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Manuscript Title:

A Transcriptomics-Based Computational Drug Repositioning Pipeline Identifies Simvastatin And Primaquine As Novel Therapeutics For Endometriosis Pain

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Keywords:

bioinformatics; endometriosis; therapeutics; transcriptomics; drug repositioning

Conflict-of-Interest Statement:

The authors have declared that no conflict of interest exists.

ABSTRACT

Introduction and Methods:

Endometriosis has limited treatment options, prompting the search for novel therapeutics. We previously used a transcriptomics-based computational drug repositioning pipeline to analyze public bulk transcriptomic data and identified several drug candidates. Fenoprofen, our top in silico candidate, was validated in a rat model. Building on this, we now evaluate two additional candidates, simvastatin (a cholesterol-lowering drug) and primaquine (an antimalarial), based on strong gene expression reversal scores and favorable safety profiles. Using a validated rat model of endometriosis and pain, we conducted behavioral testing, bulk RNA sequencing, and differential expression analysis to assess their therapeutic potential.

Results:

Of 299 drugs identified computationally, simvastatin and primaquine ranked highly for reversing gene expression signatures associated with endometriosis. In vivo validation using a rat model of endometriosis demonstrated that both drugs significantly reduced vaginal hyperalgesia, a surrogate marker of endometriosis-associated pain. RNA-seq of uteri and lesions confirmed reversal of disease-associated gene expression signatures following treatment.

Conclusion:

Simvastatin and primaquine attenuated pain behaviors and reversed endometriosis-related gene expression changes in an animal model. These findings highlight their potential as repurposed therapeutics for endometriosis-related pain and support the effectiveness of computational drug repositioning strategies in identifying new treatment strategies.

INTRODUCTION

Endometriosis is a pervasive, life-altering disease, with symptoms including severe dysmenorrhea, chronic pain, and infertility[1]. People with endometriosis can be undiagnosed for years, given non-specific symptoms that can overlap with other disorders and requirement for surgical confirmation of disease[1]. It has been suggested that 10% of reproductive-aged women have endometriosis[2], but this value may be an underestimate due to precise diagnostics requiring laparoscopy to identify and biopsy suspected endometriosis lesions. Approximately 12-32% of menstruating women who have surgery for pelvic pain and 50% who have infertility have been found to have endometriosis[3]. The disease is not only a significant public health issue, but it also has a large economic impact on health expenditures of almost $70 billion annually in the U.S[4].

Endometriosis is characterized by the growth of endometrial tissue outside of the uterus that responds to cyclical hormonal changes, similar to uterine endometrial tissue during a normal menstrual cycle[2]. Often, these lesions exist at extrauterine sites such as the pelvic peritoneum, ovaries, and bowel, where they elicit an inflammatory response, fibrosis, and pain[5]. Although endometriosis was identified over 100 years ago, this disease remains with limited medical treatment options[3]. As an estrogen-driven disease, hormonal therapies have been used to treat pain symptoms[2,6]. First-line treatments for dysmenorrhea and non-menstrual pelvic pain associated with endometriosis typically include estrogen-progestin and progestin-only contraceptives or GnRH-analogues drugs, as well as nonsteroidal anti-inflammatory drugs (NSAIDs)[3,6]. Unfortunately,

existing medical therapies for endometriosis-related pain are often ineffective, with individuals experiencing minimal or transient pain relief or intolerable side effects limiting long-term use[5]. This underscores the need for new drug treatment strategies with effective and non-hormonal therapies to control pain symptoms and to minimize local and systemic inflammation associated with pain and infertility. The development of new drugs for endometriosis has been challenging due to the heterogeneity amongst affected individuals, variable symptoms, and complex etiology, as well as historically lower investment in women's reproductive health conditions[7,8]. Additionally, drug development for endometriosis has been limited as it is both costly and time-intensive, often requiring millions of dollars and decades of work, for therapeutics to finally become commercially available[9].

Computational drug repositioning methods allow for the identification of new therapeutic applications for drugs already on the market in a fraction of the time and funds that it takes to develop and test entirely new drugs[10]. Our group developed a method that identifies potential therapeutics by identifying drugs with gene expression profiles that reverse those of a disease -- in other words, genes downregulated in the disease are upregulated by the drug, and vice versa[11]. This approach leverages transcriptomics data to generate genome-wide expression profiles from comparisons between disease and healthy samples or drug-treated and control cells[11]. In the past, this method has been successfully applied to identify both known and novel treatments for inflammatory bowel disease[12], dermatomyositis[13], liver cancer[14], preterm birth[15], and Alzheimer's disease[16]. Our recent work to identify therapeutic candidates for endometriosis leveraged publicly

available disease and drug gene-expression signatures in conjunction with a transcriptomics-based computational drug repositioning pipeline and validated the top candidate[17]. Specifically, we applied this in silico approach to endometriosis disease signatures that were unstratified and stratified by ASRM disease stage[18] (I-II and III-IV) and by menstrual cycle phase (proliferative, early secretory, and late secretory) and found 299 drugs that reversed the endometriosis disease signature[17]. Our top candidate drug fenoprofen, an uncommonly prescribed non-steroidal anti-inflammatory drug (NSAID) in the U.S., successfully alleviated vaginal hyperalgesia in a rat model of endometriosis[17].

In the current study, we selected two novel therapeutic candidates from our earlier drug repositioning work for further investigation -- the cholesterol-lowering drug simvastatin and the antimalarial agent primaquine, based on their strong reversal of the endometriosis disease signature and good safety profiles[19,20]. Statins and anti-malarial agents have previously been considered potential therapeutics for endometriosis[21–30]. Herein, we provide evidence for simvastatin and primaquine as potential endometriosis therapeutics. Specifically, we conducted behavioral studies using an established rat model of endometriosis to assess the effect of treatment with simvastatin and primaquine. Furthermore, we performed RNA sequencing on the uteri and lesions from these rats and analyzed differentially expressed genes (DEGs) for treated vs untreated (control) tissues.

RESULTS

Overview

An overview of our study is provided in Figure 1A. We previously leveraged a transcriptomics-based computational drug repositioning pipeline[11] using gene expression signatures of endometriosis queried against the CMap database[31], and identified fenoprofen as the top drug among 299 unique therapeutic candidates[17] (Table S1). Fenoprofen alleviated vaginal hyperalgesia comparably to our positive control ibuprofen in our rat endometriosis model[17]. Among the compounds of interest with the greatest disease-signature reversal, we selected two novel endometriosis therapeutic candidates for further evaluation in this current study. In our endometriosis animal study model, we assessed pain escape responses in animals who were treated with each compound and compared them to untreated animals. We then performed RNA sequencing of the uterus and endometriosis lesions to assess the gene expression from these tissues in the treated and untreated animals.

Computational Drug Repositioning Pipeline Identifies Primaquine and Simvastatin as Therapeutic Candidates for Endometriosis

Among 299 drugs identified from the unstratified and ASRM disease stage- and cycle phase-stratified signatures, the cholesterol-lowering drug simvastatin and antimalarial agent primaquine were novel endometriosis therapeutic candidates selected for further study based on criteria including their strong reversal scores, safety profiles, and

availability. When visualizing the gene expression of the six input endometriosis signatures (i.e., unstratified, and stratified by stage (I-II or III-IV) and phase (proliferative, early secretory, mid-secretory)) in comparison to the signature of simvastatin from CMap (Figure 1B), the overall reversal pattern can be observed. A similar overall pattern of reversal can be seen when visualizing the gene expression of the six input endometriosis signatures and primaquine (Figure 1C).

Primaquine and Simvastatin Attenuate Escape Responses in a Rat Model of Endometriosis

The effect of our candidate drugs on endometriosis-associated vaginal hyperalgesia, a surrogate marker for endometriosis-related pain, was assessed in a rat model of endometriosis.

Vehicle

Among rats that received endometriosis ("endo") surgery and vehicle treatment (n = 6), escape responses were significantly increased during the post-endo period compared to the baseline period, when volumes of 0.15, 0.30, 0.40, 0.55, 0.70, and 0.80 mL of water were delivered intra-vaginally (Mann–Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2B and S1)**.** During the post-treatment period for the vehicle treatment group, similar to the post-endo period, escape responses were significantly increased relative to the baseline period when volumes of 0.15, 0.30, 0.40, 0.55, 0.70,

and 0.80 mL of water were delivered (Mann–Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2B and S1). In the vehicle group, no statistically significant differences were found between the post-endo period and the post-treatment period escape responses for any volume of water delivered (Mann Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2B and S1).

Primaquine

Among rats that received endo surgery and primaquine treatment (40 mg/kg/day, p.o.) (n = 6), escape responses were significantly increased during the post-endo period compared to the baseline period, when volumes of 0.15, 0.30, 0.40, 0.55, 0.70, and 0.80 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2C and S1). During the post-treatment period, escape responses were significantly decreased compared to the post-endo period, when volumes of 0.15, 0.30, 0.40, 0.55, and 0.70 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2C and S1). Relative to the baseline period, post-treatment escape responses were significantly increased, when volumes of 0.30, 0.40, 0.55, and 0.70 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2C and S1).

Simvastatin

Among rats that received endo surgery and simvastatin treatment (40 mg/kg/day, p.o.) (n = 6), escape responses were significantly increased during the post-endo period

compared to the baseline period, when volumes of 0.15, 0.30, 0.40, 0.55, 0.70, and 0.80 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2D and S1). During the post-treatment period, escape responses were significantly decreased compared to the post-endo period when volumes of 0.15, 0.30, 0.40, 0.55, 0.70, and 0.80 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2D and S1). In the simvastatin group, no statistically significant differences were found between the baseline period and the post-treatment period escape responses for any volume of water delivered (Mann Whitney U test, Bonferroni-corrected p-value threshold of 0.05. Figures 2D and S1).

Comparison Across Groups

Overall, the pattern of escape responses for primaquine ("PRIMA") and simvastatin ("SIMVA") are similar to those previously shared for fenoprofen-treated ("FEN") and ibuprofen-treated ("IBU", positive control) rats[17] (Figures 2B and S1).

During the baseline period, there was a significant difference in escape responses between Control No endo Surgery ("CNS") and Vehicle ("VEH"), and CNS and PRIMA at 0.55mL, and between PRIMA and IBU at 0.7mL, otherwise no other significant differences were identified between the 6 animal study groups (Figure 3).

During the post-endo period, there were significant differences between the negative control CNS and the 5 other groups at volumes 0.15, 0.3, 0.4, 0.55, and 0.7 mL; and

between CNS and 3 other groups — VEH, IBU, and FEN — at 0.8mL No significant differences were identified between any of the 6 groups at 0.01 and 0.9mL (Figure 3).

