COAD-READ, 221 samples: Correlation analysis for seven miRNA-seq-based consensus clusters

G. Robertson, Andy Chu, Elizabeth Chun, Inanc Birol
21 May 2011, 13h00

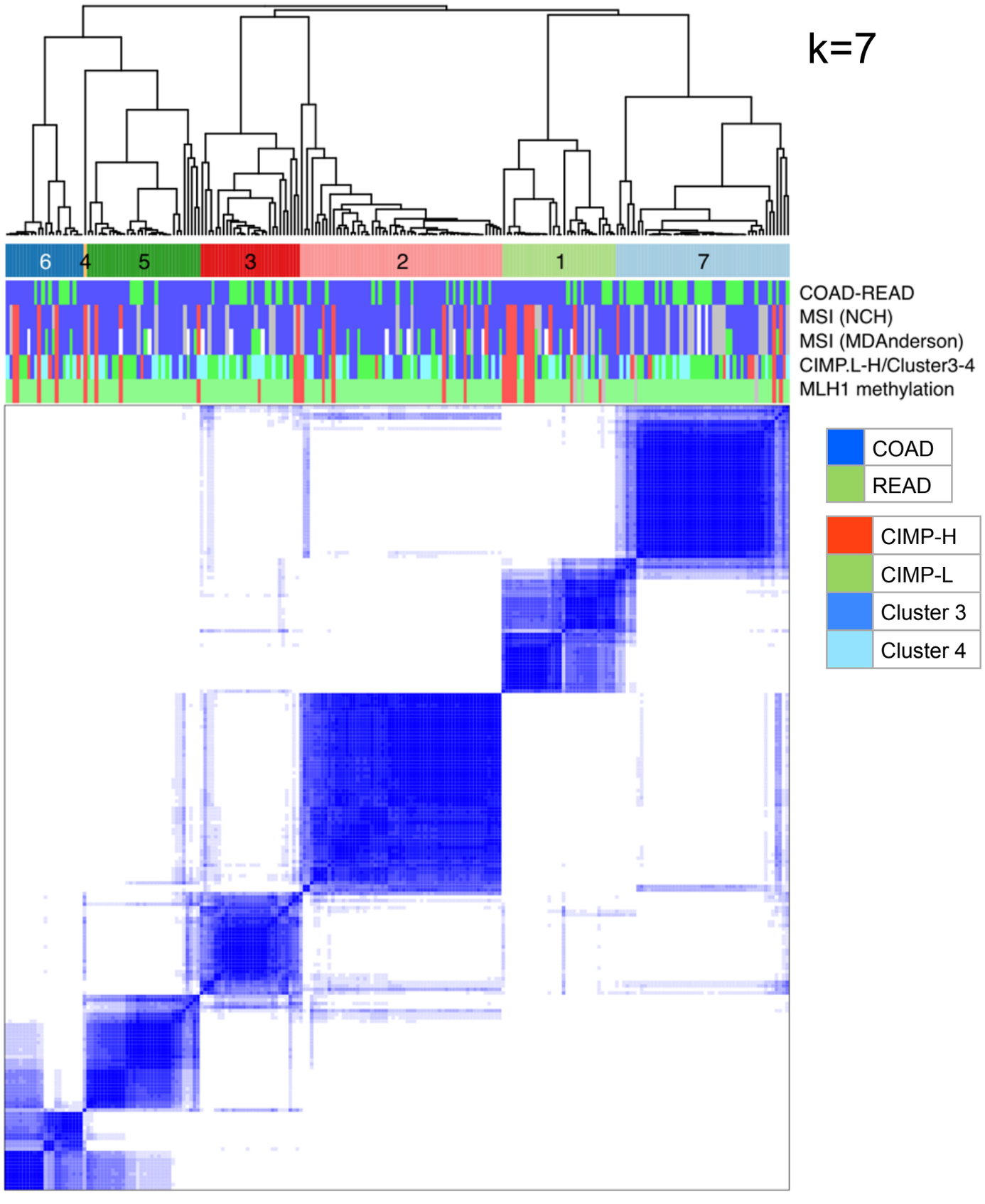
Context: we hope to send Raju something about miRNAs and the Wnt pathway.

1. A k=7 consensus clustering result that used isomiRs returns reasonably large numbers of thresholded miRNA-gene correlations. We speculate that a cluster that has few thresholded correlations has diverse (rather than consistent) correlations for almost all miRNA-gene pairs.

2. To identify stories that might be worth developing, we start to survey RPKM variation across the clusters for genes in the Wnt pathway. We hope to find large RPKM differences between clusters for genes that are known to be important for the pathway.

3. As a detailed trial, we show all thresholded mirNA-mRNA correlations for all ten Frizzled genes (FZD1-10) in the KEGG Wnt signaling pathway. Thresholds used: the 99% confidence interval on either m1 or m2 slopes does not include zero, and r^2 must be at least 0.25. The number of correlations per cluster varied from 18 to 582. The proportion of positive to negative correlations varied widely, with negatives dominant in clusters 6 and 3, and positives dominant in clusters 1, 2 and 5.

Clustering COAD-READ unfiltered isomiR data for 221 samples

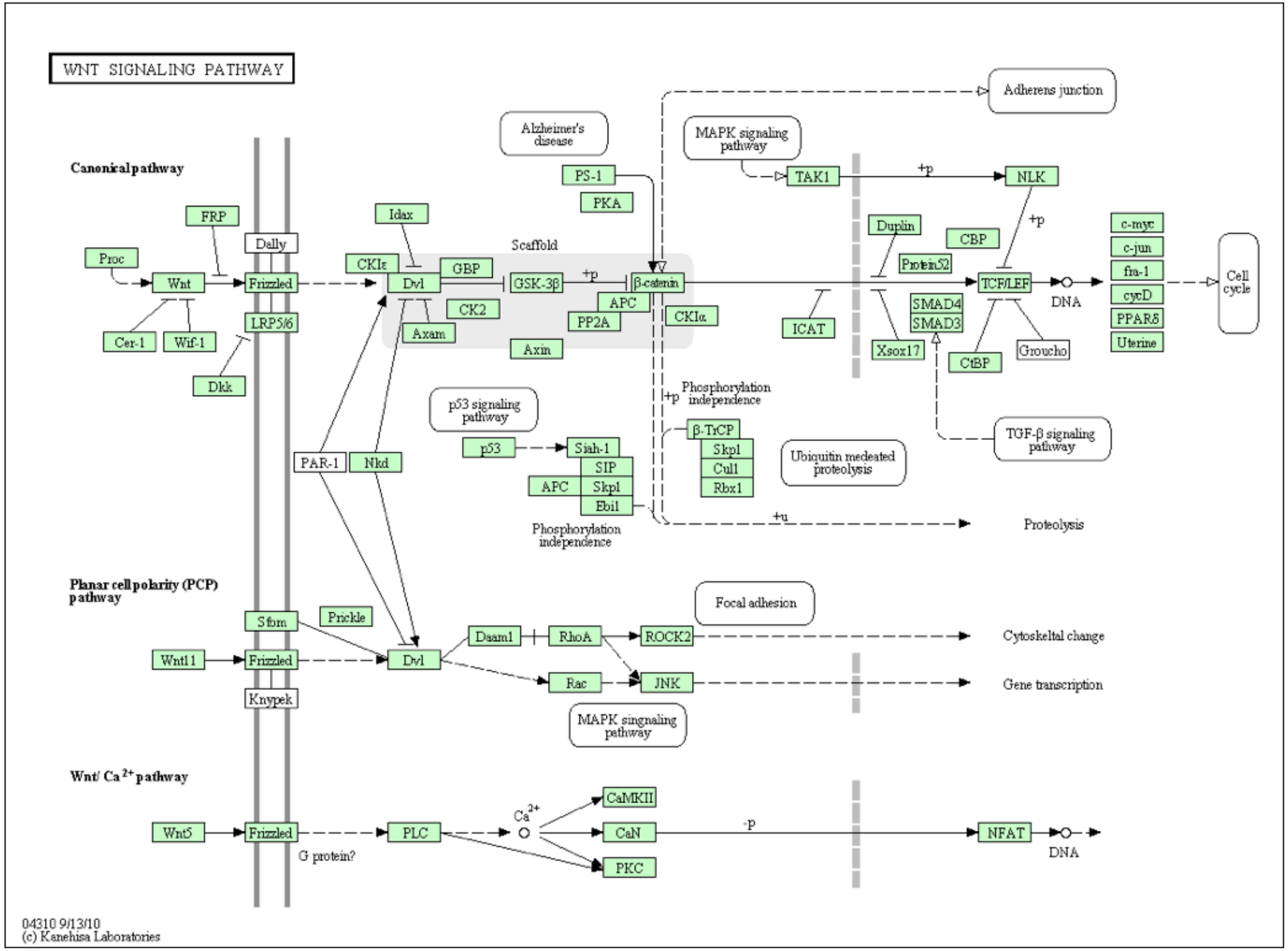


Number of thresholded miRNA-mRNA correlation records.

	negative m records		positive m records	
Clust	all	Wnt p'way	all	Wnt p'way
6	600,539	5,230	338,098	2,803
4	--		--	
5	6,653	70	9,962	104
3	240,680	2,076	296,453	2,210
2	113,668	679	133,305	1,072
1	181,005	1,282	218,039	1,809
7	329,394	2,249	356,766	2,269

