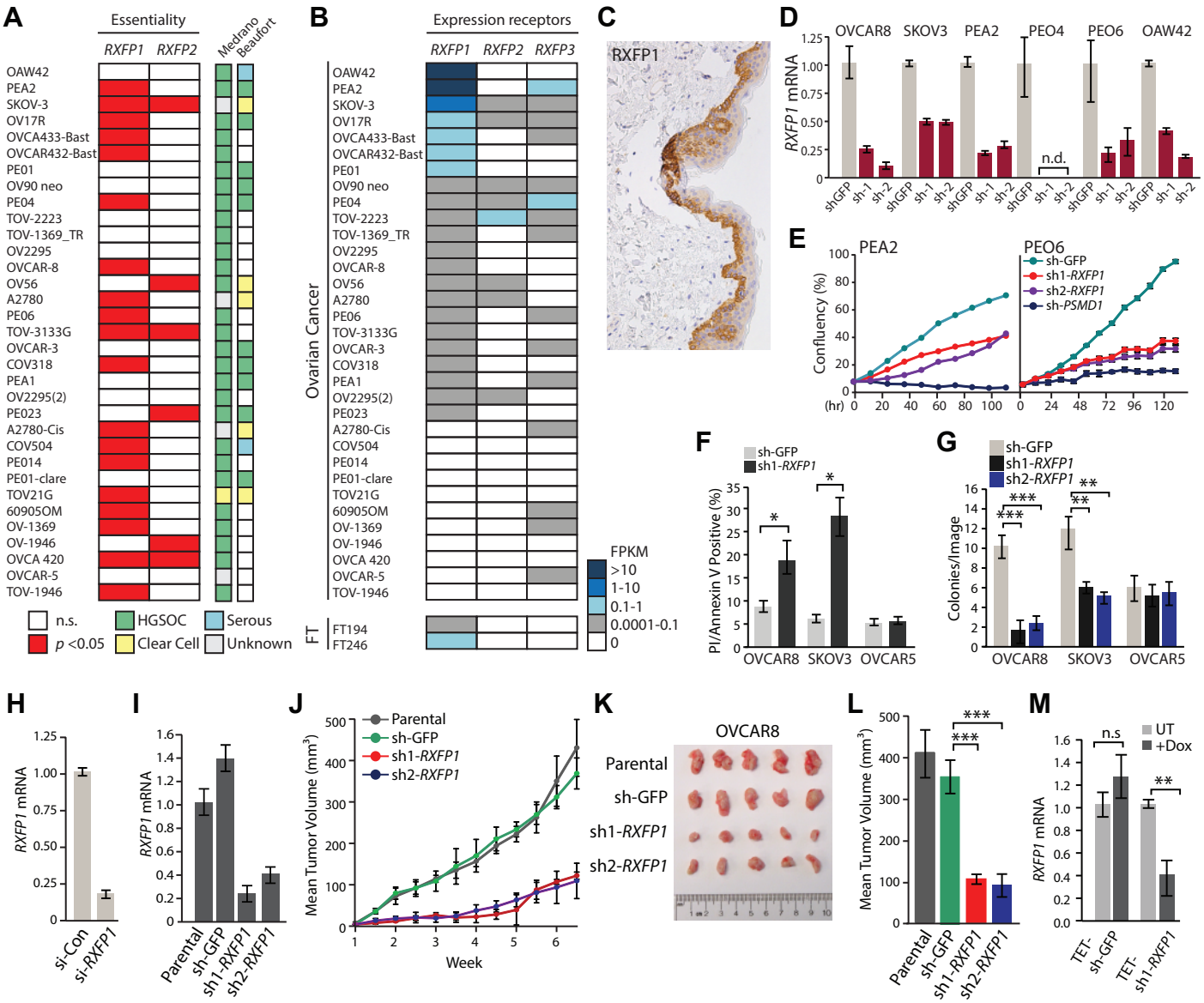


Fig.S1. *RXFP1* is an essential GPCR in a subset of HGSOC cell lines. Related to Fig.1.

(A) Essentiality score (p-value of GARP) for cell line dependency on *RXFP1* or *RXFP2*. P-value <0.05 considered essential (n.s. = not significant). Cell lines ranked based on expression of *RXFP1* (panel B). Origin of cell lines based on the suitability score (Medrano et al. Cell Reports, 2017) and putative histolgy (Beaufort et al. Plosone 2014).

(B) Representation of RNAseq expression values (FPKM) for relaxin receptors in ovarian cancer and fallopian tube (FT) cell lines.

(C) Postive control for RXFP1 staining by IHC . Human mucosa scamous epithelial cells stained with anti-RXFP1 antibody (Sigma Prestige).

(D) Validation of shRNA knockdown of *RXFP1* using two independent shRNAs targeting *RXFP1*(sh1-or sh2) or control (sh-GFP) in the indicated cell lines. For this panel and subsequent panels all shRNA are constitutively expressed.

(E) Growth curves of PEA2 and PEO6 expresssing control hairpins (sh-GFP or sh-*PSMD1*) or hairpins targeting *RXFP1* (sh1-*RXFP1* or sh2-*RXFP1*) as indicated in the legend.

(F) PI/Annexin V staining by flow cytometry 48 hours after puromycin selection in the indicated cell lines expressing sh-GFP or sh1-*RXFP1*. Error bars indicate mean ± SEM(n=3). (*p<0.01, student's t-test).

(G) Quantification of colonies formed in Fig.1E. Error bars indicate mean ± SEM (n=3). (*p<0.001, ***p<10E-06, Dunnett's test).

(H) *RXFP1* mRNA expression in OVCAR8 cells transfected with non-targeting control siRNA (si-Con) or siRNA targeting *RXFP1*.

(I) Validation of *RXFP1* knockdown in xenograft-injected OVCAR8 cells expressing constitutively active sh-GFP or two independent hairpins targeting *RXFP1* (sh1-*RXFP1*, sh2-*RXFP1*) in comparison to the parental cell line.

(J) Growth curves of the indicated OVCAR8 xenografts in mammary fat pad (error bars indicate mean +/- SEM; n=4).

(K) Excised xenografts from parental OVCAR8 or OVCAR8 expressing sh-GFP or shRNA targeting *RXFP1* (sh1-*RXFP1*, sh2-*RXFP1*).

(L) Mean tumor volume of excised xenografts described in (K). (***p<0.0001, Dunnett's test).

(M) Normalized expression (± SEM) of *RXFP1* mRNA in OVCAR8 cells expressing doxycycline-inducibleTET-shGFP or TET-sh1-*RXFP1* in the absence or presence of doxycycline (+Dox, 1 μg/mL) compared to untreated cells (UT) 48 hours post Dox treatment. (**p<0.001, student's t-test).

