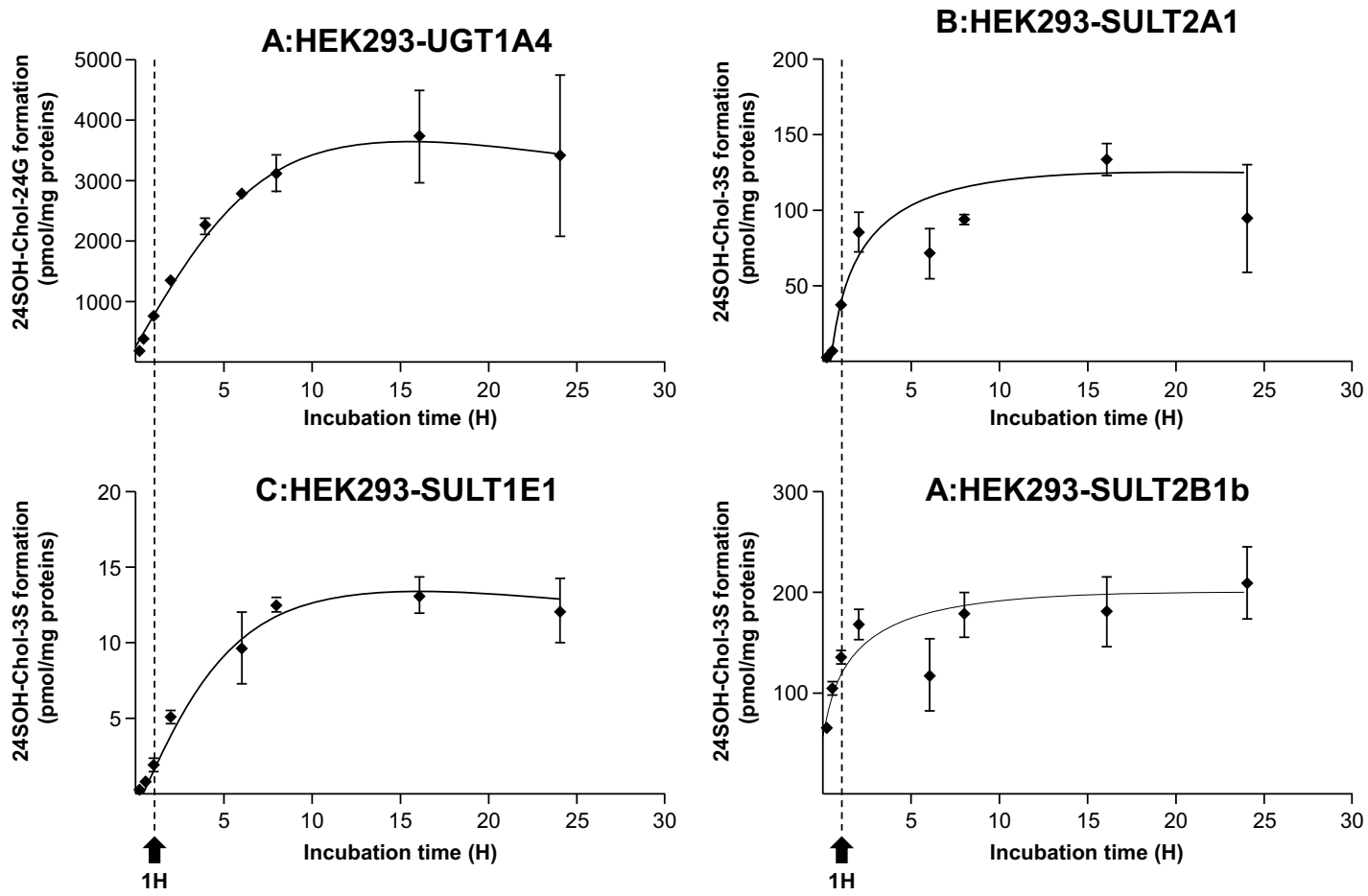
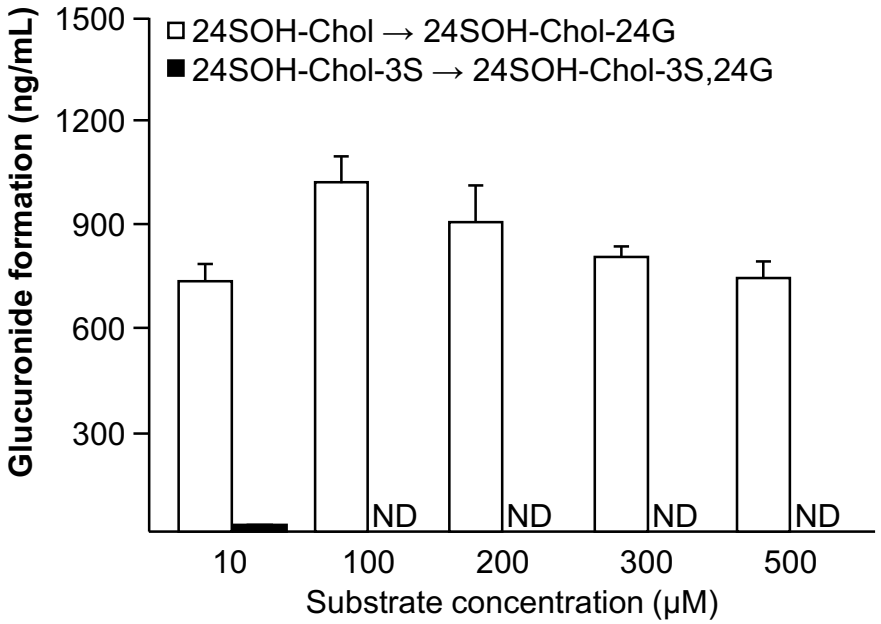
SUPPLEMENTAL MATERIALS



