Supplemental Materials

Expanded Methods

Culture of human coronary artery smooth muscle cell (HCASMC) - Primary human coronary artery smooth muscle cells (HCASMC) were purchased from Cell Applications, Inc (San Diego, CA) and were cultured in complete smooth muscle basal media (Lonza, #CC-3182) according to the manufacturer's instructions. All experiments were performed with HCASMC between passages 5–8. HEK293 cells were maintained in DMEM containing high glucose, sodium pyruvate and L-glutamine supplemented with 10% FBS.

Knockdown and overexpression - For the siRNA transfection, cells were grown to 60% confluence, then treated with siRNA or scramble control to final concentration of 20nM with RNAiMax (Invitrogen, Carlsbad, CA) for 12 hours. The siRNA for *AHR* and *TCF21* were purchased from Origene (SR300136, SR321985), and siRNA for *AC003075.4* was custom designed (Ambion/Life Technologies Corp, Supplemental Table 1). The cells were collected 48 hours after transduction. For the overexpression study, HCASMC were transduced with 2nd generation lentivirus with *AHR* cDNA (HG10456-CY, Sino Biological) and *AC003075.4* cDNA (custom gBlock fragment, IDT) cloned into pWPI (Addgene #12254) using NEBuilder HiFi cloning (New England Biolabs). Briefly, for lentiviral transduction, the cells were treated at 60% confluence with virus at MOI of 5 for 24 hours. The virus was removed and replaced with low-serum media for 48 hours prior to collection for downstream applications.

RNA isolation and qRT-PCR - RNA was isolated using RNeasy mini kit (Qiagen) and total cDNA was prepared using iScript cDNA synthesis kit (Biorad, Hercules, CA). Gene expression levels were measured using Taqman probes (Invitrogen) (*AHR, TNFRSF11B, HAPLN1*) and custom probes (*AC003075.4*, see *supplemental material*)

for SYBR Green assay and quantified on a ViiA7 Real-Time PCR system (Applied Biosystems, Foster City, CA) and normalized to *GAPDH* levels.

RNA-sequencing – Four replicates were used for each sample. The RNA were processed and analyzed as described previously. Briefly, RNAs were sent to Novogene for sample QC, library preparation, and sequencing. All samples passed QC, and 250–300 bp insert cDNA libraries were prepared for each sample. Subsequently, sequencing was performed on a Novaseq 6000 platform with paired-end 150 bp reads. The script for RNA-Seq analysis can be found in github (https://github.com/milospjanic/rnaSeqFPro). *DESeq2* was used for differential expression analysis using the Wald test. Additionally, the web-based tools iDEP.90 (http://bioinformatics.sdstate.edu/idep/) was used for analysis of the RNA-Seq data and visualization using the counts data generated from FeatureCounts.[29]

ChIP Assay - ChIP was performed using the AHR antibody (Santa Cruz, sc-5579) and TCF21 antibody (HPA013189, Sigma), which were pre-validated according to ChIP-seq guidelines. Library for ChIP-Seq was prepared using standard procedures as decribed previously.[30] Briefly, approximately 4e6 HCASMC cells were fixed with 1% formaldehyde and quenched by glycine. The cells were washed three times with PBS and then harvested in ChIP lysis buffer (50 mM Tris-HCl, pH 8, 5 mM EDTA, 0.5% SDS). Cross-linked chromatin was sheared for 3 × 1 min by sonication (Branson SFX250 Sonifier) before extensive centrifugation. Four volume of ChIP dilution buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100) was added to the supernatant. The resulting lysate was then incubated with Dynabeads™ Protein G (Thermo Scientific, 10009D) and antibodies at 4 °C overnight. Beads were washed once with buffer 1 (20 mM Tris pH 8, 2 mM EDTA, 150 mM NaCl, 1% Triton X100, 0.1% SDS), once with

buffer 2 (10 mM Tris pH 8, 1 mM EDTA, 500 mM NaCl, 1% Triton X100, 0.1% SDS), once with buffer 3 (10 mM Tris pH 8, 1 mM EDTA, 250 mM LiCl, 1% NP40, 1% sodium deoxycholate monohydrate), and twice with TE buffer. DNA was eluted by ChIP elution buffer (0.1 M $NaHCO_3$, 1% SDS, 20 µg/ml proteinase K). The elution was incubated at 65 °C overnight, and DNA was extracted with a DNA purification kit (Zymo D4013). The purified DNA was assayed by quantitative PCR with ABI ViiA 7 and Power SYBR Green Master Mix (ABI 4368706) using CYP1B1 DRE region primers (Supplemental Figure 2, Supplemental Table 5). Library for ChIP-Seq was prepared using standard procedures. Briefly, Libraries were prepared with KAPA Hyper Prep kit (KK8502). ChIPseq libraries were sequenced on HiSeq X10 for 150 bp paired-end sequencing. The script for RNA-Seq analysis can be found in github (https://github.com/zhaoshuoxp/Pipelines-Wrappers/blob/master/ChIPseq.sh). Briefly, quality control of ChIPseq data was performed using *Fastqc*, and then low-quality bases and adaptor contamination were trimmed by *cutadapt*. Filtered reads were mapped to hg19 using *BWA mem* algorithm. Duplicate reads were marked by *Picard Markduplicate* module and removed with unmapped reads by *samtools view -f 2 -F 1804*. *macs2 callpeak* was used for peaks calling and input as control. *macs2 bdgdiff* was used for differential peaks calling with default parameters.

Co-IP and western blotting - Nuclear complex Co-IP kit (Active Motif 54001) was used for co-immunoprecipitation following the manufacturer instructions. Whole cell lysate samples were harvested at 4 °C using 1× Laemmli buffer and boiled at 95 °C for 10 min. For ChIP-WB, ChIP samples were eluted with 1× Laemmli buffer (Bio-Rad 161-0747) containing Halt protease inhibitor cocktail (Thermo Fisher Scientific 78429) instead of elution buffer for western blotting. The elution was incubated at 65 °C for 6 h and then boiled at 95 °C for 2 min. Then samples were loaded onto a 4–15% gradient SDS-PAGE

gel (Bio-Rad 4561084DC). Samples were transferred to polyvinylidene difluoride membrane (Thermo Fisher Scientific LC2002) overnight at 300 mA at 4 °C and blocked with 5% milk in Tris-buffered saline and 0.1% Tween 20 (TBST, Bio-Rad 1706435) at room temperature. The membranes were hybridized with the following primary antibodies: mouse Myc-Tag antibody (CST 2276S), and HA-tag antibody (Thermo Fisher cat#26183). Rabbit GAPDH antibody (Sigma G9545-200UL) was used as the loading control. Anti-mouse HRP (Jackson ImmunoResearch 115-035-008, 115-035-003, and 115-035-174) or anti-rabbit HRP (Jackson ImmunoResearch 211-032-171, 111-035-008, and 111-035-003) secondary antibodies were used at a concentration of 1:5000-1:10000 and diluted in 5% milk containing 0.1% Tween 20. Bands were detected using SignalBoost™ Immunoreaction Enhancer Kit (Millipore 407207) per manufacturer's instructions on the LI-COR Odyssey imaging system.

