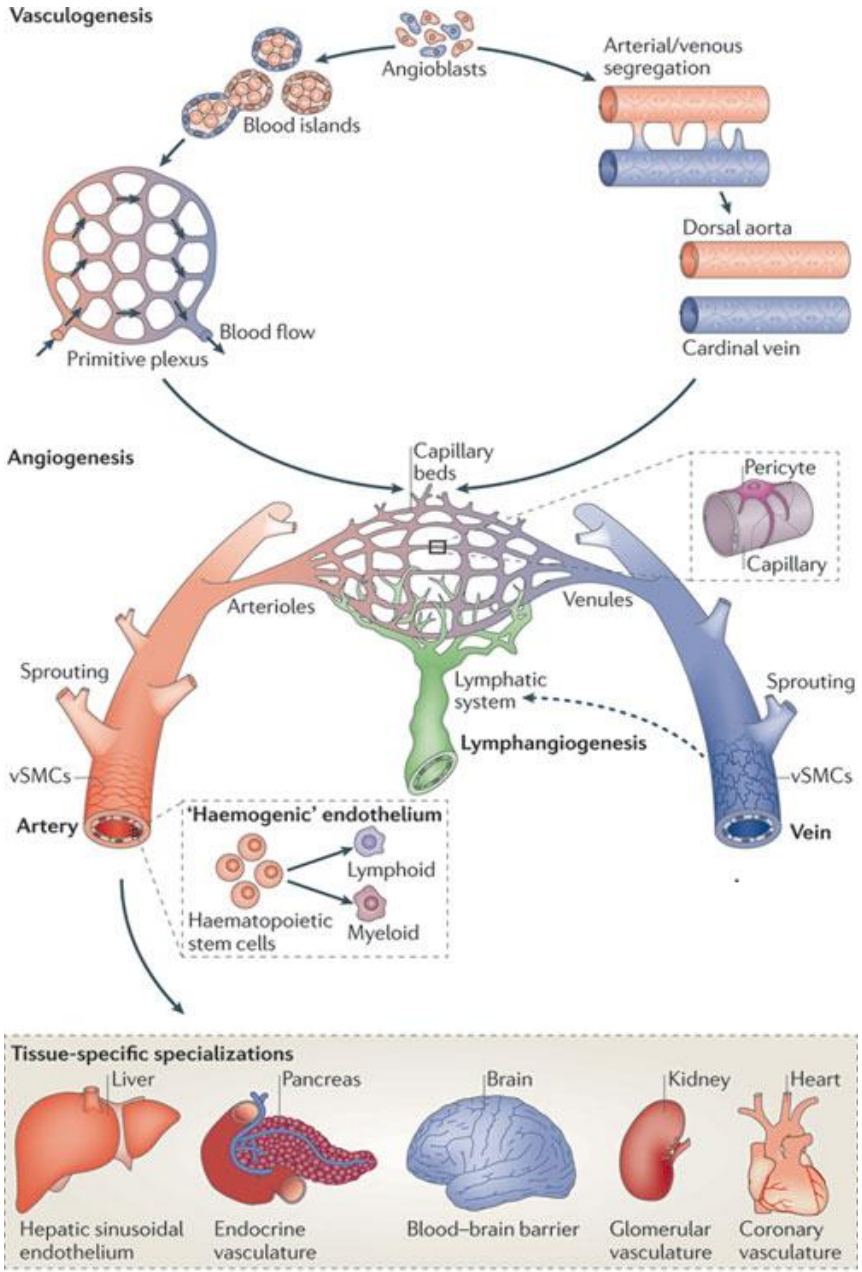
Vasculogenesis and angiogenesis

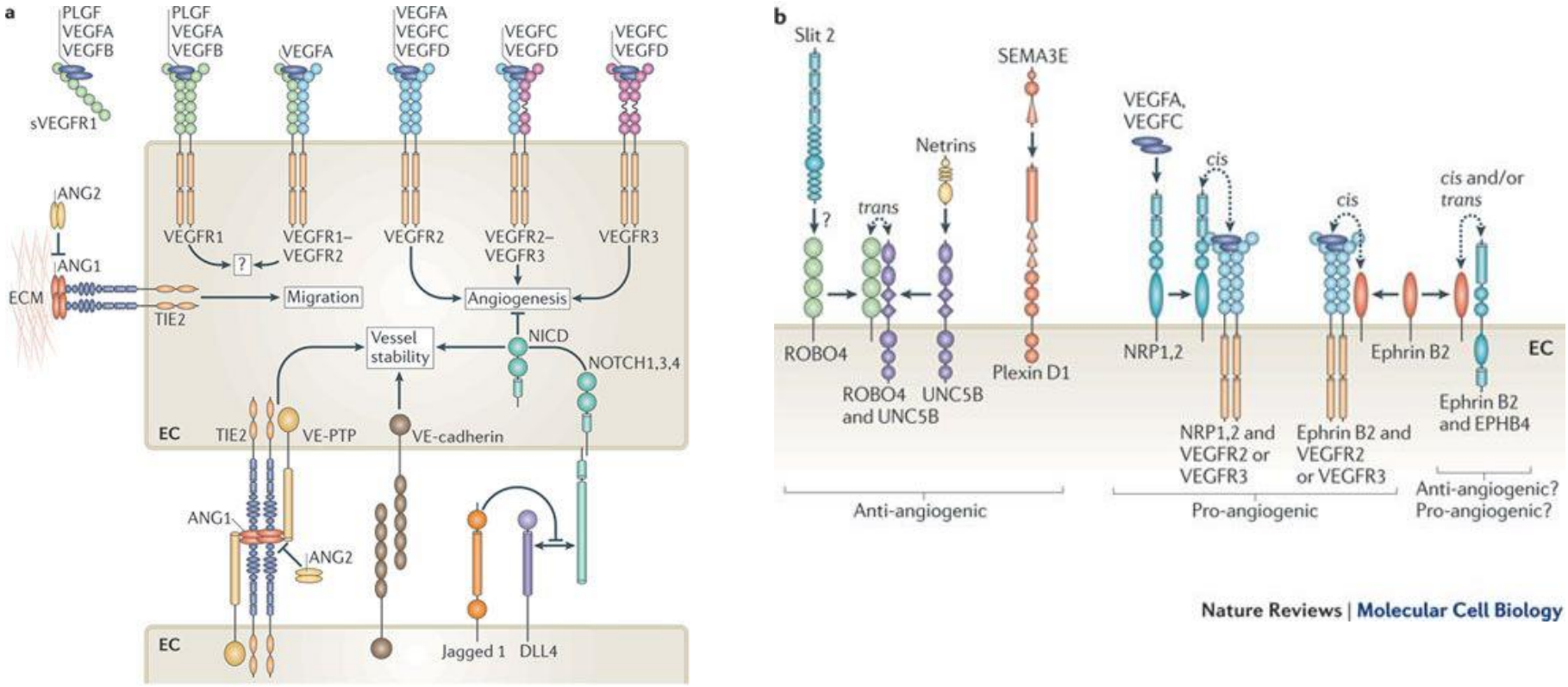


Endothelial factors and receptors

Endothelium-specific factors:
VEGF family: 5 factors
Notch family: 4 factors
Angiopoietin family : 4 factors
Ephrin family: at least 2 factor
Unc/Sema family: at least 3 factors

Non EC-specific factors :
bFGF
PDGF
TGF-β

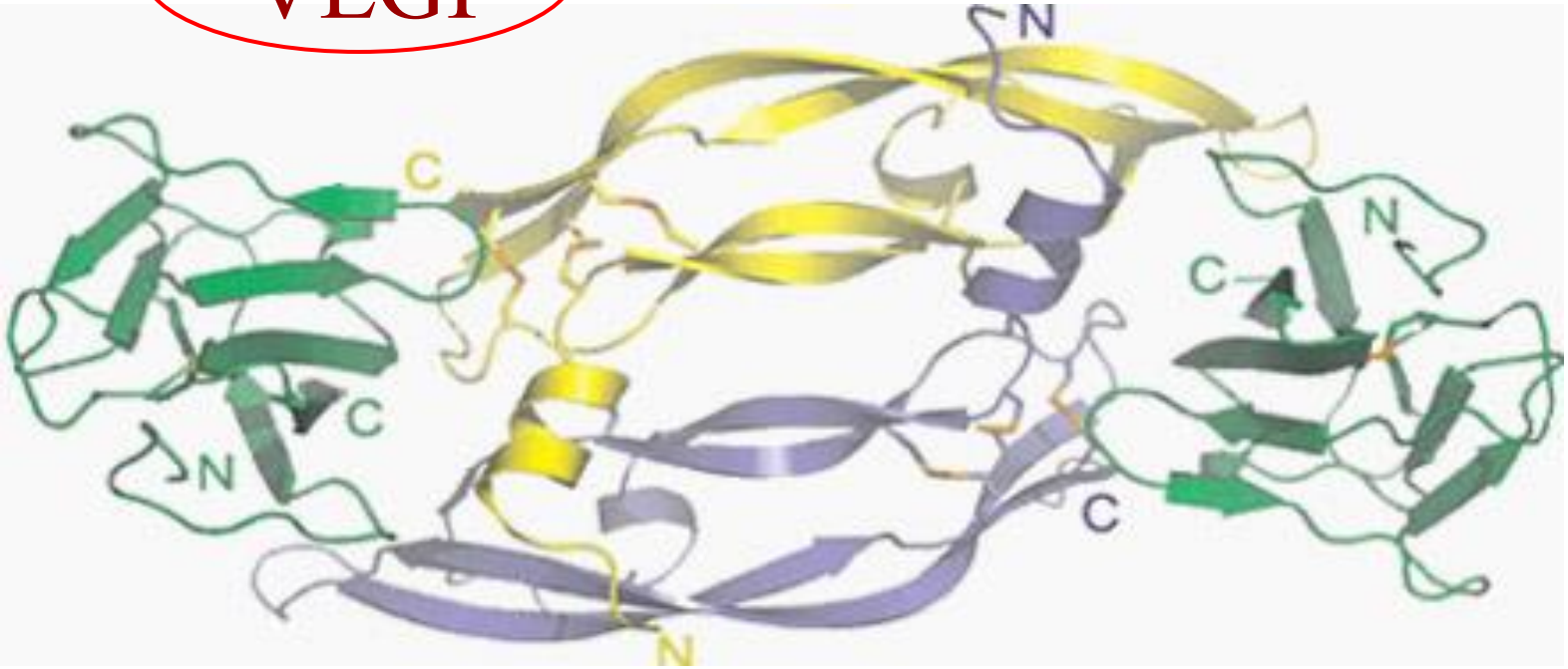
Key signaling pathways that control angiogenesis



VEGF: the predominant mediator of angiogenesis

Among the many factors implicated in angiogenesis, VEGF has been identified as the most potent and predominant. The scope of scientific research involving VEGF continues to grow exponentially. From 1995 to 2005, the number of VEGF-related abstracts presented at the annual meeting of the American Society of Clinical Oncology (ASCO) increased 50-fold, highlighting the increased focus in research upon VEGF's role in oncology.

VEGF

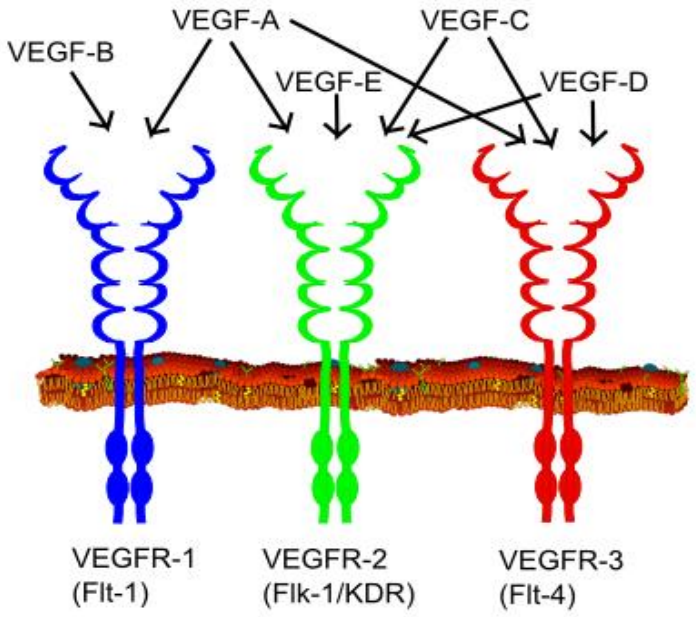


VEGF (also known as VEGF-A, but commonly referred to simply as VEGF) stands for “vascular endothelial growth factor.” This protein plays an important role in angiogenesis. As its name suggests, VEGF stimulates vascular endothelial cell growth, survival, and proliferation. As seen in preclinical models, VEGF has been shown to facilitate survival of existing vessels, contribute to vascular abnormalities (eg, tortuousness and hyperpermeability) that may impede effective delivery of antitumor compounds, and stimulate new vessel growth

The VEGF family of proteins

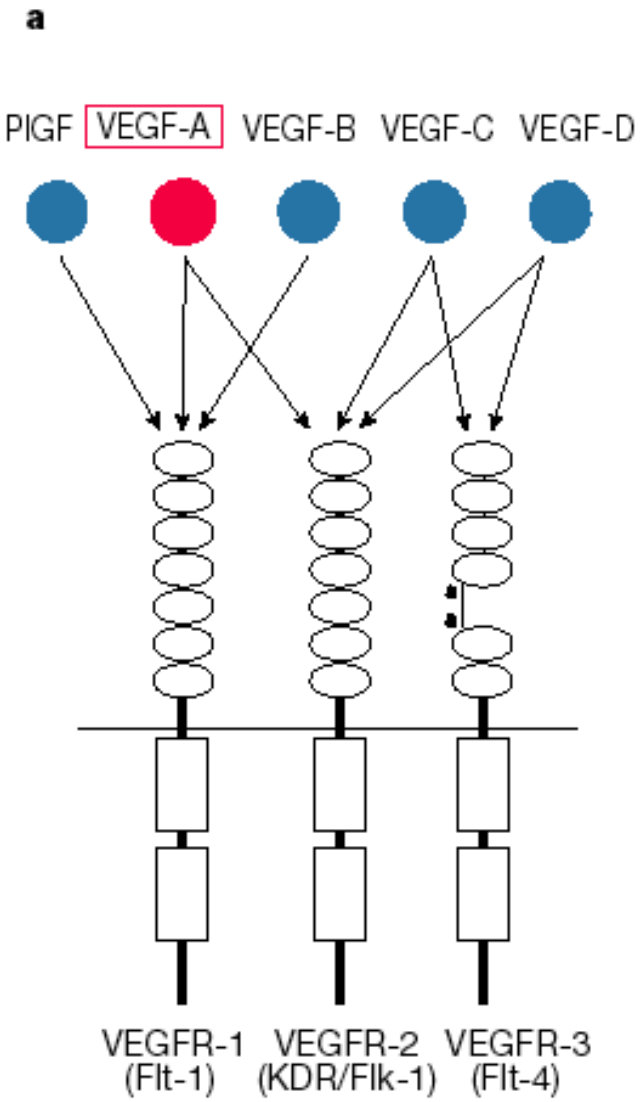
VEGF is a member of a family of 6 structurally related proteins (see table below) that regulate the growth and differentiation of multiple components of the vascular system, especially blood and lymph vessels. The angiogenic effects of the VEGF family are thought to be primarily mediated through the interaction of VEGF with VEGFR-2.

