MicroRNAs as therapeutic targets and targeted therapies in cancer

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Sept 2011
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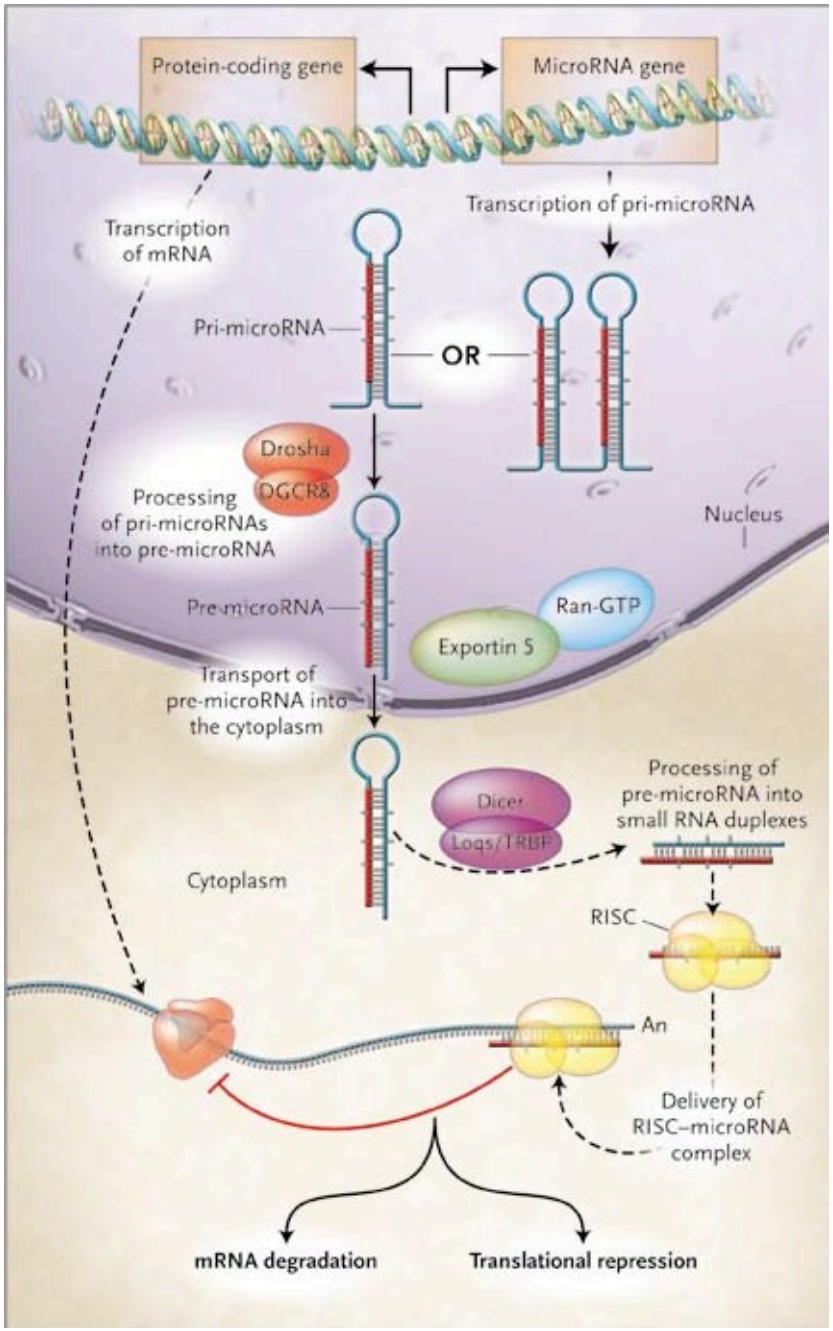
Disclosure: I am a consultant to Mirna Therapeutics and Mira Dx who have licensed IP from Yale University

MicroRNAs: biogenesis and function



- Found across all metazoans
- over 700 in humans
- estimated over 45,000 target sites
- roles in development, differentiation, homeostasis, immunity, and notably, functions related to cancer

Fig adapted from Chang-Zheng Chen, New Eng. J. Med

MicroRNAs and Cancer

- Frequently located in genomic regions linked to cancers

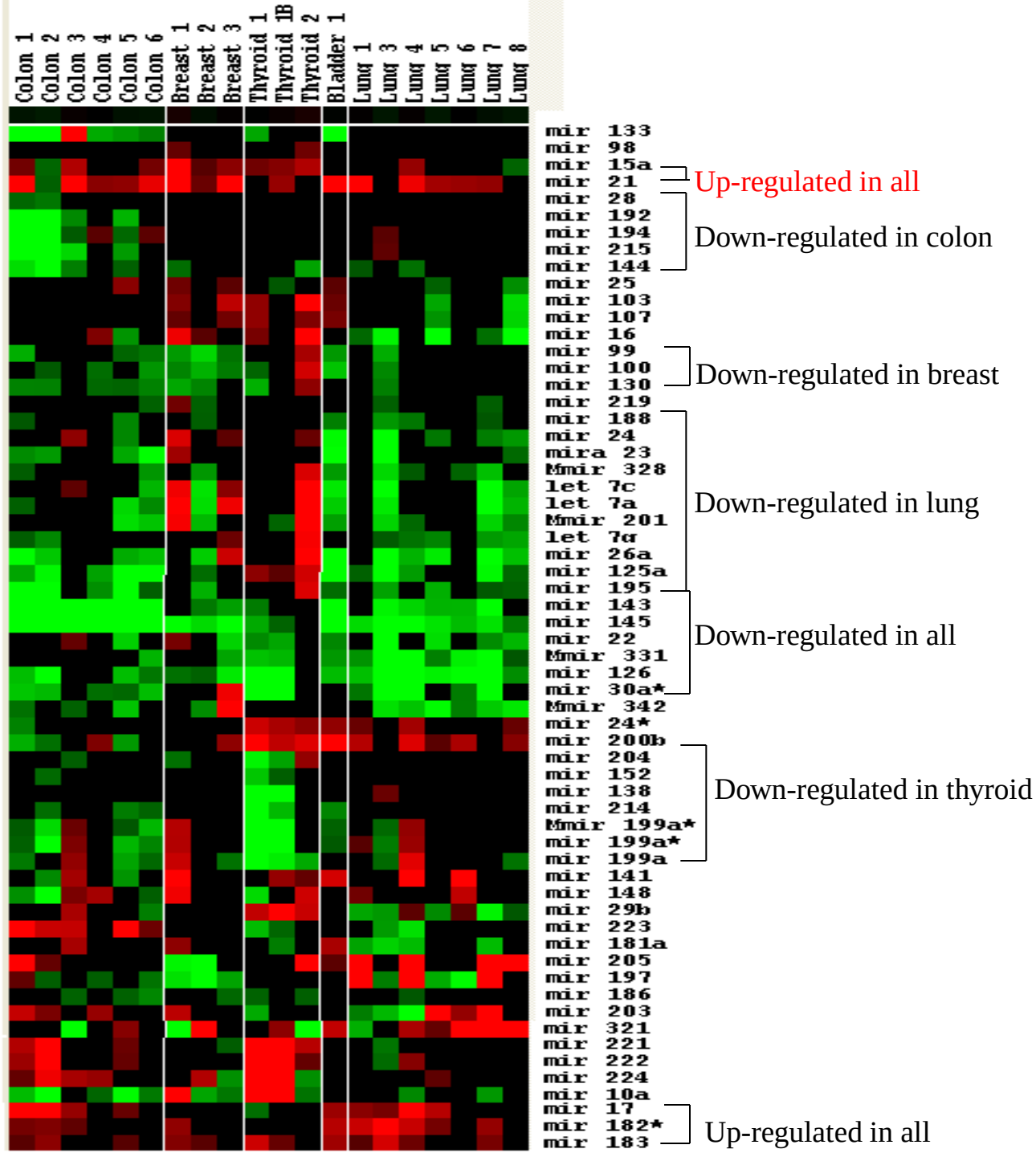
- Frequently mis-expressed in tumors versus normal tissue

- Mutation of members of the miRNA biogenesis machinery results in global down-regulation of miRNAs and contributes to transformation and tumorigenesis

- miRNAs themselves can act as tumor suppressors or oncogenes

Part A: OncomiR addiction in a mouse model of cancer- a case for antagonizing microRNAs in cancer

Potential oncomiRs with altered expression in tumors relative to normal adjacent tissue



D. Brown, 2003

mir-21 maps to a region frequently amplified in neuroblastoma

Table 2. Examples of miRs located in minimal deleted regions, minimal amplified regions, and breakpoint regions involved in human cancers

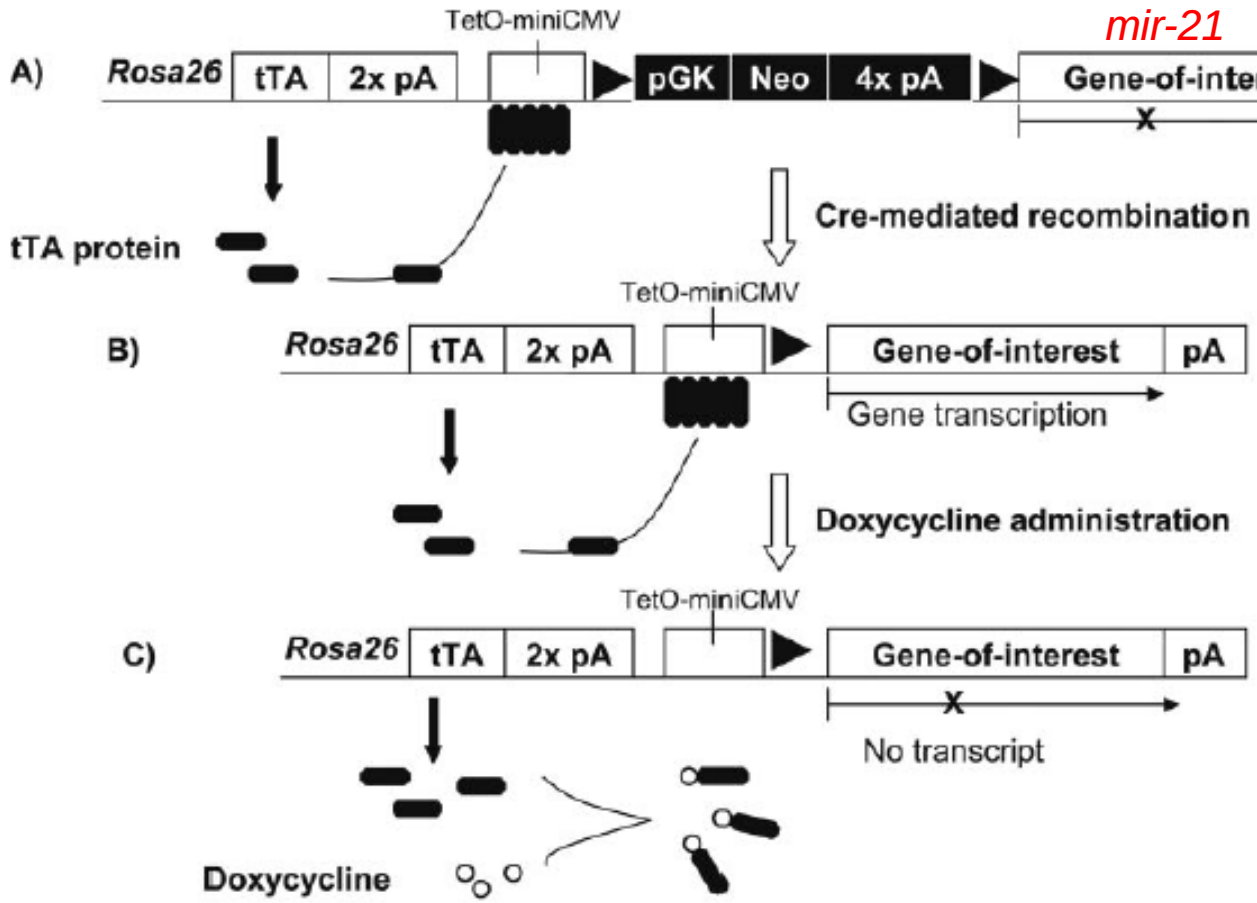
Chromosome	Location (defining markers)	Size, Mb	miR	Hystotype	Known OG/TS
3p21.1–21.2-D	ARP-DRR1	7	*let-7g/miR-135–1*	Lung, breast cancer	—
3p21.3(AP20)-D	GOLGA4-VILL	0.75	*miR-26a*	Epithelial cancer	—
3p23–21.31(MDR2)-D	D3S1768-D3S1767	12.32	*miR-26a; miR-138–1*	Nasopharyngeal cancer	—
5q32-D	ADRB2-ATX1	2.92	*miR-145/miR-143*	Myelodysplastic syndrome	—
9q22.3-D	D9S280-D9S1809	1.46	*miR-24–1/mir-27b/miR-23b; let-7a-1/let-7f-1/let-7d*	Urothelial cancer	PTC, FANCC
9q33-D	D9S1826-D9S158	0.4	*miR-123*	NSCLC	—
11q23-q24-D	D11S927-D11S1347	1.994	*miR-34a-1/miR-34a-2*	Breast, lung cancer	PPP2R1B
11q23-q24-D	D11S1345-D11S1328	1.725	*miR-125b-1/let-7a-2/miR-100*	Breast, lung, ovary, cervix cancer	—
13q14.3-D	D13S272-D13S25	0.54	*miR-15a/miR-16a*	B-CLL	—
13q32–33-A	stSG15303-stSG31624	7.15	*miR-17/miR-18/miR-19a/miR-20/ miR-19b-1/miR-92–1*	Follicular lymphoma	—
17p13.3-D	D17S1866-D17S1574	1.899	*miR-22; miR-132; miR-212*	HCC	—
17p13.3-D	ENO3-TP53	2.275	*miR-195*	Lung cancer	TP53
17q22-t(8;17)	miR-142s/c-MYC		*miR-142s; miR-142as*	Prolymphocytic leukemia	c-MYC
17q23-A	CLTC-PPM1D	0.97	*miR-21*	Neuroblastoma	—
20q13-A	FLJ33887-ZNF217	0.55	*miR-297–3*	Colon cancer	—
21q11.1-D	D21S1911-ANA	2.84	*miR-99a/let-7c/miR-125b*	Lung cancer	—

