Supplemental Data

Figure S1. **MiR-214 expression levels and p53 status in ovarian cell lines.** (**A**) qRT-PCR analysis was performed with specific miR-214 primers in indicated cell lines. (**B**) The list of p53 status in ovarian cancer cell lines.

Figure S2. MiR-214 regulates OCSC in wild-type p53 ovarian cancer cells. (**A - C**) Indicated cell lines were transfected with pre-miR-214 or ASO miR-214 and control oligo. After 72 h of transfection, cells were subjected to CD133 labeling (upper panels of A), qRT-PCR analysis of miR-214 expression level (bottom panels of A) and ALDEFLUOR analysis (B, C)

Figure S3. MiR-214 does not affect sphere growth in mutant p53 cells OV8 and SKOV3. Cells were plated in ultra-negative attachment 6-well plate (5,000 viable cells/well) and were grown in a serum-free sphere culture medium for 12 days. Sphere numbers were counted under microscopy.

Figure S4. Knockdown of miR-214 overcomes chemoresistance in ALDH1-positive cells. ALDH1-positive and -negative cells were isolated from A2780S and OV2008 cells by flow sorting and were analyzed for miR-214 levels by qRT-PCR. The ALDH1-positive cells were transfected with antisense of miR-214 (**A**). The cells were treated with or without CDDP (**B**) and doxorubicin (**C**). After 24 h of treatment, cells were subjected to MTT assay. Data shown are mean ± SD for each experimental group and all the experiments were repeated 3 times in triplicate.

Figure S5. MiR-214 does not regulate Nanog in mutant p53 cells. Indicated cells were transfected with pre-miR-214 (**A**) or ASO miR-214 (**B**) as well as control oligo. Following incubation of 72 h, cells were subjected to immunoblot and RT-PCR analyses.

Figure S6. P53 expression was negatively regulated by miR-214. (**A**) qRT-PCR and Western blot analyses show inverse correlation of miR-214 and p53 levels in a majority ovarian cancer cell lines examined. (**B**) OV2008 and A2780 cells were transfected with indicated oligos and then immuno-stained with p53 antibody after 48 h of transfection.

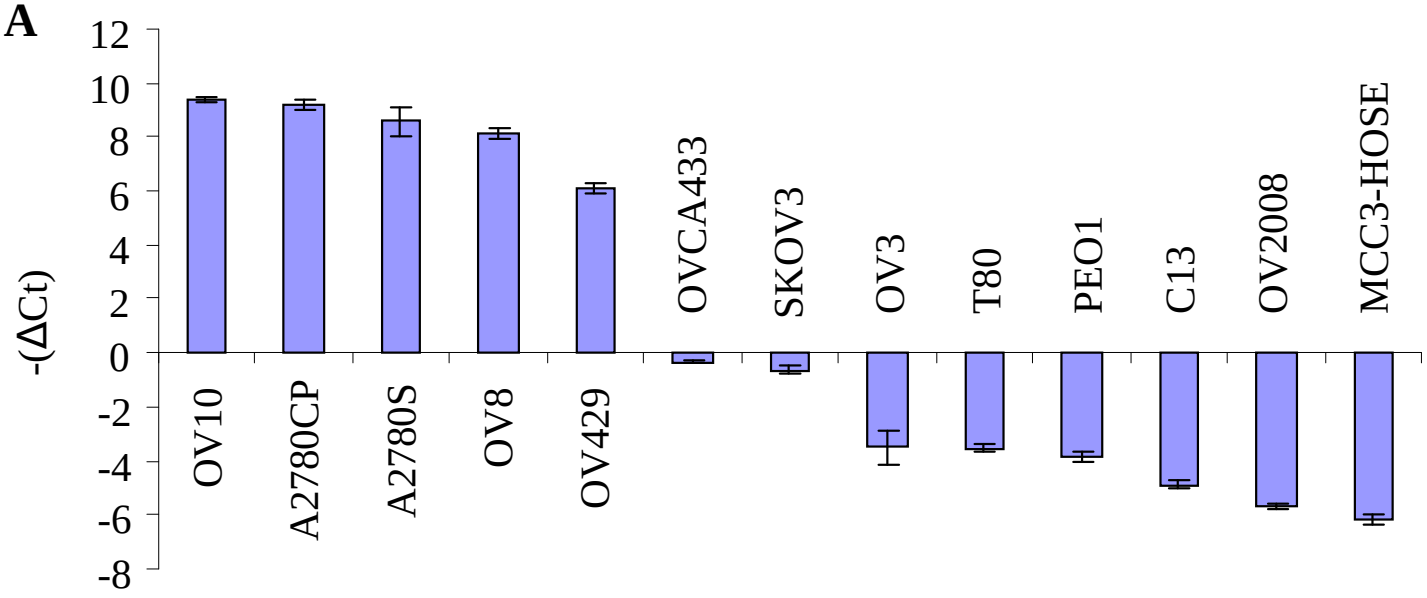
Figure S7. MiR-214 represses the expression of p53 and its downstream targets. OVCA433 cells were infected with lenti-miR-214 and lenti-miR vectors. After selection with puromycin, 3 stable miR-214 clonal cell lines were subjected to immunoblot analysis with indicated antibodies.

