

Supplementary Information

Modulation of Colon Cancer by Nutmeg

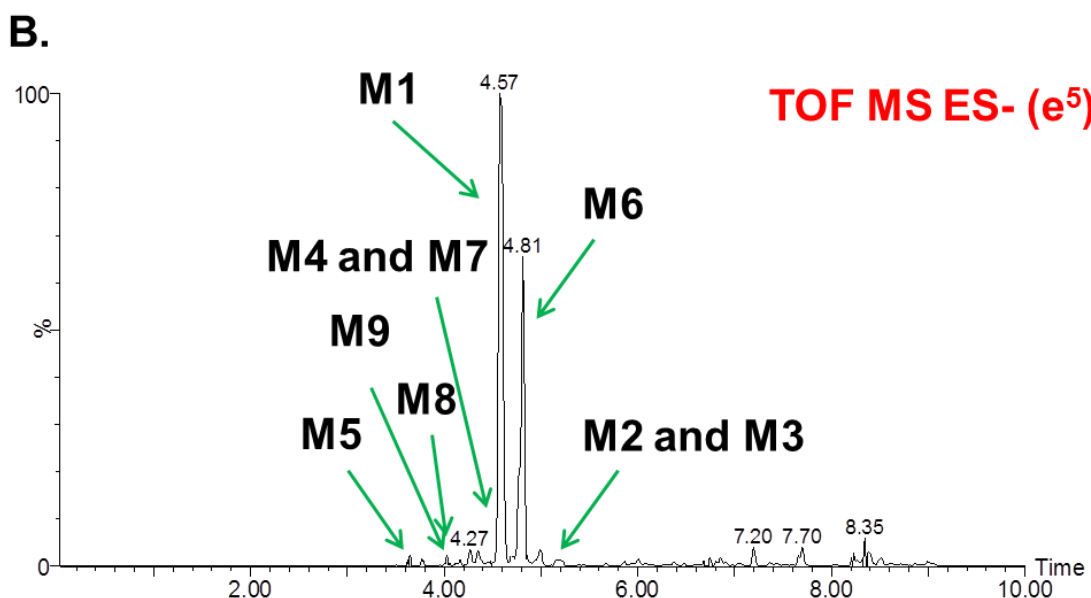
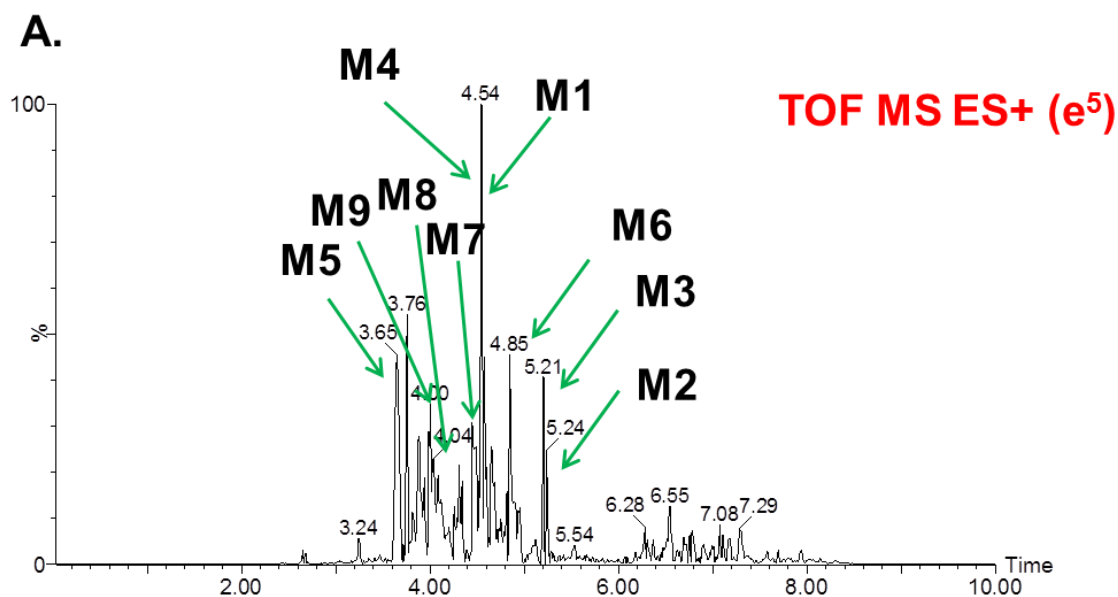
Fei Li, Xiuwei Yang, Kristopher W. Krausz, Robert G. Nichols, Wei Xu, Andrew D.