VEGF Family Members	Receptors	Functions
VEGF (VEGF-A)	VEGFR-1, VEGFR-2, neuropilin-1	Angiogenesis Vascular maintenance
VEGF-B	VEGFR-1	Not established
VEGF-C	VEGFR-2, VEGFR-3	Lymphangiogenesis
VEGF-D	VEGFR-2, VEGFR-3	Lymphangiogenesis
VEGF-E (viral factor)	VEGFR-2	Angiogenesis
Placental growth factor (PlGF)	VEGFR-1, neuropilin-1	Angiogenesis Inflammation



There are 4 major isoforms of VEGFA (VEGF), each encoded by a different portion of the *VEGF* gene. These isoforms are VEGF121, VEGF165, VEGF189, and VEGF206. Although these isoforms behave identically in solution, they differ in their ability to bind heparin and the extracellular matrix

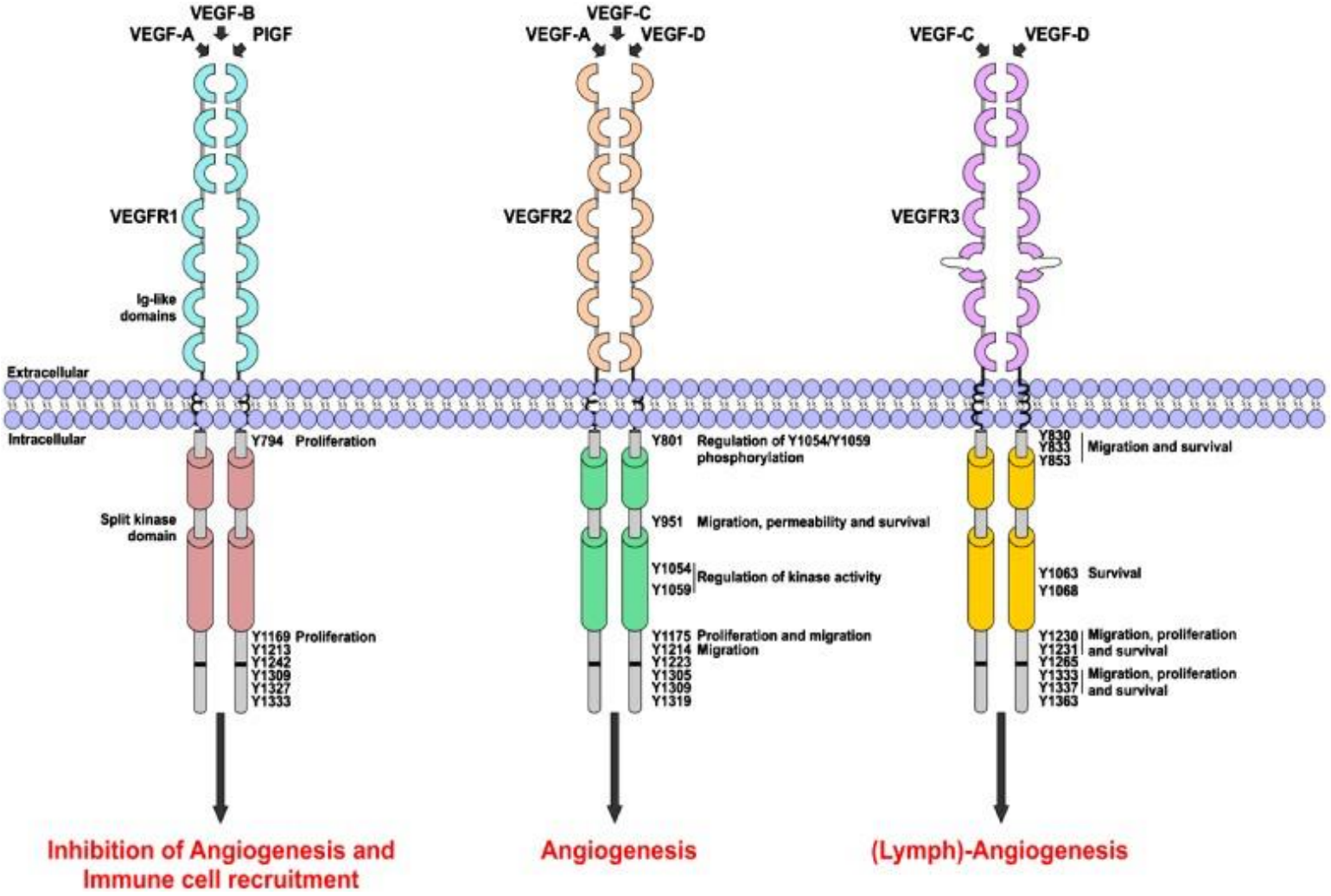
VEGF/VEGFR family



VEGFs:
VEGF-A: initiation of vasculogenesis and sprouting angiogenesis, immature vessels, Vascular permeability factor.
VEGF-B: heart vascularization
VEGF-C: lymphatic vessels
VEGF-D: lymphatic vessels
PlGF: remodeling of adult vessels

VEGFRs:
VEGFR-2: growth and permeability
VEGFR-1: negative role, decoy receptor, synergism with VEGFR-2 in tumor angiogenesis
VEGFR-3: lymphatic vessels

Receptor tyrosine kinase subfamily of VEGFR proteins



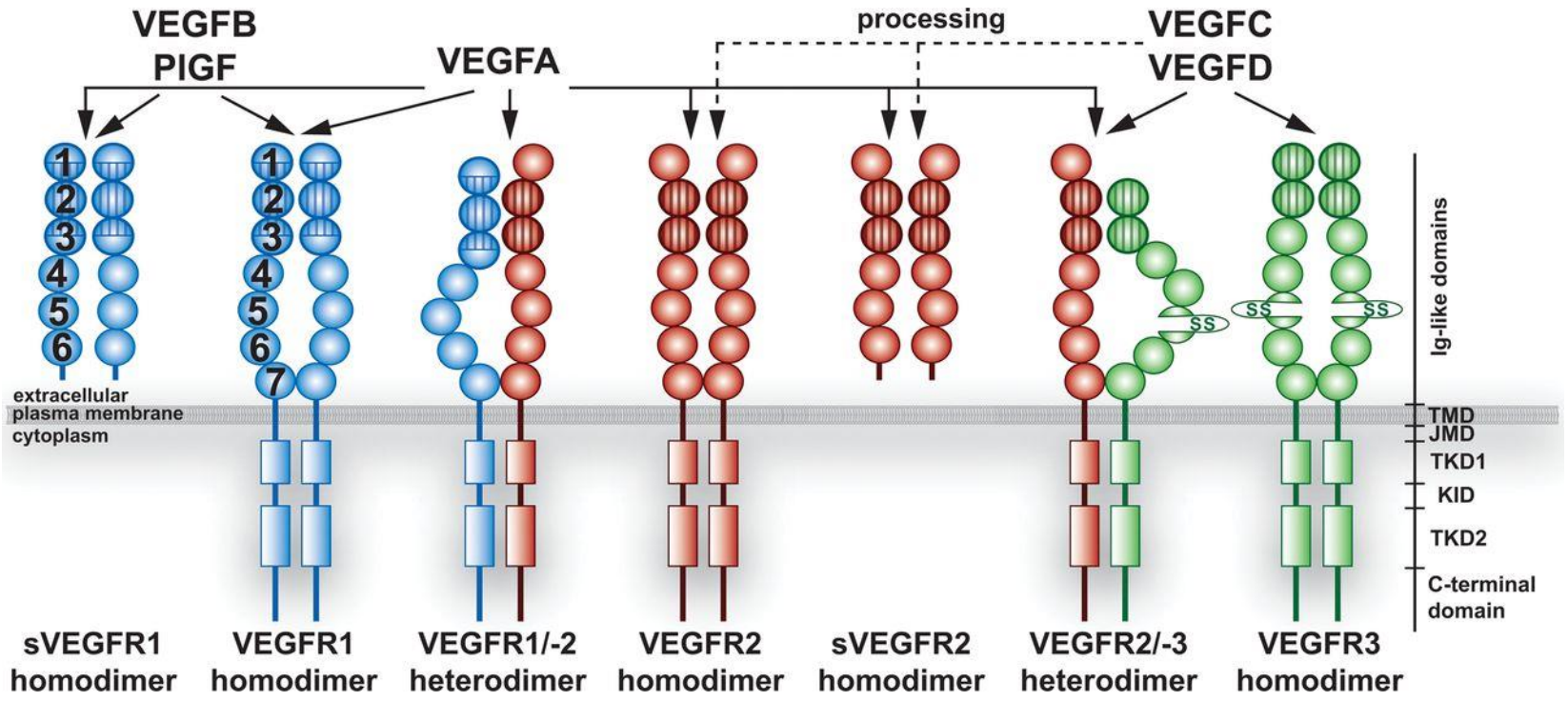
RECEPTORS OF VEGF GROWTH FACTORS

Three receptors have been identified that bind different VEGF growth factors: VEGFR1 (FLT1), VEGFR2 (Flk1/KDR), and VEGFR3 (FLT4) (initial receptor names are given in parentheses)

These receptors belong to the superfamily of receptor tyrosine kinases (RTK) and are based on their structural peculiarities, they comprise a special class within it. Like all RTK, the VEGF receptors are transmembrane proteins with a single transmembrane domain .

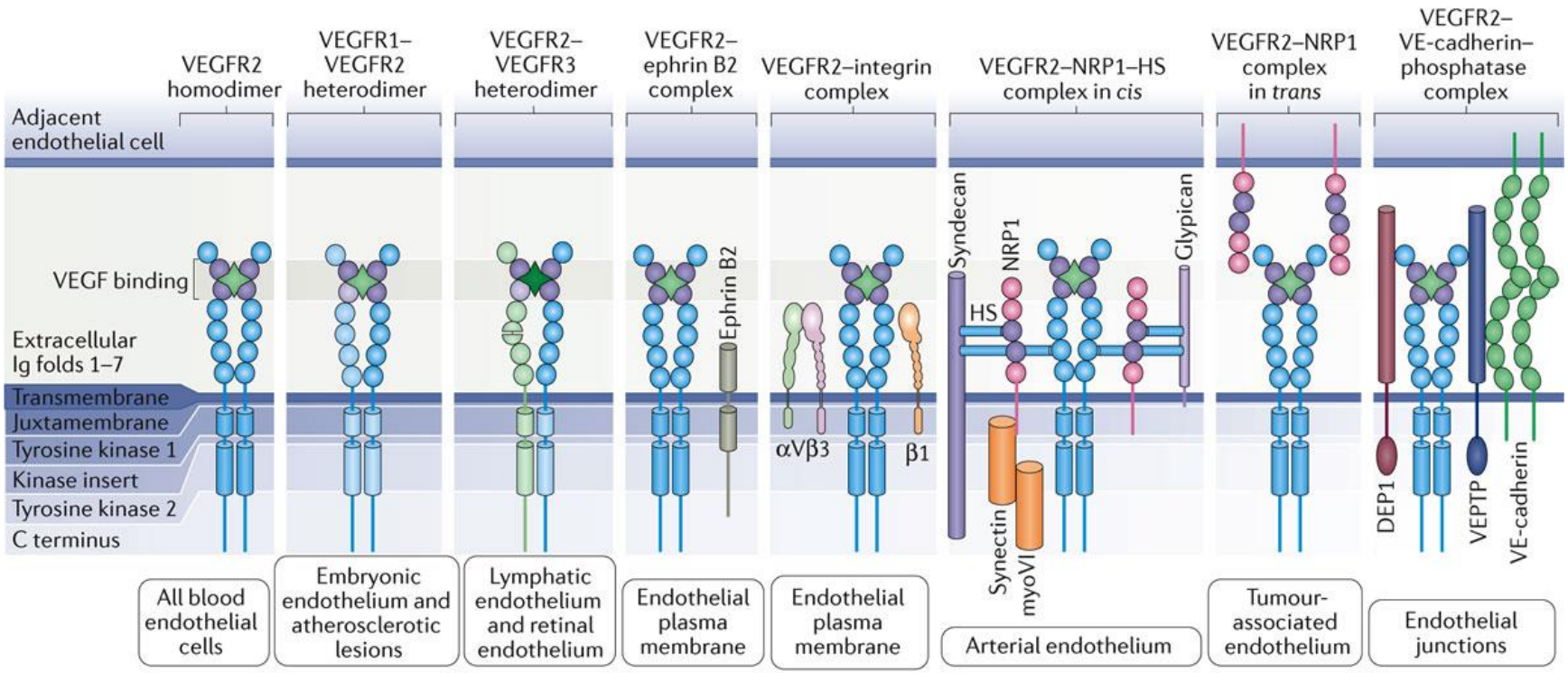
The extracellular region of VEGFR is formed by seven immunoglobulin-like domains (IG I-VII), whereas the intracellular part exhibits tyrosine kinase activity, and the tyrosine kinase domain in these receptors is separated to two fragments (TK-1 and TK-2) by an inter-kinase insert All VEGFR receptors are highly homologous .

VEGF binding specificities and VEGFR signalling complexes Schematic outline of the five VEGFs, VEGFA, VEGFB, VEGFC, VEGFD and PlGF, binding with different affinities to three VEGFRs, initiating VEGFR homo- and hetero-dimer formation.



