Immune and molecular landscape in colorectal cancer

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Center for Chemistry and Biomedicine (CCB)

Medical University of Innsbruck, Biocenter

http://icbi.at

Acknowledgement

Colorectal cancer

- Cancer with large number of somatic mutations

- 70% of the immune system is present in the gut (and 70% of all microbes)
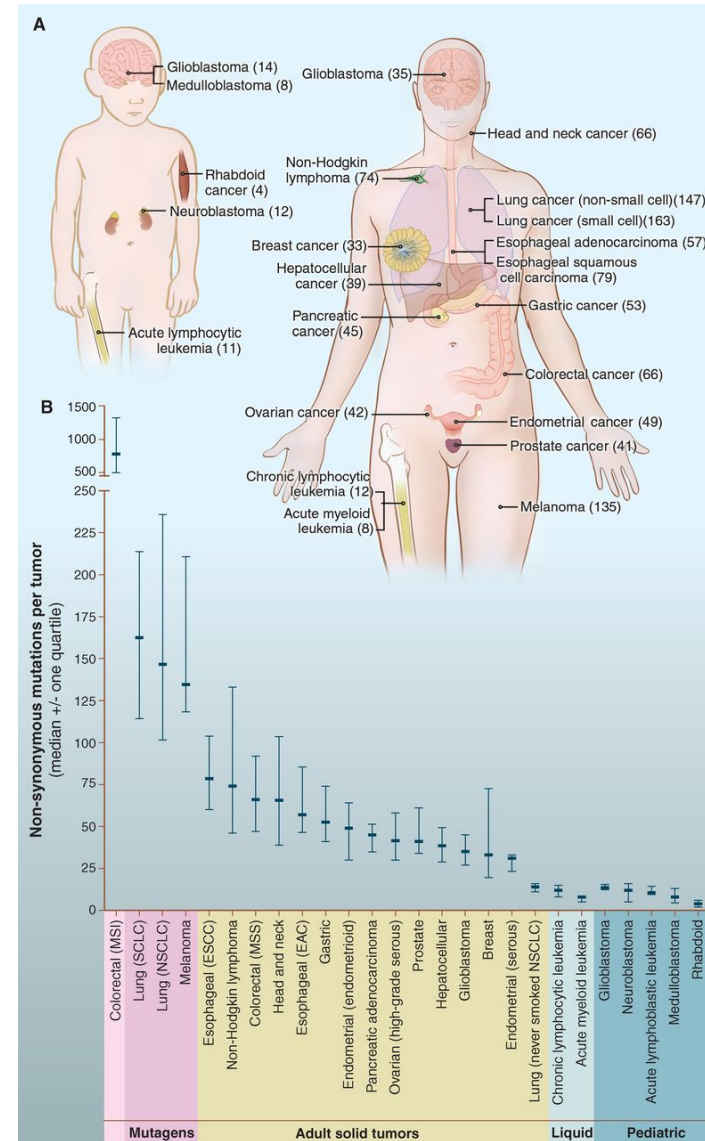
Vogelstein B *et al*. *Science* 2013. 339:1546-1558

Immune markers for patient stratification
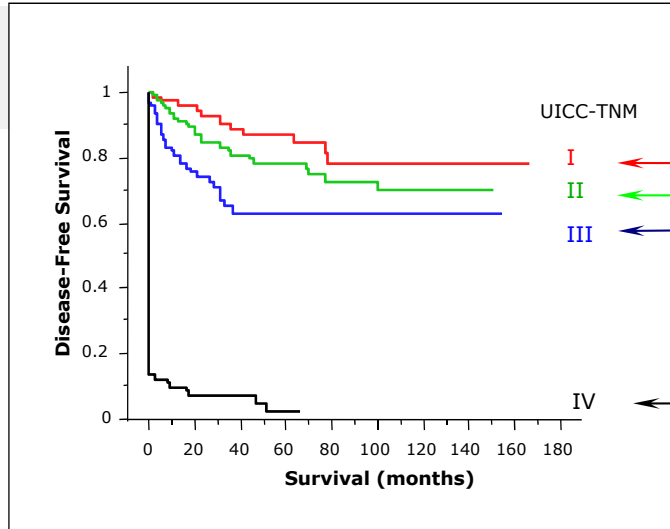
Galon et al. *Science*, 313:1960-1964, 2006

Galon et al. *J Pathology*, 232:199-209, 2014

Cancer immune cycle

Chen DS and Mellman I. *Immunity* 2013, 39:1-10
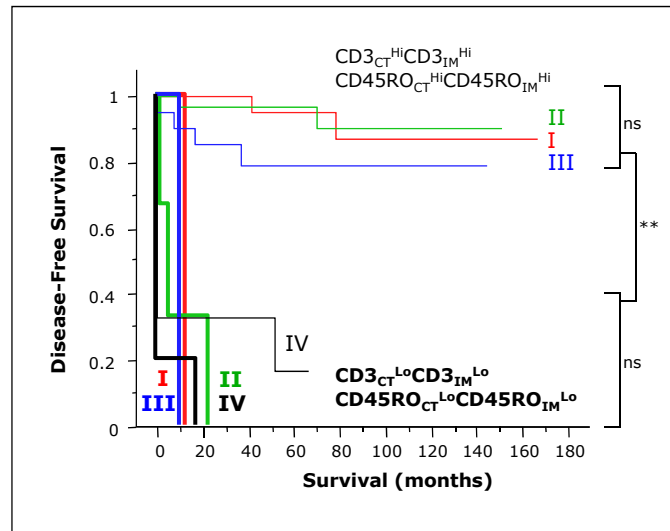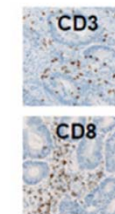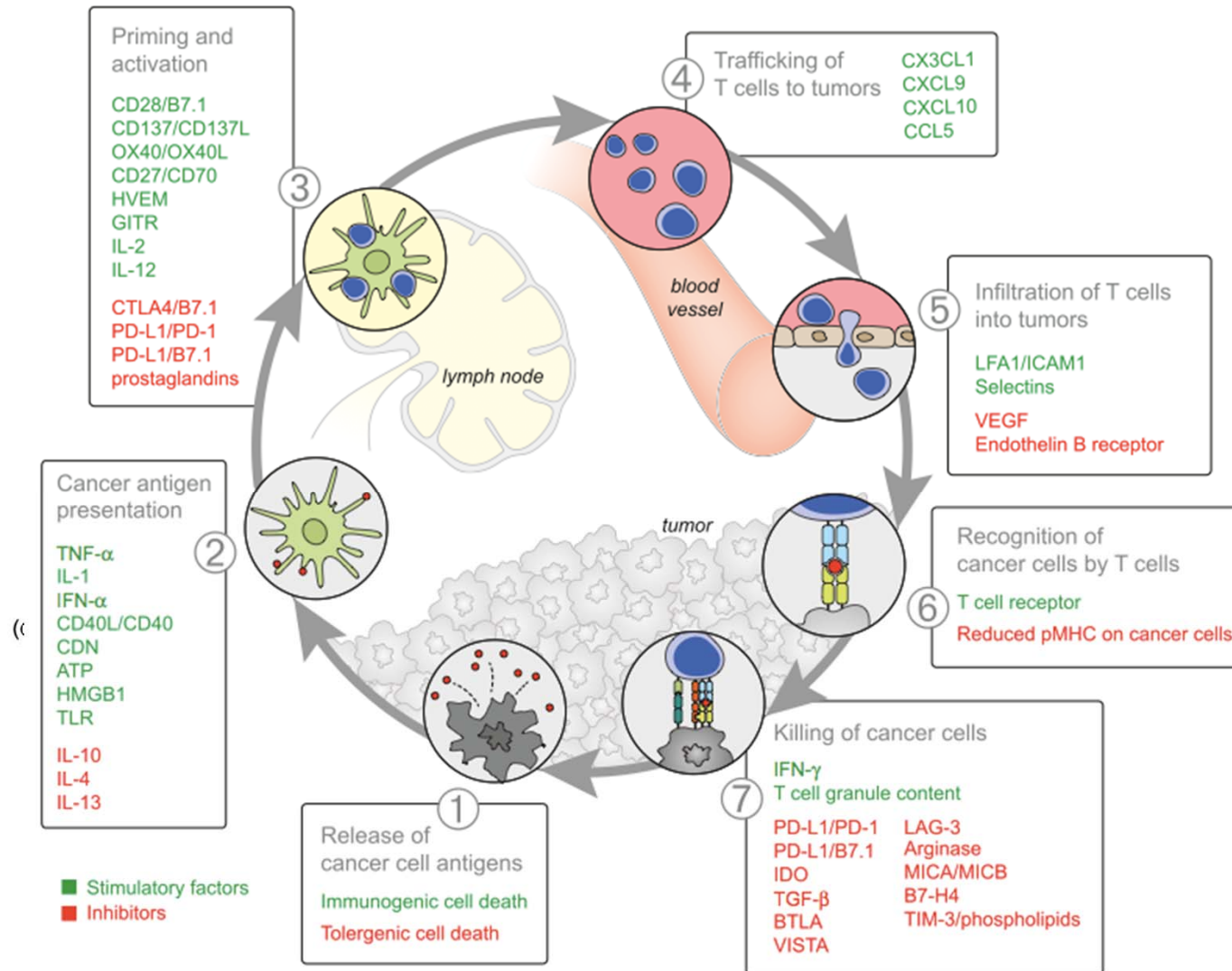
Cancer immunotherapy

- Adoptive T-cell transfer, cellular vaccines
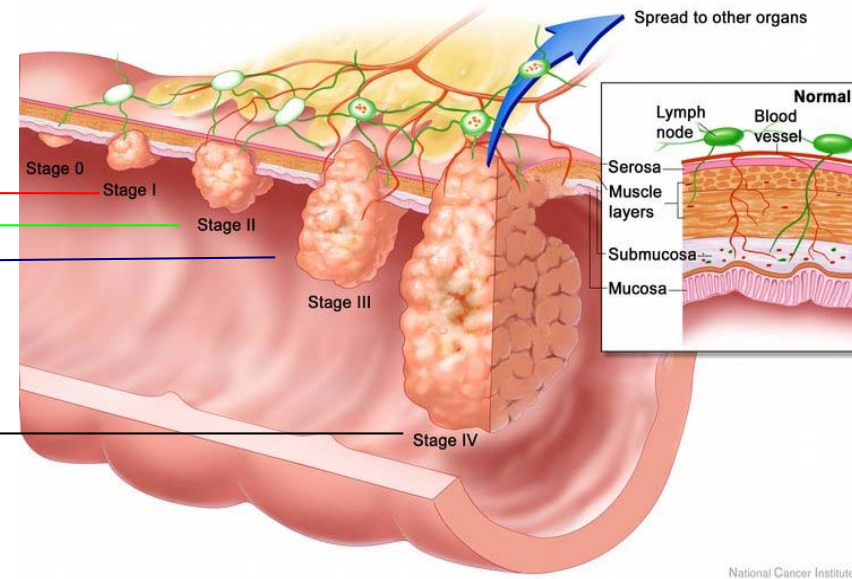- Monoclonal antibodies for immune checkpoint inhibitors

    anti-CTLA4 (ipilumab, approved 2011)

    anti-PD-1 (nivolumab, pembrolizumab approved 2014)

    anti-PD-L1 (RG7446, MEDI4736) and other checkpoint inhibitors
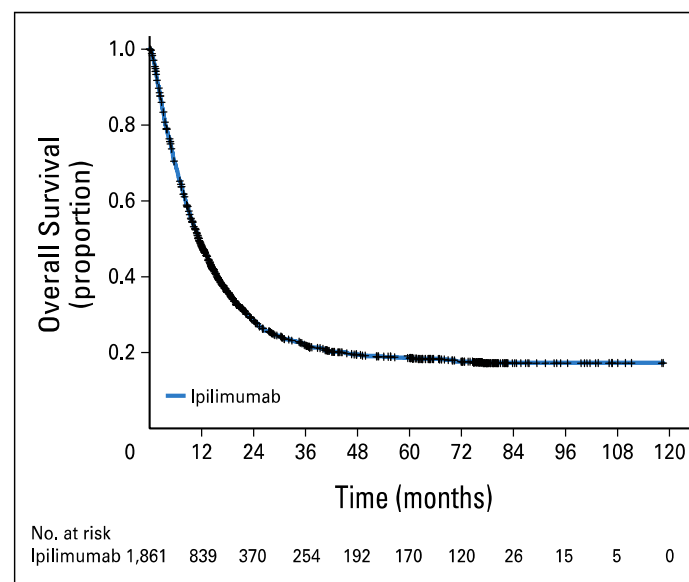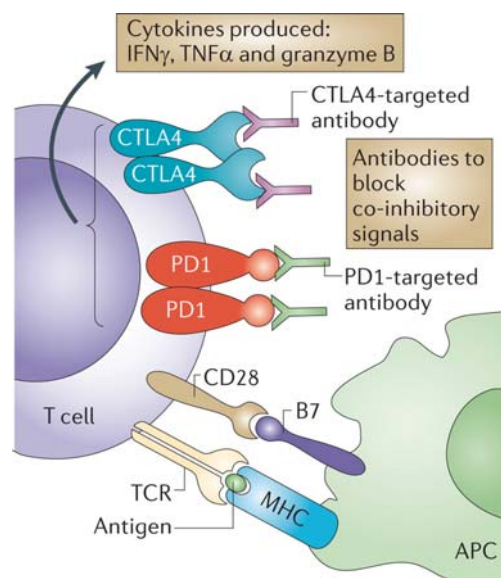
Sharma et al., *Nat Rev Cancer*, 2011; 11:805-12

Neoantigens and cancer immunotherapy
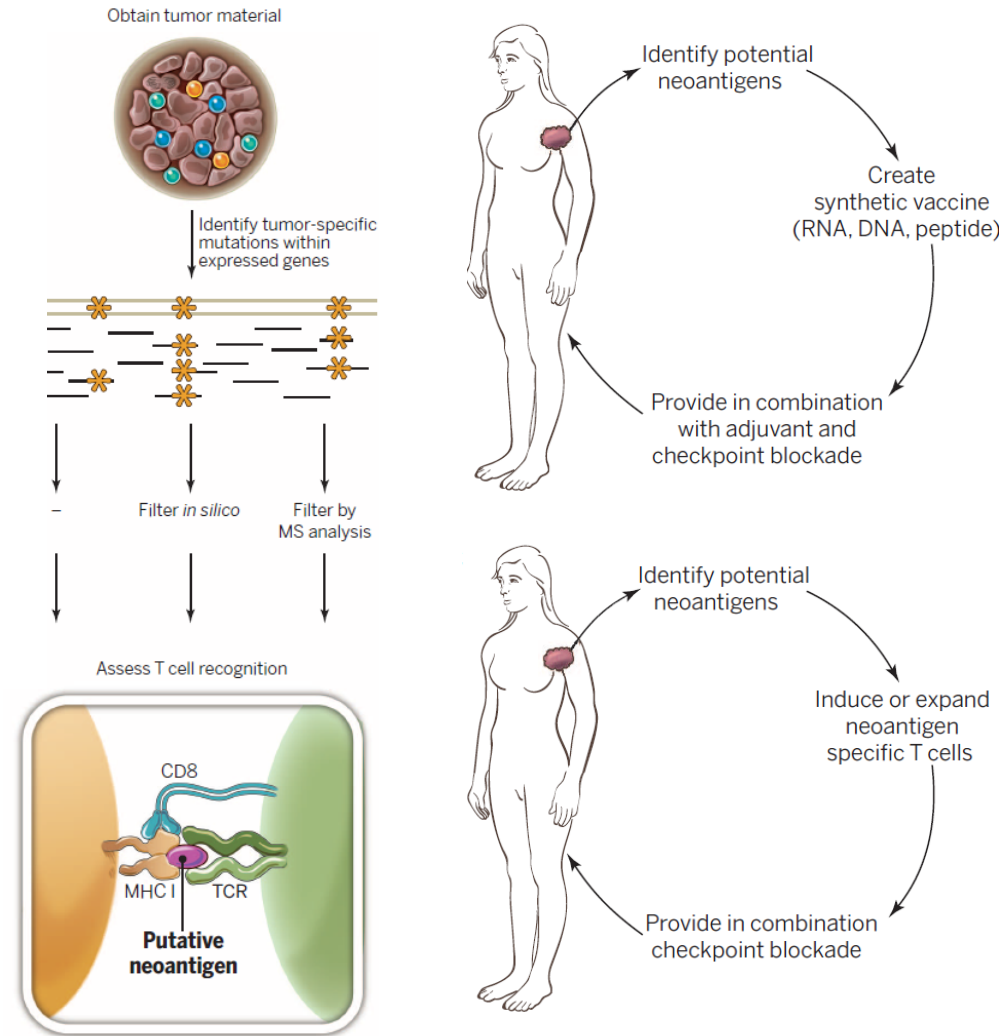
Schumacher TN, Schreiber RD. *Science* 2015

