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 ||||||| ||||| |||||| R: GGCCGAAGCTGCTGTACAAGT	CG3941	CG3629	3	0	intron	>50
Q: ACAACGACAACGACAUCGAUA ||||| ||||| ||||||| | R: ACAACAACAACAACAUCGACA	CG2253	CG4128	3	0	intron	>50
Q: CUUUUCGGCUUUGUUUUGAUU ||||| |||||||:|||| || R: CUUUUUGGCUUUGGUUUGUUU	CG11184	CG4128	2	1	intron	>50
Q: AGCACGAAAUCGAAGAGAAAC |||| ||| |||||||||| R: AGCAGAAAACCGAAGAGAAAC	CG8954	CG2507	3	0	exon	>50
Q: ACAACGACAACGACATCGATA ||||| ||||||||||:||| | R: ACAACAACAACGACAGCGACA	CG2253	CG2507	2	1	exon	33
Q: ACAACGACAACGACAUCGAUA ||||||||||| ||| || || R: ACAACGACAACAACAACGUUA	CG2253	CG3315	3	0	exon	33
Q: UCGAGGCCAAACUGAAAAUGA |||| |||||||||| || R: CCGAGCCCAAACUGAAACUGA	CG2253	CG9656	3	0	intron	20
Q: ACAUCAUGUUUGCAUUUGUUG ||| ||||||||||| |||: R: ACACCAUGUUUGCAUUCGUUU	CG11184	CG1133	2	1	intron	16
Q: AGAACGCGATCCACCCAGAAA || |||| |||| |||||||| R: AGGACGCAATCCTCCCAGAAA	CG32743	CG13185	3	0	exon	12
Q: TTATCAACCGCAAGTCGTATC |||| |||| |||||||||| R: TTATGAACCACAAGTCGTATA	CG32743	CG7978	3	0	intron	11
Q: CTTTTCGGCTTTGTTTTGATT |||||||| ||||||||| | R: CTTTTCGGTTTTGTTTTGGCT	CG11184	CG12290	3	0	exon	7

```Q: TCGGCCTGATTGGCTTTATCA```
``` ||||| || |||:||||||||```
```R: TCGGCTTGTTTGTCTTTATCA```
	CG32743	CG12819	2	1	exon	7

```Q: TGCAACAACTGCCGCAAATGG```
``` |||||||| |||||| ||||```
```R: TGCAACAAATGCCGCGAATGC```
	CG1559	CG15295	3	0	exon	6

```Q: TGCAACAACTGCCGCAAATGG```
``` |||||||| |||| |||||||||```
```R: TGCAACAAGTGCAGCAAATGG```
	CG1559	CG32046	2	0	intron	6

```Q: ACATCAAGGCCACCGAGAAGA```
``` ||||||| |:|||| |||||||```
```R: ACATCACGTCCACGGAGAAGA```
	CG2253	CG3234	2	1	exon	6

```Q: ACAACGACAACGACATCGATA```
``` ||| |||||| ||||||||||:|```
```R: ACATCGACATCGACATCGAGA```
	CG2253	CG32130	2	1	Intron	6

```Q: CTGCGTCTGTCCAAGATCATC```
``` ||| |||||||||||||||```
```R: GTGCCTCTGTCCAAGATCATA```
	CG32743	CG4678	3	0	exon	6

```Q: ATCTGCGTCTGTCCAAGATCA```
``` |||||| || |||||||||| |```
```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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