

# Felsher Liver Preliminary Data Analysis

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# Genome browser session link

- [http://genome.ucsc.edu/cgi-bin/hgTracks?db=mm10&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr17%3A47747313%2D48130099&hgqid=726431631\\_9jmlxNbVgeR4kCdWZHZsle061kqi](http://genome.ucsc.edu/cgi-bin/hgTracks?db=mm10&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr17%3A47747313%2D48130099&hgqid=726431631_9jmlxNbVgeR4kCdWZHZsle061kqi)

# Alignment and Peak calling

Downstream CHIP-seq data was processed through our Genialis platform

**Collection title** : Felsher  
**Fastq sample type** : Paired-end  
**Species** : Mus Musculus

**Alignment tool** : **Bowtie2**

Build : MM10  
Alignment mode : end to end  
Speed vs sensitivity : sensitive

(Default remaining settings)

**Peak calling tool** : **MACS 1.4**

Promoter regions Bed file : MM10\_TSS\_ALL\_-1000\_+1000.bed  
Use TagAlign files : Yes  
Quality filtering threshold : 30  
Backgrounds : Yes

(Default remaining settings)

# QC

## Post-alignment & post-peak calling

SAMPLE_NAME	GENOTYPE	DESCRIPTION	TOTAL_READS	MAPPED_READS	MAPPED_PERCENTAGE	FRIP	NUMBER_OF_PEAKS	NUMBER_OF_READS_IN_PROMOTERS
056_LIVER_MYC_H3K27AC_8CYC	MYC	H3K27ac_ChIP	23243905	20954455	90.15%	0.011	4748	651129
057_LIVER_MYC_MYC	MYC	MYC_ChIP	40840828	33362854	81.69%	0.027	8682	827282
057_LIVER_MYC_TWIST_MYC	MYC_TWIST	MYC_ChIP	36179288	32656257	90.26%	0.011	6631	656494
4134_LIVER_MYC_TWIST_H3K27AC	MYC_TWIST	H3K27ac_ChIP	15266394	14057425	92.08%	0.281	49007	811458
5039_LIVER_MYC_TWIST_H3K27AC	MYC_TWIST	H3K27ac_ChIP	11584118	10656193	91.99%	0.28	45057	728923
4134_LIVER_MYC_TWIST_MYC	MYC_TWIST	MYC_ChIP	14054121	11918675	84.81%	0.061	13553	343571
5039_LIVER_MYC_TWIST_MYC	MYC_TWIST	MYC_ChIP	14489086	13180005	90.97%	0.061	14215	587583
9204_LIVER_TWIST_H3K27AC	TWIST	H3K27ac_ChIP	10722258	9874373	92.09%	0.405	60422	715185
9203_LIVER_TWIST_H3K27AC	TWIST	H3K27ac_ChIP	15446938	14109079	91.34%	0.24	45606	719369
9203_LIVER_TWIST_MYC	TWIST	MYC_ChIP	12652939	10835771	85.64%	0.011	1337	48457
9204_LIVER_TWIST_MYC	TWIST	MYC_ChIP	16975904	14524978	85.56%	0.003	551	149229

Dropped samples (bad reads) :

- 5566\_LIVER\_MYC\_H3K27AC
- 056\_LIVER\_MYC\_H3K27AC\_4CYC
- 057\_LIVER\_MYC\_TWIST\_H3K27AC

# QC summary

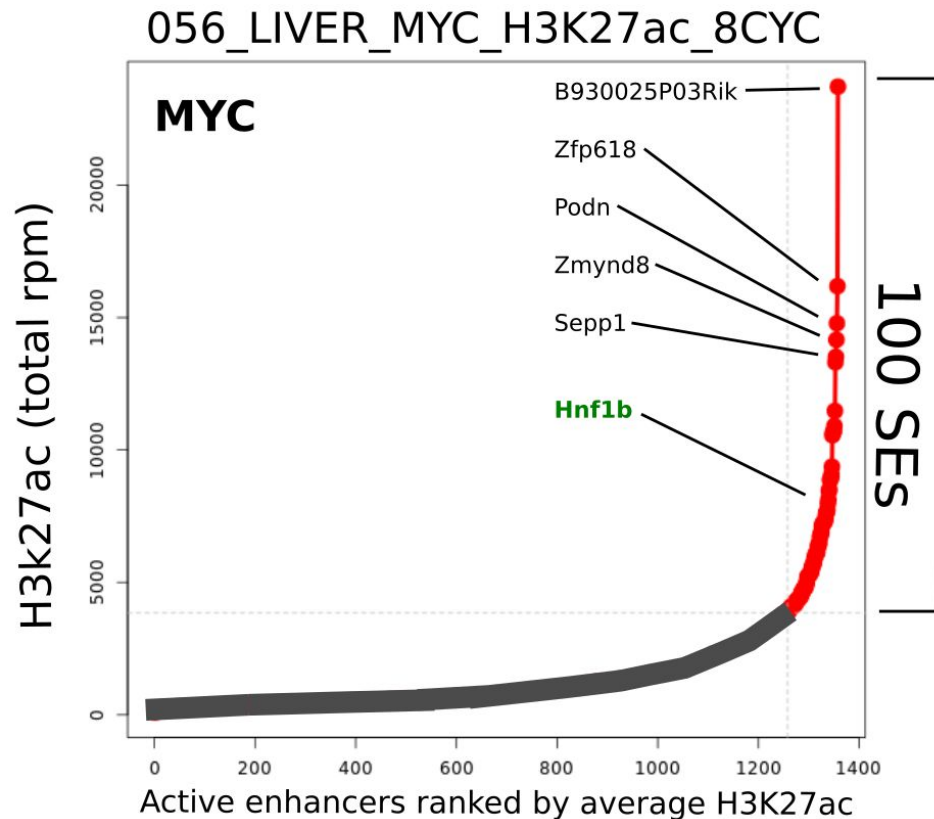
- 2 good H3K27ac and MYC datasets for the MYC+TWIST genotype
- 2 good H3K27ac datasets in the +TWIST genotype (MYC alone had very few peaks as expected)
- Only 1 high quality dataset for H3K27ac and MYC in the + MYC genotype

# H3K27ac super enhancers in +MYC genotype

## H3K27ac samples

### Parameters:

Genome Build	:	MM10
Input GFFs	:	H3K27ac beds
Ranking bams	:	H3K27ac bams
Stitching distance	:	12500 bp
TSS exclusion	:	2500 bp (default)
Background specified	:	yes
Mask blacklist regions	:	yes

