



WCGALP: Biology – Disease Resistance 2 Session

The Effects of PRRS Vaccination and WUR Genotype on Blood Gene Expression Response to Co-infection with PRRSV and PCV2 in Pigs

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Porcine Reproductive and Respiratory Syndrome (PRRS) remains a major problem to the global swine industry

- \$664 million losses/yr in the U.S. alone (Holtkamp et al. 2013)
- PRRS virus (PRRSV): RNA virus → High mutation rate
- Heterogeneity → Emergence of more virulent strains



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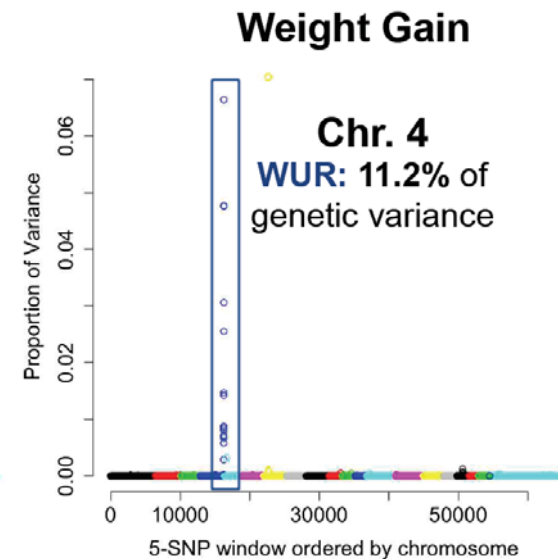
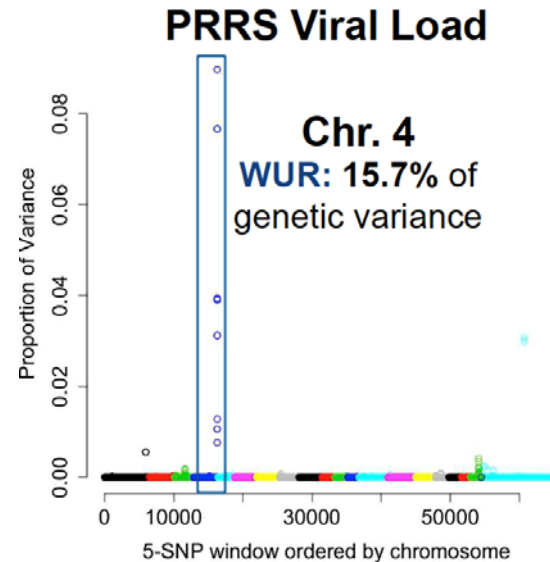
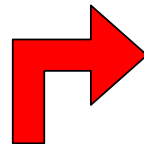
Possible solutions

❖ PRRS vaccine (Vac)

- only partially effective

❖ Genetic improvement

- **SNP marker WUR10000125 (WUR)**
B allele is favorable and dominant to A allele.



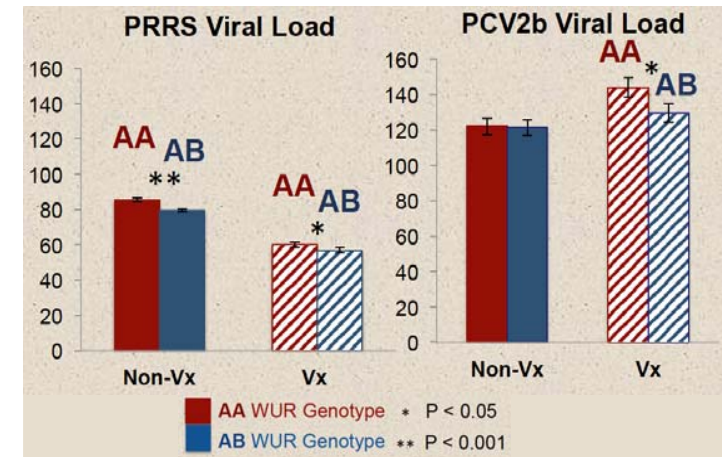
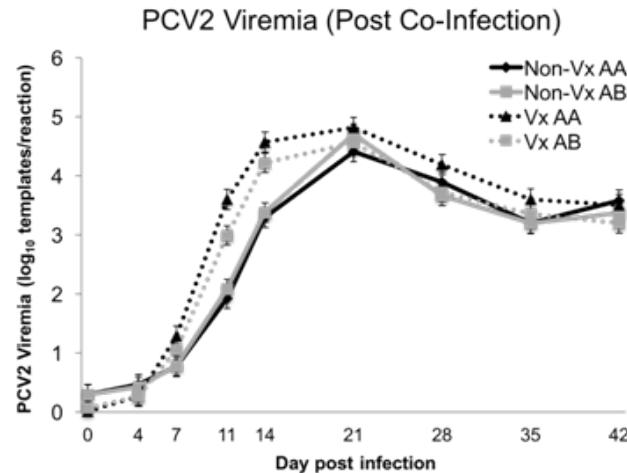
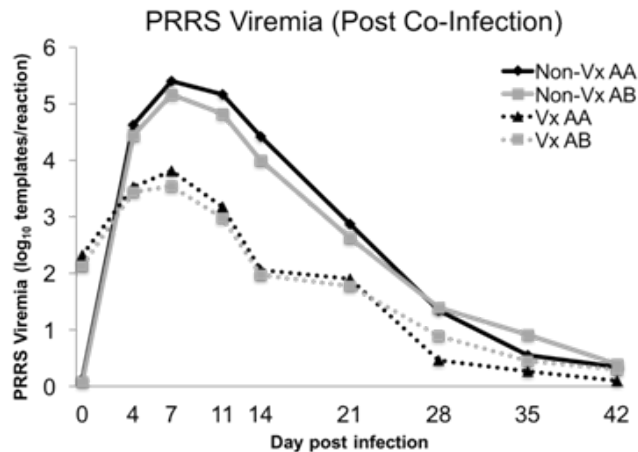
Co-infection with PRRSV and porcine circovirus type 2 (PCV2) is commonly observed in field cases

- Both PRRSV and PCV2 can **suppress** the host immune defense system.
- PRRSV can **enhance** replication of PCV2 (Allan et al., 2000).
- PCV2 can **reduce** the efficacy of PRRS modified live virus (MLV) vaccine (Opriessnig et al. 2006).

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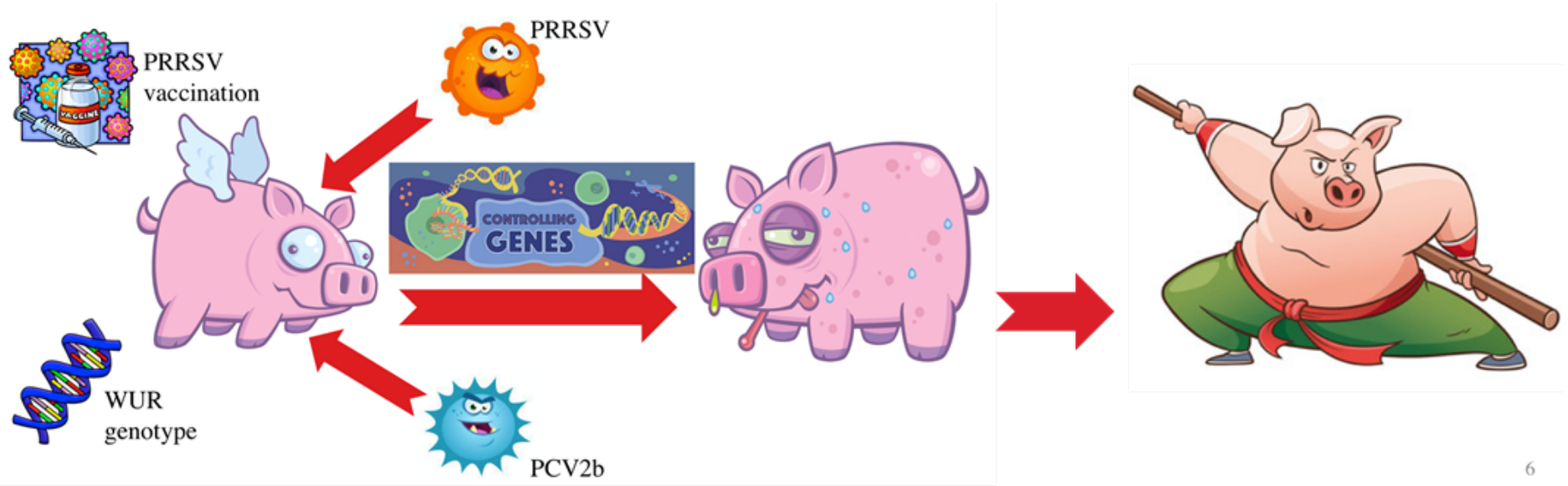
Effect of PRRS Vac and WUR on Viremia