Patterson, and Frank J. Gonzalez

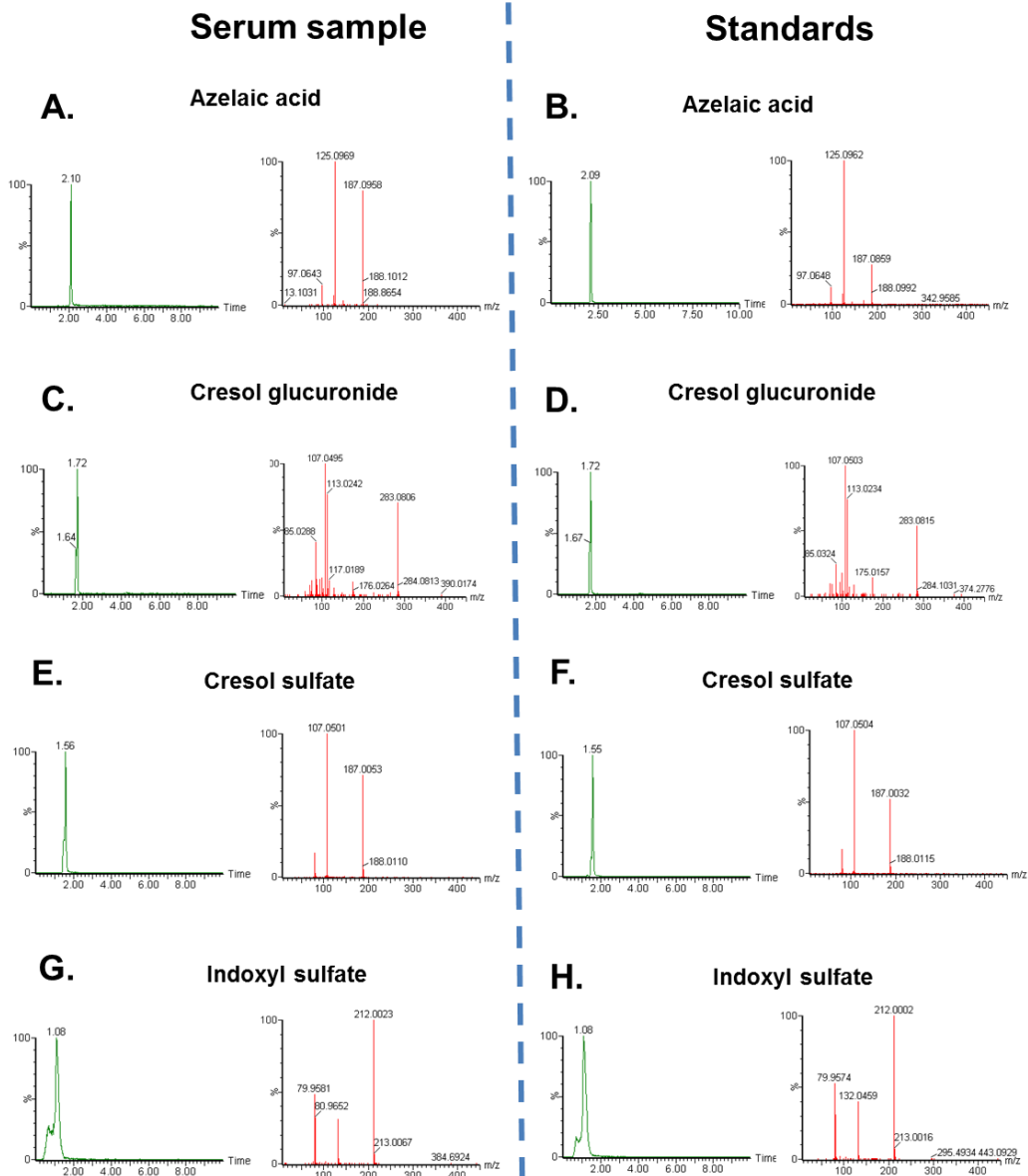
Supplementary table 1. Primer pairs used for qPCR

