“The Onassis Foundation
Science Lecture Series 2011 in Biology”

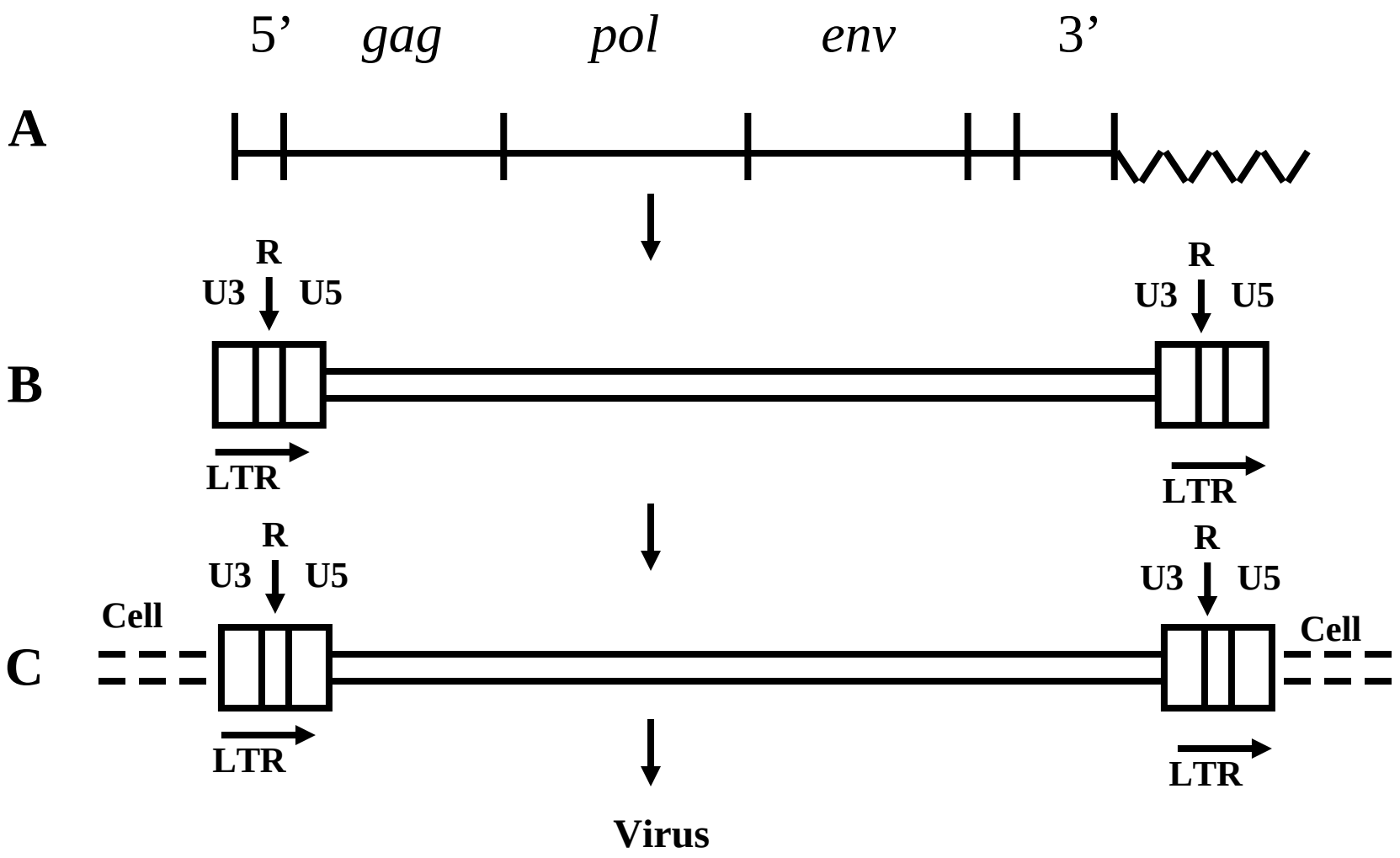
Basic and Applied Virology

July 15, 2011

Philip N. Tsichlis
Molecular Oncology Research Institute
Tufts Medical Center, Boston, MA

Oncogenes involved in the induction and progression of retrovirus-induced T cell lymphomas as probes of normal and neoplastic cell function.

RETROVIRAL REPLICATION



Validation of the Insertional Mutagenesis Model of Oncogenesis

Cell, Vol. 23, 323–334, February 1981, Copyright © 1981 by MIT

Avian Leukosis Virus-Induced Tumors Have Common Proviral Integration Sites and Synthesize Discrete New RNAs: Oncogenesis by Promoter Insertion

Benjamin G. Neel and William S. Hayward
The Rockefeller University
New York, New York 10021
Harriet L. Robinson
The Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545
Joanna Fang and Susan M. Astrin
The Institute for Cancer Research
The Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111

NATURE VOL. 302 31 MARCH 1983

A common region for proviral DNA integration in MoMuLV-induced rat thymic lymphomas

Philip N. Tsichlis, P. Gunter Strauss & Li Fu Hu

Laboratory of Tumor Virus Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

Cell, Vol. 31, 99–109, November 1982, Copyright © 1982 by MIT

Many Tumors Induced by the Mouse Mammary Tumor Virus Contain a Provirus Integrated in the Same Region of the Host Genome

Roel Nusse* and Harold E. Varmus
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San Francisco, California 94143
and *Department of Virology
Antoni van Leeuwenhoekhuis
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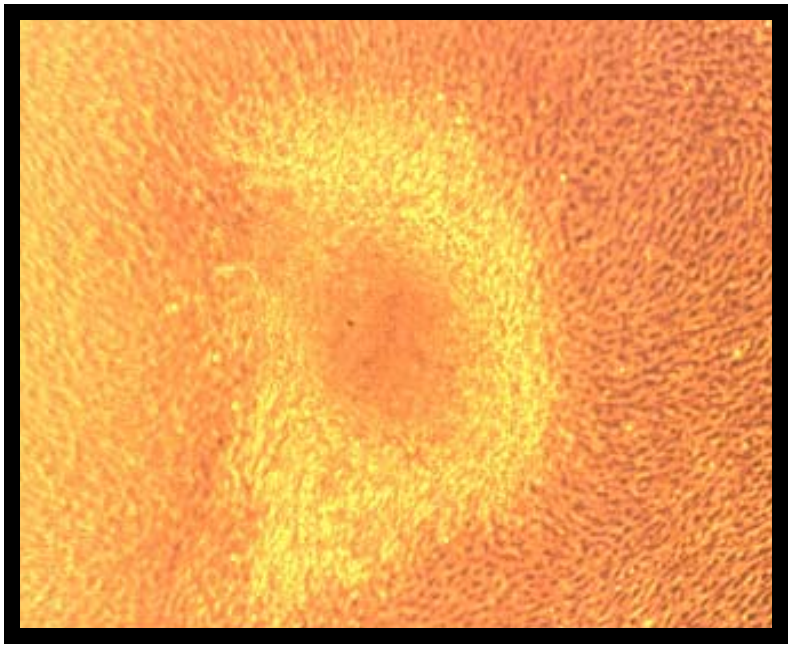
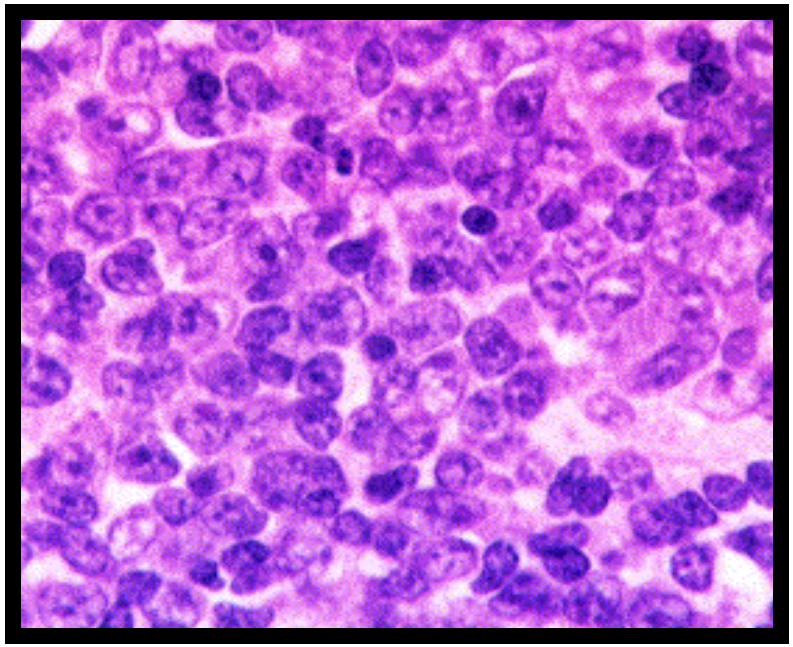
A Retroviral Oncogene, *akt*, Encoding a Serine-Threonine Kinase Containing an SH2-Like Region