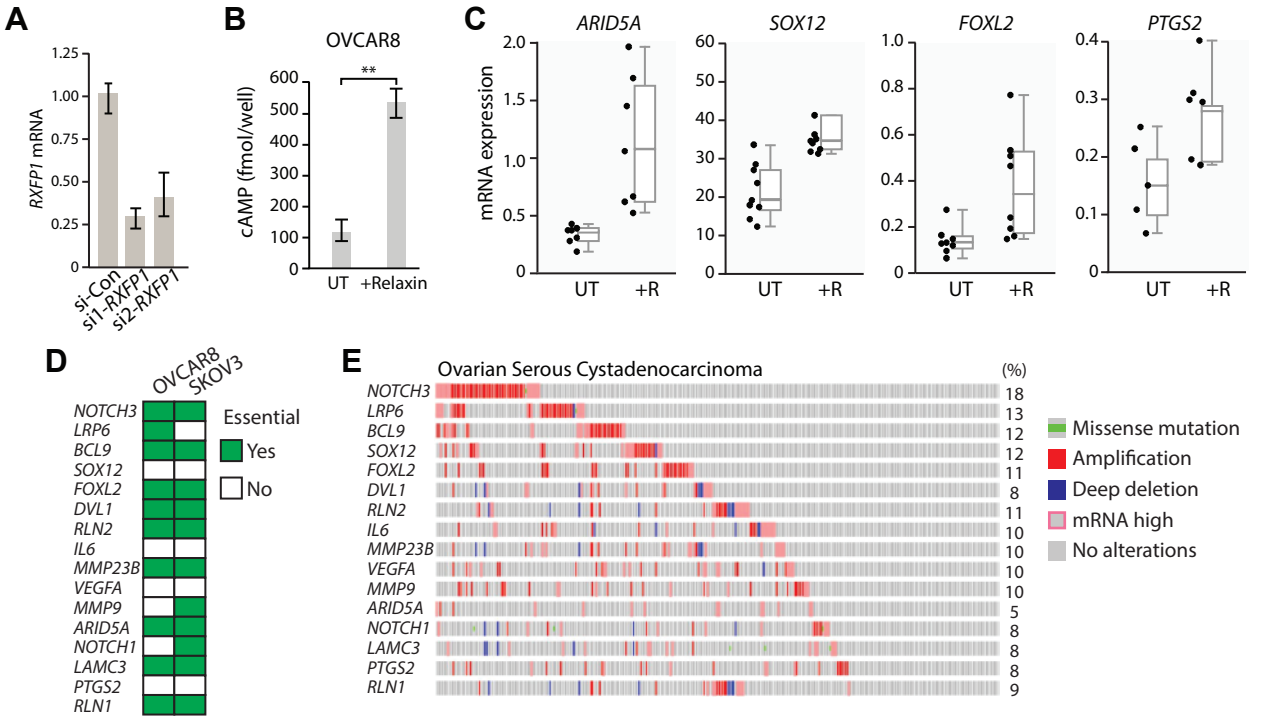


Fig.S2. Relaxin promotes proliferation and signaling in HGSOC cell lines. Related to Fig.2.

(A) *RXFP1* mRNA expression in OVCAR8 cells transfected with non-targeting control siRNA (si-Con) or siRNA targeting *RXFP1*.

(B) cAMP induction by recombinant human relaxin (+Relaxin) compare to untreated (UT) OVCAR8 cells. OVCAR8 cells were cultured in media containing low serum (1% FBS) overnight and were pre-treated with1mM IBMX for 2 hours at 37°C. Cells were cultured +/- human relaxin (50 ng/mL) for 30 minutes at 37°C. Cells were lysed and cAMP was measured by ELISA. Error bars indicate mean ± SD (n=3). (**p<0.001, student's t-test).

(C) Validation of selected relaxin target genes identified by RNAseq analysis. Transcript levels of each gene were measured in untreated cells (UT) or cells treated with recombinant human relaxin (+R, 50ng/mL) for 8h. Error bars indicate mean ± SD (n≥5).

(D) Relaxin gene signature examined for genetic vulnerability in OVCAR8 and SKOV3 cells using the CRISPR (Avana) Public 19Q4 dataset (Depmap portal).

(E) Oncoprint on ovarian serous cancer and analysis of relaxin regulated target genes indentified by RNA-seq. Data from cBioPortal.