Gene		Accession Primer Sequence (5'-3')
Mouse Primers		
IL-6	Forward	TGATGCACTTGCAGAAAACA
	Reverse	ACCAGAGGAAATTTTCAATAGGC
Ntcp	Forward	AGGGGGACATGAACCTCAG
	Reverse	TCCGTCGTAGATTCCTTTGC
Bsep	Forward	TCTGACTCAGTGATTCTTCGCA
	Reverse	GTGTAGAGTGAAGTCCTCCTTAGC
Cyp7a1	Forward	AACAACCTGCCAGTACTAGATAGC
	Reverse	GTGTAGAGTGAAGTCCTCCTTAGC
Cpt2	Forward	TGACTGGATAGGCTGCAATGTC
	Reverse	CGAGGGCATCGAACATGTCT
Fasn	Forward	GTTGGCCCAGAACTCCTGTA
	Reverse	GTCGTCTGCCTCCAGAGC
Cyp4a10	Forward	CCAGGAACTGCATTGGGAAA
	Reverse	GACCCTGGTAGGATCTGGCA
Acot1	Forward	ATGGCAGCAGCTCCAGACTT
	Reverse	CCCAACCTCCAAACCATCAT
Beta actin	Forward	TTCTTTGCAGCTCCTTCGTT
	Reverse	ATGGAGGGGAATACAGCCC
Mcad	Forward	AGCTCTAGACGAAGCCACGA
	Reverse	GCGAGCAGAAATGAAACTCC
Fasn	Forward	GCGACATGATTAATGGACA
	Reverse	CCTGCAAATGTCAGAGGAAA
Human Primers		
Beta actin	Forward	GTTGTGACGACGAGCG
	Reverse	GCACAGAGCCTCGCCTT
Cyp4a10	Forward	GGGTGGAACCTTGACCTTCAG
	Reverse	TAAGGGACGTTTCAAGTTGTG
Fasn	Forward	TCTCCGACTCTGGCAGCTT
	Reverse	GCTCCAGCCTCGCTCTC
Cpt2	Forward	CGGAGTCTCGAGCAGATAGG
	Reverse	GGAAAAGAACTGCATGAGCA

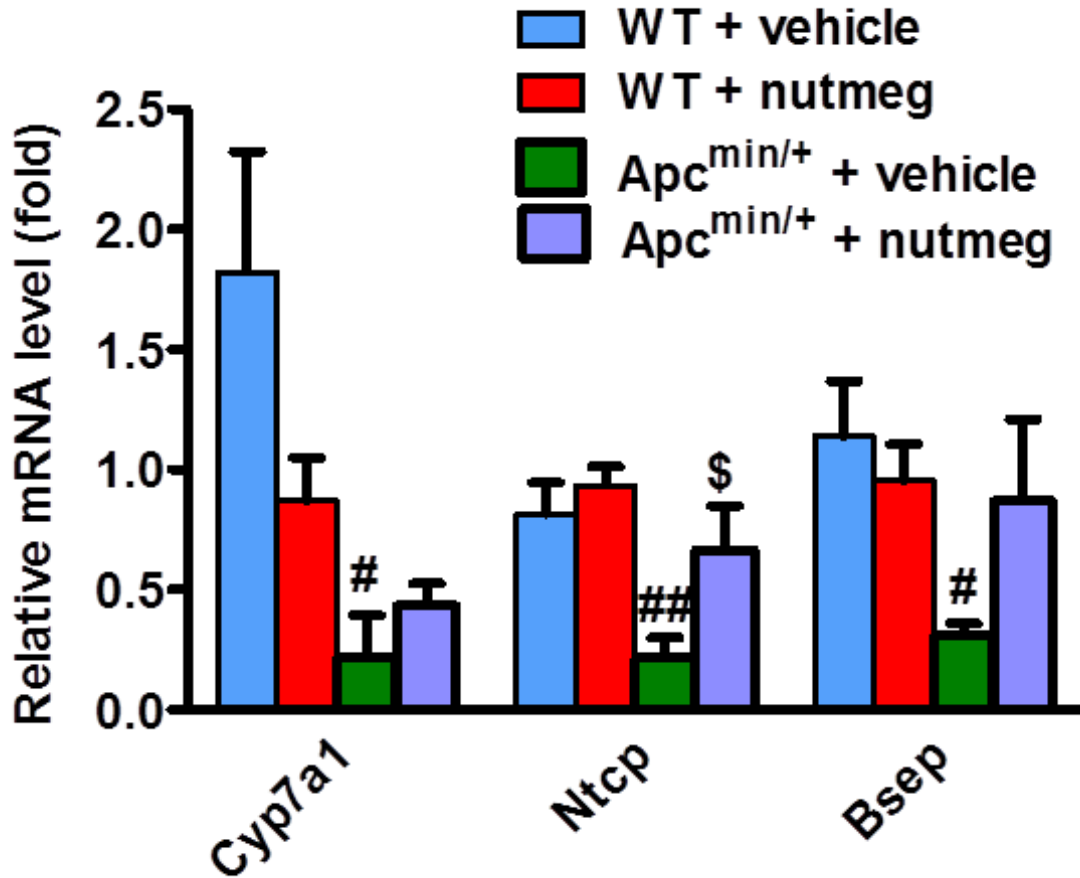
Supplementary figure 1. Chemical composition of supercritical nutmeg extract. (A) Representative positive mode (ES+) chromatograms of major lignans in nutmeg extract. (B) Representative negative mode (ES-) chromatograms of major lignans in nutmeg extract. Dehydrodiisoeugenol (M1), licarin B (M2), 2,3-dihydro-7-methoxy-2-(3-methoxy-4,5-methylenedioxyphenyl)-3-methyl-5-(E)-propenyl-benzofuran (M3), 5-methoxy-dehydrodiisoeugenol (M4), myrislignan (M5), erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl)propane (M6), erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)propane (M7), erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl)propan-1-ol (M8), erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)propan-1-ol (M9).



