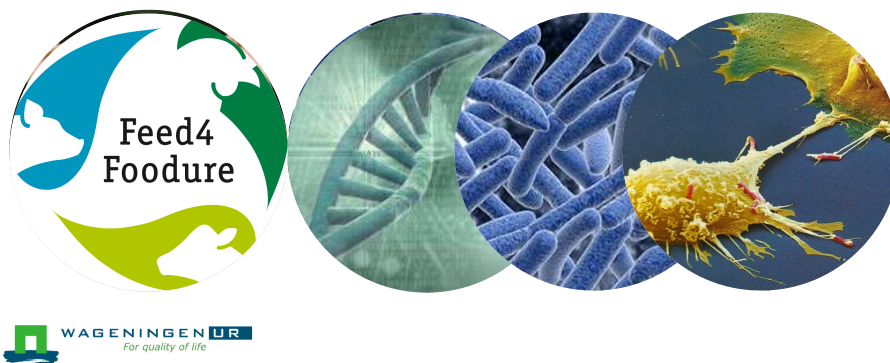


Rol van microbiota bij de ontwikkeling van immuun competentie in biggen

D. Schokker, J. Zhang, H. Smidt, J.M.J. Rebel, M.A. Smits

Livestock Research, Central Veterinary Institute, and Laboratory of Microbiology



Background (literature)



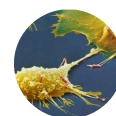
- Gut microbiota influence gut physiology and health from birth to old age.
- Environmental factors (diet, stress, disease, injury, antibiotics) can alter the diversity and composition of gut microbiota.
- Disruption of the gut microbiota (dysbiosis) can lead to a variety of immune-related diseases.
- Early maternal and environmental factors (delivery, nutrition) affect the microbial colonization of the gut.
- Changes in early life microbial colonization of the gut has an effect on the risks for developing disease later in life.



Objectives of the study



- Do early-life factors, as experienced by piglets under normal husbandry conditions, affect gut microbial colonization?
- If yes, does this also affect immune competence later in life?



Exposure to early life factors (Treatment)



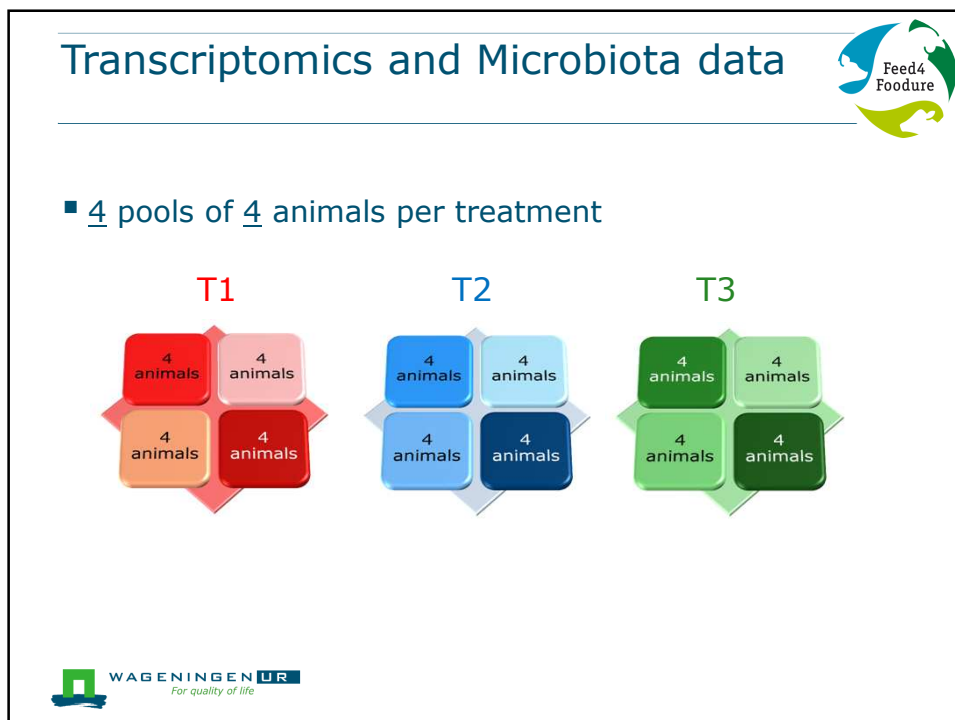
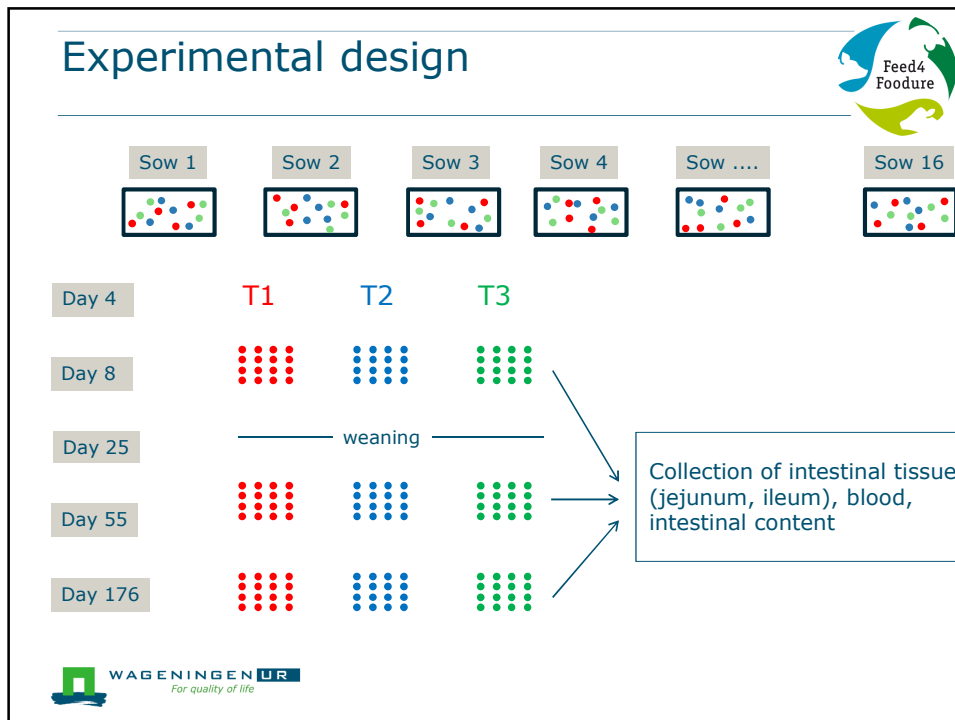
Treatment	Antibiotic*	Stressor
T1	-	-
T2	+	-
T3	+	+

