FIG. 1

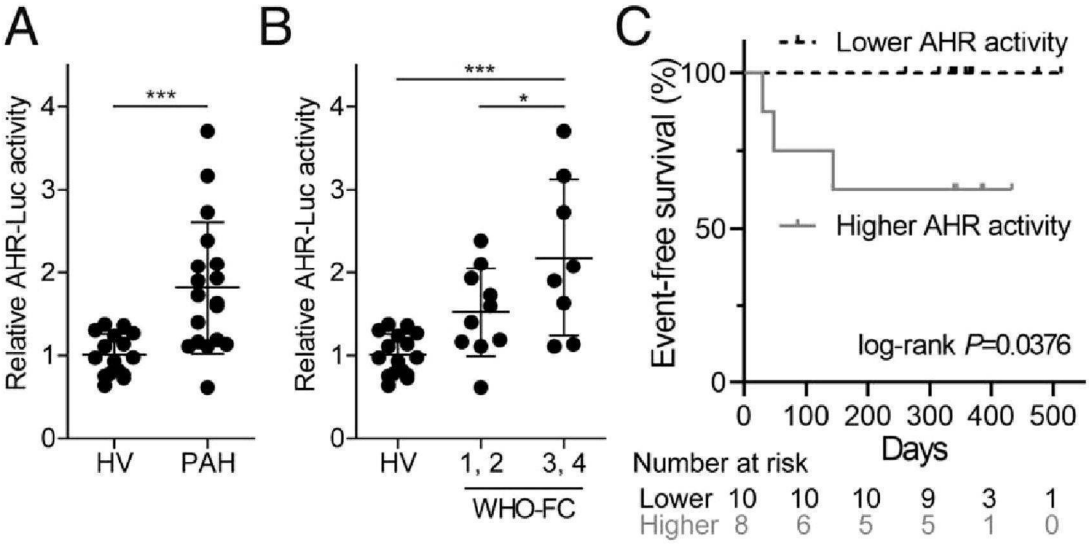

A
B
C
Relative AHR-Luc activity
HV
PAH
1, 2
3, 4
WHO-FC
Lower AHR activity
Higher AHR activity
Event-free survival (%)
log-rank P=0.0376
Days
Number at risk
Lower 10 10 10 9 3 1
Higher 8 6 5 5 1 0

FIG. 2

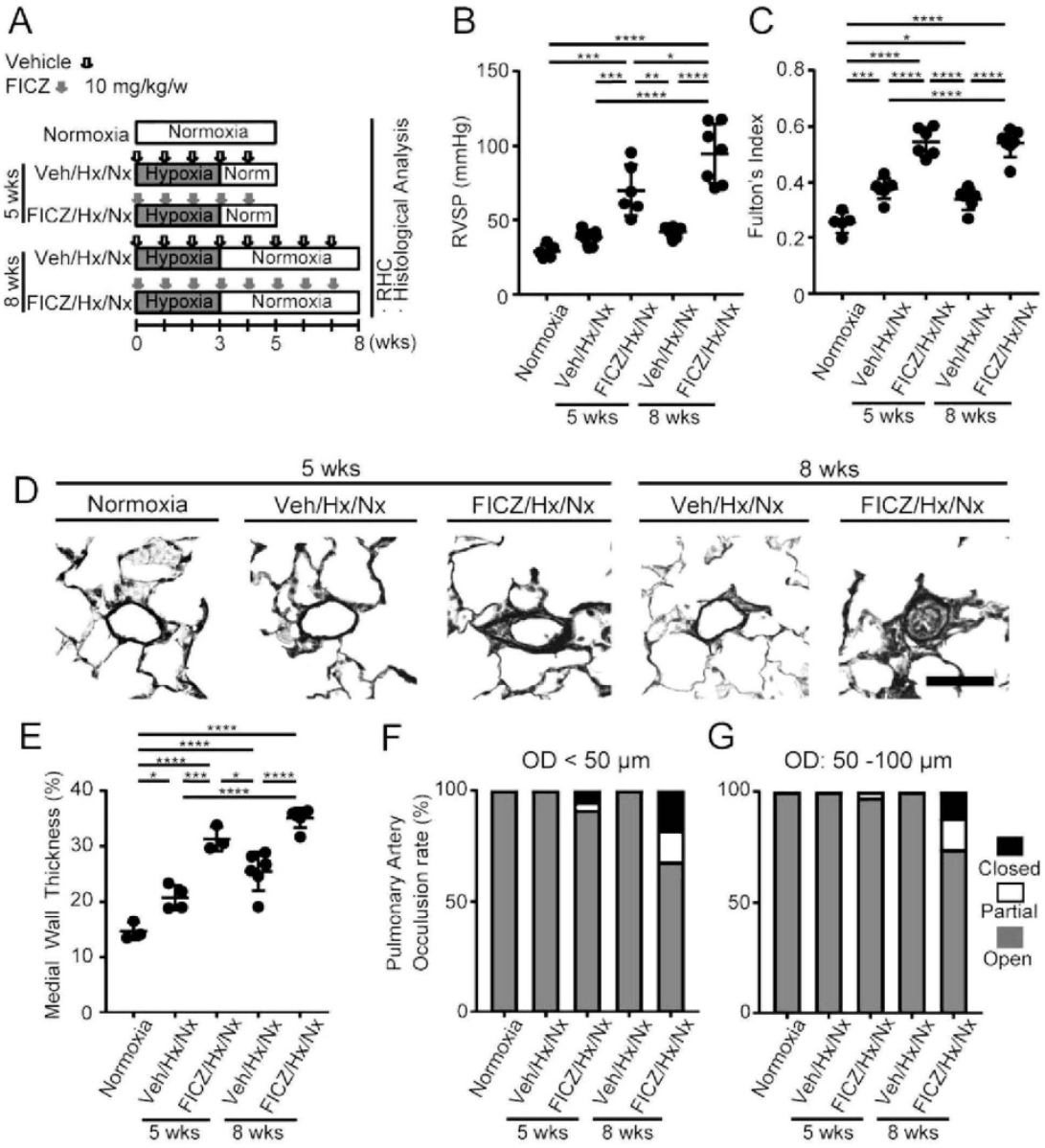

A
Vehicle
FICZ 10 mg/kg/w
Normoxia
Veh/Hx/Nx
FICZ/Hx/Nx
Hypoxia
Norm
Normoxia
5 wks
8 wks
RHC
Histological Analysis
8 (wks)
B
RVSP (mmHg)
C
Fulton's Index
5 wks
8 wks
D
E
Medial Wall Thickness (%)
F
OD < 50 μm
Pulmonary Artery Occulusion rate (%)
G
OD: 50 -100 μm
Closed
Partial
Open

