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Supplementary Material

Signal transducer and activator of transcription (STAT) 1 and STAT3 are expressed in the human ovary and have Janus kinase 1-independent functions in the COV434 human granulosa cell line

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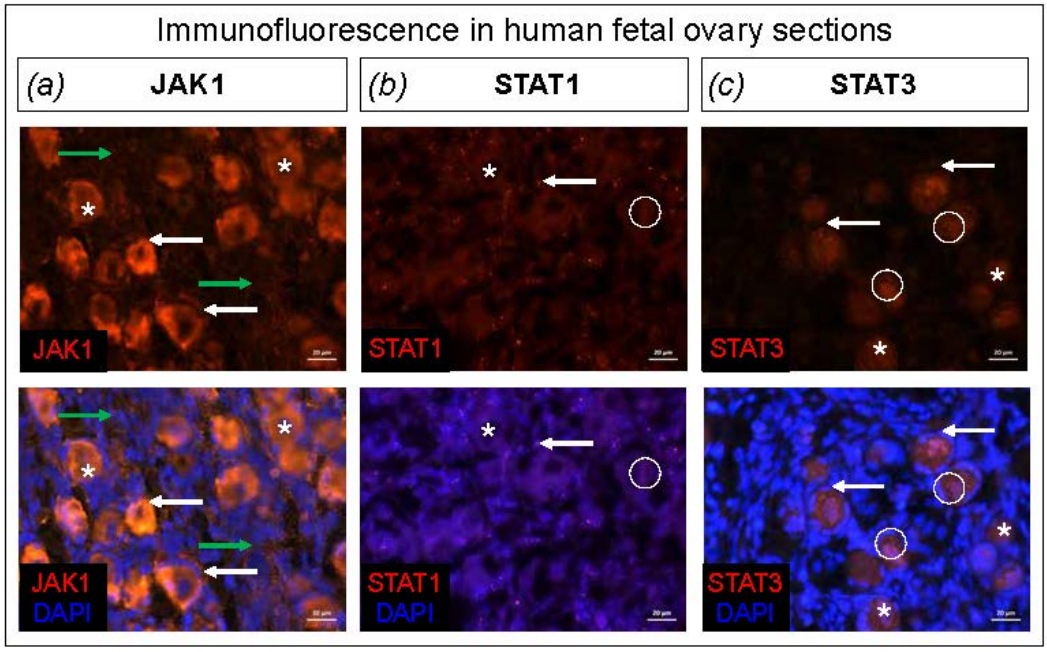

Fig. S1. Protein localisation of JAK1, STAT1 and STAT3 in human foetal ovarian sections. Representative protein localisation was analysed using immunofluorescence for JAK1, STAT1 and STAT3 in human foetal ovarian sections (Supplementary Figure 1*a*, 1*b* and 1*c*). JAK1 and STAT3 protein was expressed in both the oocyte (white asterisks) and the granulosa cells (white arrows) of primordial follicles. JAK1 was faintly detected in the stromal and interstitial cells of the foetal ovary (identified by green arrows). STAT1 showed weak protein expression in the surrounding granulosa cells, however distinct STAT1 foci were detected in the nucleus of oocytes (identified by white circles in Supplementary Figure 1*b*). Aggregates of STAT3 protein were observed in the nucleus of the oocytes (identified by white circles in Supplementary Figure 1*c*). All sections were counter-stained with DAPI (blue). Scale bar in all images is 20 μm.