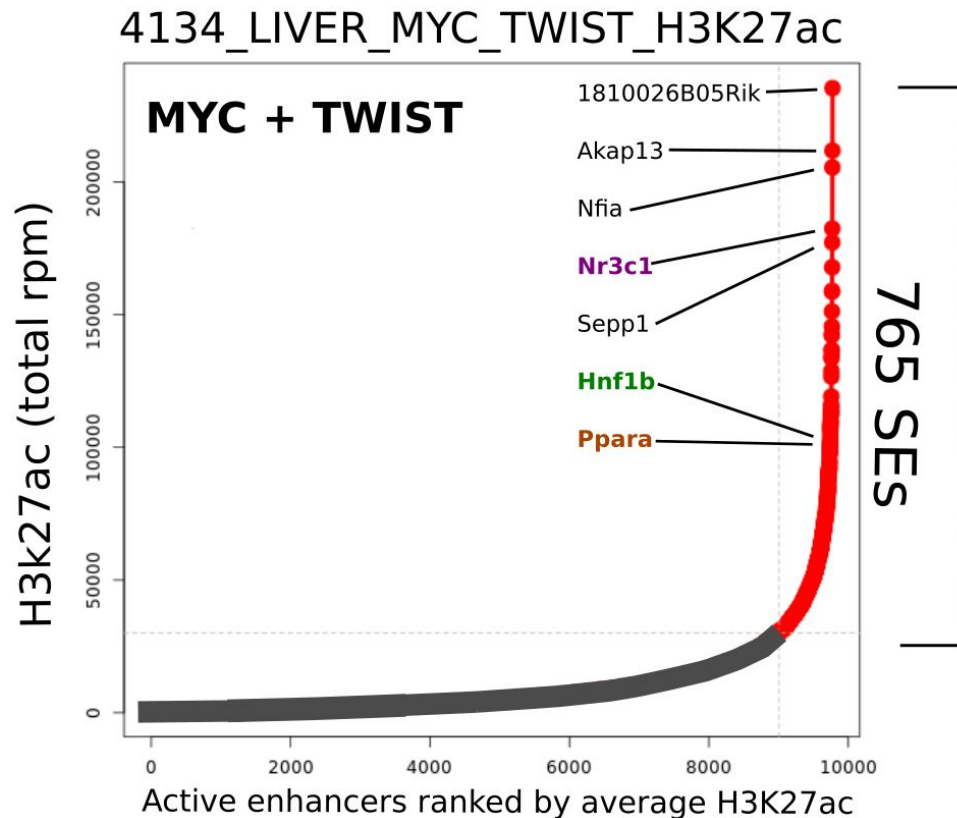


# H3K27ac super enhancers in MYC+TWIST genotype

## H3K27ac samples

### Parameters:

Genome Build	:	MM10
Input GFFs	:	H3K27ac beds
Ranking bams	:	H3K27ac bams
Stitching distance	:	12500 bp
TSS exclusion	:	2500 bp (default)
Background specified	:	yes
Mask blacklist regions	:	yes

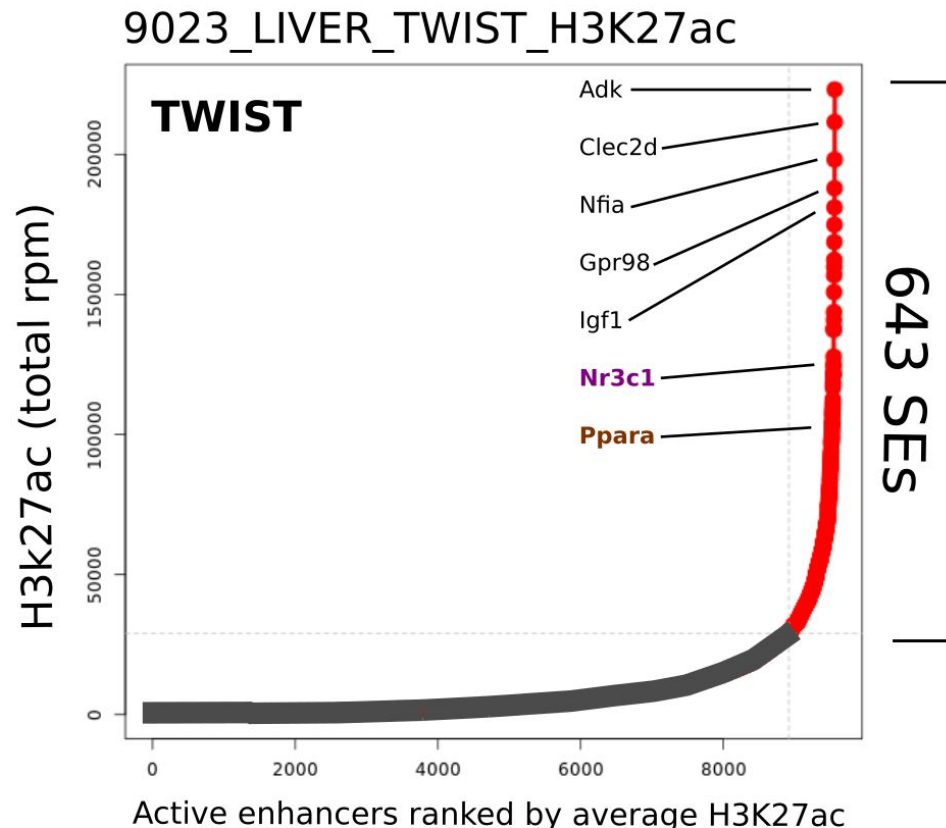


# H3K27ac super enhancers in +TWIST genotype

## H3K27ac samples

### Parameters:

Genome Build	:	MM10
Input GFFs	:	H3K27ac beds
Ranking bams	:	H3K27ac bams
Stitching distance	:	12500 bp
TSS exclusion	:	2500 bp (default)
Background specified	:	yes
Mask blacklist regions	:	yes



