Article

Cell Metabolism

Identification of Natural RORγ Ligands that Regulate the Development of Lymphoid Cells

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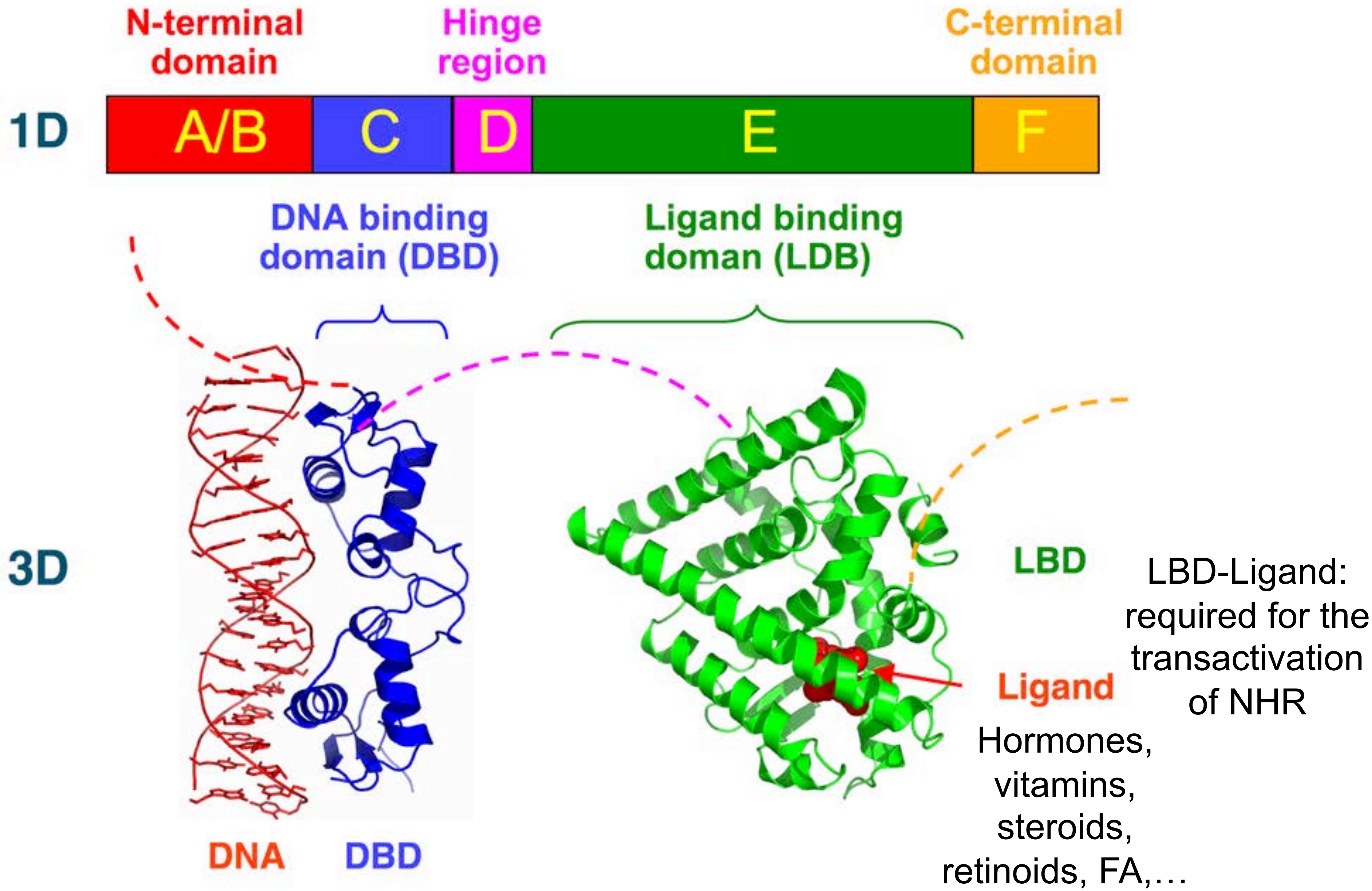
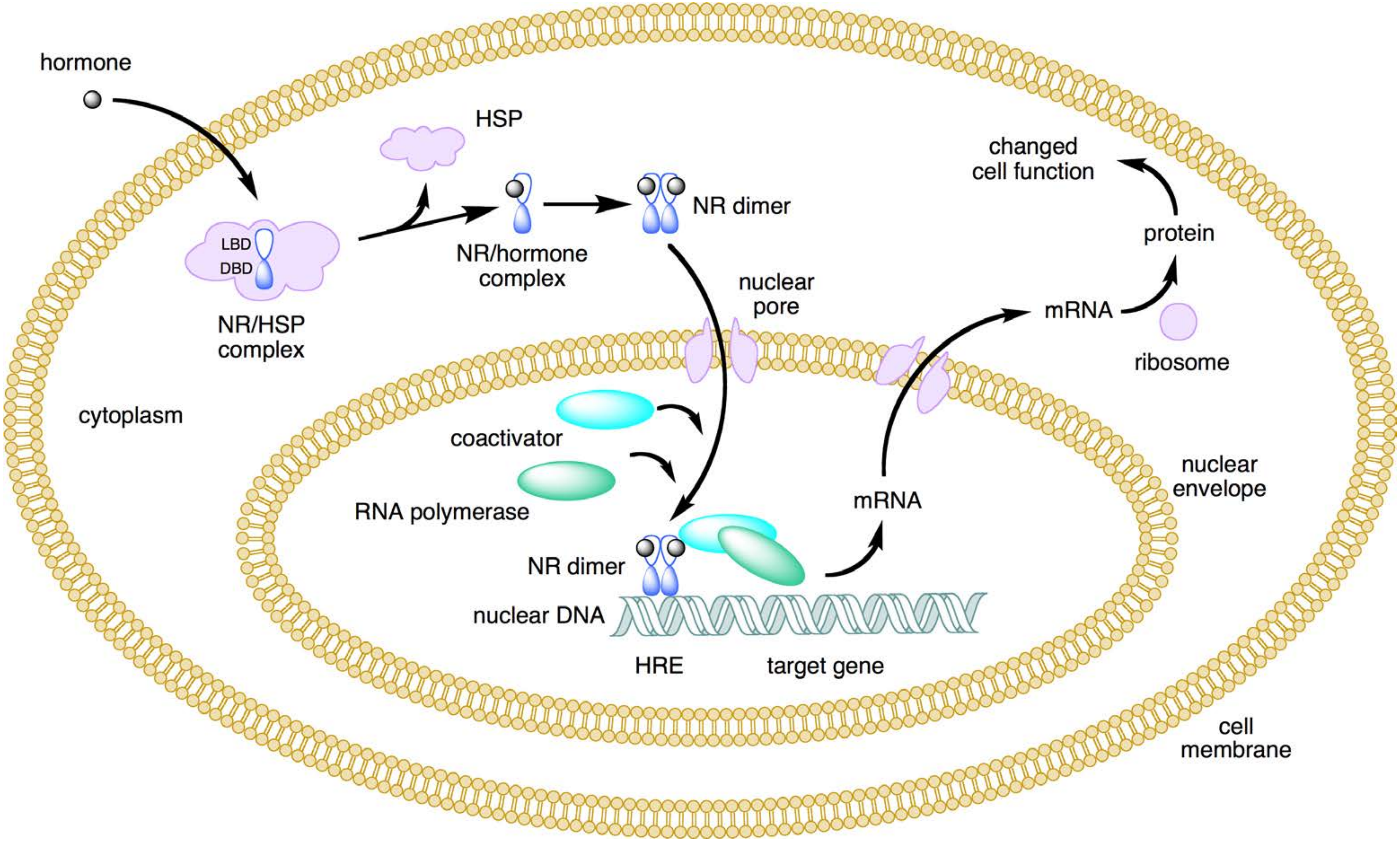
Structural Organization of Nuclear Receptors



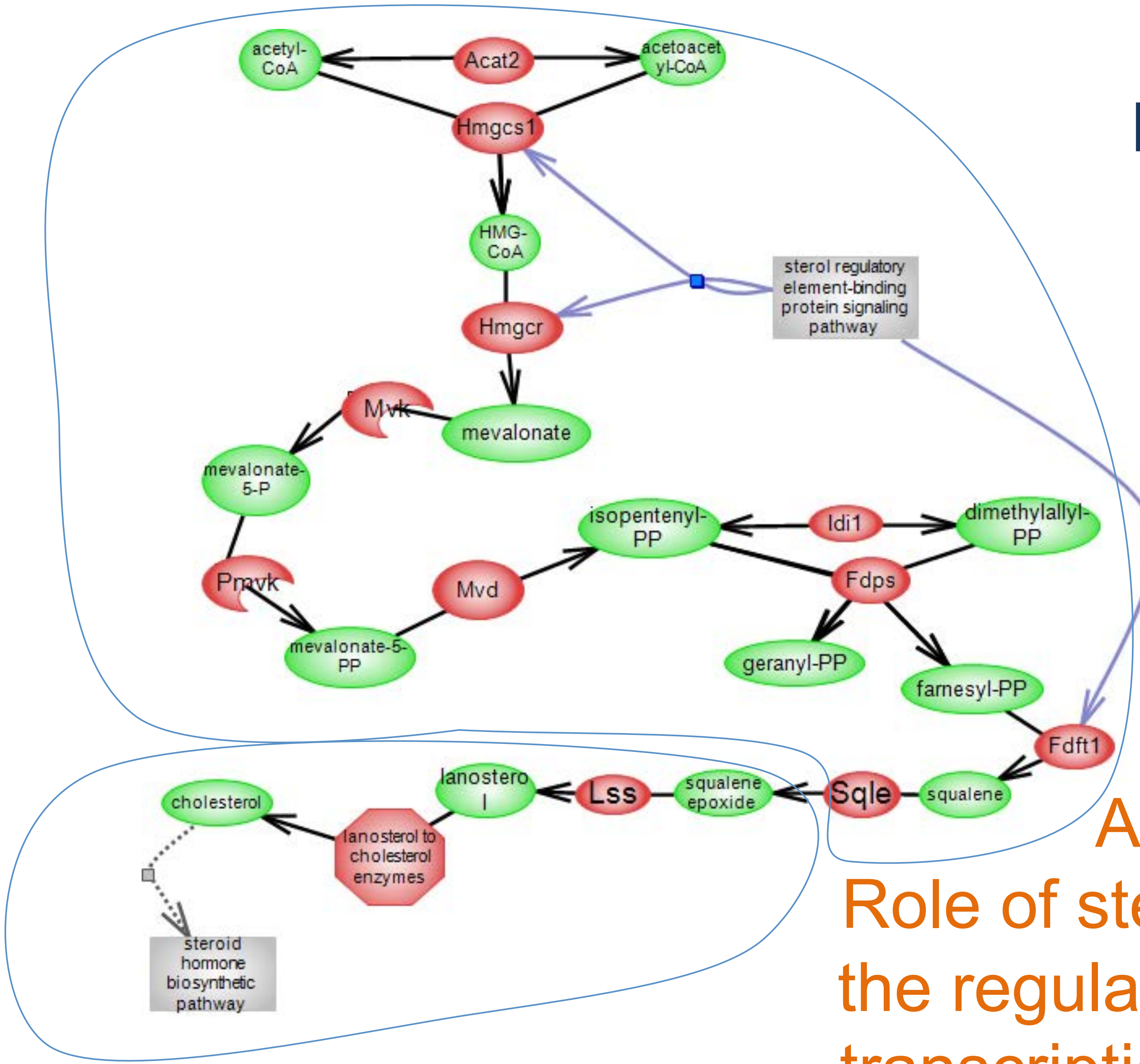
Nuclear hormone receptor transcriptional activity



RORg

- TF broadly express in human and mouse tissues
- Rorgt: isoforme express in lymphoid tissues. Essential for the development of tymocytes, LN, GALT, Th17 and some ILC
- Have a large ligand-bind pocket (>700 $Å^3$)
- RORa crystalize with the cholesterol being involve in their transcriptional activity
- RORb crystalize with stearate abd all-trans retinoic acid. And co-crystalize with FA and retinoids
- Rorg crystalize with oxysterols and vit D derivates

Cholesterol Biosynthesis



AIM:

Role of sterol lipids in the regulation of Rorg transcriptional activity

- Insect cells are auxotrophic for polysaturated fatty acids, retinoids and sterols (diet sources).
- However, some insect can grown in lipid depleted media

Chemically defined medium (CDM) to culture:
- *Drosophila melanogaster* S2 cells
- or Kc167 cells

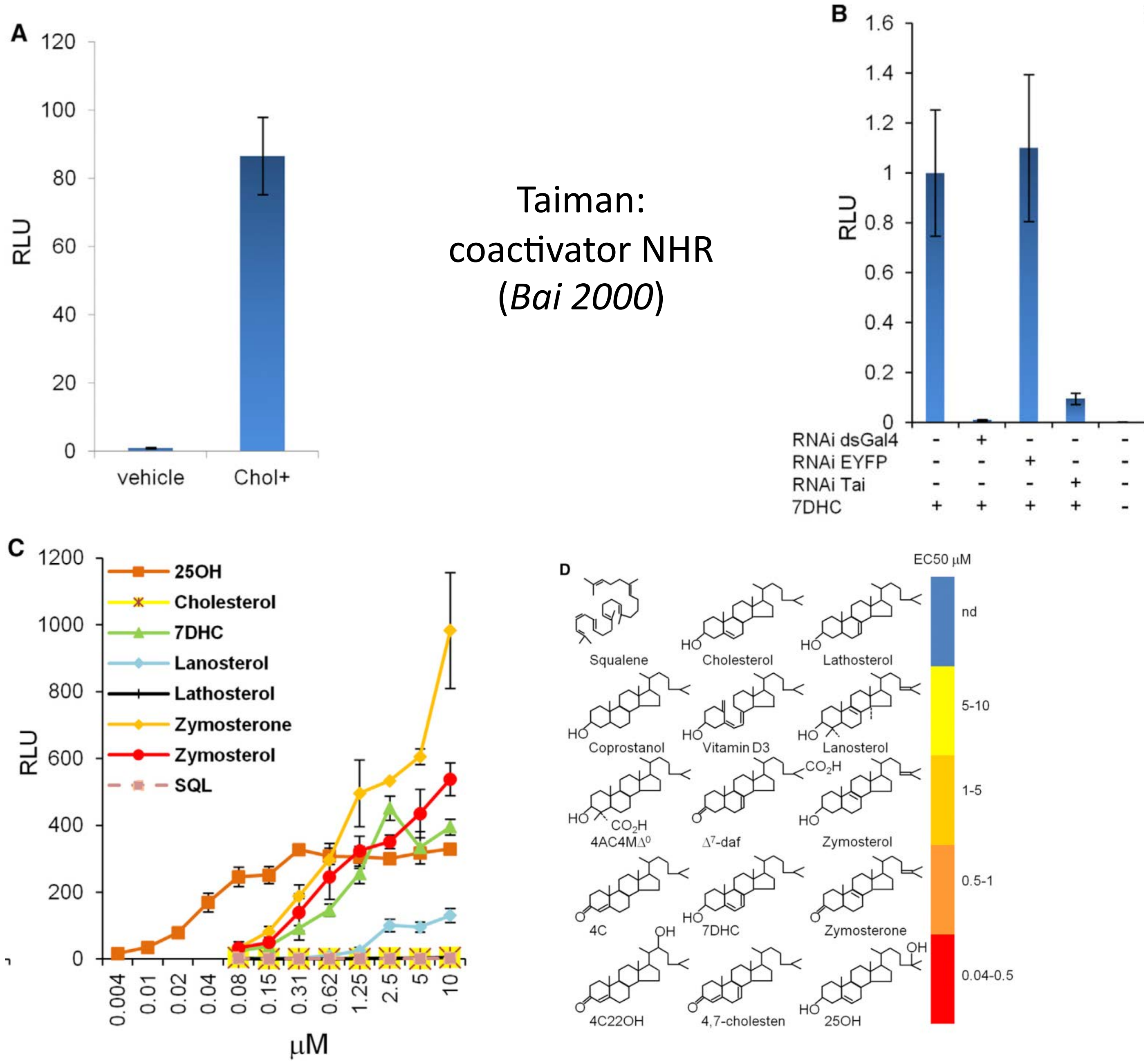
Insect grow in FSC display strong RORγ transcriptional activity (*Huh 2011*)

Which is the ligand of RORγ nuclear hormone receptor ?

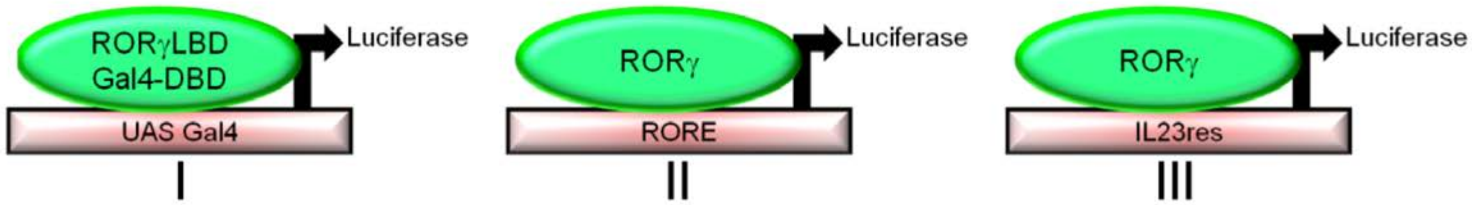
Sterols induced RORγt activity in insect cells

Rorγ-gal4/UAS
luceferase reporter
D.m Kc167 cells

Taiman:
coactivator NHR
(*Bai 2000*)

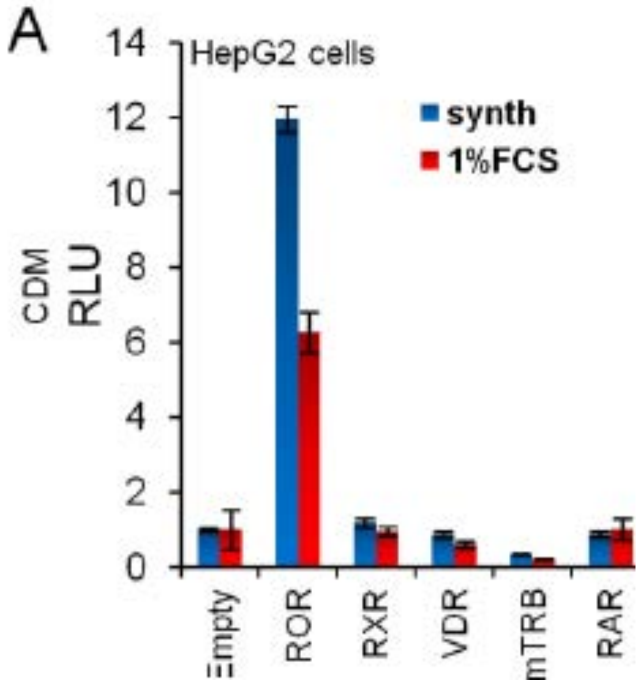


The ligand of Rorgt is a common basal metabolite

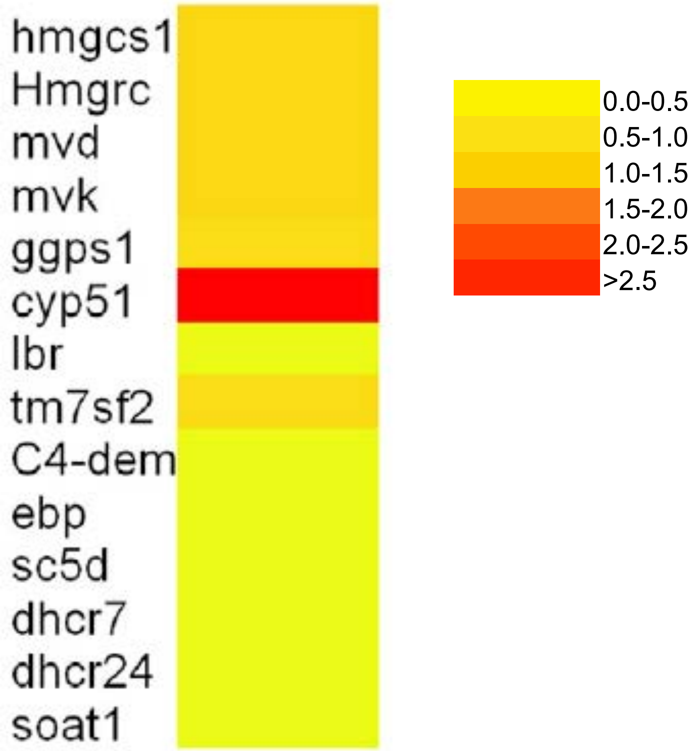


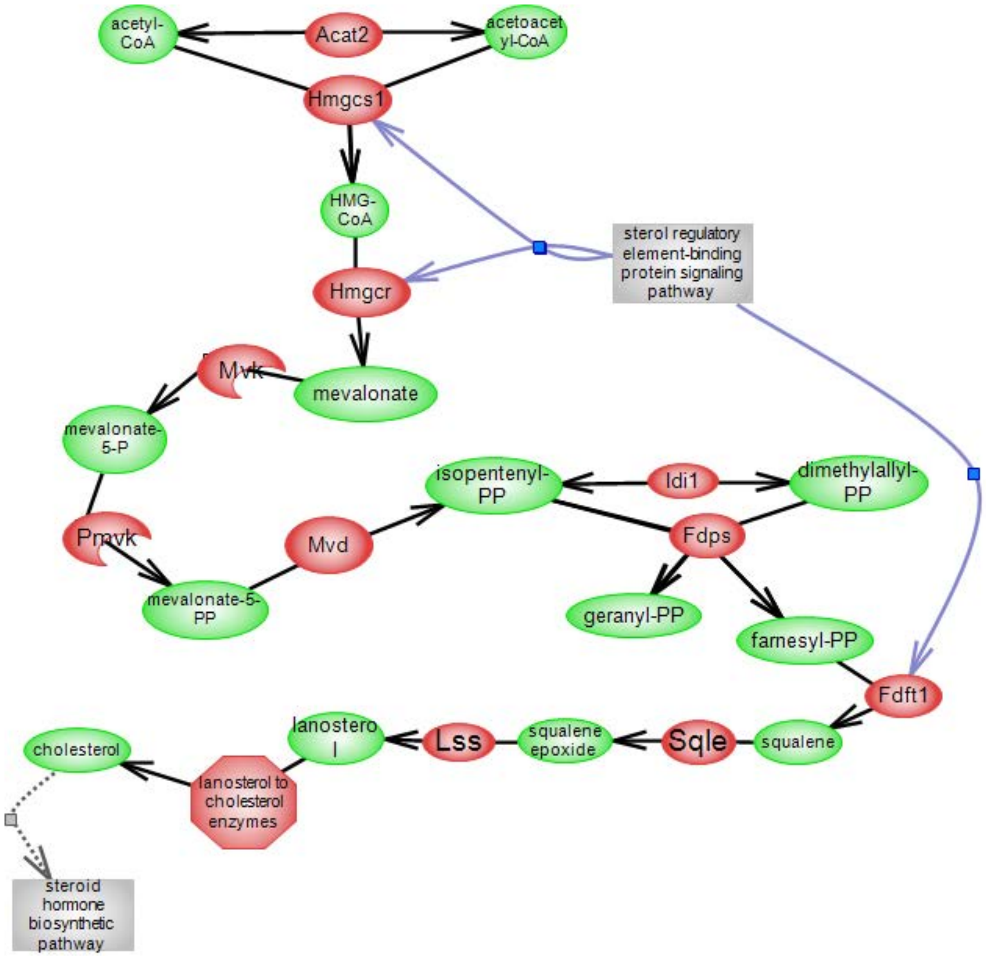
Auxotrophic and Essential Metabolic Pathways in Insect Cells	Insect Cells
fatty acid synthesis	essential in CDM >90% conserved with mammals
glycerophospholipids	essential in CDM >90% conserved with mammals
Purine biosynthesis	essential in CDM >90% conserved with mammals
Pyrimidine biosynthesis	essential in CDM >90% conserved with mammals
glycolisis	essential in CDM >90% conserved with mammals
Krebs Cycle	essential in CDM >90% conserved with mammals
PUFAs	auxotroph
retinoids	auxotroph
Cholesterol	auxotroph
Bile Acids	auxotroph
steroid hormones	auxotroph

