CANCER RESEARCH UK | BEATSON INSTITUTE

Roles of PI3K and MAPK signalling networks in melanoma development, progression and survival

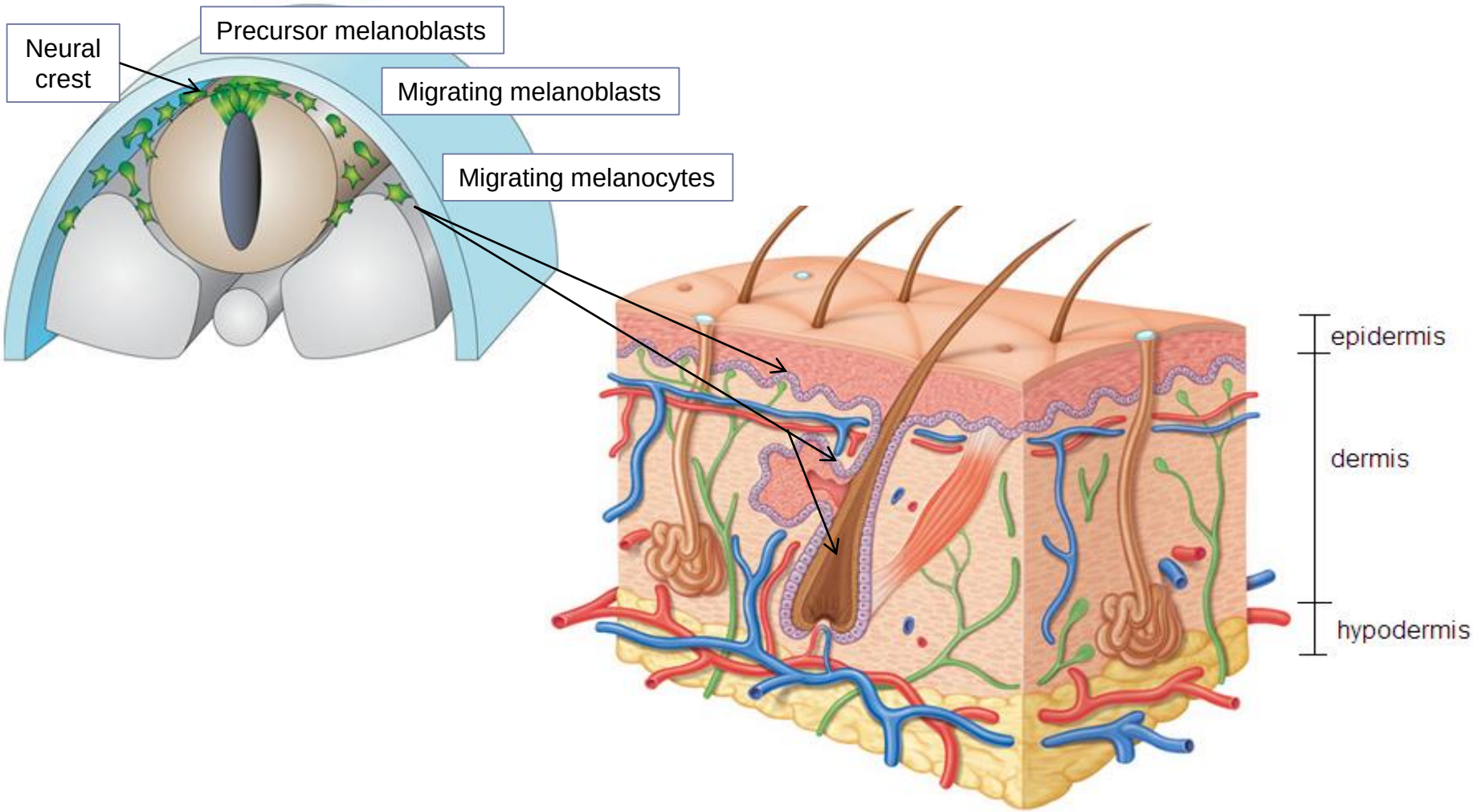
Dana Koludrovic, PhD
Beatson Institute for Cancer Research
Owen Sansom group

Scottish Association of Histotechnology (SAH) 40th scientific meeting
Friday 10th November 2017

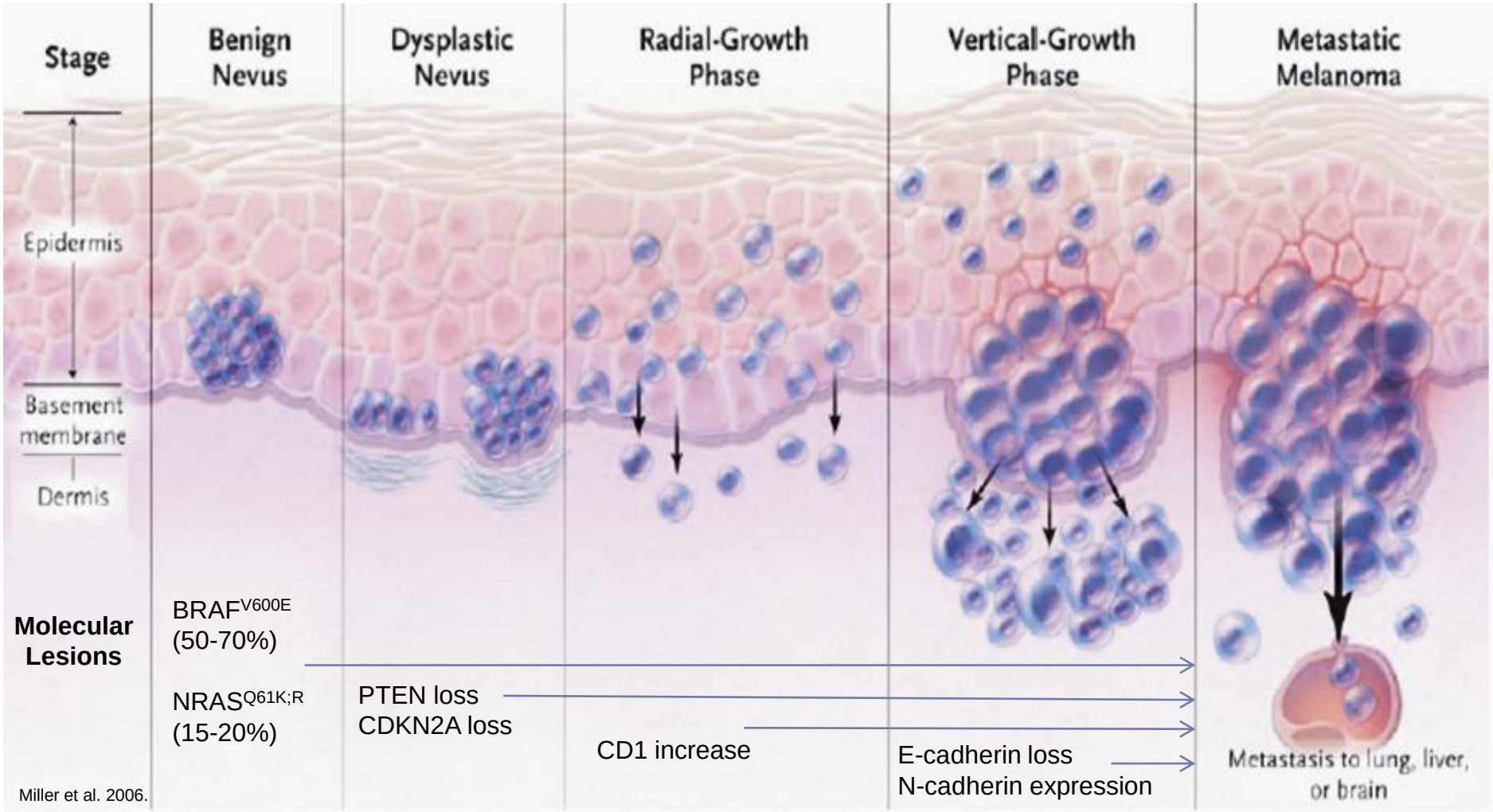
Presentation overview

- Introduction
- Results:
  - Study of melanoma progression and survival
    - Role of PREX proteins
    - Combinatory treatment of PI3K and MAPK
    - Role of mTORC2/Rictor in NRAS mediated melanoma
  - Study of melanoma development
    - Role of DUSP6
    - Role of FAK

Melanocytes colonize the epidermis and the hair follicle

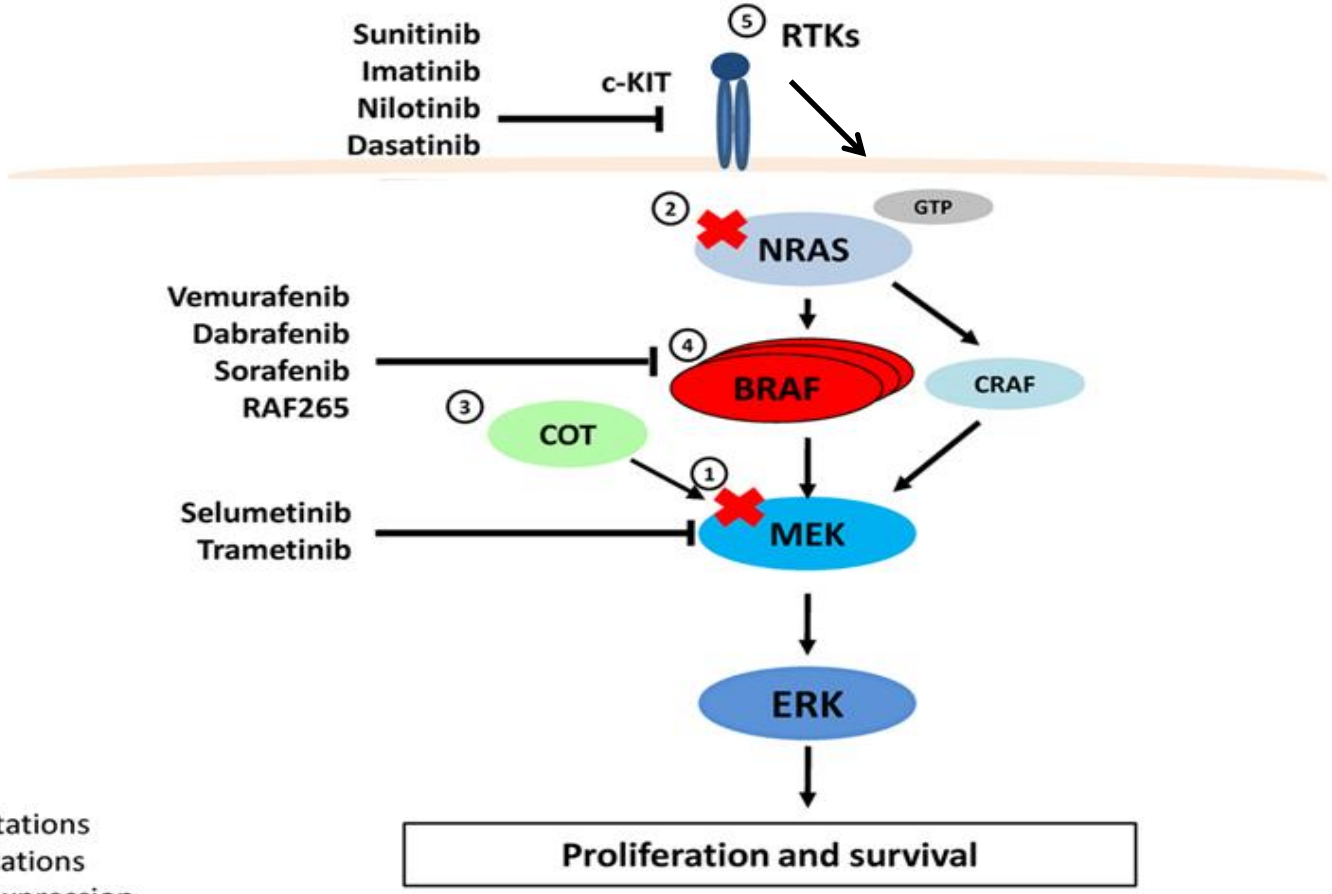


Melanoma



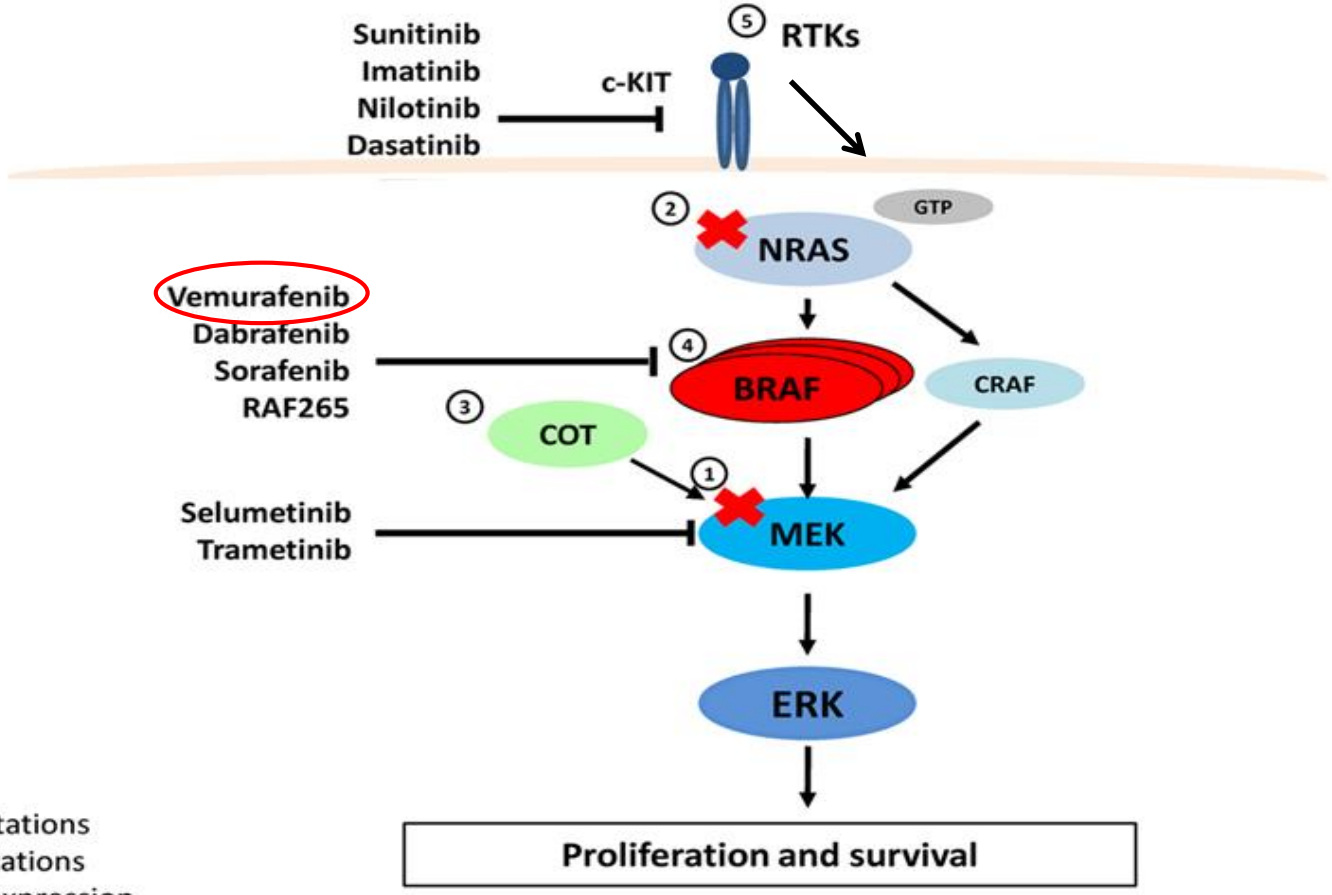
Miller et al. 2006.

Overview of therapeutic approaches in melanoma



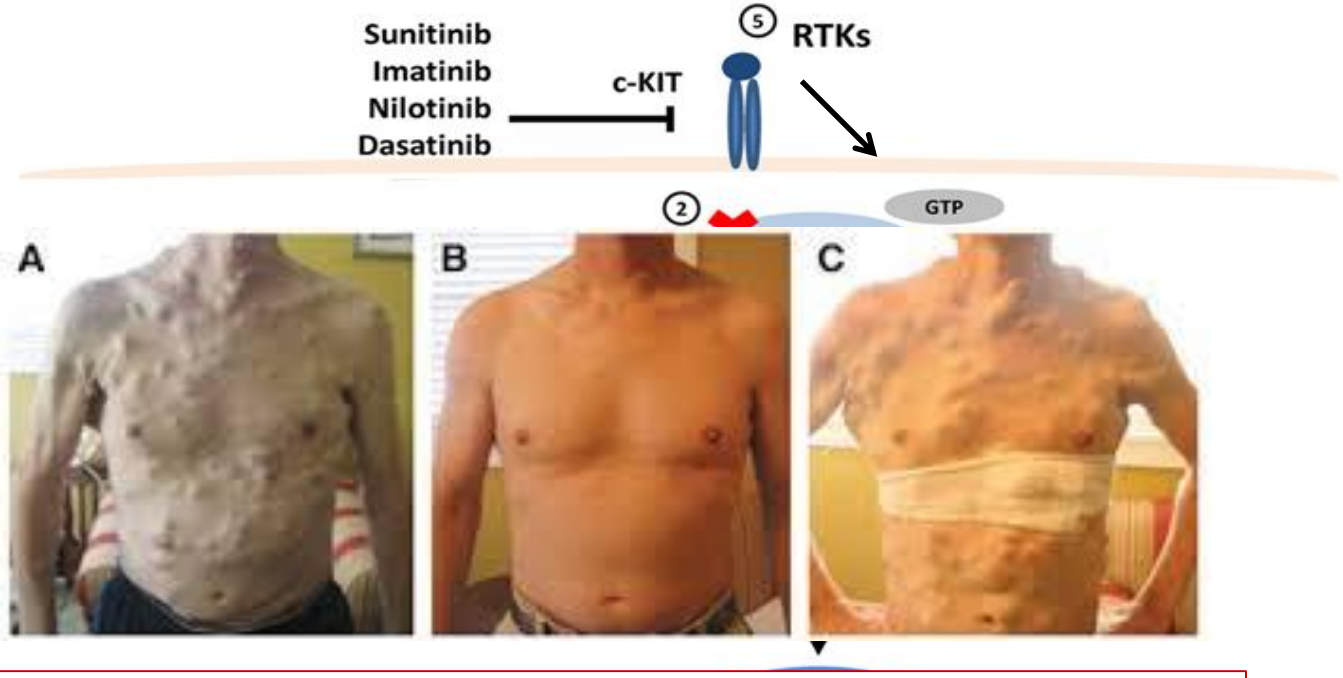
1. MEK1 mutations
2. NRAS mutations
3. COT overexpression
4. BRAF amplification/ splicing
5. RTKs overexpression/activation (PDGFRβ, IGR1F)

Overview of therapeutic approaches in melanoma



1. MEK1 mutations
2. NRAS mutations
3. COT overexpression
4. BRAF amplification/ splicing
5. RTKs overexpression/activation (PDGFRβ, IGR1F)

Overview of therapeutic approaches in melanoma

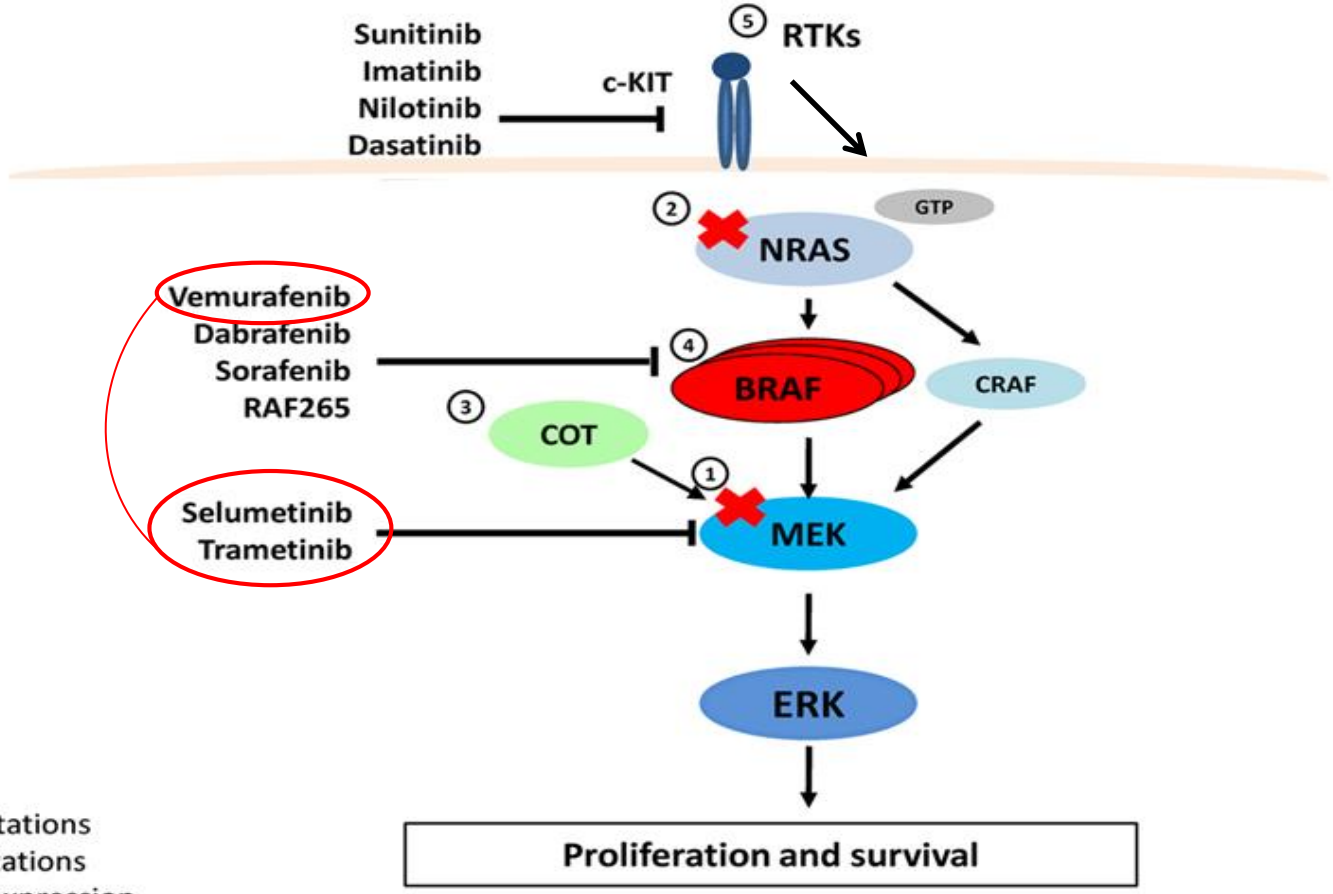


A) A metastatic melanoma patient prior to therapy.
B) Same patient after 15 weeks of therapy with the $BRAF^{V600E}$-inhibitor
C) Same patient 23 weeks after therapy

1.
2. NRAS mutations
3. COT overexpression
4. BRAF amplification/ splicing
5. RTKs overexpression/activation (PDGFRβ, IGR1F)

