Table S1. Human tumor cell lines used in this study

Cancer cell line	**Cancer types**	***IDO1* expression (transcripts per 4000 transcripts of *GAPDH*)**	**Mutations**
MZ-CHA-3	Cholangiocarcinoma	150	PIK3CA E545K*
LB1610-MEL	Melanoma	150	*BRAF* V600E, PTEN Null*
KUL98-MELA	Melanoma	200	*BRAF* V600E*
LB919-SCCHN	Head and neck carcinoma	30	ERBB2 I655V*
LB188-SAR	Sarcoma	70	None
SKOV3	Ovarian carcinoma	120	PIK3CA H1047R†[1]
NCI-H596	Non-small cell lung carcinoma	8	PIK3CA E545K†[2]
LS411N	Colorectal carcinoma	0	*BRAF* V600E*

* Data from in house RNAseq analysis.
† CCLE database from the Broad Institute.
[1] Published: Whyte DB, Holbeck SL. Correlation of PIK3Ca mutations with gene expression and drug sensitivity in NCI-60 cell lines. *Biochem Biophys Res Commun* **2006**;340(2):469-75.
[2] Published: Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, Okumura N*, et al.* PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res* **2008**;68(17):6913-21.

Suppl. Fig. 1.

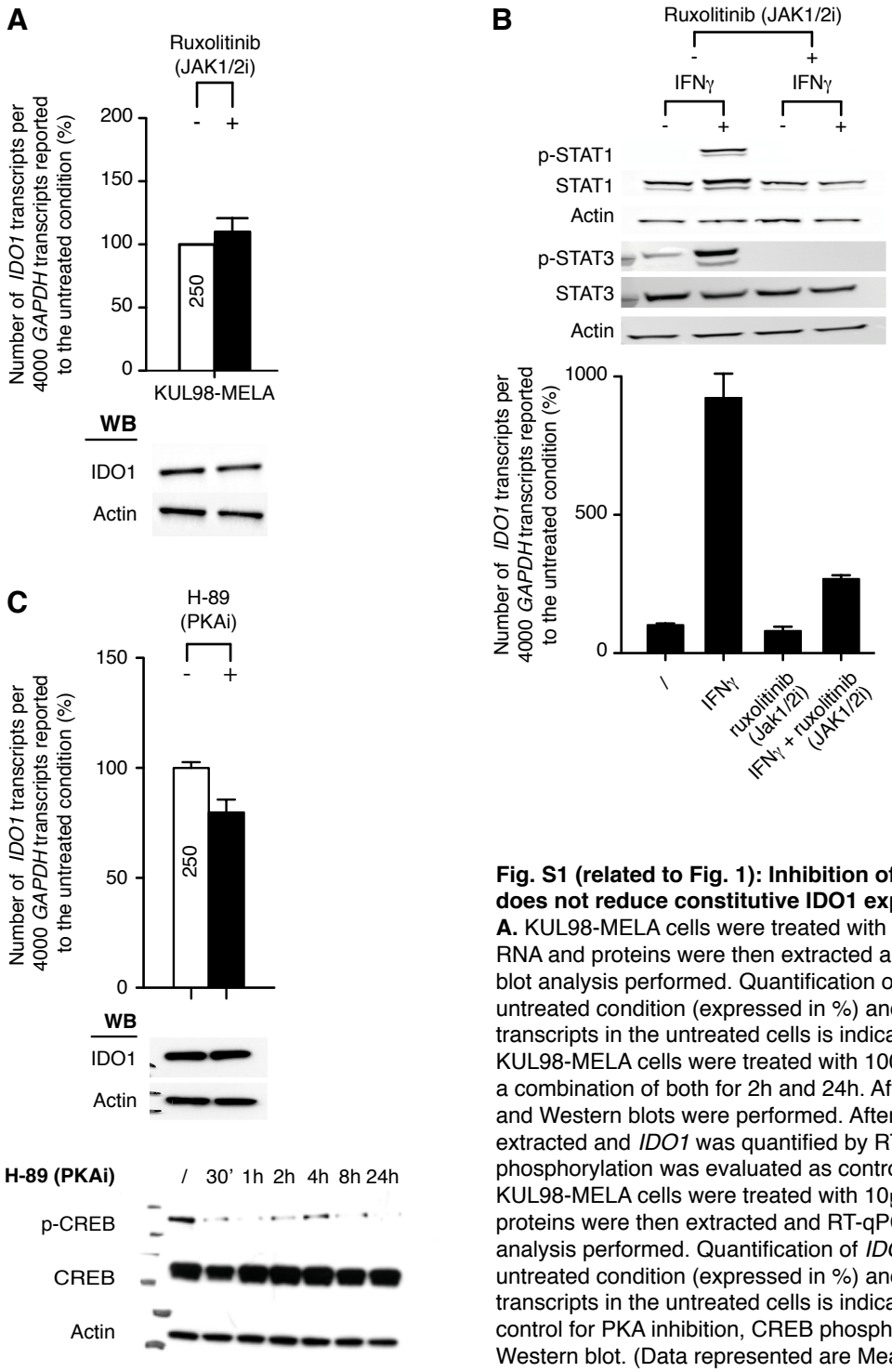


Fig. S1 (related to Fig. 1): Inhibition of the IFNγ or PKA pathway does not reduce constitutive IDO1 expression

A. KUL98-MELA cells were treated with 1μM ruxolitinib for 24h. RNA and proteins were then extracted and RT-qPCR and Western blot analysis performed. Quantification of *IDO1* was reported to the untreated condition (expressed in %) and the absolute number of transcripts in the untreated cells is indicated on the histogram. **B.** KUL98-MELA cells were treated with 100U IFNγ, 1μM ruxolitinib or a combination of both for 2h and 24h. After 2h, protein extraction and Western blots were performed. After 24h, the RNA was extracted and *IDO1* was quantified by RT-qPCR. STAT1 and STAT3 phosphorylation was evaluated as control for inhibition of JAK1/2 **C.** KUL98-MELA cells were treated with 10μM H-89 for 24h. RNA and proteins were then extracted and RT-qPCR and Western blot analysis performed. Quantification of *IDO1* was reported to the untreated condition (expressed in %) and the absolute number of transcripts in the untreated cells is indicated on the histogram. As a control for PKA inhibition, CREB phosphorylation was evaluated by Western blot. (Data represented are Mean+SD)

Suppl. Fig. 2.

