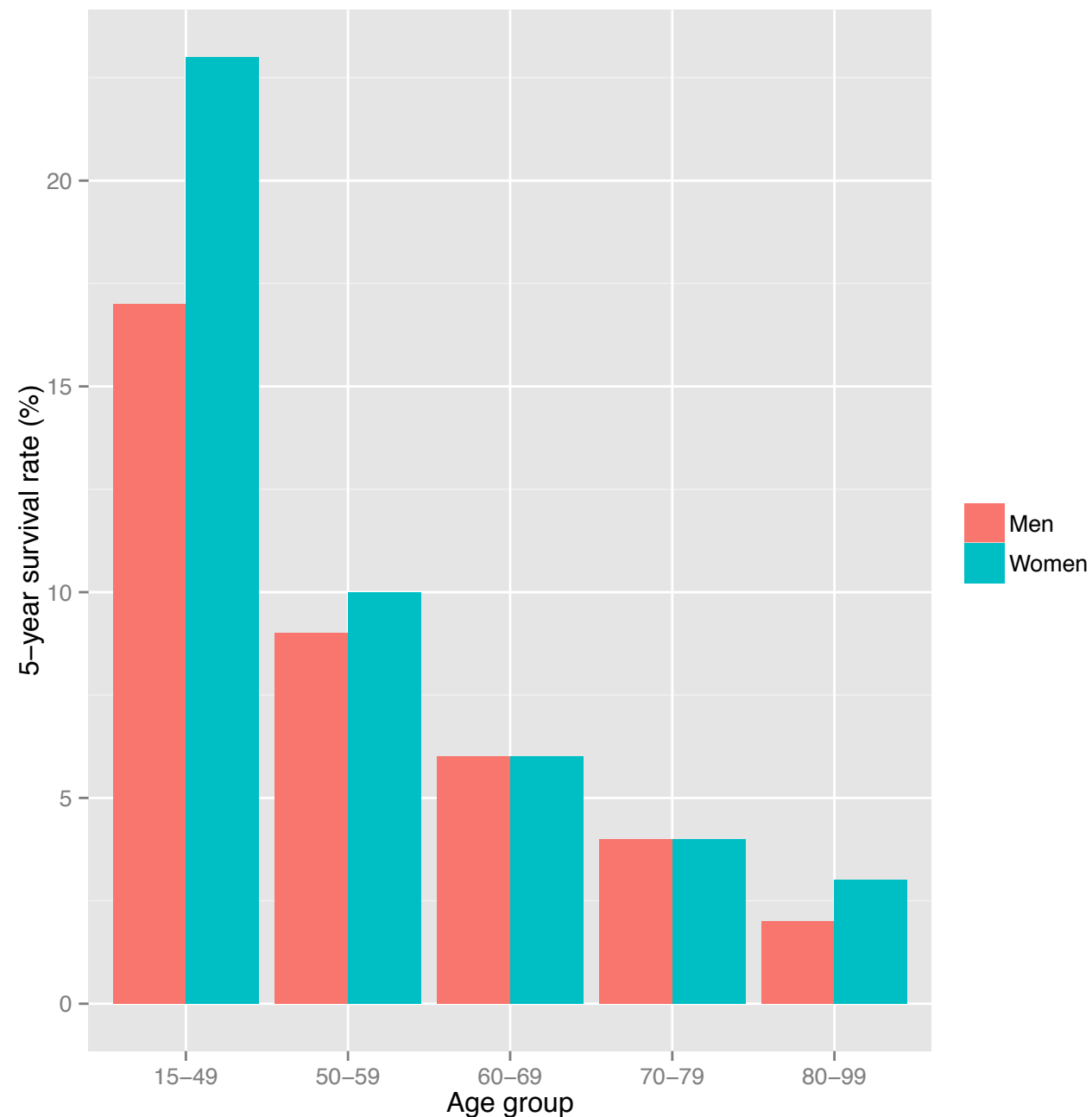




# Hypoxia and Circulating Tumor Cells in Pancreatic Cancer

Douglas Wu  
Lambowitz lab

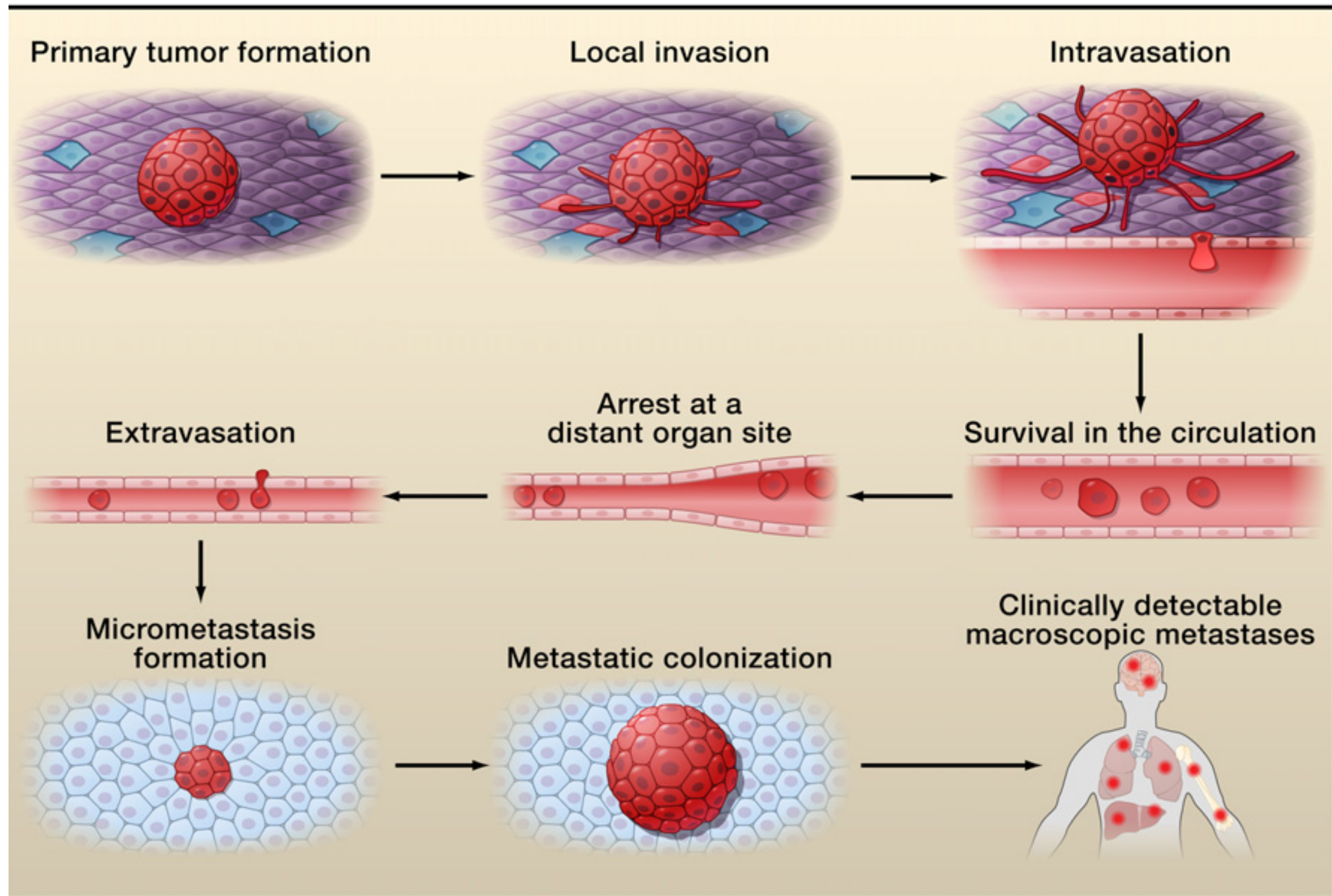
# Pancreatic cancer



- Low survival rate
- Highly metastatic

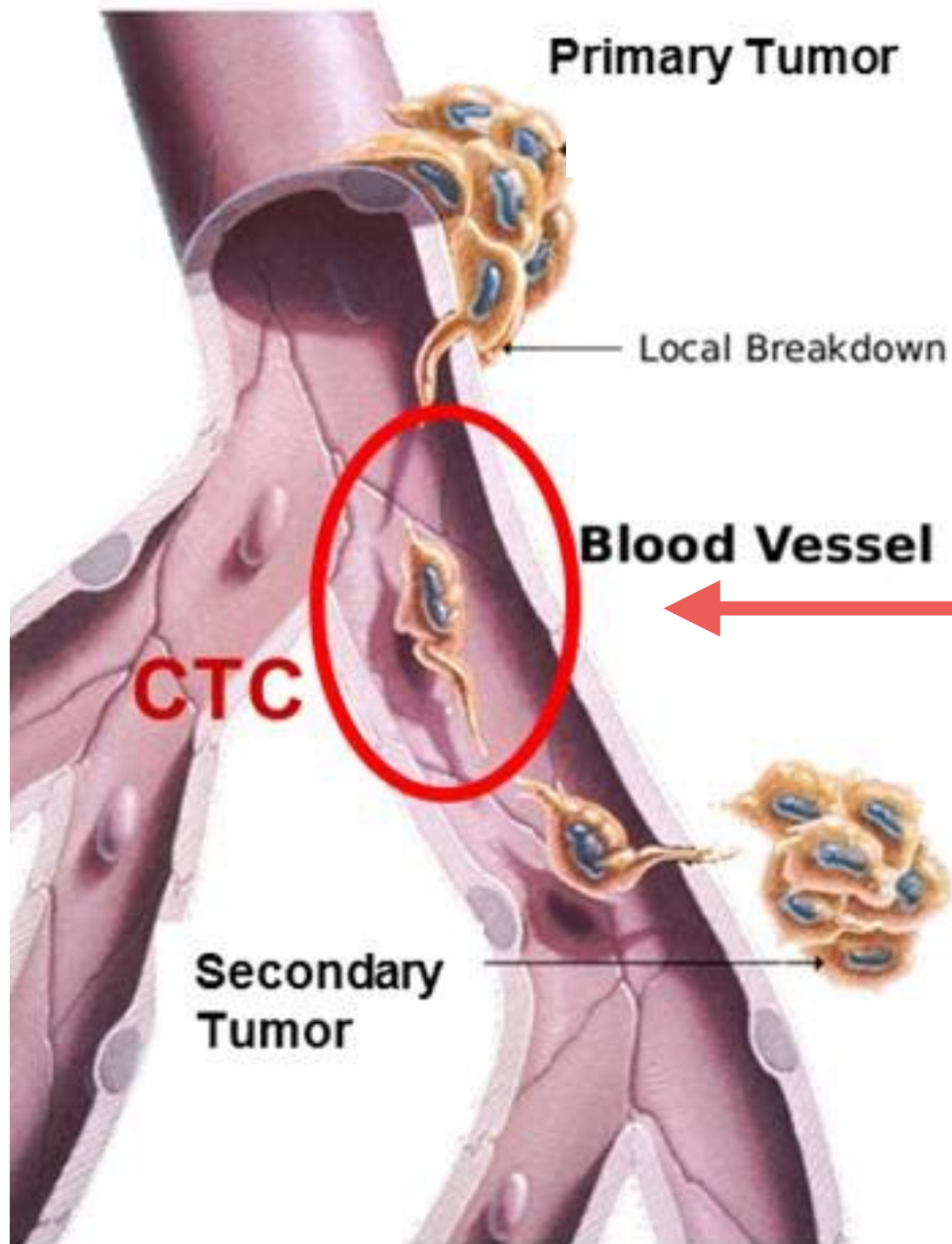


# Metastasis





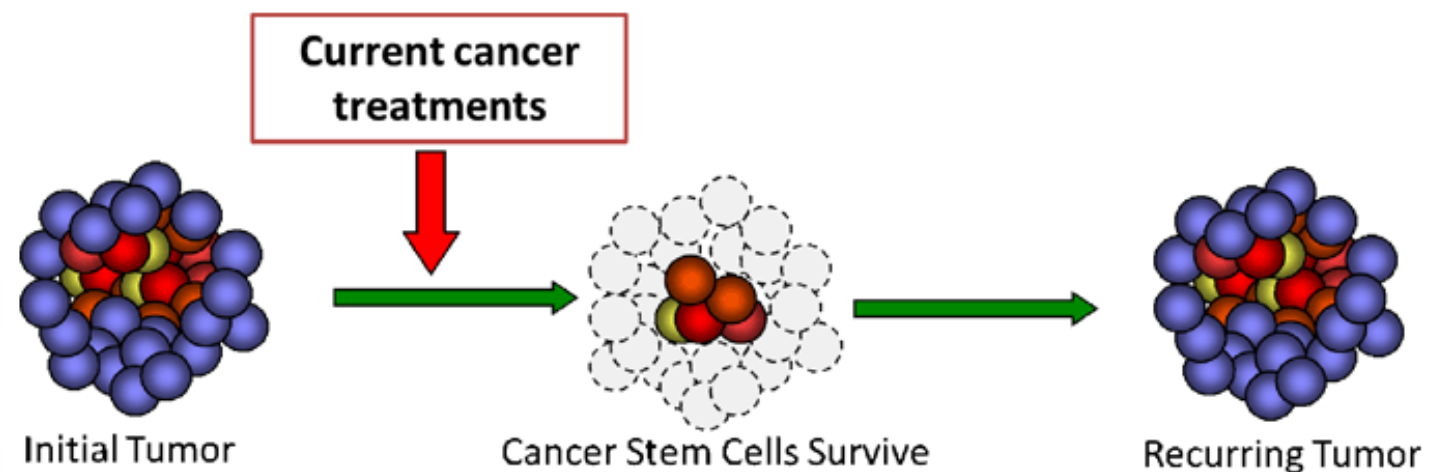
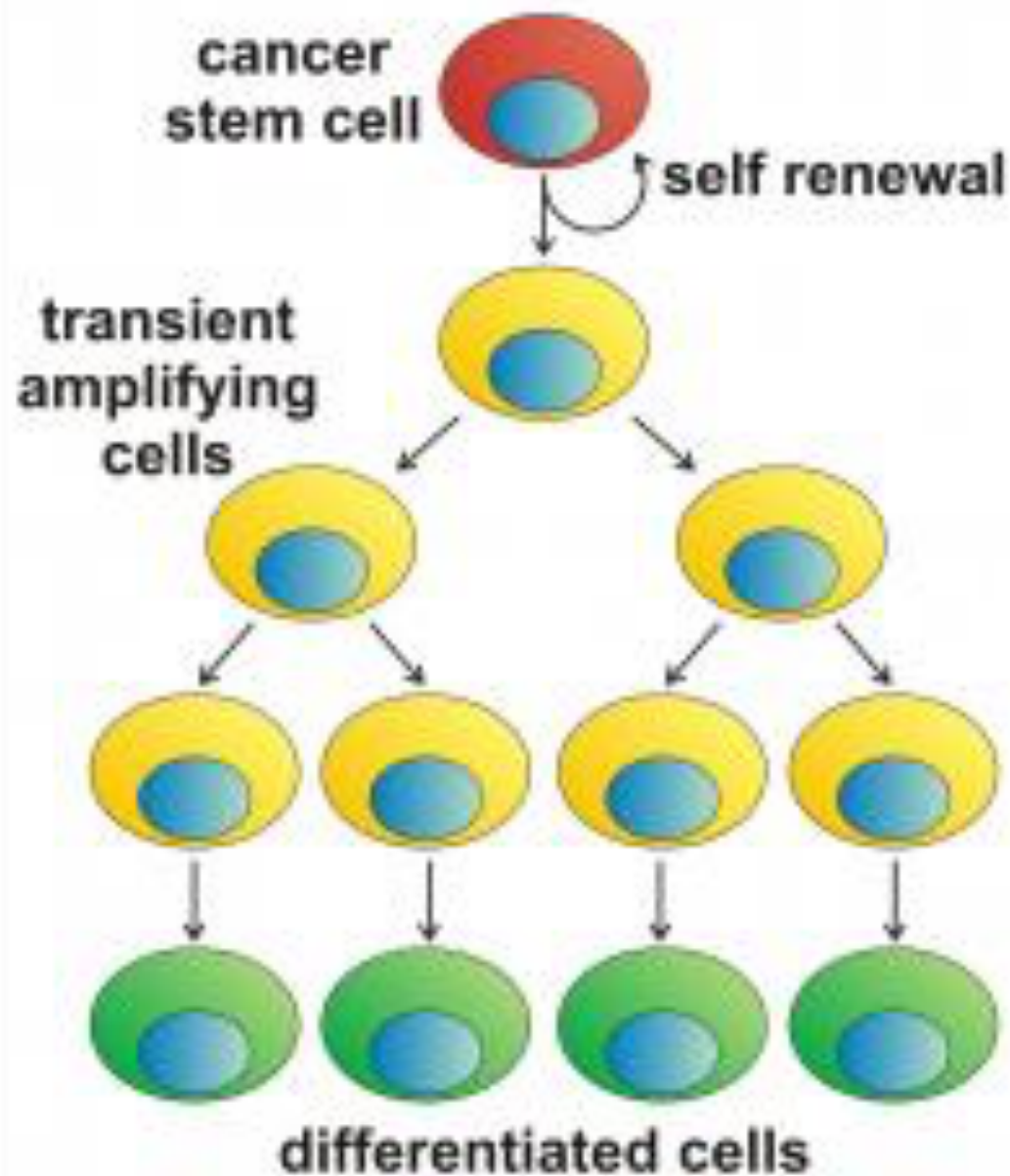