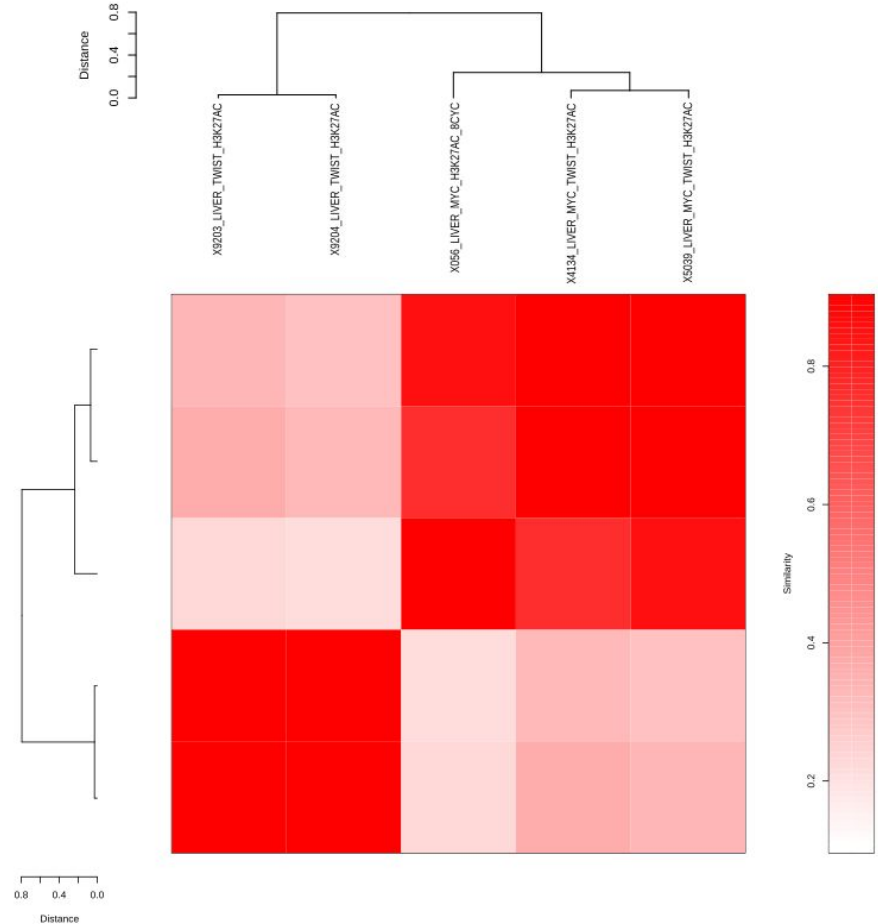


# Enhancer mapping summary

- H3K27ac dataset in the +MYC genotype isn't as high quality, not as much dynamic range
- Liver identity seems maintained in the +TWIST or MYC+TWST samples (e.g. Ppara, Hnf1a, Nr3c1)

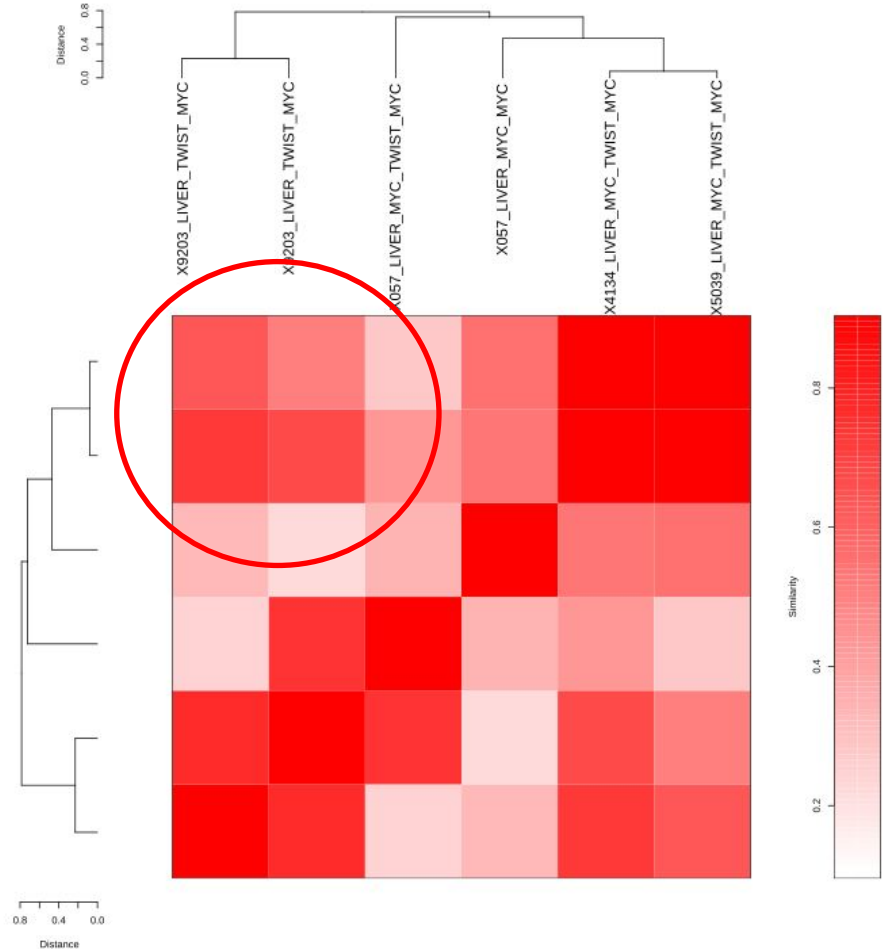
# Clustering H3K27ac super enhancer landscapes

