Supplemental Information

TRRAP Enhances Cancer Stem Cell Characteristics by Regulating NANOG Protein Stability in Colon Cancer Cells

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Supplemental Materials and Methods

Coomassie blue staining

After electrophoresis, gel was washed 3 times, 5 minutes each in deionized water. Gel was stained with Coomassie Blue R-250 Staining Solution (Enzynomics, Daejeon, Republic of Korea) for 1 hour in room temperature. Then gel was distained for 2 hours with 50% methanol, 10% acetic acid, 40% deionized water in room temperature.

Elution of TAP-NANOG and protein interactions

After pulldown of TAP-NANOG with streptavidin beads, beads were washed 3 times for 5 minutes. TAP-NANOG was eluted for 30 minutes at 4°C with streptavidin elution buffer (Agilent Technologies, Santa Clara, California, USA). Eluted TAP-NANOG was incubated with FLAG-control or FLAG-TRRAP for 2 hours followed by a pull-downed with anti-FLAG M2 affinity beads. Beads were washed 3 times and boiled for 10 minutes with sample buffer.

Supplementary Figures

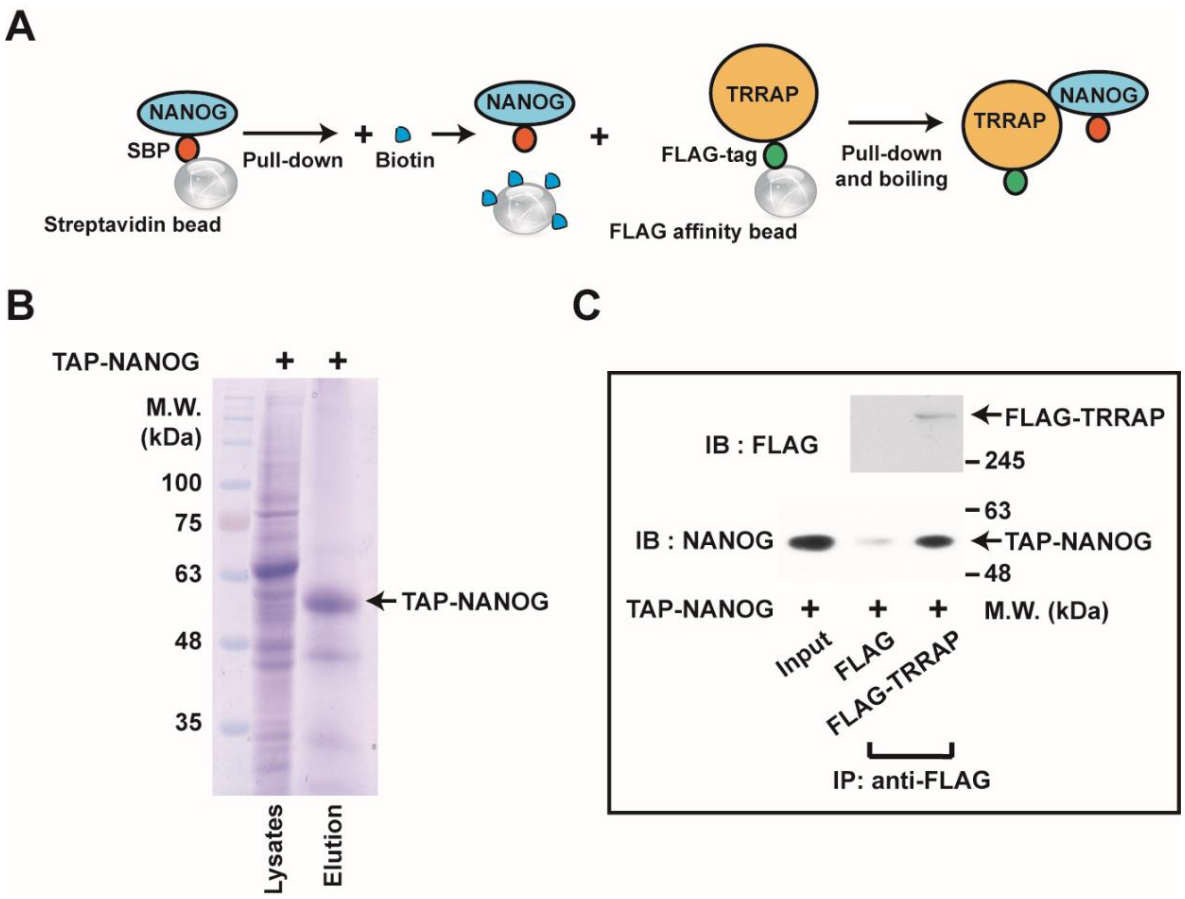

Figure S1. Direct interaction of NANOG with TRRAP. **(A)** Schematic illustration for purification of TAP-NANOG and pull-down experiment using FLAG-TRRAP immunoprecipitants. TAP-NANOG was overexpressed in HEK293 cells, purified with Streptavidin affinity purification, and the TAP-NANOG protein was eluted from the Streptavidin agarose bead by treatment with elution buffer containing biotin. The purified TAP-NANOG protein was incubated with the FLAG-TRRAP immunoprecipitants, followed by pull-down of the FLAG-TRRAP-associated TAP-NANOG protein complex by spin down experiment. SBP: Streptavidin binding peptide. **(B)** The cell lysates of TAP-NANOG-overexpressed HEK293 cells and the purified TAP-NANOG protein were separated by SDS-PAGE, followed by staining with Coomassie Blue R-250. The arrow indicates the protein band of TAP-NANOG. **(C)** Direct binding of the purified NANOG with FLAG-TRRAP. The cell lysates of TAP-NANOG-overexpressed HEK293 cells (input) were incubated with the immunoprecipitants of FLAG or FLAG-TRRAP, followed by pull-down experiment for isolation of the TRRAP-bound NANOG. The input and immunoprecipitants were analyzed by western blotting analysis of the FLAG-TRRAP and TAP-NANOG.