Supplemental Figure 1. 24SOH-Chol conjugation by human UGT and SULT enzymes is linear for up to 1H.

Glucuronidation (**A**) or sulfonation (**B-D**) assays were performed for up to 24H with homogenates from HEK293 cells overexpressing the human UGT1A4 (**A**), SULT2A1 (**B**), SULT1E1 (**C**) or SULT2B1b (**D**). 24SOH-Chol-3S and 24SOH-Chol-24G were quantified by LC-MS/MS. Data represent the mean ± SD of 2 independent experiments performed in triplicate.

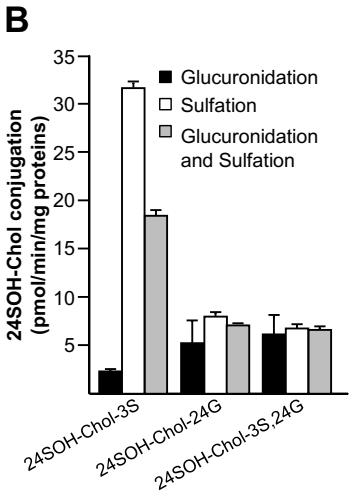


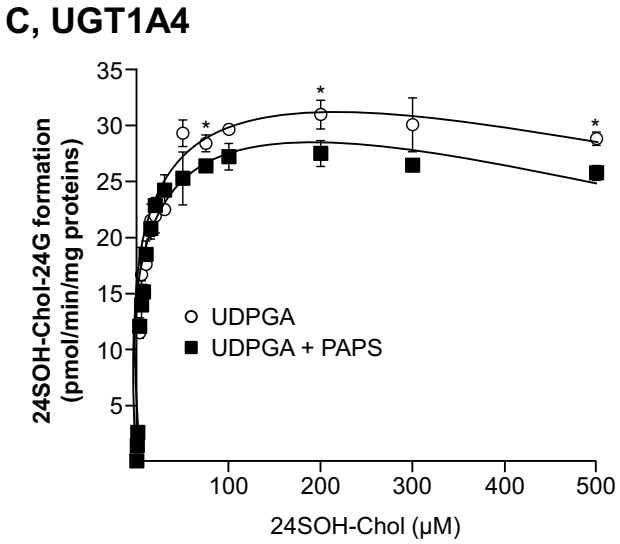
Supplemental Figure 2. Human liver homogenates fail to glucuronidate 24SOH-Chol-3S.

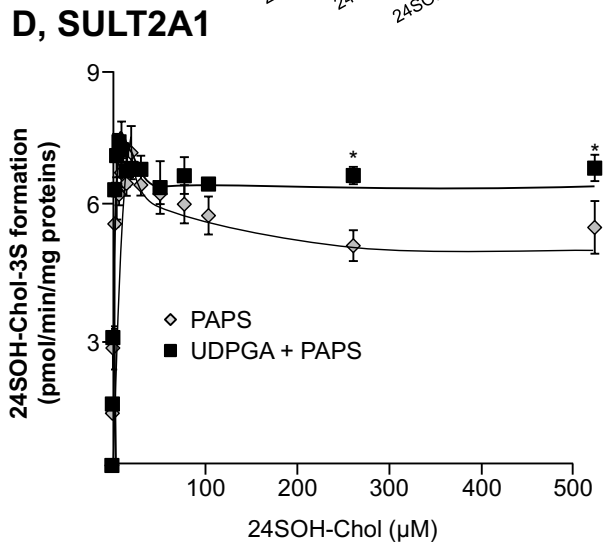
Human liver homogenates (50μg) were incubated in the presence of increasing concentrations (10 to 500μM) of 24S-hydroxycholesterol or its sulfated derivative 24SOH-Chol-3S for 1H at 37°C. 24SOH-Chol-24G and 24SOH-Chol-3S,24G were quantified by LC-MS/MS. Data represent the mean ± SD of 2 independent experiments performed in triplicate.

