Dissection of the tumor/stromal expression response to VEGF inhibition in xenograft models

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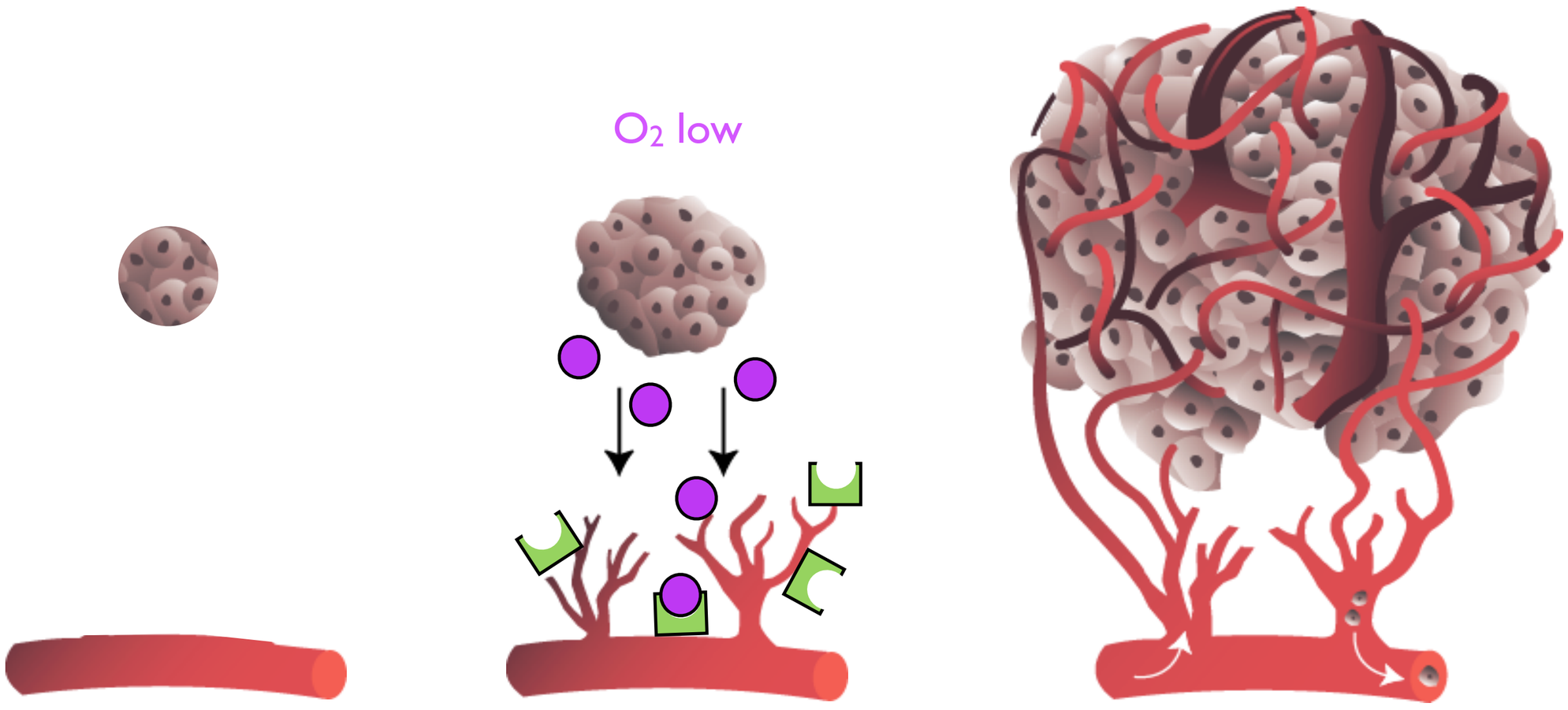
Inhibition of tumor-induced angiogenesis

Avascular tumor

Angiogenic switch

Rapid tumor growth



VEGF (Vascular Endothelial Growth Factor)

VEGFR (VEGF Receptor)

From Anthony Lama. Carmeliet and Jain (2000) Nature, Bergers and Benjamin (2003) Nat. Rev. Cancer

