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MicroRNAs and the target genes in early tumorigenesis of fallopian/ovarian carcinoma

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Robert H. Lurie Comprehensive Cancer Center

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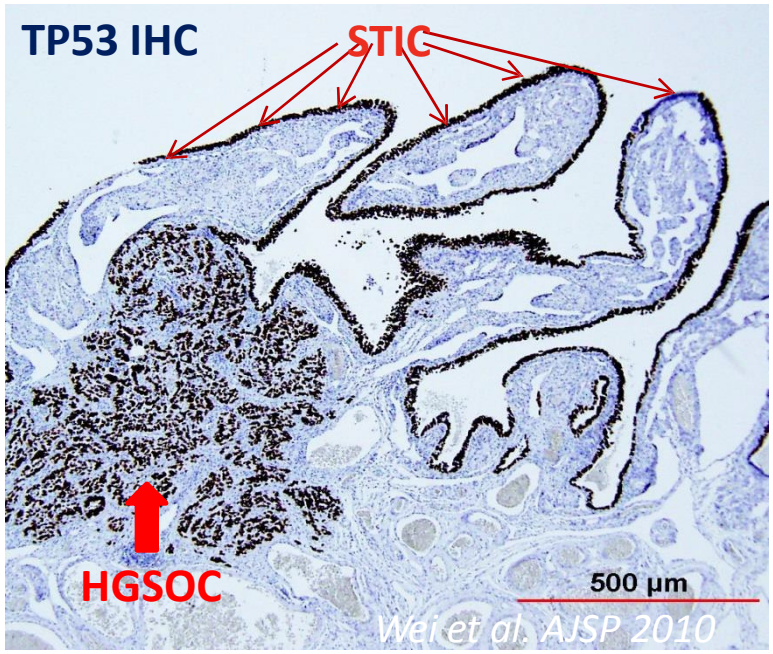
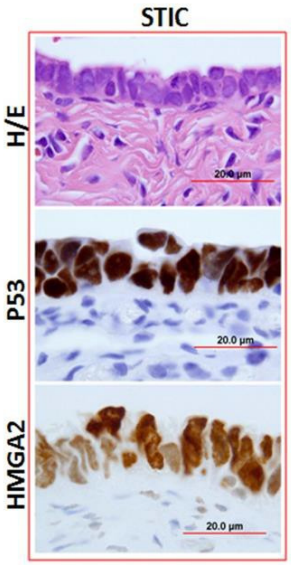
Introduction

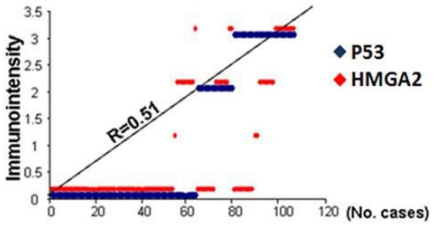
- High-grade serous ovarian carcinomas (HGSOC) are one of the most deadly and common form of ovarian cancers (>70 % of ovarian cancer).
- Recent identification of its precursor lesion, serous tubal intraepithelial carcinoma (STIC), in the fallopian tubes provides a venue for study of early tumorigenesis of HGSOC.
- Characterization of the molecular and genetic alterations in early stage of HGSOC is compelling and a few oncogenic factors have been identified:
  - ***P53*** mutations are common in STIC (>90%). However, *P53* mutations are neither sufficient to trigger a sequence of neoplasm nor rate-limiting.
  - ***BRCA1/2*** mutations are a hallmark of HGSOC. 10-20% of HGSOC have germline or somatic *BRCA1/2* mutations. Women with have a 30%-70% chance of developing PSC by age 70.
  - ***HMGA2*** is a major oncogenic factor in HGSOC. Over 70% of STIC and HGSOC have *HMGA2 overexpression. HMGA2* is defined as a key marker for ovarian cancer (The Cancer Genome Atlas Research Network).

1. Integrated genomic analyses of ovarian carcinoma. (2010) Nature **474**(7353): 609-615.
2. Bowtell, D. D. (2010). Nat Rev Cancer **10**(11): 803-808.
3. Mahajan, A., Z. Liu, et al. (2010). Mod Pathol **23**(5): 673-681.
4. Park, S. M., S. Shell, et al. (2007). Cell Cycle **6**(21): 2585-2590.
5. Press, J. Z., A. De Luca, et al. (2008). BMC Cancer **8**: 17.
6. Risch, H. A., J. R. McLaughlin, et al. (2006). J Natl Cancer Inst **98**(23): 1694-1706.
7. Salani, R., R. J. Kurman, et al. (2008). Int J Gynecol Cancer **18**(3): 487-491.
8. Wei, J. J., J. Wu, et al. (2010). Am J Surg Pathol **34**(1): 18-26.
9. Zhang, S., R. Royer, et al. (2011). Gynecol Oncol **121**(2): 353-358.





Tumor types	P53 % (cases)	HMGA2 % (cases)
STIC	70.9 (24)	75 (24)
HG-PSC	75 (54)	86 (54)
LG-PSC	20 (10)	10 (10)
Other ovarian	27 (48)	13 (48)



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Rationale and Hypothesis

- BRCA1 mutations account for 10%-20% of cases and 50-60% HGSOC have low or absence of BRCA1 expression.

1. Radosa, M. P., et al. (2011). Int J Gynecol Cancer **21**(8): 1399-1406.
2. Weberpals, J. I., D. Tu, et al. (2011). Ann Oncol **22**(11): 2403-2410.
3. McMillen B et al. (2012). Mol Path (July online publication)
4. Carser, J. E., et al. (2011). Gynecol Oncol **123**(3): 492-498.

- *P53* is a major regulator and many miRNAs are regulated by P53

1. Chang, T. C., E. et al. (2007). Mol Cell **26**(5): 745-752.
2. Suzuki, H. I., et al. (2009). Nature **460**(7254): 529-533

- HMGA2 regulates microRNA expression and is also the major target of miRNAs

1. Mayr, C., M. et al. (2007). Science **315**(5818): 1576-1579.
2. Park, S. M., et al. (2007). Cell Cycle **6**(21): 2585-2590.
3. Shell, S., et al. (2007). Proc Natl Acad Sci U S A **104**(27): 11400-11405.
4. Wu, J., et al. (2011). Cancer Res **71**(2): 349-359.

Table 5 BRCA1 protein expression analyses scored by immunohistochemistry in this study and studies published recently

No. of cases	*BRCA1 antibody*	*Immunoreactivity*				*References*
		Negative (%)	*Weak (%)*	*Moderate (%)*	*Strong (%)*	
292	MS110		41		59	Carser *et al*[21]
27	MS110		44		56	Radosa *et al*[20]
251	MS110	16	49	24	11	Weberpals *et al*[22]
117	MS110	13	38	39	10	McMillen *et al* (this study)

Hypothesis:

MicroRNA dysregulation is involved in early tumorigenesis of HGSOC

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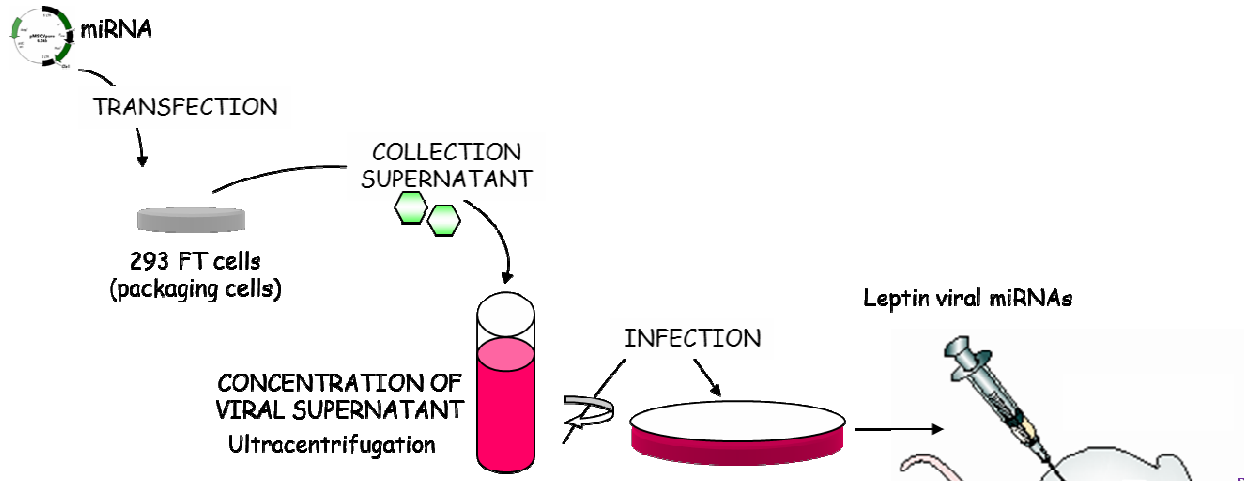
Experimental designs

- Examine global microRNA expression in FT, STIC and HGSOC
- Identify oncogenic miRNAs dysregulated in HGSOC
- Characterize the oncogenic functions of miRNAs *in vitro* and *in vivo*
- Investigate the specific target genes associated with HGSOC



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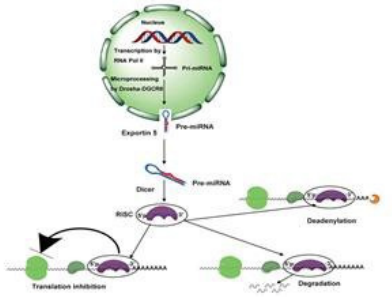
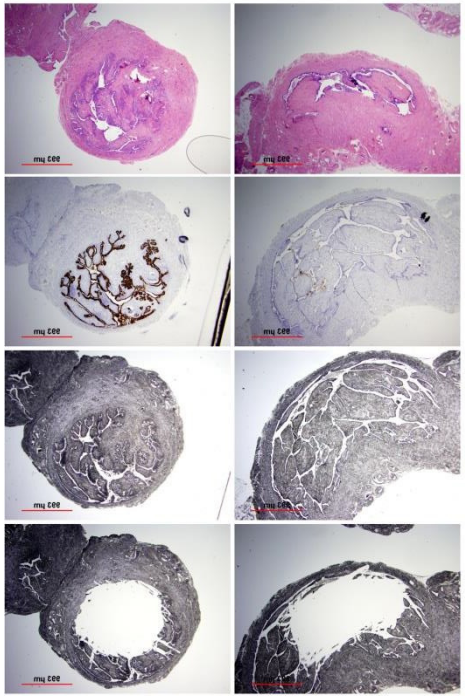
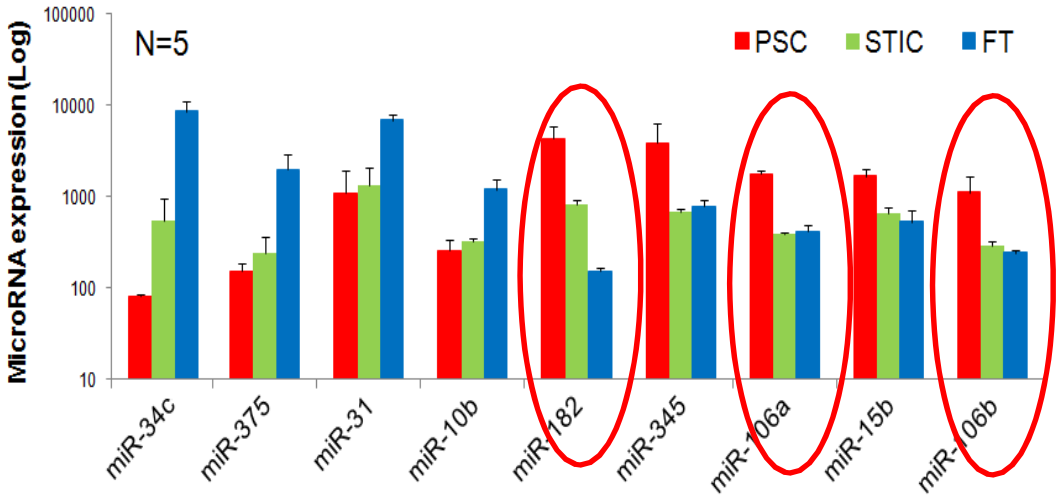
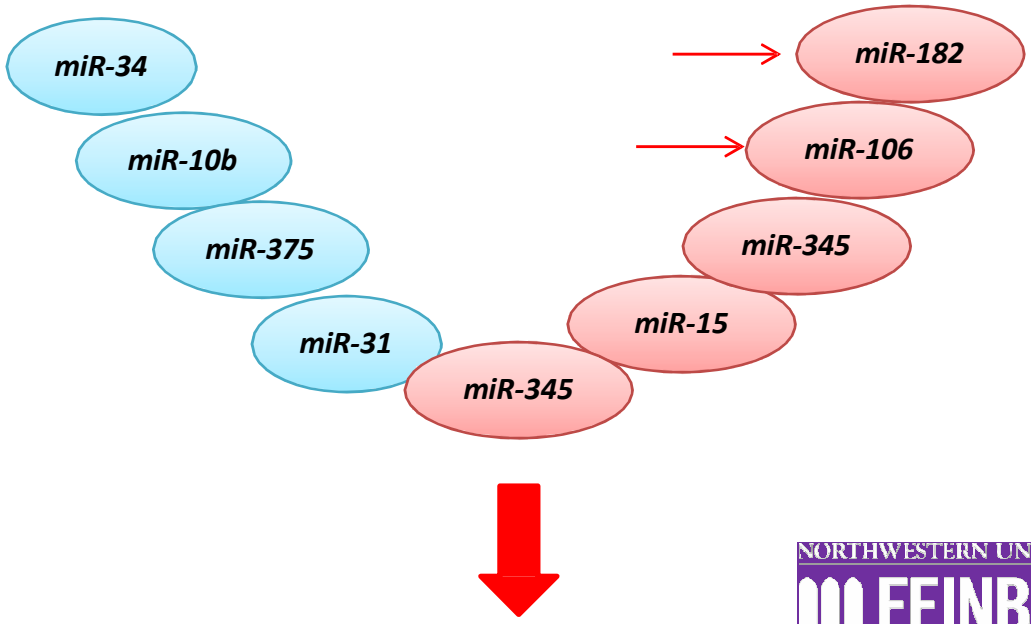
microRNA dysregulation in early and advanced ovarian cancer