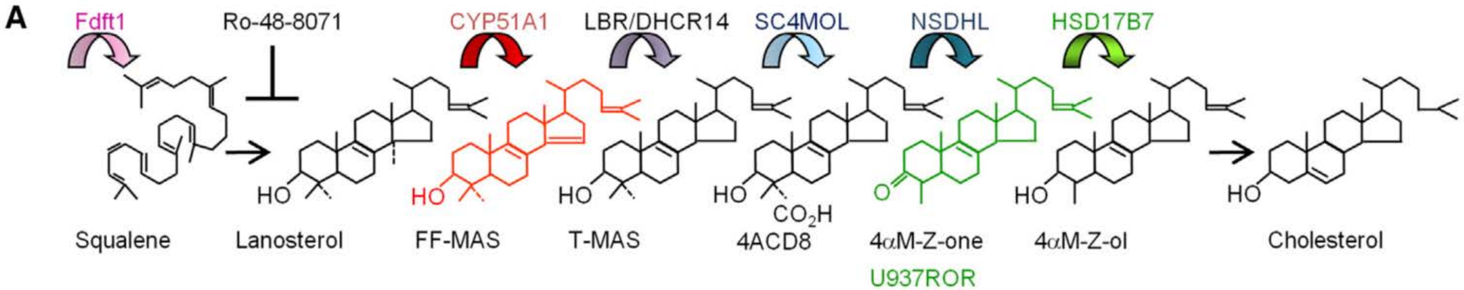
Rorγ-gal4/UAS
luceferase reporter
HepG2 cells



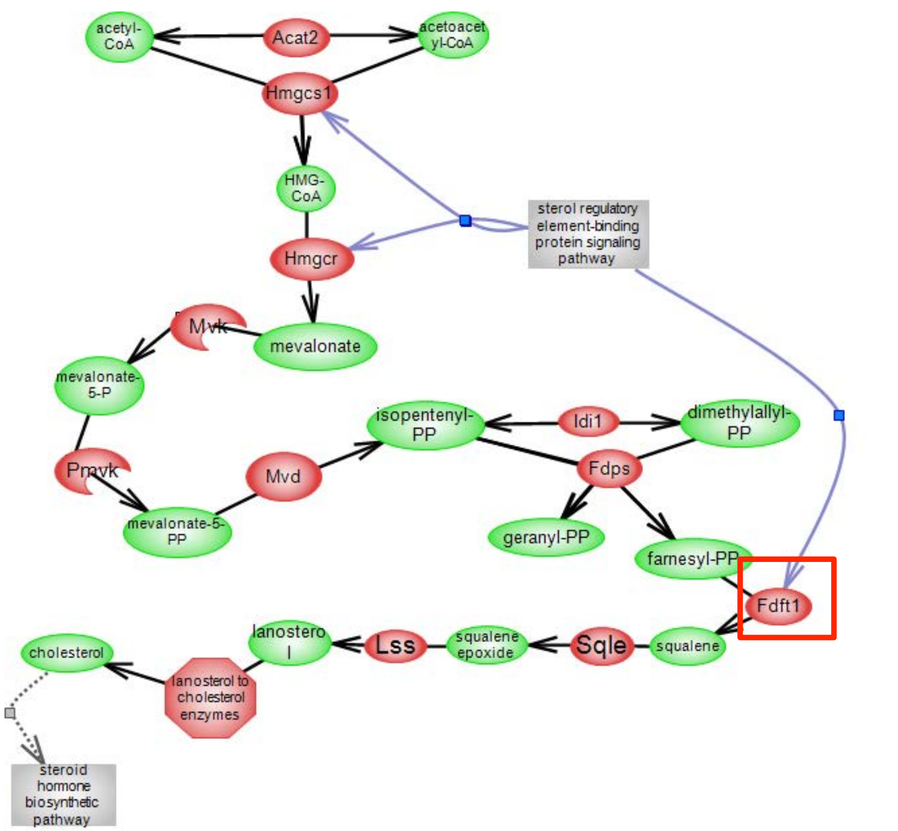
RORγt activity modulated by cholesterol biosyntheses enzymes



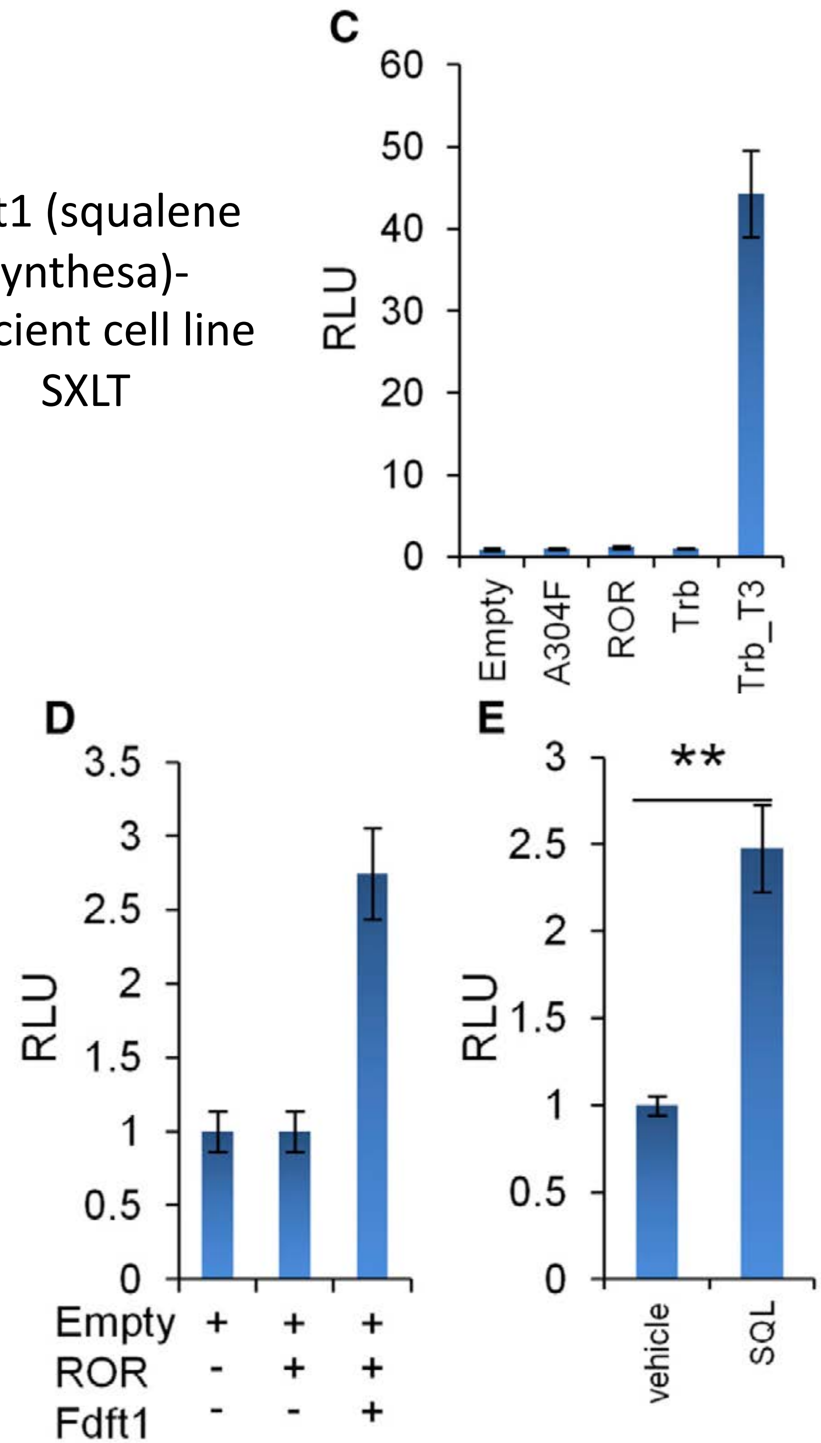




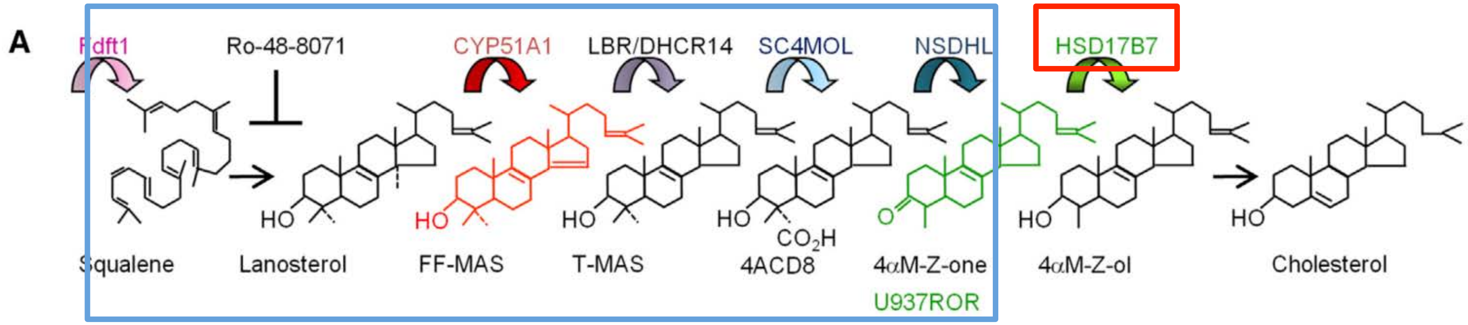
RORγt activity related to cholesterol biosyntheses pathway

