Connecting the dots: dissecting the cellular circuitry of a mouse single gene knockout, embryonic stem cell line using a combination of transcriptional analysis, bioinformatics, and drug screening.

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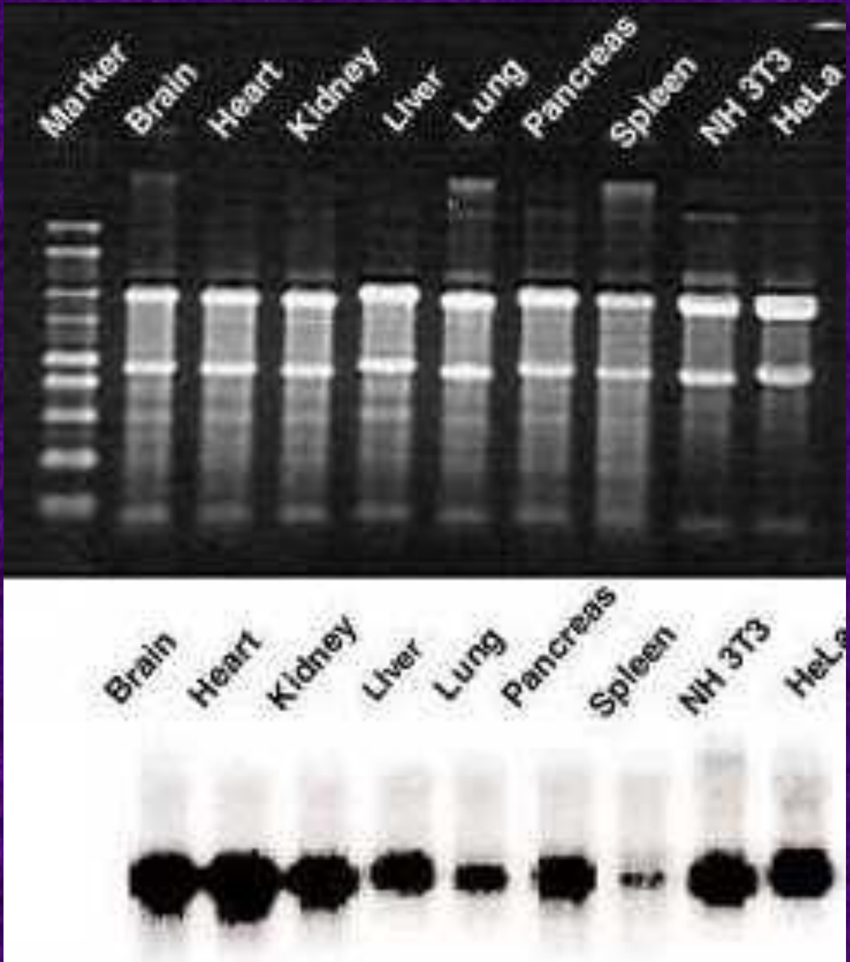
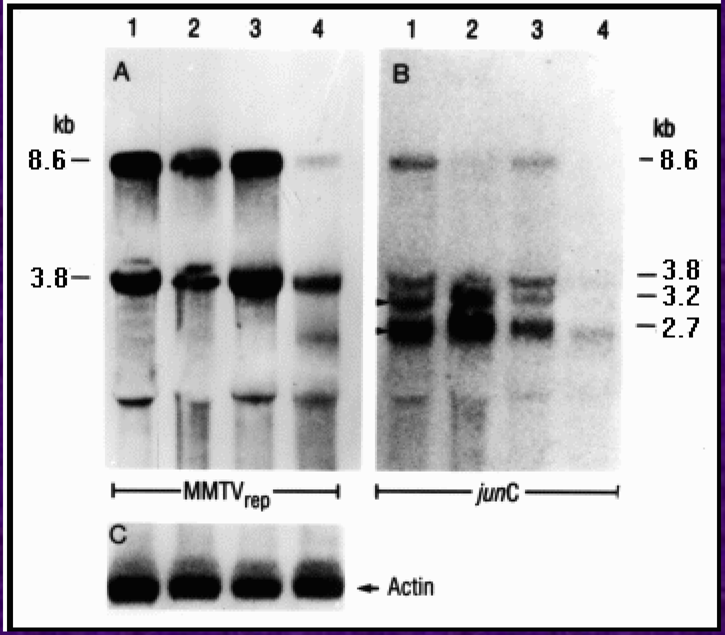
Evolution of Current Microarray Technology



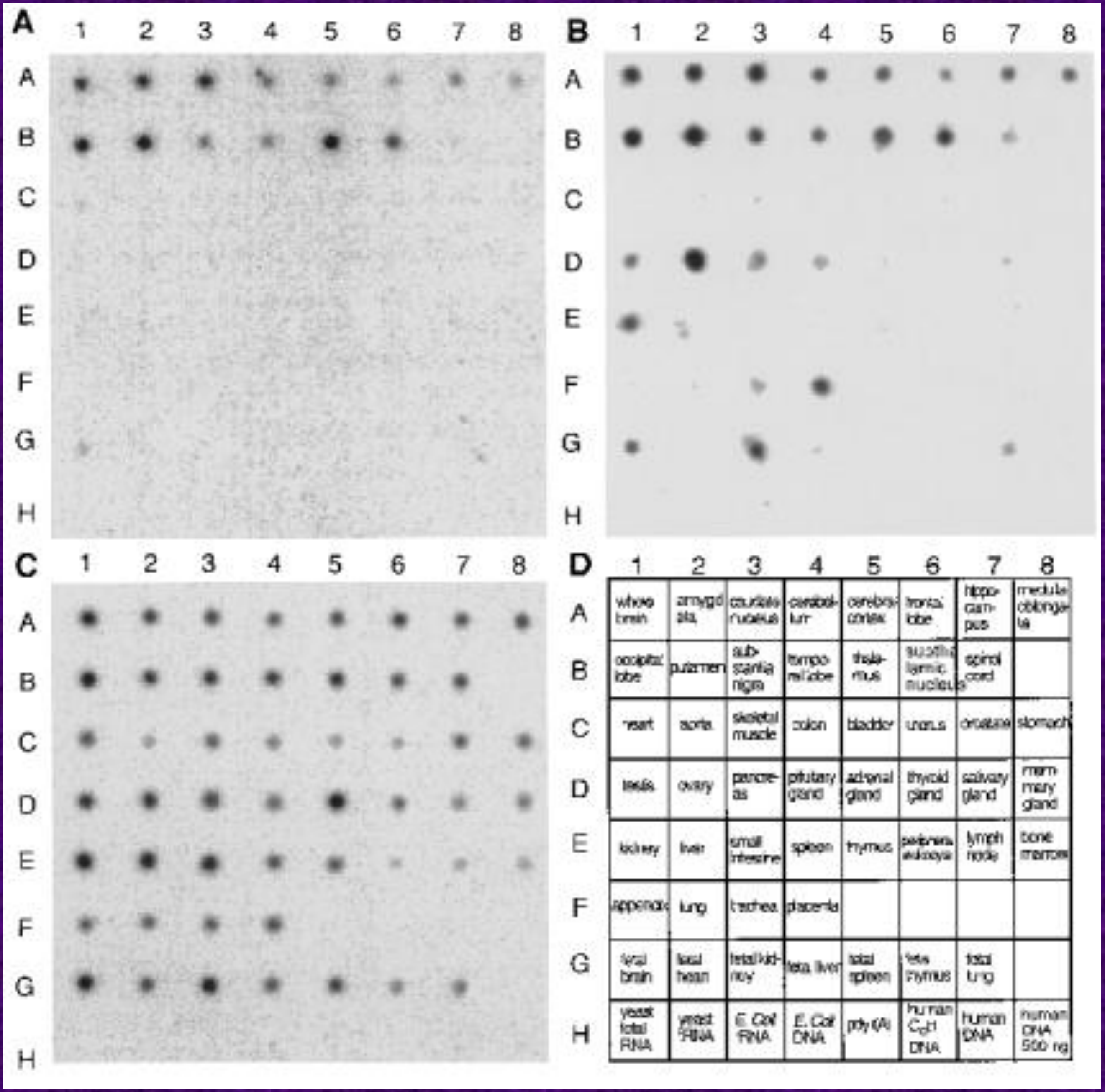
A



B

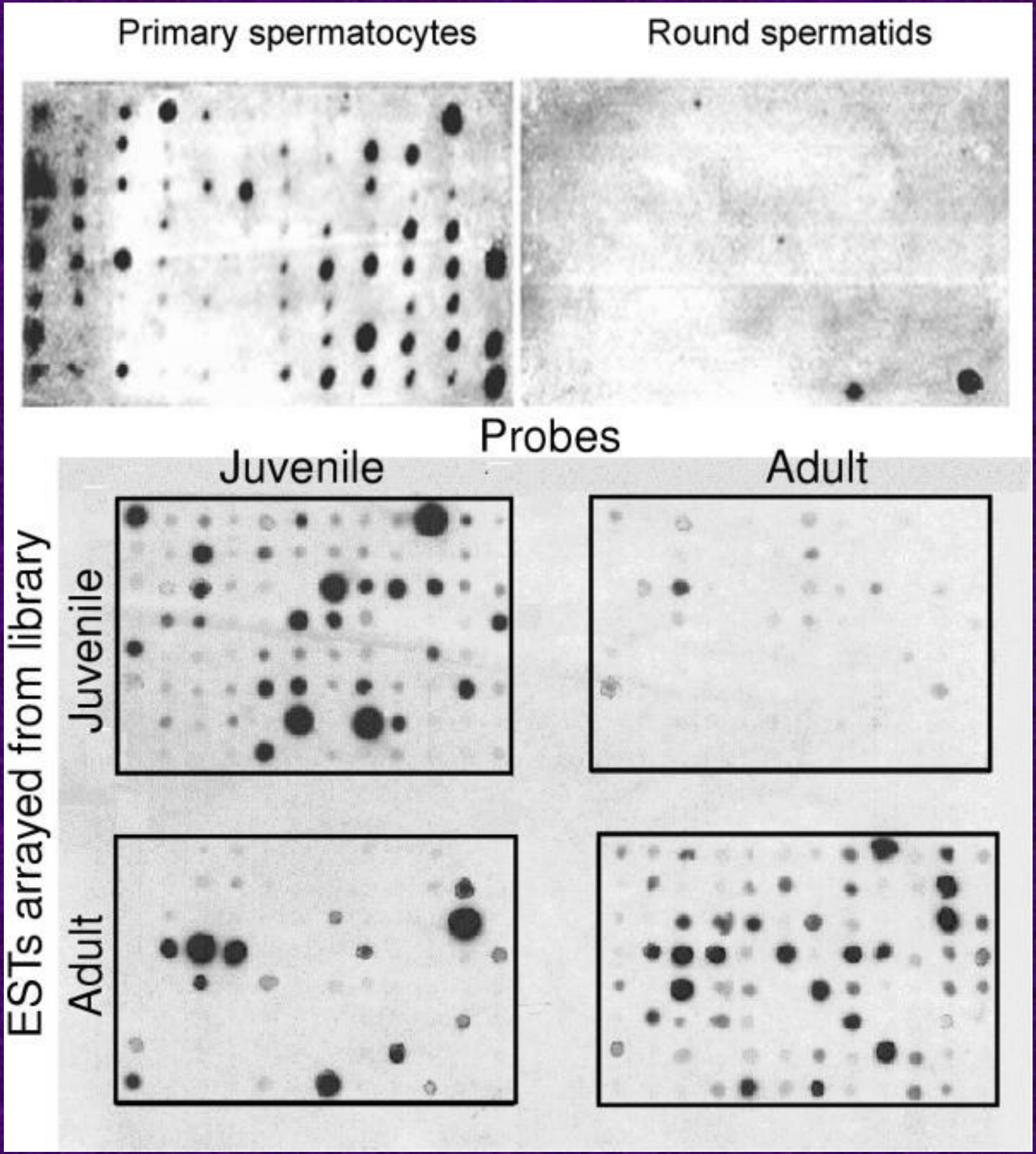
Northern blots

Dot bots- single gene, many tissues

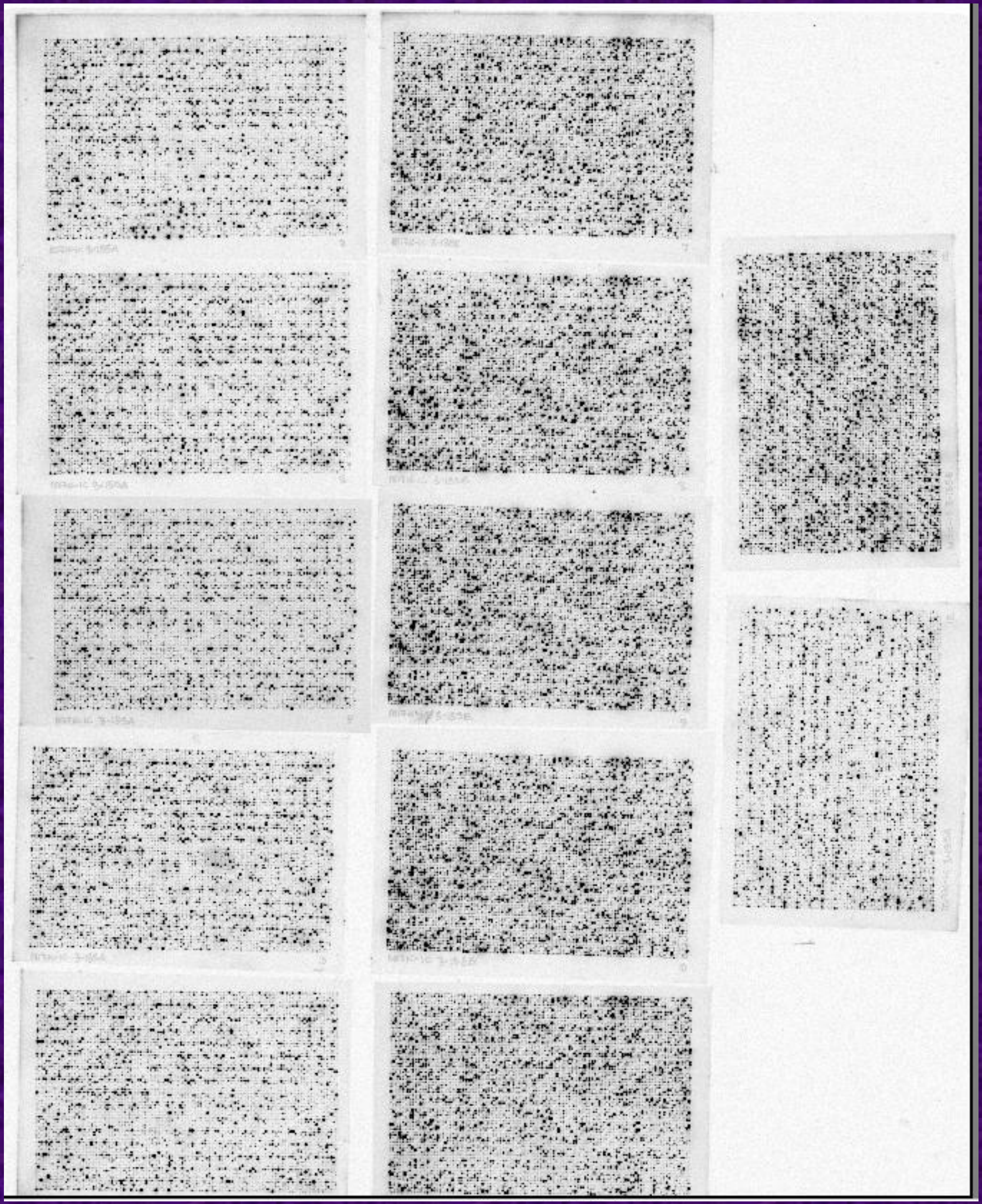


	1	2	3	4	5	6	7	8
A	whole brain	amygdala	caudate nucleus	cerebellum	cerebral cortex	frontal lobe	hippocampus	medulla oblongata
B	occipital lobe	putamen	substantia nigra	temporal lobe	thalamus	subthalamic nucleus	spinal cord	
C	heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
D	testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
E	kidney	liver	small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
F	appendix	lung	trachea	placenta				
G	fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
H	yeast total RNA	yeast tRNA	E. Coli rRNA	E. Coli DNA	poly(A)	human C_0t1 DNA	human DNA	human DNA 500 ng