FIG. 3

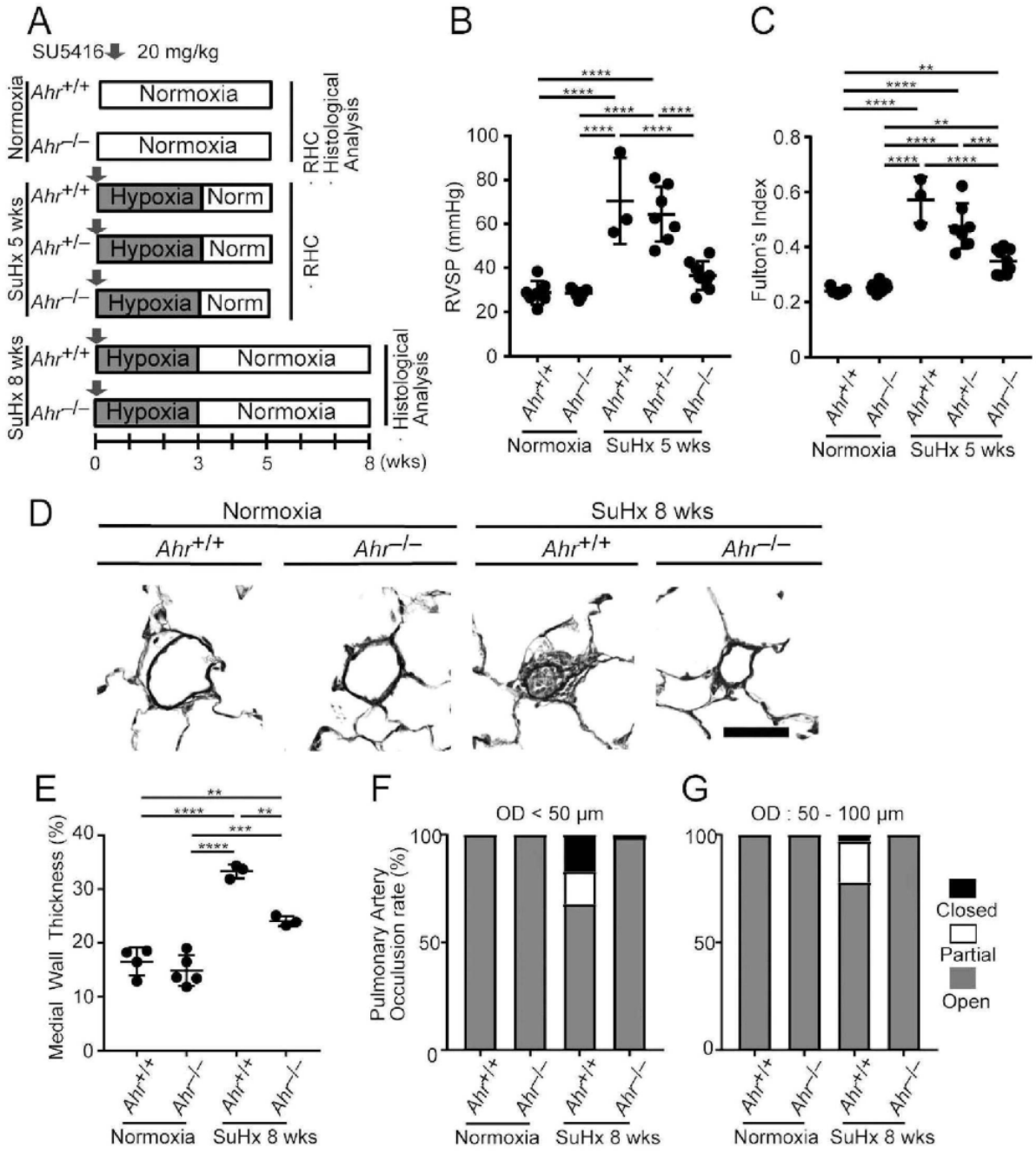

A
SU5416 20 mg/kg
Normoxia
Ahr+/+
Ahr−/−
Normoxia
Normoxia
· RHC
· Histological Analysis
SuHx 5 wks
Hypoxia
Norm
· RHC
SuHx 8 wks
Hypoxia
Normoxia
· Histological Analysis
0
3
5
8 (wks)
B
RVSP (mmHg)
Normoxia
SuHx 5 wks
C
Fulton's Index
D
Normoxia
SuHx 8 wks
E
Medial Wall Thickness (%)
F
OD < 50 μm
Pulmonary Artery Occulusion rate (%)
G
OD : 50 - 100 μm
Closed
Partial
Open

FIG. 4

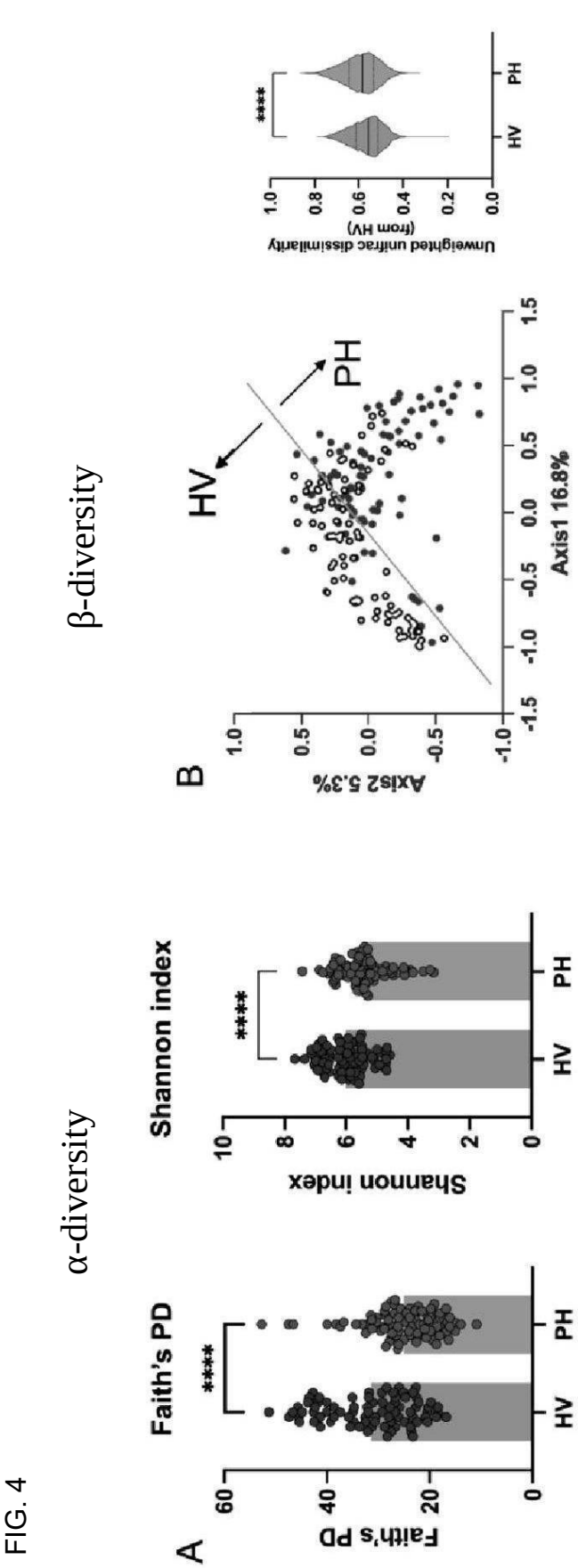

α-diversity
β-diversity
A
Faith's PD
****
HV
PH
Shannon index
B
HV
PH
Axis2 5.3%
Axis1 16.8%
Unweighted unifrac dissimilarity (from HV)

FIG. 5

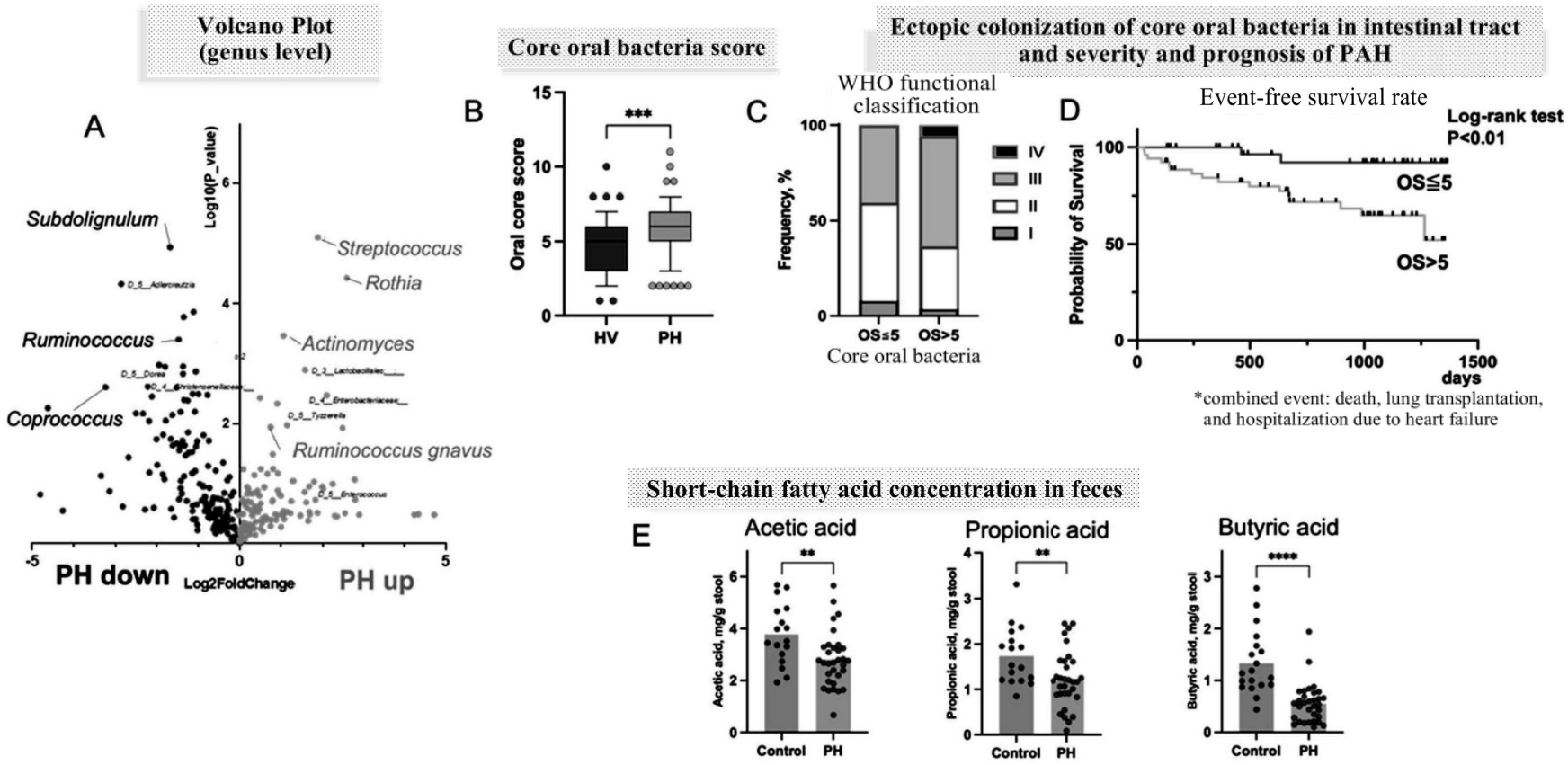

Volcano Plot
(genus level)
A
Log10(P_value)
Subdolignulum
Streptococcus
Rothia
Ruminococcus
Actinomyces
Coprococcus
Ruminococcus gnavus
PH down
Log2FoldChange
PH up
Core oral bacteria score
B
Oral core score
***
HV
PH
Ectopic colonization of core oral bacteria in intestinal tract
and severity and prognosis of PAH
C
WHO functional
classification
Frequency, %
IV
III
II
I
OS≤5
OS>5
Core oral bacteria
D
Event-free survival rate
Log-rank test
P<0.01
Probability of Survival
OS≦5
OS>5
days
*combined event: death, lung transplantation,
and hospitalization due to heart failure
Short-chain fatty acid concentration in feces
E
Acetic acid
Acetic acid, mg/g stool
**
Control
PH
Propionic acid
Propionic acid, mg/g stool
**
Control
PH
Butyric acid
Butyric acid, mg/g stool
****
Control
PH