19 May 2011, 21h00

KEGG WNT signaling pathway



14 May 2011, 20h50

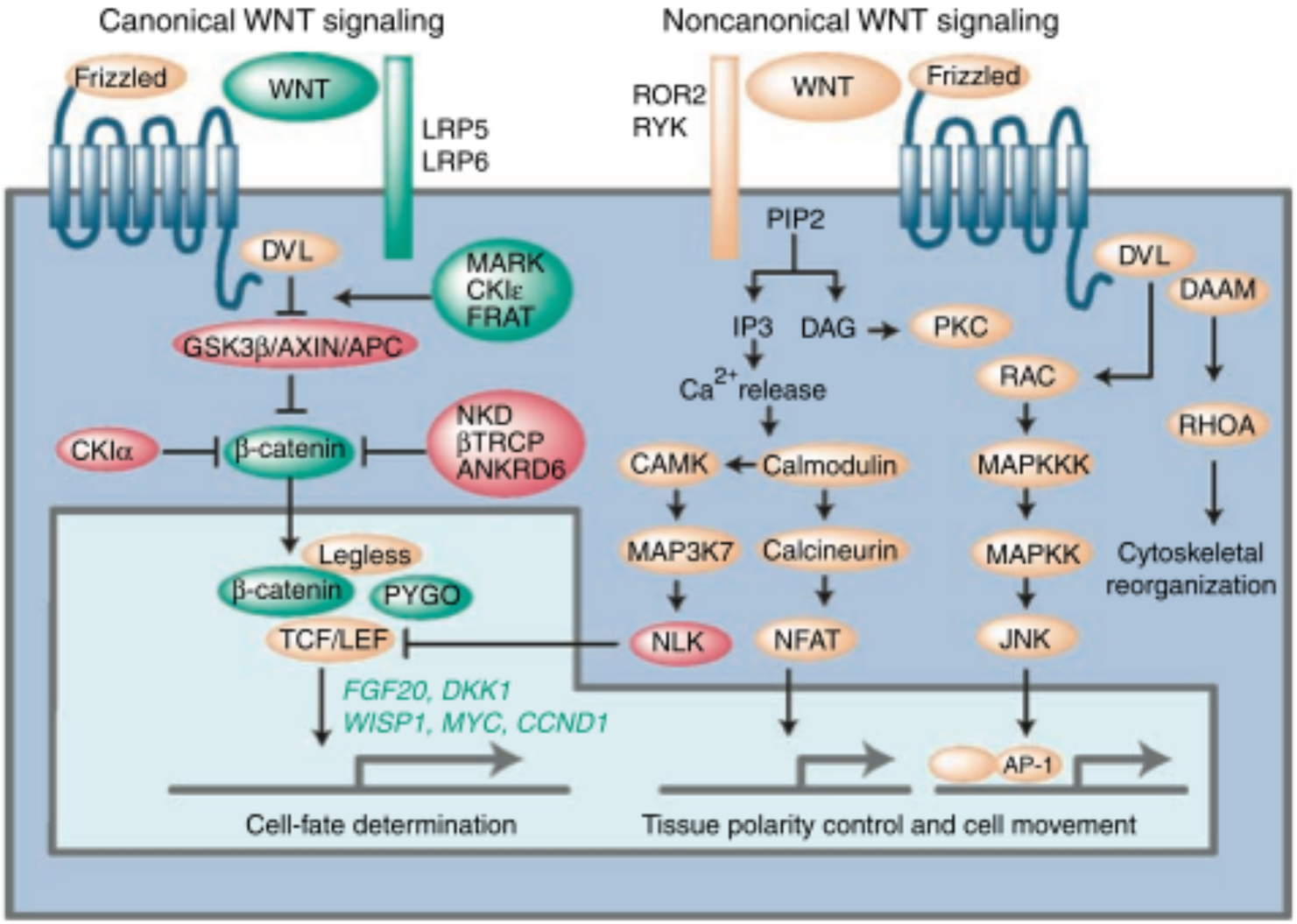


Fig.1. Landscape of WNT signaling cascades. WNT signals are transduced to the **canonical** pathway for cell fate determination, and to the **noncanonical** pathway for control of **cell movement and tissue polarity**. **Canonical** WNT signals are transduced through Frizzled family receptors and LRP5/LRP6 coreceptor to the h-catenin signaling cascade. **Noncanonical** WNT signals are transduced through Frizzled family receptors and ROR2/RYK coreceptors to the DVL-dependent (Rho family GTPases and JNK) or the Ca2+-dependent (NLK and NFAT) signaling cascades. Microtubule affinity ^ regulating kinase (MARK ; PAR-1) family kinases, CKIq, and FRAT are **positive** regulators of the **canonical** WNT pathway, whereas APC, AXIN1, AXIN2, CKIa, NKD1, NKD2, hTRCP1, hTRCP2, ANKRD6, NLK, and PPARg are **negative** regulators. FGF20, DKK1, WISP1, MYC, CCND1, and Glucagon (GCG) are **target** genes of the **canonical** WNT signaling pathway. WNT signals are context-dependently transduced to both pathways based on the expression profile of WNT, SFRP, WIF, DKK, Frizzled receptors, coreceptors, and the activity of intracellular WNTsignaling regulators. Katoh and Kato, Clin Cancer Res 2007, 13:4042.

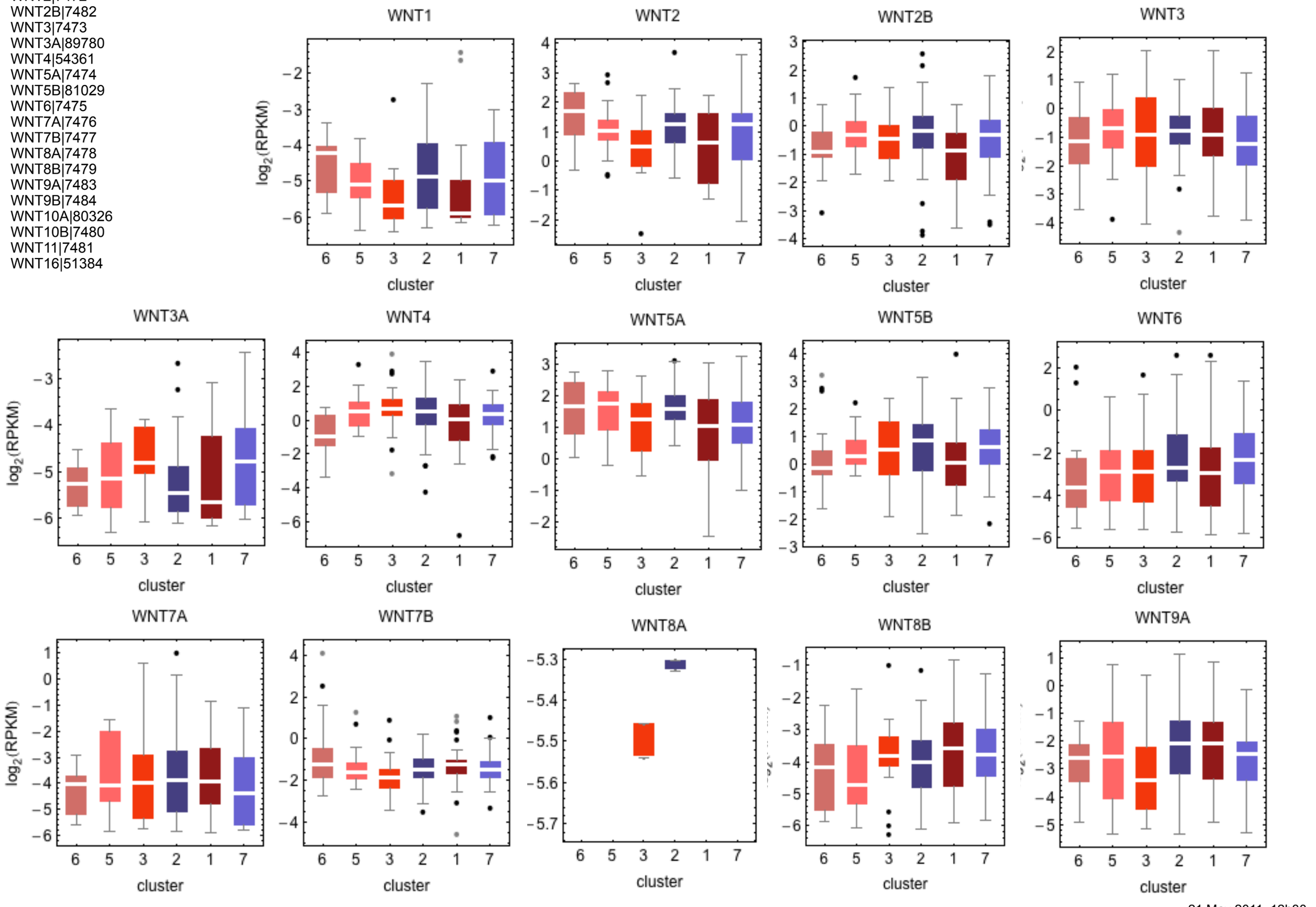
21 May 2011, 12h00

RPKM for Wnt pathway genes, across seven miRNA-seq-based consensus clusters

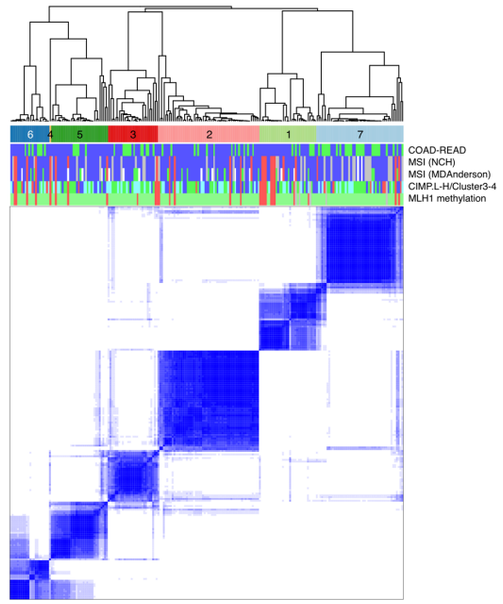
Fig.1. Landscape of WNT signaling cascades. WNT signals are transduced to the **canonical** pathway for cell fate determination, and to the **noncanonical** pathway for control of **cell movement and tissue polarity**. **Canonical** WNT signals are transduced through **Frizzled family receptors** and LRP5/LRP6 coreceptor to the h-catenin signaling cascade. **Noncanonical** WNT signals are transduced through Frizzled family receptors and ROR2/RYK coreceptors to the DVL-dependent (Rho family GTPases and JNK) or the Ca2+-dependent (NLK and NFAT) signaling cascades. Microtubule affinity ^ regulating kinase (MARK ; PAR-1) family kinases, CKIq, and FRAT are **positive** regulators of the **canonical** WNT pathway, whereas APC, AXIN1, AXIN2, CKIa, NKD1, NKD2, hTRCP1, hTRCP2, ANKRD6, NLK, and PPARg are **negative** regulators. FGF20, DKK1, WISP1, MYC, CCND1, and Glucagon (GCG) are **target** genes of the **canonical** WNT signaling pathway. **WNT signals are context-dependently transduced to both pathways based on the expression profile of WNT, SFRP, WIF, DKK, Frizzled receptors, coreceptors, and the activity of intracellular WNT signaling regulators.** Katoh and Kato, Clin Cancer Res 2007, 13:4042.

grobertson:mRNA-seq grobertson$ grep "WNT" crc_244_gene_rpkm.txt | cut -f1
WNT1|7471
WNT2|7472
WNT2B|7482
WNT3|7473
WNT3A|89780
WNT4|54361
WNT5A|7474
WNT5B|81029
WNT6|7475
WNT7A|7476
WNT7B|7477
WNT8A|7478
WNT8B|7479
WNT9A|7483
WNT9B|7484
WNT10A|80326
WNT10B|7480
WNT11|7481
WNT16|51384

WNTs: RPKM abundance across 7 clusters



SFRPs, WIF1 and DKKs: RPKM abundance across 7 clusters



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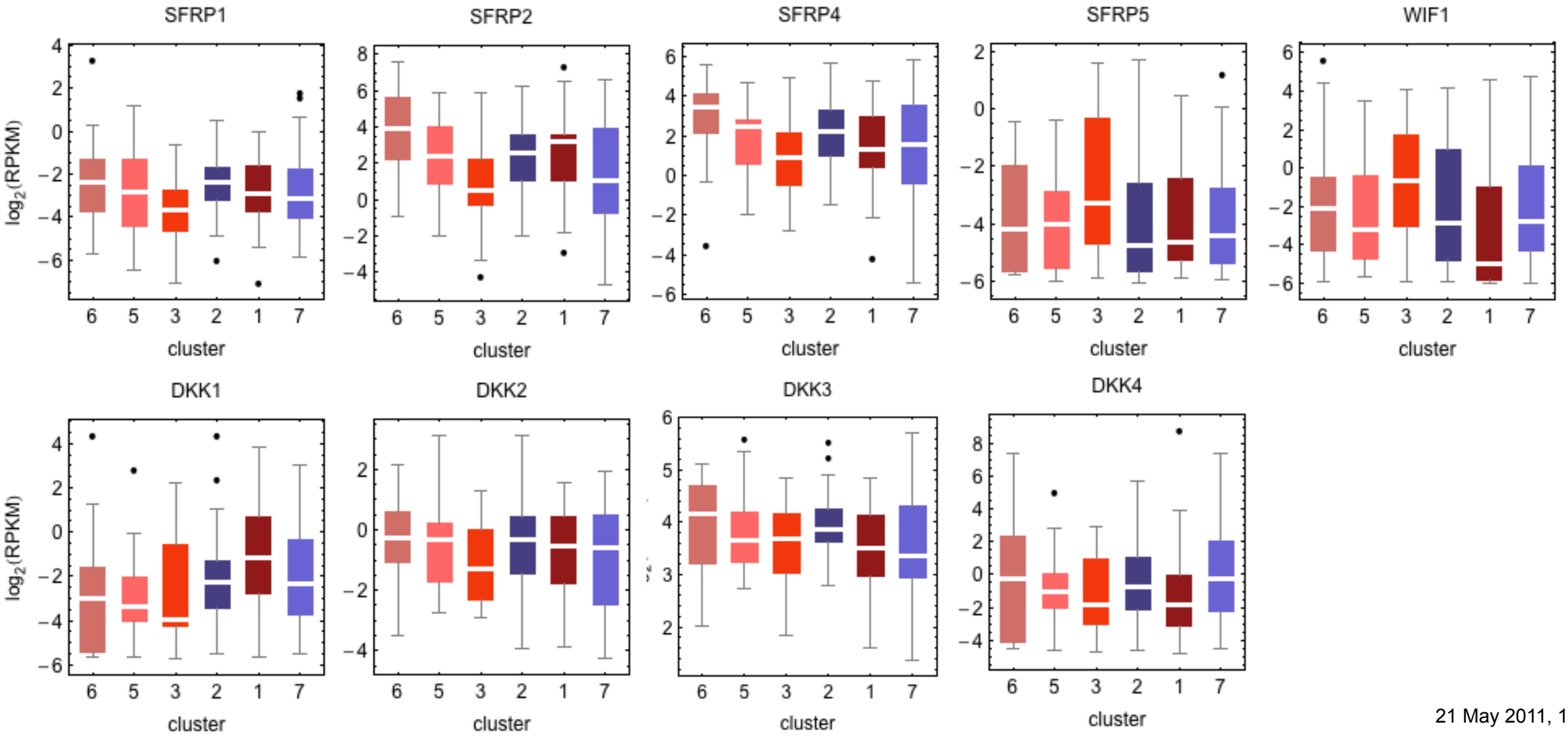