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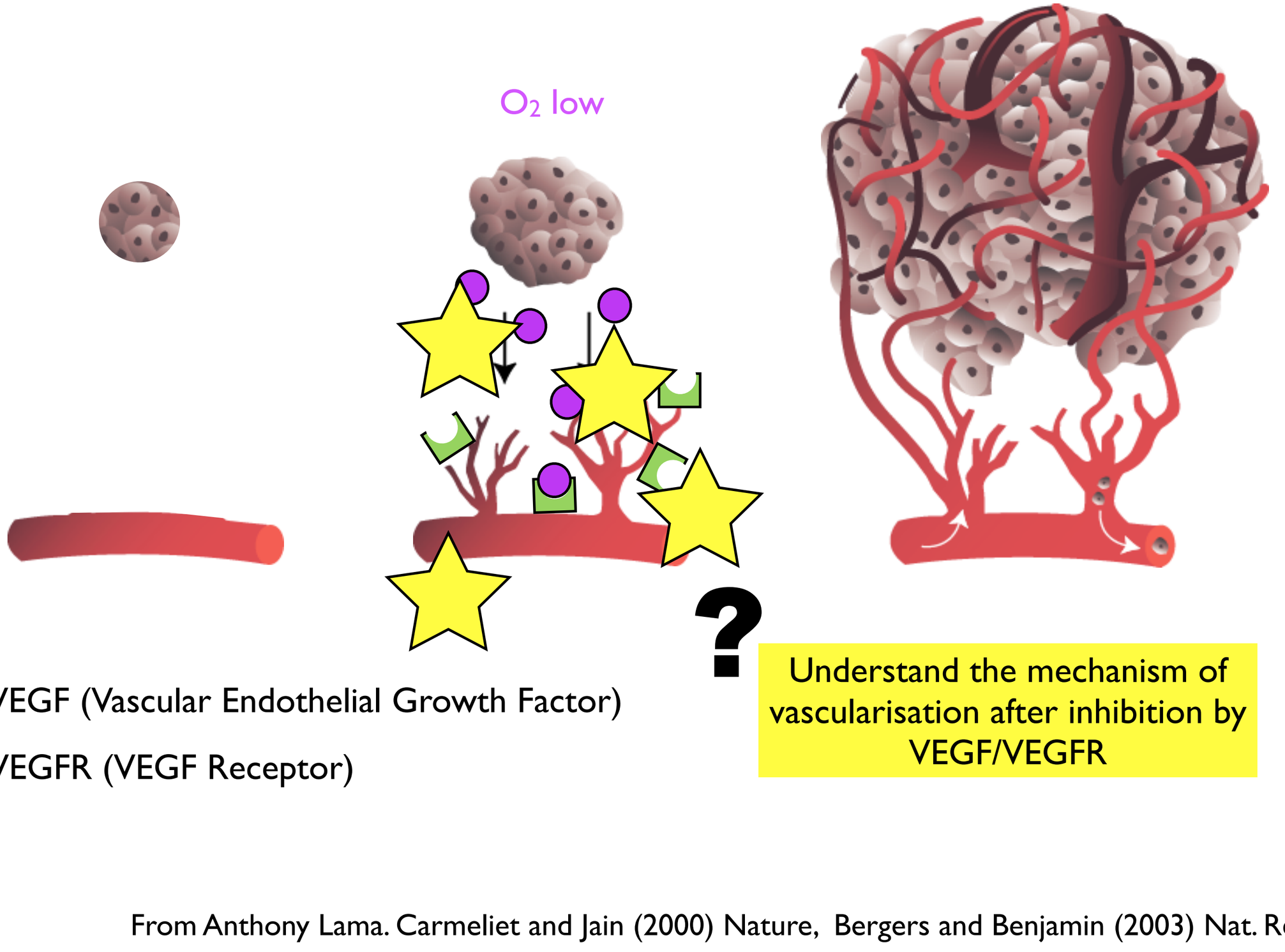


VEGF (Vascular Endothelial Growth Factor)

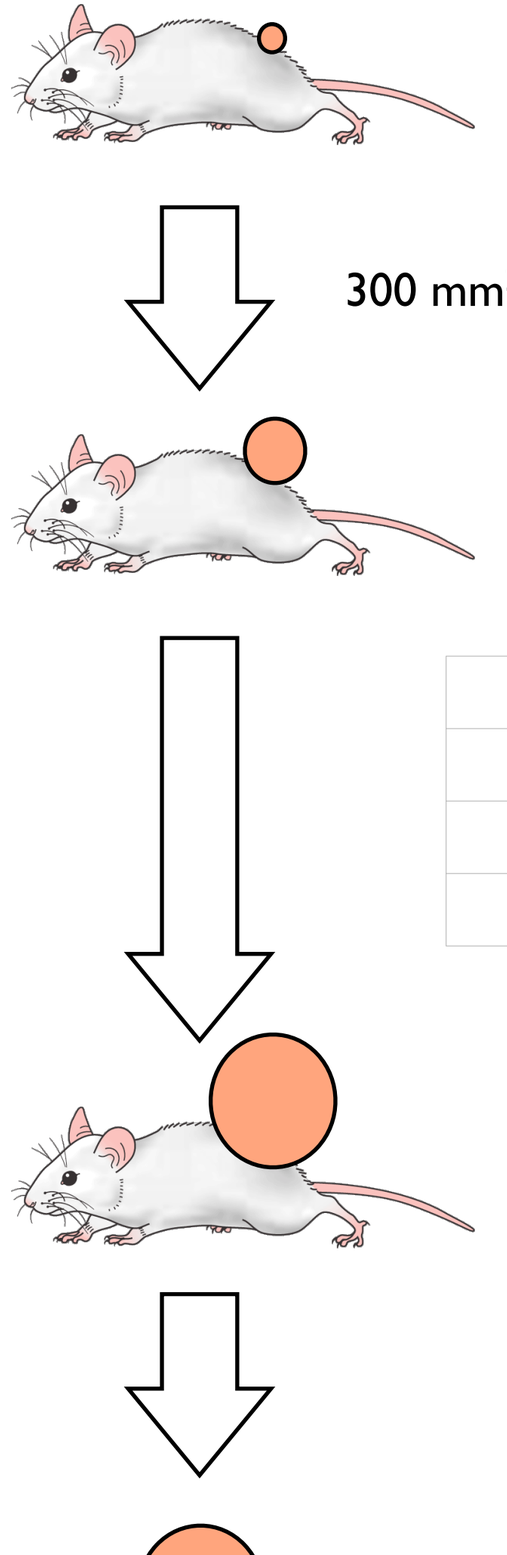
VEGFR (VEGF Receptor)

Understand the mechanism of vascularisation after inhibition by VEGF/VEGFR

From Anthony Lama. Carmeliet and Jain (2000) Nature, Bergers and Benjamin (2003) Nat. Rev. Cancer

