

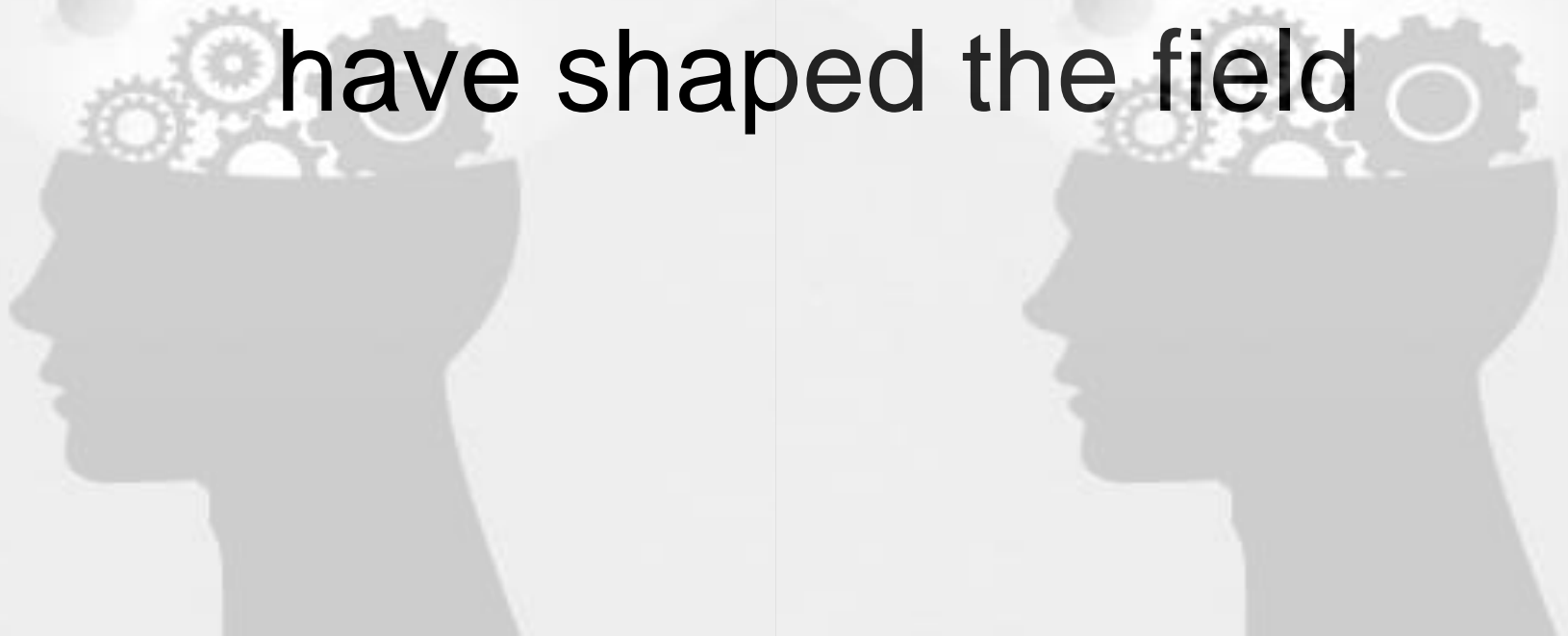
Lessons from 100 years of hypoxia research for future radiobiology



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University Health Network

Hypoxia Radiobiology

***5 conceptual* observations
have shaped the field**



1) 1909 - Hypoxia causes radiation resistance

Die Münchner Medizinische Wochenschrift erscheint wöchentlich in halber und dritteljährlicher Ausgabe. • Preis der einzelnen Nummern 50 Pf. • Abonnements in Preussland vierteljährlich 1.50 M. • Uebrigere Bezugsbedingungen siehe auf dem Umschlag.

MÜNCHENER
MEDIZINISCHE WOCHENSCHRIFT.
ORGAN FÜR AMTLICHE UND PRAKTISCHE ÄRZTE.

No. 24. 15. Juni 1909. Redaktion: Dr. B. Spate, Arnulfstrasse 26. 56. Jahrgang.
Verlag: J. F. Lehmann, Paul Heystrasse 26.

Originalien.

Ueber Desensibilisierung gegen Röntgen- und Radiumstrahlen.

Von Dr. Gottwald Schwarz.

Da sich die Wirkung der Röntgenstrahlen auf die Haut bei der grossen Empfindlichkeit und der oberflächlichen Lage dieses Organs am deutlichsten manifestierte, war die Radiotherapie ursprünglich bloss Dermatotherapie. Heute ist dies längst anders. Der ungleich bedeutungsvollere Teil des Anwendungsgebietes der X-Strahlen betrifft die malignen Blut-erkrankungen und Geschwulstbildungen, wo wir schon heute über Ergebnisse verfügen, die man vor wenigen Jahren noch als durchaus unmöglich bezeichnet hätte.

Aber gerade hier, bei den „Tiefenbestrahlungen“, wie man es nennt, erfahren wir ein sehr störendes Hemmnis, eben durch jene hohe Sensibilität der Hautdecke, die unserem therapeutischen Vorgehen ein unüberschreitbares, meist vorzeitiges Ziel setzt. Wie oft müssen wir uns bei einer leukämischen Rückfall, bei nur unvollständiger Rückbildung oder beim Rezidiv einer durch Bestrahlung schon günstig beeinflussten Geschwulst sagen: „Wir könnten mehr leisten, könnten wir nur mehr bestrahlen. Wir können es aber nicht, weil wir dann die Haut gefährden und dem Kranken durch eine „Röntgenverbrännung“ schweren und schmerzvollen Schaden zuzügen würden.“

Das Bestreben, hier Abhilfe zu schaffen, führte mich auf folgenden Weg:

Schon vor zwei Jahren hatte ich mich durch Versuche an Pflanzenkeimlingen (Mittel, a. d. Wiener Röntgenlabor., Jena, O. Fischer, 1907) darüber belehrt, dass zwischen der Stoffwechsellösse und der Röntgenlichtempfindlichkeit der Pflanzenzelle ein inniger Zusammenhang bestehe. Bestrahlte ich trockenen Samen, in welchem der Embryo sich im Zustande eines „latenten“ Lebens befindet, sein Stoffwechsel auf ein Minimum eingeschränkt ist, so blieben selbst enorme Röntgenlichtmengen (tagelange Bestrahlungen) ohne Effekt. Ausgesät, wuchsen normale Individuen heran.

Bestrahlte ich jedoch aufgequollenen Samen, war das Wachstum eingeleitet, der Wechsel der Stoffe rege geworden, so zeigten sich schon ganz kleine Röntgenlichtdosen, um hochgradige, charakteristische Veränderungen, Wachstumsstörungen und Pigmentationen zu erzeugen.

Dieser Konnex zwischen Stoffwechsellösse und Röntgenempfindlichkeit, den mir manche Beobachtung auch aus meiner radiotherapeutischen Tätigkeit zu bestätigen schien, brachte mich nun zu folgender Überlegung. War die Hypothese richtig, derzufolge ein Zellkomplex um so empfindlicher gegen Strahlenwirkung anzusehen wäre, je stärker dessen Stoffwechsel ist, so müssten alle diejenigen Agentien, die den Stoffwechsel vermindern, auch die Strahlenempfindlichkeit vermindern.

Zur Entscheidung dieser Frage unternahm ich folgenden Versuch: Bei einem 11-jährigen Mädchen, das uns wegen eines ausgedehnten Naevus flammeus des Vorderarms zur Radiumbehandlung zugewiesen worden war, bestrahlte ich zwei benachbarte Hautstellen mittels unserer verhältnismässigen Radiumkapsel, am selben Tage, gleich lange Zeit (je 1 Stunde lang),

war die Haut komprimiert, anämisiert, der Saftfluss gesperrt, der Stoffwechsel als solches gehemmt — die postulierte Bedingung somit erfüllt (Fig. 1).

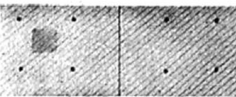
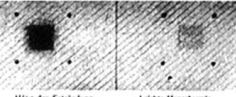

Die bestrahlte Stelle durch Kapillarkapsel komprimiert, Saftfluss gestoppt, unter Druck mit Druck



Fig. 1.

Das Ergebnis dieses Versuches war ein solches, wie ich es zu erhoffen wohl nicht gewagt hatte (Fig. 2).

Bestrahlung mittels 60 mg Radiumbromid am 16. März.

ohne Druck	mit Druck	
		Am 8. April
Digme der Entzündung.	Keine.	
		Am 12. April
Hohe der Entzündung, Oedem, colorierte Hyperämie.	Leichte Hyperämie.	
		Am 19. April
Abschwindende Entzündung, Schwellung.	Keine.	
		Am 25. April



2) 1955-Hypoxia is present in human tumors

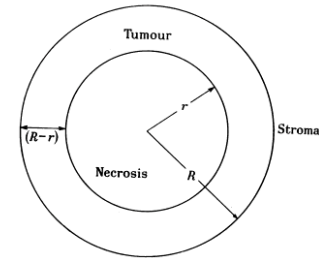
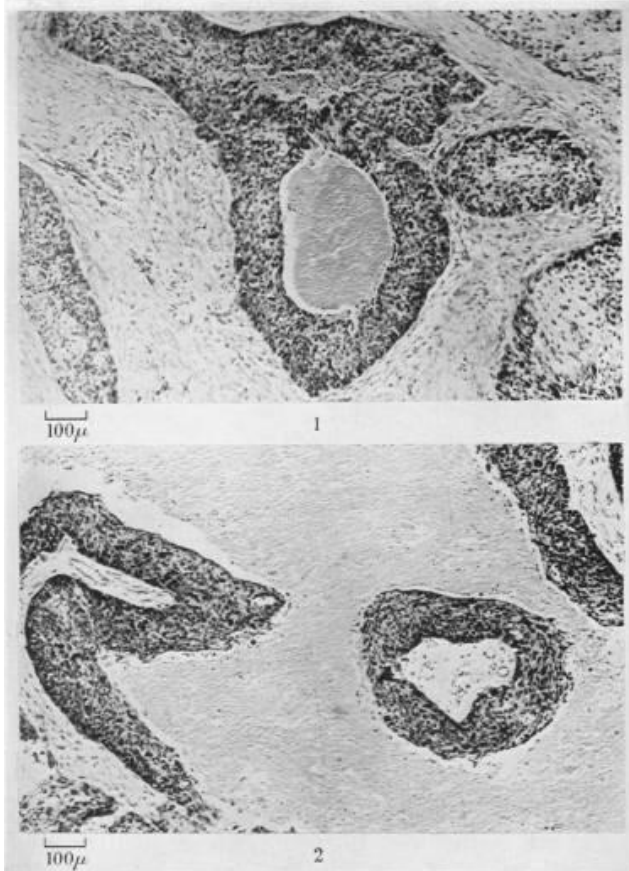


FIG. 5.—Diagrammatic representation of a tumour cord for comparison with Fig. 6-10.

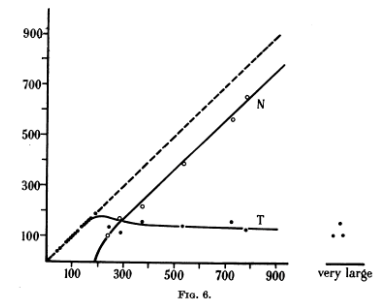
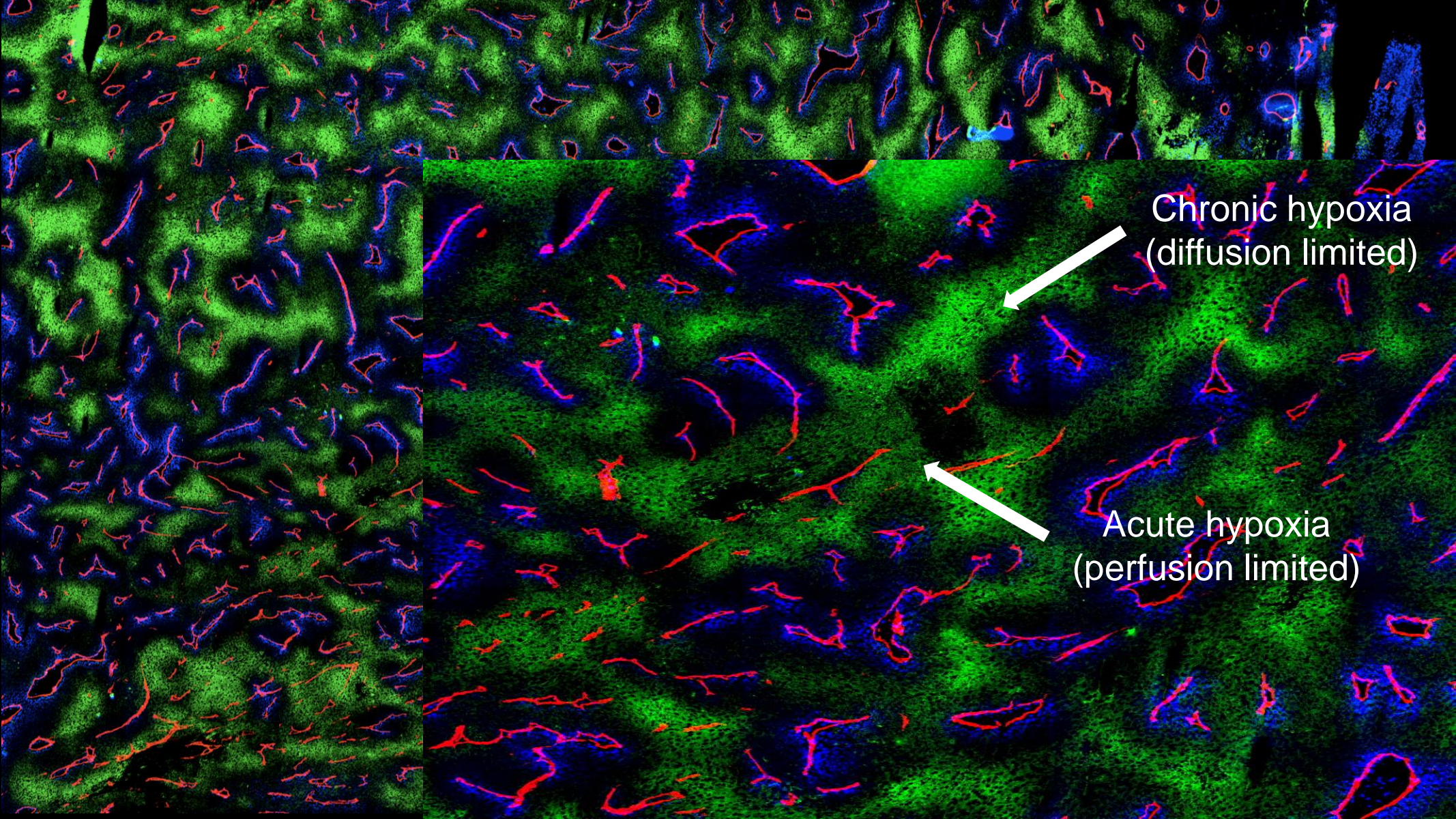


FIG. 6-10.—Dimensions of tumour cord R , central necrosis r , and region of tumour which has not yet become necrotic ($R - r$). The dimensions (in microns) are those seen in sections of carcinomas of the bronchus fixed with 5 per cent formalin and stained with haematoxylin and eosin. *Abs.*: Radius R of interface between tumour cord and stroma. *Ord.*: Radius of central necrosis r and thickness of cylindrical shell of tumour ($R - r$). ● Tumour (curve T). ○ Central necrosis (curve N). Fig. 6 case 5162/53; Fig. 7 case 4127/54; Fig. 8 case 4084/53; Fig. 9 case 889/53; Fig. 10 case 889/53, different region of the same tumour as Fig. 9.

