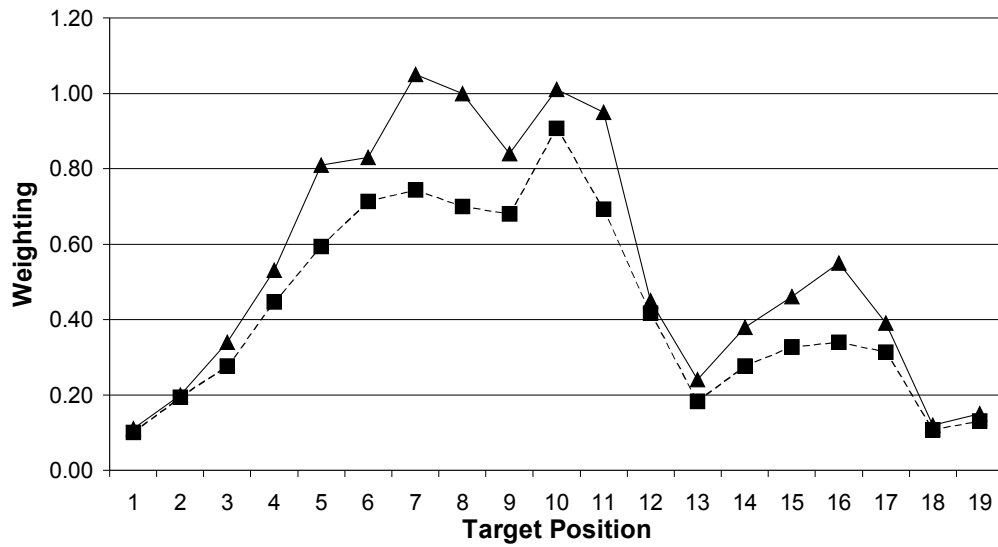


Target Gene	Target Accession	Target Start	Target End	Off-target Gene	Off-target Accession	Off-target Start	Off-target End	Hit Sequence	Score
BRAF	NM_004423	1427	1446	DVL1	NM_182779	772	790	CAAGAUCACCUUCUCCGAG	100
CDC20	NM_003380	348	367	LMNB1	NM_005573	503	521	gUACAUCGACAAGGUGCGC	98
DVL3	NM_004612	181	200	LOC401007	XM_496596	1956	1974	cUGUUUCUGCCACCUCUGU	98
HIF1A	XM_374396	970	989	DUB1A	XM_377830	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	DUB3	NM_201402	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	LOC392188	XM_373238	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	LOC401121	XM_376305	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	LOC402165	XM_377831	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	LOC402166	XM_377832	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	LOC402167	XM_377834	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	LOC402168	XM_377835	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	LOC402169	XM_377836	475	493	GAAGAUGCCCAUGAAUUuC	97
HIF1A	XM_374396	970	989	LOC402170	XM_377837	475	493	GAAGAUGCCCAUGAAUUuC	97
ICAM-1	J03132	2831	2850	A1BG	NM_130786	2057	2075	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	AIM1	NM_001624	6414	6432	gGUGAUCCUCCACCUCAG	98
ICAM-1	J03132	2731	2750	ANP32E	NM_030920	42	60	gGUGUGUGUGUGUAUGUGU	98
ICAM-1	J03132	2731	2750	APG10L	NM_031482	1768	1786	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2731	2750	B4GALT5	NM_004776	3273	3291	gGUGUGUGUGUGUAUGUGU	98
ICAM-1	J03132	2731	2750	BACH1	NM_001186*	4150	4168	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2731	2750	BAZ2A	NM_013449	6862	6880	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2831	2850	BTNL9	NM_152547	2871	2889	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	C16orf54	NM_175900	2181	2199	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	C18orf24	NM_145060	1162	1180	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2731	2750	C9orf7	NM_017586	2128	2146	UGUGUGUGUGUGUAUGUaU	97
ICAM-1	J03132	2831	2850	CA13	NM_198584	1104	1122	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	CLECTA	NM_197952*	704	722	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2731	2750	DDB1	NM_001923**	3859	3877	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2831	2850	DTX3L	NM_138287	4422	4440	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	ELF5	NM_001422*	1461	1479	AGUGAUCCUCCACCUCgG	97
ICAM-1	J03132	2731	2750	FAM61A	NM_015578	1903	1921	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2731	2750	FGF9	NM_002010	1098	1116	UGUGUGUGUGUGUAUGUGU	100
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ICAM-1	J03132	2831	2850	GRPEL2	NM_152407	2895	2913	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	HDHD4	NM_152667**	1798	1816	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2731	2750	HEMGN	NM_197978*	1766	1784	UGUGUGUGUGUGUAUGUaU	97
ICAM-1	J03132	2731	2750	KCNJ3	NM_002239	2122	2140	UGUGUGUGUGUGUAUGUaU	97
ICAM-1	J03132	2831	2850	KIAA1727	NM_033393**	4327	4345	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2731	2750	LARP4	NM_199190*	4564	4582	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2831	2850	LOC286186	XM_379586	260	278	AGUGAUCCUCCACCUCAG	100
ICAM-1	NM_000942	412	431	LOC341457	XM_292085	111	129	uGGAGAGAAAGGAAUUUGGC	98
ICAM-1	J03132	2831	2850	LOC399753	XM_378223	1323	1341	gGUGAUCCUCCACCUCAG	98
ICAM-1	J03132	2831	2850	LOC399768	XM_378228	1318	1336	gGUGAUCCUCCACCUCAG	98
ICAM-1	J03132	2731	2750	LOC400084	XM_378389	3483	3501	UGUGUGUGUGUGUAUGUGU	100
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ICAM-1	J03132	2831	2850	LOC439983	XM_495830	1328	1346	gGUGAUCCUCCACCUCAG	98
ICAM-1	J03132	2831	2850	LOC440388	XM_496170	491	509	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	LOC440426	XM_498662	1257	1275	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2731	2750	LOC440704	XM_498826	412	430	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2831	2850	LOC441438	NR_002320	9333	9351	AGUGAUCCUCCACCUCgG	97
ICAM-1	J03132	2831	2850	MHC2TA	NM_000246	5085	5103	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	MKNK1	NM_003684	738	756	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2831	2850	MRPL30	NM_145212	1345	1363	AGUGAUCCUCCACCUCAG	100
ICAM-1	J03132	2731	2750	NCOA3	NM_006534*	5902	5920	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2731	2750	NFAT5	NM_006599*	9292	9310	UGUGUGUGUGUGUAUGUGU	100
ICAM-1	J03132	2831	2850	PACRG	NM_152410	912	930	AGUGAUCCUCCACCUCAG	100