FIG. 7

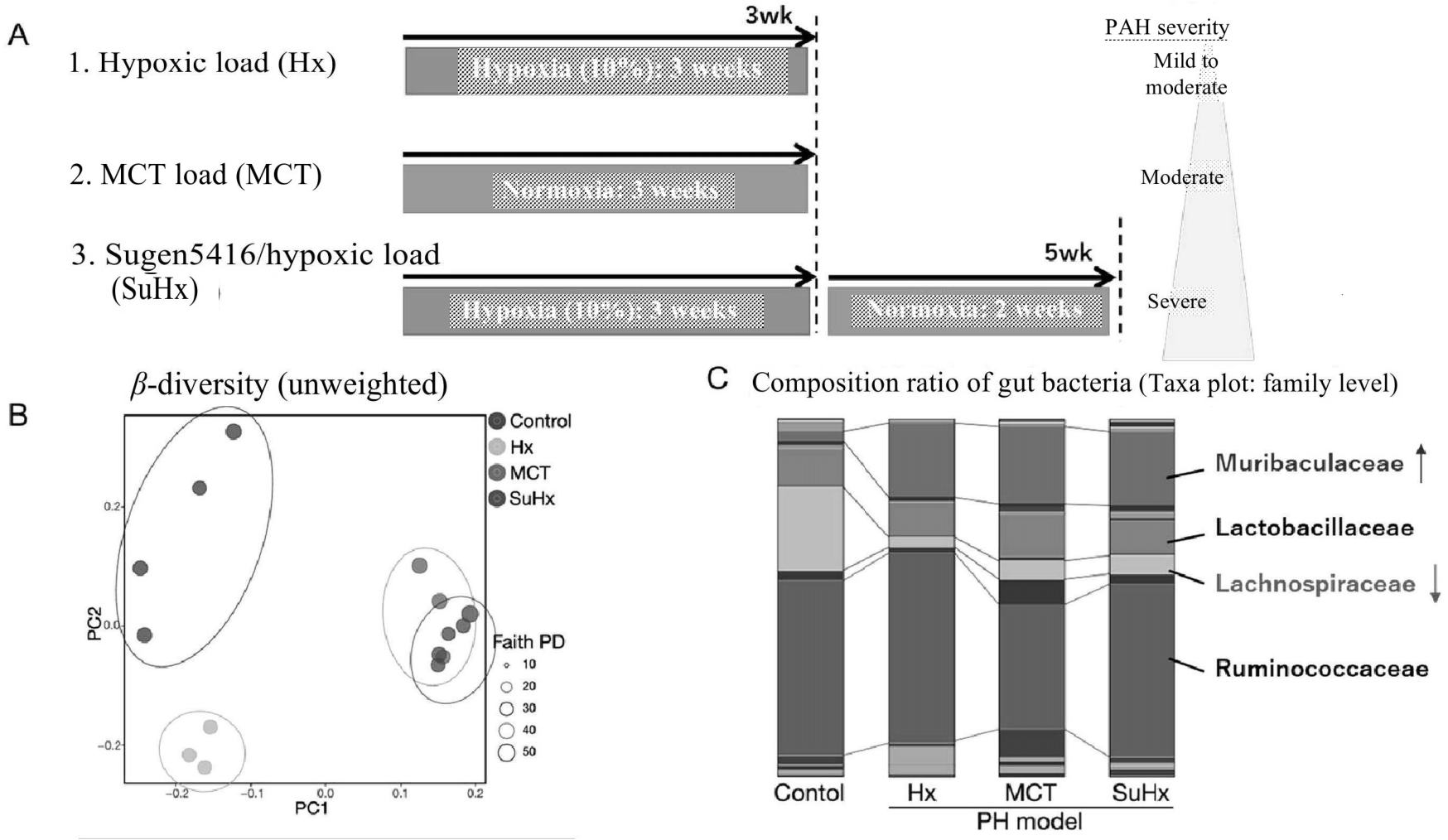

A
1. Hypoxic load (Hx)
2. MCT load (MCT)
3. Sugen5416/hypoxic load (SuHx)
3wk
5wk
Hypoxia (10%): 3 weeks
Normoxia: 3 weeks
Hypoxia (10%): 3 weeks
Normoxia: 2 weeks
PAH severity
Mild to moderate
Moderate
Severe
B
β-diversity (unweighted)
Control
Hx
MCT
SuHx
Faith PD
10
20
30
40
50
PC1
PC2
C
Composition ratio of gut bacteria (Taxa plot: family level)
Muribaculaceae
Lactobacillaceae
Lachnospiraceae
Ruminococcaceae
Contol
Hx
MCT
SuHx
PH model

FIG. 8

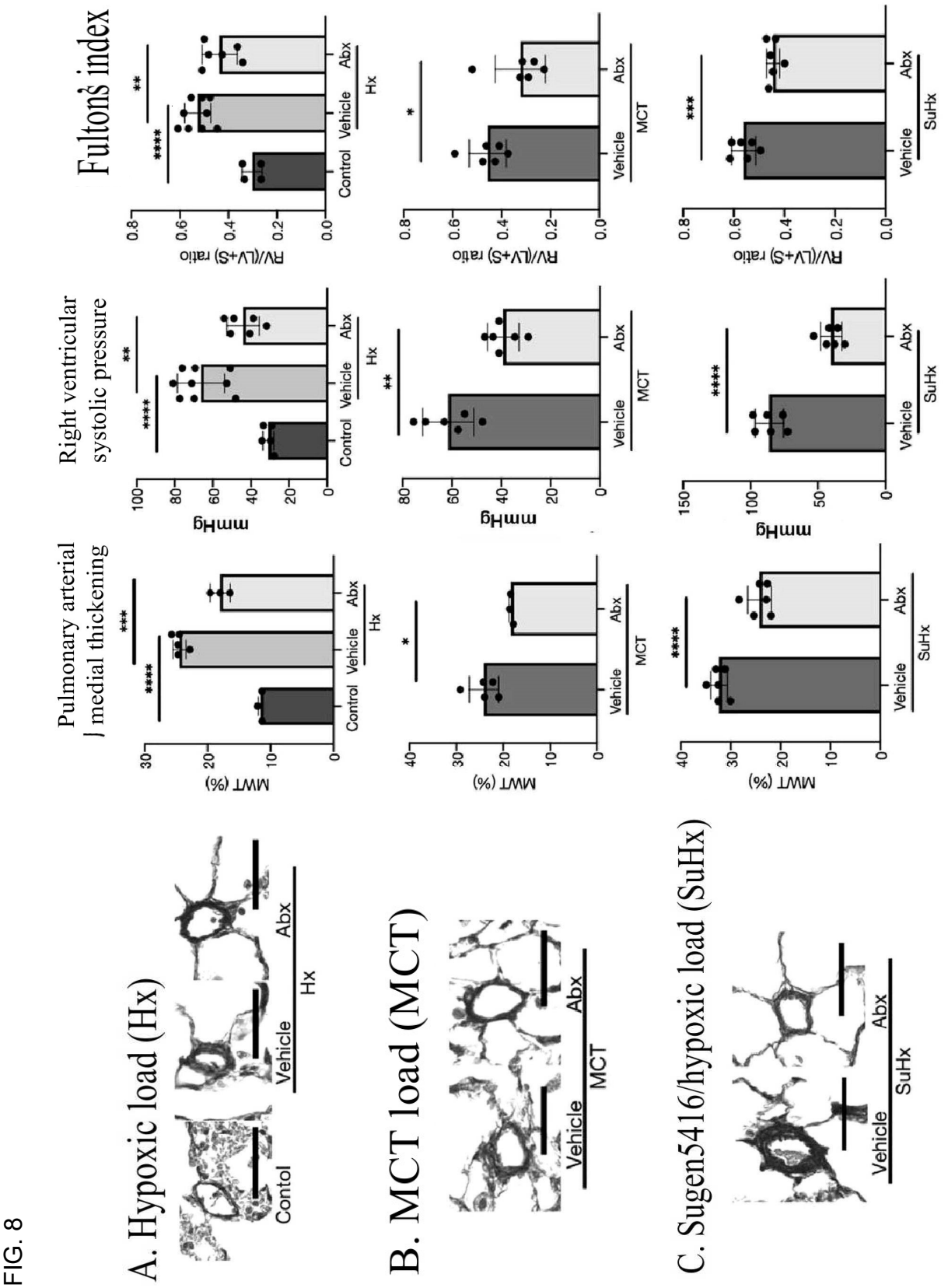

Pulmonary arterial medial thickening
Right ventricular systolic pressure
Fulton's index
A. Hypoxic load (Hx)
B. MCT load (MCT)
C. Sugen5416/hypoxic load (SuHx)
Contol
Vehicle
Abx
Hx
MCT
SuHx
MWT (%)
mmHg
RV/(LV+S) ratio
Control
Vehicle
Abx
Hx
MCT
SuHx