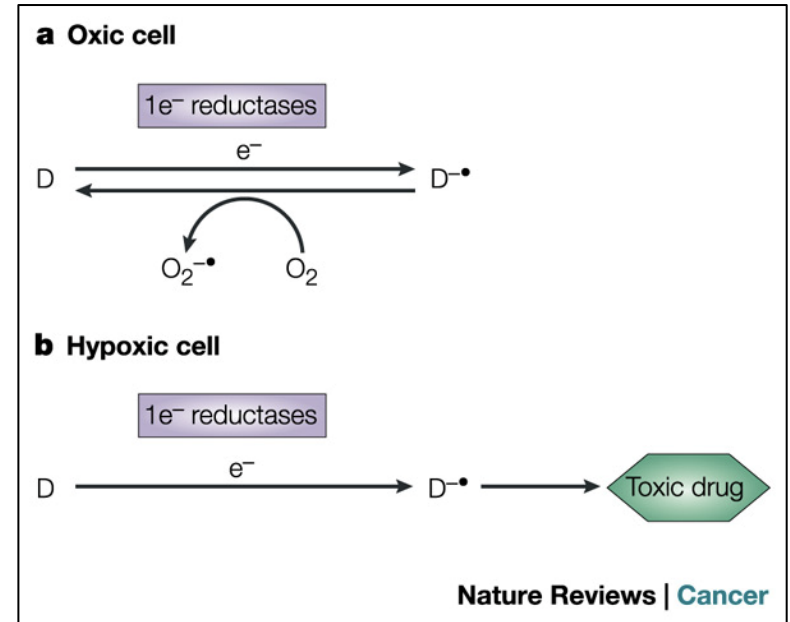
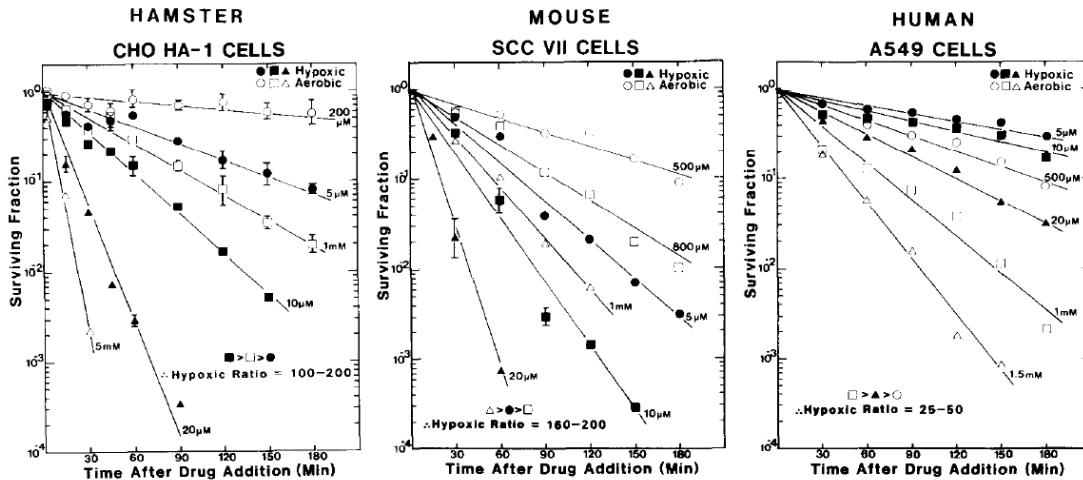
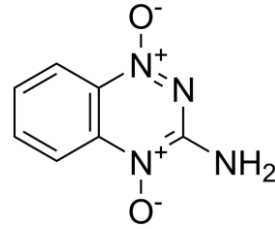


Chronic hypoxia
(diffusion limited)

Acute hypoxia
(perfusion limited)

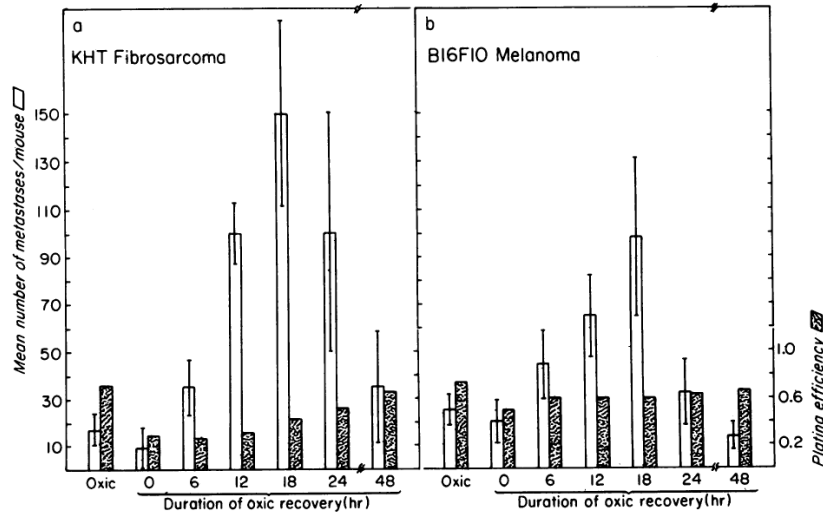
3) 1986-Hypoxic cells can be targeted

Tirapazamine

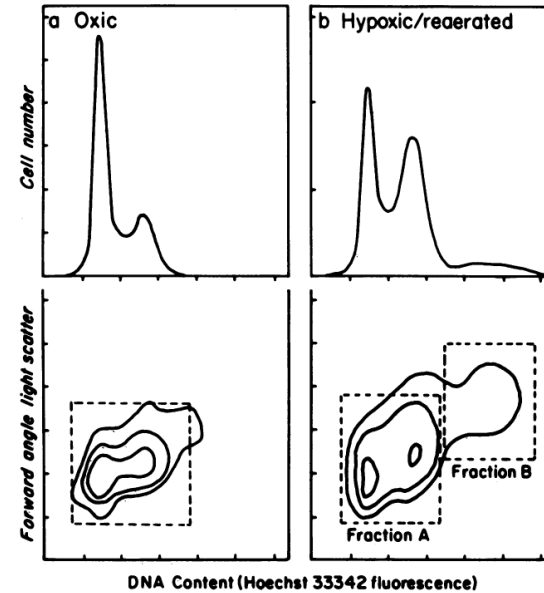


4) 1988- Hypoxia influences tumor biology

Metastasis



Genetic instability



Hypoxia drives phenotypic diversity

A fluorescence microscopy image of a tissue section. The image shows a complex network of blue-stained structures, likely representing blood vessels or a specific cell population. The background is filled with a dense field of red-stained cells, interspersed with several bright green and purple spots, indicating the presence of different cell types or markers. The overall appearance is that of a highly cellular and vascularized tissue under hypoxic conditions.

Angiogenesis

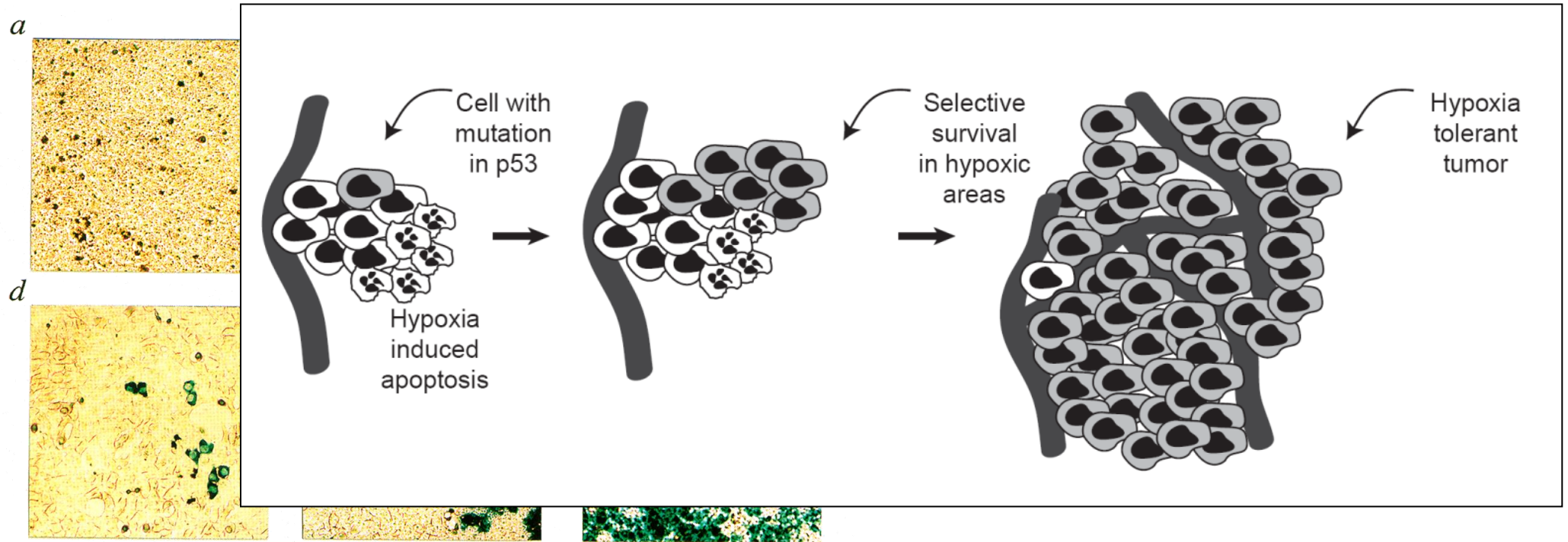
Metabolism

DNA Repair

Metastasis

Stemness

5) 1996-Mutations influence hypoxia tolerance



**Why has this not had a
bigger clinical impact?**

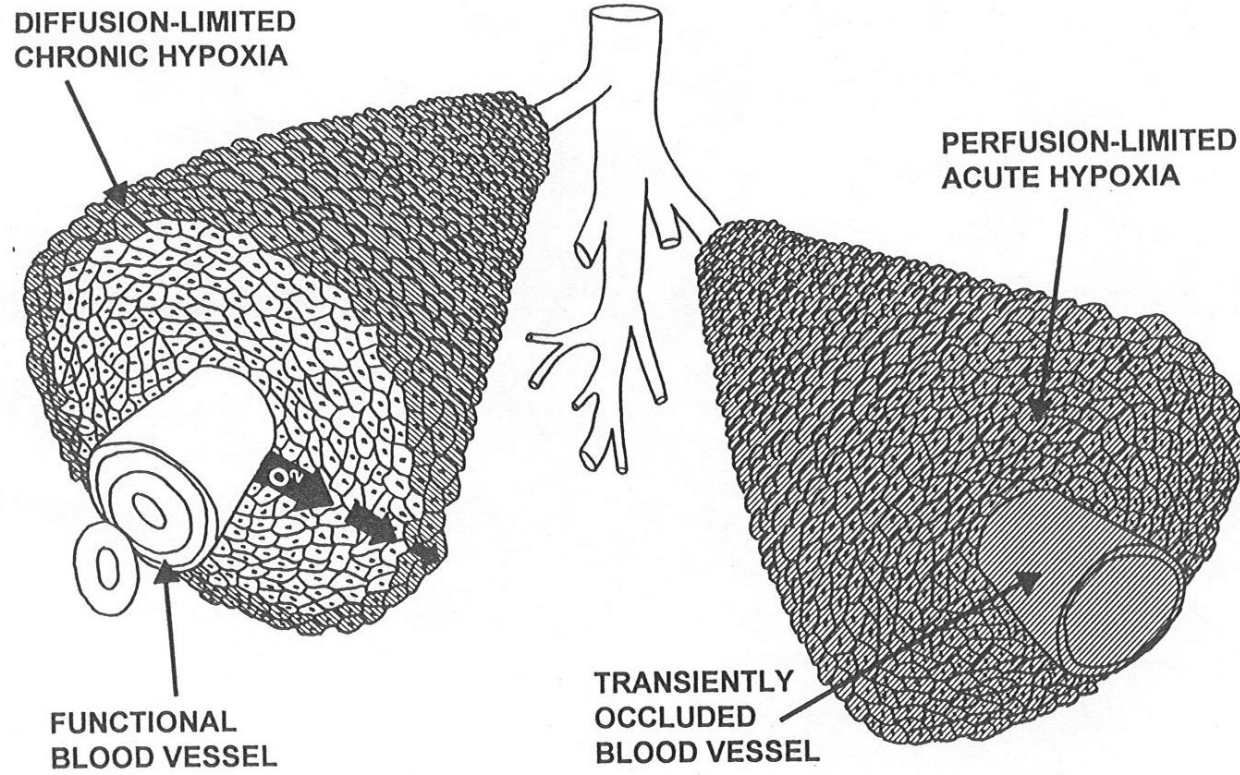
Need for personalization

The most important and interesting aspect of tumour hypoxia is its variation across patients