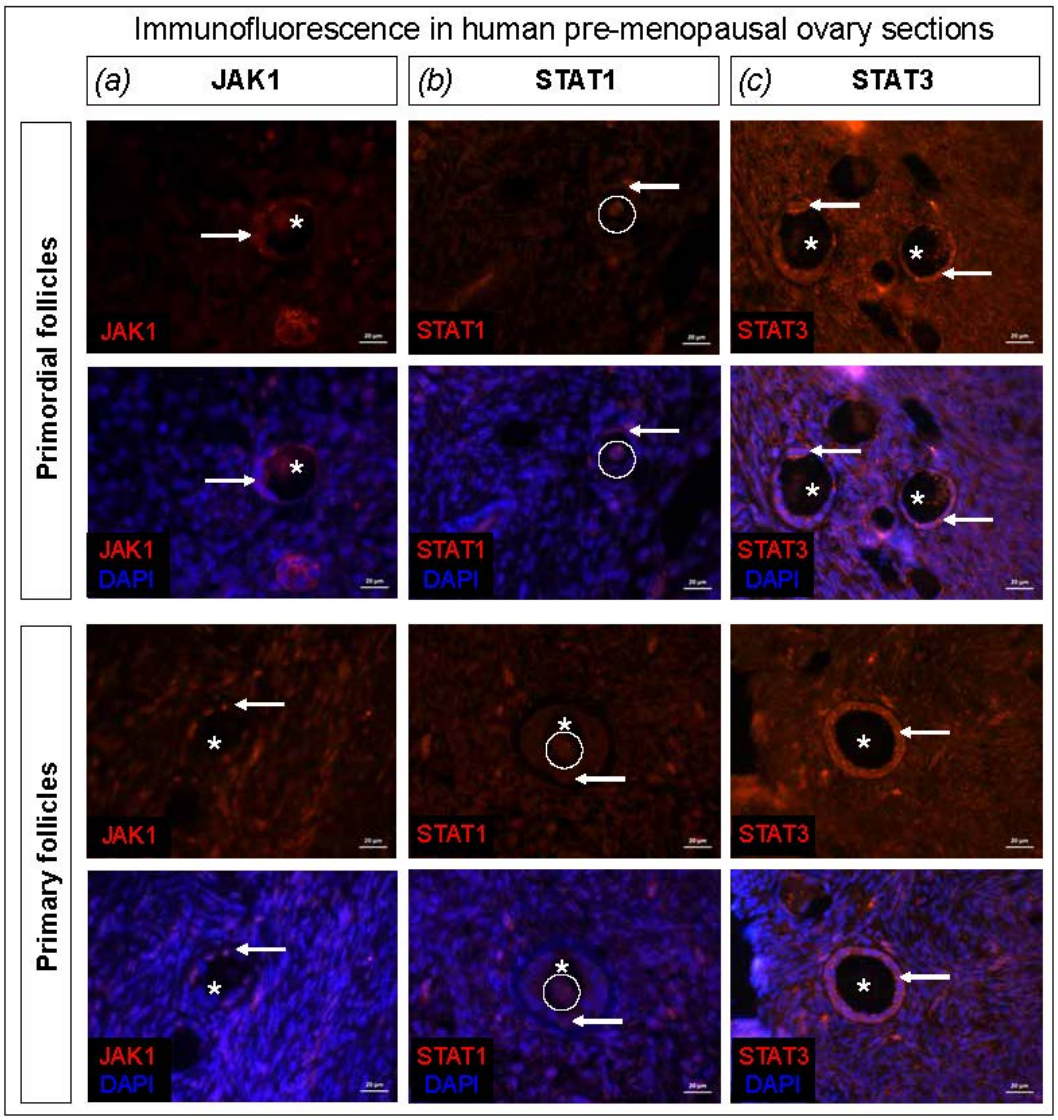

Fig. S2. Protein localisation of JAK1, STAT1 and STAT3 in human pre-menopausal ovarian sections. Representative protein localisation was analysed using immunofluorescence for JAK1, STAT1 and STAT3 in human pre-menopausal ovarian sections (Supplementary Figure 2*a*, 2*b* and 2*c*). Both primordial and primary follicles were identified in the ovary sections (according to Gougeon's criteria (Gougeon 1996)). JAK1 showed protein expression in both the oocyte (white asterisks) and the granulosa cells (white arrows) of primordial follicles (Supplementary Figure 2*a*). In primary follicles, however, JAK1 was restricted to the granulosa cells, and was undetectable in the oocyte (Supplementary Figure 2*a*). STAT1 showed weak protein expression in the

oocyte and surrounding granulosa cells (Supplementary Figure 2*b*). Aggregates of STAT1 protein were observed in the nucleus of the oocytes (identified by white circles in Supplementary Figure 2*b*). STAT3 protein was intensely localised to the pre-granulosa and granulosa cells of both follicle types (Supplementary Figure 2*c*). All sections were counter-stained with DAPI (blue). Scale bar in all images is 20 μm.