VEGF inhibition in xenograft mouse models

MDA-MB-231 human breast cell line grafted in 12 mice



300 mm^3

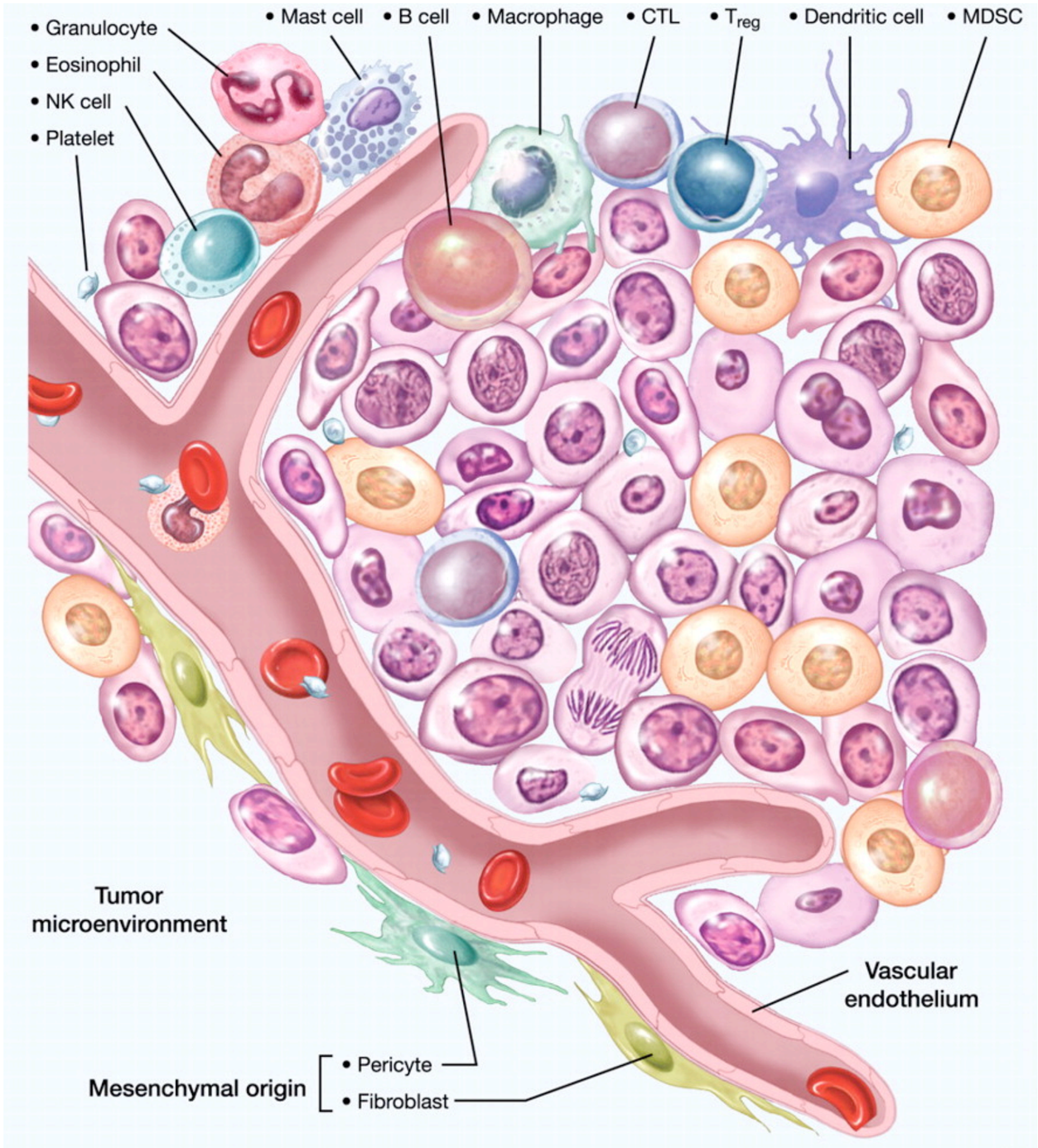
agD	Negative
B20	Anti VEGF
Axitinib	Anti VEGFR
Sunitinib	Pan RTK inhibitor

x 3 mice
72 h

RNA-Seq 75bp paired-end

Xenograft samples are heterogeneous mixtures of different cells

Tumor micro-environment (stroma)



Cancer cell

Tumor (human)