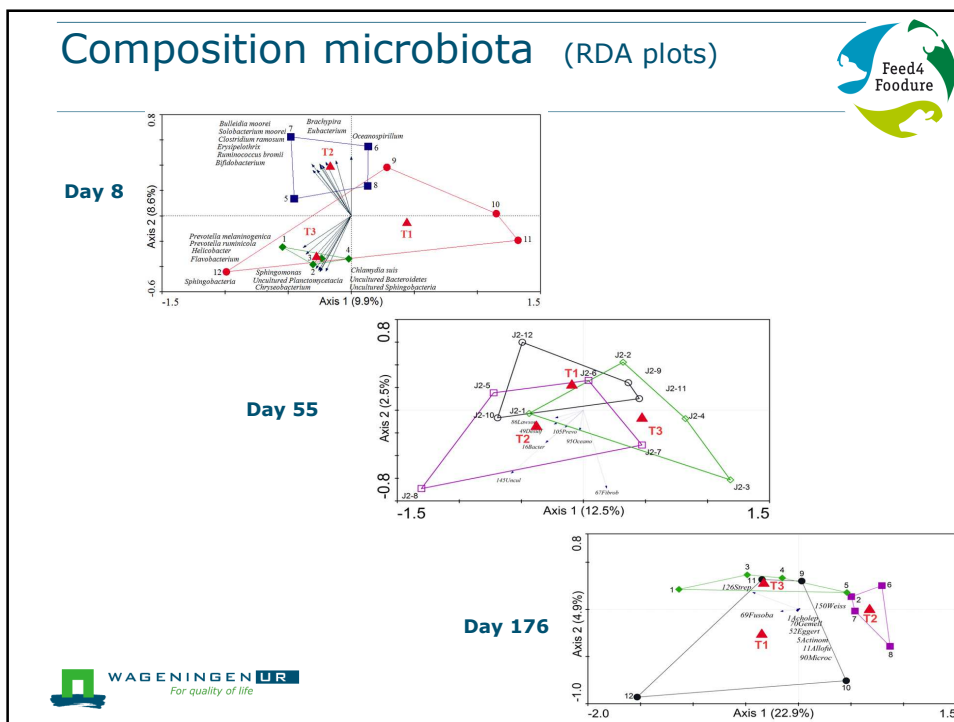
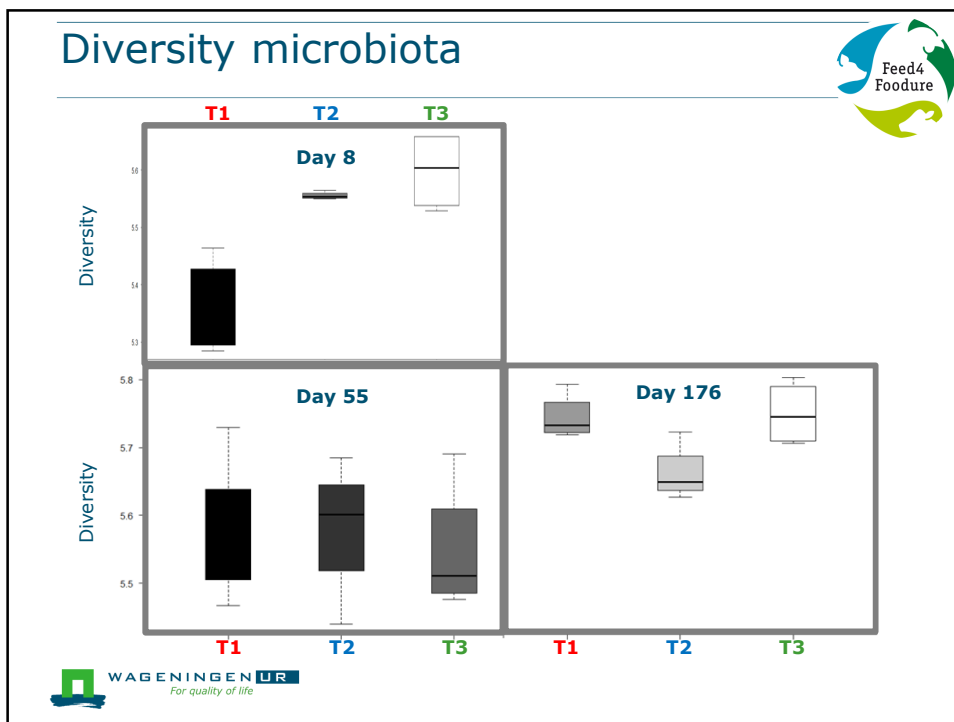
* Tulathromycine

