CMS – forskning på sykdomsmekanismer og markører

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NFR-BIP prosjekt 187301/S40: 2008-2011

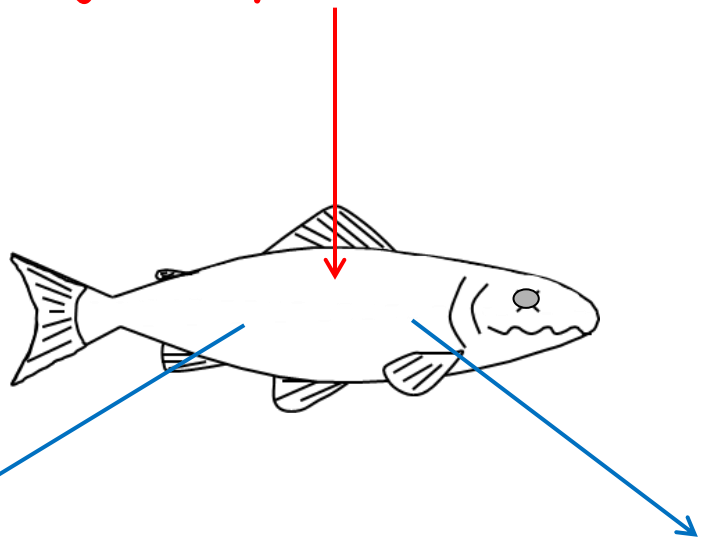
'Cardiomyopathy syndrome: A multi-task approach to reduce losses & improve knowledge'

Finansiering: NFR, industripartnere & FHF

Bakgrunn CMS - FoU utfordringer

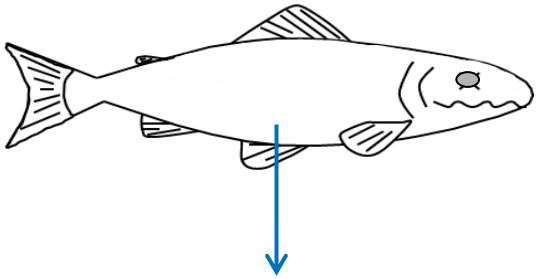
- Ukjent etiologi
  - Virus
  - Metabolsk syndrom
  - Autoimmunitet
  - Første smitteforsøk: overførbar sykdom (Fritsvold et al. 2009)
- Differensiell diagnostikk
  - Overlappende patologi med HSMB and PD
- Sykdomsovervåkning
  - Forbedre diagnostiske verktøy (tidlig fase)
  - Risiko faktorer (miljø, geno/fenotype)
- Nå: kausalt virus identifisert (PMCV) (Haugland et al., 2011)

Ukjent sykdomsfaktor



Fenotypiske responser:
-Kliniske tegn
-Patologi, vevsskade

Molekylære responser:
-Gener reguleres (av/på)
-Proteiner/enzymer reguleres



Molekylære responser – Microarray/RT-qPCR:
-Hvilke gener er regulert?
-Hvordan er de regulert?

Funksjon:
-Immunforsvaret
-Komplement respons
-IFN reseptor

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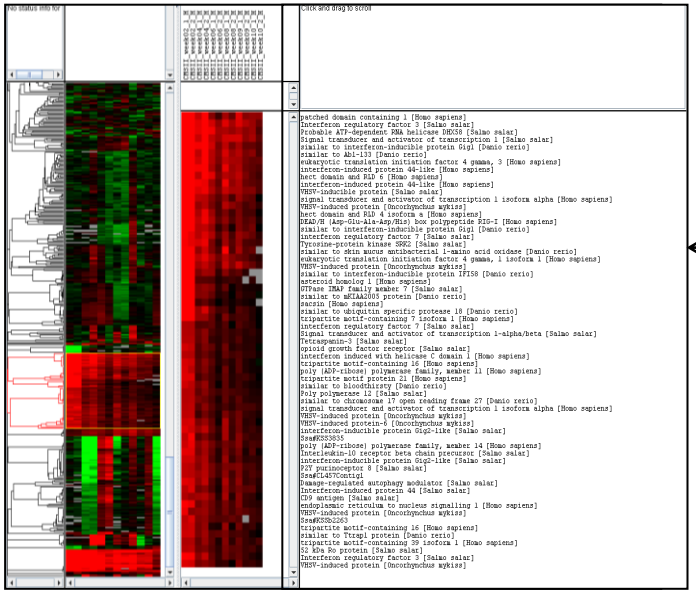
MEKANISMER



'Egenskap':
-Virus-infeksjon
-HSMB
-Temperatur
-Robusthet
-Etc..

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MARKØRER

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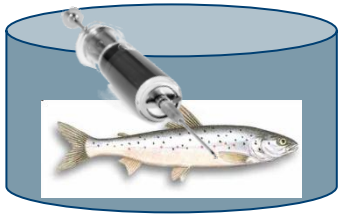
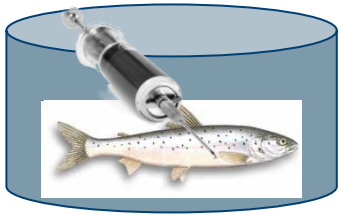
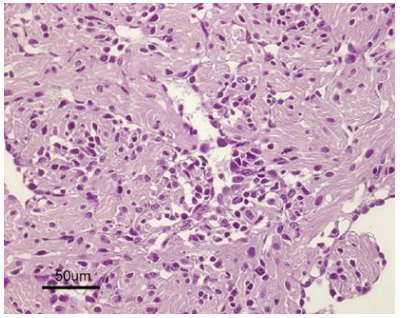
Smitteforsøk:

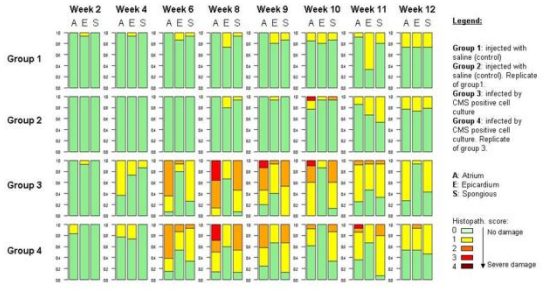


Pilotforsøk (NIVA Solbergstrand, 2008):
-Villaks Numedalslågen, 50-100 gr
-Ulike smittestoff , injeksjon/kohabitasjon
-Tidsuttak, organer etc



Hoved-smitteforsøk (VESO Vikan, 2009):
-Std uvaksinert post-smolt, 50 gr
-Celle-inokulat, ip-injeksjon
-Uttak alle vev, 8 tidspunkt

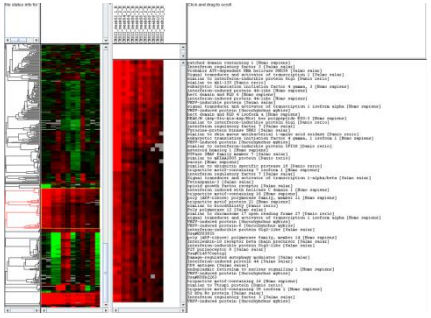




Feltmateriale:

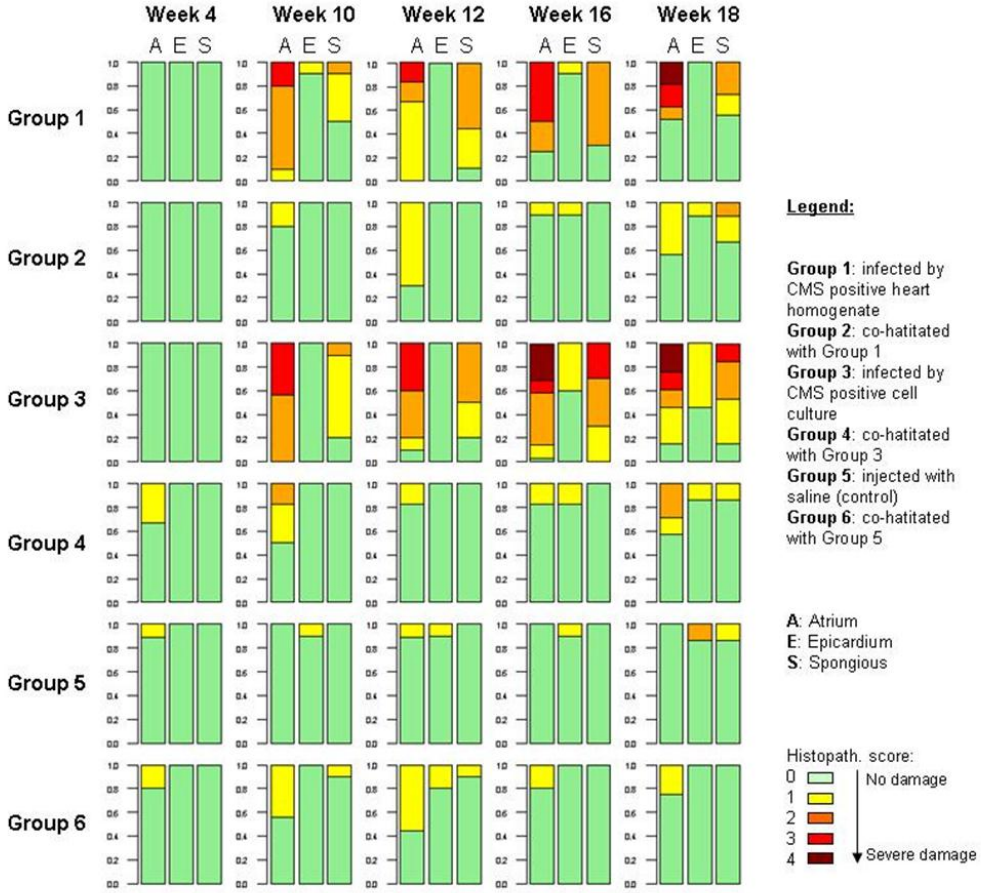
Kasus-kontroll forsøk (2008-11):
-Prøver fra CMS utbrudd og ktrl anlegg
-Felt-korrelat for resultater fra smitteforsøk

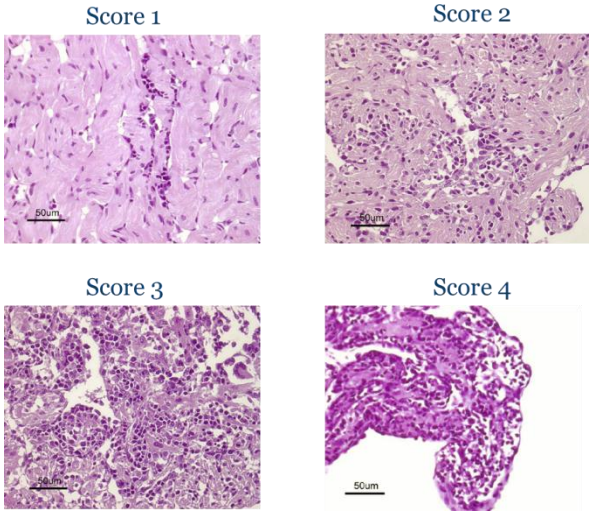
Fisk fra Aqua Gens avlskjerne



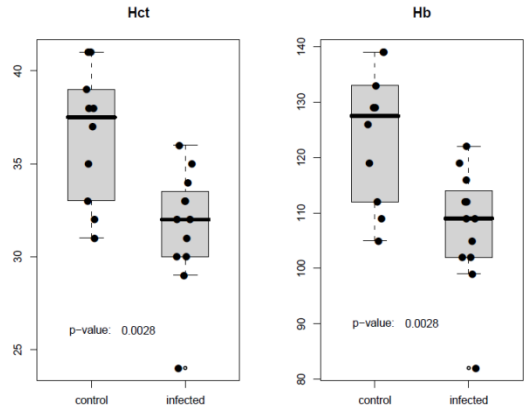
Pilot smitteforsøk

Histopatologi- NVI/NVH:

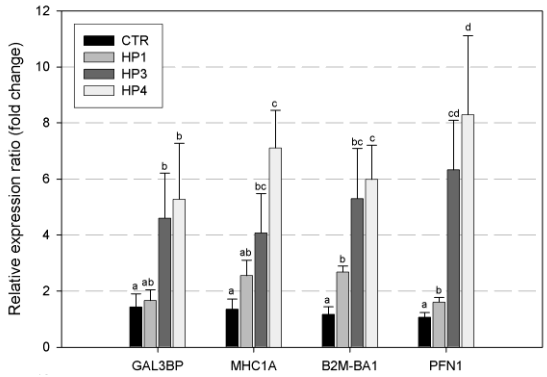




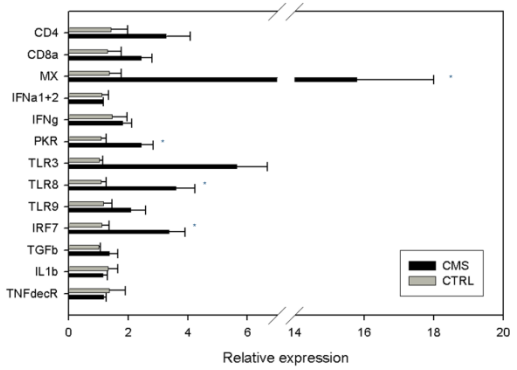
Photos: M Alarcon/TT Poppe



- CMS fisk utvikler anemi (redusert Hct/Hb)



- Gener korrelert med histopatologi score gir grunnlag for diagnostiske markører



- CMS aktiverer gener som spesifikt responderer på virus-infeksjon- indikasjon på etiologi

Hoved-smitteforsøk

Fish: Unvaccinated Atlantic salmon smolt, aver size 50 gr (VESO Vikan)

Control groups:
Injected with control inoculate

Group 1

Group 2

Tank 1

Tank 2

Test groups:
Injected with CMS inoculate (infected cell culture)