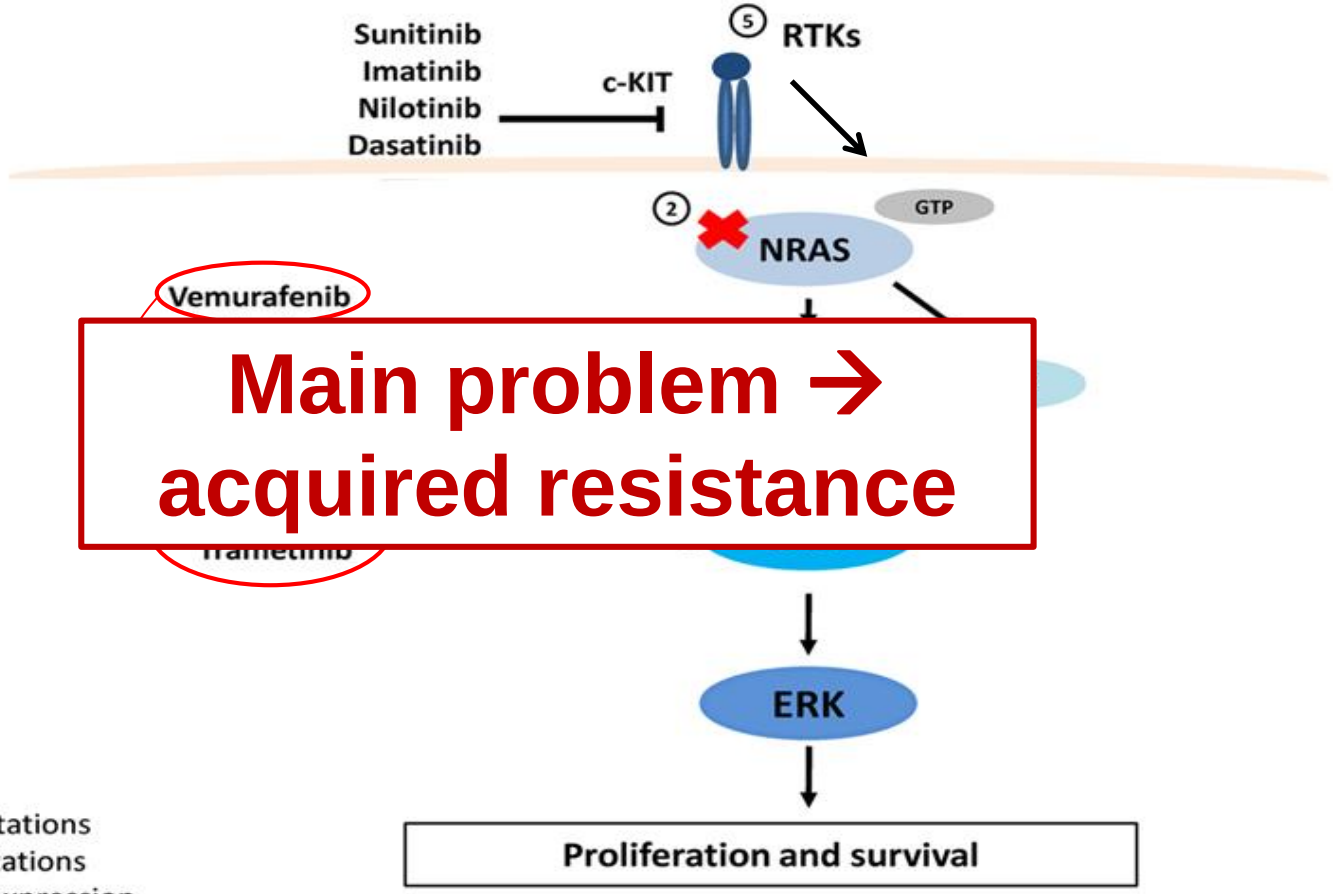
Proliferation and survival

Overview of therapeutic approaches in melanoma



1. MEK1 mutations
2. NRAS mutations
3. COT overexpression
4. BRAF amplification/ splicing
5. RTKs overexpression/activation (PDGFRβ, IGR1F)

Overview of therapeutic approaches in melanoma

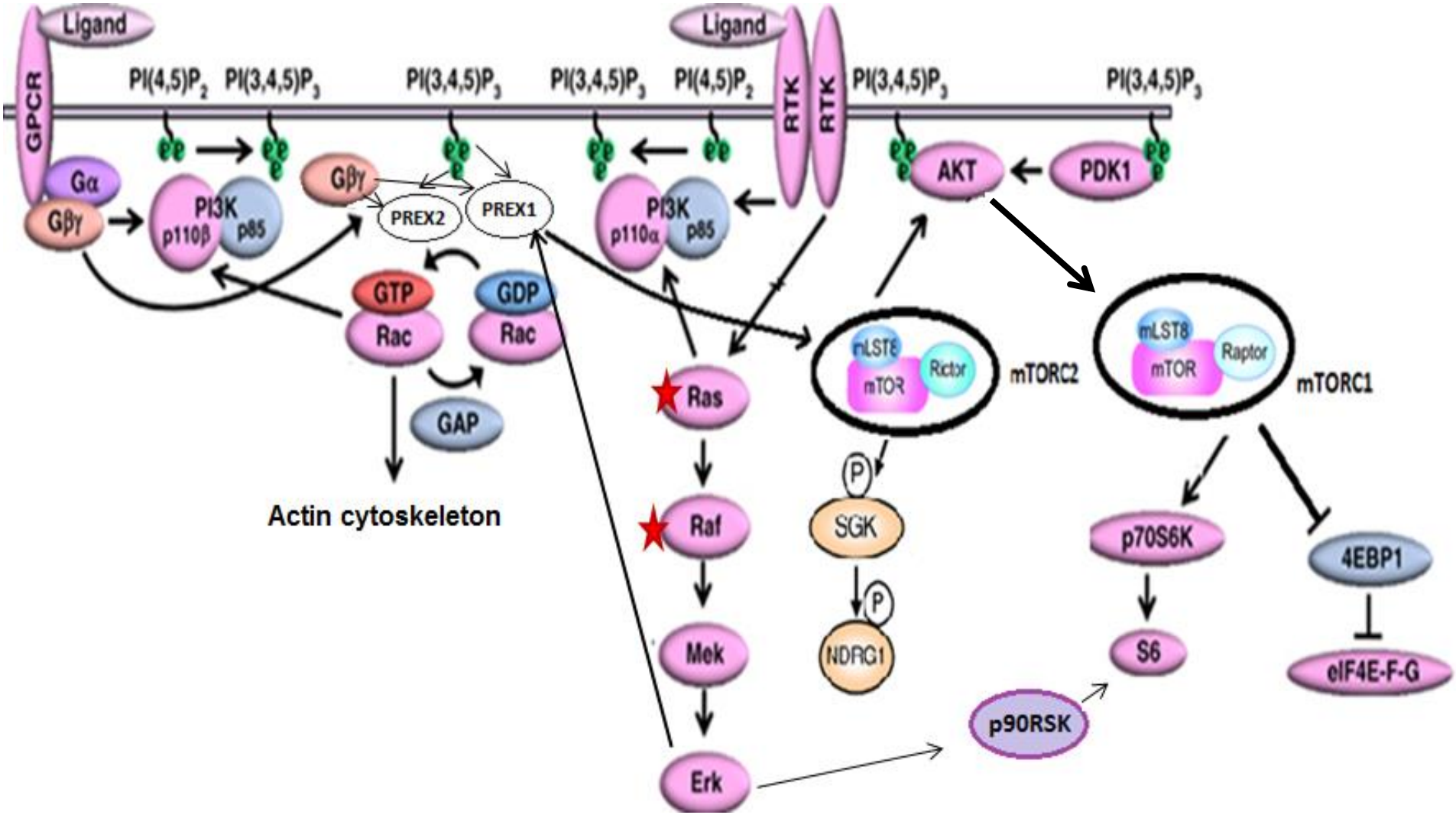


Main problem → acquired resistance

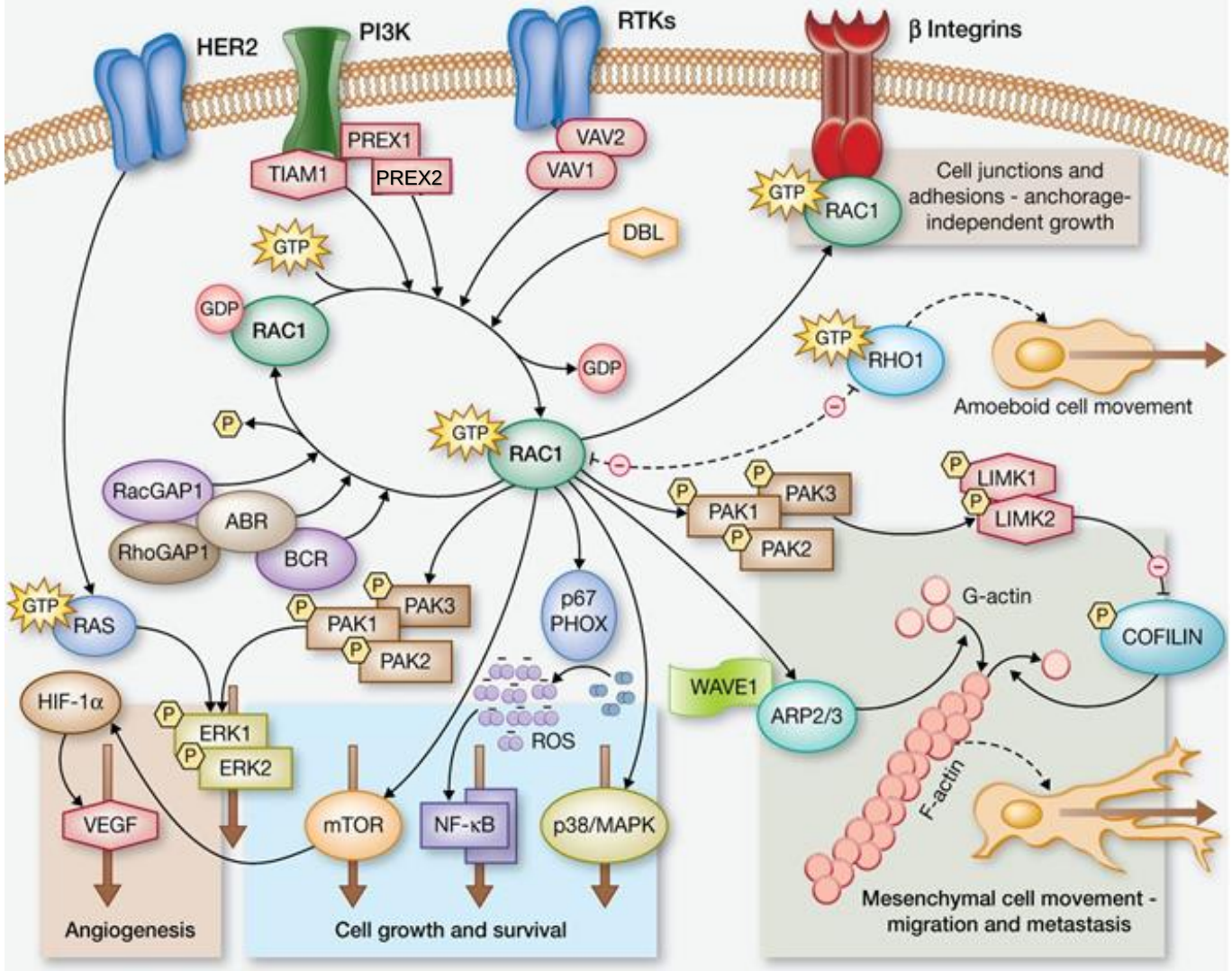
1. MEK1 mutations
2. NRAS mutations
3. COT overexpression
4. BRAF amplification/ splicing
5. RTKs overexpression/activation (PDGFRβ, IGR1F)

Study of melanoma progression and survival

Interconnections of signalling networks



RAC signalling pathway



Human sequencing indicating the importance of Rac signalling

Exome sequencing identifies recurrent somatic *RAC1* mutations in melanoma

Michael Krauthammer[1], Yong Kong[2,3], Byung Hak Ha[4], Perry Evans[1], Antonella Bacchiocchi[5], James P McCusker[1], Elaine Cheng[5], Matthew J Davis[4], Gerald Goh[6,7], Murim Choi[6,7], Stephan Ariyan[8], Deepak Narayan[8], Ken Dutton-Regester[9,10], Ana Capatana[1], Edna C Holman[5], Marcus Bosenberg[5], Mario Sznol[11], Harriet M Kluger[11], Douglas E Brash[5,6,12], David F Stern[1], Miguel A Materin[13], Roger S Lo[14], Shrikant Mane[6,15,16], Shuangge Ma[17], Kenneth K Kidd[6], Nicholas K Hayward[10], Richard P Lifton[6,7], Joseph Schlessinger[4], Titus J Boggon[4] & Ruth Halaban[5]

Human sequencing indicating the importance of Rac signalling

Exome sequencing identifies recurrent somatic *RAC1* mutations in melanoma

Michael
James P
Deepak
Mario S
Shrikan
Joseph S

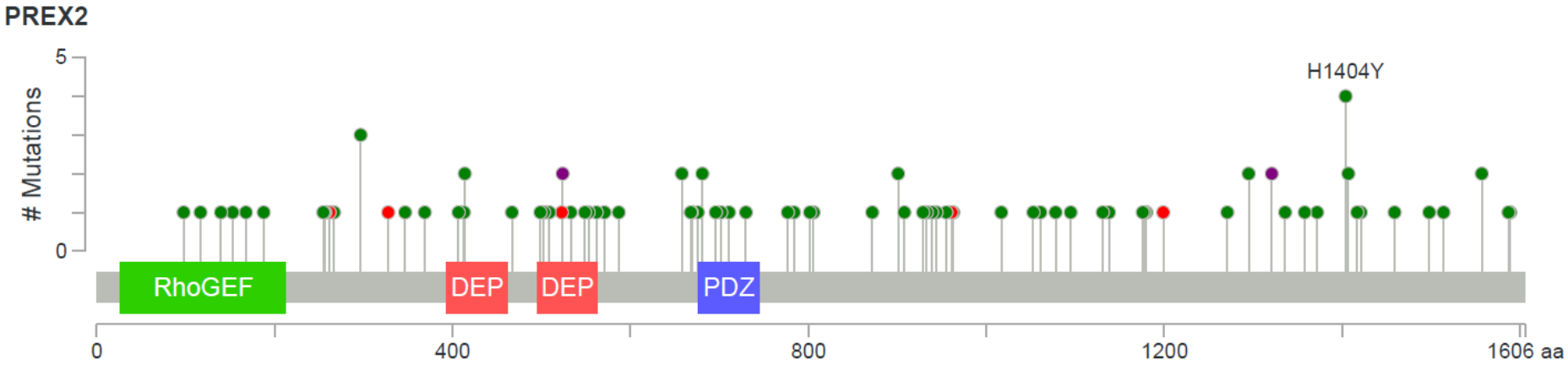
LETTER

doi:10.1038/nature11071

Melanoma genome sequencing reveals frequent *PREX2* mutations

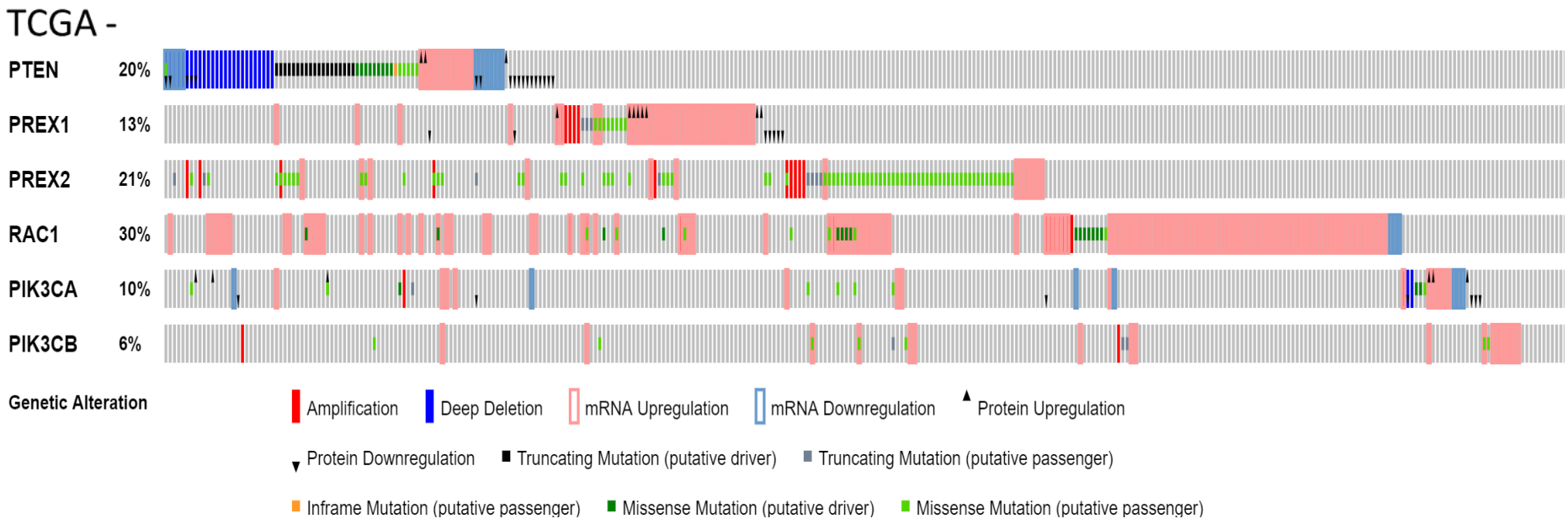
Michael F. Berger[1]†*, Eran Hodis[1]*, Timothy P. Heffernan[2]†*, Yonathan Lissanu Deribe[2]†*, Michael S. Lawrence[1], Alexei Protopopov[2]†, Elena Ivanova[2], Ian R. Watson[2]†, Elizabeth Nickerson[1], Papia Ghosh[2], Hailei Zhang[2], Rhamy Zeid[2], Xiaojia Ren[2], Kristian Cibulskis[1], Andrey Y. Sivachenko[1], Nikhil Wagle[2,3], Antje Sucker[4], Carrie Sougnez[1], Robert Onofrio[1], Lauren Ambrogio[1], Daniel Auclair[1], Timothy Fennell[1], Scott L. Carter[1], Yotam Drier[5], Petar Stojanov[1], Meredith A. Singer[2]†, Douglas Voet[1], Rui Jing[1], Gordon Saksena[1], Jordi Barretina[1], Alex H. Ramos[1,3], Trevor J. Pugh[1,2,3], Nicolas Stransky[1], Melissa Parkin[1], Wendy Winckler[1], Scott Mahan[1], Kristin Ardlie[1], Jennifer Baldwin[1], Jennifer Wargo[6], Dirk Schadendorf[4], Matthew Meyerson[1,2,3,7], Stacey B. Gabriel[1], Todd R. Golub[1,7,8,9], Stephan N. Wagner[10], Eric S. Lander[1,11]*, Gad Getz[1]*, Lynda Chin[1,2,3]†* & Levi A. Garraway[1,2,3,7]*

PREX2 mutations in human melanoma



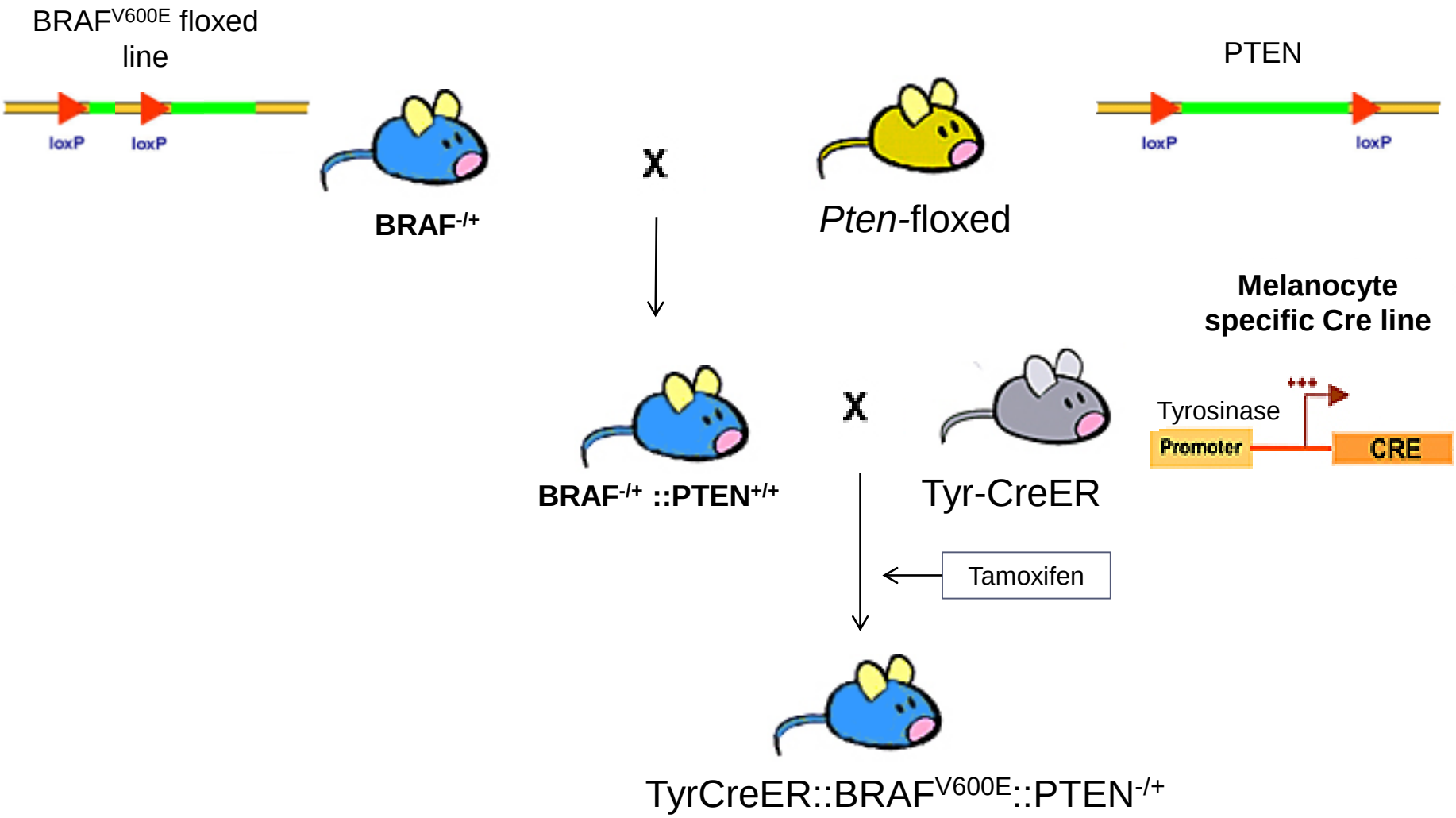
- Truncating mutations in PREX2 are hyperactivating