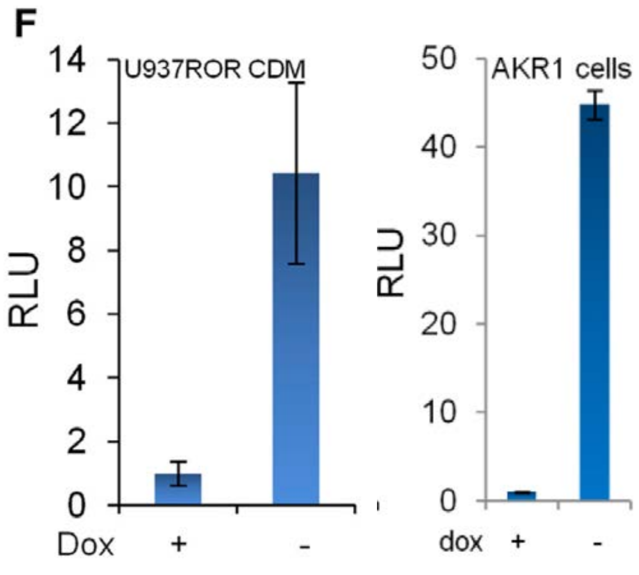


Fdft1 (squalene synthesa)-deficient cell line SXLT

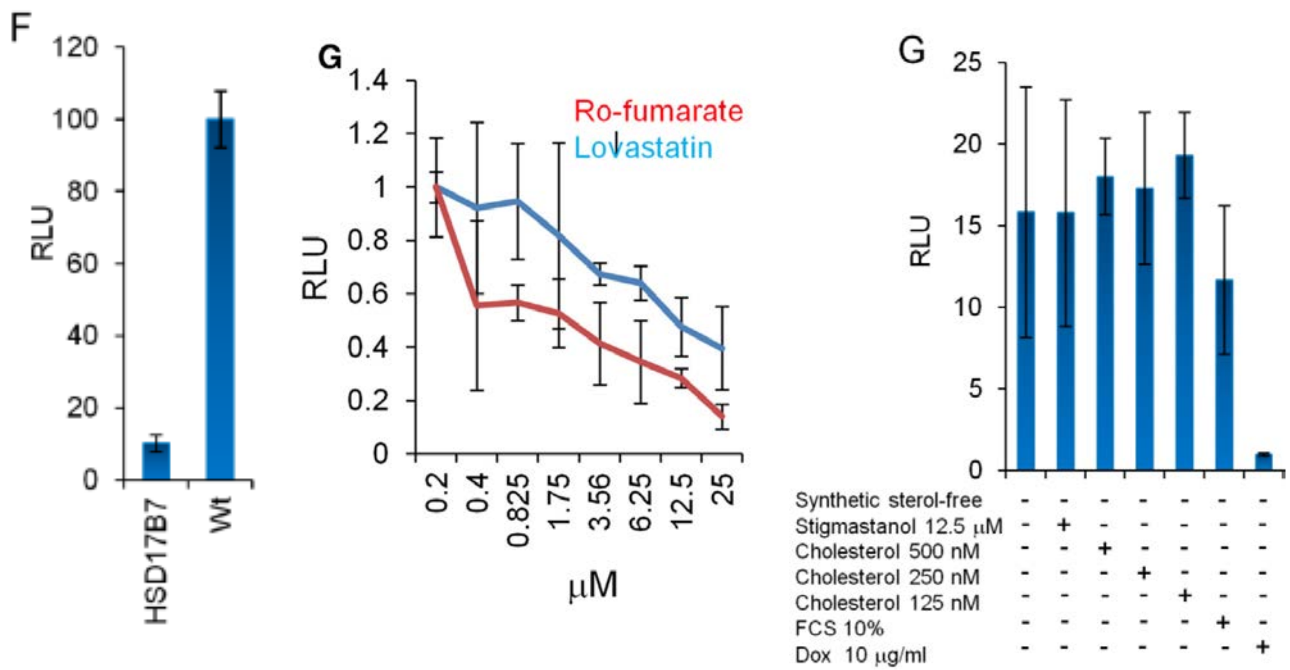


Endogenous Rorg ligand is dowstream of lanosterol and upstream of 4aM-Z.one

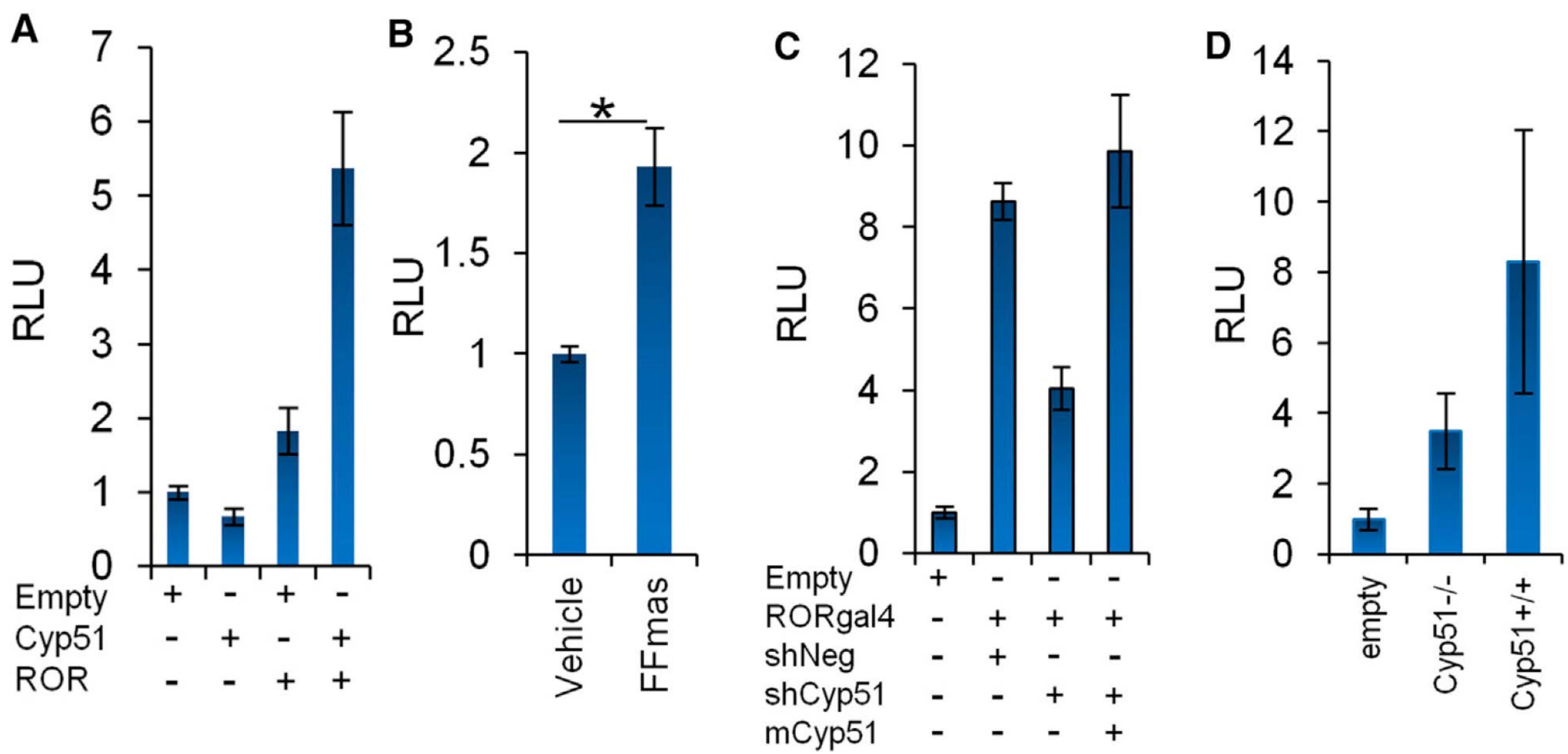


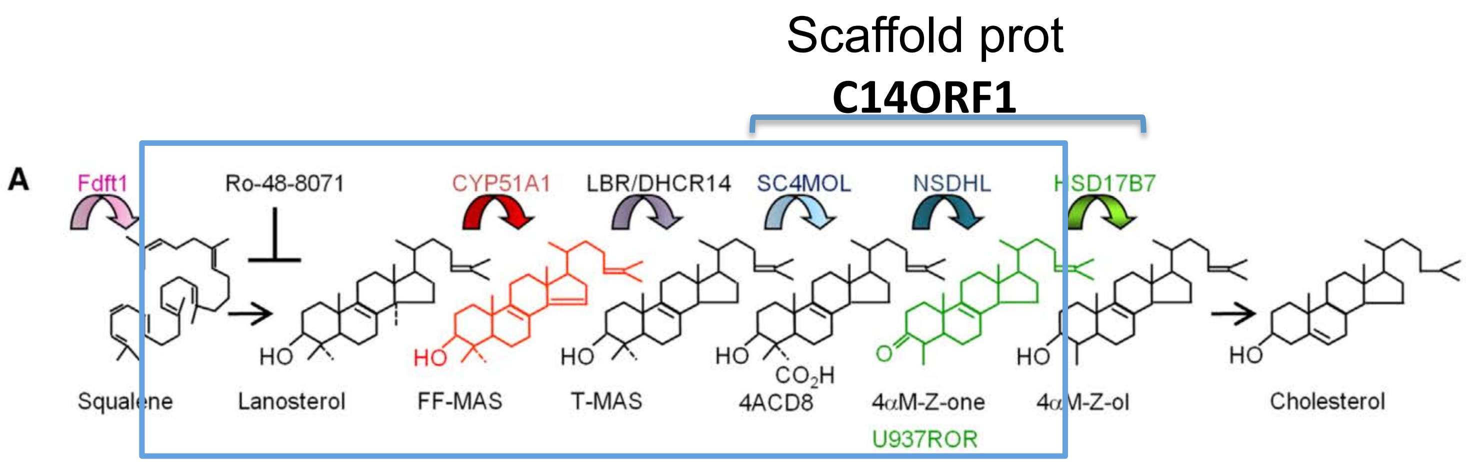


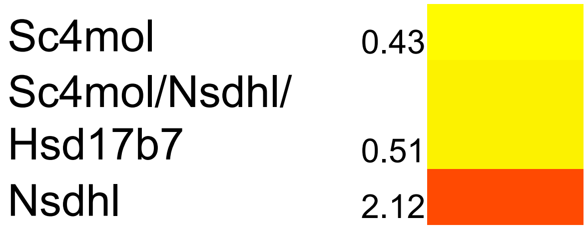
U937 deficient
in HSD17B7

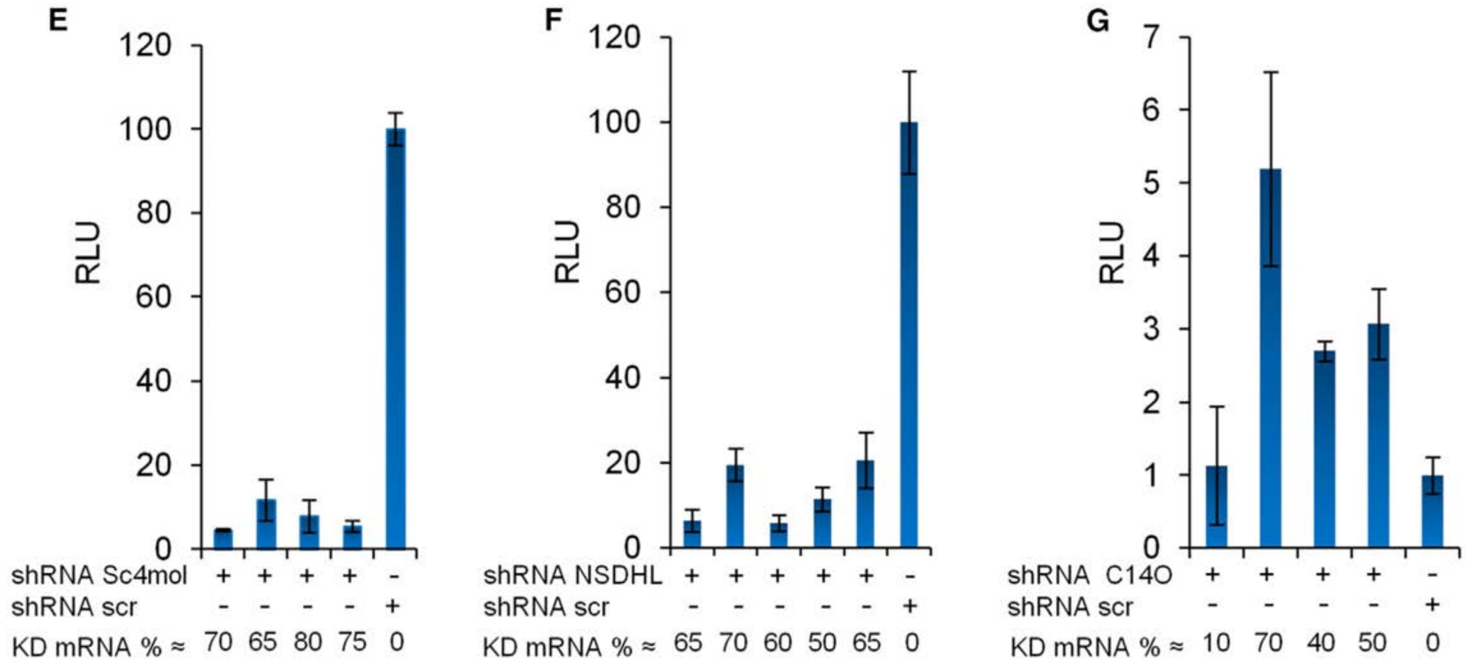


Rorgt ligand is the product of either CYP51 or a downstream enzyme

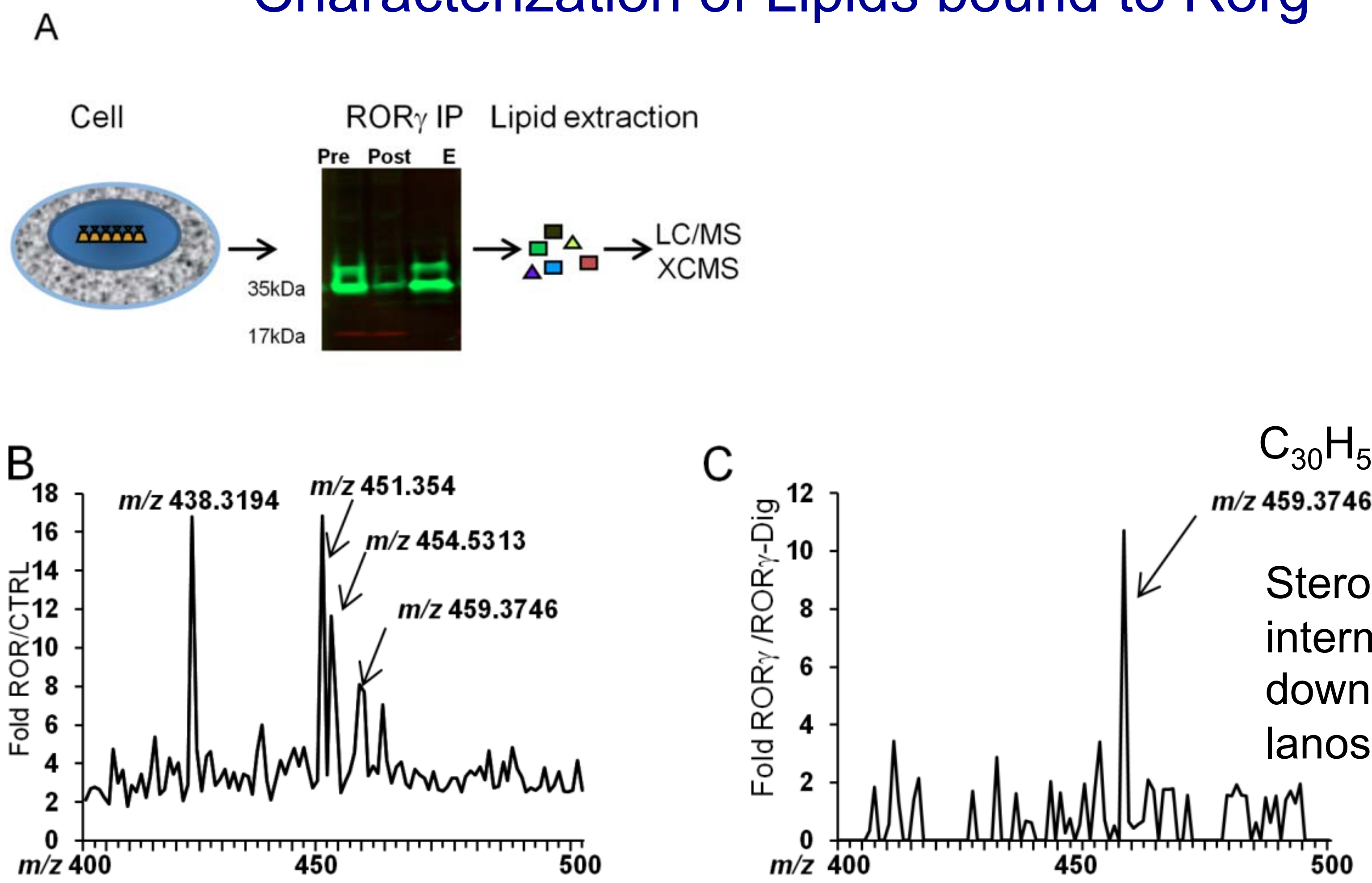



Scaffold prot
C14ORF1
A
Fdft1
Ro-48-8071
CYP51A1
LBR/DHCR14
SC4MOL
NSDHL
HSD17B7
HO
CO2H
O
Squalene
Lanosterol
FF-MAS
T-MAS
4ACD8
4αM-Z-one
U937ROR
4αM-Z-ol
Cholesterol


Sc4mol
Sc4mol/Nsdhl/
Hsd17b7
Nsdhl
0.43
0.51
2.12


E
F
G
RLU
120
100
80
60
40
20
0
7
6
5
4
3
2
1
shRNA Sc4mol + + + + -
shRNA scr - - - - +
KD mRNA % ≈ 70 65 80 75 0
shRNA NSDHL + + + + + -
shRNA scr - - - - - +
KD mRNA % ≈ 65 70 60 50 65 0
shRNA C14O + + + + -
shRNA scr - - - - +
KD mRNA % ≈ 10 70 40 50 0

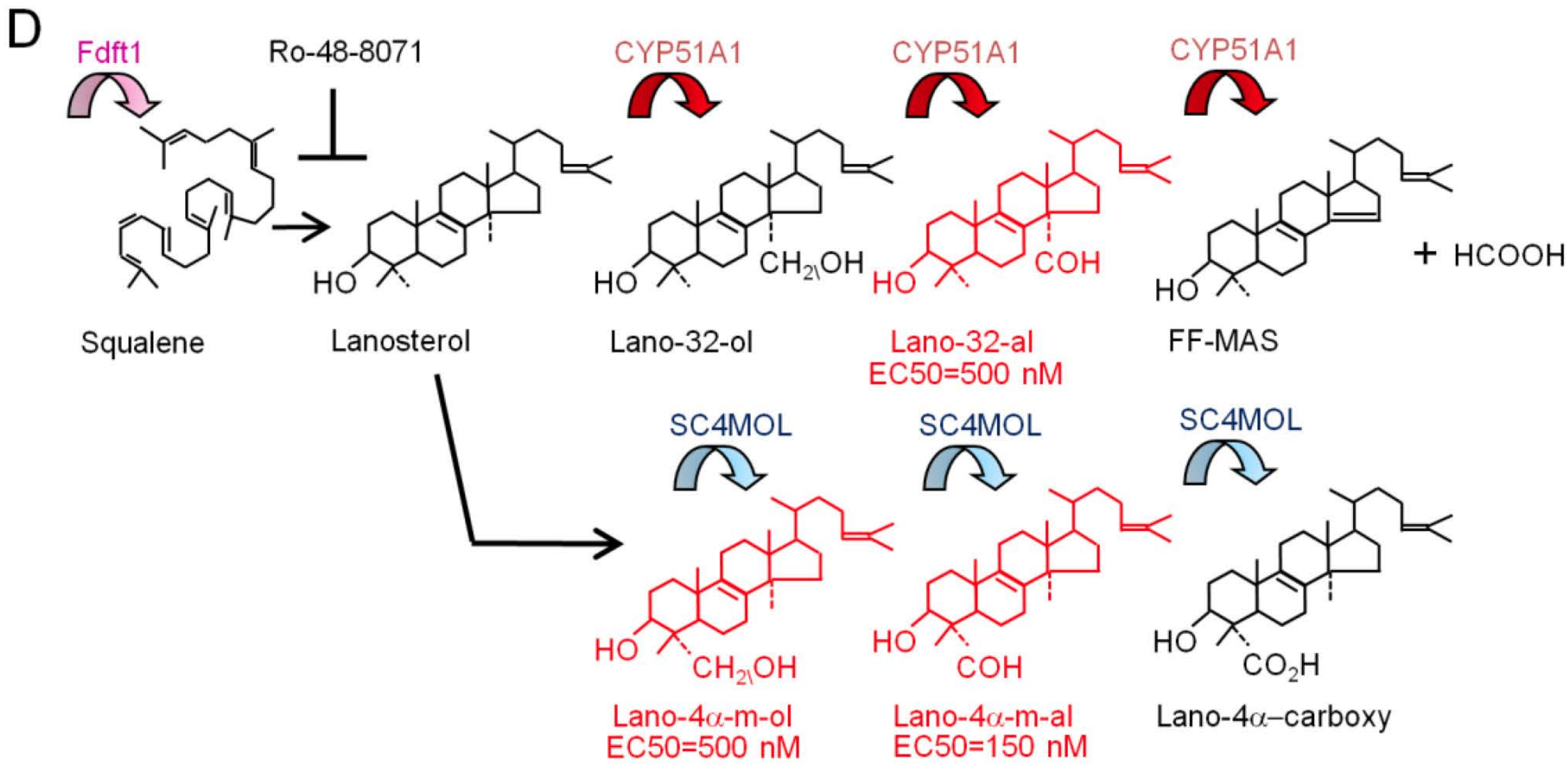
Characterization of Lipids bound to Rorg



$C_{30}H_{50}O_3$

Sterol intermediates down-stream of lanosterol

Sterol lipids are potential RORg Ligands



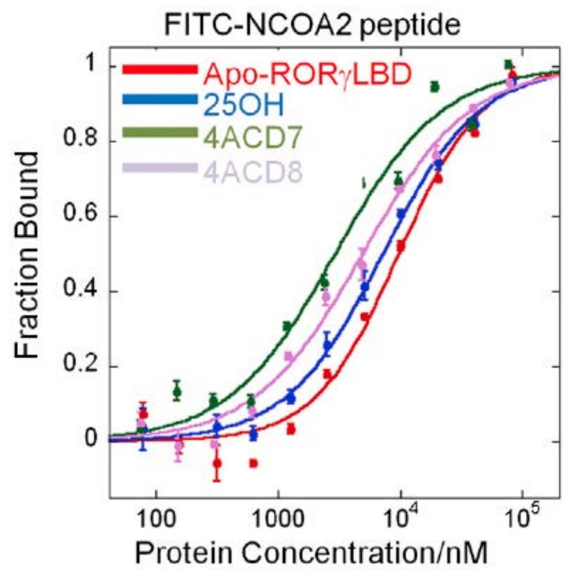
Sterol lipids are RORg Ligands

A

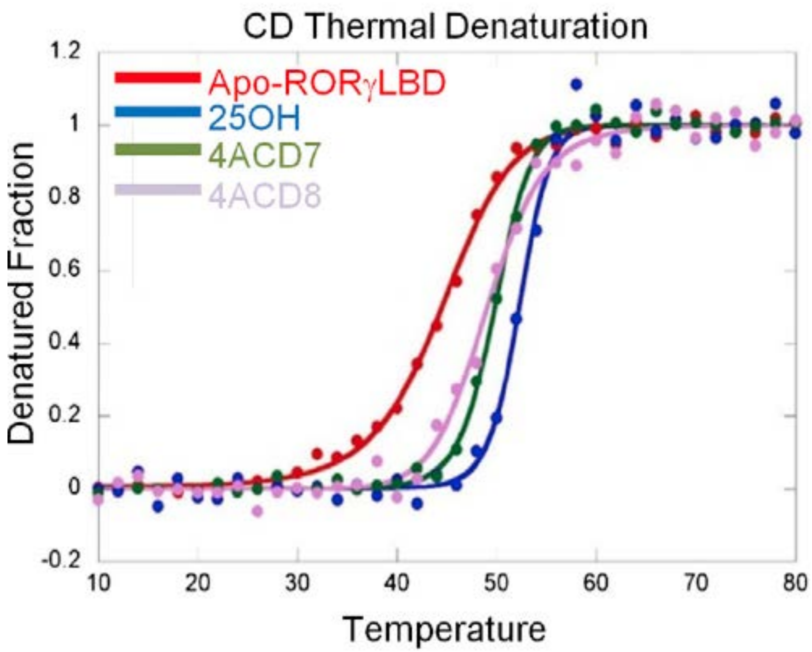
Binding affinities and tissue concentrations of CBIs

Compound	Affinity (nM)	Tissue cc (μM)*
Lanosterol	ND	1
FF-MAS	ND	0.1
T-MAS	ND	2
4ACD8	143	0.1
4AOHD7	NT	0.05
4a-M-Z-ol	>1000	NT
3KZ	50	NT
Zymosterol	50	0.65
7DHC	NT	2.5
Cholesterol	300	6500
25OH	50	0.05

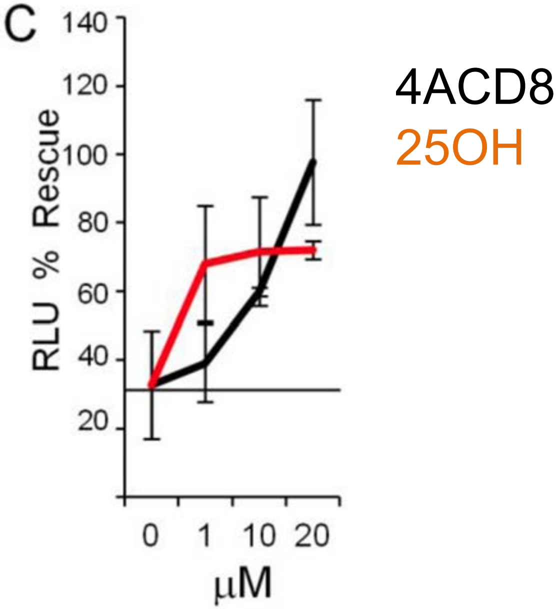
C

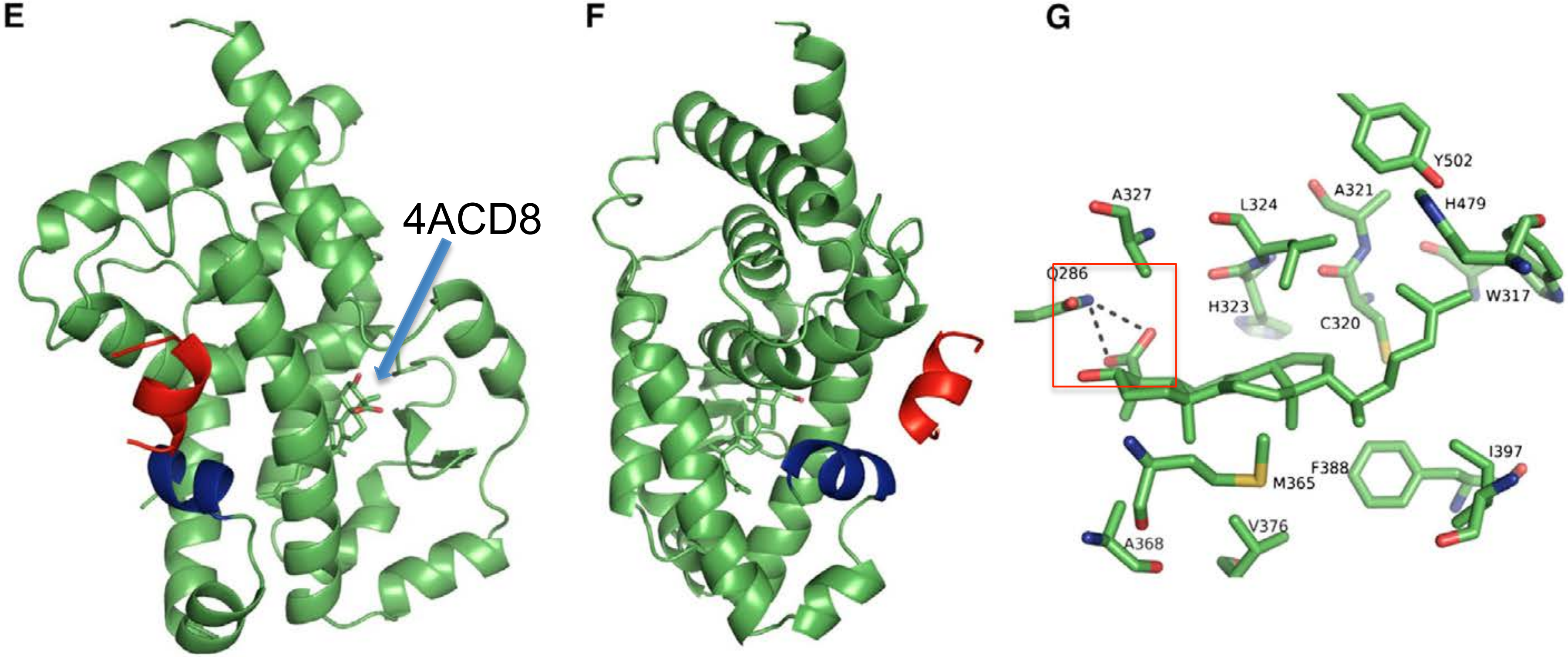


D



C

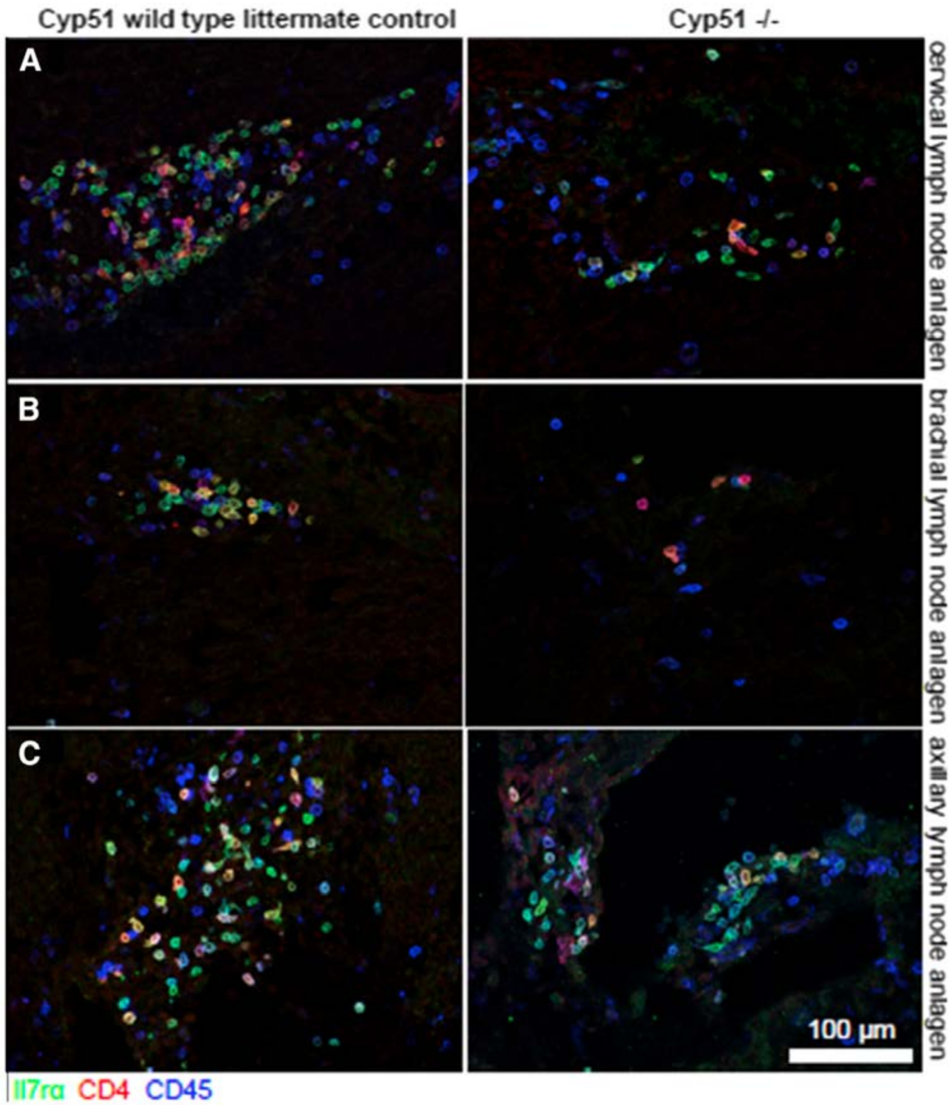


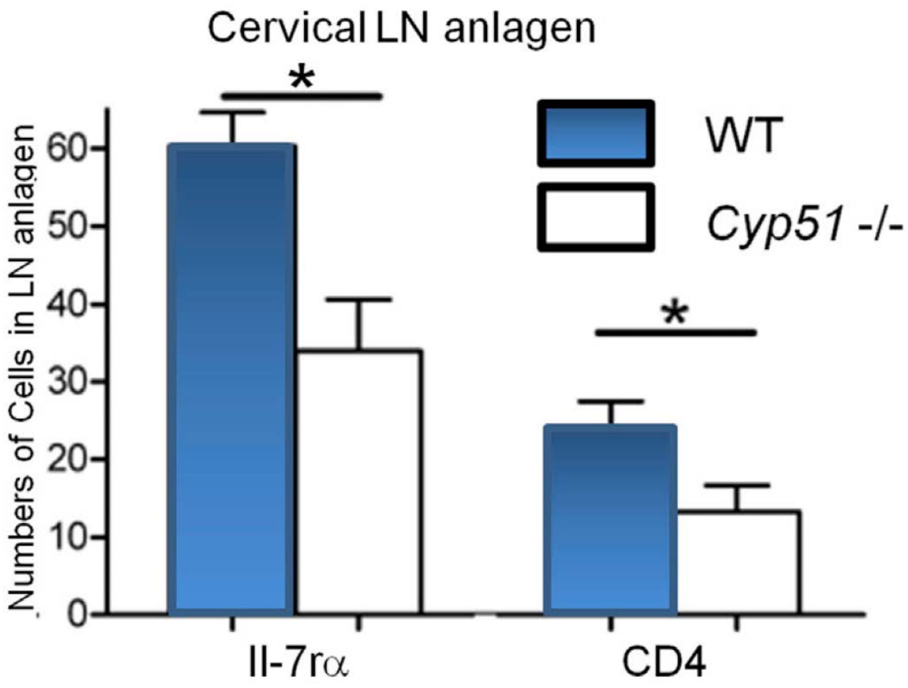


Rorg LBD
Coactivator peptide
Helix 12

Deficiencies in the cholesterol biosynthetic pathways affect lymph node development

