December 3, 2023
Initial selection: rAMP diff > 2| phase diff > 6 - since rAMP values are < 1, not filtered by rAMP here

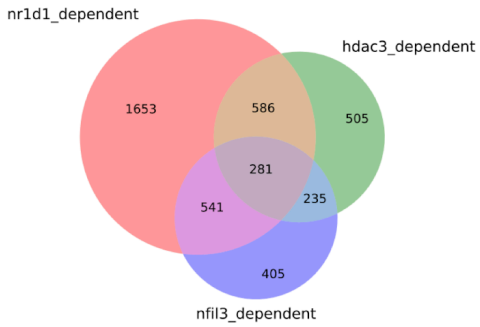
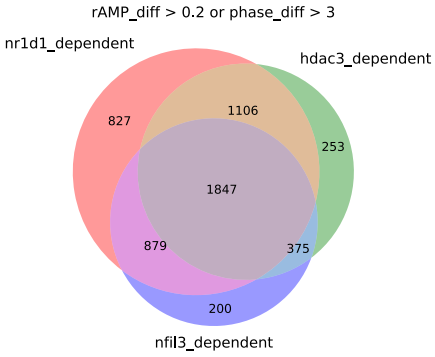


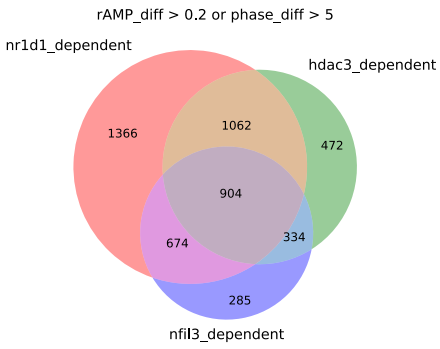
Figure 1. Phase diff > 6 only

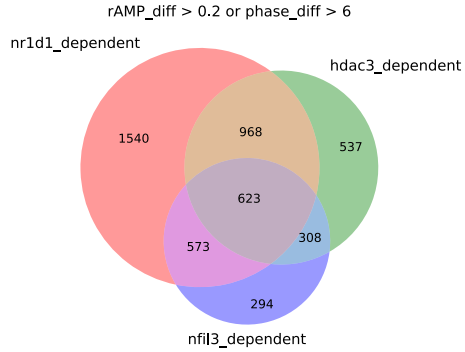
Modifying the parameters in dependent.py below

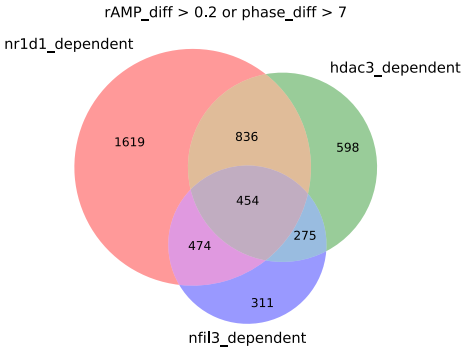
```python
import pandas as pd

gene = ["hdac3", "nfil3", "nr1d1"]

for g in gene:
    difference_df = pd.read_csv(g+"_difference_modify.txt", sep='\t')
    dependent_df = difference_df[(difference_df['meta2d_rAMP_diff'] > 0.3) | (difference_df['meta2d_phase_diff'] > 7)]

    print(len(dependent_df))

    dependent_df.to_csv(g+"_dependent_modify_0.3_7.txt", sep='\t', index=False)
```

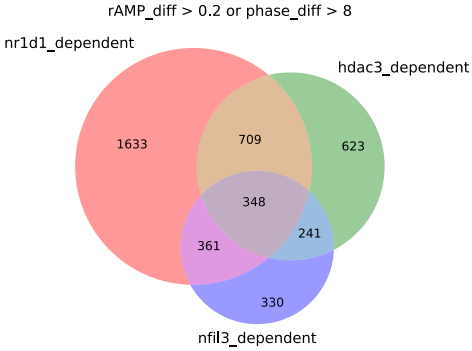
Results with different parameters in OR setting



