The Pentose Phosphate pathway

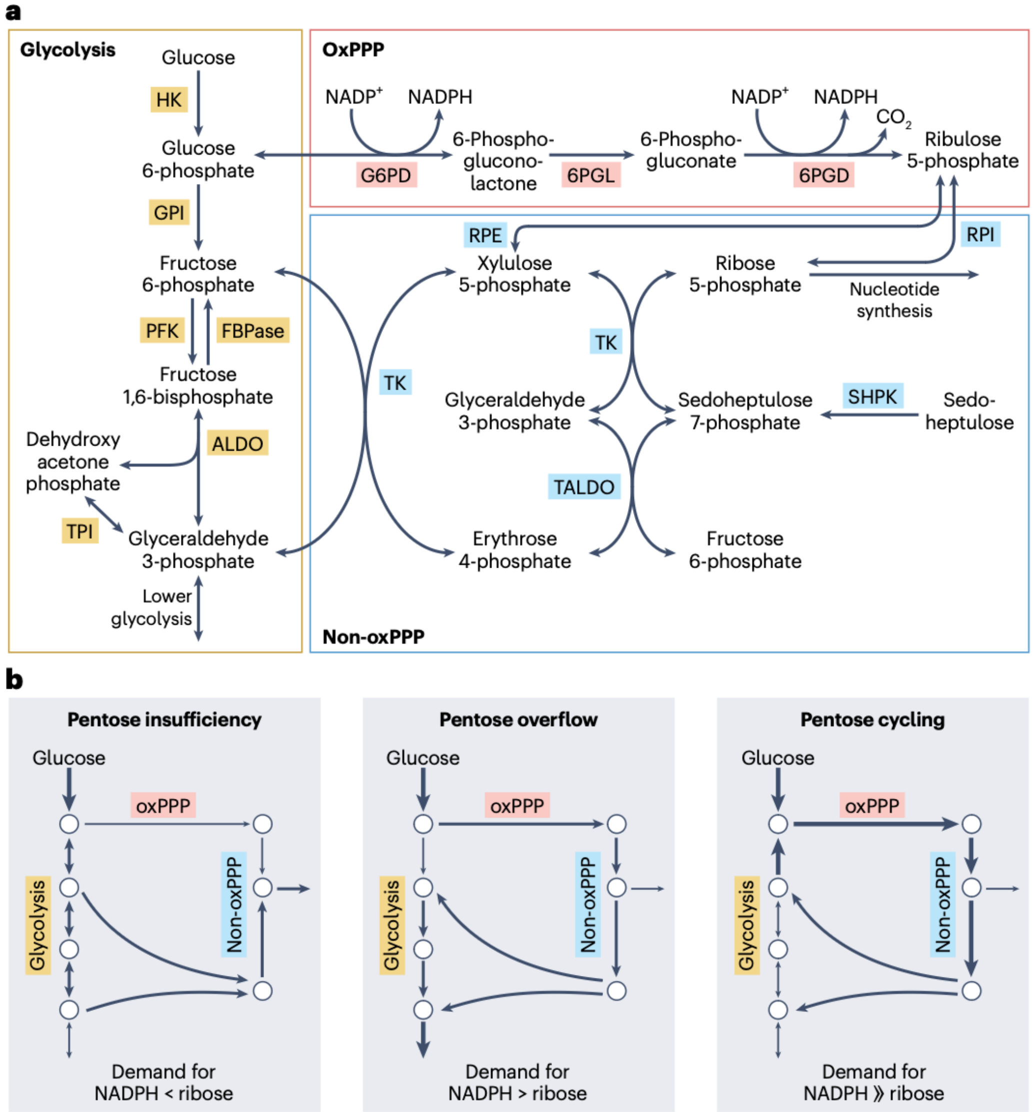


Fig. 1 | The PPP and its modes of operation. a, Overview of the oxPPP, the non-oxPPP and their connections to glycolysis. Each glucose that goes through the PPP can generate two NADPH molecules and one ribose-5-phosphate molecule. Abbreviations: HK, hexokinase; GPI, glucose-6-phosphate isomerase; RPE, ribulose-phosphate 3-epimerase; RPI, ribose-5-phosphate isomerase; FBPase, fructose 1,6-bisphophatase; ALDO, fructose-bisphosphate aldolase. **b**, Modes of PPP operation. Unmet ribose demand (that is, pentose insufficiency) leads to net non-oxPPP flux toward ribose-5-phosphate synthesis. Higher NADPH demand than ribose demand (after accounting for 2:1 pathway stoichiometry) causes non-oxPPP flux in the opposite direction, from ribose 5-phosphate toward glycolysis (that is, pentose overflow). Very high NADPH demand can lead to pentose cycling, in which the glycolytic enzyme 6-phosphate isomerase runs in reverse to make additional glucose 6-phosphate to feed the oxPPP.

NADPH consuming cellular pathways

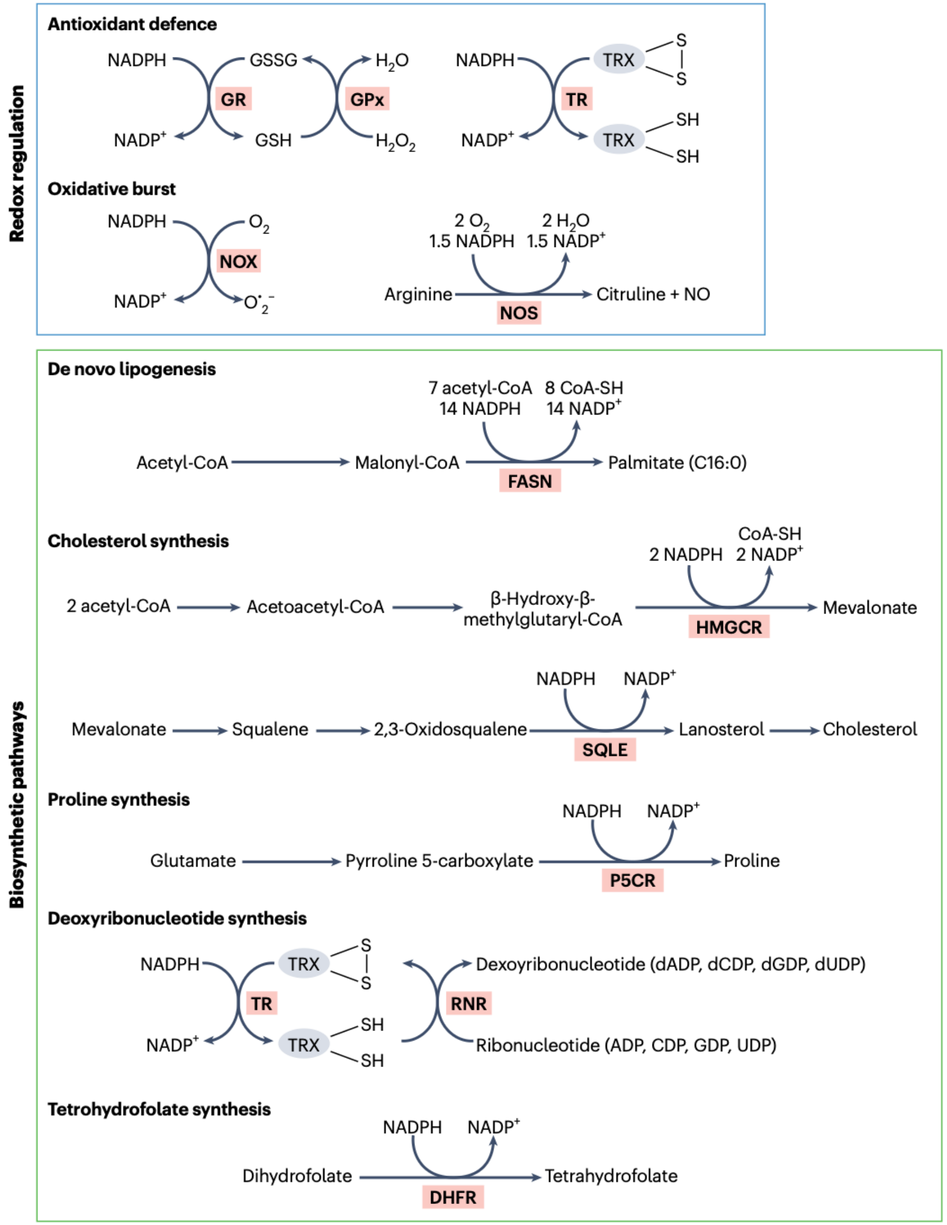


Fig. 2 | Major NADPH-consuming pathways. Abbreviations: CoA, coenzyme A; GR, glutathione reductase; GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; TR, thioredoxin reductase; FASN, fatty acid synthase; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; SQLE, squalene epoxidase; P5CR, pyrroline-5-carboxylate reductase; RNR, ribonucleotide reductase; DHFR, dihydrofolate reductase; TRX, thioredoxin.

Regulation of the Pentose Phosphate Pathway

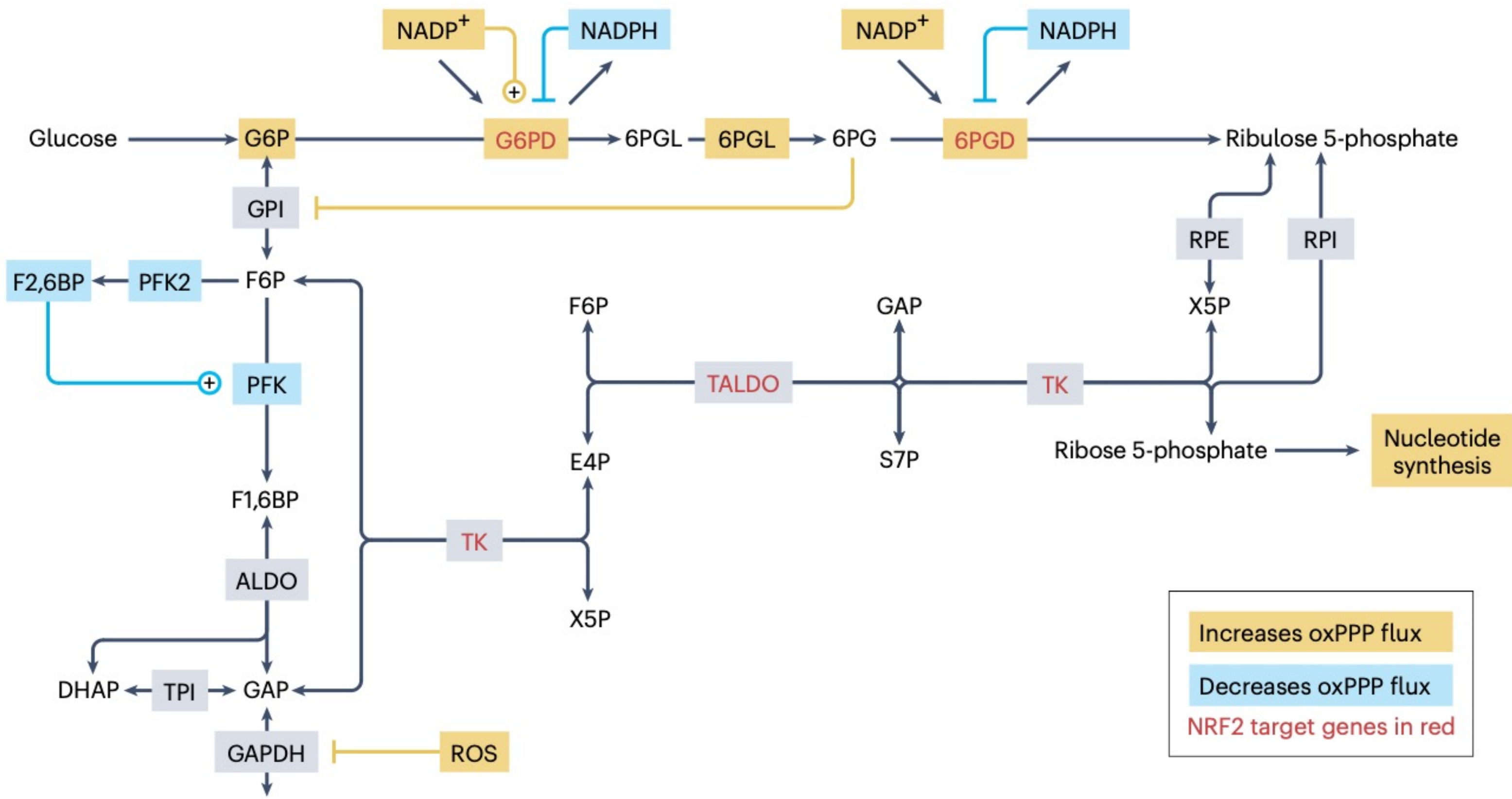


Fig. 3 | Regulation of the PPP. The PPP and glycolysis compete for carbon flux. Factors that increase oxPPP flux are highlighted in yellow, and those that decrease it are in blue. Names of enzymes induced by NRF2 are in red. E4P, erythrose 4-phosphate; F6P, fructose 6-phosphate; F1,6BP, fructose 1,6-biphosphate; F2,6BP, fructose 2,6-biphosphate; G6P, glucose 6-phosphate; 6PG, 6-phosphogluconate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; S7P, sedoheptulose 7-phosphate; X5P, xylulose 5-phosphate.

G6PD deficiency and hemolytic anemia

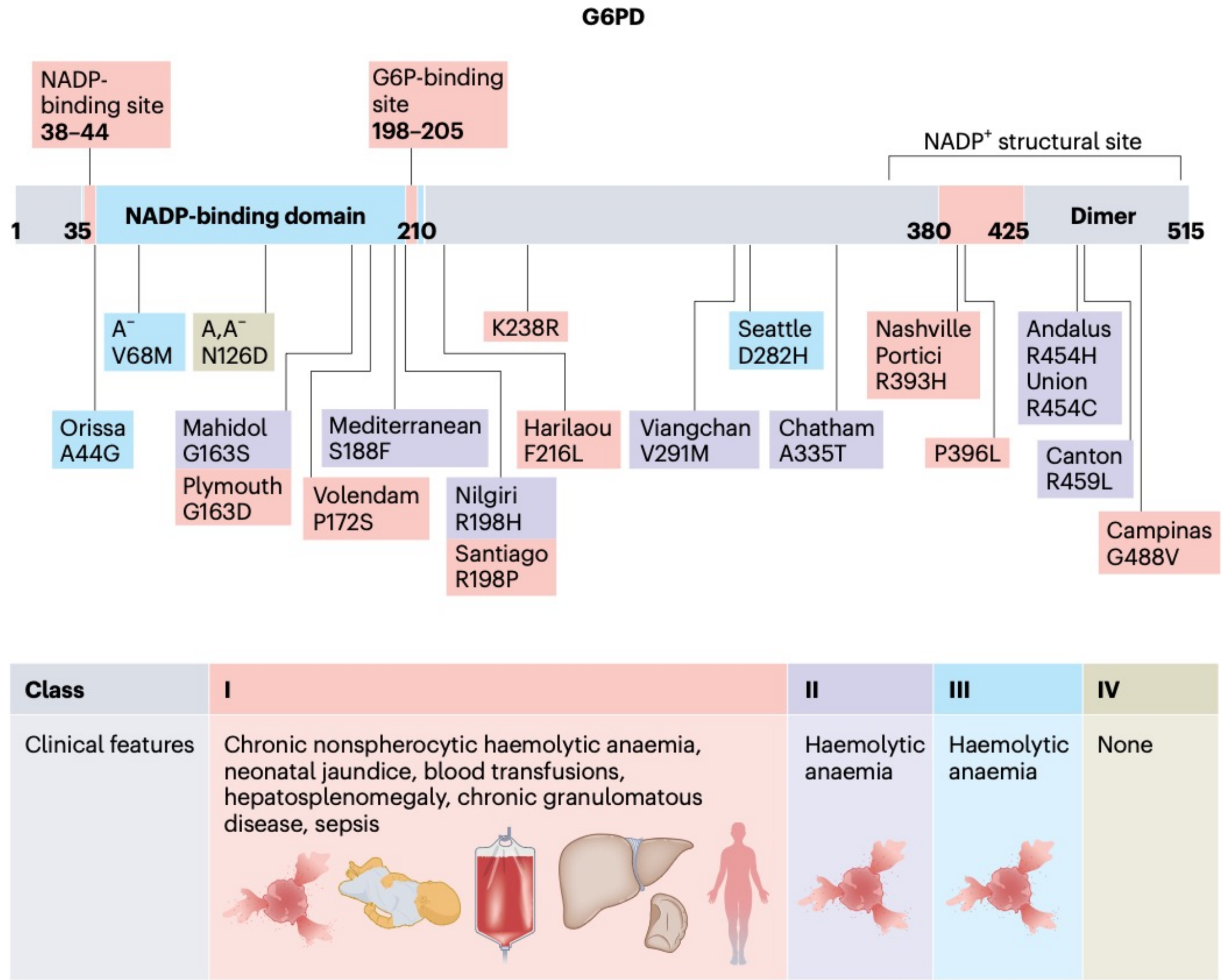


Fig. 4 | G6PD deficiency leads to RBC and immune dysfunction. The best-studied mutations in G6PD and their locations within the protein. Mutations are coloured according to their clinical phenotype from most to least severe: class I mutations in red, class II in purple, class III in blue and class IV in beige. The most severe class 1 mutations cluster around the glucose-6-phosphate (G6P) binding site, the dimer interface and the NADP$^+$ structural site that is involved in allosteric activation and homotetramer formation.

