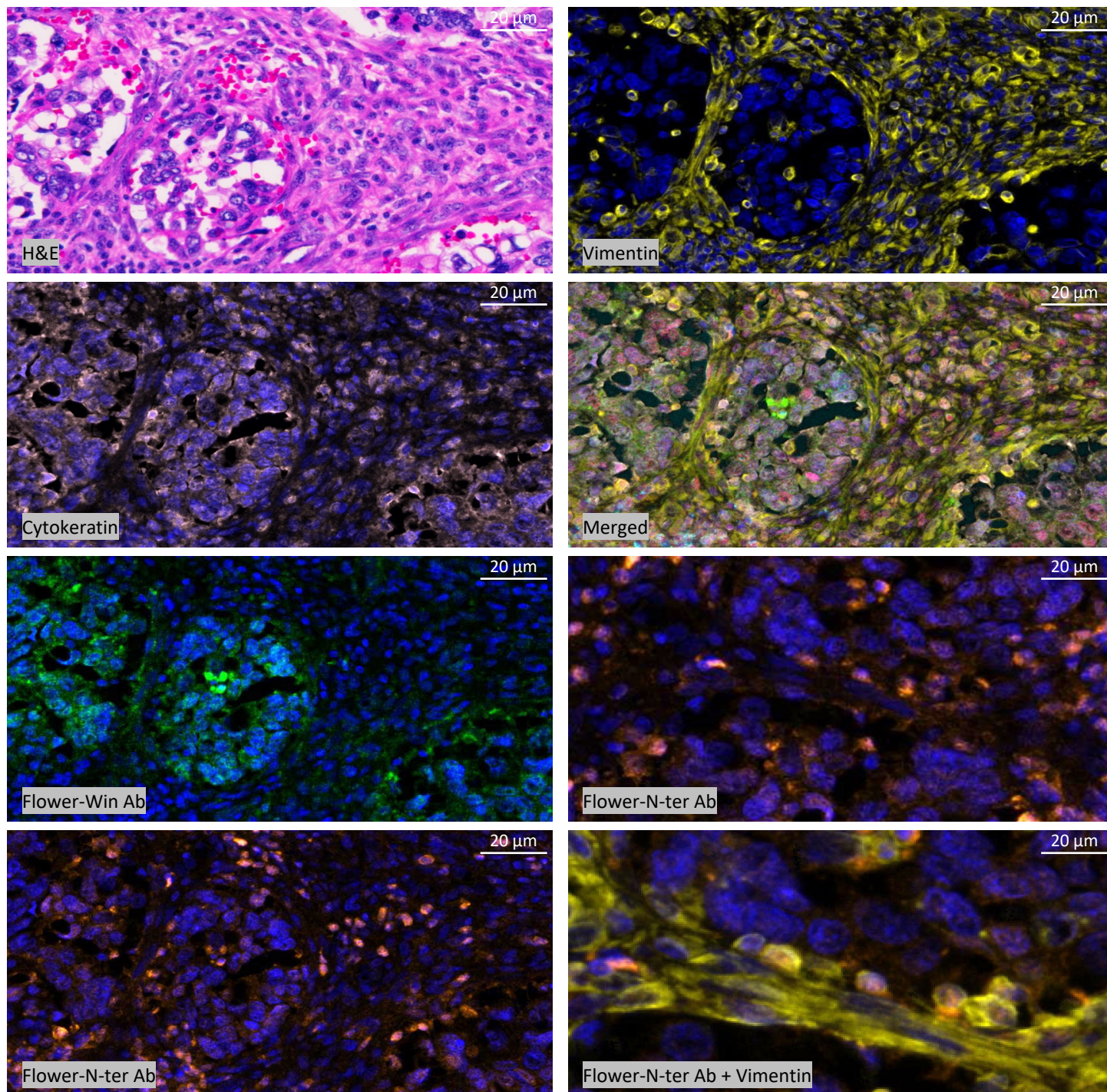


Ovarian tumor cells gain competitive advantage by actively reducing the cellular fitness of microenvironment cells

In the format provided by the
authors and unedited

Supplementary Figure 1A



Supplementary Figure Legends

Figure S1: Multiplexed immunohistochemistry demonstrating location of Flower Win and Lose expression in HGSC tissue samples

Routine immunohistochemistry using Flower N-terminus and Win Ab and control with secondary IgG is shown in the left panel. Multiplex IHC (right panel) was used to define the precise location of Flower Win and Lose expression in stromal and cancer components of a second HGSC tissue sample. Similar to **Figure 1C**, staining was performed with the Flower N-terminus Ab and Flower Win Ab. Staining with Vimentin Ab and Cytokeratin was again used to mark the presence of fibroblasts and epithelial cells in the HGSC samples respectively.

Supplementary Figure 2A

Cytokeratin +
cancer cells



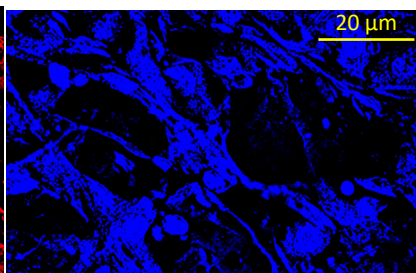
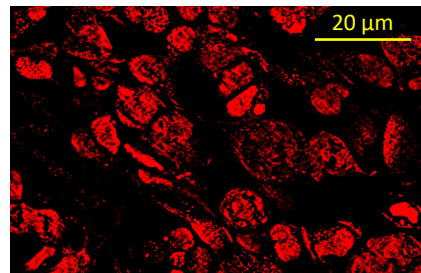
Vimentin +
Fibroblasts



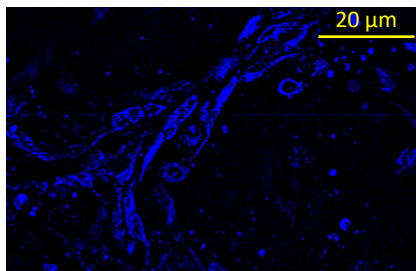
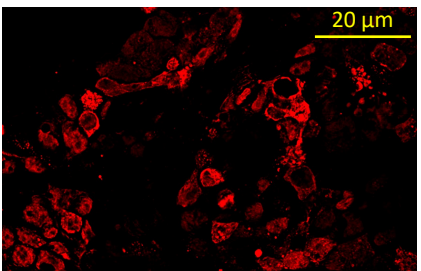
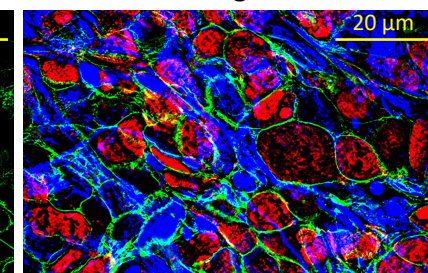
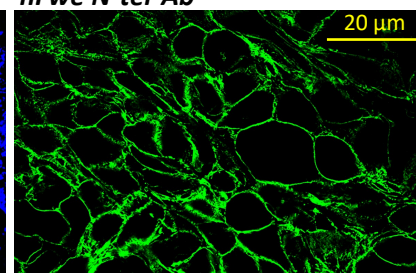
Flower (hFwe)



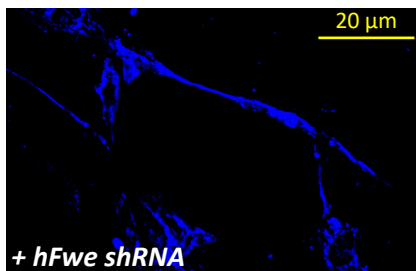
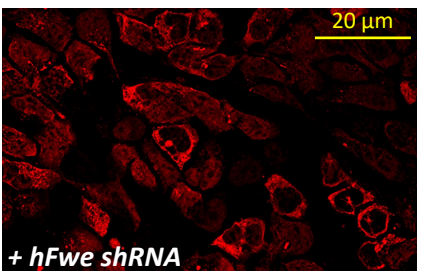
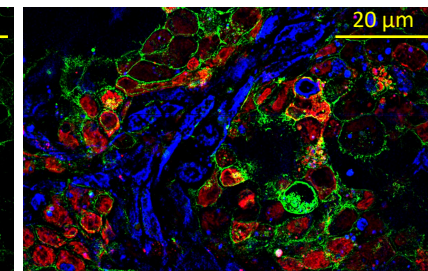
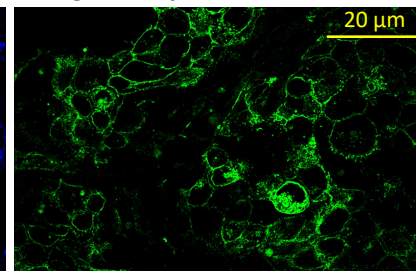
Merged



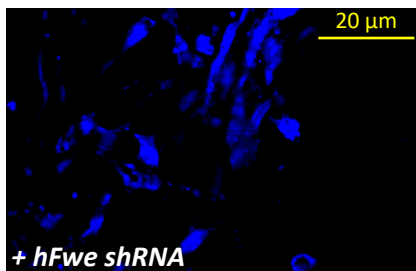
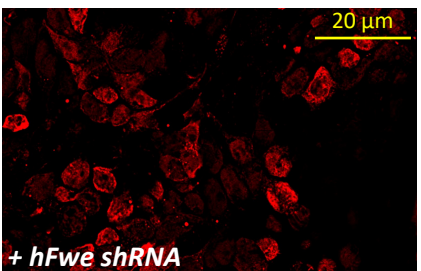
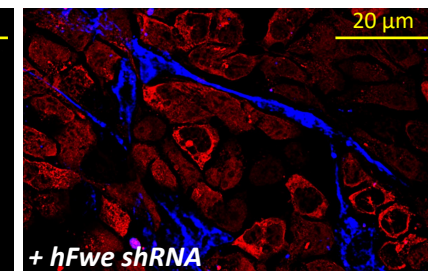
hFwe N-ter Ab



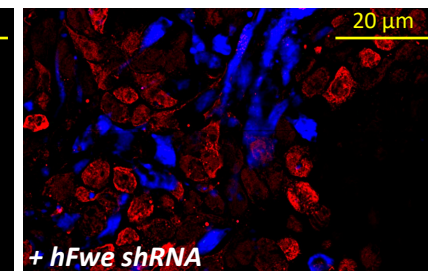
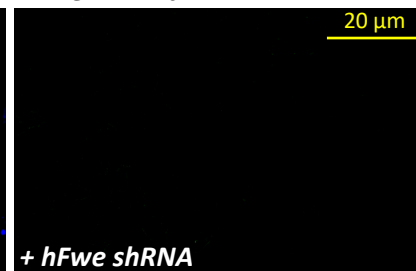
hFwe Win Ab



hFwe N-ter Ab



hFwe Win Ab



+ *hFwe shRNA*

+ *hFwe shRNA*

+ *hFwe shRNA*

+ *hFwe shRNA*

+ *hFwe shRNA*

+ *hFwe shRNA*

+ *hFwe shRNA*

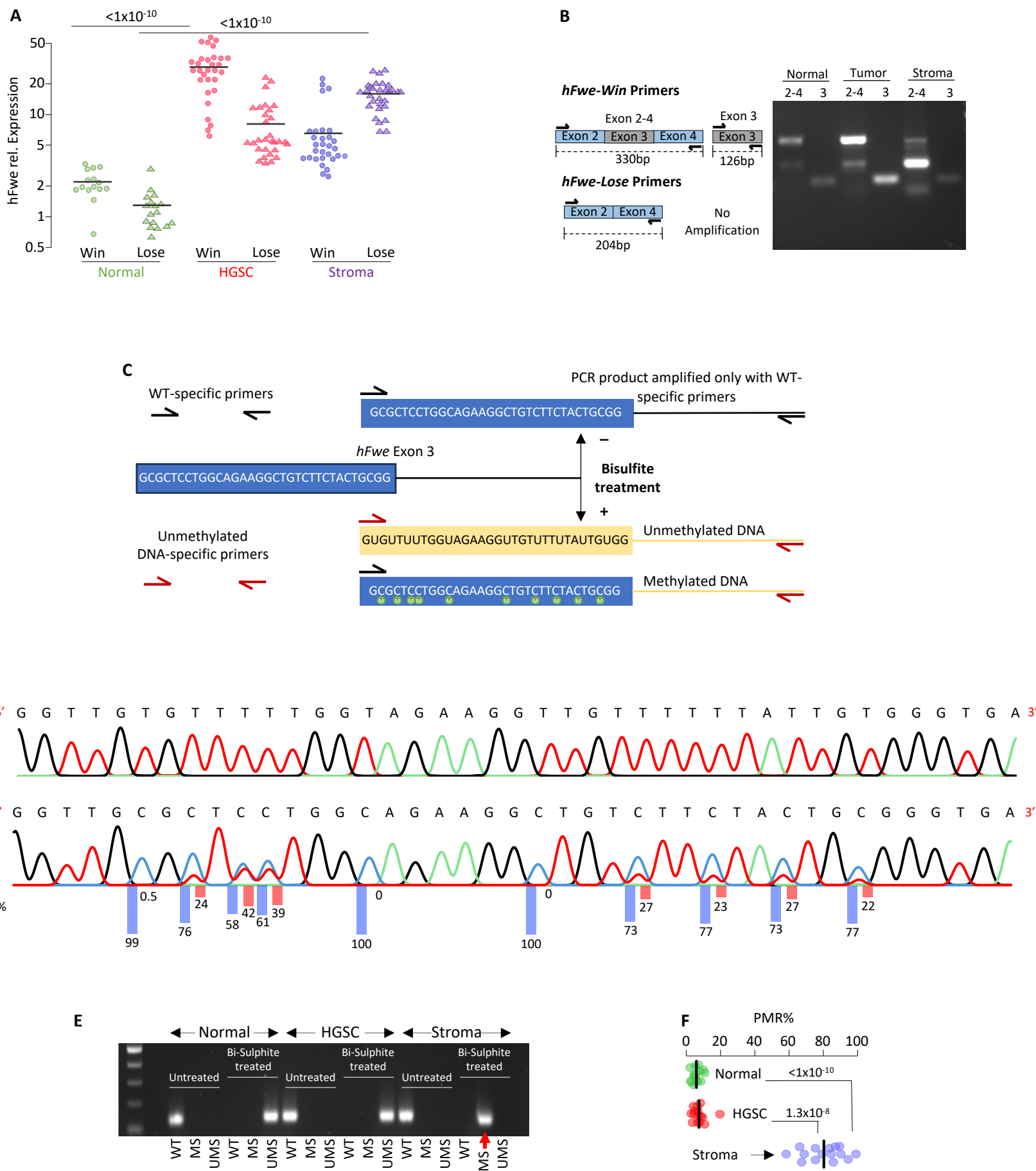
+ *hFwe shRNA*

Supplementary Figure Legends

Figure S2: Immunofluorescence experiment demonstrating location of Flower Win and Lose expression