Cancer genomics data

– 2006: The Cancer Genome Atlas (TCGA)

   Comprehensively characterize 20 cancer types

– 2011: International Cancer Genome Consortium (ICGC)

   Comprehensively characterize 50 cancer types
   and/or subtypes (25,000 genomes)

Deep mining of genomic data

36 studies, 813 microarrays

Immune cell microarray expression profiles

TCGA cancer genomics data (n=598)

RNA-Seq | Exome-Seq | SNP Arrays | Promoter methylation data

11 studies (n~2000)

Colorectal cancer expression profiles

Gene expression

Sequenced reads

Somatic mutations

Copy number

Compendium of immune genes[1]

HLA haplotype estimation[2]

Methylation subtypes

Validation in independent cohorts

CRC immune landscape

CT Database

Epitope prediction[3]

Tumor heterogeneity[4]

Clinical information
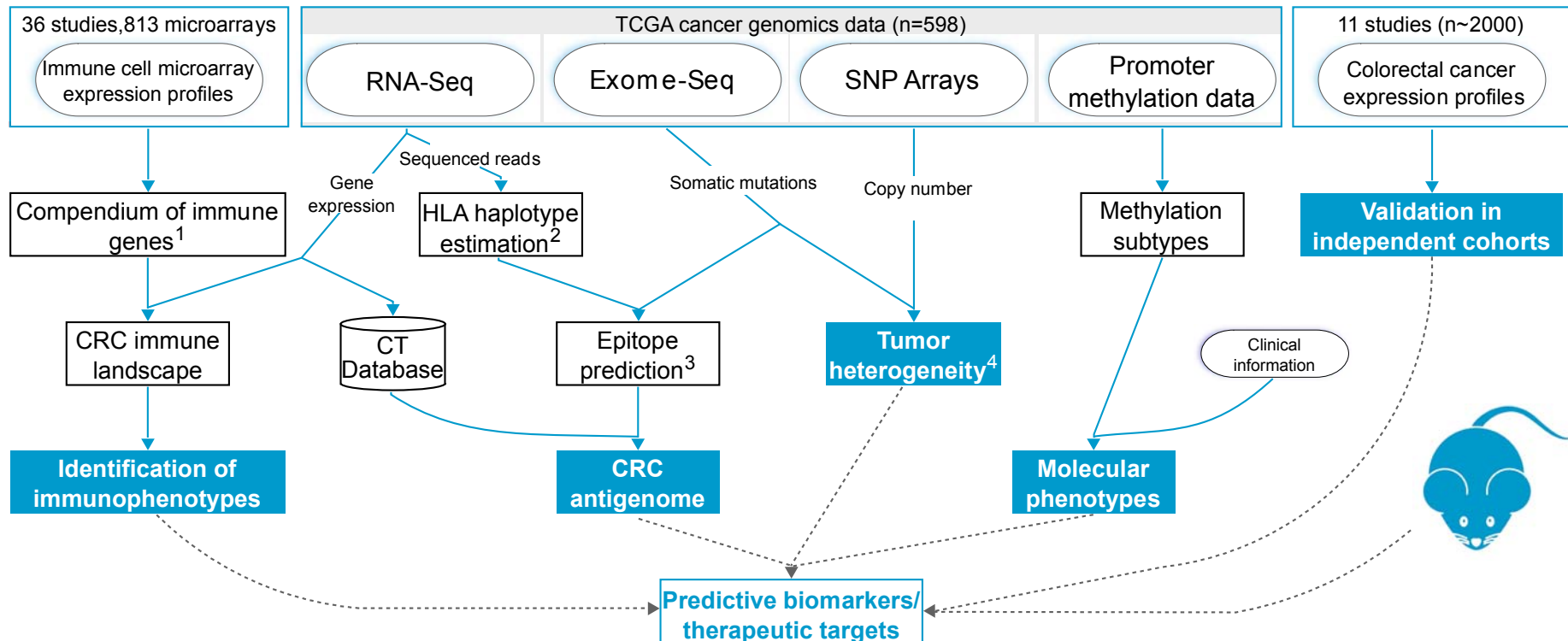
Identification of immunophenotypes

CRC antigenome

Molecular phenotypes

Predictive biomarkers/ therapeutic targets

Tools/Methods
1. Bindea G, *et al. Immunity* 2013; 39:782-795
2. Warren R L *et al. Genome Medicine* 2012; 4**:** 95.
3. Nielsen M *et al. PLoS ONE* 2007; 2**:** e796
4. Carter SL *et al. Nat Biotech* 2012; 30**:** 413–421

Data
The Cancer Genome Atlas Network. *Nature*; 487: 330-7
16.5 TB   microarrays: 25 GB, SNP-arrays 250 GB, exome-Seq: 9 TB, RNA-seq: 7.2 TB

Angelova et al. *Genome Biol.* 2015.16:64

Compendium of genes enriched in immune cells
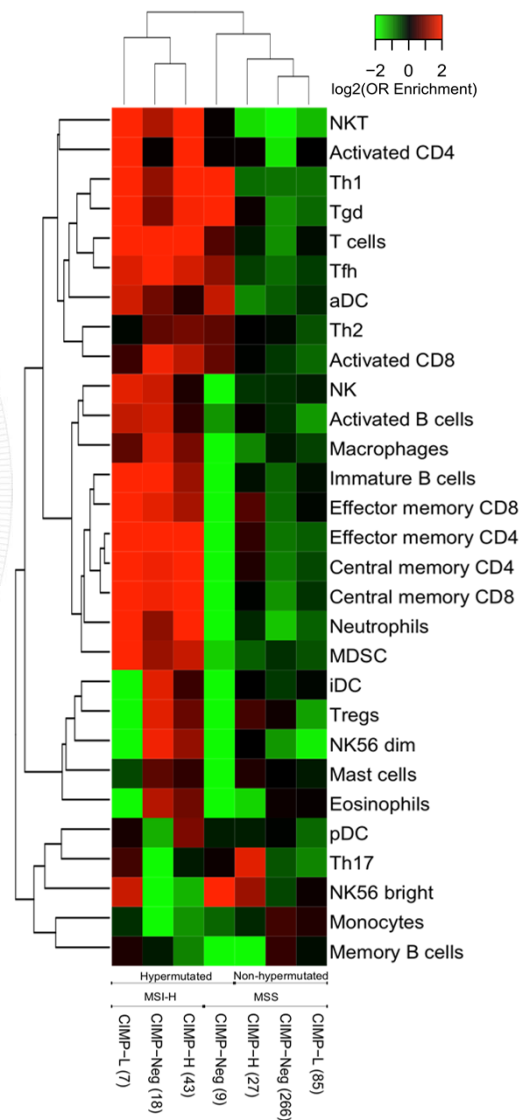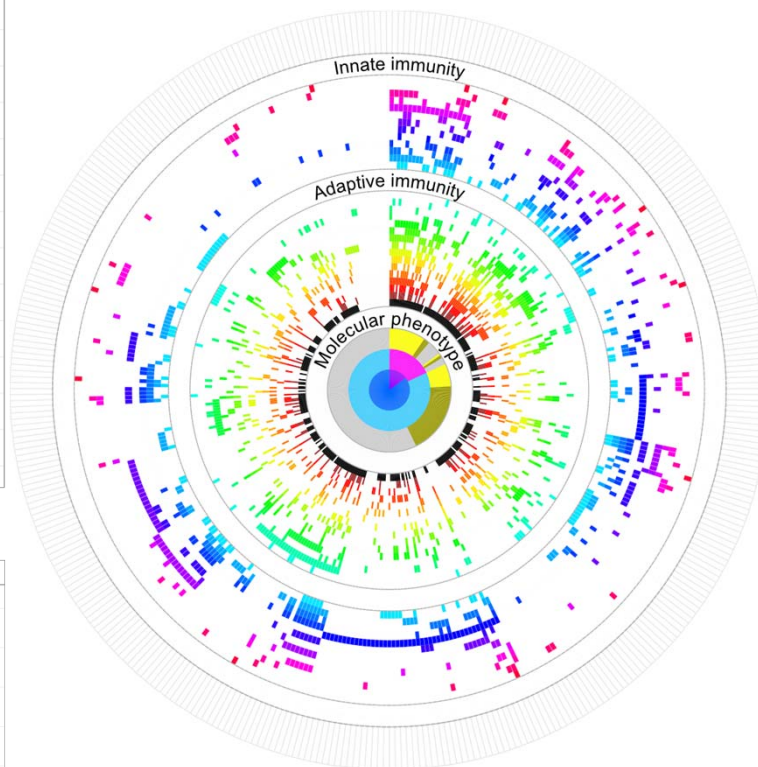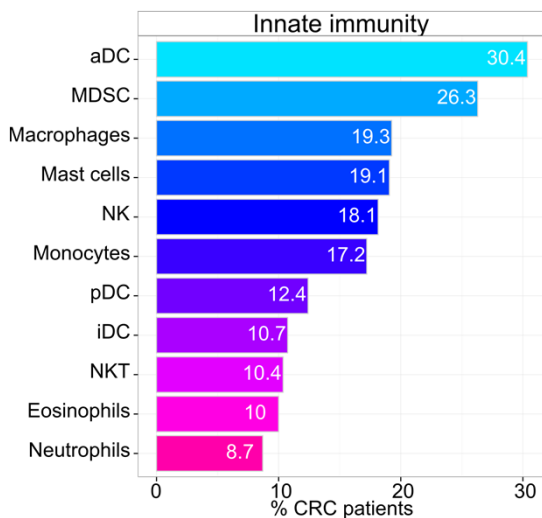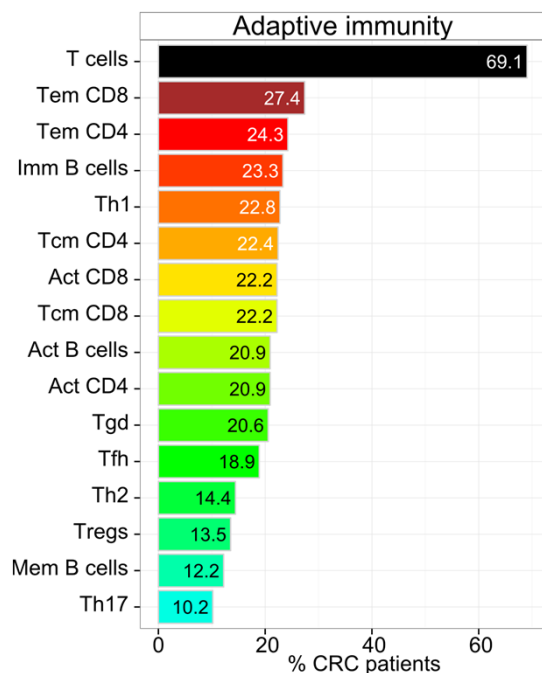
813 Affy HG-U133A arrays
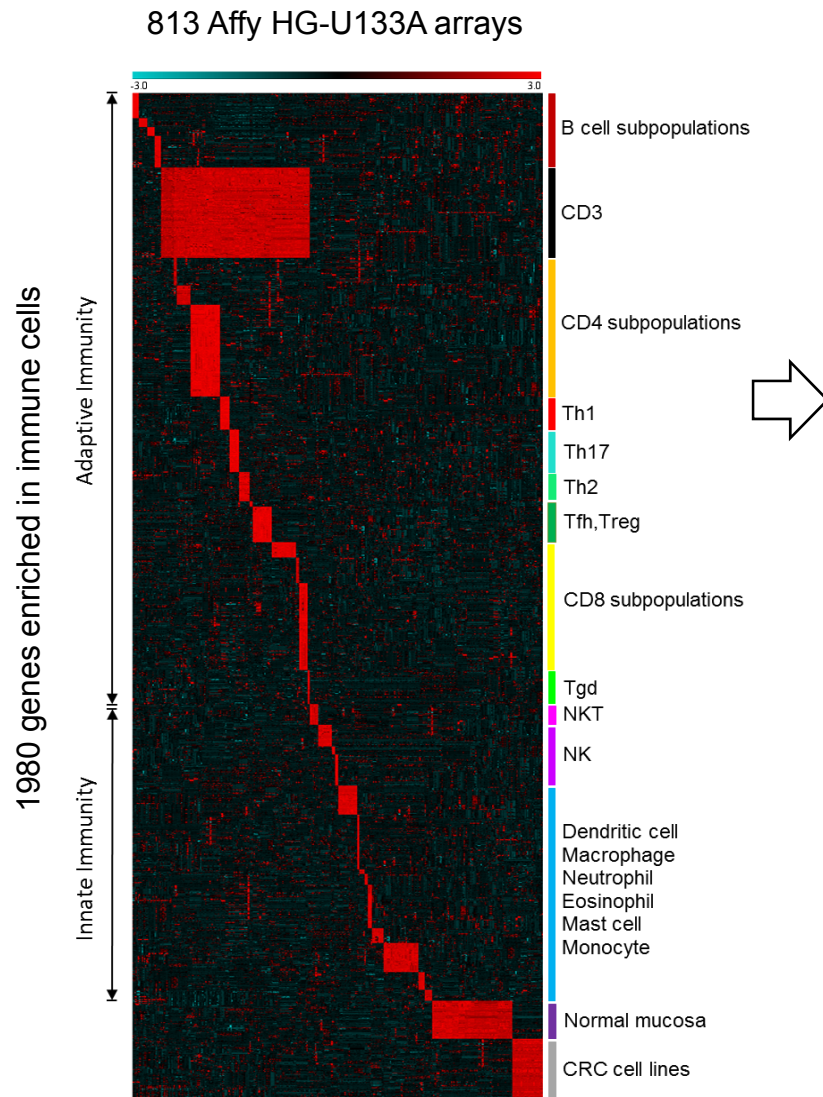
Metagenes:

Genes from the same cell type similarly expressed across all samples (patients) in the TCGA RNAseqV2 data (normalized counts) (corr≥0.6; p<0.01)

Single sample gene set enrichment analysis (ssGSEA) (NES>0; FDR<0.1)