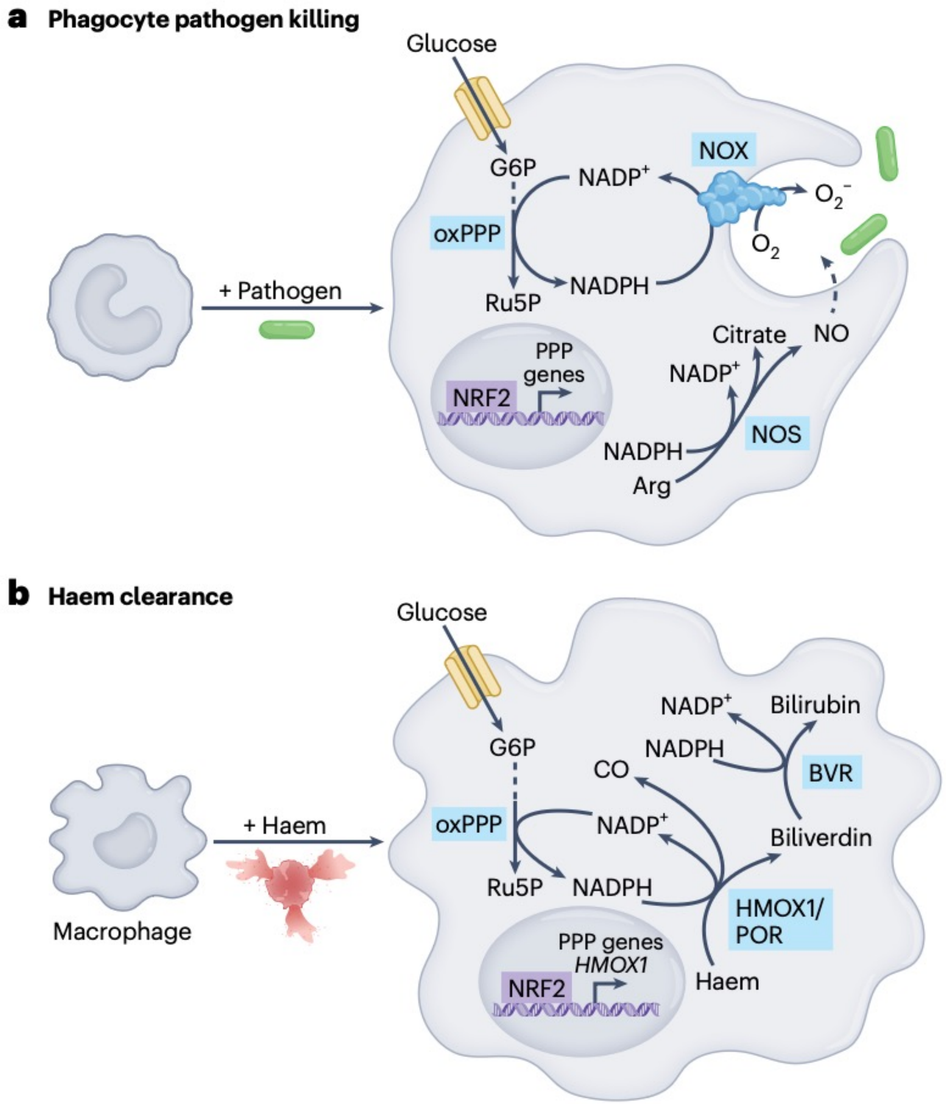


Fig. 5 | The role of oxPPP-produced NADPH in phagocyte function.
a, In phagocytic cell types including macrophages and neutrophils, NADPH production by the oxPPP supports production of superoxide by NOX and nitric oxide by NOS for killing pathogens in the phagosomes and extracellular space. Ru5P, ribulose 5-phosphate. **b**, In macrophages involved in haem clearance, NADPH supports the breakdown of haem into biliverdin by HMOX with the help of p450 oxoreductase (POR) and biliverdin into bilirubin by biliverdin reductase (BVR).

Pentose Phosphate Pathway and oncogenic activation

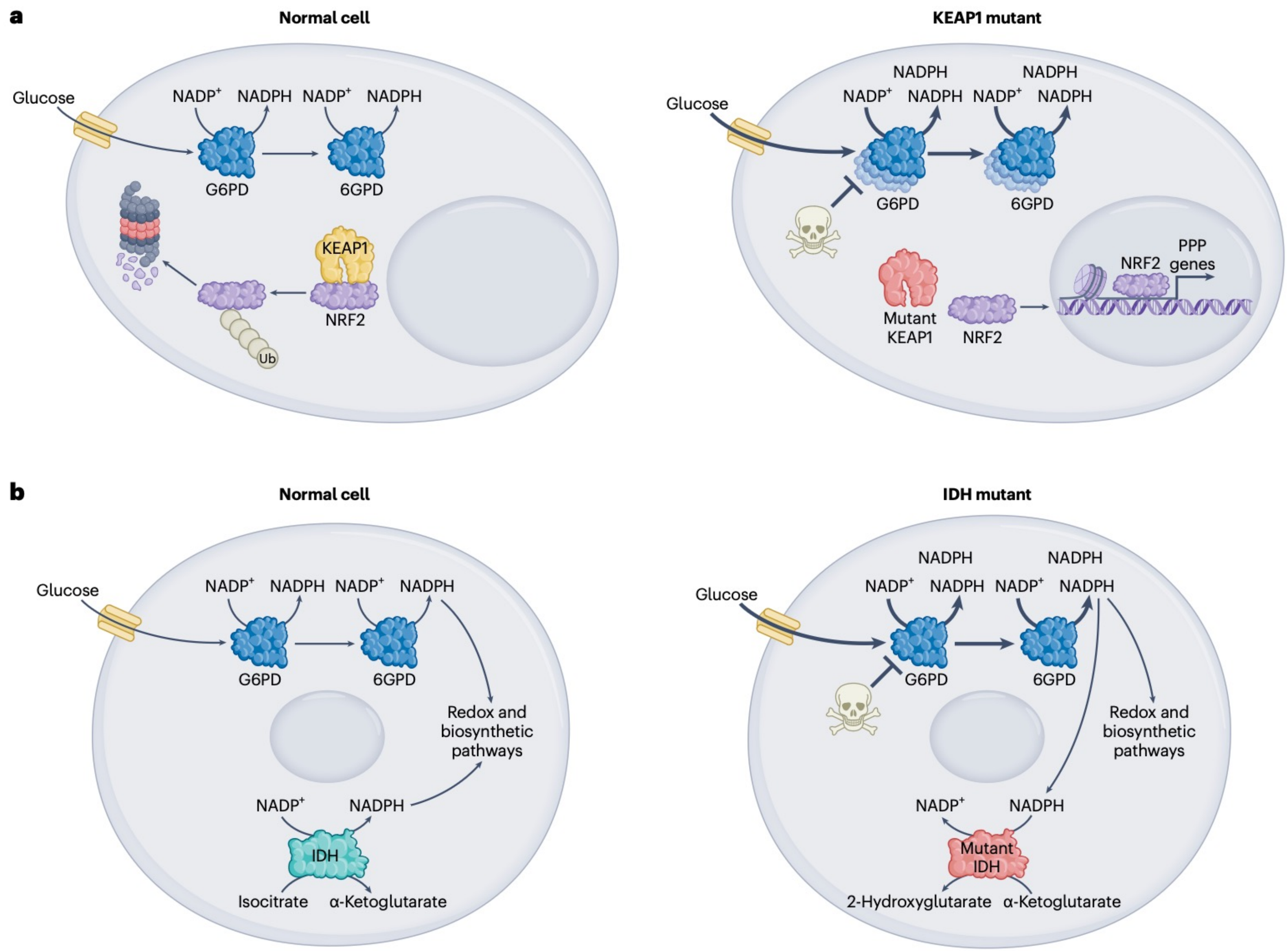
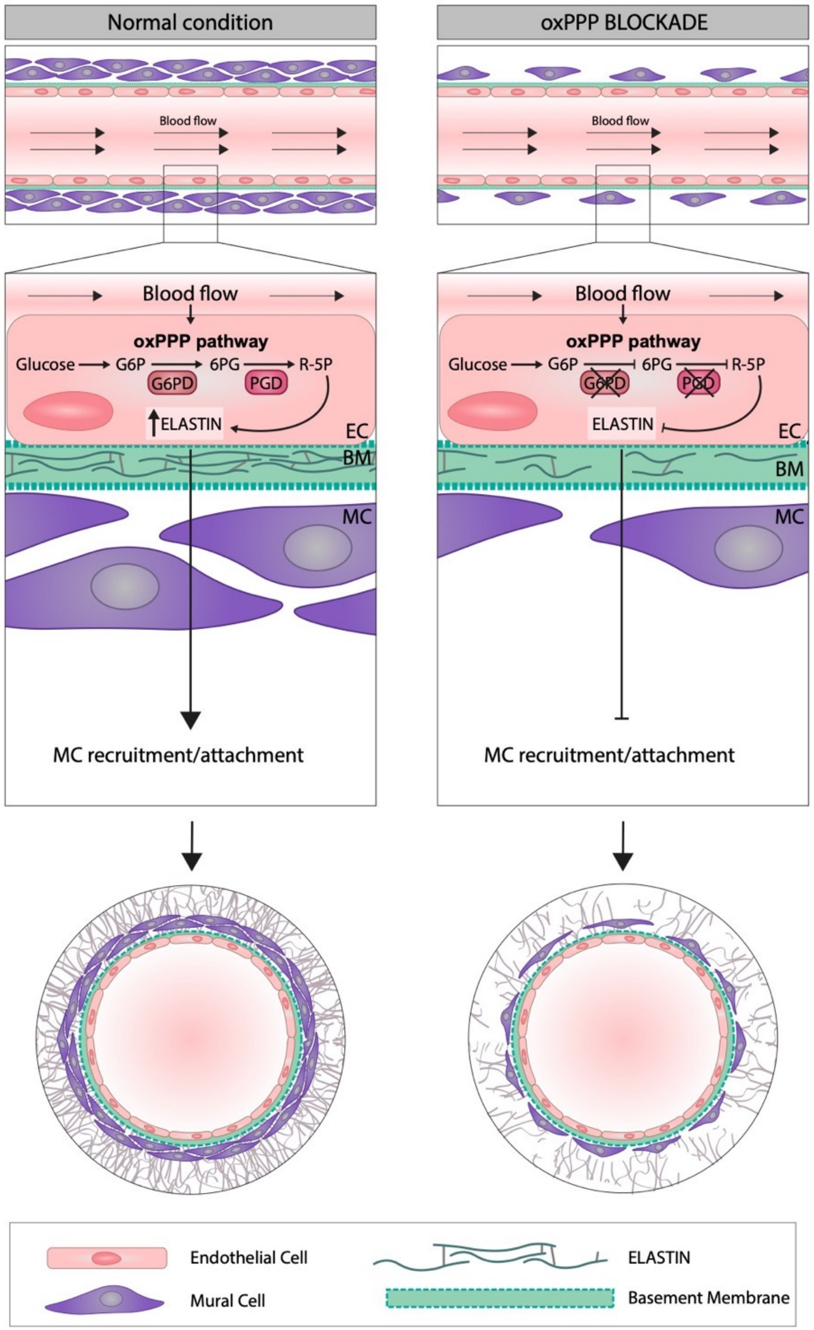
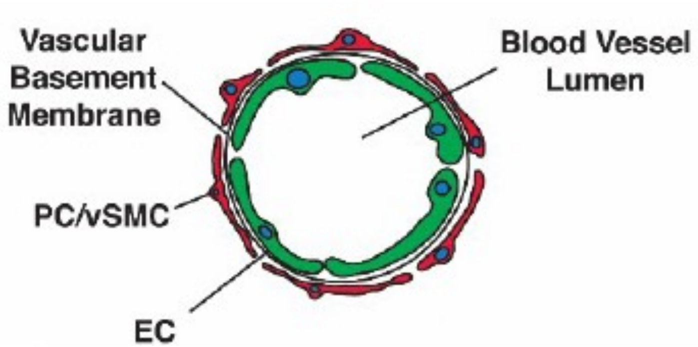


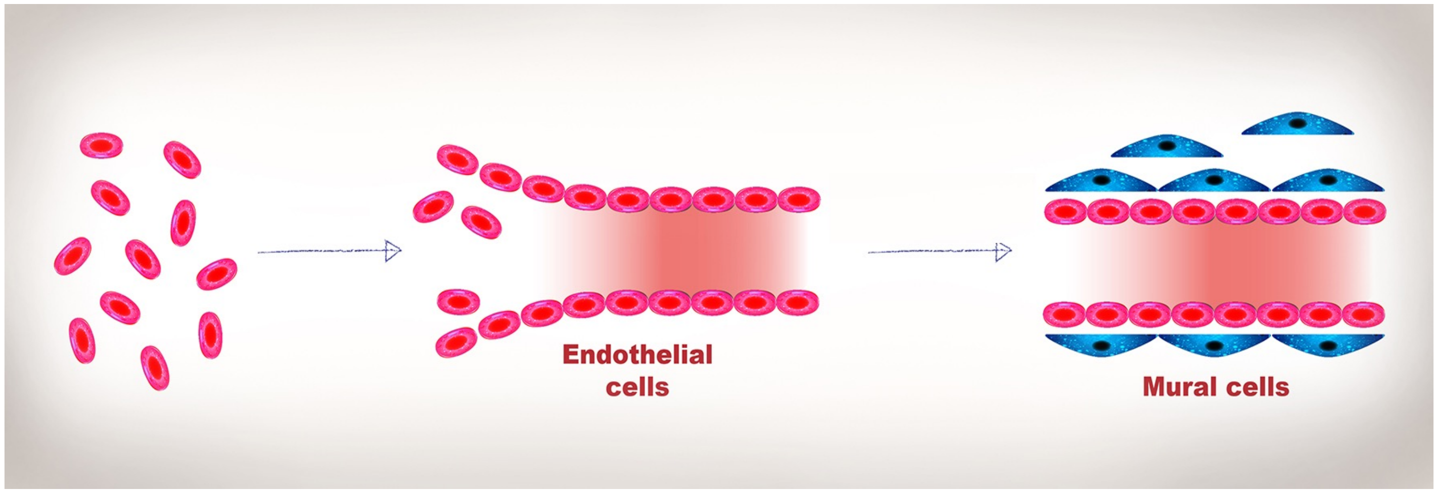
Fig. 6 | Oncogenic contexts for targeting the PPP. a, *KEAP1* mutations lead to stabilization of NRF2, which promotes transcription of PPP genes and leads to dependency on their enzyme activity. Ub, ubiquitin. **b**, Mutations in IDH1 convert IDH from an NADPH producer into a consumer. Therefore, the oxPPP becomes a more important source of NADPH with mutant IDH.

Pentose Phosphate Pathway and vascular functions



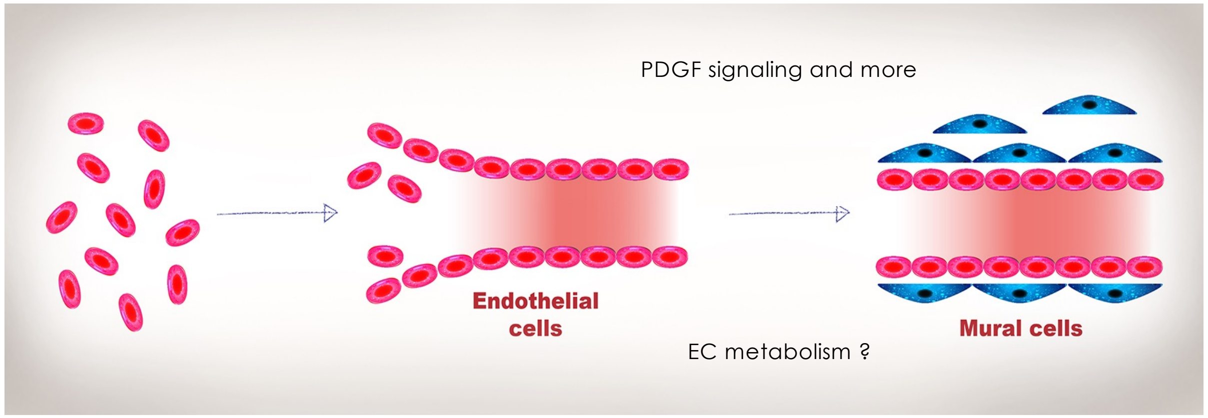
mural cell (vMC) recruitment leads to vascular maturation during CV development

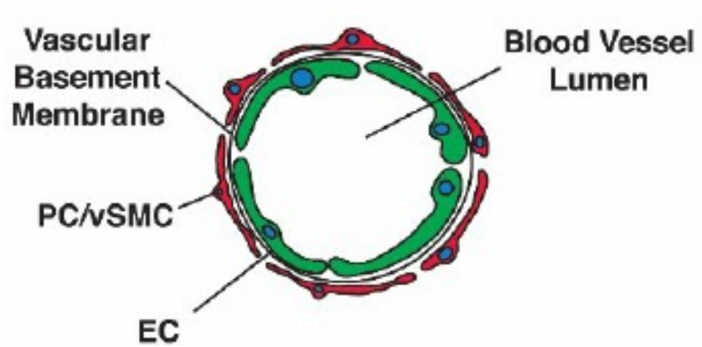




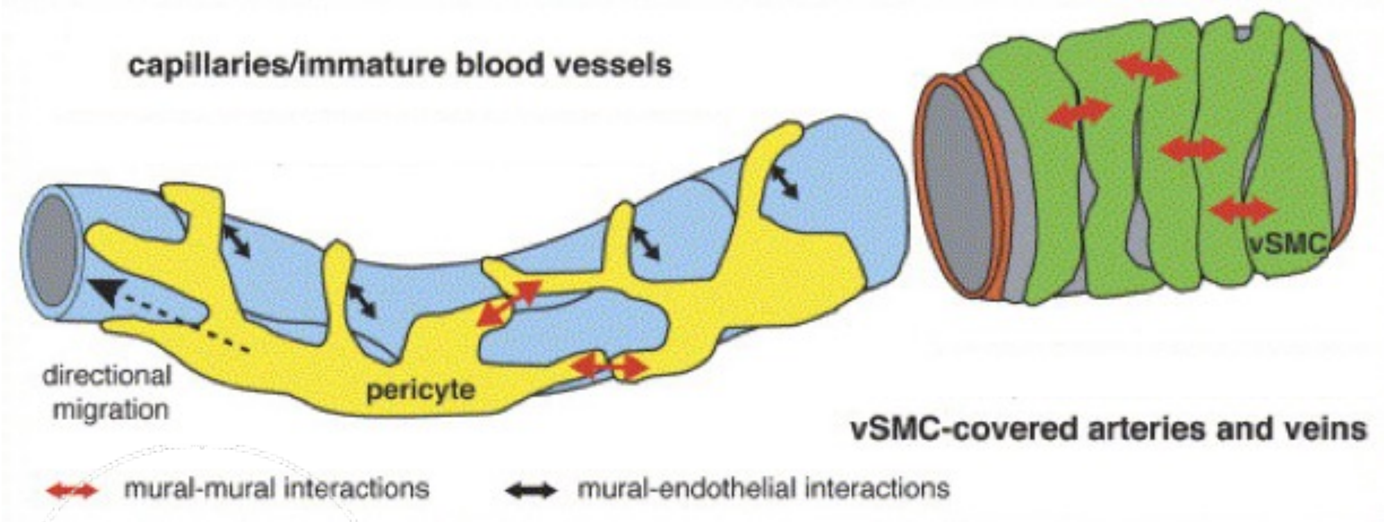
QUESTION:

Can endothelial *metabolism* regulate *vascular maturation (i.e. mural cell recruitment)* ?

