# RNAi-induced off-target effects in Drosophila melanogaster

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# Supplementary Table 1

| 21-nt sequence from dsRNA | Targeted gene | Off-targeted gene | Regular mismatches | G:U mismatches | exon/intron | fold downregulation |
|---|---|---|---|---|---|---|
| Q: AGCCGAAGGTGCTGAACAAGT<br>R: GGCCGAAGCTGCTGTACAAGT | CG3941 | CG3629 | 3 | 0 | intron | >50 |
| Q: ACAACGACAACGACAUCGAUA<br>R: ACAACAACAACAACAUCGACA | CG2253 | CG4128 | 3 | 0 | intron | >50 |
| Q: CUUUUCGGCUUUGUUUUGAUU<br>R: CUUUUUGGCUUUGGUUUGUUU | CG11184 | CG4128 | 2 | 1 | intron | >50 |
| Q: AGCACGAAAUCGAAGAGAAAC<br>R: AGCAGAAAACCGAAGAGAAAC | CG8954 | CG2507 | 3 | 0 | exon | >50 |
| Q: ACAACGACAACGACATCGATA<br>R: ACAACAACAACGACAGCGACA | CG2253 | CG2507 | 2 | 1 | exon | 33 |
| Q: ACAACGACAACGACAUCGAUA<br>R: ACAACGACAACAACAACGUUA | CG2253 | CG3315 | 3 | 0 | exon | 33 |
| Q: UCGAGGCCAAACUGAAAAUGA<br>R: CCGAGCCCAAACUGAAACUGA | CG2253 | CG9656 | 3 | 0 | intron | 20 |
| Q: ACAUCAUGUUUGCAUUUGUUG<br>R: ACACCAUGUUUGCAUUCGUUU | CG11184 | CG1133 | 2 | 1 | intron | 16 |
| Q: AGAACGCGATCCACCCAGAAA<br>R: AGGACGCAATCCTCCCAGAAA | CG32743 | CG13185 | 3 | 0 | exon | 12 |
| Q: TTATCAACCGCAAGTCGTATC<br>R: TTATGAACCACAAGTCGTATA | CG32743 | CG7978 | 3 | 0 | intron | 11 |
| Q: CTTTTCGGCTTTGTTTTGATT<br>R: CTTTTCGGTTTTGTTTTGGCT | CG11184 | CG12290 | 3 | 0 | exon | 7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Q: TCGGCCTGATTGGCTTTATCA<br>R: TCGGCTTGTTTGTCTTTATCA | CG32743 | CG12819 | 2 | 1 | exon | 7 |
| Q: TGCAACAACTGCCGCAAATGG<br>R: TGCAACAAATGCCGCGAATGC | CG1559 | CG15295 | 3 | 0 | exon | 6 |
| Q: TGCAACAACTGCCGCAAATGG<br>R: TGCAACAAGTGCAGCAAATGG | CG1559 | CG32046 | 2 | 0 | intron | 6 |
| Q: ACATCAAGGCCACCGAGAAGA<br>R: ACATCACGTCCACGGAGAAGA | CG2253 | CG3234 | 2 | 1 | exon | 6 |
| Q: ACAACGACAACGACATCGATA<br>R: ACATCGACATCGACATCGAGA | CG2253 | CG32130 | 2 | 1 | Intron | 6 |
| Q: CTGCGTCTGTCCAAGATCATC<br>R: GTGCCTCTGTCCAAGATCATA | CG32743 | CG4678 | 3 | 0 | exon | 6 |
| Q: ATCTGCGTCTGTCCAAGATCA<br>R: ATCTGCCTCCGTCCAAGATGA | CG32743 | CG3359 | 3 | 0 | intron | 5 |

