Functional screening identifies miR-315 as a potent activator of Wingless signaling Silver *et al.* 10.1073/pnas.0706673104.

Supporting Information

Files in this Data Supplement: <u>SI Table 1</u> <u>SI Table 2</u> <u>SI Figure 5</u> **Fig. 5.** Phylogenetic conservation of miR-315-binding sites in *Axin* and *Notum*. Shown are alignments of the 3' regions of *Axin* and *Notum* across the 12 sequenced Drosophilids as provided by the University of California Santa Cruz Genome Center. The positions of the stop codons and the miR-315-binding sites are indicated by arrows. In higher magnification are the local nucleotide regions surrounding each miR-315-binding site. The sites in *Axin* are perfectly conserved in 10 and 12 Drosophilids, respectively; the large sequence gap in the vicinity of the first site in *Drosophilia simulans* makes it likely that this is either an alignment artifact or sequencing error. Note that all sites conserve t1a features. The first site in *Notum* is relatively newly evolved and found in only 5 species, whereas the second site is perfectly conserved in 11 species.





Table 1. Many components of *Drosophila* Wg--Wnt signaling are predicted as miRNA targets

Gene	Predicted regulatory miRNAs	
APC	miR-210, miR-315	
Armadillo (ß- catenin)	miR-10 ¹ , miR-310 ¹ /miR-313 ¹ /miR-311/miR-312, miR-3 ¹ /miR-318 ¹	
Arrow (LRP)	miR-263b, miR-277, miR-305 ²	
Axin	miR-277, miR-315, miR-311/miR-310 ¹ /miR-313 ¹ /miR-312	
Dally	miR-79/miR-4, miR-5	
Dally-like protein	miR-283, miR-289, miR-315	
Disheveled	miR-307, miR-315	
Frizzled	miR-277, miR-14, miR-34, miR-4/miR-79, miR-33 ¹ , miR-315, miR-289 ¹ , miR- 288 ¹	
Fz2	miR-34, miR-286, miR-14, miR-263b, miR-303, ² miR-279, miR-315	
Fz4	miR-6/miR-13a/miR-2b/miR-2c ¹ /miR-13b/miR-2a/miR-308/miR-11, miR- 31a/miR-31b, miR-4/miR-79	
GSK3-ß ³	miR-289, miR-283	
Hyrax/Parafibromin	no predictions	
Legless ³	miR-7	
Naked	miR-14, miR-277, miR-316 ² , miR-283 ¹ , miR-289 ¹	
Nemo	miR-1, miR-278	
Notum	miR-306, miR-275, let-7, miR-14, miR-314 ¹ , miR-315, miR-316, miR-3 ²	
Pangolin ³ (dTCF)	miR-311/miR-310/miR-313/miR-312/miR-92a/miR-92b	
Porcupine	miR-289 ¹ , miR-92a	
Pygopus	miR-307, miR-87 ¹	
SImb	miR-313 ¹ /miR-311/miR-310 ¹ /miR-312	
Wingless	miR-8, miR-14 ²	

Wntl	ess/Evi
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miR-31a/miR-31b, miR-8²

All miRNA:mRNA target pairs listed are predicted by both recent prediction algorithms (1-3) unless otherwise indicated. 1, predicted by Pictar (1) only; 2, predicted by EMBL (2, 3) only; 3, these genes were only present in one of the two prediction data sets. Members of miRNA families are grouped together with backslashes. Note that not all members of a given family were necessarily predicted in the different algorithms.

- 1. Lall S, Grun D, Krek A, Chen K, Wang YL, Dewey CN, Sood P, Colombo T, Bray N, Macmenamin P, *et al.* (2006) *Curr Biol* 16:460-471.
- 2. Brennecke J, Stark A, Russell RB, Cohen SM (2005) PLoS Biol 3:e85.
- 3. Stark A, Brennecke J, Bushati N, Russell RB, Cohen SM (2005) Cell 123:1133-1146.

UAS-miRNA construct	Fold change TCF- luc (-) Wg	Fold change TCF-luc (+) Wg
UAS-dsRed	1.00	1.00
let-7	1.45	1.37
miR-1	2.67	1.40
miR-10	1.61	1.68
miR-100	1.30	1.16
miR-100/let-7/miR-125	0.78	1.17
miR-12	1.62	1.69
miR-12/ miR-283/ miR-304	1.38	1.41
miR-124	2.03	1.24
miR-125	1.42	1.23
miR-133	2.11	1.82
miR-133/ miR-288	2.13	1.91

Table 2. Primary data for the miRNA TCF-luc screen

miR-13b-1/ miR-13a/ miR-2c	0.90	1.29
miR-14	1.61	1.18
miR-184	2.65	1.25
miR-184	2.36	1.46
miR-210	1.87	2.74
miR-219	1.89	1.59
miR-252	3.07	2.41
miR-263a	1.77	1.61
miR-274	2.18	1.82
miR-275	1.12	1.09
miR-275/ miR-305	1.73	1.66
miR-276a	1.82	1.40
miR-276b	1.86	1.25
miR-277	1.55	1.48
miR-278	2.26	1.15
miR-279	1.43	1.07
miR-280	1.37	1.23
miR-281-1/ miR-281-2	0.93	1.21
miR-282	2.07	1.35
miR-283	2.04	1.47
miR-284	2.07	1.32
miR-285	1.78	0.91

miR-286	1.40	1.10
miR-287	1.37	1.19
miR-288	1.68	1.58
miR-289	1.09	1.01
miR-2a-2/ miR-2a-1/ miR-2b-1	1.18	1.24
miR-3	1.65	1.74
miR-303	1.72	1.13
miR-304	1.46	1.11
miR-305	2.08	1.54
miR-306	1.76	0.93
miR-307	1.11	1.15
miR-308	1.43	0.98
miR-309	1.62	1.19
miR-309/ miR-3/ miR-286/ miR-4/ miR-6- 1,6-2,6-3	0.89	1.13
miR-31	1.44	1.12
miR-310	1.52	1.09
miR-310/ miR-311/ miR-312/ miR-313	1.57	1.13
miR-311	1.04	1.21
miR-312	1.20	0.95
mIR-313	1.21	0.99
miR-314	1.16	1.37
miR-315	8.86	1.96

miR-316	2.48	1.53
miR-316	2.13	1.31
miR-317	1.75	1.31
miR-318	1.99	1.74
miR-33	1.36	1.43
miR-34	2.31	1.71
miR-34/ miR-277	2.19	1.67
miR-4	1.54	1.40
miR-5	1.46	0.94
miR-6-1,6-2,6-3	1.19	1.42
miR-7	0.68	1.18
miR-79	1.41	1.28
miR-8	2.13	1.00
miR-87	1.49	1.18
miR-92a	1.82	1.22
miR-92b	1.70	1.06
miR-9a	1.99	1.17
miR-9b/ miR-306/ miR-79/ miR-9c	1.03	1.27
miR-9c	1.16	1.26
miR-iab-4	1.97	1.04

Quantitative effects of ectopic miRNAs on TCF-luc in clone 8 cells were assayed in the absence (-) or presence (+) of Wg. Data of both screens were normalized by setting the ratio of TCF-luc to control pol III-renilla-luc in the presence of ub-Gal4 and UAS-DsRed to 1. Fold changes to this ratio in the presence of UAS-DsRed-miRNA plasmids are shown.