Human sequencing data

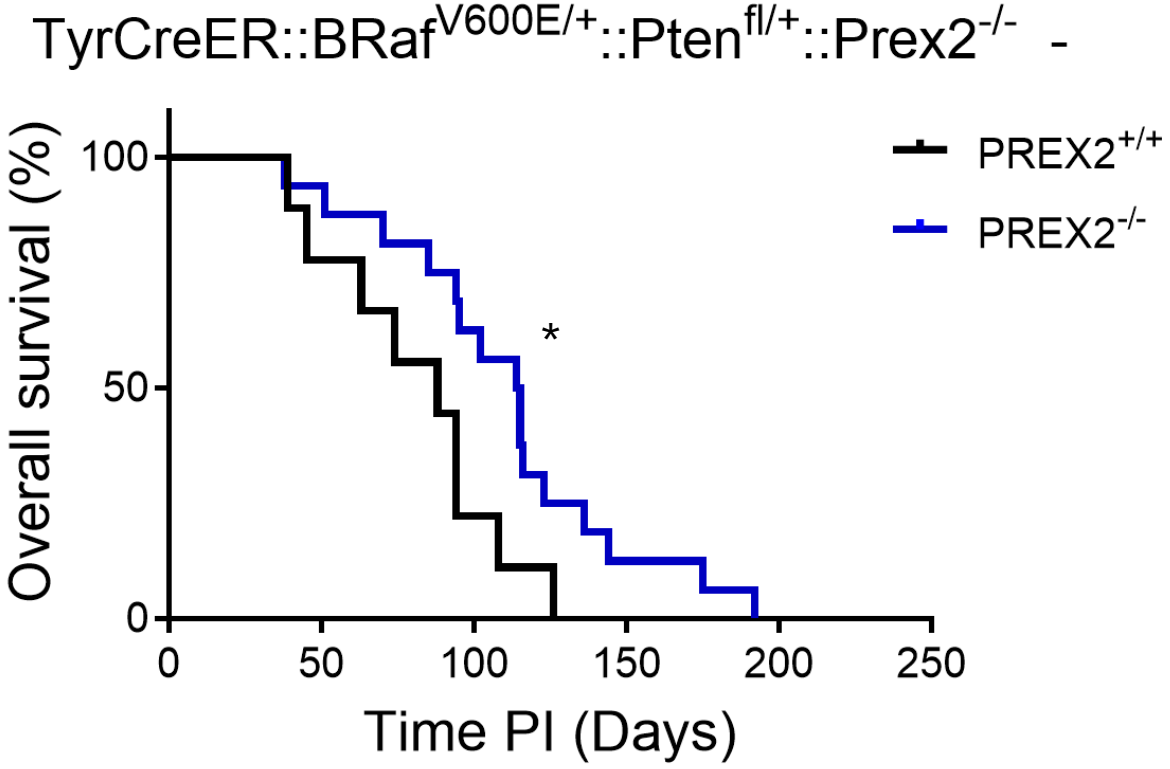


PTEN, Rac1, PREX2 , PREX1 mutations are mutually exclusive

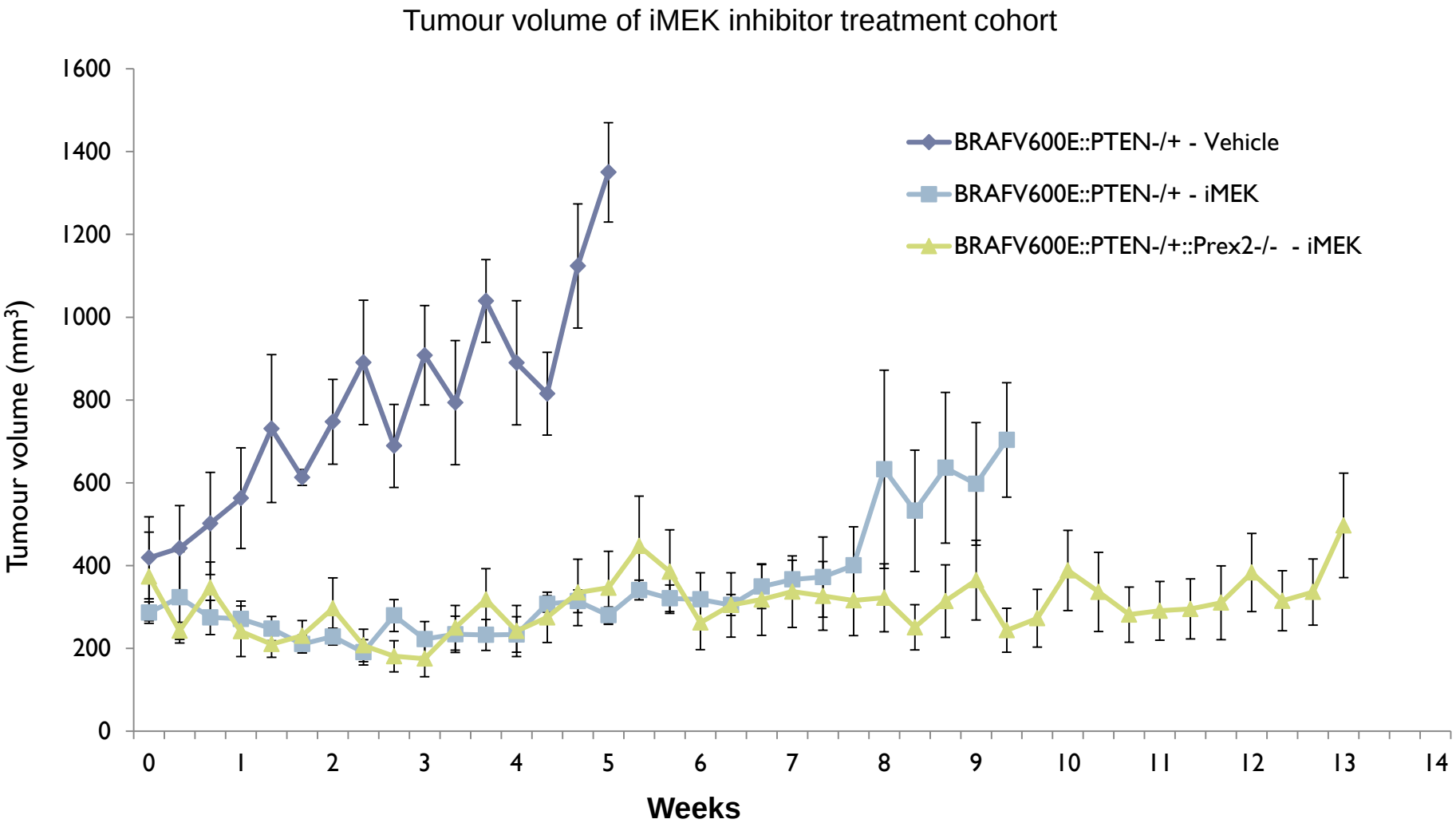
BRAF mutant mouse model of melanoma



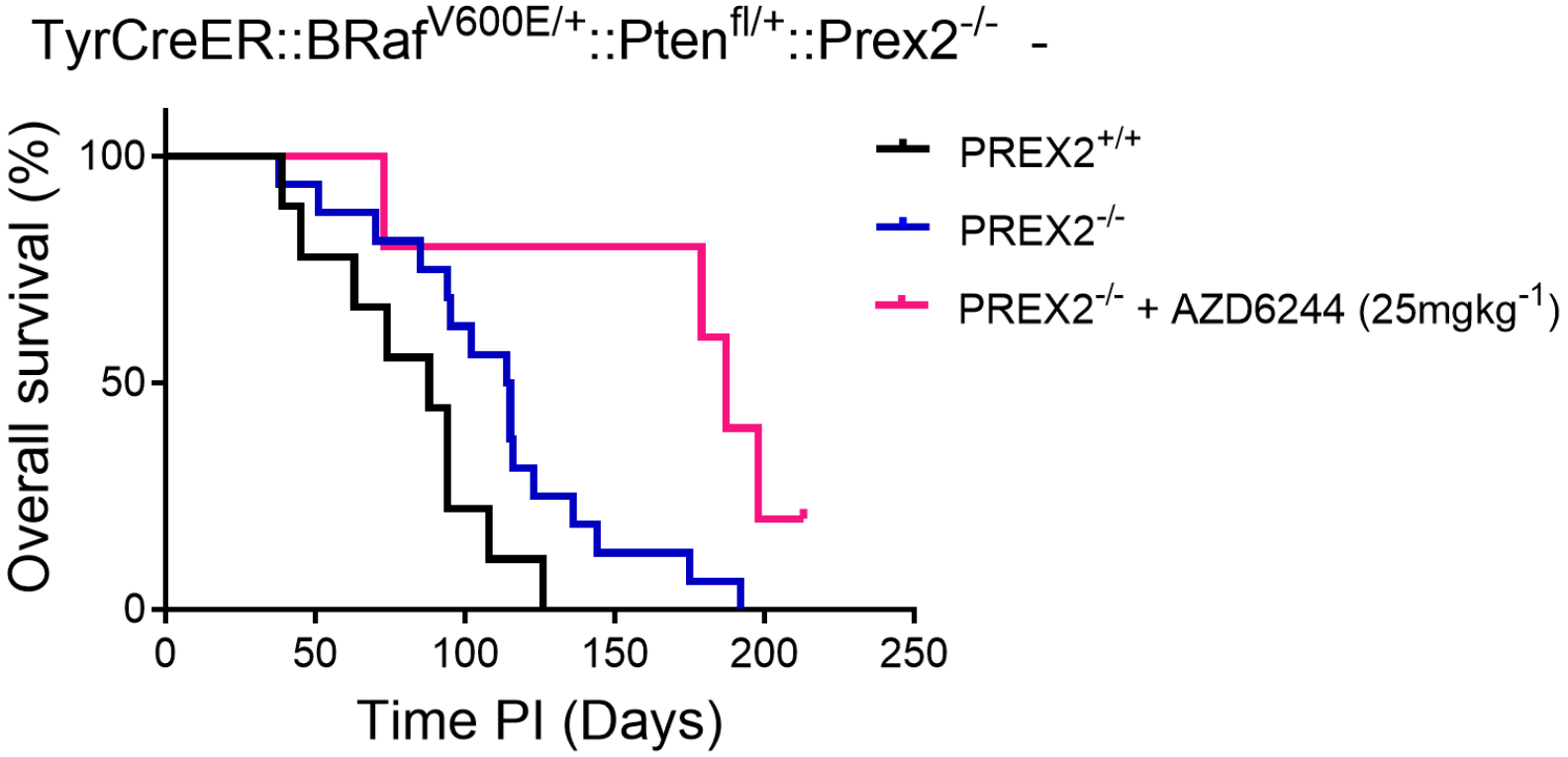
PREX2 loss extends survival in BRAF mutant melanoma



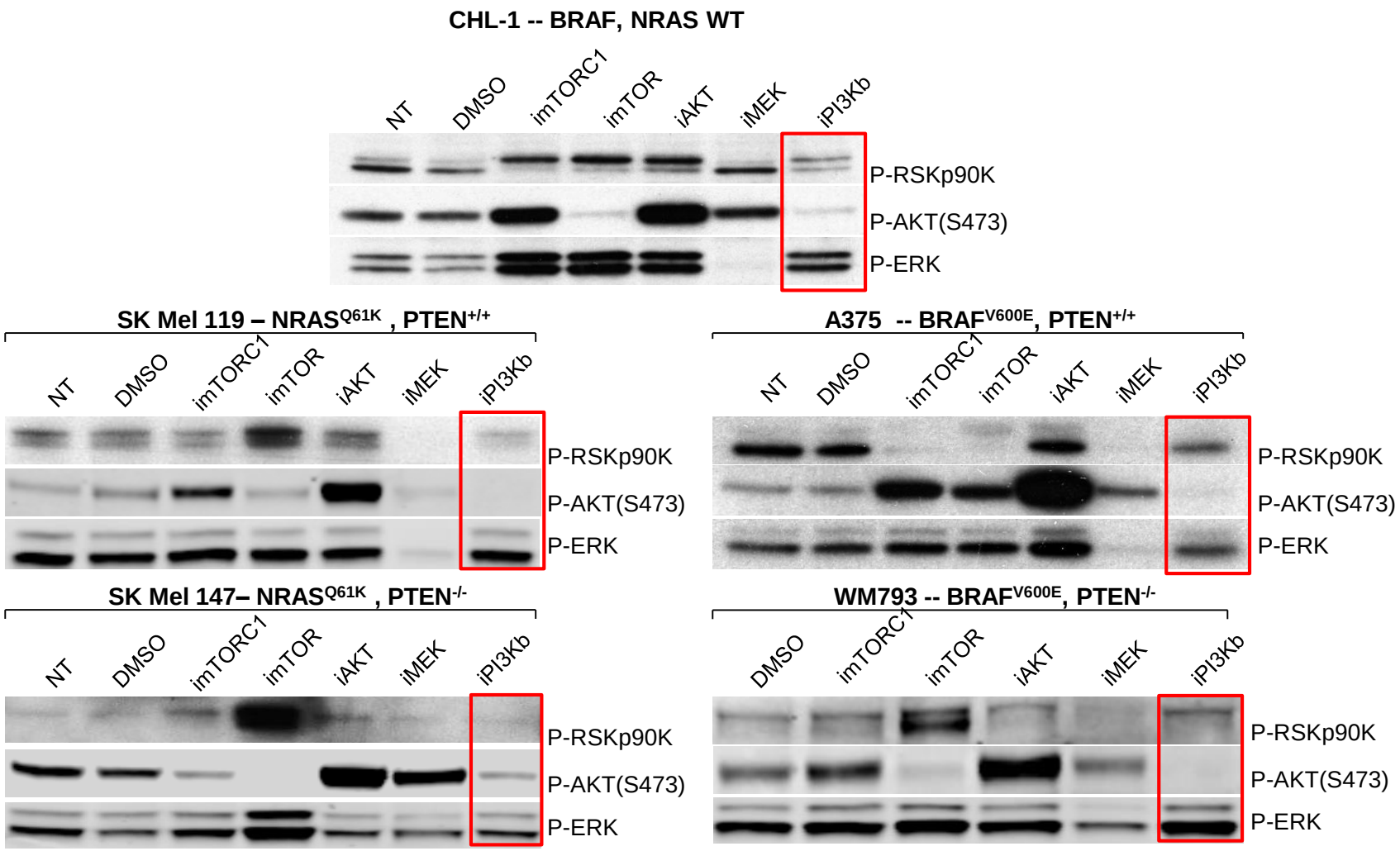
PREX2 combined with MEK inhibition extends survival



PREX2 combined with MEK inhibition extends survival

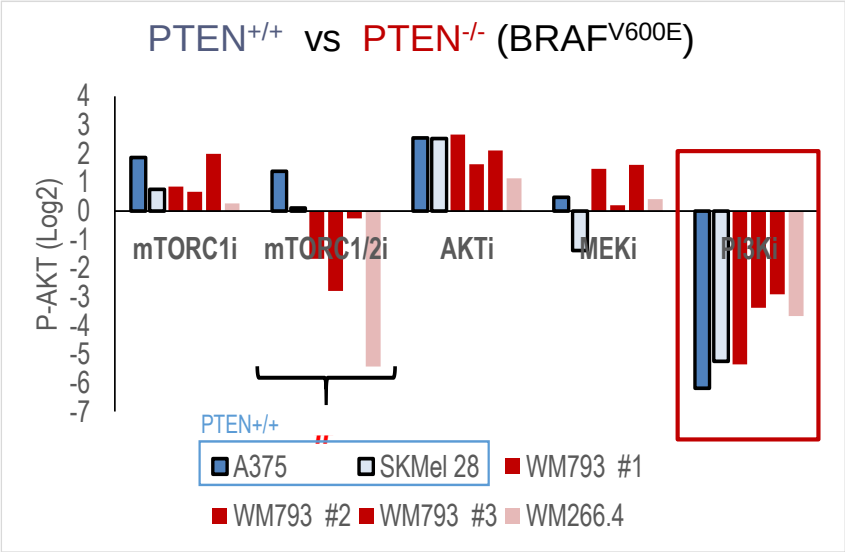


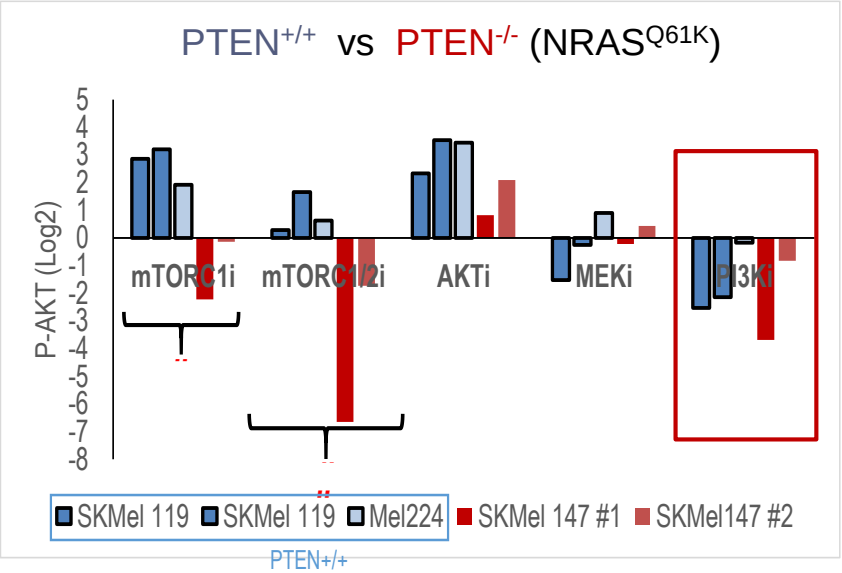
Screen of inhibitors of main signalling nodes in melanoma cell lines



Results: Combinatory treatment of PI3K and MAPK

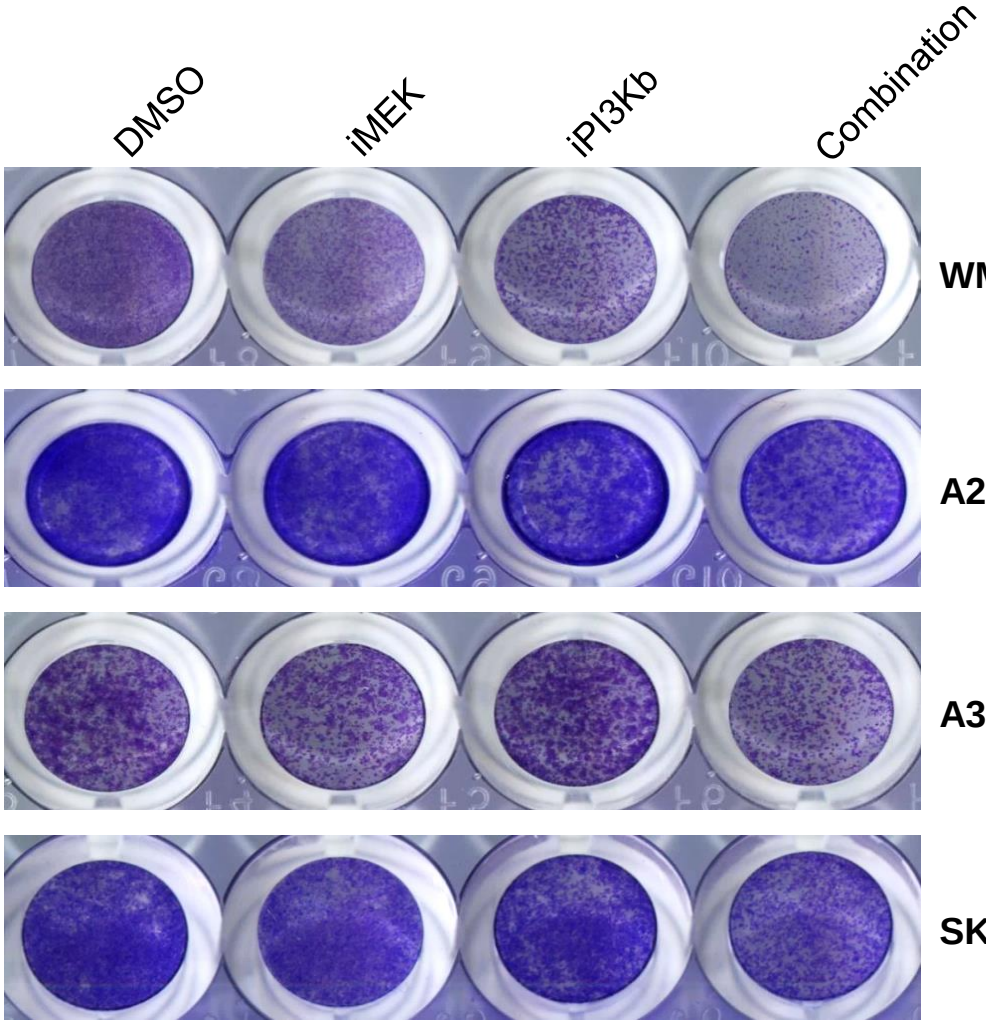
Screen of inhibitors of main signalling nodes in melanoma cell lines





