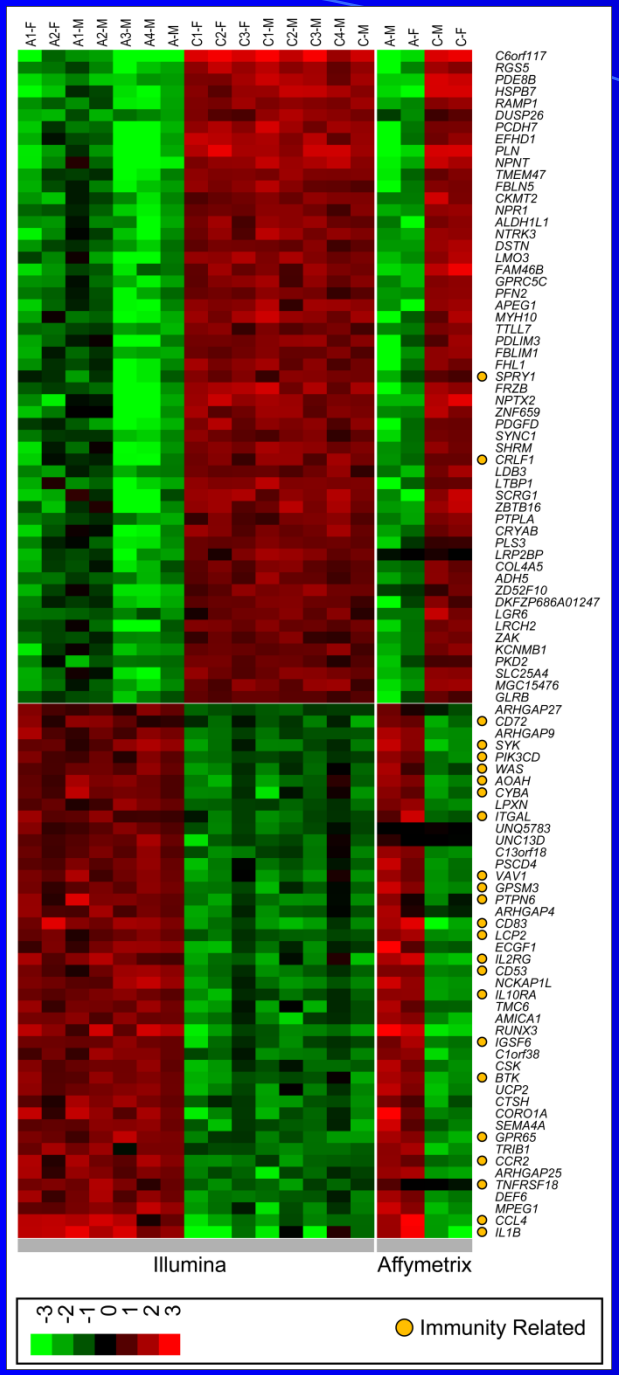
Transcriptional Genomics of AAA

IMAD 3, Liège 2012-10-05

mRNA Expression

- Isolate RNA from control and AAA tissue
- Microarray (printed, Affymetrix, Illumina)
  - Hybridization
  - Signal intensity ~ proportional to copy number
- Sequence (RNA-Seq)
  - Count of reads ~ proportional to copy number



Differential Expression Patterns

- Global gene expression profiles for AAA and control abdominal aorta samples
- Two different microarray platforms
  - Illumina
  - Affymetrix
- 3,274 genes with significantly different expression levels

Lenk et al. BMC Genomics 2007

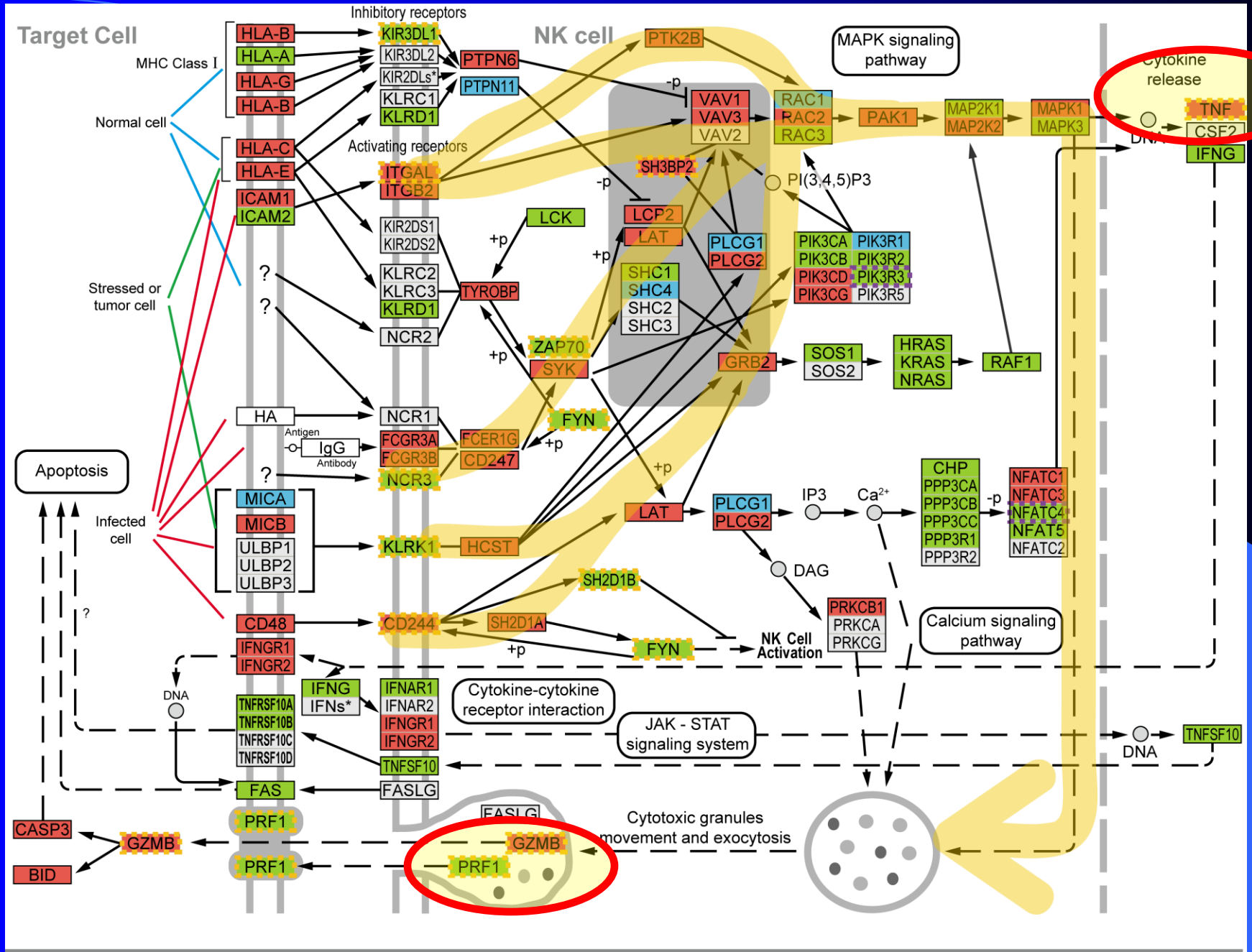
Making sense of complexity

How does one comprehend 3,274 genes

Pick and choose my favorite genes?