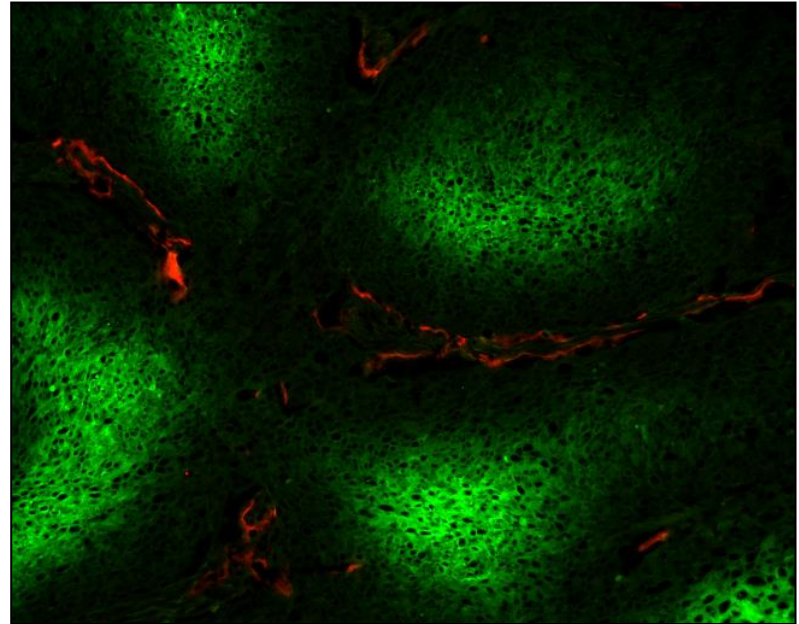
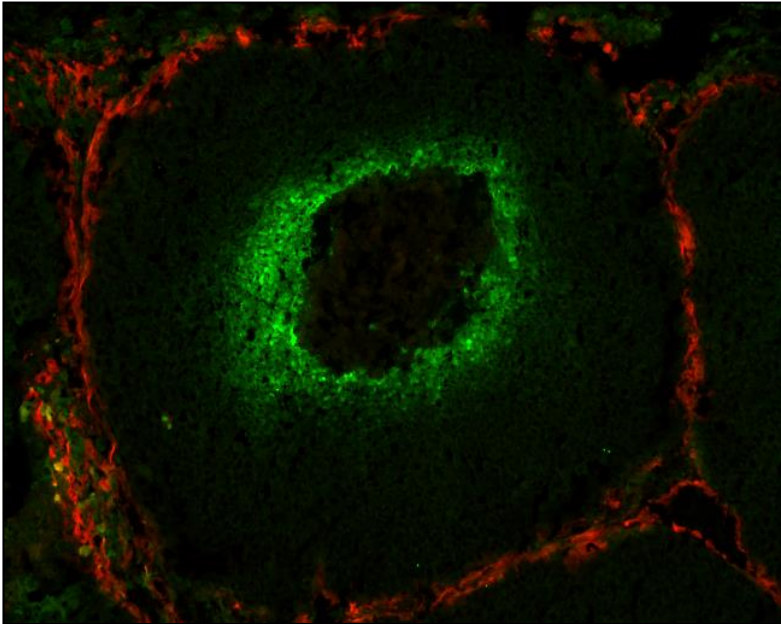
1) We don't understand what causes the different patterns of hypoxia from patient to patient

2) We don't base therapeutic approaches to hypoxia on any patient specific feature

Determinants of tumour hypoxia – oxygen delivery/vasculature



Tumour hypoxia is about more than delivery



Courtesy of Bert van der Kogel

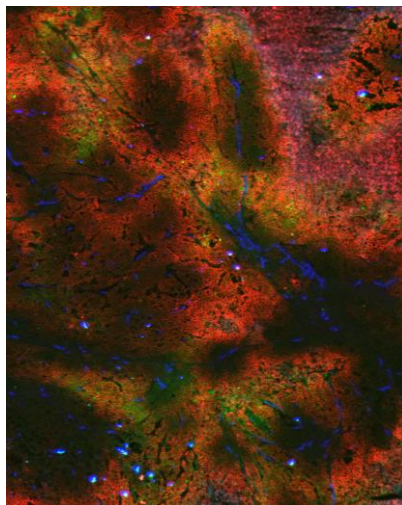
Oxygen metabolism and hypoxia tolerance influence tumor hypoxia

Oxygen delivery/demand

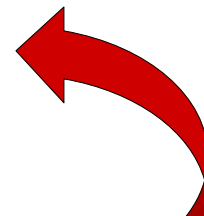
- 1) Angiogenesis/perfusion
- 2) Oxygen metabolism



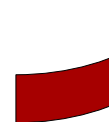
Microenvironment
dynamic equilibrium



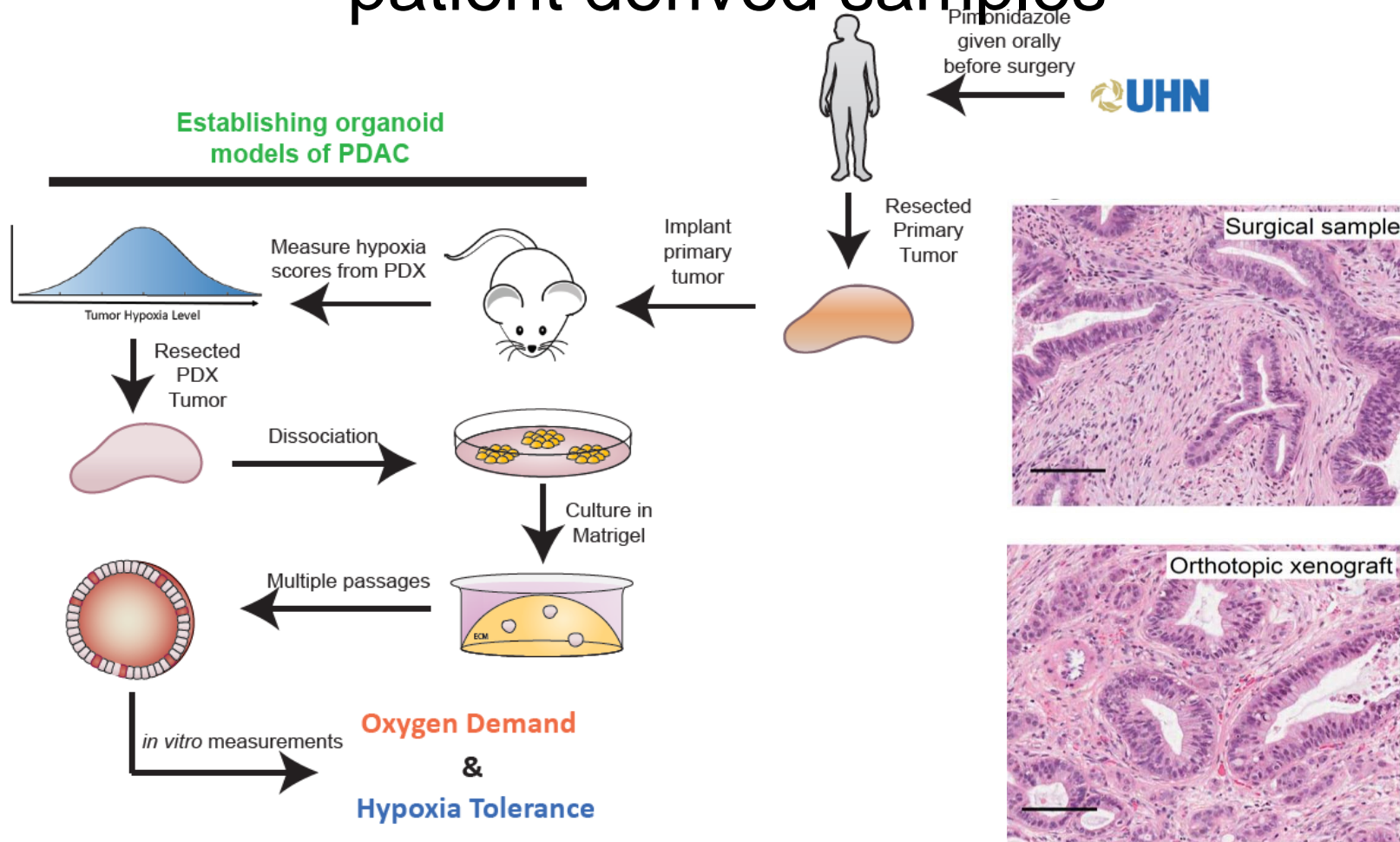
3) Hypoxia
Tolerance



Adaptive
pathways

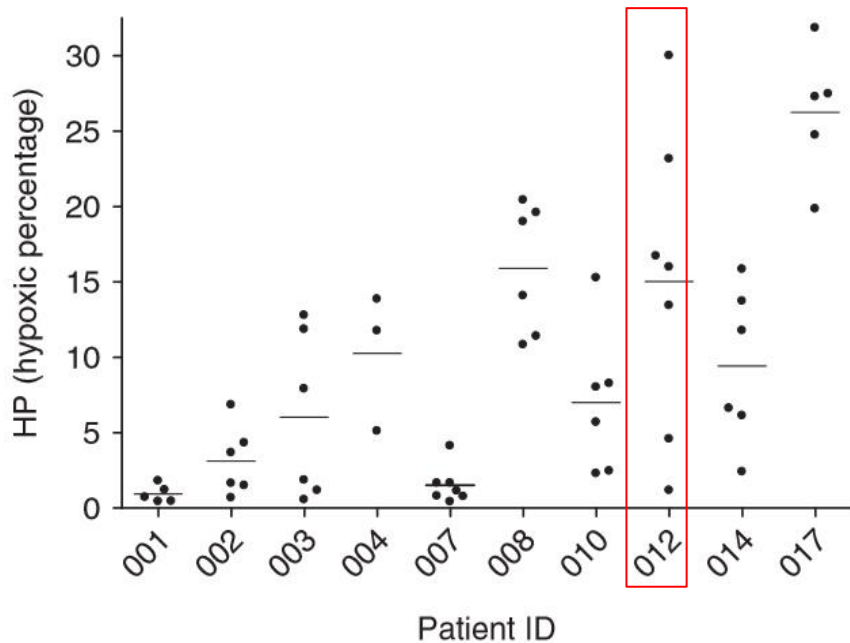


Analysis of oxygen metabolism and hypoxia tolerance in patient derived samples

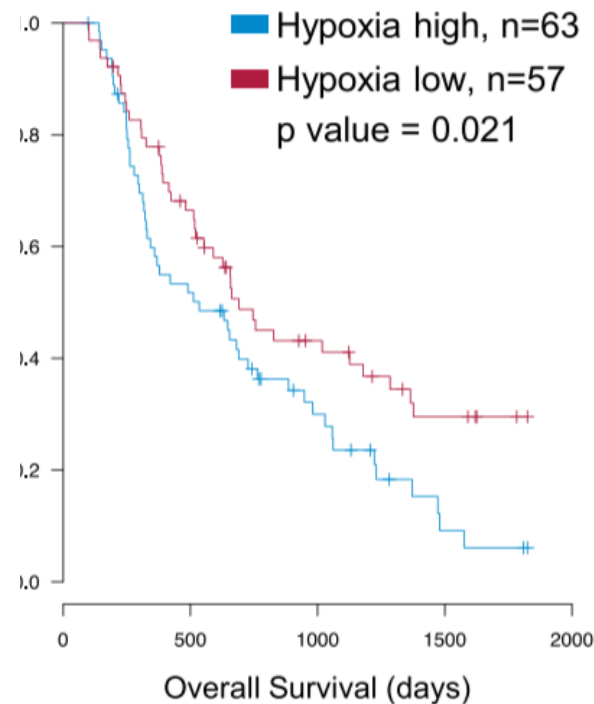
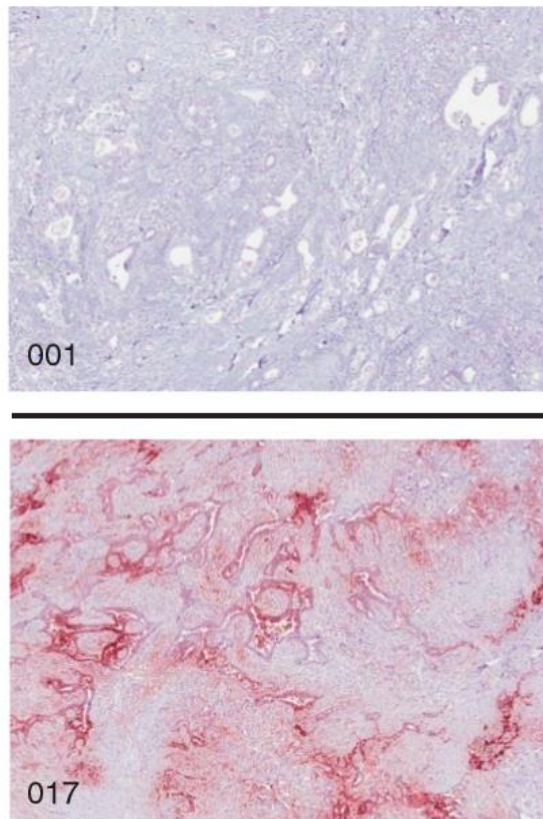


How does oxygen metabolism and hypoxia tolerance drive variation in hypoxia across patients?

