Supplemental Material

Vascular endothelial Tissue Factor contributes to trimethylamine N-oxide-enhanced arterial thrombosis

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Supplemental Table 1. Patient characteristics for GeneBank cohort on anti-platelets

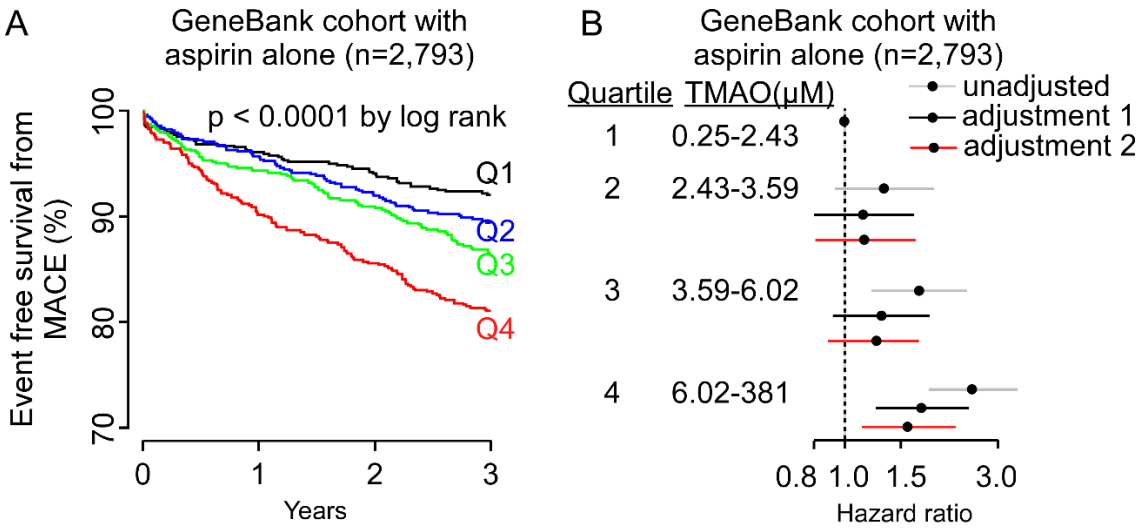
Characteristic	Anti-platelet therapy (N=2989)	Aspirin alone (N=2793)
Age (yr)	62.8±10.8	63.0±10.7
Male (%)	67.2	66.7
Diabetes (%)	32.2	32.9
Hypertension (%)	73.5	73.0
Smoking (%)	65.5	64.6
MACE 3 Years (%)	12.3	12.2
CVD (%)	84.5	83.4
CHF (%)	16.3	15.4
History of MI (%)	46.1	43.7
History of stroke (%)	6.3	6.1
BMI (kg/m^2)	28.7(25.8-32.5)	28.7(25.8-32.6)
LDL cholesterol (mg dl^{-1})	94.0(77.0-114.0)	95.0(77.0-114.0)
HDL cholesterol (mg dl^{-1})	33.8(28.1-40.2)	33.9(28.2-40.4)
Total cholesterol (mg dl^{-1})	160.0(139.0-186.1)	160.5(139.6-186.6)
Triglycerides (mg dl^{-1})	121.0(86.0-174.0)	121.0(86.0-173.0)
Follow-up anti-platelet regimen		
Aspirin (%)	99.0	100.0
Clopidogrel (%)	6.6	0.0
Ticagrelor	0.0	0.0
Prasugrel	0.0	0.0

Characteristics of patients in GeneBank receiving anti-platelet drugs or aspirin alone for whom TMAO levels were available. Continuous data are presented as mean ± standard deviation or median (interquartile range), categorical variables are presented as %. BMI, body mass index; CHF, congestive heart failure; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MACE, major adverse cardiac events; MI, myocardial infarction.