21 May 2011, 12h30

Frizzled 1 to 10: RPKM abundance across 7 clusters

grobertson:mRNA-seq grobertson$ grep "FZD" crc_244_gene_rpkm.txt | cut -f1
FZD1|8321
FZD2|2535
FZD3|7976
FZD4|8322
FZD5|7855
FZD6|8323
FZD7|8324
FZD8|8325
FZD9|8326
FZD10|11211

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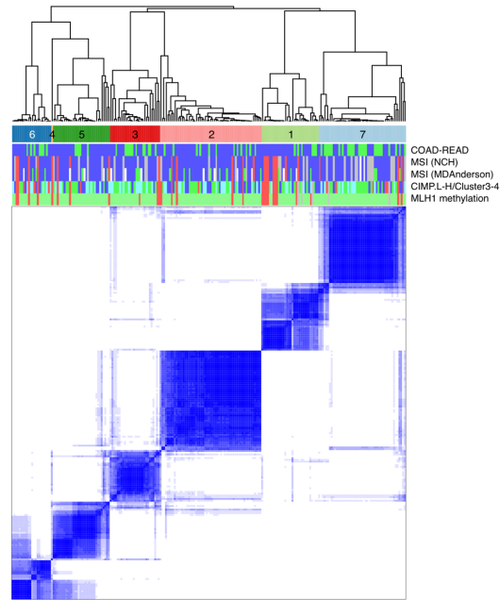
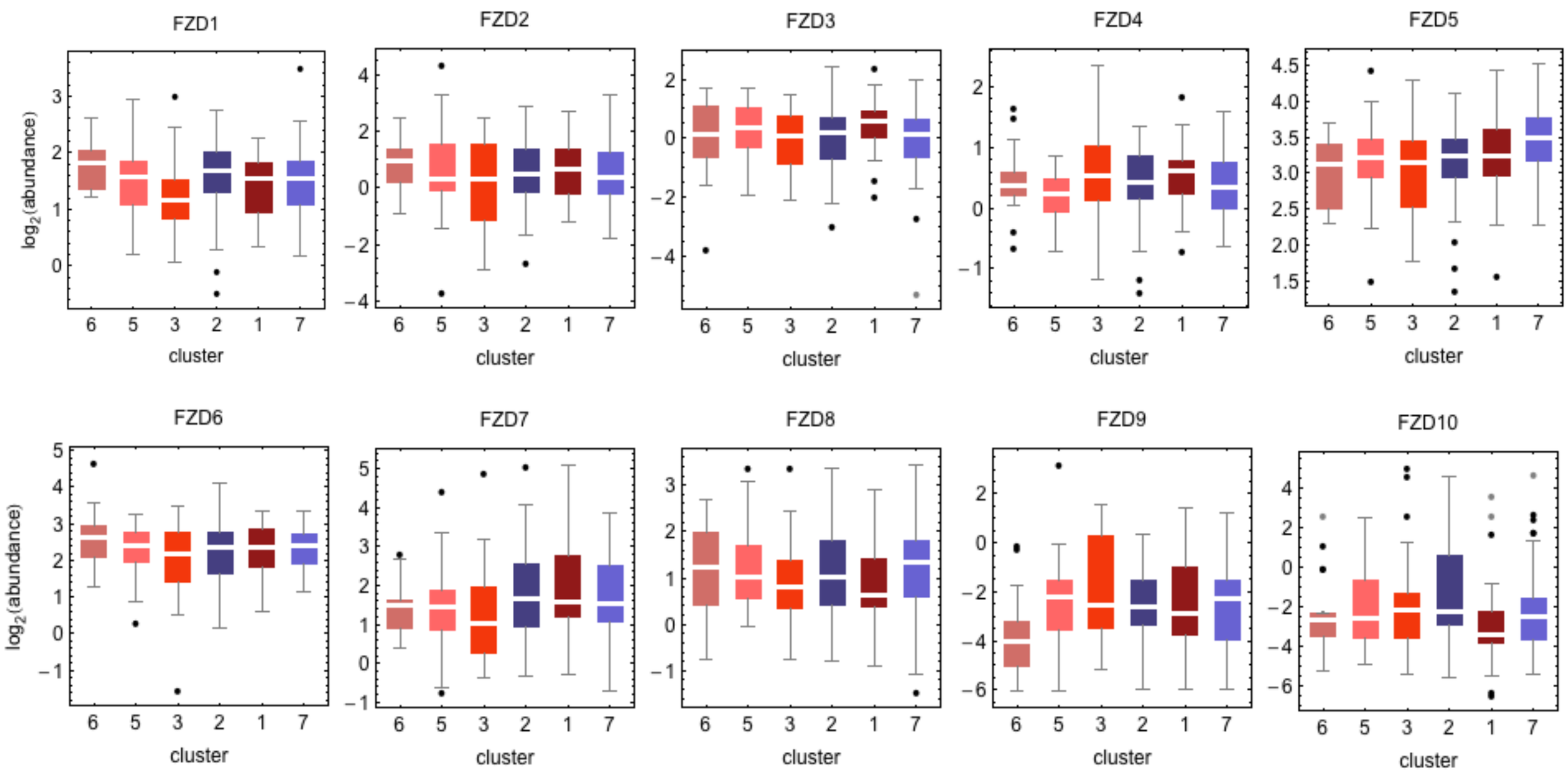




21 May 2011, 13h00

RPKM abundance across 7 clusters

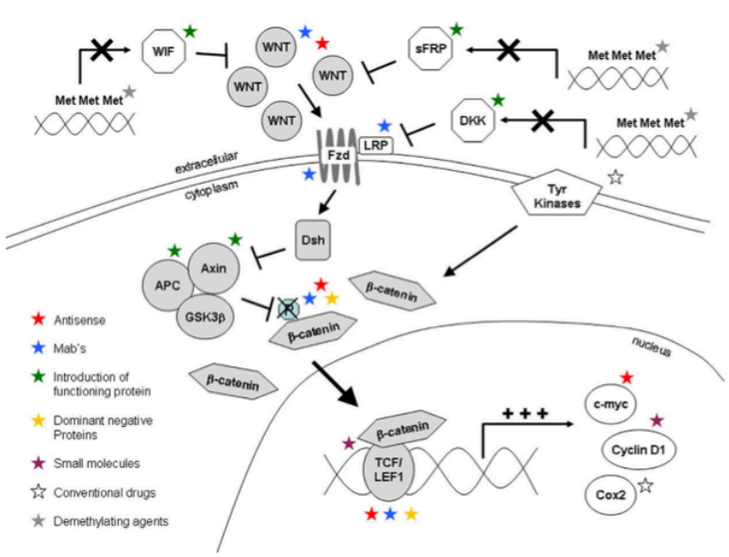
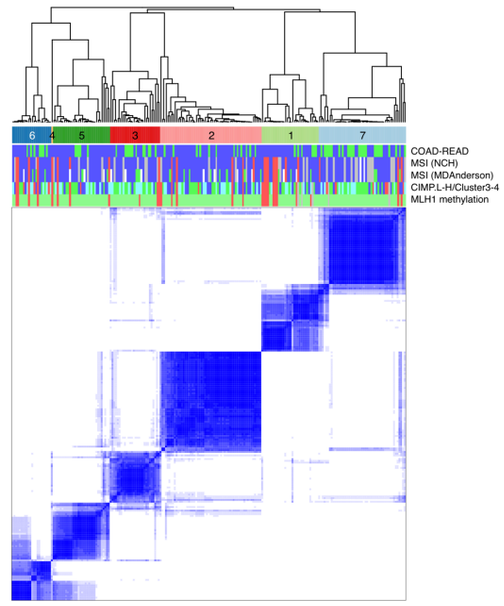
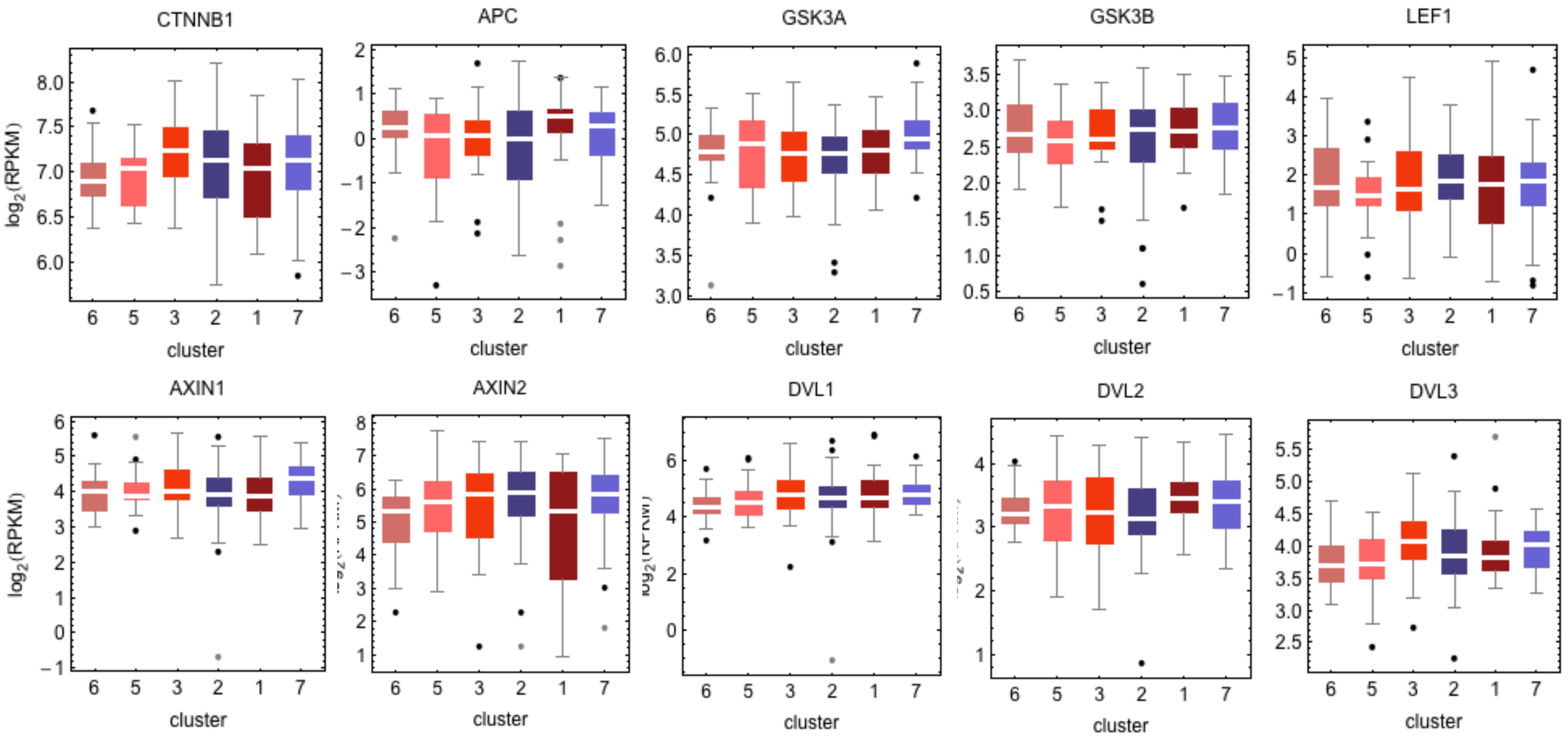