ALFONSO BELLACOSA, JOSEPH R. TESTA, STEPHEN P. STAAL,* PHILIP N. TSICHLIS†

***Science*, 254: 274, 1991.**

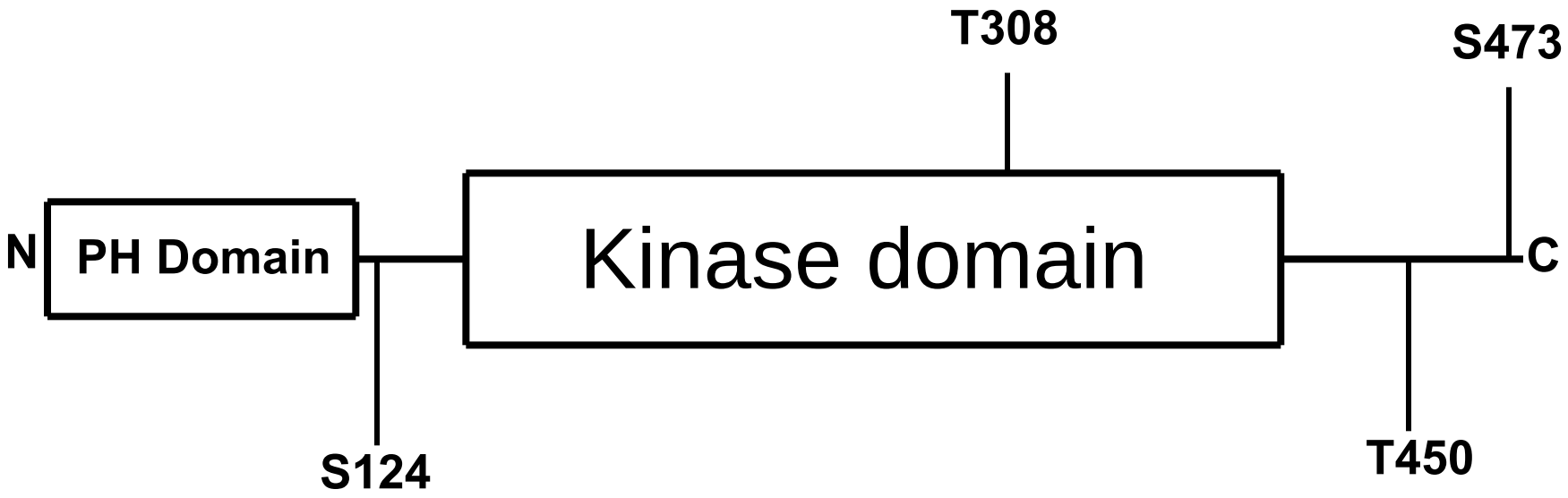


Akt transforms cultured cells

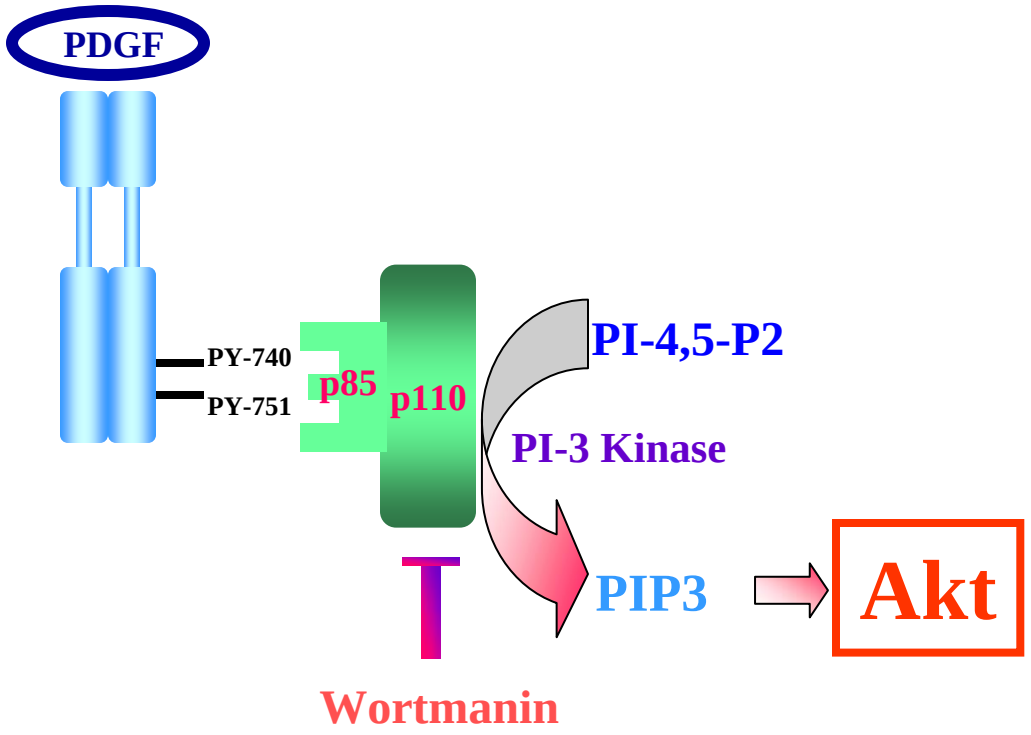


Akt induces Lymphoid Tumors

Genetic structure of the Akt Kinase

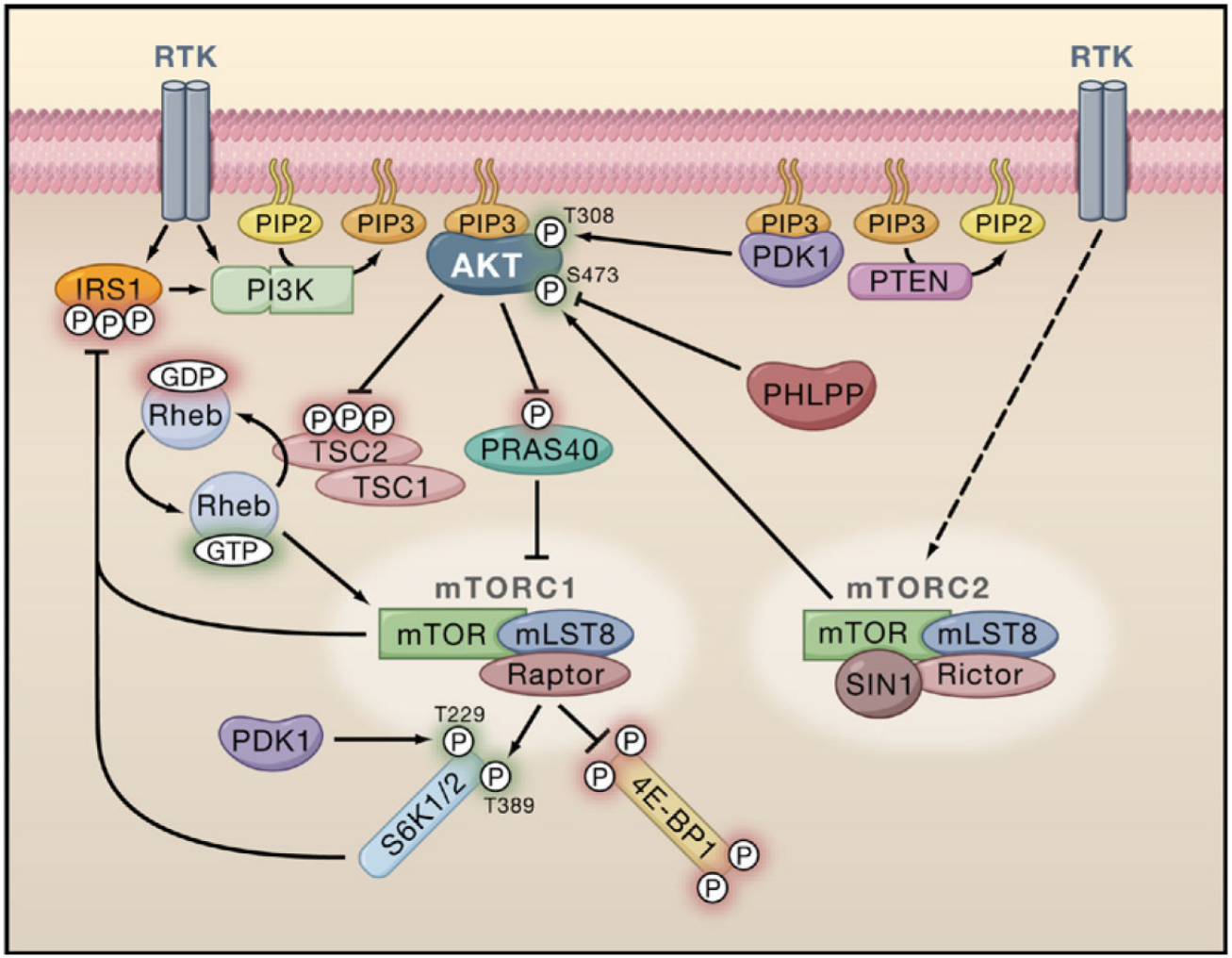


AKT IS A TARGET OF THE PI-3 KINASE



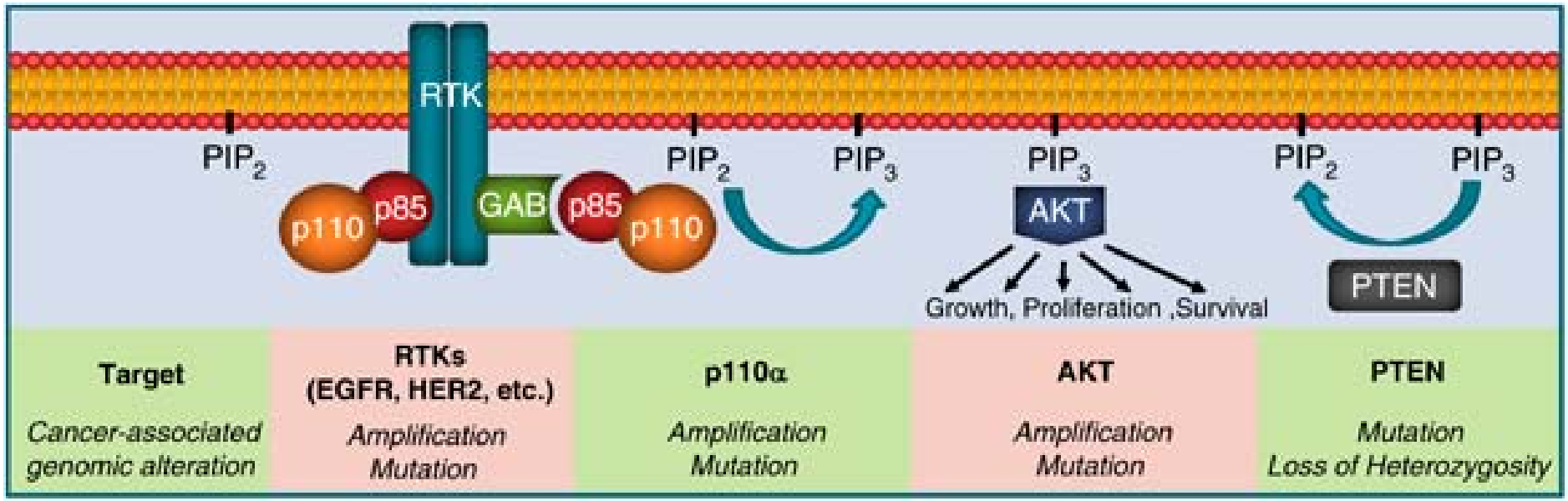
Cell, 81: 727, 1995

Akt activation by growth factors



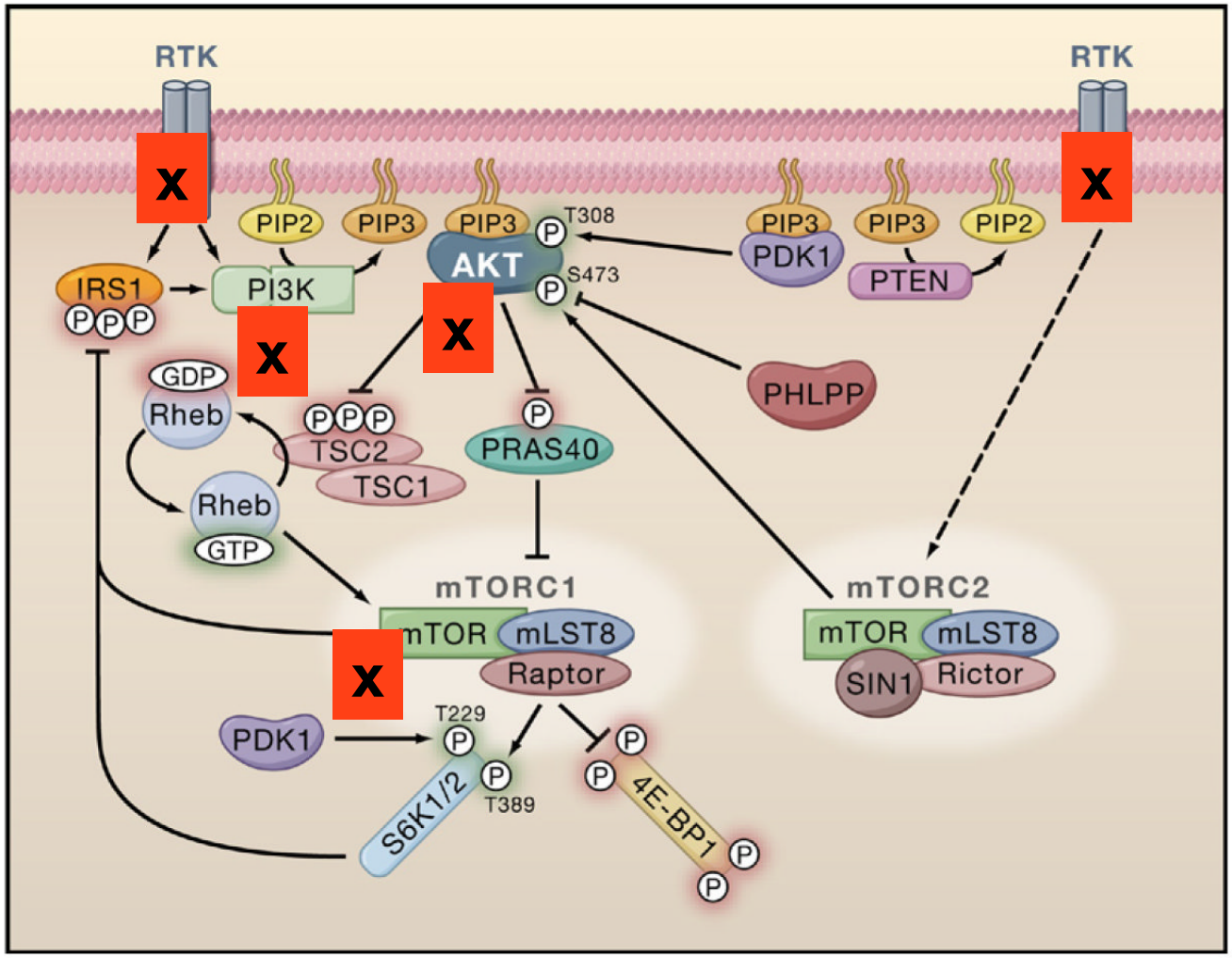
Cell 129:1261, 2007

The PI-3K/Akt pathway in human cancer



Oncogene 27: 5497, 2008

Targeting the PI-3K/Akt pathway



Cell 129:1261, 2007

Inhibitors of the PI-3K/Akt pathway under development

Inhibitor	Company	Phase of clinical trial	Refs
Dual PI3K and mTOR inhibitors			
BEZ235	Novartis	Phase I/II	37,92,96,103,149
BGT226	Novartis	Phase I/II	NS
XL765	Exelixis	Phase I	NS
SF1126	Semafore	Phase I/II	NS
GSK1059615	GSK	Preclinical	150
PI3K inhibitors			
XL147	Exelixis	Phase I	NS
PX866	Oncothyreon	Phase I	100,151,152
GDC0941	Genentech/Piramed/Roche	Phase I	NS
BKM120	Novartis	Phase I	NS
CAL101 (targets p110δ)	Calistoga Pharmaceuticals	Phase I	NS
Akt inhibitors			
Perifosine	Keryx	Phase I/II	153–156
GSK690693	GSK	Phase I	157,158
VQD002	Vioquest	Phase I	NS
MK2206	Merck	Phase I	NS
mTOR inhibitors (catalytic site)			
OSI027	OSI Pharmaceuticals	Phase I	NS
AZD8055	AstraZeneca	Phase I/II	NS

NS, not stated.

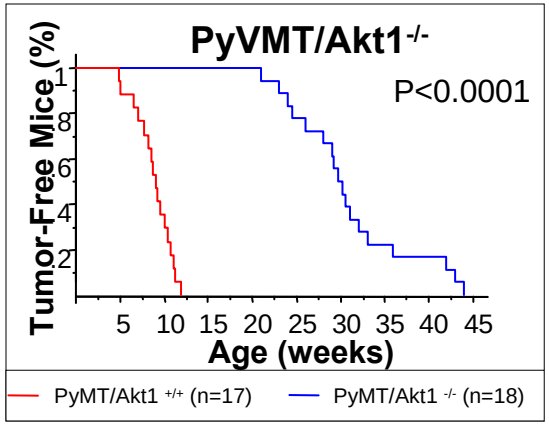
Nature Reviews, Cancer 9: 550, 2009

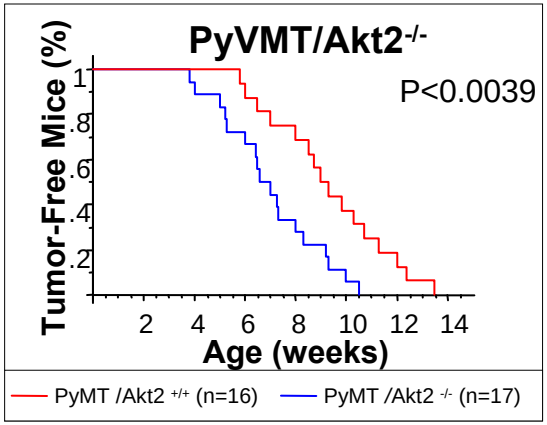
There are three Akt isoforms, Akt1, Akt2 and Akt3

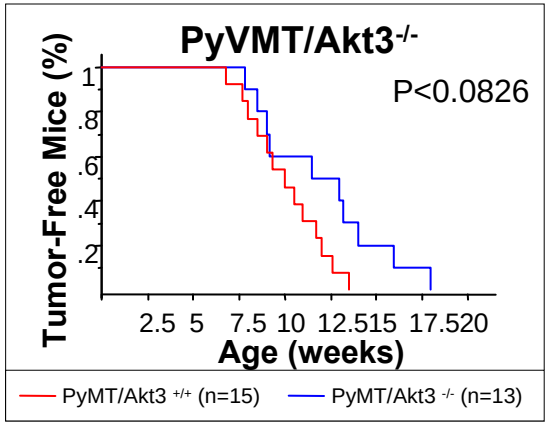
Akt1 is not Akt2 is not Akt3

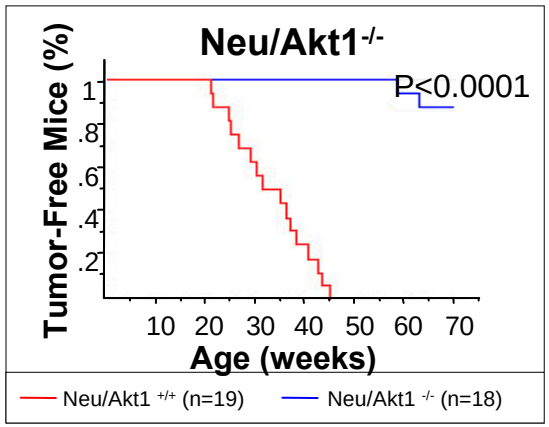
For at least some of the Akt functions, the balance between isoforms is more important biologically, than the overall Akt activity

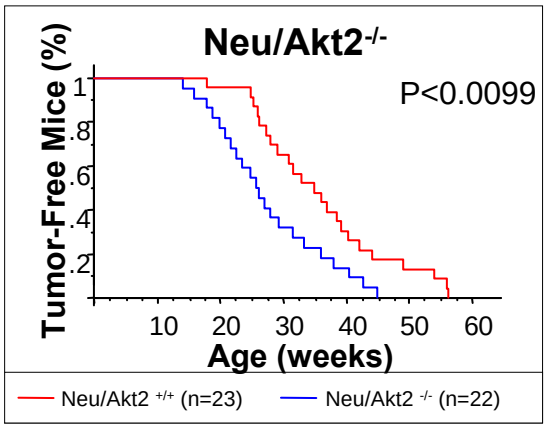
Akt1 ablation significantly delays, while Akt2 ablation accelerates the development of mammary adenocarcinomas in MMTV-Neu and MMTV-PyMT transgenic mice.

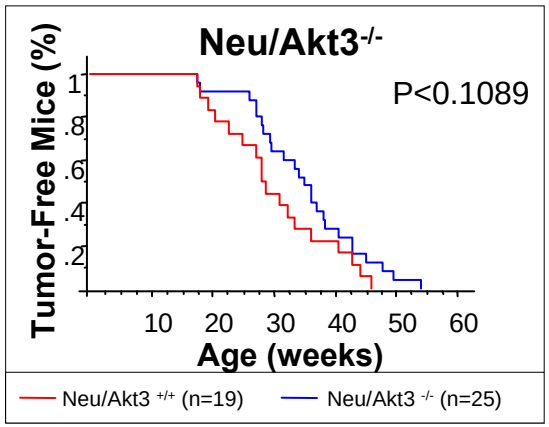








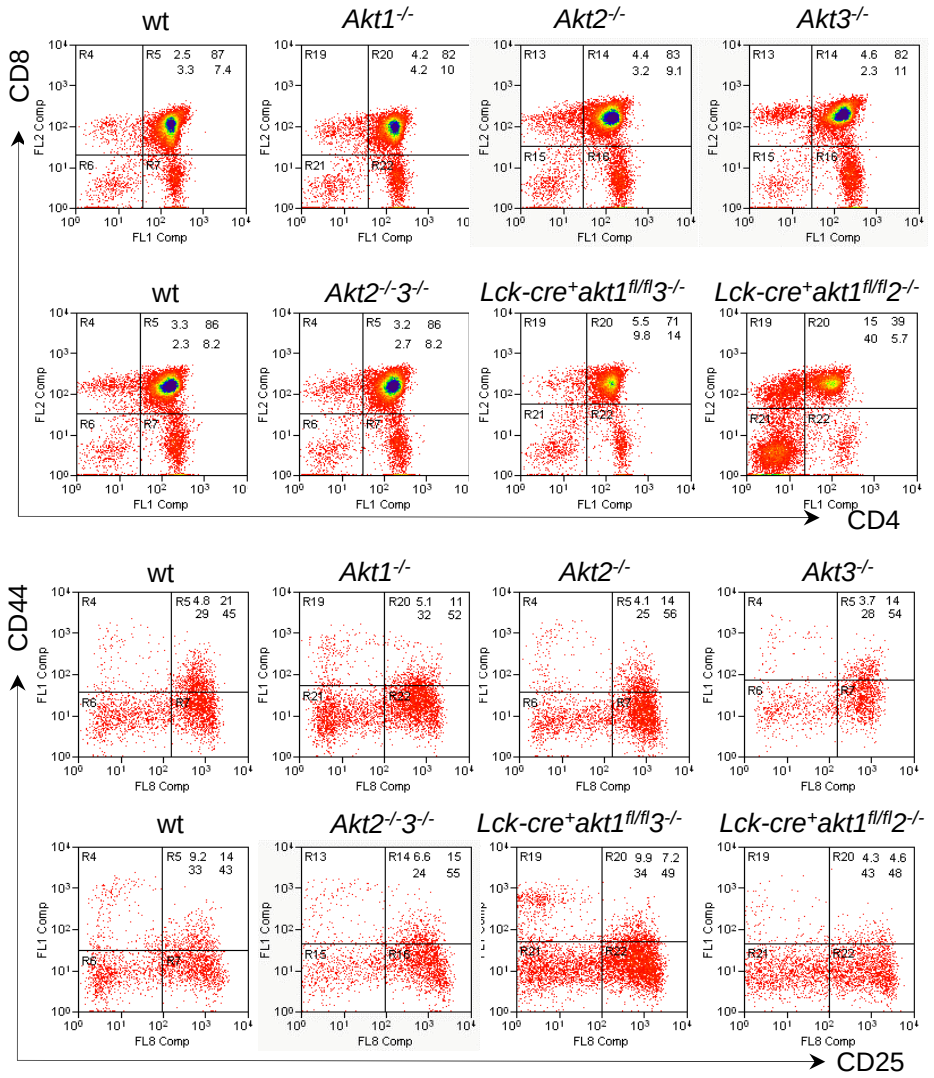




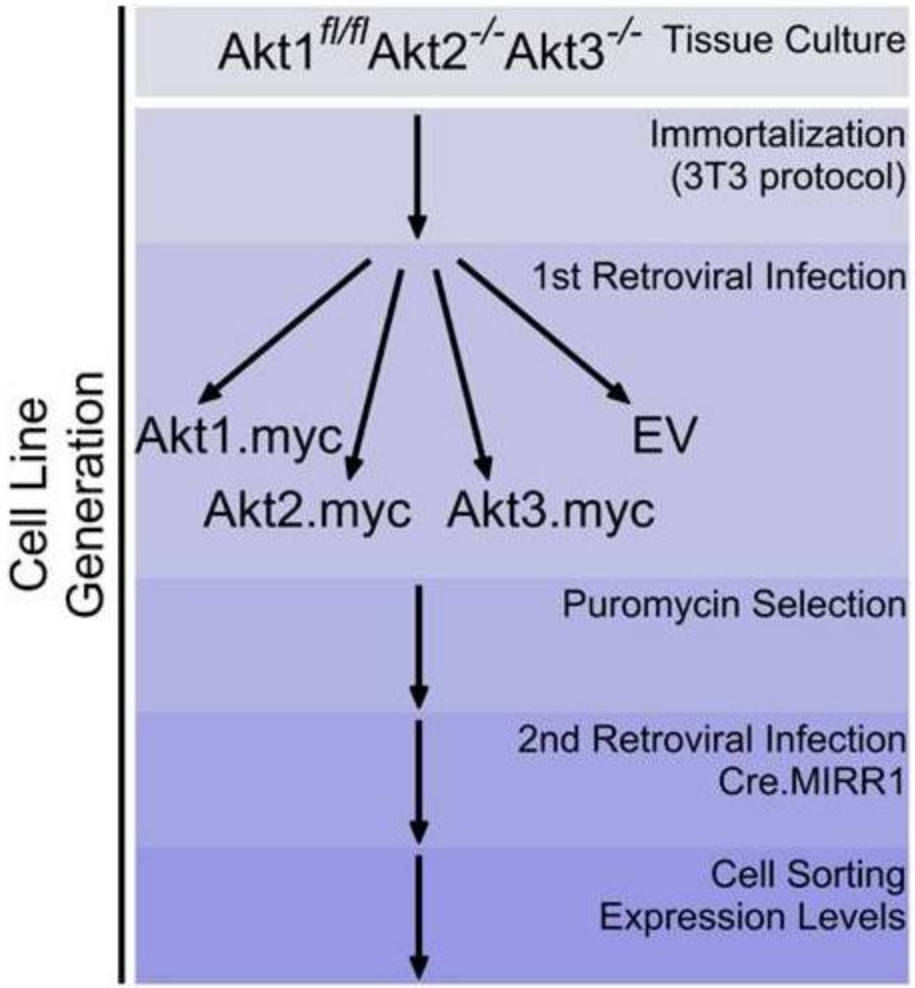
Mammary adenocarcinomas in Akt1 knockout mice are highly invasive

