Essential role for oncogenic Ras in tumour maintenance

Nature: Volume 400(6743), 29 July 1999, pp 468-472

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Statement of the Problem

- Established tumors harbor many mutations in oncogenes and tumor suppressor genes
- Which are necessary for genesis and which for maintenance?
- Are the mutations leading to genesis dispensable after a tumor is formed?

Hypothesis

Activated Ras is required for tumor genesis and maintenance

The Model: Tyr/Tet±Ras transgenic in an INK4a-deficient background

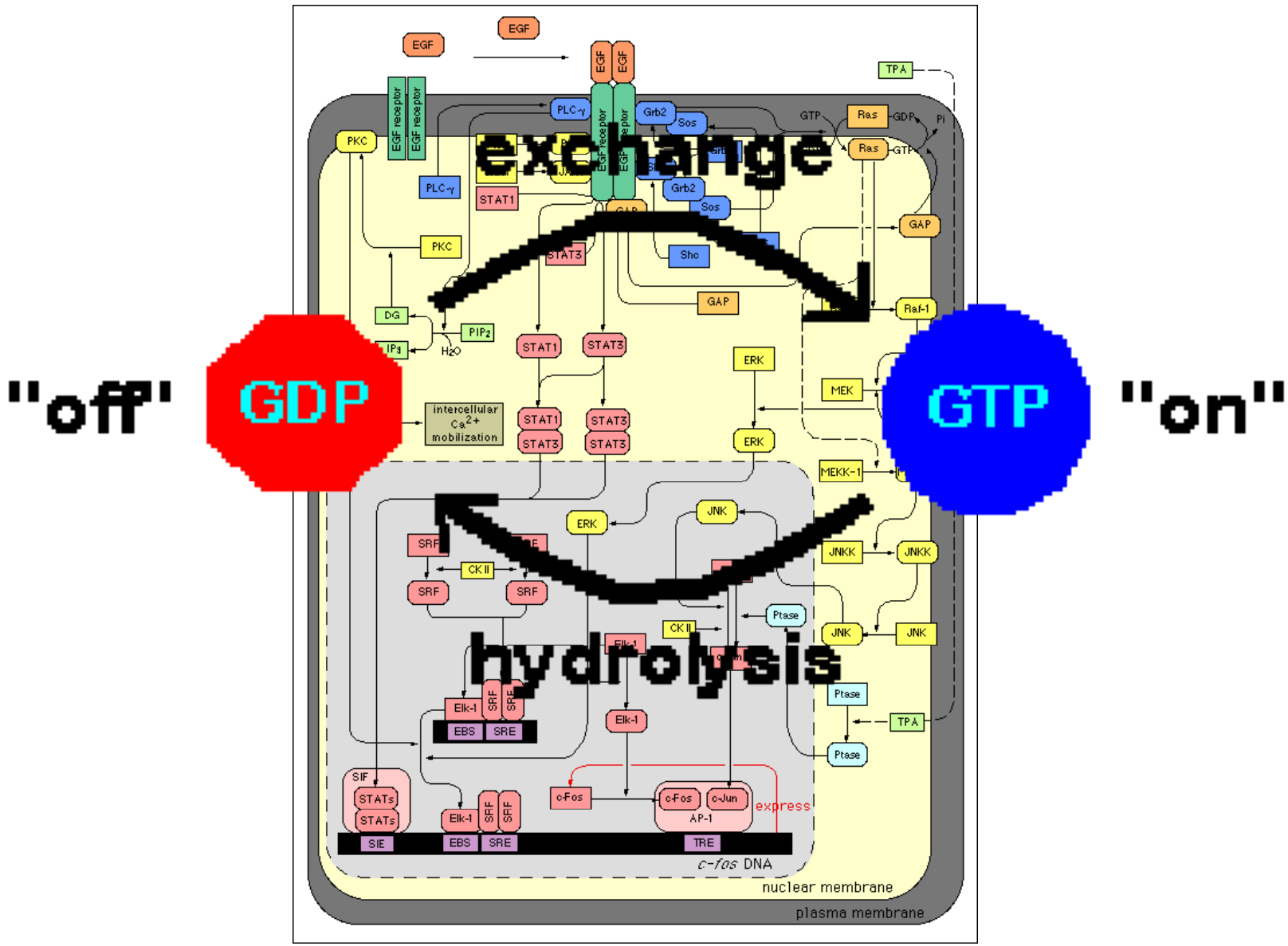
Tyrosinase is the rate limiting enzyme in pigment synthesis

Why choose Ras and Ink 4?