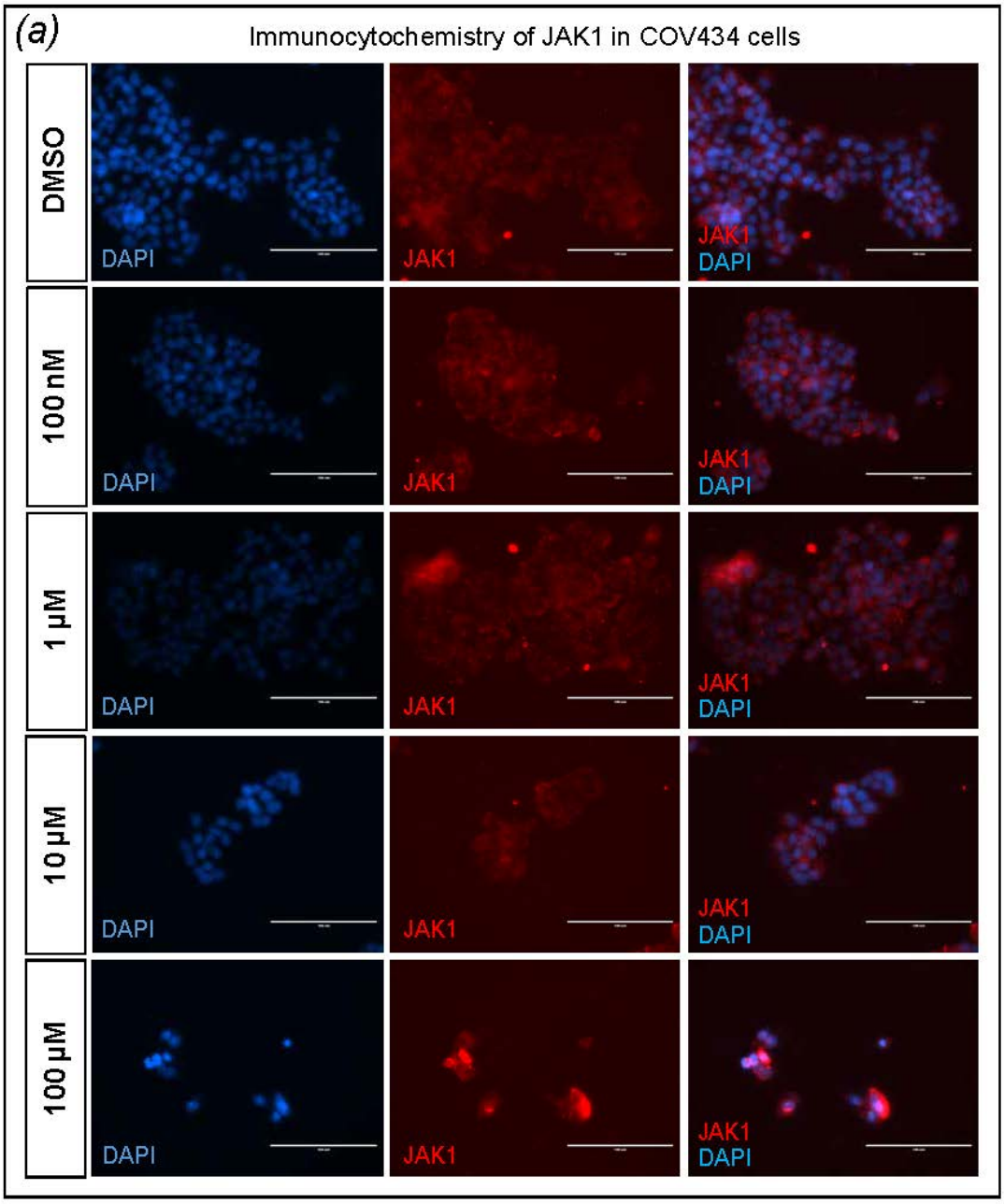

Fig. S3. Optimisation of Ruxolitinib treatment doses for JAK1 inhibition. The doses of Ruxolitinib trialled were based on the IC50 value of Ruxolitinib to inhibit JAK1 and previous studies using Ruxolitinib in whole ovary culture (Sutherland *et al.* 2018). Representative JAK1 protein localisation was analysed in COV434 cells following treatment with DMSO as a vehicle control, and increasing doses of Ruxolitinib (labelled) (Supplementary Figure 4*a*). Cells were counterstained with DAPI to mark the nucleus (blue). The scale bar in all images is 100 µm.

(a) qPCR analysis of potential house-keeping genes

	Raw Cq values					
	Vehicle Rep = 1	Vehicle Rep = 2	Vehicle Rep = 3	100nM Ruxolitinib Rep = 1	100nM Ruxolitinib Rep = 2	100nM Ruxolitinib Rep = 3
ACTB	12.74	12.69	13.28	12.41	14.19	13.02
HPRT1	21.24	20.68	20.88	21.22	21.1	20.83
PPIA	19.05	18.04	18.35	18.55	18.61	19.23
GAPDH	16.61	16.19	16.55	16.05	18.66	16.44

(b) Relative quantities of potential house-keeping genes for NormFinder analysis

	Relative Quantity values					
	Vehicle Rep = 1	Vehicle Rep = 2	Vehicle Rep = 3	100nM Ruxolitinib Rep = 1	100nM Ruxolitinib Rep = 2	100nM Ruxolitinib Rep = 3