Approaches for Follow-up

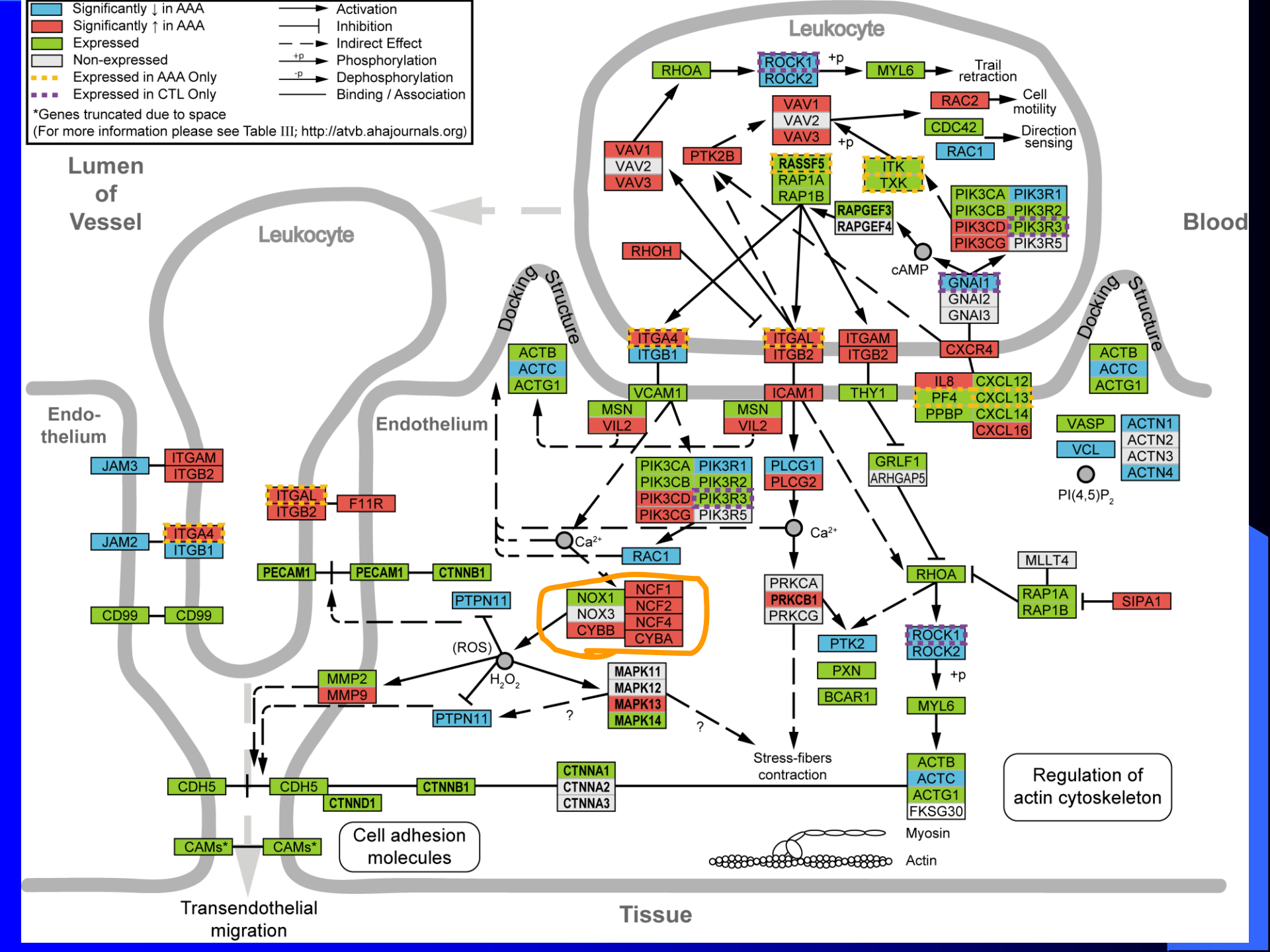
- *In silico* functional analysis
  - Functional classification: Gene Ontology (GO)
  - Pathway analysis: Kyoto Encyclopedia of Genes and Genomes (KEGG)
- *In silico* transcriptional genomics
  - Search for Transcription Factor Binding Sites (TFBS) in promoter sequences



Target Cell
NK cell
Inhibitory receptors
Activating receptors
MHC Class I
Normal cell
Stressed or tumor cell
Infected cell
Apoptosis
HLA-B
HLA-A
HLA-G
HLA-B
HLA-C
HLA-E
ICAM1
ICAM2
HA
Antigen
IgG
Antibody
MICA
MICB
ULBP1
ULBP2
ULBP3
CD48
IFNGR1
IFNGR2
DNA
TNFRSF10A
TNFRSF10B
TNFRSF10C
TNFRSF10D
FAS
CASP3
BID
GZMB
PRF1
KIR3DL1
KIR3DL2
KIR2DLs*
KLRC1
KLRD1
ITGAL
ITGB2
KIR2DS1
KIR2DS2
KLRC2
KLRC3
KLRD1
NCR2
NCR1
FCGR3A
FCGR3B
NCR3
KLRK1
CD244
IFNG
IFNs*
IFNAR1
IFNAR2
IFNGR1
IFNGR2
TNFSF10
FASLG
PTPN6
PTPN11
LCK
TYROBP
ZAP70
SYK
FCER1G
CD247
FYN
HCST
SH2D1A
SH2D1B
FYN
PTK2B
VAV1
VAV3
VAV2
SH3BP2
LCP2
LAT
SHC1
SHC4
SHC2
SHC3
PLCG1
PLCG2
GRB2
LAT
PLCG1
PLCG2
IP3
Ca2+
DAG
PRKCB1
PRKCA
PRKCG
NK Cell Activation
MAPK signaling pathway
RAC1
RAC2
RAC3
PAK1
MAP2K1
MAP2K2
MAPK1
MAPK3
PI(3,4,5)P3
PIK3CA
PIK3CB
PIK3CD
PIK3CG
PIK3R1
PIK3R2
PIK3R3
PIK3R5
SOS1
SOS2
HRAS
KRAS
NRAS
RAF1
CHP
PPP3CA
PPP3CB
PPP3CC
PPP3R1
PPP3R2
NFATC1
NFATC3
NFATC4
NFAT5
NFATC2
Calcium signaling pathway
Cytokine release
TNF
CSF2
IFNG
DNA
TNFSF10
Cytokine-cytokine receptor interaction
JAK - STAT signaling system
Cytotoxic granules movement and exocytosis
Significantly ↓ in AAA
Significantly ↑ in AAA
Expressed
Non-expressed
Expressed in AAA Only
Expressed in CTL Only
Activation
Inhibition
Indirect Effect
Phosphorylation
Dephosphorylation
Binding / Association
*Non-expressed gene symbols not included to save space (For more information Table II; please see http://atvb.ahajournals.org)

NK Cytotoxicity Pathway

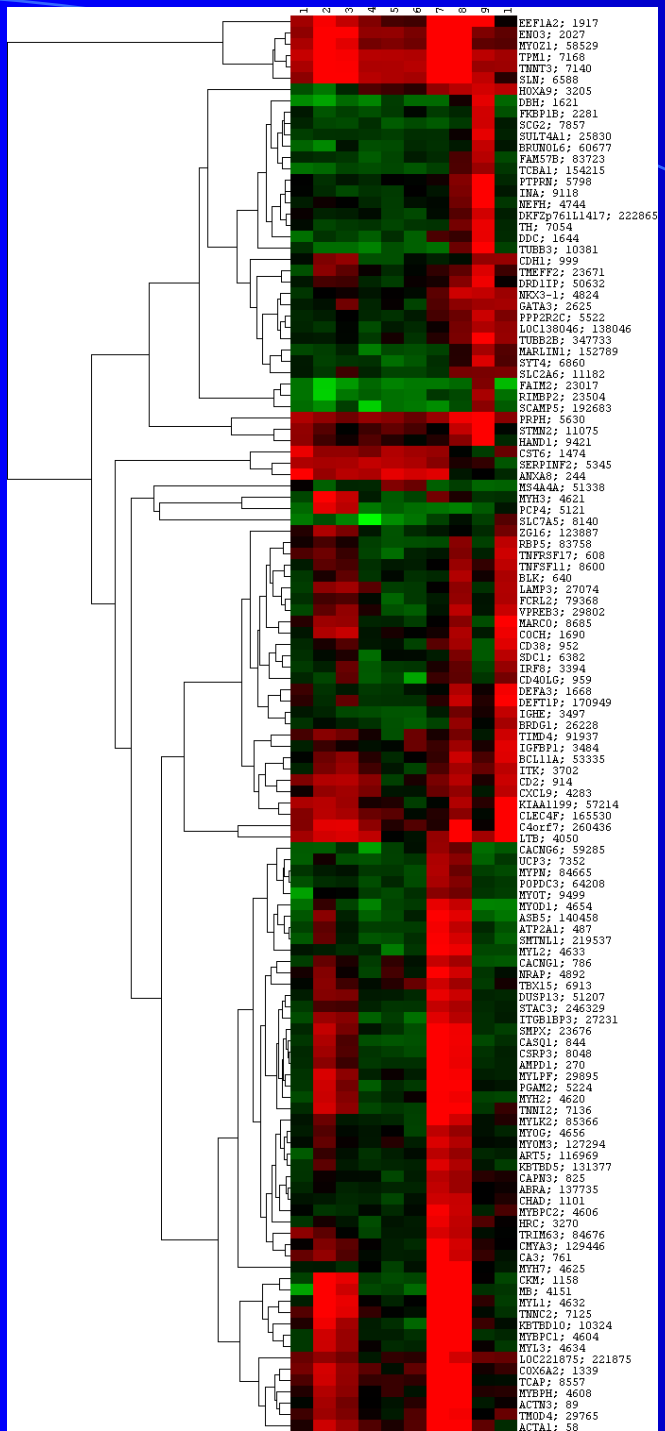
- Apoptosis of the SMCs in AAA is well known
- Perforin (as well as other proteins) have been shown in AAA using histology previously
- Increased cytotoxicity in AAA


