RNAi screen for piRNA pathway genes

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2019-11-26

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I. Summary

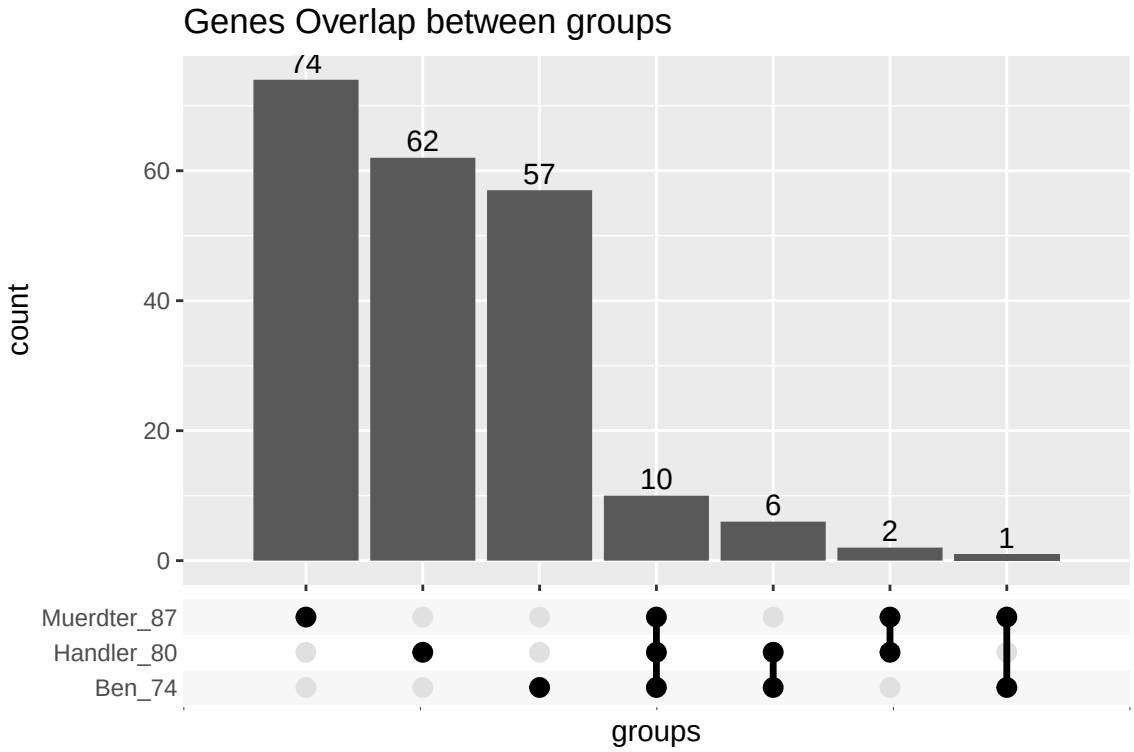
II. Results

1. Group1: 74-49-87

Candidate genes:

- 74 genes, Czech Benjamin (Greg lab)
- 49 genes, Handler Dominik (Julius lab)
- 87 genes, Muerdter Felix (Greg lab)

The positive genes reported in each screen.



Overlapped genes:

- Overlap in all screens:

num	FBID	CG	Symbol
1	FBgn0041164	CG11513	armi
2	FBgn0036826	CG3893	arx
3	FBgn0033273	CG2183	Gasz
4	FBgn0016034	CG11254	mael
5	FBgn0034617	CG9754	Panx
6	FBgn0037707	CG16788	RnpS1
7	FBgn0003401	CG4735	shu
8	FBgn0263143	CG4771	vret
9	FBgn0027499	CG12340	wde
10	FBgn0261266	CG12314	zuc

