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STAT3, a Hub Protein of Cellular Signaling Pathways, Is Triggered by β-Hexaclorocyclohexane

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Appendix A

Supplementary data.

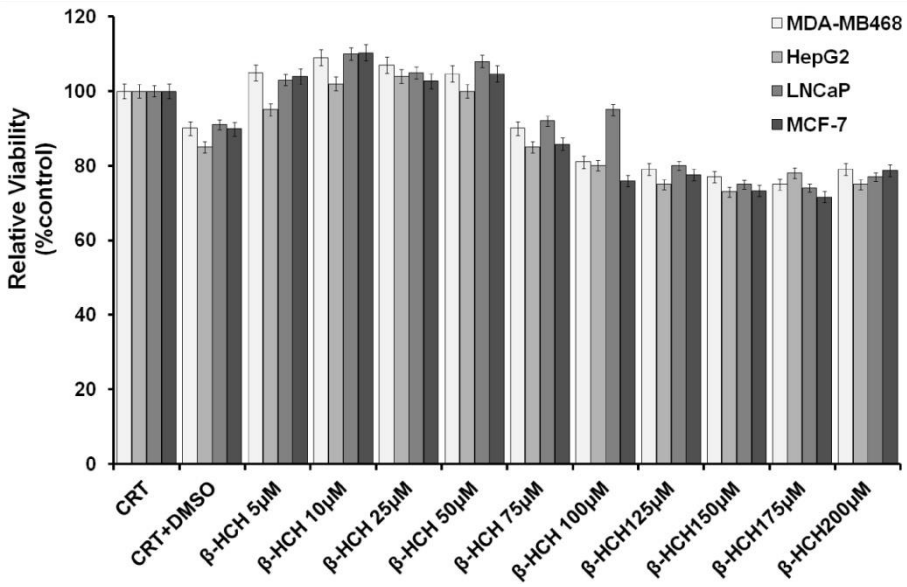

Figure S1. Cell viability assay performed on human prostate cancer (LNCaP), human breast cancer (MCF-7 and MDA-MB 468), and human hepatoma (HepG2) cell lines after 48h treatment with increasing concentration of β-hexaclorocyclohexane (β-HCH). 0.3% DMSO has also been tested as control.

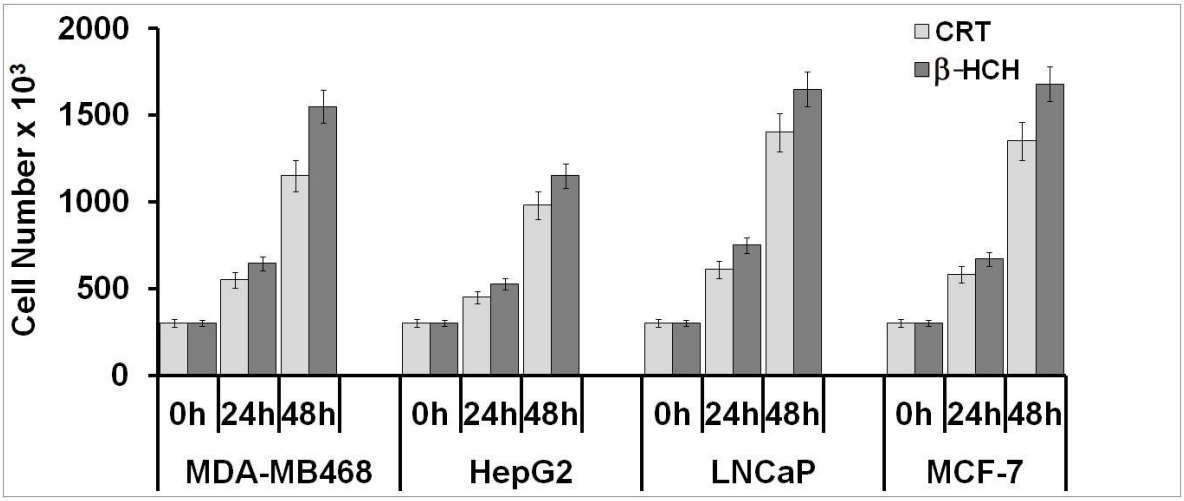

Figure S2 Evaluation of MDA-MB 468, HepG2, MCF-7, and LNCaP cell proliferation at 24h and 48h of treatment with 10 μM β-HCH.

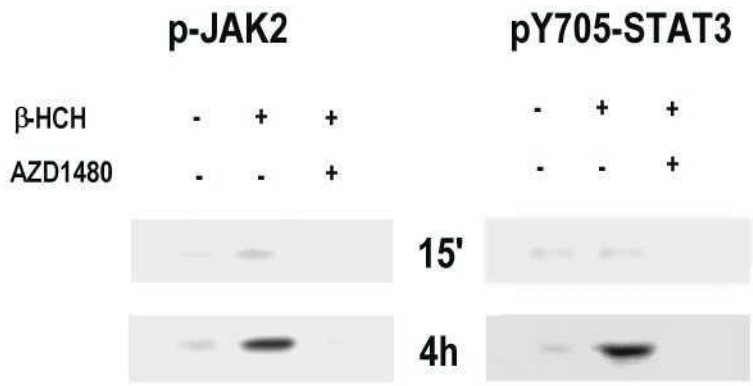

Figure S3. Evaluation of JAK2 and signal transducer and activator of transcription 3 (STAT3) phophorylation level in MCF-7 cell line treated with 10 μM β-HCH. Cellular extracts were obtained from MCF-7 cells incubated for 15 minutes and 4 hours in the absence or presence of JAK2 inhibitor (AZD1480), and then subjected to immnunoblot analysis.