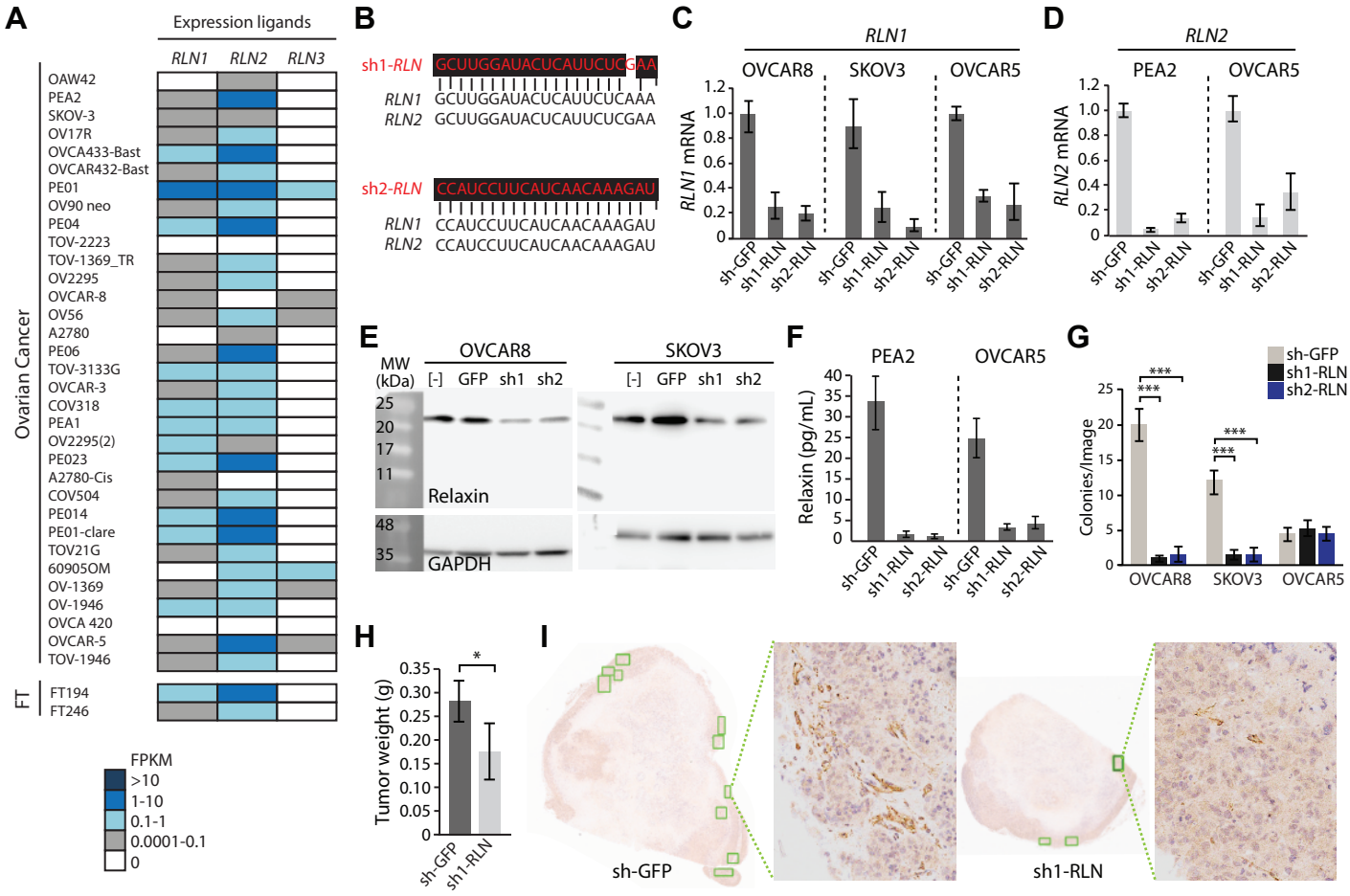


Fig.S3. Expression of relaxin in HGSOC cell lines is essential for survival. Related to Fig.3.

(A) Representation of RNAseq expression values (FPKM) for relaxin ligands in ovarian cancer and fallopian tube (FT) cell lines.

(B) Alignment of sh1-RLN and sh2-RLN target sequences to *RLN1* and *RLN2* mRNA.

(C-D) Evaluation of *RLN1* mRNA (C) and *RLN2* mRNA (D) expression following knockdown by sh1-RLN and sh2-RLN in the indicated cell lines compared to expression in sh-GFP cells.

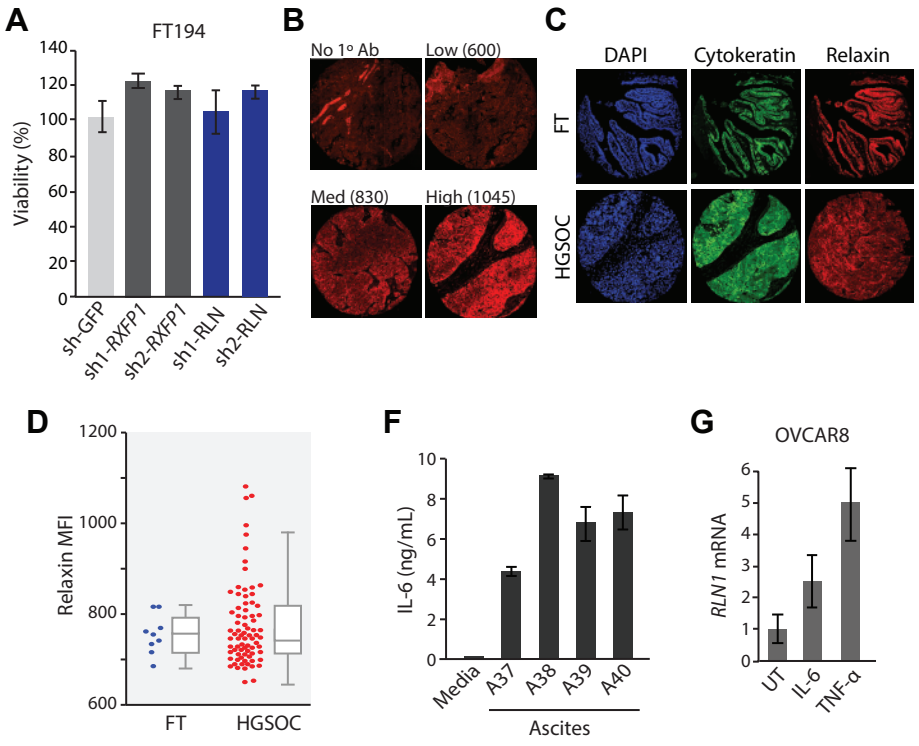
(E) Uncropped blot showing pro-relaxin in OVCAR8 and SKOV3 parental cells [-] or cells expressing sh-GFP or shRNA targeting RLN (sh1- or sh2-) 48 hours following selection. GAPDH served as a loading control. Photograph of the molecular weight markers obtained with Microchemi-4.2 (DNR Bio-imaging Systems) software. Data represents the images cropped in Figure 3E.

(F) Relaxin levels measured by ELISA in cell culture supernatants derived from PEA2 and OVCAR5 cells expressing sh-GFP or shRNA targeting relaxin (sh1-RLN or sh2-RLN).

(G) Quantification of colonies formed in Fig.3F. Error bars indicate mean ± SEM (n=3). (***p<0.0001, Dunnett's test.).

(H) Average tumor weight (grams =g) of excised xenografts described in Figure 3G. Error bars represent mean +/- SEM. (*p<0.01, Student's t-test).

(I) IHC of CD31 staining in tumors dervied from OVCAR8 cells expressing sh-GFP control of shRNA targeting relaxin (sh1-RLN). Green boxes denote regions used for quantification based on visual detection of positive CD31 staining. The expanded regions are also shown in Fig3.



E

Parameter		Number
Number of patients (n)		48
Age (years)	Median	63
	STD	10.9412
Debulking	Primary	26
	Secondary	29
	Tertiary	1
	Interval	4
Pathology (n = 48)	High Grade Serous*	38
	Clear Cell**	3
	Other***	7
Stage	IA	2
	IIB	1
	IIIB	6
	IC	1
	IIC	2
	IIIC	31
	III	5
	IV	5
	Unknown	7
CA125		
HGSOC	Mean (μ)	827.7
	Median	222
Clear Cell	Mean (μ)	45.3
	Median	30
Other	Mean (μ)	178.9
	Median	148
Relaxin (pg/mL)		
High Grade Serous	Mean	85.3
	Median	41.9
Clear Cell	Mean	80.5
	Median	102.7
Other	Mean	114.7
	Median	53.7
Control	Mean	30.5
	Median	28.5

Fig.S4. Examination of relaxin expression in tumors and ascites. Related to Fig.4.