ACTB	0.7955365	0.823591	0.5471469	1	0.291183397	0.655196702
HPRT1	0.0021974	0.0032395	0.0028202	0.002228054	0.002421304	0.002919627
PPIA	0.0100268	0.020193	0.0162885	0.014179987	0.013602353	0.008850655
GAPDH	0.0544094	0.0727958	0.0567199	0.080214119	0.013139006	0.061213769

(c) NormFinder analysis of potential house-keeping genes

Gene name	Stability value
ACTB	0.146
HPRT1	0.148
PPIA	0.249
GAPDH	0.234

Best gene	ACTB
Stability value	0.146
Best combination of two genes	ACTB and HPRT1
Stability value for best combination of two genes	0.118

Fig. S4. Selection of human house-keeping primers for qPCR analysis. COV434 cells were treated for 72 h with 100 nM Ruxolitinib or vehicle control. The raw Cq values for *ACTB, HPRT1, PPIA* and *GAPDH* are shown in Supplementary Figure 3a, along with the relative quantities of mRNA in Supplementary Figure 3b. The relative quantities were used in the NormFinder analysis (Andersen *et al.* 2004) to identify the most suitable house-keeping gene for normalisation of the COV434 cell data. Three biological replicates of vehicle control and Ruxolitinib-treated cells were used in the analysis.

