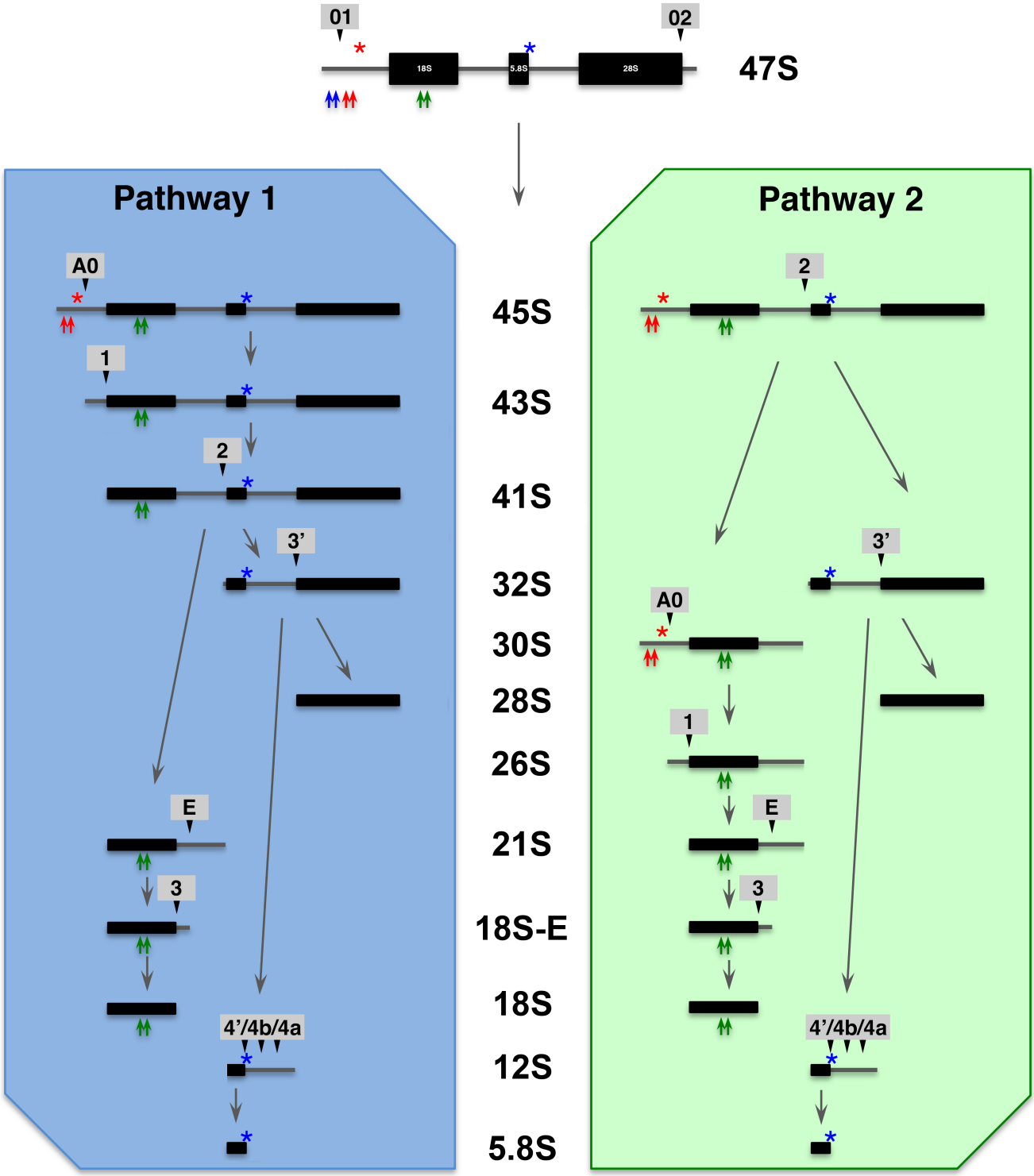

Fig. S1. Scheme of the two pre-rRNA processing pathways in humans. The processing of the primary pre-rRNA transcript (47S) into mature rRNAs involves numerous endonucleotic cleavages occurring at specific sites indicated in grey boxes. Initial cleavages (01 and 02) lead to 45S pre-rRNA (45S) whose maturation may follow either of two alternative pathways. The pathways 1 and 2 do not differ in the sites of cleavage but in the order in which cleavages take place. They generate common (32S, 21S, 18S-E and 12S) and specific (43S, 41S, 30S and 26S) intermediate pre-rRNAs in addition to mature rRNAs (28S, 18S and 5.8S). The primers used for RTqPCR experiments are indicated as double arrows in blue (primers ETS1 and ETS2), red (primers ETS3 and ETS4) and green (primers 18S1 and 18S2), and the probes used for northern blots are indicated as red (ETS probe) and blue (5.8S+ probe) asterisks.

