Supplements to the manuscript

Regulation of interferon-inducible proteins by doxorubicin via IFNγ-JAK-STAT signaling in tumor cells

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Journal: Molecular Pharmacology

Supplemental Table 1. Functional categorization of differentially expressed genes identified in HeLa cells upon doxorubicin treatment after 24 and 48 h determined by microarray analysis (>2 fold change).

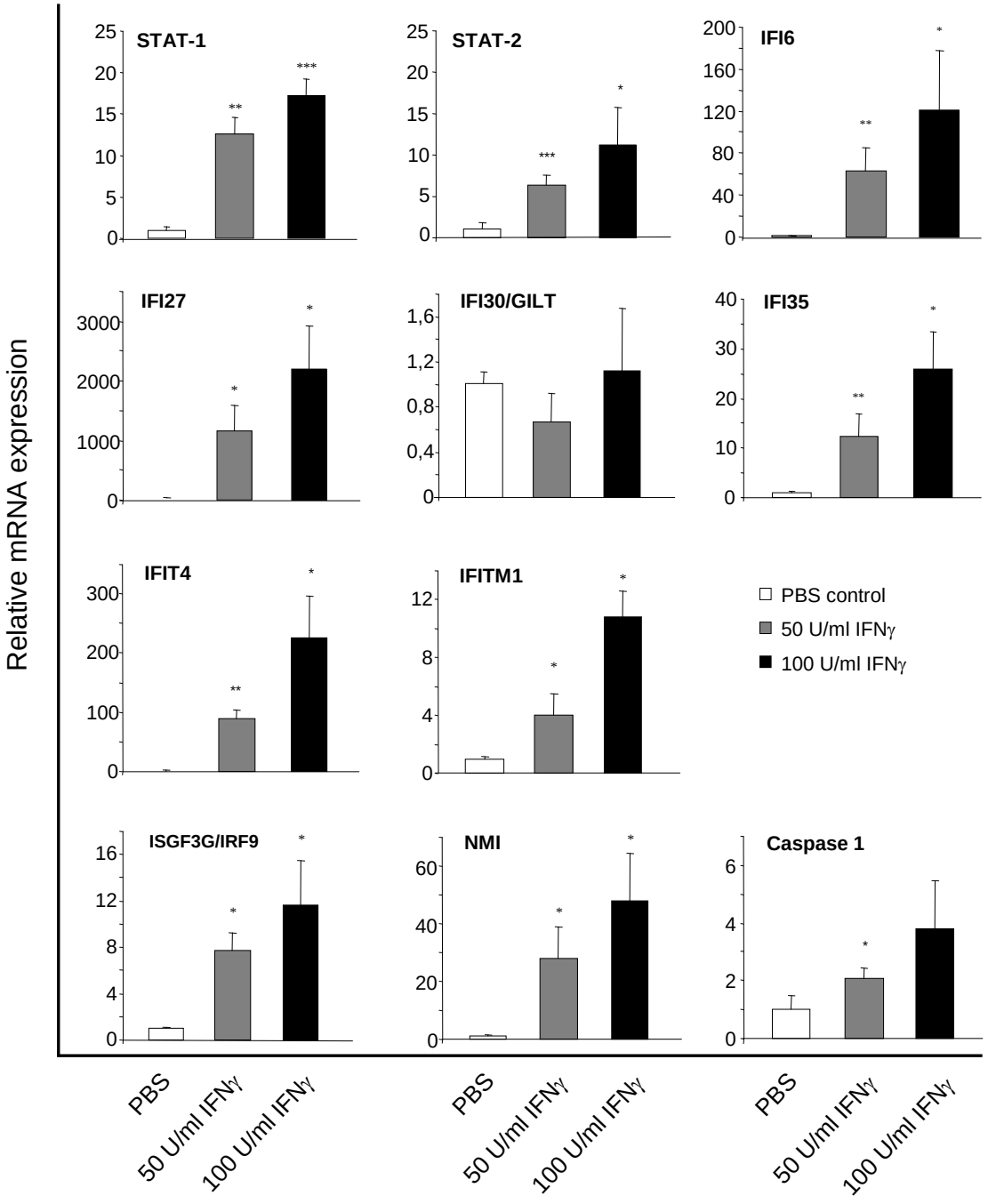
Ingenuity Canonical Pathways (*p*<0.001)	total molecules known to be associated to pathway	-log (p-value)	number of repressed genes upon Dox treatment	% down*	number of induced genes upon Dox treatment	% up**
Interferon Signaling	30	4.41	3	10	16	37
HGF Signaling	100	4.01	28	28	19	53
Cdc42 Signaling	120	3.37	29	24	18	61
Activation of IRF by Cytosolic Pattern Recognition Receptors	69	3.05	6	9	24	57
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	160	3.01	31	19	33	60
LPS-stimulated MAPK Signaling	75	2.87	17	23	16	56
IL-17 Signaling	74	2.82	14	19	20	54
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	334	2.73	42	13	69	67
PPAR Signaling	97	2.69	21	22	16	62
Prolactin Signaling	72	2.65	20	28	12	56
IL-6 Signaling	91	2.62	13	14	26	57
Glucocorticoid Receptor Signaling	268	2.6	42	16	52	65
Antigen Presentation Pathway	39	2.6	0	0	16	59
Molecular Mechanisms of Cancer	359	2.45	70	19	52	66
Type I Diabetes Mellitus Signaling	111	2.43	8	7	34	62
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	75	2.41	6	8	26	57
4-1BB Signaling in T Lymphocytes	33	2.39	5	15	11	52
mTOR Signaling	145	2.39	38	26	17	62
HMGB1 Signaling	96	2.38	20	21	20	58
PXR/RXR Activation	72	2.37	12	17	18	58
p53 Signaling	92	2.31	19	21	20	58
HER-2 Signaling in Breast Cancer	79	2.26	20	25	13	58
PPARα/RXRα Activation	174	2.26	33	19	27	66
AMPK Signaling	141	2.18	31	22	21	63
TR/RXR Activation	90	2.16	27	30	10	59
IL-8 Signaling	173	2.16	31	18	33	63
Dendritic Cell Maturation	167	2.14	18	11	38	66
CD40 Signaling	66	2.12	10	15	17	59