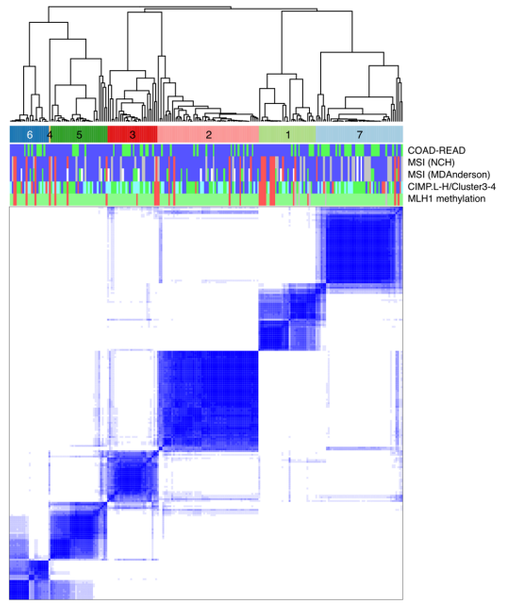
Fig. 1 – Active WNT/b-catenin signalling cascade: WNT proteins bind the receptor complex and activate DSH, which inhibits the APC/Axin/GSK3b complex, preventing phosphorylation of b-catenin, which accumulates in the cytoplasm, translocates into the nucleus and activates TCF/LEF1 family transcription factors. Stars indicate possible intervention targets and approaches following shown colour scheme. **APC**: adenomatous polyposis coli; Cox2: cyclooxygenase 2; DKK: Dickkopf; **DSH**: Dishevelled; FZD: Frizzled; GSK3: glycogen synthase kinase 3; LEF1: Lymphoid enhancer-binding factor 1; LRP: low-density lipoprotein receptor-related protein, Mabs: Monoclonal antibodies, Met: hypermethylation; P: phosphorylation, sFRP: secreted frizzled related protein; TCF: T-cell factor; Tyr kinase: Tyrosine kinase; WIF1: WNT inhibitory factor 1. Gehrke et al, 2009. Eur J Cancer 25:2759-2767.



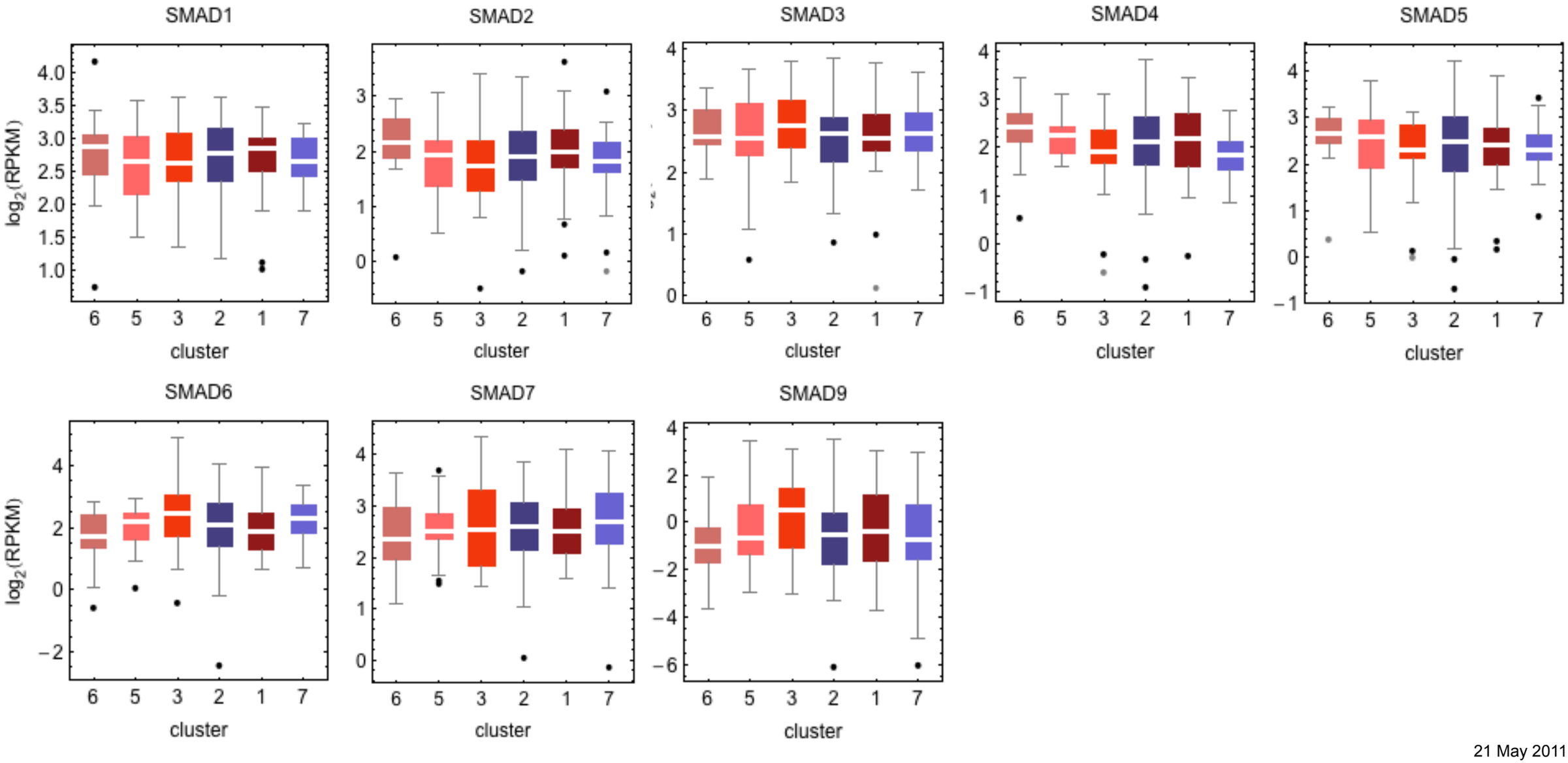
21 May 2011, 12h00



SMADs: RPKM abundance across 7 clusters

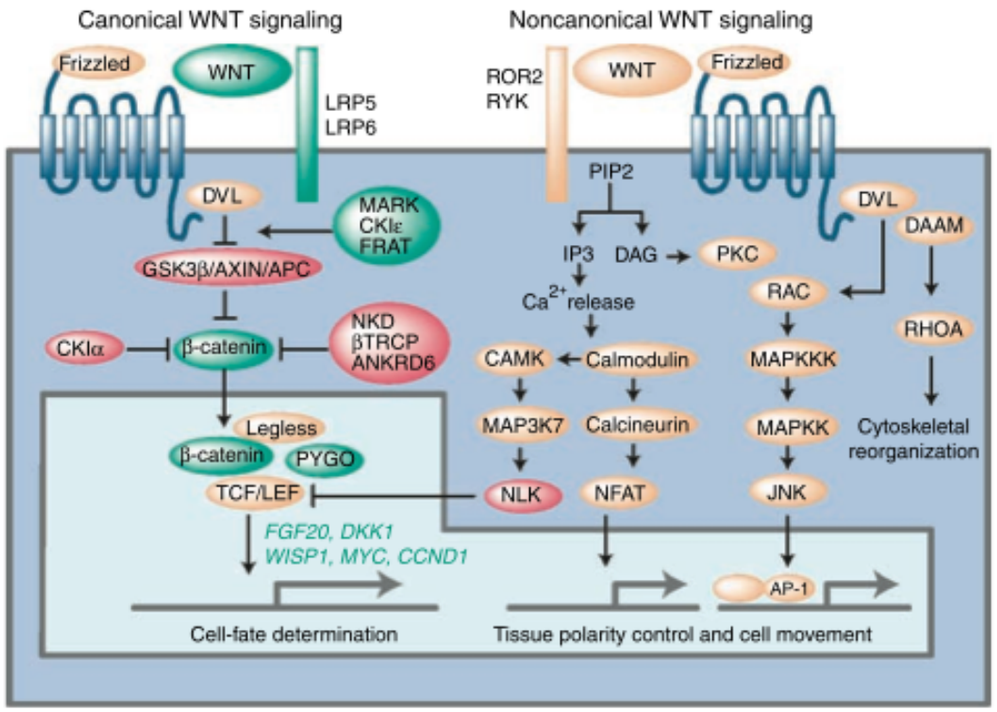


grobertson:mRNA-seq grobertson$ grep "SMAD" crc_244_gene_rpkm.txt | cut -f1
SMAD1|4086
SMAD2|4087
SMAD3|4088
SMAD4|4089
SMAD5|4090
SMAD6|4091
SMAD7|4092
SMAD9|4093



21 May 2011, 12h00

LRP5,6, ROR2, RYK coreceptors: RPKM abundance across 7 clusters



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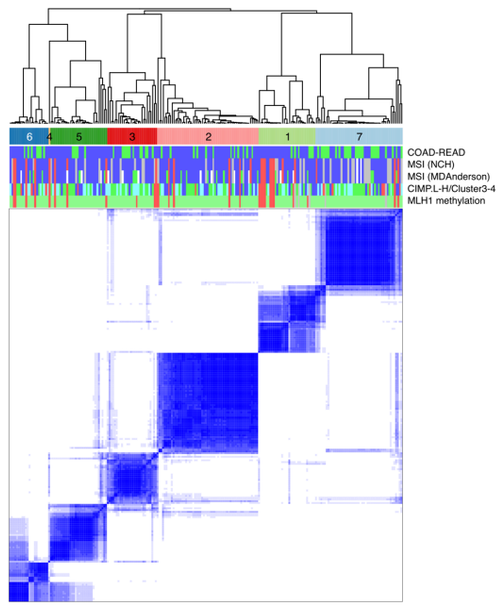
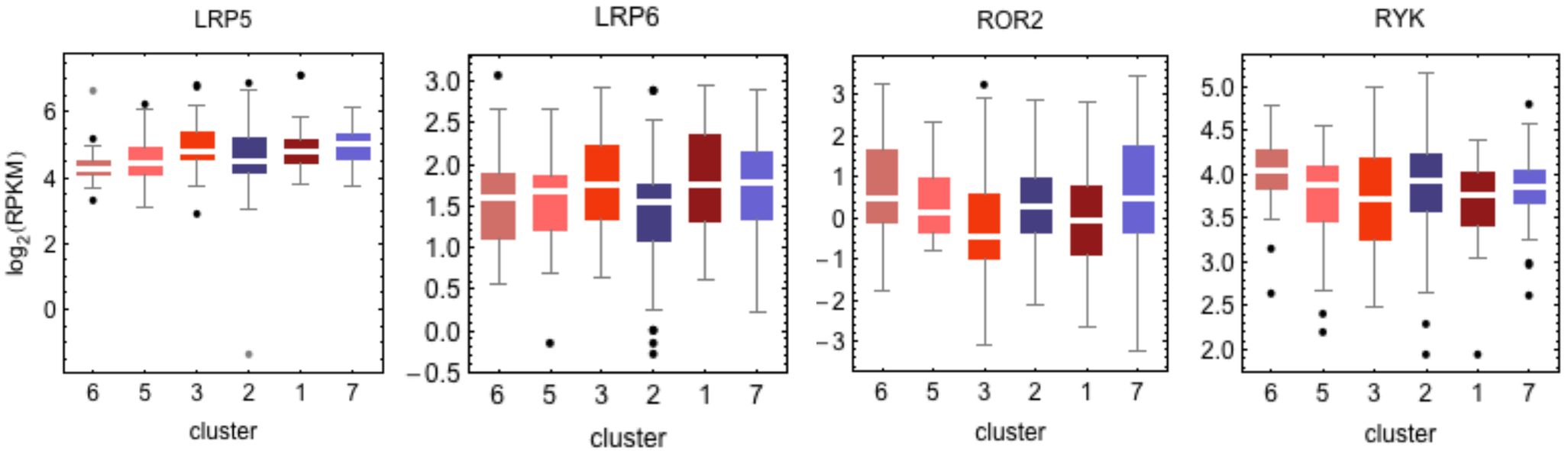
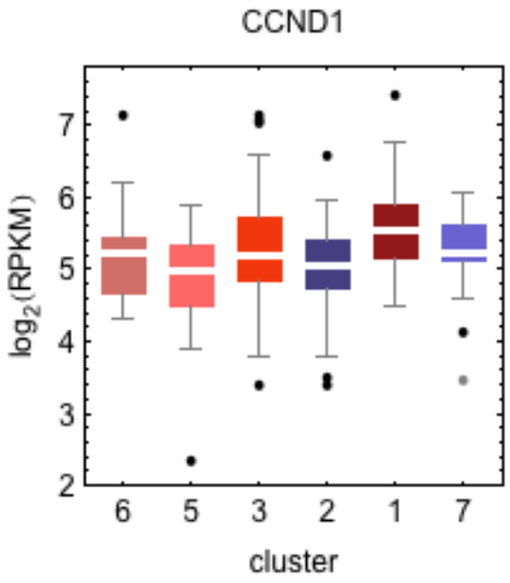
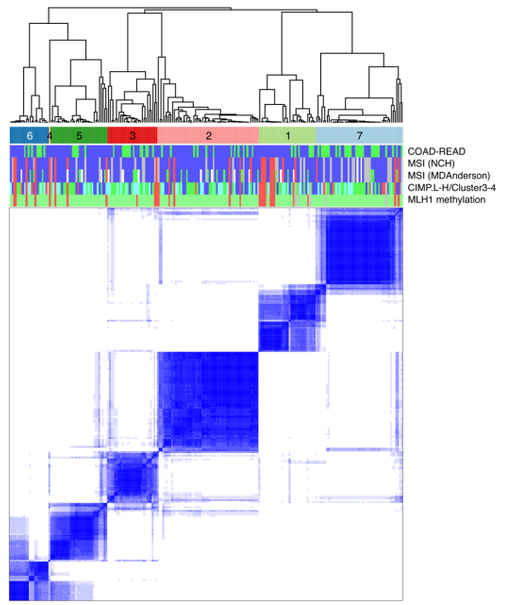