Group 3

Group 4

Tank 3

Tank 4

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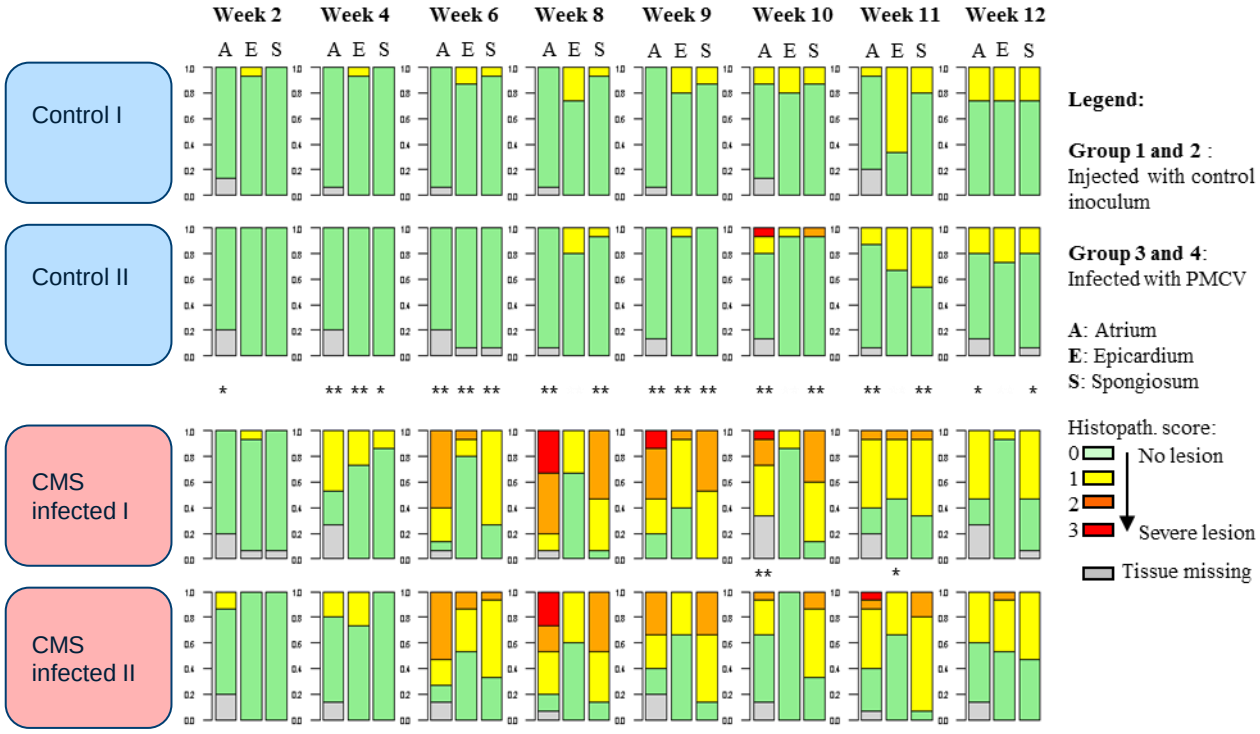
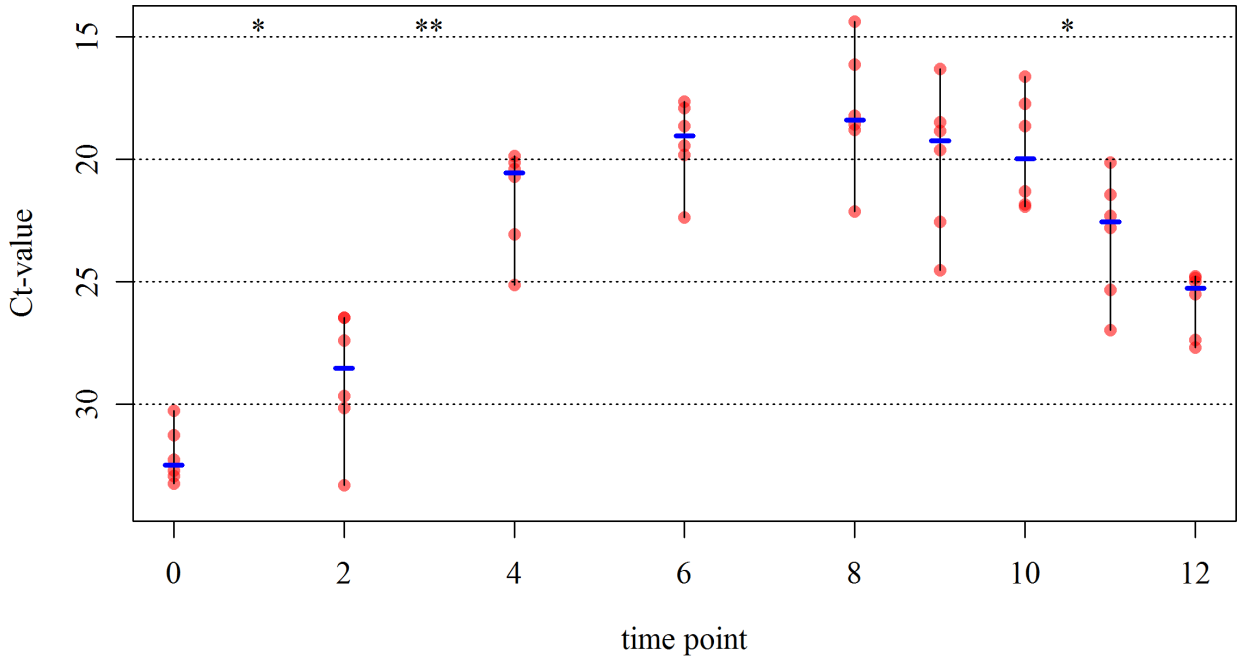
Histopatologi – hjertevev*:



- Ingen dødelighet
- Patologi uke 6-11, maks uke 8-9

*Utført av VI/NVH

Mengde PMCV virus (RNA) i hjerte over tid (qPCR)*



*utført av PHARMAQ

- Viruset formerer seg kraftigst i tidlig fase (fra uke 2-4)
- Platå-fase sammenfaller med histopatologi-topp
- Verten kvitter seg med virus fra uke 10-12

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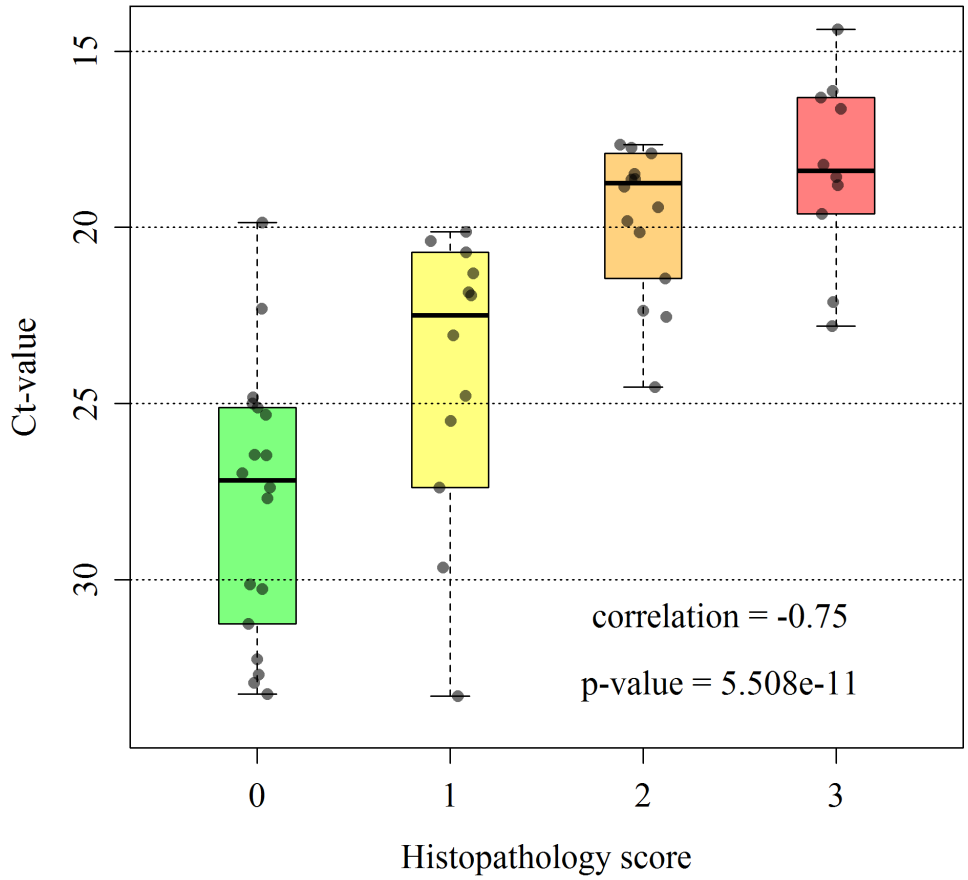
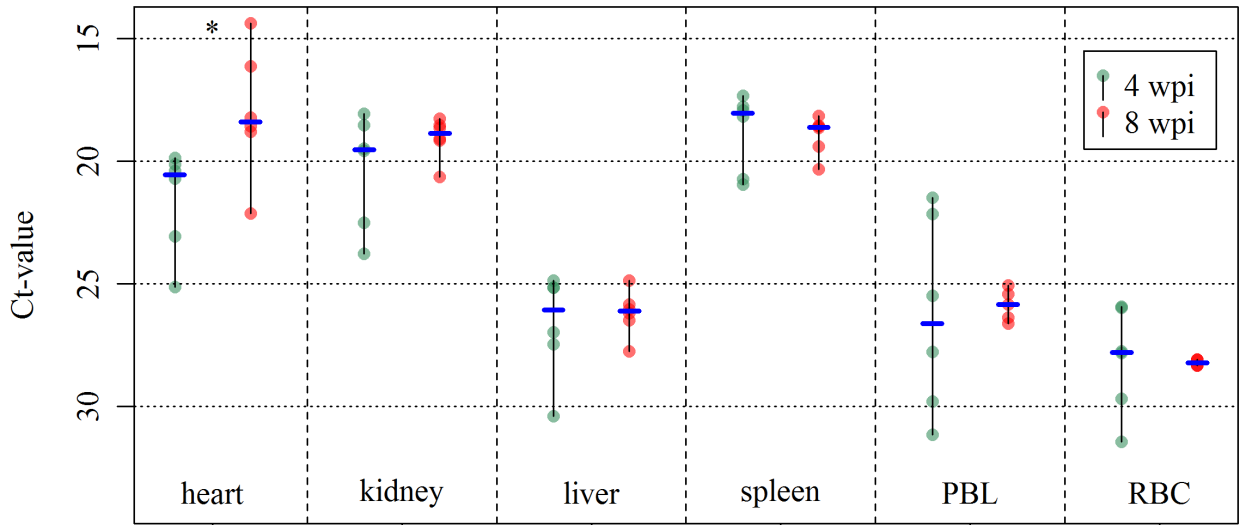
Korrelasjon hjerte histopatologi og virus-mengde