- Overlap in at least two screens

num	FBID	CG	Symbol
1	FBgn0263198	CG10473	Acn
2	FBgn0033526	CG12892	Caf1-105
3	FBgn0035842	CG7504	CG7504
4	FBgn0000928	CG2706	fs(1)Yb
5	FBgn0001197	CG5499	His2Av
6	FBgn0035357	CG1244	MEP-1
7	FBgn0036640	CG4118	nxf2

num	FBID	CG	Symbol
8	FBgn0028411	CG12752	Nxt1
9	FBgn0004872	CG6122	piwi

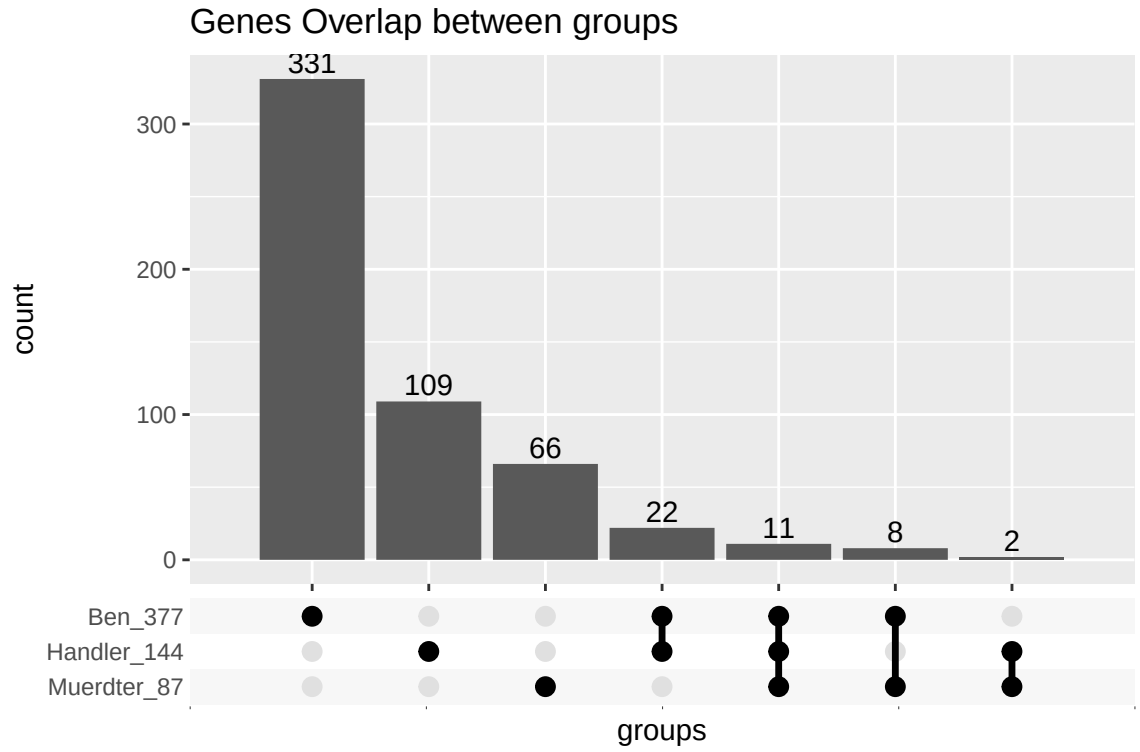
2. Group2: 377-144-87

Candidate genes:

- 377 genes, Czech Benjamin (Greg lab)
- 144 genes, Handler Dominik (Julius lab)
- 87 genes, Muerdter Felix (Greg lab)

For Benjamin screen, instead use average Z-score of four TEs <= -2.0 (74 genes), at least Z-score <= -2.0 for **one** TE. (377 genes).

For Dominik screen, change the criteria of staining from 2.0 (49 genes) to 1.0 (144 genes).



- Overlaped in all three screens

num	FBID	CG	Symbol
1	FBgn0041164	CG11513	armi
2	FBgn0036826	CG3893	arx
3	FBgn0033273	CG2183	Gasz
4	FBgn0016034	CG11254	mael

num	FBID	CG	Symbol
5	FBgn0028411	CG12752	Nxt1
6	FBgn0034617	CG9754	Panx
7	FBgn0037707	CG16788	RnpS1
8	FBgn0003401	CG4735	shu
9	FBgn0263143	CG4771	vret
10	FBgn0027499	CG12340	wde
11	FBgn0261266	CG12314	zuc

