

1 **Titles and Legends to Supplementary Figures**

2 **Figure S1: HITS-CLIP Antibody verification and AGO expression in mouse**
3 **liver during development**

4 (A, B, C) Detection of the specificity of AGO antibodies by Western blot from Wako
5 (A), Abnova (B) or MBL (C).

6 (D, E, F) Detection of antibodies by silver stain (D), and immunoprecipitation (E,F).

7 (G, H) Detection of mRNA (G) and protein (H) expression of Ago among different
8 development stages of mouse liver.

9 **Figure S2: The crosslinking and digestion conditions of HITS-CLIP were**
10 **optimized to obtain high quality and suitable length products**

11 (A) Detection of Uv cross-linking efficiency by autoradiogram.

12 (B) Detection of RNase digestion by autoradiogram.

13 (C, D) Detection of PCR product by PAGE electrophoresis (C) and Agilent 2100(D)
14 analysis.

15 **Figure S3: Analysis of AGO-bound miRNA expression profile of mouse liver**
16 **from various developmental stages**

17 (A) Length distribution of fragment produced by HITS-CLIP.

18 (B) Correlation analysis of miRNA expression among different developmental stages
19 (log2 normalized miRNA reads)

20 (C) Genome location distribution of Ago binding RNA reads.

21 **Figure S4: Analysis of miRNA targets identified by HITS-CLIP**

22 (A) Gene expression differences assessed by microarray of background genes, or

miR-122 targets identified by Ago HITS-CLIP in the coding region between WT and miR-122 KO mice, p values were calculated with a one-sided Kolmogorov-Smirnov test.

(B, C) Cumulative distribution curve of target mRNA level change after miR-142 knock out (B) 3'UTR targets and (C) CDS targets. The gene expression data of WT and miR-142 KO mice T cells were obtained from the GEO dataset (GSE20610). p values were calculated with a one-sided Kolmogorov-Smirnov test.

(D) IGV showed the change of Ago binding miR-122 targeting site of the Scarb1 gene during mouse liver development.

(E) Gene Ontology (GO) analysis of miR-122 targetome.

Figure S5: KEGG pathway analysis of targets of development-upregulated miRNAs with high abundance

Figure S6: The establishment of inducible overexpression miR-122 cell line and Hippo pathway reporter plasmid

(A) Change of GFP fluorescence before and after inducible expressing miR-122 in Hepa1-6 cell line.

(B) Schematic diagram of the skeleton of Hippo pathway reporter plasmid.

(C) Detection of the effectiveness 8xGTIIC-luciferase Hippo pathway reporter in and HeLa cell lines by YAP siRNAs, values are mean $\pm$ SEM. (D) The effect of miR-122 mimics on Hippo pathway activities, which was measured by 8xGTIIC-luciferase reporter in HepG2 cells. (E) The effect of miR-122 inhibitors on Hippo pathway activities, which was measured by 8xGTIIC-luciferase reporter in Huh7 cells.

Figure 7: The expression the Hippo pathway downstream genes in miR-122 KO

46 **mice liver.** Public microarray dataset (GSE20610) of miR-122 KO mice were
47 analyzed to detect the influence of miR-122 on genes Hippo pathway-target genes *in*
48 *vivo*.

49 **Figure 8: Predicted miR-122 binding sites of the targets.** The putative miR-122
50 target base pair interactions were predicted by miRanda.

51 **Figure 9: influence of candidate targets on phosphorylation of YAP.** (A). Western
52 Blot showing the impact of silencing candidate genes on phosphorylation of YAP.

53 (B) Ago HITS-CLIP showing the miR-122 target site at the 3'UTR of Taz (Wwtr1).

54 (C) Ago HITS-CLIP showing the miR-122 target site at the 3'UTR of Ppp1cc.