- PI3Kb inhibitor is **highly effective in all mutation backgrounds**

Combination treatment using iMEK and iPI3Kb in melanoma cell lines



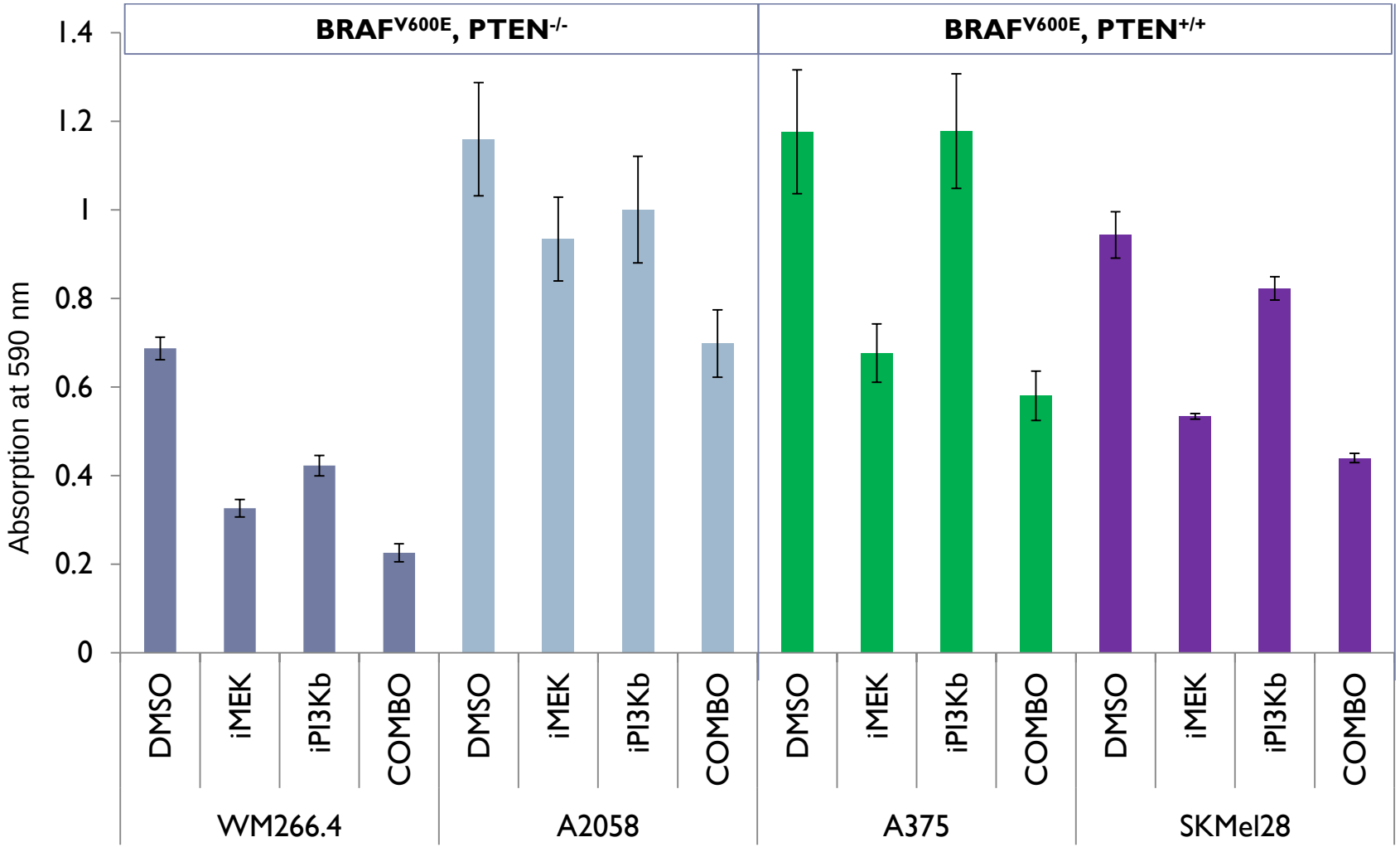
WM266.4 -- BRAF^{V600E}, PTEN$^{-/-}$

A2058 -- BRAF^{V600E}, PTEN$^{-/-}$

A375 -- BRAF^{V600E}, PTEN$^{+/+}$

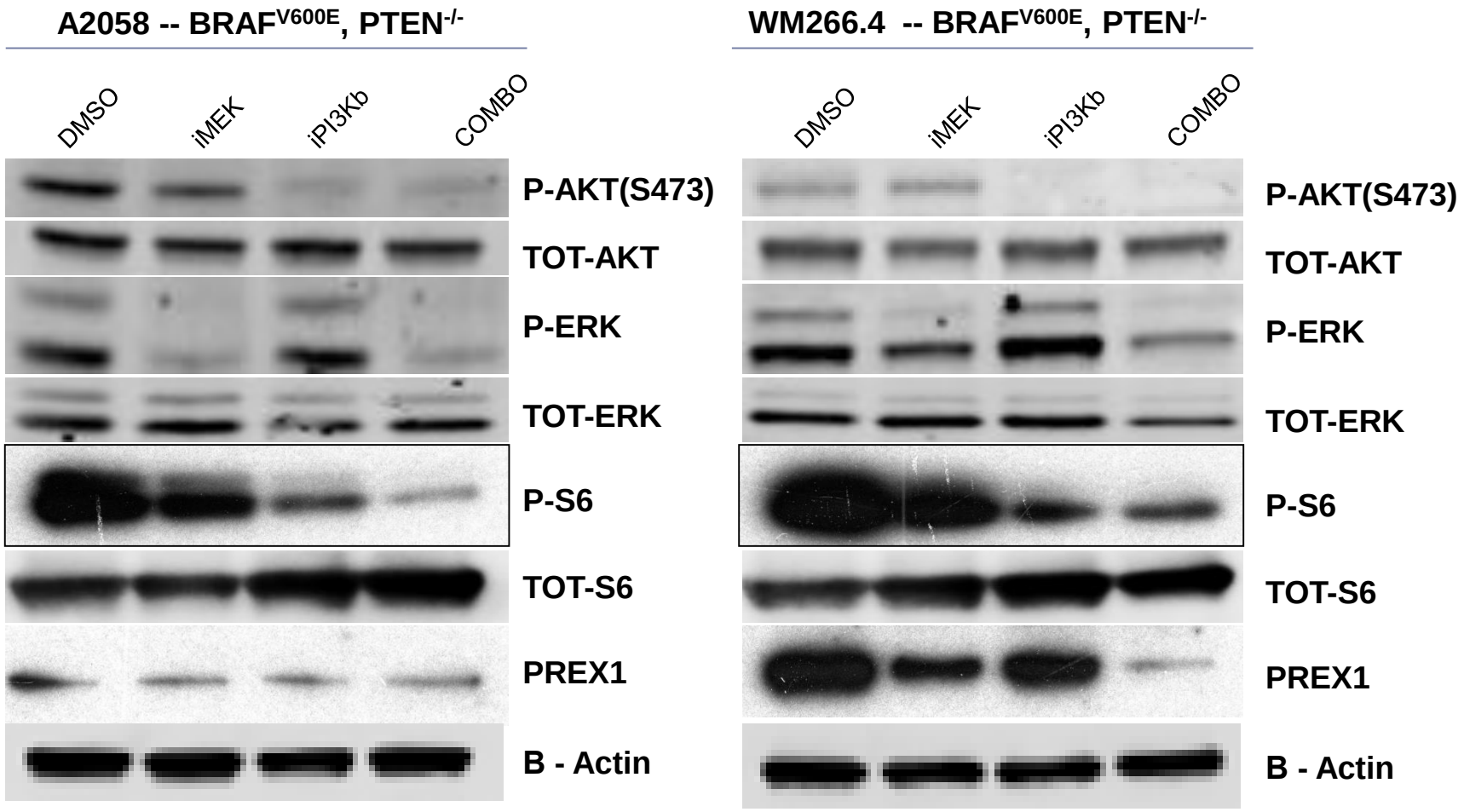
SKMel -- BRAF^{V600E}, PTEN$^{+/+}$

Combination treatment using iMEK and iPI3Kb in melanoma cell lines



Results: Combinatory treatment of PI3K and MAPK

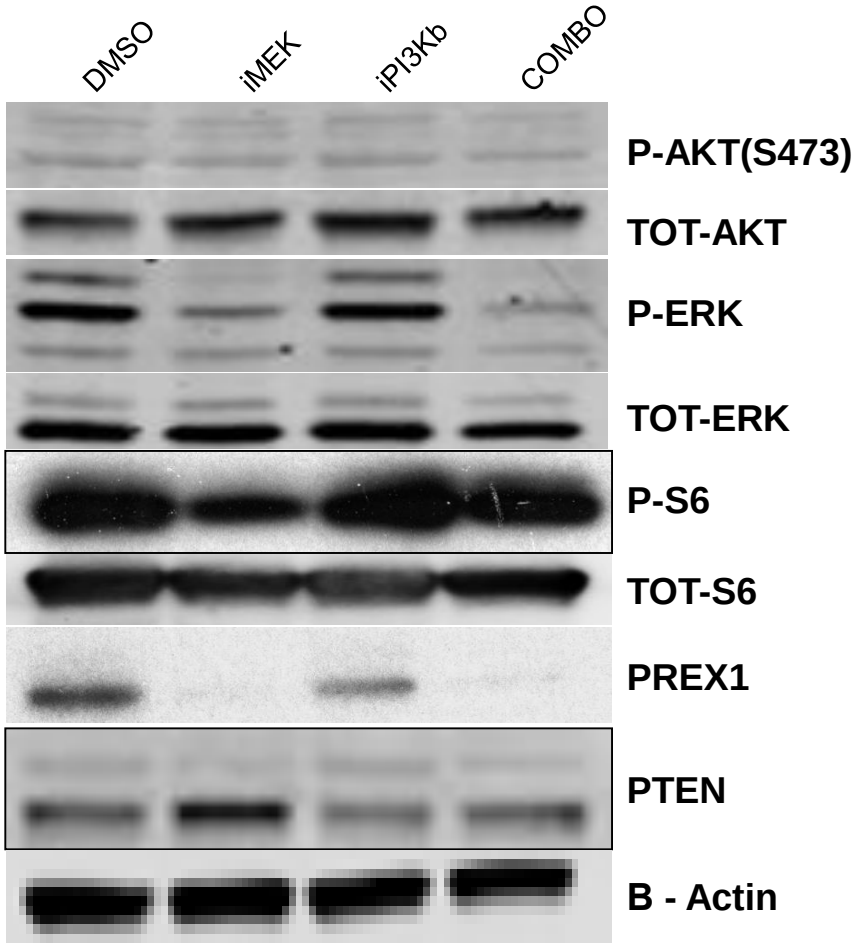
Treatment of BRAF^{V600E}, PTEN$^{-/-}$ cell lines



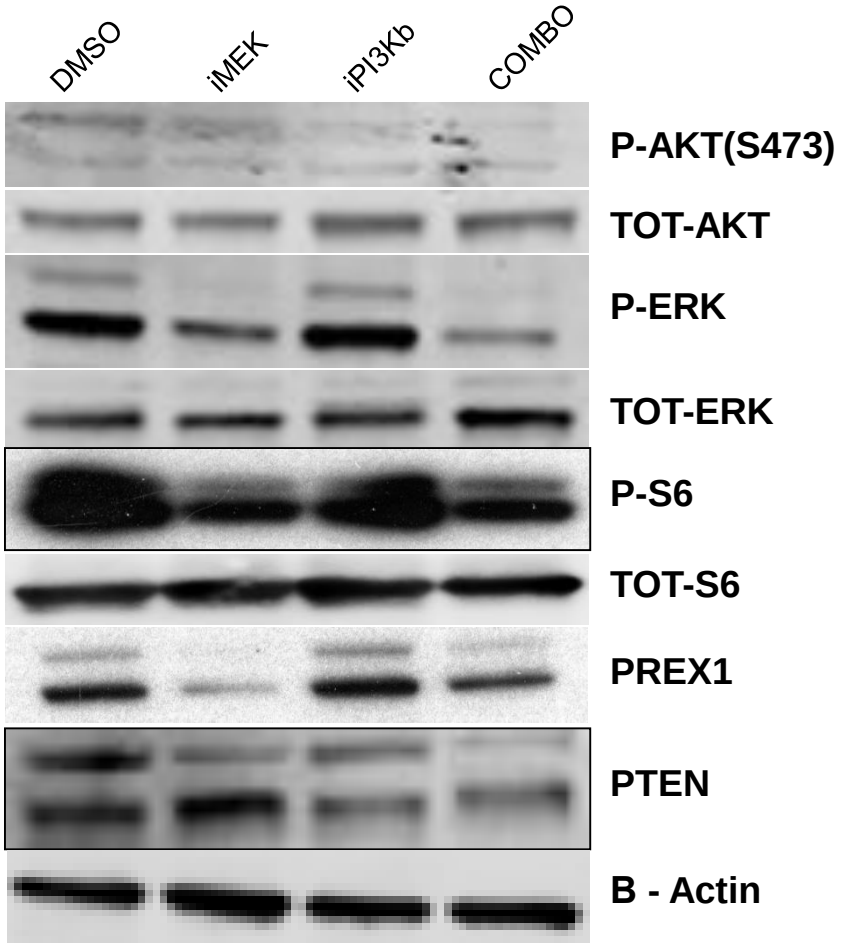
Results: Combinatory treatment of PI3K and MAPK

Treatment of $BRAF^{V600E}$, $PTEN^{+/+}$ cell lines

A375 -- $BRAF^{V600E}$, $PTEN^{+/+}$

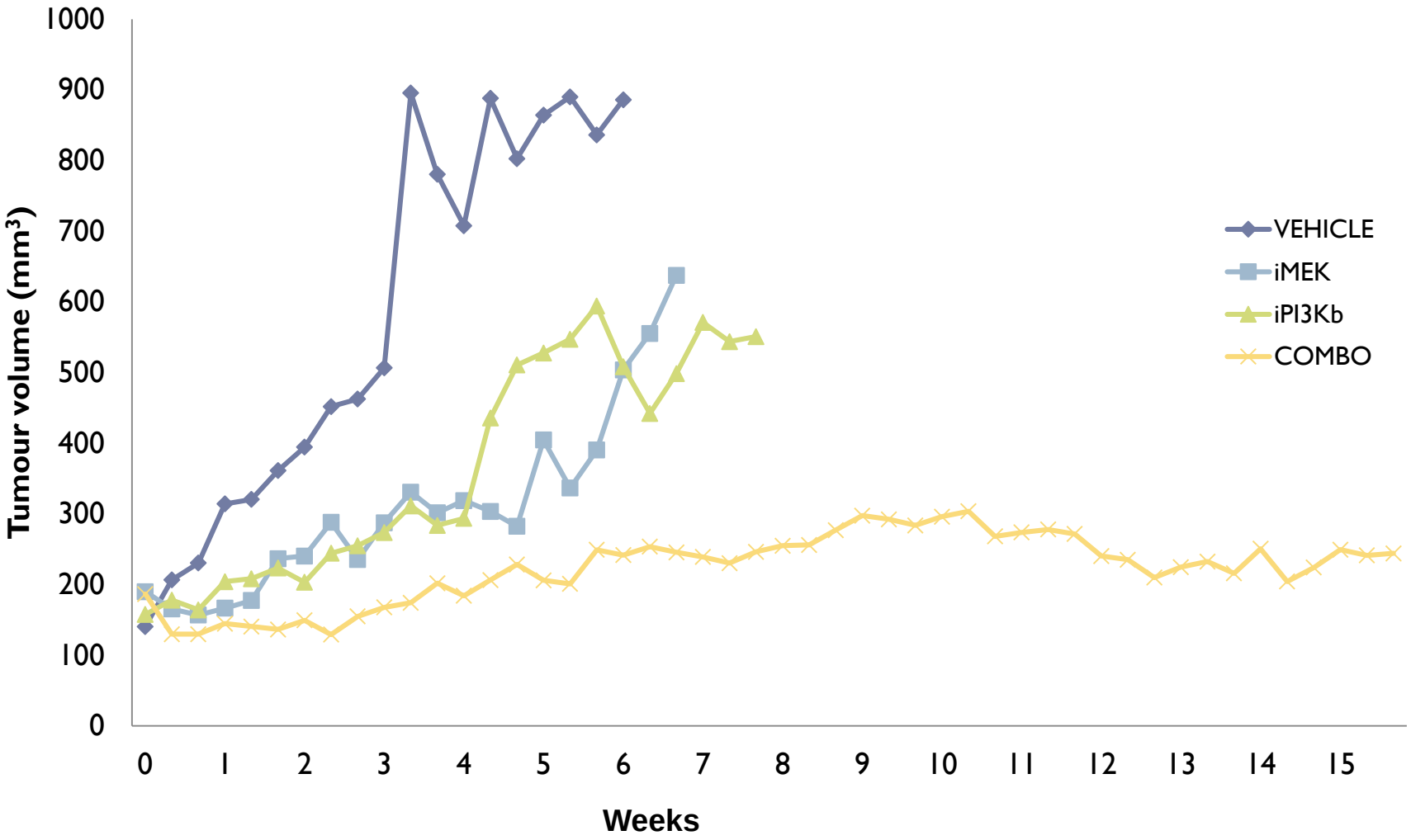


SKMel 28 -- $BRAF^{V600E}$, $PTEN^{+/+}$



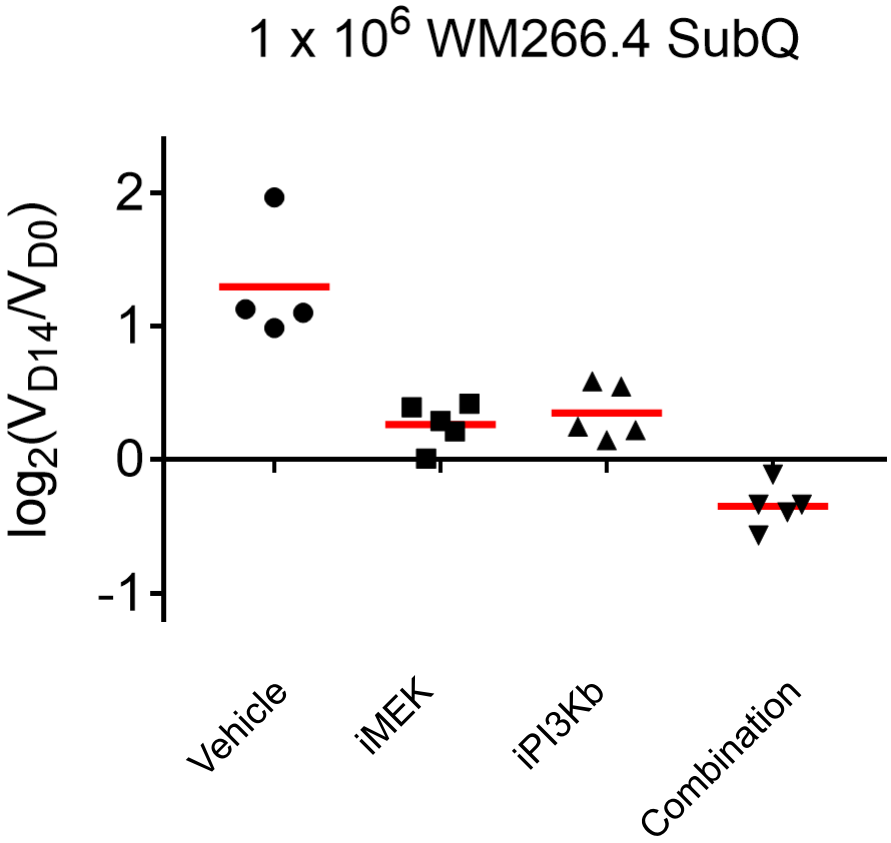
Results: Combinatory treatment of PI3K and MAPK

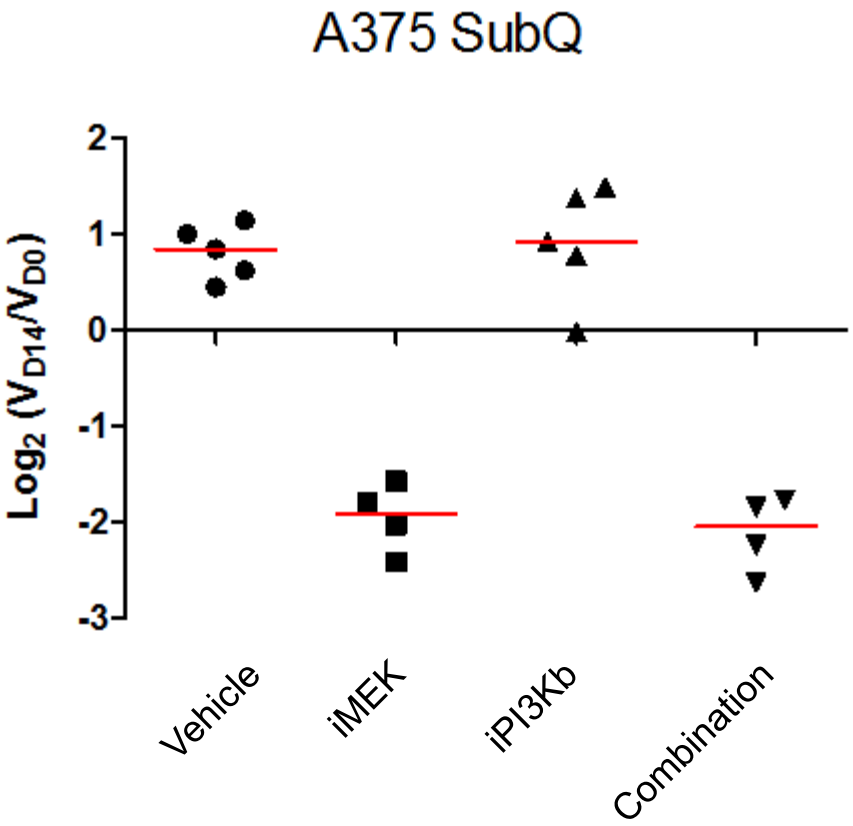
Xenograft experiments using WM266.4 human cell line



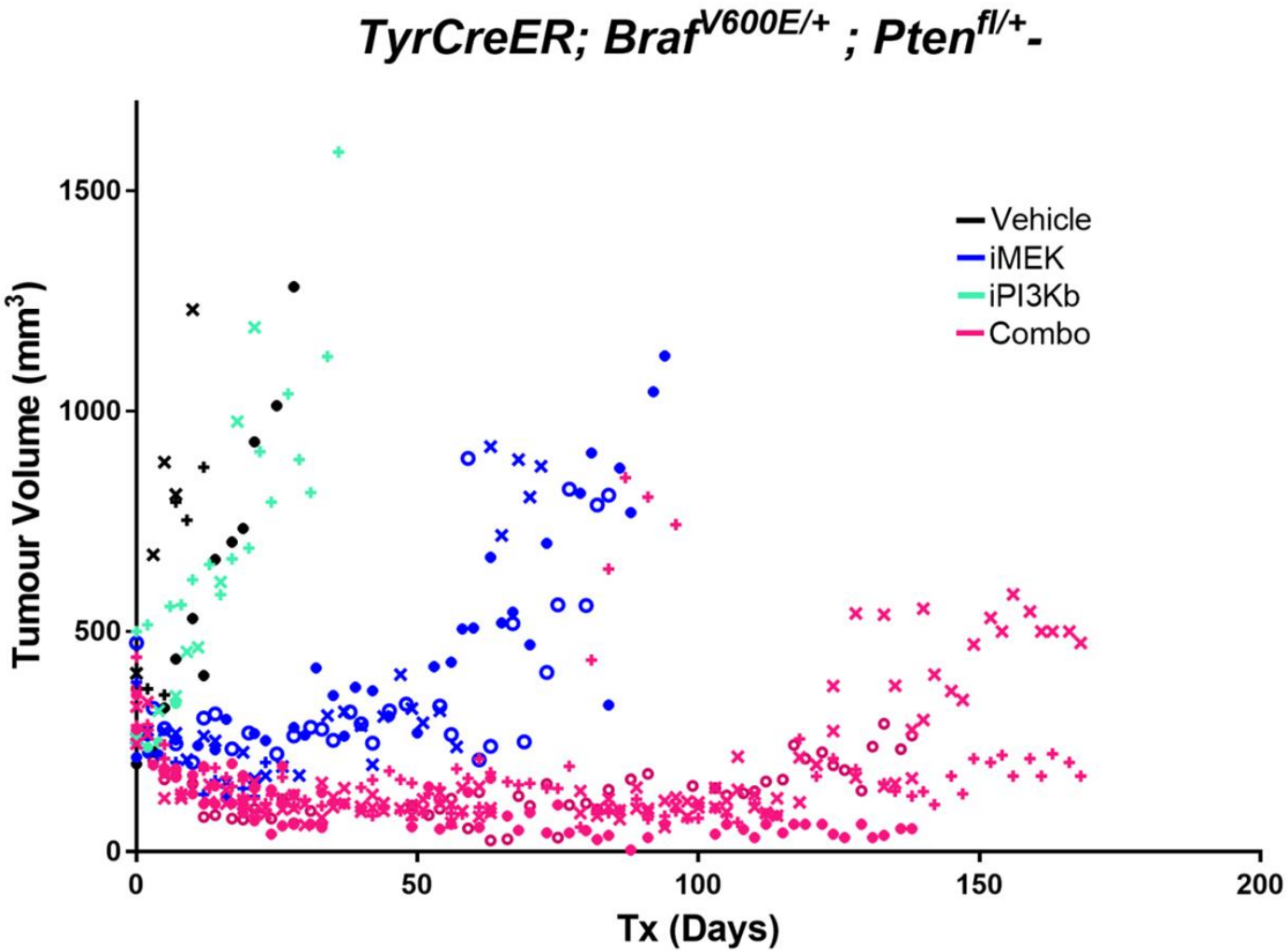
Results: Combinatory treatment of PI3K and MAPK

Xenograft experiments – tumour volume



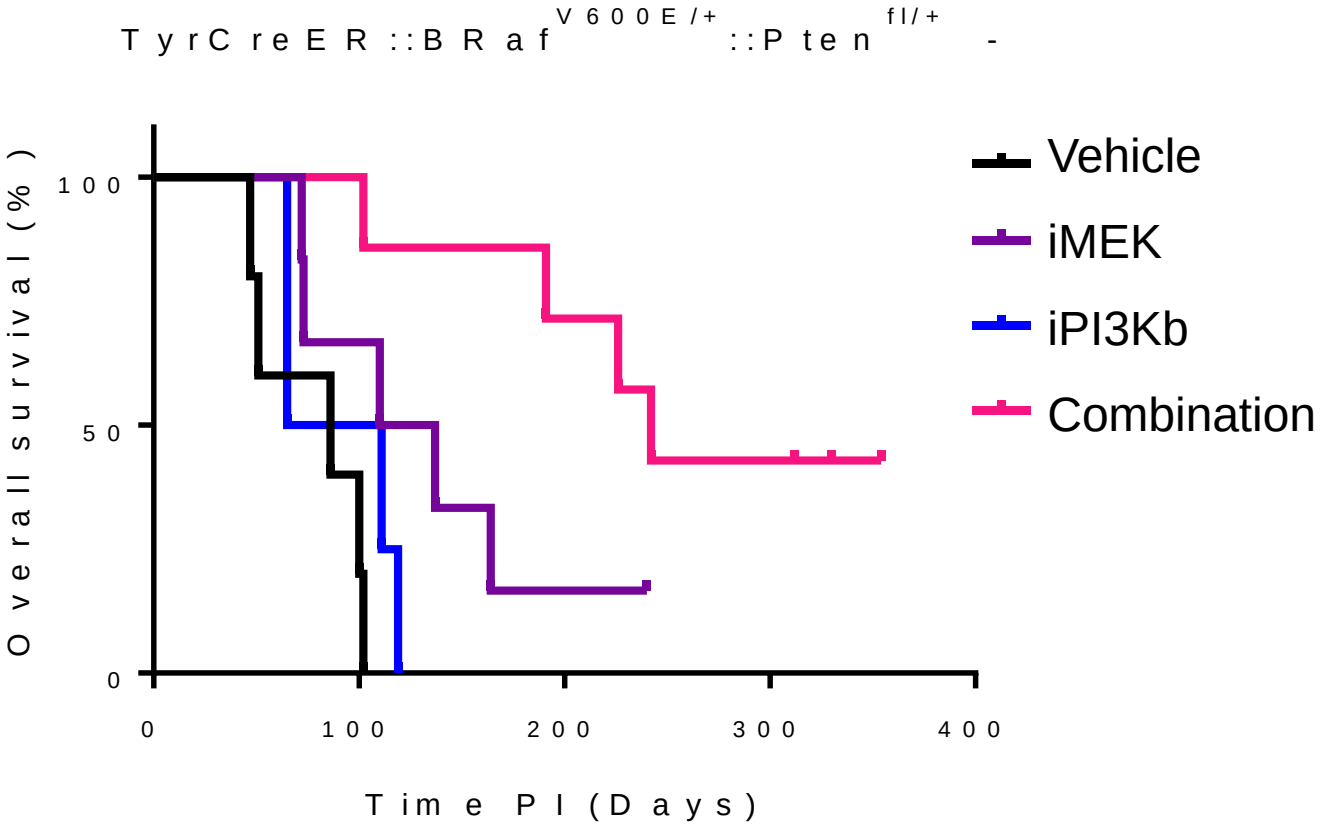


iMEK and iPI3Kb combination treatment in genetic mouse melanoma model



Results: Combinatory treatment of PI3K and MAPK

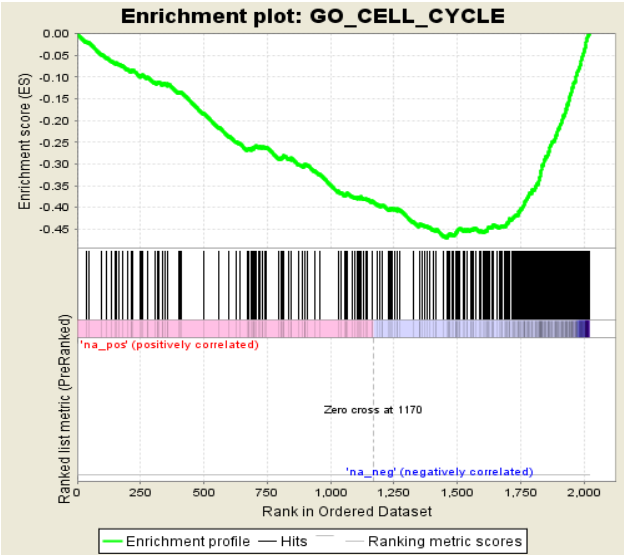
iMEK and iPI3Kb combination treatment in genetic mouse melanoma model