Immunofluorescence from co-cultures of HGSC patient-derived cancer cells and HGSC patient-derived fibroblasts. HGSC patient-derived cancer cells are marked with Alexa Fluor® 594 anti-Cytokeratin-19, shown in the first column in red. HGSC patient-derived fibroblasts are marked Alexa Fluor® 647 anti-Vimentin, shown in the second column in blue. Flower was targeted with anti-Flower N-terminus (targeted in both hFwe Lose and Win) and anti-Flower Win, which was marked with goat anti-mouse IgG AlexaFluor 488, show in the third column in green. The three-color channels were merged to identify which cell type expresses Flower (fourth column). The first row represents a co-culture of HGSC patient-derived cancer cells and HGSC patient-derived fibroblasts incubated with anti-Flower N-terminus. Here, both cell types express hFwe since we can observe that the membrane staining specific of the Flower N-terminus antibody (green) co-localizes with both the expression of cytokeratin (cancer cell marker in red) and vimentin (fibroblast marker in blue). The second row represents a co-culture of HGSC patient-derived cancer cells and HGSC patient-derived fibroblasts incubated with anti-Flower Win, which shows that Flower Win expression is specific for cancer cells since membrane staining only co-localizes with the expression of cytokeratin (red). As controls, co-cultures of HGSC patient-derived *hFwe* KD cancer cells and HGSC patient-derived *hFwe* KD fibroblasts were used and incubated either with anti-Flower N-terminus (third row), or anti-Flower Win (fourth row). The figure shows that the expression of hFwe is not observed in the membrane of both cancer cells and fibroblasts regardless of the hFwe antibody used.

Supplementary Figure 3

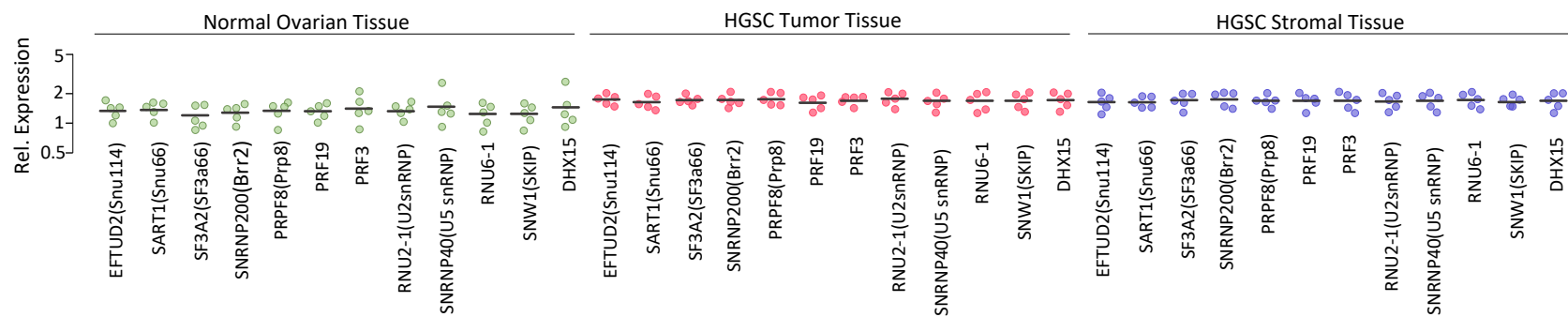


Supplementary Figure Legends

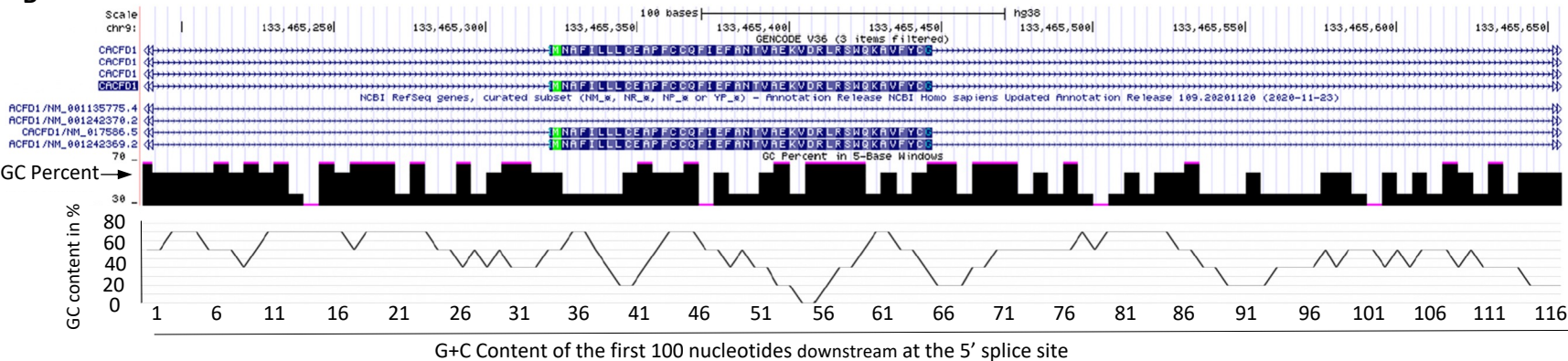
Figure S3: *Flower* Lose Expression and Exon 3 Methylation occurs in the stroma of HGSC tissue

- A) *hFwe-Win* is expressed abundantly in HGSC tumor while *hFwe-Lose* isoforms is expressed in stromal tissue. *hFwe-Win* and *hFwe-Lose* expression was analyzed by qRT-PCR in 30 matched HGSC FFPE samples and 15 normal ovarian FFPE tissue samples. *hFwe-Win* were significantly abundant in HGSC tumor versus their adjacent normal ovarian tissues (unmatched and matched t-test $p < 1 \times 10^{-10}$). While *hFwe-Lose* were abundant in stromal tissue (unmatched and matched t-test $p < 1 \times 10^{-10}$).
- B) RT-PCR was used to demonstrate Exon 3 skipping and synthesis of *hFwe-Lose* expression. The locations of the four PCR primers used for amplifications are represented schematically on the structure of the human *hFwe* gene. Total RNA was extracted from normal, HGSC tumor and stromal tissue and amplified by nested RT-PCR using primers annealing to exons 2 and 4. The 330-bp and shorter product of 204 bp (corresponding to the removal of Exon 3) transcript was detected together in all samples with higher expression in tumor. A shorter product of 126 bp, shows higher expression in stromal tissue.
- C) Model depicting methylation-specific PCR of *hFwe* Exon 3 is shown. Briefly, we designed two sets of primers targeting the same genomic region of *hFwe* Exon 3: one for WT DNA and another for unmethylated DNA. Without bisulfite treatment, only the WT primers can generate a PCR product. Upon bisulfite treatment, all unmethylated cytosines are converted to uracil. In that case, if *hFwe* Exon 3 is fully unmethylated only the unmethylated DNA-specific primers show amplification. If *hFwe* Exon 3 is methylated, bisulfite treatment cannot convert cytosine to uracil, thus maintaining the same sequence of the WT DNA. Since the potential methylation region of *hFwe* Exon 3 is small, there will only be amplification if we combine the forward WT-specific primer targeting *hFwe* Exon 3 with the reverse unmethylated DNA-specific primer targeting *hFwe* Intron3.
- D) DNA was extracted from 15 matched normal, stromal, and tumor tissue samples and subjected to bisulfite treatment. Sequencing results of bisulfite treated HGSC (top) and stroma (bottom) DNA are shown. The PCR products amplified from bisulfite-treated tissue DNA were cloned into the pUC57 vector via the restriction site and then sequenced. The plot under the sequencing results for bi-sulfite-treated stromal samples identifies the methylated cytosines and their percentage of occurrence.
- E) Methylation-specific PCR (MSP) was performed on both the control and the bisulfite-treated DNA (pooled from 15 matched normal, stromal, and tumor tissue samples). Results of the MSP for normal (lanes 1-6), tumor (lanes 7-12), and stroma (lanes 13-18) tissue are shown. Each tissue type is further subdivided into untreated (first 3 lanes) and bisulfite treated (last 3 lanes). The distribution shown further validates the methylation pattern in the stromal tissue. In all three tissue types, the WT band is present in the untreated group. For the normal and HGSC tissue, the band in the treated group appears only in the presence of unmethylation primers. However, in the bisulfite treated DNA from stromal tissue, amplification occurred only with methylation-specific primers.
- F) The methylation levels (percentage of methylated reference, or PMR) observed by qPCR were significantly different between Normal, HGSC and Stroma. Significantly higher methylation level was observed in the case of the Stroma compared to the normal ($p < 1 \times 10^{-10}$) and HGSC (unmatched t-test $p < 1.3 \times 10^{-8}$; matched t-test $p < 1 \times 10^{-10}$).

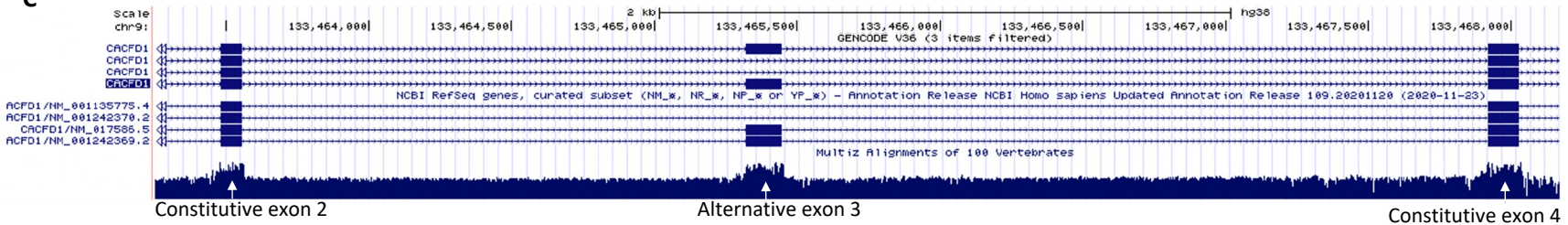
A



B



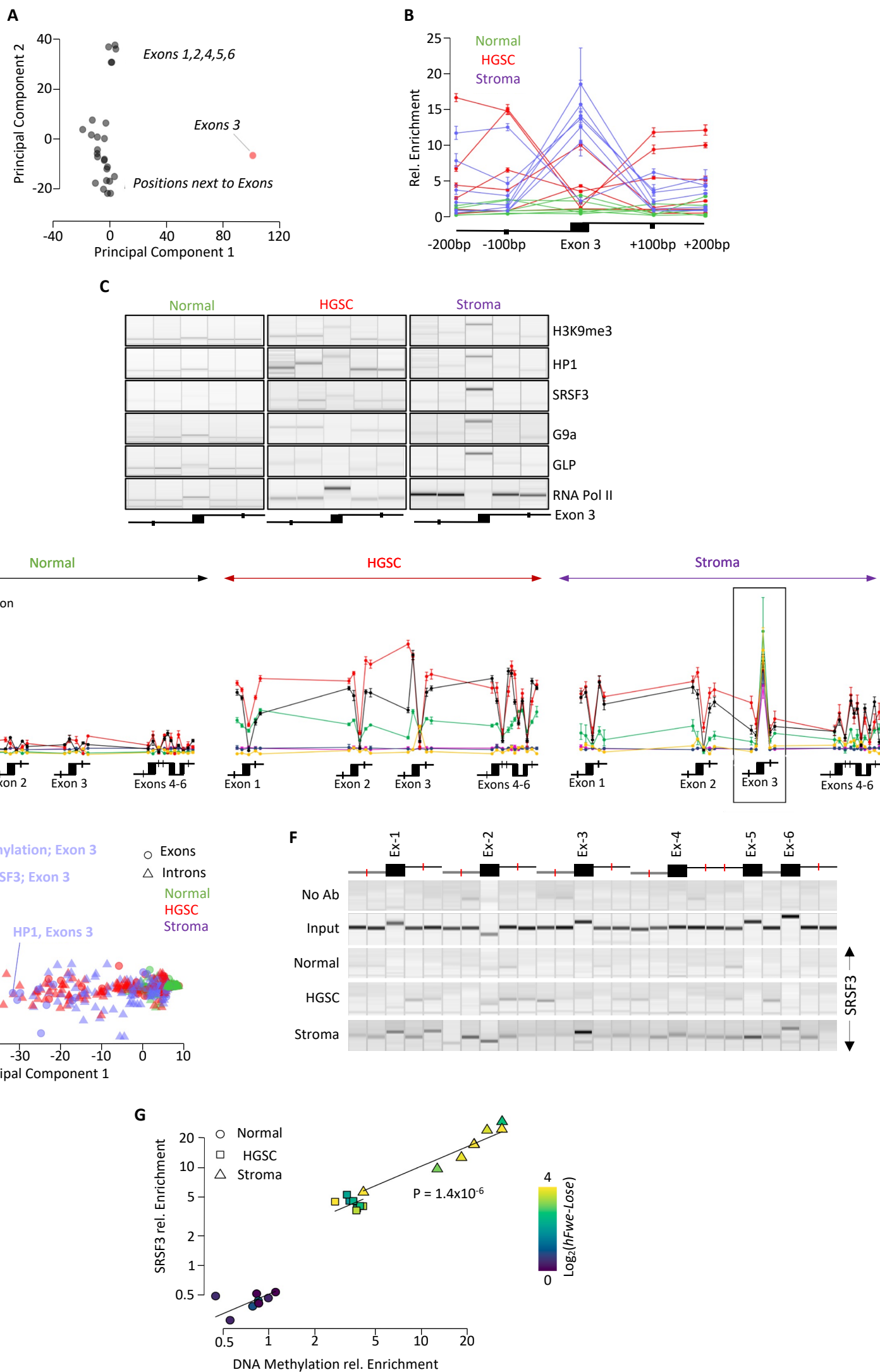
C



Supplementary Figure Legends

Figure S4: Characterization of all *Flower* exons.