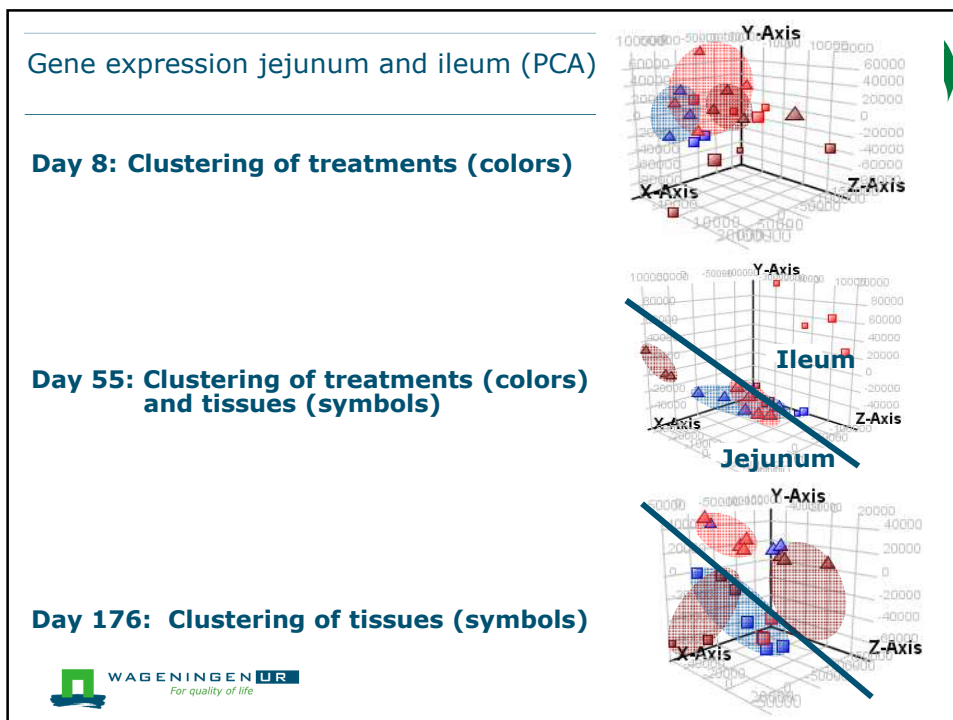
Treatment: At day 4 after birth.

