

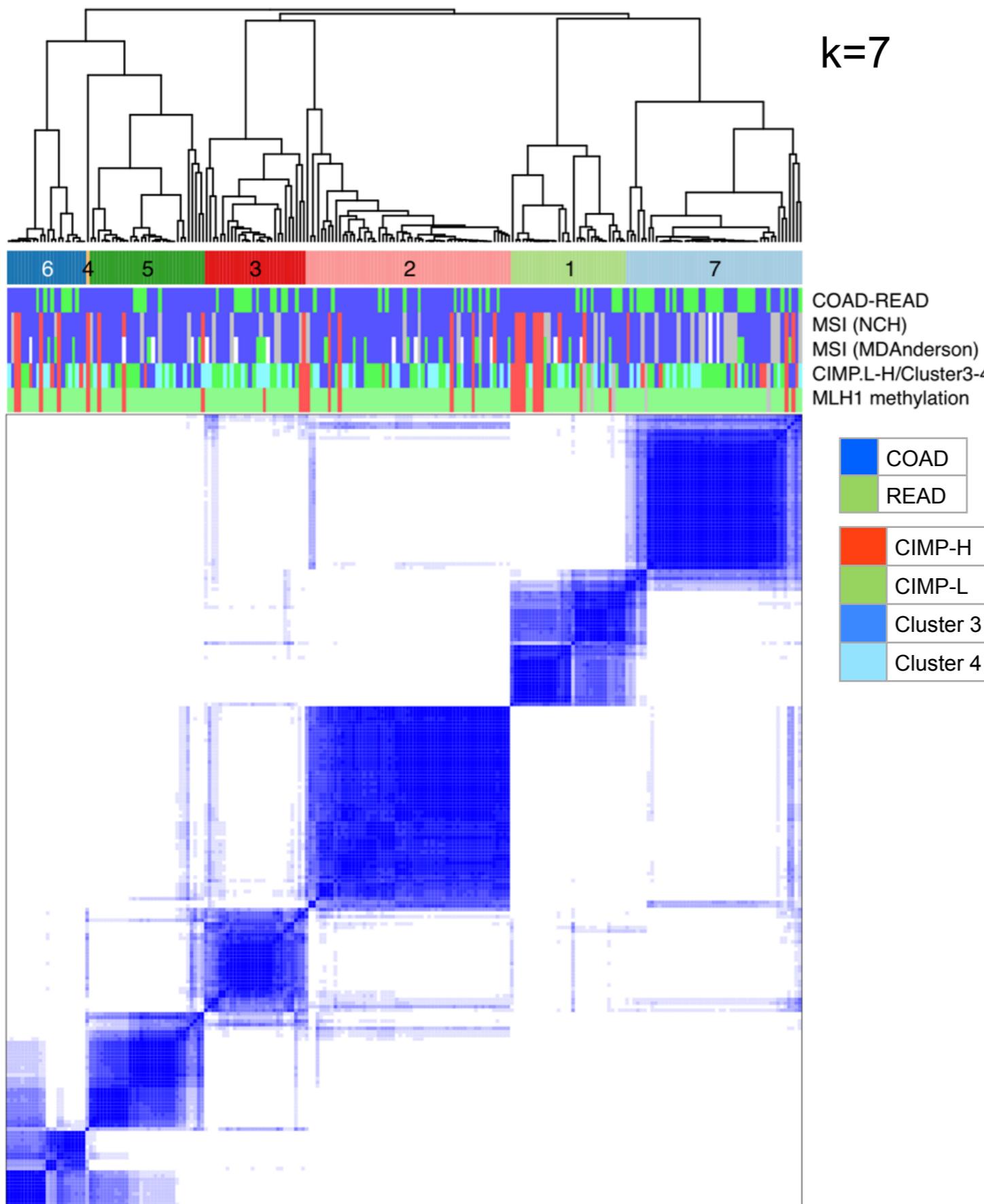
# COAD-READ, 221 samples: Correlation analysis for seven miRNA- seq-based consensus clusters

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



k=7

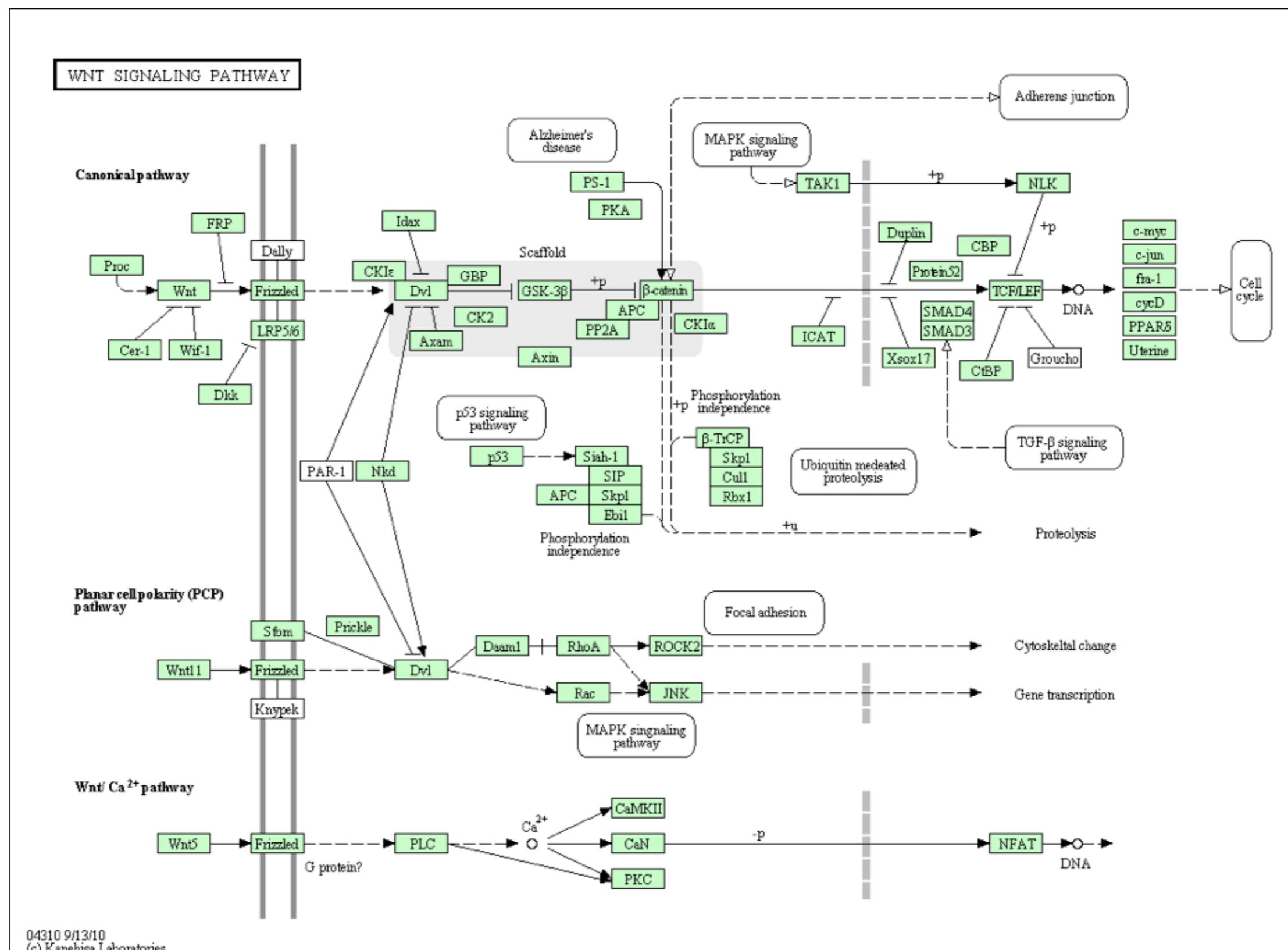
COAD-READ  
MSI (NCH)  
MSI (MDAnderson)  
CIMP.L-H/Cluster3-4  
MLH1 methylation

COAD  
READ  
CIMP-H  
CIMP-L  
Cluster 3  
Cluster 4

Number of thresholded miRNA-mRNA correlation records.

Clust	negative m records		positive m records	
	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

# KEGG WNT signaling pathway



14 May 2011, 20h50

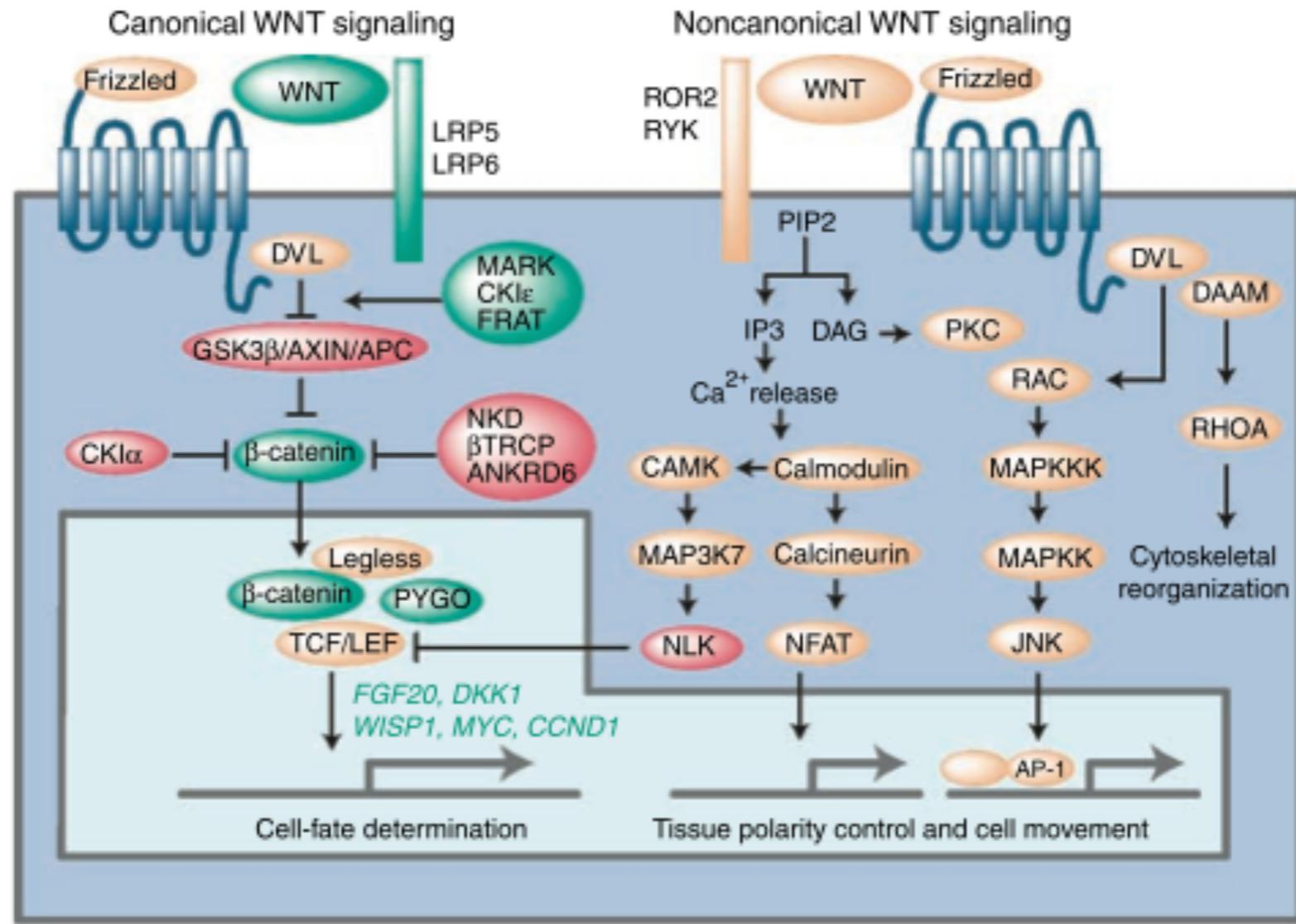


Fig.1. Landscape of WNT signaling cascades. WNT signals are transduced to the **canonical** pathway for cell fate determination, and to the **noncanonical** pathway for control of **cell movement and tissue polarity**. **Canonical** WNT signals are transduced through Frizzled family receptors and LRP5/LRP6 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 Ca<sup>2+</sup>-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, CKIα, NKD1, NKD2, hTRCP1, hTRCP2, ANKRD6, NLK, and PPARγ 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.