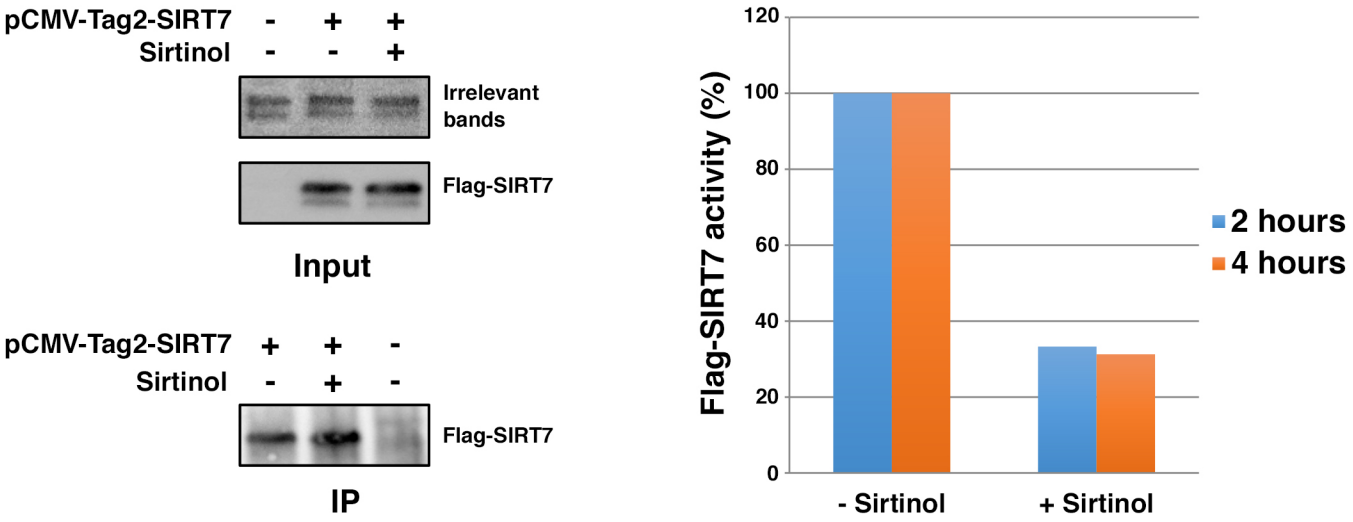

Fig. S2. Sirtuin 7 deacetylase activity is inhibited in cells treated with sirtinol. Flag-SIRT7 immunoprecipitation was performed on extracts prepared from HEK293T cells, or HEK293T cells expressing Flag-SIRT7 treated or not treated with sirtinol. A quarter of the immunoprecipitate was used to quantitatively analyze the Flag-SIRT7 immunoprecipitation. The immunoblotting was performed using anti-SIRT7 antibody for immunoprecipitation (IP) as well as for cell extracts (Input). The remaining immunoprecipitate was used for in vitro assaying Flag-SIRT7 deacetylase activity at 2 and 4 h. Flag-SIRT7 activity is normalized according to the level of immunoprecipitated Flag-SIRT7 and results obtained for untreated cells (- Sirtinol), arbitrarily normalized to 100, compared to those obtained for treated cells (+ Sirtinol). Cropped immunoblots are shown.

