SUPPLEMENTARY MATERIAL

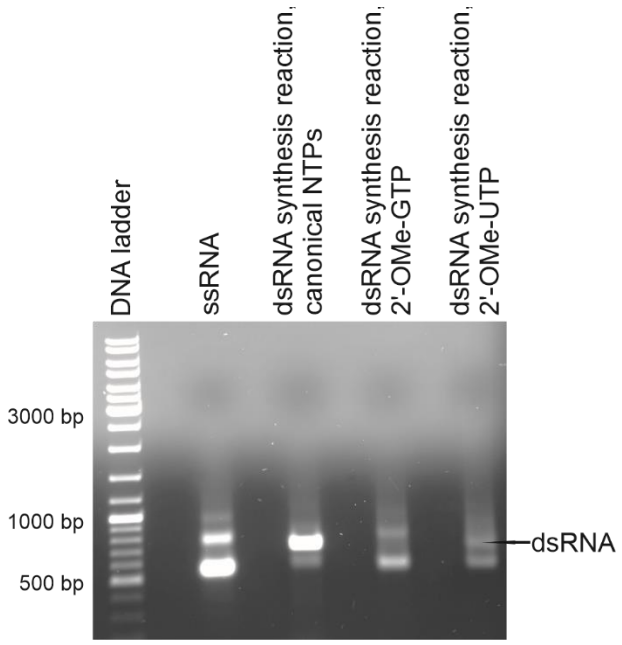

Fig. S1. DsRNA synthesis reactions using 2'-OMe-NTPs. Three identical dsRNA synthesis reactions were carried out in parallel using 1) canonical unmodified NTPs, 2) 2'-OMe-GTP instead of canonical GTP, and 3) 2'-OMe-UTP instead of canonical UTP. After 6 h incubation of ssRNA with a mixture of NTPs and phi6 RdRp at 30°C, equal amounts from the reactions were loaded on agarose gel. An input ssRNA was loaded as a control (second lane). GeneRuler DNA ladder mix (Thermo Scientific) was used as a standard (first lane).

