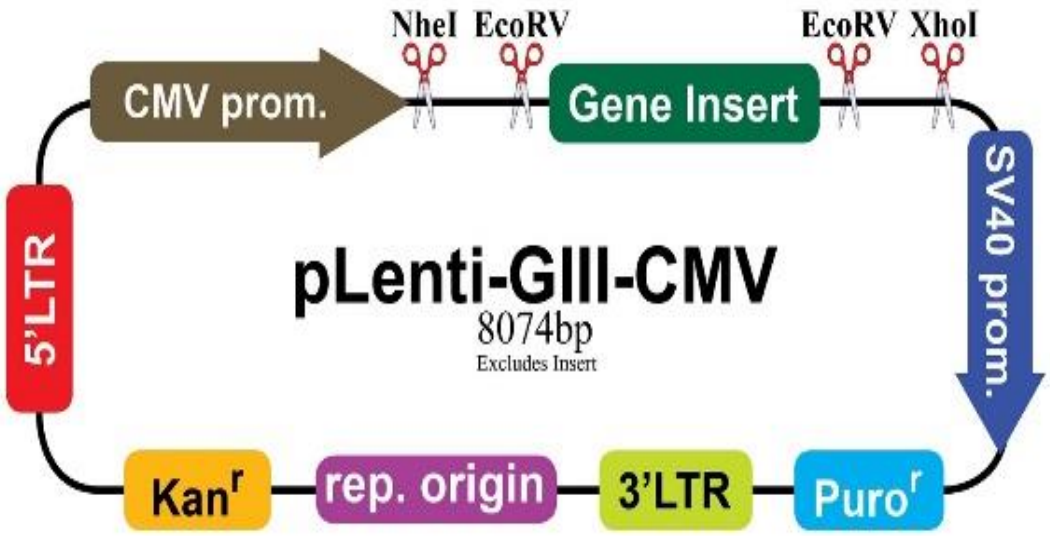
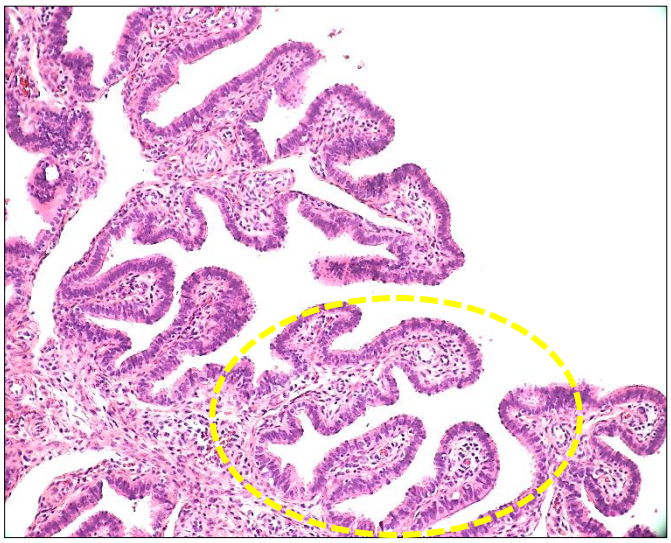


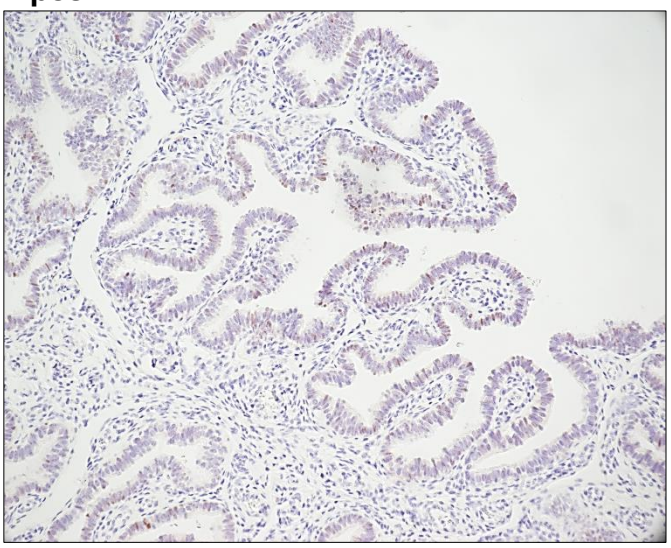
Vector construct map for STAT3 overexpression plasmid used in the current study

SUPPLEMENTARY FIG . 1: STAT3 overexpression construct *:* STAT3 overexpression was achieved using Lentivirus (Human) (CMV) bearing STAT3 overexpression construct (pLenti-GIII-CMV) (abm Inc., Richmond BC, Canada, Cat. No. LVP323577). Plasmid pLenti-GIII-CMV is 8074 base pairs and STAT3 gene insert is 2310 bp. Lentiviruses have the ability to integrate into the host genome and generate a stable cell line expressing the gene of interest using the recommended protocol. The target cells were plated in a 6-well plate, 24 hours prior to viral infection at a density of 3×10^5 cells per well. After 24 hours, the media from the wells was replaced with 2 ml of the polybrene-media-mix per well (8 µg/ml) and ViralPlus Transduction Enhancer (abm Inc., Cat. No. G698) at 1:100 along with the viral titer at an MOI of 5 to infect the cells. A transduction well with a positive GFP control virus and one well of uninfected cells were used as controls. Following the infection-incubate the cells at 37°C with 5% CO2 overnight. The STAT3 overexpressing cells, post transduction were selected on puromycin enriched medium and confirmed by western blot.

