

Supplemental Material

Selonsertib Enhances Kidney Protection Beyond Standard of Care in a Hypertensive, Secondary Glomerulosclerosis CKD Model

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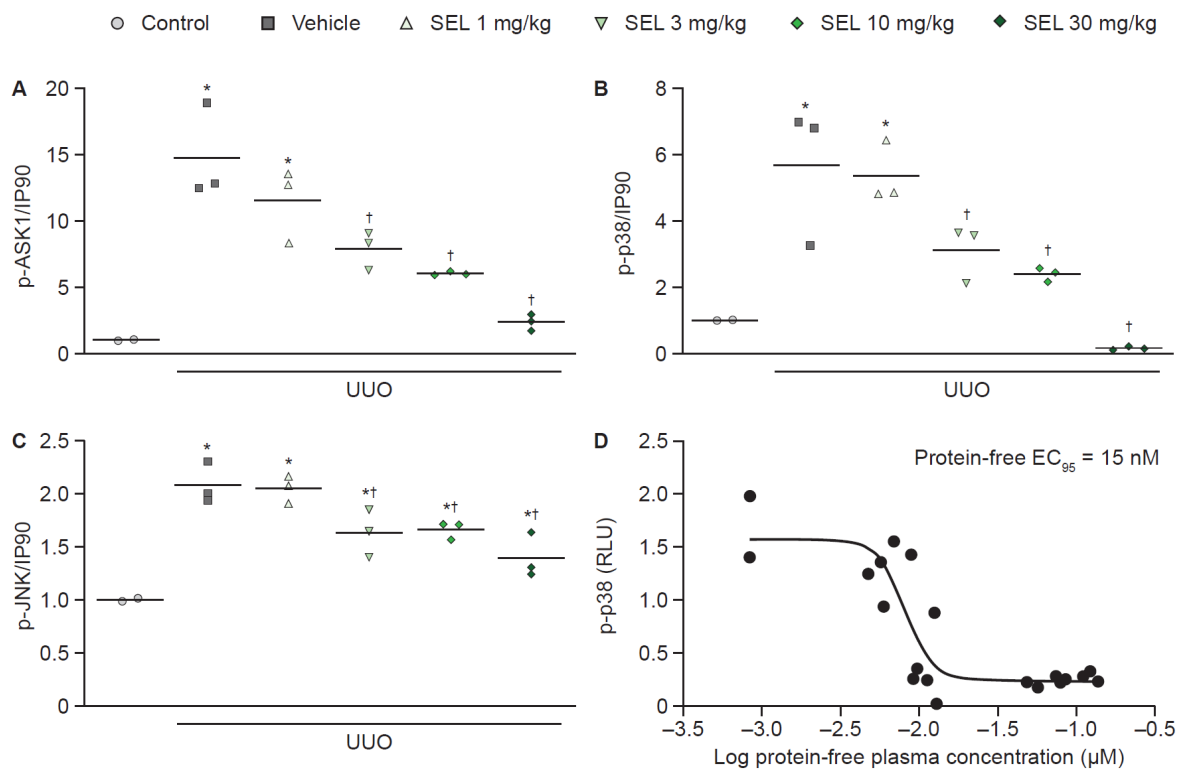
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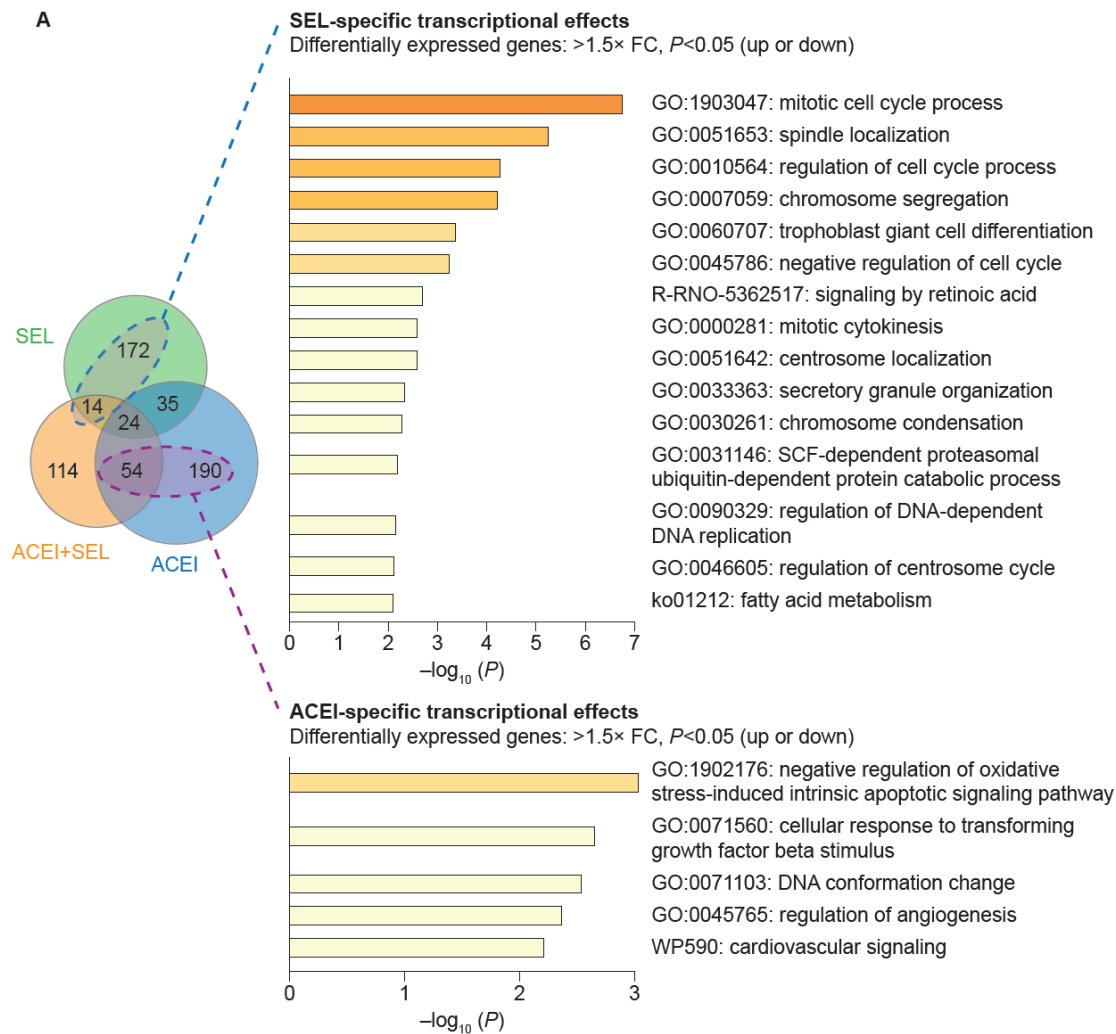
Supplemental Table 1. Hematocrit and hemoglobin at week 12