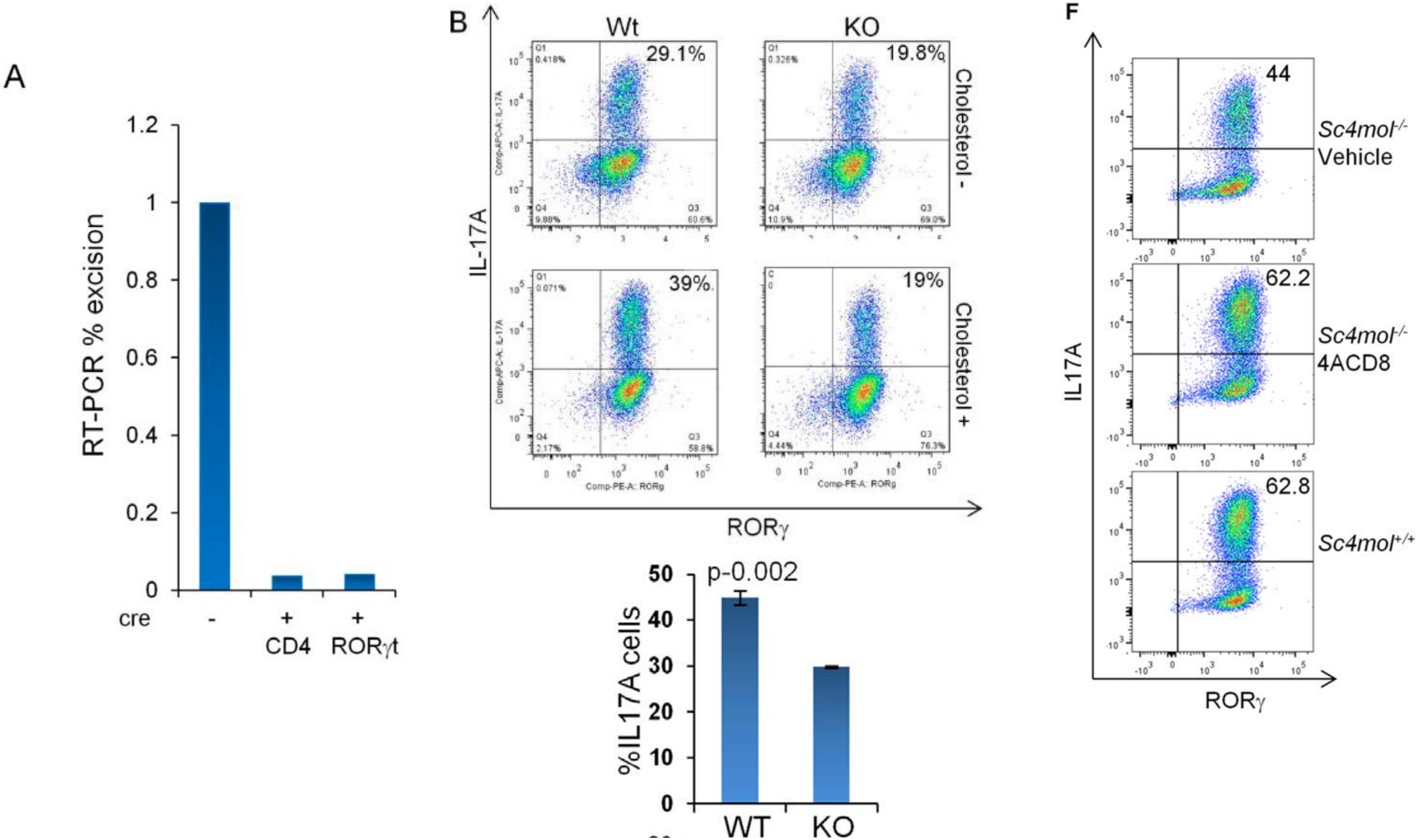
E14.5

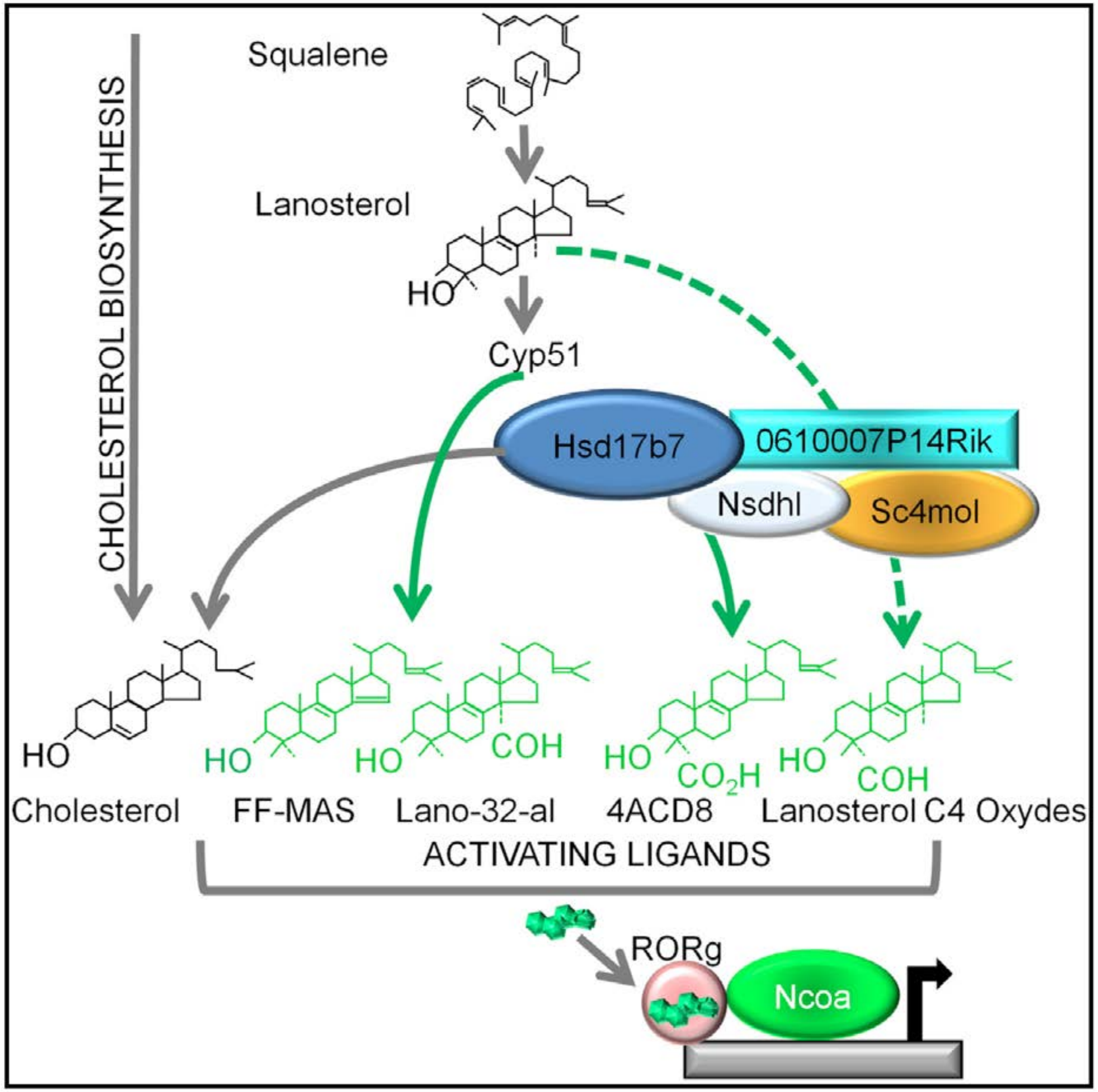




Deficiencies in the cholesterol biosenthetic patways affect Th17 cell differentiation

Sc4mol$^{f/f}$ mice x CD4cre & Rorgt-cre
(T and LTi cells deficiency)

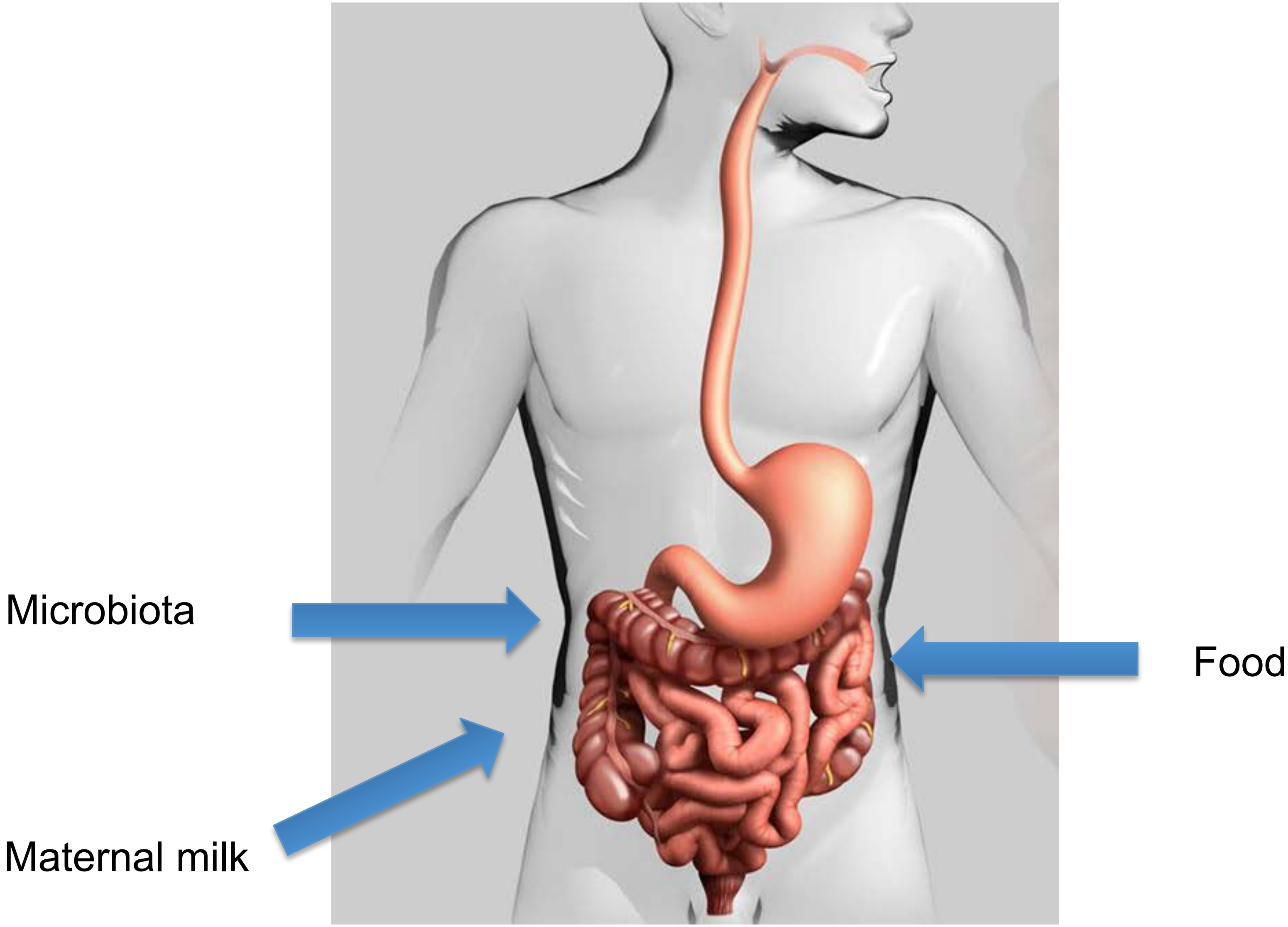


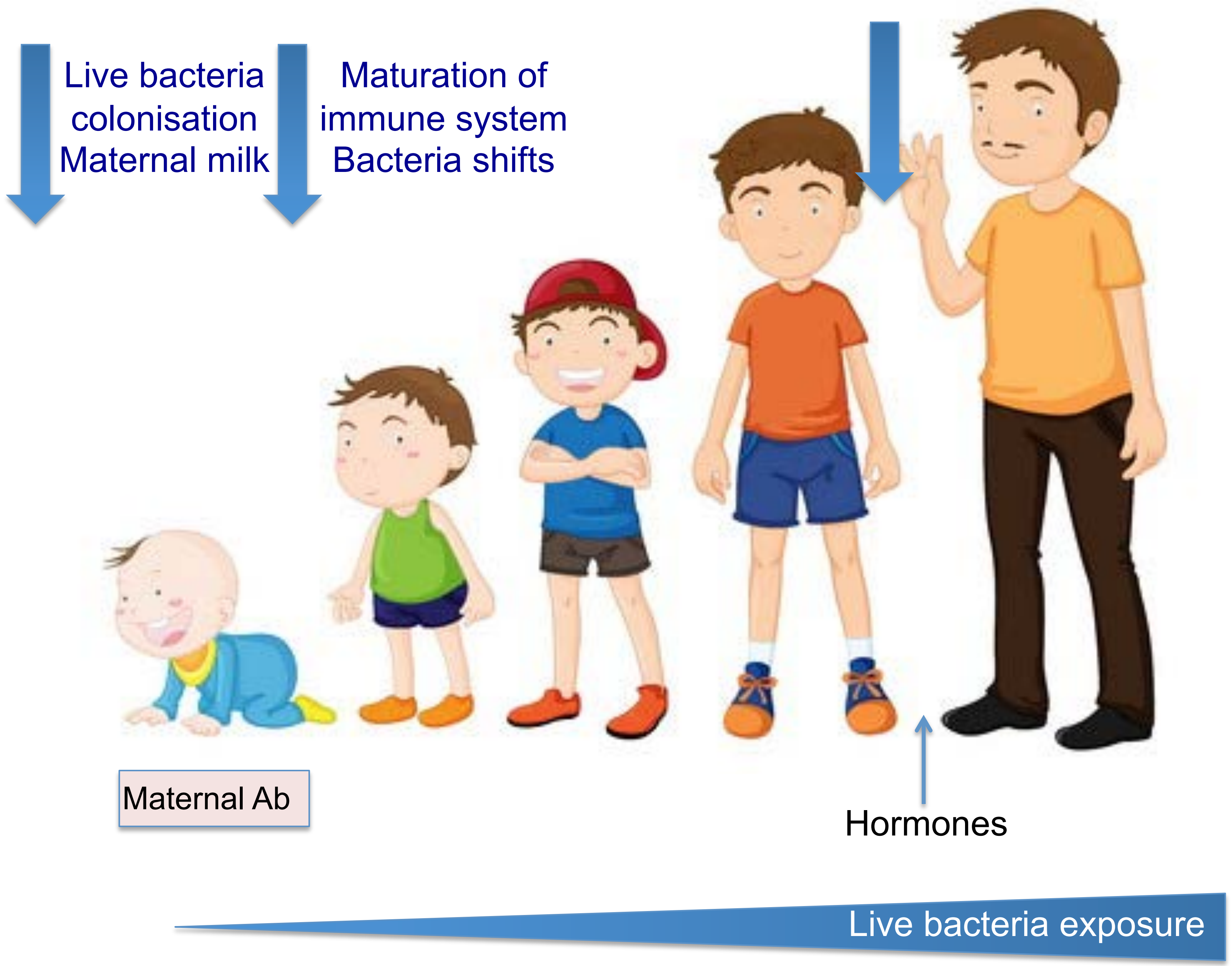


CHOLESTEROL BIOSYNTHESIS
Squalene
Lanosterol
HO
Cyp51
Hsd17b7
0610007P14Rik
Nsdhl
Sc4mol
HO
HO
HO
COH
HO
CO2H
HO
COH
Cholesterol
FF-MAS
Lano-32-al
4ACD8
Lanosterol C4 Oxydes
ACTIVATING LIGANDS
RORg
Ncoa

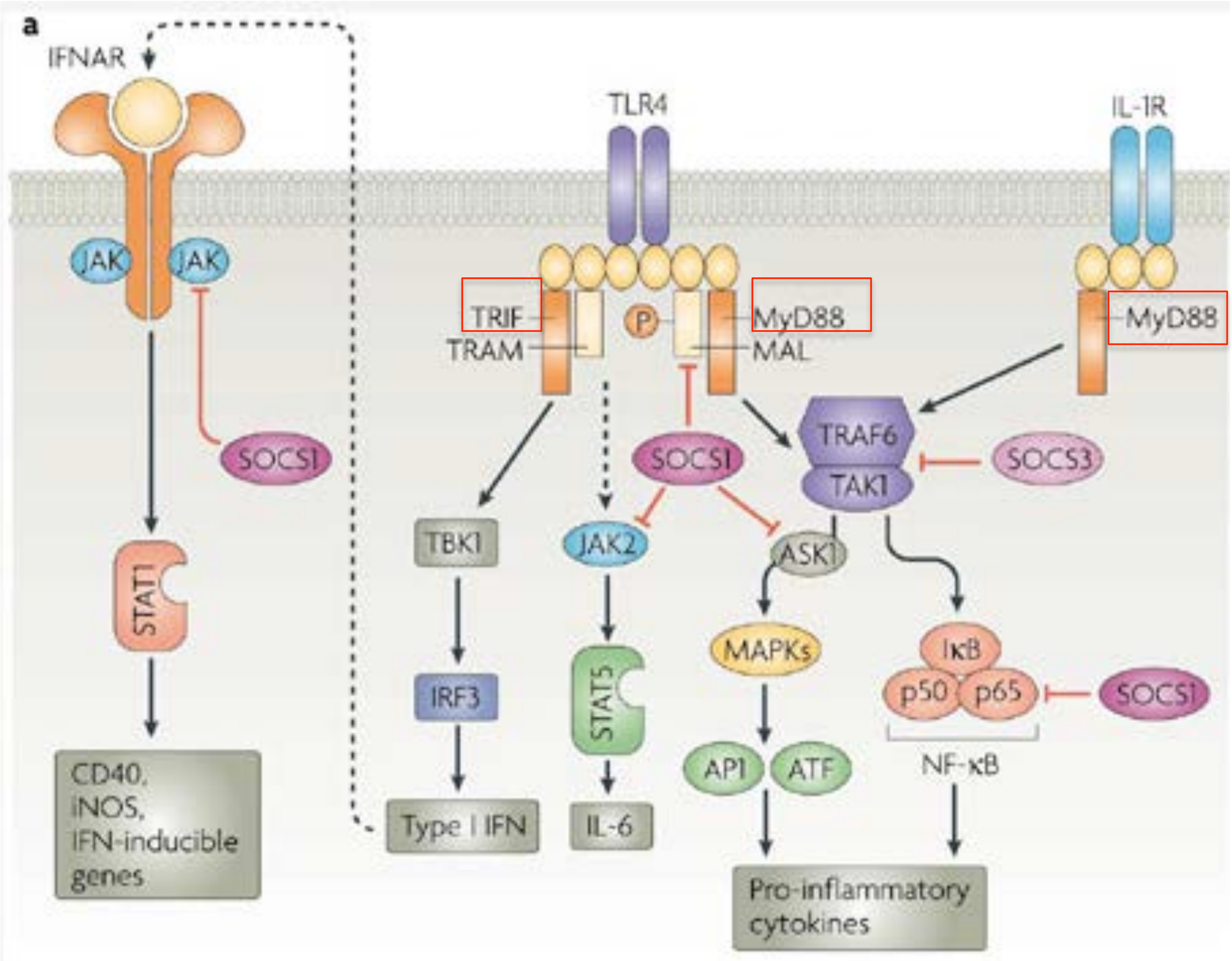
Analysis of gene–environment interactions in postnatal development of the mammalian intestine

Seth Rakoff-Nahoum[a,b,c,1], Yong Kong[d], Steven H. Kleinstein[e], Sathish Subramanian[f], Philip P. Ahern[f], Jeffrey I. Gordon[f], and Ruslan Medzhitov[a,b,1]