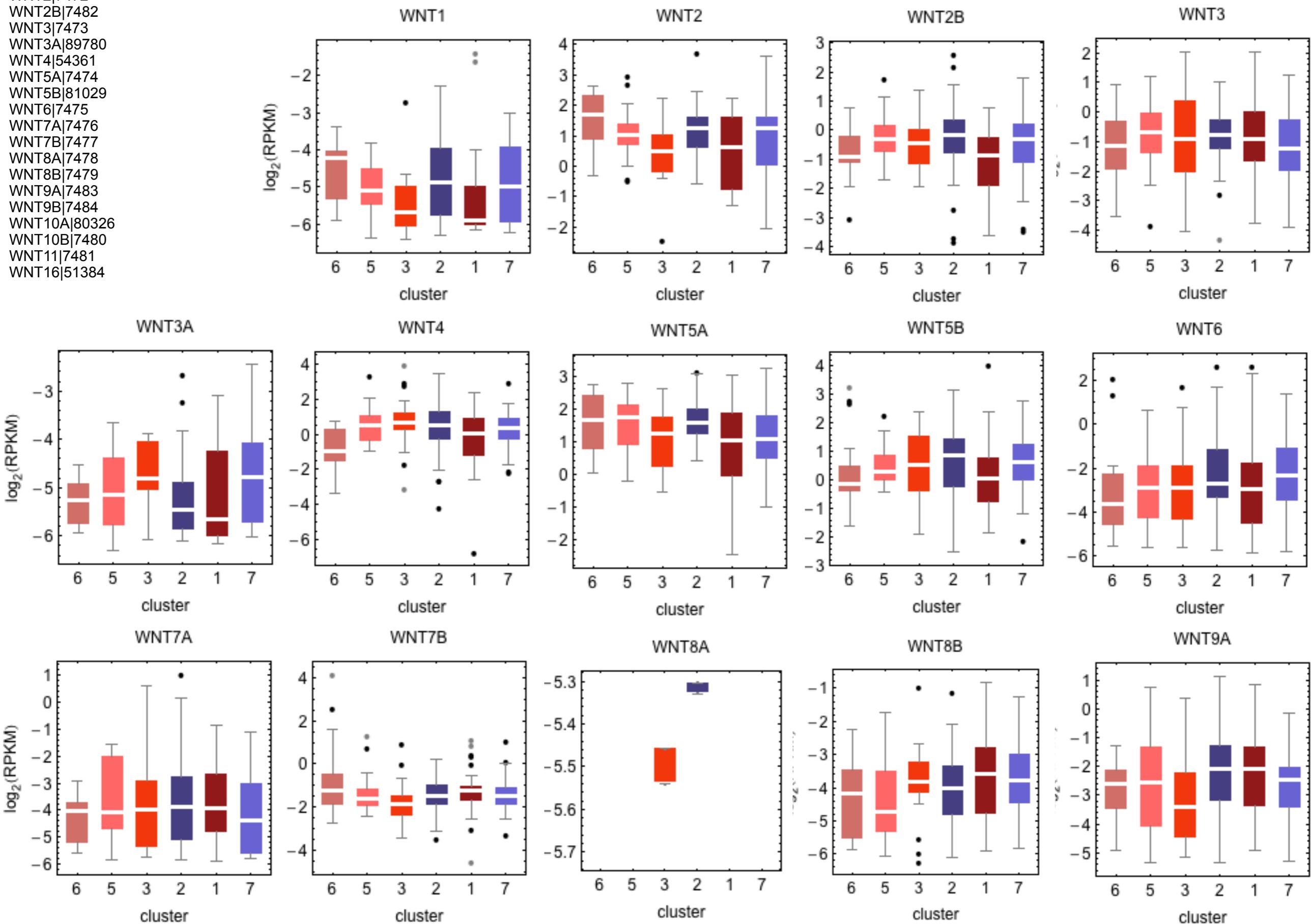
# RPKM for Wnt pathway genes, across seven miRNA-seq-based consensus clusters

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 Ca<sup>2+</sup>-dependent (NLK and NFAT) signaling cascades. Microtubule affinity ^ regulating kinase (MARK ; PAR-1) family kinases, CKI<sup>q</sup>, and FRAT are **positive** regulators of the **canonical** WNT pathway, whereas APC, AXIN1, AXIN2, CKI<sup>a</sup>, NKG1, NKG2, hTRCP1, hTRCP2, ANKRD6, NLK, and PPAR<sup>g</sup> 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

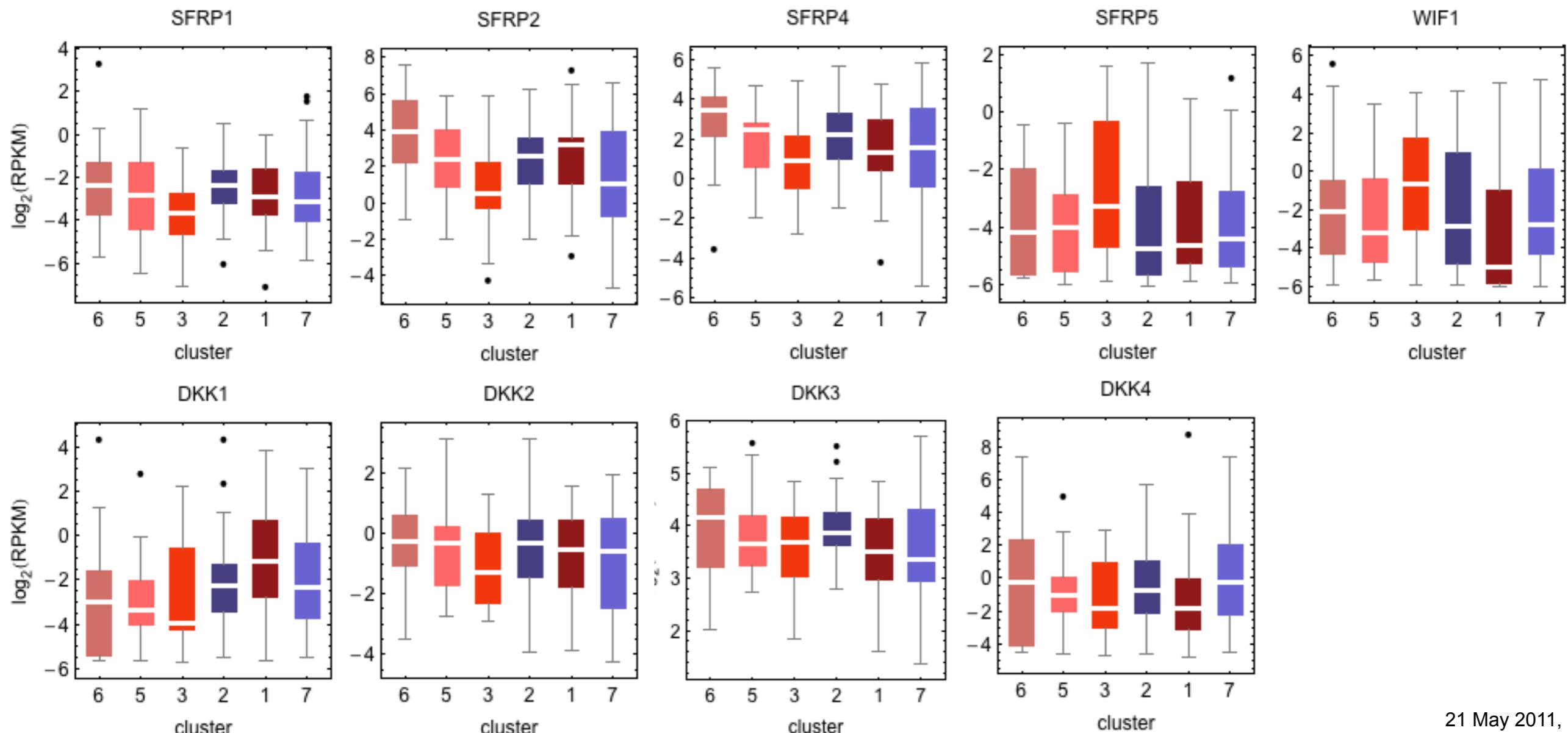
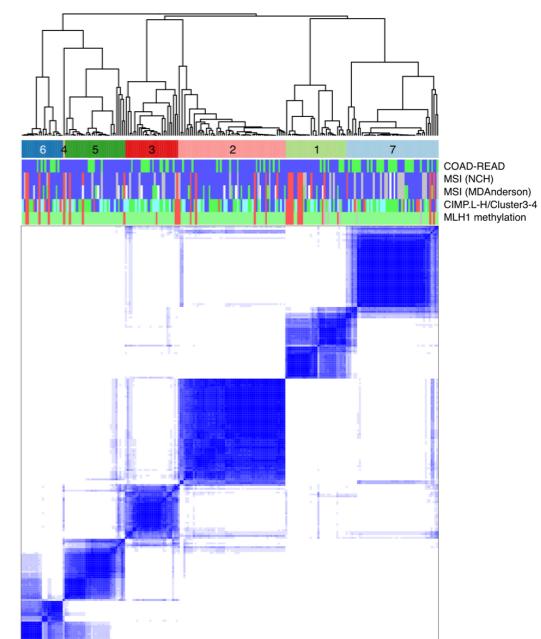
# WNTs: RPKM abundance across 7 clusters



21 May 2011, 12h30

# 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 Ca<sup>2+</sup>-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.



