The regulatory landscape of intestinal cells

Investigating the transcriptional effect of autophagy impairment observed in Crohns disease using organoid and network biology approaches

Agatha Treveil, March 06 2019

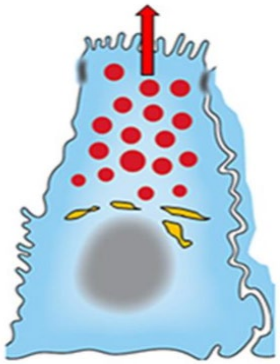
Disclosures:

Conflict of interest:

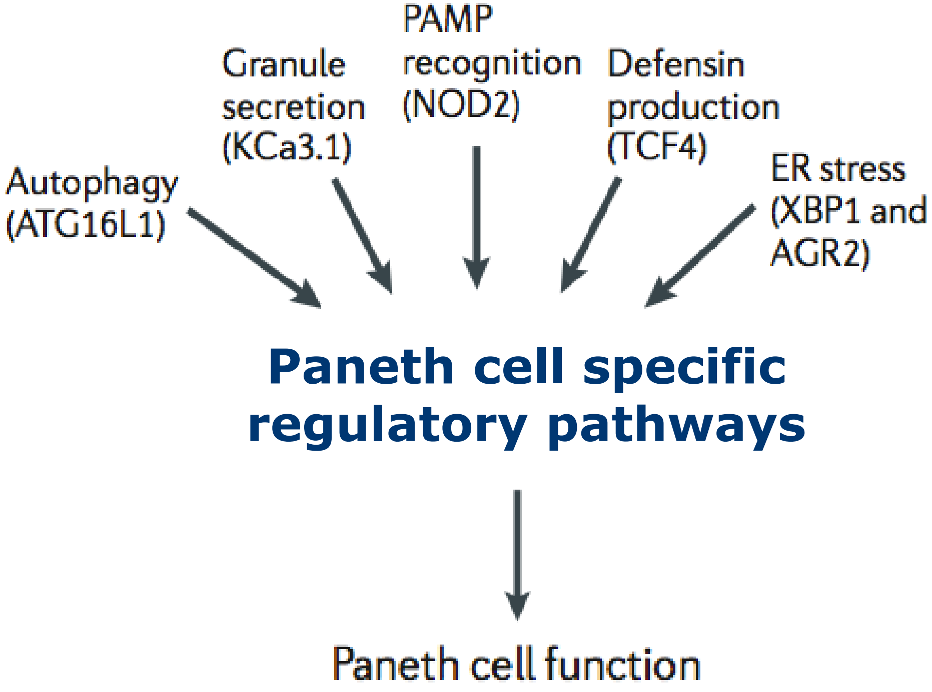
- Grants and personal fees: Biotechnology and Biological Sciences Research Council (UK), during the conduct of the study

Paneth cells in gut health

Antimicrobial peptides



Paneth cells



CD susceptibility genes

Autophagy (ATG16L1)

Granule secretion (KCa3.1)

PAMP recognition (NOD2)

Defensin production (TCF4)

ER stress (XBP1 and AGR2)

Paneth cell specific regulatory pathways

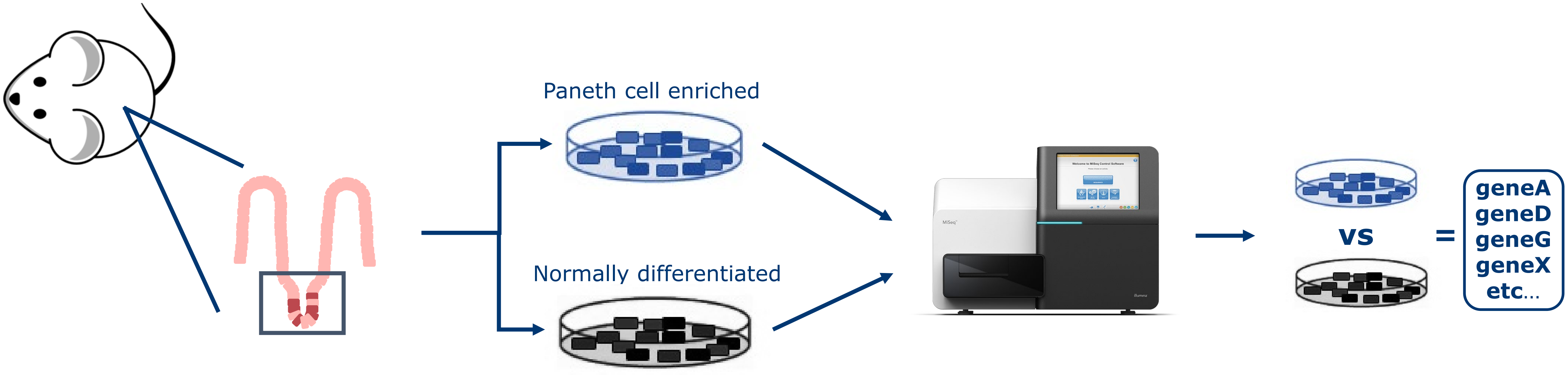
Paneth cell function

Aims:

1. Develop a workflow to study the regulatory mechanisms of key gut secretory cells (incl. Paneth cells).
2. Apply workflow to study:
    a. Regulatory differences between cell types
    b. How regulatory mechanisms are altered in particular genetic backgrounds

Moal & Servin, Microbiol Mol Biol Rev, 2013

Workflow 1/2 : data generation



Paneth cell enriched

Normally differentiated

vs

=

geneA
geneD
geneG
geneX
etc…

Extraction of small intestinal crypts

Wild type mice

3D organoid differentiation & enrichment

Whole organoid RNA sequencing

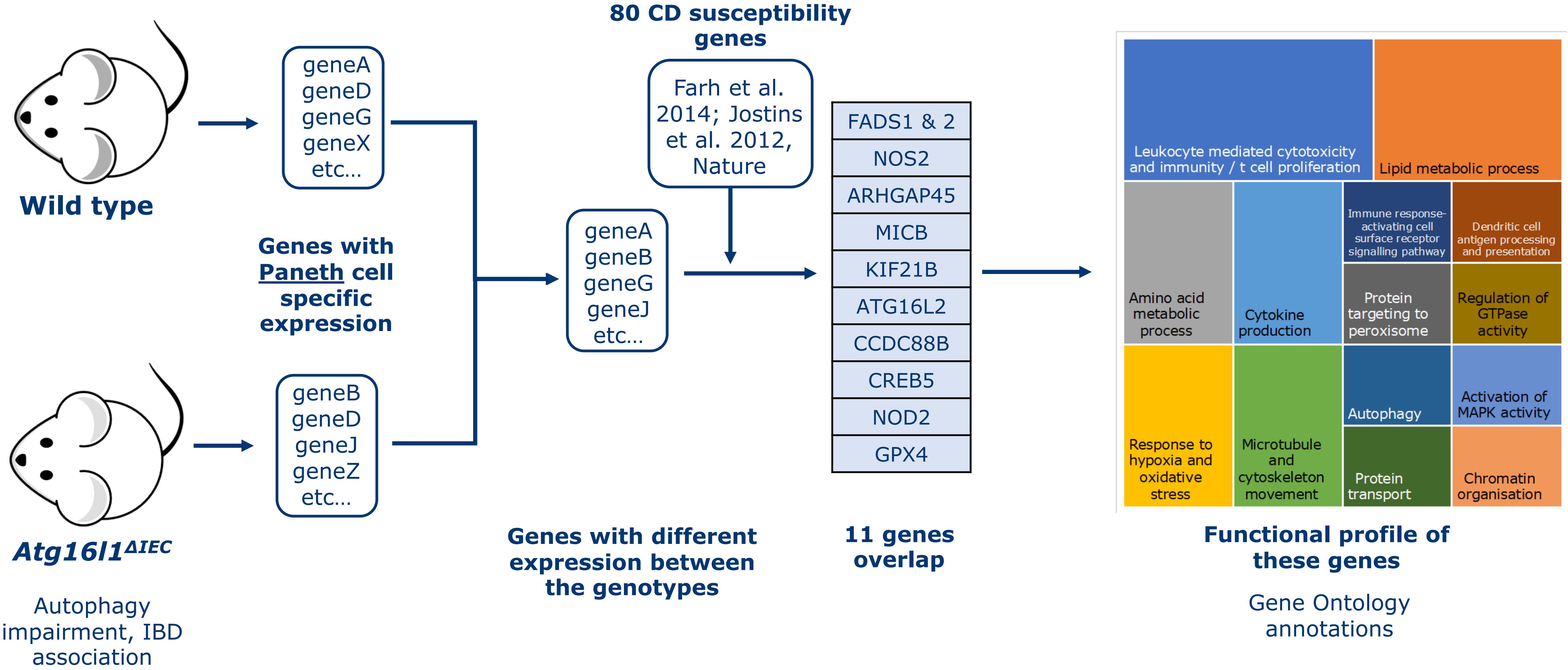
miRNA, mRNA & lncRNA expression profiling

Differential expression testing

Outputs list of genes with Paneth cell specific expression

Autophagy impairment

Autophagy impairment can affect the expression of a number of Crohns associated genes, even when not mutated.