MicroRNA maturation and its functions









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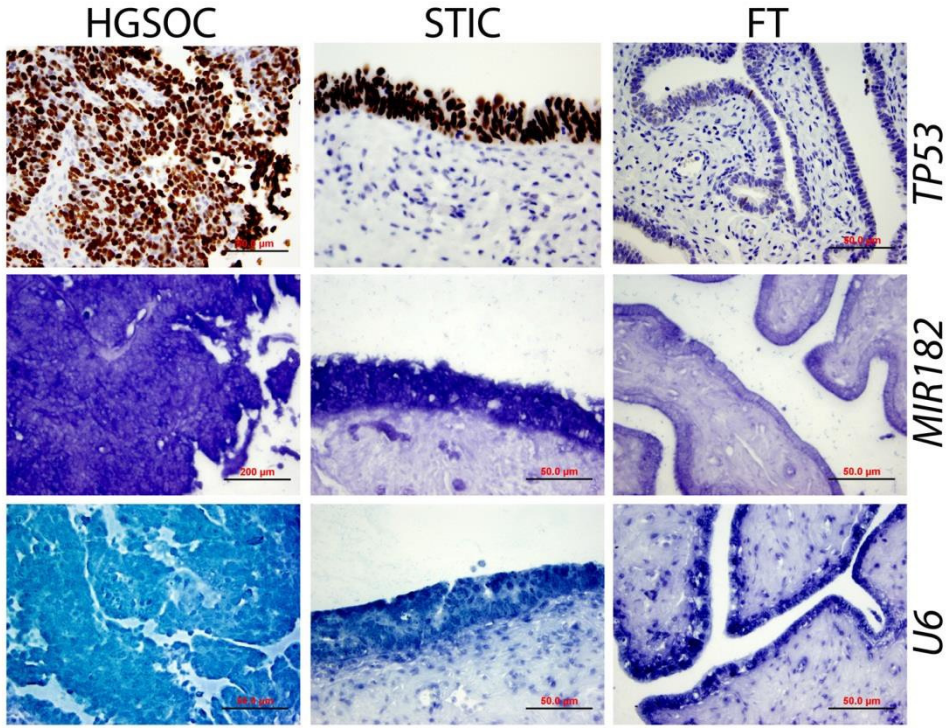
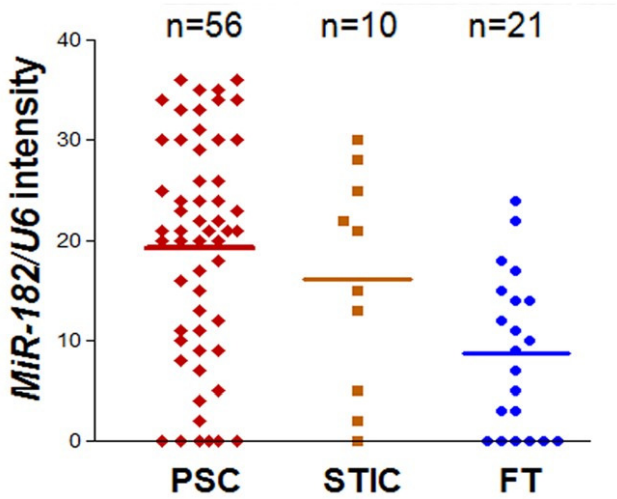
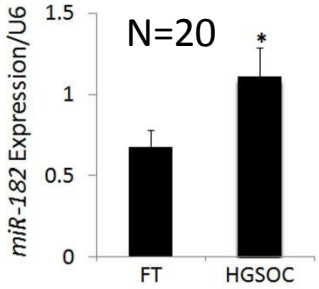
MIR182 expression is validated by TaqMan RT-PCR and miRNA *in situ* hybridization in FT, STIC and HGSOC



MIR182 in situ hybridization in high grade serous ovarian carcinoma (right), serous tubal intraepithelial carcinoma (mid) and fallopian tube (right). U6 as RNA loading control and TP53 IHC for mutant TP53.



Dot plot analysis of relative MIR182 expression in HGSOC, STIC and FT, normalized by U6.



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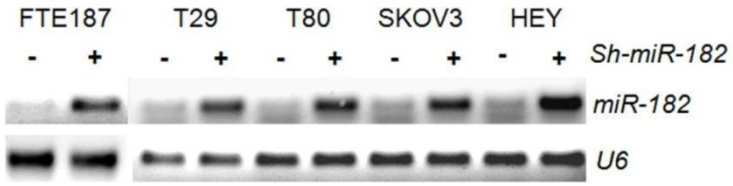
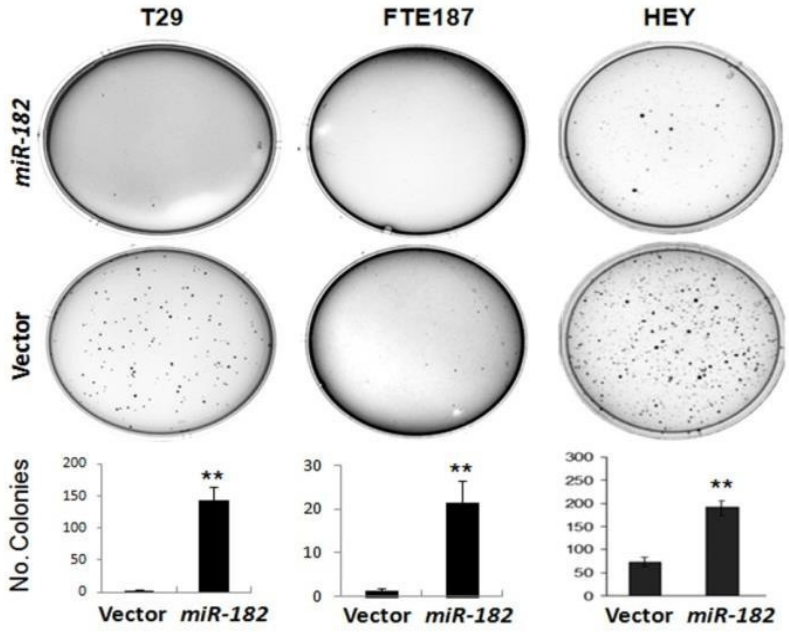
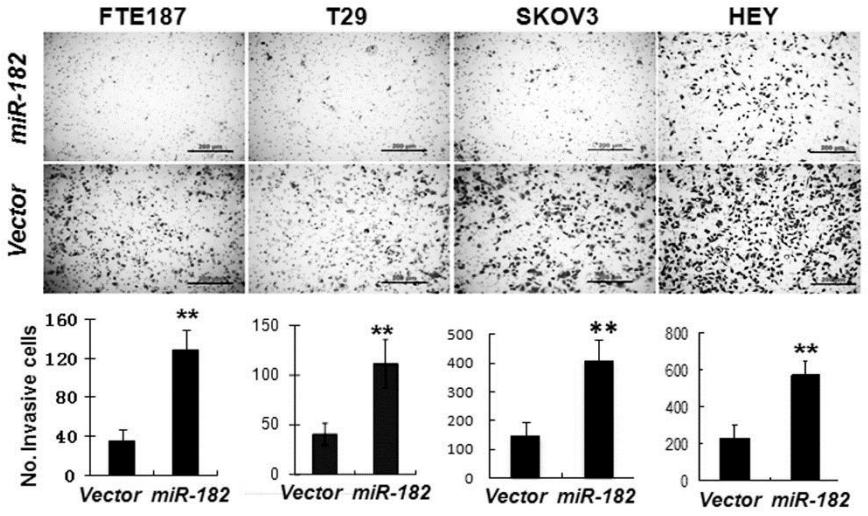
Oncogenic properties of *MIR182* in benign and malignant ovarian cancer cell lines

- Stable *MIR182* overexpression was established by lentiviral transfections in 3 normal and 3 cancer cell lines



- Introducing *MIR182* overexpression results in increased anchorage independent growth in normal ovarian surface epithelial (T29, T80), fallopian tube secretory epithelial (FTE187) and ovarian cancer (HEY, OVCAR3 and SKOV3) cell lines





- Introducing *MIR182* overexpression enhances cell migration/invasion in Mitragel

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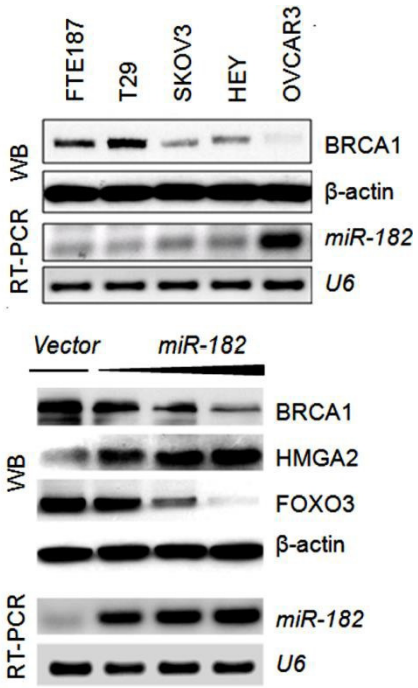
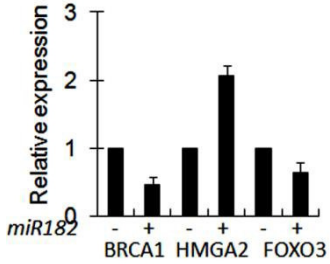
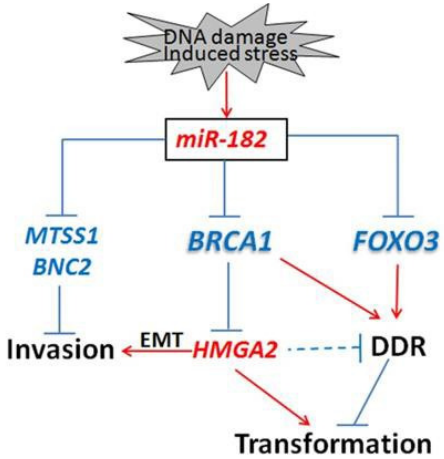
HMGA2 is regulated by *MIR182* through its negative regulation of *BRCA1*

- Two independent studies show:
  - *BRCA1* is negatively regulated by *MIR182 (Moskwa, P., et al. (2011). Mol Cell **41**(2): 210-220)*
  - BRCA1 inhibits *HMGA2* expression at transcriptional level (*Ahmed, K. M., et al. (2010). J Biol Chem **285**(7): 4464-4471.)*
- We confirmed that *MIR182* enhances *HMGA2* expression through double negative regulation and this regulation is dose-dependent in both benign and malignant ovarian cell lines
  - *Liu Z et al. (2012). J*







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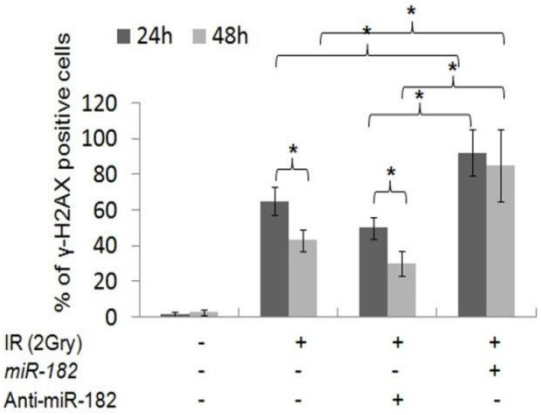
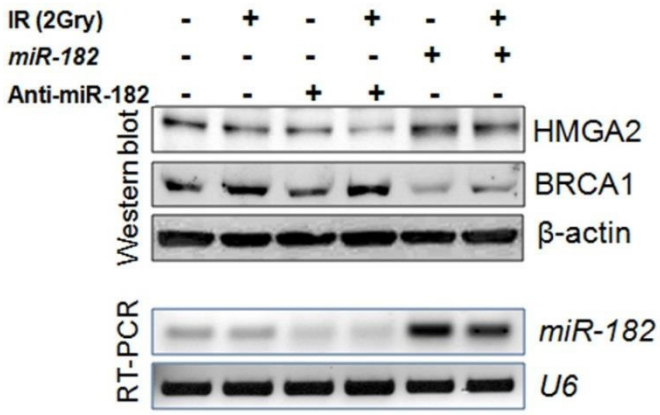
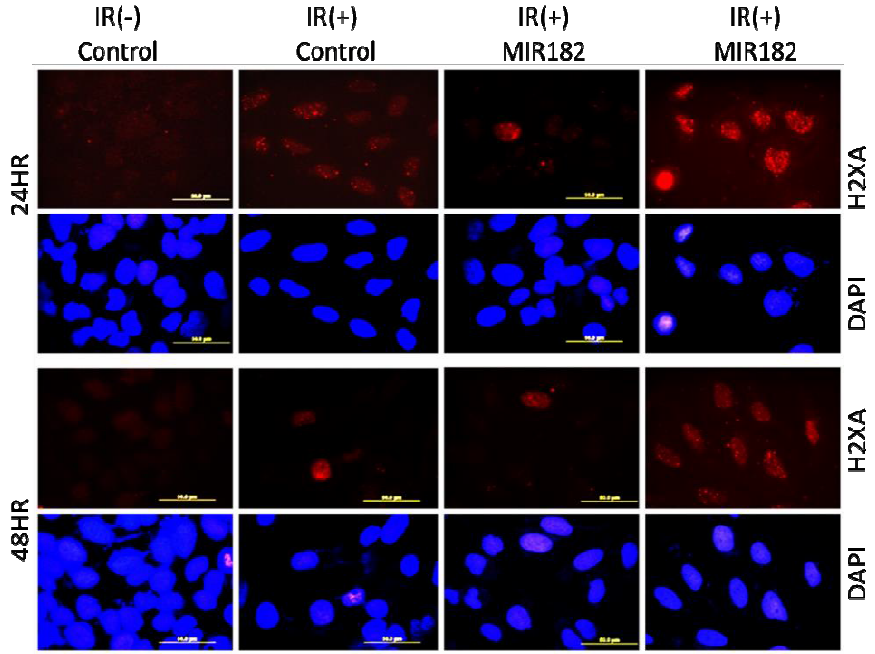
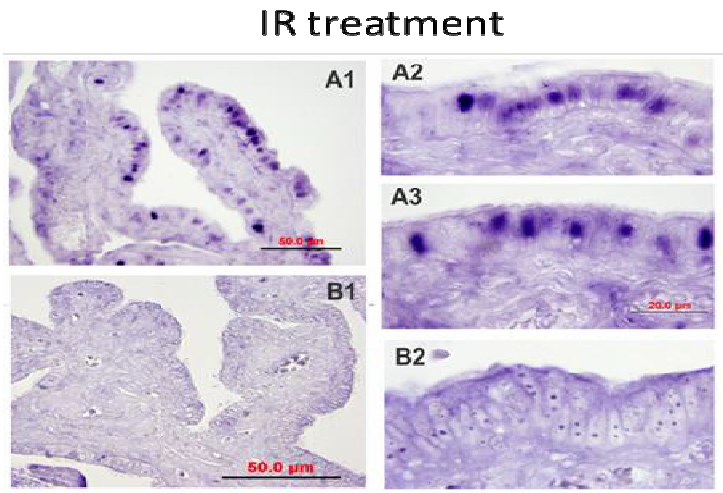
MIR182 overexpression impairs DNA damage response (DDR) by IR exposure