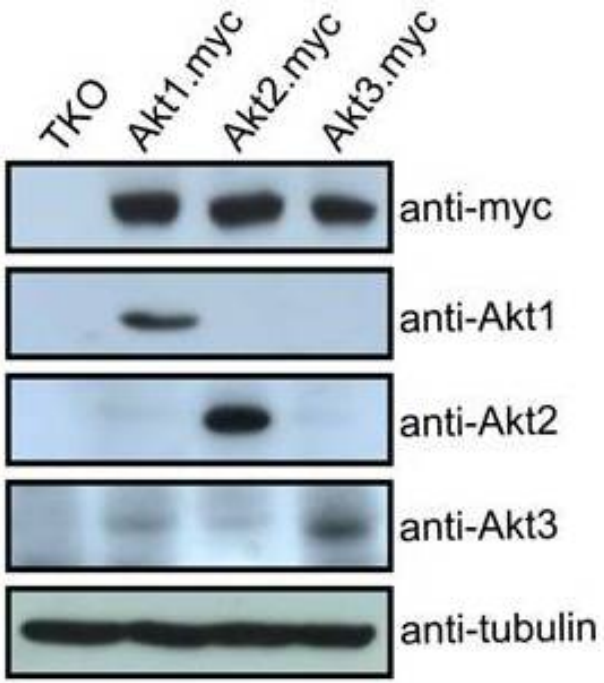
	MMTV Neu-induced mammary adenocarcinomas		
	WT	*Akt1*$^{-/-}$	*Akt2*$^{-/-}$
Invasion	2/10	8/8	5/12

Combined ablation of Akt1 and Akt2 inhibits β-selection and the transition of DN thymocytes to the DP stage

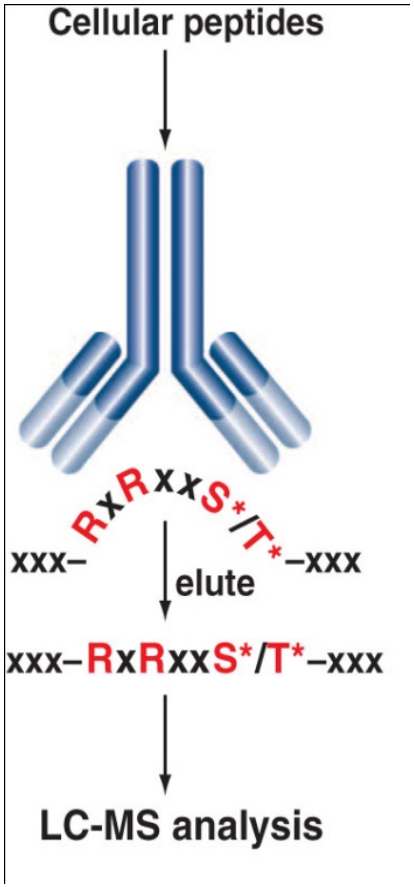


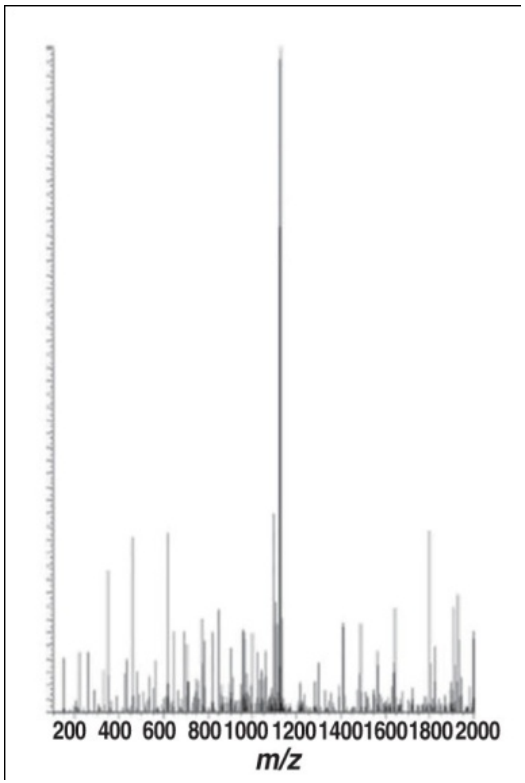
Development of a new platform for the study of Akt isoform-specific properties





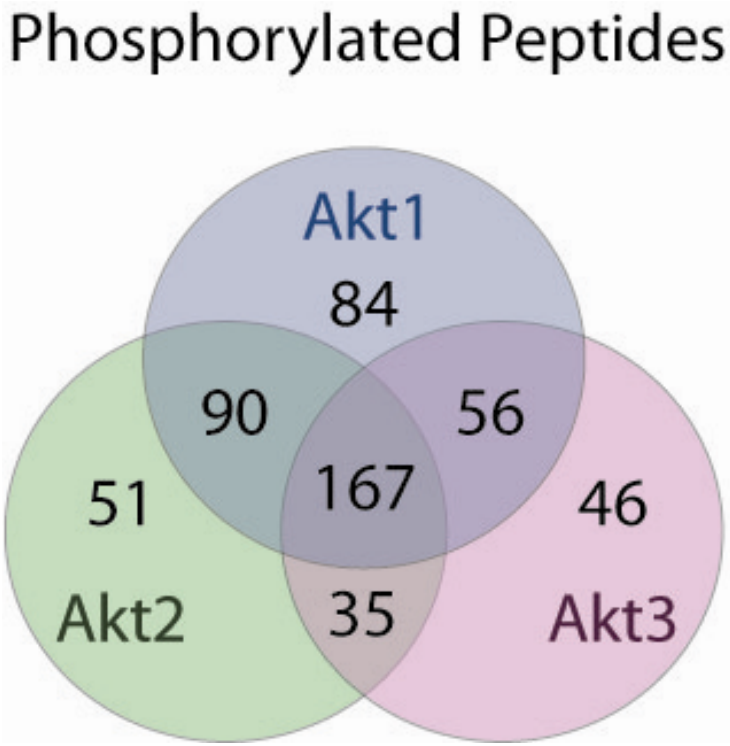
Quantitative profiling of post-translational modifications (phosphorylation)

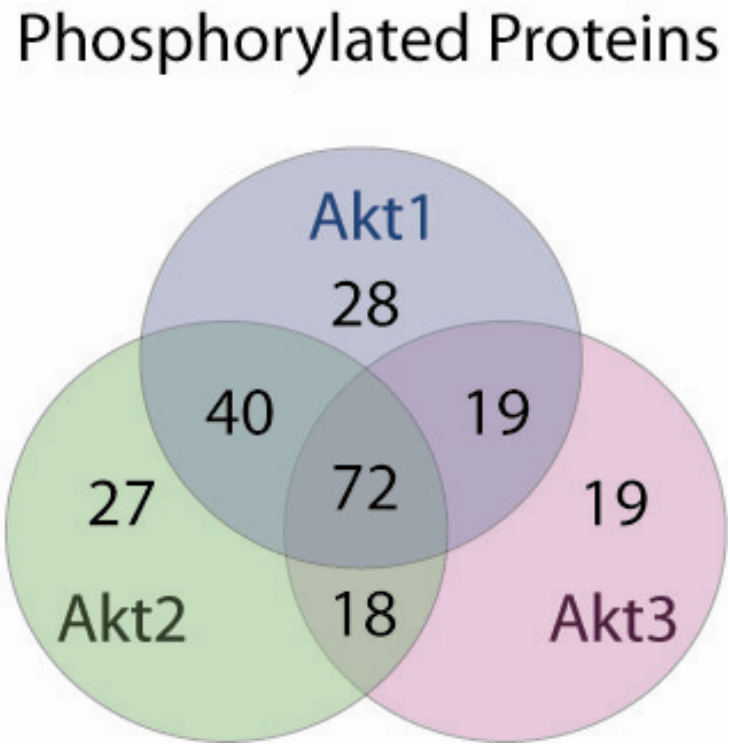




Akt1 is not Akt2
Akt1 is not Akt3
Akt2 is not Akt3

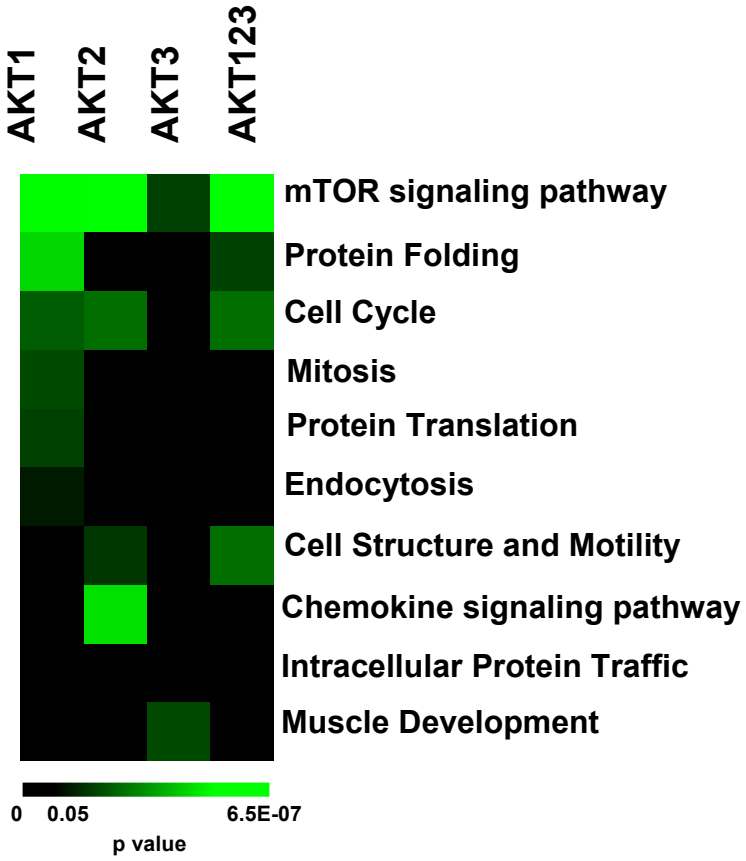
Common-Individual Targets



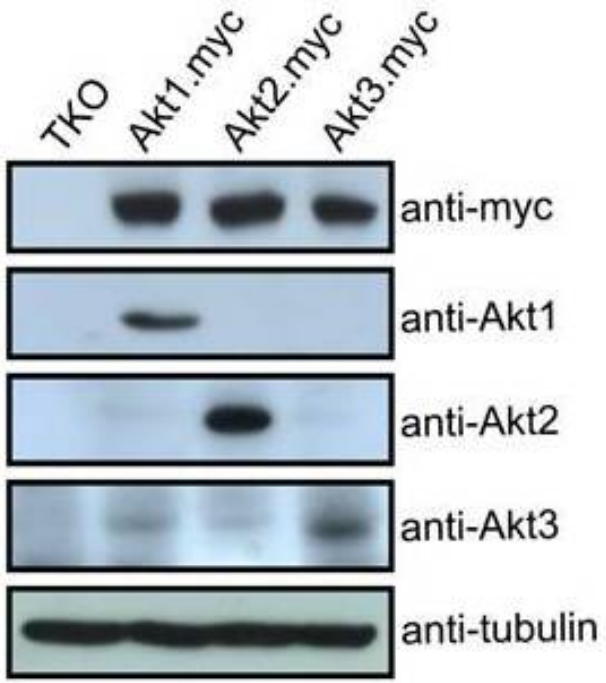


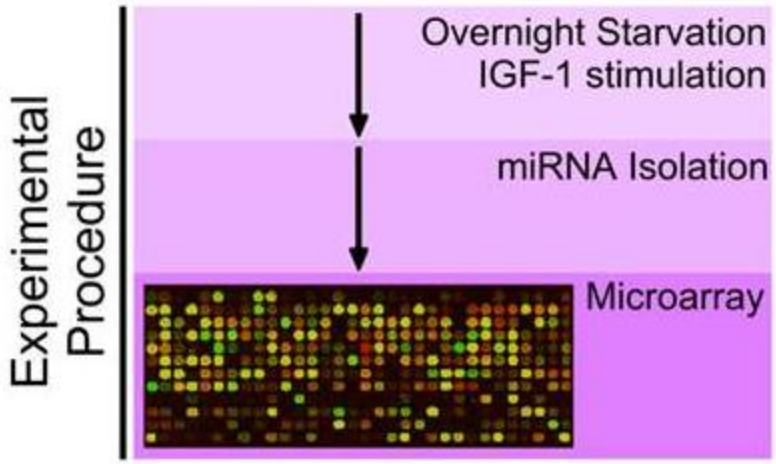
Akt1 is not Akt2
Akt1 is not Akt3
Akt2 is not Akt3

