

LV-Nr.:

861.011

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Ankersmit H, Mildner M

# CURRENT TOPICS IN APPLIED IMMUNOLOGY

# Global microRNA depletion suppresses tumor angiogenesis

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Massachusetts, USA

***Genes Dev.* 2014 28: 1054-1067**



Christian  
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Laboratory

for  
Cardiac and Thoracic  
Diagnosis & Regeneration

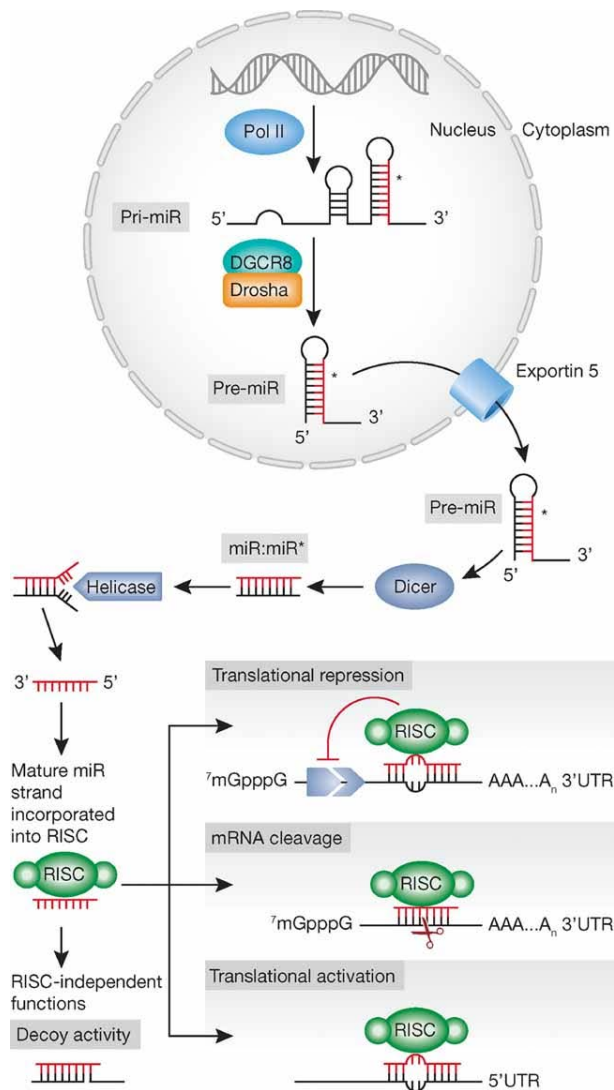


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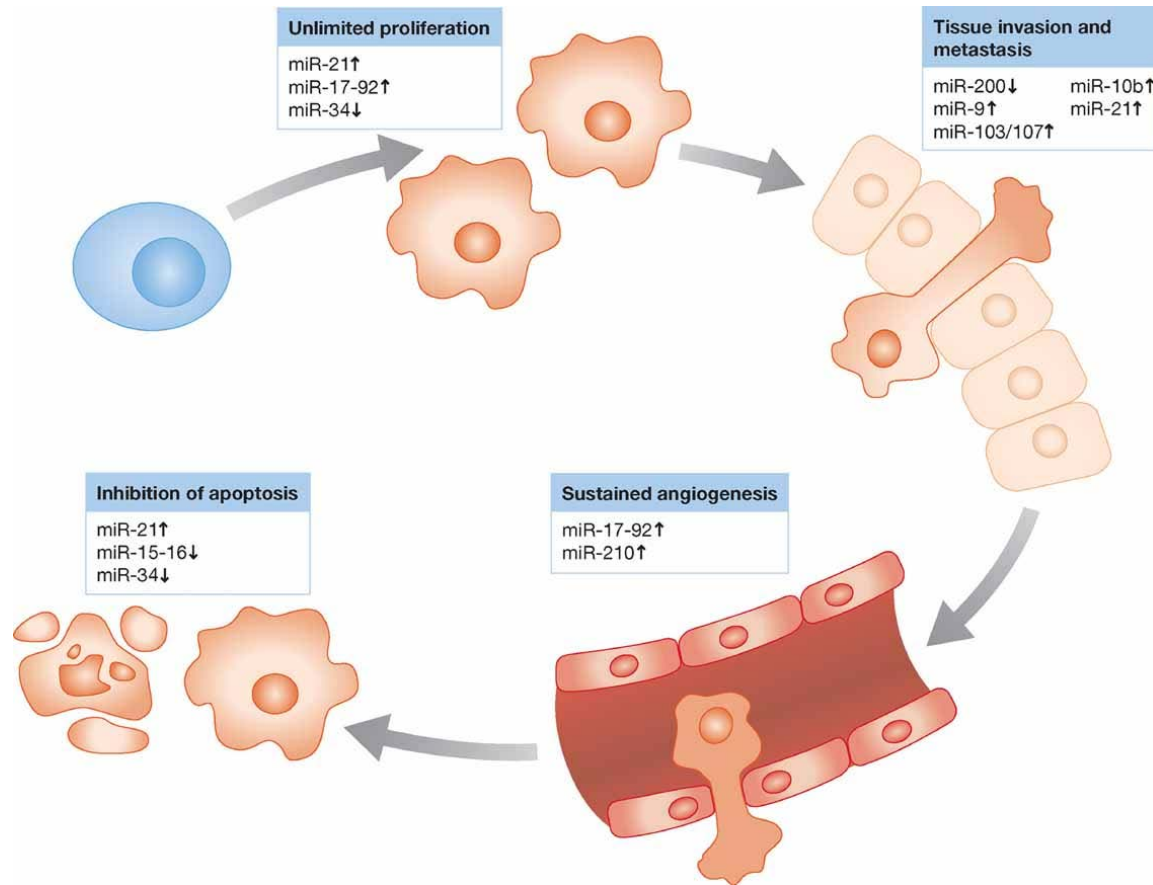
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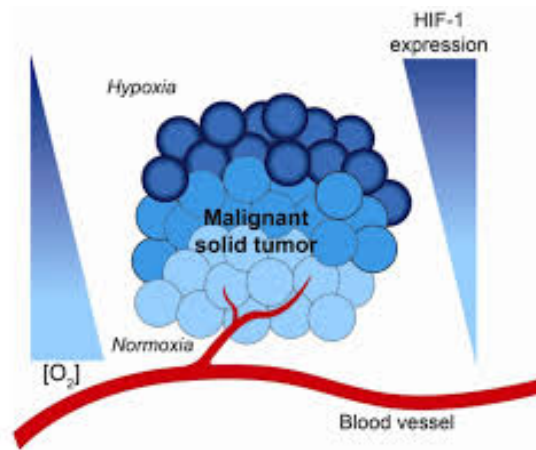
# microRNAs



miRNA	Tissue type specificity	Chromosomal location	Property	Malignancy
<i>let-7</i> family	Ubiquitous	Multiple members (chromosomes 3, 9, 11, 19, 21, 22)	TS	CLL [119], lymphoma [120], gastric [121], lung [122], prostate [9], breast [123], ovarian [121], colon [121], leiomyoma [121], melanoma [121]
<i>miR-15a/16-1</i> cluster	Ubiquitous	13q14.2	TS	CLL [124], lymphoma [9], multiple myeloma [9], pituitary adenoma [125], prostate [125], pancreatic [125]
<i>miR-17-92</i> family	Ubiquitous	Multiple members (chromosomes 7, 13, X)	OG	Lymphoma [126], multiple myeloma [9], lung [122], colon [126], medulloblastoma [127], breast [123], prostate [128]
<i>miR-21</i>	Ubiquitous	17q23.1	OG	Lymphoma, breast, lung, prostate, gastric, cervical, head and neck, colorectal, glioblastoma (for all: [129])
<i>miR-26a</i>	Ubiquitous	3p22.2 (-1) 12q14.1 (-2)	TS OG	Lymphoma [130], hepatocellular carcinoma [131], thyroid carcinoma [132] Glioblastoma [44,133]
<i>miR-34a/b/c</i>	Ubiquitous	1p36.22 (a) 11q23.1 (b) 11q23.1 (c)	TS	CLL [119], lymphoma [9] Pancreatic [9], colon [9], neuroblastoma [134] Glioblastoma [135]
<i>miR-155</i>	Haematopoietic system	21q21.3	OG	Lymphoma (ie Burkitt's, Hodgkin's, non-Hodgkin's) [9], CLL [9,18], breast [123], lung [9], colon [9], pancreatic [9]
<i>miR-200/141</i> family	Epithelial-specific	Multiple members (chromosomes 1, 12)	TS	Breast [123,136], renal clear cell carcinoma [137], gastric [138], bladder [139]
<i>miR-205</i>	Epithelial-specific	1q32.2	OG/TS TS	Ovarian [140-142] Prostate [143,144], bladder [145], breast [136,146,147], oesophageal [148]
<i>miR-206</i>	Skeletal muscle-specific	6p12.2	OG TS	Ovarian [149] Rhabdomyosarcoma [150], breast [151]
<i>miR-9</i>	Nervous system-specific	1q22 (-1) 5q14.3 (-2) 15q26.1 (-3)	TS OG/TS	Medulloblastoma [152], ovarian [153] Breast [57,154,155]