- A) qPCR experiment is performed to study the expression of spliceosome components (12) in normal ovarian (white bar plot), HGSC tumor (red bar plot), and stromal (blue bar plot) tissue samples (5 HGSC patients). Results show no significant difference in the expression profile of the spliceosome proteins in all the three tissue samples (all p-values > 0.05).
- B) The G+C content (as a percentage) signal 100 nucleotides downstream of the 5' splice site at Exon 3 of *hFwe* gene locus is depicted.
- C) The PhastCons score of the *hFwe* gene at Exons 2, 3 and 4, which represents gene conservation, is depicted. Conservation peaks at Exons 2, 3, and 4 are highlighted with white arrows.



Supplementary Figure Legends

Figure S5: HGSC stromal tissue shows a specific enrichment of splicing machinery components at *Flower* Exon 3.

A) The principal component analysis (PCA), focusing on the genomic positions, shows clusters of exons and positions next to exons (± 200 bp), as well as the remote position of the Exon 3 in the dimensional reduction space. The red-highlighted dot implies the distinctiveness of the Exon 3 body of the *hFwe* from all other positions on the *hFwe* gene, explaining ~90% of the first principal component variance.

B) The line plot summarizes the *hFwe* Exon 3 body and the adjacent 5' & 3' flanking 200bp regions from matched normal, tumor, and stromal tissue. The colors green, red, and blue represent the tissue origin (representing pooled normal, tumor, and stroma tissue samples respectively). The plot shows increased DNA methylation and H3K9Me3, and binding of the SRSF3, GLP, G9a, and HP1, with decreased RNA Pol II occupancy at the *hFwe* Exon 3 in the stromal samples (all data points have $p < 0.05$; the error bars represent standard errors centered around the mean, biological replicates, $n=8$).

C) ChIP is performed in matched normal ovarian, HGSC tumor, and stromal tissue for H3K9Me3, HP1, SRSF3, G9a, GLP, and RNA Pol II occupancy on the *hFwe* Exon 3 and the adjacent 5' & 3' 200bp flanking regions. Amplicons are analyzed by electrophoresis using the QIAxcel instrument. Results show a poor association of all the epigenetic modifiers across all Exons and the flanking regions. The HGSC tumor and stromal tissue show a reverse binding pattern showing high binding exclusively on Exon 3 and low association at the 5' & 3' flanking regions in the stromal tissue samples and vice versa for H3K9Me3, HP1, SRSF3, G9a, GLP, and RNA Pol II. RNA Pol II occupancy on contrary is found to be high on Exon 3 on the HGSC tumor tissue compared with the stromal tissue.

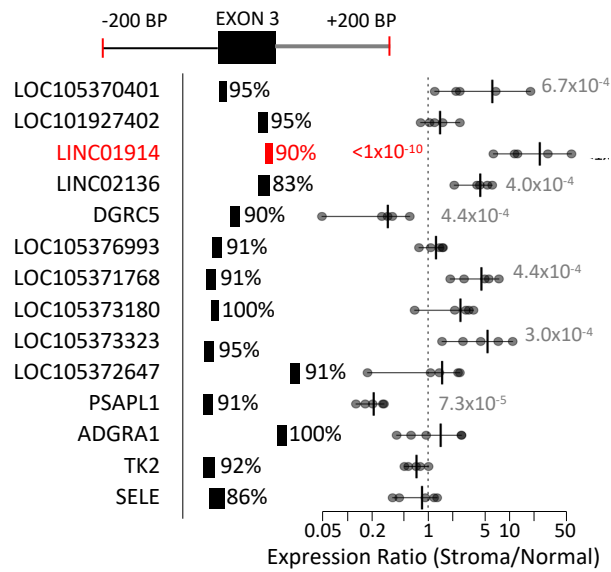
D) The line plot shows aggregated traces of DNA methylation and H3K9Me3, as well as binding of HP1, SRSF3, RNA Pol II, G9a, and GLP across all the six Exons and flanking regions of the *hFwe* gene locus in the matched normal ovarian, HGSC tumor, and stromal tissue samples from 8 HGSC patients. Normal ovarian tissue shows a relatively poor association of the epigenetic modifiers across all the six exons and the flanking regions in comparison to the HGSC tumor tissue. A unique association of epigenetic modifiers with the exception of RNA Pol II binding on the Exon 3 body in the stromal tissue is outlined within the black box. Each error bar represents the standard error, centered around the mean (Biological replicates, $n=8$).

E) The principal component analysis, using all available ChIP-qPCR data as input (genomic location + all modifications/binding), shows the distinctiveness of the DNA Methylation (~50% and ~25% of the first and second principle components variances explained, respectively) and SRSF3 binding (~40% and ~20% of the first and second principle components variances explained, respectively) exclusively on the *hFwe* Exon 3 (blue dots outlined within the red box corresponding to the stromal tissue samples). Their remote position on the PCA dimensional reduction suggests their crucial role in making the *hFwe* Exon 3 genomic region epigenetically distinct. Colors green, red, and blue correspond to the matched normal ovarian, HGSC tumor, and stromal tissue samples from 8 HGSC patients.

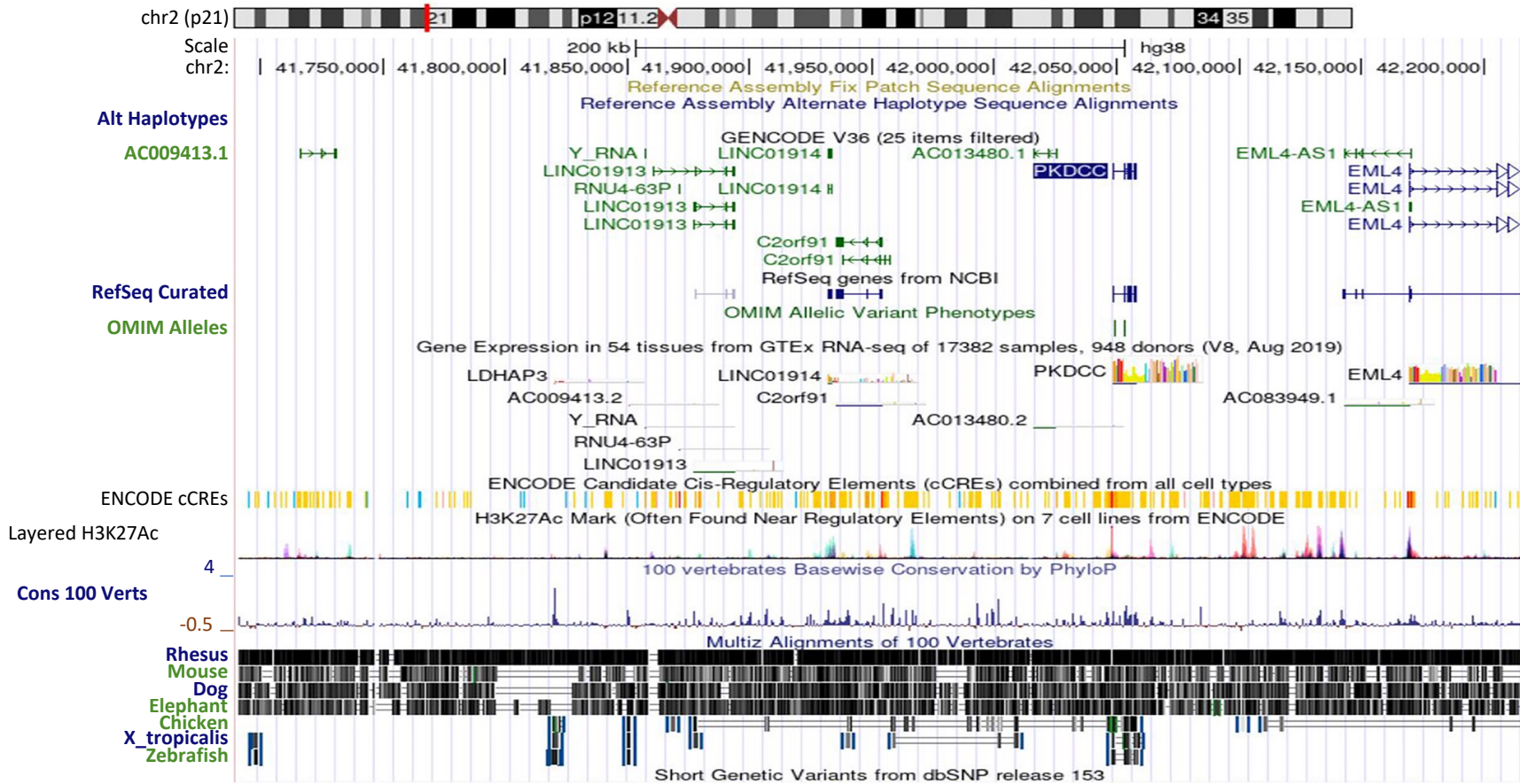
F) Exon walking is performed on the *hFwe* gene and 200bp flanking regions upstream (E1-6 -200bp, -100bp) and downstream (Ex1-6 +100bp, +200bp) of each Exon for SRF3 binding by ChIP analyzed by electrophoresis using QIAxcel instrument. Image displays no antibody (negative control, row1); Input (positive control, row 2); Normal ovarian (row 3); HGSC tumor (row 4); and Stromal (row 5) matched tissue samples from 8 HGSC patients, depicting a site-specific association of SRSF3 exclusively to the Exon 3 body in the stromal tissue with no binding observed on any Exons in the normal ovarian or the HGSC tumor tissue.

G) The scatter plot shows a clear separation of the normal (green), tumor (red), and stromal (blue) sample clusters based on DNA Methylation and SRSF3 enrichment (the black line represents the linear regression with ($p = 1.4 \times 10^{-6}$) between the tumor and stroma). The size of the colored dots represents the corresponding increase in the expression or the *hFwe-Lose* (fold change ranging from 5 to 15) with respect to the DNA Methylation and SRSF3 fold enrichment.

A



B



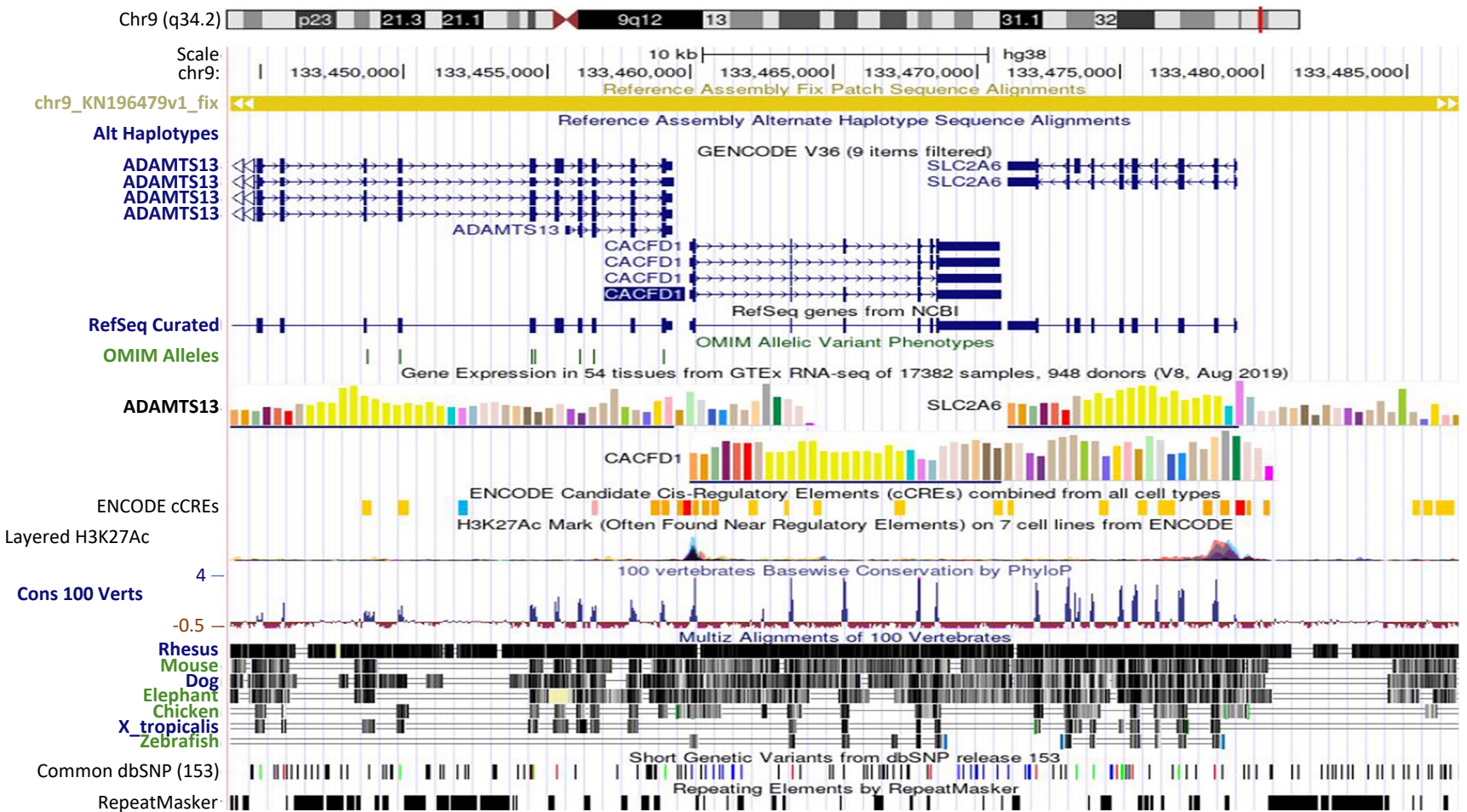
Supplementary Figure Legends

Figure S6: Characterization of *LINC01914* locus.