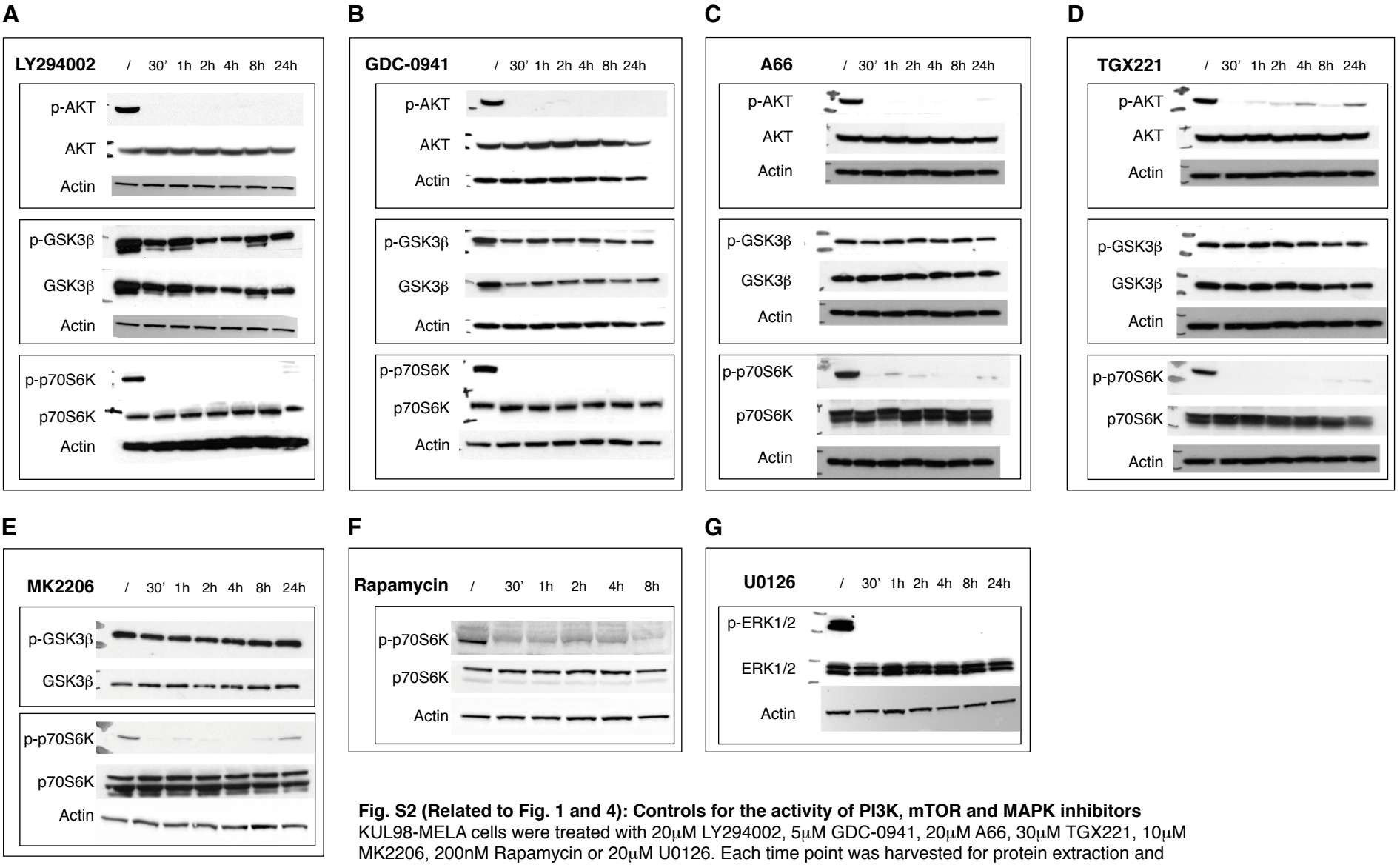


Fig. S2 (Related to Fig. 1 and 4): Controls for the activity of PI3K, mTOR and MAPK inhibitors
KUL98-MELA cells were treated with 20μM LY294002, 5μM GDC-0941, 20μM A66, 30μM TGX221, 10μM MK2206, 200nM Rapamycin or 20μM U0126. Each time point was harvested for protein extraction and Western blot analysis.

Suppl. Fig. 3.

