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INTRODUCTION

- Gastrointestinal nematode (GIN) infections are a major concern that is affecting sheep production and also causing economic loss.
- Small ruminant farming plays an important role in South African agriculture and holds promise in mitigating challenges associated with unsustainable farming practices that contribute to global food insecurity.
- The South African Dohne Merino sheep was bred to maximize wool and meat production and represent an important genetic resource that can be harnessed to breed for resistance to GIN as an alternative strategy to chemical control of nematodes.
- The productivity of the livestock's is mainly limited by diseases, parasites, poor nutrition, poor management and poor breeding policies.


AIM

The study used RNA-Seq and differential gene expression profiling to understand the underlying molecular mechanism associated with the infection of *H. contortus* in Dohne Merino sheep.

METHODOLOGY



Six adult Dohne Merino sheep were categorised into resistant (n=3) and susceptible (n = 3) to *Haemonchus contortus* based on Estimated Breeding Values derived from faecal egg count phenotypes.



Total RNA was extracted from the abomasum, ileum, jejunum and duodenum tissues samples of 3 resistance and 3 susceptible Dohne Merino sheep tissue samples using RNeasy Mini Kit (Qiagen)

RNA sequencing using Illumina HiSeq 2500

Data analysed using Hisat2, Stringtie2, DESeq2

David and WEGO were used for functional annotation analysis



RESULTS AND DISCUSION

RNA SEQUENCING AND READS ALIGNMENTS

- The average of 6 691 243 reads with a length of 125bp paired-end from 24 tissue samples
- The overall alignment rate of the quality-controlled reads to the reference genome (*Ovis aries* 4.0) was 94.44 % [resistant (94.27 %); susceptible (94.61 %); abomasum (95.75 %); jejunum (94.1 %); ileum (94.7 %) and duodenum (93.6 %)]

DIFFERENTIALS EXPRESSED GENES (DEGs)

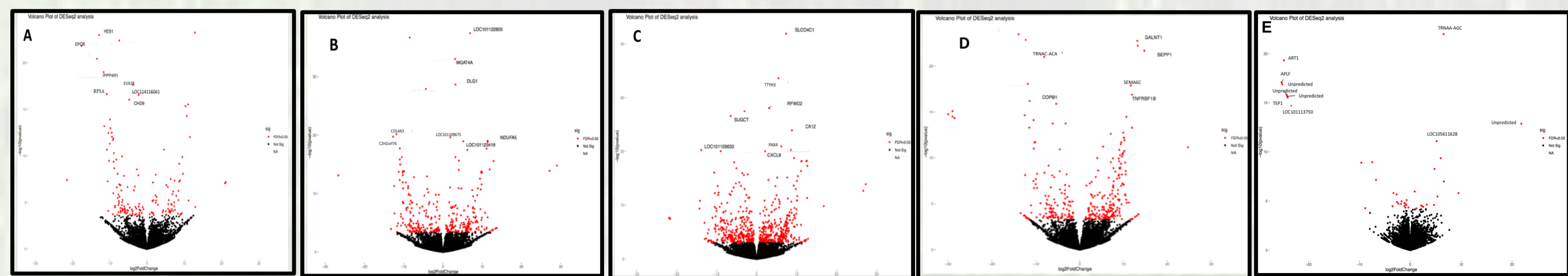


Figure 1. Volcano plot showing genes with significant differences in the relative abundance of study animals. The dot colour denotes significantly differentially expressed (red dot) at FDR<0.05, non-significant (black dot), down-regulated (<0) and up-regulated (> 0) with (A)abomasum, (B) ileum, (C) jejunum, (D) duodenum and (E) Animals infected with *H. contortus*

