Figure S1. TRAIL combined with Andro inhibits OS-RC-2 cell proliferation, colony formation, and migration. (A) Images (magnification, x200) show OS-RC-2 cell morphology after treatment with various concentrations of TRAIL and/or Andro for 24 h. (B) Effects of TRAIL (50 ng/ml) and Andro (10 μM) on the clonogenic formation of OS-RC-2 cells. The histogram (right) indicates the colony number as a percentage of the control group. (C) Images (magnification, x100) show the effects of TRAIL (50 ng/ml) and Andro (1 μM) on the migration of OS-RC-2 cells. The histogram (right) indicates the ratio of migration of the initial width (one-way ANOVA, Tukey). Data are shown as mean ± SD; ***P<0.001, n=3. Andro, andrographolide; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

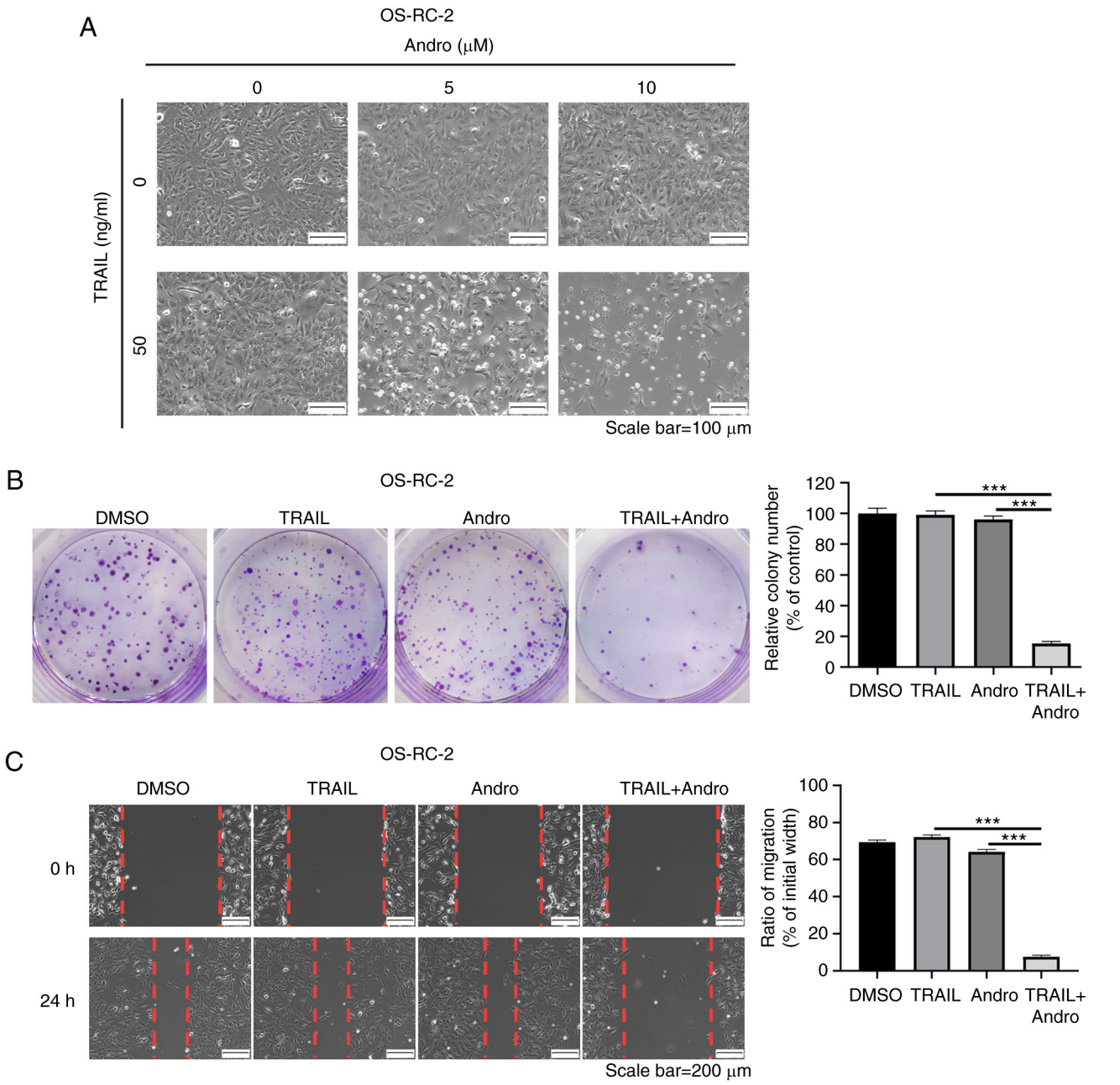

Figure S2. TRAIL combined with Andro inhibits ACHN cell proliferation, clone formation, and migration. (A) Images (magnification, x200) show ACHN cell morphology after treatment with various concentrations of TRAIL and/or Andro for 24 h. (B) Effects of TRAIL (50 ng/ml) and Andro (0.5 μM) on the clonogenic formation of ACHN cells. The histogram (right) indicates the colony number as a percentage of the control group. (C) Images (magnification, x100) show the effects of TRAIL (50 ng/ml) and Andro (5 μM) on the migration of ACHN cells. The histogram (right) indicates the ratio of migration of the initial width (one-way ANOVA, Tukey). Data are shown as mean ± SD; ***P<0.001, n=3. Andro, andrographolide; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

