Endothelial fatty acid-binding proteins contribute to the pathogenesis of pulmonary hypertension

Bin Liu[1,2,3], Dan Yi[1,2,3], Shuai Li[1,2], Karina Ramirez[1,2], Xiaomei Xia[1,2], Yanhong Cao[1,2], Hanqiu Zhao[1,2], Ankit Tripathi[1,2], Shenfeng Qiu[3], Mrinalini Kala[2], Ruslan Rafikov[5], Haiwei Gu[6], Vinicio de jesus Perez[7], Sarah-Eve Lemay[8], Christopher C. Glembotski[2,3], Kenneth S Knox[1,2], Sebastien Bonnet[8], Vladimir V. Kalinichenko[9,10], Michael B. Fallon[2], Olivier Boucherat[8], and Zhiyu Dai[1,2,3,11,12*]

[1]Division of Pulmonary, Critical Care and Sleep, [2]Department of Internal Medicine, College of Medicine-Phoenix, University of Arizona, Phoenix, Arizona, USA

[3]Translational Cardiovascular Research Center, College of Medicine-Phoenix, University of Arizona, Phoenix, Arizona, USA

[4]Basic Medical Sciences, College of Medicine-Phoenix, University of Arizona, Phoenix, Arizona, USA

[5]Department of Medicine, Indiana University College of Medicine, Indianapolis, IN, USA

[6]College of Health Solutions, Arizona State University, Phoenix, AZ, USA

[7]Division of Pulmonary and Critical Care, Stanford University, Palo Alto, CA, USA

[8]Pulmonary Hypertension and Vascular Biology Research Group, Faculty of Medicine, Laval University, Quebec, QC, Canada

[9]Division of Neonatology, Phoenix Children's Hospital, Phoenix, Arizona, USA

[10]Phoenix Children's Health Research Institute, College of Medicine-Phoenix, University of Arizona, Phoenix, Arizona, USA

[11]BIO5 Institute, University of Arizona, Tucson, Arizona, USA

[12]Sarver Heart Center, University of Arizona, Tucson, Arizona, USA

[*]**Correspondence to:** Zhiyu Dai, Ph.D. (zhiyudai@arizona.edu), Department of Internal Medicine, College of Medicine-Phoenix, University of Arizona, Phoenix, Arizona. 475 N. 5th Street, Phoenix, AZ 85004, USA. Phone: +1-(602) 827-2982.

Short title

Role of FABP4-5 in PAH

Abstract

Background: Pulmonary arterial hypertension (PAH) is a devastating disease characterized by obliterative vascular remodeling and persistent increase of vascular resistance, leading to right heart failure and premature death. Understanding the cellular and molecular mechanisms will help develop novel therapeutic approaches for PAH patients. Recent studies showed that FABP4 and FABP5 were expressed in ECs across multiple tissues and circulating FABP4 level was elevated in the PAH patients. However, the role of endothelial FABP4/5 in the pathogenesis of PAH remains undetermined.

Methods: FABP4/5 expression was examined in pulmonary arterial endothelial cells (PAECs) and lung tissues from patients with idiopathic PAH (IPAH) and pulmonary hypertension (PH) rat models. Plasma proteome analysis was performed in human PAH samples. Echocardiography, hemodynamics, histology, and immunostaining were performed to evaluate the lung and heart PH phenotypes in *Egln1*^{Tie2Cre} (CKO) mice and *Egln1*^{Tie2Cre}/*Fabp4-5*$^{-/-}$ (TKO) mice. Bulk RNA sequencing (RNA-seq) and single-cell RNA-seq (scRNA-seq) analysis were performed to understand the cellular and molecular mechanisms of endothelial FABP4/5 mediated PAH pathogenesis.

Results: Both FABP4 and FABP5 were highly induced in ECs of CKO mice and PAECs from IPAH patients, and in whole lungs of PH rats. Plasma levels of FABP4/5 were upregulated in IPAH patients and directly correlated with severity of hemodynamics and biochemical parameters. Genetic deletion of both *Fabp4* and 5 in CKO mice caused a reduction of right ventricular systolic pressure (RVSP) and RV hypertrophy, attenuated pulmonary vascular remodeling and prevented the right heart failure. *Fabp4/5* deletion also normalized EC glycolysis, reduced ROS and HIF-2α expression, and decreased aberrant EC proliferation in CKO lungs.

Conclusions: PH causes aberrant expression of FABP4/5 in pulmonary ECs which leads to enhanced ECs glycolysis and hyperproliferation, contributing to the accumulation of arterial ECs and vascular remodeling and exacerbating the disease.

Keywords: lipid metabolism, glycolysis, right heart failure, oxidative, hypoxia

Introduction

PAH is a progressive, complex, and devastating disease arising from a variety of pathogenic and genetic causes. Excessive vasoconstriction and abnormal vascular remodeling have been considered as the major factors contributing to the complicated pathogenesis of PAH[1,2]. Owing to the poor understanding of the underlying mechanisms of obliterative vascular remodeling, current therapies result in only modest improvement in morbidity and mortality with 5 years survival rate around 50%[3]. Accumulating evidence shows that PAH is a systemic metabolic disease[4–6]. Previous studies showed that non-esterified free fatty acids and acylcarnitines in the circulation are upregulated in patients with PAH compared to healthy controls, which was linked to RV lipotoxicity[7–10]. Using a combination of high-throughput liquid-and-gas-chromatography-based mass spectrometry analysis on human PAH lung, Zhao et al demonstrated an increase of β-oxidation in fatty acids and upregulation of lipid oxidation in PAH lungs[11]. However, these studies are mainly observational and the role of fatty acid metabolism during PAH development and progression remains elusive[12].

Blood vessels deliver oxygen and nutrients including lipids. Circulating fatty acids are transported in the forms of triglycerides, which are released by lipoprotein lipase (LPL) anchoring in the lumen side of the endothelium[13]. Mounting evidence demonstrated that fatty acid chaperones mediate transendothelial fatty acid transport[14]. Fatty acid-binding protein (FABP) 4 and 5 are members of intracellular FABPs, which bind long-chain fatty acids and facilitate lipids transport in and between cells. FABP4 is firstly identified in mature adipocytes, while FABP5 is expressed abundantly in the skin epidermal cells[15]. Recent studies showed that FABP4 and FABP5 are expressed in ECs across multiple tissues[16,17], and circulating FABP4 level is elevated in the PAH patients[18]. However, the role of endothelial FABP4/5 in the pathogenesis of PAH remains undetermined.

Loss of distal capillaries and accumulation of pathogenic pulmonary arterial ECs with proliferative, anti-apoptotic and glycolytic phenotypes are hallmarks of PAH development[19–23]. In the present study, we found that among fatty acid chaperones, both FABP4 and FABP5 are dramatically induced in mouse PAECs from *Egln1*^{Tie2Cre} mice, a novel mouse model with severe PH and right heart failure[24,25], and in human PAECs isolated from IPAH patients. Based on scRNA-seq analysis, genetic deletion of *Fabp4/5* dramatically reduced arterial ECs population and vascular remodeling in *Egln1*^{Tie2Cre} mice. We aimed to determine how lung endothelial FABP4/5 regulate arterial ECs accumulation in the pathogenesis of PAH.

Methods

Human Samples

The use of archived human lung tissues and cells from Pulmonary Hypertension Breakthrough Initiative (PHBI) was granted by the UA Institutional Review Board (IRB#1907824872)**.** Human plasma proteomics analysis was described previously[1]. All experimental procedures were performed with the approval of Laval University and the IUCPQ Biosafety and Ethics Committees (CER#20735). All patients provided and signed informed consent form.

Mice

Egln1^{Tie2Cre} (CKO) and *Egln1/Hif2a*^{Tie2Cre} (EH2) mice were generated as described previously[2]. *Fabp4-5*$^{-/-}$ mice were obtained from Gökhan S. Hotamisligil, Harvard School of Public Health[3]. CKO mice were bred with *Fabp4-5*$^{-/-}$ mice to generate *Egln1*^{Tie2Cre}/*Fabp4-5*$^{-/-}$ (TKO) and *Egln1*$^{f/f}$/*Fabp4-5*$^{-/-}$ (DKO) mice. Both male and female *Egln1*$^{f/f}$ (designed as WT), CKO, TKO and DKO little mates at the age of 5 weeks to 3.5 months were used in these studies. The animal care and study protocols were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Arizona (#19-513).

Data availability

Scripts used for bulk RNA sequencing and single-cell RNA sequencing (scRNA-seq) analysis and analyzed data in R objects are available in GitHub (https://github.com/DaiZYlab/Fabp45). Other data that support the findings of this study are available from the corresponding author upon reasonable request.

Statistical Analysis

The statistical analysis for human plasma proteomic study was described previously[1]. Normally distributed variables were compared by Student's t-test. Non-normally distributed variables were compared by Mann-Whitney U-test. For more than two groups, one-way ANOVA for normally distributed variables or the Kruskal-Wallis test for non-normally distributed variables was performed for comparison. The correlation analysis between circulating FABP4 and 5 and hemodynamics (mean PAP, PVR, CVI and stroke volume), NT-proBNP and eGFR were performed by Pearson's correlation test. For other studies, two-group comparisons were compared by the unpaired 2-tailed Student t test for equal variance or the Welch t test for unequal variance. Multiple comparisons were performed by one-way ANOVA with a Tukey post hoc analysis that calculates corrected P values. P less than 0.05 indicated a statistically significant difference. Differential gene expression analyses on the scRNA-seq were performed using the Wilcoxon Rank Sum Test algorithm. Adjusted P less than 0.05 indicated a statistically significant difference. All bars in dot plot figures represent mean. All bar graphs represent mean±SD.

For expanded methods please see **Supplemental Material**.

Results

FABP4/5 were upregulated in PAECs from human PAH and rodent PH

We recently performed scRNA-seq analysis to profile pulmonary cells in *Egln1*$^{f/f}$ (WT) and *Egln1*^{Tie2Cre} (CKO) mice at the age of 3.5 months, at which age the CKO mice develop severe PH[25–27]. Using scRNA-seq analysis[28], we found that FABP4 was expressed at relatively high levels in ECs and macrophages (expression level: ECs > macrophages) in native (WT) lungs, whereas FABP5 was expressed in alveolar type 2 cells (AT2), alveolar macrophages (aMac), and ECs (expression level: AT2 > macrophage > ECs) in the native lung (**Fig.1A**). *Fabp4* and *Fabp5* expression was significantly higher in ECs but not aMac of CKO mice compared to WT mice (**Fig. 1A**). Immunostaining confirmed that FABP4 and FABP5 were highly expressed in highly remodeled and occlusive vessels of CKO lungs (**Fig. 1B**). Moreover, Western blotting showed a significant increase in FABP4 and FABP5 protein amounts in lungs of PH rodent models

including CKO mice, monocrotaline (MCT)[29] and Sugen5416/hypoxia(SuHx)-induced PH rats[30] (**Fig. 1C and 1D**). In addition to the animal models, endothelial FABP4/5 expression was also significantly increased in isolated PAECs from patients with IPAH compared to PAECs from healthy donors (**Fig. 1E and 1F**). Our data were consistent with a recent study showing the upregulation of circulating FABP4 in PAH patients and lung *Fabp4* mRNA in PH rodent models[18]. Thus, endothelial FABP4/5 are induced in mouse, rat, and human PAH lungs.

Plasma FABP4/5 levels were upregulated in PAH patients

We previously employed plasma proteome analysis in patients with PAH using a high-throughput multiplex proximity extension assay (PEA) using Canada cohorts[31]. We found that plasma FABP4 and FABP5 protein levels were upregulated in patients with PAH (**Fig 2A and 2B**). FABP4 protein was positively correlated with FABP5 protein level in PAH samples (**Fig 2C**). Comparison of FABP4 and FABP5 levels with hemodynamics and biochemical parameters in the human subjects showed that levels of FABP5 were positively correlated with mean pulmonary arterial pressure (mPAP), pulmonary vascular resistance (PVR) and NT-proBNP, the latter of which is a well-established and powerful marker of heart failure risk. The levels of FABP4 were also positively correlated with PVR, and NT-proBNP, but not mPAP (**Fig 2D-2F**). The levels of FABP4 and FABP5 were negatively correlated with the cardiac index, stroke volume, and estimated glomerular filtration rate (eGFR) (**Fig 2G-2I**). These data suggest that plasma FABP4 and FABP5 were elevated in the PAH patients.

***Fabp4/5* deletion protected *Egln1* deficiency-induced PH in mice**

To gain insight into the role of FABP4/5 in the development of PAH, we obtained a mouse model with *Fabp4* and *Fabp5* double knockout in the whole-body ($Fabp4/5^{-/-}$) from Gökhan S. Hotamisligil, Harvard School of Public Health[32]. We first exposed $Fabp4/5^{-/-}$ mice to chronic hypoxia for 3 weeks. We found that $Fabp4/5^{-/-}$ mice were protected from hypoxia-induced PH development (**Supplemental Fig 1**). Next, we generated $Egln1^{Tie2Cre}/Fabp4/5^{-/-}$ (TKO) mice by breeding $Fabp4/5^{-/-}$ mice with $Egln1^{Tie2Cre}$ mice (**Fig 3A**). Western blotting confirmed that FABP4/5 proteins were undetectable in lungs of TKO mice but increased in CKO mice compared to WT controls (**Fig 3B**). As CKO mice develop spontaneously progressive PH[26], we then evaluated the PH phenotype in TKO mice. Age and gender-matched TKO mice were compared with control $Egln1^{f/f}/Fabp4\text{-}5^{-/-}$ (DKO) mice at the ages of between 2~3.5 months. CKO mice showed increases in RVSP and right heart vs left heart and septum ratio (indicative of RV hypertrophy), whereas TKO mice exhibited a reduction of RVSP and RV hypertrophy compared to CKO mice (**Fig 3C and 3D**). These data indicated that FABP4/5 contribute to the pathogenesis of severe PH in *Egln1*-deficient mice.