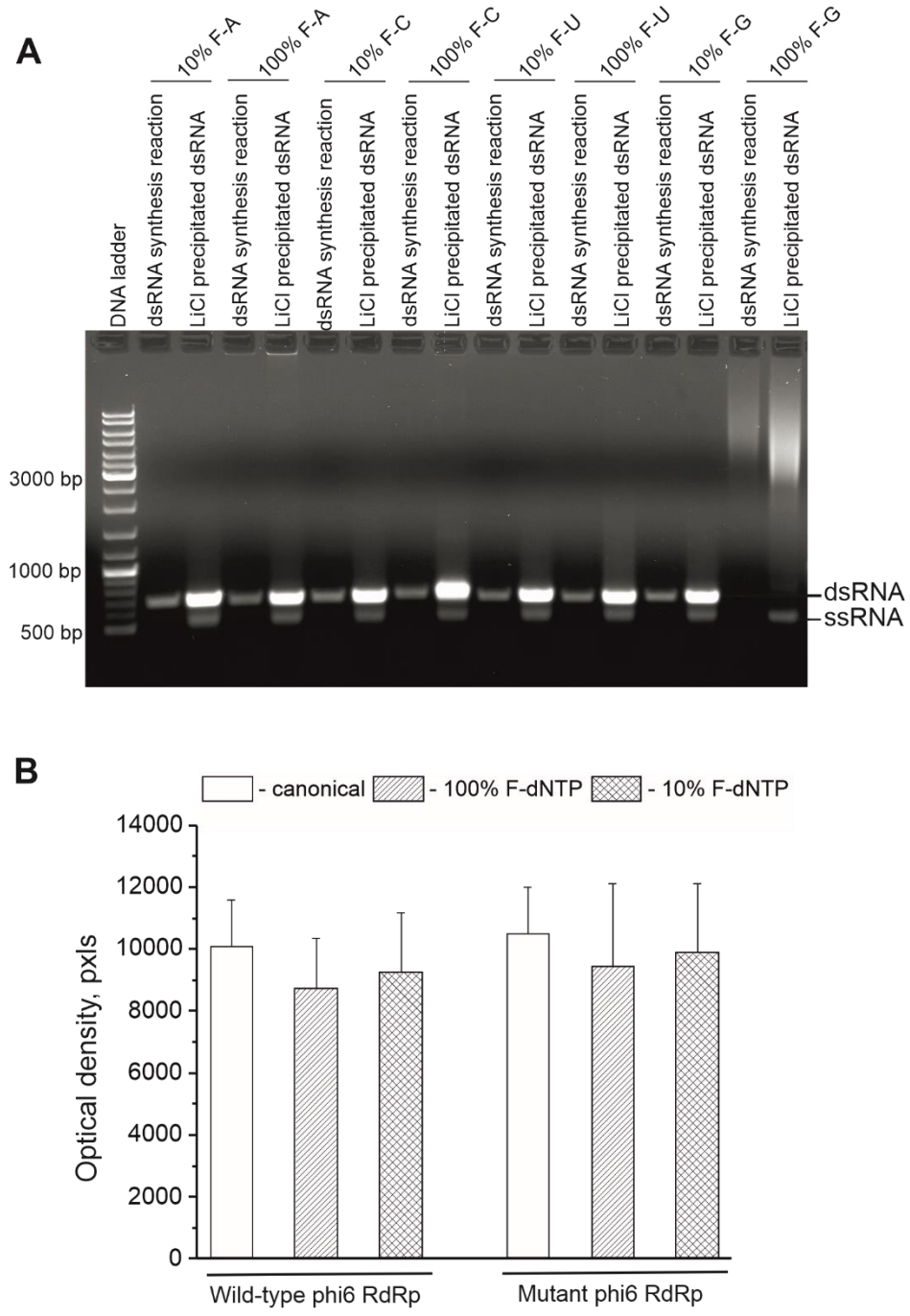

Fig. S2. DsRNA synthesis reactions using 2'-F-dNTPs. Equivalent dsRNA synthesis reactions were set up. In reactions marked with 100%, a canonical NTP was replaced with the corresponding 2'-F-dNTP (F-A/C/U/G). Alternatively, only a 10% fraction of the canonical NTP was replaced. After 6 h incubation at 30°C, dsRNAs were precipitated with 4 M LiCl and samples were analyzed

by agarose gel electrophoresis. (**A**) Agarose gel electrophoresis of representative dsRNA synthesis reactions in the presence of the indicated 2'-F-dNTPs before and after LiCl precipitation. GeneRuler DNA ladder mix (Thermo Scientific) was used as a standard (first lane). (**B**) Comparison of wild-type and mutant phi6 RdRp. Three independent reactions including 2'-F-dATP, 2'-F-dCTP, or 2'-F-dUTP were set up using either wild-type or mutant (K147A) phi6 RNA polymerase. The resulting gel pictures were analyzed with Fiji software. The data on optical density are presented as mean ± S.D. The groups were compared between each other using Mann-Whitney's nonparametric test and no statistically significant difference was discovered.

