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

G. Robertson, Andy Chu, Elizabeth Chun, Inanc Birol
23 May 2011, 11h00

Context: we hope to send Raju a figure for miRNAs, 5'isomiRs and the Wnt pathway.

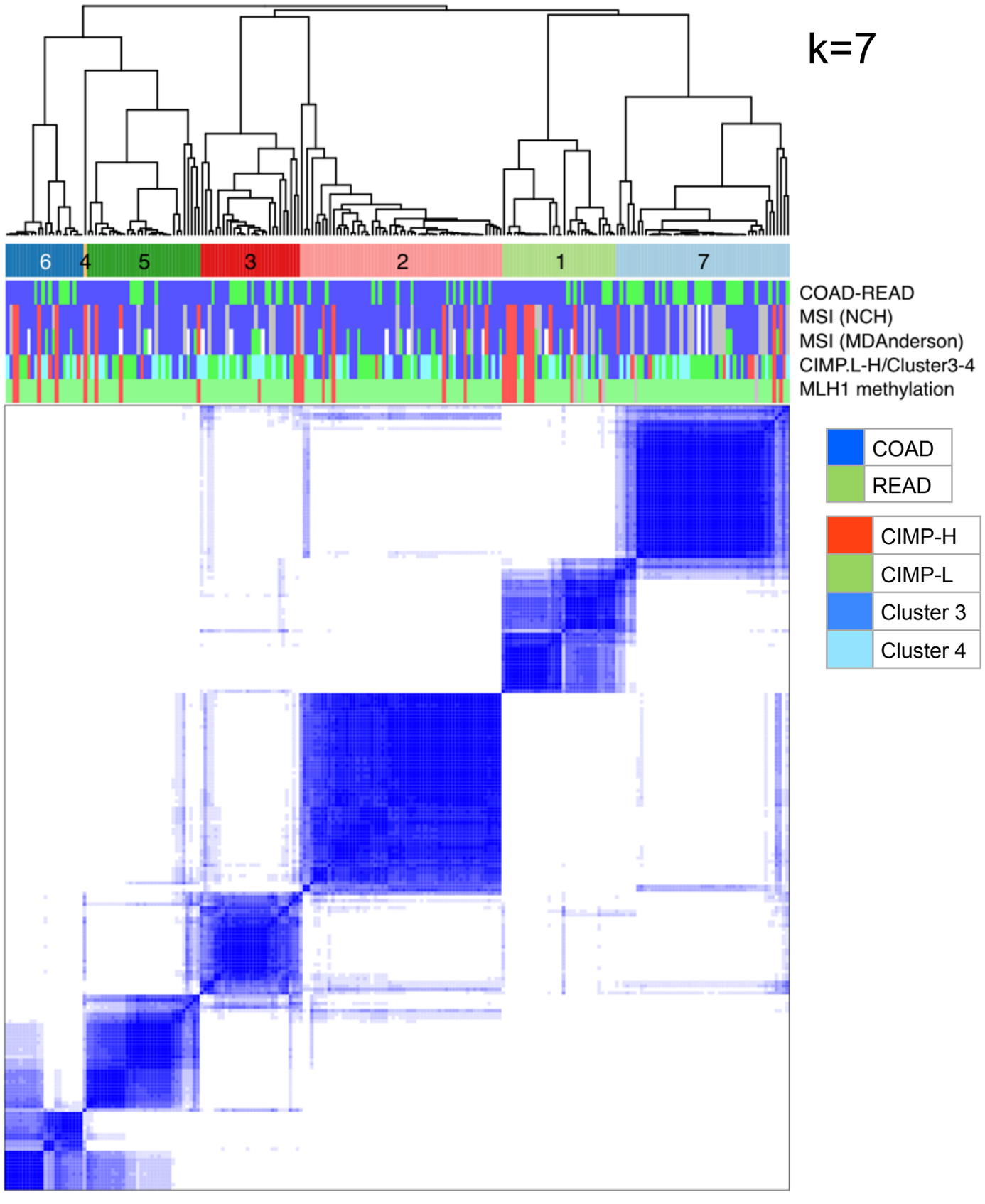
1. A k=7 consensus clustering result that used isomiRs returns reasonably large numbers of thresholded miRNA-gene correlations. We speculate that a cluster that has few thresholded correlations has diverse (rather than consistent) correlations for almost all miRNA-gene pairs.

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.

4. As a second detailed trial, we show all thresholded correlations for SFRP1,2,4,5.

Clustering COAD-READ unfiltered isomiR data for 221 samples

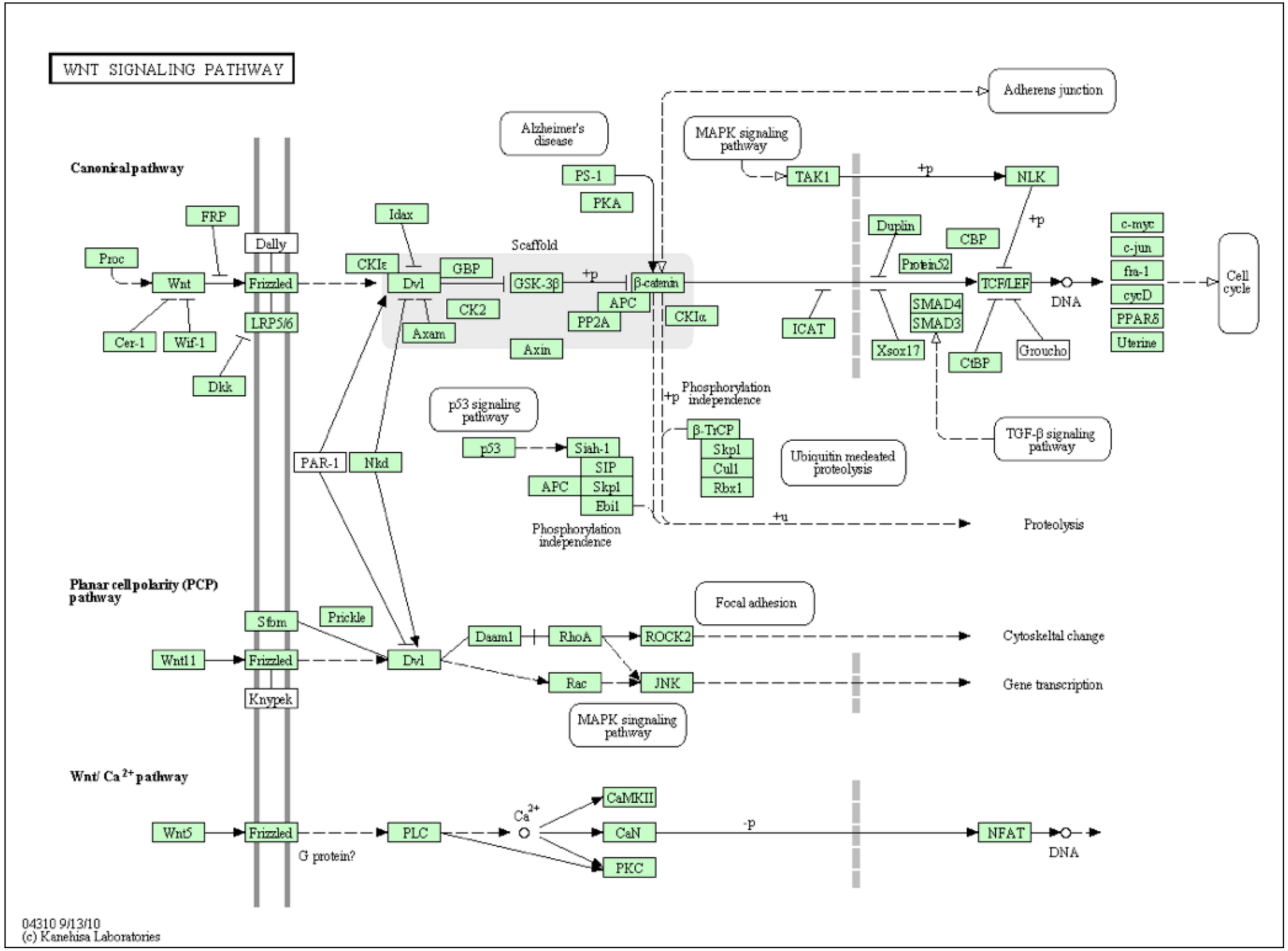


Number of thresholded miRNA-mRNA correlation records.

	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

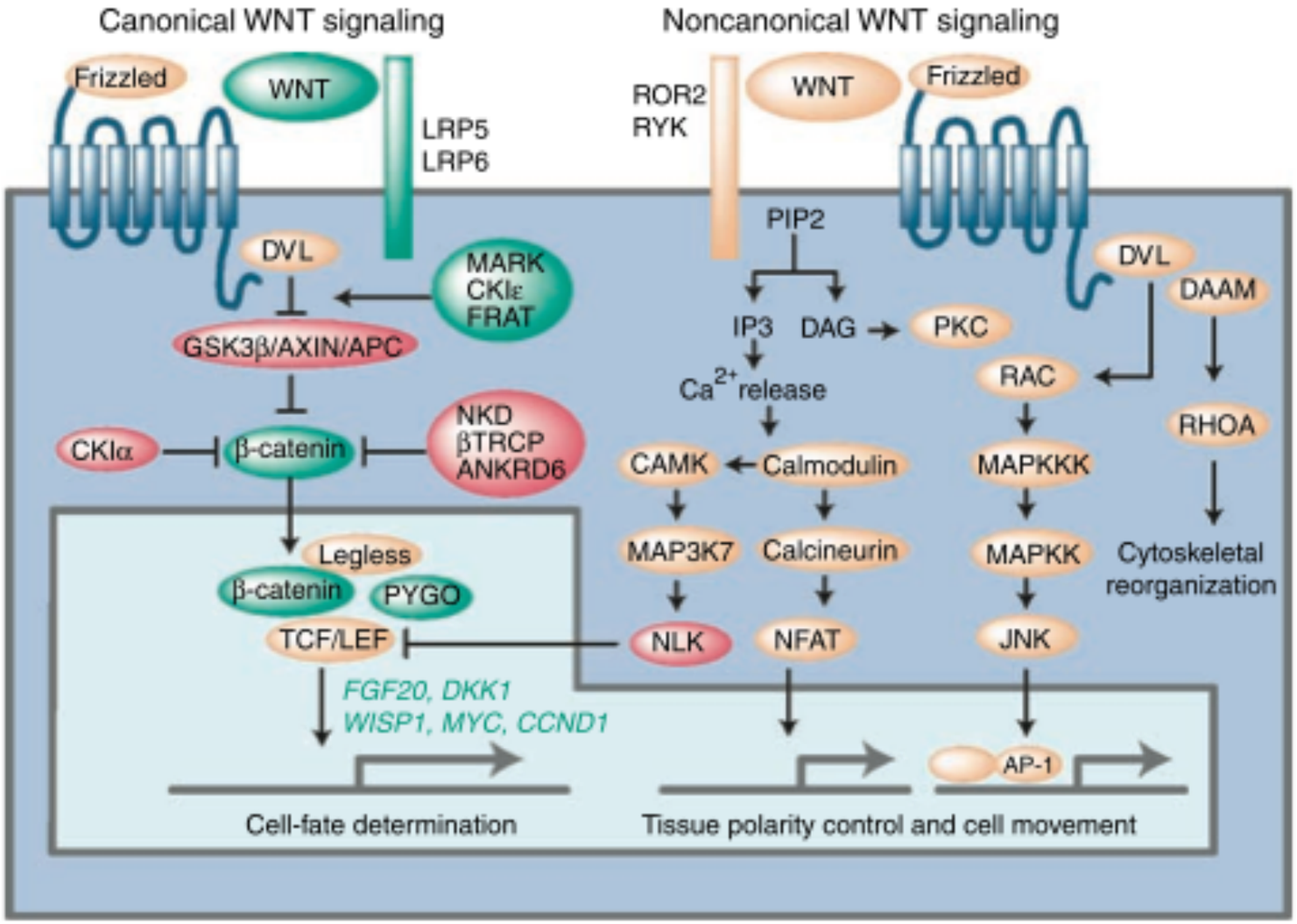


Fig.1. Landscape of WNT signaling cascades. WNT signals are transduced to the **canonical** pathway for cell fate determination, and to the **noncanonical** pathway for control of **cell movement and tissue polarity**. **Canonical** WNT signals are transduced through Frizzled family receptors and LRP5/LRP6 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

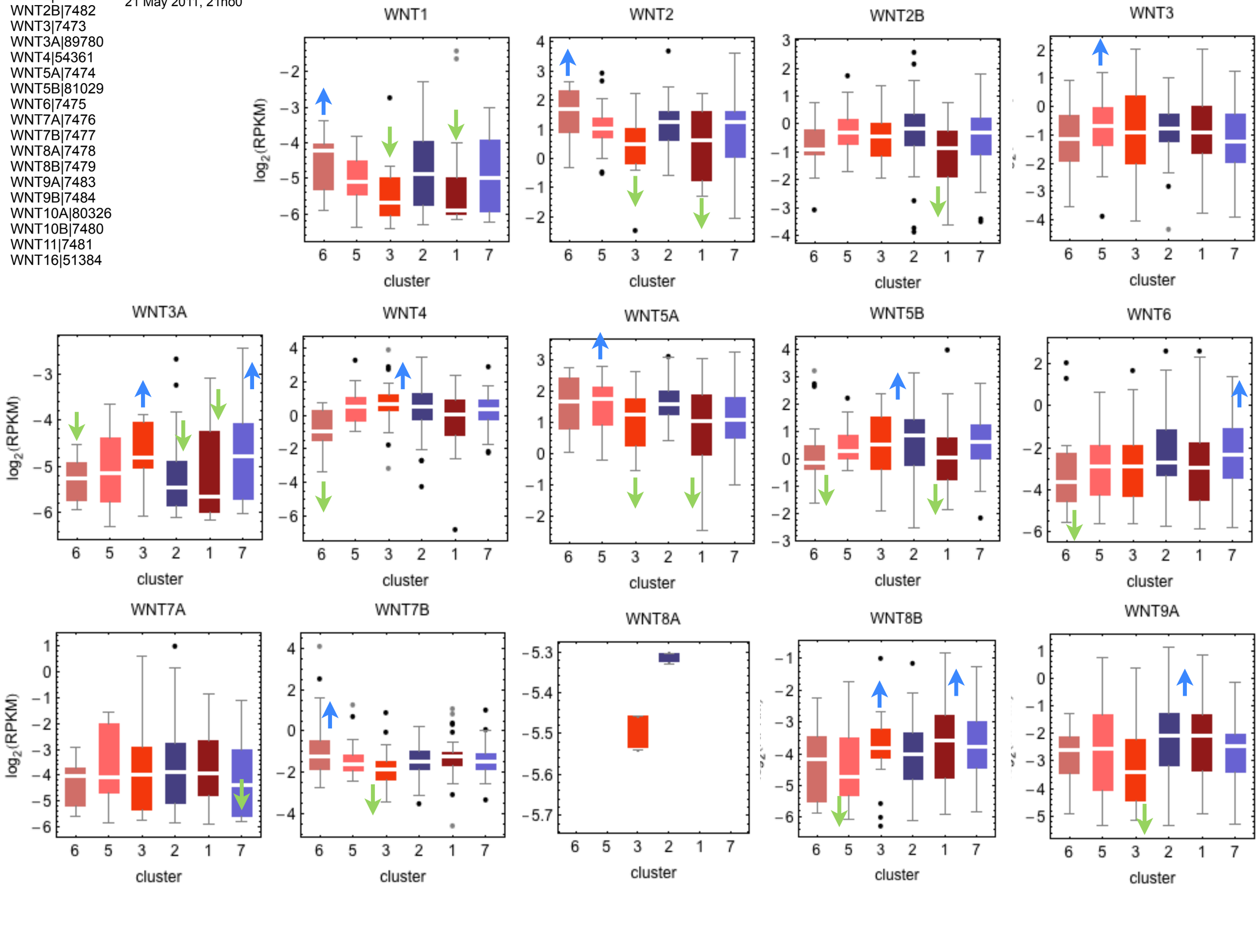
RPKM for Wnt pathway genes, across seven miRNA-seq-based consensus clusters

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

21 May 2011, 21ho0

WNTs: RPKM abundance across 7 clusters



SFRPs, WIF1 and DKKs: RPKM abundance across 7 clusters

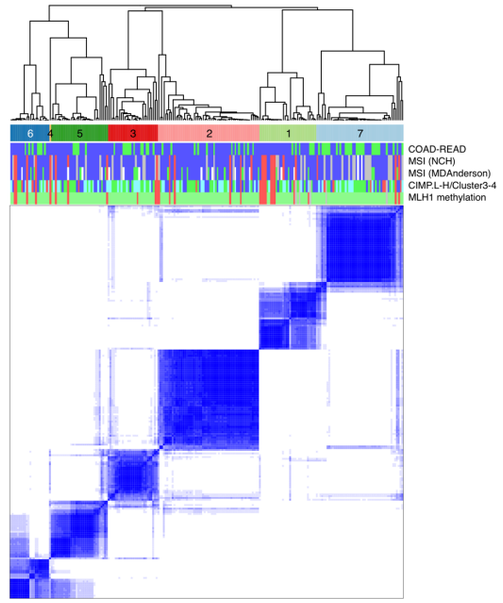
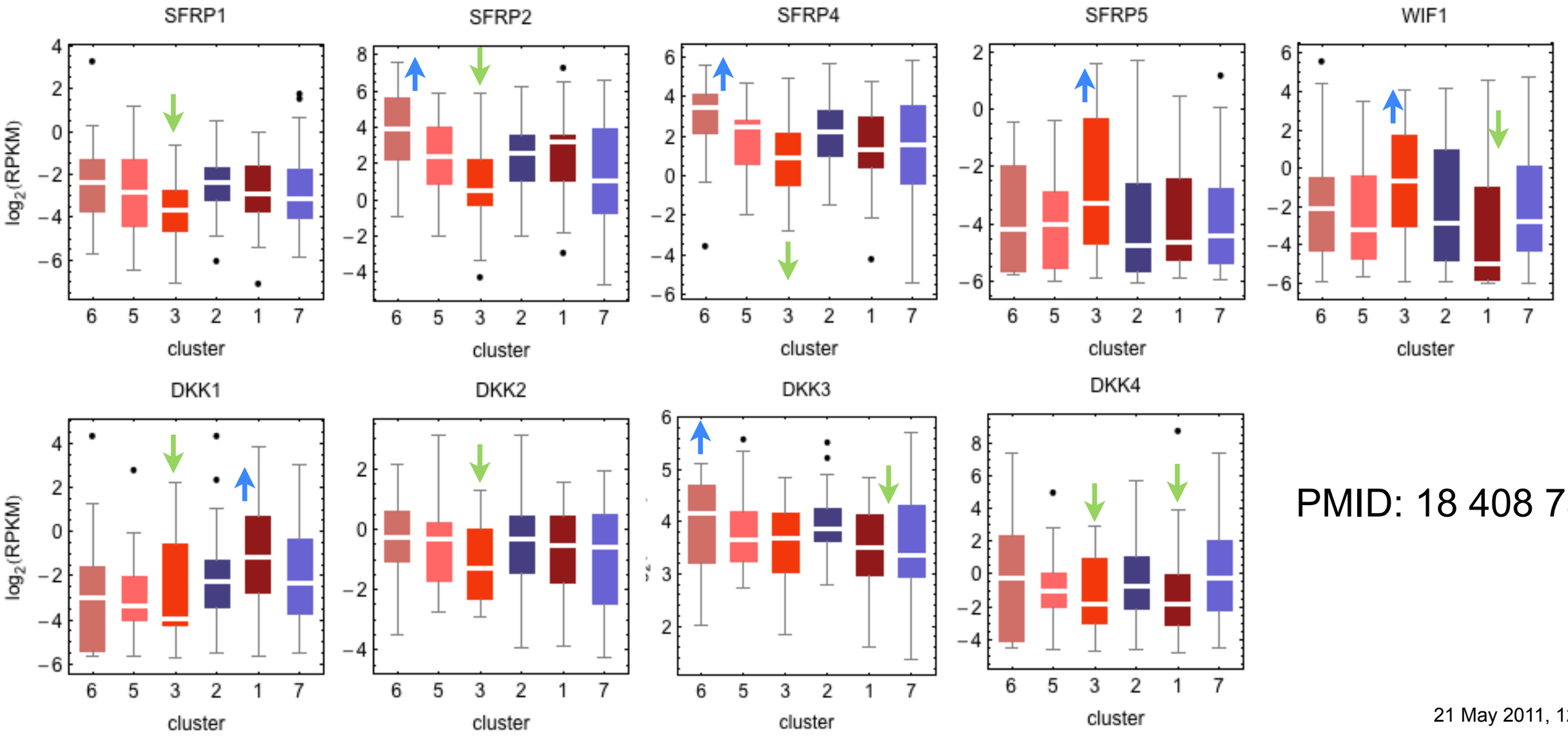


Fig.1. Landscape of WNT signaling cascades. WNT signals are transduced to the **canonical** pathway for cell fate determination, and to the **noncanonical** pathway for control of **cell movement and tissue polarity**. **Canonical** WNT signals are transduced through **Frizzled family receptors** and 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.