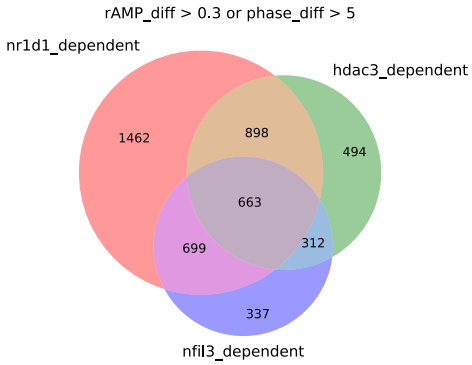


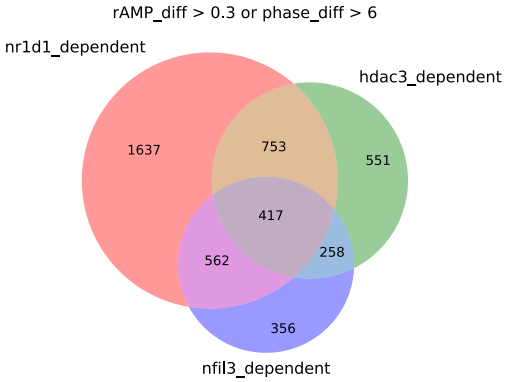

rAMP_diff > 0.2 or phase_diff > 6
nr1d1_dependent
hdac3_dependent
nfil3_dependent
1540
968
537
623
573
308
294

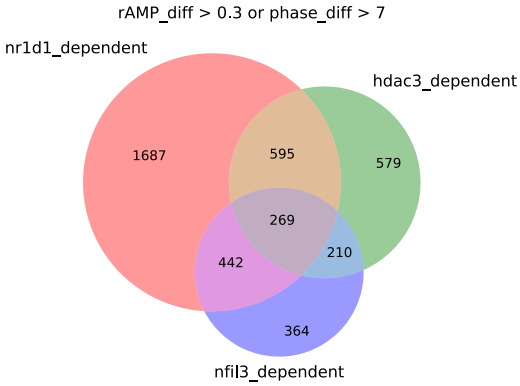

rAMP_diff > 0.2 or phase_diff > 7
nr1d1_dependent
hdac3_dependent
nfil3_dependent
1619
836
598
454
474
275
311


rAMP_diff > 0.2 or phase_diff > 8
nr1d1_dependent
hdac3_dependent
nfil3_dependent
1633
709
623
348
361
241
330