- Grad av hjerteskade bestemmes av mengde virus (cytopatiske effekter)

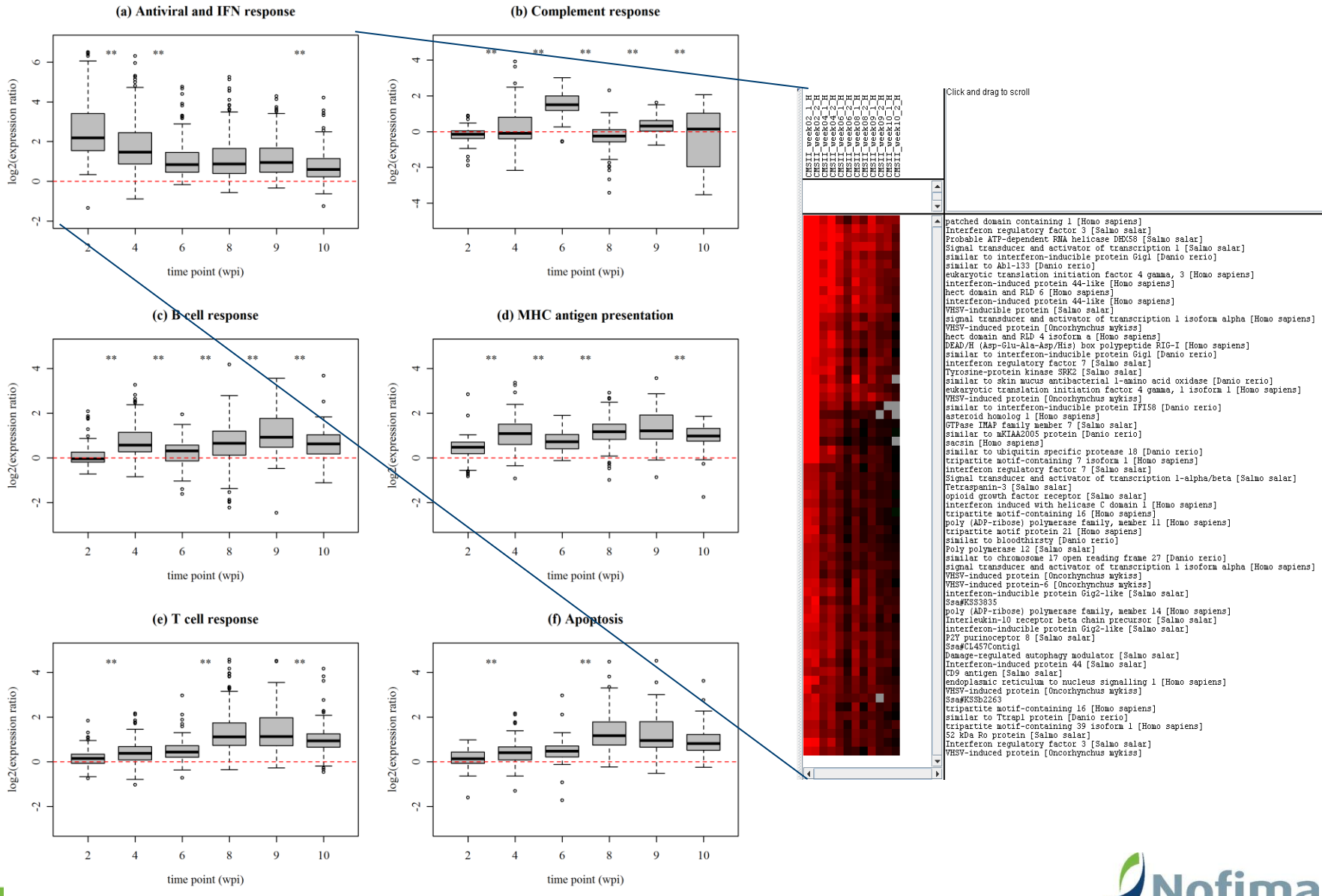
Mengde virus i ulike organer, tidlig og sen fase*



*utført av PHARMAQ

- Viruset finnes i alle organer
- Dominerer i hjerte, nyre og milt
- Patologi i andre vev enn hjerte?
- Tidlig påvisning i blod – utg pkt for diagnostikk/overvåkning

Immunologiske responser i hjerte (microarray)



CMS sykdoms-profil: Mekanismer

Antiviral/IFN-respons

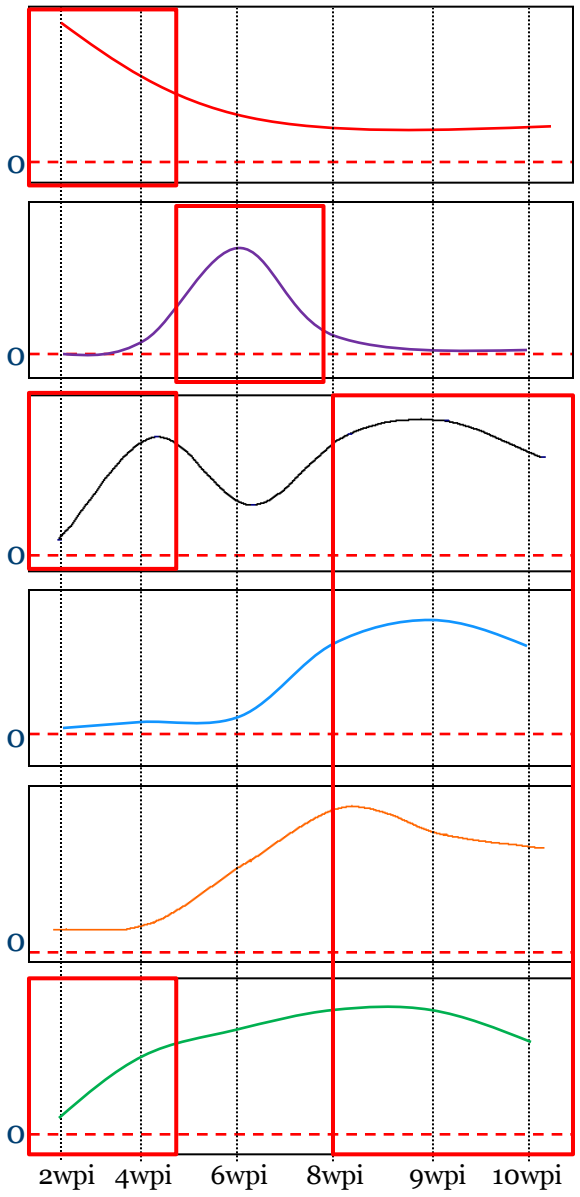
Komplement-respons

Ag-presentasjon/
B celle-respons

T celle-respons

Histopatologi

Virus



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CMS sykdoms-profil: Markører