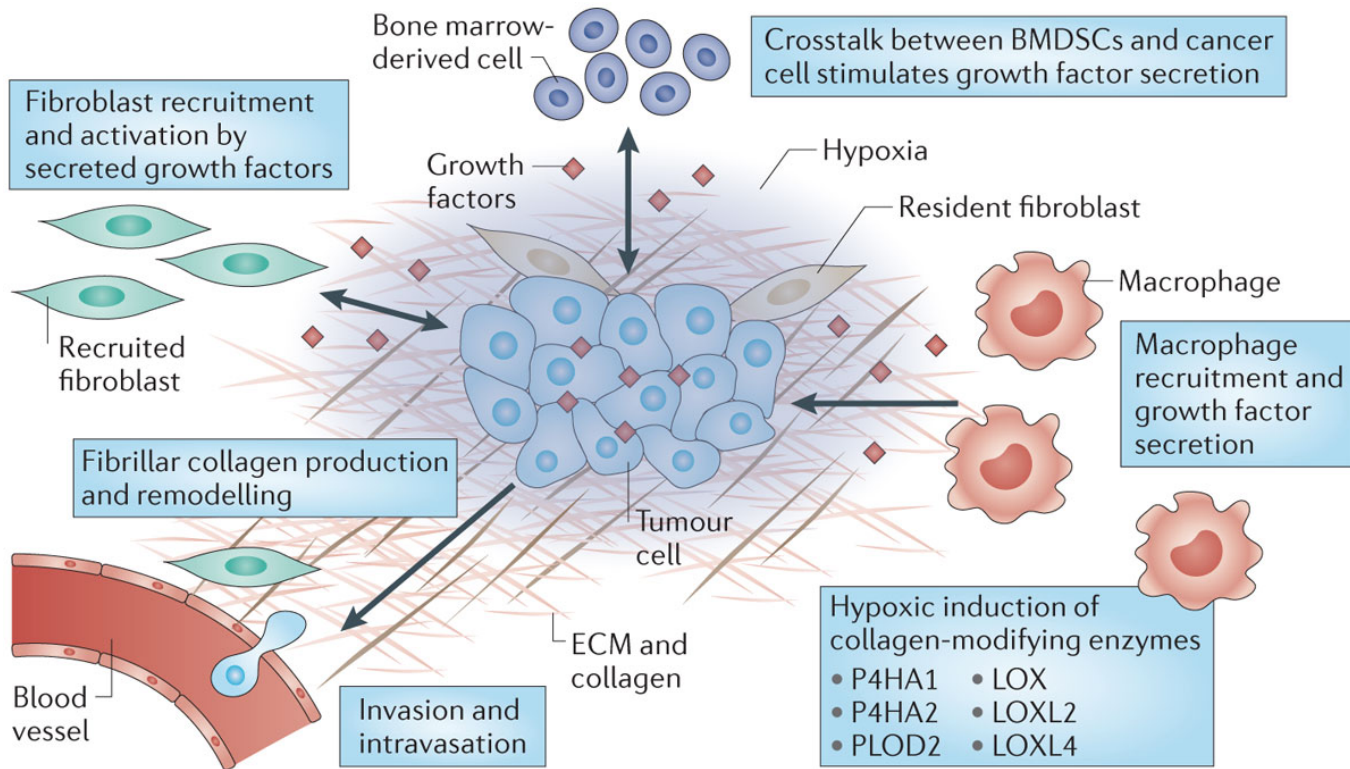


# Tumour & Hypoxia





# Cancer & Hypoxia



Nature Reviews | **Cancer**



Study aim:

Investigation of the effects of miRNA knockout in NSCLC



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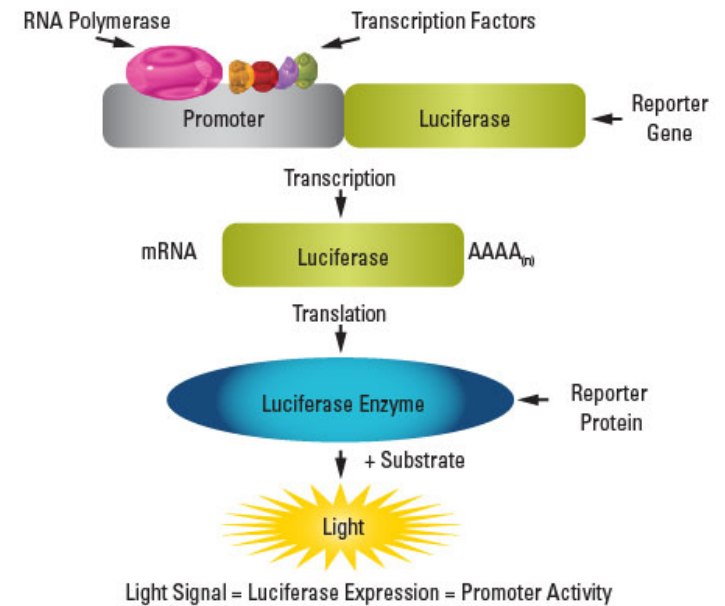
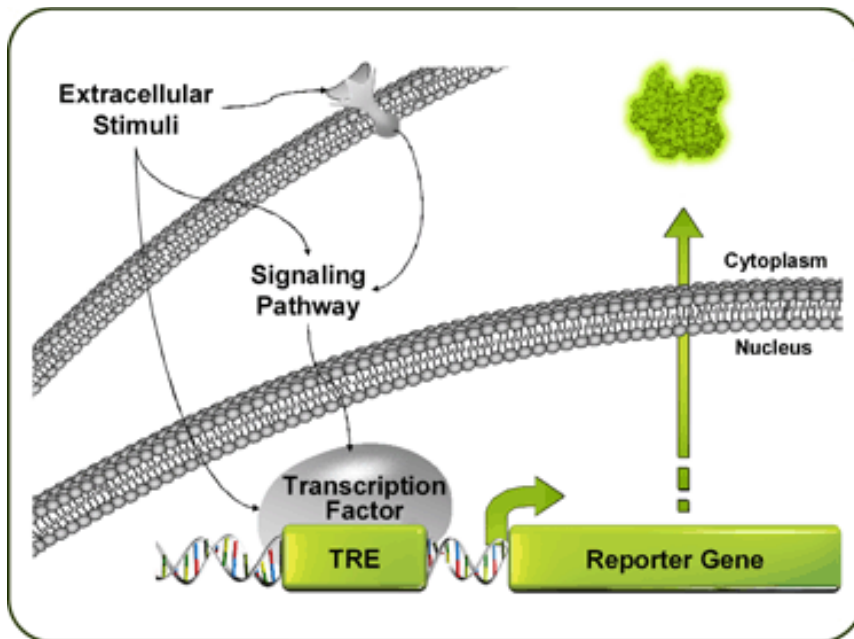
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# METHODS

# Luciferase Assay

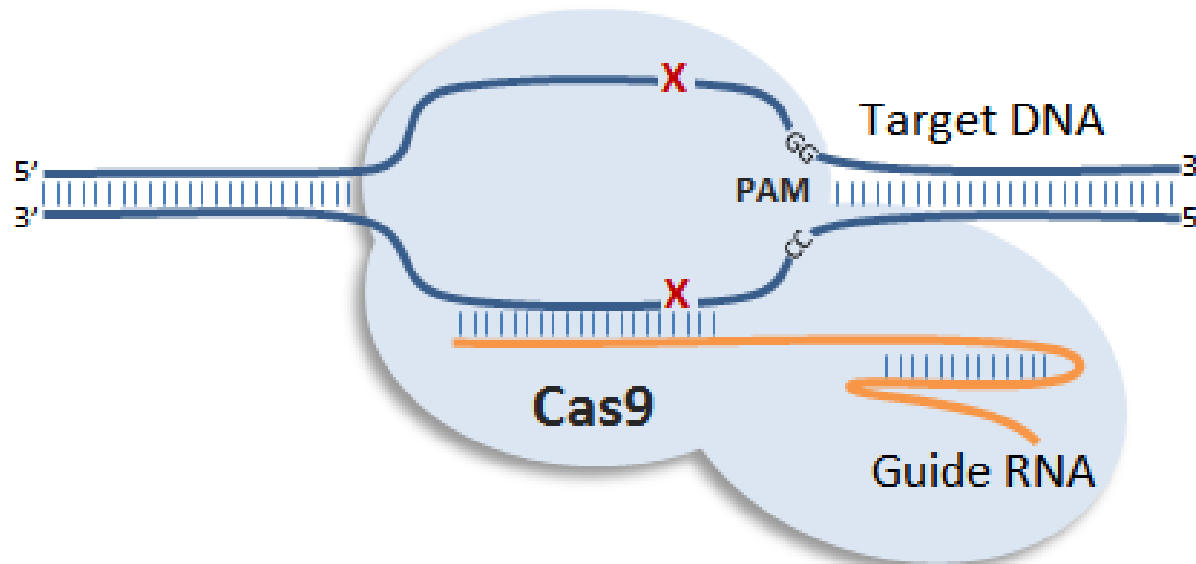


# CRISPR/Cas9 system

**CRISPR**

(Clustered Regularly  
Interspaced Short Palindromic Repeats)  
endonuclease

**Cas9**





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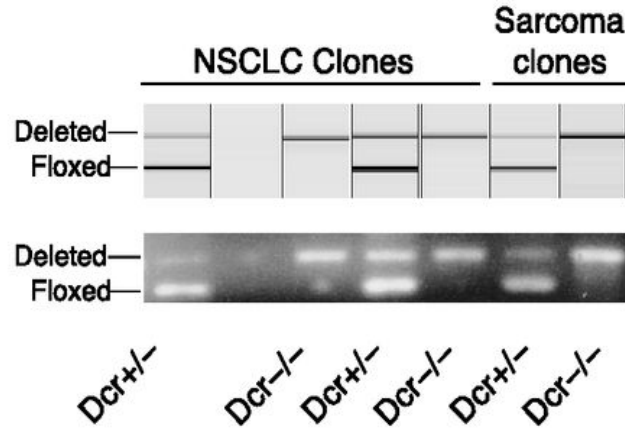