Dot bots- many genes, single tissue



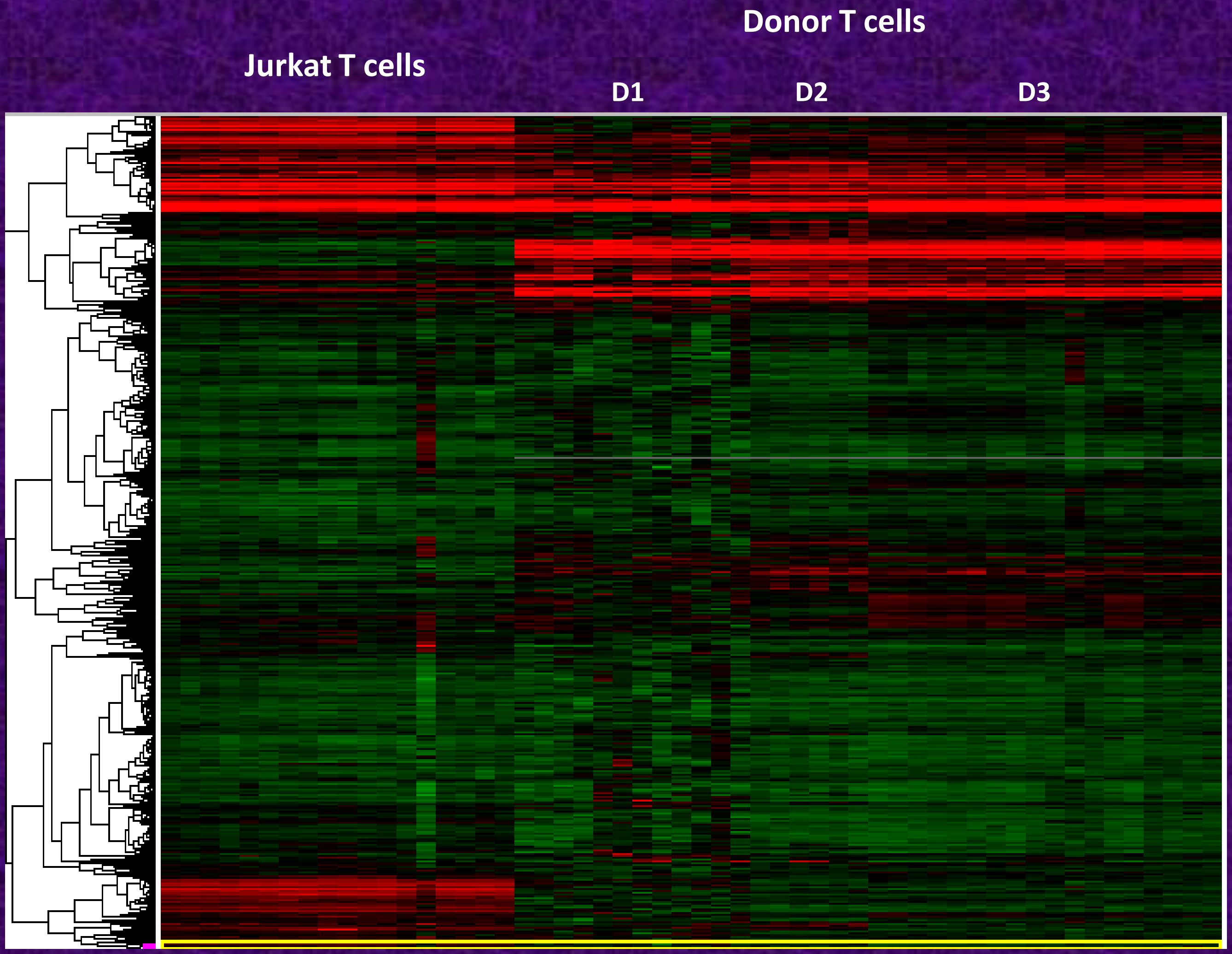
Microarrays-
radioactivity
on nylon



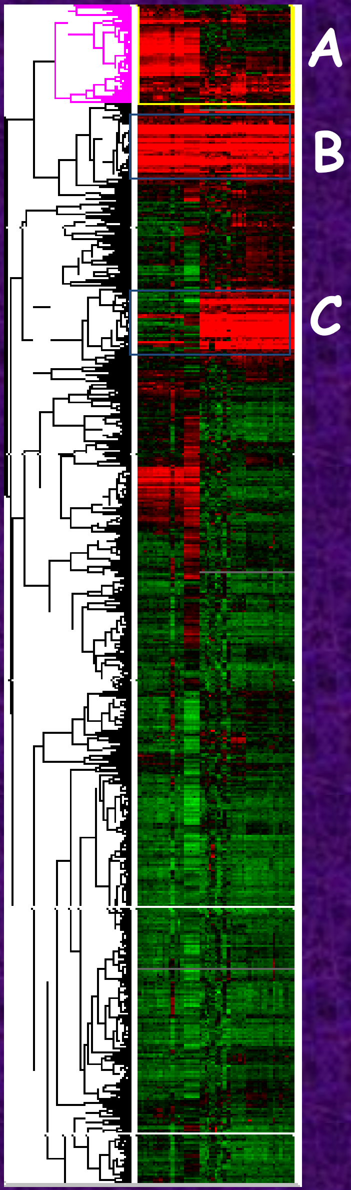
Advantages:
v. inexpensive

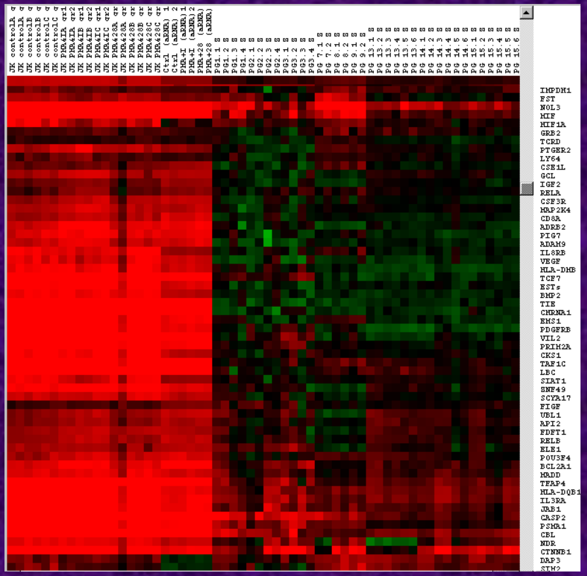
Disadvantages:
size limits,
specificity of
cDNA probes,
clone handling

Cluster Analysis of Z Transformed Data
(experiment number = 54)

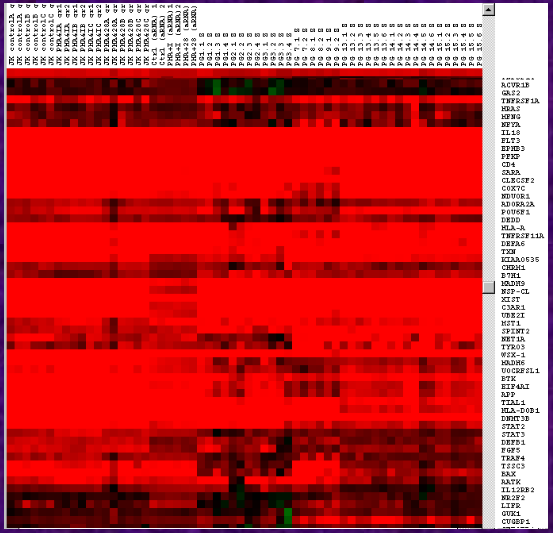


Genes differentially expressed in Jurkat vs Donor T cells

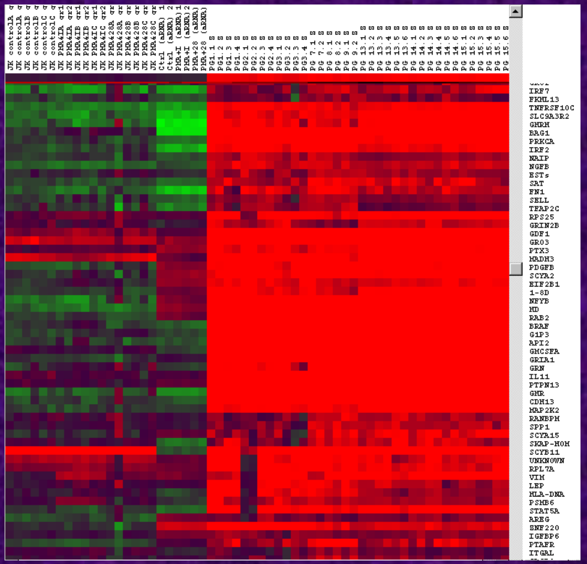




A. Genes up-regulated in Jurkat only



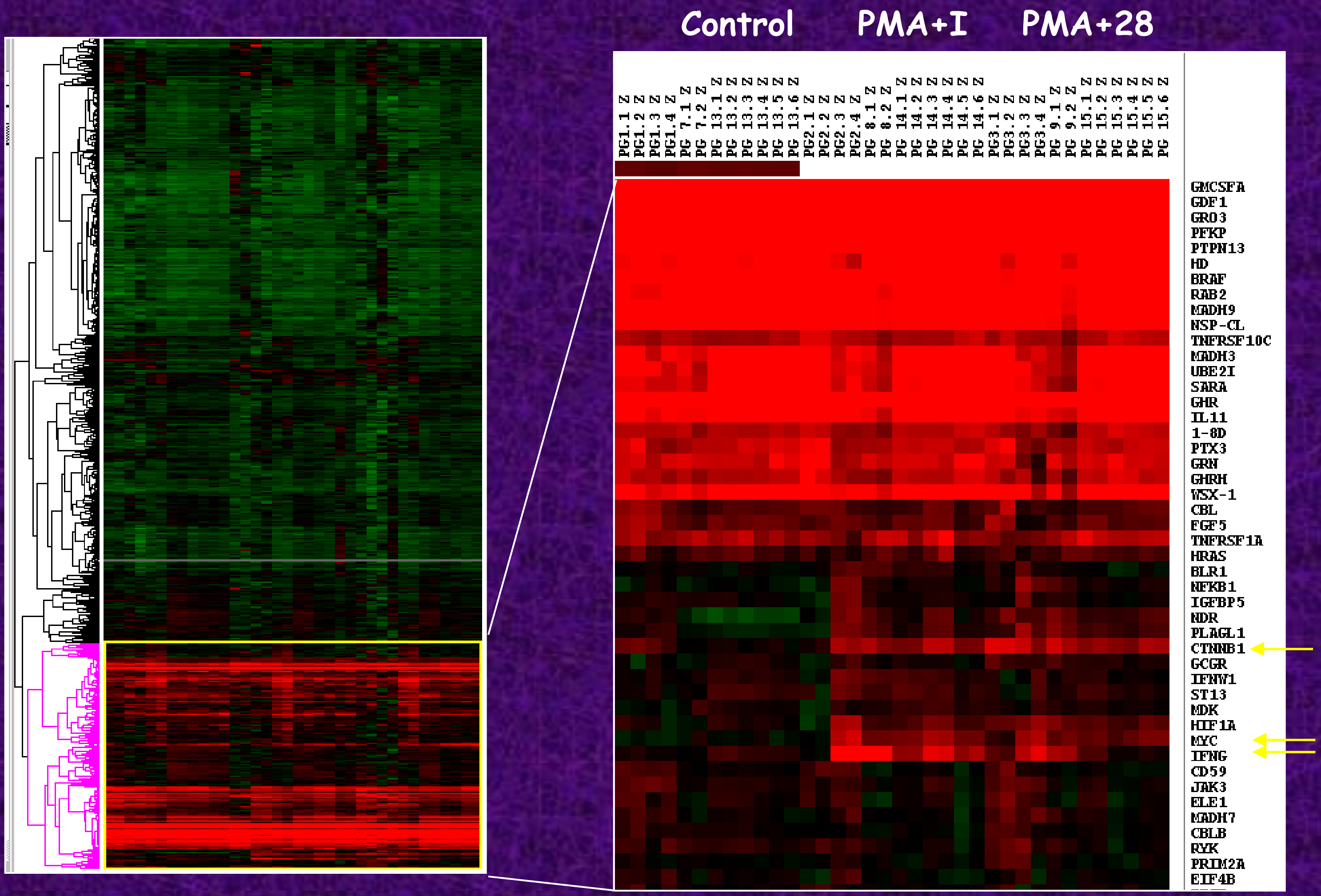
B. Genes highly expressed in Jurkat and Donor T cells



C. Genes up-regulated in Donor T cells only

NIA ImmunoArray

Condition specific regulation of CTNNB1, Myc and IFNG expression in stimulated donor T cells



Rescued……

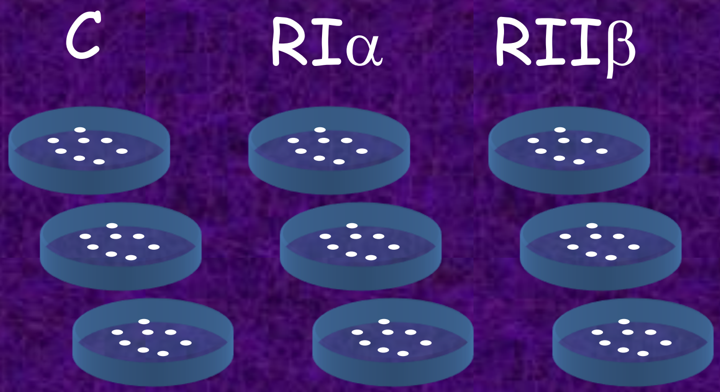
……by BeadArrays!