Sina Koch et al. Biochem. J. 2011;437:169-183

Overview of VEGFR2 receptor and its biological signaling output



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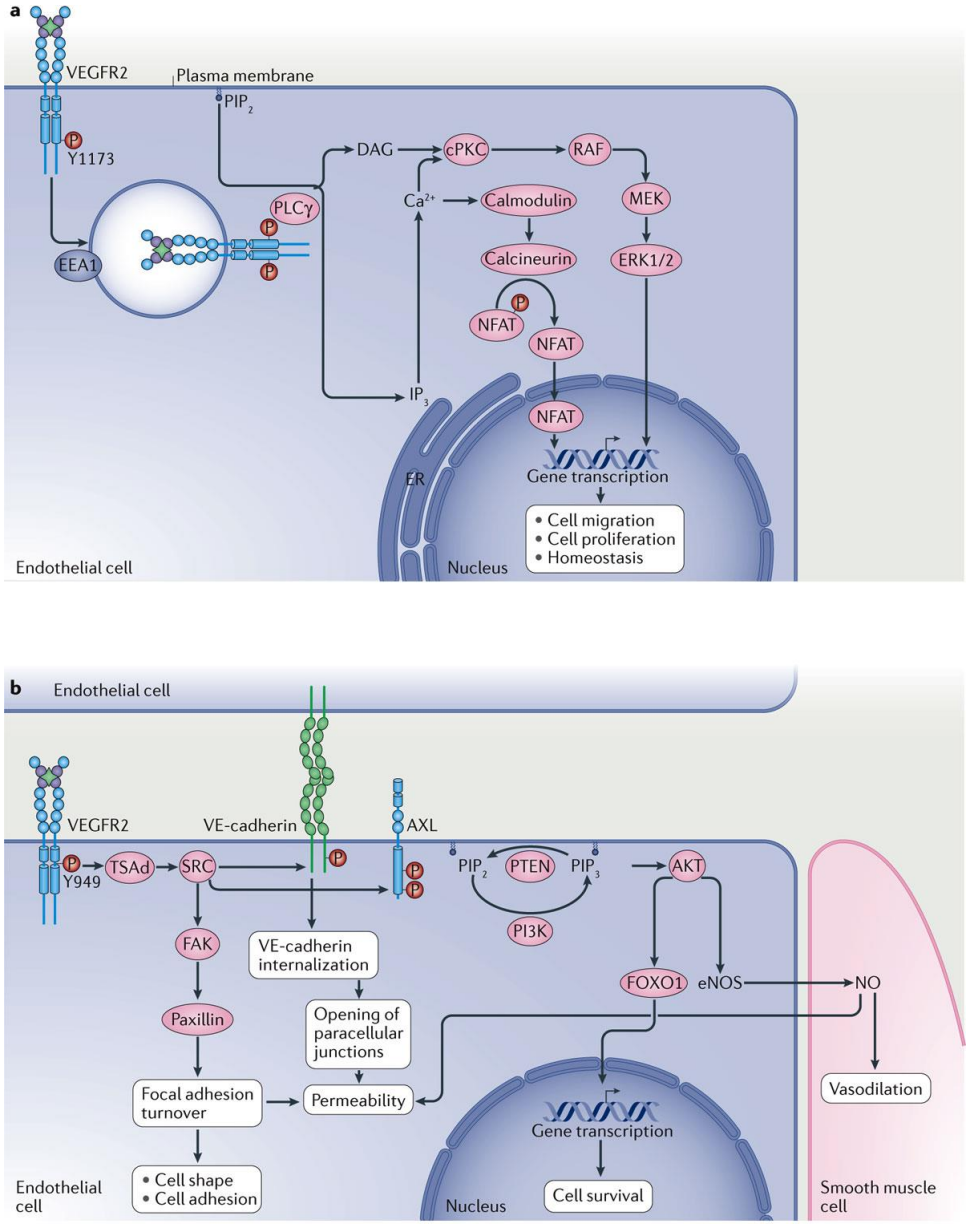
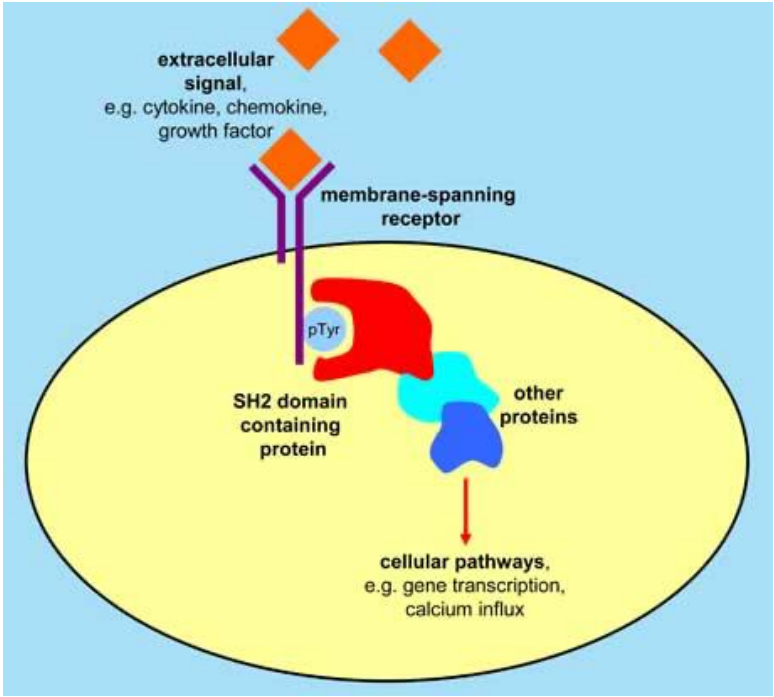
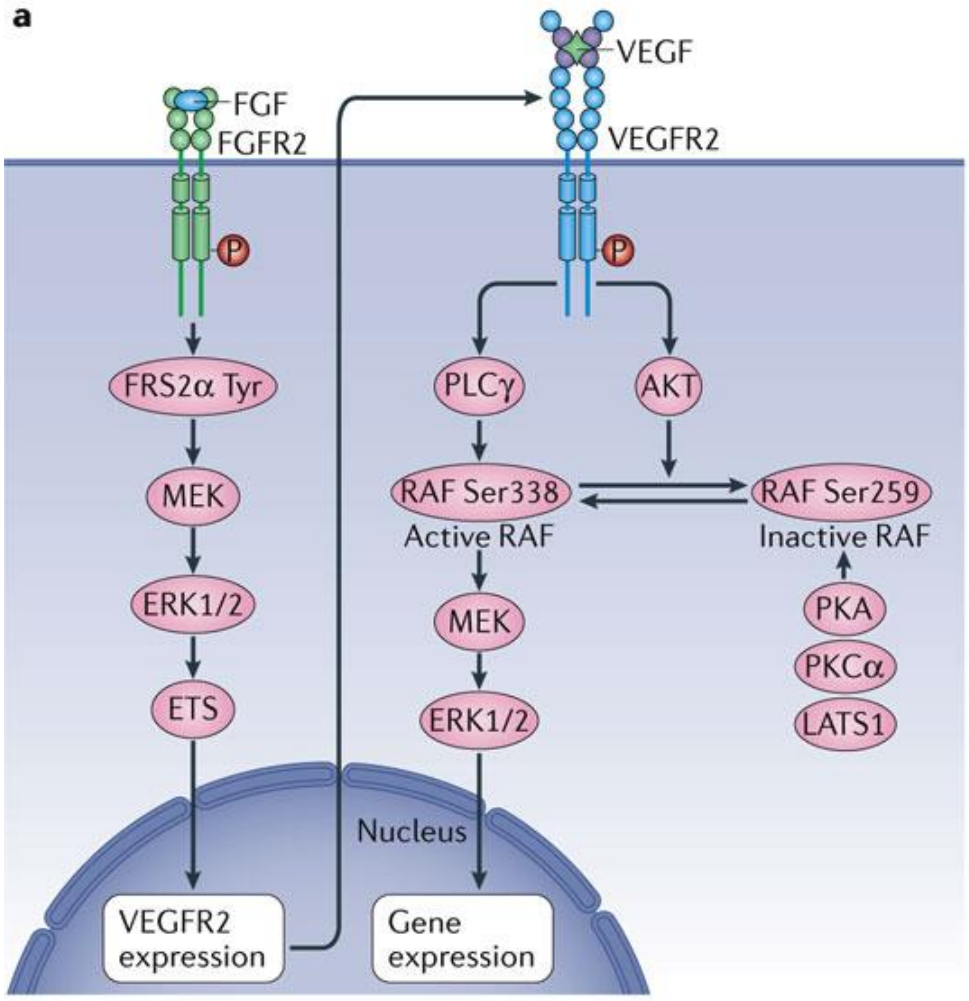
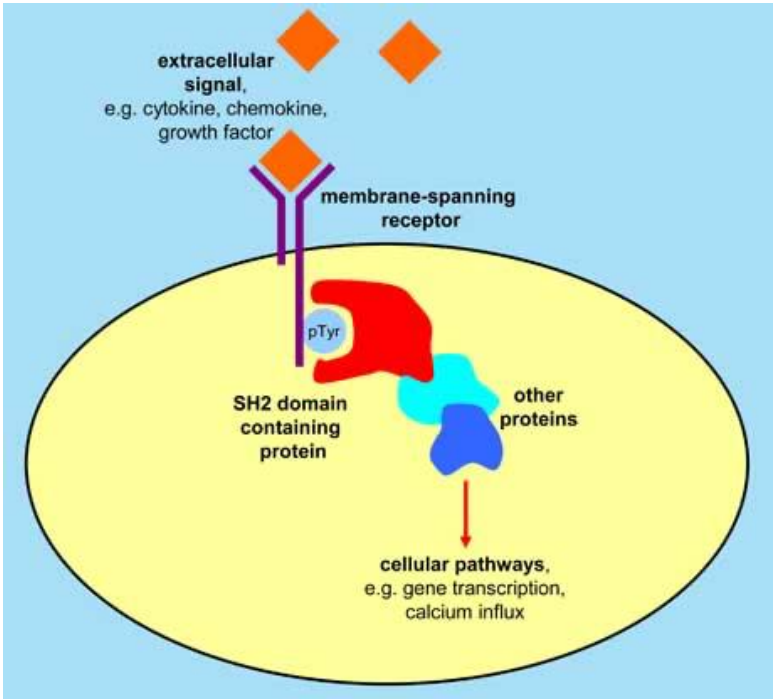
Schematic representation of vascular endothelial growth factor (VEGF)-activated VEGF receptor 2 (VEGFR2) signalling pathways



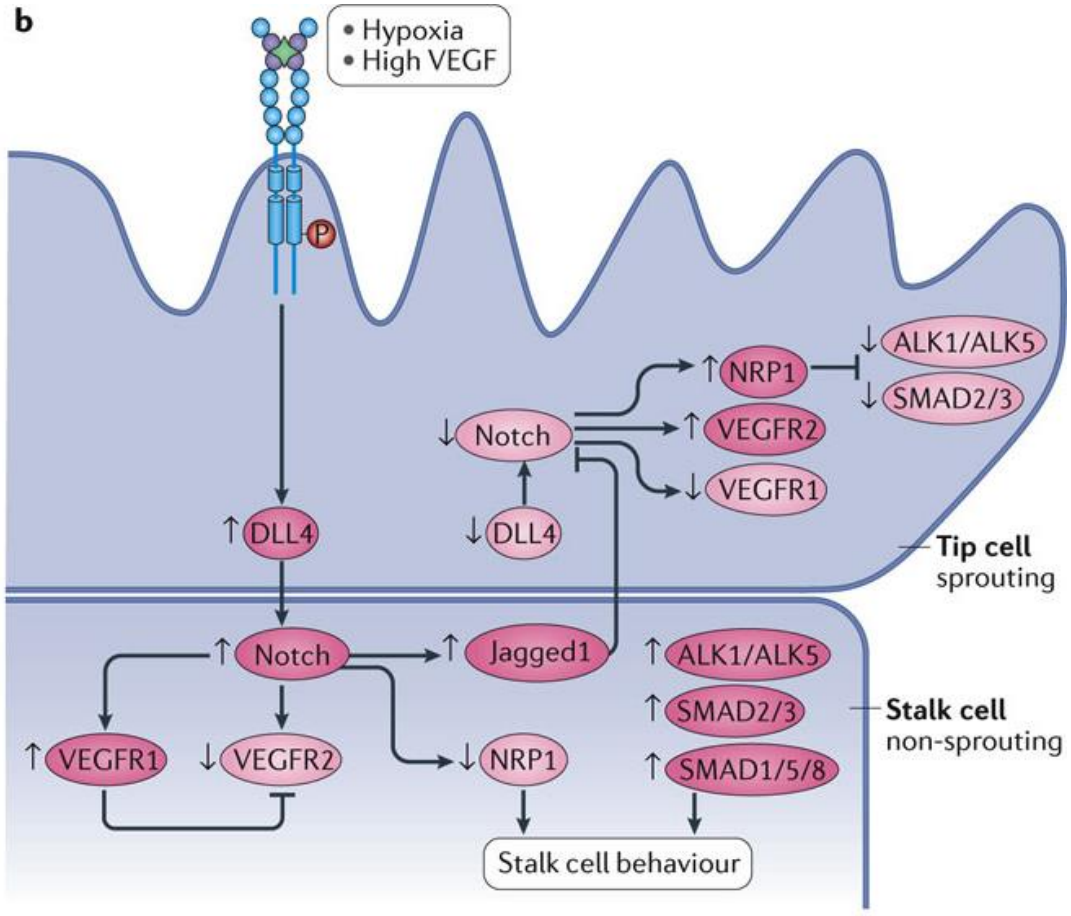


Fibroblast growth factor (FGF) signalling modulates the sensitivity of endothelial cells to vascular endothelial growth factor (VEGF)





Notch signalling acts both upstream and downstream of VEGFR2



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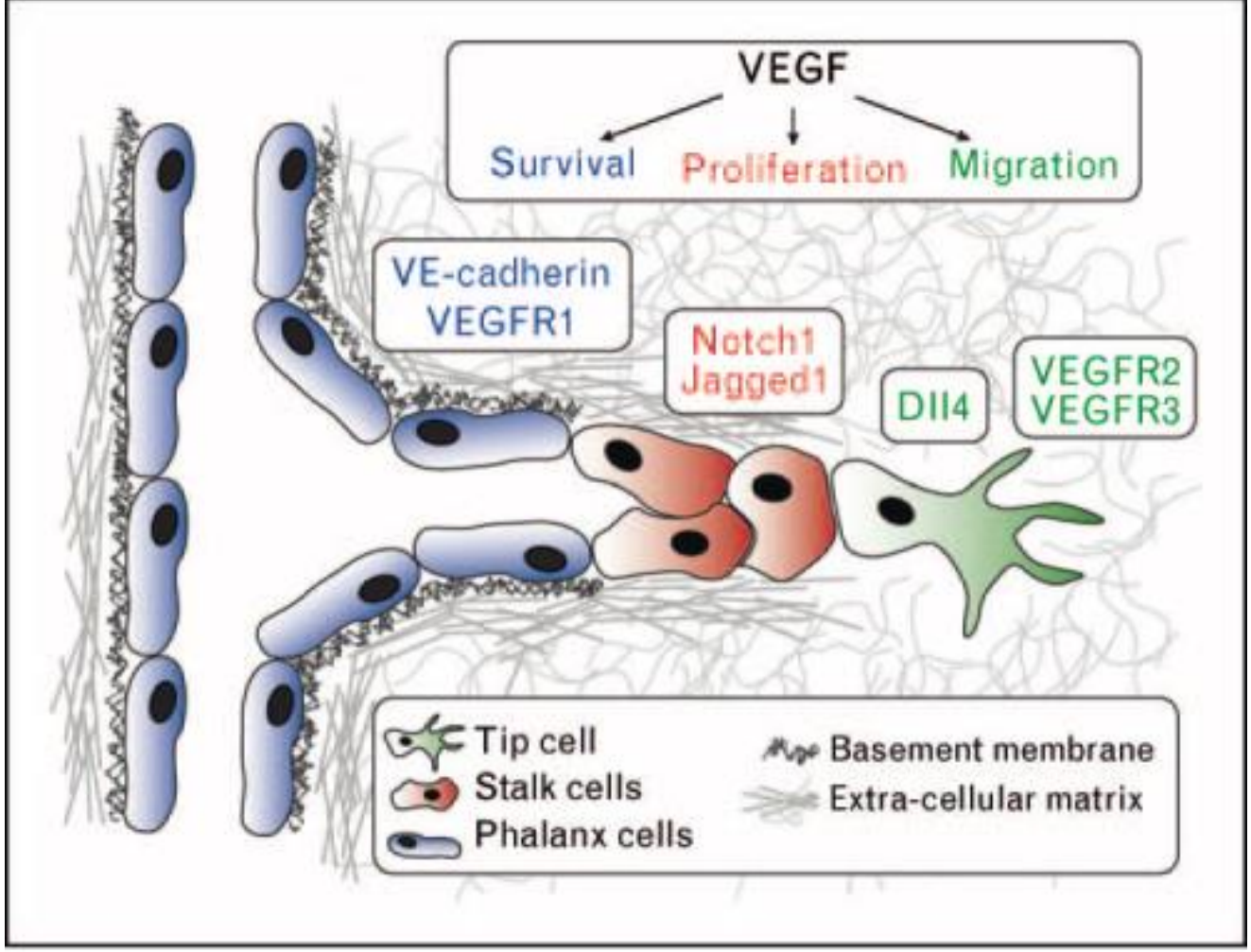
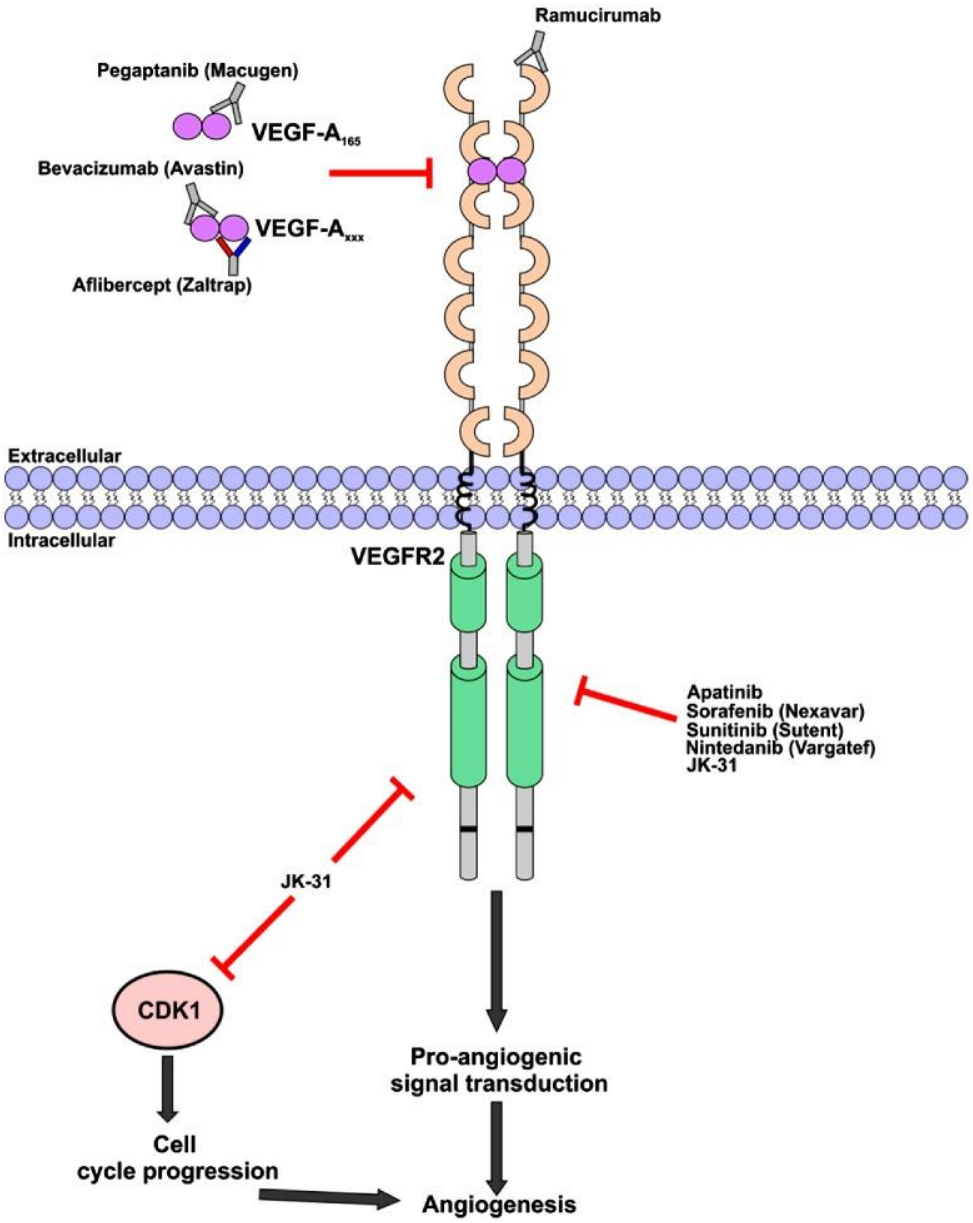
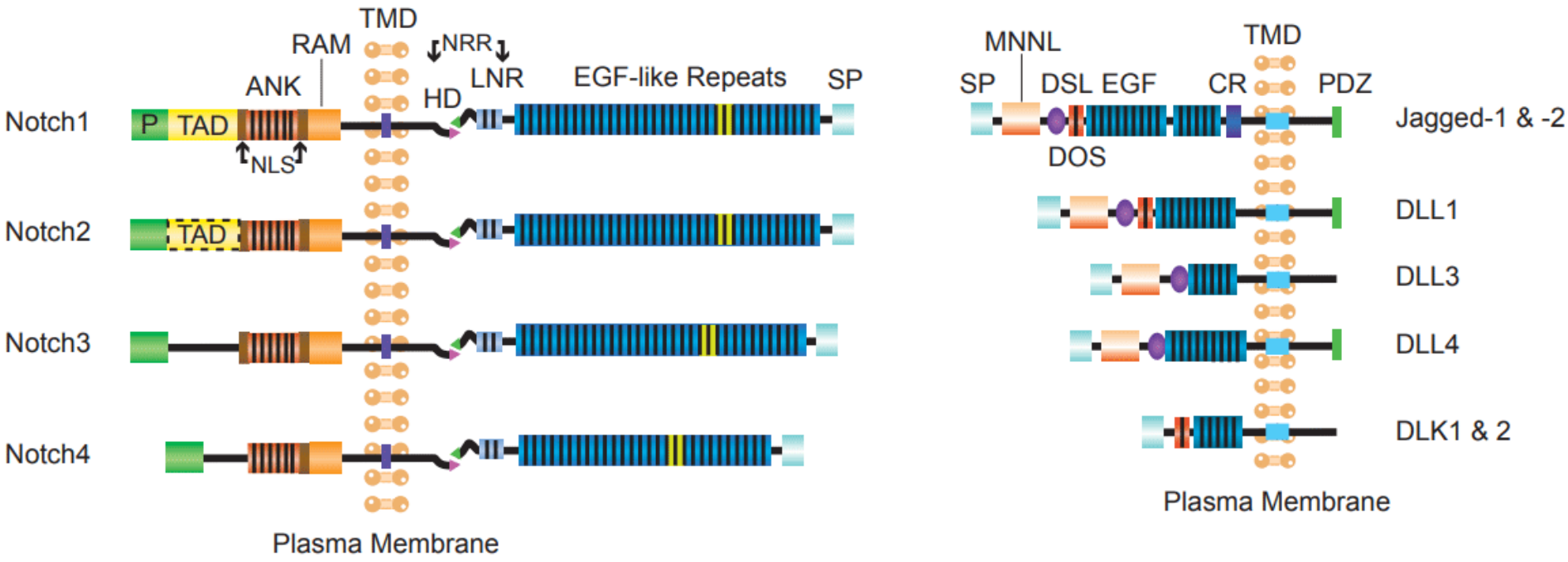