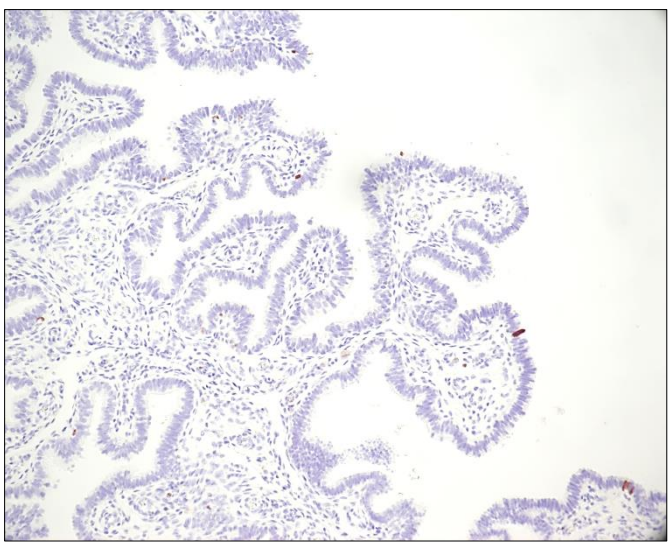
H&E



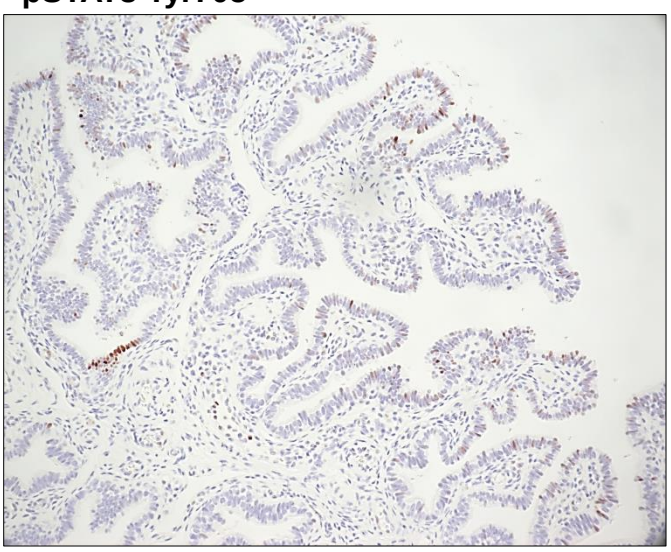
p53



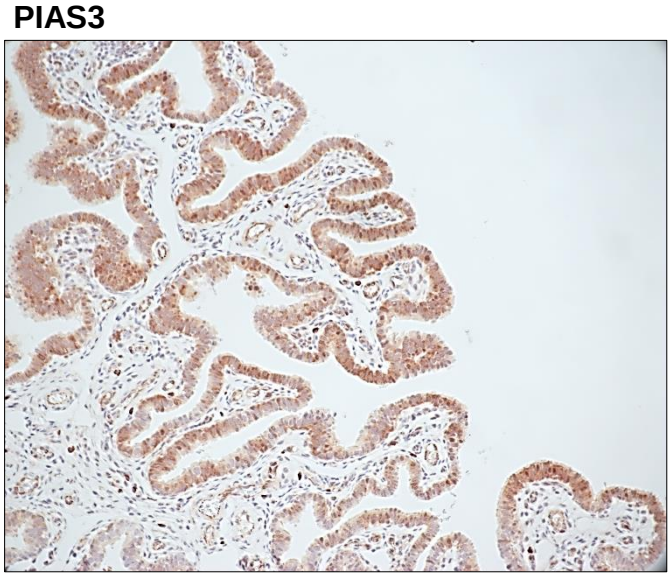
Ki67



pSTAT3 Tyr705

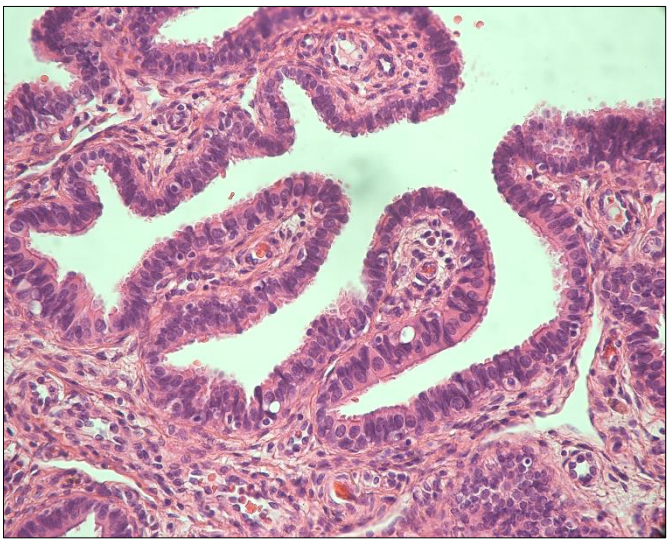


PIAS3

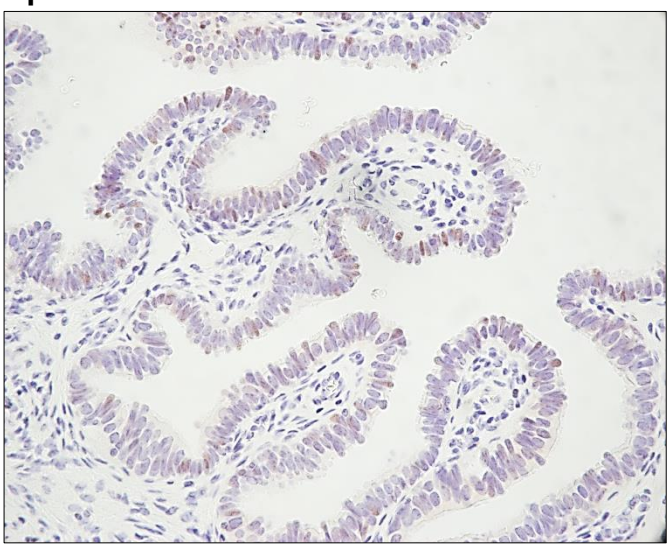


SUPPLEMENTARY FIG. 2A: BENIGN human FALLOPIAN TUBE (20x). The figure serves as a higher magnification (20X) of main Figure 1A. Consecutive sections of benign human FT samples were stained for p53, Ki67, pSTAT3 Tyr705, and PIAS3. P53, Ki67, and pSTAT3 Tyr705 are negative, but PIAS3 shows dense staining. The portion marked with the yellow dotted oval represents the area which is magnified using a 40X in Supplementary figure 2B for all the slides.