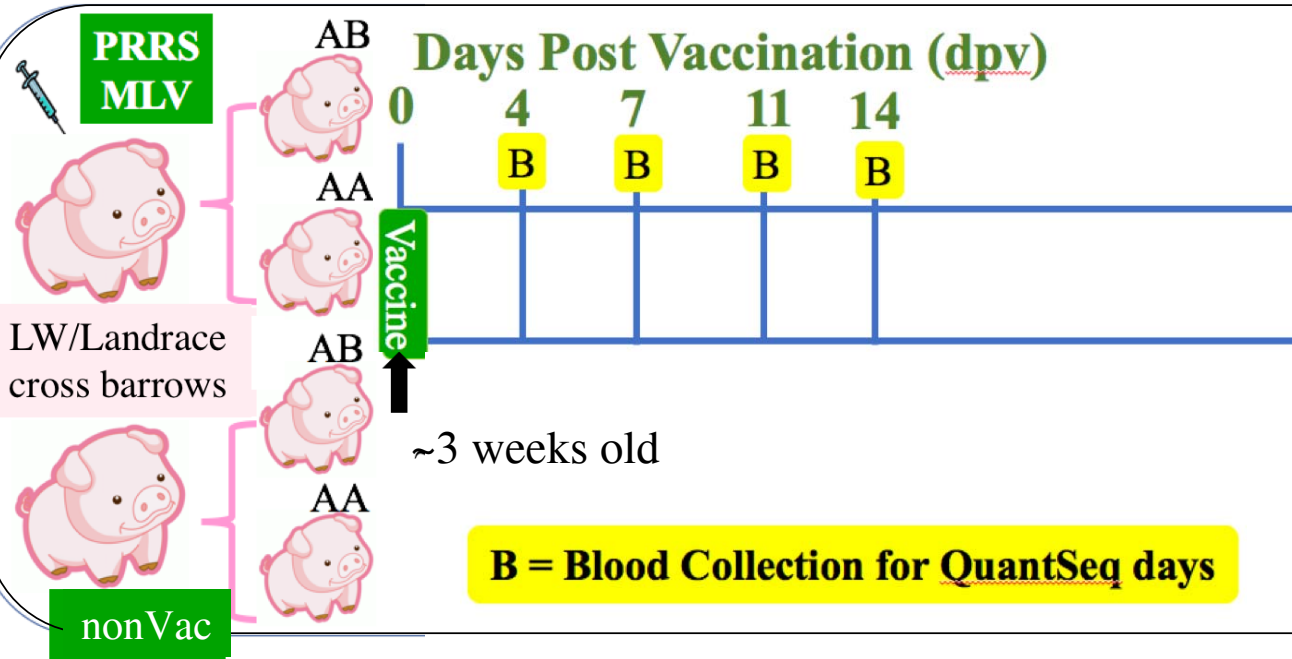
((Dunkelberger et al. 2017)⁵

Objectives

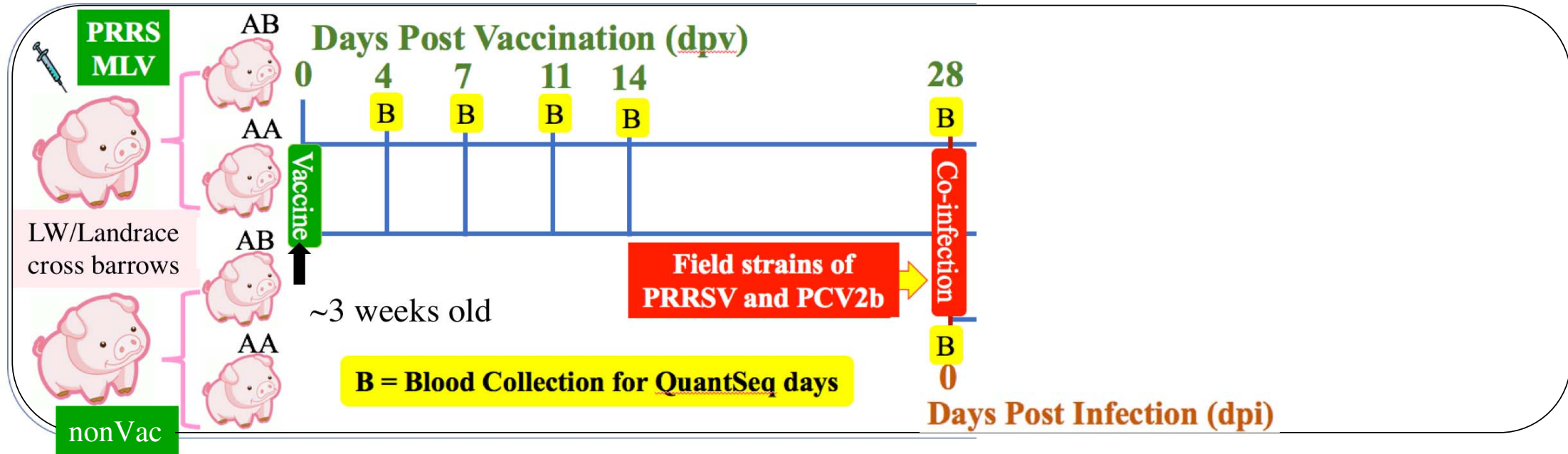
- ❖ To evaluate the effect of PRRS vaccination and WUR genotype on pig **blood transcriptome response** following the co-infection with PRRSV and PCV2.
- ❖ To identify mechanisms involved.



Blood Transcriptome Experimental design

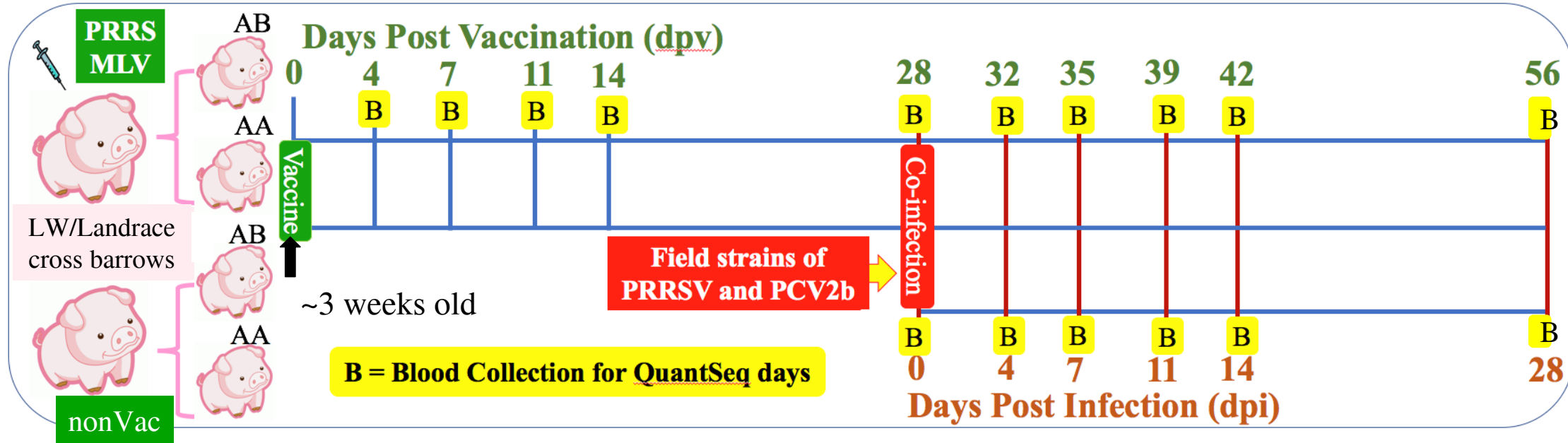


Blood Transcriptome Experimental design



- PRRSV infection (KS62) and PRRS MLV strains are heterologous.

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Methods and Materials for Blood Transcriptome

- Animal:**

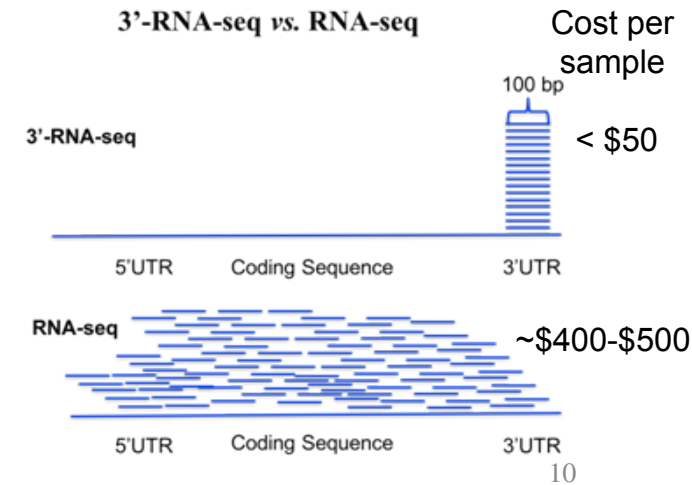
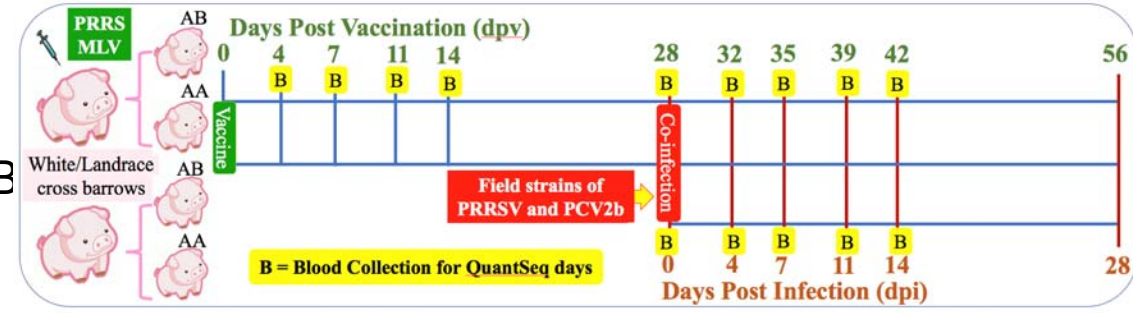
- 7 pigs for each treatment group:
 - Vac-AA, Vac-AB, nonVac-AA, nonVac-AB

- Samples:**

- 191 Blood samples
- at 4, 7, 11, 14 day post vaccination(dpv, Vac pigs only) and 0, 4, 7, 11, 14, 28 day post infection(dpi)

- Genome-wide analysis of gene expression:**

- QuantSeq (3'RNA-seq)



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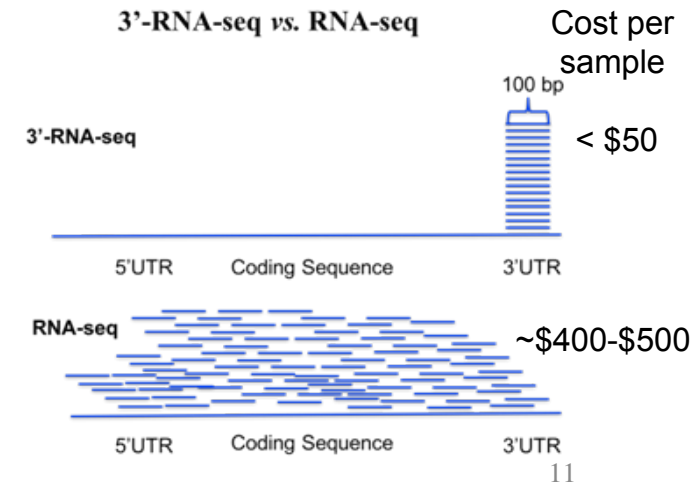
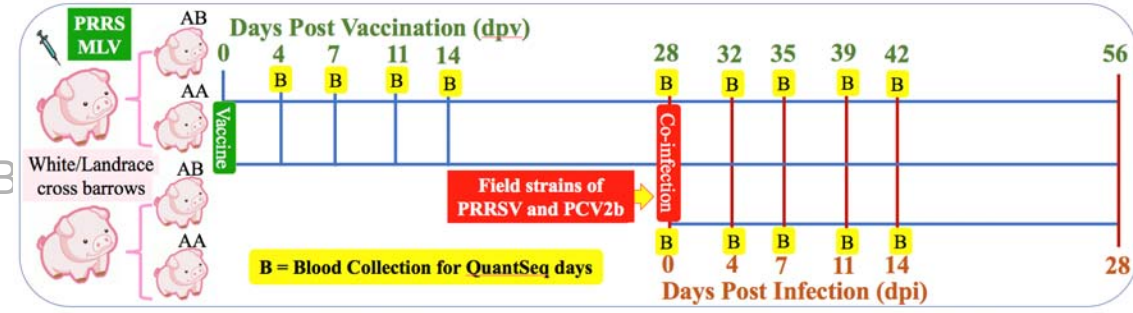
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- Bluebee (pig 10.2 genome) → gene expression counts