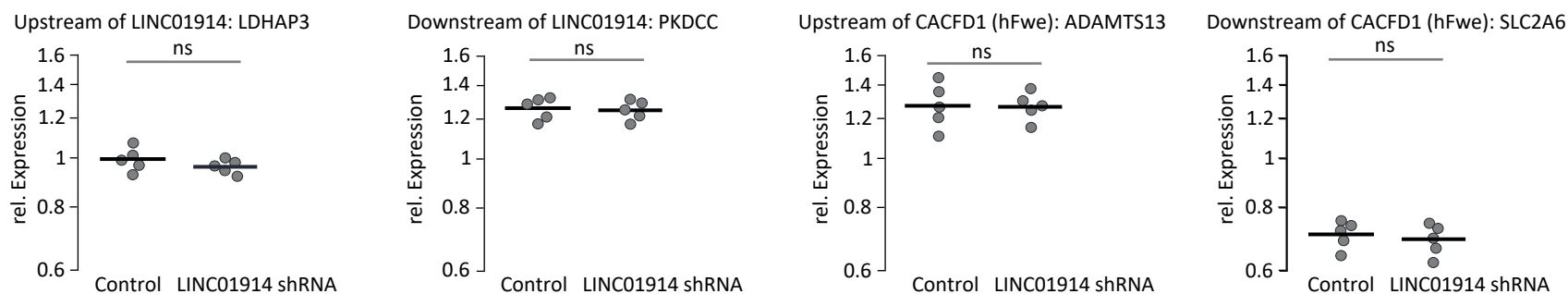
A) An analysis derived from sequence-specific BLAST between Exon 3 and transcripts with potential complementarity with *hFwe* Exon 3 and flanking regions shows a list of potential candidates which may influence DNA methylation in a site-specific manner. The percent complementarity and location of complementarity relative to Exon 3 is shown (left panel). Box plot (right panel) shows expression of these transcripts in matched stromal and normal tissue. *LINC01914* shows 90% sequence homology with the core element of *hFwe* Exon 3 and has high expression in the stromal tissue ($p < 1 \times 10^{-10}$) (the error bars represent standard errors centered around the mean, biological replicates, $n=5$).

B) UCSC Genome Browser maps the human *LINC01914* gene on chromosome 2. The map provides annotations of genes upstream and downstream of the *LINC01914* gene marked as *LDHAP3* and *PKDCC* respectively.

A



B



Supplementary Figure Legends

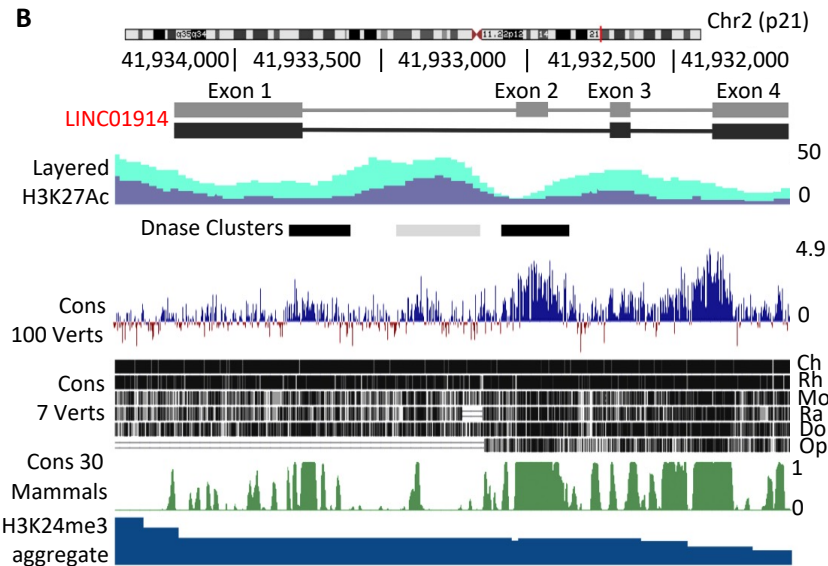
Figure S7: The knockdown of *LINC01914* does not impact the expression of the genes upstream and downstream of *LINC01914* and *CACFD1*.

- A) UCSC Genome Browser maps the human *CACFD1* gene on chromosome 9. The map provides annotations of genes upstream and downstream of the *CACFD1* gene marked as *ADAMTS13* and *SLC2A6* respectively.
- B) RT-qPCR experiment is conducted to assess the cis- or trans-regulatory role of *LINC01914* lncRNA. The experiment is performed in the control primary cultured stromal cells and experimental stromal cells upon silencing of *LINC01914* lncRNA. Results show no effect on the expression of the nearby genes on chromosome 2 (*LINC01914*) and chromosome 9 (*CACFD1*), showing it works in trans (all p-values > 0.05) (Biological replicates, n=5).

A



B

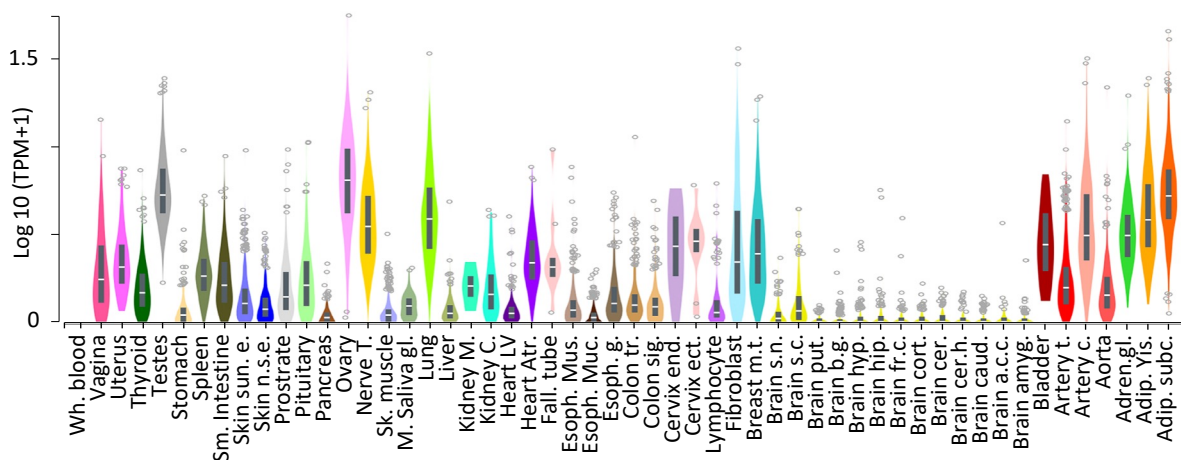


C

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Exon 1 Exon 3 Exon 4

D

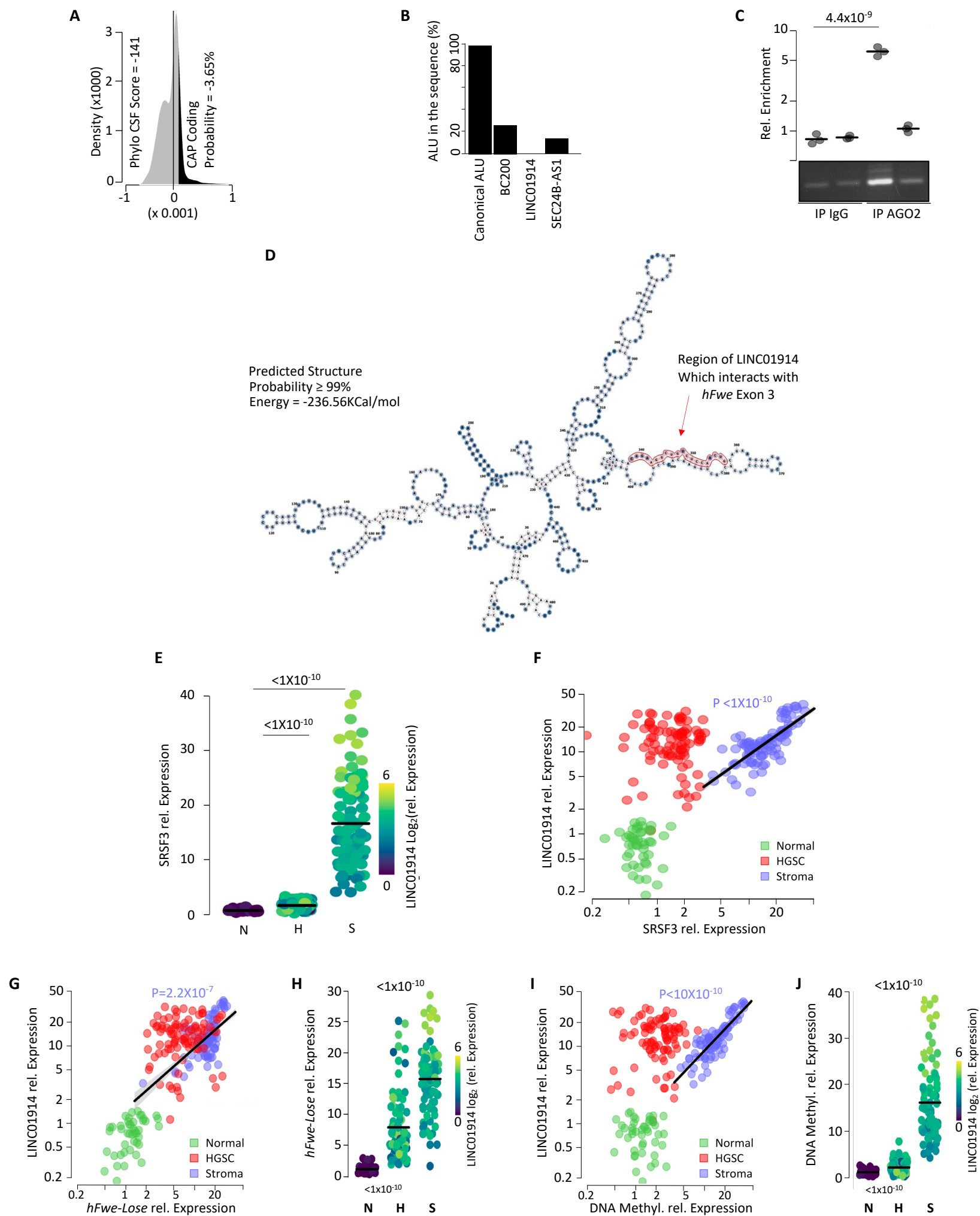


Supplementary Figure Legends

Figure S8: Characterization of *LINC01914* exons and expression of *LINC01914* in different tissue types.

- A) Heatmap representation of the expression of *LINC01914* exons 1-4 (Ex1-4) in indicated tissues calculated by median count per base. Among other tissues, ovaries express large amounts of *LINC01914*.
- B) ENCODE data mining for gene characterization of *LINC01914*. Two isoforms of *LINC01914* on Chromosome 2 are depicted. The isoform with 4 exons (top; grey color) is not synthesized as Exon 2 is not included in the principal transcript. The isoform (bottom; black color) with 3 Exons is the primary transcript that is expressed in HGSC stromal tissue. H3K27Ac ChIP shows active gene promoter, DNase I activity also shows active transcription at this gene. Gene conservation mapping shows that this lncRNA is conserved between vertebrates.
- C) The sequence and the color-coded exon structure of the spliced, functional variant of *LINC01914* is shown.
- D) Violin plots show the expression of *LINC01914* across indicated tissue types. Among other tissues, *LINC01914* is highly expressed in ovaries.