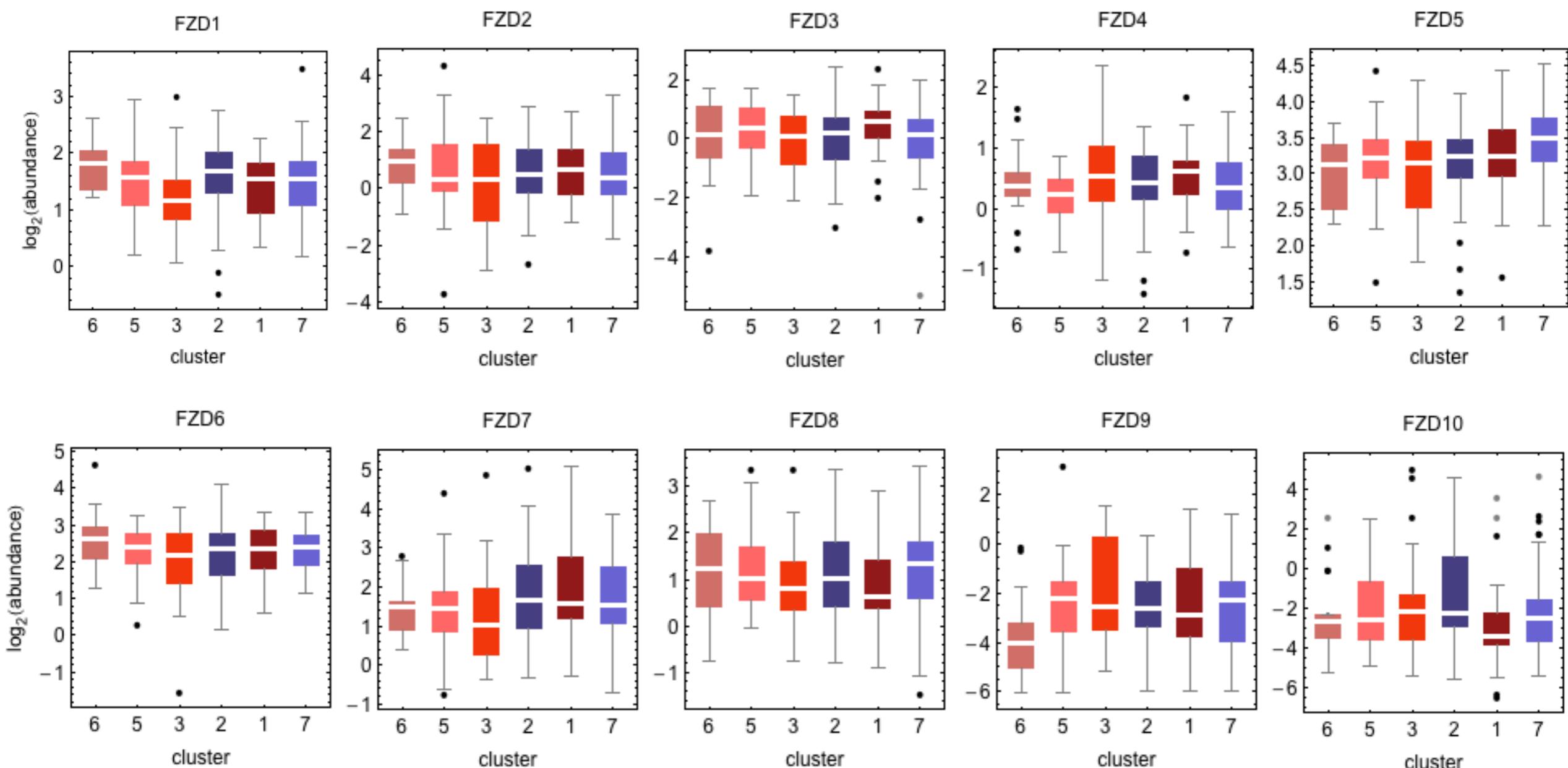
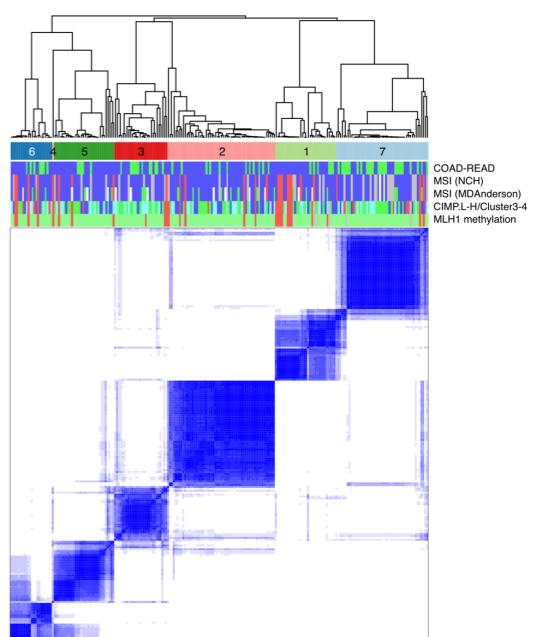
21 May 2011, 12h30

# 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

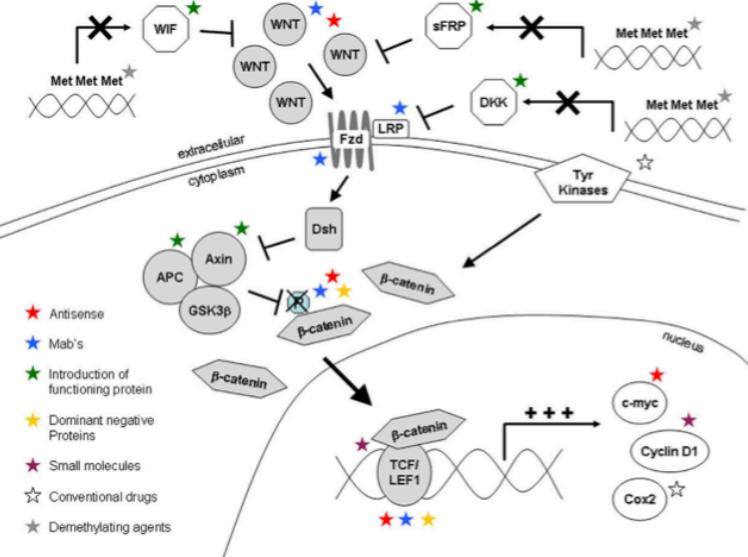
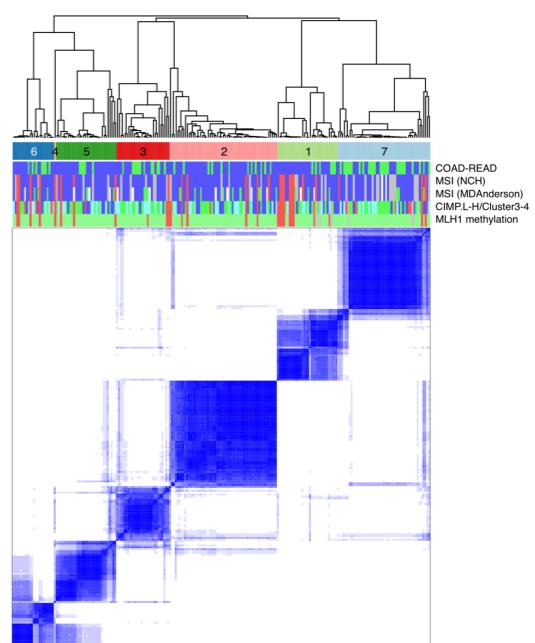
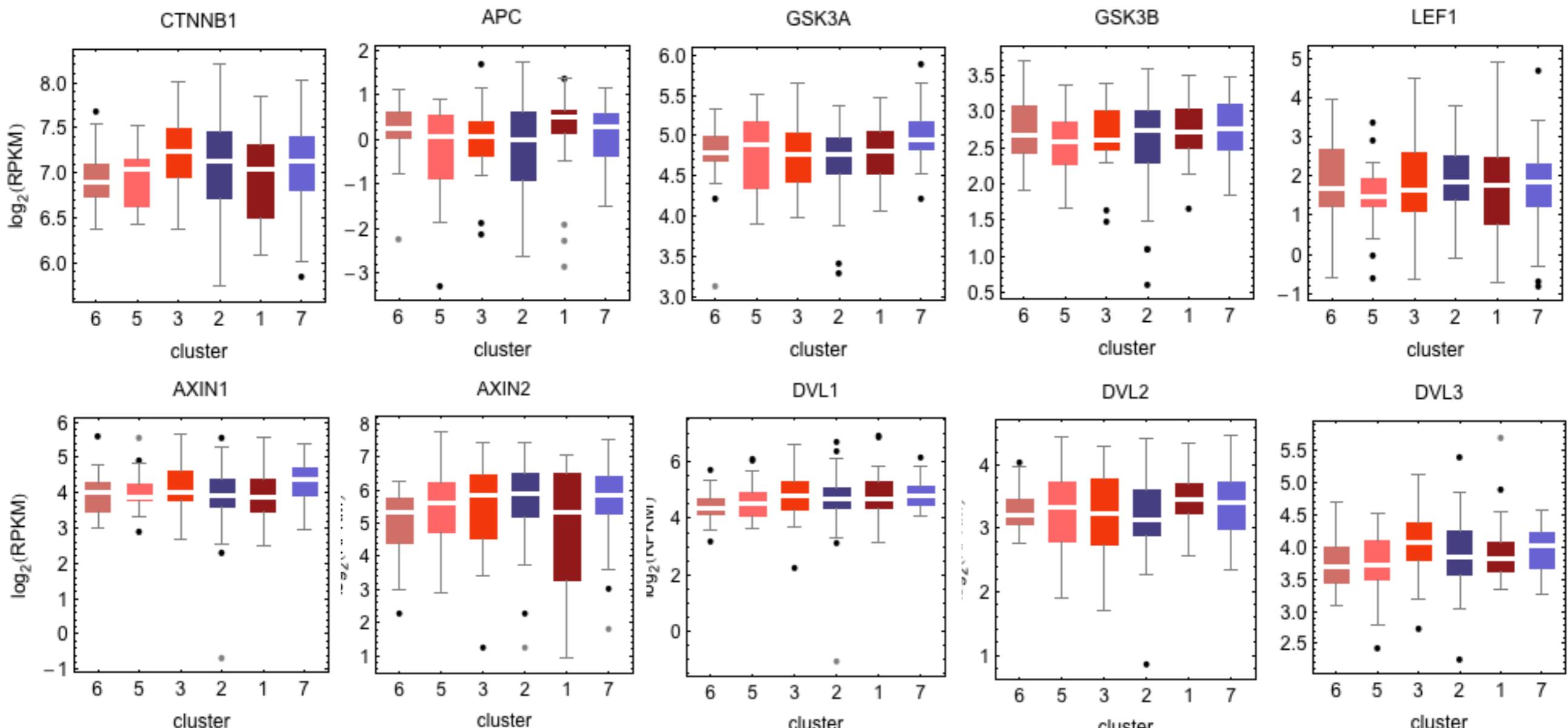


Fig. 1 – Active WNT/b-catenin signalling cascade: WNT proteins bind the receptor complex and activate DSH, which inhibits the APC/Axin/GSK3b complex, preventing phosphorylation of b-catenin, which accumulates in the cytoplasm, translocates into the nucleus and activates TCF/LEF1 family transcription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



