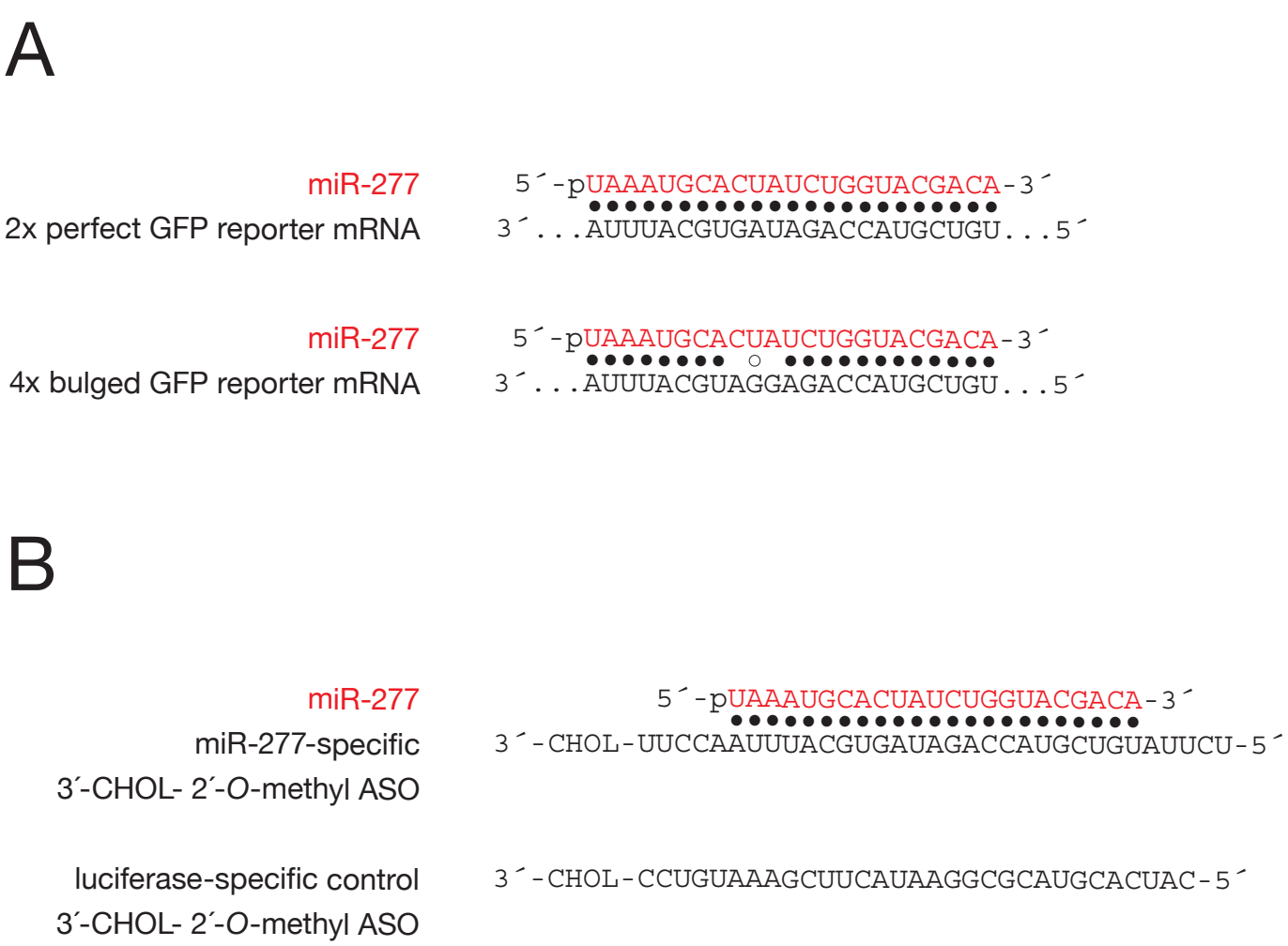

Figure S1. (A) Structure of the miR-277-binding sites in 3´ UTR of the perfectly complementary or the partially complementary GFP reporter mRNAs. (B) Structure of the 3´-cholesterol conjugated, 2´-*O*-methyl modified, antisense oligonucleotides (ASOs) used as a control (luciferase-specific) or used to inhibit miR-277 expression in cultured S2 cells. Every ribose 2´ hydroxyl in each ASO was replaced with a methoxy group.

