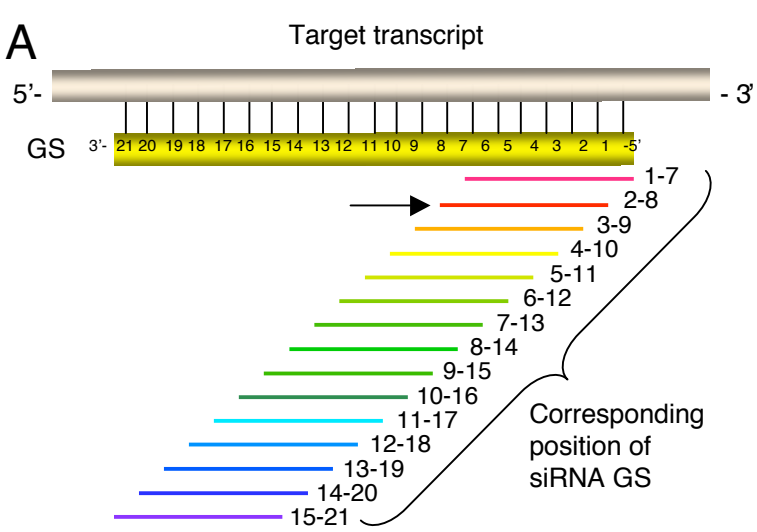
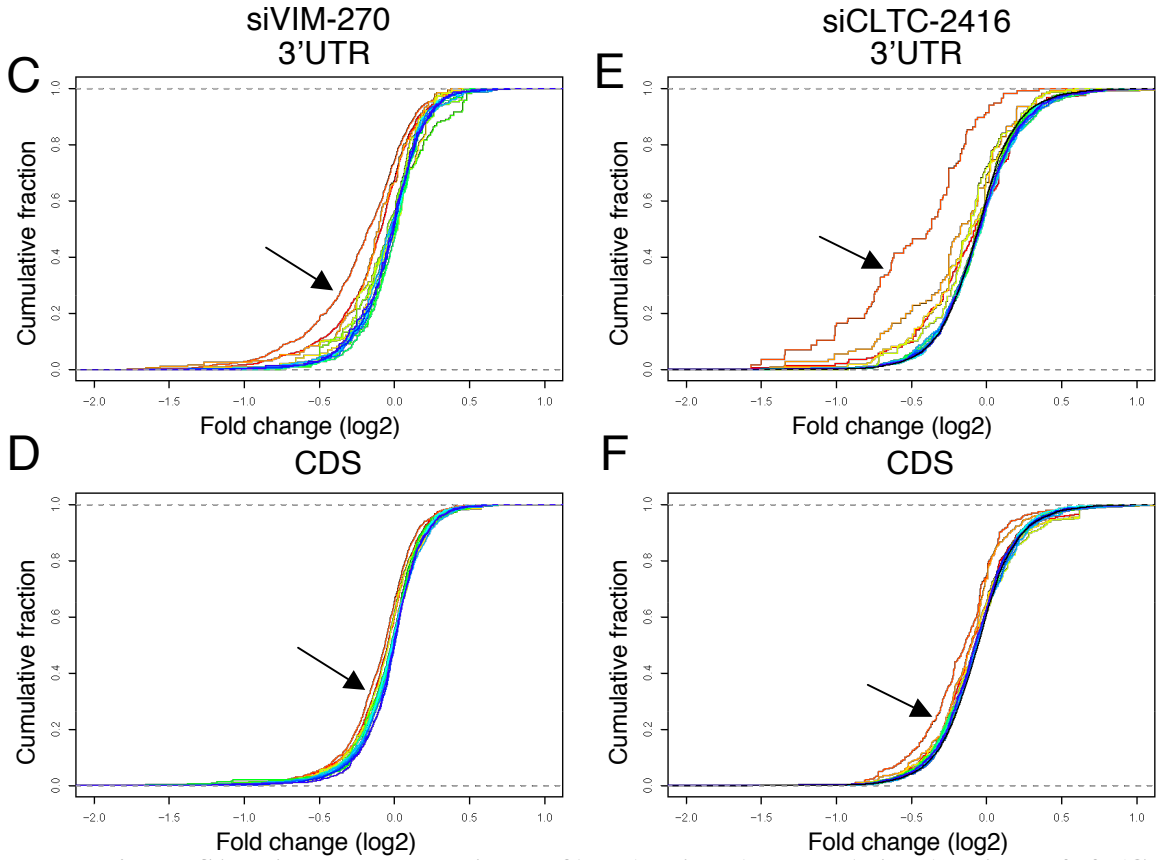
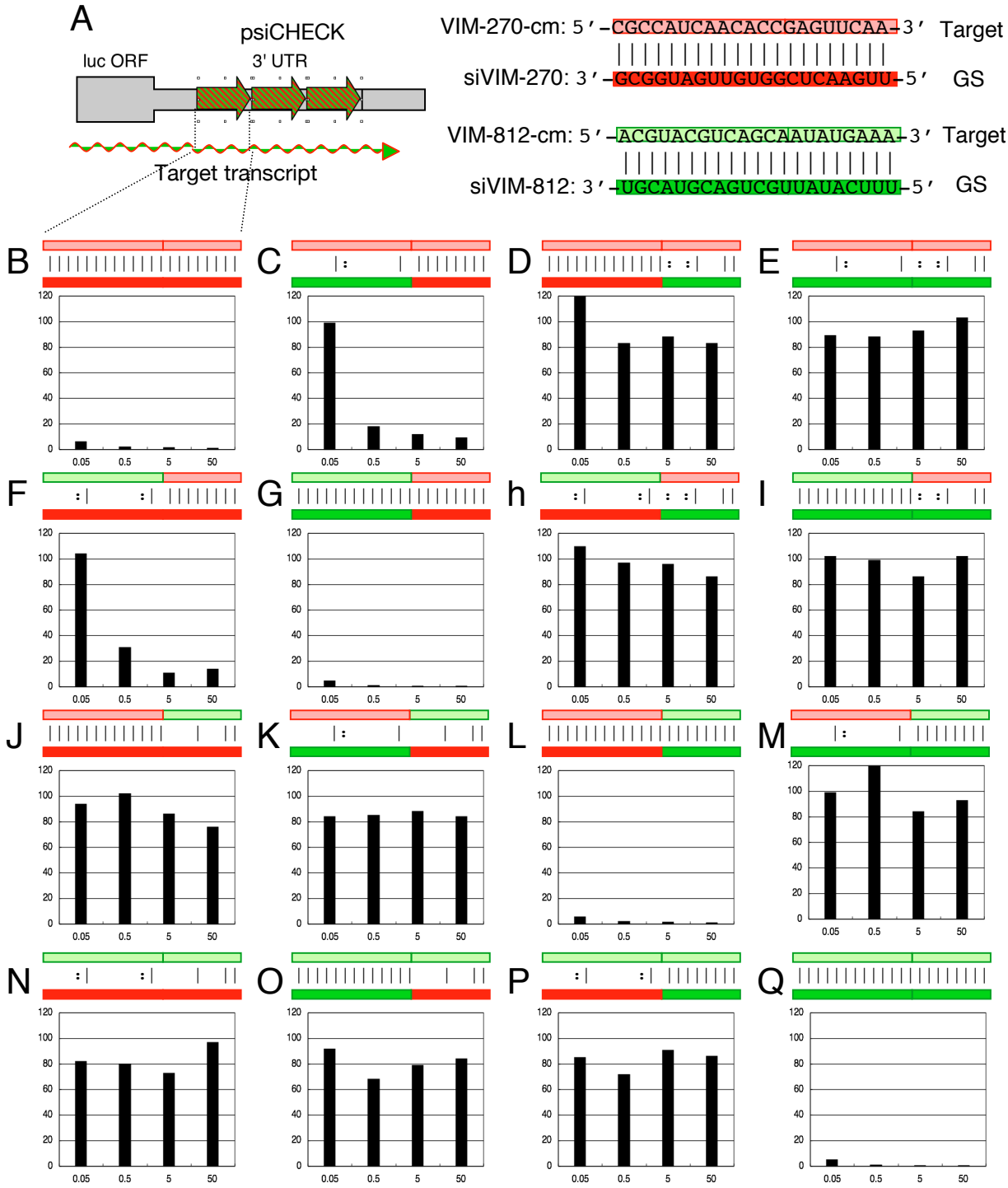


B

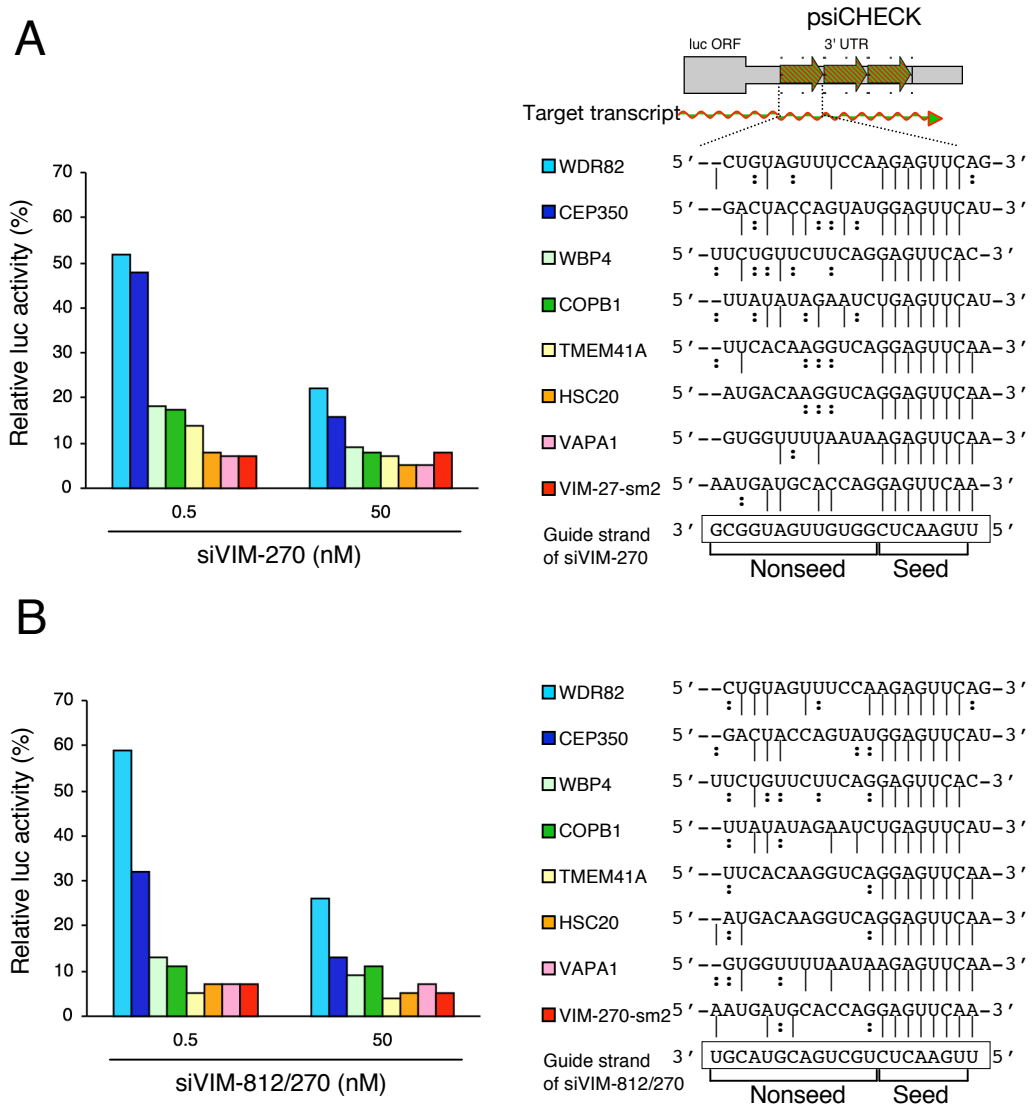
Homologous position	siVIM-270		siCLTC-2416	
	3'UTR	CDS	3'UTR	CDS
1-7	1890	2664	277	868
2-8	1734	2397	116	467
3-9	192	1108	110	478
4-10	244	808	205	817
5-11	276	766	157	741
6-12	404	1535	1262	2286
7-13	217	846	1685	2105
8-14	274	606	1241	2226
9-15	899	2555	1619	2347
10-16	993	1891	1335	2303
11-17	1099	2846	1321	1617
12-18	790	2766	1014	1392
13-19	1053	2888	1443	1725
14-20	1176	3126	1796	3385
15-21	295	1381	1663	3215