Adipocyte

Leucocyte

Vascular endothelial cell

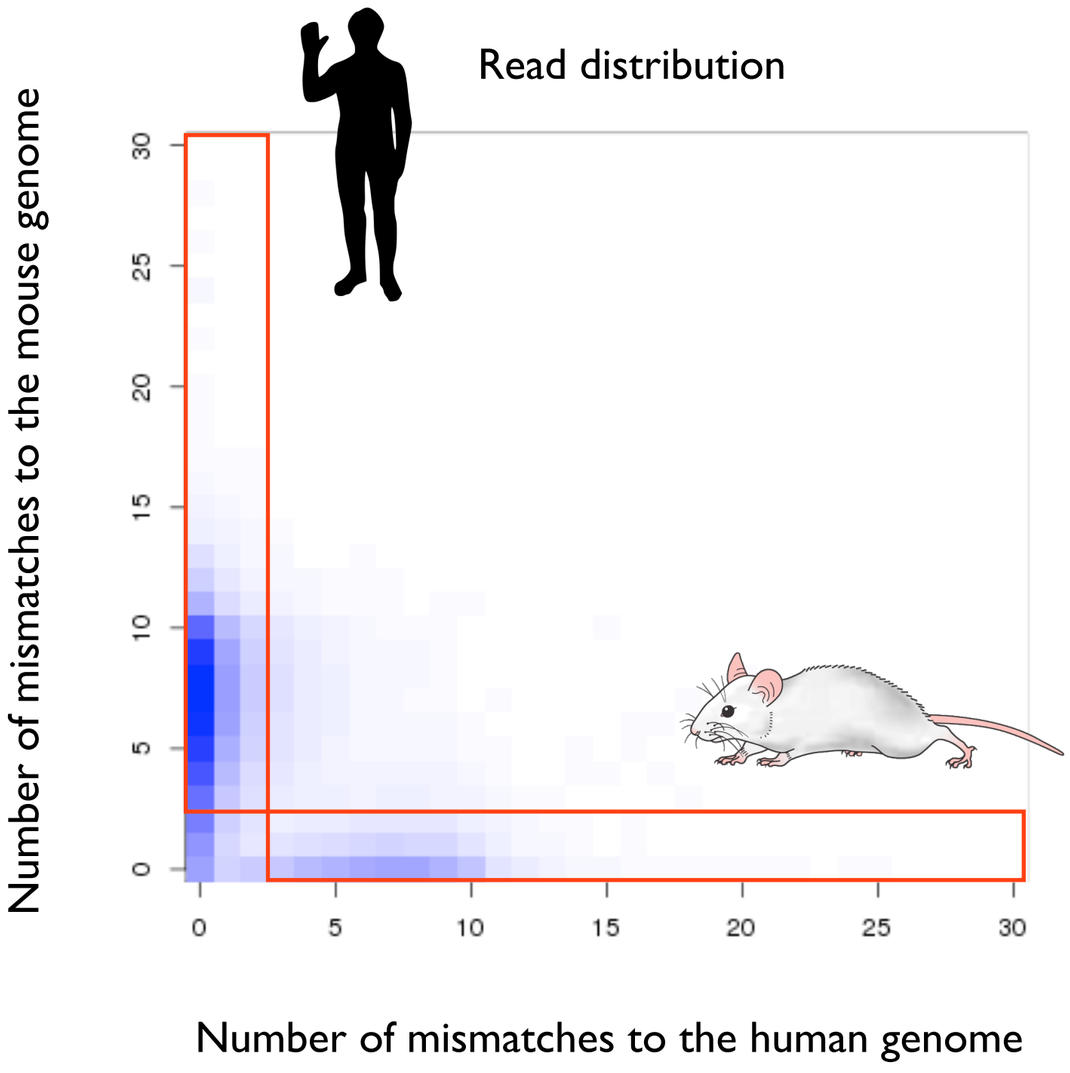
... other cell types

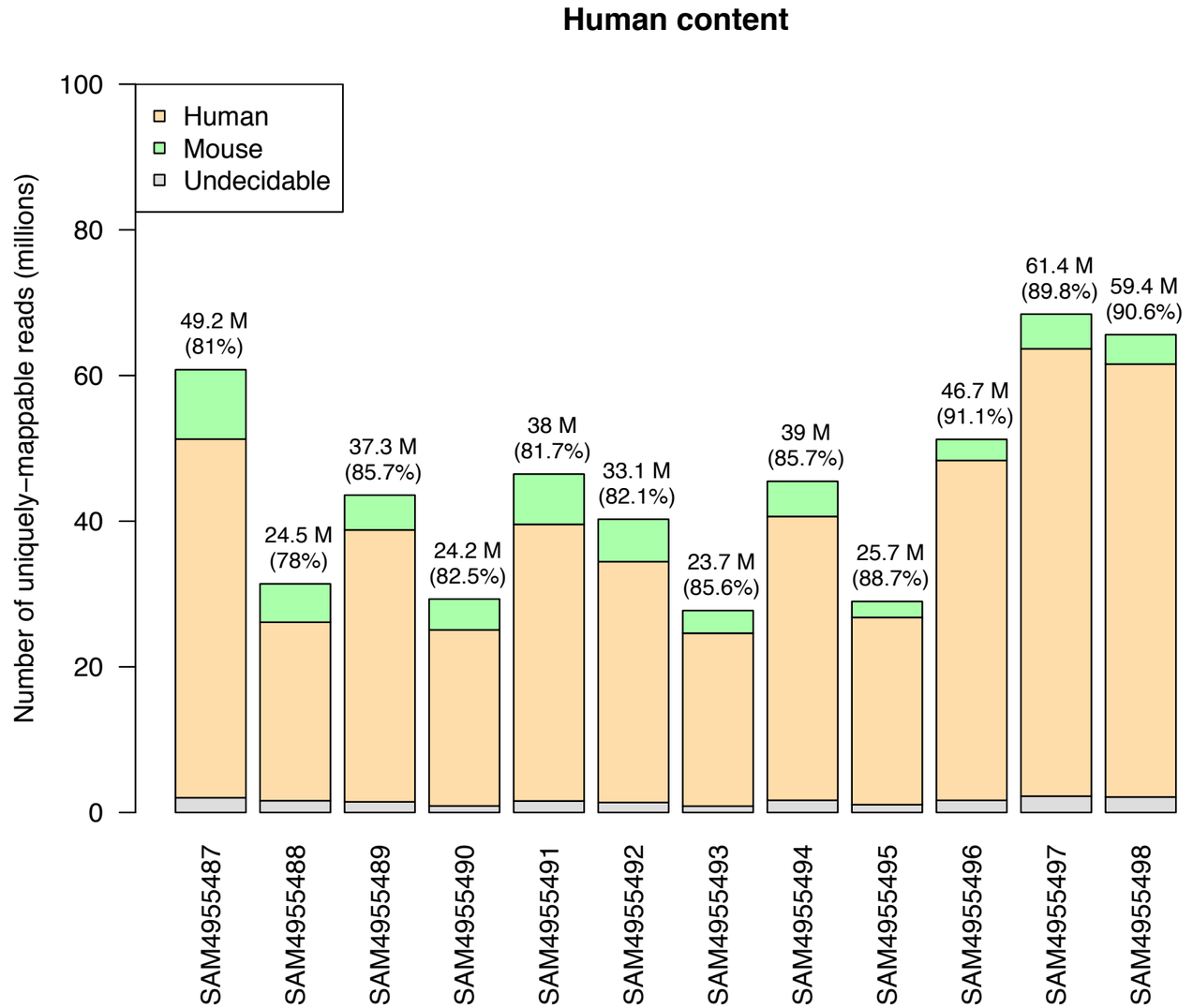
Stroma (mouse)

