Low MAD2 expression levels associate with reduced progression-free survival in patients with high-grade serous epithelial ovarian cancer

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1a) MAD2 nuclear immunohistochemical staining

Staining intensity = +2  Staining intensity = +3  Staining intensity = +4

1b) PFS in months

% survival

--- Low MAD2
--- High MAD2
2a) MAD2 mRNA expression

2b) MAD2 and β-actin protein levels

2c) Cell viability assay with paclitaxel
3a) Cyclin B1
- 10 50 - 10 50 - 10 50 - 10 50 - 10 50 nM paclitaxel 24 h
Cyclin B1
A2780 ovcar7 UPN251 ovca432 ovca433

3b) PARP
- 10 50 - 10 50 - 10 50 - 10 50 - 10 50 nM paclitaxel 48 h
PARP
A2780 ovcar7 UPN251 ovca432 ovca433

3c) Cell viability

Furlong et al. Figure 3
4a) miR-433 expression

miRNA sequence:

guggcucccgg→guauacu

taccatggataa→catcatgaatttattgacattgt

designed miR-433 sequence:
	Iglaglactaataacgltgtaaca

MAD2 morpholino

4b) miR-433 expression

relative quantification

|        | ovca432 | ovca433 | ovca7 | UPN251 | A2780 |
|--------|---------|---------|-------|--------|-------|
| + pre-miR-scr |       |         |       |        |       |
| + pre-miR-433 |       |         |       |        |       |
| - pre-miR-scr |       |         |       |        |       |
| - pre-miR-433 |       |         |       |        |       |

4c) densitometry

|        | MAD2 | β-actin |        | MAD2 | β-actin |        |
|--------|------|---------|--------|------|---------|--------|
| + scr-morpho |      |         |        |      |         |        |
| - scr-morpho |      |         |        |      |         |        |
| + pre-miR-433 |    |         |        |      |         |        |
| - pre-miR-433 |    |         |        |      |         |        |

4d) luciferase activity

|        | luciferase activity % of control |        |        |
|--------|---------------------------------|--------|--------|
| + pre-miR-433 | *                                |        |        |
| - pre-miR-433 |                                |        |        |
5a)

![Bar graph showing cell viability with different treatments and miRNA expressions.](image)

5b)

![Western blot images of various proteins with molecular weights indicated.](image)

**Furlong et al. Figure 5**
Furlong et al. Supplemental Figure 1

MAD2

β-actin

pre-miR-scr  pre-miR-433

20 kDa

45 kDa

48  72  96

48  72  96

h
Supplemental Figure 2

PFS in months

% survival

Low miR-433
Intermediate miR-433
High miR-433

S2