Figure S1

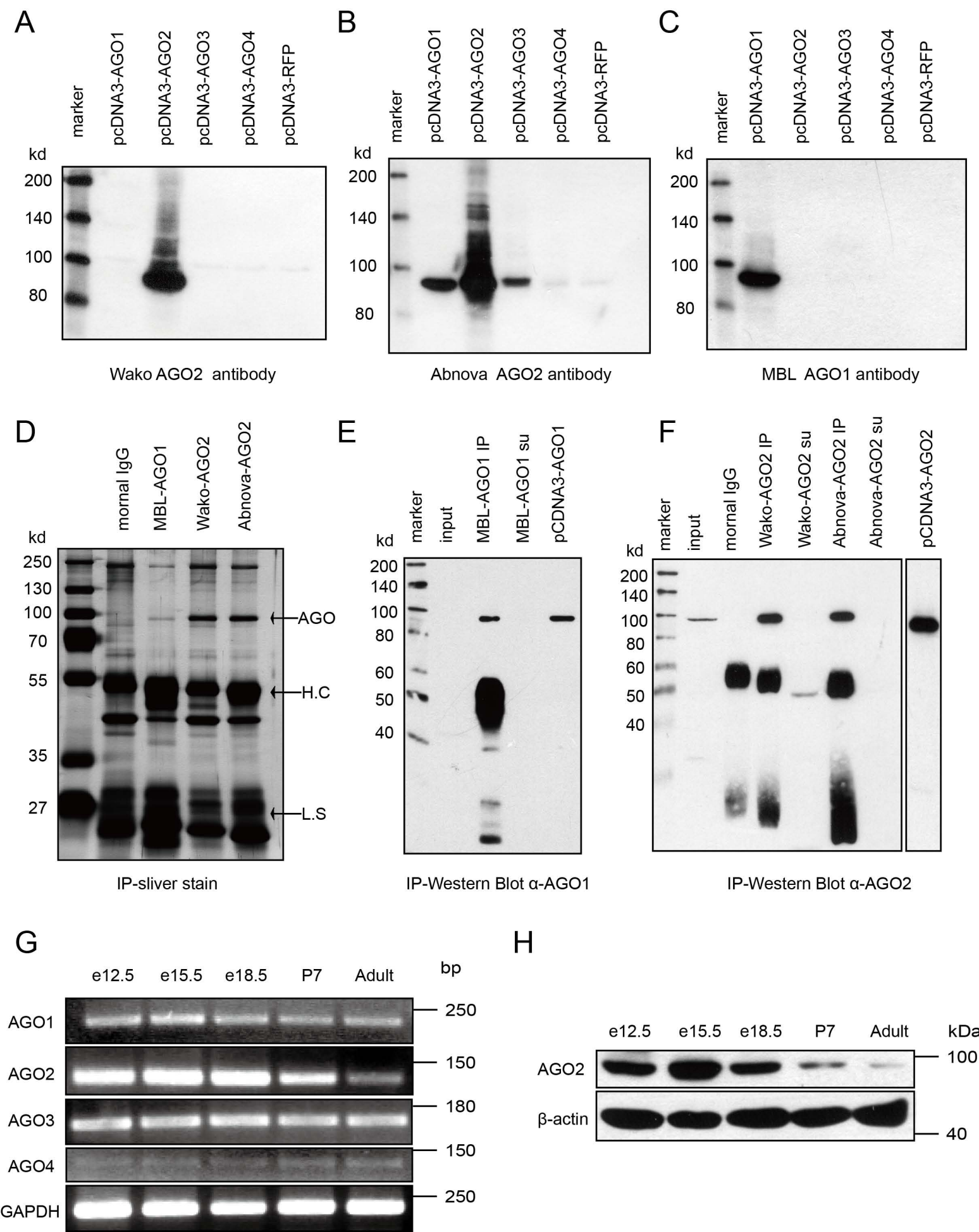
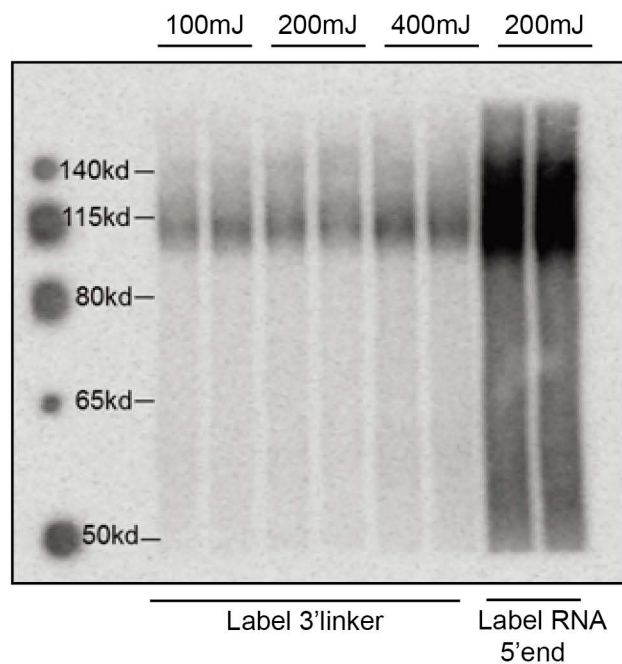
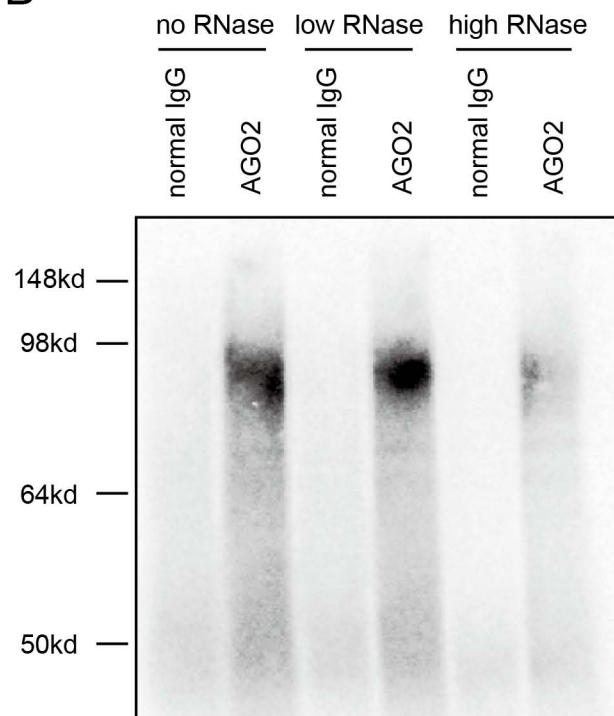


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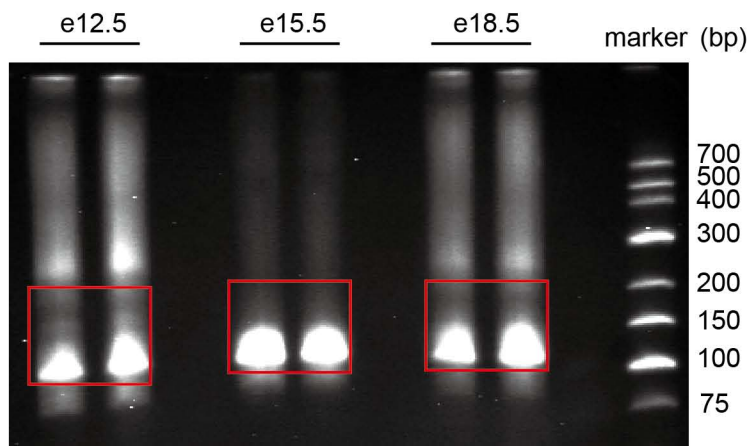
A