Supplementary Figure 9



Supplementary Figure Legends

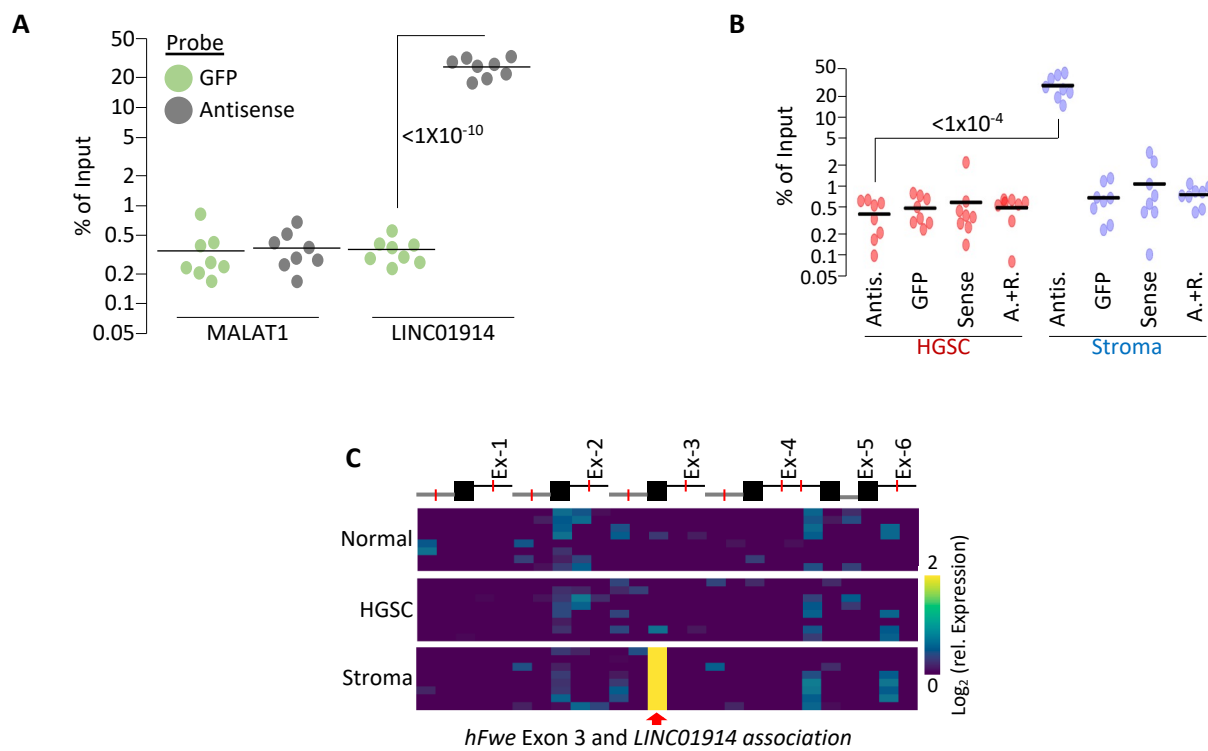
Figure S9: Characterization of *LINC01914* structure and correlation of *LINC01914* expression with markers of *Flower Lose* generation.

- A) *LINC01914* was analyzed for conserved protein-coding probability using Phylo CSF. A Phylo CSF score of -141.44 was obtained showing low protein-coding probability.
- B) *LINC01914* sequence (lane 3) was analyzed for the presence of Alu elements relative to other lncRNAs (*BC200* and *SEC24B-AS1*) and canonical Alu sequence (lane 1).
- C) Immunoprecipitation was performed using IgG or AGO2 antibodies and fold enrichments of *SOCS1* (a protein-coding gene) and *Tu-Stroma* (non-protein coding) RNA were analyzed by qPCR. The fold enrichment is depicted as a bar plot and the gel image is presented below. The error bar represents the standard error ($p = 4.4 \times 10^{-9}$) (Technical replicates, $n=3$).
- D) The RNA structure of *LINC01914* was predicted using RNAWebSuite, ViennaRNA Web Services and minimum free energy structure is depicted. Base colors indicate probability of folding. The 21 bp complementary sequence to *hFwe* Exon 3 is highlighted in red.
- E) The box plot shows increased SRSF3 binding to the *hFwe* Exon 3 in the stromal samples (unmatched $p < 1 \times 10^{-10}$ and matched $p = 7.2 \times 10^{-7}$ normal vs. HGSC; unmatched and matched $p < 1 \times 10^{-10}$ HGSC vs. stroma). The color of the dot represents the log2 fold change of the *LINC01914* expression.
- F) The scatter plot shows a positive correlation of *LINC01914* expression and SRSF3 binding to the *hFwe* Exon 3 (the black line represents linear regression with $R^2 = 0.67$ in the stromal samples; $p < 1 \times 10^{-10}$).
- G) The scatter plot shows positive correlation of *LINC01914* expression and *hFwe-Lose* (the black line represents linear regression in the stromal samples; $p = 2.2 \times 10^{-7}$).
- H) The box plot shows increased *hFwe-Lose* expression in the stromal samples (unmatched and matched $p < 1 \times 10^{-10}$ normal vs. HGSC; unmatched $p < 1 \times 10^{-10}$ and matched $p = 9.6 \times 10^{-7}$ HGSC vs. stroma). The color of the dot represents the log2 fold change of the *LINC01914* expression.
- I) The scatter plot shows positive correlation of *LINC01914* expression and *hFwe* Exon 3 DNA Methylation (the black line represents linear regression with $R^2 = 0.82$ in the stromal samples; $p < 1 \times 10^{-10}$).
- J) The box plot shows increased DNA Methylation of the *hFwe* Exon 3 in the Stromal samples (unmatched $p < 1 \times 10^{-10}$ and matched $p = 1.1 \times 10^{-3}$ normal vs. HGSC; matched and unmatched $p < 1 \times 10^{-10}$ HGSC vs. stroma). The color of the dot represents the log2 fold change of the *LINC01914* expression.

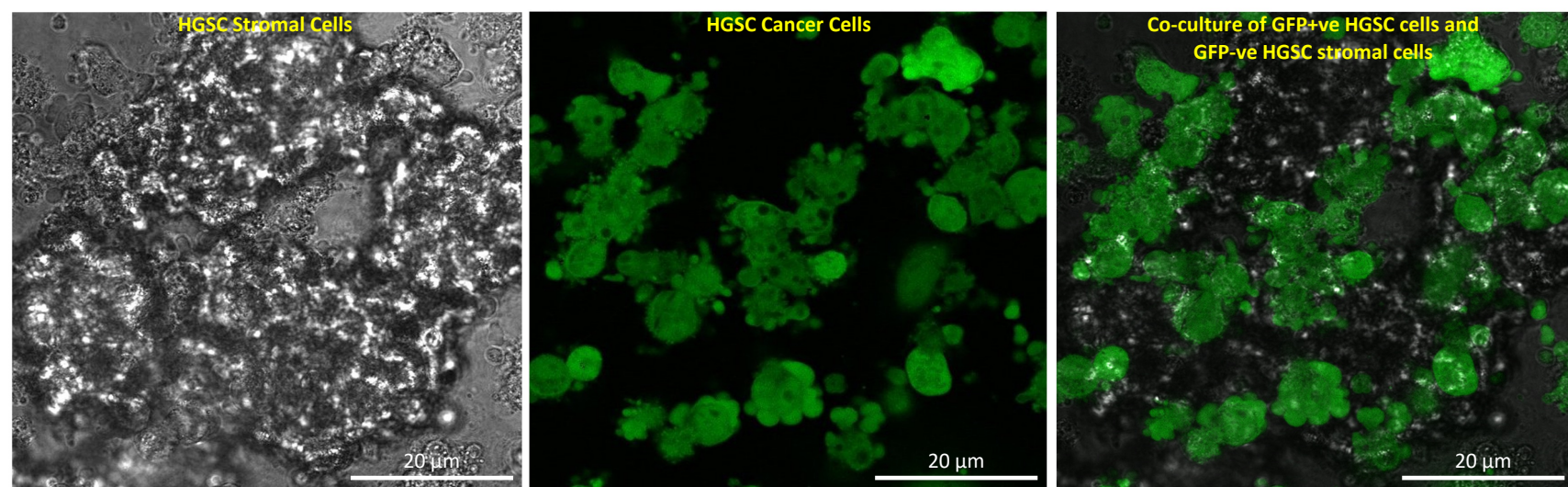
Supplementary Figure Legends

Figure S10: Elevated *LINC01914* expression in stroma leads to increased enrichment of splicing machinery at *Flower* Exon 3.

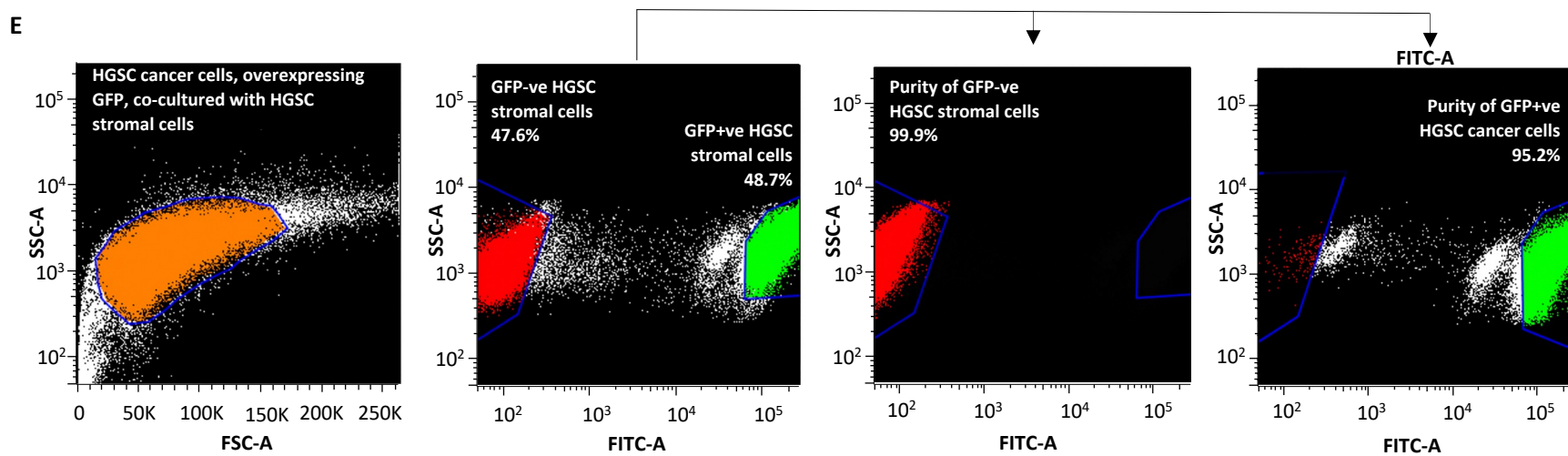
- A) The distribution of PMR values demonstrating *hFwe* Exon 3 methylation in a new cohort of HGSC tumor, stromal, and normal ovarian tissue is shown. Significantly higher methylation level was observed in the case of the Stroma compared to the normal ($p < 1 \times 10^{-10}$) and HGSC (unmatched and matched $p < 1 \times 10^{-10}$) (Biological replicates for normal tissue $n=15$, biological replicates for tumor and stroma tissue $n=30$).
- B) MethyLight assay shows *Flower* Exon 3 DNA methylation in WT (row 1) and *LINC01914* overexpressing (row 2) stromal cells. Data significantly higher methylation in the presence of *LINC01914* overexpression ($p = 1.7 \times 10^{-4}$) (Technical replicates, $n=5$).
- C) RT-PCR confirmed overexpression of *LINC01914* upon transfection compared to control in WT stromal cells and increase in *hFwe-Lose* transcript upon *LINC01914* overexpression in in WT stromal cells.
- D) ChIP on Exon 3 and 100 bp flanking regions in WT stromal cells and *LINC01914* overexpressing stromal cells shows significantly increased association of DNA methylation, DNMT3A, G9a, GLP, H3K9Me3, HP1, and SRSF3 after *LINC01914* overexpression.
- E) The model depicts the method used to silence *LINC01914* expression. A Poly-A sequence was inserted in the *LINC01914* locus, specifically before the region that binds to *hFwe* Exon 3. Briefly, CRISPR-Cas9 homology repair directs the insertion of a Poly-A sequence prior to the binding site sequence of *LINC01914* to *hFwe* Exon 3, thus blocking *LINC01914* transcription by RNA polymerase.



D



E



Supplementary Figure Legends

Figure S11: *LINC01914* binds to *Flower* at Exon 3.

