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Supplementary Materials

Table S1. Results of the NPs library screening assay. The inhibitor (50 μM) was incubated with reaction buffer and equally concentrated XT-I protein solutions derived from CHO-K1 pgsA745 cell line complemented with full-length *XYLT1* expressing plasmid. The XT-I activities shown are means from one experiment performed in technical duplicates and calculated relative to the XT-I activity of the negative control sample containing DMSO.

Compound	CAS Number	Molecular Weight	Name	XT-I Activity [%]
1	53123-88-9	914.18	Rapamycin (Sirolimus)	70
2	56390-09-1	579.98	Epirubicin HCl	95
3	18883-66-4	265.22	Streptozotocin (STZ)	101
4	364622-82-2	438.52	Doripenem Hydrate	86
5	78110-38-0	435.43	Aztreonam	94
6	114-07-8	733.93	Erythromycin	105
7	2022-85-7	129.09	Flucytosine	93
8	72559-06-9	847	Rifabutin	112
9	5536-17-4	267.24	Vidarabine	103
10	486-66-8	254.24	Daidzein	115
11	145-13-1	316.48	Pregnenolone	95
12	467214-21-7	653.21	Alvespimycin (17-DMAG) HCl	120
13	33419-42-0	588.56	Etoposide	114
14	553-21-9	232.32	Costunolide	121
15	4759-48-2	300.44	Isotretinoin	86
16	4618-18-2	342.3	Lactulose	122
17	1397-89-3	924.08	Amphotericin B	25
18	317-34-0	420.43	Aminophylline	122
19	59-67-6	123.11	Nicotinic Acid	126
20	80621-81-4	785.88	Rifaximin	83
21	70458-95-6	429.46	Pefloxacin Mesylate	108
22	723-46-6	253.28	Sulfamethoxazole	121
23	114977-28-5	807.88	Docetaxel	104
24	362-07-2	302.41	2-Methoxyestradiol (2-MeOE2)	114
25	50-02-2	392.46	Dexamethasone (DHAP)	106
26	96036-03-2	383.46	Meropenem	107
27	7681-93-8	665.73	Natamycin	95
28	54965-21-8	265.33	Albendazole	77
29	78613-38-4	353.97	Amorolfine HCl	70
30	61379-65-5	877.03	Rifapentine	50
31	79902-63-9	418.57	Simvastatin	82
32	124832-27-5	360.8	Valaciclovir HCl	74
33	127-69-5	267.3	Sulfisoxazole	95
34	33069-62-4	853.91	Paclitaxel	79
35	2068-78-2	923.04	Vincristine sulfate	87
36	86386-73-4	306.27	Fluconazole	75
37	501-36-0	228.24	Resveratrol	51
38	50-55-5	608.68	Reserpine	89
39	128-13-2	392.57	Ursodiol	98
40	56-75-7	323.13	Chloramphenicol	87
41	13292-46-1	822.94	Rifampin	77
42	59277-89-3	225.2	Aciclovir	71

43	110871-86-8	392.4	Sparfloxacin	89
44	62997-67-5	1056.24	Nystatin (Fungicidin)	52
45	187235-37-6	359.26	PA-824	75
46	56180-94-0	645.6	Acarbose	92
47	112811-59-3	375.39	Gatifloxacin	98
48	220620-09-7	585.65	Tigecycline	59
49	91832-40-5	395.41	Cefdinir	72
50	59-87-0	198.14	Nitrofural	104
51	63-74-1	172.2	Sulfanilamide	118
52	117467-28-4	620.72	Cefditoren Pivoxil	104
53	83905-01-5	748.98	Azithromycin	109
54	98-92-0	122.12	Nicotinamide (Vitamin B3)	116
55	100986-85-4	361.37	Levofloxacin	111
56	73-31-4	232.28	Melatonin	99
57	63968-64-9	282.33	Artemisinin	93
58	446-72-0	270.24	Genistein	94
59	165800-03-3	337.35	Linezolid	103
60	23593-75-1	344.84	Clotrimazole	101
61	58-61-7	267.24	Adenosine	103
62	50-23-7	362.46	Hydrocortisone	122
63	68-35-9	250.28	Sulfadiazine	108
64	773-76-2	214.05	Chloroxine	121
65	68-19-9	1355.37	Vitamin B12	94
66	58-27-5	172.18	Menadione	106
67	25316-40-9	579.98	Doxorubicin (Adriamycin) HCl	113
68	34157-83-0	450.61	Celastrol	38
69	15291-77-7	424.4	Ginkgolide B	100
70	137234-62-9	349.31	Voriconazole	94
71	62893-19-0	645.67	Cefoperazone	83
72	302-79-4	300.4	Tretinoin	84
73	57-83-0	314.46	Progesterone	113
74	79-57-2	460.43	Oxytetracycline (Terramycin)	17
75	474-25-9	392.57	Chenodeoxycholic Acid	113
76	443-48-1	171.15	Metronidazole	109
77	298-81-7	216.19	Methoxsalen	107
78	51-21-8	130.08	Fluorouracil (5-Fluoracil, 5-FU)	137
79	152044-53-6	493.66	Epothilone A	119
80	103060-53-3	1620.67	Daptomycin	86
81	70356-03-5	385.82	Cefaclor	71
82	651-06-9	280.3	Sulfameter	126
83	53-16-7	270.37	Estrone	120
84	50-28-2	272.38	Estradiol	128
85	536-33-4	166.24	Ethionamide	130
86	458-37-7	368.38	Curcumin	43
87	65899-73-2	387.71	Tioconazole	136
88	22832-87-7	479.14	Miconazole Nitrate	149
89	144-82-1	270.33	Sulfamethizole	111
90	68373-14-8	233.24	Sulbactam	138
91	122-11-2	310.33	Sulphadimethoxine	132
92	10212-25-6	261.66	Cyclocytidine HCl	142
93	2922-28-3	171.59	Adenine HCl	146
94	404-86-4	305.41	Capsaicin (Vanilloid)	135