Significantly ↓ in AAA
Significantly ↑ in AAA
Expressed
Non-expressed
Expressed in AAA Only
Expressed in CTL Only
*Genes truncated due to space
(For more information please see Table III; http://atvb.ahajournals.org)
Activation
Inhibition
Indirect Effect
Phosphorylation
Dephosphorylation
Binding / Association
Lumen of Vessel
Leukocyte
Blood
Endo-thelium
Endothelium
Tissue
Docking Structure
RHOA
ROCK1
ROCK2
MYL6
Trail retraction
RAC2
Cell motility
CDC42
Direction sensing
RAC1
VAV1
VAV2
VAV3
PTK2B
RASSF5
RAP1A
RAP1B
ITK
TXK
RAPGEF3
RAPGEF4
PIK3CA PIK3R1
PIK3CB PIK3R2
PIK3CD PIK3R3
PIK3CG PIK3R5
RHOH
cAMP
GNAI1
GNAI2
GNAI3
ITGA4
ITGB1
ITGAL
ITGB2
ITGAM
ITGB2
CXCR4
ACTB
ACTC
ACTG1
VCAM1
ICAM1
THY1
IL8
PF4
PPBP
CXCL12
CXCL13
CXCL14
CXCL16
MSN
VIL2
VASP
VCL
PI(4,5)P2
ACTN1
ACTN2
ACTN3
ACTN4
JAM3
JAM2
CD99
F11R
PECAM1
CTNNB1
Ca2+
PLCG1
PLCG2
GRLF1
ARHGAP5
PTPN11
NOX1
NOX3
CYBB
NCF1
NCF2
NCF4
CYBA
(ROS)
H2O2
PRKCA
PRKCB1
PRKCG
PTK2
PXN
BCAR1
MLLT4
SIPA1
MMP2
MMP9
MAPK11
MAPK12
MAPK13
MAPK14
?
Stress-fibers contraction
MYL6
CDH5
CTNND1
CTNNA1
CTNNA2
CTNNA3
FKSG30
Myosin
Actin
CAMs*
Cell adhesion molecules
Regulation of actin cytoskeleton
Transendothelial migration

Leukocyte TransEndothelial Migration (LTEM)

- Immune infiltrates are present in AAA
- Increased expression of “leukocyte” genes fits well with previous data
- Increase in expression of NADPH oxidase
  - Work by others indicates involvement of ROS in AAA

KEGG pathway	Probed*	Observed	Expected	FDR
Regulation of actin cytoskeleton (RAC)	204	76	36.99	4.71e-09
Cell adhesion molecules (CAMs)	127	51	23.03	1.63e-07
Focal adhesion (FA)	208	72	37.71	1.98e-07
Natural killer cell mediated cytotoxicity (NK)	127	47	23.03	5.94e-06
Leukocyte transendothelial migration (LTEM)	115	43	20.85	1.06e-05
Type I diabetes mellitus (T1DM)	42	20	7.62	1.29e-04
ECM-receptor interaction (ECM)	86	31	15.59	5.69e-04
T cell receptor signaling pathway (T cell)	99	34	17.95	6.93e-04
Hematopoietic cell lineage (HCL)	84	30	15.23	7.03e-04
B cell receptor signaling pathway (B cell)	70	26	12.69	8.65e-04
Adherens junction (AJ)	76	27	13.78	1.38e-03
Gap junction (GJ)	96	32	17.41	1.38e-03
Calcium signaling pathway (Ca2+)	175	50	31.73	2.12e-03
Antigen processing and presentation (AP&P)	79	27	14.32	2.12e-03
Cytokine-cytokine receptor interaction (CCRI)	247	66	44.78	2.12e-03
Tight junction (TJ)	116	36	21.03	2.12e-03
Alzheimer's disease (AD)	22	11	3.99	2.60e-03
MAPK signaling pathway (MAPK)	270	69	48.95	5.10e-03
Long-term potentiation (LTP)	68	21	12.33	2.58e-02
Wnt signaling pathway (WNT)	144	38	26.11	2.87e-02
Axon guidance (AG)	129	34	23.39	4.02e-02



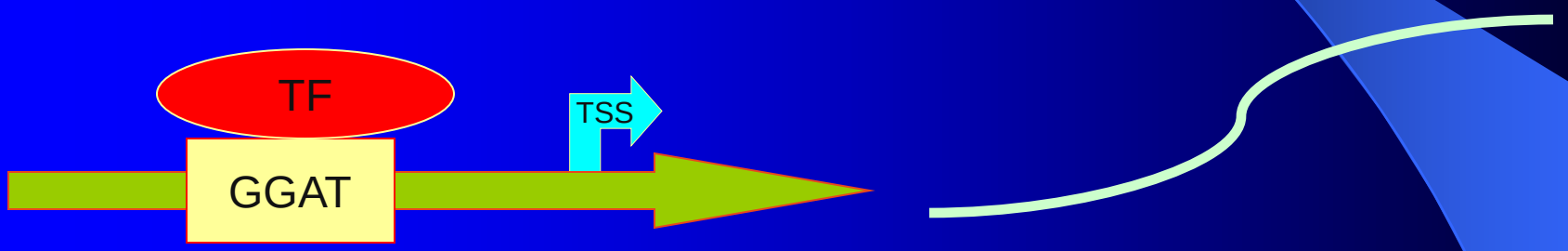
Regional Variation of mRNA Expression In the Aorta

- Baboon euthanized at Southwest National Primate Research Center by collaborator Laura A. Cox
- from the heart to the femoral arteries

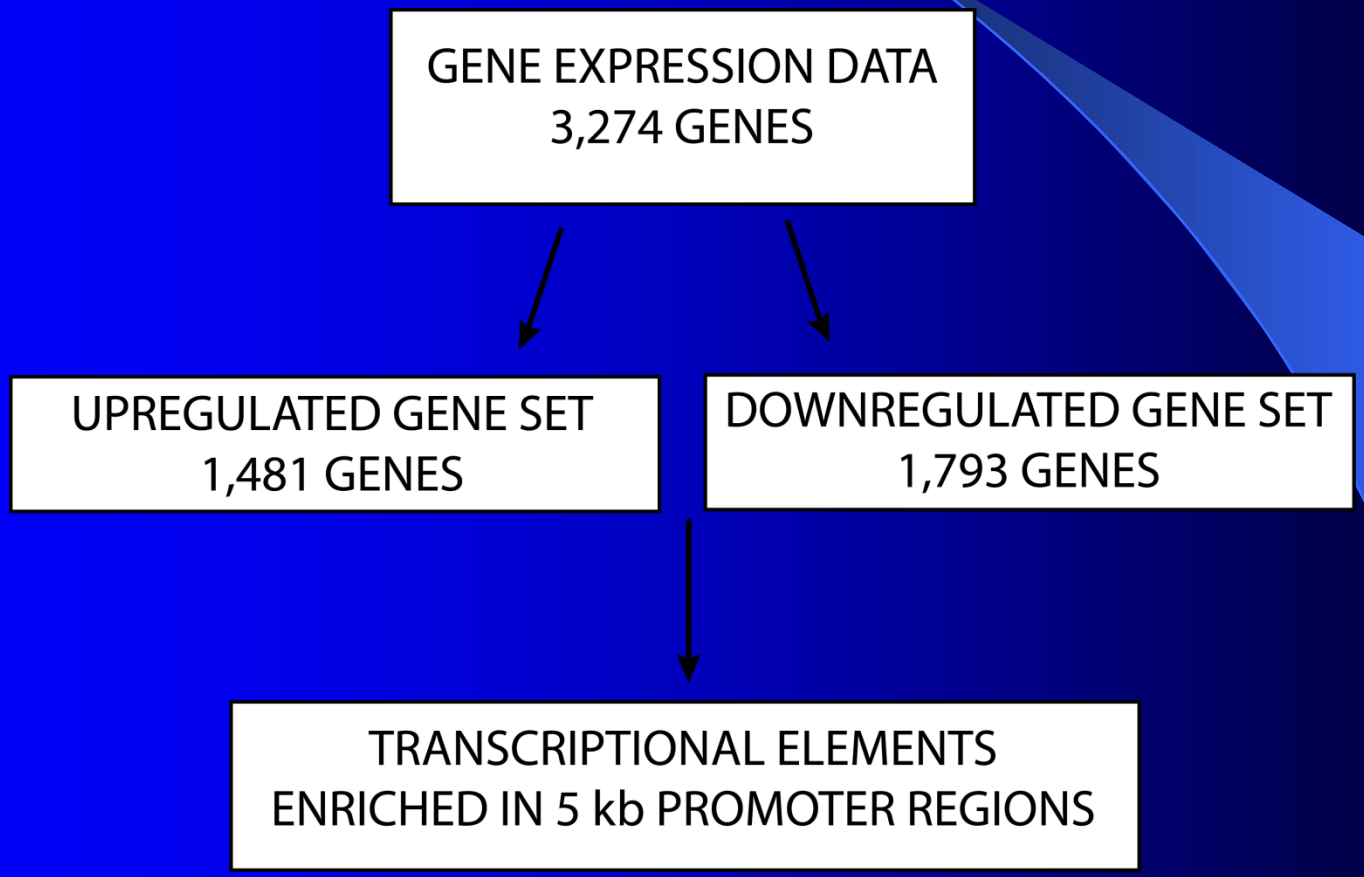
Approaches for Follow-up

- *In silico* functional analysis
  - Functional classification: Gene Ontology (GO)
  - Pathway analysis: Kyoto Encyclopedia of Genes and Genomes (KEGG)
- ***In silico* transcriptional genomics**
  - **Search for Transcription Factor Binding Sites (TFBS) in promoter sequences**