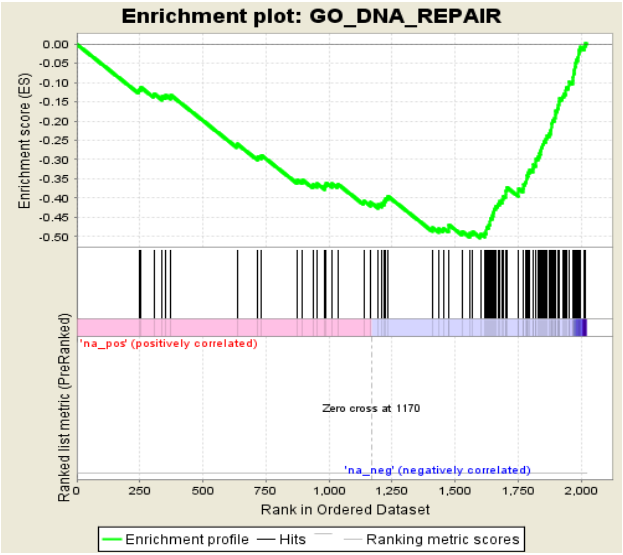


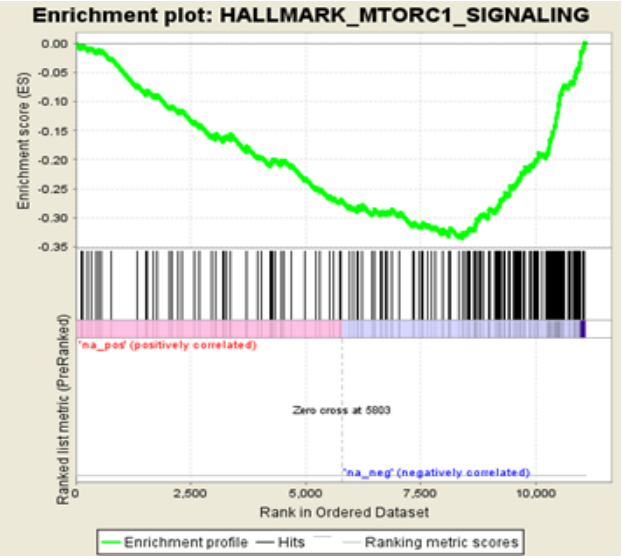
RNA sequencing

- Tumours from GEM model were treated short term (72h) and sampled for RNA sequencing

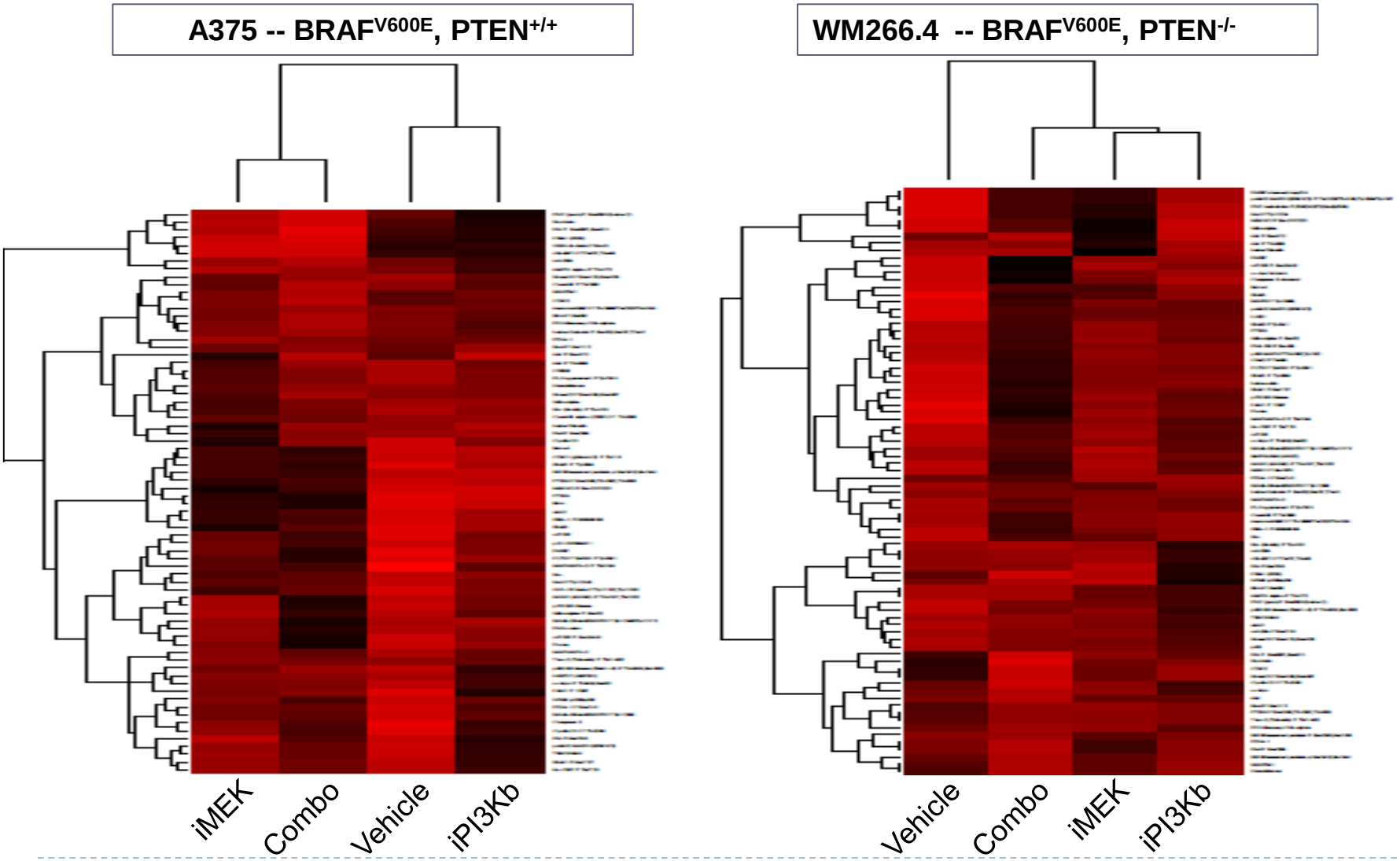
- Among significantly altered transcriptional programmes:
  - Cell cycle control
    - E2F and Myc targets, G2M checkpoint, mitotic spindle
  - DNA repair
  - Control of protein translation







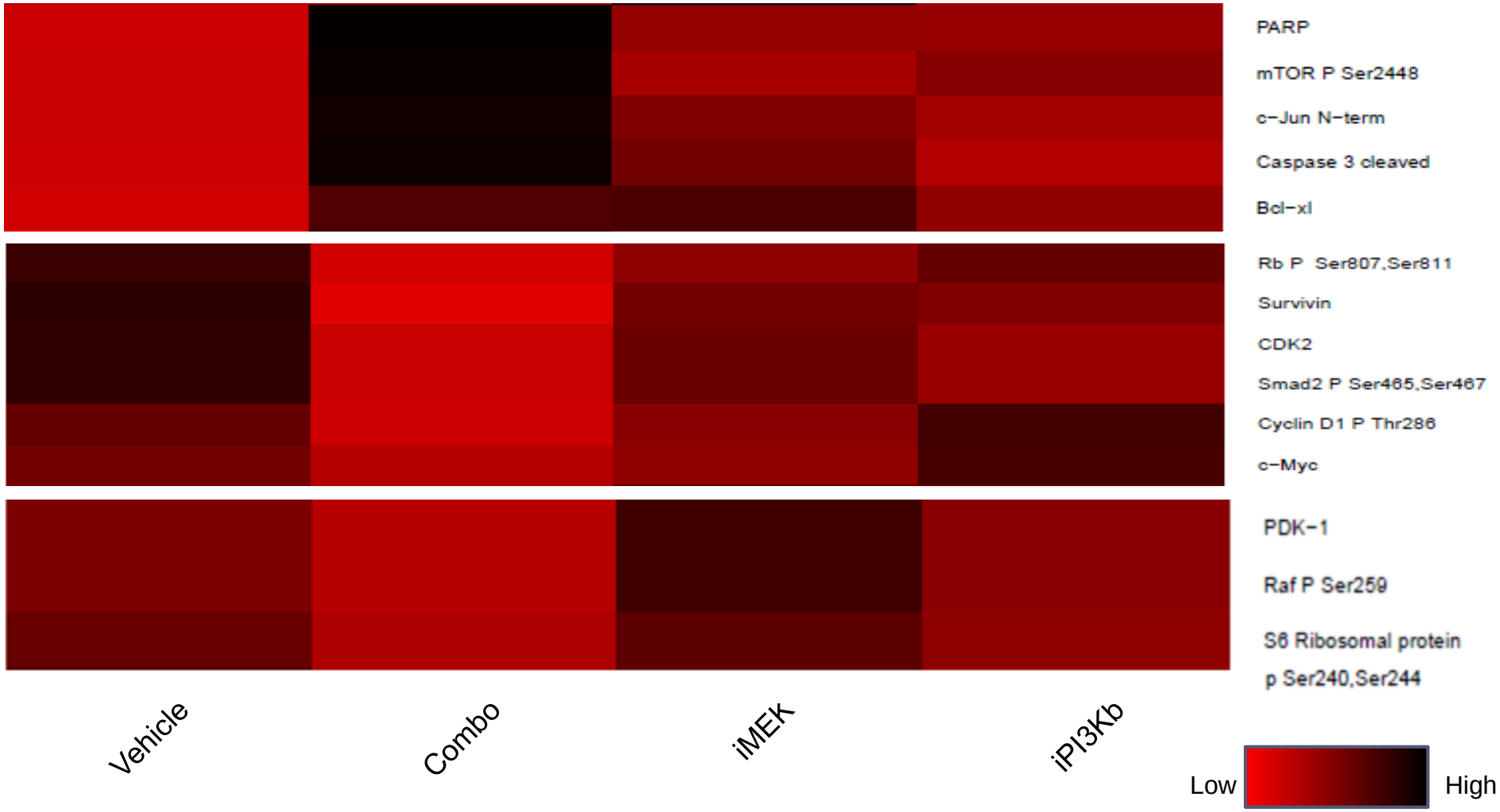
RPPA analysis - hierarchical clustering of individual treatments and combination



- Matt Neilson

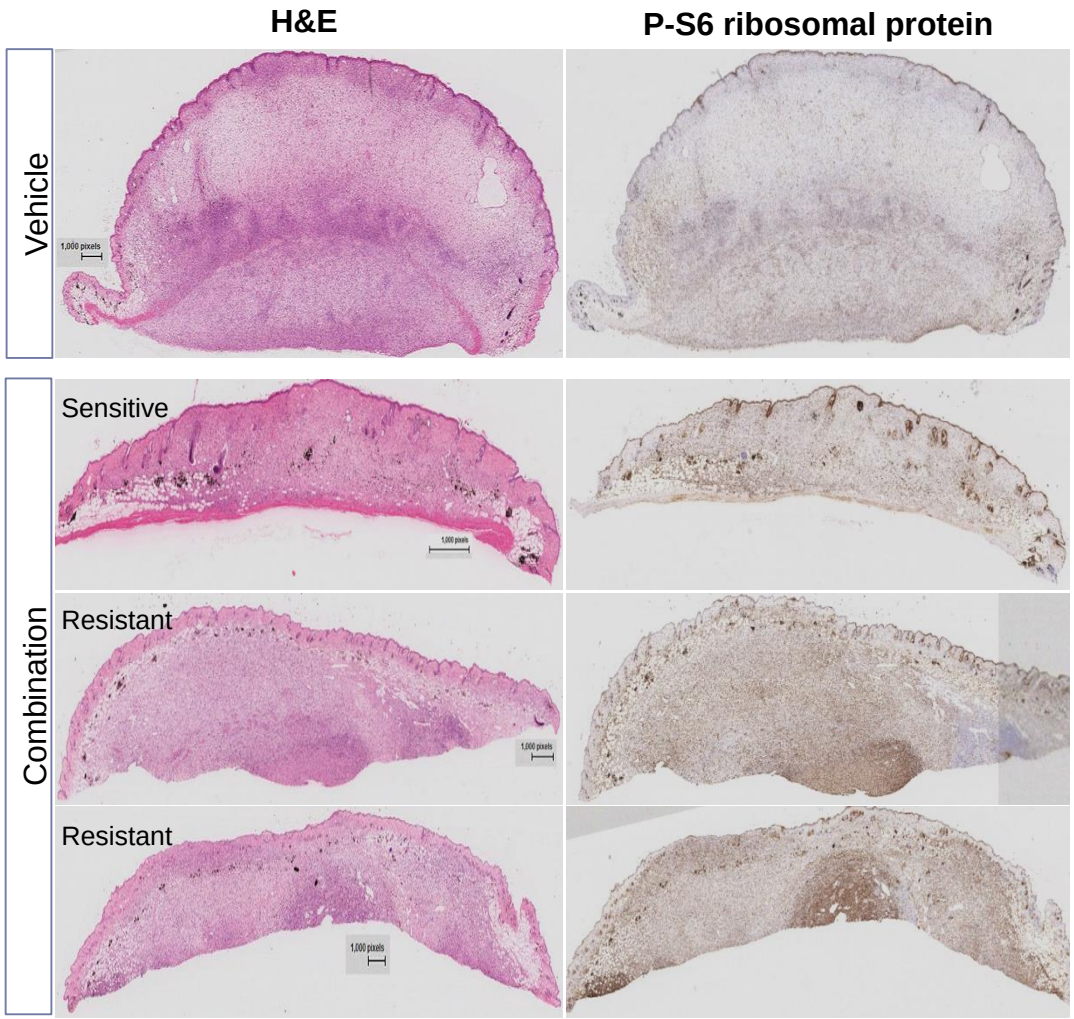
Low High

Details of RPPA analysis of the BRAF^{V600E},PTEN$^{-/-}$ cell line



- Matt Neilson

Histological analysis of tumours treated with iMEK and iPI3Kb combination treatment

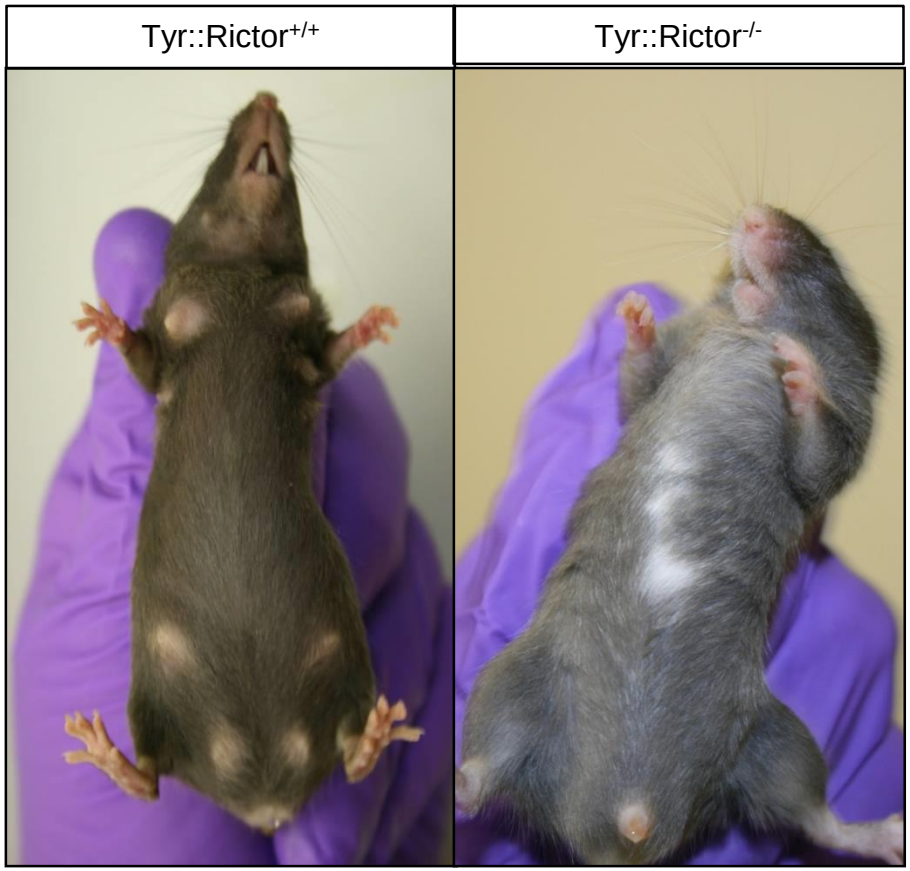


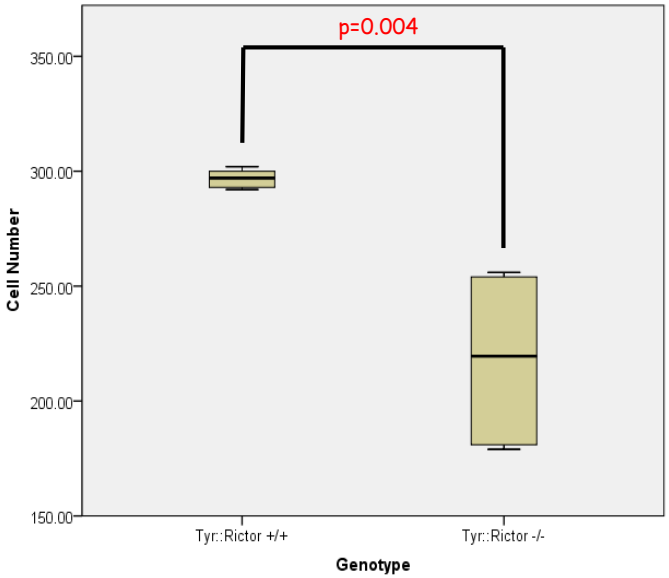
Results: Combinatory treatment of PI3K and MAPK

Future directions 1

- Combinatory treatment with PI3Kb and MEK
  - Application of the treatment within existing clinical setting, in combination with Vemurafenib and Dabrafenib
  - Generating treatment – resistant cell lines; exploring further options for resistant tumors (imTOR, Rapamycin)
  - Uveal (eye) melanoma; non – responsive to treatment (conventional or targeted), testing whether the combination treatment would be beneficial

mTORC2 subunit Rictor deletion reduced number of melanoblasts

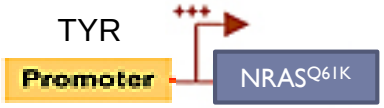




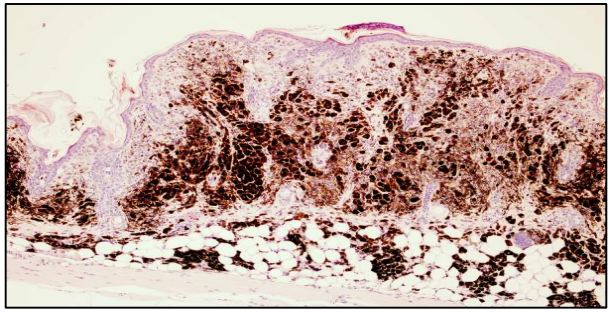
William Faller

NRAS mutant mouse model of melanoma

NRAS^{Q61K} knock-in line



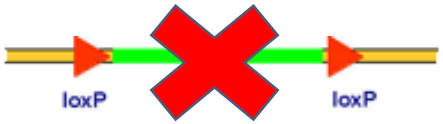
Tyr-NRAS$^{Q61K/+}$



X

INK4A-null

INK4A full body knock – out

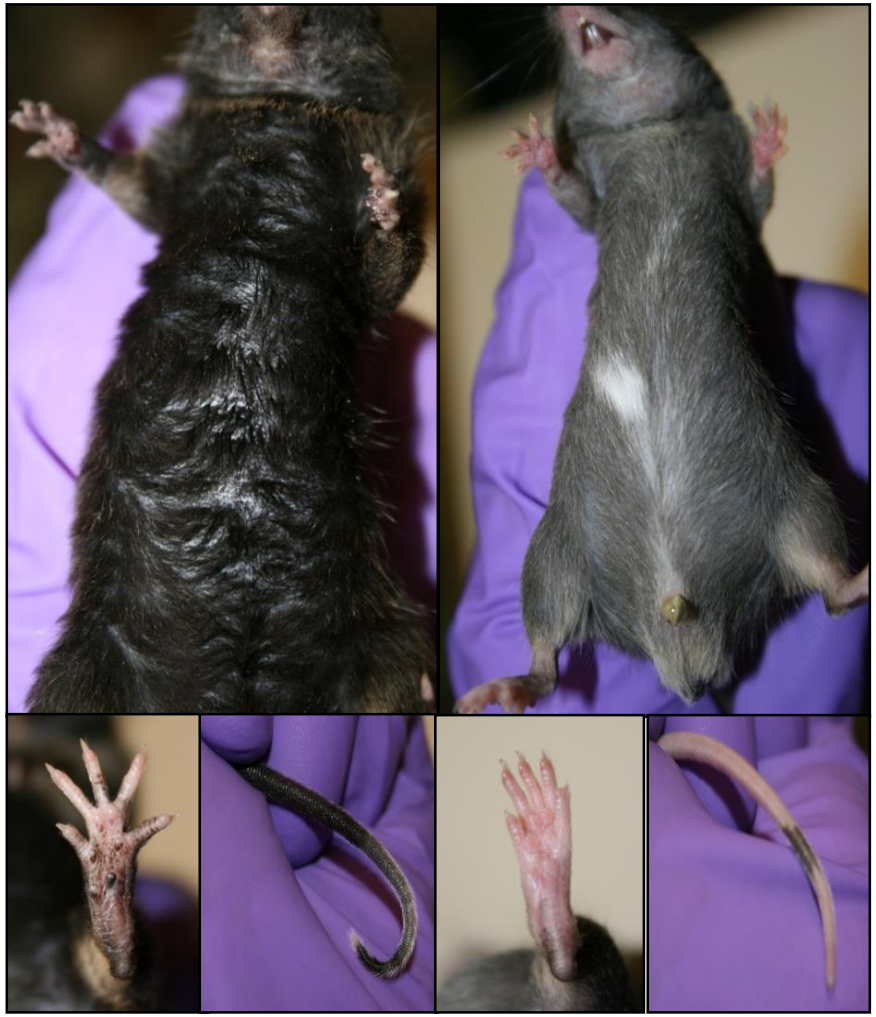


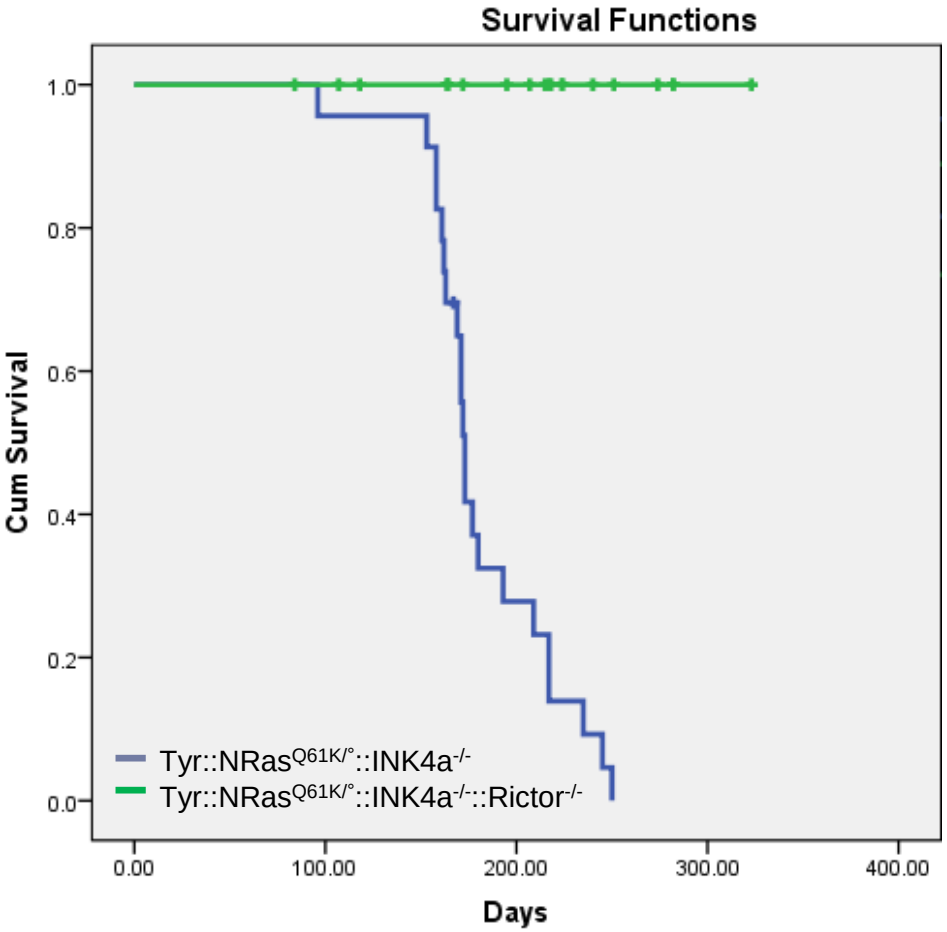
Causes lymphoma, sarcoma

Tyr-NRAS$^{Q61K/+}$ ::INK4A$^{-/-}$

Rictor deletion rescues the NRAS mutant phenotype

Tyr::NRas$^{Q61K/°}$; INK4a$^{-/-}$ Tyr::NRas$^{Q1K/°}$; INK4a$^{-/-}$; Rictor$^{-/-}$