- Low dose IR (2Gy) exposure in FTE187 cells with *MIR182* overexpression revealed significantly delayed DDR. This results can be repeated in T29 cell line.
- Delayed DDR maybe related to *MIR182*-mediated BRCA1 and HMGA2 dysregulation









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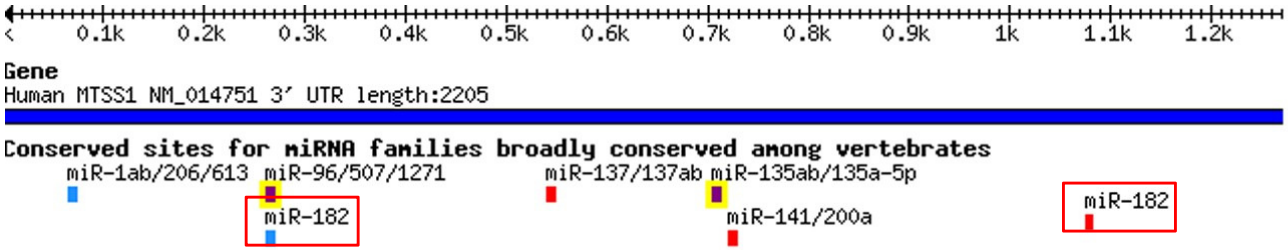
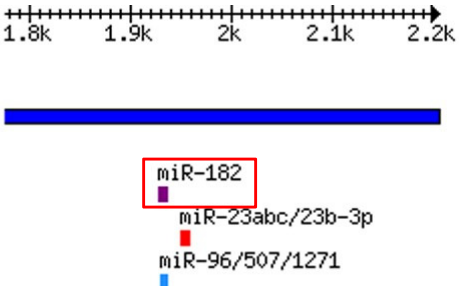
Characterizing miR-182 target gene MTSS1





- *MTSS1* (metastasis suppressor 1) is a tumor suppressor.
- Downregulation of *MTSS1* enhances the growth, invasion and mobility of ovarian and breast cancer cells and poor clinical outcome.
  - *Huynh, C., et al. (2011). Oncogene **30**(12): 1481-1488.*
  - *Liu, Z., J. Liu, et al. (2012). J Pathol.*
  - *Parr, C. and W. G. Jiang (2009). Eur J Cancer **45**(9): 1673-1683.*

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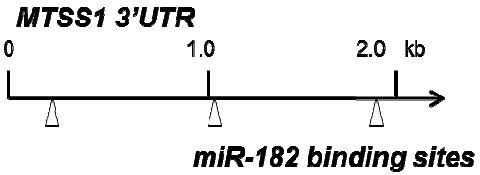

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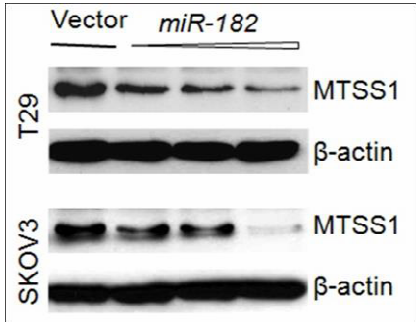

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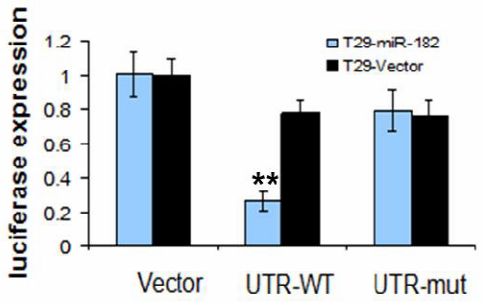

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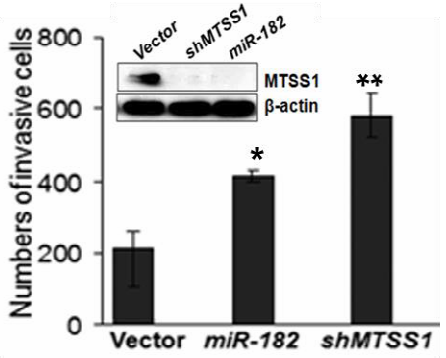

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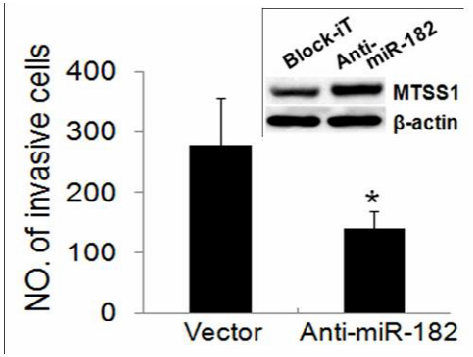

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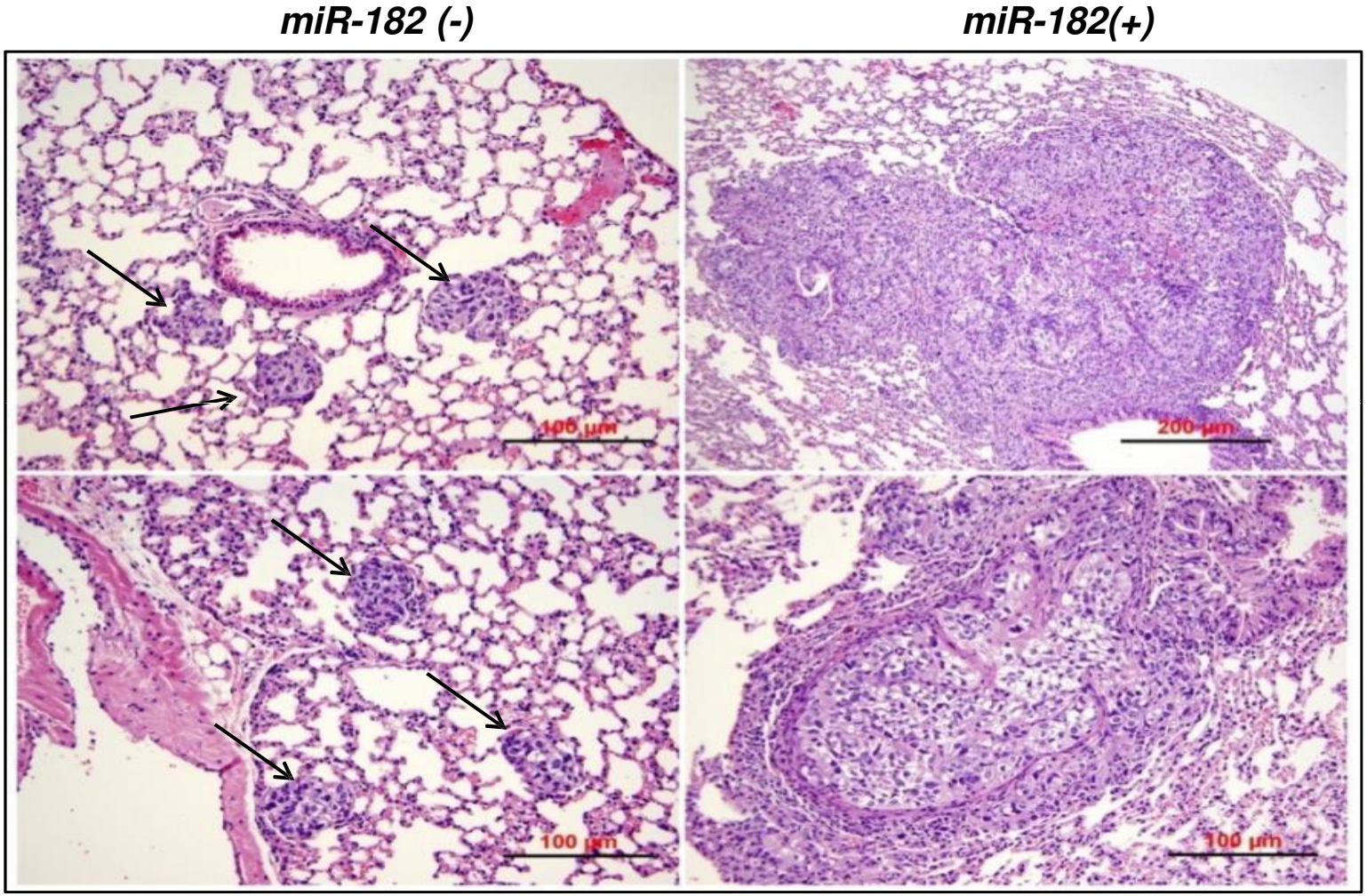
Tumor burden for miR-182