Assay for transposome accessible chromatin (ATAC) assays - For ATAC-Seq, we followed the published Omni-ATAC-seq protocol.[31] Approximately 5xe4 fresh HCAMSC cells were collected by centrifugation at 500 g and washed twice with cold PBS. Nuclei-enriched fractions were extracted with cold resuspension buffer (0.1% NP-40, 0.1% Tween 20, and 0.01% Digitonin) and washed out with 1 ml of cold resuspension buffer containing 0.1% Tween 20 only. Nuclei pellets were collected by centrifugation and resuspended with transposition reaction buffer containing Tn5 transposases (Illumina Nextera). Transposition reactions were incubated at 37 °C for 30 min, followed by DNA purification using the DNA Clean-up and Concentration kit (Zymo D4013). Libraries were amplified using Nextera barcodes and high-fidelity polymerase (NEB M0541S) and purified using Agencourt Ampure XP beads (Beckman Coulter A63880) double-size selection (0.5X:0.9X). Libraries were sequenced on HiSeq X10 for 150-bp paired-end sequencing and analyzed as described previously.[30] The script for ATAC-Seq analysis

can be found in github (https://github.com/zhaoshuoxp/Pipelines-Wrappers/blob/master/ATACseq.sh). Libraries were sequenced on HiSeq X10 for 150-bp paired-end sequencing. Raw fastq files were evaluated with *fastqc,* and then low-quality bases and adaptor contamination were trimmed by *cutadapt*. Reads were mapped to hg19 using *bowtie2*. Duplicate reads were marked by *Picard Markduplicate* module and removed with unmapped or mitochondrial reads by *samtools*. *bedtools* was used to generate BED file from filtered reads followed by Tn5 shifting with *awk*. *macs2 callpeak* with *--broad* parameter was used for peak calling. *macs2 bdgdiff* with *FDR* cutoff 0.05 were used for differential peak comparison in *AHR* disrupted samples. Bigwig files were generated for University of California Santa Cruz (UCSC) Genome Browser visualization.

For qPCR experiments, the purified DNA was quantified with ABI ViiA 7 and Power SYBR Green Master Mix (ABI 4368706) and normalized by genomic DNA which extracted using Quick-DNA Microprep Kit (Zymo D3020). Assays were repeated at least three times. Data shown were average values ± SD of representative experiments.

Cis-regulatory functional enrichment and network analysis - We utilized the Genomic Regions Enrichment of Annotations Tool (GREAT 3.0) to analyze the detected peaks for Gene Ontology. The *HOMER findMotifsGenome.pl* script was employed to search for known TRANSFAC motifs and to generate de novo motifs. The *intersectBed* was used to find at least 1 bp overlapped peaks between AHR and TCF21. *P* values were calculated by *Fisher's exact test with the whole genome as the background*.

Mouse strains - The animal study protocol was approved by the Administrative Panel on Laboratory Animal Care (APLAC) at Stanford University. To enact SMC-specific lineage tracing and *Tcf21* knockout, we used mice containing a well-characterized BAC

transgene that expresses a tamoxifen-inducible Cre recombinase driven by the SMC-specific *Myh11* promoter (*Tg*$^{Myh11\text{-}CreERT2}$, JAX# 019079)[16]. These mice were bred with a floxed tandem dimer tomato (tdT) fluorescent reporter line (B6.Cg-*Gt(ROSA)26Sor*$^{tm14(CAGtdTomato)Hze}$/J, JAX# 007914)[17] to allow SMC-specific lineage tracing to generate the SMC$^{LnT/WT}$ mice. All mice were bred onto the C56BL/6, *ApoE*$^{-/-}$ background. Final genotypes of SMC lineage-tracing (SMC^{lin}) mice were: *Tg*$^{Myh11\text{-}CreERT2}$, *Ahr*$^{+/+}$, *ROSA*$^{tdT/+}$, *ApoE*$^{-/-}$. An *Ahr*$^{flox/flox}$ mice were obtained from Jackson Labs (JAX#006203), which was constructed by placing lox-P sites flanking the second exon of the *Ahr* gene. The *Ahr*$^{flox/flox}$ mice were then bred to the SMC$^{LnT/WT}$ mice. Final genotypes of SMC lineage-tracing, Ahr knockout (SMC$^{LnT/KO}$) mice were: *Tg*$^{Myh11\text{-}CreERT2}$, *Ahr*$^{\Delta SMC/\Delta SMC}$, *ROSA*$^{tdT/+}$, *ApoE*$^{-/-}$. As the Cre-expressing BAC was integrated into the Y chromosome, all lineage tracing mice in the study were male.

Induction of lineage marker and Ahr knockout by Cre recombinase - Two doses of tamoxifen, at 0.2mg/gm bodyweight, were administered by oral gavage at 8 weeks of age, with each dose separated by 48 hours. Approximately 48 hours after the second dose of tamoxifen, high fat diet (HFD) was started (Dyets #101511, 21% anhydrous milk fat, 19% casein, 0.15% cholesterol). The Cre-mediated excision of exon 2 of *Ahr* was confirmed in the DNA obtained from vascular tissues (Supplemental Figure 1, Supplemental Table 5 for primers).

Single-cell RNAseq – Mouse aortic root dissociation was performed as described previously.[19] To increase biological replication, multiple mice were used to obtain single cell suspensions. For both SMC$^{LnT/WT}$ and SMC$^{LnT/KO}$ genotype, three mice were used at 16 weeks of disease. Cells were sorted on a BD Aria II instrument. Dissociated cells were sorted for live/dead signal and tdTomato^{+} signal. tdTomato+ cells (considered to be

of SMC lineage) were then captured for all subsequent analyses. All single-cell capture and library preparation was performed at the Stanford Functional Genomics Facility (SFGF). Cells were loaded into a 10X Genomics microfluidics chip and encapsulated with barcoded oligo-dT-containing gel beads using the 10X Genomics Chromium controller according to the manufacturer's instructions. Single-cell libraries were then constructed according to the manufacturer's instructions. Libraries from individual samples were multiplexed into one lane prior to sequencing on an Illumina HiSeq4000 instrument.

Analysis of Single-Cell RNAseq Data - Fastq files from each experimental time point and mouse genotype were aligned to the reference genome individually using CellRanger Software (10X Genomics). Individual datasets were aggregated using the CellRanger *aggr* command without subsampling normalization. The aggregated dataset was then analyzed using the R package Seurat.[34] The dataset was trimmed of cells expressing fewer than 500 genes, and genes expressed in fewer than 5 cells. The number of genes, the number of unique molecular identifiers (UMIs) and the percentage of mitochondrial genes were examined to identify outliers. As an unusually high number of genes can result from a "doublet" event, in which two different cell types are captured together with the same barcoded bead, cells with > 4000 genes were discarded. Cells containing >10% mitochondrial genes were presumed to be of poor quality and were also discarded. The gene expression values then underwent library size normalization, using the published *sctranform* function in the Seurat R package.[35] Principal component analysis was used for dimensionality reduction, followed by clustering in PCA space using a graph-based clustering approach.[35,36] UMAP was then used for two-dimensional visualization of the resulting clusters. The "Endochondral Score" was constructed using average expression of the following genes – *Bmp2, Alpl, Nab2, Pex7, Mgp, Smpd3,*

Sox9, Runx2, Col2a1, Acan, Col1a1, Mef2c, and Mmp2. Raw data from single-cell RNAseq data that support the findings of this study will be deposited in the GEO database. Primary accession codes are pending. Wilcoxon rank sum test was used for identification of differential markers between clusters.