To further determine whether FABP4/5 regulate pulmonary vascular remodeling in CKO mice, we performed Russel-Movat pentachrome staining and immunostaining of lung tissues for α-smooth muscle actin (SMA)[10,11,22]. Examination of lung pathology showed that in comparison to CKO mice, TKO mice exhibited a marked reduction of pulmonary wall thickness and distal pulmonary arterial muscularization as assessed by α-SMA immunostaining (**Fig 3E-3H**). Taken together, genetic deletion of *Fabp4/5* attenuated pulmonary vascular remodeling and PH in *Egln1*-deficient mice.

Genetic deletion of *Fabp4/5* improved right heart function in PH mice

Patients with severe PAH die of right heart hypertrophy and failure. We found that plasma level of FABP4 was increased in decompensated RV failure, whereas FABP5 level was elevated in both compensated and decompensated RV failure in human PAH patients (**Fig 2A** and **2B**). Therefore, we tested if genetic *Fabp4/5* deletion will prevent right heart failure in CKO mice. Echocardiography measurements showed that RV wall thickness and RV chamber size were decreased in TKO mice compared to CKO mice (**Fig 4A**). RV

contractility, assessed by RV fraction area change, was also improved in TKO mice compared to CKO mice (**Fig 4B**), suggesting that RV function was better in CKO mice after deletion of *Fabp4/5*. In addition, pulmonary arterial function, determined by PA AT/ET ratio, was improved after *Fabp4/5* deletion (**Fig 4C**). We did not observe any significant alterations in heart rate, cardiac output, left ventricular fractional shortening among these mice (**Fig 4D-4F**). These data demonstrated that FABP4/5 contributed to right heart dysfunction in PH induced by *Egln1* deficiency.

Fabp4/5 deletion normalized the altered expression of PH-causing and glycolytic genes

To delineate the molecular mechanisms mediated by FABP4/5 in PH development, we performed whole-transcriptome RNA sequencing analysis. Principal component analysis and the representative heatmap demonstrated that *Fabp4/5* deletion altered the overall gene expression signature in CKO lungs toward gene signature in WT lungs (**Fig 5A and 5B**). We found that 2,727 genes (adjusted $p < 0.05$) were significantly altered in CKO lungs compared with WT lungs (**Fig 5C** and **Supplemental Fig 2A**). Among these genes, there were 1,455 upregulated and 1,272 downregulated genes in CKO lungs. 63% of the dysregulated genes were normalized when *Fabp4/5* were deleted (**Fig 5C and Supplemental Fig 2B and 2C**). Next, we performed the enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Set Enrichment Analysis (GESA) analysis based on the list of genes upregulated in CKO lungs and downregulated in TKO lungs. The KEGG analysis revealed that Epithelial mesenchymal transition, Coagulation, Hypoxia, Inflammatory response, Glycolysis, and Complement pathways were positively regulated by FABP4/5 (**Fig 5D**). All these pathways have been shown to mediate PH development. The GESA analysis showed that the enrichment of Hypoxia, and glycolysis pathways were positively regulated by FABP4/5 (**Fig 5E**). We also observed that expression levels of many genes related to PH development (*Bmpr2, Cav1, Cxcl12, Pdgfb*) and glycolysis (*Eno1, Gapdh, Ldha*) were dysregulated in CKO lungs but normalized in TKO lungs. (**Fig 5F**). qRT-PCR and western blotting were performed to confirm that mRNAs and proteins of PH and glycolysis-associated genes were altered in CKO lungs but normalized in TKO lungs (**Fig 5G and 5H**). Taken together, these data demonstrate that genetic deletion of *Fabp4/5* altered PH-associated gene expression in *Egln1*-deficient mice.

scRNA-seq and spatial transcriptomics analyses of gene expression regulated by FABP4/5

To gain an understanding of cellular and molecular mechanisms of FABP4-5 upregulation as a contributing factor to pathogenesis of severe PH, we performed whole lung scRNA-seq analysis of WT, CKO, TKO at the age of between 2 to 3.5 months. Our data showed that ECs were one of the major cell types with significant changes in CKO mice compared to WT mice[28] (**Fig 6A** and **6B**, and **Supplemental Fig 3A-3C**). Recent studies employing scRNA-seq analysis demonstrated that lung ECs contained arterial (expressing markers *Cxcl12, Sparcl1, Edn1, Hey1*)[33], venous (*Prss23*, *Vwf*)[33], general capillary ECs (*Gpihbp1*, *Plvap1*), and aerocytes (*Car4, Ednrb*)[33,34]. Using similar markers to define EC subpopulations, our scRNA-seq data showed remarkable decreases in both general capillary EC and aerocyte number and proportion, but an increase in arterial EC number and proportion in CKO mice compared to WT mice (**Supplemental Fig 4A-4D**). These data indicate the loss of capillary ECs and enrichment of arterial ECs in PH. Importantly, the number and proportion of arterial ECs were dramatically reduced in TKO mice compared to CKO mice (**Supplemental Fig 4A-4D**).

To determine the molecular mechanisms regulated by FABP4/5 in pulmonary ECs, we performed a focused transcriptomic analysis of ECs from the scRNA-seq data. We found that 58.6% of the upregulated genes and 67.5% of the downregulated genes in the CKO mice were normalized by TKO ECs. **(Fig 6C**). Similar to the bulk RNA-seq analysis, pathway enrichment analysis on the ECs transcriptomes showed that Hypoxia, Epithelial mesenchymal transition, Glycolysis pathways were enriched in the upregulated DEGs in the CKO ECs and normalized in TKO ECs (**Fig 6D**). Expression of many ECs genes related to glycolysis

(including *Eno1*, *Pgk1*, *Tpi1*, *Pkm*, *Ldha*, *Pfkl*) were upregulated in pulmonary ECs of CKO mice, but the expression of these genes were normalized in TKO mice (**Fig 6E**). scRNA-seq data showed that the alteration of glycolysis score was increased in ECs but also detectable in other pulmonary cell types such as macrophages, pericytes and T cells (**Fig 6F and Supplemental Fig 5**). Taken together, our data suggest that upregulation of glycolysis-associated gene expression by FABP4/5 contributes to pathogenesis of severe PH in in *Egln1*-deficient mice.

FABP4/5 induced ECs dysfunction in PH

To understand the direct impact of FABP4/5 on EC glycolysis *in vitro*, we overexpressed FABP4/5 in hPAECs using lentivirus-mediated gene overexpression (**Fig 7A**) and performed glycolytic function analysis on Seahorse XF equipment. FABP4/5 overexpression stimulated glycolysis and increased cell proliferation in hPAECs (**Fig 7B and 7C**). Glycolysis has been demonstrated as an important mechanism in the development of PAH[20–23]. Pulmonary vascular ECs sustain the proliferative and anti-apoptotic phenotypes that are depended on glycolysis. To determine whether upregulation of glycolysis by FABP4/5 overexpression contributes to EC proliferation, we assessed EC proliferation (BrdU incorporation assay) in the presence of the glycolysis inhibitor, 2-DG (5 μM). Our data showed that 2-DG treatment significantly inhibited FABP4/5-induced PAEC proliferation (**Fig. 7D**). Consistent with the glycolytic status, TKO mice exhibited a reduction in EC proliferation in the lung tissue compared to CKO mice (**Fig. 7E**).

The classical function of FABP4/5 is related to fatty acid binding for intracellular delivery into intracellular compartments, including mitochondria for fatty acid oxidation (FAO)[35]. Endothelial loss of CPT1α, a rate-limited enzyme for FAO, has been shown to cause impaired vascular sprouting via blocking *de novo* nucleotide synthesis required for DNA replication[36]. Our data showed that overexpression of FABP4 or FABP5 in hPAECs increased mitochondrial respiration in the presence of palmitate (the salts and esters of palmitic acid), which was blocked by CPT1α inhibitor Etomoxir (ETO)[37], indicating that FABP4/5 promoted FAO in hPAECs (**Fig 7F and 7G**). To further determine whether FAO and FAO-derived dNTPs are required for FABP4/5 induced ECs proliferation, we treated FABP4/5 overexpressing hPAECs with ETO and 5-FU (DNA synthesis inhibitor). Our data showed that both inhibitors reduced FABP4/5-induced ECs proliferation (**Fig 7H**). Furthermore, we compared the FAO capacity between control and IPAH PAECs using Seahorse FAO assay. Our data showed that 5 of 6 lines of PAECs from IPAH exhibited upregulation of FAO compared to control PAECs (**Fig 7I and 7J**), consistent with previous publication that upregulation of FAO in lungs of PAH patients[38].

Recent studies showed that the neointima in PH is derived from PASMCs using a house dust mite mouse model[39], and our previous studies demonstrated that PAH ECs promoted PASMC proliferation via paracrine effect[27]. Thus, we co-cultured FABP4/5-overexpressing hPAECs with human PASMCs and found that FABP4/5-overexpressing ECs promoted PASMCs proliferation evident by increased BrdU incorporation measuring DNA replication **(Supplemental Fig 6**).

HIF-2α mediates glycolysis induced by FABP4/5 in pulmonary ECs

Multiple transcriptional factors such as FOXO1, FOXM1, c-MYC, KLF2, HIF-1/2α, YAP1, and Notch are involved in activation of metabolism in pulmonary ECs[40,41]. To define the molecular mechanisms responsible for FABP4/5-mediated activation of EC glycolysis, we performed the iRegulon analysis (a method to detect transcriptional factors, targets and motifs from a set of genes)[42] based on the overlapping genes upregulated (>2 fold) in CKO mice and normalized in TKO mice. We found that both HIF-2α and HIF-1α were enriched based on published literature and iRegulon analysis (**Fig 8A).** Western Blotting analysis confirmed that HIF-2α protein expression was upregulated in CKO lungs and normalized in TKO lungs (**Fig 8B**). HIF-1α was not considered for further investigations because our previous study showed

that HIF-1α deletion in CKO mice did not protect from severe PH[26]; and therefore, it is unlikely that HIF-1α is involved in FABP4/5-mediated PH pathogenesis. To reduce the risk of excluding potential factors in addition to HIF-2α, we also validated p53 and c-Myc expression. Our data showed that neither p53 nor c-Myc was upregulated in CKO lungs and normalized in TKO lungs (**Fig 8B**). Based on RNA-seq data from WT, CKO mice, and *Egln1*^{Tie2Cre}/*Hif2a*^{Tie2Cre} (EH2) double knockout mice[26], glycolysis-related genes (including *Eno1*, *Ldha*, *Pkm2*) were highly induced in the CKO lungs and normalized in *EH2* lungs (**Fig. 8C**), suggesting that HIF-2α is a key factor which regulates glycolysis downstream of FABP4/5 in CKO mice. To determine whether HIF-2α is involved in FABP4/5-induced cell proliferation, we knocked down HIF-2α in FABP4-5-overexpressing PAECs using HIF-2α specific siRNA. HIF-2α inhibition reduced FABP4/5-induced EC proliferation (**Fig 8D**).

Since FAO increased mitochondrial ROS levels which promotes stabilization of HIF-α through prolyl hydroxylases inhibition[43,44], we hypothesize that FABP4/5 upregulate HIF-2α via FAO/mitochondrial ROS generation in PAECs. To test this hypothesis, we first performed staining against oxidative/nitrative stress markers[45] such as nitrotyrosine[46] in the lungs from WT, CKO, and TKO mice and found that *Fabp4/5* deletion reduced nitrotyrosine expression (**Fig 8E**). Next, we measured mitochondrial ROS levels using MitoSOX Red Mitochondrial Superoxide Indicator[47] in FABP4/5-overexpressing hPAECs and found that FABP4/5 overexpression upregulated the mitochondrial ROS levels (**Fig 8F**). Taken together, these studies demonstrate that ROS/HIF-2α signaling is required for FABP4/5 to induce ECs dysfunction in PH.

Discussion

In this study, we demonstrated that FABP4 and FABP5 were markedly upregulated in pulmonary ECs of mice with severe PH, lung tissue of IPAH patients, human PAECs, and in lung tissues of rodent PH models, including *Egln1*^{Tie2Cre} mice, MCT-treated rats, and SuHx-treated rats. Plasma levels of FABP4 and 5 were also elevated in patients with PAH from two cohorts and correlated with hemodynamics and biochemical parameters in PAH patients. Genetic deletion of *Fabp4* and *Fabp5* protected from severe PH and right heart failure in *Egln1*^{Tie2Cre} mice, supporting the critical role of FABP4/5 in pathogenesis of PH. We also observed that FABP4/5 promoted glycolysis in ECs both *in vivo* and *in vitro*, and regulated ECs proliferation in HIF-2α-dependent manner. Deletion of *Hif2a* in ECs blocked glycolysis and ECs proliferation. Furthermore, we demonstrated FABP4/5 induced FAO and ROS generation in pulmonary ECs, which in turn contributes to HIF-2α activation (**Fig 8G**).

