Supplemental data

Enhanced activation of human NK cells by drug-exposed hepatocytes

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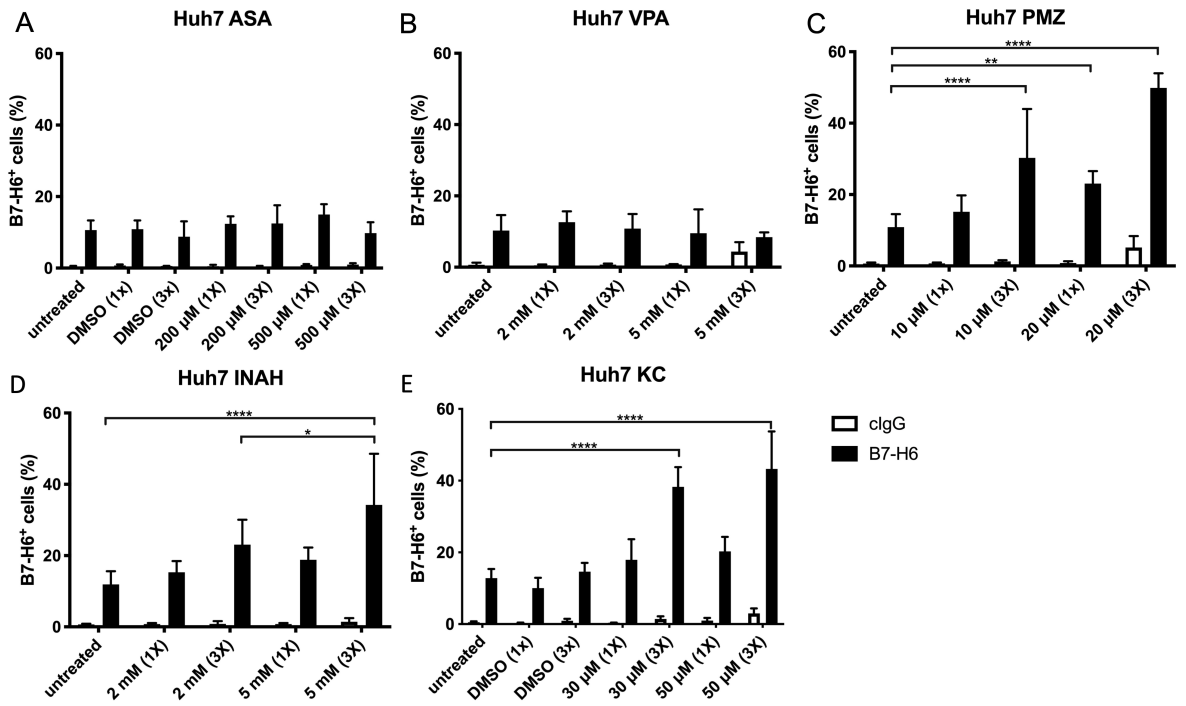
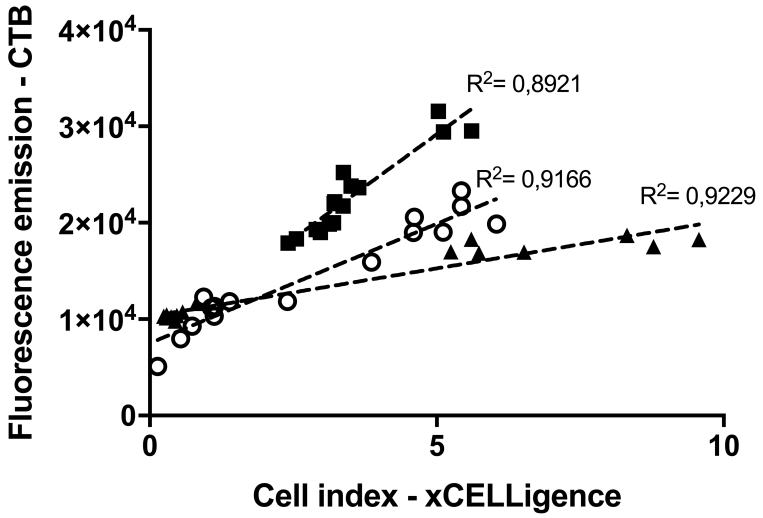
Gene	Drugs which induce more than 2-fold changes in expression **(p<0.05; fdr adjusted)**
MICA	acetaminophen, phenobarbital, valproic acid, methapyrilene, propylthiouracil, ketoconazole
MICB	allyl alcohol, propylthiouracil, ketoconazole, diethyl maleate, N-methyl-N-nitrosourea
B7-H6	isoniazid, chlorpromazine, methapyrilene, adapin, labetalol, flutamide, perhexiline, ketoconazole, iproniazid, hydroxyzine, ethambutol, ranitidine, diltiazem, disopyramide, mexiletine, promethazine, sulpiride,ajmaline, venlafaxine, diethyl maleate, butylated hydroxyanisole, amphotericin B, propranolol, aflatoxin B1
ULBP-2	valproic acid