Molecular phenotypes and TILs in CRC

Infiltrated immune cells are associated with survival
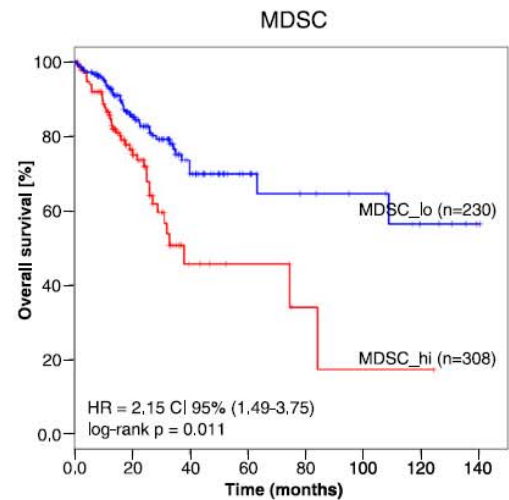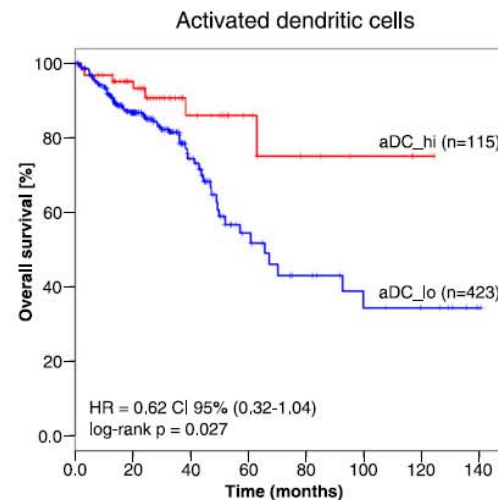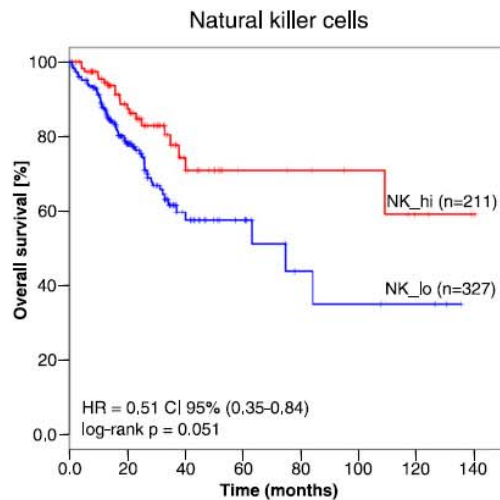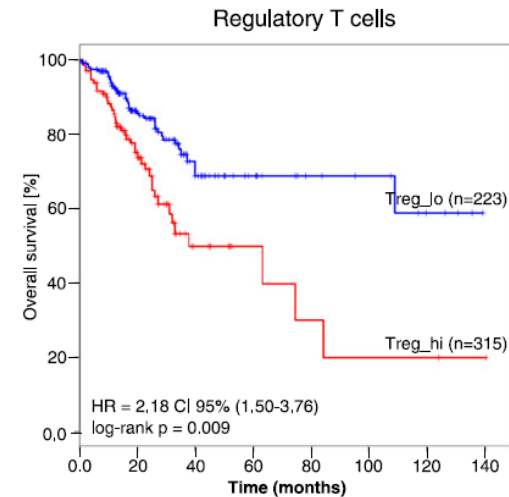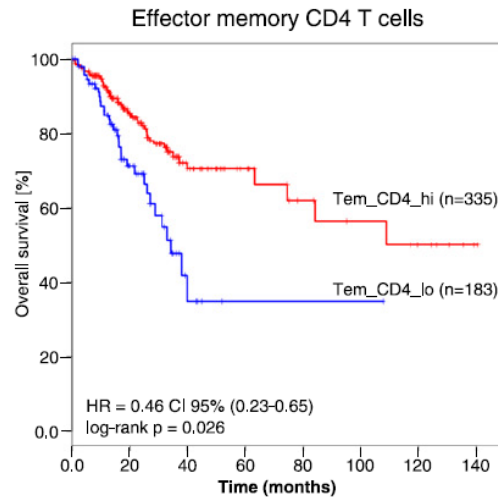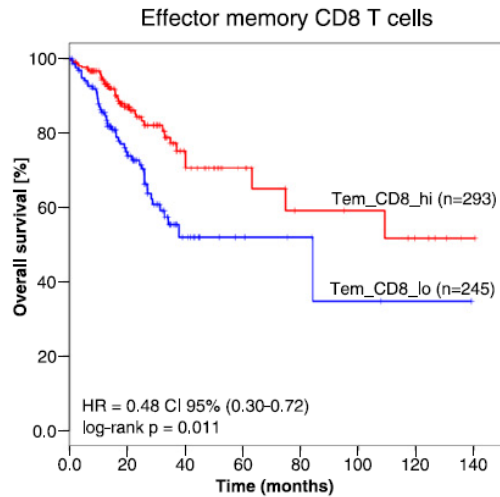
Tumor heterogeneity

Based on whole-exome NGS data and SNP arrays cancer cell fractions and tumor heterogenity (clones and subclones) were calculated by the ABSOLUTE algorithm. MSS patients were clustered into 4 groups
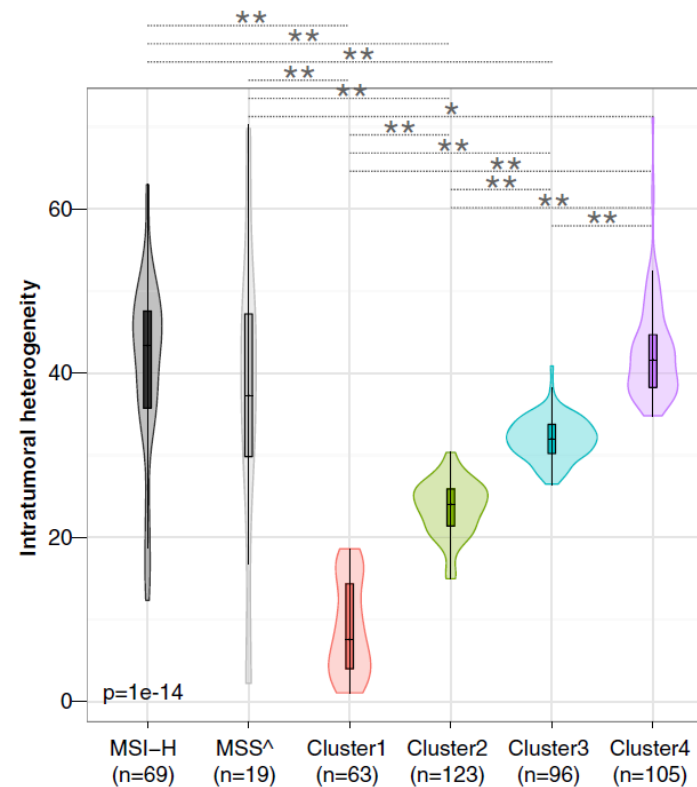
Association of TILs with tumor heterogenity

Gene expression of immunomodulators

Tumor versus normal log2 fold expression change

Network reconstruction reveals candidate genes for immunotherapy

TNFRSF17 is druggable target for cancer immunotherapy in CRC patients

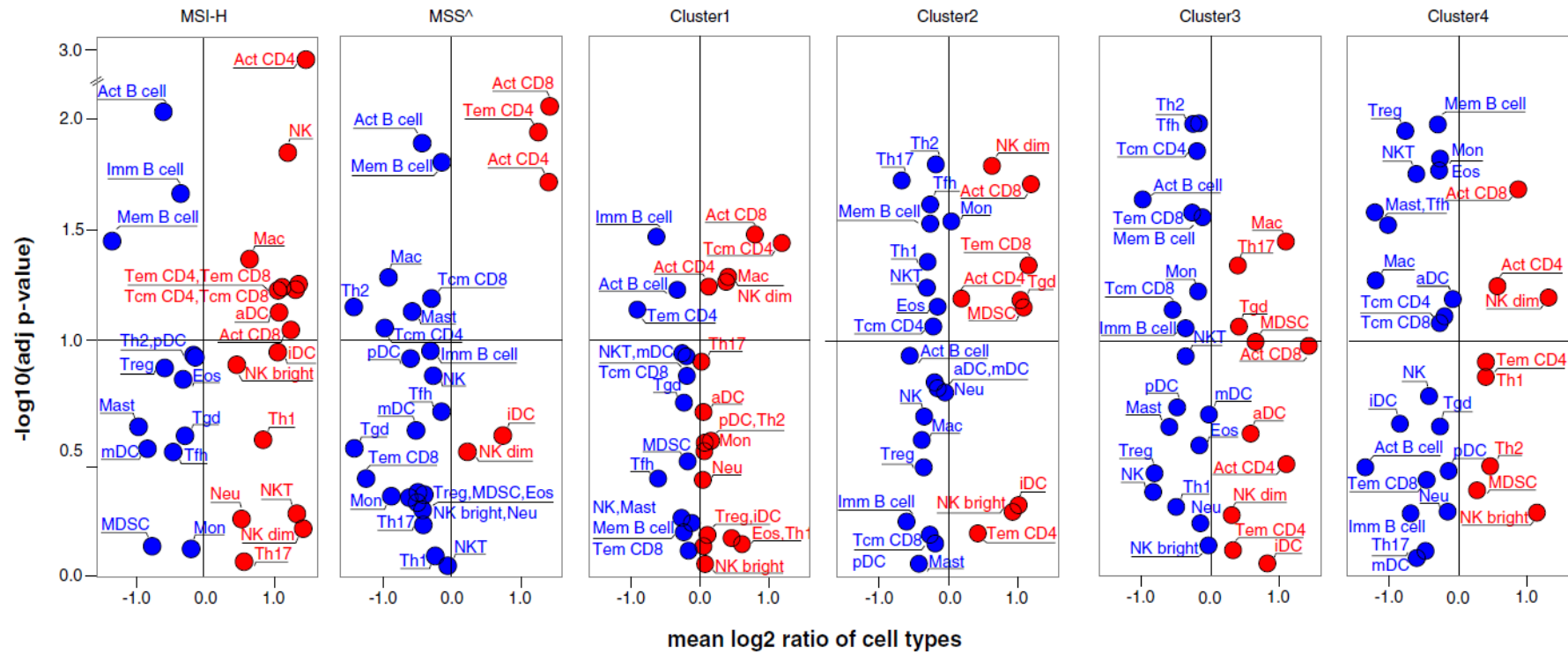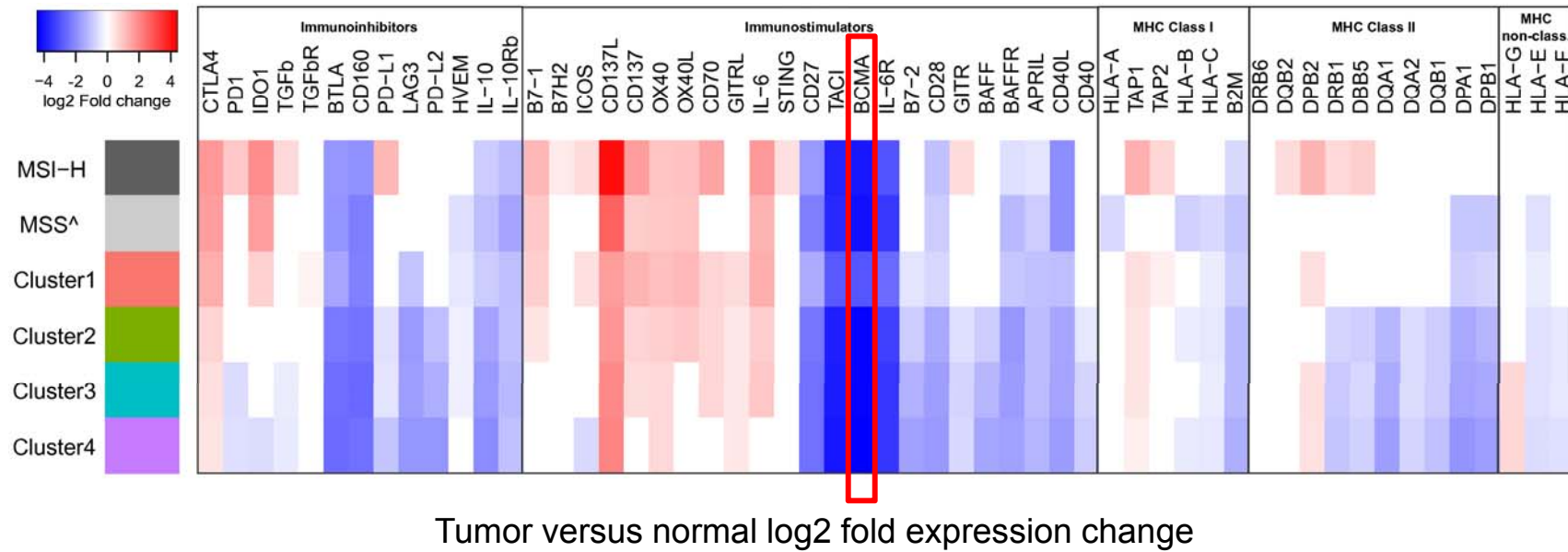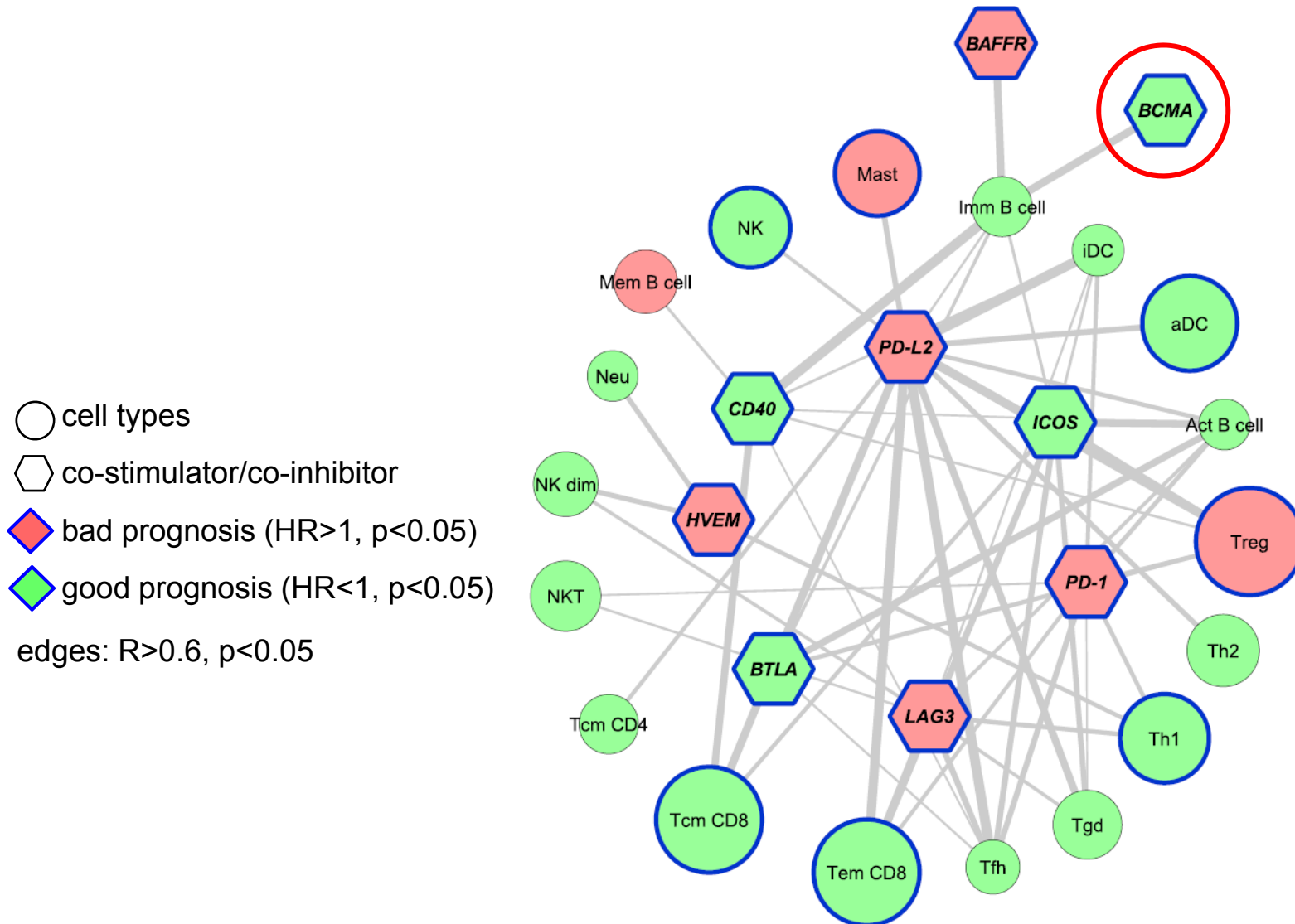| BCMA Survival	Cohort	Log2 (Hazard Ratio)	95% CI	p-value
OS	TCGA (n=538)	-1.25	0.16-0.72	0.02
	GSE12945 (n=62)	-1.36	0.10-1.19	0.21
	GSE17538 (n=240)	-1.09	0.47-0.91	0.06
	GSE29623 (n=130)	-1.74	0.14-0.58	0.32
	GSE33113 (n=90)	-1.06	0.12-1.13	0.39
	GSE28722 (n=125)	-1.22	0.15-1.17	0.05
DFS	GSE12945 (n=62)	-2.12	0.12-1.20	0.15
	GSE41258 (n=187)	-1.84	0.19-0.89	0.12
	GSE14333 (n=290)	-0.92	0.28-0.90	0.24
	GSE17538 (n=240)	-2.02	0.14-0.34	0.00
	GSE29623 (n=130)	-1.94	0.11-1.23	0.28
	GSE39582 (n=566)	-0.84	0.34-0.72	0.03
	GSE24549 (n=83)	-1.56	0.22-0.81	0.23
	GSE24550 (n=77)	-1.69	0.01-1.16	0.99
	GSE30378 (n=95)	-1.64	0.21-0.97	0.93
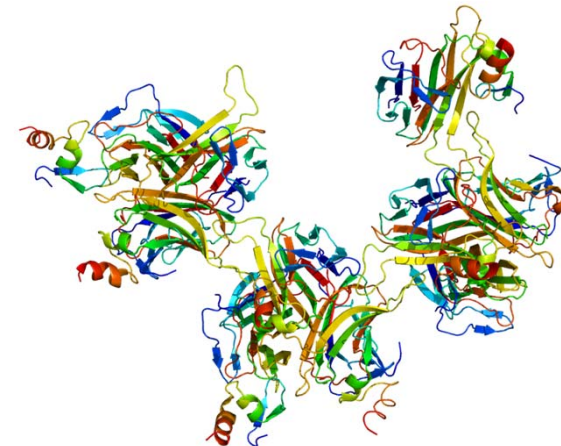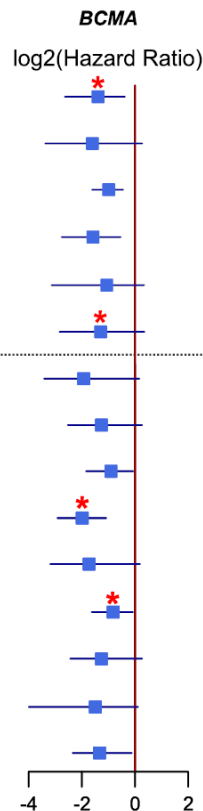
TNFRSF17 (BCMA,CD269)
Induces antigen presentation in B cells; promotes B cell survival, activates NF-kappaB and JNK

