

Figure S1. Schematic graph of constructs of 5A-Full, 5A-ECD and 5A-ICD. *pNI-6xHis/5A-Full/FLAG/Kan* was constructed to overexpress SEMA5A full length with a His-tag at the N-terminus and a Flag-tag at the C-terminus. *pNI-18xHis/5A-ECD/Kan* was constructed to overexpress the extracellular domain with a His-tag at the N-terminus, and *pNI-5A-ICD/18xHis/Kan* was constructed to overexpress the intracellular domain with a His-tag at the C-terminus. Red arrows indicate primer direction and position. 5A-ECD, SEMA5A extracellular domain; 5A-Full, SEMA5A full length; 5A-ICD, SEMA5A intracellular domain; SEMA5A, semaphorin 5A.

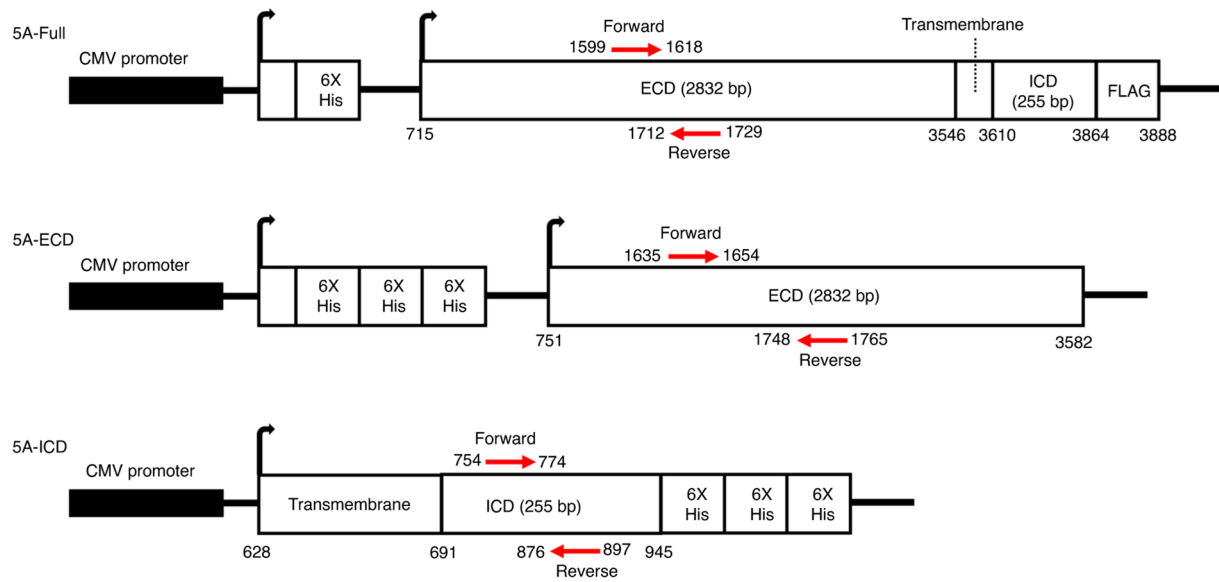


Figure S2. Different domains of SEMA5A do not affect the cell cycle distribution and apoptosis. (A) Representative diagrams of cell cycle and apoptosis analysis using flow cytometry in A549 cells overexpressing different SEMA5A domains. (B) Representative diagrams of cell cycle and apoptosis analysis using flow cytometry in H1299 cells overexpressing different SEMA5A domains. (C) Quantification of A549 cells in different cell cycle stages. (D) Quantification of H1299 cells in different cell cycle stages. (E) Quantification of apoptosis in A549 cells. (F) Quantification of apoptosis in H1299 cells. (G) Western blot analysis of caspase-3 and cleaved caspase-3 in A549 cells overexpressing different SEMA5A domains. (H) Western blot analysis of caspase-3 and cleaved caspase-3 in H1299 cells overexpressing different SEMA5A domains. Experiments were repeated in triplicate, and the results are presented as the mean $\pm$ SD. 5A-ECD, SEMA5A extracellular domain; 5A-Full, SEMA5A full length; 5A-ICD, SEMA5A intracellular domain; ACTB, actin β ; SEMA5A, semaphorin 5A.