Figure S8. Nanog is regulated by miR-214 in wild-type p53 cells. OV10 cells were transfected with indicated oligos and then subjected to RT-PCR (upper panels) and immunoblot analyses (bottom panels).

Figure S9. ALDH-positive cells express CSC markers. ALDH-negative and –positive cells were sorted and then immunoblotted with indicated antibodies.

Figure S10. **MiR-214 represses p53 but does not induce Nanog expression in p53-mutant cells.** OV3 and A2780CP cells were transfected with pre-miR-214 (left) and ASO miR-214 (right), respectively. After incubation for 72 h, cells were analyzed by immunoblot (upper panels) and semi-quantitative RT-PCR (bottom panels) for p53 and Nanog expression.

A



B

Cell line	p53 status
A2780S	Wild type
OV10	Wild type
OV433	Wild type
OV2008	Wild type
OV429	Wild type
A2780CP	Mutation
OV 8	Mutation
OV 3	Mutation
SKOV 3	Null
C13	Wild type
PEO1	Mutation

Figure S1

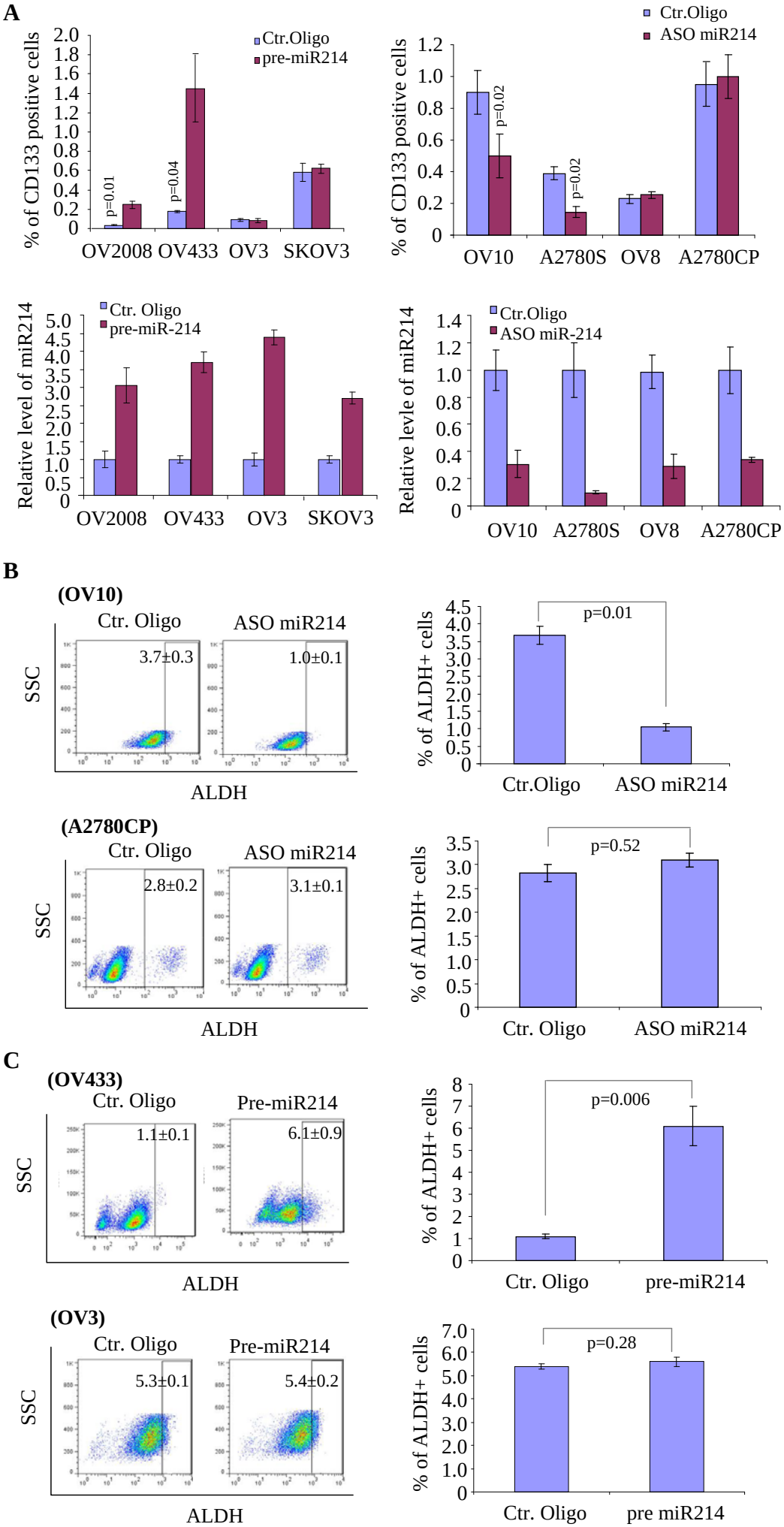