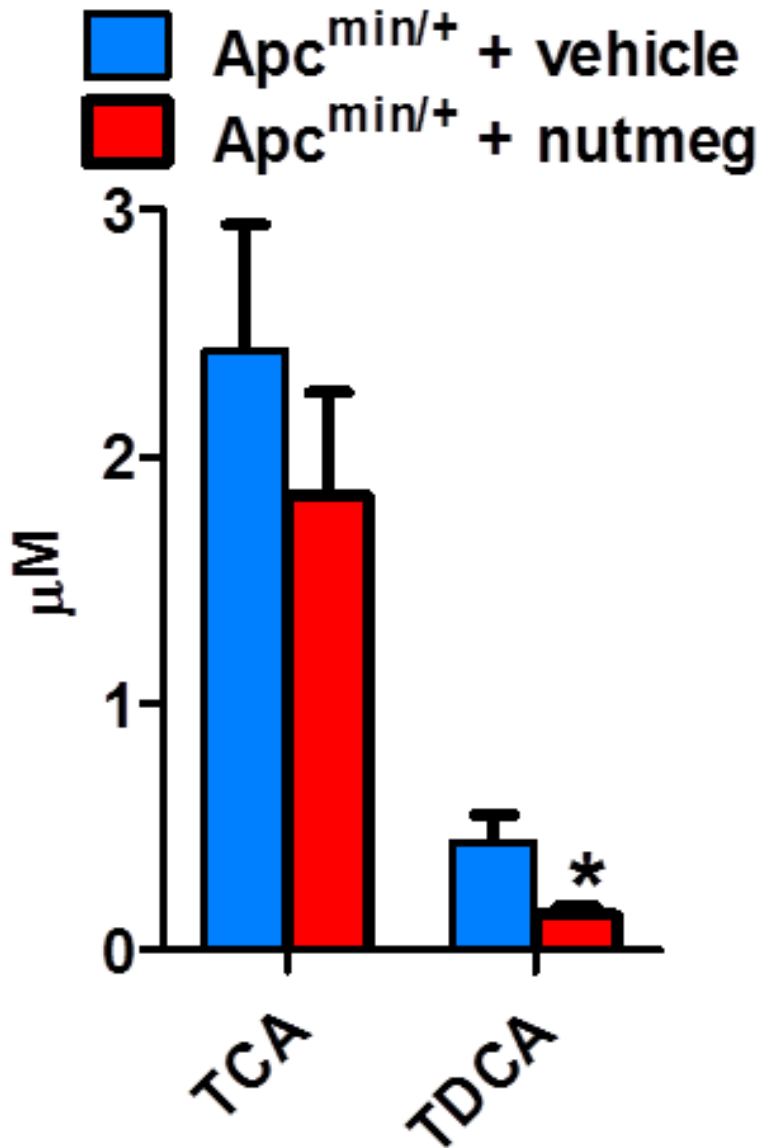
Supplementary figure 2. Identification of the altered metabolites in the serum of *Apc^{min/+}* mice. (A-B) Retention time and MS/MS fragmentation patterns of azelaic acid in the serum and standard. (C-D) Retention time and MS/MS fragmentation patterns of cresol glucuronide in the serum and standard. (E-F) Retention time and MS/MS fragmentation patterns of cresol sulfate in the serum and standard. (G-H) Retention time and MS/MS fragmentation patterns of indoxyl sulfate in the serum and standard.