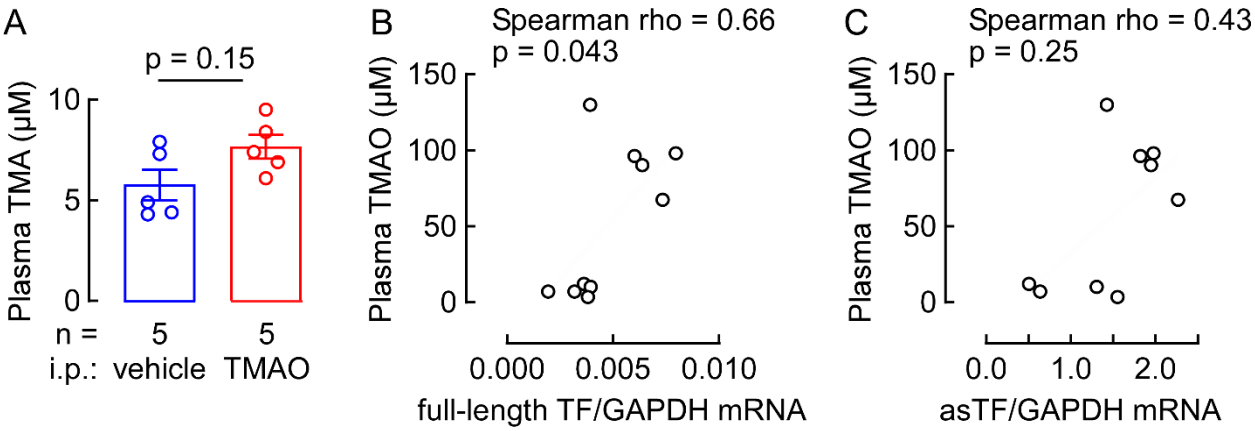
Supplemental Table 2. Patient characteristics for multi-center Swiss ACS cohort on dual anti-platelet therapy

Characteristic	Dual anti-platelet therapy (N=1469)
Age (yr)	63.4±12.3
Male (%)	77.5
Diabetes (%)	16.9
Hypertension (%)	57.5
Smoking (%)	69.9
MACE 1 Years (%)	10.2
CVD (%)	36.8
CHF (%)	1.2
History of MI (%)	14.7
History of stroke (%)	1.97
BMI (kg/m^2)	26.5(24.3-29.3)
LDL cholesterol (mg dl^{-1})	119.6(92.0-149.4)
HDL cholesterol (mg dl^{-1})	43.3(36.4-53.4)
Total cholesterol (mg dl^{-1})	189.5(158.6-220.4)
Triglycerides (mg dl^{-1})	90.3(61.1-139.1)

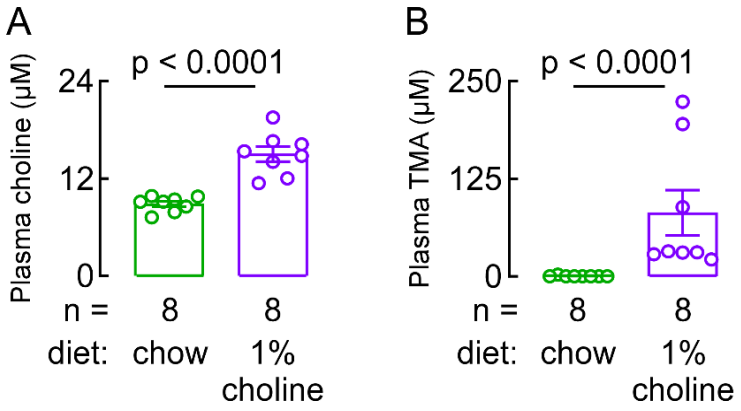
Characteristics of patients in the Swiss ACS cohort on dual anti-platelet therapy. Continuous data are presented as mean ± standard deviation or median (interquartile range), categorical variables are presented as %. BMI, body mass index; CHF, congestive heart failure; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MACE, major adverse cardiac events; MI, myocardial infarction.