SFRP1 SFRP2 SFRP4 SFRP5



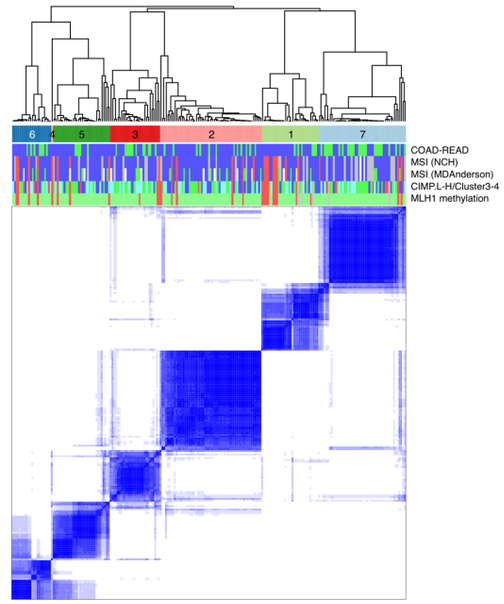
PMID: 18 408 752

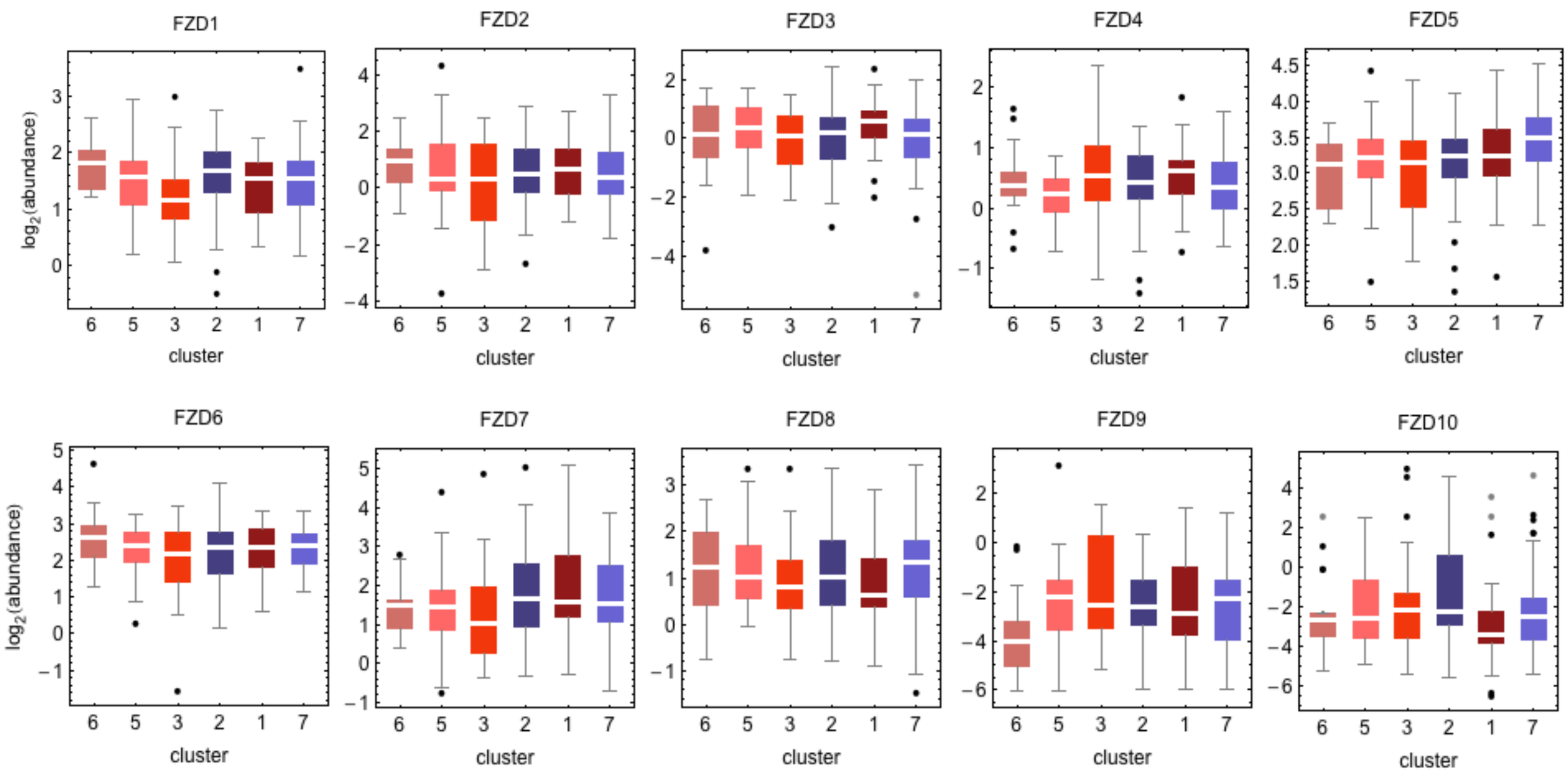
21 May 2011, 12h30

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

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, 13h00

RPKM abundance across 7 clusters

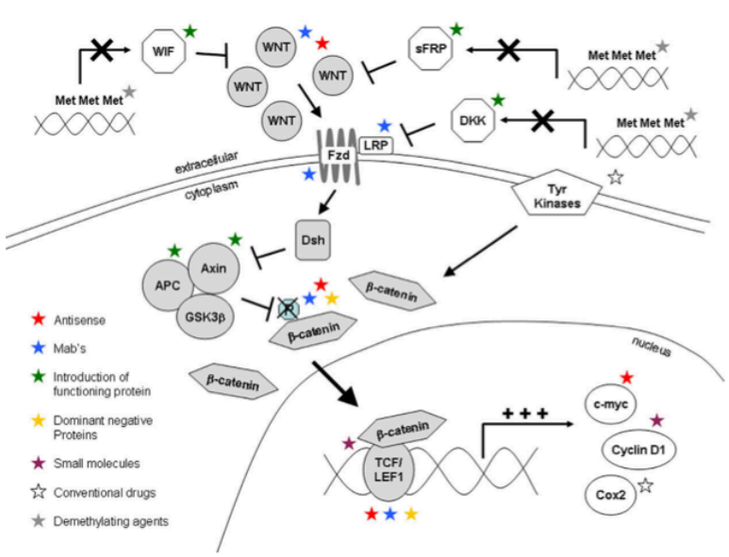
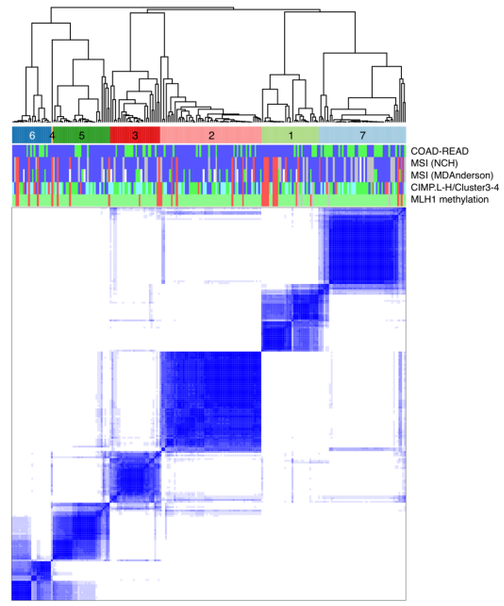
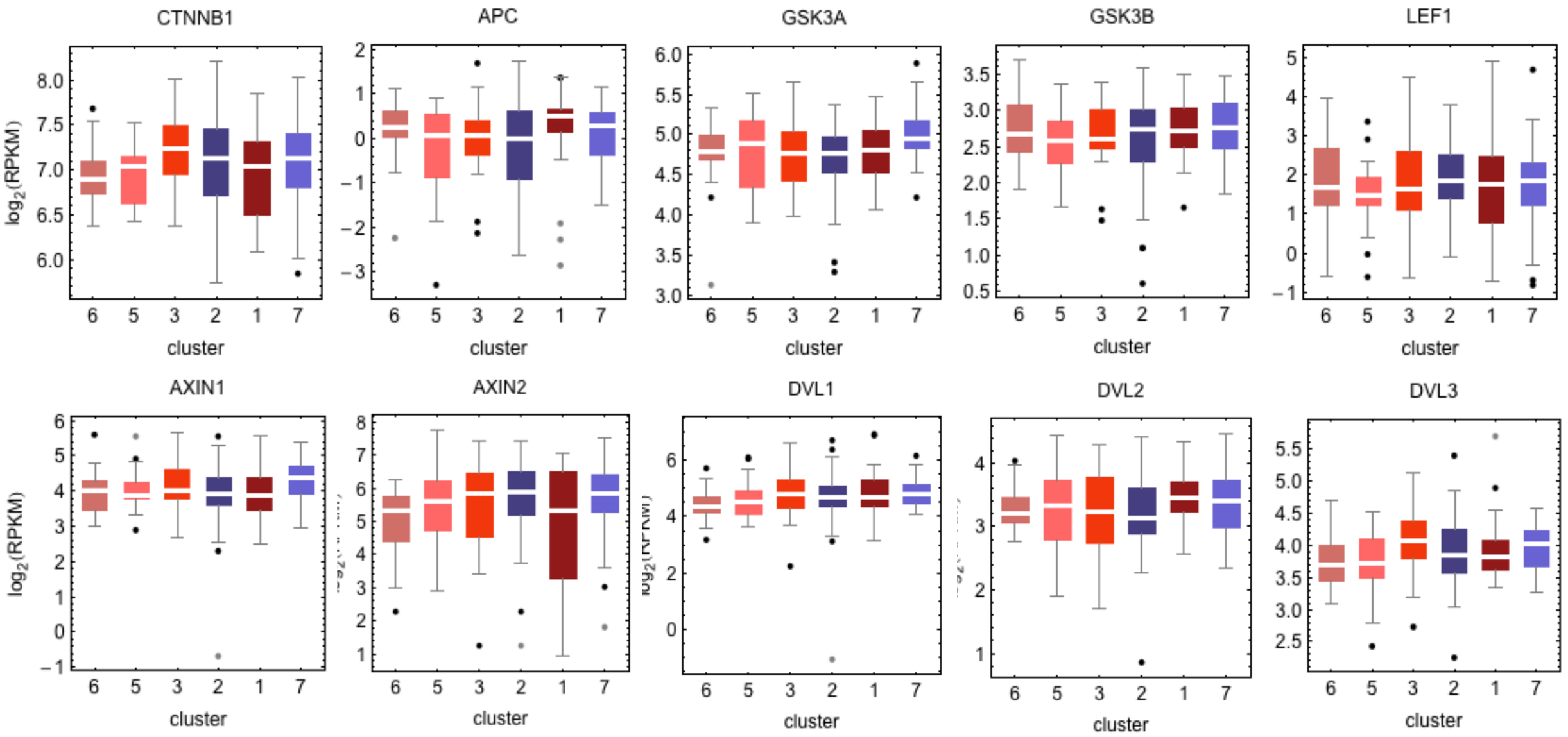


Fig. 1 – Active WNT/b-catenin signalling cascade: WNT proteins bind the receptor complex and activate DSH, which inhibits the **APC/Axin/GSK3b** complex, preventing phosphorylation of b-catenin, which accumulates in the cytoplasm, translocates into the nucleus and activates TCF/**LEF1** family transcription 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.

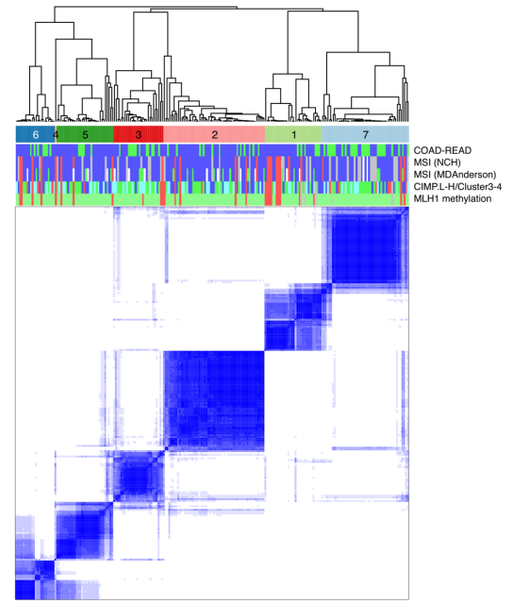


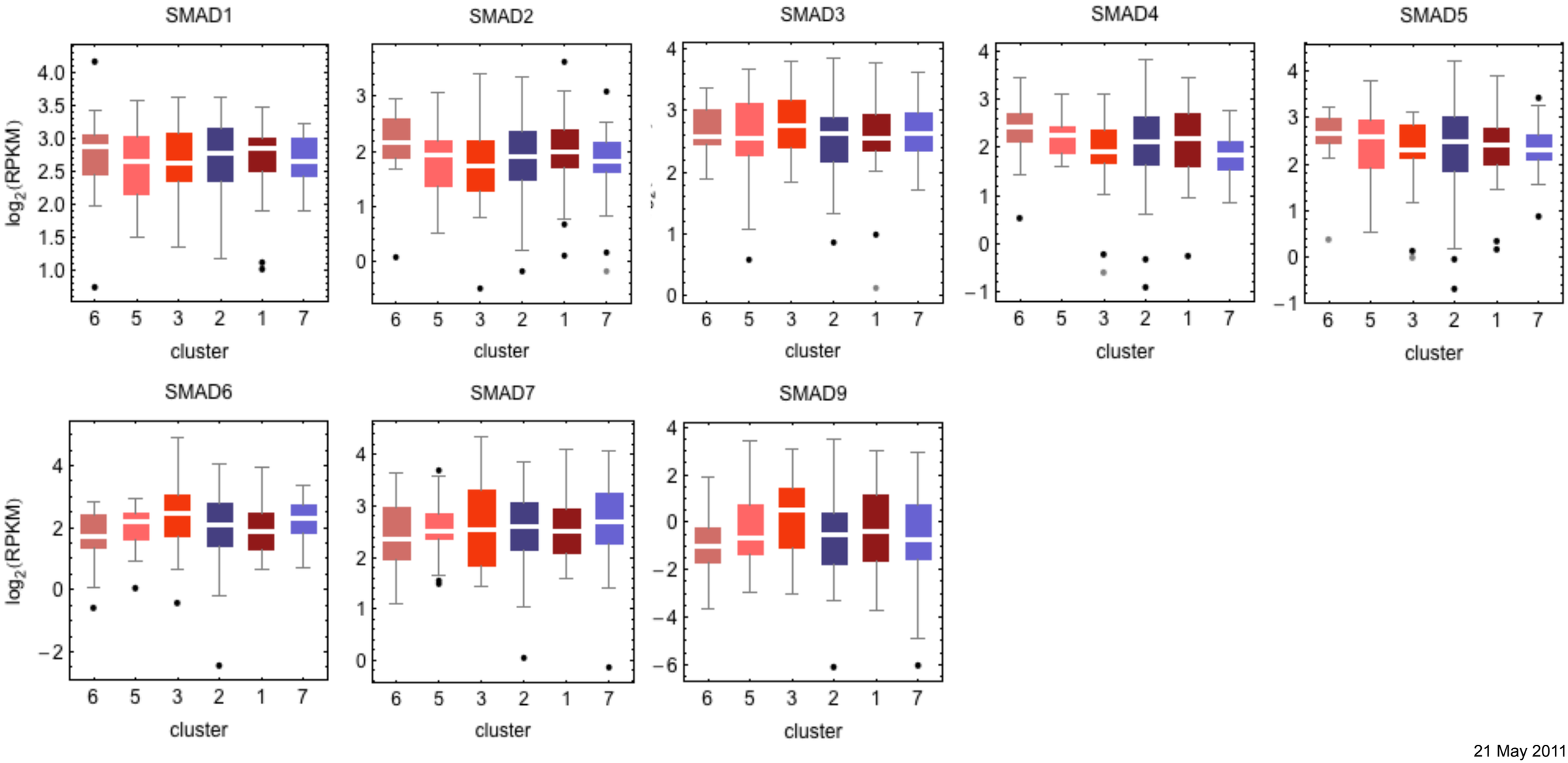
21 May 2011, 12h00



SMADs: RPKM abundance across 7 clusters

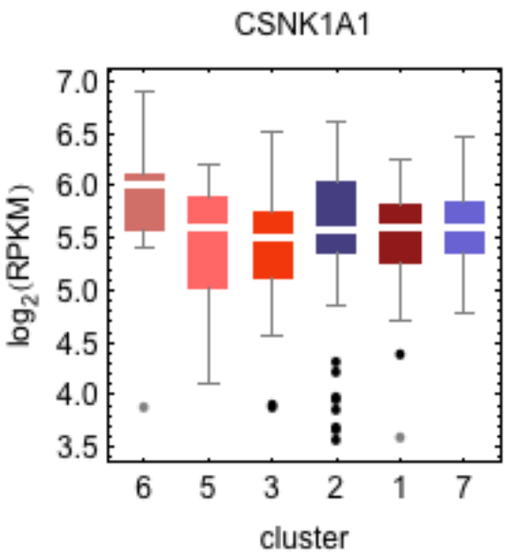
grobertson:mRNA-seq grobertson$ grep "SMAD" crc_244_gene_rpkm.txt | cut -f1
SMAD1|4086
SMAD2|4087
SMAD3|4088
SMAD4|4089
SMAD5|4090
SMAD6|4091
SMAD7|4092
SMAD9|4093



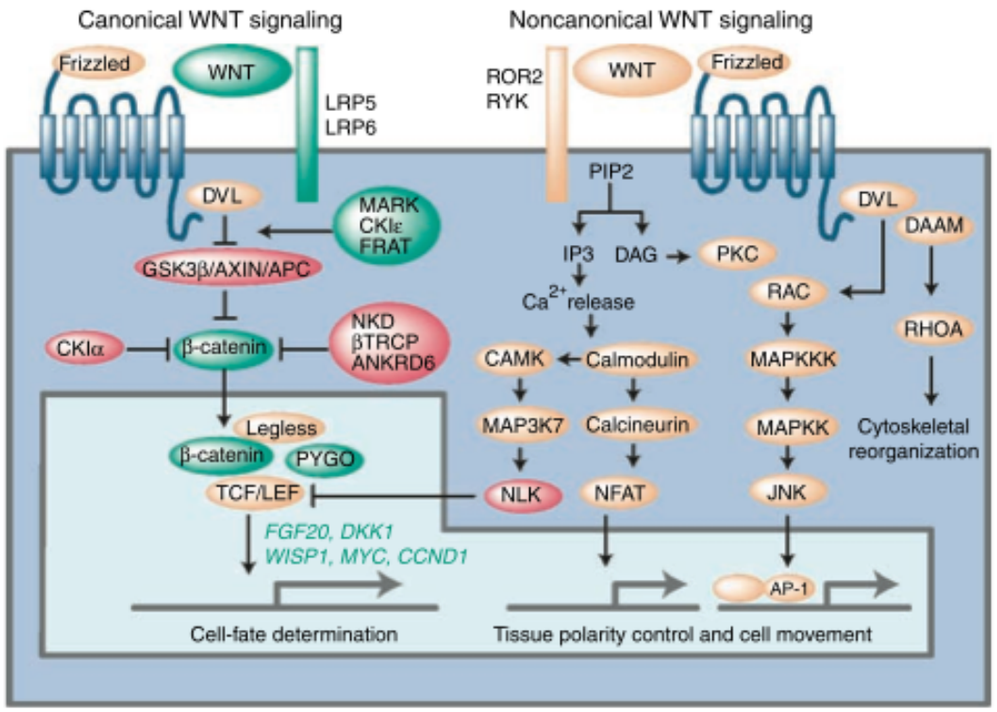


21 May 2011, 12h00

Canonical WNT signals are transduced through Frizzled (FZD) family receptors and LRP5/LRP6 coreceptor to the h-catenin signaling cascade (2, 3). In the absence of canonical WNT signaling, h-catenin complexed with APC and AXIN is phosphorylated by casein kinase Ia (CKIa) [**CSNK1A1**] and glycogen synthase kinase 3h (GSK3h) [**GSK3B**] in the NH2-terminal degradation box, which is polyubiquitinated by hTRCP1 or hTRCP2 complex for the following proteasome- mediated degradation (4). In the presence of canonical WNT signaling, Dishevelled (DVL) is phosphorylated by CKIa for high-affinity binding to FRAT. Because canonical WNT signal induces the assembly of FZD-DVL complex and LRP5/6-AXIN- FRAT complex (5, 6), h-catenin is released from phosphoryla- tion by CKIa and GSK3h for stabilization and nuclear accumulation. Nuclear h-catenin is complexed with T-cell factor/lymphoid enhancer factor (TCF/LEF) family transcrip- tion factors and also with Legless family docking proteins (BCL9 and BCL9L) associated with PYGO family coactivators (PYGO1 and PYGO2; refs. 7, 8). The TCF/LEF-h-catenin- Legless-PYGO nuclear complex is the effector of the canonical WNT signaling pathway to activate the transcription of target genes such as FGF20, DKK1, WISP1, MYC, and CCND1 (9–12). Katoh



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



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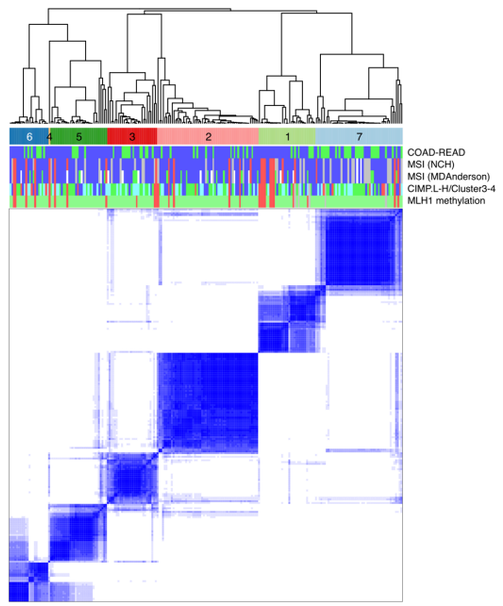
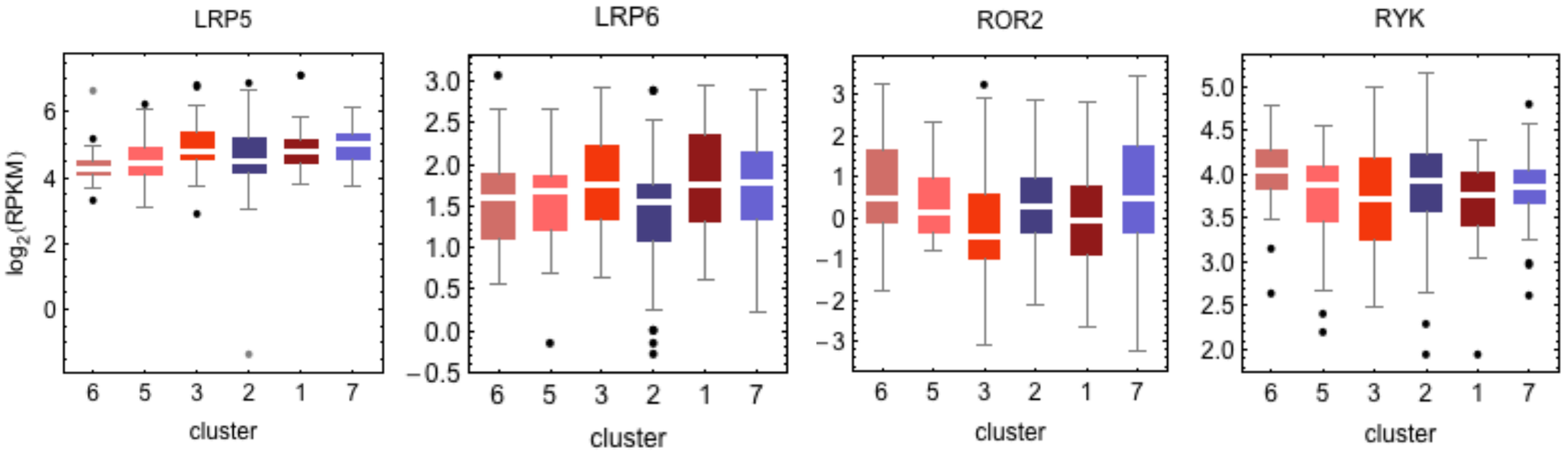
Katoh and Kato, Clin Cancer Res 2007, 13:4042.