Glioma Signaling	107	2.1	28	26	12	63
B Cell Receptor Signaling	148	2.07	29	20	27	62
SAPK/JNK Signaling	97	2.06	24	25	13	62

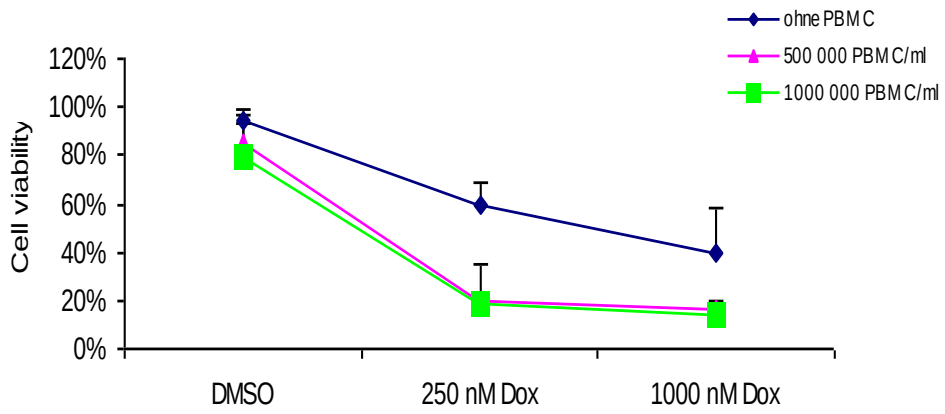
* percentage of all known molecules belonging to the pathway that is down regulated
** percentage of all known molecules belonging to the pathway that is up regulated

Supplemental Table 2. Expression levels of IFN-stimulatable genes in the presence or absence of doxorubicin. HeLa cells were incubated for 24 or 48 h with DMSO as solvent or 1 µM doxorubicin. Gene expression was determined either using DNA microarray or qRT-PCR relative to the DMSO control at the respective time point. Abbreviations: Dox, Doxorubicin; f.c., fold change

Gene symbol	Annotation	Probe set	f.c. 24 h Dox microarray	f.c. 24 h Dox qRT-PCR	f.c. 48 h Dox microarray	f.c. 48 h Dox qRT-PCR
STAT1	signal transducer and activator of transcription 1	AFFX-HUMISGF3A/M97935_5_at	5.4	3.9	10.6	9.6
STAT2	signal transducer and activator of transcription 2	225636_at	3.9	3.4	6.9	6.3
IFI6	interferon, alpha-inducible protein 6 (G1P3)	204415_at	8.0	14.3	23.3	58.9
IFI27	interferon, alpha-inducible protein 27	202411_at	15.0	89.9	1020	210
IFI30	interferon, alpha-inducible protein 30	201422_at	2.5	2.1	4.0	5.1
IFI35	interferon, alpha-inducible protein 35	209417_s_at	3.8	4.2	27.9	4.3
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	229450_at	33.7	48.3	84.0	30.4
IFITM1	interferon induced transmembrane protein 1 (9-27)	214022_s_at	2.3	6.3	7.5	13.8
IRF9	interferon regulatory factor 9	203882_at	4.3	5.2	9.5	21.0
NMI	N-myc (and STAT) interactor	203964_at	3.4	7.0	9.2	7.4
CASP1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	211367_s_at	3.4	0.5	23.9	51.0



Supplemental figure 1. Transcriptional regulation of doxorubicin stimulated genes upon IFNγ treatment. HeLa cells were treated with PBS (solvent), 50 U/ml or 100 U/ml IFNγ for 48 h, RNA was extracted, reverse transcribed and analyzed by qRT-PCR. Mean+SD, n=3, * $p<0.01$, ** $p<0.05$ and *** $p<0.001$.



Supplemental figure 2. Influence of PBMCs (peripheral blood mononuclear cells) isolated from healthy volunteers on doxorubicin induced HeLa cell killing in dependence of treatment with doxorubicin (Dox) at 250 nM and 1 µM. HeLa cells were seeded at a density of 5000 cells per well in 96-well plate and treated 24 h later with DMSO or Dox for further 24 h. Thereafter, IL-2 activated (48 h) PBMCs were added to HeLa cells at the indicated dilutions and 48 h later the supernatant containing PBMCs were removed, HeLa cells were washed with PBS for elimating all of the PBMCs and HeLa cell viability was determined using AlamarBlue assay according to the manufacturer´s instructions (Invitrogen). Mean and SD, n=3.