**Supplementary Table 1. Examples of predicted and downregulated off-targets containing various types of mismatches**

Collection of 18 identified potential off-targets from the six available datasets which appeared to be 5-fold or more downregulated. The first column shows the alignments as found by predicting off-targets using a previously described method [15]. The target genes as well as the predicted off-target genes are listed. The number of regular mismatches and the number of G:U mismatches is given for each off-target. It is listed whether the off-target sequence is present within intronal or exonal sequences of the gene. The fold downregulation compared to the control group (as derived from the available dataset) is presented for each predicted off-target. Functional comparison (using the UniProt Protein knowledgebase; http://www.uniprot.org) did not indicate any functional relation between the targeted gene and these 18 off-targeted genes

(Supplementary Table 2). Because up to three mismatches containing siRNA constructs can be active, these predicted genes that were also actually downregulated, should be considered as possible off-target effects while interpreting the microarray data.

# Supplementary Table 2

| ***Gene*** | ***Description (Uniprot)*** |
|---|---|
| CG2253, CG11184, CG8954, | nuclear-transcribed mRNA catabolic process, nonsense-mediated decay |
| CG32743 | mRNA surveillance |
| CG1559 | hydrolase activity |
| CG3941 | DNA binding |
| CG3315 | Belongs to the thioredoxin family. |
| CG4128 | Ion transport |
| CG9656 | Transcription factor that is vital to the development of multiple organ systems. |
| CG1133 | Transcription factor essential for parasegmental subdivision of the embryo. |
| CG2507 | Putative epidermal cell surface receptor |
| CG3629 | Transcription factor that plays a role in larval and adult appendage development. |
| CG4128 | Ionic channel |
| CG13185 | Hydrolase |

| | |
|---|---|
| CG7978 | This is a membrane-bound, calmodulin-insensitive adenylyl cyclase |
| CG12290 | G-protein coupled receptor protein signaling pathway |
| CG12819 | nucleolus organization and biogenesis |
| CG15295 | protein binding |
| CG32046 | Unknown |
| CG3234 | Forms a heterodimer with period (PER); the complex then translocates into the nucleus. Required for the production of circadian rhythms. |
| CG32130 | Apoptosis |
| CG4678 | Carboxypeptidase |
| CG3359 | Unknown |

**Supplementary Table 2. UniProt analysis for targeted genes**

List of functions (as defined by UniProt) of the on-targeted genes from the 6 analyzed dsRNAs and the 18 potential off-target genes listed in Supplementary Table 1.