TCFs, NKDs: RPKM abundance across 7 clusters

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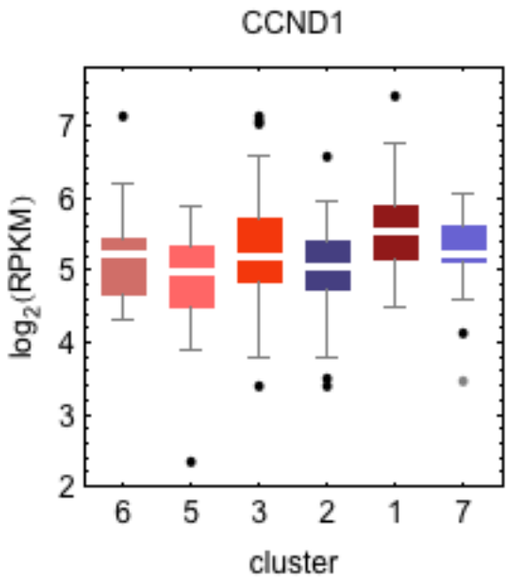
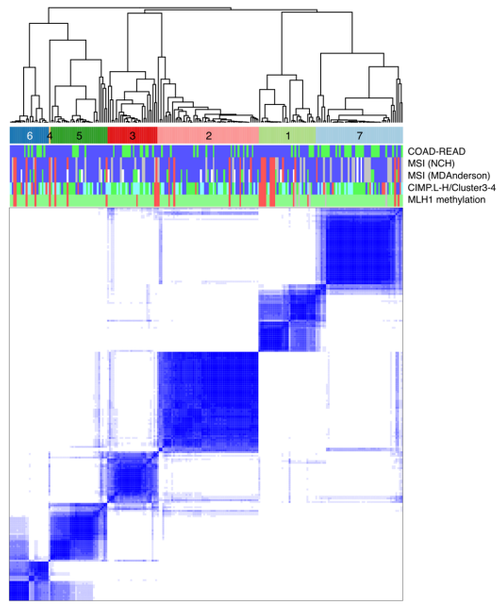
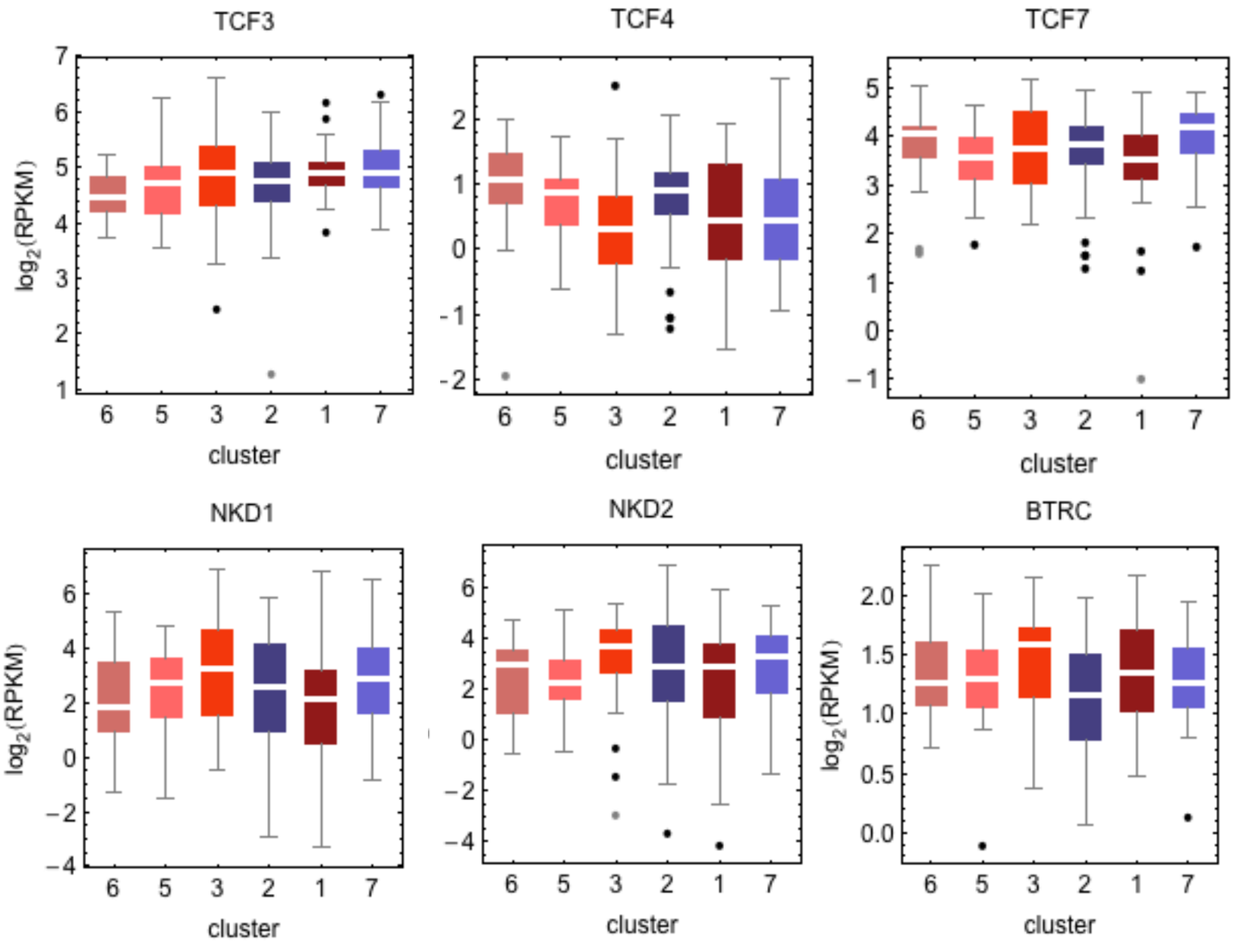
grobertson:mRNA-seq grobertson$ grep "TCF" crc_244_gene_rpkm.txt | (
TCF3|6929
TCF4|6925
TCF7|6932
TCF12|6938
TCF15|6939
TCF19|6941
TCF20|6942
TCF21|6943
TCF23|150921
TCF25|22980
TCF7L1|83439
TCF7L2|6934
TCFL5|10732
```







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21 May 2011, 12h30

Bengochea A, de Souza MM, Lefrançois L, Le Roux E, Galy O, Chemin I, Kim M, Wands JR, Trepo C, Hainaut P, Scoazec JY, Vitvitski L, Merle P. **Common dysregulation of Wnt/Frizzled receptor elements in human hepatocellular carcinoma**. Br J Cancer. 2008 Jul 8;99(1): 143-50.

Dysregulation of growth factors and their receptors is central to human hepatocellular carcinoma (HCC). We previously demonstrated that the Frizzled-7 membrane receptor mediating the Wnt signalling can activate the β-catenin pathway and promotes malignancy in human hepatitis B virus-related HCCs. Expression patterns of all the 10 Frizzled receptors, and their extracellular soluble autoparacrine regulators (19 Wnt activators and 4 sFRP inhibitors) were assessed by real-time RT–PCR in 62 human HCC of different etiologies and their matched peritumorous areas. Immunostaining was performed to localise Frizzled on cell types in liver tissues. Regulation of three known Frizzled-dependent