A
% of CD133 positive cells
Ctr.Oligo
pre-miR214
p=0.01
p=0.04
OV2008 OV433 OV3 SKOV3
% of CD133 positive cells
Ctr.Oligo
ASO miR214
p=0.02
p=0.02
OV10 A2780S OV8 A2780CP
Relative level of miR214
Ctr. Oligo
pre-miR-214
OV2008 OV433 OV3 SKOV3
Relative levle of miR214
Ctr.Oligo
ASO miR-214
OV10 A2780S OV8 A2780CP
B
(OV10)
Ctr. Oligo
ASO miR214
3.7±0.3
1.0±0.1
SSC
ALDH
% of ALDH+ cells
p=0.01
Ctr.Oligo
ASO miR214
(A2780CP)
Ctr. Oligo
ASO miR214
2.8±0.2
3.1±0.1
SSC
ALDH
% of ALDH+ cells
p=0.52
Ctr. Oligo
ASO miR214
C
(OV433)
Ctr. Oligo
Pre-miR214
1.1±0.1
6.1±0.9
SSC
ALDH
% of ALDH+ cells
p=0.006
Ctr. Oligo
pre-miR214
(OV3)
Ctr. Oligo
Pre-miR214
5.3±0.1
5.4±0.2
SSC
ALDH
% of ALDH+ cells
p=0.28
Ctr. Oligo
pre miR214

Figure S2

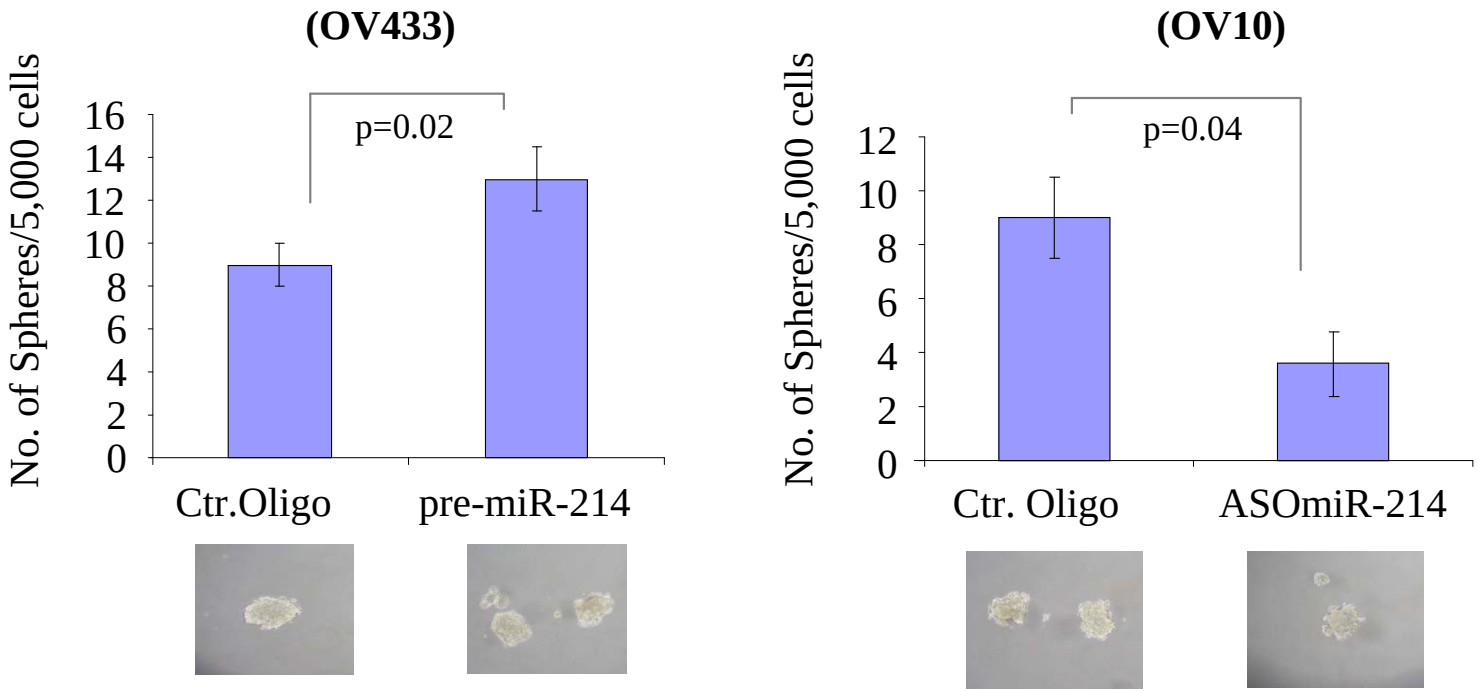

(OV433)
No. of Spheres/5,000 cells
16
14
12
10
8
6
4
2
0
p=0.02
Ctr.Oligo
pre-miR-214
(OV10)
No. of Spheres/5,000 cells
12
10
8
6
4
2
0
p=0.04
Ctr. Oligo
ASOmiR-214

