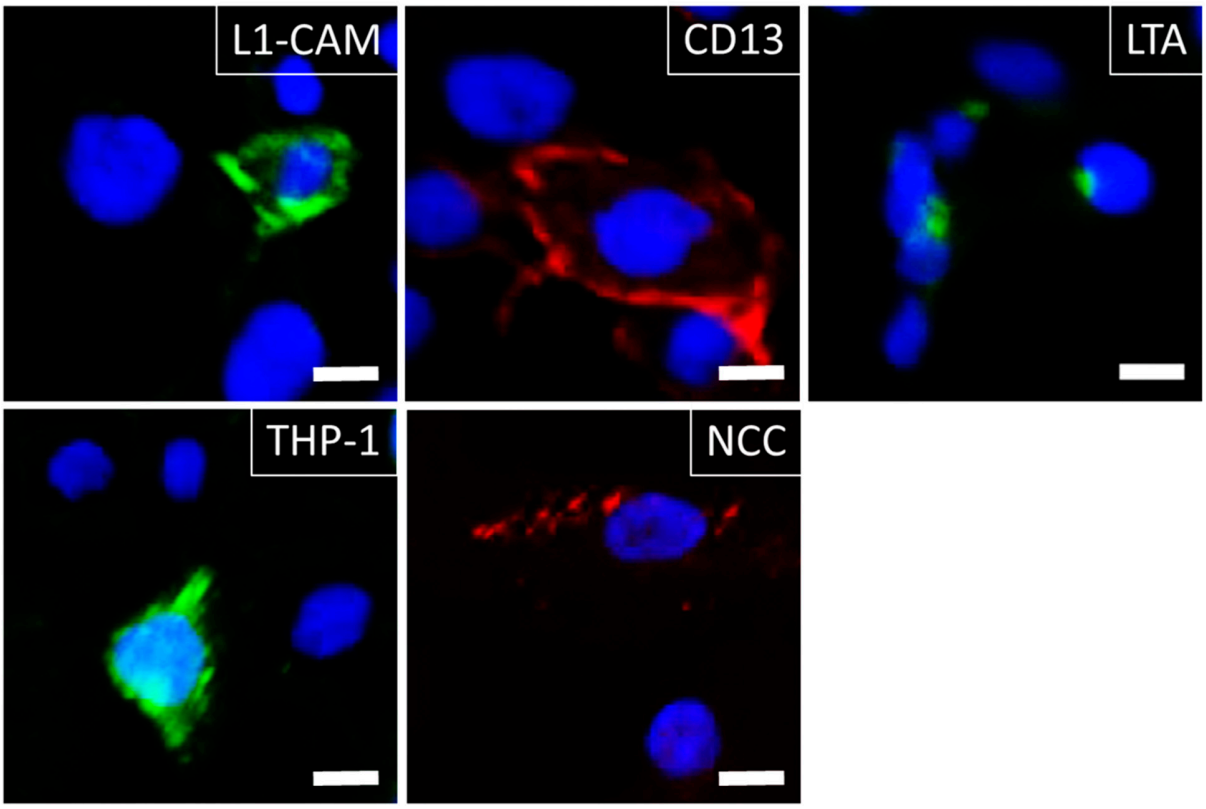
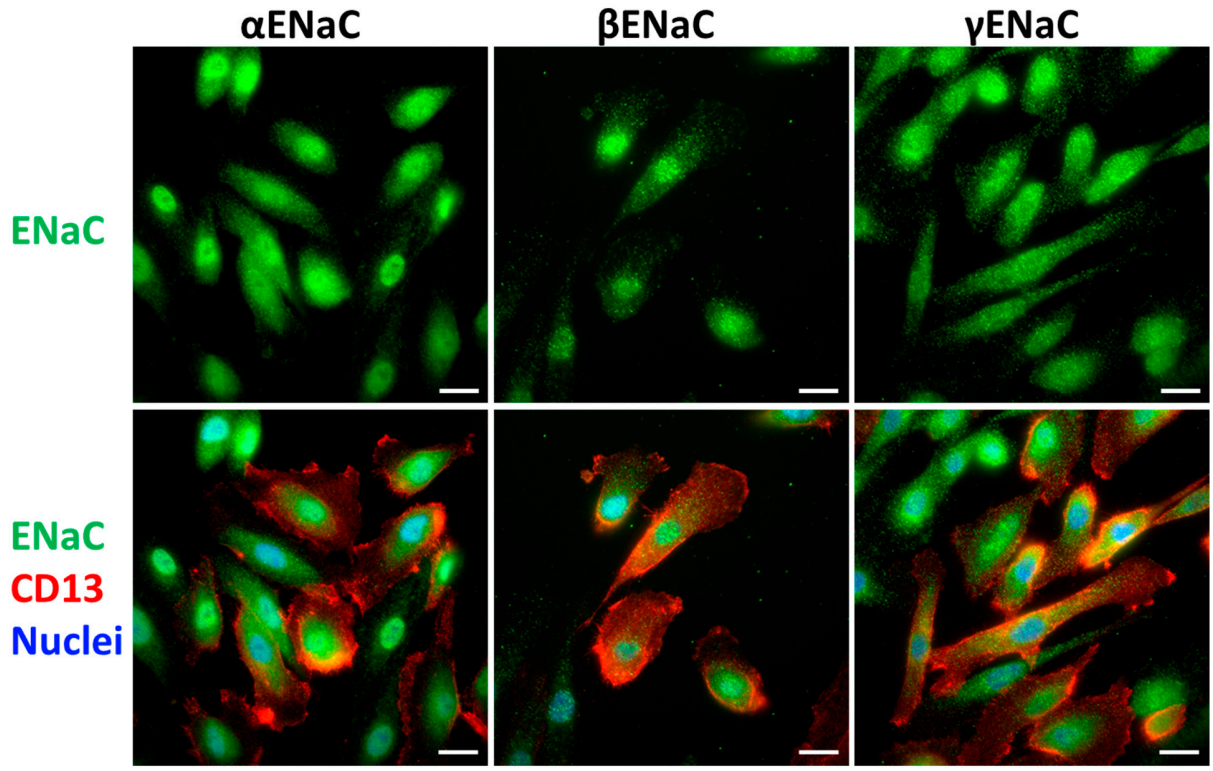