D, deleted region; A, amplified region; NSCLC, non-small-cell lung cancer; HCC, hepatocellular carcinoma; PTC, patched homolog (*Drosophila*); FANCC, Fanconi anemia, complementation group C; PPP2R1B, protein phosphatase 2, regulatory subunit A (PR 65), β isoform, miRs in a cluster are separated by a slash. For references, see Table 6.

miR-21 is over-expressed in many cancers

Transgenic Mice to Over-express *mir-21*: Tissue specific & DOX inducibility (tet-off)

Tetracycline transactivator (tTA) is expressed by the ubiquitously expressed *ROSA26* promoter



Endogenous *ROSA26* promoter will express the tTA (Tet transactivator) necessary for the function of miniCMV promoter.

mir-21 gene expression is driven by the Tet-Operator-miniCMV that need, tTA for their expression

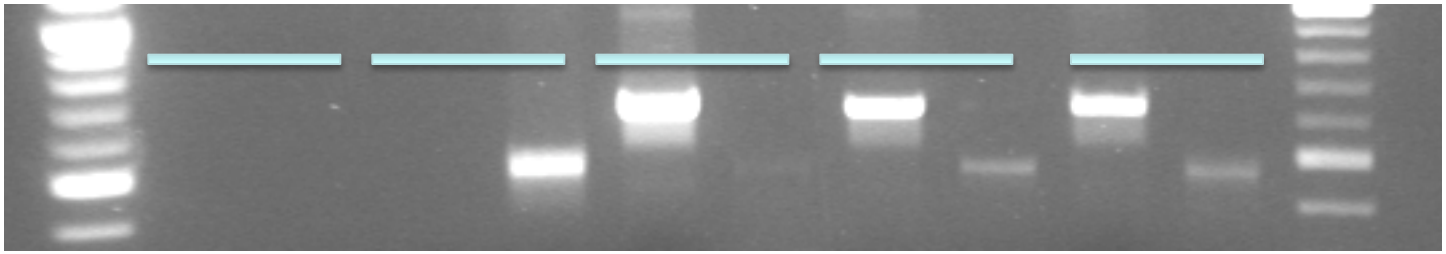
Doxycycline binds to tTA and inactivates it: expression is lost.

Medina et al. *Nature* 2010

LoxP recombination and higher miR-21 levels

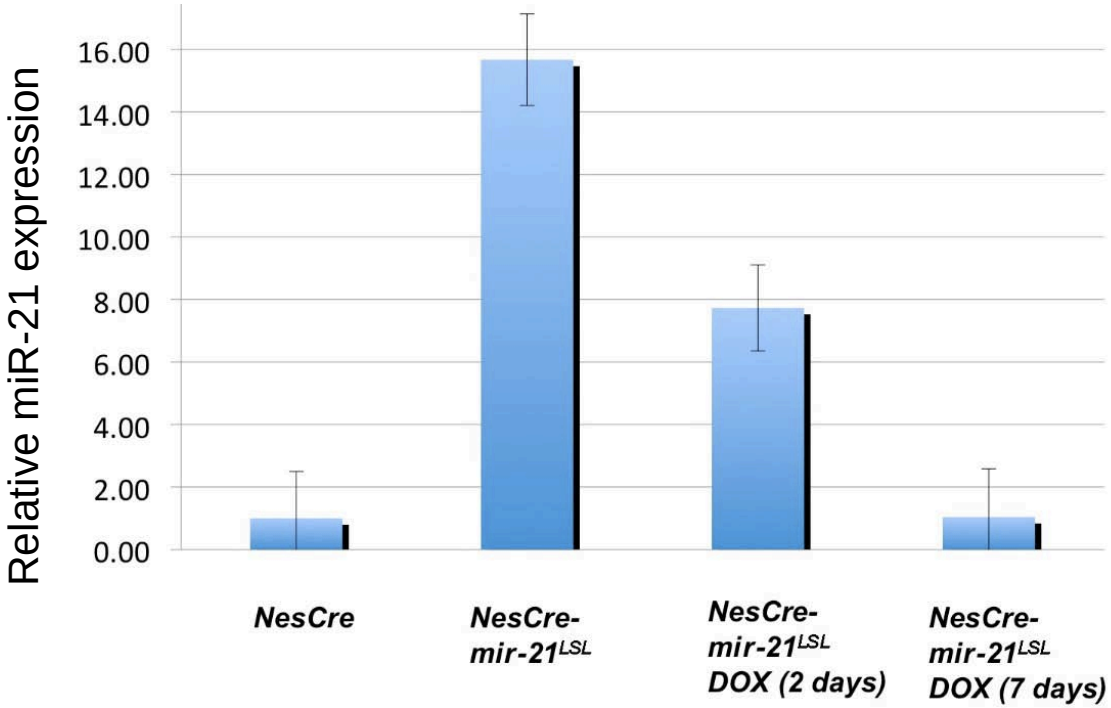
WT ***mir-21*** ***NesCre-mir-21$^{LSL\text{-}Tetoff}$***

Thymus Lymph Spleen

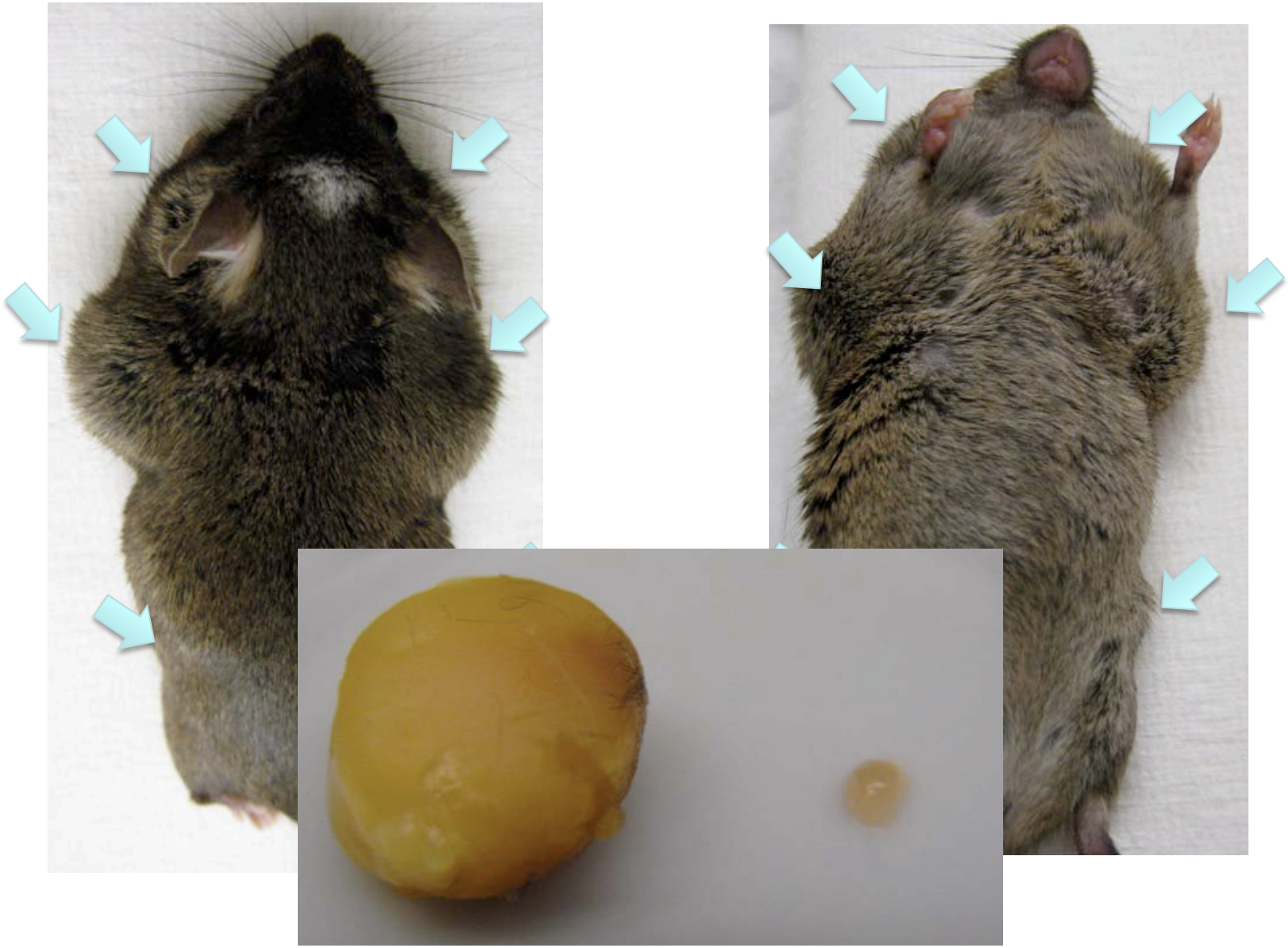


Cre-recombined (606bp)

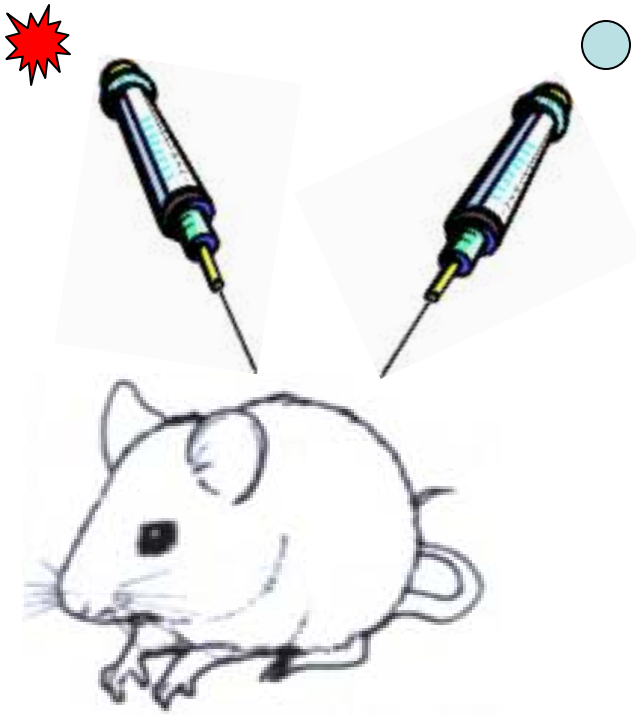
Cre-non recombined (461bp)



