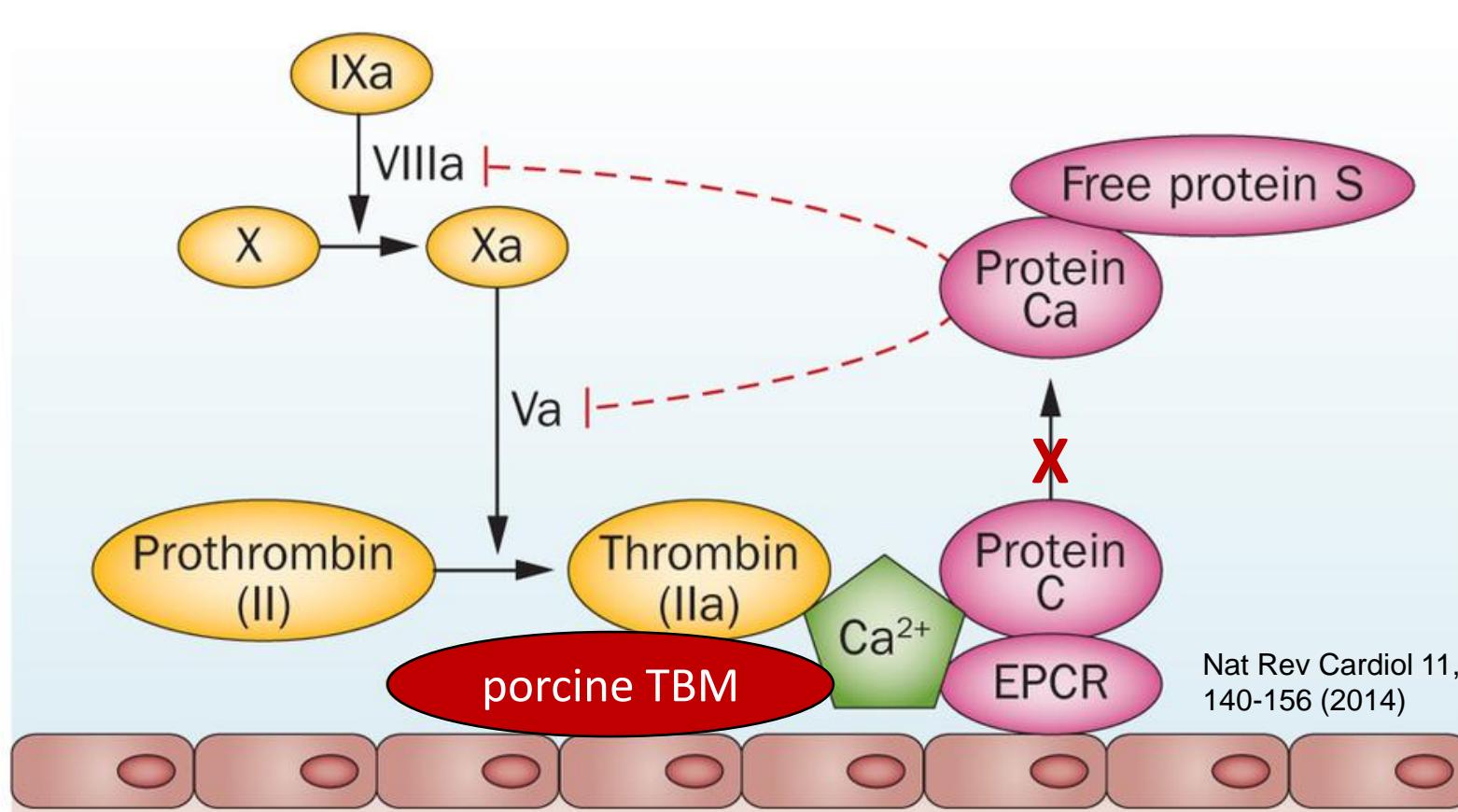
**Knockdown of TF**

**Expression of CTLA-4Ig, LEA29Y**

**Knockout of SLA class I**



# Xenogeneic coagulation disorder



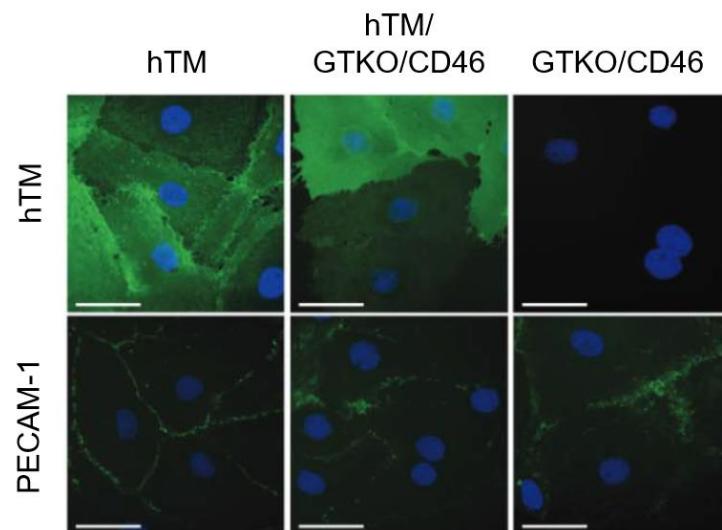
Porcine thrombomodulin binds human thrombin, but is a poor cofactor for the activation of human protein C