- +MYC and MYC+TWIST samples distinctly cluster together



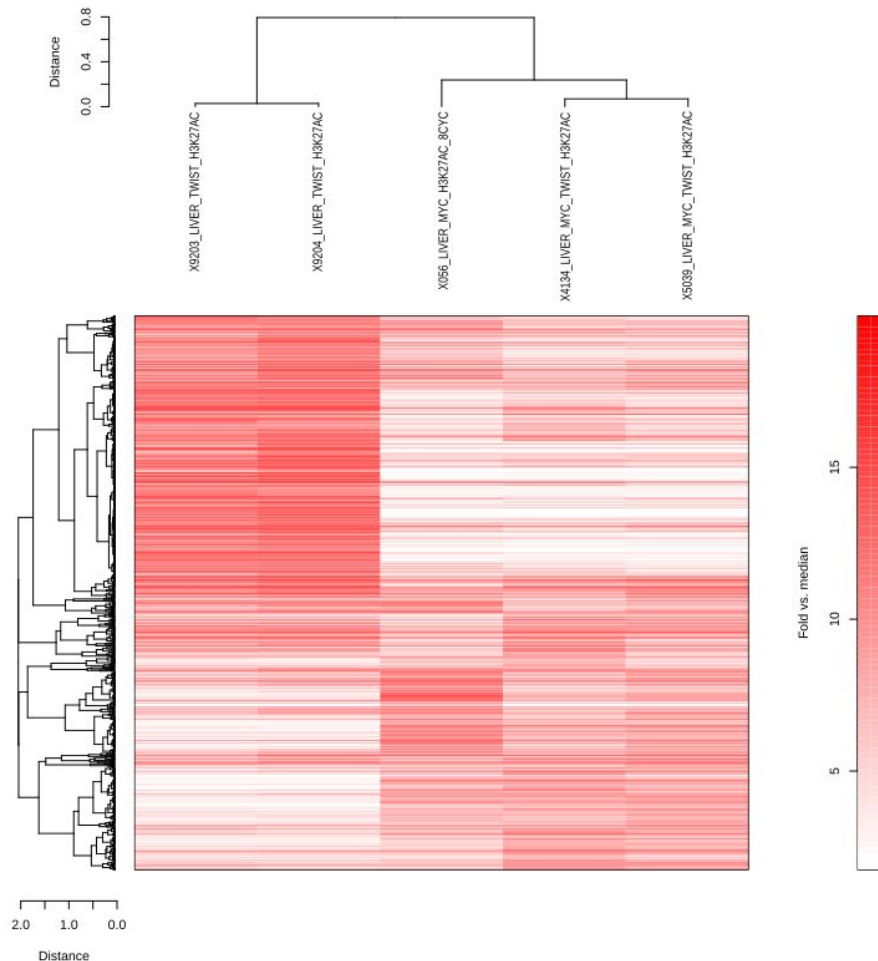
# Clustering MYC landscapes

- MYC+TWIST shows more similarity to +TWIST than to +MYC alone
- Consistent with MYC following other pioneering like factors



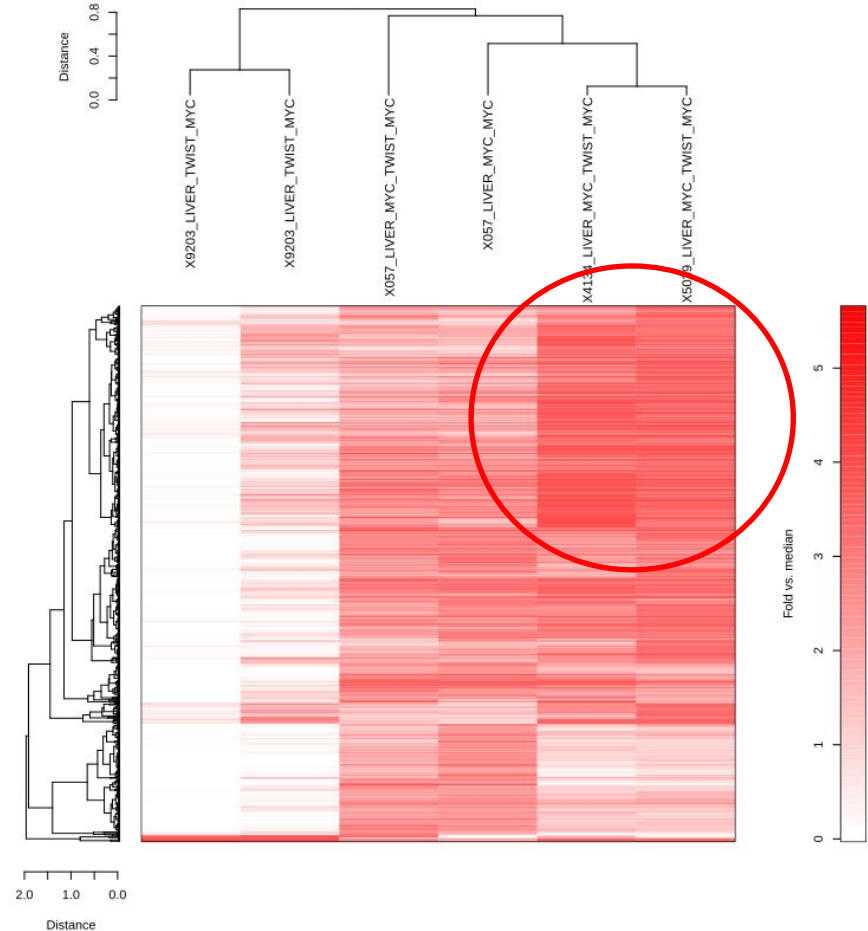
# Clustering H3K27ac super enhancer landscapes – all super enhancer regions as rows

- Enhancers in the +MYC state cluster with MYC+TWIST more than +TWIST
- TWIST only seems to define a large cohort of distinct enhancers



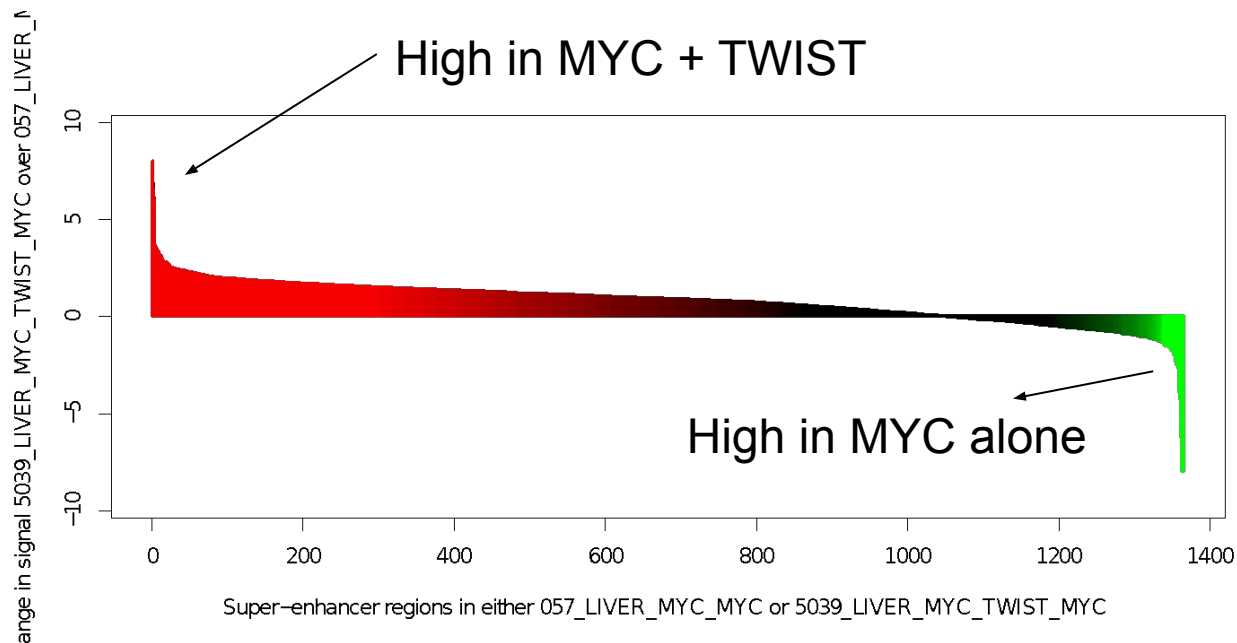
# Clustering MYC landscapes – all regions as rows