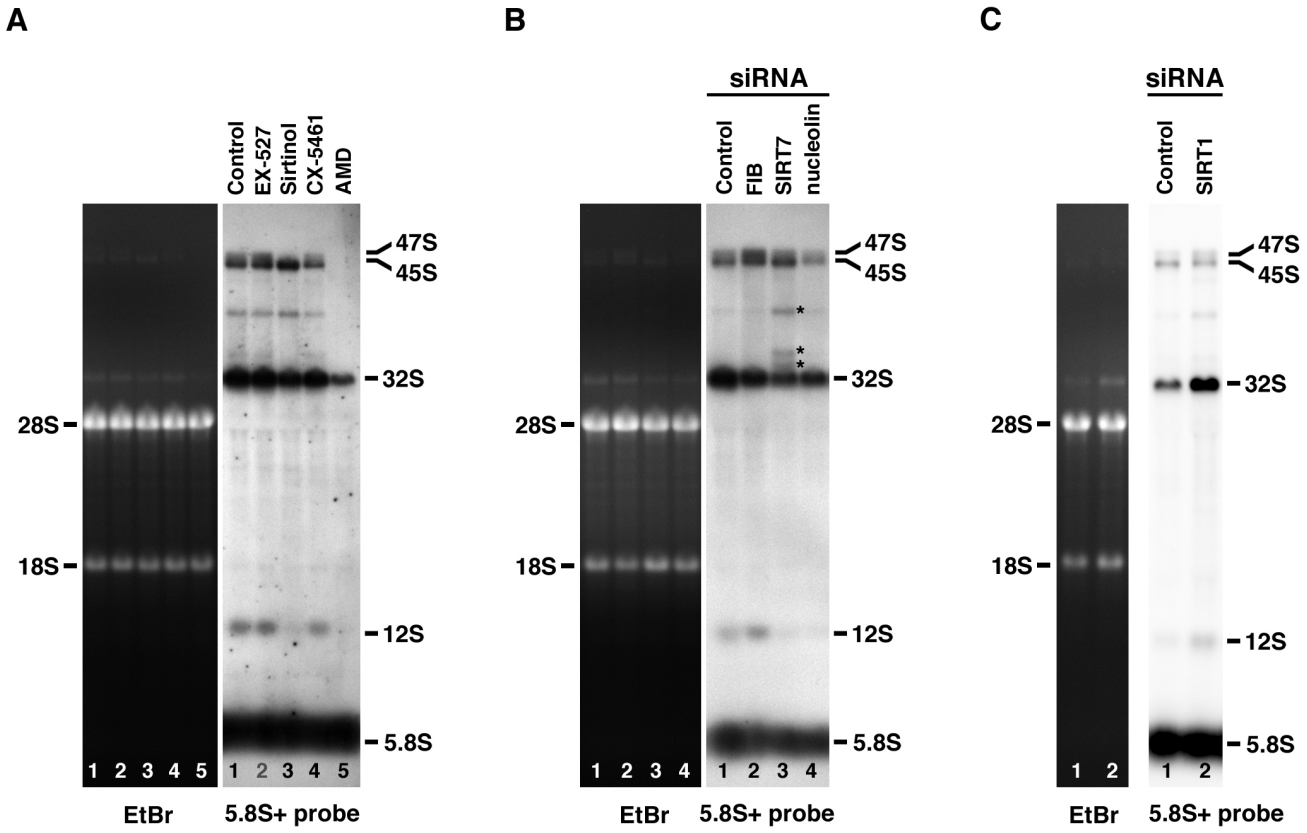

Fig. S3. The extended forms of 32S pre-rRNAs are not observed in sirtinol-treated HeLa cells but are observed in HeLa cells depleted for Sirtuin 7. (A) Total RNAs were prepared from HeLa cells treated with EX-527, sirtinol, CX-5461 or AMD. (B) Total RNAs were prepared from HeLa cells transfected with Control siRNAs, or with siRNAs targeting either fibrillarin (FIB), Sirtuin 7 (SIRT7) or nucleolin. (C) Total RNAs were prepared from HeLa cells transfected with Control siRNAs or with siRNAs targeting Sirtuin 1 (SIRT1). Northern blot analyses were performed using the 5.8S+ probe. Asterisks in B point the extended forms of 32S pre-rRNAs.