Preparation of mouse aortic root sections - Immediately after sacrifice, mice were perfused with 0.4% PFA. The mouse aortic root and proximal ascending aorta, along with the base of the heart, was excised and immersed in 4% PFA at 4C for 12 hours (for immunohistochemisty) to 24 hours (for RNAscope). After passing through a sucrose gradient, tissue was frozen in OCT to make blocks. Blocks were cut into 7um-thick sections for further analysis.

Immunohistochemistry - Slides were prepared and processed as described previously.[12] Sections were then incubated overnight at 4C with anti-SM22alpha rabbit polyclonal primary antibody (Abcam #ab14106, 1:300 dilution), or CD68 rabbit polyclonal antibody (Abcam #ab125212, 1:300 dilution). Sections were washed for 5 minutes x2 with TBS and then incubated with the Rabbit-on-Rodent HRP Polymer (Biocare Medical # RMR622) for 30 minutes at room temperature (RT). Sections were washed x2 with TBS and then incubated with the Betazoid DAB chromogen reagents (Biocare Medical #BDB2004) for 4 minutes at RT. Sections were washed x2 in DI water and air-dried, followed by mounting with Fluoroshiled with 4′,6-diamidino-2-phenylindole (DAPI) (Sigma #F6057). For AHR immunofluorescence, antigen retrieval with sodium citrate buffer was performed, and AHR antibody (Santa Cruz, sc-5579) and goat anti-RFP antibody (MyBioSource #MBS448122) was used as primary antibody for tdTomato expression. For the alkaline phosphatase enzymatic assay, Ferangi Blue Chromogen kit (SKU#FB813H, Biocare Medical) was used as instructed. Researchers were blinded to

the genotype of the animals until completion of the analysis. The processed sections were visualized using Leica DM5500 microscope and images were obtained using Leica Application Suite X software. Areas of interest were quantified using *ImageJ* (NIH) software, and compared using a two-sided t-test. The lesion cap was defined as 30μm segment from the luminal surface as previously described.[6]

RNAscope - Slides were processed according to the manufacturer's instructions, and all reagents were obtained from ACD Bio. Slides were washed once in PBS, then immersed in 1Å~ Target Retrieval reagent at 100 °C for 5 min. Slides were washed twice in deionized water, immersed in 100% ethanol and air dried, and sections were encircled with a liquid-blocking pen. Sections were incubated with Protease Plus reagent for 30 min at 40°C, then washed twice with deionized water. Sections were incubated with probes against mouse *Cyp1b1*, *Ahr*, human *AHR* or a negative control probe for 2 h at 40 °C. RNAscope HD assay (RED) and Multiplex colorimetric assay were performed per the manufacturer's instructions.

HCASMC Phenotypic Assays - The Radius Cell Migration Assay kit (Cell Biolabs, Inc) was used for the gap closure assay. Briefly, HCASMC was plated at 80% confluence, then following the protocol, the polymer at the center of each well were dissolved to allow migration. Migration was quantified by remaining area after migration, compared to original area. For the proliferation assay, EdU was introduced into the cell culture 3 hours before assay for uptake. The protocol for Click-iT Plus EdU proliferation kit (Thermo Fisher) was followed as instructed. For the apoptosis assay, HCASMC was treated with Doxorubicin (1uM) for 24 hours to induce apoptosis. The RealTime-Glo Annexin V Apoptosis kit (Promega) was used as instructed to quantify the degree of apoptosis in HCASMC. To assay for calcification, HCASMC was exposed to calcification

media with 10mM beta-glycerophophate and 100ug/ml ascorbic acid as described previously.[36] The HCASMC were treated with siRNA and lentivirus for AHR as described in "Knockdown and overexpression" and then exposed to calcification media with 1% FBS for 10 days. The calcified cells were treated with 0.6N hydrochloric acid for 24 hours, then the supernatant was collected for calcium assay, and the cell layer was collected for protein quantification. The Calcium Colorimetric Assay Kit (Sigma-Aldrich) was used for quantification of calcium, then normalized to total protein content.

Statistics - For the statistical analyses not discussed above, methods were as follows. R or GraphPad Prism 7.0 was used for statistical analysis. For motif and gene enrichment analyses, we used the binomial test. For overlapping of genomic regions or gene sets, we used *Fisher's exac*t test. For comparisons between two groups of equal variance, an unpaired two-tailed Student's *t-test* was performed or in cases of unequal variance a Welch's unequal variances t-test was performed, as indicated. *P* values <0.05 were considered statistically significant. For multiple comparison testing, one-way analysis of variance (ANOVA) accompanied by Tukey's *post hoc* test were used as appropriate. All error bars represent standard error of the mean (SE).

Number of stars for the *P* values in the graphs: ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Supplemental Table Legends (Excel Files)

Table I. Methods – Primers and custom siRNA, cDNA sequences
Tab 1. qPCR primer sequences
Tab 2. AC003075.4 custom siRNA sequences
Tab 3. AC003075.4 cDNA gBlock fragment sequence

Table II. RNA-Seq outputs from *AHR* and *TCF21* modulation
Tab 1. *AHR* knockdown differentially expressed gene list (adjusted p<e-5, fold change >1.3)
Tab 2. *AHR* overexpression differentially expressed gene list (adjusted p<0.05, fold change >1.3)
Tab 3. Enriched GO biological pathways from *AHR* knockdown differentially expressed genes
Tab 4. Enriched GO biological pathways from *AHR* overexpression differentially expressed genes
Tab 5. TCF21 knockdown differentially expressed gene list (adjusted p<e-5, fold change >1.3)

Table III. AHR ChIP-Seq and ATAC-Seq outputs

Tab 1. AHR ChIP-Seq motifs (HOMER known motifs)
Tab 2. AHR ChIP-Seq motifs (HOMER de novo motifs)
Tab 3. AHR ChIP-Seq GREAT enriched pathways (GO biological pathways)
Tab 4. *AHR* overexpression ATAC-Seq Enriched pathways (GO Biological pathways)

Table IV. Comparison of AHR and TCF21 epigenomics

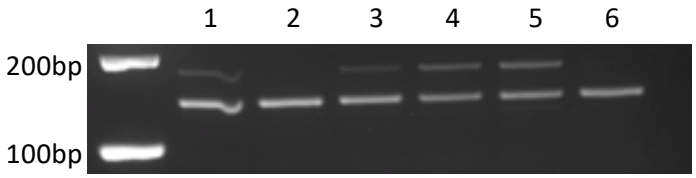
Tab 1. Enriched GO biological pathways from genes differentially expressed by both AHR knockdown and TCF21 knockdown
Tab 2. Enriched KEGG pathways from genes differentially expressed by both AHR knockdown and TCF21 knockdown
Tab 3. GO biological pathways enriched from genes differentially regulated by AHR knockdown only (and not by TCF21 knockdown).
Tab 4. Enriched GO biological pathways from peaks common to AHR ChIP-Seq and TCF21 ChIP-Seq