Pancreatic cancer



(Dhani et al., 2015, British Journal of Cancer)

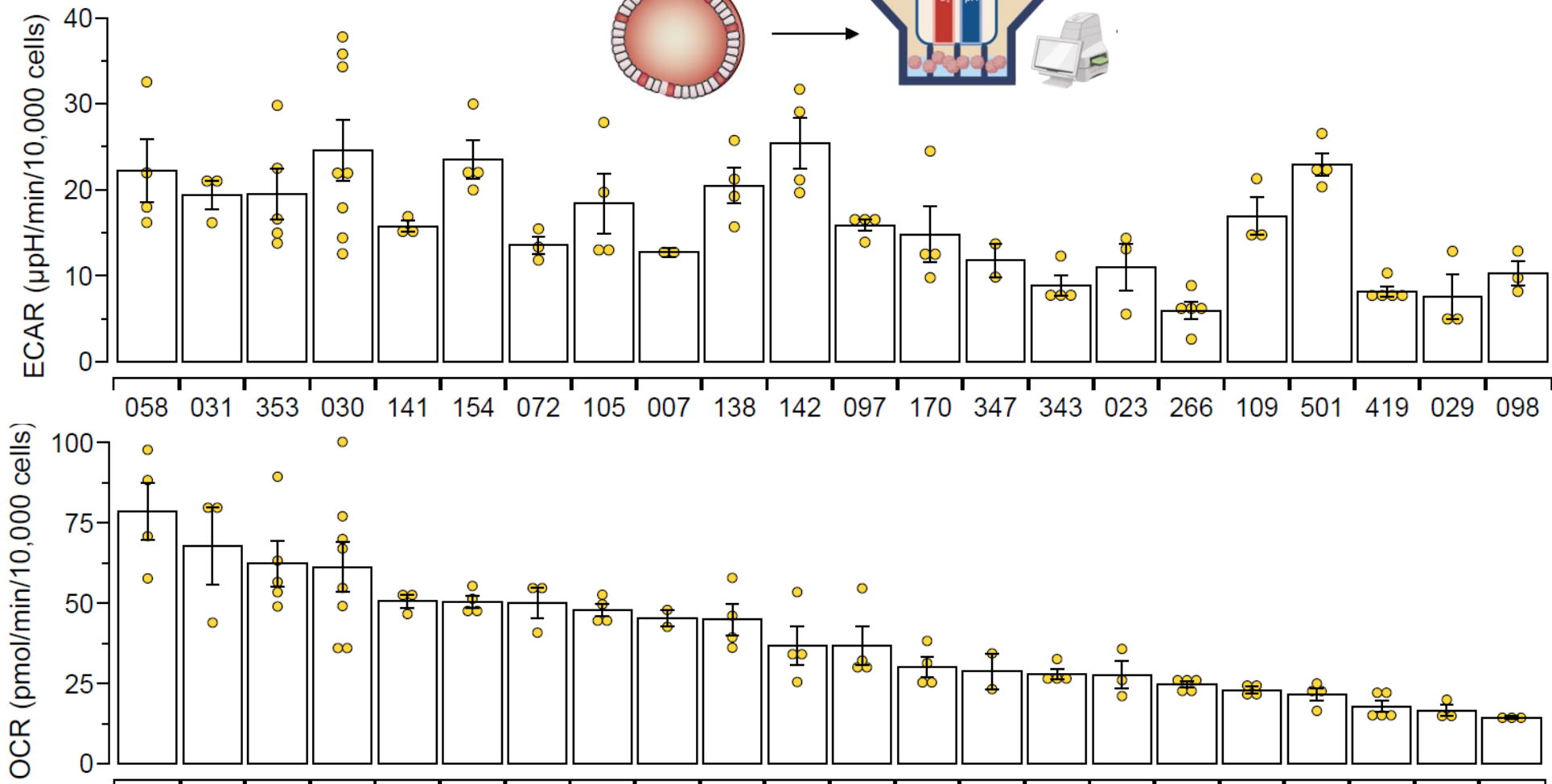
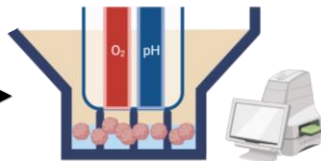
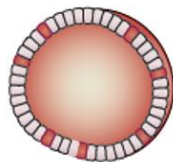


(Connor et al., 2019, Cancer Cell)

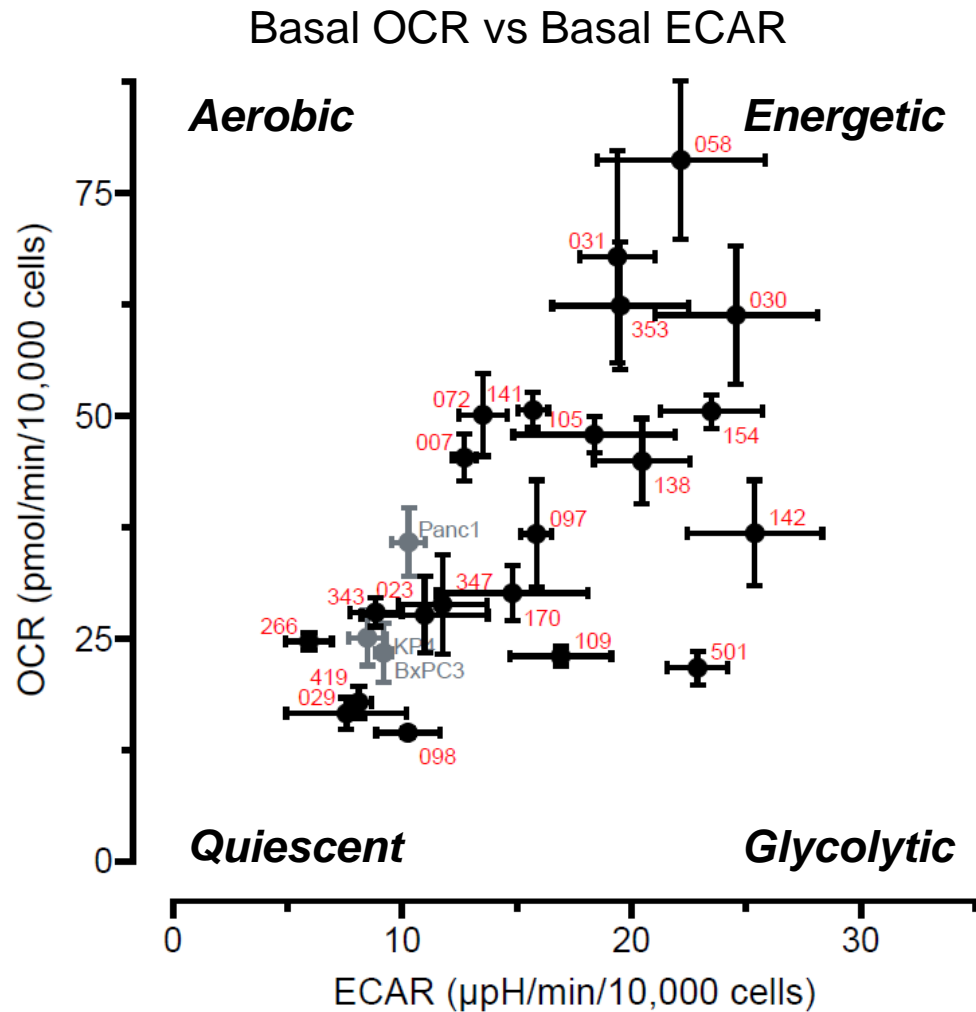
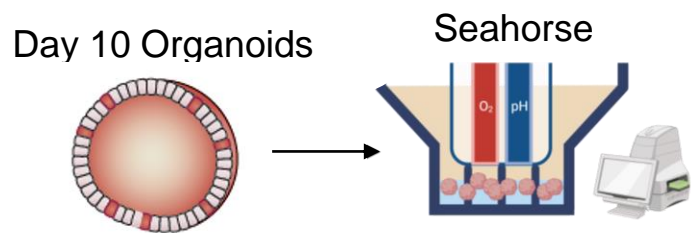
High range in oxygen consumption in PDO

Day 10 Organoids

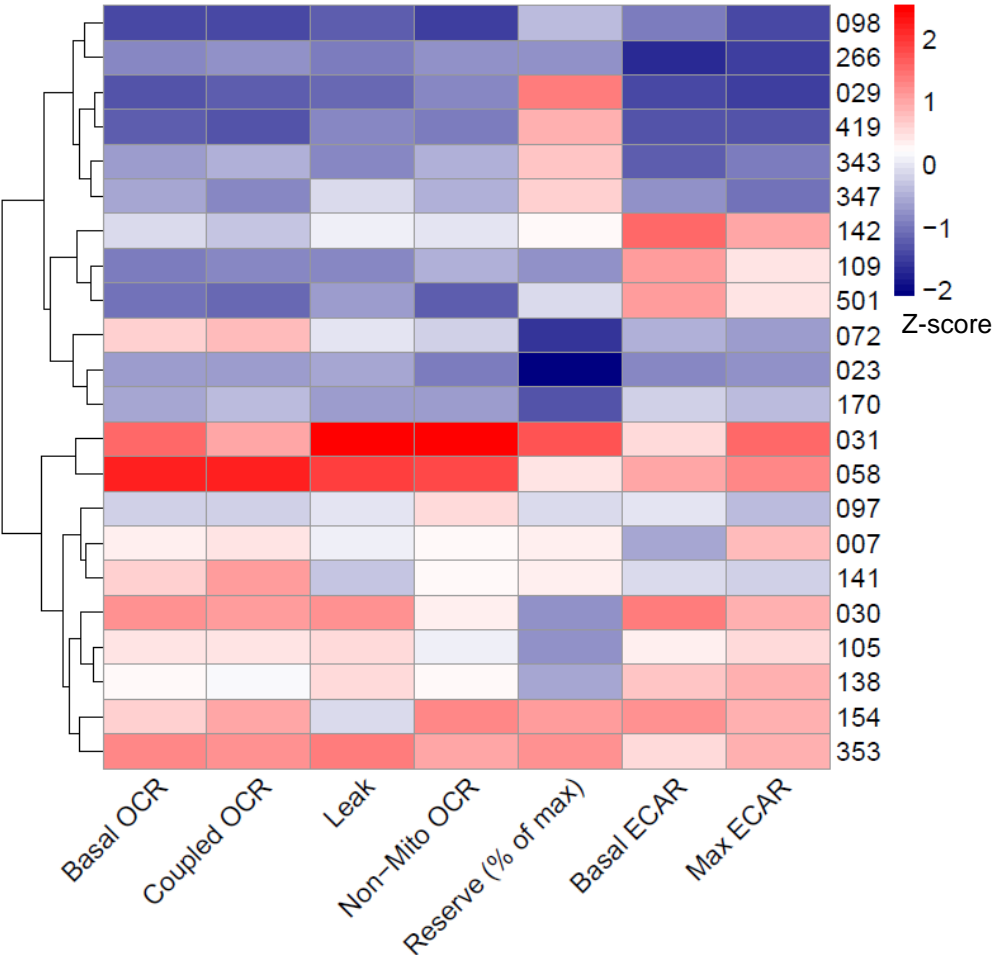
Seahorse



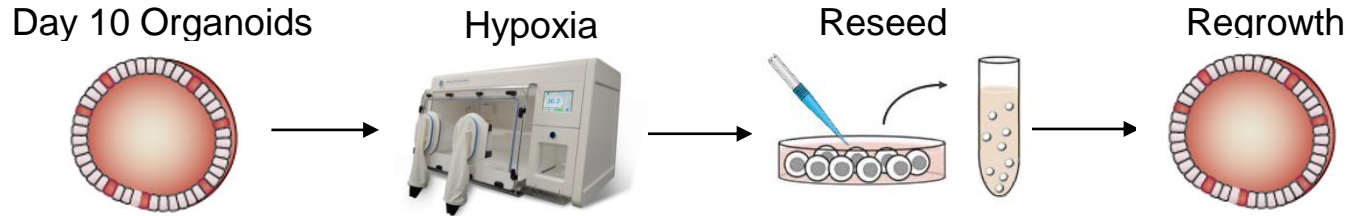
PDOs exhibit a wide range of metabolic phenotypes