# Circulating tumor cells



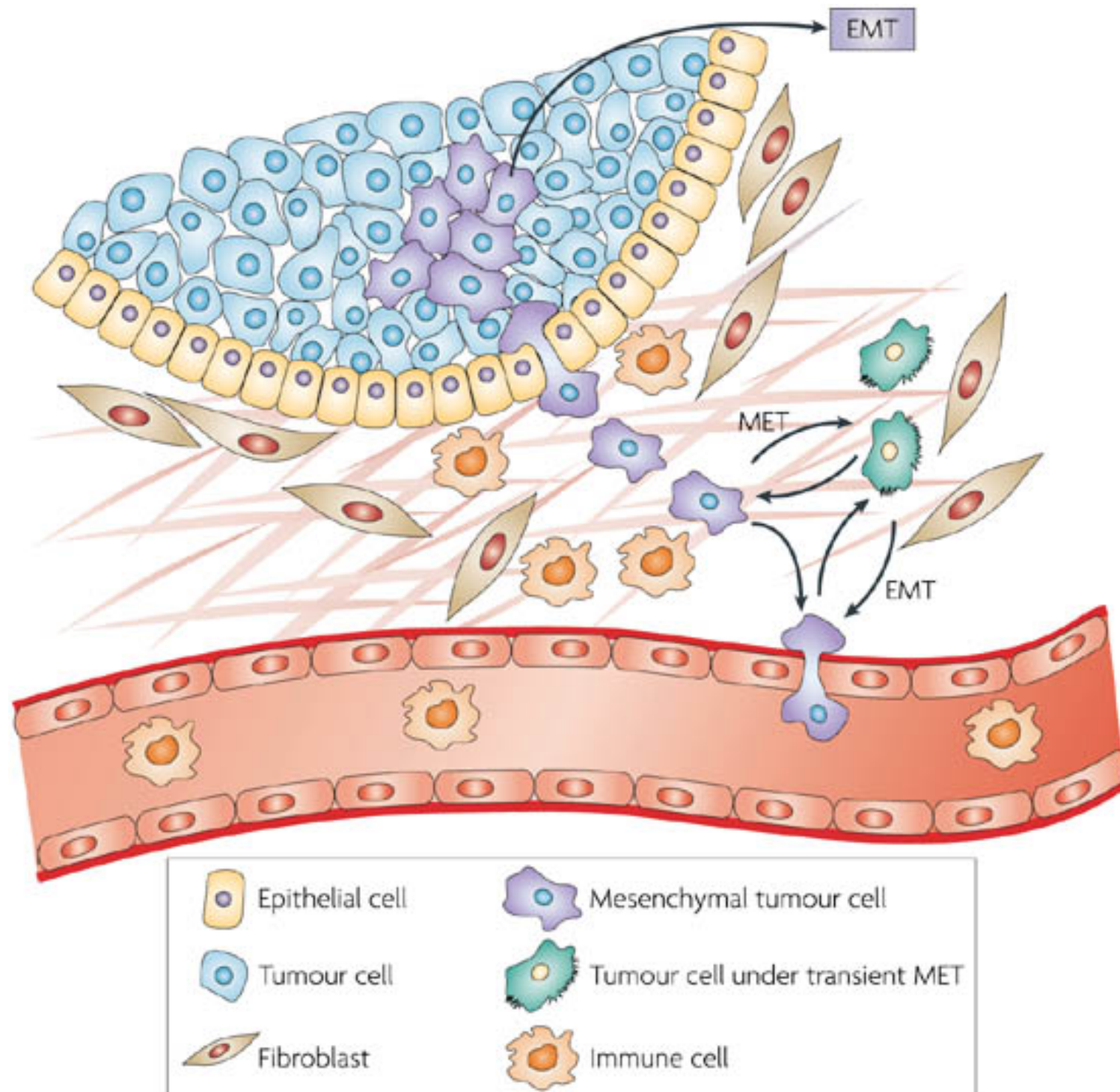
Adapted from National Cancer  
Institute

# Cancer stem cells

## The cancer stem cell model



# Epithelial-mesenchymal transition promotes invasion



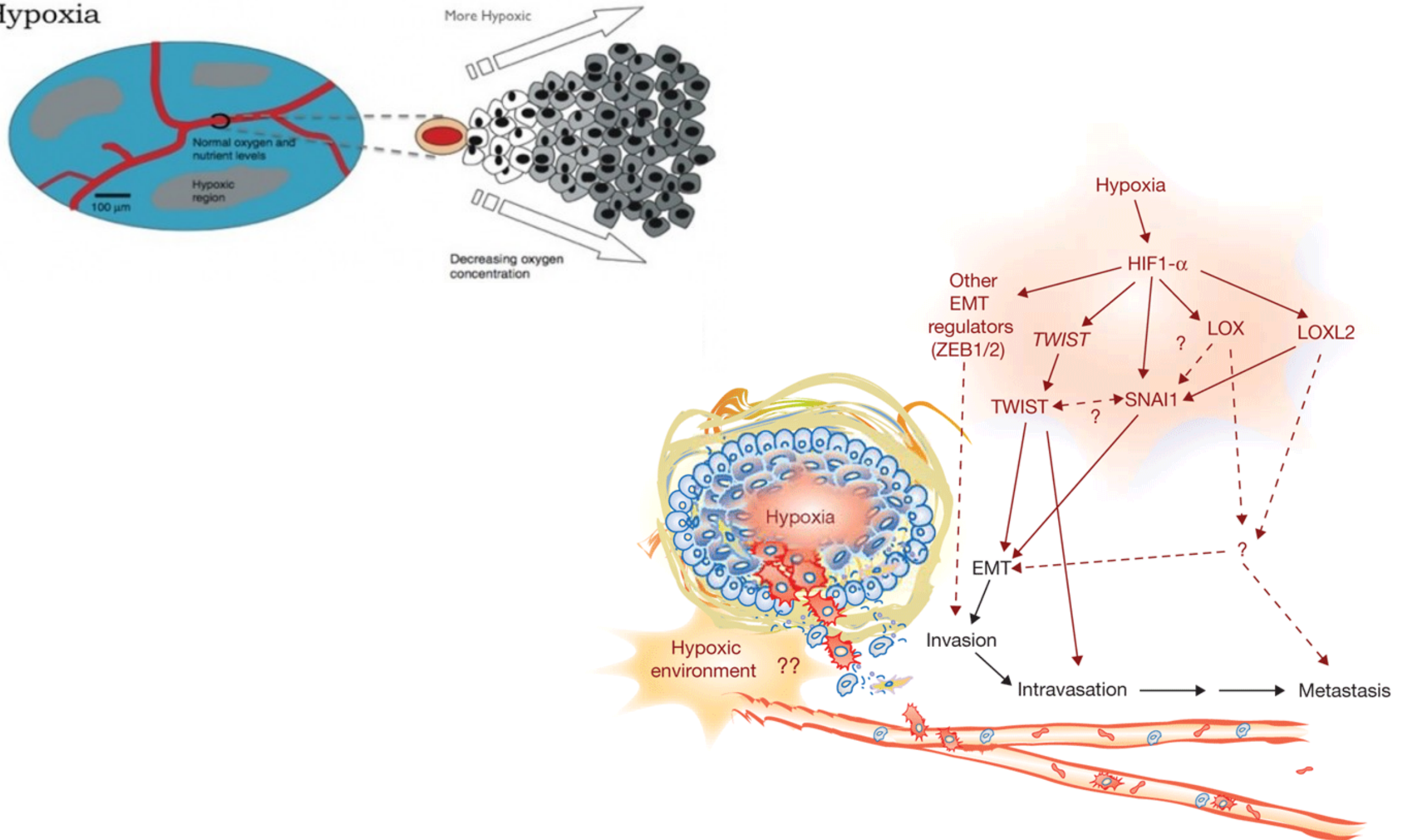
Nature Reviews | Cancer

Peinado, et al. *Nat Rev.* 2007  
<http://blogs.scientificamerican.com/guest-blog/2013/10/30/the-hallmarks-of-cancer-6-tissue-invasion-and-metastasis/>