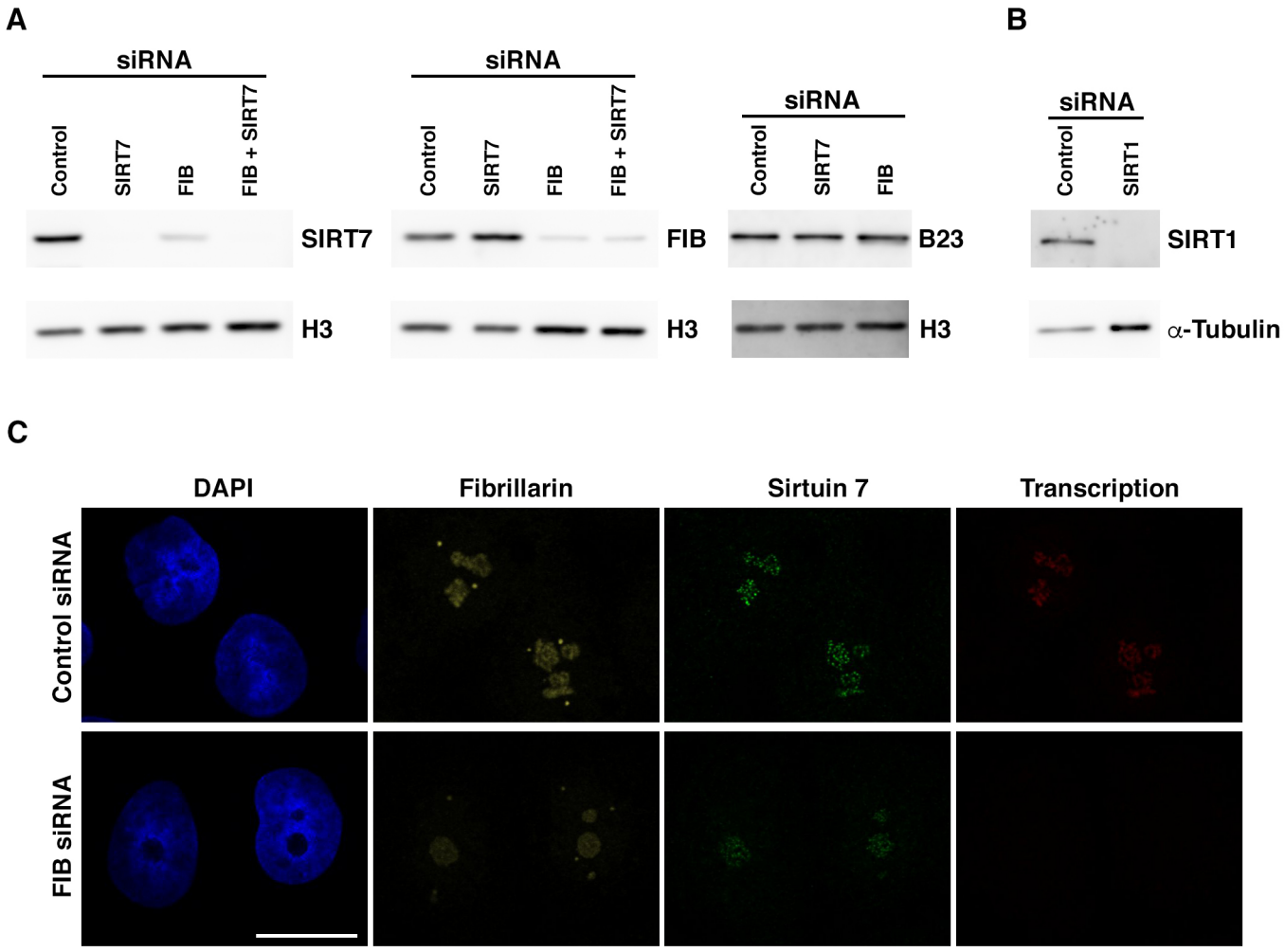

Fig. S4. siRNA-mediated fibrillarin depletion induces also Sirtuin 7 depletion and inhibition of rDNA transcription. (A) Protein extracts were prepared from HeLa cells transfected with Control siRNA, SIRT7 siRNA or FIB siRNA, or with siRNAs targeting both fibrillarin and Sirtuin 7. Immunoblotting was performed using anti-SIRT7, anti-FIB and anti-B23 antibodies, and using anti-histone H3 antibody as loading control. (B) Protein extracts were prepared from HeLa cells transfected with Control siRNA, and SIRT1 siRNA. Immunoblotting was performed using anti-SIRT1 antibody and anti-α-tubulin antibody as loading control. Cropped immunoblots are shown in A and B. (C) HeLa cells were transfected with control siRNAs (Control siRNA) or with siRNAs targeting fibrillarin (FIB siRNA) and cultured in medium containing FU for the last 20 min of culture before being processed to reveal FU incorporation (Transcription) and to observe fibrillarin and Sirtuin 7. Optical sections are shown. Scale bar: 10 μm.