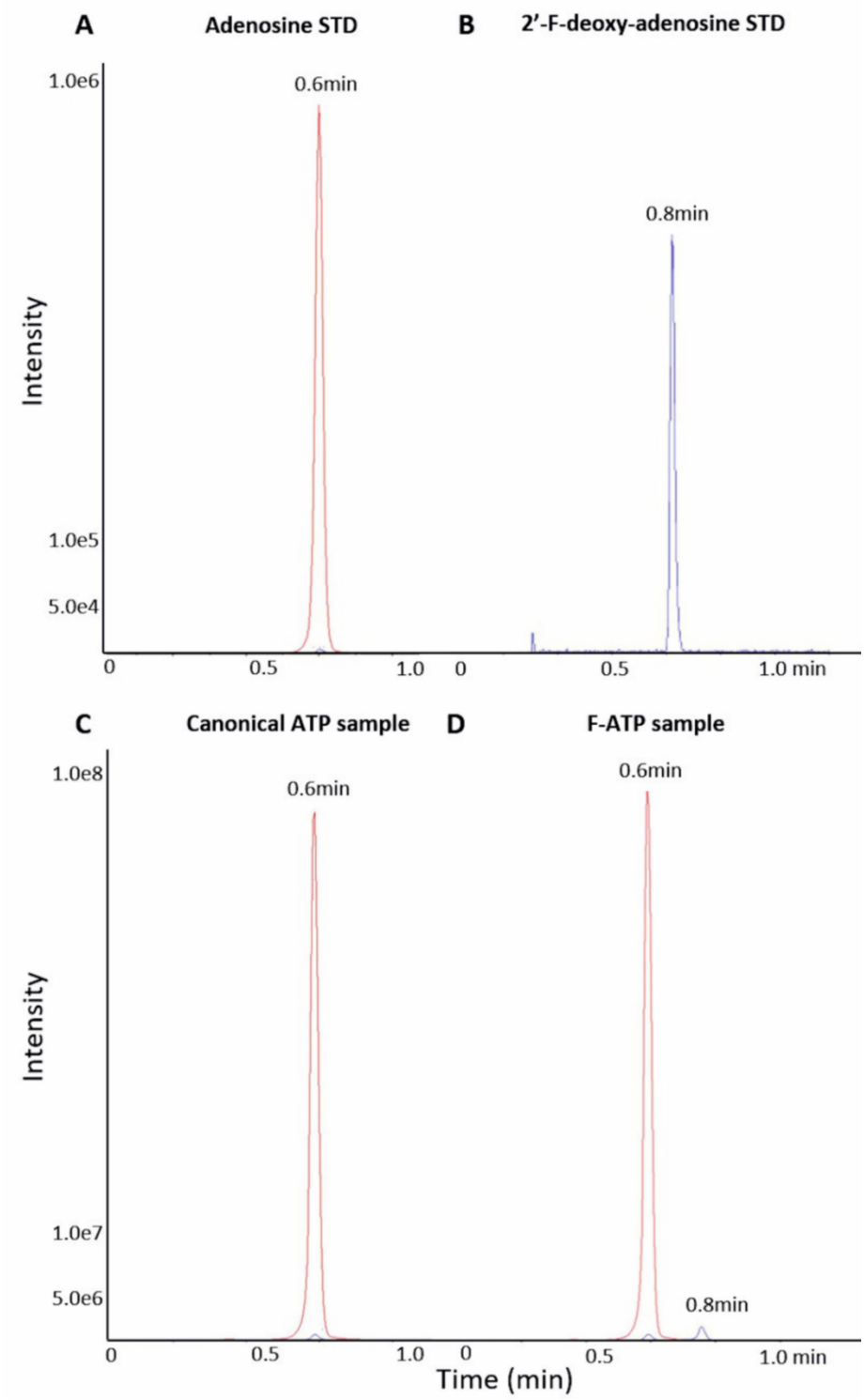

Fig. S3. Mass spectrometry analysis of siRNA swarms. Samples of siRNA were incubated at 100°C for 10 min and transferred on ice. The RNA was digested with nuclease P1 (Sigma-Aldrich, # N8620) and dephosphorylated into nucleosides with Shrimp Alkaline Phosphatase (New

England Biolabs, #M0371). The produced nucleosides were applied to Hypersep Hypercarb SPE extraction tips (Thermo Fisher Scientific, #60109-404), washed with 0.1% formic acid (Riedel-de Haën, # 33015), eluted with 0.1% formic acid in 80% acetonitrile (Sigma-Aldrich, #34851), lyophilized and resuspended in 5 mM ammonium formate (Sigma-Aldrich, # 516961) pH 5.3. The subsequent mass spectrometral analysis of the adenosine and 2-deoxy-fluoro-adenosine were executed with ExionLC UPLC- 6500+ QTRAP/MS (ABSciex). Chromatographic separation was performed in Waters Acquity UPLC BEH C18 column (2.1 mm × 50 mm) at 40°C with 0.1% formic acid in MQ water (A) and in acetonitrile (B), with a flow of 0.6 ml/min. The linear gradient started at 2% of B and increased to 30% B in 5 min. The samples were analyzed in positive ionization mode with Multiple Reaction Monitoring (MRM): 268 →136 for adenosine and 270 →136 (2-deoxy-fluoro-adenosine). The data acquisition was performed with the Analyst Software (AB Sciex). (**A**) Adenosine standard (Sigma-Aldrich); (**B**) 2'-fluoro-deoxy-adenosine standard (Carbosynth); (**C**) unmodified siRNA swarm; (**D**) siRNA swarm produced in the reaction, in which 10% of the required rATP was replaced with 2'-F-dATP.