Adapted from Kerkar and Restifo (2012) Cancer Research

Separation of mouse and human mRNAs

- Co-alignement of reads with the mouse and human genome
- Separation based on the number of alignment mismatches





Tumor response to VEGF inhibition

B20 (anti VEGF)

- 41 DE genes
- Heatshock
- Hypoxia

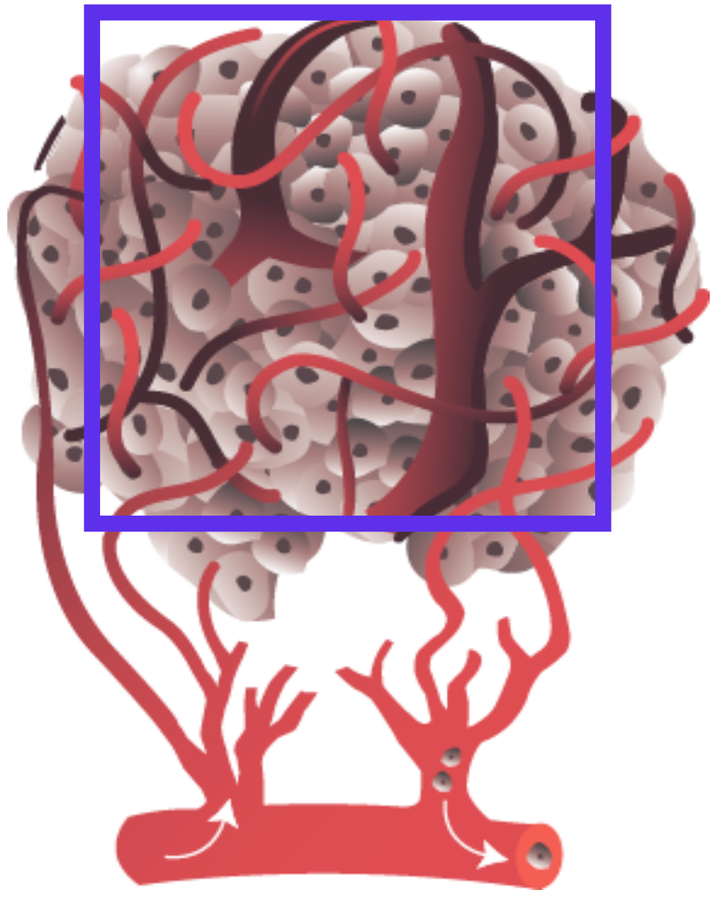
Axitinib (anti VEGFR)

- 0 DE genes
- No effects
- Dose too low

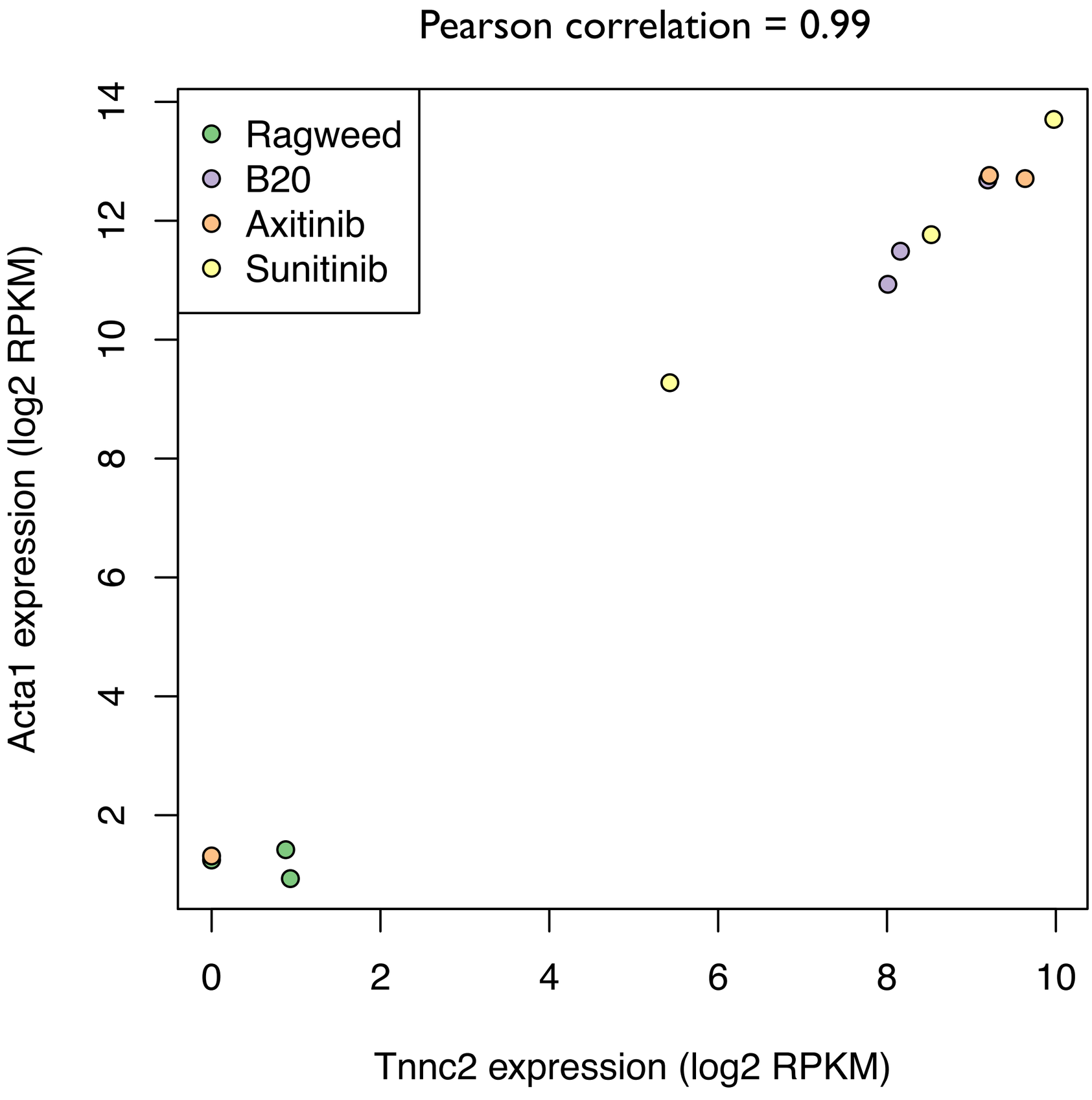
Sunitinib (Pan-RTK inh.)