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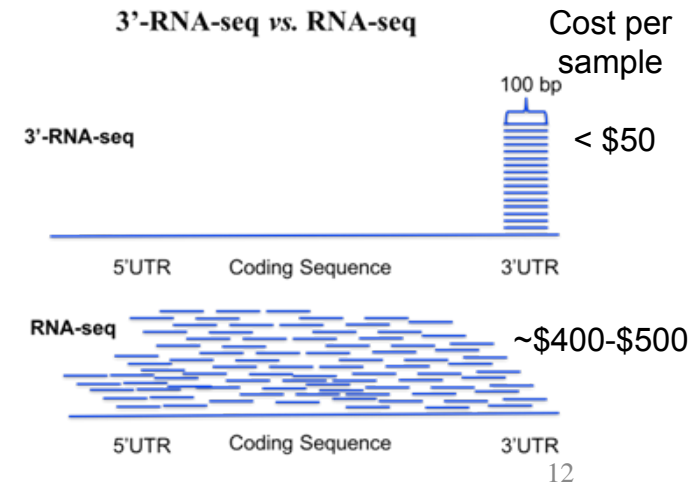
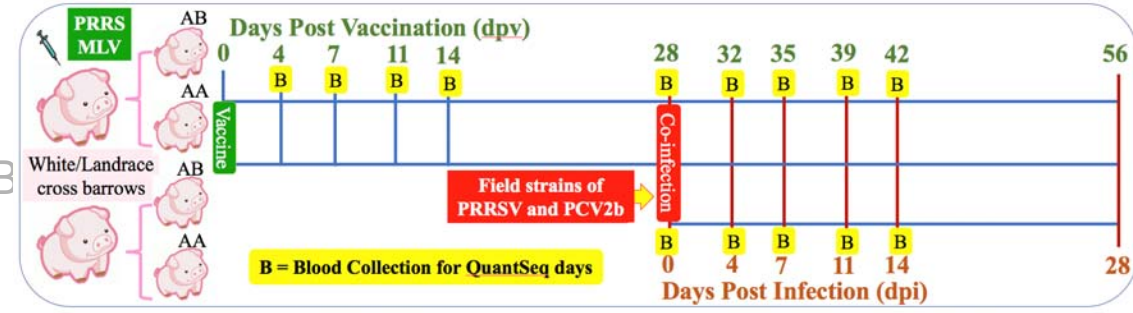
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- Filtering globin and genes with low read counts → 5445 genes



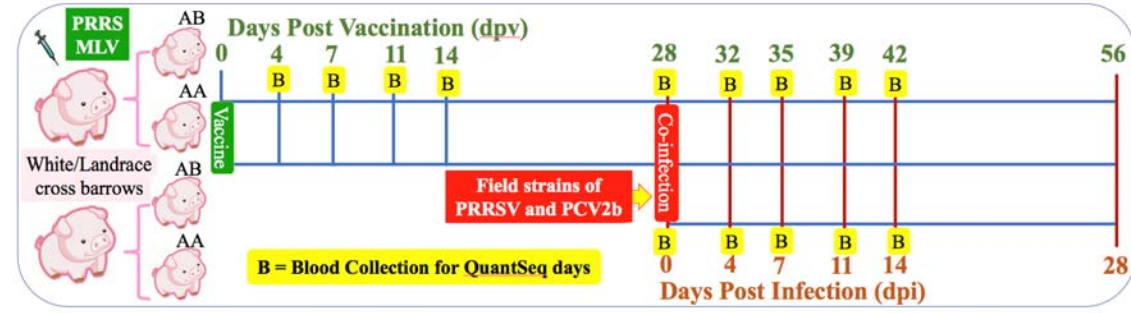
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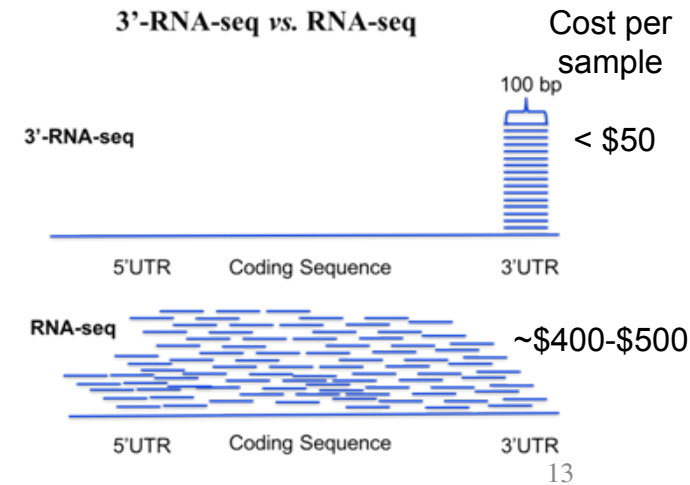
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 - Gene expr. = WUR+Vac+WUR*Vac+RIN+Lane



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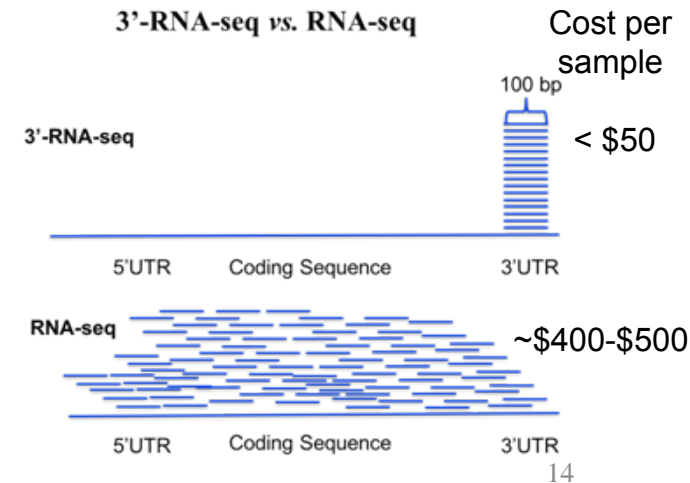
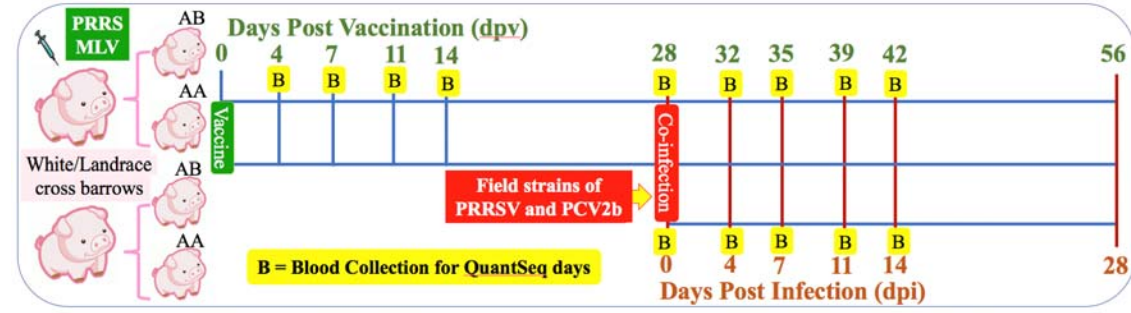
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- Ingenuity pathway analysis (IPA) → functional analyses

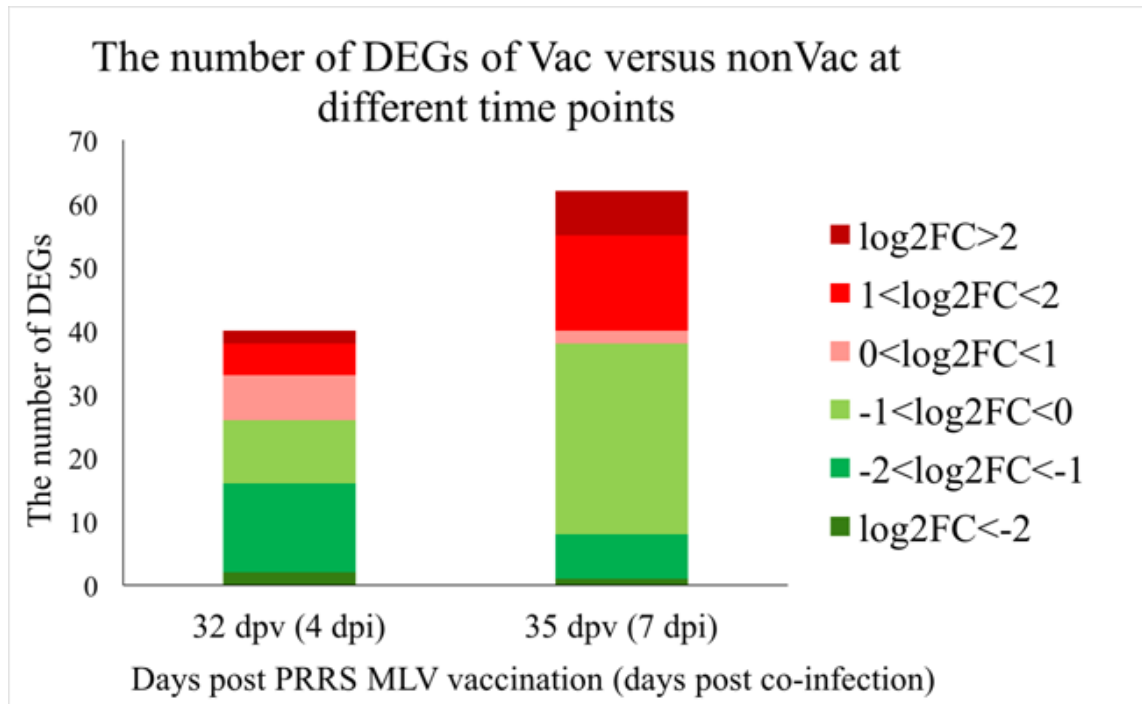


Results and Discussion: number of DEGs ($q < 0.2$)

❖ No DEGs for WUR or for WUR \times VxStatus.