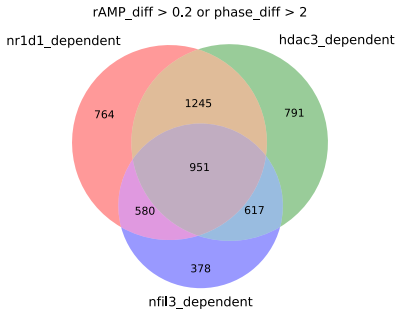
skip 7

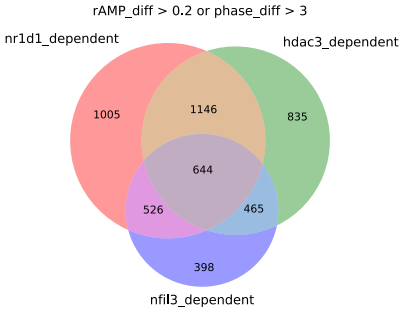


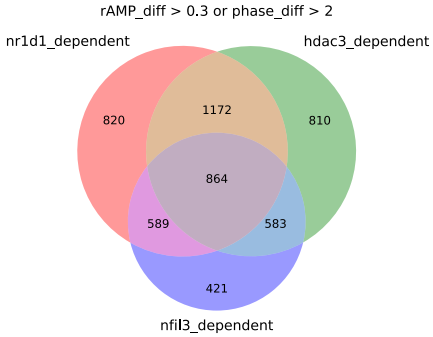


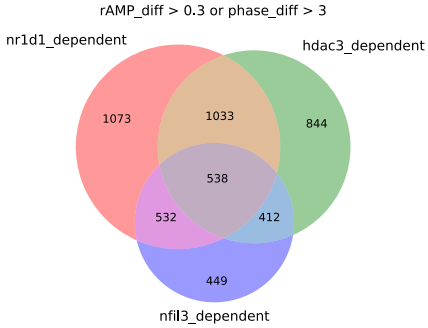


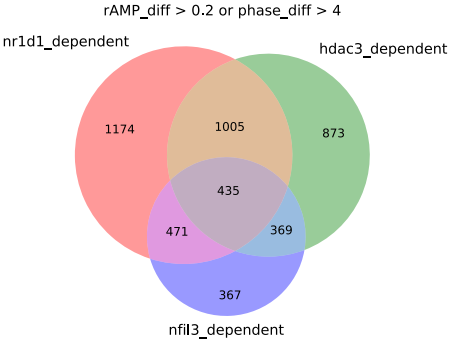
December 11, 2023
From meta2d data, select genes that have p val < 0.05 in WT (filter out the rest in both WT and KO). P-value determines if the genes are cycling (are circadian genes)

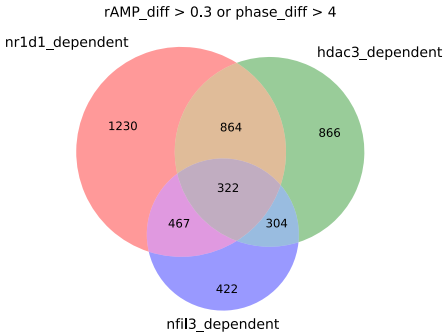


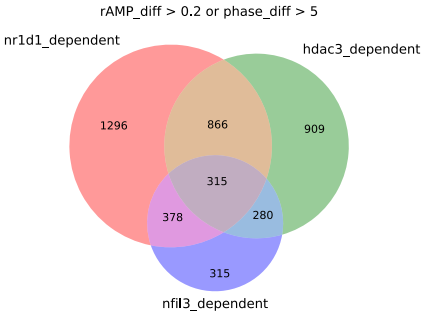




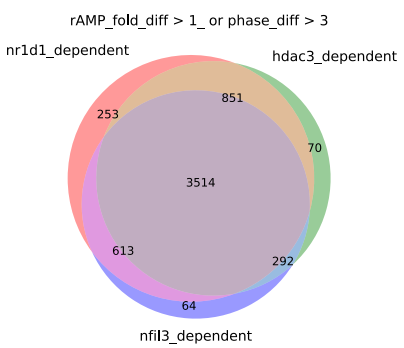


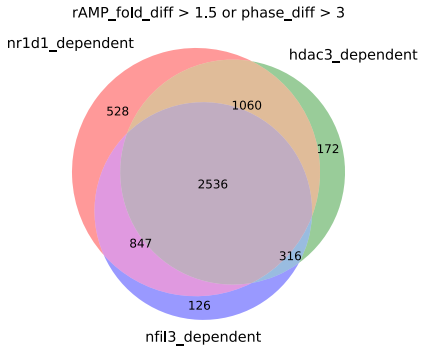






Modified rAMP difference calculation from absolute (abs(KO-WO)) to relative (WO/KO)