B



C



D

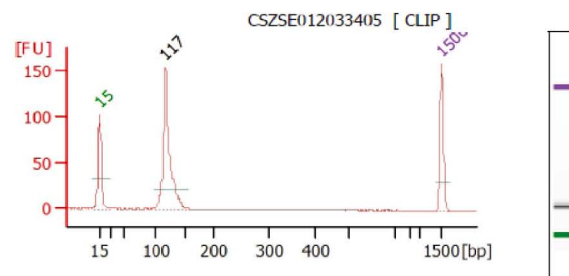
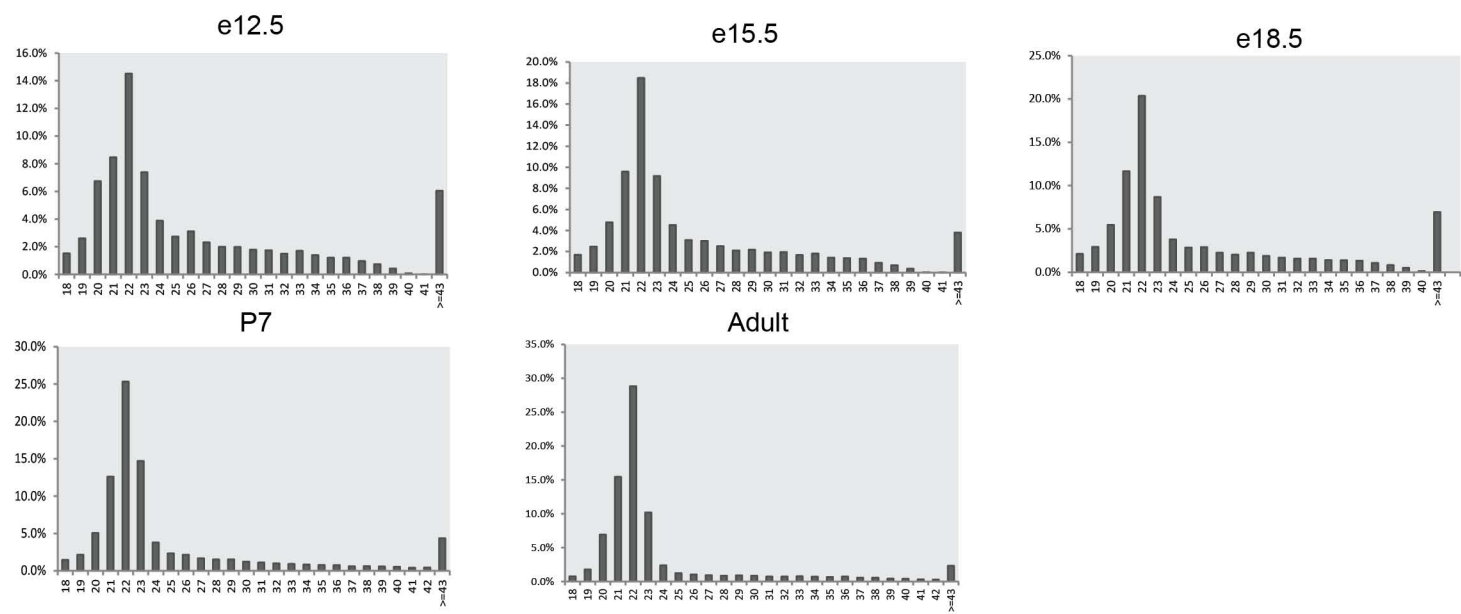
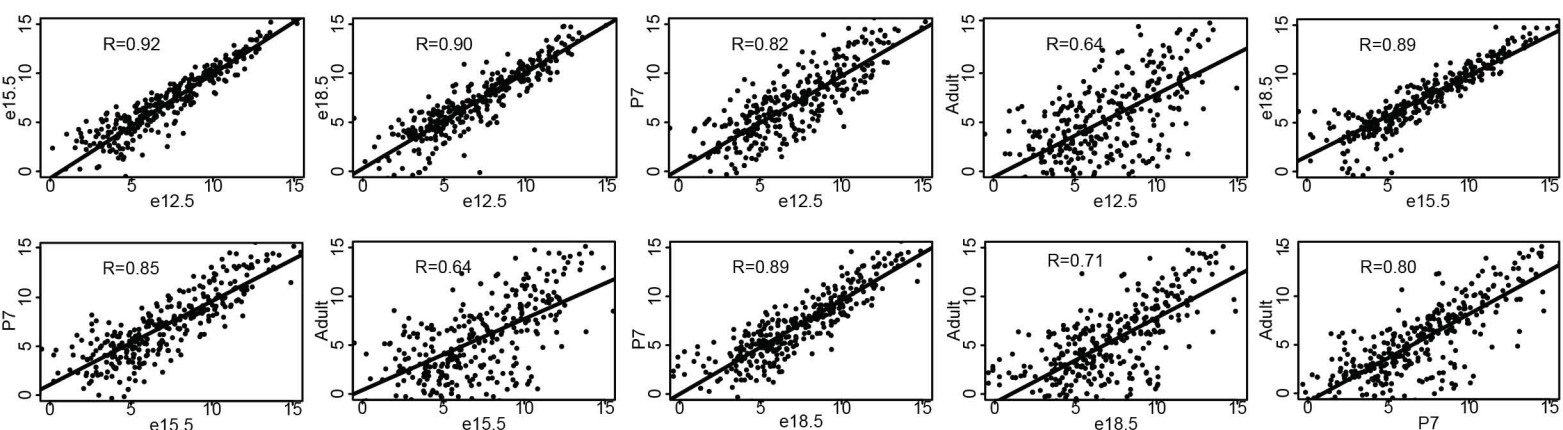