Pathology-IV: Histology and IHC



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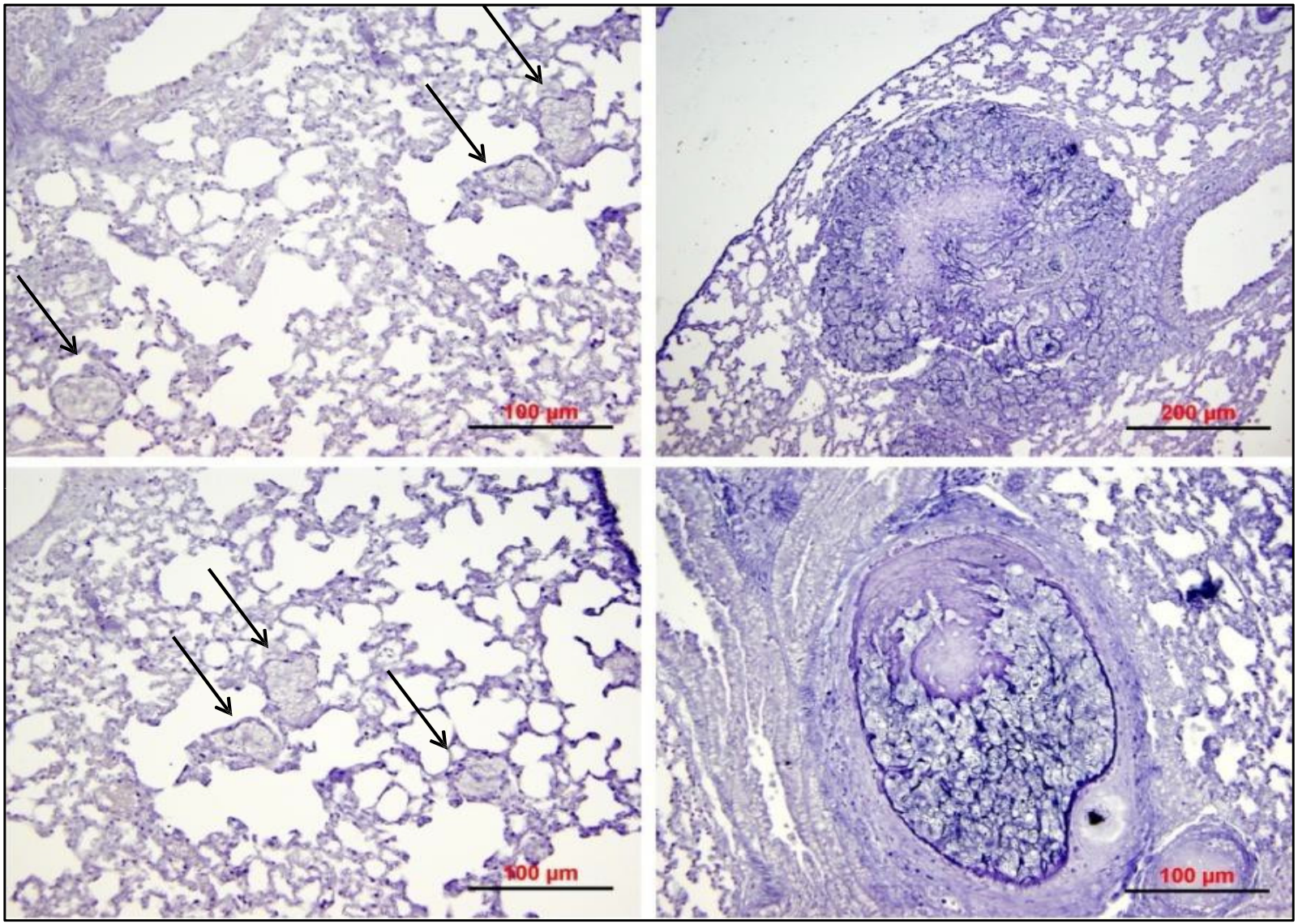
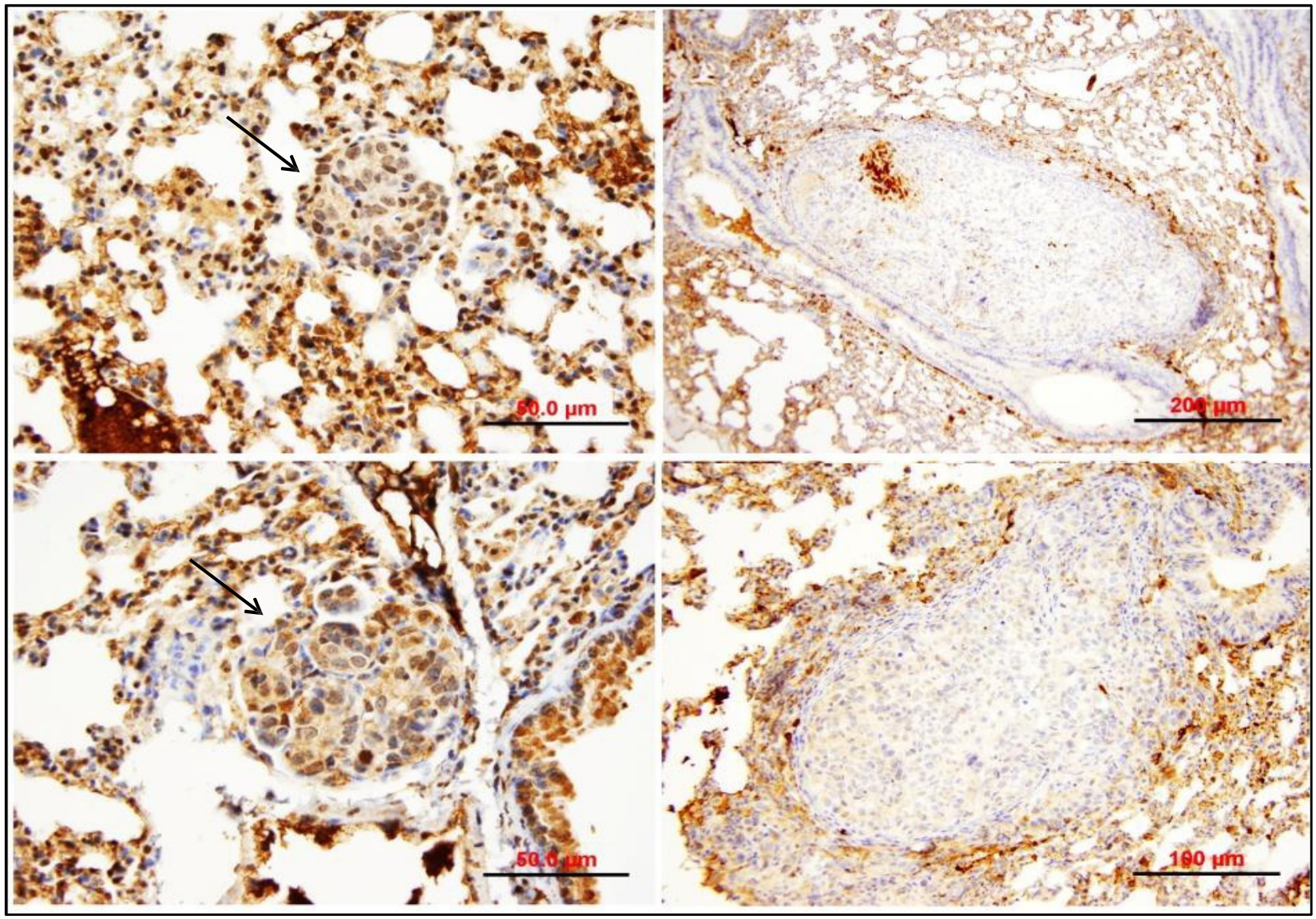
miR-182 (-)

miR-182(+)



MTSS1

miR-182 (-) ***miR-182(+)***



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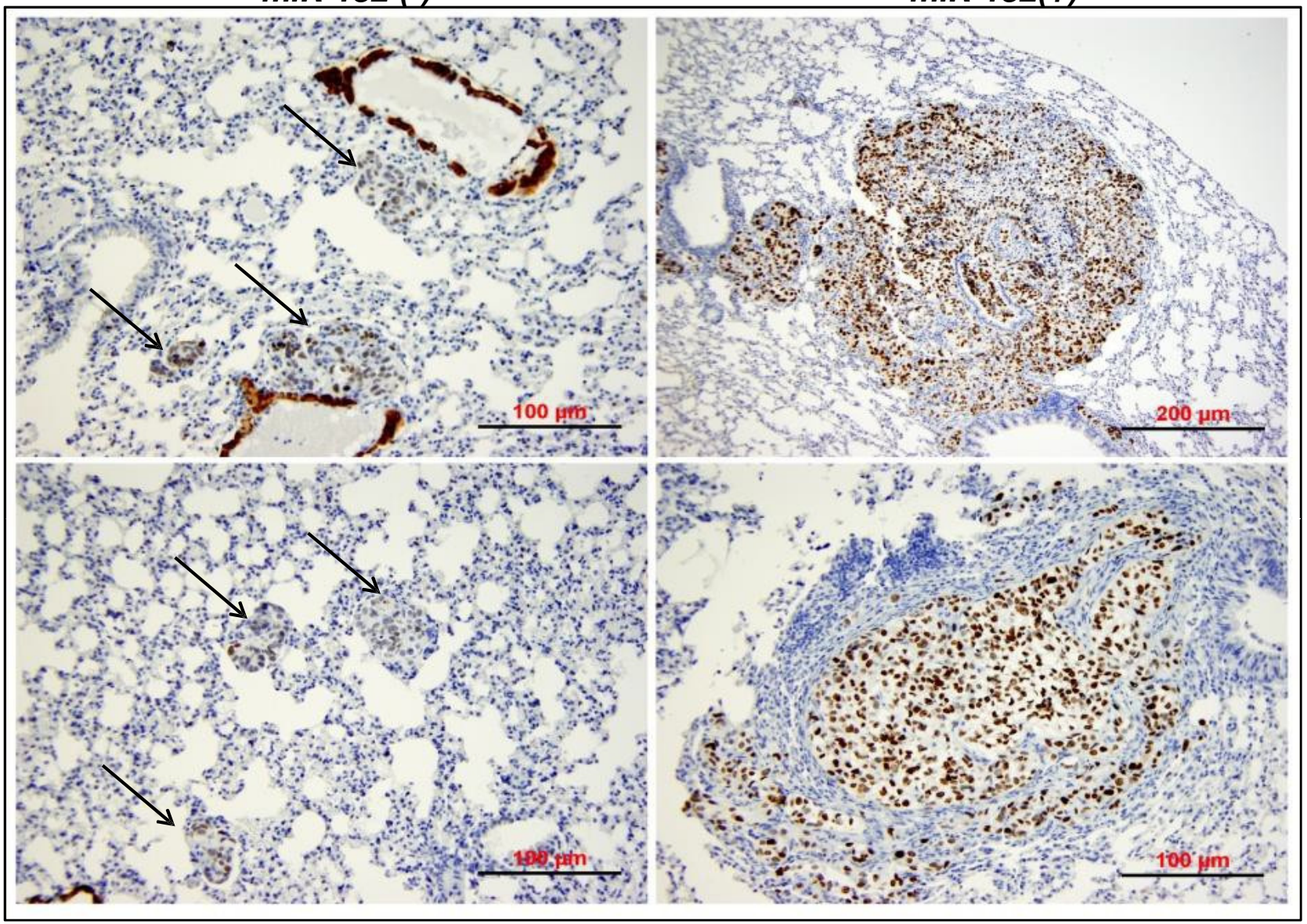
miR-182 (-)

miR-182(+)

HMGA2



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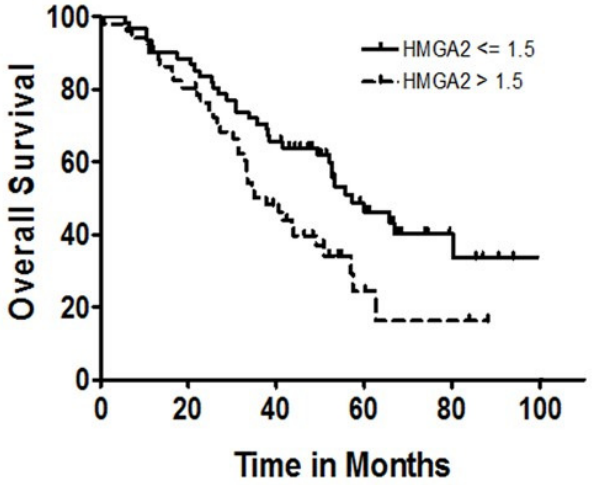
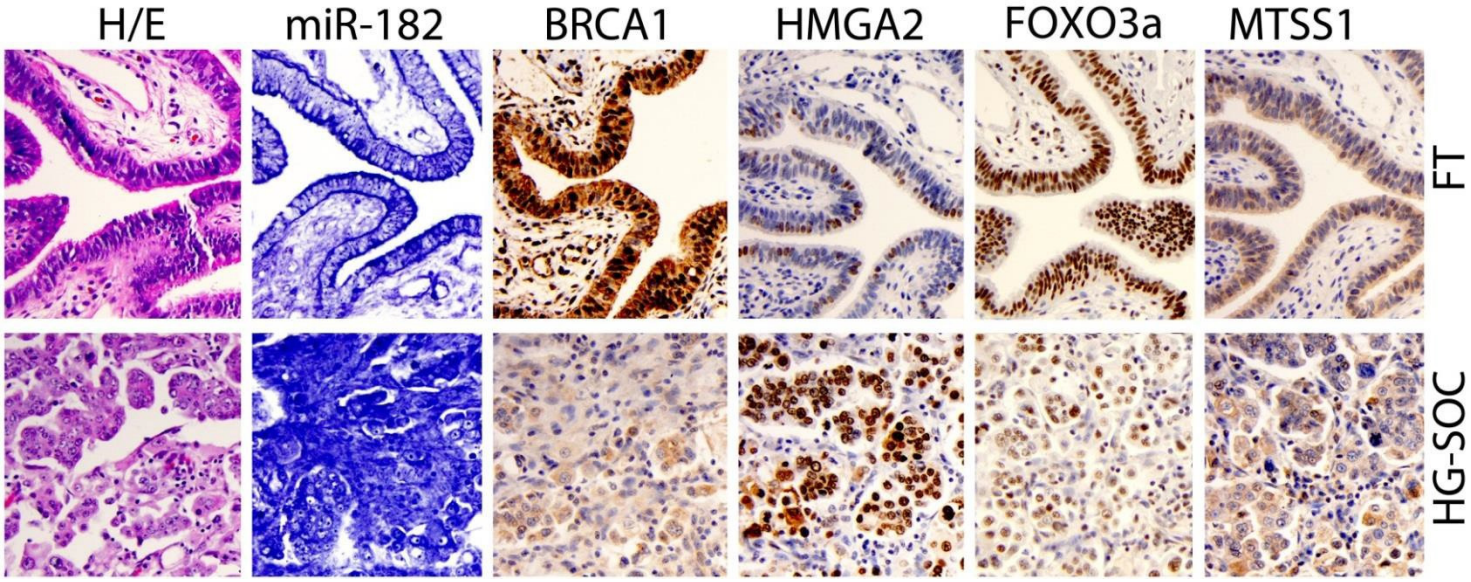
MiR-182 and its target gene expression in high grade serous ovarian cancer

Markers	Fallopian Tube		High grade serous ovarian carcinoma		
	50th P	min-max	50th P	min-max	*p* values
No. cases	30		117		
MiR-182	1	1.00-3.00	2	0.50-3.00	**0.0003**
BRCA1	2	1.00-3.00	1	0.00-3.00	**< 0.0001**
FOX03a	3	0.00-3.00	1.5	0.00-3.00	**< 0.001**
MTSS1	1	0.00-2.00	1	0.00-2.50	0.21
HMGA2	1	0.00-3.00	1.5	0.00-3.00	**0.04**