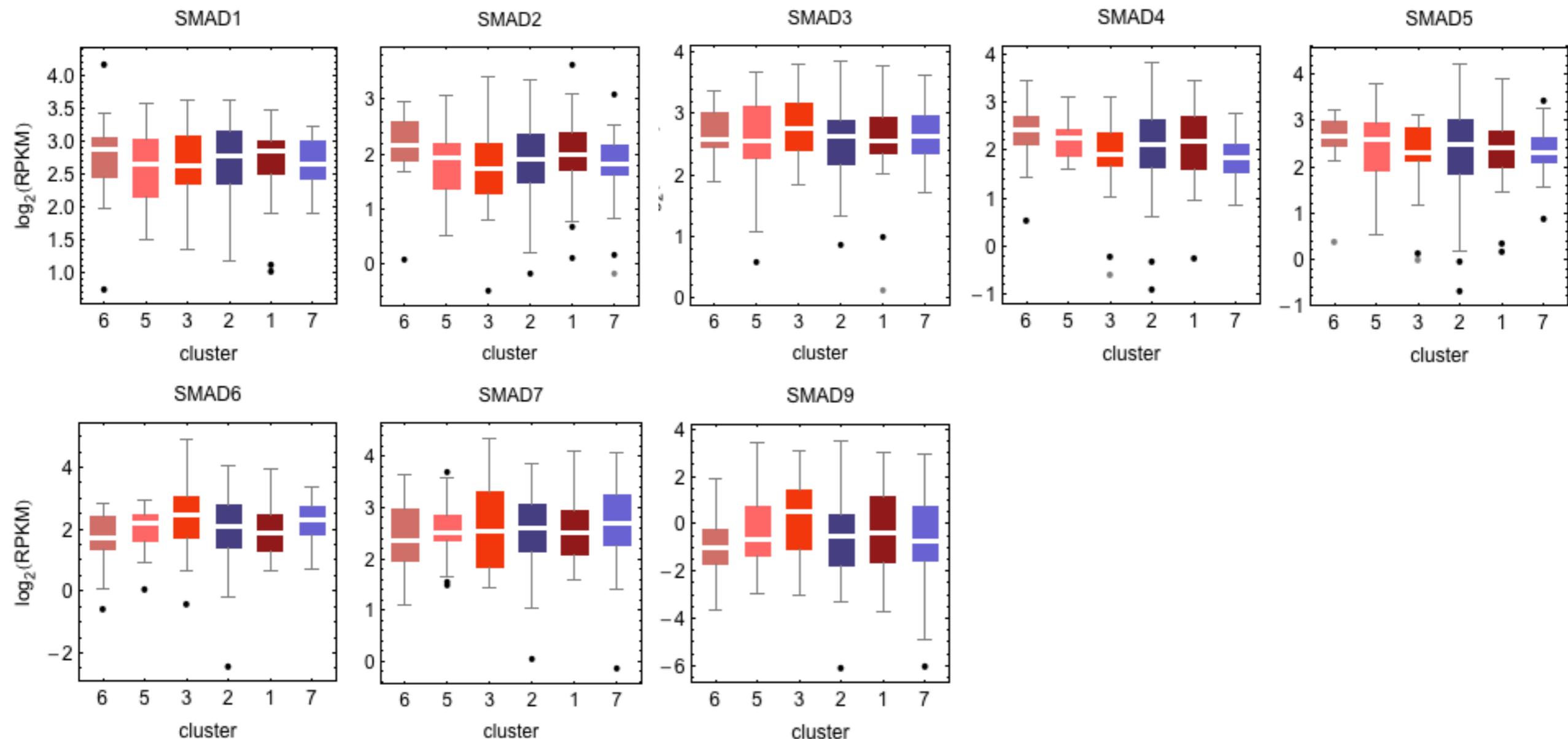
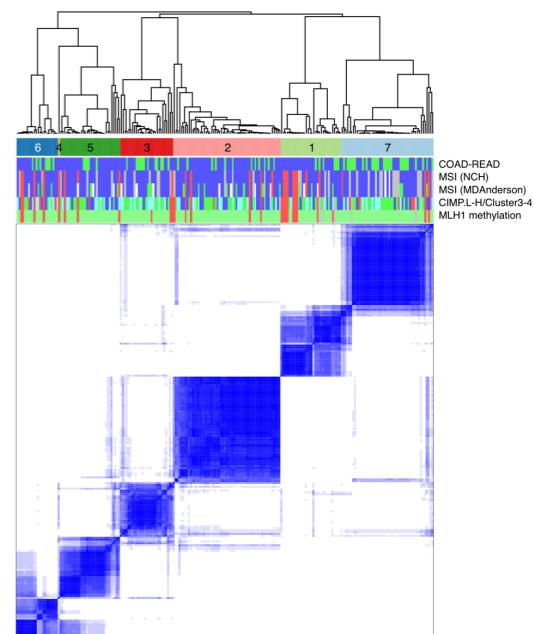
21 May 2011, 12h00



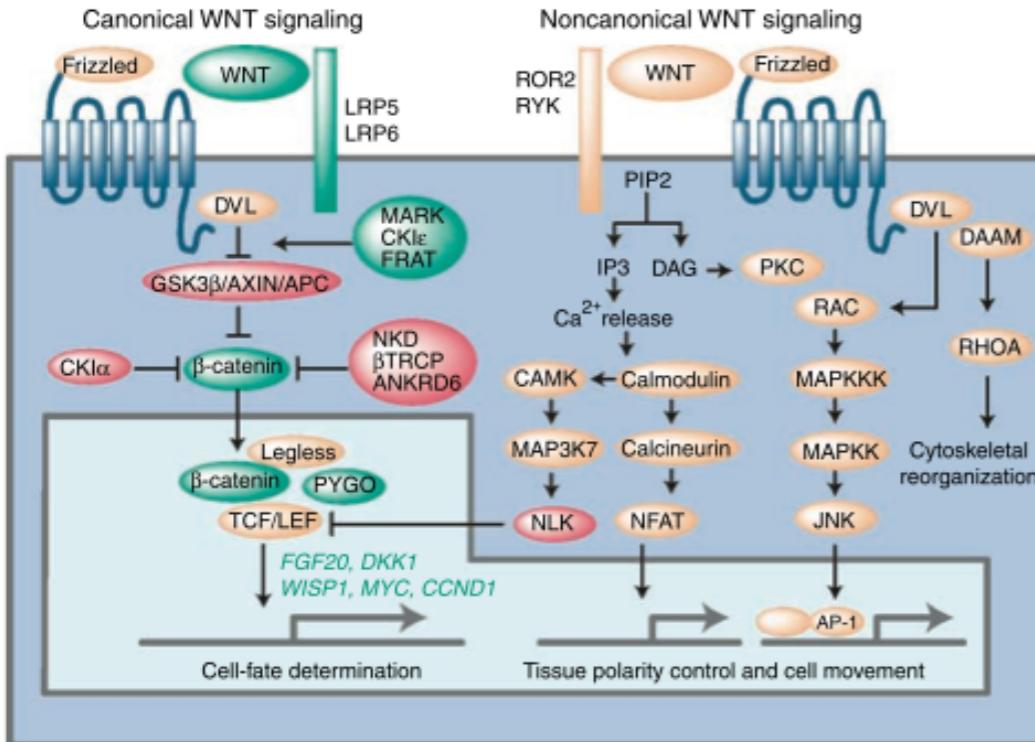
# SMADs: RPKM abundance across 7 clusters

```
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

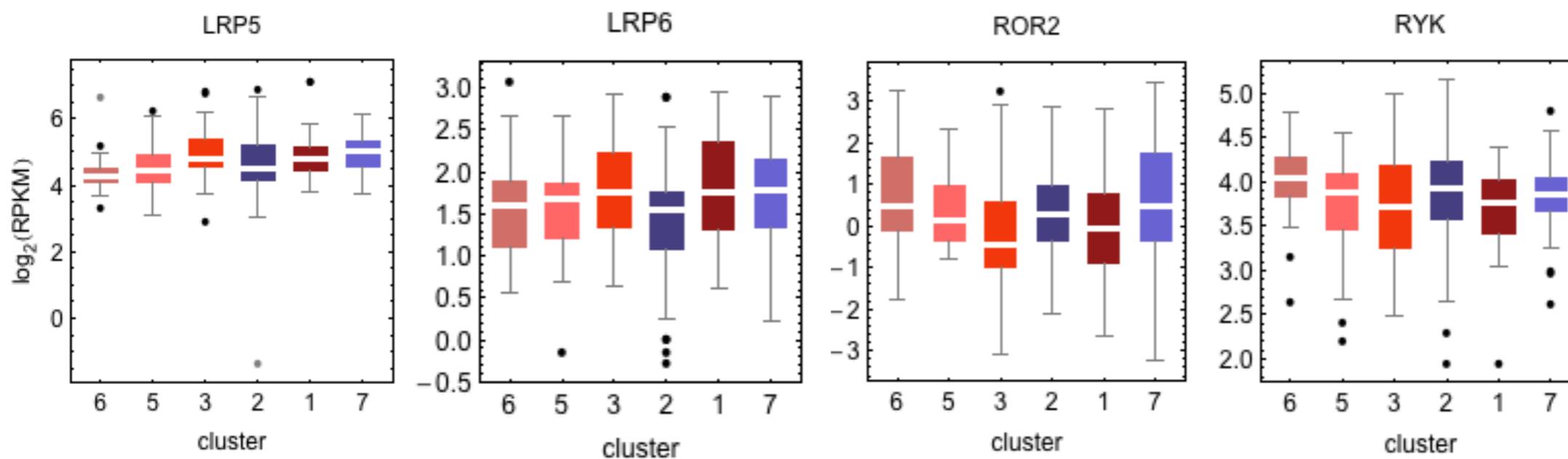
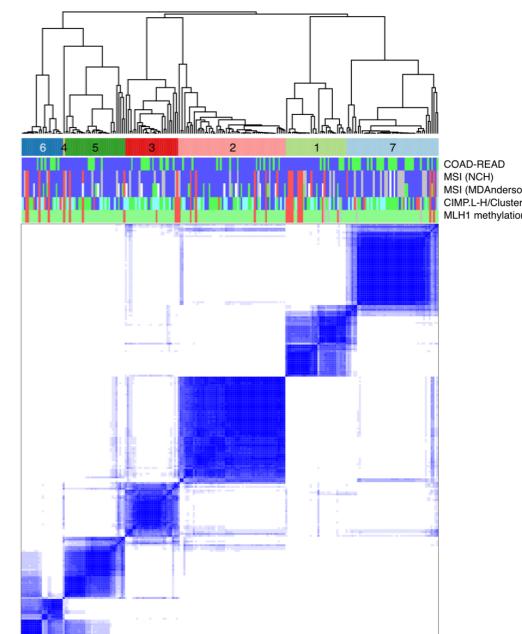


# LRP5,6, ROR2, RYK coreceptors: RPKM abundance across 7 clusters



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



# TCFs, NKDs: RPKM abundance across 7 clusters