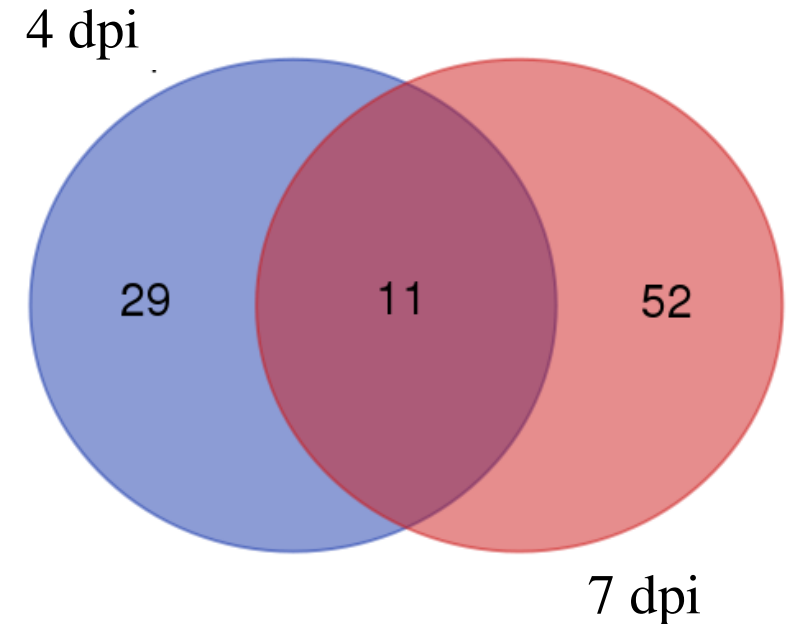
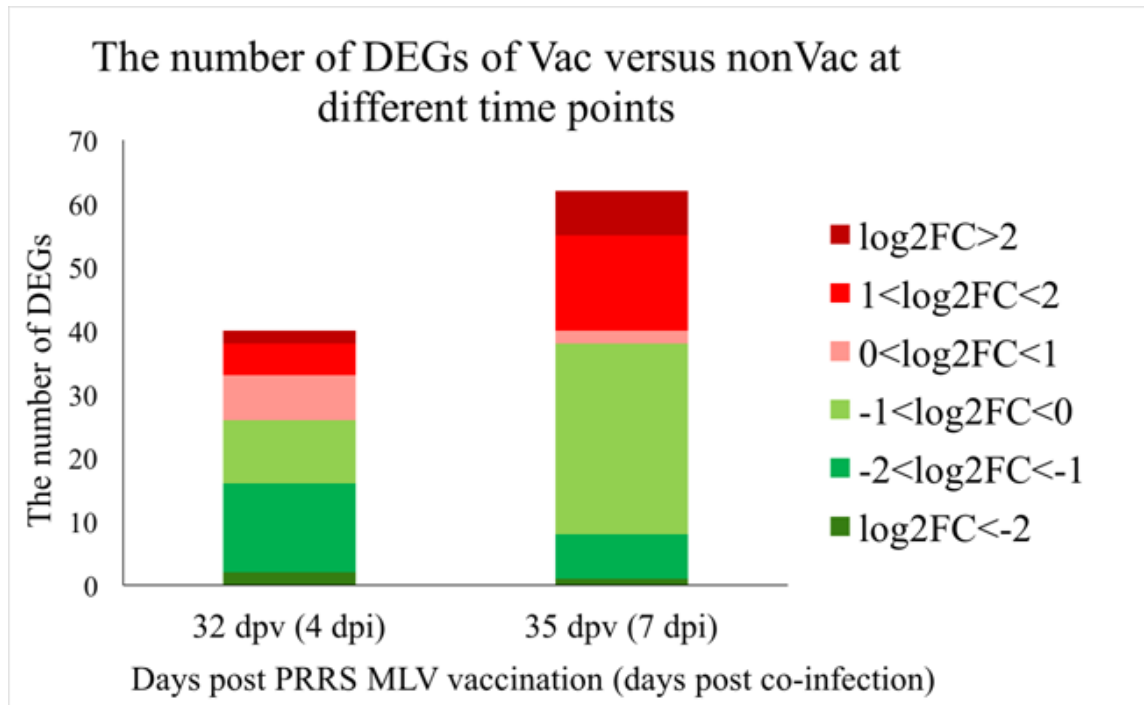
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- ❖ No DEGs for WUR or for WUR \times VxStatus.
- ❖ For **VxStatus**, DEGs identified at **4 dpi** (n=40) and **7 dpi** (n=63).



All 11 overlapping DEGs were less expressed in vaccinated pigs and most related to viral immune response , e.g. MX1, MX2, CXCL10, ISG12(A), CD169, CD64.

IPA results: Transcriptomic Response in Vac/nonVac

4 dpi

Endocytosis by cells

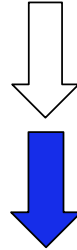


IPA results: Transcriptomic Response in **Vac/nonVac**

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Endocytosis by cells

Phagocytosis of phagocytes



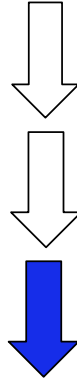
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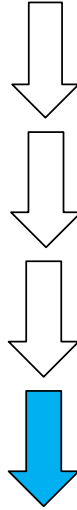
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Endocytosis by cells

Phagocytosis of phagocytes

Immune response of cells

Apoptosis



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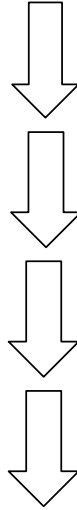
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7 dpi

Replication of PRRSV



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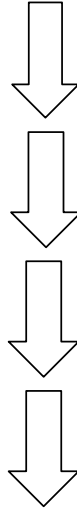
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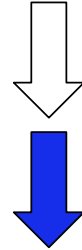
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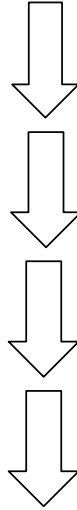
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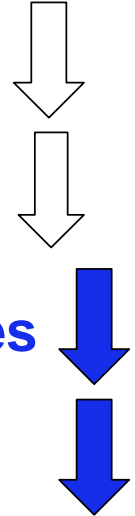
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Replication of PRRSV

Inflammatory response

Chemotaxis of phagocytes

Leukocyte migration



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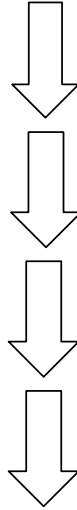
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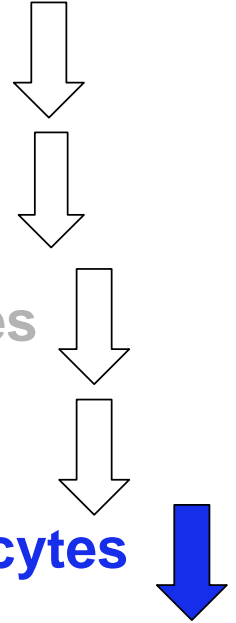
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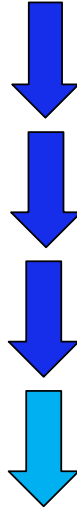
Immune response of phagocytes



IPA results: Transcriptomic Response in Vac/nonVac

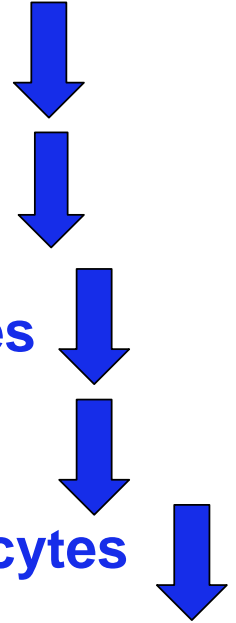
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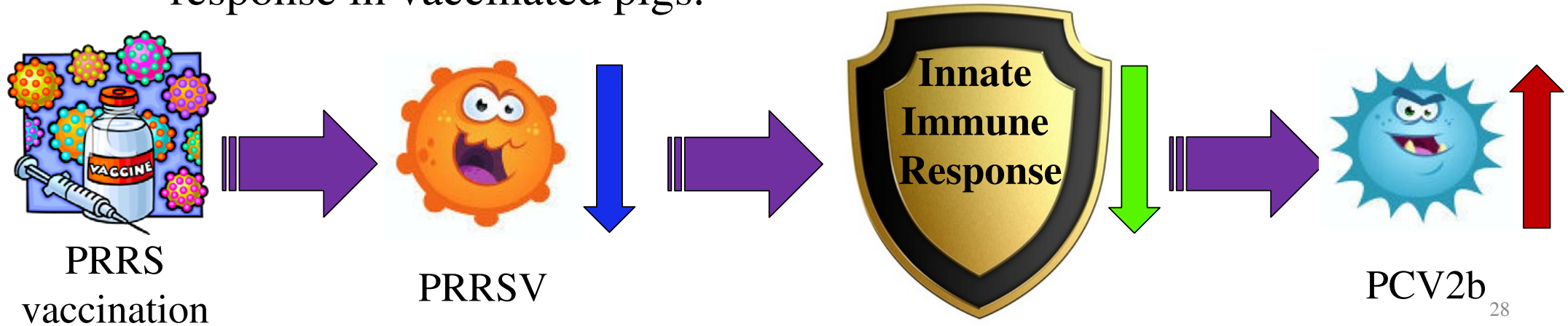


Conclusions

- ❖ The effects of WUR and interactions between WUR and vaccination status were not significant.
 - GBP5 causative gene (Koltes et al., 2015) is not in pig genome build 10.2

Conclusions

- ❖ The effects of WUR and interactions between WUR and vaccination status were not significant.
- ❖ The effects of PRRS vaccination were significant at 4 dpi (32 dpv) and 7 dpi (35 dpv), which may represent lower innate immune response in vaccinated pigs.



Acknowledgements

USDA-NIFA grant # 2013-68004-20362



Jack Dekkers
(My supervisor)



Joan Lunney



Christopher Tuggle



James Reecy



Bob Rowland



Yet Nguyen



Hyeonseon Jeon

Future work

- Pig genome build 10.2 → 11.1 (GBP5)
- +Blood transcriptome assembly data (better 3' end)
- +Annotation of Iso-Seq data (more isoforms)

Future work

- Pig genome 10.2 → 11.1 version
- +Blood transcriptome assembly data (better 3' end)
- +Annotation of Iso-Seq data (more isoforms)
- Combine all time points QuantSeq data
- +2nd run QuantSeq
- +QuantSeq with globin block

DEG (q<0.1) at 4 dpi

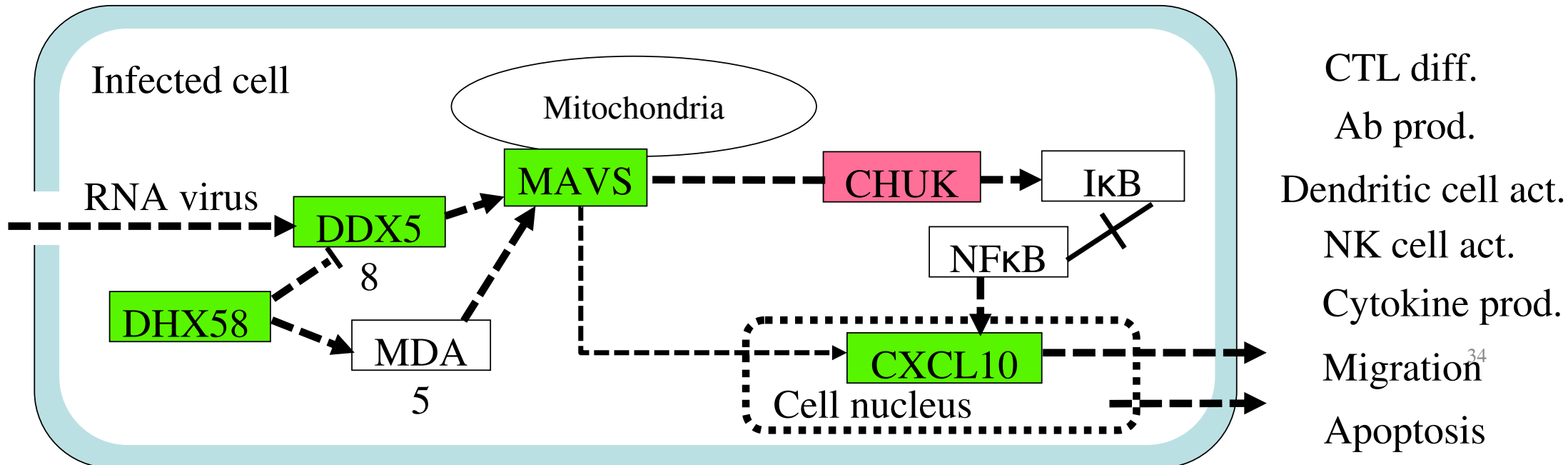
Gene stable ID	Gene description	Chrom.	Gene name	FDR	log2fc
ENSSSCG00000007146	sialic acid binding Ig like lectin 1 (CD169)	17	SIGLEC1	0.0015	-2.12
ENSSSCG00000002471	interferon, alpha-inducible protein 27 (IFI27L2)	7	ISG12(A)	0.0015	-2.46
ENSSSCG00000017087	GM2 ganglioside activator	16	GM2A	0.0015	-0.86
ENSSSCG00000006678	high affinity immunoglobulin gamma Fc receptor I precursor (CD64)	4	FCGR1A	0.0034	-1.91
ENSSSCG00000018005	myosin-1	12	MYH1	0.0034	5.92
ENSSSCG00000008647	cytidine/uridine monophosphate kinase 2	3	CMPK2	0.0034	-1.43
ENSSSCG00000012077	MX dynamin like GTPase 1	13	MX1	0.0143	-0.98
ENSSSCG00000010190	actin, alpha 1, skeletal muscle	14	ACTA1	0.0467	4.98
ENSSSCG00000017416	DExH-box helicase 58	12	DHX58	0.0467	-1.09
ENSSSCG00000029414	novelgene	1	novelgene	0.0467	-1.15
ENSSSCG00000018094	cytochrome b (mitochondrion)	MT	CYTB	0.0467	0.75
ENSSSCG00000018087	NADH-ubiquinone oxidoreductase chain 4	MT	MT-ND4	0.0481	0.70
ENSSSCG00000018084	NADH-ubiquinone oxidoreductase chain 3	MT	MT-ND3	0.0501	0.77
ENSSSCG00000009720	DExD/H-box helicase 60	14	DDX60	0.0572	-1.21
ENSSSCG00000025533	Cytochrome c oxidase subunit 7A1, mitochondrial	6	COX7A1	0.0610	-1.73
ENSSSCG00000022258	novelgene	17	novelgene	0.0715	-1.21