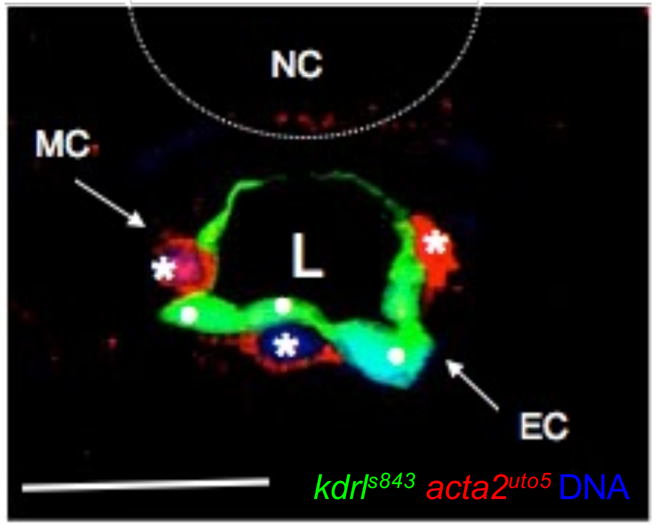




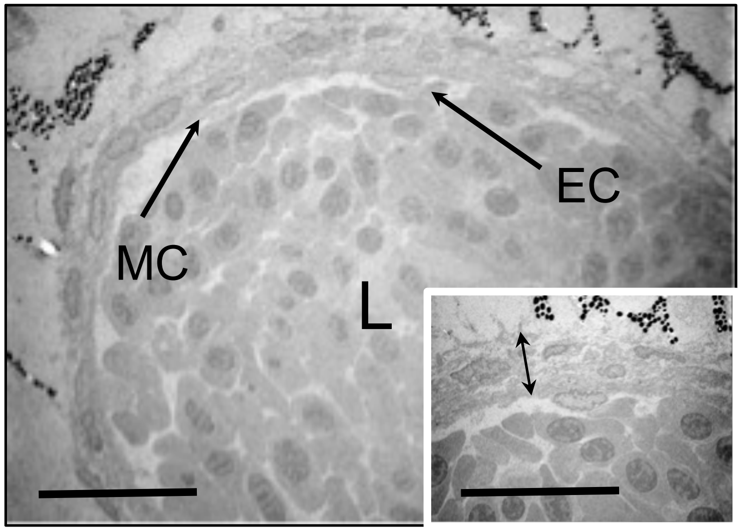
Zebrafish mural cells location and morphology are similar to mammalian pericytes and vaSMC



3dpf and small vessels (pericytes-like)

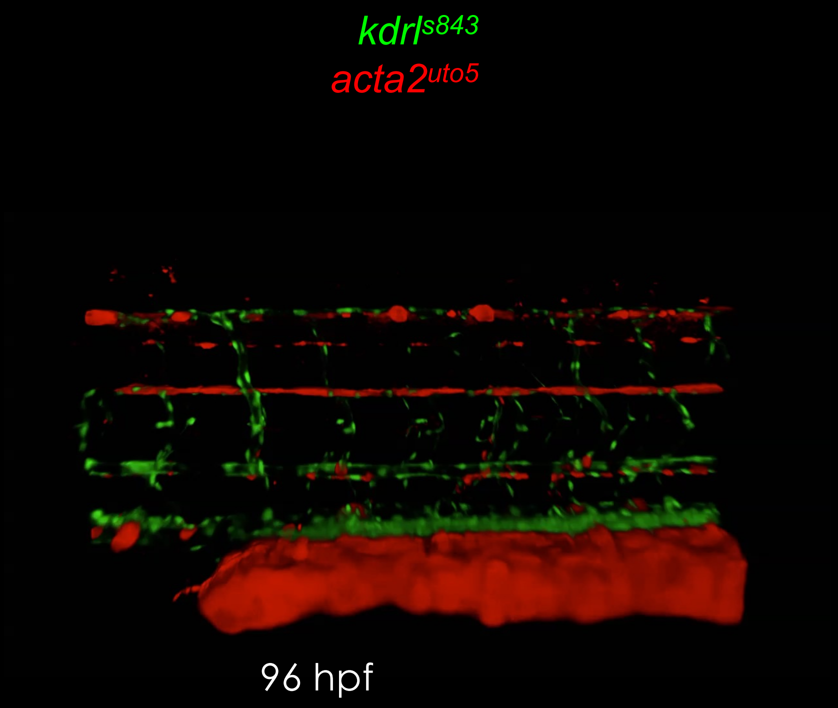


21dpf (vascular smooth muscle cells)

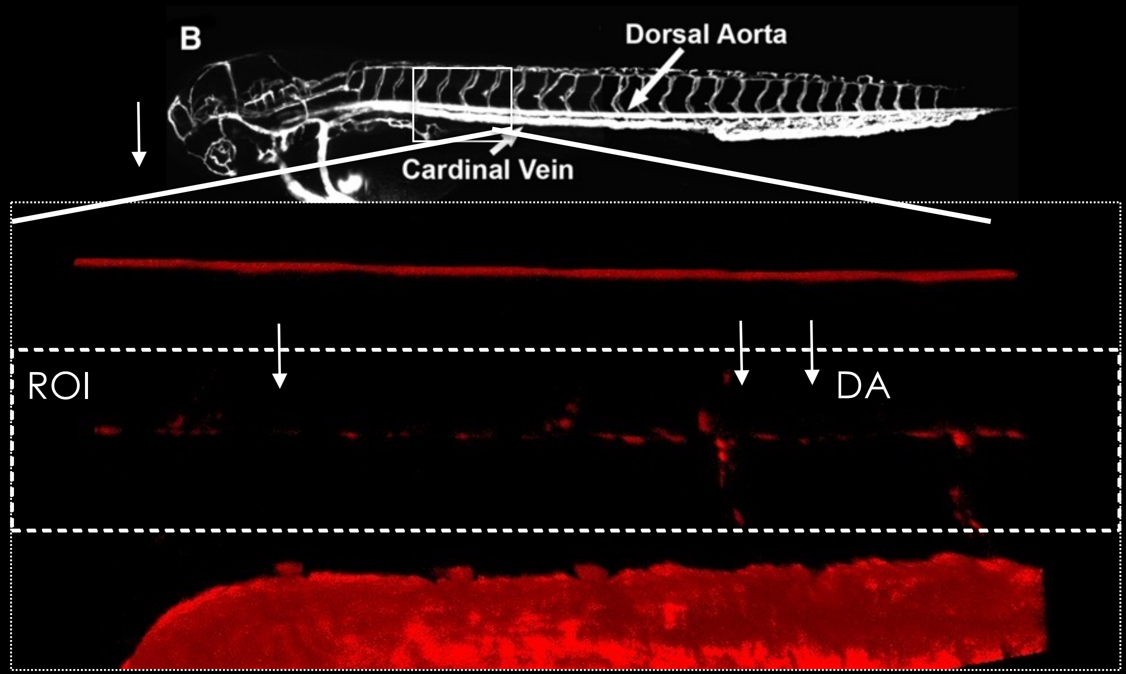


Santoro et al., 2009; Whitesell et al., 2014; Wang et al., 2014; Fortuna et al., 2015; Ando et al., 2016, 2019; Stratman et al., 2017, 2020; Donadon and Santoro, 2021

Generation of zebrafish transgenic lines to study vascular MCs development



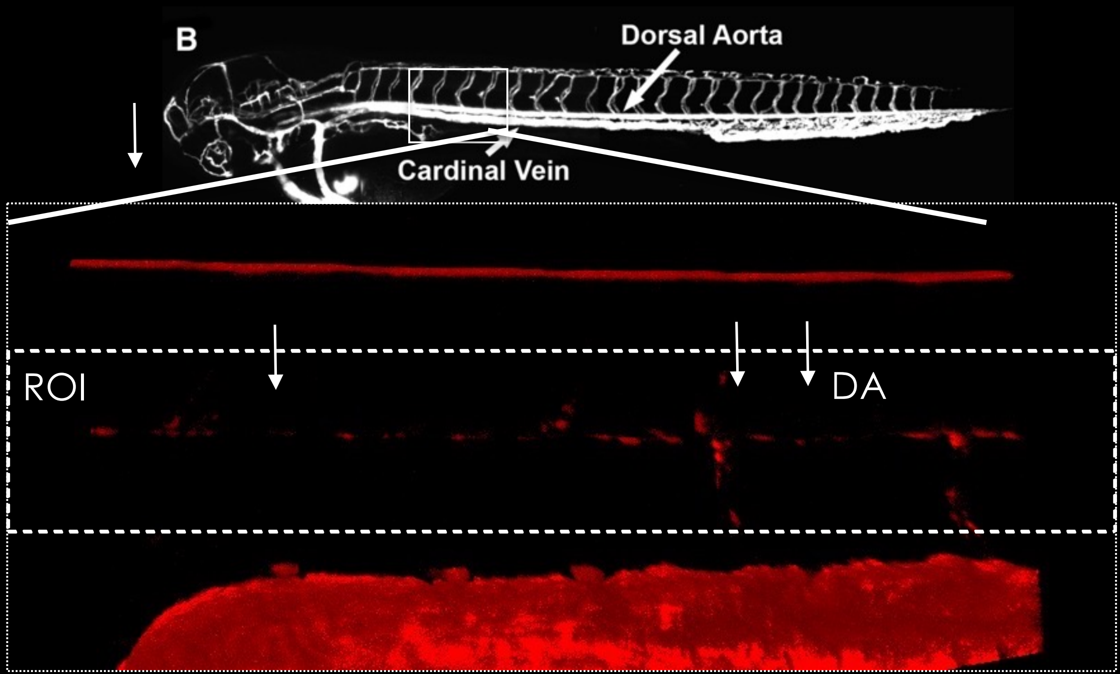
Intensity of fluorescence



Chen et al., 2017 *Cell Reports*
Gays et al. 2017 *Development*

Generation of zebrafish transgenic lines to study vascular mural cell (vMC) recruitment

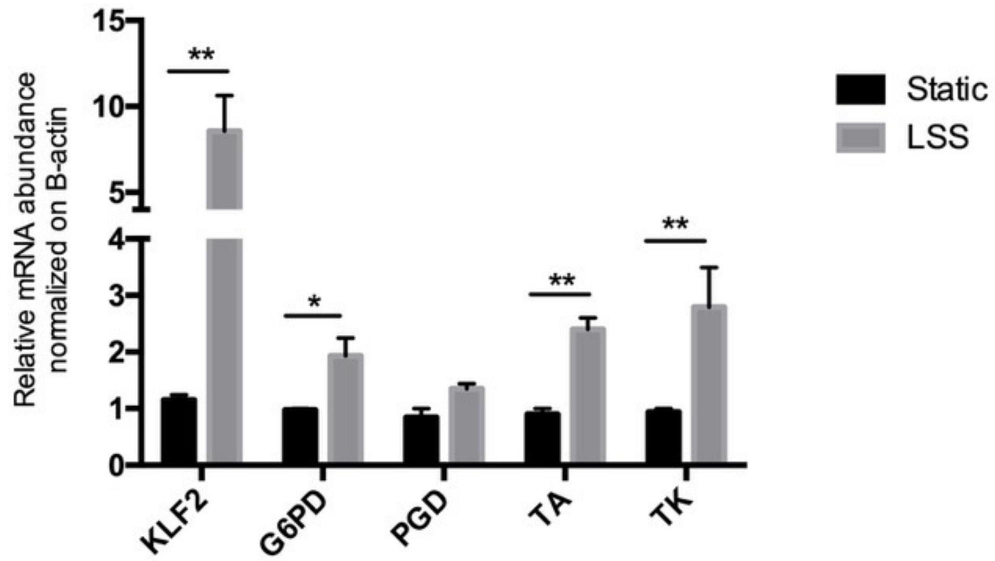
Intensity of fluorescence



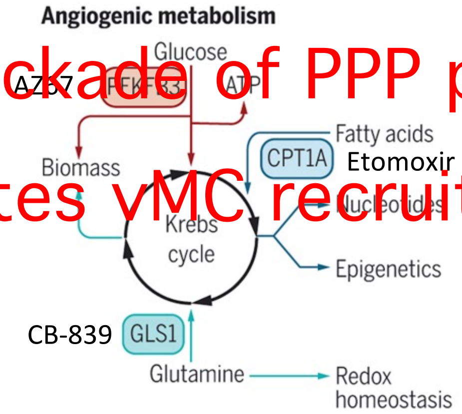
Chen et al., 2017 *Cell Reports*
Gays et al. 2017 *Development*

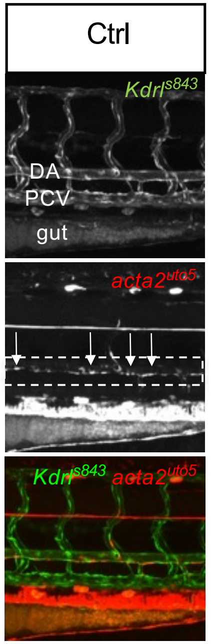
LSS positive regulates PPP enzymes

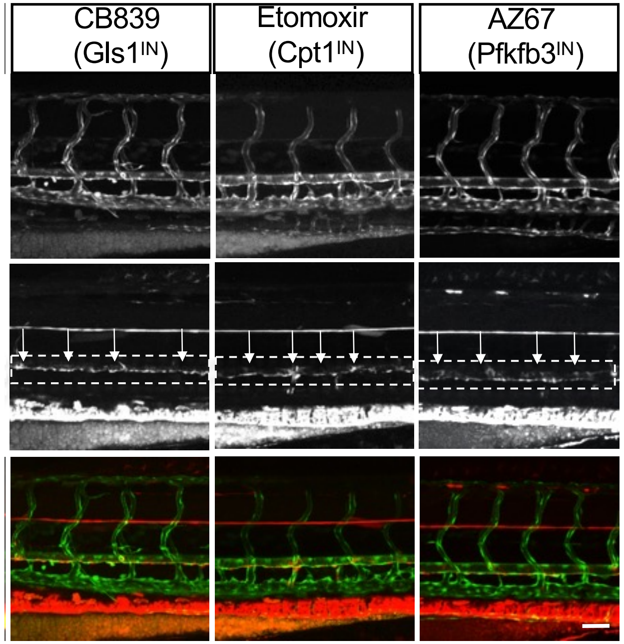
Ajami NE et al 2017 PNAS

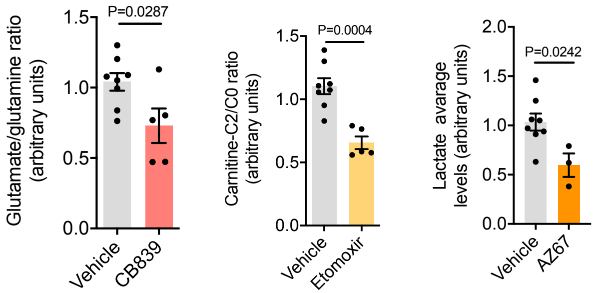


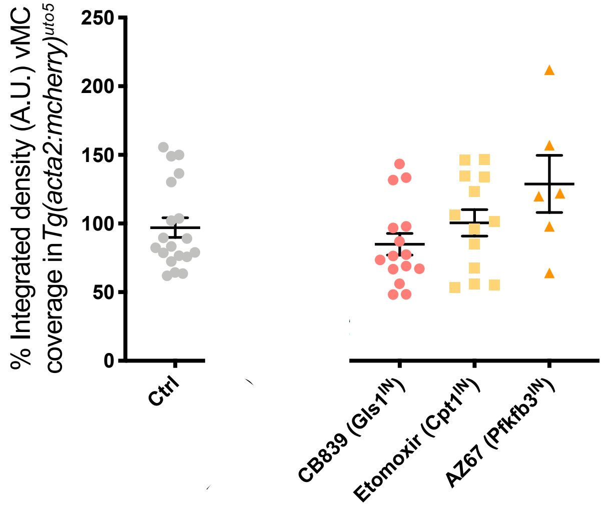
Pharmacological blockade of PPP pathway negatively regulates vMC recruitment