Figure S3

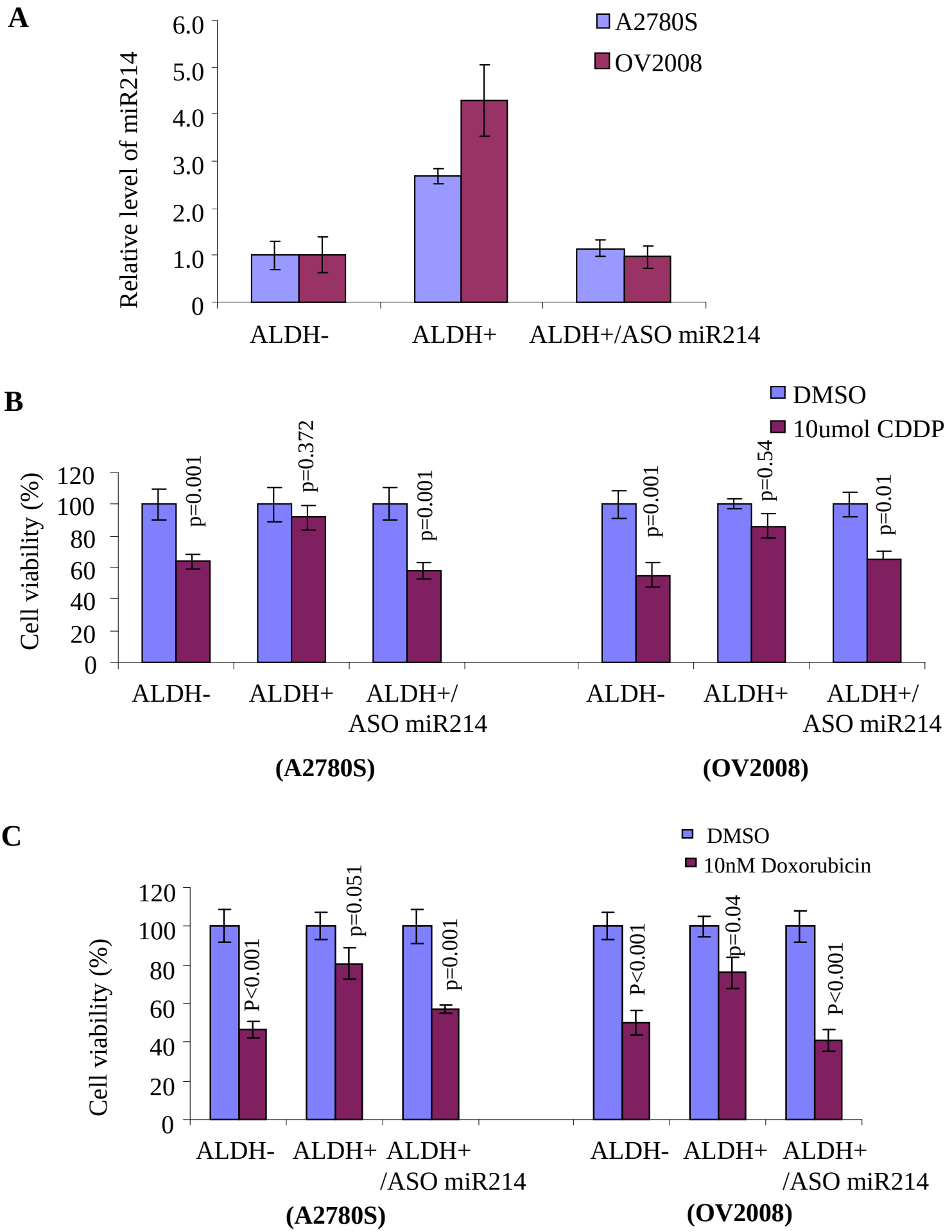

A
A2780S
OV2008
Relative level of miR214
6.0
5.0
4.0
3.0
2.0
1.0
0
ALDH-
ALDH+
ALDH+/ASO miR214
B
DMSO
10umol CDDP
Cell viability (%)
120
100
80
60
40
20
0
p=0.001
p=0.372
p=0.001
p=0.001
p=0.54
p=0.01
ALDH-
ALDH+
ALDH+/ ASO miR214
ALDH-
ALDH+
ALDH+/ ASO miR214
(A2780S)
(OV2008)
C
DMSO
10nM Doxorubicin
Cell viability (%)
120
100
80
60
40
20
0
P<0.001
p=0.051
p=0.001
P<0.001
p=0.04
P<0.001
ALDH-
ALDH+
ALDH+ /ASO miR214
ALDH-
ALDH+
ALDH+ /ASO miR214
(A2780S)
(OV2008)