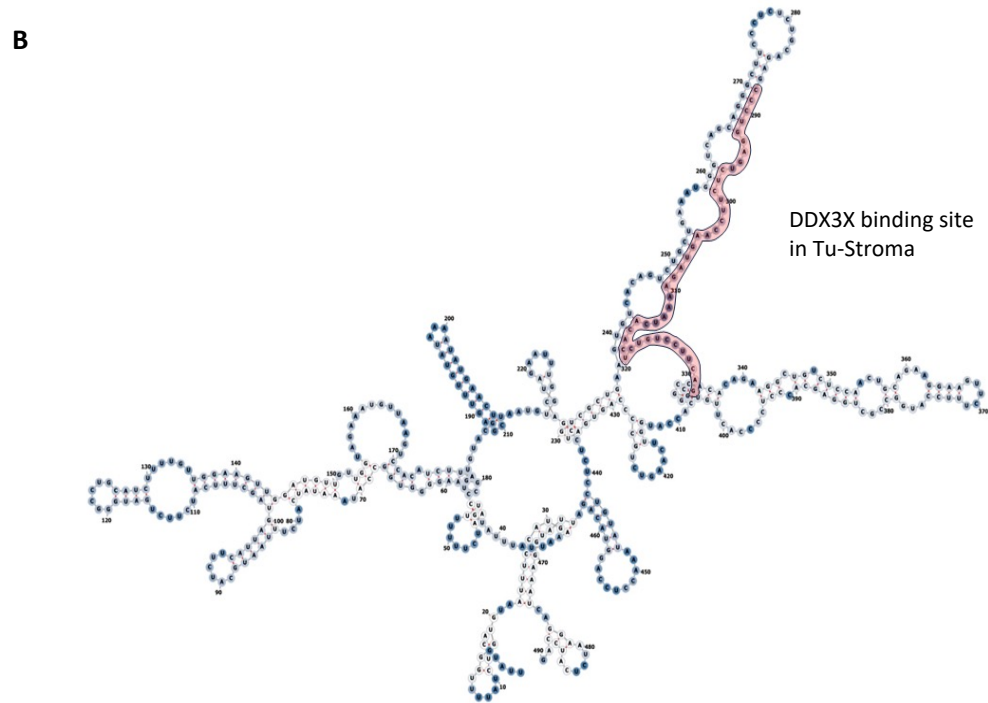
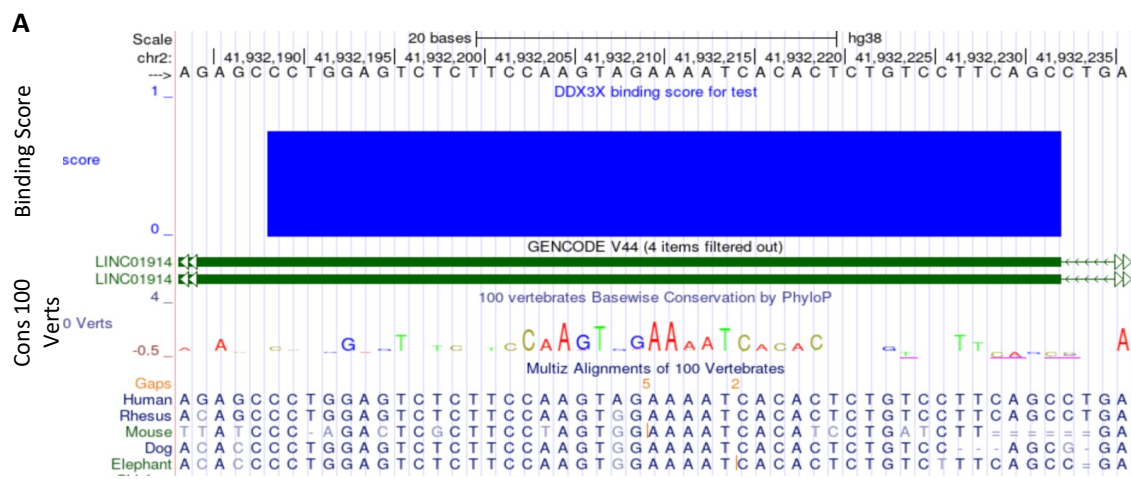
A) ChOP pull-down assay is performed in the pooled set of 8 stromal tissue fractions collected from the HGSC patients to determine the physical association of *LINC01914* on the Exon 3 body on the *hFwe* gene locus. The assay includes the use of biotin-labeled antisense DNA oligos spanning across the region complementary to the Exon 3 body to allow capture of *LINC01914* RNA-associated Exon 3 body of the *hFwe* gene loci with streptavidin beads. qPCR analysis shows the enrichment of *LINC01914* RNA on the Exon 3 body using the *LINC01914* anti-sense oligos (*LINC01914* Pr) (red and black dots indicates the experiment repeated 8 times) but not for the positive control, *MALAT1* (nuclear-enriched Lnc-RNA) using *MALAT1* anti-sense oligos (*MALAT1* Pr) (represented as a percentage of input). Pull-down with biotin probes against the green fluorescent protein (GFP) (used for both *MALAT1* and *LINC01914*) RNA is used as a negative control, as it shows no enrichment on the Exon 3 body. All the data shows the mean values $\pm$ SE (error bars) from eight replicates. P-value is shown in the figure ($p < 1 \times 10^{-10}$).

B) ChOP pull-down assay is performed using sense and antisense oligos to determine the physical association of *LINC01914* on the Exon 3 body on the *hFwe* gene locus in the pooled set of HSGC tumor (red) and stromal (blue) tissue fractions collected from 8 HGSC patients. Data shows no enrichment of *LINC01914* RNA on the Exon 3 body using the *LINC01914* anti-sense and sense oligos in the HGSC tumor fractions. No enrichment is observed with the *LINC01914* anti-sense oligos upon pretreating the chromatin with RNase A (R). On the other hand, enrichment of *LINC01914* RNA on the Exon 3 body is observed exclusively upon using the *LINC01914* anti-sense oligos. Pull-down with biotin probes against the GFP (used for both *MALAT1* and *LINC01914*) RNA is used as a negative control. All the data shows the mean values $\pm$ SE (error bars) from eight replicates. P-value is shown in the figure ($p < 1 \times 10^{-4}$).

C) ChOP analysis is performed on each Exon (1-6) and 200bp flanking regions upstream (5') and downstream (3') from each exon of the *hFwe* gene locus in 8 normal ovarian, HGSC tumor, and stromal tissue samples. Results reveal the physical association of *LINC01914* exclusively to the Exon 3 body and not to any other exons or their adjacent 5' or 3' flanking regions in the stromal tissue samples (highlighted blue color). Normal ovarian and HGSC tumor tissue show no physical interaction on either exons or the adjacent flanking regions. The heatmap depicts individual data points showing color deviation from low to high expression (yellow-black-blue).

D) The efficiency of GFP-lentivirus infection is confirmed by detecting GFP expression using fluorescence microscopy in primary cultured HGSC cancer cells. Images represent the primary co-culture of non-GFP labeled stromal cells and GFP-labeled cancer cells extracted from the HGSC patient sample.

E) HGSC GFP-positive cancer cells and HGSC GFP-negative stromal cells are co-cultured for 8 days, and then isolated by BD FACSAria™. HGSC cancer cells were isolated as GFP positive cells with a purity of 99.9%, while HGSC stromal cells were sorted out as GFP negative cells with a purity of 95.2%.



Supplementary Figure Legends

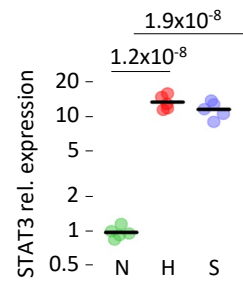
Figure S12: Identification of DDX3X binding site within LINC01914.

A) A visual representation is presented to evaluate the binding affinity of DDX3X in the LINC01914 context. The figure includes a genome browser depiction featuring conservation and base-wise conservation tracks at the LINC01914 locus³⁷. The "Cons 100 Verts" track illustrates conservation across 100 vertebrate species, measured by PhastCons. Additionally, conservation comparisons between humans and other species are demonstrated in tracks labeled with the respective species names.

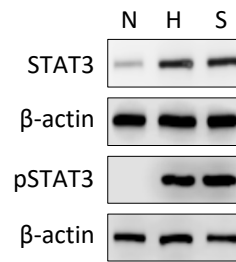
B) A visual representation highlighting the significance of the LINC01914 site when in a double-stranded conformation is shown. This configuration is predicted to be a binding site for the DDX3X protein (shown in red). The map offers insights into the regions or features that are most influential or critical for the predicted interaction between DDX3X and the LINC01914 site in its double-stranded conformation.

C) The figure visually represents the sequence of the DDX3X binding site, highlighted in brown, within the genomic context of LINC01914. In this illustration, the exonic regions of LINC01914 are distinguished: Exon 1 is depicted in green, Exon 3 in purple, and Exon 4 in gray. The purpose of this representation is to provide a clear and detailed view of the spatial arrangement of the DDX3X binding site in relation to the specific exonic regions of the LINC01914.

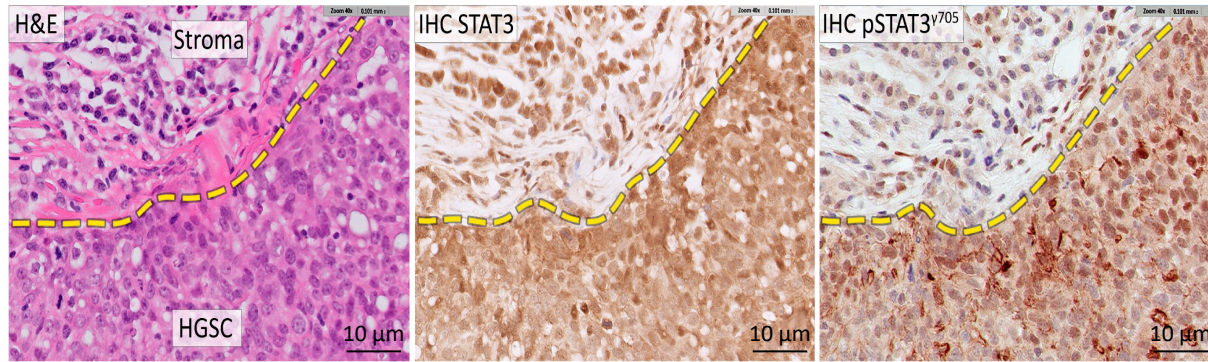
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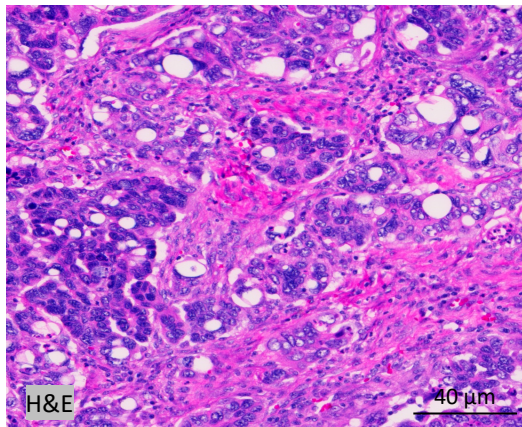
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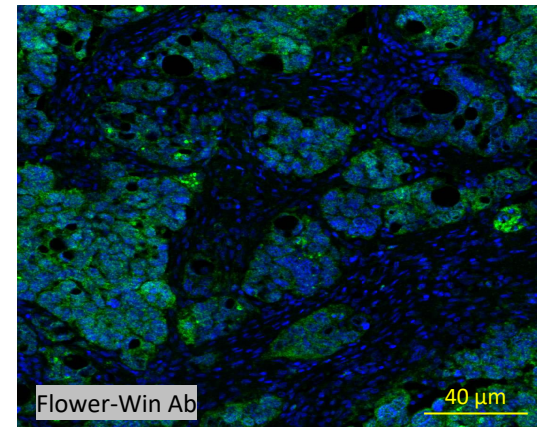
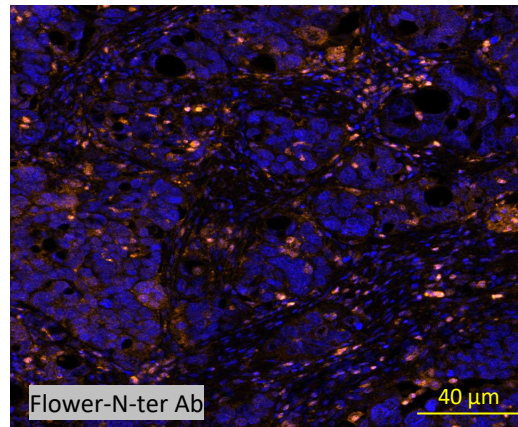
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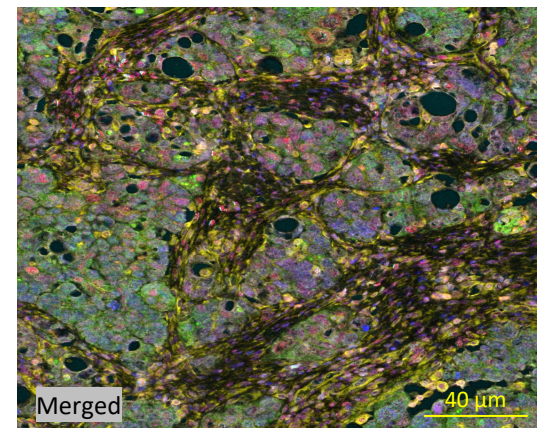
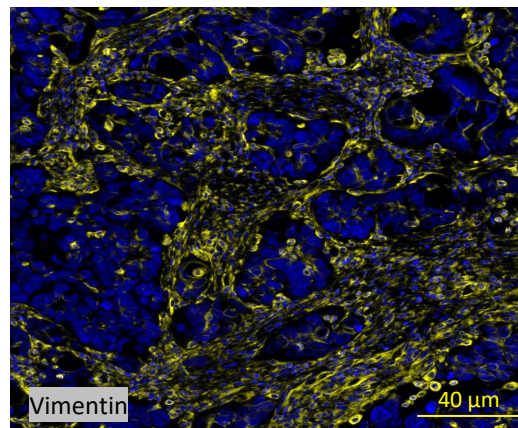
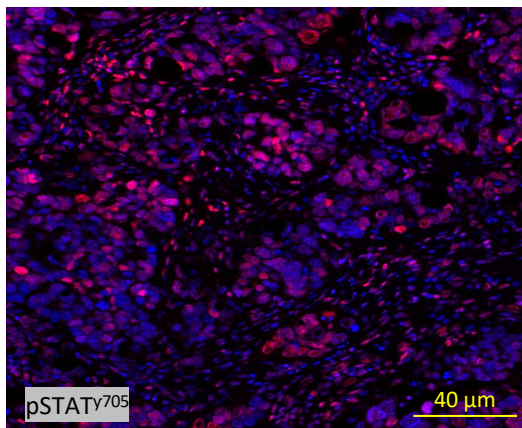
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E