Wild type

geneA
geneD
geneG
geneX
etc…

Genes with Paneth cell specific expression

$\mathit{Atg16l1}^{\Delta IEC}$

Autophagy impairment, IBD association

geneB
geneD
geneJ
geneZ
etc…

geneA
geneB
geneG
geneJ
etc…

Genes with different expression between the genotypes

80 CD susceptibility genes

Farh et al. 2014; Jostins et al. 2012, Nature

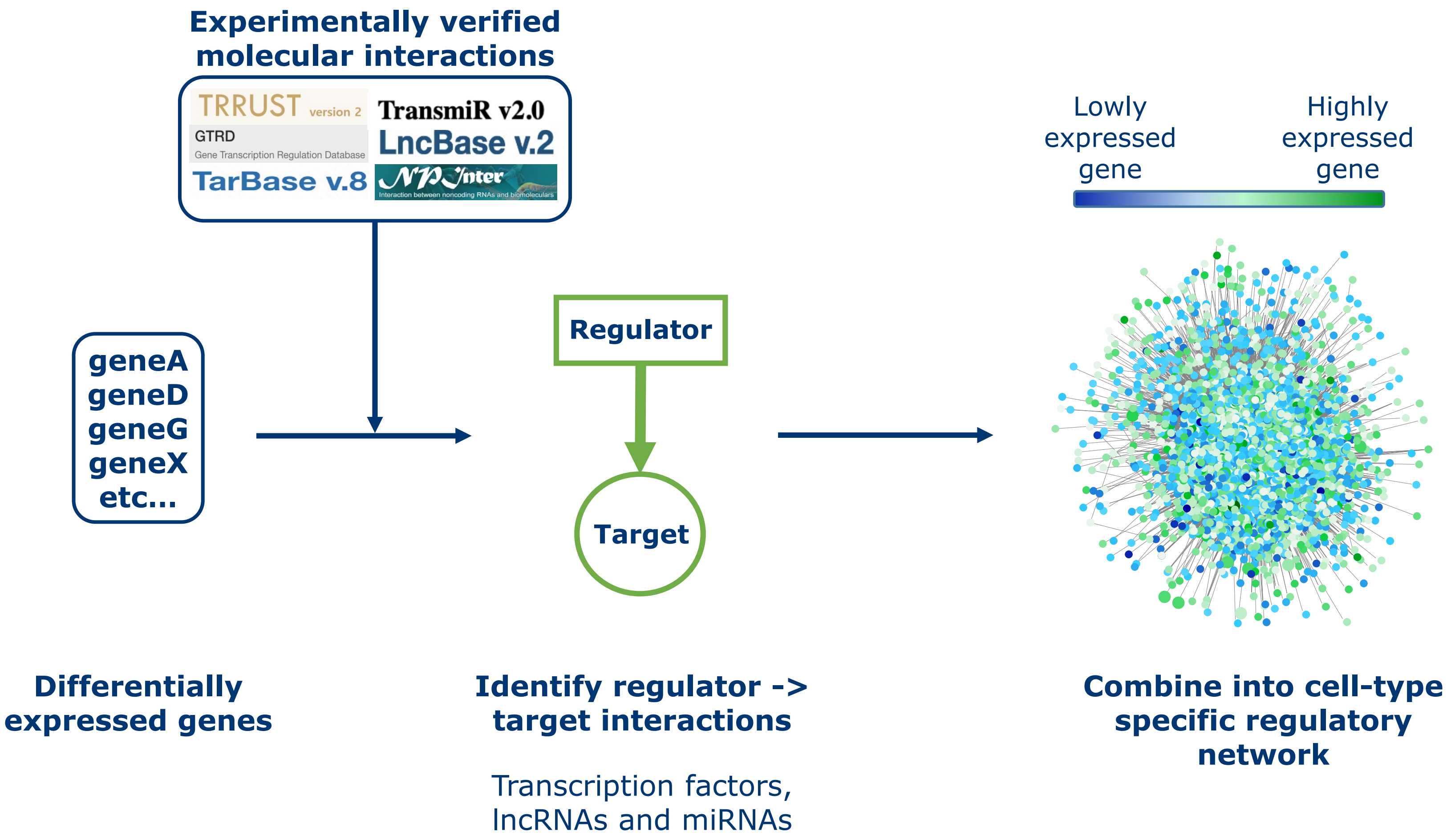
- FADS1 & 2
- NOS2
- ARHGAP45
- MICB
- KIF21B
- ATG16L2
- CCDC88B
- CREB5
- NOD2
- GPX4

11 genes overlap

Functional profile of these genes

Gene Ontology annotations

Workflow 2/2 : data analysis



Experimentally verified molecular interactions

TRRUST version 2
TransmiR v2.0
GTRD
Gene Transcription Regulation Database
LncBase v.2
TarBase v.8
NPInter
Interaction between noncoding RNAs and biomolecules

geneA
geneD
geneG
geneX
etc…

Regulator

Target

Lowly expressed gene

Highly expressed gene

Differentially expressed genes

Identify regulator -> target interactions

Transcription factors, lncRNAs and miRNAs

Combine into cell-type specific regulatory network

Paneth cell regulatory network:

37,062 interactions
208 regulators
3,023 targets

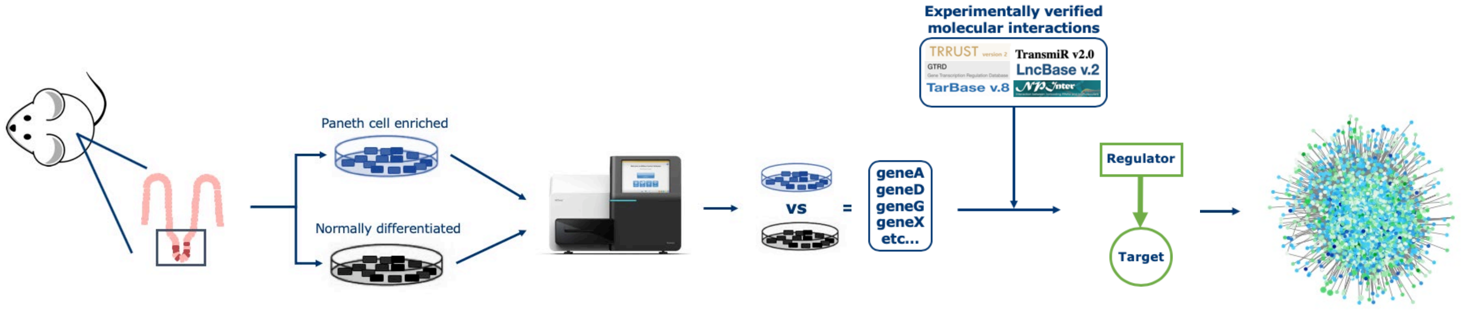
Putative master regulators:

Ets1
Cebpa
Jun
Nr1d1
Nr3c1
Rxra
Vdr

Nuclear hormone receptors with connections to inflammation, autophagy and IBD

Summary + Future

- Novel & reusable workflow to generate cell type specific regulatory networks. Can be applied to:
  - Different cell types
  - Different genetic backgrounds



- Workflow improvements:
  - Fluorescence-activated cell sorting (FACS) instead of cell type enrichment of organoids
  - Apply the workflow to patient derived organoids (Quadram Institute)

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BBSRC bioscience for the future

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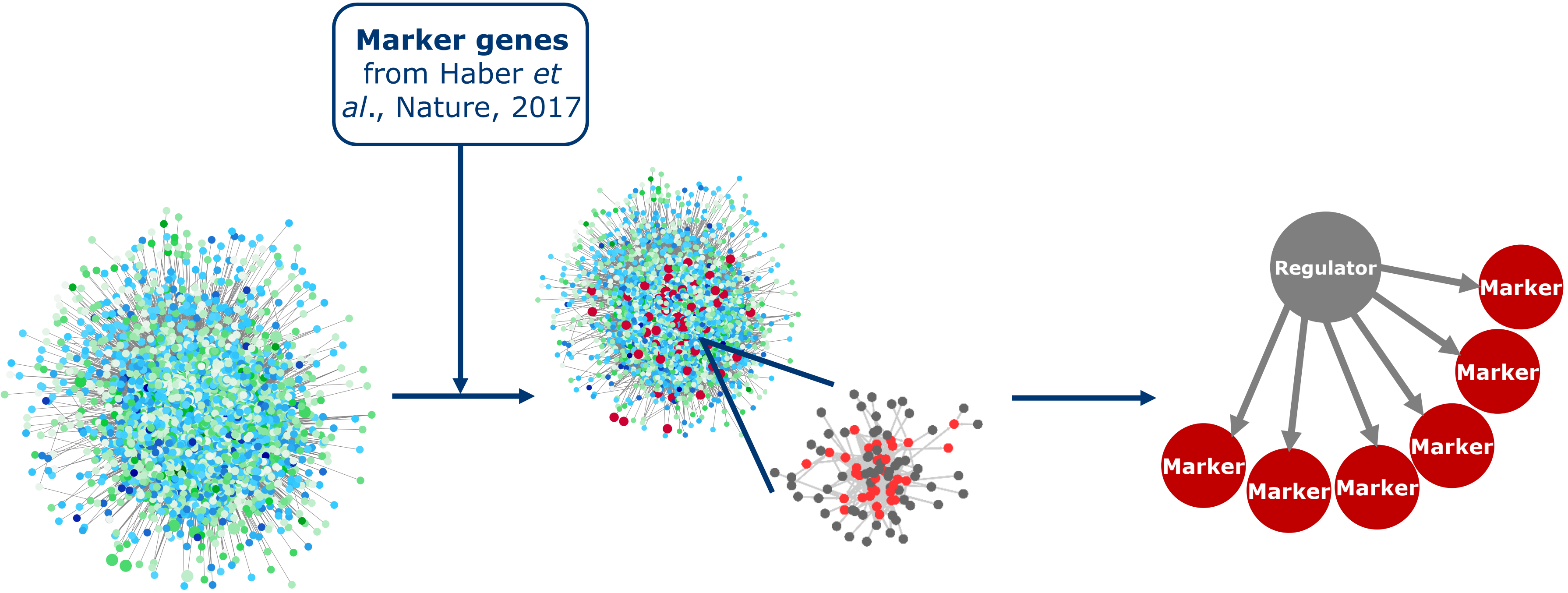
Further analysis: master regulators



Marker genes from Haber *et al.*, Nature, 2017

Cell-type specific regulatory network

Extract cell-type specific marker genes and their regulators

Observe regulators with many targets

Putative master regulators of the cell type

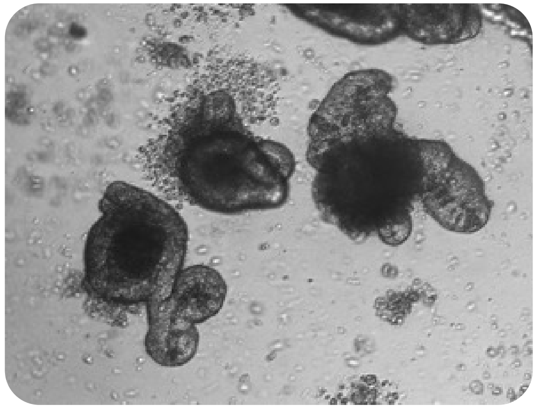
Paneth cell putative master regulators:

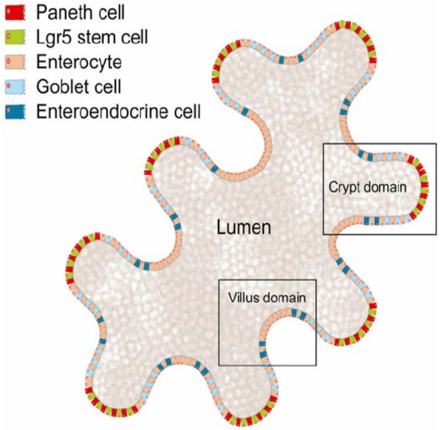
Ets1
Cebpa
Jun
Nr1d1
Nr3c1
Rxra
Vdr

Nuclear hormone receptors with connections to inflammation, autophagy and IBD

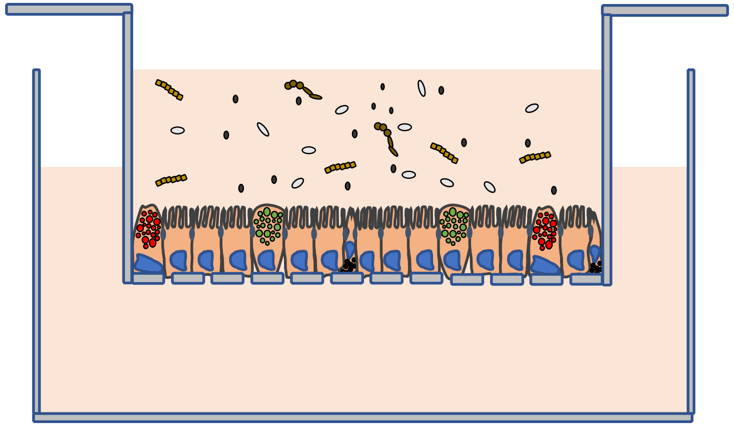
Organoids

- Tiny, self-organized three-dimensional tissue cultures that are derived from stem cells.





Lukova & Roeselers rev., 2015, Book chapter



Putative master regulators

Putative master regulator	Autophagy-inflammation-IBD associations	References
NR1D1 (REV-ERBa)	Modulates autophagy and lysosome biogenesis in macrophages leading to antimycobacterial effects	Chandra et al. 2015
	SNP rs12946510 which has associations to IBD, acts as a cis-eQTL for NR1D1	Mirza et al. 2015
NR3C1 (glucocorticoid receptor)	Associations with cellular proliferation and anti-inflammatory responses	Oakley and Cidlowski, 2013
	Exogenous glucocorticoids are heavily used as anti-inflammatory therapy for IBD	Rutgeerts 1998; Prantera and Marconi 2013
	ATG16L1, an autophagy related gene, was down-regulated in patients who do not respond to glucocorticoid treatment	De Iudicibus et al. 2011; Dubois-Camacho et al. 2017
	Transcriptionally regulates NFKβ1, a SNP affected gene in ulcerative colitis	Yemelyanov et al. 2007; Dinkel et al. 2016; Han et al. 2018
VDR (Vitamin D Receptor)	Regulates autophagy in Paneth cells through ATG16L1 – dysfunction of autophagy in Paneth cells has been linked to Crohn's disease	Sun 2016; Bakke et al. 2018
	Induces antimicrobial gene expression in other cell lines	Wang et al. 2004; Gombart et al. 2005
	specific polymorphisms in the VDR genes have been connected to increased susceptibility to IDB	Pei et al. 2011
	A study looking at colonic biopsies of IBD patients observed reduced VDR expression compared to healthy biopsies	Abreu et al. 2004
	Interacts with five SNP affected UC genes	Bovolenta et al. 2012; Lesurf et al. 2016; Dinkel et al. 2016; Han et al. 2018
RXRa (Retinoid X Receptor Alpha)	Heterodimerizes with VDR (see above)	

Other putative master regulators

- **Ets1**
  - Proto oncogene - DNA synthesis and damage repair
  - Important role in the development of hematopoietic cells and Th1 inflammatory responses (Grenningloh et al. 2005)
  - IBD associated gene (Konno et al., 2004; Li et al, 2018)

- **Cebpa**
  - Transcription factor involved in the differentiation of certain blood cells.

