Supplementary information

Table S1. Primers for RT-PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>APC</td>
<td>GCAACAAGAGTGCGTTTCCC</td>
<td>CACCTTGAGAAACATATTGG</td>
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<tr>
<td>Axin</td>
<td>CAGTACCACAGAGGATGCAGAGA AGAAC</td>
<td>AAGAGCTCGTGACAATCTTTGTGT TGTTTC</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>TCGAGCCAAGCAGACACTCC</td>
<td>CGGCTACACAGTGCCATTGC</td>
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</tbody>
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Fig. S1. Action of dicer on the trimer siRNA nanostructure and monomer siRNA

RNAs were analyzed for the dicer cleavage reaction at 37°C for 5 h. The dicer reaction was stopped, and samples were collected at various time intervals (0, 0.5, 1, 2 and 5 h). Each mixture was analyzed by 15% native PAGE and SYBER green I staining.
The theoretical model of the 3-in-1 concept

The most simple assumption is that only one siRNA completely knocks down its corresponding target, and two or more of the same kind of siRNA has no additional effect. Under this assumption, if one trimer exists in a cell, the signal is silenced. The possibility of one or more trimers in a cell, $p_{tr}$, can be expressed based on the Poisson process as follows:

$$p_{tr} = 1 - \exp[-\lambda_{tr}] \quad (1)$$

where $\lambda_{tr}$ is the average number of trimers in a cell, which is (most simply) linearly proportional to the trimer concentration. This is the probability of having non-zero trimer in a cell, because the probability of no trimer in a cell is $\exp[-\lambda_{tr}]$. In the case of the mixture, the possibility of one or more of each siRNA, $p_{i}$, is:

$$p_{i} = 1 - \exp[-\lambda_{i}] \quad (2)$$

where $i = 1, 2, 3$ represents different kinds of (monomeric) siRNA targeting Axin, APC or GSK-3, respectively, and $\lambda_{i}$ represents the average number of monomeric siRNAs, $i$. Thus, the possibility of having all three different siRNAs in a cell, $p_{mix}$, is:

$$p_{mix} = p_{1} p_{2} p_{3} \quad (3)$$

Because the concentration of each siRNA is the same as each other, and the same as that of the trimer, $\lambda = \lambda_{1} = \lambda_{2} = \lambda_{3} = \lambda_{tr}$ and $p_{1} = p_{2} = p_{3} = p_{tr}$ results in

$$p_{mix} = p_{i}^{3} \quad (4)$$

Figure 7(a) shows (S1) and (S4) in the range of $0 < \lambda < 3$. Apparently, this model indicates the saturating behavior. In Fig. 7(b), very low, low, intermediate, and high concentrations correspond to $\lambda = 0.15$, $0.75$, $1.50$, and $3.00$, respectively. In the Monte Carlo simulation, a random number was generated for each dot (cell) to determine whether the cell contains trimer(s) or not (for trimer simulation), or each siRNA(s) or not (for mixture simulation). The results were coded to the color. Because this figure is aimed to present intuitively understandable schematics, only 900 cells were simulated for each case.