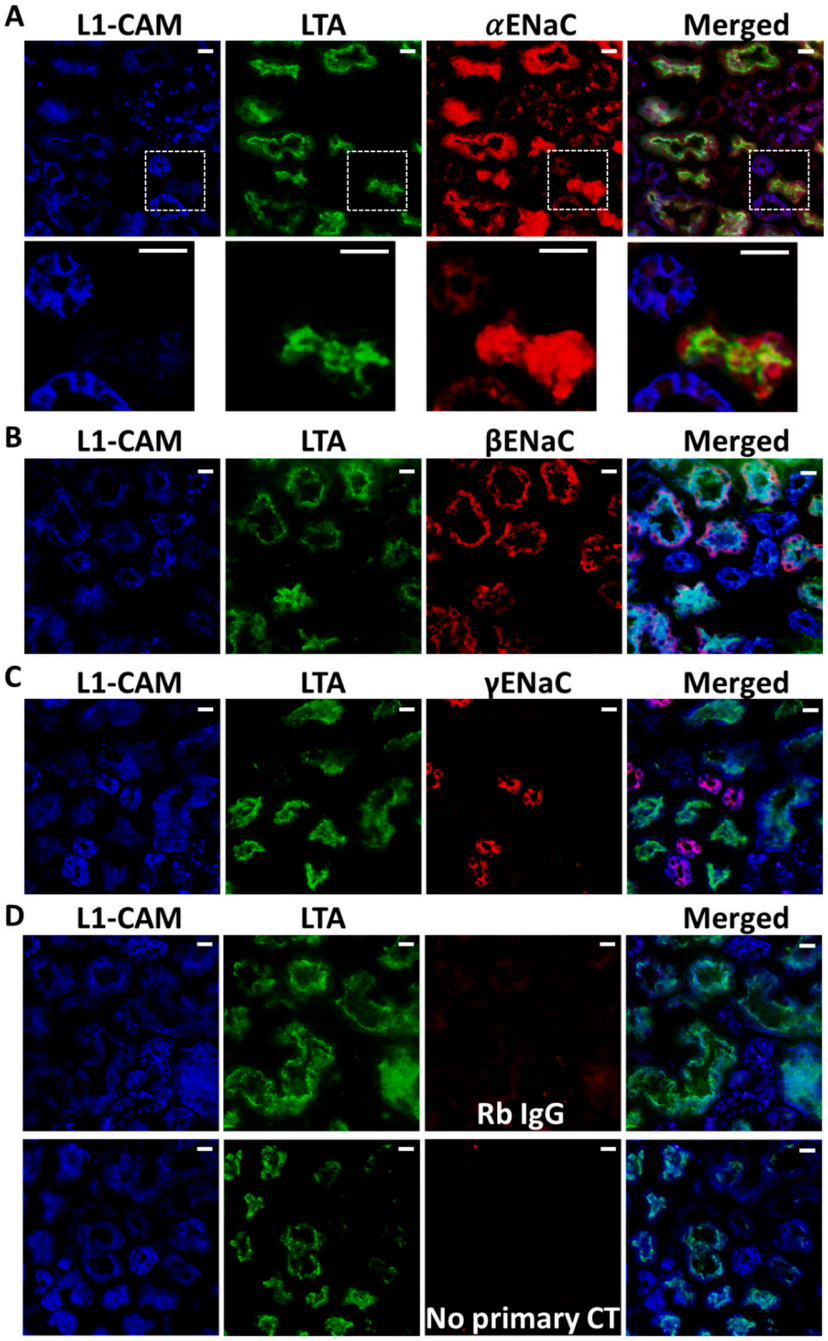