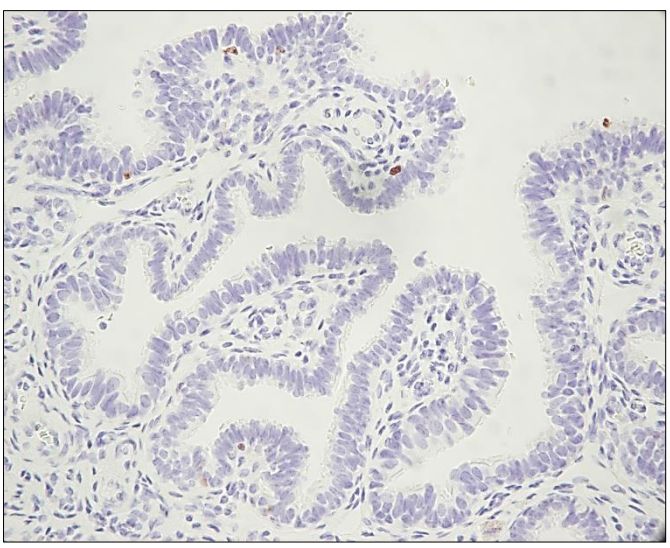
H&E



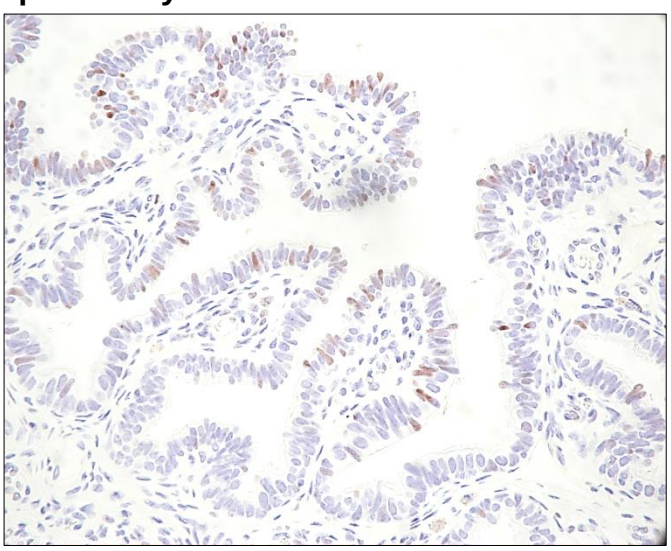
p53



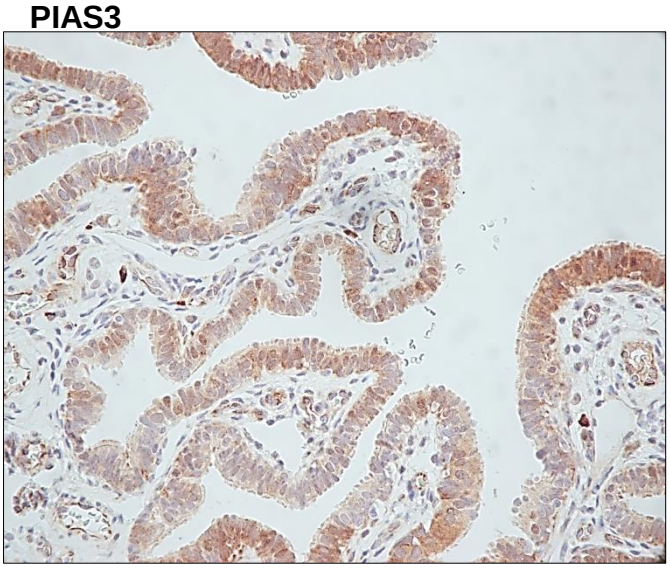
Ki67



pSTAT3 Tyr 705

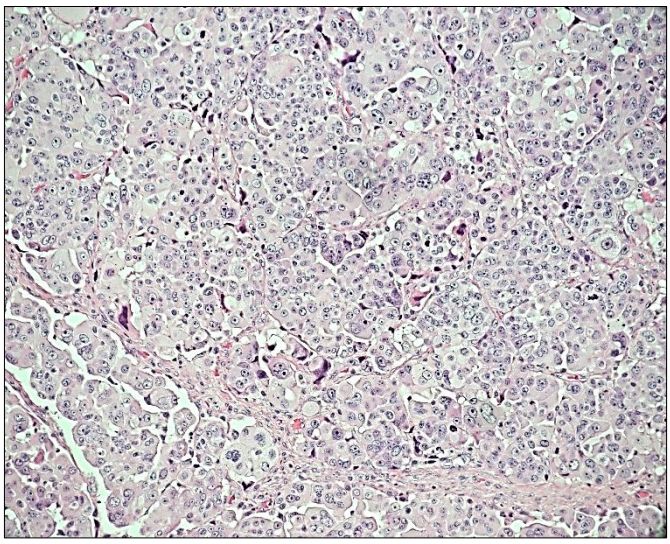


PIAS3

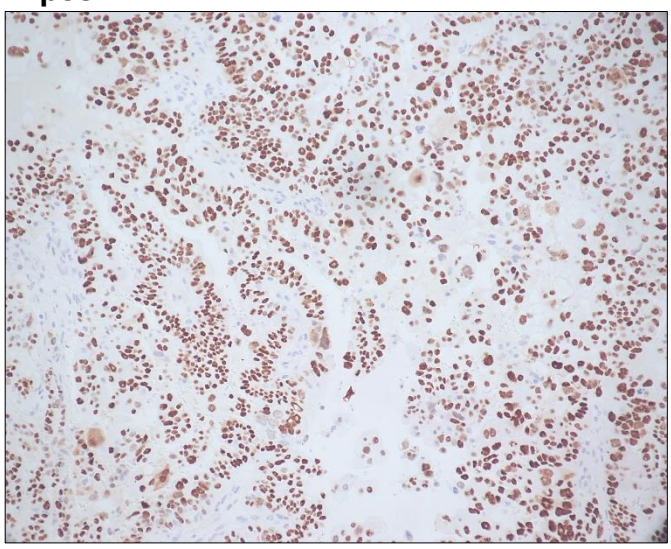


SUPPLEMENTARY FIG. 2B: BENIGN human FALLOPIAN TUBE (40x). The figure serves as a higher magnification (40X) of main Figure 1A and suppl. Fig. 2A. Consecutive sections of benign human FT samples were stained for p53, Ki67, pSTAT3 Tyr705, and PIAS3. P53, Ki67, and pSTAT3 Tyr705 are negative, but PIAS3 shows dense staining.