OVCAR8 human ovarian cancer

OVCAR cells were transfected with one of two key regulatory subunits of Protein Kinase A (PKA).

PKA Regulatory Subunit 1A (RIα) induces a hyper-proliferative state

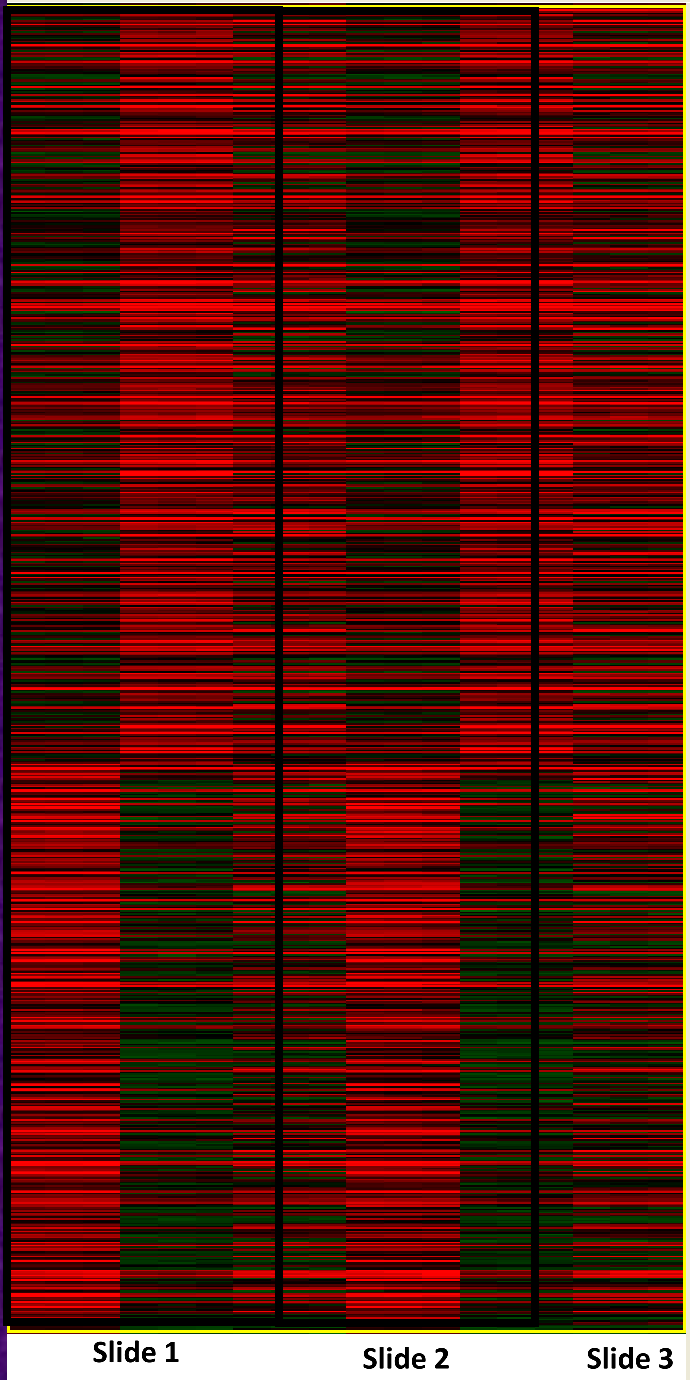
PKA Regulatory Subunit 2B (RIIβ) causes a quasi-differentiated phenotype

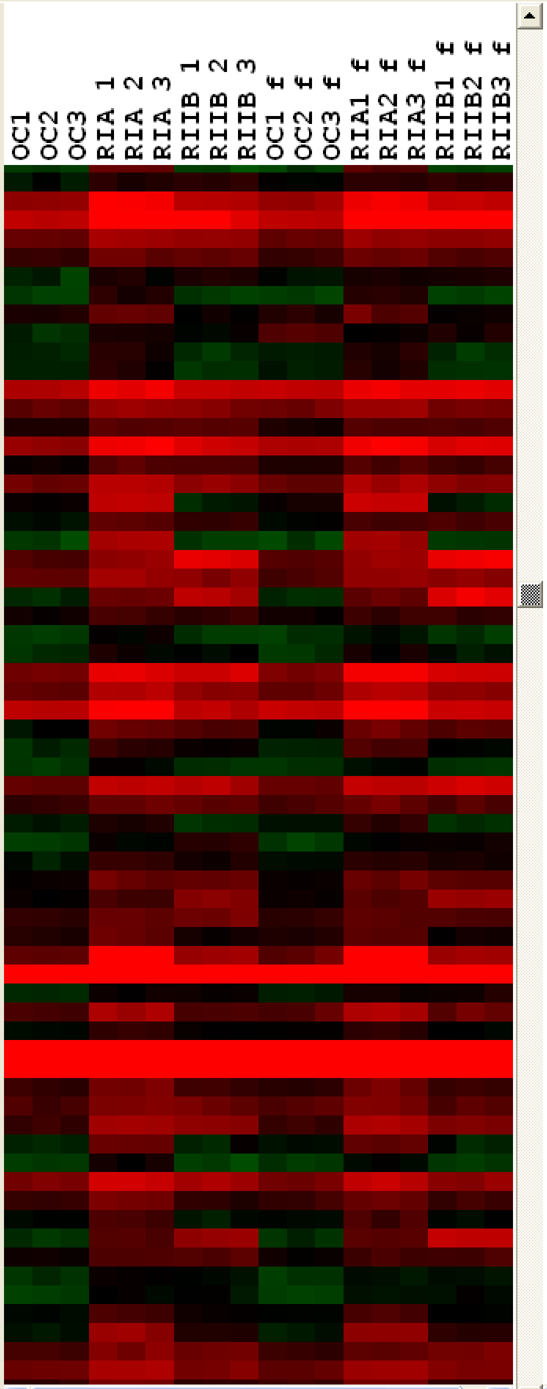


9 RNA samples
27 independent labelings
27 arrays (9 Affymetrix, 9 Agilent, 9 Illumina)
Data was normalized appropriately for the specific array type.

Cheadle, C., Nesterova, M., Johnson, N., Becker, K. and Y-S. Cho-Chung. (2008), *PKA Regulatory Subunits Define an Axis of Cellular Proliferation/Differentiation in Ovarian Cancer Cells*. BMC Med Genomics. 2008 Sep 26;1:43.

Illumina OVCAR RIA
All sig N&P up&down





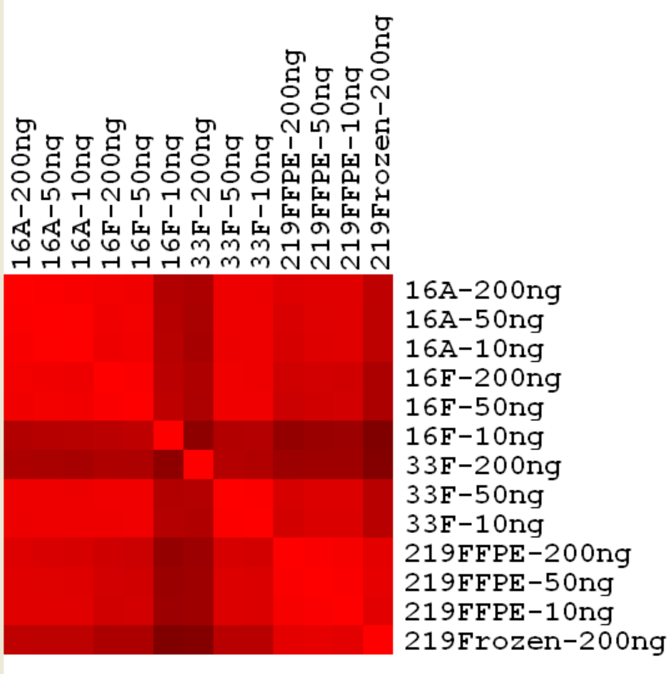
PRSS8 - protease, serine, 8 (prostasin) (PRSS8), mRNA.
PSEN2 - presenilin 2 (Alzheimer disease 4) (PSEN2), trans
PSMA4 - proteasome (prosome, macropain) subunit, alpha ty
PSMC2 - proteasome (prosome, macropain) 26S subunit, ATPa
PSMD7 - proteasome (prosome, macropain) 26S subunit, non-
PSPC1 - paraspeckle component 1 (PSPC1), mRNA.
PSPH - phosphoserine phosphatase (PSPH), mRNA.
PSTPIP2 - proline-serine-threonine phosphatase interactin
PTER - phosphotriesterase related (PTER), mRNA.
PTGES - prostaglandin E synthase (PTGES), transcript vari
PTGFRN - prostaglandin F2 receptor negative regulator (PT
PTGFRN - prostaglandin F2 receptor negative regulator (PT
PTK9 - PTK9 protein tyrosine kinase 9 (PTK9), transcript
PUS1 - pseudouridylate synthase 1 (PUS1), mRNA.
PWP2H - PWP2 periodic tryptophan protein homolog (yeast)
PYCR1 - pyrroline-5-carboxylate reductase 1 (PYCR1), tran
PYCR1 - pyrroline-5-carboxylate reductase 1 (PYCR1), tran
PYCS - pyrroline-5-carboxylate synthetase (glutamate gamm
QPCT - glutaminyl-peptide cyclotransferase (glutaminyl cy
RAB20 - RAB20, member RAS oncogene family (RAB20), mRNA.
RAB25 - RAB25, member RAS oncogene family (RAB25), mRNA.
RAB3A - RAB3A, member RAS oncogene family (RAB3A), mRNA.
RAB8A - RAB8A, member RAS oncogene family (RAB8A), mRNA.
RAMP1 - receptor (calcitonin) activity modifying protein
RANBP6 - RAN binding protein 6 (RANBP6), mRNA.
RARRES2 - retinoic acid receptor responder (tazarotene in
RB1 - retinoblastoma 1 (including osteosarcoma) (RB1), mR
RCN2 - reticulocalbin 2, EF-hand calcium binding domain (
REC14 - recombination protein REC14 (REC14), mRNA.
RER1 - RER1 homolog (S. cerevisiae) (RER1), mRNA.
RG9MTD1 - RNA (guanine-9-) methyltransferase domain conta
RGS16 - regulator of G-protein signalling 16 (RGS16), mRN
RLN2 - relaxin 2 (H2) (RLN2), transcript variant 1, mRNA.
RNASET2 - ribonuclease T2 (RNASET2), mRNA.
RNU3IP2 - RNA, U3 small nucleolar interacting protein 2 (
RODH - 3-hydroxysteroid epimerase (RODH), mRNA.
ROR1 - receptor tyrosine kinase-like orphan receptor 1 (R
RPC8 - RNA polymerase III subunit RPC8 (RPC8), mRNA.
RPE - ribulose-5-phosphate-3-epimerase (RPE), transcript
RPH3AL - rabphilin 3A-like (without C2 domains) (RPH3AL),
RPIA - ribose 5-phosphate isomerase A (ribose 5-phosphate
RPL13 - ribosomal protein L13 (RPL13), transcript variant
RPL17 - ribosomal protein L17 (RPL17), mRNA.
RPL24 - ribosomal protein L24 (RPL24), mRNA.
RPL28 - ribosomal protein L28 (RPL28), mRNA.
RPL34 - ribosomal protein L34 (RPL34), transcript variant
RPP38 - ribonuclease P/MRP 38kDa subunit (RPP38), transcr
RPS17 - ribosomal protein S17 (RPS17), mRNA.
RPS20 - ribosomal protein S20 (RPS20), mRNA.
RPS6KB2 - ribosomal protein S6 kinase, 70kDa, polypeptide
RRP46 - exosome component Rrp46 (RRP46), mRNA.
RTKN - rhotekin (RTKN), mRNA.
S100A14 - S100 calcium binding protein A14 (S100A14), mRN
SAA1 - serum amyloid A1 (SAA1), transcript variant 1, mRN
SAP30 - sin3-associated polypeptide, 30kDa (SAP30), mRNA.
SARA2 - SAR1a gene homolog 2 (S. cerevisiae) (SARA2), mRN
SATB2 - SATB family member 2 (SATB2), mRNA.
SC5DL - sterol-C5-desaturase (ERG3 delta-5-desaturase hom
SC65 - synaptonemal complex protein SC65 (SC65), mRNA.
SCARA3 - scavenger receptor class A, member 3 (SCARA3), t
SCARA3 - scavenger receptor class A, member 3 (SCARA3), t
SCLY - selenocysteine lyase (SCLY), mRNA.
SCNN1A - sodium channel, nonvoltage-gated 1 alpha (SCNN1A
SDC4 - syndecan 4 (amphiglycan, ryudocan) (SDC4), mRNA.
SDCBP - syndecan binding protein (syntenin) (SDCBP), mRNA