Ligands: BAFF, APRIL

Antibodies, agonists, and mouse models available

Antagonist: atacicept (PhaseII/III for rheumatoid arthritis, MS, multiple myeloma)

Linear modeling identifies CCR8 as target

Test 221 parameters including chemokines, cytokines, immunomodulators, MHC molecules, Tregs, MDSC to predict cytotoxic cells (CD8,NK,Tgd cells)
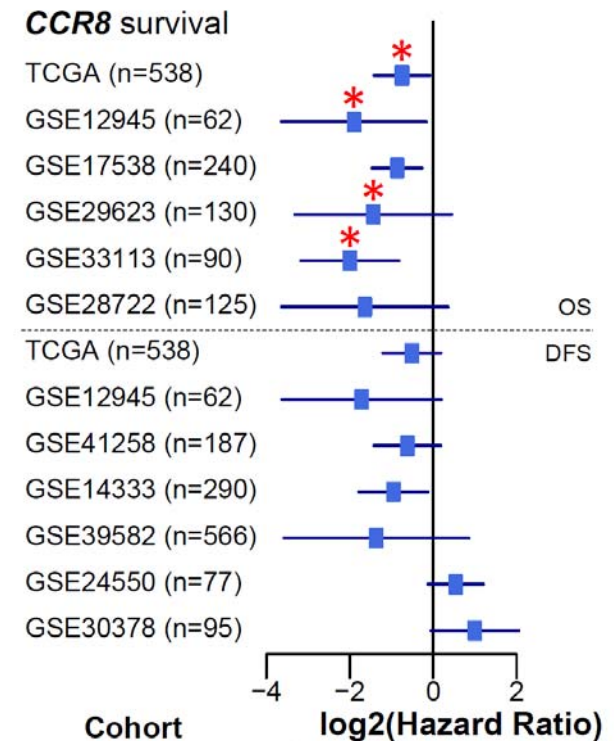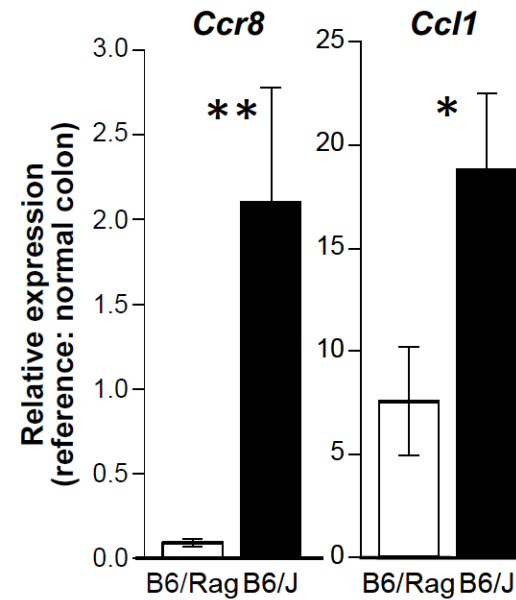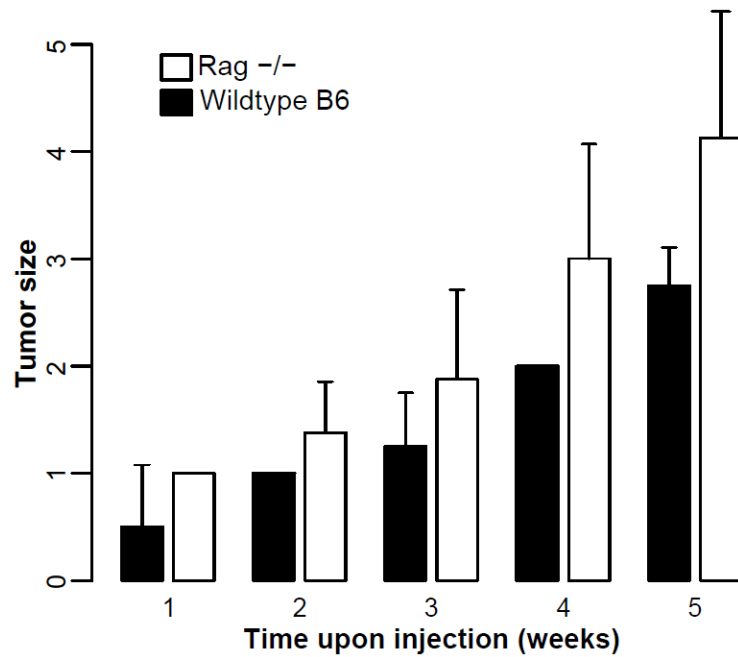
Rank	Parameters	Coefficient	Adjusted p-value	OS Hazard Ratio (CI 95%)	p-value
1	MDSC	-0.325	3.19E-28	2.96 (1.98-7.52)	0.007
2	Treg	-0.301	4.82E-17	1.87 (1.04-3.77)	0.065
3	*CCR8*	0.421	1.55E-15	0.62 (0.37-0.96)	0.045
4	*CXCR5*	0.402	4.32E-15	0.62 (0.35-0.99)	0.994
5	*CD86*	0.351	7.14E-09	1.58 (1.00-2.80)	0.594
6	*PD-L2*	0.195	6.99E-07	2.03 (1.27-5.00)	0.044

CCR8 survival

Cohort	
TCGA (n=538)	OS
GSE12945 (n=62)	
GSE17538 (n=240)	
GSE29623 (n=130)	
GSE33113 (n=90)	
GSE28722 (n=125)	
TCGA (n=538)	DFS
GSE12945 (n=62)	
GSE41258 (n=187)	
GSE14333 (n=290)	
GSE39582 (n=566)	
GSE24550 (n=77)	
GSE30378 (n=95)	

log2(Hazard Ratio)

Ccr8 is less expressed in an orthotopic induced tumor (MC38) in Rag1-/- mouse model.
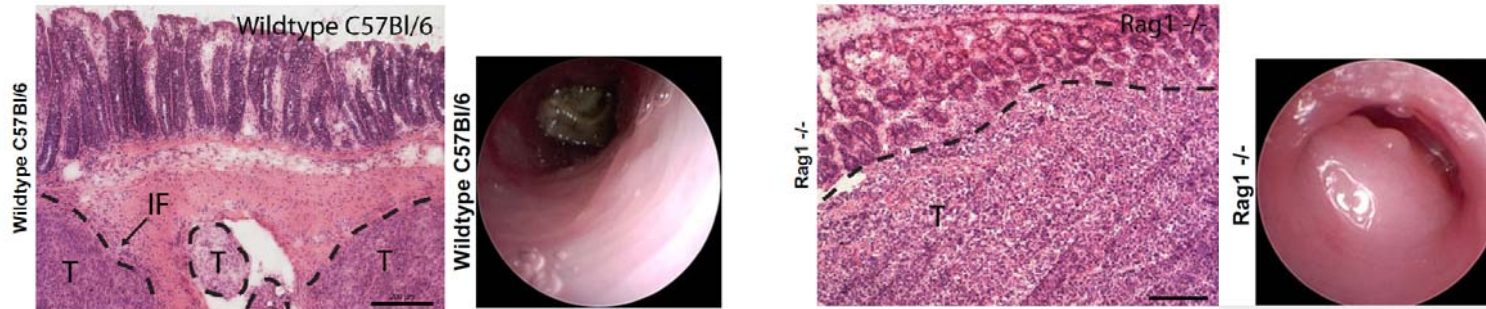
Deep mining of genomic data
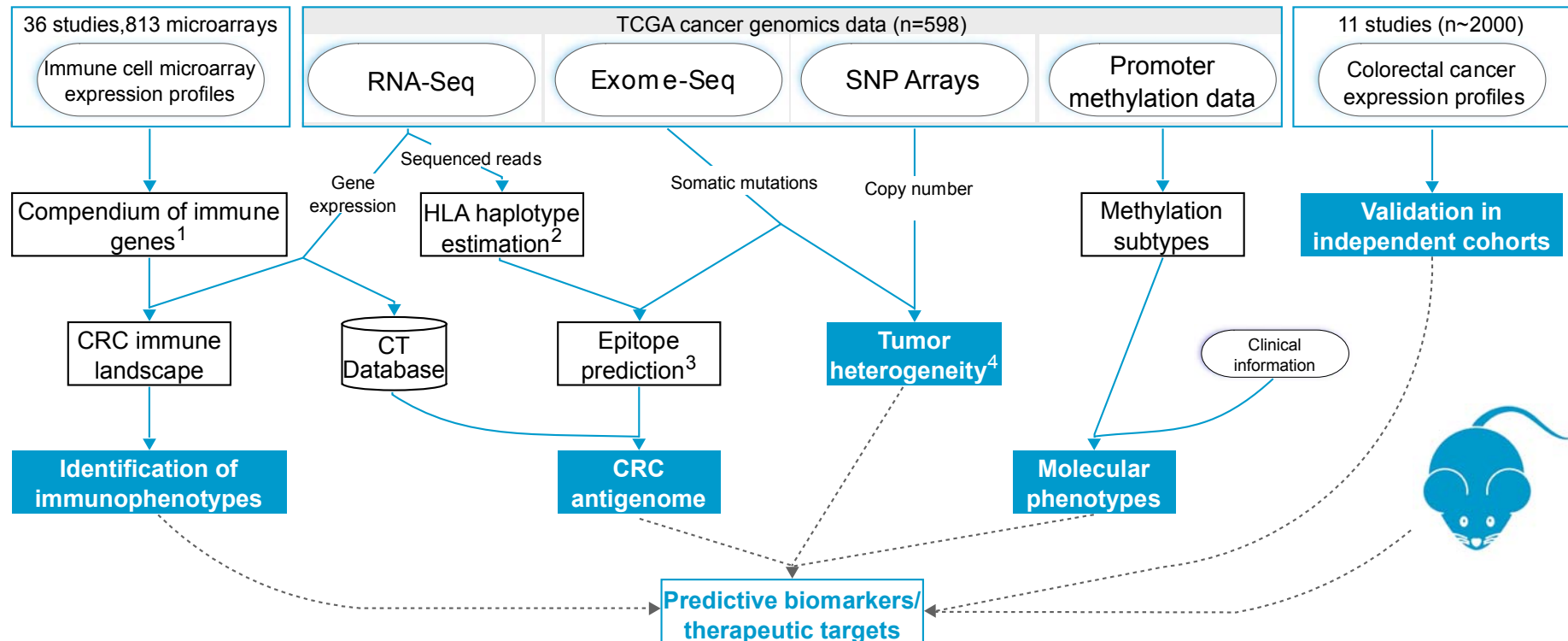
Tools/Methods
1. Bindea G, *et al. Immunity* 2013; 39:782-795
2. Warren R L *et al. Genome Medicine* 2012; 4**:** 95.
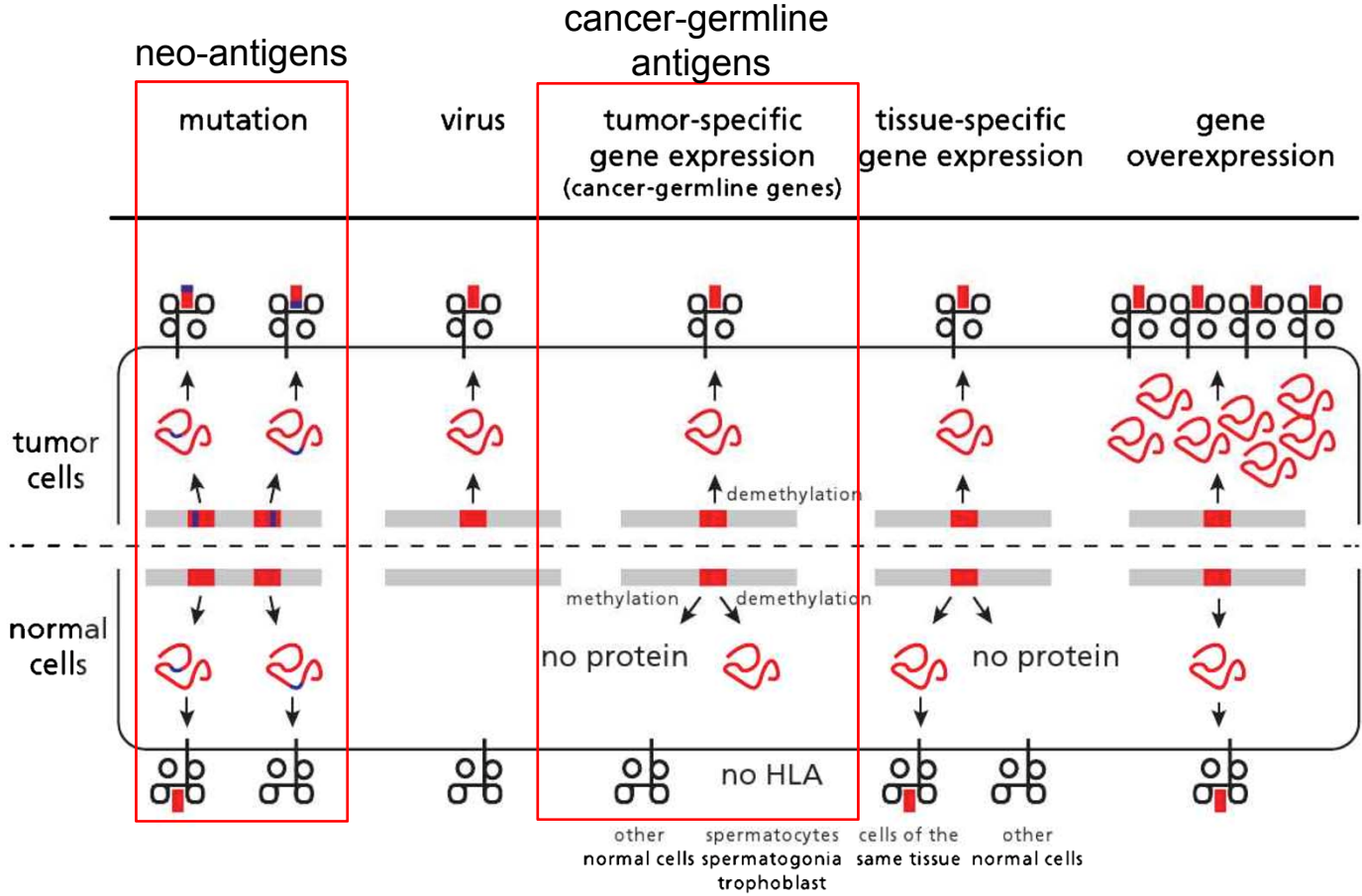3. Nielsen M *et al. PLoS ONE* 2007; 2**:** e796
4. Carter SL *et al. Nat Biotech* 2012; 30**:** 413–421