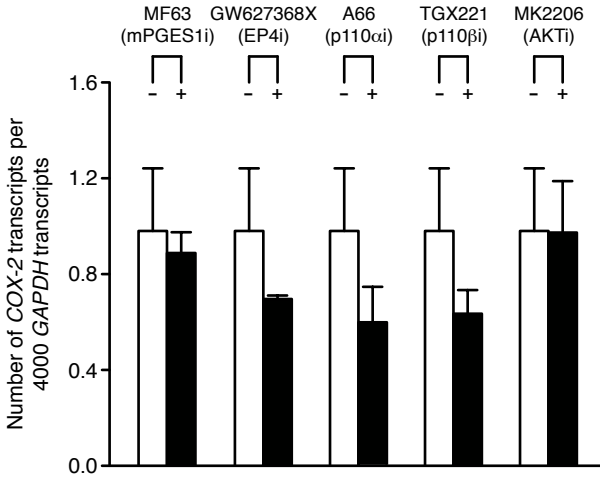


Fig. S3 (related to Fig 4) : Effect of PI3K inhibitors on COX-2 expression in KUL98-MELA cells.
KUL98-MELA cells were treated with 10μM MF63, 5 μg/ml GW627368X, 20μM A66, 30μM TGX221 or 10μM MK2206 for 72h. RNA was extracted and expression of *COX-2* was analyzed by RT-qPCR. (Data represented are Mean+SD)