Gene Ontology HeatMap of AKT Phosphoproteomics

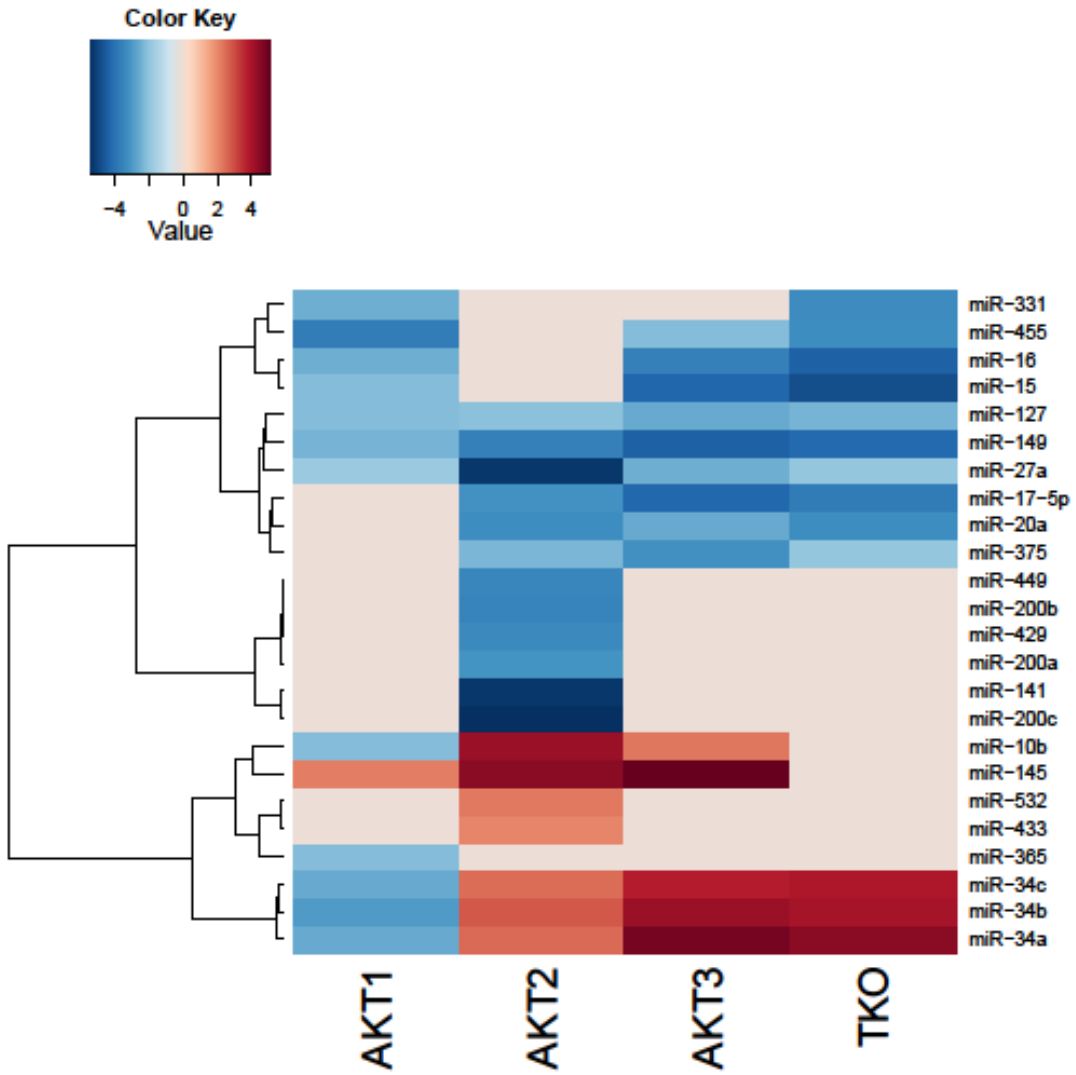


MicroRNA profiling of cells expressing indvidual Akt isoforms

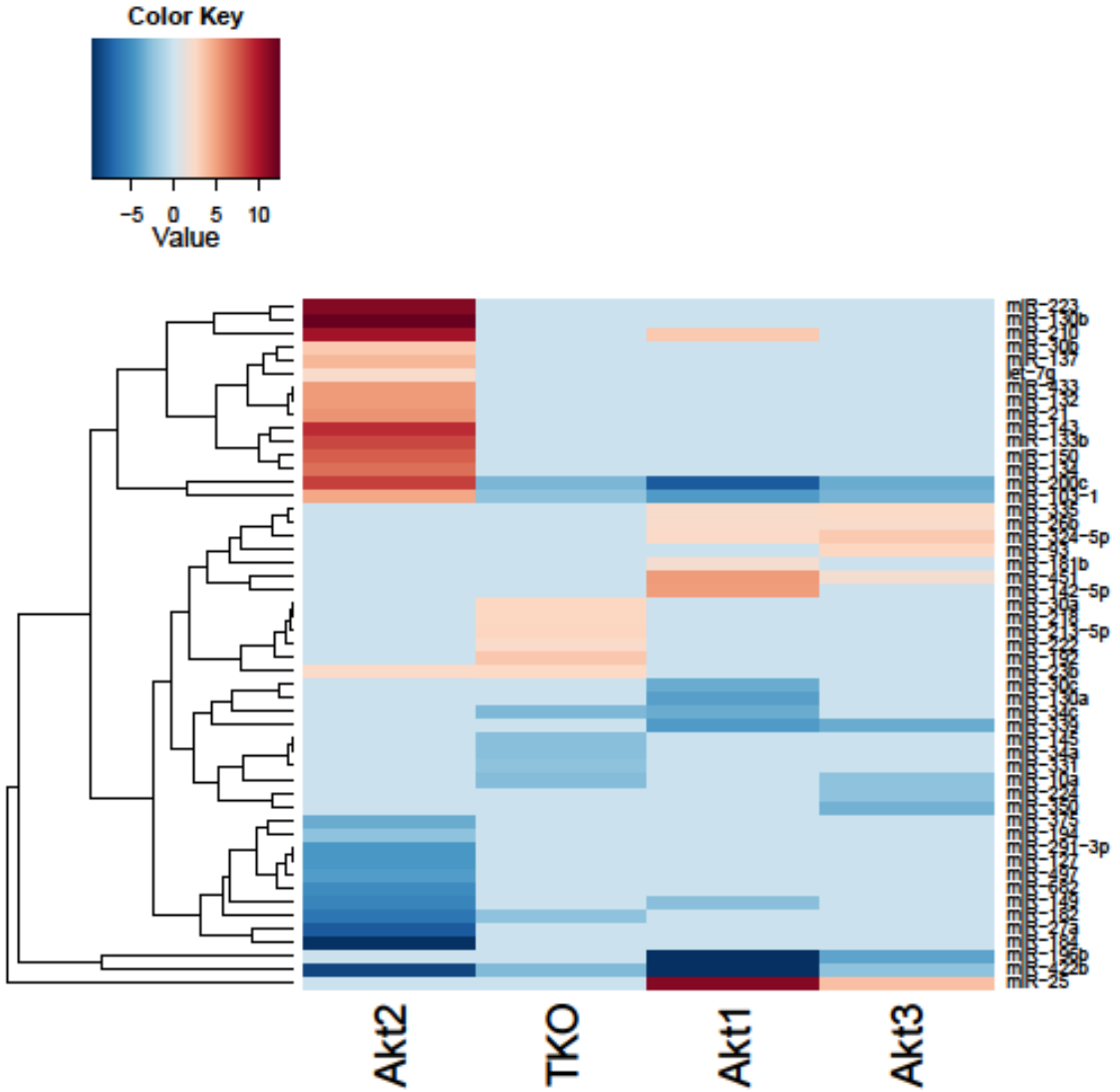




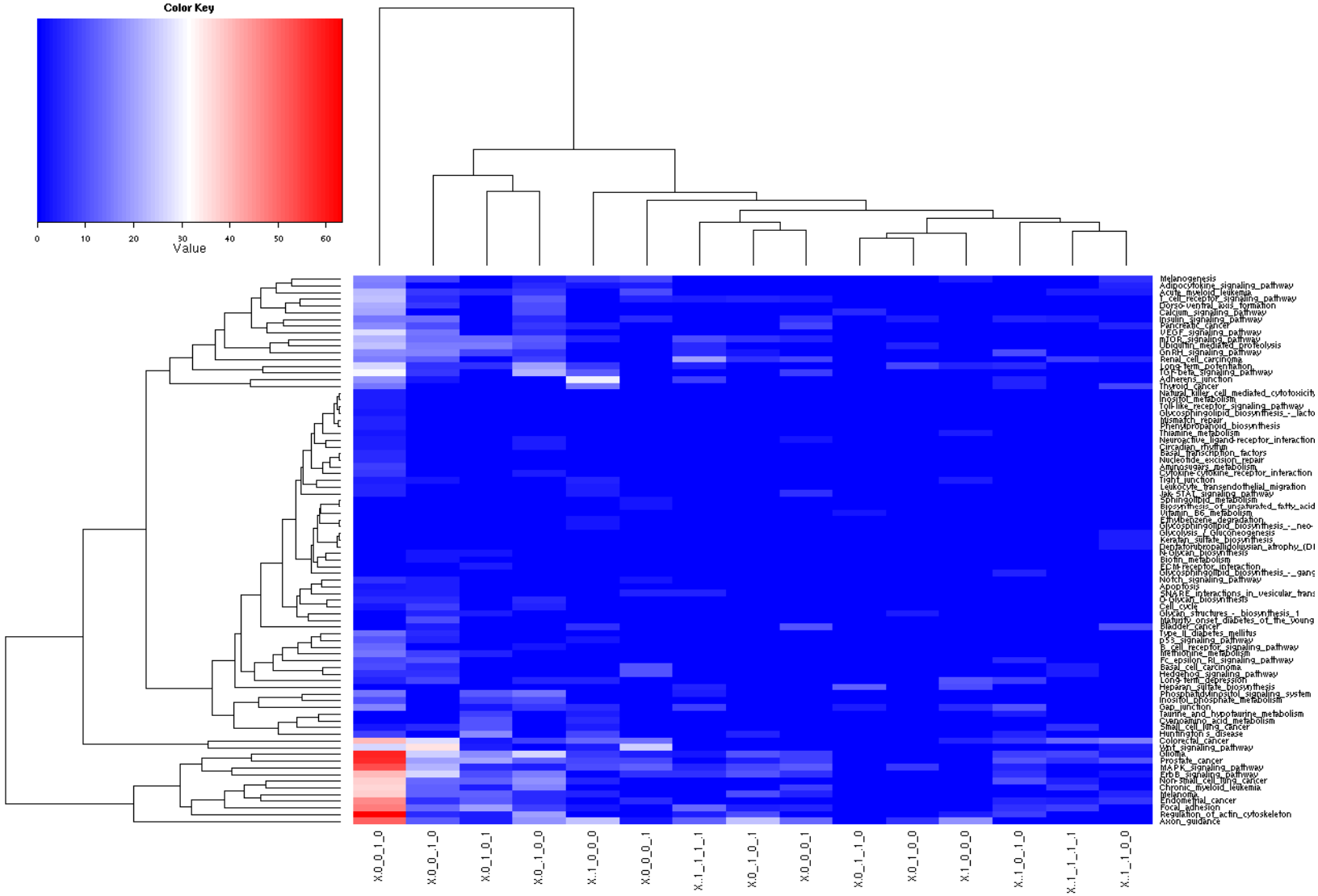
MicroRNAs differentially regulated by Akt isoforms during IGF stimulation



MicroRNAs differentially regulated by Akt isoforms during Hypoxia

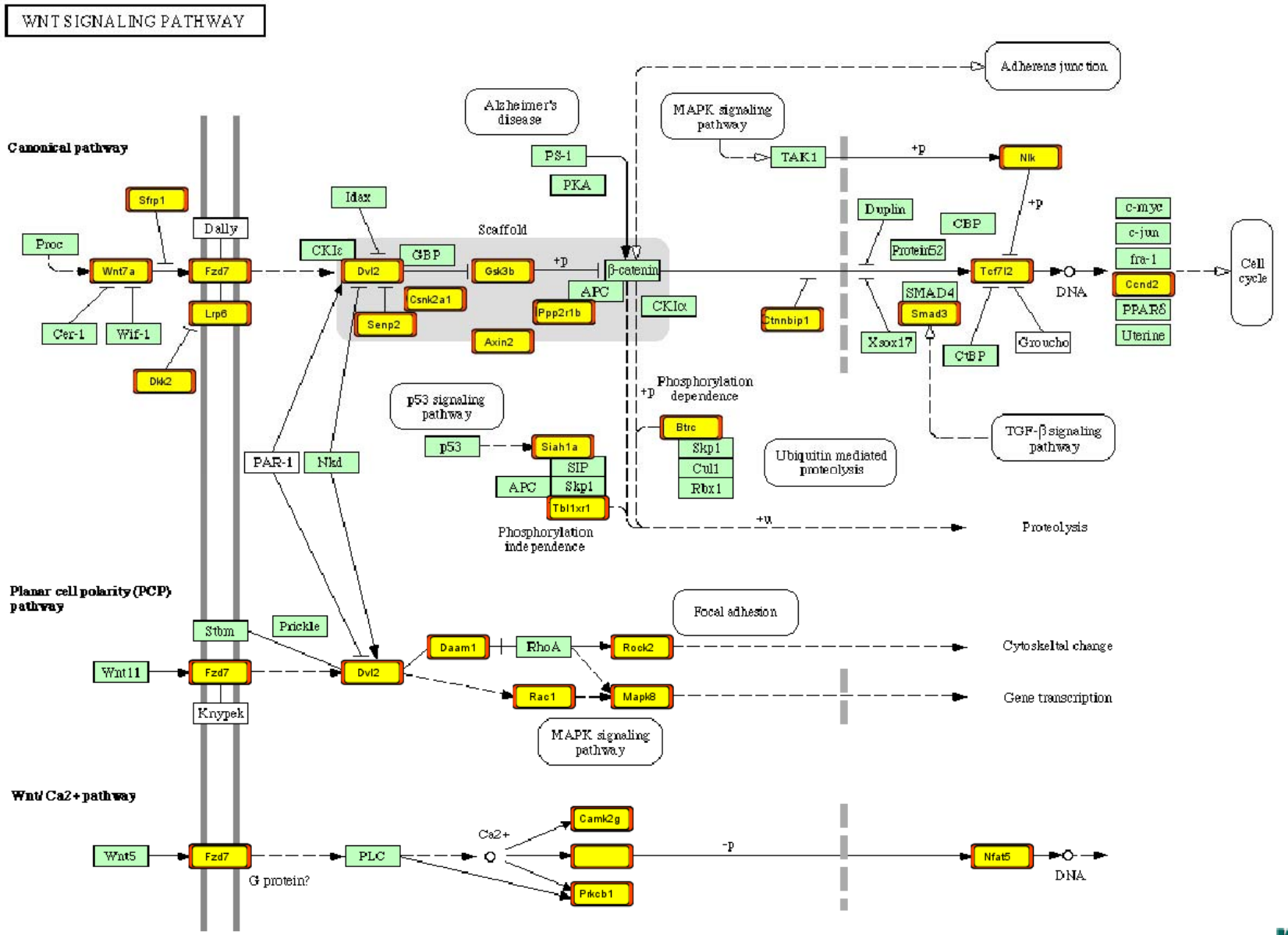


Pathways predicted to be differentially regulated by Akt isoforms during hypoxia, based on differences in microRNA gene expression