A growing sprout is composed of tip cell (green), stalk cells (red), and phalanx cells (blue). Each cell type is characterized by a unique molecular signature, resulting in a differential response to VEGF. Tip cells exhibit a migratory response to VEGF and show an upregulation of Dll4, VEGFR3, and VEGFR2. Stalk cells undergo proliferation and show upregulation of Notch1 and Jagged1. VEGF signaling in phalanx cells leads to a survival response mediated by increased levels of VE–cadherin and VEGFR1. VEGF, vascular endothelial growth factor.

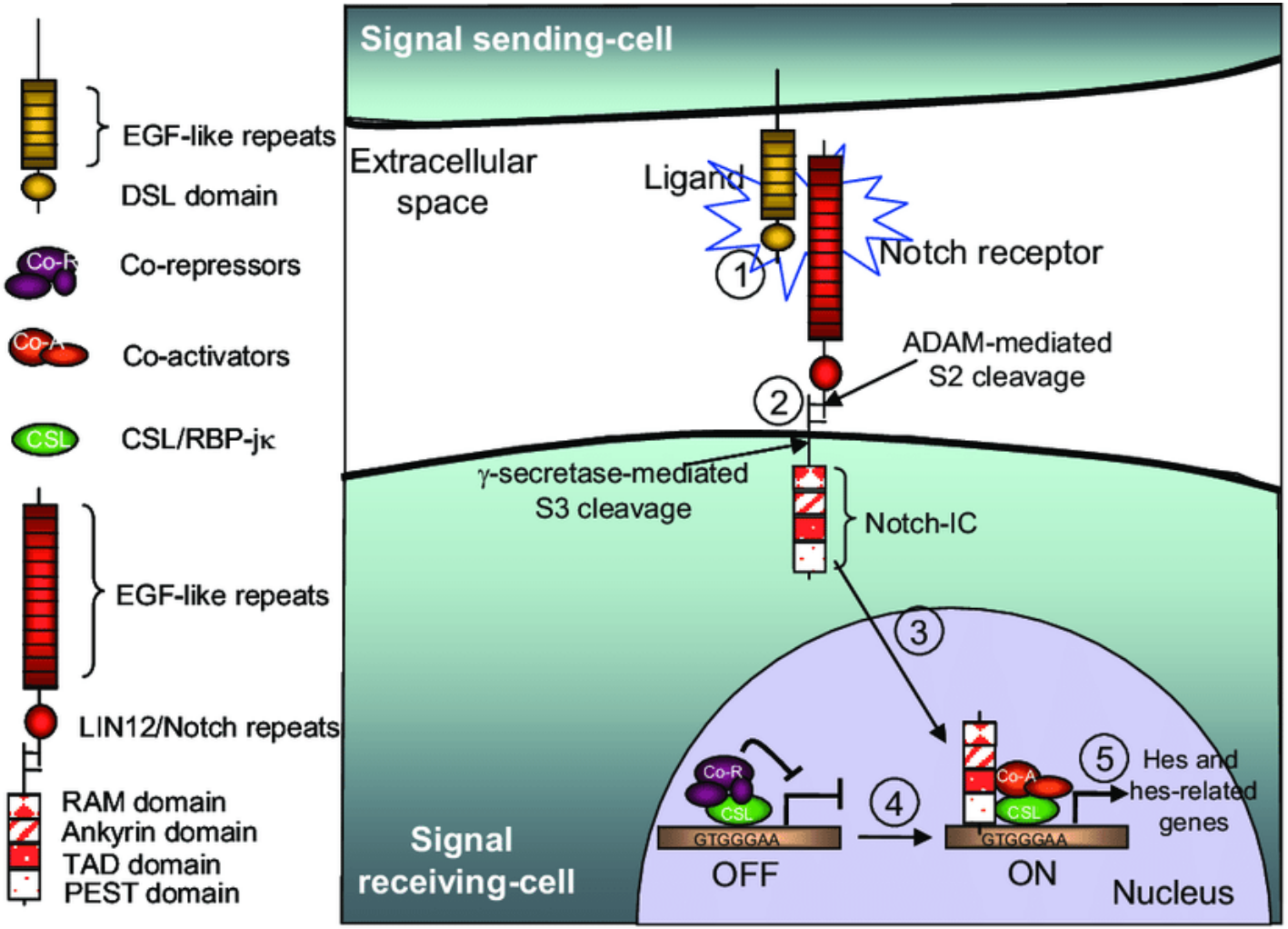
Therapeutic inhibitors of VEGFR2 signal transduction