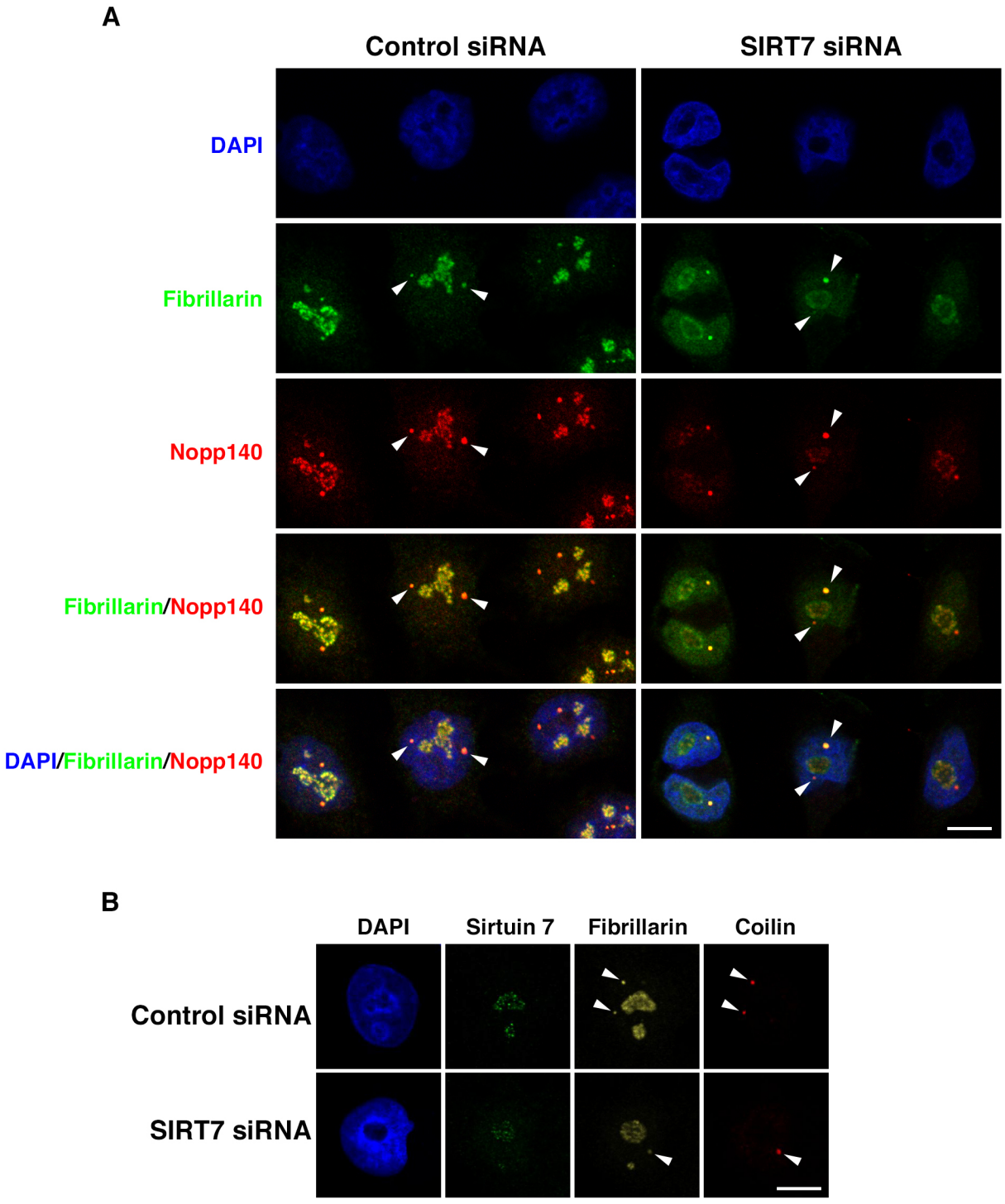

Fig. S5. Sirtuin 7 influences the localization of fibrillarin. (A) HeLa cells were transfected with control siRNAs (Control siRNA) or with siRNAs targeting Sirtuin 7 (SIRT7 siRNA) before being processed to observe fibrillarin and Nopp140. (B) HeLa cells transfected with control siRNAs (Control siRNA) or with SIRT7 siRNAs (SIRT7 siRNA) were processed to observe Sirtuin 7, fibrillarin and the CB marker coilin. Optical sections and merge images are shown. The arrowheads denote CBs. Optical sections are shown. Scale bars: 10 μm.