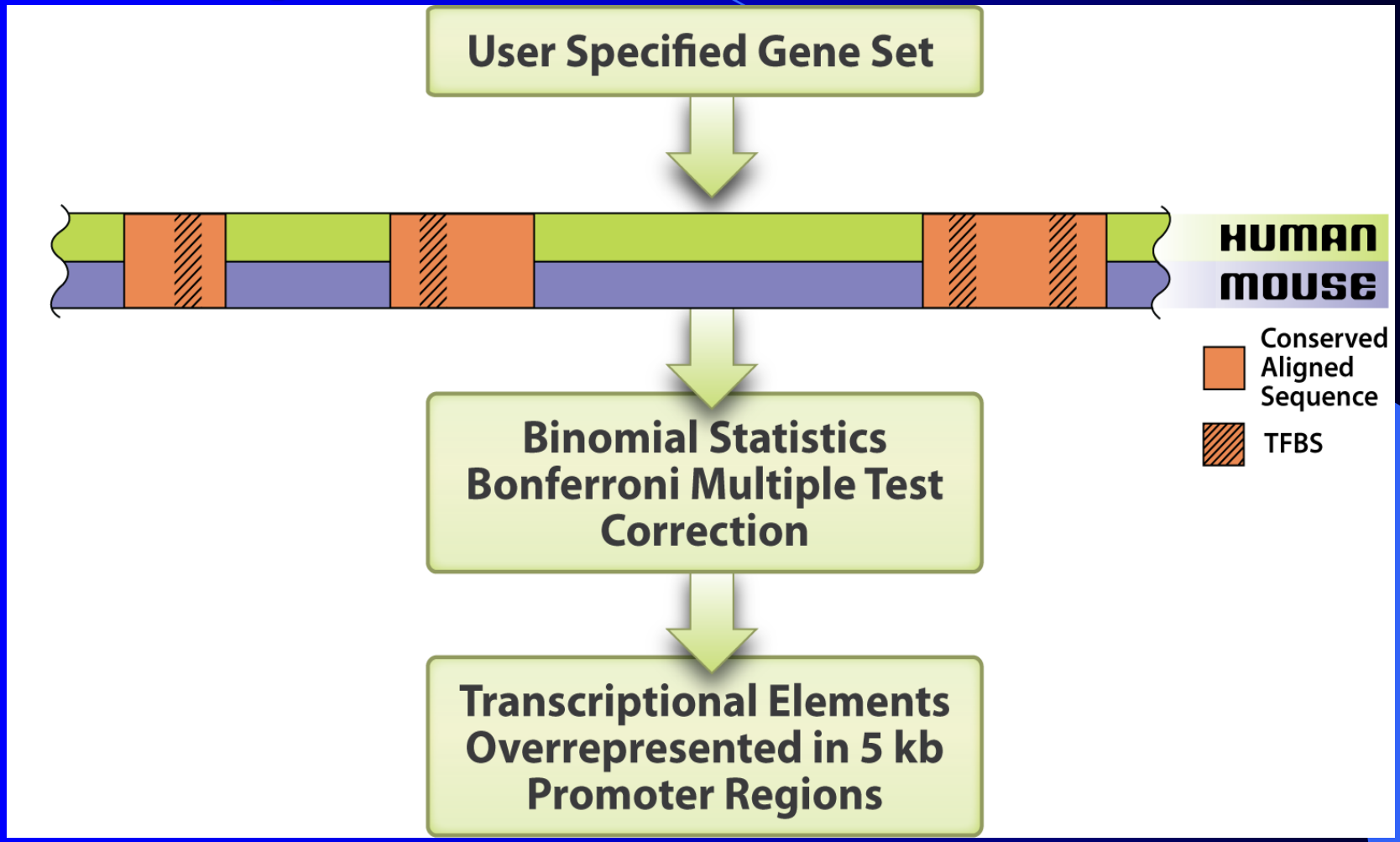
Transcription



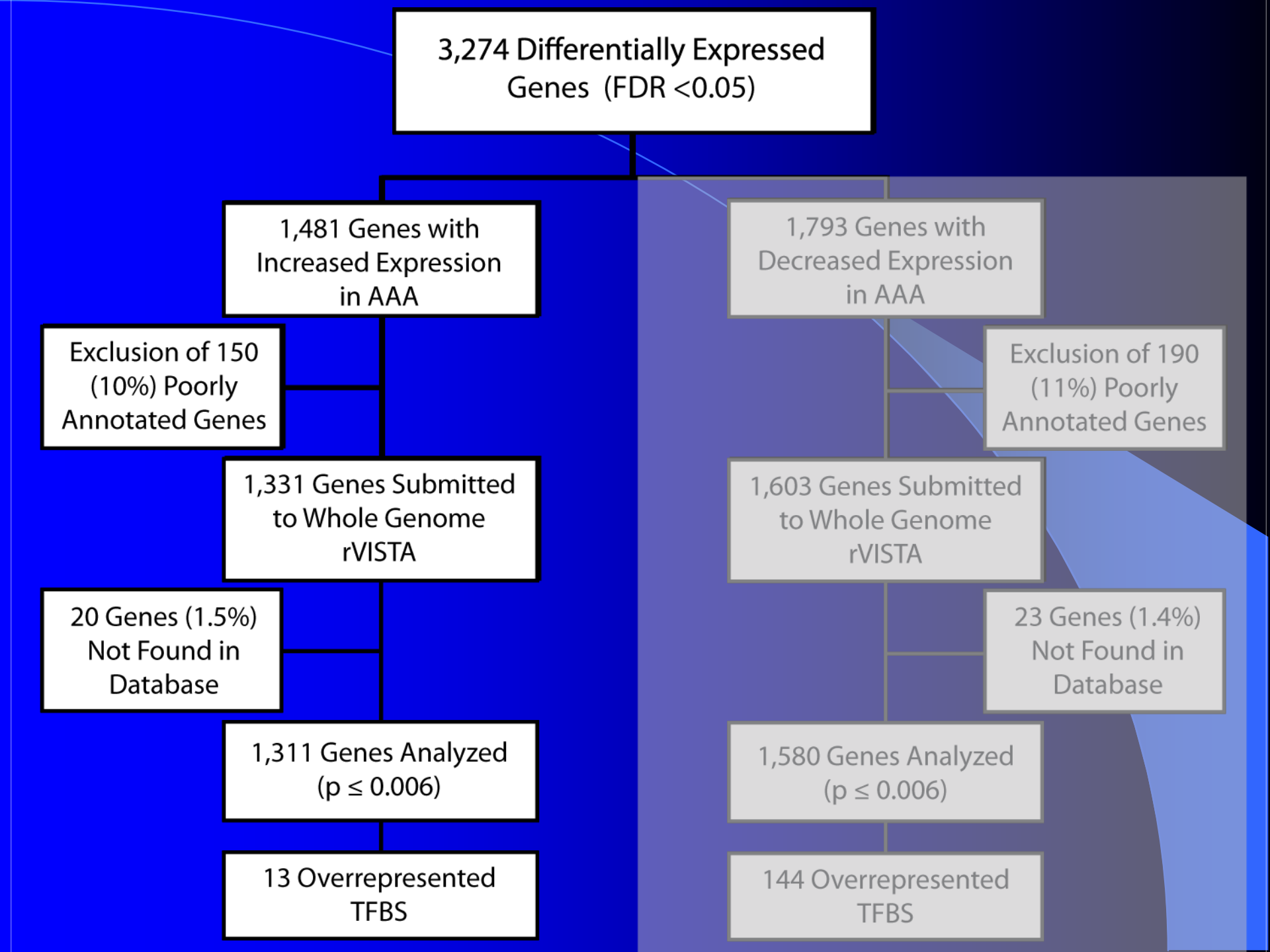
Transcription of Target Gene


GENE EXPRESSION DATA
3,274 GENES
UPREGULATED GENE SET
1,481 GENES
DOWNREGULATED GENE SET
1,793 GENES
TRANSCRIPTIONAL ELEMENTS
ENRICHED IN 5 kb PROMOTER REGIONS