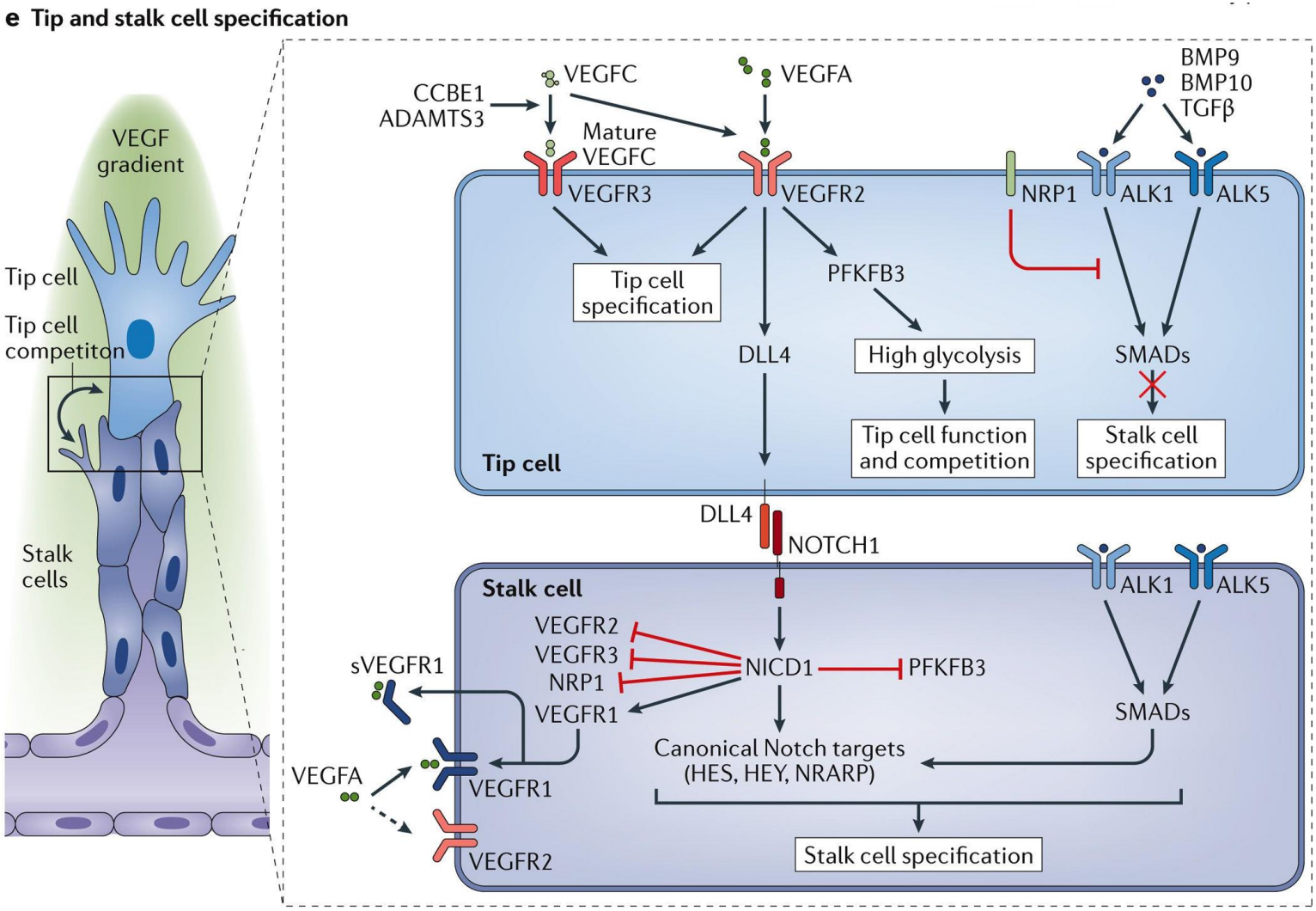
Notch receptors in angiogenesis



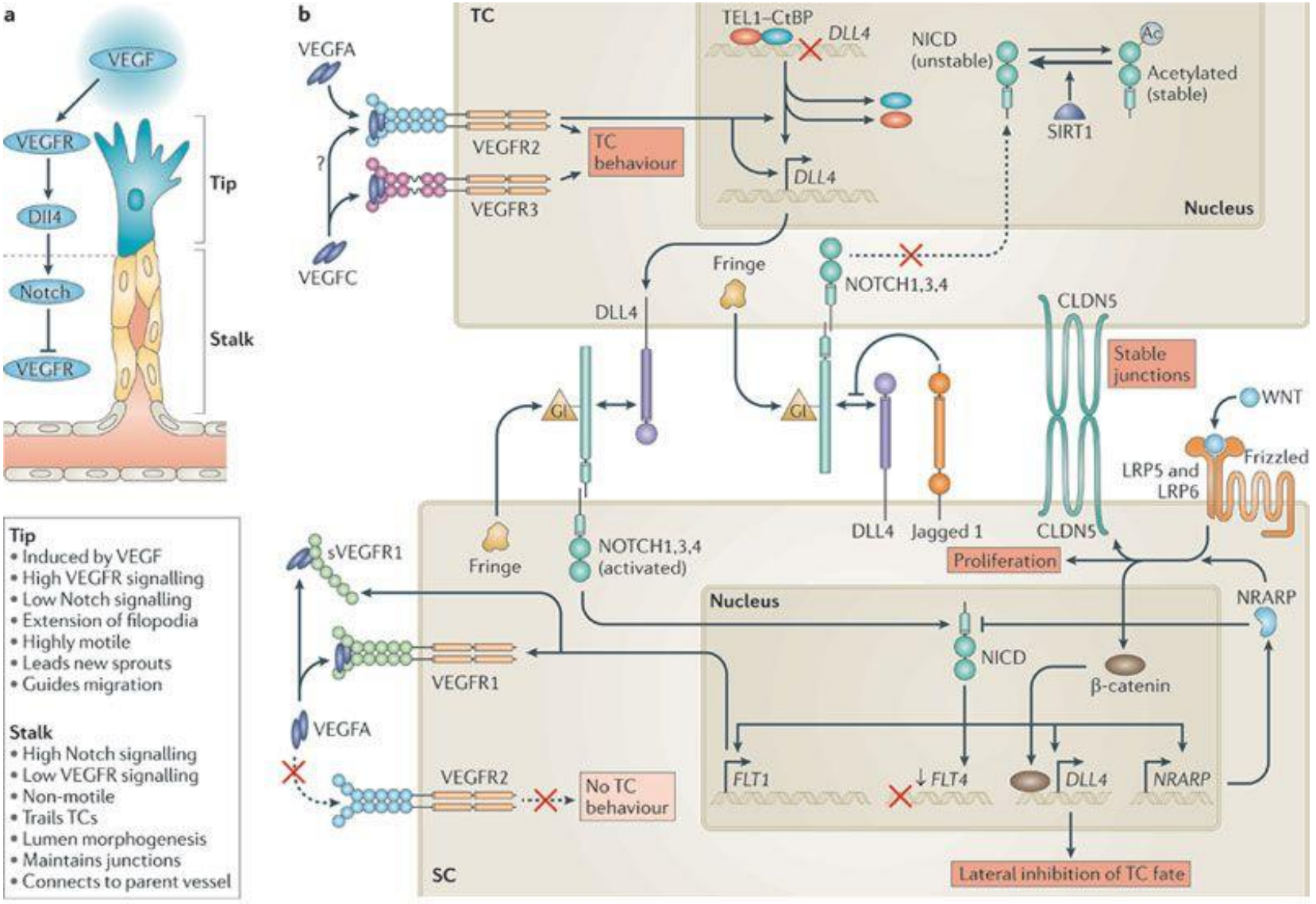
Notch receptors activation



Mechanisms of vascular development

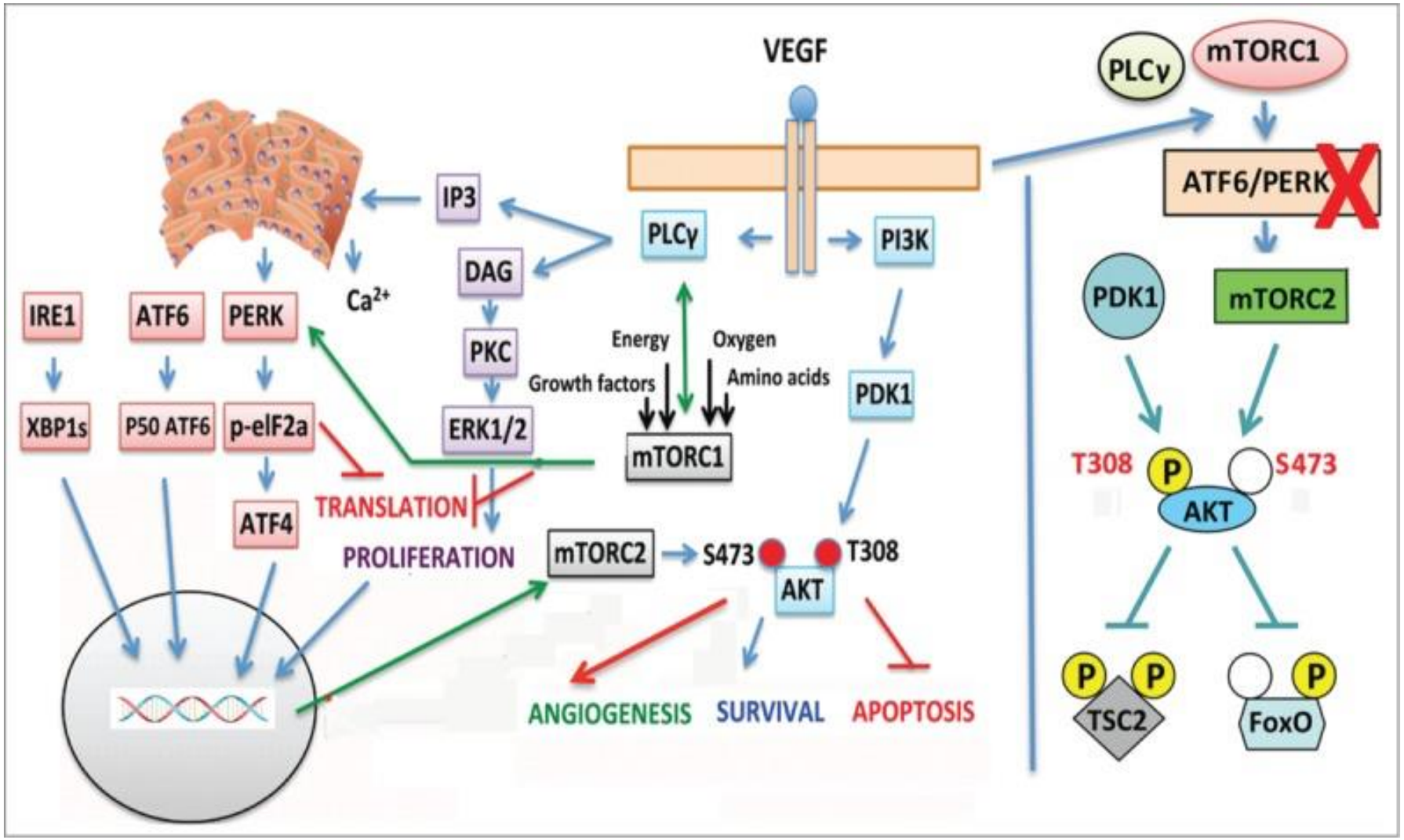


Molecular mechanisms of endothelial tip cell selection

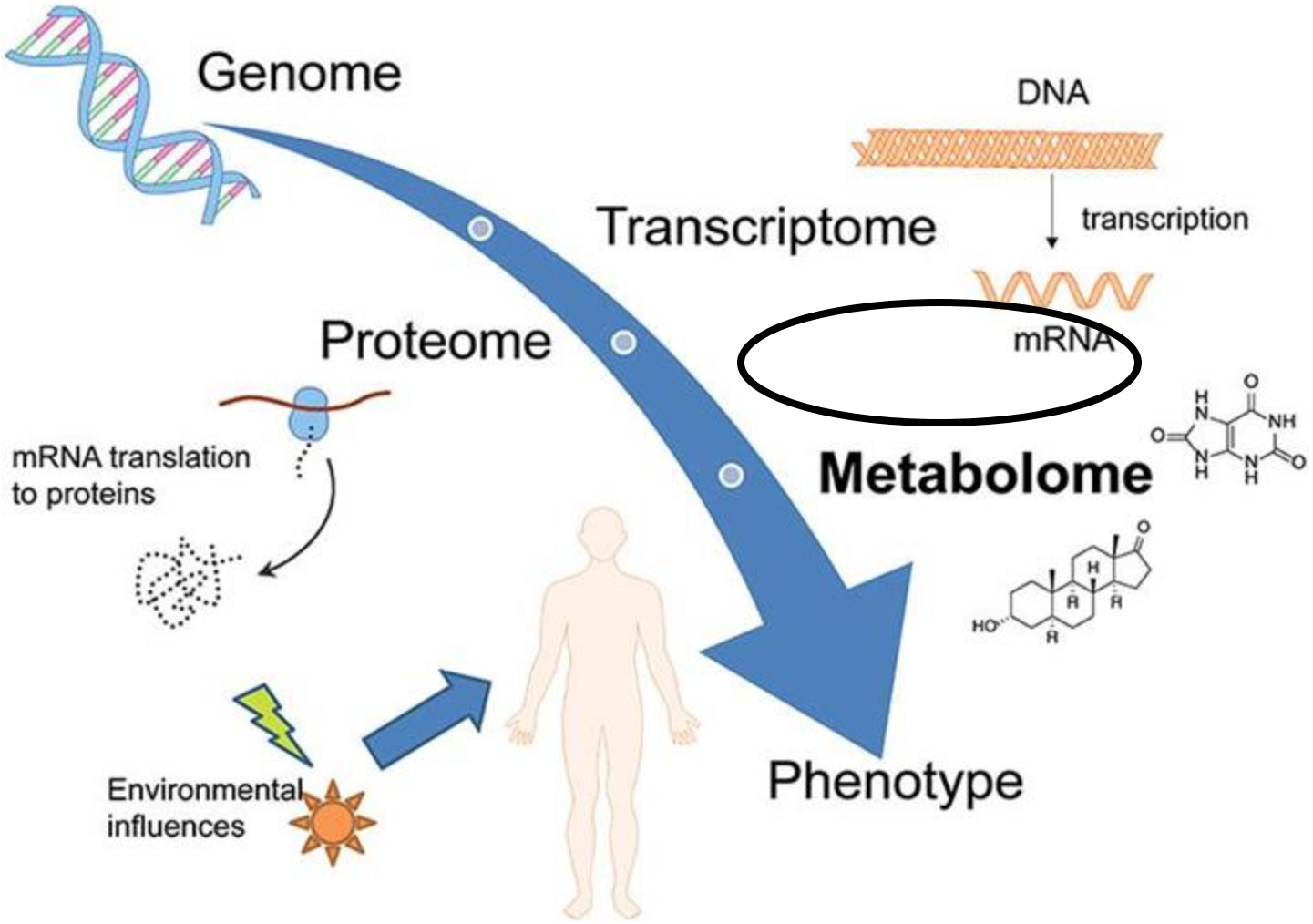


VEGF signaling and mTOR complexes:

Towards a role of metabolic sensing in the regulation of angiogenesis

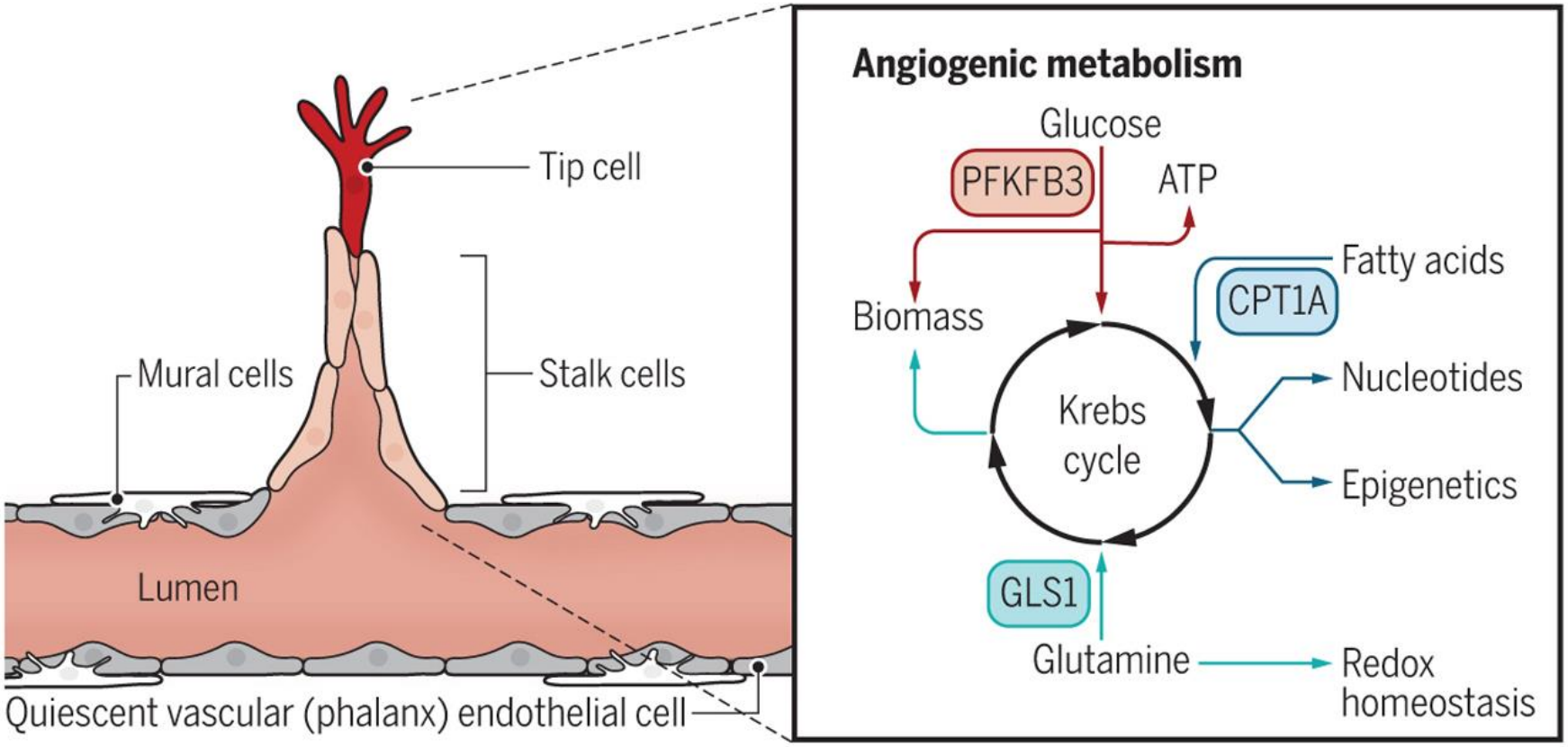


Angiogenesis and metabolism

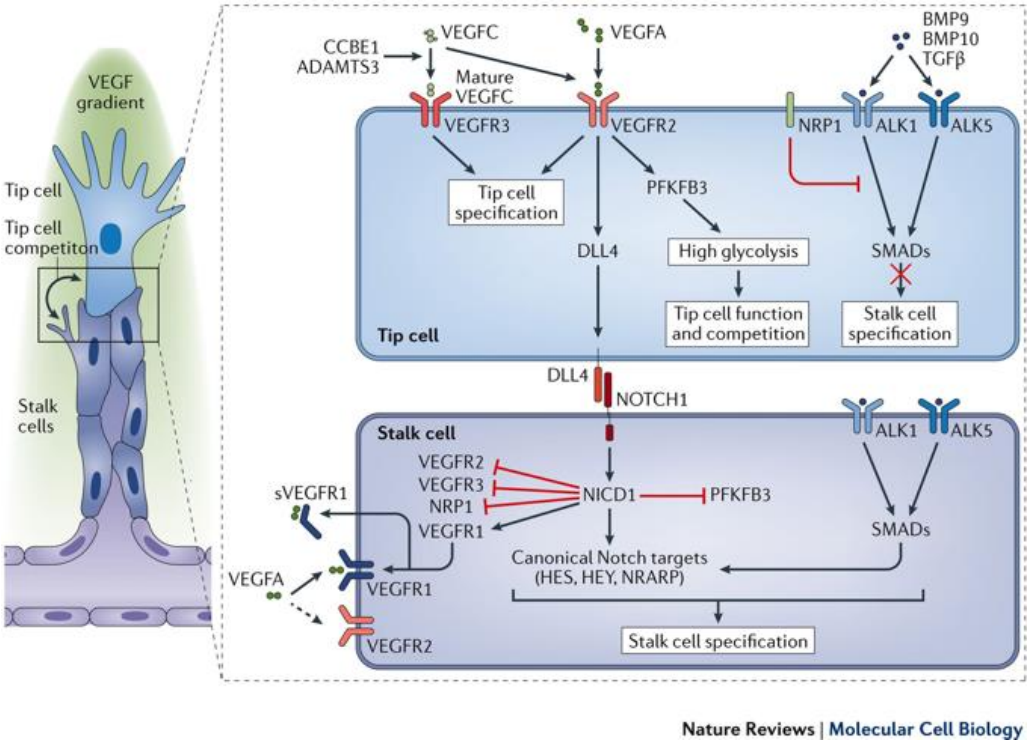


Metabolic pathways in angiogenesis

During angiogenesis, endothelial cells undergo metabolic changes that facilitate the formation of a sprout by stalk cells, which is directed by the tip cell. Key regulators of endothelial cell metabolism, PFKFB3, CPT1A, and GLS1, might be new therapeutic targets for various conditions.