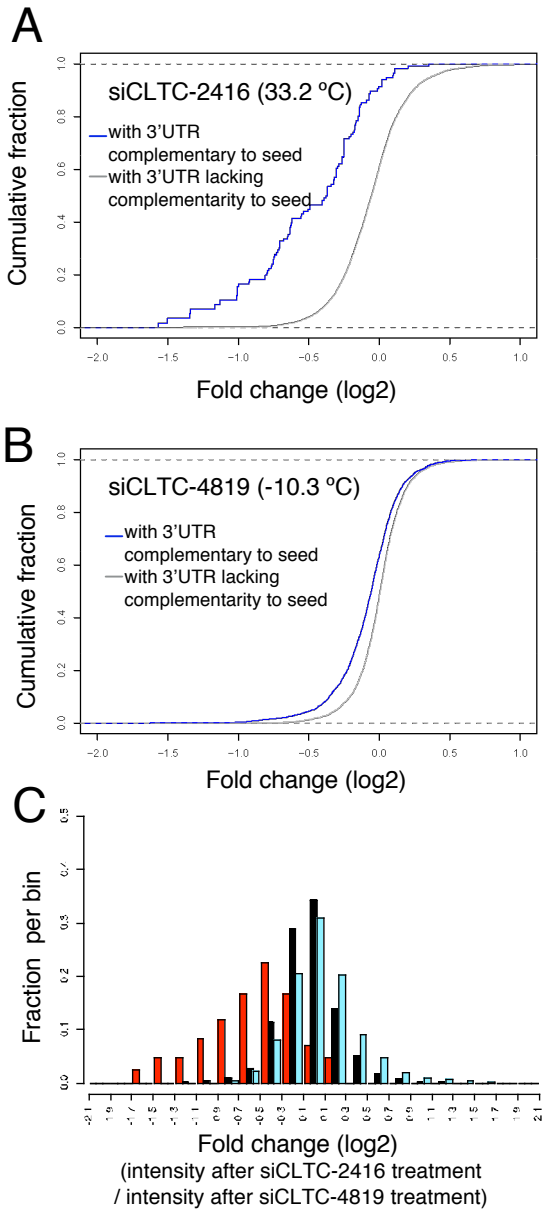
Supplementary Figure S1. Microarray transcript profiles showing downregulation by siVIM-270 (**C, D**) and siCLTC-2416 (**E, F**) 24 h after transfection. (**A**) Transcripts possessing the complementarity to a 7-bp-long GS sequence of a given siRNA were divided into15 groups based on the position of the complementary sequence in the siRNA GS. Transcripts labelled with 1-7 possess the complementarity to nucleotides 1-7 of siRNA GS and vice versa. Horizontal arrow, transcript group with seed complementarity. (**B**) The number of transcripts belonging to each group, which is