Feb 3rd 2024

Tophat – align -> cuffdiff to calculate FPKM values

Dr. Kuang ran cuffdiff with 32 files total – 4 GF, 8 Hdac3, 8 Nr1d1, 12 Nfil3

```
cuffdiff -o ZK_4group -p 30 -u genes.gtf ./GF_mm10/ZK1_tophat_out/accepted_hits.bam ./GF_mm10/ZK2_tophat_out/accepted_hits.bam .
/GF_mm10/ZK3_tophat_out/accepted_hits.bam ./GF_mm10/ZK4_tophat_out/accepted_hits.bam ./Hdac3_mm10/ZK1_tophat_out/accepted_hits.bam ./Hdac3_mm10/
ZK2_tophat_out/accepted_hits.bam ./Hdac3_mm10/ZK3_tophat_out/accepted_hits.bam ./Hdac3_mm10/ZK4_tophat_out/accepted_hits.bam ./Hdac3_mm10/ZK5_to
phat_out/accepted_hits.bam ./Hdac3_mm10/ZK6_tophat_out/accepted_hits.bam ./Hdac3_mm10/ZK7_tophat_out/accepted_hits.bam ./Hdac3_mm10/ZK8_tophat_o
ut/accepted_hits.bam ./Nr1d1_mm10/ZK1_tophat_out/accepted_hits.bam ./Nr1d1_mm10/ZK2_tophat_out/accepted_hits.bam ./Nr1d1_mm10/ZK3_tophat_out/acc
epted_hits.bam ./Nr1d1_mm10/ZK4_tophat_out/accepted_hits.bam ./Nr1d1_mm10/ZK5_tophat_out/accepted_hits.bam ./Nr1d1_mm10/ZK6_tophat_out/accepted_
hits.bam ./Nr1d1_mm10/ZK7_tophat_out/accepted_hits.bam ./Nr1d1_mm10/ZK8_tophat_out/accepted_hits.bam ./Nfil3_mm10/ZK1_tophat_out/accepted_hits.b
am ./Nfil3_mm10/ZK2_tophat_out/accepted_hits.bam ./Nfil3_mm10/ZK3_tophat_out/accepted_hits.bam ./Nfil3_mm10/ZK4_tophat_out/accepted_hits.bam ./N
fil3_mm10/ZK5_tophat_out/accepted_hits.bam ./Nfil3_mm10/ZK6_tophat_out/accepted_hits.bam ./Nfil3_mm10/ZK7_tophat_out/accepted_hits.bam ./Nfil3_m
m10/ZK8_tophat_out/accepted_hits.bam ./Nfil3_mm10/ZK9_tophat_out/accepted_hits.bam ./Nfil3_mm10/ZK10_tophat_out/accepted_hits.bam ./Nfil3_mm10/Z
L11_tophat_out/accepted_hits.bam ./Nfil3_mm10/ZK12_tophat_out/accepted_hits.bam
```

output file to XueK/ZK_4group -> genes.fpkm_tracking

remove rows with FAIL in status column > genesFailRemoved.txt

1	tracking_id	class_code	nearest_ref_	gene_id	gene_short_name	tss_id	locus	length	coverage	q1_FPKM	q1_conf_lo	q1_conf_hi	q1_status
5809	Cyp4f13	-	-	Cyp4f13	Cyp4f13	TSS10638	chr17:32924	-	-	51.2917	11.4555	91.1279	OK
5810	Cyp4f14	-	-	Cyp4f14	Cyp4f14	TSS18626,TS	chr17:32905	-	-	1002.84	0	1036.21	FAIL
5811	Cyp4f15	-	-	Cyp4f15	Cyp4f15	TSS25797	chr17:32685	-	-	0.111026	0	0.337876	OK
5812	Cyp4f16	-	-	Cyp4f16	Cyp4f16	TSS12179	chr17:32536	-	-	218.487	41.3726	395.602	OK
5813	Cyp4f17	-	-	Cyp4f17	Cyp4f17	TSS2923	chr17:32506	-	-	0.239663	0	0.694555	OK
5814	Cyp4f18	-	-	Cyp4f18	Cyp4f18	TSS15053	chr8:719884	-	-	1.76286	0	4.23868	OK
5815	Cyp4f39	-	-	Cyp4f39	Cyp4f39	TSS16876	chr17:32452	-	-	0.443671	0	1.07173	OK
5816	Cyp4f40	-	-	Cyp4f40	Cyp4f40	TSS5038	chr17:32659	-	-	39.1799	8.29535	70.0644	OK

```
(base) tinaryu@Tinas-MacBook-Pro-3 KuangLab % grep FAIL genes.txt| cu
4930513N10Rik
Actr3
B230312A22Rik
BC065397
Ccdc93
Cnot1
Ctage5
Cyp4f14
Etv3
Galnt6
Glra4
Gm11602
Gng12
Heph
Ldlr
Mir1942
Morf4l2
Ndufs1
Ogdh
Otud4
Plec
Plekhg2
Rpl34
Rpl34-ps1
Sept7
(base) tinaryu@Tinas-MacBook-Pro-3 KuangLab % grep -c FAIL genes.txt
25
```