2. Tip and stalk cell specification



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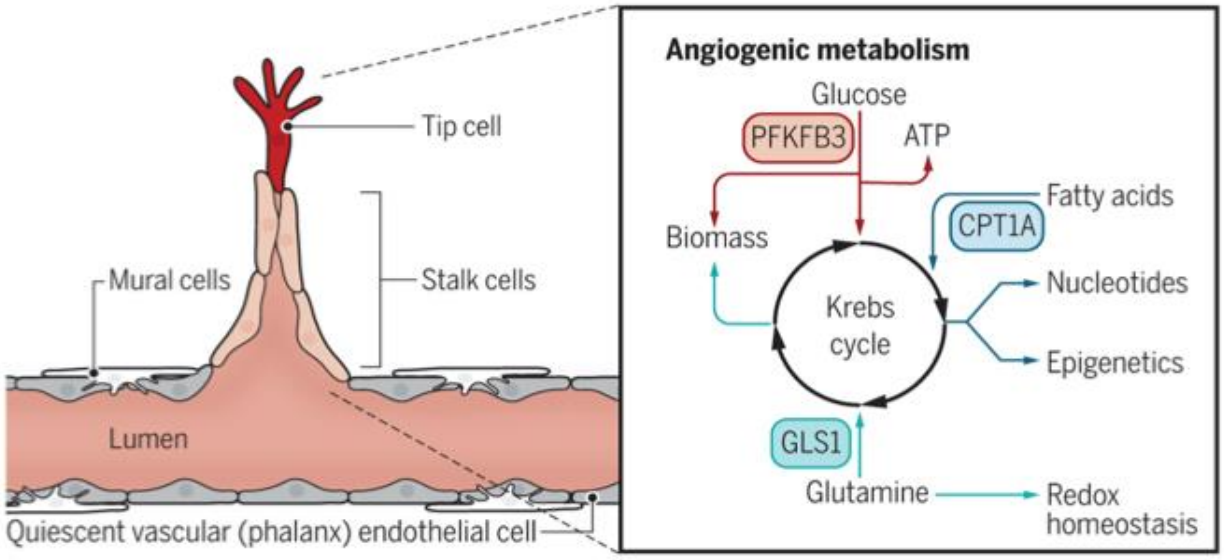
→ MIGRATION

→ PROLIFERATION

Different METABOLISM

3. Metabolic pathways in angiogenesis



Xuri Li and Peter Carmeliet, 2018

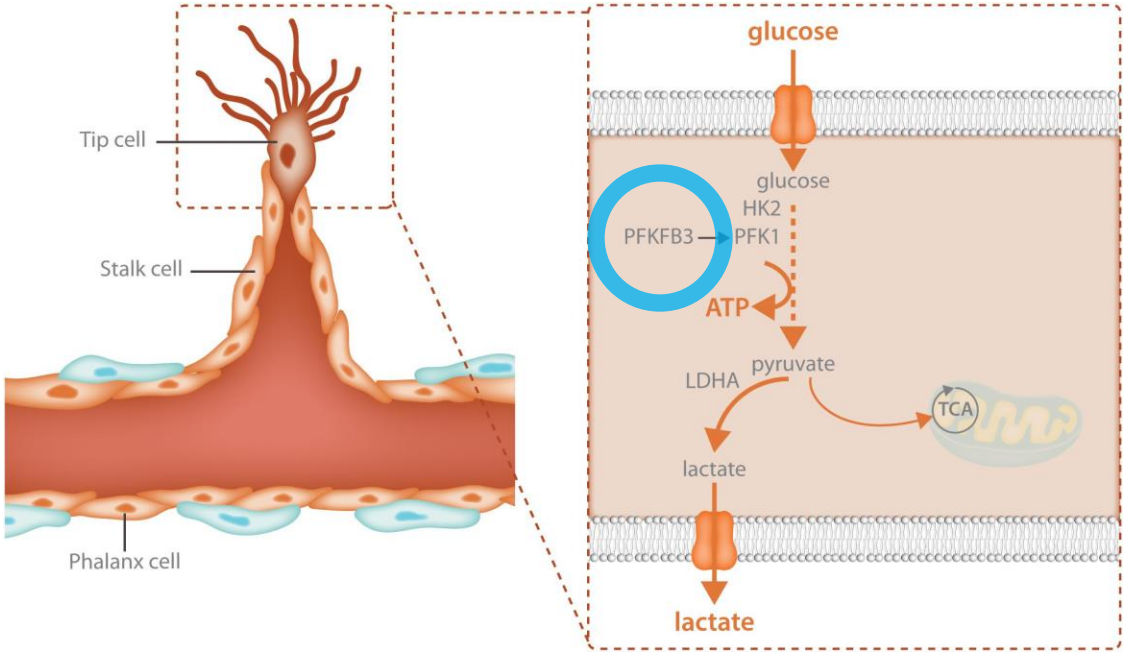
85% of ATP from Glycolysis
15% of ATP from OXPHOS

ADVANTAGES?

Glycolysis vs OXPHOS

1. Shorter time
2. Revascularization of ischemic tissues
3. Reduction of ROS

3. Metabolic pathway in **tip** cell: Glycolysis



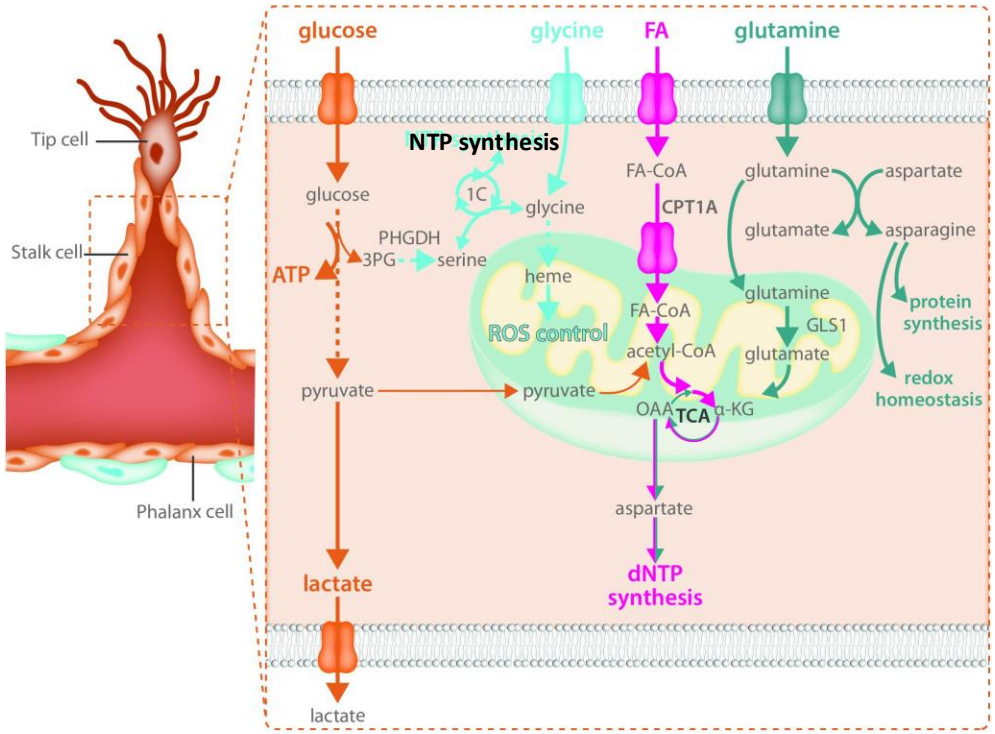
Fitzgerald et al., 2018

Energy for migration

WHY?