Antiviral/IFN-respons

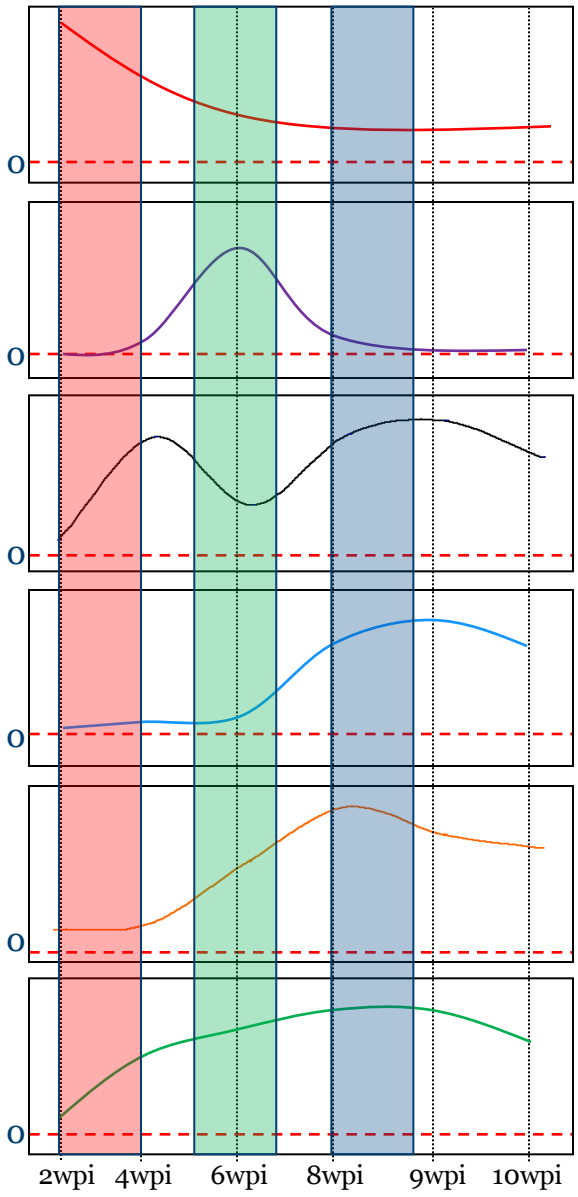
Komplement-respons

Ag-presentasjon/
B celle-respons

T celle-respons

Histopatologi

Virus



Tidlig viremi & antiviral

Komplement

Patologisk fase/CMI

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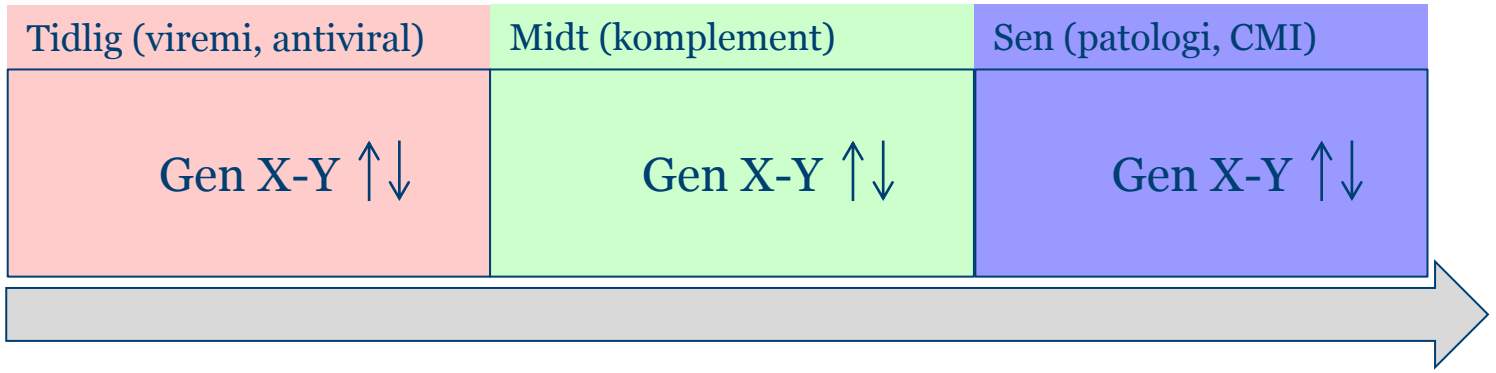
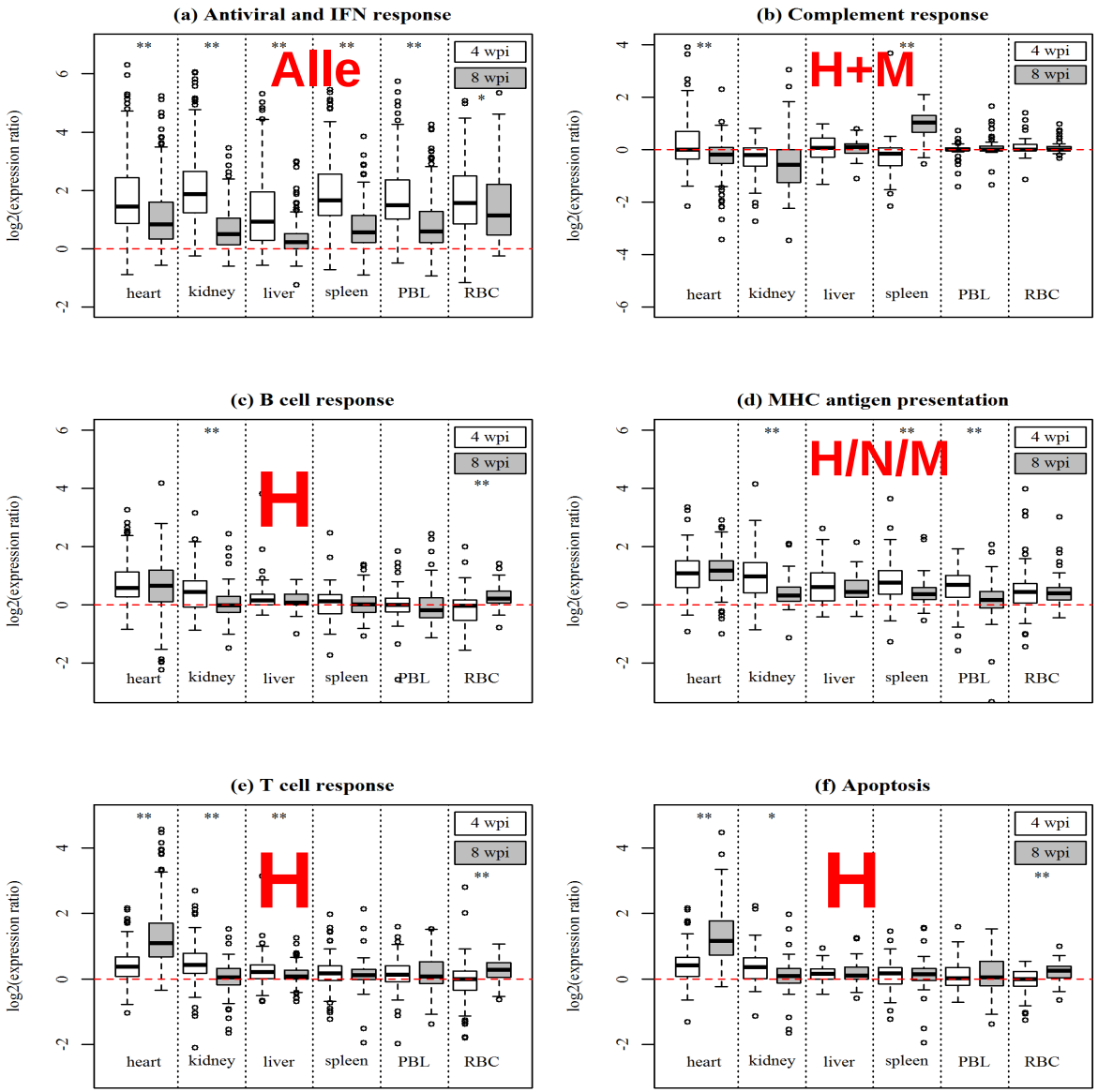
Etablert basis profil for sykdomsstadier