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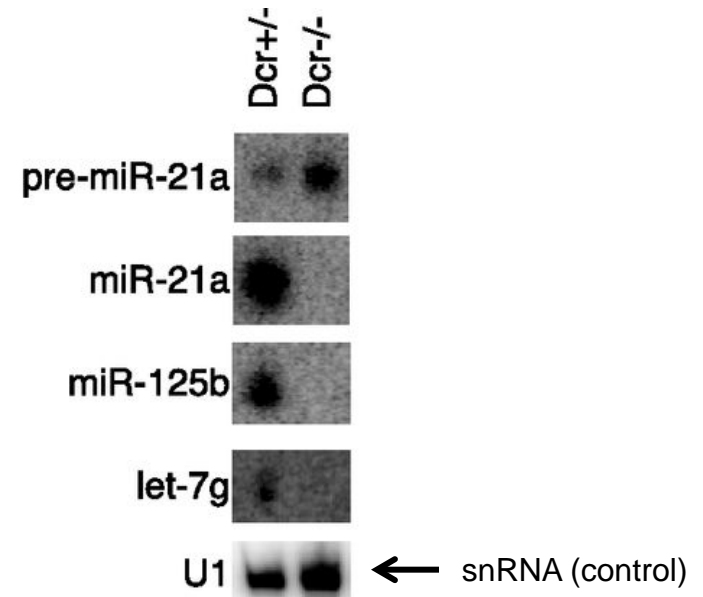
# RESULTS

# Dicer1 knockout

## Norther blot

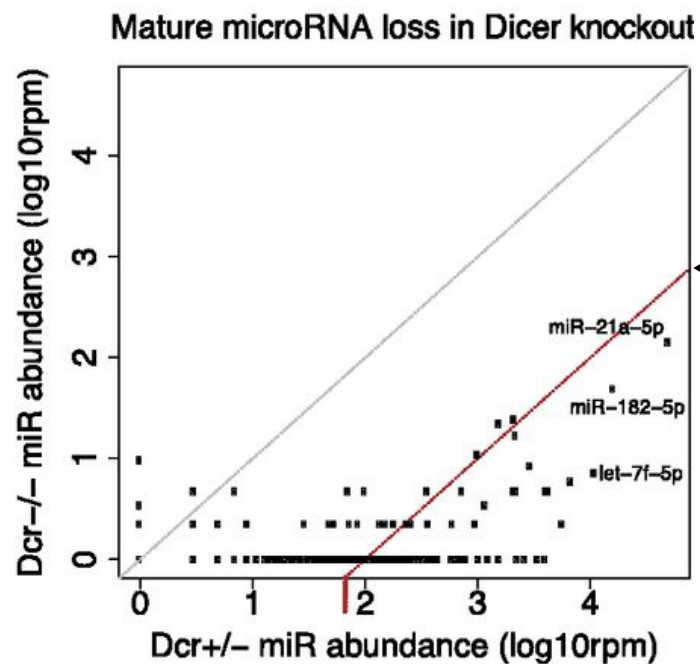
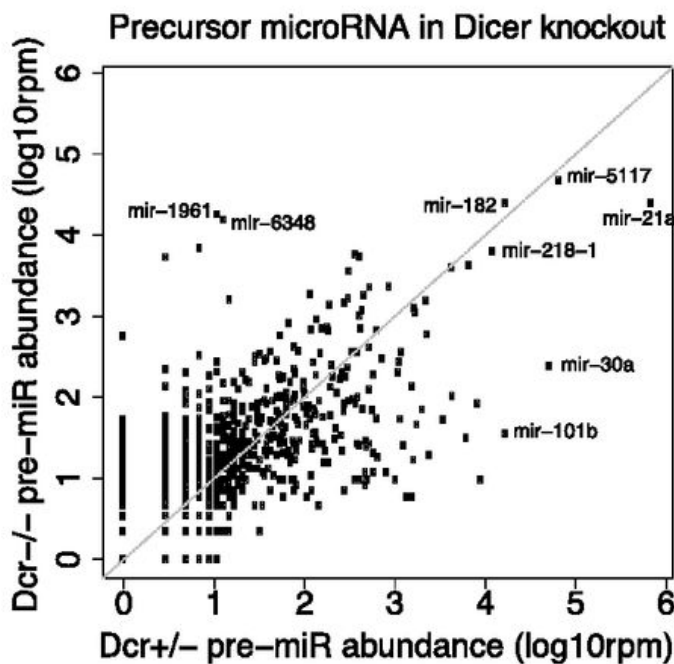


## Norther blot





# Dicer1 knockout



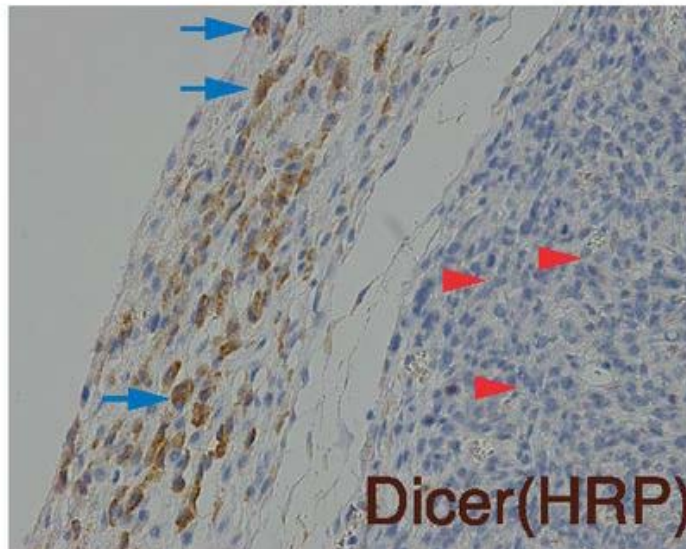




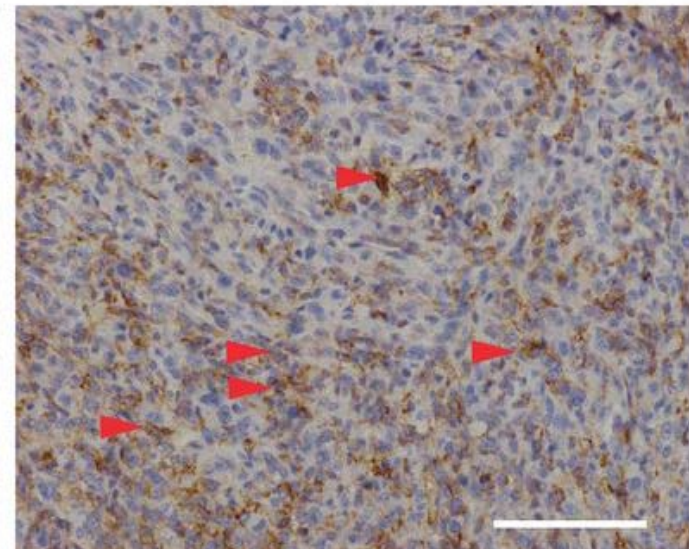
# *Dicer1* knockout



**Dcr<sup>-/-</sup>**



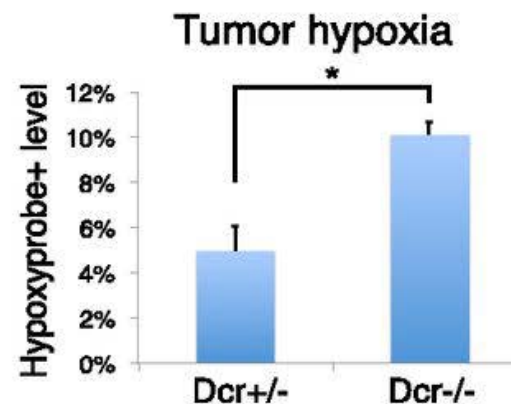
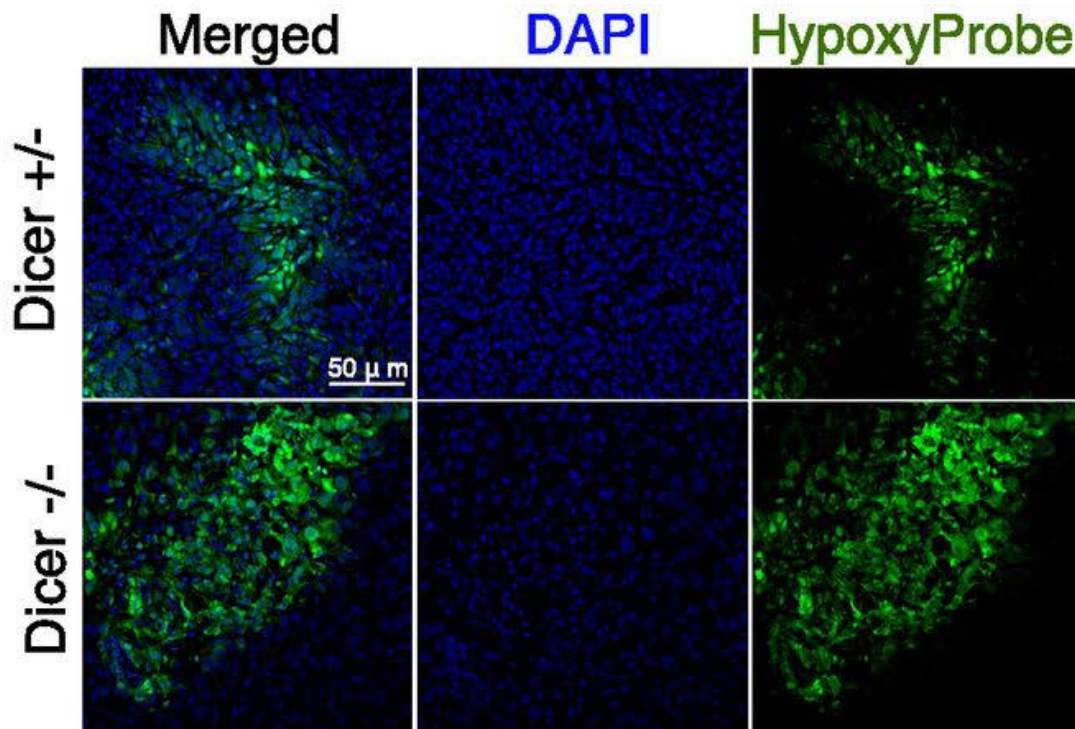
**Dcr<sup>+/-</sup>**