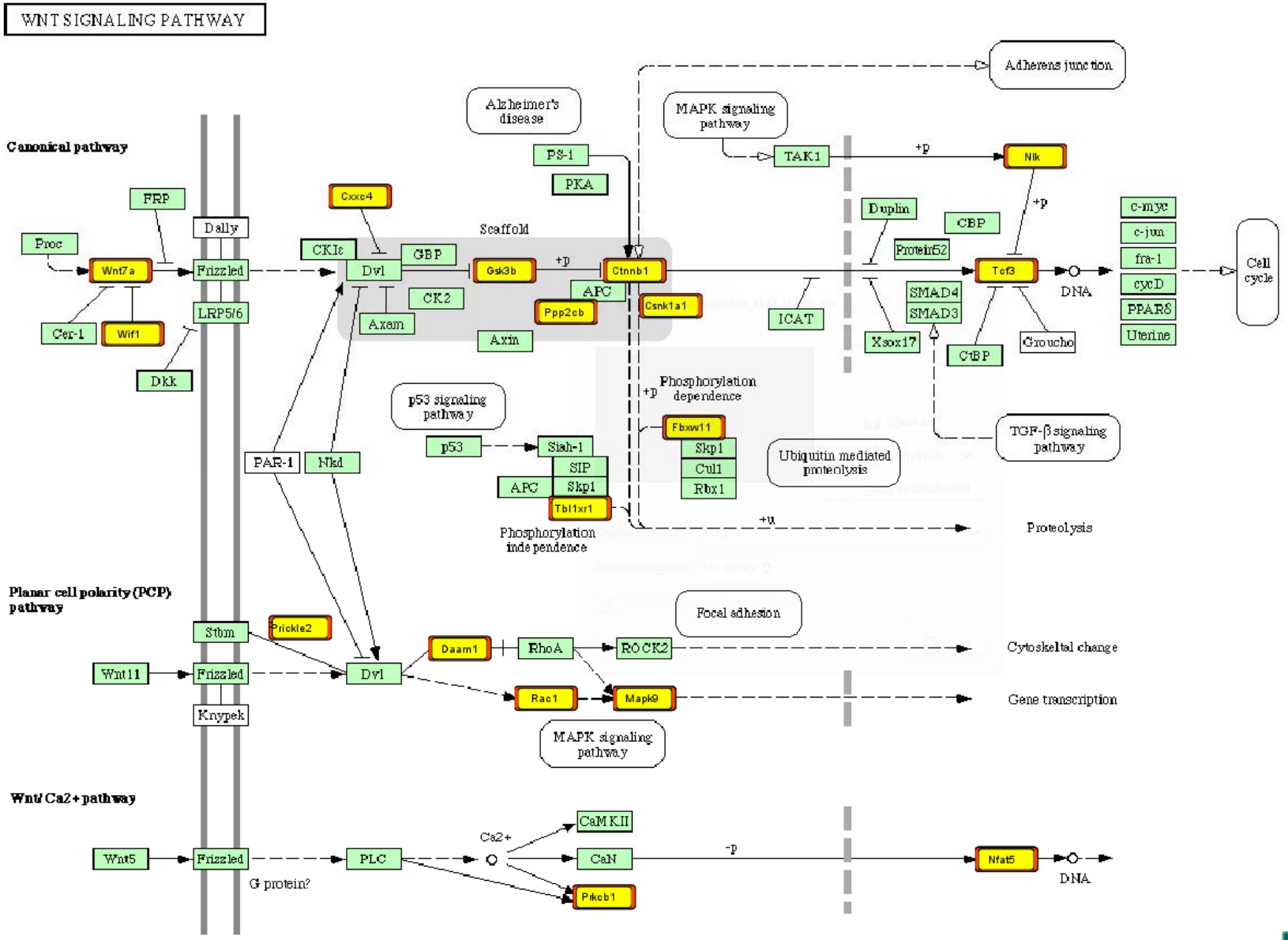


RNA/Pathway
Cut off
3

Targets of upregulated miRs

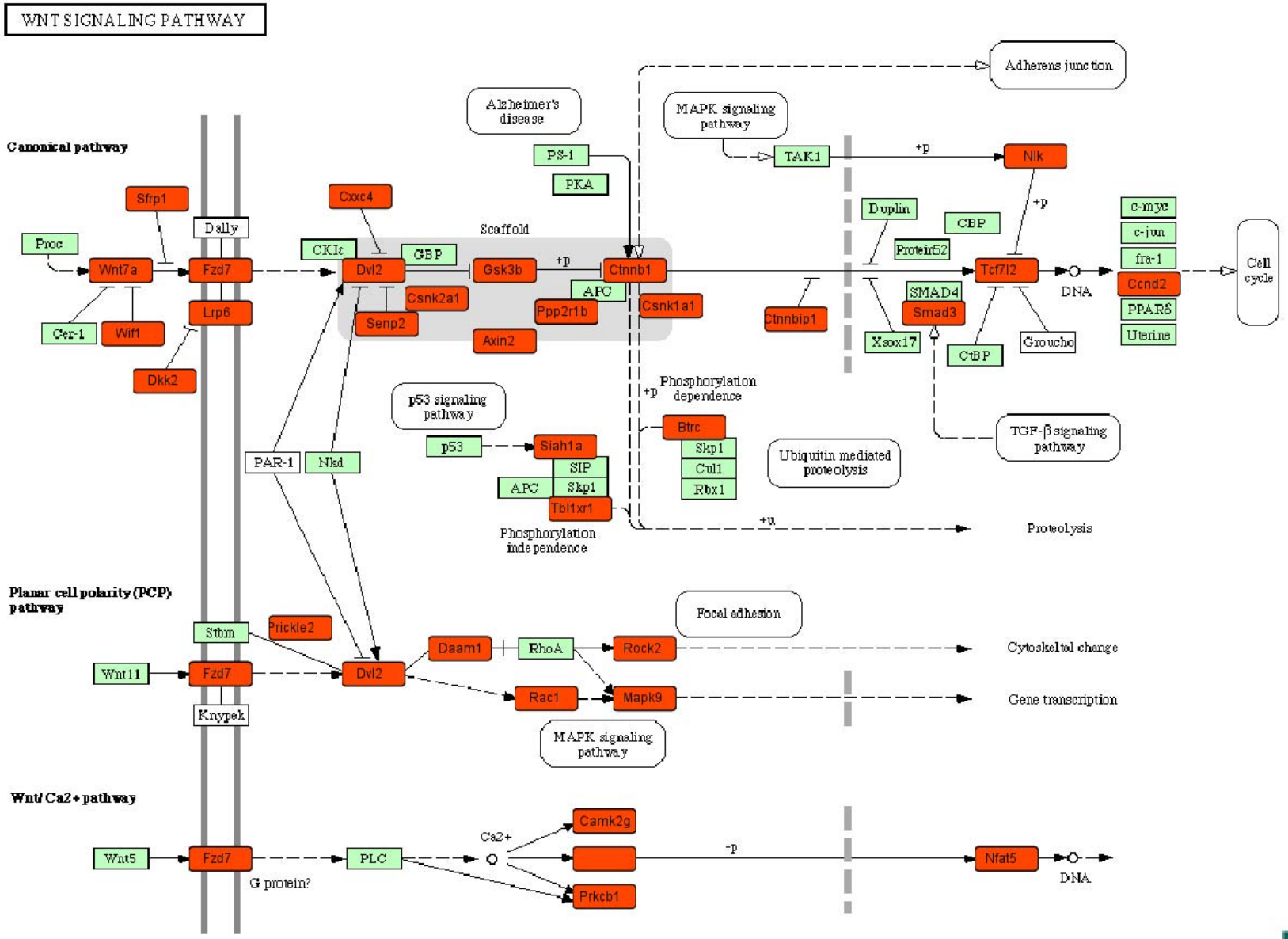


Targets of downregulated miRs



04310 7/23/07

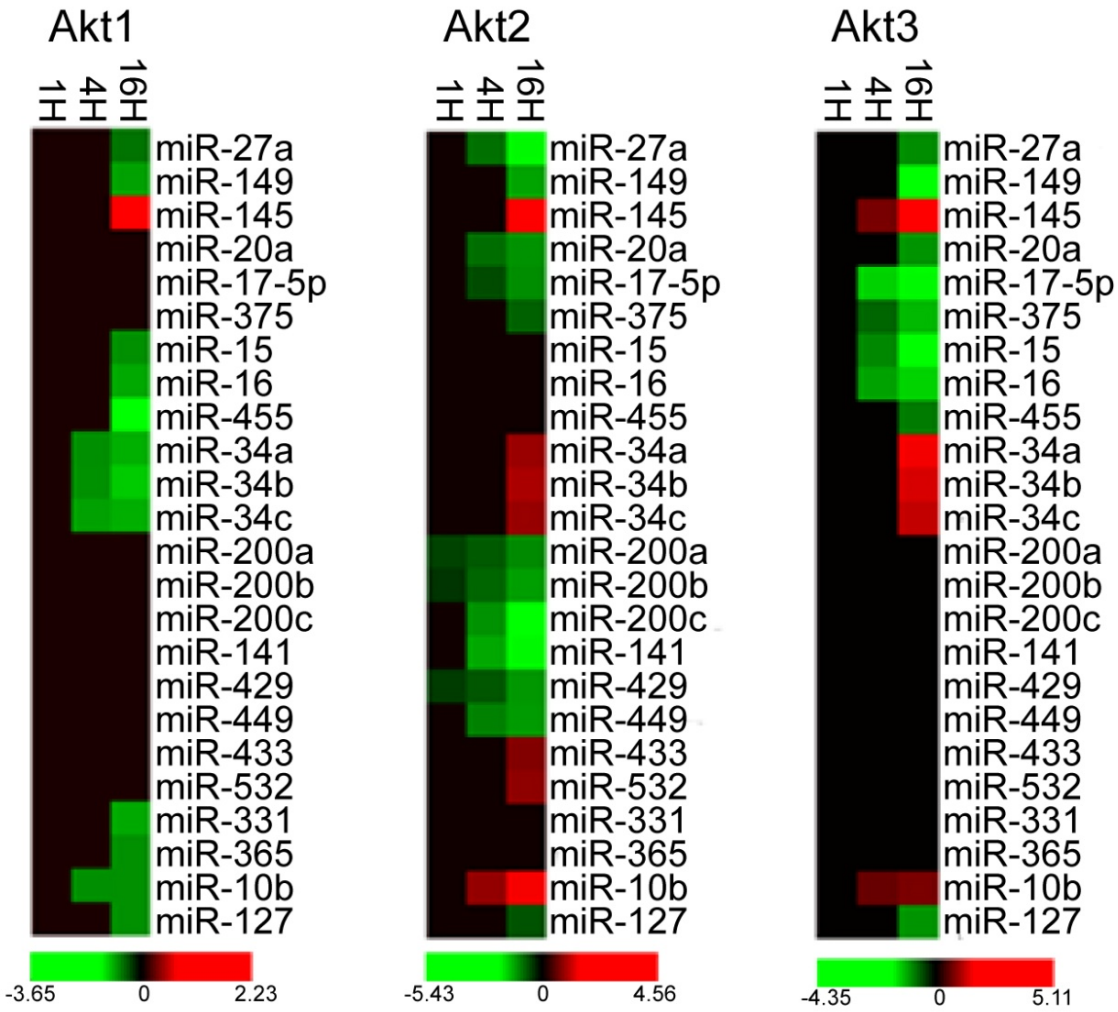
Targets of all dysregulated miRs



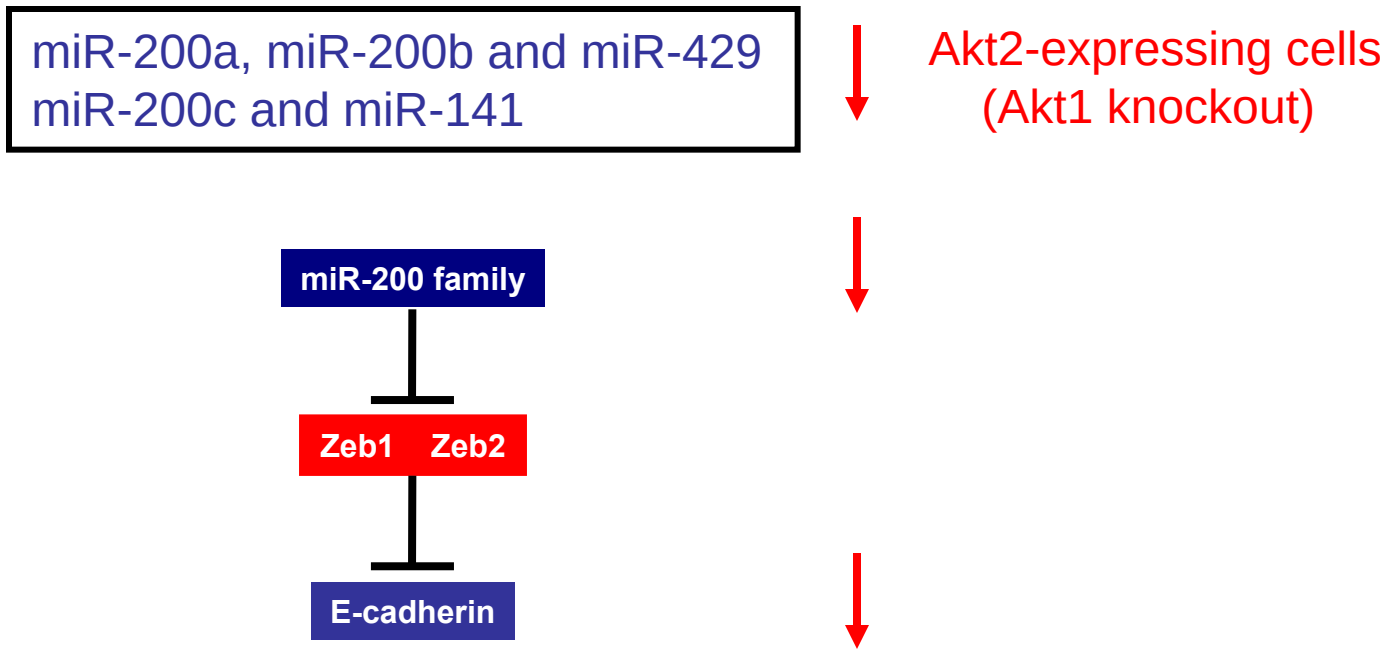
Focus on Akt2

- **MicroRNAs differentially regulated by Akt2 control EMT and stem cell renewal in cancer cells**
- MicroRNAs differentially regulated by Akt2 render cells resistant to hypoxia
- Akt2, in the absence of Akt1 promotes inflammation

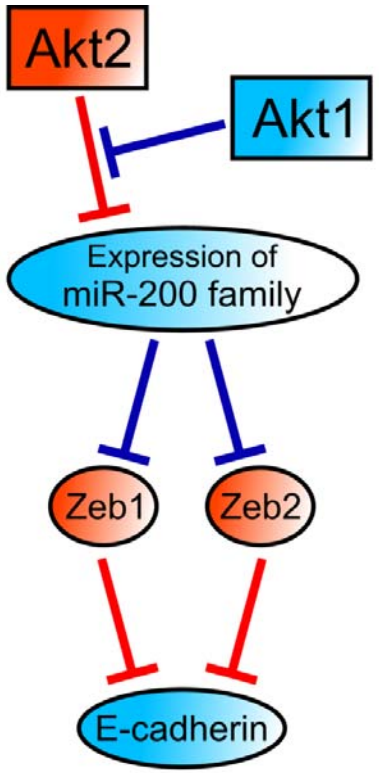
Akt isoforms have different microRNA gene signatures



Downregulation of the microRNAs of the miR-200 family in Akt2-expressing cells, may cause EMT and promote invasiveness

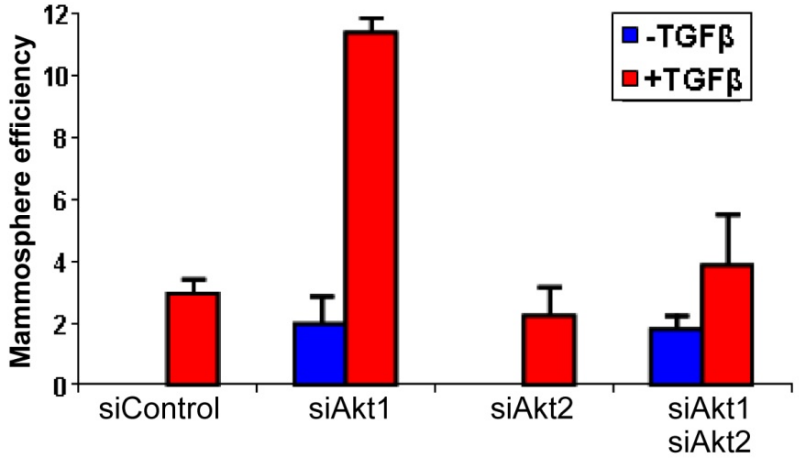


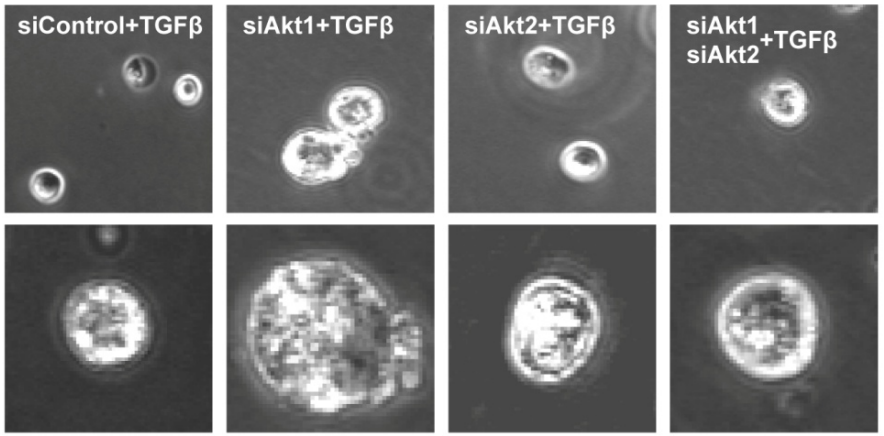
Model of EMT regulation by Akt1 and Akt2



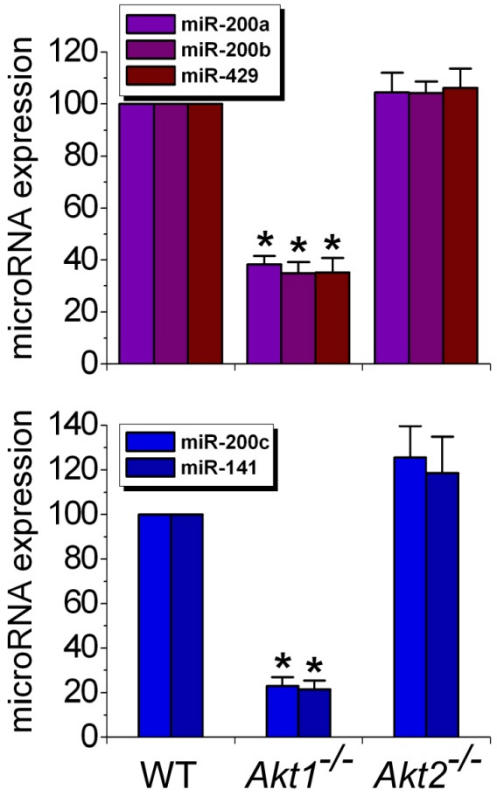
EMT/Acquisition of Stem cell properties

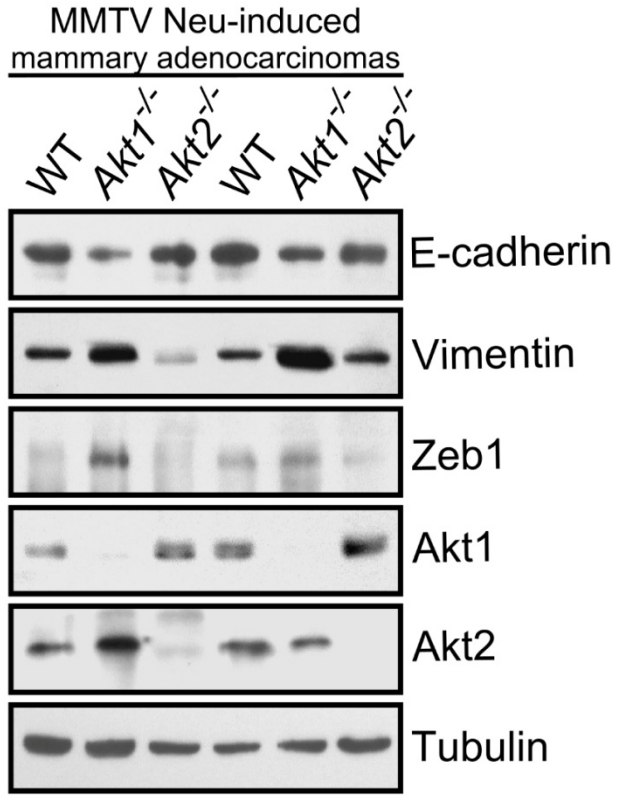
Cells undergoing EMT via miR-200 downregulation, in response to TGFβ treatment and Akt1 knockdown, exhibit stem cell properties



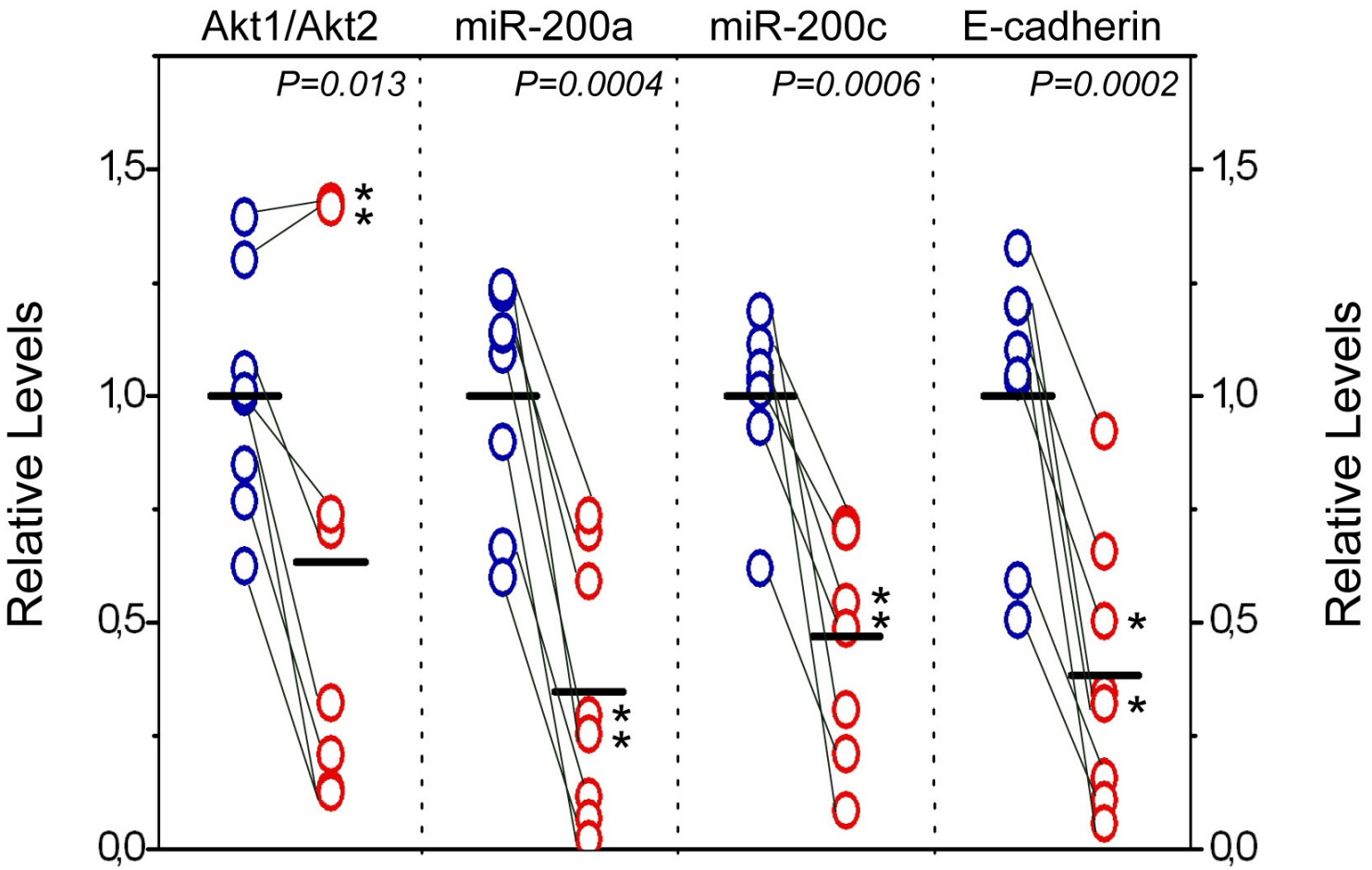


Mammary adenocarcinomas developing in MMTV-cErbB2/Akt1$^{-/-}$ mice express low levels of the miR-200 family members, high levels of Zeb1 and low levels of E-cadherin

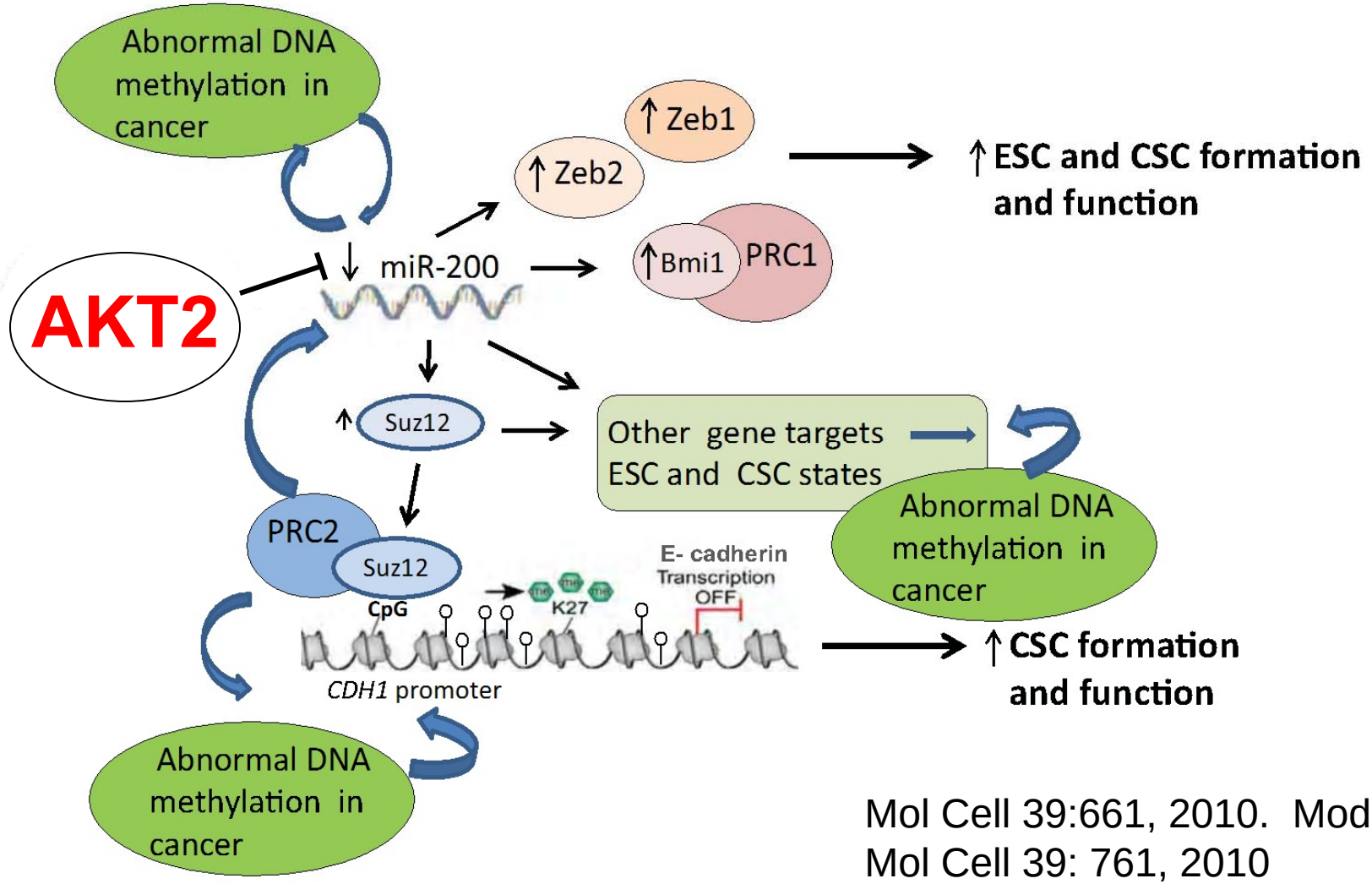




The Akt/miR-200/E-cadherin axis contributes to the metastatic phenotype in the majority of human mammary adenocarcinomas.