Lymphoadenopathy: enlargement of lymph nodes



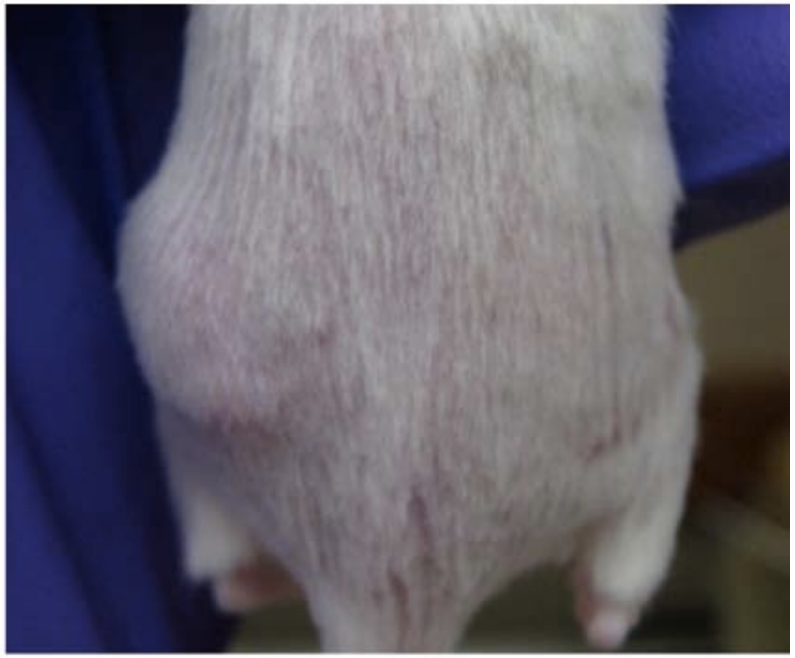
Nestin-Cre/*mir-21*$^{LSL\text{-}Tetoff}$ Lymphocyte cells



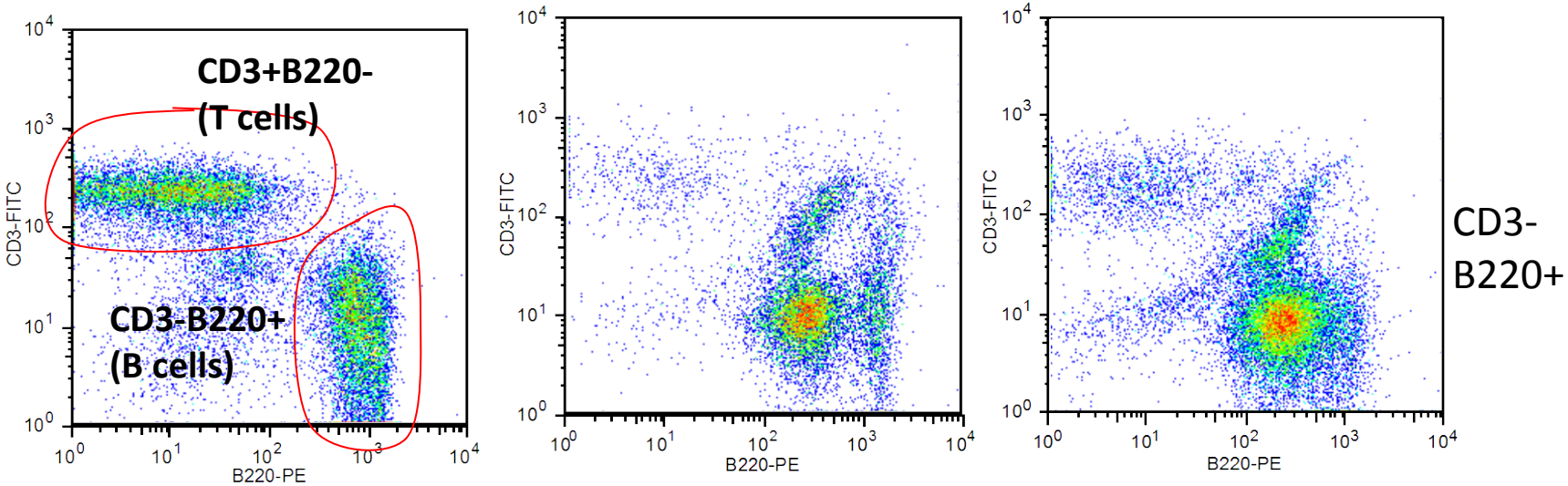
Normal Lymphocyte cells

7 immunodeficient mice were inoculated with 5 million lymphoid cells.

All the mice developed conspicuous tumors in less than 2.5 weeks

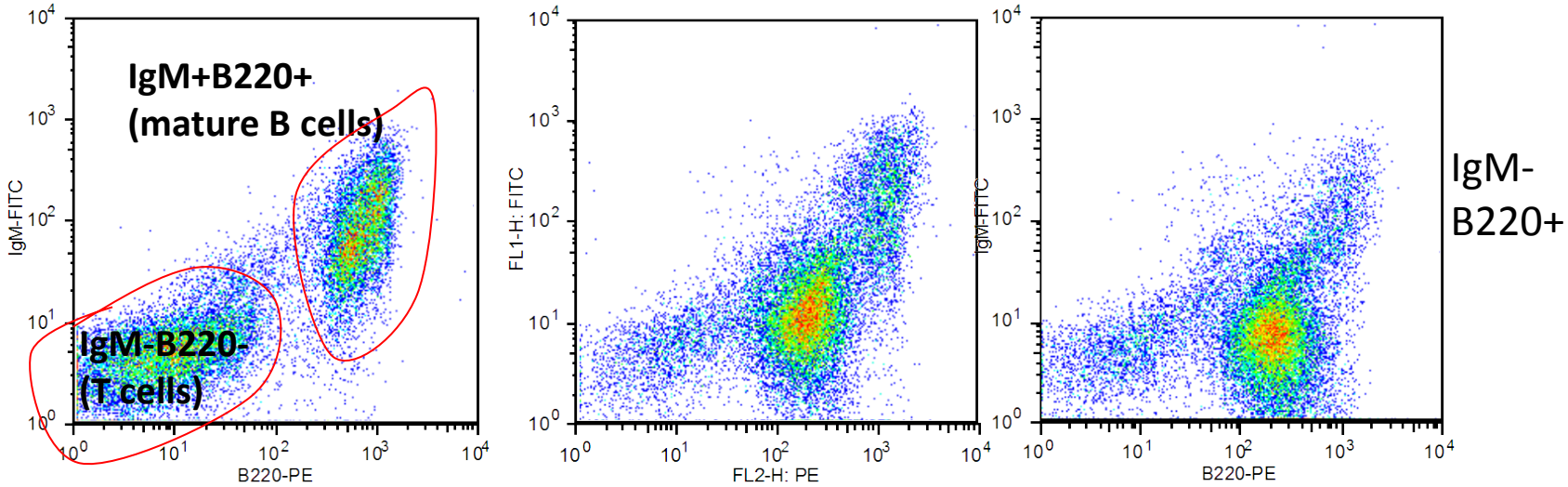


Mice develop a Pre-B-Cell Lymphoblastic Lymphoma/Leukemia



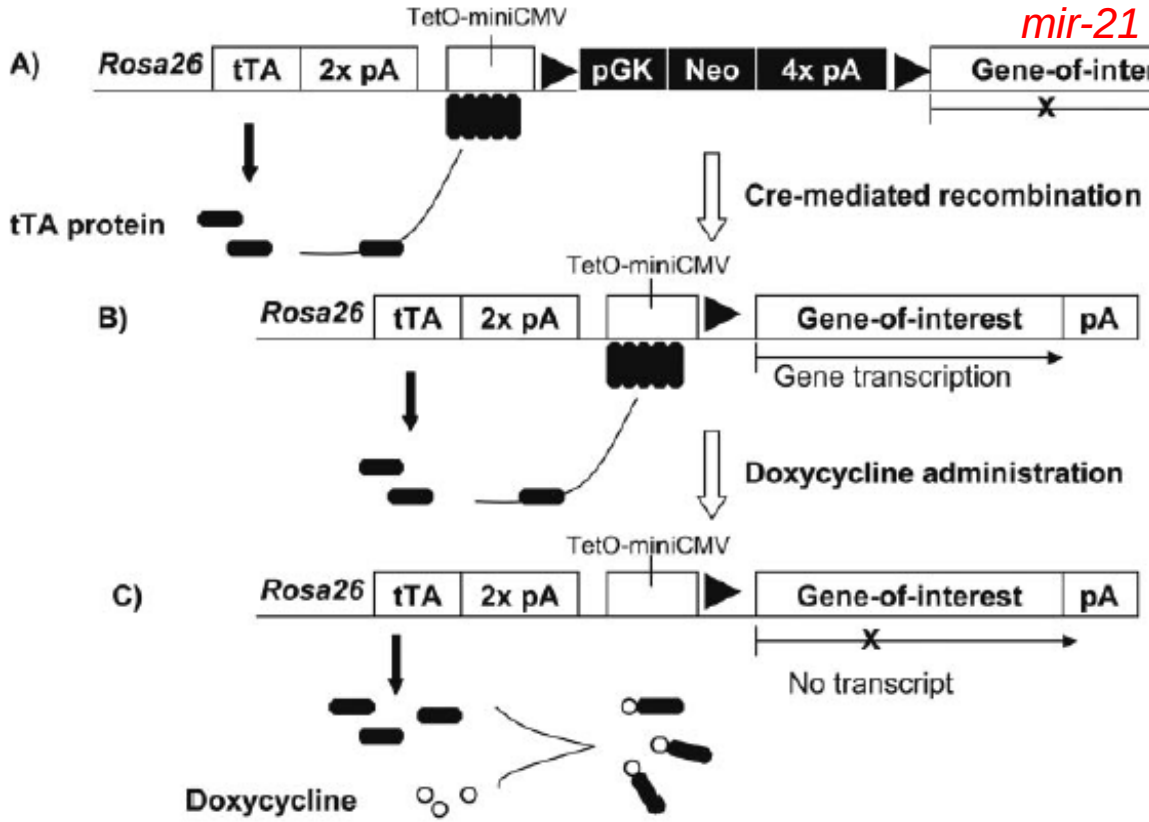
mir-21$^{LSL\text{-}Tetoff}$/Nestin-Cre

Spleen wt | Spleen #247 | Spleen #332



Transgenic mice to over-express *mir-21*:
Tissue specific & DOX inducibility (tet-off)

Tetracycline transactivator (tTA) is expressed by the ubiquitously expressed ROSA26 promoter