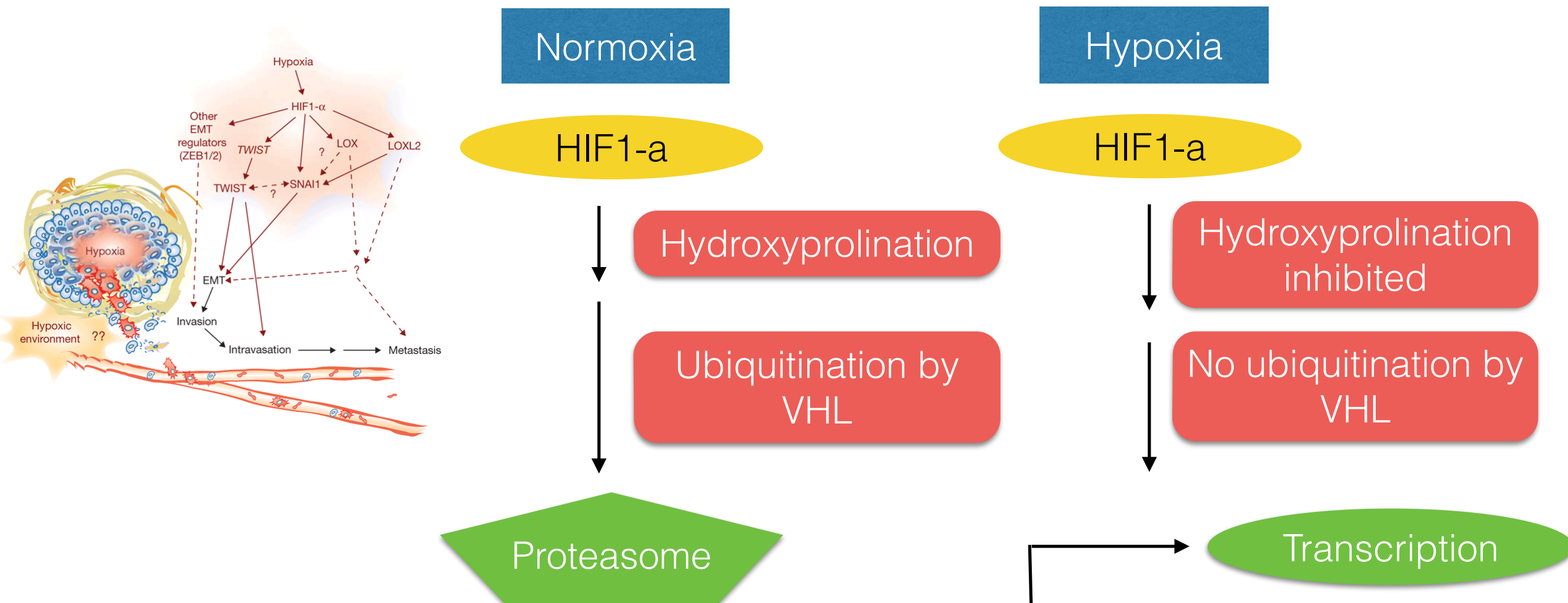


# Hypoxic environment promotes EMT

## Hypoxia



# Hypoxic environment promotes EMT





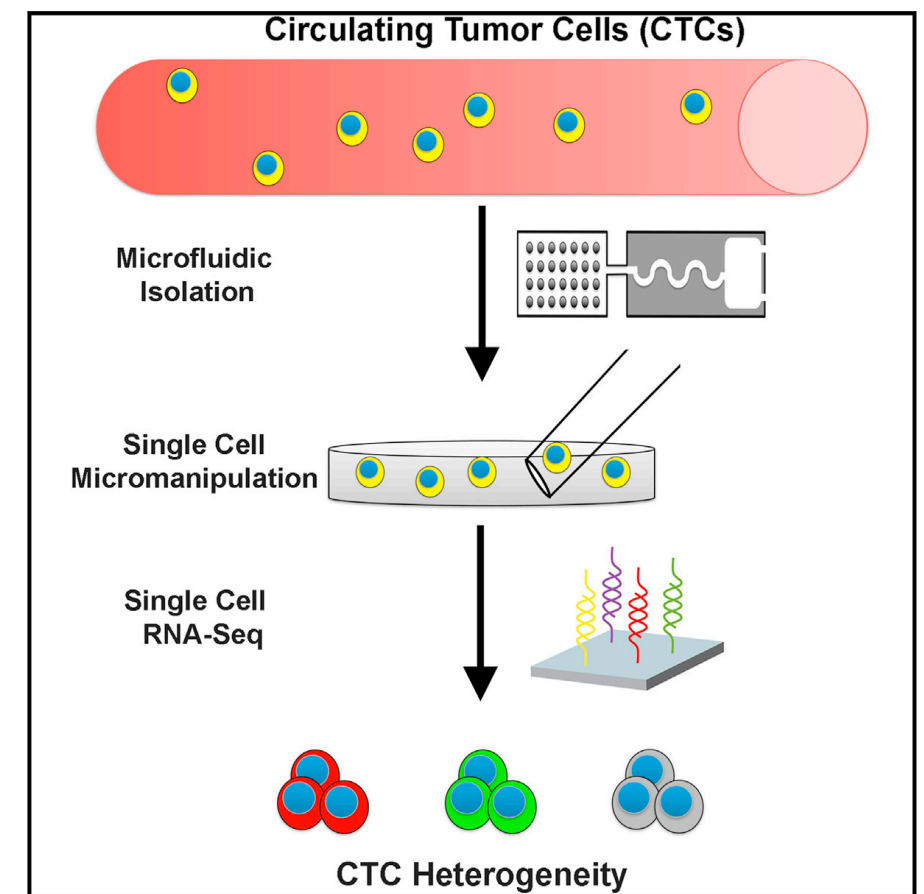
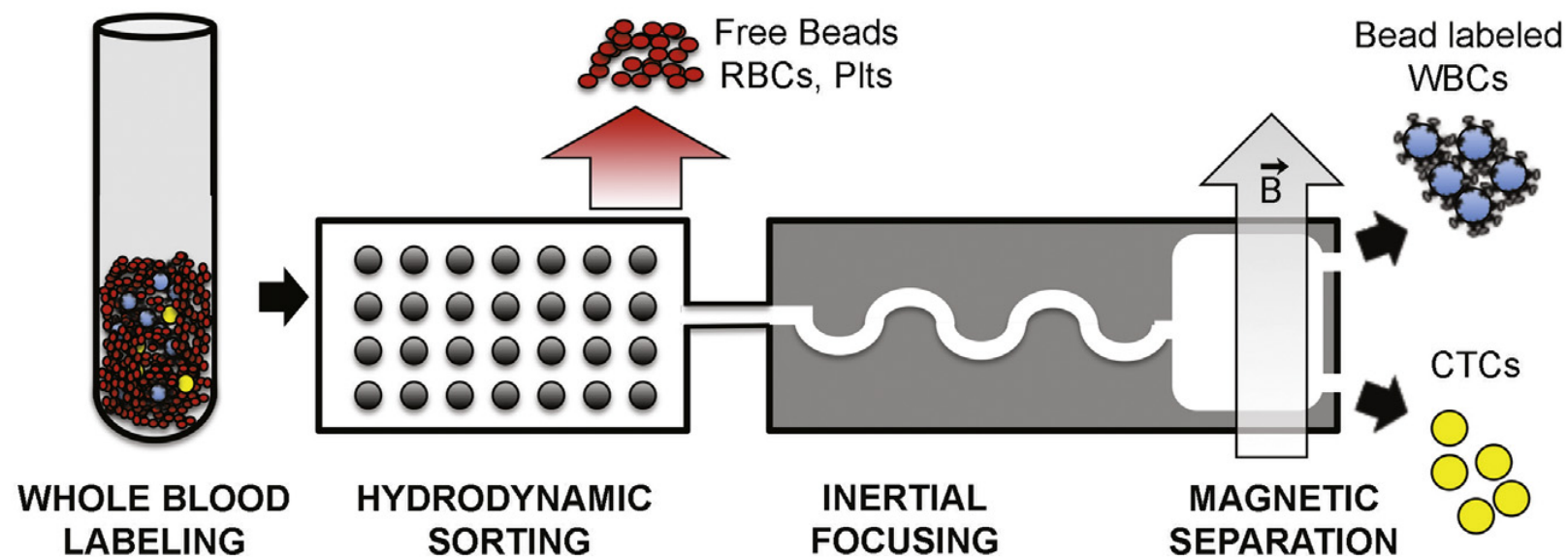
# Hypothesis