# Human thrombomodulin expression in transgenic pigs

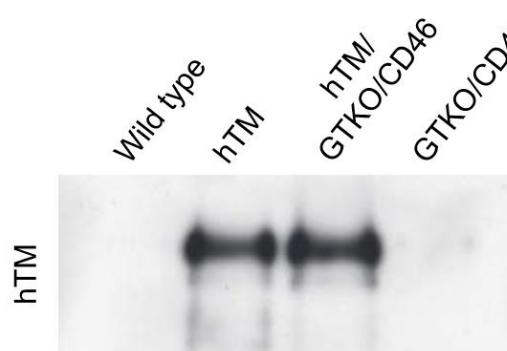
A



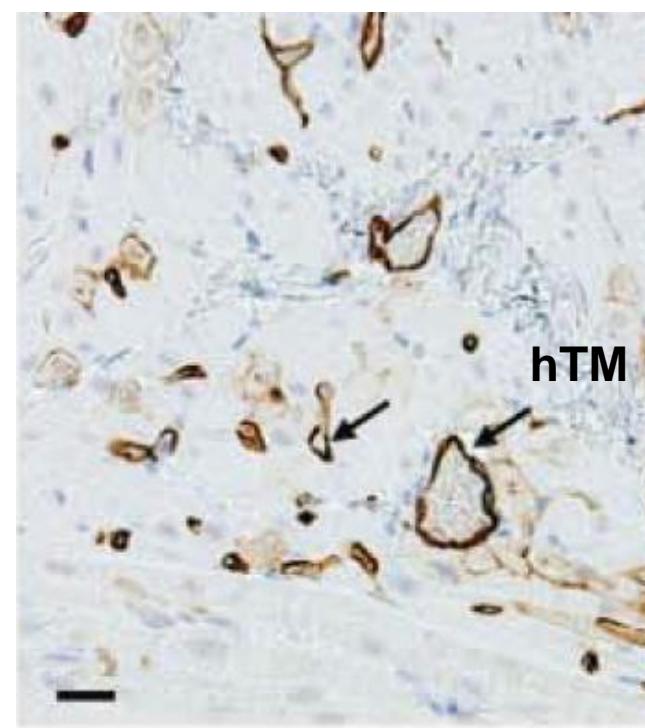
B



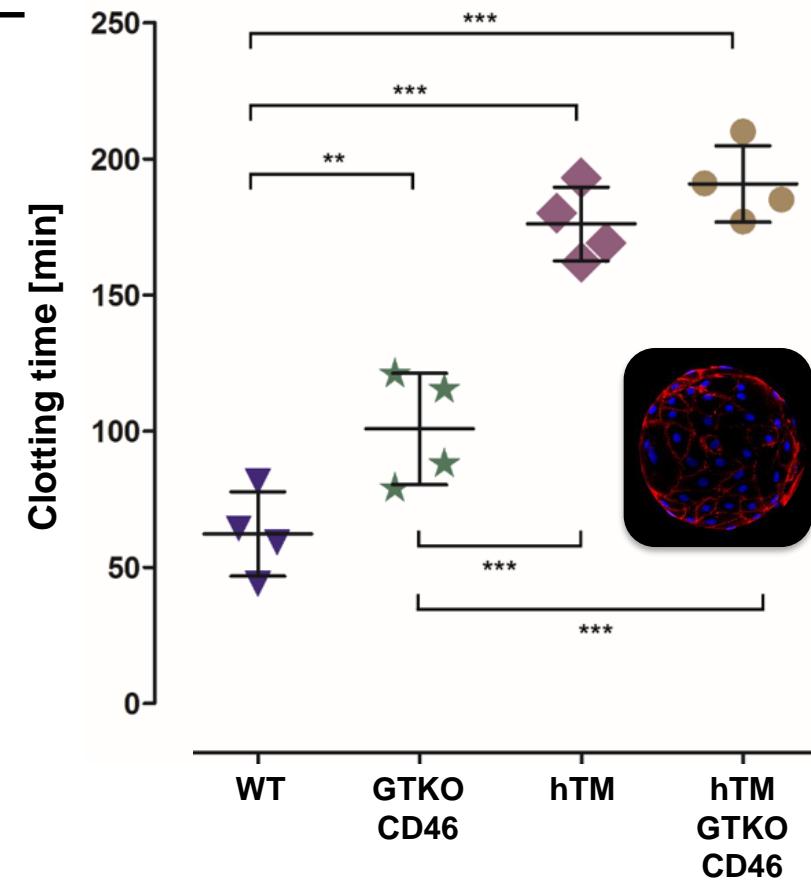
C



D

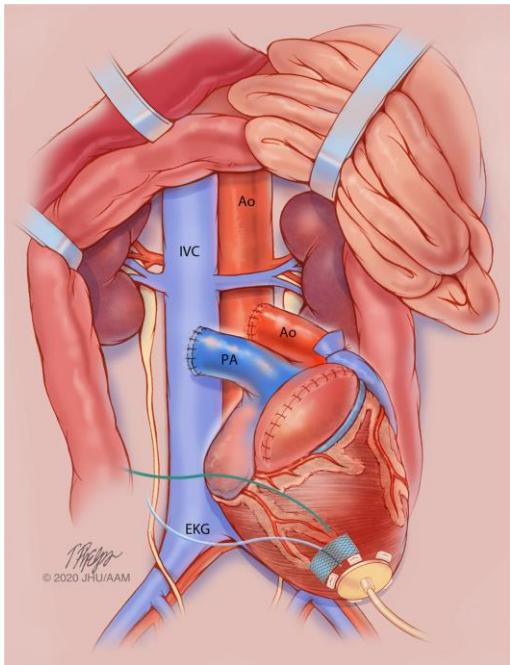


E

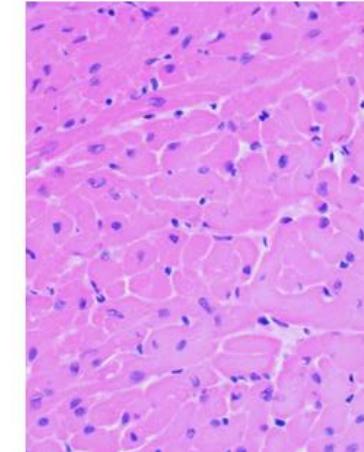
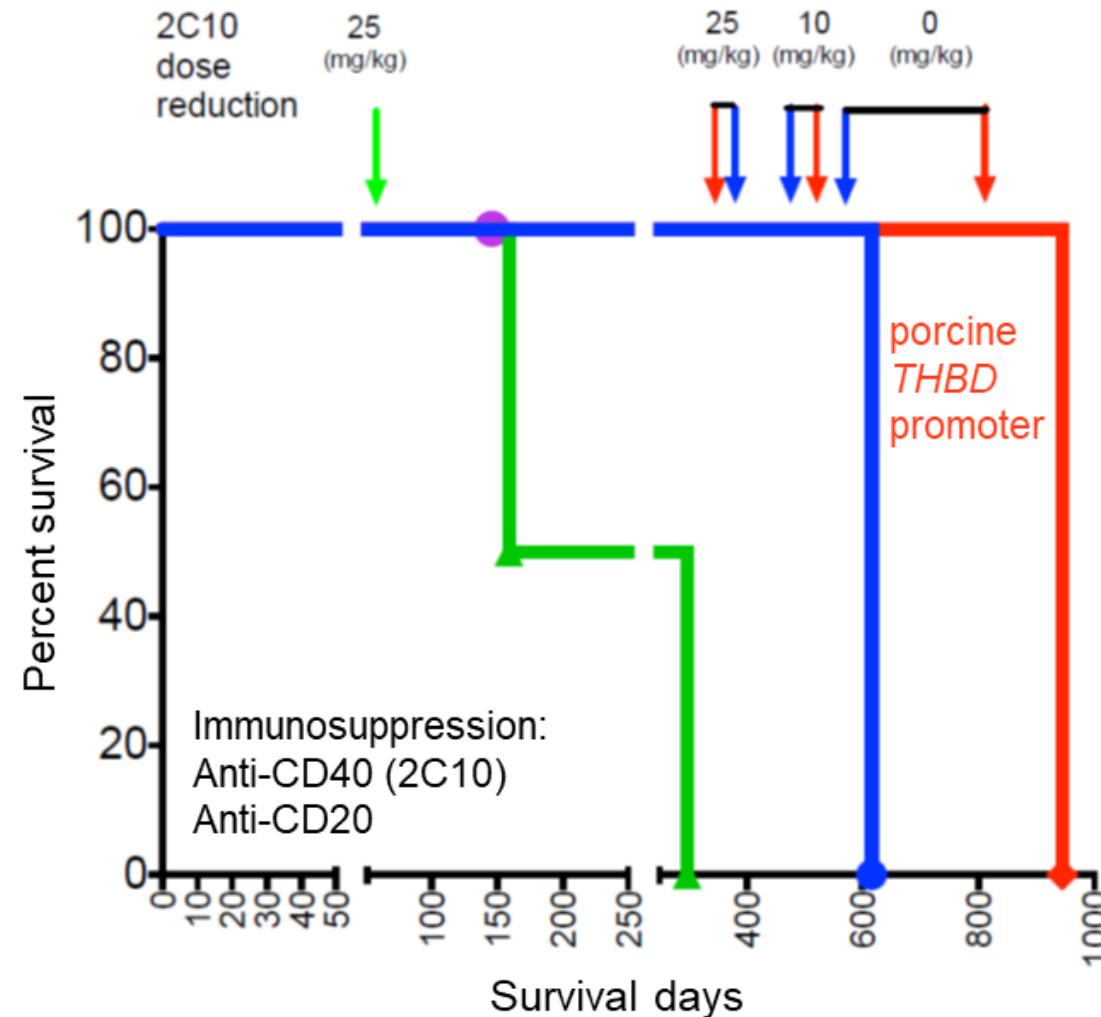