# Supplementary Table 3

| siRNA | gene | PLIER Log Transformed Sample Averages ([GFP]) | PLIER Log Transformed Sample Averages ([CG3941]) | Change |
|---|---|---|---|---|
| CCTCGATTAGGATCTTGAACA | CG10011 | 7,0758576 | 7,0793757 | 0,0035181 |
| TGTTGGTCGTGCCAGCAAAGA | CG10055 | 8,287945 | 8,3318405 | 0,0438955 |
| CAAGATGATCCGACCAAGAAC | CG10231 | 8,324593 | 8,185164 | -0,139429 |
| ATGAGCTGAAGGTGGATAACA | CG10510 | 6,770418 | 6,6032586 | -0,1671594 |
| TGTTGGTCGTGCCAGCAAAGA | CG10630 | 12,259856 | 12,235466 | -0,02439 |
| ATCTGCCATTGGACGATCAAG | CG10631 | 7,631436 | 7,733879 | 0,102443 |
| CGATGGTGGCTTCCAAGAAGT | CG11926 | 7,4555225 | 7,4250464 | -0,0304761 |
| TCAAGCCGAAGGTGCTGAACA | CG12296 | 10,202237 | 10,1837435 | -0,0184935 |
| AACAAGTCCTCGATTAGGATC | CG12690 | 7,144793 | 7,012076 | -0,132717 |
| AGAGCATGCTGGAGGATATGG | CG1271 | 4,292626 | 3,7691085 | -0,5235175 |
| AGAGCATGCTGGAGGATATGG | CG12725 | 2,7071903 | 2,5614817 | -0,1457086 |
| TCAAGCCGAAGGTGCTGAACA | CG13739 | 2,2208786 | 1,9240206 | -0,296858 |
| AAAAGCTGCTGAACACAATGG | CG14023 | 5,2321324 | 4,903741 | -0,3283914 |
| AAAAGCTGCTGAACACAATGG | CG14026 | 7,3602443 | 7,192494 | -0,1677503 |
| GAGAAGGAGTTCCCCGATATC | CG14945 | 8,09917 | 7,9079347 | -0,1912353 |
| ACAATGGCCAAATCCAGTAGT | CG14961 | 4,2906985 | 4,311647 | 0,0209485 |
| TGAACACAATGGCCAAATCCA | CG15552 | 2,0585396 | 2,5654852 | 0,5069456 |
| TGAACACAATGGCCAAATCCA | CG15624 | 2,4751813 | 2,5457335 | 0,0705522 |
| AGAGCATGCTGGAGGATATGG | CG15753 | 4,6198773 | 4,777483 | 0,1576057 |
| TGAAGGTGGATAACAACCAGG | CG15824 | 3,869911 | 3,7345135 | -0,1353975 |
| CCTCGATTAGGATCTTGAACA | CG1624 | 7,6595893 | 7,5801277 | -0,0794616 |
| AGAGCATGCTGGAGGATATGG | CG18076 | 8,082535 | 7,920265 | -0,16227 |
| CCAGTAGTCAGGTGATCATCG | CG18135 | 8,052136 | 7,924259 | -0,127877 |
| AAAAGCTGCTGAACACAATGG | CG1825 | 8,556577 | 8,360581 | -0,195996 |
| TCAAGCCGAAGGTGCTGAACA | CG1877 | 3,0509758 | 1,8145055 | -1,2364703 |

| | | | | |
|---|---|---|---|---|
| CGATGGTGGCTTCCAAGAAGT | CG2209 | 3,6375864 | 3,2531707 | -0,3844157 |
| CGAGATCCATCGGCTGAATCA | CG2368 | 7,782516 | 7,5833716 | -0,1991444 |
| TGAAGGTGGATAACAACCAGG | CG2668 | 2,7809408 | 2,270806 | -0,5101348 |
| GGATATGGCCAGTGAGCTAGA | CG30147 | 6,926825 | 6,688499 | -0,238326 |
| CCAGTGAGCTAGAGAAGGAGT | CG31224 | 8,195302 | 8,20954 | 0,014238 |
| AGATCCATCGGCTGAATCACA | CG31873 | 7,432766 | 7,451584 | 0,018818 |
| ATGAGCTGAAGGTGGATAACA | CG32112 | 7,5451527 | 7,3287683 | -0,2163844 |
| AGAGCATGCTGGAGGATATGG | CG32169 | 3,113495 | 1,8426205 | -1,2708745 |
| CGATGGTGGCTTCCAAGAAGT | CG32445 | 3,1145496 | 3,1020272 | -0,0125224 |
| AGAGCATGCTGGAGGATATGG | CG32713 | 2,2185984 | 1,8787171 | -0,3398813 |
| AAAAGCTGCTGAACACAATGG | CG32732 | 7,677002 | 7,6682153 | -0,0087867 |
| TGTTGGTCGTGCCAGCAAAGA | CG32773 | 2,4855716 | 2,2324898 | -0,2530818 |
| AGAGCATGCTGGAGGATATGG | CG33208 | 1,3887143 | 2,3559258 | 0,9672115 |
| AGAGCATGCTGGAGGATATGG | CG33223 | 2,2185984 | 1,8787171 | -0,3398813 |
| ATGAGCTGAAGGTGGATAACA | CG33519 | 2,5681283 | 2,4791658 | -0,0889625 |
| GAGAAGGAGTTCCCCGATATC | CG33545 | 0,041791994 | 0,044357974 | 0,00256598 |
| AGCTGAAGGTGGATAACAACC | CG33970 | 8,103564 | 8,07772 | -0,025844 |
| AGAAGGAGTTCCCCGATATCC | CG3427 | 6,9449096 | 6,45593 | -0,4889796 |
| CGATGGTGGCTTCCAAGAAGT | CG3564 | 10,394695 | 10,24418 | -0,150515 |
| AGCCGAAGGTGCTGAACAAGT | CG3629 | 1,9607176 | 1,1180875 | -0,8426301 |
| AGAGCATGCTGGAGGATATGG | CG3926 | 4,0784774 | 3,906498 | -0,1719794 |
| AGTATCCGCTCACGGGTAACT | CG3941 | 8,774968 | 5,967096 | -2,807872 |
| CTGAACAAGTCCTCGATTAGG | CG3942 | 3,5230682 | 3,6358502 | 0,112782 |
| AGCTGAAGGTGGATAACAACC | CG3980 | 7,309879 | 7,360625 | 0,050746 |
| AAAAGCTGCTGAACACAATGG | CG4181 | 5,4072113 | 5,019833 | -0,3873783 |
| CGATGGTGGCTTCCAAGAAGT | CG4847 | 4,5920334 | 4,7530084 | 0,160975 |
| ATCTGCCATTGGACGATCAAG | CG4894 | 2,3624065 | 2,459273 | 0,0968665 |
| ACAATGGCCAAATCCAGTAGT | CG5290 | 9,067573 | 9,152059 | 0,084486 |
| ACAATGGCCAAATCCAGTAGT | CG5481 | 6,778341 | 6,212174 | -0,566167 |