Table V. Single-cell RNA-Seq analyses

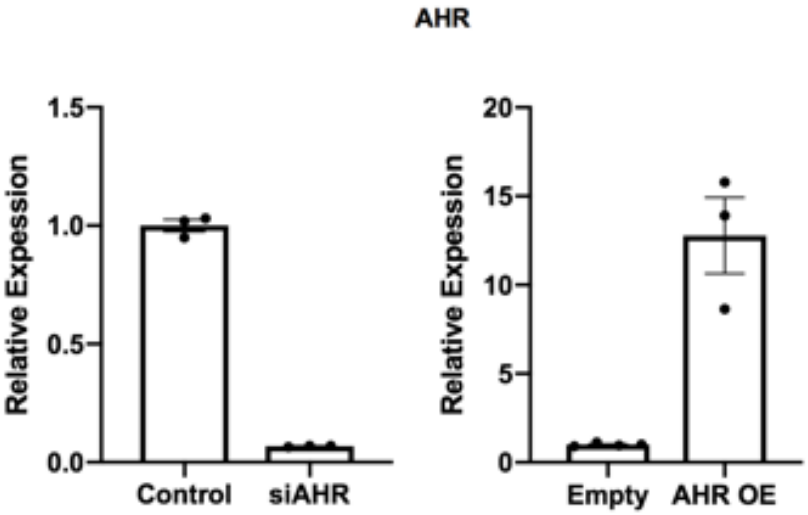
Tab 1. Top 50 markers of all cell types from tdTomato+ cells of $SMC^{LnT/WT}$ mice
Tab 2. Differential markers of FMC1 vs. FMC2 (avg_logFC = average log(Fold Change), percentage of cells expressing gene in FMC1 (pct.1), FMC2 (pct.2))
Tab 3. Enriched GO biological pathways from the top 100 upregulated genes in FMC2 compared to FMC1.
Tab 4. Upstream regulator analysis of the top 100 activated genes in FMC2 compared to FMC1 using Ingenuity pathway analysis (IPA).
Tab 5. STRING analysis of the upstream regulators identified from IPA
Tab 6. Top enriched KEGG/Reactome from top upstream regulators of FMC2 vs. FMC1.
Tab 7. Differentially expressed genes between CMC of WT vs. CMC of KO.
Tab 8. Enriched GO biological pathways from differentially expressed genes CMC of WT vs. CMC of KO.

Figure I

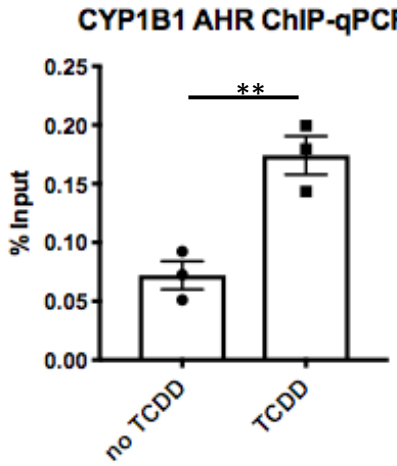
a.


1
2
3
4
5
6
200bp
100bp

b.


AHR
Relative Expression
1.5
1.0
0.5
0.0
Control
siAHR
Relative Expression
20
15
10
5
0
Empty
AHR OE

c.


CYP1B1 AHR ChIP-qPCR
% Input
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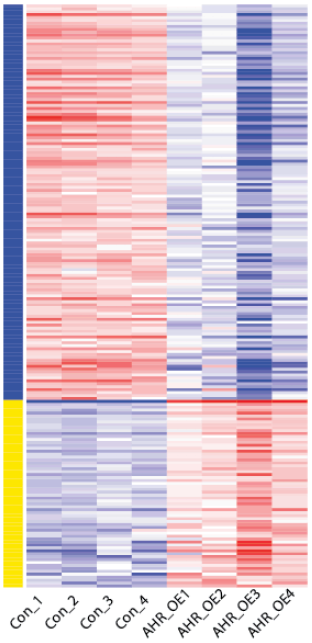
0.20
0.15
0.10
0.05
0.00
**
no TCDD
TCDD

Figure II

a.


Con_1
Con_2
Con_3
Con_4
AHR_OE1
AHR_OE2
AHR_OE3
AHR_OE4

b.

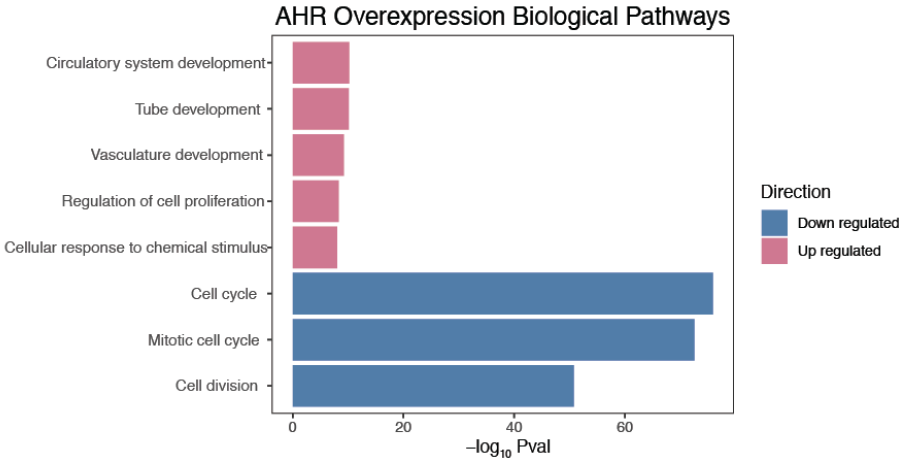
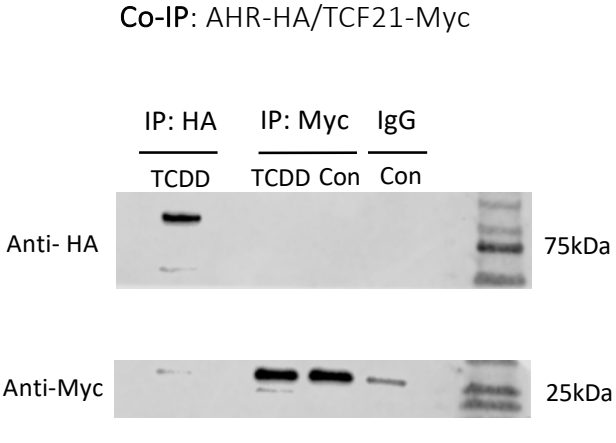

AHR Overexpression Biological Pathways
Circulatory system development
Tube development
Vasculature development
Regulation of cell proliferation
Cellular response to chemical stimulus
Cell cycle
Mitotic cell cycle
Cell division
0
20
40
60
–log10 Pval
Direction
Down regulated
Up regulated

Figure III

a.



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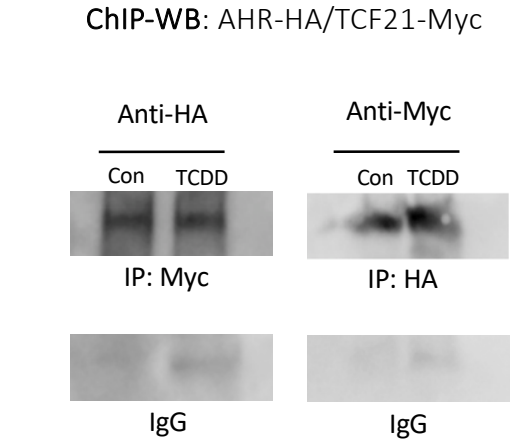
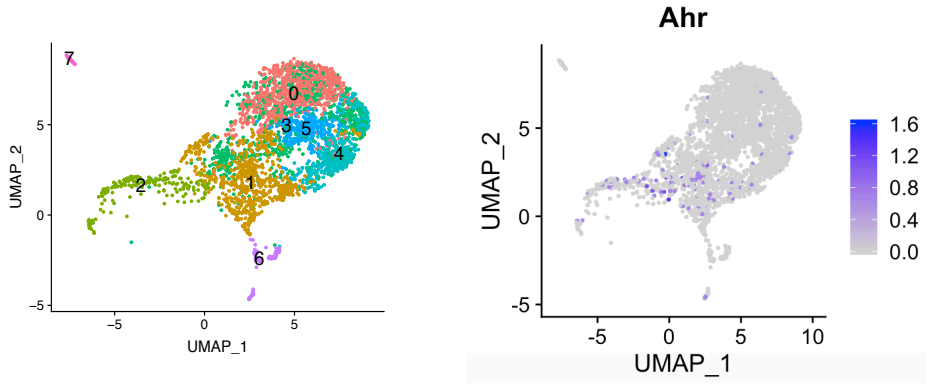


Figure IV

a.