Gen-profiler = sykdomsmarkører

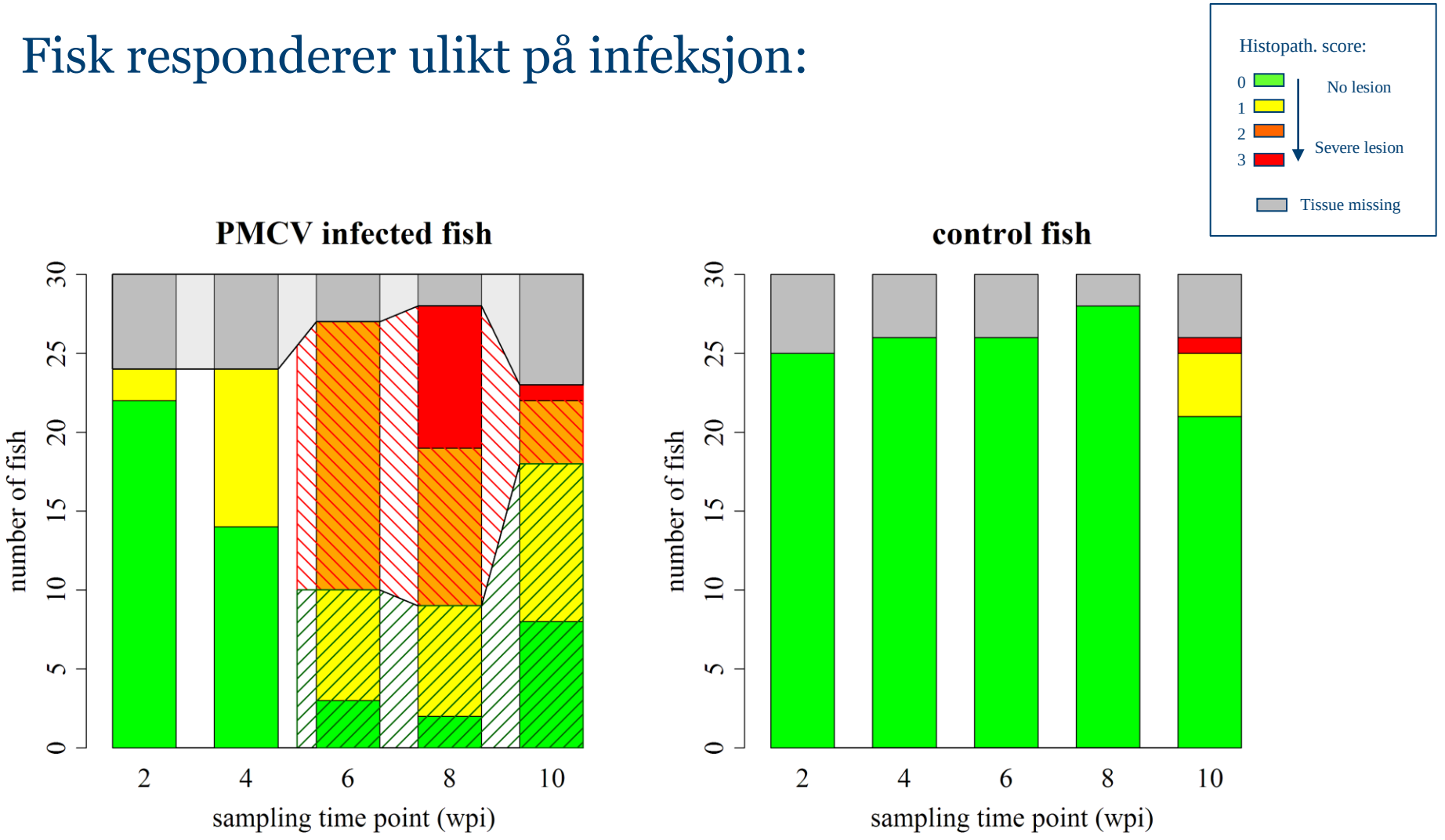


Immunologiske responser i ulike organer, uke 4+8



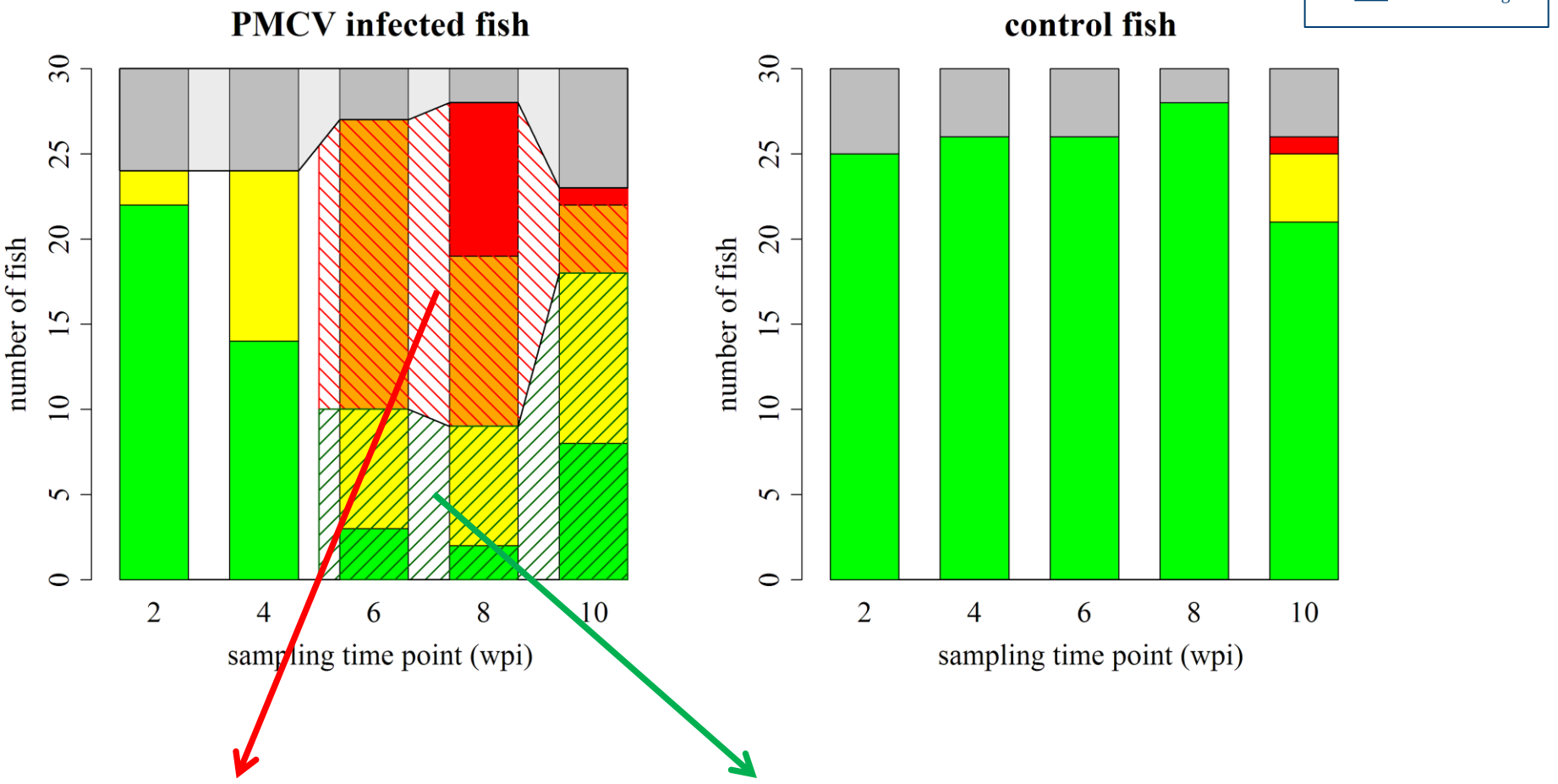
H - hjerte
N - nyre
M - milt

Fisk responderer ulikt på infeksjon:



- *Betydelig andel fisk utvikler ikke hjerteskade*

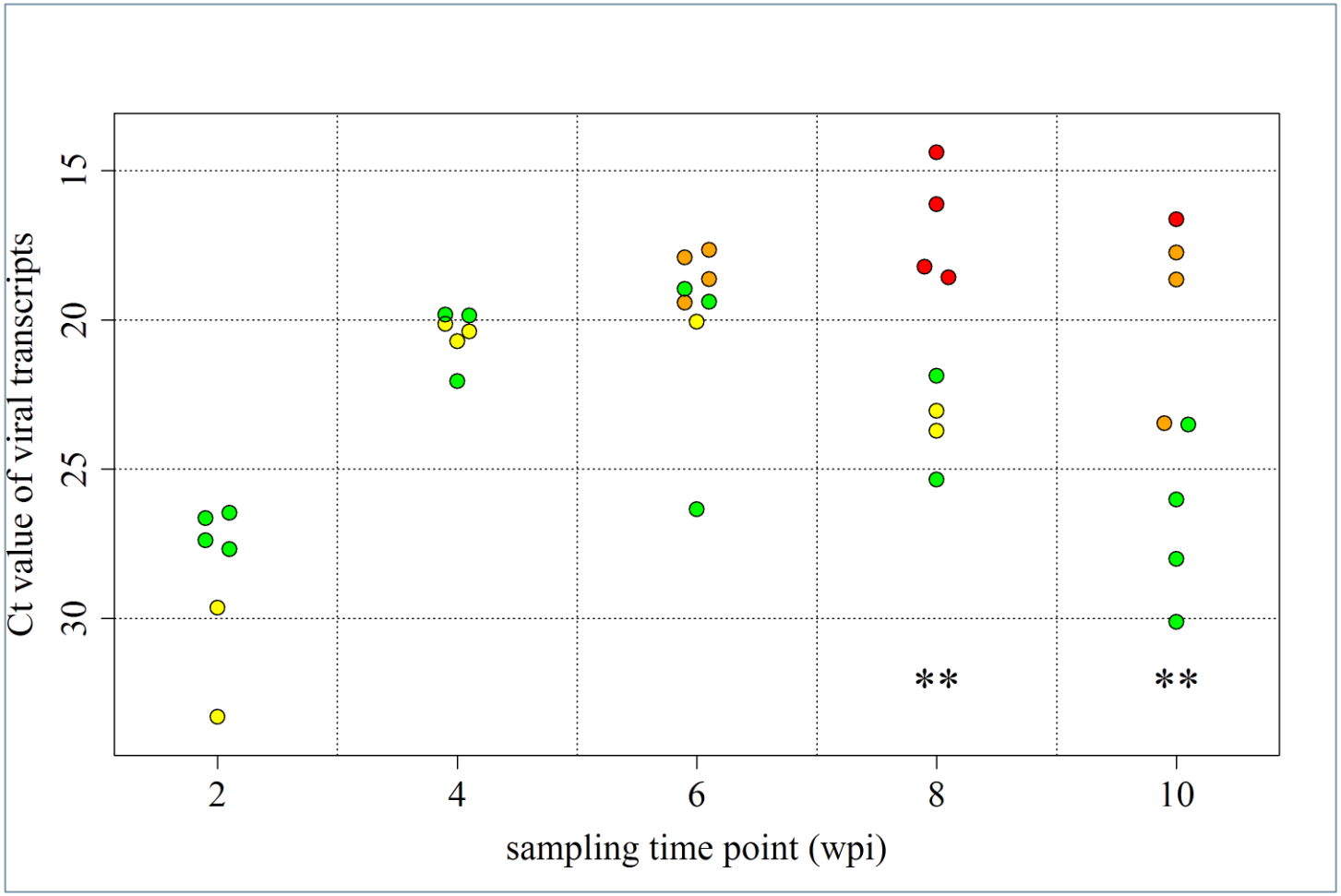
Resistens (immunologi) eller adferdsstrategi?

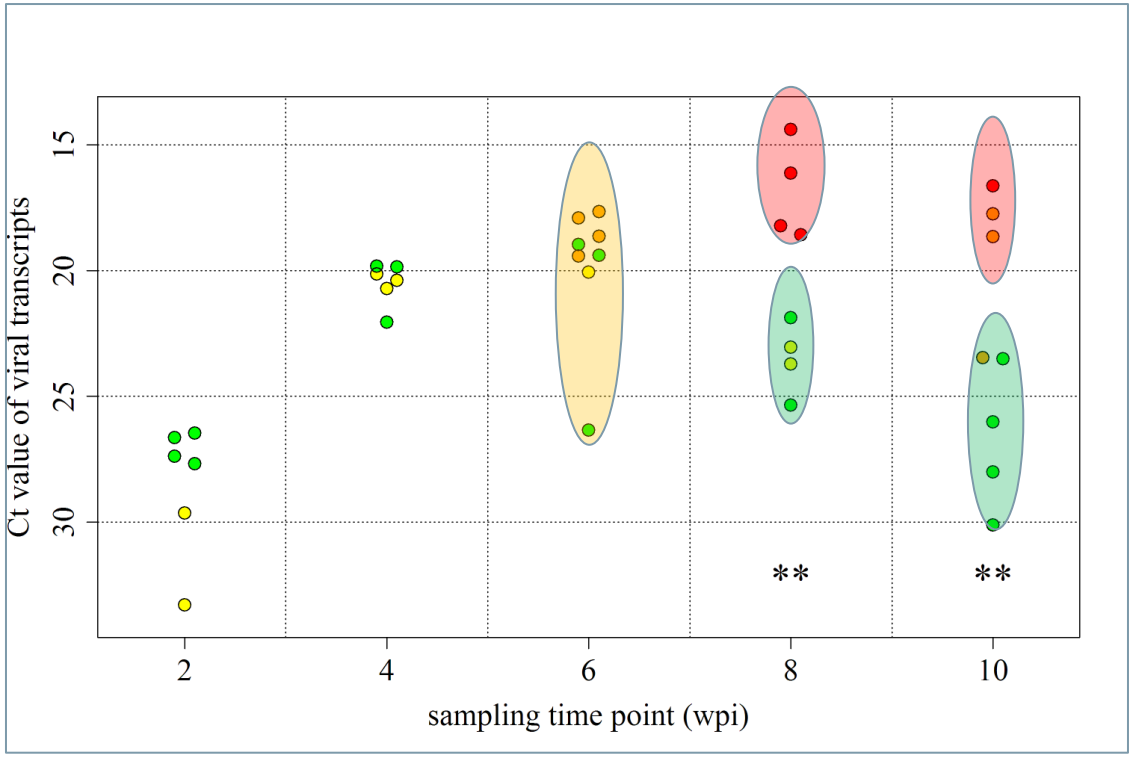


High Responders (HR)

Low Responders (LR)

Ulik mengde virus i HR vs LR?