```
grobertson:mRNA-seq grobertson$ grep "TCF" crc_244_gene_rpkm.txt | sort -t| -k1,1 -k2,2 -k3,3 -k4,4 -k5,5 -k6,6 -k7,7 -k8,8 -k9,9 -k10,10 -k11,11 -k12,12 -k13,13 -k14,14 -k15,15 -k16,16 -k17,17 -k18,18 -k19,19 -k20,20 -k21,21 -k22,22 -k23,23 -k24,24 -k25,25 -k26,26 -k27,27 -k28,28 -k29,29 -k30,30 -k31,31 -k32,32 -k33,33 -k34,34 -k35,35 -k36,36 -k37,37 -k38,38 -k39,39 -k40,40 -k41,41 -k42,42 -k43,43 -k44,44 -k45,45 -k46,46 -k47,47 -k48,48 -k49,49 -k50,50 -k51,51 -k52,52 -k53,53 -k54,54 -k55,55 -k56,56 -k57,57 -k58,58 -k59,59 -k60,60 -k61,61 -k62,62 -k63,63 -k64,64 -k65,65 -k66,66 -k67,67 -k68,68 -k69,69 -k70,70 -k71,71 -k72,72 -k73,73 -k74,74 -k75,75 -k76,76 -k77,77 -k78,78 -k79,79 -k80,80 -k81,81 -k82,82 -k83,83 -k84,84 -k85,85 -k86,86 -k87,87 -k88,88 -k89,89 -k90,90 -k91,91 -k92,92 -k93,93 -k94,94 -k95,95 -k96,96 -k97,97 -k98,98 -k99,99 -k100,100 -k101,101 -k102,102 -k103,103 -k104,104 -k105,105 -k106,106 -k107,107 -k108,108 -k109,109 -k110,110 -k111,111 -k112,112 -k113,113 -k114,114 -k115,115 -k116,116 -k117,117 -k118,118 -k119,119 -k120,120 -k121,121 -k122,122 -k123,123 -k124,124 -k125,125 -k126,126 -k127,127 -k128,128 -k129,129 -k130,130 -k131,131 -k132,132 -k133,133 -k134,134 -k135,135 -k136,136 -k137,137 -k138,138 -k139,139 -k140,140 -k141,141 -k142,142 -k143,143 -k144,144 -k145,145 -k146,146 -k147,147 -k148,148 -k149,149 -k150,150 -k151,151 -k152,152 -k153,153 -k154,154 -k155,155 -k156,156 -k157,157 -k158,158 -k159,159 -k160,160 -k161,161 -k162,162 -k163,163 -k164,164 -k165,165 -k166,166 -k167,167 -k168,168 -k169,169 -k170,170 -k171,171 -k172,172 -k173,173 -k174,174 -k175,175 -k176,176 -k177,177 -k178,178 -k179,179 -k180,180 -k181,181 -k182,182 -k183,183 -k184,184 -k185,185 -k186,186 -k187,187 -k188,188 -k189,189 -k190,190 -k191,191 -k192,192 -k193,193 -k194,194 -k195,195 -k196,196 -k197,197 -k198,198 -k199,199 -k200,200 -k201,201 -k202,202 -k203,203 -k204,204 -k205,205 -k206,206 -k207,207 -k208,208 -k209,209 -k210,210 -k211,211 -k212,212 -k213,213 -k214,214 -k215,215 -k216,216 -k217,217 -k218,218 -k219,219 -k220,220 -k221,221 -k222,222 -k223,223 -k224,224 -k225,225 -k226,226 -k227,227 -k228,228 -k229,229 -k230,230 -k231,231 -k232,232 -k233,233 -k234,234 -k235,235 -k236,236 -k237,237 -k238,238 -k239,239 -k240,240 -k241,241 -k242,242 -k243,243 -k244,244
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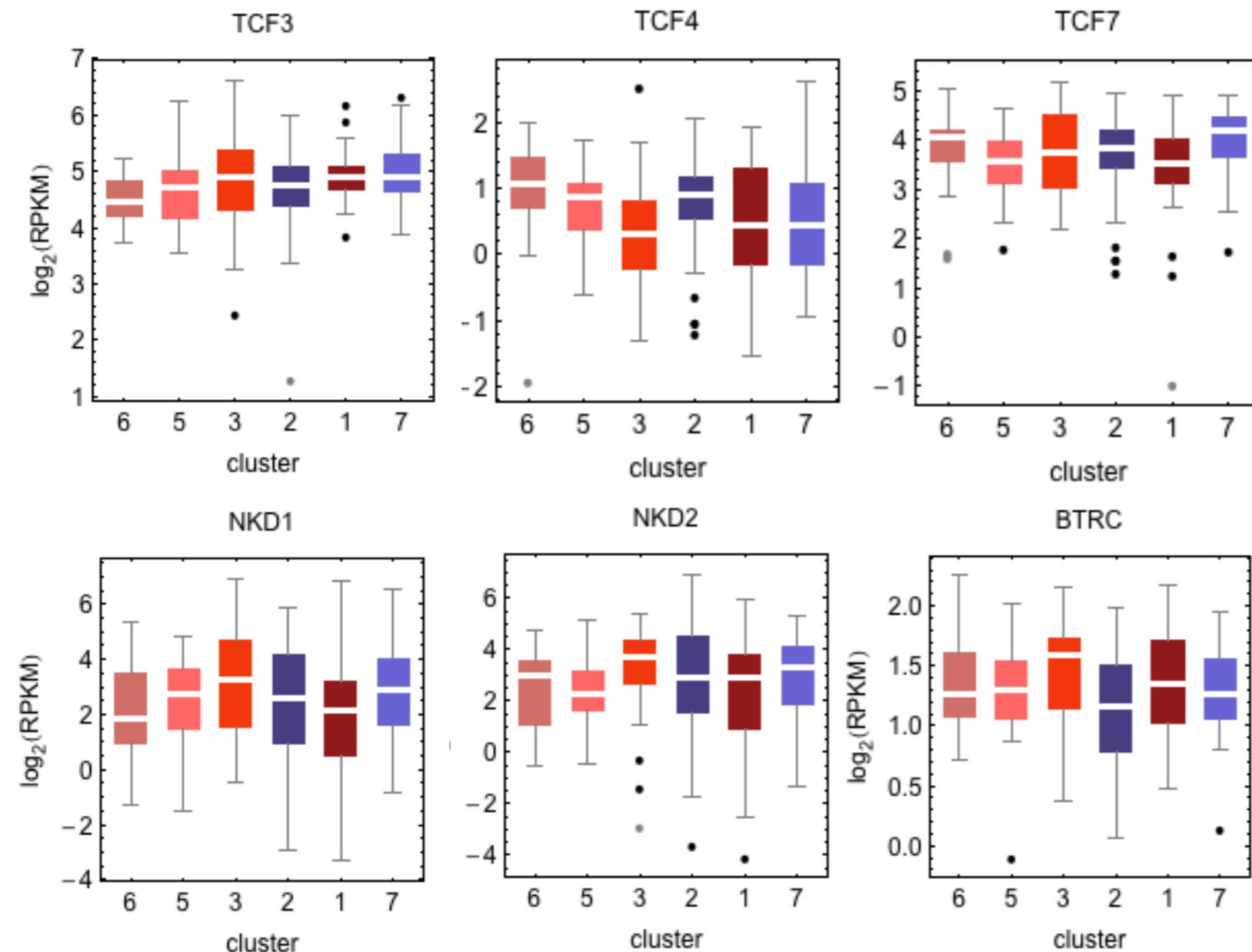
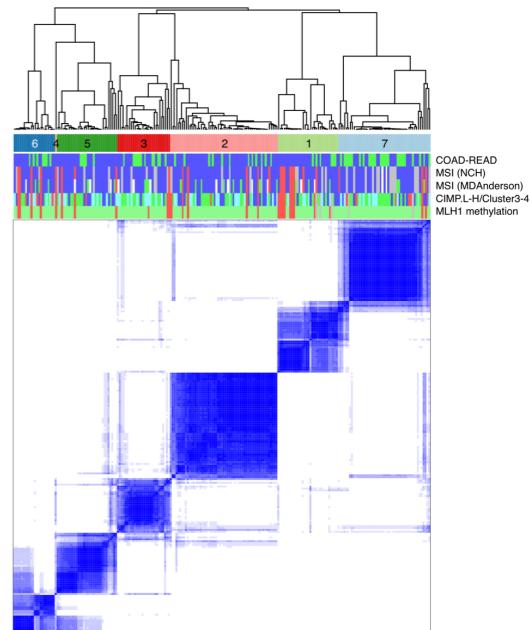
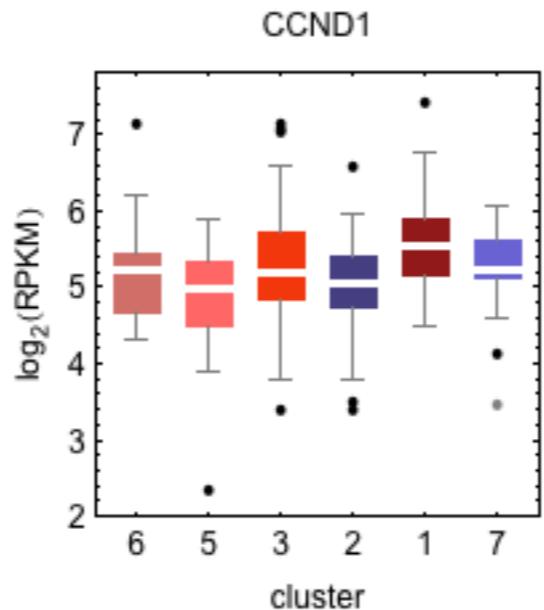


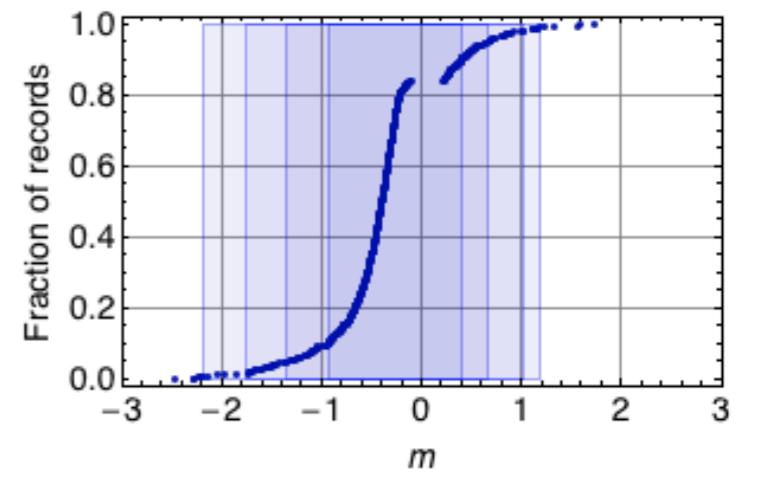
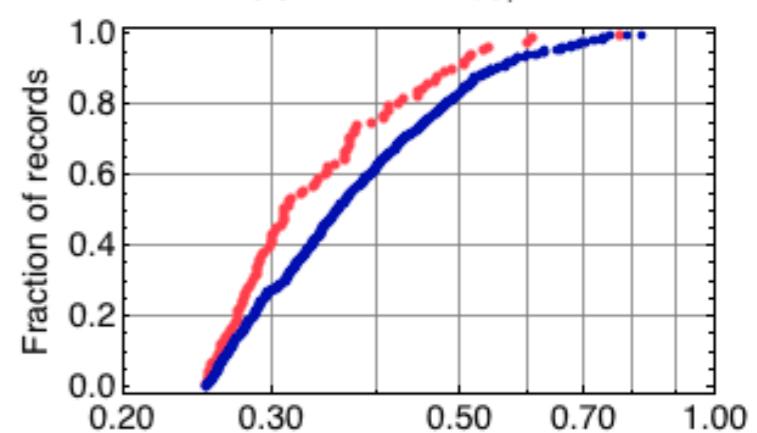
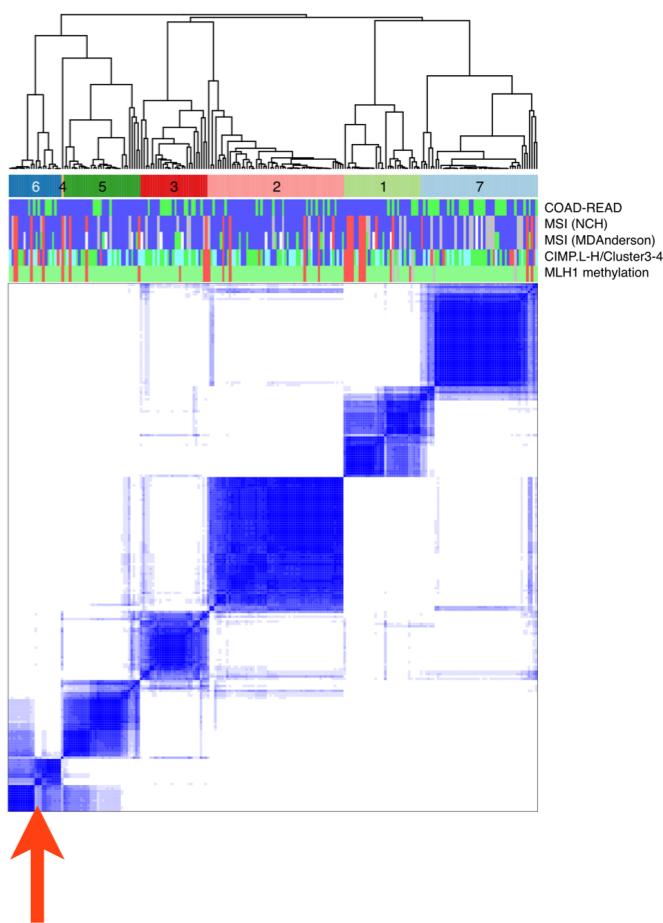
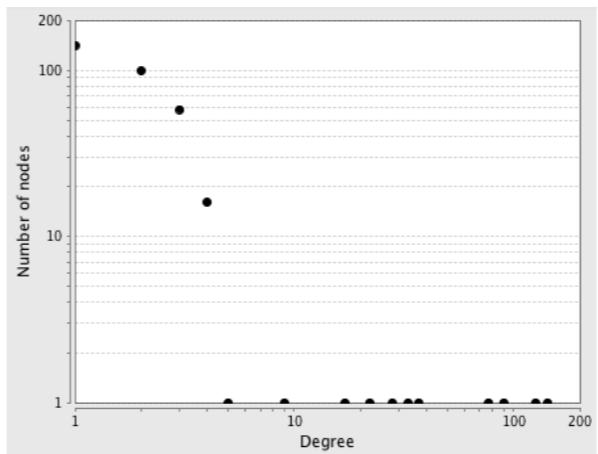
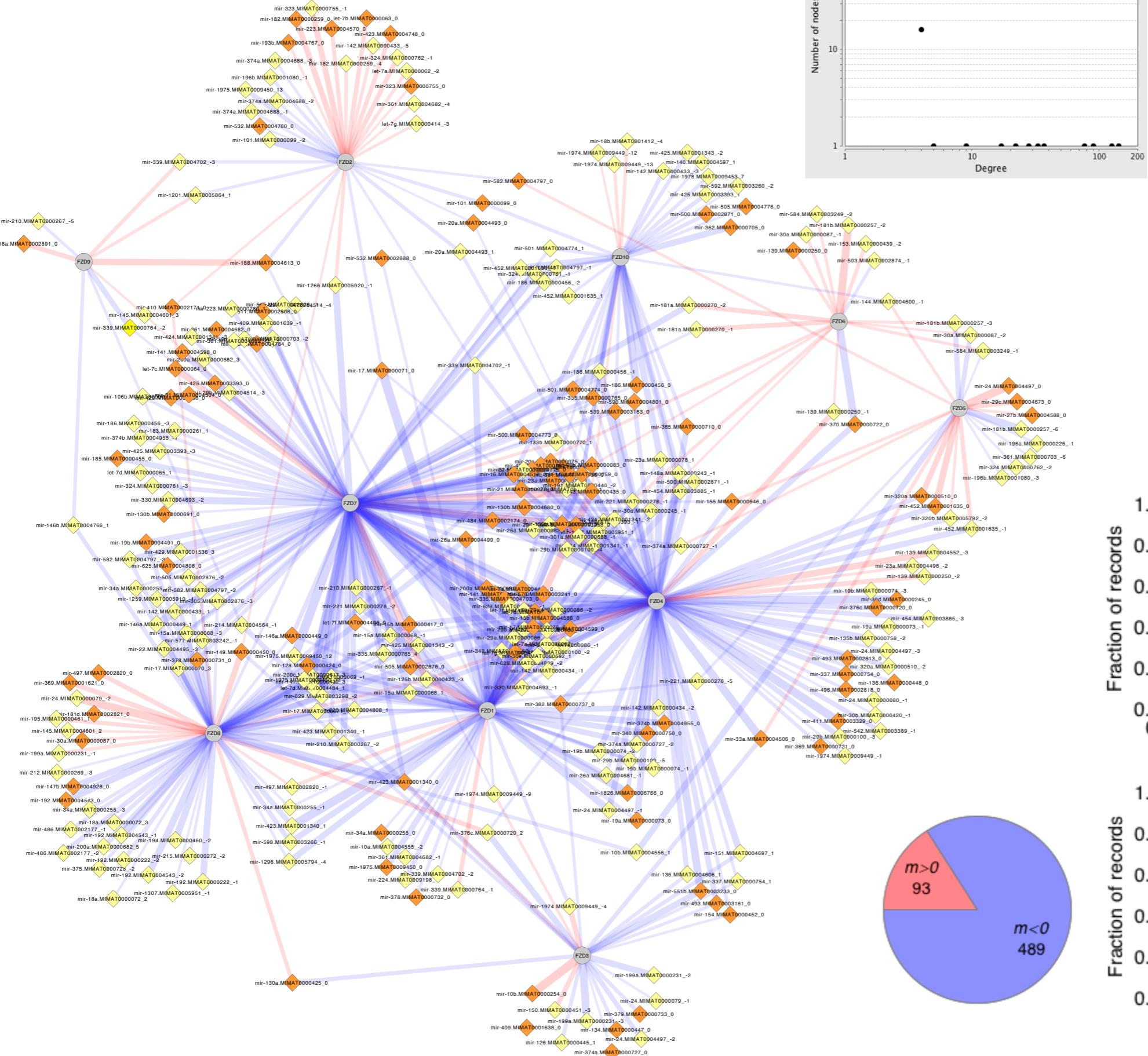
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21 May 2011, 12h30