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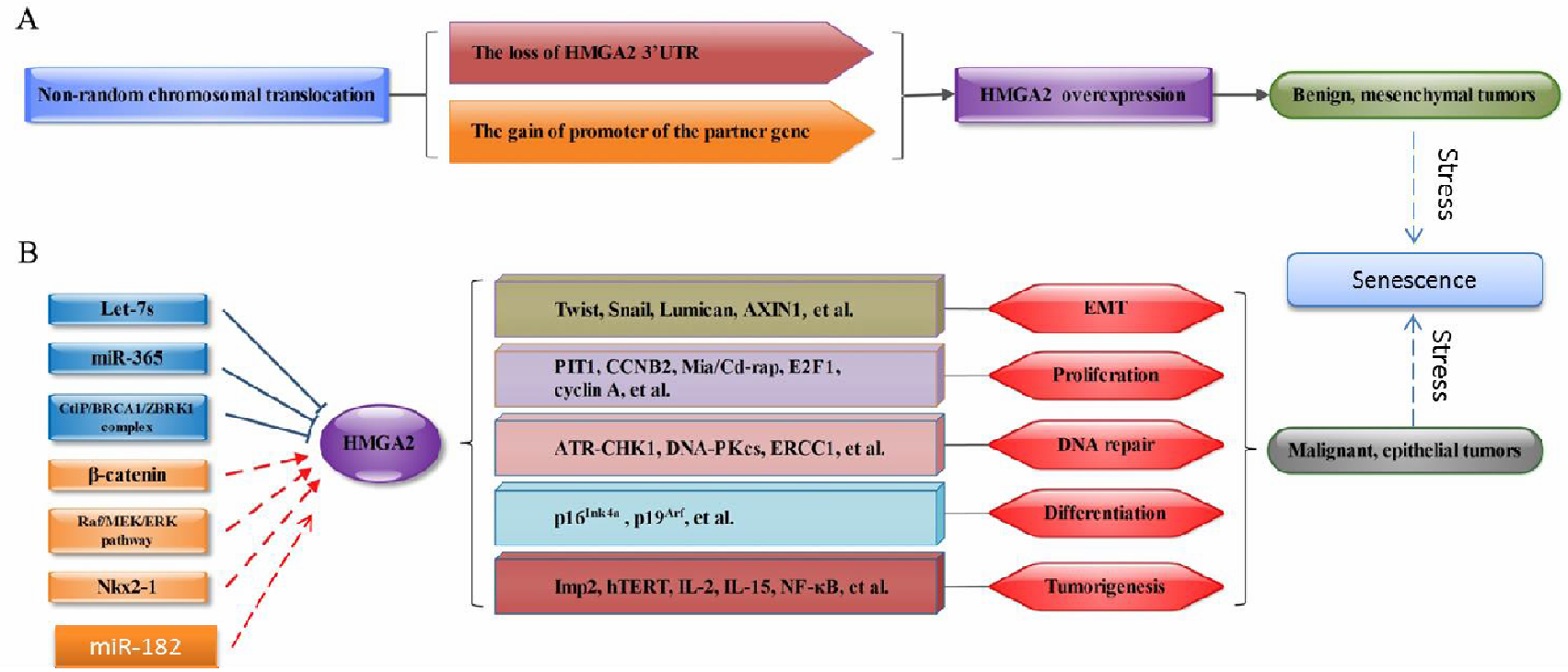
HMGA2 and cancer



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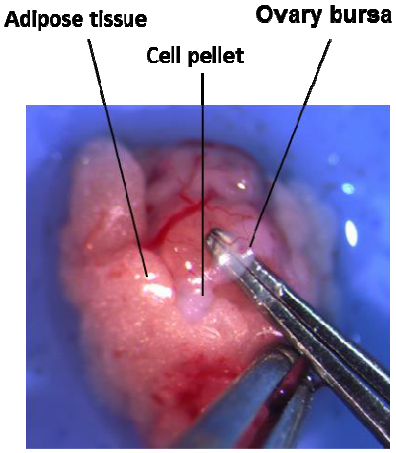
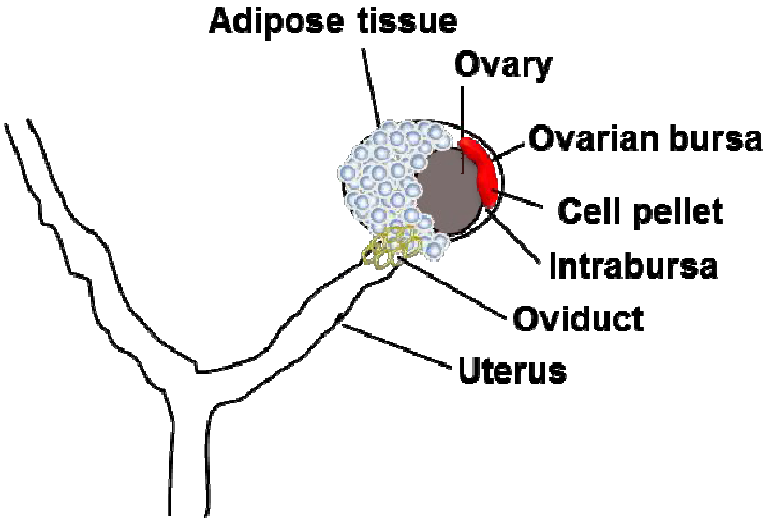
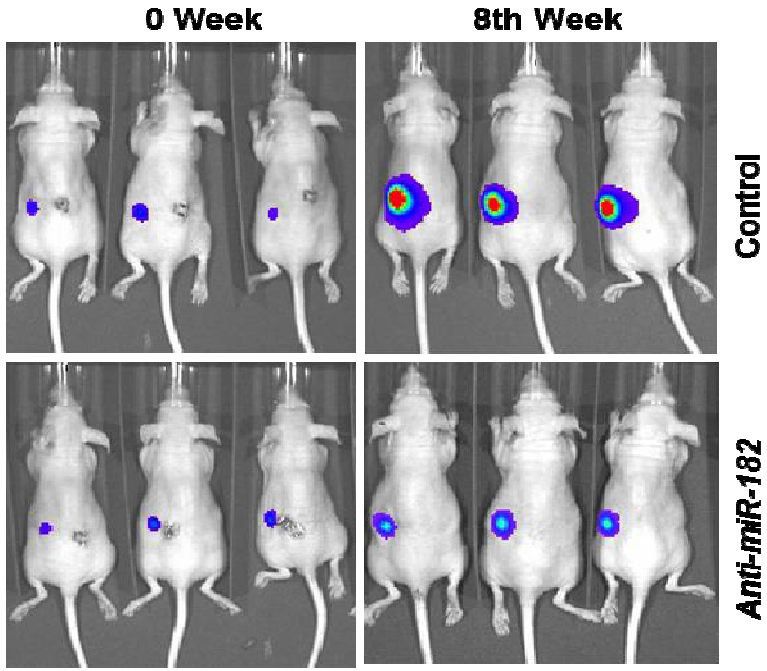
anti-miR-182 in vivo

Orthotopic mouse model of ovarian cancer







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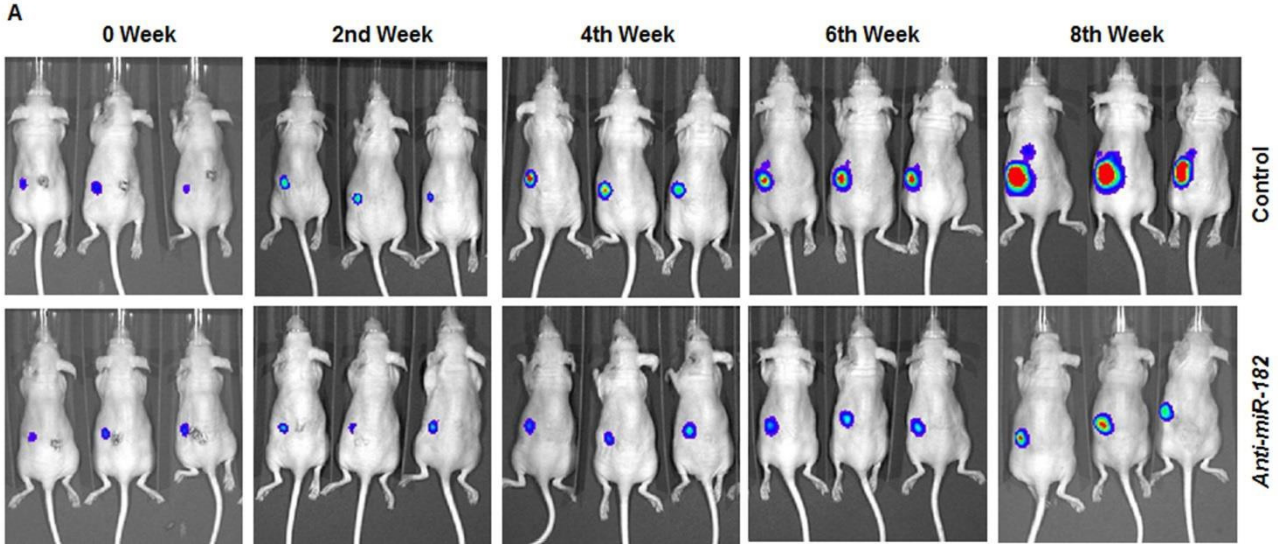
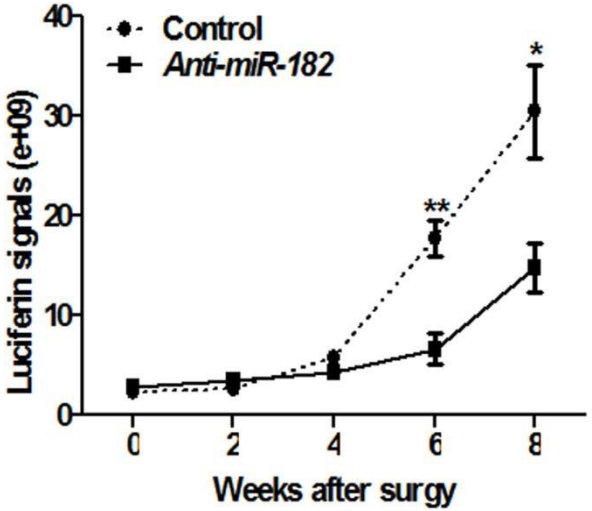
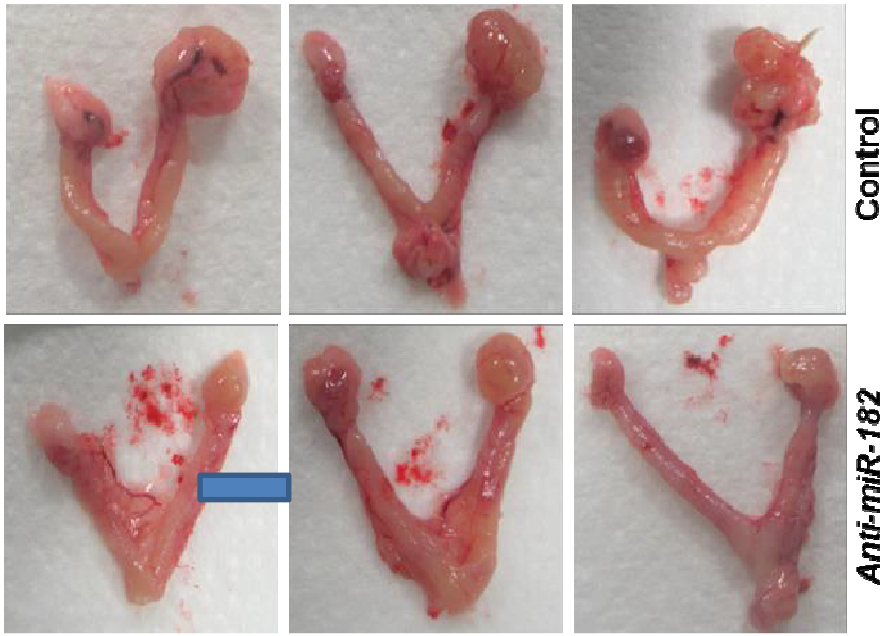
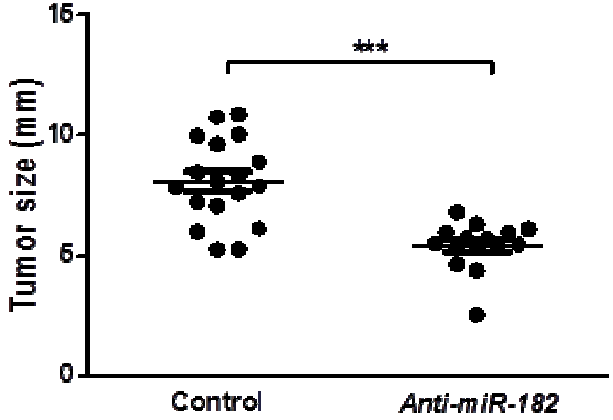
Therapeutic potential of *anti-miR-182 in vivo*