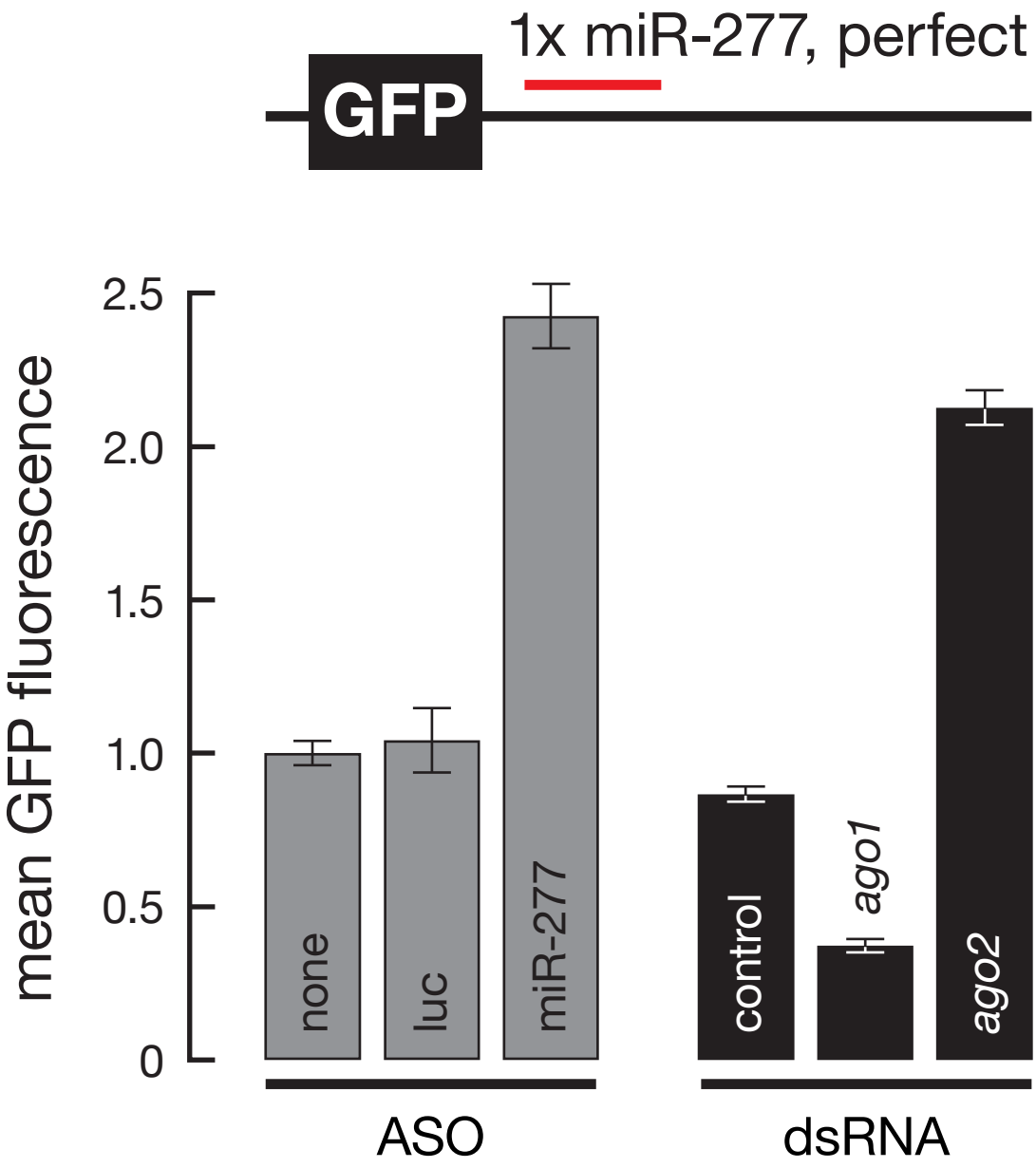

Figure S2. Endogenous miR-277-programmed Ago2, not Ago1, represses a GFP reporter containing a single, perfectly complementary site in its 3´ UTR. At left, clonally derived S2 cells bearing the GFP reporter were transfected with the indicated antisense oligonucleotide (ASO), including an ASO complementary to miR-277. At right, the cells were transfected with dsRNA corresponding to the indicated gene. Each bar represents the average ± standard deviation for three independent experiments.