We provide evidence that FABP4/5 were upregulated in the lung ECs and tissue in PH rodents and IPAH patients. Overexpression of FABP4/5 was also identified in the systemic circulation of PAH patients. Although an increase in FABP4 in circulation in PAH patients was previously identified[18], and serum FABP4 concentrations were significantly higher in patients with HF than those in non-HF subjects[48], our study is the first report to link circulating levels of FABP4 and 5 to hemodynamics and RV failure in patients with PAH. We also determined the causative role of FABP4/5 in PH using genetic mouse models and identified the underlying molecular mechanisms whereby FABP4/5 regulate lung remodeling in PH. Circulating FABPs are seen as markers of cell injury or death. During PAH development, endothelial injury is accompanied by tissue hypoxia and inflammation. Both HIF-1α and HIF-2α expression is upregulated in PAH ECs.[25,49,50] Previous studies also showed that FABP4 was induced during hypoxia treatment in hepatocytes in HIF-1α-dependent manner during liver ischemia/reperfusion[51]. Our data showed marked upregulation of FABP4/5 in CKO lungs with severe PH and the normalization of PH parameters in EH2 mice *in vivo* (**Supplemental Fig 7**), suggesting that FABP4/5 are novel downstream targets of HIF-2α in PAECs. FABP4 is regulated by VEGF and contributes to EC proliferation, migration, and angiogenesis[52,53]. Hwangbo et al showed that endothelial Apelin/Apelin receptor signaling through FoxO1 regulated FABP4 but not FABP5 during the tissue fatty acid uptake in skeletal muscles[16]. Further studies are warranted to determine the upstream signaling regulating FABP4/5 in PH.

Previous studies showed that both FABP4 and FABP5 played important roles in the pathogenesis of chronic metabolic diseases, including obesity and diabetes[35]. The *Fabp4-Fabp5* double knockout mice (*Fabp4/5*$^{-/-}$) were protected from high fat diet-induced weight gain and insulin sensitivity, as well as hepatic steatosis[54]. Accumulating evidence shows that PAH is a systemic metabolic disease[4–6]. The prevalence of obesity in PAH doubled between 2006 and 2016[55], and the insulin resistance is likely a risk factor for PAH[6]. In the present study, we provide the first genetic evidence that *Fabp4/5* deletion attenuated PH development, including inhibition of RVSP and pulmonary vascular remodeling, as well as prevented right heart failure induced by *Egln1* deficiency. Our data are consistent with previous studies showing that FABP5/7 inhibition using SBFI-26 aggravated pulmonary arterial fibrosis and PH secondary to left heart disease induced by ligation of left main artery in mice[56]. Taken together, our data indicate that FABP4/5 contribute to the pathogenesis of PAH and right heart failure.

FABP4 and 5 are expressed in multiple PH-related cell types, including ECs and macrophages in human and mouse lungs based on LungMAP datasets (**Supplemental Fig 8**). Our data showed that *Fabp4 and 5* were mainly upregulated in ECs in CKO mice. We then demonstrated that FABP4/5 promoted endothelial dysfunction, including augmentation of glycolysis and proliferation, and altered paracrine effects in ECs *in vitro* and *in vivo*. In general, FABPs function as lipid chaperones to facilitate fatty acid trafficking. Increased intracellular influx of fatty acids facilitated by FABP4/5 upregulates the fatty acid oxidation in ECs, which likely provides the sources of dNTPs for DNA synthesis and ECs hyperproliferation in PH condition. This finding is consistent with previous study showing that heterozygous deletion of *Cpt1a*, the rate limited enzyme for fatty acid oxidation, protected from hypoxia-induced PH in mice[38]. Moreover, our studies identified a novel role of FABP4/5 in regulation of endothelial glycolysis through activation of ROS/HIF-2 signaling in PH. Previous studies showed that free fatty acids upregulated protein level of HIF-2α in clear cell renal cancer cells[57]. It is likely that FAO derived from FABP4/5 binding of fatty acid upregulates HIF-2α expression. Moreover, our study also found that overexpression of FABP4/5 reduced endothelial PPARγ protein levels whereas knockdown of FABP4/5 upregulated endothelial PPARγ protein but not mRNA expression (**Supplemental Fig 9**), which might further lead to EC dysfunction and PH development.

Both FABP4 and FABP5 are expressed in alveolar macrophages[58]. However, previous studies showed that FABP4 and FABP5 regulated different signaling pathways in macrophages compared to other cell types. For example, FABP4 was highly expressed in alveolar macrophages in COVID-19 patients, which could contribute to obesity-associated severity of COVID-19[59]. Another study demonstrated that loss of FABP5 promoted pro-inflammatory reprogramming of macrophages, increasing the expression of pro-inflammatory cytokines and transcription factors[60]. One might speculate that macrophage FABP4 and FABP5 might play a role in the pathogenesis of PH. To answer this question, we analyzed the macrophage populations in *Fabp4/5*-deficient mice. Our data showed that *Fabp4/5* deletion did not change the cellular distribution in macrophage subsets (**Supplemental Fig 10**), suggesting that macrophage FABP4/5 might play an opposite role in PH development. Future studies are needed to employ macrophage-specific deletion of *Fabp4/5* in mice to determine the role of macrophage FABP4 and FABP5 in PH development.

FABPs are traditionally considered cytoplasmic proteins. FABPs were identified in the circulation under obesity-associated diseases, cancer and in PAH[35]. A previous study showed that circulating FABP4 released from adipose tissue directly enhances tumor stemness and aggressiveness through activation of the IL-6/STAT3/ALDH1 pathway[61]. Exogenous FABP4 can induce human coronary artery smooth muscle cells proliferation and migration, as well as suppress eNOS expression in human umbilical vein ECs and cardiomyocyte contraction in vitro. However, the role of exogenous FABP5 is largely unknown. Future studies are needed to investigate whether blocking exogenous FABP4/5 will inhibit PH development in preclinical models.

There are several limitations in the current studies. Firstly, it remains unknown whether the upregulation of plasma FABP4 and 5 are secreted from lung ECs, other lung cell types or adipose tissue in PAH patients. Secondly, although our data point out that *Fabp4/5* are primarily upregulated in the lung ECs in PH, our

study employed a global loss of *Fabp4/5* (deletion of *Fabp4/5* in all cells of the body). Therefore, the cell specific roles of FABP4 or FABP5 in PAH remain to be studied.

In summary, our studies showed that plasma levels of FABP4 and 5 were elevated in patients with PAH and positively correlated with hemodynamics, as well as inversely correlated with RV function. Our data support a pathogenic role of FABP4/5 in mediating EC glycolysis, FAO, and pulmonary arterial remodeling in PH. Additionally, our data suggest that targeting FABP4/5 or FAO signaling may represent a novel therapeutic approach to inhibit pulmonary vascular remodeling in human PAH.

Disclosure: None.

Sources of Funding: This work was supported in part by NIH grant R00HL138278, R01HL158596, R01HL62794, R01HL169509, R01HL170096, AHA Career Development Award 20CDA35310084, The Cardiovascular Research and Education Foundation, Arizona Biomedical Research Centre funding (RFGA2022-01-06), and University of Arizona institution funding to Z.D.

Author contributions: Z.D. conceived the experiments and interpreted the data. B.L., D.Y., S.L., Y. C., K.R., X.X., A.T., Y.C., Z.H., M.K., R.R, H.G., and Z.D. designed, performed experiments, and analyzed the data. Z.D. wrote the manuscript. K.S.K, V.P., C.C.G, V. V.K. revised the manuscript. S.B., O.,V., and M.B.F. provided key experimental materials.

Acknowledgements: We thank Dr. Gökhan S. Hotamisligil (Harvard School of Public Health) for providing the *Fabp4-5*$^{-/-}$ mice. The authors thank the Pulmonary Hypertension Breakthrough Initiative for providing the Data/tissue samples. Funding for the Pulmonary Hypertension Breakthrough Initiative is provided under an NHLBI R24 grant (R24HL123767) and by the Cardiovascular Medical Research and Education Fund.

Reference

1. Stacher E, Graham BB, Hunt JM, Gandjeva A, Groshong SD, McLaughlin VV, Jessup M, Grizzle WE, Aldred MA, Cool CD, Tuder RM. Modern age pathology of pulmonary arterial hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2012;186:261–272.

2. Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, Olschewski AJ, Pullamsetti SS, Schermuly RT, Stenmark KR, Rabinovitch M. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *The European respiratory journal*. 2019;53:1801887.

3. Thenappan T, Ormiston ML, Ryan JJ, Archer SL. Pulmonary arterial hypertension: Pathogenesis and clinical management. *BMJ (Online)*. 2018;360:j5492.

4. Paulin R, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. *Circulation Research*. 2014;115:148–164.

5. Nickel NP, Yuan K, Dorfmuller P, Provencher S, Lai YC, Bonnet S, Austin ED, Koch CD, Morris A, Perros F, Montani D, Zamanian RT, De Jesus Perez VA. Beyond the lungs: Systemic manifestations of pulmonary arterial hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2020;201:148–157.

6. Zamanian RT, Hansmann G, Snook S, Lilienfeld D, Rappaport KM, Reaven GM, Rabinovitch M, Doyle RL. Insulin resistance in pulmonary arterial hypertension. *European Respiratory Journal*. 2009;33:318–324.

7. Brittain EL, Talati M, Fessel JP, Zhu H, Penner N, Calcutt MW, West JD, Funke M, Lewis GD, Gerszten RE, Hamid R, Pugh ME, Austin ED, Newman JH, Hemnes AR. Fatty acid metabolic defects and right ventricular lipotoxicity in human pulmonary arterial hypertension. *Circulation*. 2016;133:1936–1944.

8. Talati M, Hemnes A. Fatty acid metabolism in pulmonary arterial hypertension: Role in right ventricular dysfunction and hypertrophy. *Pulmonary Circulation*. 2015;5:269–278.

9. Hemnes AR, Brittain EL, Trammell AW, Fessel JP, Austin ED, Penner N, Maynard KB, Gleaves L, Talati M, Absi T, Disalvo T, West J. Evidence for right ventricular lipotoxicity in heritable pulmonary arterial hypertension. *American journal of respiratory and critical care medicine*. 2014;189:325–34.

10. Hemnes AR, Fessel JP, Chen X, Zhu S, Fortune NL, Jetter C, Freeman M, Newman JH, West JD, Talati MH. BMPR2 dysfunction impairs insulin signaling and glucose homeostasis in cardiomyocytes. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 2020;318:L429–L441.

11. Zhao Y, Peng J, Lu C, Hsin M, Mura M, Wu L, Chu L, Zamel R, Machuca T, Waddell T, Liu M, Keshavjee S, Granton J, De Perrot M. Metabolomic heterogeneity of pulmonary arterial hypertension. *PLoS ONE*. 2014;9:e88727.

12. Liu B, Dai Z. Fatty Acid Metabolism in Endothelial Cell. *Genes*. 2022;13:2301.

13. Li X. VEGF-B: A thing of beauty. *Cell Research*. 2010;20:741–744.

14. Hagberg CE, Falkevall A, Wang X, Larsson E, Huusko J, Nilsson I, Van Meeteren LA, Samen E, Lu L, Vanwildemeersch M, Klar J, Genove G, Pietras K, Stone-Elander S, Claesson-Welsh L, Ylä-Herttuala S, Lindahl P, Eriksson U. Vascular endothelial growth factor B controls endothelial fatty acid uptake. *Nature*. 2010;464:917–921.

15. Storch J, Corsico B. The emerging functions and mechanisms of mammalian fatty acid-binding proteins. *Annual Review of Nutrition*. 2008;28:73–95.

16. Hwangbo C, Wu J, Papangeli I, Adachi T, Sharma B, Park S, Zhao L, Ju H, Go GW, Cui G, Inayathullah M, Job JK, Rajadas J, Kwei SL, Li MO, Morrison AR, Quertermous T, Mani A, Red-Horse K, Chun HJ. Endothelial APLNR regulates tissue fatty acid uptake and is essential for apelin's glucose-lowering effects. *Science Translational Medicine*. 2017;9:eaad4000.

17. Fuseya T, Furuhashi M, Matsumoto M, Watanabe Y, Hoshina K, Mita T, Ishimura S, Tanaka M, Miura T. Ectopic fatty acid-binding protein 4 expression in the vascular endothelium is involved in neointima formation after vascular injury. *Journal of the American Heart Association*. 2017;6:1–15.

18. Papathanasiou AE, Spyropoulos F, Michael Z, Joung KE, Briana DD, Malamitsi-Puchner A, Mantzoros CS, Christou H. Adipokines and metabolic regulators in human and experimental pulmonary arterial hypertension. *International Journal of Molecular Sciences*. 2021;22:1–16.

19. Condon D, Agarwal S, Chakraborty A, de Jesus Perez VA. The cancer hypothesis of pulmonary arterial hypertension: The next ten years. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 2020;318:L1138–L1139.