DEG (q<0.1) at 7 dpi

	A	B	C	D	E	F
1	Gene stable ID	Gene description	Chrom.	Gene name	qvalue.Vx	log2fc.Vx
2	ENSSSCG00000002471	interferon, alpha-inducible protein 27 (IFI27L2)	7	ISG12(A)	0.006	-2.39
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4	ENSSSCG00000006678	novelgene	4	FCGR1A	0.015	-1.76
5	ENSSSCG000000012076	MX dynamin like GTPase 2	13	MX2	0.058	-1.23
6	ENSSSCG00000008977	C-X-C motif chemokine 10 precursor	8	CXCL10	0.006	-1.08
7	ENSSSCG000000010452	interferon induced protein with tetratricopeptide repeats 3	14	IFIT3	0.035	-1.04
8	ENSSSCG00000006588	S100 calcium binding protein A9	4	S100A9	0.033	-1.00
9	ENSSSCG00000000774	novelgene	5	USP18	0.059	-0.93
10	ENSSSCG000000029414	novelgene	1	novelgene	0.010	-0.82
11	ENSSSCG000000030108	novelgene	17	novelgene	0.029	-0.80
12	ENSSSCG00000000654	novelgene	5	novelgene	0.049	-0.72
13	ENSSSCG000000014136	versican	2	VCAN	0.092	-0.70
14	ENSSSCG000000016265	novelgene	15	novelgene	0.035	-0.68
15	ENSSSCG000000028448	novelgene	8	novelgene	0.045	-0.62
16	ENSSSCG000000014920	frizzled class receptor 4	9	FZD4	0.093	-0.59
17	ENSSSCG000000001408	allograft inflammatory factor 1	7	AIF1	0.065	-0.55
18	ENSSSCG000000001667	zinc finger protein 318	7	ZNF318	0.065	0.63
19	ENSSSCG000000012841	patatin like phospholipase domain containing 2	2	PNPLA2	0.064	0.68
20	ENSSSCG000000023710	receptor accessory protein 1	3	REEP1	0.093	0.69
21	ENSSSCG000000004554	novelgene	1	novelgene	0.049	0.73
22	ENSSSCG000000021803	novelgene	6	novelgene	0.096	0.79
23	ENSSSCG000000021812	membrane-spanning 4-domains, subfamily A, member 1	2	MS4A1	0.012	0.80
24	ENSSSCG000000007423	ubiquitin conjugating enzyme E2 C	17	UBE2C	0.035	0.88
25	ENSSSCG000000029901	ubiquitin conjugating enzyme E2 O	12	UBE2O	0.029	1.17

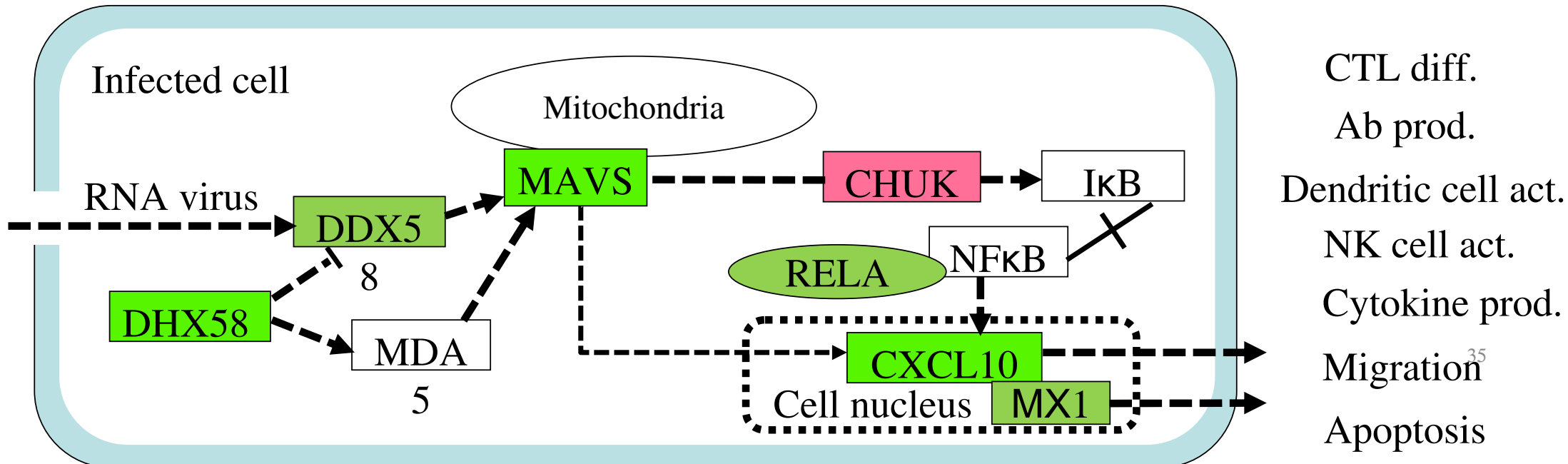
WGCNA results at 4 and 7 dpi

- **Module at 4 dpi (cor.=-0.8, p value=2e-06, #genes=166)**
 - Four genes are involved in “**RIG-I-like receptor signaling pathway**” and **less expressed in Vac**, especially **CXCL10**, **DHX58** as DEG.



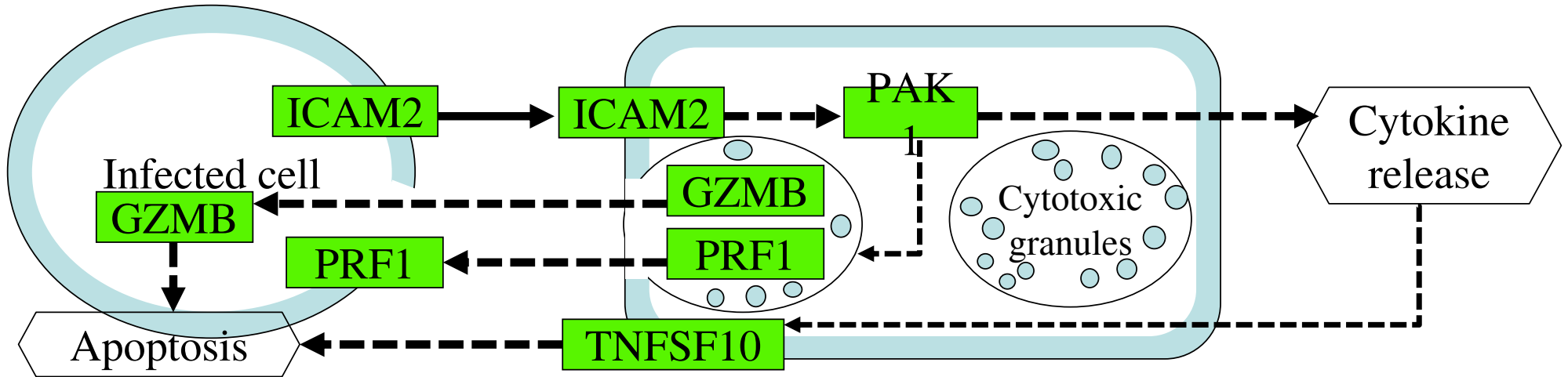
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- **Module at 4 dpi (cor.=-0.8, p value=2e-06, #genes=166)**
 - Four genes are involved in “RIG-I-like receptor signaling pathway” and less expressed in Vac, especially CXCL10, DHX58 as DEG.
- **Module at 7 dpi (cor.=-0.52, p value=0.01, #genes=105)**
 - In total, nine genes are involved in “cytokine-cytokine receptor interaction”, “chemokine signaling pathway” “NFκB signaling pathway” and “influenza A” and less expressed in Vac, especially DDX58 and MX1 as DEG.



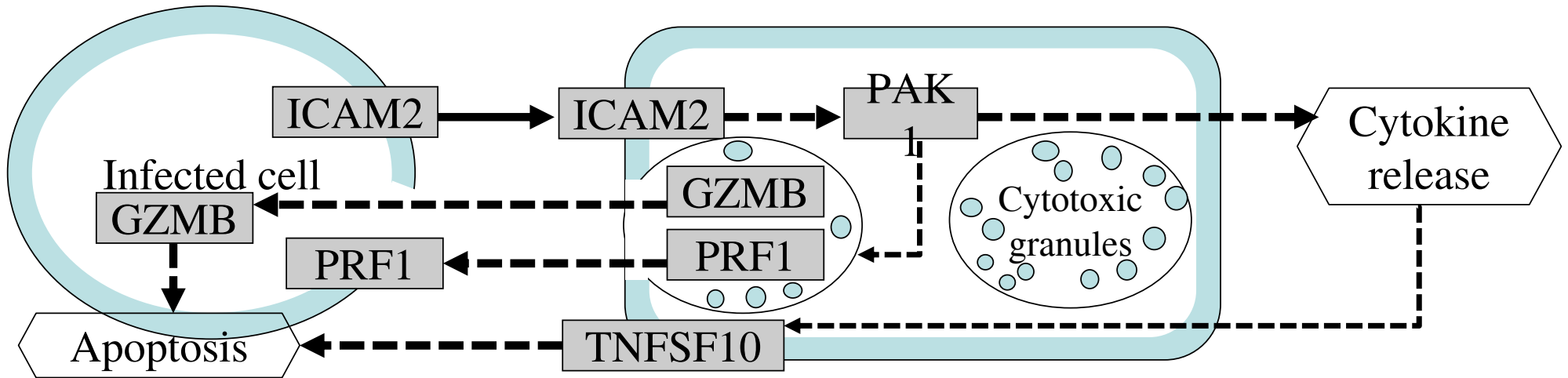
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- **Module at 4 dpi (cor.=-0.8, p value=2e-06, #genes=166)**
 - seven genes are involved in “natural killer cell mediated cytotoxicity” and less expressed in *Vac*, especially **TNFSF10** as DEG.