# Heterotopic abdominal cardiac xenotransplantation in baboons

<https://www.nature.com/articles/s41598-020-66430-x>



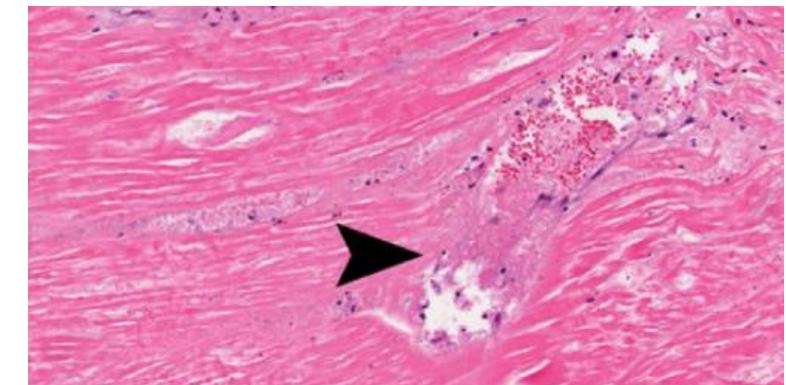
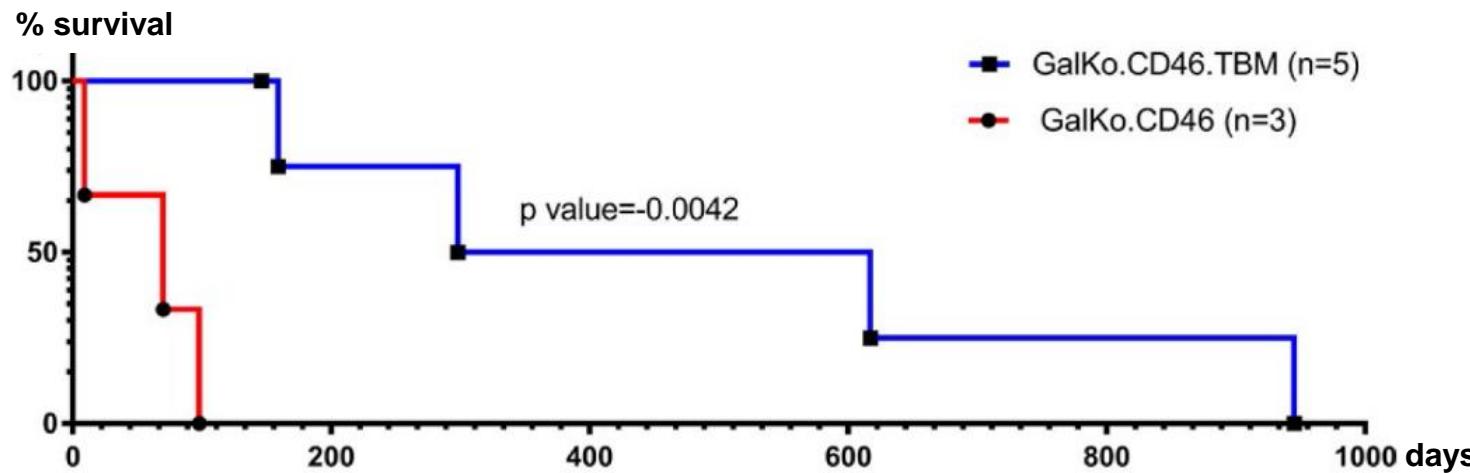
GGTA1 knockout  
hCD46 transgenic  
hTHBD transgenic