pathways (β-catenin, protein kinase C, and C-Jun NH2-terminal kinase) was measured in tissues by western blot. We found that eight Frizzled-potentially activating events were pleiotropically dysregulated in 95% HCC and 68% peritumours as compared to normal livers (upregulations of Frizzled-3/6/7 and Wnt3/4/5a, or downregulation of sFRP1/5), accumulating gradually with severity of fibrosis in peritumours and loss of differentiation status in tumours. The hepatocytes supported the Wnt/Frizzled signalling since specifically overexpressing Frizzled receptors in liver tissues. Dysregulation of the eight Frizzled-potentially activating events was associated with differential activation of the three known Frizzled-dependent pathways. This study provides an extensive analysis of the Wnt/Frizzled receptor elements and reveals that the dysregulation may be one of the most common and earliest events described thus far during hepatocarcinogenesis.

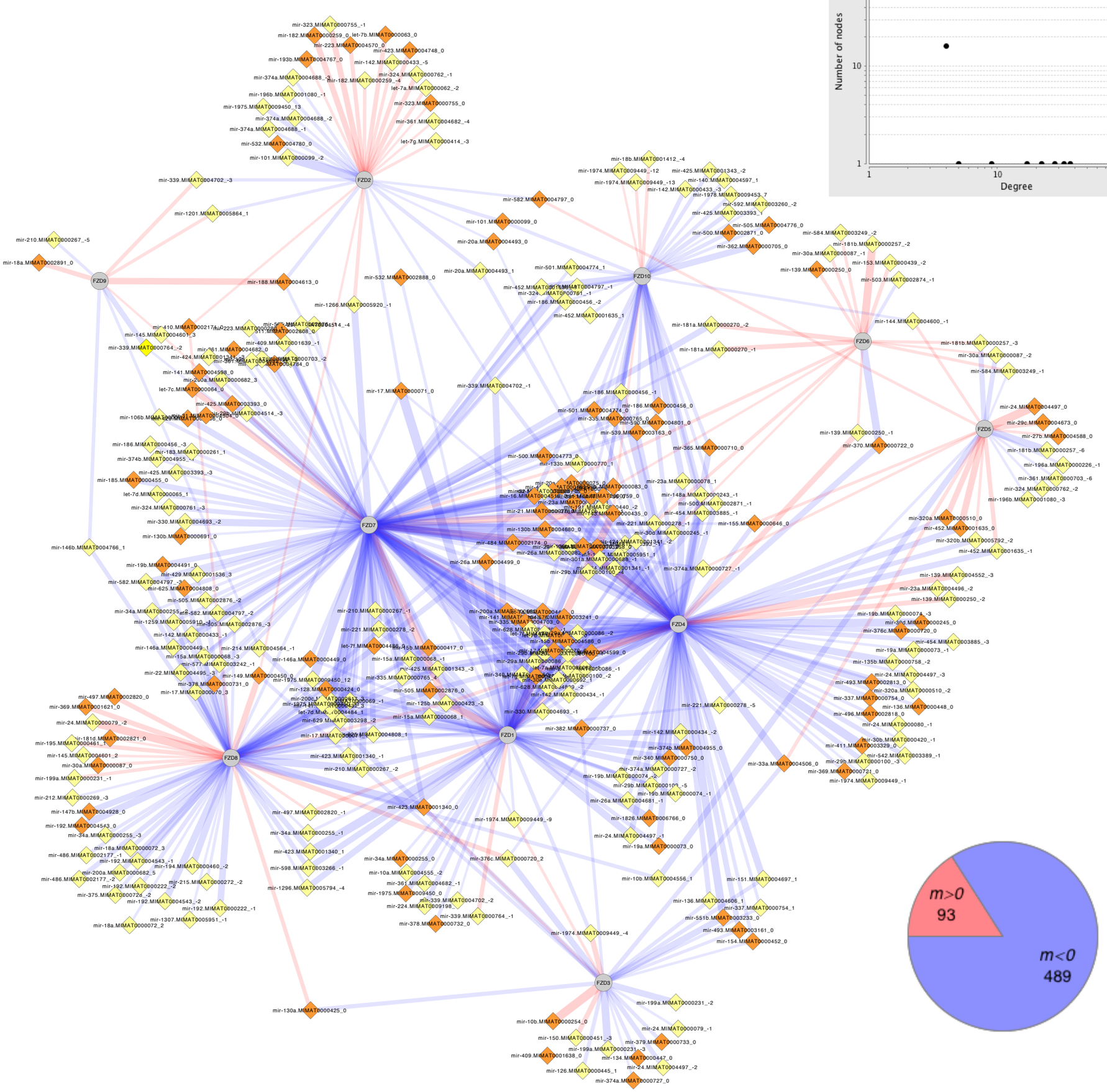
DICKKOPF-4 is induced by TCF/beta-catenin and upregulated in human colon cancer, promotes tumour cell invasion and angiogenesis and is repressed by 1alpha,25-dihydroxyvitamin D3.
Pendás-Franco N, García JM, Peña C, Valle N, Pálmer HG, Heinäniemi M, Carlberg C, Jiménez B, Bonilla F, Muñoz A, González-Sancho JM.
Oncogene. 2008 Jul 24;27(32):4467-77.

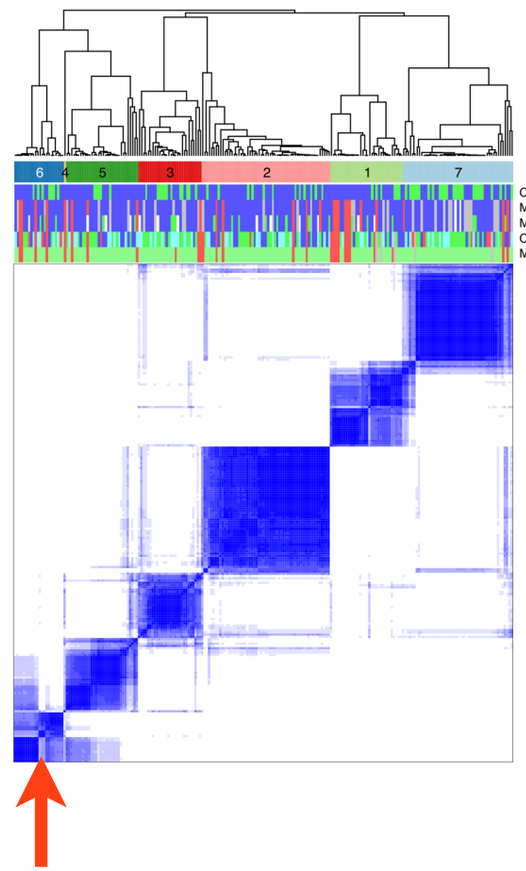