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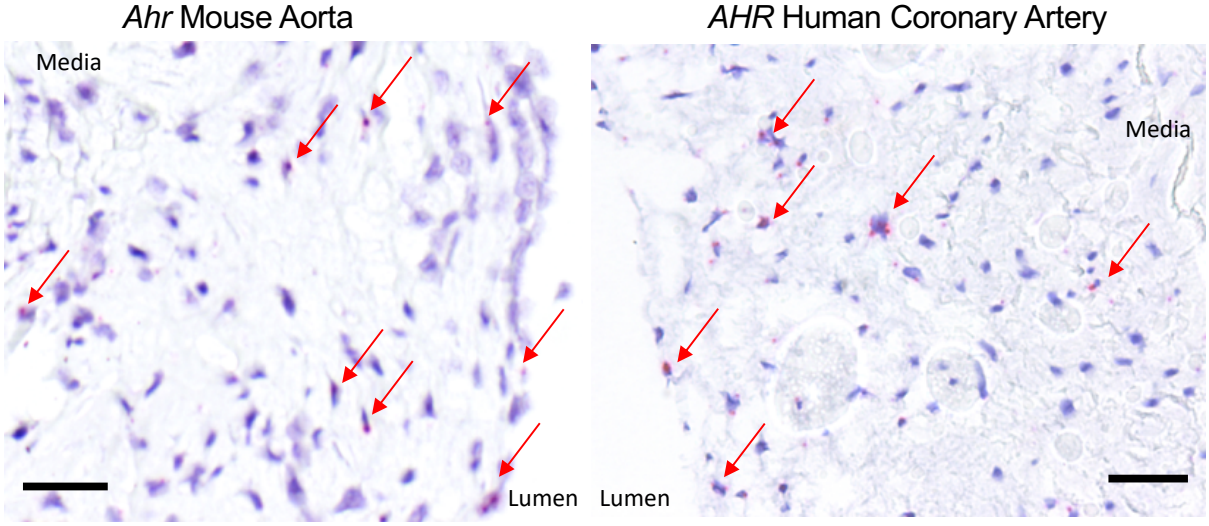

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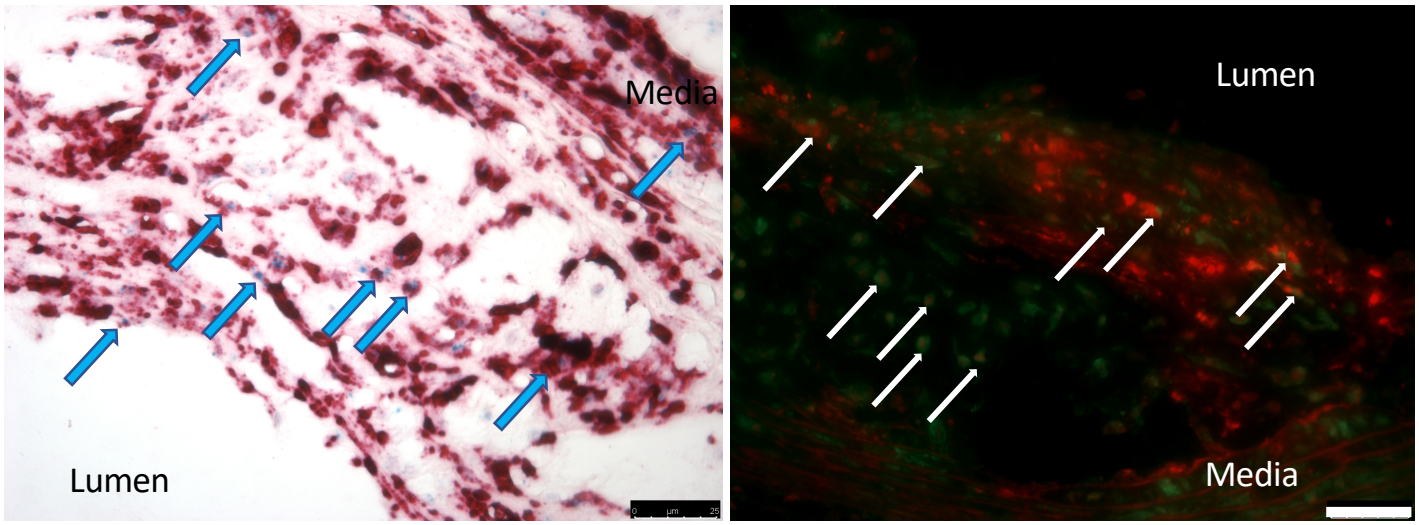

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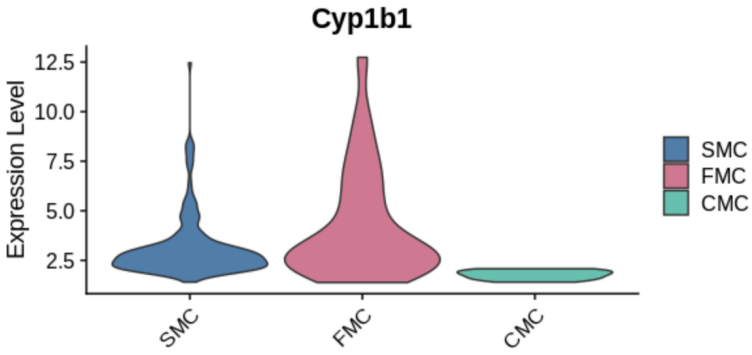

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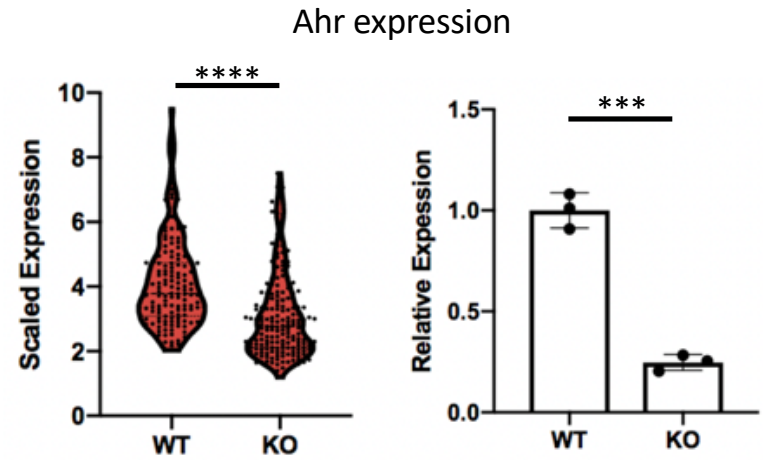