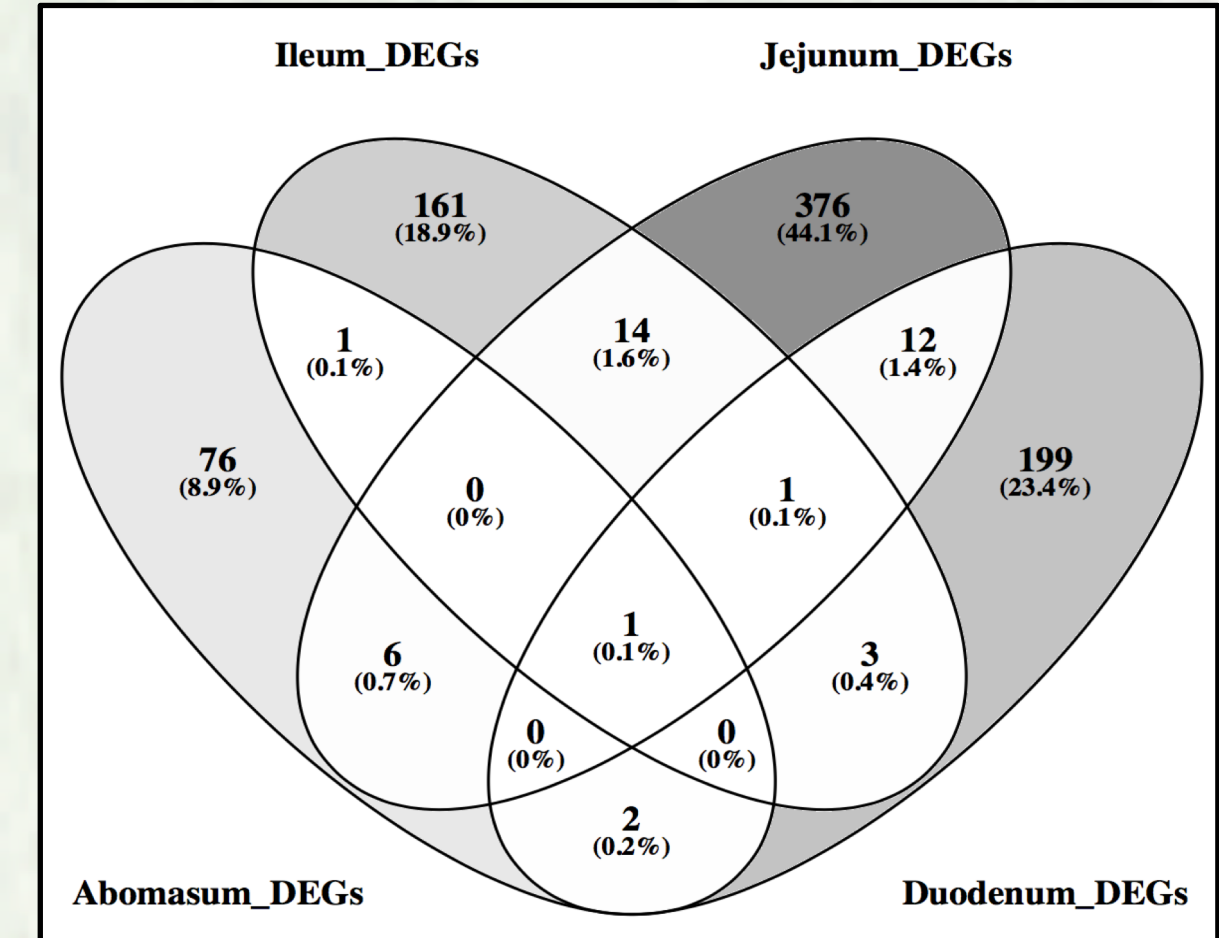


Figure 2. Venn diagram plot showing the number of all DEGs of each segment using Venny 2.1 .

Table 1. Up- and down-regulated DEGs of the study animals and all the 4 segment at FDR<0.05

	Total DEGs	Up-regulated	Down-regulated
Resistant and susceptible animals	34	20	14
Abomasum	146	58	88
Ileum	302	161	141
Jejunum	584	302	281
Duodenum	332	206	126