Aberrant activation of the Wnt/beta-catenin signaling pathway is a hallmark of colon cancer. We show that the Wnt antagonist DICKKOPF-4 (DKK-4) gene is repressed by 1alpha,25-dihydroxyvitamin D3 (1,25(OH)2D3) in human colon cancer cells. This effect correlated with the inhibition of the DKK-4 promoter. Chromatin immunoprecipitation assays revealed that 1,25(OH)2D3 induces early and transient binding of the vitamin D receptor (VDR) and the SMRT corepressor to the region adjacent to the transcription start site of DKK-4. Additionally, we demonstrate that the DKK-4 gene is a new downstream target of TCF/beta-catenin. Remarkably, expression of DKK-4 messenger RNA (mRNA) was not detected in a series of 29 human normal (N) colon biopsies but expression was upregulated in all the matched tumour (T) tissues. An inverse correlation existed between the expression of DKK-4 and VDR RNA in the Ts. Ectopic DKK-4 expression increased the migration and invasion properties of colon cancer cells and conditioned media (CM) from DKK-4-expressing cells enhanced the capacity to migrate and form capillary-like tubules of human primary microvascular endothelial cells. In conclusion, DKK-4 is upregulated in colon cancer and is associated with the acquisition of malignant properties by neoplastic cells. DKK-4 downregulation is a novel effect of 1,25(OH)2D3 that may contribute to its anticancer action.

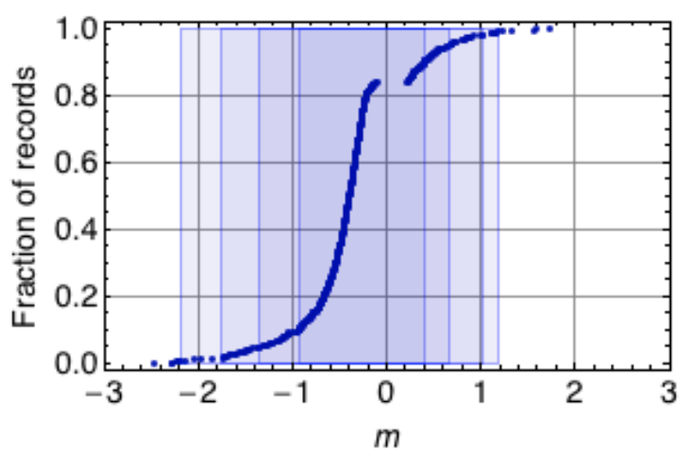
Correlations for Frizzled genes FZD1 to 10

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

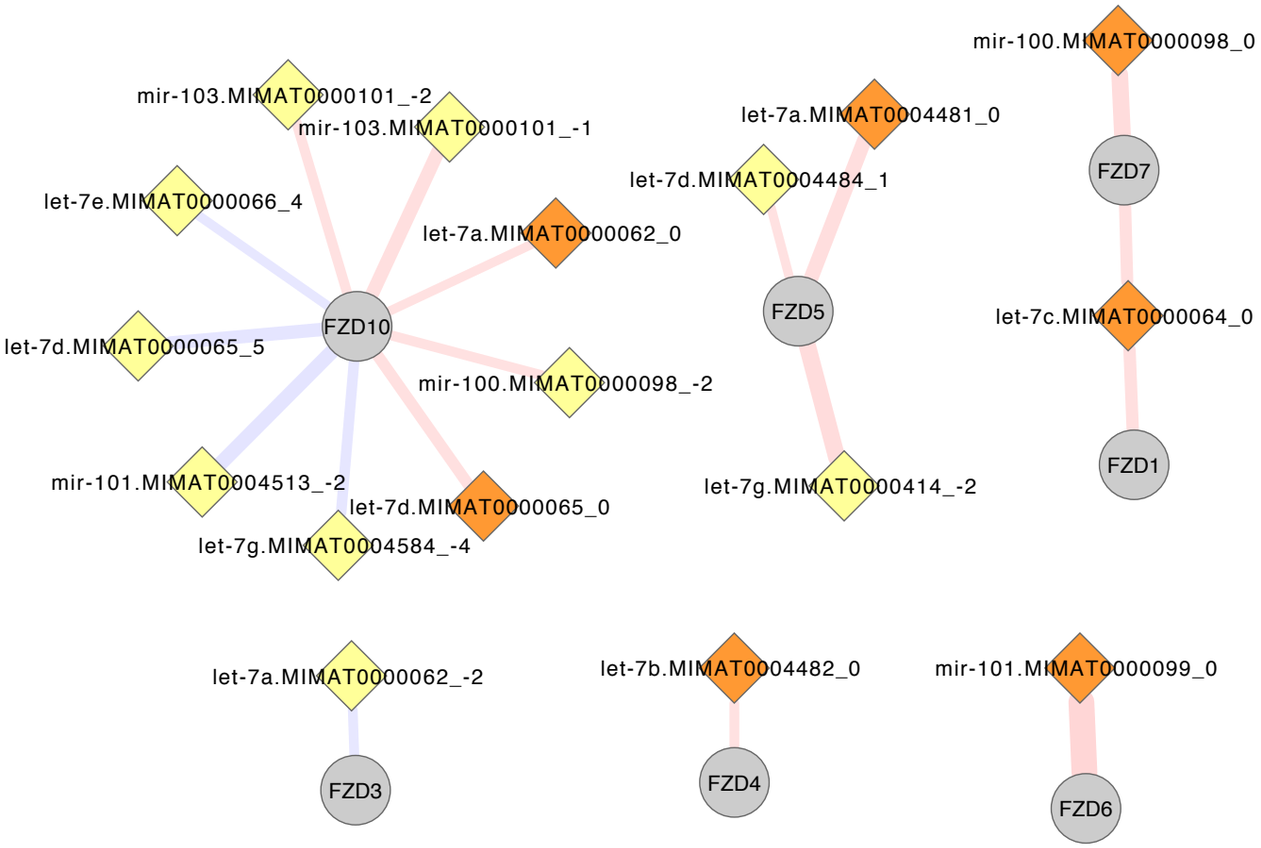