20. Caruso P, Dunmore BJ, Schlosser K, Schoors S, Dos Santos C, Perez-Iratxeta C, Lavoie JR, Zhang H, Long L, Flockton AR, Frid MG, Upton PD, D'Alessandro A, Hadinnapola C, Kiskin FN, Taha M, Hurst LA, Ormiston ML, Hata A, Stenmark KR, Carmeliet P, Stewart DJ, Morrell NW. Identification of MicroRNA-124 as a Major Regulator of Enhanced Endothelial Cell Glycolysis in Pulmonary Arterial Hypertension via PTBP1 (Polypyrimidine Tract Binding Protein) and Pyruvate Kinase M2. *Circulation*. 2017;136:2451–2467.

21. Fessel JP, Hamid R, Wittmann BM, Robinson LJ, Blackwell T, Tada Y, Tanabe N, Tatsumi K, Hemnes AR, West JD. Metabolomic analysis of bone morphogenetic protein receptor type 2 mutations in human pulmonary endothelium reveals widespread metabolic reprogramming. *Pulmonary circulation*. 2012;2:201–13.

22. Cao Y, Zhang X, Wang L, Yang Q, Ma Q, Xu J, Wang J, Kovacs L, Ayon RJ, Liu Z, Zhang M, Zhou Y, Zeng X, Xu Y, Wang Y, Fulton DJ, Weintraub NL, Lucas R, Dong Z, Yuan JXJ, Sullivan JC, Meadows L, Barman SA, Wu C, Quan J, Hong M, Su Y, Huo Y. PFKFB3-mediated endothelial glycolysis promotes pulmonary hypertension. *Proceedings of the National Academy of Sciences of the United States of America*. 2019;116:13394–13403.

23. Yu Q, Tai YY, Tang Y, Zhao J, Negi V, Culley MK, Pilli J, Sun W, Brugger K, Mayr J, Saggar R, Saggar R, Wallace WD, Ross DJ, Waxman AB, Wendell SG, Mullett SJ, Sembrat J, Rojas M, Khan OF, Dahlman JE, Sugahara M, Kagiyama N, Satoh T, Zhang M, Feng N, Gorcsan J, Vargas SO, Haley KJ, Kumar R, Graham BB, Langer R, Anderson DG, Wang B, Shiva S, Bertero T, Chan SY. BOLA (BolA Family Member 3) Deficiency Controls Endothelial Metabolism and Glycine Homeostasis in Pulmonary Hypertension. *Circulation*. 2019;139:2238–2255.

24. Dai Z, Li M, Wharton J, Zhu MM, Zhao Y-Y. Prolyl-4 Hydroxylase 2 (PHD2) Deficiency in Endothelial Cells and Hematopoietic Cells Induces Obliterative Vascular Remodeling and Severe Pulmonary Arterial Hypertension in Mice and Humans Through Hypoxia-Inducible Factor-2α. *Circulation*. 2016;133:2447–2458.

25. Dai Z, Zhu MM, Peng Y, Machireddy N, Evans CE, Machado R, Zhang X, Zhao YY. Therapeutic targeting of vascular remodeling and right heart failure in pulmonary arterial hypertension with a HIF-2a inhibitor. *American Journal of Respiratory and Critical Care Medicine*. 2018;198:1423–1434.

26. Dai Z, Li M, Wharton J, Zhu MM, Zhao YY. Prolyl-4 Hydroxylase 2 (PHD2) deficiency in endothelial cells and hematopoietic cells induces obliterative vascular remodeling and severe pulmonary arterial hypertension in mice and humans through hypoxia-inducible factor-2α. *Circulation*. 2016;133:2447–2458.

27. Dai Z, Zhu MM, Peng Y, Jin H, Machireddy N, Qian Z, Zhang X, Zhao YY. Endothelial and smooth muscle cell interaction via FoxM1 signaling mediates vascular remodeling and pulmonary hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2018;198:788–802.

28. Dai Z, Dai J, Zhao Y-Y. Single-cell Transcriptomes Identify Abnormal Endothelial Subpopulation in Pulmonary Arterial Hypertension. *Circulation*. 2019;140:A11227–A11227.

29. Stenmark KR, Meyrick B, Galie N, Mooi WJ, McMurtry IF. Animal models of pulmonary arterial hypertension: The hope for etiological discovery and pharmacological cure. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 2009;297:L1013-32.

30. Taraseviciene‐Stewart L, Kasahara Y, Alger L, Hirth P, Mahon GM, Waltenberger J, Voelkel NF, Tuder RM. Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. *The FASEB Journal*. 2001;15:427–438.

31. Boucherat O, Yokokawa T, Krishna V, Kalyana-Sundaram S, Martineau S, Breuils-Bonnet S, Azhar N, Bonilla F, Gutstein D, Potus F, Lawrie A, Jeyaseelan J, Provencher S, Bonnet S. Identification of LTBP-2 as a plasma biomarker for right ventricular dysfunction in human pulmonary arterial hypertension. *Nature Cardiovascular Research*. 2022;1:748–760.

32. Furuhashi M, Tuncman G, Görgün CZ, Makowski L, Atsumi G, Vaillancourt E, Kono K, Babaev VR, Fazio S, Linton MF, Sulsky R, Robl JA, Parker RA, Hotamisligil GS. Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature*. 2007;447:959–965.

33. Kalucka J, de Rooij LPMH, Goveia J, Rohlenova K, Dumas SJ, Meta E, Conchinha NV, Taverna F, Teuwen LA, Veys K, García-Caballero M, Khan S, Geldhof V, Sokol L, Chen R, Treps L, Borri M, de Zeeuw P, Dubois C, Karakach TK, Falkenberg KD, Parys M, Yin X, Vinckier S, Du Y, Fenton RA, Schoonjans L, Dewerchin M, Eelen G, Thienpont B, Lin L, Bolund L, Li X, Luo Y, Carmeliet P. Single-Cell Transcriptome Atlas of Murine Endothelial Cells. *Cell*. 2020;180:764-779.e20.

34. Gillich A, Zhang F, Farmer CG, Travaglini KJ, Tan SY, Gu M, Zhou B, Feinstein JA, Krasnow MA, Metzger RJ. Capillary cell-type specialization in the alveolus. *Nature*. 2020;586:785–789.

35. Hotamisligil GS, Bernlohr DA. Metabolic functions of FABPs - Mechanisms and therapeutic implications. *Nature Reviews Endocrinology*. 2015;11:592–605.

36. Schoors S, Bruning U, Missiaen R, Queiroz KCS, Borgers G, Elia I, Zecchin A, Cantelmo AR, Christen S, Goveia J, Heggermont W, Goddé L, Vinckier S, Van Veldhoven PP, Eelen G, Schoonjans L, Gerhardt H, Dewerchin M, Baes M, De Bock K, Ghesquière B, Lunt SY, Fendt SM, Carmeliet P. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature*. 2015;520:192–197.

37. Divakaruni AS, Hsieh WY, Minarrieta L, Duong TN, Kim KKO, Desousa BR, Andreyev AY, Bowman CE, Caradonna K, Dranka BP, Ferrick DA, Liesa M, Stiles L, Rogers GW, Braas D, Ciaraldi TP, Wolfgang MJ, Sparwasser T, Berod L, Bensinger SJ, Murphy AN. Etomoxir Inhibits Macrophage Polarization by Disrupting CoA Homeostasis. *Cell Metabolism*. 2018;28:490-503.e7.

38. Lee MH, Sanders L, Kumar R, Hernandez-Saavedra D, Yun X, Ford JA, Perez MJ, Mickael C, Gandjeva A, Koyanagi DE, Harral JW, Irwin DC, Kassa B, Eckel RH, Shimoda LA, Graham BB, Tuder RM. Contribution of fatty acid oxidation to the pathogenesis of pulmonary hypertension. *American journal of physiology Lung cellular and molecular physiology*. 2022;323:L355–L371.

39. Steffes LC, Froistad AA, Andruska A, Boehm M, McGlynn M, Zhang F, Zhang W, Hou D, Tian X, Miquerol L, Nadeau K, Metzger RJ, Spiekerkoetter E, Kumar ME. A Notch3-marked subpopulation of vascular smooth muscle cells is the cell of origin for occlusive pulmonary vascular lesions. *Circulation*. 2020;1545–1561.

40. Evans CE, Cober ND, Dai Z, Stewart DJ, Zhao YY. Endothelial cells in the pathogenesis of pulmonary arterial hypertension. *European Respiratory Journal*. 2021;58:2003957.

41. Li X, Sun X, Carmeliet P. Hallmarks of Endothelial Cell Metabolism in Health and Disease. *Cell Metabolism*. 2019;30:414–433.

42. Janky R, Verfaillie A, Imrichová H, van de Sande B, Standaert L, Christiaens V, Hulselmans G, Herten K, Naval Sanchez M, Potier D, Svetlichnyy D, Kalender Atak Z, Fiers M, Marine JC, Aerts S. iRegulon: From a Gene List to a Gene Regulatory Network Using Large Motif and Track Collections. *PLoS Computational Biology*. 2014;10:e1003731.

43. Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, Schumacker PT. Reactive oxygen species generated at mitochondrial Complex III stabilize hypoxia-inducible factor-1α during hypoxia: A mechanism of O2sensing. *Journal of Biological Chemistry*. 2000;275:25130–25138.

44. Gerald D, Berra E, Frapart YM, Chan DA, Giaccia AJ, Mansuy D, Pouysségur J, Yaniv M, Mechta-Grigoriou F. JunD reduces tumor angiogenesis by protecting cells from oxidative stress. *Cell*. 2004;118:781–794.

45. Bowers R, Cool C, Murphy RC, Tuder RM, Hopken MW, Flores SC, Voelkel NF. Oxidative Stress in Severe Pulmonary Hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2004;169:764–769.

46. Dai Z, Zhu MM, Zhao Y-Y. PHD2 Deficiency Induces Obliterative Vascular Remodeling and Severe PAH through Nitrative Stress and Caveolin-1 Downregulation. *The FASEB Journal*. 2017;31:1016.8-1016.8.

47. Sun X, Kumar S, Sharma S, Aggarwal S, Lu Q, Gross C, Rafikova O, Lee SG, Dasarathy S, Hou Y, Meadows ML, Han W, Su Y, Fineman JR, Black SM. Endothelin-1 induces a glycolytic switch in

pulmonary arterial endothelial cells via the mitochondrial translocation of endothelial nitric oxide synthase. *American Journal of Respiratory Cell and Molecular Biology*. 2014;50:1084–1095.

48. Liu M, Zhou M, Bao Y, Xu Z, Li H, Zhang H, Zhu W, Zhang J, Xu A, Wei M, Jia W. Circulating adipocyte fatty acid-binding protein levels are independently associated with heart failure. *Clinical Science*. 2013;124:115–122.

49. Tang H, Babicheva A, McDermott KM, Gu Y, Ayon RJ, Song S, Wang Z, Gupta A, Zhou T, Sun X, Dash S, Wang Z, Balistrieri A, Zheng Q, Cordery AG, Desai AA, Rischard F, Khalpey Z, Wang J, Black SM, Garcia JGN, Makino A, Yuan JXJ. Endothelial HIF-2α contributes to severe pulmonary hypertension due to endothelial-to-mesenchymal transition. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 2018;314:L256–L275.

50. Fijalkowska I, Xu W, Comhair SAA, Janocha AJ, Mavrakis LA, Krishnamachary B, Zhen L, Mao T, Richter A, Erzurum SC, Tuder RM. Hypoxia inducible-factor1α regulates the metabolic shift of pulmonary hypertensive endothelial cells. *American Journal of Pathology*. 2010;176:1130–1138.

51. Hu B, Guo Y, Garbacz WG, Jiang M, Xu M, Huang H, Tsung A, Billiar TR, Ramakrishnan SK, Shah YM, Lam KSL, Huang M, Xie W. Fatty acid binding protein-4 (FABP4) is a hypoxia inducible gene that sensitizes mice to liver ischemia/reperfusion injury. *Journal of Hepatology*. 2015;63:855–862.

52. Elmasri H, Karaaslan C, Teper Y, Ghelfi E, Weng MQ, Ince TA, Kozakewich H, Bischoff J, Cataltepe S. Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells. *FASEB Journal*. 2009;23:3865–3873.

53. Elmasri H, Ghelfi E, Yu CW, Traphagen S, Cernadas M, Cao H, Shi GP, Plutzky J, Sahin M, Hotamisligil G, Cataltepe S. Endothelial cell-fatty acid binding protein 4 promotes angiogenesis: Role of stem cell factor/c-kit pathway. *Angiogenesis*. 2012;15:457–468.

54. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA, Hotamisligil GS. Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metabolism*. 2005;1:107–119.

55. Weatherald J, Huertas A, Boucly A, Guignabert C, Taniguchi Y, Adir Y, Jevnikar M, Savale L, Jaïs X, Peng M, Simonneau G, Montani D, Humbert M, Sitbon O. Association Between BMI and Obesity With Survival in Pulmonary Arterial Hypertension. *Chest*. 2018;154:872–881.

56. Lei Q, Yu Z, Li H, Cheng J, Wang Y. Fatty acid-binding protein 5 aggravates pulmonary artery fibrosis in pulmonary hypertension secondary to left heart disease via activating wnt/β-catenin pathway. *Journal of Advanced Research*. 2022;40:197–206.