Antibiotic: Regularly used in intensive farming systems to prevent respiratory diseases.

Stressor: Common in intensive farming systems: weighing, numbering, and tail docking.








Number of regulated genes in jejunum and ileum

ANOVA $p < 0.05$ & $|FC| > 1.5$



Day / Tissue	T2 vs T1		T3 vs T1		T3 vs T2	
	Down	UP	Down	Up	Down	Up
8 / Jejunum	63	24	29	38	6	55
8 / Ileum	80	49	80	76	45	76
55 / Jejunum	182	823	1042	1277	701	296
55 / Ileum	306	315	663	725	371	284
176 / Jejunum	19	6	6	1	6	19
176 / Ileum	1	4	3	4	0	0

WAGENINGEN UR
For quality of life

Functional analysis of regulated genes. Day 8: **T2 vs T1**

Tissue	Down	Up
Jejunum	# Name	# Name
	1 chemotaxis	1 -
	2 cytokine activity	2 -
	3 chemokine activity	3 -
	4 reg. of secretion /immune effector process	4 -
	5 cell migration/motion (leukocyte)	5 -
Ileum	# Name	# Name
	1 cytokine activity	1 cell fraction
	2 chemotaxis	2 -
	3 second-messenger-mediated signaling (cAMP)	3 -
	4 chemokine activity	4 -
	5 response to bacterium/reg. systemic process	5 -

Decrease in activity of immune related processes in piglets treated with antibiotics

Functional analysis of regulated genes. Day 8: **T3 vs T1**

Tissue	Down	Up
Jejunum	# Name	# Name
	1 chemotaxis	1 nucleotide binding
	2 cytokine activity	2 membrane fraction
	3 extracellular region	3 ATP binding
	4 chemokine activity	4 -
	5 second-messenger-mediated signaling (cAMP)	5 -
Ileum	# Name	# Name
	1 response to wounding/defense response	1 nucleotide binding
	2 cytokine activity	2 positive reg. of catalytic activity/signaling cascade
	3 chemotaxis	3 plasma membrane
	4 extracellular region	4 -
	5 chemokine activity	5 -

Decrease in activity of immune related processes in piglets treated with antibiotics + stressors

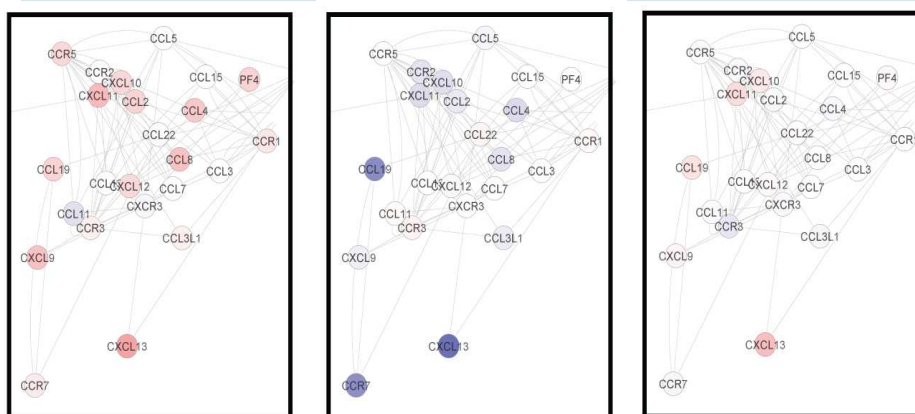
Functional analysis of regulated genes. Day 8: **T3 vs T2**



Tissue	Down	Up
Jejunum	# Name	# Name
	1 -	1 chemotaxis
	2 -	2 nucleotide binding
	3 -	3 -
	4 -	4 -
5 -	5 -	
Ileum	# Name	# Name
	1 -	1 Immunoglobulin
	2 -	2 nucleotide binding
	3 -	3 plasma membrane
	4 -	4 nucleotide/ATP binding
5 -	5 positive reg. of catalytic activity/signaling cascade	

