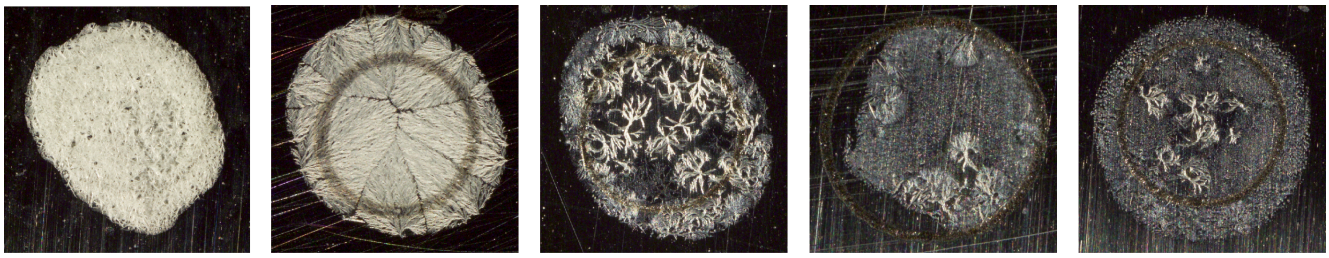
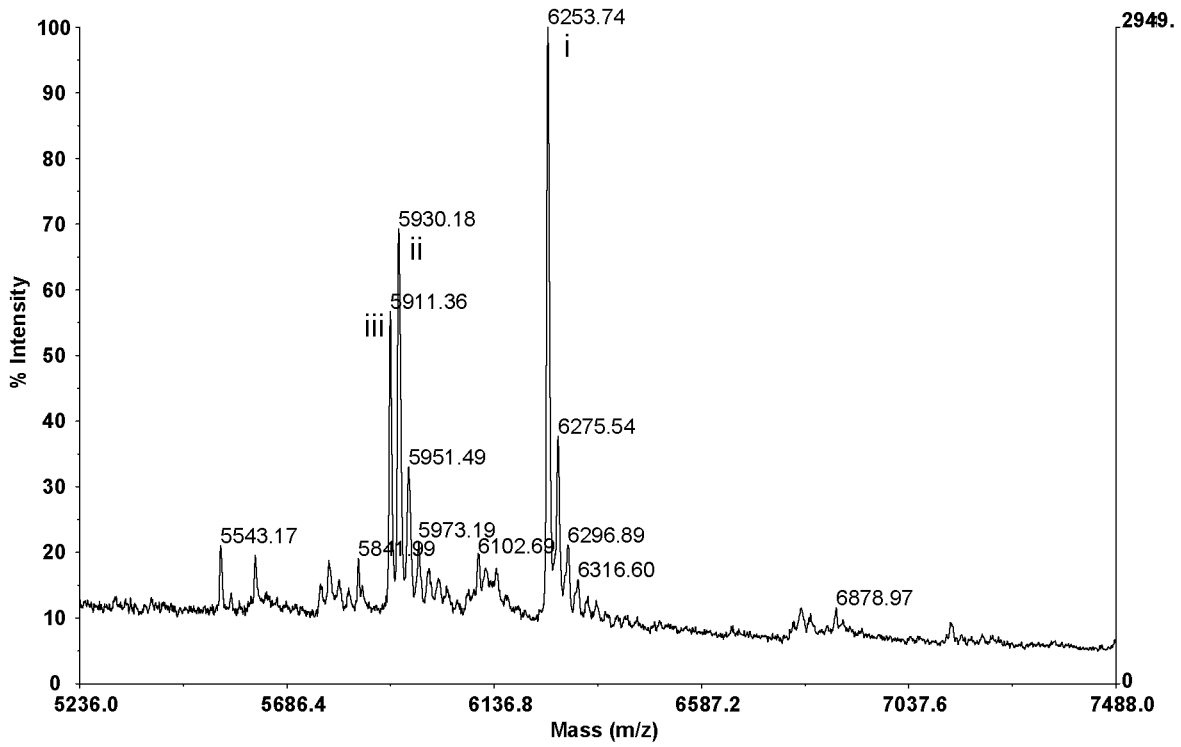
# Supplementary Information

## MALDI sample preparation and data acquisition

Crucial to the successful and reproducible use of this technique is the preparation of the MALDI samples. Samples that are not prepared correctly will either require increased laser power for data acquisition or data cannot be acquired. It is important to obtain a thin layer of crystallised matrix when using matrices **b** and **c**. For preparation of samples, see experimental section. It is important to note that applying more than 0.1 $\mu$l of serum stability reaction mixture (with siRNA at 50 $\mu$M) is not necessary and can be detrimental to data acquisition. Sample preparations using matrix **c** enabled mouse and human serum concentrations of 30 % to be tolerated (data not shown). Below, are examples of sample preparation that led to either good or bad spectra. Typically, using the edges of the well gave the best results.