An epigenetic network of neoplastic stem cells

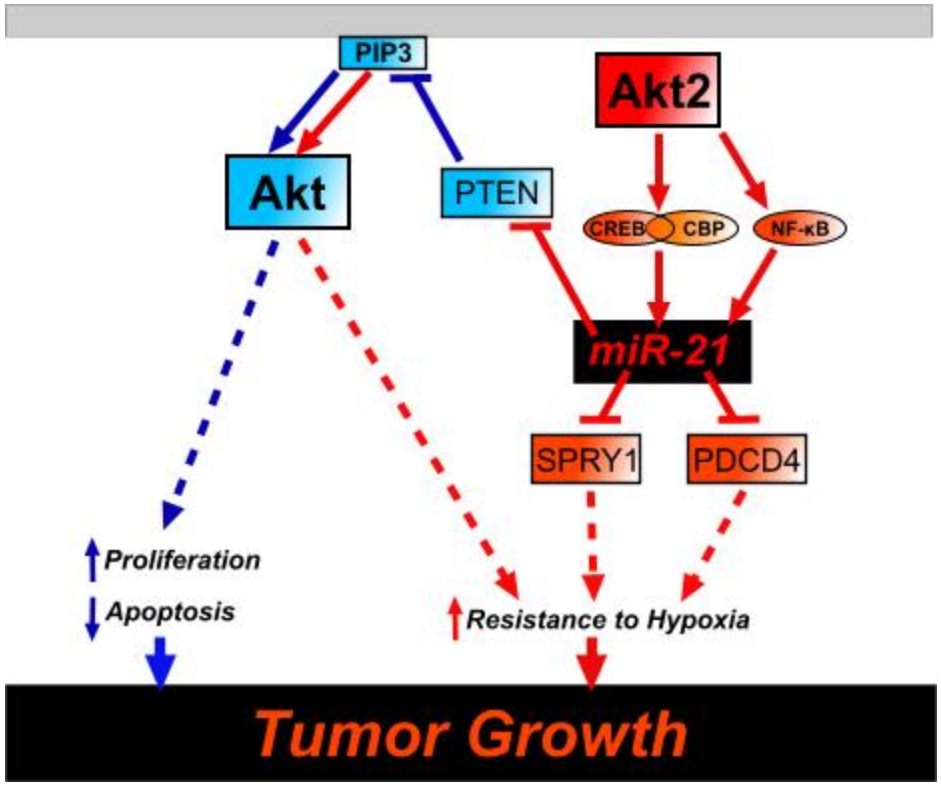


Mol Cell 39:661, 2010. Modified
Mol Cell 39: 761, 2010

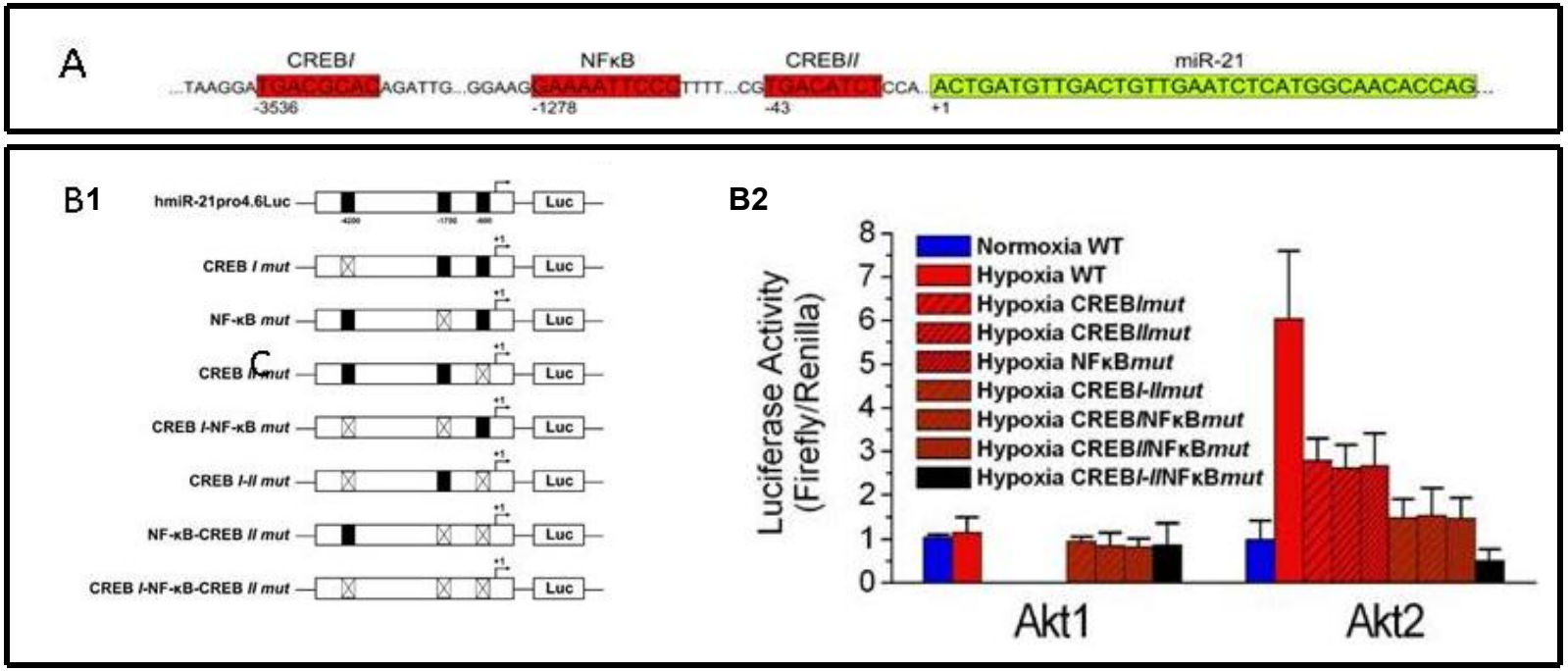
Focus on Akt2

- MicroRNAs differentially regulated by Akt2 control EMT and stem cell renewal in cancer cells
- **MicroRNAs differentially regulated by Akt2 render cells resistant to hypoxia**
- Akt2, in the absence of Akt1 promotes inflammation

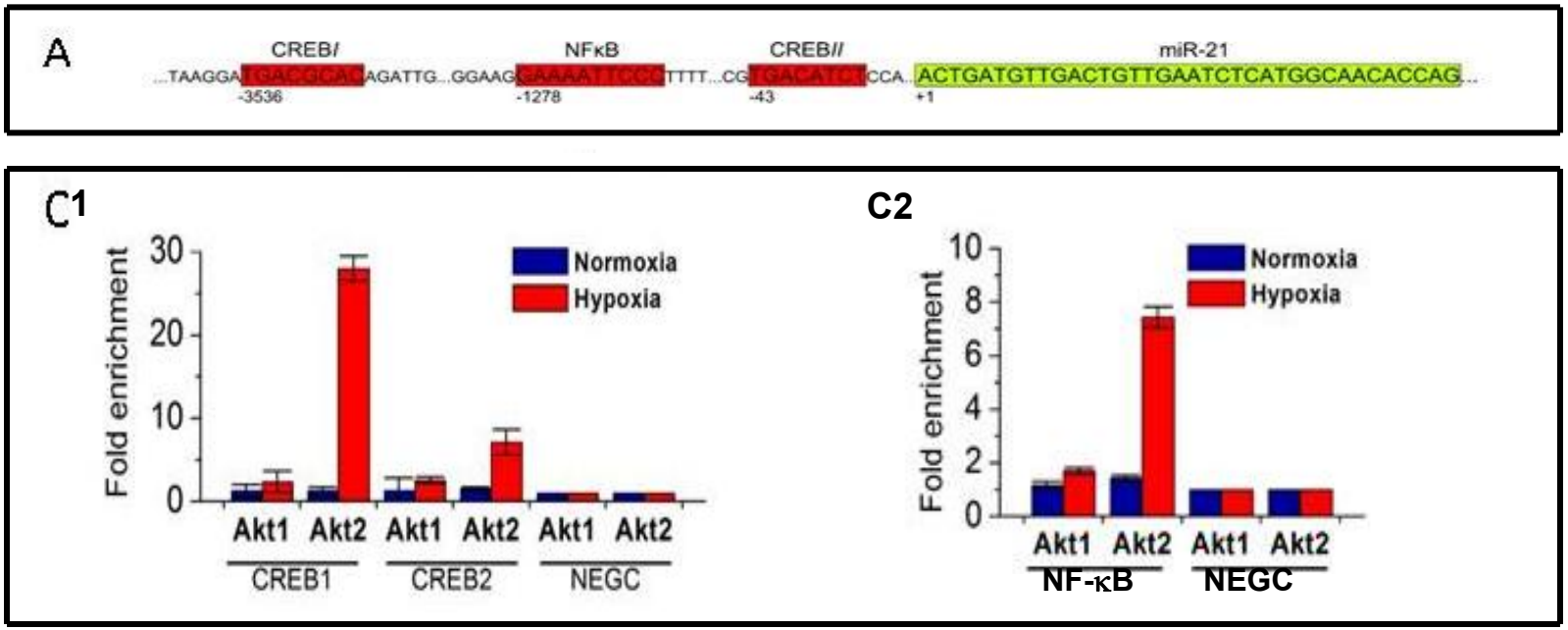
The hypoxia-activated Akt2-miR-21 PTEN axis



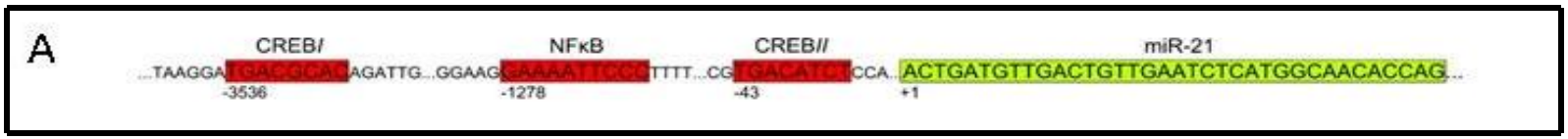
Both CREB binding and NF-κB binding to the miR-21 promoter are required for the activation of the promoter in Akt2-expressing cells exposed to hypoxia

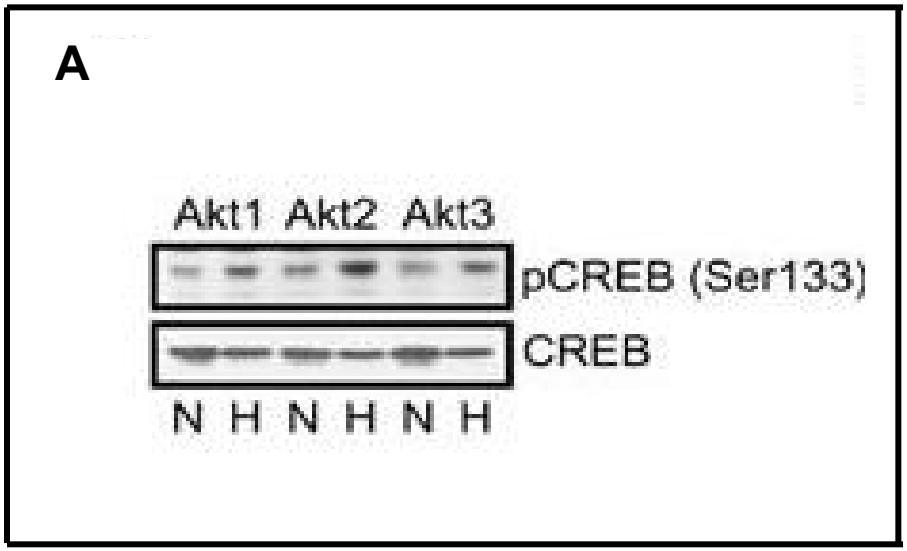


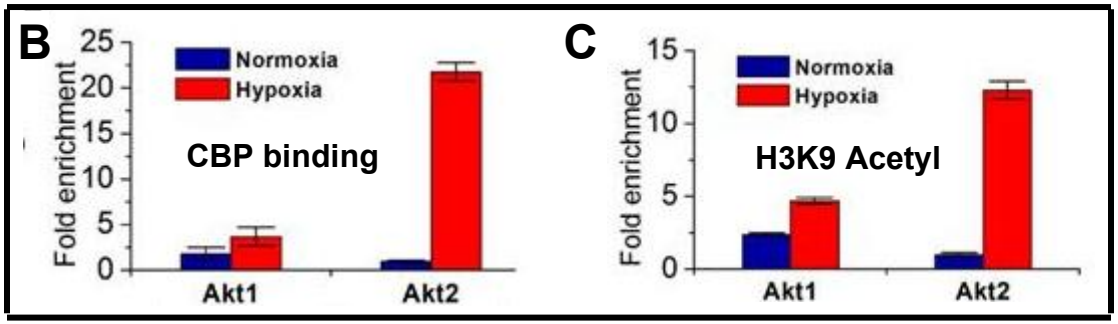
CREB and NF-κB binding on the miR-21 promoter in cells exposed to hypoxia depend on Akt2 and not on Akt1



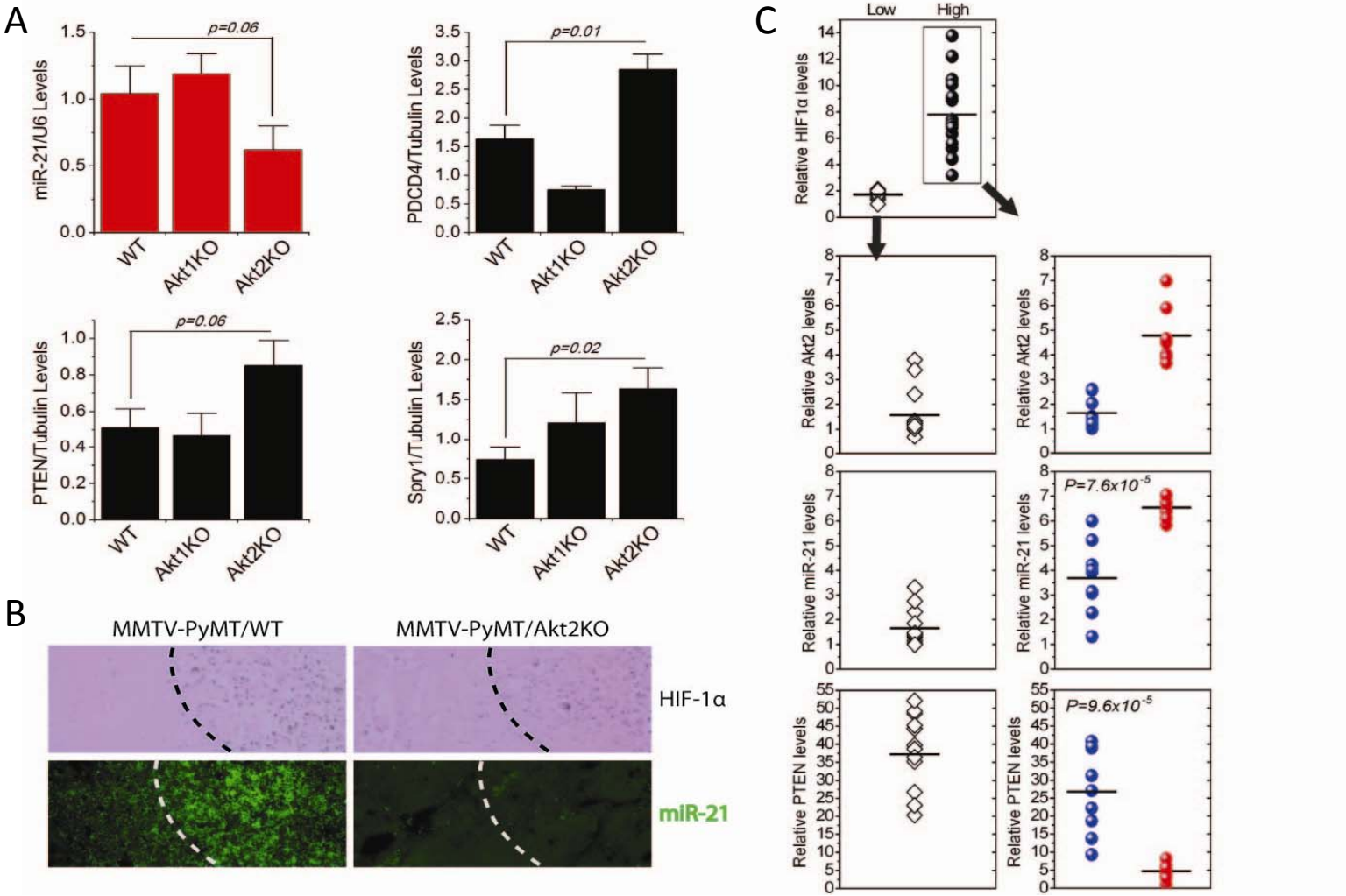
CBP binding and histone H3 acetylation at K9 in the miR-21 promoter in cells exposed to hypoxia depends primarily on Akt2