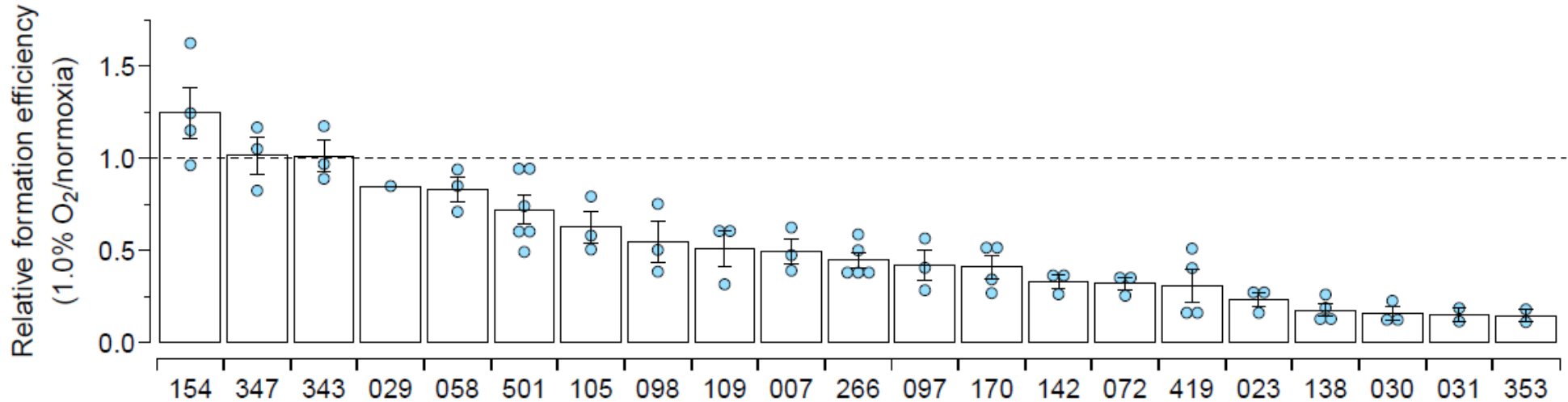
Metabolic subtypes in PDAC



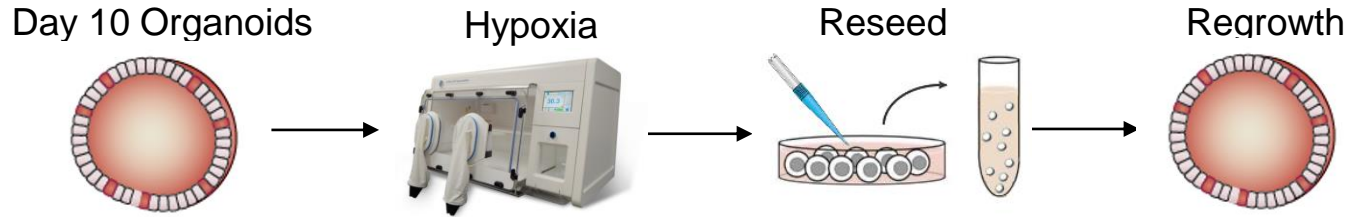
High range of hypoxia tolerance in PDO



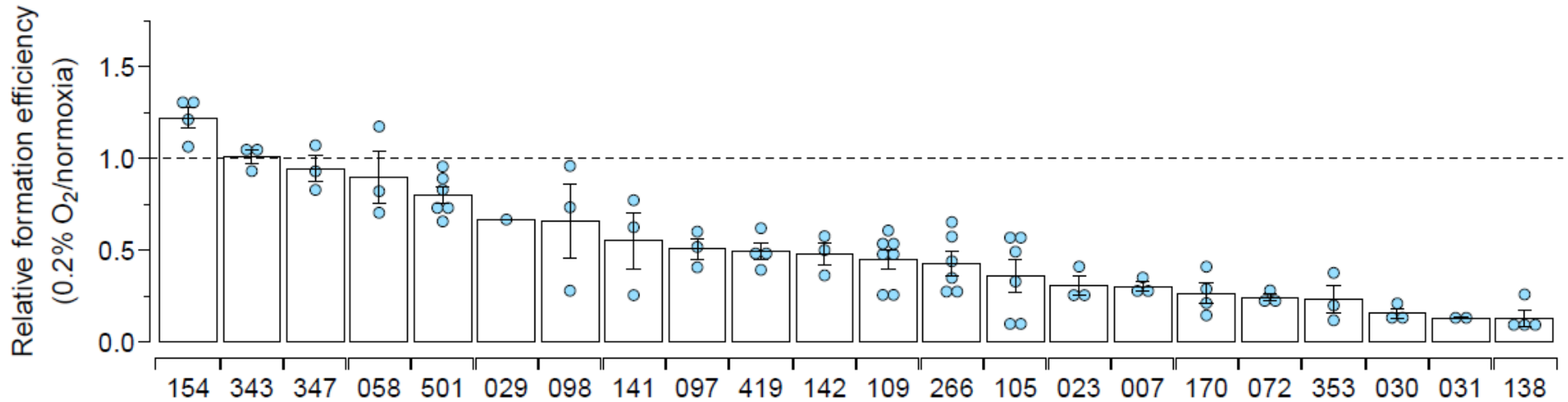
48hr 1% oxygen



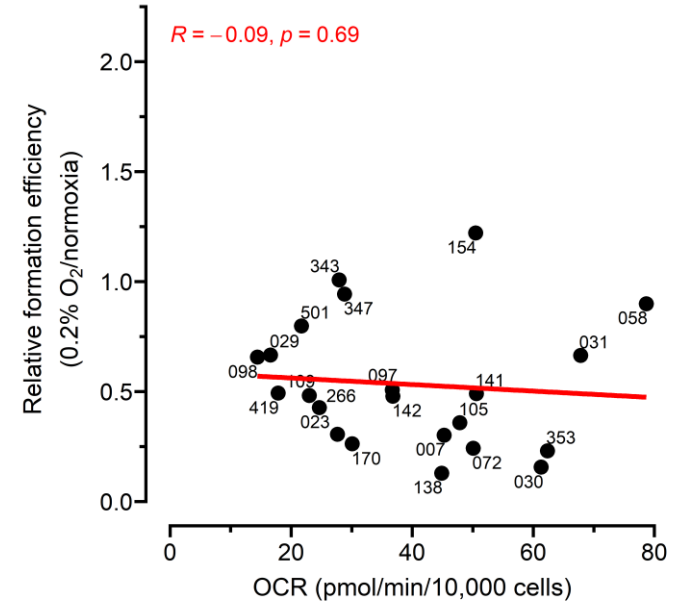
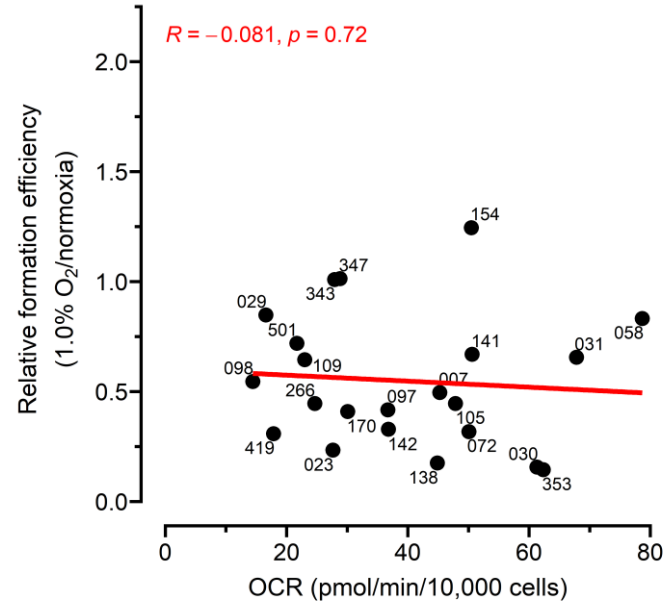
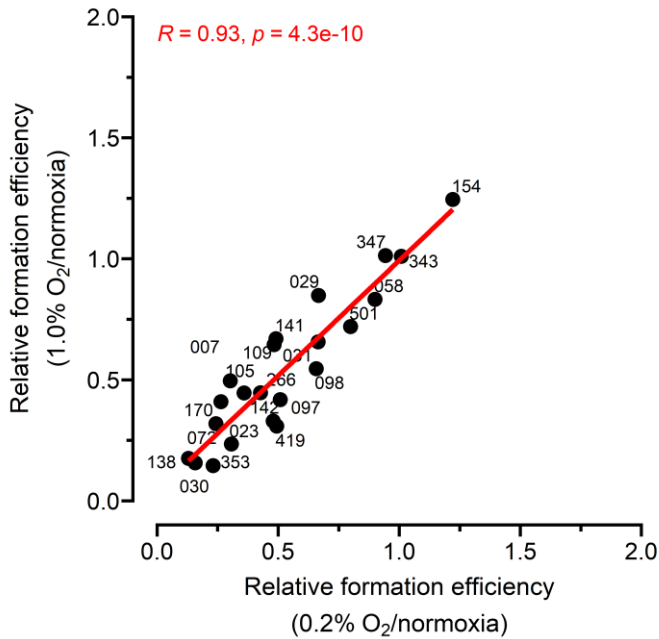
High range of hypoxia tolerance in PDO



48hr 0.2% oxygen



Correlation between hypoxia tolerance and basal OCR



Metabolic and hypoxic phenotypes captured *in vivo*



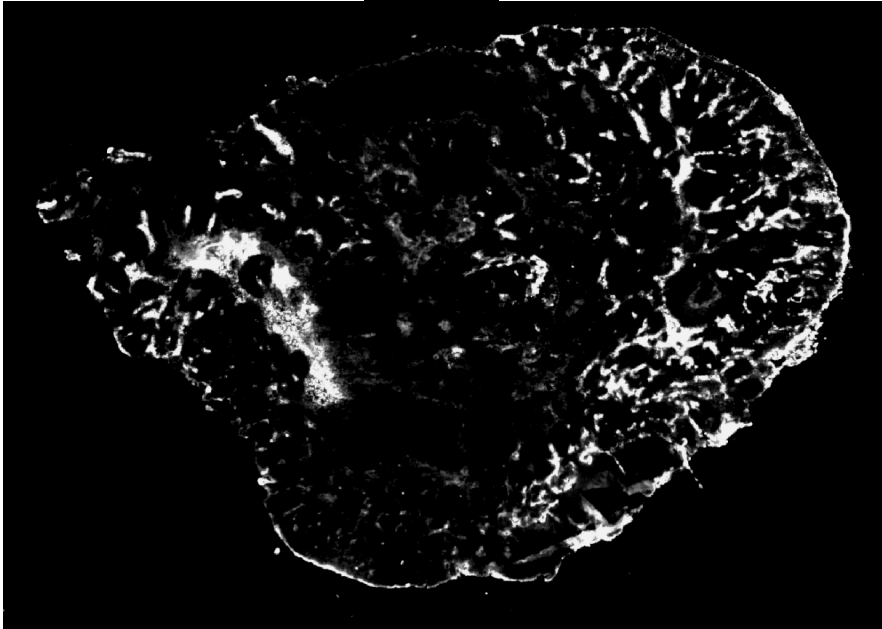
Injections:
PIMO-180min
EdU-30min
Hoechst-1min



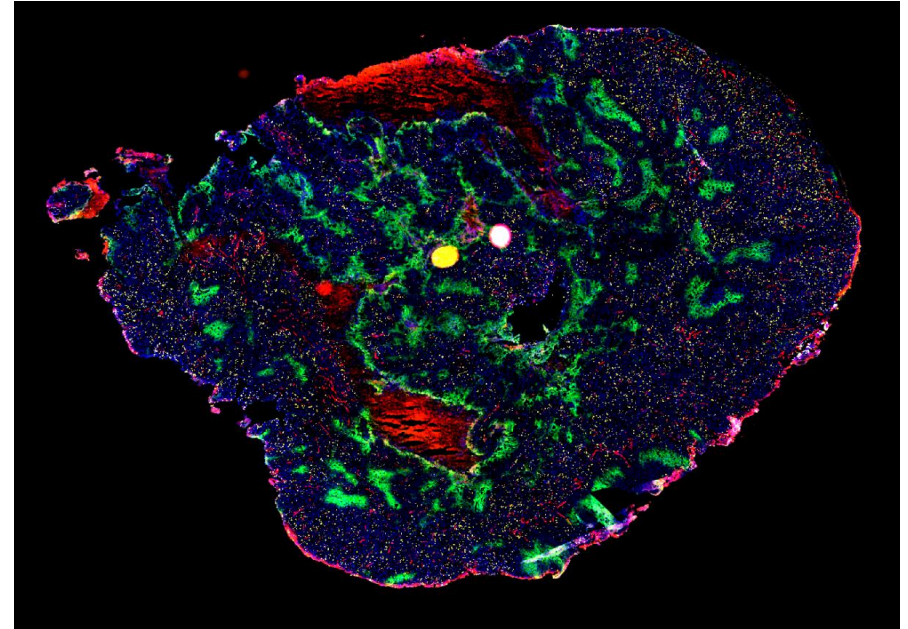
Evaluate the variation of phenotypes *in vivo*

- oxygen metabolism
- hypoxia tolerance
- vasculature

Hoechst



DAPI CD31 PIMO EdU



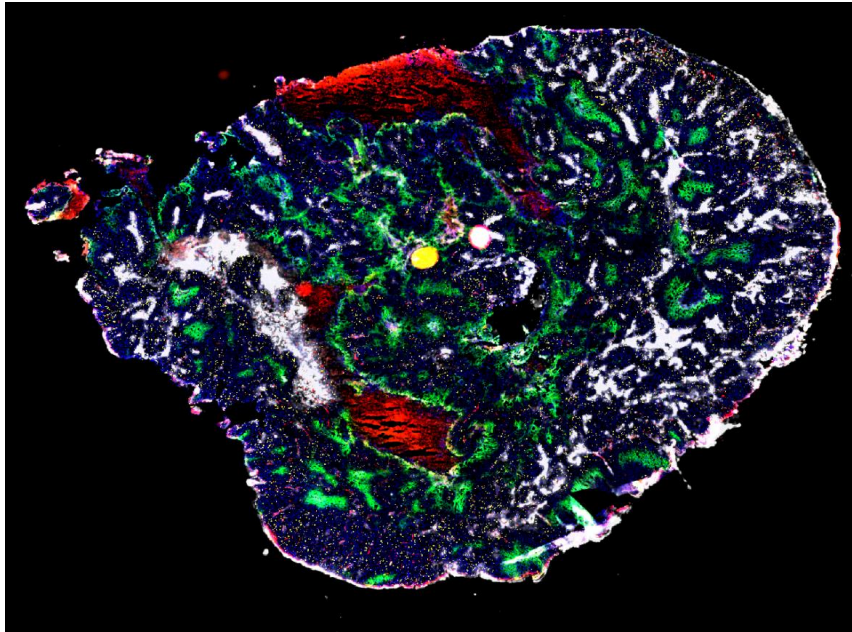
Analysis pipeline – live/dead



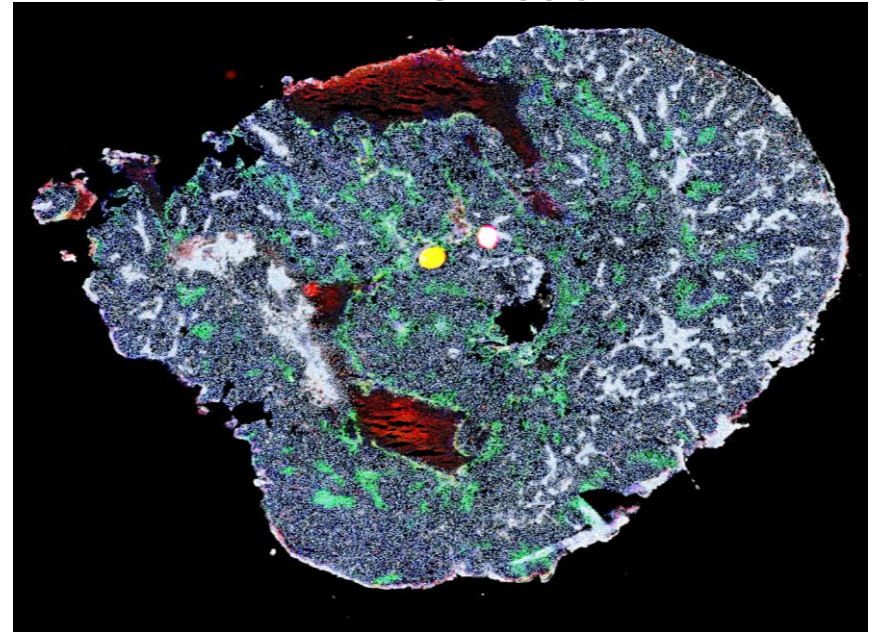
Injections:
PIMO-180min
EdU-30min
Hoechst-1min

- Cell segmentation on DAPI using StarDist deep learning
- Live/dead cells identified using an object classifier trained from manually annotated images