- Overlaped, in at least two screens

num	FBID	CG	Symbol
1	FBgn0263198	CG10473	Acn
2	FBgn0029512	CG12276	Aos1
3	FBgn0011741	CG11678	Arp6
4	FBgn0033526	CG12892	Caf1-105
5	FBgn0038768	CG4936	CG4936
6	FBgn0038546	CG7379	CG7379
7	FBgn0035842	CG7504	CG7504
8	FBgn0035987	CG3689	Cpsf5
9	FBgn0000376	CG2714	crm
10	FBgn0260634	CG10192	eIF4G2
11	FBgn0033806	CG4616	FLASH
12	FBgn0000928	CG2706	fs(1)Yb
13	FBgn0051992	CG31992	gw
14	FBgn0001197	CG5499	His2Av
15	FBgn0087013	CG1059	Karybeta3
16	FBgn0002736	CG9401	mago
17	FBgn0038016	CG10042	MBD-R2
18	FBgn0035357	CG1244	MEP-1
19	FBgn0032720	CG10603	mRpL13
20	FBgn0038923	CG13410	mRpL35
21	FBgn0021761	CG4579	Nup154
22	FBgn0033737	CG8831	Nup54
23	FBgn0036640	CG4118	nxf2
24	FBgn0020261	CG3291	pcm
25	FBgn0004872	CG6122	piwi
26	FBgn0028982	CG12225	Spt6
27	FBgn0027865	CG6120	Tsp96F
28	FBgn0033378	CG8781	tsu
29	FBgn0029113	CG7528	Uba2
30	FBgn0032030	CG17293	Wdr82
31	FBgn0032321	CG4621	YL-1
32	n.d.	NA	NA

3. Group2: 137-144-87

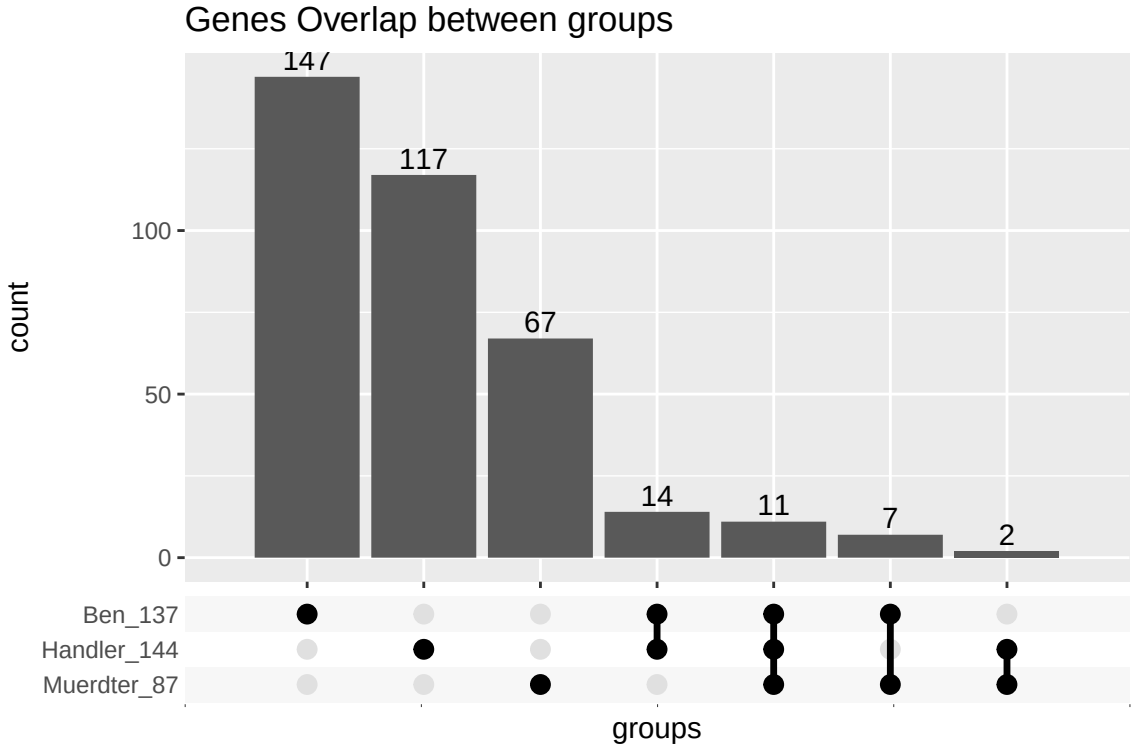
Candidate genes:

- 137 genes, Czech Benjamin (Greg lab)

- 144 genes, Handler Dominik (Julius lab)
- 87 genes, Muerdter Felix (Greg lab)

For Benjamin screen, instead use average Z-score of four TEs <= -2.0 (74 genes), at least Z-score <= -2.0 for **two** TEs. (137 genes).