Figure S3

A



B



C

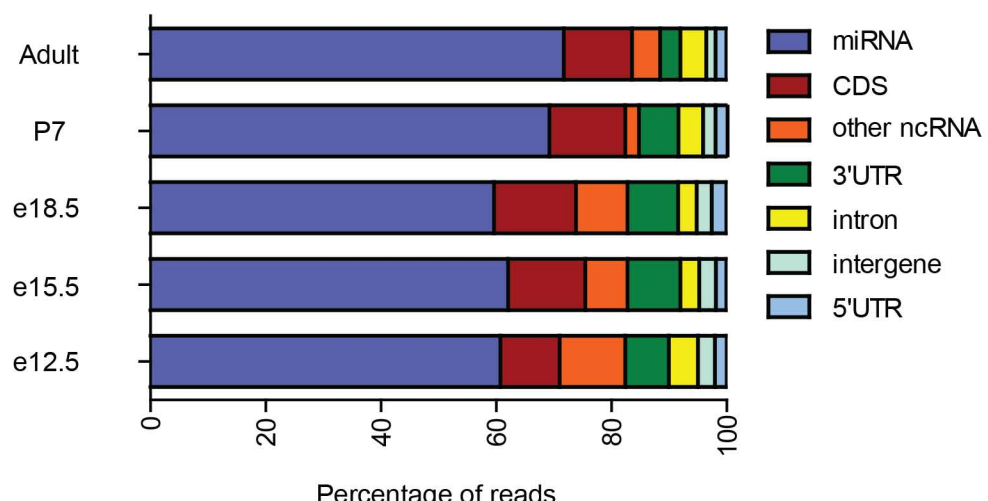
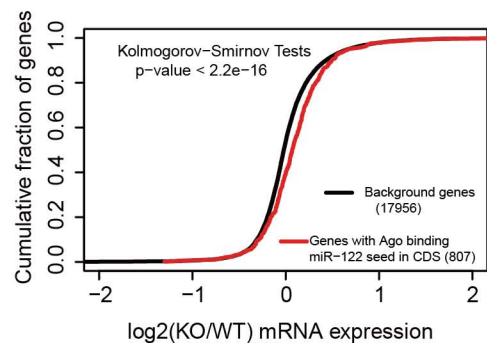
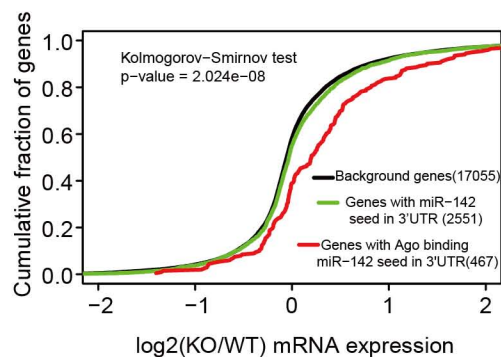


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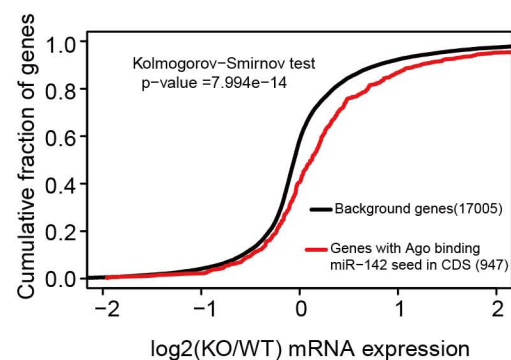
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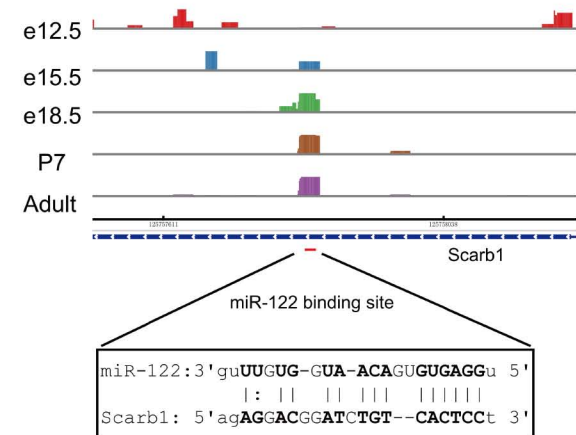
B