- 73 DE genes
- Innate immune response

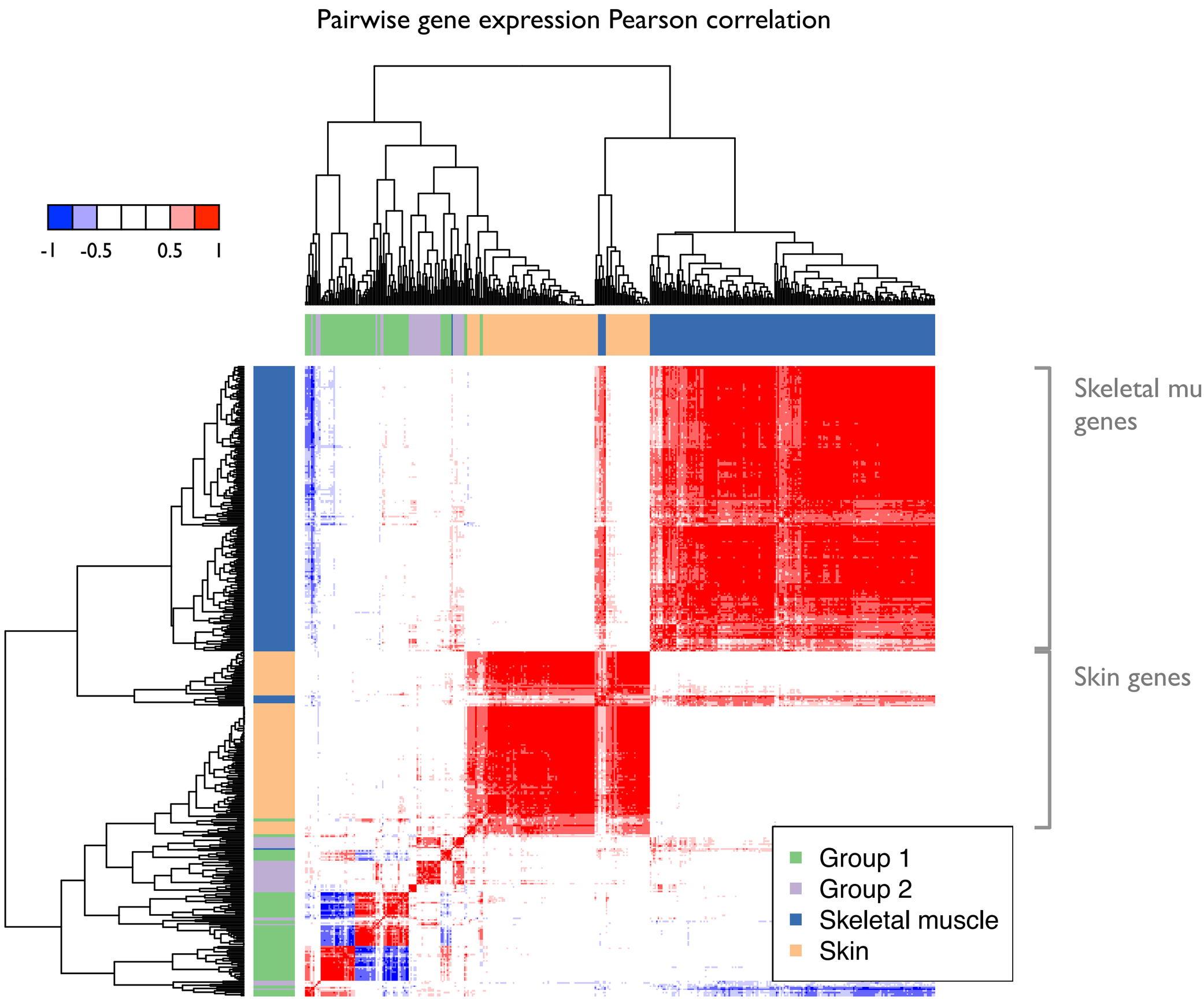
DESeq2, default parameters



Gene signature suggests