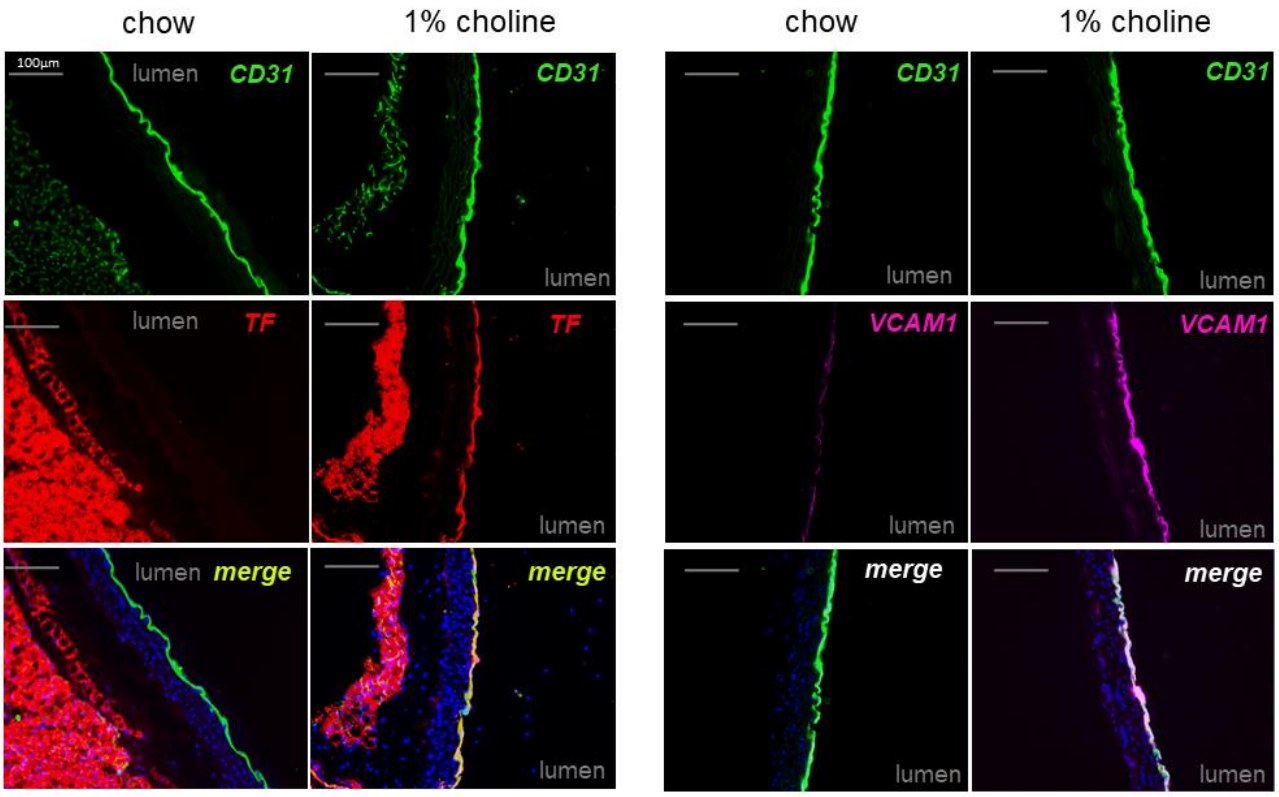
Supplemental Figure 1. A, Kaplan-Meier estimates and the risk of incident major adverse cardiac events (MACE) (MI, stroke, or death) over follow-up periods ranked by quartiles of TMAO levels in Genebank subjects with aspirin alone. **B**, Forest plots indicating the risks of incident MACE at 3 years stratified by quartiles of TMAO levels, multivariable Cox model for hazard ratio included adjustments for age, gender, hypertension, smoking, diabetes, HDL, LDL, TG (adjustment1) and in addition creatinine (adjustment2). The 5–95% confidence interval is indicated by line length.



Supplemental Figure 2. (A) TMA plasma levels of C57/BL6 mice 1.5h following i.p. injection with either vehicle or TMAO. (B and C) Correlation of plasma TMAO with aortic mRNA expression of full length TF and alternatively-spliced (as)TF. Results are presented as mean ± SEM. Both n and each data point represent individual biological replicates (animals). Differences between 2 groups were assessed using a Mann-Whitney test. Association of TMAO with TF mRNA was analyzed by Spearman correlation.