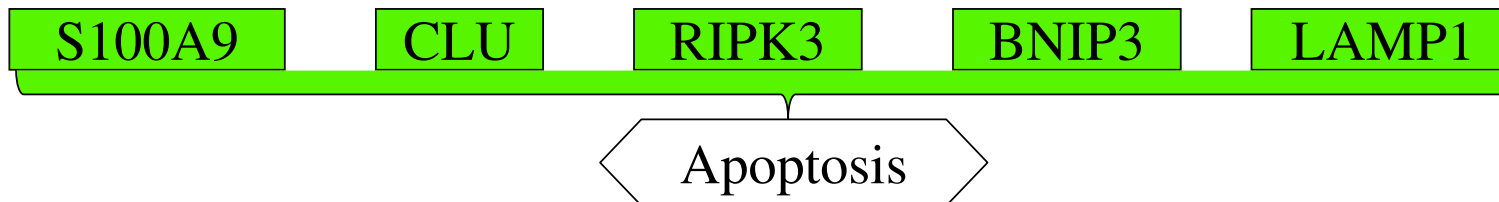


WGCNA results at 4 and 7 dpi

- **Module at 4 dpi (cor.=-0.8, p value=2e-06, #genes=166)**
 - seven genes are involved in “natural killer cell mediated cytotoxicity” and less expressed in Vac, especially TNFSF10 as DEG.



- **Module at 7 dpi (cor.=0.6, p value=0.003, #genes=229)**
 - In total, six genes are involved in apoptosis and less expressed in Vac, especially S100A9 as DEG.



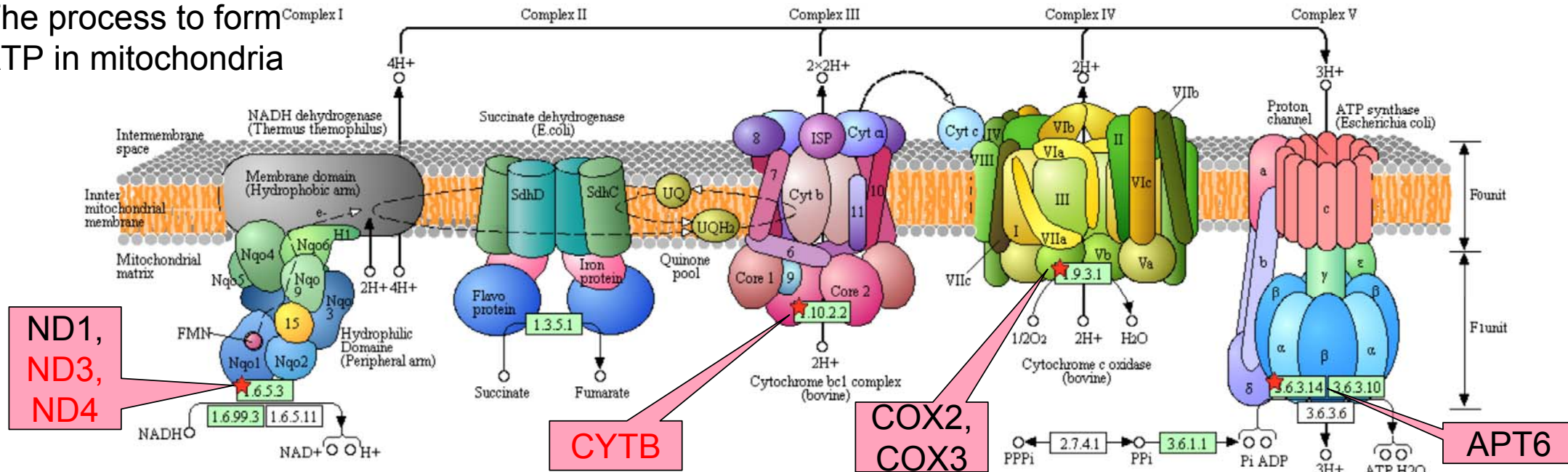
WGCNA results at 4 dpi (32 dpv)

- Module 2 (cor.=0.64, p value=7e-04, #genes=39)
 - the most significantly **positively** correlated with vaccination status
 - seven genes are involved in “**oxidative phosphorylation**” and **more expressed in Vac**, especially **ND3, ND4, CYTB** as DEG.

OXIDATIVE PHOSPHORYLATION

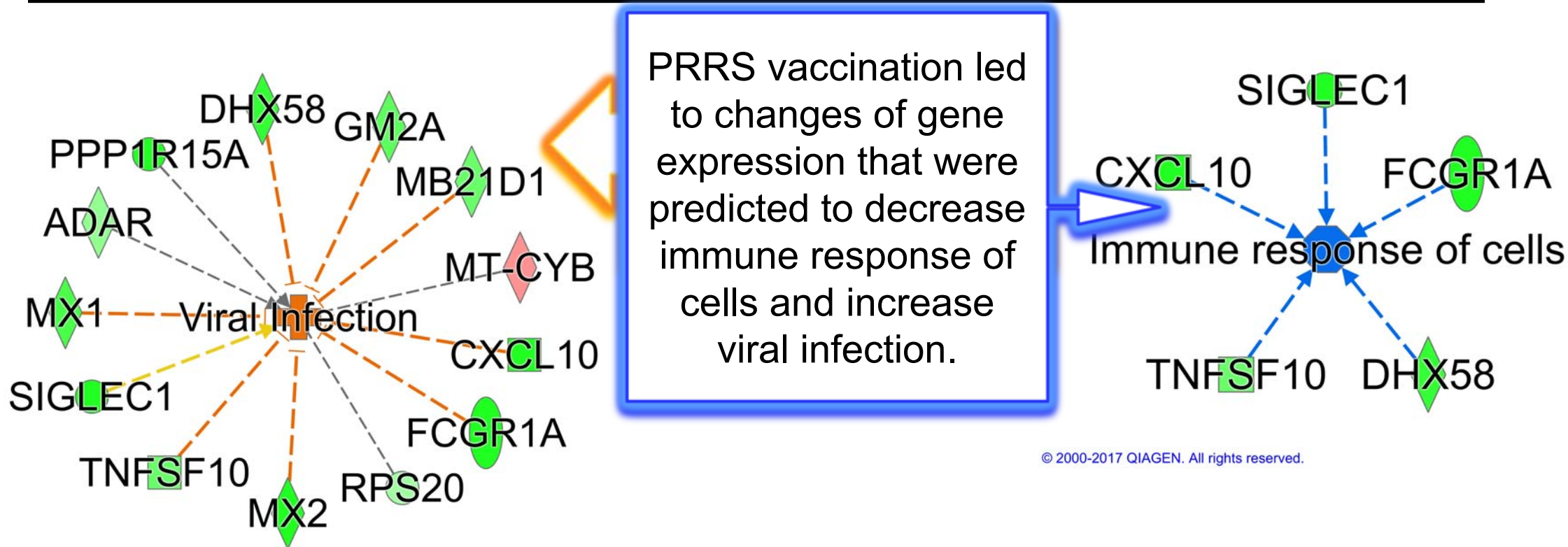
COX7A1

The process to form
ATP in mitochondria



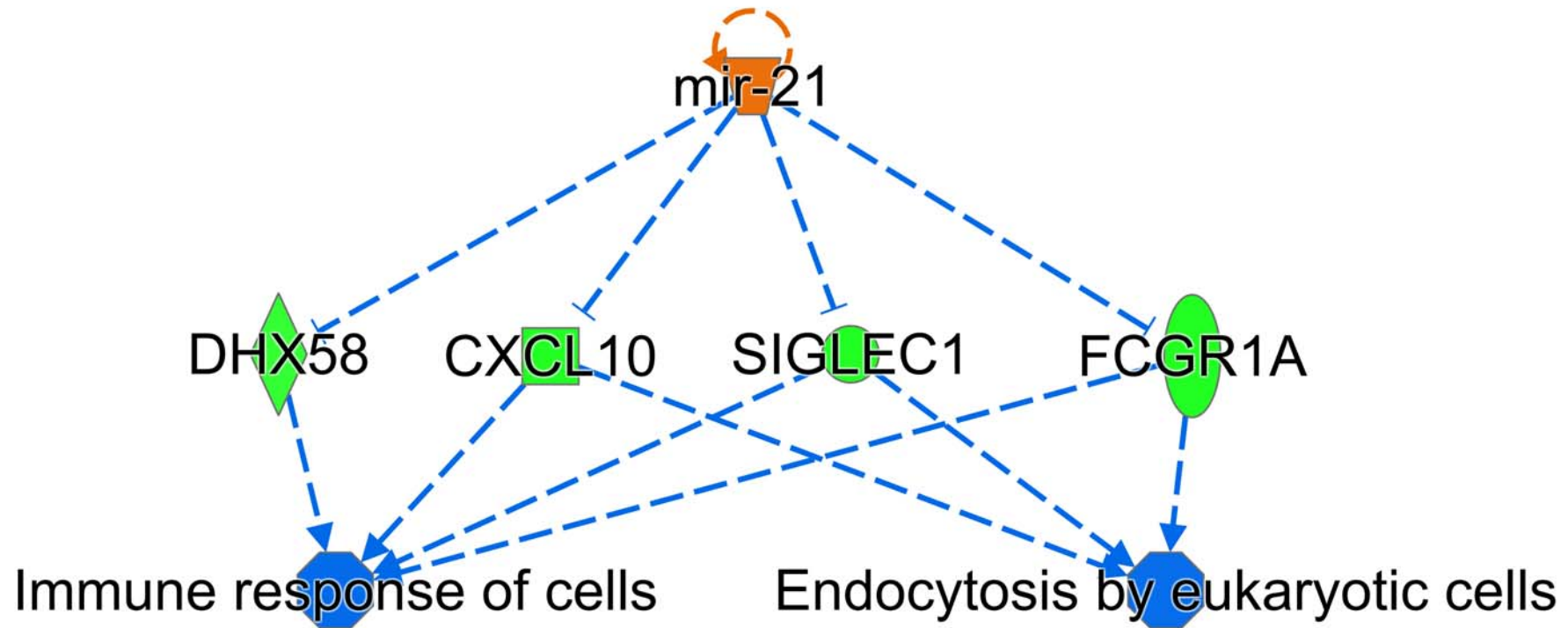
IPA results at 4 dpi (32 dpv)

-  DEGs ($q < 0.2$) less expressed in PRRSV vaccinated pigs than non-vaccinated pigs
-  DEGs ($q < 0.2$) more expressed in PRRSV vaccinated pigs than non-vaccinated pigs