Extract FPKM values for each group (7 files) > GF.txt, hdac3_WT.txt, hdac3_KO.txt, nr1d1_WT.txt, nr1d1_KO.txt, nfil3_WT.txt, nfil3_KO.txt

2 versions: one original and one replicated

gene_id	q1_FPKM	q2_FPKM	q3_FPKM	q4_FPKM
0610005C13I	42.3248	90.0548	58.472	33.0997
0610007C21I	69.5387	65.9222	64.5194	53.7944
0610007L01F	9.0729	9.29366	8.62519	7.60749
0610007N19I	6.30459	9.80456	7.29856	6.4871
0610007P08I	1.01314	1.1118	1.01785	1.14966

gene_id	q1_FPKM	q2_FPKM	q3_FPKM	q4_FPKM	q1_FPKM_re	q2_FPKM_re	q3_FPKM_re	q4_FPKM_rep
0610005C13	42.3248	90.0548	58.472	33.0997	42.3248	90.0548	58.472	33.0997
0610007C21	69.5387	65.9222	64.5194	53.7944	69.5387	65.9222	64.5194	53.7944
0610007L01	9.0729	9.29366	8.62519	7.60749	9.0729	9.29366	8.62519	7.60749
0610007N19	6.30459	9.80456	7.29856	6.4871	6.30459	9.80456	7.29856	6.4871
0610007P08	1.01314	1.1118	1.01785	1.14966	1.01314	1.1118	1.01785	1.14966

Feb 23rd 2024

1. Filter out genes that are not expressed well at all time points fpkm val < 1

Before 23263 genes nfil3_KO.txt
After 11249 genes nfil3_KO_expressed.txt

(base) tinaryu@Tinas-MacBook-Pro-3 replicated % awk '$2 > 1 || $3 > 1 || $4 > 1 || $5 > 1' GF.txt > GF_expressed.txt

2. Run Metacycle using the groupname_expressed.txt files above > meta2d_GF_expressed.txt

3. Filter out genes with meta2d pval > 0.05. From the Metacycle output file with LS, ARS, JTK, and meta2d values, extract the columns with meta2d data only

```
(base) tinaryu@Tinas-MacBook-Pro-3 meta2d_rep_expressed % cut -f 1,17-23 meta2d_GF_expressed.txt| awk '$2 < 0.05' > meta2d_GF_sig.txt
```

2 types of files:
file 1 – list of gene names with meta2d pval < 0.05
file 2 – meat2d pval < 0.05 genes and meta2d values

Before 11249 genes meta2d_nfil3_KO_expressed.txt
After 6111 genes meta2d_sig/meta2d_nfil3_KO_sig.txt

4. Extract geneIDs of genes that are dependent on hdac3, nr1d1, or nfil3 by subtracting gene list of WT – KO using unix

```
(base) tinaryu@Tinas-MacBook-Pro-3 meta2d_sig_genelist % grep -v -xFf meta2d_hdac3_KO_sig_genelist.txt  meta2d_hdac3_WT_sig_genelist.txt > hdac3_dependent_genelist.txt
```

6230 genes in hdac3_WT -> 3355 genes which are hdac3 dependent

Compare the gene overlap between 3 groups