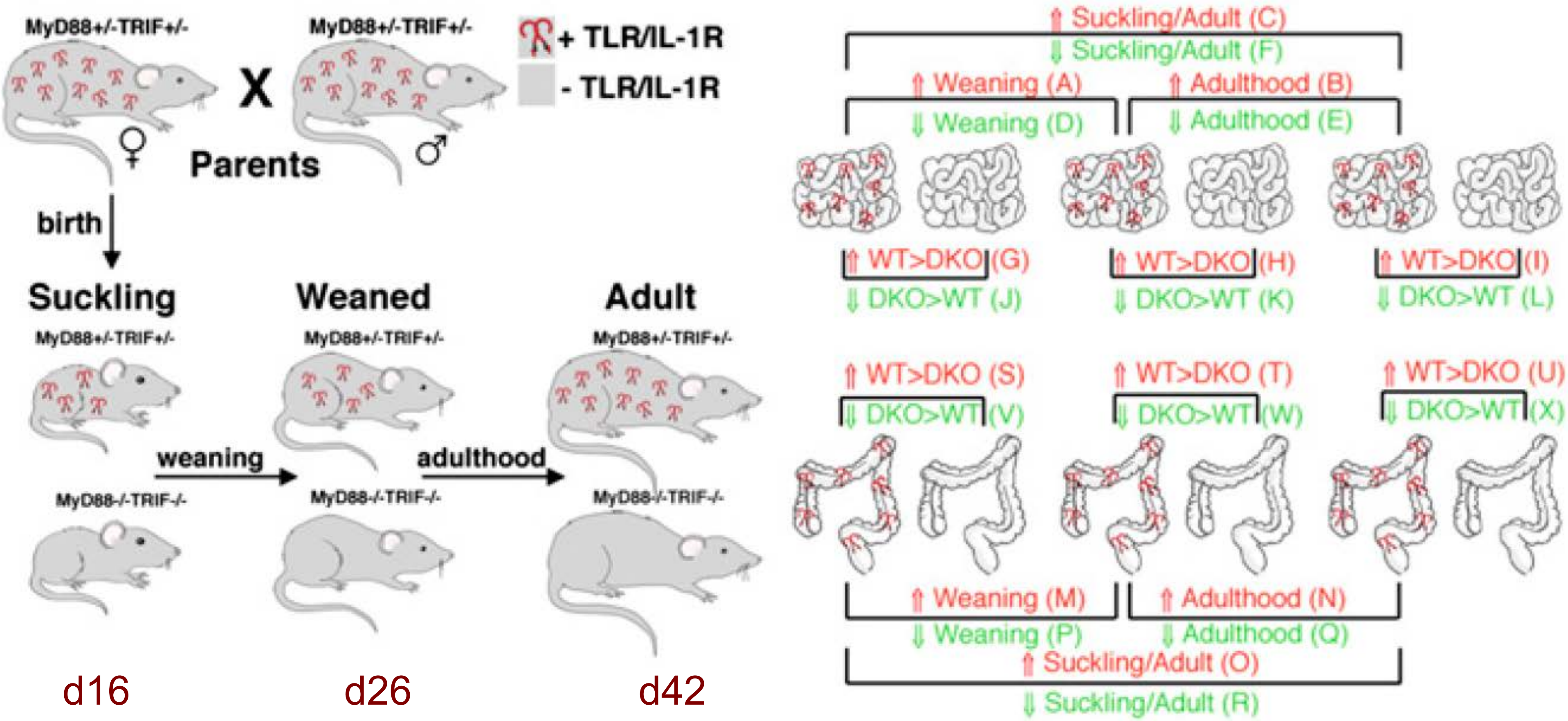

Microbiota
Food
Maternal milk


Live bacteria colonisation
Maternal milk
Maturation of immune system
Bacteria shifts
Maternal Ab
Hormones
Live bacteria exposure

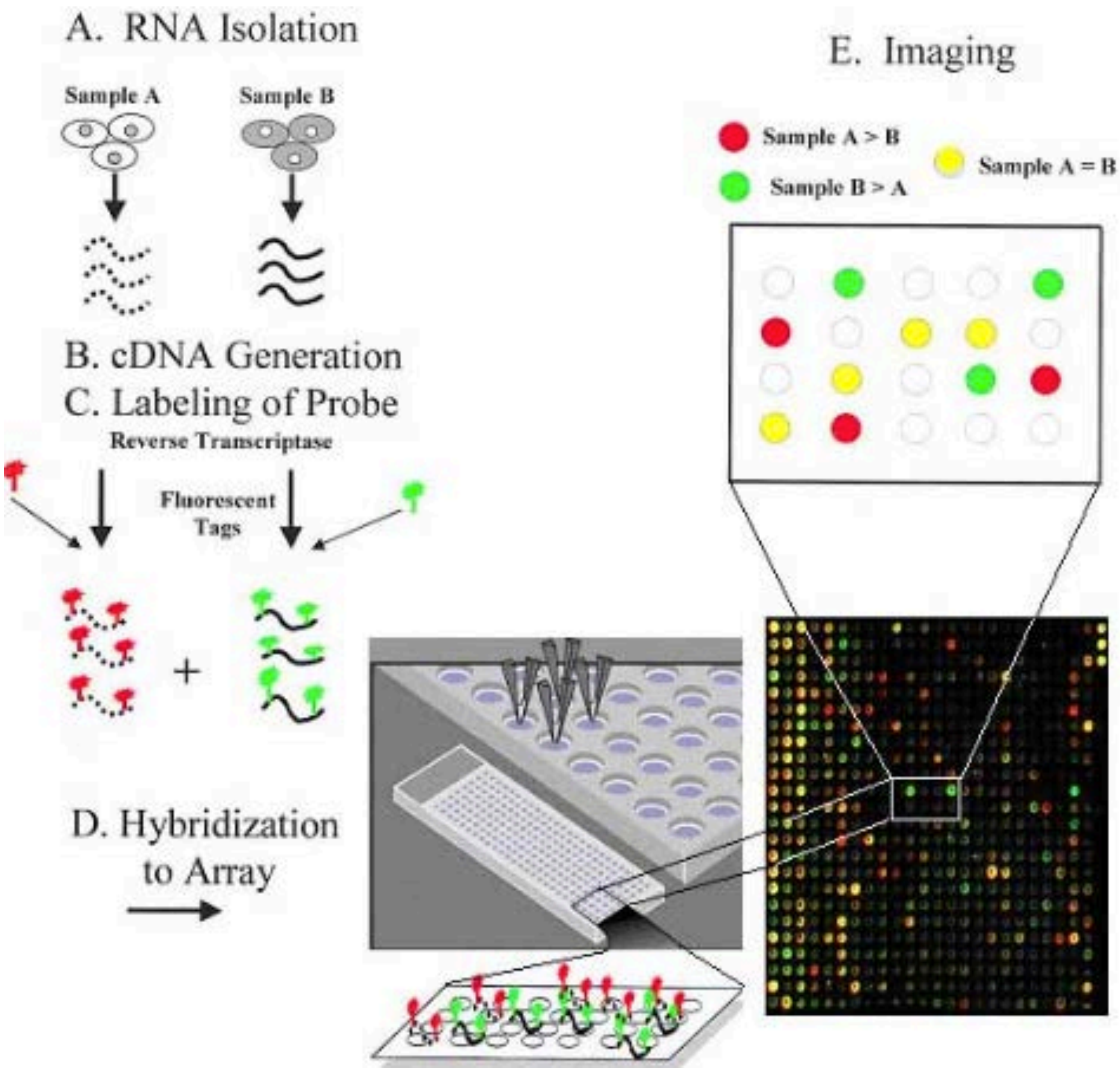
Role of TLR and IL-1R signaling in the postnatal gene expression



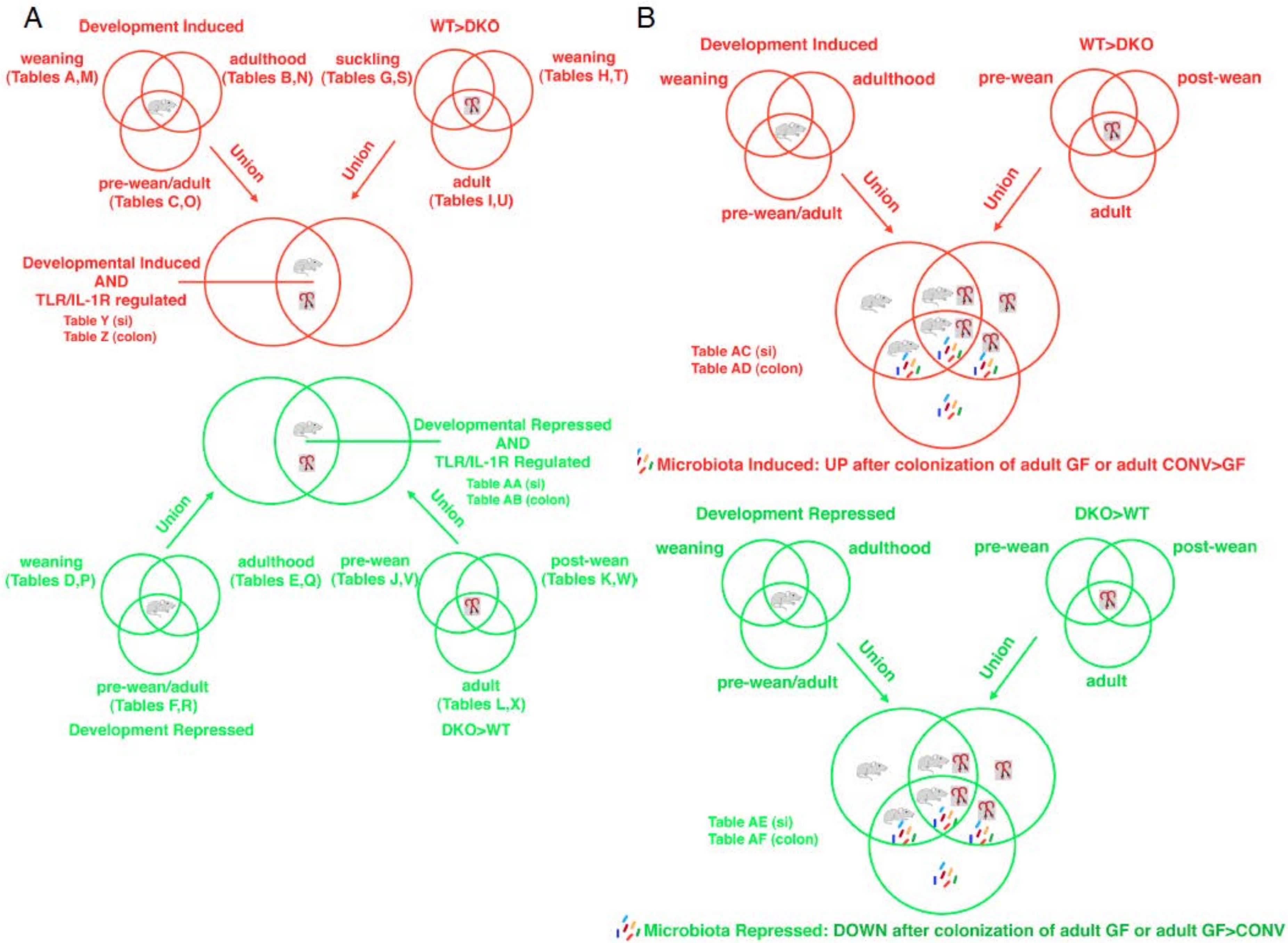
Experimental design



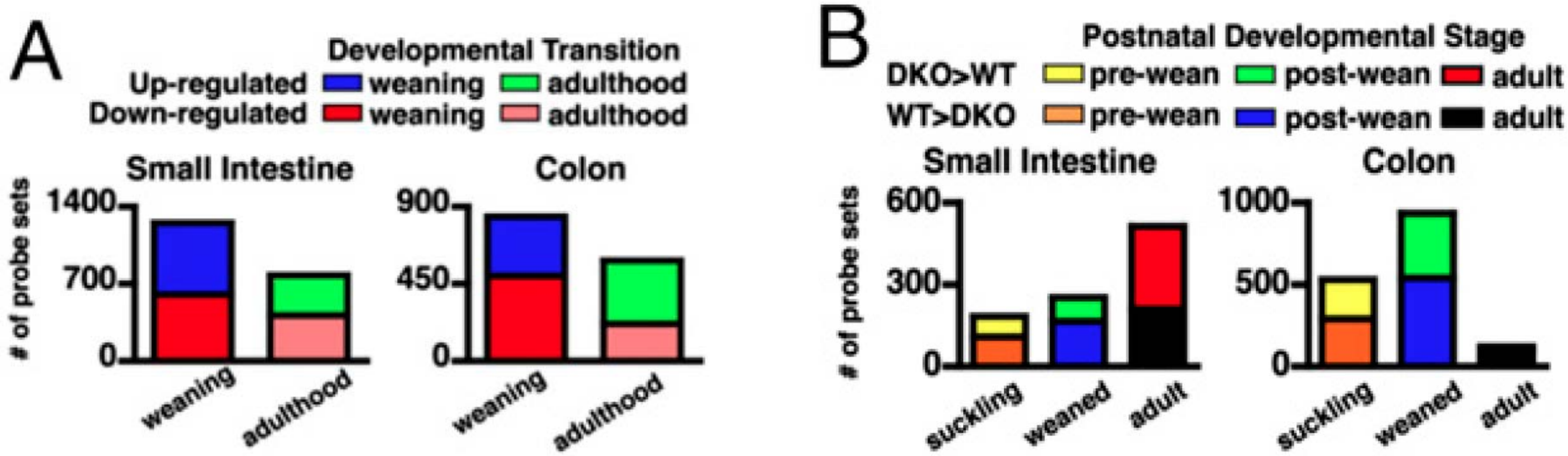
Illumina Mouse WG-6 v2.0 Expression beadchips for whole-genome expression prolife



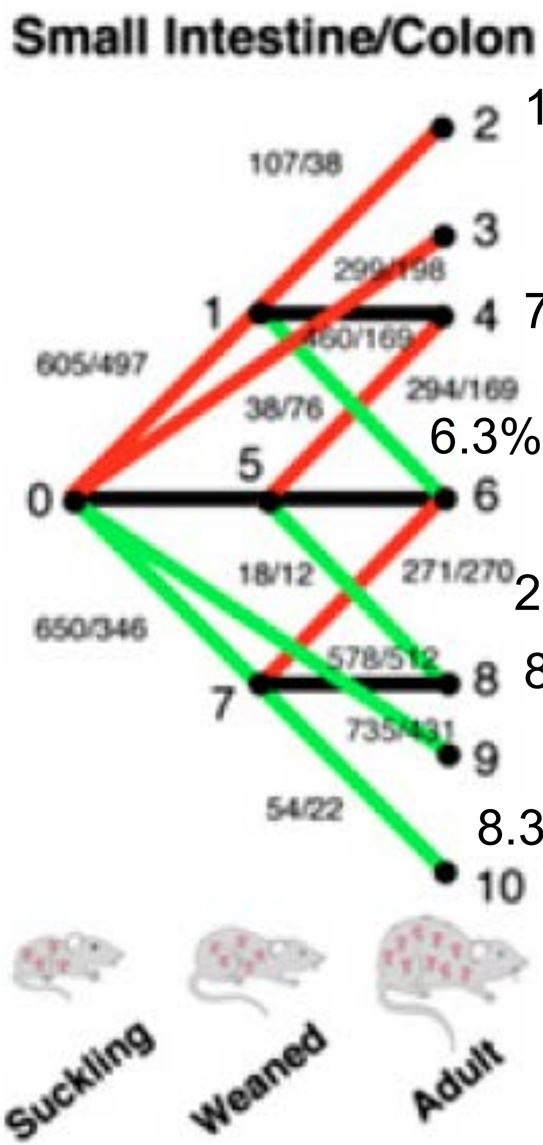
≥1.5 fold-change
p-value
duplicate


A
Development Induced
weaning (Tables A,M)
adulthood (Tables B,N)
pre-wean/adult (Tables C,O)
WT>DKO
suckling (Tables G,S)
weaning (Tables H,T)
adult (Tables I,U)
Union
Union
Developmental Induced AND TLR/IL-1R regulated
Table Y (si)
Table Z (colon)
Developmental Repressed AND TLR/IL-1R Regulated
Table AA (si)
Table AB (colon)
Union
Union
weaning (Tables D,P)
adulthood (Tables E,Q)
pre-wean/adult (Tables F,R)
Development Repressed
pre-wean (Tables J,V)
post-wean (Tables K,W)
adult (Tables L,X)
DKO>WT
B
Development Induced
weaning
adulthood
pre-wean/adult
WT>DKO
pre-wean
post-wean
adult
Union
Union
Table AC (si)
Table AD (colon)
Microbiota Induced: UP after colonization of adult GF or adult CONV>GF
Development Repressed
weaning
adulthood
pre-wean/adult
DKO>WT
pre-wean
post-wean
adult
Union
Union
Table AE (si)
Table AF (colon)
Microbiota Repressed: DOWN after colonization of adult GF or adult GF>CONV

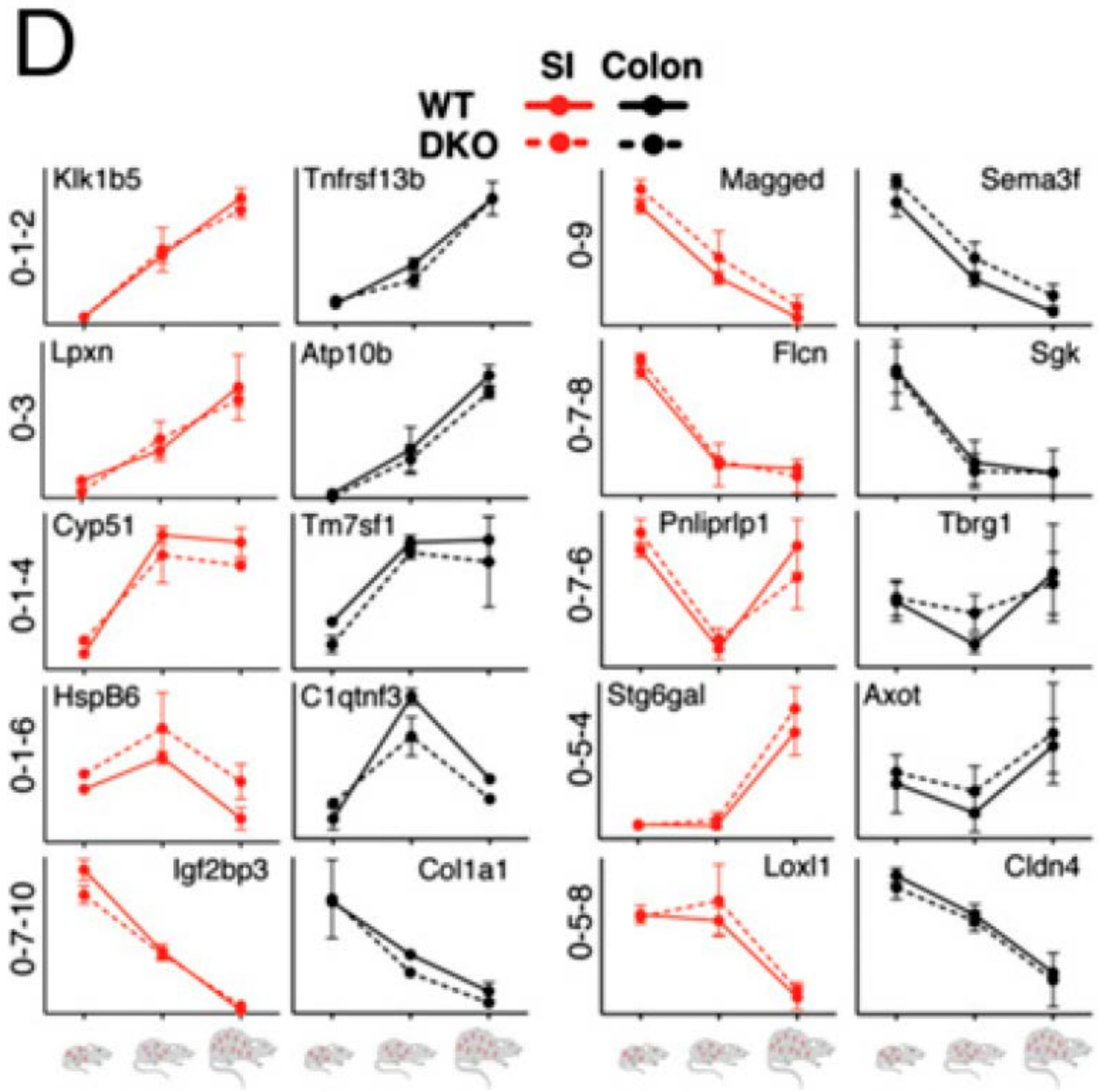
Differential roles of TIR signaling in colon and SI



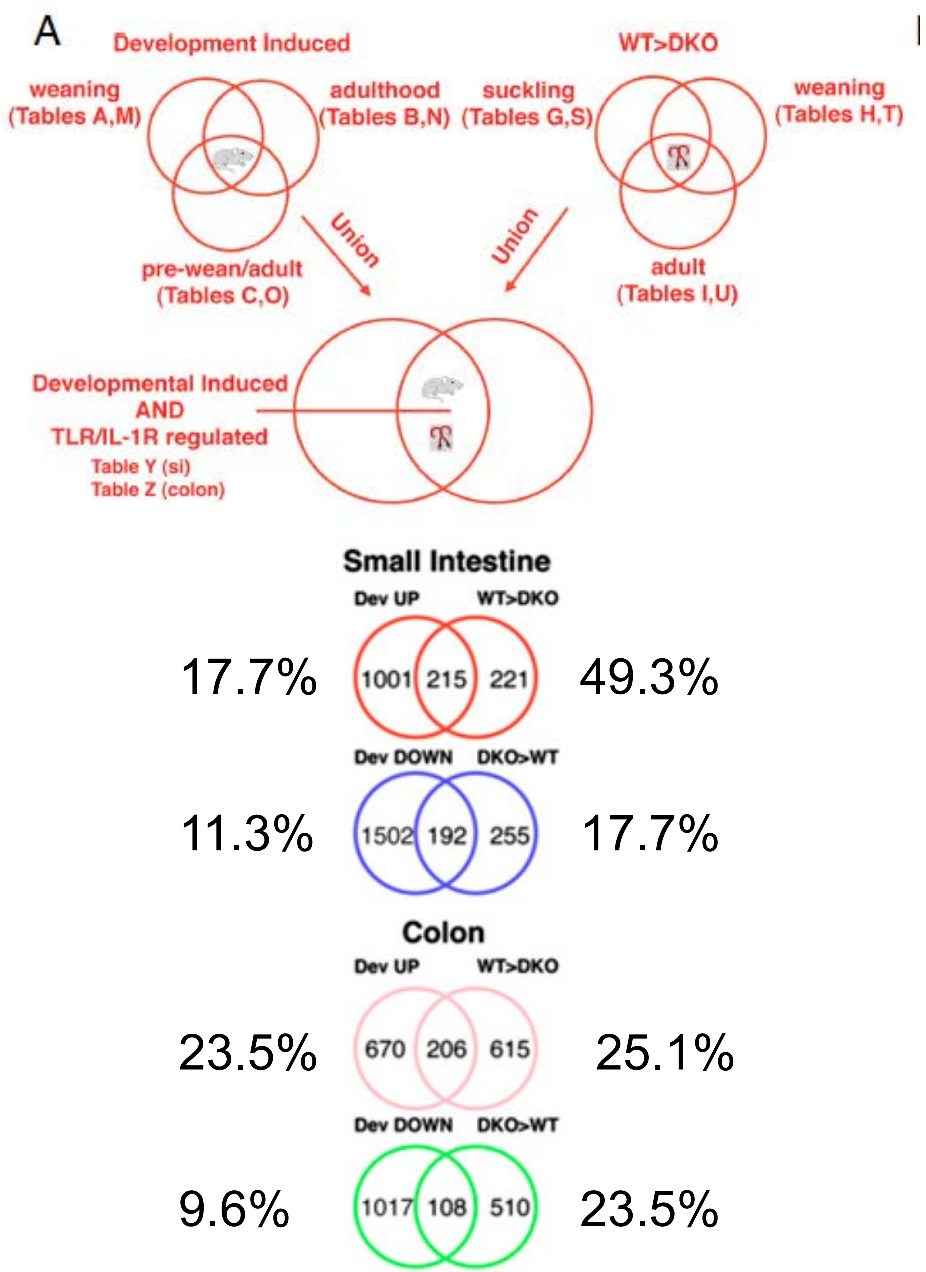
The majority of genes undergo transcriptional stabilization after weaning