**Hypoxic environment promotes CTC production,  
intravasation and tumorigenicity**

# Hypothesis: Hypoxic environment promotes CTC production, intravasation and tumorigenicity

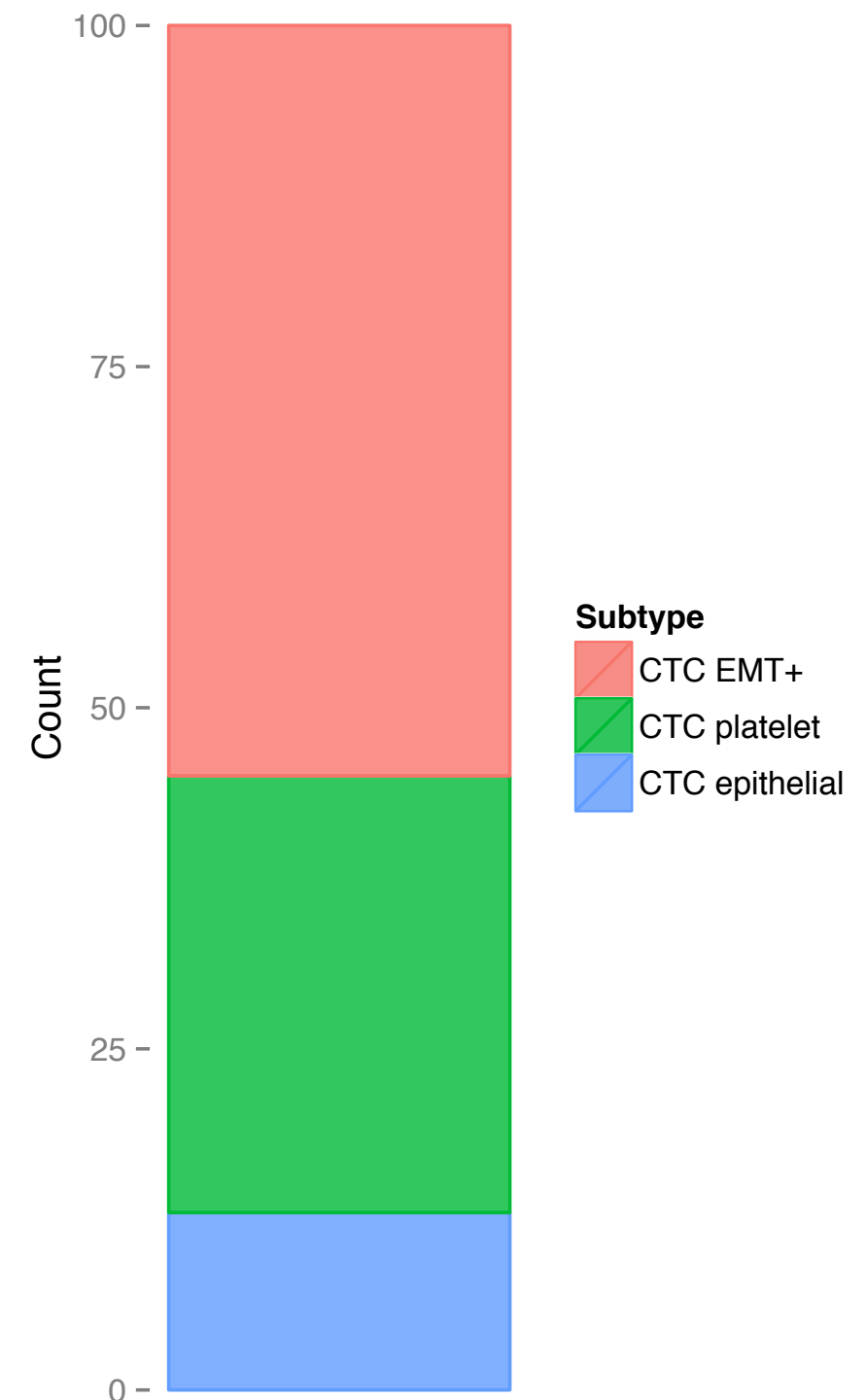
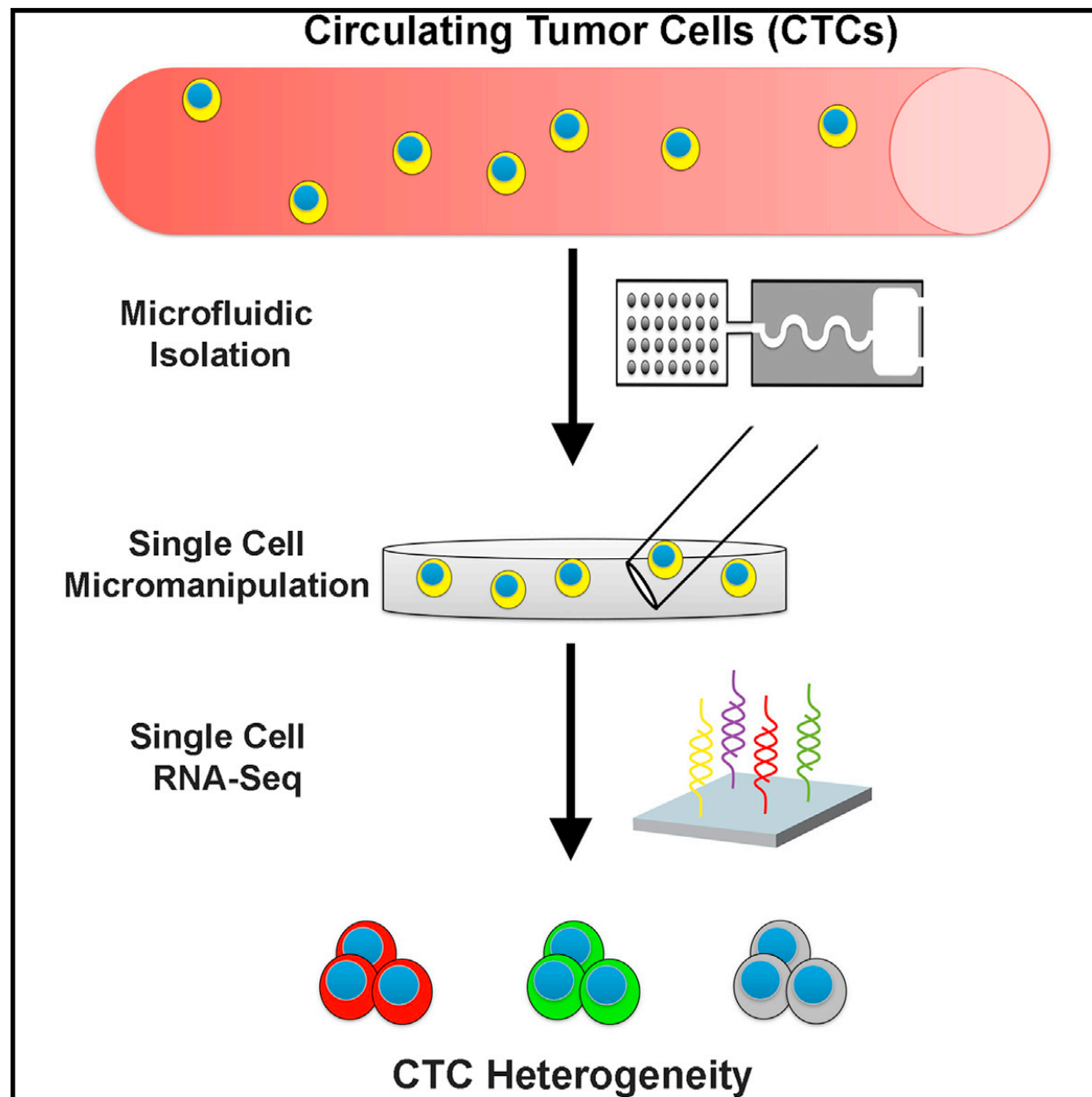
## Challenge: No clear definition of CTCs

### CTC-iChip



# Hypothesis: Hypoxic environment promotes CTC production, intravasation and tumorigenicity

## CTC heterogeneity





# HIF1-a mRNA is more abundant in CTCs



- HIF1-a can be post-transcriptionally regulated through its 3' UTR

Yasuda et al. *BBRC*. 2014

## annotation

- circulating tumor cells (n=109)
- primary tumor (TuGMP3) (n=38)

Thanks to Pubmed's GEO Datasets!

# Hypothesis: Hypoxic environment promotes CTC production, intravasation and tumorigenicity

## Specific aims

1. Investigate up-regulation of molecules in the hypoxia signaling pathway in CTCs.
2. Identify the effects of hypoxia on CTC biogenesis.
3. Investigate hypoxia-mediated enhanced tumorigenicity of CTCs.

**Aim 1: Investigate up-regulation of molecules in the hypoxia signaling pathway.**

## **Hypothesis**

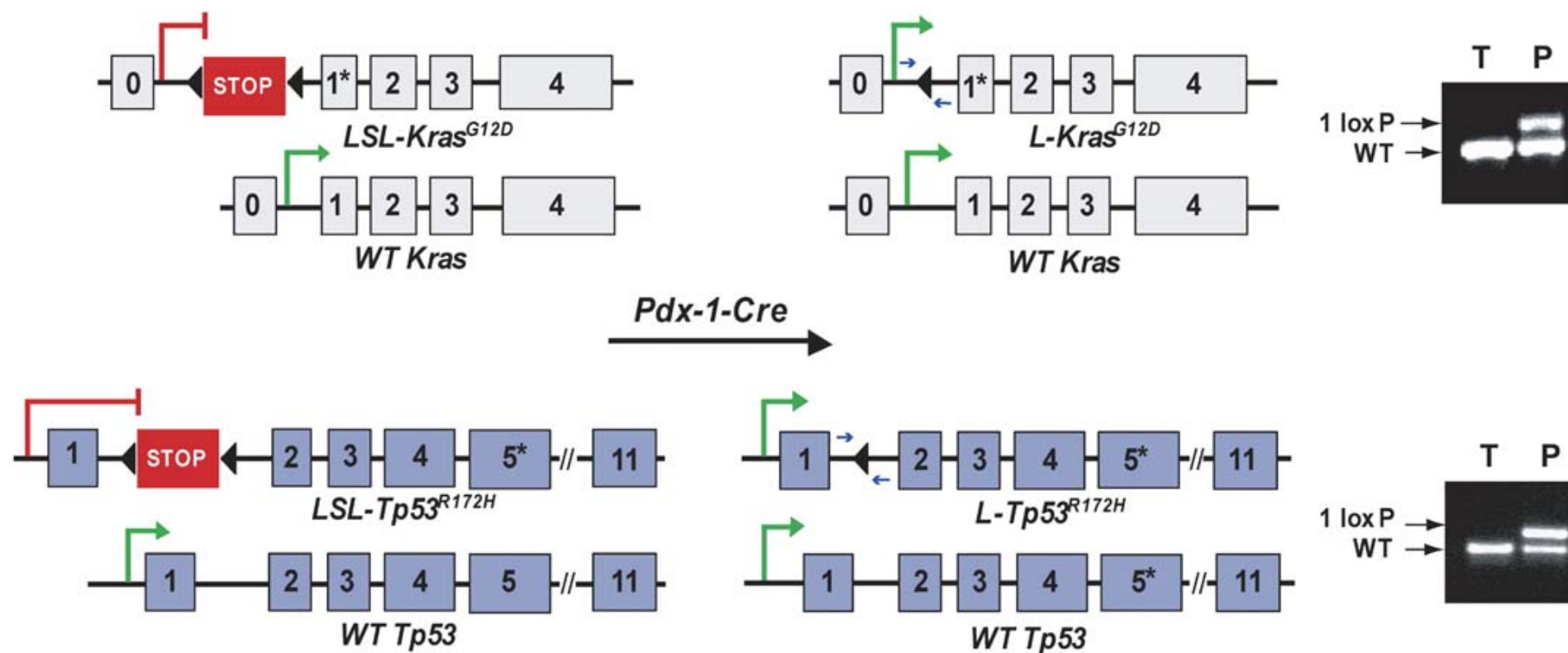
Hypoxia signaling is up-regulated in CTCs



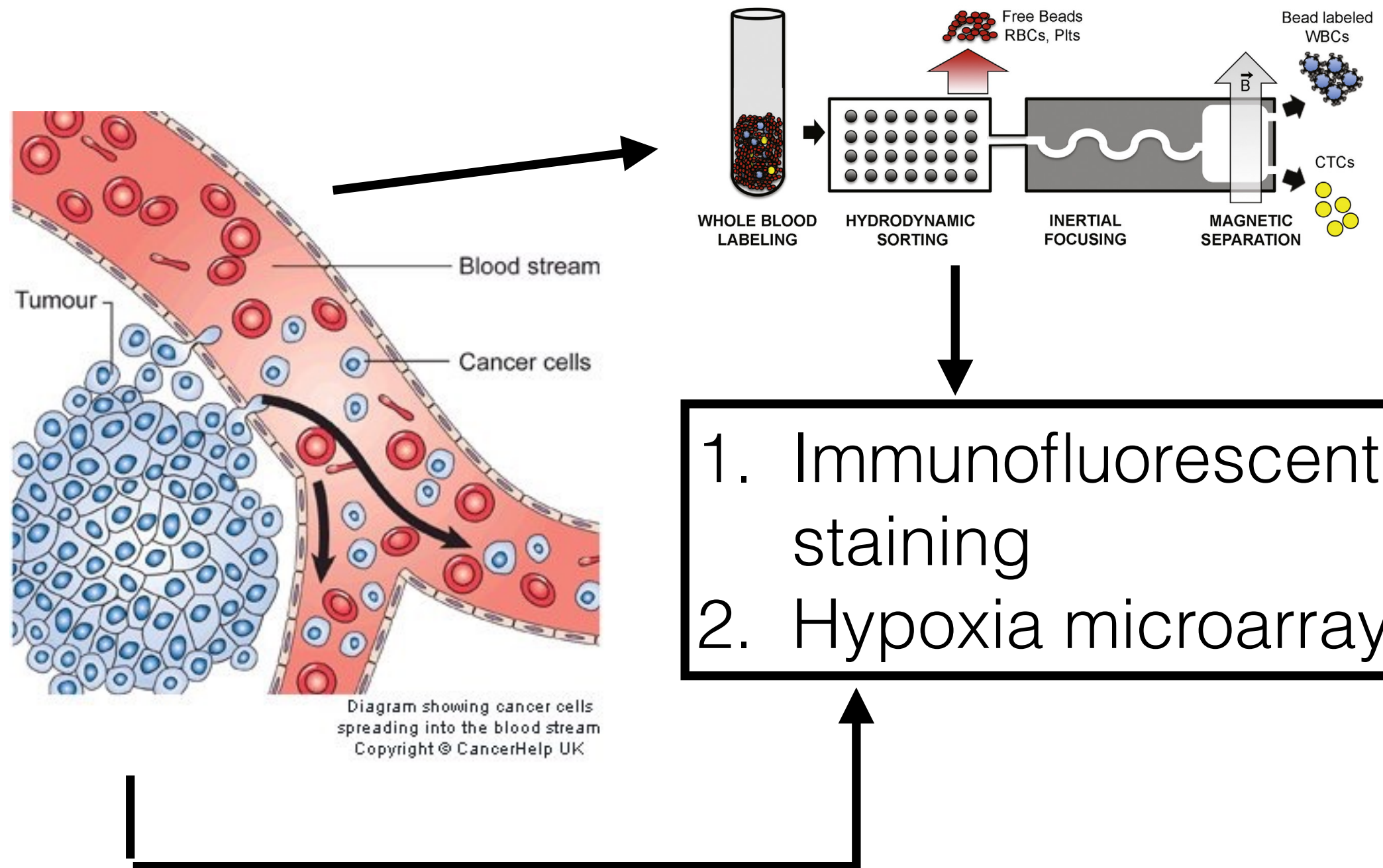
# Aim 1: Investigate up-regulation of molecules in the hypoxia signaling pathway.