Supplemental Table 1: Excerpt of data from gene expression analysis of primary human hepatocytes incubated with different hepatotoxic drugs at in vivo relevant concentrations (1).

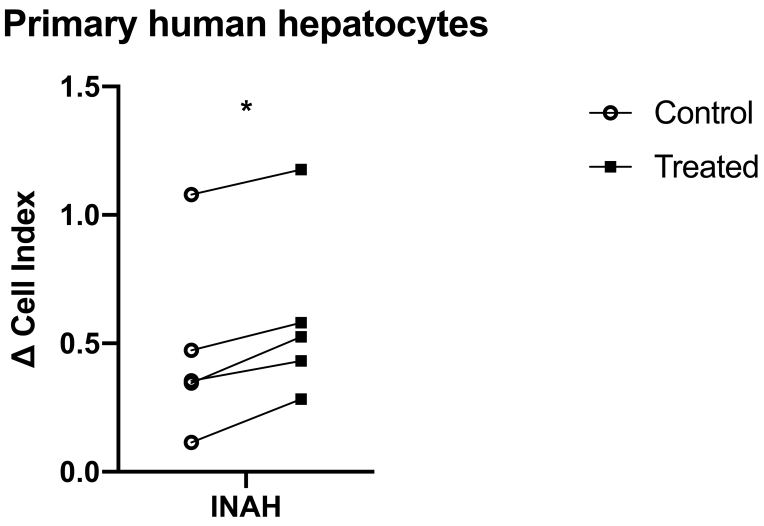
1. Grinberg, M., R. M. Stober, K. Edlund, E. Rempel, P. Godoy, R. Reif, A. Widera, K. Madjar, W. Schmidt-Heck, R. Marchan, A. Sachinidis, D. Spitkovsky, J. Hescheler, H. Carmo, M. D. Arbo, B. van de Water, S. Wink, M. Vinken, V. Rogiers, S. Escher, B. Hardy, D. Mitic, G. Myatt, T. Waldmann, A. Mardinoglu, G. Damm, D. Seehofer, A. Nussler, T. S. Weiss, A. Oberemm, A. Lampen, M. M. Schaap, M. Luijten, H. van Steeg, W. E. Thasler, J. C. Kleinjans, R. H. Stierum, M. Leist, J. Rahnenfuhrer, and J. G. Hengstler. 2014. Toxicogenomics directory of chemically exposed human hepatocytes. *Arch Toxicol* 88: 2261-2287.



