Supporting Information

A functionalized gold nanoparticles-assisted universal carrier for antisense DNA**

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Preparation of AuNP GDS-AD conjugates

AuNP GDS (10 nM) was mixed with the antisense oligonucleotide (4 μM) in annealing buffer (1× phosphate-buffered saline containing 0.3 M NaCl) by gentle shaking for 10 min, incubated at 55°C for 10 min, and cooled to room temperature for ~1 hr. The resulting conjugates were spun down at 10,000 ×g for 10 min, the supernatant was removed, and the conjugate pellet was resuspended in DMEM medium. This precipitation and resuspension step was carried out three times. The AuNP GDS-AD conjugates (10 nM) was added to a tissue culture media, such that the final concentration was 1 nM

Western blot analysis

293T (4.5×10^5) or HeLa cells (3.0×10^5) were incubated with antisense oligos functionalized with gold-nanoparticles in six-well dishes. Cell lysates were prepared in NP-40 lysis buffer (50 mM Tris-HCl, pH 8.0, 0.15 M NaCl, and 1% NP-40) containing 10% protease inhibitor cocktail (Sigma). For quantitative protein analysis, a standard curve was established with the standard BSA solution (Pierce, Rockford, IL, USA), and cell lysates containing equal amounts of total protein were subjected to

sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were transferred onto nitrocellulose membrane and western blot analyses were performed. Anti-MCL-1L and anti-p53 monoclonal antibodies (both from Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used to detect MCL-1L and p53, respectively.

Semi-quantitative RT PCR

Total RNA was extracted from the cell lines with TRI REAGENT(Invitrogen; Caelsbad, CA, USA) according to the manufacturer's instructions. Synthesis of cDNA was performed using the SuperScript™ First-Strand Synthesis System (Invitrogen). PCR was carried out in a total volume of 20 μl, using 1 μl of RT reaction. The PCR products were analyzed in 2% agarose gels. Primers used for CADPH were GAPDH-F (5′-AGCCAAAAGGGTCATCATCTCT) and GAPDH-R (5′-AGGGGCCATCCACAGTCTT), MCL-1L-F (5′-TGGTCGGGGAATCTGGTAAT) and MCL-1L-R (5′-GTAAGGTCTCCAGCGCCTTC for MCL-1L, and p53-F (5′-AGCTTTGAGGTGCGTGTTTG and p53-R (5′-TCAGCTCTCGGAACATCTCG).

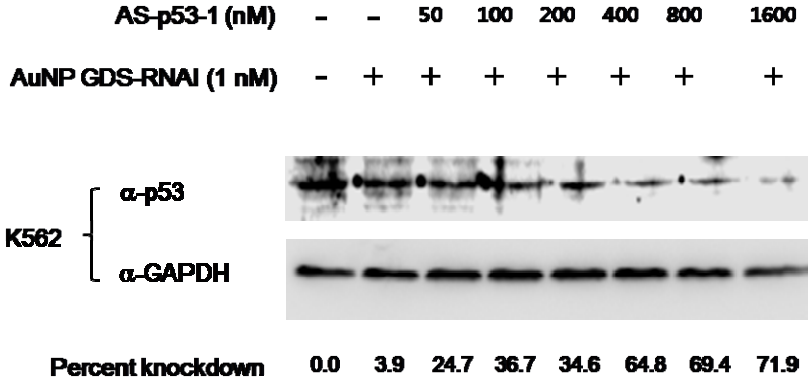

Figure S1. Dose response of Au NP-GDS-AS-p53 conjugates to p53 gene knockdown in K562.

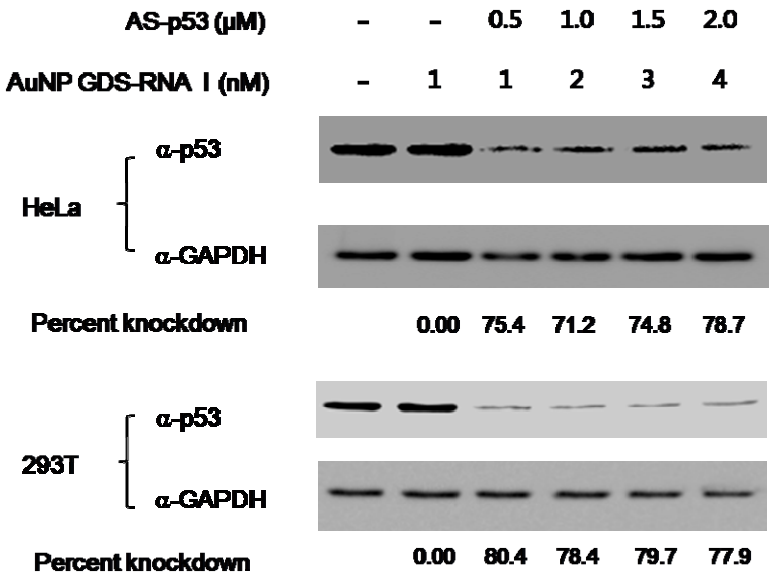

Figure S2. Measurement of saturating concentrations of Au NP-GDS-AS-p53 conjugates to p53 gene knockdown in HeLa and 293T cells.