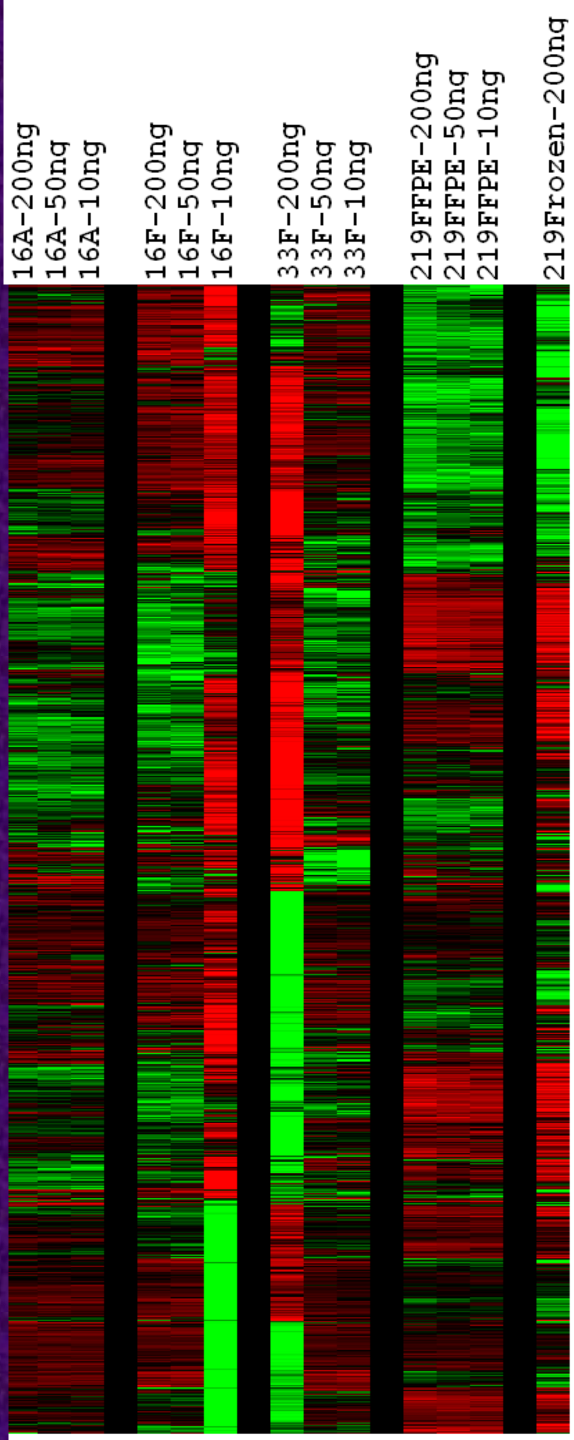
Emerging technologies

Correlation matrix – all samples

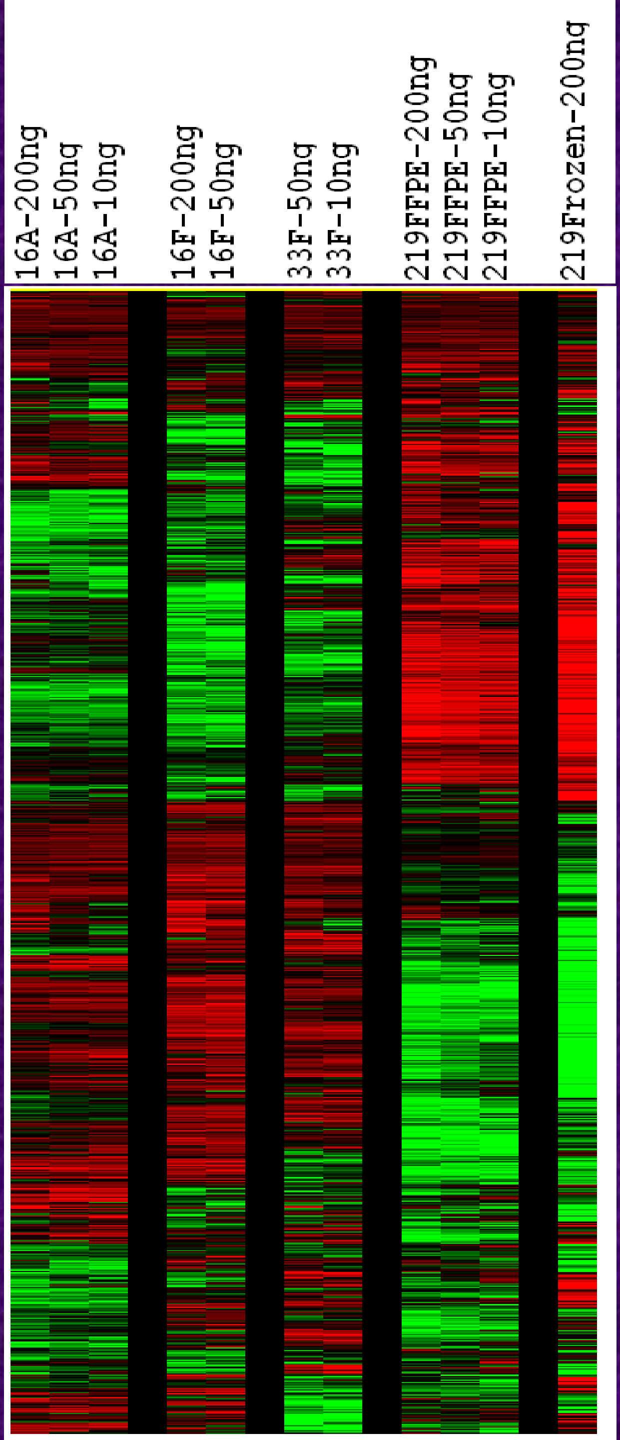
Correlations

	16A-200ng	16A-50ng	16A-10ng	16F-200ng	16F-50ng	16F-10ng	33F-200ng	33F-50ng	33F-10ng	219FFPE-200ng	219FFPE-50ng	219FFPE-10ng	219Frozen-200ng
16A-200ng	1	0.9883	0.9782	0.9532	0.9464	0.6989	0.6773	0.9365	0.9273	0.8729	0.8853	0.8798	0.7491
16A-50ng	0.9883	1	0.9929	0.946	0.9535	0.7138	0.6645	0.9369	0.9363	0.8496	0.8764	0.8792	0.7449
16A-10ng	0.9782	0.9929	1	0.9363	0.9496	0.7178	0.6563	0.9319	0.9353	0.8409	0.8729	0.8789	0.7444
16F-200ng	0.9532	0.946	0.9363	1	0.9866	0.7299	0.6901	0.9465	0.9352	0.8092	0.8251	0.822	0.6751
16F-50ng	0.9464	0.9535	0.9496	0.9866	1	0.7477	0.6788	0.9456	0.9439	0.7963	0.8229	0.8261	0.6769
16F-10ng	0.6989	0.7138	0.7178	0.7299	0.7477	1	0.5572	0.7009	0.7063	0.5832	0.6084	0.6156	0.5045
33F-200ng	0.6773	0.6645	0.6563	0.6901	0.6788	0.5572	1	0.7072	0.6932	0.6187	0.6234	0.6214	0.5203
33F-50ng	0.9365	0.9369	0.9319	0.9465	0.9456	0.7009	0.7072	1	0.9887	0.8419	0.8631	0.8629	0.7279
33F-10ng	0.9273	0.9363	0.9353	0.9352	0.9439	0.7063	0.6932	0.9887	1	0.8281	0.8557	0.86	0.7218
219FFPE-200ng	0.8729	0.8496	0.8409	0.8092	0.7963	0.5832	0.6187	0.8419	0.8281	1	0.9872	0.9675	0.9003
219FFPE-50ng	0.8853	0.8764	0.8729	0.8251	0.8229	0.6084	0.6234	0.8631	0.8557	0.9872	1	0.9878	0.904
219FFPE-10ng	0.8798	0.8792	0.8789	0.822	0.8261	0.6156	0.6214	0.8629	0.86	0.9675	0.9878	1	0.8939
219Frozen-200ng	0.7491	0.7449	0.7444	0.6751	0.6769	0.5045	0.5203	0.7279	0.7218	0.9003	0.904	0.8939	1



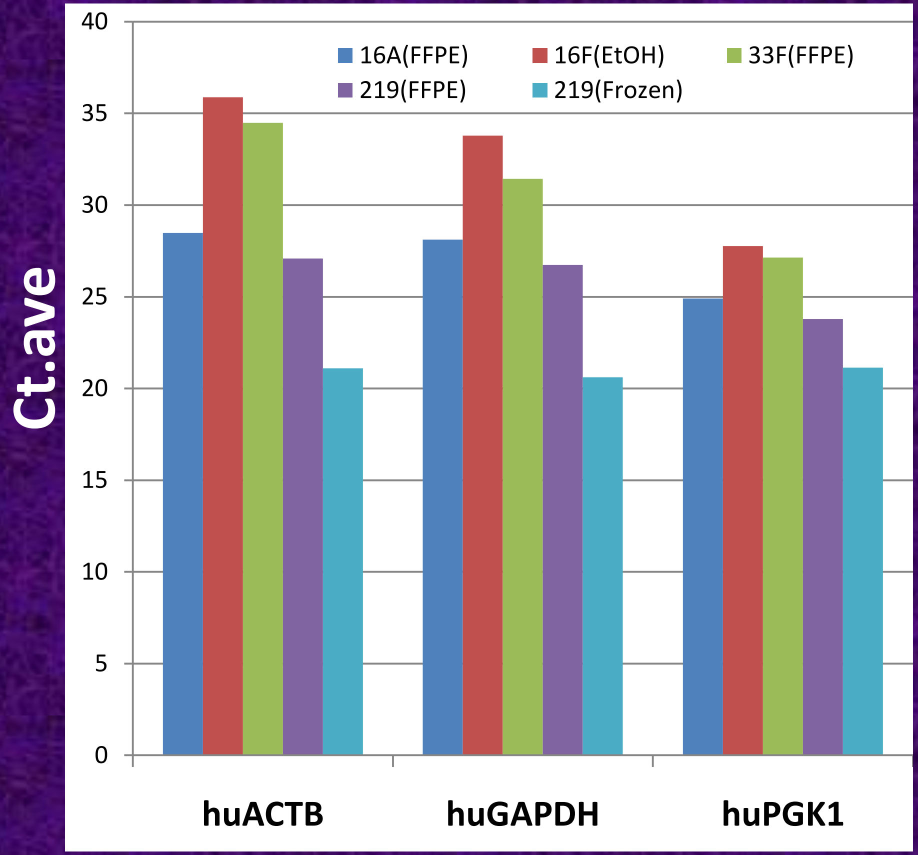


outliers removed



FFPE RNA – RT-PCR estimates of degradation

- QPCR was performed using 1 ng RNA per reaction.
- Ct. ave is the mean of duplicates for each sample.



Amplicon size 171 bp 122 bp 75 bp

	Amplicon	219(Frozen)	219(FFPE)	16A(FFPE)	16F(EtOH)	33F(FFPE)
huACTB	171 bp	21.09	27.08	28.48	35.89	34.49
huGAPDH	122 bp	20.61	26.74	28.12	33.78	31.44
huPGK1	75 bp	21.14	23.79	24.9	27.77	27.14

A combination of transcriptional analysis, bioinformatics, and drug screening reveals mechanisms of global dysregulation in a single gene knock-out mouse embryonic stem cell line.

Tristetraprolin (TTP) - aka ZFP36

•a basic proline-rich 33.6 KD protein.

•TTP destabilizes TNF-alpha mRNA after binding directly to the ARE of the 3-prime UTR of the TNA-alpha mRNA.

•AU-rich elements (AREs) in the 3-prime untranslated regions (UTRs) of unstable mRNAs dictate their degradation.

•Human miR16 containing an UAAAUAUU sequence that is complementary to ARE sequences, is required for ARE-RNA turnover and requires the ARE-binding protein TTP.

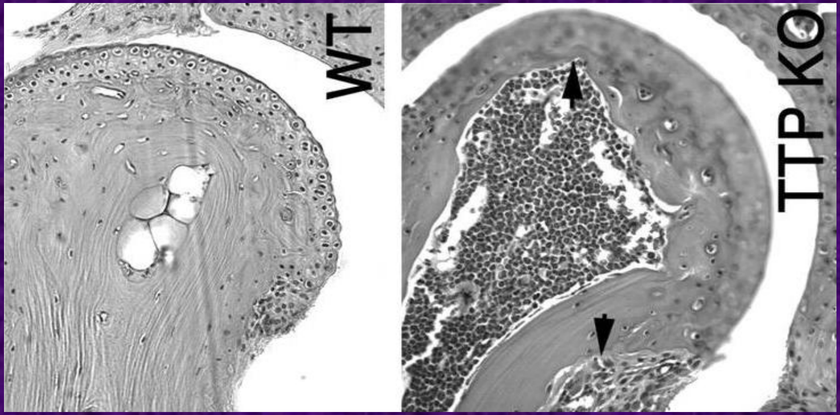
TTP deficient knock-out mice

Mice made deficient in TTP by gene targeting appeared normal at birth, but soon manifested marked medullary and extramedullary myeloid hyperplasia associated with cachexia, erosive arthritis, dermatitis, conjunctivitis, glomerular mesangial thickening, and high titers of anti-DNA and antinuclear antibodies.

Treatment of young TTP-deficient mice with antibodies to tumor necrosis factor α (TNFα) prevented the development of essentially all aspects of the phenotype. These results indicate a role for TTP in regulating TNFα synthesis, secretion, turnover, or action.

A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. Taylor GA, Carballo E, Lee DM, Lai WS, Thompson MJ, Patel DD, Schenkman DI, Gilkeson GS, Broxmeyer HE, Haynes BF, Blackshear PJ. Immunity. 1996 May;4(5):445-54

TTP deficient knock-out mice



Histology of finger joints (6 month)

	WT	TTP KO
Synovium:	smooth	inflamed
Bone marrow cavity:	small, few cells	neutrophil-packed
Bone within marrow cavity:	smooth	severe erosions
Juxtarticular bone surface:	smooth	significant erosions
TNF mRNA & Protein Levels:	low	significantly elevated
Phenotype:	normal	similar to human Rheumatoid Arthritis

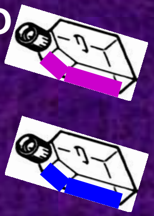
1. The mouse arthritis phenotype could be almost entirely prevented by injecting TNF antagonist;
2. TNF and TTP play pivotal roles in the pathogenesis of rheumatoid arthritis;
3. TTP binds TNF mRNA 3'UTR to promote its degradation; TTP is the key arthritis suppressor;
4. TNF antagonist has been approved by FDA as the first-choice drug to treat human rheumatoid arthritis.