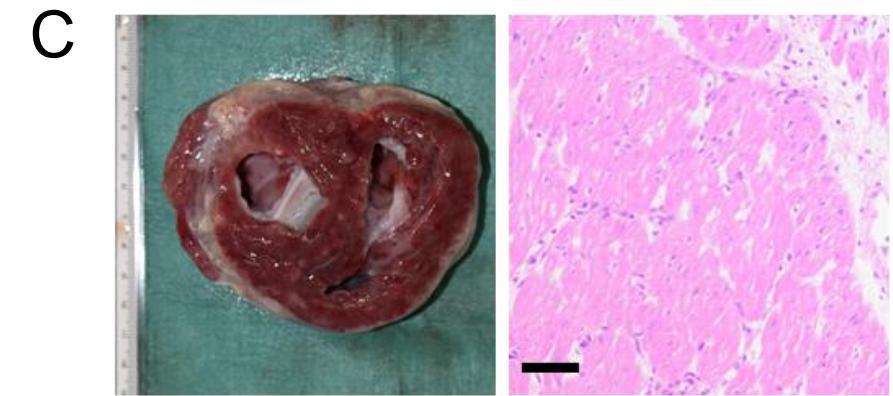
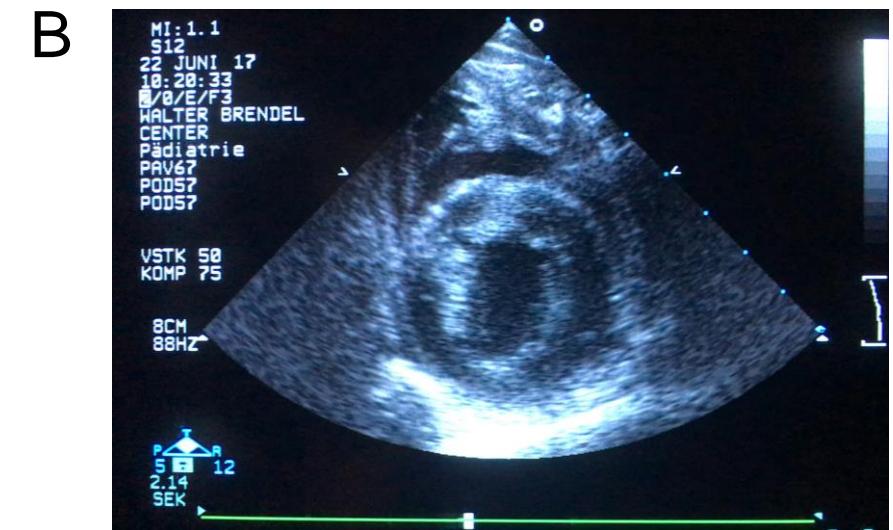
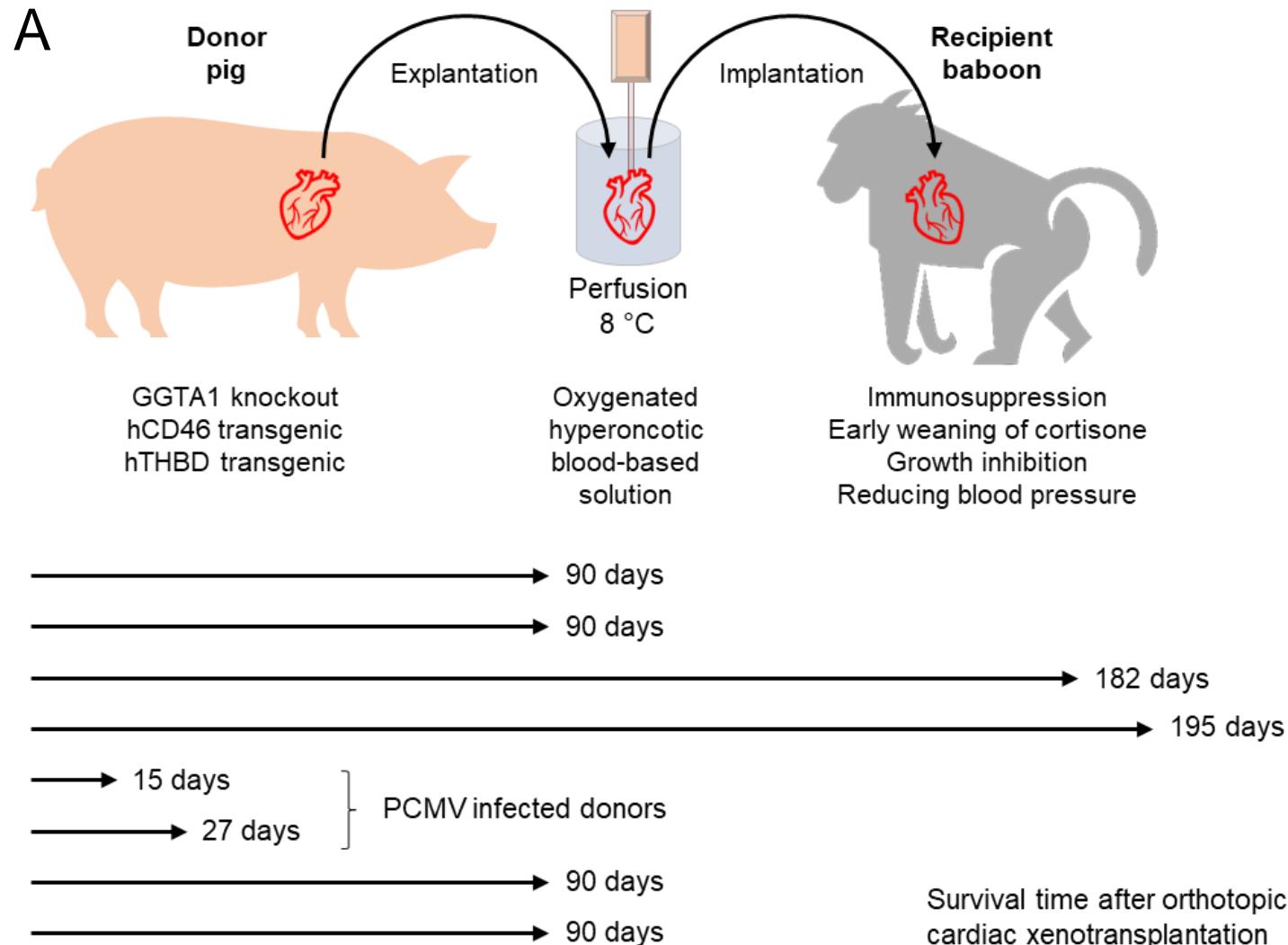
# Expression of human thrombomodulin was key to success