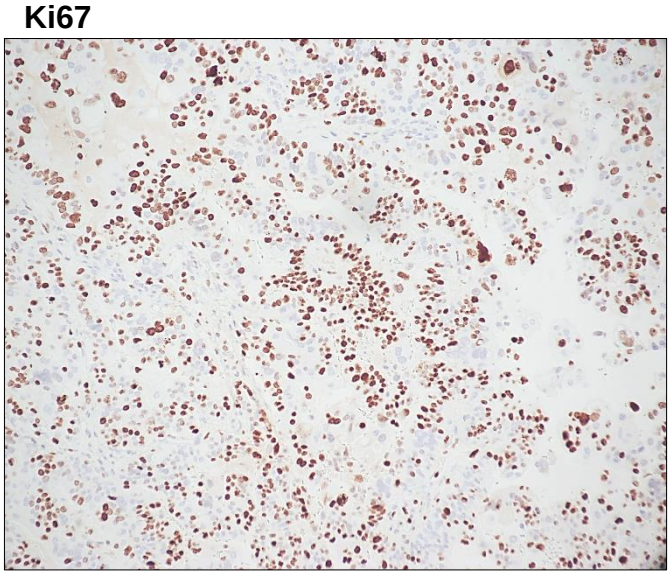
H&E



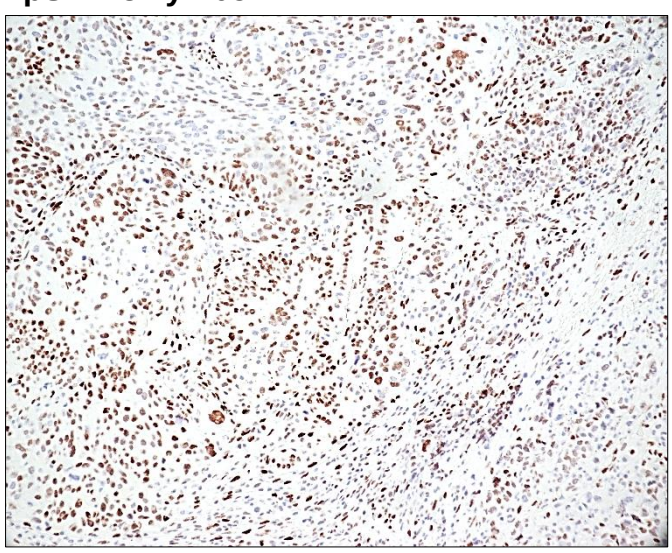
p53



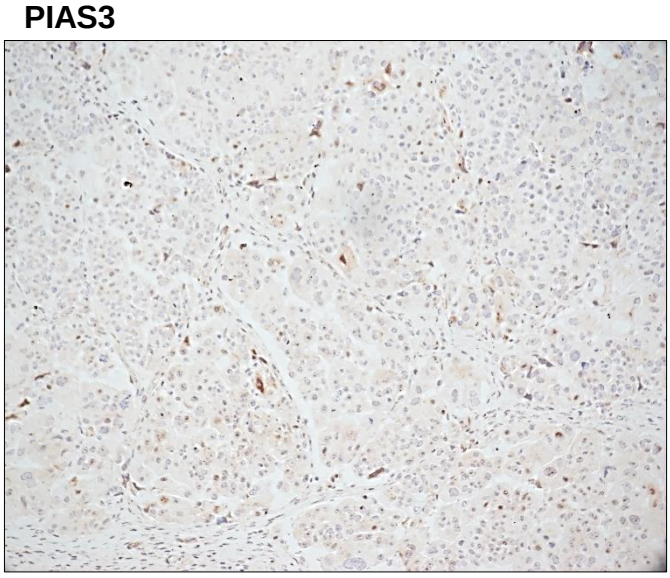
Ki67



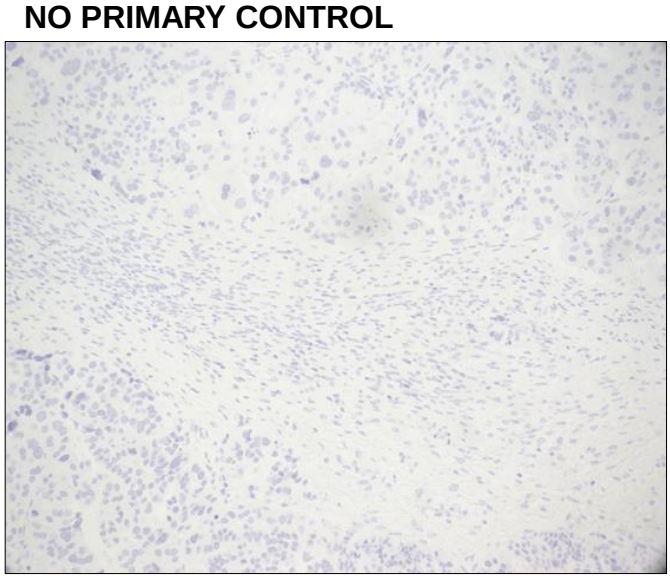
pSTAT3 Tyr705



PIAS3

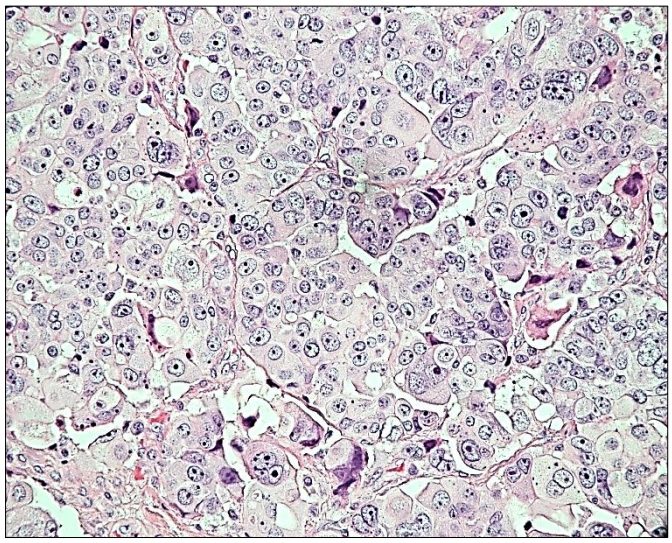


NO PRIMARY CONTROL

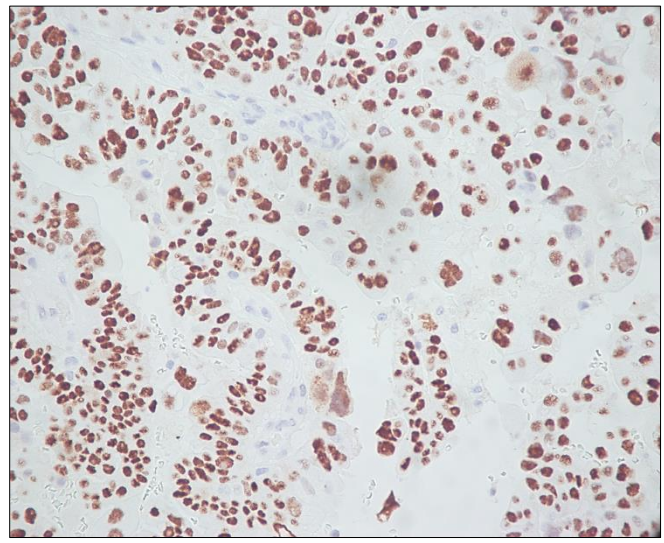


SUPPLEMENTARY FIG. 3A: Malignant human HGSOC (20x). The figure serves as a higher magnification (20X) of main Figure 1A. Consecutive sections of malignant human HGSOC samples were stained for p53, Ki67, pSTAT3 Tyr705, PIAS3, and no primary antibody control. P53, Ki67, and pSTAT3 Tyr705 are positive but, PIAS3 shows negative staining. This is exactly opposite to what was observed in the benign human FT tissue. Note that the absence of any staining in the no primary control ensures that the staining is produced from the detection of the antigen by the primary antibodies and not by the detection system or the specimen.