During the post-treatment period, there were significant differences in escape responses between the negative control VEH and the other 4 drug-treatment groups — IBU, FEN, PRIMA, and SIMVA — at volumes 0.15, 0.3, 0.4, 0.55, 0.7 and 0.8 mL; however, no significant differences between the groups were identified at 0.01 or 0.9 mL. There was a significant difference between CNS and IBU at 0.55 mL; otherwise, there were no significant differences in escape responses between CNS and the 4 drug-treatment groups — IBU, FEN, PRIMA, and SIMVA. Between the drug-treatment groups, there were significant differences in escape responses between PRIMA and SIMVA at 0.3 mL, PRIMA and positive control IBU at volumes 0.4 and 0.55 mL, and FEN and positive control IBU at volume 0.55 mL; otherwise no other significant differences were identified between positive control IBU, FEN, PRIMA, and SIMVA (Figure 3).

RNA-Seq Analysis of Treated Animals Confirms Reversal of Disease Signatures (Figures 4 and 5)

To assess the impact of our candidate drugs on gene expression, bulk RNA sequencing was performed on the uterus and endometriosis-like “lesions” from rats (n = 6 per group per sample source).

Principal component analysis

Principal component analysis (PCA) revealed that samples from different treatment groups did not show distinct clustering (Figure 4A). Instead, the sample source (i.e., uterus or lesion) was the primary factor contributing to the differences between samples (Figure 4B). PCA was subsequently conducted within each sample source which still did not show distinct clustering based on treatment groups (Figure S2).

Differential gene expression

Differential gene expression analysis of the lesion and uterus samples between endometriosis rats with and without treatment revealed varying numbers of differentially expressed genes (DEGs) (Figures S3 and S4, Tables S2-S10). The comparison of the uterus samples from the wild-type (WT) rats and the rats that underwent endometriosis surgery and no drug treatment (i.e., vehicle) showed the gene expression patterns associated with the disease (Figure 4C, left). Further comparisons of the disease-associated gene expression patterns with those from drug-treated rats demonstrated that the four drugs -- primaquine and simvastatin, as well as fenoprofen and positive control ibuprofen -- effectively reversed the gene expression profile in the uterus (Figure 4C, middle), with significant negative correlations (Figure 4D, bottom right). However, no strong reversal was observed in the lesions (Figure 4C, right), with only weak negative correlations (Figure 4D, top left).

Gene set enrichment analysis

From gene set enrichment analysis, pathways enriched in the uterus before and after drug treatment were identified. *Cytokine-cytokine receptor interaction* and *osteoclast differentiation* were upregulated KEGG pathways in the uteri of endometriosis rats without drug treatment (i.e., vehicle), while *cytoskeleton in muscle cells* was a downregulated KEGG pathway. Simvastatin was able to reverse the *cytokine-cytokine receptor interaction* and *osteoclast differentiation* pathways in the uterus. Additionally, fenoprofen, simvastatin, and ibuprofen were able to reverse the *cytokine-cytokine receptor interaction* and *cytoskeleton in muscle cell pathways* in the lesion, although these effects were not strongly reflected at the overall gene expression level. (Figure 4E).

Network analysis

To explore the similarities between the rat model and humans, the rat endometriosis gene signatures were compared to the human signatures reported in previous work[17,32], resulting in the identification of 55 overlapping DEGs (Figure 5A). A hypergeometric test on rat DEGs with human homologs confirmed their overrepresentation in human signatures. The expression profiles of these overlapping genes in the uterus and the lesion samples with and without drug treatment are visualized in Figure 5B. Despite the foreseeable discrepancies in the expression profiles in humans and rats, several genes, such as Fos Proto-Oncogene (human *FOS*, rat *Fos)*, FosB Proto-Oncogene (human

FOSB, rat Fosb*)*, Interleukin 17C (Human *IL17C*, rat *Il17c)*, Nuclear Receptor Subfamily 4 Group A Member 1 (human *NR4A1*, rat *Nr4a1)*, Dual Specificity Phosphatase 1 (human *DUSP1*, rat *Dusp1)*, lymphocyte antigen 75 (human *LY75* or *DEC-205*, rat *Ly75*), and FRAS1 Related Extracellular Matrix 2 (human *FREM2*, rat *Frem2)* showed consistency across species. *FOS* and *FOSB*, members of the Fos gene family, encode Fos proteins which have previously been associated with endometriosis[33,34]. *NR4A1*[35,36] and *LY75*[37] have also been linked with endometriosis, whereas *IL17C*, *DUSP1*, and *FREM2* are more novel for this condition. Notably, the four drugs reversed or partially reversed the expression of these genes in the uterus of endometriosis rats (Figure 5B). To identify pathways associated with these 55 genes, we conducted a network analysis, revealing *cytokine-cytokine receptor interaction*, *IL-17 signaling*, and *MAPK signaling* as the primary pathways involved (Figure 5C).

DISCUSSION

Effective therapeutics for endometriosis are in great need; however, developing new drugs for endometriosis is challenging due to the disease's heterogeneity and complex symptoms, and the requirement of substantial financial and time commitments to bring new treatments to market[7,9]. Bioinformatics approaches can help identify existing drugs that can be repurposed to treat this condition[10]. Our work leveraging publicly available endometrial disease and drug gene-expression signatures and a transcriptomics-based computational drug repositioning pipeline identified several FDA-approved drugs (for

other diseases) including simvastatin and primaquine[17]. While statins have been considered for some time as a potential treatment for endometriosis[21–28], anti-malarial agents are more novel in this regard[29,30]. In vitro studies evaluating the effect of simvastatin on human endometrial stromal cell cultures have found that simvastatin inhibits cell growth in a concentration-dependent manner[23–25], and that lipid-soluble statins including simvastatin decrease stromal cell invasiveness[26,27]. A small clinical trial (n = 60) comparing treatment with simvastatin versus a gonadotropin-releasing hormone (GnRH) agonist in women who underwent laparoscopic surgery for pelvic endometriosis found significant reductions in dyspareunia, dysmenorrhea, and pelvic pain within both treatment groups over the 6-month postoperative period[28]. No statistically significant differences were observed between the two treatment groups[28]. Another study of hydroxychloroquine found that treatment with this anti-malarial in vitro reduced endometrial and endometriotic cell survival, and decreased the number of lesions in a mouse endometriosis model[29]. Another study of the anti-malarial chloroquine and MK2206, an inhibitor of the serine/threonine protein kinase Akt (protein kinase B), found that combination treatment more effectively inhibited deep endometriotic stromal cell growth and reduced implant size in a mouse endometriosis model than either drug alone[30].

We tested primaquine and simvastatin in a rat model of endometriosis, and found that treatment with each drug significantly alleviated vaginal hyperalgesia, a surrogate marker for endometriosis-associated pain, comparable to our prior findings with fenoprofen, the top drug candidate from our drug repositioning work, and with the

positive control ibuprofen[17]. There was a significant decrease in pain responses in the post-treatment period compared to the post-endo period. Furthermore, the degree to which simvastatin treatment attenuated the pain response was essentially the same as treatment with fenoprofen or ibuprofen, where there were no statistically significant differences between the baseline period and the post-treatment period[17]. In endometriosis rats with no treatment, vaginal hyperalgesia was maintained, confirming that the reduction in hyperalgesia seen in the treatment groups was not a result of additional vaginal nociceptive testing following the establishment of endometriosis. Overall, the findings from our current animal study work support our novel drug candidates, especially simvastatin, as therapeutics that could be used for endometriosis-associated pain.

The comparative analysis of gene expression patterns from our animal model showed that treatment with primaquine, simvastatin, fenoprofen, and ibuprofen effectively reversed the disease-associated profile in the uterus, with significant negative correlations; however, this reversal was not strong in the lesions, indicating that the transcriptional response to treatment varies between different tissues. Gene set enrichment analysis identified pathways affected in our endometriosis animal model, including *cytokine-cytokine receptor interaction* and *osteoclast differentiation* which were upregulated in the uterus, and *cytoskeleton in muscle cells* which was downregulated, and that simvastatin reversed the *cytokine-cytokine receptor interaction* and *osteoclast differentiation* pathways in the uterus, and fenoprofen, simvastatin, and

ibuprofen reversed the *cytokine-cytokine receptor interaction* and *cytoskeleton in muscle cell* pathways in the lesions.

Among 55 endometriosis disease-associated genes that were found in common between humans and the rat model, several including *Fos*, *Fosb*, *Nr4a1*, *and Ly75*, showed consistency in the direction of their expression across species signaling the critical role of these genes in disease pathology, and a pattern of reversal of expression after treatment by one or more of our drugs. *FOS* and *FOSB* are members of the Fos gene family, which encode Fos proteins, components of the AP-1 transcription factor complex that regulates key cellular processes such as proliferation, migration, and transformation. *FOS* has been found to be upregulated during endometriosis establishment in a baboon endometriosis model[33]. *FOS* gene and Fos protein expression were found to be higher in endometriotic tissue and eutopic endometrium in women with endometriosis relative to the eutopic endometrium of patients without endometriosis[34]. NR4A1 has been found to be overexpressed in endometriosis and NR4A1 antagonists inhibit the growth of endometriotic lesions[35,36]. Expression of *LY75*, involved in immune and inflammatory responses, has been found to be higher in patients with endometriosis compared to healthy control patients[37].

Network analysis identified pathways associated with the endometriosis disease-associated genes common across the two species, revealing *cytokine-cytokine receptor interaction*, *IL-17 signaling*, and *MAPK signaling* as primary pathways involved. *Cytokine-cytokine receptor interaction* and *IL-17 signaling* play important roles in host

immunity and inflammation, and have been considered among targets for immune-mediated inflammatory disorders such as rheumatoid arthritis[38]. *MAPK signaling* is crucial in regulating cellular processes such as proliferation, differentiation, development, transformation, and apoptosis.[39] Interestingly, these pathways have also previously been identified as enriched in studies of ectopic endometrium surrounding ovarian cysts compared to eutopic endometrium of patients with endometriosis (*cytokine-cytokine receptor interaction*, *IL-17 signaling*, and *MAPK signaling*)[40] and human endometrial endothelial cells derived from eutopic endometrium of patients with and without endometriosis (*cytokine-cytokine receptor interaction*)[41]. The enrichment of these pathways, particularly among conserved DEGs, suggests their critical role in endometriosis. Moreover, these pathways may indicate the mechanistic targets of candidate drugs. Endometriosis is characterized by the growth of endometrial tissue outside of the uterus and inflammation; thus, targeting these pathways could provide therapeutic strategies to manage the disease and alleviate its symptoms.