57. Tan SK, Mahmud I, Fontanesi F, Puchowicz M, Neumann CKA, Griswold AJ, Patel R, Dispagna M, Ahmed HH, Gonzalgo ML, Brown JM, Garrett TJ, Welford SM. Obesity-Dependent Adipokine Chemerin Suppresses Fatty Acid Oxidation to Confer Ferroptosis Resistance. *Cancer Discovery*. 2021;candisc.1453.2020.

58. Li B, Hao J, Zeng J, Sauter ER. SnapShot: FABP Functions. *Cell*. 2020;182:1066-1066.e1.

59. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, Cheng L, Li J, Wang X, Wang F, Liu L, Amit I, Zhang S, Zhang Z. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med*. 2020;26:842–844.

60. El Kharbili M, Aviszus K, Sasse SK, Zhao X, Serban KA, Majka SM, Gerber AN, Gally F. Macrophage programming is regulated by a cooperative interaction between fatty acid binding protein 5 and peroxisome proliferator-activated receptor γ. *The FASEB Journal* [Internet]. 2022 [cited 2023 Jul 29];36. Available from: https://onlinelibrary.wiley.com/doi/10.1096/fj.202200128R

61. Hao J, Zhang Y, Yan X, Yan F, Sun Y, Zeng J, Waigel S, Yin Y, Fraig MM, Egilmez NK, Suttles J, Kong M, Liu S, Cleary MP, Sauter E, Li B. Circulating Adipose Fatty Acid Binding Protein Is a New Link Underlying Obesity-Associated Breast/Mammary Tumor Development. *Cell Metabolism*. 2018;28:689-705.e5.

Figures and legends

Figure 1. Upregulation of FABP4 and 5 in the PAH patients and PH rodents.

(A) Single-cell RNA sequencing analysis showed that FABP4 and 5 were upregulated in the lung ECs of *Egln1*^{Tie2Cre} (CKO) mice compared to WT mice. Wilcoxon Rank Sum Test, **** adjusted P < 0.0001; ns=no significance. **(B)** Representative images of immunostaining against FABP4 and 5 showed that FABP4 and 5 were upregulated in the lung ECs of *Egln1*^{Tie2Cre} mice compared to WT mice. Lung tissues were co-stained with anti-FABP4 or anti-FABP5 and anti-CD31 (marker for ECs). Nuclei were counterstained with DAPI. FABP4 and 5 expression was upregulated in pulmonary vascular ECs in CKO mice. V, vessel. More than 3 lungs from each group of mice were checked. (**C**) Representative Western Blotting demonstrating upregulation of FABP4 and 5 protein expression in the lung lysate isolated from CKO lungs compared with WT mice. (**D**) Representative Western Blotting showing an increase of FABP4 and 5 protein expression in the lung lysate isolated from Sugen5416/hypoxia (SuHx) -exposed lungs or monocrotaline (MCT) -exposed lungs compared with basal rats. (**E, F**) QRT-PCR analysis and Western Blotting demonstrating an upregulation of FABP4 and 5 in the isolated PAECs from IPAH patients compared with healthy donor lungs. Each sample represents PAECs from individual patients or healthy controls (n=3). t test (**C, D, E**). *P<0.05; **P<0.01; Scale bar, 50 μm.

Figure 2. Upregulation of the plasma FABP4 and 5 in patients with PAH.

(**A, B**) Plasma protein NPX levels of the FABP4 (**A**) and 5 (**B**) in donors (CTRL) and patients with PAH from Canada cohorts. Scatter dot plots show individual values (n). According to contemporary Cardiac Index (CI) measured by right heart catheterization at the time the blood sample was drawn. Patients with PAH were categorized as cRV (CI> 2.2 L/min/m^2) and dRV (CI ≤2.2 L/min/m^2). CTRL N=28, cRV N= 31, dRV N=29. (**C**) Plasma protein NPX level of FABP4 were correlated with plasma protein NPX level of FABP5 in the human subjects including both donors and PAH patients. (**D-I**) Pearson's correlation coefficient of the levels of FABP4 and 5 and hemodynamics/biomedical parameters of PAH patients with associated P value is shown in each graph. mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; SV, strove volume; eGFR, estimating glomerular filtration rate. t test (left panels of **A, B**). ANOVA followed by Turkey post hoc analysis was used for statistical analysis (right panels of **A, B**). Pearson's correlation test (**C-I**). **P<0.01; ****P<0.0001. ns=not significant.

Figure 3. FABP4 and 5 deletions protected from *Egln1*-deficiency-induced PH in mice.

(A) A diagram showing the strategy for generation of FABP4 and 5 double knockouts in *Egln1*^{Tie2Cre} mice (TKO). **(B)** Representative Western blotting demonstrating diminished FABP4 and 5 protein expression in lung lysate isolated from TKO lungs compared with CKO lungs. (**C**) Dramatic reduction in RVSP was seen in TKO mice compared with CKO mice. Bars represent the mean. (**D**) Marked inhibition of RV hypertrophy in TKO mice compared with CKO mice. (**E, F**) Representative images from Russel-Movat pentachrome staining and quantification of wall thickness of PA exhibited reduced thickness in the intima, media, and adventitia layers of tissue of TKO mice compared with CKO mice. Occlusive lesions were diminished in TKO mice compared with CKO mice. Br, bronchus; V, vessel. (**G**) Exemplary images showed a reduction in muscularization of distal pulmonary vessels in TKO mice. Lung sections were subjected to immunostaining using anti-smooth muscle α-actin (SMA). (**H**) Muscularization quantification was performed by tallying SMA-positive vessels across 40 fields (at ×20 magnification) within every lung section. The results were presented as the mean value along with the standard deviation (mean ± SD) based on a sample size of 5 to 10 mice per group. ANOVA followed by Turkey post hoc analysis was used for statistical analysis (**B, C, D, F** and **H**). *P<0.05; ***P<0.001, and ****P<0.0001.

Figure 4. **Genetical deletion of Fabp4-5 improved right heart function in PH mice**

(**A**) Representative echocardiography images revealed a reduction of RV wall thickness during diastole (RVWTD) in TKO mice compared with CKO mice. (**B**) Improved RV fraction area change (RVFAC), indicating enhancing RV contractility, in TKO mice compared with CKO mice. (**C**) An increased ratio of pulmonary artery acceleration time to ejection time (PA AT/ET) in TKO mice compared with CKO mice. (**D-F**) There is no significant change of heart rate, cardiac output and left ventricular fractional shorting. ANOVA followed by Turkey post hoc analysis was used for statistical analysis (**A, B, C**). Significance levels were denoted as **P<0.01, ***P<0.001, and ****P<0.0001.

Figure 5. FABP4-5 regulated genes related to PH and glycolysis

(A) Principal component analysis showed that FABP4-5 deletion normalized the altered gene signature by *Egln1* deficiency in mice. (**B**) A representative heatmap showed that Fabp4-5 deletion altered the overall gene signature of CKO lungs toward to WT lungs. (**C**) Venn diagrams indicated FABP4-5 controlled the expression of the dysregulated genes in CKO mice. (**D**) The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the genes upregulated in CKO lungs and normalized by FABP4-5 deletion. (E) Gene Set Enrichment Analysis (GESA) analysis showed the enrichment of Hypoxia and Glycolysis pathways based on the gene sets regulated by FABP4/5. (**F**) A panel of representative genes related to PH pathogenesis and glycolysis were normalized by FABP4-5 deletion in CKO mice. (**G**) qPCR analysis confirmed the PH causing genes and glycolysis related genes were altered in CKO lungs and normalized in the TKO mice by RNA-seq data. (**H**) Western blotting showing FABP4-5 deletion reduced the protein level of glycolytic genes in CKO mice. ANOVA followed by Turkey post hoc analysis was used for statistical analysis (**G** and **H**). *P<0.05; **P<0.01; ***P<0.001, and ****P<0.0001.

Figure 6. scRNA-seq and spatial transcriptomics analysis on FABP4-5 regulated PH development.

(A, B) ScRNA-seq analysis demonstrated that FABP4-5 deletion partially normalized the alteration of EC subpopulations in CKO mice. **(C)** Venn diagrams indicated FABP4-5 controlled the expression of the dysregulated genes in ECs. (**D**) The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the genes upregulated in CKO ECs and normalized by FABP4-5 deletion in ECs. (**E**). A heatmap analysis based on the scRNA-seq analysis on ECs population showed that the glycolytic genes were upregulated in CKO lungs and normalized in TKO ECs. (**F**) The glycolytic score calculation showed that glycolysis is controlled by FABP4-5 in ECs.

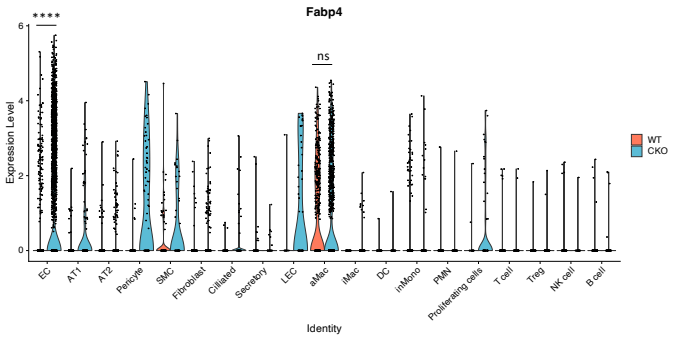
Figure 7. Overexpression of FABP4-5 induced ECs dysfunction

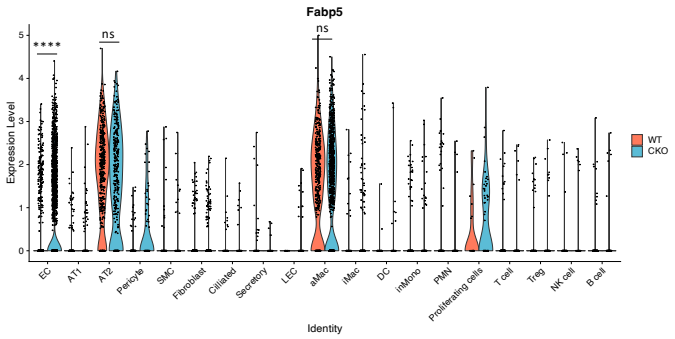
(**A**) Overexpression of FABP4 and 5 fused with Flag tag in hPAECs using lentiviruses mediated gene overexpression. (**B**, **C**) FABP4-5 overexpression promoted ECs glycolysis and proliferation assessed by BrdU incorporation assay. (**D**) Glycolytic inhibitor 2-DG treatment inhibited FABP4-5 induced PAEC proliferation. (**E**) FABP4-5 deletion in vivo reduced lung PAECs proliferation in TKO mice compared with CKO mice. (**F** and **G**) Overexpression of FABP4-5 promoted fatty acid oxidation (FAO) in hPAECs in the presence of palmitic acid. The double arrows label the OCR generation contributed by FAO. (**H**) Both CPT1α inhibitor Etomoxir (ETO, 20 μM) and DNA synthesis inhibitor (5-FU, 20 μM) blocked FABP4-5 induced ECs proliferation. (**I** and **J**) A representative seahorse data showing upregulation of FAO in PAECs

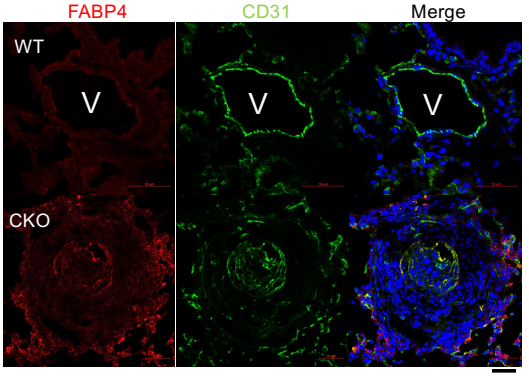
isolated from IPAH patients compared to control donors. Six lines of control or IPAH patients PAECs were tested. (**C, D, H**) For BrdU assay, each dot represents a biological replicate. The experiments were performed at least three times. Student t test (**D**). ANOVA followed by Turkey post hoc analysis was used for statistical analysis (**B, C, E, F, G, H, J**). Significance levels were denoted as *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

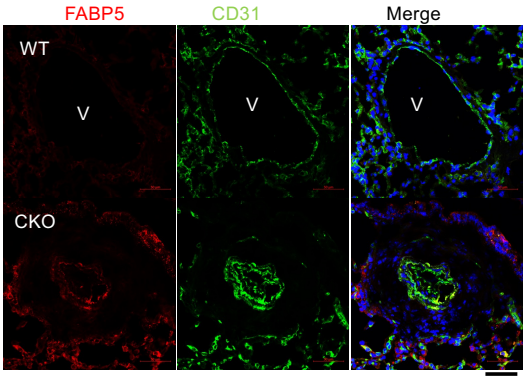
Figure 8. HIF-2α mediated glycolysis induced by FABP4-5 in pulmonary ECs