Suppl. Fig. 4

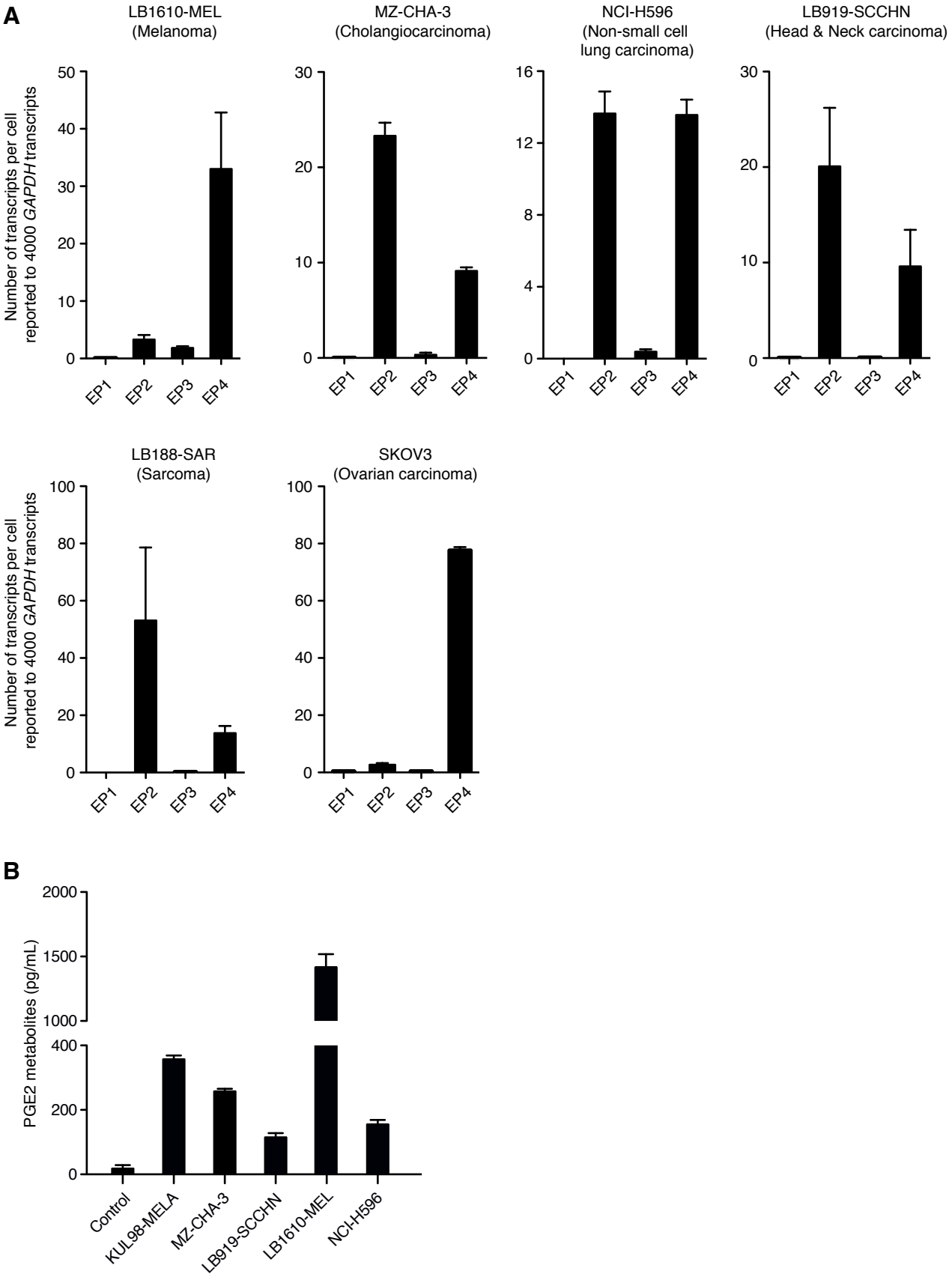


Fig. S4 (Related to Fig. 6): Quantification of EP receptors and PGE2 production in different cancer cell lines
A. Quantification by RT-qPCR of the EP receptor transcripts (EP1-4) was performed on RNA extracted from LB1610-MEL, MZ-CHA-3, NCI-H596, LB919-SCCHN, LB188-SAR and SKOV3 cells. **B.** LB1610-MEL, KUL98-MELA, MZ-CHA-3, NCI-H596 and LB919-SCCHN cells were cultured in X-VIVO 15 medium for 5 days before harvesting the supernatant and testing for the presence of PGE_2 metabolites. (Data represented are Mean+SD)

Suppl. Fig. 5.