FIG. 9

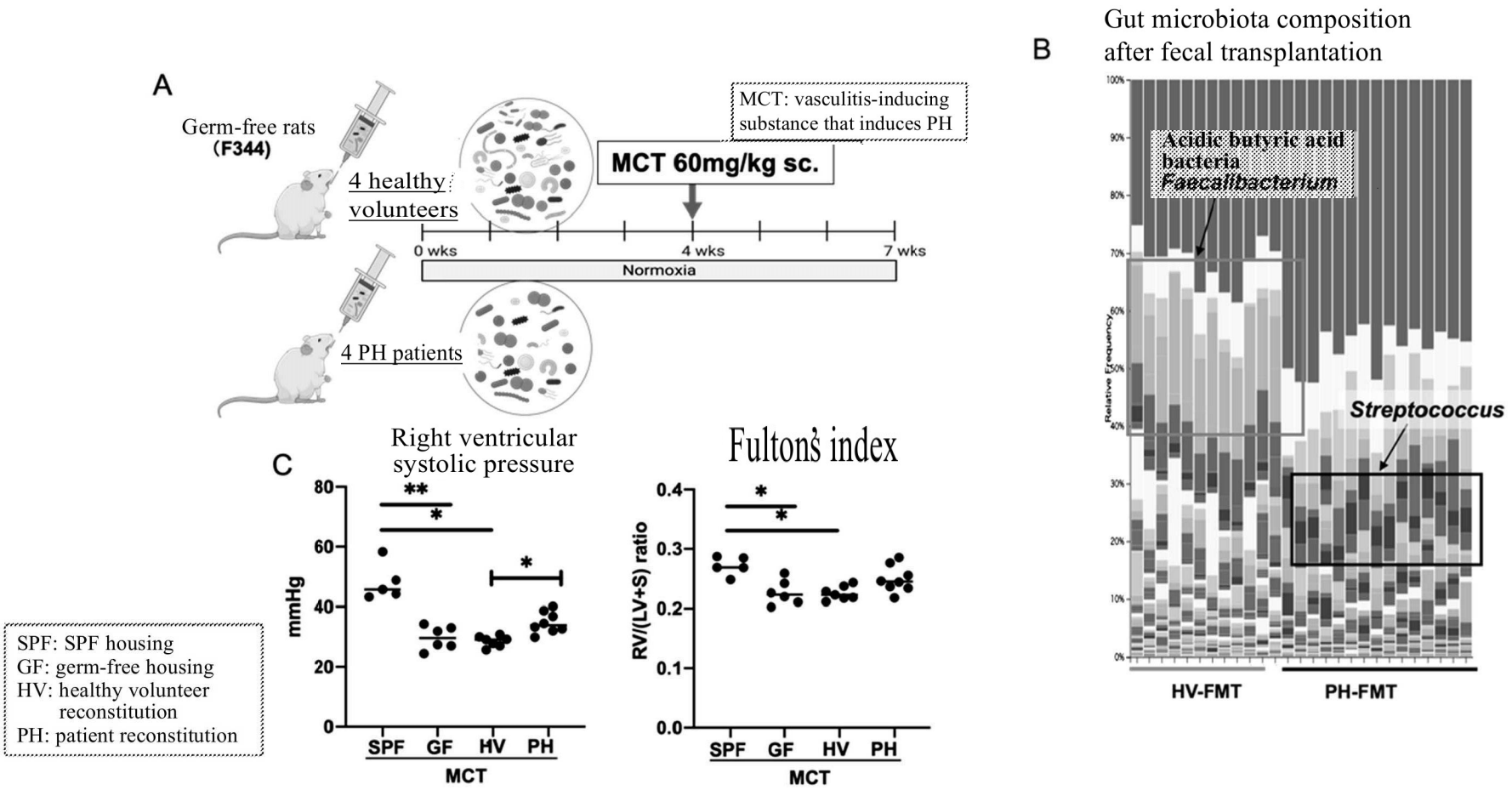

A
Germ-free rats
(F344)
4 healthy volunteers
4 PH patients
MCT: vasculitis-inducing substance that induces PH
MCT 60mg/kg sc.
0 wks
4 wks
7 wks
Normoxia
B
Gut microbiota composition after fecal transplantation
Acidic butyric acid bacteria
Faecalibacterium
Streptococcus
Relative Frequency
HV-FMT
PH-FMT
C
Right ventricular systolic pressure
mmHg
SPF GF HV PH
MCT
Fulton's index
RV/(LV+S) ratio
SPF GF HV PH
MCT
SPF: SPF housing
GF: germ-free housing
HV: healthy volunteer reconstitution
PH: patient reconstitution

FIG. 10

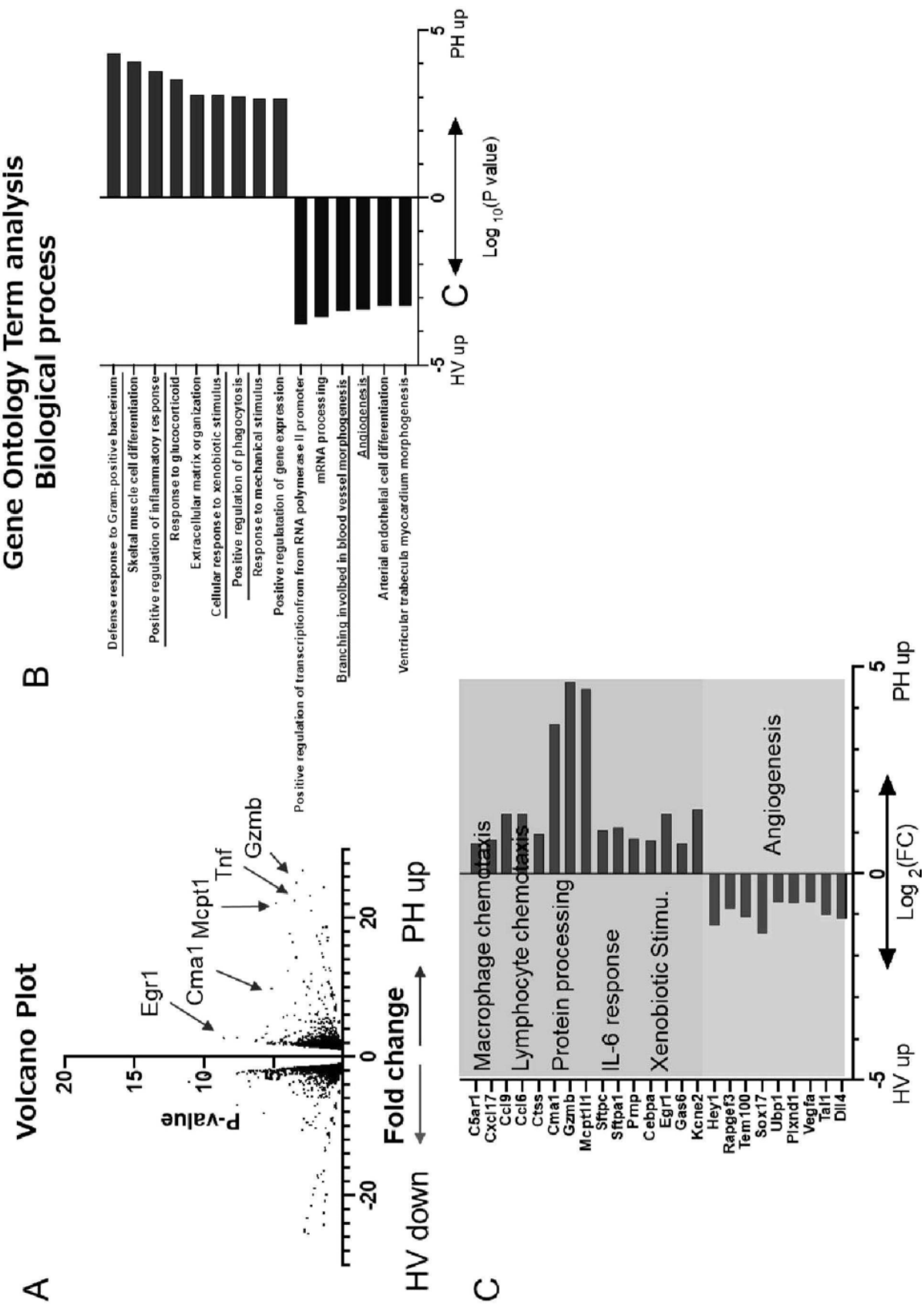

A
Volcano Plot
P-value
Egr1
Cma1
Mcpt1
Tnf
Gzmb
HV down
Fold change
PH up
B
Gene Ontology Term analysis
Biological process
Defense response to Gram-positive bacterium
Skeltal muscle cell differentiation
Positive regulation of inflammatory response
Response to glucocorticoid
Extracellular matrix organization
Cellular response to xenobiotic stimulus
Positive regulation of phagocytosis
Response to mechanical stimulus
Positive regulatation of gene expression
Positive regulation of transcriptionfrom from RNA polymerase II promoter
mRNA processing
Branching involbed in blood vessel morphogenesis
Angiogenesis
Arterial endothelial cell differentiation
Ventricular trabecula myocardium morphogenesis
HV up
PH up
Log 10(P value)
C
C5ar1
Cxcl17
Ccl9
Ccl6
Ctss
Cma1
Gzmb
Mcpt1l1
Sftpc
Sftpa1
Pmp
Cebpa
Egr1
Gas6
Kcne2
Hey1
Rapgef3
Tem100
Sox17
Ubp1
Plxnd1
Vegfa
Tal1
Dll4
Macrophage chemotaxis
Lymphocyte chemotaxis
Protein processing
IL-6 response
Xenobiotic Stimu.
Angiogenesis
HV up
PH up
Log 2(FC)