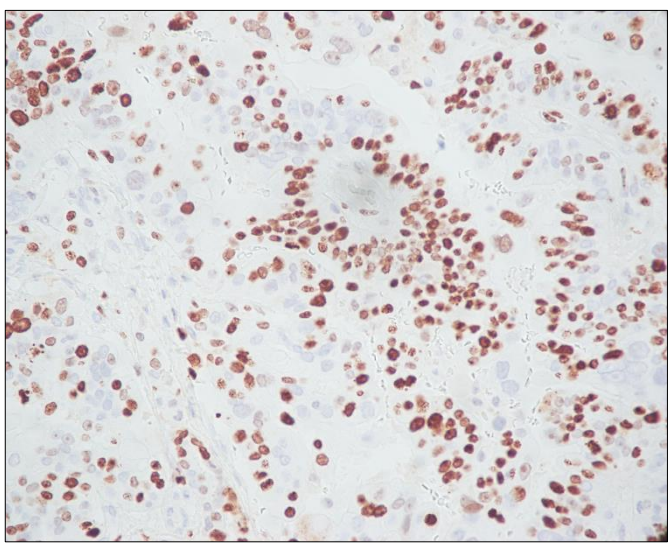
H&E



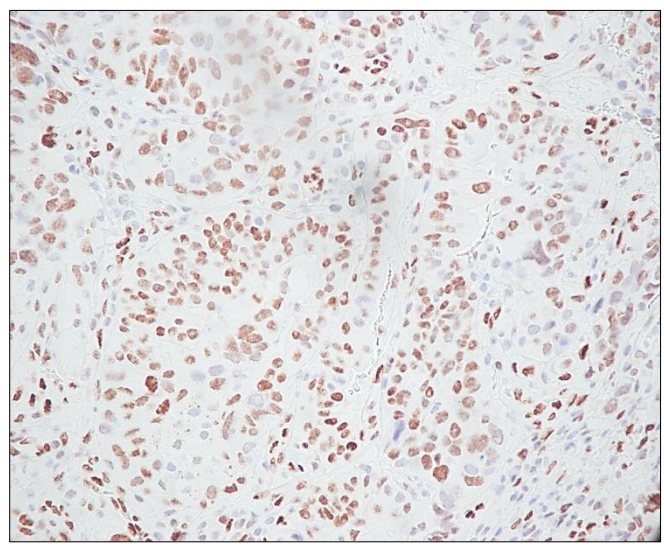
p53



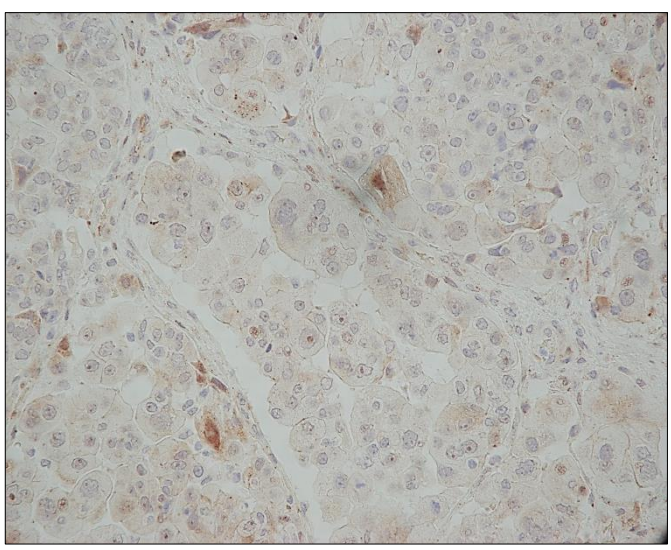
Ki67



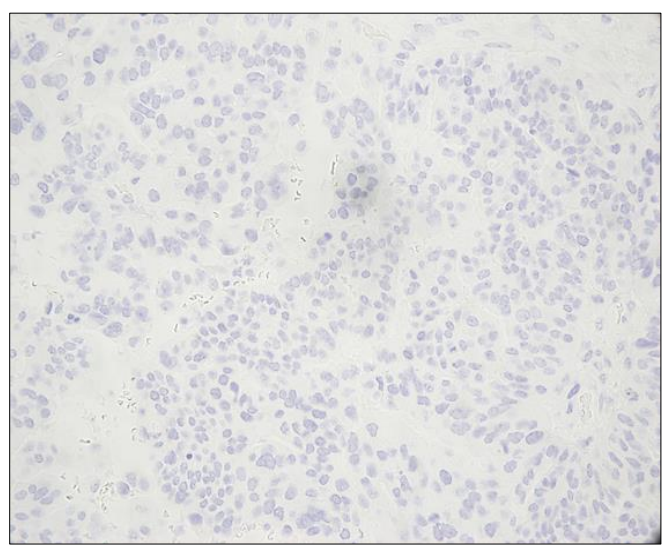
pSTAT3 Tyr705



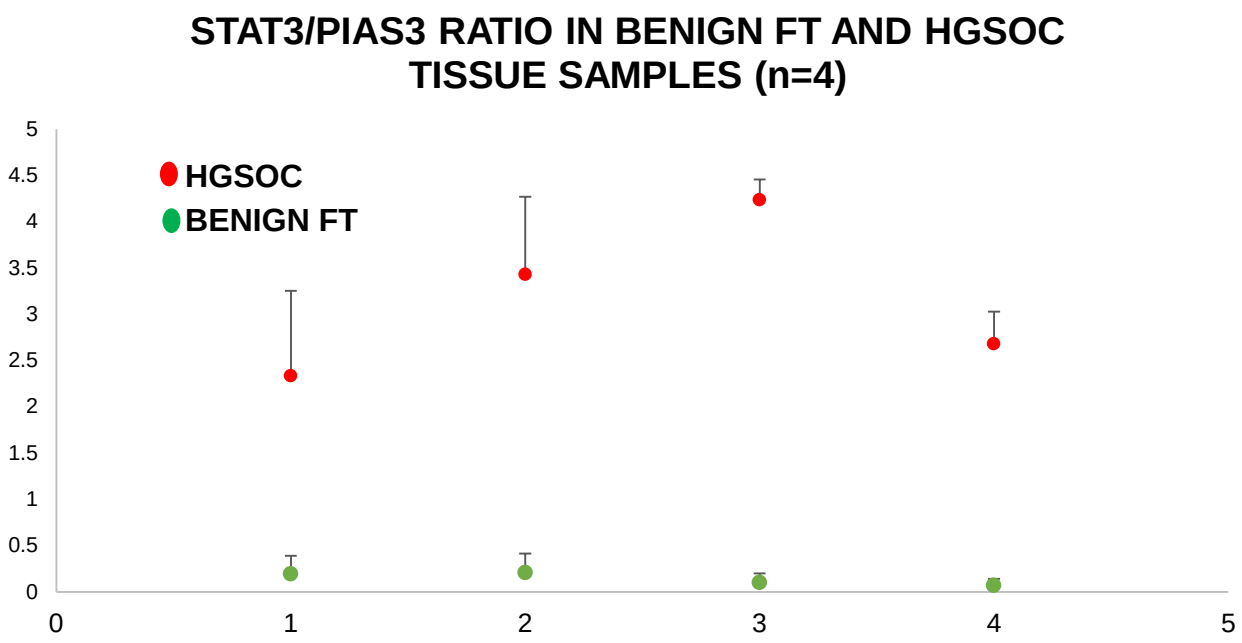
PIAS3



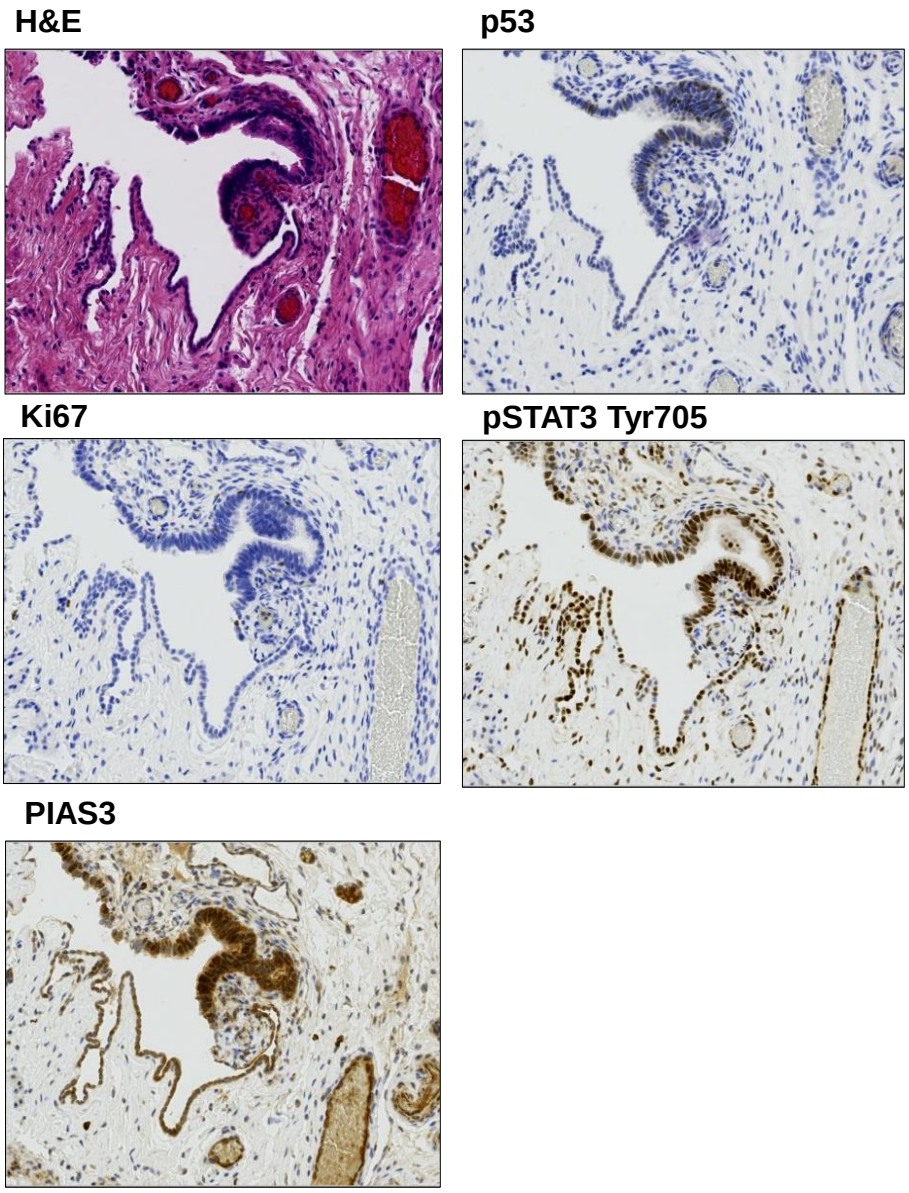
NO PRIMARY CONTROL



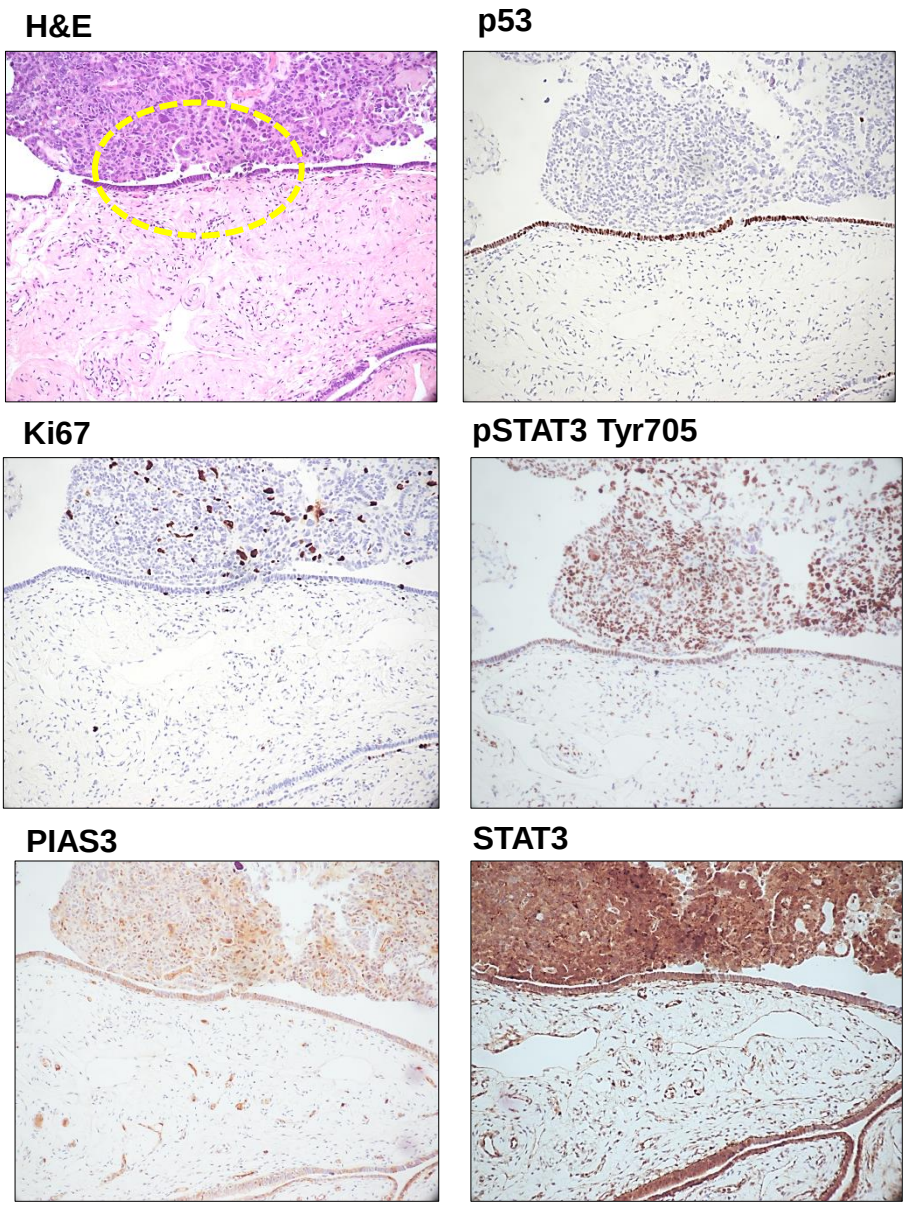
SUPPLEMENTARY FIG. 3B: Malignant human HGSOC (40x). The figure serves as a higher magnification (40X) of main Figure 1A and suppl. Fig. 2A. Consecutive sections of malignant human HGSOC samples were stained for p53, Ki67, pSTAT3 Tyr705, PIAS3, and no primary antibody control. P53, Ki67, and pSTAT3 Tyr705 are positive but, PIAS3 shows negative staining. This is exactly opposite to what was observed in the benign human FT tissue. Note that the absence of any staining in the no primary control ensures that the staining is produced from the detection of the antigen by the primary antibodies and not by the detection system or the specimen.