Hoechst **DAPI** **CD31** **PIMO**



Live Dead



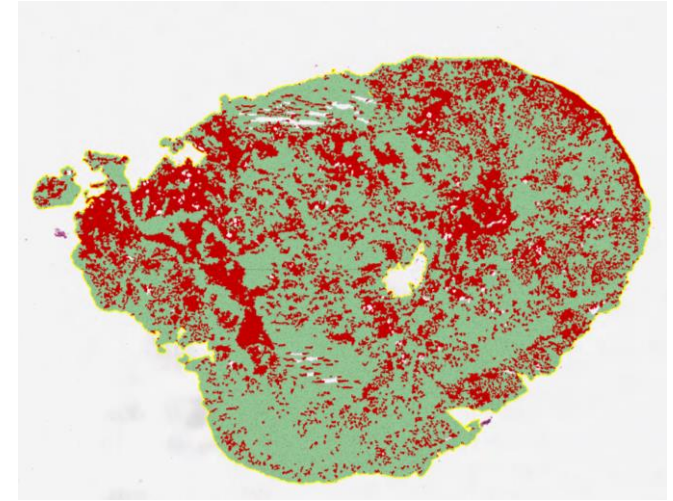
Analysis pipeline – stroma/tumour



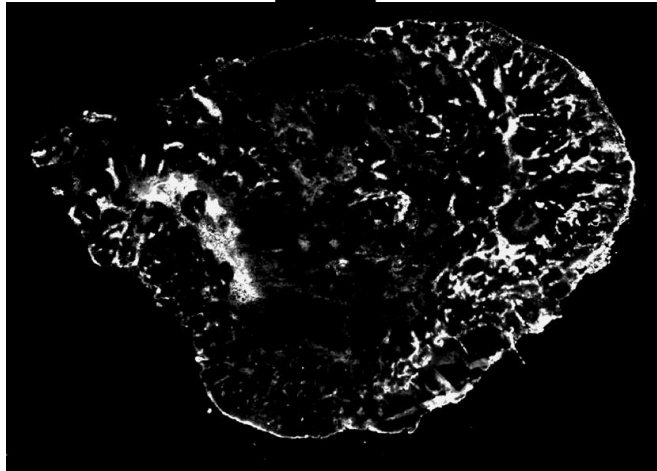
Injections:
PIMO-180min
EdU-30min
Hoechst-1min



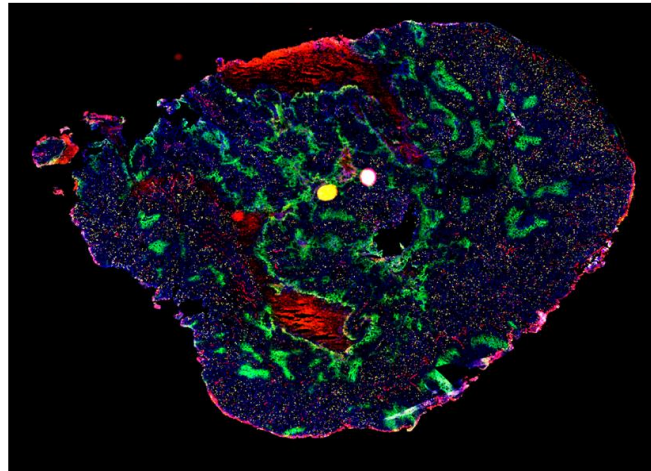
- Epithelia (tumour) and stroma regions distinguished using an object classifier based on pathologist annotated images.



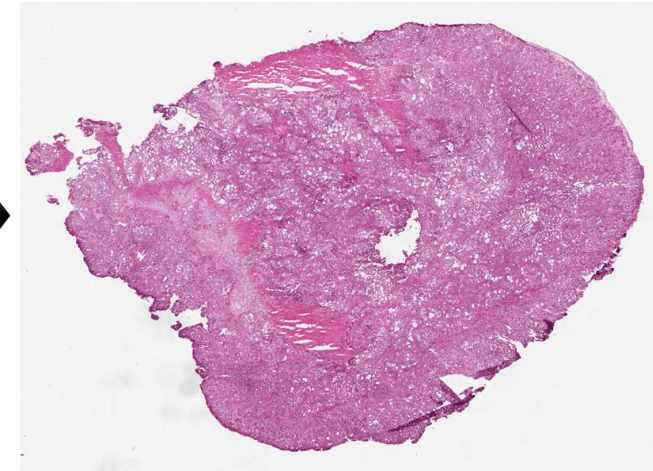
Hoechst



DAPI CD31 PIMO EdU

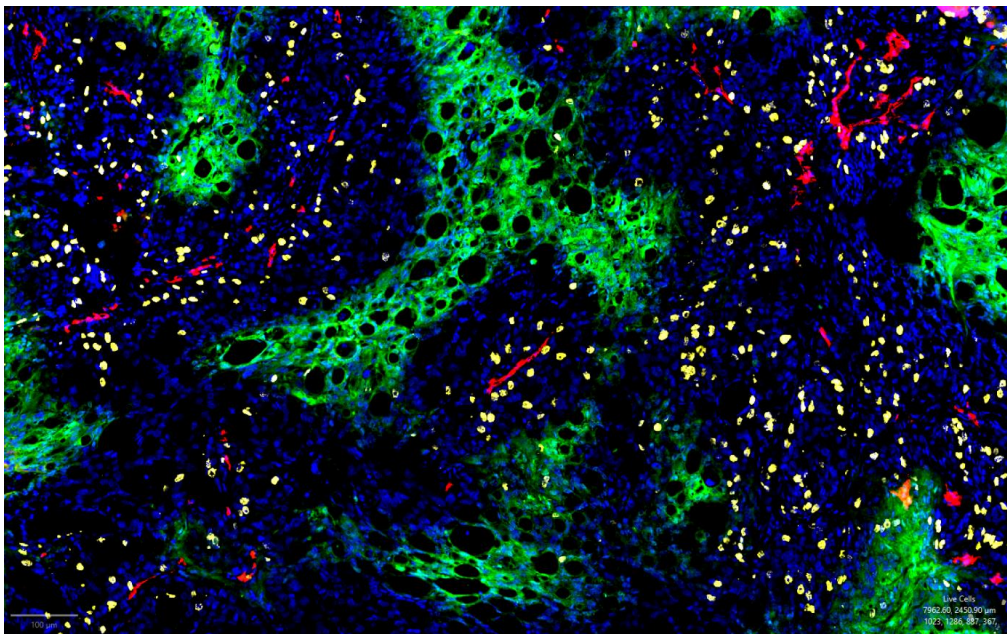


HE

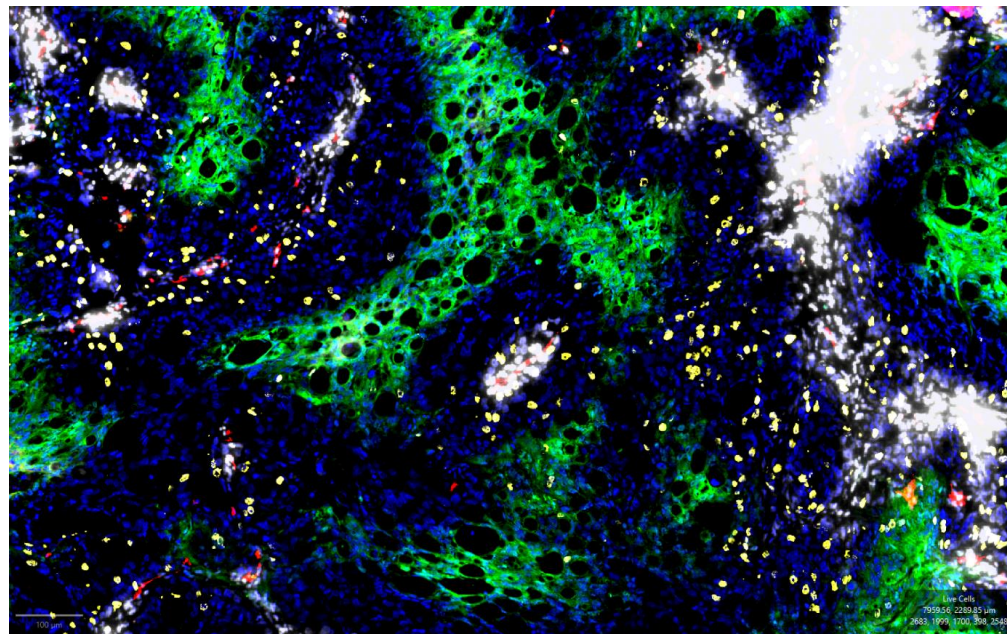


Delivery - Vessel detection and perfusion

DAPI CD31 PIMO EdU



Hoechst DAPI CD31 PIMO EdU

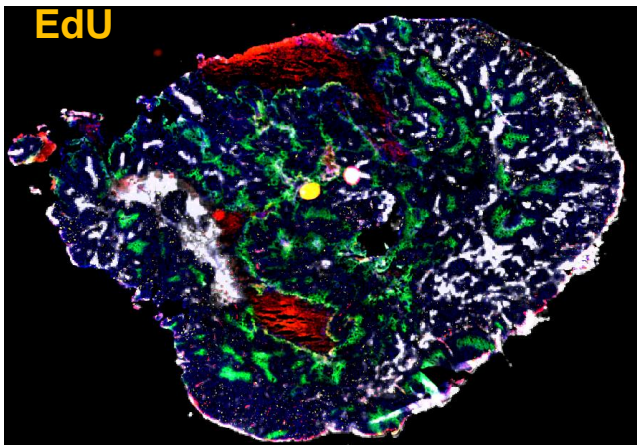


Perfusion metrics - example

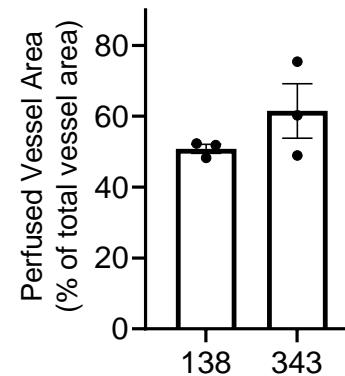
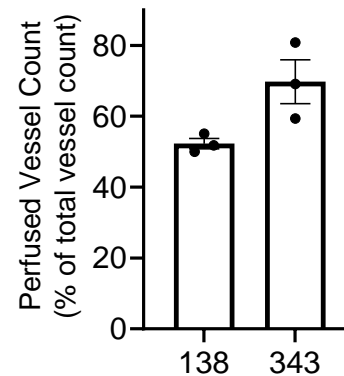
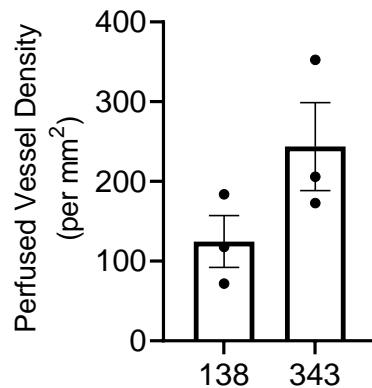
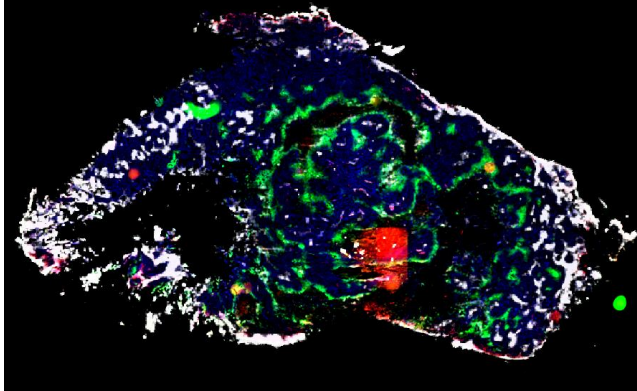
Hoechst DAPI CD31 PIMO

EdU

138



343

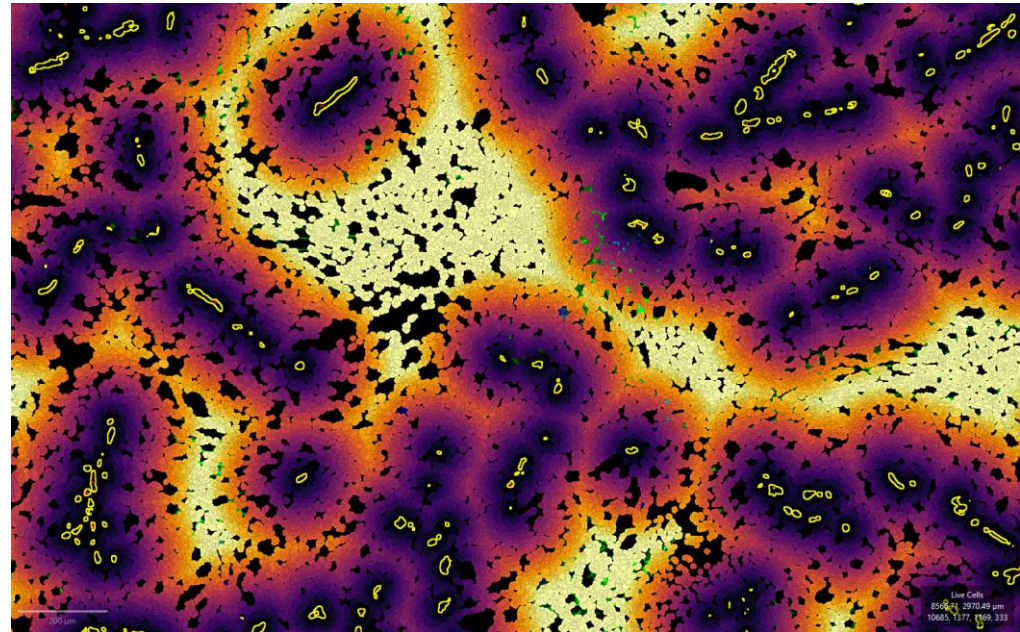
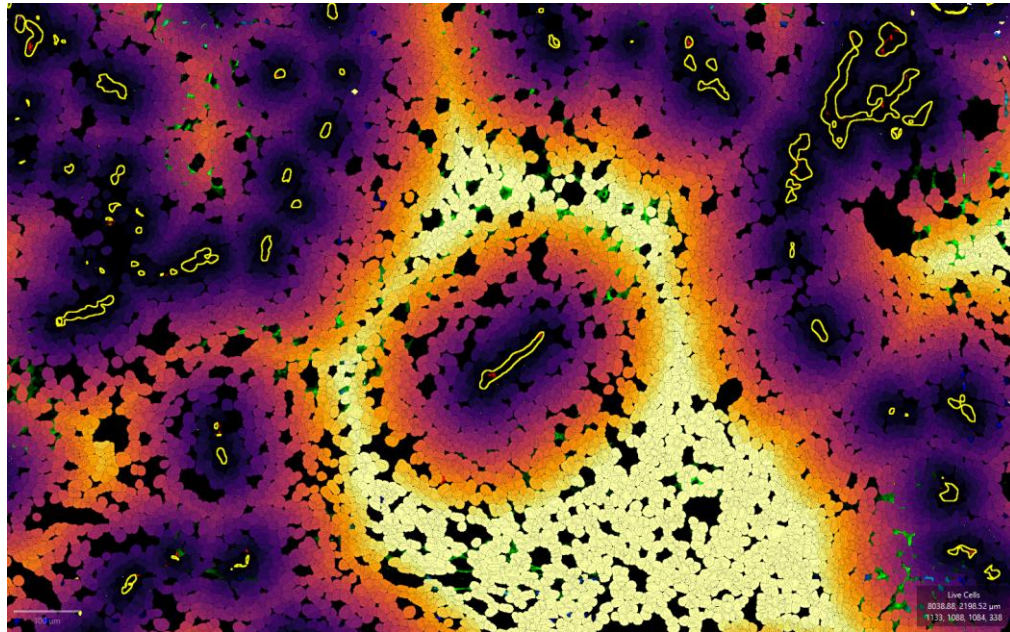


Hypoxia spatial metrics

- Distance to centroid of the nearest perfused vessel was calculated for each cell.