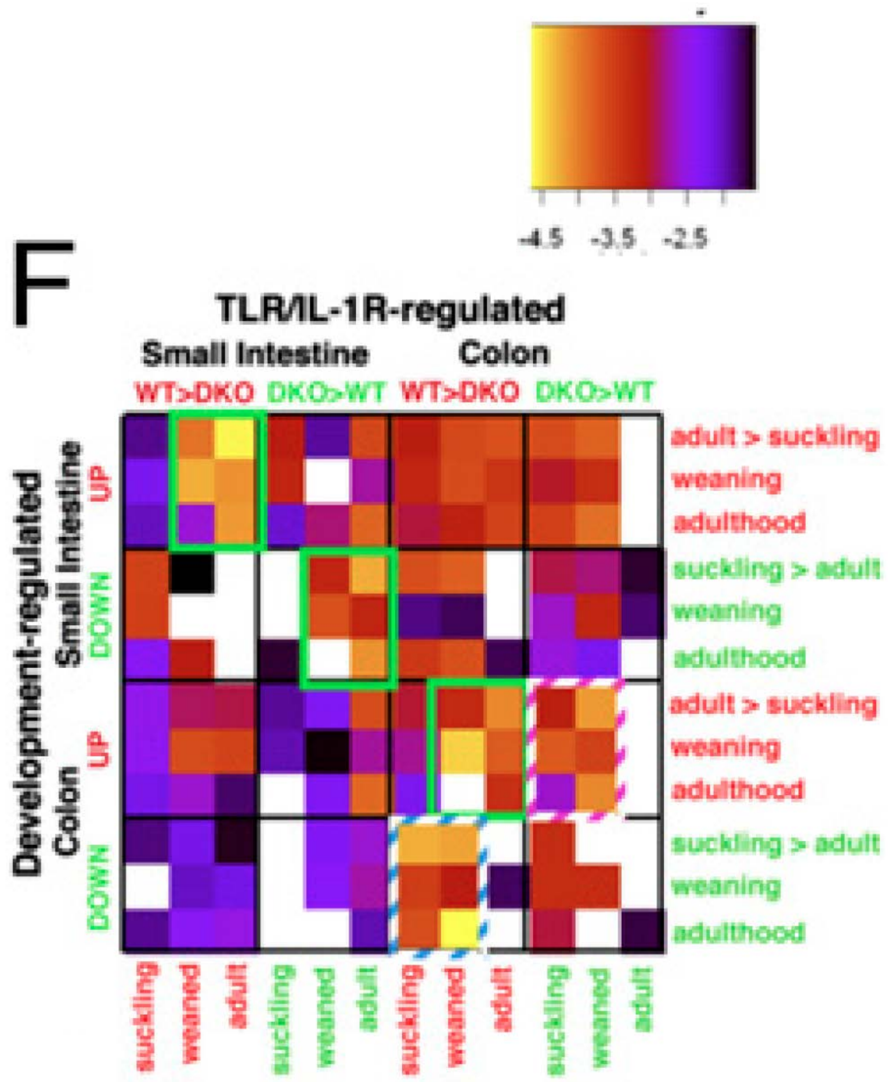
17.7% ↑
76% =
6.3% ↓
2.7% ↑
88.9% =
8.3% ↓



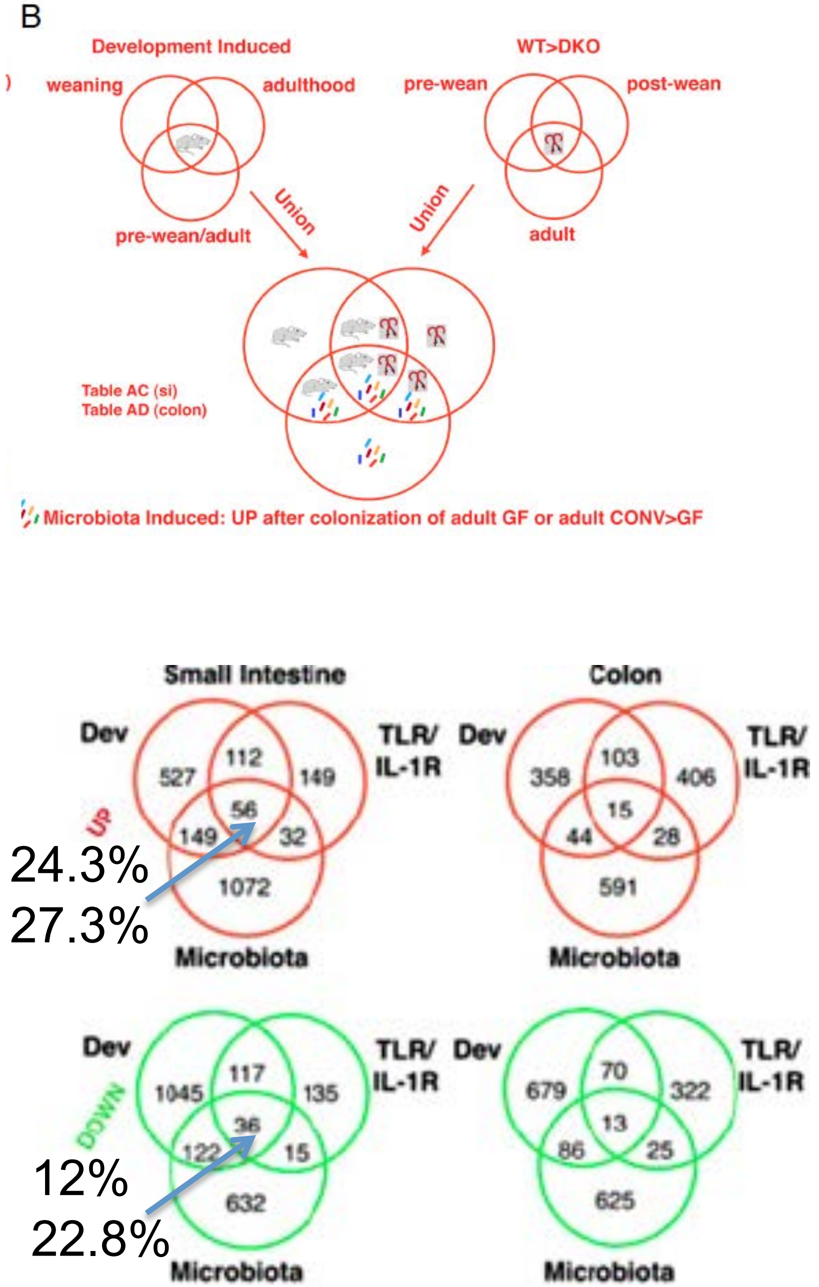
Correlation between the genes induced or repressed by the development and the TIR

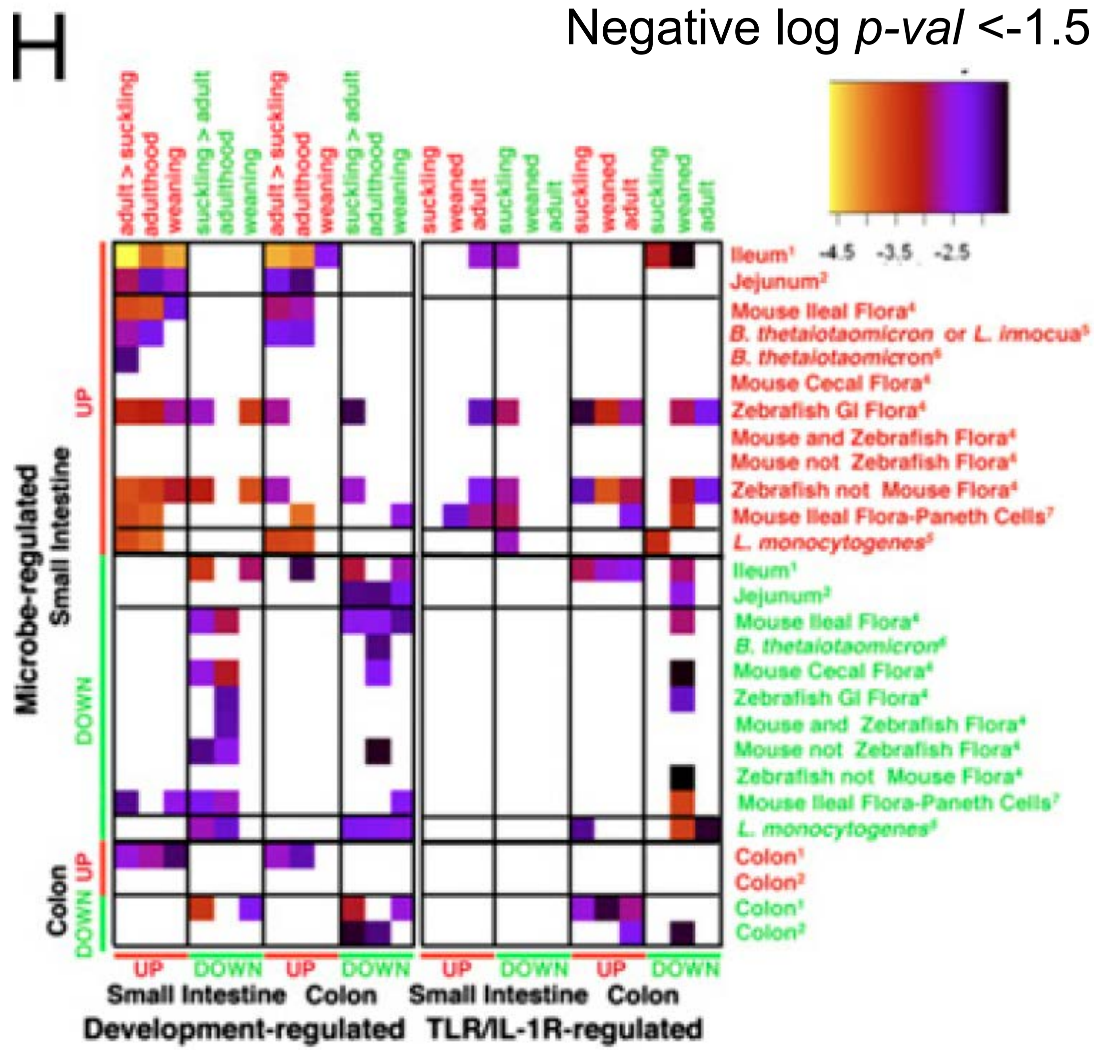


Negative log *p-val* <-1.5

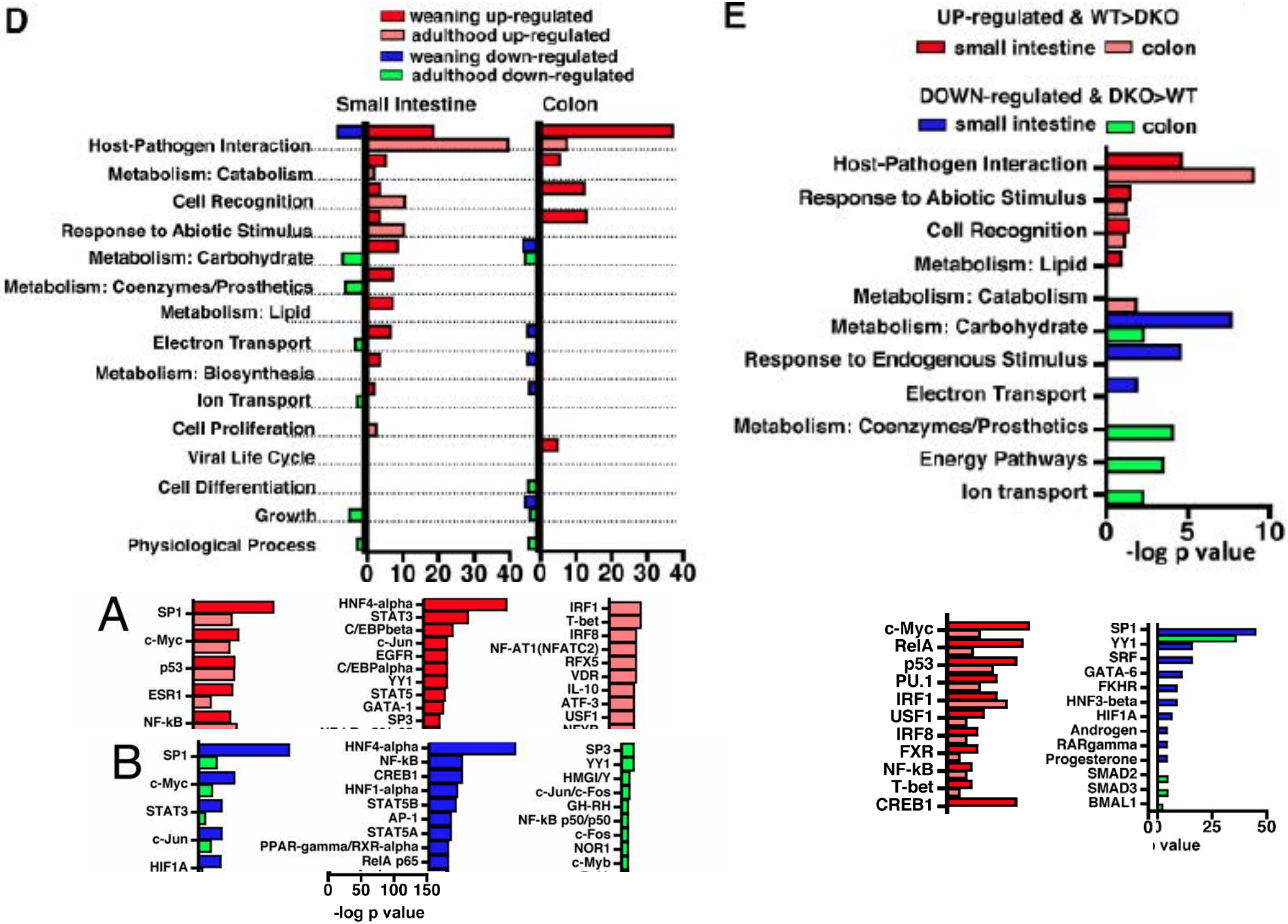


Developmental and the TIR genes are modified by the microbiota during intestinal ontogeny

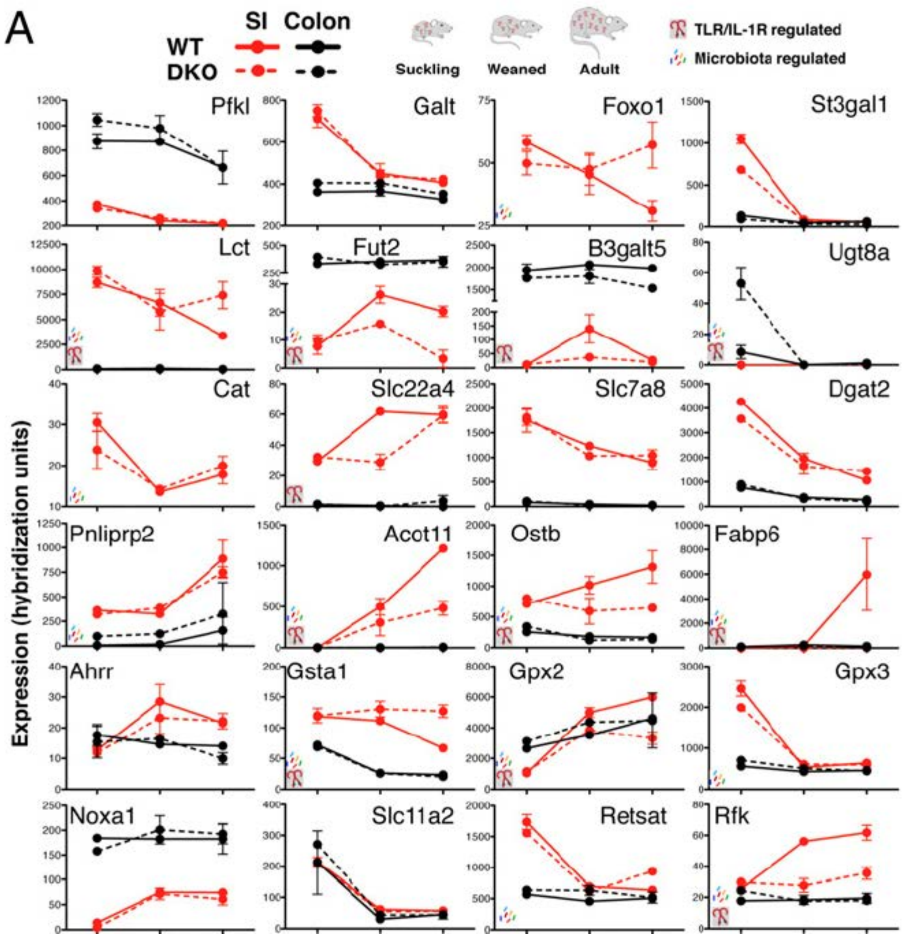




TF act as a master regulators of postnatal intestinal development programs



Regulation of metabolism and host response



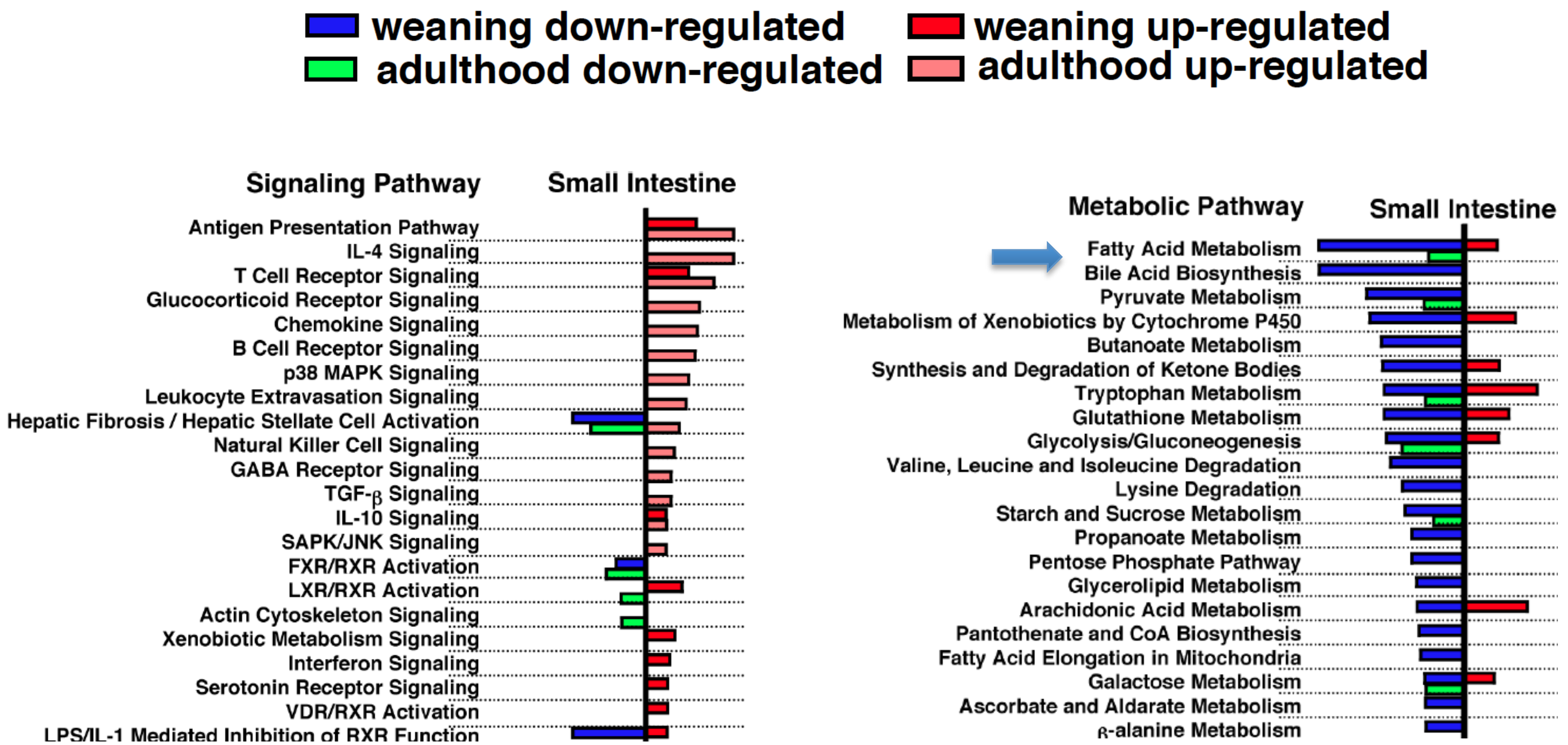
Weaning:

- Pyruvate metabolism
- Glucolys/gluconeogenesis
- Disaccharide utilization
- ↓Galactose (Galt, Pfkl and Gaa)
- ↓Starch/Sucrose (Slc37a4)
- ↑Fructose/mannose utilisation (Hk2 Nudt5, Gmds, Mpi, Sord, Glut5/Scl2a5)
- ↑Sodium/Glucose cotransport (SGLT3a/-b, Slc5a4a, Slc5a4b)