Figure S4

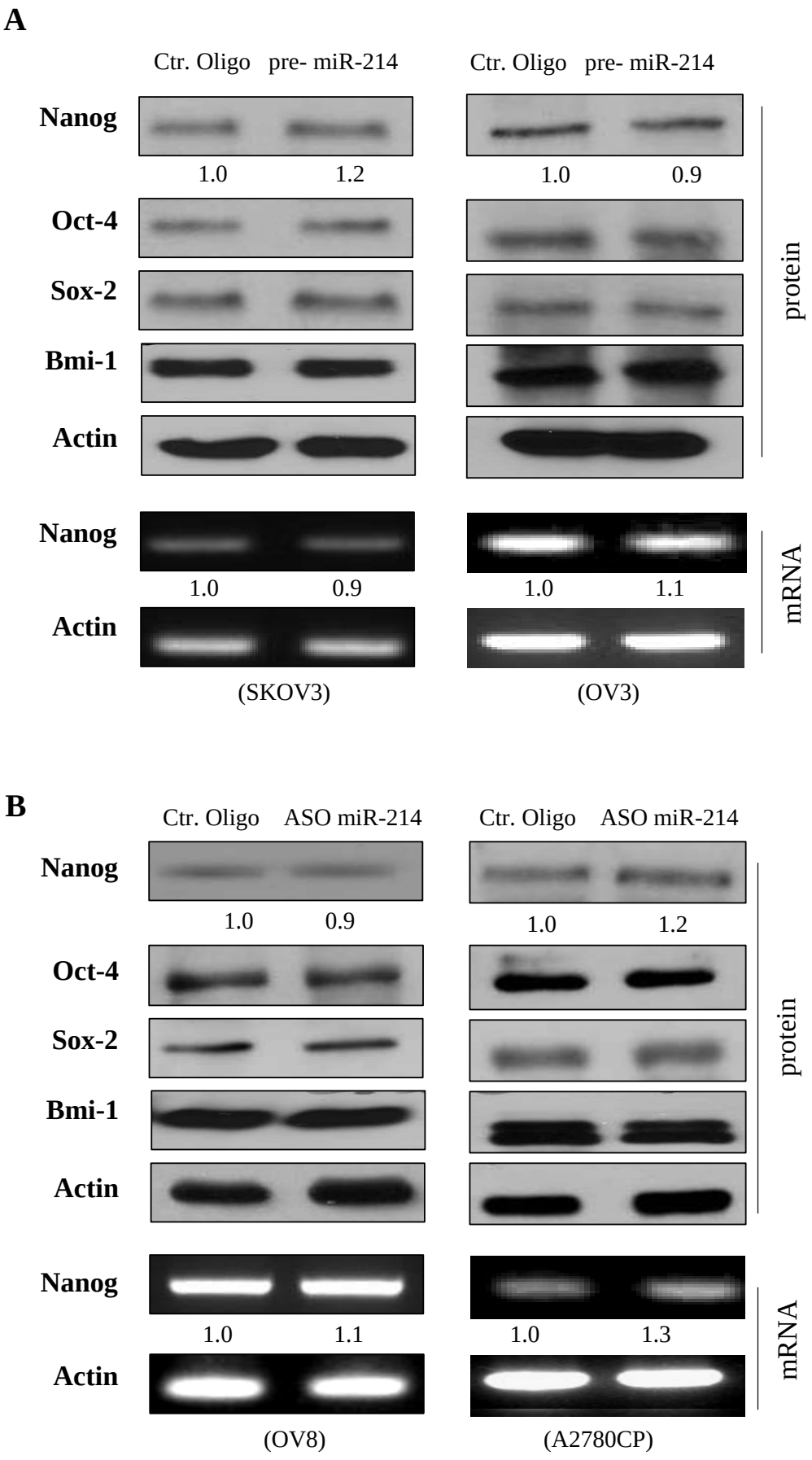
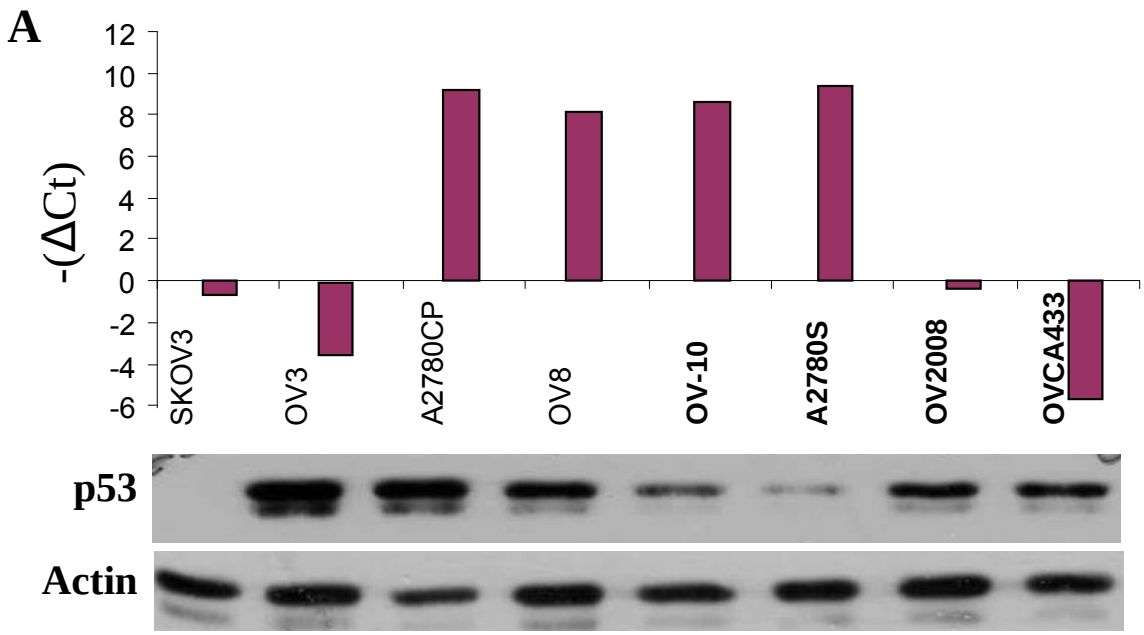