# 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.

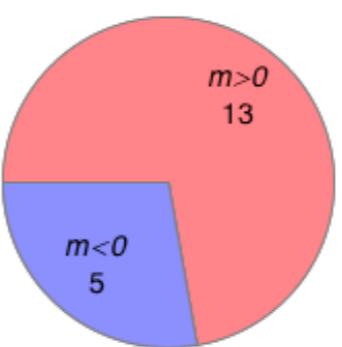
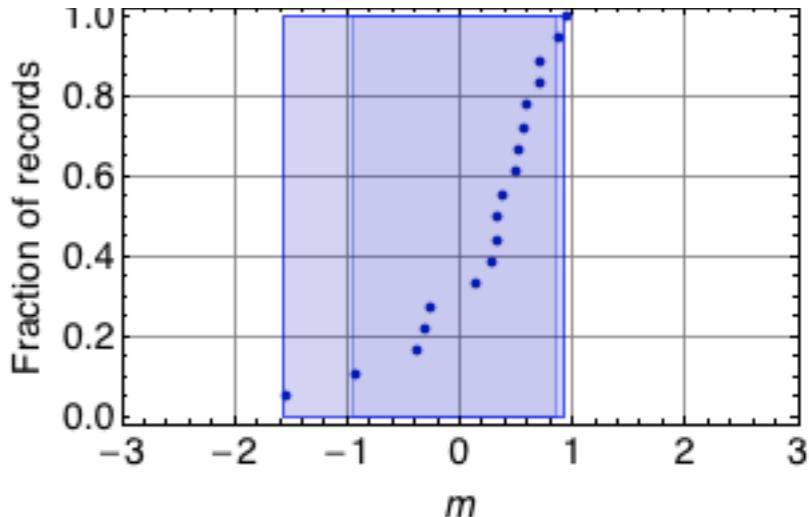
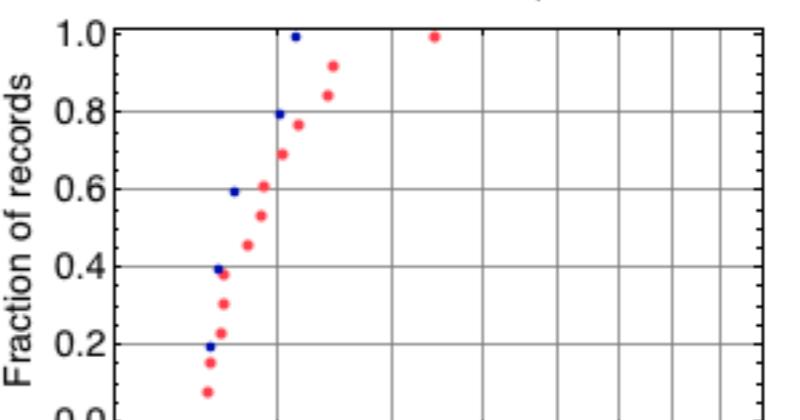
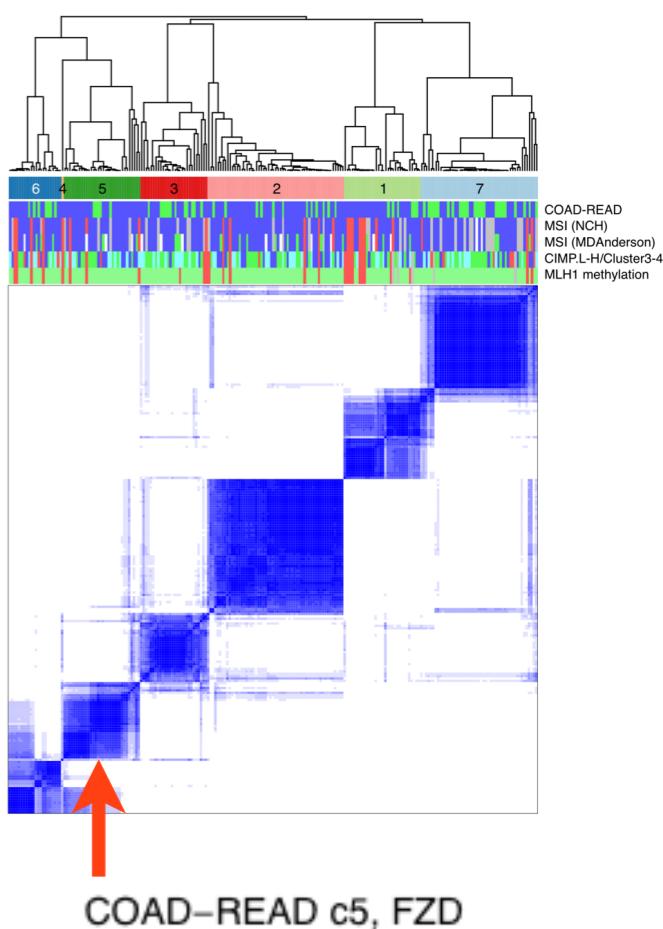
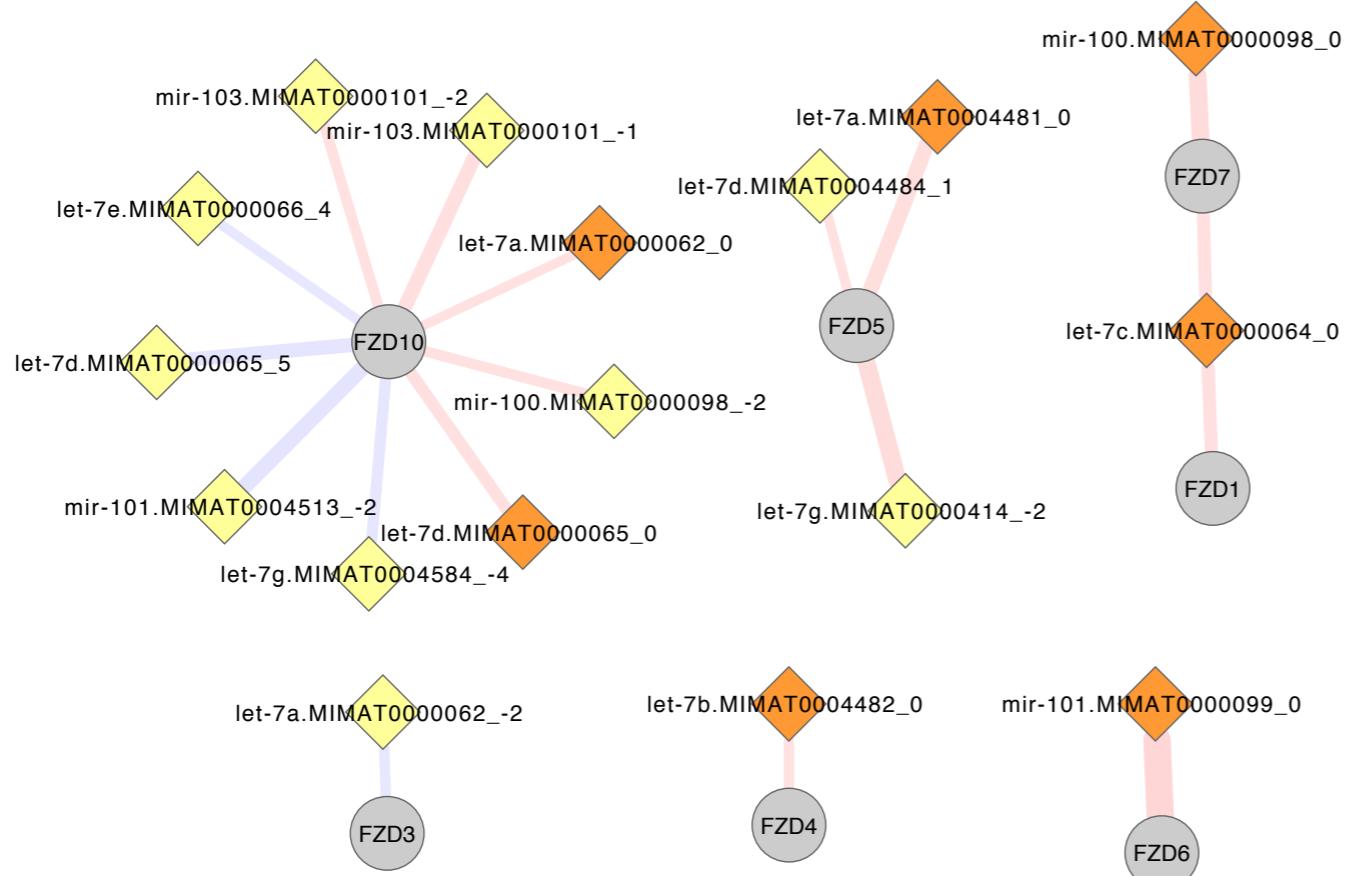
# 582 FZD records, cluster 6