Phillips k, et al. PNAS, 2004, 101: 2011; *Kontoyiannis et al. Immunity, 1999,10:387*

Mouse Embryonic Fibroblasts (MEFs) +/- TTP

Cell Line Characterization:

TTP KO MEFs



WT MEFs

10% FBS treatment → Protein Extraction → Western Blot to detect TTP protein Expression

Induction of TTP Protein upon TNF Stimulation:

TTP KO MEFs

WT MEFs

TNF treatment → Protein Extraction → Western Blot to detect TTP protein Expression

Microarray Analysis:

TTP KO MEFs

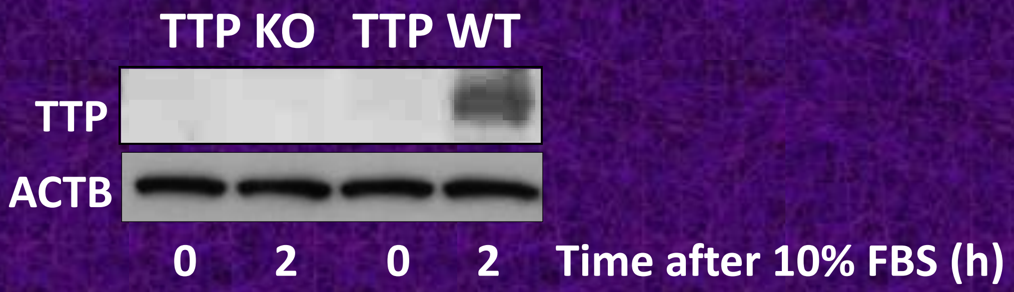
WT MEFs

TNF treatment → Total RNA Extraction → cDNA synthesis cRNA amplification → Data Analysis

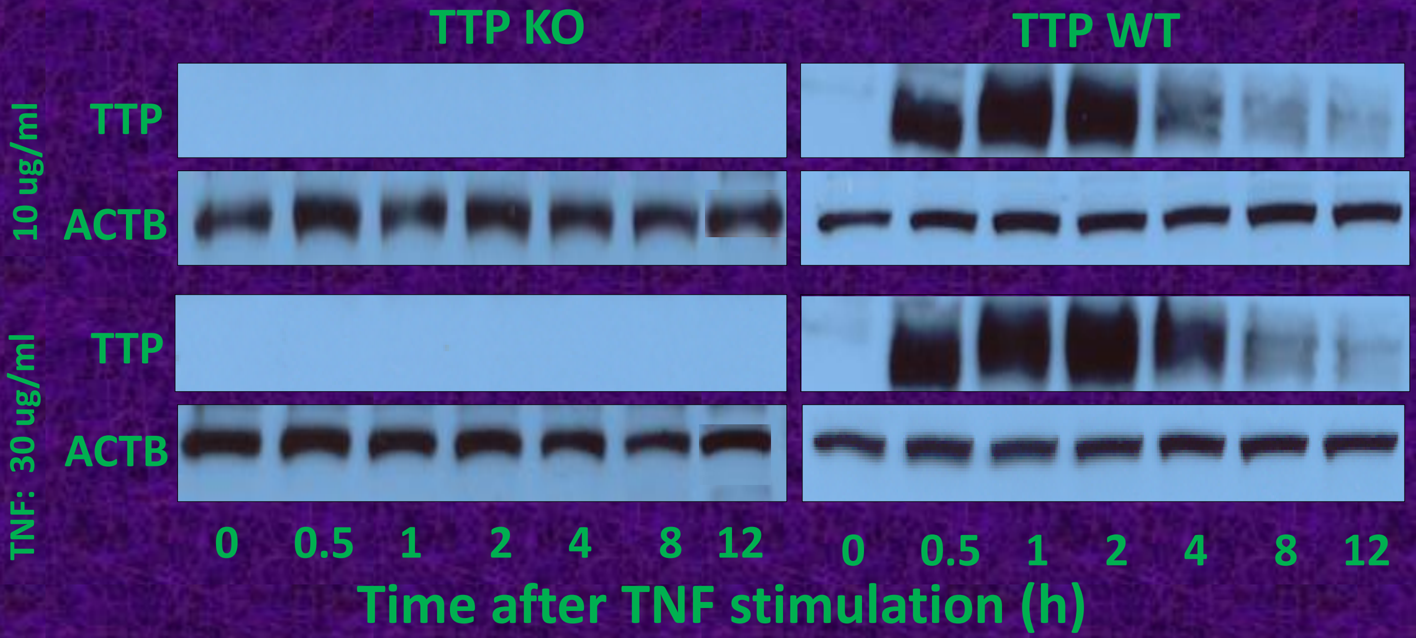
Illumina Microarray

Cell Line Characterization & TNF-induced TTP Expression

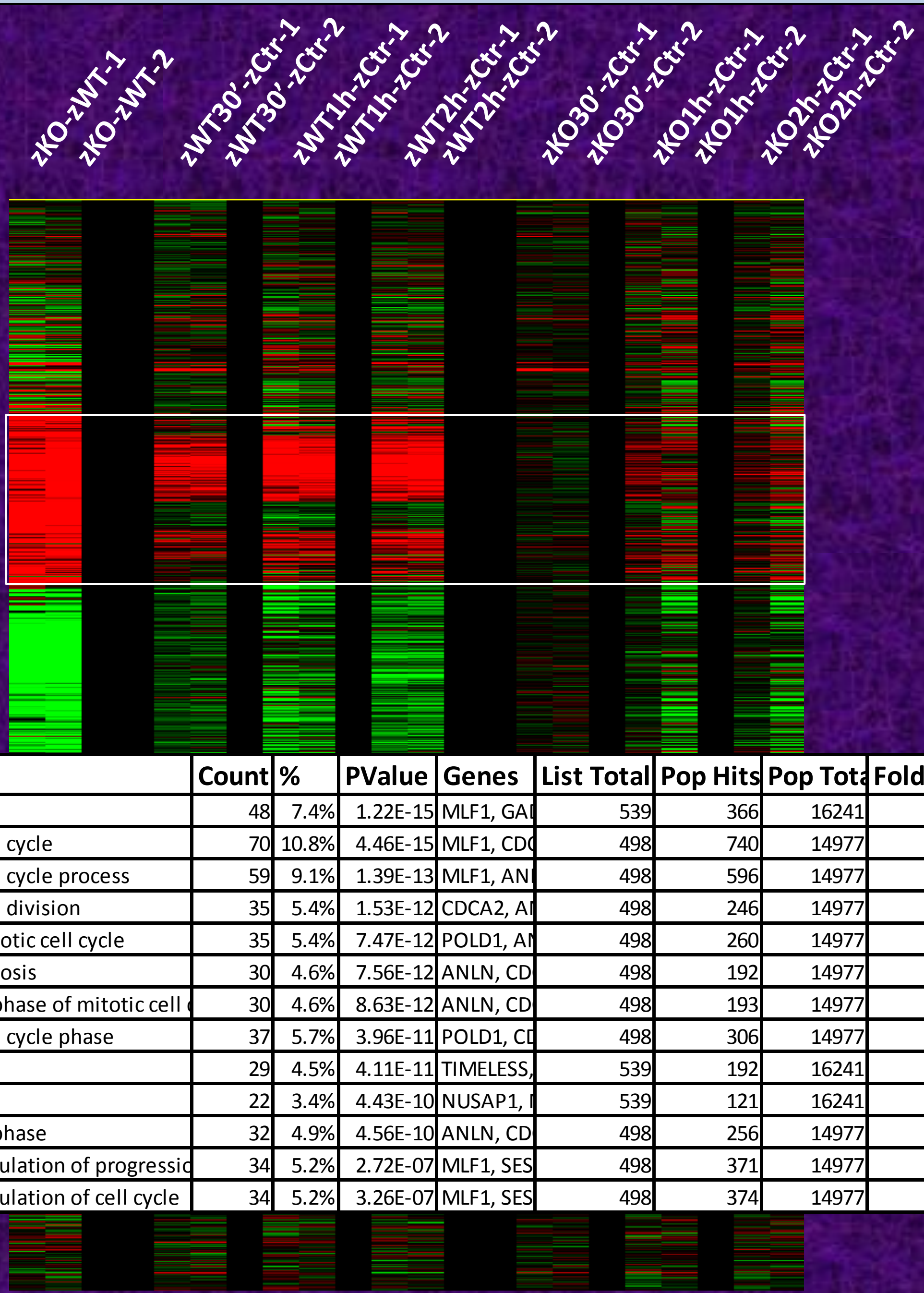
Cell Line Characterization:



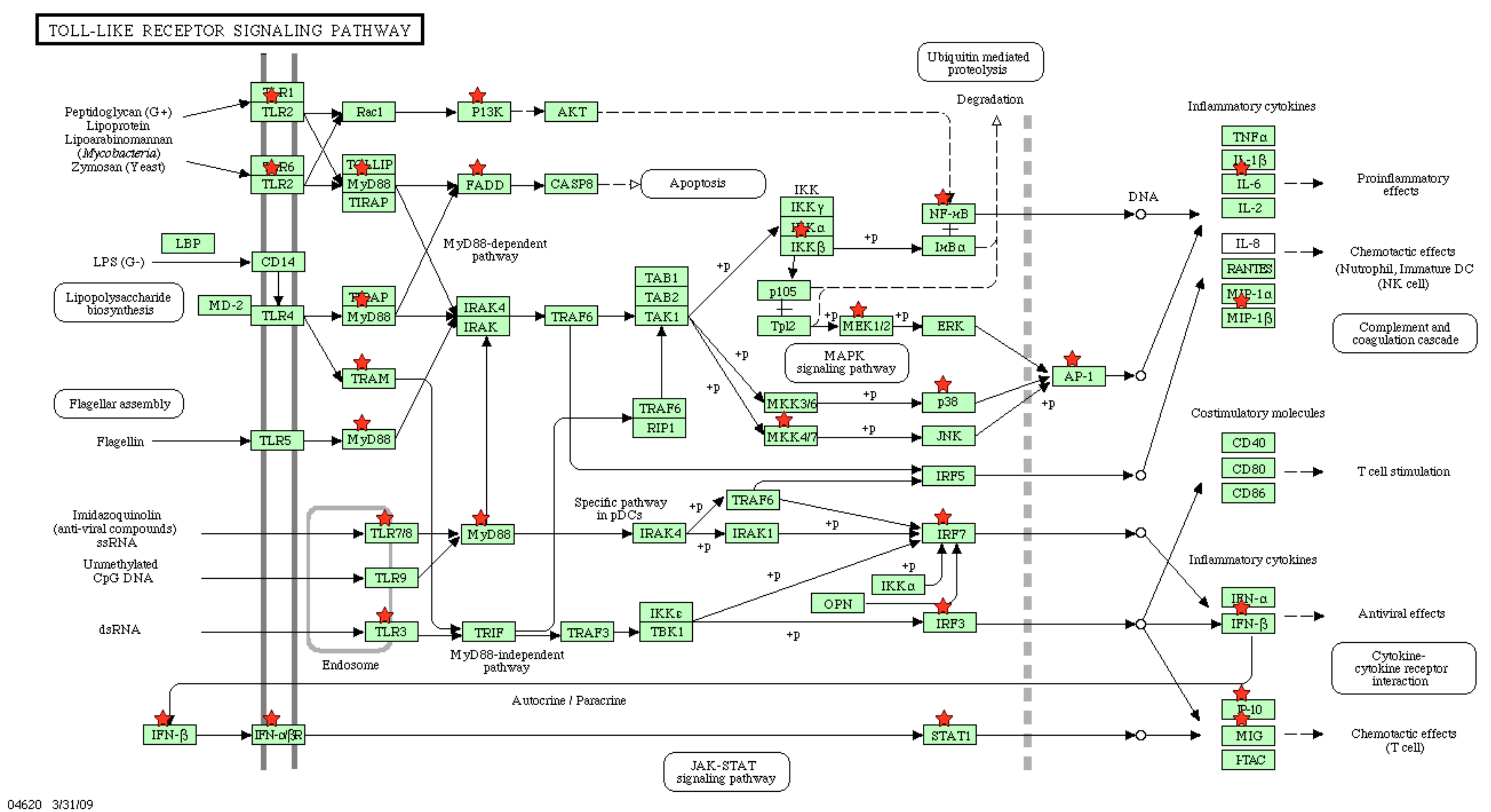
Induction of TTP Protein upon TNF Stimulation:



TNF-mediated Gene Expression in TTP KO & WT MEFs



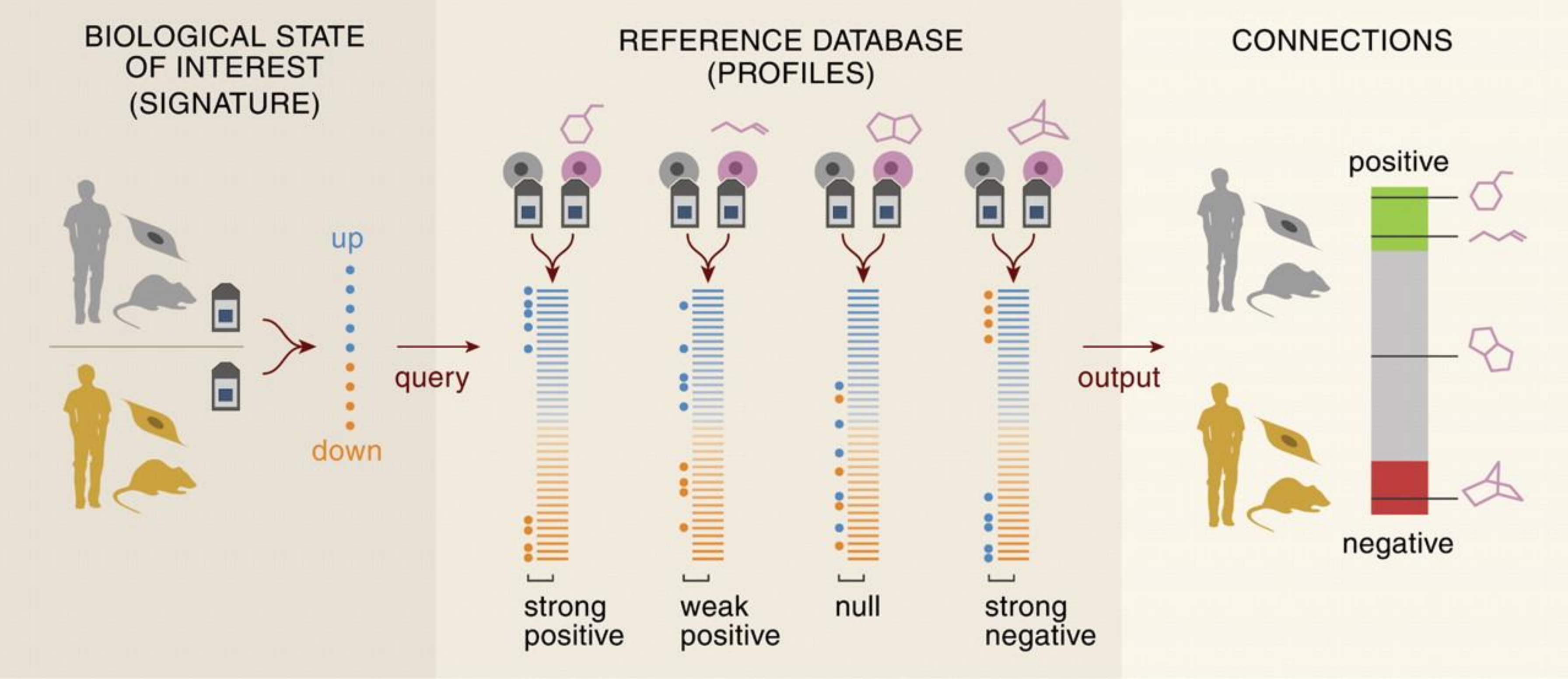
Category	Term	Count	%	PValue	Genes	List Total	Pop Hits	Pop Tota	Fold Enr	Bonferr	Benjam	FDR
SP_PIR_KEYWORDS	cell cycle	48	7.4%	1.22E-15	MLF1, GA	539	366	16241	3.95	1.06E-12	1.06E-12	1.90E-12
GOTERM_BP_ALL	GO:0007049~cell cycle	70	10.8%	4.46E-15	MLF1, CD	498	740	14977	2.84	2.31E-11	2.31E-11	8.48E-12
GOTERM_BP_ALL	GO:0022402~cell cycle process	59	9.1%	1.39E-13	MLF1, AN	498	596	14977	2.98	7.22E-10	3.61E-10	2.66E-10
GOTERM_BP_ALL	GO:0051301~cell division	35	5.4%	1.53E-12	CDCA2, A	498	246	14977	4.28	7.94E-09	2.65E-09	2.92E-09
GOTERM_BP_ALL	GO:0000278~mitotic cell cycle	35	5.4%	7.47E-12	POLD1, A	498	260	14977	4.05	3.88E-08	9.70E-09	1.43E-08
GOTERM_BP_ALL	GO:0007067~mitosis	30	4.6%	7.56E-12	ANLN, CD	498	192	14977	4.70	3.93E-08	7.85E-09	1.44E-08
GOTERM_BP_ALL	GO:0000087~M phase of mitotic cell	30	4.6%	8.63E-12	ANLN, CD	498	193	14977	4.67	4.48E-08	7.47E-09	1.65E-08
GOTERM_BP_ALL	GO:0022403~cell cycle phase	37	5.7%	3.96E-11	POLD1, C	498	306	14977	3.64	2.06E-07	2.94E-08	7.58E-08
SP_PIR_KEYWORDS	cell division	29	4.5%	4.11E-11	TIMELESS,	539	192	16241	4.55	3.56E-08	1.19E-08	6.37E-08
SP_PIR_KEYWORDS	mitosis	22	3.4%	4.43E-10	NUSAP1,	539	121	16241	5.48	3.84E-07	6.40E-08	6.87E-07
GOTERM_BP_ALL	GO:0000279~M phase	32	4.9%	4.56E-10	ANLN, CD	498	256	14977	3.76	2.37E-06	2.96E-07	8.70E-07
GOTERM_BP_ALL	GO:0000074~regulation of progressi	34	5.2%	2.72E-07	MLF1, SES	498	371	14977	2.76	0.00141	1.09E-04	5.19E-04
GOTERM_BP_ALL	GO:0051726~regulation of cell cycle	34	5.2%	3.26E-07	MLF1, SES	498	374	14977	2.73	0.00169	1.21E-04	6.22E-04

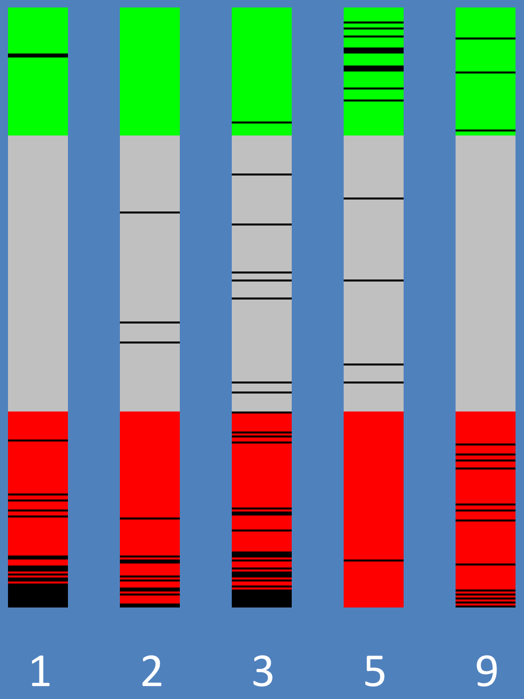

TOLL-LIKE RECEPTOR SIGNALING PATHWAY
Peptidoglycan (G+)
Lipoprotein
Lipoarabinomannan
(Mycobacteria)
Zymosan (Yeast)
TLR1
TLR2
TLR6
TLR2
Rac1
P13K
AKT
TOLLIP
MyD88
TIRAP
FADD
CASP8
Apoptosis
LBP
LPS (G-)
CD14
Lipopolysaccharide biosynthesis
MD-2
TLR4
TIRAP
MyD88
MyD88-dependent pathway
IRAK4
IRAK
TRAF6
TAB1
TAB2
TAK1
TRAM
Flagellar assembly
Flagellin
TLR5
MyD88
TRAF6
RIP1
IKK
IKKγ
IKKα
IKKβ
p105
Tpl2
MEK1/2
ERK
MAPK signaling pathway
MKK3/6
p38
MKK4/7
JNK
Ubiquitin mediated proteolysis
Degradation
NF-κB
IκBα
DNA
AP-1
IRF5
+p
Specific pathway in pDCs
TRAF6
IRAK4
IRAK1
IRF7
Imidazoquinolin
(anti-viral compounds)
ssRNA
Unmethylated CpG DNA
dsRNA
TLR7/8
TLR9
TLR3
Endosome
MyD88
IKKα
OPN
TRIF
TRAF3
IKKε
TBK1
IRF3
MyD88-independent pathway
Autocrine / Paracrine
IFN-β
IFN-α/βR
STAT1
JAK-STAT signaling pathway
Inflammatory cytokines
TNFα
IL-1β
IL-6
IL-2
Proinflammatory effects
IL-8
RANTES
MIP-1α
MIP-1β
Chemotactic effects
(Nutrophil, Immature DC
(NK cell)
Complement and coagulation cascade
Costimulatory molecules
CD40
CD80
CD86
T cell stimulation
Inflammatory cytokines
IFN-α
IFN-β
Antiviral effects
Cytokine-cytokine receptor interaction
IP-10
MIG
I-TAC
Chemotactic effects
(T cell)
04620 3/31/09
(c) Kanehisa Laboratories

The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease.

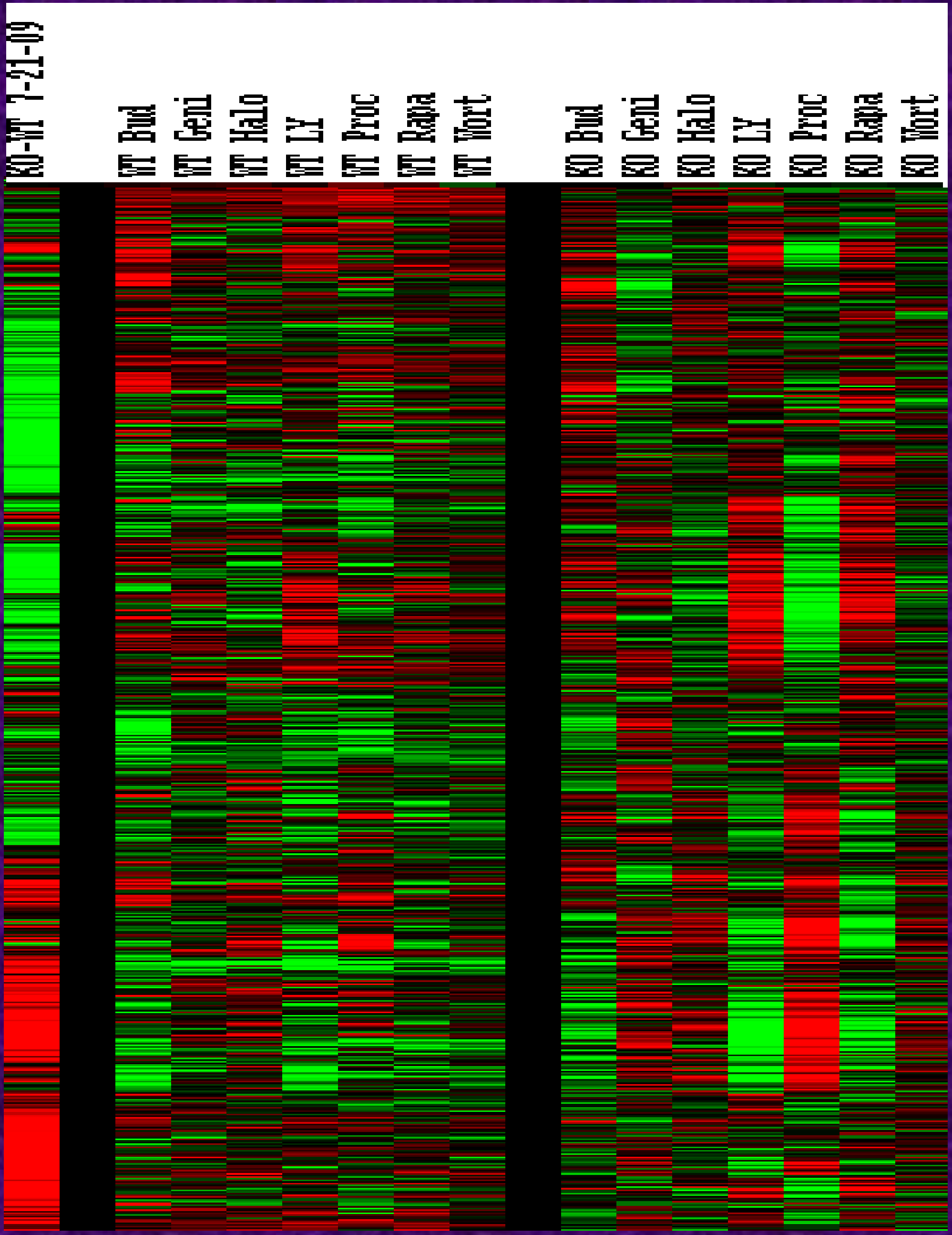
Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet JP, Subramanian A, Ross KN, Reich M, Hieronymus H, Wei G, Armstrong SA, Haggarty SJ, Clemons PA, Wei R, Carr SA, Lander ES, Golub TR.

Science. 2006 Sep 29;313(5795):1929-35.

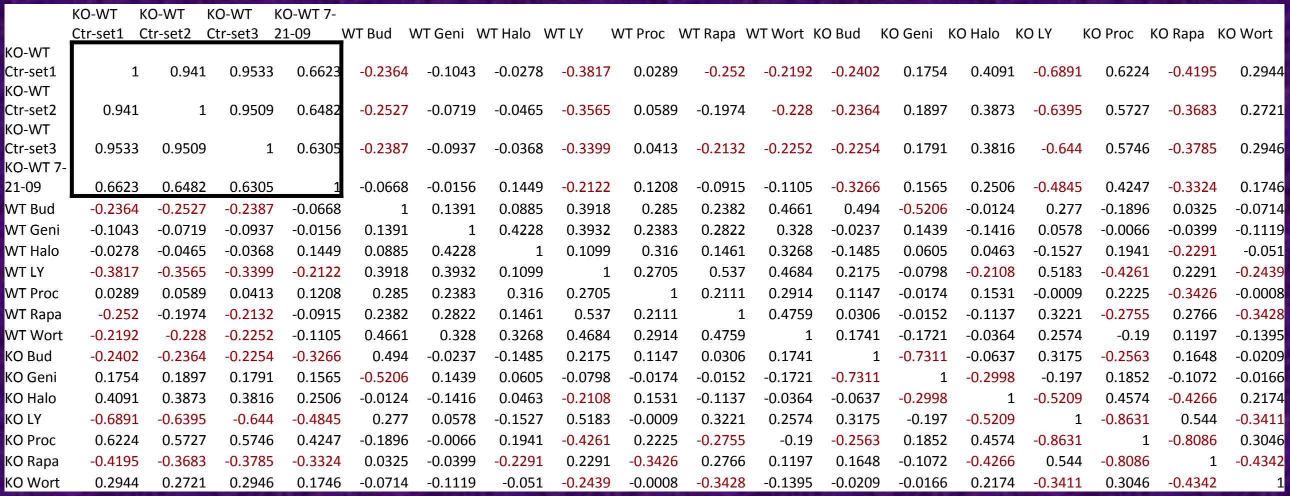




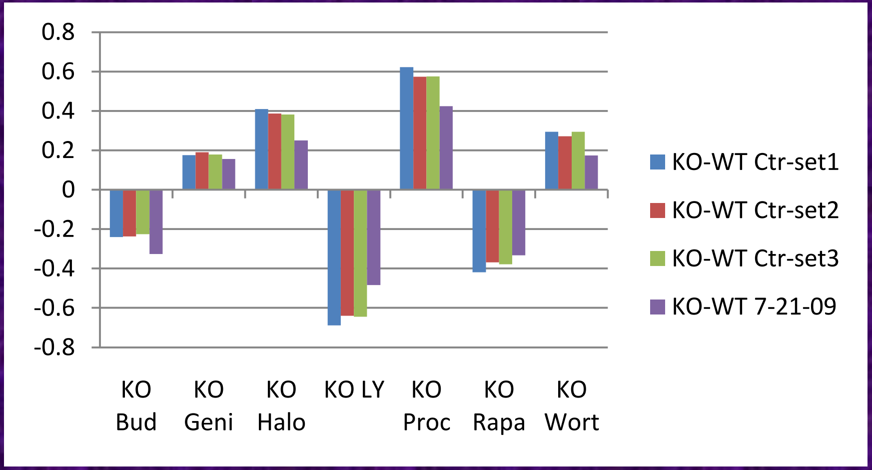
rank	cmap name	mean	n	enrichment	p	specificity	percent non-null
1	**sirolimus**	-0.633	44	-0.748	0	0	95
2	**wortmannin**	-0.598	18	-0.694	0	0.0127	83
3	**LY-294002**	-0.574	61	-0.621	0	0.0123	81
4	monorden	-0.337	22	-0.581	0	0	68
5	**genistein**	0.339	17	0.552	0	0.0058	70
6	tanespimycin	-0.361	62	-0.523	0	0.042	69
7	thioridazine	-0.43	20	-0.515	0	0.129	75
8	fulvestrant	-0.264	40	-0.396	0	0.0216	57
9	**prochlorperazine**	-0.372	16	-0.543	0.00002	0.0377	81
10	valproic acid	-0.253	57	-0.31	0.00002	0.0563	61