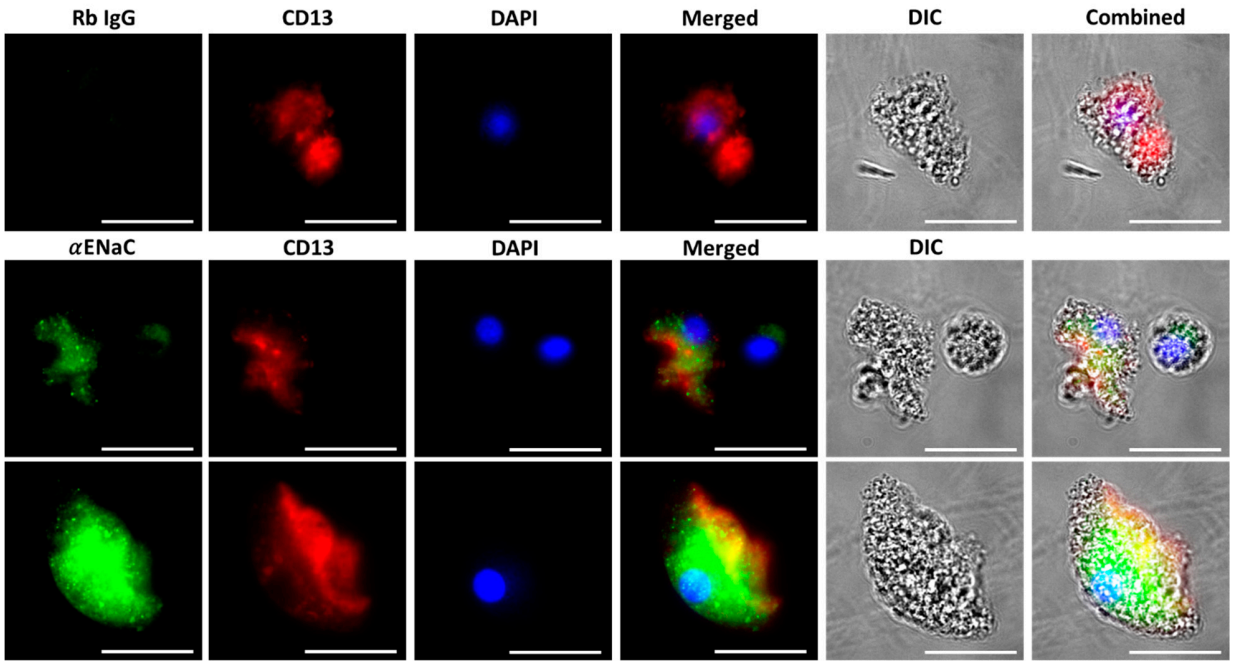
Supplemental Figure S1: Representative images show different types of renal cells in the urine. L1-Cam is collecting duct cell (CDC) marker, CD13 and LTA are proximal tubule cell (PTC) markers. THP is a thick ascending limb marker. NCC is distal tubule cell marker. Scale bar=10 µm