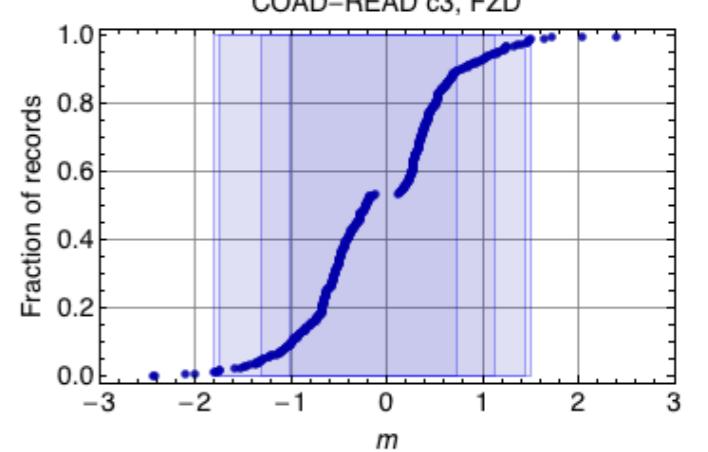
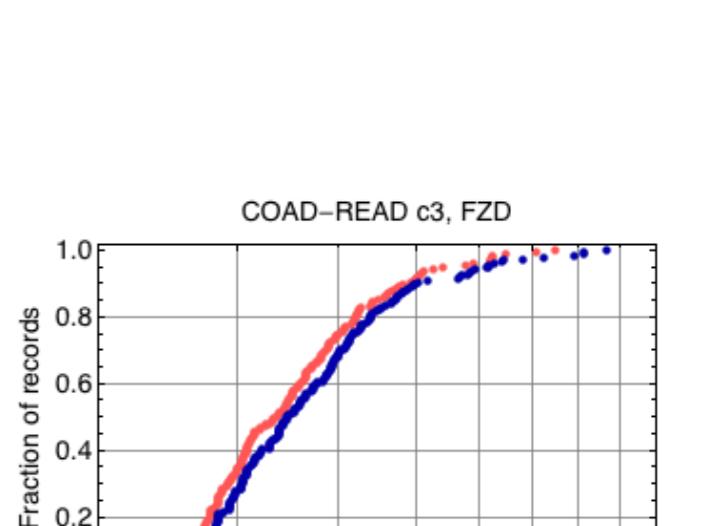
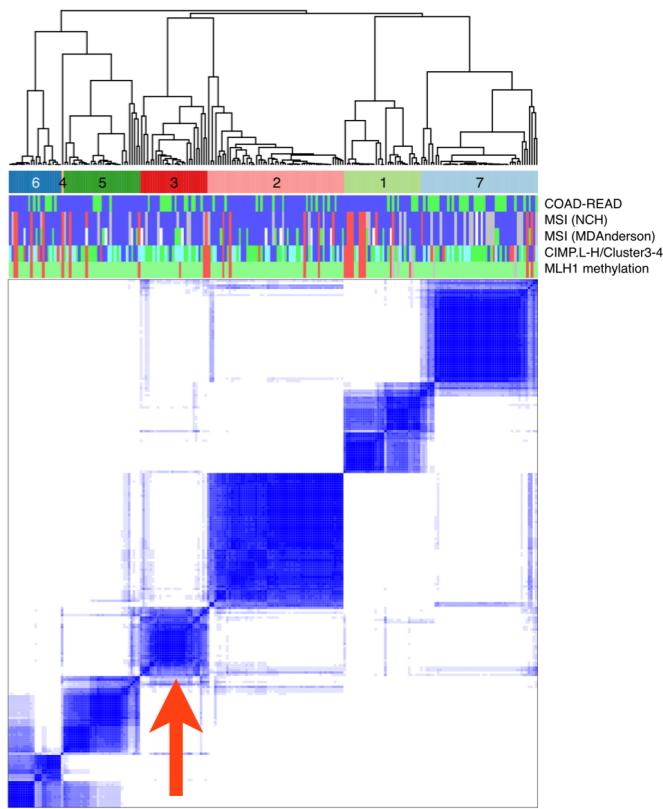
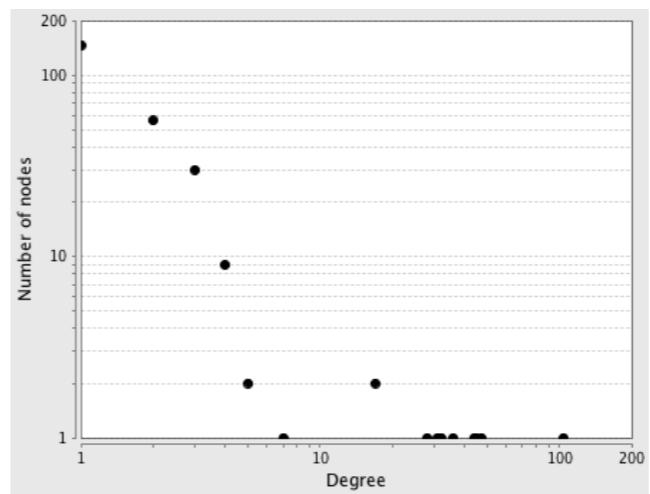
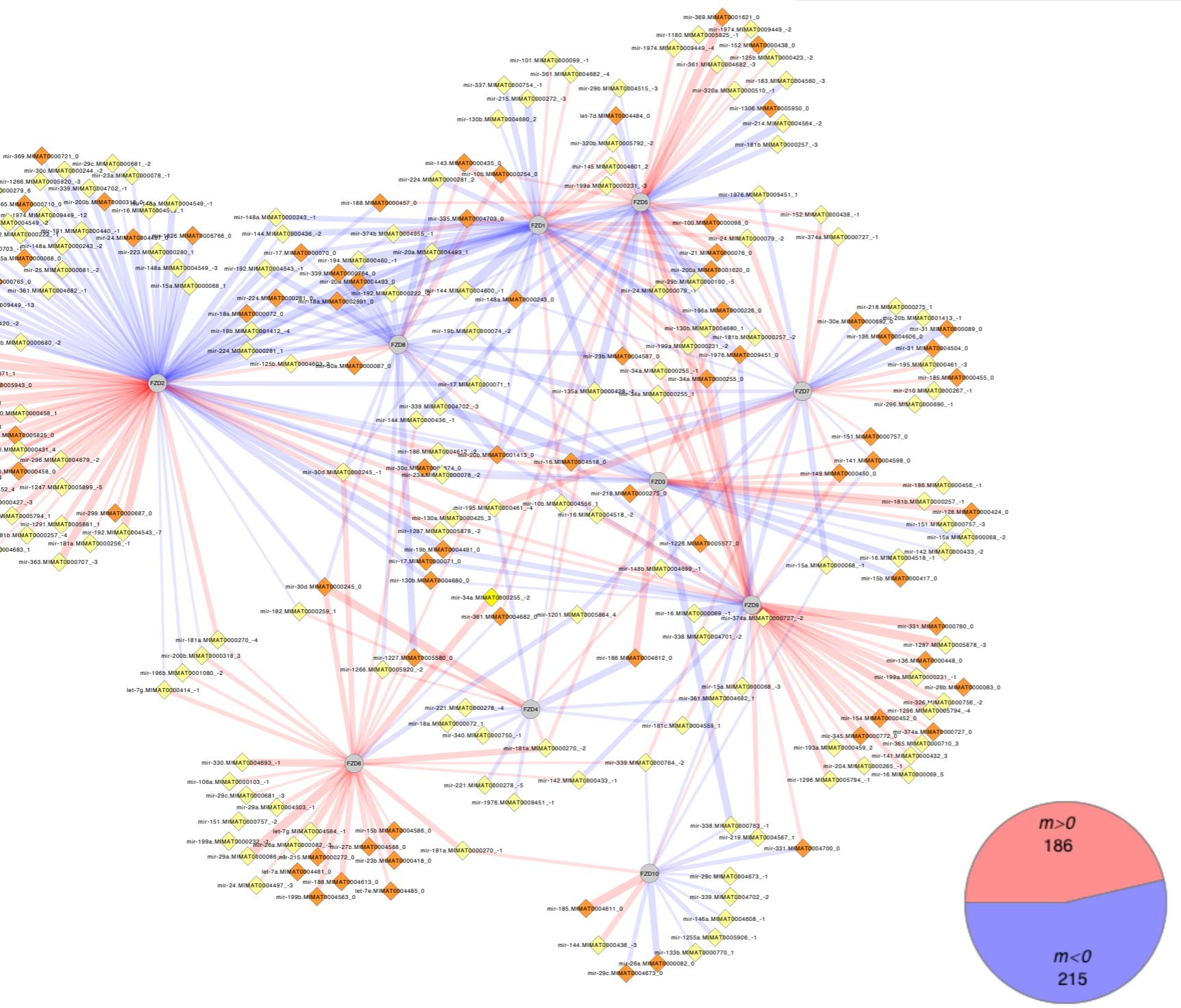
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93