C



D



E

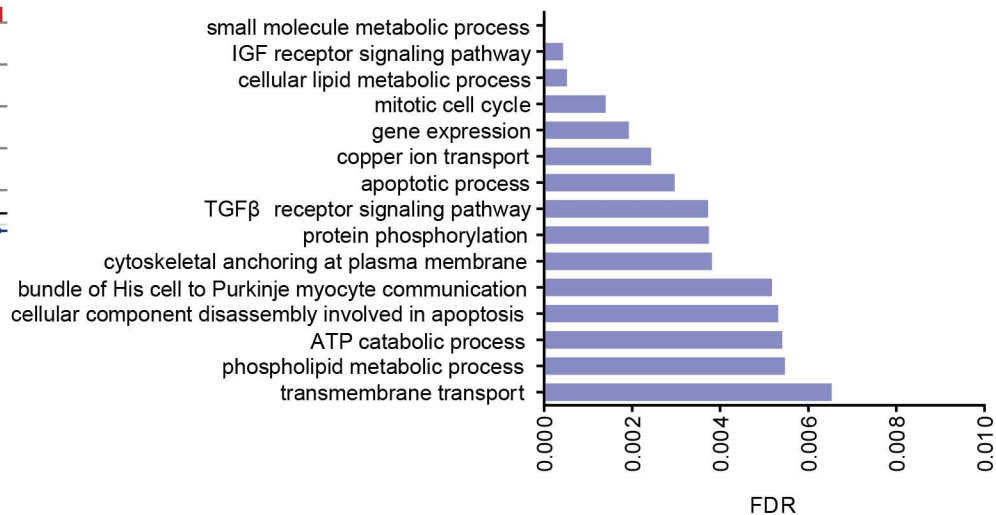


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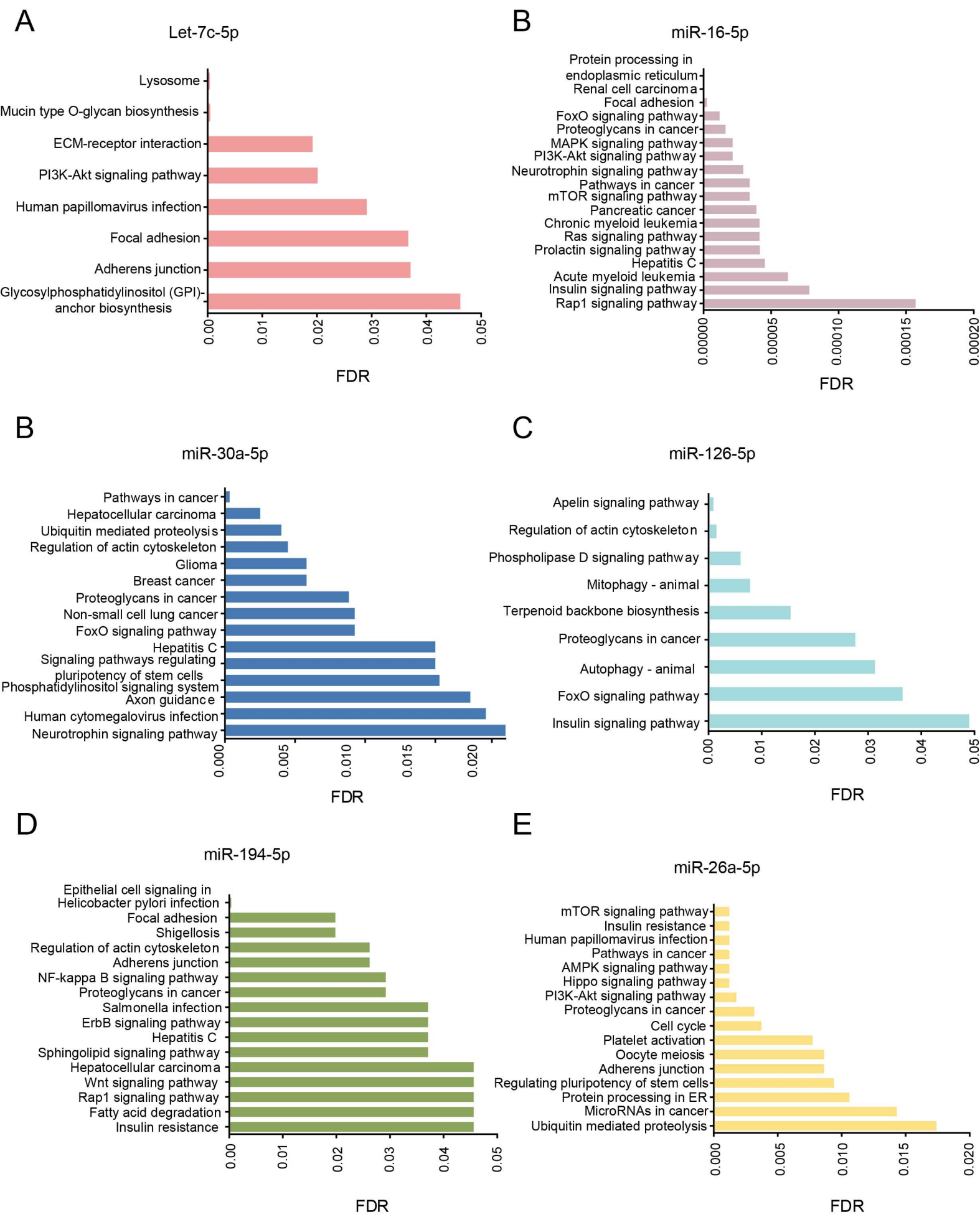
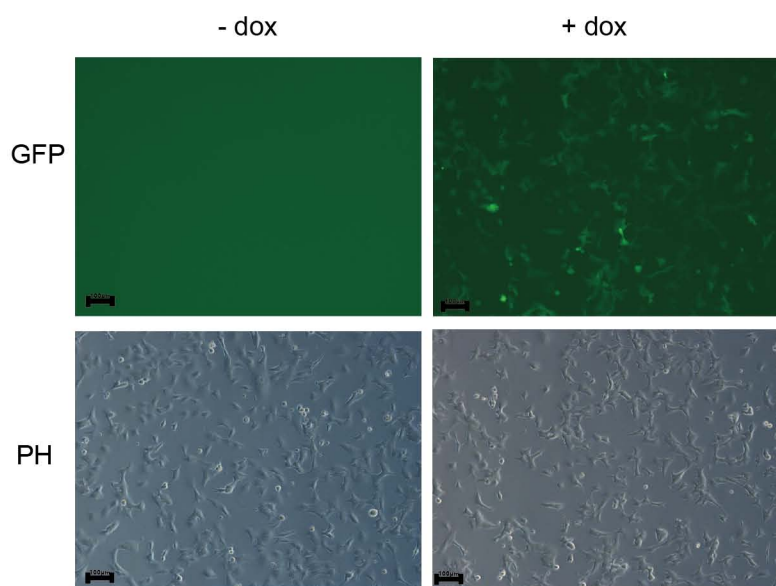
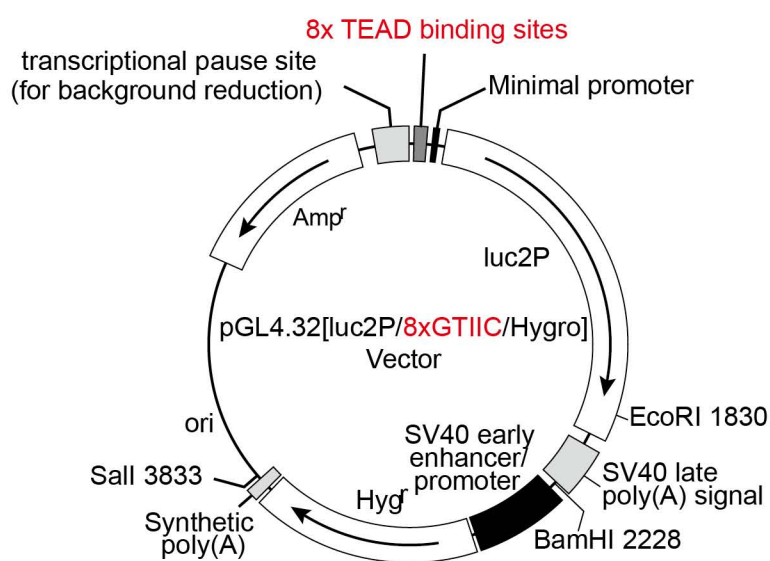