## Tumor model

- KPC mice



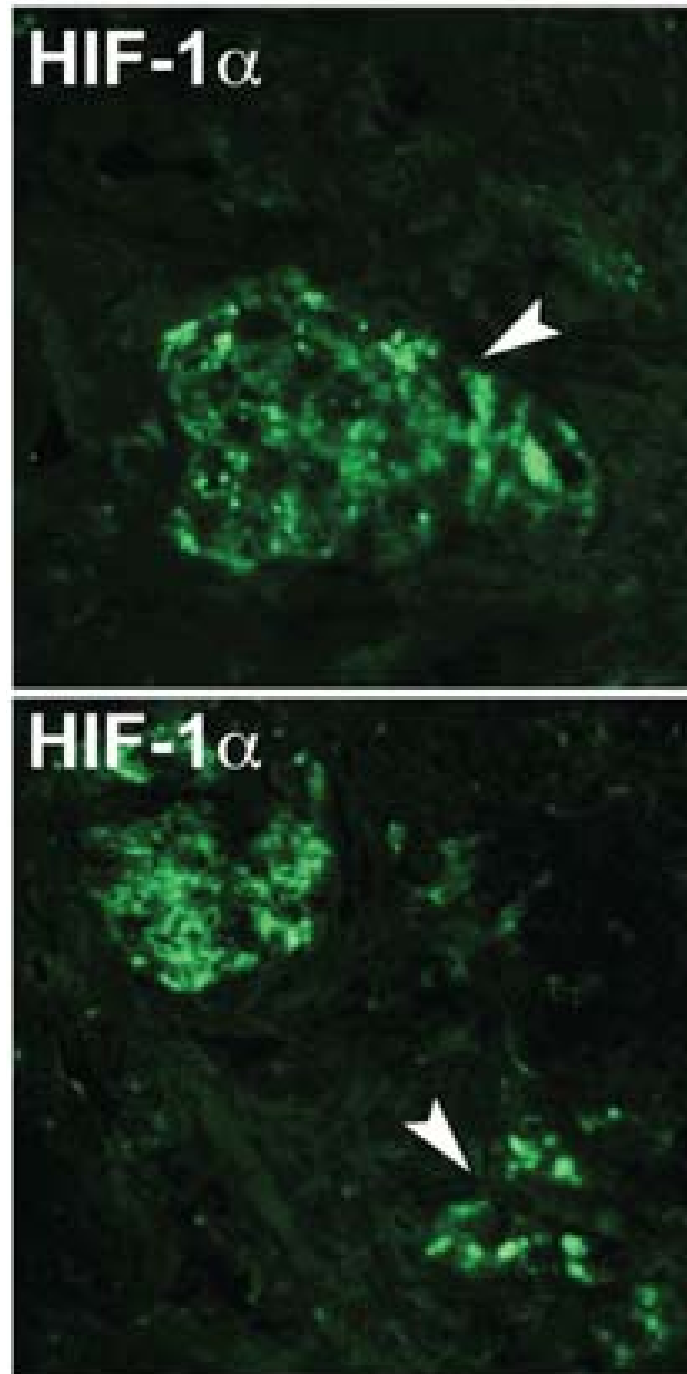
# Aim 1: Approach



Ting et al., *Cell Reports*, 2014

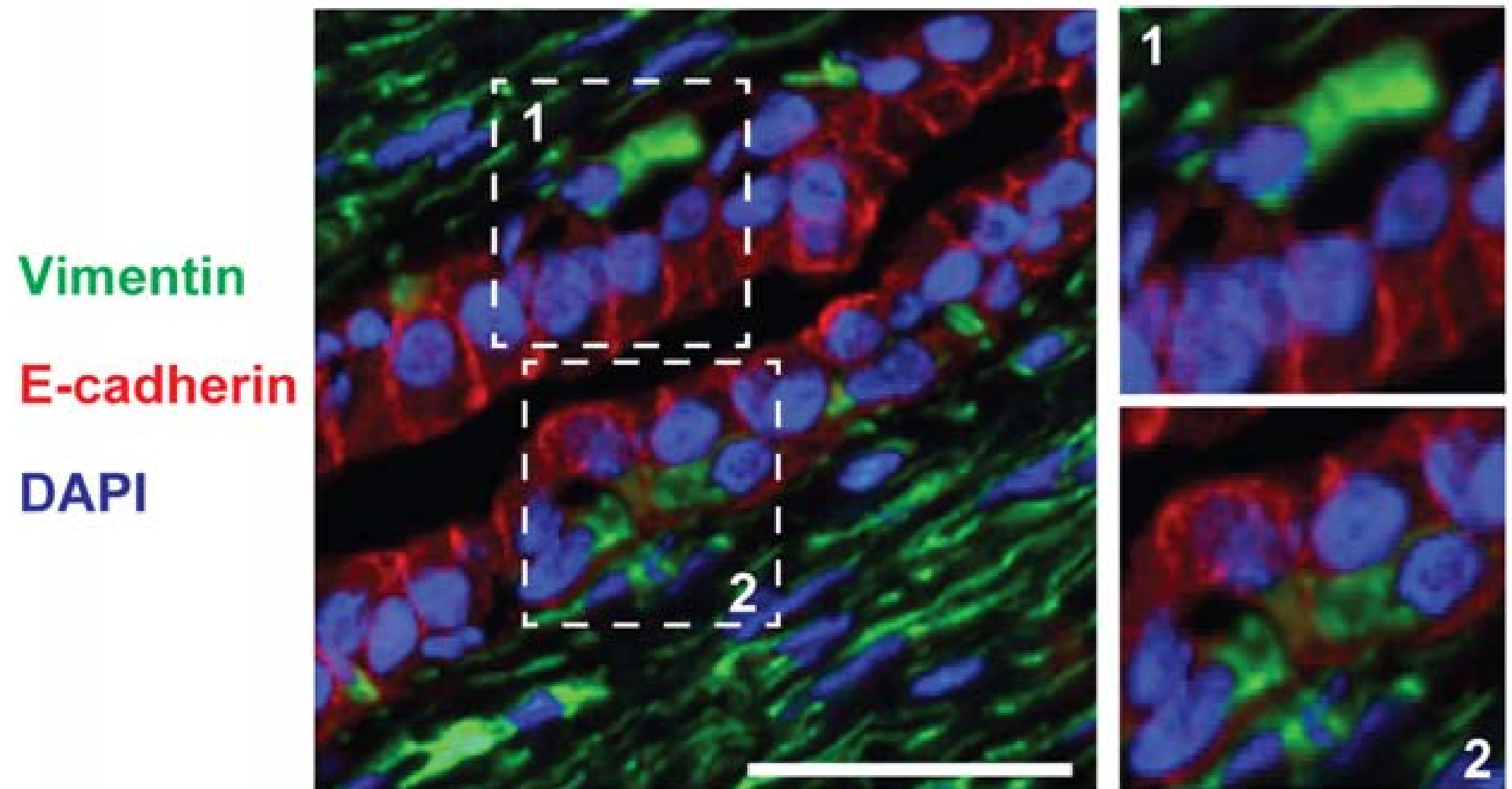
[http://www.cancerresearchuk.org/prod\\_consump/groups/cr\\_common/@cah/@gen/documents/image/crukimg\\_1000img-12231.jpg](http://www.cancerresearchuk.org/prod_consump/groups/cr_common/@cah/@gen/documents/image/crukimg_1000img-12231.jpg)

