Supplementary method

Primer list for mutagenesis

Site A of p27 (Forward)	5’-CTA AAA GCG TTG GAT AGT ACA TTA TGC AAT TAG G-3’
Site A of p27 (Reverse)	5’-CCT AAT TGC ATA ATG TAC TAT CCA ACG CTT TTA G-3’
Site B of p27 (Forward)	5’-GTT TTT ACC TTT TAT AGT ACA CAT AAA CTT TGG G-3’
Site B of p27 (Reverse)	5’-CCC AAA GTT TAT GTG TAC TAT AAA AGG TAA AAA C-3’
miR-222~221 binding site in p57 (Forward)	5’-GCT GGG ACC GTT CAT GTA GCA GCA ACC GGC GGC G-3’
miR-222~221 binding site in p57 (Reverse)	5’- CGC CGC CGG TTG CTG CTA CAT GAA CGG TCC CAG C-3’
miR-25 binding site in p57 (Forward)	5’-AAT TTT GAA AAC TGT AAC GTG TAT TAA TAA CGT-3’
miR-25 binding site in p57 (Reverse)	5’-ACG TTA TTA ATA CAC GTT ACA GTT TTC AAA ATT-3’
Site A of p21 (Forward)	5’-AAG TAA ACA GAT GGC TTC ATG AAG GGG CCT CAC-3’
Site A of p21 (Reverse)	5’-GTG AGG CCC CTT CAT GAA GCC ATC TGT TTA CTT-3’
Site B of p21 (Forward)	5’-TCC CCA GTT CAT TGC TTC ATG ATT AGC AGC GGA A-3’
Site B of p21 (Reverse)	5’-TTC CGC TGC TAA TCA TGA AGC AAT GAA CTG GGG A-3’

Kinase assay

HeLa cells were transfected with siRNA, and two days later, cells were lysed with 1% Triton-X lysis buffer. The clear lysate was obtained by centrifugation and Cdk2 protein was immunoprecipitated with anti-Cdk2 antibody immobilized on protein A/G sepharose beads. Beads were washed with lysis buffer twice and with kinase reaction

buffer twice. After washing, beads were incubated with H1 protein and gamma-P^{32}-ATP in kinase buffer. After 30min of incubation, protein sample buffer was added into kinase reaction tubes and the protein was separated by PAGE. The gel was directly exposed to X-ray film.

Supplementary figure legends

Supplementary Table 1. miRNA expression profiling of human gastric cancer tissues.

Supplemantary Table 2. Quantitation of the northern blotting in Figure 1B. Multi Gauge V3.0 software was used for quantitation, and expression ratio (tumor/normal) normalized against tRNA (or rRNA) was presented in the 'Norm' column.

Supplementary Table 3. The predicted target genes of each member of miR-222 and miR-106b clusters. Target genes were extracted from TargetScan 4.1 server (www.targetscan.org), and their GO terms were analyzed. GO terms with 'cell cycle', 'cell division', 'cell proliferation', 'apoptosis', and 'replication' were selected and combined.

Supplementary Figure 1. Sequence alignment of miR-106b~93~25 and related miRNA clusters. Sequences are retrieved from miRBase (Release 10.1). The chromosomal locations of the clusters are shown below. The seed sequences are highlighted in yellow.

Supplementary Figure 2. miRNA target sites in the 3'-UTR of Cdk inhibitors. Boxed sequences were mutated into the sequences in red.

Supplementary Figure 3. Regulation of p27 by miR-222 or miR-221. Small RNA duplex was transfected into (A) HeLa or (B) MCF7 cells. Two days later, protein samples were prepared and western blot analysis was carried out.

Supplementary Figure 4. Kinase assay. Control siRNA or miR-222~221 was transfected into HeLa cells. Two days later, protein was extracted and 30μg of protein was reserved for western blotting of p27 and GAPDH protein. Five hundred μg of protein was used for immunoprecipitation using antibody against Cdk2 protein. 10% of the immunoprecipitated sample was used for Cdk2 western blotting, and kinase assay was carried out using 90% of the sample.

Supplementary Figure 5. Induction of miR-222~221 cluster by serum stimulation. (A) Mature miR-222~221 is induced following serum stimulation. Exponentially growing AGS or MKN-28 cells were deprived of serum for 24hrs and used for Ser (-) sample. For Ser (-/+) sample, the cells were serum-deprived for 24hrs, and then were re-added with fresh media supplemented with serum and were incubated for additional 24hrs. (B) The same cells as in (A) were used for protein preparation, and western blotting against p27 was carried out.