Ras

- Mutated in up to 50% of epithelial malignancies into a “locked on” state
- A critical integrator of growth factor proliferative and survival signaling

Why Ras?

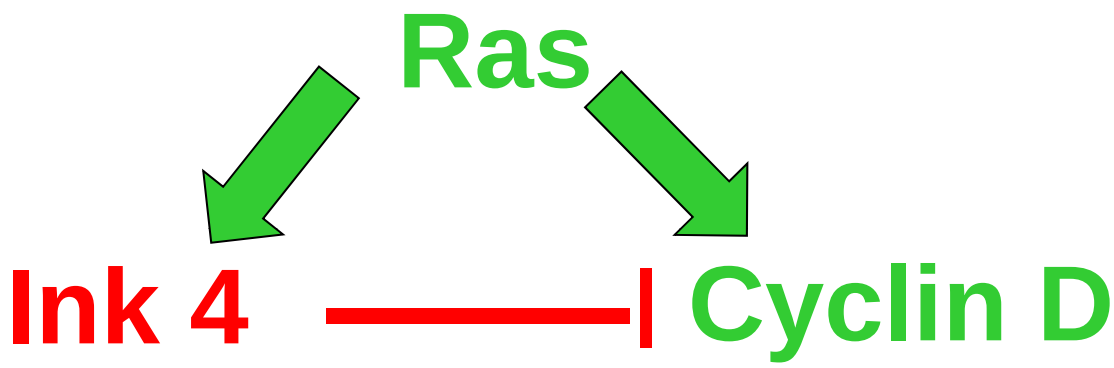

"off"
GDP
exchange
hydrolysis
GTP
"on"
EGF
EGF receptor
PLC-γ
PKC
STAT1
STAT3
Grb2
Sos
Ras
GTP
GDP
Pi
GAP
Shc
Raf-1
MEK
ERK
MEKK-1
JNK
JNKK
Ptase
TPA
DG
IP3
PIP2
H2O
intercellular Ca2+ mobilization
CK II
SRF
Elk-1
EBS
SRE
SIF
STATs
SIE
c-Fos
c-Jun
AP-1
express
TRE
c-fos DNA
nuclear membrane
plasma membrane