$m < 0$   
489

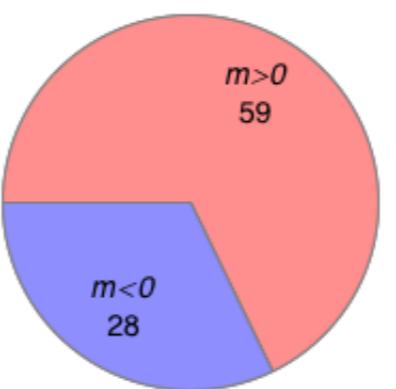
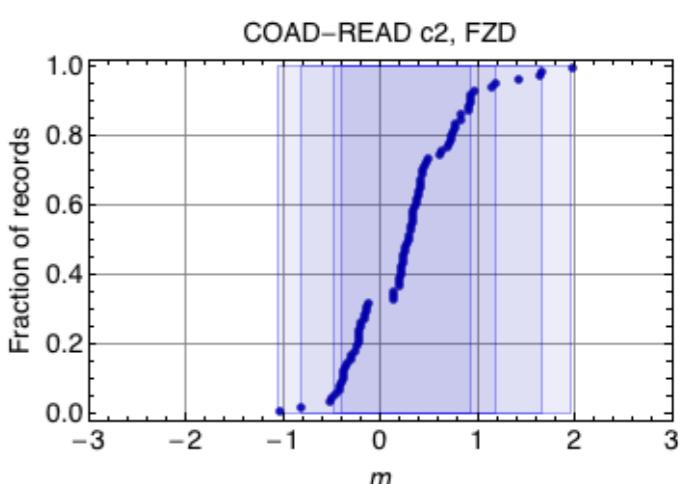
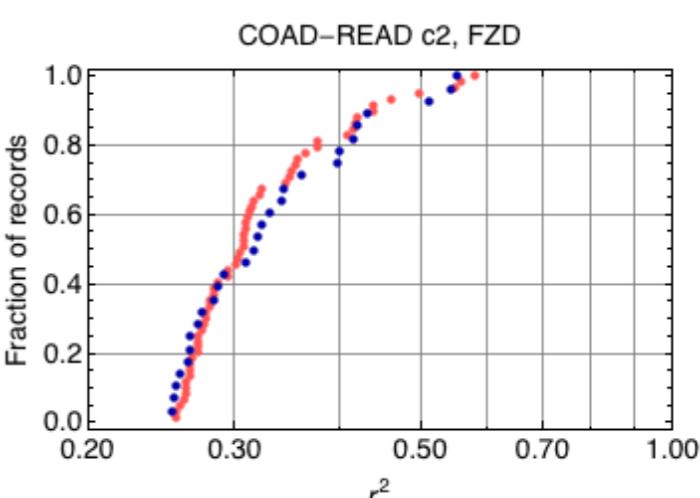
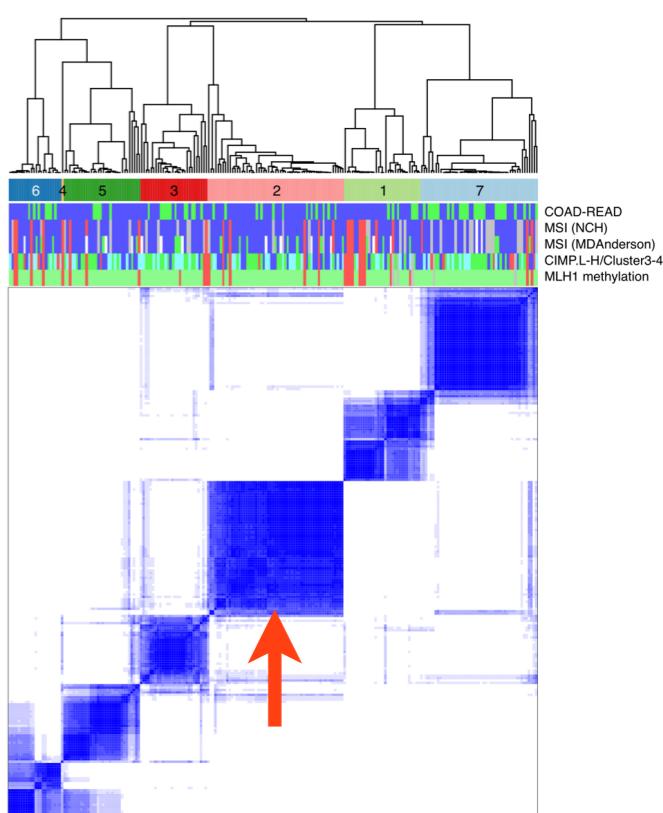
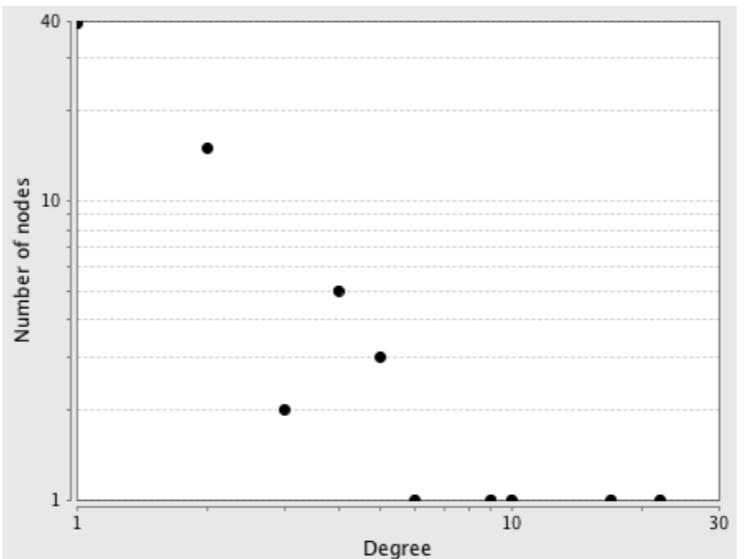
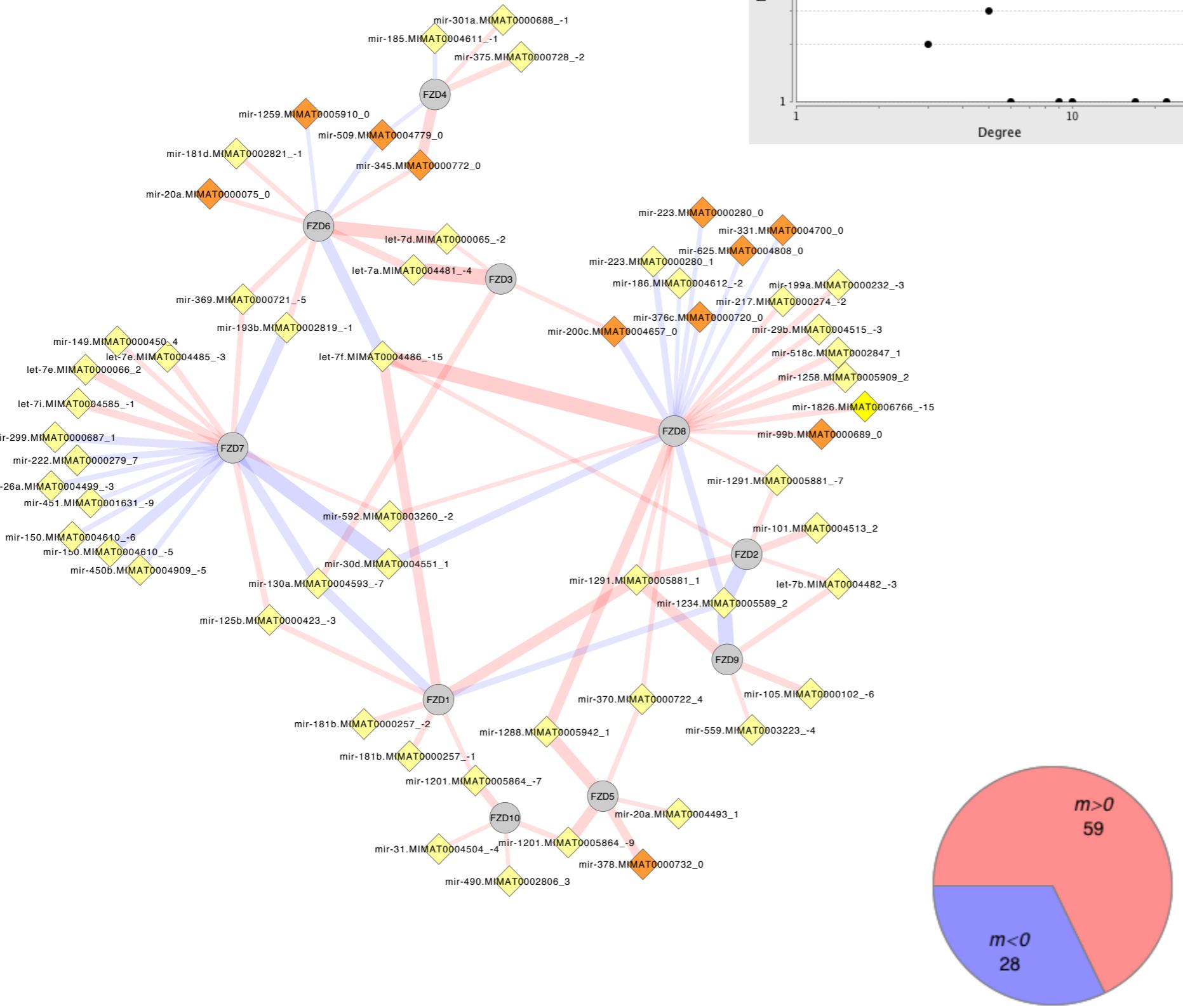
# 18 FZD records, cluster 5



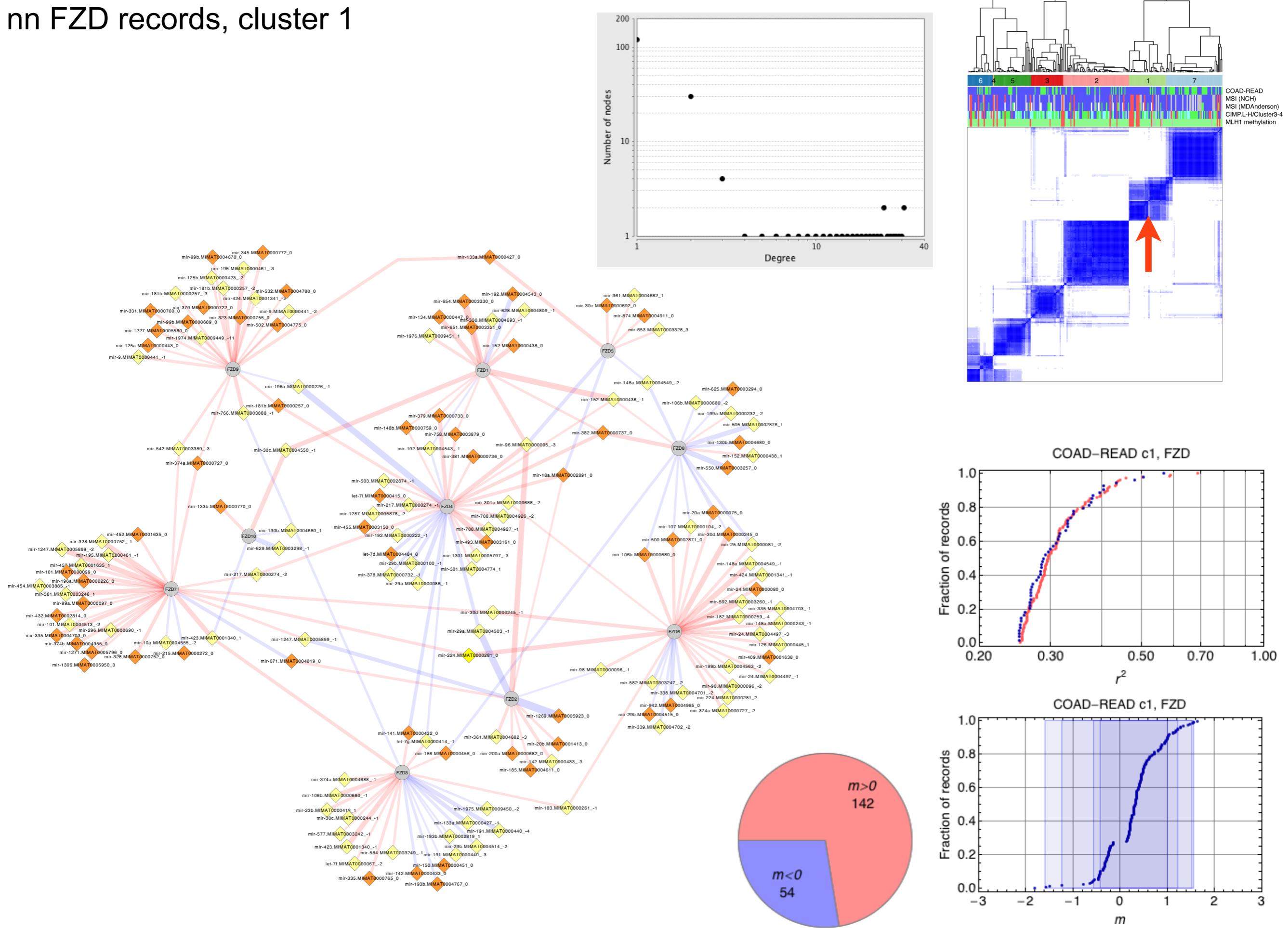
# 401 FZD records, cluster 3



# 87 FZD records, cluster 2



# nn FZD records, cluster 1



# 399 FZD records, cluster 7