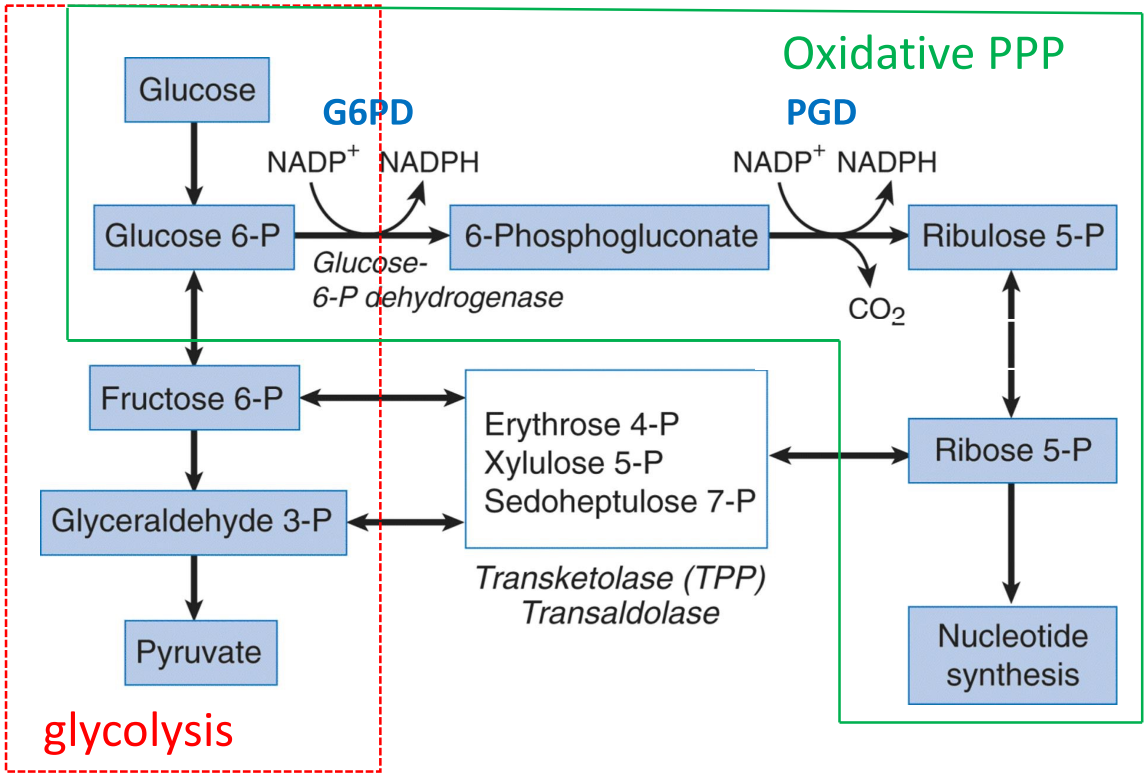








Glycolysis and Pentose Phosphate Pathway (PPP)

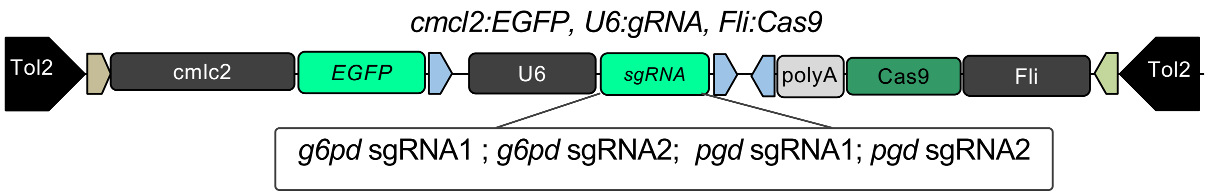


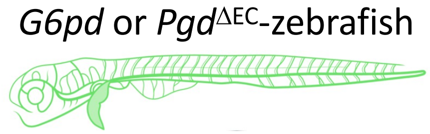
- Glucose can be shunted into oxidative pentose phosphate pathway (oxPPP)

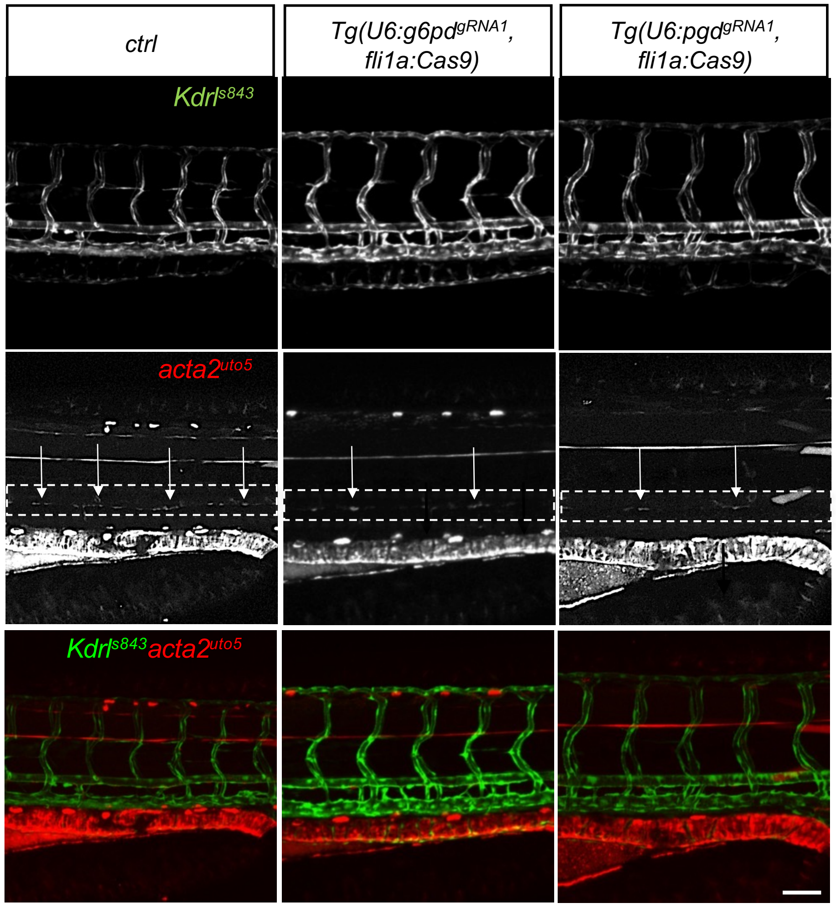
- The 2 oxPPP enzymes are:
  - **G6PD** (glucose-6-phosphate dehydrogenase)
  - **PGD** (6-phosphogluconate dehydrogenase)

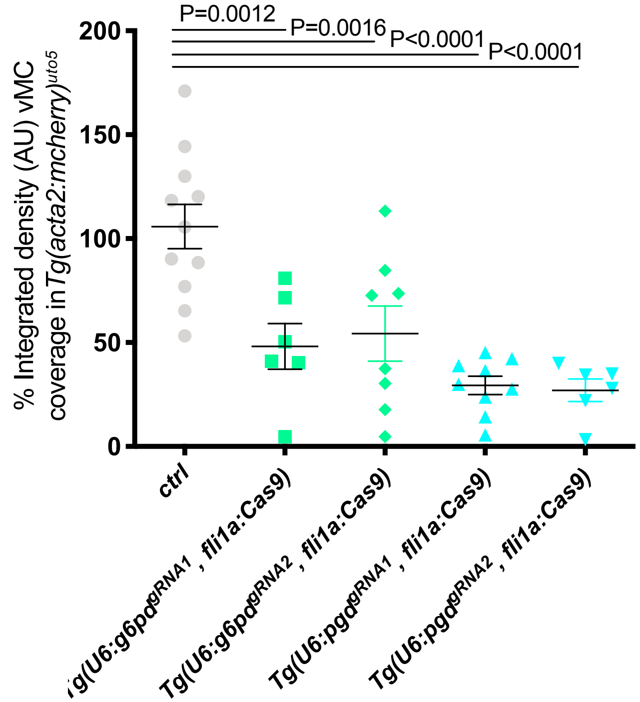
- More complex pathway than glycolysis and it regulates
  - formation of **NADPH** for synthesis of fatty acids, steroids, and maintaining reduced **glutathione** for antioxidant activity (redox balance)
  - synthesis of **ribose** for nucleotide synthesis

Genetic blockade of oxPPP pathway in ECs affects vMC recruitment in zebrafish embryos