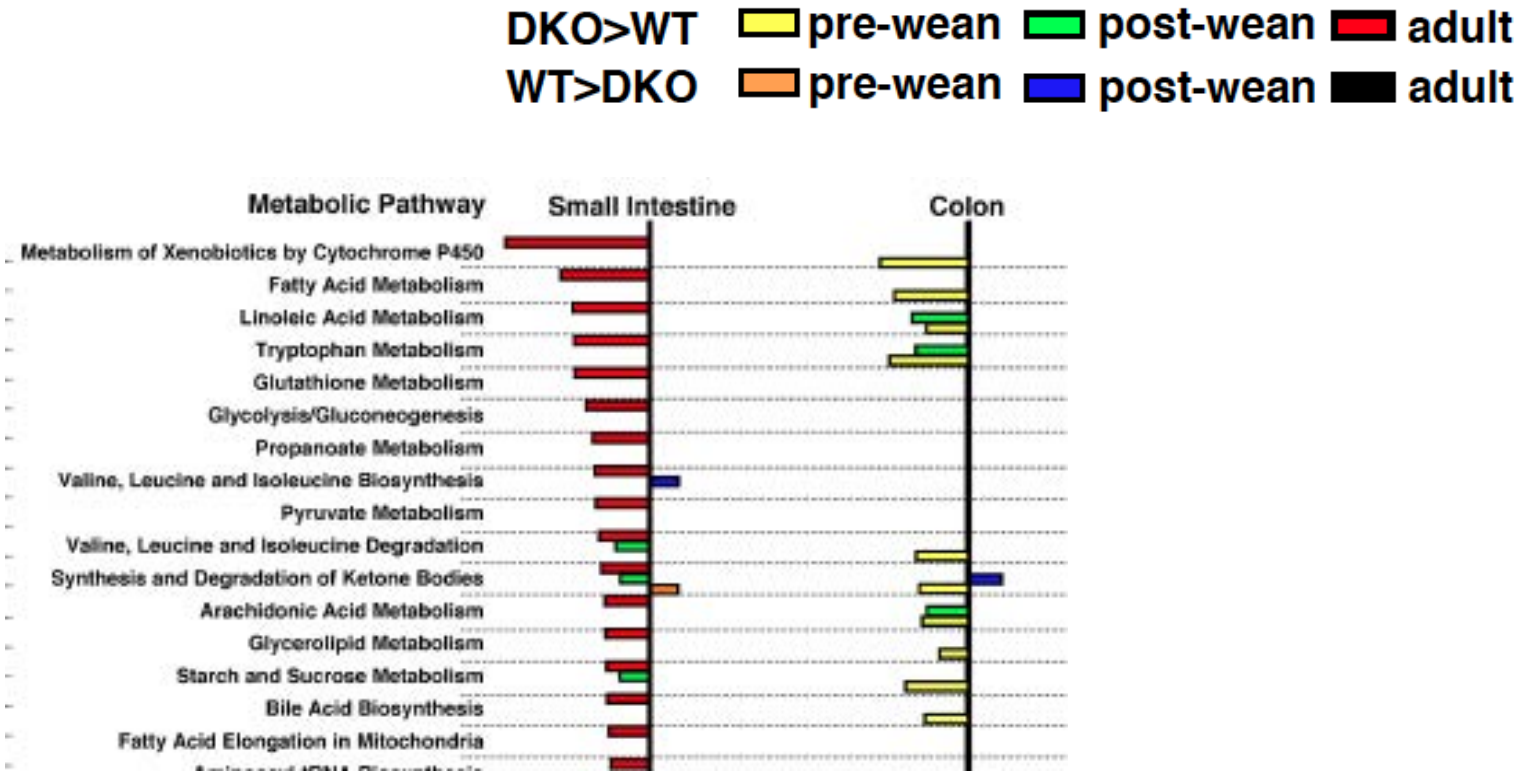
Depend on TIR for postnatal repression:

- FOXO1/FKHR, PdH, G6PC

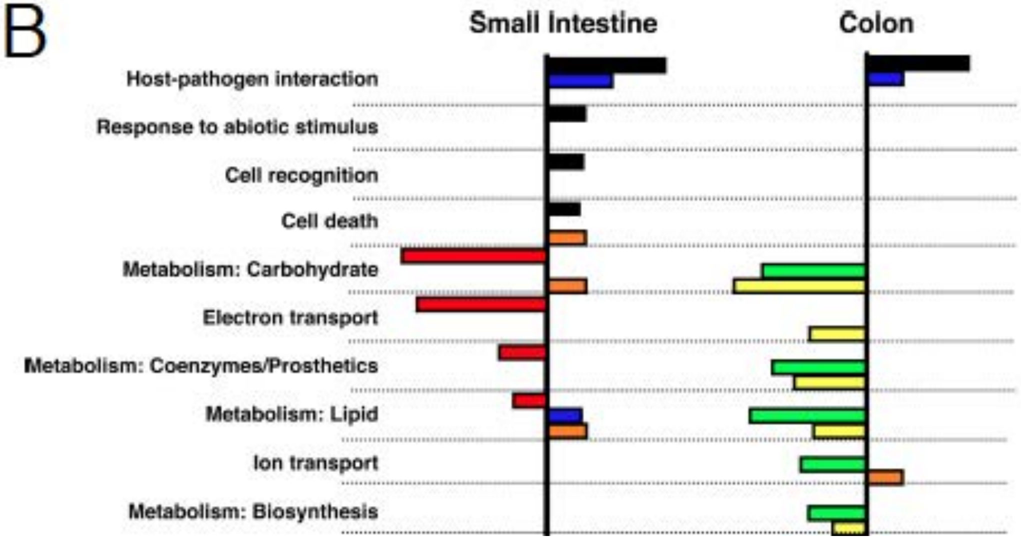
Regulation of metabolism and host response



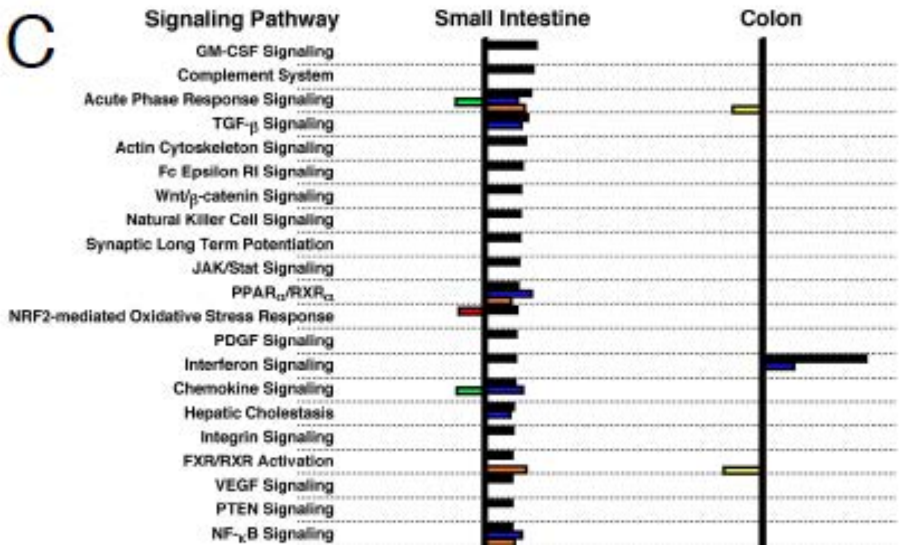
TIR signaling coordinate metabolomics pathways



B

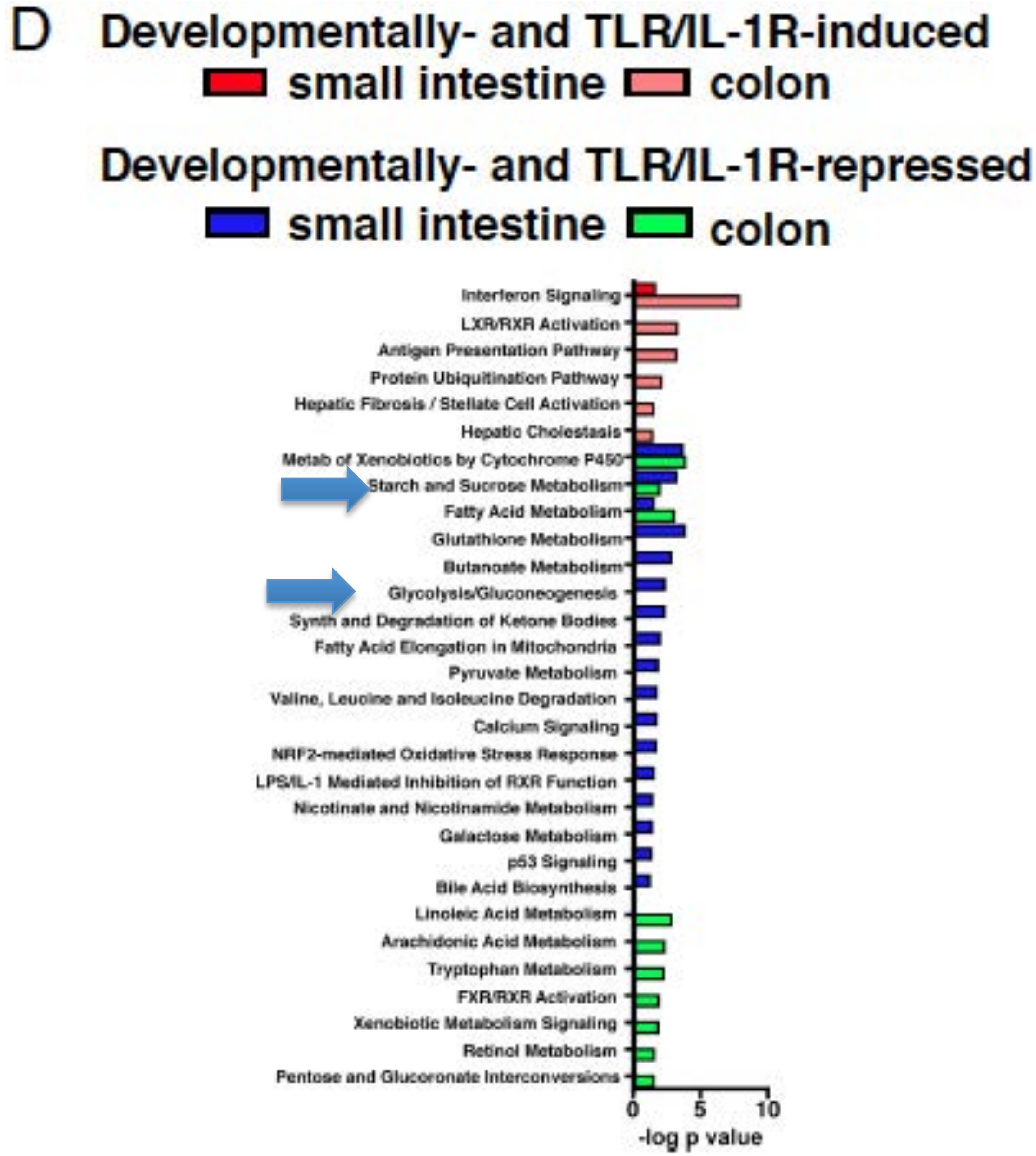


C

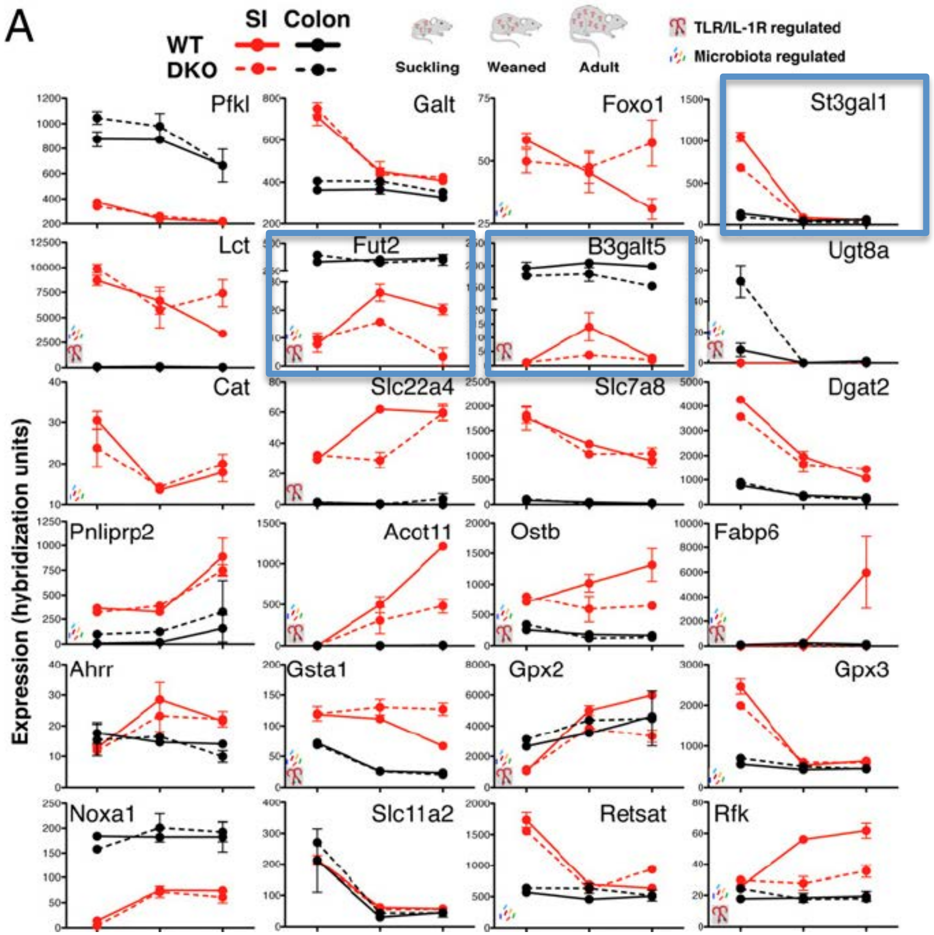


TIR signaling coordinate metabolomics pathways

(TIR repression)



Regulation of metabolism and host response



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- ↑Sodium/Glucose cotransport (SGLT3a/-b, Slc5a4a, Slc5a4b)

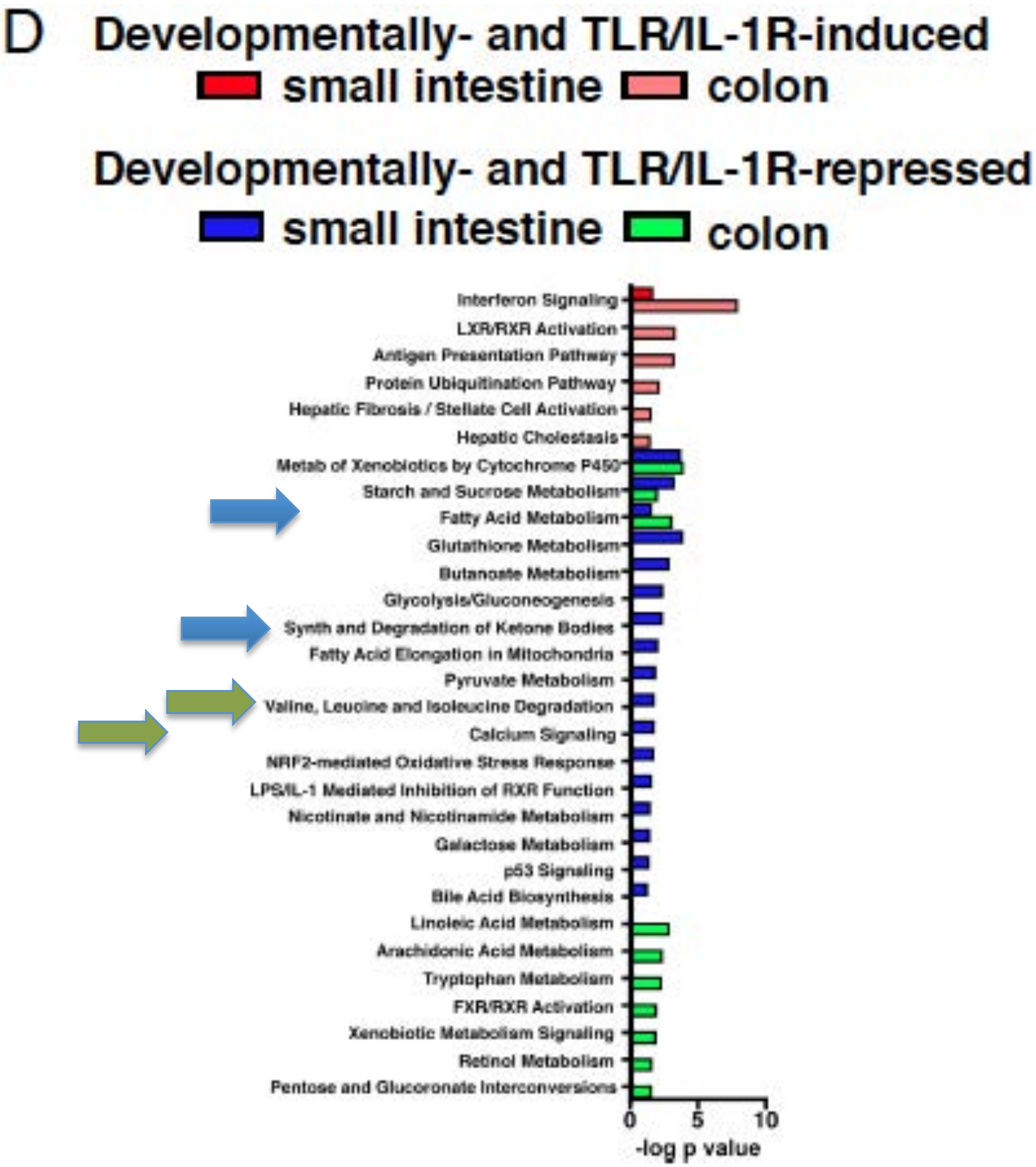
Postnatal:

- Glucosylation states of mucus:
  - ↑Fut2, B3galt5 (↑in colonized adult mice and dep in TIR)
  - ↓St3gal (↑St6gal in adult)

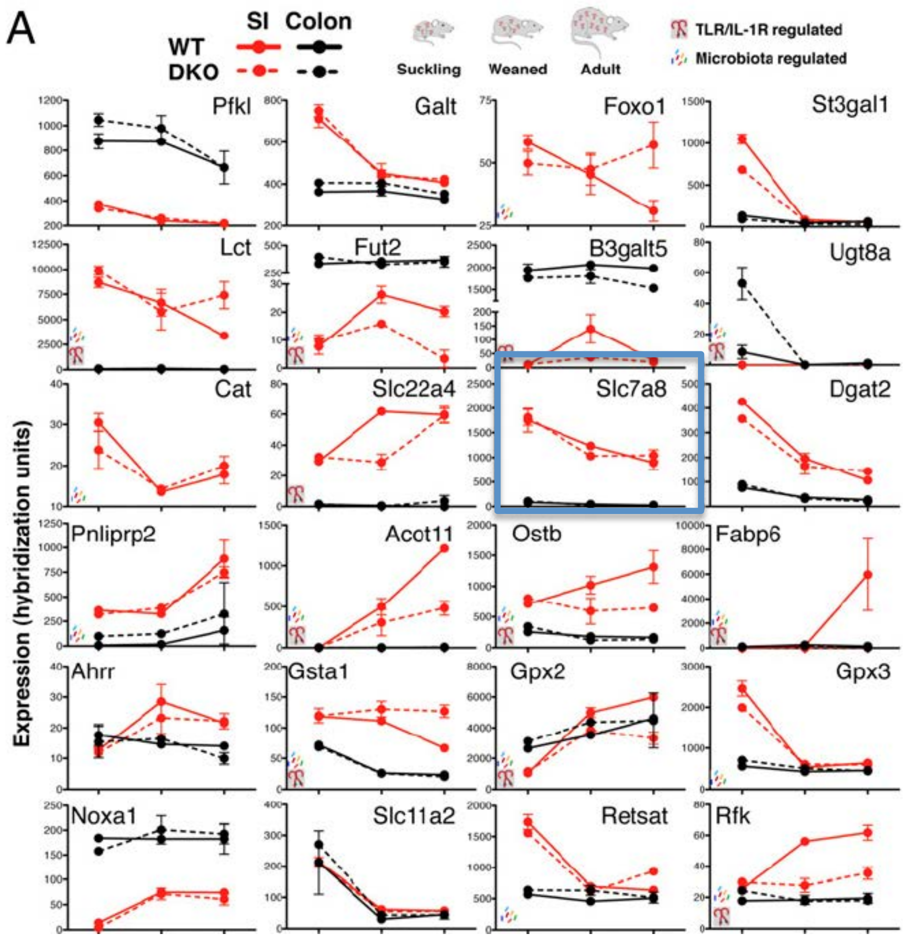
Colon & dep on TIR:

- UDP glucuronoosyltranferase (Ugt)

TIR signaling coordinate metabolomics pathways



Regulation of metabolism and host response



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- ↓Galactose (Galt, Pfkl and Gaa)
- ↓Starch/Sucrose (Slc37a4)
- ↑Fructose/mannose utilisation (Hk2 Nudt5, Gmds, Mpi, Sord, Glut5/Scl2a5)
- ↑Sodium/Glucose cotransport (SGLT3a/-b, Slc5a4a, Slc5a4b)