INK4

- **Lost in familial malignant melanoma.**

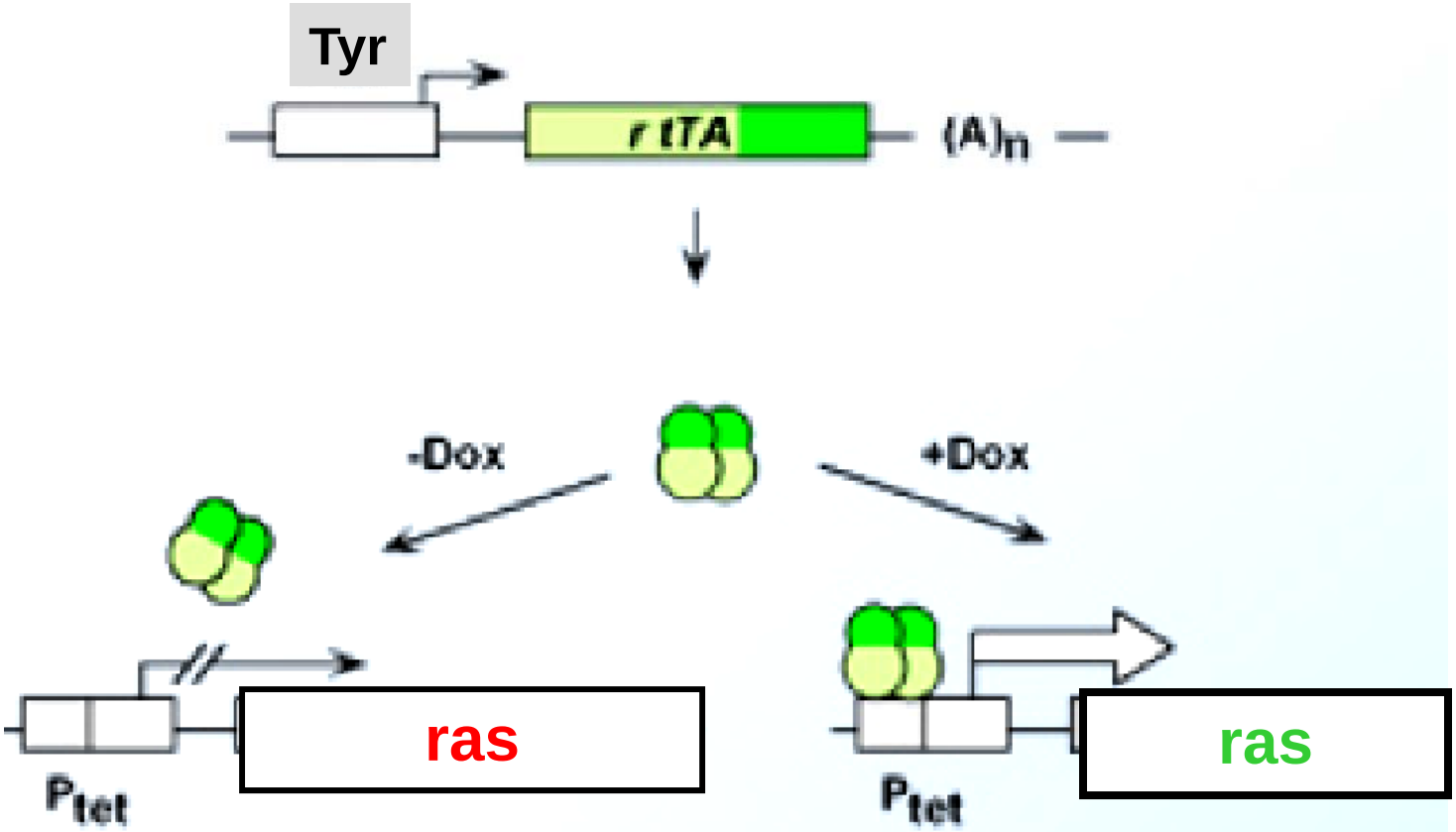
- **Deleted/inactivated in :**
  - **- 50% of T-cell Leukemia**
  - **- 20% of B-cell Leukemia**
  - **- many solid tumors:**
  - **e.g. lung,pancreas,bladder, ovary**

Ras is restrained by Ink 4



The INK4 inhibitors inhibit Cdks allosterically. They induce conformational changes that propagate to the cyclin-binding site and interfere with cyclin binding. The INK4 inhibitors also distort the kinase active site and interfere with ATP binding.