FIG. 11

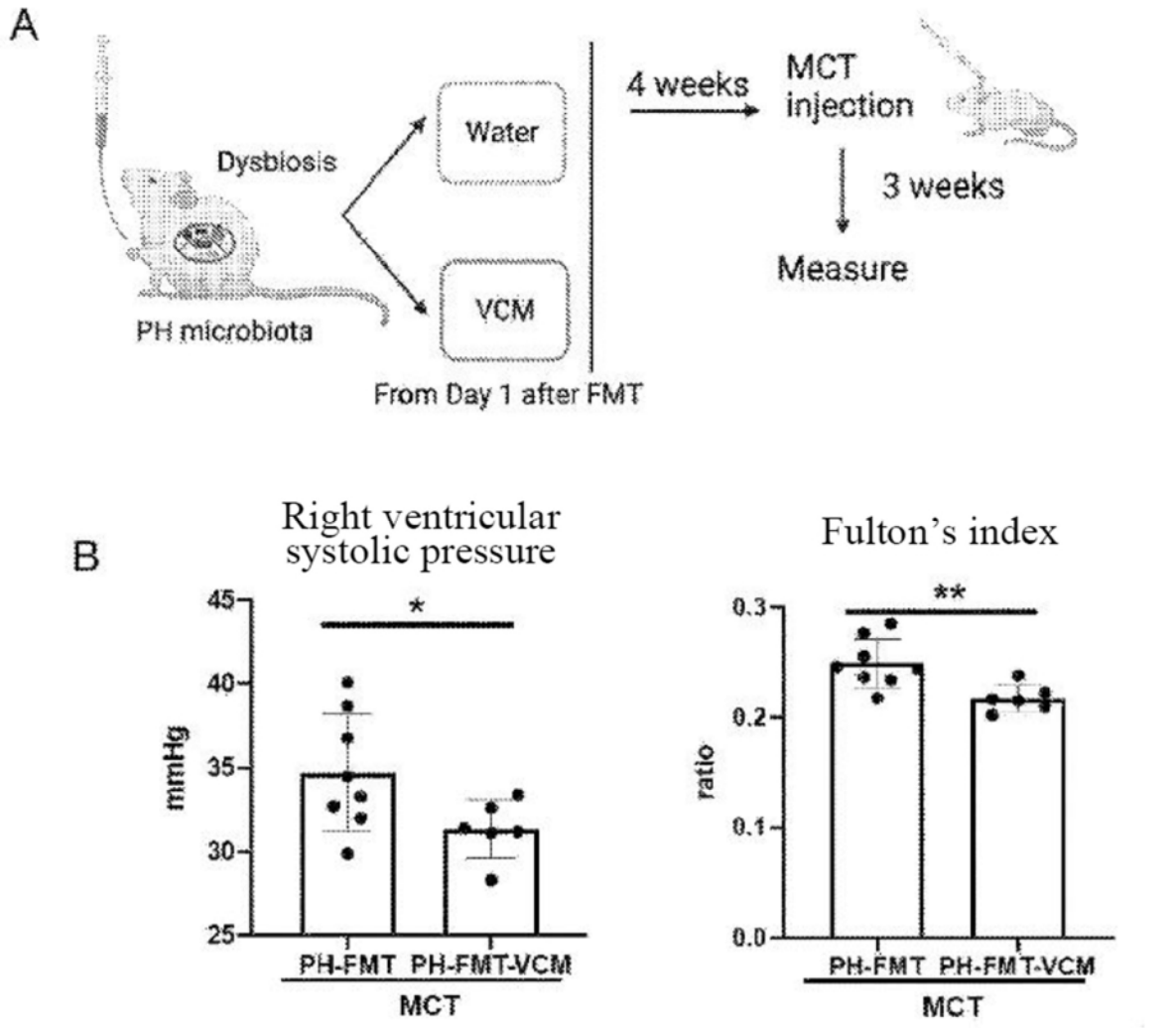
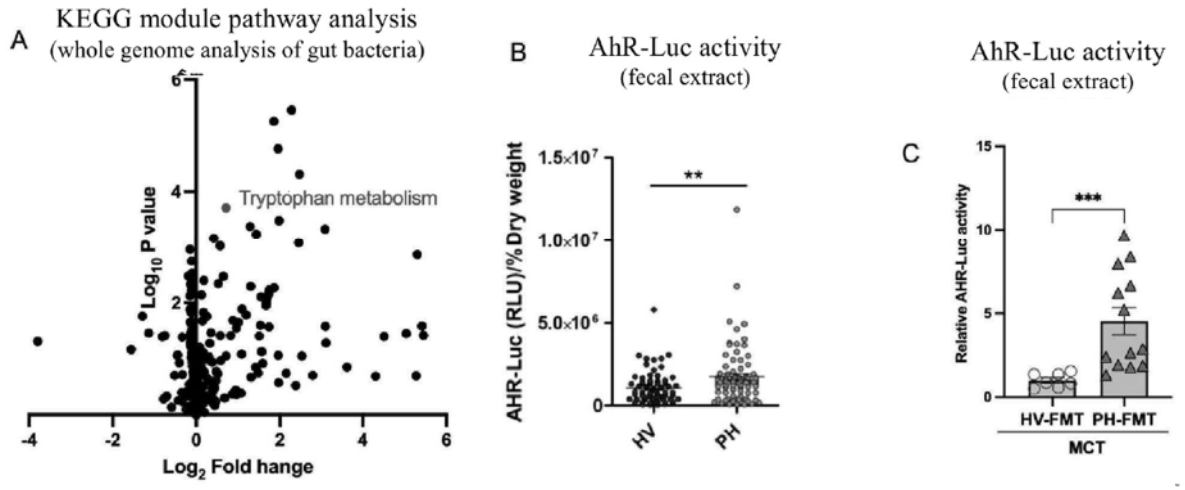

A
Dysbiosis
PH microbiota
Water
VCM
From Day 1 after FMT
4 weeks
MCT injection
3 weeks
Measure
B
Right ventricular systolic pressure
mmHg
45
40
35
30
25
*
PH-FMT
PH-FMT-VCM
MCT
Fulton's index
ratio
0.3
0.2
0.1
0.0
**
PH-FMT
PH-FMT-VCM
MCT

FIG. 12


A
KEGG module pathway analysis
(whole genome analysis of gut bacteria)
Tryptophan metabolism
Log10 P value
Log2 Fold hange
B
AhR-Luc activity
(fecal extract)
AHR-Luc (RLU)/% Dry weight
1.5×10⁷
1.0×10⁷
5.0×10⁶
0
**
HV
PH
AhR-Luc activity
(fecal extract)
C
Relative AHR-Luc activity
***
HV-FMT
PH-FMT
MCT

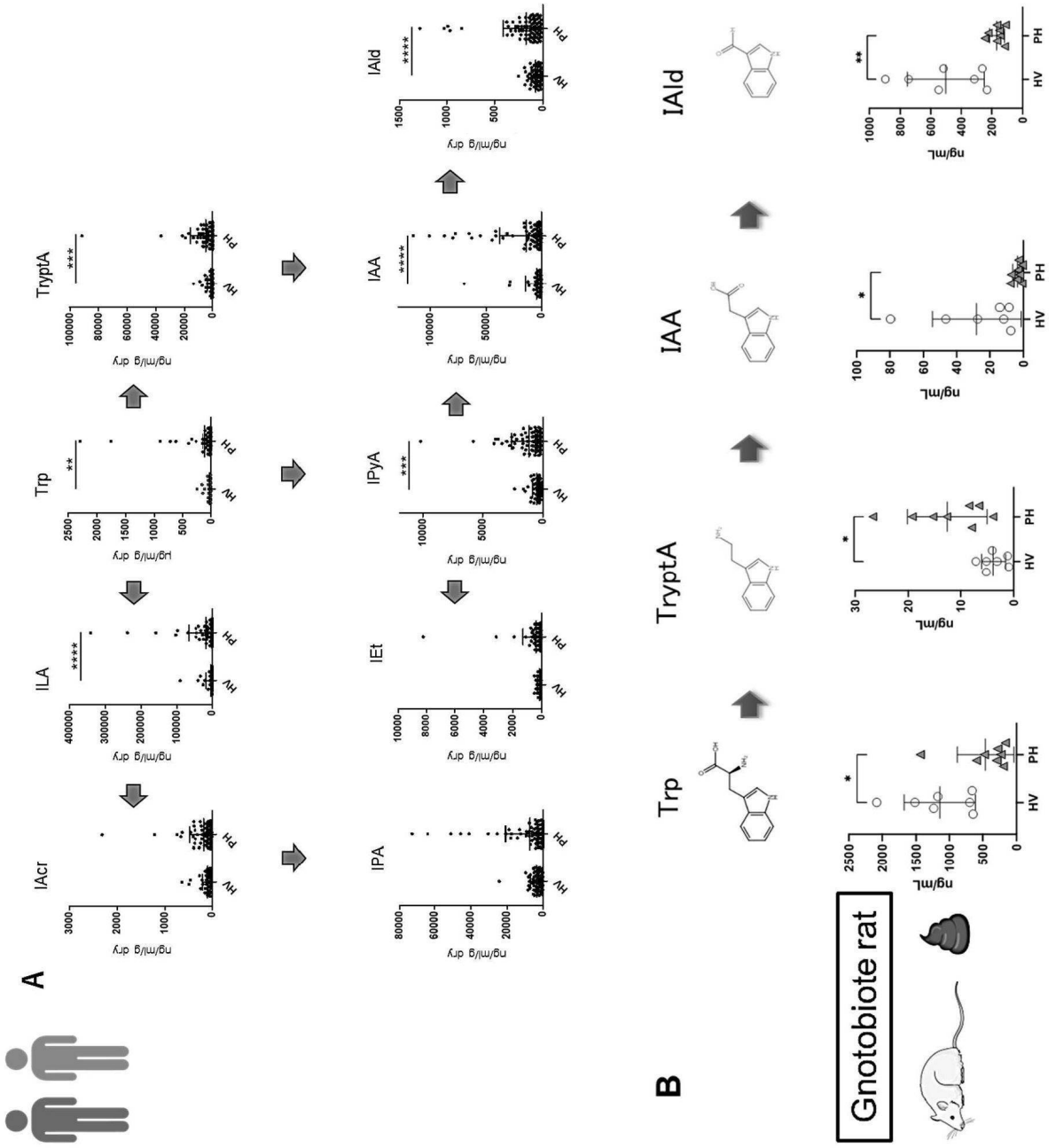

A
IAcr
ILA
Trp
TryptA
IPA
IEt
IPyA
IAA
IAld
ng/mL/g dry
HV
PH
B
Trp
TryptA
IAA
IAld
ng/mL
HV
PH
Gnotobiote rat