Table 2. Differential expressed gene (excluding unpredicted genes), FDR<0.05

Gene name	Function
Cyclin-dependent kinase inhibitor 1A (<i>CDKN1A</i>)	<ul style="list-style-type: none">Cell cycle regulatorPlays a role in various cell death conditions such as apoptosis and ferroptosis
Aprataxin and PNKP Like Factor (<i>APLF</i>)	<ul style="list-style-type: none">Is one of the adaptors or modulators of the non-homologous end joining (NHEJ) scaffold which is a DNA repair protein
Apoptosis regulatory protein Siva (<i>SIVA1</i>)	<ul style="list-style-type: none">Plays a role in the programmed cell death or cellular death mechanismsPromotes T-cell receptor-mediated apoptosis which is highly expressed in the small intestine
YES proto-oncogene 1 (<i>YES1</i>)	<ul style="list-style-type: none">Member of the SRC family which function as the cell growth, adhesion, survival, and differentiation
Ribosomal protein SA (<i>RPSA</i>)	<ul style="list-style-type: none">Plays a role in the process of pathogenic bacteria adhering to host cellsContributes to the migration invasion, angiogenesis, and part of tumour cells
CASP8 and FADD like apoptosis regulator (<i>CFLAR</i>)	<ul style="list-style-type: none">Plays a role in fundamental intracellular processes like inflammation and apoptosisFunction as the connection between cell survival and cell death pathways in mammalian cells