A
Ctr. Oligo
pre- miR-214
Ctr. Oligo
pre- miR-214
Nanog
1.0
1.2
1.0
0.9
Oct-4
Sox-2
Bmi-1
Actin
protein
Nanog
1.0
0.9
1.0
1.1
Actin
mRNA
(SKOV3)
(OV3)
B
Ctr. Oligo
ASO miR-214
Ctr. Oligo
ASO miR-214
Nanog
1.0
0.9
1.0
1.2
Oct-4
Sox-2
Bmi-1
Actin
protein
Nanog
1.0
1.1
1.0
1.3
Actin
mRNA
(OV8)
(A2780CP)

Figure S5


A
12
10
8
6
4
2
0
-2
-4
-6
-(ΔCt)
SKOV3
OV3
A2780CP
OV8
OV-10
A2780S
OV2008
OVCA433
p53
Actin

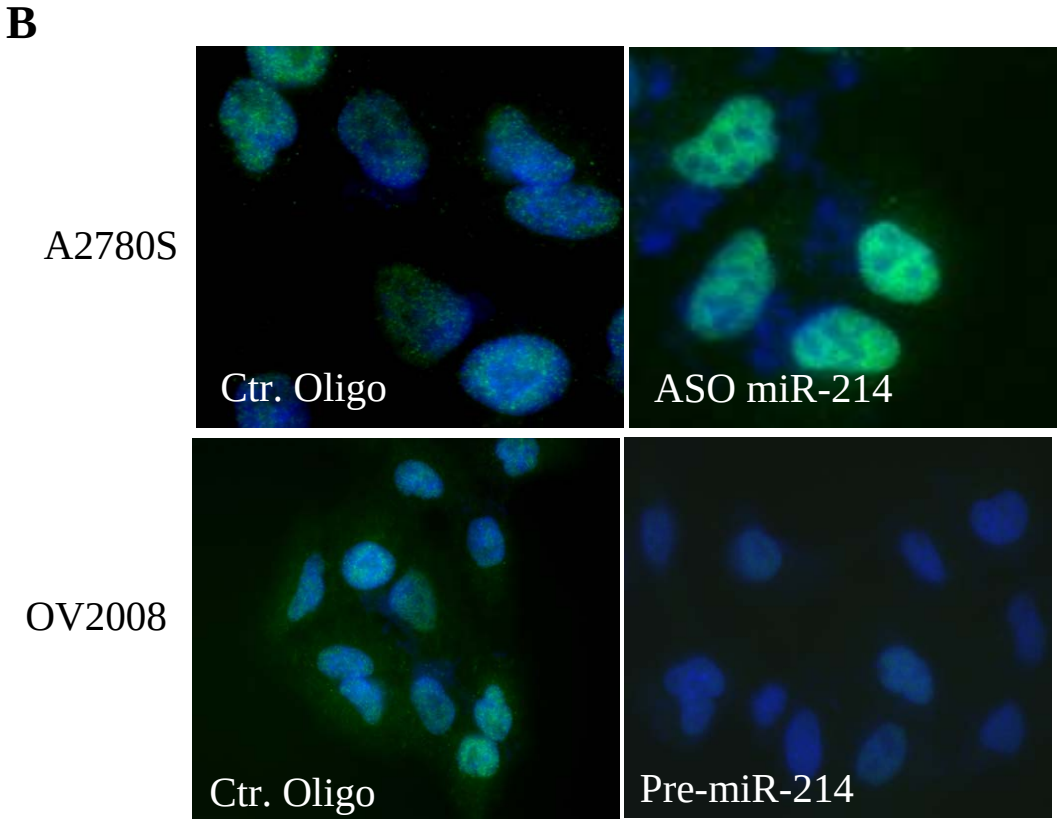

B
A2780S
Ctr. Oligo
ASO miR-214
OV2008
Ctr. Oligo
Pre-miR-214

Figure S6

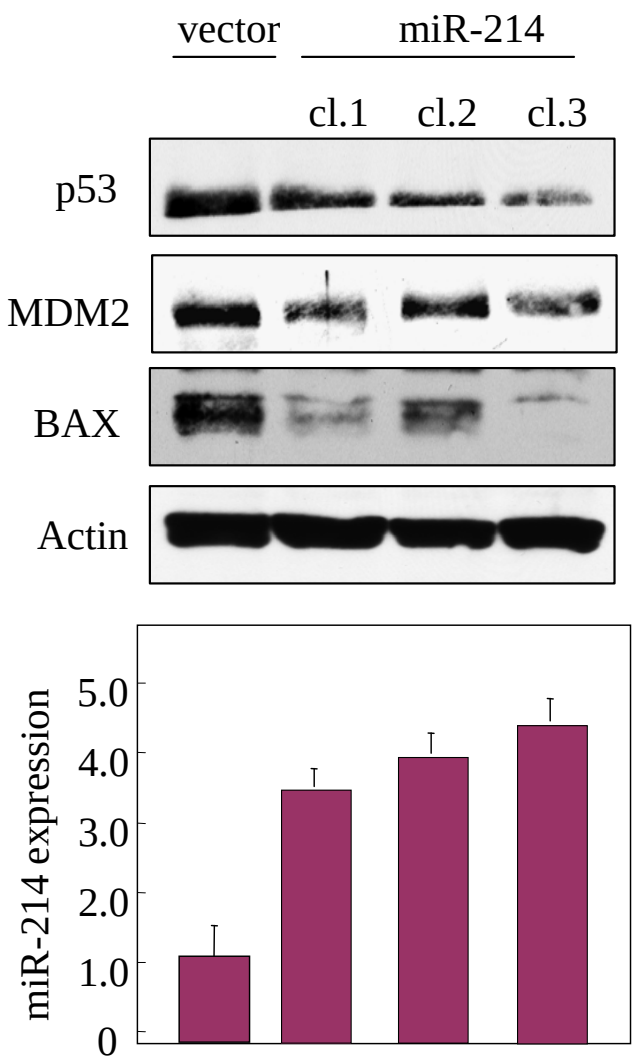
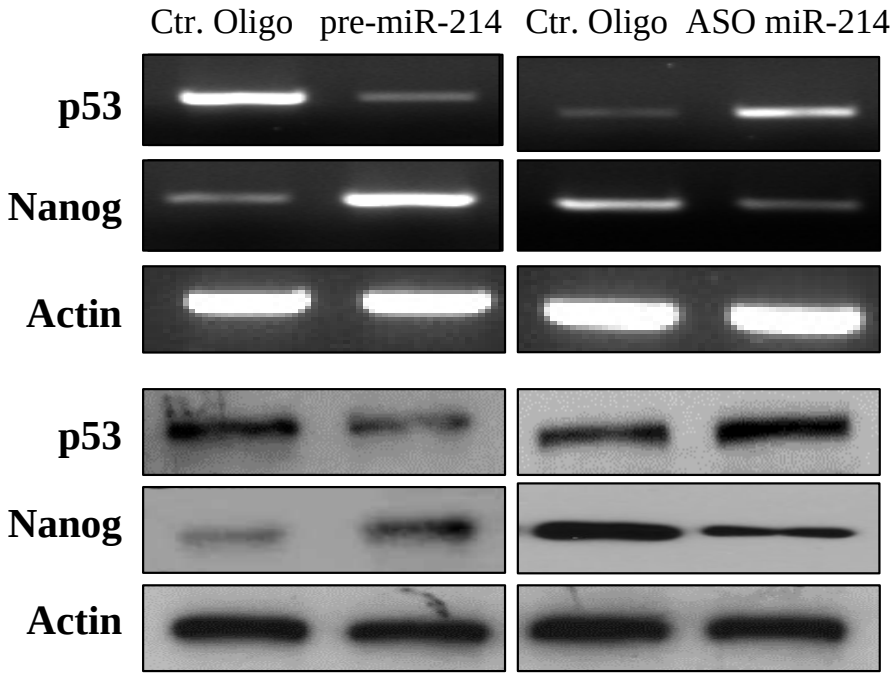

vector
miR-214
cl.1
cl.2
cl.3
p53
MDM2
BAX
Actin
miR-214 expression
5.0
4.0
3.0
2.0
1.0
0