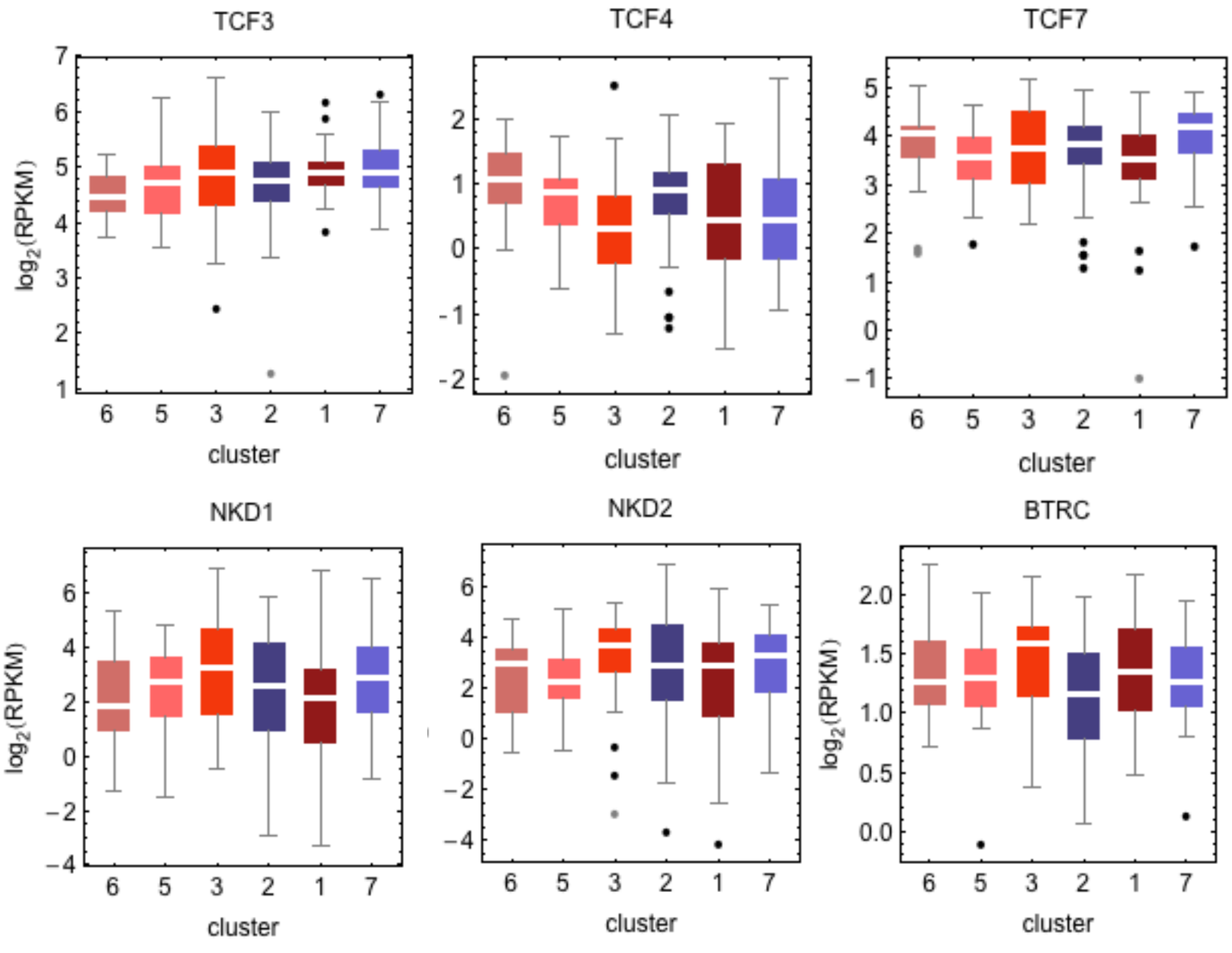


TCFs, NKDs: RPKM abundance across 7 clusters

grobertson:mRNA-seq grobertson$ grep "TCF" crc_244_gene_rpkm.txt |
TCF3|6929
TCF4|6925
TCF7|6932
TCF12|6938
TCF15|6939
TCF19|6941
TCF20|6942
TCF21|6943
TCF23|150921
TCF25|22980
TCF7L1|83439
TCF7L2|6934
TCFL5|10732







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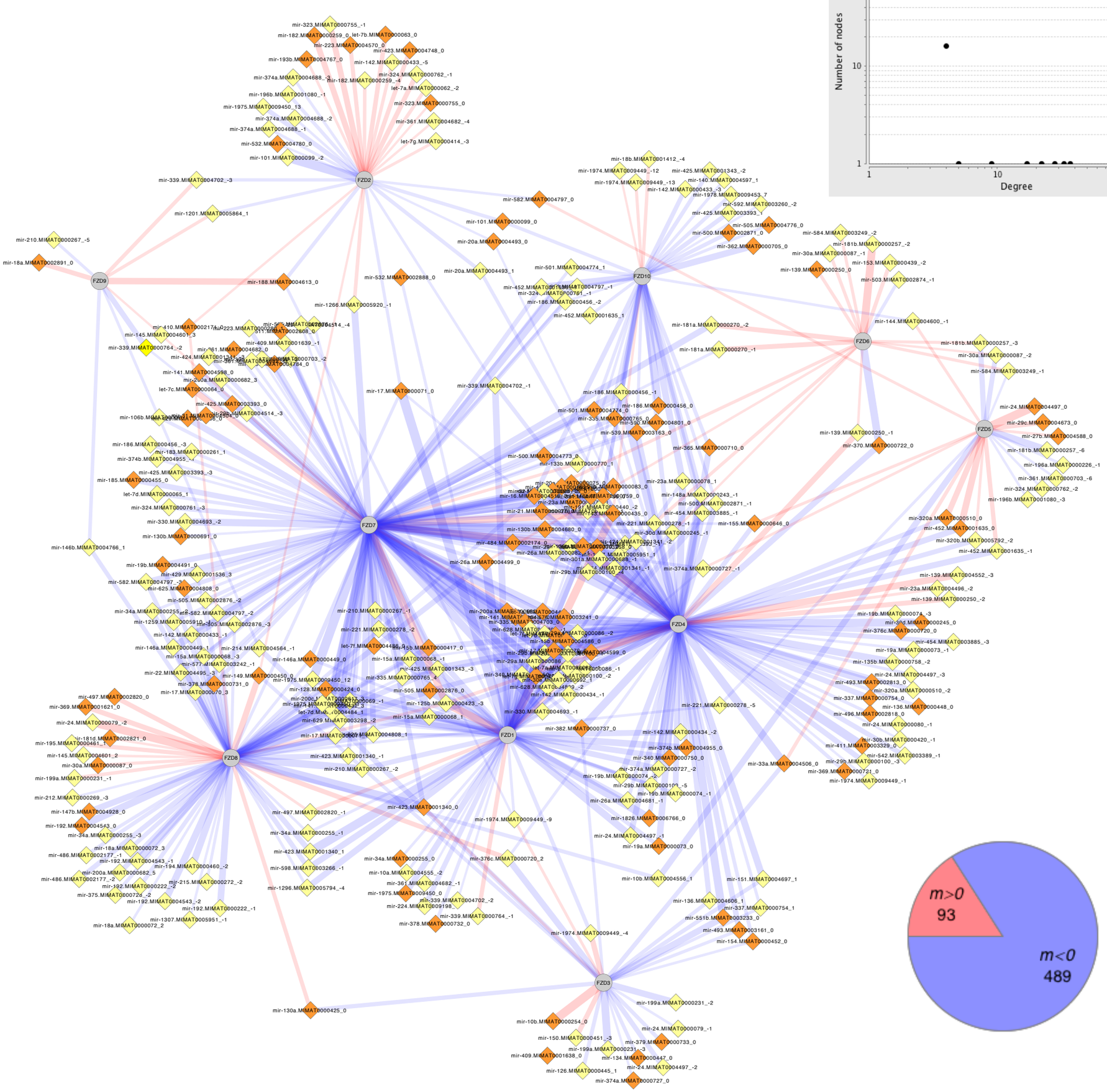
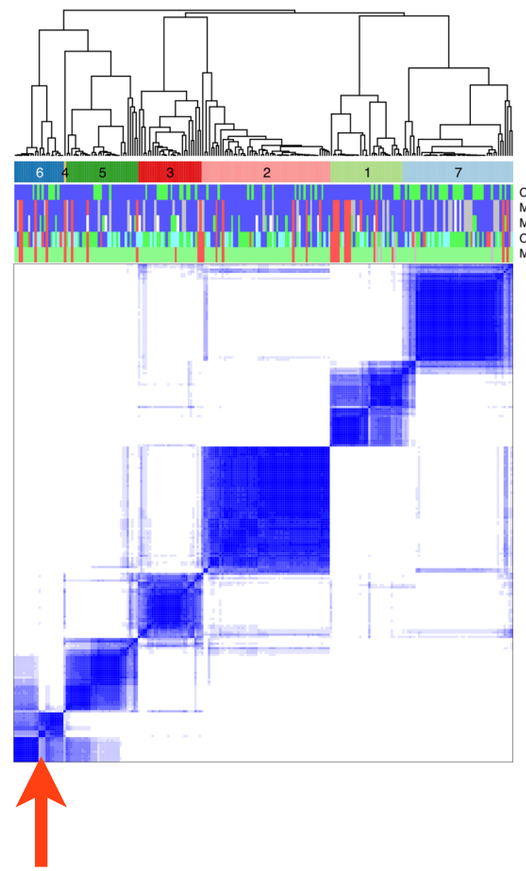
21 May 2011, 12h30

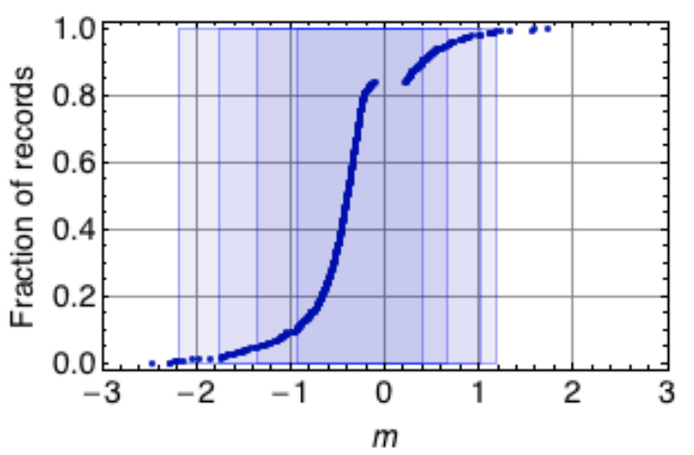
Correlations for Frizzled genes FZD1 to 10

We speculate that a cluster that has few thresholded correlations has diverse (rather than consistent) correlations for almost all miRNA-gene pairs.