FIG. 13

FIG. 14

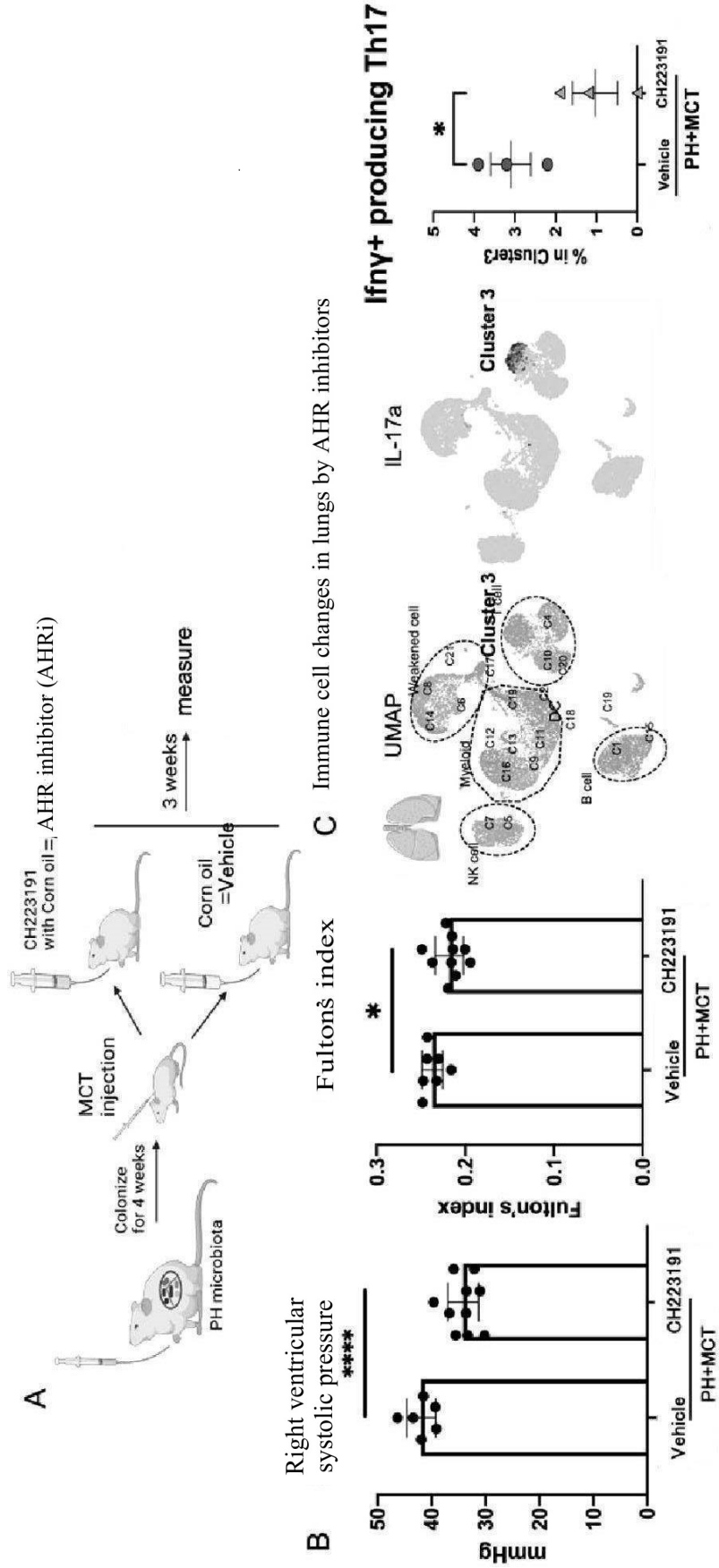

A
CH223191
with Corn oil =, AHR inhibitor (AHRi)
Colonize
for 4 weeks
PH microbiota
MCT
injection
Corn oil
=Vehicle
3 weeks
measure
B
Right ventricular
systolic pressure
****
mmHg
Vehicle
CH223191
PH+MCT
Fultonŝ index
*
Fulton's index
Vehicle
CH223191
PH+MCT
C
Immune cell changes in lungs by AHR inhibitors
UMAP
IL-17a
Cluster 3
Ifnγ+ producing Th17
% in Cluster3
*
Vehicle
CH223191
PH+MCT

FIG. 20

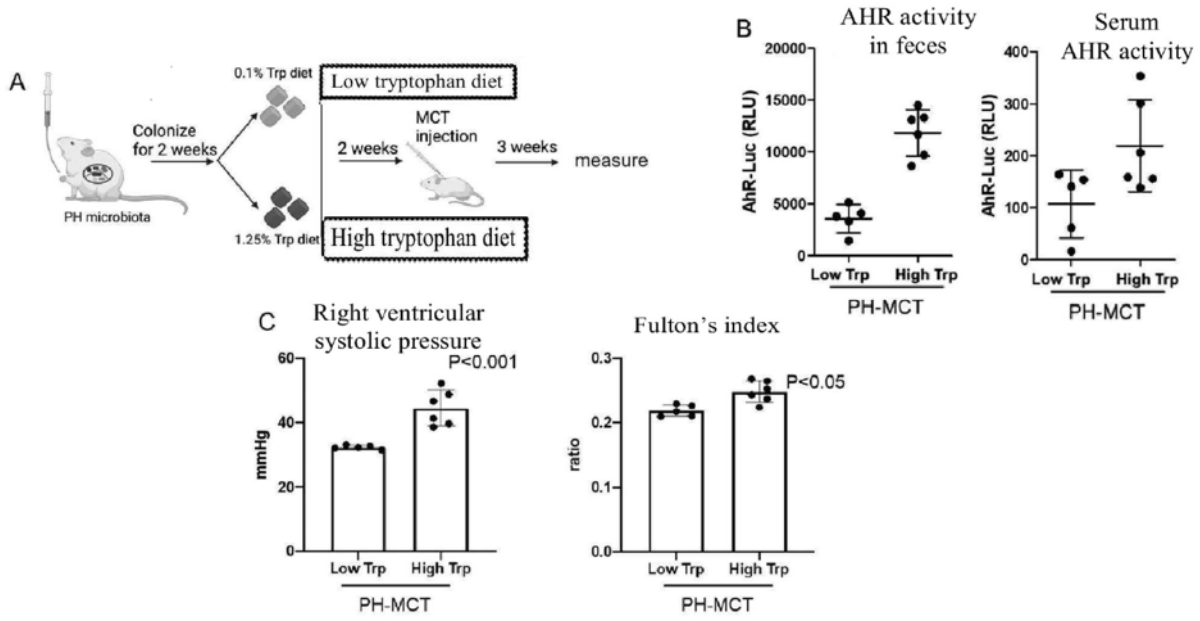

A
0.1% Trp diet
Low tryptophan diet
Colonize
for 2 weeks
MCT
injection
2 weeks
3 weeks
measure
PH microbiota
1.25% Trp diet
High tryptophan diet
B
AHR activity
in feces
AhR-Luc (RLU)
20000
15000
10000
5000
0
Low Trp
High Trp
PH-MCT
Serum
AHR activity
AhR-Luc (RLU)
400
300
200
100
0
Low Trp
High Trp
PH-MCT
C
Right ventricular
systolic pressure
P<0.001
mmHg
60
40
20
0
Low Trp
High Trp
PH-MCT
Fulton's index
P<0.05
ratio
0.3
0.2
0.1
0.0
Low Trp
High Trp
PH-MCT