- **Jun**
  - Proto-oncogene
  - Associated with apoptosis

Atg16l1 analysis

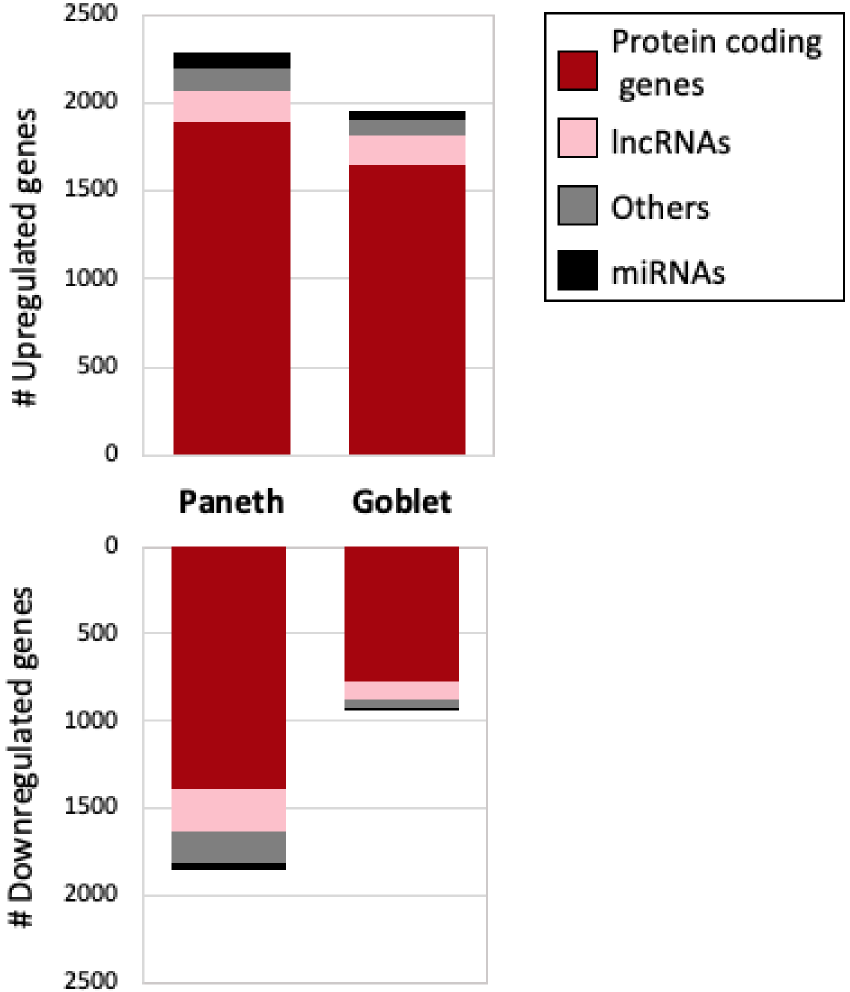
- Atg16l1
  - Paneth cell dysfunction
    - Autophagy defects
    - Reduced Amp secretion
    - Aberrant granule size, number and location

- Crohns genes
  - 3040 genes different between genotypes (64%)
  - 11/80 Crohns genes found in our dataset – hypergeometric p val = 0.0857

Genes
FADS1 & 2
NOS2
ARHGAP45
MICB
KIF21B
ATG16L2
CCDC88B
CREB5
NOD2
GPX4

A summary of the physical interactions compiled to generate the background network.