Pathology V: *Experimental animal pathology*









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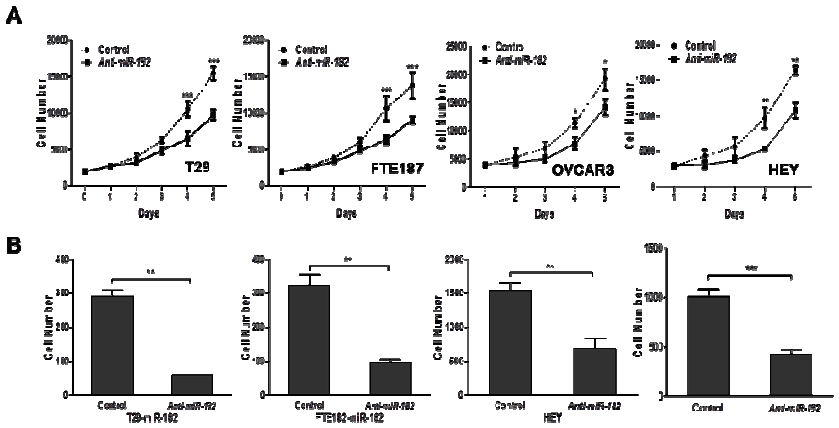
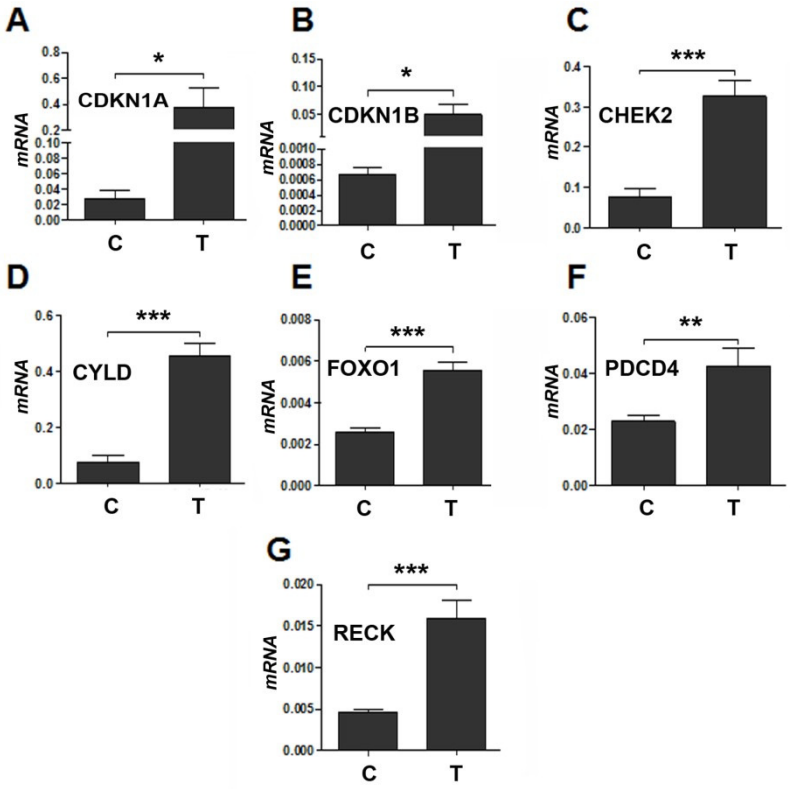
Anti-miR-182 inhibit tumor proliferation





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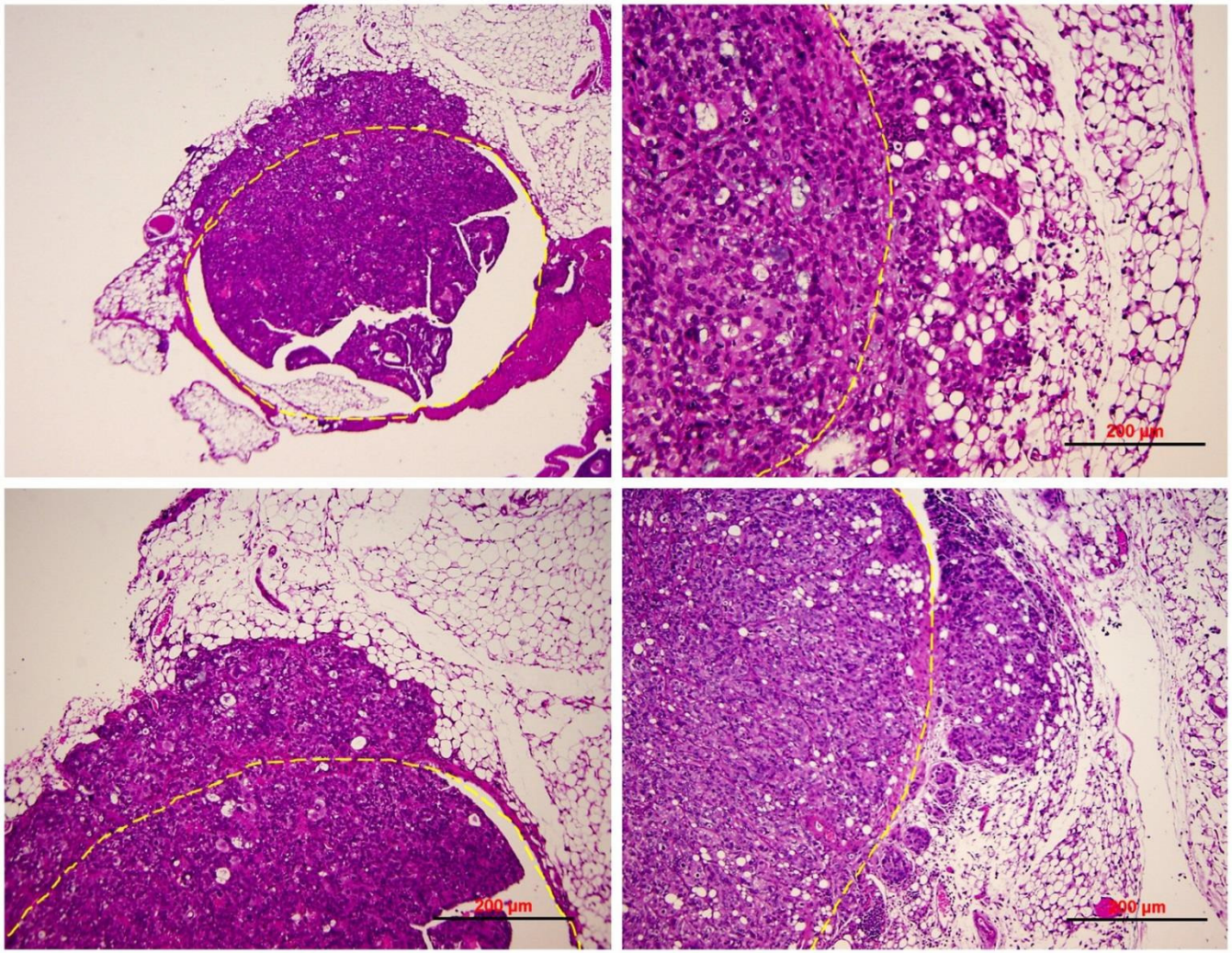
Ovarian cancer invasion through intrabursa



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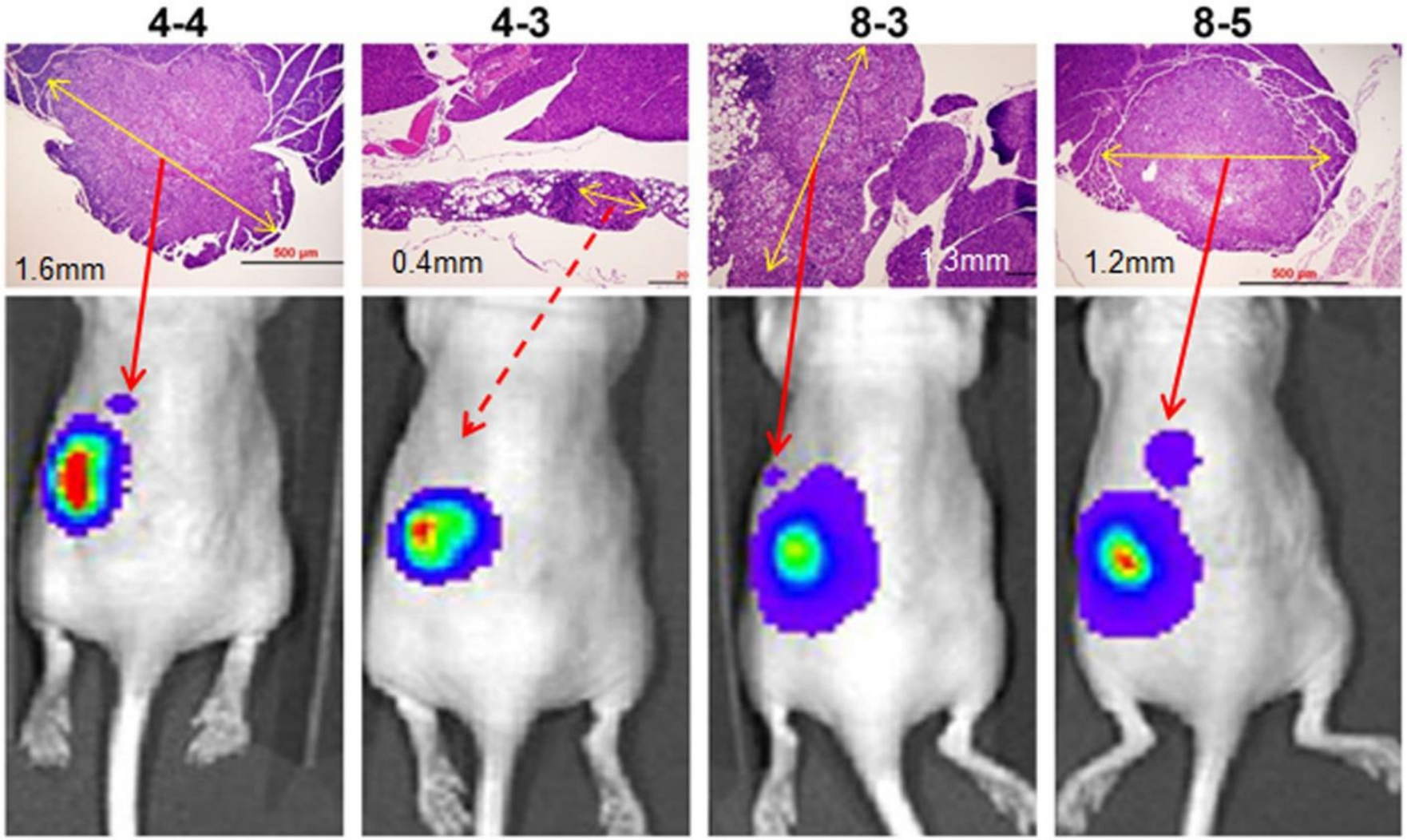
Ovarian cancer metastasis in pancreas



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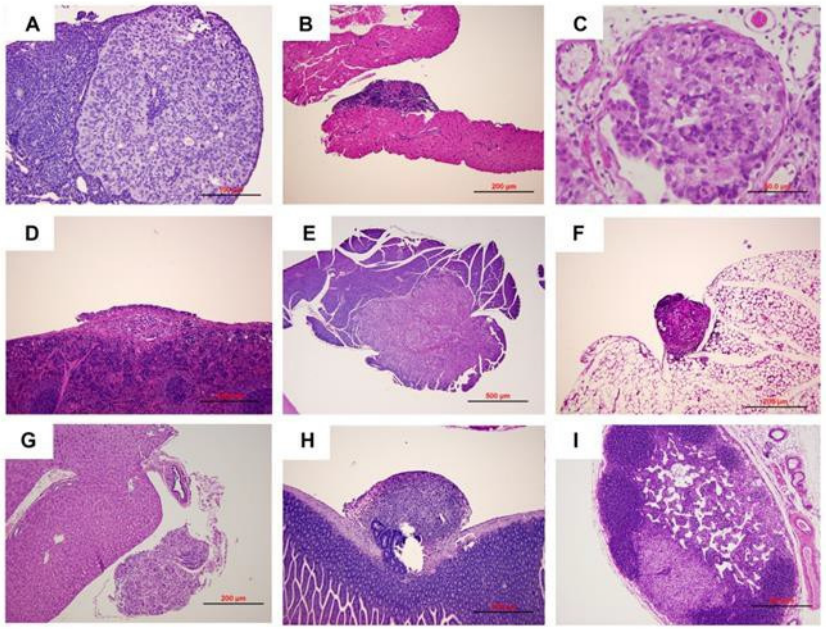
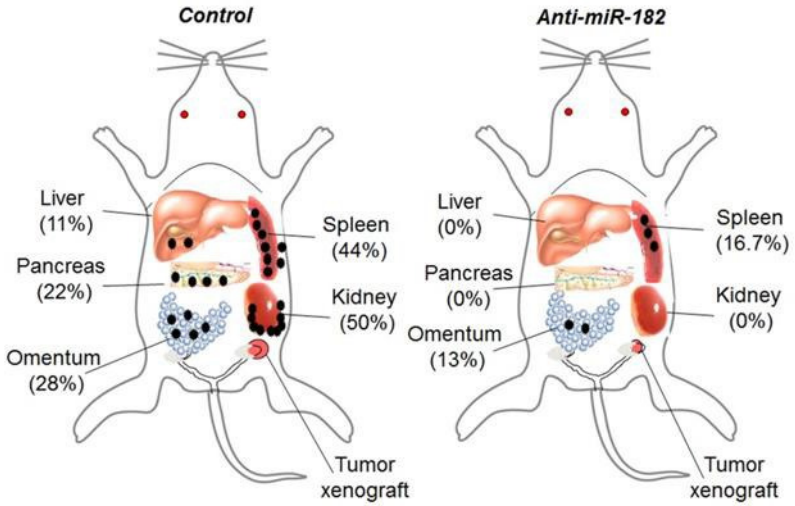
Anti-miR-182 reduces metastasis