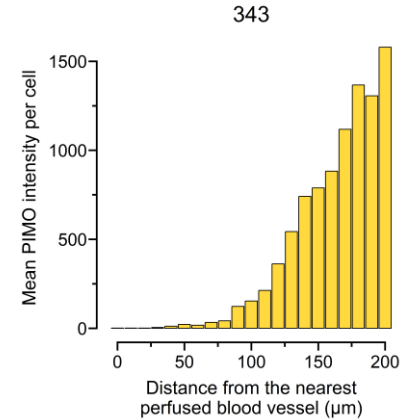
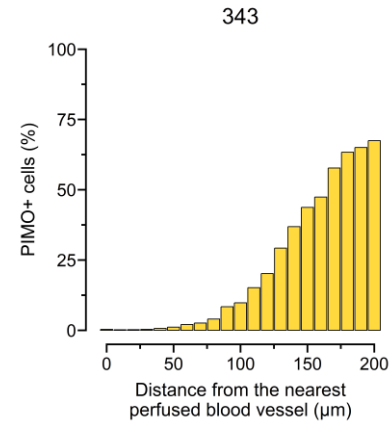
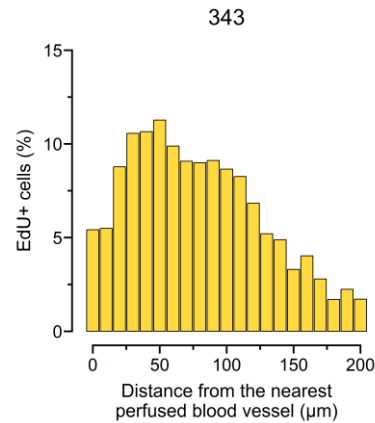
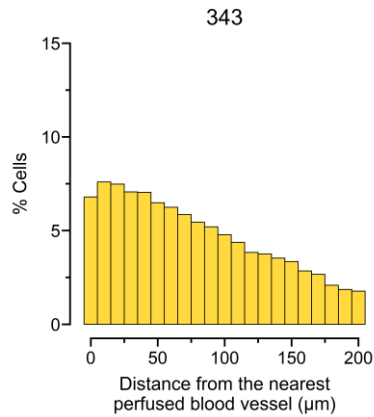
Heatmap Scale (μm)  0.00 200.00

Distance map

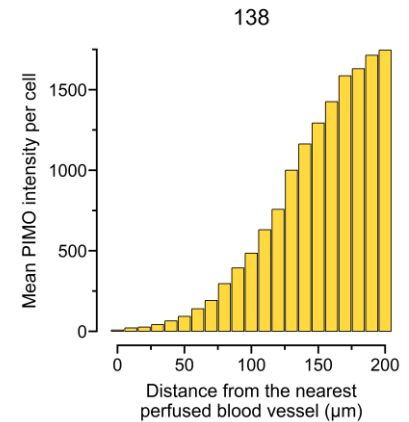
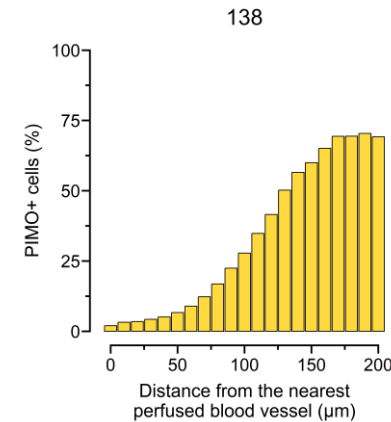
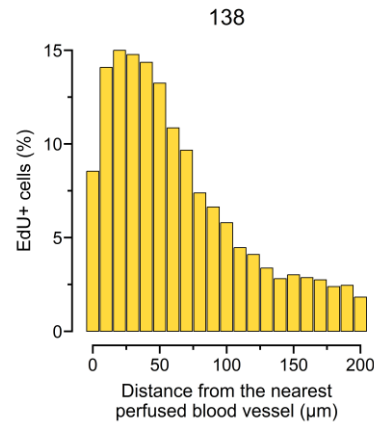
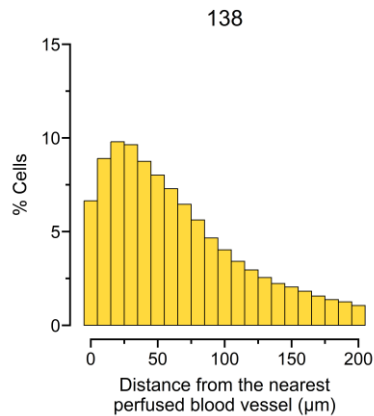


Spatial gradients in hypoxia and proliferation

- Individual cell parameters can be analyzed relative to their spatial position



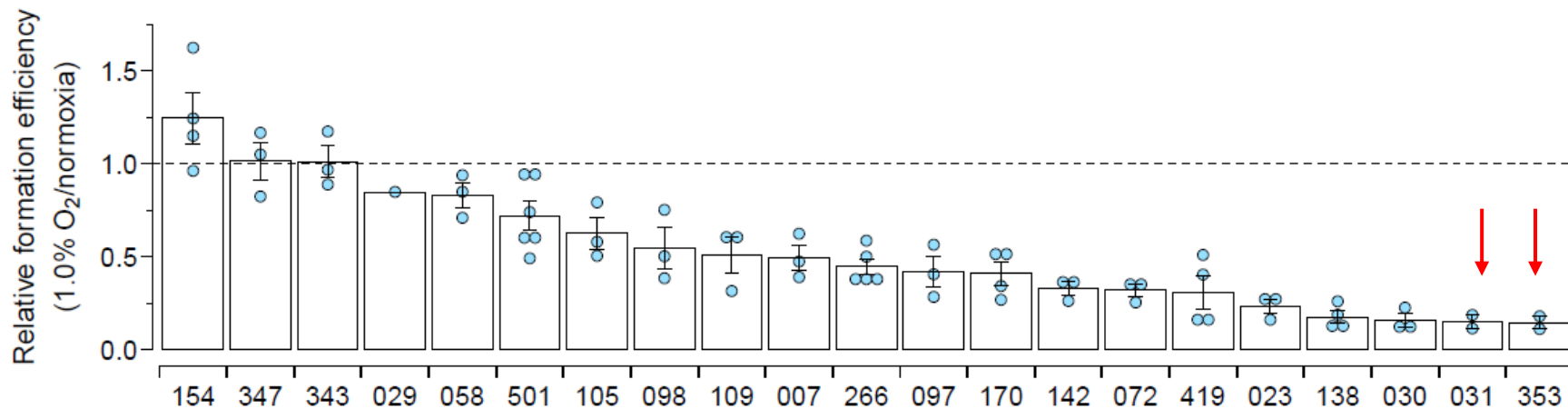
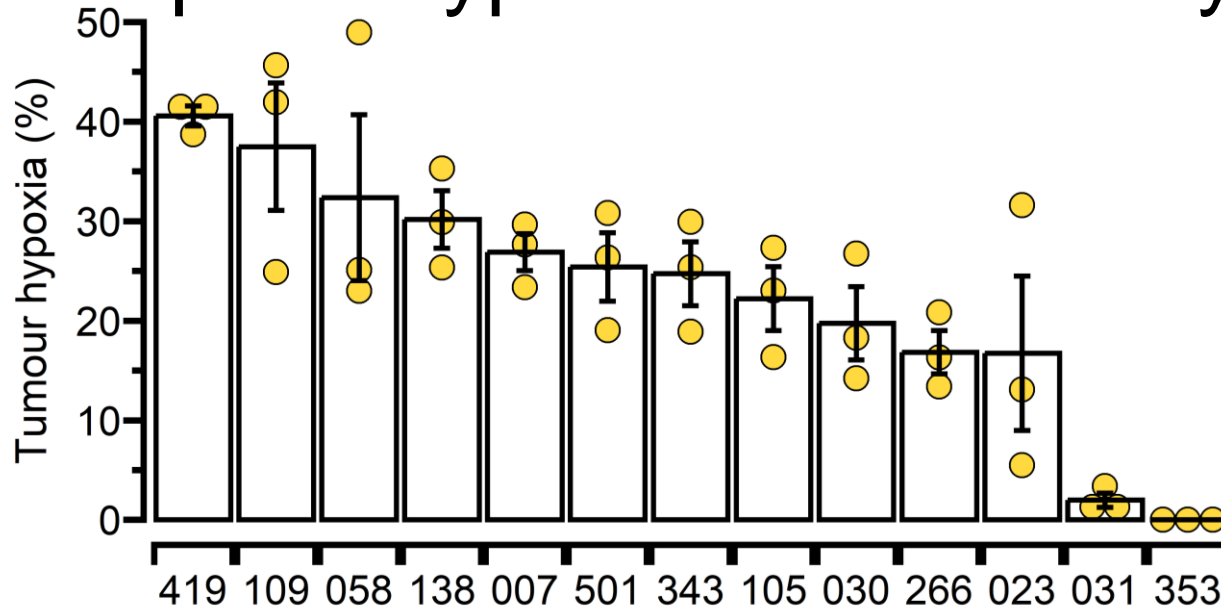
- In this example, each parameter is grouped into 10 μm distance bins from perfused vessels



Metabolic and hypoxic phenotypes captured *in vivo*

Category	Parameters
Individual cell classification	Live/dead (%Necrosis) Epithelial/stroma
Single-channel analysis	Hypoxic fraction (PIMO+ cells) Proliferative fraction(Edu+ cells)
Perfusion	CD31+ vessel density Perfused vessel density (per tumour area) Perfused vessel count (% of total) Perfused vessel area (% of total)
Spatial gradients based on distance metrics to perfused vessels	Cell density gradients Oxygen gradient (oxygen consumption) Pimo intensity gradients (tolerance) Acute hypoxia Proliferation gradients

Do *in vitro* phenotypes drive *in vivo* hypoxia?

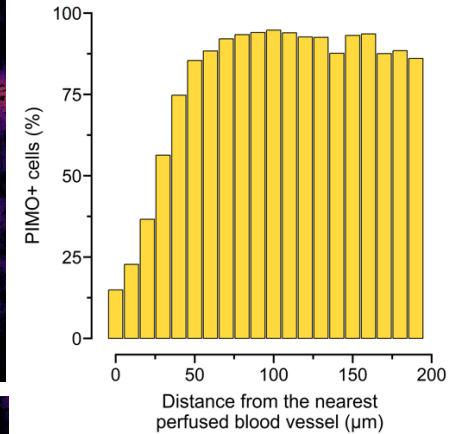
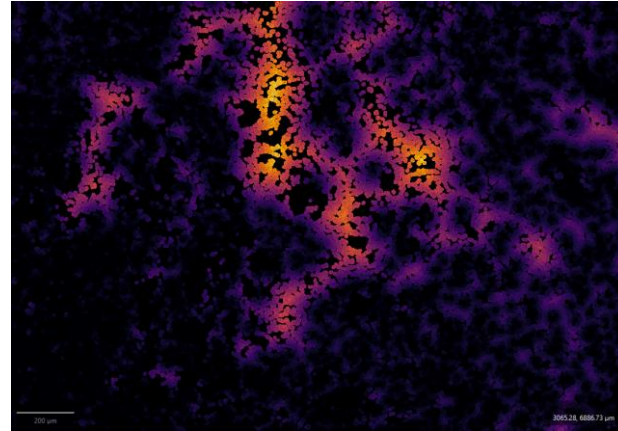
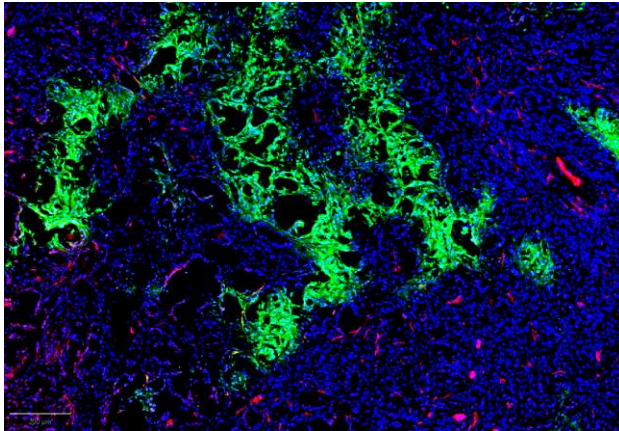


In vitro OCR and hypoxia tolerance correlate with oxygen gradients and oxygen levels *in vivo*

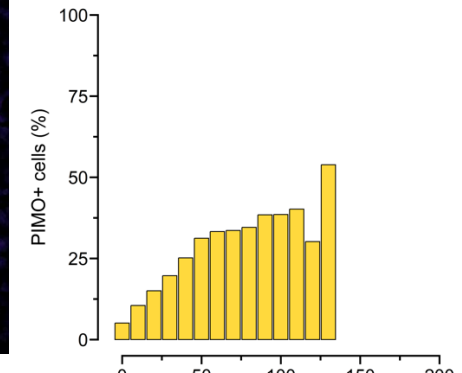
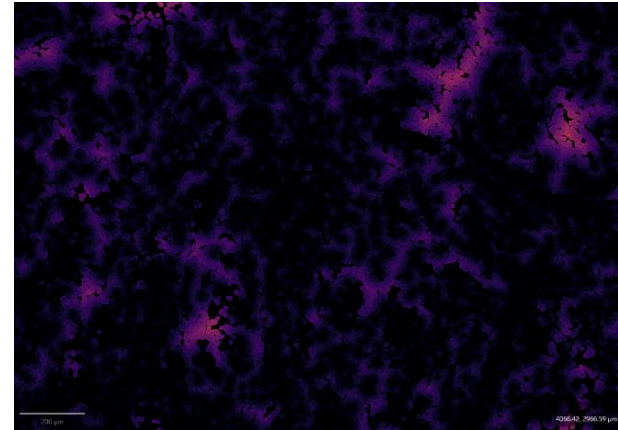
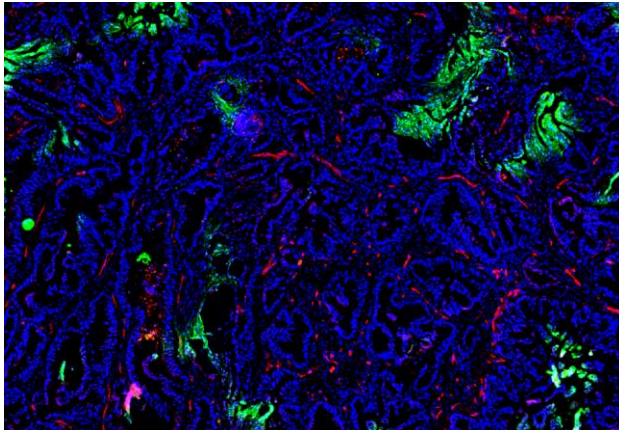
CD31 PIMO