- Very strong set of regions with high MYC in the MYC+TWIST genotype

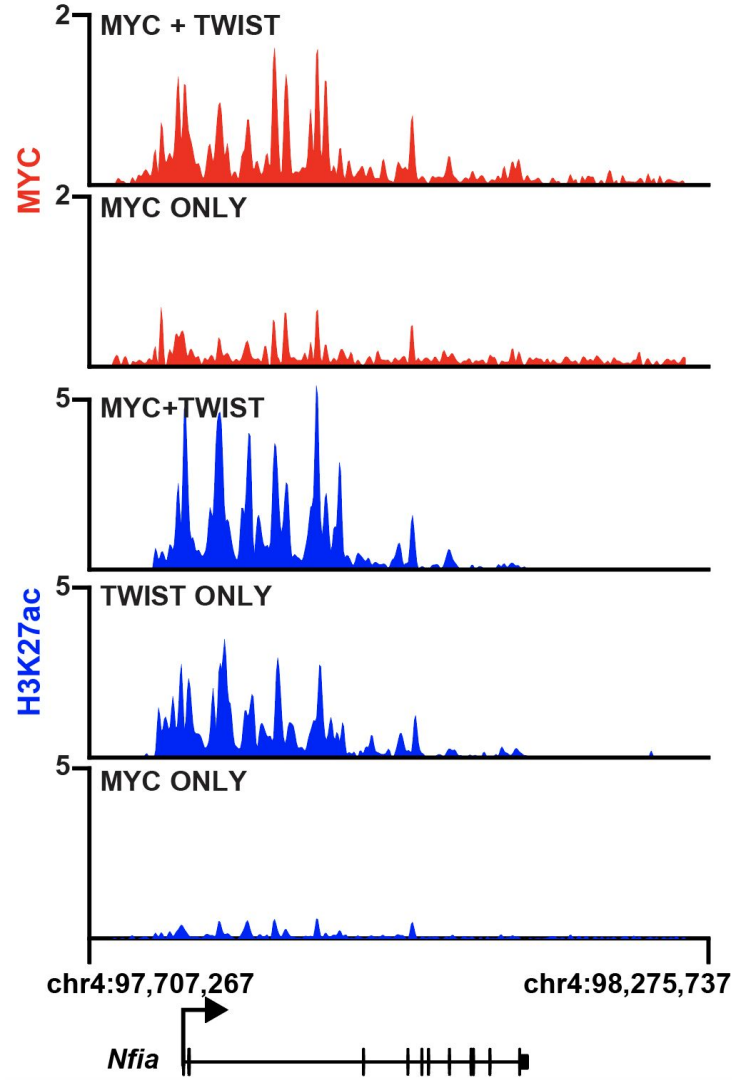


# Dynamic comparison of MYC binding

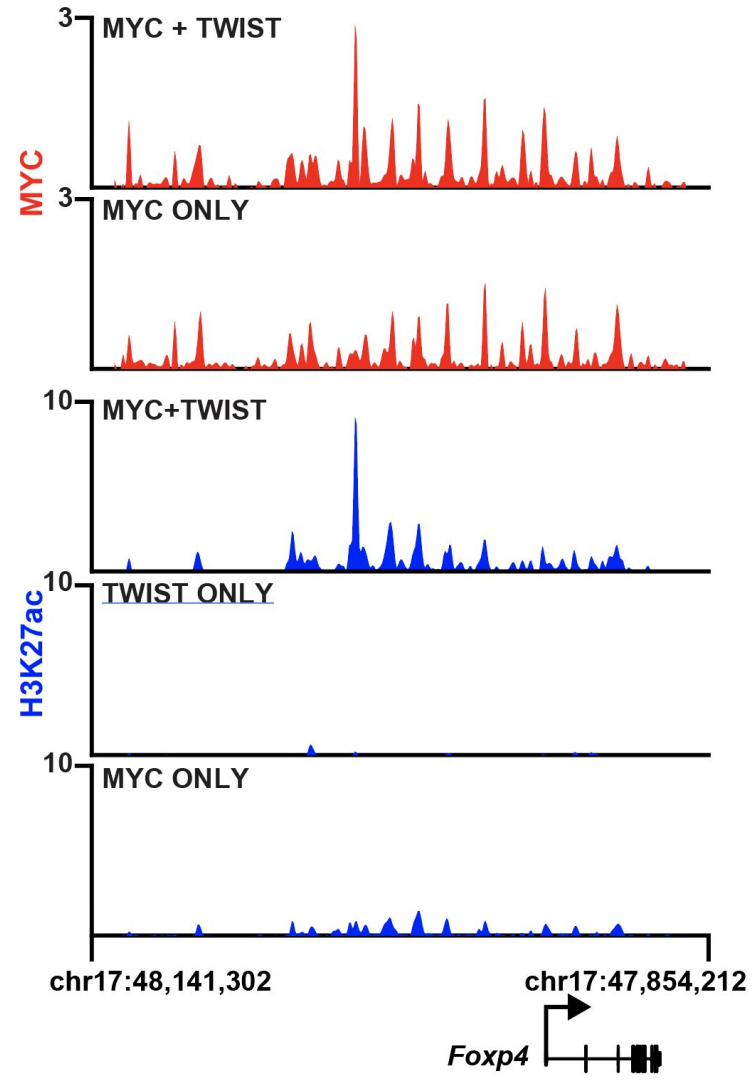
Top MYC peaks in either MYC + TWIST versus MYC alone