95	15291-75-5	408.4	Ginkgolide A	150
96	9041-93-4	1512.62	Bleomycin sulfate	149
0	67-68-5	78.13	Dimethyl sulfoxide	100

Table S2. Experimental analysis of human XT-I inhibition by celastrol and amphotericin B. K_m and V_{max} values shown were calculated from nonlinear regression (Michaelis-Menten plot) and the corresponding Y-intercept and slope values from the simple linear regression (Lineweaver-Burk plot). Data are means ± SEM of triplicate data points per experiment.

Compound.	**Michaelis-Menten plot**		**Lineweaver-Burk plot**	
DMSO	V_{max} [AU]	12124 ± 626	Y-intercept	$7.8 \pm 1.3 \cdot 10^{-5}$
	K_m [µM]	31.2 ± 3	slope	$2.6 \cdot 10^{-3} \pm 5.09 \cdot 10^{-5}$
Celastrol	V_{max} [AU]	11826 ± 551	Y-intercept	$7.1 \pm 1.1 \cdot 10^{-5}$
	K_m [µM]	53.3 ± 3	slope	$4.5 \cdot 10^{-3} \pm 7.4 \cdot 10^{-5}$
Amphotericin B	V_{max} [AU]	10031 ± 388	Y-intercept	$9.1 \pm 1.5 \cdot 10^{-5}$
	K_m [µM]	27.5 ± 3	slope	$2.8 \cdot 10^{-3} \pm 9.6 \cdot 10^{-5}$