Distance

058
High OCR
High tolerance

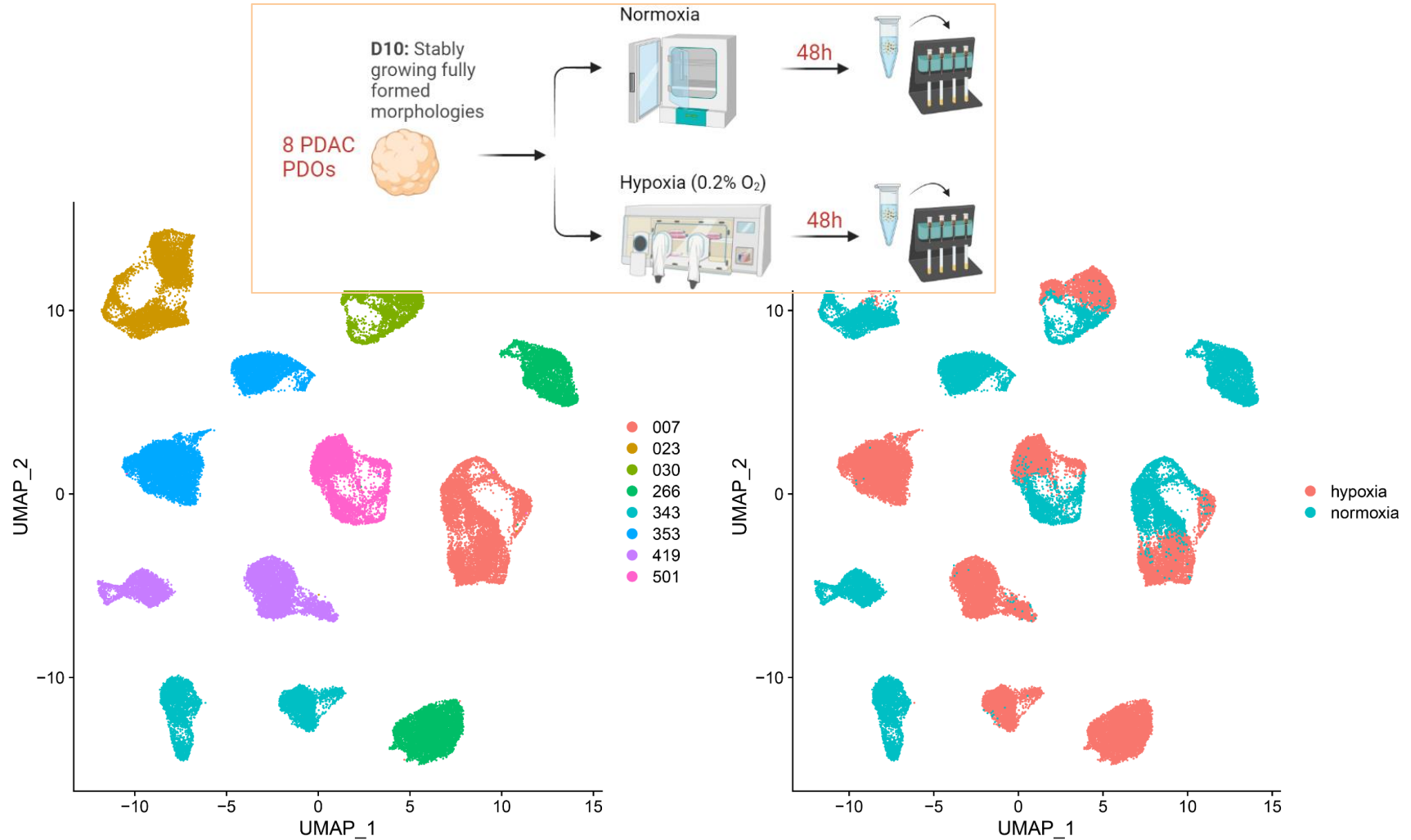


023
Low OCR
Low tolerance

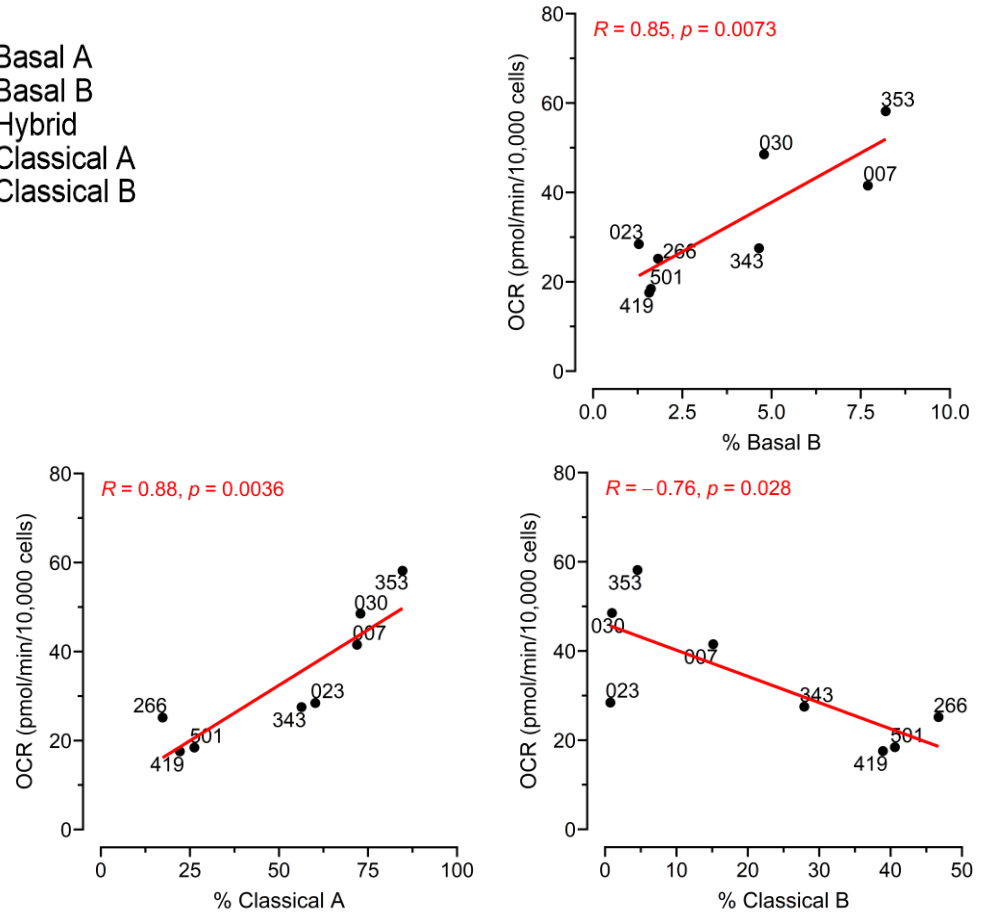
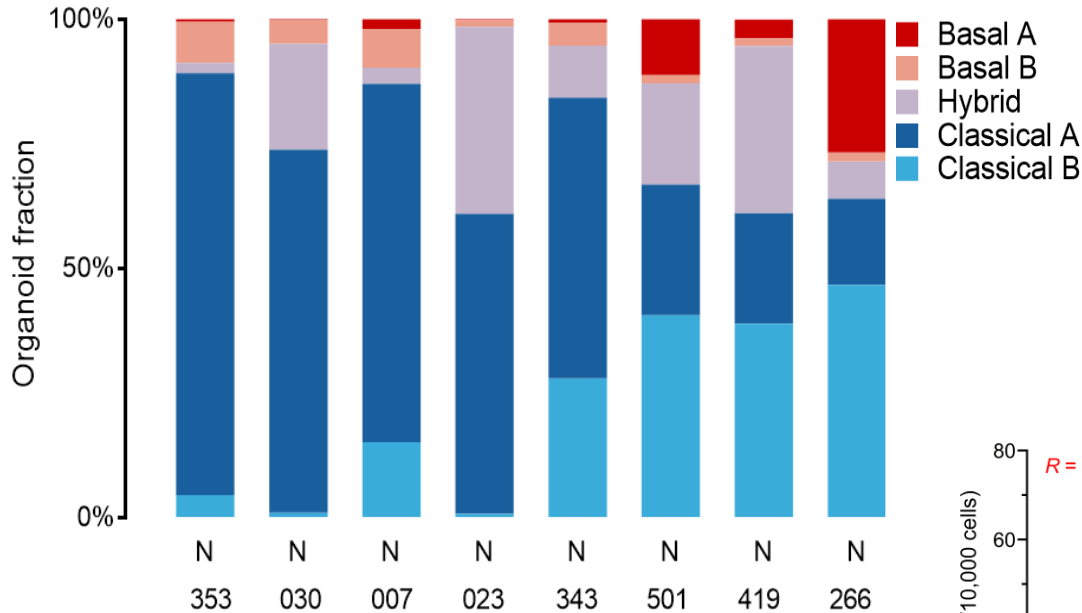


What are the underlying molecular determinants of oxygen metabolism and hypoxia tolerance?

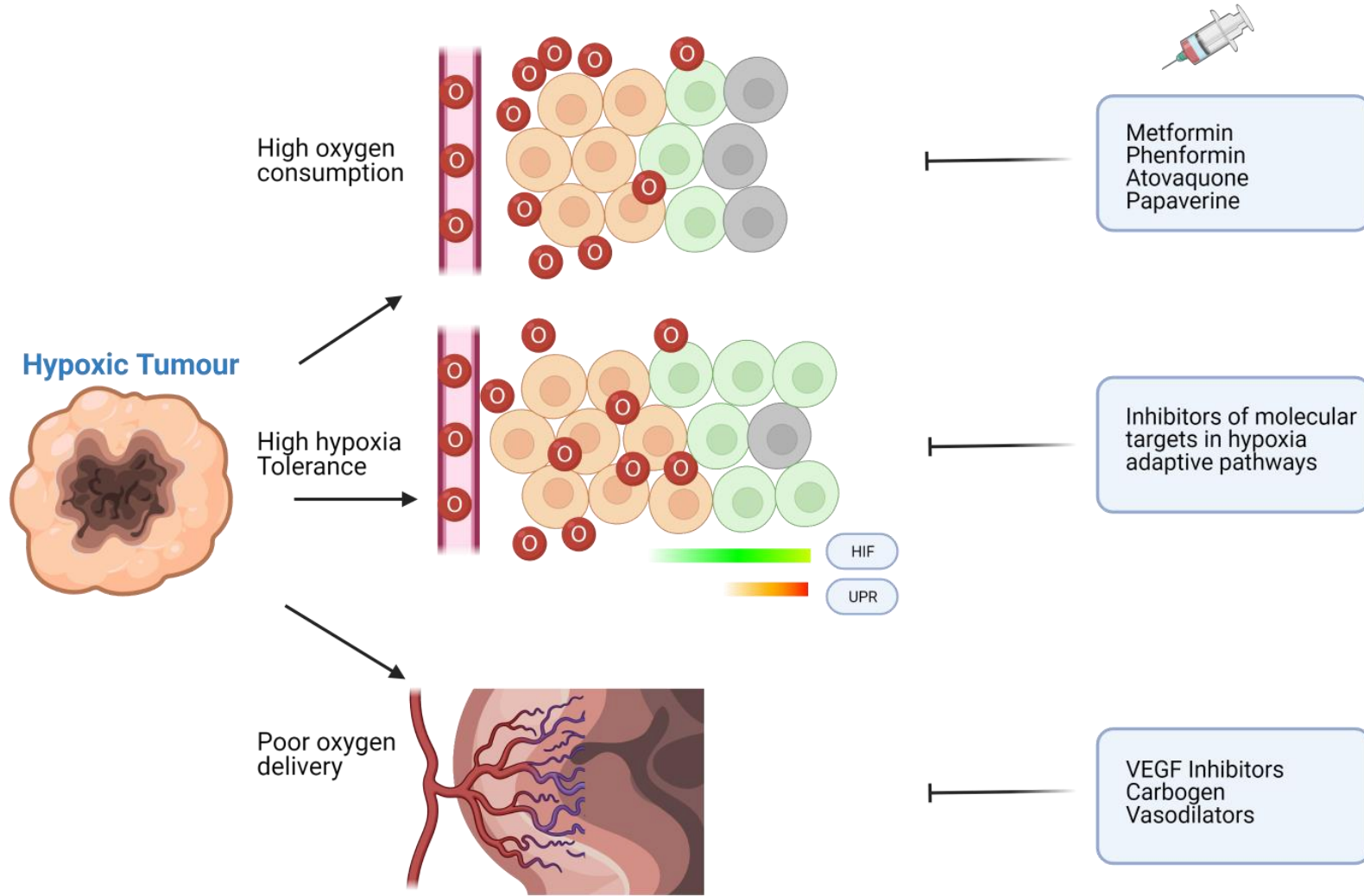
Transcriptional subtypes in PDAC organoids



PDOs transcriptional subtypes associate with oxygen metabolism



Personalized targeting of hypoxia



Lessons

- Key biological properties that impact radiation response are highly tumour specific
 - All approaches to change RT schedules, combinations will have differential effects on patients
- Therapeutic approaches must be developed in concert with patient selection
 - Treatment strategy addresses both causes and consequences of hypoxia at the patient level
- Understanding the contributors to patient variation in tumor biology are needed to inform future radiation treatment
 - Protons, ions, dose/fx, SBRT, drug combinations



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