Limitations

Our study has several limitations. Transcriptomics from endometrium rather than endometriosis lesions was used to identify drug repositioning candidates. Future work should include signatures of the lesions themselves to query the drug data for therapeutic discovery. Drugs not represented in the CMap dataset (e.g., ibuprofen and GnRH antagonists) would be missed by the drug repurposing pipeline. Similarly, drugs present in CMap but excluded during preprocessing due to inconsistent expression

profiles also would not be identified[14]. The drug repurposing pipeline focuses on identifying drugs that significantly reverse the disease signature. This method does not necessarily consider whether the transcriptional effects of the drug are confined to the genes impacted by the disease. Therefore, a drug that induces extensive gene changes, including reversing the gene alterations caused by endometriosis, might be highlighted as a potential therapeutic option; however, such widespread gene changes may lead to unwanted side effects unrelated to the disease, which could limit the clinical usefulness of these drugs depending on the specificity and severity of the side effects. Moreover, reversal of disease signatures by our drug candidates was in the uteri, and the effects on fertility were not assessed. While we and others have extensively utilized the CMap dataset for therapeutic discovery in various non-cancer conditions,[12,13,15–17] it is important to note that the compounds in the CMap dataset were tested on cancer cell lines. The effects of these drugs on endometrial tissue would provide a more accurate assessment of their potential applications for treating endometriosis. Unfortunately, a large number of these compounds have not yet been tested on relevant endometrial tissues. However, as new datasets become available, we will incorporate them into our future drug discovery efforts for endometriosis. A further limitation of our study is that we assessed our potential endometriosis therapeutics in a rodent model. Although this model mimics several disease characteristics observed in women with endometriosis, rats do not menstruate or develop endometriosis naturally. Research involving menstruating non-human primates would provide a more suitable model since they develop endometriosis spontaneously; however, due to their close genetic relationship to humans, these models come with unique ethical considerations. To determine the

efficacy of our drug candidates in treating endometriosis, clinical trials would be necessary.

Conclusion

Our work demonstrates that simvastatin and primaquine, therapeutic candidates identified by our transcriptomics-based drug repositioning computational pipeline, effectively alleviate pain response in an animal model of endometriosis. Additionally, these drugs successfully reversed the disease signature at the gene expression level in this animal model. Simvastatin, in particular, shows promise as a potential therapeutic for endometriosis-related pain. The overlapping genes and pathways between our animal model and endometriosis patients offer insights into the biological processes associated with the disease and potential therapeutic targets. Our findings highlight the value of computational approaches in identifying therapeutic strategies for endometriosis.

METHODS

Computational Drug Repositioning

In previous work[17], our computational drug repositioning pipeline was applied to bulk transcriptomic data, which consisted of 105 samples from eutopic endometrial tissues of women with and without endometriosis. On the drug side, the Connectivity Map (CMap) dataset was used to obtain gene expression profiles from cell lines treated with existing small-molecule drugs. Through this approach, potential therapeutics were identified based on reversal of endometriosis gene expression signatures that were unstratified as well as stratified by ASRM disease stage (ASRM stages I/II and III/IV) and menstrual cycle phase (proliferative, early secretory, and mid-secretory phases) as described in Bunis et al. [32]. As a proof of principle, the top therapeutic candidate fenoprofen, an NSAID infrequently prescribed for endometriosis, was validated in an animal model of endometriosis.

In this study, among the 299 unique drug candidates that were previously identified, those among the top-ranked (i.e., among the top 10%) were evaluated for further consideration, which included primaquine and simvastatin. Factors such as safety profiles, availability (i.e., on the World Health Organization list of essential medicines[42]), and ease of administration (e.g., does not require intravenous administration) were taken into consideration.

Animal Study Design

The main goal of the animal study was to explore the effects of simvastatin and primaquine treatment on pain-related behavior in a rat model of endometriosis. Initially, baseline behavioral assessments were performed on all animals, including monitoring

estrous cycles, and establishing pain thresholds. Following this, endometriosis was surgically induced in rats, and treatment with simvastatin, primaquine, or vehicle (negative control) was administered over a 4-week period. Behavioral assessments of vaginal nociception were conducted post-endometriosis and post-treatment to determine how these treatments impacted pain-associated behaviors. Groups of six rats were allocated to each treatment group, and the study employed a within-subject design to allow comparisons across different phases (baseline, post-endometriosis induction, and post-treatment). All behavioral training and testing were performed 3–8 hours after lights were turned on. These sessions were run 3 times/week on non-consecutive days for a total of 3–4 sessions per week.

Animal Subjects and Conditions

A total of 18 adult virgin female Sprague-Dawley rats were used in this study, each weighing between 175 and 225 grams at the onset of the study, and were sourced from Charles River Laboratory (Wilmington, MA; Raleigh, NC facility). The animals were housed individually in standard rodent cages with access to water and chow ad libitum. They were kept under controlled conditions with a 12-hour light/dark cycle (lights on at 07:00), and the room temperature was maintained at ~22°C. Estrous cycle stages were monitored and documented daily 2 hours after lights on via vaginal lavage.

Endometriosis Induction

Endometriosis was surgically induced in rats as described by Vernon and Wilson. Animals were anesthetized with a combination of ketamine (73 mg/kg) and xylazine (8.8 mg/kg), and a midline abdominal incision was made. A 1 cm segment of the left uterine

horn with surrounding fat was excised and transferred to sterile saline. This tissue was cut into four 2x2 mm fragments and sutured onto the mesenteric arteries supplying the small intestine. After the surgical procedure, the incision was closed, and the rats were closely monitored during recovery. The operation had no complications and estrous cycles resumed within a few days.

Drug Administration

The experimental groups were treated with one of three conditions: simvastatin (40 mg/kg/day) (n=6), primaquine (40 mg/kg/day) (n=6), or a vehicle solution (control) (n=6). Treatments were administered via daily oral gavage for a 4-week period following the induction of endometriosis. Simvastatin and primaquine were selected based on their potential anti-inflammatory and analgesic properties, respectively. The vehicle control group received the same volume of vehicle solution but no active drug.

Assessment of Vaginal Nociception

Vaginal nociception was assessed using a behavioral test where rats were exposed to vaginal distention through the inflation of a latex balloon inserted into the vaginal canal. The primary endpoint measured was the percentage of successful escape responses, with animals trained to break a light beam by extending their heads into a tube when they experienced discomfort from the distension. This procedure was repeated at eight different balloon volumes three times each in random order to assess the threshold at which nociceptive responses (escape response) were evoked.

Behavioral Testing Apparatus

The testing apparatus was a rectangular Plexiglas chamber equipped with a grid floor, which restricted the rat from turning around. A hollow tube containing light-emitting diodes and a photosensor extended from the front of the chamber. When the rat extended its head, it interrupted the light beam, resulting in the cessation of the stimulus and the provision of peanut butter as a reward by the experimenters. This action, referred to as an escape response, was used to measure behavioral responses to the vaginal distention.

An opening in the rear of the chamber allowed the balloon-tipped catheter to be connected to the computer-controlled and automated stimulus-delivery device. The latex balloon (10 mm long, 1.5 mm wide when uninflated) was lubricated with K-Y® jelly and inserted into the vaginal canal of the rat before each session. It was then inflated to different volumes through the computer-controlled pump to induce vaginal distention at 60-second intervals, with the pressure produced by each volume monitored via a small-volume Cobe pressure transducer and the escape response to each volume recorded.

Behavioral Training

Training began by acclimating the rats to the testing chamber for 10 minutes daily for 3–4 days, during which small amounts of peanut butter were provided on a wooden stick. The rats were then trained by using tail pinches with padded forceps, which prompted them to extend their head into the tube to break the light beam. Once the light beam was broken, the tail pinch was released to reinforce the "escape response," and the rats were rewarded with peanut butter if they successfully interrupted the light beam to escape the tail pinch. This process was repeated for 10 tail pinches at 1-minute

intervals over 3 training sessions each week on non-consecutive days. Training was completed (>80% escape behavior) in 4–8 sessions.

The rats were next trained to make identical escape responses to deflate vaginal distention stimuli. These sessions were run 3 times/week on non-consecutive days for a total of 3–5 sessions. Ten large distention volumes (0.80 ml – 1.0 ml, inflation rate 1 ml/s) were delivered for a maximum of 15 s at 1-min intervals. All rats showed some behavioral response to these stimuli, which allowed the experimenter to use deflation of the vaginal balloon to shape the rat's escape responses. All rats learned the escape response within 2–4 sessions. Once trained, testing sessions began.

Experimental Procedure

Behavioral testing was conducted across three phases: baseline (prior to endometriosis induction), post-endo (after endometriosis induction but before treatment), and post-treatment (after drug/control administration). In each phase, rats underwent a series of 24 trials (8 different balloon inflation volumes delivered 3 times each). Each trial involved inflating the balloon at a constant rate of 1 mL/s and maintaining the inflation for up to 15 seconds unless an escape response occurred. The balloon was then deflated at 0.5 mL/s if an escape response occurred or if 15 seconds elapsed. The percentage of successful escape responses was recorded for analysis.

RNA Seq Experimental

RNAs were extracted using miRNeasy mini Kit (Qiagen), and 1.5ug of total RNA was used for library construction. The libraries for RNA sequencing were prepared using the

Illumina Stranded Total RNA Prep with Ribo-Zero™ Plus kit. We sequenced libraries using Novaseq X-Plus (Illumina), paired-end, 100 reads up to 50M paired reads per sample.

Statistics

Animal Study

For each rat group (VEH: with endo surgery, vehicle treatment (vehicle control) (n=6); (SIMVA: with endo surgery, treated with simvastatin (n=6); PRIMA: with endo surgery, treated with primaquine (n=6), a non-parametric test was performed to compare within groups, the escape responses of the 3 testing periods: baseline (24 data points/rat), post-endo surgery (64 data points/rat), and post-treatment (32 data points/rat). Mann Whitney U (MWU) tests were performed to compare the escape responses of (a) the baseline period and the post-endo period, (b) the post-endo period and the post-treatment period, and (c) the baseline period and the post-treatment period at each volume of water (0.0, 0.15, 0.30, 0.40, 0.55, 0.70, 0.80, and 0.90 mL) delivered to the balloon placed within the mid-vaginal canal of the rats. Moreover, Mann Whitney U (MWU) tests were performed to compare between groups, the escape responses of (a) VEH vs. SIMVA, (b) VEH vs. PRIMA, and (c) SIMVA vs. PRIMA for each of the three condition periods (the baseline period, the post-endo period, and post-treatment period) at each volume of water (0.01, 0.15, 0.30, 0.40, 0.55, 0.70, 0.80, and 0.90 mL) delivered to the balloon placed within the mid-vaginal canal of the rats. Escape responses reflect the percentage of times rats extended their head into the tube to interrupt the light beam to terminate the stimulus (balloon deflates). A significance threshold of 0.05 was applied

to Bonferroni–corrected p-values. Data are presented as median and interquartile range (IQR).