Data
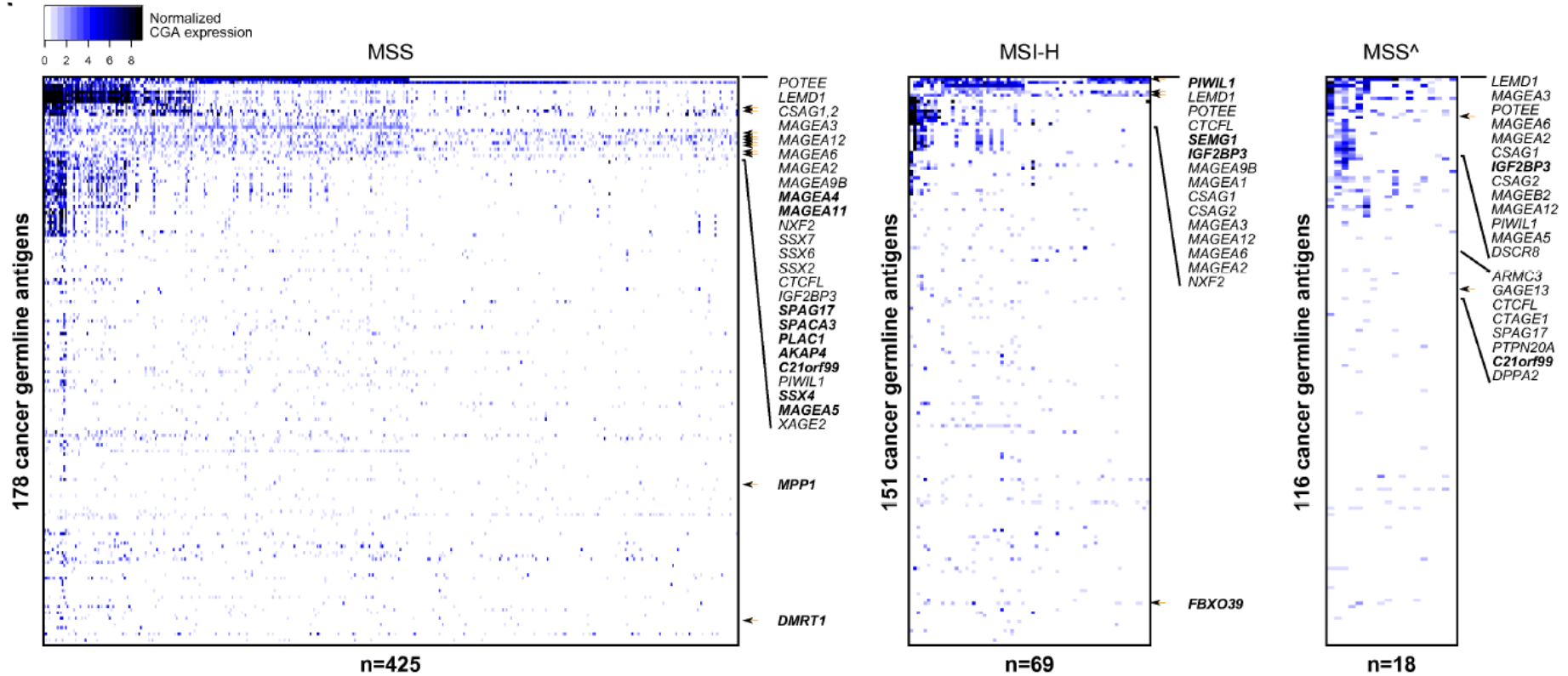The Cancer Genome Atlas Network. *Nature*; 487: 330-7
16.5 TB   microarrays: 25 GB, SNP-arrays 250 GB,
                    exome-Seq: 9 TB, RNA-seq: 7.2 TB

Classes of tumor antigens recognized by T-cells

Romero P, Coulie PG. Adaptive T-cell immunity and tumor antigen recognition. Tumor immunology and immunotherapy, Rees RC (Ed). Oxford University Press

Cancer-germline antigens in CRC

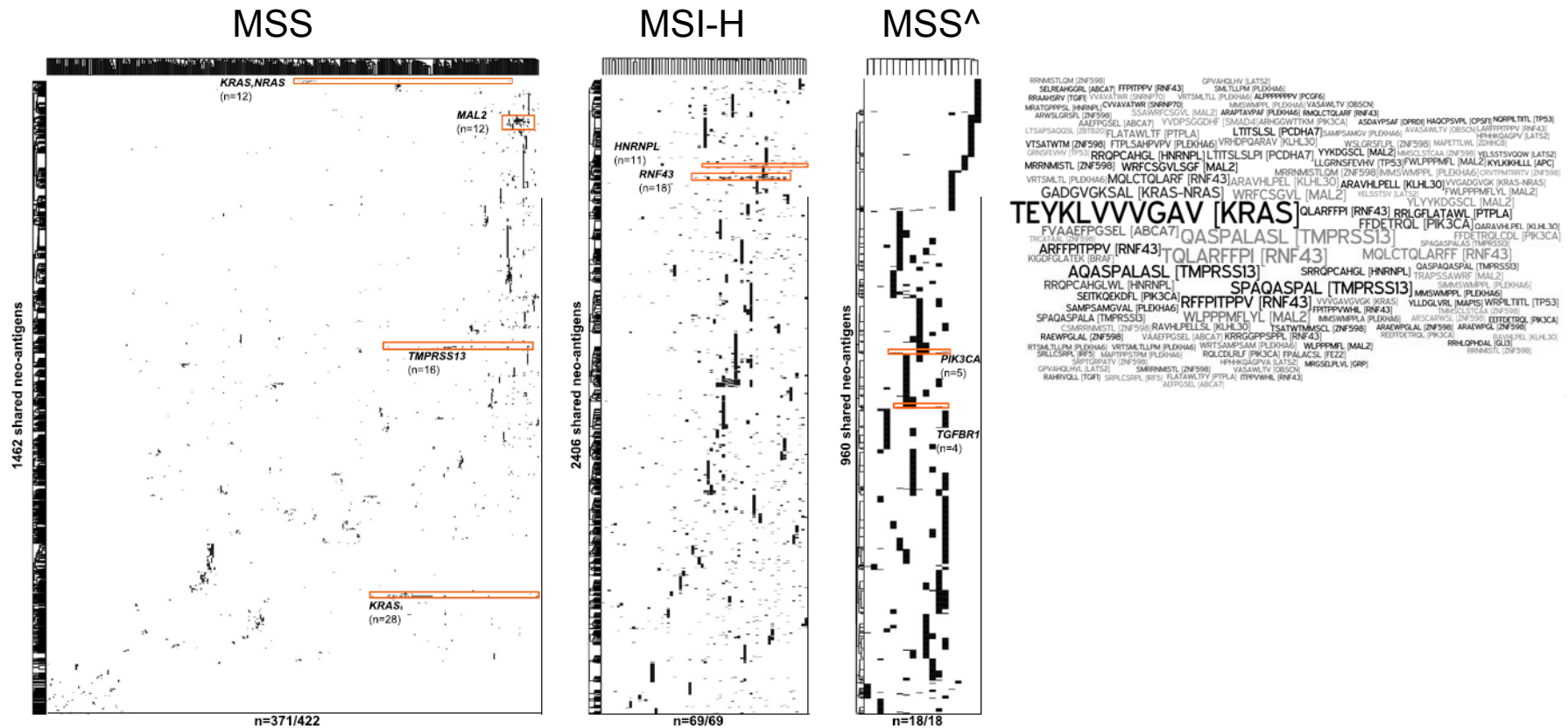# CRC landscape of neo-antigens is sparse

222.169 somatic mutations

Peptide length 8-11
HLA-miner => predict four-digit HLA class I allele ⎤⎦ NetMHCpan

Immune Epitope Database, Dana Faber repository, CIG-DB, MHCBN, PeptideDatabase,
SYFPEITHY, TANTIGEN only 13 were experimentally confirmed

Conclusion

Adaptive immunity is associated with lower tumor aggression and prolonged survival in CRC patients
Novel prognostic markers and identification of individuals at high risk for metastasis (TNM-I)

Immune landscape and tumor immunogenicity are evolving during progression of CRC
Development of strategies for modulating the immune landscape

2 novel targets for therapy:
- TNFRSF17(BCMA) identified using network approach and validated in 11 independent cohorts.
- CCR8 identified by linear modeling and feature reduction method. Ccr8 is not expressed in Rag1-/- mice indicating that is expressed in immune cells (CCR8-CCL1 axis might be different in endothelial tumors).

CRC antigenome is sparse (small number of neo-antigens are shared between patients)
Cancer vaccination strategy requires individualized multiepitope vaccines

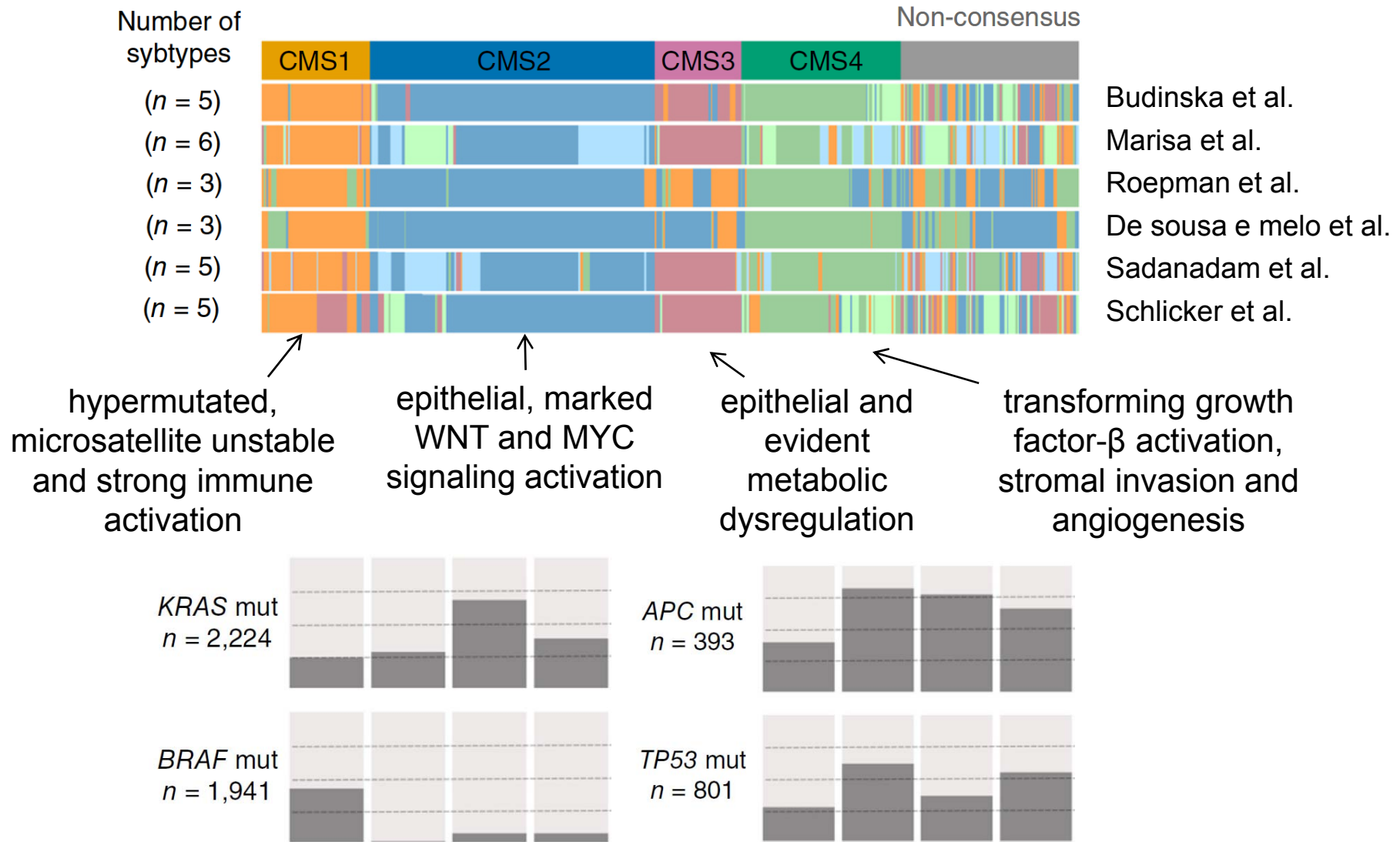Cancer germline antigens are expressed within more patients

Patients for cancer vaccination should be stratified rather on intratumoral immune landscape than on molecular phenotype

Inverse association between tumor heterogenity and immune responses

Conclusion

Angelova et al., *Oncoimmunology*, 2015
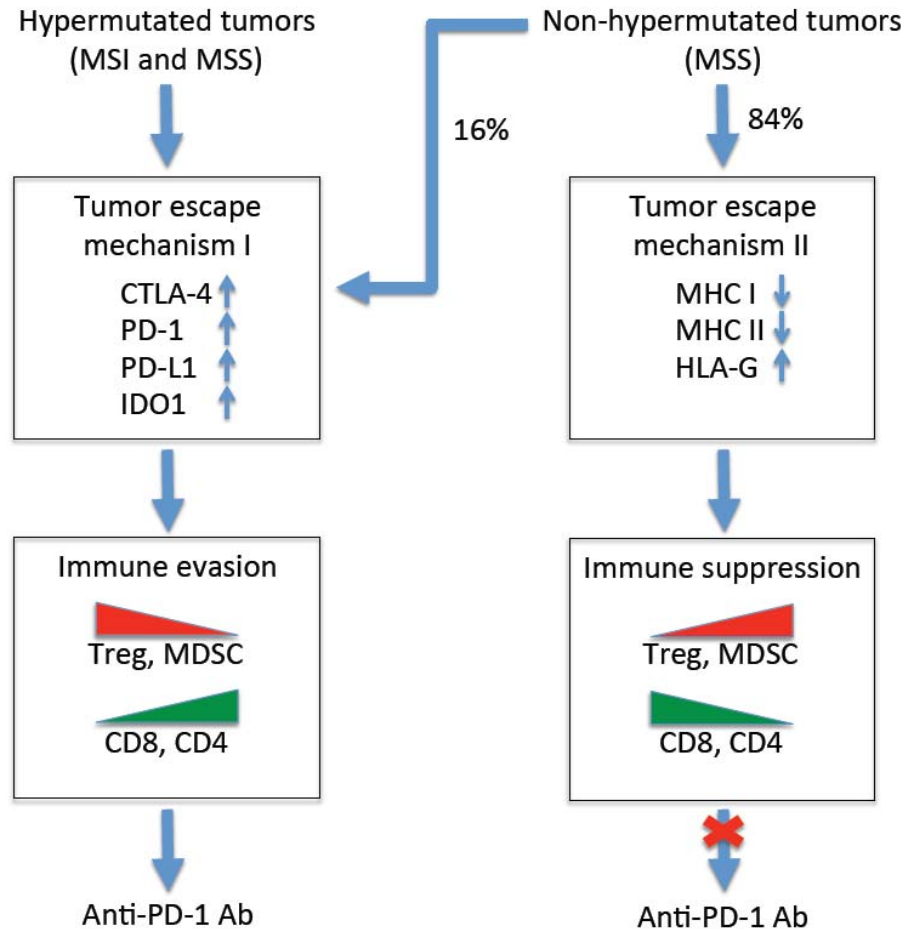
Consensus CRC molecular subtypes

Guinney et al. *Nature Med*. 2015. 21:1350-1356

Can microRNA expression signatures and there (predicted) target landscape be used to discern these molecular and cellular subtypes?