## Cardiac xenografts show reduced survival in the absence of transgenic human thrombomodulin expression in donor pigs

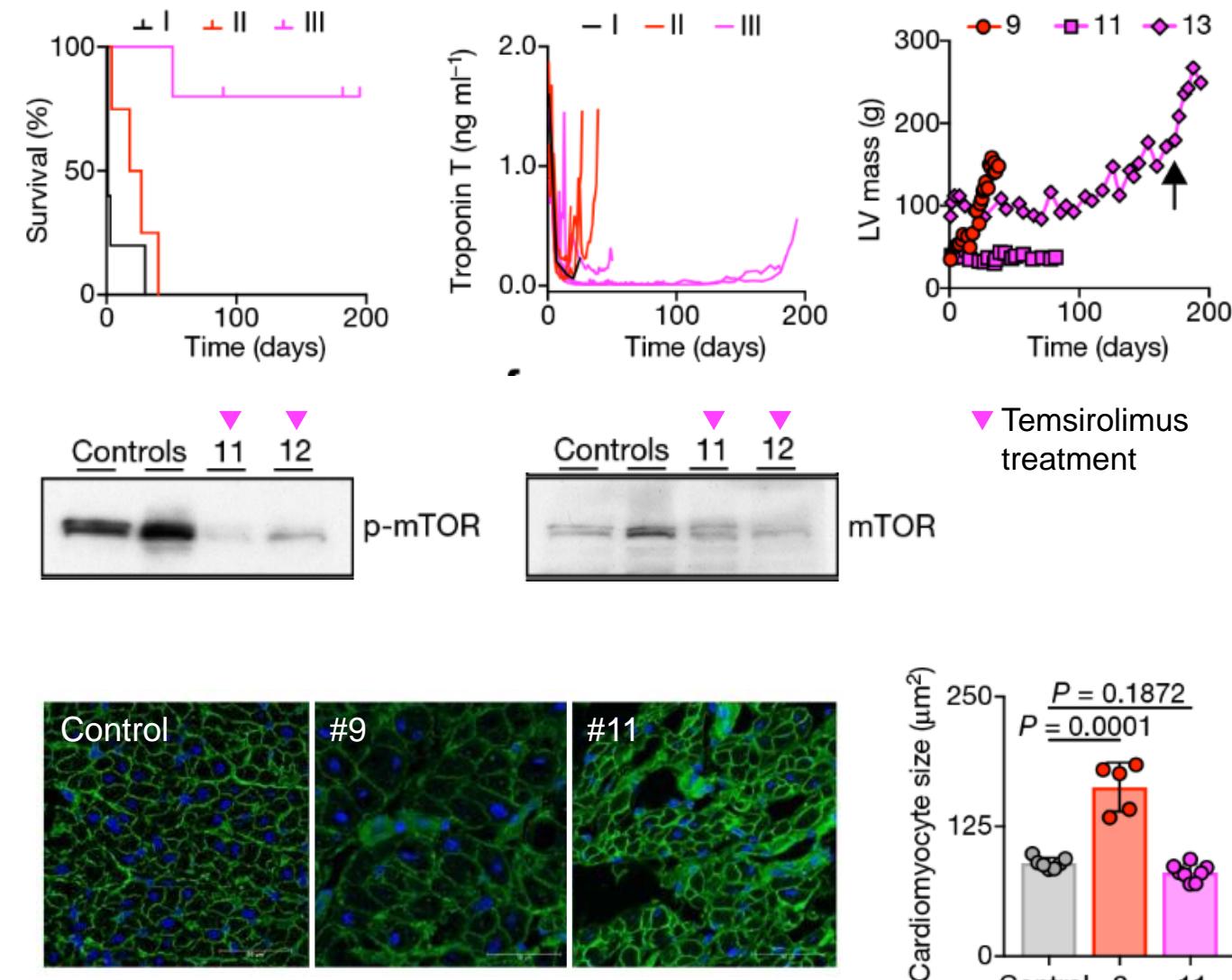
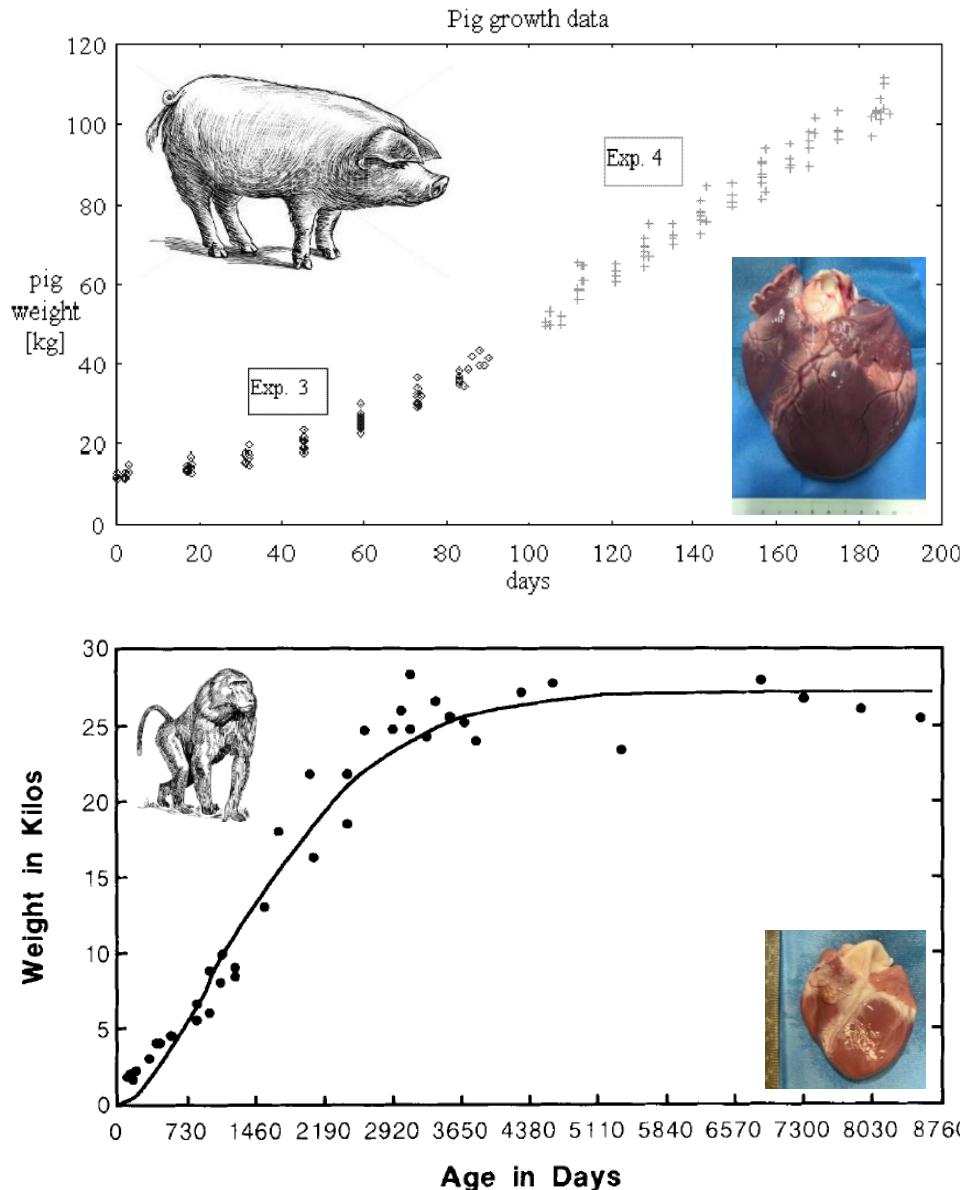
- Results from a preclinical pig-to-baboon heterotopic cardiac xenotransplantation model suggest that a three-pronged approach is successful in extending xenograft survival:
  - (a)  $\alpha$ -1,3-galactosyl transferase gene knockout pigs (GKTO) to prevent Gal-specific antibody-mediated rejection;
  - (b) transgenic expression of hCD46 and hTM to avoid complement activation and coagulation dysregulation; and
  - (c) effective induction and maintenance of immunomodulation (co-stimulation blockade of CD40-CD40L pathways with anti-CD40 (2C10R4) monoclonal antibody (mAb)).
- **Xenografts from pigs without hTM expression (GKTO.CD46) underwent rejection at an early time point (median 70 days)** despite utilization of our previously reported successful immunosuppression regimen ...