RNA Seq

Adapter sequences in the raw read files were trimmed using cutadapt (v4.9) with an error rate of 0.1, minimum overlap of 3 bps, minimum 3' base quality score of 25, and minimum length after trimming of 55 bps. We also set to remove poly-A tails and ploy-G with lengths over 20 bps. The trimmed reads were mapped to Rattus Norvegicus reference genome GRCr8 with exon and splice site information using Hisat2 (v2.2.0). The SAM files were converted to BAM files and sorted using samtools (v1.21). The gene counts were summarized by featureCounts (v2.0.6).

For the dimensionality reduction, gene counts were $\log_2$ transformed and the top 3000 most variable genes based on their standard deviation were used to perform principal component analysis (PCA).

The differential gene expression analysis comparing treatment groups was performed using the edgeR package (v4.4.1) in R 4.4.2. We first excluded genes that were uncharacterized from further analysis. Low-expressed genes were dropped using the filterByExpr() function based on the treatment factors. After filtering, we recalculated the library sizes. To account for sample-specific effects, we applied the trimmed mean of

M-values (TMM) normalization. We then estimated the dispersions for all genes and fitted a negative binomial generalized log-linear model to the gene counts. This model included an interaction term between the sample source and treatment, with the RNA Integrity Number (RIN) included as a covariate. The differential expression of genes between treatment groups within each sample source was tested with genewise quasi F-tests for the coefficient contrasts of the model. P-values were subjected to the Benjamini-Hochberg (BH) method to control the false positive rate. A Benjamini-Hochberg (BH)-adjusted p-value threshold of 0.05 was used to determine significance. Gene set enrichment analysis and over-representation analysis were performed using the clusterProfiler package (version 4.14.4). Pearson correlation coefficients were calculated to assess gene expression correlations between treatment groups. The overlap between human disease signatures and rat differentially expressed genes was evaluated using the hypergeometric test.

Study Approval

The study and procedures were approved by the Emory University Institutional Animal Care and Use Committee (IACUC) as protocol #2021000201. All laboratory animal experimentation adhered to the NIH Guide for the Care and Use of Laboratory Animals.

Data availability

The animal study RNA seq data are available through the Gene Expression Omnibus (GEO), accession ID GSE296883 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE296883)

AUTHOR CONTRIBUTIONS

Designing research studies: TTO, XT, LCG, SLM, MS. Conducting experiments: EA, AG, AL, SLM. Acquiring data: EA, AG, AL, SLM. Analyzing data: TTO, XT, EA, AG, AL, SLM. Writing manuscript: TTO, XT, EA, AG, FA, SLM. Authorship was determined by the definitions of the International Committee of Medical Journal Editors (ICJME). Authorship order was determined by the relative contribution of each author to the research and manuscript.

ACKNOWLEDGMENTS

The work was in part supported by NIH NICHD P01 HD106414 (T.T.O., A.B., J.C.I., B.G., D.K.S., L.C.G., S.L.M., M.S.), NIH NICHD P50 HD055764 (A.B., J.C.I., L.C.G., M.S.), and NIH NICHD R00 HD093858 (S.L.M), as well as by the March of Dimes Prematurity Research Center at UCSF (T.T.O., M.S.), the March of Dimes Prematurity Research Center at Stanford University (B.G., D.K.S.), and the Stanford Maternal and Child Health Research Institute (B.G., D.K.S.).

This study was also supported in part by the Emory Integrated Genomics Core (EIGC) (RRID:SCR_023529), which is subsidized by the Emory University School of Medicine and is one of the Emory Integrated Core Facilities. Additional support was provided by the Georgia Clinical & Translational Science Alliance of the National Institutes of Health under Award Number UL1TR002378. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institutes of Health.

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FIGURES

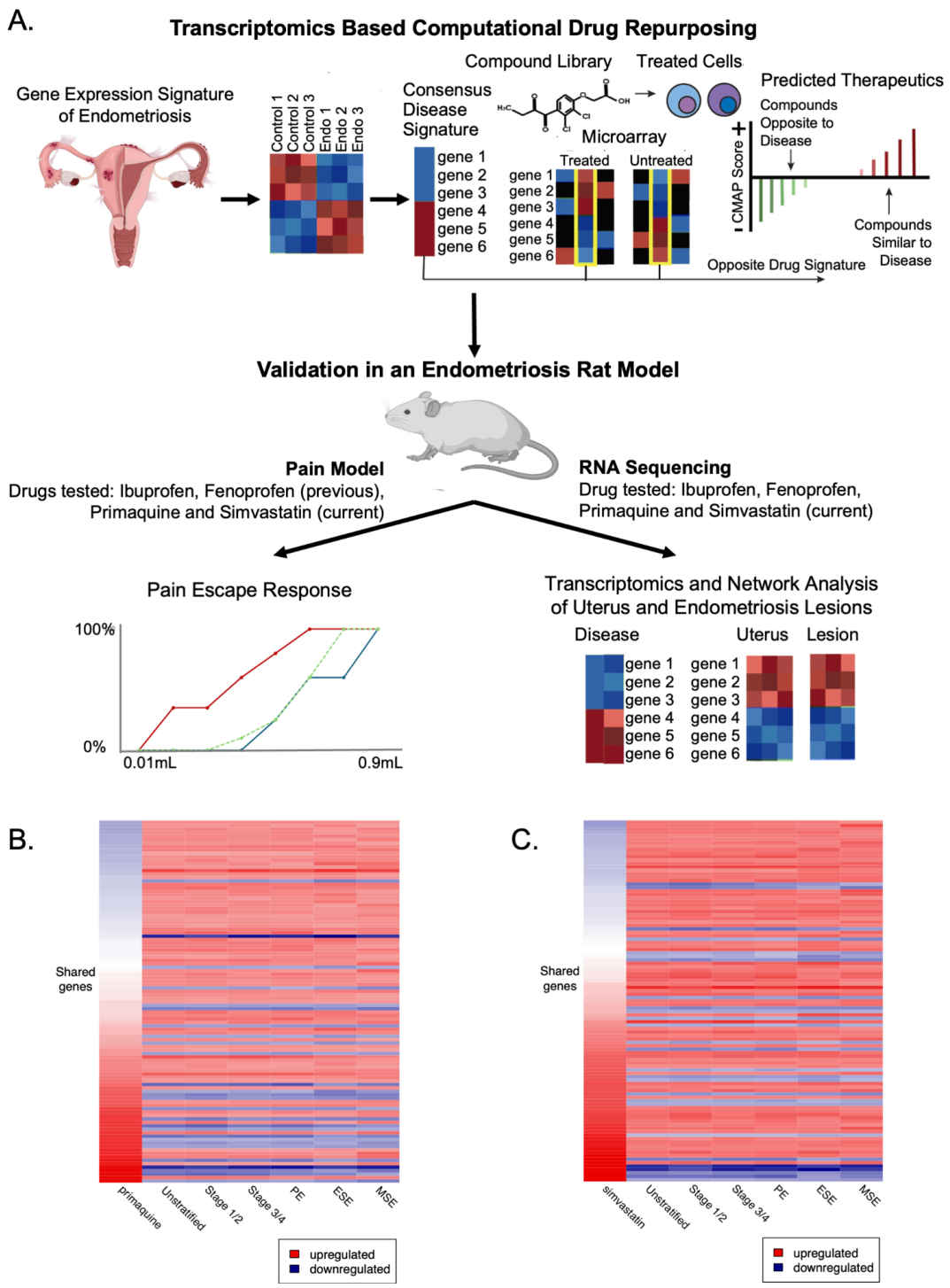
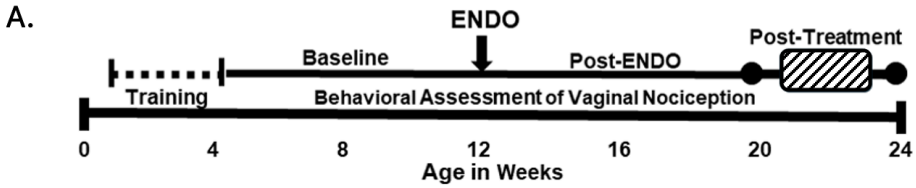


Figure 1. Study overview and disease signature reversal by primaquine and simvastatin. (A) Overview of the study that identified therapeutic candidates through a transcriptomics-based computational drug repositioning pipeline and then validated drug candidates in a rat model of endometriosis. Bulk RNA-sequencing was conducted of the uterus and endometriosis lesions from the rats, and differentially expressed genes and enriched pathways were analyzed. **(B,C)** Heatmap showing **(B)** primaquine and **(C)** simvastatin drug signature vs. unstratified and stratified endometriosis disease signatures.



B.