Figure V

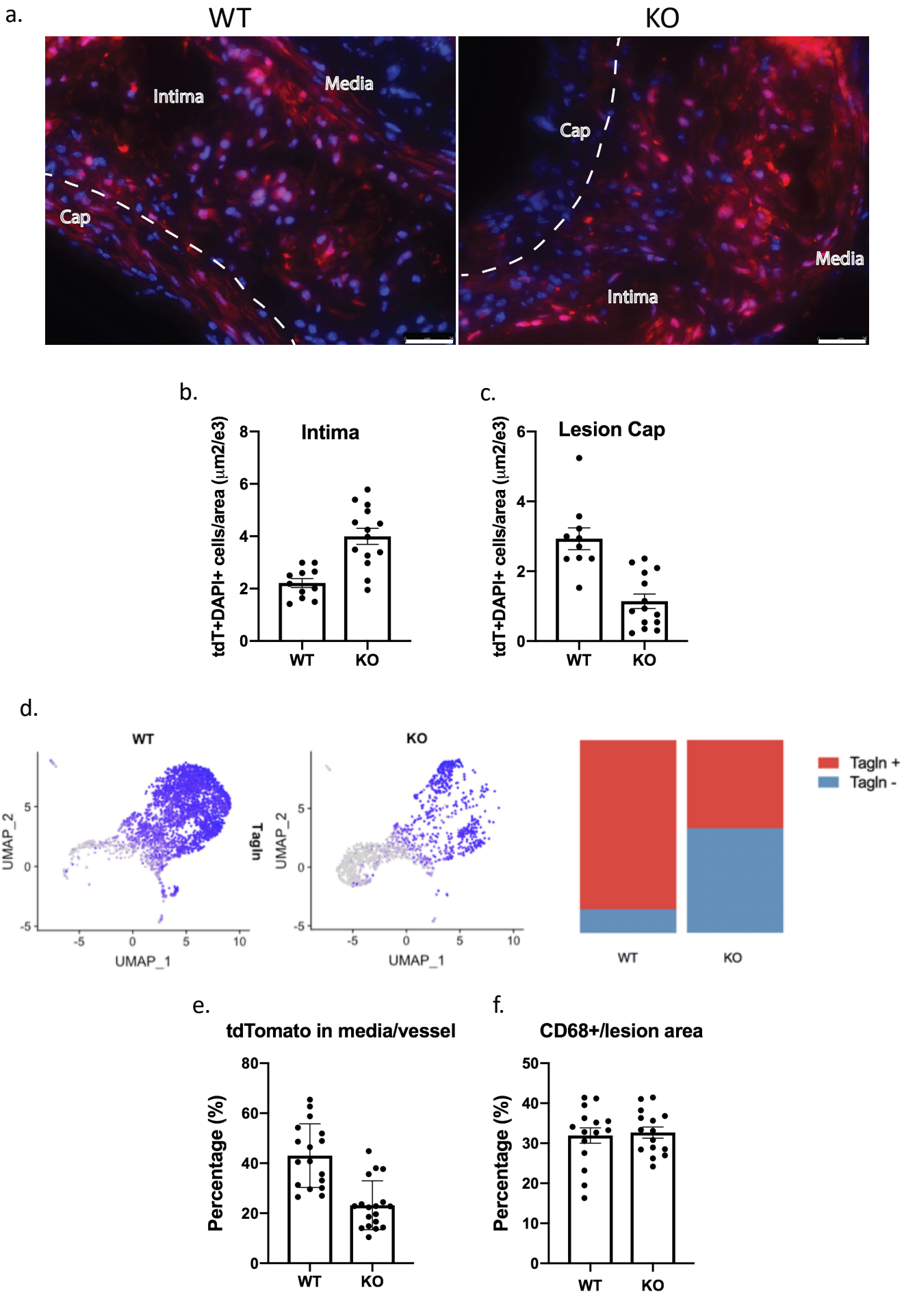
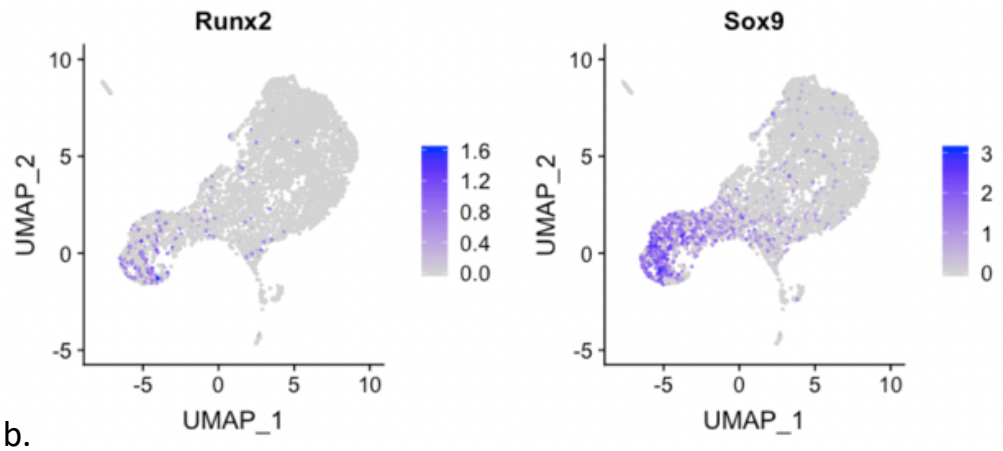
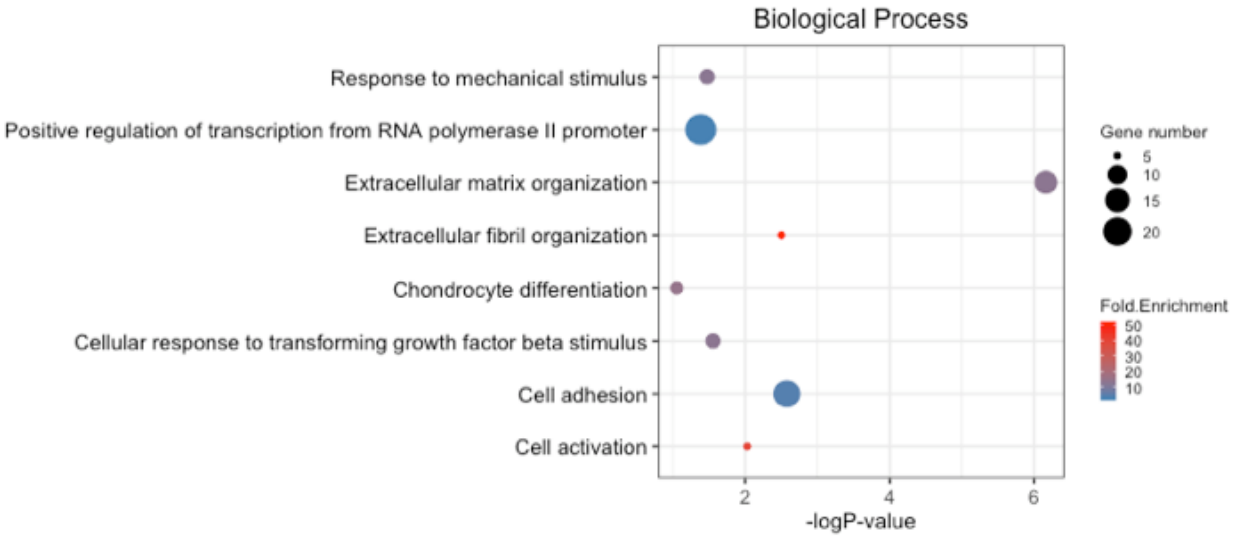

a.
WT
KO
Intima
Media
Cap
Cap
Media
Intima
b.
Intima
tdT+DAPI+ cells/area (µm2/e3)
WT
KO
c.
Lesion Cap
tdT+DAPI+ cells/area (µm2/e3)
WT
KO
d.
WT
KO
UMAP_2
UMAP_1
Tagln
Tagln +
Tagln -
WT
KO
e.
tdTomato in media/vessel
Percentage (%)
WT
KO
f.
CD68+/lesion area
Percentage (%)
WT
KO

Figure VI

a.



b.



c.

