



### Knock-down rate: 86%

**Lanes:** 1. HeLa cells transfected with Renilla Luciferase esiRNA (RLUC, Cat.No. EHURLUC) as negative control. 2. HeLa cells transfected with NCSTN esiRNA (Cat.No. EHU078021).

**Quantitative-Immunoblotting:** Lysates were separated on SDS-Page and probed with mouse anti-GFP (1:3000) (Roche) and goat anti-GAPDH (1:7000) (Acris) antibodies. The signal intensities of indicated bands were quantified using an Odyssey infrared imaging system (Li-COR) and normalized to the GAPDH signal. At 72h post transfection, a 86% NCSTN-GFP protein reduction was measured between the negative control RLUC and the sample treated with NCSTN esiRNA.

**HeLa cells:** NCSTN was LAP tagged (including GFP) on a bacterial artificial chromosome (BAC) and stably integrated into the genome of HeLa cells (MCB: ky\_3576). The BAC preserves the genomic context of the gene, thereby ensuring near physiological expression (Poser I. et al Nat Methods. 2008 May;5(5):409-15).