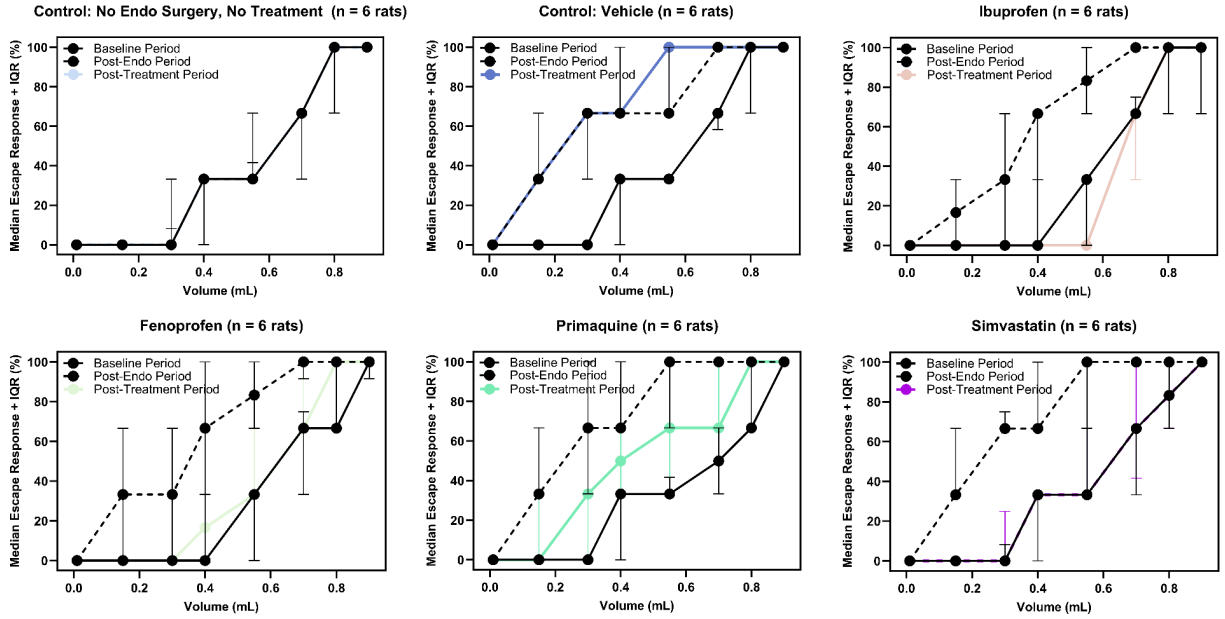


Figure 2. Endometriosis pain-associated escape behaviors in a rat model. (A) Female Sprague Dawley rats (n=18) were trained over 4 weeks to perform an escape response to terminate a noxious vaginal stimulus (water-filled balloon). Vaginal nociception was assessed as % escape response to varying balloon volumes in 1-hour sessions, 3 times/week, over 24 weeks. Each session included 8 volumes (0.01–0.90 mL), each tested 3 times in randomized, blinded order. Responses to all 3 trials of a volume were counted as 100% response. Nociception was measured at Baseline (8 weeks), after endometriosis induction (Post-Endo, 8 weeks), and during a 4-week treatment period. **(B)** Animal model median escape response (%) with interquartile range (IQR; error bars)(y-axis) for each delivered volume (0.01, 0.15,0.30, 0.40, 0.55, 0.70, 0.80, and 0.90 mL)(x-axis) during the baseline, post-endo surgery, and post-treatment periods for the 6 animal study groups; Control No endo Surgery; Vehicle; Ibuprofen; Fenoprofen; Primaquine; and Simvastatin. (Note: Control No endo Surgery, Ibuprofen, and Fenoprofen escape responses results are from previous work.[17])

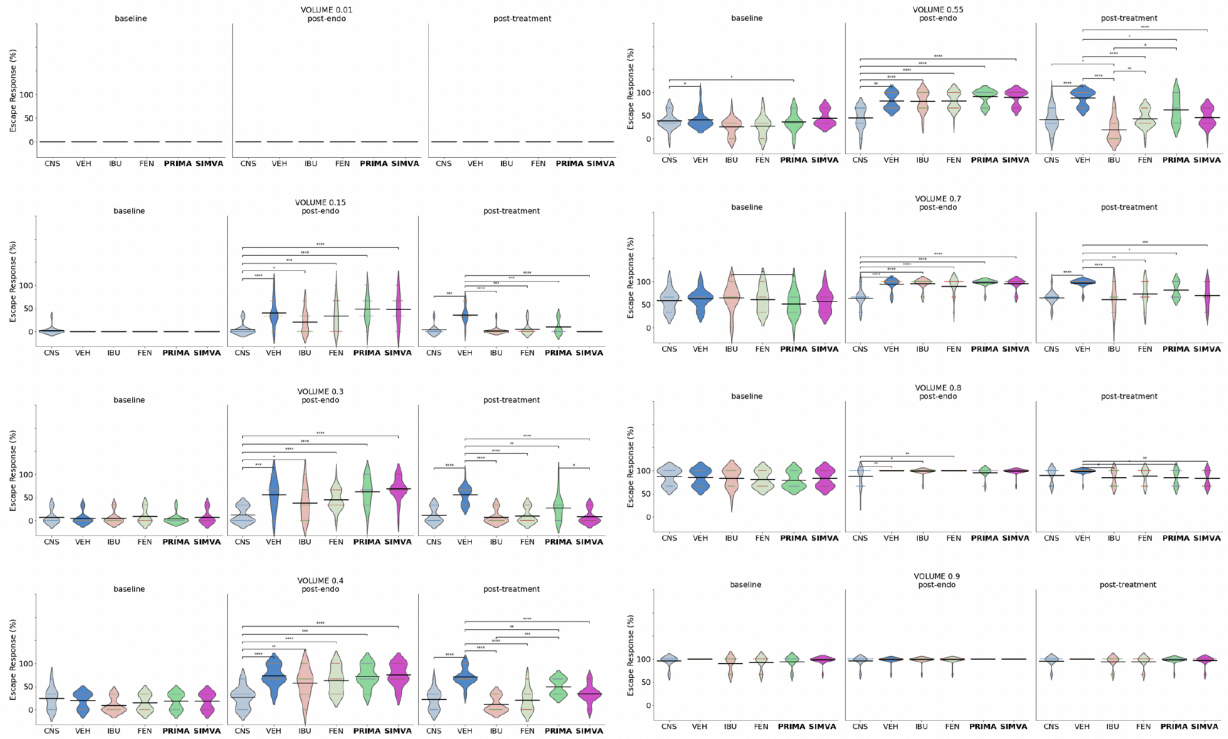


Figure 3. Strip and Violin plots with Medians and Significance Bars of Escape Responses to vaginal balloon distention at volumes of 0.01, 0.15, 0.30, 0.40, 0.55, 0.70, 0.80, and 0.90 mL for at baseline (Baseline), post-endometriosis (Post-Endo), and post-treatment (Post-Treatment) periods in a rat endometriosis model comparing 6 animal study groups — Control No Surgery ("CNS"), Vehicle ("VEH"), Ibuprofen ("IBU"), Fenoprofen ("FEN"), Primaquine ("PRIMA"), Simvastatin ("SIMVA"). Mann-Whitney test; Bonferonni adjusted p-values from Mann-Whitney U comparisons of groups. (Note: CNS, FEN, and IBU escape responses results are from previous work.[17])

p-value annotation legend:
ns: 5.00e-02 < p <= 1.00e+00
*: 1.00e-02 < p <= 5.00e-02
**: 1.00e-03 < p <= 1.00e-02
***: 1.00e-04 < p <= 1.00e-03
****: p <= 1.00e-04

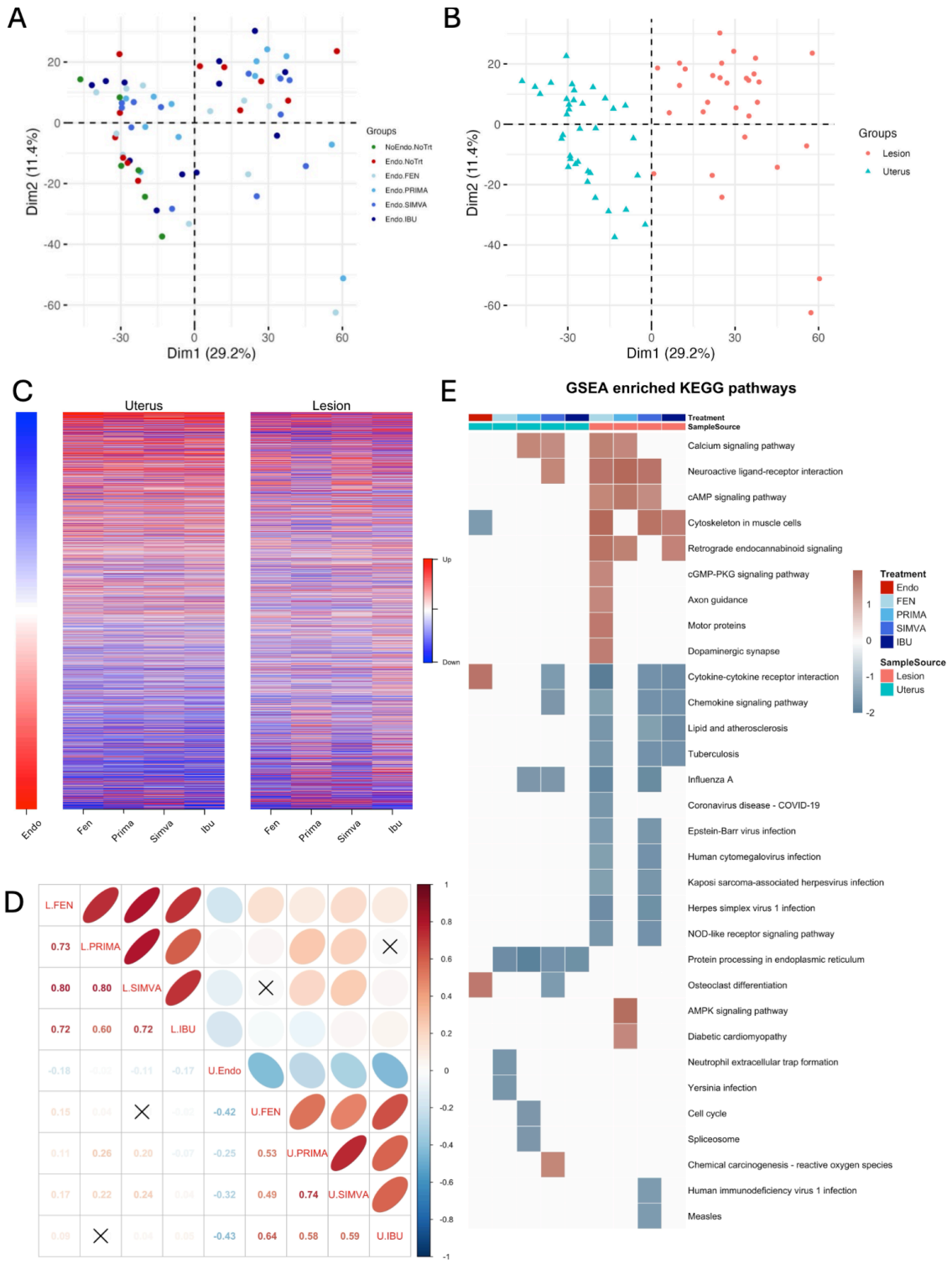


Figure 4. RNA-Seq analysis of treated animals. Principal component analysis (PCA) of all animal study samples, with each dot representing a sample, colored **(A)** by treatment group or **(B)** by sample source. **(C)** Heatmap of disease-associated and treatment-associated gene signatures (Left: vehicle-treated uterus samples. Middle: drug-treated uterus samples. Right: lesion samples). Red indicates up-regulated genes and blue indicates downregulated genes. **(D)** Pearson correlation analysis between disease-induced ("U.Endo") and drug-induced gene expression changes. Red indicates positive correlations and blue indicates negative correlations. **(E)** Gene Set Enrichment Analysis (GSEA) of KEGG pathways in disease and drug-treated groups. Red indicates up-regulated pathways and blue indicates downregulated pathways.