A

IDO1 expression

Cell lines	RT-qPCR: Number of *IDO1* transcripts per cell reported to 4000 *GAPDH* transcripts	RNAseq: Gene-centric RMA-normalized mRNA expression data	
NCIH520	0.006	4.08	
MCF7	0.002	4.08	
HUPT4	4.4	4.38	Percentile 20 = 4.2
NCIH661	0.04	4.41	
A375	0.03	4.43	
JEKO1	0.006	4.44	
A172	1.8	4.44	
CALU6	0.014	4.45	
NCIH460	0	4.49	
OCILY3	0.012	4.52	
U87MG	0.002	4.53	
EB2	0.002	4.6	
U251	0.15	4.75	
BT20	0.016	4.78	
SKMES1	0.02	4.85	Percentile 80 = 4.85
CALU1	0.14	4.88	
HS578T	0.24	4.94	
NCIH441	13.54	6.33	
HUPT3	3.44	6.55	
NCI-H596	23.44	9.12	
SKOV3	305.5	11.01	

B

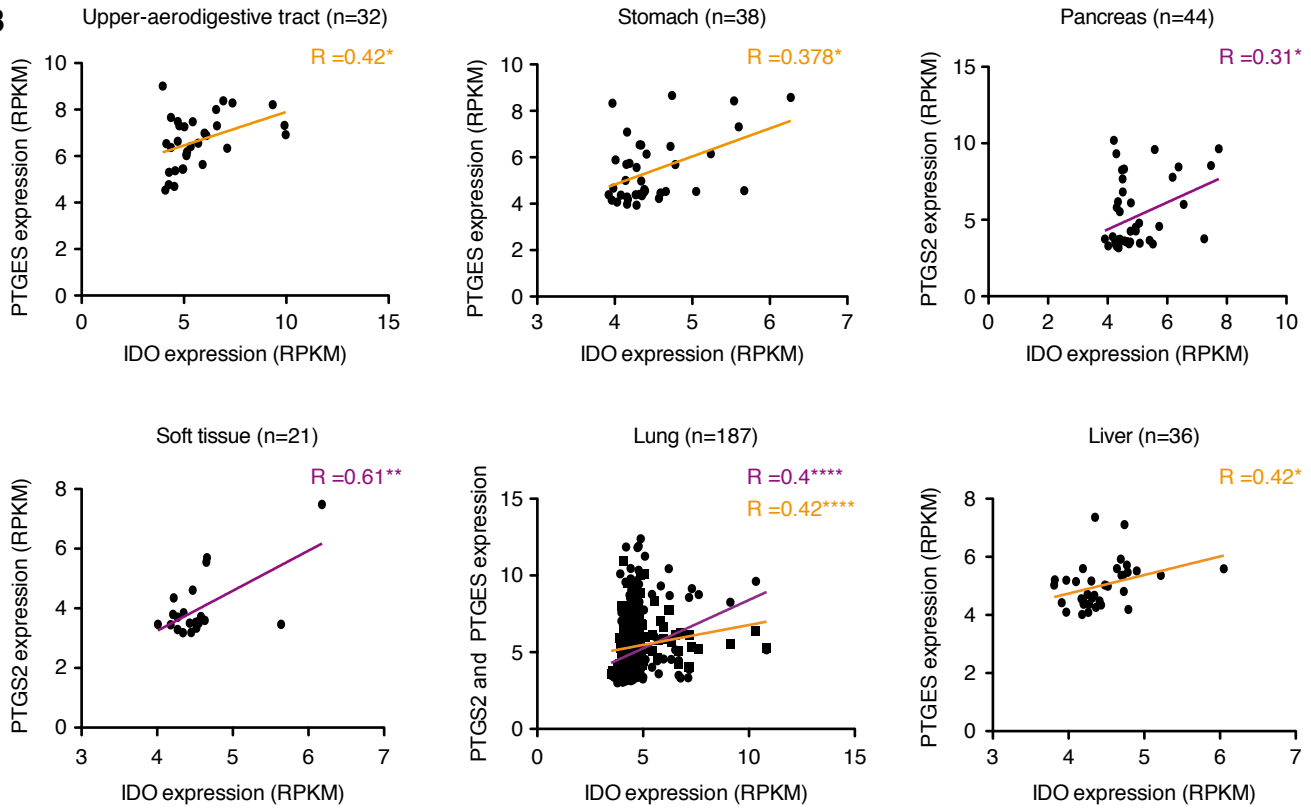


Fig. S5 (Related to Fig. 6): In silico analysis of the CCLE database
A. RNA from the selected 21 cell lines was extracted and *IDO1* expression was measured by RT-qPCR. The result was compared with the RNAseq score retrieved from the CCLE database. **B.** The CCLE database of human cancer cell lines was clustered by the different histologies and Spearman correlations between *IDO1* and *COX-2* (*PTGS2*) (violet) or *IDO1* and *mPGES1* (*PTGES*) (orange) were calculated.

Suppl. Fig. 6.