# Aim 1: Immunofluorescence stain



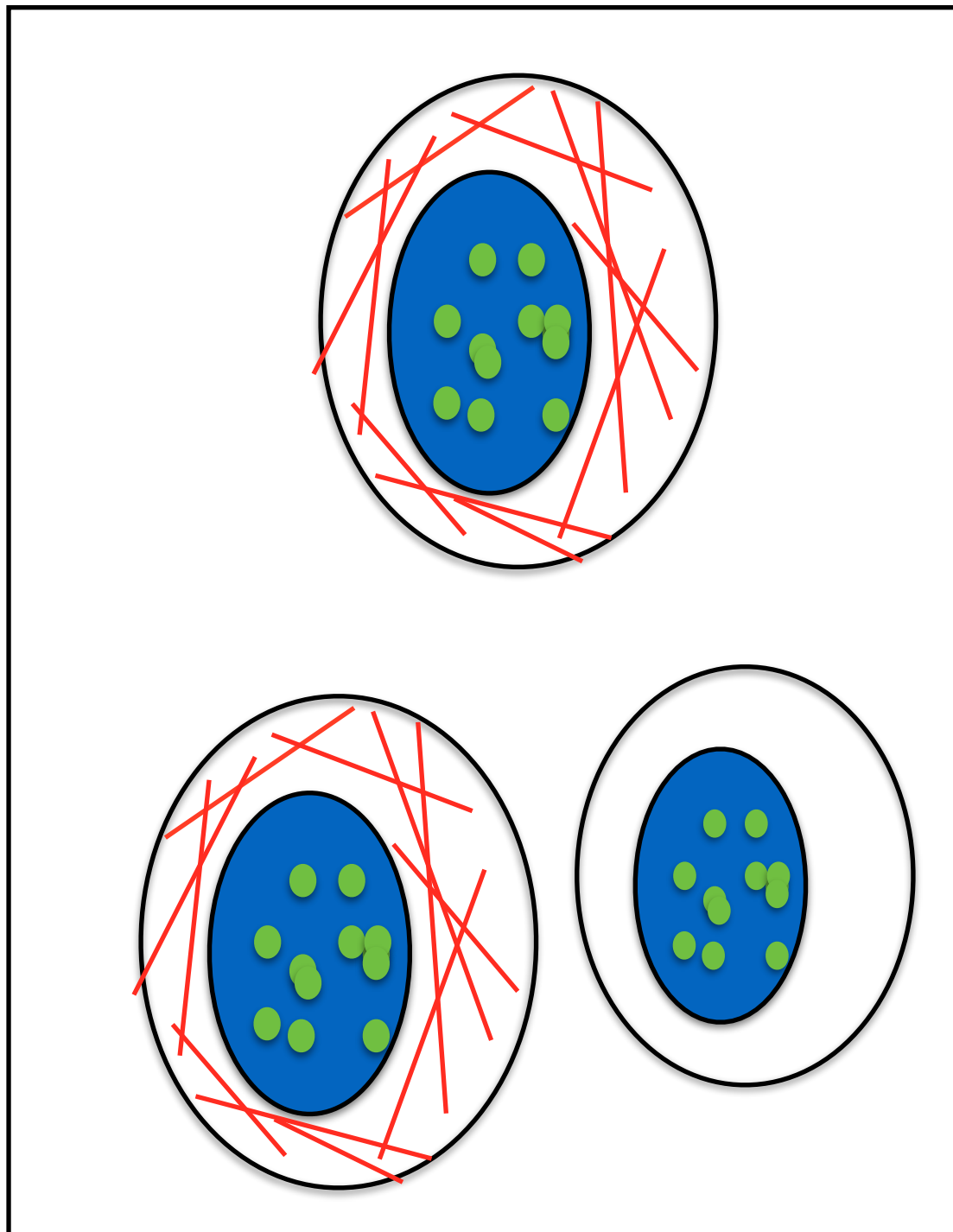


# Aim 1: Immunofluorescence stain



# Aim 1: Immunofluorescence stain

## Expected results in CTCs



- Vimentin
- HIF1-a
- DAPI

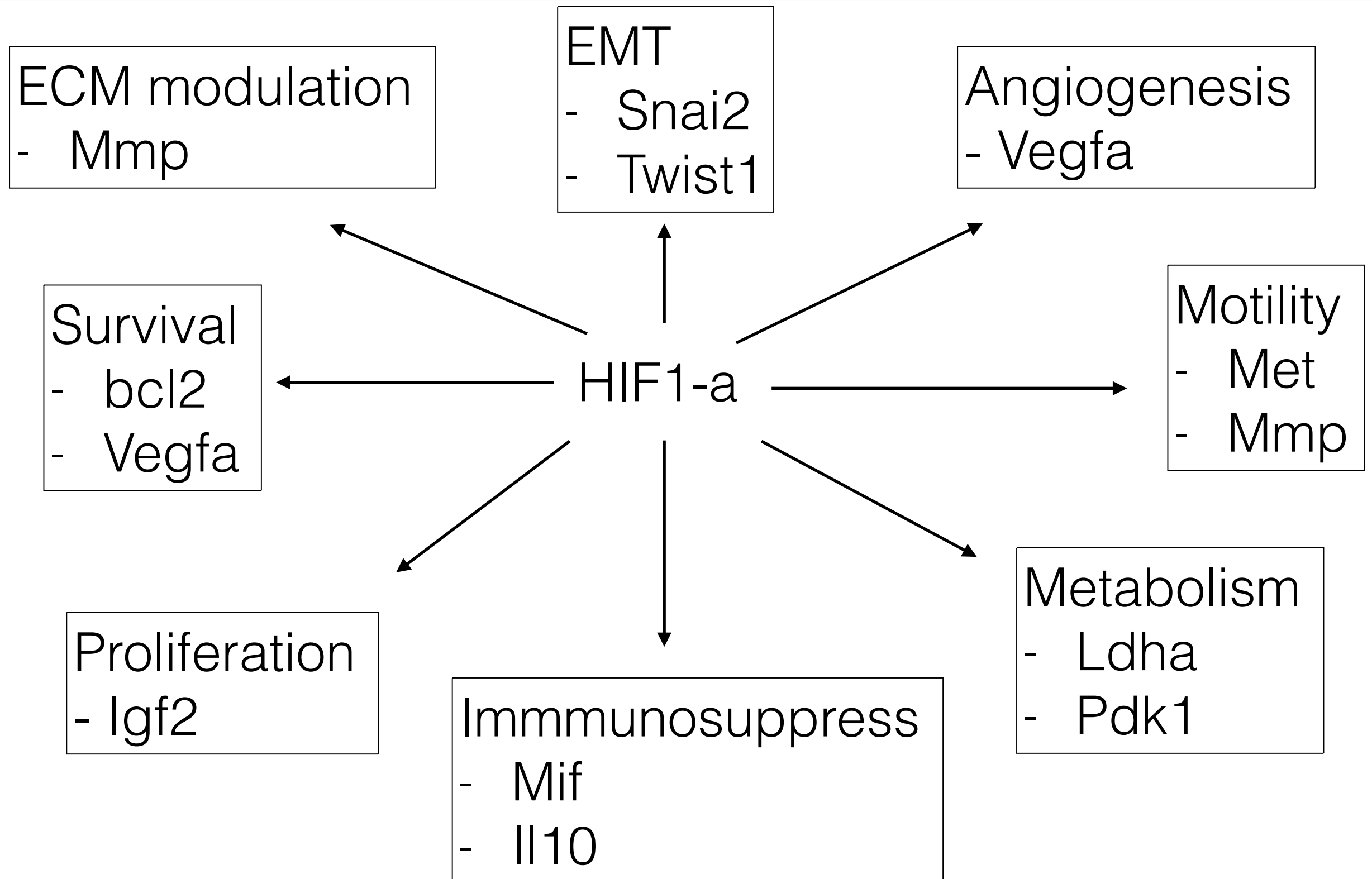
# **Aim 1: Investigate up-regulation of molecules in the hypoxia signaling pathway.**

## **Predictions**

1. Immunofluorescence staining shows HIF1- $\alpha$  in CTCs
2. Downstream targets also up-regulated

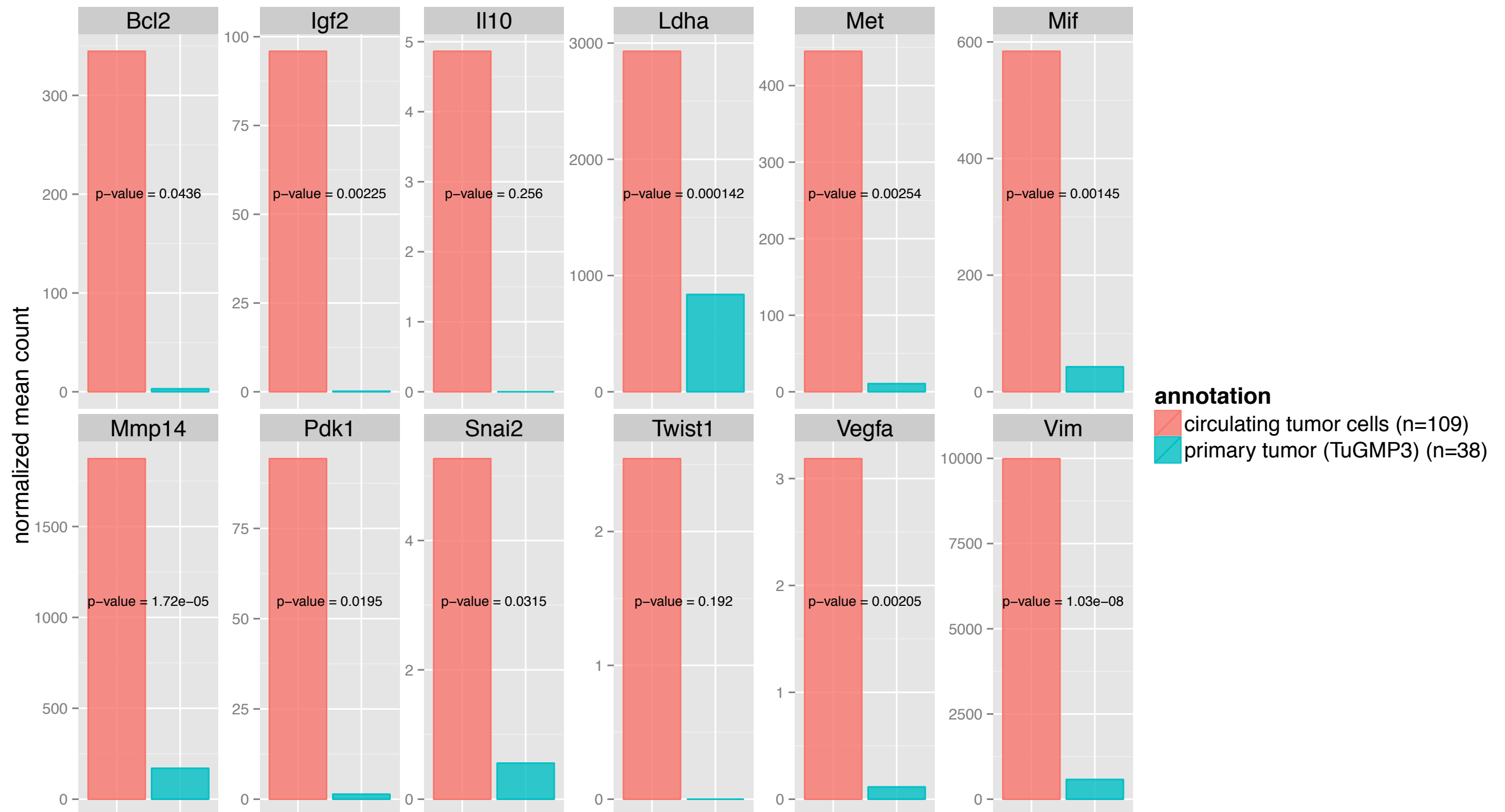


# HIF1-a is a master regulator in hypoxia signaling



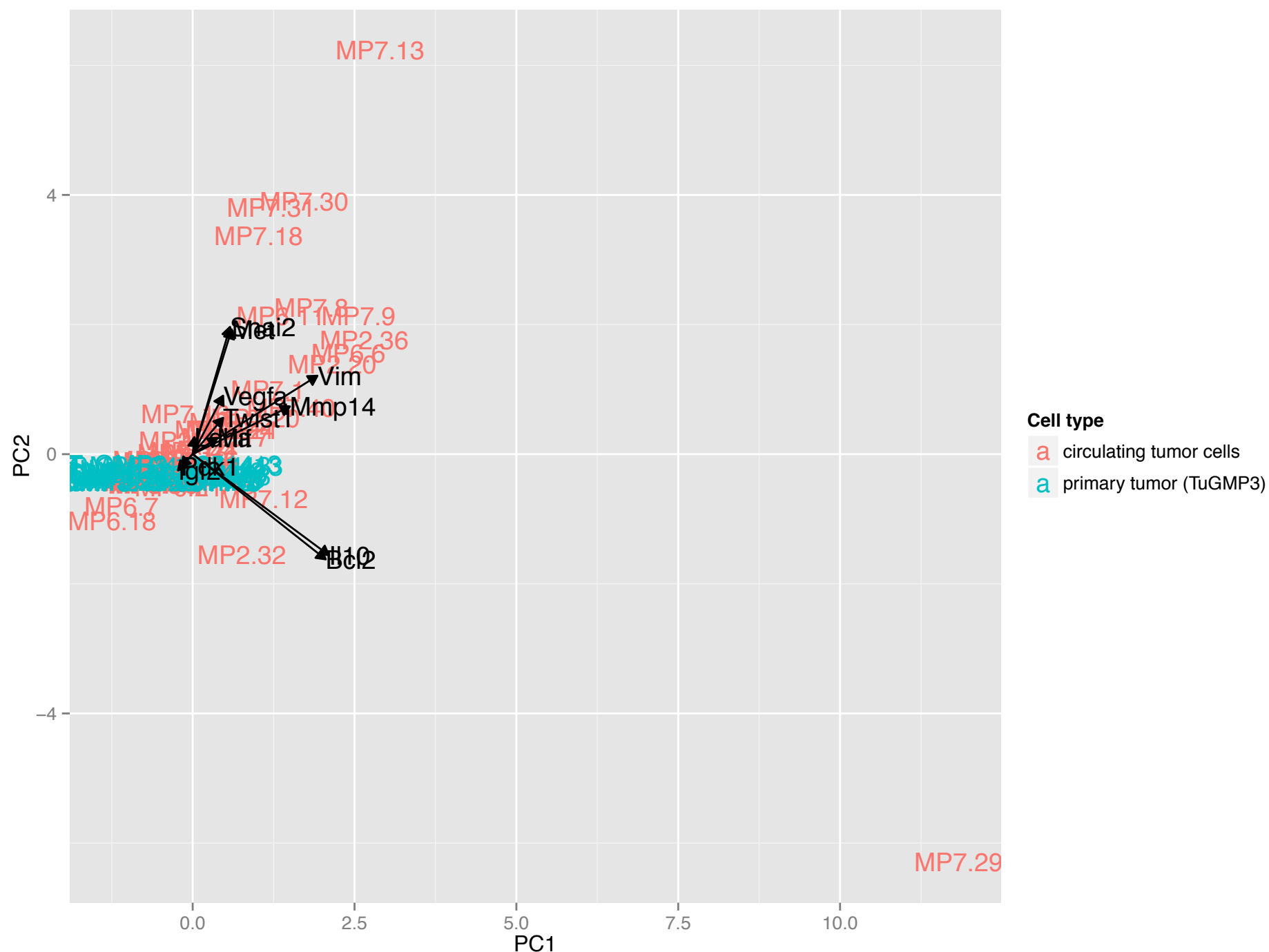
# Single cell RNA-seq of CTCs had active hypoxia signaling

Downstream targets of HIF1- $\alpha$  are also up-regulated



# Single cell RNA-seq of CTCs had active hypoxia signaling

Biplot: HIF1-a downstream targets introduce variations between CTCs and primary tumor cells



# **Aim 1: Investigate up-regulation of molecules in the hypoxia signaling pathway.**

## **Summary**

- CTCs show EMT markers with hypoxia phenotype
- Downstream targets of HIF1- $\alpha$  up-regulated in CTCs

# Hypothesis: Hypoxic environment promotes CTC production, intravasation and tumorigenicity

## Specific aims

1. Investigate up-regulation of molecules in the hypoxia signaling pathway in CTC.
2. Identify the effects of hypoxia on CTC biogenesis.
3. Investigate hypoxia-mediated enhanced tumorigenicity of CTCs.