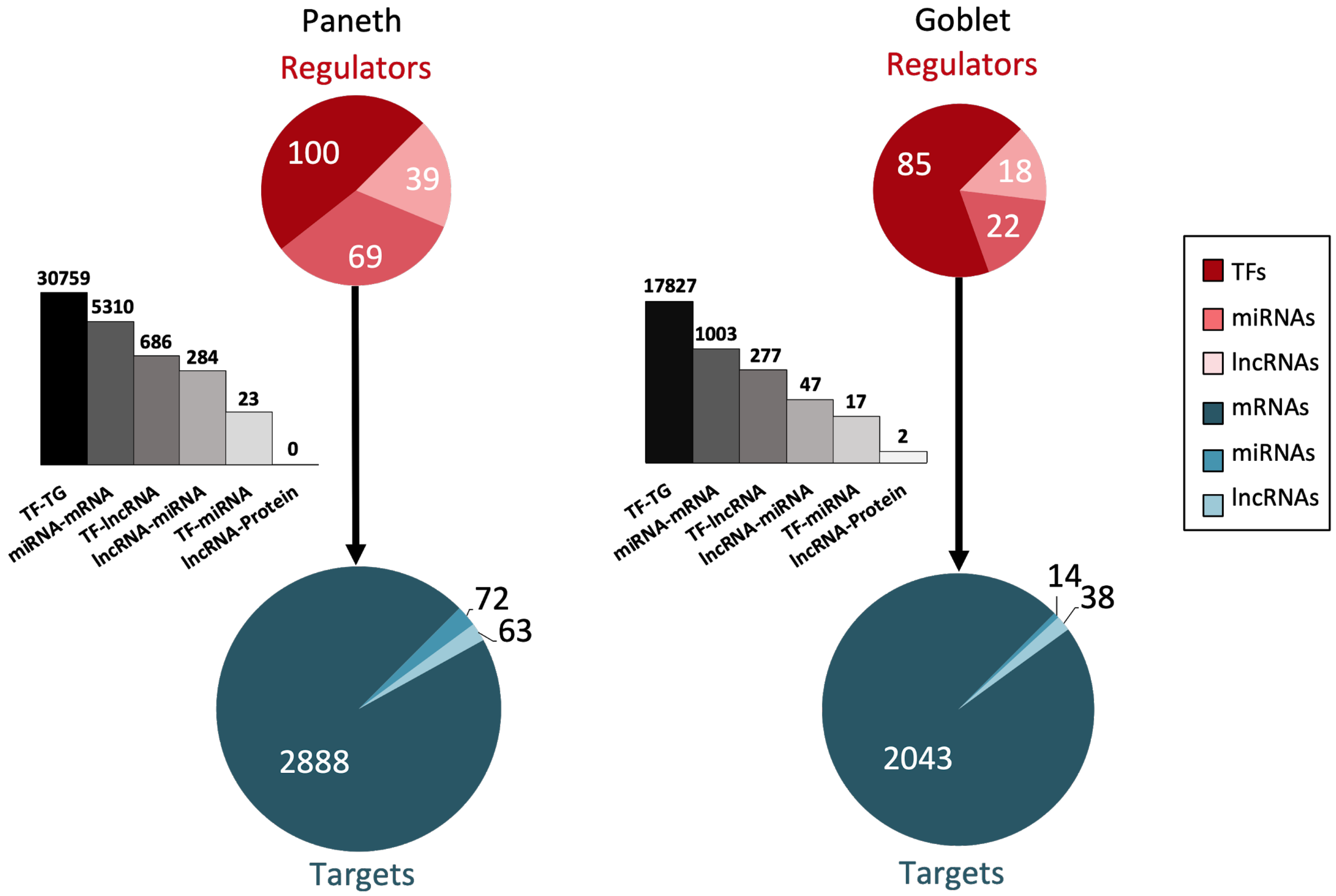
Interaction type	Source(s)	Number of unique interactions	Quality control criteria
TF-TG	TRRUST v2 GTRD	1066383	ChIP-Seq peaks should not overlap any gene annotation; if peak on + strand, only the first gene downstream to the gene or if peak on - strand, only first gene upstream to the peak is considered.
TF-lncRNA	GTRD	159055	ChIP-Seq peaks should not overlap any gene annotation; if peak on + strand, only the first gene downstream to the gene or if peak on - strand, only first gene upstream to the peak is considered.
miRNA-mRNA	TarBase v7.0	141892	Only HITS-CLIP based experimental evidence considered
TF-miRNA	TransmiR v1.2 TRRUST v2 GTRD	9204	ChIP-Seq peaks should not overlap any gene annotation; if peak on + strand, only the first gene downstream to the gene or if peak on - strand, only first gene upstream to the peak is considered.
lncRNA - miRNA	lncBase2	6637	Only HITS-CLIP based experimental evidence considered
lncRNA - protein	NPINTER v3.0	725	Only physical interactions considered; interactions involving RNA binding proteins removed



Number of differentially expressed genes.

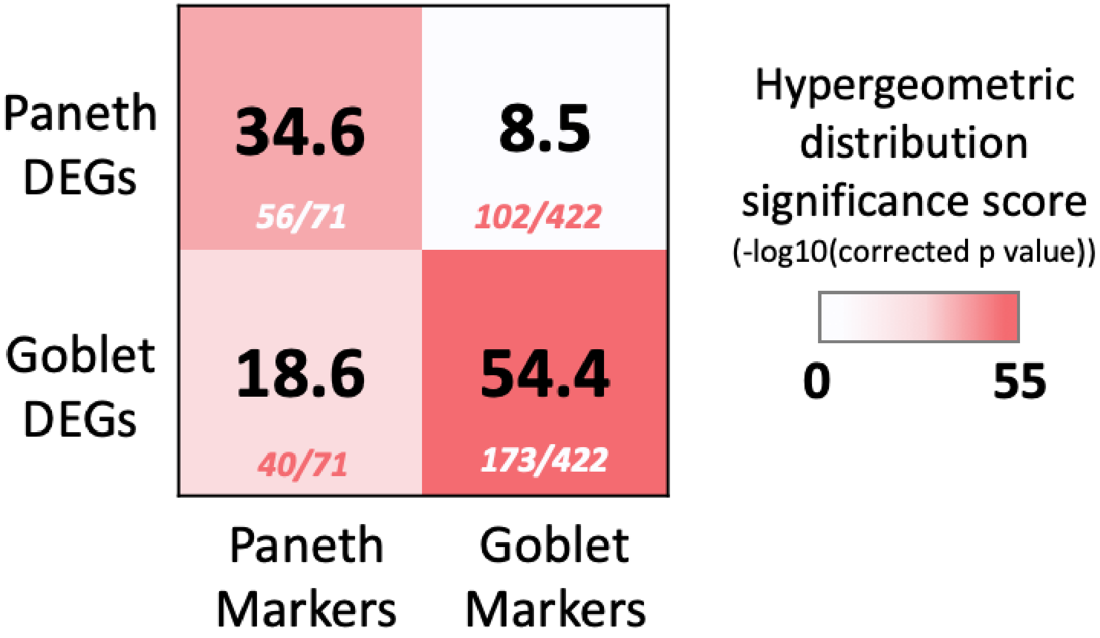
Differential expression was defined as log2 fold change > |1| and false discovery rate <= 0.05. Goblet = goblet enriched organoids compared to normally differentiated; Genes annotated as ‘other’ include pseudogenes and antisense genes.