Melanocyte



Tet on +

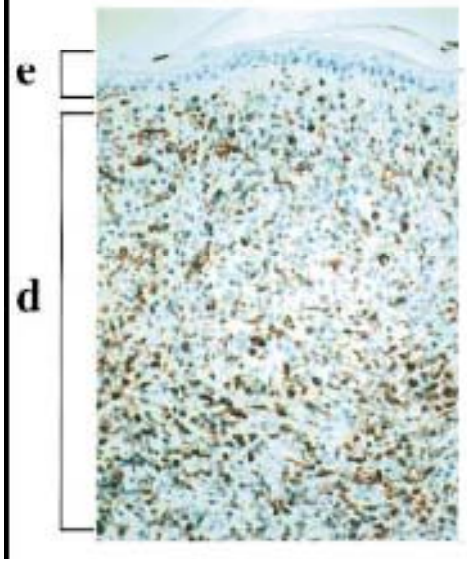
Lacks rtTA

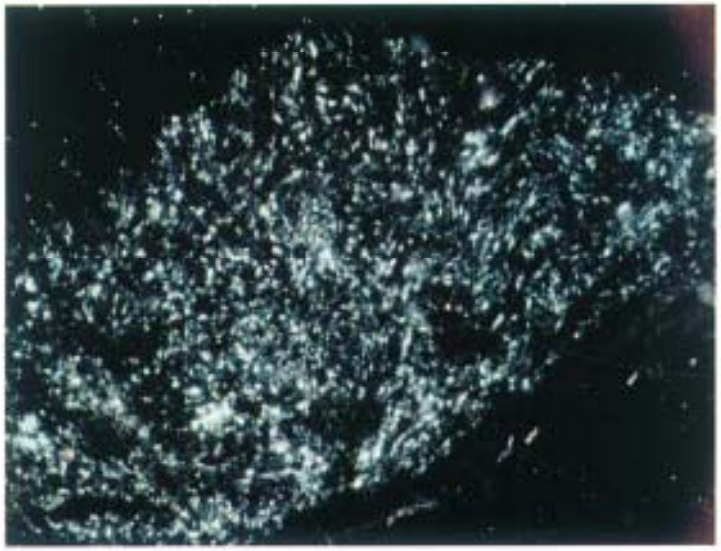
Tet on -

a

Number of mice	Genotype Tyr–rtTA	Tet–Ras	INK4a	Doxycycline induction	Number of mice with melanoma	Latency (days ± s.d.)
40	+	+	–/–	Yes	10	60 ± 26
23	–	+	–/–	Yes	0	—
12	+	+	–/–	No	0	—