Increase in activity of some immune related processes in piglets treated with antibiotics + stressor (compared to antibiotic alone)

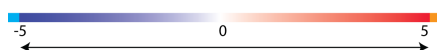
Development chemokine signalling network: day 8



T1

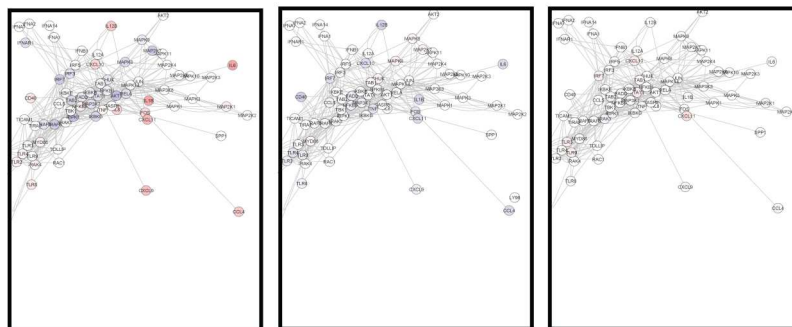
T2

T3



Similar observations for TLR network

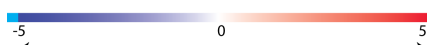
Development TLR network: day 8



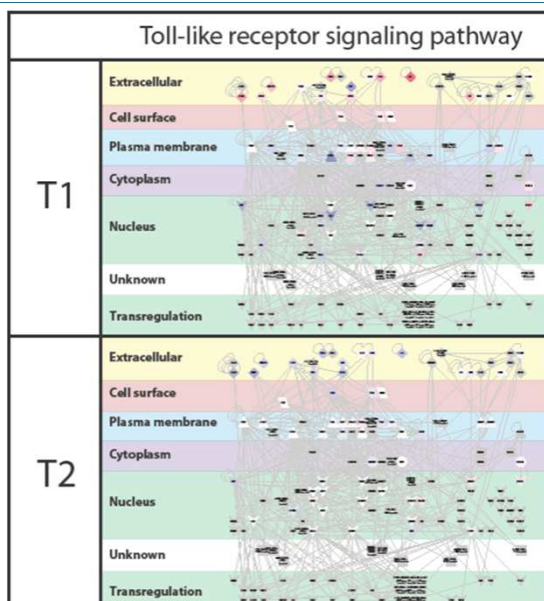
T1

T2

T3



TLR network - Ileum Day 8



Functional analysis Day 55

	T3vsT1 T3	T3vsT1 T1	T2vsT1 T2	T2vsT1 T1	T3vsT2 T3	T3vsT2 T2
j	membrane (ER,golgi,plasma,etc)	virus	metabole (lipid,FA)	virus	immune (innate,complement,TCR)	transporter and hormone activity
e	metabole (lipid,FA)	ribosome	localization/transport	ribosome	metabole	
j	apoptosis	beta cells	membrane (ER,golgi,plasma,etc)	apoptosis		
u	localization/transport		cell cycle		membrane	
n	immune (TLR3,TCR,innate,etc)		immune (TLR,TCR,innate,etc)		cell cycle	
u	cytoskeleton (light junctions,actin)		cytoskeleton (light junctions,actin)		cancer	
m	virus (HIV)		apoptosis			
	cell cycle					
	cancer					
	infection bacteria					
	(NOTE: 600 terms FDR < 0.1)		(NOTE: 500 terms FDR < 0.1)		(NOTE: 300 terms FDR < 0.1)	

	T3vsT1 T3	T3vsT1 T1	T2vsT1 T2	T2vsT1 T1	T3vsT2 T3	T3vsT2 T2
i	CYTOSKELETON (hema, actin,)	x	immune (cytokine, TLR3), innate, TCR	x	immune (TCR, BCR, NFkB, NK, IL2RB)	behavior (locomotory)
l	APOPTOSIS		CYTOSKELETON (ECM, hema, actin, cell junction)		cell cycle	Chemokine
e	immune (TCR,BCR,TLR, innate, NK, CD28, NFkB)		metabole (FA)		cancer	
u	metabole (FA)		apoptosis		metabole	
m	cancer		cell cycle		apoptosis	
	virus (HIV)					
	membrane (ER, golgi, etc)					
	cell cycle					