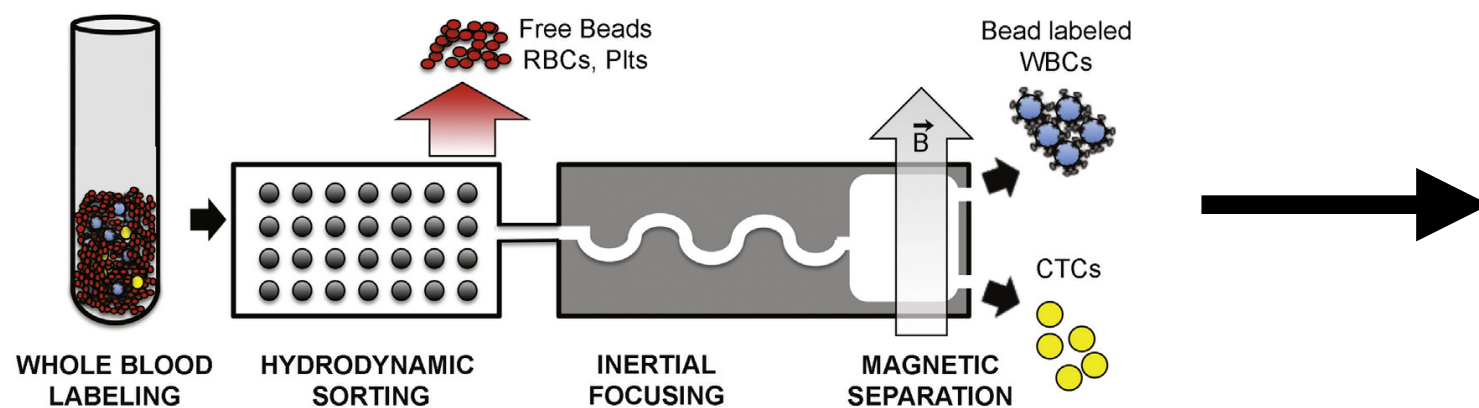
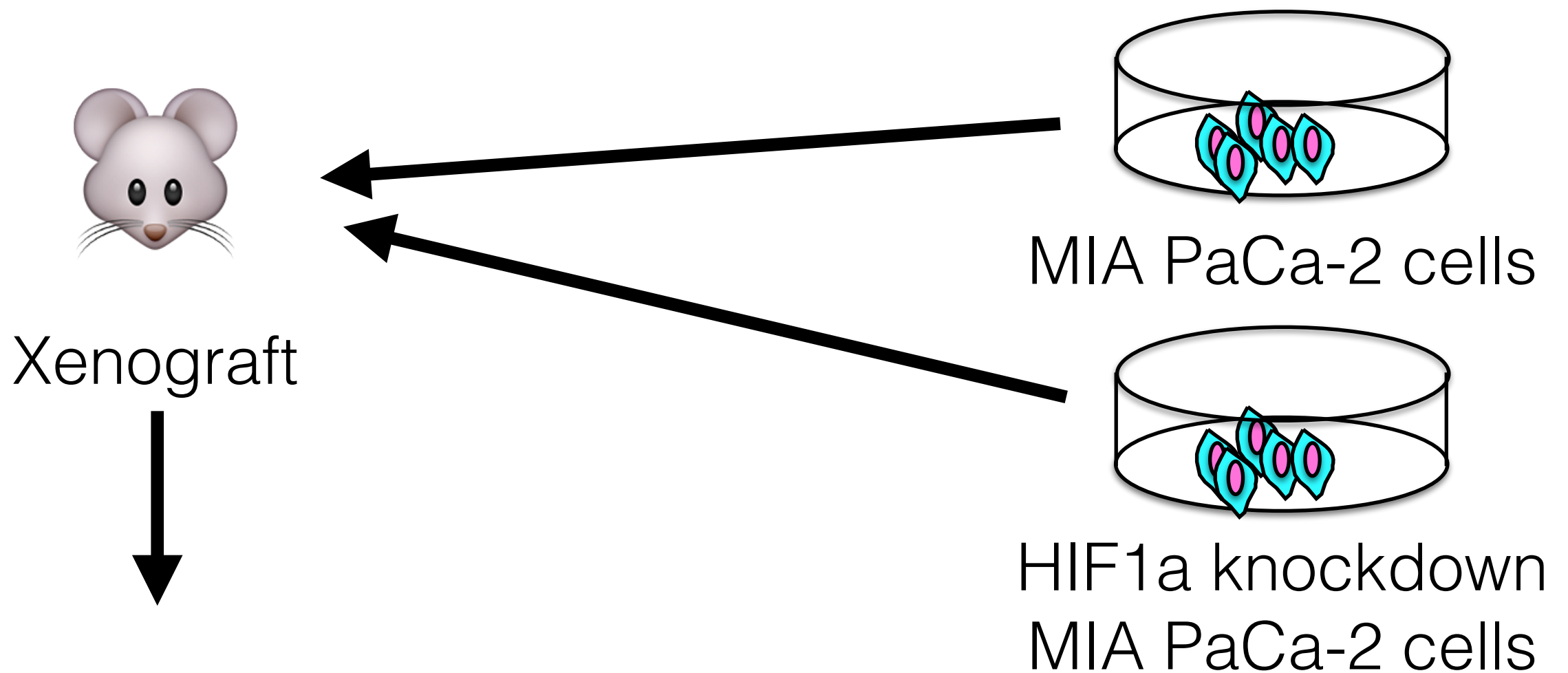


# **Aim 2: Identify the effects of hypoxia on CTC biogenesis**

## **Hypothesis**

Loss of HIF1- $\alpha$  reduces CTCs production

# Aim 2: Approach

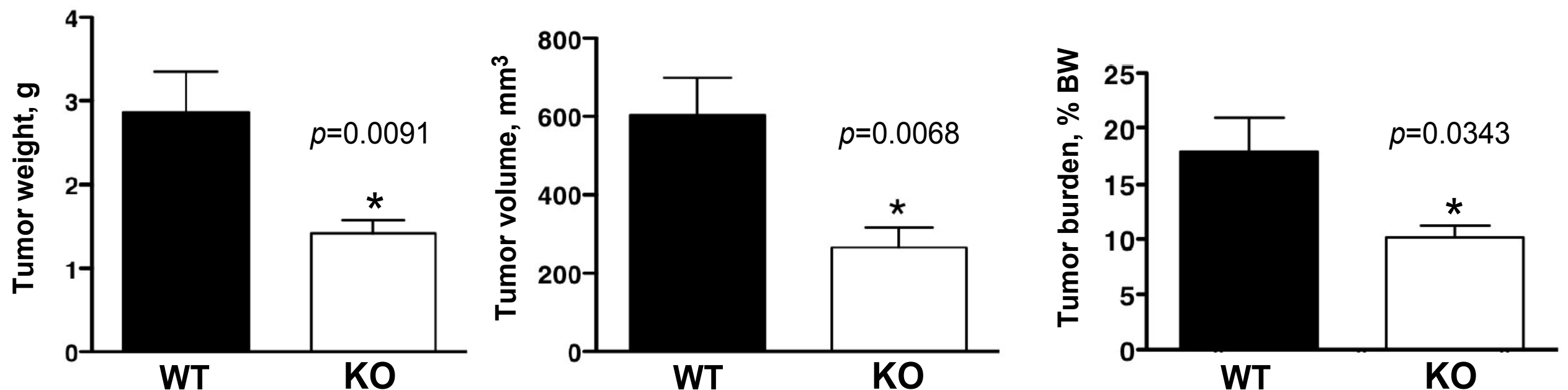


1. Single cell RNA-seq
2. CTC count
3. Tumor Size

## Aim 2: Predictions

# HIF1-a knockdown cells tumor xenograft shows slower growth rate

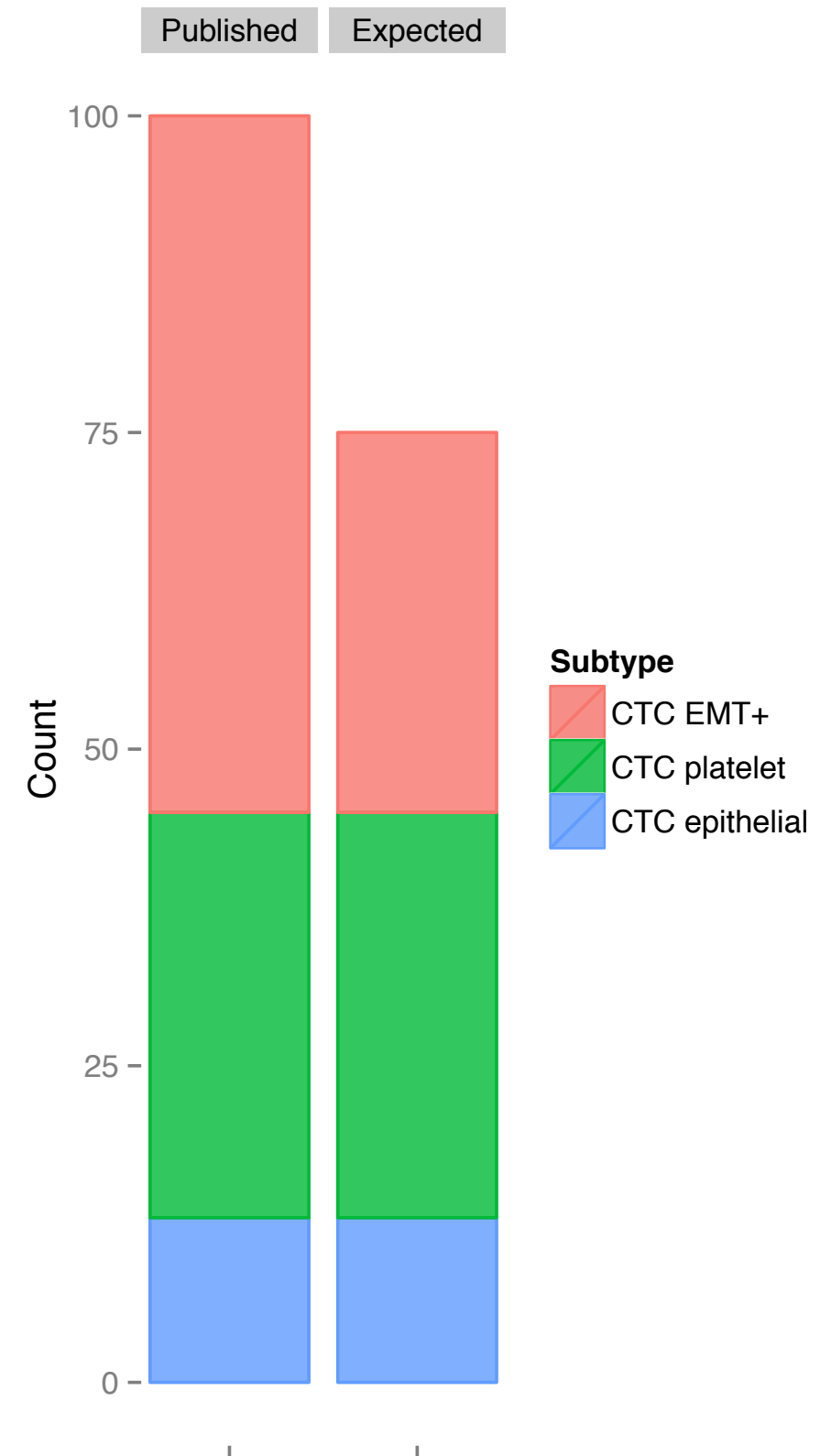
- **Tumor size**
  - correlate to previous study



# Aim 2: Predictions

## Mice implanted with HIF1-a knockdown cells have fewer CTCs

- **CTC count**
  - Decrease in HIF1-a knockout
- **Change in CTC subtypes**
  - Decrease in CTC EMT+ subtype



# Aim 2: Identify the effects of hypoxia on CTC biogenesis

## Summary

- Tumor size decreases
- Fewer CTCs
- Change in CTC count is due to loss of EMT<sup>+</sup> subtype