Cis-Response Element Site Mapping



Matrices provided by TRANSFAC® Professional


3,274 Differentially Expressed Genes (FDR <0.05)
1,481 Genes with Increased Expression in AAA
Exclusion of 150 (10%) Poorly Annotated Genes
1,331 Genes Submitted to Whole Genome rVISTA
20 Genes (1.5%) Not Found in Database
1,311 Genes Analyzed (p ≤ 0.006)
13 Overrepresented TFBS
1,793 Genes with Decreased Expression in AAA
Exclusion of 190 (11%) Poorly Annotated Genes
1,603 Genes Submitted to Whole Genome rVISTA
23 Genes (1.4%) Not Found in Database
1,580 Genes Analyzed (p ≤ 0.006)
144 Overrepresented TFBS

TFBSs Enriched in Upregulated Genes

Transcription Factor Binding Site	Number of hits in the submitted regions	Total number of hits on the genome	$-\log_{10}$(p-value)
PEA3	1456	21187	17.8
ELF1	809	11549	14.8
ETS2	831	11968	14.3
ETS1	1406	22501	9.8
NFKB	1472	23956	8.5
NFKB 65	180	2232	7.6
NFKB 50	183	2308	7.2
C-ETS1 68	799	12878	5.5
GABP	284	4098	5.4
TEL2	39	385	4
ISRE	45	485	3.7
RUNX1	182	2740	2.9
NERF	36	433	2.3

TFBSs Enriched in Upregulated Genes

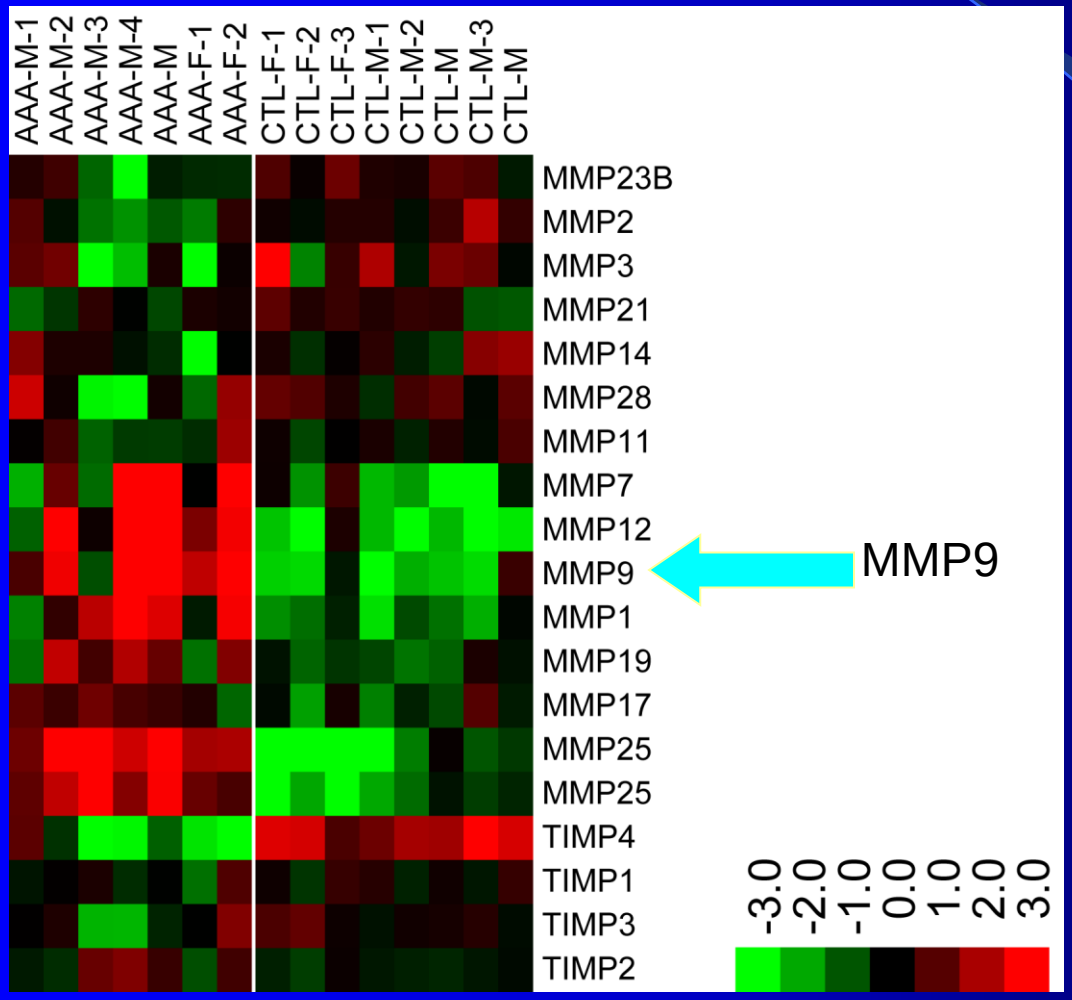
Transcription Factor Binding Site	$-\log_{10}$(p-value)	Binding Factor	TRANSFAC Family
PEA3	17.8	PEA3	ETS-type
ELF1	14.8	ELF-1	ETS-type
ETS2	14.3	ETS2	ETS-type
ETS1	9.8	c-Ets-1	ETS-type
NFKB	8.5	NF-kappaB/RelA-p65/p50	Rel-related factor
NFKB 65	7.6	RelA-p65	Rel-related factor
NFKB 50	7.2	p50	Rel-related factor
C-ETS1 68	5.5	c-Ets-1 68	ETS-type
GABP	5.4	GABP-alpha:GABP-beta	ETS-type
TEL2	4.0	TEL-2	ETS-type
ISRE	3.7	ISGF-3	Interferon-regulating
RUNX1	2.9	RUNX1	Runt-homology Domain
NERF	2.3	NERF-1a	ETS-type

TFBSs Enriched in Upregulated Genes

- Of 1,311 genes, 981 (74.8%) contain one or more binding sites of at least one of the 13 enriched TFBSs
- Range: 1 TFBS (158 genes) to 12 TFBSs
- Average: 4.2 TFBSs/gene

(*MMP9*)