b

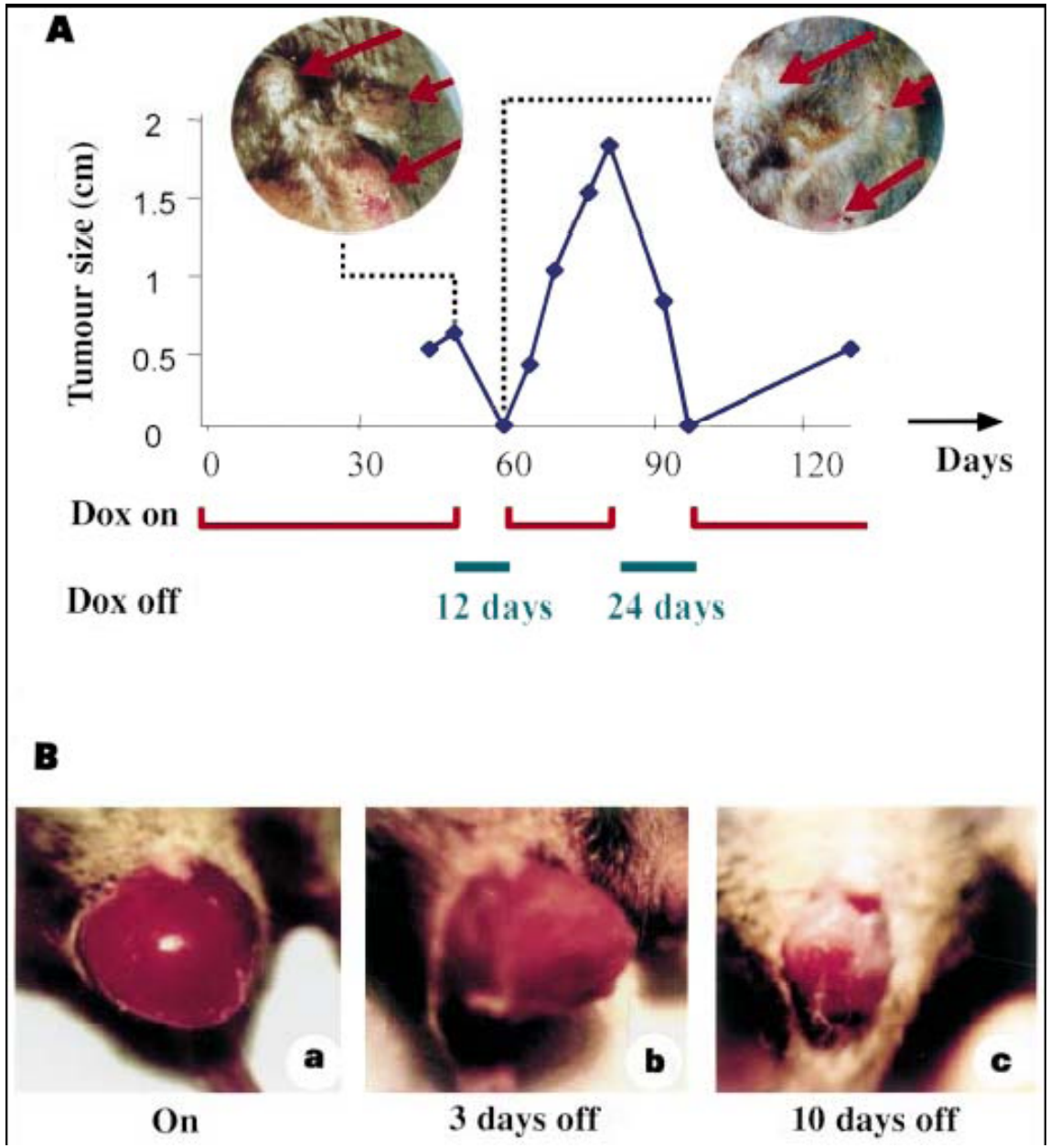




Tyrosinase related protein 1

Ras in situ (mRNA)

Figure 2. Activated Ras expression is necessary to maintain growth of established cutaneous melanomas in vivo.