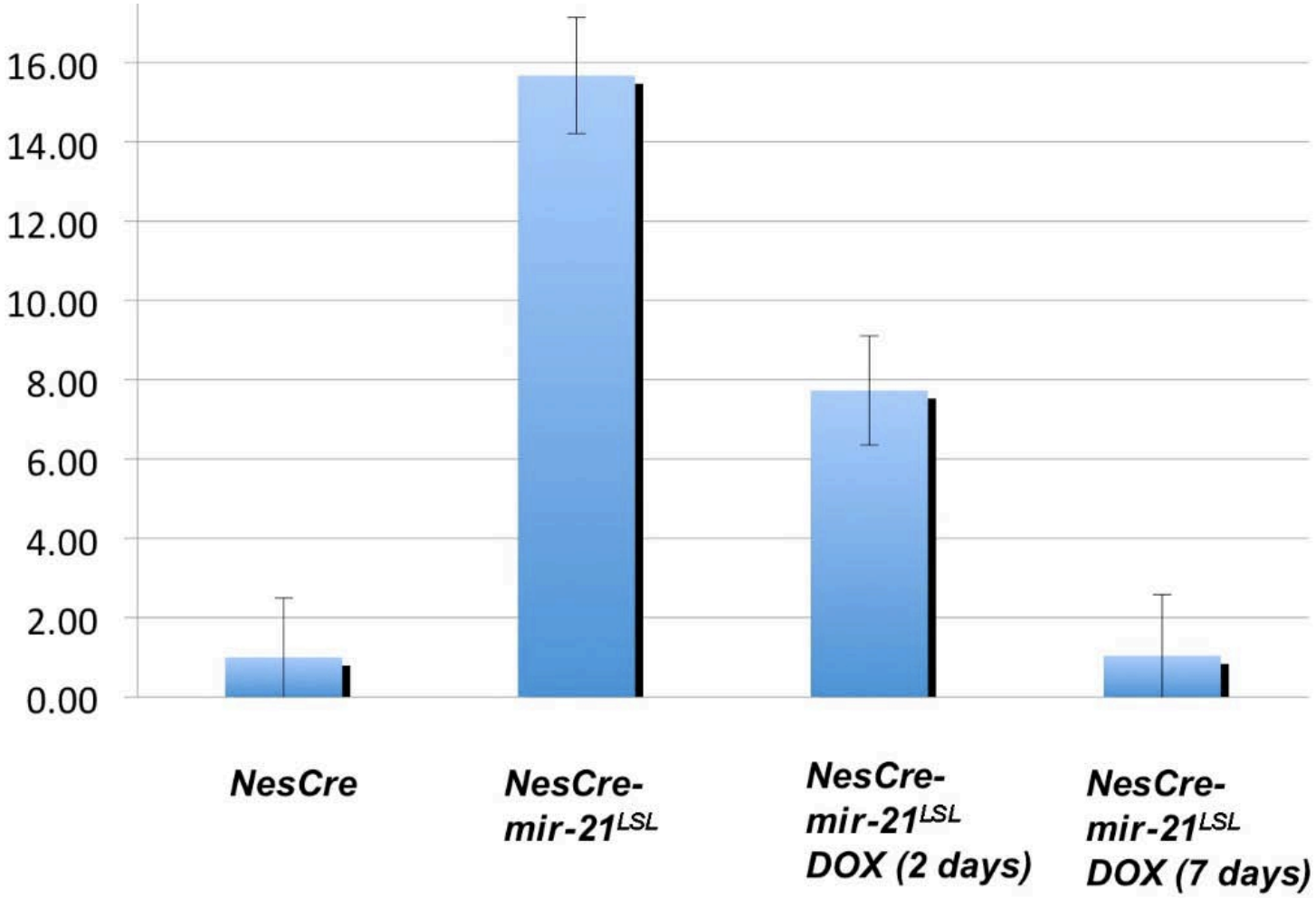
Endogenous ROSA26 promoter will express the tTA (Tet transactivator) necessary to the function of miniCMV promoter.

The *mir-21* gene expression is driven by the Tet-Operator-miniCMV that is inactivated by Dox.

Doxycycline binds to the tTA and inactivates it: the expression is lost.

(Mao et al., Nucleic Acids Research, 2006)

miR-21 levels in Nestin-Cre/mir-21 mice

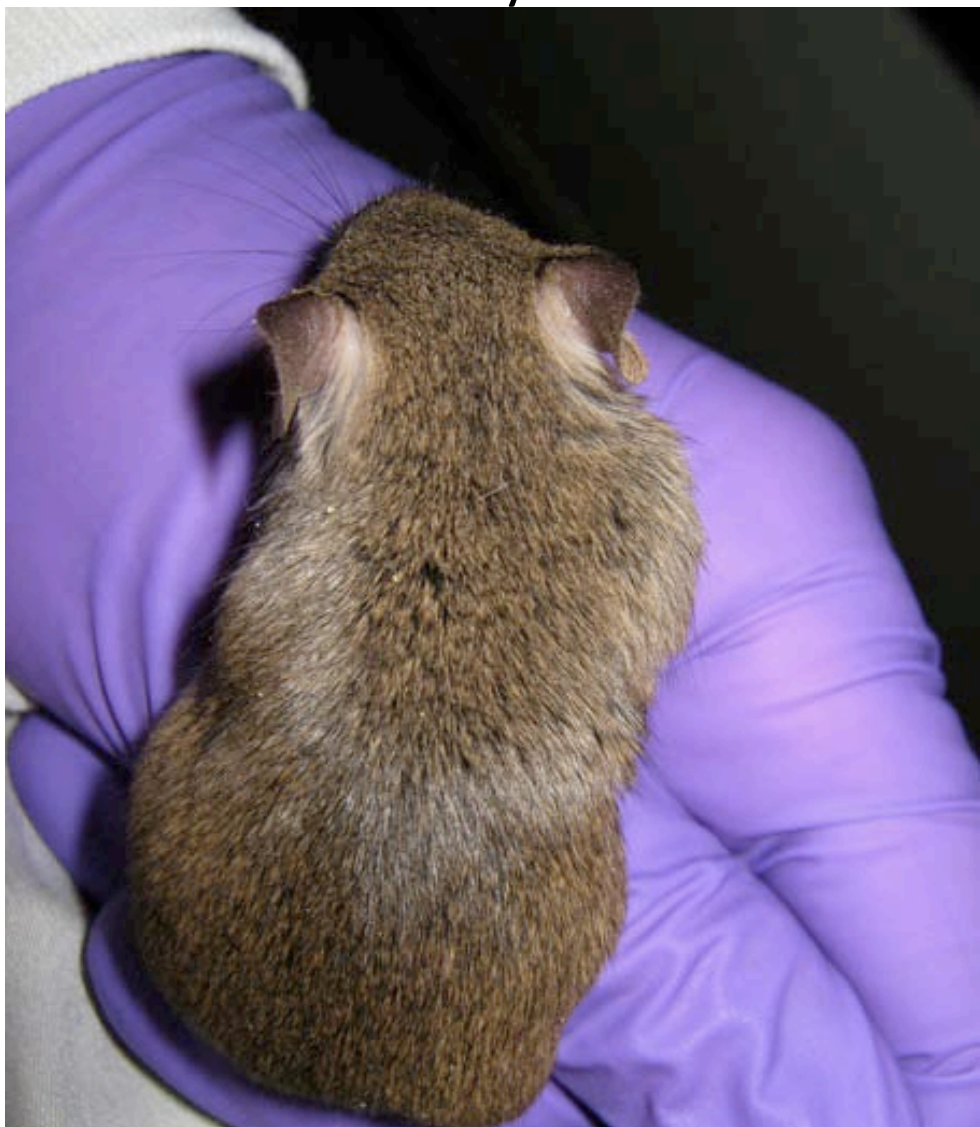


Mice fed with doxycycline food

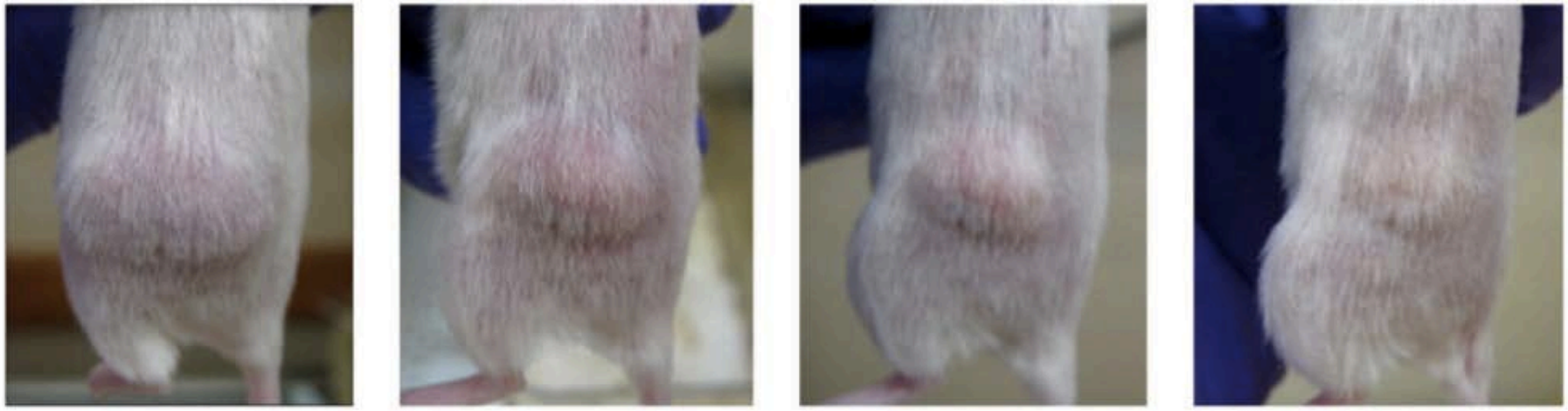
DOX treatment leads to regression of lymphoadenopathy