further subdivided into 3' UTR and CDS based on the location of the complementary sequence on mRNA. Transcripts possessing the GS complementarity in 5' UTR were neglected. (**C, D, E, F**) Change in gene expression is shown by log2 of fold change ratio to mock transfection. Arrow, cumulative distribution of transcript group labelled with 2-8. Cumulative distribution of 15 groups are colored as shown in (**A**). Note that the groups of transcripts labelled with 2-8/3' UTR are the most sensitive to the off-target effects due to siVIM-270 or siCLTC-2416, suggesting GS nucleotides 2-8 to serve as a seed. Results of Wilcoxon's rank-sum test for seed-dependent off-target effect is as follows: transcripts with 3' UTR complementary to the siVIM-270 seed, $p \leq 10^{-154}$; transcripts with CDS complementary to the siVIM-270 seed, $p \leq 10^{-60}$; transcripts with 3' UTR complementary to the siCLTC-2416 seed, $p \leq 10^{-32}$; transcripts with CDS complementary to the siCLTC-2416 seed, $p \leq 10^{-19}$. Differences in mean value between normalized log2 fold change of mRNA with seed match and that of all mRNA are: 0.20 ± 0.01 (**C**), 0.08 ± 0.01 (**D**), 0.44 ± 0.04 (**E**), and 0.12 ± 0.02 (**F**).