- ✓ Faster ATP production
- ✓ Occurs in $-O_2$
- ✓ Limited ROS production

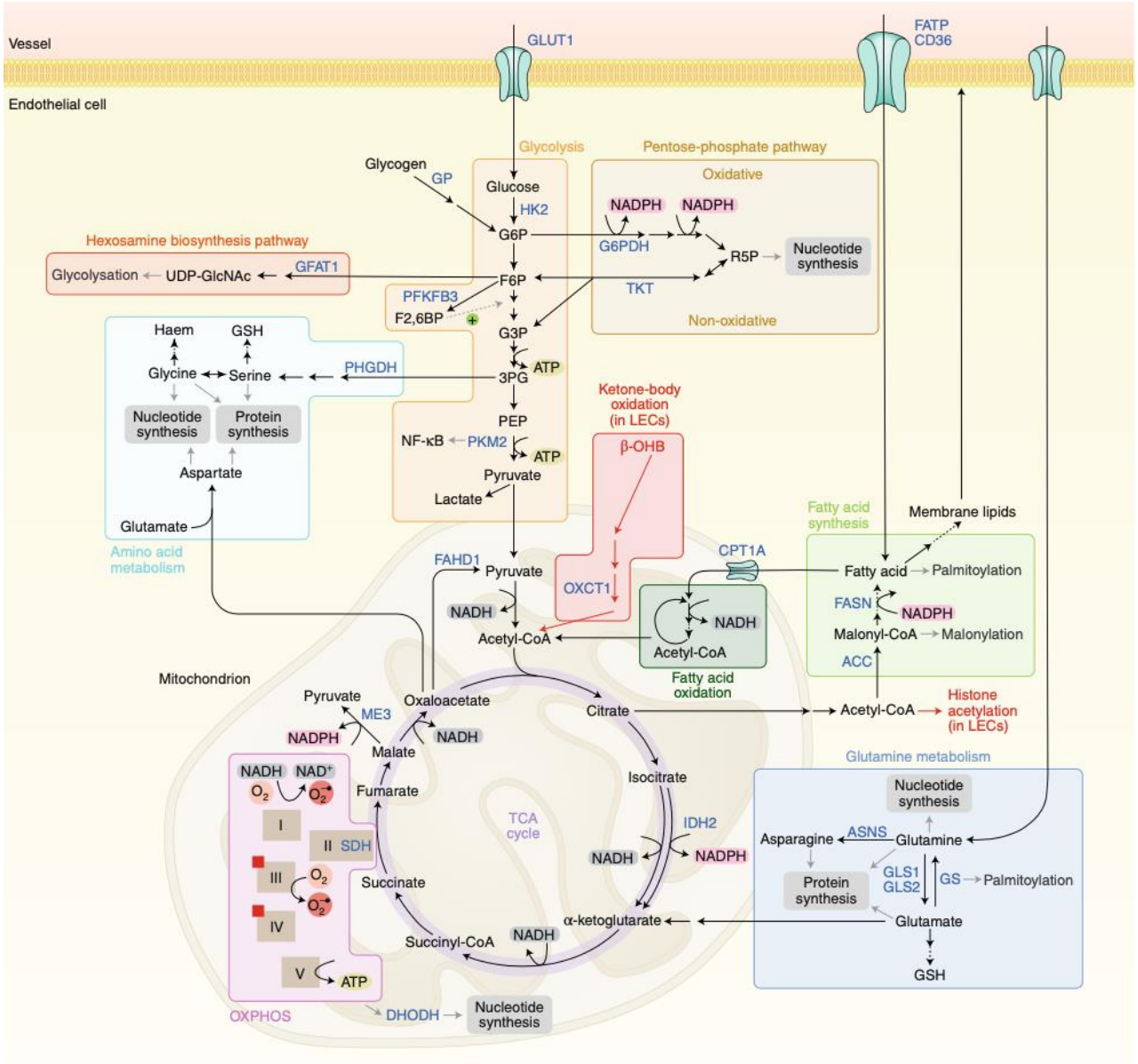
3.1 Metabolism at the Stalk



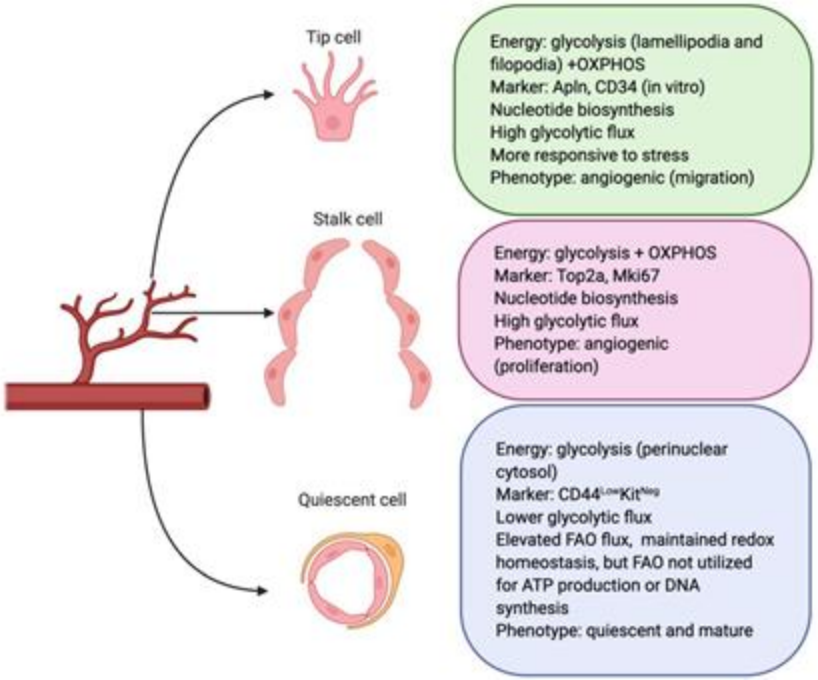
Fitzgerald et al., 2018

Energy for proliferation and biomass synthesis

Key metabolic pathways in EC



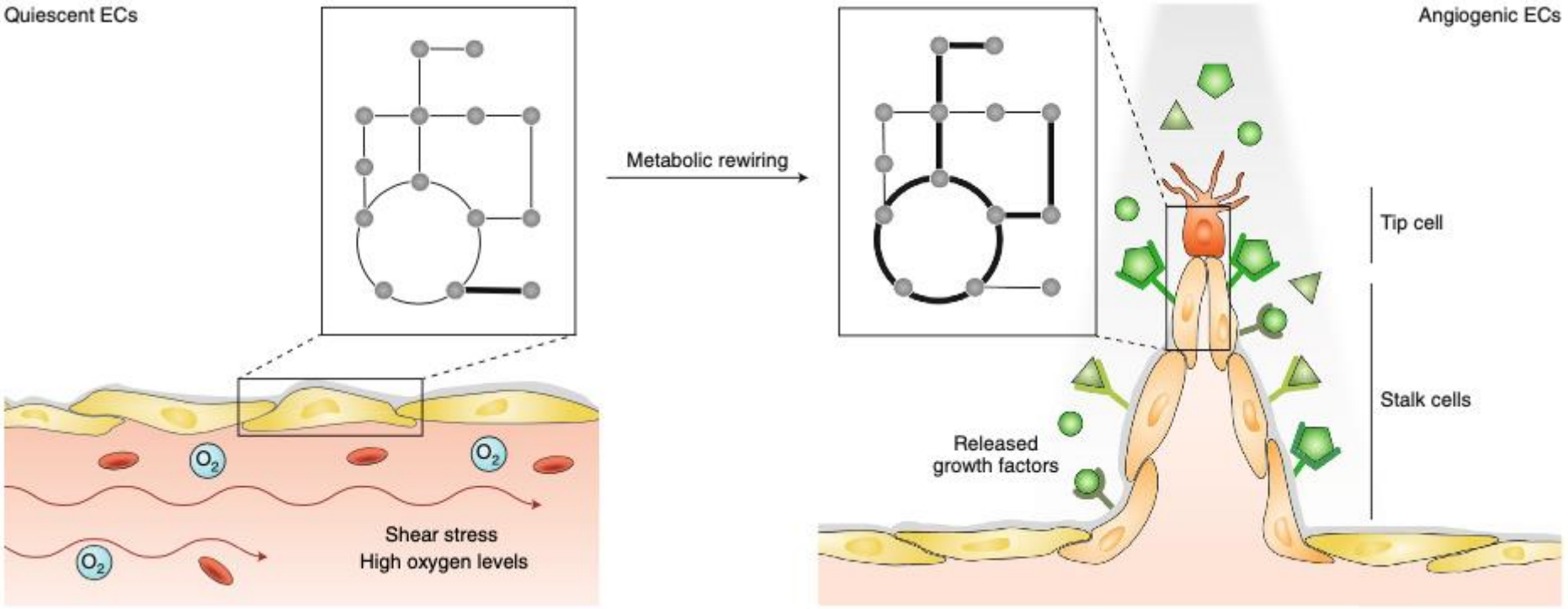
EC metabolism



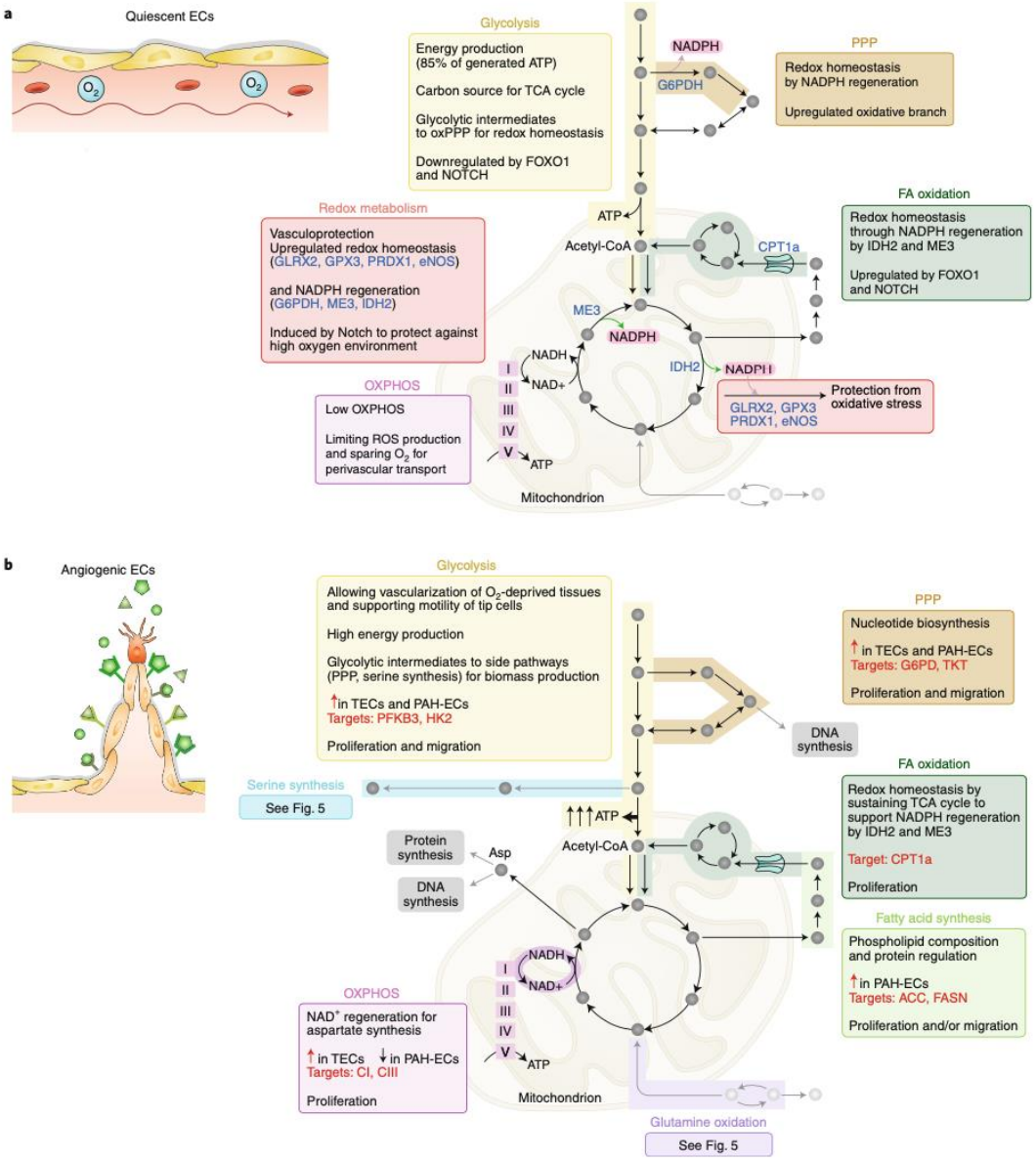
Angiogenic ECs show upregulated glycolysis gene signatures during the angiogenic switch to meet their metabolic demands. Quiescent ECs lower their glycolytic flux (35–40%) and use fatty acid oxidation (FAO) flux to maintain energy homeostasis.

Du, W., Ren, L., Hamblin, M. H., & Fan, Y. (2021). Endothelial Cell Glucose Metabolism and Angiogenesis. *Biomedicines*. https://doi.org/10.3390/biomedicines9020147

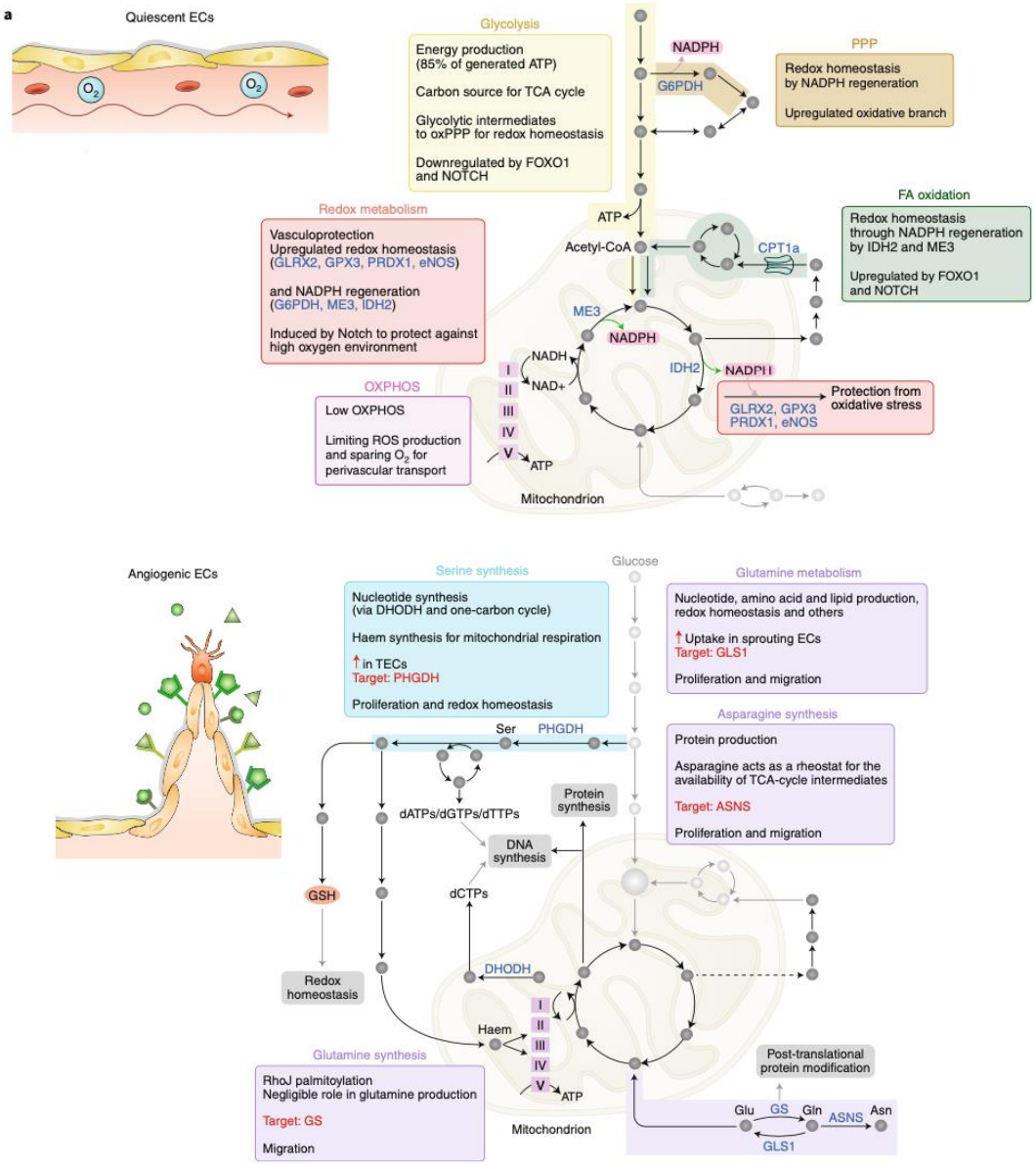
ECs rely on metabolic rewiring during active (tumor) angiogenesis.



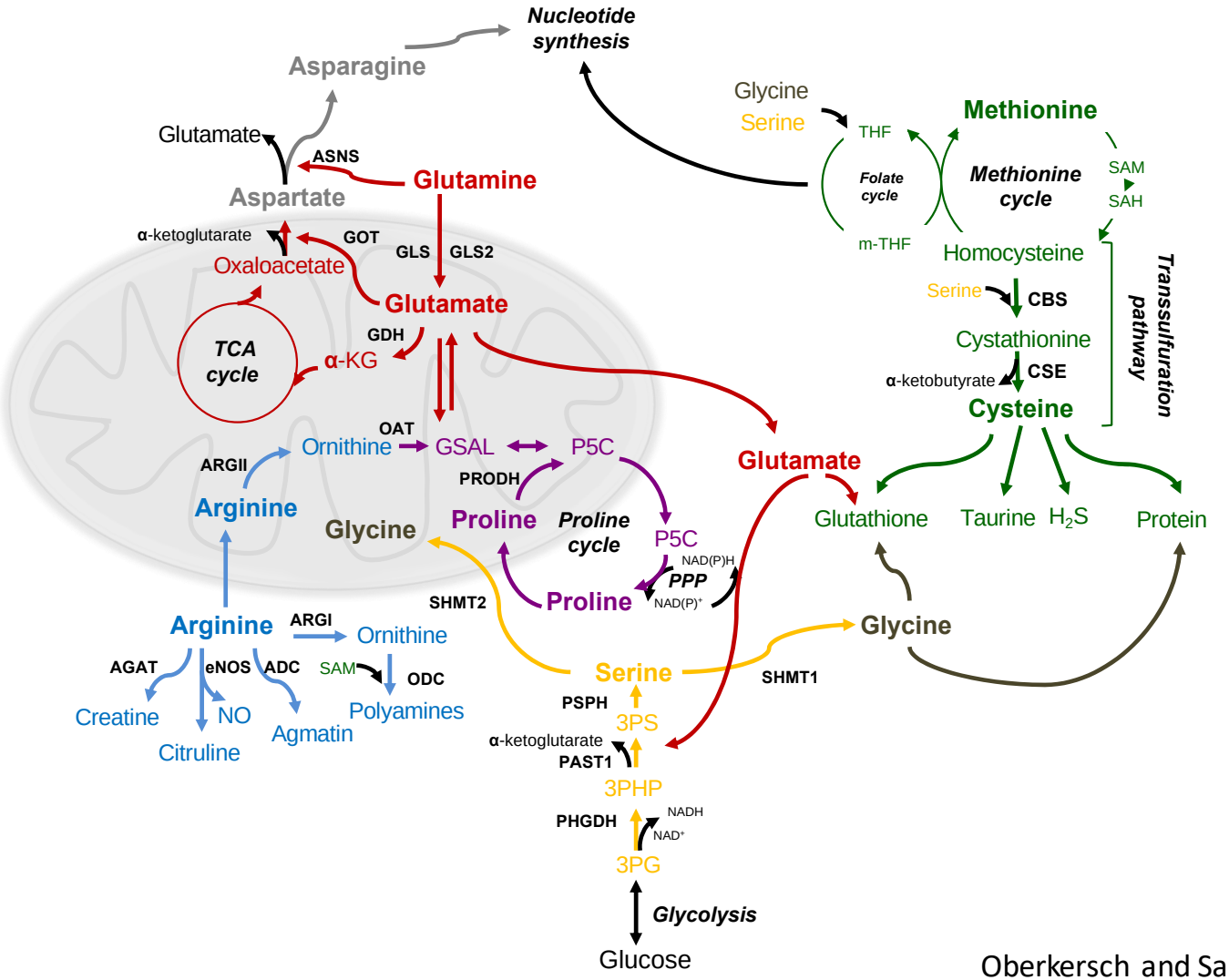
Adaptations of metabolic pathways in quiescent versus angiogenic ECs



Amino acid metabolism in angiogenic ECs



Amino acid metabolism in angiogenic ECs



Oberkersch and Santoro, 2017

Aminoacid metabolism support mTOR activation

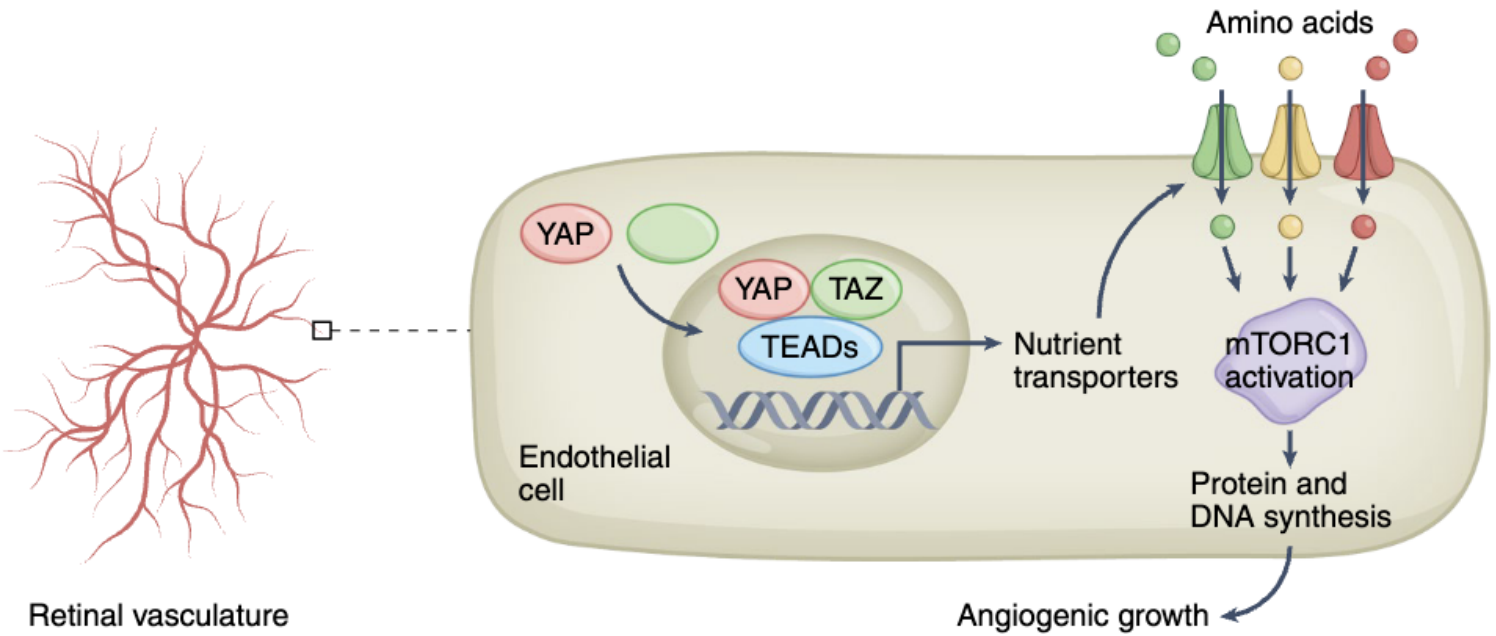
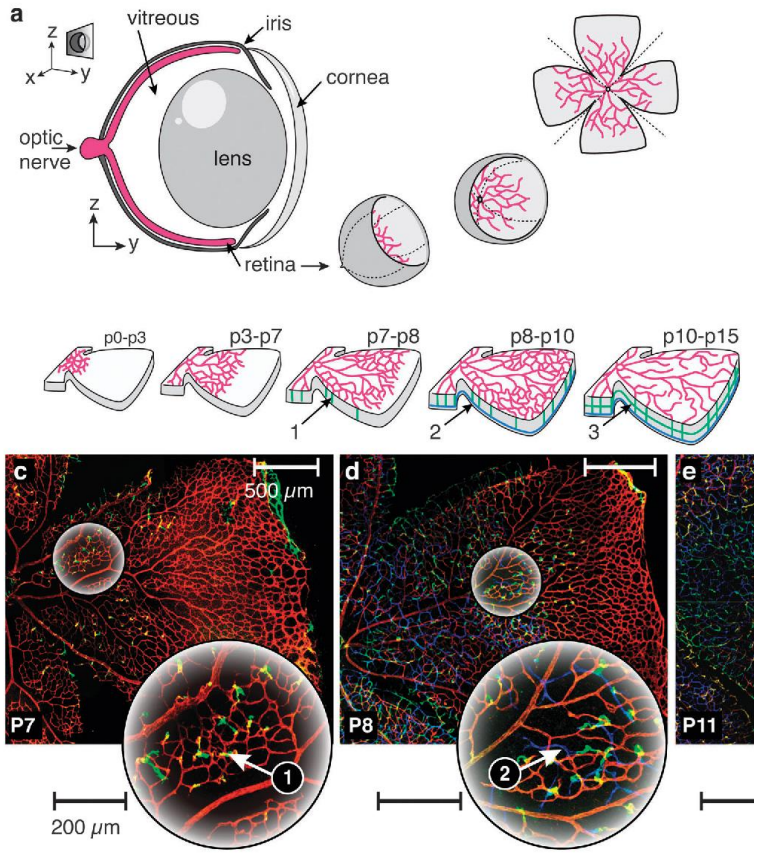
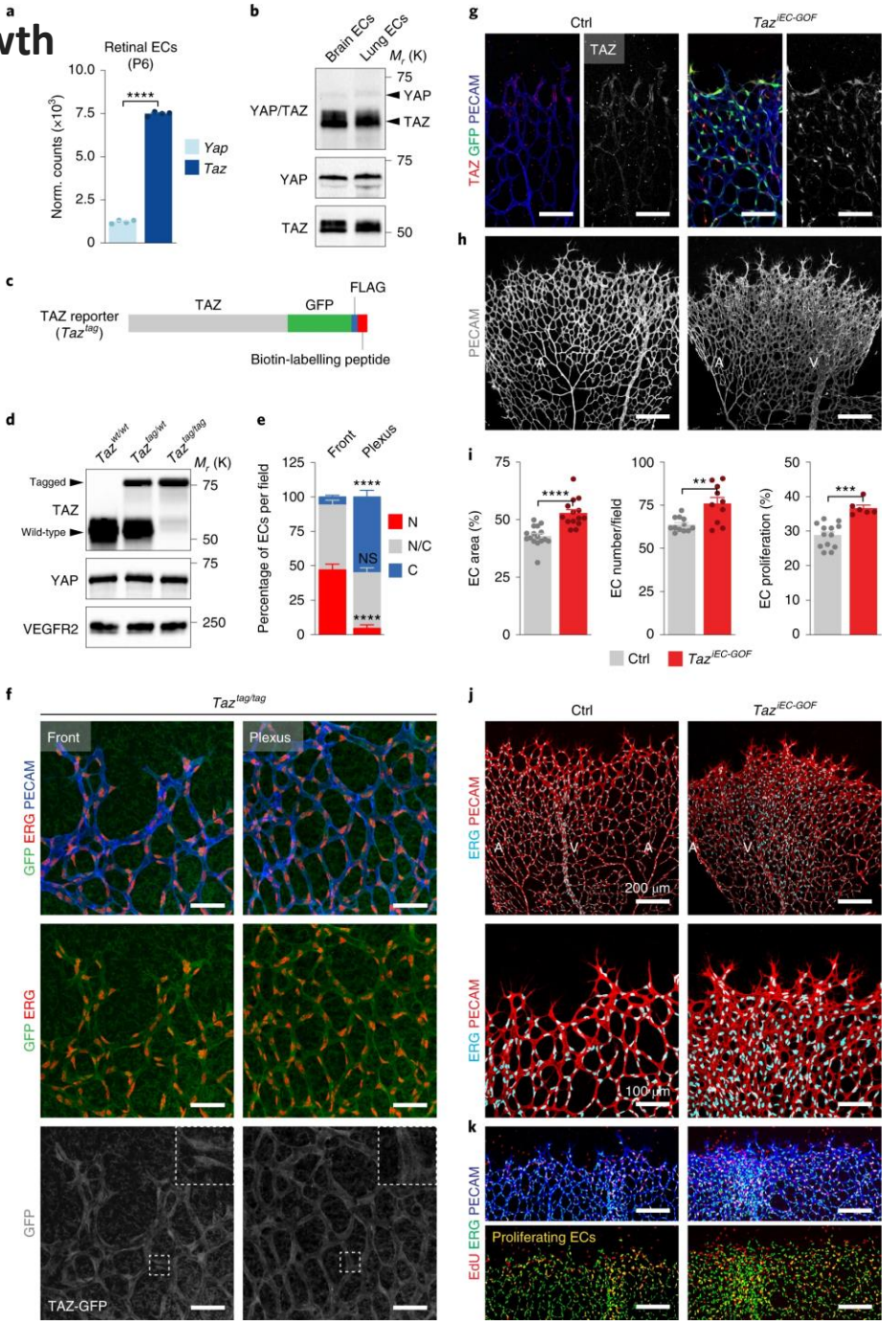