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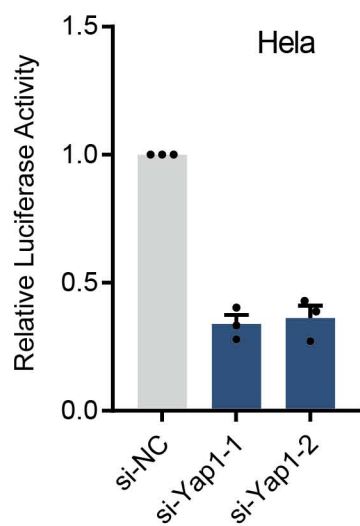
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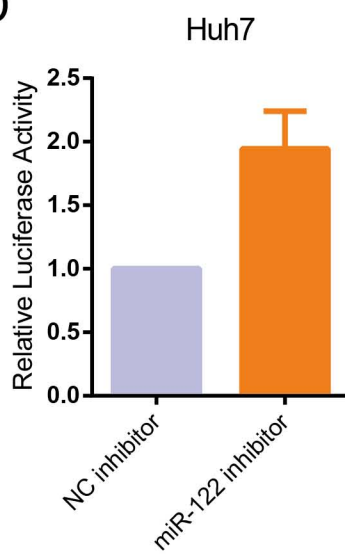
B