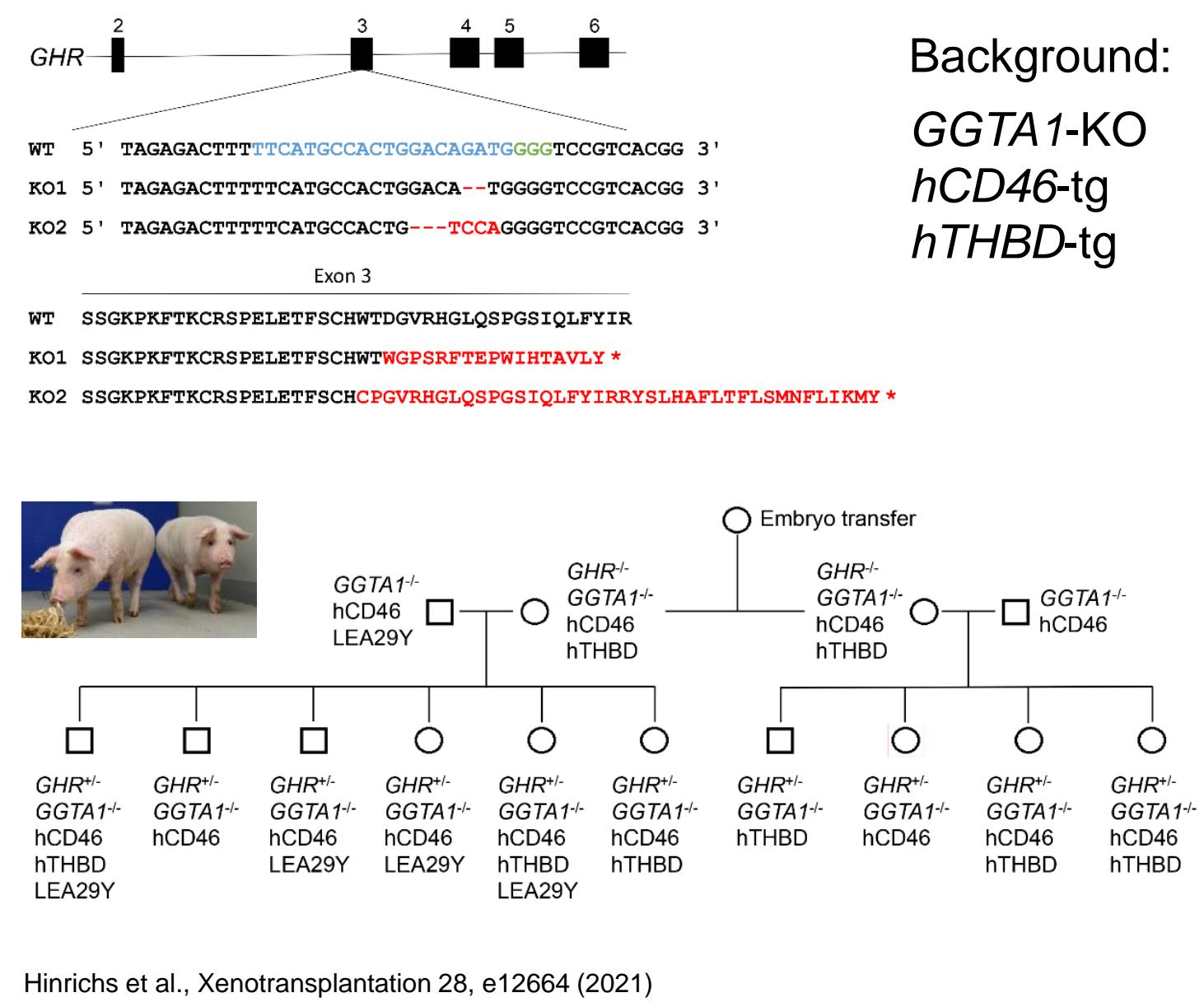
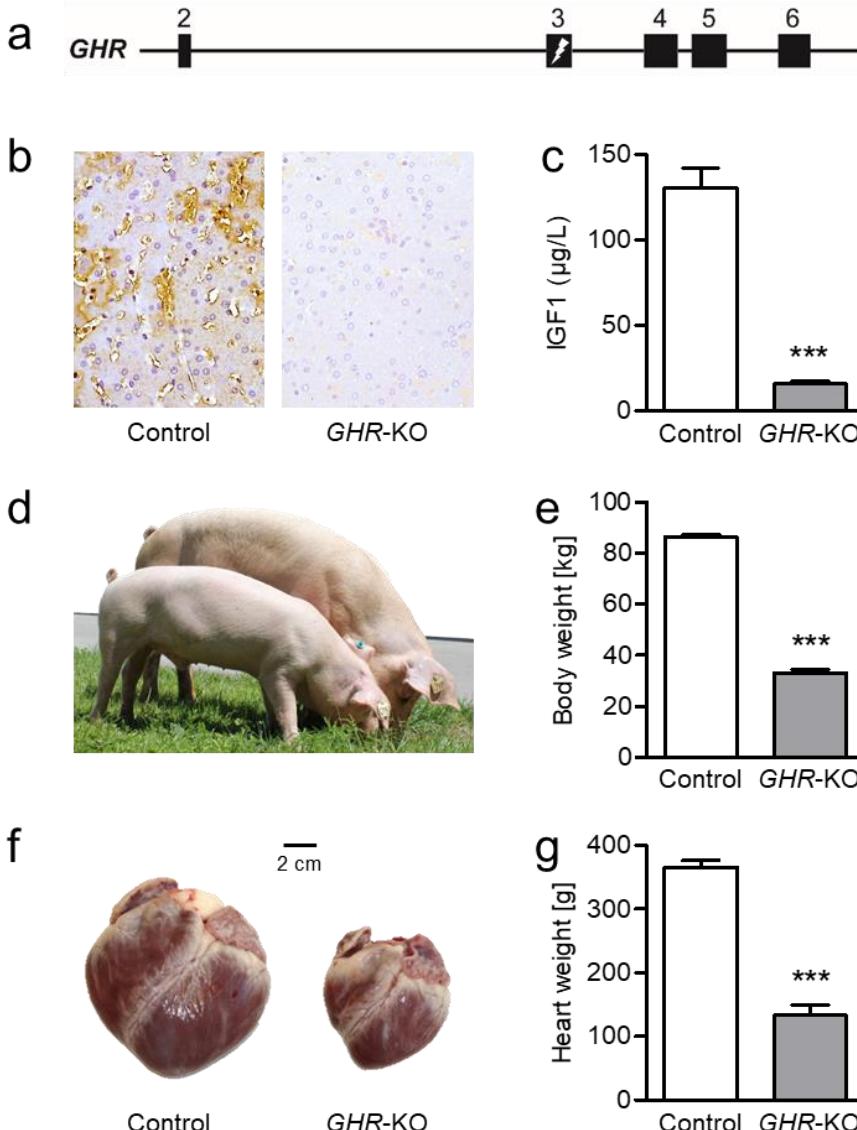
# Consistent success in life-supporting cardiac xenotransplantation