0 days

4 days

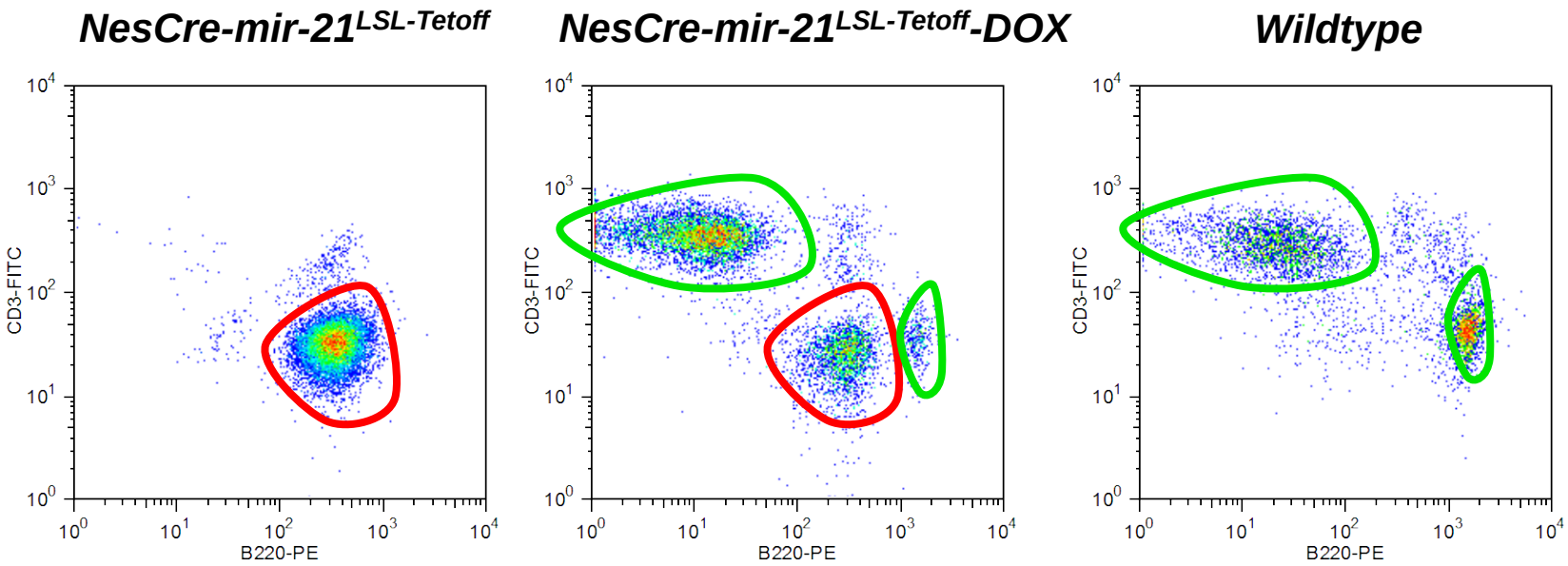


Doxycycline treatment leads to regression of transplanted tumors



0 days 2 days 4 days 6 days

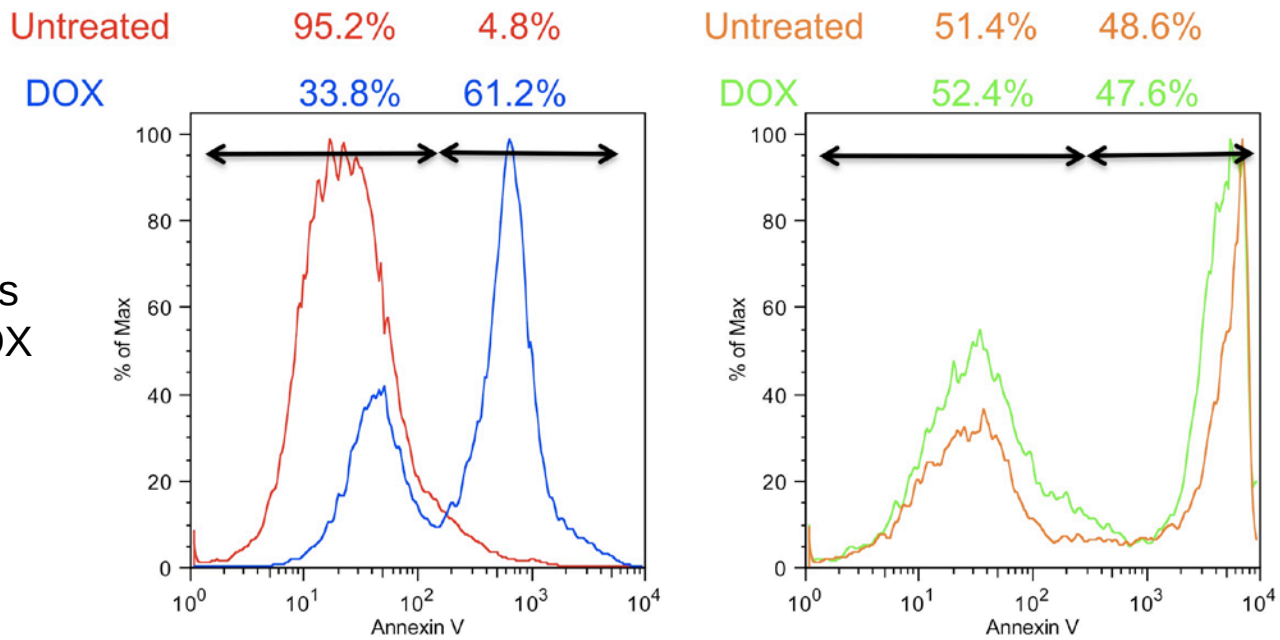
Hematopoietic recovery as seen by FACS



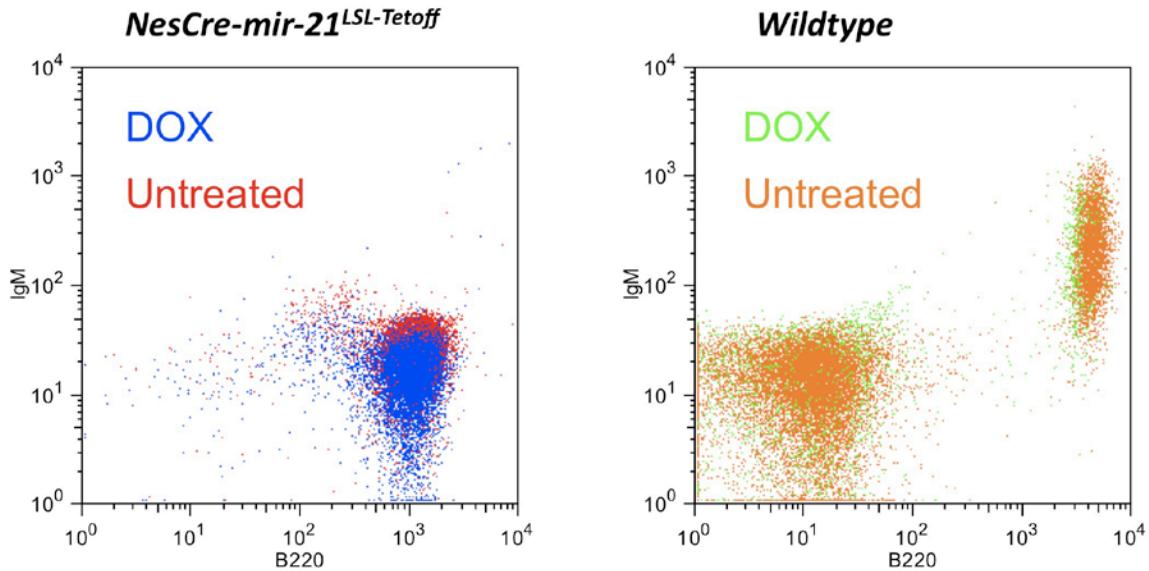
DOX 72h

Dox treatment leads to apoptosis but not differentiation

Apoptosis levels modified by DOX in *mir-21* mice, not in wt mice.



Immunotype (related to cell differentiation) Unchanged by DOX



Conclusions

- *mir-21* is a bona-fide oncogene.

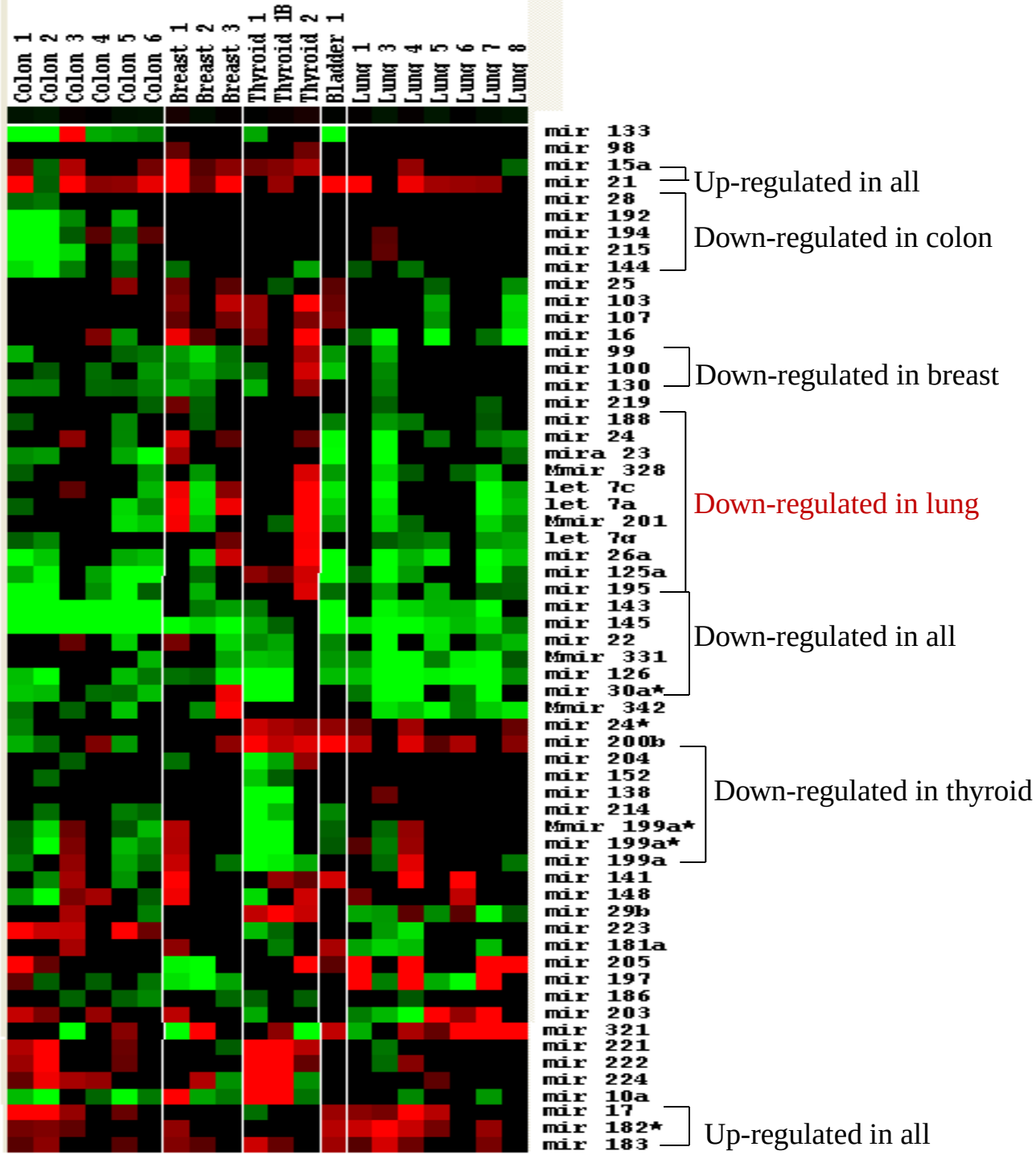
- The tumors are addicted to miR-21 over-expression (this is the first demonstration of oncomiR addiction).

- miR-21 is an excellent target for anticancer therapy.

Part B: MicroRNA therapeutics - a case for microRNA therapeutics in cancer

Collaboration with Andy Bader and David Brown at Mirna Rx

Potential oncomirs with altered expression in tumors relative to normal adjacent tissue



D. Brown, 2003

let-7 genes map to "fragile regions" associated with various cancers

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17p13.3-D	D17S1866-D17S1574	1.899	*miR-22; miR-132; miR-212*	HCC	—
17p13.3-D	ENO3-TP53	2.275	*miR-195*	Lung cancer	TP53
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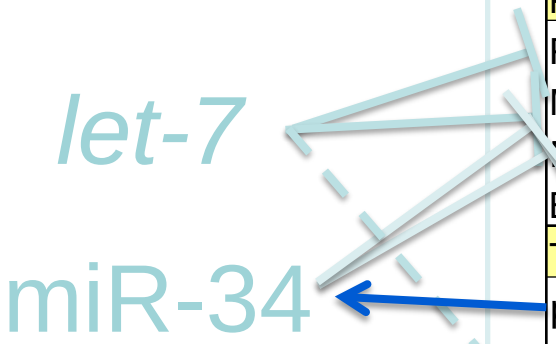
D, deleted region; A, amplified region; NSCLC, non-small-cell lung cancer; HCC, hepatocellular carcinoma; PTC, patched homolog (*Drosophila*); FANCC, Fanconi anemia, complementation group C; PPP2R1B, protein phosphatase 2, regulatory subunit A (PR 65), β isoform, miRs in a cluster are separated by a slash. For references, see Table 6.

Calin, G.A., *et al.*, 2004. *PNAS* 101:2999-3004

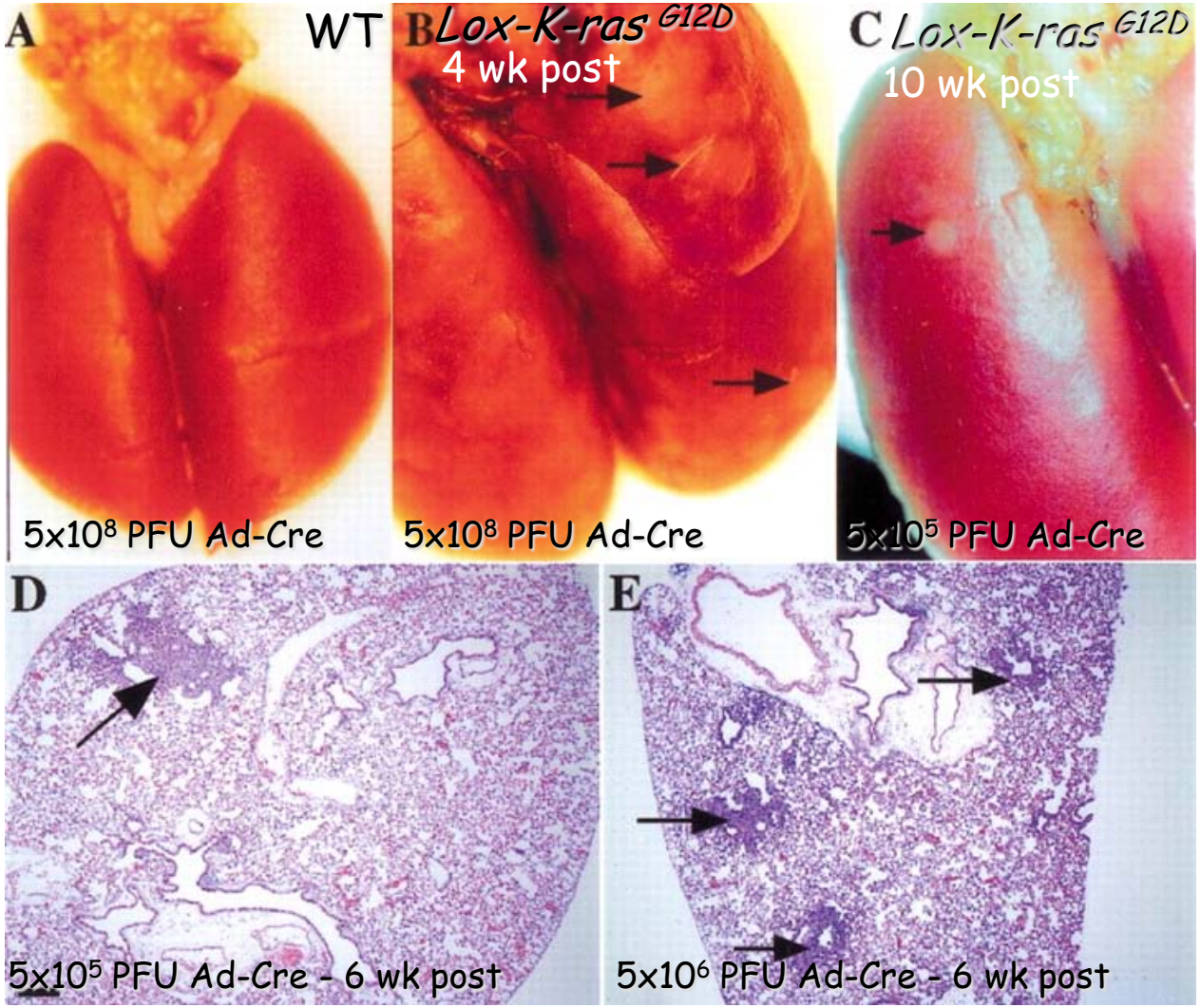
Combined, let-7 and miR-34 target the major oncogenes in lung cancer