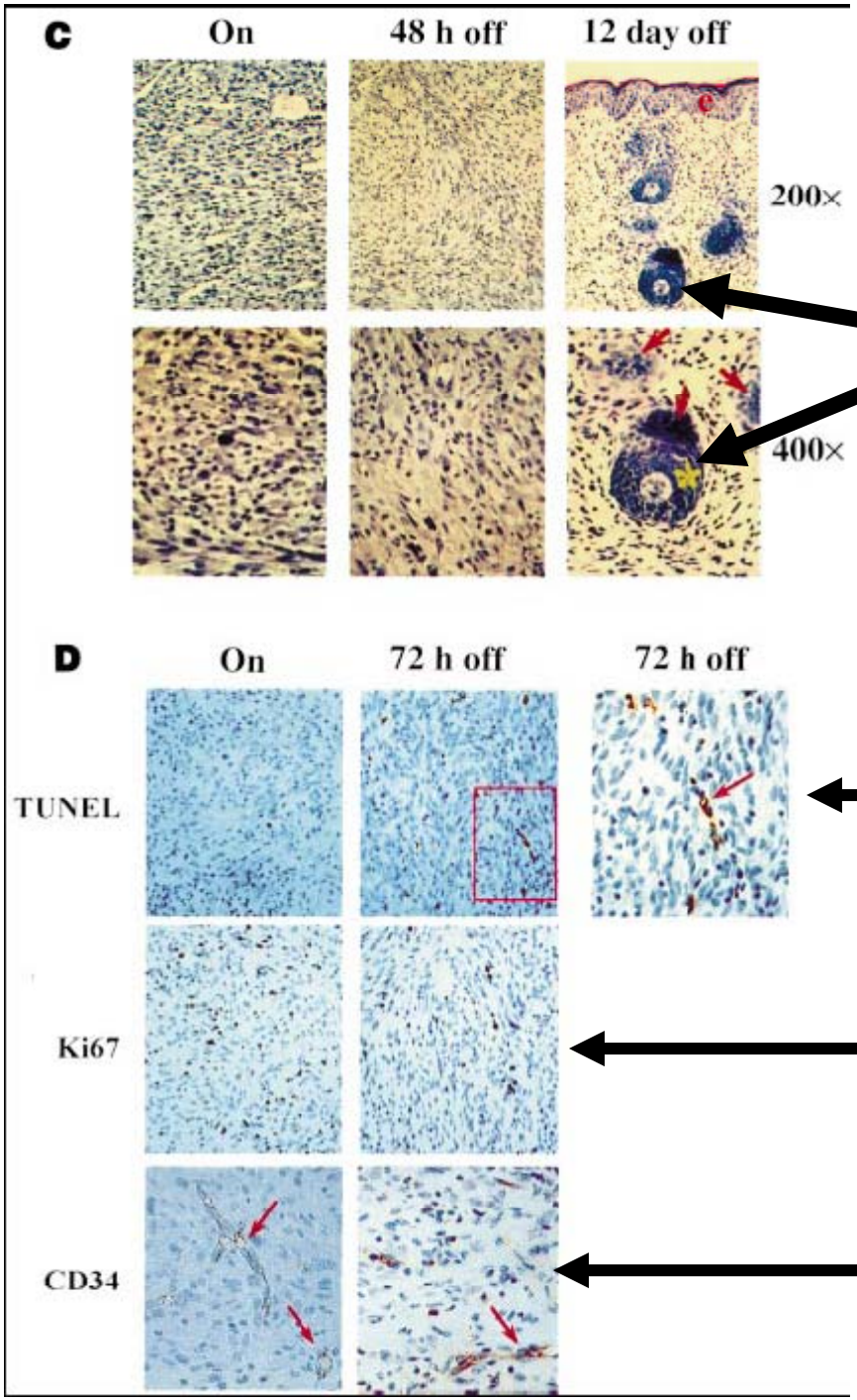
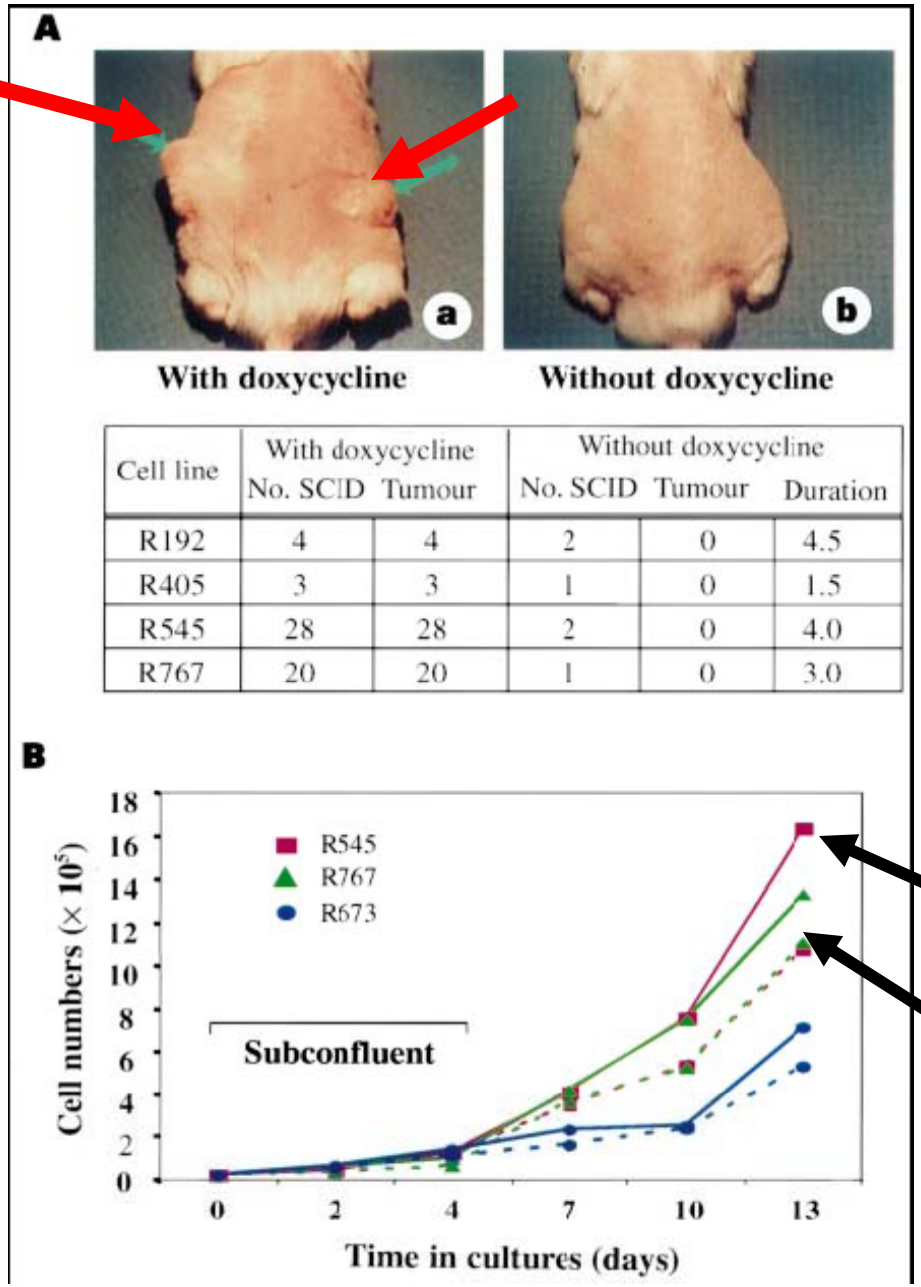
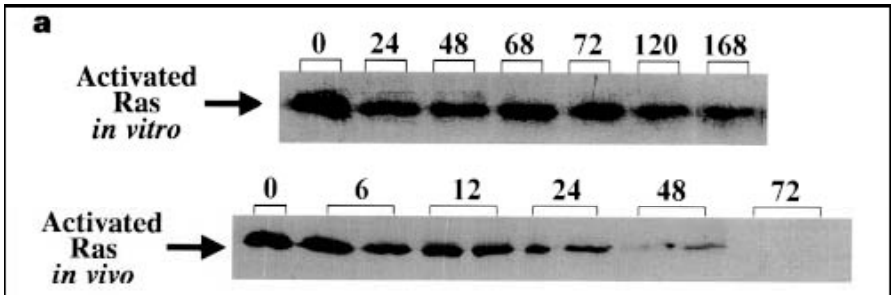