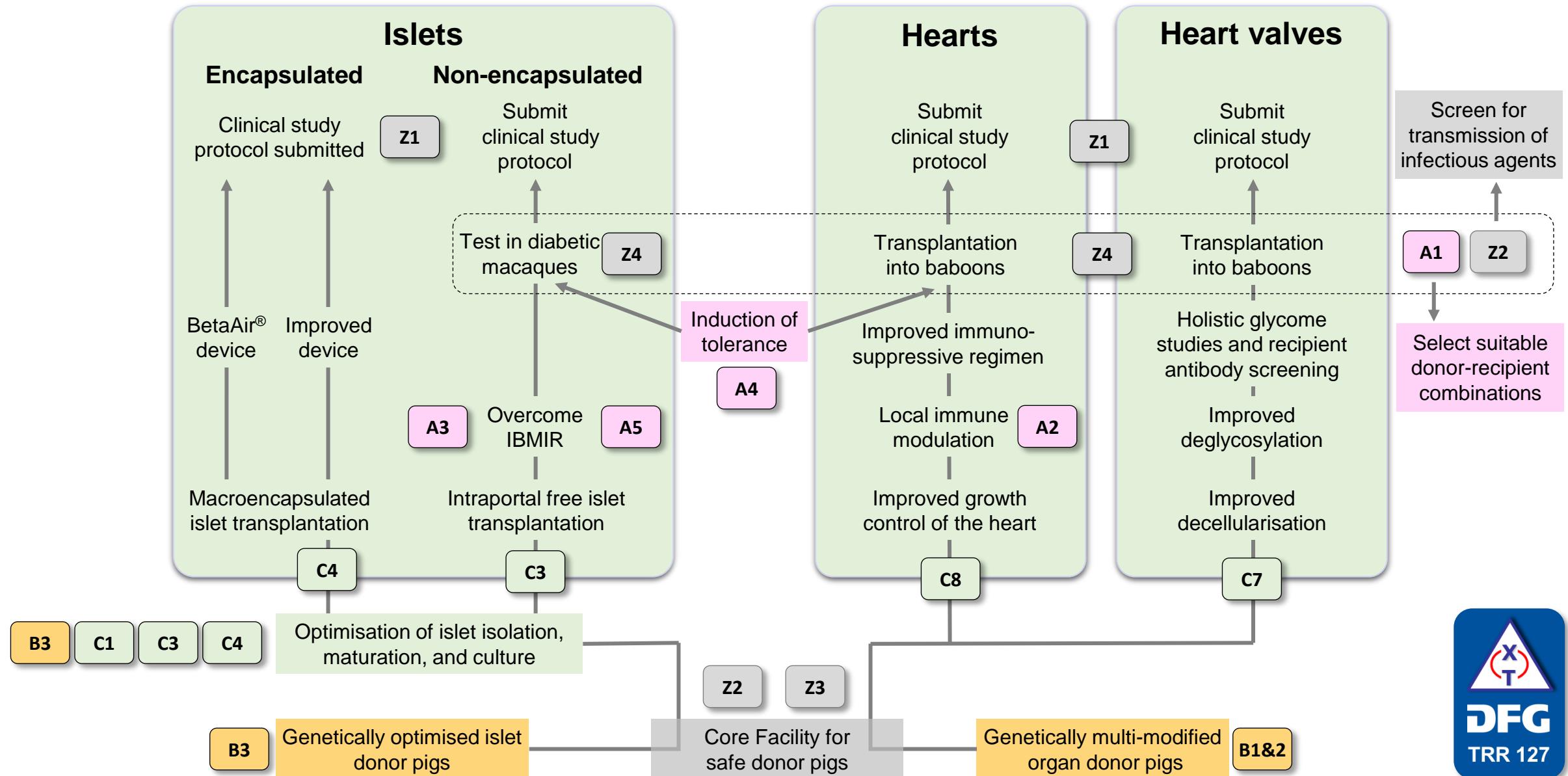
# Size matters in pig-to-baboon cardiac xenotransplantation trials



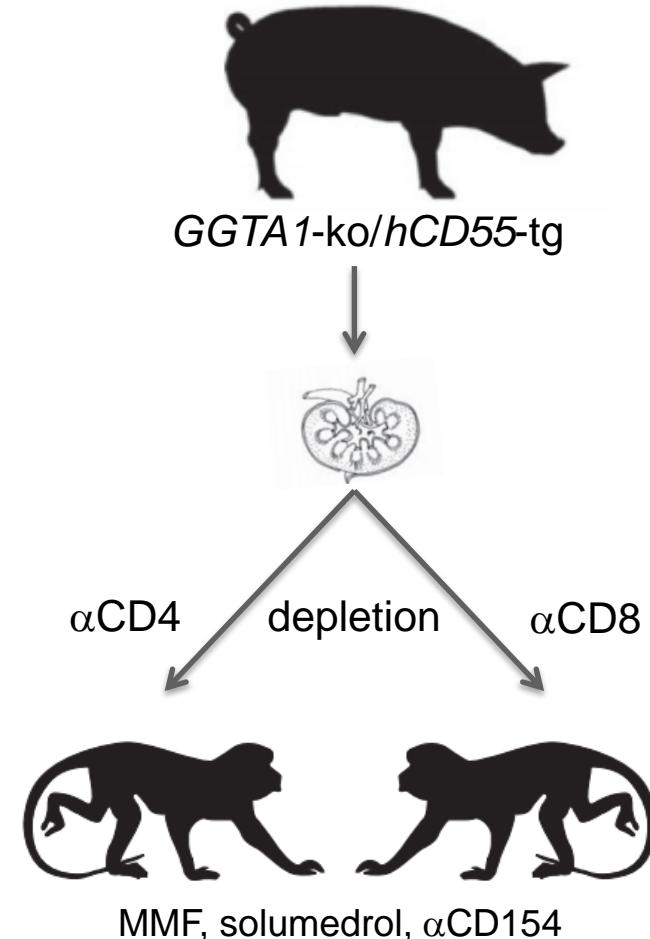
# Size reduction of donor pigs by KO of the *GHR* gene