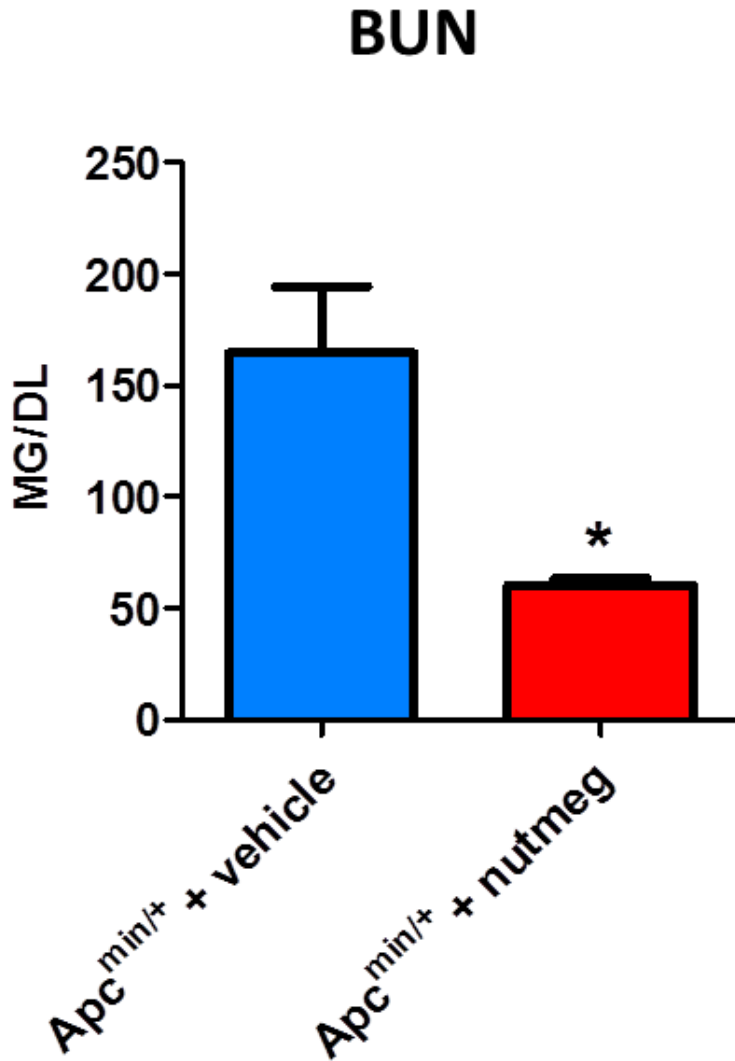
Supplementary figure 3. The metabolism of bile acid is inhibited in *Apc^{min/+}* mice and repaired in nutmeg-fed *Apc^{min/+}* mice. Hepatic mRNA analysis of the genes associated with bile acid synthesis and transport in *Apc^{min/+}* mice and nutmeg-fed *Apc^{min/+}* mice compared to the control group. The mRNA levels were normalized to those of β -actin mRNA. Statistical analysis was performed using two-tailed Student's t-test and ANOVA (n = 4/5 in each group). #, $P < 0.05$ and ##, $P < 0.01$ compared with WT mice; \$, $P < 0.05$ compared with vehicle-fed *Apc^{min/+}* mice.



Supplementary figure 4. Bile acids in *Apc^{min/+}* mice are improved by nutmeg treatment. Serum levels of TCA and TDCA in nutmeg-fed *Apc^{min/+}* mice compared with vehicle-fed *Apc^{min/+}* mice. Statistical analysis was performed using two-tailed Student's t-test and ANOVA (n = 4/5 in each group). *, $P < 0.05$ compared with vehicle-fed *Apc^{min/+}* mice.