Supplemental Figure S2: αENaC, βENaC and γENaC staining in permeabilized urine-derived renal tubule cells. Nuclei are in blue. αENaC, βENaC and γENaC are in green. CD13 is in red. Scale bar =10 µm

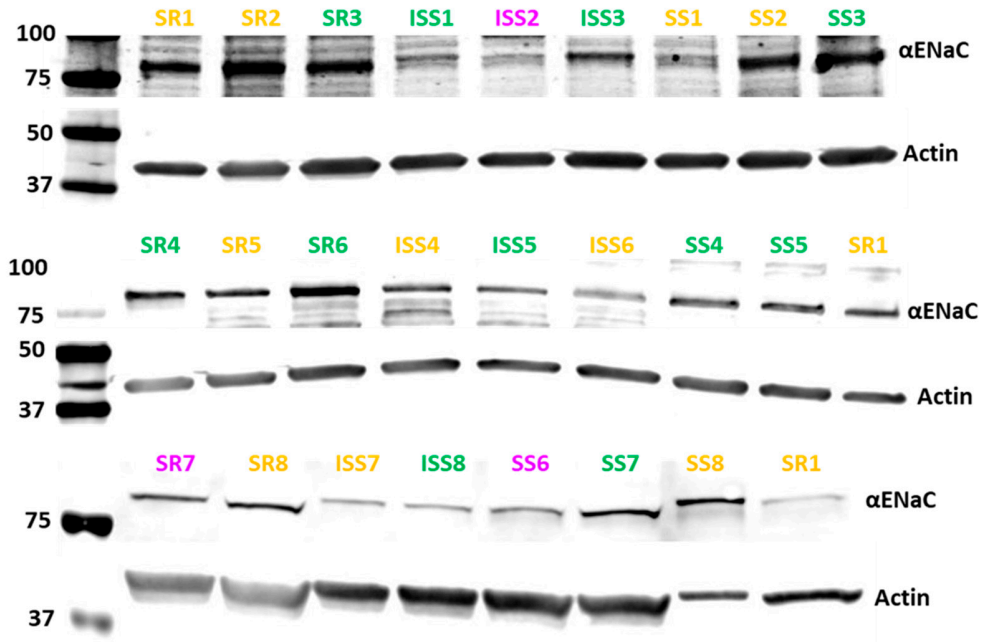


Supplemental Figure S3. αENaC, βENaC and γENaC (StressMarq antibodies) staining in human renal cortex. Both αENaC and βENaC are present in PTC. γENaC is restricted within CDCs. αENaC, βENaC and γENaC are in red. LTA, a PTC marker, is in green. L1-CAM, a CDC marker, is in blue. Rb (rabbit) IgG and no primary antibody are tested as negative controls. The lower panel of (A) is zoomed-in views of boxed regions of the upper panel. Scale bar = 10 µm

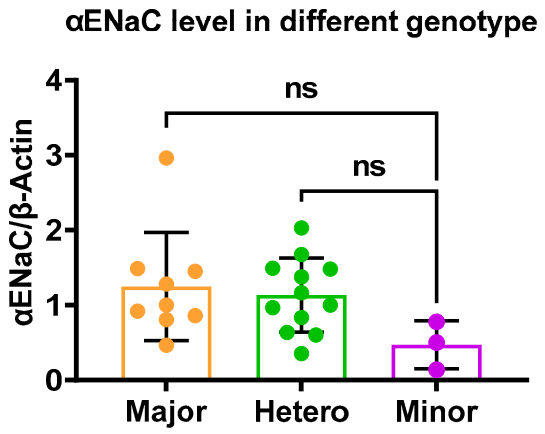


Supplemental Figure S4: αENaC was stained in cells digested from fresh human renal cortex in suspension. Rabbit IgG was used for negative control. CD13 positive cells also shows strong αENaC staining on their apical side. Scale bar = 10 µm