100  $\mu$ m

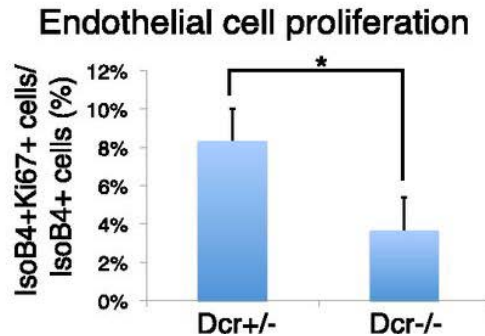
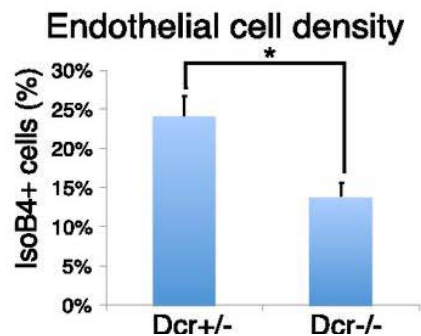
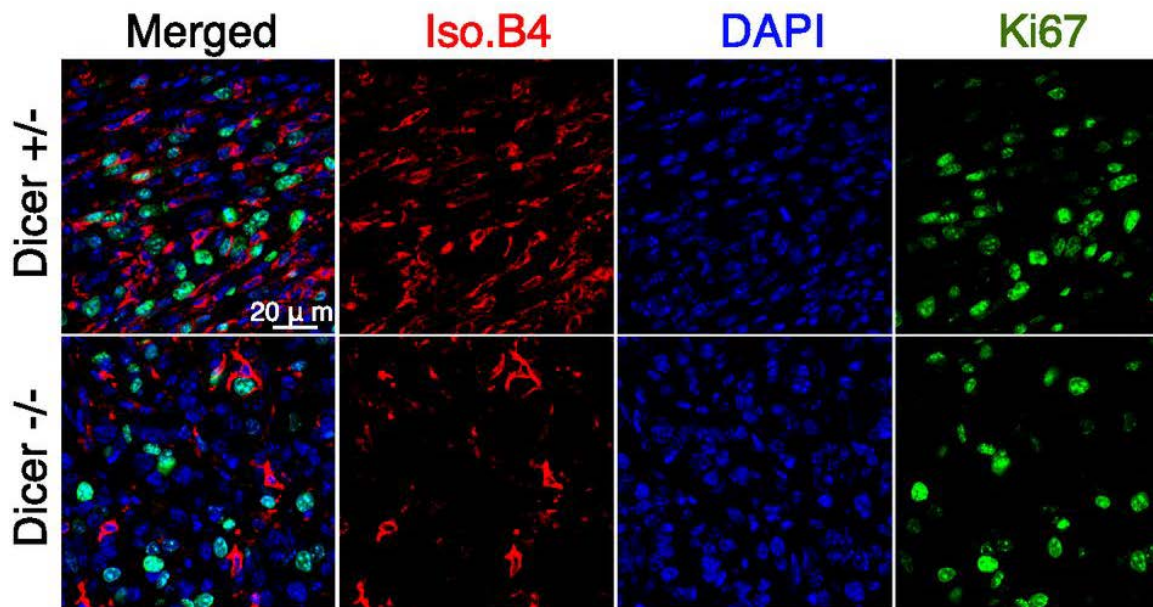


# Hypoxia in *Dicer1*<sup>-/-</sup> tumours





# Reduced EC Proliferation in *Dicer1*<sup>-/-</sup> tumours

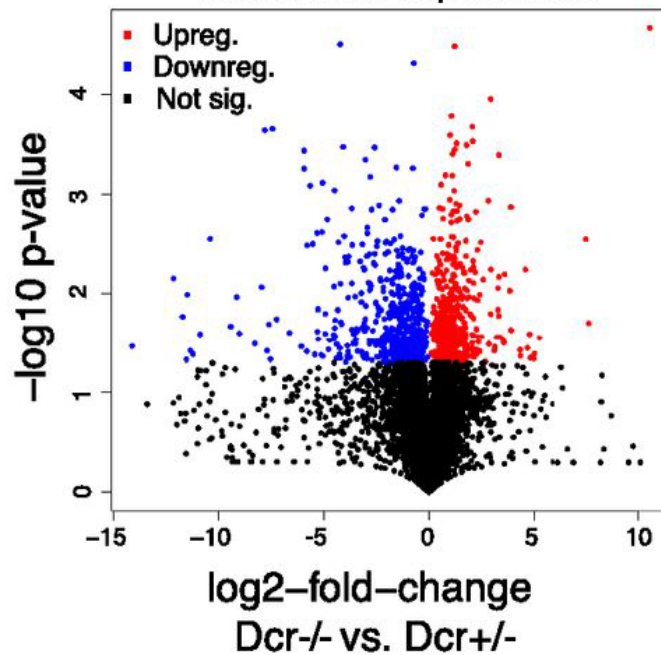




# mRNA derepression in *Dicer1*<sup>-/-</sup> cells

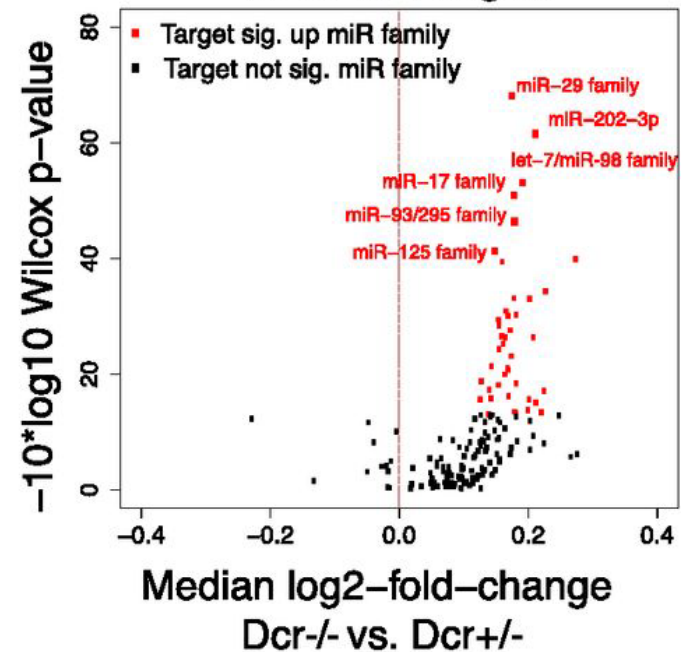
## Vulcano Plot mRNA

Dicer<sup>-/-</sup> vs. Dicer<sup>+/-</sup>  
differential expression



## Vulcano Plot of miRNA target genes

MicroRNA family activity  
on mRNA targets

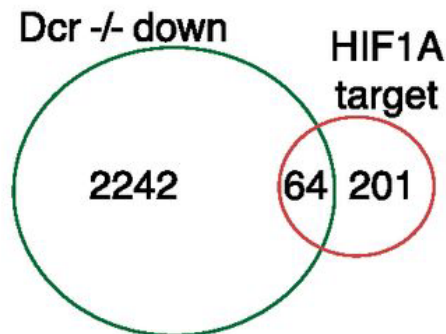


# Gene expression in *Dicer1*<sup>-/-</sup> cells

## GO analyses of *Dicer*<sup>-/-</sup> down genes

Biological process	Adj. p-value
Translation	2.05E-05
Blood vessel morphogenesis	4.44E-05
Cell adhesion	6.70E-05
Biological adhesion	6.86E-05
Blood vessel development	7.40E-05
Vasculature development	1.04E-04
Regulation of cell proliferation	1.47E-04
Angiogenesis	2.47E-04
Embryonic limb morphogenesis	3.11E-03
Embryonic appendage morphogenesis	3.11E-03
Cell migration	3.34E-03