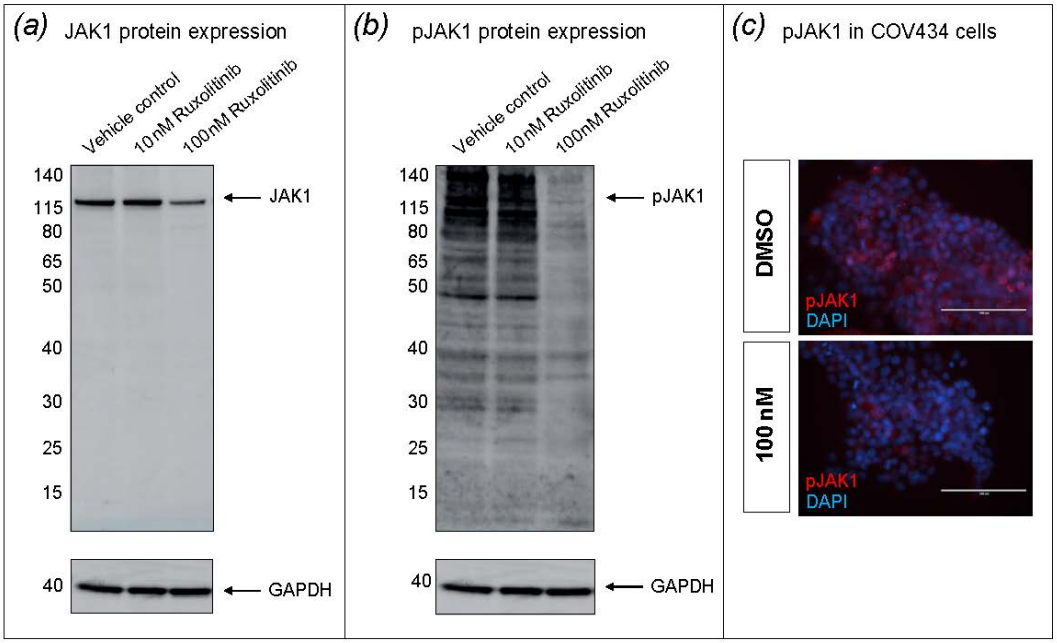

Fig. S5. Optimisation of Ruxolitinib treatment doses for JAK1 inhibition using immunoblotting and immunocytochemistry. As Ruxolitinib is a pan JAK1/JAK2 inhibitor, JAK1 and JAK2 protein levels with Ruxolitinib treatment were examined to determine the efficacy of the inhibitor. Representative blots showing protein expression levels in COV434 cells treated with 100 nM Ruxolitinib or vehicle control for JAK1 (Supplementary Figure 5*a*) and pJAK1 (Supplementary Figure 5*b*) are shown. Visible decreases in both JAK1 and pJAK1 are observed at the 100 nM Ruxolitinib dose (Supplementary Figure 5*a* and Supplementary Figure 5*b*). Representative pJAK1 protein localisation was analysed in cultured COV434 cells following 100 nM Ruxolitinib treatment, with DMSO as a vehicle control (Supplementary Figure 5*c*). Decreases in phosphorylated JAK1 were observed in Ruxolitinib treatment conditions. The scale bar in all images is 100 μm.

Table S1. Primer sequences for real-time polymerase chain reaction

JAK1, Janus kinase 1; *STAT1*, signal transducer and activator of transcription 1; *STAT3*, signal transducer and activator of transcription 3; *ACTB*, β-actin

Gene	Primer sequence (5′–3′)	Annealing temperature (°C)	Efficiency in COV434 cells (AU)
JAK1	Forward: AGACTTGTGAATACGTTAAAAGAAGGA Reverse: AAAGCTTGTCCGATTGGATG	59	2.03
STAT1	Forward: CTTACCCAGAATGCCCTGAT Reverse: CGAACTTGCTGCAGACTCTC	65	1.78
STAT3	Forward: GGTCTGGCTGGACAATATCATT Reverse: GAGGCTTAGTGCTCAAGATGG	65	1.87
ACTB	Forward: TGTGGCATCCACGAAACTACC Reverse: ACATCTGCTGGAAGGTGGACA	65	1.96

Table S2. Immunofluorescence results showing relative expression of candidate proteins in COV434 cells

JAK1, Janus kinase 1; STAT1, signal transducer and activator of transcription 1; STAT3, signal transducer and activator of transcription 3

Protein	Cell compartment	Relative expression of each protein compared to the cell region (immunofluorescence)
JAK1	Nucleus	High
	Cytoplasm	Medium
STAT1	Nucleus	High
	Cytoplasm	High
STAT3	Nucleus	Medium
	Cytoplasm	Medium