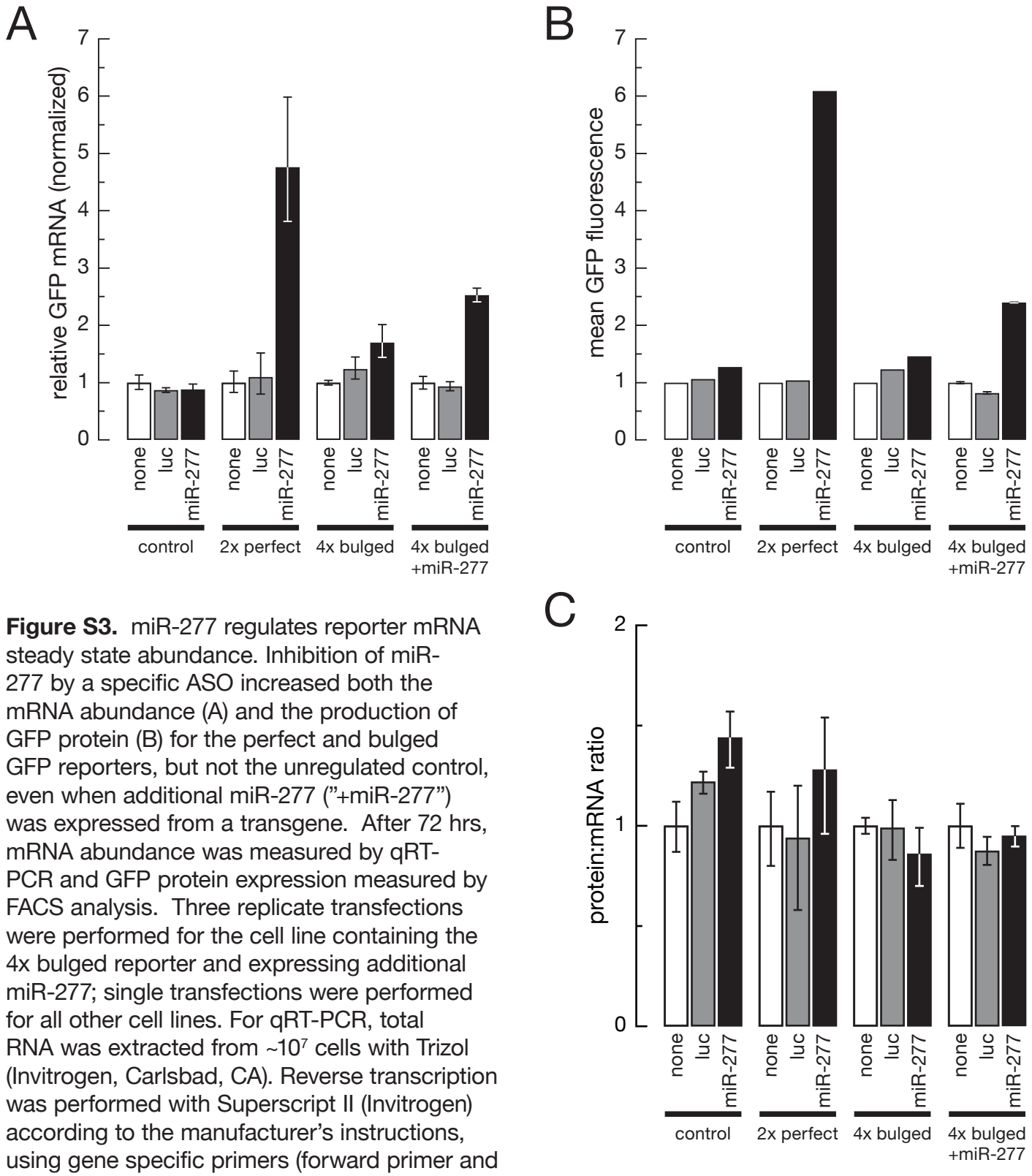

Figure S3. miR-277 regulates reporter mRNA steady state abundance. Inhibition of miR-277 by a specific ASO increased both the mRNA abundance (A) and the production of GFP protein (B) for the perfect and bulged GFP reporters, but not the unregulated control, even when additional miR-277 ("+miR-277") was expressed from a transgene. After 72 hrs, mRNA abundance was measured by qRT-PCR and GFP protein expression measured by FACS analysis. Three replicate transfections were performed for the cell line containing the 4x bulged reporter and expressing additional miR-277; single transfections were performed for all other cell lines. For qRT-PCR, total RNA was extracted from ~10^7 cells with Trizol (Invitrogen, Carlsbad, CA). Reverse transcription was performed with Superscript II (Invitrogen) according to the manufacturer's instructions, using gene specific primers (forward primer and RT/reverse primer) for the GFP and RP49 coding regions: 5´-CCG CTT CAA GGG ACA GTA TCT G-3´ and 5´-ATC TCG CCG CAG TAA ACG C-3´ for RP49; 5´-TGT CGG GCA GCA GCA C-3´ and 5´-AAC GGC ATC AAG GTG AAC TTC-3´ for GFP. Relative GFP mRNA abundance was calculated using the $2^{-\Delta\Delta Ct}$ method. Values were normalized to the no treatment control. Error bars represent the standard deviation of three PCR replicates. For the 4x bulge+miR277 sample, error bars represent standard deviation of the means of three independent transfection experiments. (C) miR-277 does not affect the ratio of reporter GFP protein to RNA. For each cell line tested, the ratio of the mean GFP fluorescence to the relative GFP mRNA abundance is shown, normalized to the no treatment control.