(A) Viability assay of FT194 cells expressing control shRNA (sh-GFP) or shRNA targeting *RXFP1* (sh1-*RXFP1*, sh2-*RXFP1*) or shRNA targeting relaxin (sh1-RLN, sh2-RLN).

(B) Validation of intensity of relaxin immunofluorescence in HGSOC tissue microarrays. Low, medium and high values estimated based on mean fluoresence intensity (MFI). Background staining indicated in the no primary antibody (No 1° Ab) panel.

(C) Immunofluorescence of relaxin expression in normal fallopian tube (FT) and HGSOC tissue microarrays. Epithelial cells are marked by cytokeratin and DAPI marks the nucleus.

(D) Quantification of Relaxin levels in normal fallopian tube and HGSOC tissue microarrays (MFI = mean floresence intensity).

(E) Table of parameters associated with patient cohort data in Figure 4. Cancer antigen 125 (CA125). Control for relaxin elisa healthy donor serum (n=14).

*High Grade Serous (Serous, Serous primary peritoneal, Serous Fallopian tube , Borderline Serous Papillary, Serous Papillary, Primary peritoneal)

**Clear Cell (Clear Cell ovarian carcinoma, High grade Clear Cell carcinoma, Clear Cell ovarian adenocarcinoma)

***Other (Granulosa cell tumor, Mixed Serous and endometroid ovarian adenocarcinoma, Pelvic mass: Polypoid Endometriosis and Fibroadipose tissue, Metastatic adenocarcinoma (BRCA1+), Mucinous adenocarcinoma of the ovary, Serous borderline, Borderline Serous with numerous psammoma bodies).

(F) IL-6 protein levels in patient derived ascites supernatant (samples A37-40) measured by ELISA.

(G) *RLN1* mRNA expression in OVCAR8 cells treated with IL-6 or TNF-α (50 μg/mL).

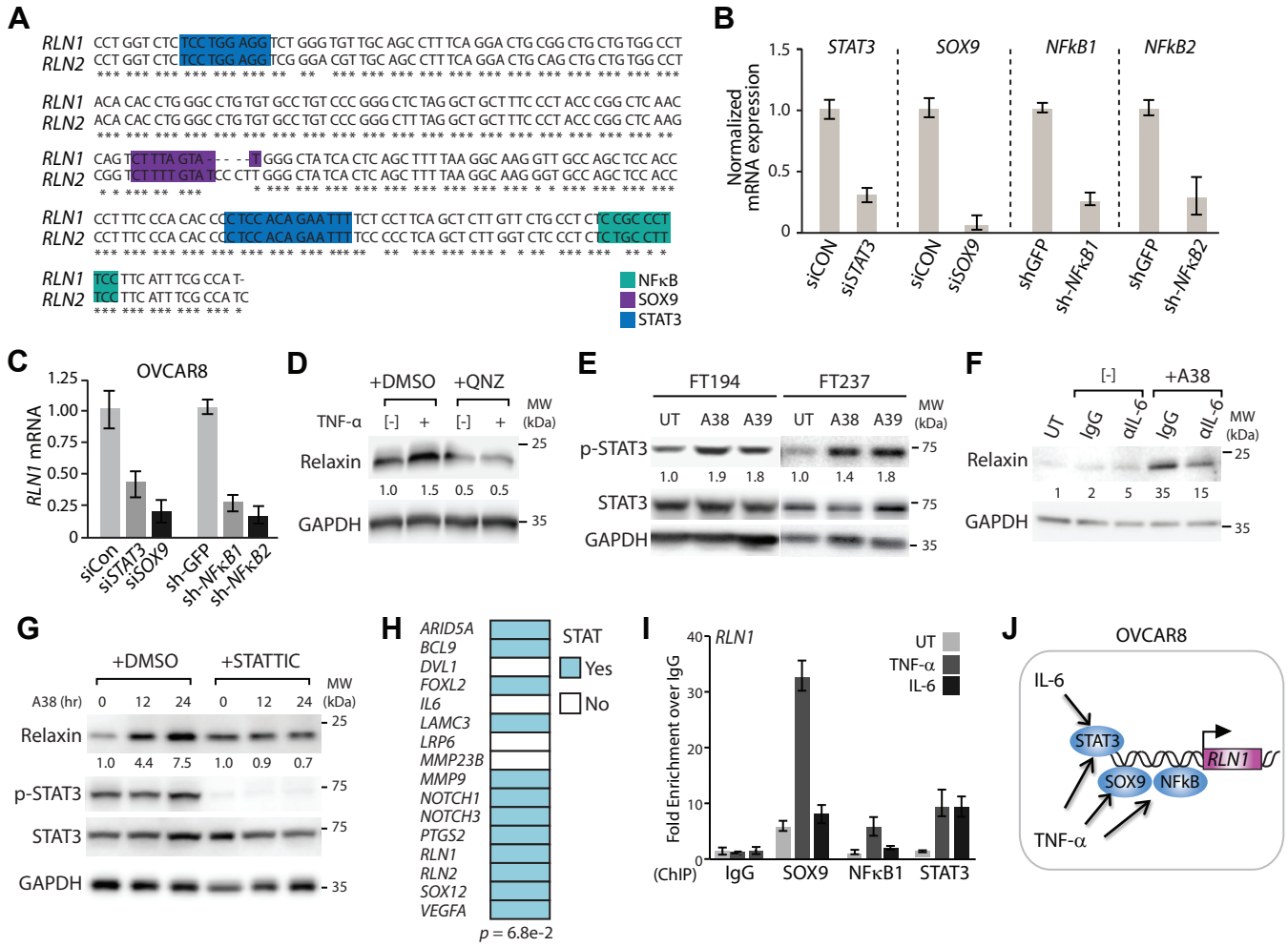


Fig.S5. Relaxin is a tanscriptional target of NFKB, STAT3 and SOX9. Related to Fig.5.

(A) Clustal alignment of *RLN1* and *RLN2* genomic regions corresponding to the proximal promoter and the minimal promoter RP-3 as reported in Fig.6. Predicted STAT3, SOX9, and NFKB binding elements defined by Transfac database are highlighted.

(B) Validation of the indicated transcription factor knockdown in OVCAR8 cells by qPCR.

(C) *RLN1* mRNA expression measured by Taqman qPCR in OVCAR8 cells transfected with nontargeting siRNA (siCon) or siRNA targeting STAT3 or SOX9 and OVCAR8 cells expressing shRNA control (sh-GFP) or shRNA targeting NFκB subunits B1 or B2.

(D) Relaxin protein levels in OVCAR8 cells treated with TNF-α (50 ng/mL) for 24 hours pre-treated with NFκB inhibitor QNZ (+QNZ) compared to cells treated with DMSO.