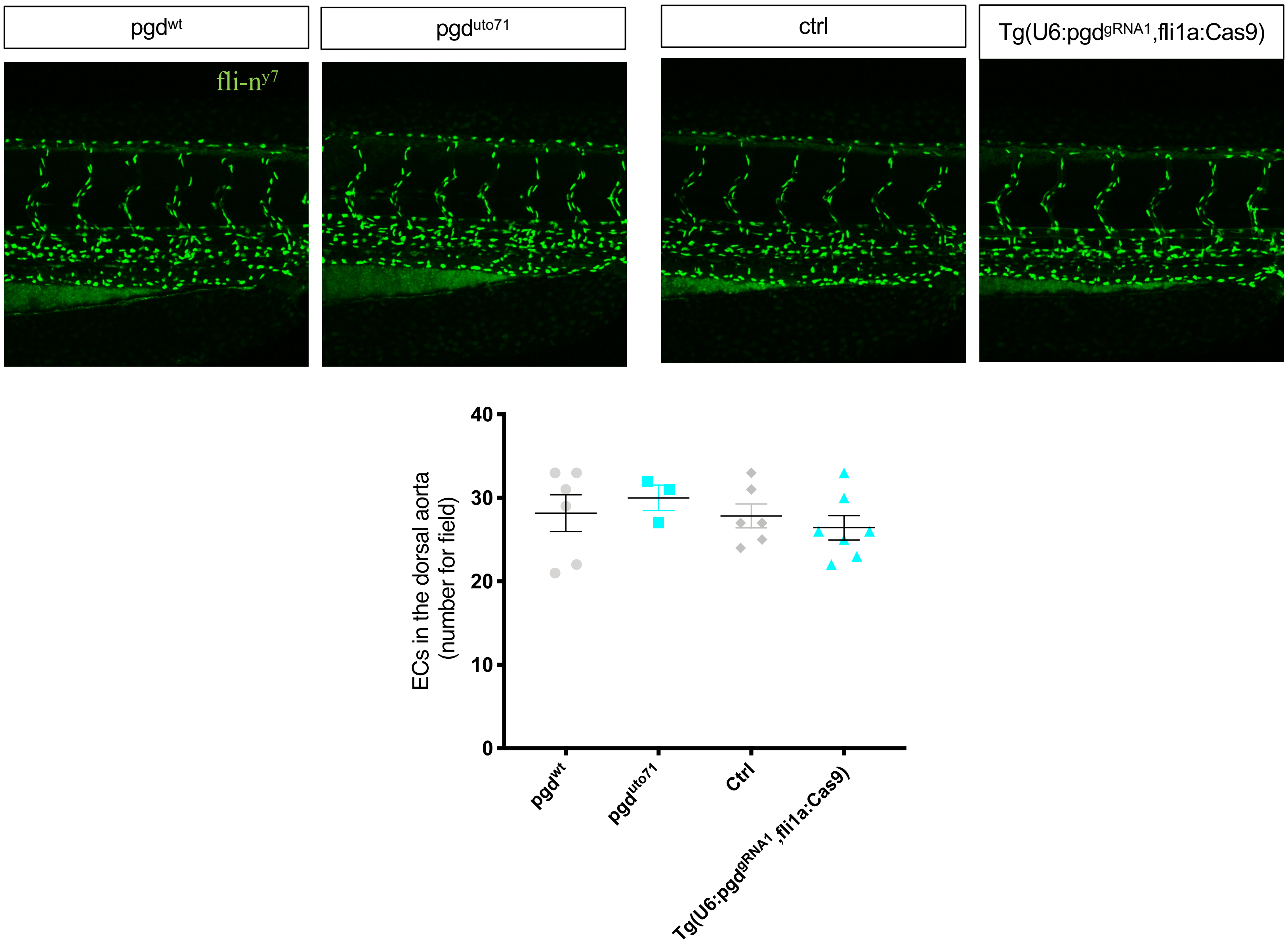




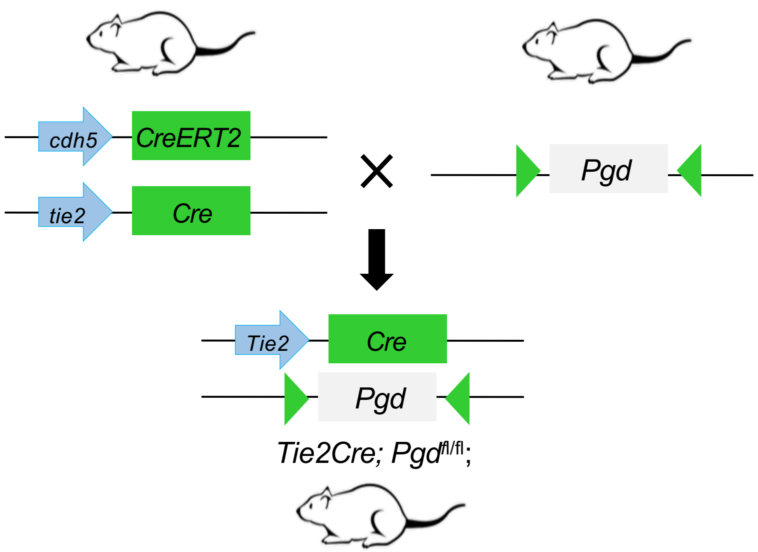


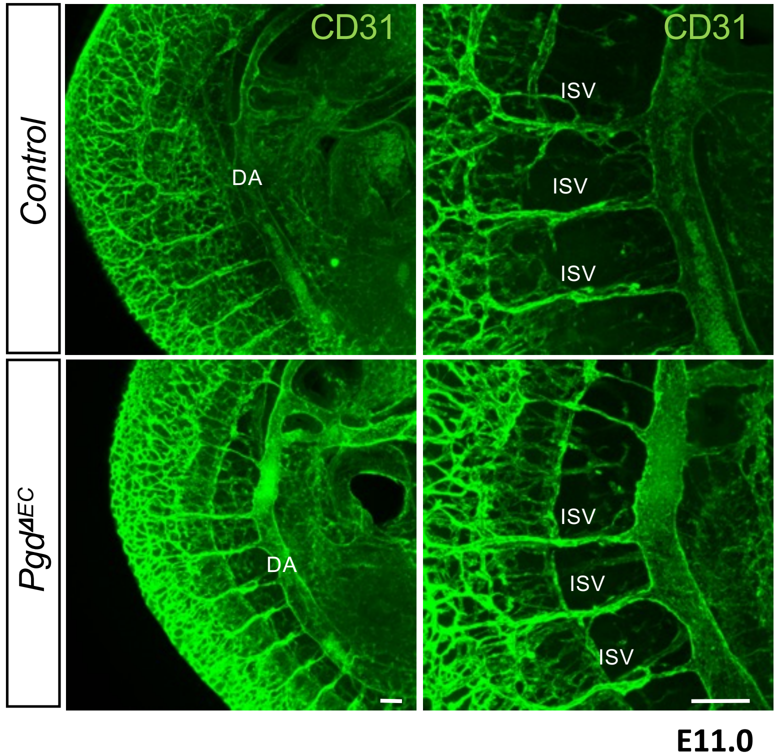


Pgd mutants do not show trunk vasculature defects

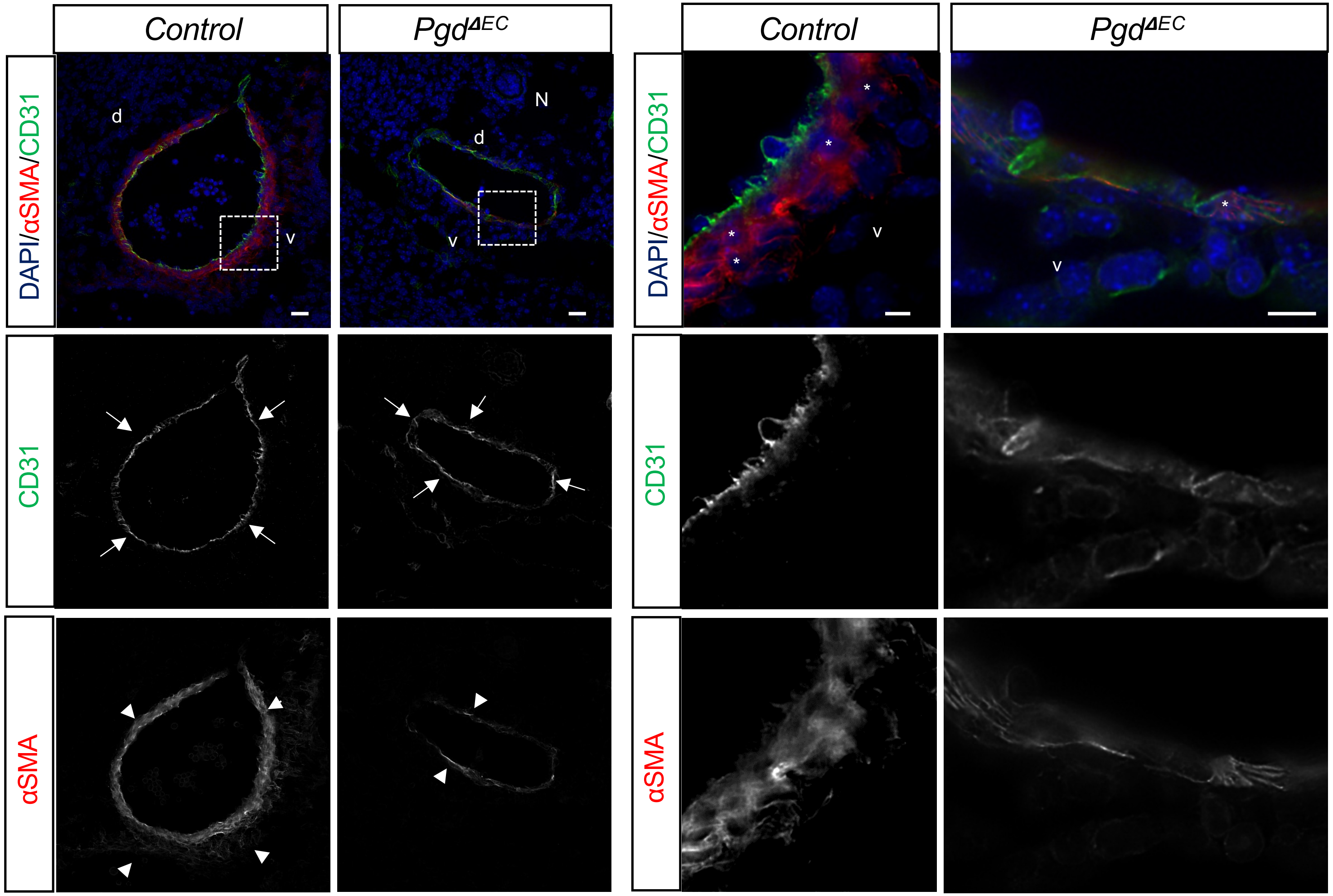


$PGD^{\Delta EC}$ display *fairly* normal trunk vasculature during early development

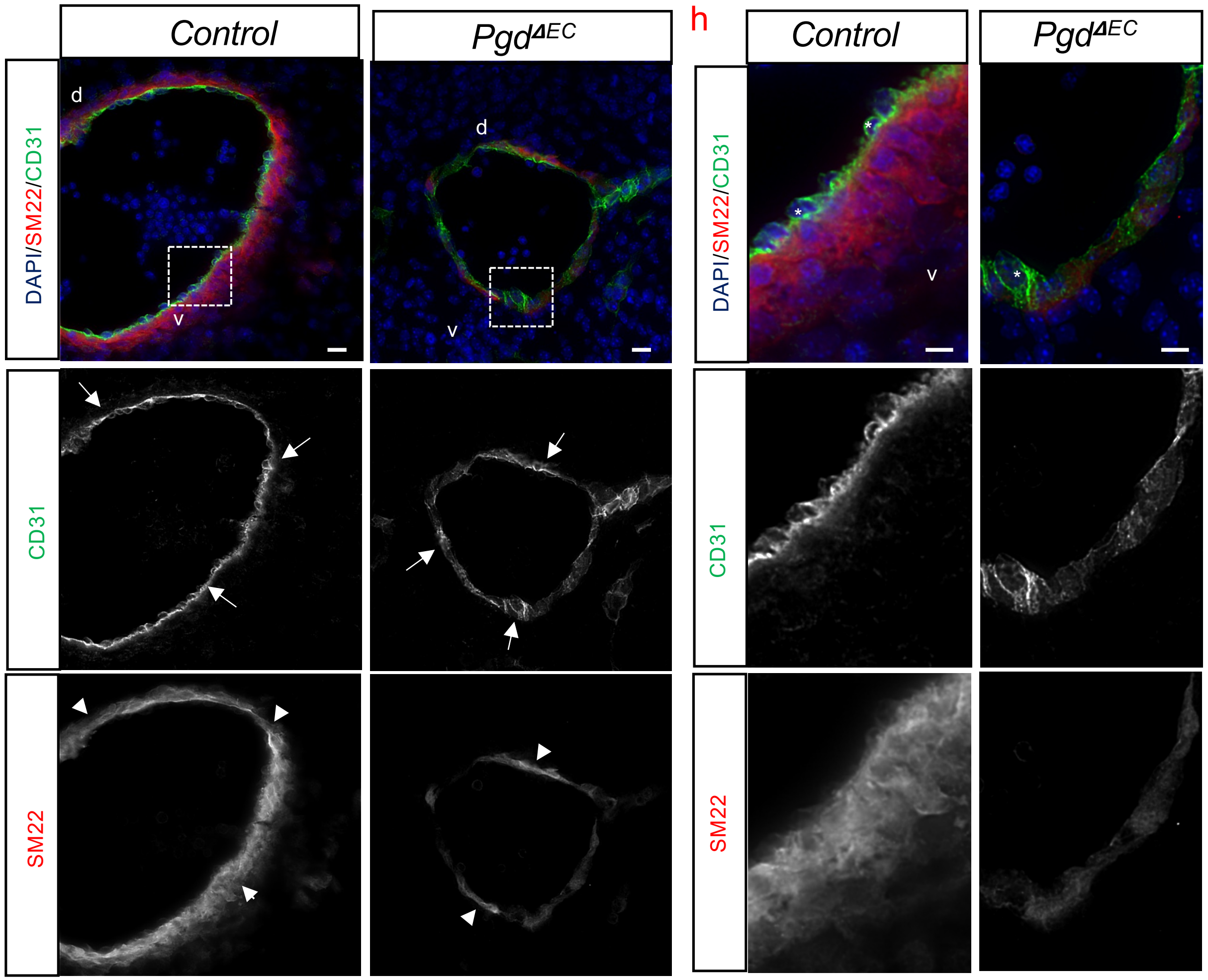




PGD$^{\Delta EC}$ display vMC recruitment defects around DA

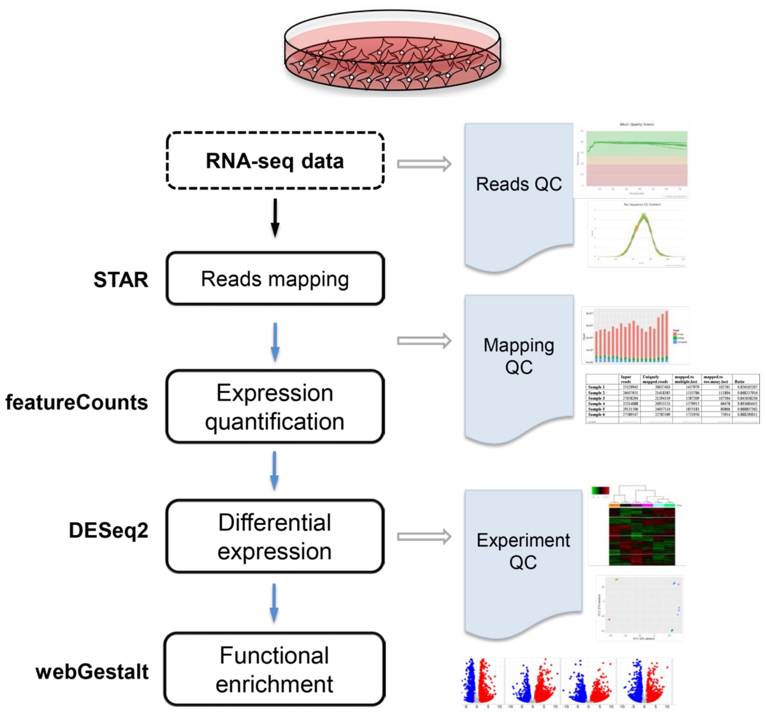


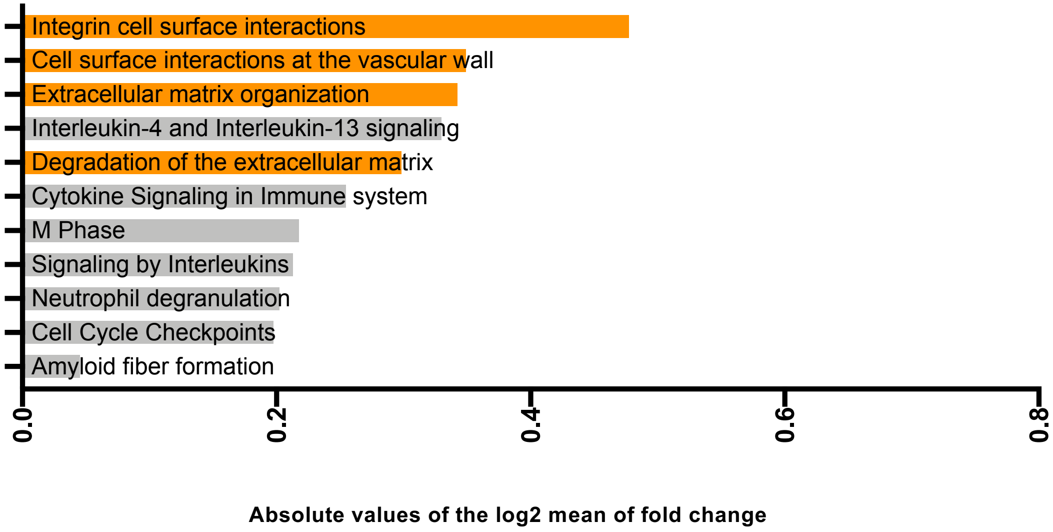
PGD$^{\Delta EC}$ display vMC recruitment defects around DA



EC TRANSCRIPTOMICS PROFILE ANALYSIS ON PPP-DEFICIENT CELLS SHOW DIFFERENTIALLY EXPRESSED GENES RELEVANT TO ECM ORGANIZATION

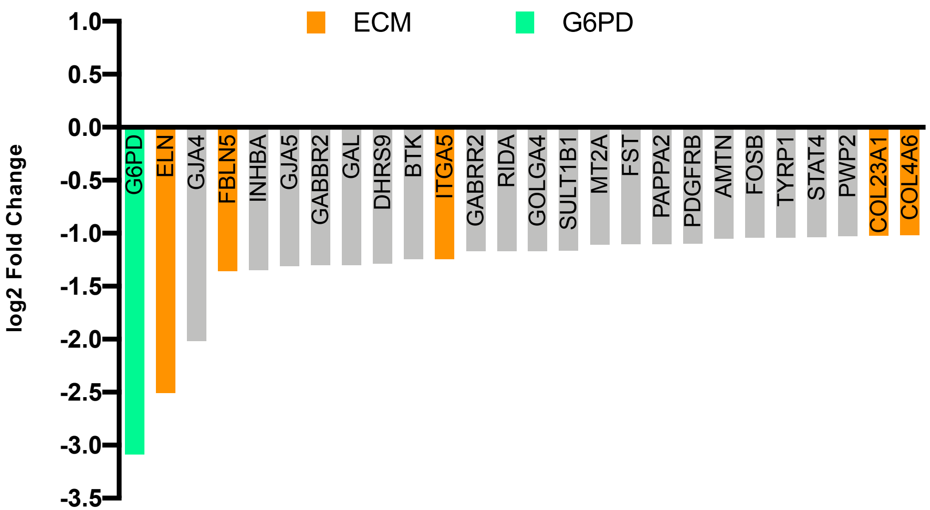
RNA-seq from EC-treated
with shRNAs for *G6PD* and *PGD.*