582 FZD records, cluster 6

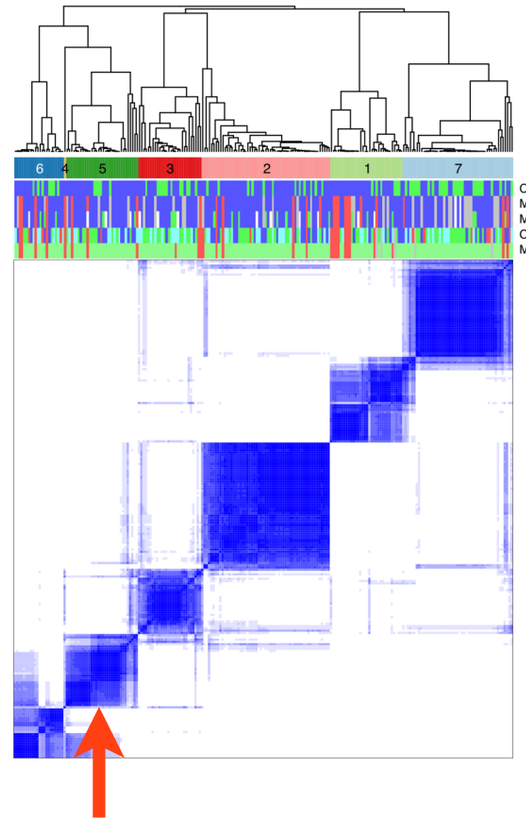


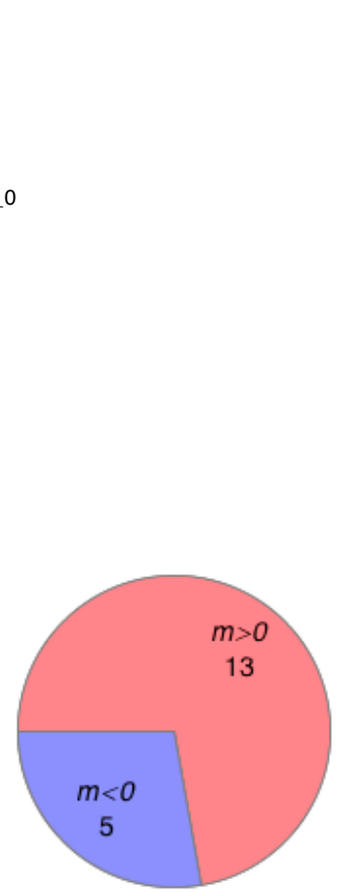




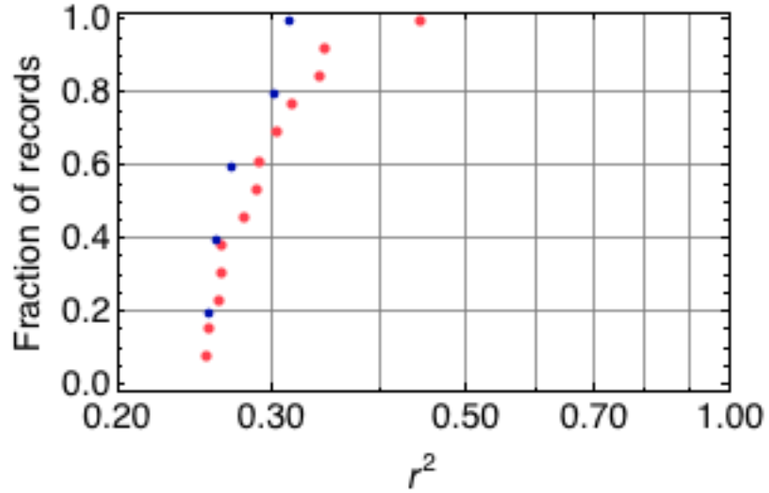
18 FZD records, cluster 5

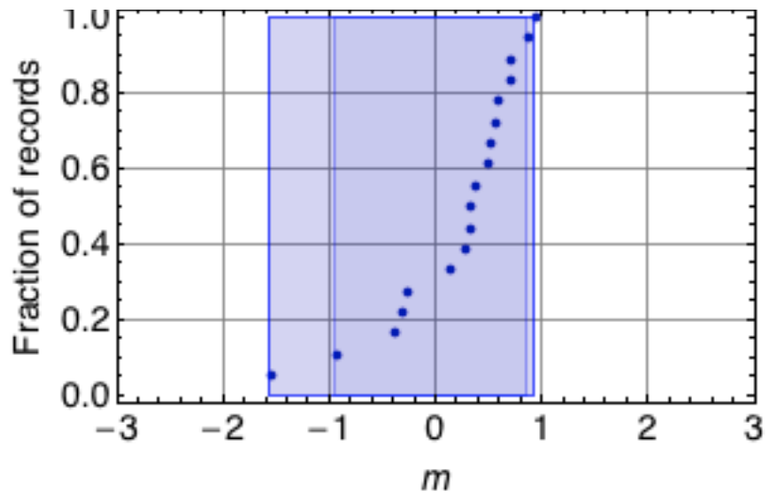




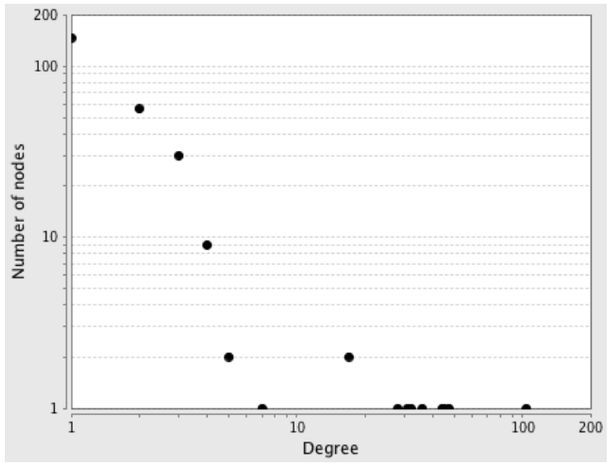


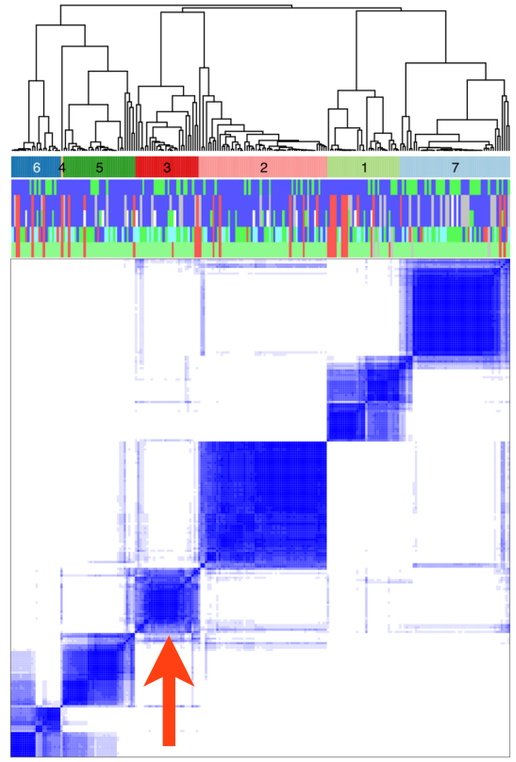
COAD–READ c5, FZD

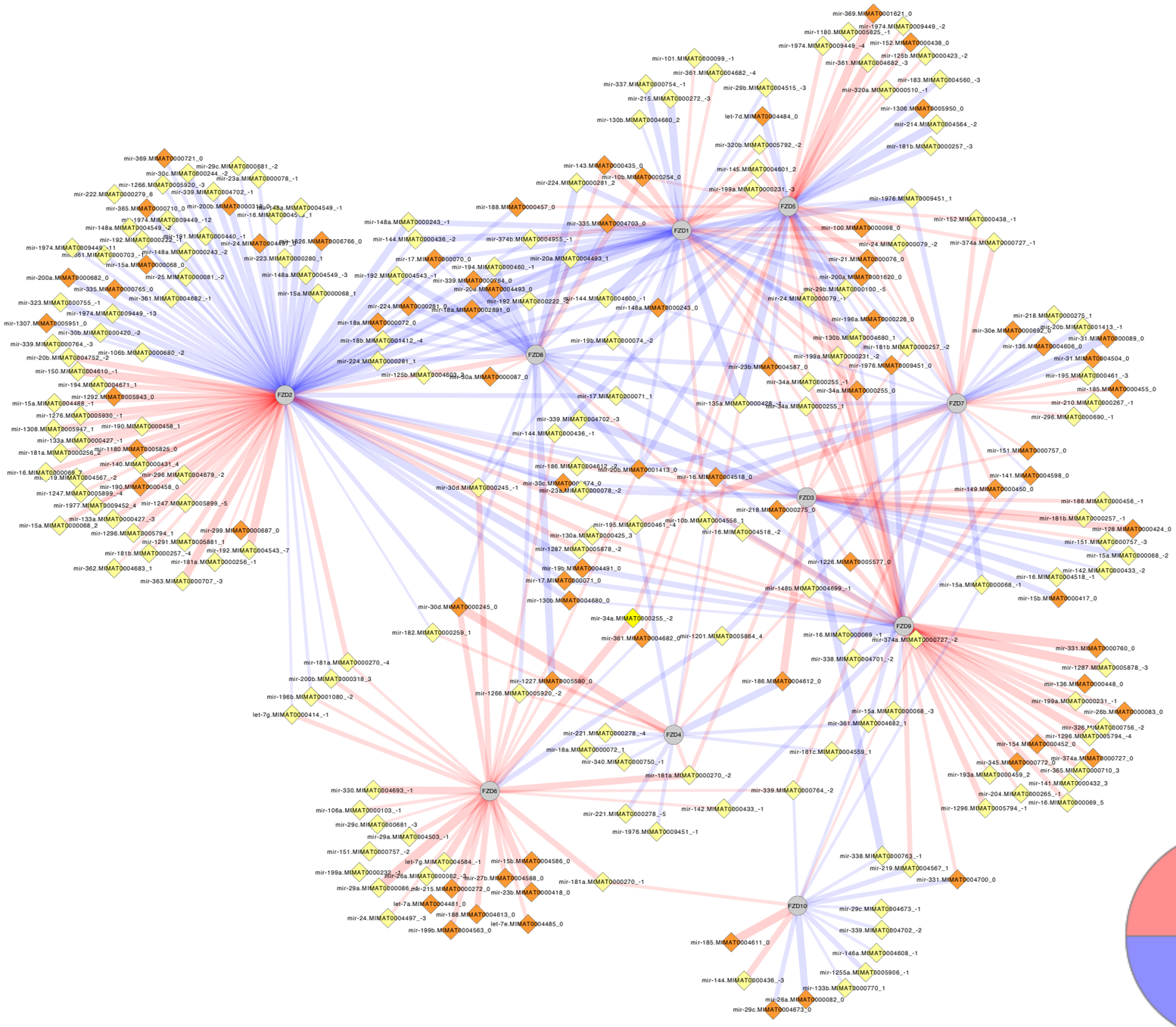


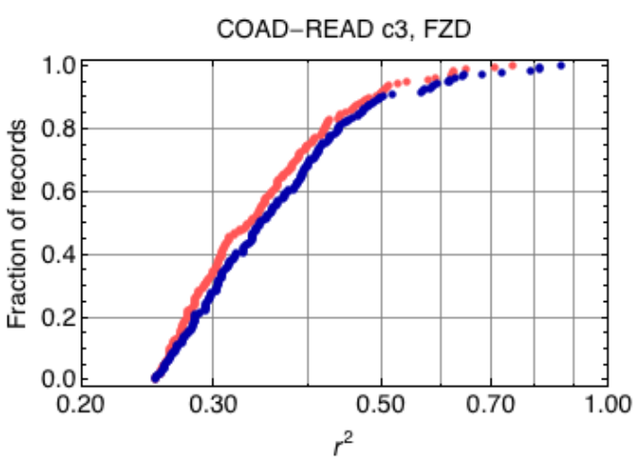


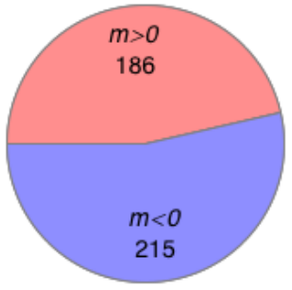
401 FZD records, cluster 3

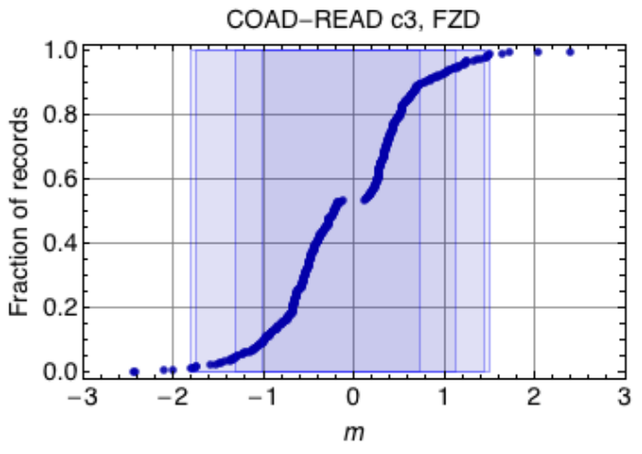




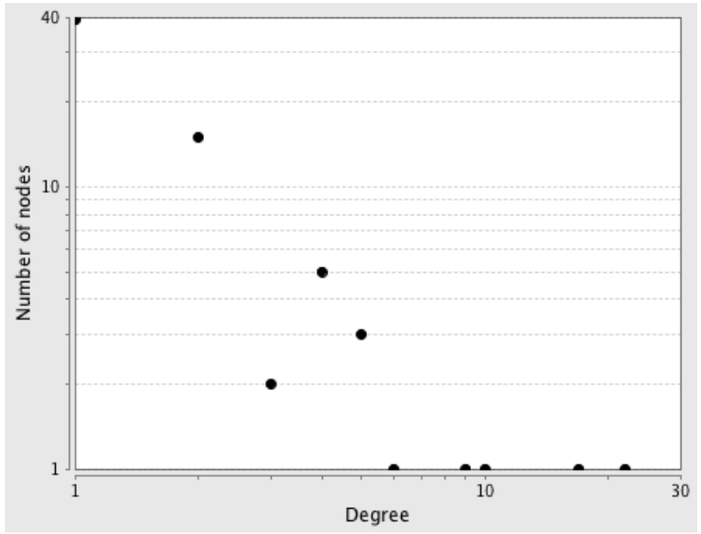


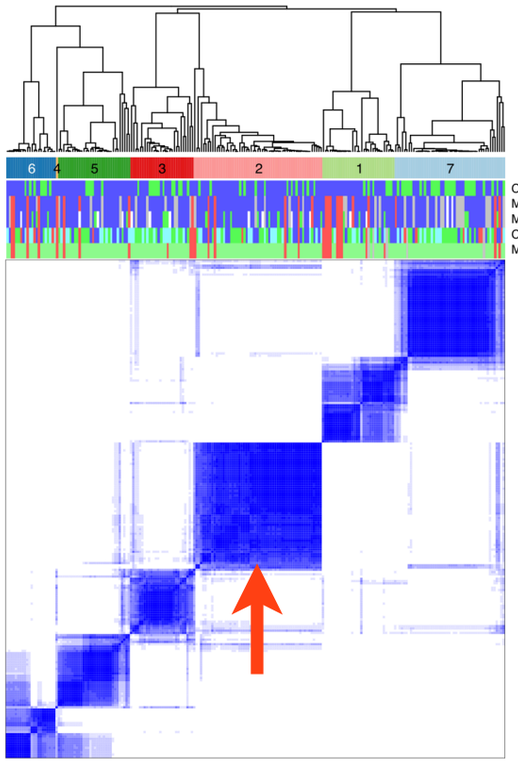


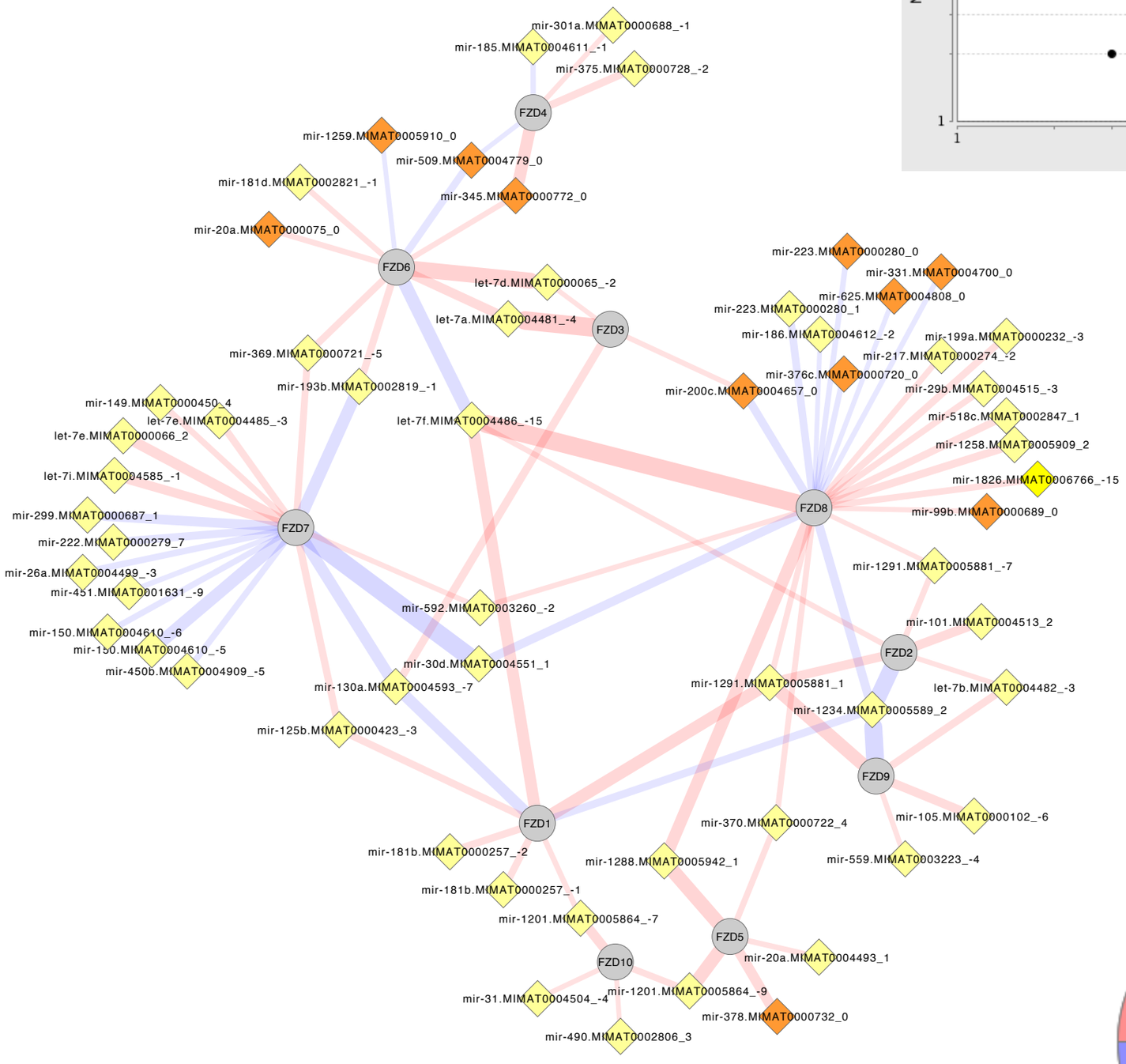


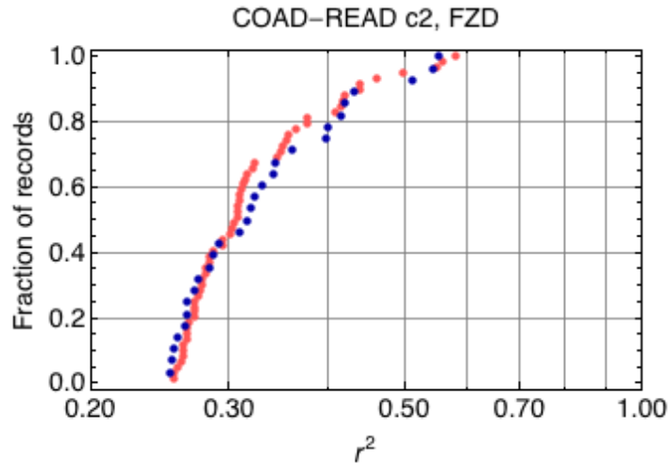


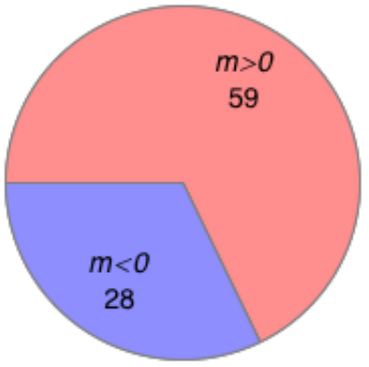
87 FZD records, cluster 2

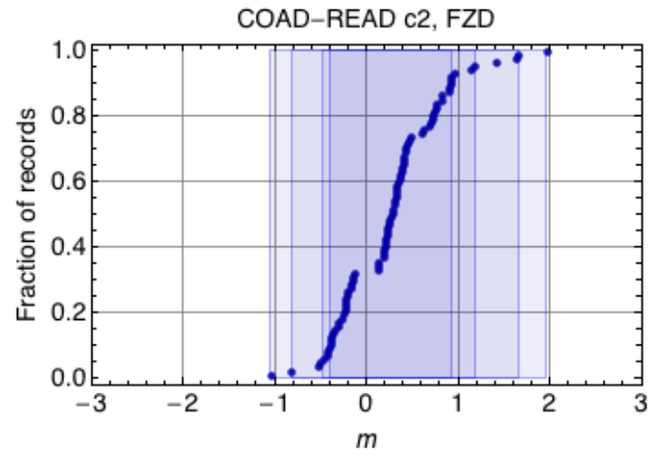




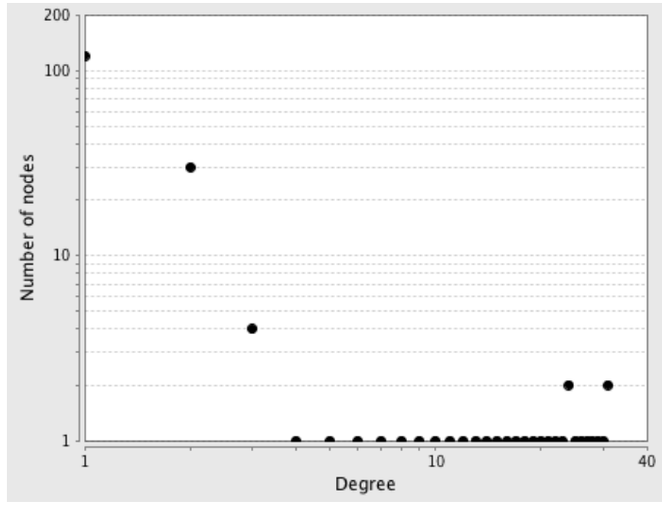


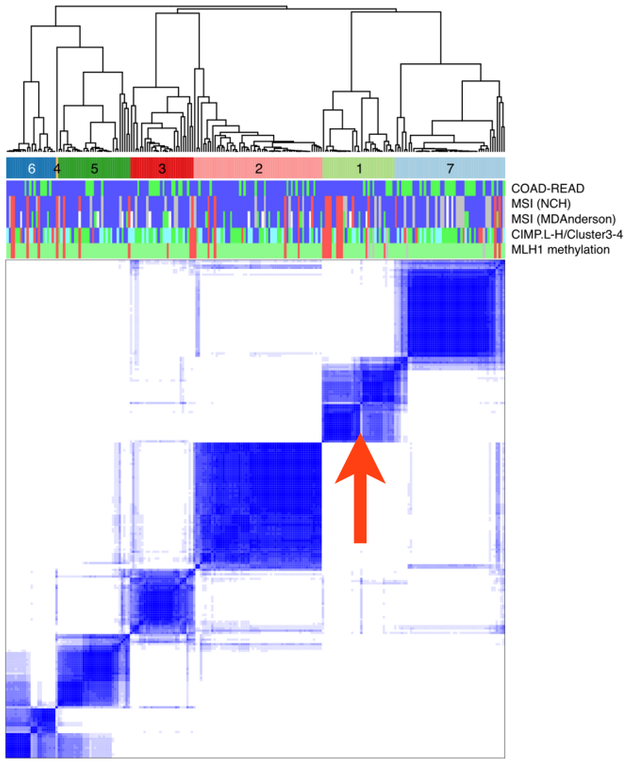


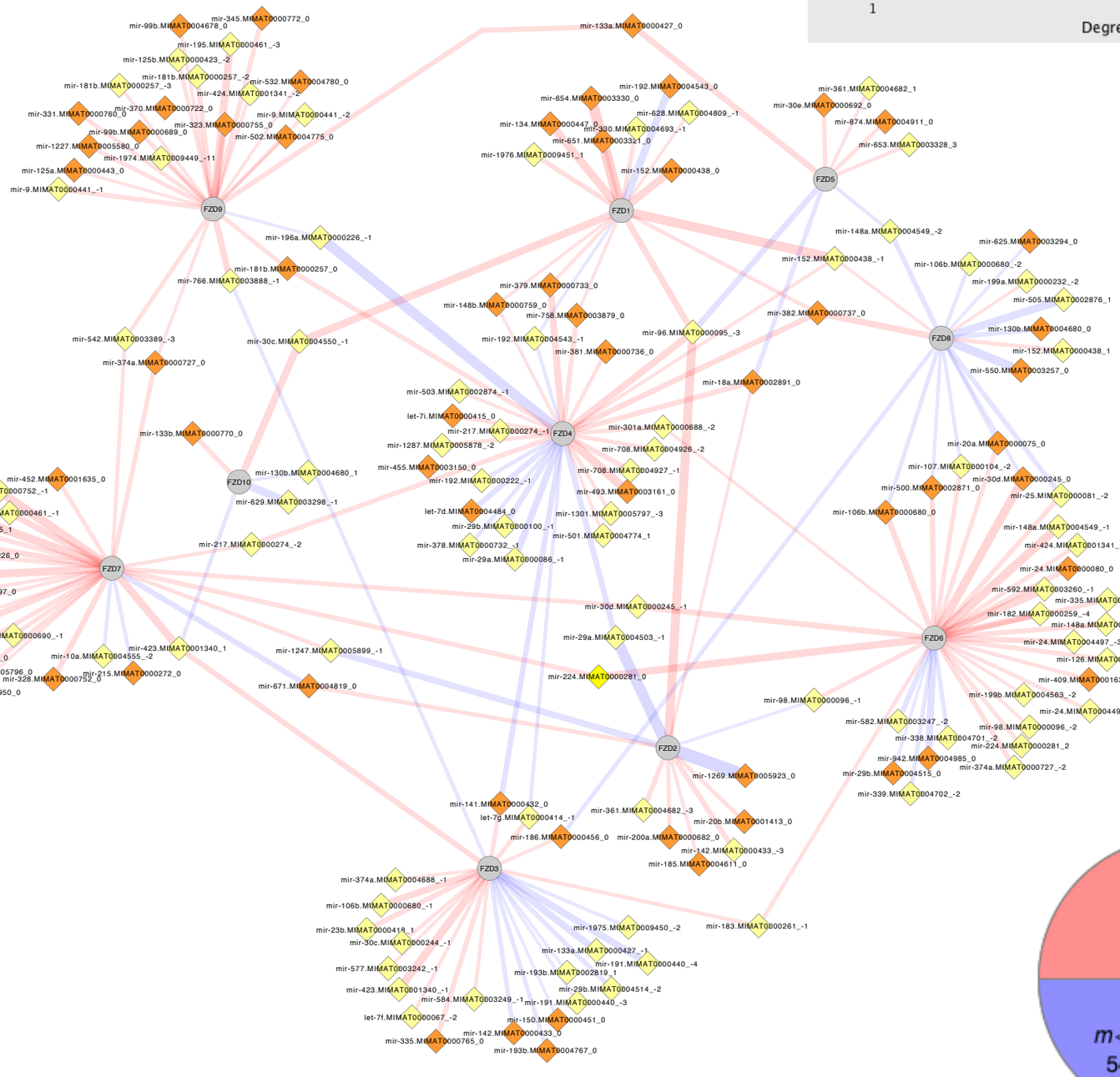


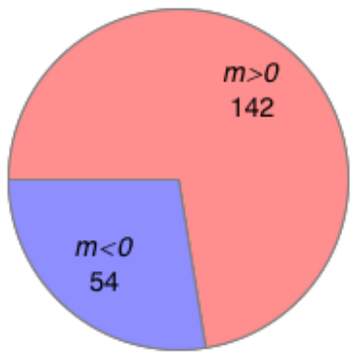


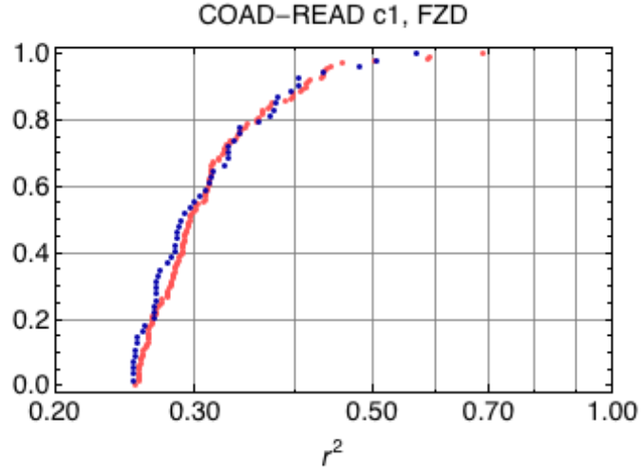
nn FZD records, cluster 1

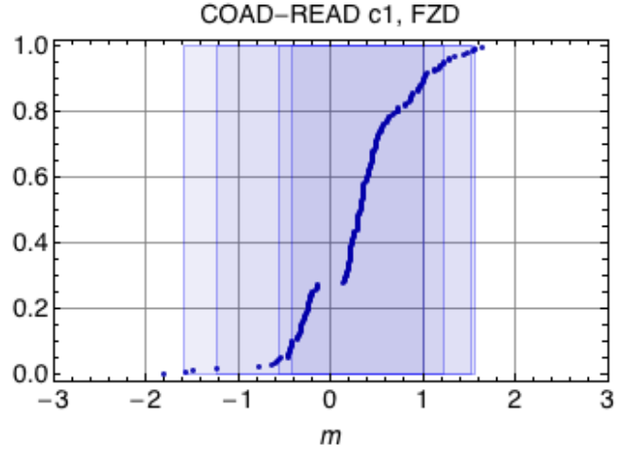




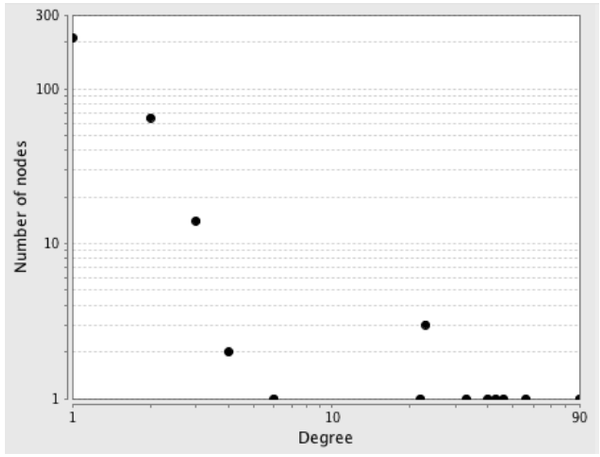


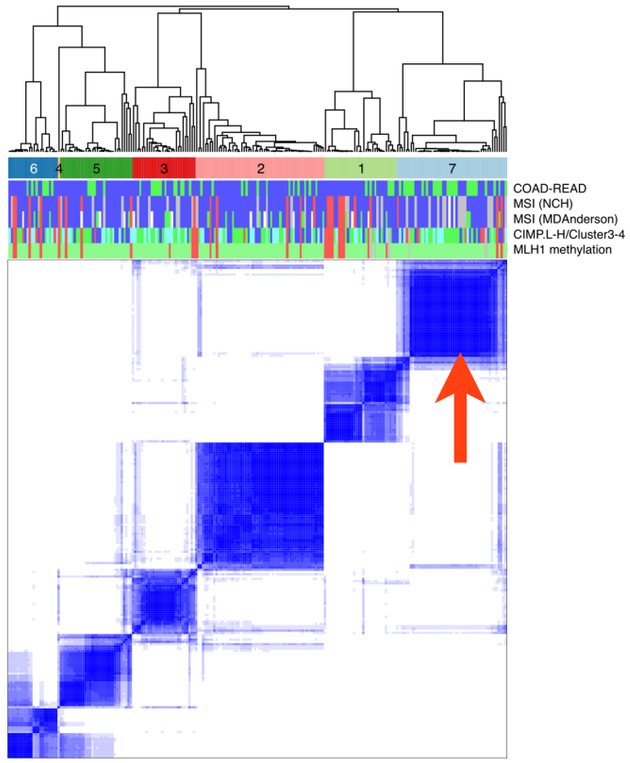


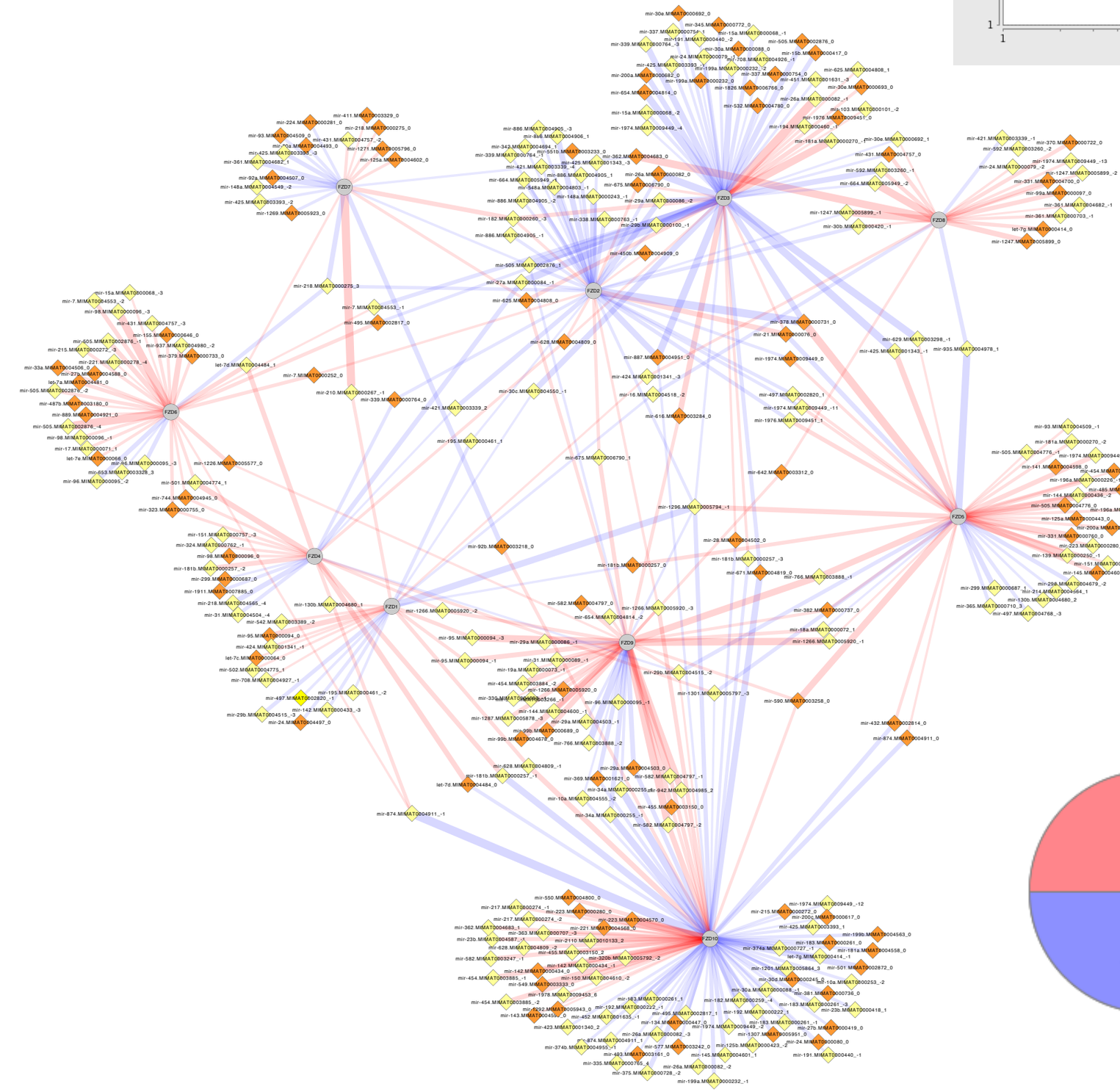


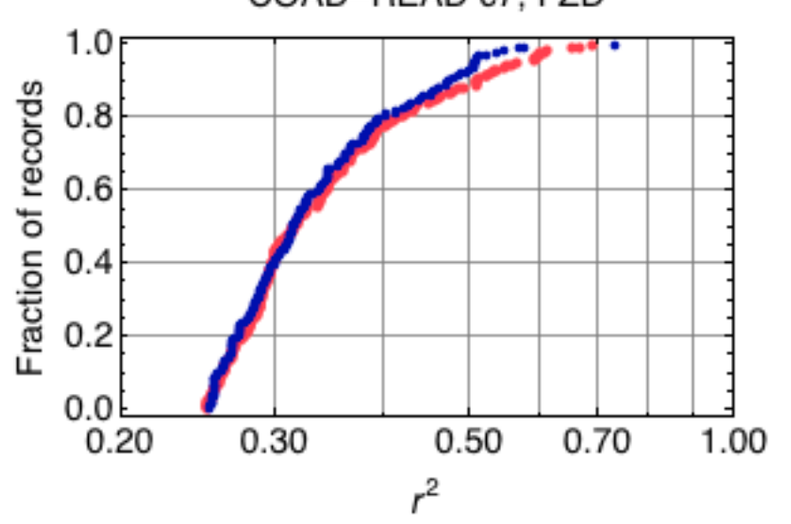


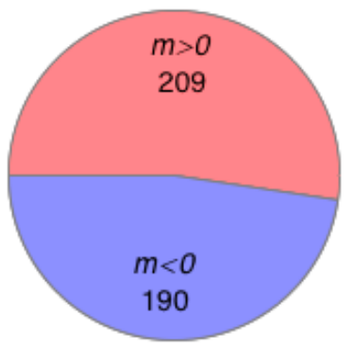
399 FZD records, cluster 7

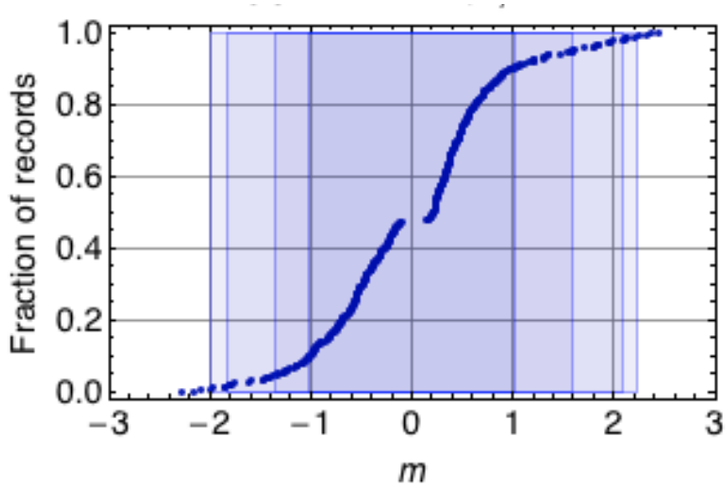




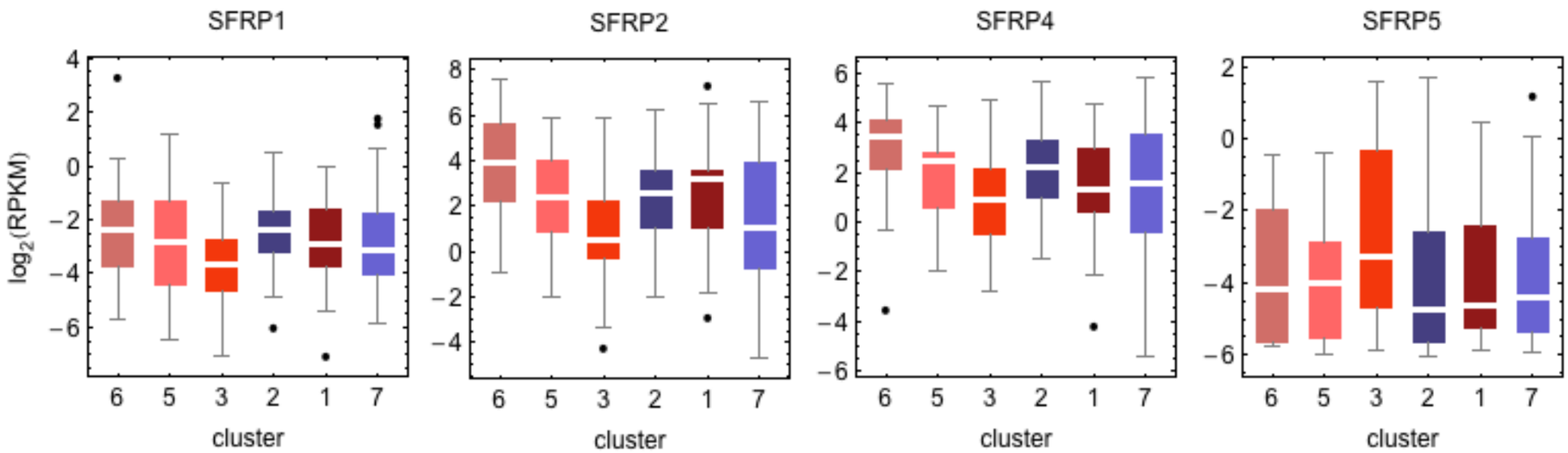






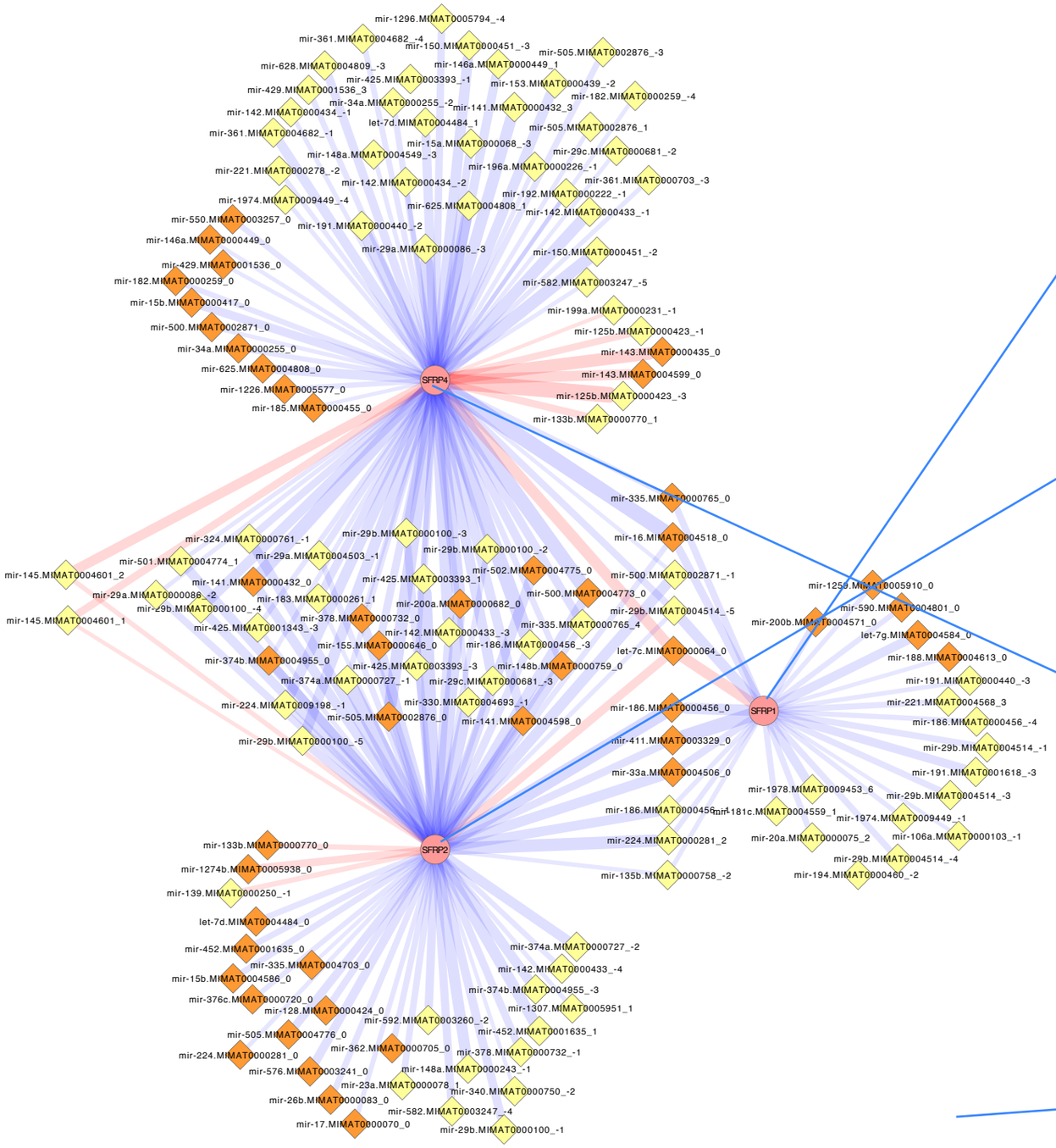