Genomic data

TCGA	GEO GSE35602

546 CRC tumors 11 tumor-normal pairs	14 tumor 4 normal epithelium epithelium stroma stroma

miRNAseq	miRNA microarrays (Agilent)

mRNAseqV2	mRNA microarrays (Agilent)

CNA	Methylation

Clinical information

TCGA network et al. *Nature.* 487:330-337

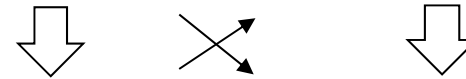Nishida N et al. *Clin Cancer Res.* 2012.18:3054-70

microRNA-target genes

In silico prediction tools

- miRanda (miRBase)
- miRanda (microrna.org)
- PITA (all targets 3/15 flank)
- PicTar (4-way)
- PicTar (5-way)
- TargetScan42 (conserved)
- TargetScan42 (noncons.)
- DIANA-microT
- EIMMo
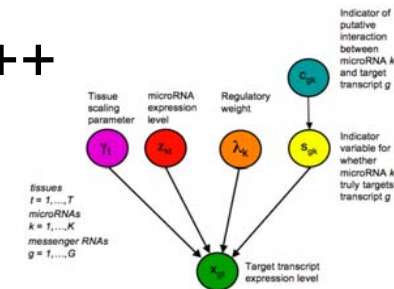
AGO-CLIP data sets

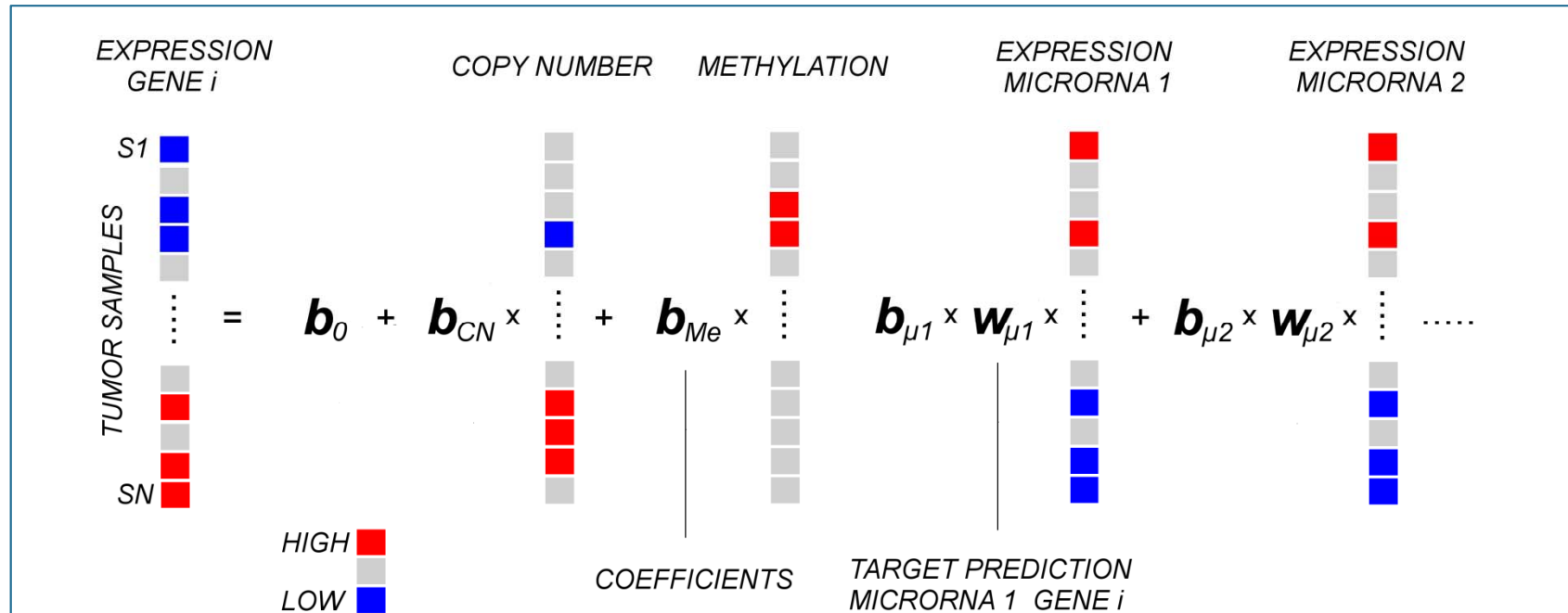**microRNA expression**

mRNA expression

Priors

GenMir++

Huang et al. *Nat Methods* 2007

Bayesian prediction score

Gene expression model
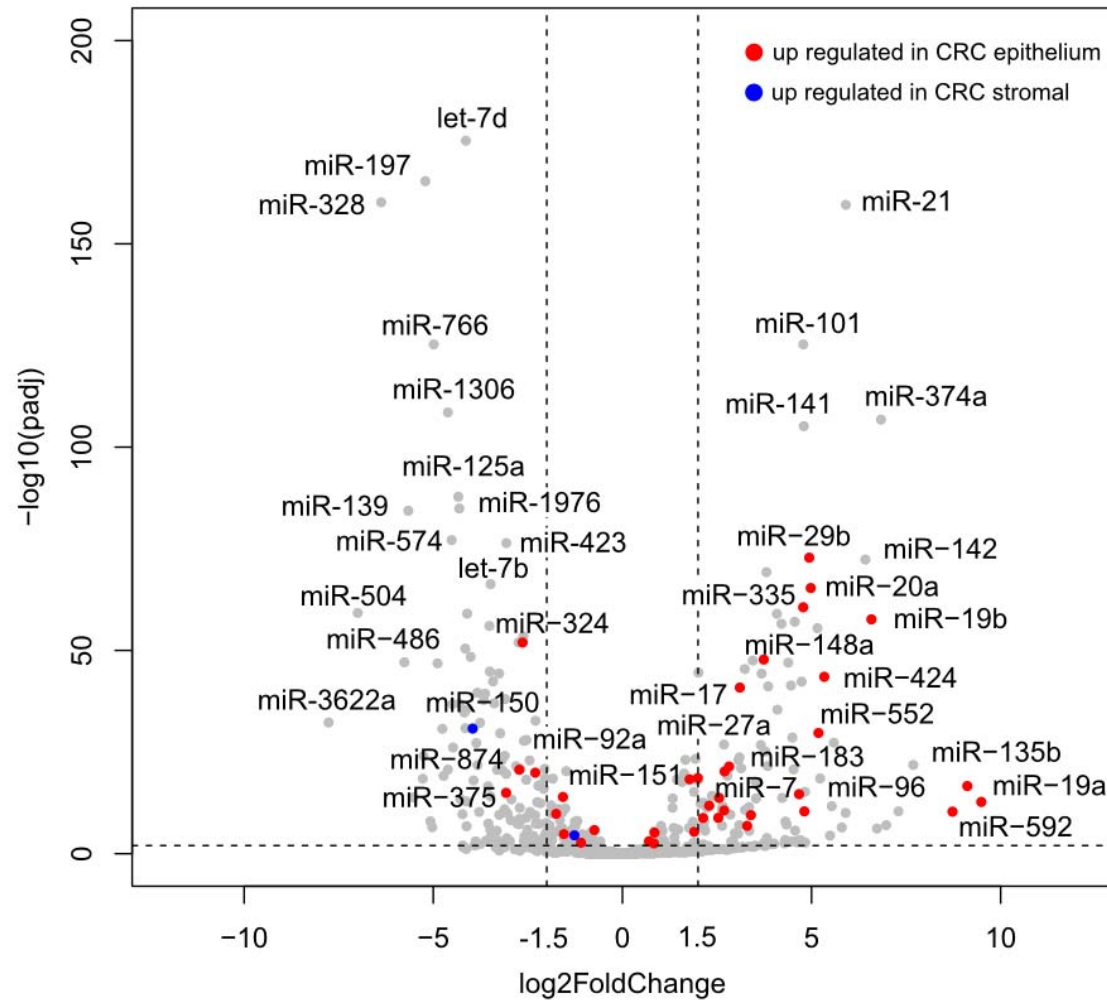
– Linear model of gene$_i$ expression in tumor samples

Least absolute shrinkage and selection operator (LASSO) and cross validation

Jacobsen et al. *Nature Struct Mol Biol* 2013, Setty et al. *Mol Sys Biol* 2012

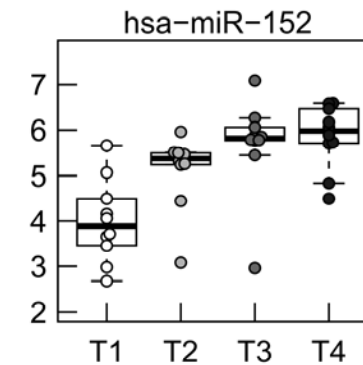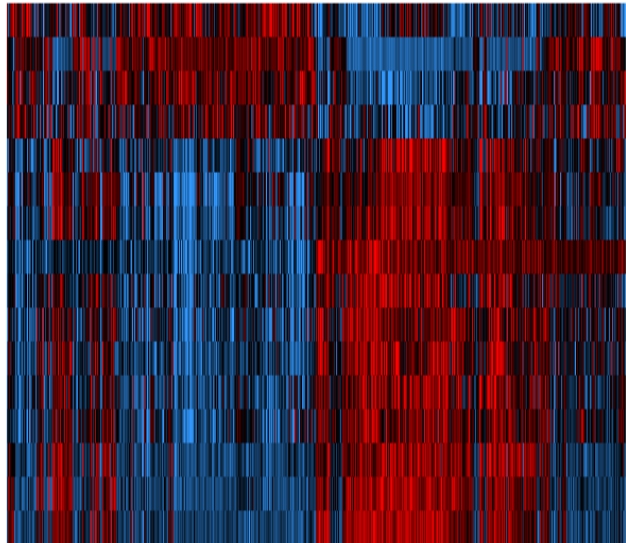# microRNAs differentially expressed between tumor and normal tissue
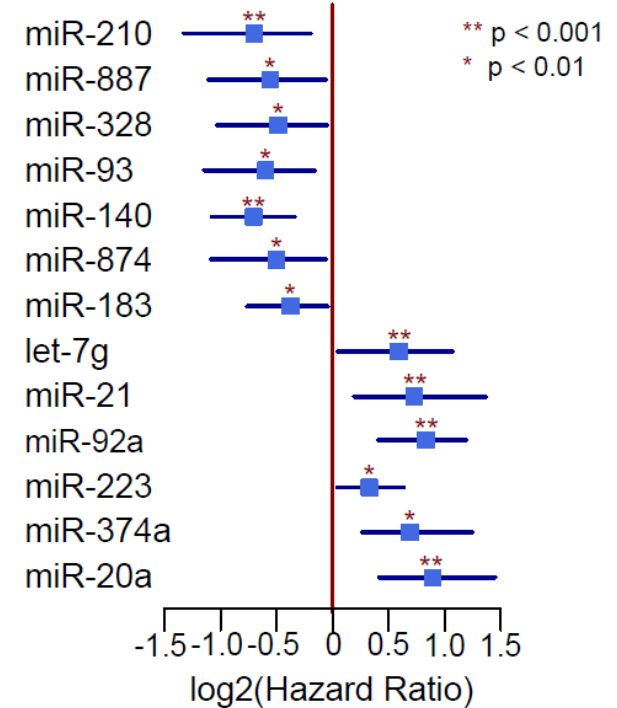
microRNAs differentially expressed between between tumor stages
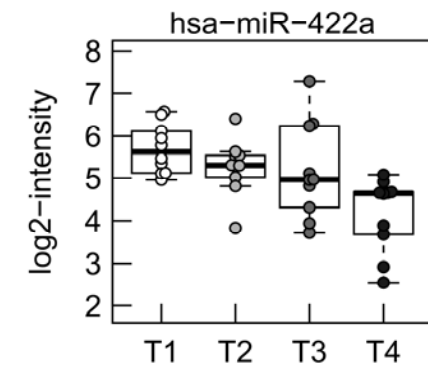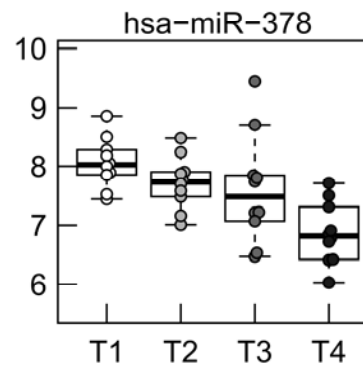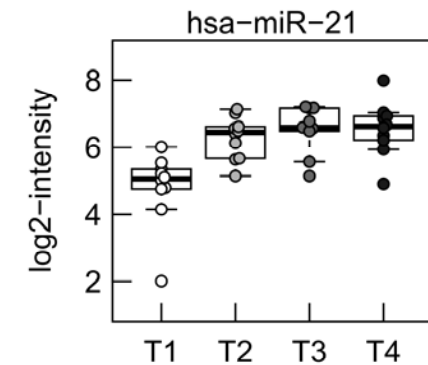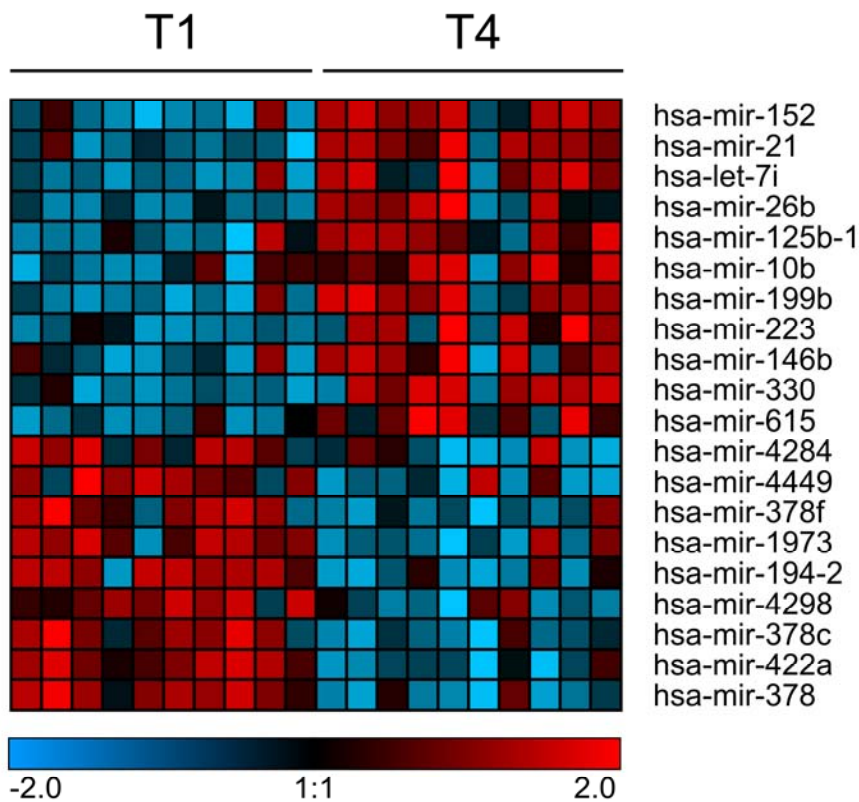
Log2-FC (T4vsT1)>1.5; adj. p<0.
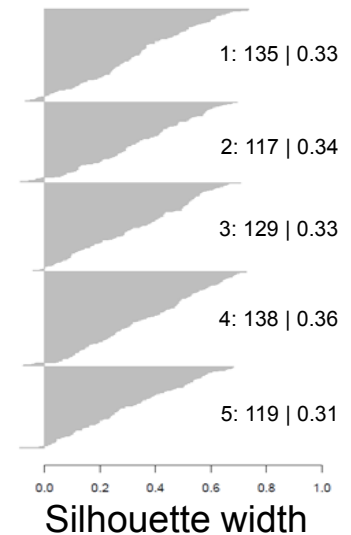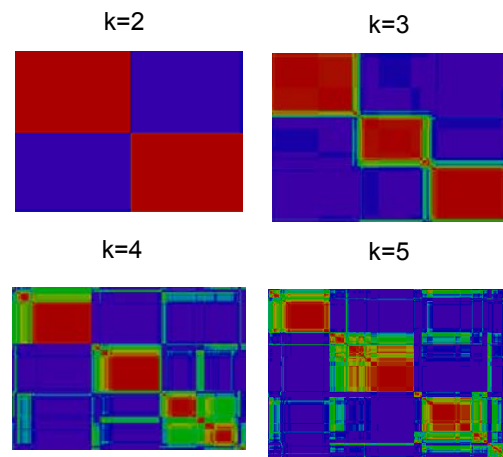 Spearman rank correlation (T1,T2,T3,T4) rho>0.5 (<-0.5)
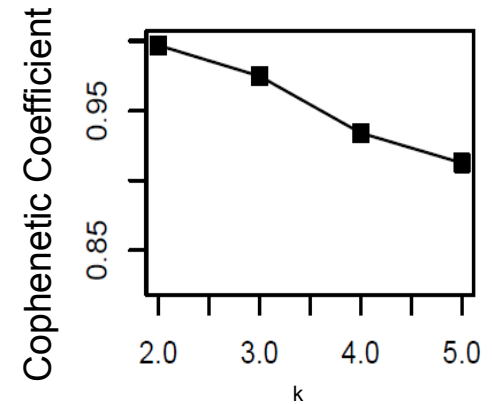
Identification of microRNA based CRC classification
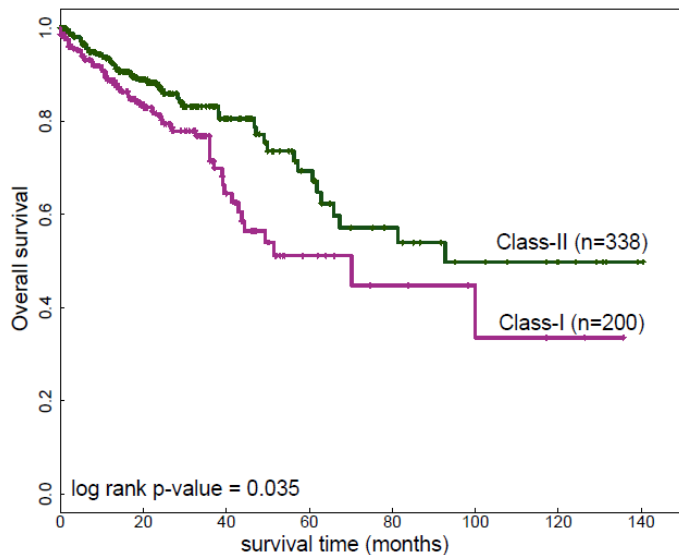
MicroRNA based CRC subtype classification
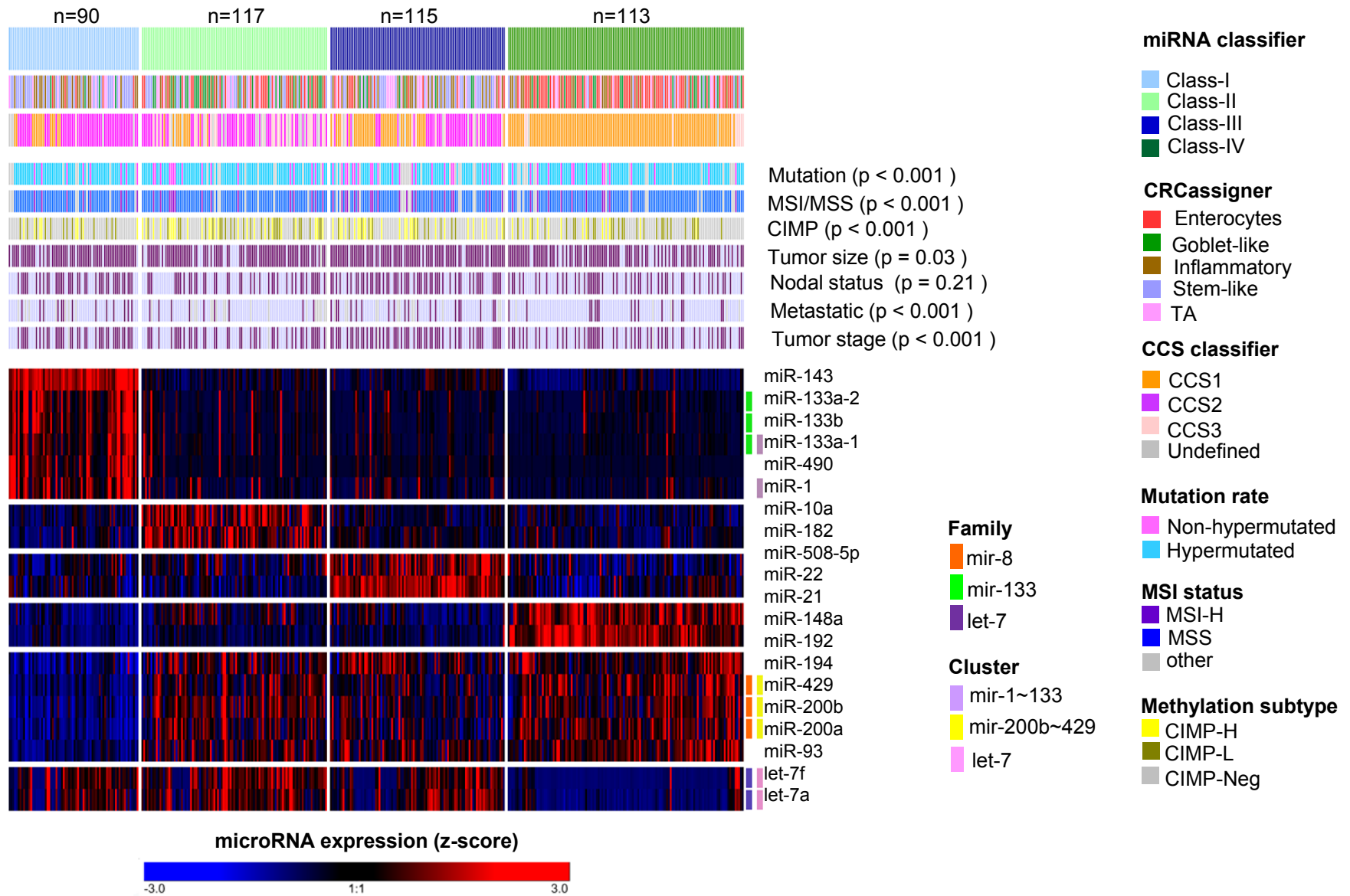