Supplementary Figure S2. Effects on gene silencing of seed/non-seed swapping. Using siVIM-270 and siVIM-812, swap siRNA constructs were generated between the seed with the 5' end (nucleotide position 1-8) and non-seed region (9-21). Similarly, corresponding swap target oligonucleotides were constructed and inserted into psiCHECK and HeLa cells were transfected with resultant plasmids. Gene silencing effects of all 16 combinations were examined using a dual luciferase assay. (**A**) Left, structure of psiCHECK. Right, parental target and GS sequences used. **(B, G, L, Q)** , Control experiments (gene silencing due to completely matched GS). All parental and chimeric siRNAs, belonging to class I, were highly functional. **(E, H, K, N)**, Negative control (gene silencing due to GS with no appreciable complementarity to target). Virtually no gene silencing was observed. **(C, F, M, P)**, Seed-dependent gene silencing. siVIM-270 is an siRNA causing seed-dependent gene silencing effectively **(F)** while siVIM-812 is almost incapable of inducing seed-dependent gene silencing **(M)**. A chimeric siRNA possessing the siVIM-270 seed **(C)** still maintained a strong seed-dependent gene silencing activity, whereas little gene silencing activity was found subsequent to transfection of a chimeric siRNA possessing the siVIM-812 seed **(P)**. **(D, I, J, O)**, Any appreciable gene silencing activity appeared absent from cells transfected with siRNAs with only non-seed region complementarity to the target, indicating that the non-seed region cannot function as a substitute for the seed.

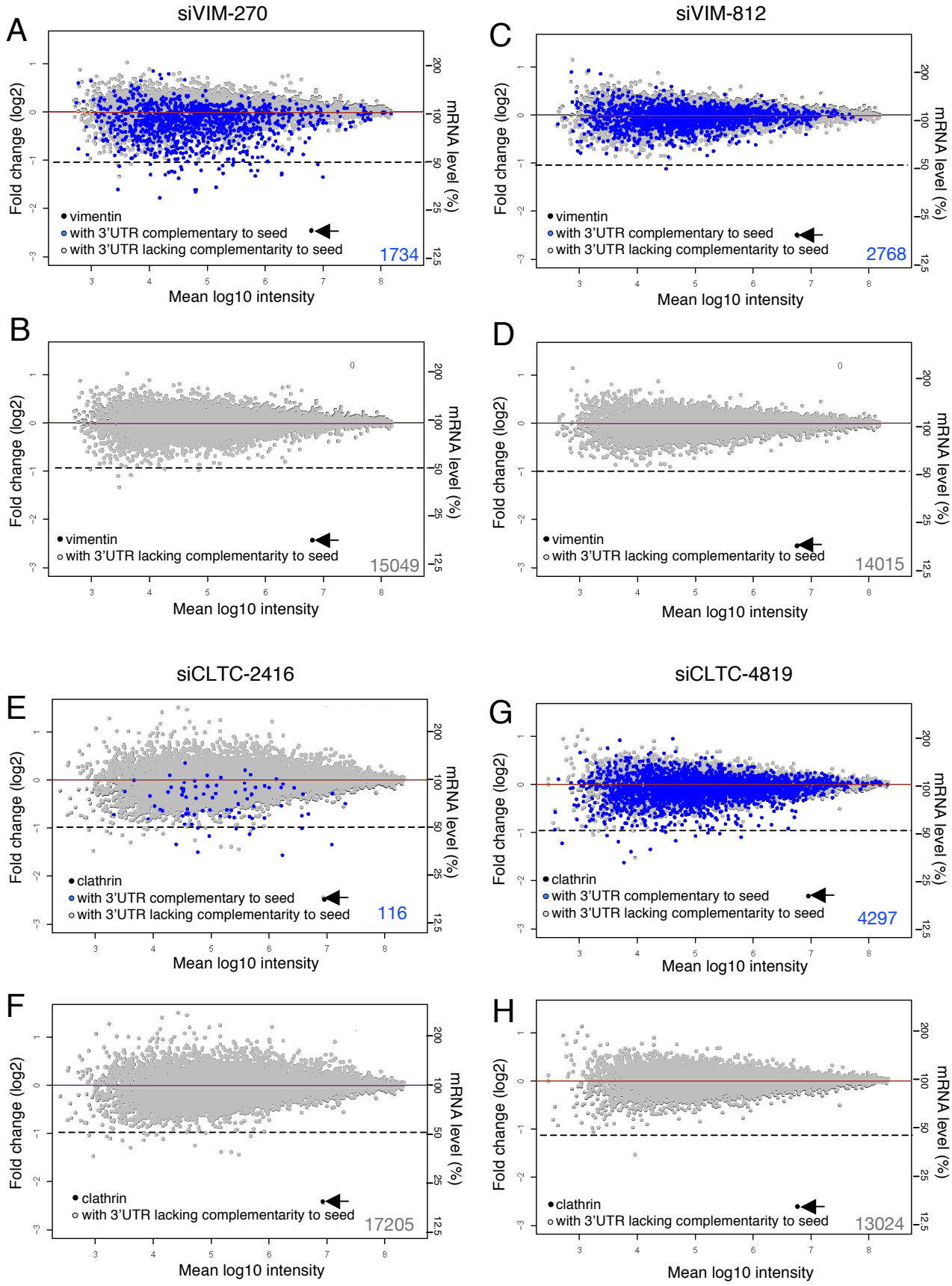


Supplementary Figure S3. Possible involvement of siRNA non-seed region and/or its target counterpart in off-target effect. Complementarity of used 8 target sequences to siVIM-270 GS (**A**) and siVIM-812/270 GS (**B**) are shown in the right margin. Note that the 8 targets possess non-seed sequences unhomologous from one another while siVIM-270 GS and siVIM-812/270 GS share in common an identical seed sequence. There is no appreciable homology in non-seed between siVIM-270 and siVIM-812/270. That off-target effects considerably vary depending on sequences of either GS non-seed or its target counterpart may indicate their possible involvement in modulation of seed-dependent gene silencing.