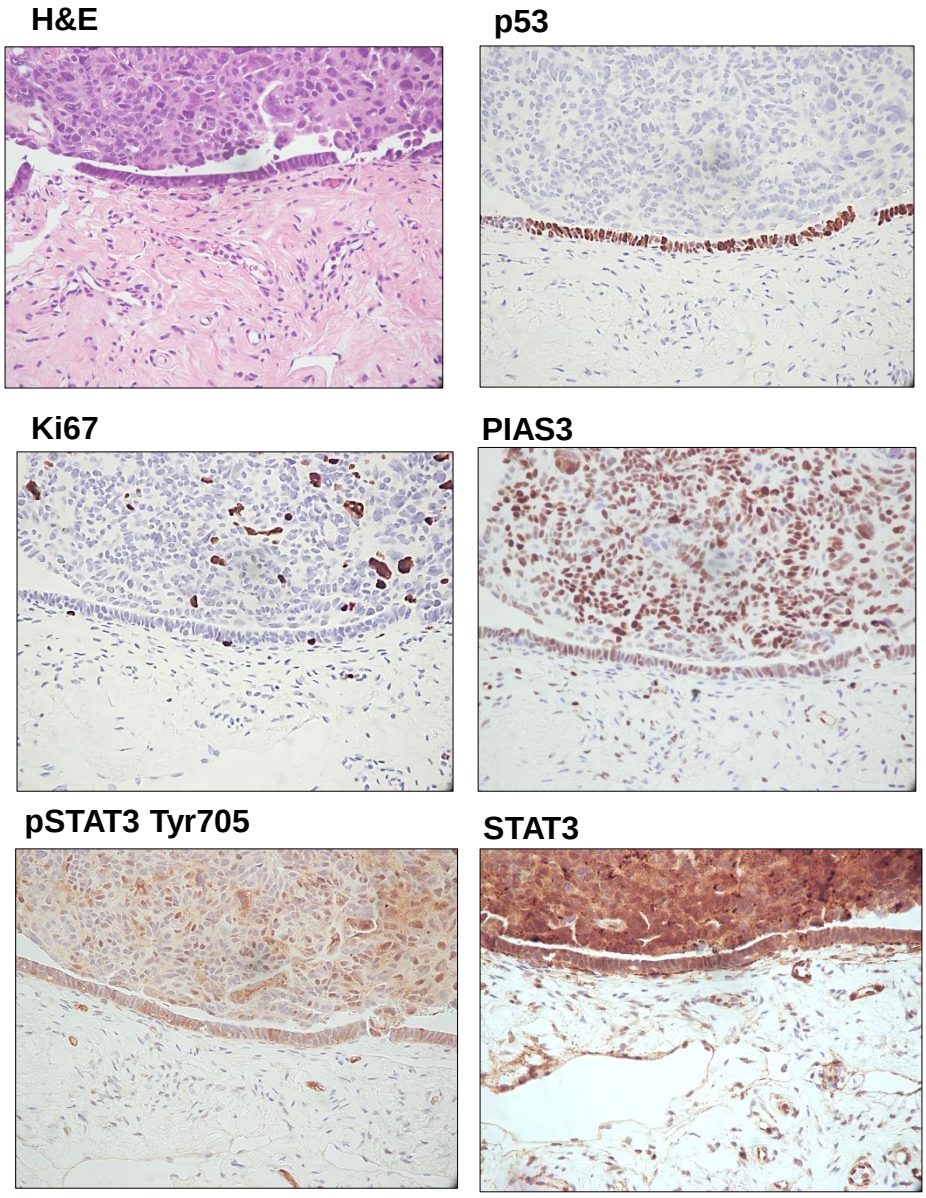
SUPPLEMENTARY FIG. 4: Ratio of STAT3/PIAS3 in benign human FT vs malignant human HGSOC tissue. RNA from 4 tissue samples of benign and malignant human tissues was subjected to real time qPCR and relative mRNA expression of STAT3 and PIAS3 was calculated by normalizing to the housekeeping gene, GAPDH**.** The values were thereafter used to calculate STAT3/PIAS3 ratios for each tissue sample.



SUPPLEMENTARY FIG. 5: Tubal peritoneal junction (TPJ): The figure shows human tubal peritoneal junction and serves as an additional sample to the one displayed in main Fig. 2. P53 and Ki67 are negative, while some pSTAT3 Tyr705 positive cells are observed, and PIAS3 maintains intense positive staining.

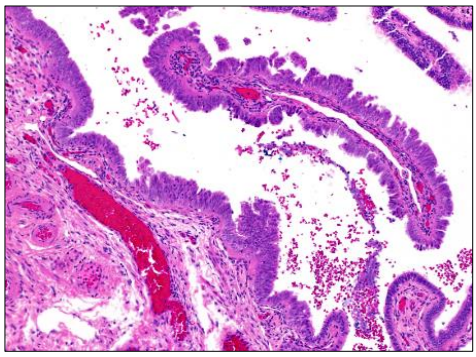


SUPPLEMENTARY FIG. 6 A: p53 Signature (20X): The figure shows p53 signature (20X) and serves as an additional sample to the one displayed in main Fig. 3. P53 positive cells are observed in an area of the sample, but Ki67 is negative. There is increased pSTAT3 Tyr705 (in comparison to benign or TPJ) along with diminished PIAS3, similar to what was seen in main Fig.3. Strong total STAT3 is also observed in the sample. The portion marked with the yellow dotted oval represents the area which is magnified using a 40X in Supplementary figure 6B for all the slides.

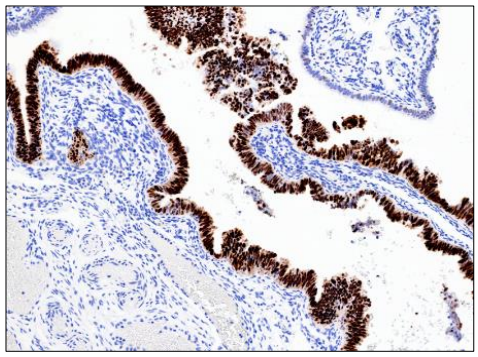


SUPPLEMENTARY FIG. 6B: p53 Signature (40X): The figure shows p53 signature (40X) and serves as an additional sample to the one displayed in main Fig. 3. P53 positive cells are observed in an area of the sample, (add comma) but Ki67 is negative. There is ~~an~~ increased pSTAT3 Tyr705 (in comparison to benign or TPJ) along with diminished PIAS3, similar to what was seen in main Fig.3. Strong total STAT3 is also observed in the sample.

