SUPPLEMENT Appendix A

PKCα and Src-kinase support human prostate-distributed dihydrotestosterone-metabolizing UDP- glucuronosyltransferase-2B15 activity

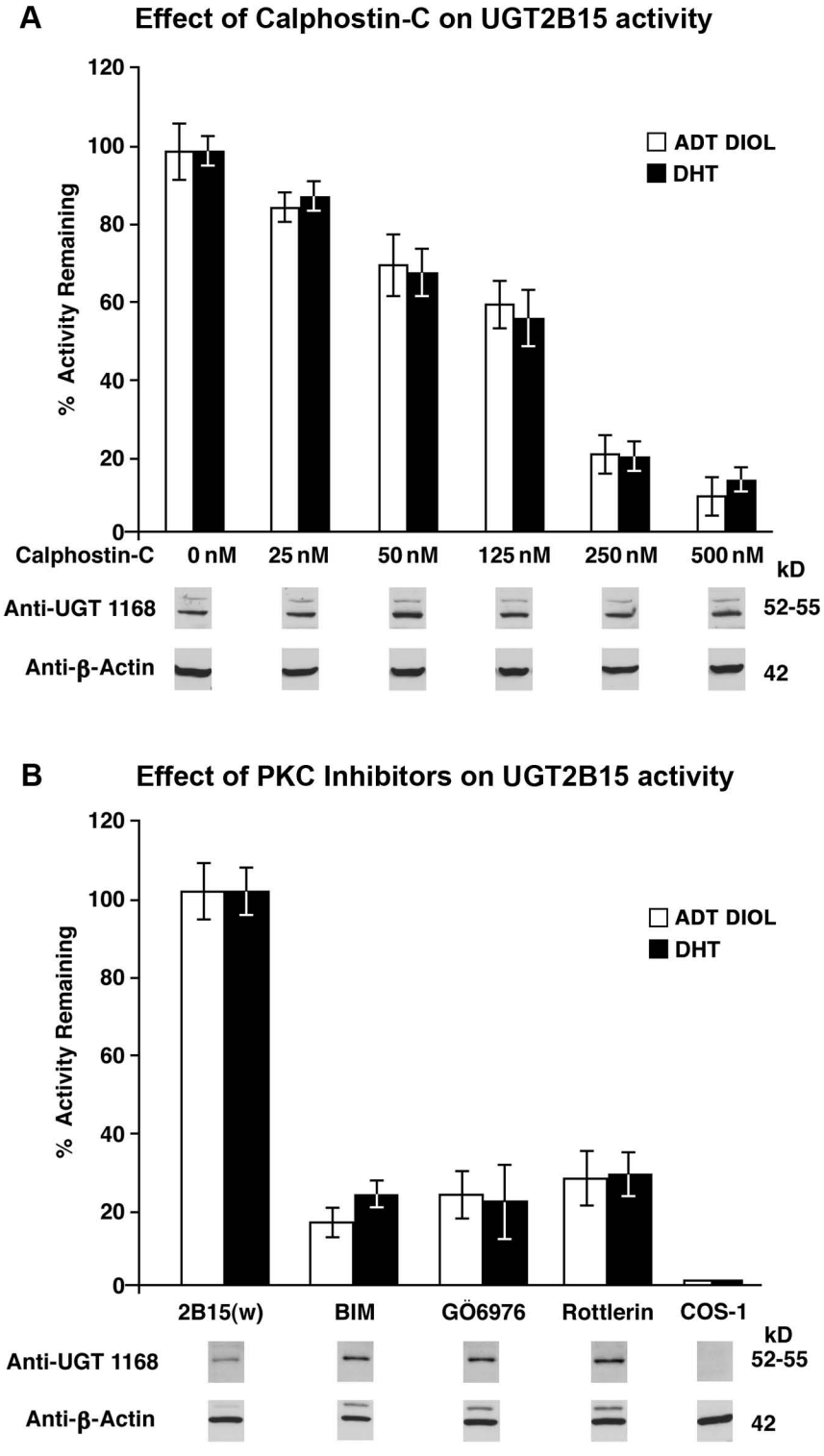
Sunit K. Chakraborty*, Nikhil K. Basu*, Sirsendu Jana, Mousumi Basu, Amit Raychoudhuri and Ida S. Owens‡