Gene	Incidence
Proto-oncogenes	
RAS	15-20%
MYC	5-10%
BCL-2	10-35%
EGFR	7%
Tumor Suppressors	
p53	>50%
RB	15-30%
p16	10-40%

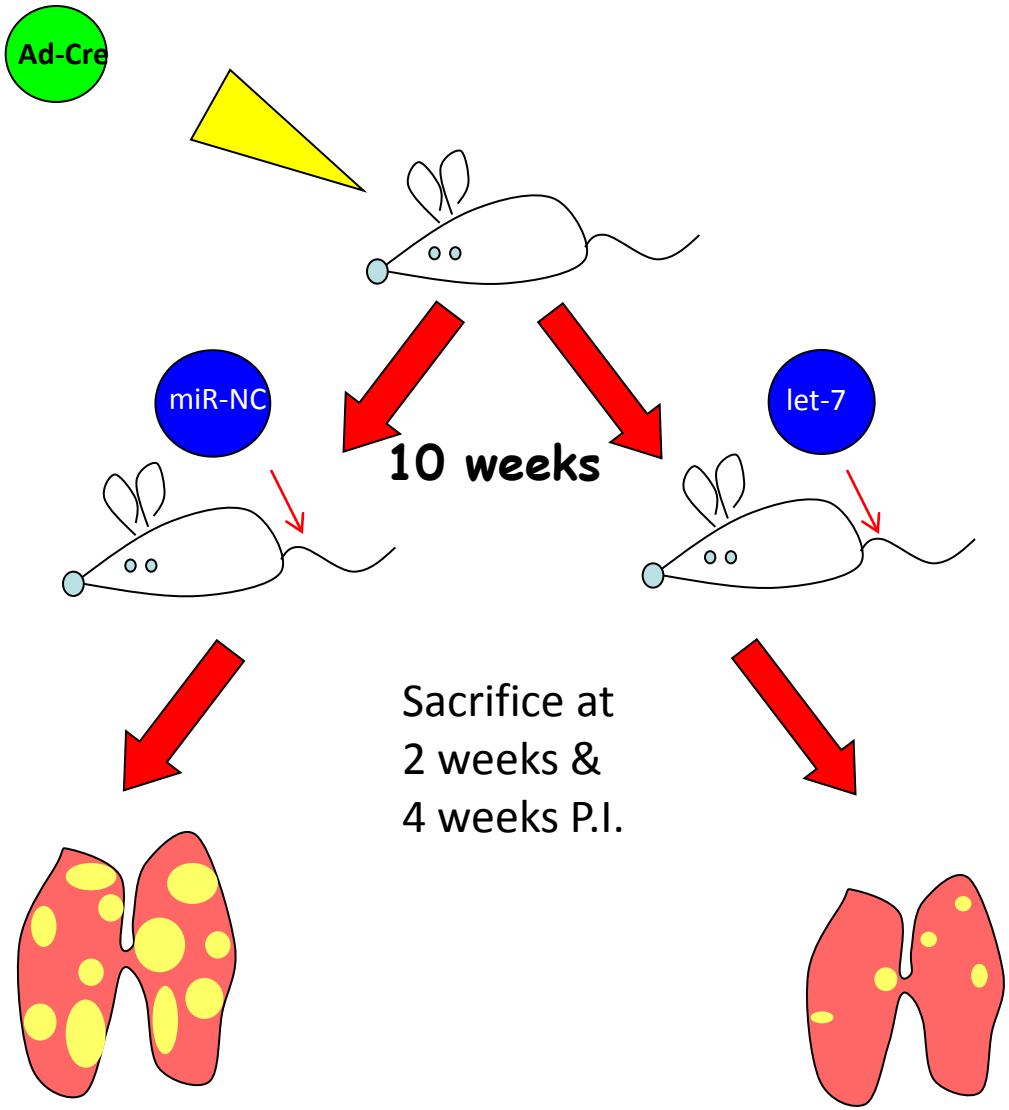
Can loss of *let-7* function increase tumor load in an activated *K-ras* lung cancer mouse model?

25%-50% of all human lung adenomas carry activating mutations in *KRAS*



Jackson, et al., (2001) Genes Dev.

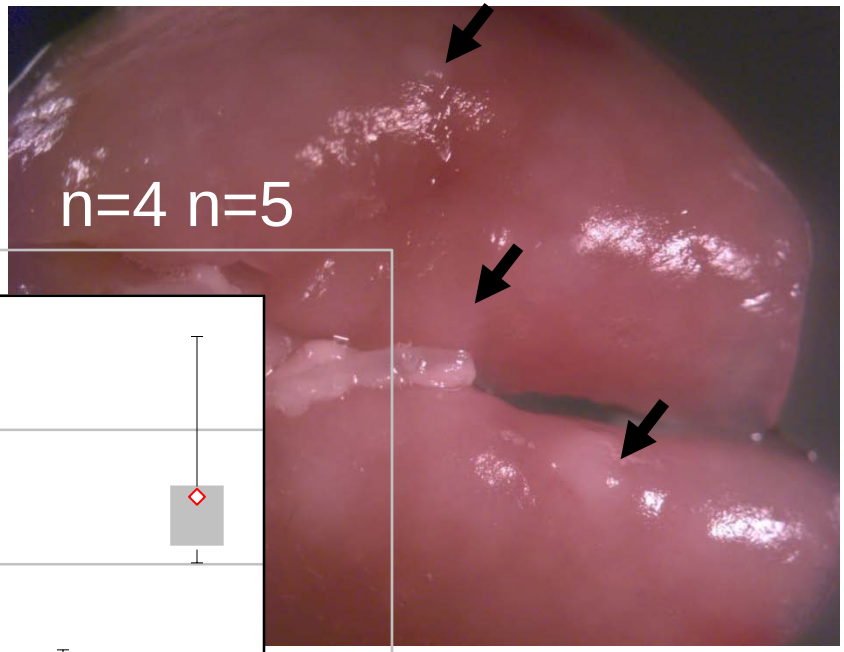
Can systemic *let-7* delivery reduce lung tumor burden?

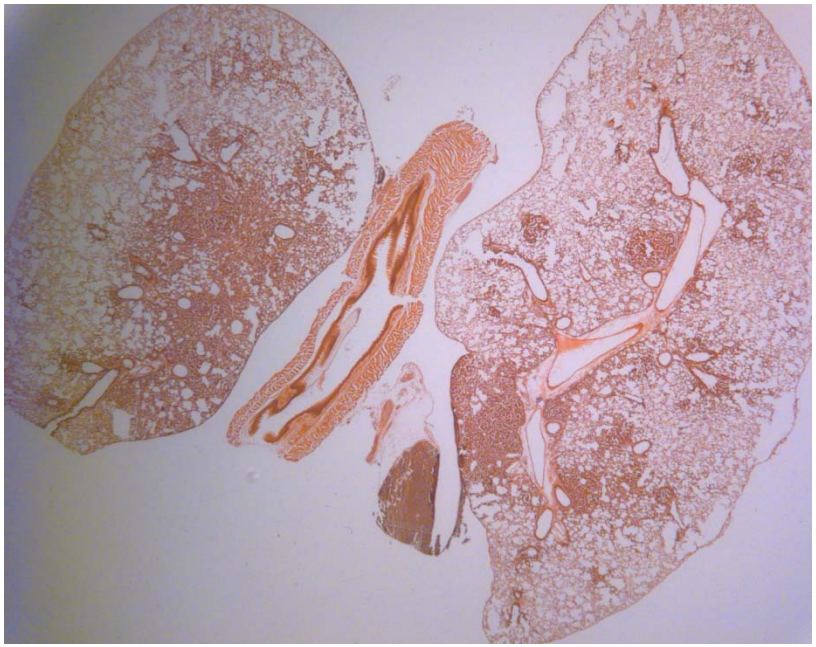


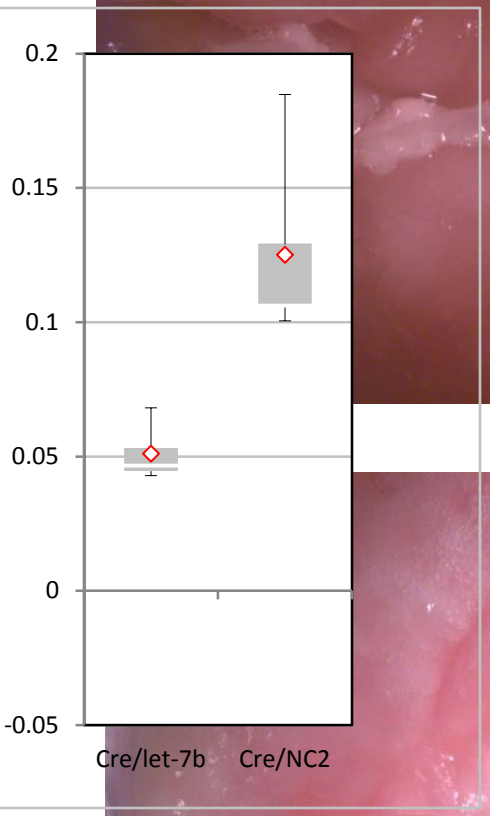
- 1mg/kg for a total of 8 tail vein injections every other day.
- Treated animals were sacrificed 48 hrs after the last injection

Trang et al, 2011 *Mol. Ther.*

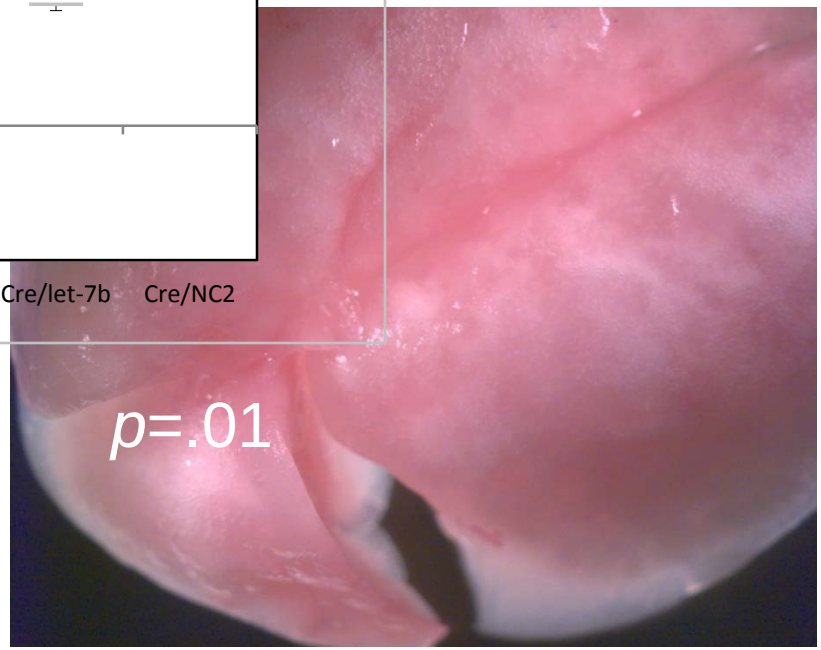
Negative control (NC2)