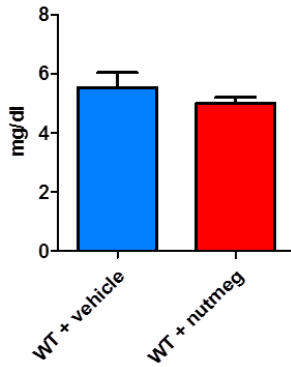


Supplementary figure 5. Improvement of serum chemistry parameter in nutmeg-fed *Apc^{min/+}* mice. The levels of BUN in the serum of nutmeg-fed *Apc^{min/+}* mice and vehicle-fed *Apc^{min/+}* mice. Statistical analysis was performed using two-tailed Student's t-test and ANOVA (n = 4/5 in each group). *, $P < 0.05$ compared with vehicle-fed *Apc^{min/+}* mice.

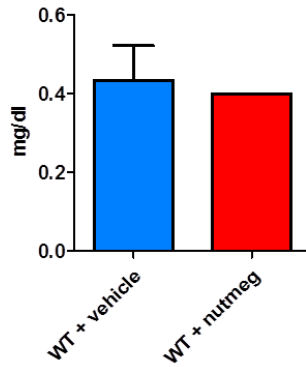


Supplementary figure 6. The change of serum parameters following 4 month treatment with nutmeg extracts. The levels of total protein (A), total bilirubin (B), phosphorus (C), calcium (D), potassium (E), total fatty acids (F), and triglyceride (G) in the serum of nutmeg-fed WT mice (WT + nutmeg) and vehicle-fed WT mice (Vehicle + nutmeg).

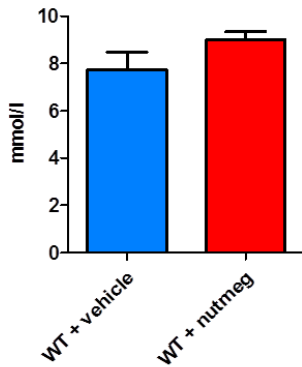
A. Total protein



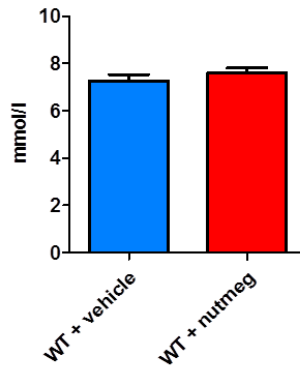
B. Total bilirubin



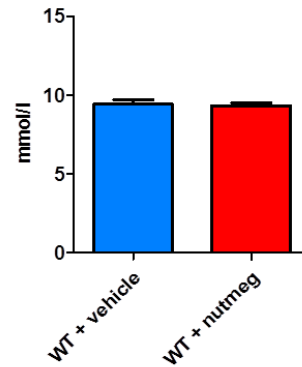
C. Phosphorus



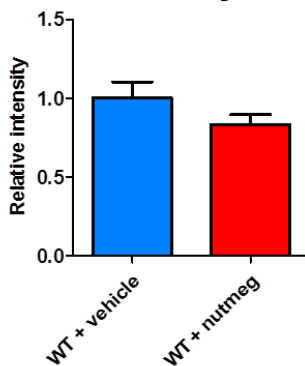
D. Calcium



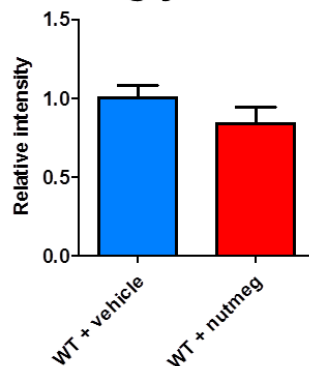
E. Potassium



F. Total fatty acids



G. Triglyceride



Supplementary figure 7. Urinary excretion of uremic toxins in *Apc^{min/+}* mice. The levels of cresol sulfate, cresol glucuronide, indoxyl sulfate, and phenyl sulfate in the urine of *Apc^{min/+}* and wild-type (WT) mice. Statistical analysis was performed using two-tailed Student's t-test and ANOVA (n = 4/5 in each group). *, $P < 0.05$ compared with WT mice.