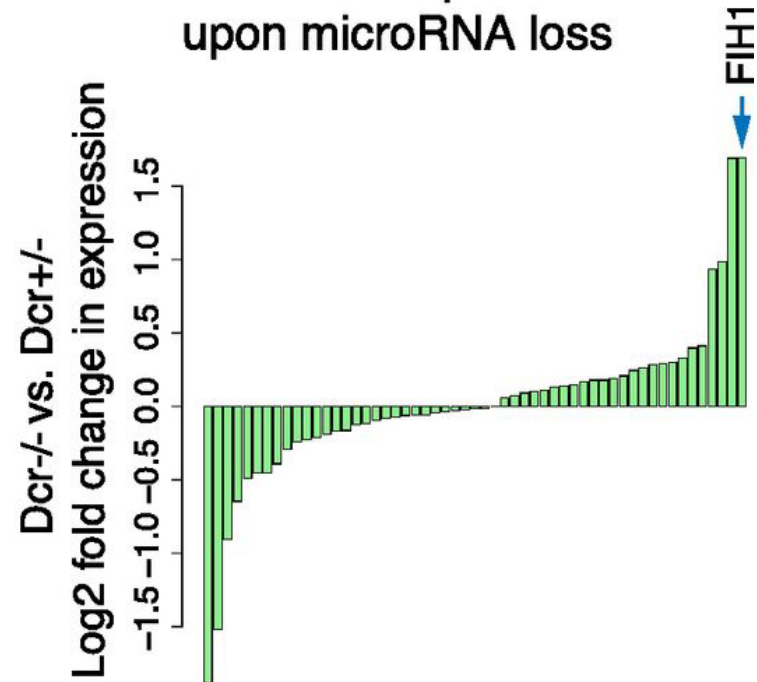
## Venn diagram



p = 3.2e-10

## Waterfall plot

HIF antagonists differential expression upon microRNA loss





# FIH1 targeting using CRISPR/Cas9 system

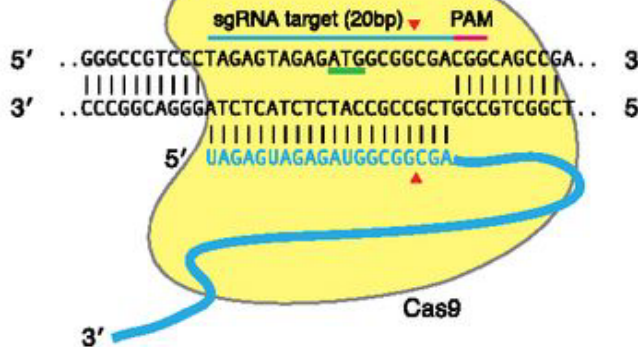
## FIH1 knockout

## Western blot

Mouse FIH1 locus (chr. 19)

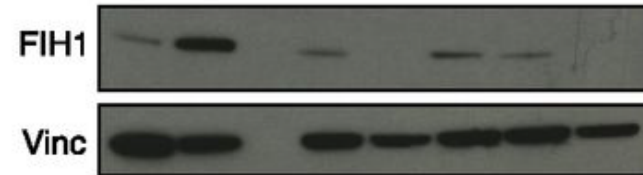


FIH1 exon1 target region

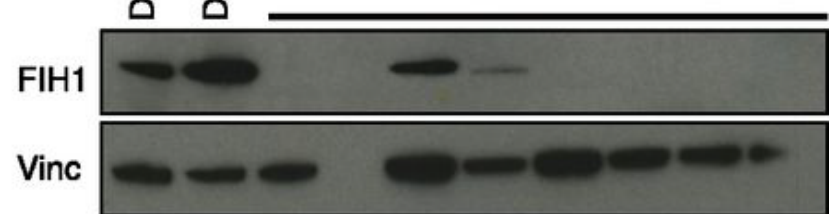


single guide RNA targeting FIH1 (sg-FIH1)

Dcr +/- Dcr -/ Dcr -/; + sg-FIH1 Clones (set 1)

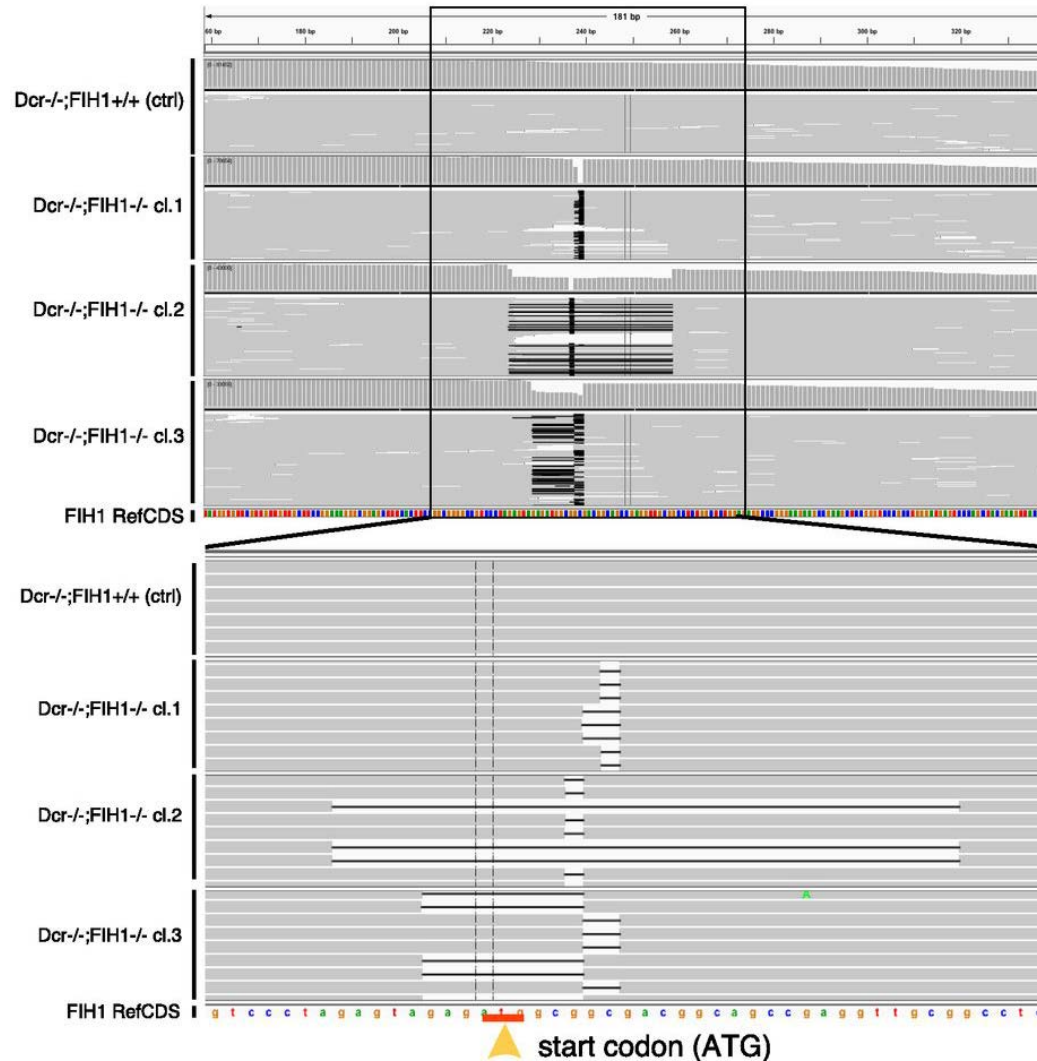


Dcr +/- Dcr -/ Dcr -/; + sg-FIH1 Clones (set 2)