Genes with elevated  
MYC and H3K27ac  
in the +TWIST genotype

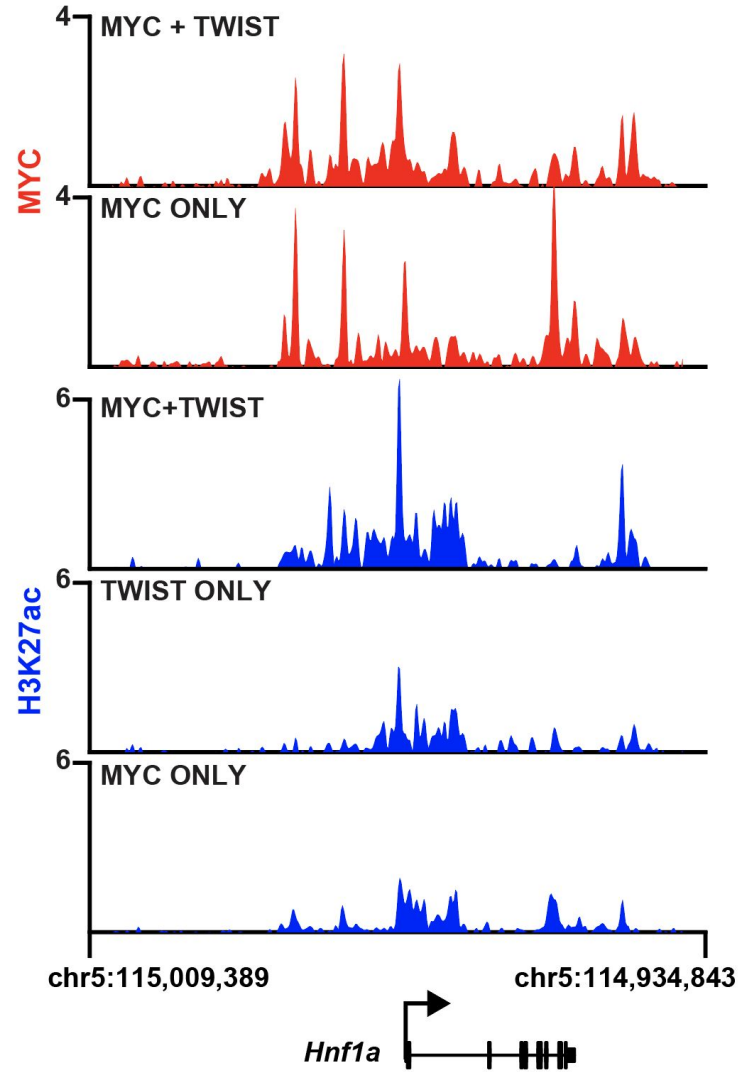


Genes with elevated  
MYC and H3K27ac  
only in MYC + TWIST





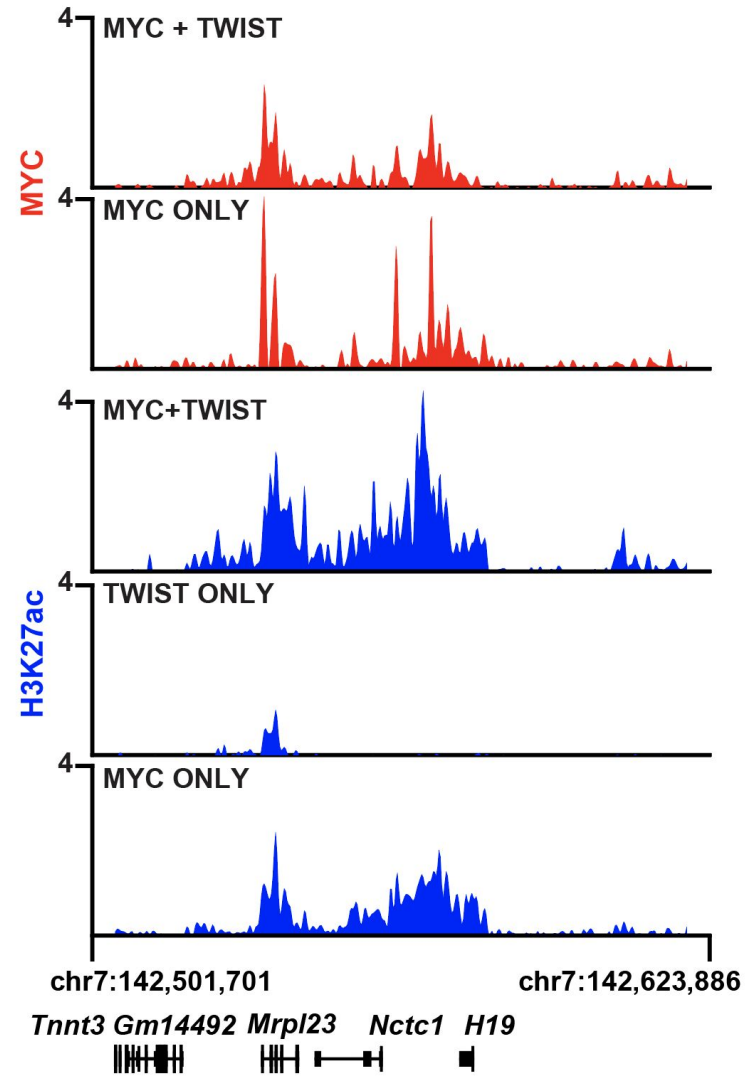
Genes with stable MYC  
and H3K27ac in all  
genotypes



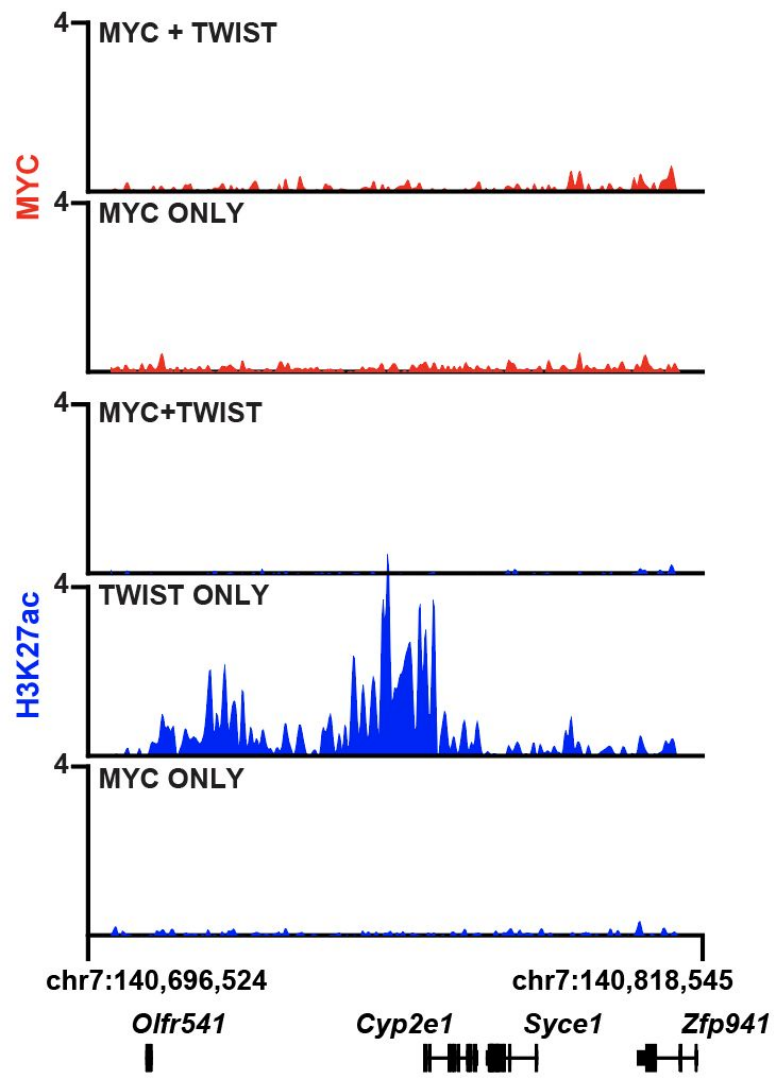
# Pathways associated with high MYC in MYC+TWIST vs. MYC only