FIG. 21

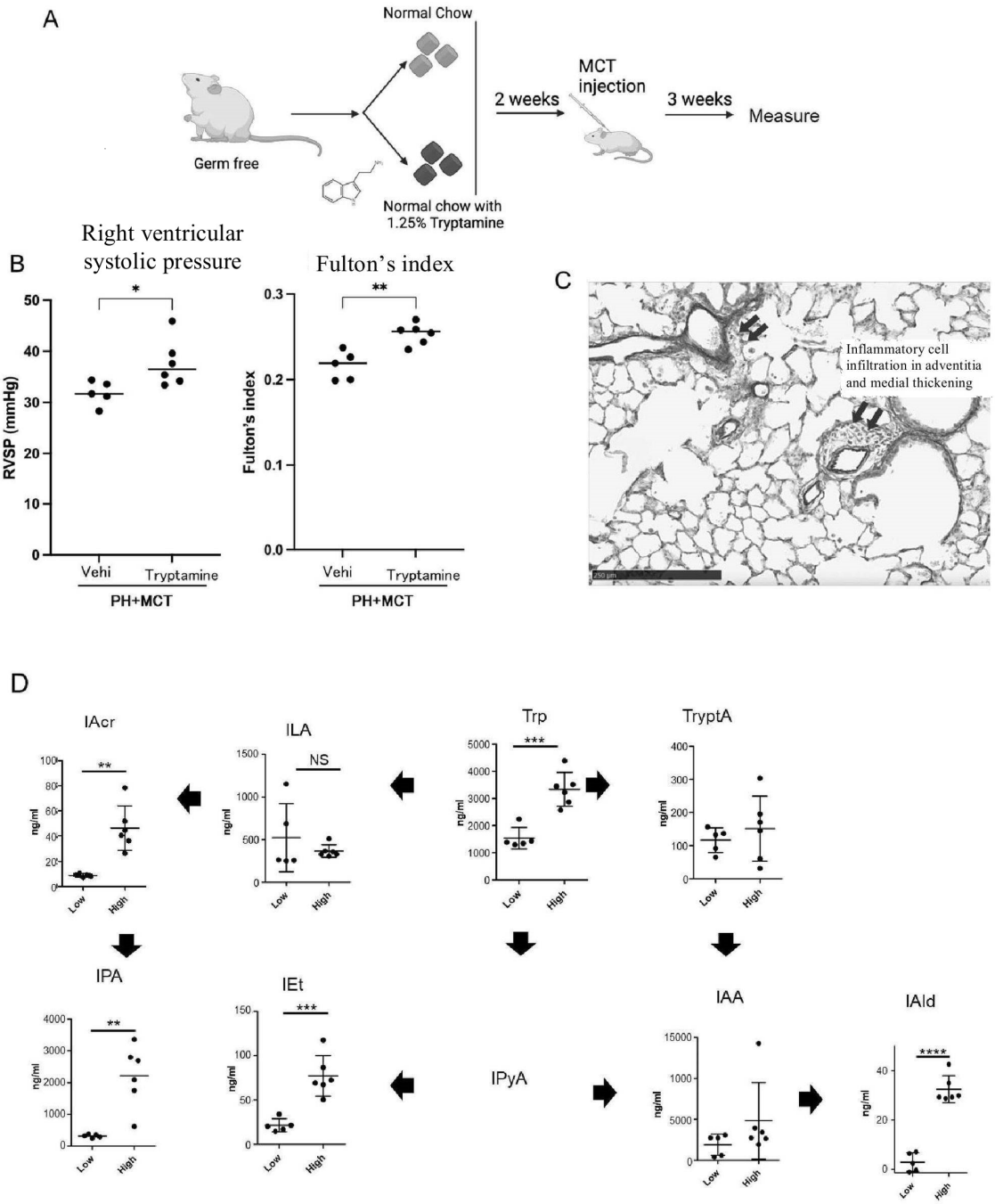
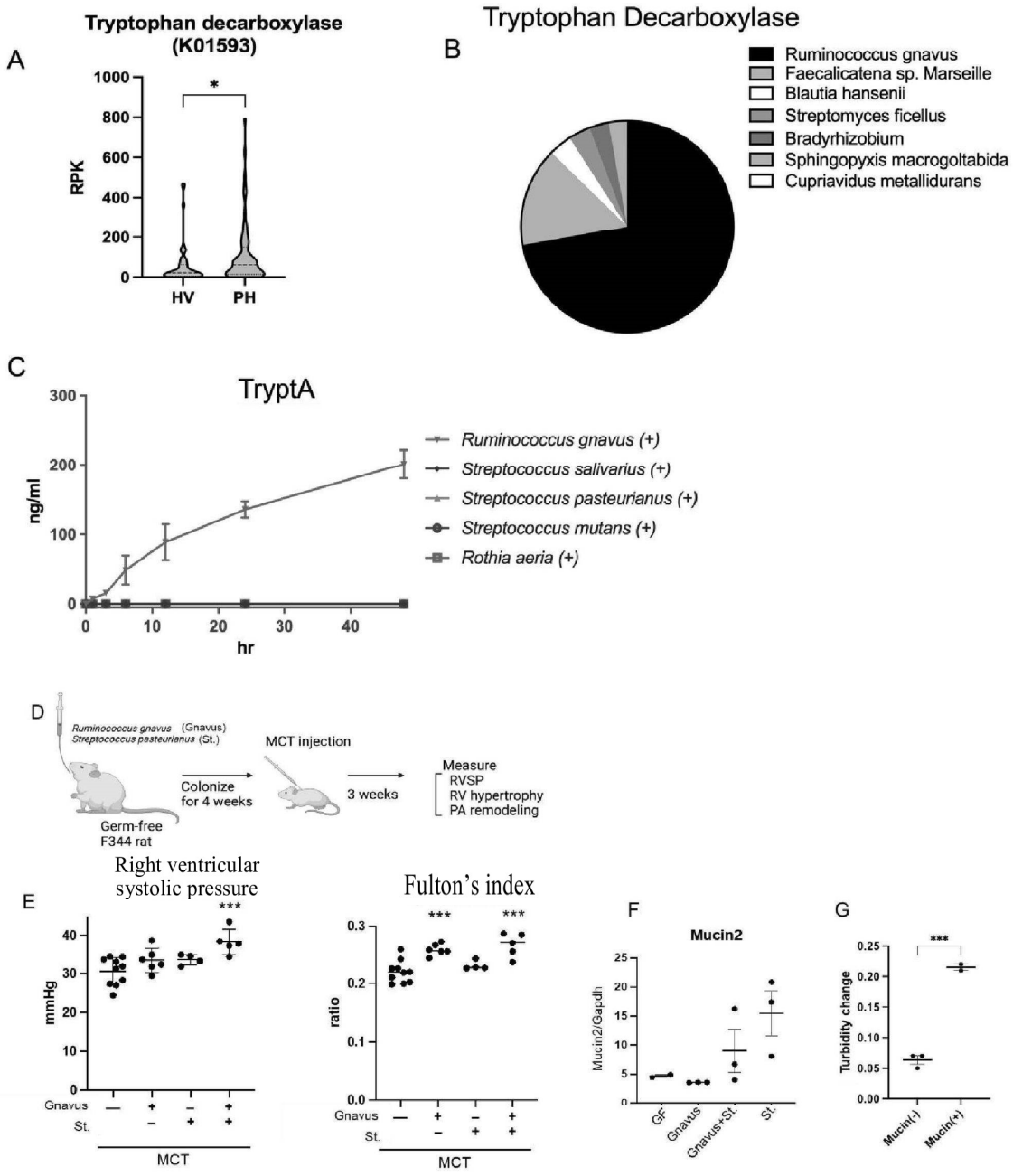

A
Normal Chow
Germ free
Normal chow with 1.25% Tryptamine
2 weeks
MCT injection
3 weeks
Measure
B
Right ventricular systolic pressure
RVSP (mmHg)
Vehi
Tryptamine
PH+MCT
Fulton's index
Fulton's index
Vehi
Tryptamine
PH+MCT
C
Inflammatory cell infiltration in adventitia and medial thickening
D
IAcr
ILA
NS
Trp
TryptA
IPA
IEt
IPyA
IAA
IAld
ng/ml
Low
High

FIG. 22-1


A
Tryptophan decarboxylase
(K01593)
RPK
1000
800
600
400
200
0
*
HV
PH
B
Tryptophan Decarboxylase
Ruminococcus gnavus
Faecalicatena sp. Marseille
Blautia hansenii
Streptomyces ficellus
Bradyrhizobium
Sphingopyxis macrogoltabida
Cupriavidus metallidurans
C
TryptA
ng/ml
300
200
100
0
0
10
20
30
40
hr
Ruminococcus gnavus (+)
Streptococcus salivarius (+)
Streptococcus pasteurianus (+)
Streptococcus mutans (+)
Rothia aeria (+)
D
Ruminococcus gnavus (Gnavus)
Streptococcus pasteurianus (St.)
Germ-free
F344 rat
Colonize
for 4 weeks
MCT injection
3 weeks
Measure
RVSP
RV hypertrophy
PA remodeling
E
Right ventricular
systolic pressure
mmHg
40
30
20
10
0
***
Gnavus
St.
MCT
Fulton's index
ratio
0.3
0.2
0.1
0.0
***
***
Gnavus
St.
MCT
F
Mucin2
Mucin2/Gapdh
25
20
15
10
5
0
GF
Gnavus
Gnavus+St.
St.
G
Turbidity change
0.25
0.20
0.15
0.10
0.05
0.00
***
Mucin(-)
Mucin(+)