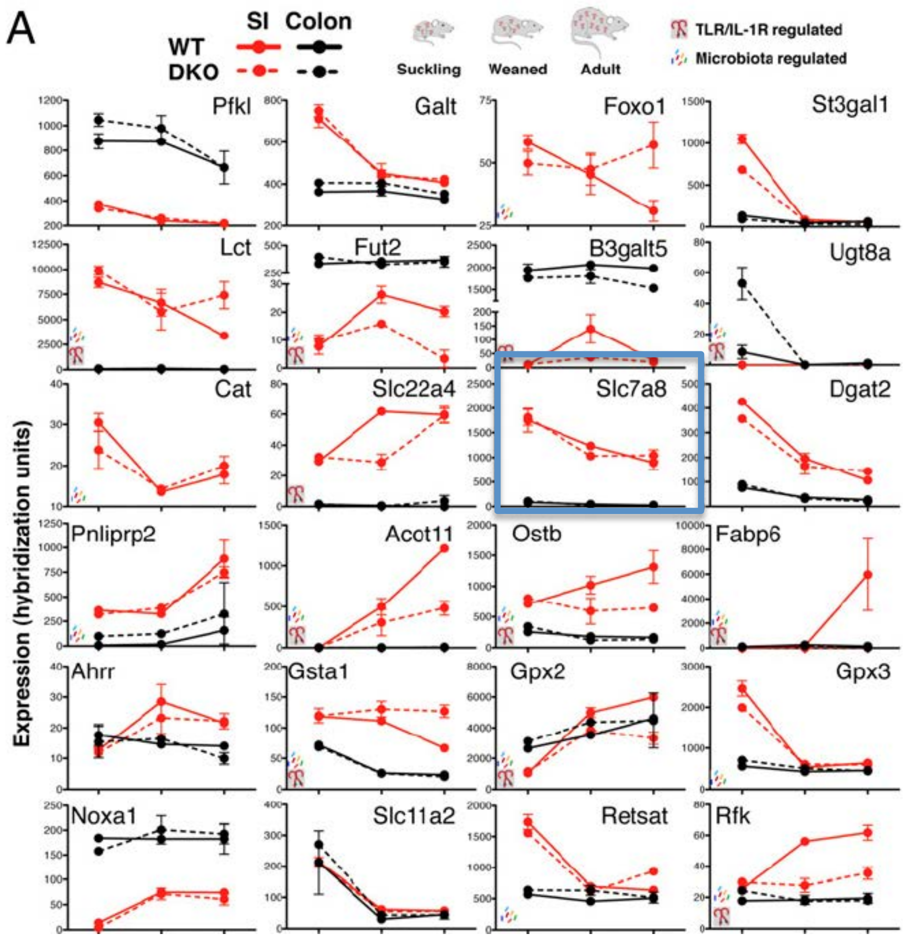
Postnatal:

- Glucosylation states of mucus:
  - ↑Fut2, B3galt5 (↑in colonized adult mice and dep in TIR)
  - ↓St3gal (↑St6gal in adult)
  - Aa/peptide & organic transporters:

Colon & dep on TIR:

- UDP glucuronoosyltranferase (Ugt)

Regulation of metabolism and host response



Weaning:

- Pyruvate metabolism
- Glucolys/gluconeogenesis
- Disaccharide utilization
- ↓Galactose (Galt, Pfkl and Gaa)
- ↓Starch/Sucrose (Slc37a4)
- ↑Fructose/mannose utilisation (Hk2 Nudt5, Gmds, Mpi, Sord, Glut5/Scl2a5)
- ↑Sodium/Glucose cotransport (SGLT3a/-b, Slc5a4a, Slc5a4b)

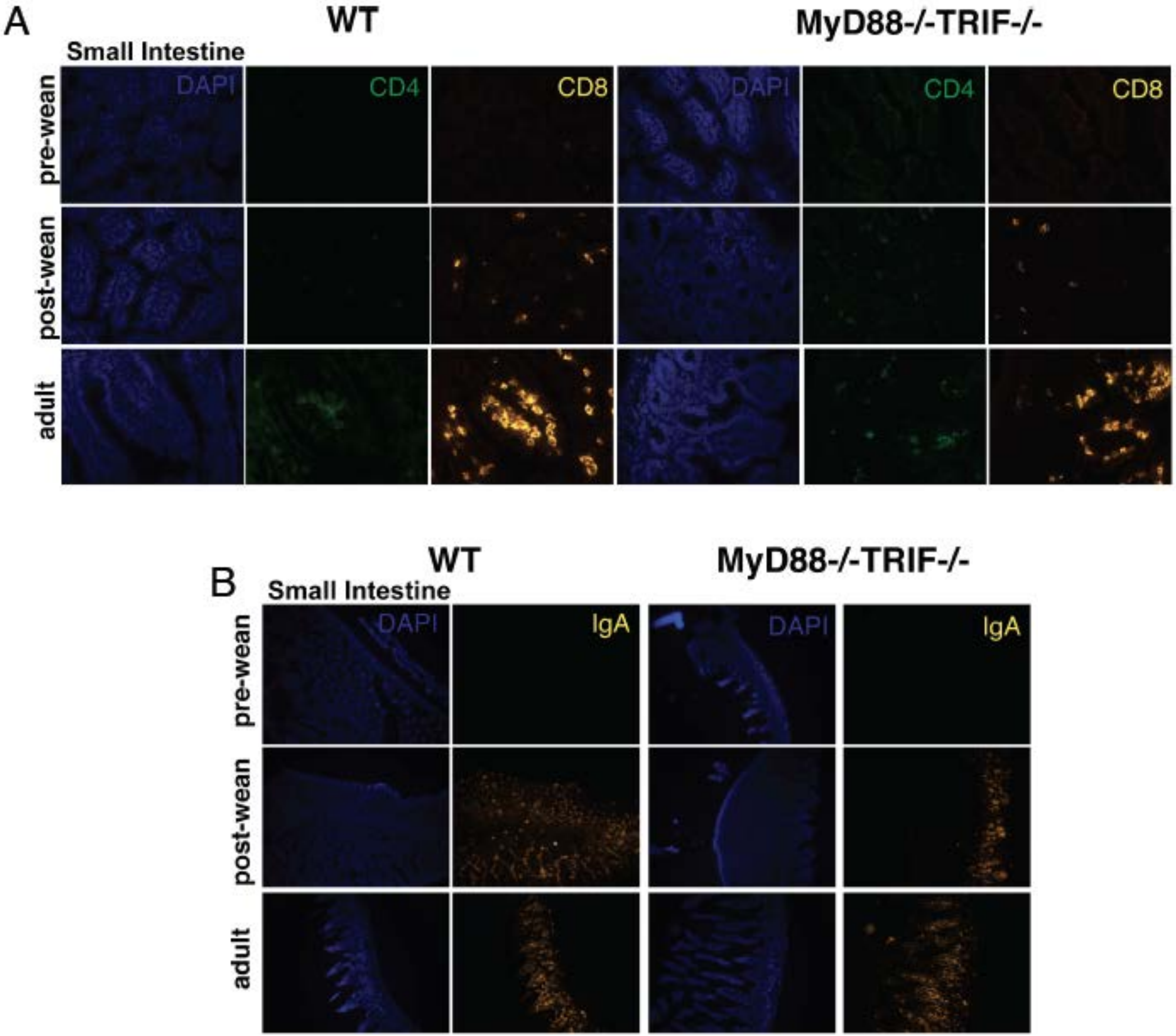
Postnatal:

- Glucosylation states of mucus:
  - ↑Fut2, B3galt5 (↑in colonized adult mice and dep in TIR)
  - ↓St3gal (↑St6gal in adult)
  - Aa/peptide & organic transporters:

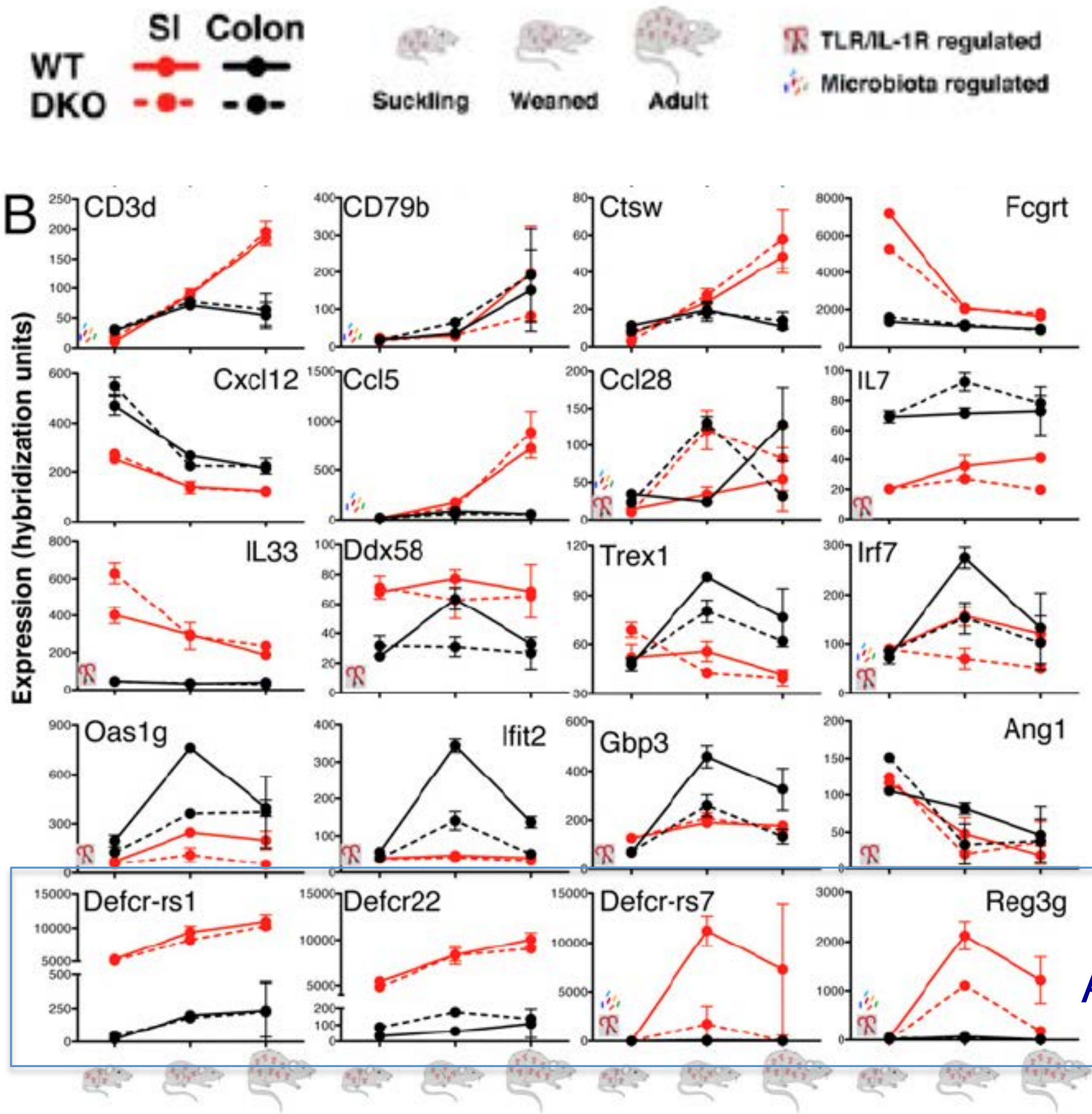
Colon & dep on TIR:

- UDP glucuronoosyltranferase (Ugt)

Host defense regulation TIR independant

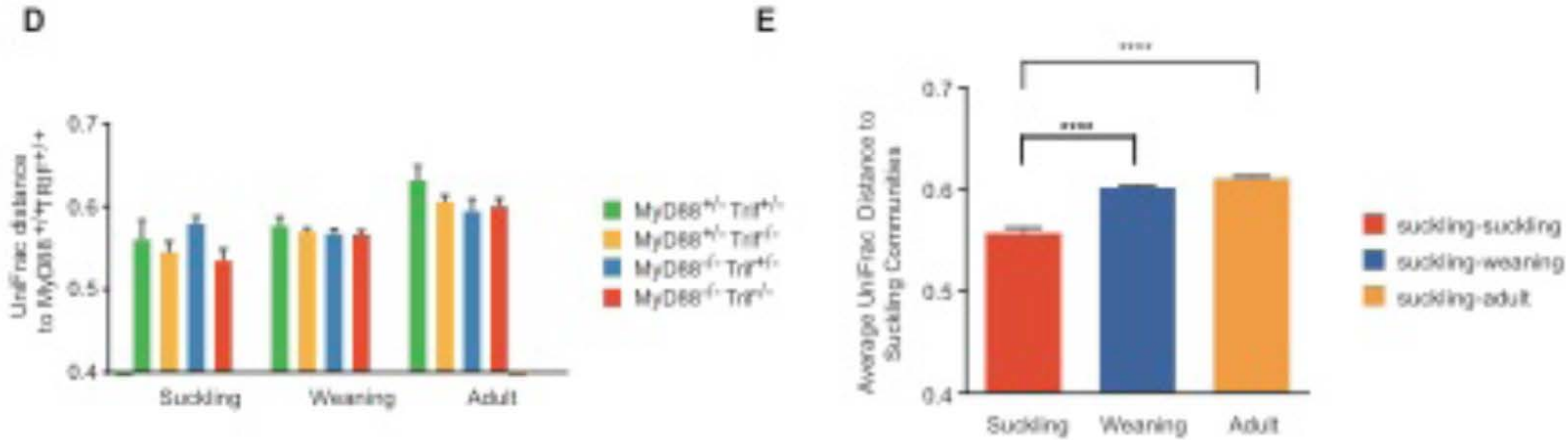


Host defense regulation



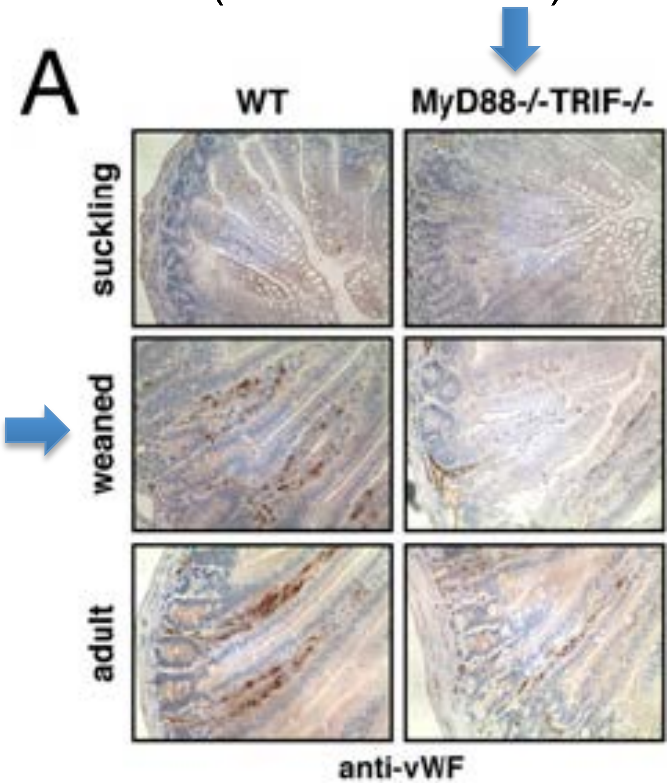
Antimicrobial products

Lack of dramatic changes in microbiota composition

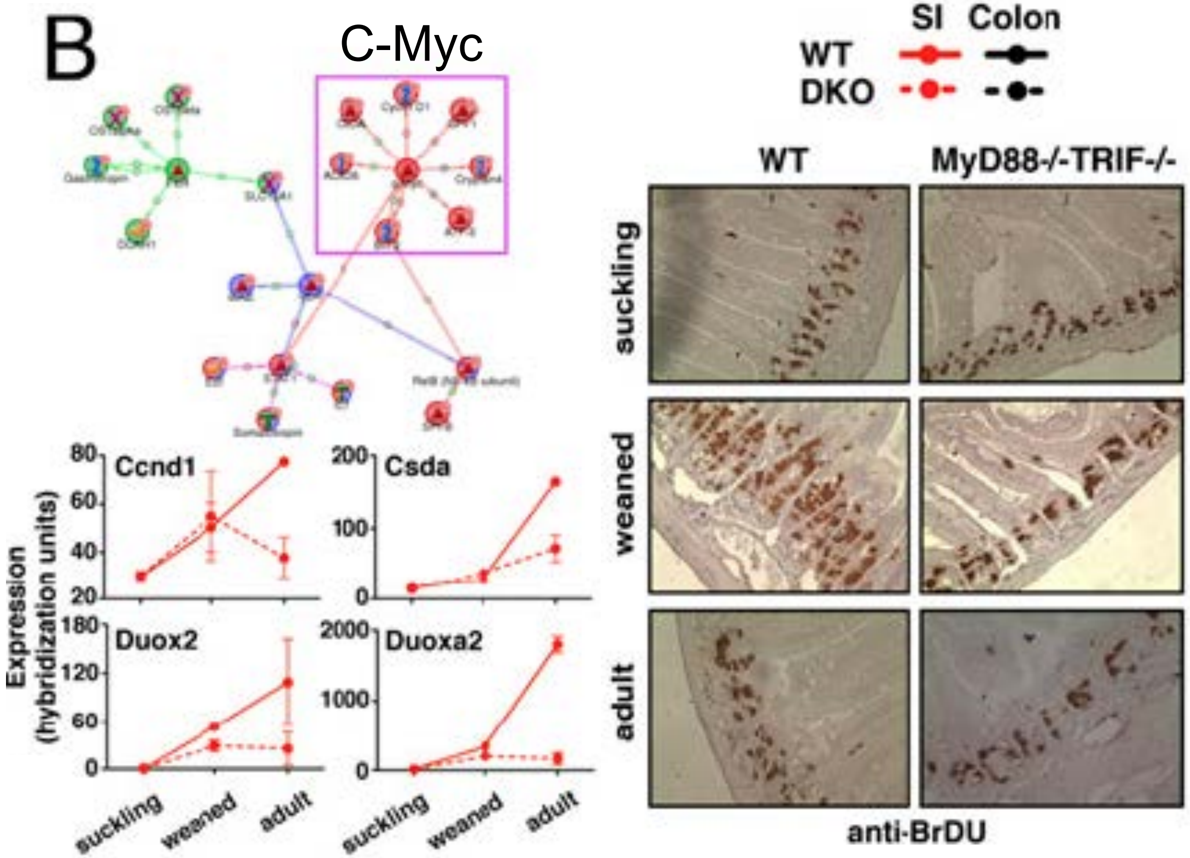


Phenotype effect of TIR in postnatal developmental

Vascular endothelium tissue
(Small intestine)

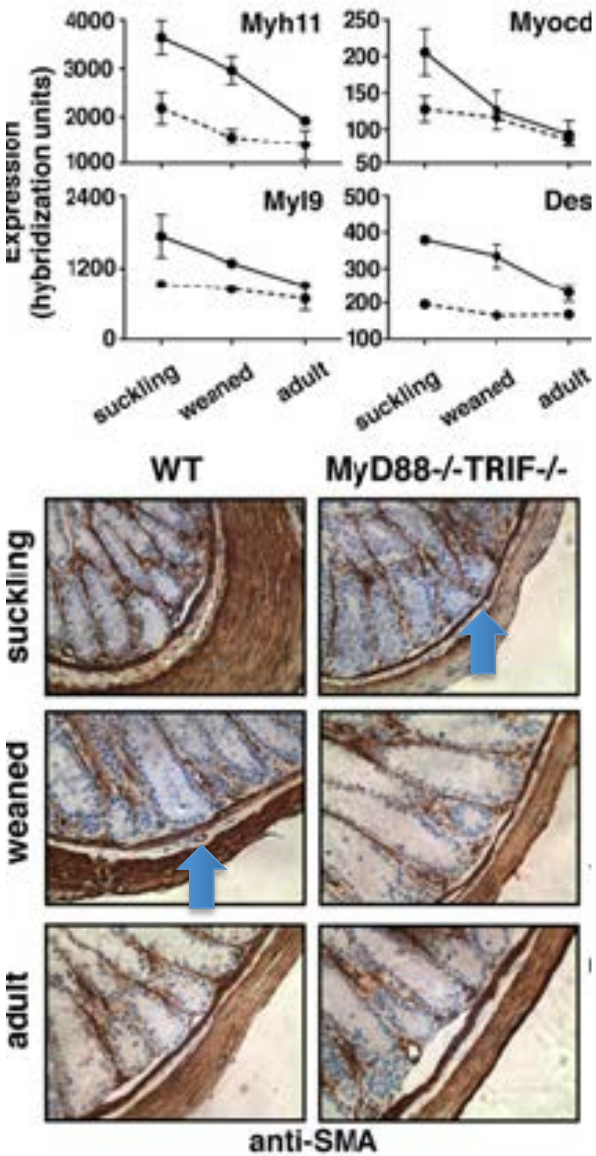


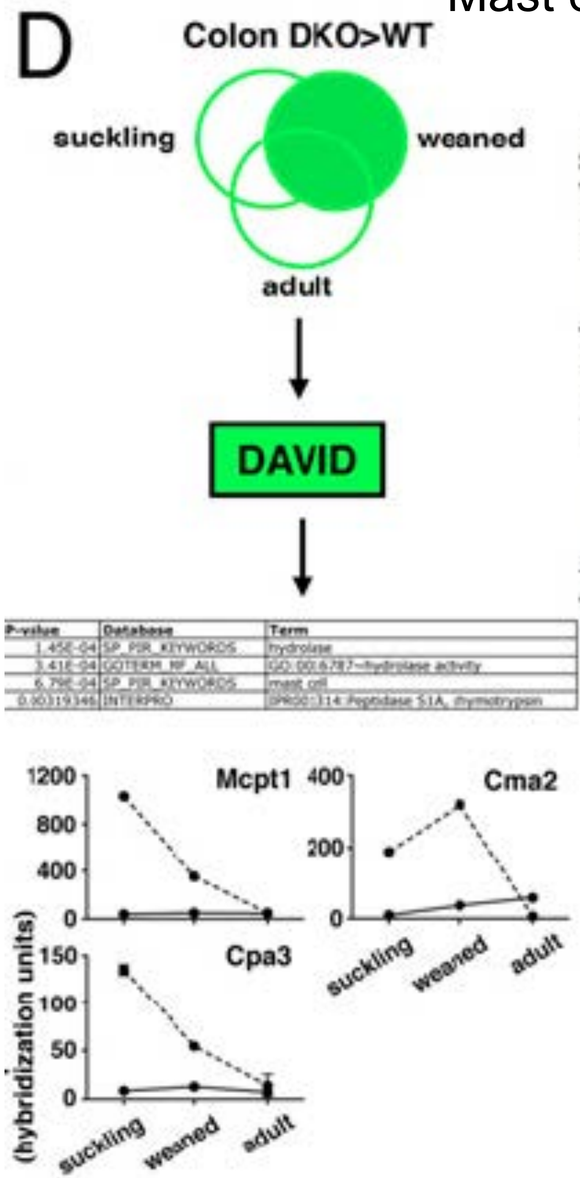
Epithelial cells proliferation
(Small intestine)



Phenotype effect of TIR in postnatal developmental

Smooth muscle cells colon





P-value	Database	Term
1.45E-04	SP_PIR_KEYWORDS	hydrolase
3.41E-04	GOTERM_MF_ALL	GO:0016787~hydrolase activity
6.79E-04	SP_PIR_KEYWORDS	mast cell
0.00319346	INTERPRO	IPR001314:Peptidase S1A, chymotrypsin

Mast cells in the colon