C



D



E

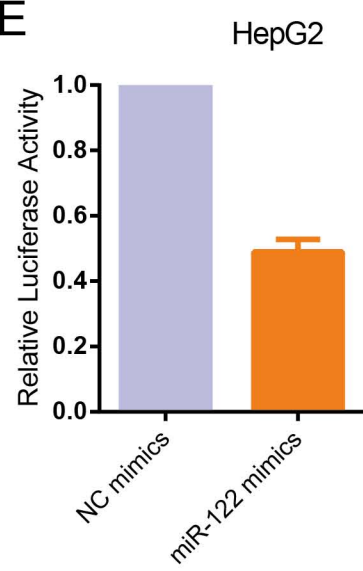


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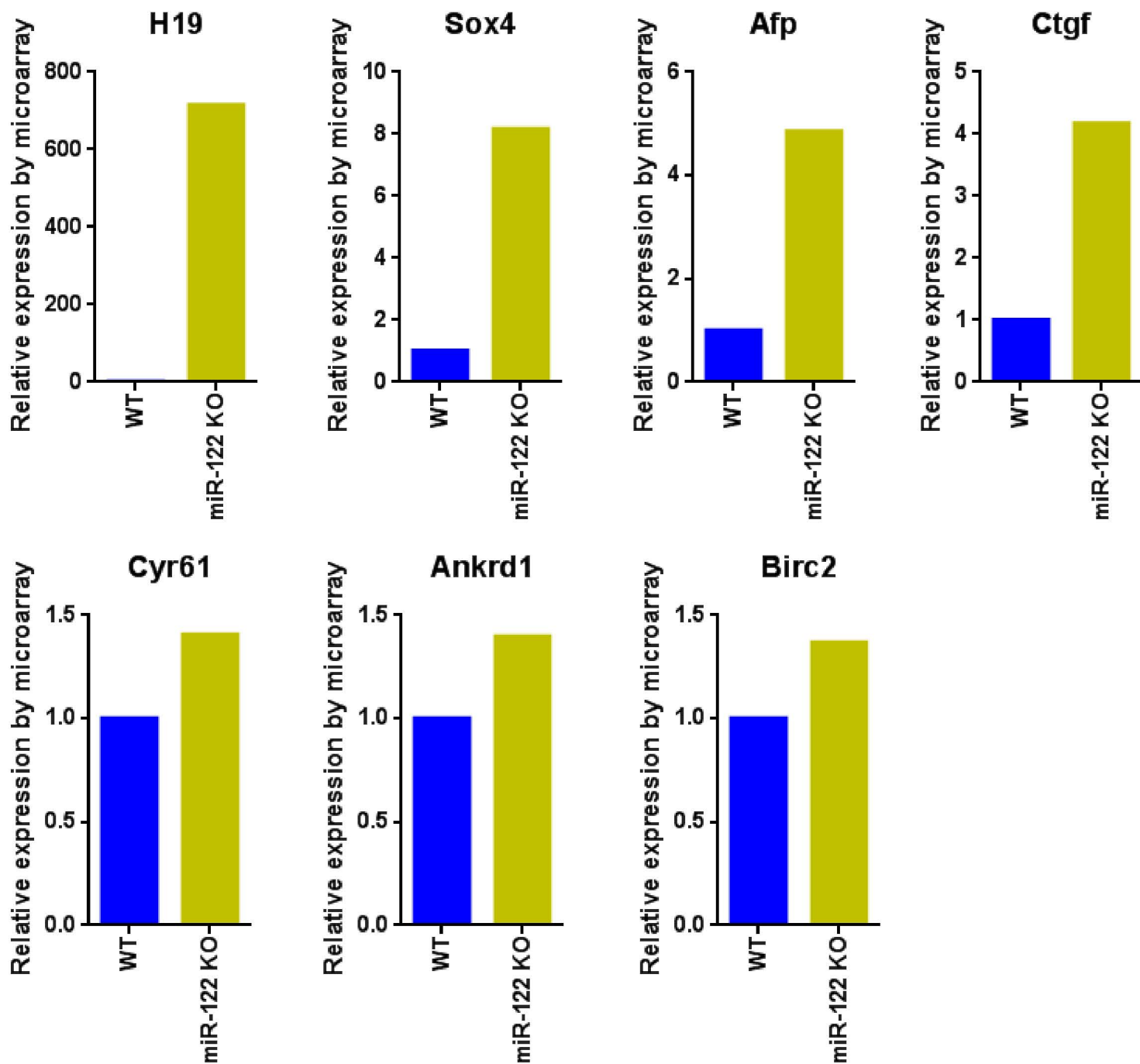


Figure S8

Taz-MT: 5' GUCUGCCUUUGUACUCAGUGAGGU 3'
 : || | | | | | | |
miR-122: 3' UUGUGGUAAC ---AGUGUGAGGU 5'
 || | | | | | | | | | | | | | | | | |
Taz-WT: 5' GUCUGCCUUUGUACUCACACUCCU 3'

Ppp1cc-MT: 5' CCCAGACUAUCUGAAGCAGUGAGGU 3'
 | | | | | : | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUA-AC--AGUGUGAGGU 5'
 | | | | | : | | | | | | | | | | | | | | | | | |
Ppp1cc-WT: 5' CCCAGACUAUCUGAAGCACACUCCU 3'

Net1-MT: 5' AGAAAUCCUUAGACAGUGAGGC 3'
 : || | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 : || | | | | | | | | | | | | | | | | |
Net1-WT: 5' AGAAAUCCUUAGACACACUCCC 3'

Arhgap32-MT: 5' GAAACAUGUCUGGACACGUGAGGA 3'
 | | | | | : : | | | | | | | | | | | |
miR-122: 3' GUUUGUG-GUAACAGU-GUGAGGU 5'
 | | | | | : : | | | | | | | | | | | | | | | | |
Arhgap32-WT: 5' GAAACAUGUCUGGACACCACUCCA 3'

Ppp2r3a-MT: 5' CUCUCACUAU--ACAGUGAGGU 3'
 | | | | | : | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 | | | | | : | | | | | | | | | | | | | | | | | |
Ppp2r3a-WT: 5' CUCUCACUAU--ACACACUCCG 3'

Arhgap5-MT: 5' AGUAUCCUGUUCAUAUGUGAGGU 3'
 : | | | | | : | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 : | : : : : | | | | | | | | | | | | | | | | | |
Arhgap5-WT: 5' AGUAUCCUGUUCAUAGCACUCCA 3'

Rock2-MT: 5' GCACUGGAUGCAAUAGUGAGGU 3'
 | : : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 | : : | | | | | | | | | | | | | | | | | |
Rock2-WT: 5' GCACUGGAUGCAAUACACUCCA 3'

Smurf2-MT: 5' AGGACA-CACUACAGUGAGGU 3'
 : : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 : : | | | | | | | | | | | | | | | | | |
Smurf2-WT: 5' AGGACA-CACUACACACUCCU 3'

Scrib-MT: 5' GUAGGAGGUUCUGAUGUGAGGU 3'
 | : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 | : | | | | | | | | | | | | | | | | | |
Scrib-WT: 5' GUAGGAGGUUCUGAUCACUCCA 3'

Arhgap11a-MT: 5' AAGGAACCAGCUAAGGUGAGGU 3'
 | | | | | : | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 | | | | | : | | | | | | | | | | | | | | | | | |
Arhgap11a-WT: 5' AAGGAACCAGCUAAGCACUCCA 3'

Ppp1r9a-MT: 5' CUGUGACUUUAUUAAGUGAGGU 3'
 | : : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 | : : | | | | | | | | | | | | | | | | | |
Ppp1r9a-WT: 5' CUGUGACUUUAUUAACACUCCA 3'