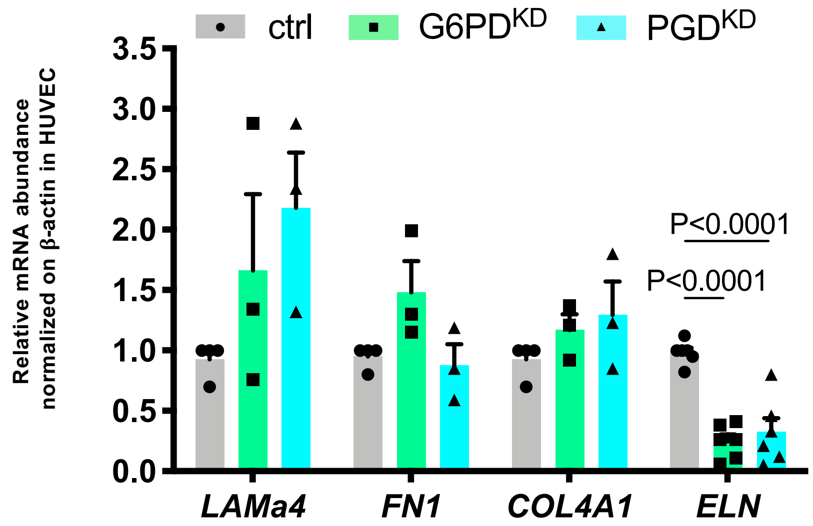




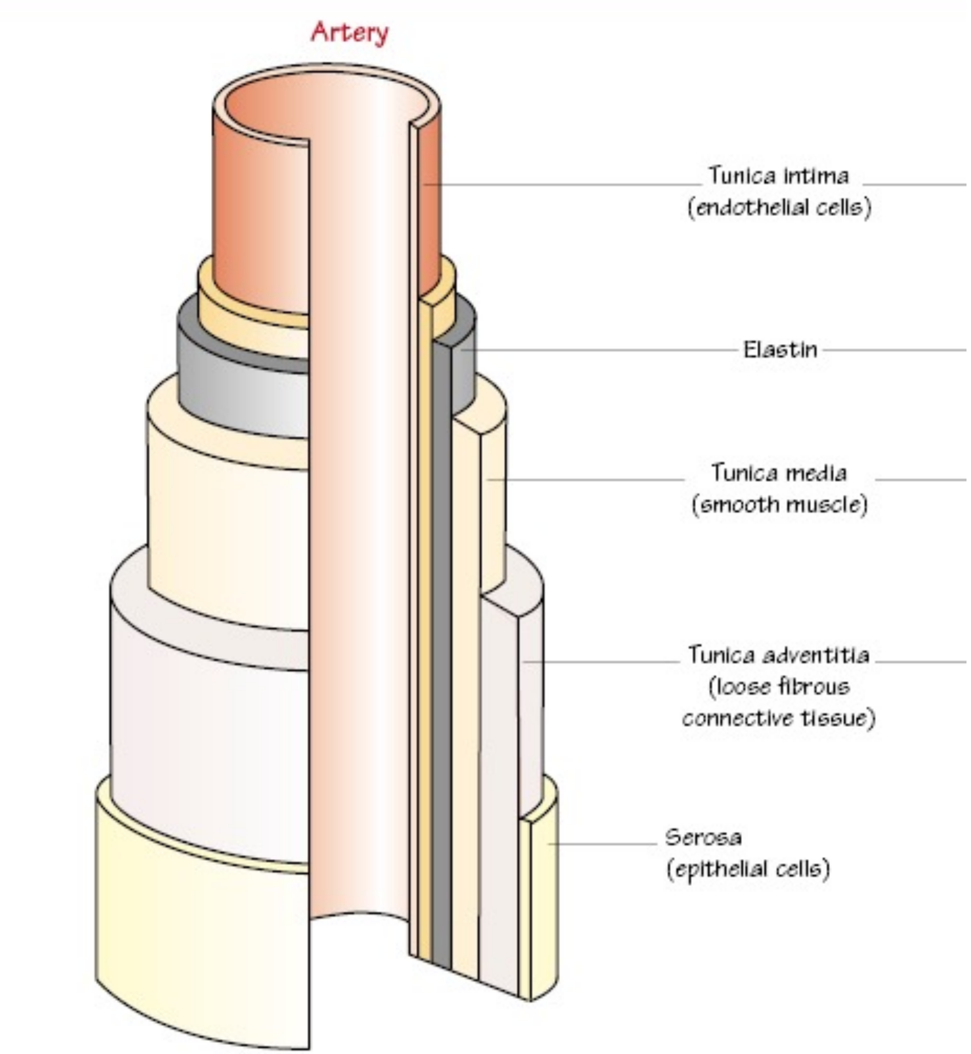
REACTOME PATHWAY ENRICHEMENT ANALYSES

RNA-seq analyses identified elastin (ELN) as a PPP-regulated gene





Elastin is a critical determinant of arterial vessels



Elastin is an essential determinant of arterial morphogenesis

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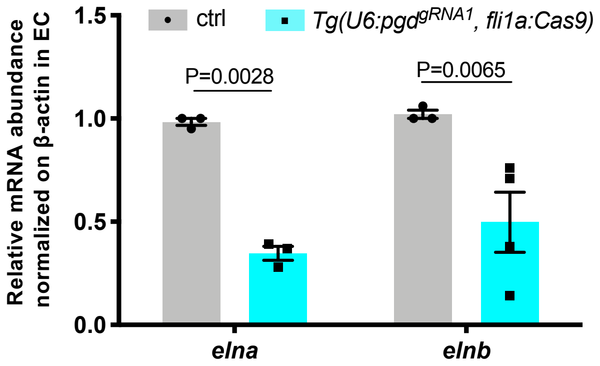
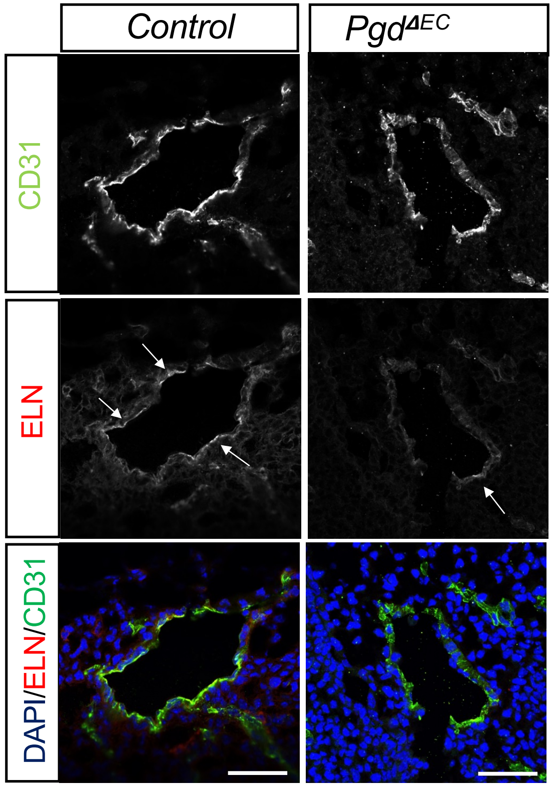
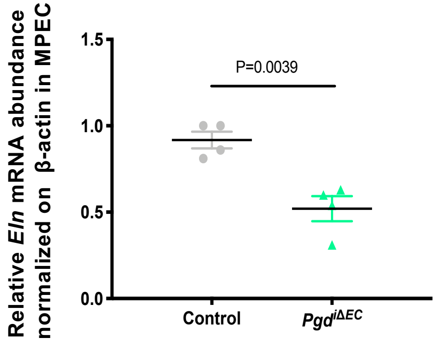
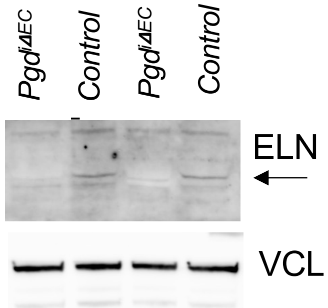
ELN expression is impaired in PGD$^{\Delta EC}$ embryos





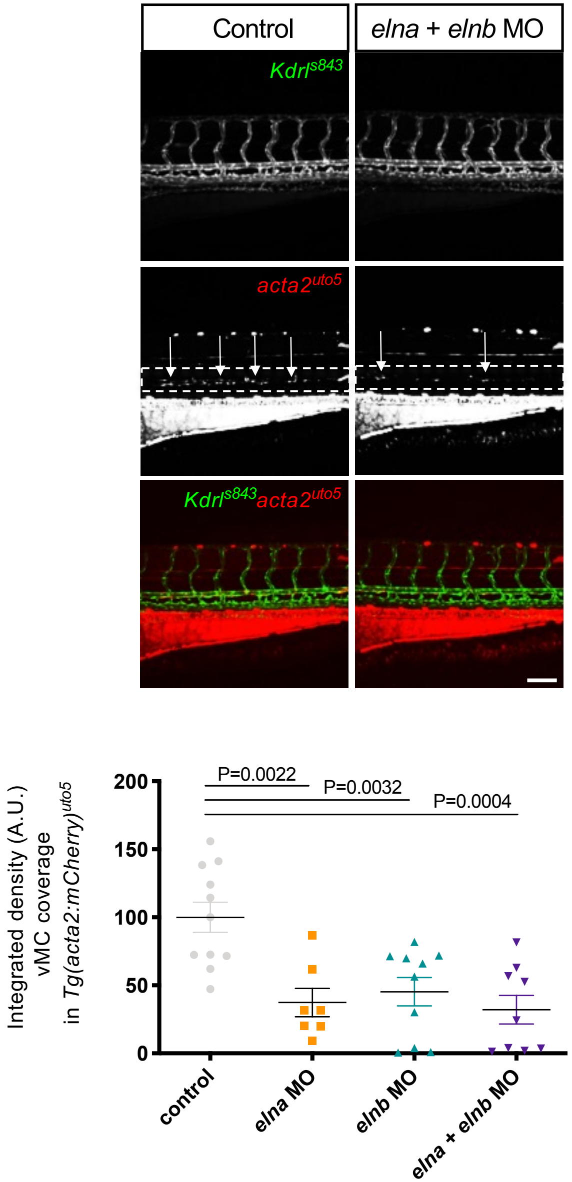


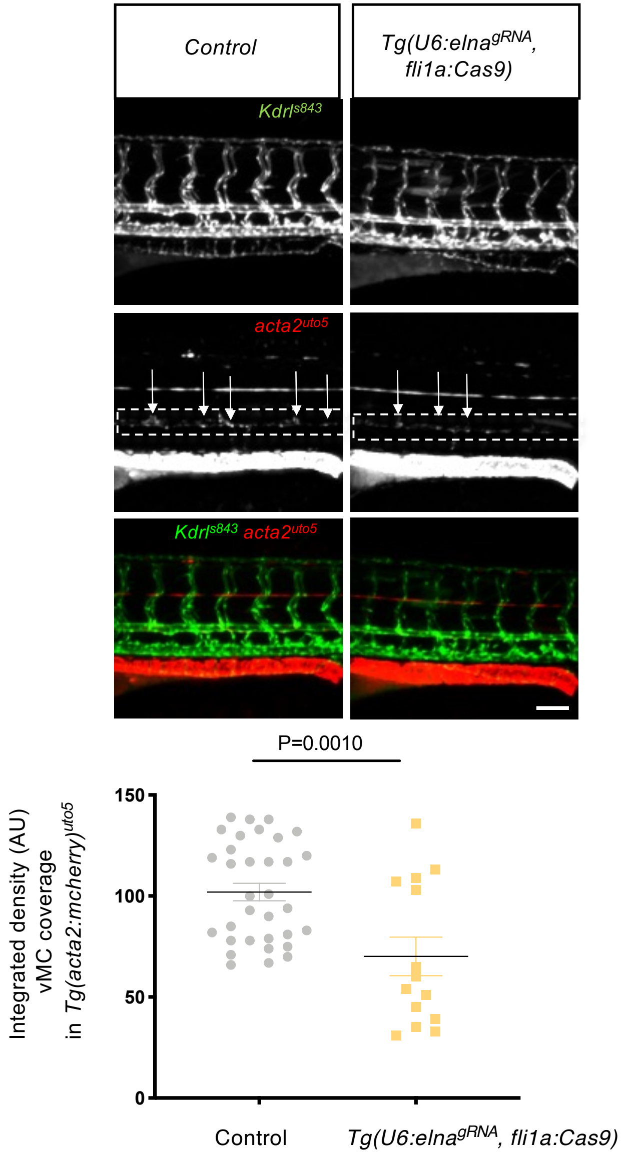


zebrafish embryos

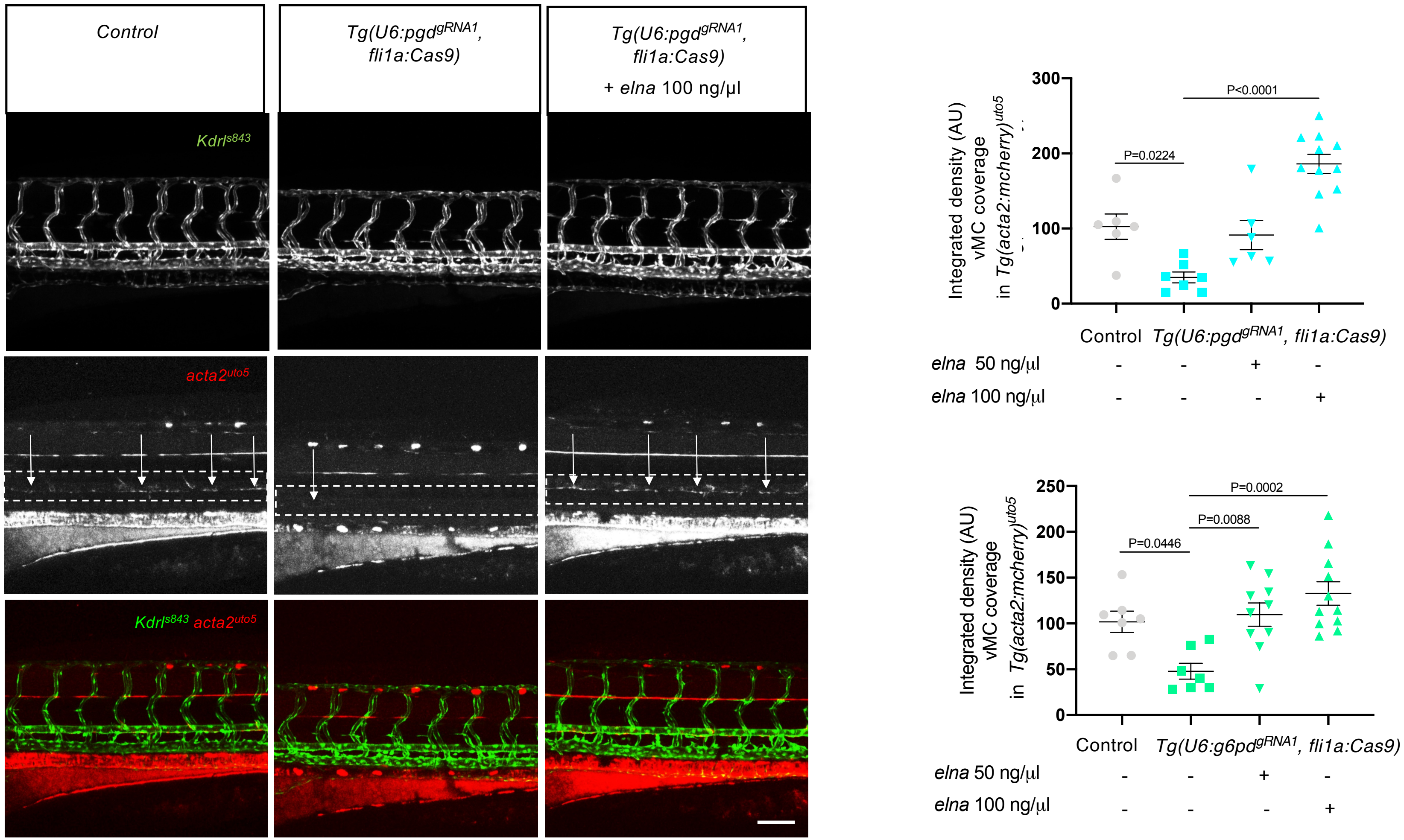
mouse embryos

ELN-loss in ECs affects vMC recruitment

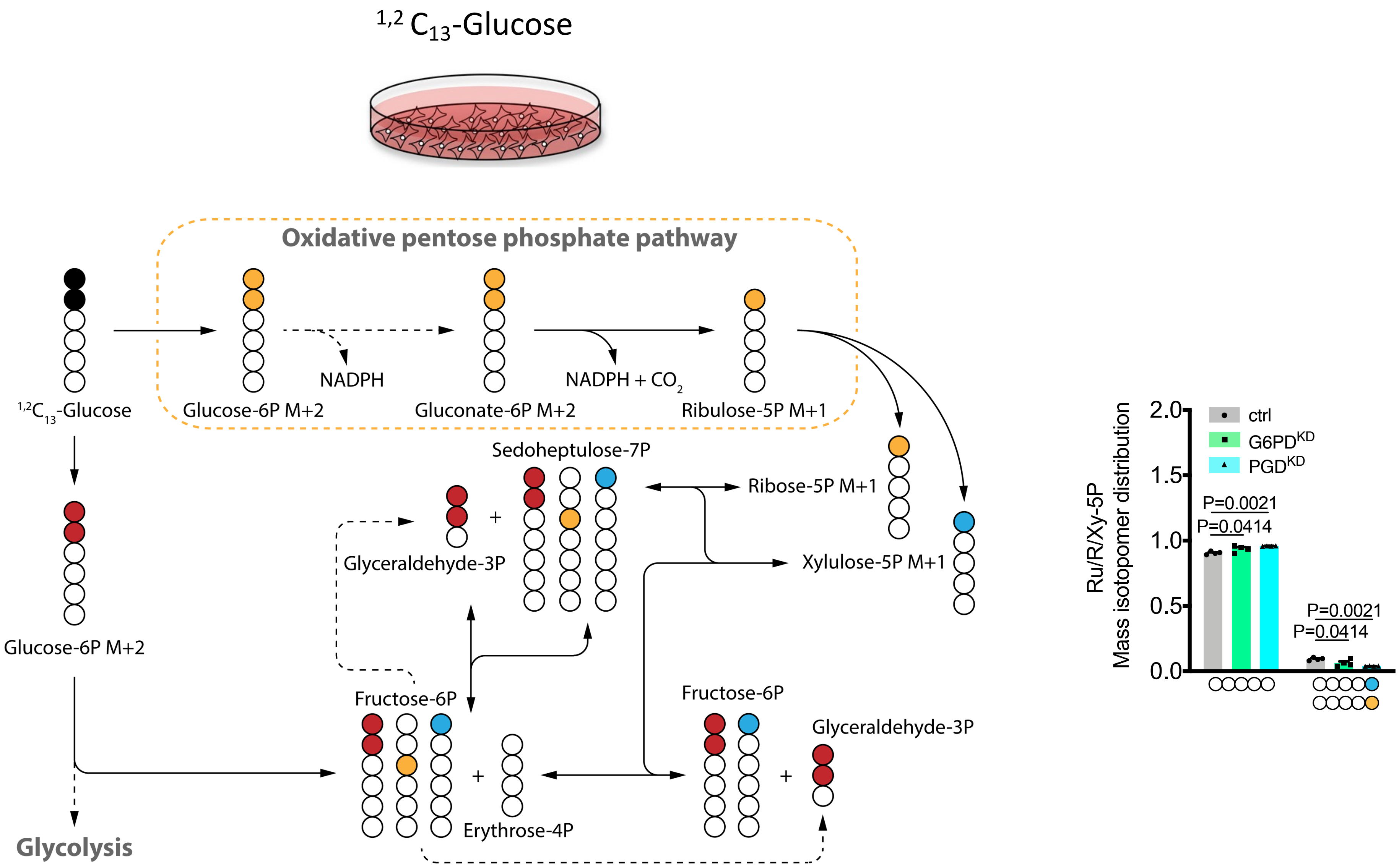




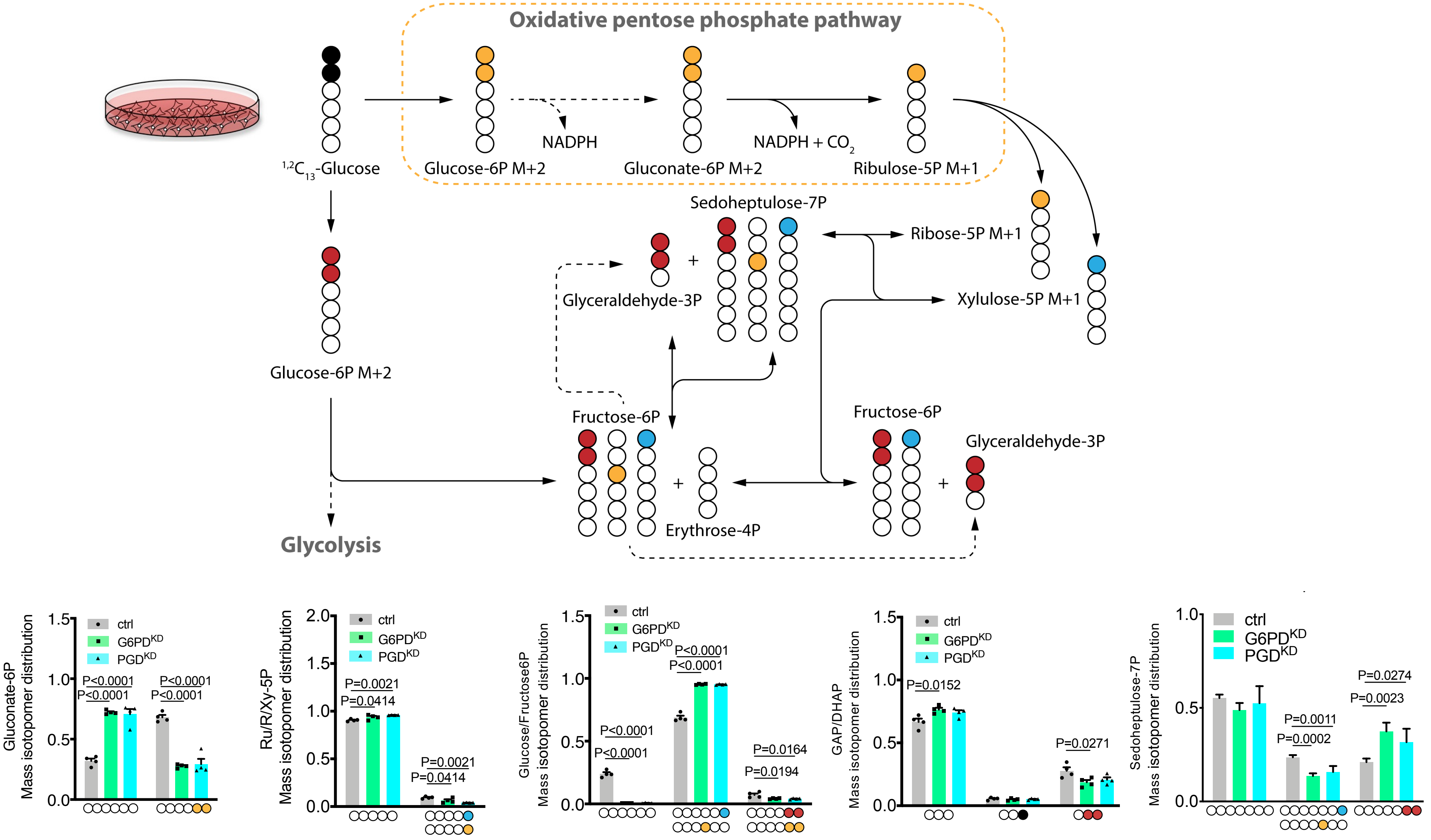
ELN expression rescue vMC recruitment in PPP mutants