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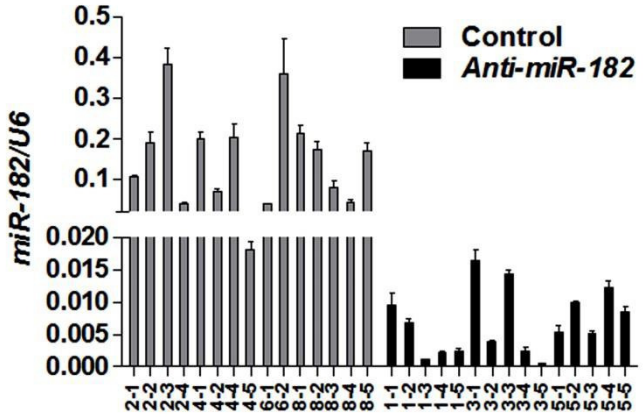
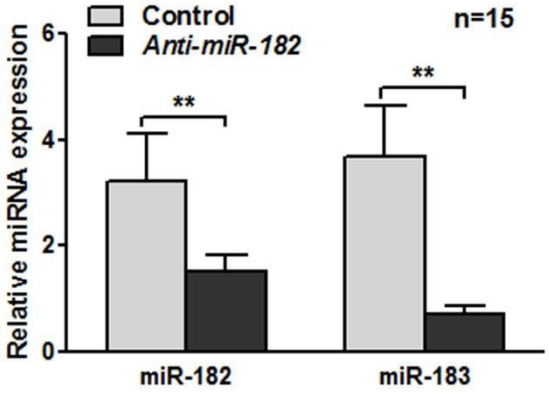
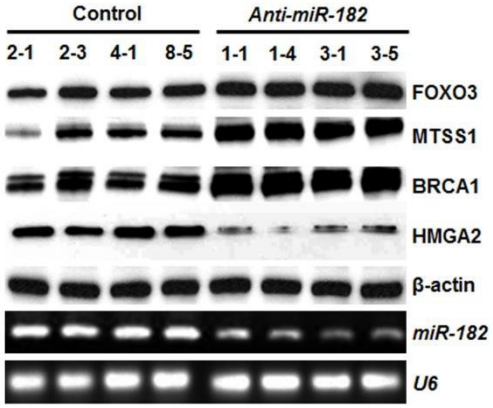
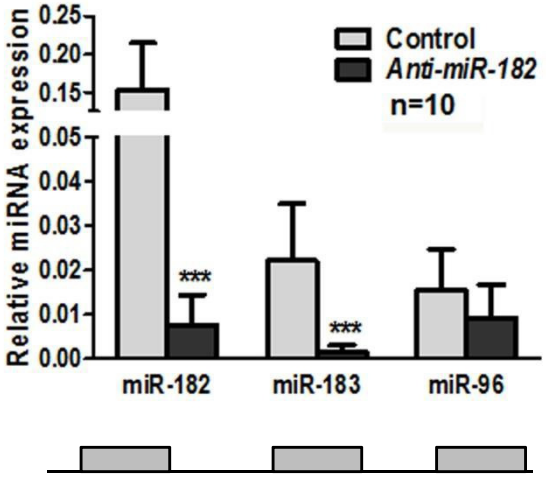
Anti-*miR-182* blocks *miR-182* expression and restores tumor suppressor genes in tumor xenograft

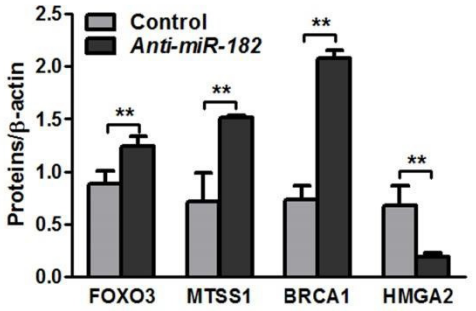




Mouse serum







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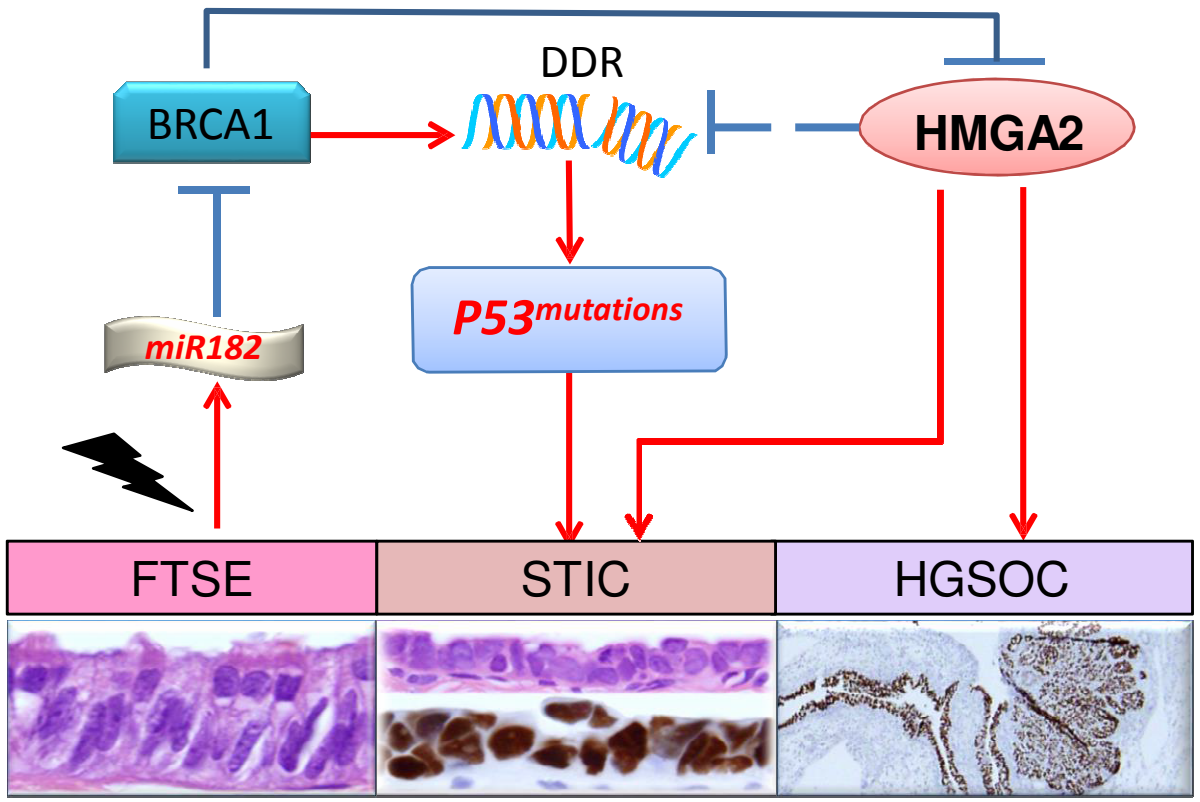

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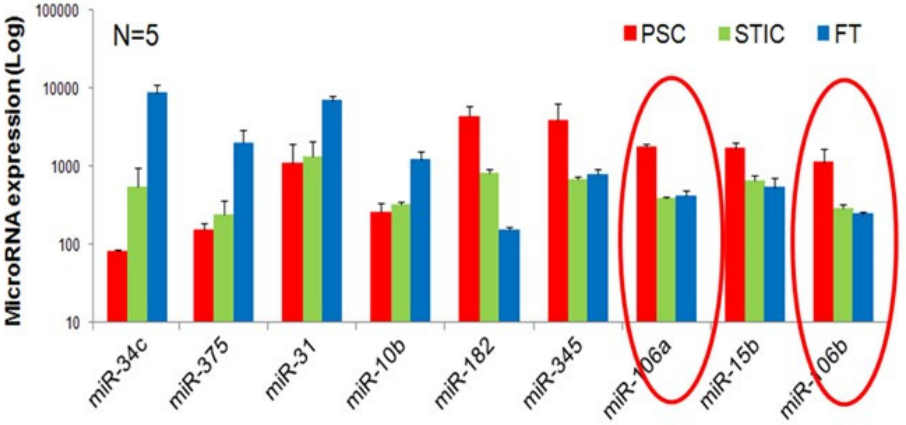
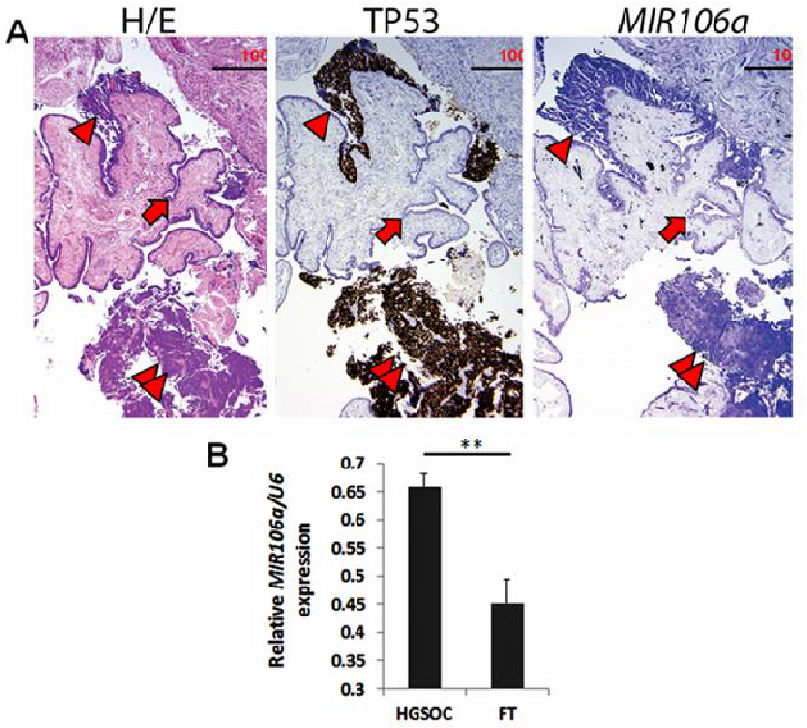
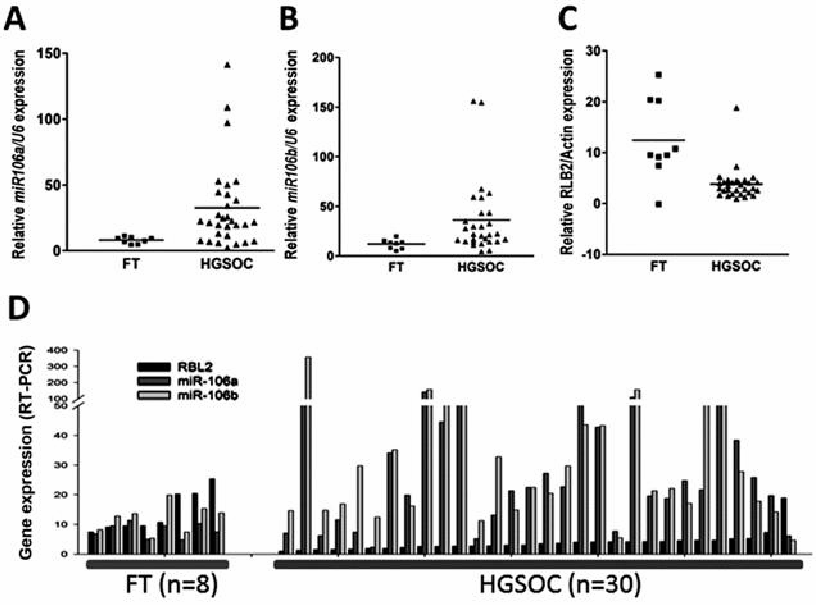
miR-106a expression in HGSOC



microRNA in situ hybridization



Real-time RT-PCR



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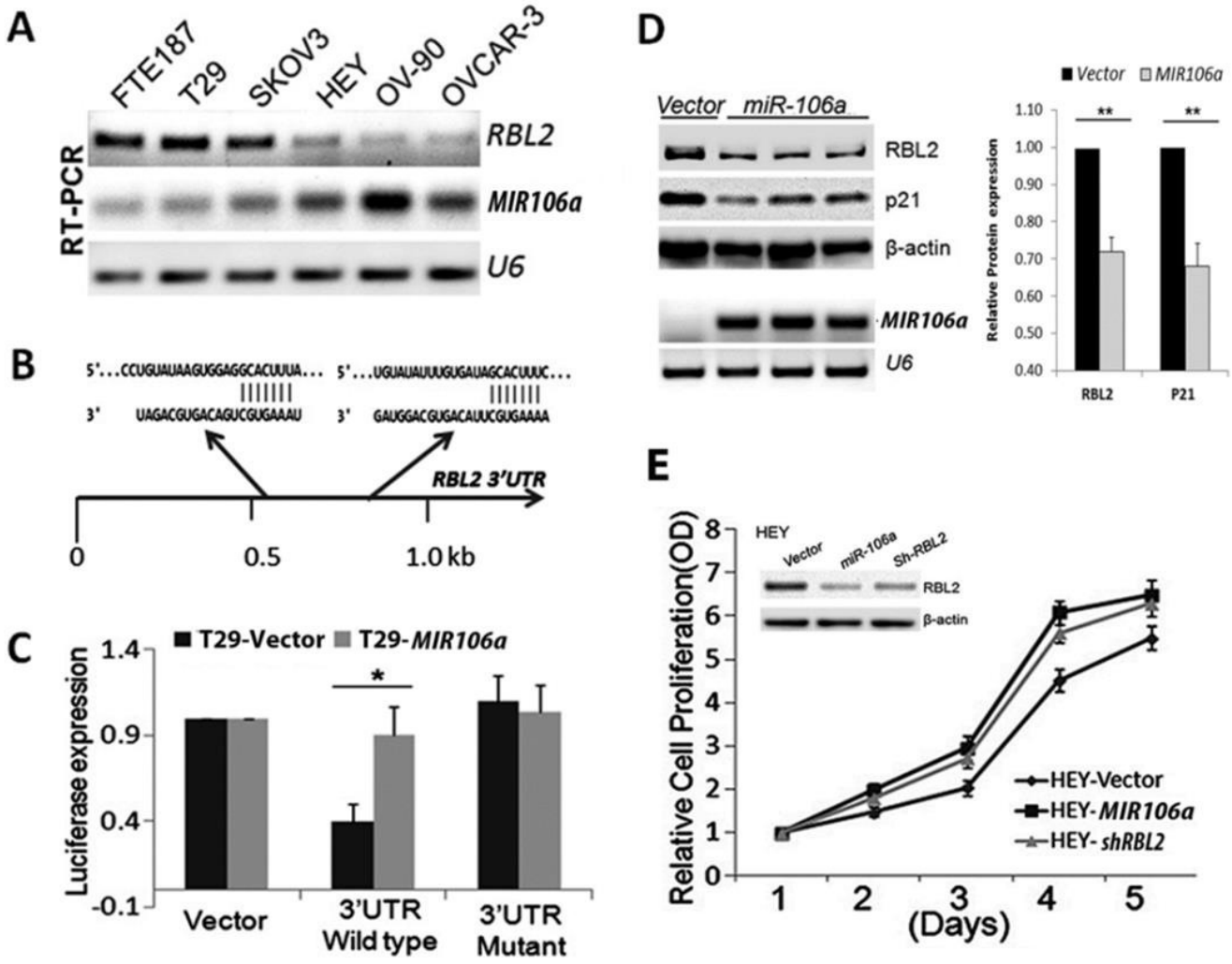
Characterizing *miR-106* target gene *RBL2*