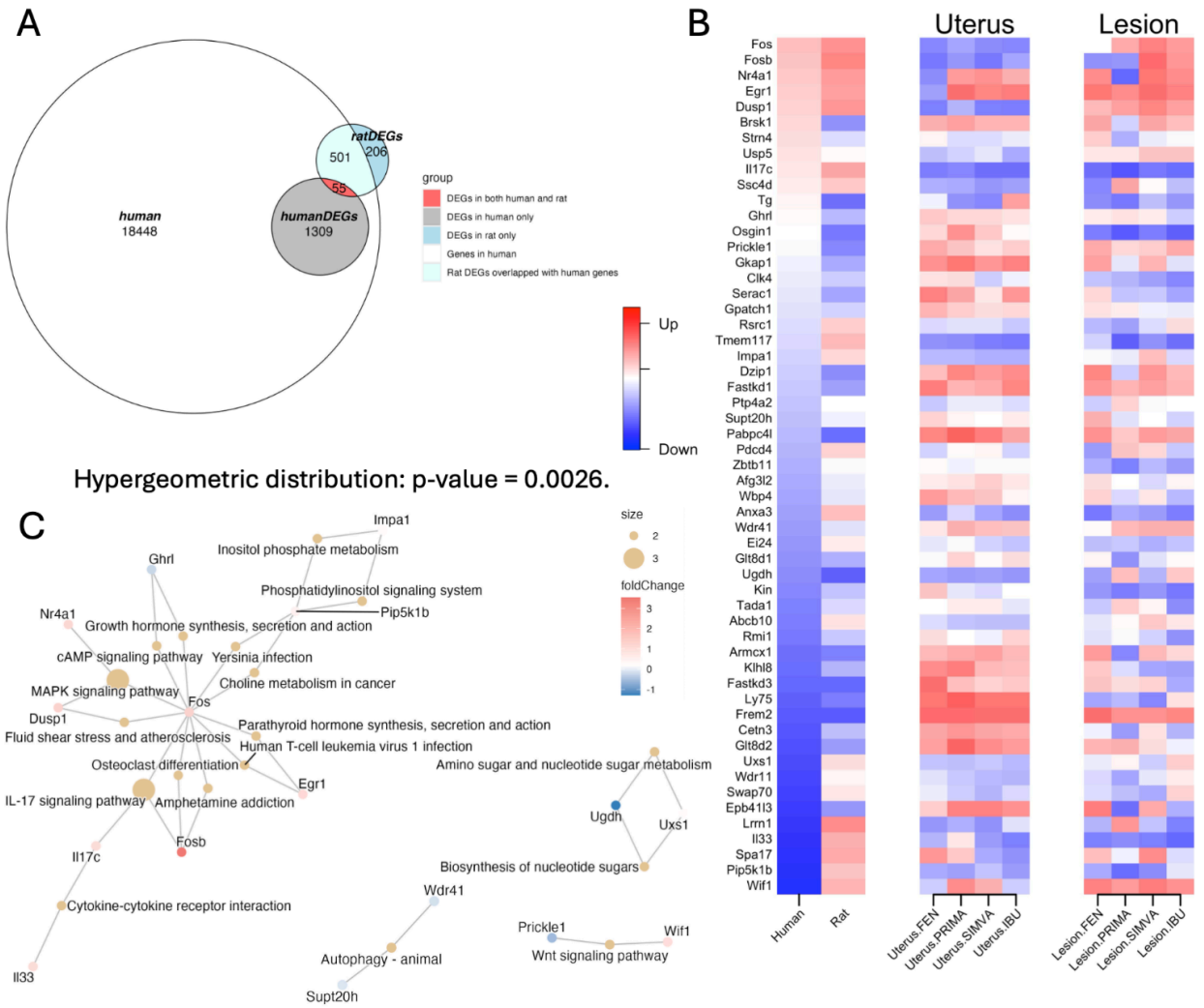


Figure 5. Correlation with human data. (A) Venn Diagram, **(B)** Heatmap, and **(C)** Network analysis to identify pathways associated with the 55 differentially expressed genes (DEGs) that overlap between our human and rat transcriptomics analysis, and their networks.

SUPPLEMENTARY FIGURES

Volume (mL)	Baseline period (BL) median	BL IQR	Post-Endo period (PE) median	PE IQR	Post-Treatment period (PT) median	PT IQR	BL vs PE period MWU test Bonferroni-adjusted p-values	PE vs PT period MWU test Bonferroni-adjusted p-values	BL vs PT period MWU test Bonferroni-adjusted p-values
CONTROL NO SURGERY (Negative control)									
0.01	0	(0-0)	0	(0-0)	0	(0-0)	1	1	1
0.15	0	(0-0)	0	(0-0)	0	(0-0)	1	1	1
0.30	0	(0-0)	0	(0-33.3)	0	(0-33.3)	1	1	1
0.40	33.3	(0-33.3)	33.3	(0-33.3)	33.3	(0-33.3)	1	1	1
0.55	33.3	(33.3-33.3)	33.3	(33.3-66.6)	33.3	(33.3-66.6)	1	1	1
0.70	66.6	(33.3-66.6)	66.6	(66.6-66.6)	66.6	(66.6-66.6)	1	1	1
0.80	100	(66.6-100)	100	(66.6-100)	100	(66.6-100)	1	1	1
0.90	100	(100-100)	100	(100-100)	100	(100-100)	1	1	1
VEHICLE (Negative control)									
0.01	0	(0-0)	0	(0-0)	0	(0-0)	1	1	1
0.15	0	(0-0)	33.3	(33.3-66.6)	33.3	(33.3-33.3)	1.7E-05 ****	1	1.1E-05 ****
0.30	0	(0-33.3)	66.6	(33.3-66.6)	66.6	(33.3-66.6)	9.8E-06 ****	1	7.0E-06 ****
0.40	33.3	(0-33.3)	66.6	(66.6-100)	66.6	(66.6-66.6)	4.1E-08 ****	1	1.1E-06 ****
0.55	33.3	(33.3-41.6)	100	(66.6-100)	100	(75.0-100)	1.7E-11 ****	1	4.8E-09 ****
0.70	66.6	(58.3-75.0)	100	(100-100)	100	(100-100)	4.3E-06 ****	1	2.3E-05 ****
0.80	100	(66.6-100)	100	(100-100)	100	(100-100)	6.1E-05 ****	1	0.02 *
0.90	100	(100-100)	100	(100-100)	100	(100-100)	1	1	1
IBUPROFEN (Positive control)									
0.01	0	(0-0)	0	(0-0)	0	(0-0)	1	1	1
0.15	0	(0-0)	16.65	(0-33.3)	0	(0-0)	3.5E-03 **	2.3E-03 **	1
0.30	0	(0-0)	33.3	(0-66.6)	0	(0-0)	2.0E-03 **	9.7E-04 **	1
0.40	0	(0-25)	66.6	(33.3-66.6)	0	(0-33.3)	1.5E-05 ****	2.9E-06 ****	1
0.55	33.3	(8.3-33.3)	83.3	(66.6-100)	0	(0-33.3)	2.5E-07 ****	3.4E-08 ****	1
0.70	66.6	(66.6-66.6)	100	(100-100)	66.6	(33.3-66.6)	5.8E-05 ****	5.1E-06 ****	1
0.80	100	(66.6-100)	100	(100-100)	100	(66.6-100)	3.9E-03 **	1.8E-03 **	1
0.90	100	(74.9-100)	100	(100-100)	100	(100-100)	0.12	0.8	1
FENOPROFEN									
0.01	0	(0-0)	0	(0-0)	0	(0-0)	1	1	1
0.15	0	(0-0)	33.3	(0-66.6)	0	(0-0)	2.7E-04 ***	2.3E-03 **	0.61
0.30	0	(0-25)	33.3	(33.3-66.6)	0	(0-33.3)	2.0E-05 ****	3.0E-06 ****	1
0.40	0	(0-33.3)	66.6	(33.3-91.7)	16.65	(0-33.3)	4.0E-06 ****	1.8E-05 ****	1
0.55	33.3	(8.3-33.3)	83.3	(66.6-100)	33.3	(33.3-66.6)	2.7E-07 ****	7.9E-07 ****	0.16
0.70	66.6	(33.3-66.6)	100	(100-100)	66.6	(66.6-100)	1.5E-03 **	4.4E-02 *	0.97
0.80	66.6	(66.6-100)	100	(100-100)	100	(66.6-100)	4.8E-05 ****	5.8E-03 *	1
0.90	100	(100-100)	100	(100-100)	100	(100-100)	0.34	0.8	1
PRIMAQUINE									
0.01	0	(0-0)		(0-0)	0	(0-0)	1	1	1
0.15	0	(0-0)	33.3	(0-66.6)	0	(0-33.3)	3.7E-06 ****	1.4E-03 **	0.24
0.30	0	(0-0)	50	(33.3-66.6)	33.3	(0-33.3)	1.2E-07 ****	1.7E-02 *	2.6E-03 **
0.40	33.3	(0-33.3)	66.6	(41.6-100)	50	(33.3-66.6)	4.5E-09 ****	3.3E-02 *	1.6E-05 ****
0.55	33.3	(33.3-41.6)	100	(66.6-100)	66.6	(33.3-100)	3.4E-10 ****	1.0E-03 **	0.03 *
0.70	50	(33.3-66.6)	100	(100-100)	66.6	(66.6-100)	6.5E-09 ****	5.8E-03 *	9.8E-04 **
0.80	66.6	(66.6-100)	100	(100-100)	100	(66.6-100)	6.2E-03 *	9.3E-02	1
0.90	100	(100-100)	100	(100-100)	100	(100-100)	0.47	1	1
SIMVASTATIN									
0.01	0	(0-0)	0	(0-0)	0	(0-0)	1	1	1
0.15	0	(0-0)	33.3	(33.3-66.6)	0	(0-0)	6.8E-08 ****	1.7E-09 ****	1
0.30	0	(0-8.3)	66.6	(41.6-66.6)	0	(0-25.0)	2.0E-11 ****	1.0E-12 ****	1
0.40	33.3	(0-33.3)	66.6	(66.6-100)	33.3	(33.3-33.3)	6.0E-11 ****	3.9E-08 ****	0.08
0.55	33.3	(33.3-66.6)	100	(66.6-100)	33.3	(33.3-66.6)	5.4E-09 ****	1.1E-07 ****	1
0.70	66.6	(33.3-66.6)	100	(100-100)	66.6	(41.6-100)	5.0E-07 ****	2.3E-04 ***	1
0.80	83.3	(66.6-100)	100	(100-100)	83.3	(66.6-100)	9.0E-05 ****	1.5E-05 ****	1
0.90	100	(100-100)	100	(100-100)	100	(100-100)	1	1	1