FUCNTIONAL ANNOTATION

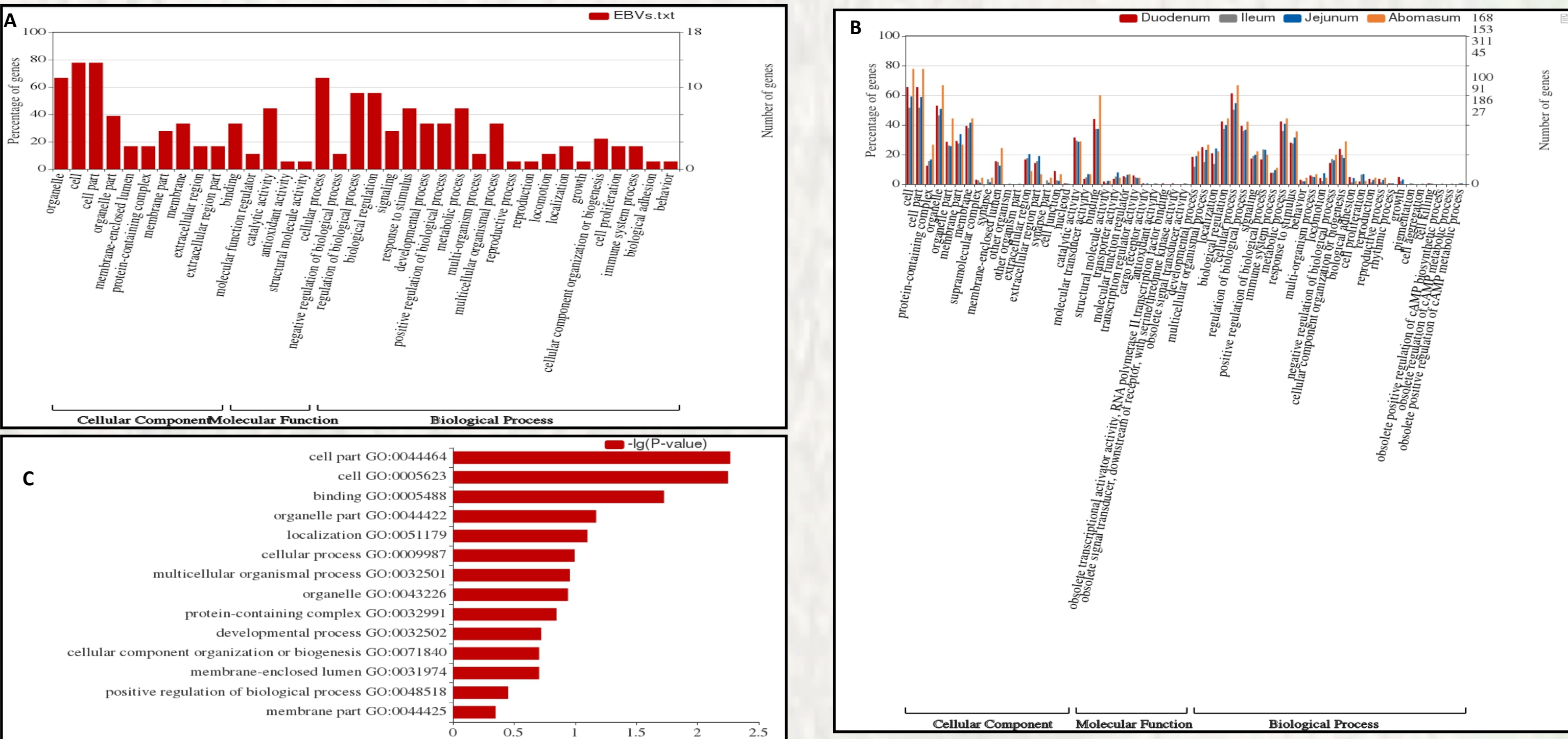


Figure 3. GO analysis describing the properties of DEGs of the (A) gastrointestinal tract, (B) abomasum, duodenum, ileum and jejunum of resistant and susceptible sheep, (C)The -log10 of P-values obtained from all datasets of major functional annotation clusters in descending order, indicating the data significant differences of differentially expressed genes between the segments of resistant and susceptible sheep.

BIOLOGICAL PATHWAYS

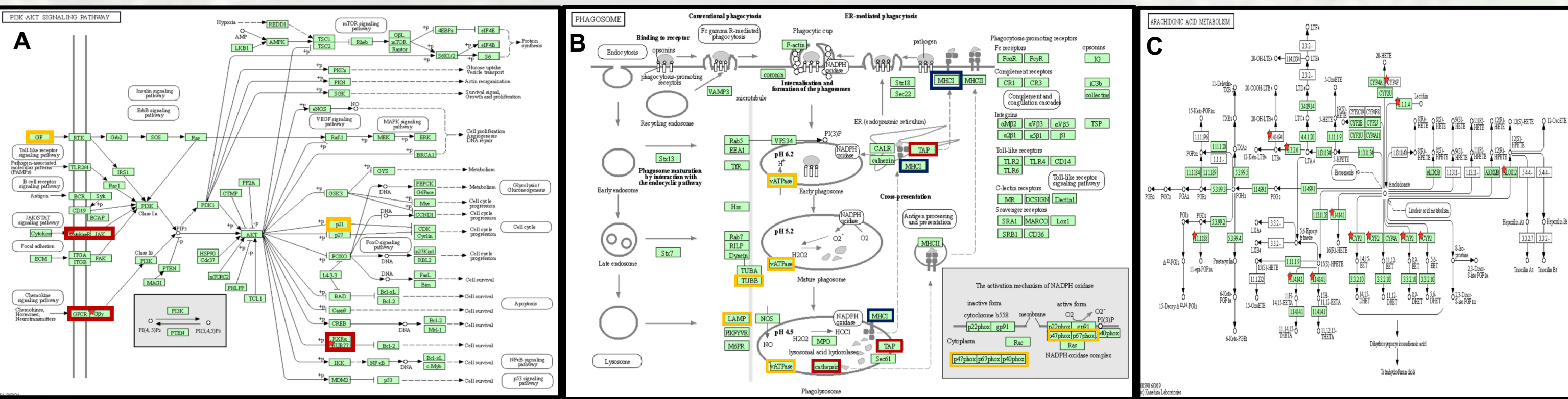


Figure 4. Significantly enriched KEGG pathways using up- and down-regulated DEGs. (A) PI3K-Akt enriched in the abomasum (red squares with red stars) and sheep naturally infected with *H. contortus* (yellow squares), (B) Phagosome pathway enriched in ileum (red) and jejunum (yellow) and purple (genes shared on both segments), (C) Arachidonic acid metabolism pathways enriched in duodenum (blue squares with red stars) and jejunum (red stars) are genes involved.

CONCLUSIONS

- RNA approach enabled us to uncover genes and pathways that are implicated in sheep resistant or susceptible to *H. contortus*.
- Genes belonging to immune and stimulus-response were identified which could be significant to resistance to nematodes infection.
- Demonstrated tissue-specific response mechanisms to *H. contortus* infection in sheep
- This study provides information that form basis of selection of animals understanding the genetics for resistance to GINs.