Sadanandam et al. 2013 *Nature Med* 19:619-25. (2013) , De Sousa EM et al. 19:614-8 *Nature Med.* (2013)

TCGA (n=403)

miRNA	
mir-143	*
mir-133a-2	*
mir-133b	*
mir-133a-1	*
mir-490	*
mir-1	*
mir-10	*
mir-182	
mir-22	
mir-21	
mir-148a	
mir-192	*
mir-194	*
mir-429	
mir-200b	
mir-200a	*
mir-93	*
let-7f	*
let-7a	*

−1.5 −1 −0.5 0 0.5 1 1.5 2

log2 Hazard ratio (95% CI)

GSE29622 (n=65)

miRNA	
mir-143	
mir-133a	
mir-133b	
mir-490-3p	
mir-1	
mir-10a	*
mir-182	
mir-22	
mir-21	
mir-148a	
mir-192	
mir-194	*
mir-429	*
mir-200a	*
mir-200b	
mir-93	*
let-7a	
let-7f	

−2 −1 0 1 2 3

log2 Hazard ratio (95% CI)

MicroRNAs discerning cellular CRC subtypes
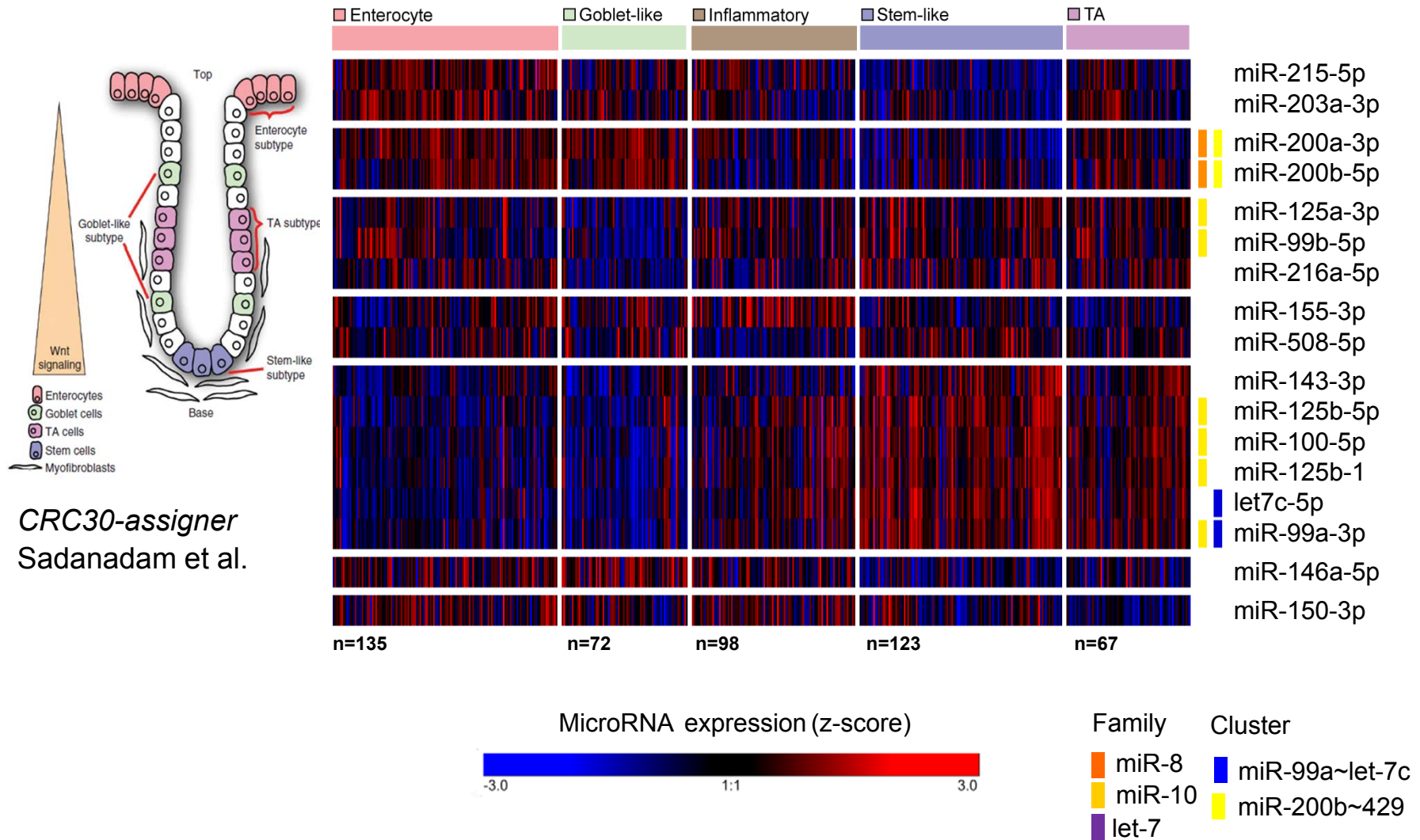
CRC30-assigner
Sadanadam et al.

Enterocyte | Goblet-like | Inflammatory | Stem-like | TA

miR-215-5p
miR-203a-3p
miR-200a-3p
miR-200b-5p
miR-125a-3p
miR-99b-5p
miR-216a-5p
miR-155-3p
miR-508-5p
miR-143-3p
miR-125b-5p
miR-100-5p
miR-125b-1
let7c-5p
miR-99a-3p
miR-146a-5p
miR-150-3p

n=135   n=72   n=98   n=123   n=67

MicroRNA expression (z-score)