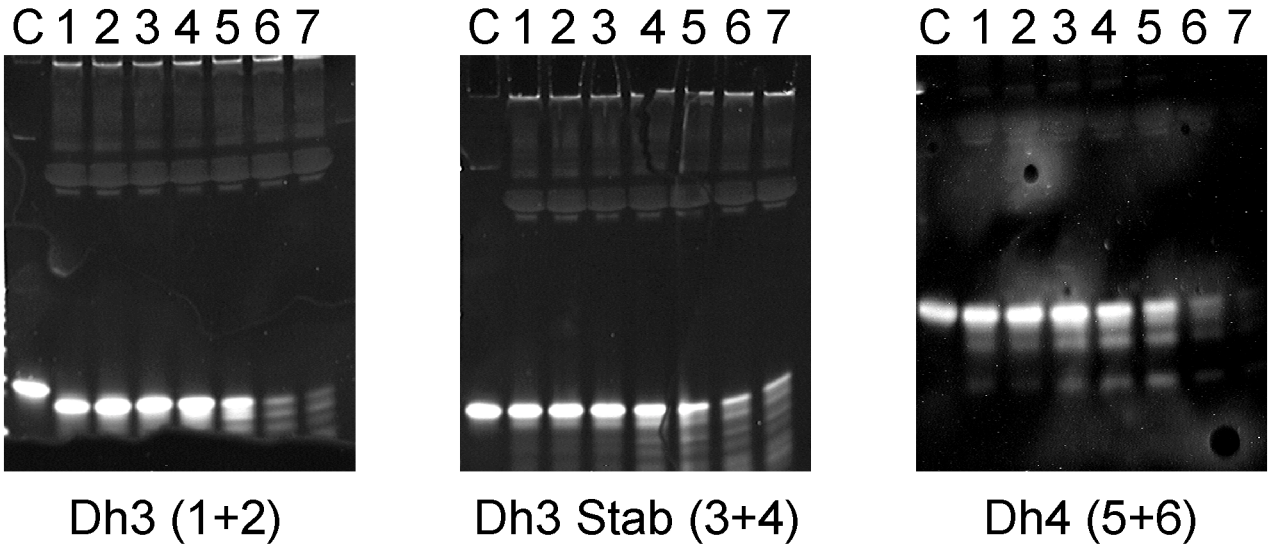



# Supplementary Figures:



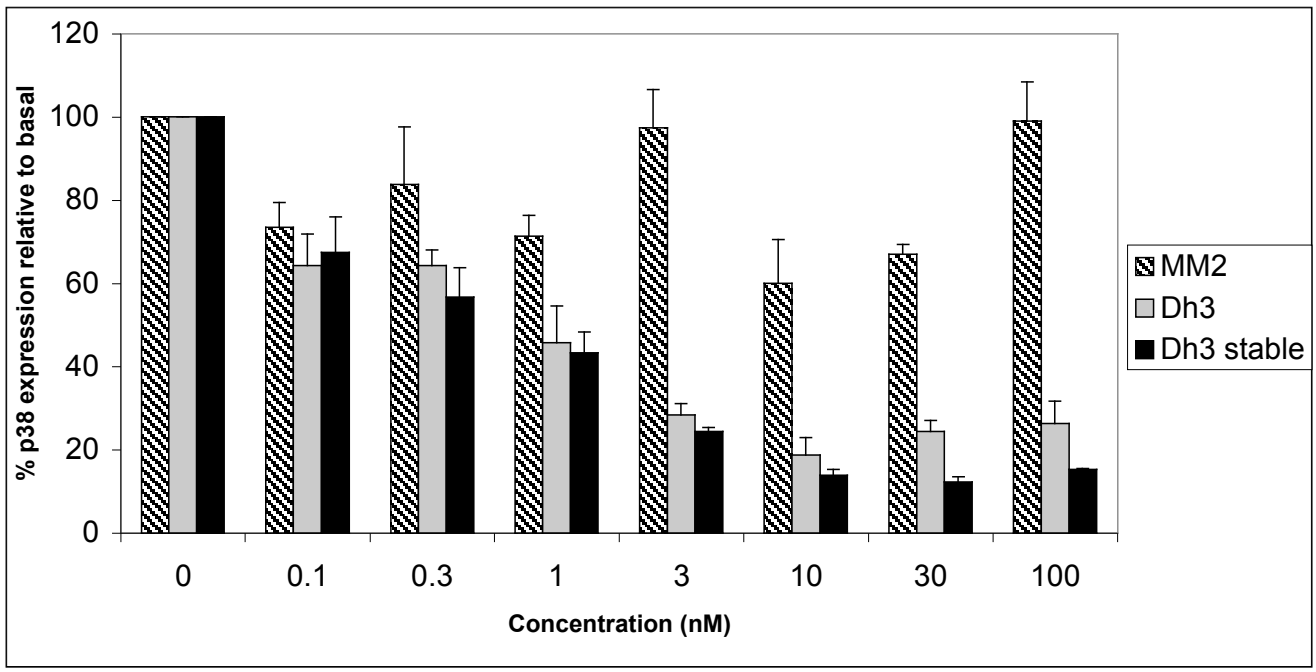

i) Antisense clipped
ii) Sense clipped phosphate
iii) Sense clipped 2',3' cyclic phosphate

**Supplementary Figure 1.** MALDI-TOF spectrum of siRNA Dh3 (RNA 1+2, 50 $\mu$M) incubated with 10% mouse serum for 26 h. Initial 2',3' cyclic phosphate clipped species (iii) is hydrolysed to phosphate species (ii) over extended periods of time.

