Figure S1. Effect of siCypA_2 on the proliferation and tumorsphere-forming ability of AGS GCSCs. AGS GCSCs were transfected with either siCypA_2 (sense 5'-AAGAUGAGAACUUCAUCCU-3'; antisense 5'-AGGAUGAAGUUCUCAUCUU-3') or a non-targeting siRNA control. (A) Cell proliferation was assessed using an ATP-based luminescence assay. (B) Tumorspheres that formed were observed and quantified. Scale bar, 200 μm. (C) Tumorsphere formation frequency was measured using a limiting dilution assay. (D) Cells underwent staining with Muse® Cell Cycle reagent, and subsequent analysis of cell cycle phases was conducted using the Muse Cell Analyzer. (E) Cells were treated with Muse® Annexin V & Dead Cell reagent for staining, and the Muse Cell Analyzer was employed to quantify the percentage of apoptotic cells. *P<0.05, **P<0.001 vs. control. 7-AAD, 7-amino-actinomycin D; Apop., apoptosis; CypA, cyclophilin A; GCSC, gastric cancer stem cell; NT, non-targeting small interfering RNA control; siCypA, CypA-specific small interfering RNA; siRNA, small interfering RNA.

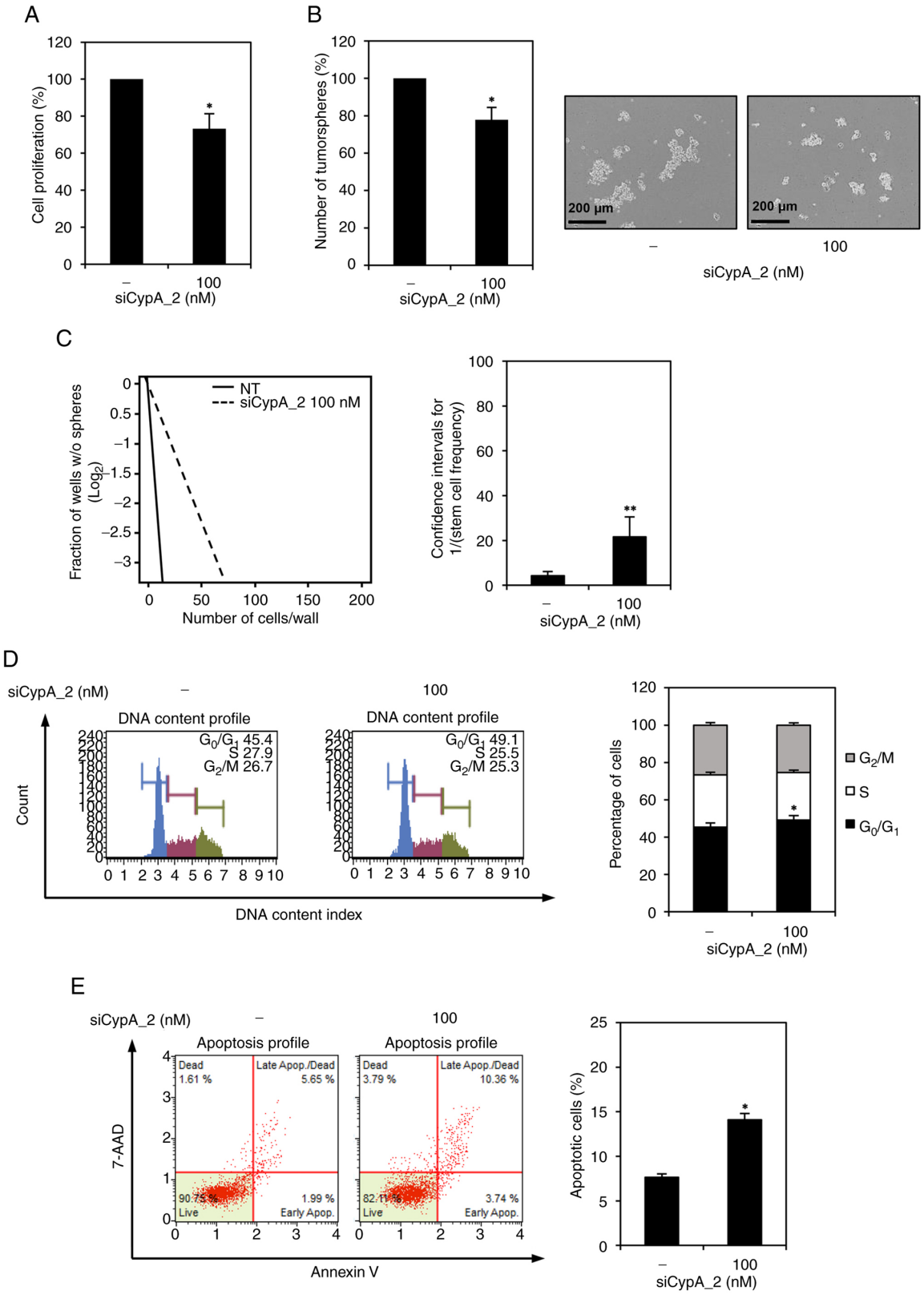