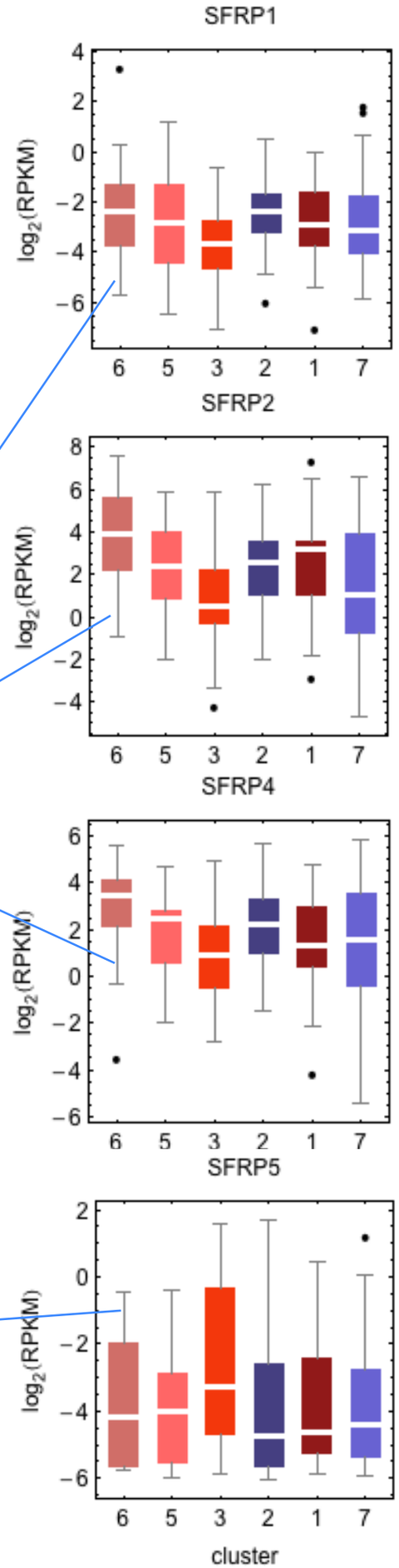
Correlations for SFRPs

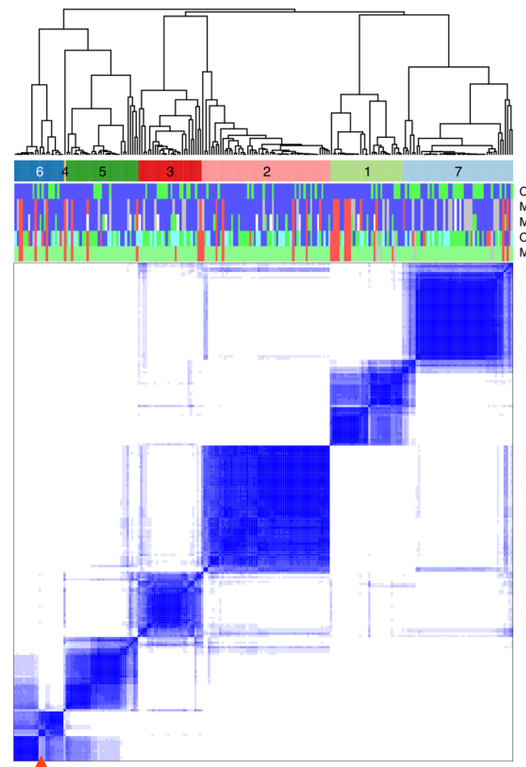


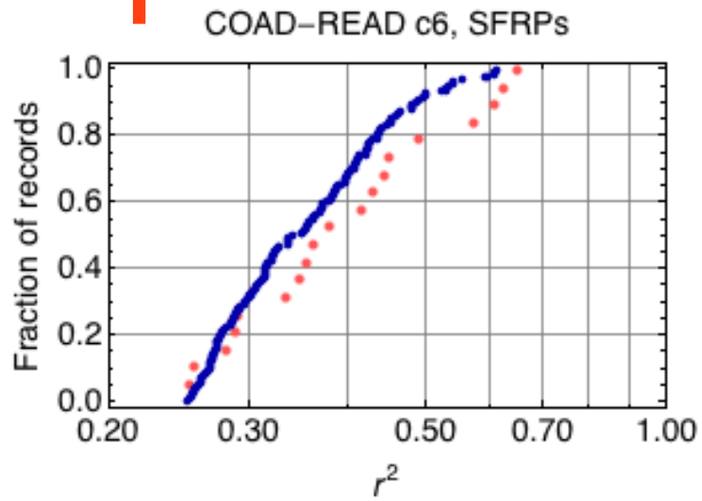
Levels of SFTP1, 2 and 4 progressively decrease in clusters 6, 5 and 3.

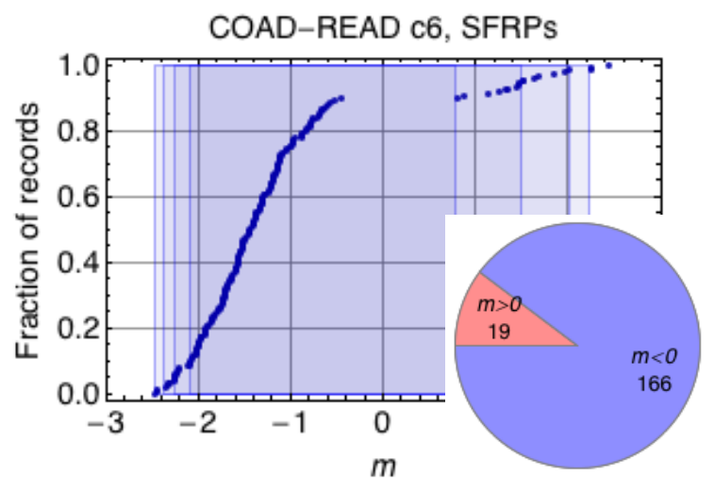
185 SRFP correlations, cluster 6



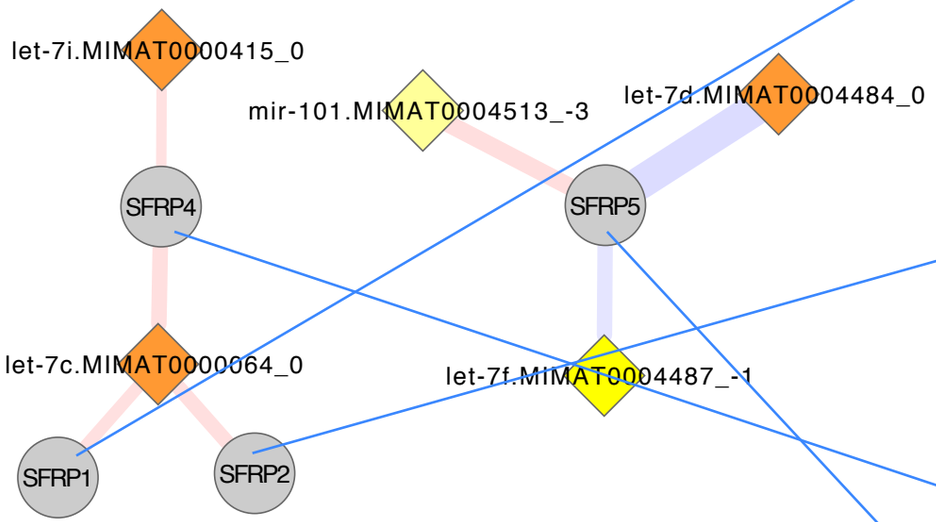


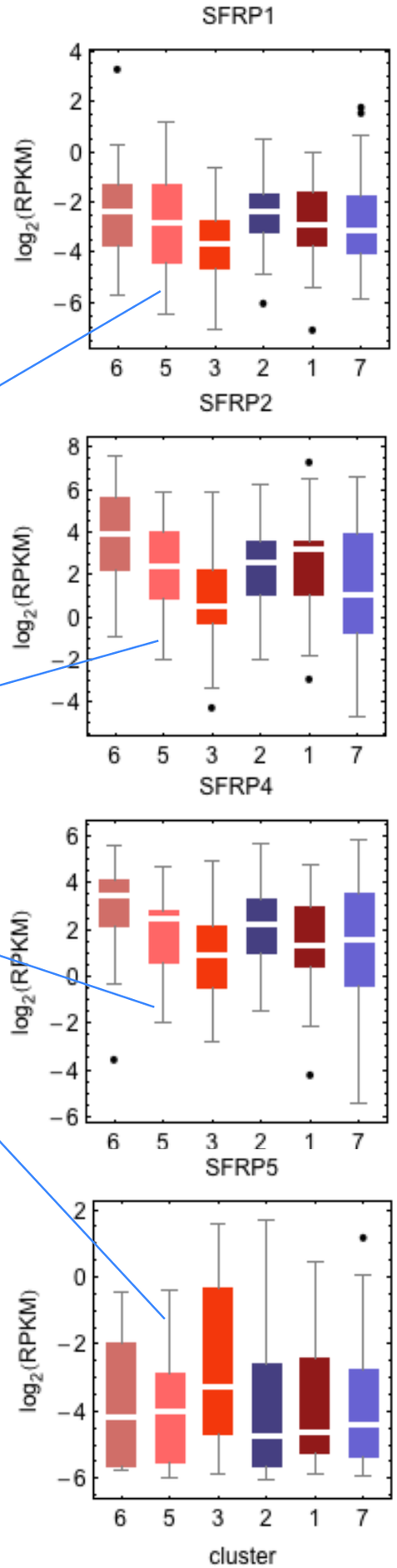


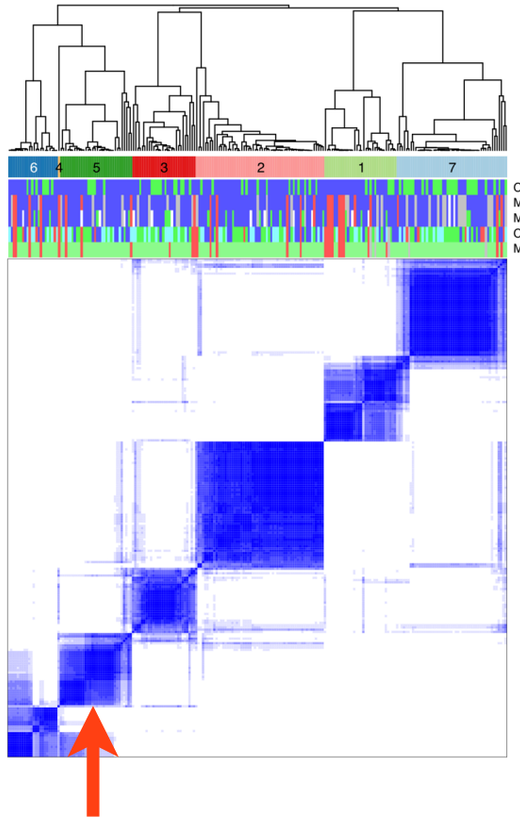


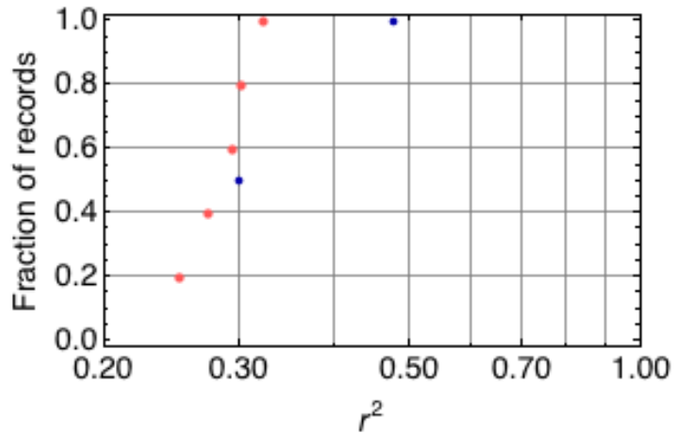


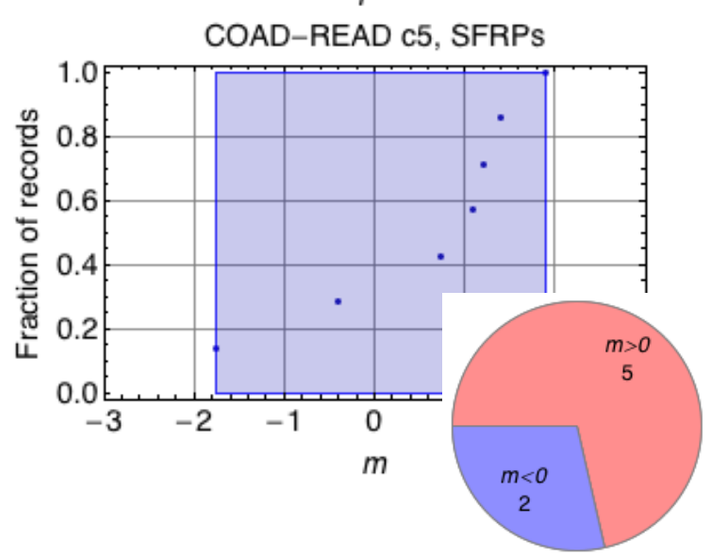
7 SRFP correlations, cluster 5



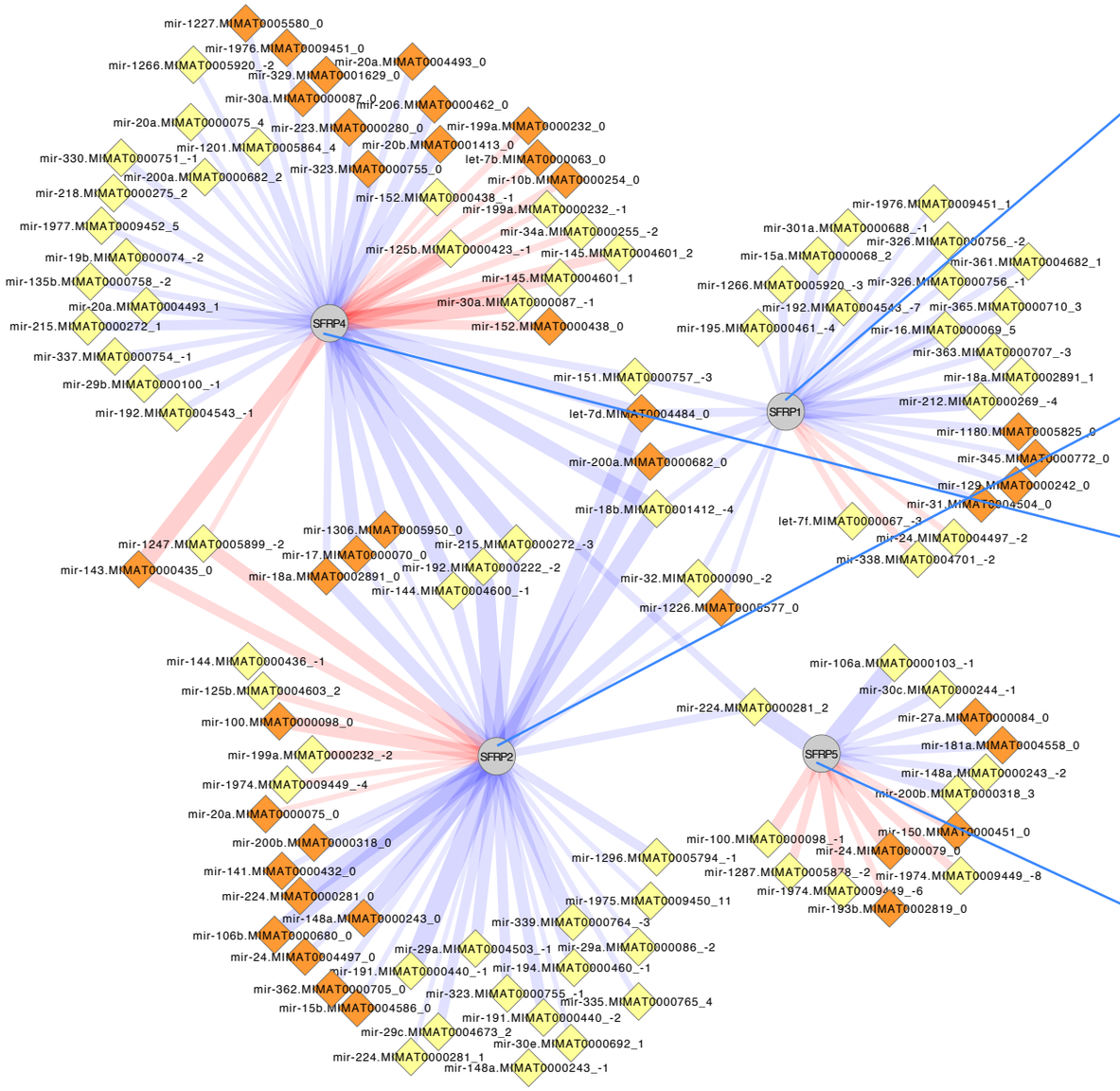


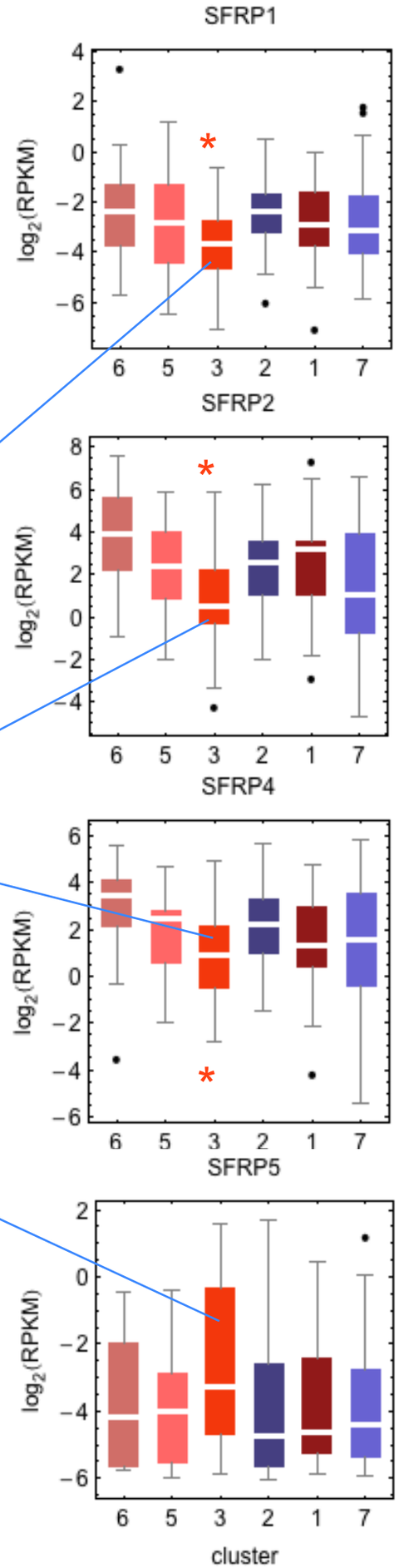


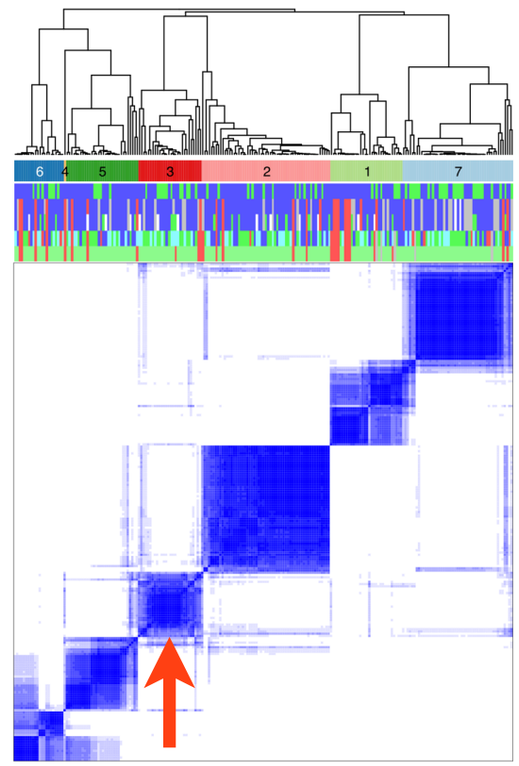


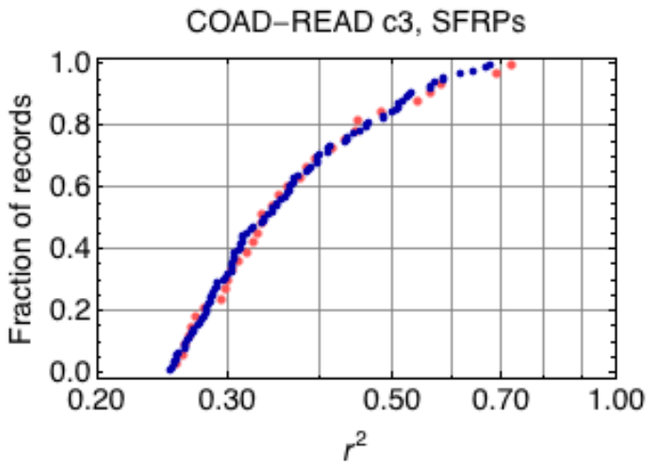


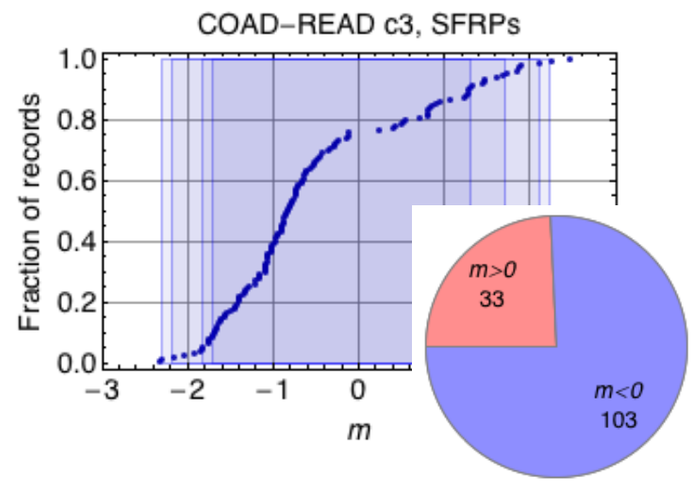
136 SRFP correlations, cluster 3



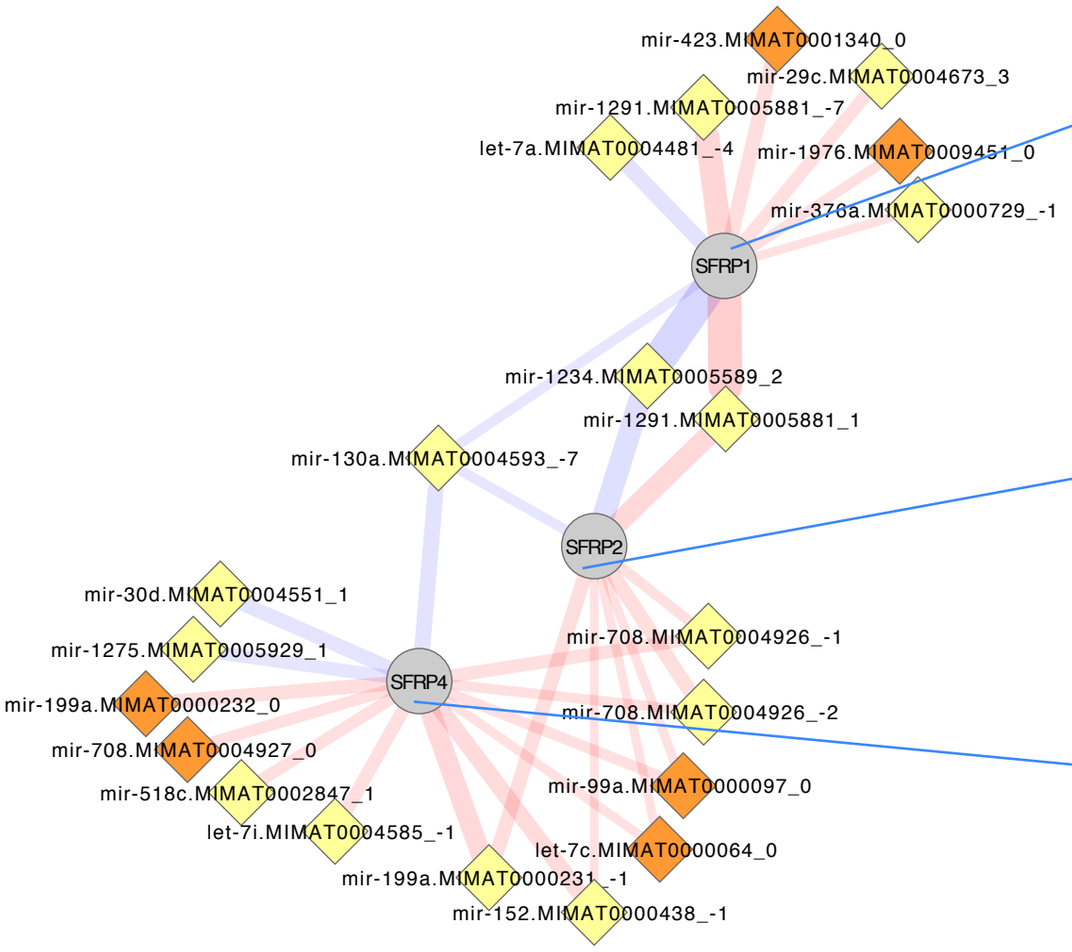


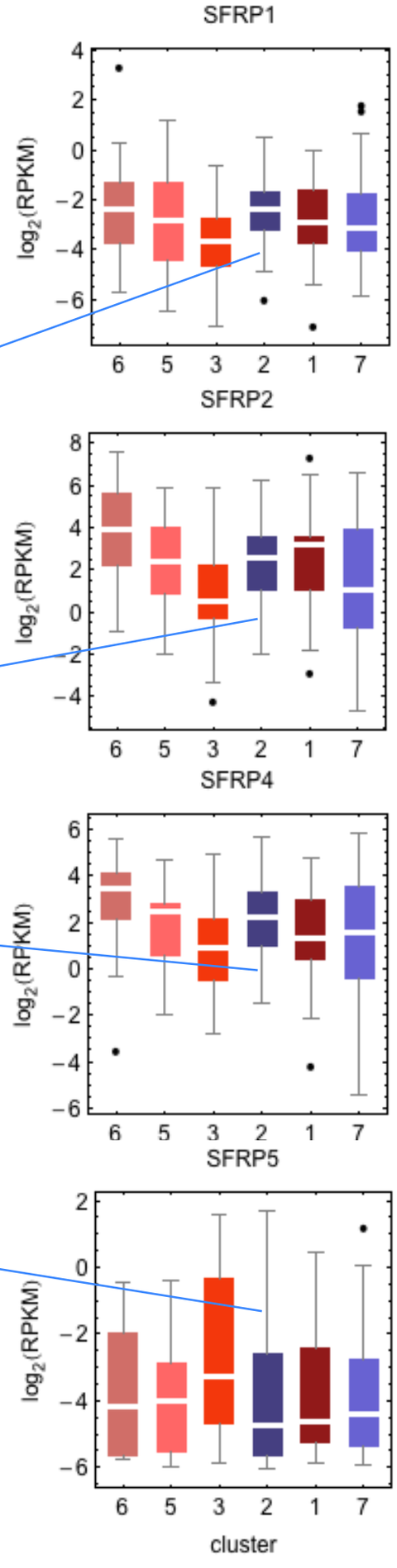


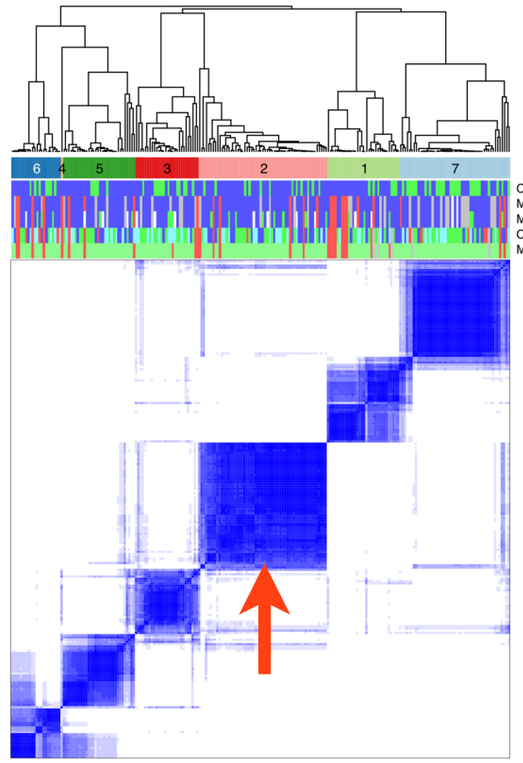


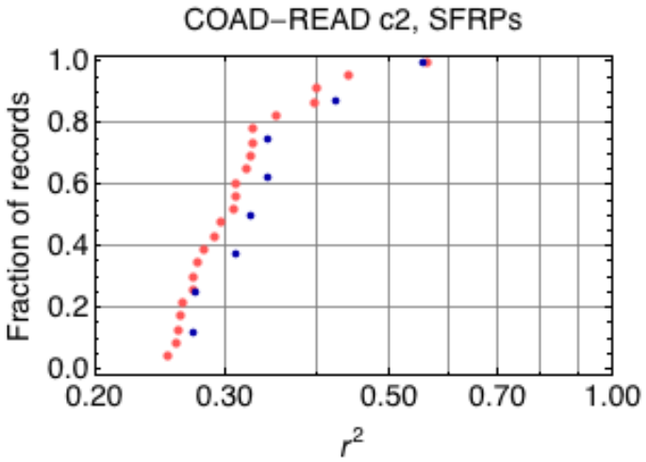


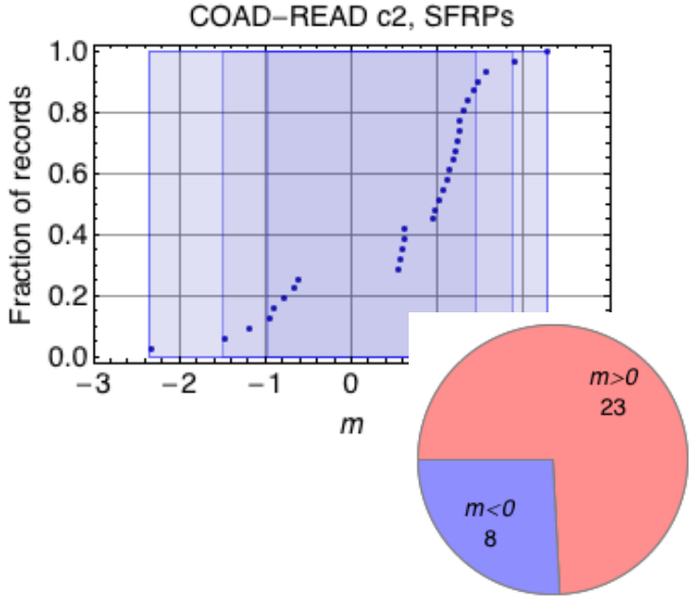
31 SRFP correlations, cluster 2



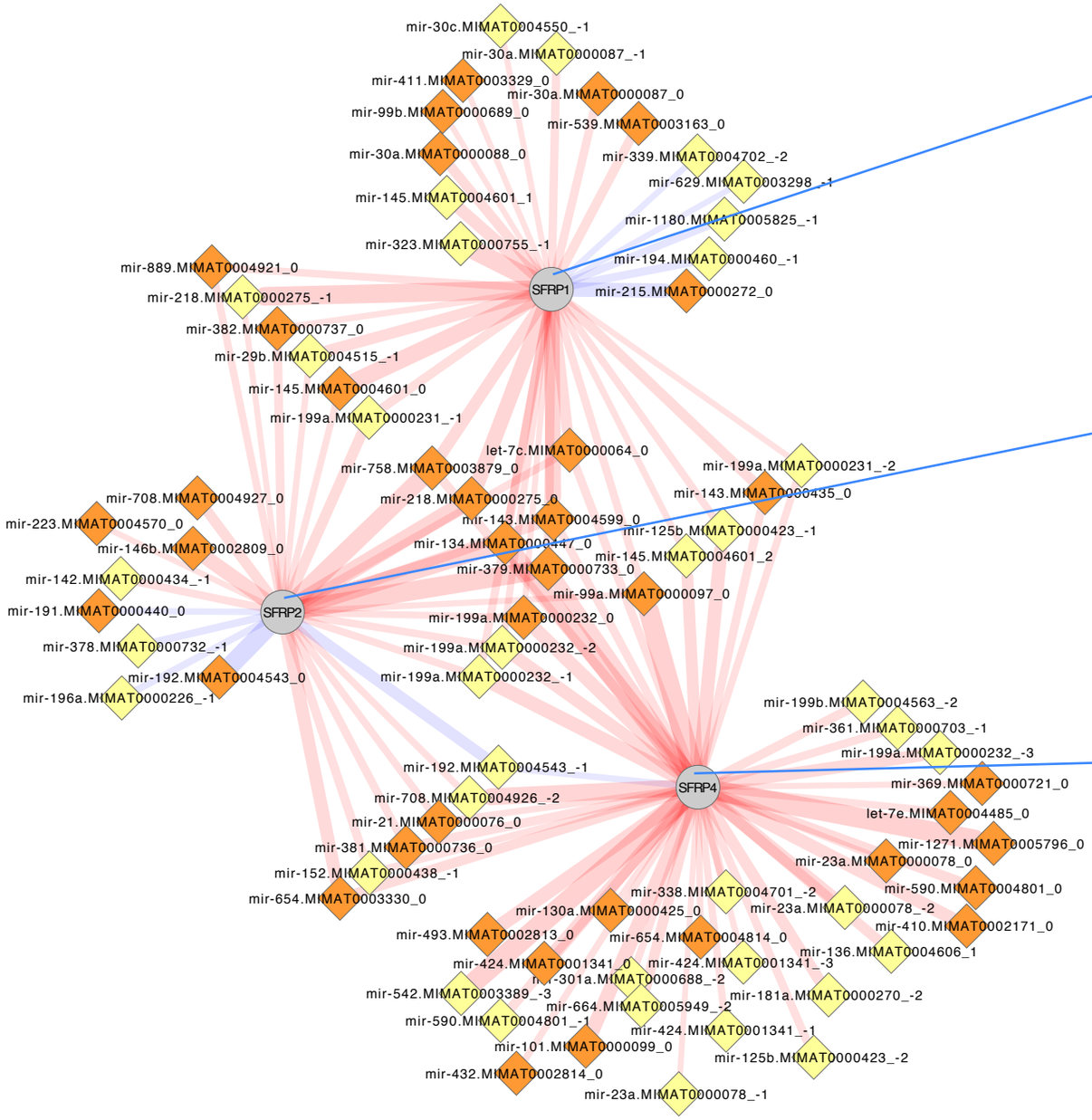


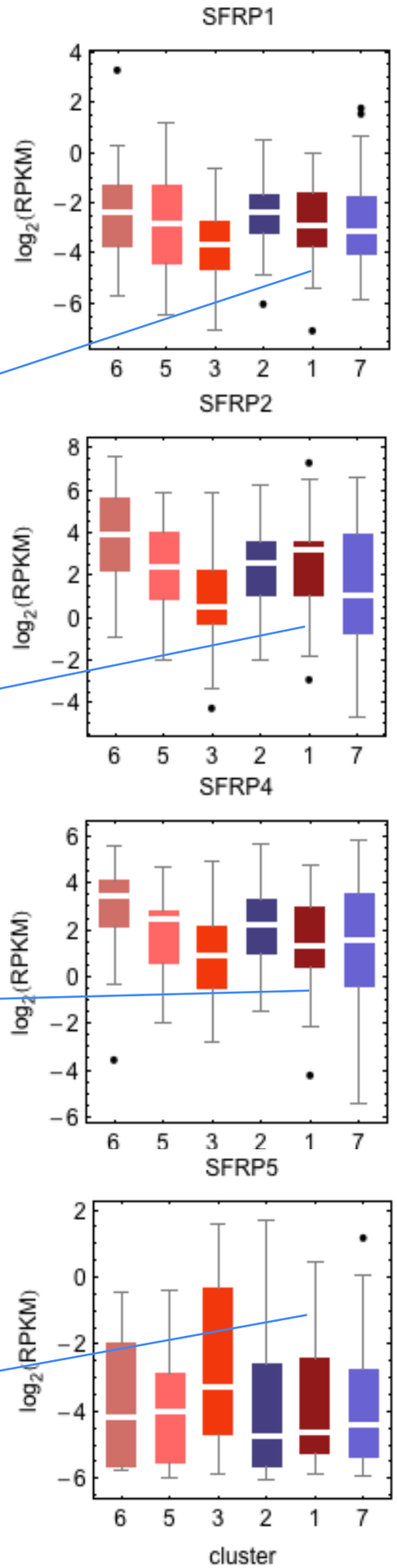


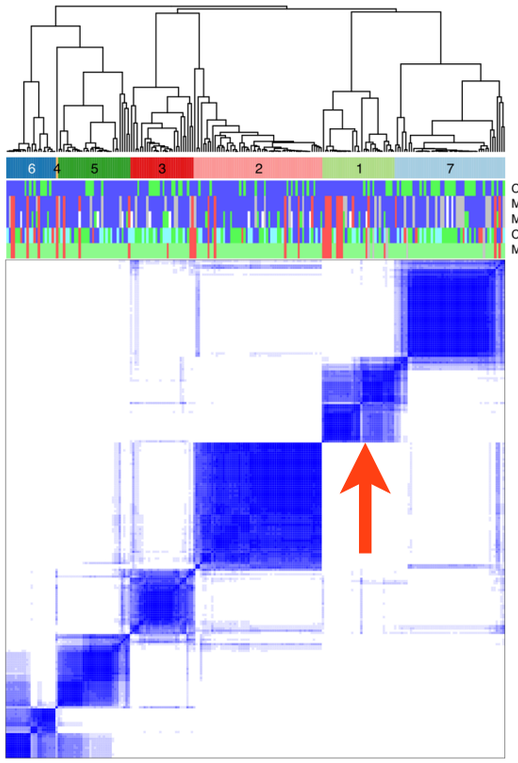


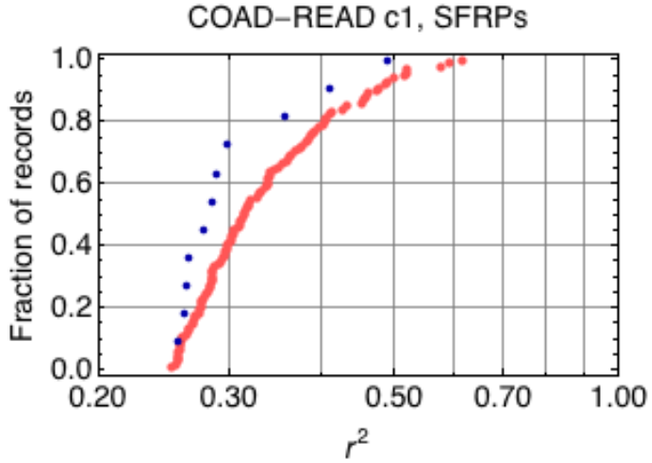


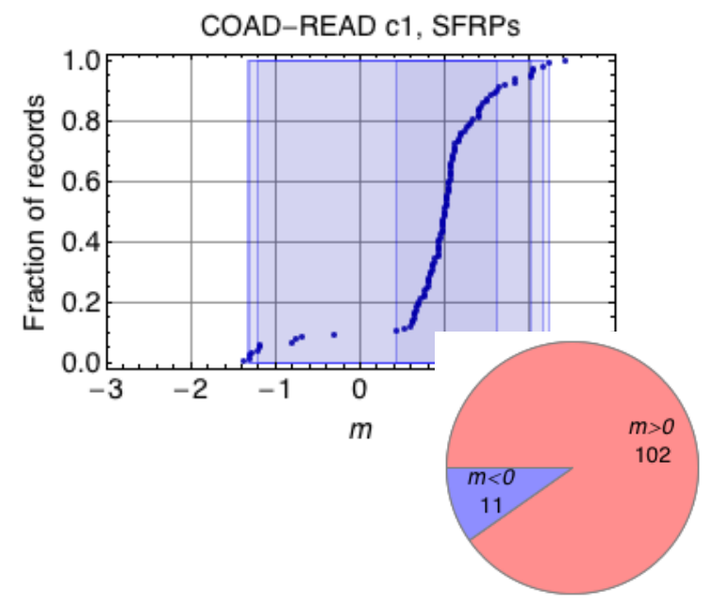
113 SRFP correlations, cluster 1



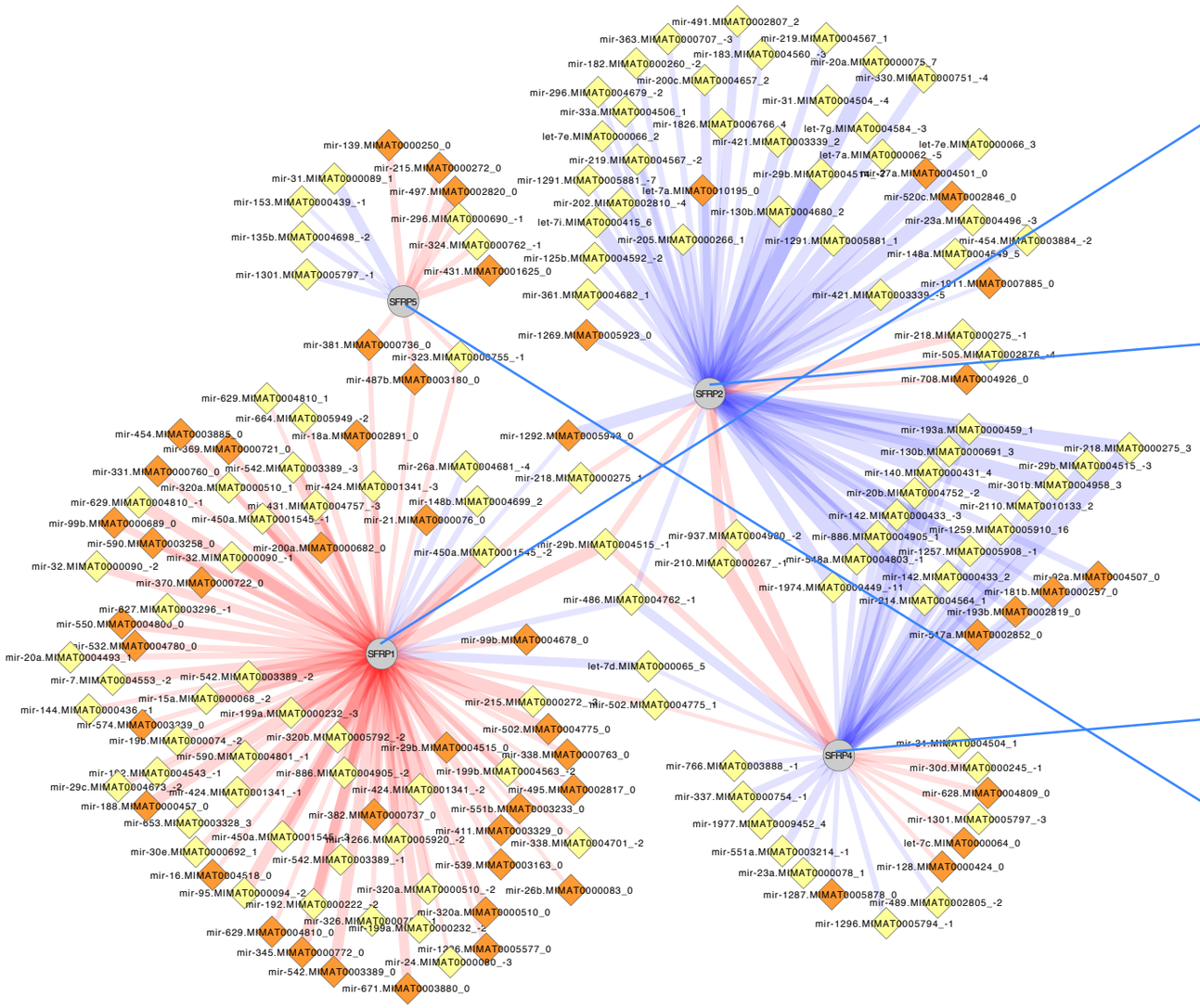


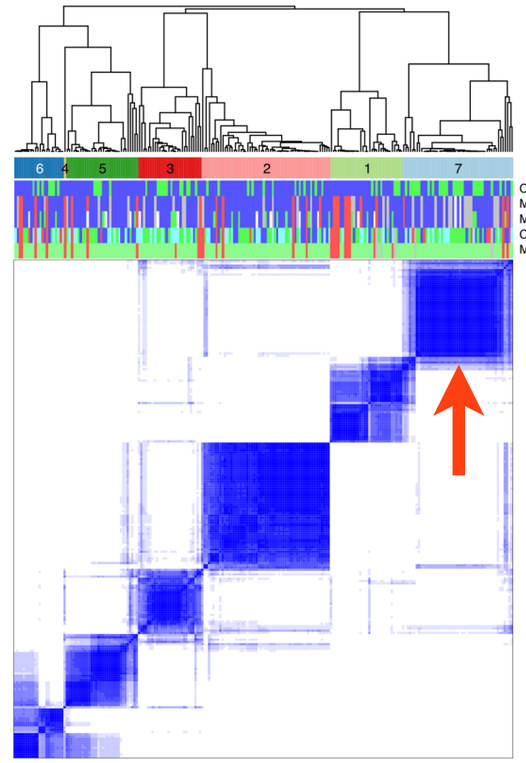


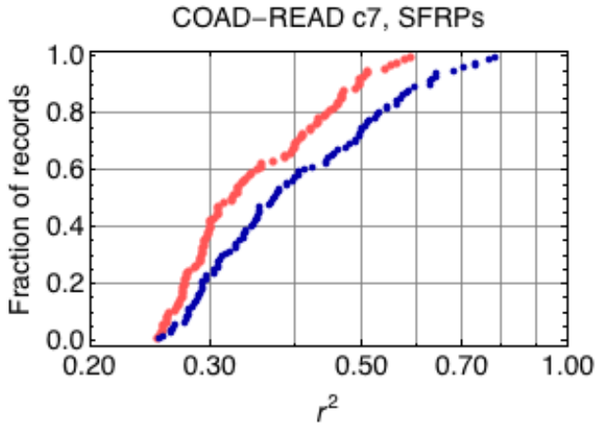


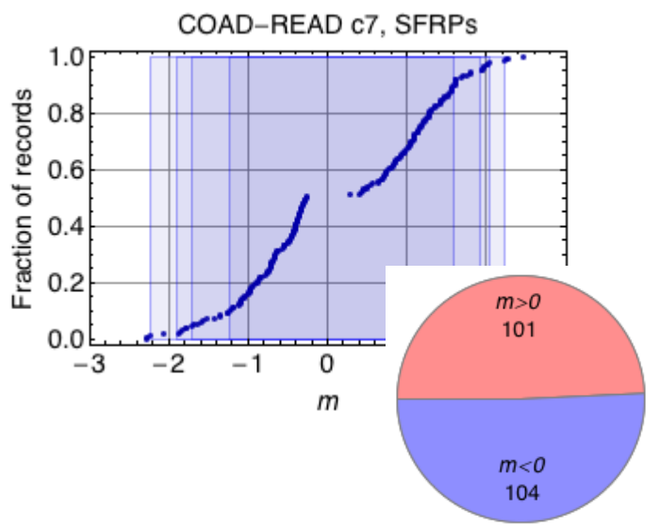