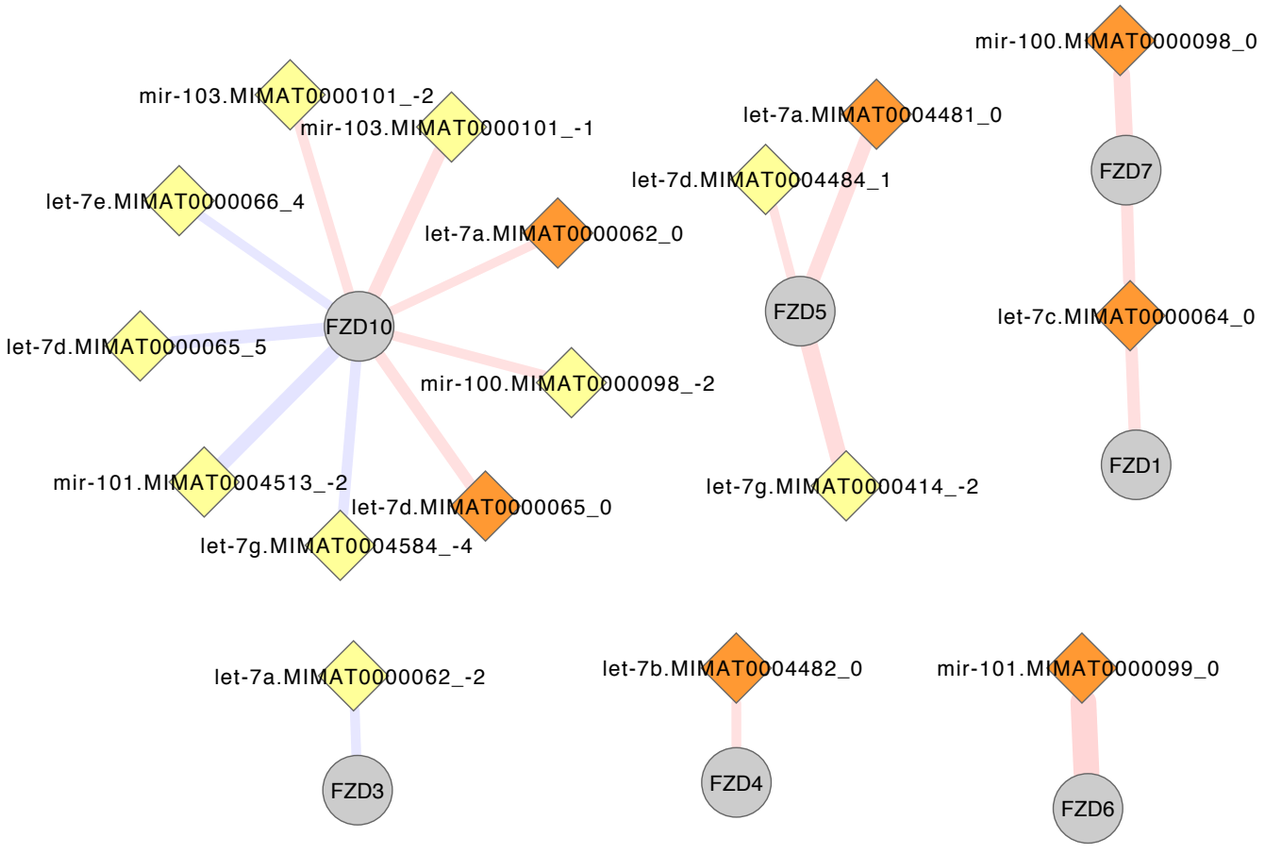
582 FZD records, cluster 6

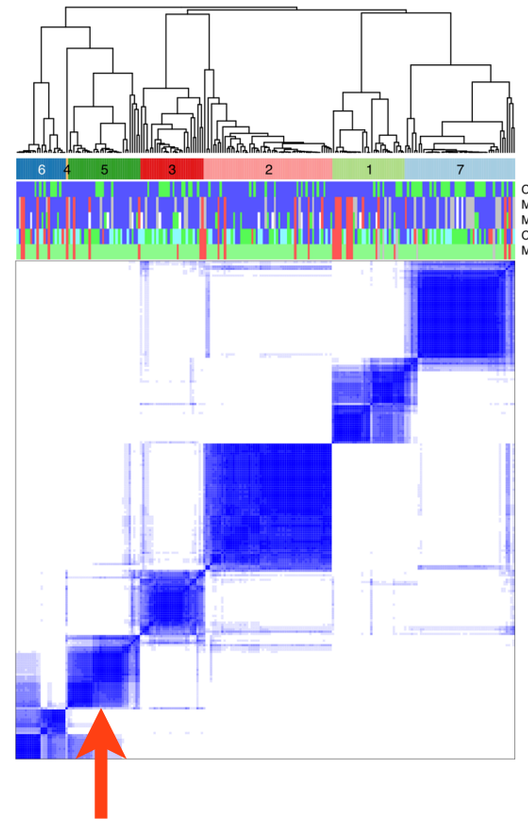


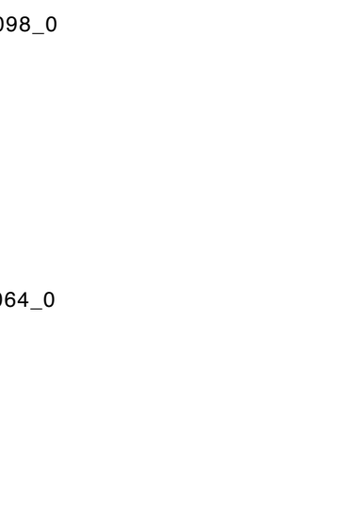




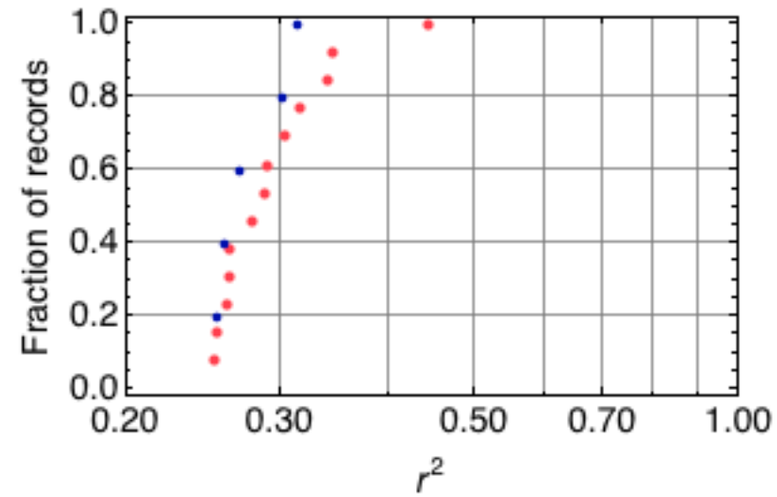
18 FZD records, cluster 5

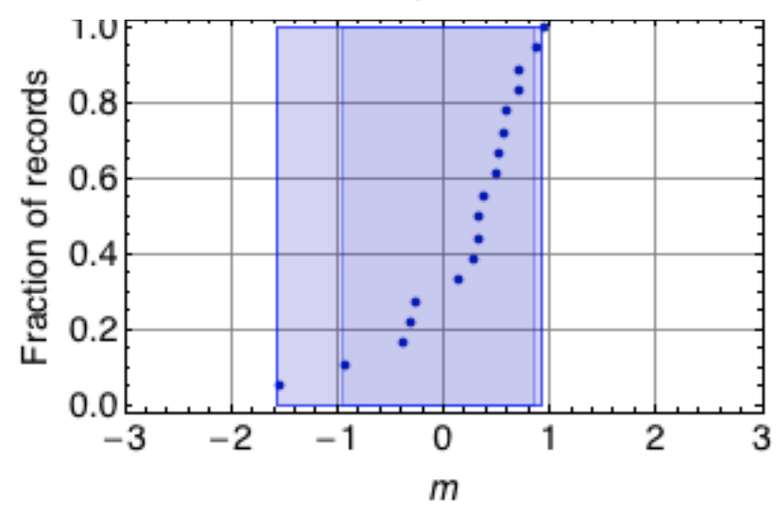




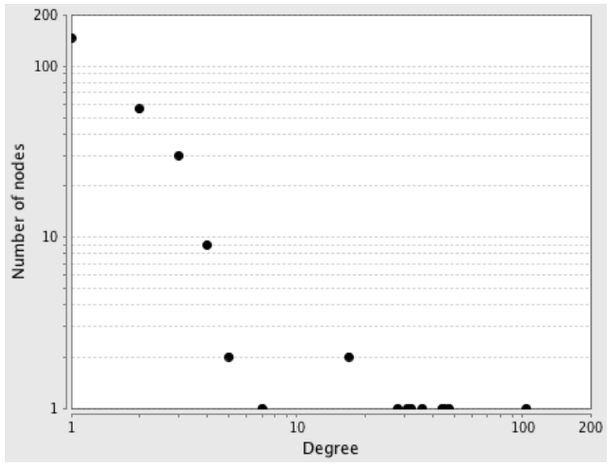


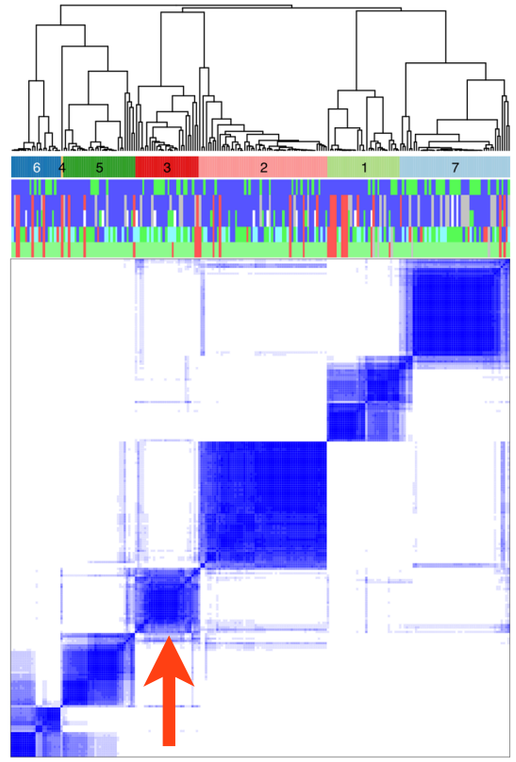
COAD–READ c5, FZD