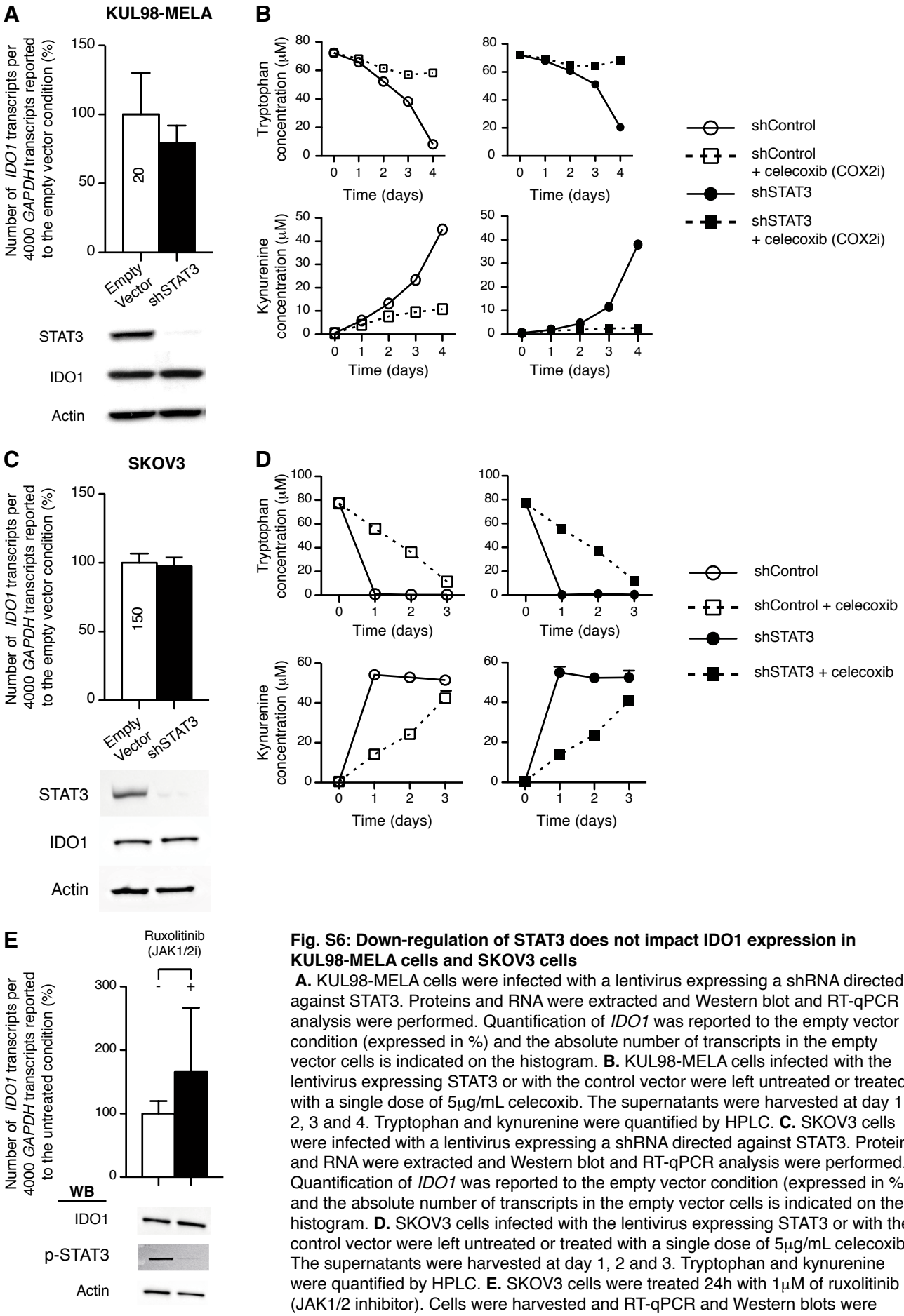


Fig. S6: Down-regulation of STAT3 does not impact IDO1 expression in KUL98-MELA cells and SKOV3 cells

A. KUL98-MELA cells were infected with a lentivirus expressing a shRNA directed against STAT3. Proteins and RNA were extracted and Western blot and RT-qPCR analysis were performed. Quantification of *IDO1* was reported to the empty vector condition (expressed in %) and the absolute number of transcripts in the empty vector cells is indicated on the histogram. **B.** KUL98-MELA cells infected with the lentivirus expressing STAT3 or with the control vector were left untreated or treated with a single dose of 5μg/mL celecoxib. The supernatants were harvested at day 1, 2, 3 and 4. Tryptophan and kynurenine were quantified by HPLC. **C.** SKOV3 cells were infected with a lentivirus expressing a shRNA directed against STAT3. Proteins and RNA were extracted and Western blot and RT-qPCR analysis were performed. Quantification of *IDO1* was reported to the empty vector condition (expressed in %) and the absolute number of transcripts in the empty vector cells is indicated on the histogram. **D.** SKOV3 cells infected with the lentivirus expressing STAT3 or with the control vector were left untreated or treated with a single dose of 5μg/mL celecoxib. The supernatants were harvested at day 1, 2 and 3. Tryptophan and kynurenine were quantified by HPLC. **E.** SKOV3 cells were treated 24h with 1μM of ruxolitinib (JAK1/2 inhibitor). Cells were harvested and RT-qPCR and Western blots were performed. (Data represented are Mean+SEM)

Suppl. Fig. 7.