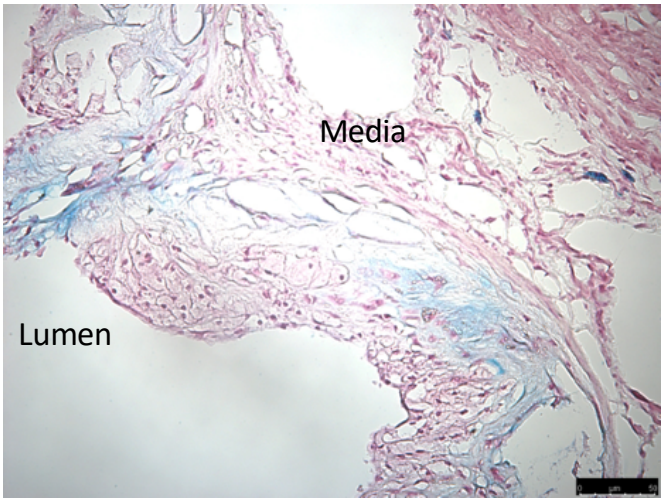


Figure VII

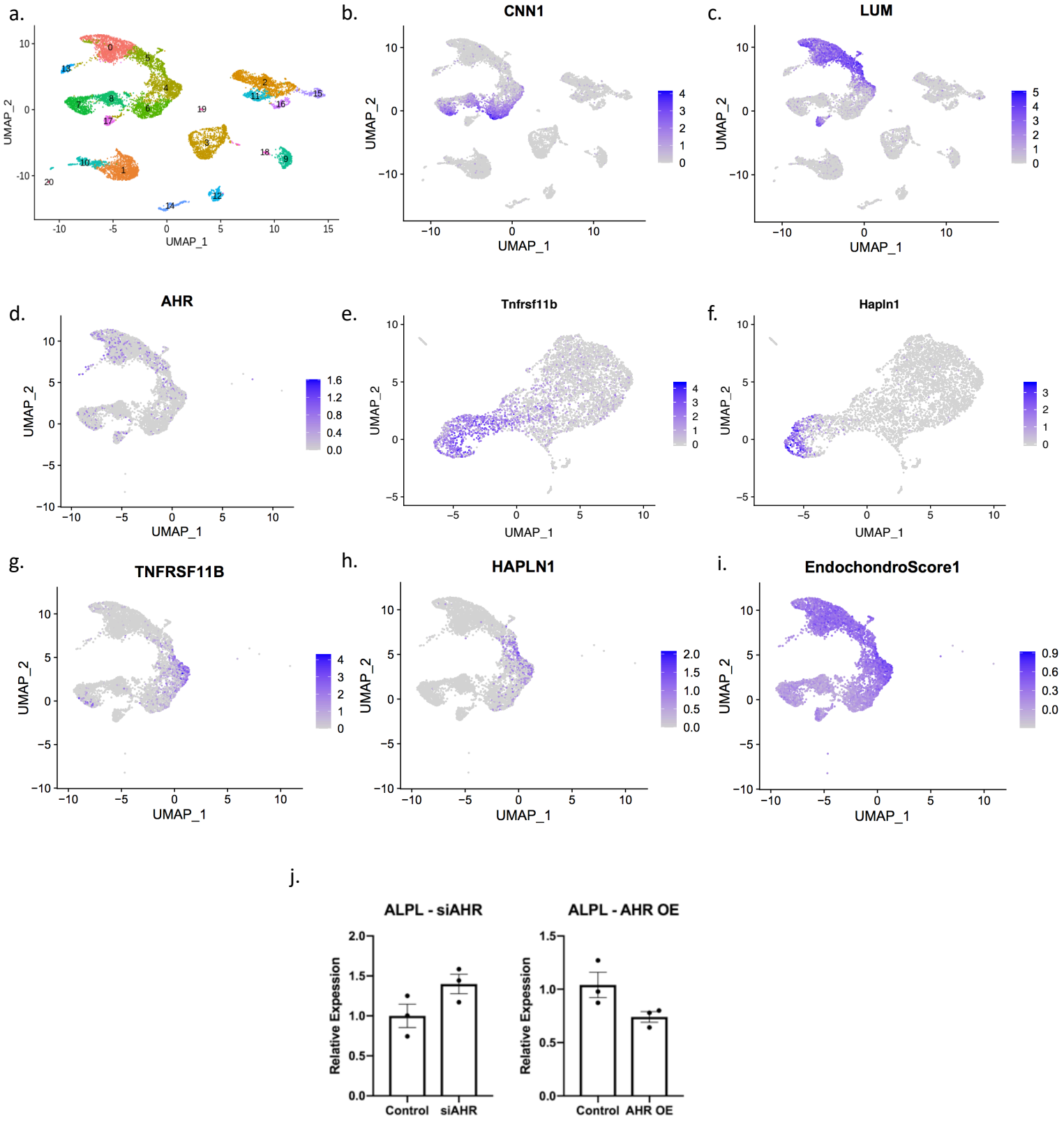
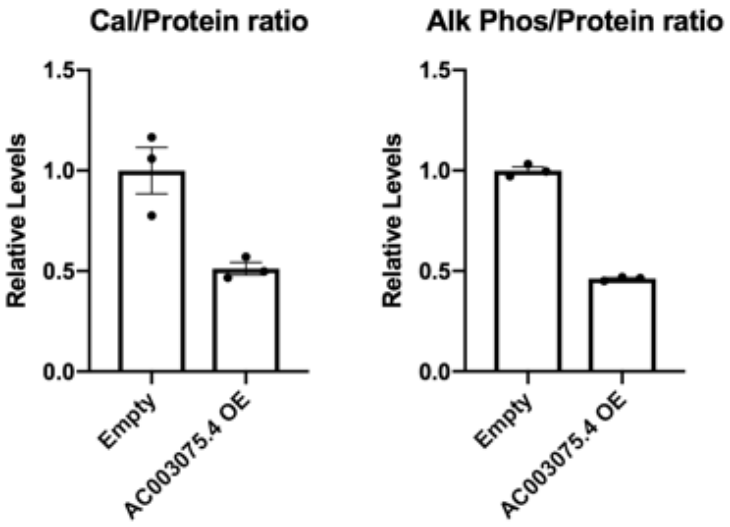
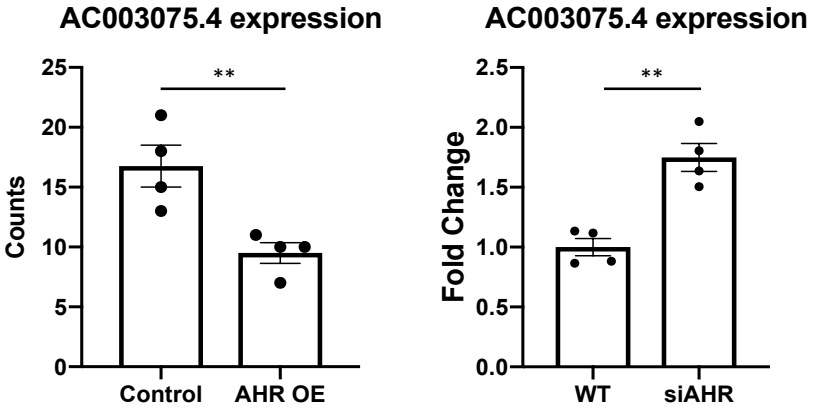

a.
b.
CNN1
c.
LUM
d.
AHR
e.
Tnfrsf11b
f.
Hapln1
g.
TNFRSF11B
h.
HAPLN1
i.
EndochondroScore1
UMAP_1
UMAP_2
j.
ALPL - siAHR
ALPL - AHR OE
Relative Expression
Control
siAHR
AHR OE

Figure VIII

a.



b.