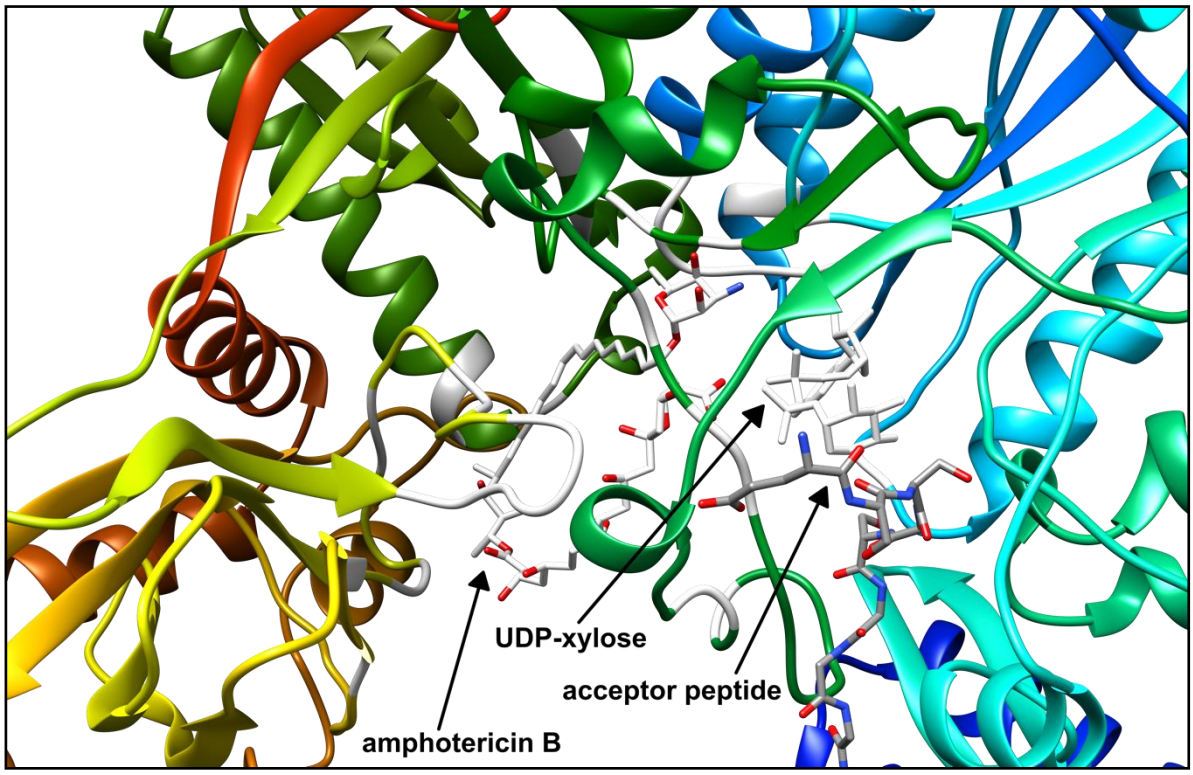

Figure S1. Structure of human XT-I complexed with amphotericin B, UDP-D-xylose and the acceptor peptide. Crystal structure of human XT-I [5] (rainbow colored from the N terminus (blue) to C terminus (red)) complexed with the chimera models #**2** of amphotericin B (white colored), UDP-D-xylose and modified acceptor peptide (atoms: C (grey), N (blue), O (red), P (orange)) are shown in stick representation. All atoms and bonds that meet the criteria < 5.0 Å from amphotericin B were colored in white.

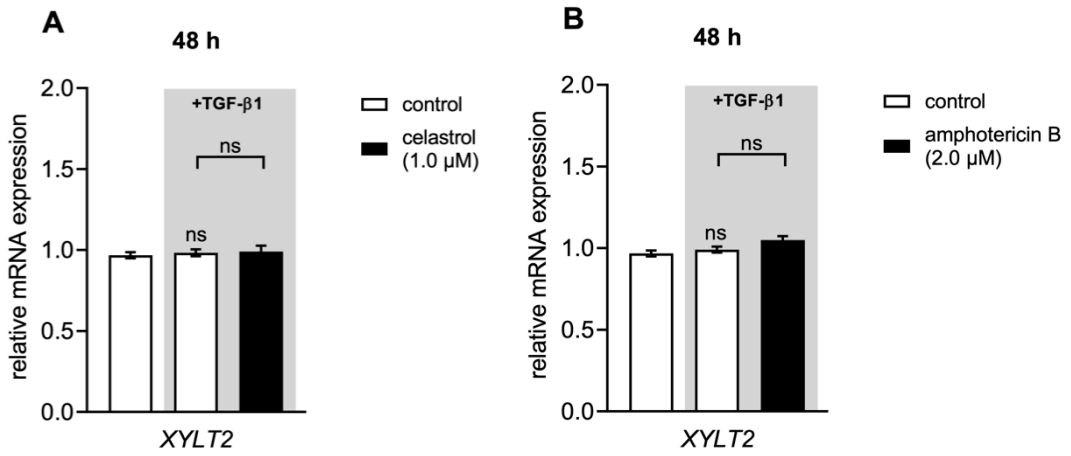

Figure S2. *XYLT2* mRNA expression is not affected by inhibitor or cytokine treatment of NHDF. Human primary dermal fibroblasts (n = 3) were cultured the day before the experiment. Cells were treated for 48 h with vehicle only (control), vehicle or (**A**) 1.0 μM celastrol or (**B**) 2.0 μM amphotericin B with additional TGF-β1 (5 μg/L) supplementation (highlighted in grey). Relative *XYLT2* mRNA expression levels were analyzed by quantitative real-time PCR. Data are means ± SEM of three biological and three technical replicates per experiment. Mann-Whitney U test: not significant (ns).

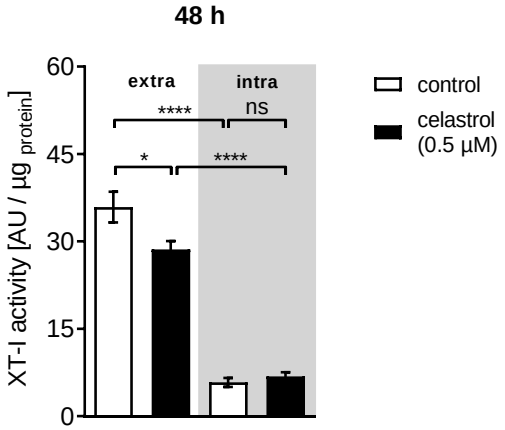

Figure S3. Extracellular XT-I activity reduction by celastrol was not caused by the downregulation of *XYLT1* mRNA expression. Human primary dermal fibroblasts (n = 3) were cultured the day before the experiment. Cells were treated with vehicle (control) or 0.5 μM celastrol for 48 h. Intracellular XT activity (intra, grey) was determined from the cell lysates and the corresponding supernatants were utilized for extracellular XT activity (extra) determination by UPLC-ESI-MS/MS XT-I assay. The XT activity is expressed as arbitrary units (AU) per μg of protein in 1 mL sample. Data are means ± SEM of three biological and three technical replicates per experiment. Mann-Whitney U test: not significant (ns), $p < 0.05$ (*), $p < 0.0001$ (****).