In vitro and In vivo models for studying endothelial cell development and hereditary hemorrhagic telangiectasia

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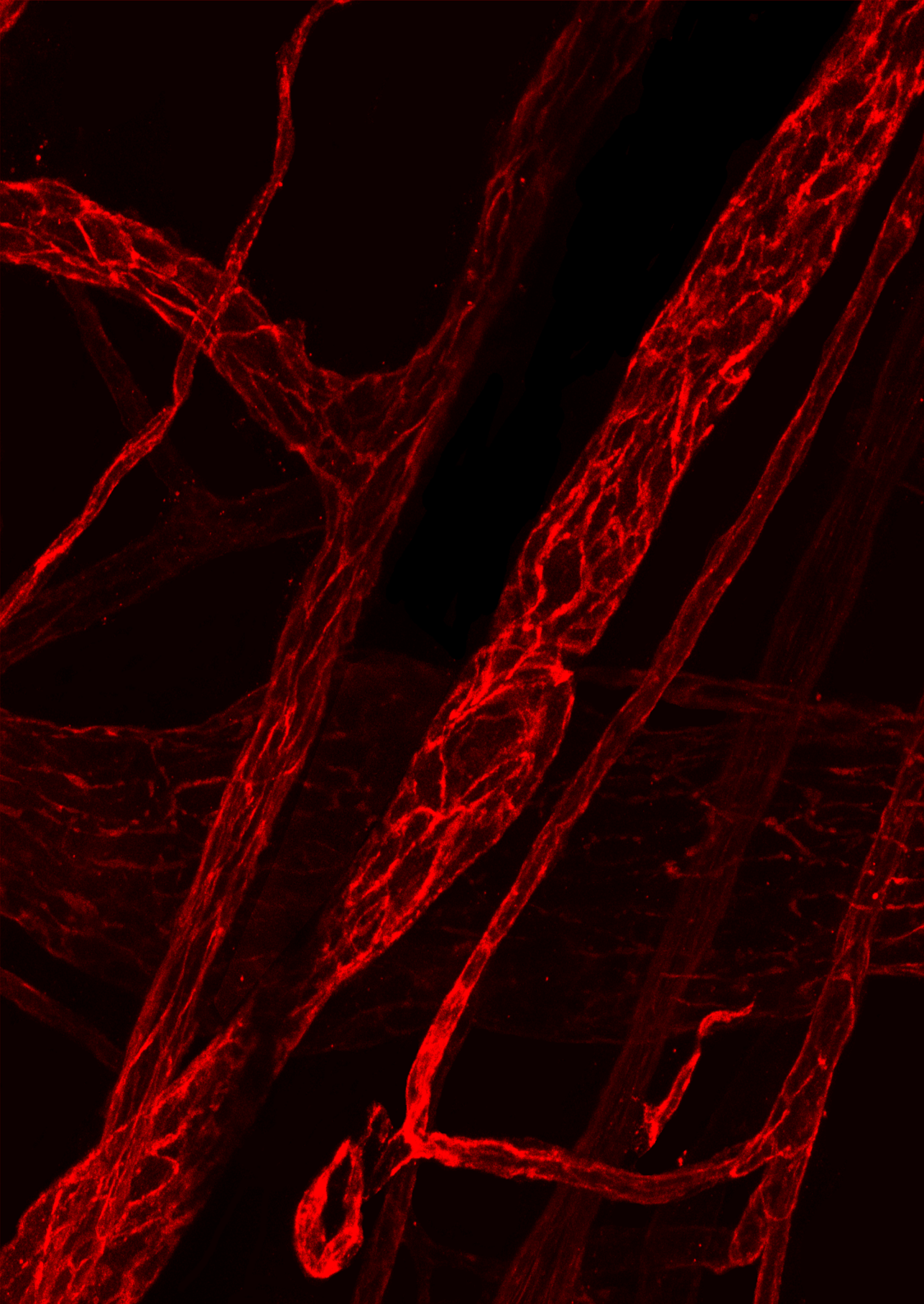