- Like nivåer inntil uke 6
- Redusert virus-infeksjon assosiert med mangel på hjerteskade (evnt recovery)
- Underliggende mekanismer?

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Verts-responser i HR vs LR:

	2 wpi	4 wpi	6 wpi	8 wpi	10 wpi	
HR	13 GO: 2376: immune system process 8 GO: 9607: response to biotic stimulus 5 GO: 19882: antigen processing and presentation	34 GO: 2376: immune system process 20 GO: 6955: immune response 12 GO: 19882: antigen processing and presentation 6 GO: 18212: peptidyl-tyrosine modification	17 GO: 2376: immune system process 11 GO: 6955: immune response 9 GO: 19882: antigen processing and presentation 3 GO: 2474: antigen processing and presentation of peptide antigen via MHC class I 3 GO: 48002: antigen processing and presentation of peptide antigen 57 GOs significant: 5 GO: 14866: skeletal myofibril assembly 6 GO: 55002: striated muscle cell development 3 GO: 48739: cardiac muscle fiber development 3 GO: 30241: skeletal muscle myosin thick filament assembly 3 GO: 30240: skeletal muscle thin filament assembly 52 more ...	21 GO: 50896: response to stimulus 18 GO: 2376: immune system process 12 GO: 19882: antigen processing and presentation 12 GO: 6955: immune response 4 GO: 2474: antigen processing and presentation of peptide antigen via MHC class I 4 GO: 48002: antigen processing and presentation of peptide antigen 3 GO: 2483: antigen processing and presentation of endogenous peptide antigen 3 GO: 19885: antigen processing and presentation of endogenous peptide antigen via MHC class I 3 GO: 19883: antigen processing and presentation of endogenous antigen	5 GO: 6955: immune response 3 GO: 19882: antigen processing and presentation	Histopathology 2 or 3
LR	6 GO: 55001: muscle cell development	7 GO: 51056: regulation of small GTPase mediated signal transduction 6 GO: 35023: regulation of Rho protein signal transduction 5 GO: 14866: skeletal myofibril assembly 5 GO: 30239: myofibril assembly 5 GO: 31032: actomyosin structure organization 5 GO: 10927: cellular component assembly involved in morphogenesis	15 GO: 2376: immune system process 11 GO: 6955: immune response 8 GO: 19882: antigen processing and presentation 61 GOs significant: 5 GO: 14866: skeletal myofibril assembly 3 GO: 48739: cardiac muscle fiber development 3 GO: 30241: skeletal muscle myosin thick filament assembly 3 GO: 30240: skeletal muscle thin filament assembly 3 GO: 71688: striated muscle myosin thick filament assembly 5 GO: 30239: myofibril assembly 3 GO: 7076: mitotic chromosome condensation 5 GO: 31032: actomyosin structure organization 53 more...		6 GO: 2376: immune system process 5 GO: 19882: antigen processing and presentation 5 GO: 6955: immune response	Histopathology 0 or 1

*GO enrichment (funksjonelle gen-klasser)

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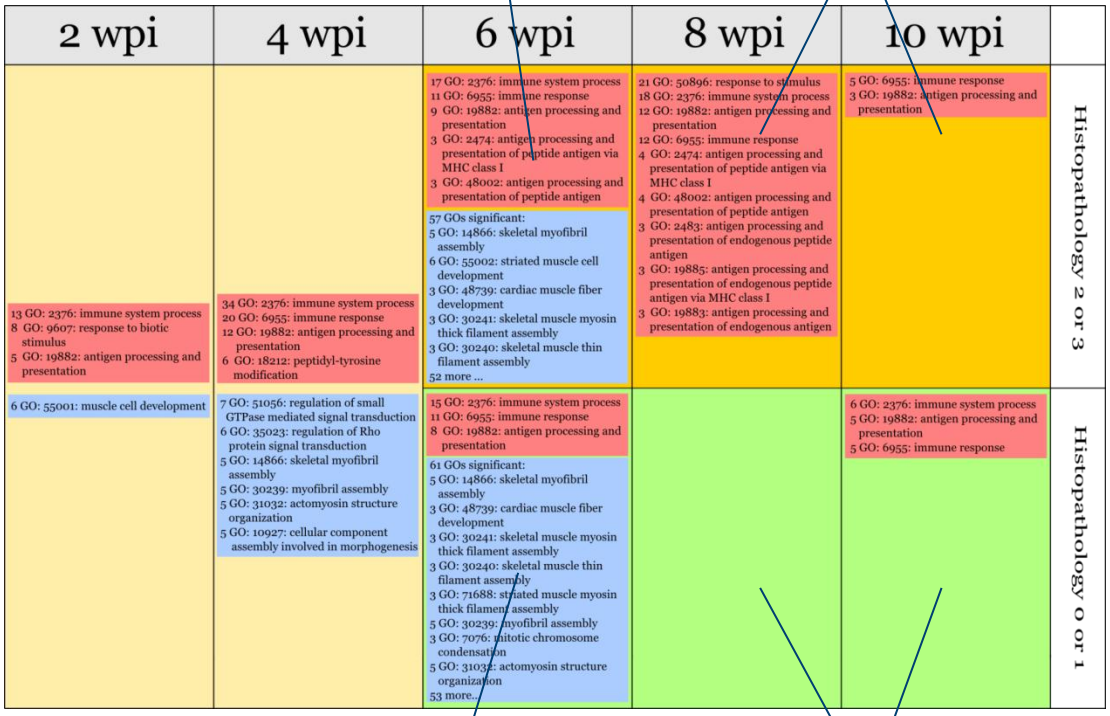
Verts-responser i HR vs LR:

Immun-responser OPP Hjertemuskulatur NED

Immun-responser OPP T celle-respons



Celle-mediert immun respons:
-Reduserer virus
-Forsterker patologi

Immun-responser OPP Hjertemuskulatur NED

Ingen immun-respons

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Betydning for industrien?

- Basis profil - sykdomsmarkører
- Evaluere effekter av sykdomsbegrensende tiltak
  - Vaksinering
  - Genetikk
  - Ernæring
  - Miljø
- Overvåkning
  - Rutineprøver fra blod
  - Diagnostikk, sykdomsfase
  - Virus vs infeksjon – er fisken virkelig syk?

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Anvendelser

- Analyse av sykdomsstatus i familiemateriale
  - Avlskjerne fra felt med CMS (Aqua Gen)
  - Histopat + virus (uført), sykdomsmarkører (pågår)

- Evaluering av funksjonelle fôr på CMS
  - Diett/smitteforsøk i regi av EWOS (start april 2011)
  - Histopat, virus, sykdomsmarkører/mekanismer

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Takk for oppmerksomheten!

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