MMP and TIMP Expression In AAA vs. Control Aorta

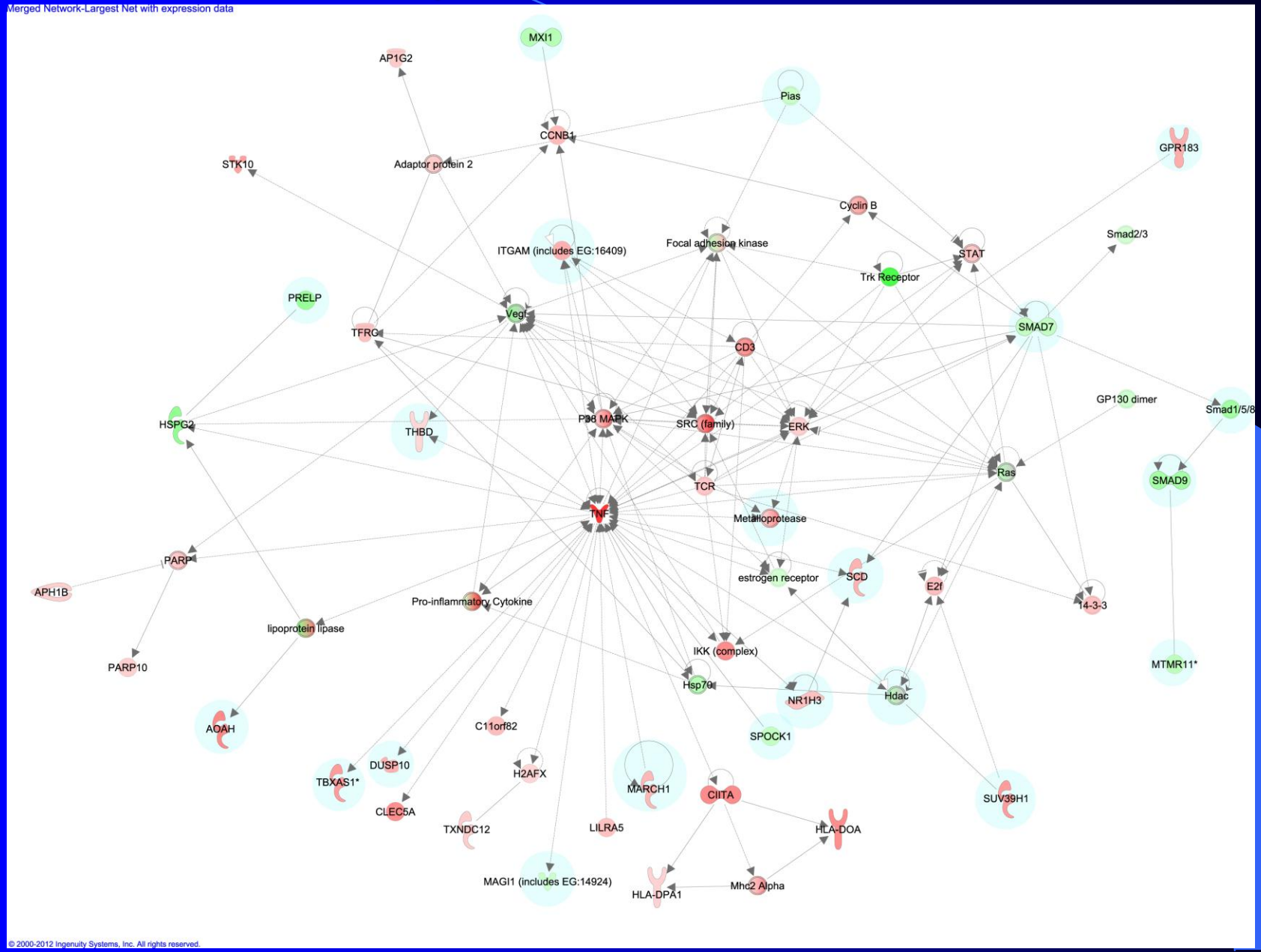


Chromatin Immunoprecipitation

- Isolate nuclei from control and AAA tissue
- Crosslink proteins to DNA
- Immunoprecipitate with Ab to DNA-binding protein

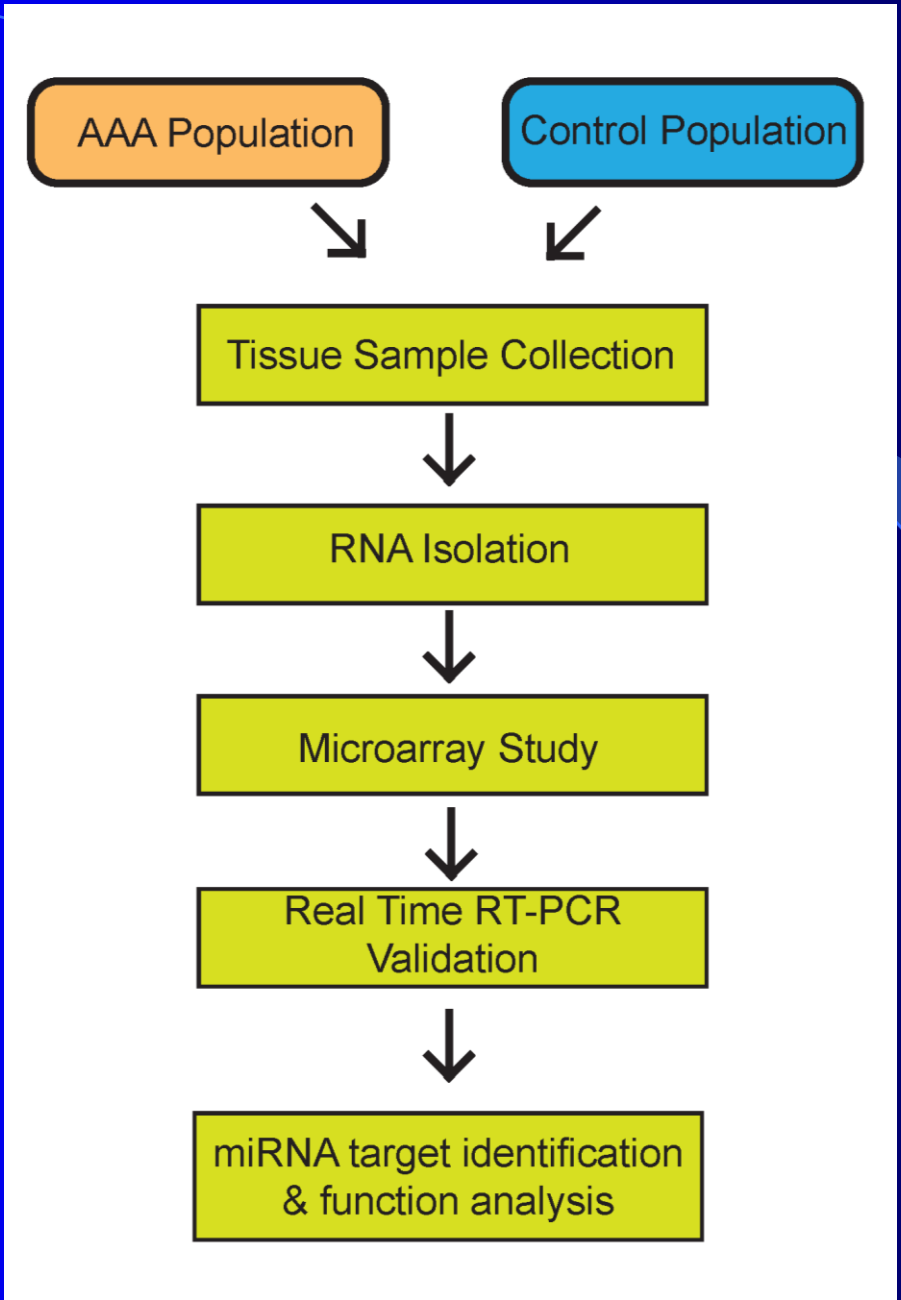
- Hybridize to microarray (ChIP-chip)
  - Signal intensity ~ proportional to occupancy
- Sequence (ChIP-Seq)
  - Count of reads ~ proportional to occupancy

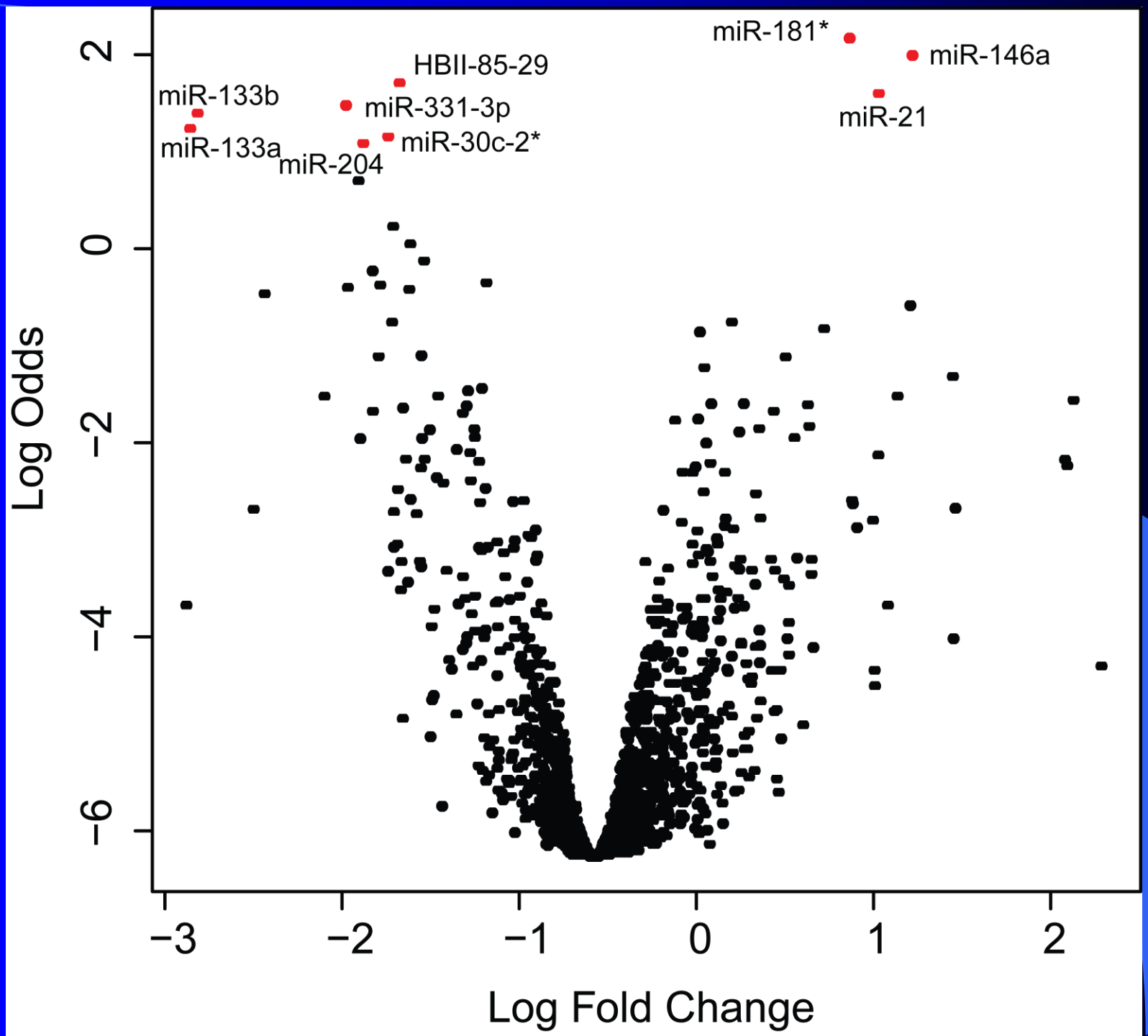
Network (ETS2, ELF1, RUNX1, STAT5)