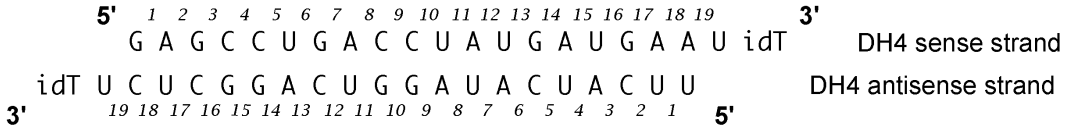


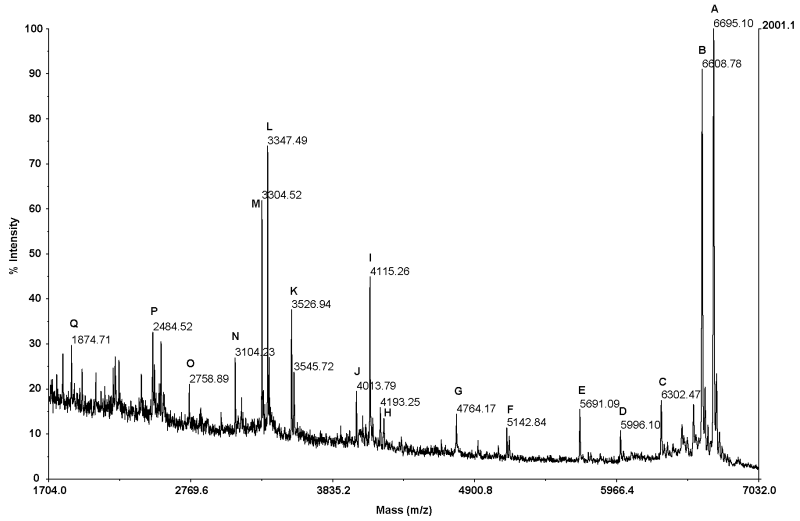

C = control
1-7: 5 min, 15 min, 1 h, 3 h, 6 h, 12 h, 15 h

**Supplementary Figure 2.** Stability of Dh3 (RNA 1+2), Dh3 Stab (RNA 3+4) and Dh4 (5+6) in 90 % mouse serum at 37 °C. siRNA was incubated with 90 % mouse serum at 37 °C at various time intervals and then separated on a 20% PAGE gel with siRNA products detected using a Sybr Gold nucleic acid stain. Lane description see figure. Full length Dh3 Stab is still visible after 15 h serum treatment compared to < 5min for Dh3, and 12 h for Dh4.



**Supplementary Figure 3.** P38 expression knockdown of siRNA in mouse L929 cells. Dh3 (RNA 1+2), Dh3 stabilised (RNA 3+4) and MM2 (mismatch sequence, see experimental) were transfected using Lipofectamine 2000. P38 expression was measured by taqman real-time PCR and normalized to 18S. Biological activity of Dh3 stabilised has not been detrimentally affected by the 2'OMe modifications on both strands.




| Peak | Description | Observed (Da) | Calculated (Da) |
|---|---|---|---|
| A | Sense full length | 6695.1 | 6695.0 |
| B | AS full length | 6608.8 | 6608.9 |
| C | 3' AS Frag: $U_1$ | 6302.5 | 6302.7 |
| D | 3' AS Frag: $U_2$ | 5996.1 | 5996.6 |
| E | 3' AS Frag: $C_3$ | 5691.1 | 5691.4 |
| F | 5' Sense Frag: $U_{16}$ cP | 5142.8 | 5143.0 |
| G | 3' Sense Frag: $U_6$ | 4764.2 | 4758.9 |
| H | Mouse serum | 4193.3 | - |
| I | 3' AS Frag: $U_8$ | 4115.3 | 4115.5 |
| J | Mouse serum | 4013.8 | - |
| K | 5' Sense Frag: $U_{11}$cP | 3526.9 | 3527.1 |
| L | Sense full length $^{2+}$ | 3347.5 | 3347.5 |
| M | AS full length $^{2+}$ | 3304.5 | 3304.5 |
| N | 5' AS Frag: $G_{10}$ minus P | 3104.2 | 3105.9 |
| O | 5' AS Frag: $A_9$ minus P | 2758.9 | 2760.7 |
| P | 3' AS Frag: $C_{13}$ | 2484.5 | 2484.5 |
| Q | 5' AS Frag: $C_6$ P | 1874.7 | 1876.1 |

**Supplementary Figure 4.** MALDI-TOF spectrum of Dh4 after 26 h incubation with 10% mouse serum at room temperature. Several cleavage sites can be determined. Most occur at 3' pyrimidine positions.