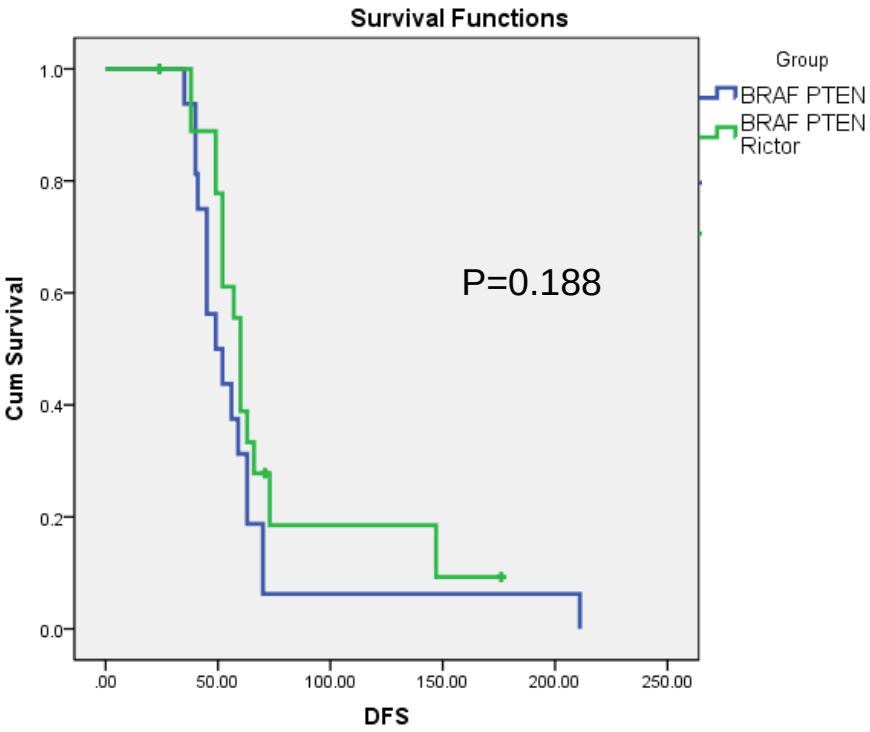




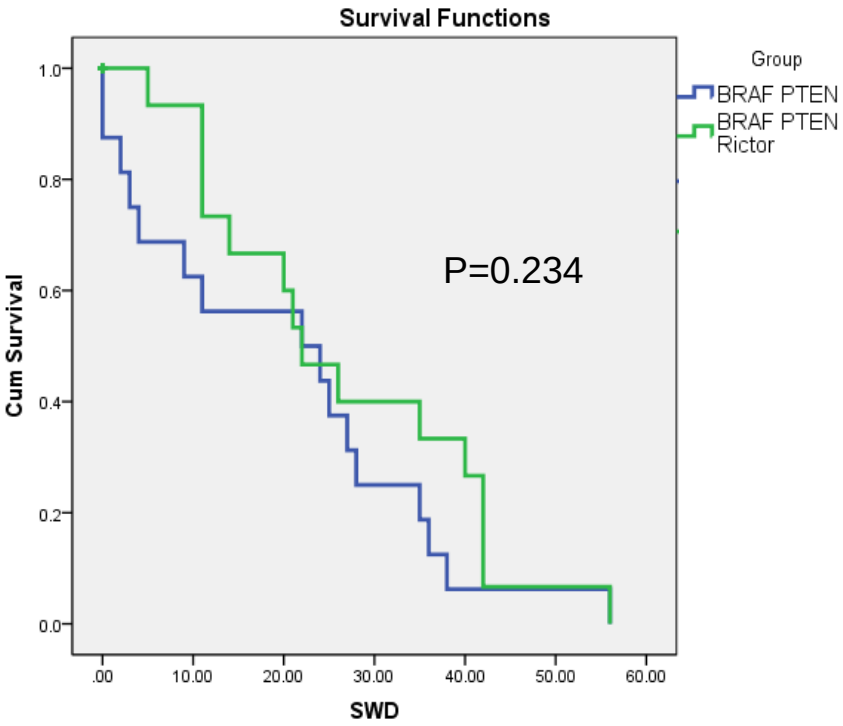
William Faller

Rictor deletion has no effect in $BRAF^{V600E}$::$PTEN^{+/-}$ mediated melanoma

Time from induction to melanoma appearance



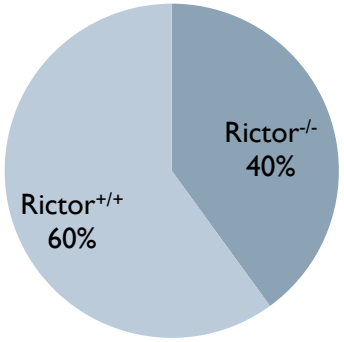
Time from melanoma appearance to death



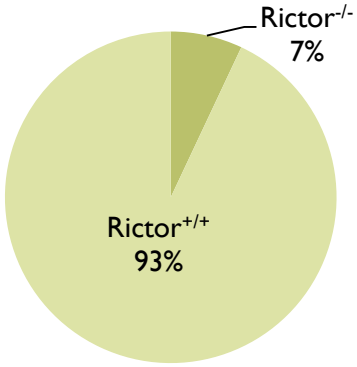
William Faller

Rictor deletion in human melanoma cell lines

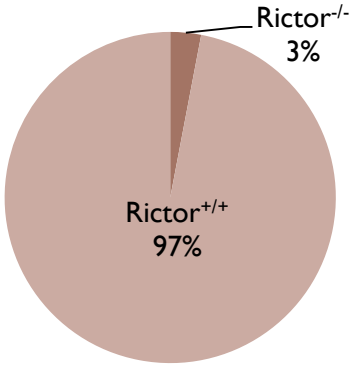
BRAF^{V600E}::PTEN$^{-/-}$

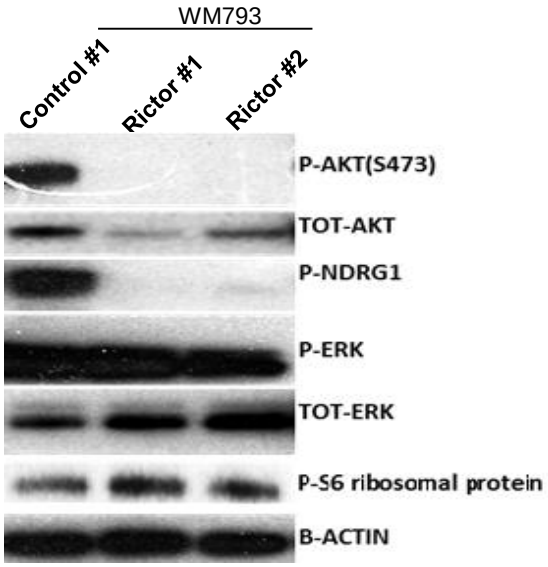


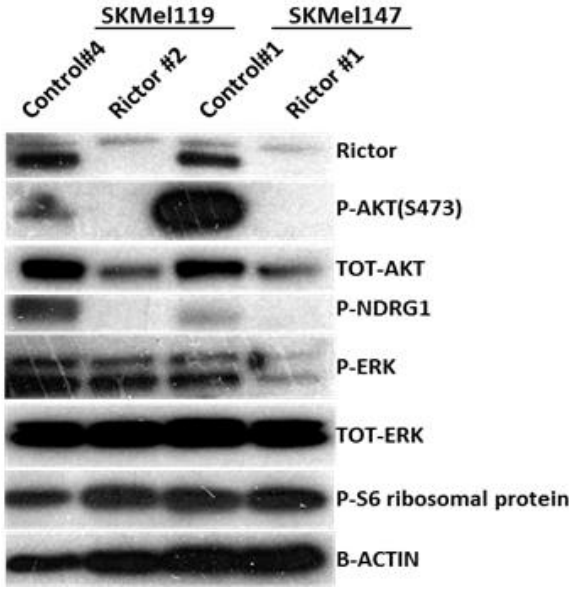
NRAS^{Q61K}::PTEN$^{+/+}$



NRAS^{Q61K}::PTEN$^{-/-}$



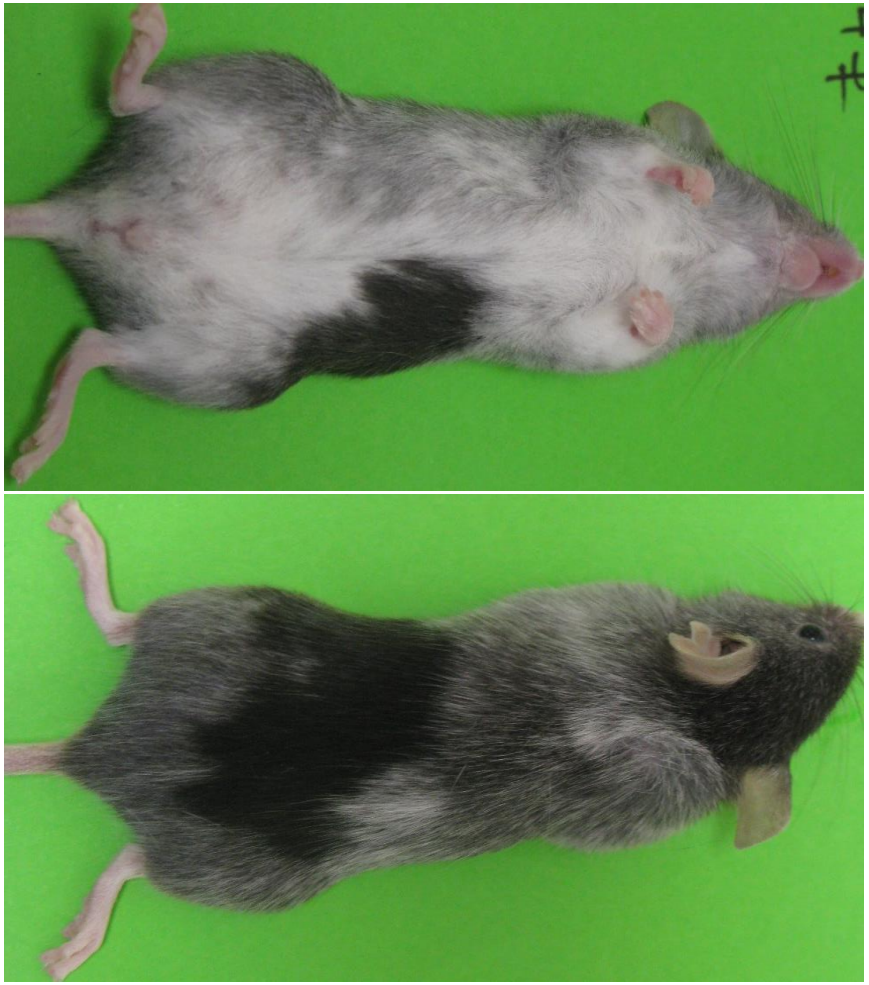




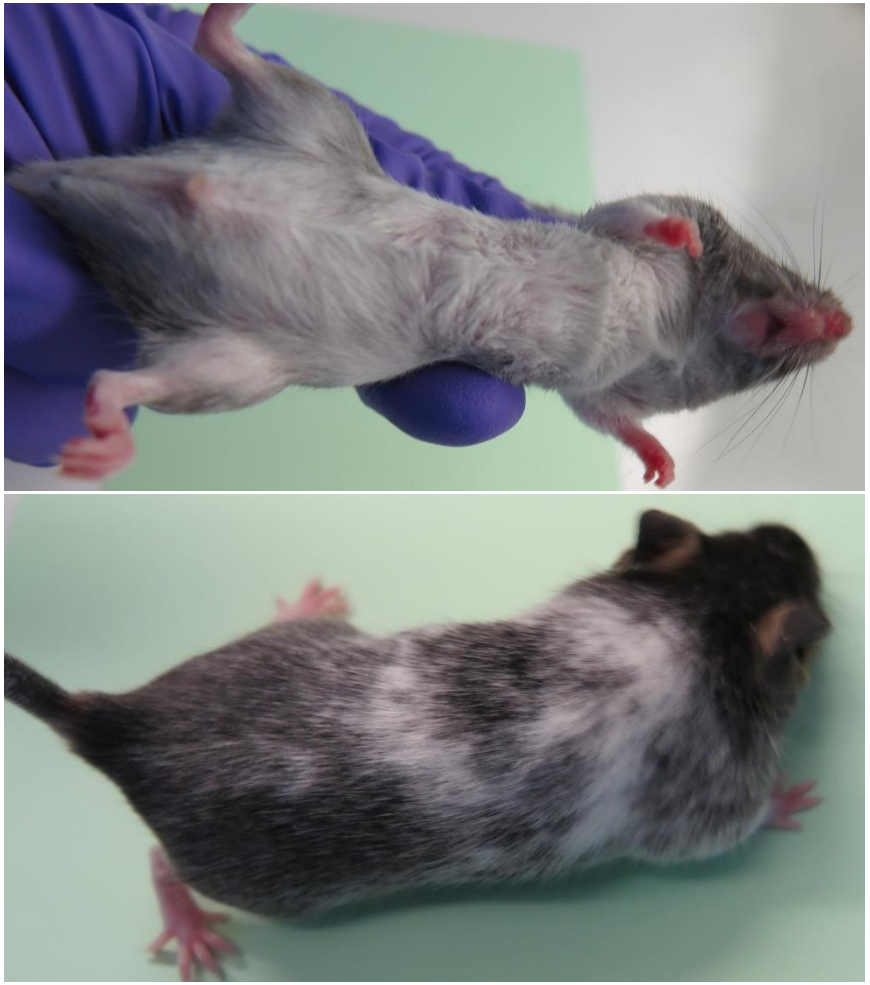
Results: Role of mTORC2/Rictor

Triple deletion of Rictor, PREX1 and PREX2

TyrCre::Rictor$^{-/-}$::PREX1$^{-/-}$::PREX2$^{-/+}$



TyrCre::Rictor$^{-/-}$::PREX1$^{-/-}$::PREX2$^{-/-}$

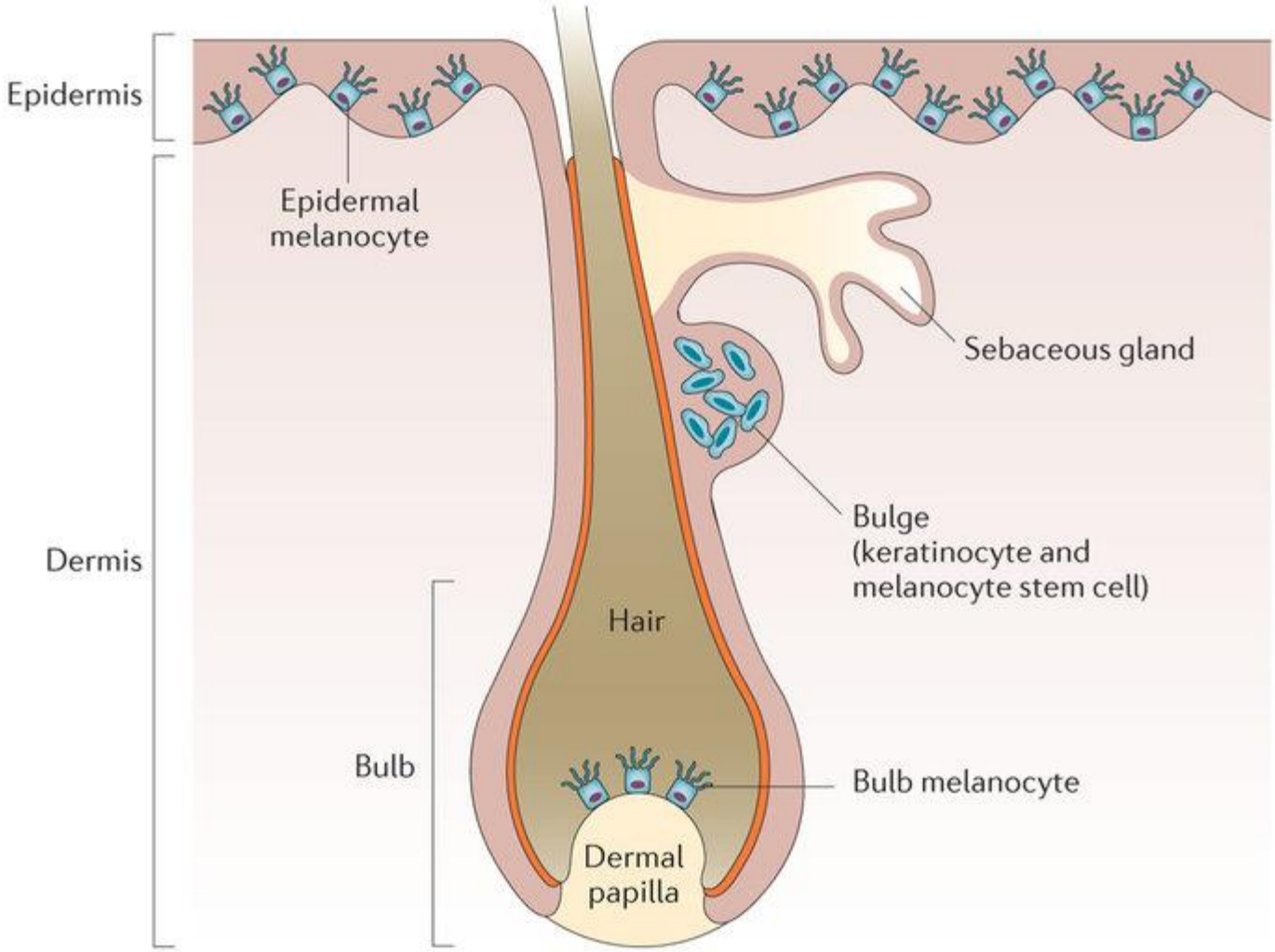


Future directions 2

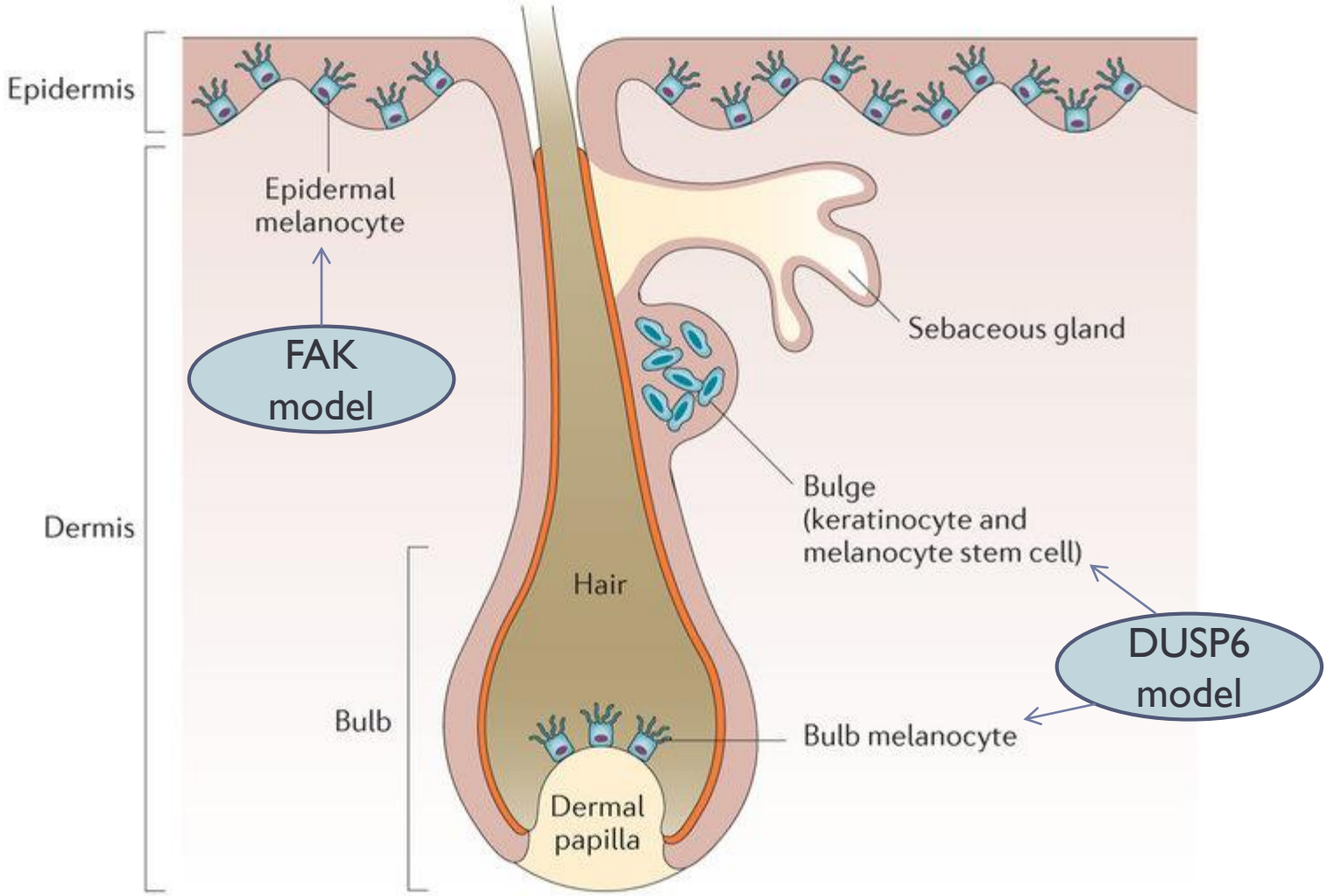
- Role of Rictor in melanoma
  - Investigation of mTORC2 regulation in NRAS and BRAF melanoma
  - Further elucidation of mTORC2 and PREX signaling cross-regulation