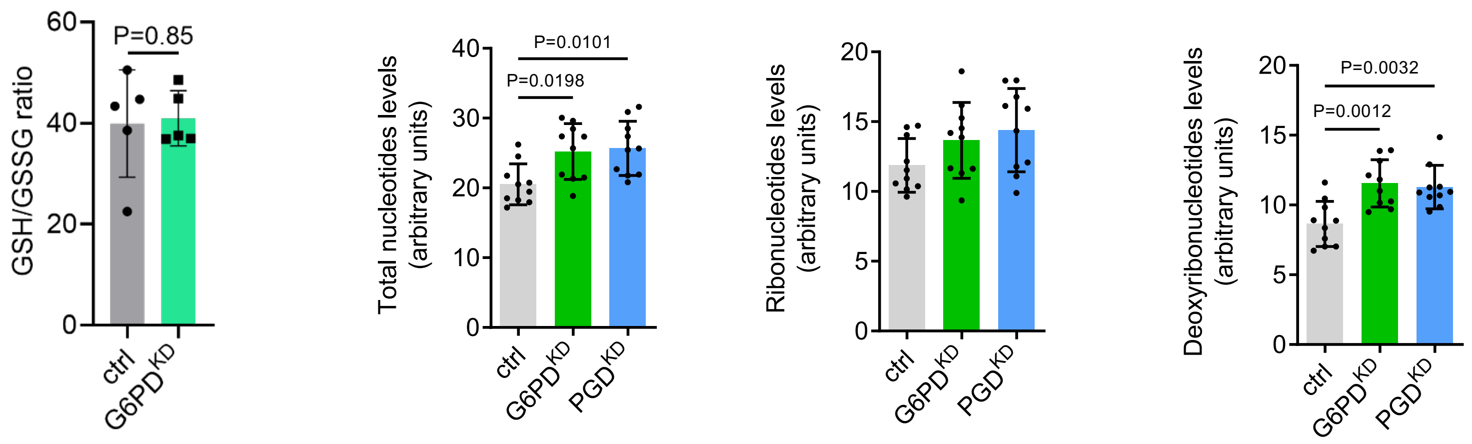
Pentose phosphate pathway flux analyses in ECs



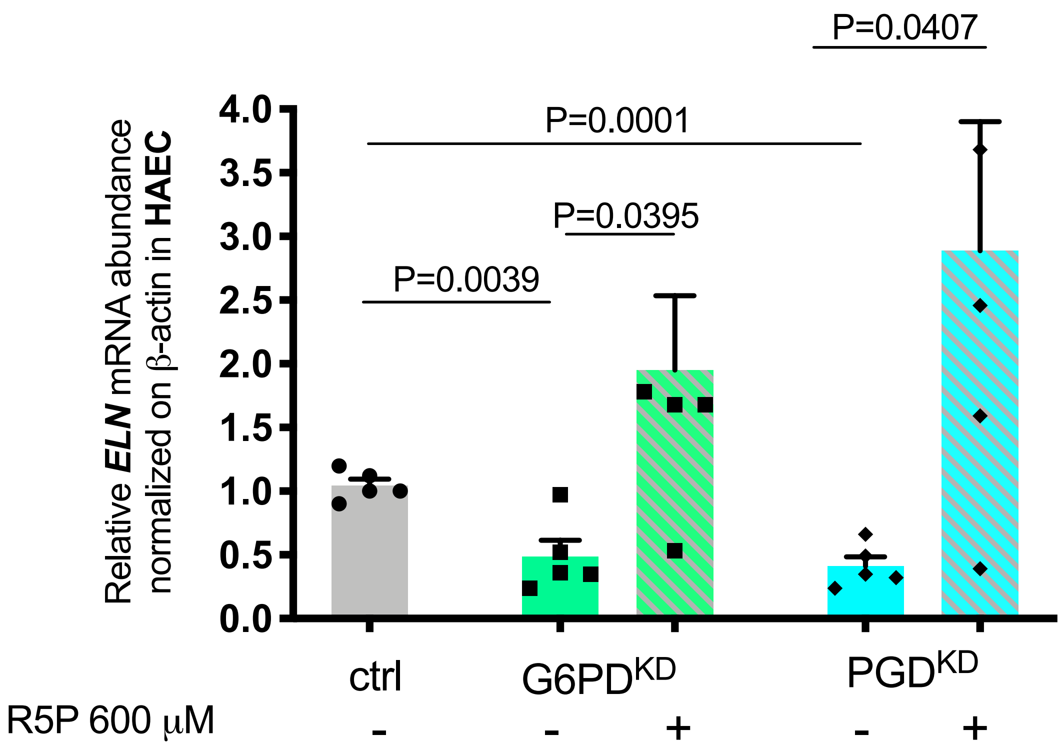
Pentose phosphate pathway flux in ECs



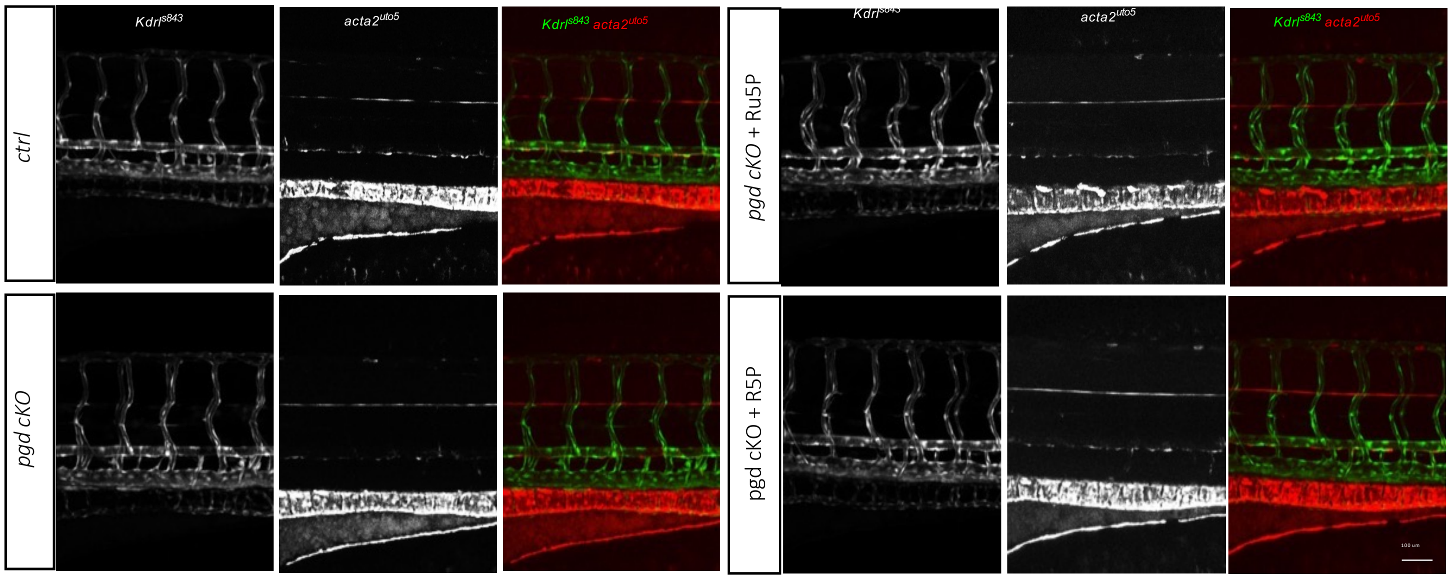
GSH and nucleotide levels do not drop in PPP^{KD} EC

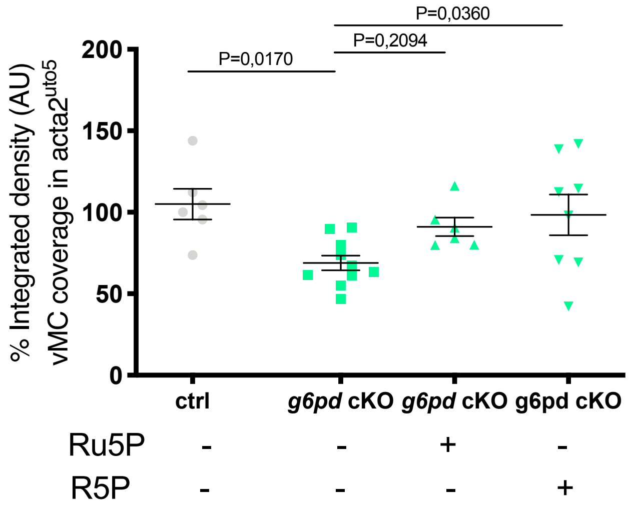


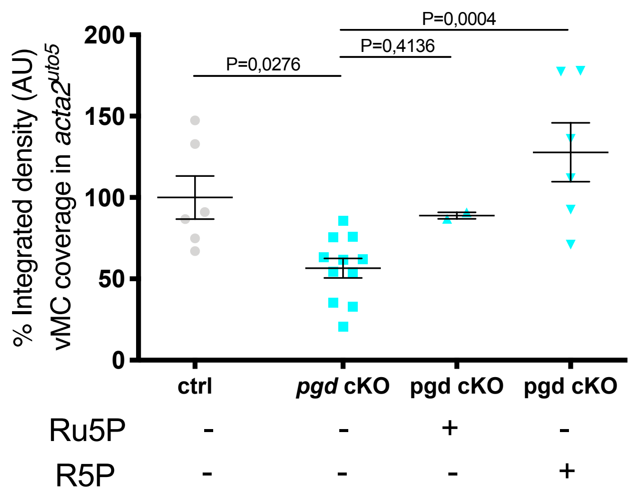
ELN expression can be rescue by R5P treatment in PPP-loss ECs



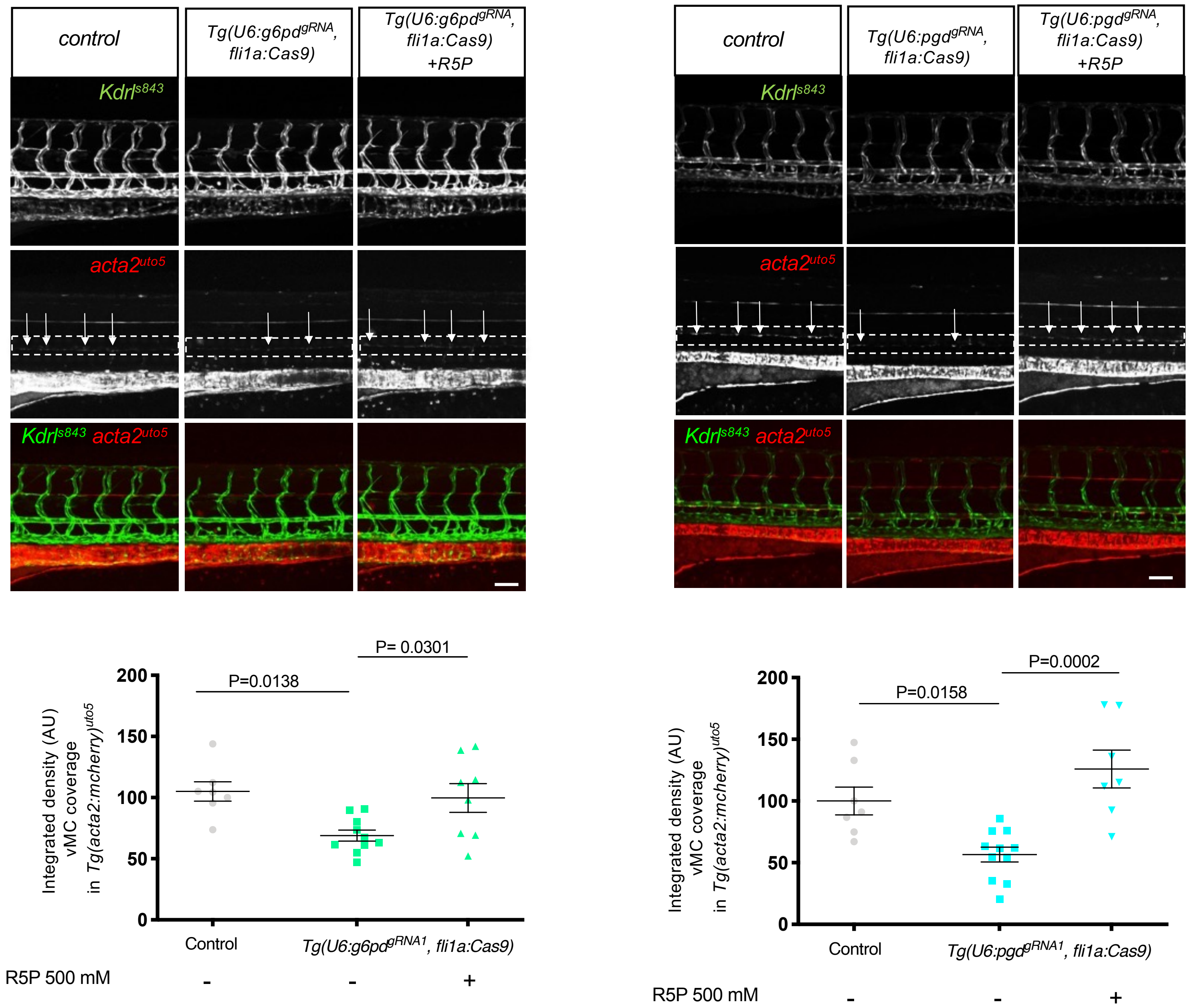
vMC recruitment can be rescue by R5P administration