(E) Immunoblot of phosphorylated (p)-STAT3 (p-STAT3) in FT194 and FT237 cells 72h growth post culture in 10% ascites supernatant (a38, A39) in comparison to untreated cells (UT).

(F) Immunoblot and quantification of relaxin levels in FT-194 cells cultured in 10% ascites supernatant (+A38) compared to control treated cells (-) examined 72h post treatment. Cells were co-treated with IL-6 neutralizing antibody (αIL-6) or IgG isotype control (IgG) as indicated.

(G) Western blot of Relaxin protein levels in FT-194 cells cultured in 10% ascites supernatant (A38) for the indicated time in hours (hr) in cells pre-treated for 6 hours with STATTIC (10μM) or DMSO control.

(H) Relaxin gene signature examined for STAT binding elements in the putative promoter of the indicated genes using the motif identifed by the transfac matrix database (v7.0) annotated in human, mouse, rat alignment (DAVID and UCSC genome browser).

(I) ChIP analysis of NFκB, STAT3 and SOX9 binding on the promoter region of *RLN1* in OVCAR8 cells mock treated (UT) or treated with TNF-α (50ng/mL) or IL-6 (50ng/mL). Fold enrichment is relative to IgG.

(J) Summary of *RLN1* gene activation in OVCAR8 cells following stimulation as determined by ChIP.

A

ID #	Grade:	Stage:	Time of sampling:	Sampling Time (Months after T0):
1	4	IIIC	1st day of chemo, post surgery	T0
			End of chemo because progression	4.5
2	3	IIIC	At surgery, pre-chemo	T0
			At 2nd surgery, post-chemo	2.75
3	4	IIIC	At surgery, pre-chemo	T0
			Post-chemo, last day of treatment	4.75
4	3	IIIC	At surgery, pre-chemo	T0
			At 2nd surgery, post-chemo	4
5	3	IIIC	At surgery, pre-chemo	T0
			At 2nd surgery, post-chemo	2.25
6	4	IIIC	At surgery, pre-chemo	T0
			At 2nd surgery, post-chemo	4
7	4	II	Pre-chemo, pre-surgery	T0
			Post-chemo	4.75
8	3	IIB	At surgery, pre-chemo	T0
			At progression, 2nd surgery, post-chemo	2
9	4	IIIC	At surgery, pre-chemo	T0
			At 2nd surgery, post-chemo	2.5
10	4	IVA	At surgery, pre-chemo	T0
			At 2nd surgery, post-chemo	4.75
11	4	IIIC	At surgery, pre-chemo	T0
			At 2nd surgery, post-chemo	5.75
12	4	IIIC	At surgery, pre-chemo	T0
			At 2nd surgery, post-chemo	4.25

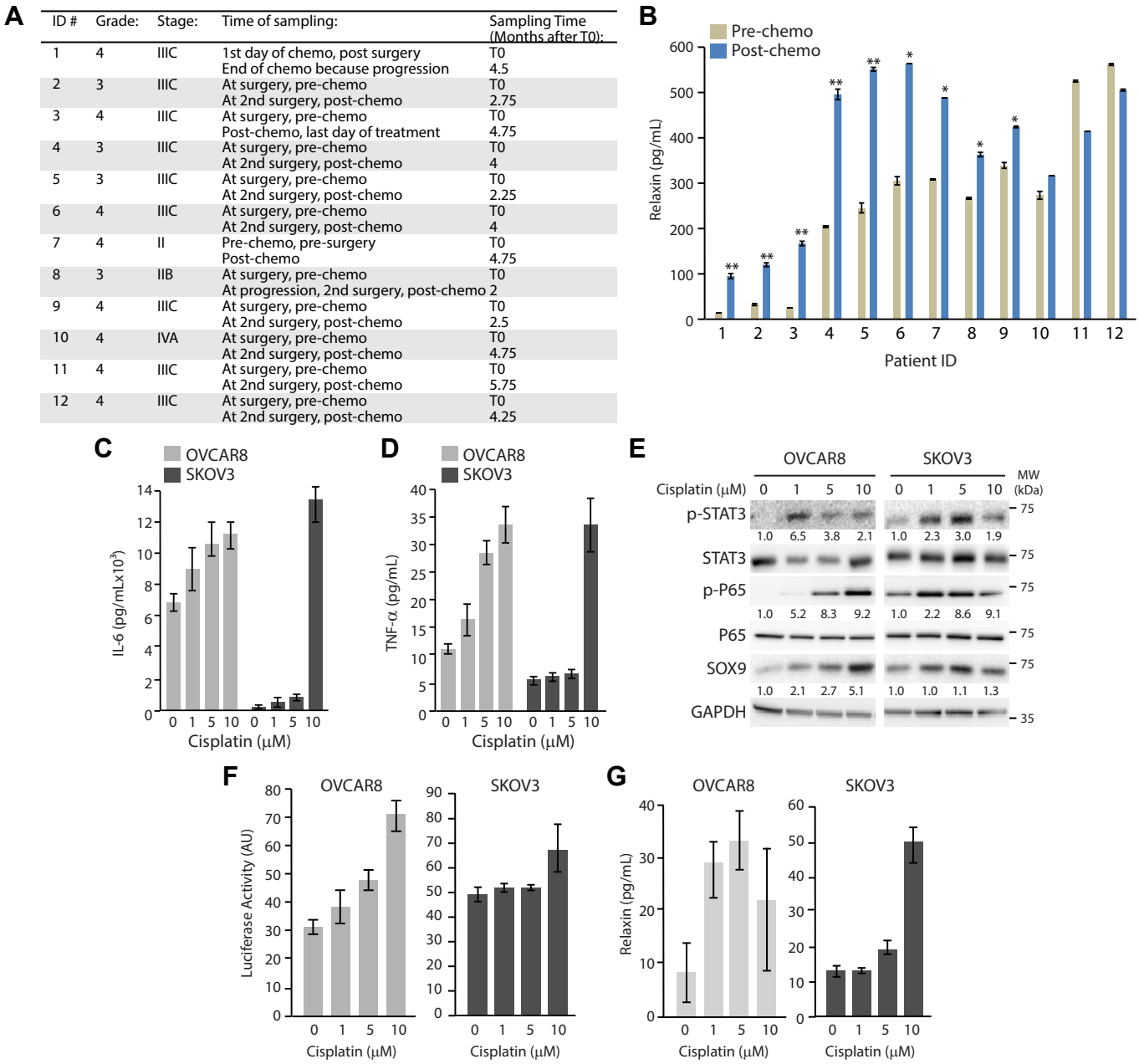


Fig.S6. Depletion of *RXFP1*-relaxin sensitizes HGSOC cells and tumors to cisplatin. Related to Fig.6.