Study of melanoma development

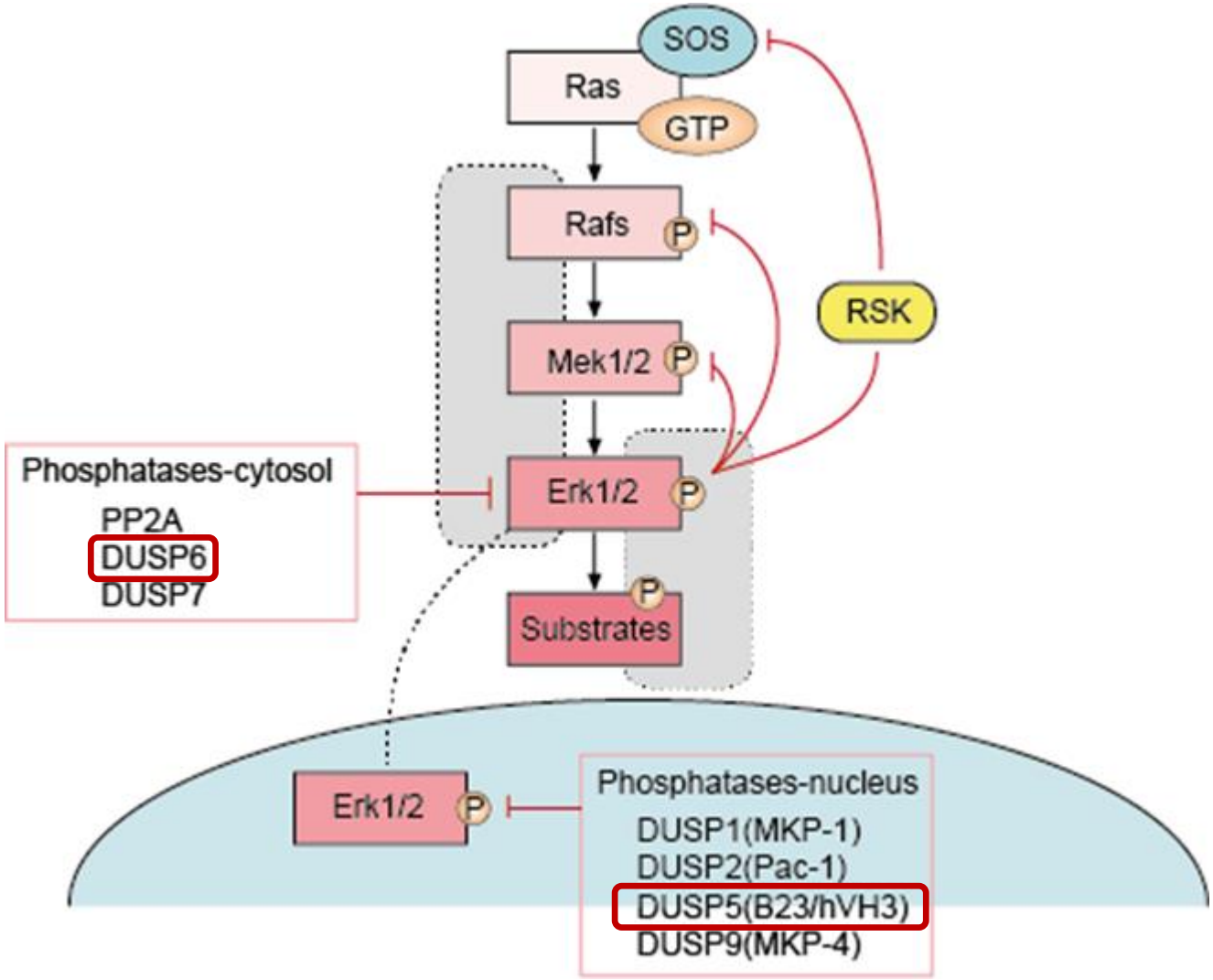
Cell of origin of melanoma



Cell of origin of melanoma



Role of DUSP proteins in MAPK signaling



Extensive greying in Tyr-NRAS^{Q61K}::DUSP6

Tyr::DUSP6$^{+/+}$

Tyr::N-Ras^{Q61K} DUSP6$^{+/-}$

Tyr::N-Ras^{Q61K} DUSP6$^{-/-}$

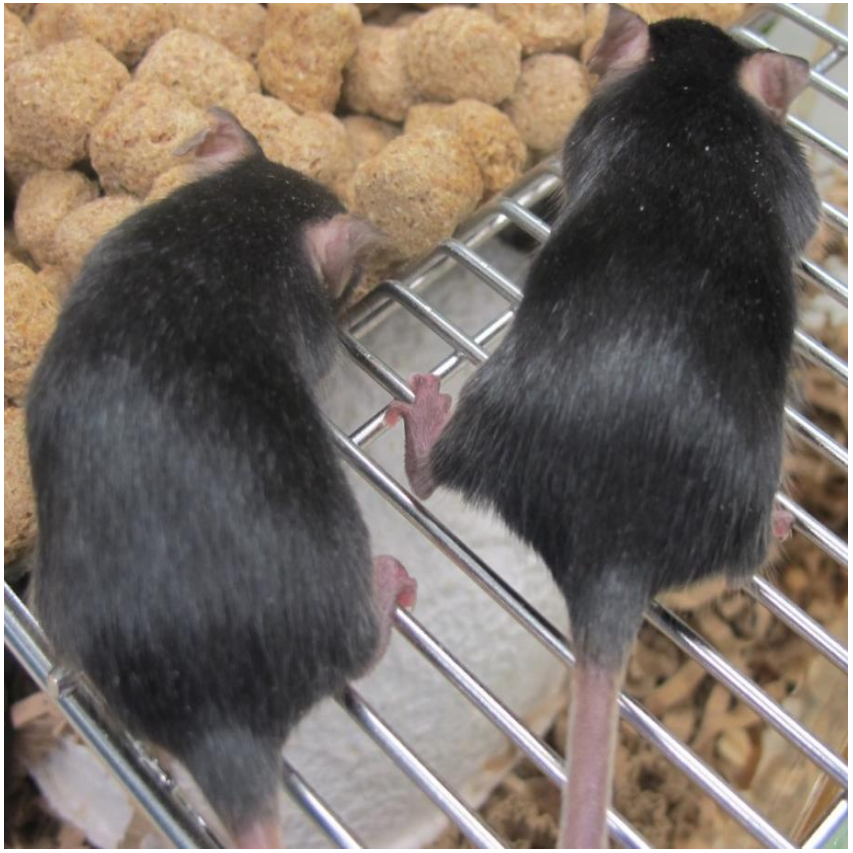
Tyr::N-Ras^{Q61K} DUSP6$^{+/+}$

Tyr::N-Ras^{Q61K} DUSP6$^{-/-}$

DUSP6 has no effect on Tyr-NRAS^{WT}

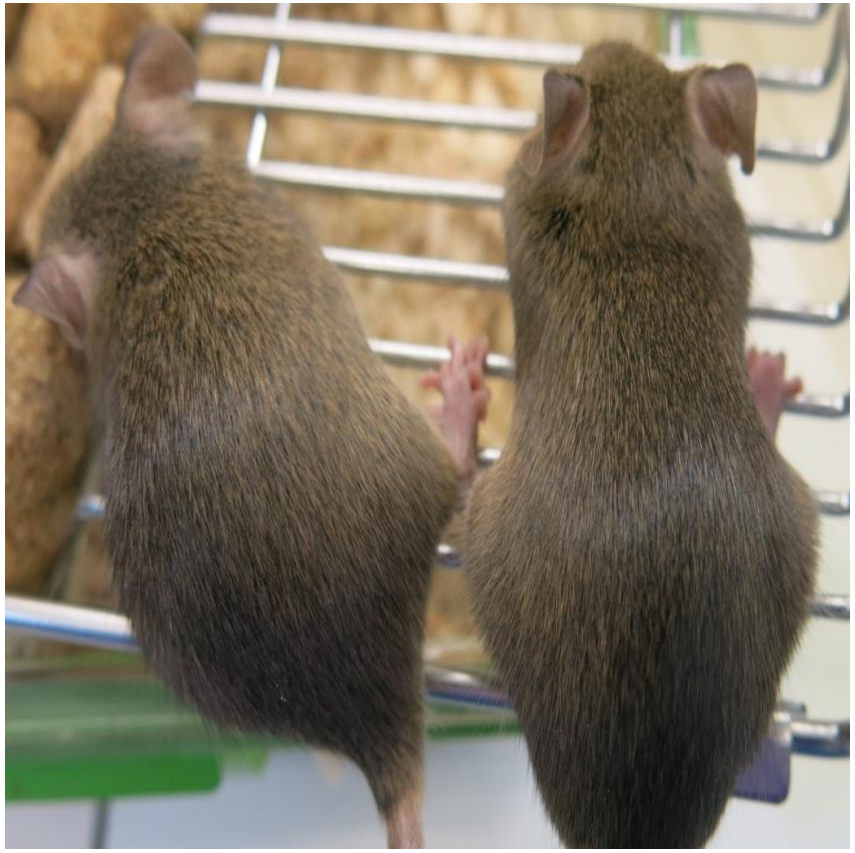
Tyr::N-Ras^{WT} ::DUSP6$^{+/+}$

Tyr::N-Ras^{WT} ::DUSP6$^{-/-}$

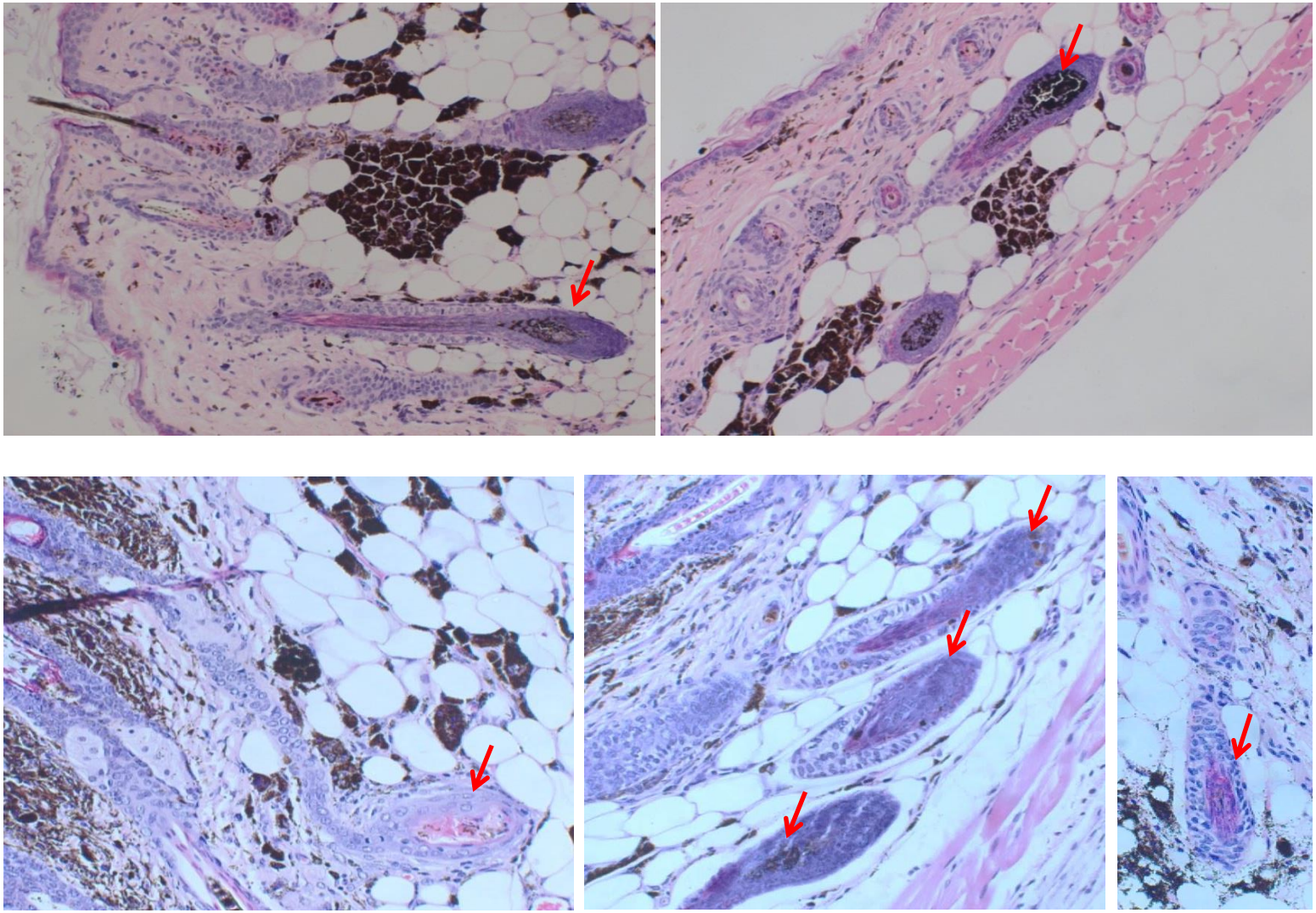


Tyr::N-Ras^{WT} ::DUSP6$^{+/+}$

Tyr::N-Ras^{WT} ::DUSP6$^{-/-}$



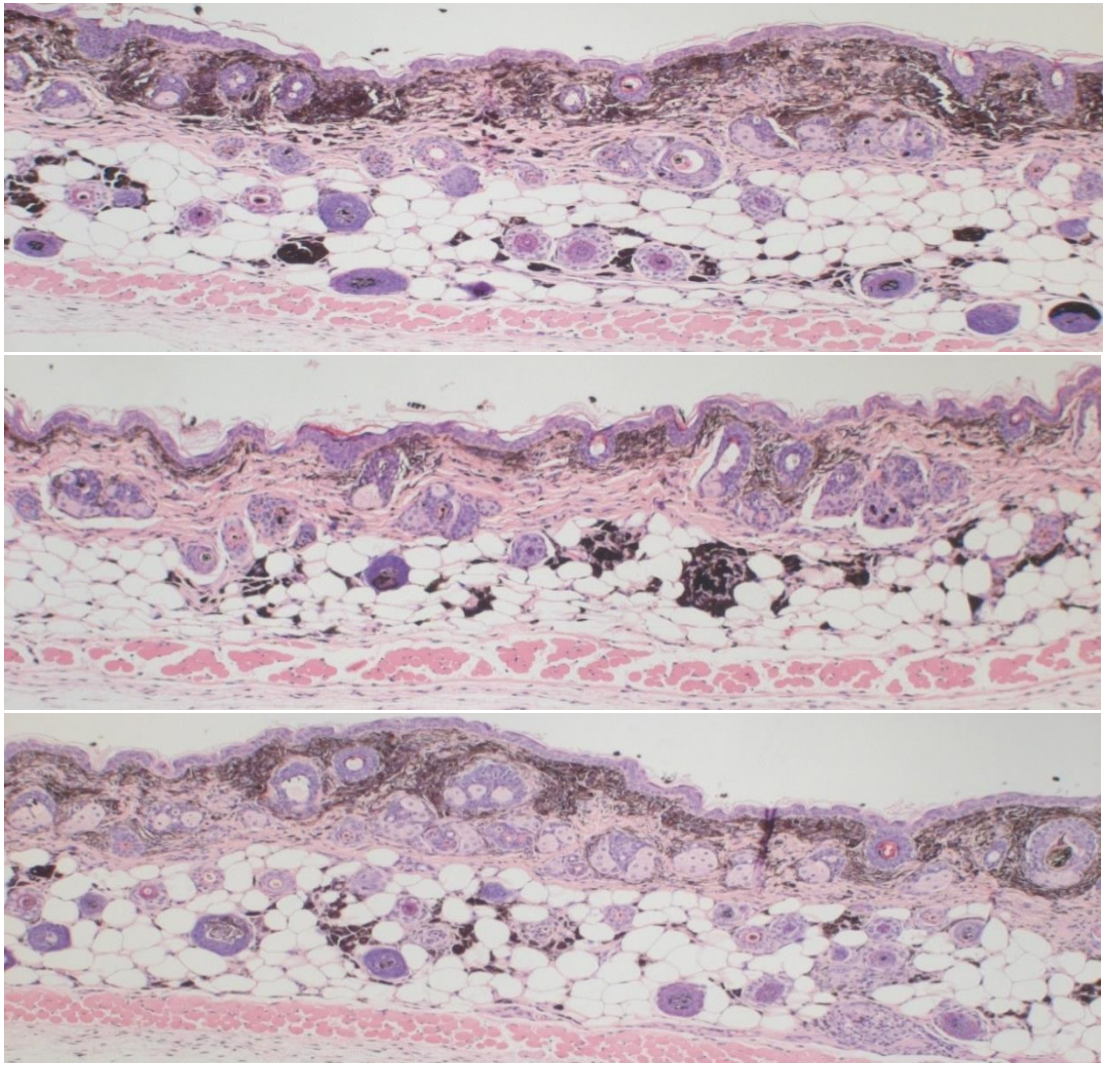
Melanocytes absent form Tyr::NRAS^{Q61K}::DUSP6$^{-/-}$ hair follicles



Tyr::N-Ras^{Q61K} DUSP6$^{+/+}$

Tyr::N-Ras^{Q61K} DUSP6$^{-/-}$

Dermal melanocyte proliferation in Tyr-NRAS^{Q61K} not regulated by DUSP6



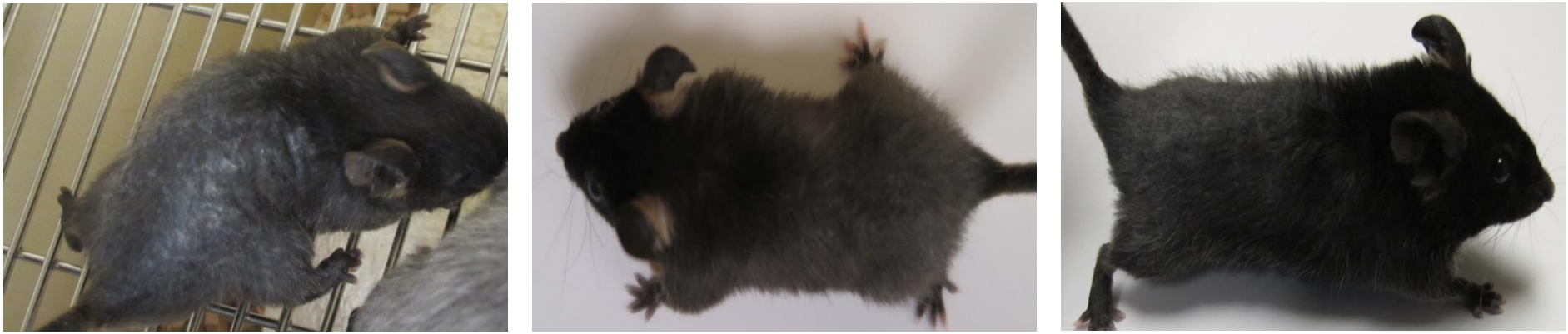
Tyr::N-Ras^{Q61K} ::DUSP6$^{+/+}$

Tyr::N-Ras^{Q61K} ::DUSP6$^{+/-}$

Tyr::N-Ras^{Q61K} ::DUSP6$^{-/-}$

MEK inhibition rescues the Tyr-NRAS^{Q61K}::DUSP6$^{-/-}$ phenotype

Before treatment | Treated for 1 month | Treated for 2 months



Tyr::N-Ras^{Q61K}::DUSP6$^{+/+}$

Tyr::N-Ras^{Q61K} ::DUSP6$^{-/-}$

Rescue of the Tyr-NRAS^{Q61K}::DUSP6$^{-/-}$ phenotype is transient

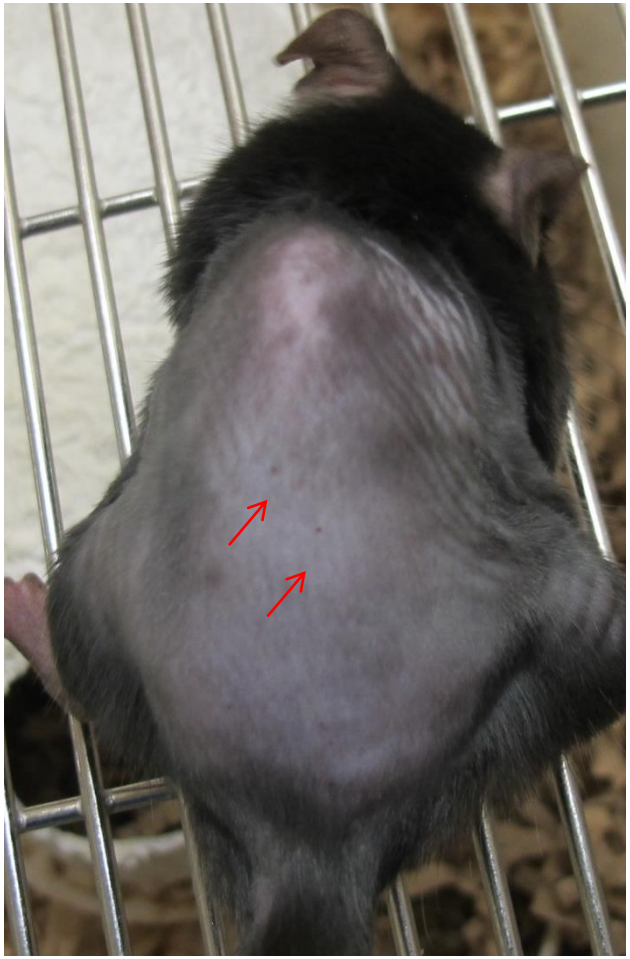
Treated for 1 month

Treated for 2 months

Off treatment for 2 months

DUSP6 deletion in BRAF^{V600E}::PTEN$^{-/+}$ melanoma model

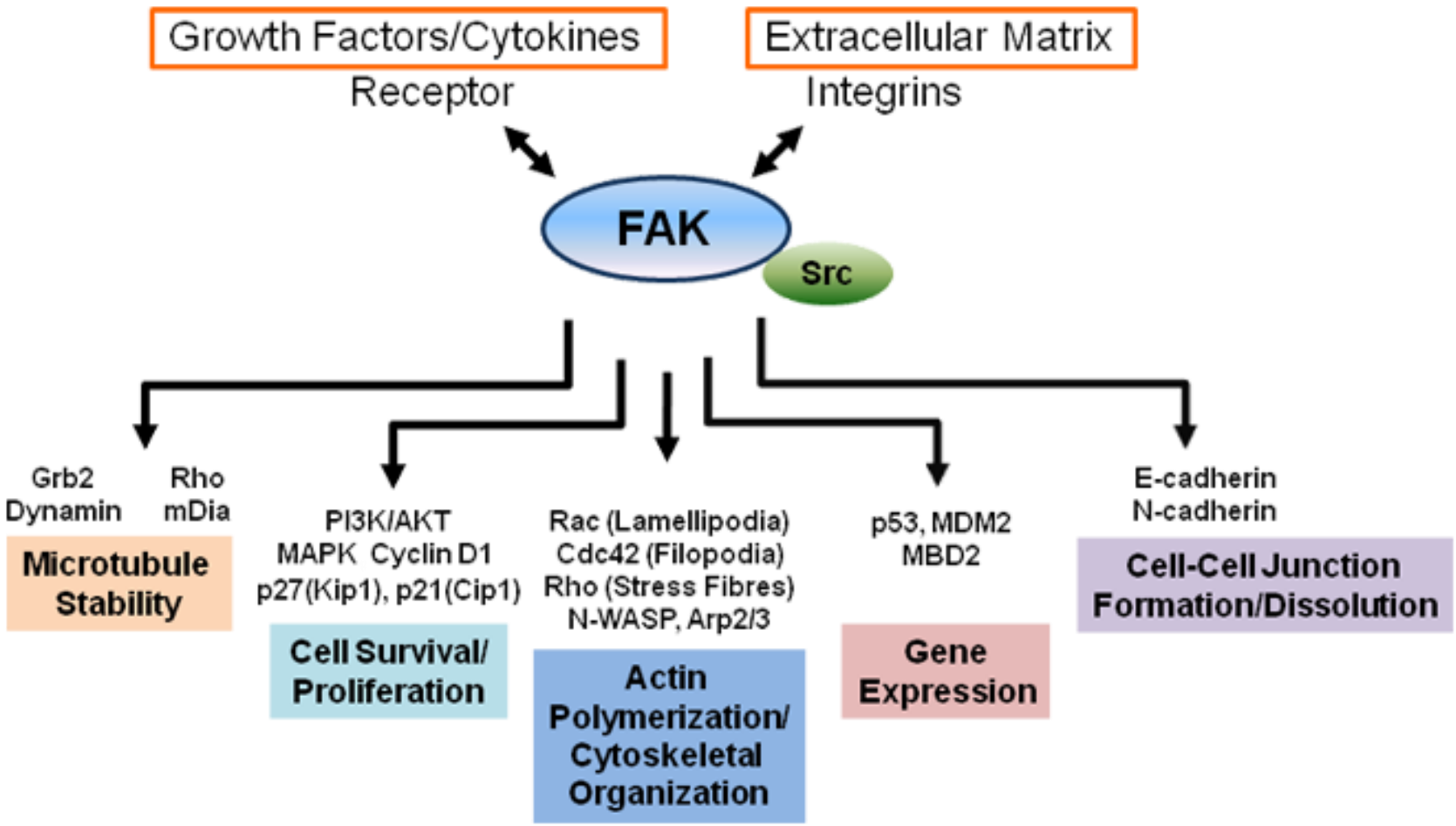
BRAF$^{V600E/+}$::PTEN$^{FL/+}$::DUSP6$^{+/+}$