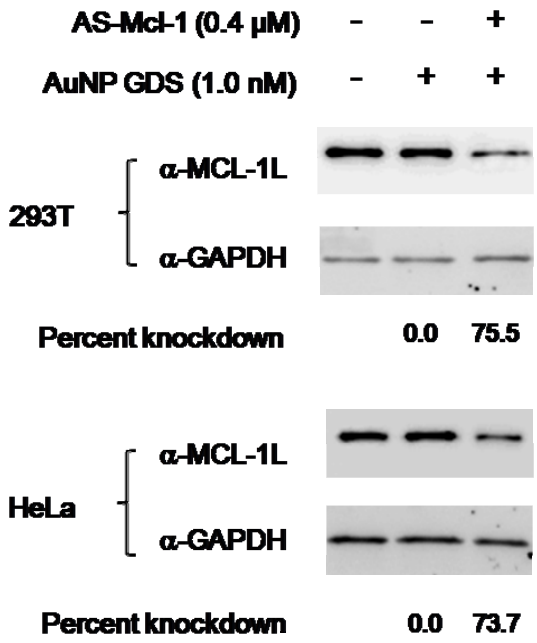

Figure S3. Knockdown of Mcl-1Lexpression by AuNP-GDS-AS-Mcl-1L in HeLa and 293T cells.

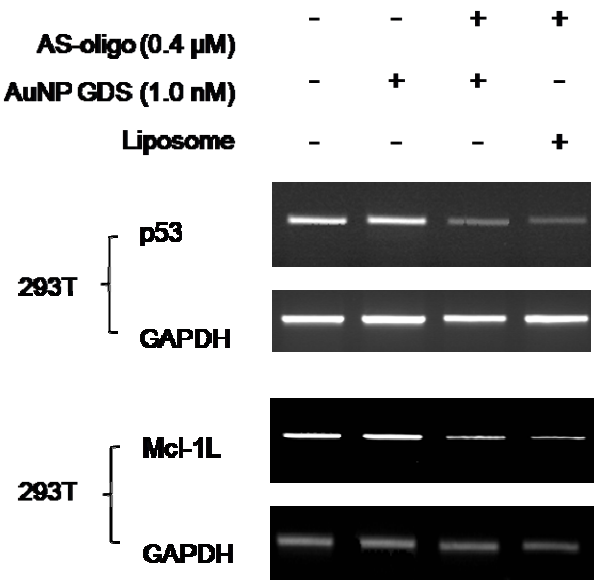

Figure S4. Effects of AuNP-GDS-AS on the steady state levels of target mRNA in 293T cells.

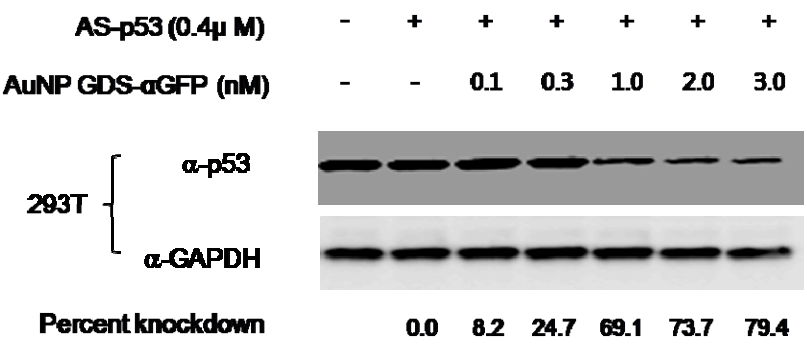

Figure S5. Dose response of Au NP-GDS-αGFP-AS-p53 conjugates to p53 gene knockdown in K562.

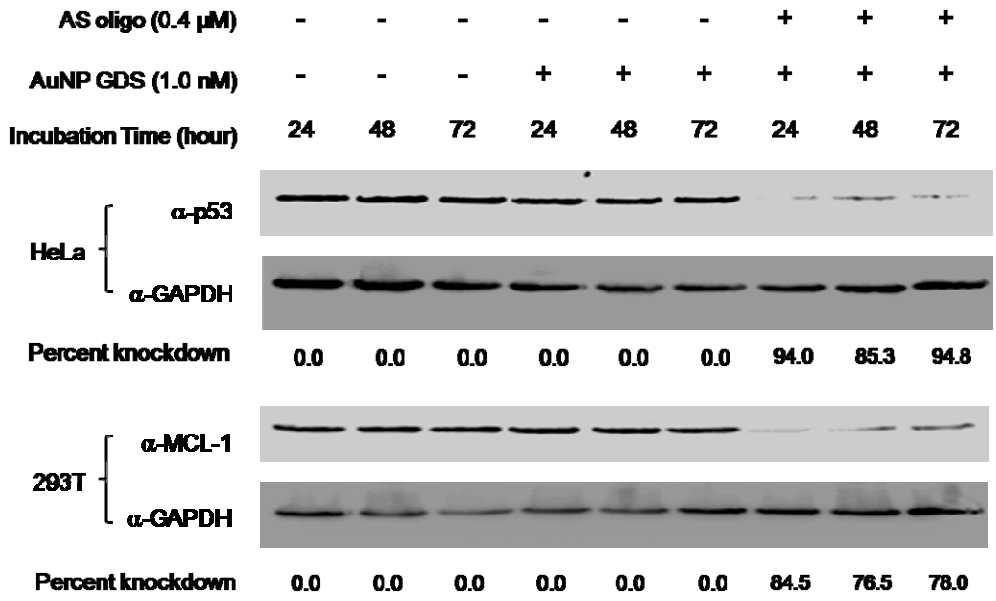

Figure 6S. Duration of knockdown effects on target gene expression by AuNP-GDS-AS.