For Dominik screen, change the criteria of staining from 2.0 (49 genes) to 1.0 (144 genes).



- Overlaped in all three screens

num	FBID	CG	Symbol
1	FBgn0041164	CG11513	armi
2	FBgn0036826	CG3893	arx
3	FBgn0033273	CG2183	Gasz
4	FBgn0016034	CG11254	mael
5	FBgn0028411	CG12752	Nxt1
6	FBgn0034617	CG9754	Panx
7	FBgn0037707	CG16788	RnpS1
8	FBgn0003401	CG4735	shu
9	FBgn0263143	CG4771	vret
10	FBgn0027499	CG12340	wde
11	FBgn0261266	CG12314	zuc

- Overlaped, in at least two screens

num	FBID	CG	Symbol
1	FBgn0263198	CG10473	Acn
2	FBgn0029512	CG12276	Aos1
3	FBgn0011741	CG11678	Arp6
4	FBgn0033526	CG12892	Caf1-105
5	FBgn0038546	CG7379	CG7379
6	FBgn0035842	CG7504	CG7504
7	FBgn0260634	CG10192	eIF4G2
8	FBgn0033806	CG4616	FLASH
9	FBgn0000928	CG2706	fs(1)Yb
10	FBgn0001197	CG5499	His2Av
11	FBgn0087013	CG1059	Karybeta3
12	FBgn0002736	CG9401	mago
13	FBgn0038016	CG10042	MBD-R2
14	FBgn0035357	CG1244	MEP-1
15	FBgn0033737	CG8831	Nup54
16	FBgn0036640	CG4118	nxf2
17	FBgn0004872	CG6122	piwi
18	FBgn0028982	CG12225	Spt6
19	FBgn0027865	CG6120	Tsp96F
20	FBgn0033378	CG8781	tsu
21	FBgn0029113	CG7528	Uba2
22	FBgn0032030	CG17293	Wdr82
23	n.d.	NA	NA

III. Materials

1. Czech paper (Greg lab)

Where

- Ovary, Germline specific genes

How

- TE derepression upon knockdown of given genes.

What

- knock down genes by dsRNA;
- Check the expression of TEs (HetA, TAHRE, blood, burdock); (derepression); (LINE:HeT-A, TAHRE; LTR: blood, burdock), armi=positive, white=negative;
- Filter by Z-score;

Results

- A total of 8171 genes were screened. table S1.
- 74 genes (Average Z-score <= -2.0) ;

- 216 genes (Average Z-score <= -1.5) ;
- 137 genes (Z-score <= -2.0, at least in two TEs) ;
- 377 genes (Z-score <= -2.0, at least in one TE) ;

See table S1 for details.

2. Handler paper (Julius lab)

Where

- Ovary; somatic piRNA pathway

What

- reporter: gypsy-LTR + beta-gal (controled by flam derived piRNAs)
- X-gal staining

How

- The expression of flam was controled by piRNA pathway. (repression, no staining, white)
- The repression of flam was disrupted by specific gene knockdown (staining, blue)

Results

- 49 genes, (staining >=2) ;
- 80 genes, (staining >= 1.5) ;
- 144 genes, (staining >= 1) ;

Notes

- 7257 genes expressed in OSC (RPKM >= 1).
- VDRC 6818 genes, (2 crosses per gene).

See tabld S1 for details.

3. Muerdter paper (Greg lab)

Where

- OSS celline

What

- Check gypsy, ZAM, gypsy3 expression by qPCR ;
- TE derepression upon knockdown (KD) of any given genes

How

- genes were knockdown by dsRNA

Results

- 87 genes, validated
- 52 genes, development defect
- 149 genes, non validated

Notes

- H3K9me3 decreased, piRNA levels not changed.

see table S3 for details.