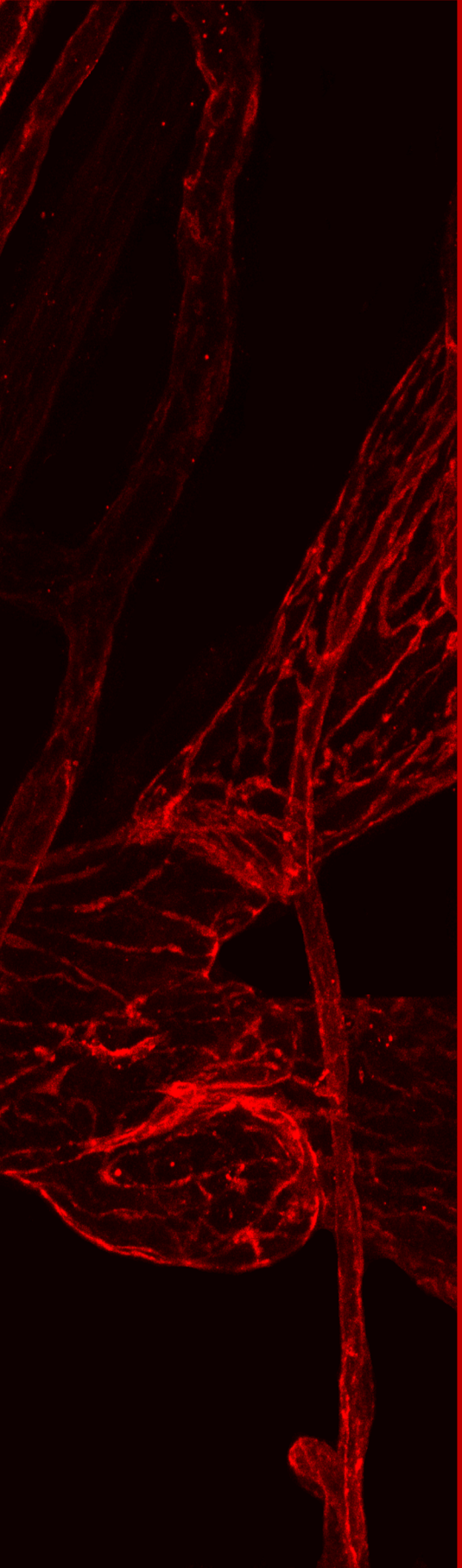

SUMMARY
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Summary

Over the last two decades, significant advances have been made in better understanding the molecular and cellular mechanisms of vascular cell development and disease. PSCs are proving to be a useful and renewable source to study the molecular mechanisms underlying physiologic endothelial cell development and pathophysiologic states of a disease *in vitro*, such as HHT. In the same line, mouse models carrying mutations in HHT-related genes are also essential to increase our understanding of the disease mechanisms.

Chapter 2 investigated the generation of knockin mESC line in order to study the biallelic distribution of Nanog expression at the protein level. Nanog is a key transcription factor expressed in pluripotent cells and is required for self-renewal *in vitro.* We found that this knock-in fusion strategy allows the generation of functional Nanog fusion proteins mirroring normal protein distribution *in vivo* and *in vitro.* This approach will be of value in creating new genetic vascular disease models and reporter lines for vascular lineage specification.

Chapter 3 investigated the generation of human ECs from hESCs using a spin-EB differentiation approach. Although significant advances have been made in inducing endothelial differentiation from hPSCs, new methods would be useful to identify the critical regulators guiding commitment and diversification of endothelial progenitors and allow the derivation of tissue type specific ECs. Latest studies have described ETV2 as one of the master regulators in the development of ECs. In this chapter, we showed that ECs originate from a VEGF-treated mesodermal population and that *ETV2* expression is enriched on day 4 of differentiation in endothelial progenitors in both $PDGFR\alpha^{+}APJ^{+}KDR^{+}$ and $PDGFR\alpha^{-}APJ^{+}KDR^{+}$ fractions. Furthermore, we provided a new knock-in fusion strategy that allows non-invasive quantification of ETV2-expressing cells.

Chapter 4 described the first derivation of three human iPS cell lines in the Netherlands: two from healthy individuals and one from a patient with HHT. All of these human iPSC lines were derived from fibroblasts grown from skin samples and were able to give rise to multiple cardiovascular cell types including beating cardiomyocytes, when seeded on visceral endoderm-like (END-2) cells.

Chapter 5 investigated the use of hiPSC-EC as a model for studying HHT1 *in vitro.* HHT is an autosomal dominant vascular disorder caused by mutations in one of two receptors of the TGFβ family, ENG or ACVRL1, which are mainly expressed by ECs and lead to HHT1 or HHT2, respectively. We found that cell density culture conditions are coupled to SMAD phosphorylation in hiPSC-ECs and that ENG haploinsufficency can affect TGFβ signaling. We further identified MTUS1, as a new TGFβ signaling candidate gene that is highly upregulated when ENG levels are reduced.

Chapter 6 investigated the role of Gja5 that encodes for the gap junction protein Cx40 in the development of AVMs in the HHT2 mouse model. We identified *GJA5* as a downstream target gene of BMP9/ALK1 signaling pathway and also found reduced Cx40 levels in a small group of HHT2 patients. In the $Acvrl1^{+/-}$ mice, *Gja5* haploinsufficiency led to the rarefaction of the capillary bed and the vasodilation of the arteries, in which ROS production was elevated. We showed that altered hemodynamic forces in $Acvrl1^{+/-}$;$Gja5^{EGFP/+}$ mice could develop transient arteriovenous shunts in the capillaries, where large malformations can be developed upon exposed to environmental insults, such as wounding.