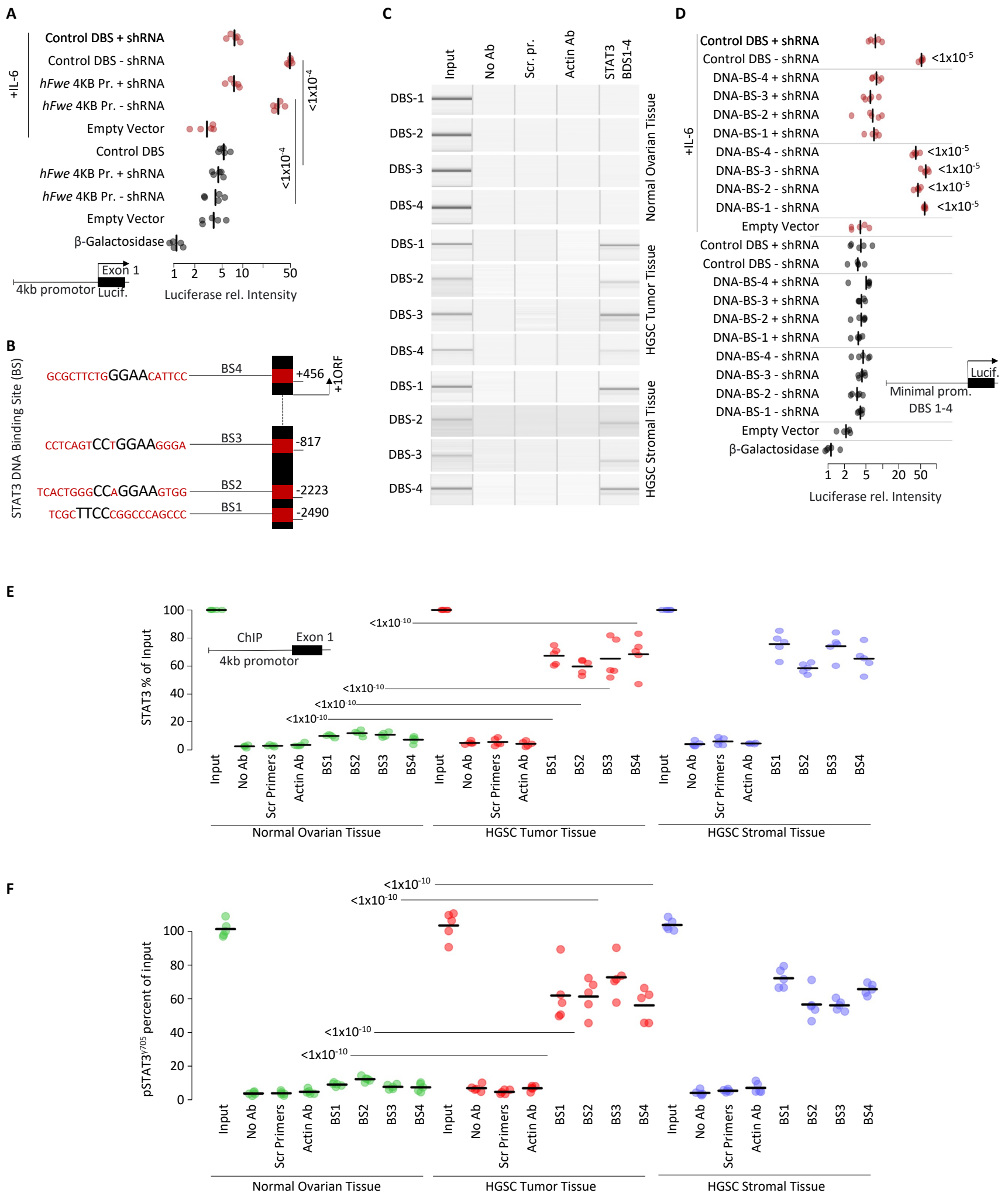
F



Supplementary Figure Legends

Figure S13: STAT3 and phospho-STAT3 expression is elevated in the HGSC compared to normal ovarian tissue.

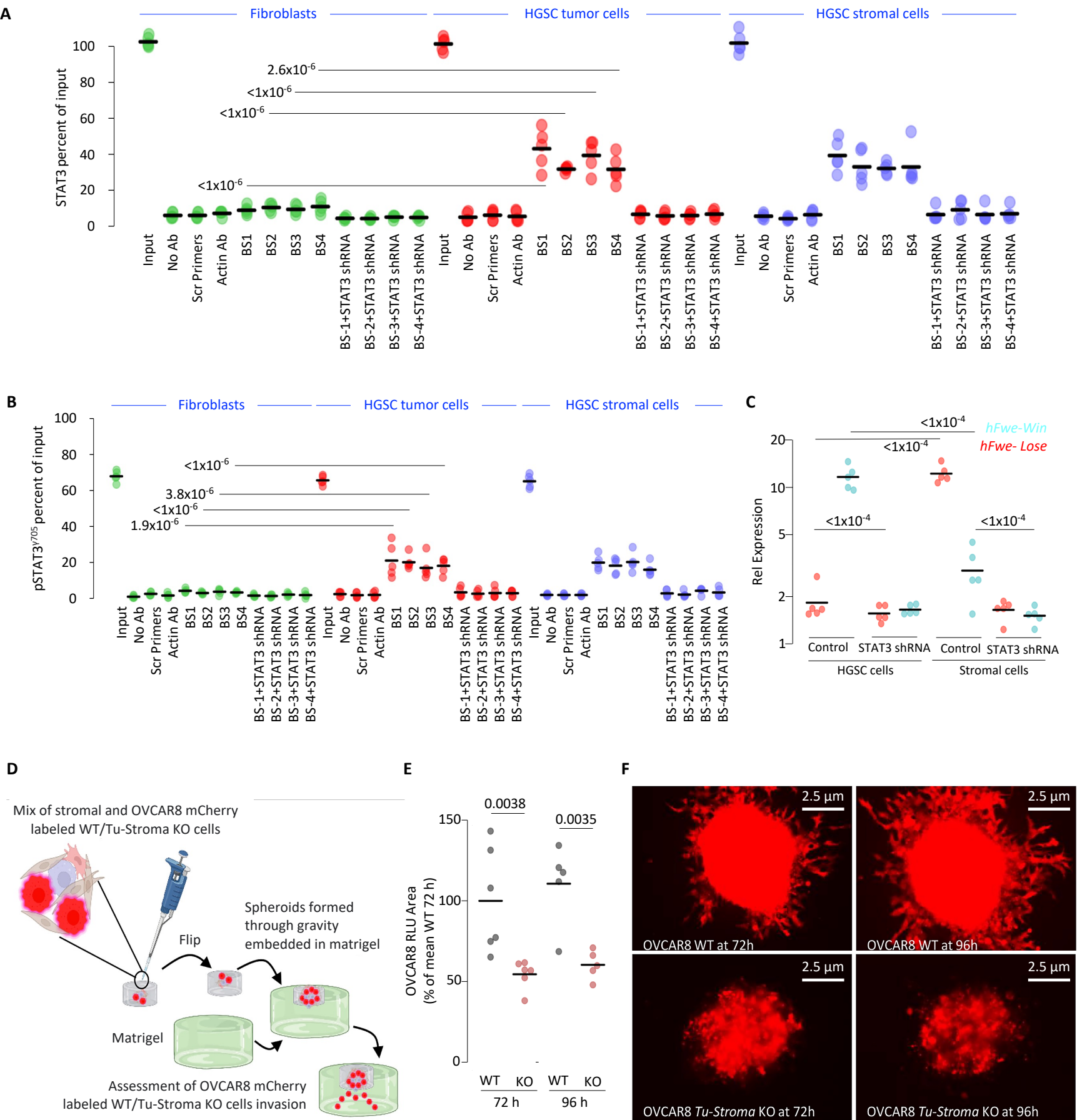
- A) *STAT3* mRNA expression was analyzed in pooled normal ovarian, HGSC cancer and stromal matched tissue from 8 patients using RT-qPCR and relative fold expression $\pm$ SE is depicted (unmatched $p = 1.2 \times 10^{-8}$ and matched $p < 1 \times 10^{-10}$ normal vs. HGSC; unmatched $p = 1.9 \times 10^{-8}$ and matched p HGSC vs. stroma).
- B) STAT3 and STAT3^{Y705} protein levels were analyzed in pooled normal ovarian, HGSC cancer and stromal matched tissue from 8 patients using western blot analysis and a representative blot is depicted.
- C) STAT3 and STAT3^{Y705} levels were analyzed stroma and HGSC cancer tissue sections. H&E staining shows stroma-tumor boundary. IHC staining using STAT3 and pSTAT3^{Y705} antibodies shows high expression both in the stroma and tumor tissue.
- D) Hematoxylin and eosin staining of a HGSC sample with both tumor and stromal regions is shown.
- E) Multiplexed IHC of the same HGSC sample from **Figure S13D** using Flower Win Ab (panel 2), and Flower N-terminus Ab (panel 1) is shown.
- F) Multiplexed IHC of the same HGSC sample (**Figure S13D**) using pSTAT3^{Y705} Ab (left panel), Vimentin Ab (middle panel) and a merge (right panel) of all antibodies used in **Figures S13E & F** are shown.



Supplementary Figure Legends

Figure S14: STAT3 controls transcription at the *hFwe* promoter in both cancer and stromal cells

- A) Luciferase assay is performed on the 4kb promoter of *hFwe* gene cloned in pGL4 vector transfected in OVCAR8 cells in the presence or absence of IL-6. *c-Fos* promoter (control STAT3 DBS) is used as positive control whereas empty vector and β -galactosidase are used as negative controls. The specificity of IL-6 mediated STAT3-dependent activation of *hFwe* is examined by shRNA mediated silencing of STAT3 in IL-6 treated cells (STAT3 shRNA; $p < 1 \times 10^{-4}$) (Technical replicates, n=5).
- B) Schematic representation of the four DNA binding sites (DBS 1-4) on the *hFwe* promoter region is presented along with their location relative to the ORF. Notably, site 4 lies downstream of the *hFwe* ORF.
- C) The occupancy of STAT3 on *hFwe* DBS is measured by ChIP using indicated antibodies in pooled normal ovarian, HGSC cancer and stromal matched tissue. A band in STAT3 DBS1-4 confirms the presence of STAT3 on all four DBS in HGSC cancer and HGSC stroma but absent in normal ovarian tissue.
- D) Luciferase assay is performed on minimal *hFwe* promoter with each of the four putative DBS sites (DBS 1-4) in the presence or absence of IL-6. *c-Fos* promoter (control STAT3 DBS) is used as positive control whereas empty vector and β -galactosidase are negative controls. The specificity of IL-6 mediated STAT3-dependent activation of *hFwe* is examined by shRNA mediated silencing of STAT3 in IL-6 treated cells (STAT3 shRNA; $p < 1 \times 10^{-5}$) (Technical replicates, n=5).
- E) The occupancy of STAT3 on each of the four putative DBS sites on *hFwe* promoter is measured by ChIP using indicated antibodies in pooled normal ovarian, HGSC cancer and stromal matched tissue. The result of fold enrichment $\pm$ SE relative to input is depicted $p < 1 \times 10^{-10}$ (Biological replicates, n=5).
- F) The occupancy of pSTAT3^{Y705} on each of the four putative DBS sites on *hFwe* promoter is measured by ChIP using indicated antibodies in pooled normal ovarian, HGSC cancer and stromal matched tissue. Results show that pSTAT3^{Y705} binds to all the four STAT3 DBS (STAT3 DNA-BS-1-4) in the HGSC tumor and stroma tissue samples. Data from qPCR-based analysis is depicted as fold enrichment relative to input. The respective p-values are shown on the plot ($p < 1 \times 10^{-10}$) (Biological replicates, n=5).



Supplementary Figure Legends

Figure S15: Transcriptional and epigenetic regulation of *Flower* isoform expression promotes tumor growth