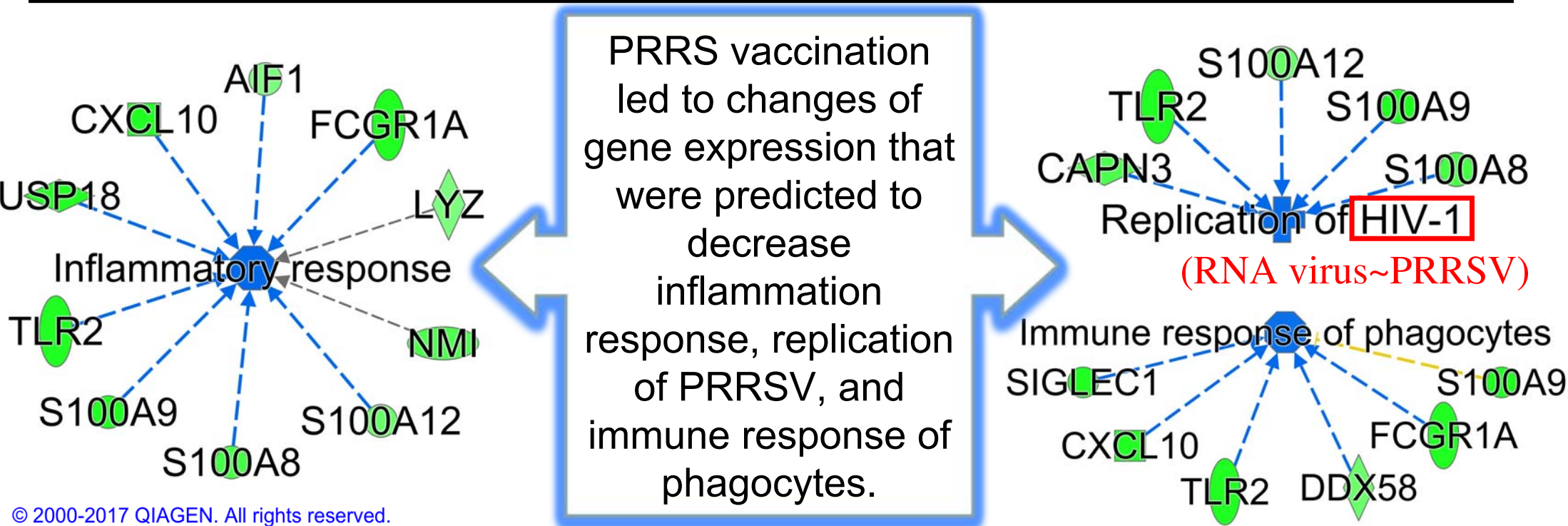
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IPA results at 4 dpi (32 dpv)



IPA results at 7 dpi (35 dpv)

- DEGs ($q < 0.2$) less expressed in PRRSV vaccinated pigs than non-vaccinated pigs
- DEGs ($q < 0.2$) more expressed in PRRSV vaccinated pigs than non-vaccinated pigs

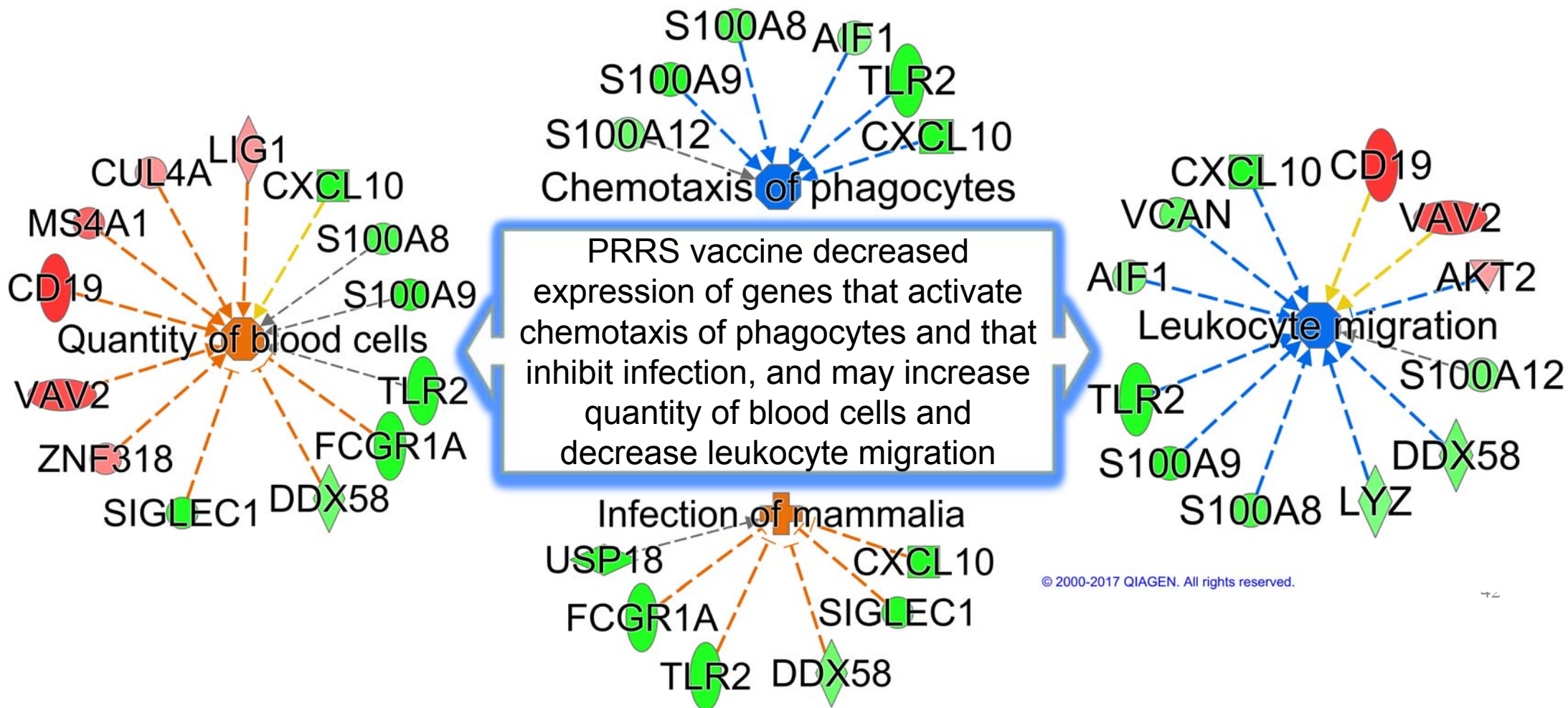


PRRS vaccine → Genes

- Inflammatory response
- Immune response of phagocytes
- Replication of PRRSV

IPA results at 7 dpi (35 dpv)

- DEGs (q<0.2) less expressed in PRRSV vaccinated pigs than non-vaccinated pigs
- DEGs (q<0.2) more expressed in PRRSV vaccinated pigs than non-vaccinated pigs



Viral load (area under the curve)

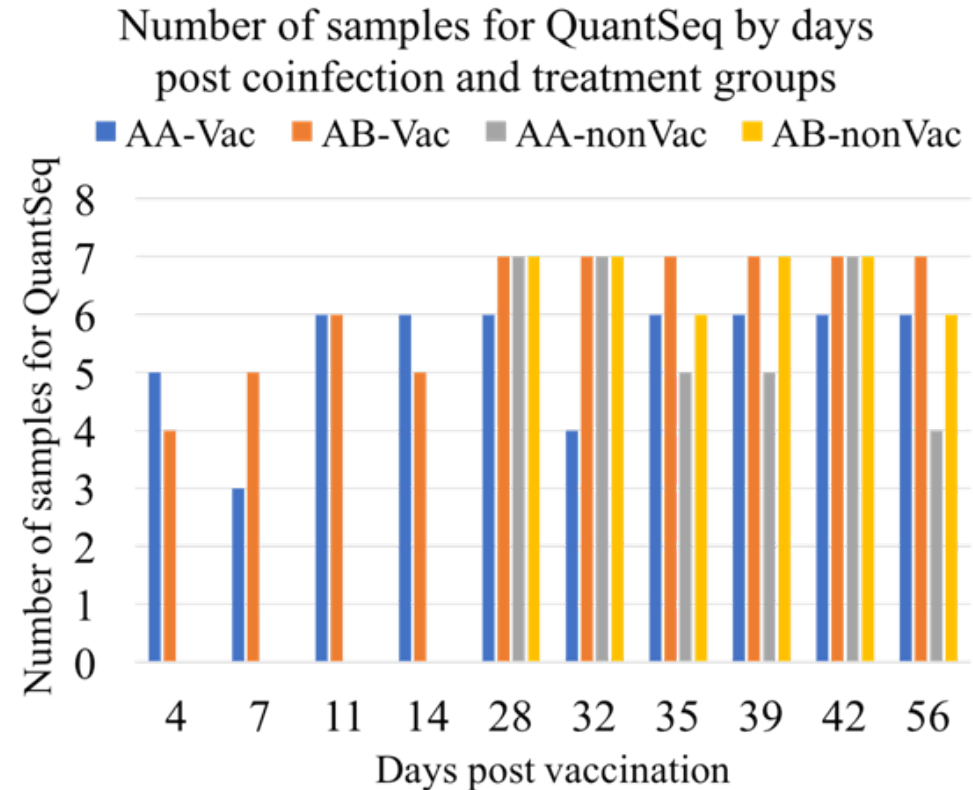
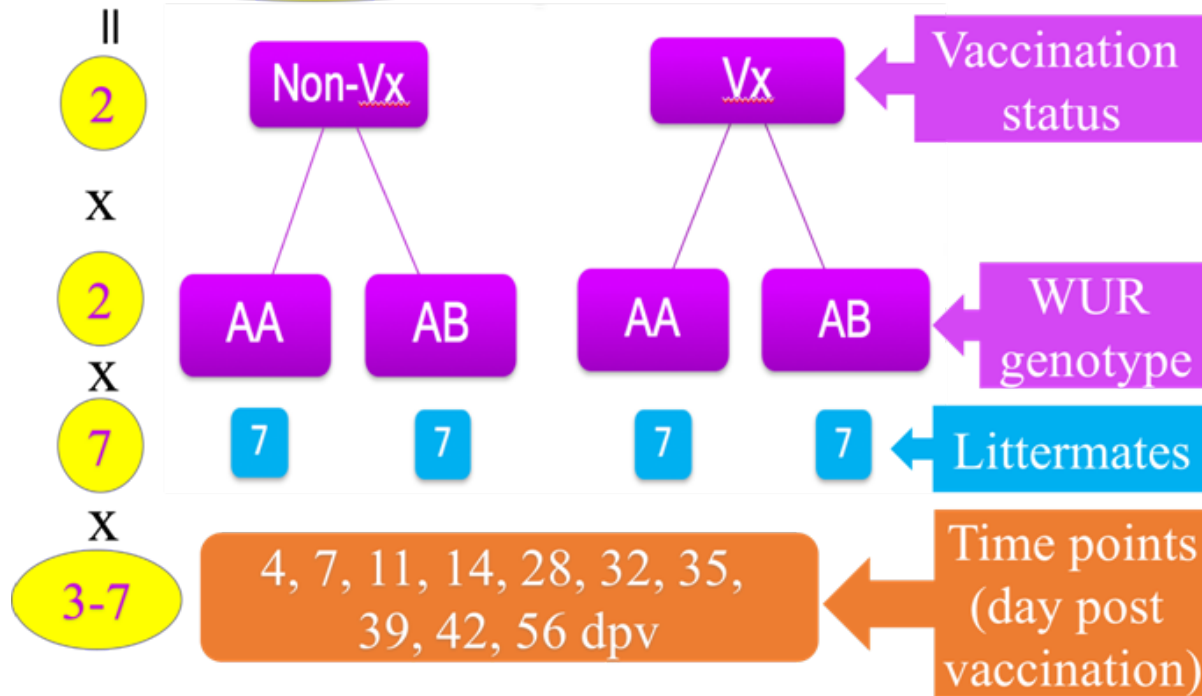
- PPRSV viremia: 0-21 dpi
 - A portion of pigs enter a rebound phase after 21 dpi
 - A property of the virus rather than host genetics
- PCV2 viremia: 0-42 dpi
 - Much noisier
 - No clear evidence of rebound