(A) Table of patient clinical data. All patient cases are from serous cystadenocarcinoma and were treated with Taxol/Carboplatin. Time zero (T0) represents data points labeled "pre-chemo" and second sampling represent "post chemo" in panel B.

(B) Relaxin protein levels determined by ELISA in patient serum samples pre- and post-chemo. (*p<0.05 and **p<0.01, Student's t-test).

(C) IL-6 protein levels determined by ELISA in supernatants derived from OVCAR8 cells treated with increasing doses of cisplatin.

(D) TNF-α protein levels determined by ELISA in supernatants derived from OVCAR8 cells treated with increasing doses of cisplatin.

(E) Immunoblot of phosphorylated (p) STAT3 (p-STAT3), p-NFKB (p-P65) and SOX9 in OVCAR8 and SKOV3 cells following treatment with increasing doses of cisplatin.

(F) Luciferase activity (arbitrary units = AU) driven by the Relaxin promoter (RP-3) in OVCAR8 and SKOV3 cells following treatment with increasing doses of cisplatin.

(G) Relaxin protein levels determined by ELISA in supernatants derived from OVCAR8 and SKOV3 following treatment with increasing doses of cisplatin.

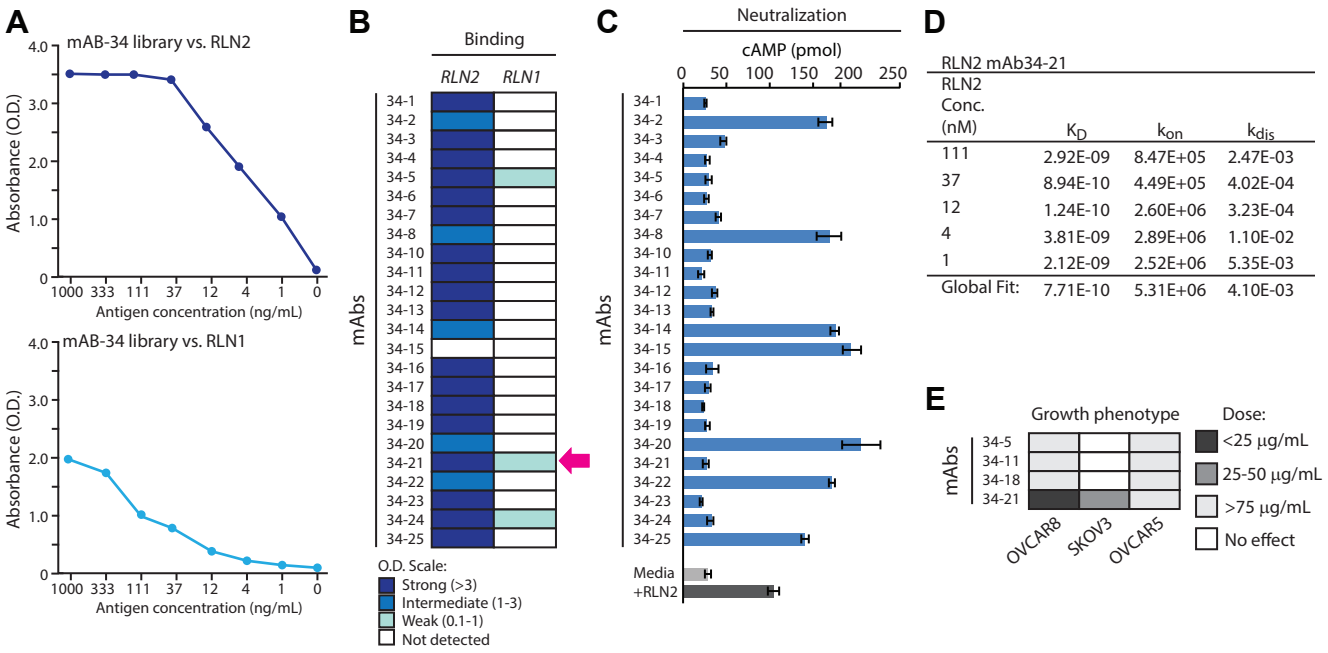


Fig.S7. Relaxin neutralizing monoclonal antibody abrogates HGSOC cell growth. Related to Fig.7.

(A) Antigen titre of hybridoma library RLN2Am34. The pooled polyclonal hybridoma library was screened for binding against serial dilutions of either RLN1 or RLN2 by ELISA.

(B) Binding analysis of anti-relaxin monoclonal antibodies (mAbs). Binding of each mAb to recombinant RLN1 and RLN2 was determined by ELISA, with colors indicating relative strength of binding to each ligand (OD value). OD values ranged from background (OD=0.05) to a maximal OD value of 3.37. Strong binding represents OD values >60X background, intermediate binding represents OD values 20-60X background OD, and weak binding represents OD values 2-20X background OD. Pink arrow highlights mAb-34-21 used in subsequent experiments.

(C) Neutralization analysis of anti-relaxin monoclonal antibodies (mAbs). Neutralizing activity represents ability to reduce cAMP accumulation in THP-1 cells by recombinant relaxin (rhRLN2). Media alone and media plus rhRLN2 (+RLN2) are indicated.

(D) Binding kinetics of purified m34-21 antibody to relaxin-2 (RLN2) at the indicated concentraions (nM = nanomolar) and assessed by Bio-Layer interferometry. Meaasure of the parameters for on rates (kon), off rates (kdis) and the overall molar affinity constant (KD) for M21 binding to each concentration of RLN2 are indicated.

(E) Ability of select anti-relaxin mAbs to reduce growth of relaxin dependent (OVCAR8 and SKOV3) and independent (OVCAR5) cells. Dose represents the minimum concentration of each mAb required to observe a growth defect.