| | | | | |
|---|---|---|---|---|
| CCAGTGAGCTAGAGAAGGAGT | CG5884 | 9,966991 | 9,8642845 | -0,1027065 |
| GTAGTCAGGTGATCATCGAGG | CG6026 | 2,4597237 | 1,7917448 | -0,6679789 |
| AGAAGGAGTTCCCCGATATCC | CG6043 | 7,40094 | 6,726528 | -0,674412 |
| GAGAAGGAGTTCCCCGATATC | CG6383 | 7,4093204 | 7,4287987 | 0,0194783 |
| TGAAGGTGGATAACAACCAGG | CG6659 | 9,057574 | 9,091976 | 0,034402 |
| GGATATGGCCAGTGAGCTAGA | CG6963 | 8,6487875 | 8,581727 | -0,0670605 |
| GAGAAGGAGTTCCCCGATATC | CG7433 | 5,9774795 | 5,7592072 | -0,2182723 |
| CGATTAGGATCTTGAACAAGG | CG7918 | 3,636245 | 3,5232751 | -0,1129699 |
| AGAGCATGCTGGAGGATATGG | CG8552 | 9,607126 | 9,534208 | -0,072918 |
| GAGAAGGAGTTCCCCGATATC | CG8849 | 8,487894 | 8,129428 | -0,358466 |
| CCTCGATTAGGATCTTGAACA | CG9151 | 5,682402 | 6,223883 | 0,541481 |
| AGATCCATCGGCTGAATCACA | CG9198 | 8,33042 | 8,235469 | -0,094951 |
| GAGAAGGAGTTCCCCGATATC | CG9267 | 7,6554627 | 7,6183248 | -0,0371379 |
| AGAAGGAGTTCCCCGATATCC | CG9450 | 9,601012 | 9,559306 | -0,041706 |
| CAAAGTCAAGCGAGATGATAG | CG9559 | 8,970767 | 9,061164 | 0,090397 |

**Supplementary Table 3 – Detailed information for off-target analysis of CG3941 dsRNA**

Example output from RNAi-Select for the CG3941 dsRNA. First column specifies the potential siRNAs found in the dsRNA for CG3931 from which potential off-targets where found that are listed in the second column. Data derived from the micro-array are listed in the 3th and 4th column after PLIER normalization, log transformation and averaging. The last column shows the change in expression between the control array and the array of the CG3941 dsRNA treated samples.

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