205 SRFP correlations, cluster 7







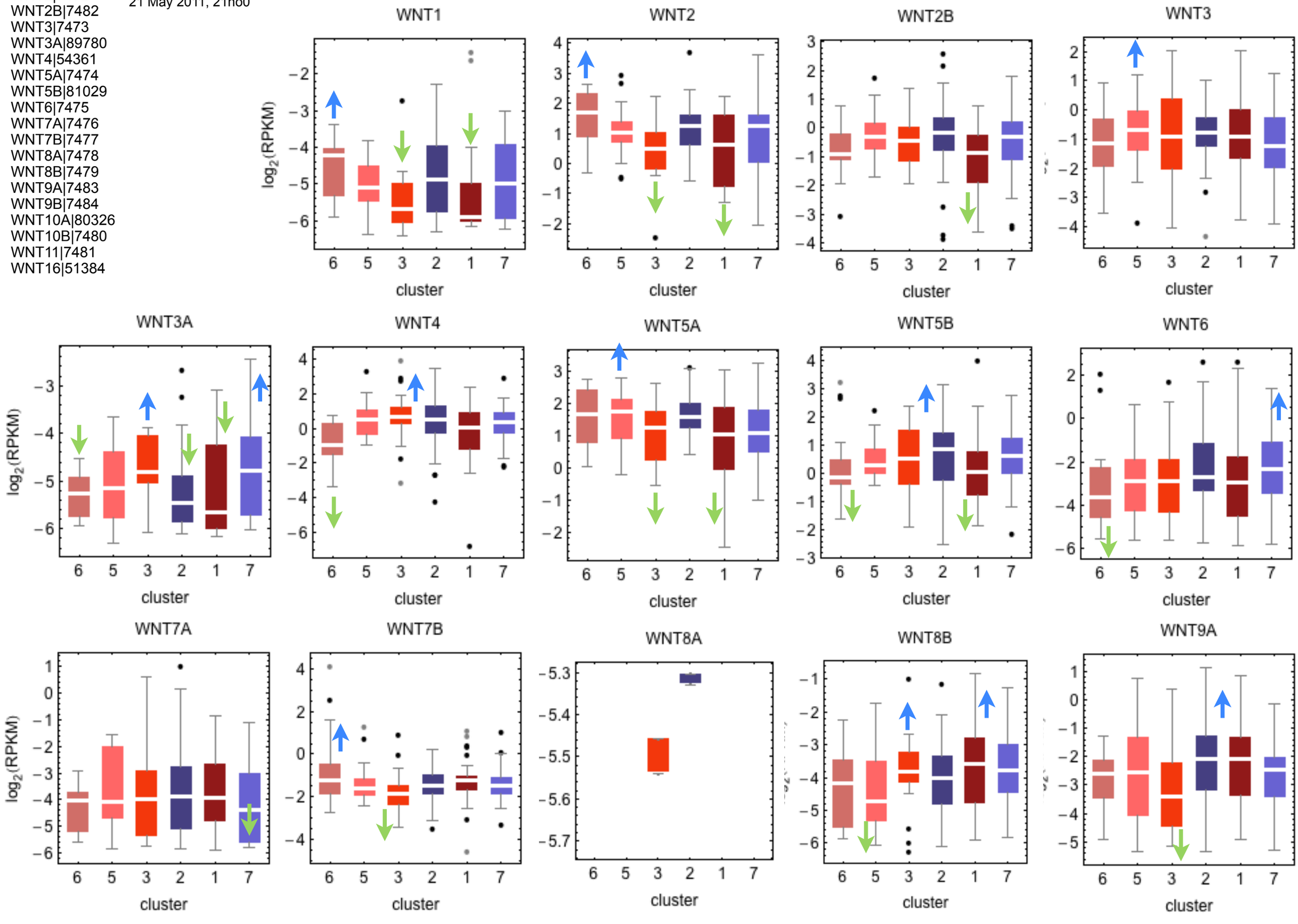


Correlations for WNTs

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

21 May 2011, 21ho0

WNTs: RPKM abundance across 7 clusters

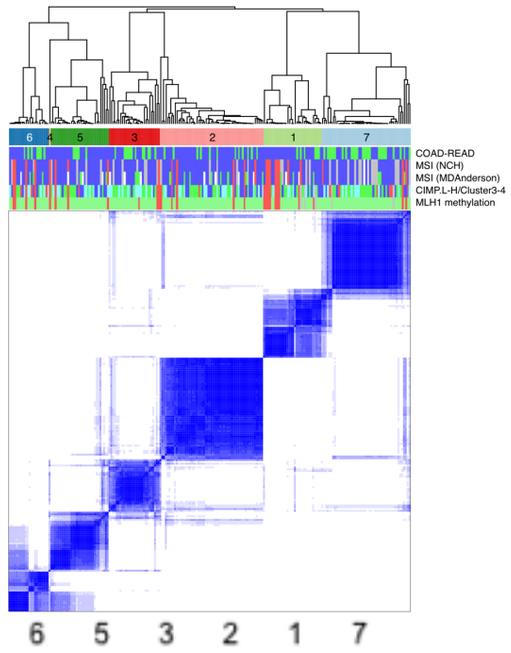


Insulin/IGF2, ASCL2? Insulin pathway, receptors?

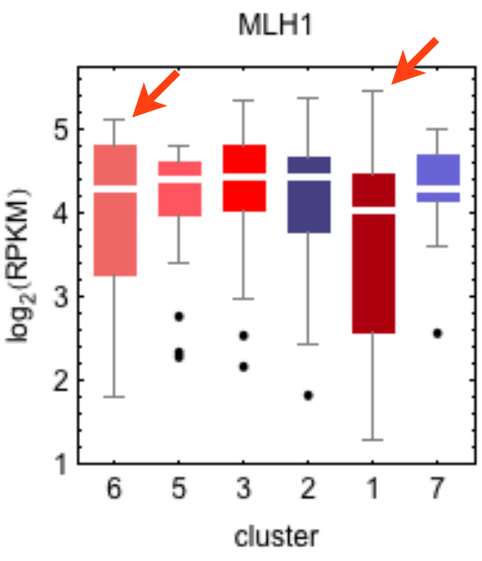
DNA methylation CIMP-H?
BRAF-positive? MSI-high CIMP tumors?
mismatch repair: MLH1,3; MSH2,3,4,5,6; PMS1,2
PARADIGM - HIF1A/ARNT; Delta-Notch

Microsatellite instability (MSI)

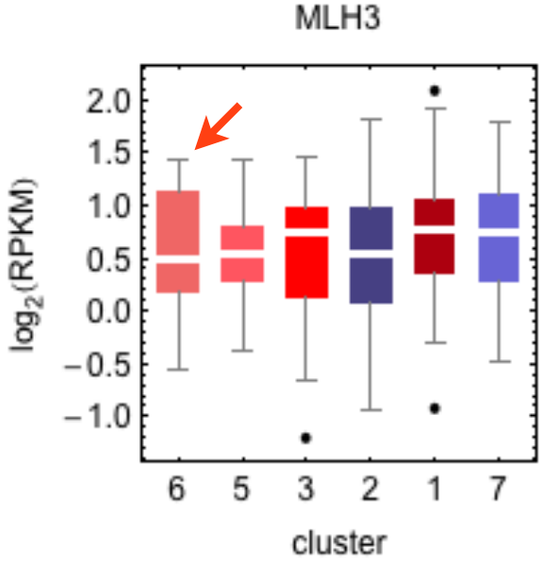
23 May 2011, 12h35

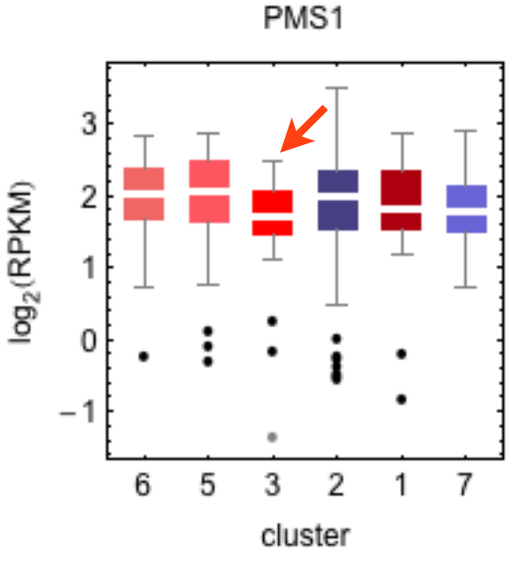


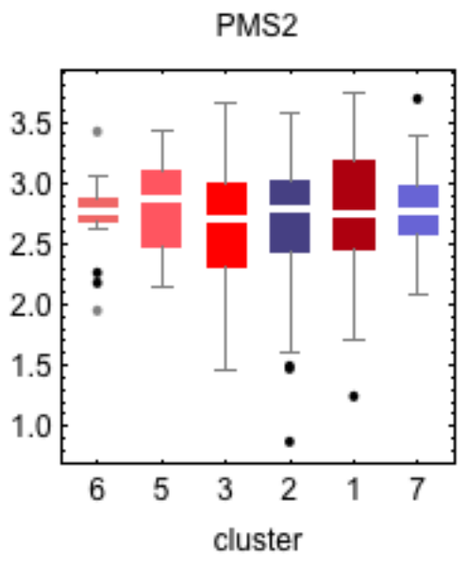
MLH1 MLH2 MLH3 MSH2 MSH3 MSH4 MSH5 MSH6 PMS1 PMS2

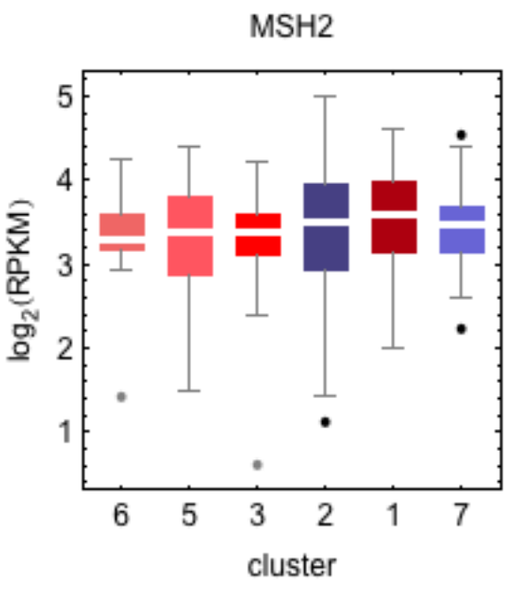


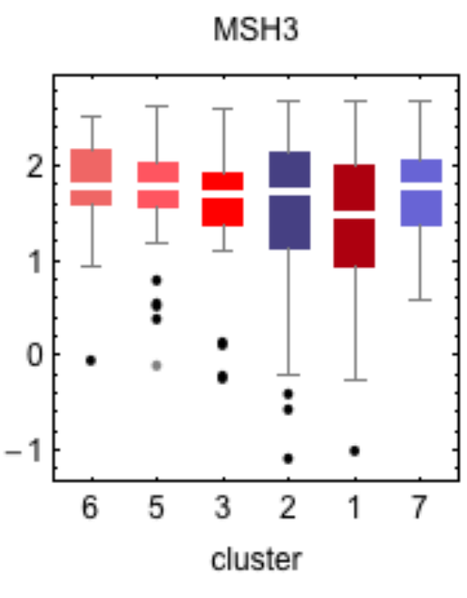
MLH2

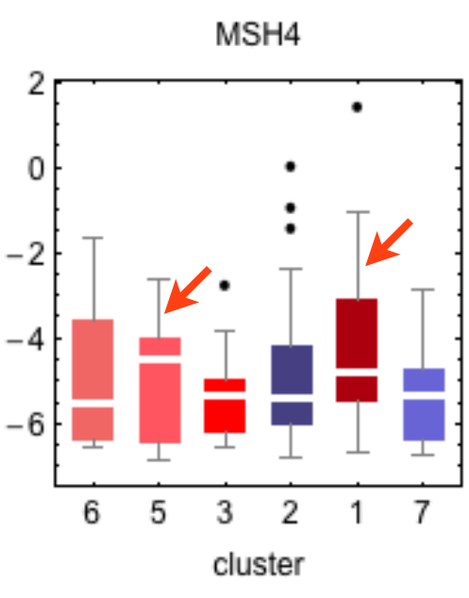


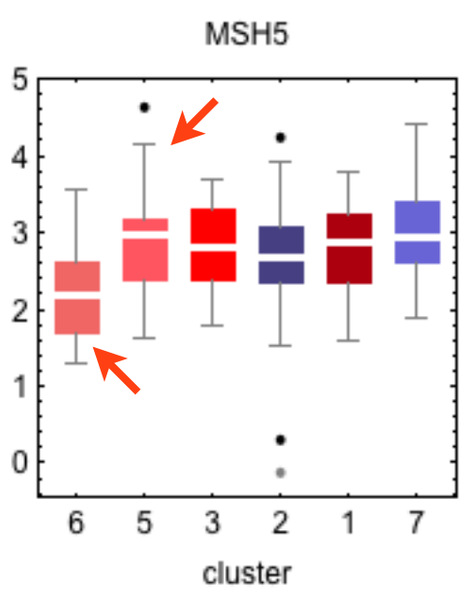


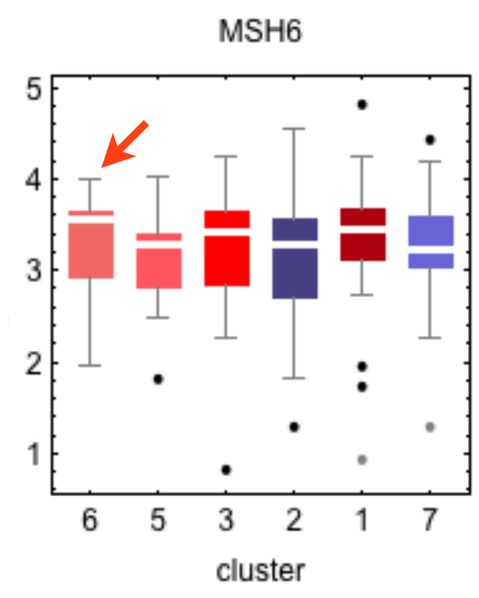




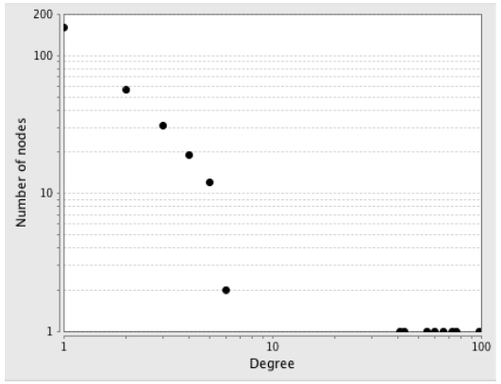


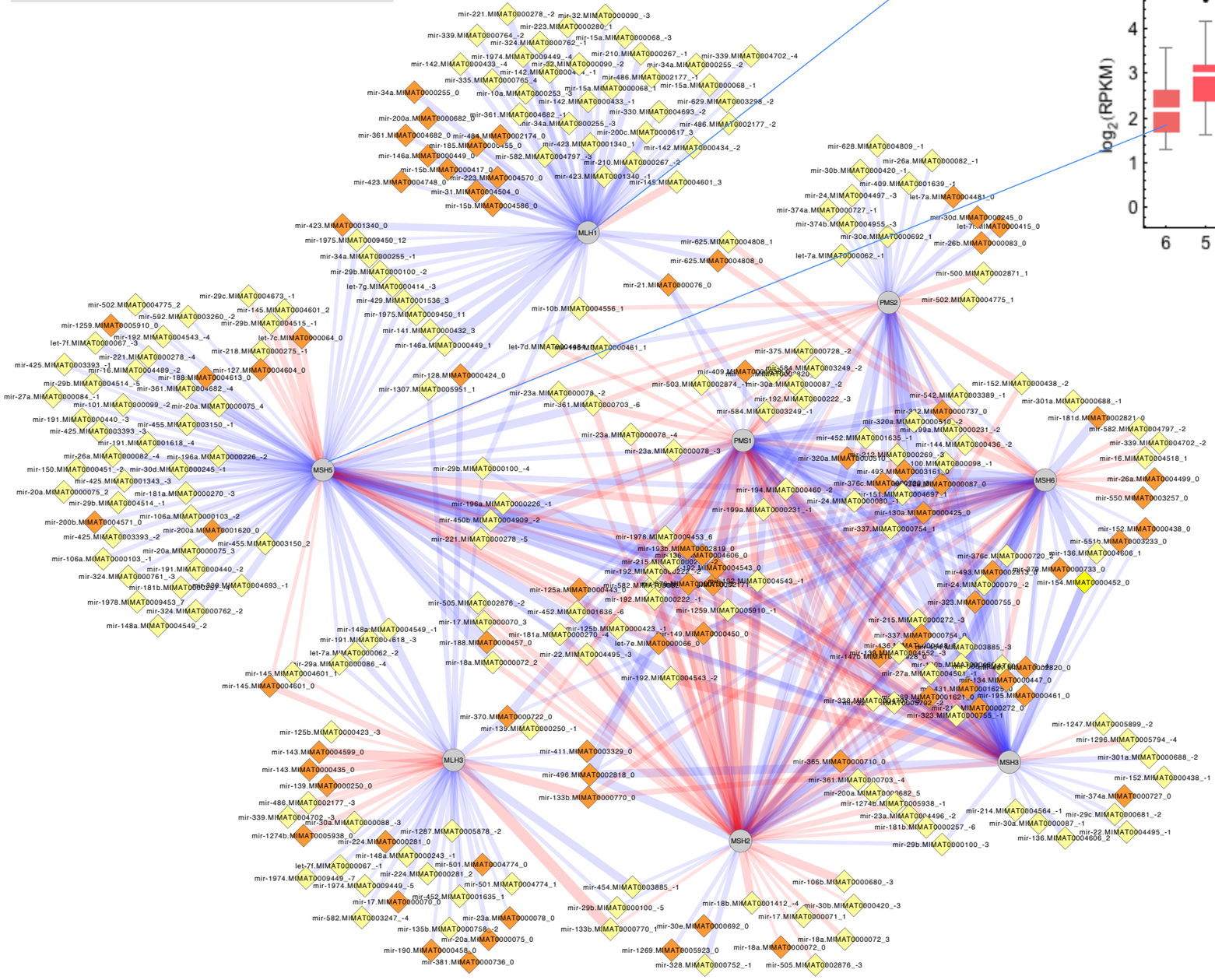


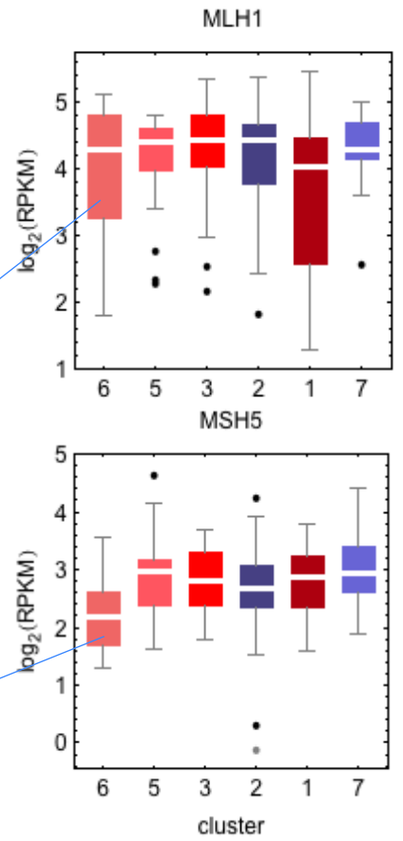


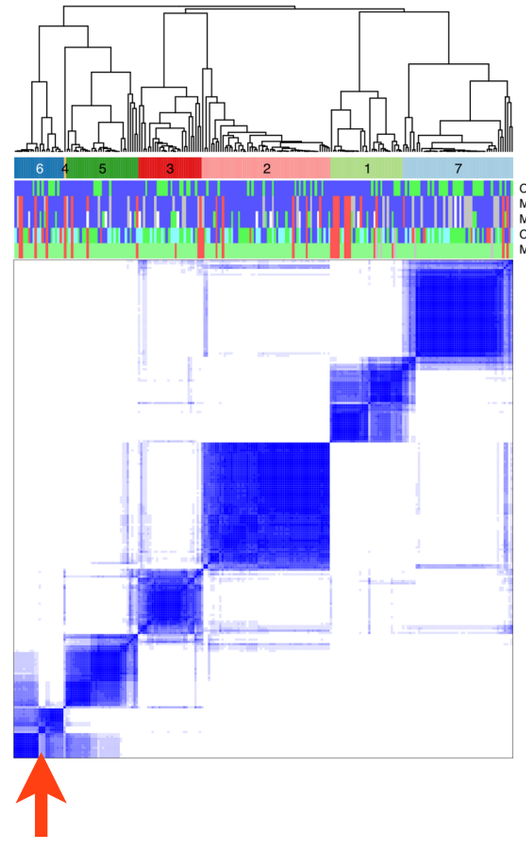


Microsatellite instability (MSI): c6



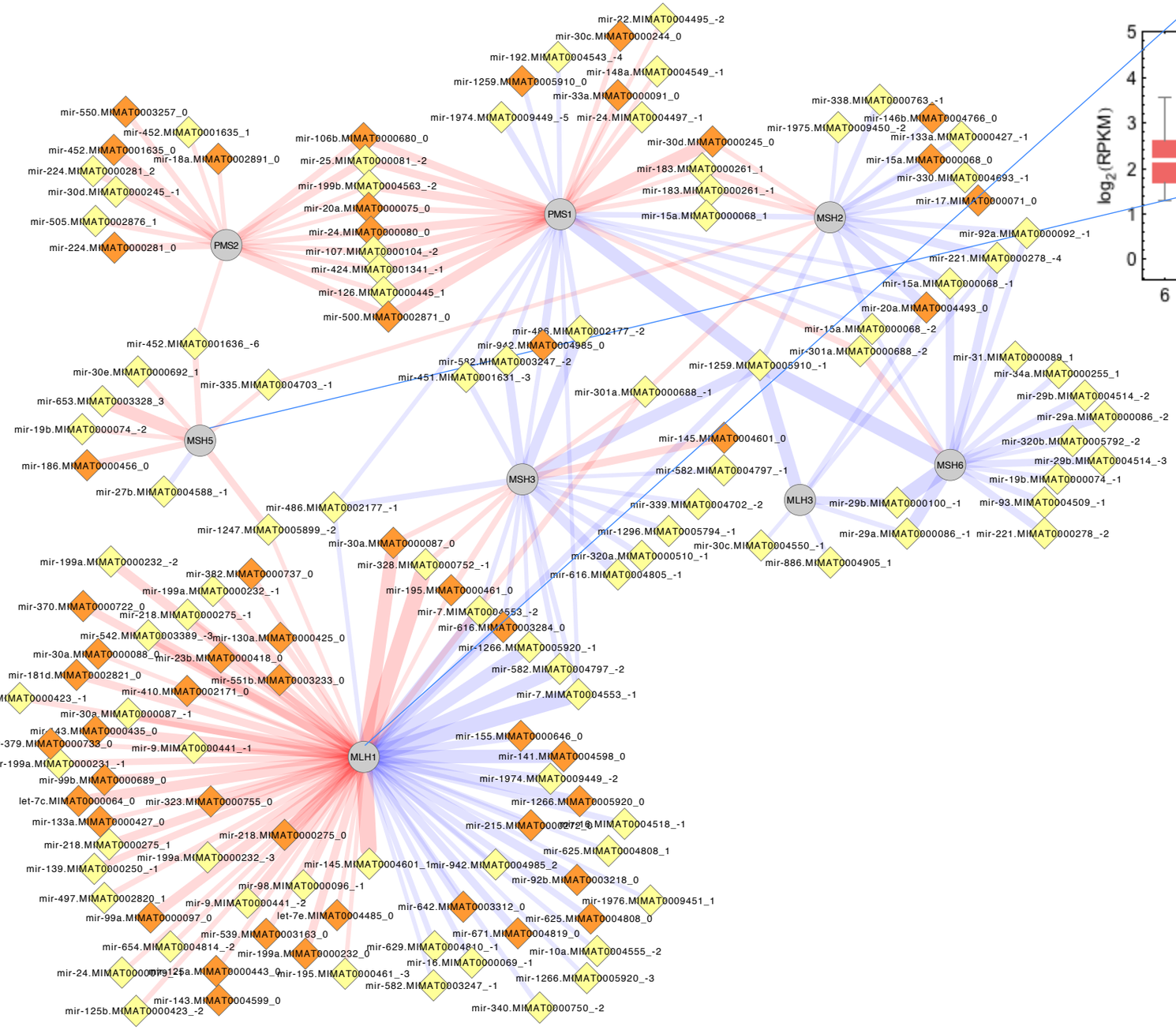


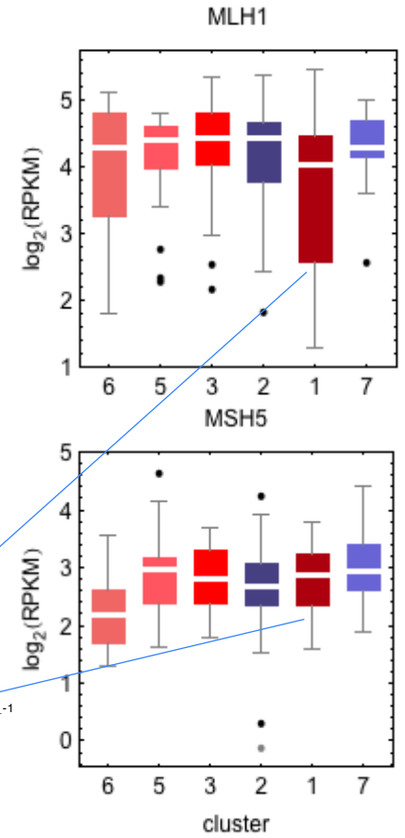


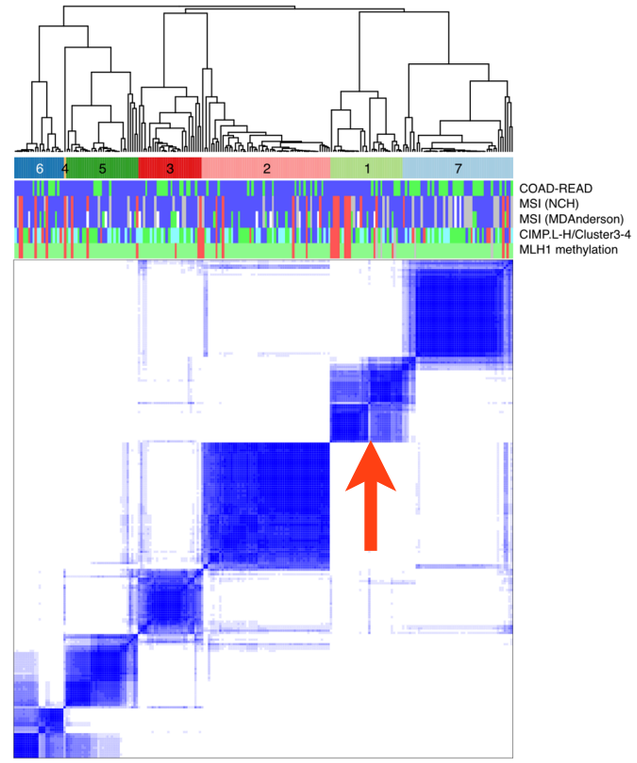


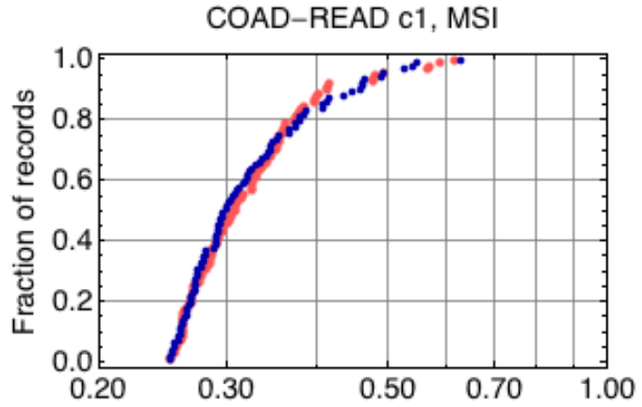
23 May 2011, 16h50

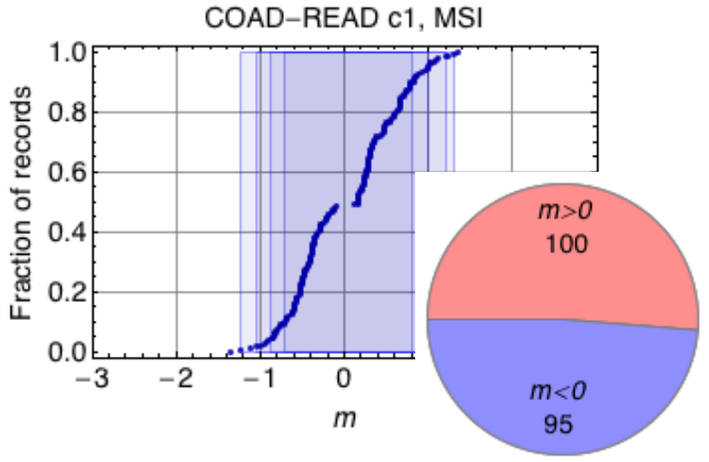
Microsatellite instability (MSI): c1, 195 correlations











23 May 2011, 16h50