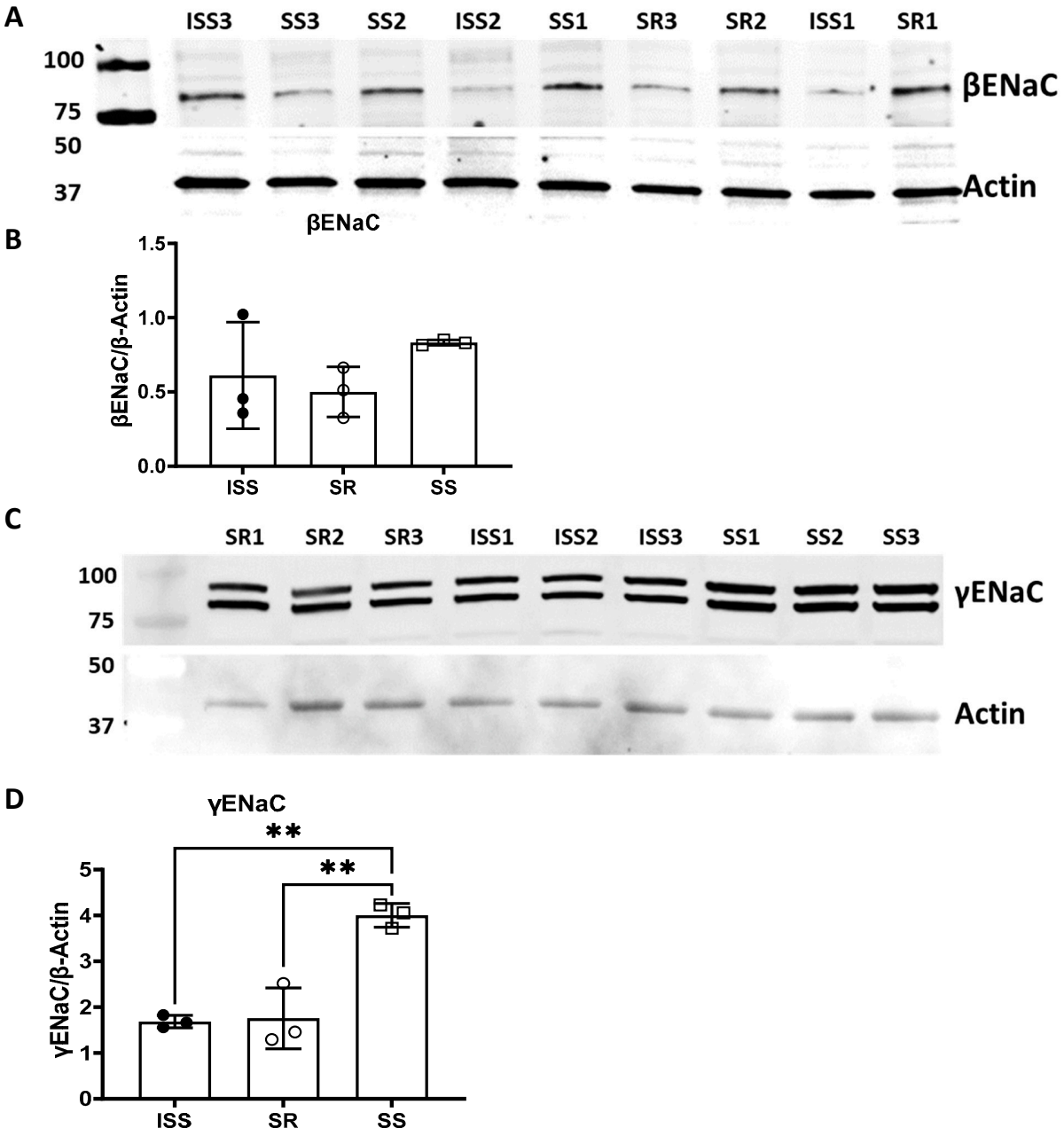
A



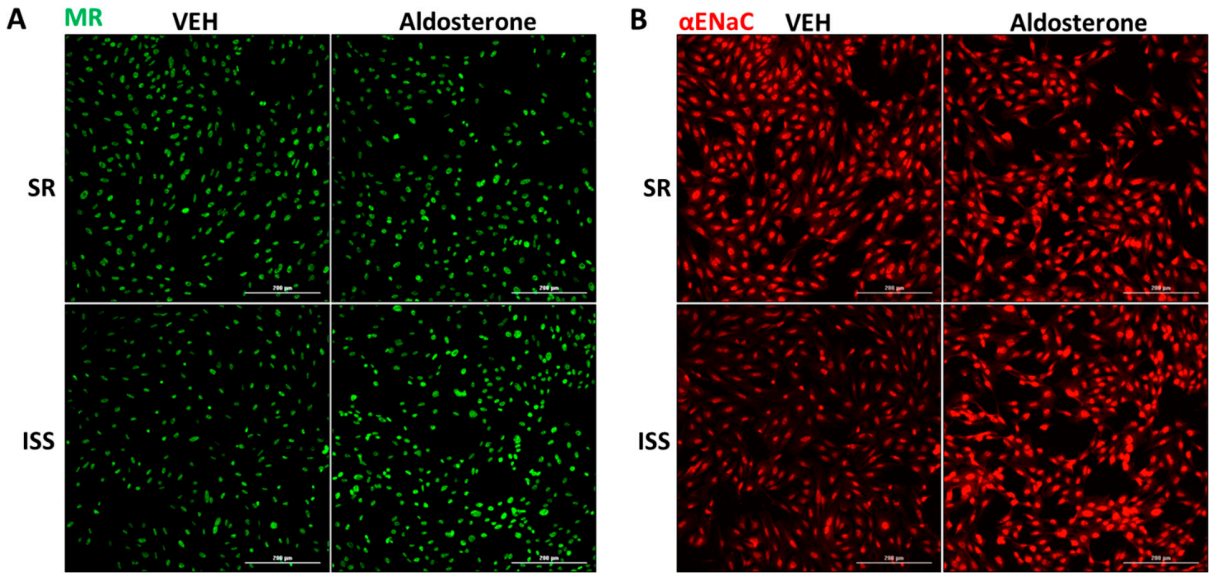
B



Supplemental Figure S5. αENaC western blot analysis in more urine-derived hRTC lines. (A) αENaC western blot in another 15 urine-derived hRTC lines. SR, salt resistant, SS salt sensitive; ISS inverse salt sensitive. The calculation was normalized by SR1 on each blot. (B) αENaC level in different genotype. Homozygous major variants are in orange; heterozygous samples are in green; homozygous minor variants are in purple.



Supplemental Figure S6: βENaC and γENaC are found in CD13^{+} urine-derived hRTCs. Actin was used as loading control in all western blot analysis. A and B) There is no significant difference of βENaC among different phenotypes. C and D) γENaC is significantly higher in SS than both SR and ISS.



Supplemental Figure S7. Representative images of mineralocorticoid receptor (MR) and αENaC immunostaining under aldosterone treatment. MR staining is in green (A), and αENaC is stained in red (B). Scale bar = 200 µm

Supplemental Table S1

Characteristics of UVA Study Subjects (Mean±SD)