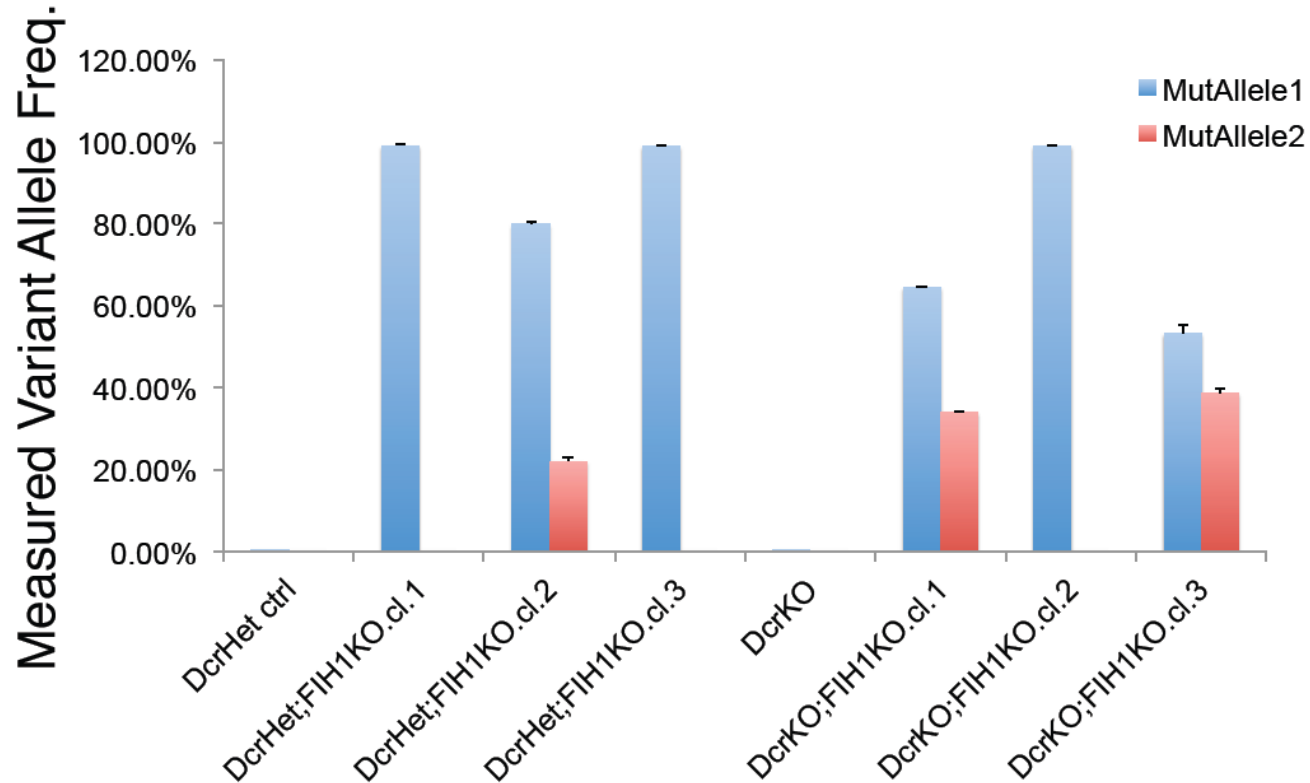




# Genotyping of FIH1-null mutants

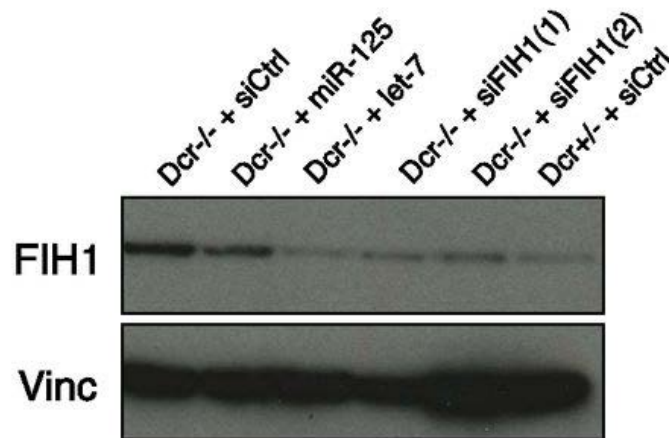


# Genotyping of FIH1-null mutants



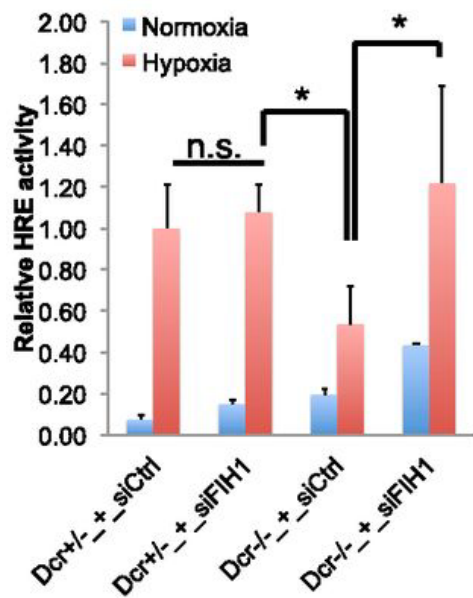
# FIH1 – HIF interaction

## Western blot



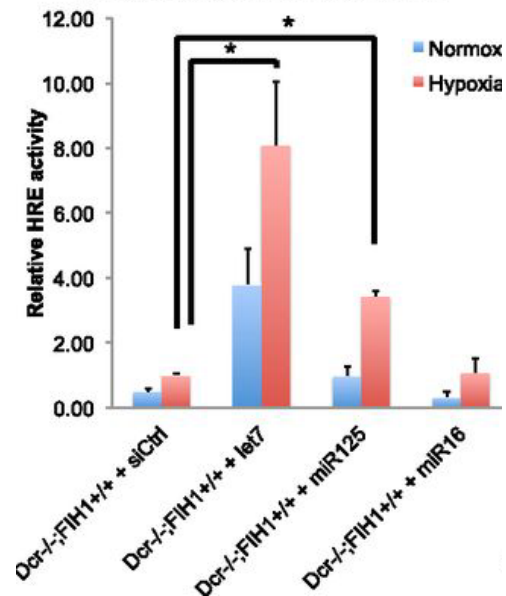
## HIF-responsive element reporter assay

### FIH1 knockdown



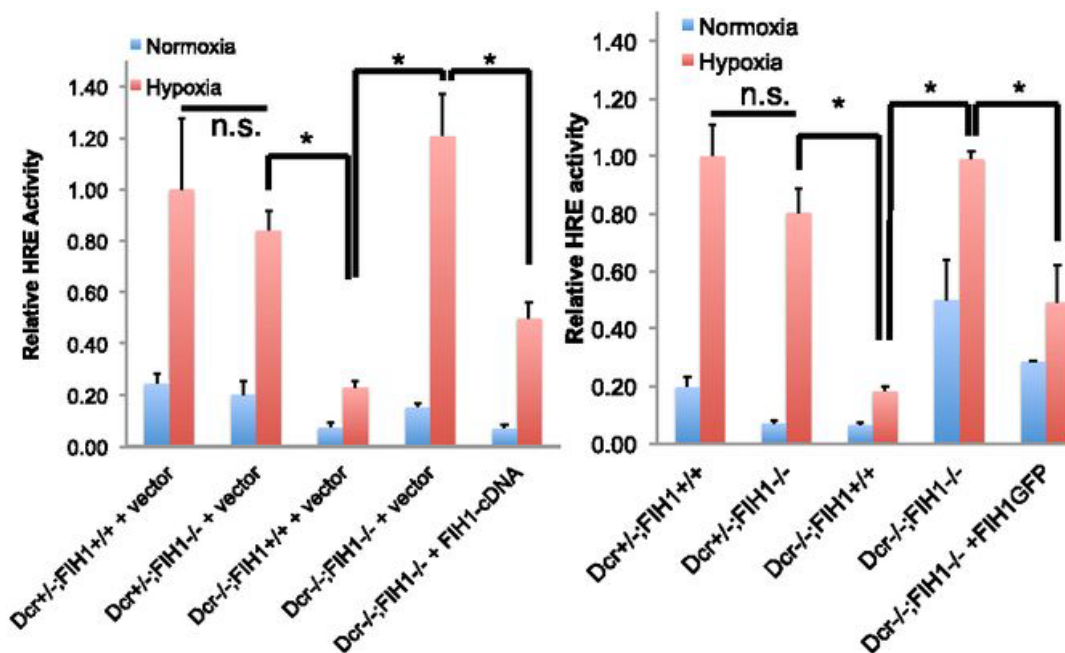
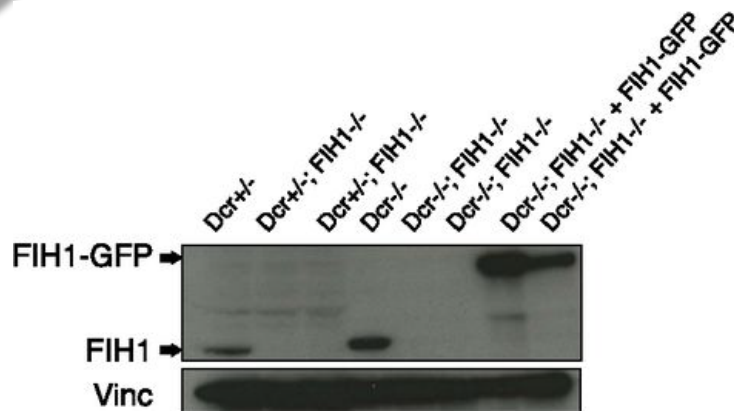
## miRNA-addback