Figure S1: Endometriosis animal model escape responses. Median escape response with interquartile range (IQR) for each delivered volume (0.01, 0.15,0.30, 0.40, 0.55, 0.70, 0.80, and 0.90 mL) during the baseline, post-endo surgery, and post-treatment periods, with Bonferroni-corrected p-values from Mann-Whitney U test for baseline period vs post-endo surgery period, post-endo surgery vs post-treatment period, and baseline period vs post-treatment period (* denotes <0.05, ** denotes <0.005, *** denotes <0.0005, and **** denotes <0.0001). Endo rats (n=6/group) vaginal nociception assessed. Treated with vehicle (negative control), primaquine, simvastatin, fenoprofen, or ibuprofen (positive control). Note: Fenoprofen and ibuprofen data are from prior work[17]

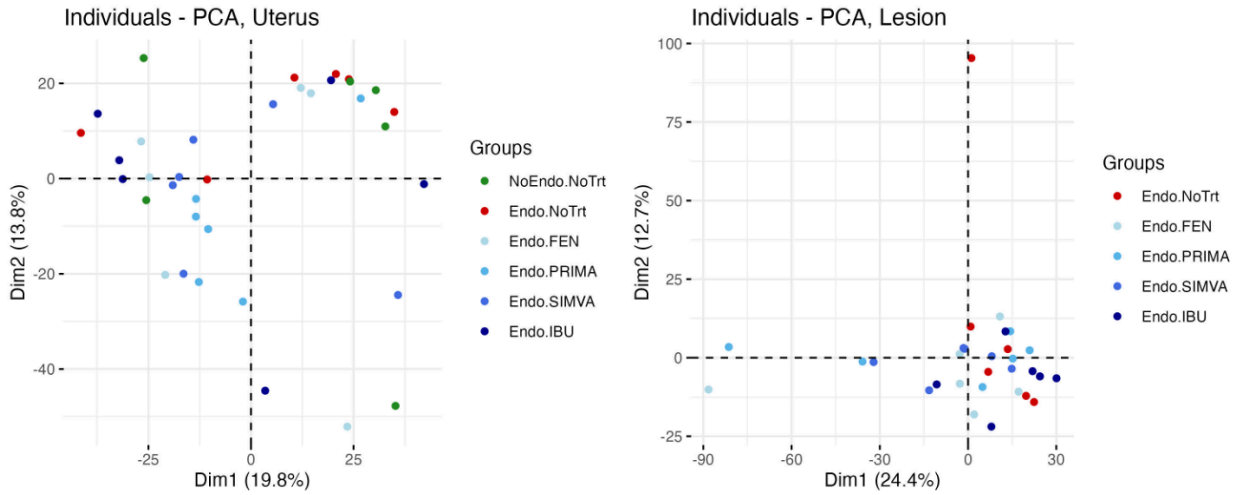


Figure S2. **Principal component analysis** (PCA) for each sample source -- uterus (left) and lesion (right). Each dot represents a sample

Lesion	FEN	PRIMA	SIMVA	IBU
Total Genes	17504	17504	17504	17504
DEG P<0.05	1621	1496	974	1073
DEG FDR<0.05	190	233	105	116

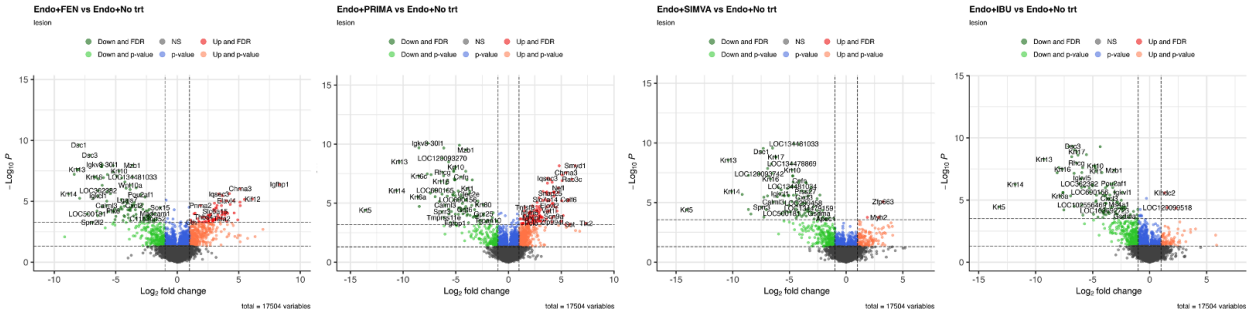
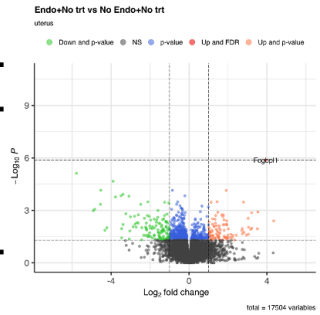


Figure S3. Volcano plots of individual differentially expressed genes (DEGs) in lesion samples by treatment group. The x-axis reflects the negative (-) $\log_{10}$ of the Benjamini-Hochberg (BH)-adjusted p-value, and the y-axis reflects the $\log_2$ fold change.

Uterus	Endo	FEN	PRIMA	SIMVA	IBU
Total Genes	17504	17504	17504	17504	17504
DEG P<0.05	762	1566	3481	1983	1420
DEG FDR<0.05	1	19	168	2	0



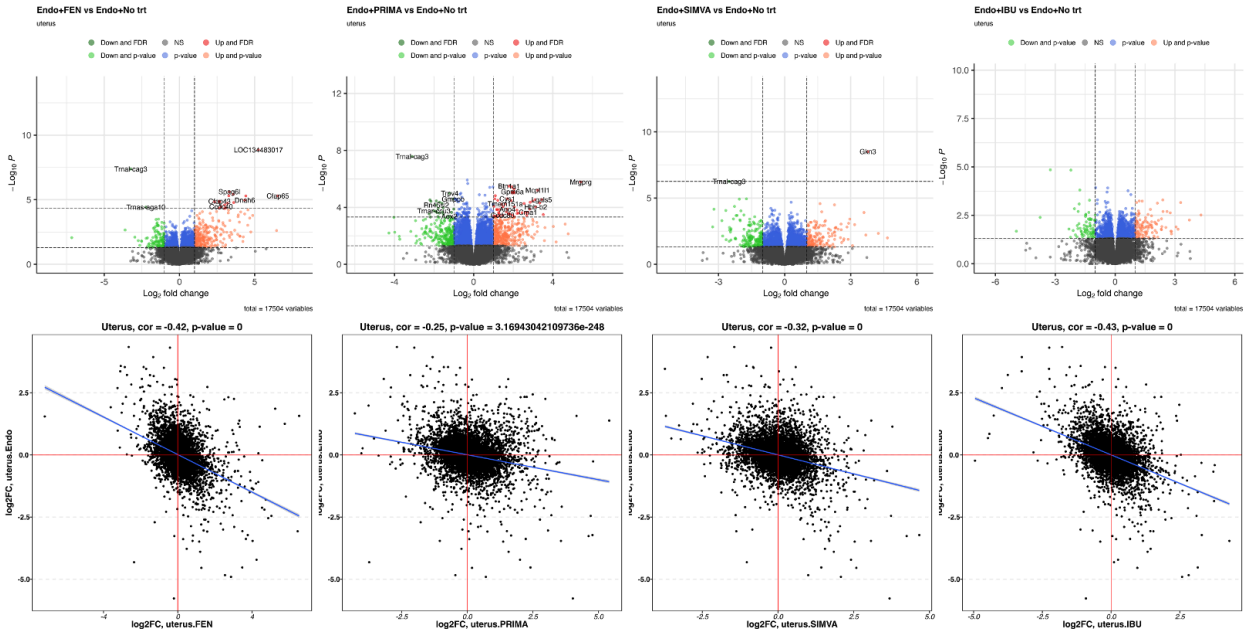


Figure S4. Volcano plots of individual differentially expressed genes (DEGs) in uterus samples by treatment group. The x-axis reflects the negative (-) $\log_{10}$ of the Benjamini-Hochberg (BH)-adjusted p-value, and the y-axis reflects the $\log_2$ fold change.

SUPPLEMENTARY TABLES

Table S1. Drug candidates identified via computational drug repositioning pipeline using unstratified and stratified bulk transcriptomic signatures, https://ars.els-cdn.com/content/image/1-s2.0-S2589004224006096-mmc3.xlsx