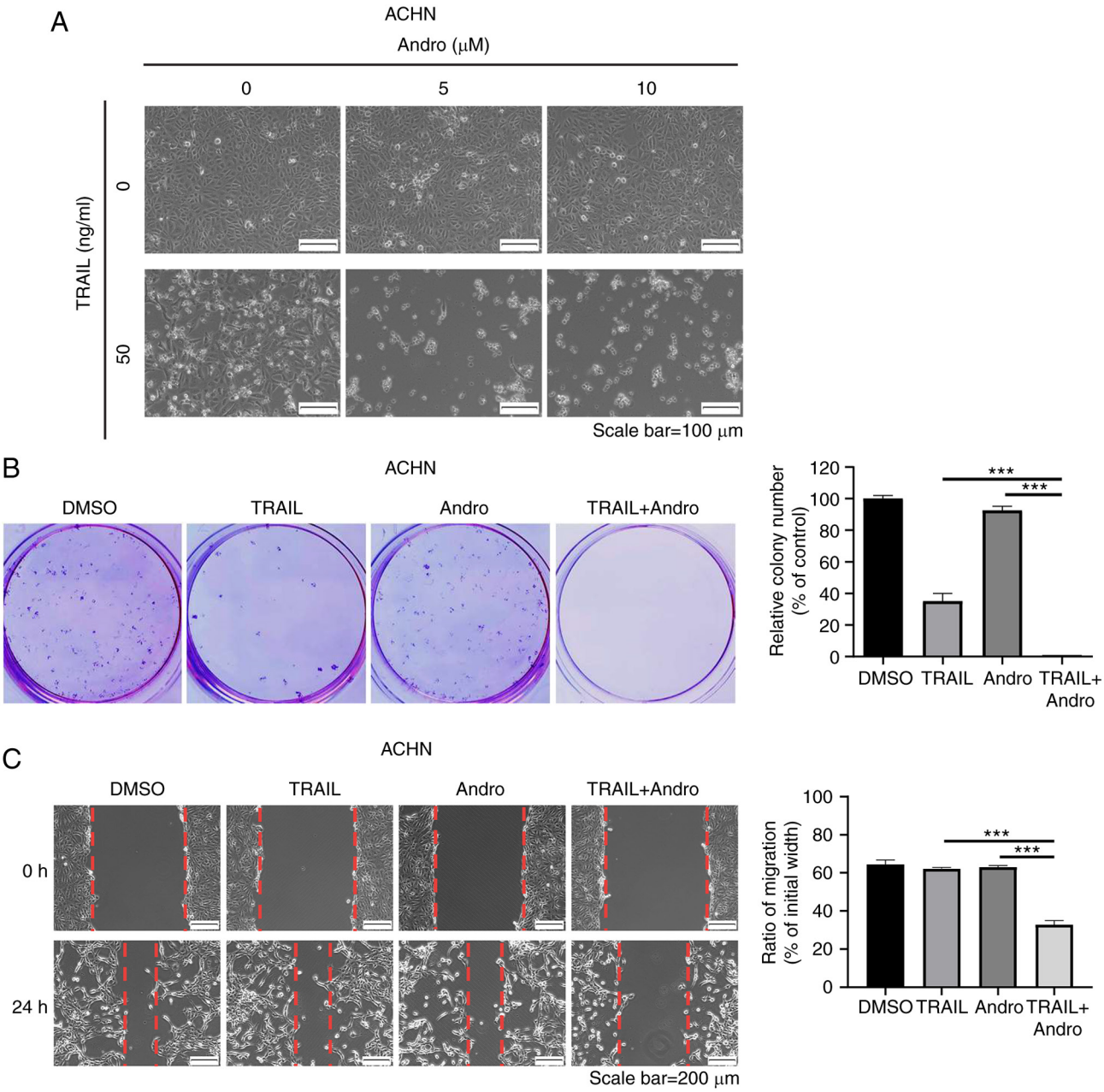

Figure S3. Combined treatment of Andro and TRAIL induces caspase-dependent apoptosis in OS-RC-2 and ACHN cells. (A) Cell apoptosis was determined by Annexin V-FITC after DMSO, TRAIL (50 ng/ml), and/or Andro (10 μM) treatment for 24 h in OS-RC-2 cells. (B) Indicated protein levels in OS-RC-2 cells treated with TRAIL (50 ng/ml) and/or Andro (10 μM) for 24 h as detected by immunoblotting. (C) Cell apoptosis was determined by Annexin V-FITC after DMSO, TRAIL (50 ng/ml), and/or Andro (5 μM) treatment for 24 h in ACHN cells. (D) Indicated protein levels in ACHN cells treated with TRAIL (50 ng/ml) and/or Andro (5 μM) for 24 h as detected by immunoblotting. Andro, andrographolide; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; PI, propidium iodide; PARP1, poly(ADP ribose) polymerase 1; Bax, Bcl-2 associated X, apoptosis regulator, DR, death receptor.