C
On
48 h off
12 day off
200×
400×
Hair follicle
D
On
72 h off
72 h off
TUNEL
apoptosis
Ki67
proliferation
CD34
endothelial
determinant

Figure 3. Derived melanoma cell lines remain doxy responsive and form tumors in SCID mice

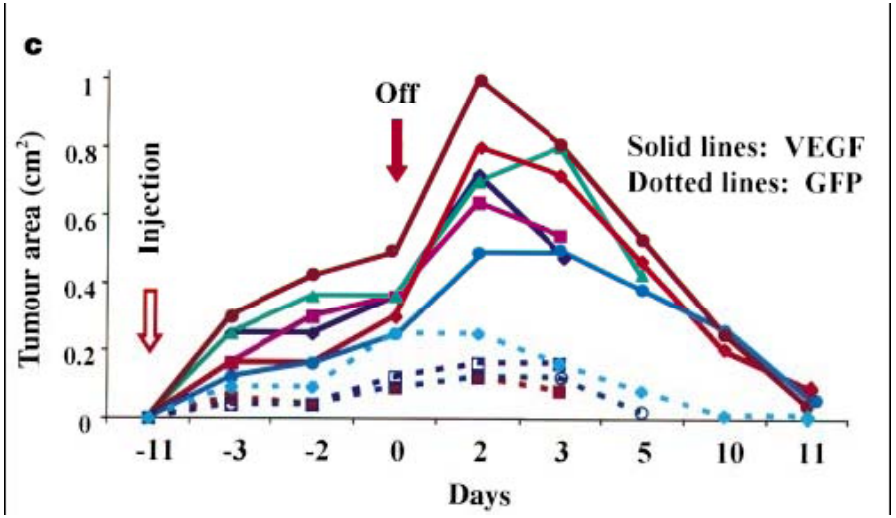


Cell line	With doxycycline No. SCID	Tumour	Without doxycycline No. SCID	Tumour	Duration
R192	4	4	2	0	4.5
R405	3	3	1	0	1.5
R545	28	28	2	0	4.0
R767	20	20	1	0	3.0