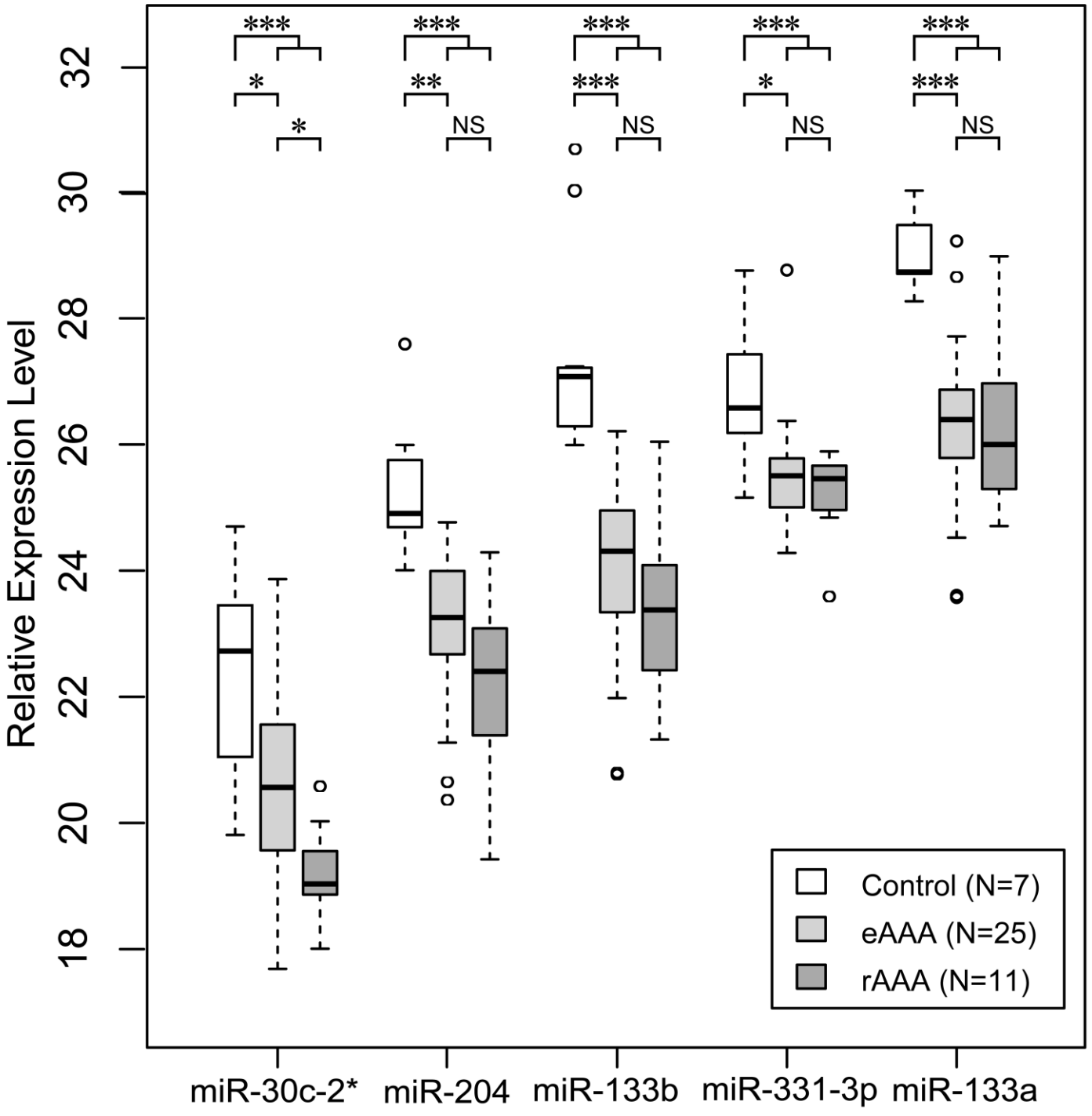


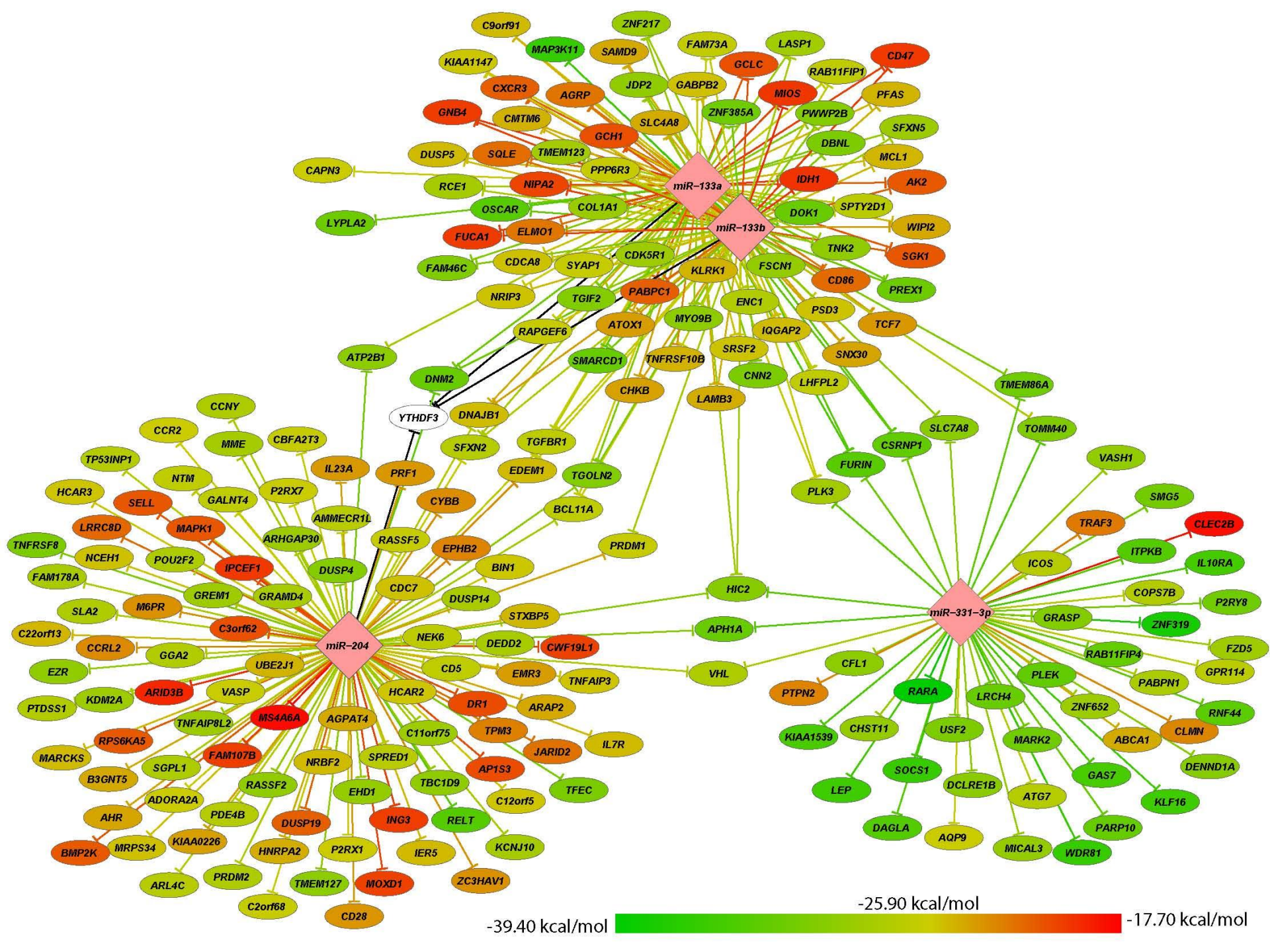
microRNA Expression

- Isolate RNA from control and AAA tissue
- Microarray (printed, Affymetrix, Illumina)
  - Hybridization
  - Signal intensity ~ proportional to copy number
- Sequence (RNA-Seq)
  - Count of reads ~ proportional to copy number