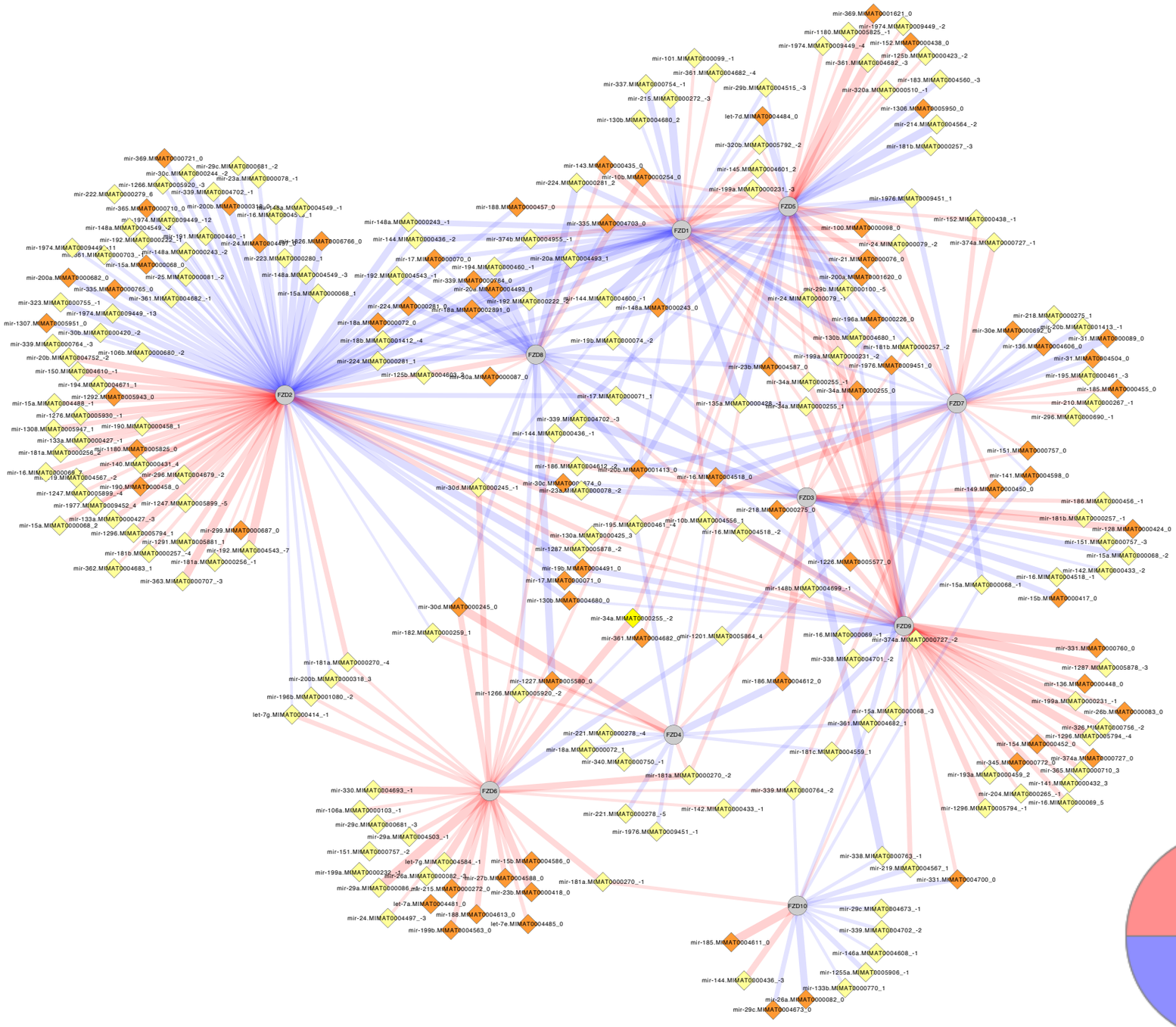


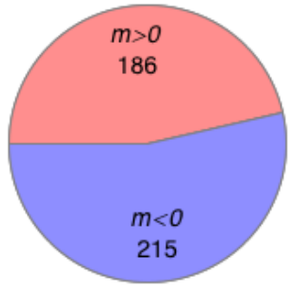


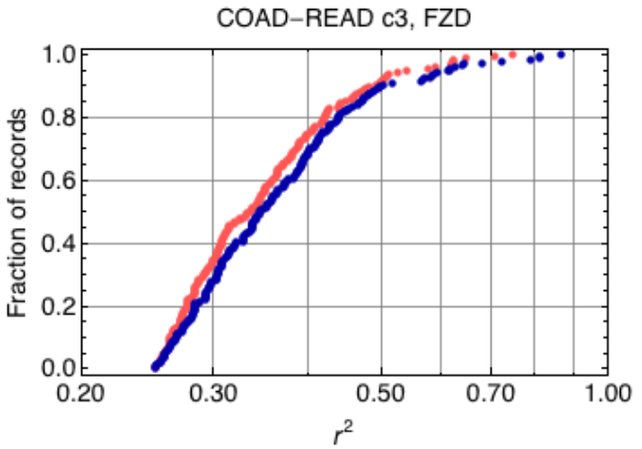
401 FZD records, cluster 3

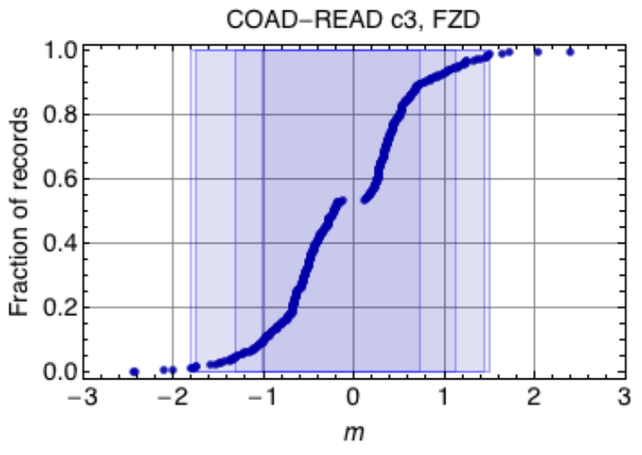




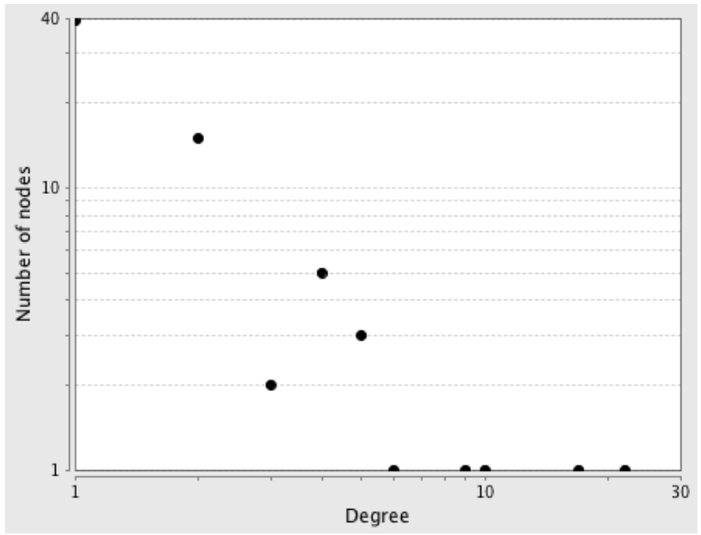


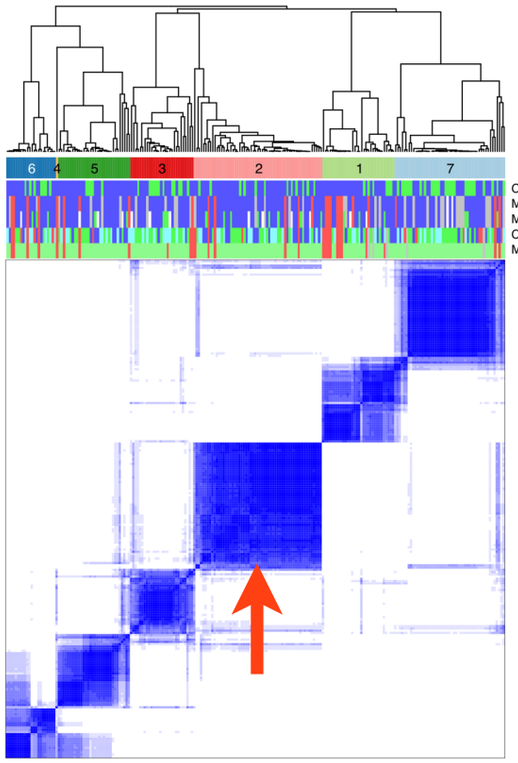


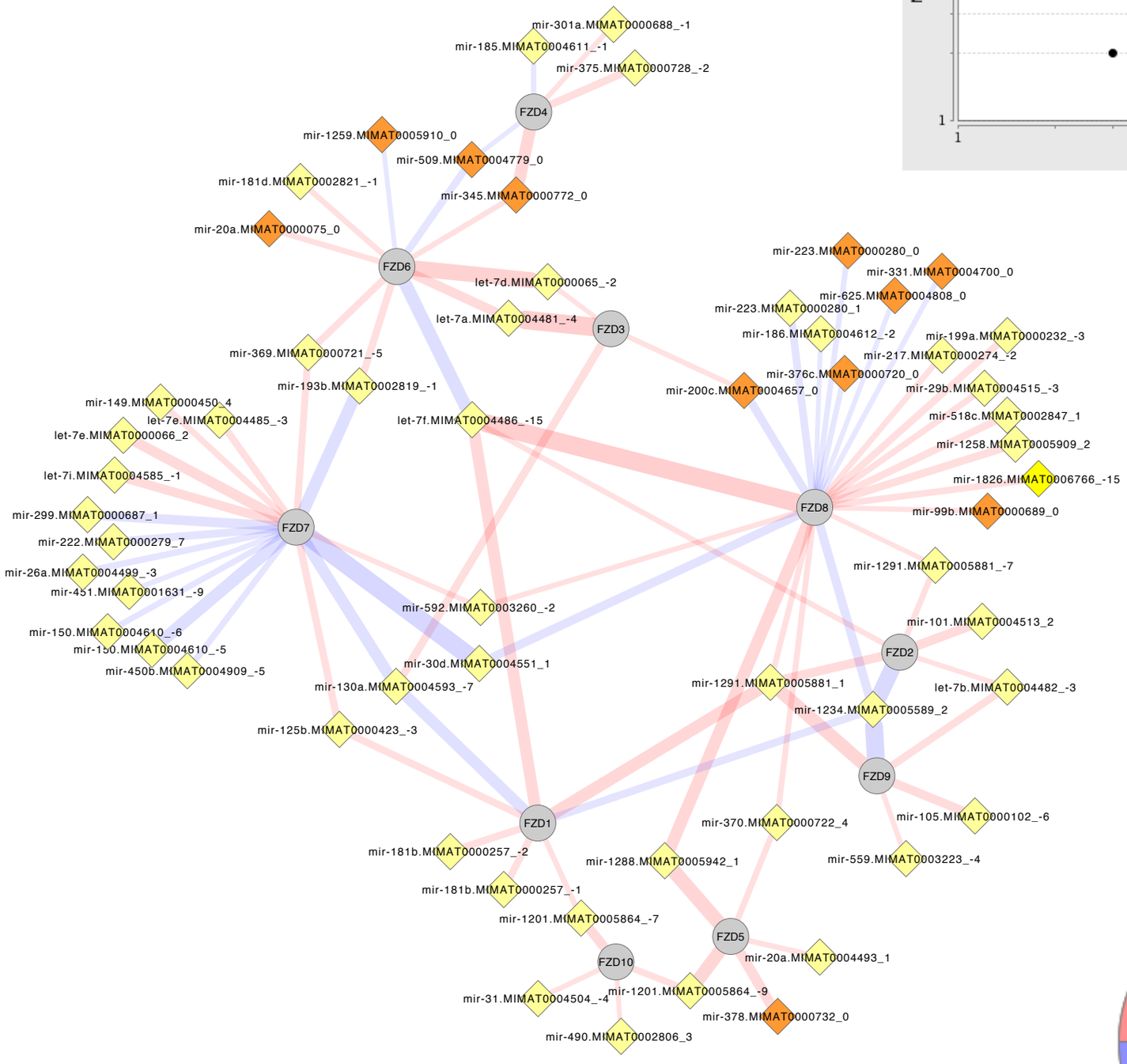


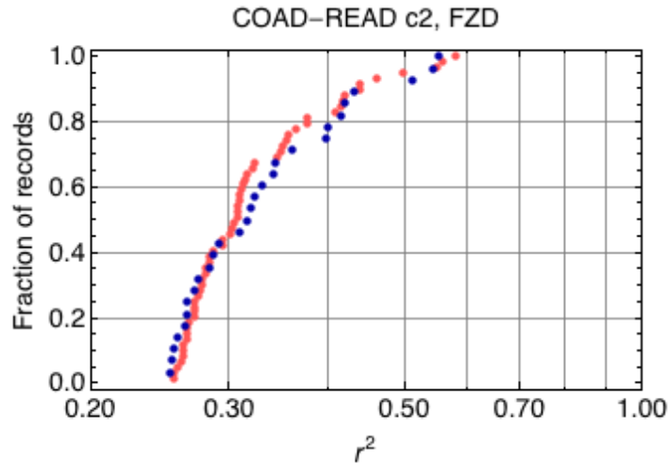


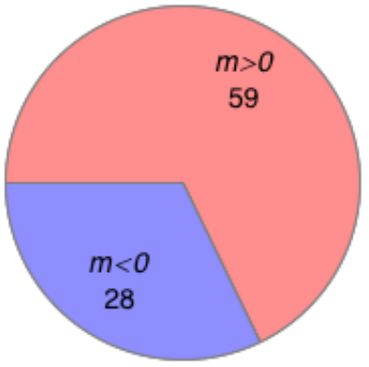