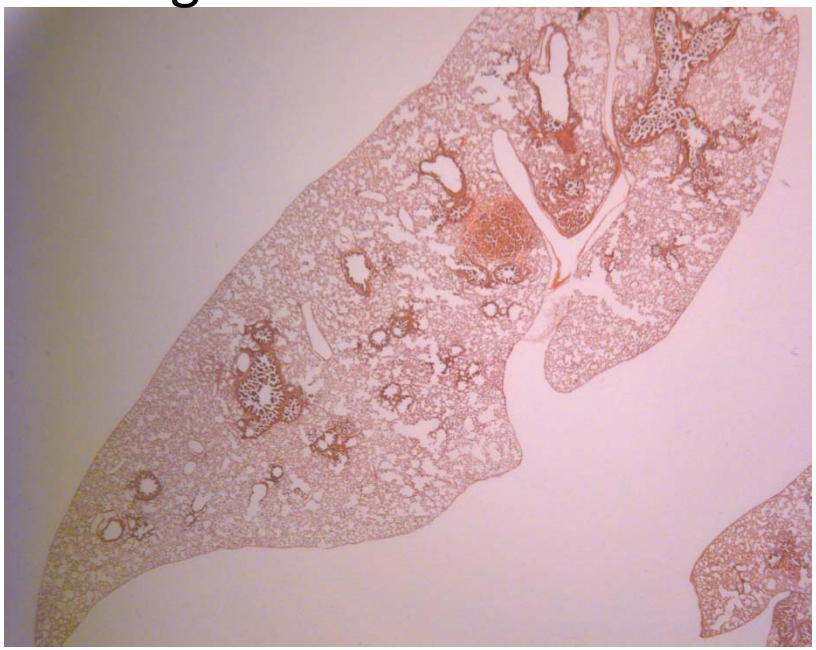




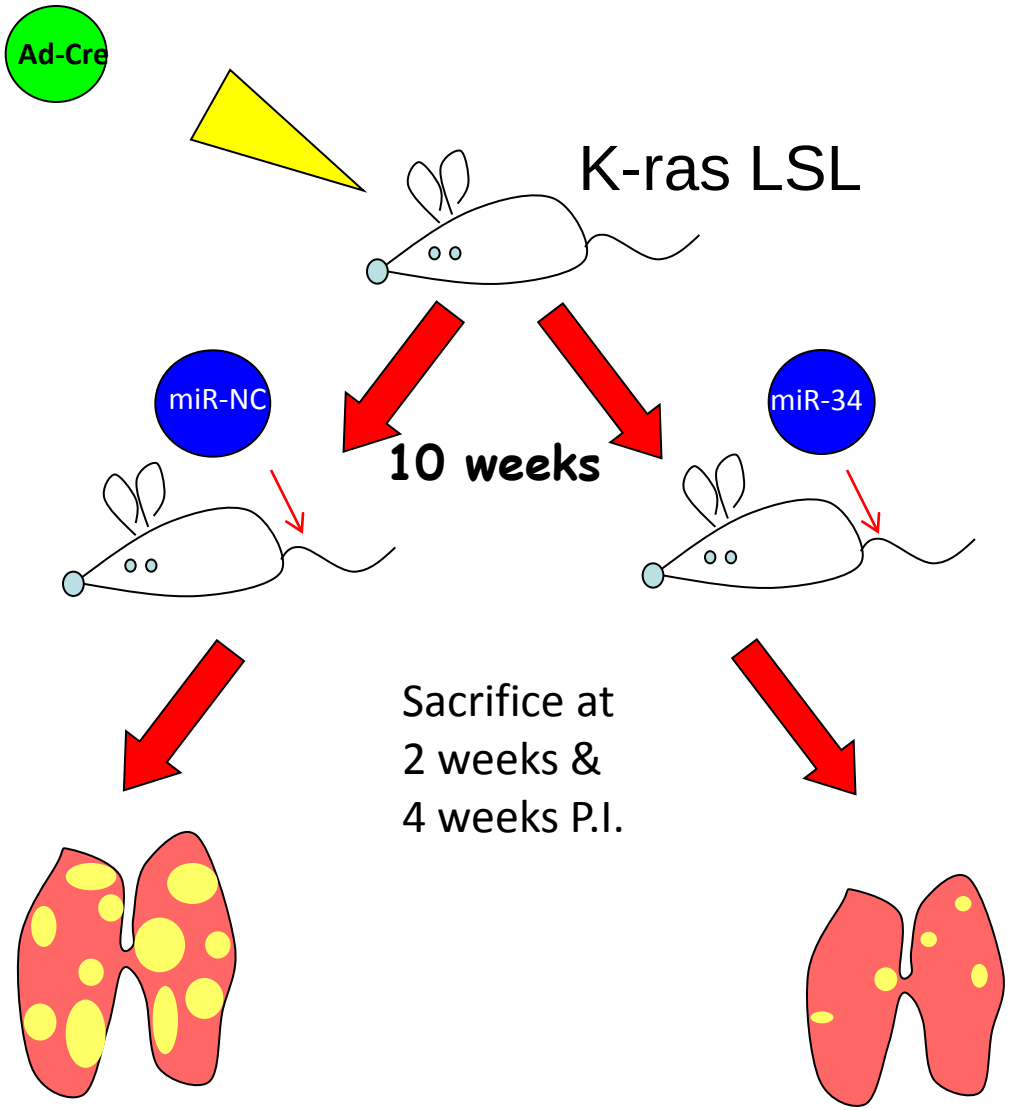


Let-7b treated lungs





Can systemic miR-34 delivery reduce lung tumor burden?



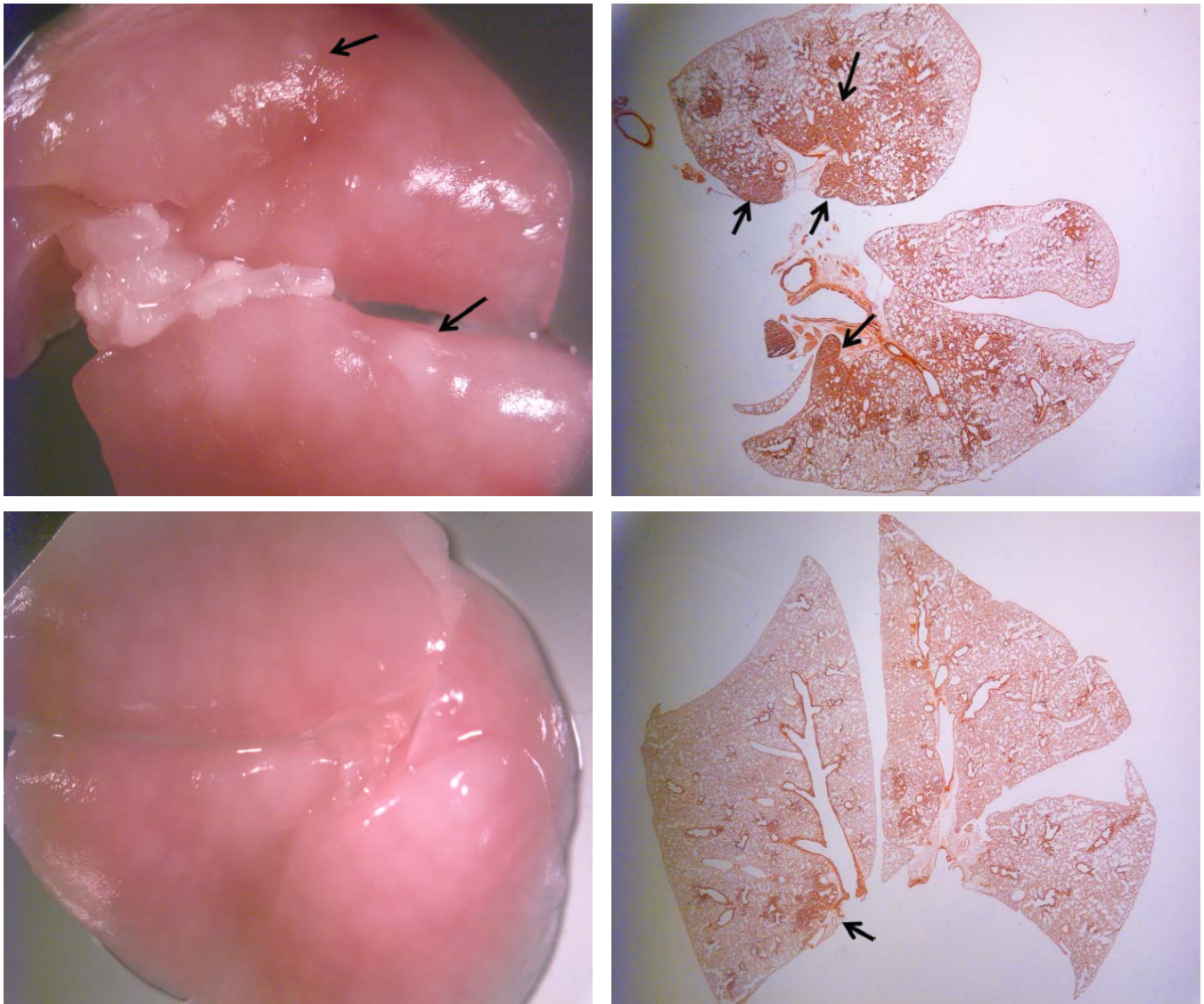
- 1mg/kg for a total of 8 tail vein injections every other day.
- Treated animals were sacrificed 48 hrs after the last injection