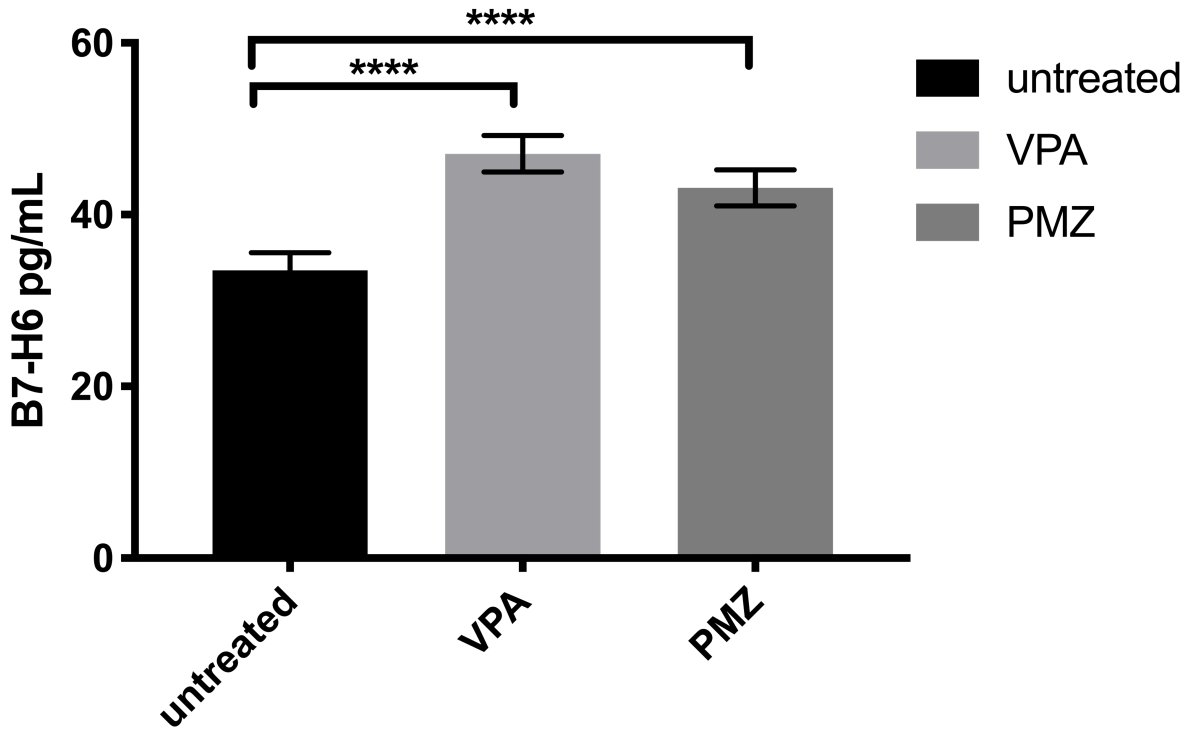
Supplemental Figure 1: Expression of the activating NK cell ligand B7-H6 after drug exposure of Huh7 cells. Huh7 cells were seeded in 6 well plates and then treated with the indicated concentrations of (A) acetylsalicylic acid (ASA), (B) valproic acid (VPA), (C) promethazine (PMZ), (D) isoniazid (INAH), or (E) ketoconazole (KC) for 24 hours (1x) or for 72 hours once every day (3x). Cells were then harvested, stained with specific antibodies against B7-H6 or an appropriate isotype control and analyzed by flow cytometry. Shown is the quantification of FACS analysis (n = 3-4 per drug). Analyzed for statistical significance by 2way ANOVA.



Supplemental Figure 2: Cell Index correlates with Cell Titer Blue assay. Huh7 cells were analyzed by CTB assay after the RTCA co-culture assay for 3 independent experiments. CTB values correlated with endpoint CI values.



Supplemental Figure 3: Enhanced cytotoxicity of NK cells against INAH-treated primary human hepatocytes. Cytotoxicity assay conducted as shown in Fig. 3A. PHH cells were seeded in E-Plates, treated with the 2 mM isoniazid and NK cells were added after washing for 24 hours of co-culture. Cell Index was recorded every 5 min. Delta Cell Index was calculated for each drug as depicted in Fig 3A. Data points represent biological replicates with different NK cell donors conducted in technical duplicates or triplicates. Analyzed for statistical significance by Paired t test. A p of <0.05 was considered significant (* $p < 0.05$).



Supplemental Figure 4: Increased shedding of B7-H6 after drug treatment.
Huh7 cells were treated with INAH or PMZ and then supernatants were analyzed for B7-H6 by ELISA.