Ppp2r4-MT: 5' CUGGCCCCUUUGCGGAAGUGAGGU 3'
 : : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 : : | | | | | | | | | | | | | | | | | |
Ppp2r4-WT: 5' CUGGCCCCUUUGCGGAACACUCCA 3'

lqgap1-MT: 5' GGUGCCUCAGU-UCAGUGAGGU 3'
 | : : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 : | : | | | | | | | | | | | | | | | | | |
lqgap1-WT: 5' GGUGCCUCAGU-UCACACUCCC 3'

Foxo1-MT: 5' UGACUACCUUUGUUUGGGGUGAGGU 3'
 : | : | | | | | | | | : | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAG---UGUGAGGU 5'
 : | : | | | | | | | | : | | | | | | | | | | | |
Foxo1-WT: 5' UGACUACCUUUGUUUGGGCACUCCA 3'

Ppp2r2a-MT: 5' ACAGUUGGCUGUAAUGUGAGGU 3'
 | : : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 | : : | | | | | | | | | | | | | | | | | |
Ppp2r2a-WT: 5' ACAGUUGGCUGUAAUCACUCCU 3'

Agt-MT: 5' UGACUACCUUUGUUUGGGGUGAGGU 3'
 : | : | | | | | | | | : | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAG---UGUGAGGU 5'
 : | : | | | | | | | | : | | | | | | | | | | | |
Agt-WT: 5' agAACCCCAGTGTGGAGACACTCCc 3'

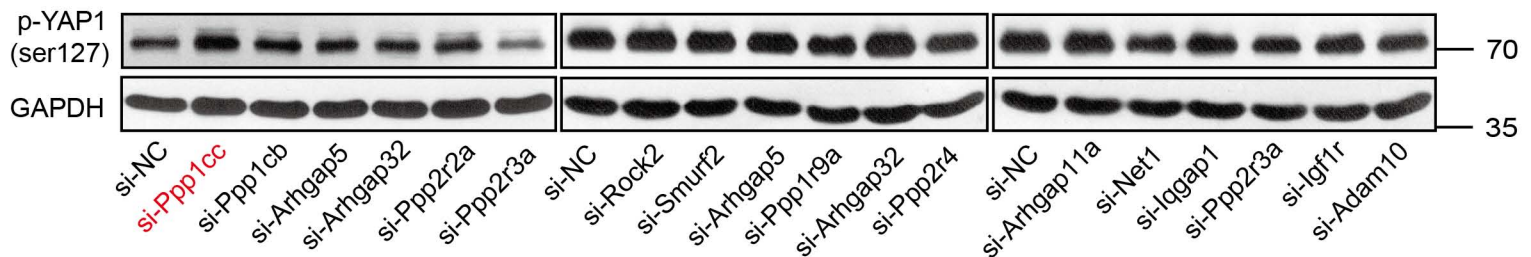
Rapgef6-MT: 5' ACAGUUGGCUGUAAUGUGAGGU 3'
 | : : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 | : : | | | | | | | | | | | | | | | | | |
Rapgef6-WT: 5' ACAGUUGGCUGUAAUCACUCCU 3'

Ppp1cb-MT: 5' UCCUGACCUUUGAGUAUCUGGUGAGGU 3'
 |
miR-122: 3' GUUUGUGGUAAC---AG-UGUGAGGU 5'
 | | | | | | | | | | | | | | : | | | | | | | | | | | |
Ppp1cb-WT: 5' UCCUGACCUUUGAGUAUCUGCACUCCC 3'

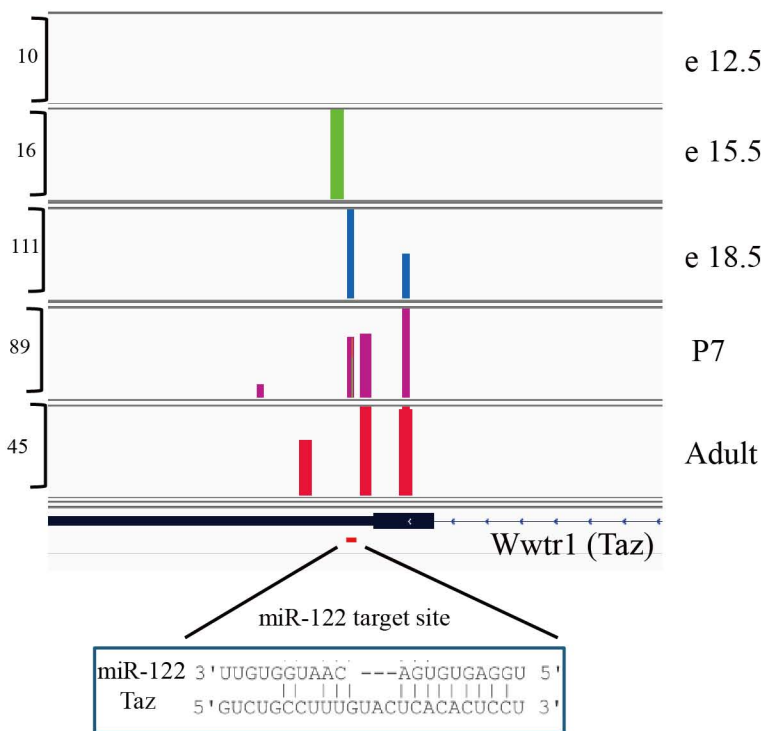
Magi3-MT: 5' CAAGAAAGCACCCAUCACUCCA 3'
 | : | | | | | | | | | | | | | | | | | |
miR-122: 3' GUUUGUGGUAACAGUGUGAGGU 5'
 | : | | | | | | | | | | | | | | | | | |
Magi3-WT: 5' CAAGAAAGCACCCAUCACUCCA 3'

Figure S9

A



B



C