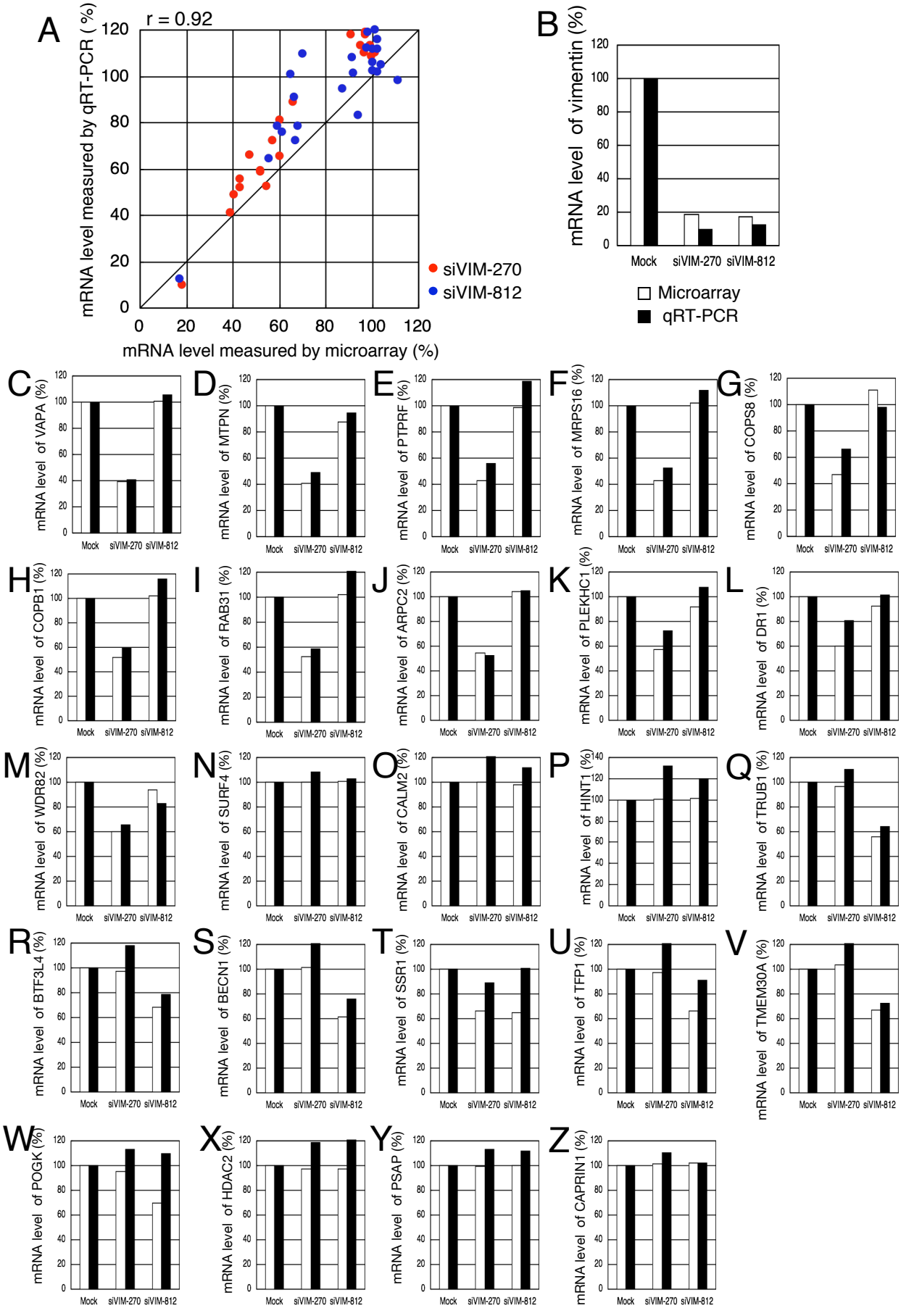
Supplementary Figure S4. Microarray-based off-target-effect profiles of transcripts downregulated by siCLTC-2416 and siCLTC-4819, which, respectively, generate seed duplexes with high (33.2 ºC) and low (-10.3 ºC) Tm values upon association of cognate target mRNAs. Changes in expression, shown as the log2 of the ratio of the fold change to the mock transfection, were determined using microarray data for 17,321 human transcripts obtained from HeLa cells transfected with cognate siRNAs. The blue line indicates cumulative distribution of transcripts with 3' UTR complementary to the seed of cognate siRNA. The gray line shows transcripts with no seed complementarity. Differences in mean value between normalized log2 fold change of mRNA with 3' UTR complementary to the seed and mRNA lacking seed match are 0.45 ± 0.04 (**A**) and 0.08 ± 0.005 (**B**). **(C)** Comparison of microarray profiles of transcripts downregulated by siCLTC-2416 and ciCLTC-4819. All transcripts with 3' UTR complementarity to siRNA seed sequences were examined. Ordinate, fraction. Abscissa, signal intensity obtained after siCLTC-2416 treatment divided by that after siCLTC-4819 treatment. The red line indicates distribution of transcripts complementary to the siCLTC-2416 seed sequence. The blue line denotes the distribution of transcripts complementary to the siCLTC-4819 seed sequence. The black line shows transcripts with no seed complementarity.