Figure S2. Effect of siCypA_2 on the migration and invasion of AGS gastric cancer stem cells. (A) Cell migration was analyzed using a wound closure assay. Cells that moved into the gap were monitored at the specified time points using light microscopy. To quantify cell migration, the gap area was measured. Scale bar, 200 μm. (B) Cell invasion was assayed using Transwell chambers with extracellular matrix gel-coated membrane inserts (8.0-μm pores). Cells that had invaded were stained with hematoxylin and eosin and quantified under a light microscope. Scale bar, 200 μm. (C) Expression levels of epithelial-mesenchymal transition markers were confirmed by western blotting and determined as the normalized ratio of each target protein relative to β-actin. *P<0.05 vs. control. CypA, cyclophilin A; NT, non-targeting small interfering RNA control; siCypA, CypA-specific small interfering RNA.

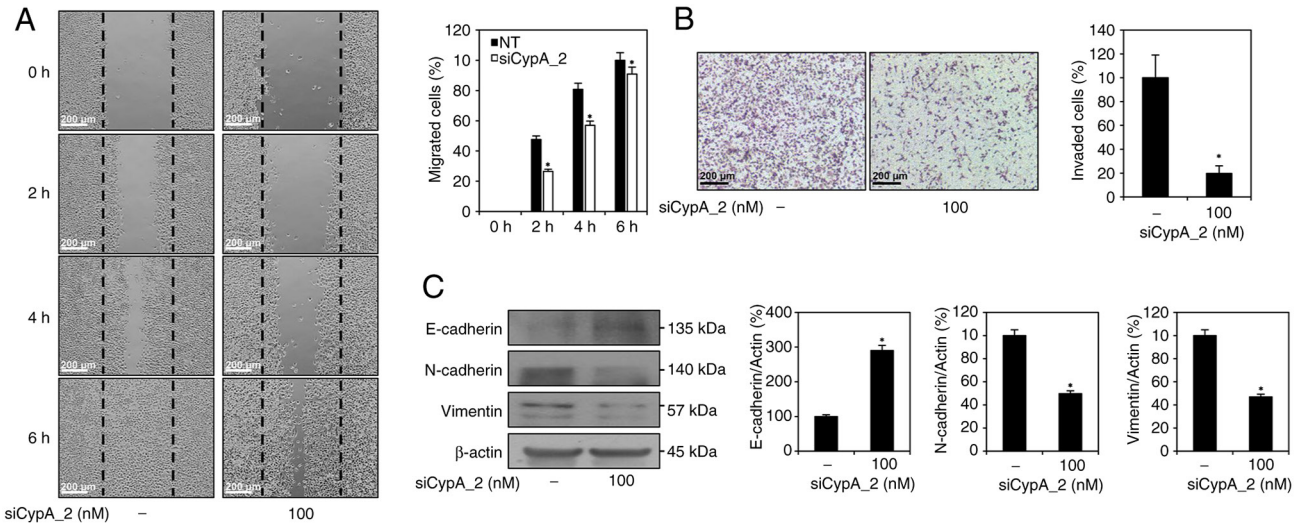

Figure S3. Impact of siCypA_2 on the (A) CD147/STAT3/AKT/ERK pathway and (B) stemness regulators in AGS gastric cancer stem cells. Western blotting was used to confirm the expression levels. Results are presented as the normalized ratio of each target protein (or phosphorylated protein) relative to β-actin (or total protein). *P<0.05 vs. control. ALDH1A1, aldehyde dehydrogenase 1 family member A1; CypA, cyclophilin A; p-, phosphorylated; siCypA, CypA-specific small interfering RNA.