Additional slides for potential questions

- PRRS MLV:
 - a 2-ml dose administered intramuscularly
 - Ingelvac PRRS MLV; Boehringer Ingelheim Animal Health; GenBank accession no. AF159149
- Co-infection on 28 dpv:
 - PRRSV:
 - 2-ml dose of 10^5 TCID₅₀ PRRSV
 - isolate KS62; GenBank accession no. KM035803
 - PCV2:
 - $10^{3.6}$ TCID₅₀ PCV2b (GenBank accession no. JQ692110)
 - administered intranasally and intramuscularly

Blood QuantSeq Samples

191 samples for
FWD QuantSeq



QuantSeq data analysis pipeline

Bluebee

- Read QC → Trimming → Alignment → Mapping → Gene Read Counting
- Pig 10.2 version Genome

QuasiSeq

The **generalized linear model at each time point** for differential expression analysis:

(1) For 4, 7, 11, 14 dpv: Gene expression=WUR+RIN+Plate

(2) For 28, 32, 35, 39, 42, 56 dpv: Gene expr.=WUR+**Vac**+ **Vac x WUR** +RIN+Plate

IPA

- Differential expressed gene (DEG): $q < 0.2$
- Ingenuity Pathway Analysis (IPA) for biological function analysis

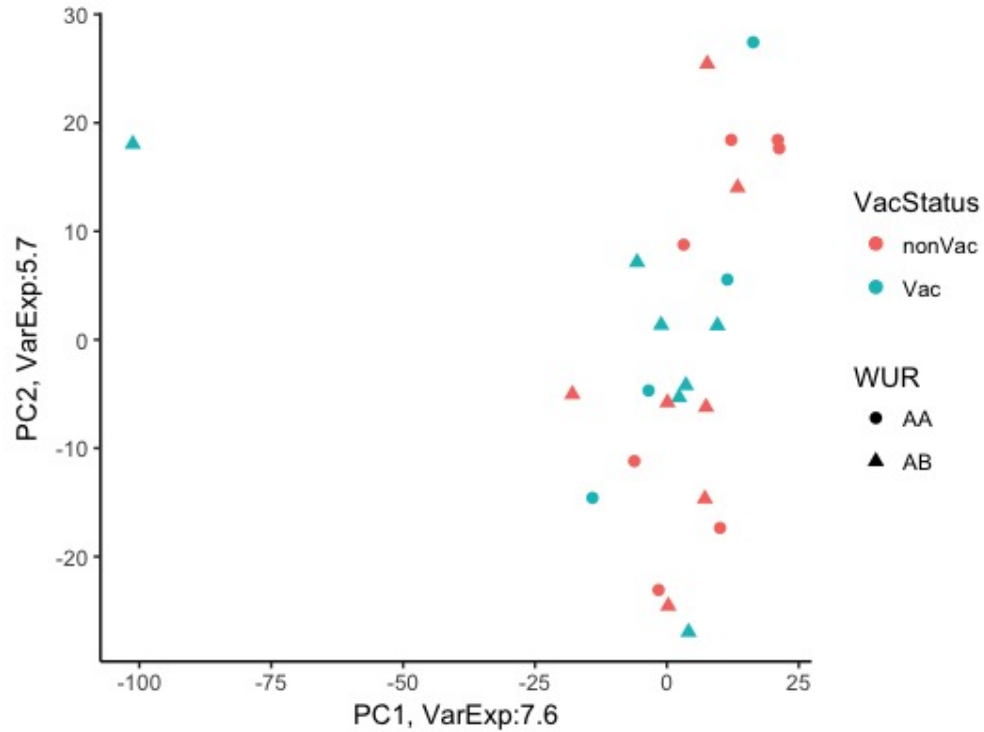
Filtering:

- Removed Globin reads
- Removed reads from genes with average read count < 2 across samples
- Removed reads from genes with read counts > 0 for less than 3 samples ⁴⁶

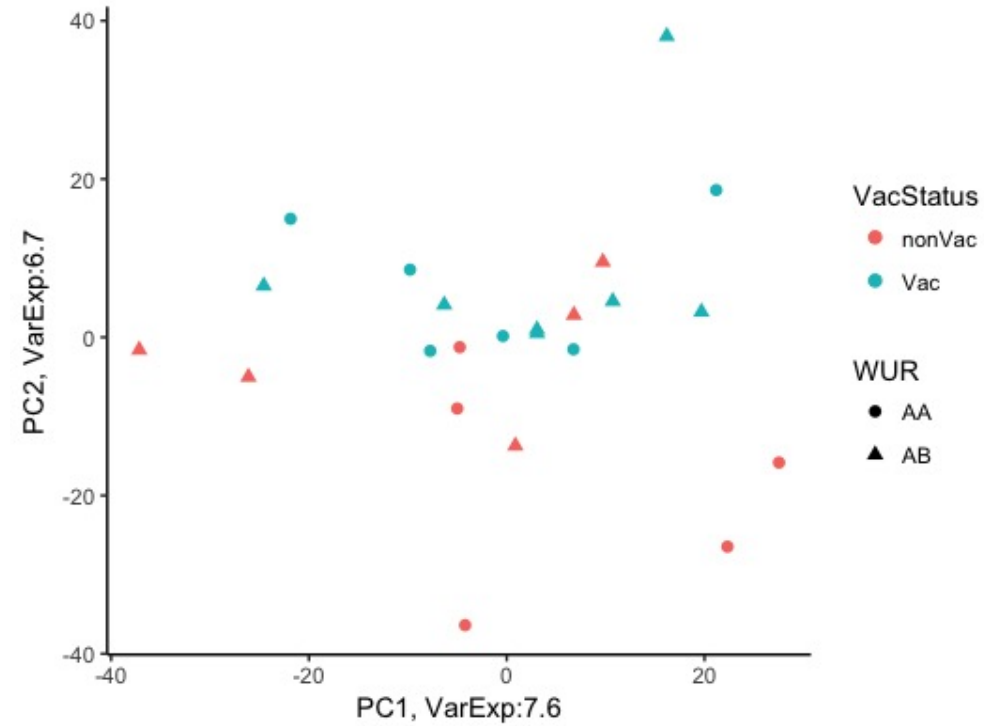
→ 5,445 genes expressed in blood on average

PCA plots

4 dpi



7 dpi



DEGs between **Vaccinated** vs. **Non-vaccinated**

**Red = more highly
expressed in the
vaccinated pigs at
time point X**

upregulated

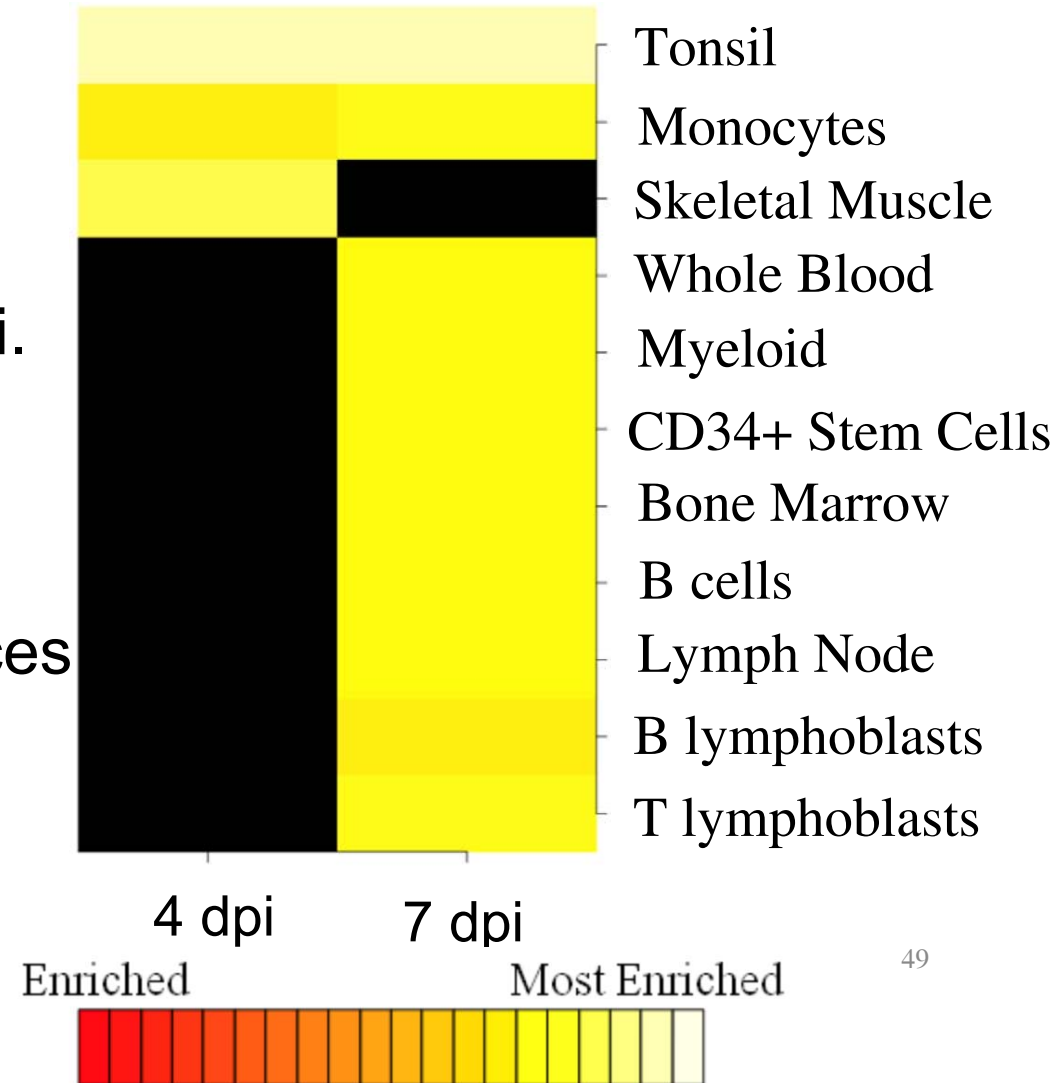
**Green = more highly
expressed in the non-
vaccinated pigs at
time point X**

downregulated

Cell enrichment analyses results

❖ Cten was used to predict enriched cell types from DEG between Vac and nonVac pigs within 4 and 7 dpi.

→ The observed differences in gene expression may result from differences in immune cell composition in blood.



WGCNA results at 7 dpi (35 dpv)

- Module 1 (cor.=-0.52, p value=0.01, #genes=105)
- In total, nine genes are involved in “cytokine-cytokine receptor interaction”, “chemokine signaling pathway” “NFκB signaling pathway” and “influenza A” and less expressed in Vac, especially DDX58 and MX1 as DEG.