# Strategy towards clinical xenotransplantation

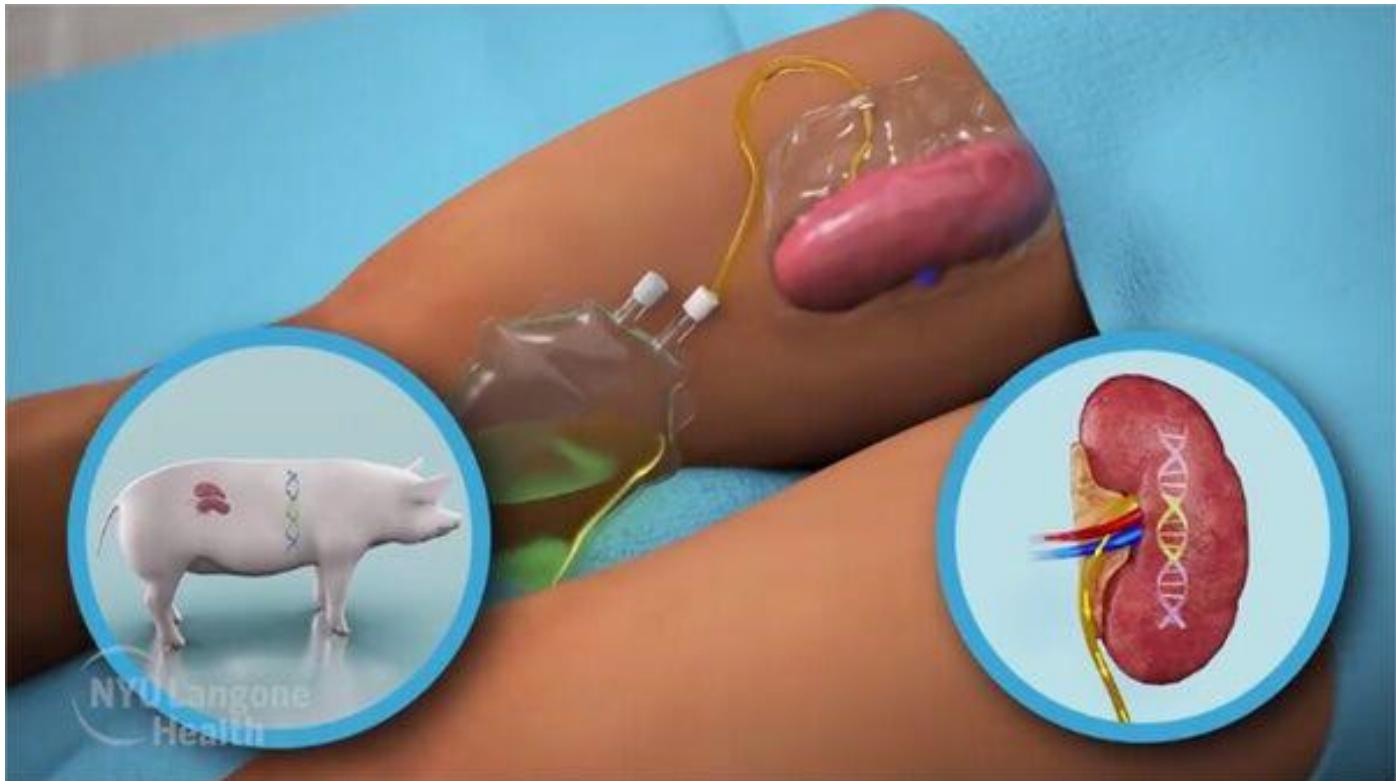


# Long-term survival of pig-to-rhesus macaque kidney xenografts after CD4<sup>+</sup> T cell depletion



Treatment group	Donor ID	Donor weight (kg)	Recipient weight (kg)	Recipient pretransplant IgG mean fluorescent intensity (MFI)	Cold ischemia time (min)	Survival (d)
High titer	D1	16.8	3.60	8983	195	6
αCD4 <sup>+</sup> αCD8 <sup>+</sup> anti-CD154	D1	16.8	3.65	2960	90	310
	D2	15.0	3.20	1041	93	160
	D3	25.5	4.42	1699	45	406
			3.70	1049	147	18
	D4	35.8	4.04	2273	51	115
			4.11	966	180	>400
αCD4 <sup>+</sup> anti-CD154	D5	25.0	3.92	1195	200	499
	D6	30.6	4.07	1340	235	414
	D7	37.0	5.87	1446	62	>70
αCD8 <sup>+</sup> anti-CD154	D5	25.0	5.18	1969	40	15
	D6	30.6	4.41	2159	150	6
	D7	37.0	8.58	979	132	6

# Pig Kidney Successfully Transplanted From Hog to Human

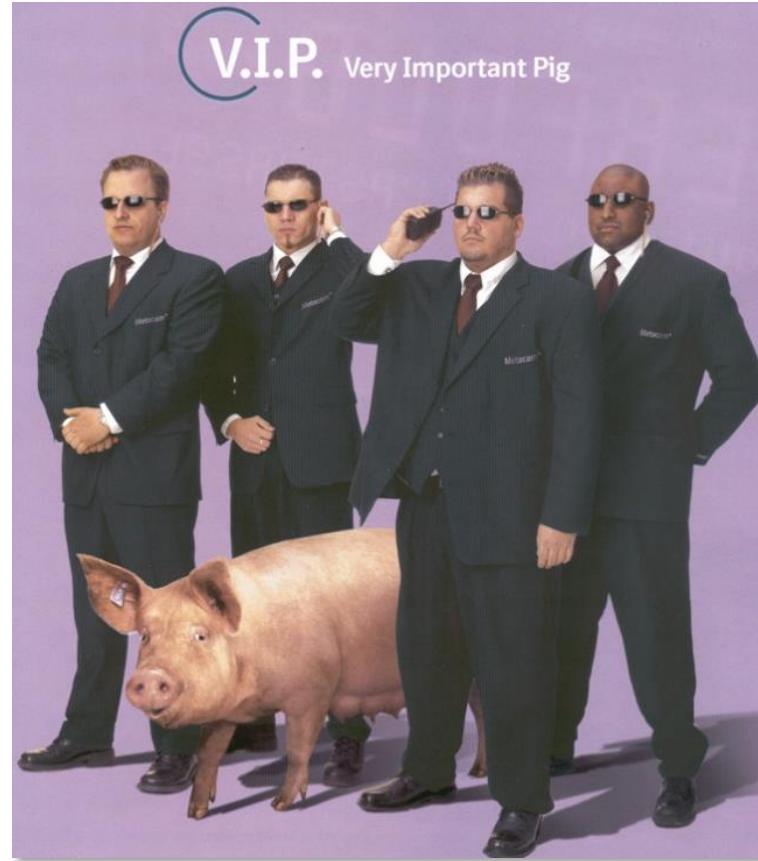


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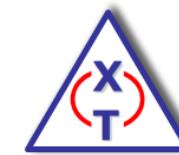
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