Addendum

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Genome-wide RNAi analysis of JAK/STAT signaling components in *Drosophila*

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Recently it was shown that long double-stranded RNAs (dsRNAs) can lead to "off-target effects" (OTE) in *Drosophila* cells (Kulkarni et al. 2006; Ma et al. 2006). We therefore generated one or two additional independent dsRNAs for each of the 121 candidate genes of the JAK/STAT signaling pathway that we initially reported (Baeg et al. 2005). Each of the newly generated dsRNAs was designed to be free of 19 base pairs (bp) or longer overlap with other genes. We retested these new dsRNAs in parallel with the original dsRNAs identified from the screen and found that 111 original dsRNAs scored, and among them, 50 could be further confirmed by one or two independent dsRNAs (Table 1). Of interest, we note that 17 of the original dsRNAs that were devoid of any 19-bp homology with other genes failed to be confirmed by additional dsRNAs. This finding suggests that other OTE rules that we have not been able to identify (such as interference with miRNA function through potential seed regions found in small interfering RNAs [siRNAs] [Lewis et al. 2003]) may also lead to false positives in large-scale screens in *Drosophila* cells. Alternatively, it is possible that knockdown efficiency varies among different long dsRNAs. In addition, nine of the dsRNAs in the initial 121 positives that, based on our in silico analysis, were predicted to have off-target sequences targeting 15 or more other genes could be confirmed with a second or third dsRNA. Taken together, our data strongly support the recommendation made by Echeverri et al. (2006) that testing of two or more independent dsRNAs should be performed, and will help minimize the risk of reporting false positives in RNA interference (RNAi)-based assays. In conclusion, cell-based assays and RNAi, when well controlled, constitute a valid approach for identification of genes potentially involved in a given biological process, but more detailed biochemical and genetic analyses will be necessary to validate these candidate genes.

Table 1. *List of genes that were confirmed by two or three independent dsRNAs in the JAK/STAT assay*

Negative regulators			Positive regulators		
Amplicon	**Gene**	**Fold change**	**Amplicon**	**Gene**	**Fold change**
DRSC11325	ash1	3.43	DRSC03504	cdc2	0.40
DRSC32654	ash1	3.81	DRSC30705	cdc2	0.39
DRSC32655	ash1	2.64	DRSC30706	cdc2	0.38
DRSC19337	Bap60	1.47	DRSC17794	CG11700	0.27
DRSC32656	Bap60	1.70	DRSC31545	CG11700	0.22
DRSC32657	Bap60	1.53	DRSC08254	CG12104	0.65
DRSC11330	brm	1.89	DRSC32318	CG12104	0.67
DRSC30901	brm	1.32	DRSC32317	CG12104	0.55
DRSC03287	Cas	1.43	DRSC15283	CG17836	0.24
DRSC32658	Cas	1.48	DRSC32680	CG17836	0.29
DRSC32659	Cas	1.76	DRSC32681	CG17836	0.29
DRSC04085	CG10955	1.43	DRSC18386	CG32767	0.30
DRSC30727	CG10955	1.55	DRSC32396	CG32767	0.54
DRSC30728	CG10955	1.81	DRSC13053	CG3563	0.54
DRSC09878	CG12310	2.22	DRSC30936	CG3563	0.73
DRSC25358	CG12310	1.48	DRSC10516	CG5546	0.44
DRSC04191	CG13550	2.44	DRSC30863	CG5546	0.34
DRSC31776	CG13550	1.37	DRSC30862	CG5546	0.49
DRSC31777	CG13550	1.50	DRSC10563	CG5971	0.78
DRSC00447	CG15432	1.48	DRSC32714	CG5971	0.63
DRSC32675	CG15432	1.25	DRSC19969	CG5988	0.26
DRSC06127	CG30089	3.36	DRSC32715	CG5988	0.23
DRSC32689	CG30089	1.27	DRSC32716	CG5988	0.35
DRSC10977	CG32365	1.74	DRSC18427	CG8636	0.35
DRSC32700	CG32365	1.29	DRSC32087	CG8636	0.41
DRSC11697	CG32428	2.80	DRSC32088	CG8636	0.55
DRSC32701	CG32428	1.24	DRSC19583	dome	0.17
DRSC06562	CG33455	2.01	DRSC32731	dome	0.16
DRSC32709	CG33455	1.47	DRSC32732	dome	0.13

(*continued*)

Table 1. (*continued*)

Negative regulators			Positive regulators		
Amplicon	**Gene**	**Fold change**	**Amplicon**	**Gene**	**Fold change**
DRSC32710	CG33455	1.38	DRSC16704	Hmgcr	0.34
DRSC04360	CG3363	1.37	DRSC31628	Hmgcr	0.77
DRSC32064	CG3363	1.90	DRSC20340	hop	0.23
DRSC18349	CG4136	3.83	DRSC32739	hop	0.25
DRSC32406	CG4136	3.27	DRSC32740	hop	0.23
DRSC32407	CG4136	3.59	DRSC00708	lilli	0.56
DRSC10635	CG6434, CG5585	1.51	DRSC32745	lilli	0.49
DRSC32720	CG6434	1.24	DRSC32746	lilli	0.65
DRSC11848	CG7752	1.53	DRSC11251	Pdp1	0.58
DRSC31702	CG7752	1.29	DRSC32517	Pdp1	0.77
DRSC31701	CG7752	1.48	DRSC11285	Snap	0.54
DRSC20132	CG8949	1.89	DRSC31261	Snap	0.20
DRSC32727	CG8949	1.44	DRSC16870	Stat92E	0.17
DRSC32728	CG8949	1.43	DRSC32773	Stat92E	0.23
DRSC04096	enok	2.51	DRSC32774	Stat92E	0.27
DRSC32735	enok	1.81	DRSC00843	ush	0.14
DRSC32736	enok	2.38	DRSC32226	ush	0.42
DRSC16651	jumu	1.59	DRSC32227	ush	0.34
DRSC32741	jumu	2.02			
DRSC32742	jumu	1.81			
DRSC04696	ken	4.83			
DRSC31748	ken	1.69			
DRSC06948	lolal	1.76			
DRSC32751	lolal	1.64			
DRSC32752	lolal	1.78			
DRSC15378	mor	1.35			
DRSC32754	mor	1.30			
DRSC14209	Nup98	1.38			
DRSC31803	Nup98	1.29			
DRSC11874	Pitslre	1.55			
DRSC31971	Pitslre	1.56			
DRSC08683	Ptp61F	4.39			
DRSC32761	Ptp61F	4.52			
DRSC32762	Ptp61F	4.01			
DRSC17034	puc	1.84			
DRSC31024	puc	1.34			
DRSC02455	Socs36E	5.46			
DRSC30658	Socs36E	2.32			
DRSC30659	Socs36E	5.54			
DRSC16211	Ssdp	1.46			
DRSC31311	Ssdp	1.29			
DRSC31310	Ssdp	1.51			
DRSC11309	Trn	1.53			
DRSC32778	Trn	1.22			
DRSC17089	trx	4.90			
DRSC32779	trx	4.20			
DRSC32780	trx	5.87			
DRSC20381	unc-4	1.99			
DRSC32781	unc-4	1.61			
DRSC03641	zf30C	1.55			
DRSC32783	zf30C	1.83			
DRSC32784	zf30C	1.77			

The average reporter activity from multiple control samples treated with *lacZ* dsRNA was set as 1 and those from samples treated with test dsRNAs were calculated accordingly. Results shown were the average values from three independent experiments conducted in duplicate. The cut-off values are 1.2 and 0.8 for negative and positive regulators, respectively. The original amplicons are shaded.

References

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