Iv. Code

1. Czech (Greg lab)

```
## Benjamin Czech, 2013 (Greg lab)
## table S1: https://ars.els-cdn.com/content/image/1-s2.0-S1097276513002888-mmc2.xlsx
##
## Table S1. Raw Data for Primary Screen, Related to Figure 2. Shown are transformant IDs (of VDRC stoc
##
## derepression threshold, Z-score
## genes; Z-score
## 74    -2.0
## 216   -1.5
## 137   -2.0 in at least two TE
## 377   -2.0 in at least one TE
fileURL <- "https://ars.els-cdn.com/content/image/1-s2.0-S1097276513002888-mmc2.xlsx"
fileTableS1  <- "data/1.Ben_2013_Greg_lab/TableS1.xlsx"
# download.file(fileURL, fileTableS1, method = "curl")

## including NA values (n.d.)
## 8171 records
df <- readxl::read_xlsx(fileTableS1, na = "n.d.") %>%
  dplyr::mutate(HeTA = as.numeric(HeTA),
                TAHRE = as.numeric(TAHRE),
                blood = as.numeric(blood),
                burdock = as.numeric(burdock))

## 8133 records
```

```
df$mean <- df[, 5:8] %>%
  rowMeans(na.rm = TRUE)
df <- dplyr::arrange(df, mean)

## df <- df[complete.cases(df), ]
df[is.na(df)] <- 0

## threshold: -2.0 for each condition
df$status <- purrr::map(df[, 5:8], function(x) x <= -2.0) %>%
  as.data.frame() %>%
  rowSums()

## threshold: -2.0, -1.5
sum(df$mean <= -2.0) # 74
```

```
## [1] 74
```

```
sum(df$mean <= -1.5) # 216
```

```
## [1] 216
```

```
sum(df$status >= 2)  # 137, at least in two TE
```

```
## [1] 137
```

```
sum(df$status >= 1)  # 377, at least in two TE
```

```
## [1] 377
```

```
## total
df1 <- df %>%
  dplyr::filter(status >= 1) %>%
  dplyr::mutate(FBID = mapIds(org.Dm.eg.db, keys = Gene, keytype = "FLYBASECG", column = "FLYBASE"),
                Symbol = mapIds(org.Dm.eg.db, keys = Gene, keytype = "FLYBASECG", column = "SYMBOL"),
                CG = Gene) %>%
  dplyr::select(FBID, CG, Symbol, mean, status)
```

```
## 'select()' returned 1:1 mapping between keys and columns
## 'select()' returned 1:1 mapping between keys and columns
```

```
## save to table
file1 <- "data/1.Ben_2013_Greg_lab/01_screen_Ben_377.txt"
readr::write_delim(df1, file1, delim = "\t", na = "n.d.", col_names = TRUE)

## 74 genes
df2 <- df1 %>%
  dplyr::filter(mean <= -2.0)

file2 <- "data/1.Ben_2013_Greg_lab/01_screen_Ben_74.txt"
readr::write_delim(df2, file2, delim = "\t", na = "n.d.", col_names = TRUE)
```

```
## 137 genes
df3 <- df1 %>%
  dplyr::filter(mean <= -1.5)

file3 <- "data/1.Ben_2013_Greg_lab/01_screen_Ben_137.txt"
readr::write_delim(df3, file3, delim = "\t", na = "n.d.", col_names = TRUE)
```