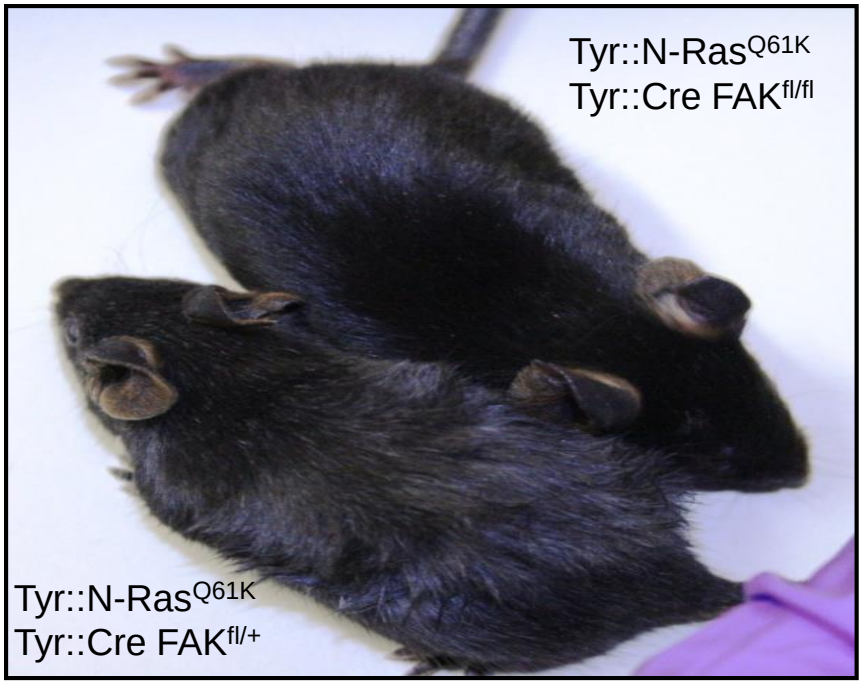
BRAF$^{V600E/+}$::PTEN$^{FL/+}$::DUSP6$^{FL/+}$

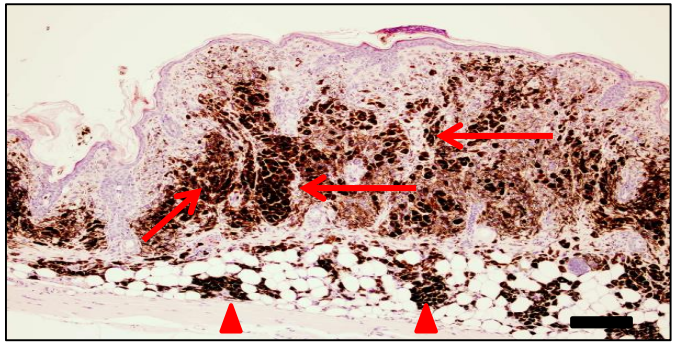
BRAF$^{V600E/+}$::PTEN$^{FL/+}$::DUSP6$^{FL/FL}$

Role of focal adhesion kinase – FAK in melanoma

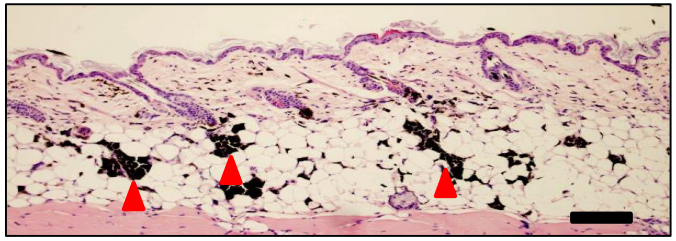


FAK deletion reduces dermal melanocyte proliferation in NRAS mutant mice

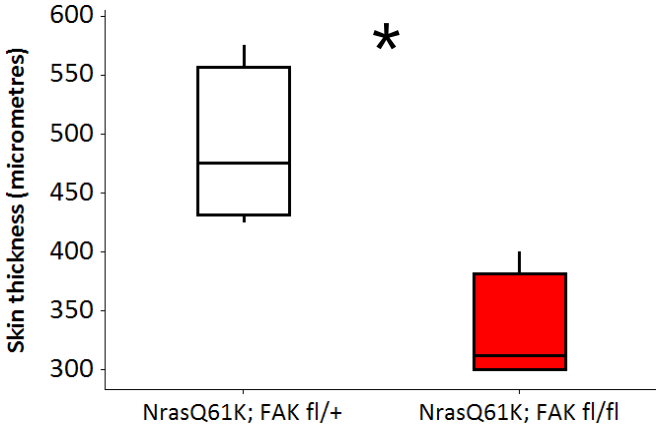




Tyr::N-Ras^{Q61K}
Tyr::Cre FAK$^{fl/+}$

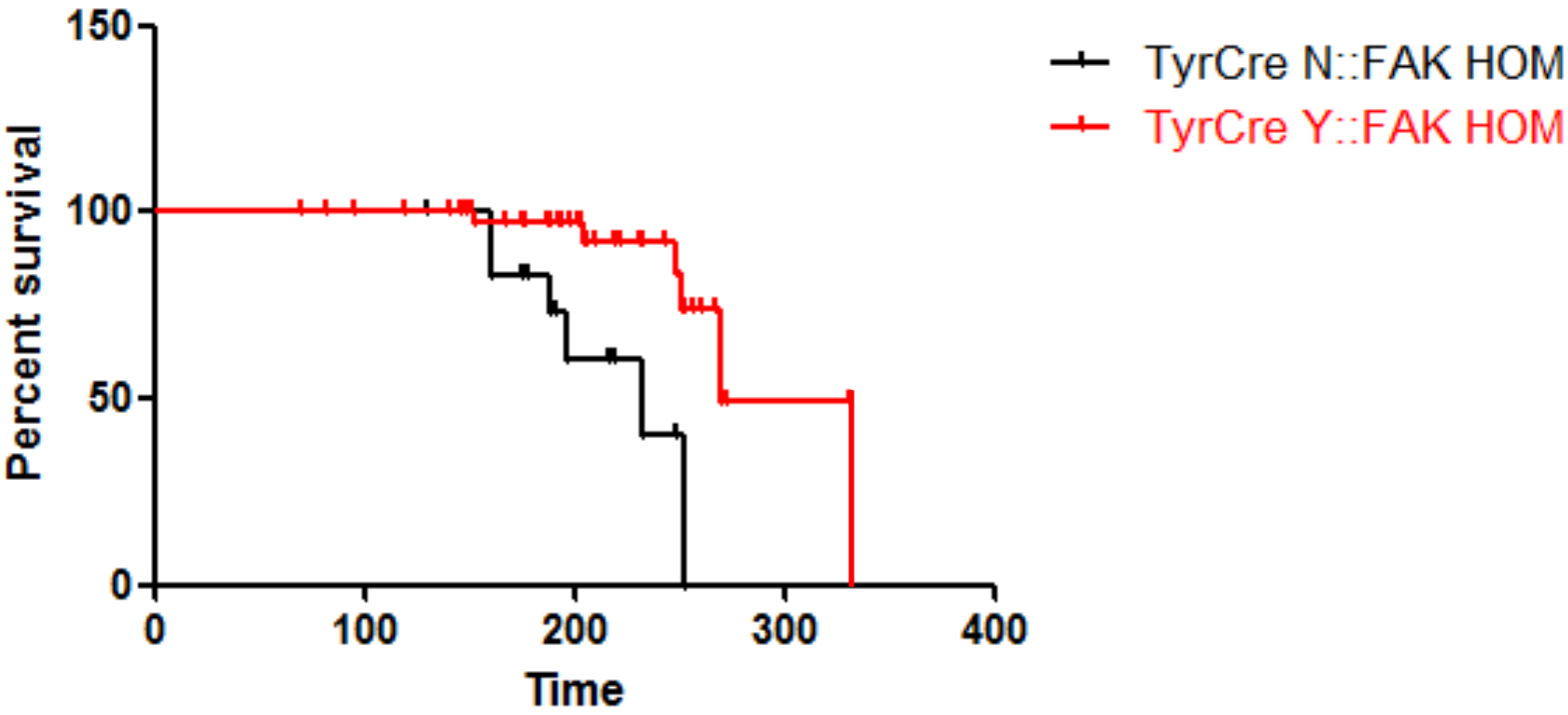


Tyr::N-Ras^{Q61K}
Tyr::Cre FAK$^{fl/fl}$



Colin Lindsey

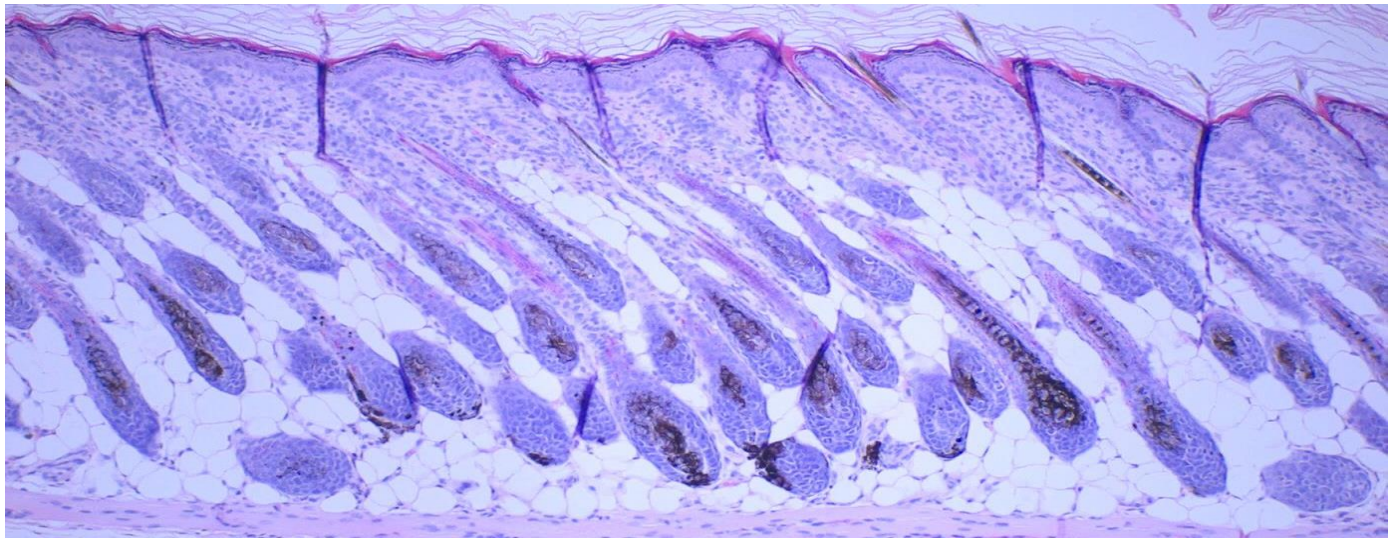
FAK deletion extends survival in the NRAS mutant melanoma



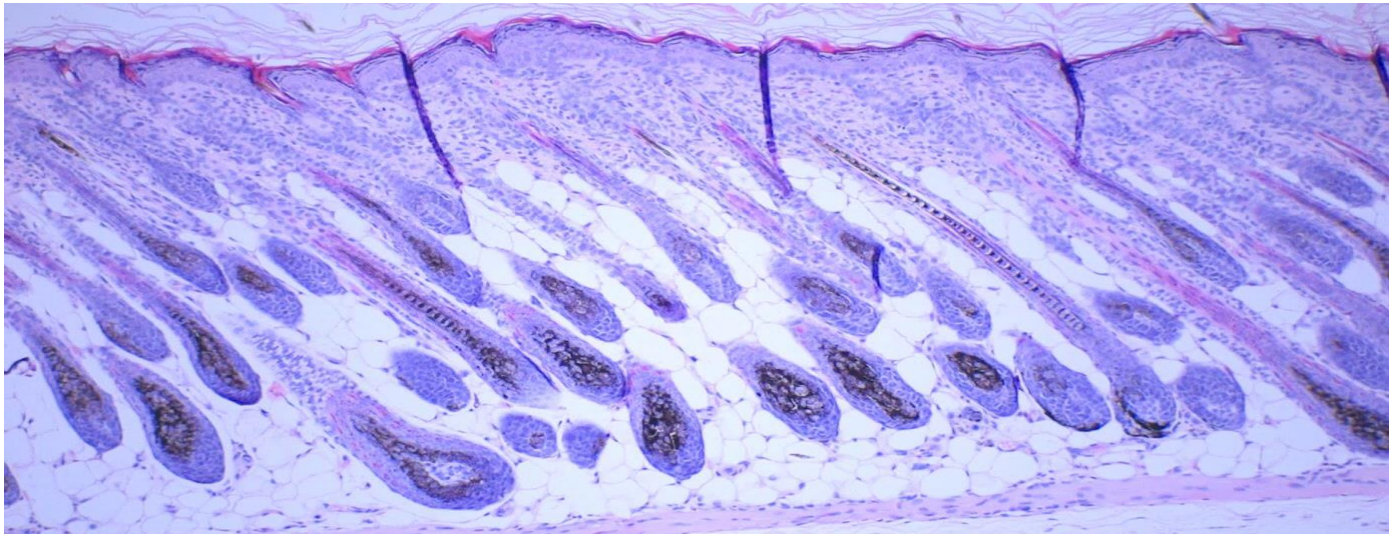
Tyr::N-RasQ61K::FAKfl/fl (Cre-N)	6/14 → 41%
Tyr::N-RasQ61K::TyrCre::FAKfl/fl	6/42 → 12%

William Faller

Effect of FAK deletion on early dermal mislocalisation of melanocytes – day 5

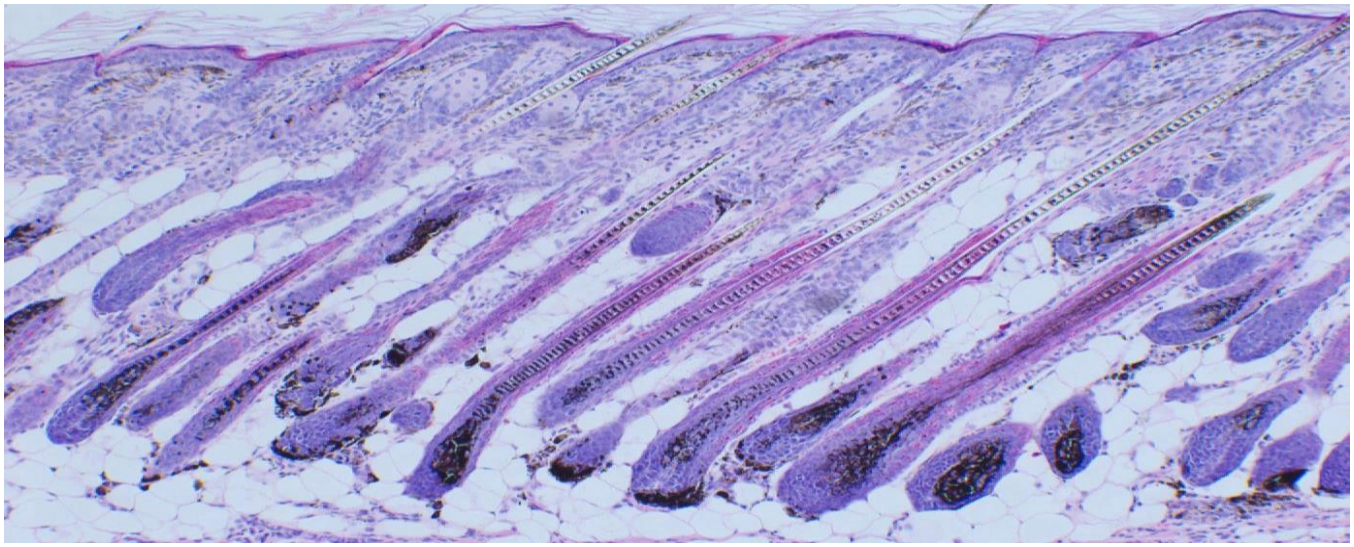


Tyr::N-Ras^{Q61K}
Tyr::Cre FAK$^{+/+}$

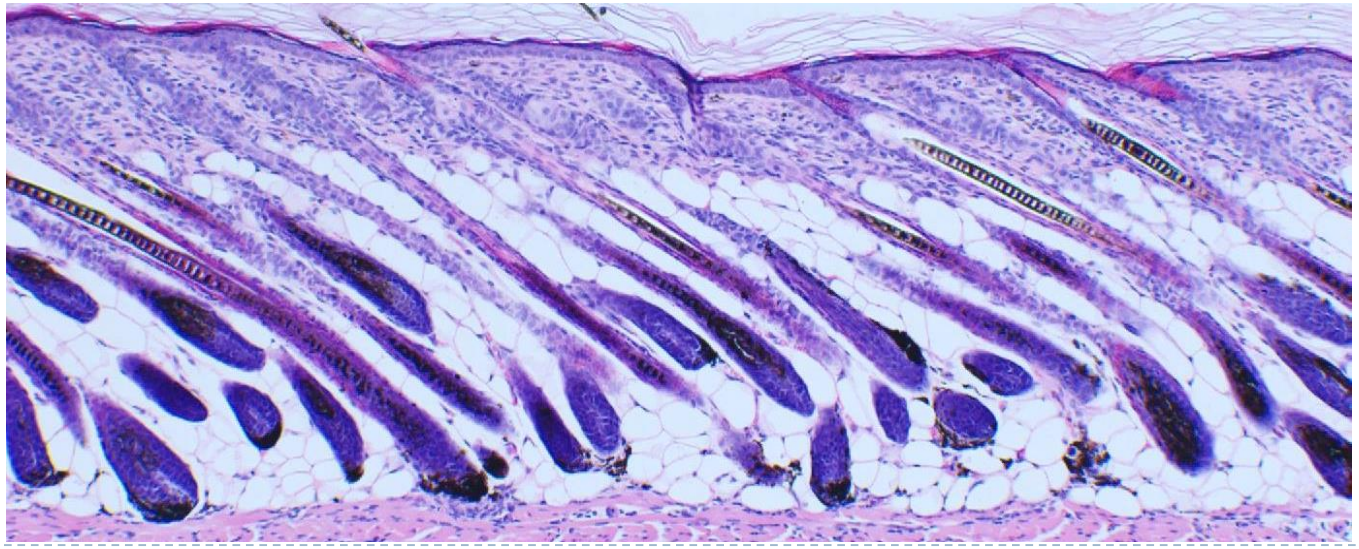


Tyr::N-Ras^{Q61K}
Tyr::Cre FAK$^{fl/fl}$

Effect of FAK deletion on early dermal mislocalisation of melanocytes – day 7

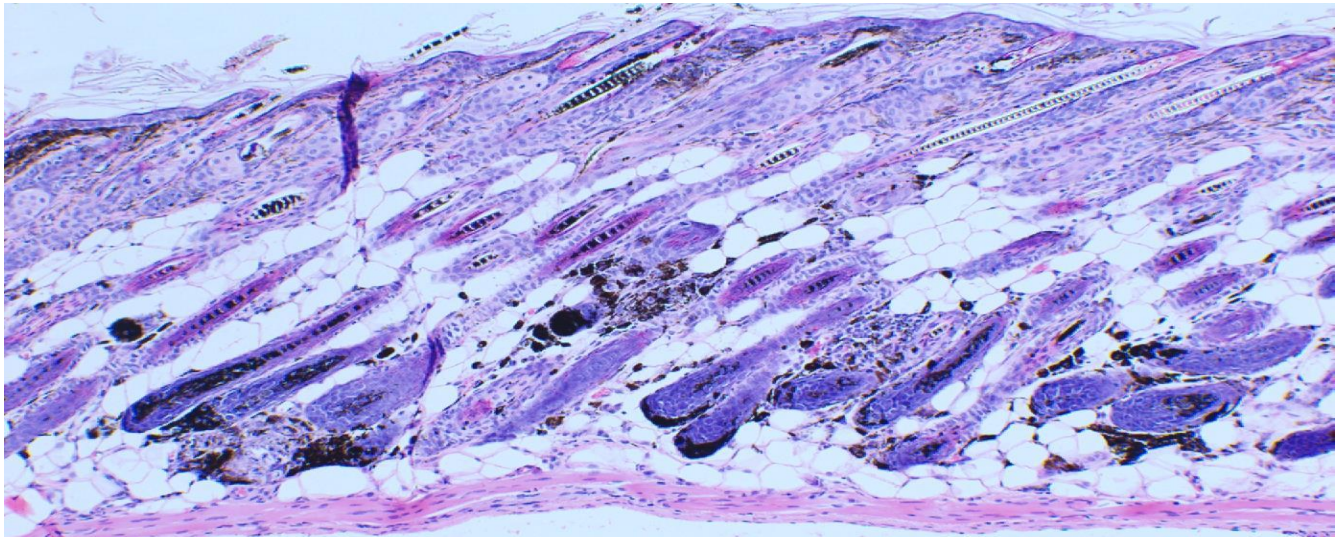


Tyr::N-Ras^{Q61K}
Tyr::Cre FAK$^{+/+}$

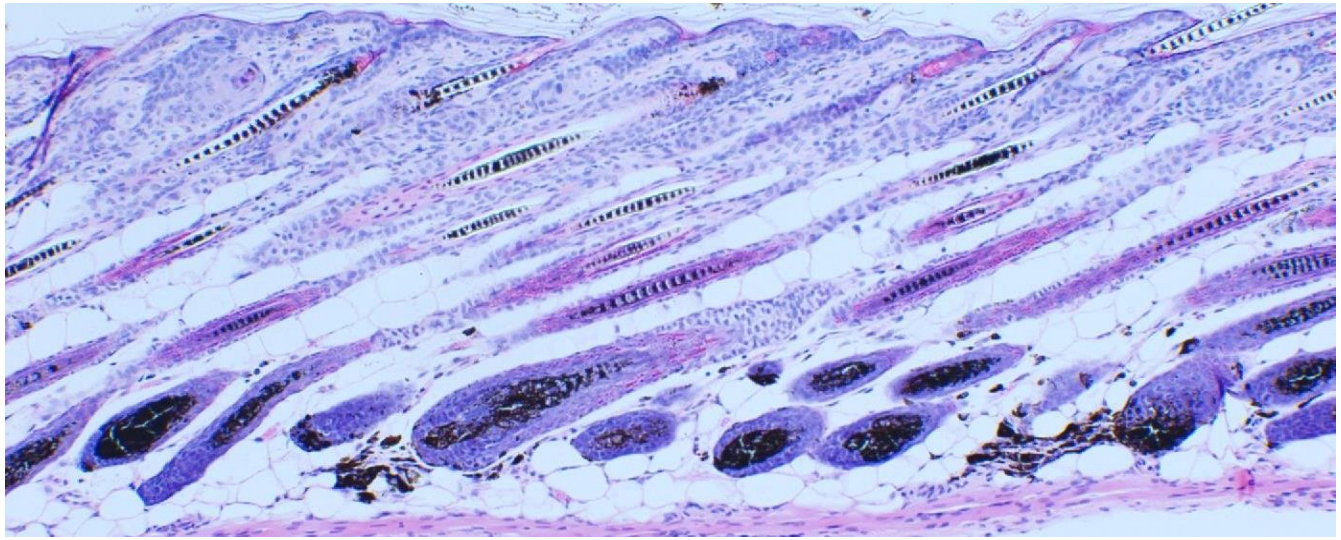


Tyr::N-Ras^{Q61K}
Tyr::Cre FAK$^{fl/fl}$

Effect of FAK deletion on early dermal mislocalisation of melanocytes – day 14



Tyr::N-Ras^{Q61K}
Tyr::Cre FAK$^{+/+}$



Tyr::N-Ras^{Q61K}
Tyr::Cre FAK$^{fl/fl}$

Future directions

- Role of DUSP proteins in melanoma
  - Setup TyrCreER-BRAF^{V600E}::PTEN$^{-/+}$ ::DUSP6 melanoma colony – role of DUSP6 in BRAF mutant melanoma
  - Setup Tyr-NRAS^{Q61K}::Cdkn2a$^{-/-}$ ::DUSP6 melanoma colony – role of DUSP6 in early NRAS melanoma
  - Setup TyrCreER-NRAS^{Q61R}::Cdkn2a$^{-/-}$ ::DUSP6 melanoma colony – role of DUSP6 in adult NRAS melanoma
  - Role of DUSP5 in melanoma development, alone and together with DUSP6
- Role of FAK in melanoma
  - Expanding the investigation of the effect of FAK on immune infiltration
  - Setup TyrCreER-BRAF^{V600E}::PTEN$^{-/+}$ ::FAK melanoma colony – role of FAK in BRAF mutant melanoma

Acknowledgements

Owen Sansom
Andrew Campbell
Mona Foth
William Faller

All R18 & R14

Collaborators:

- William Gallagher, Walter Kolch, Lan Nguyen; Sungyoung Shin
  Conway Institute, Dublin;
  University of Sydney
- Lionel Larue
  Institut Curie, Orsay, France
- Simon Barry
  Astra Zeneca

Cell sorting and BAIR platforms:
Tom Glibey
Tim Harvey
Margaret O'Prey
Histology:
Colin Nixon and co.
Common services:
Andrew Keith
Billy Clark
Bioinformatics:
Ann Hedley
Matt Neilson
BSU, BRU staff

Thank you for your attention!!!

CANCER RESEARCH UK
AstraZeneca
MARIE CURIE ACTIONS
SEVENTH FRAMEWORK PROGRAMME
Disease Models & Mechanisms
The Company of Biologists