	Control	ACEI	ACEI+SEL	SEL
Hct, %	39.49±2.26	35.85±1.09	42.34±2.15	36.38±2.85
RBC, 10 ⁶ /mm ³	7.60±0.41	6.88±0.19	8.27±0.47	6.96±0.53
Hb, g/dl	13.84±0.79	12.44±0.45	14.87±0.83	12.68±1.04
Retic, %	0.07±0.03	0.06±0.03	0.04±0.02	0.14±0.07

Data are shown as mean±SEM. n = 9–12 animals per group. ACEI, angiotensin converting enzyme inhibitor; Hb, hemoglobin; Hct, hematocrit; RETIC, reticulocyte count; RBC, red blood cells; SEL, selonsertib; SEM, standard error of the mean.



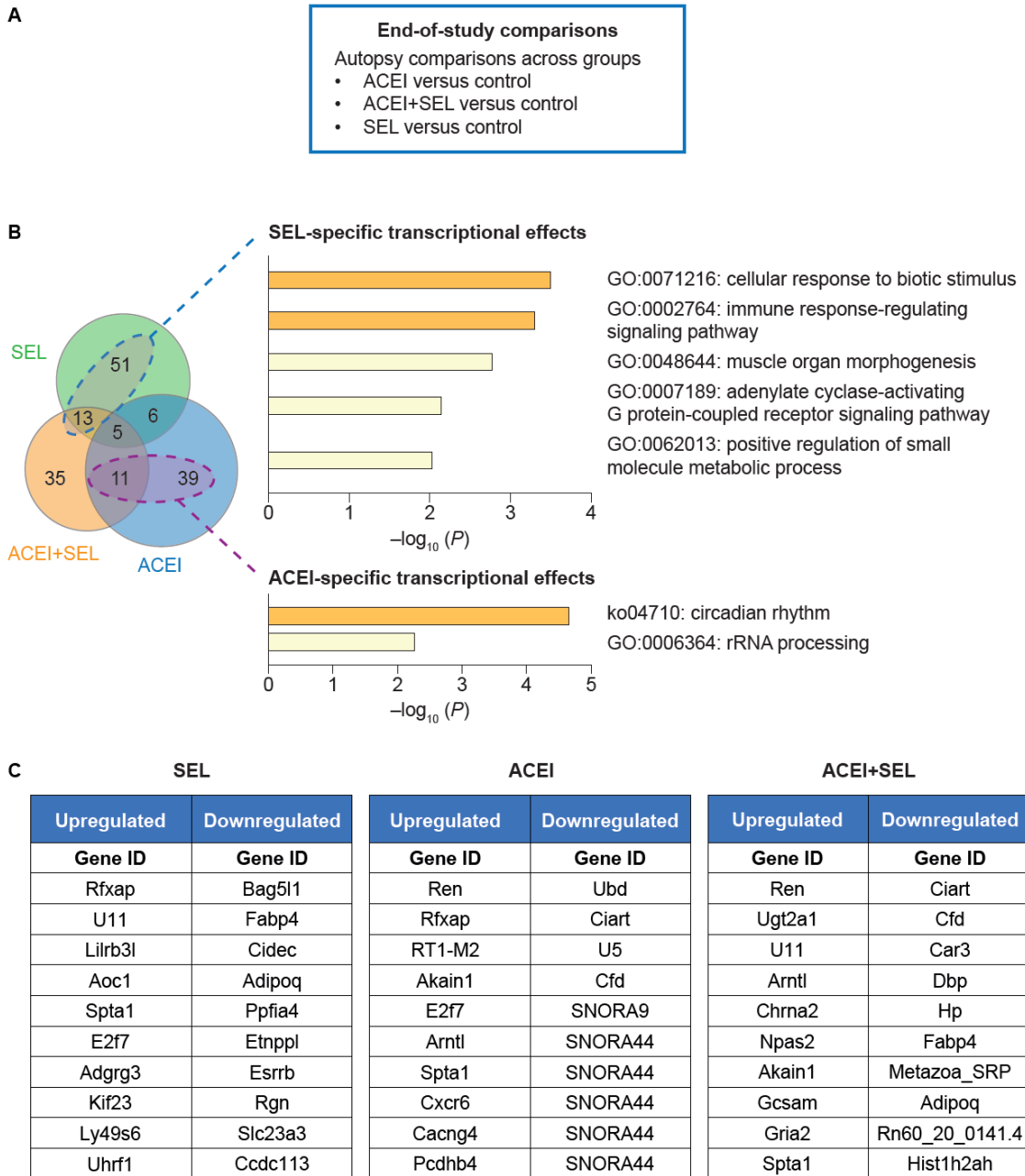
Supplemental Figure 1. Selonsertib PK/PD relationship in a rat model of tubulointerstitial fibrosis. Selonsertib (1–30 mg/kg, orally, twice daily) was administered to Sprague-Dawley rats for 7 days following unilateral ureteral obstruction (UUO) surgery. Quantification of western blot signals demonstrated significant decreases in (A) p-ASK1, (B) p-p38 and (C) p-JNK in kidney cortex as compared to UUO animals treated with vehicle (n = 6–10). Data are presented as individual data points per animal (horizontal bar shows mean). **P*<0.05 vs control (sham surgery), †*P*<0.05 vs vehicle; ANOVA followed by Newman-Keuls multiple comparison test. (D) Correlation between the level of p-p38 in the kidney cortex and the plasma concentration of selonsertib in individual animals at trough on day 7. p-p38 was measured by an ELISA. Data were analyzed by GraphPad Prism 5 software using nonlinear regression curve fitting with variable slope to determine the EC₉₅ value. ANOVA, analysis of variance; ASK1, apoptosis signal-regulating kinase 1; EC₉₅, effective concentration required to inhibit 95%; ELISA, enzyme-linked immunosorbent assay; JNK, c-Jun N-terminal kinase; PD, pharmacodynamic; PK, pharmacokinetic; RLU, relative light unit; SD, standard deviation; SEL, selonsertib.