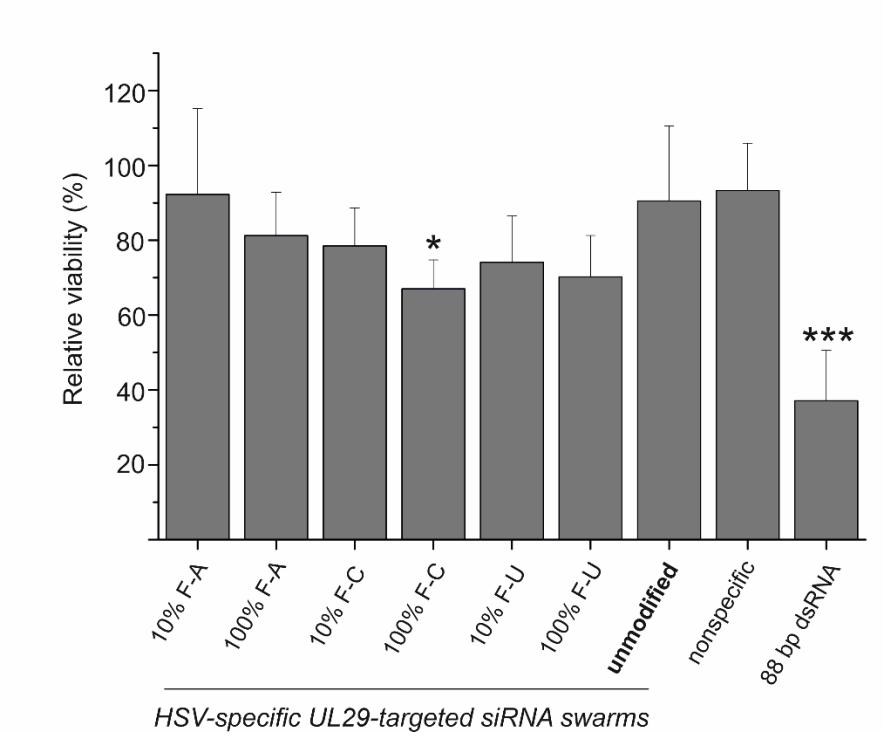

Fig. S4. The effect of 2'-F-modifications in siRNA swarms on cellular viability of human corneal epithelial cells. Corneal epithelial cells [HCE, kindly provided by prof. Arto Urtti (University of Helsinki and University of Eastern Finland, Finland)] were transfected with 50 nM of unmodified or modified siRNA swarms. The antisense strand of the modified siRNA swarms was fully (referred to as 100%) or partially (referred to as 10%) modified containing either 2'-F-dAMP, 2'-F-dCMP or 2'-F-dUMP as the modified nucleotide (10% or 100% F-A, F-C or F-U, respectively). 88-bp-long phi6-derived dsRNA with known cytotoxicity was used as a control RNA, at a concentration of 10 nM. The viability of the cells was quantified at 48 hpt and shown as relative viability compared to mock- and untreated samples. The error bars represent the standard deviation of the mean from two separate experiments with 3 biological replicates each. Significant difference to the unmodified UL29-specific swarm is marked with asterisks (* : $p \leq 0.05$, *** : $p \leq 0.001$, not shown : non-significant).

The HCE cells were maintained in DMEM with Hepes (Gibco), supplemented with 7% FBS.