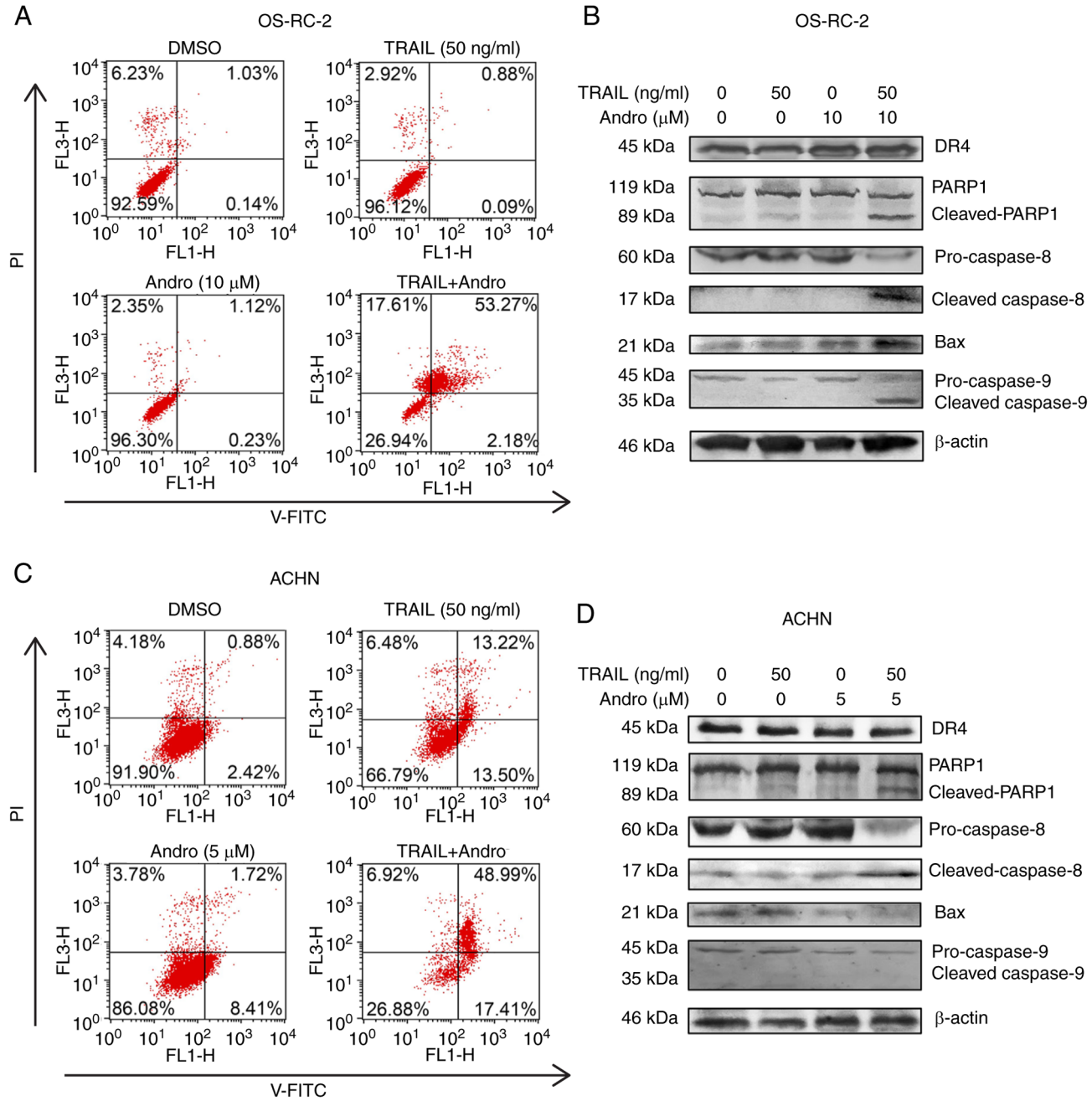

Table SI. Sequences of the siRNAs.

RNA oligos	Forward (5'-3')	Reverse (5'-3')
Negative-control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
DR4	GCUGUUCUUUGACAAGUUUTT	AAACUUGUCAAAGAACAGCTT
DR5	CAGCCGUAGUCUUGAUUGUTT	ACAAUCAAGA CUACGGCUGTT