Supplementary Figure S5.



Supplementary Figure S5. Microarray profiles of transcripts downregulated by transfection of siRNAs. Four different siRNAs were used. (**A**, **B**) siVIM-270 and (**E, F**) siCLTC-2416 are capable forming seed duplexes with high Tm values (26.2 and 33.2 ºC, respectively) with the 3' UTR counterparts of target mRNAs. siVIM-812 (**C, D**) and siCLTC-4819 (**G, H**) are capable forming seed duplexes with low Tm values (8.8 and -10.3 ºC, respectively) with the 3' UTR counterparts of target mRNAs. Microarray-based expression profiles were examined 24 h after transfection. Change in gene expression is shown by log2 of fold change ratio (left ordinate) and mRNA level (right ordinate) relative to mock transfection. Abscissa, signal intensity of the transcript (log10). Blue and gray dots, respectively, represent transcripts complementary to the seed and those with no seed complementarity. Transcripts with no seed complementarity were plotted separately in **B, D, F** and **H**. Signals for cm target genes, vimentin (**A**-**D**) and clathrin heavy chain (**E**-**H**), are colored black and indicated by arrows. Their expression levels were reduced to 18 % (siVIM-270; **A, B**), 17 % (siVIM-812; **C, D**), 17 % (siCLTC-2416; **E, F**) and 17 % (siCLTC-4819; **G, H**). The number of genes examined is shown on the bottom-right edge in each panel. The expression of transcripts with 3' UTR complementary to siVIM-270 (**A**) or siCLTC-2416 (**E**) seeds was significantly reduced subsequent to the transfection of the cognate siRNA. In contrast, the reduction of transcripts with 3' UTR complementary to siVIM-812 (**C**) or siCLTC-4819 (**G**) seed sequences was little, if any. These findings may support the notion that seed-dependent gene silencing activity of siRNA is determined by the stability of the seed duplex formed between siRNA GS and 3' UTR of target mRNA.

Supplementary Figure S6.



Supplementary Figure S6. Comparison of microarray data with those of qRT-PCR. (**A**) Twenty four genes whose transcripts possess complementarity to either siVIM-270 and siVIM-812 were arbitrarily chosen and change in expression level of their mRNA along with that of vimentin RNA in siRNA-treated cells, compared with mock transfection, were examined by qRT-PCR and microarray (**B-Z**; see also **Supplementary Figure S5**). Red dots, data obtained subsequent to siVIM-270 treatment. Blue dots, data obtained subsequent to siVIM-812 treatment. Note that microarray data are almost linear with those of qRT-PCR, indicating that results of microarray analysis and those of qRT-PCR are essentially identical to each other. The correlation coefficient was estimated at 0.92. Comparison at the level of individual gene is shown in **(B-Z)**; (**B**) vimentin, (**C**) VAPA, (**D**) MTPN, (**E**) PTPRF, (**F**) MRPS16, (**G**) COPS8, (**H**) COPB1, (**I**) RAB31, (**J**) ARPC2, (**K**) PLEKHC1, (**L**) DR1, (**M**) WDR82, (**N**) SURF4, (**O**) CALM2, (**P**) HINT1, (**Q**) TRUB1, (**R**) BTF3L4, (**S**) BECN1, (**T**) SSR1, (**U**) TFP1, (**V**) TMEM30A, (**W**) POGK, (**X**) HDAC2, (**Y**) PSAP and (**Z**) CAPRIN1. Target transcripts in (**C**-**O**) contain 3' UTRs possessing complementarity to the siVIM-270 seed, while those in (**P**-**Z**) contain 3' UTRs possessing complementarity to the siVIM-812 seed.