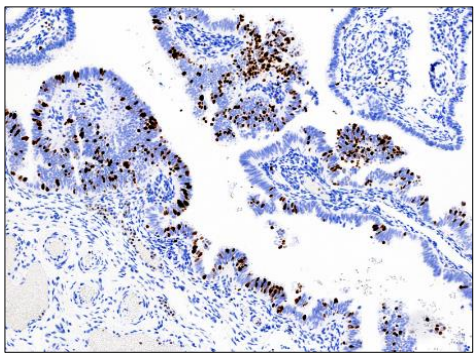
H&E



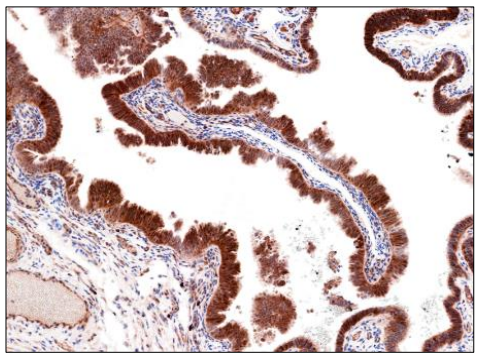
p53



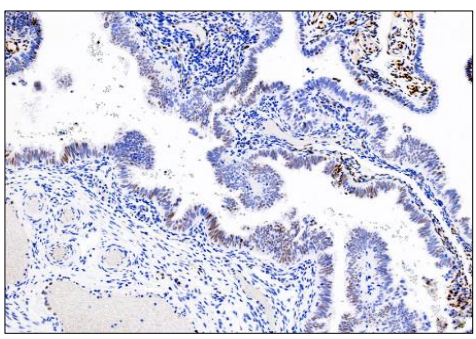
Ki67



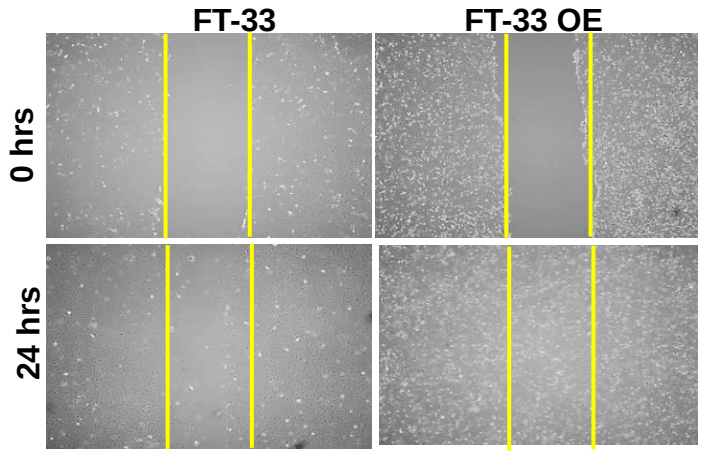
pSTAT3 Tyr705



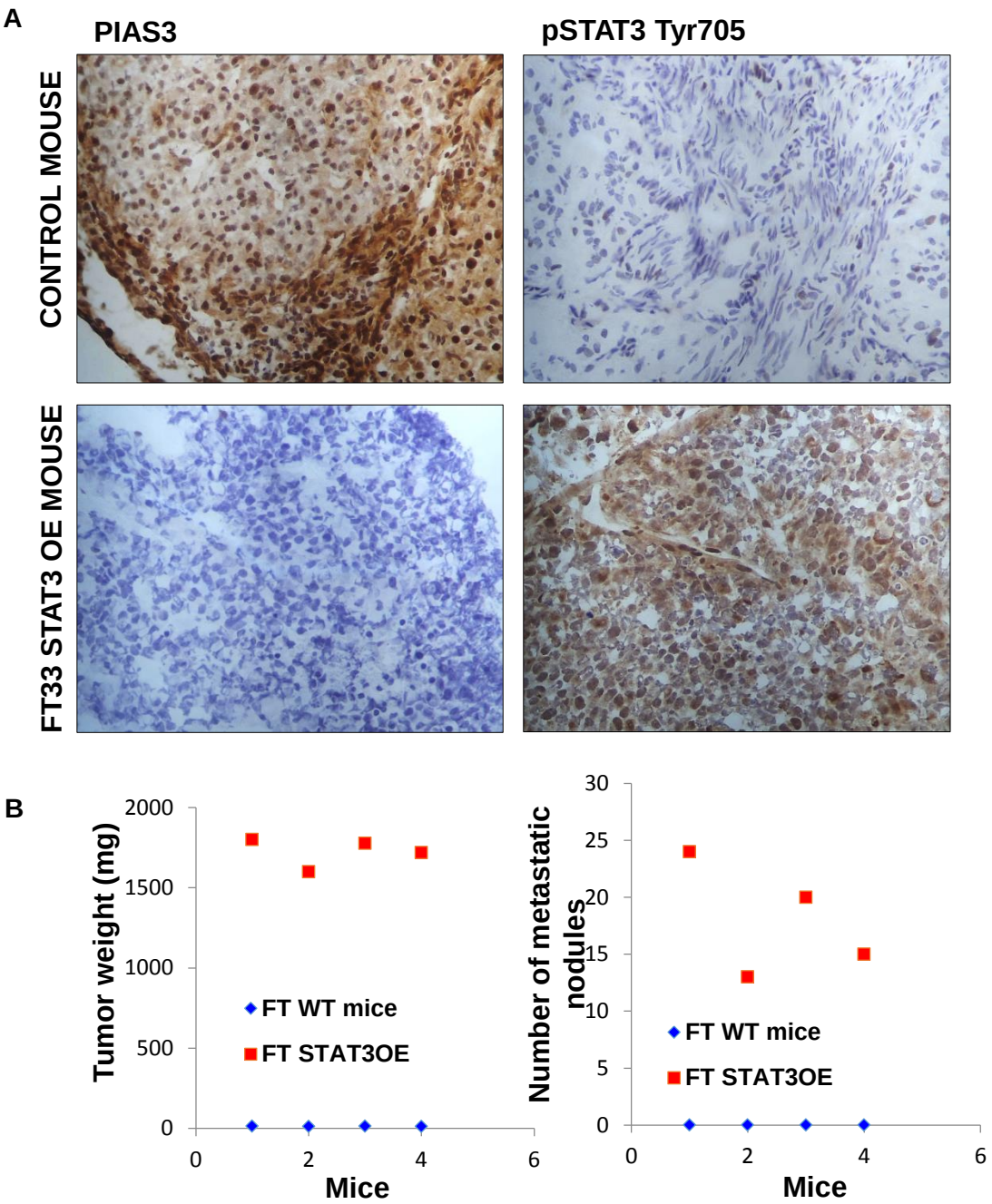
PIAS3



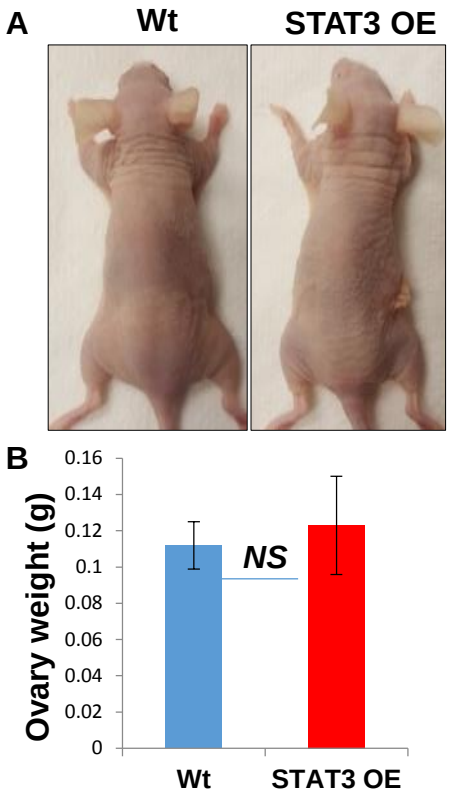
SUPPLEMENTARY FIG. 7:Tubal intraepithelial lesion in transition (**TILT**) 20X**:** The figure shows human Tubal intraepithelial lesion in transition" (**TILT**)and serves as 20X magnification to main Fig. 4.