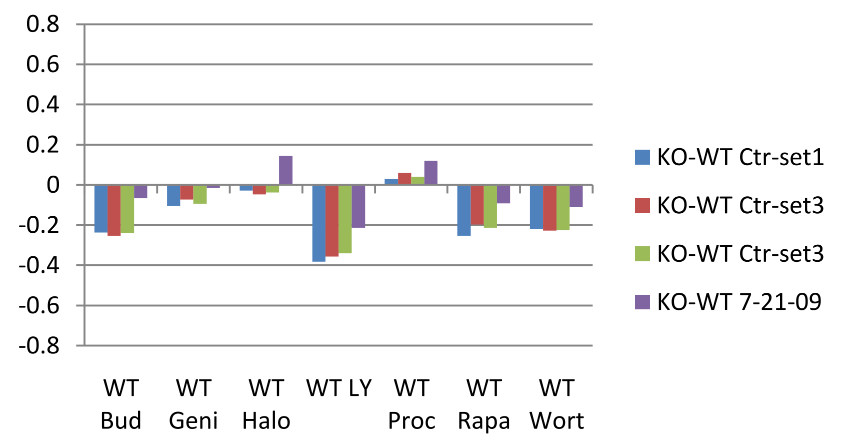


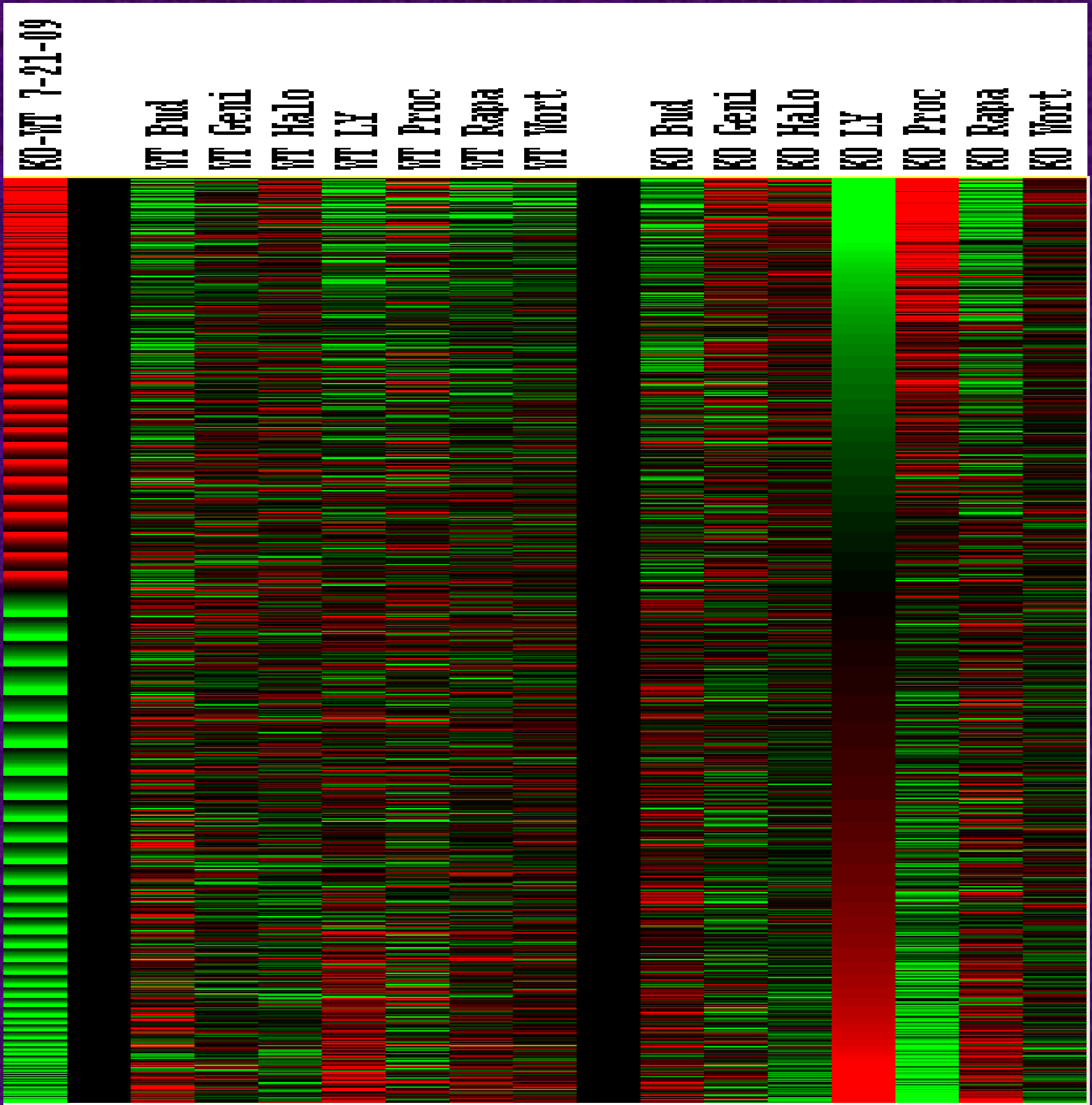
all present genes
- 13,659



	KO-WT Ctr-set1	KO-WT Ctr-set2	KO-WT Ctr-set3	KO-WT 7-21-09	WT Bud	WT Geni	WT Halo	WT LY	WT Proc	WT Rapa	WT Wort	KO Bud	KO Geni	KO Halo	KO LY	KO Proc	KO Rapa	KO Wort
KO-WT Ctr-set1	1	0.941	0.9533	0.6623	-0.2364	-0.1043	-0.0278	-0.3817	0.0289	-0.252	-0.2192	-0.2402	0.1754	0.4091	-0.6891	0.6224	-0.4195	0.2944
KO-WT Ctr-set2	0.941	1	0.9509	0.6482	-0.2527	-0.0719	-0.0465	-0.3565	0.0589	-0.1974	-0.228	-0.2364	0.1897	0.3873	-0.6395	0.5727	-0.3683	0.2721
KO-WT Ctr-set3	0.9533	0.9509	1	0.6305	-0.2387	-0.0937	-0.0368	-0.3399	0.0413	-0.2132	-0.2252	-0.2254	0.1791	0.3816	-0.644	0.5746	-0.3785	0.2946
KO-WT 7-21-09	0.6623	0.6482	0.6305	1	-0.0668	-0.0156	0.1449	-0.2122	0.1208	-0.0915	-0.1105	-0.3266	0.1565	0.2506	-0.4845	0.4247	-0.3324	0.1746
WT Bud	-0.2364	-0.2527	-0.2387	-0.0668	1	0.1391	0.0885	0.3918	0.285	0.2382	0.4661	0.494	-0.5206	-0.0124	0.277	-0.1896	0.0325	-0.0714
WT Geni	-0.1043	-0.0719	-0.0937	-0.0156	0.1391	1	0.4228	0.3932	0.2383	0.2822	0.328	-0.0237	0.1439	-0.1416	0.0578	-0.0066	-0.0399	-0.1119
WT Halo	-0.0278	-0.0465	-0.0368	0.1449	0.0885	0.4228	1	0.1099	0.316	0.1461	0.3268	-0.1485	0.0605	0.0463	-0.1527	0.1941	-0.2291	-0.051
WT LY	-0.3817	-0.3565	-0.3399	-0.2122	0.3918	0.3932	0.1099	1	0.2705	0.537	0.4684	0.2175	-0.0798	-0.2108	0.5183	-0.4261	0.2291	-0.2439
WT Proc	0.0289	0.0589	0.0413	0.1208	0.285	0.2383	0.316	0.2705	1	0.2111	0.2914	0.1147	-0.0174	0.1531	-0.0009	0.2225	-0.3426	-0.0008
WT Rapa	-0.252	-0.1974	-0.2132	-0.0915	0.2382	0.2822	0.1461	0.537	0.2111	1	0.4759	0.0306	-0.0152	-0.1137	0.3221	-0.2755	0.2766	-0.3428
WT Wort	-0.2192	-0.228	-0.2252	-0.1105	0.4661	0.328	0.3268	0.4684	0.2914	0.4759	1	0.1741	-0.1721	-0.0364	0.2574	-0.19	0.1197	-0.1395
KO Bud	-0.2402	-0.2364	-0.2254	-0.3266	0.494	-0.0237	-0.1485	0.2175	0.1147	0.0306	0.1741	1	-0.7311	-0.0637	0.3175	-0.2563	0.1648	-0.0209
KO Geni	0.1754	0.1897	0.1791	0.1565	-0.5206	0.1439	0.0605	-0.0798	-0.0174	-0.0152	-0.1721	-0.7311	1	-0.2998	-0.197	0.1852	-0.1072	-0.0166
KO Halo	0.4091	0.3873	0.3816	0.2506	-0.0124	-0.1416	0.0463	-0.2108	0.1531	-0.1137	-0.0364	-0.0637	-0.2998	1	-0.5209	0.4574	-0.4266	0.2174
KO LY	-0.6891	-0.6395	-0.644	-0.4845	0.277	0.0578	-0.1527	0.5183	-0.0009	0.3221	0.2574	0.3175	-0.197	-0.5209	1	-0.8631	0.544	-0.3411
KO Proc	0.6224	0.5727	0.5746	0.4247	-0.1896	-0.0066	0.1941	-0.4261	0.2225	-0.2755	-0.19	-0.2563	0.1852	0.4574	-0.8631	1	-0.8086	0.3046
KO Rapa	-0.4195	-0.3683	-0.3785	-0.3324	0.0325	-0.0399	-0.2291	0.2291	-0.3426	0.2766	0.1197	0.1648	-0.1072	-0.4266	0.544	-0.8086	1	-0.4342
KO Wort	0.2944	0.2721	0.2946	0.1746	-0.0714	-0.1119	-0.051	-0.2439	-0.0008	-0.3428	-0.1395	-0.0209	-0.0166	0.2174	-0.3411	0.3046	-0.4342	1






KO-WT 7-21-09
WT Bud
WT Geni
WT Halo
WT LY
WT Proc
WT Rapa
WT Wort
KO Bud
KO Geni
KO Halo
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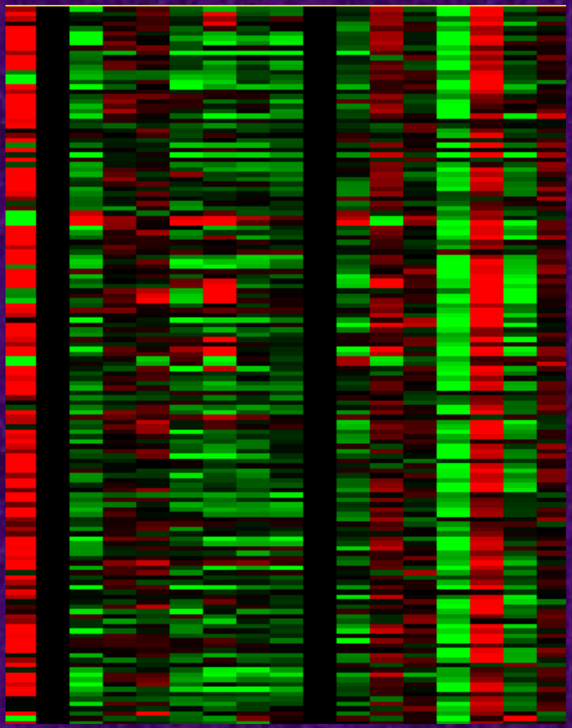
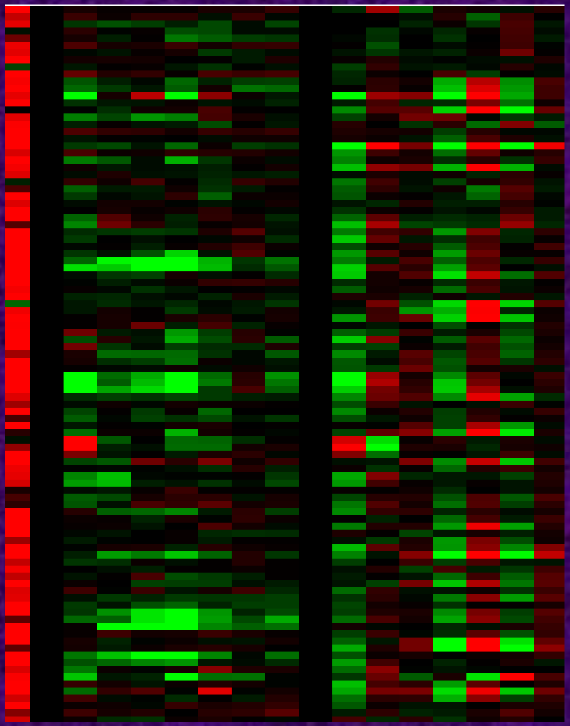