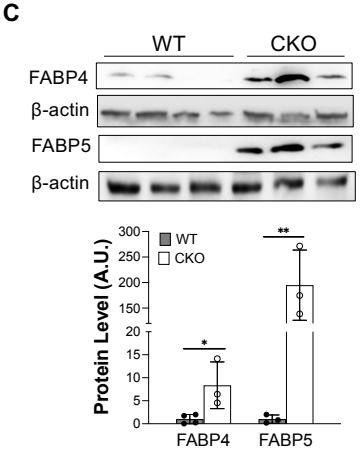
(**A**) A diagram showing the predicted transcription factors based on the DEGs and literature. (**B**) Western blotting demonstrated HIF-2α but not p53 or C-Myc was upregulated in CKO lungs and normalized in TKO lungs. (**C**) A representative heatmap showed that glycolytic genes were depended on HIF-2α using WT, CKO mice, and *Egln1*^{Tie2Cre}/*Hif2a*^{Tie2Cre} (EH2) mice. (**D**) HIF-2α knockdown inhibited FABP4-5 induced endothelial proliferation. (**E**) Nitrative stress assessed by protein nitrotyrosine modification was reduced in TKO lungs compared to CKO lungs. (**F**) Overexpression of FABP4 and 5 promoted mitochondrial ROS levels in HPAECs. (**G**) A diagram showing our proposed model. Our study addresses a novel role of lung endothelial FABP4-5 controlling PAECs accumulation through increased glycolysis in the pathogenesis of PAH. By facilitating fatty acid uptake and translocation into mitochondria, FABP4-5 promote FAO and ROS generation, which activates HIF-2α signaling to promote endothelial glycolysis. For BrdU assay in (**D**) and mitochondrial ROS assay in (**F**), each dot represents a biological replicate. The experiments were performed at least three times. ANOVA followed by Turkey post hoc analysis was used for statistical analysis (**B, D, E, F**). Significance levels were denoted as *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

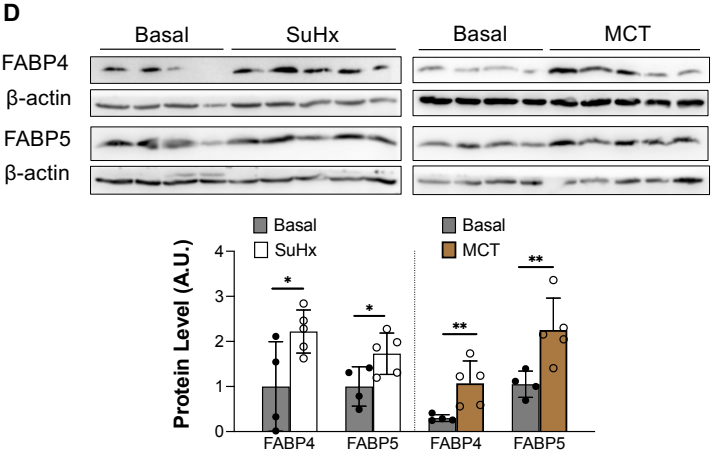

A
Fabp4
Expression Level
Identity
WT
CKO
EC
AT1
AT2
Pericyte
SMC
Fibroblast
Ciliated
Secretory
LEC
aMac
iMac
DC
inMono
PMN
Proliferating cells
T cell
Treg
NK cell
B cell
****
ns

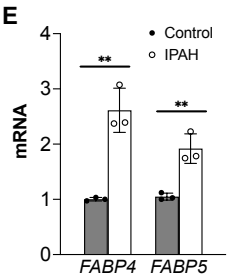

Fabp5
Expression Level
Identity
WT
CKO
EC
AT1
AT2
Pericyte
SMC
Fibroblast
Ciliated
Secretory
LEC
aMac
iMac
DC
inMono
PMN
Proliferating cells
T cell
Treg
NK cell
B cell
****
ns