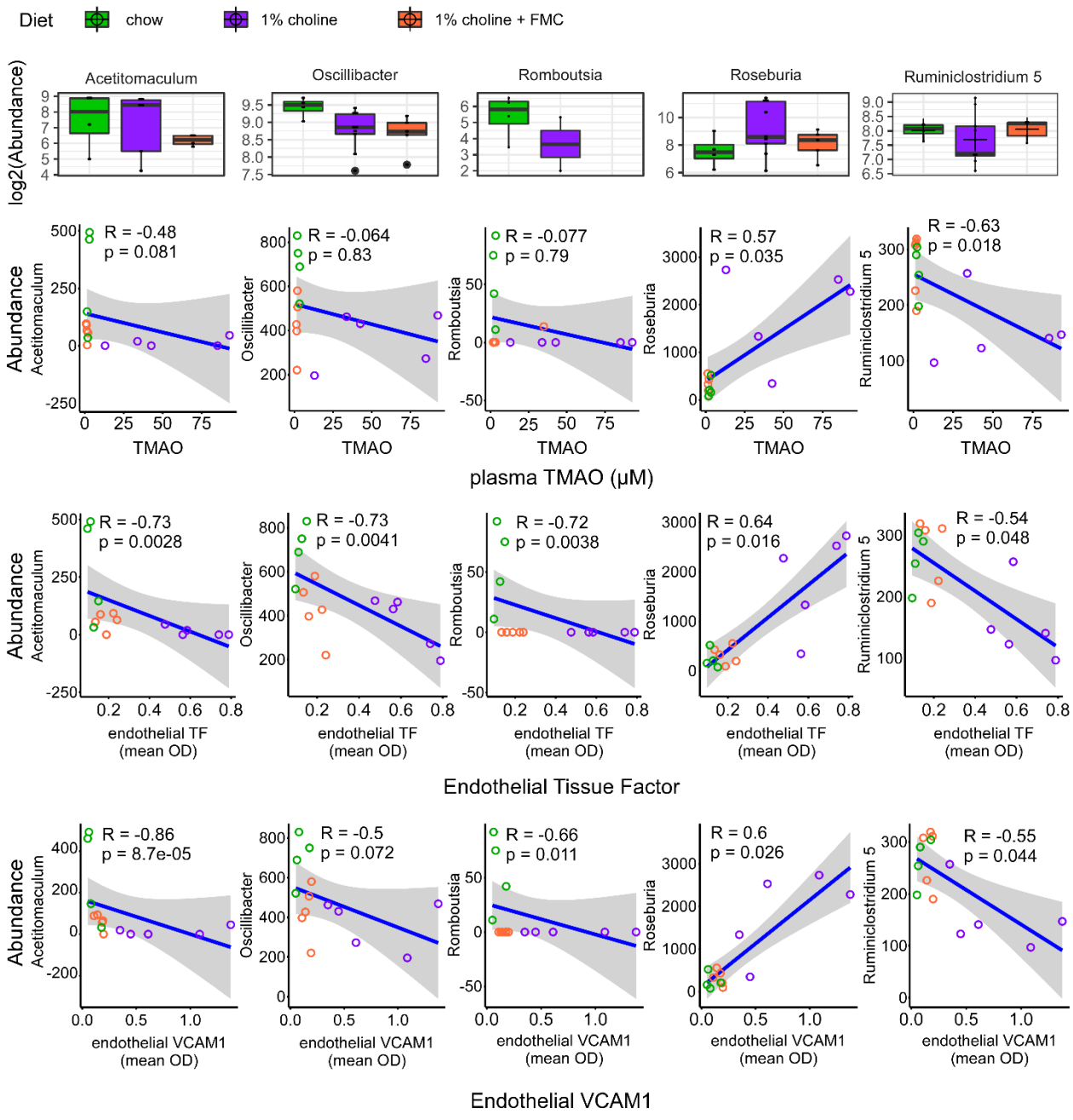


Supplemental Figure 3. (A), Plasma levels of choline and (B) TMA from C57/BL6 mice that were maintained on a control chow diet or a 1 g% choline-supplemented diet (Methods) for 10 days. Results are presented as mean ± SEM for the indicated numbers of mice. Differences between 2 groups were analyzed using a Mann-Whitney test.