	Inverse Salt Sensitive Subjects	**Salt Resistant Subjects**	**Salt Sensitive Subjects**	**P Value**
Number of subjects	37	178	65	
Male	12	78	23	0.2837[+]
Female	25	100	42	
Age (yrs)	40.8±15.2	42±15.1	50.5±14.2	0.0002
BMI	25.1±3.0	24.8±2.9	25.3±2.9	0.4716
After Low Sat Diet				
Systolic (mmHg)	121±13.3	117.2±13.8	114.9±12.7	0.0915
Diastolic (mmHg)	76.2±8.4	72.9±9.1	71.7±6.5	0.0345
MAP (mmHg)	91.1±9.5	87.7±10	86.1±7.8	0.0382
After High Sat Diet				
Systolic (mmHg)	110.3±12.2	118.5±14.1	129.5±12.9	<0.0001
Diastolic (mmHg)	66±7.3	71.9±9.6	80±8.2	<0.0001
MAP (mmHg)	80.7±8.6	87.4±10.5	87.4±10.5	0.0013

For the continuous variables, one-way ANOVA was used to compare the three groups.

+ Comparison of distribution of gender in three groups was done by Chi-square test.

Supplemental Table S2: Odds Ratio

	ISS	NON-ISS (SR+SS)
SNPed (Homozygous minor variant and heterozygous)	23	112
NON-SNPed (homozygous major variant)	14	130

Odds ratio = 23*130/(14*112)=1.91, indicating the odds are 1.91 times higher that people who have rs4764586 will become ISS compare to those who are homozygous major variant.