Systemic miR-34a treatment causes regression of lung tumors

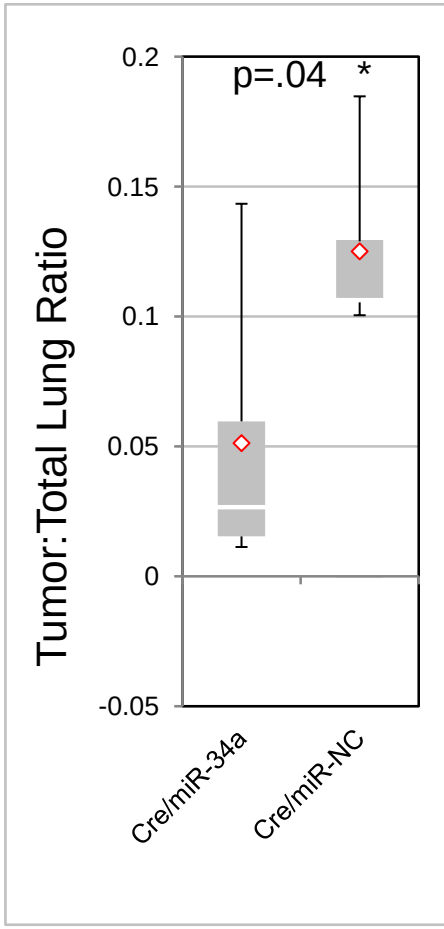
A

Whole lung

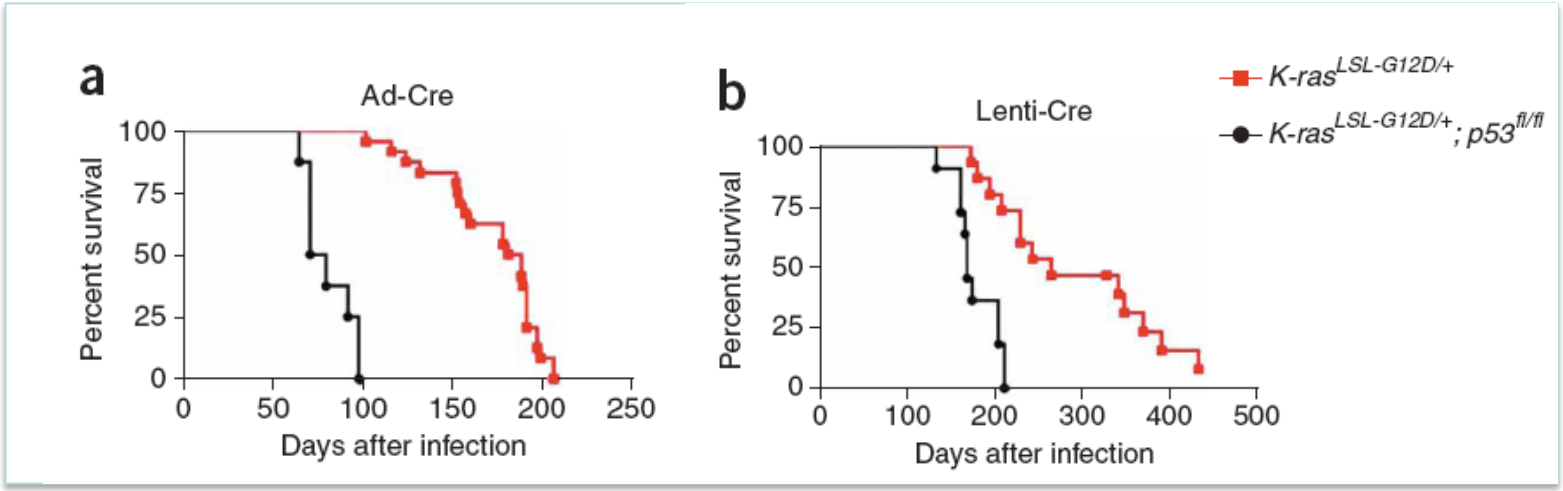
H&E

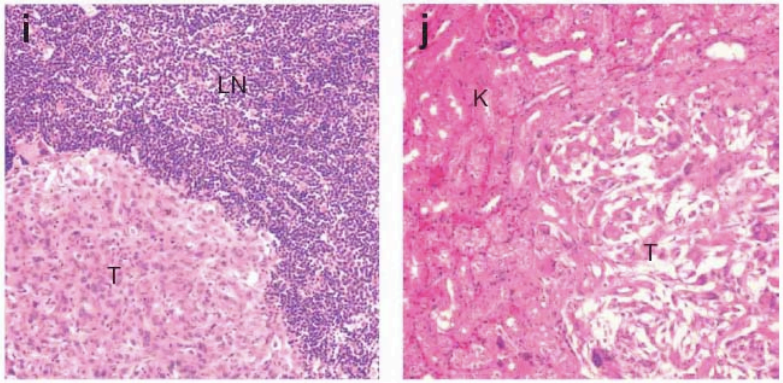


B



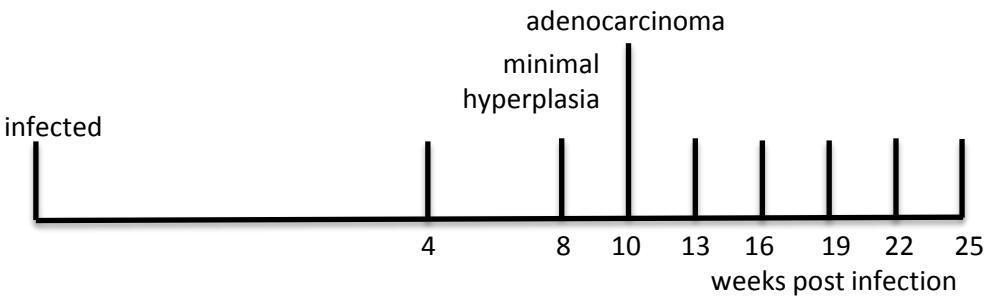
Kras^{G12D}; *p53*$^{fl/fl}$ mice have advanced disease and live half as long as *Kras*^{G12D} animals

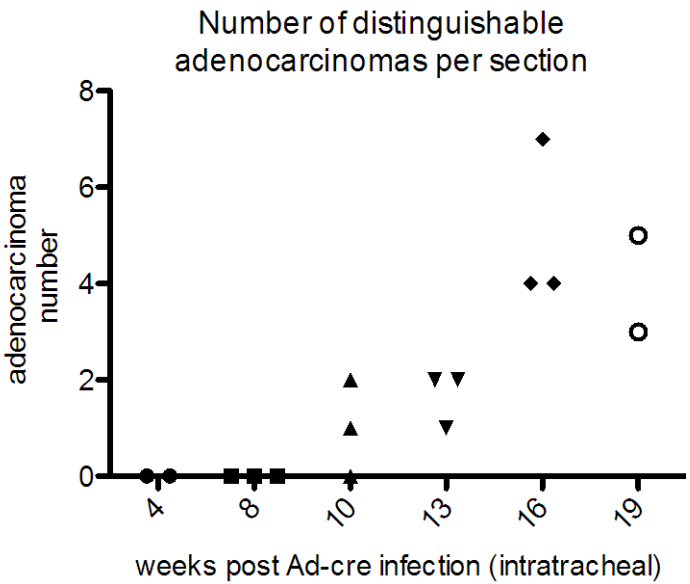


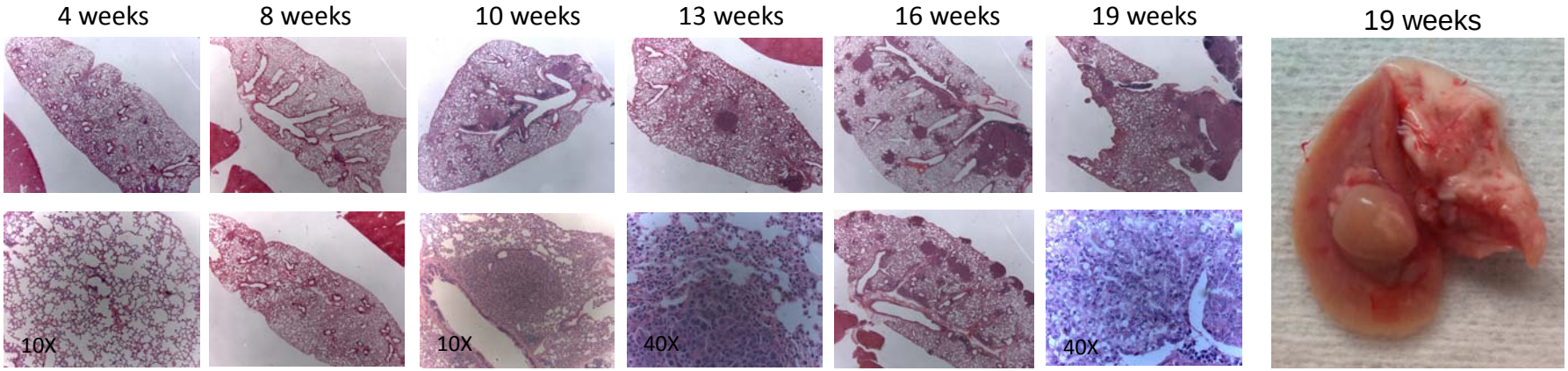


DuPage et al. Nature Protocols 2009

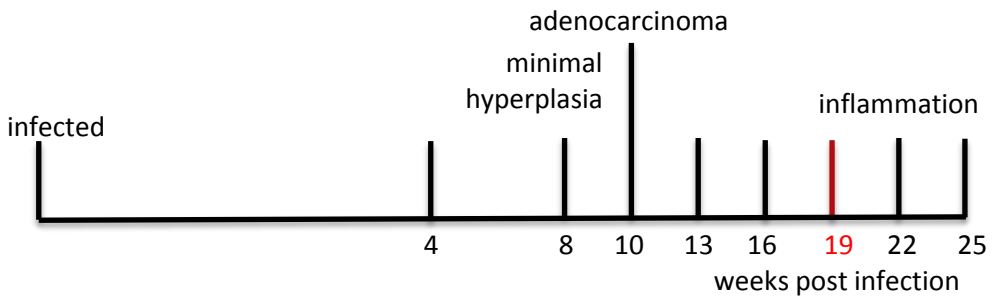
Tumor burden increase over time in *Kras*$^{G12D/+}$;*p53*$^{R172/+}$ mice



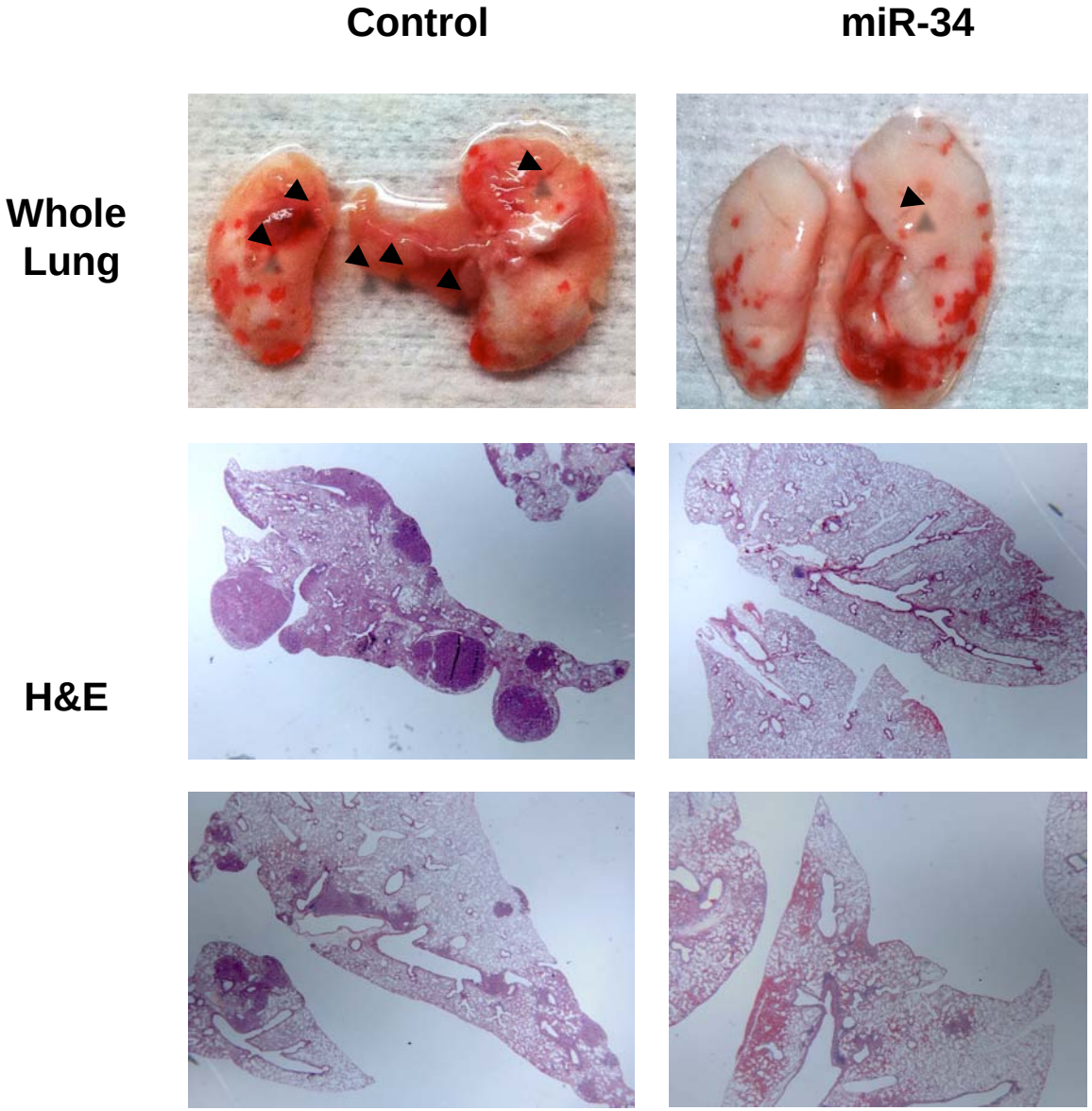




Restoration of miR-34 in *Kras*$^{G12D/+}$;*p53*$^{R172/+}$ mice (study design)



miR-34 pretreatment prevents *Kras*$^{G12D/+}$;*p53*$^{R172/+}$ lung tumorigenesis



Conclusions

- Local or systemic delivery of tumor suppressor miRNAs leads to organ-specific tumor regression.
- microRNAs and antimiRs should prove useful as therapeutics in difficult to treat cancers.

Current members of the Slack Lab

Post-docs:
Alex deLencastre
Masaomi Kato
Pedro Medina
Zach Pincus
Giovanni Stefani
Phong Trang
Andrea Kasinski
Samrat Kundu

Graduate students:
Imran Babar
Xiaowei Chen
Tang Metheetrairut
Sachi Inukai
Thalyana Smith-Vikos
Mike Turner

Technician:
Xianping Liang

Former Graduate students:
Steven Johnson, Monica Vella
Betsy Schulman, Mona Nolde
Michelle Boehm, Lena Chin,
Sarah Roush, Sirie Godshalk,
Katherine Carter

Former Postdocs:
Helge Grosshans, Aurora Kerscher
Gopi Ramaswamy, Ryusuke Niwa
Diya Banerjee, E-Y. Choi, Rachael Nimmo
Shih-Peng Chan

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