Gene Set Name [# Genes (K)]	Description	# Genes in Overlap (k)	k/K	p-value ?	FDR q-value ?
MARSON_BOUND_BY_FOXP3_STIMULATED [1022]	Genes with promoters bound by FOXP3 [GeneID=50943] in hybridoma cells stimulated by PMA [PubChem=4792] and ionomycin [PubChem=3733].	45	<div></div>	8.62 e-28	4.11 e-24
MARSON_BOUND_BY_FOXP3_UNSTIMULATED [1229]	Genes with promoters bound by FOXP3 [GeneID=50943] in unstimulated hybridoma cells.	45	<div></div>	1.66 e-24	3.96 e-21
FISCHER_DREAM_TARGETS [929]	Target genes of the DREAM complex.	33	<div></div>	1.01 e-17	1.61 e-14
REACTOME_AMYLOIDS [83]	Genes involved in Amyloids	14	<div></div>	1.97 e-17	2.34 e-14
REACTOME_RNA_POL_I_PROMOTER_OPENING [62]	Genes involved in RNA Polymerase I Promoter Opening	12	<div></div>	6.86 e-16	6.53 e-13
REACTOME_MEIOTIC_RECOMBINATION [86]	Genes involved in Meiotic Recombination	13	<div></div>	1.26 e-15	9.98 e-13
REACTOME_RNA_POL_I_TRANSCRIPTION [89]	Genes involved in RNA Polymerase I Transcription	13	<div></div>	2 e-15	1.35 e-12
REACTOME_TRANSCRIPTION [210]	Genes involved in Transcription	17	<div></div>	2.26 e-15	1.35 e-12
PUJANA_BRCA1_PCC_NETWORK [1652]	Genes constituting the BRCA1-PCC network of transcripts whose expression positively correlated (Pearson correlation coefficient, PCC >= 0.4) with that of BRCA1 [GeneID=672] across a compendium of normal tissues.	39	<div></div>	6.99 e-15	3.7 e-12
REACTOME_MEIOSIS [116]	Genes involved in Meiosis	13	<div></div>	6.9 e-14	3.29 e-11