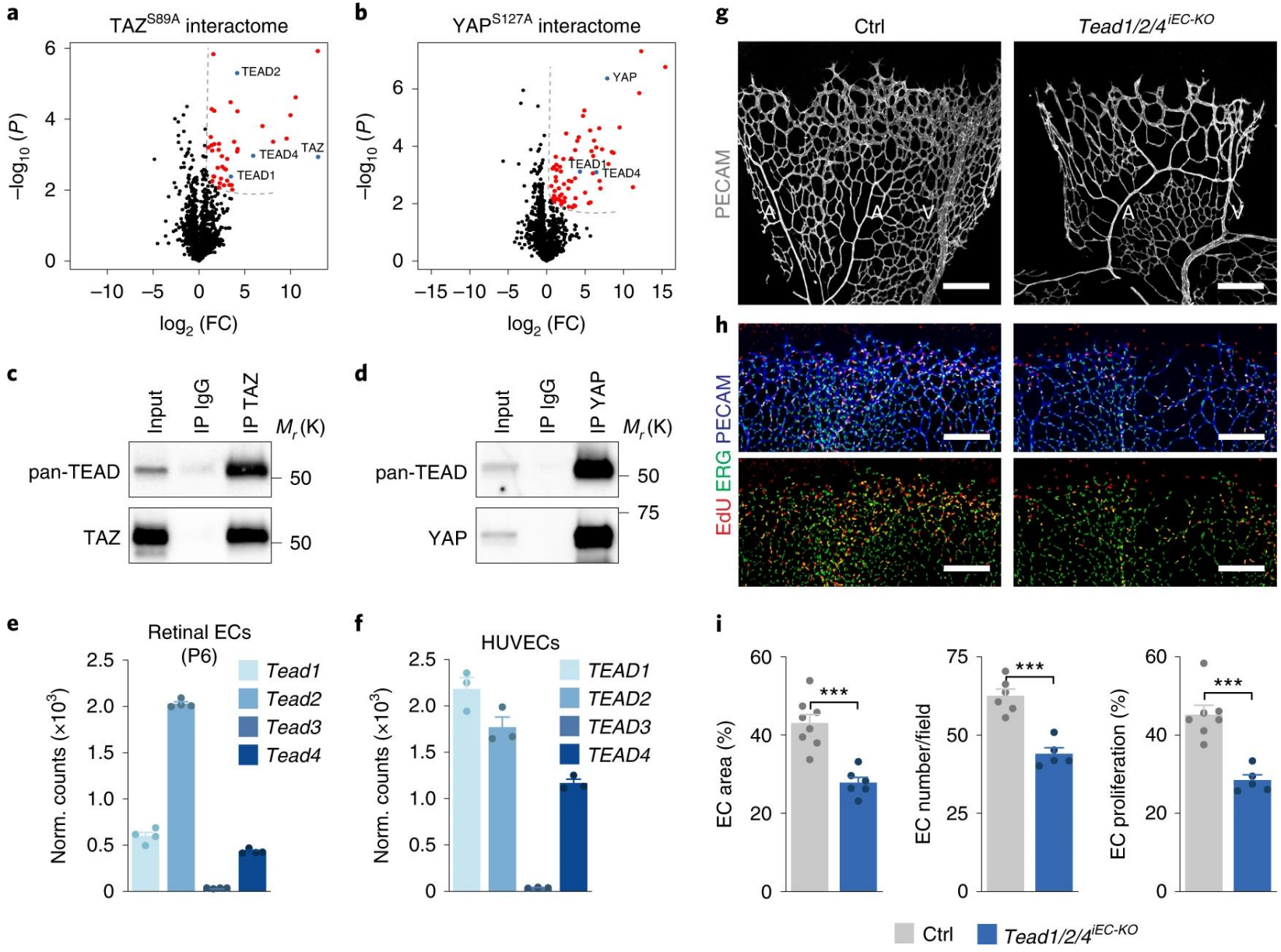
Fig. 1 | YAP/TAZ–TEAD pathway supports retinal angiogenesis by controlling nutrient transporter levels and mTORC1 activation. Endothelial cells (ECs) present a nuclear localization of the Hippo pathway components Yes-associated protein 1 (YAP)/ WW-domain-containing transcription regulator 1 (TAZ) at the angiogenic front of the retinal vasculature. In the nucleus, TAZ binds transcriptional enhancer factor domain (TEAD) TEAD1, 2 and 4, inducing the transcription of nutrient transporters (for example, solute carrier family 7 members 5 and 2 (SLC7A5 and SLC7A2)), which control amino acid availability. Increased amino acid levels in ECs signal to mechanistic target of rapamycin complex 1 (mTORC1) through the lysosome-associated Rag GTPases and other regulators, leading to activation of mTORC1 signalling. mTORC1 activity controls the major anabolic processes, including protein and nucleotide synthesis, that are crucial for angiogenic growth.

Nuclear TAZ signalling drives vascular growth

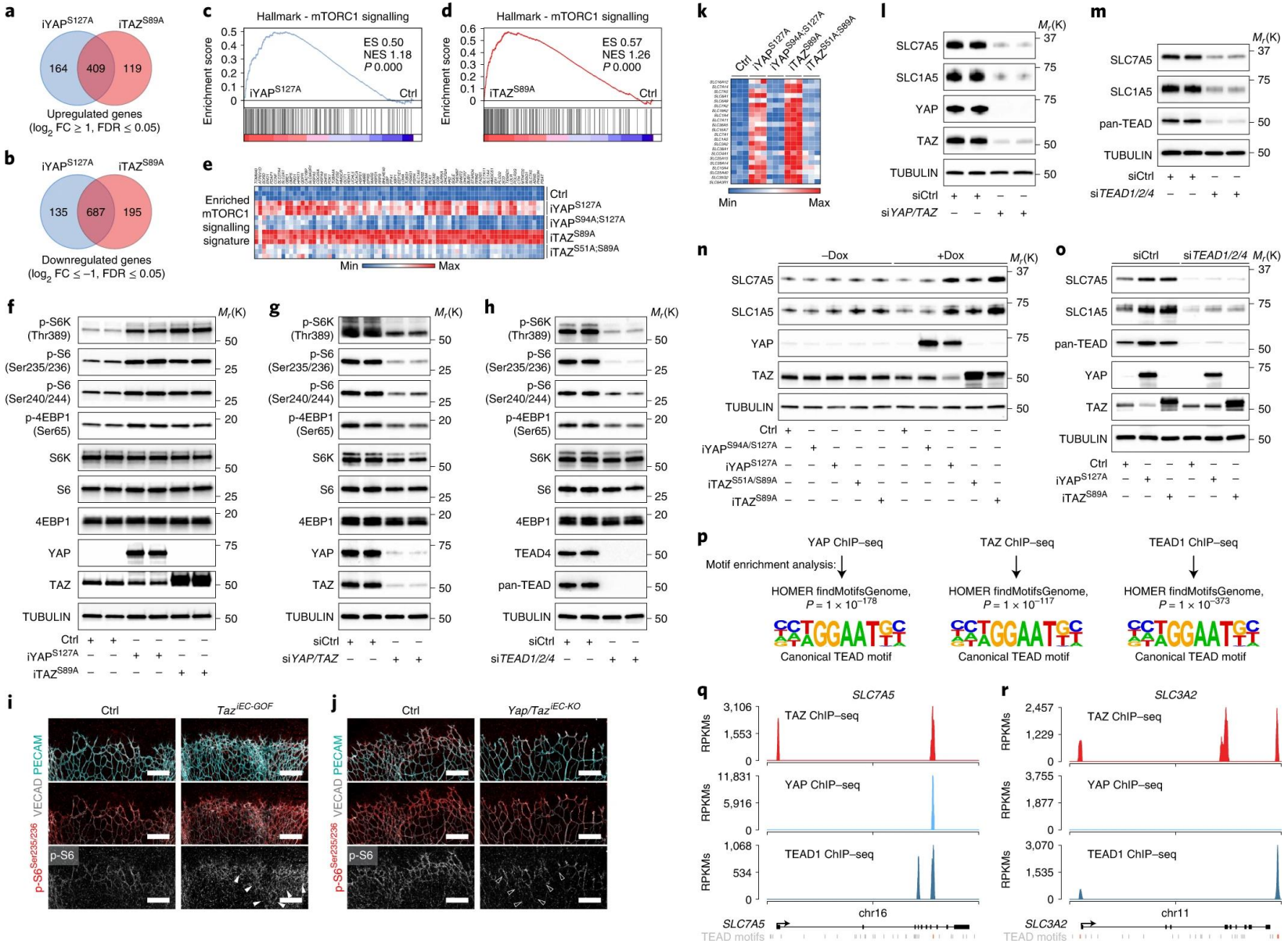




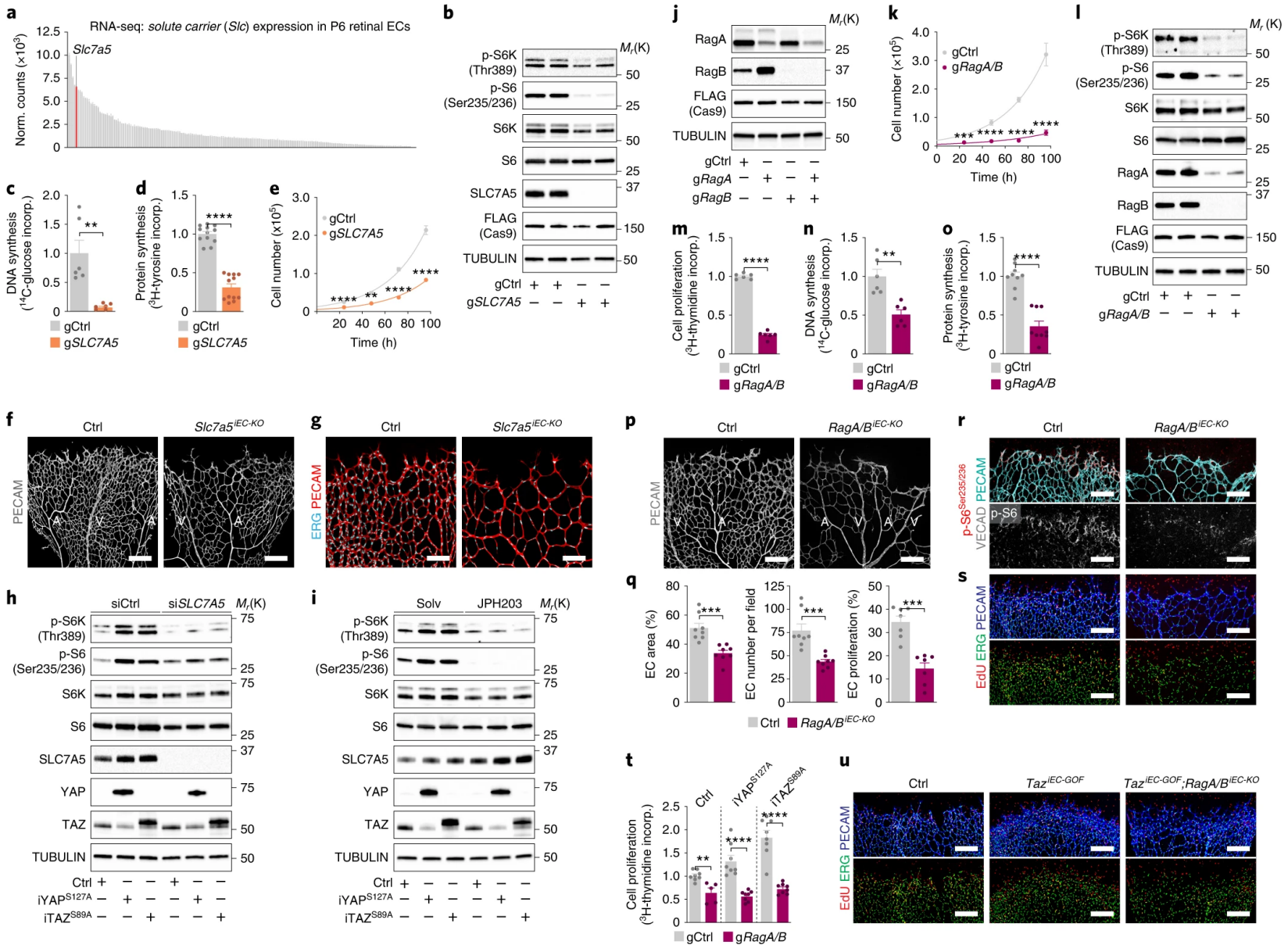
TEADs are redundant transcriptional effectors of endothelial YAP/TAZ signalling.



YAP/TAZ-TEAD fuel endothelial mTORC1 activity by orchestrating the transcription of nutrient transporters.



Nutrient-mediated mTORC1 signalling is critical for YAP/TAZ-induced vascular growth.



Principles of Targeting EC Metabolism in pathological angiogenesis