2. Handler (Julius lab)

```
## Handler, 2013 (Julis lab)
## table S1: https://ars.els-cdn.com/content/image/1-s2.0-S1097276513003365-mmc2.xlsx
##
## Table S1. Primary and Verified Results of the Screen.
##
## genes (staining)
## 49 (>=2)
## 80 (>=1.5)
## 144 (>=1)
fileURL <- "https://ars.els-cdn.com/content/image/1-s2.0-S1097276513003365-mmc2.xlsx"
fileTableS1 <- "data/2.Handler_2013_Julius_lab/TableS1.xlsx"
# download.file(fileURL, fileTableS1, method = "curl")

## including blanks
df <- readxl::read_xlsx(fileTableS1, trim_ws = TRUE, skip = 1) %>%
  dplyr::select(c(1, 2, 4, 6, 7, 9:14))
```

```
## New names:
## * `` -> ...3
## * `` -> ...5
## * staining -> staining...6
## * morphology -> morphology...7
## * `` -> ...8
## * ... and 8 more problems
```

```
names(df) <- c("Symbol", "CG", "RPKM", "staining", "morphology",
               "VDRC_1", "staining_1", "morphology_1",
               "VDRC_2", "staining_2", "morphology_2")
## staining
df_staining <- data.frame(
  value = c(0, 1, 1.5, 2, 2.5, 3),
  status = c("no staining", "weak staining",
             "intermediate-weak staining", "intermediate staining",
             "strong-intermediate staining", "strong staining"),
  stringsAsFactors = FALSE
)

df <- df %>%
  dplyr::mutate(status = plyr::mapvalues(staining,
                                         from = df_staining$value,
```

```
                                          to = df_staining$status))
# df <- df[complete.cases(df), ]
df[is.na(df)] <- 0

sum(df$staining >= 2)   # 49
```

```
## [1] 49
```

```
sum(df$staining >= 1.5) # 80
```

```
## [1] 80
```

```
sum(df$staining >= 1)   # 144
```

```
## [1] 144
```

```
## pick 144 genes
df <- df %>%
  dplyr::filter(staining >= 1)

df1 <- df %>%
  dplyr::mutate(
    FBID = mapIds(org.Dm.eg.db, keys = CG, keytype = "FLYBASECG", column = "FLYBASE"),
    Symbol = mapIds(org.Dm.eg.db, keys = CG, keytype = "FLYBASECG", column = "SYMBOL")) %>%
  dplyr::select(FBID, CG, Symbol, staining, status)
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
## save to table
file1 <- "data/2.Handler_2013_Julius_lab/02_screen_Handler_144.txt"
readr::write_delim(df1, file1, delim = "\t", na = "n.d.", col_names = TRUE)

## 80 genes
df2 <- df1 %>%
  dplyr::filter(staining >= 1.5)
##
file2 <- "data/2.Handler_2013_Julius_lab/02_screen_Handler_80.txt"
readr::write_delim(df2, file2, delim = "\t", na = "n.d.", col_names = TRUE)
```

3. Muerdter (Greg lab)

```
## Muerdter, 2013 (Greg lab)
## table S1: https://ars.els-cdn.com/content/image/1-s2.0-S1097276513002876-mmc2.xlsx
## table S3: https://ars.els-cdn.com/content/image/1-s2.0-S1097276513002876-mmc3.xlsx
## Table S1. Primary Screen Results, Related to Figure 1.
## Table S3. Validation Screen Results, Related to Figure 2.
```

```
##
## genes (validated)
## 86 (va)
##
fileURL <- "https://ars.els-cdn.com/content/image/1-s2.0-S1097276513002876-mmc3.xlsx"
fileTableS3 <- "data/3.Muertder_2013_Greg_lab/TableS3.xlsx"
# download.file(fileURL, fileTableS3, method = "curl")

## parsing data
df <- readxl::read_xlsx(fileTableS3, trim_ws = TRUE)
colnames(df) <- c("FBID", "TRANSID", "Symbol", "Fertility",
                  "fc_gypsy", "fc_gypsy3", "fc_ZAM", "status")

## 87 validated genes
length(
  df %>%
    dplyr::filter(status %in% c("va")) %>%
    dplyr::pull("FBID") %>%
    unique())
```

```
## [1] 87
```

```
## 52 dev defect
length(
  df %>%
    dplyr::filter(status %in% c("dd")) %>%
    dplyr::pull("FBID") %>%
    unique())
```

```
## [1] 52
```

```
## only va genes
df1 <- df %>%
  dplyr::filter(status %in% c("va")) %>%
  dplyr::mutate(
    CG = mapIds(org.Dm.eg.db, keys = FBID, keytype = "FLYBASE", column = "FLYBASECG")
  ) %>%
  dplyr::select(FBID, CG, Symbol, status)
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
## save to table
file <- "data/3.Muertder_2013_Greg_lab/03_screen_Muerdter_87.txt"
readr::write_delim(df1, file, delim = "\t", na = "n.d.", col_names = TRUE)
```

V. Reference

1. Czech, B., Preall, J. B., McGinn, J. & Hannon, G. J. A Transcriptome-wide RNAi Screen in the Drosophila Ovary Reveals Factors of the Germline piRNA Pathway. Mol. Cell 50, 749–761 (2013). DOI: 10.1016/j.molcel.2013.04.007

2. Handler, D. et al. The Genetic Makeup of the Drosophila piRNA Pathway. Mol. Cell 50, 762–777 (2013). DOI: 10.1016/j.molcel.2013.04.031

3. Muerdter, F. et al. A Genome-wide RNAi Screen Draws a Genetic Framework for Transposon Control and Primary piRNA Biogenesis in Drosophila. Mol. Cell 50, 736–748 (2013). DOI: 10.1016/j.molcel.2013.04.006