Regions with high  
acetylation only in  
+MYC or  
MYC+TWIST

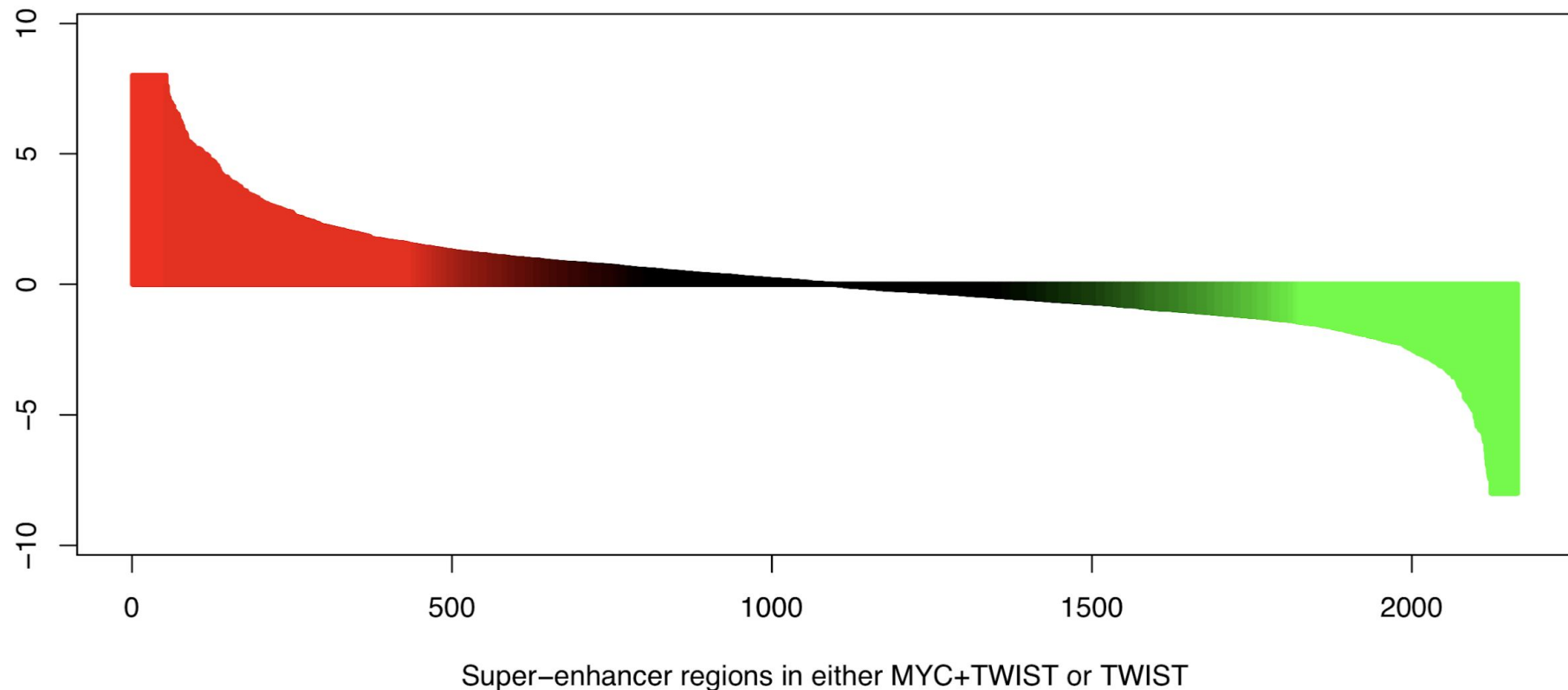


Regions with high  
acetylation only in  
+TWIST



# Comparing H3K27ac in MYC+TWIST vs. +TWIST













Log2 change in signal TWIST over MYC+TWIST



# Pathways with high H3K27ac in the MYC+TWIST vs. +TWIST

Gene Set Name [# Genes (K)]	Description	# Genes in Overlap (k)	k/K	p-value ?	FDR q-value ?
<a href="#">DIAZ_CHRONIC_MEYLOGENOUS_LEUKEMIA_UP [1382]</a>	Genes up-regulated in CD34+ [GeneID=947] cells isolated from bone marrow of CML (chronic myelogenous leukemia) patients, compared to those from normal donors.	61	<div></div>	1.43 e <sup>-19</sup>	6.79 e <sup>-16</sup>
<a href="#">GRAESSMANN_APOPTOSIS_BY_DOXORUBICIN_DN [1781]</a>	Genes down-regulated in ME-A cells (breast cancer) undergoing apoptosis in response to doxorubicin [PubChem=31703].	67	<div></div>	7.73 e <sup>-18</sup>	1.84 e <sup>-14</sup>
<a href="#">PILON_KLF1_TARGETS_DN [1972]</a>	Genes down-regulated in erythroid progenitor cells from fetal livers of E13.5 embryos with KLF1 [GeneID=10661] knockout compared to those from the wild type embryos.	69	<div></div>	9.62 e <sup>-17</sup>	1.53 e <sup>-13</sup>
<a href="#">GRYDER_PAX3FOXO1_ENHANCERS_IN_TADS [975]</a>	Expressed genes (FPKM>1) associated with high-confidence PAX3-FOXO1 sites with enhancers in primary tumors and cell lines, restricted to those within topological domain boundaries	46	<div></div>	4.8 e <sup>-16</sup>	5.71 e <sup>-13</sup>
<a href="#">GEORGES_TARGETS_OF_MIR192_AND_MIR215 [893]</a>	Genes down-regulated in HCT116 cells (colon cancer) by expression of MIR192 or MIR215 [GeneID=406967;406997] at 24 h.	43	<div></div>	2.23 e <sup>-15</sup>	1.92 e <sup>-12</sup>
<a href="#">DACOSTA_UV_RESPONSE_VIA_ERCC3_DN [855]</a>	Genes down-regulated in fibroblasts expressing mutant forms of ERCC3 [GeneID=2071] after UV irradiation.	42	<div></div>	2.42 e <sup>-15</sup>	1.92 e <sup>-12</sup>
<a href="#">JOHNSTONE_PARVB_TARGETS_3_DN [918]</a>	Genes down-regulated upon overexpression of PARVB [GeneID=29780] in MDA-MB-231 cells (breast	43	<div></div>	5.82 e <sup>-15</sup>	3.96 e <sup>-12</sup>

# Pathways with high H3K27ac in the MYC+TWIST vs. +TWIST

Gene Set Name [# Genes (K)]	Description	# Genes in Overlap (k)	k/K	p-value 	FDR q-value 
<a href="#">BOCHKIS_FOXA2_TARGETS [425]</a>	Direct targets of FOXA2 [GeneID=3170] in liver, according to a ChIP-chip analysis.	42		2.55 e <sup>-28</sup>	1.21 e <sup>-24</sup>
<a href="#">REACTOME_BIOLOGICAL_OXIDATIONS [139]</a>	Genes involved in Biological oxidations	26		3.31 e <sup>-25</sup>	7.89 e <sup>-22</sup>
<a href="#">OHGUCHI_LIVER_HNF4A_TARGETS_DN [149]</a>	Genes down-regulated in liver samples of liver-specific knockout of HNF4A [GeneID=3172].	26		2.2 e <sup>-24</sup>	3.49 e <sup>-21</sup>
<a href="#">HSIAO_LIVER_SPECIFIC_GENES [244]</a>	Liver selective genes	30		2.57 e <sup>-23</sup>	3.06 e <sup>-20</sup>
<a href="#">SHETH_LIVER_CANCER_VS_TXNIP_LOSS_PAM4 [261]</a>	Cluster PAM4: genes down-regulated in hepatocellular carcinoma (HCC) vs normal liver tissue from mice deficient for TXNIP [GeneID=10628].	29		2.5 e <sup>-21</sup>	2.38 e <sup>-18</sup>
<a href="#">LEE_LIVER_CANCER_MYC_TGFA_DN [65]</a>	Genes down-regulated in hepatocellular carcinoma (HCC) tissue of MYC and TGFA [GeneID=4609;7039] double transgenic mice.	18		3.47 e <sup>-21</sup>	2.75 e <sup>-18</sup>
<a href="#">YOSHIMURA_MAPK8_TARGETS_UP [1305]</a>	Genes up-regulated in vascular smooth muscle cells (VSMC) by MAPK8 (JNK1) [GeneID=5599].	57		4.04 e <sup>-20</sup>	2.75 e <sup>-17</sup>
<a href="#">LEE_LIVER_CANCER_DENA_DN [74]</a>	Genes down-regulated in hepatocellular carcinoma (HCC) induced by diethylnitrosamine (DENA) [PubChem=5921].	18		4.65 e <sup>-20</sup>	2.77 e <sup>-17</sup>
<a href="#">HOSHIDA_LIVER_CANCER_SUBCLASS_S3 [266]</a>	Genes from 'subtype S3' signature of hepatocellular carcinoma (HCC): hepatocyte differentiation.	28		5.34 e <sup>-20</sup>	2.82 e <sup>-17</sup>
<a href="#">LEE_LIVER_CANCER_MYC_E2F1_DN [64]</a>	Genes down-regulated in hepatocellular carcinoma (HCC) from MYC and E2F1 [GeneID=4609;1869] double transgenic mice.	17		9.74 e <sup>-20</sup>	4.64 e <sup>-17</sup>

# Initial conclusions

- Most of the signal coming from enhancer gain in the MYC+TWIST genotype with acetylation present already in the +TWIST genotype (e.g. NFIA)
- Some enhancer regions only hyper-acetylated in MYC+TWIST as opposed to TWIST alone or MYC alone
- Coherent pathway activation for Foxp3 targets... Foxp4 is the TF activated in the tumors
- Strong CD34+ signature in the MYC+TWIST (by H3K27ac)
- Loss of liver identity in the MYC+TWIST vs. TWIST