T2 vs T1

T3 vs T1

T3 vs T2

Differences in activity of several processes including immune related processes



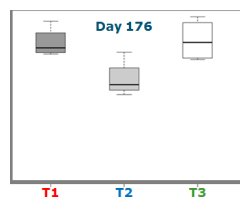
Functional analysis Day 176



T3 vs T1

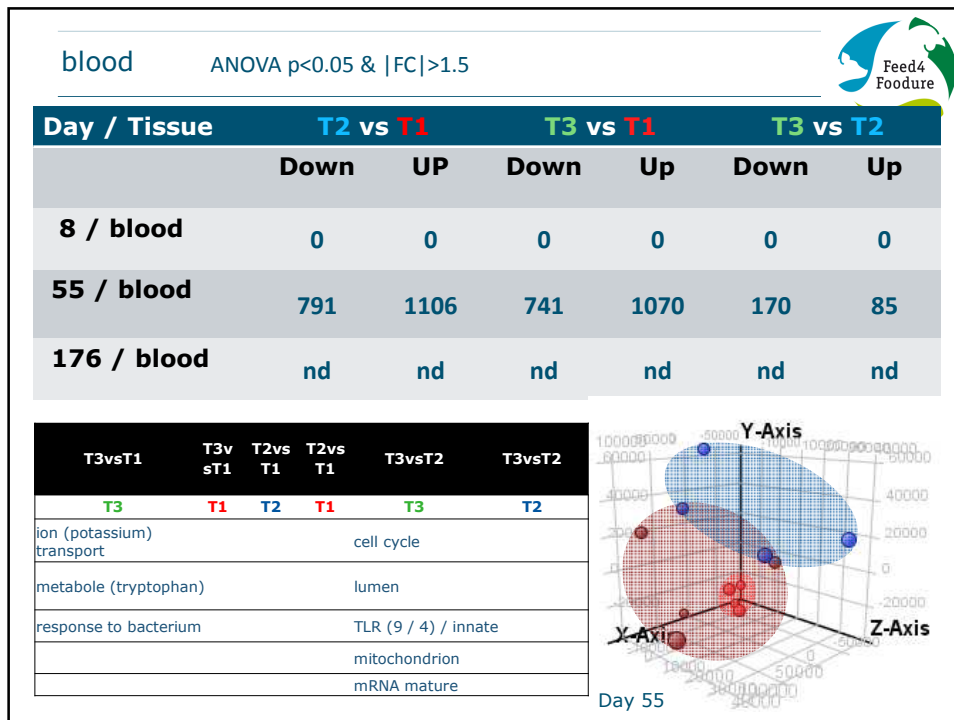
T2 vs T1

T3 vs T2



Differences in activity of a small number of processes in T2, some of which are immune related





Conclusies

Betekenis



- Vroege kolonisatie van de darm met microbiota is van belang voor de ontwikkeling en "status" van het immuunsysteem.
- Veranderingen in microbiota gaan gepaard met veranderingen in de immuun "status".
- Omdat diervoeding de samenstelling en diversiteit van microbiota moduleert is het een belangrijke factor voor (de ontwikkeling van) immuun competentie.

Dank voor uw aandacht



mari.smits@wur.nl

Functional analysis of regulated genes. Blood, day 55:

Down**#Name**

- 1 pos. reg. transcription/met. process
- 2 reg. phosphorylation / met. Process
- 3 pos. reg. transcription
- 4 pos. reg. synaptic transmission
- 5 kinase activity

#Name

- 1 pos. reg. transcription/met. process
- 2 pos. reg. transcription
- 3 pos. reg. synaptic transmission
- 4 reg. kinase activity / met. process
- 5 reg. synaptic transmission

#Name

- 1 reg. phosphorylation / met. process
- 2 reg. caspase/(endo)peptidase
- 3 (neg.) reg. (hormone) secretion
- 4 reg. apoptosis
- 5 pos. reg. hydrolase/catalytic activity

Up**#Name**

- 1 plasma membrane
- 2 blood circulation/pressure
- 3 nucleotide biosynthetic process
- 4 binding; retinoid, isoprenoid, retinol
- 5 melanocortin receptor

#Name

- 1 plasma membrane
- 2 nucleotide biosynthetic process
- 3 binding; retinoid, isoprenoid, retinol
- 4 melanocortin receptor
- 5 neg. reg. (immune) cell activation

#Name

- 1 reg. apoptosis
- 2 pos. reg. apoptosis
- 3
- 4
- 5

T2 vs. T1**T3 vs. T1****T3 vs. T2**