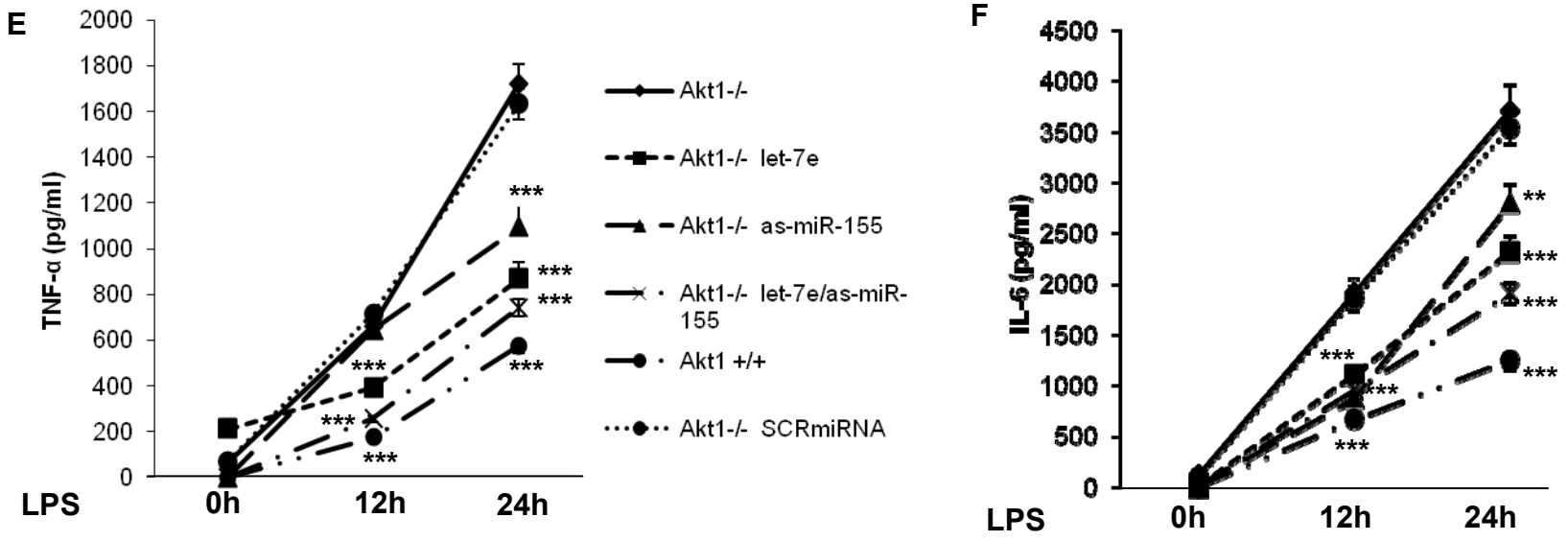
The hypoxia activated Akt2-miR-21-PTEN axis is functional in MMTV-PyMT-induced murine mammary adenocarcinomas and human ovarian carcinomas



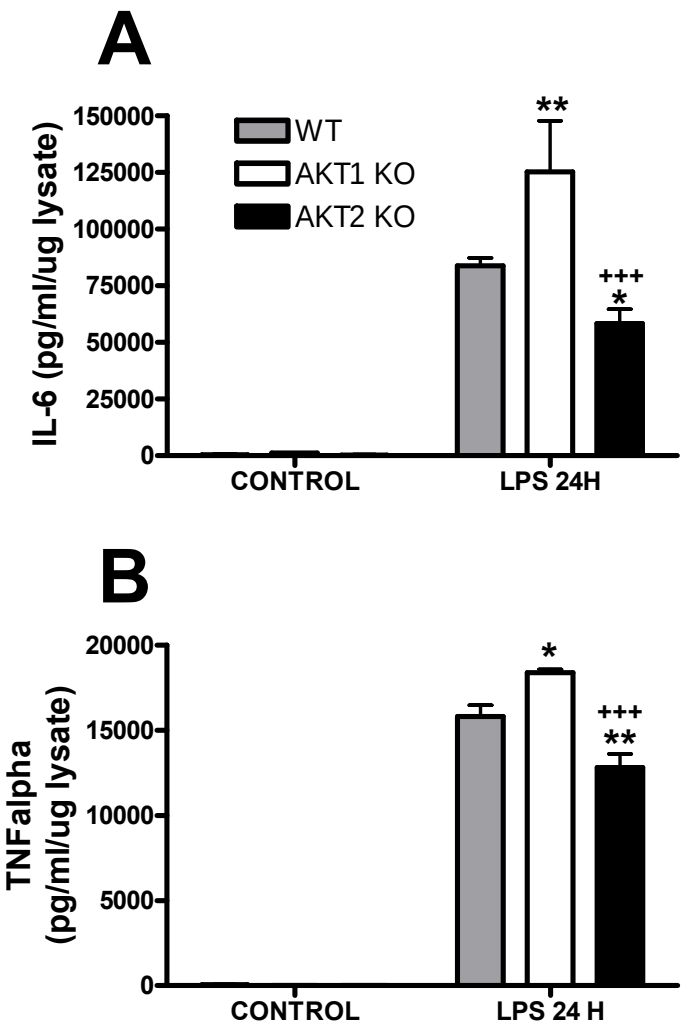
Focus on Akt2

- MicroRNAs differentially regulated by Akt2 control EMT and stem cell renewal in cancer cells
- MicroRNAs differentially regulated by Akt2 render cells resistant to hypoxia
- **Akt2 promotes, while Akt1 inhibits inflammation**

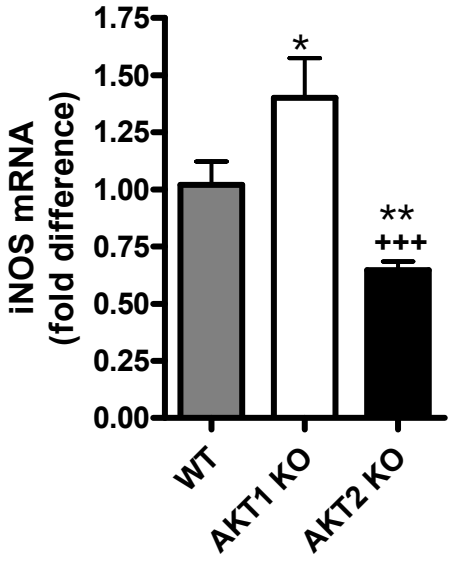
The induction of pro-inflammatory mediators by LPS in Akt1-/- macrophages is normalized by the combination of let-7e and as-miR-155

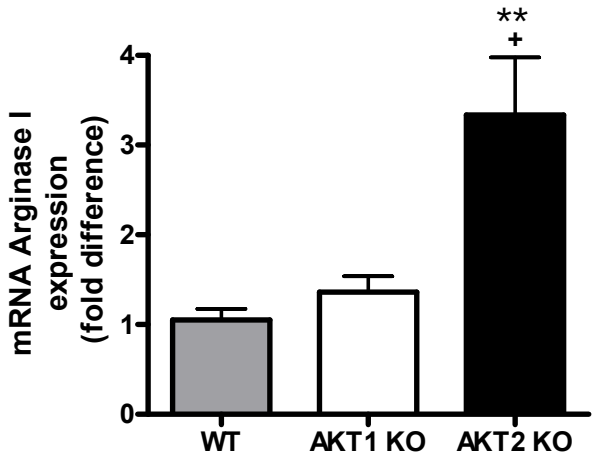


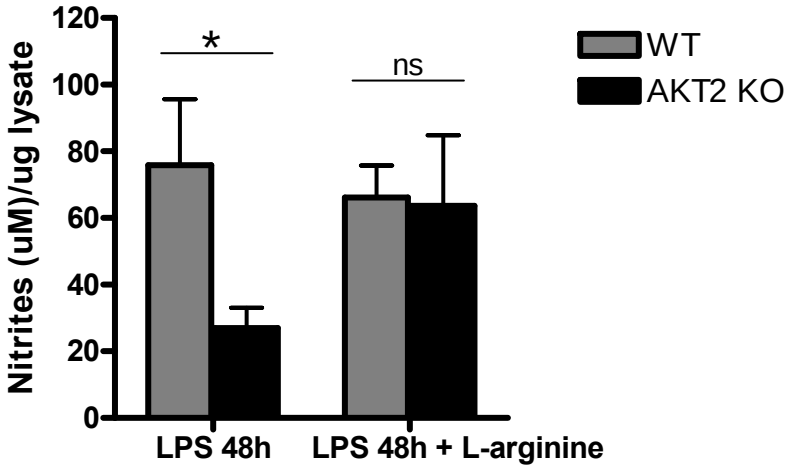
Akt2 ablation downregulates the expression of proinflammatory mediators in macrophages in response to LPS



Akt1 and Akt2 isoforms differentially regulate macrophage polarization





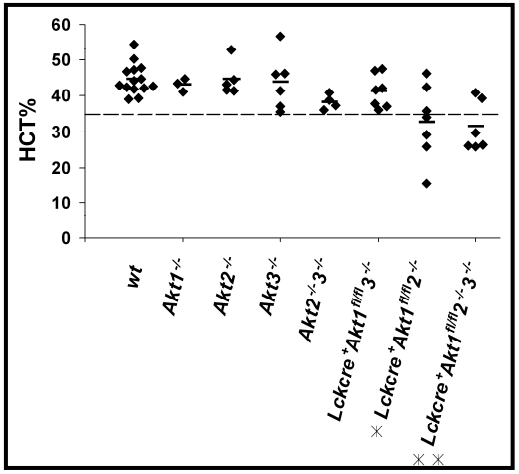


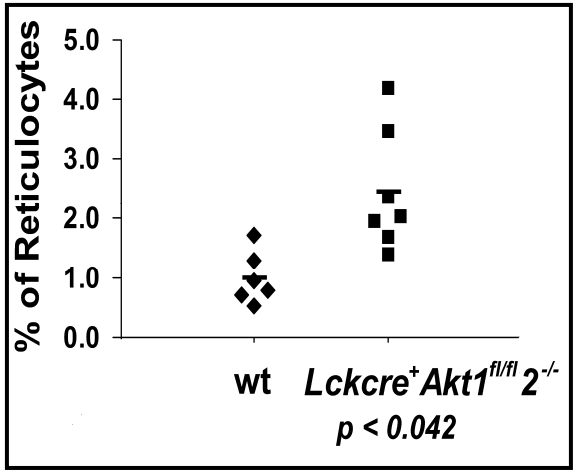
Focus on Akt2

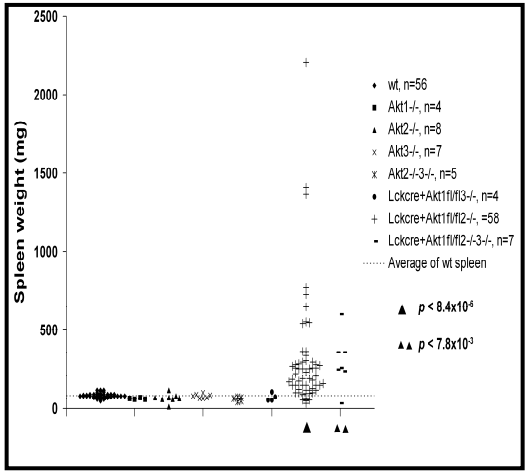
- MicroRNAs differentially regulated by Akt2 control EMT and stem cell renewal in cancer cells
- MicroRNAs differentially regulated by Akt2 render cells resistant to hypoxia
- Akt2, in the absence of Akt1, promotes inflammation

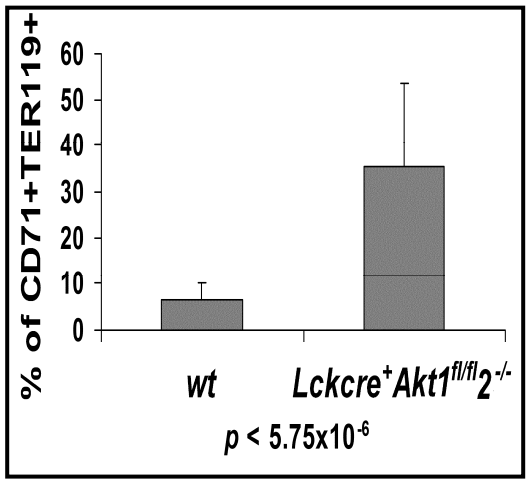
- **Akt1 and Akt2 maintain self-tolerance by controlling the development of regulatory T cells.**

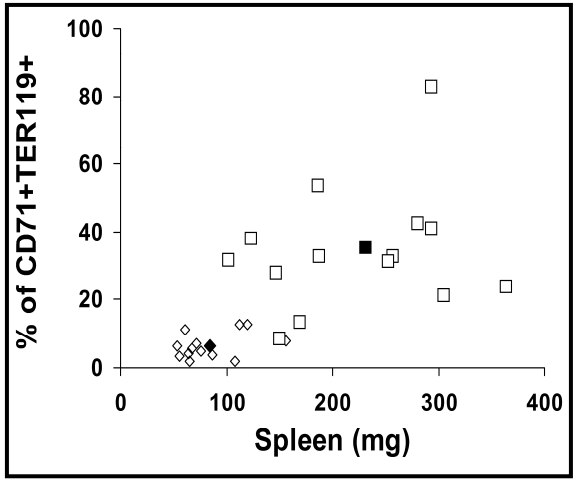
Akt1/Akt2 double knockout mice develop hemolytic anemia