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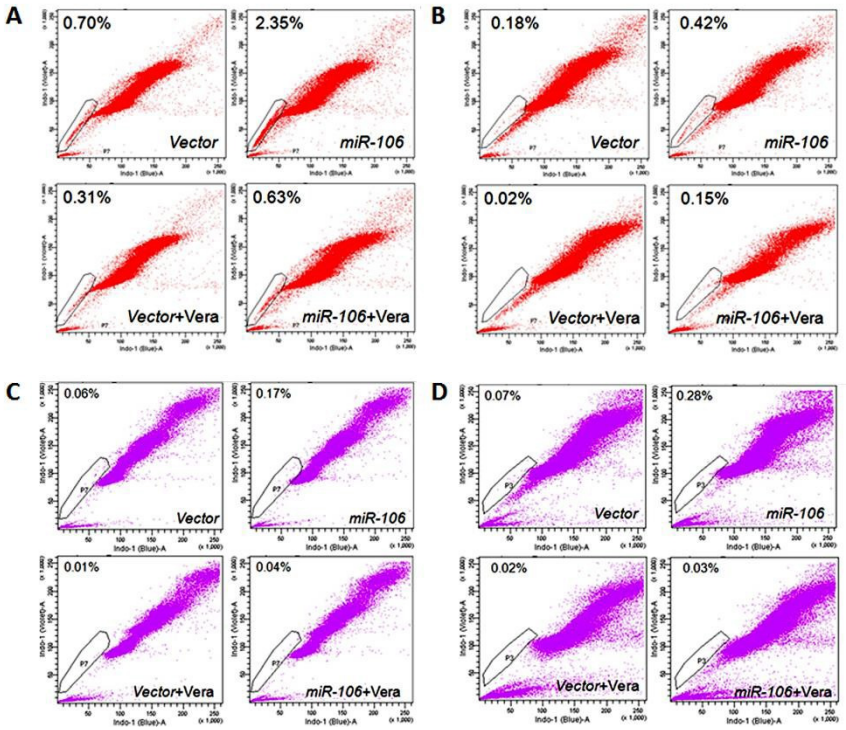
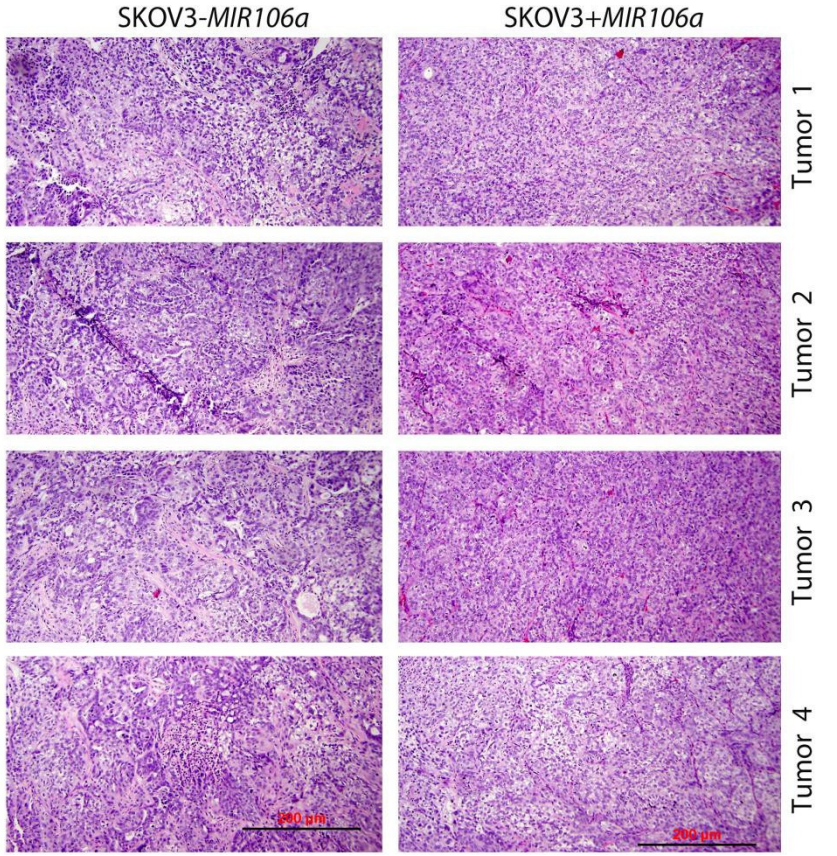
miR-106 mediated tumor growth through stem cell regulation





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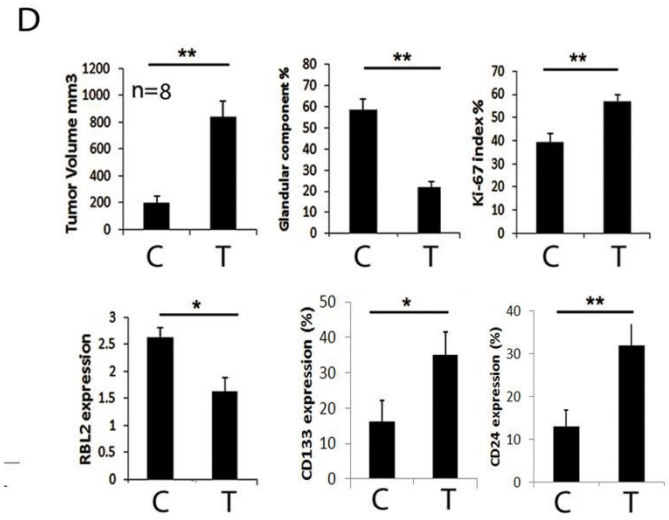
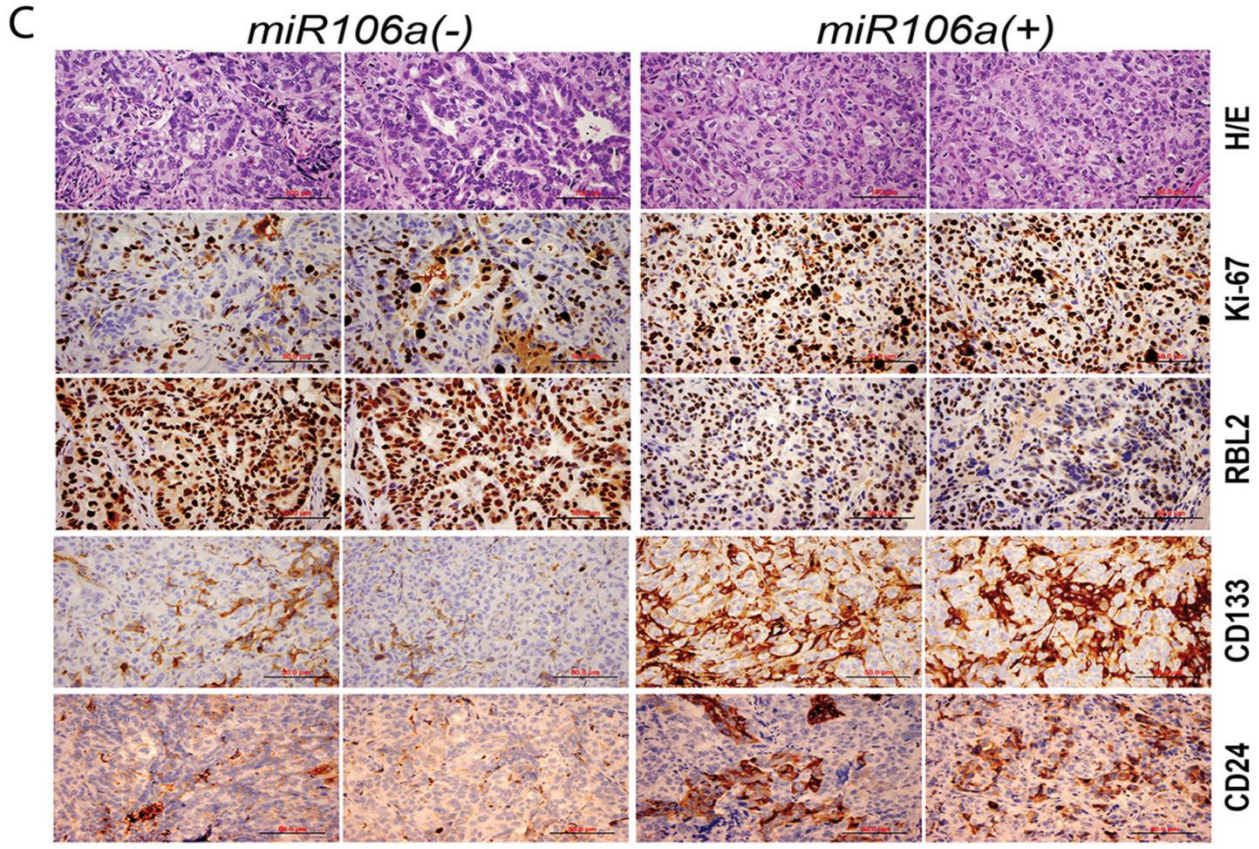
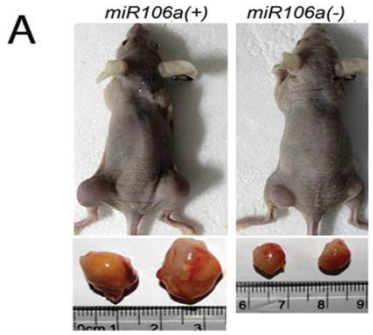
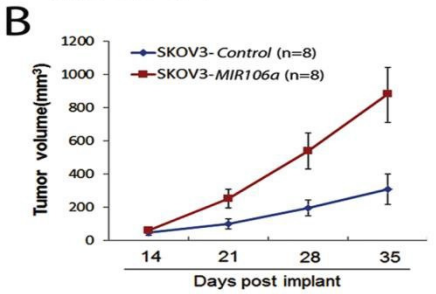
miR-106 promotes tumor growth and dedifferentiation









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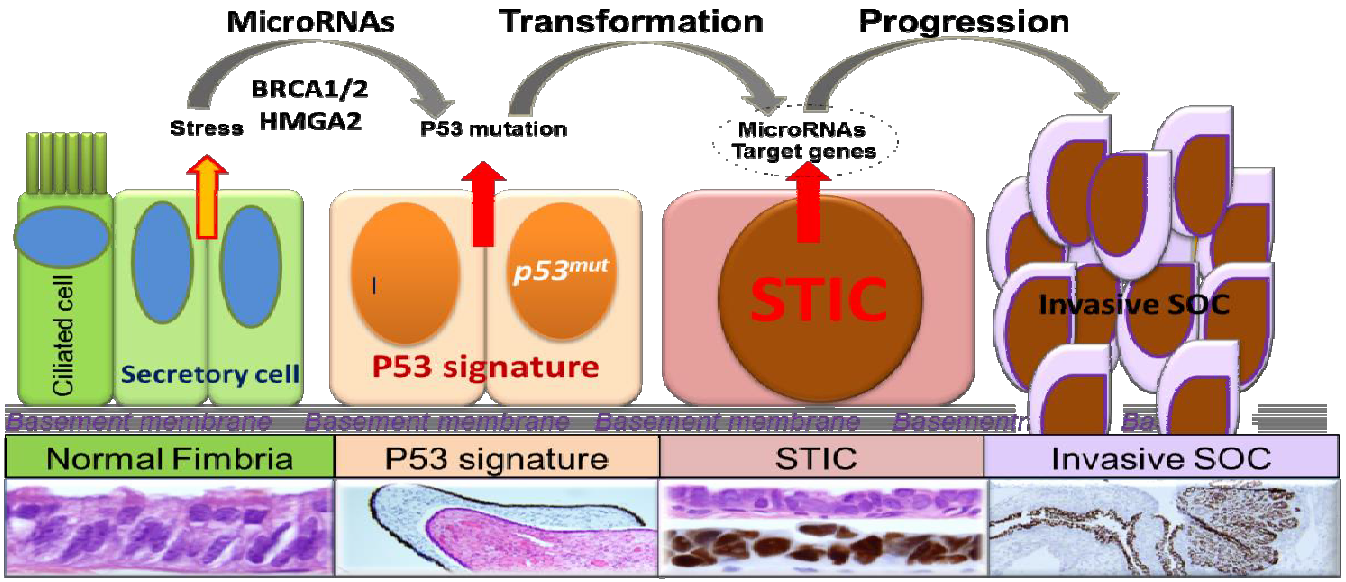
Future directions

- The genetic mechanism(s) for *MIR182* upregulation in early and late stages of HGSOC
- The relationship between *MIR182*-mediated DDR defects and *P53* mutations in tumorigenesis of HGSOC
- Animal model of *MIR182*-mediated tumorigenesis in mice
- Therapeutic potential of *anti-MIR182* to treat HGSOC



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- Eva Hernando

Thanks!

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