87 FZD records, cluster 2

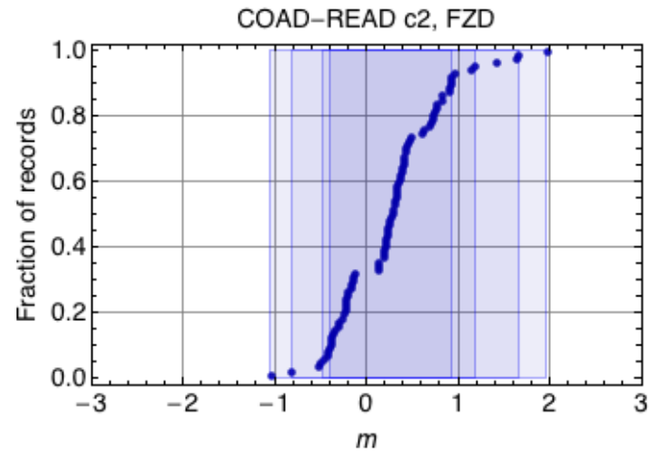




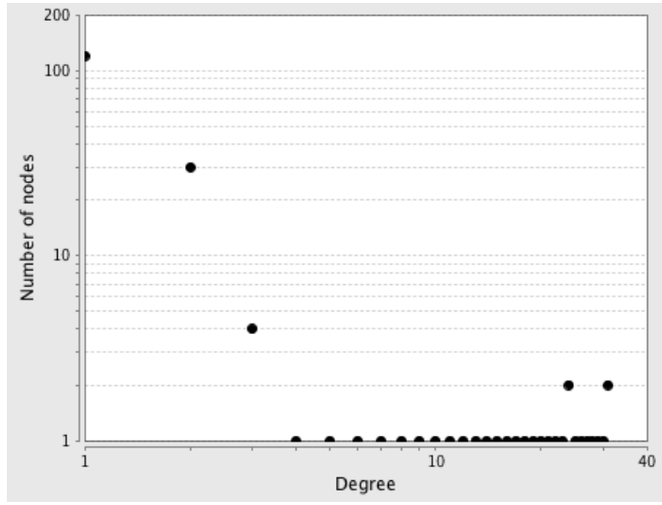


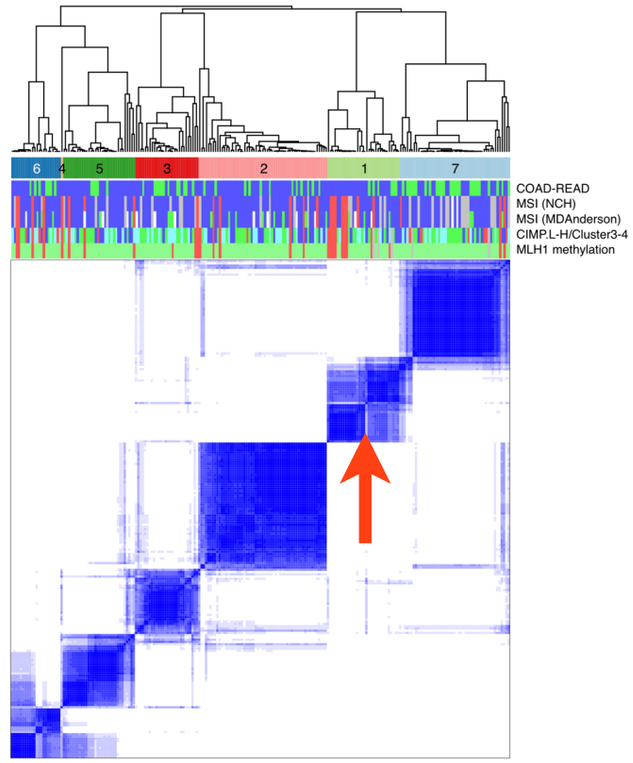


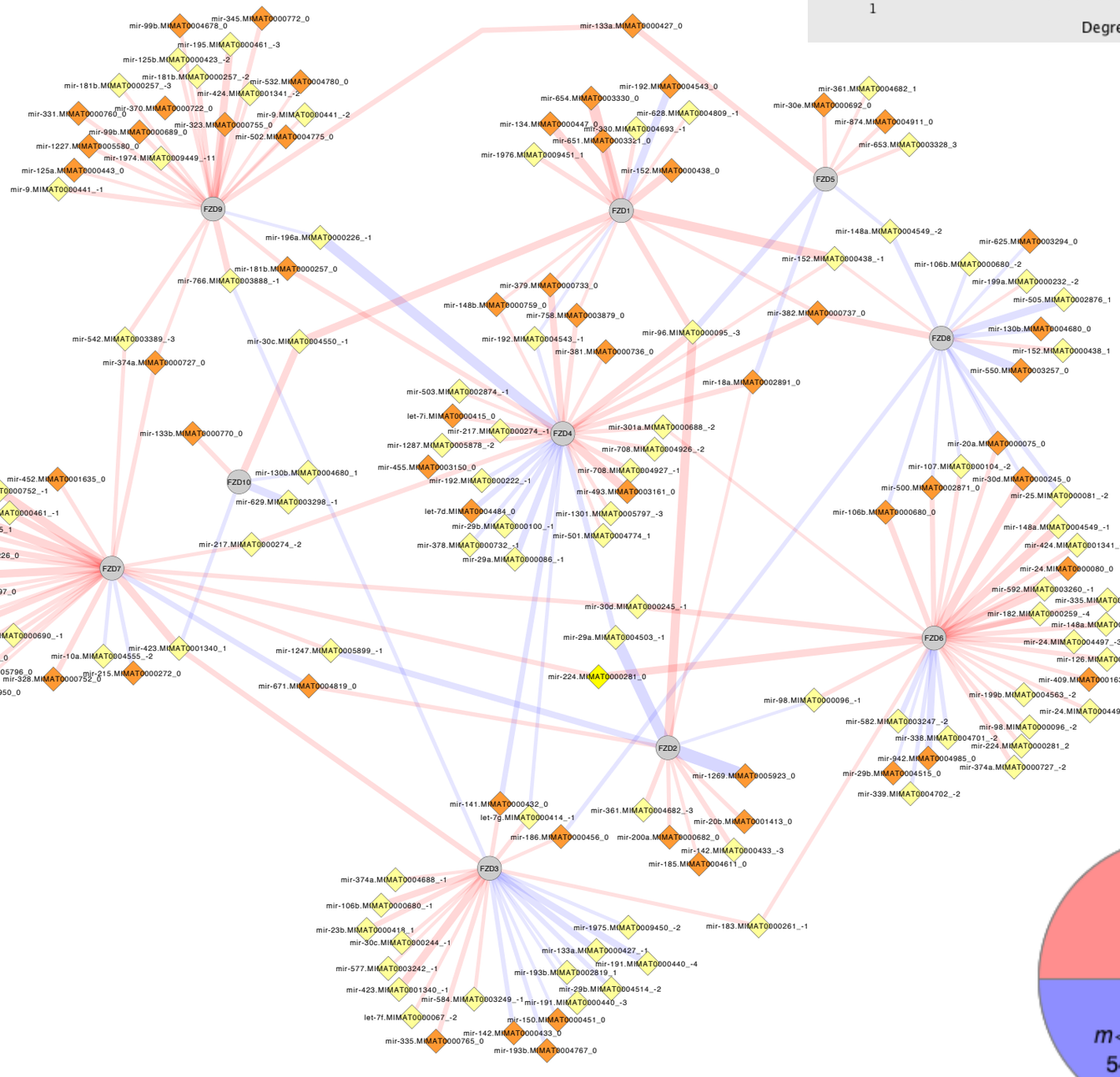


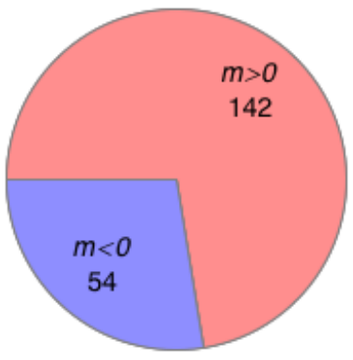


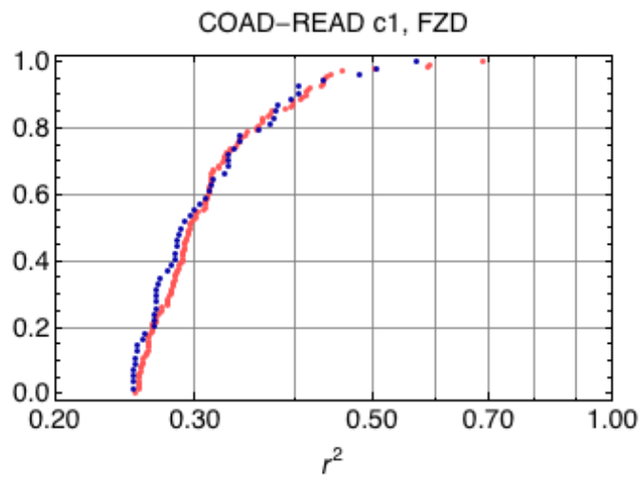
nn FZD records, cluster 1

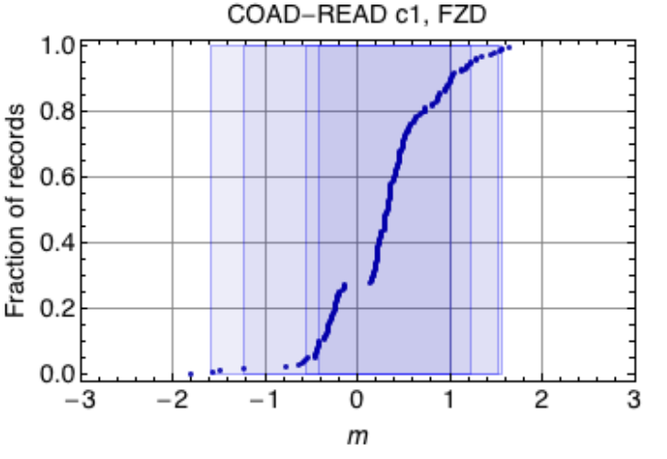












399 FZD records, cluster 7