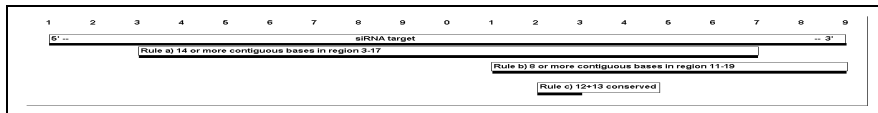
**Table S1** – Nonspecific siRNAs in siRNadb. The table lists matches with score > 95 when screening siRNadb for off-target matches in RefSeq after hits to similar genes have been removed. Three siRNAs account for 88 of the 97 total matches. Redundant off-target matches have been collapsed: \* denotes matches to multiple accession numbers; \*\* denotes multiple matches to the same accession number. Lowercase nucleotides denote mismatches.

Database	Hit criterion			
	a	b	c	a AND b
Hits in siRNAdb	877	10877	36516	171
Genes hit in siRNAdb	381	1069	1143	51

**Table S2** - Off-target siRNA hits in RefSeq according to the non-specificity rules based on the data from Jackson et al. (2003). Criteria a, b and c represent different levels of similarity between query and target, which are described in detail in the supplementary text.



(a)

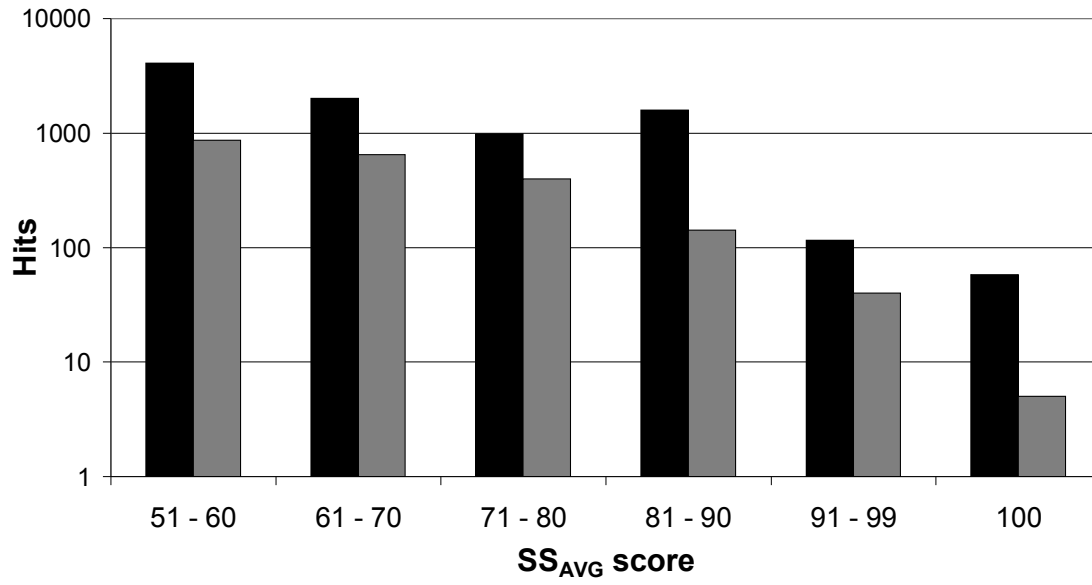


(b)

**Figure S1** - Rules for determining the likelihood of a non-specific effect.

(a) Rules based on experimental data from Du *et al.* (2005). Effect of single mismatches on efficacy vs. position:  $W_{AVG}$  (black squares) and  $W_{MAX}$  (black triangles). The penalty for mismatches in each position is shown.  $W_{AVG}$  corresponds to the average of the 3 possible mismatches;  $W_{MAX}$  corresponds to maximum penalty from the 3 possible matches. Terminal bases are generally penalized less than mismatches towards the centre of the siRNA.

(b) Rules generated from microarray driven specificity discovery.



**Figure S2** - Distribution of off-target  $SS_{AVG}$  scores for 1188 experimentally verified siRNAs after filtering of hits to genes with high similarity. Total siRNA hits to Refseq are represented by solid bars. Gray bars show the number of unique non-specific siRNAs in the score interval.