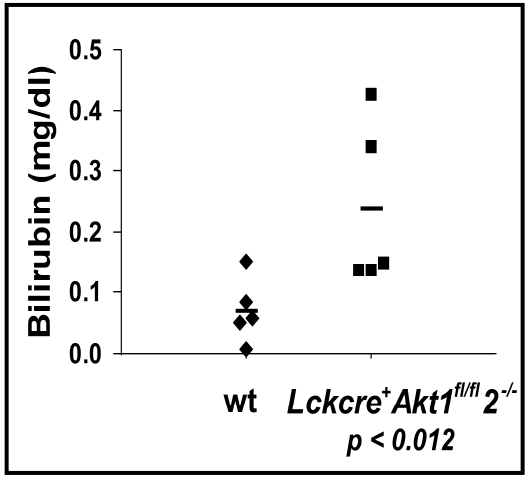




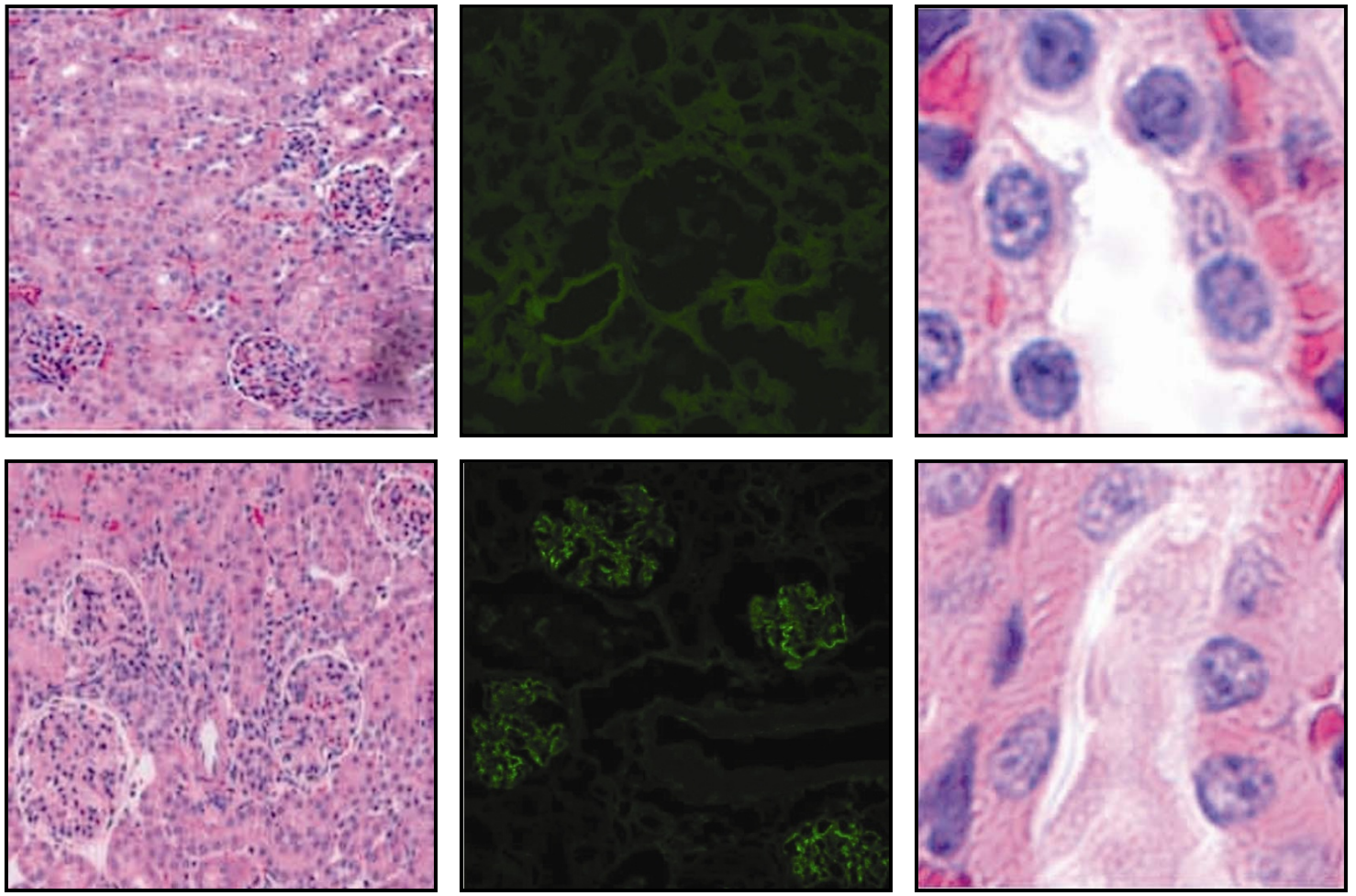




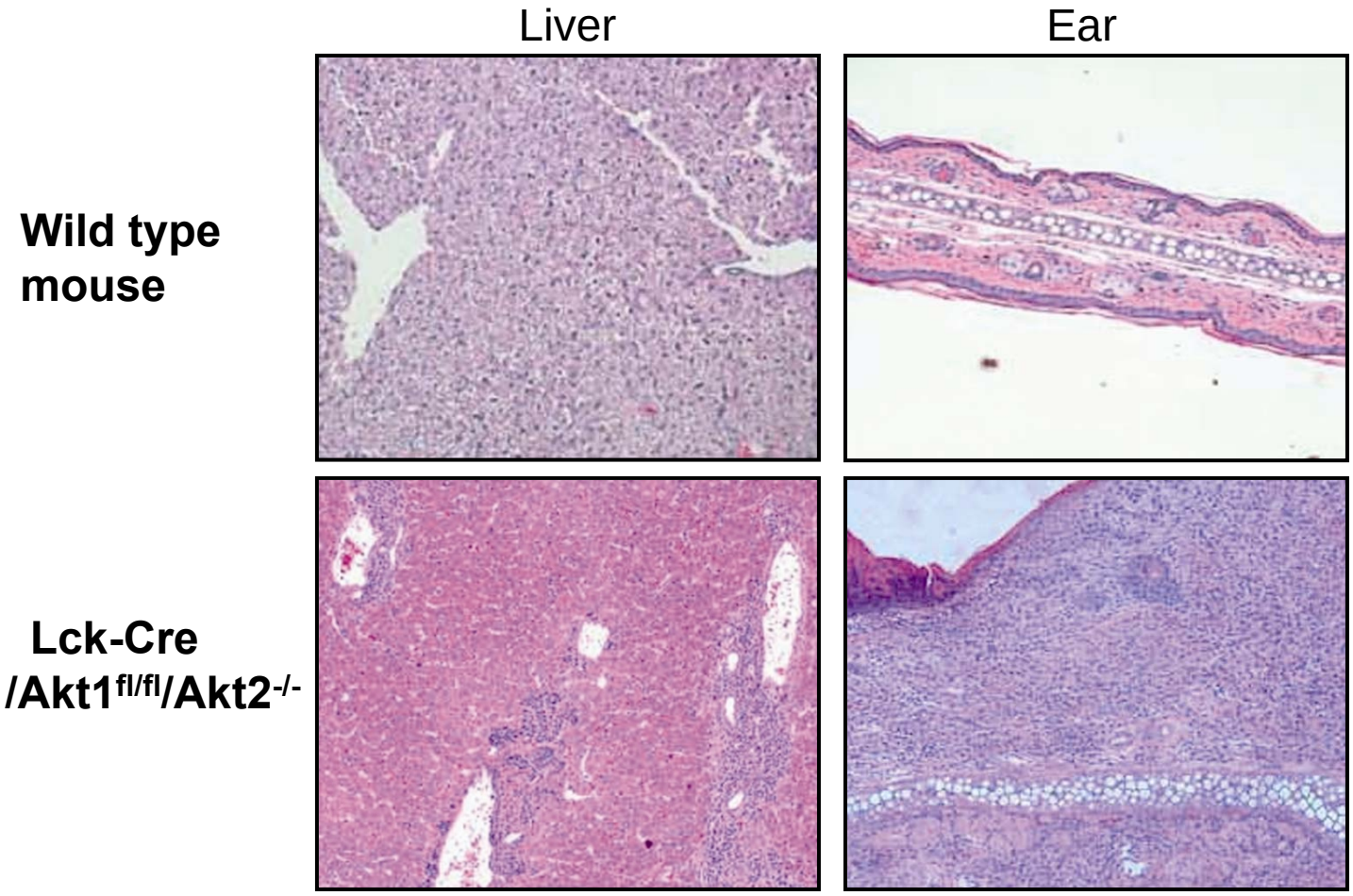




Akt1/Akt2 double knockout mice develop glomerulonephritis

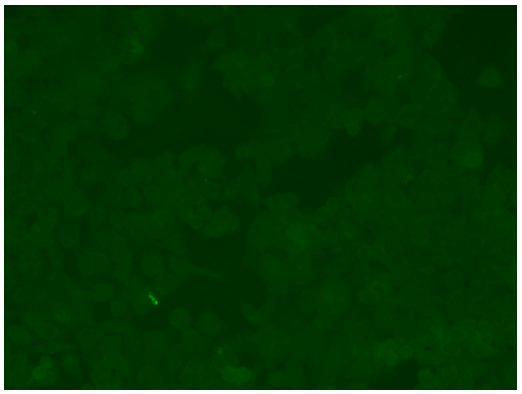


Akt1/Akt2 double knockout mice develop widespread inflammation

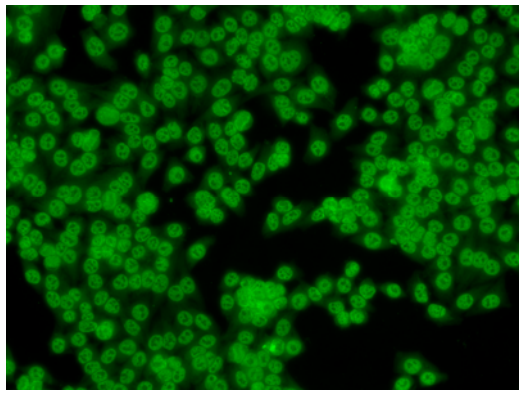


The serum of Akt1/Akt2 double knockout mice contains antinuclear antibodies

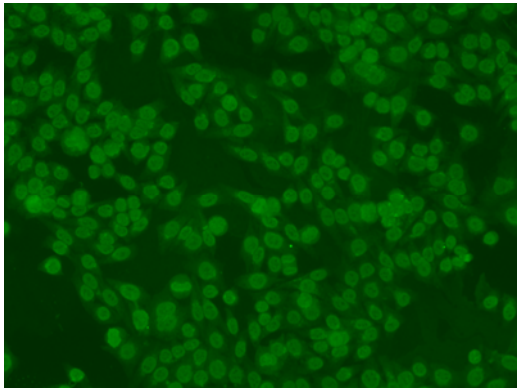
C57BL/6



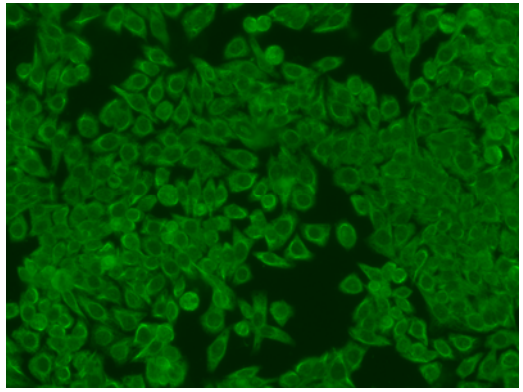
MRL/lpr



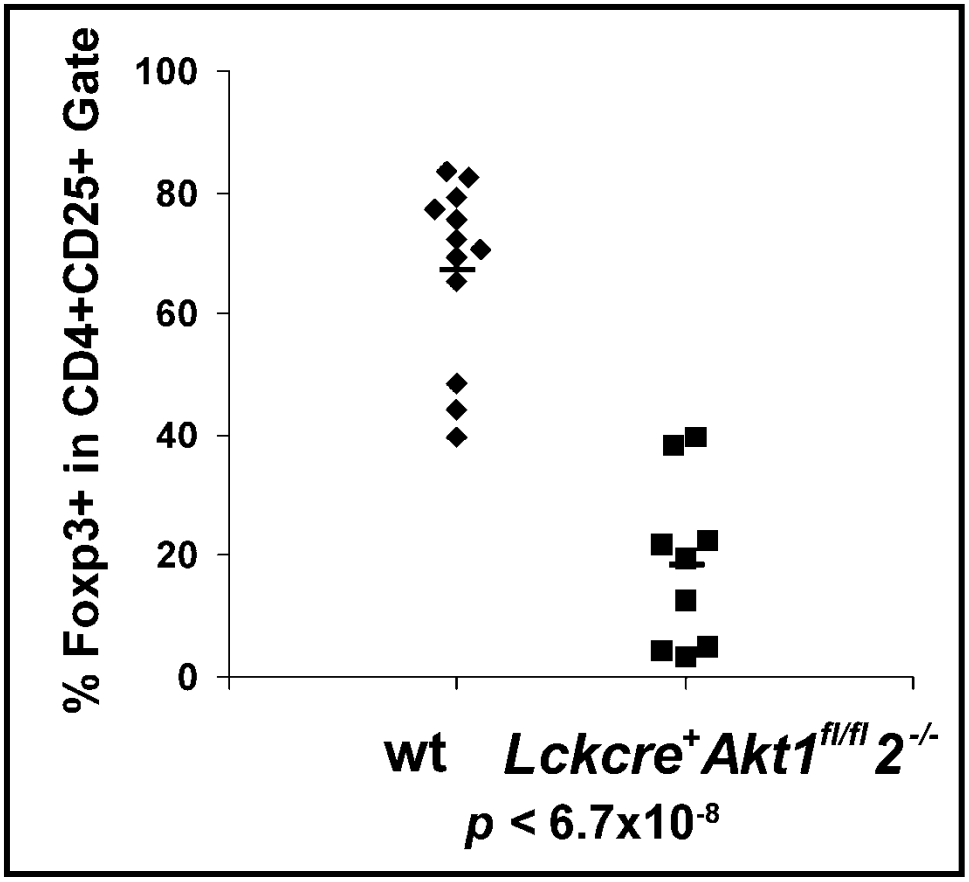
Lck-cre^{+}*Akt1*$^{fl/fl}$*2*$^{-/-}$ n=3



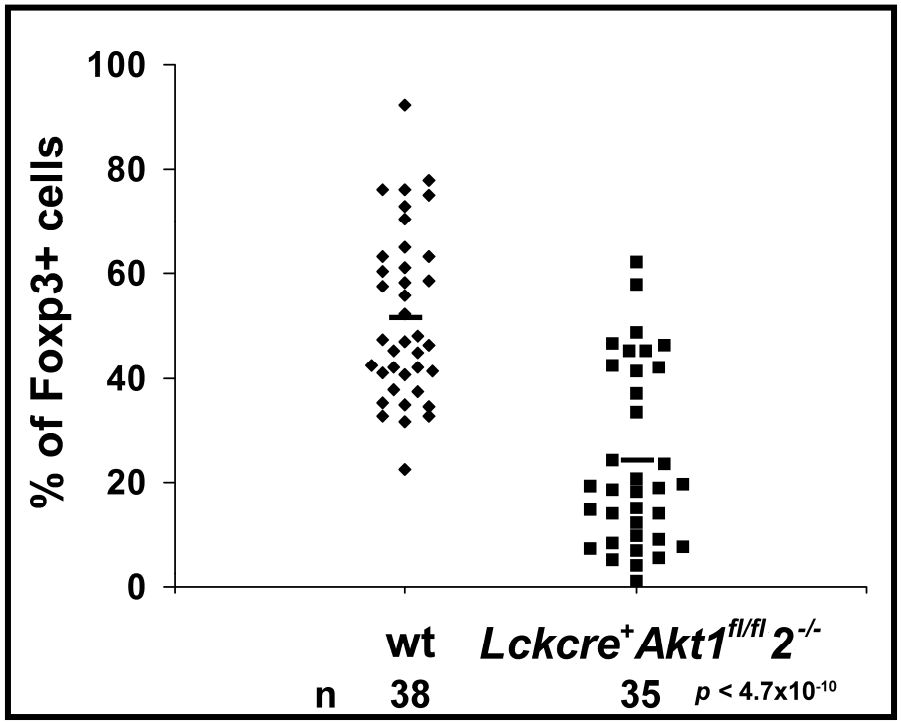
Lck-cre^{+}*Akt1*$^{fl/fl}$*2*$^{-/-}$*3*$^{-/-}$ n=2



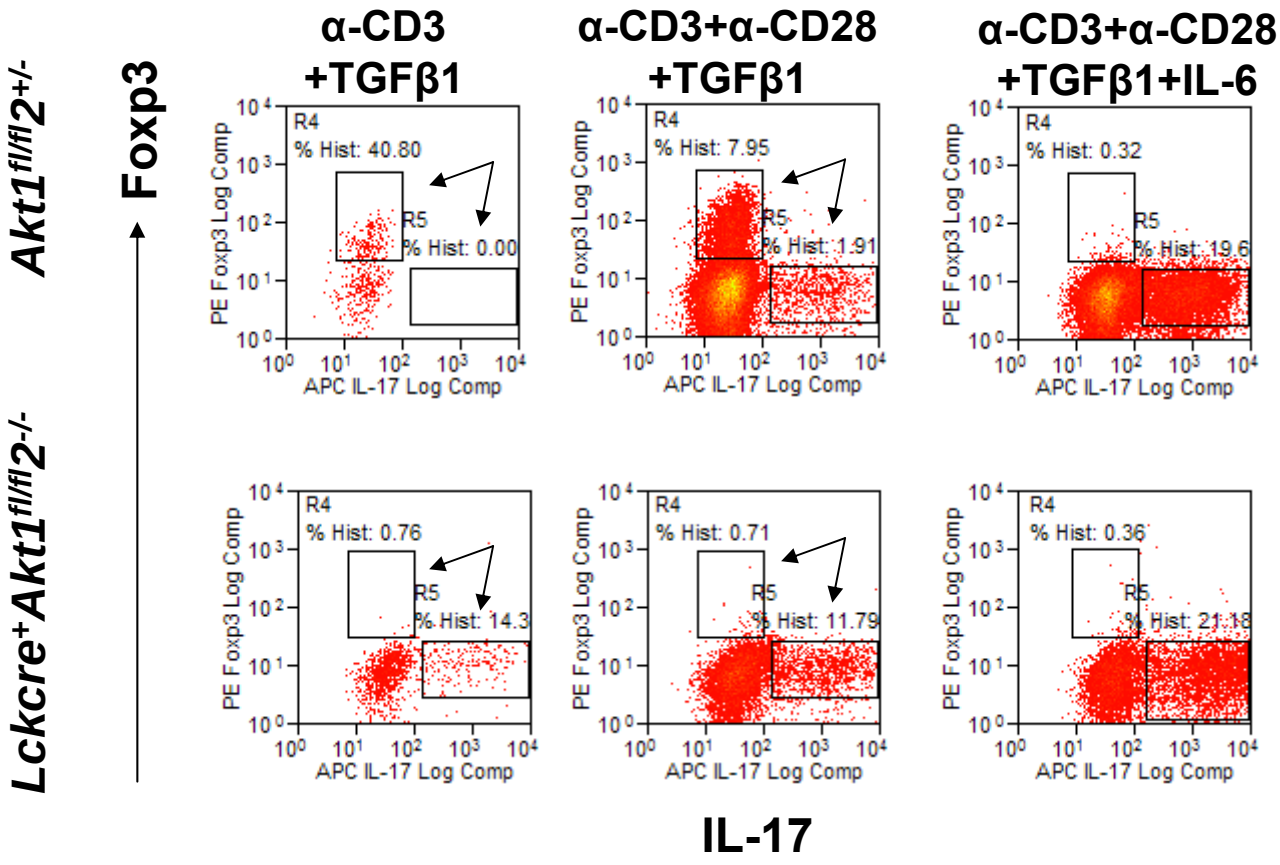
Foxp3 + cells in the thymus of wt and Lck-Cre/Akt1fl/fl/Akt2-/- mice



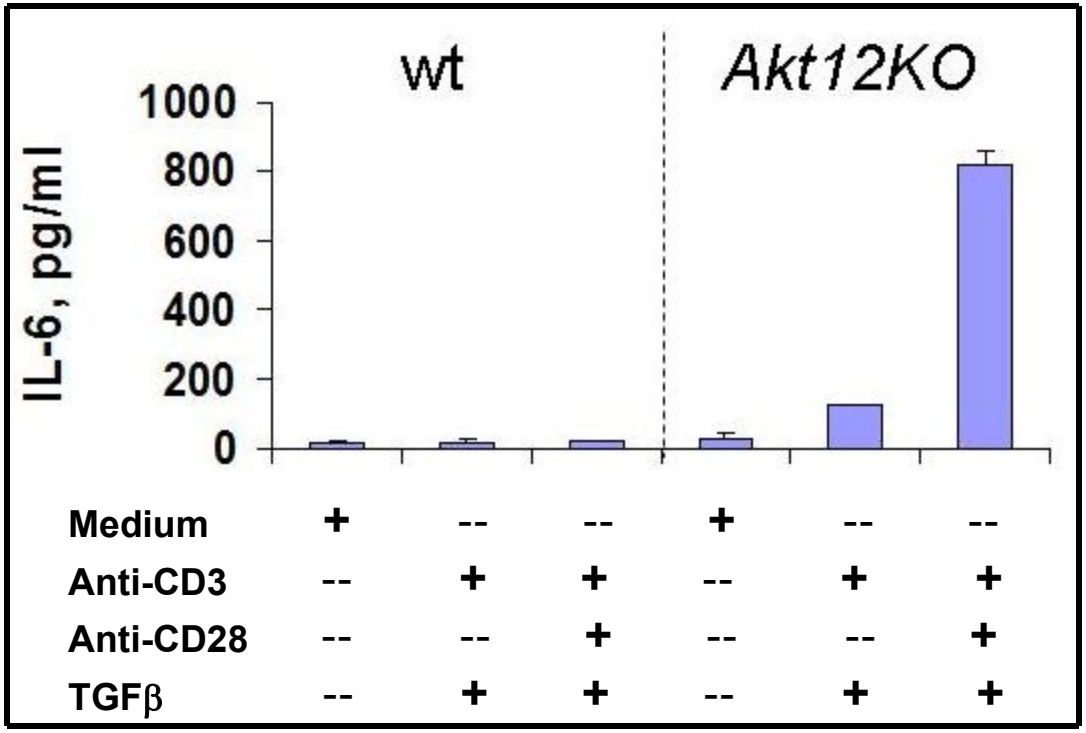
CD4+/CD25+ cells in the spleen of Akt1/Akt2 double knockout mice express low levels of Foxp3

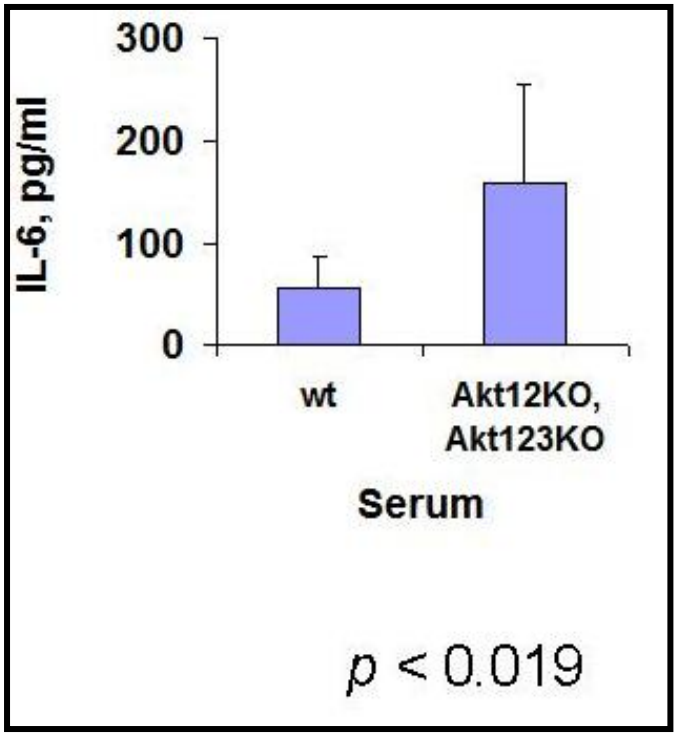


TCR and TGFβ stimulation of CD4+ T cells from Akt1/Akt2 double knockout mice induces Th17 cells rather than Tregs



Akt1/Akt2 DKO cell cultures and Akt1/Akt2 DKO animals produce high levels of IL-6.





Summary

- Identification of pathways activated or inhibited by sets of microRNAs, that are upregulated or downregulated by different Akt isoforms during IGF stimulation or hypoxia.
- Akt2 inhibits the expression of microRNAs of the miR-200 family. As a result, Akt2 promotes EMT, tumor cells invasiveness and the acquisition of stem cell properties by the tumor cells.
- Akt1 inhibits the effects of Akt2 on the expression of microRNAs that regulate EMT. As a result, it is the balance between Akt1 and Akt2, rather than the overall Akt activity, that regulates this process.
- Akt2 enhances resistance to hypoxia via microRNA-dependent mechanisms.
- Akt2 promotes inflammation. The differential effects of Akt1 and Akt2 on inflammation depend on differential microRNA regulation by the two isoforms

- Akt1 and Akt2 maintain self-tolerance by controlling the development of regulatory T cells.

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