Composition of regulatory networks



All regulators in the networks are shown in the red circles, and all targets in the blue circles.

In black bar charts are the types of regulatory interactions, most of which are transcription factor target gene interactions.

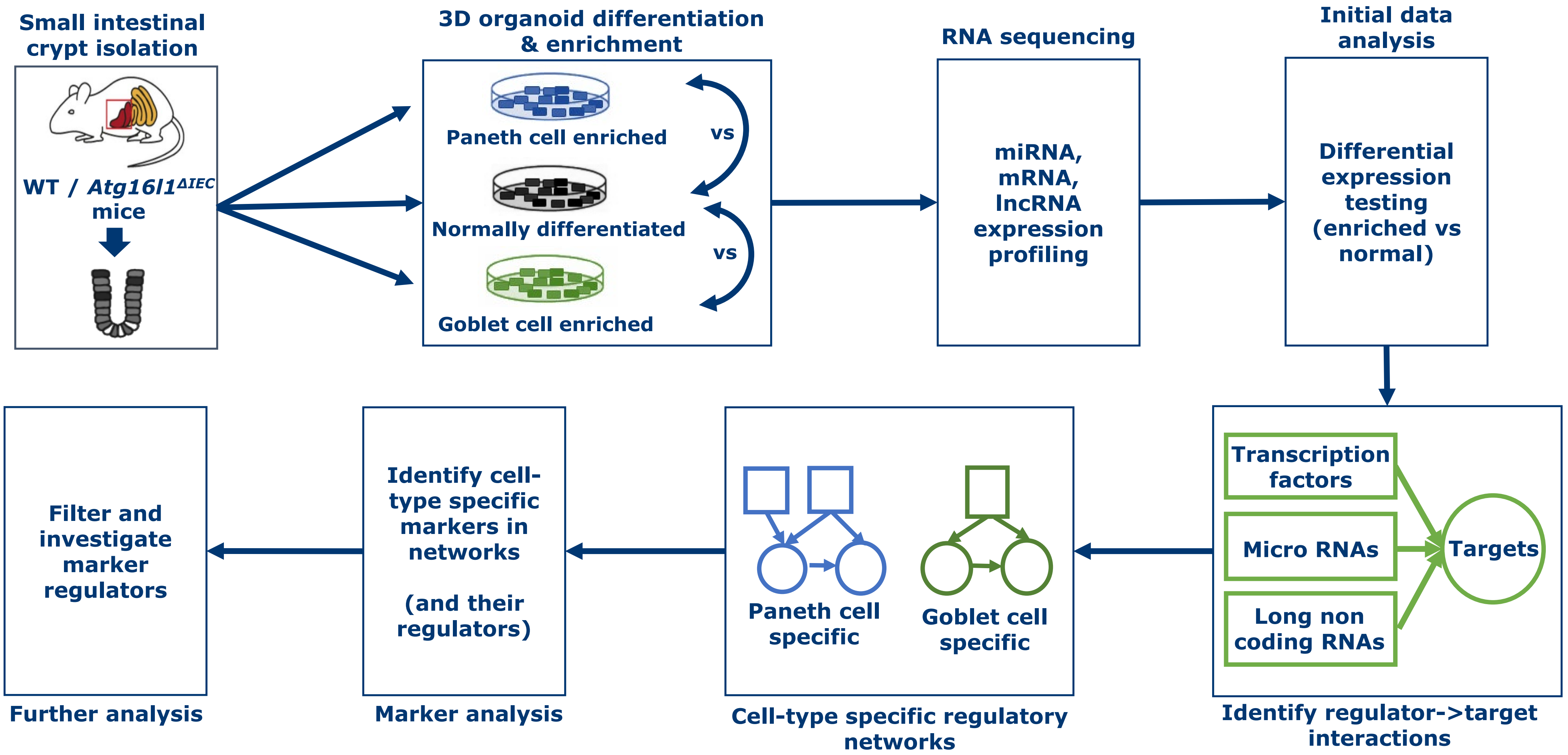


	Paneth Markers	Goblet Markers
Paneth DEGs	34.6 56/71	8.5 102/422
Goblet DEGs	18.6 40/71	54.4 173/422

Enrichment of cell-type specific marker genes in the differentially expressed gene (DEG) lists.

Higher significance scores indicate greater enrichment. Number of markers in DEG list out of total number of markers shown below significance score.

Workflow



Small intestinal crypt isolation

WT / *Atg16l1*$^{\Delta IEC}$ mice

3D organoid differentiation & enrichment

Paneth cell enriched

vs

Normally differentiated

vs

Goblet cell enriched

RNA sequencing

miRNA, mRNA, lncRNA expression profiling

Initial data analysis

Differential expression testing (enriched vs normal)

Transcription factors

Micro RNAs

Long non coding RNAs

Targets

Identify regulator->target interactions

Paneth cell specific

Goblet cell specific

Cell-type specific regulatory networks

Identify cell-type specific markers in networks

(and their regulators)

Marker analysis

Filter and investigate marker regulators

Further analysis