### miR addback in DcrKO;FIH1WT

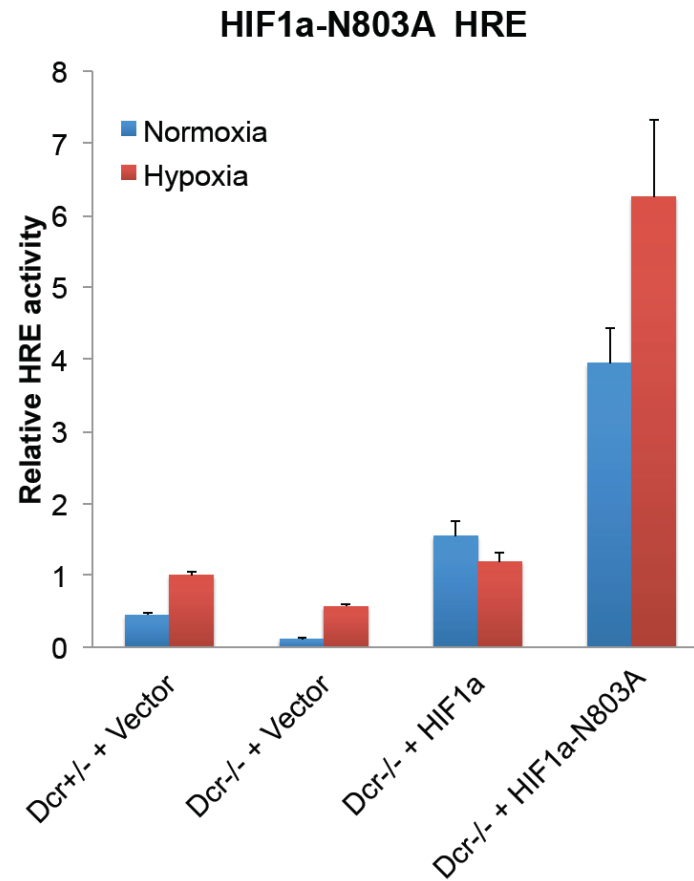




# FIH1 suppresses HIF activity

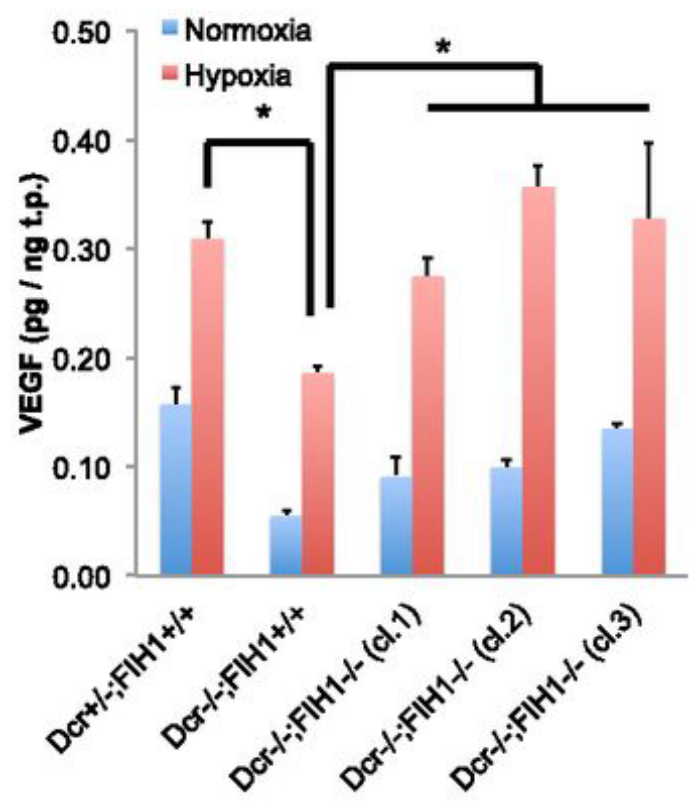


# HIF1a mutant unresponsive to FIH1



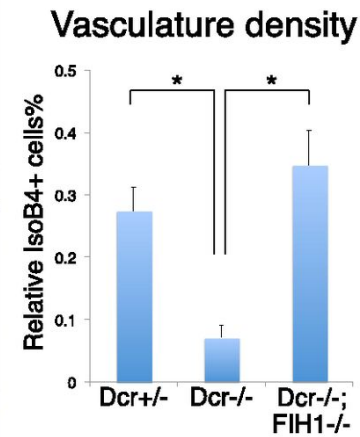
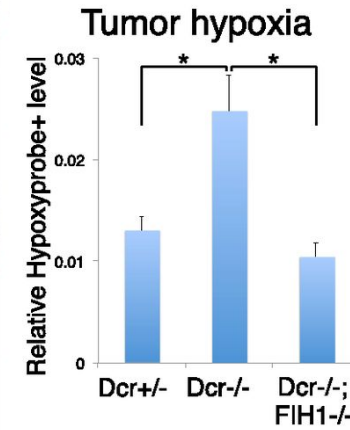
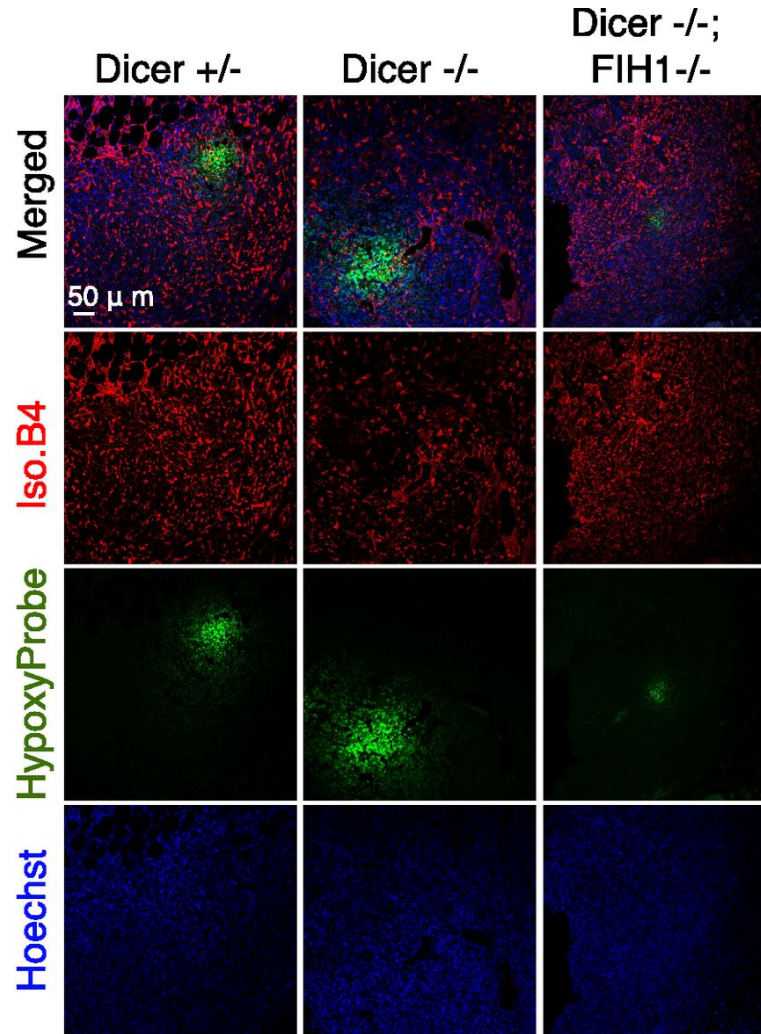
# miRNA knockdown reduces VEGF concentrations

**VEGF production**



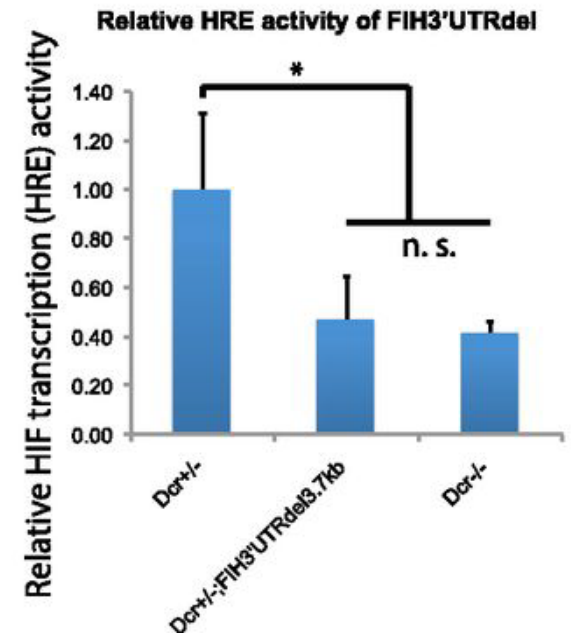
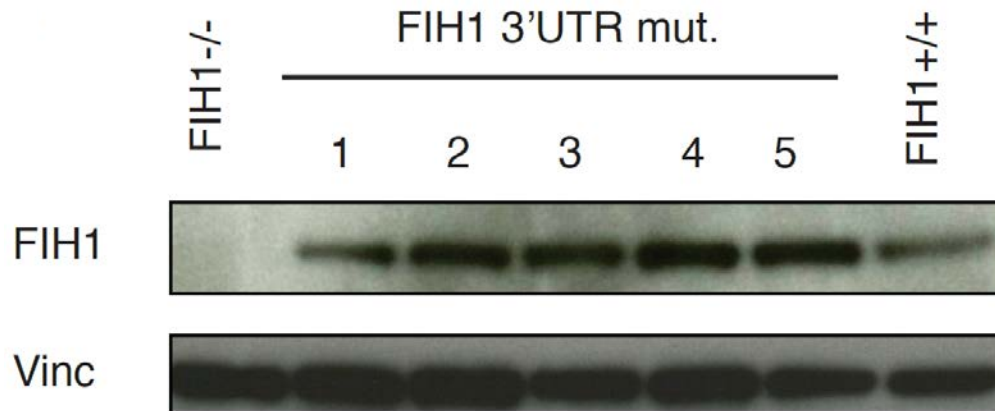
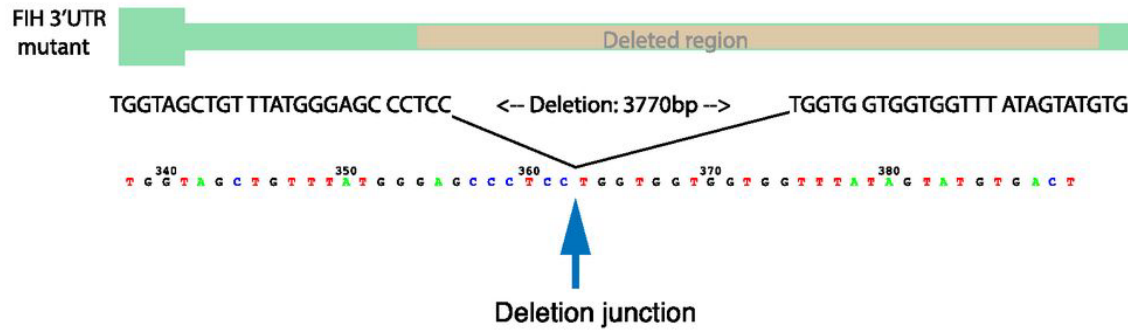