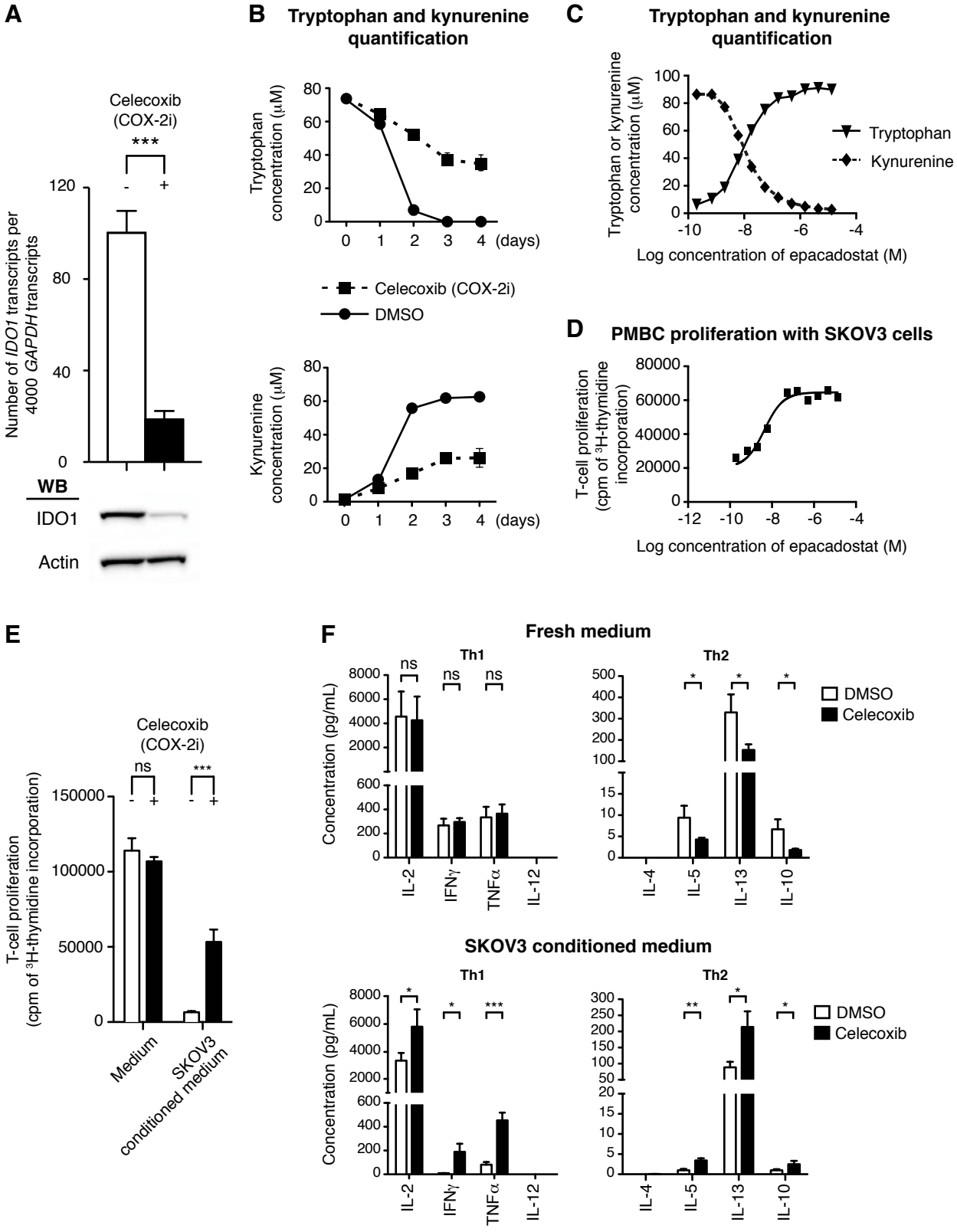


Fig. S7 (Related to Fig. 7): Treatment of SKOV3 cells with celecoxib represses IDO1 expression and tryptophan degradation, and restores proliferation of co-incubated allogeneic lymphocytes (PBMC)

A. SKOV3 cells were treated with 5 μg/ml celecoxib for 72h. RNA and proteins were extracted and Western blot and RT-qPCR were performed. **B.** SKOV3 cells were left untreated or treated with 5 μg/ml celecoxib. The supernatants were harvested every day up to day 4. Tryptophan and kynurenine were then quantified by HPLC. **C.** SKOV3 cells were treated 24h with a range of different concentrations of epacadostat. Supernatants were harvested and tryptophan and kynurenine were quantified by HPLC. **D.** Irradiated SKOV3 cells were pre-treated with a range of different concentrations of epacadostat and co-cultured with human allogeneic PBMCs stimulated with CD3/CD28 beads and IL-2. ^{3}H-thymidine was added and proliferation was measured. **E.** Human allogeneic PBMCs stimulated with CD3/CD28 beads and IL-2 were cultivated in fresh medium containing or not 5 μg/ml celecoxib or in SKOV3 conditioned medium from SKOV3 cells (treated or not 72h with 5 μg/ml celecoxib). ^{3}H-thymidine was added and proliferation was measured 24h later. **F.** Supernatants of the PBMCs from panel E were used to quantify production of Th1 and Th2 cytokines by the PBMCs. (Data represented are Mean+SD)