AAA Population
Control Population
Tissue Sample Collection
RNA Isolation
Microarray Study
Real Time RT-PCR Validation
miRNA target identification & function analysis

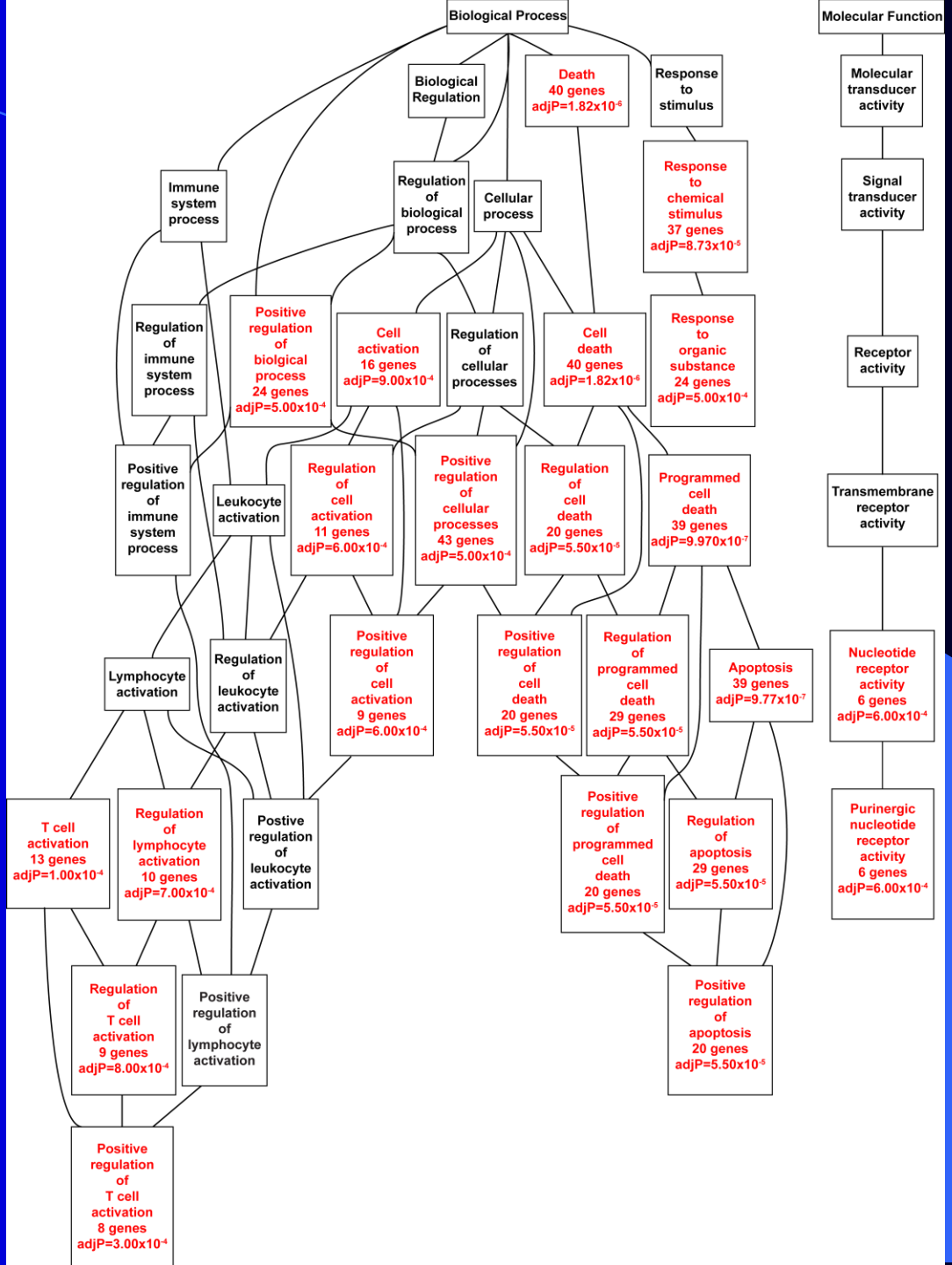

miR-181*
miR-146a
HBII-85-29
miR-133b
miR-331-3p
miR-21
miR-133a
miR-30c-2*
miR-204
2
0
−2
−4
−6
Log Odds
−3
−2
−1
0
1
2
Log Fold Change


Relative Expression Level
18
20
22
24
26
28
30
32
***
*
*
***
**
NS
***
***
NS
***
*
NS
***
***
NS
miR-30c-2*
miR-204
miR-133b
miR-331-3p
miR-133a
Control (N=7)
eAAA (N=25)
rAAA (N=11)



miR-133a
miR-133b
miR-204
miR-331-3p
C9orf91
ZNF217
MAP3K11
SAMD9
FAM73A
LASP1
CD47
KIAA1147
GCLC
RAB11FIP1
CXCR3
AGRP
JDP2
GABPB2
MIOS
PFAS
GNB4
CMTM6
SLC4A8
ZNF385A
PWWP2B
SFXN5
GCH1
DBNL
DUSP5
SQLE
TMEM123
MCL1
CAPN3
PPP6R3
IDH1
AK2
RCE1
NIPA2
OSCAR
COL1A1
DOK1
SPTY2D1
LYPLA2
ELMO1
WIPI2
FUCA1
TNK2
SGK1
CDK5R1
FAM46C
CDCA8
SYAP1
KLRK1
FSCN1
CD86
PREX1
NRIP3
TGIF2
PABPC1
ENC1
PSD3
TCF7
ATOX1
MYO9B
RAPGEF6
IQGAP2
SRSF2
SNX30
ATP2B1
SMARCD1
TNFRSF10B
CNN2
LHFPL2
TMEM86A
DNM2
CHKB
LAMB3
CCNY
YTHDF3
DNAJB1
SLC7A8
TOMM40
CCR2
MME
CBFA2T3
SFXN2
TGFBR1
CSRNP1
TP53INP1
IL23A
PRF1
EDEM1
TGOLN2
FURIN
VASH1
NTM
P2RX7
HCAR3
GALNT4
CYBB
PLK3
SMG5
SELL
AMMECR1L
BCL11A
TRAF3
CLEC2B
LRRC8D
MAPK1
ARHGAP30
RASSF5
EPHB2
PRDM1
ITPKB
TNFRSF8
NCEH1
POU2F2
IPCEF1
DUSP4
BIN1
ICOS
IL10RA
FAM178A
CDC7
HIC2
COPS7B
GREM1
GRAMD4
DUSP14
P2RY8
SLA2
M6PR
STXBP5
GRASP
ZNF319
C22orf13
C3orf62
APH1A
NEK6
DEDD2
CWF19L1
FZD5
CCRL2
GGA2
RAB11FIP4
EZR
UBE2J1
CD5
EMR3
TNFAIP3
VHL
CFL1
GPR114
PLEK
PABPN1
ARID3B
VASP
HCAR2
PTPN2
RARA
LRCH4
PTDSS1
KDM2A
DR1
ARAP2
ZNF652
RNF44
TNFAIP8L2
MS4A6A
AGPAT4
TPM3
KIAA1539
CHST11
USF2
MARK2
ABCA1
CLMN
RPS6KA5
C11orf75
IL7R
JARID2
MARCKS
FAM107B
NRBF2
SPRED1
SOCS1
DENND1A
SGPL1
AP1S3
GAS7
B3GNT5
RASSF2
TBC1D9
TFEC
LEP
DCLRE1B
ATG7
KLF16
ADORA2A
EHD1
C12orf5
AHR
PDE4B
DUSP19
ING3
RELT
DAGLA
AQP9
PARP10
BMP2K
MRPS34
KIAA0226
HNRPA2
P2RX1
IER5
KCNJ10
MICAL3
WDR81
ARL4C
PRDM2
TMEM127
MOXD1
ZC3HAV1
C2orf68
CD28
-25.90 kcal/mol
-39.40 kcal/mol
-17.70 kcal/mol

GO functional annotation



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