Supplemental File: Uncropped gels from the main figures:

Figure 1E

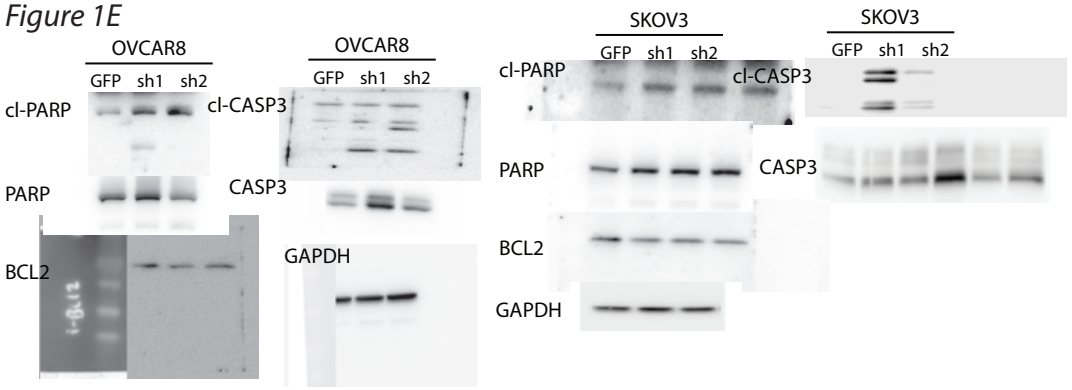


Figure 2C

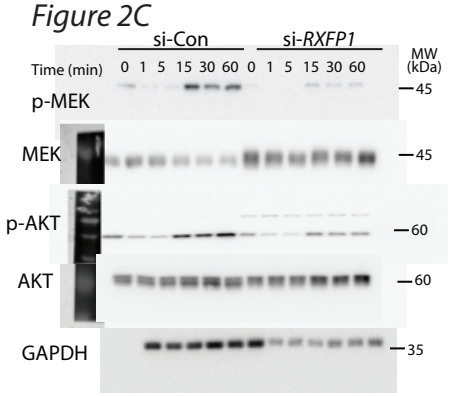


Figure 3D

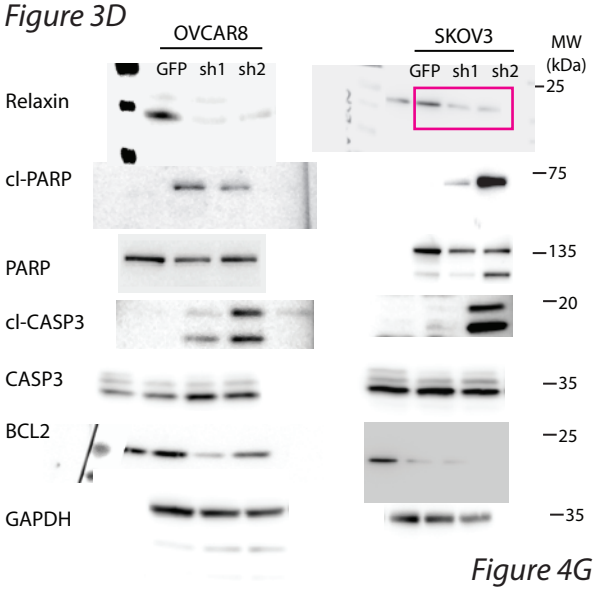


Figure 4A

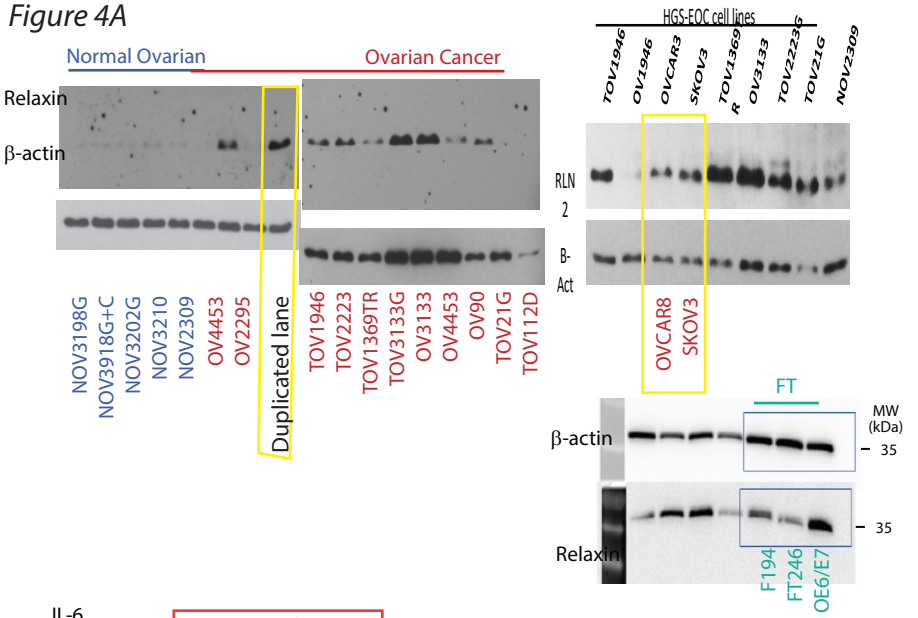


Figure 4E

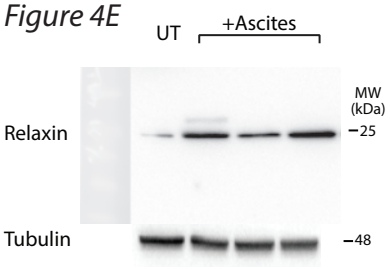


Figure 4G

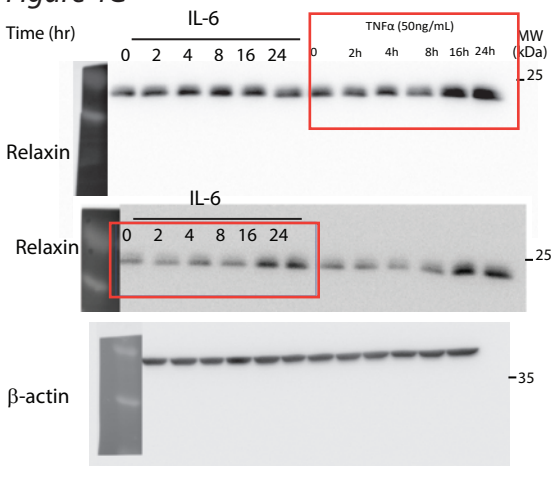


Figure 4H

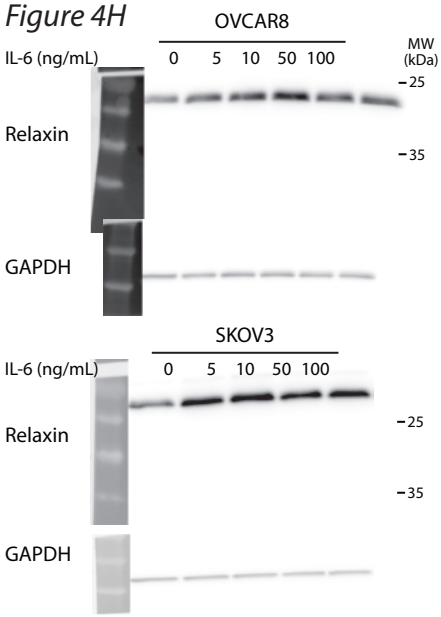


Figure 5H