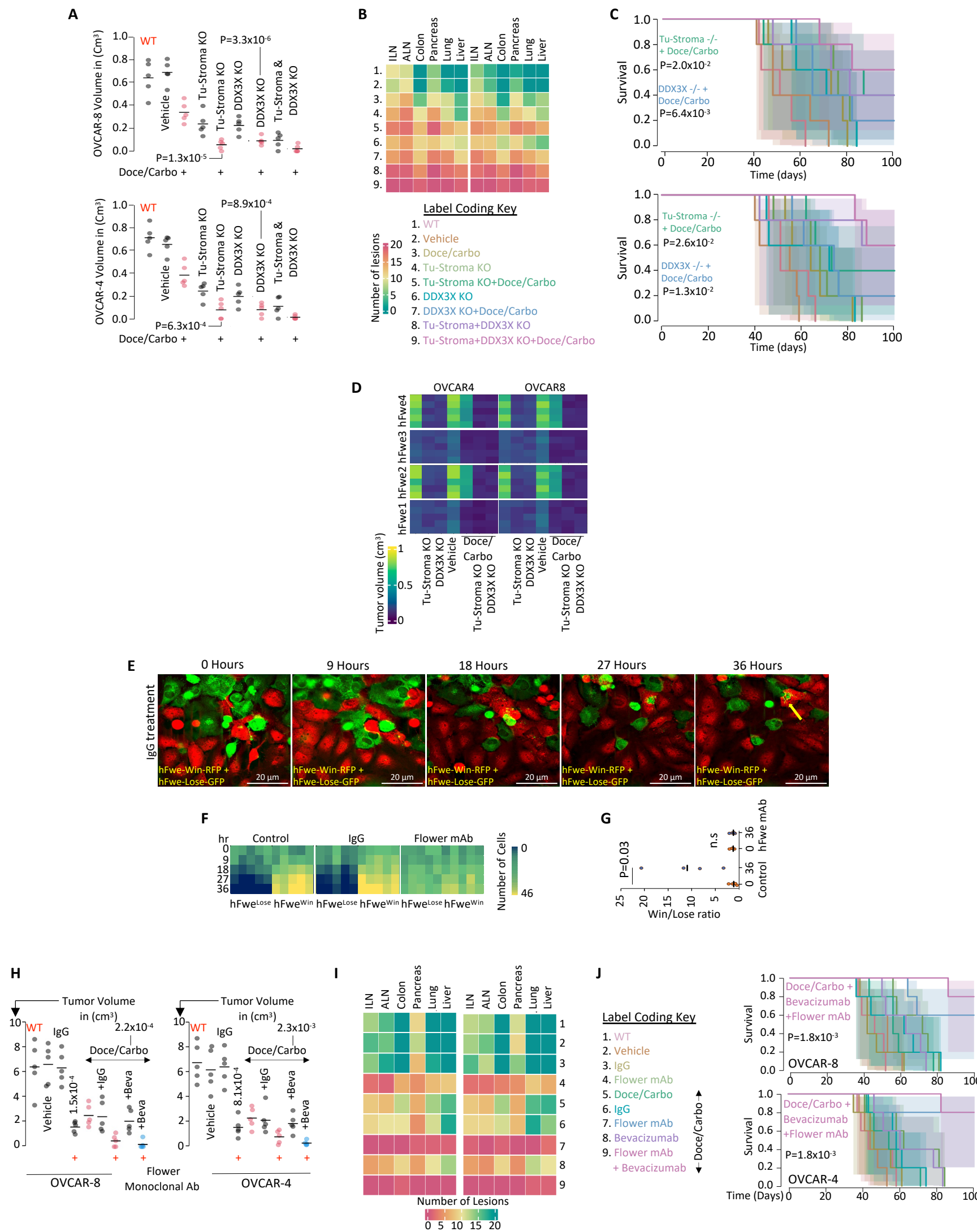
A&B) Relative occupancy of STAT3 (A) and pSTAT3^{Y705} (B) is measured by qChIP for the indicated antibodies on DBS1-4 sites in primary cultured fibroblasts, HGSC tumor cells and HGSC stromal cells in the presence or absence of STAT3 shRNA. Results show a high fold enrichment on all the four STAT3-DBS in co-cultured and separated HGSC tumor and HGSC stromal cells, however, silencing of STAT3 using STAT3 shRNA, abrogates the effect. No antibody (no Ab), scrambled primer (scr. prim.) and actin antibody are used as negative controls. Data from qPCR-based analysis is depicted as fold enrichment relative to input. The respective p-values are shown on the plot (Technical replicates, n=5).

A) HGSC and stromal cells are co-cultured for 8 days in the presence or absence of STAT3 shRNA. HGSC and stromal cells are then sorted. *hFwe-Win* and *hFwe-Lose* expression is analyzed using RT-qPCR and the relative fold change $\pm$ SE is depicted $p < 1 \times 10^{-4}$ (Technical replicates, n=5).

B) Schematic outline depicting the OVCAR8 growth and invasion assay. Briefly, spheroids containing *Tu-Stroma* WT or KO OVCAR8 cells labeled with mCherry were grown in hanging drops and subsequently embedded in Matrigel.

C) The capacity of OVCAR8 WT and *Tu-Stroma* KO cells to invade the surrounding Matrigel is assessed (n = 5). OVCAR8 growth and invasion is measured at 72h and 96h and is expressed as relative light units (RLU) as percent WT cells at 72h and 96h. *Tu-Stroma* KO significantly reduces the invasiveness of OVCAR8 after 72 ($p = 3.8 \times 10^{-3}$) and 96 hours ($p = 3.5 \times 10^{-3}$).

D) Representative images of data in **Figure S15E** are shown.



Supplementary Figure Legends

Figure S16: *Tu-Stroma* KO, DDX3X KO, and Flower monoclonal antibody therapy reduce tumor size and improve survival in orthotopic HGSC models.

- A) Tumor volumes of each of the cohorts in **Extended Figure 10D** are shown for both OVCAR8 (top) and OVCAR4 (bottom) groups. Red dots identify the groups treated with Docetaxel/Carboplatin therapy. The p-values compare the tumor volume at the endpoint of *Tu-Stroma* KO in combination with chemotherapy (OVCAR8: $p = 1.3 \times 10^{-5}$; OVCAR4: 6.3×10^{-4}) and DDX3X KO in combination with chemotherapy (OVCAR8: $p = 3.3 \times 10^{-6}$; OVCAR4: 8.9×10^{-4}) with the control (Biological replicates, $n=5$).
- B) The heatmaps show the number of lesions detected in the liver, axillary lymph nodes, colon, pancreas, lung, and inguinal lymph nodes at the endpoint of the experiment described in **Extended Figure 10D** and **Figure S16A** (top). A colored and numbered label coding key is shown to identify the groups used in the experiment (bottom).
- C) Kaplan-Meier curves of each group used in OVCAR8 and OVCAR4 orthotopic experiment shown in **Extended Figure 10D**. Each group line is colored as per the label coding key shown in **Figure S16B**. The p-values show the comparison in survival between *Tu-Stroma* KO in combination with chemotherapy (OVCAR8: $p = 2.0 \times 10^{-2}$; OVCAR4: 6.4×10^{-3}) and DDX3X KO in combination with chemotherapy (OVCAR8: $p = 2.6 \times 10^{-2}$; OVCAR4: 1.3×10^{-2}) with the WT. The number of mice use in each group was equal to 5.
- D) The heatmap shows the tumor volumes of each group at the endpoint of the experiment described in **Figure 4D**.
- E) Live-cell imaging demonstrates the efficacy of the Flower mAb to protect hFwe-Lose expressing cells from competition-induced apoptosis. Top row shows a co-culture of GFP and hFwe-Lose expressing cells and RFP and hFwe-Win expressing epithelial cancer cells at 0, 9, 18, 27, and 36 hours, as demonstrated previously¹¹. The bottom row shows the same co-culture with application of the Flower mAb, demonstrating the rescue of hFwe-Lose expressing cells from competition-induced apoptosis.
- F) Heatmap showing a time-dependent elimination of hFwe-Lose expressing cells in the cell-competition assay in the presence and absence of Flower monoclonal antibody.
- G) The impact of Flower mAb in protecting hFwe-Lose cells from apoptosis during cell-competition is highlighted by the change in ratio of hFwe-Win and hFwe-Lose expressing cells in presence and absence of Flower mAb. The ratio of Win/Lose isoforms changes significantly over 36 hours in the absence of Flower mAb ($p = 3 \times 10^{-2}$) but remains unaffected with Flower mAb (Biological replicates, $n=5$).
- H) Tumor volumes of each of the cohorts in **Figure 6A** are shown for both OVCAR4 (left) and OVCAR8 (right) groups. Groups that received the Flower mAb are designated with a red plus under the corresponding data points. As shown, addition of Flower mAb significantly reduces tumor volume compared to the control groups and groups that did not receive Flower mAb. The p-values shown compare tumor volumes at the endpoint of the experiment of mice untreated mice with Docetaxel/Carboplatin and Bevacizumab treated (OVCAR8: $p = 2.2 \times 10^{-4}$; OVCAR4: $p = 2.3 \times 10^{-3}$), and Flower mAb (OVCAR8: $p = 1.5 \times 10^{-4}$; OVCAR4: $p = 8.1 \times 10^{-4}$). The number of mice use in each group was equal to 5.
- I) Heatmaps demonstrate the number of metastatic lesions in each of the treatment groups that received OVCAR4 (left) and OVCAR8 (right) implants. Each row represents a treatment group that is designated on the right of the heatmaps. These results further demonstrate the ability of Flower mAb to attenuate metastatic spread both as a monotherapy and in combination with standard chemotherapy. Kaplan-Meier analysis of each of the OVCAR4 and OVCAR8 cohorts demonstrates the impact of Flower mAb treatment on survival. The color of each line represents a treatment cohort corresponding to the labeling key. Mice that received treatment with Docetaxel/Carboplatin/Bevacizumab/Flower mAb had the highest survival rate, with 80% survival at the end of the experiment (OVCAR8: $p = 1.8 \times 10^{-3}$; OVCAR4: $p = 1.8 \times 10^{-3}$).

	Test cohort (n=99, 72 events)			Validation cohort (n=296, 121 events)		
	Hazard ratio	χ^2 value	p-value	Hazard ratio	χ^2 value	p-value
Flower-Loose	6.2 (3.4, 11.1)	55.7	$8.46 \cdot 10^{-14}$	2.0 (1.6, 2.4)	54.0	$1.99 \cdot 10^{-13}$
Tu-Stroma	4.8 (3.1, 7.6)	71.2	$<2.2 \cdot 10^{-16}$	2.0 (1.6, 2.4)	50.5	$1.20 \cdot 10^{-12}$

Results from the Cox proportional hazards models for the relative expression of Flower-Lose and Tu-Stroma, respectively. Hazard ratios (estimate and 95% confidence interval) are for $\log_2$ relative expression and are adjusted for age and stage. P-values are from type-II likelihood ratio tests based on the χ^2 statistic with 1 degree of freedom.

Supplementary Table Legends

Table S1: Multivariate Analysis in HGSC patients.

Survival from HGSC patients was analyzed in proportional hazard models to estimate the association of hFwe and *Tu-Stroma* expression on survival. The table shows the results for the test cohort and for the validation cohort. Hazard ratios (estimate and 95% confidence interval) are for $\log_2$ relative expression and are adjusted for age and stage. P-values are from type-II likelihood ratio tests based on the χ^2 statistic with 1 degree of freedom. The estimated hazard ratio refers to a doubling of the relative expression of the respective gene. The values above 1.0 indicate a higher hazard or a worse survival with increasing expression.

Supplementary Video Legends

Supplementary Videos-SV1: Live-cell imaging shows OVCAR8 DDX3X-RFP cells exporting exosomes to primary stromal cells.

Live-cell imaging of co-culture with OVCAR8 DDX3X-RFP cells and primary stromal cells for 24 hours. In SV1 the data shows OVCAR8 cells marked with red resulting from the expression of a RFP fused to DDX3X, and untagged primary stromal cells. The 24 hours live-cell imaging shows untagged primary stromal cells starting to express RFP. This data shows that primary stromal cells uptake DDX3X exported through exosomes by OVCAR8 cells.

Supplementary Videos-SV2: 360° visualization of co-cultures of GFP-labeled WT OVCAR8 and primary stromal cells in patients-derived 3-D scaffolds.

A 360° visualization of light sheet microscopy imaging data showing GFP-labeled WT OVCAR8 cells in patient derived 3D scaffolds carrying primary stromal cells extracted from patients. In SV2 the data is presented for a 3D scaffold where the cancer cells are marked with green, and the 3D scaffold structure and stromal cell populations are marked in purple. The scaffolds are scanned with light-sheet microscopy and videos were rendered to highlight the structure of the 3D scaffold.

Supplementary Videos-SV3: 360° visualization of co-cultures of GFP-labeled *Tu-Stroma* KO OVCAR8 and primary stromal cells in patients-derived 3-D scaffolds.

A 360° visualization of light sheet microscopy imaging data showing GFP-labeled *Tu-Stroma* KO OVCAR8 cells in patient-derived 3D scaffolds carrying primary stromal cells extracted from patients. The data is presented for a 3D scaffold where the cancer cells are marked with green, and the 3D scaffold structure and stromal cell populations are marked in purple. The 3D scaffold set carrying the *Tu-Stroma* KO OVCAR8 cells are scanned with light-sheet microscopy and videos were rendered to highlight the structure of the 3D scaffold. The comparison of data in SV2 and SV3 clearly shows significantly reduced ability of *Tu-Stroma* KO OVCAR8 cells to spread in the 3-D scaffold and replace stromal cells, when compared with the WT OVCAR8 cells.

Supplementary Videos-SV4: Live-cell imaging shows efficiency of Flower monoclonal antibodies in protecting Flower-Lose expressing cells.

Live-cell imaging of Flower-Win expressing (RFP) cells in presence of Flower-Lose expressing (GFP) cells in a cell competition assay as described earlier⁴. Data shows that Flower-Lose expressing GFP cells do not undergo apoptosis in presence of Flower monoclonal antibody. Thus, the monoclonal antibody successfully blocks Flower-Win-Lose competitive interaction.

Supplementary Video-SV5: HGSC tumor cells manipulate the transcription and alternative splicing of *Flower* in fibroblasts by exporting STAT3 and *Tu-Stroma* via exosomes.

An animated presentation of the main findings from our study are summarized as SV5. HGSC tumor cells export STAT3 to fibroblasts via exosomes. Once inside these cells, STAT3 enters the nucleus and binds to the *hFwe* promoter, which induces the expression of *Flower* isoforms. The difference between *hFwe-Win* and *hFwe-Lose* isoforms is the presence of Exon 3 in *hFwe-Win* isoforms. Tumor cells produce a lncRNA containing a 21 bp region with 90% complementarity to *hFwe* Exon 3, named *Tu-Stroma*. The binding of DDX3X with *Tu-Stroma* packs this lncRNA into exosomes, which then are exported to fibroblasts. Once there, *Tu-Stroma* translocate to the nucleus and binds to *hFwe* DNA at Exon 3, leading to Exon 3 trimethylation by recruiting the DNA methyltransferases G9a, GLP, and DNMT3A. Consequently, HP1 binds to the methylated DNA and recruits SRSF3, which excludes *hFwe* Exon 3 from the final mRNA transcript of *hFwe*. This chain of events results in the upregulation of *hFwe-Lose* isoforms in fibroblasts and culminates with their elimination by cell competition with tumor cells.