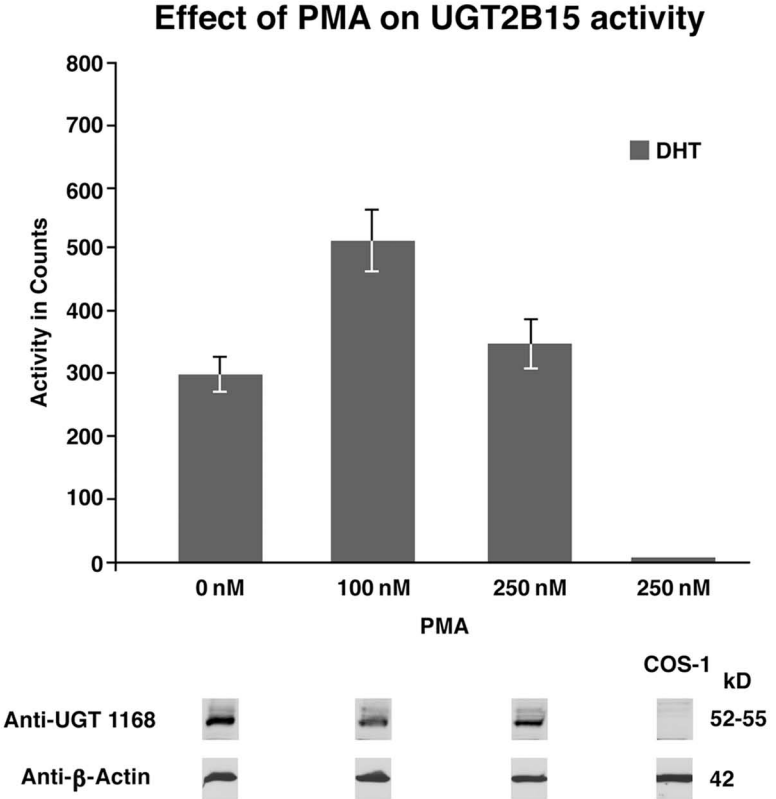
Figure Legends

Fig. 1S (A) Effect of different concentrations of Calphostin-C on 2B15 activity expressed in COS-1 cells. Seventy hr after transfection with pSVL-UGT2B15 with the last 12 hr in phosphate-free medium, cells were treated with increasing concentrations of calphostin-C between 25 and 500nM for 2 hr. 2B15 glucuronidation of either DHT and 3α-diol was carried out at 37ºC for 6 hr. Western blot analysis was carried out with anti-UGT-1168 and anti-β-actin as described under Experimental Procedures. Data represent 3 experiments with standard errors of ±2-6%. (B) Effects of select PKC inhibitors on UGT2B15 expressed in COS-1 cells. Seventy hr after transfection with the pSVL-UGT2B15 construct with the last 12 hr in phosphate-free medium, COS-1 cells were treated with select PKC inhibitors: 250 nM BIM (Bis-indolemaleimide), 1.0 μM Gö6976 or 10 μM Rottlerin for 2 hr before harvesting. Homogenates were assayed in vitro using DHT or ADT DIOL. Western blot analysis was carried out with anti-UGT-1168 and anti-β-actin as described. Data represent 3 experiments with standard errors of ±2-4%.

Fig. 2S Effect of phorbal myristate acetate (PMA) on 2B15 expressed in COS-1 Cells. Seventy hr after transfection with pSVL-UGT2B15, COS-1 cells were treated with 100 or 250 nM PMA that continued for one hr. Upon harvesting cells, homogenates were assayed in vitro for glucuronidation of DHT (shown) or ADT DIOL. Western blot analysis was carried out with anti-UGT-1168 and anti-β-actin as described under Methods. Data represent 3 experiments with standard errors of ±2-6%.

Fig. 3S Effect of Src activator, 1, 25 di-hydroxy-vitamin D3, on UGT2B15 expressed in COS-1 cells. Seventy two hr after transfection in COS-1 cells with with pSVL-UGT2B15, 1,25 di-hydroxy-vitamin D3 was added as described in Experimental Procedures. Cells were harvested, stored and assayed using DHT (shown) and ADT DIOL. Western blot analysis was carried out with anti-UGT-1168, anti-Src and anti-β-actin as described Experimental Procedures. Data represent 3 experiments with standard errors of ±2-6%


A
Effect of Calphostin-C on UGT2B15 activity
% Activity Remaining
120
100
80
60
40
20
0
ADT DIOL
DHT
Calphostin-C
0 nM
25 nM
50 nM
125 nM
250 nM
500 nM
kD
Anti-UGT 1168
52-55
Anti-β-Actin
42
B
Effect of PKC Inhibitors on UGT2B15 activity
% Activity Remaining
120
100
80
60
40
20
0
ADT DIOL
DHT
2B15(w)
BIM
GÖ6976
Rottlerin
COS-1
kD
Anti-UGT 1168
52-55
Anti-β-Actin
42


Effect of PMA on UGT2B15 activity
800
700
600
500
400
300
200
100
0
Activity in Counts
DHT
0 nM
100 nM
250 nM
250 nM
PMA
COS-1
kD
Anti-UGT 1168
52-55
Anti-β-Actin
42

Effect of Vitamin D treatment on UGT2B15 activity