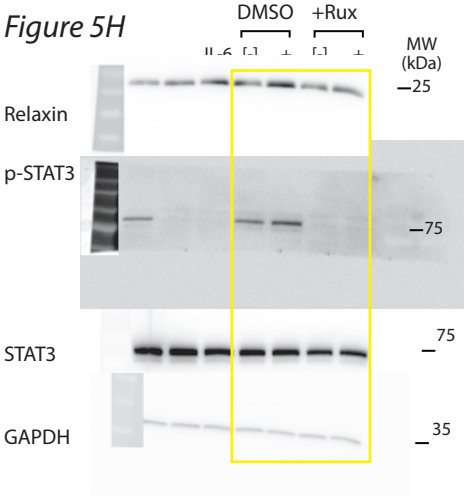
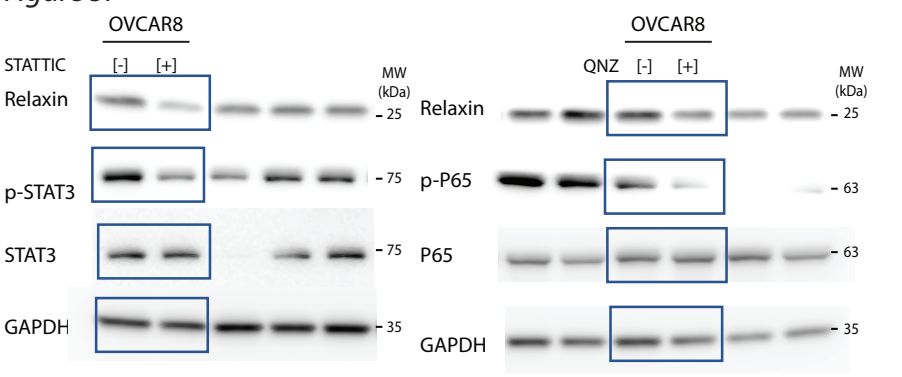
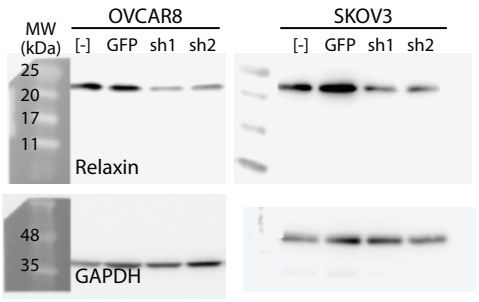


Figure 5F

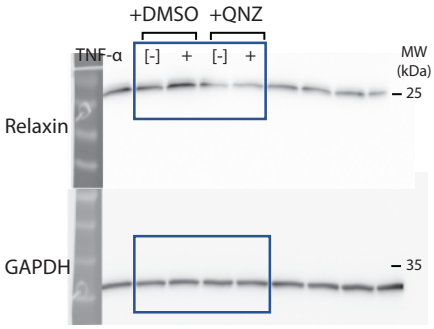


Supplemental File: Uncropped gels from the supplemental figures:

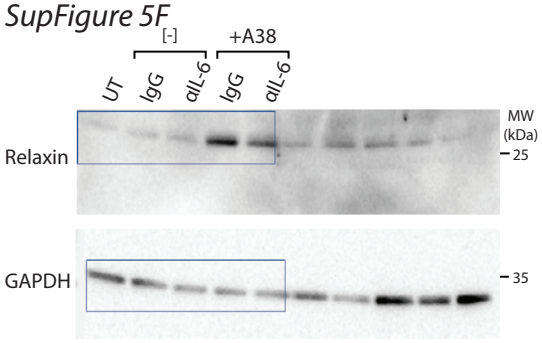
SupFigure 3E



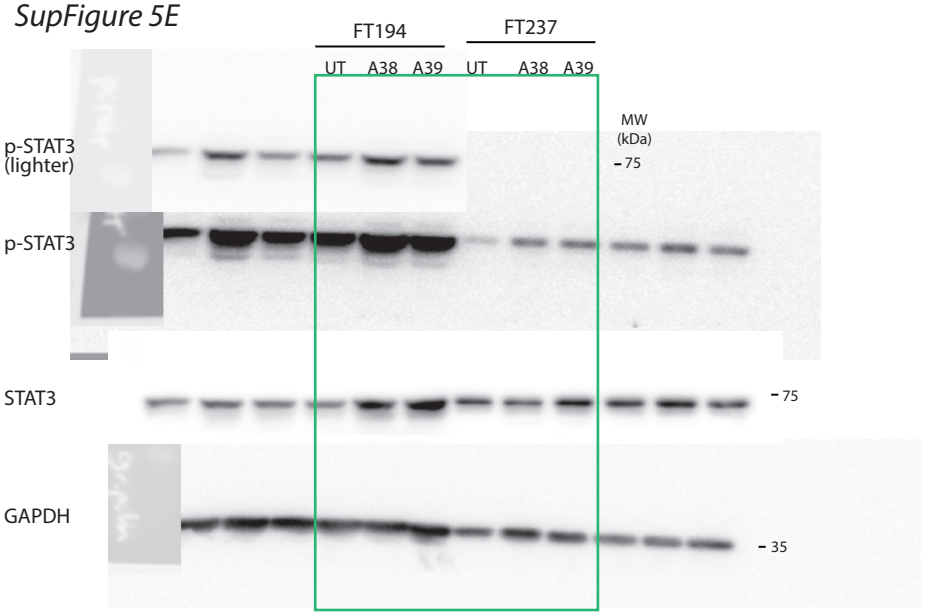
SupFigure 5D



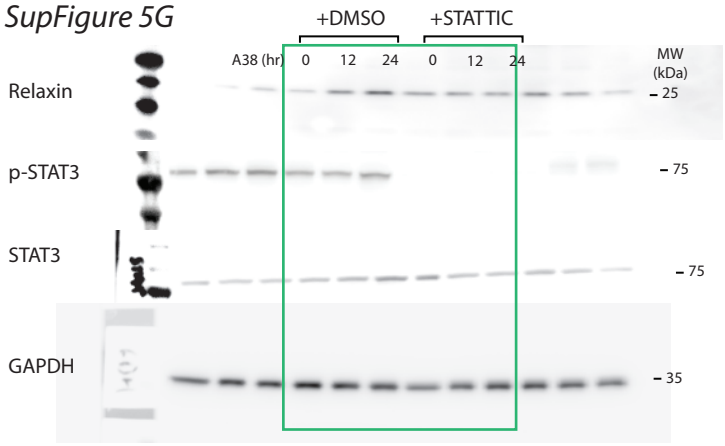
SupFigure 5F



SupFigure 5E



SupFigure 5G



SupFigure 6E