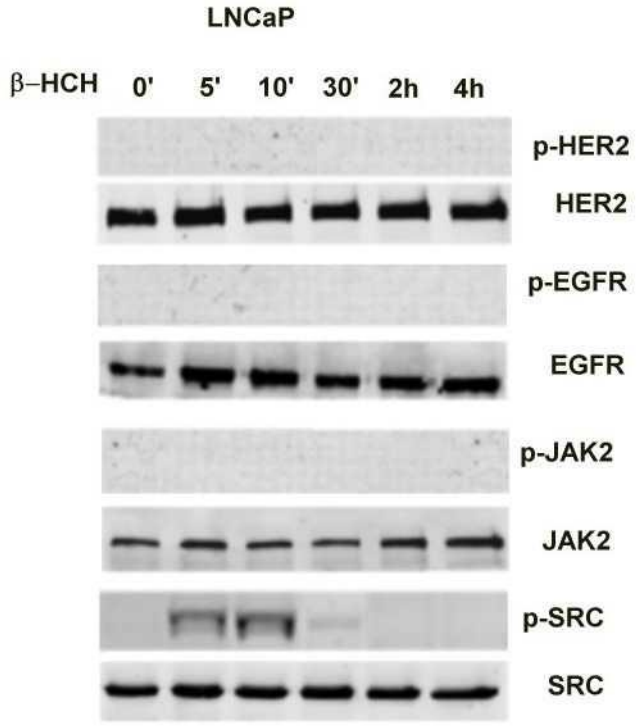

Figure S4. Analysis of the signaling pathways triggered by β-HCH in LNCaP cells. Immunoblot analysis of HER2, EGFR, JAK2, and SRC protein in a time-course assay. Both the unmodified and phosphorylated forms of each protein were evaluated.

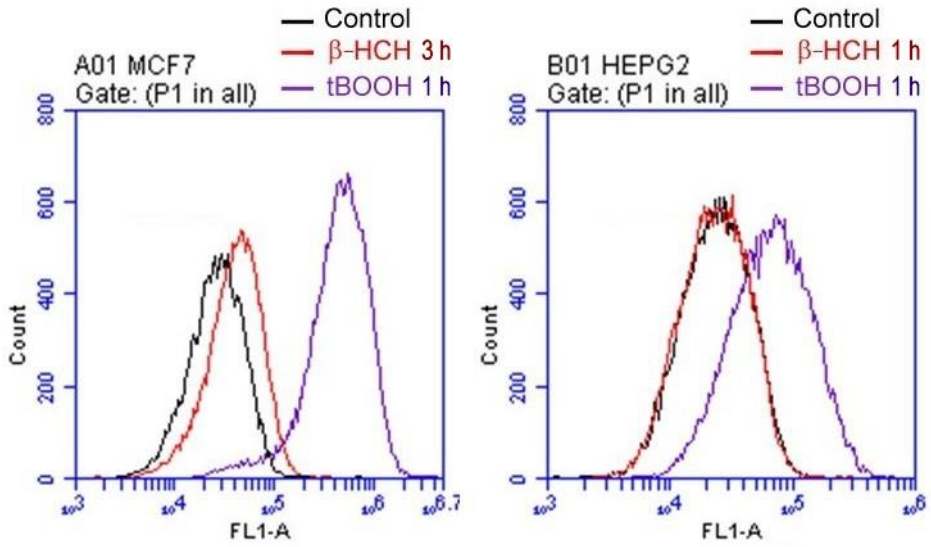

Figure S5. Quantification of ROS generated in MCF-7 (left) and HepG2 (right) cells treated with 10 μM β-HCH and 75 μM tert-butyl hydroperoxyde.

Table S1. Quantification of reduced glutathione (GSH), oxidized glutathione (GSSG), and ROS in human breast cancer (MCF-7) and human hepatoma (HepG2) cells treated for different times with 10 μM β-hexaclorocyclohexane (β-HCH).

	GSH (nmol)	**GSSG** (nmol)	**GSH/GSSG** ratio	**Cell ROS** (mean fluorescence)
HepG2 Control	30.16	4.31	7.00	28313.32
HepG2 β-HCH 10 **μM 1h**	30.28	6.20	4.88	27622.26
HepG2 β-HCH 10 **μM 3h**	30.65	6.47	4.74	26095.56
MCF-7 Control	40.03	4.71	8.49	30535.25
MCF-7 β-HCH 10 **μM 3h**	48.43	4.02	12.06	46279.62
MCF-7 β-HCH 10 **μM 6h**	46.58	7.55	6.17	39146.35