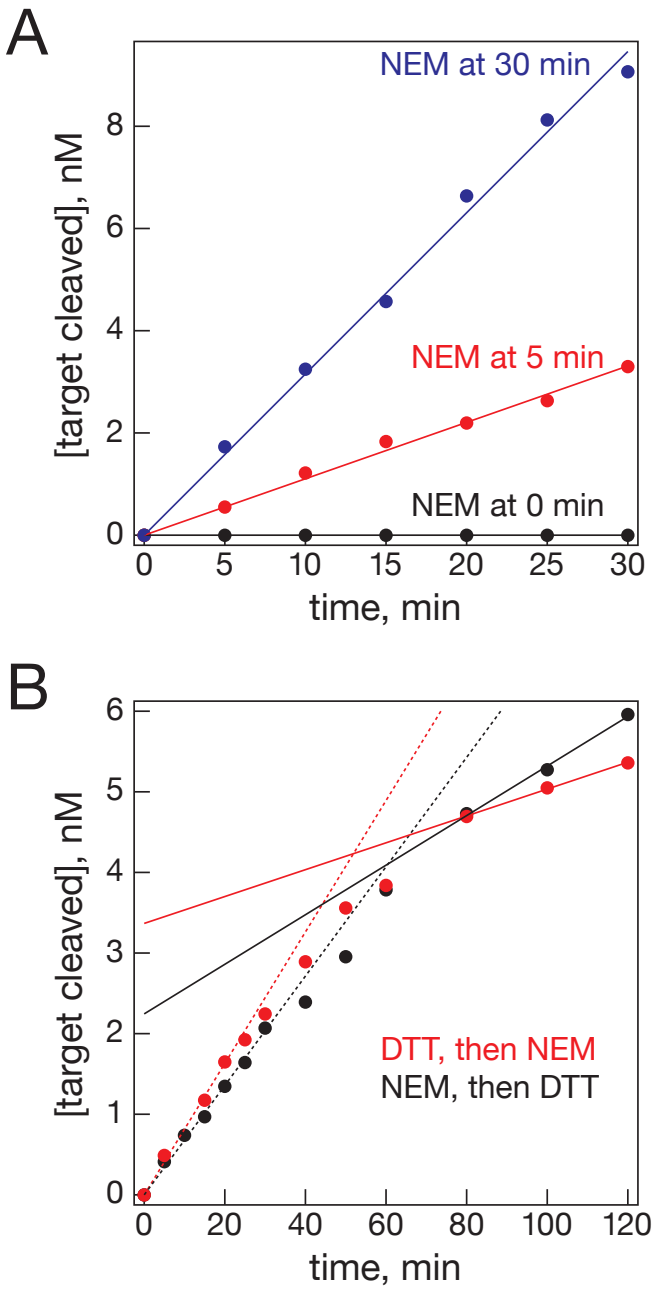

Figure S4. *N*-ethyl maleimide (NEM) inactivates assembly of Ago1-RISC, but does not alter the kinetics of Ago1-mediated target cleavage. (A) Ago1 was programmed with *let-7* in vitro using 50 nM *let-7*/*let-7** duplex, then the reaction treated with NEM at the indicated time. Unreacted NEM was quenched with DTT. The relative amount of Ago1-RISC assembled was then determined by measuring the rate of cleavage of 50 nM target RNA containing a single *let-7*-complementary site. (B) 50 nM*let-7*/*let-7** duplex was incubated with embryo lysate for 60 min to program Ago1-RISC, then the reaction treated with NEM, followed by DTT. As a control, DTT was added before the NEM. The cleavage of 200 nM target RNA by *let-7*-programmed Ago1-RISC was then assayed. Both reactions displayed the burst kinetics characteristic of Ago1-mediated target cleavage.