contamination by muscle cells



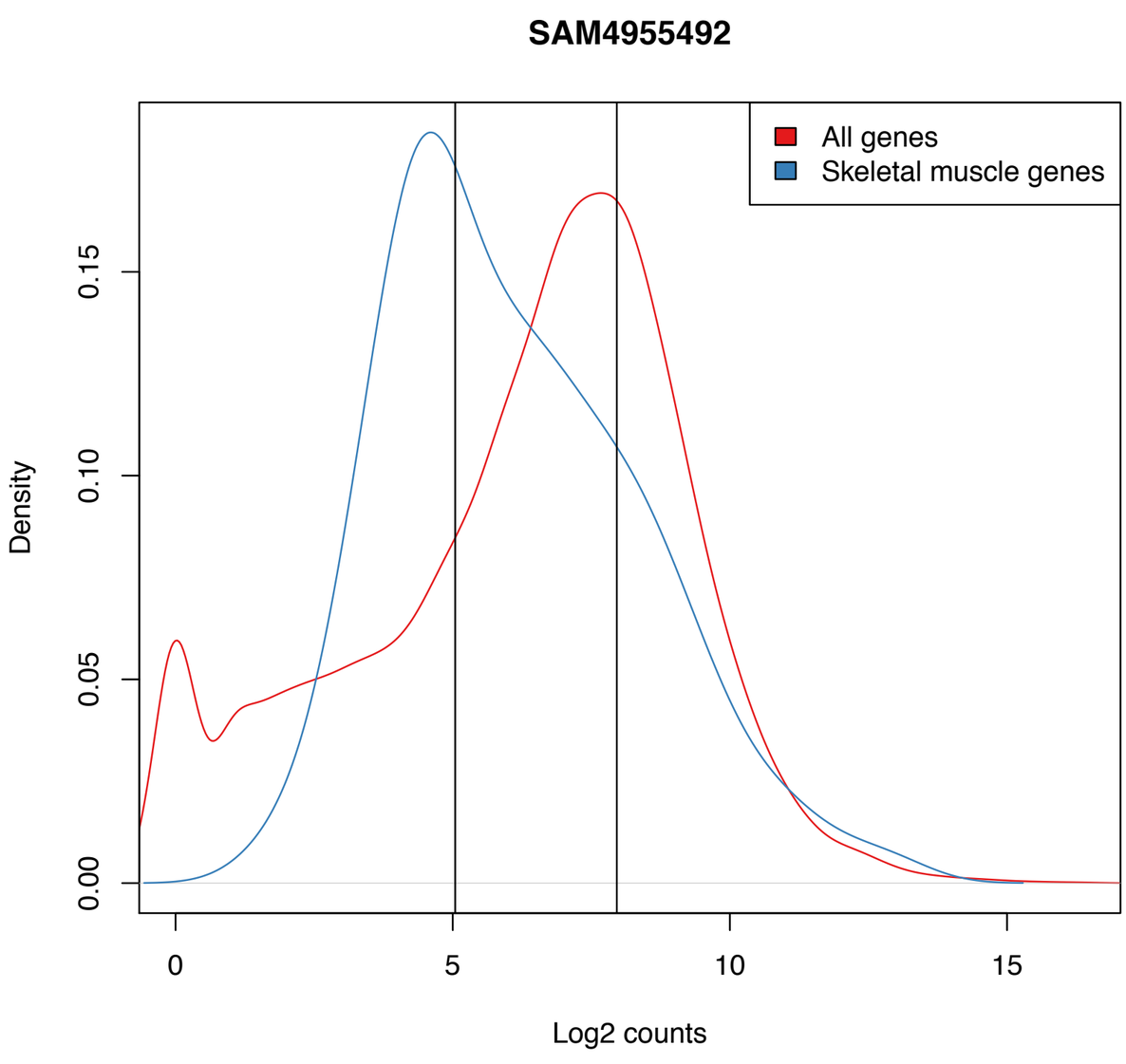
Correlation analysis in stroma shows existence of tissue-specific genes