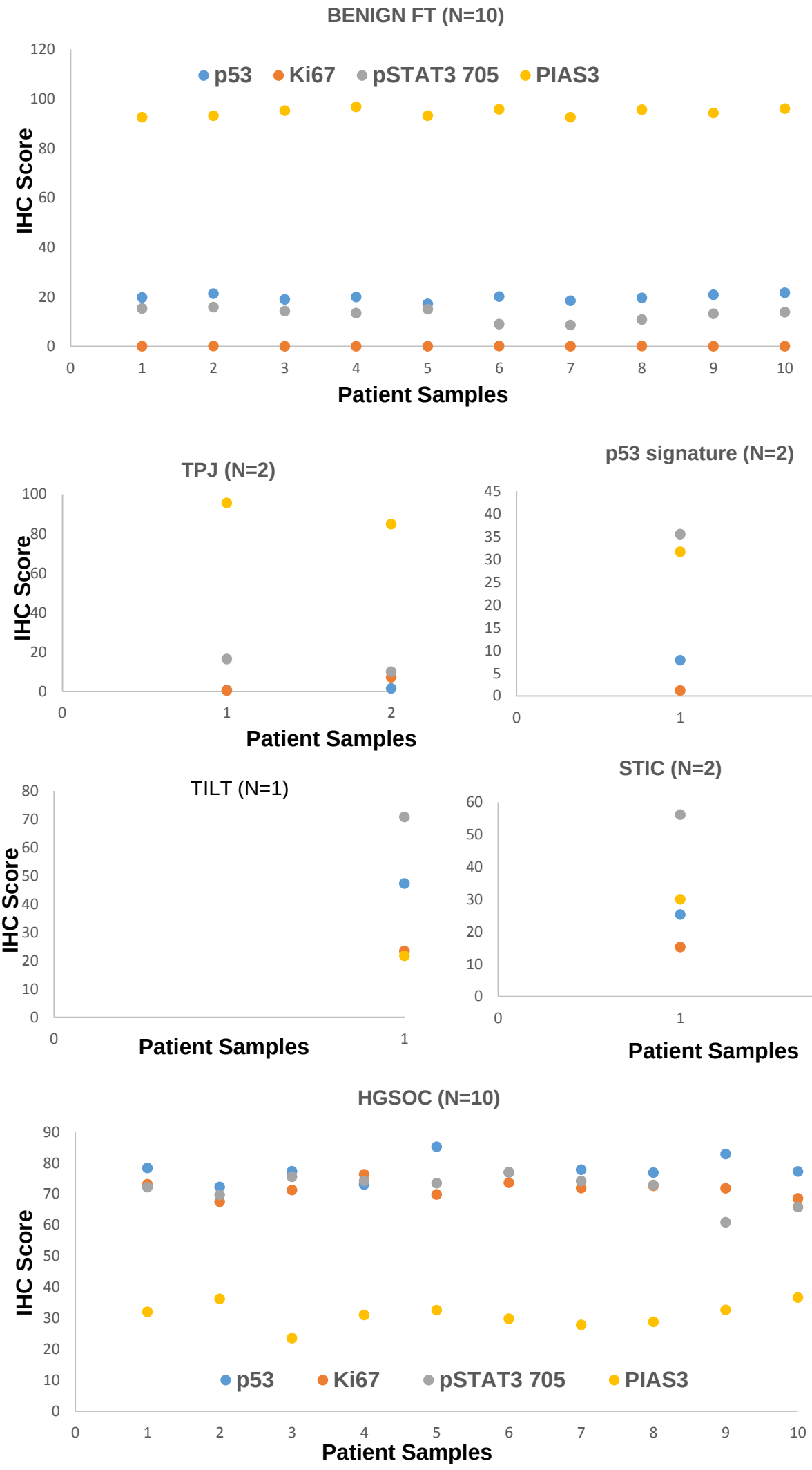
SUPPLEMENTARY FIG: 8: FT 33 cells with or without STAT3 overexpression were subjected to wound healing assays in order to understand the correlation between STAT3 expression level and migration capability in FT cells. The panel on the top shows the plates at 0 hr after scratch and the panel on the bottom shows the migration pattern after 24 hours. The cells over-expressing STAT3 migrate and close the gap (99%) within the first 24 hours, while the control FT33 cells migrate to fill 10-15% of the scratched area.



SUPPLEMENTARY FIG. 9: Tumor burden in the mice injected with FT33 control or FT33 cells overexpressing STAT3. A) IHC of ovaries from control mice showed intense PIAS3 staining and no pSTAT3 Tyr705 staining (representative, top panel). When compared to the staining of the control mice, the trend of staining from the STAT3 OE mice was reversed for both proteins. B) Tumor burden, in terms of tumor weight and metastatic nodules, was significantly higher in the mice injected with STAT3 OE cells than in the mice injected with normal FT33 cells.



SUPPLEMENTARY FIG: 10. A. Normal human ovarian surface epithelial cells (hOSE) or hOSE cells with STAT3 overexpression (OE) were transplanted into nude mice . **B**. The graph shows a comparison of ovary weights from the WT and hOSE STAT3 OE mice. There was not a significant increase in ovary weights between the two groups.



SUPPLEMENTARY FIG: 11. Quantification of gene expression for p53, pSTAT3 Tyr705, and PIAS3 calculated from IHC of paraffin embedded sections using Image J software. The images were adjusted using “color threshold” and counted using “analyze particles.” 40X images were used for the quantifications of all the pictures and the ratio of the number of positive cells to the total number of cells was plotted on the scatter plots.

Sup. Table 1 – Antibody list

Antibody name	Company (catalogue no.)	Dilution information
pSTAT3 705	Cell Signalling Technology (9145)	IHC: 2.5:1000 ICC: 5:1000
PIAS3	Abcam(ab58406)	4:1000
P53	Abcam(ab31333)	10:1000
Ki67	Cell Signalling Technology (12202S)	2.5:1000
C myc	Abcam (ab32072)	ICC: 1:1000 IHC: 2:1000
Anti rabbit IgG (HRP)	Abcam(ab6721)	1:1000
Anti mouse IgG (HRP)	Abcam (ab6789)	1:1000
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen (A-11034)	2:1000
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	Invitrogen (A-11031)	2:1000

Sup. Table 2 – Primers list

PRIMER NAME	SEQUENCE
STAT3	Sense: ATGGCCCAATGGAATCAGC Antisense: TTATTTCCAAACTGCATCAA
C-MYC	Sense: AATGAAAAGGCCCCCAAGGTAGTTATCC Antisense: GTCGTTTCCGCAACAAGTCCTCTTC
CYC D1	Sense: AGCTCCTGTGCTGCGAAGTGGAAAC Antisense: AGTGTTCAATGAAATCGTGCG GGGT
PIAS3	Sense: GATTCTTACATCCAGAGAGGTTC Antisense: ACCTGTATGGTATAATCACATTTGG
StIP1	Sense: CCAGAAGTAATGTGCCTTCAGA Antisense: CCAGTCTTCCAGGTAACATCAG

Sup. Table 3. Summarizing the range of percentage of positive cells in patient samples

(number of cases used)	p53 (%)	Ki67(%)	pSTAT3 Tyr 705(%)	PIAS3(%)
Benign Fallopian Tube (FT) (n=10)	15-20	< 0.1	5-15	90-100
Tubal Peritoneal Junction (TPJ) (n=2)	< 2	< 10	< 20	>80
P53 Signature (n=3)	5-10	< 5	35-40	30-40
Tubal intraepithelial lesion in transition (TILT) (n=1)	47	23	70	21
Serous tubal intraepithelial carcinoma (STIC) (n=3)	25-30	15-20	46-56	30-40
High Grade Serous Ovarian Cancer (HGSOC) (n=10)	>75	>70	>70	< 30

Table 3: Table summarizing the range of percentage of positive cells for p53, Ki67, pSTAT3 705 and PIAS3 in Benign FT, TPJ, p53 signature, TILT, STIC and HGSOC. For pSTAT3 705 and PIAS3, <20% was deemed as low, between 20-50% as moderate, between 20-50% as moderate, 50-75% as high and above 75% as very high. In case of p53, >75% of at least 12 epithelial cells (with or without intervening ciliated cells) was considered p53(+). Foci showing a Ki-67 labeling index $\geq$20% were considered “Ki-67 high” since normal tubal mucosa typically has a labeling index <2%, whereas a Ki-67 proliferation index of <10% was considered “low”.