# Hypothesis: Hypoxic environment promotes CTC production, intravasation and tumorigenicity

## Specific aims

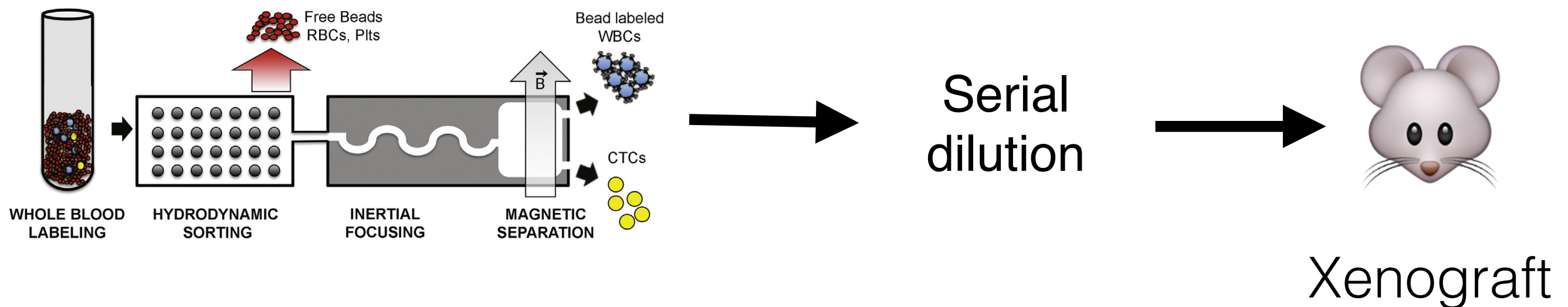
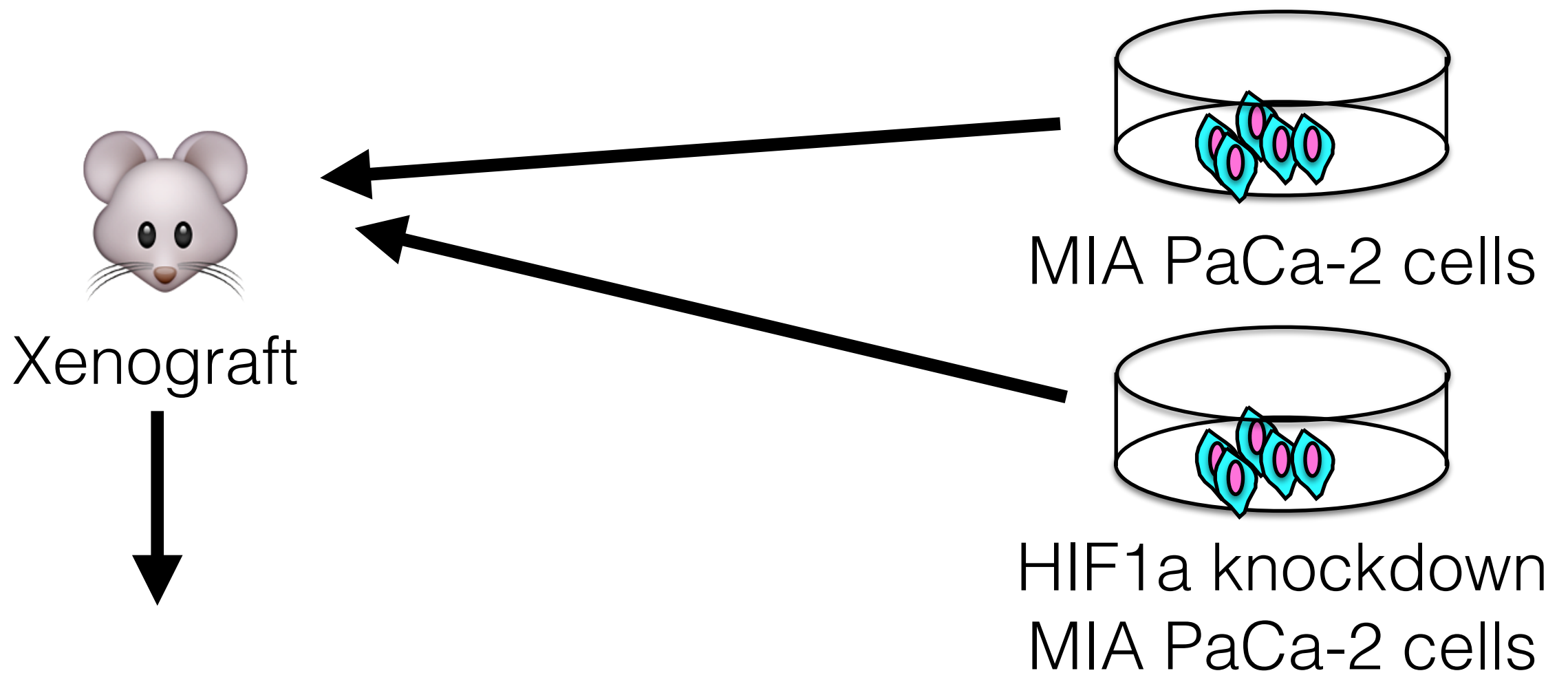
1. Investigate up-regulation of molecules in the hypoxia signaling pathway in CTC.
2. Identify the effects of hypoxia on CTC biogenesis.
3. Investigate hypoxia-mediated enhanced tumorigenicity of CTCs.

# **Aim 3: Investigate hypoxia-mediated enhanced tumorigenicity of CTCs.**

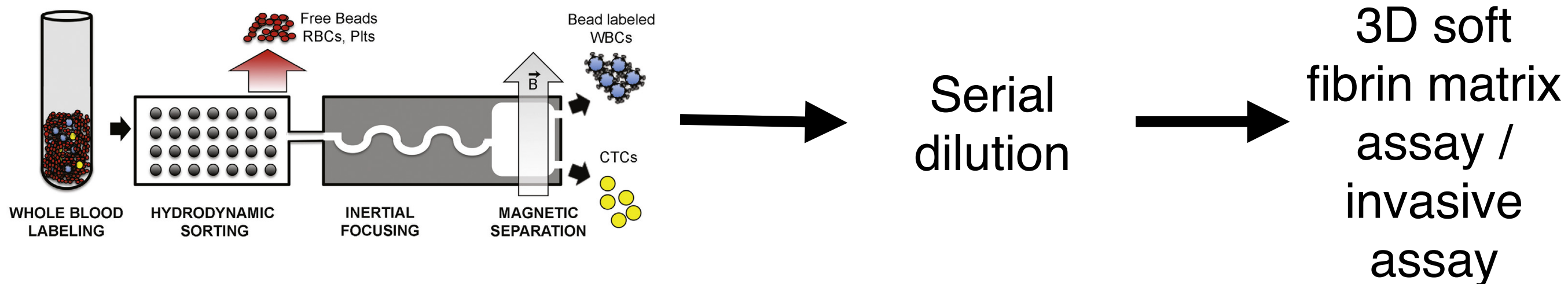
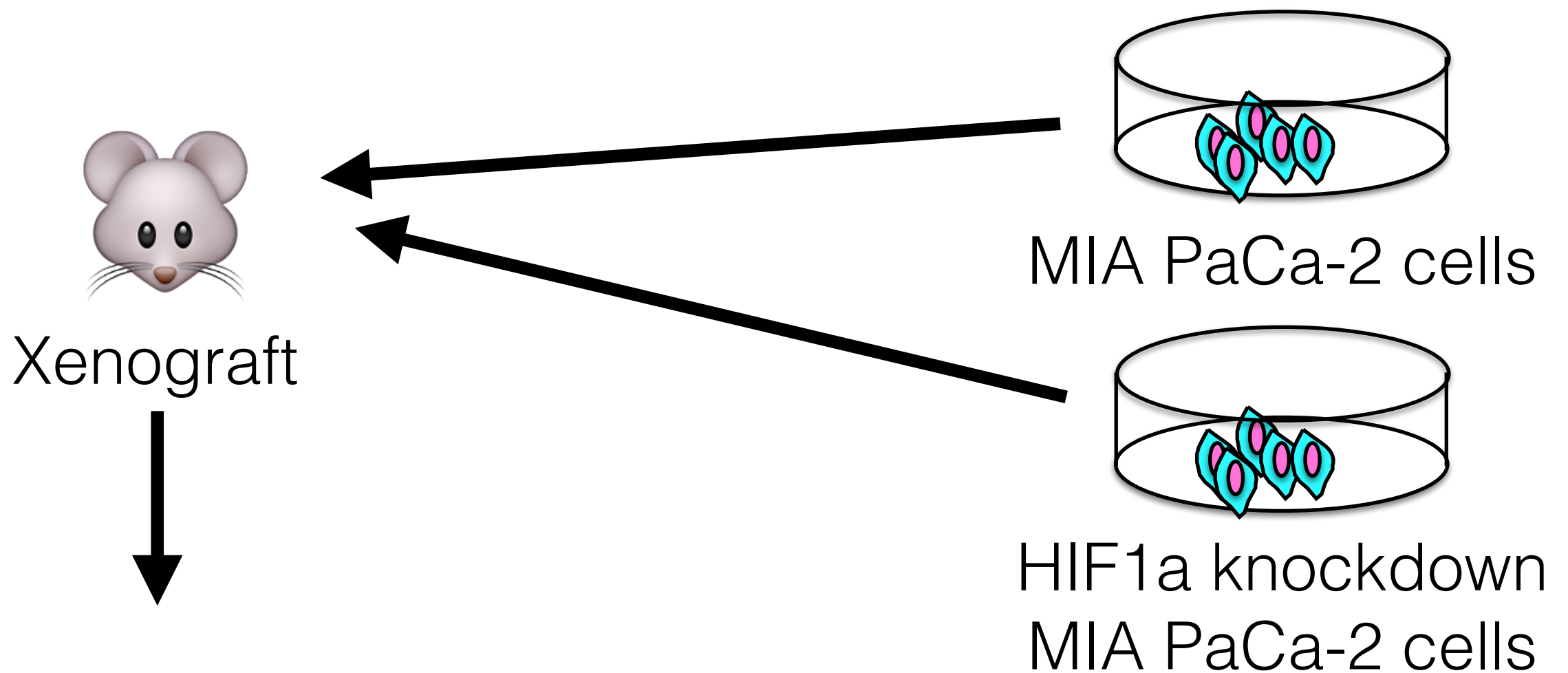
## **Hypothesis**

Loss of HIF1- $\alpha$  reduces CTC tumorigenicity

# Aim 3: Approach

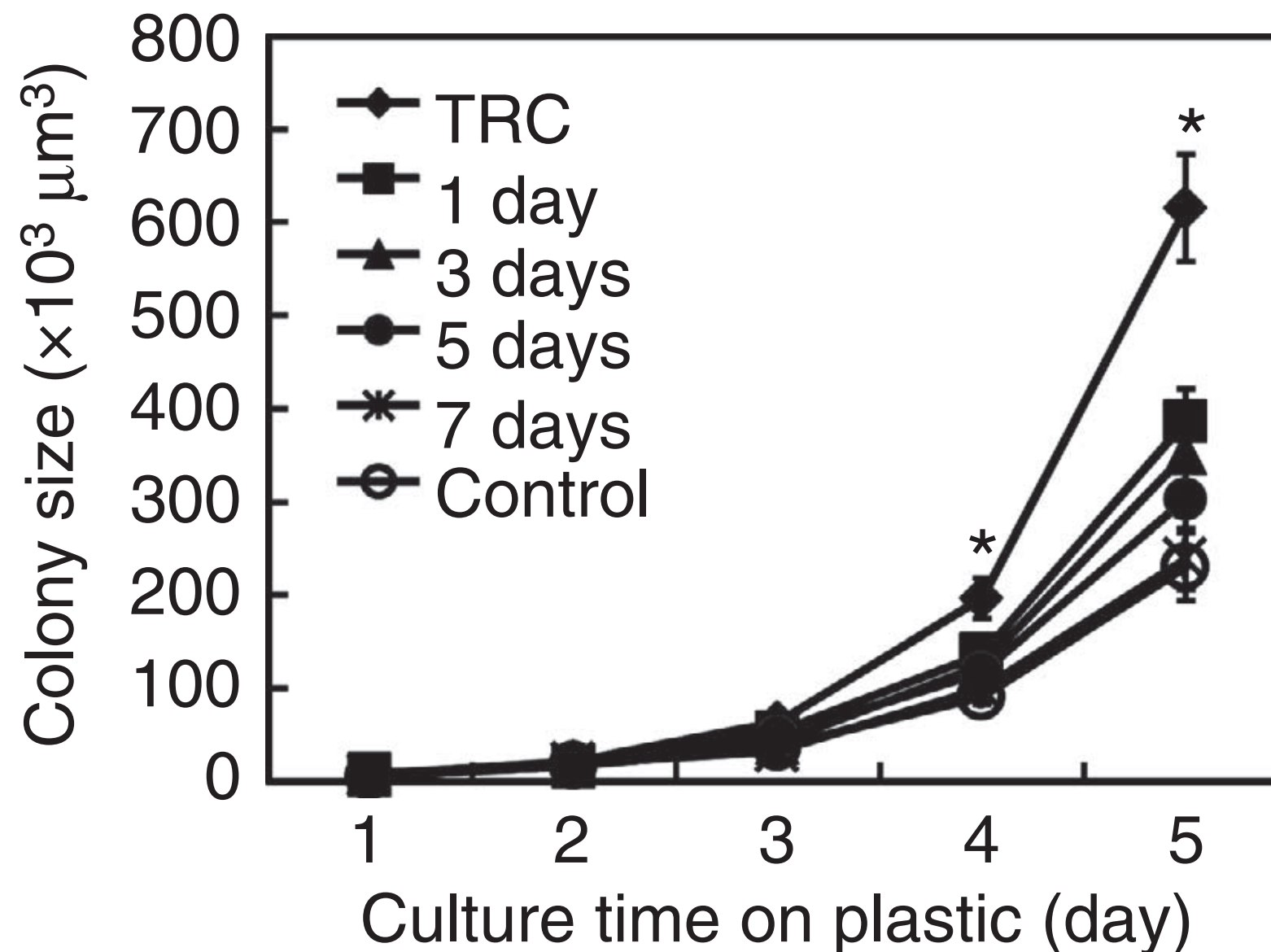


# Aim 3: Approach



# Aim 3: Investigate hypoxia-mediated enhanced extravasation of CTCs.

## Alternative: 3D soft fibrin matrix assay





# Aim 3: Expected result

- Decrease in tumorigenicity from CTCs collected from mice implanted with HIF1-a knockdown MIA PaCa-2 cells

# Summary