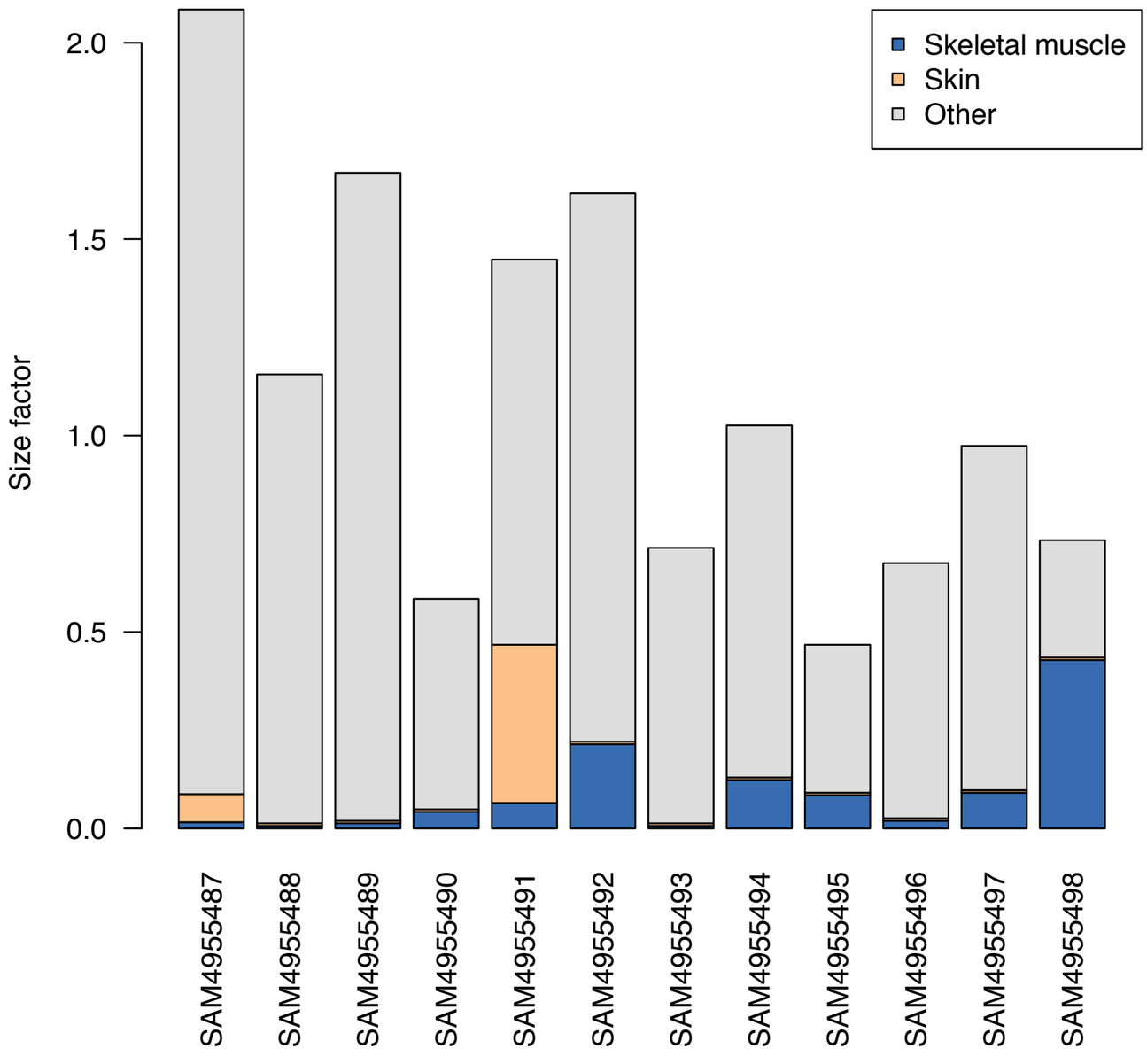
Stromal composition and size factor re-estimation

- Hypotheses
  - Tissue gene distribution is similar to the overall gene distribution
  - Tissue composition is independent of treatment

Size factor estimation



Stromal composition



Stromal response to VEGF inhibition

B20 (anti VEGF)

- 189 DE genes
- Endothelial VEGF response

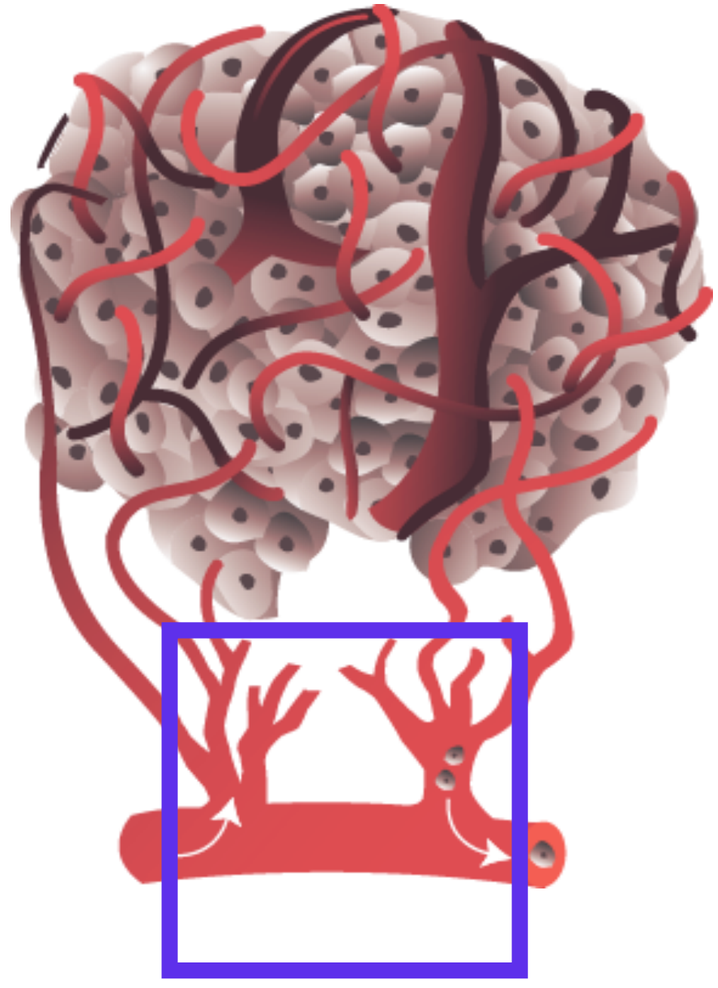
Axitinib (anti VEGFR)

- 41 DE genes
- Endothelial VEGF response

Sunitinib (anti RTK)

- 378 DE genes
- Endothelial VEGF response
- Cell cycle arrest

DESeq2, with re-estimated size factors



Conclusion

- Xenograft mouse models are hetereogeneous mixture of cells

- Method to separate stromal (mouse) from tumor (human) response
- Estimation of stromal tissue composition
- Re-estimation of size factors to sharpen differential expression analysis

- Generalizable to filter/estimate non-human fraction (bacteria, viruses...)
- To study host-pathogen interactions, virus integration, cell-cell signalling...

- Soon in Bioconductor