	exp_id	cmap_score	p	q	subset_comp	analysis_id	name	concentratio	duration	cell_line	array_platfo	vehicle	vendor	vendor_catal	vendor_catal	drug_concep	cas_number	DrugBank.ID
1	3279	-0.7654613	0	0	unstratified_	cmap	fenoprofen	7.20E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	754	Fenoprofen (	C0015837	53746-45-5	DB00573
2	3277	-0.7511449	0	0	unstratified_	cmap	flumetasone	9.80E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	734	Flumethasor	NULL	2135-17-3	DB00663
3	3311	-0.7157295	0	0	unstratified_	cmap	promazine	1.24E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	693	Promazine h	C0546875	53-60-1	DB00420
4	6039	-0.711365	0	0	unstratified_	cmap	irinotecan	1.00E-04	6	MCF7	HT_HG-U133	DMSO	Sigma-Aldric	I1406	irinotecan H	C0594375	NULL	DB00762
5	3269	-0.7021255	0	0	unstratified_	cmap	primaquine	8.80E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	476	Primaquine (	C0033126	63-45-6	DB01087
6	3225	-0.6954178	0	0	unstratified_	cmap	scopolamine	1.18E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	877	Scopolamine	C0036442	55-16-3	DB00747
7	3267	-0.6892034	0	0	unstratified_	cmap	zuclopenthixol	9.20E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	998	Zuclopenthix	C0350505	633-59-0	DB01624
8	3275	-0.65312	0	0	unstratified_	cmap	levonorgestrel	1.28E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	773	Norgestrel-(	C0023566	797-63-7	DB00367
9	3306	-0.6530724	0	0	unstratified_	cmap	flunisolide	9.20E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	643	Flunisolide [	C0060501	3/3/85	DB00180
10	3276	-0.6378132	0	0	unstratified_	cmap	cloperastine	1.10E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	793	Cloperastine	MMS_608	14984-68-0	DB09002
11	2694	-0.6148223	0	0	unstratified_	cmap	cycloserine	3.92E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	1086	D-cycloserine	C0010590	68-41-7	DB00260
12	3597	-0.6134951	0	0	unstratified_	cmap	bepridil	1.00E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	368	Bepridil hydr	C0005116	74764-40-2	DB01244
13	519	-0.6083808	0	0	unstratified_	cmap	trifluoperazine	1.00E-05	6	PC3	HT_HG-U133	DMSO	Sigma-Aldric	T6062	trifluoperazi	C0304381	NULL	DB00831
14	3272	-0.6069295	0	0	unstratified_	cmap	medrysone	1.16E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	743	Medrysone [	C0065865	2668-66-8	DB00253
15	1159	-0.590782	0	0	unstratified_	cmap	metolazone	1.10E-05	6	PC3	HG-U133A	DMSO	Prestwick Ch	112	Metolazone	C0025854	17560-51-9	DB00524
16	2136	-0.5900829	0	0	unstratified_	cmap	iopanoic acid	7.00E-06	6	HL60	HT_HG-U133	DMSO	Prestwick Ch	1052	Iopanoic acic	C0022028	96-83-3	DB08946
17	3796	-0.5892555	0	0	unstratified_	cmap	procyclidine	1.24E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	844	Procyclidine	C0033254	1508-76-5	DB00387
18	2758	-0.5860093	0	0	unstratified_	cmap	sulfamethoxazole	1.58E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	177	Sulfamethox	MMS_115	723-46-6	DB01015
19	3474	-0.5842548	0	0	unstratified_	cmap	sertaconazole	8.00E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	1045	Sertaconazol	C0074391	99592-39-9	DB01153
20	5117	-0.5837657	0	0	unstratified_	cmap	adipiodone	3.60E-06	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	939	Iodipamide [	NULL	606-17-7	DB04711
21	2965	-0.5805996	0	0	unstratified_	cmap	camptothecin	1.14E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	200	Camptotheci	NULL	3/4/89	DB04690
22	4569	-0.5796991	0	0	unstratified_	cmap	isoconazole	9.60E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	127	Isoconazole [	C0700489	27523-40-6	DB08943
23	3807	-0.5768143	0	0	unstratified_	cmap	simvastatin	9.60E-06	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	865	Simvastatin	C0074554	79902-63-9	DB00641
24	3725	-0.5712908	0	0	unstratified_	cmap	fenbufen	1.58E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	218	Fenbufen [3	C0060156	36330-85-5	DB08981
25	3966	-0.5689187	0	0	unstratified_	cmap	sulfadiazine	1.60E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	23	Sulfadiazine	C0723509	68-35-9	DB00359
26	356	-0.5685765	0	0	unstratified_	cmap	pyrvinium	1.25E-06	6	MCF7	HT_HG-U133	DMSO	MP Biomedi	2156475	pyrvinium pa	C0072804	NULL	DB06816
27	5387	-0.5655987	0	0	unstratified_	cmap	nocodazole	1.32E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	100	Nocodazole [	NULL	31430-18-9	DB08313
28	3127	-0.5643829	0	0	unstratified_	cmap	ginkgolide A	9.80E-06	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	444	Ginkgolide A	NULL	15291-75-5	DB06743
29	3908	-0.5633159	0	0	unstratified_	cmap	ouabain	5.40E-06	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	471	Strophantine	C0029904	11018-89-6	DB01092
30	4907	-0.5524134	0	0	unstratified_	cmap	tretinoin	1.34E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	257	Retinoic acid	C0040845	302-79-4	DB00755
31	1150	-0.5403643	0	0	unstratified_	cmap	apomorphine	6.40E-06	6	PC3	HG-U133A	DMSO	Prestwick Ch	101	Apomorphine	C0237406	41372-20-7	DB00714
32	5003	-0.5351502	0	0	unstratified_	cmap	remoxipride	9.80E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	971	Remoxipride	C0771022	73220-03-8	DB00409
33	4837	-0.5345855	0	0	unstratified_	cmap	valproic acid	2.00E-04	6	HL60	HT_HG-U133	DMSO	Sigma-Aldric	P4543	2-propylpent	NULL	NULL	DB00313
34	3963	-0.5326876	0	0	unstratified_	cmap	thiocolchicoside	7.00E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	539	Thiocolchico	C0076456	602-41-5	DB11582
35	4136	-0.5324069	0	0	unstratified_	cmap	hesperetin	1.32E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	124	Hesperetin [	APRD00117	520-33-2	DB01094
36	3273	-0.5316383	0	0	unstratified_	cmap	demeclocycline	8.00E-06	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	753	Demeclocycl	C0011276	64-73-3	DB00618
37	2385	-0.5273743	0	0	unstratified_	cmap	methyldopa	1.90E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	326	Methyldopa	C0304527	555-30-6	DB00968
38	3486	-0.5251063	0	0	unstratified_	cmap	clomipramine	1.14E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	269	Clomipramin	C0282107	17321-77-6	DB01242
39	3842	-0.5231805	0	0	unstratified_	cmap	miconazole	9.60E-06	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	67	Miconazole [	MMS_579	22916-47-8	DB01110
40	343	-0.5223235	0	0	unstratified_	cmap	resveratrol	1.00E-05	6	MCF7	HT_HG-U133	DMSO	Sigma-Aldric	R 5010	resveratrol	EXPT02968	NULL	DB02709
41	4826	-0.520045	0	0	unstratified_	cmap	mesalazine	2.62E-05	6	HL60	HT_HG-U133	DMSO	Prestwick Ch	1069	Mesalamine	NULL	89-57-6	DB00244
42	3646	-0.5186353	0	0	unstratified_	cmap	menadione	2.32E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	459	Menadione [	C0025270	58-27-5	DB00170
43	6052	-0.515398	0	0	unstratified_	cmap	daunorubicin	1.00E-06	6	PC3	HT_HG-U133	DMSO	Sigma-Aldric	D8809	daunorubicin	C0724567	NULL	DB00694
44	4732	-0.5135385	0	0	unstratified_	cmap	ethotoin	1.96E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	696	Ethotoin [86-	C0015046	86-35-1	DB00754
45	3478	-0.5092354	0	0	unstratified_	cmap	zomepirac	1.28E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	779	Zomepirac s	C0149477	64092-48-4	DB04828
46	1005	-0.5088352	0	0	unstratified_	cmap	ipratropium bromide	9.80E-06	6	HL60	HG-U133A	DMSO	Prestwick Ch	575	Ipratropium	C0700580	22254-24-6	DB00332
47	3960	-0.506961	0	0	unstratified_	cmap	rimexolone	1.08E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	1020	Rimexolone	C0140594	49697-38-3	DB00896
48	3752	-0.5063315	0	0	unstratified_	cmap	galantamine	1.08E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	588	Galanthamir	C0949312	4/4/53	DB00674
49	1454	-0.5045577	0	0	unstratified_	cmap	terfenadine	8.40E-06	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	138	Terfenadine	C0085173	50679-08-8	DB00342
50	1904	-0.5021935	0	0	unstratified_	cmap	methazolamide	1.70E-05	6	HL60	HT_HG-U133	DMSO	Prestwick Ch	802	Methazolam	C0025631	554-57-4	DB00703
51	1292	-0.5018846	0	0	unstratified_	cmap	canrenoic acid	1.00E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	148	Canrenoic ac	C0282085	4/6/81	DB09015
52	2499	-0.5016635	0	0	unstratified_	cmap	azacitidine	1.64E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	866	Azacytidine-!	C0004475	320-67-2	DB00928
53	4487	-0.5015514	0	0	unstratified_	cmap	fluorometholone	1.06E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	718	Fluorometho	C0016351	426-13-1	DB00324
54	4492	-0.500319	0	0	unstratified_	cmap	hexestrol	1.48E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	699	Hexestrol [8	NULL	84-16-2	DB07931
55	1175	-0.4987932	0	0	unstratified_	cmap	diltiazem	8.80E-06	6	PC3	HG-U133A	DMSO	Prestwick Ch	134	Diltiazem hy	C0700579	33286-22-5	DB00343
56	4080	-0.4936755	0	0	unstratified_	cmap	felodipine	1.04E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	478	Felodipine [7	C0015772	72509-76-3	DB01023
57	4716	-0.4935348	0	0	unstratified_	cmap	sulfamerazine	1.52E-05	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	694	Sulfamerazir	C0038684	127-79-7	DB01581
58	2196	-0.4919332	0	0	unstratified_	cmap	latamoxef	7.00E-06	6	HL60	HT_HG-U133	DMSO	Prestwick Ch	819	Moxalactam	NULL	64953-12-4	DB04570
59	3977	-0.4909196	0	0	unstratified_	cmap	mefenamic acid	1.66E-05	6	PC3	HT_HG-U133	DMSO	Prestwick Ch	54	Mefenamic a	C0025152	61-68-7	DB00784
60	3169	-0.4905221	0	0	unstratified_	cmap	trimipramine	9.80E-06	6	MCF7	HT_HG-U133	DMSO	Prestwick Ch	806	Trimipramin	C0041056	521-78-8	DB00726

Table S2. Differentially expressed genes (DEGs) in lesion samples in FEN treatment group. lesion.FEN.csv
Table S3. Differentially expressed genes (DEGs) in lesion samples in IBU treatment group. lesion.IBU.csv
Table S4. Differentially expressed genes (DEGs) in lesion samples in PRIMA treatment group. lesion.PRIMA.csv
Table S5. Differentially expressed genes (DEGs) in lesion samples in SIMVA treatment group. lesion.SIMVA.csv
Table S6. Differentially expressed genes (DEGs) in uterus samples in VEH treatment group. uterus.Endo.csv
Table S7. Differentially expressed genes (DEGs) in uterus samples in FEN treatment group. uterus.FEN.csv
Table S8. Differentially expressed genes (DEGs) in uterus samples in IBU treatment group. uterus.IBU.csv
Table S9. Differentially expressed genes (DEGs) in uterus samples in PRIMA treatment group. uterus.PRIMA.csv
Table S10. Differentially expressed genes (DEGs) in uterus samples in SIMVA treatment group. uterus.SIMVA.csv