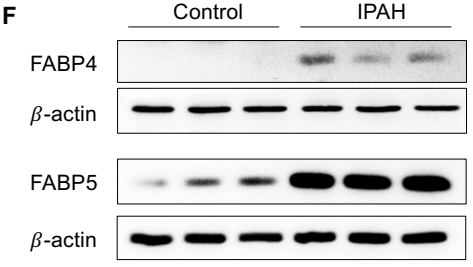

B
FABP4
CD31
Merge
WT
CKO
V

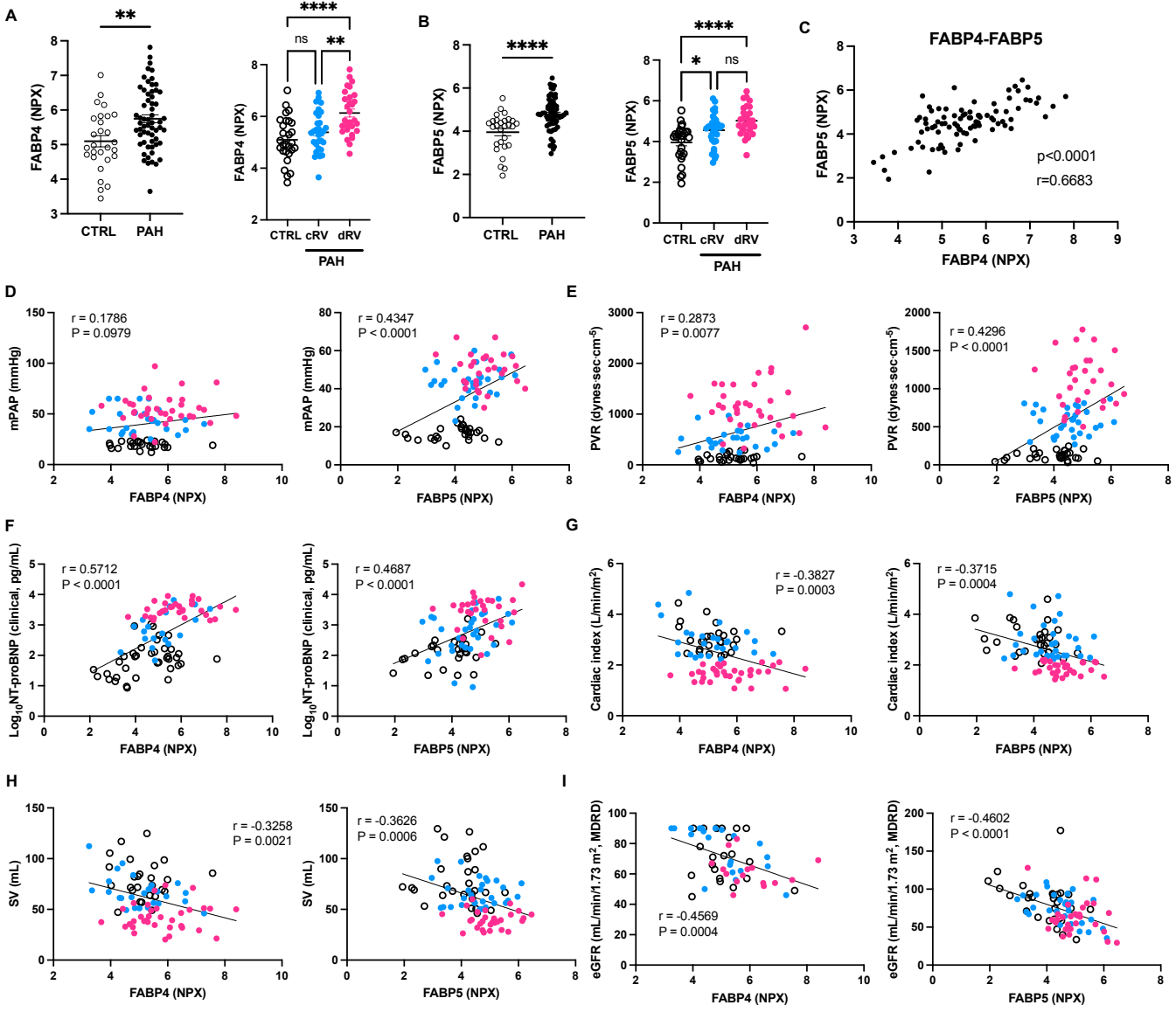

FABP5
CD31
Merge
WT
CKO
V

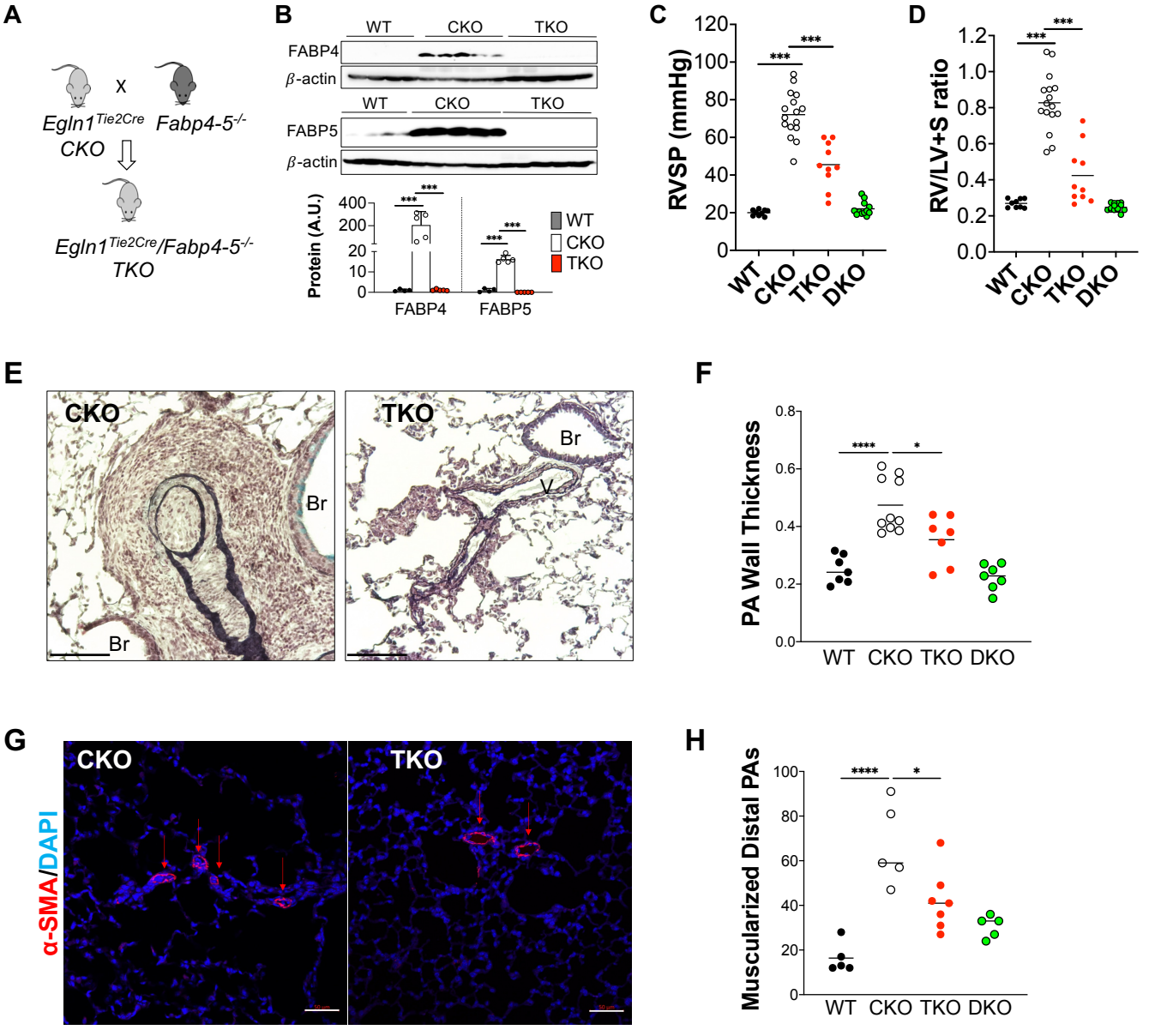

C
WT
CKO
FABP4
β-actin
FABP5
β-actin
Protein Level (A.U.)
WT
CKO
FABP4
FABP5

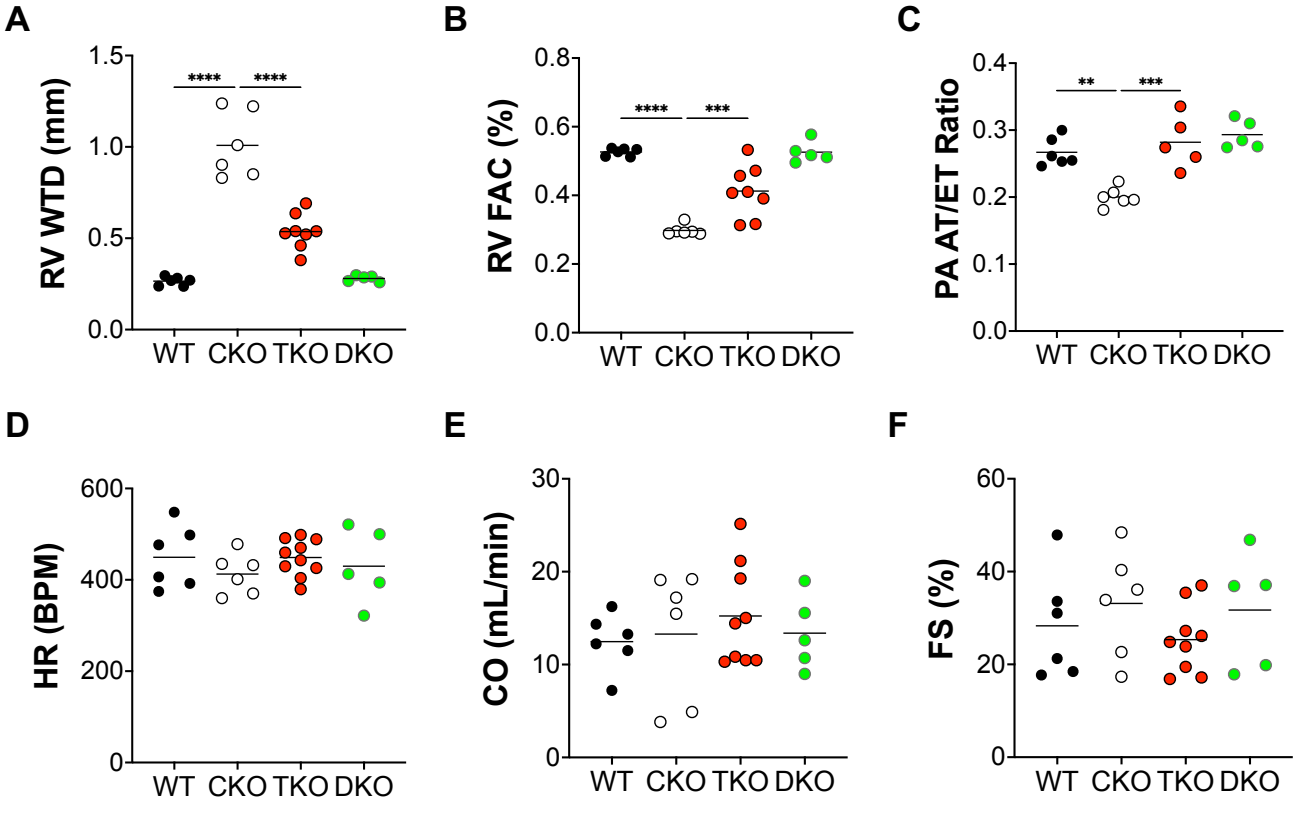

D
Basal
SuHx
Basal
MCT
FABP4
β-actin
FABP5
β-actin
Protein Level (A.U.)
Basal
SuHx
Basal
MCT
FABP4
FABP5
FABP4
FABP5