Figure S7



(OV10)

Figure S8

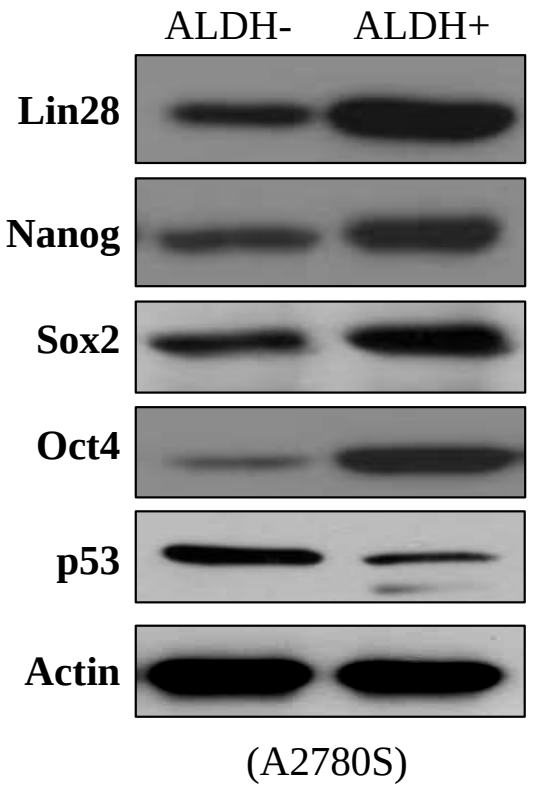


Figure S9

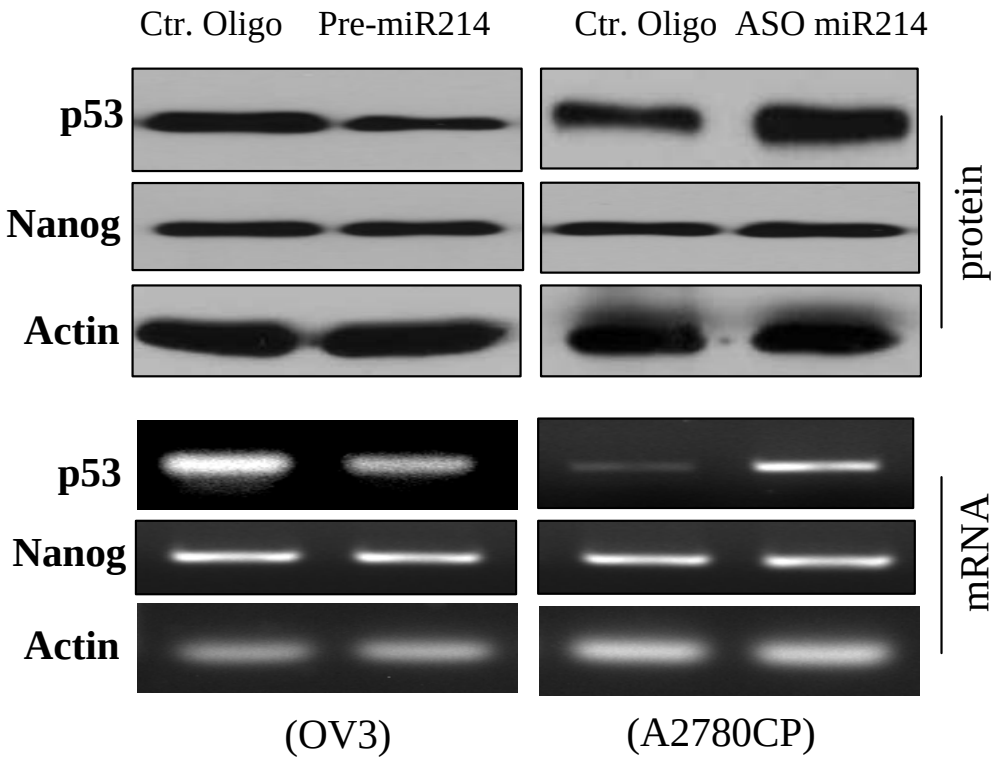

Ctr. Oligo
Pre-miR214
Ctr. Oligo
ASO miR214
p53
Nanog
Actin
protein
p53
Nanog
Actin
mRNA
(OV3)
(A2780CP)

Figure S10