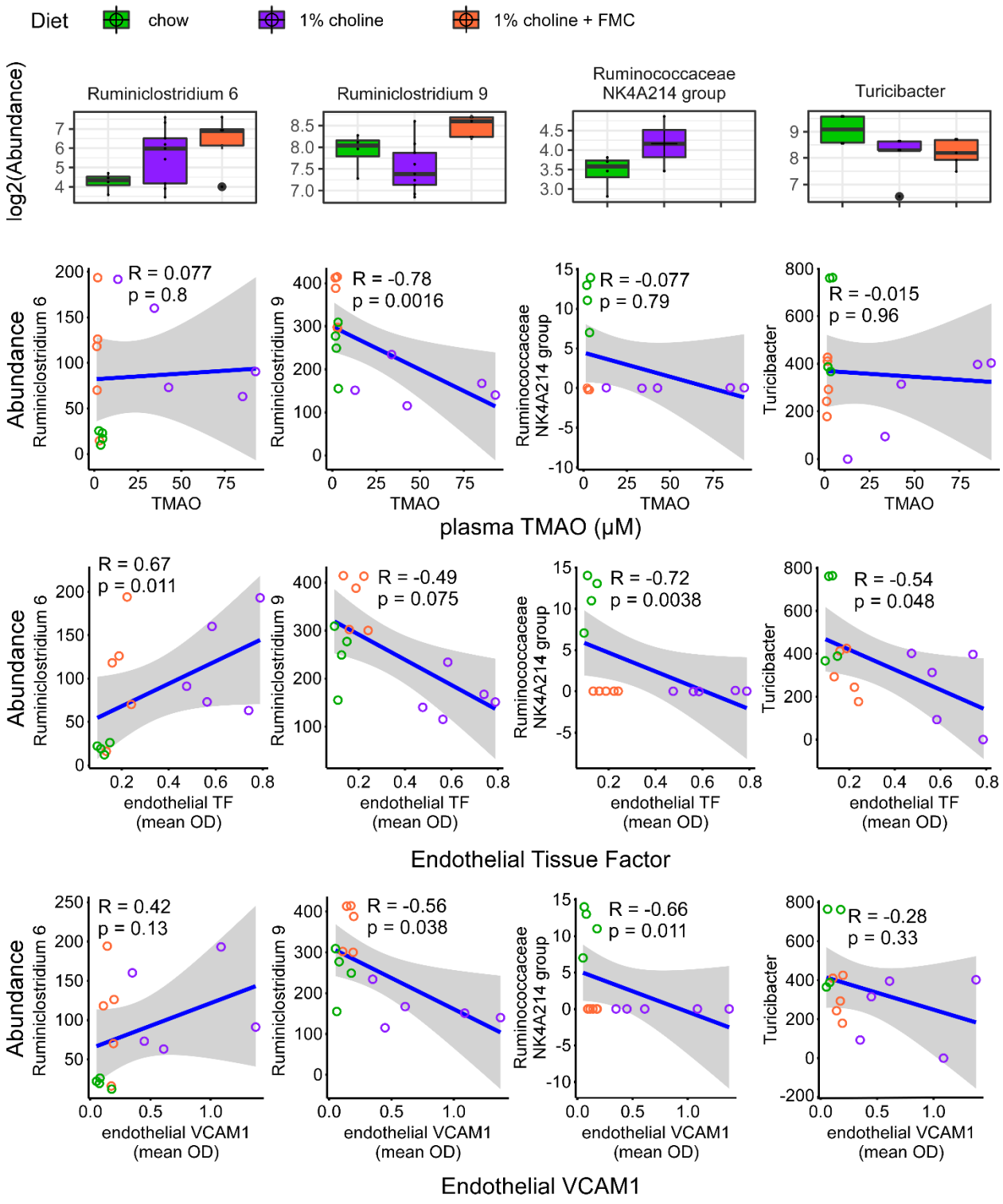
Supplemental Figure 4.

Mouse aortic tissue retrieved from animals on a chow vs. 1% choline diet was stained for TF (red), VCAM1 (purple), CD31 (green) and counterstained with DAPI (blue) as outlined in Methods. Merging of the signals confirmed that a choline-supplemented diet induced TF and VCAM1 protein in the endothelium, evidenced by co-localization with CD31.



Supplemental Figure 5. Upper panel, Statistically significant (Benjamini-Hochberg False Discovery Rate P<0.05) genera differentiating 3 groups (chow, choline, and choline+FMC). Plotted are interquartile range(IQR)s (boxes). The dark line in the box is the median, lower whiskers represent smallest observation (≥ 25% quantile - 1.5 * IQR), upper whiskers largest observation (≤ 75% quantile - 1.5 * IQR) with outliers as dots

outside of the box. **Second panel**, Scatter plots based on linear regression showing correlation between abundance of indicated genera with plasma TMAO (μM) levels, (**third panel**) endothelial TF protein and (**fourth panel**) endothelial VCAM1 protein in mouse aortas on the indicated diets, expressed as optical density (OD) within the annotated endothelial layer quantified by immunofluorescence (as described in Methods). R^2 and p values are indicated in each panel. The grey area shows the 95% confidence interval.



Supplemental Figure 6 (Extension of Supplemental Figure 5). Upper panel,

Statistically significant (Benjamini-Hochberg False Discovery Rate P<0.05) genera

differentiating 3 groups (chow, choline, and choline+FMC). Plotted are interquartile range(IQR)s (boxes). The dark line in the box is the median, lower whiskers represent smallest observation (≥ 25% quantile - 1.5 * IQR), upper whiskers largest observation (≤ 75% quantile - 1.5 * IQR) with outliers as dots outside of the box. **Second panel**, Scatter plots based on linear regression showing correlation between abundance of indicated genera with plasma TMAO (μM) levels, (**third panel**) endothelial TF protein and (**fourth panel**) endothelial VCAM1 protein in mouse aortas on the indicated diets, expressed as optical density (OD) within the annotated endothelial layer quantified by immunofluorescence (as described in Methods). R^2 and p values are indicated in each panel. The grey area shows the 95% confidence interval.