A

Solutions	Buffer		
	Glucuronidation	Sulfation	Glucuronidation and sulfation
Tris-HCl pH 7.5 50mM	X	X	X
$MgCl_2$	X (10mM)	X (1mM)	X (10mM)
Phosphatidylcholine 10µg/mL	X		X
Pepstatine 2.5µg/mL	X		X
Leupeptine 0.5µg/mL	X		X
Alamethicin 0.025µg/mL	X		X
BSA 1mg/mL		X	X
DTT 7.5mM		X	X







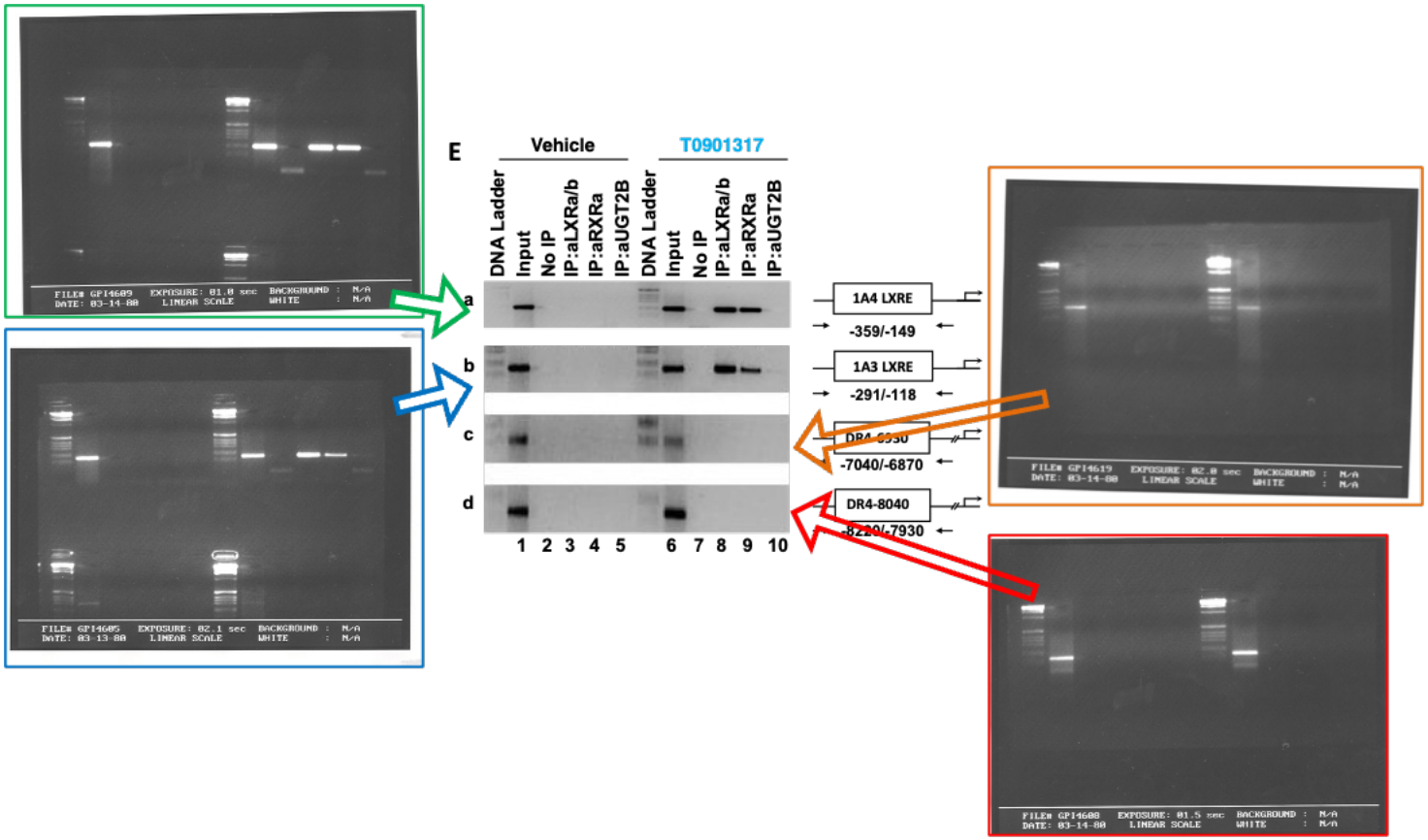
Supplemental Figure 3. The glucuronidation/sulfonation assay buffer (A) minimally impacts the respective conjugation reactions (B-D).

(A) Composition of the enzymatic assay buffers.

(B) Conjugation assays were performed using liver homogenates (50µg) and 24S-hydroxycholesterol (100µM) which were incubated for 1H at 37°C in the corresponding assay buffer (**A**).

(**C-D**) Glucuronidation (**C**) and sulfonation (**D**) assays were performed for 1H at 37°C using either the glucuronidation (**C**), sulfonation (**D**) or glucuronidation/sulfonation (**C&D**) buffers, and homogenates (50µg) from HEK293 cells expressing the human UGT1A4 (**C**) or SULT2A1 (**D**) enzymes in the presence of increasing concentrations (0.5 to 500µM) of 24S-hydroxycholesterol.

The formation of conjugated forms of 24SOH-Chol was resolved by LC-MS/MS. Data represent the mean ± SD of 2 independent experiments performed in triplicate. Statistically significant differences are indicated by asterisks (*:$p<0.05$).



Supplemental Figure 4. Full-length gels version of the gelshift experiment depicted in panel F of the Figure 5.