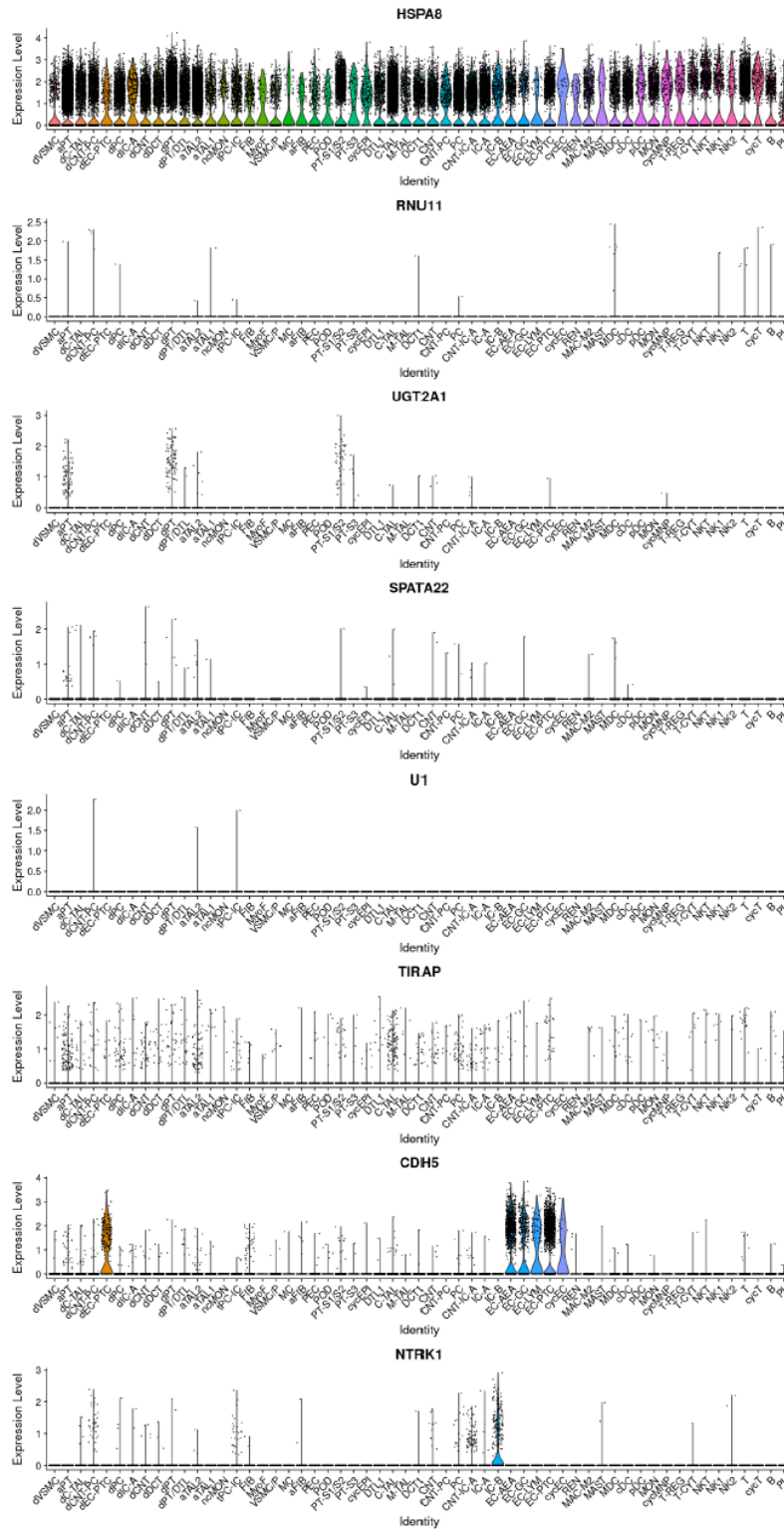
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SEL		ACEI	
Upregulated	Downregulated	Upregulated	Downregulated
Gene ID	Gene ID	Gene ID	Gene ID
Fam122b	Rtkn	Hmgn5	Zmiz2
Moap1	Slco1a6	Fam122b	Hnrnpab
Cr2	Nat8b	Ugt2a1	Bag511
Scd	Fabp4	U11	Ebna1bp2
Olr300	Tmem132b	Pcsk9	Ciart
Nudt10	Pdk4	U5	Abl1
Snap91	Zbtb8b	Olr338	Chi3l1
Hmgn5	Iqch	Fbp2	Rnd1
Plk4	Cndp1	Chchd7	Tmem266
Kif2c	Kcnh1	Hacd1	Fer1l6

Supplemental Figure 2. Differentially expressed genes with selonsertib and ACEI treatment from longitudinal comparisons. (A) Significantly affected molecular pathways (B) Genes specifically modified by each treatment. ACEI, angiotensin-converting enzyme inhibitor; FC, fold change; SEL, selonsertib.



Supplemental Figure 3. Differentially expressed genes with selonsertib, ACEI or combination treatment from end-of-study comparisons. (A) Comparisons across groups. (B) Overlap of differentially expressed genes (B) Genes specifically modified by each treatment. ACEI, angiotensin-converting enzyme inhibitor; FC, fold change; SEL, selonsertib.



Supplemental Figure 5. Single-Cell RNA-Seq violin plots of genes up-regulated in selonsertib and enalapril combination longitudinal comparisons. Data obtained from Kidney Precision Medicine Project Atlas (www.kpmp.org/atlas).