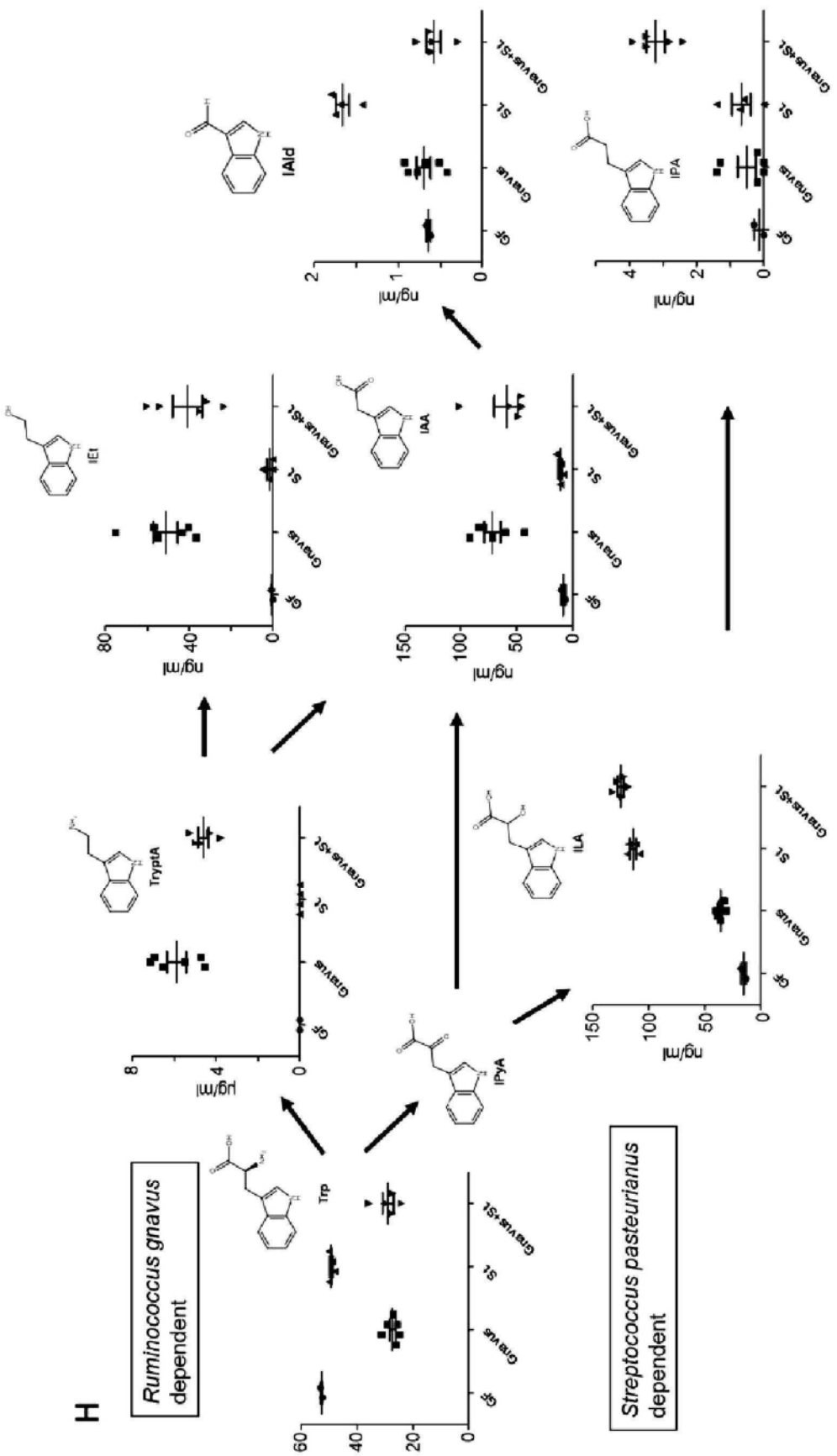

H
Ruminococcus gnavus dependent
Streptococcus pasteurianus dependent
Trp
TryptA
IPyA
ILA
IAA
IEt
IAId
IPA
μg/ml
ng/ml
GF
Gna Vus
St
Gna Vus+St

FIG. 22-2

FIG. 23

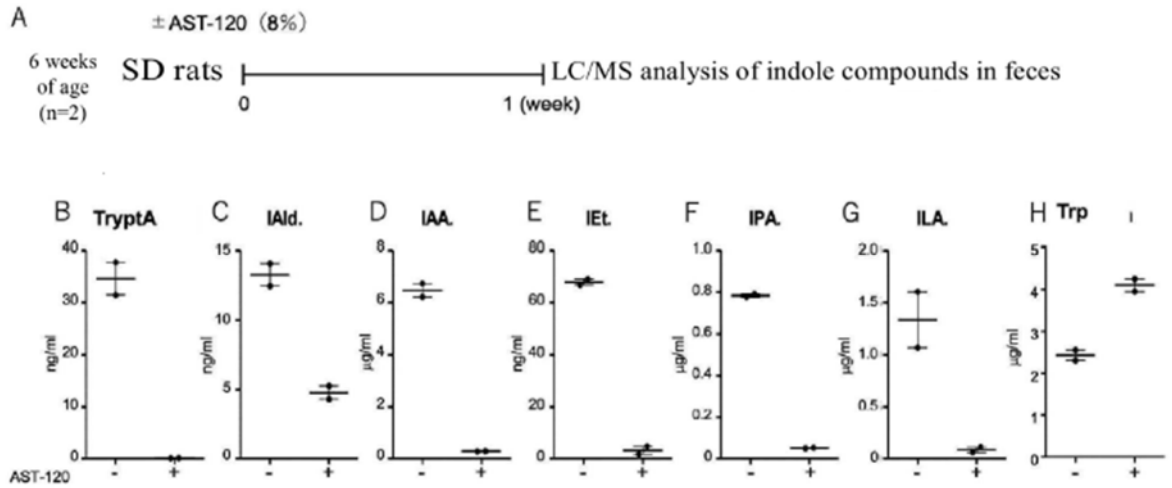
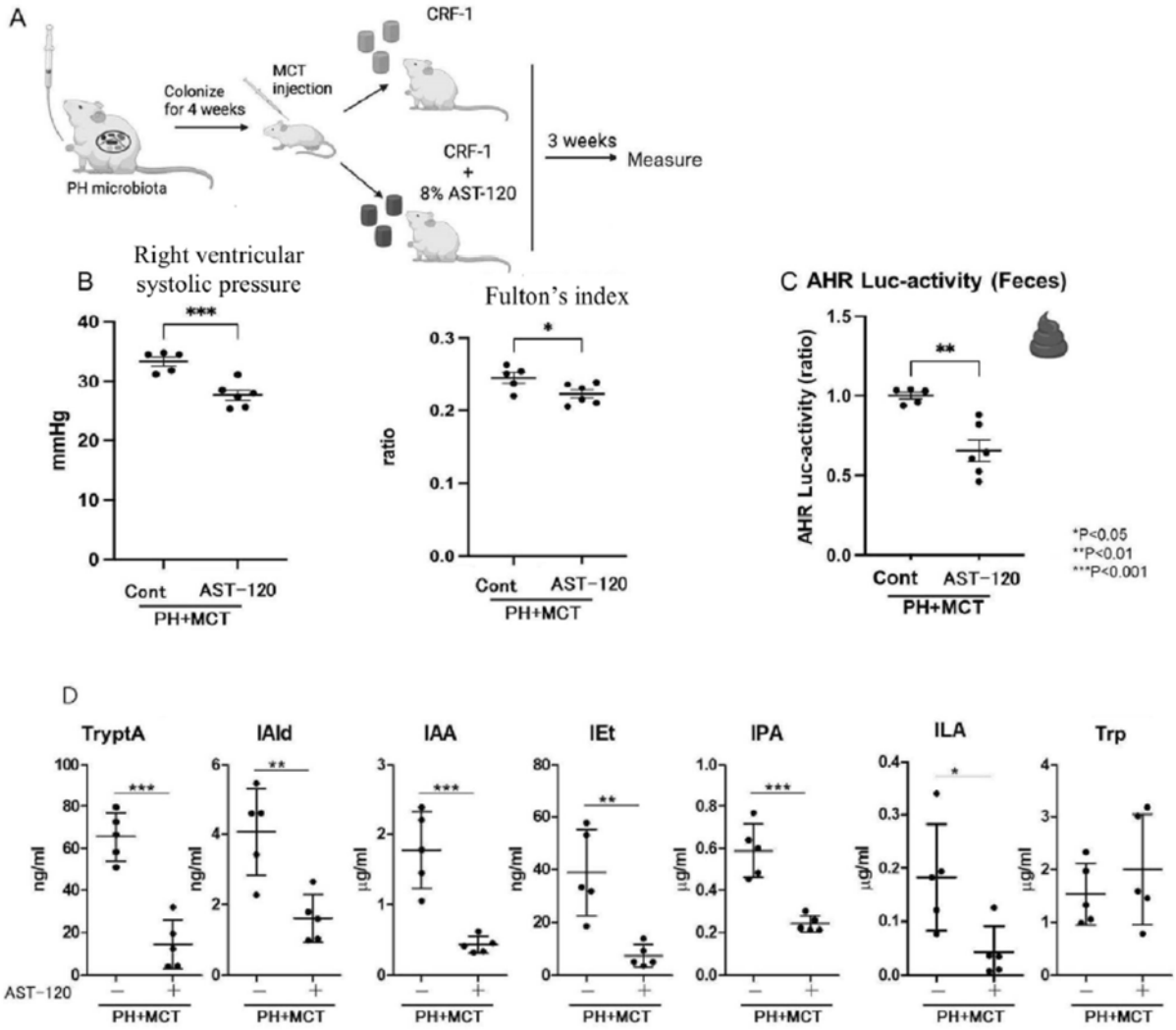

A
±AST-120 (8%)
6 weeks of age (n=2)
SD rats
0
1 (week)
LC/MS analysis of indole compounds in feces
B TryptA
C IAld.
D IAA.
E IEt.
F IPA.
G ILA.
H Trp
ng/ml
μg/ml
AST-120

FIG. 24


A
CRF-1
Colonize for 4 weeks
MCT injection
PH microbiota
CRF-1 + 8% AST-120
3 weeks
Measure
B
Right ventricular systolic pressure
mmHg
Cont
AST−120
PH+MCT
Fulton's index
ratio
C AHR Luc-activity (Feces)
AHR Luc-activity (ratio)
*P<0.05
**P<0.01
***P<0.001
D
TryptA
IAld
IAA
IEt
IPA
ILA
Trp
ng/ml
μg/ml
AST−120
PH+MCT