-3.0   1:1   3.0

Family
miR-8
miR-10
let-7

Cluster
miR-99a~let-7c
miR-200b~429
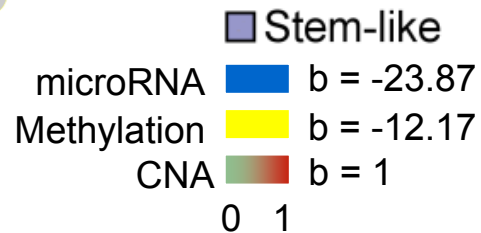
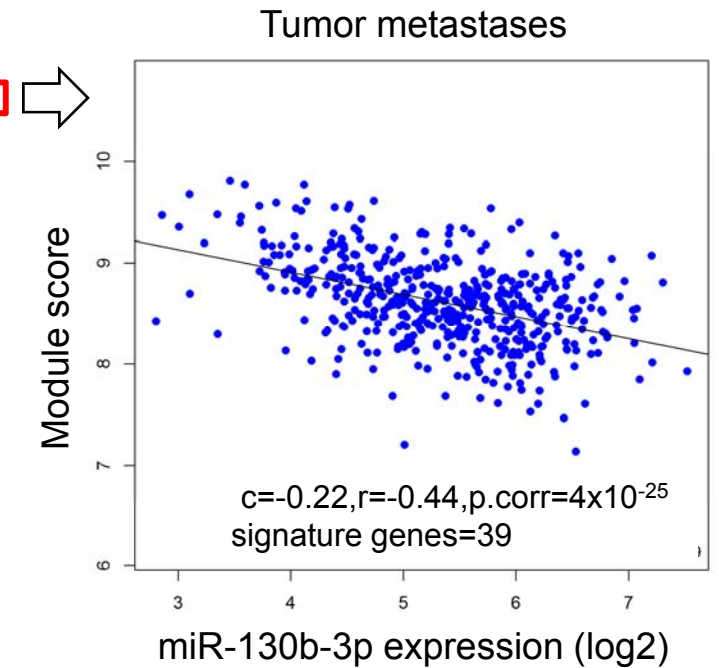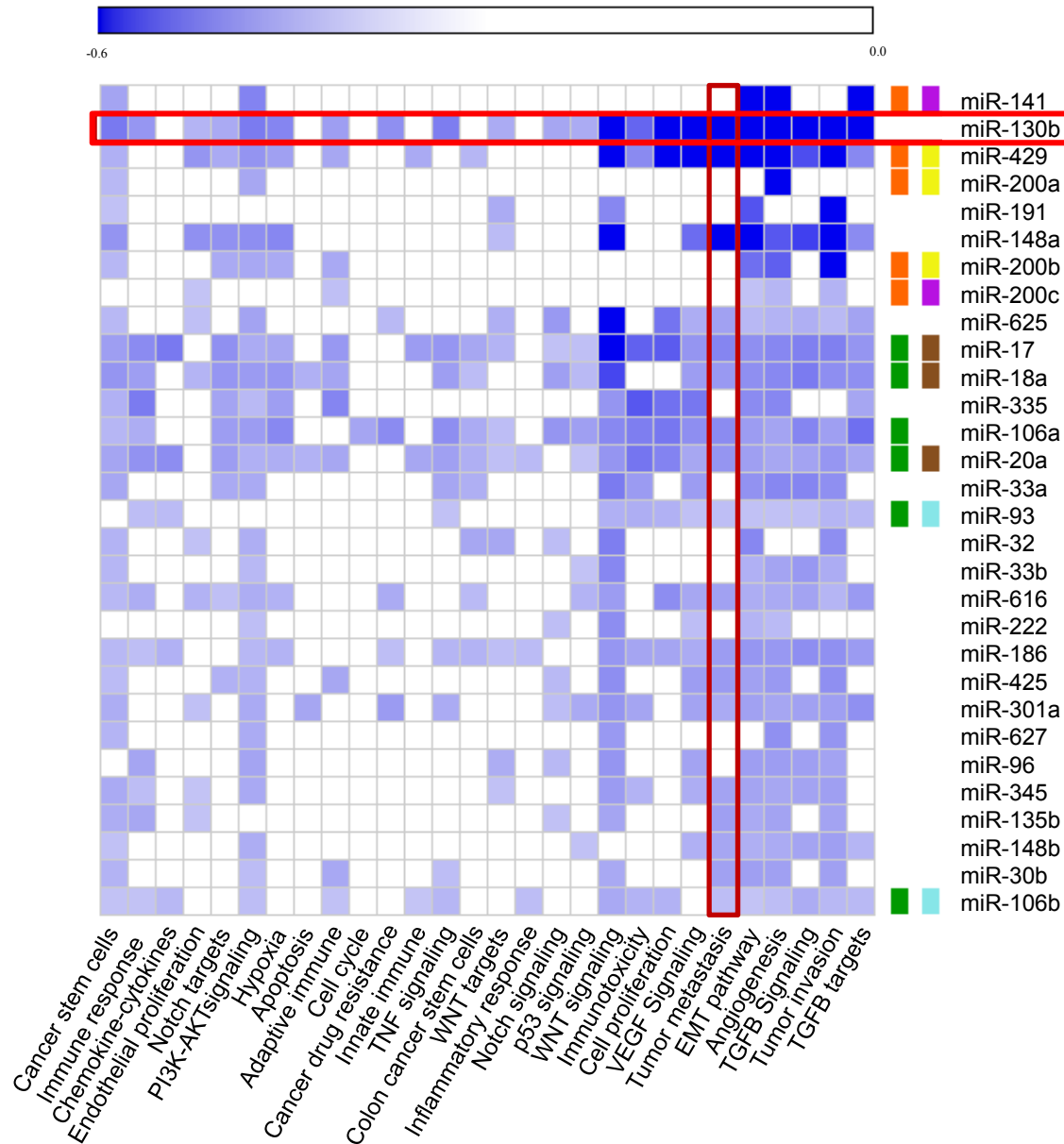# microRNAs targeting CRC30 assigner genes
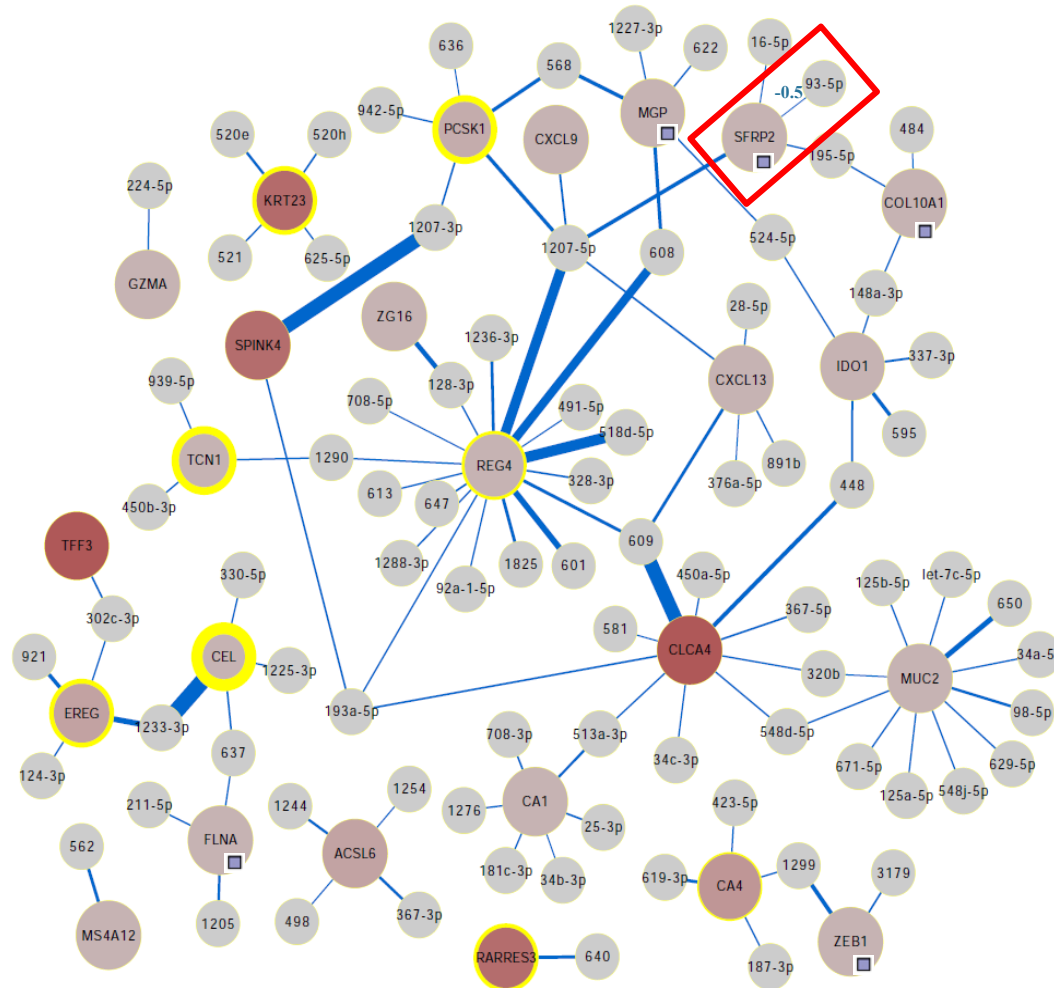
hsa-miR-93 down-regulated in CRC stem cells (SW1116csc)
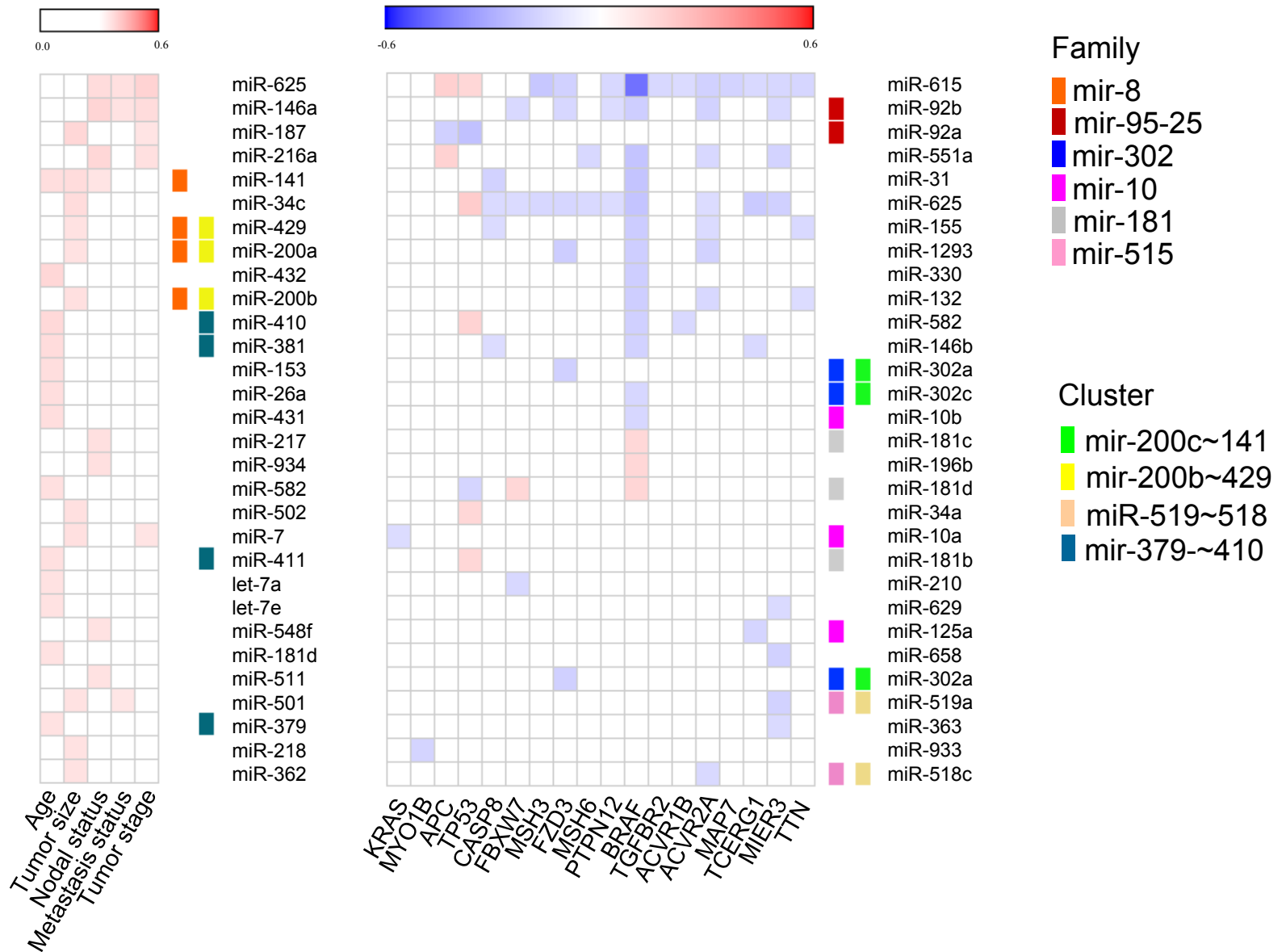
Yu XF et al. *WJ Gastroenterol*. 2011

Biological processes associated with miRNAs
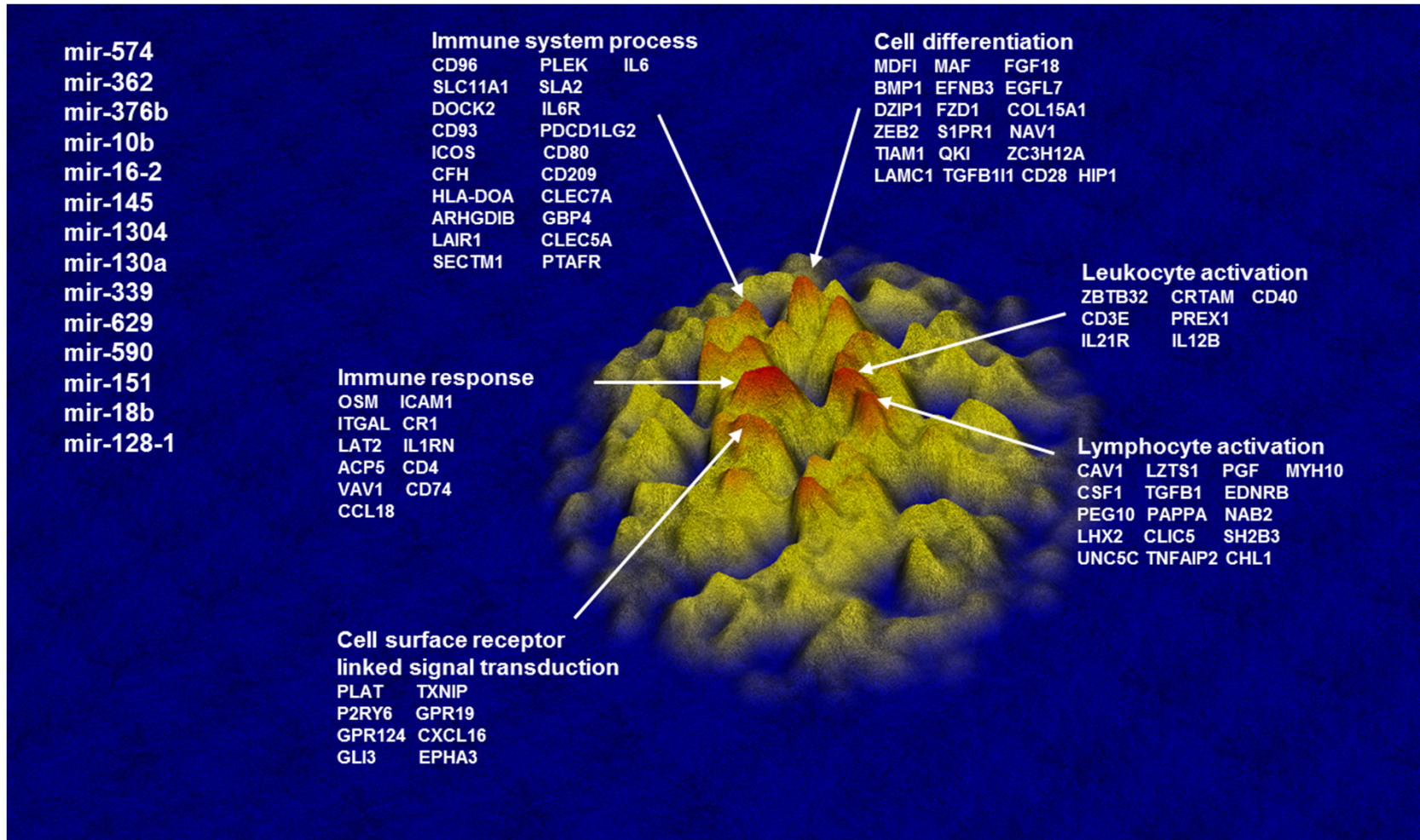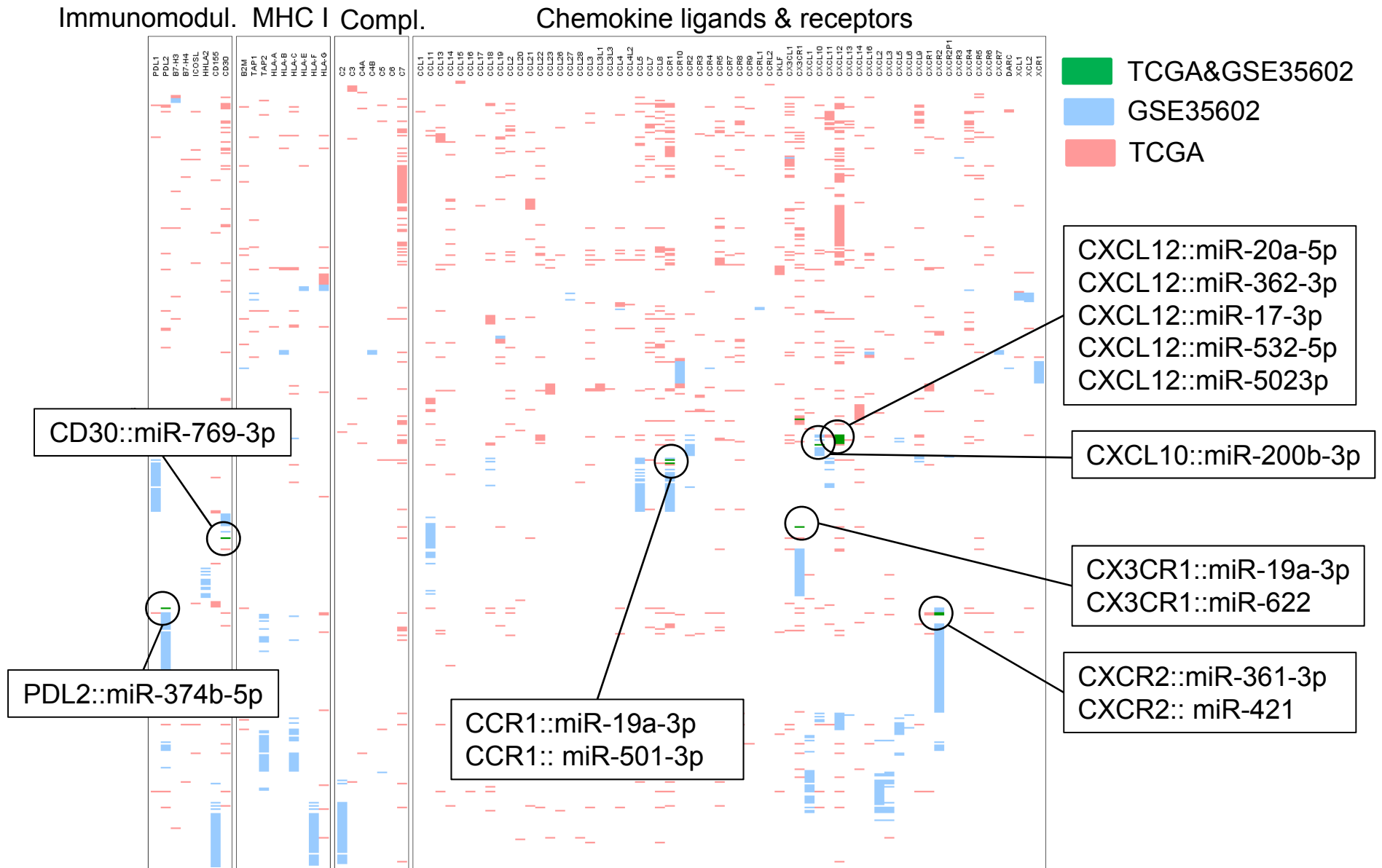
Clinicopathologic factors and mutated genes associated with miRNAs

MicroRNA target landscape

MicroRNAs targeting immune related genes in CRC epithelium

Summary

MicroRNA signatures discerning molecular and cellular subtypes

Cluster

mir-200c~141
mir-200b~200a~429
mir-17~92
mir-106b~25

miR-93
– part of mir-106b~25
– potentially targeting SFRP2 (CRC30 assigner, Wnt signaling)
– down-regulated in CRC stem cells
– in 4 group classification signature
– significant good OS prognosis

miR-130b
– in module score associated with many cancer processes

let-7 family
– let-7f in 4&2 group classification signature, bad OS prognosis
– let-7a in 4 group classification signature, bad OS prognosis
– let-7c (cluster with miR-99) in cellular subtypes signature
– let-7g bad OS prognosis
– let-7i associated with T-stages

PDL2::miR-374b-5p (in epithelium)
– target for immunotherapy

miR-21
– tumor-normal, associated with T-stages, bad OS prognosis

miR-143
– bad OS prognosis, in all classification signatures

Immune and molecular landscape in colorectal cancer

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