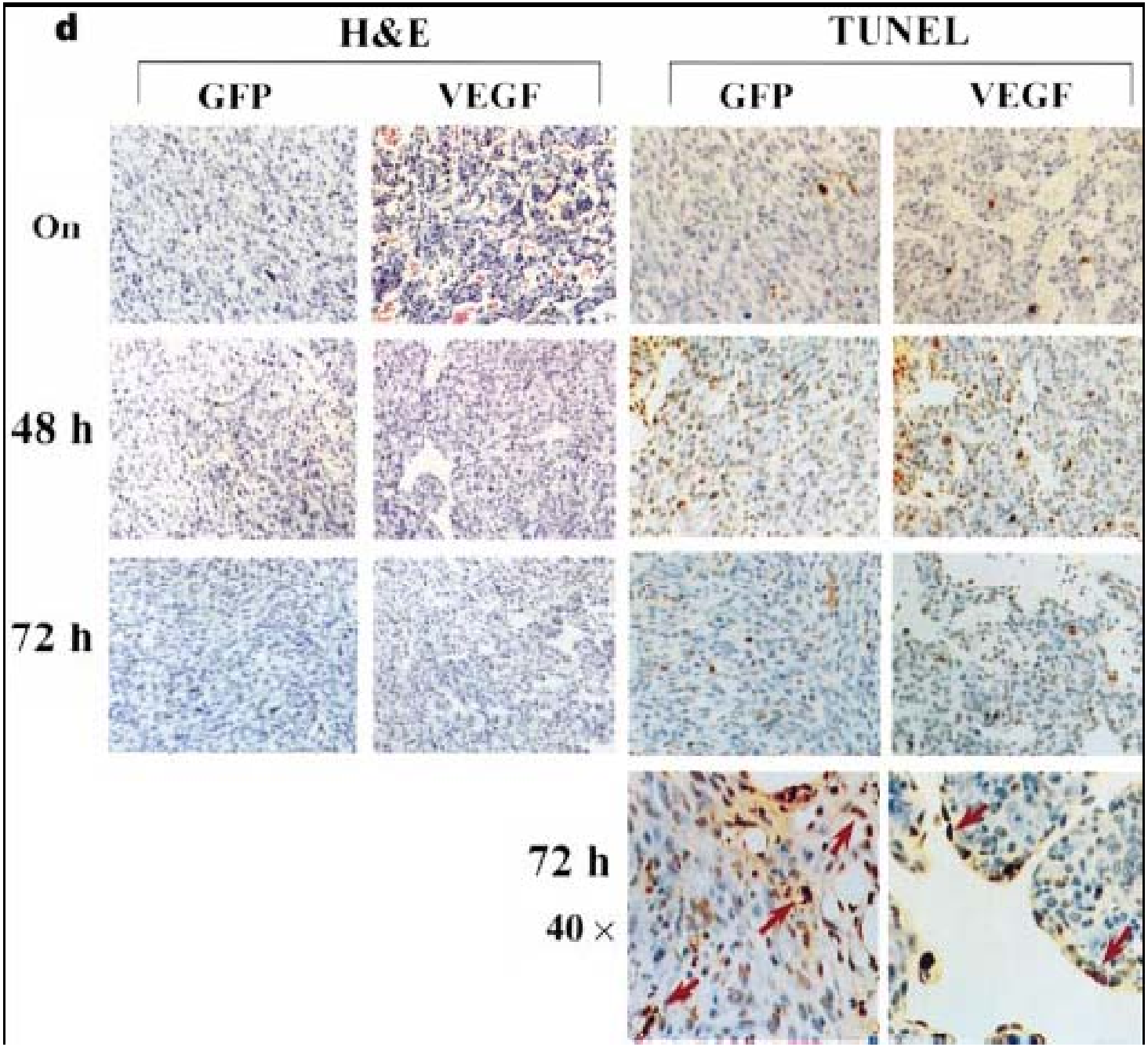
Figure 4. VEGF does not sustain tumour viability following doxy withdrawal



R545 melanoma cells in cultures or R545-derived SCID tumours were subjected to doxycycline withdrawal.



VEGF tumors grew faster but collapsed 48h after doxy was off


d
H&E
TUNEL
GFP
VEGF
GFP
VEGF
0h
48 h
72 h
72 h
40 ×

Conclusions

- Melanoma genesis and maintenance is strictly dependent on activated Ras
- It’s not just VEGF mediating the tumor maintenance effect of Ras