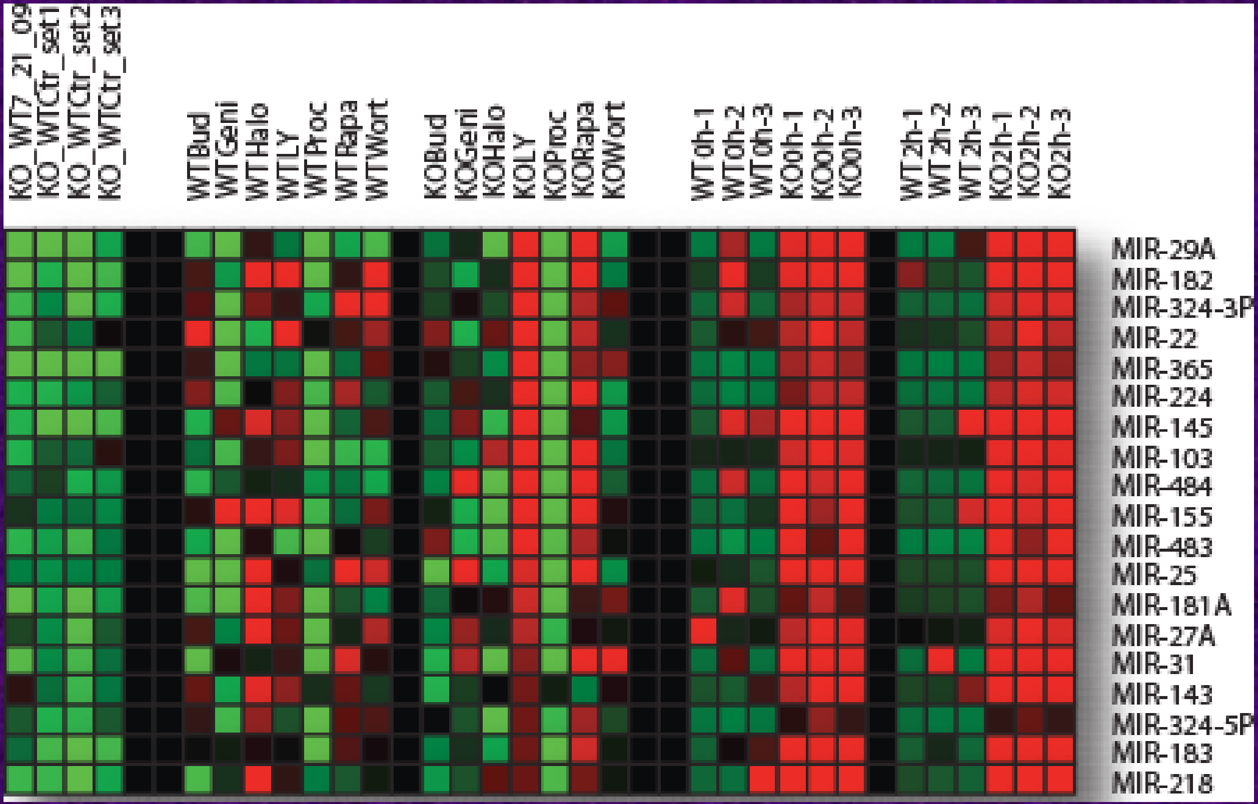
Cell cycle genes



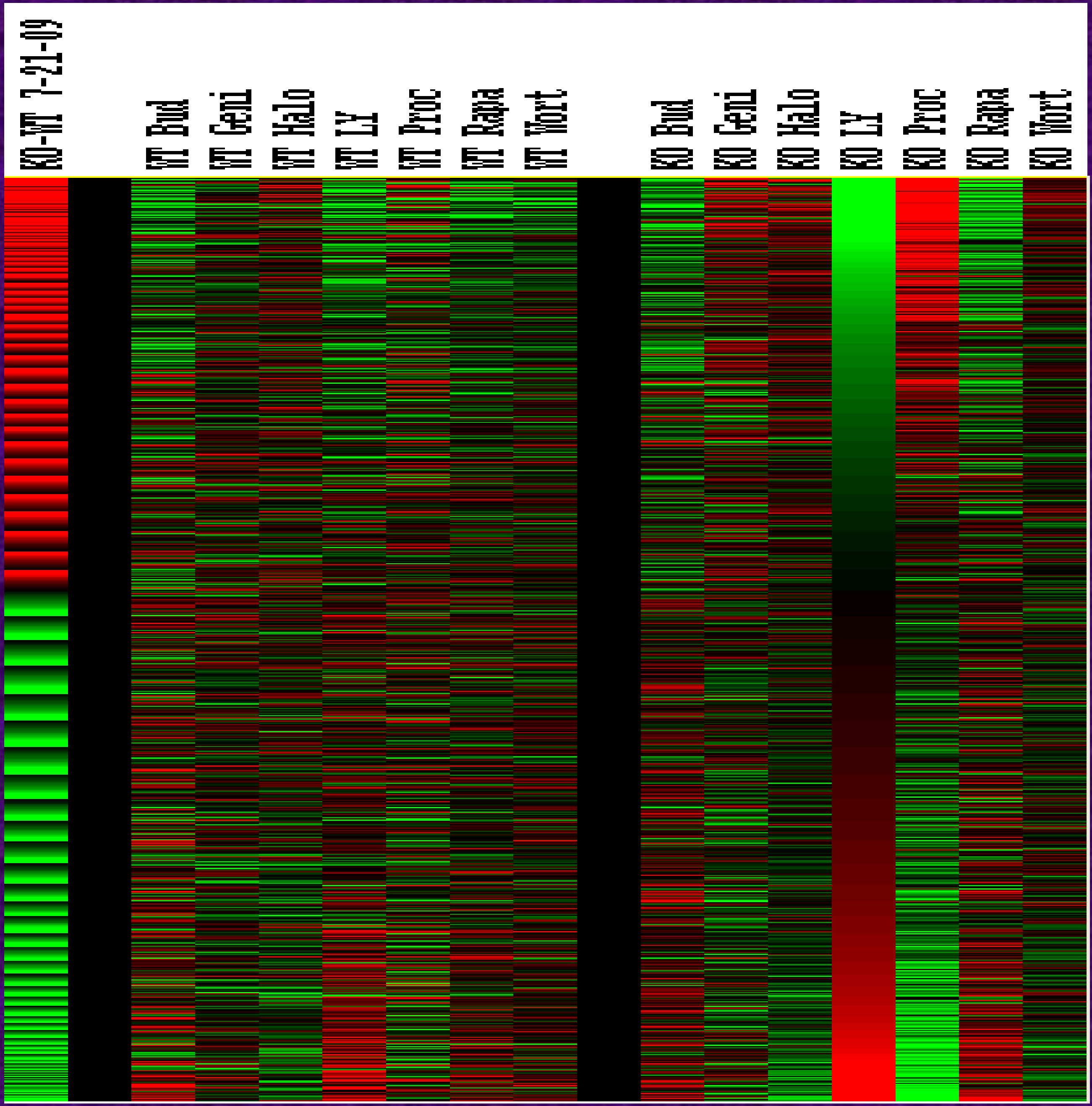
Immune response genes

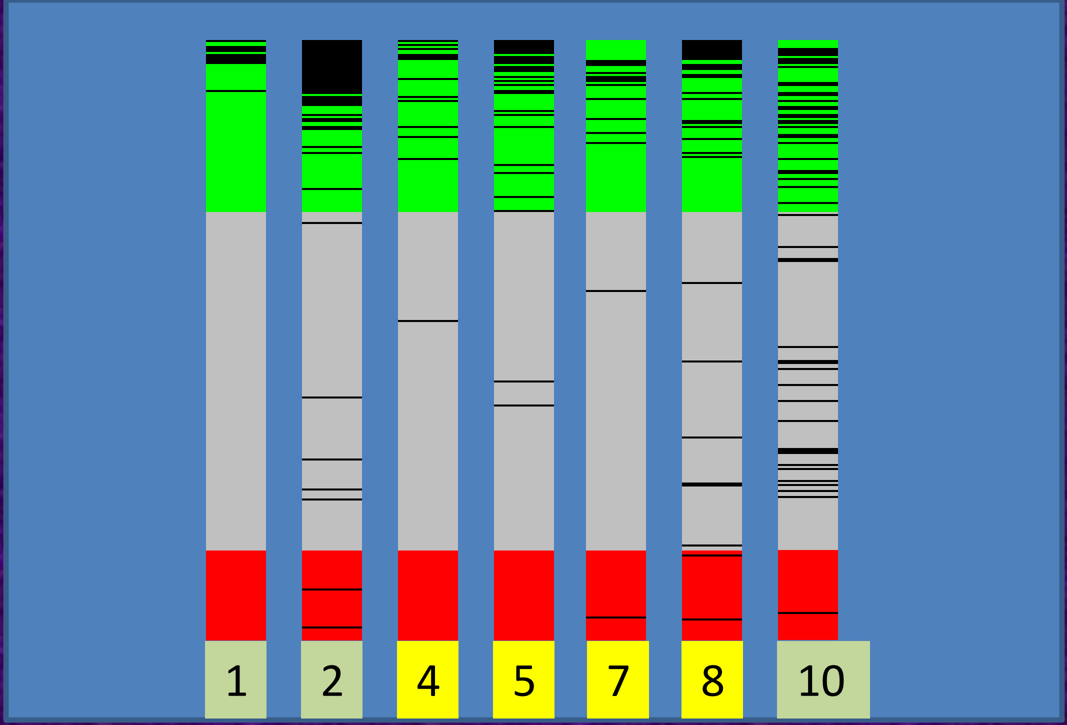
Expression of microRNA-targeted gene sets is decreased in KO/WT

microRNA expression is upregulated in KO/WT



Expression of microRNA-targeted gene sets is reversed by drug treatment


KO-WT 7-21-09
WT Bud
WT Geni
WT Halo
WT LY
WT Proc
WT Rapa
WT Wort
KO Bud
KO Geni
KO Halo
KO LY
KO Proc
KO Rapa
KO Wort



rank	cmap name	mean	n	enrichment	p	specificity	percent non-null
1	vorinostat	0.664	12	0.918	0	0.0503	100
2	trichostatin A	0.582	182	0.827	0	0.0332	96
3	trifluoperazine	0.534	16	0.785	0	0	93
4	wortmannin	0.602	18	0.749	0	0	94
5	sirolimus	0.589	44	0.723	0	0.006	95
6	thioridazine	0.484	20	0.715	0	0.0274	90
7	prochlorperazine	0.425	16	0.705	0	0.0097	87
8	LY-294002	0.614	61	0.684	0	0.0067	86
9	fluphenazine	0.455	18	0.662	0	0.0052	94
10	valproic acid	0.276	57	0.32	0	0.0395	57

Tristetraprolin regulates Cyclin D1 and c-Myc mRNA stability in response to rapamycin in an Akt-dependent manner via p38 MAPK signaling.

Marderosian M, Sharma A, Funk AP, Vartanian R, Masri J, Jo OD, Gera JF.

Oncogene. 2006 Oct 12;25(47):6277-90. Epub 2006 May 15.

A novel mechanism of tumor suppression by destabilizing AU-rich growth factor mRNA.

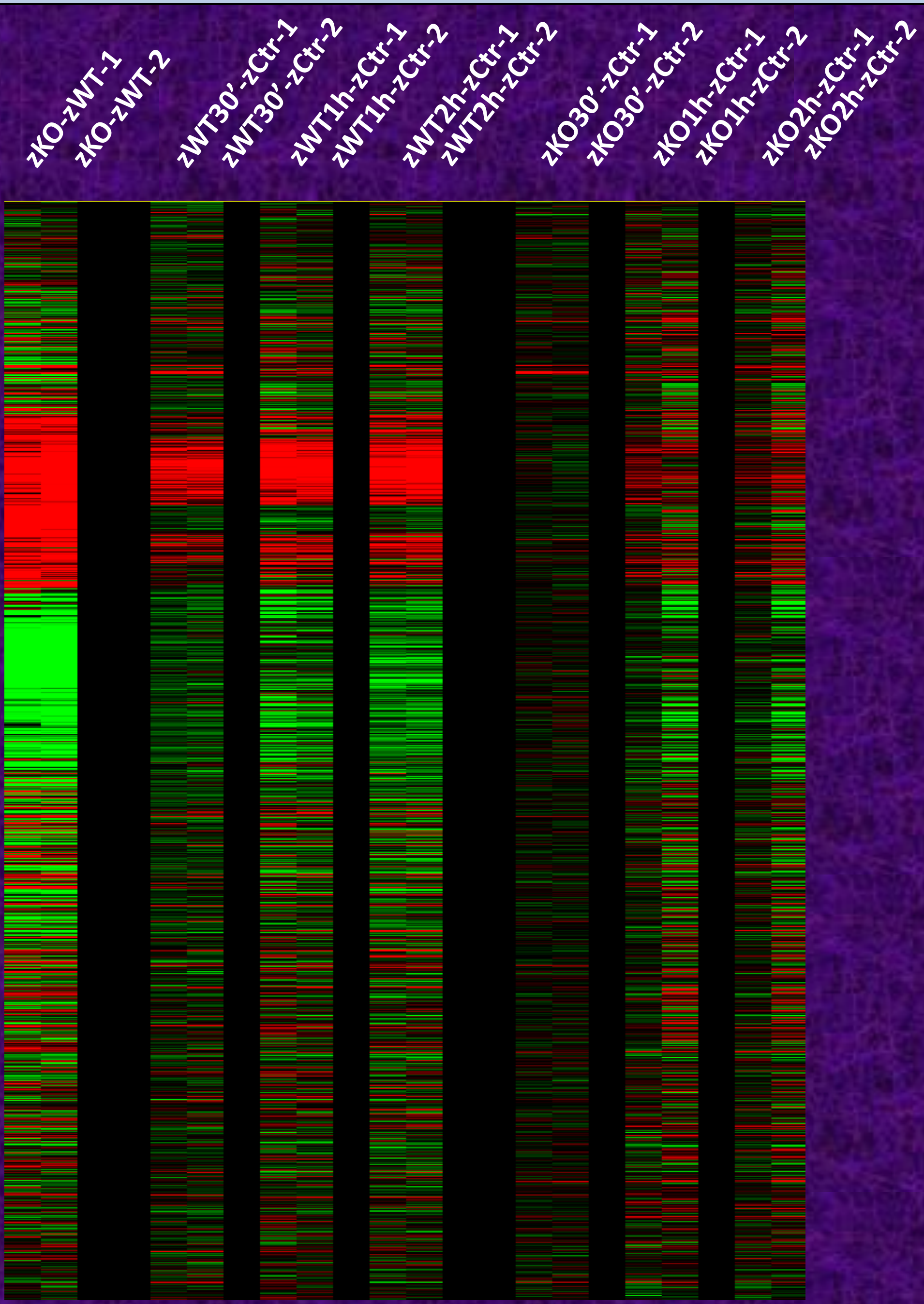
Stoecklin G, Gross B, Ming XF, Moroni C.

Oncogene. 2003 Jun 5;22(23):3554-61.

The occurrence of pathologically stable mRNAs of proto-oncogenes, growth factors and cyclins has been proposed to contribute to experimental and human oncogenesis. In normal resting cells, mRNAs containing an AU-rich element (ARE) in their 3' untranslated region are subjected to rapid degradation. Tristetraprolin (TTP) is an RNA-binding zinc-finger protein that promotes decay of ARE-containing mRNAs. Here we report that TTP acts as a potent tumor suppressor in a v-H-*ras*-dependent mast cell tumor model, where tumors express abnormally stable interleukin-3 (IL-3) mRNA as part of an oncogenic autocrine loop. Premalignant v-H-*ras* cells were transfected with TTP and injected into syngeneic mice. TTP expression delayed tumor progression by 4 weeks, and late appearing tumors escaped suppression by loss of TTP. When transfected into a fully established tumor line, TTP reduced cloning efficiency *in vitro* and growth of the inoculated cells *in vivo*. Transgenic TTP interfered with the autocrine loop by enhancing the degradation of IL-3 mRNA with concomitant reduction of IL-3 secretion. Our data establish the ARE as an antioncogenic target in a model situation, underline the importance of mRNA stabilization in oncogenesis and show for the first time that tumor suppression can be achieved by interfering with mRNA turnover.

Here we report that TTP acts as a potent tumor suppressor…

TNF-mediated Gene Expression in TTP KO & WT MEFs



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