Supplementary Figure Legends

Figure I. (a) Verification of Cre-induced excision of *Ahr* Exon 2. DNA was extracted from different tissues one day after the second tamoxifen gavage to confirm the Cre-induced recombination event with PCR (primers in Supplemental Table 5). Wildtype allele band is 130 bp, recombined allele 180 bp. Lanes 1) lung, 2) liver, 3) esophagus, 4) aortic sinus, 5) descending aorta, and 6) heart. No recombination is seen in the liver and the heart. (b) *AHR* siRNA transfection and lentiviral transduction leads to significant knockdown and overexpression of AHR transcript. (b) AHR ChIP shows enrichment at the CYP1B1 dioxin response element following 1 hour of dioxin exposure (0.07% vs. 0.17% of Input chromatin, triplicates, p=0.007). The sample was processed for library preparation and sequencing. $^{**}P < 0.01$.

Figure II. (a) Heatmap of differentially expressed genes in *AHR* overexpression RNA-Seq data in HCASMC show a significant impact of *AHR* modulation on the transcriptome. (b) Top enriched biological pathways from differentially expressed genes of *AHR* overexpression.

Figure III. AHR and TCF21 do not interact directly at the protein level, but through chromatin binding. Using HEK293 cells transfected with HA-tagged AHR construct and Myc-tagged TCF21 construct; (a) co-immunoprecipitation (IP) was performed on the cell lysates as described. IP with anti-HA antibody (Ab) followed by western blot (WB) with Myc Ab (top), or IP with anti-Myc Ab followed by WB with anti-HA Ab (bottom) showed no interaction. (b) Chromatin immunoprecipiation (ChIP) followed by western blot was performed on lysates after chromatin crosslinking. There is evidence of AHR and TCF21 interaction facilitated by the protein-chromatin interaction. ChIP with anti-HA Ab was followed by WB with anti-Myc Ab (left), and

ChIP with anti-Myc Ab was followed by WB with HA Ab (right). IgG was used as control for both co-IP and ChIP-WB experiments. (Con = DMSO control, TCDD = Dioxin treatment)

Figure IV. The *AHR* pathway is activated in FMC. (a) Ahr is expessed in the tdTomato+ cell population, and concentrated in the FMC population. (b) RNAscope of Ahr in (*left*) mouse aorta and (*right*) human coronary artery shows Ahr expression in the cells present in the intima and the cap of the lesion (red = *Ahr*, blue = Hematoxyln stain, bar = 25μm). (c) Ahr expression is co-localized with tdTomato expressing cells (bar = 25μm). Dual probe RNAscope shows Ahr expression (green) co-localizing with tdTomato (red) signal (blue arrows) (*Left*); Immunofluorescence of Ahr (red) and tdTomato (green) is colocalized in the lesion cap and intema (white arrows) (*Right*). (d) Average expression of *Cyp1b1*, a canonical downstream gene of the *Ahr* pathway is increased in FMC compared to SMC (p=1.84e-5). (e) Expression of *Ahr* is significantly lower in the KO compared to WT as seen by (*left*) scaled data from scRNA-seq (4.11±0.12 vs. 3.08±0.09, p=6.9e-11), and (*right*) qPCR of mouse aorta (1.0±0.05 vs. 0.25±0.02, p=0.0002). ****$P < 0.0001$, ***$P < 0.001$.

Figure V. Shifting of tdTomato+ cells from lesion cap to intima in AHR KO mice. (a) Number of cells co-staining for tdTomato and DAPI signals were obtained using *ImageJ* software, and normalized by the size of area queried to obtain the density of these cells in both the intima and the lesion cap (*left* – WT, *right* – KO, white bar = 25μm). (b) Density of tdTomato+ cells significantly increased in the intima of KO compared to WT (WT 2.21±0.17 vs. KO 4.00±0.31 cells per 10^{-3} μm^2, p=0.0001), and (c) a significant decrease in the density of tdTomato+ cells in the lesion cap in the KO vs. WT (WT 2.93±0.31 vs. KO 1.14±0.21 cells per 10^{-3} μm^2, p<0.0001). (d) There was a significant reduction in the proportion of *Tagln*+ cells among the tdTomato+ lineage traced cells in the KO compared to WT (WT 87.9% vs. KO 45.9%, p=1.57e-215; *Tagln+ defined as above 25%percentile expression*), (e) There was

reduction in the tdTomato+ area in the media in the KO, suggesting phenotypic modulation and shifting of medial cells into the lesion (WT 43.02±3.09 vs. KO 23.18±2.30 %, p<0.0001). (f) There was no significant difference in the Cd68+ area in the lesion (p=NS).

Figure VI. (a) Chondromyocytes are enriched for cartilage/chondrocyte specific transcription factors, including Runx2 and Sox9. (b) Differential expression analysis comparing CMC of WT vs. KO enrich for pathways relevant to ossification/cartilage production including ECM organization, chrondrocyte differentiation, and cellular response to TGF-beta stimulus. (c) Alcian blue staining shows proteoglycan components of the extracellular matrix associated with chondrocytes in the intima (bar = 50μm).

Figure VII. Chondromyocytes are present in human coronary arteries. (a) UMAP of scRNA-Seq of diseased human right coronary artery using previously published dataset[12] (b) Mature SMC clusters are visualized by *CNN1* expression (clusters 6, 7, 8) and (c) fibroblasts, FMC and CMC clusters are visualized by *LUM* expression (clusters 0, 4, 5). (d) *AHR* is expressed in the fibroblast and FMC/CMC clusters. (e, f) *Tnfrsf11b* and *Hapln1* are both specific for the CMC cluster in mice; (g, h) The CMC markers *TNFRSF11B* and *HAPLN1* are localized in the region between the SMC and fibroblasts in human cells, and (i) the endochondral score is highest in the human CMC region.(j) *ALPL* expression is regulated by AHR modulation in HCASMC (p=0.1 for siAHR, and p=0.08 for AHR OE).

Figure VIII. (a) *AC003075.4* inhibits calcification of HCASMC. Overexpression of *AC003075.4* resulted in decreased relative total calcium level (1.0±0.12 vs. 0.51±0.03, p=0.015) and alkaline phosphatase activity (1.0±0.02 vs. 0.46±0.01, p<0.0001), normalized to total protein content. (b) *AHR* negatively regulates lncRNA AC003075.4 expression. *AHR* overexpression results in decreased expression of *AC003075.4,* data from RNA-seq experiments (p=0.0099). The

negative regulation was confirmed with qPCR where *AHR* knockdown resulted in increased *AC003075.4* expression (p=0.0016). ***P* < 0.01.