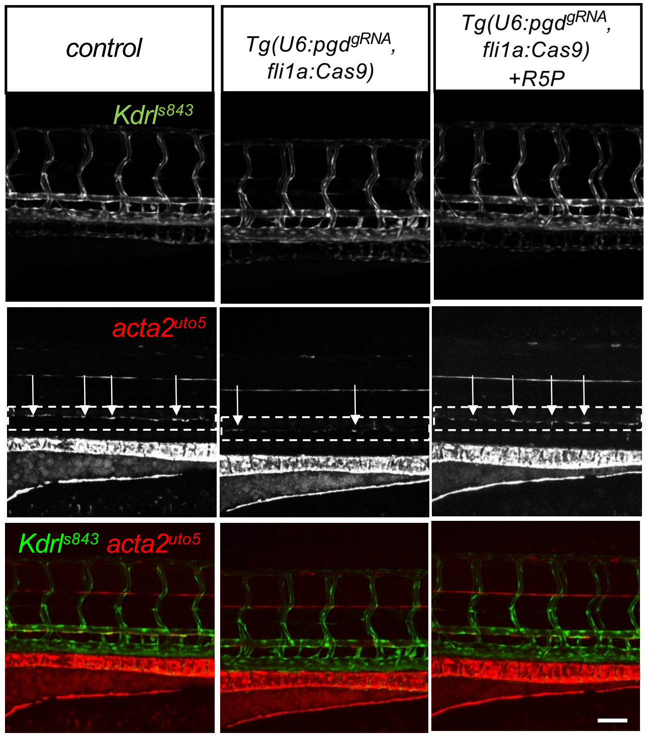


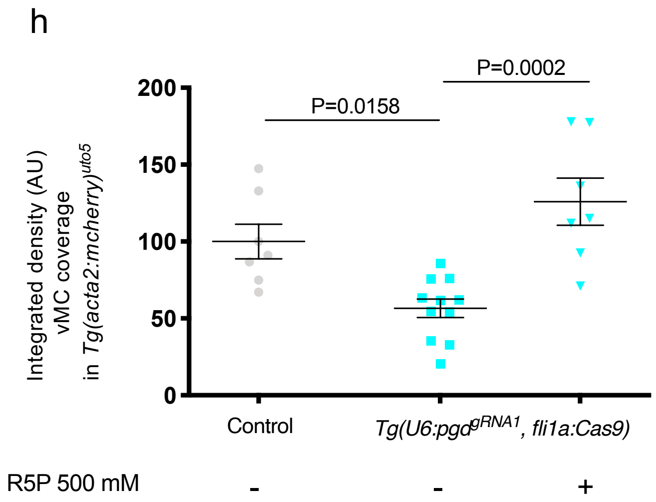


vMC recruitment can be rescued by R5P administration in PPP zebrafish mutants

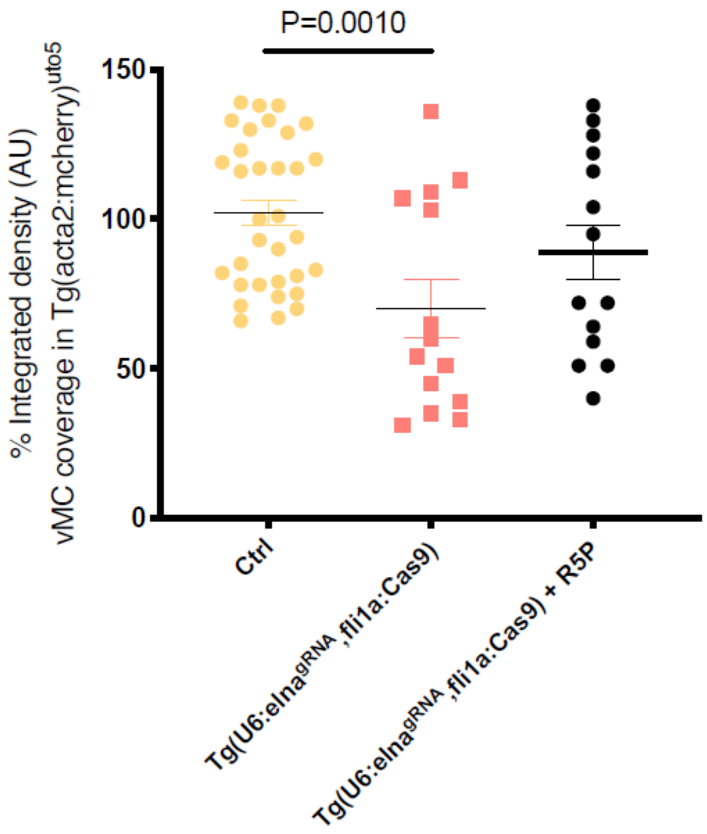


vMC recruitment can be rescued by R5P administration in PPP zebrafish mutants

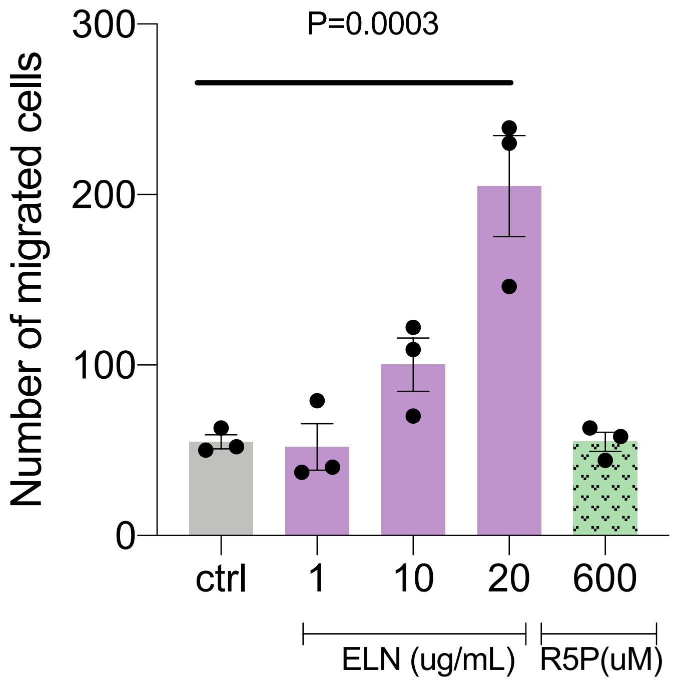


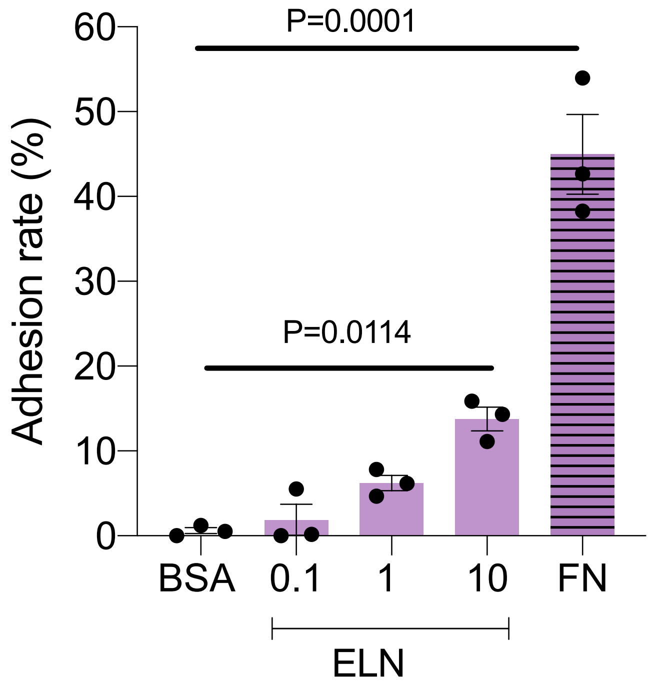


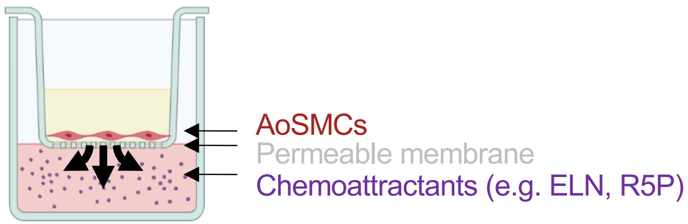
R5P treatment does not rescue vMC recruitment in ELN-deficient animals

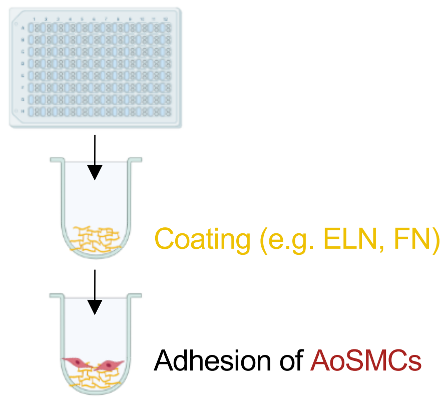


ELN supports vMC migration and adhesion

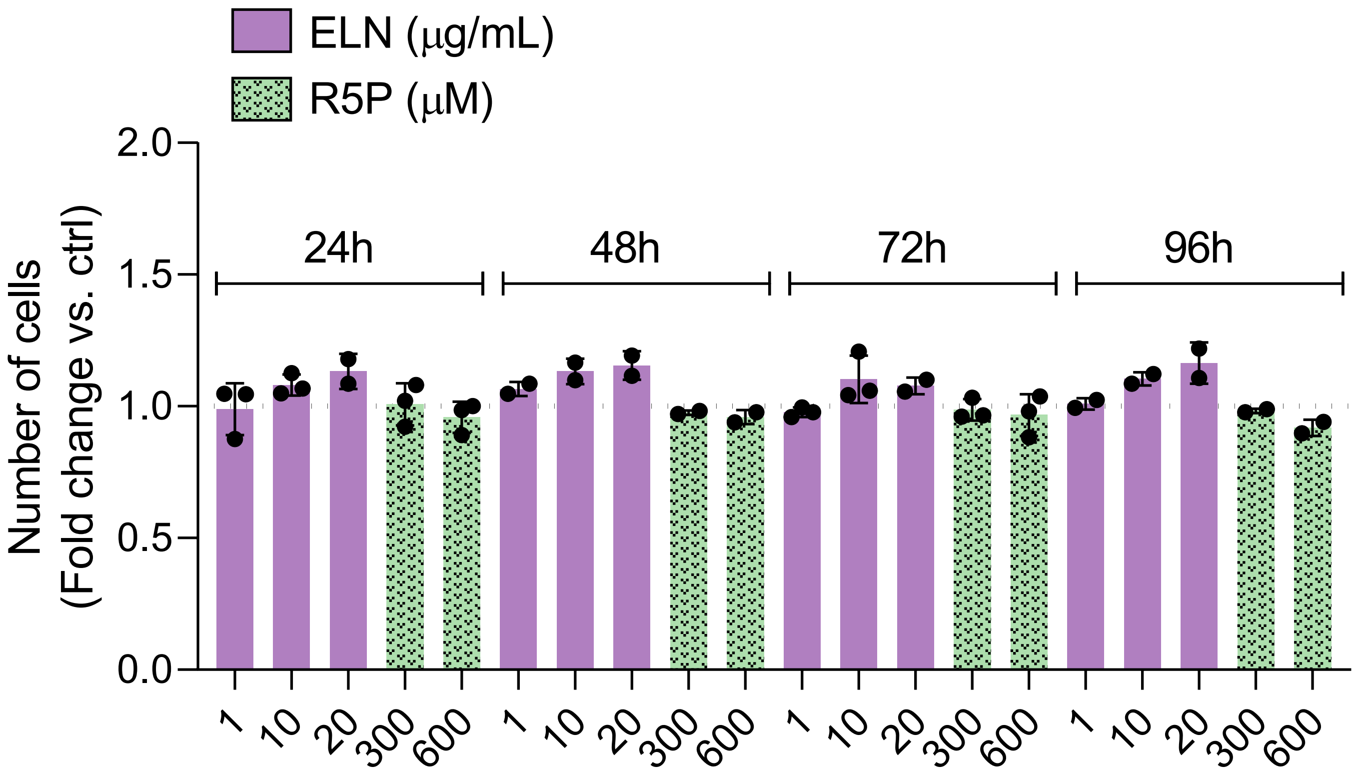




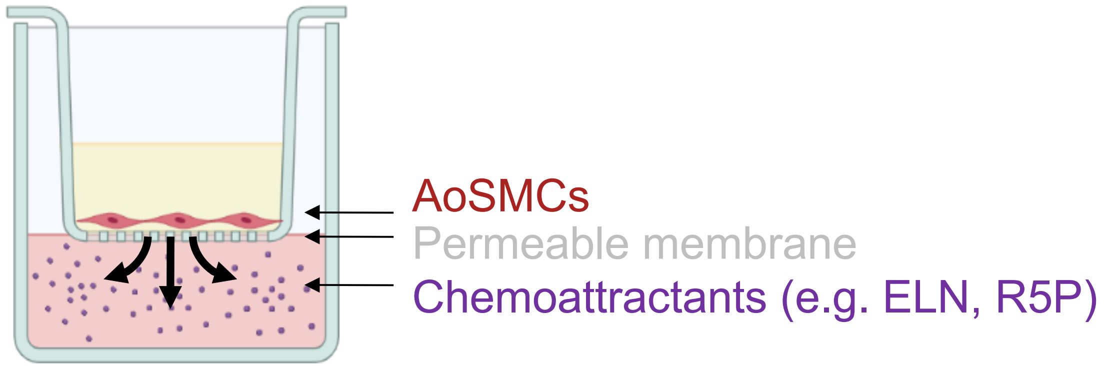


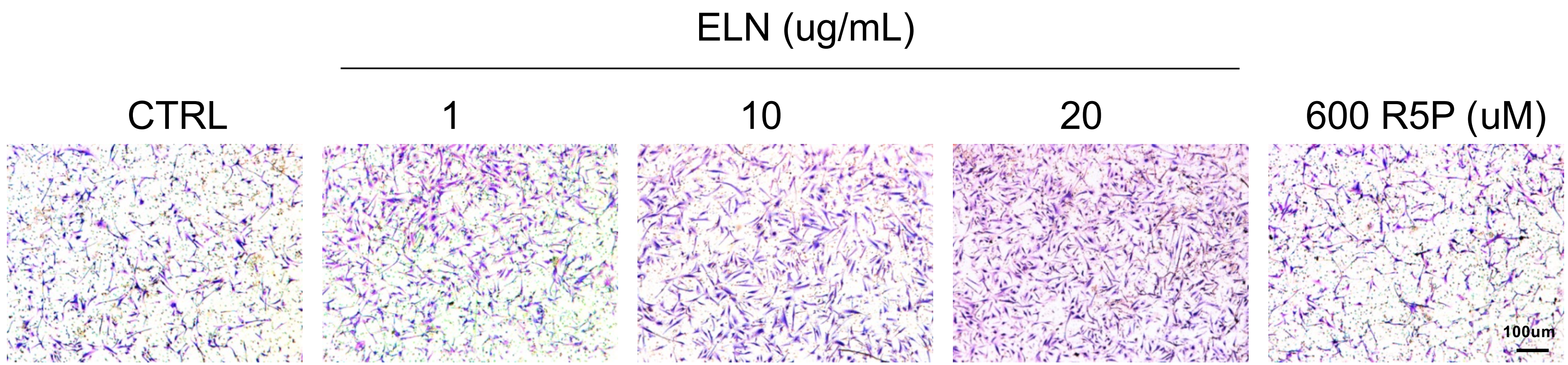


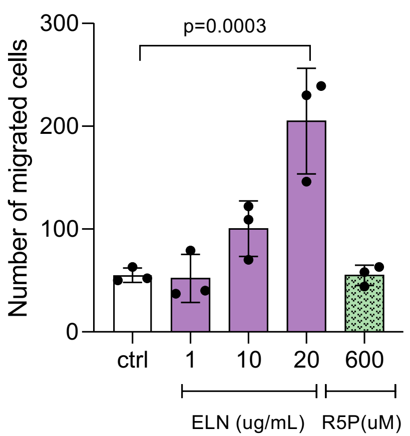
ELN and R5P does not supports vMC proliferation

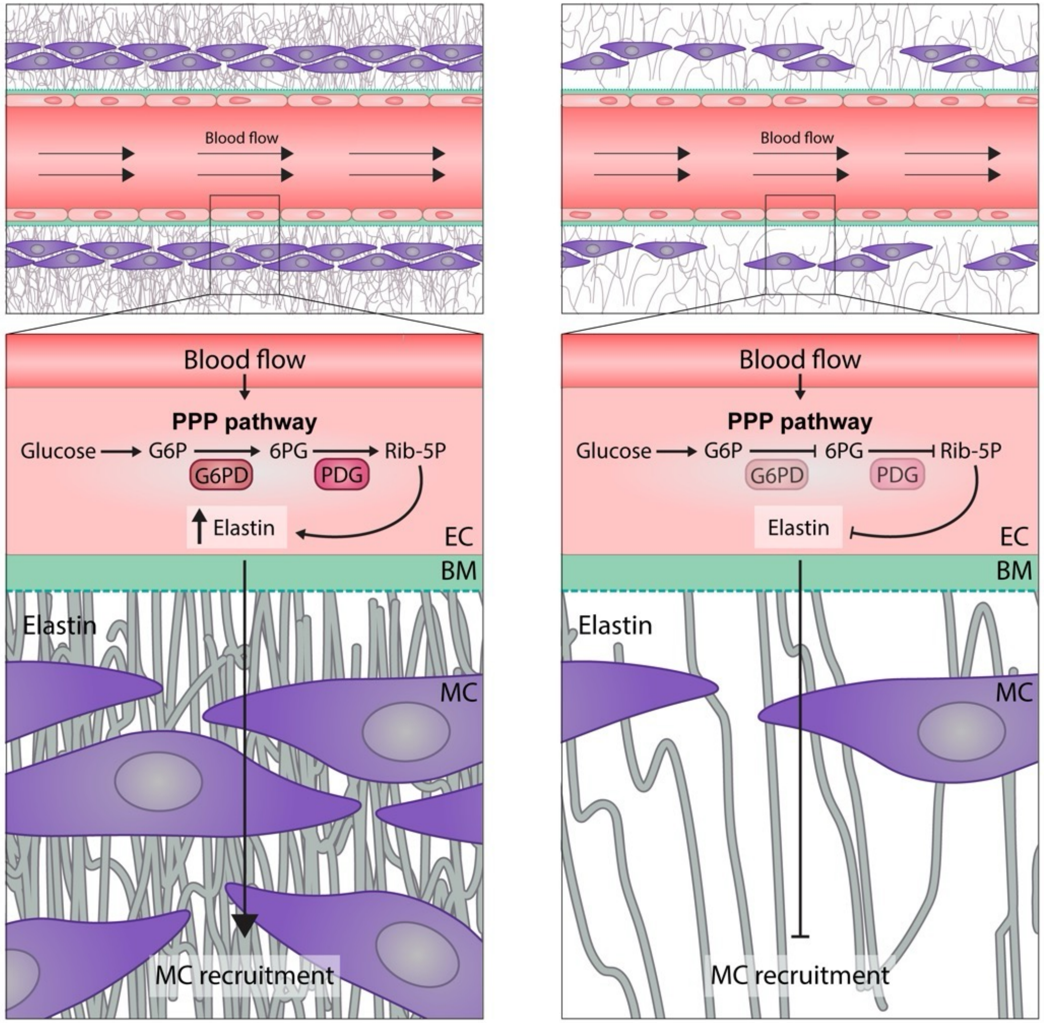


Transwell migration assay









Blood flow
Blood flow
PPP pathway
Glucose
G6P
6PG
Rib-5P
G6PD
PDG
Elastin
EC
BM
Elastin
MC
MC recruitment
Blood flow
Blood flow
PPP pathway
Glucose
G6P
6PG
Rib-5P
G6PD
PDG
Elastin
EC
BM
Elastin
MC
MC recruitment

How does R5P regulate elastin expression ?