# FIH1 knockout attenuates tumor-hypoxia





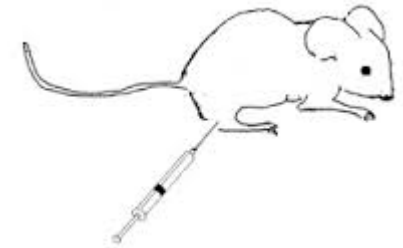
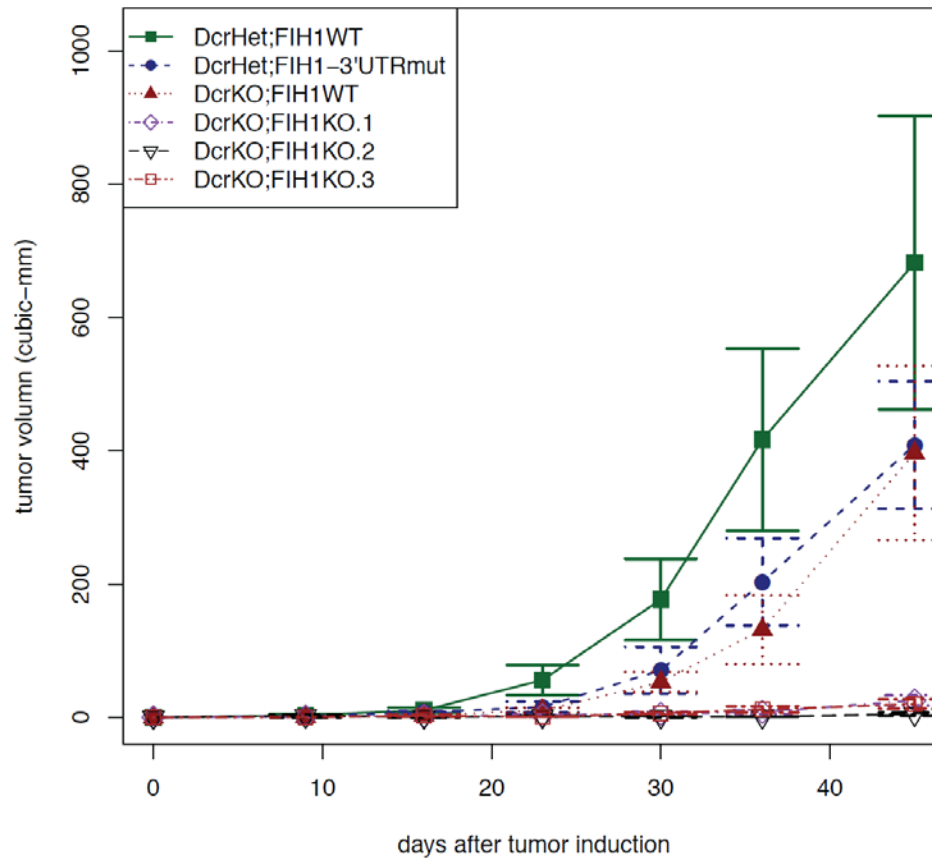
# Modulation of FIH1 at 3' UTR



# Dicer1<sup>-/-</sup> and FIH1-3'UTR mutant repress tumor growth

WT = wild type  
KO = knockout

FIH1 Mutant tumor growth

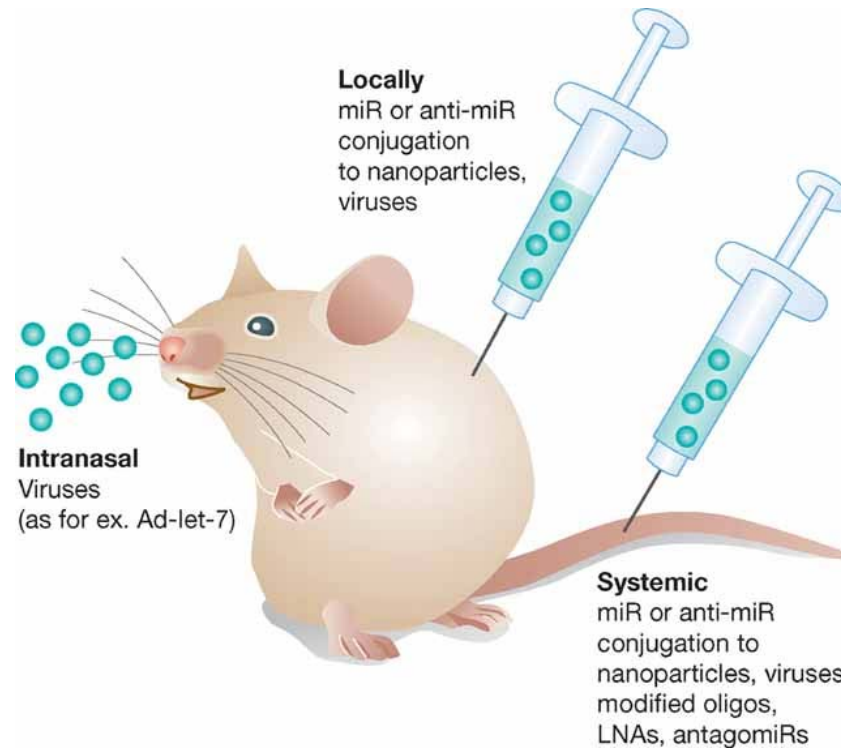


[www.pediatricgist.cancer.gov](http://www.pediatricgist.cancer.gov)

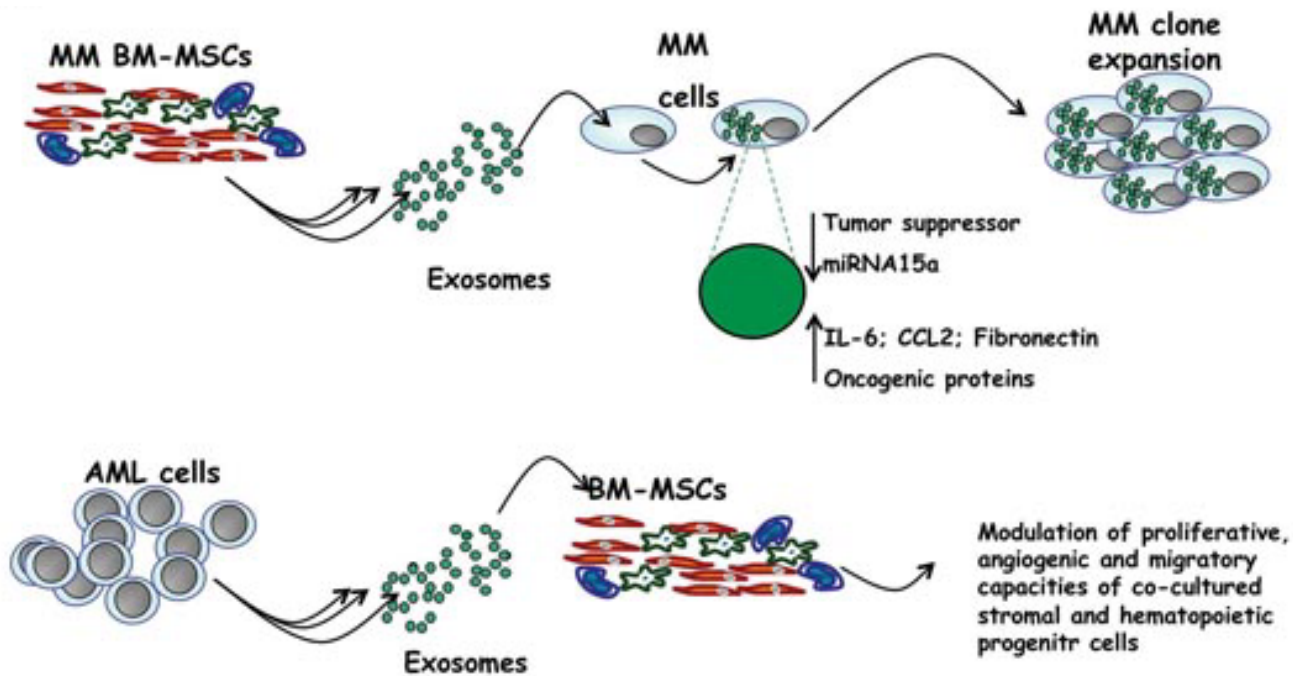
# Summary

1. **Dicer** knockdown attenuates **miRNA** expression
2. “**Derepression**” of mRNAs in *Dicer*<sup>-/-</sup> cells
3. *Dicer*<sup>-/-</sup> tumours are **hypoxic** and poorly **vascularized**
4. Anti-angiogenic **FIH1** is induced and pro-angiogenic **HIF1A** is repressed in *Dicer*<sup>-/-</sup> cells
5. **FIH1** is regulated by miRNAs – loss of miRNAs derepresses FIH1
6. **HIF1A** is an target of **FIH1**
7. *Dicer*<sup>-/-</sup> or inhibition of miRNA mediated FIH1 degradation **inhibits tumour growth**

## Targeting miRNA as tumor therapies



## Exosomes as carriers for micRNAs





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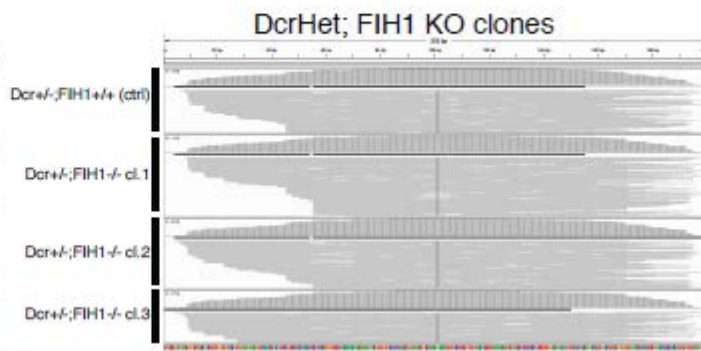
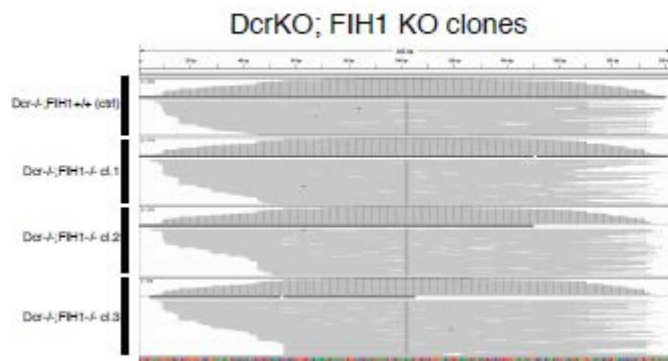


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**THANK YOU FOR YOUR  
ATTENTION**



### Offtarget 1: Evc2 locus



### Offtarget 2: Fam126 locus