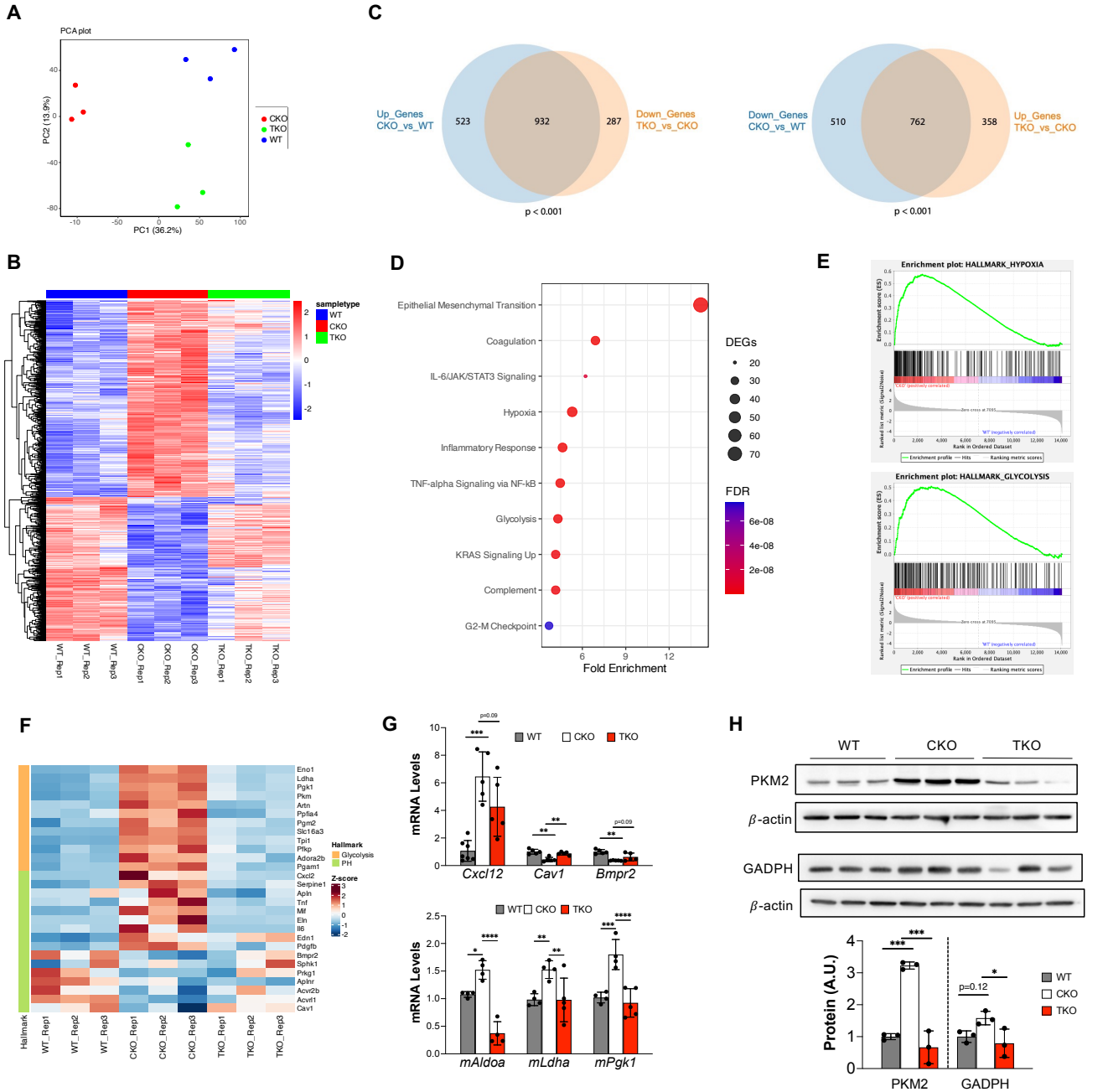

E
Control
IPAH
mRNA
FABP4
FABP5

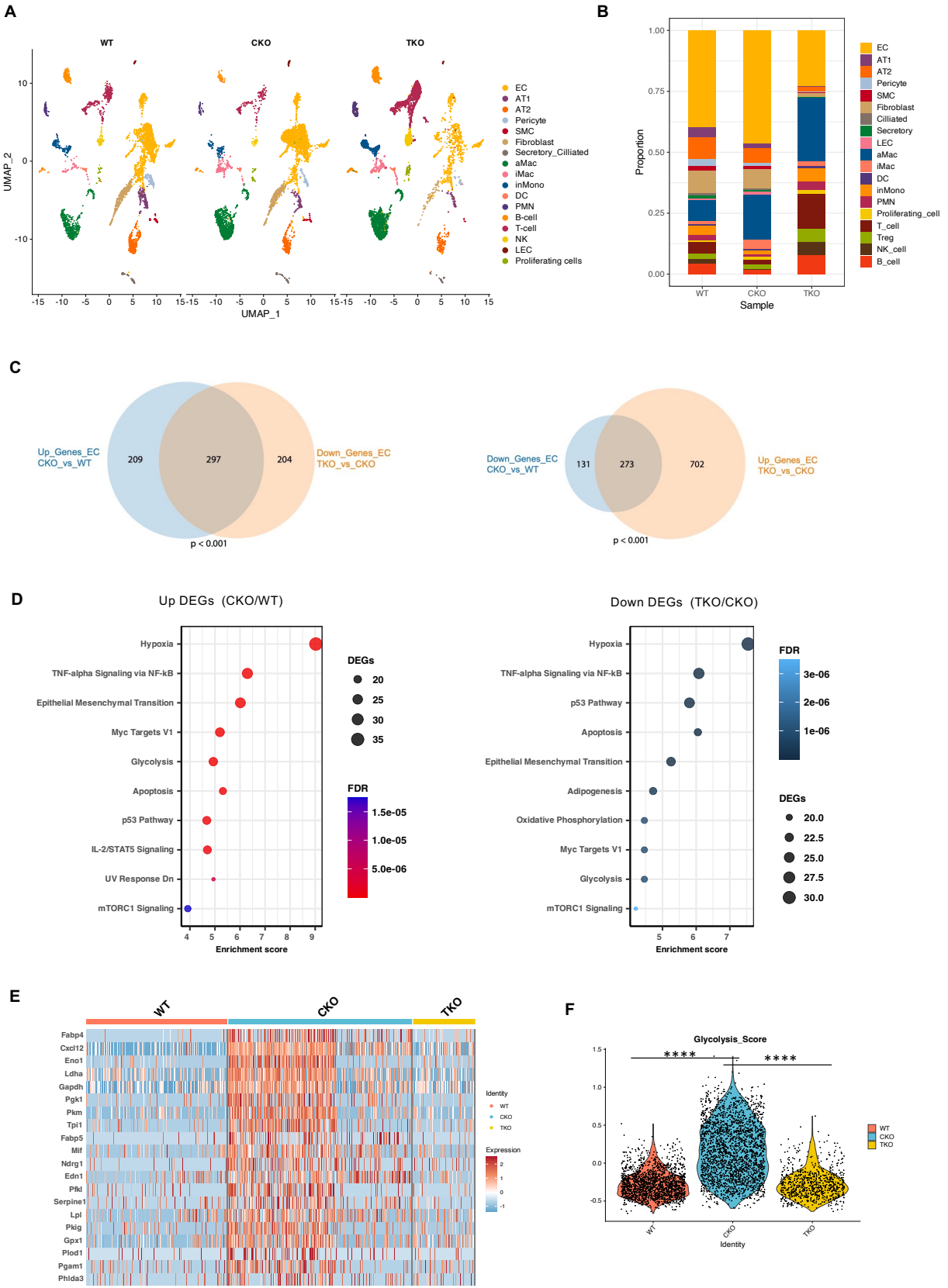

F
Control
IPAH
FABP4
β-actin
FABP5
β-actin

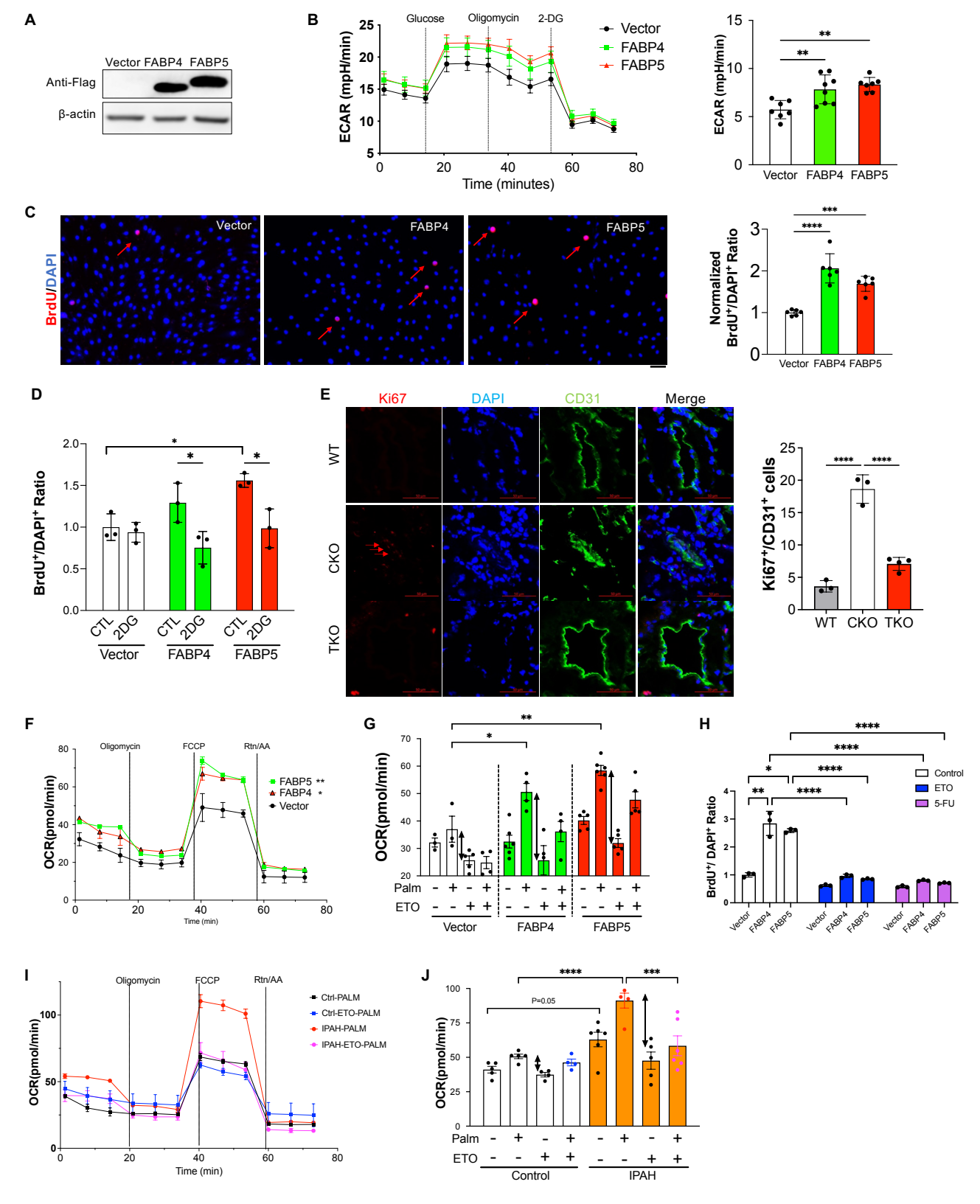

A
**
FABP4 (NPX)
CTRL
PAH
****
ns
**
CTRL cRV dRV
PAH
B
****
FABP5 (NPX)
CTRL
PAH
****
*
ns
CTRL cRV dRV
PAH
C
FABP4-FABP5
FABP5 (NPX)
FABP4 (NPX)
p<0.0001
r=0.6683
D
r = 0.1786
P = 0.0979
mPAP (mmHg)
FABP4 (NPX)
r = 0.4347
P < 0.0001
mPAP (mmHg)
FABP5 (NPX)
E
r = 0.2873
P = 0.0077
PVR (dynes·sec·cm-5)
FABP4 (NPX)
r = 0.4296
P < 0.0001
PVR (dynes·sec·cm-5)
FABP5 (NPX)
F
r = 0.5712
P < 0.0001
Log10NT-proBNP (clinical, pg/mL)
FABP4 (NPX)
r = 0.4687
P < 0.0001
Log10NT-proBNP (clinical, pg/mL)
FABP5 (NPX)
G
r = -0.3827
P = 0.0003
Cardiac index (L/min/m2)
FABP4 (NPX)
r = -0.3715
P = 0.0004
Cardiac index (L/min/m2)
FABP5 (NPX)
H
r = -0.3258
P = 0.0021
SV (mL)
FABP4 (NPX)
r = -0.3626
P = 0.0006
SV (mL)
FABP5 (NPX)
I
r = -0.4569
P = 0.0004
eGFR (mL/min/1.73 m2, MDRD)
FABP4 (NPX)
r = -0.4602
P < 0.0001
eGFR (mL/min/1.73 m2, MDRD)
FABP5 (NPX)

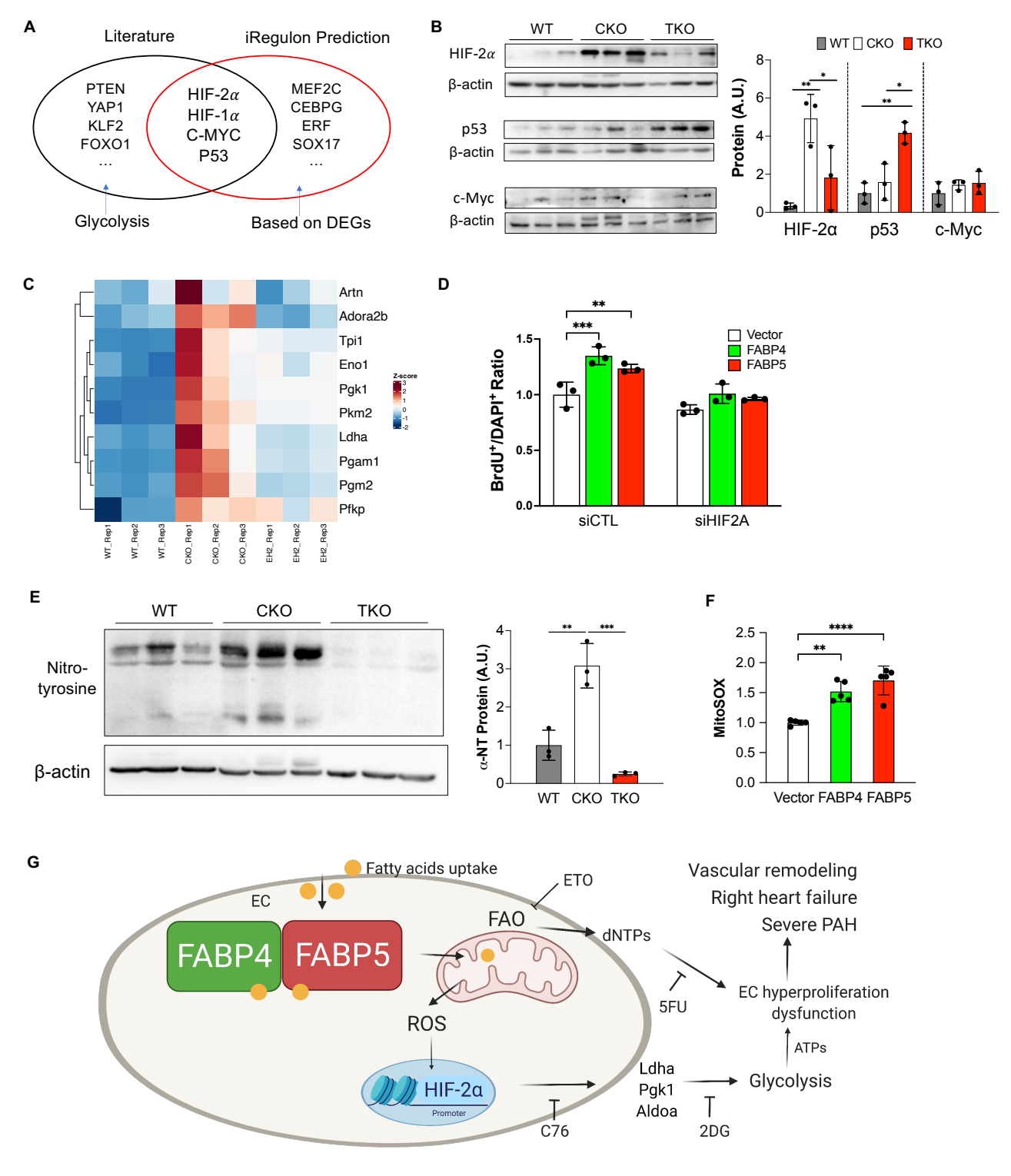

A
X
Egln1Tie2Cre CKO
Fabp4-5-/-
Egln1Tie2Cre/Fabp4-5-/- TKO
B
WT
CKO
TKO
FABP4
β-actin
FABP5
Protein (A.U.)
FABP4
FABP5
C
RVSP (mmHg)
WT
CKO
TKO
DKO
D
RV/LV+S ratio
E
CKO
TKO
Br
V
F
PA Wall Thickness
G
α-SMA/DAPI
H
Muscularized Distal PAs


A
RV WTD (mm)
1.5
1.0
0.5
0.0
****
****
WT CKO TKO DKO
B
RV FAC (%)
0.8
0.6
0.4
0.2
0.0
****
***
WT CKO TKO DKO
C
PA AT/ET Ratio
0.4
0.3
0.2
0.1
0.0
**
***
WT CKO TKO DKO
D
HR (BPM)
600
400
200
0
WT CKO TKO DKO
E
CO (mL/min)
30
20
10
0
WT CKO TKO DKO
F
FS (%)
60
40
20
0
WT CKO TKO DKO


A
PCA plot
PC2 (13.9%)
PC1 (36.2%)
CKO
TKO
WT
C
Up_Genes CKO_vs_WT
523
932
287
Down_Genes TKO_vs_CKO
p < 0.001
Down_Genes CKO_vs_WT
510
762
358
Up_Genes TKO_vs_CKO
p < 0.001
B
sampletype
WT
CKO
TKO
WT_Rep1
WT_Rep2
WT_Rep3
CKO_Rep1
CKO_Rep2
CKO_Rep3
TKO_Rep1
TKO_Rep2
TKO_Rep3
D
Epithelial Mesenchymal Transition
Coagulation
IL-6/JAK/STAT3 Signaling
Hypoxia
Inflammatory Response
TNF-alpha Signaling via NF-kB
Glycolysis
KRAS Signaling Up
Complement
G2-M Checkpoint
Fold Enrichment
DEGs
FDR
E
Enrichment plot: HALLMARK_HYPOXIA
Enrichment plot: HALLMARK_GLYCOLYSIS
F
Hallmark
Glycolysis
PH
Z-score
Eno1
Ldha
Pgk1
Pkm
Artn
Ppfia4
Pgm2
Slc16a3
Tpi1
Pfkp
Adora2b
Pgam1
Cxcl2
Serpine1
Apln
Tnf
Mif
Eln
Il6
Edn1
Pdgfb
Bmpr2
Sphk1
Prkg1
Aplnr
Acvr2b
Acvrl1
Cav1
G
WT
CKO
TKO
mRNA Levels
Cxcl12
Cav1
Bmpr2
mAldoa
mLdha
mPgk1
H
WT
CKO
TKO
PKM2
β-actin
GADPH
β-actin
Protein (A.U.)
PKM2
GADPH


A
WT
CKO
TKO
UMAP_2
UMAP_1
EC
AT1
AT2
Pericyte
SMC
Fibroblast
Secretory_Cilliated
aMac
iMac
inMono
DC
PMN
B-cell
T-cell
NK
LEC
Proliferating cells
B
Proportion
Sample
EC
AT1
AT2
Pericyte
SMC
Fibroblast
Cilliated
Secretory
LEC
aMac
iMac
DC
inMono
PMN
Proliferating_cell
T_cell
Treg
NK_cell
B_cell
C
Up_Genes_EC
CKO_vs_WT
209
297
204
Down_Genes_EC
TKO_vs_CKO
p < 0.001
Down_Genes_EC
CKO_vs_WT
131
273
702
Up_Genes_EC
TKO_vs_CKO
p < 0.001
D
Up DEGs (CKO/WT)
Hypoxia
TNF-alpha Signaling via NF-kB
Epithelial Mesenchymal Transition
Myc Targets V1
Glycolysis
Apoptosis
p53 Pathway
IL-2/STAT5 Signaling
UV Response Dn
mTORC1 Signaling
Enrichment score
DEGs
FDR
Down DEGs (TKO/CKO)
Hypoxia
TNF-alpha Signaling via NF-kB
p53 Pathway
Apoptosis
Epithelial Mesenchymal Transition
Adipogenesis
Oxidative Phosphorylation
Myc Targets V1
Glycolysis
mTORC1 Signaling
Enrichment score
FDR
DEGs
E
WT
CKO
TKO
Fabp4
Cxcl12
Eno1
Ldha
Gapdh
Pgk1
Pkm
Tpi1
Fabp5
Mif
Ndrg1
Edn1
Pfkl
Serpine1
Lpl
Pkig
Gpx1
Plod1
Pgam1
Phlda3
Identity
Expression
F
Glycolysis_Score
****
****
Identity


A
Vector FABP4 FABP5
Anti-Flag
β-actin
B
Glucose
Oligomycin
2-DG
Vector
FABP4
FABP5
ECAR (mpH/min)
Time (minutes)
ECAR (mpH/min)
Vector FABP4 FABP5
C
BrdU/DAPI
Vector
FABP4
FABP5
Normalized BrdU+/DAPI+ Ratio
Vector FABP4 FABP5
D
BrdU+/DAPI+ Ratio
CTL 2DG CTL 2DG CTL 2DG
Vector FABP4 FABP5
E
Ki67
DAPI
CD31
Merge
WT
CKO
TKO
Ki67+/CD31+ cells
WT CKO TKO
F
Oligomycin
FCCP
Rtn/AA
FABP5 **
FABP4 *
Vector
OCR(pmol/min)
Time (min)
G
OCR(pmol/min)
Palm
ETO
Vector FABP4 FABP5
H
Control
ETO
5-FU
BrdU+/ DAPI+ Ratio
Vector FABP4 FABP5 Vector FABP4 FABP5 Vector FABP4 FABP5
I
Oligomycin
FCCP
Rtn/AA
Ctrl-PALM
Ctrl-ETO-PALM
IPAH-PALM
IPAH-ETO-PALM
OCR(pmol/min)
Time (min)
J
P=0.05
OCR(pmol/min)
Palm
ETO
Control IPAH


A
Literature
iRegulon Prediction
PTEN
YAP1
KLF2
FOXO1
...
HIF-2α
HIF-1α
C-MYC
P53
MEF2C
CEBPG
ERF
SOX17
...
Glycolysis
Based on DEGs
B
WT
CKO
TKO
HIF-2α
β-actin
p53
β-actin
c-Myc
β-actin
WT CKO TKO
Protein (A.U.)
HIF-2α
p53
c-Myc
C
Artn
Adora2b
Tpi1
Eno1
Pgk1
Pkm2
Ldha
Pgam1
Pgm2
Pfkp
Z-score
WT_Rep1
WT_Rep2
WT_Rep3
CKO_Rep1
CKO_Rep2
CKO_Rep3
Eth2_Rep1
Eth2_Rep2
Eth2_Rep3
D
Vector
FABP4
FABP5
BrdU+/DAPI+ Ratio
siCTL
siHIF2A
E
WT
CKO
TKO
Nitro-
tyrosine
β-actin
α-NT Protein (A.U.)
WT CKO TKO
F
MitoSOX
Vector FABP4 FABP5
G
Fatty acids uptake
EC
ETO
FAO
dNTPs
FABP4
FABP5
ROS
HIF-2α
Promoter
5FU
C76
Ldha
Pgk1
Aldoa
2DG
Glycolysis
ATPs
EC hyperproliferation
dysfunction
Vascular remodeling
Right heart failure
Severe PAH