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AP-1 subunit JUNB promotes invasive phenotypes in endometriosis

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Abstract

Endometriosis is a disease defined by the presence of abnormal endometrium at ectopic sites, causing pain and infertility in 10% of women. Mutations in the chromatin remodeling protein ARID1A (AT-rich interactive domain-containing protein 1A) have been identified in endometriosis, particularly in the more severe deep infiltrating endometriosis and ovarian endometrioma subtypes. ARID1A has been shown to regulate chromatin at binding sites of the Activator Protein 1 (AP-1) transcription factor, and AP-1 expression has been shown in multiple endometriosis models. Here, we describe a role for AP-1 subunit JUNB in promoting invasive phenotypes in endometriosis. Through a series of knockdown experiments in the 12Z endometriosis cell line, we show that JUNB expression in endometriosis promotes the expression of epithelial-to-mesenchymal transition genes co-regulated by ARID1A including transcription factors SNAI1 and SNAI2, cell adhesion molecules ICAM1 and VCAM1 and extracellular matrix remodelers LOX and LOXL2. In highly invasive ARID1A-deficient endometriotic cells, co-knockdown of JUNB is sufficient to suppress invasion. These results suggest that AP-1 plays an important role in the progression of invasive endometriosis, and that therapeutic inhibition of AP-1 could prevent the occurrence of deep infiltrating endometriosis.

Keywords

endometriosis; endometrium; invasion; EMT; JUNB; AP-1; ARID1A

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Author Contributions

Conceptualization, M.R.W. and R.L.C.; Investigation, M.R.W.; Methodology, M.R.W., J.J.R., and R.L.C.; Resources, R.L.C.; Formal Analysis, M.R.W. and J.J.R..; Data Curation, M.R.W. and J.J.R.; Writing – Original Draft, M.R.W.; Writing – Review & Editing, M.R.W., J.J.R. and R.L.C.; Funding Acquisition, M.R.W. and R.L.C.; Supervision, R.L.C.

Conflict of Interest

The authors declare no competing interests.

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Not applicable.

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Code availability

Not applicable.

Introduction

Endometriosis is a condition defined by benign growth of uterine endometrium outside of the uterus, causing pain and infertility in up to 10% of women [1–5]. The endometrium is the layer of cells lining the inside of the uterus composed of stroma and epithelia [6, 7]. During the menstrual cycle, the endometrium undergoes proliferation, differentiation and shedding [7]. Endometriosis is thought to occur following the translocation of endometrium to ectopic cites via retrograde menstruation [8]. The clinical observation that the incidence of endometriosis is increased by the obstruction of menstrual flow supports the role for retrograde menstruation in the development of endometriosis [8]. After translocation to distal tissues via retrograde menstruation, endometrial cells which have undergone epithelial-to-mesenchymal transition (EMT) are capable of invading other tissues [9]. EMT markers expressed highly among endometriotic lesions in comparison to eutopic endometrium [10], particularly among the deep infiltrating endometriosis subtype [11]. In our previous work, we identified the protein ARID1A (AT-rich interactive domain-containing protein 1A, also called BAF250A) as a master regulator of EMT and epithelial identity in the endometrial epithelium [12, 13].

ARID1A is a subunit in the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex, is known to be mutated at a high rate in endometriosis [14]. ARID1A plays a role in several cancers derived from the endometrial epithelium and was first identified as a tumor suppressor in two ovarian cancer subtypes associated with endometriosis: ovarian clear-cell carcinoma and ovarian endometrioid carcinoma [15, 16]. ARID1A mutations have also been observed in endometrial hyperplasia and endometrial cancer [17–19]. Although cancer-associated mutations in multiple genes found in endometriotic lesions are observed in the normal endometrium without endometriosis, ARID1A mutations have not been observed in the normal endometrium and are only associated with the disease [20, 21]. ARID1A loss may indicate malignancy in atypical endometriosis [18]. Furthermore, ARID1A mutations are observed in the more severe deep ovarian and deep infiltrating endometriosis subtypes [14, 21–25], suggesting an enhanced invasive capability of ARID1A-deficient endometriosis.

We previously described the role for ARID1A in the suppression of invasive phenotypes in the endometrial epithelium [12, 13, 26–28]. In a mouse model of ARID1A mutant endometrial epithelium, endometrial tissue can spread throughout the peritoneal cavity and form endometriosis following salpingectomy in mice [13]. Loss of ARID1A in the endometrial epithelium results in EMT and invasion resulting from changes to chromatin accessibility and histone acetylation [12, 26, 29]. Specifically, ARID1A regulates cell identity through its repressive role at super-enhancers, and loss of ARID1A allows for hyperacetylation of super-enhancers by P300 resulting in EMT and invasion [26]. Additionally, ARID1A promotes the expression of progesterone receptor in response to estrogen [30], a feature which is lost in endometriosis and endometrial cancers [31–33]. Among sites of differentially accessible chromatin following ARID1A loss in the endometrial epithelium, we have identified Activator Protein 1 (AP-1) as the top transcription factor binding motif [12]. Additionally, ARID1A binds AP-1 sites across the

genome [12]. AP-1 is a dimeric transcription factor composed of homodimers between JUN family members, JUN/FOS heterodimers with FOS family members, and others, that regulate a variety of processes including proliferation, migration and transformation [34–36]. Previous studies have suggested a relationship between AP-1 and SWI/SNF through interactions with BAF60A [37], and repression of Cyclin D1 by the SWI/SNF ATPase domain BRG1 requires AP-1 sites at the Cyclin D1 promoter [38]. Moreover, AP-1 recruits SWI/SNF complexes to AP-1 sites [39], and ARID1A facilitates AP-1 binding [40]. The FOS AP-1 subunit has been shown to be upregulated both during establishment of endometriosis in a baboon endometriosis model [41] and in pelvic endometriotic implants relative to the eutopic endometrium [42]. Endometrial FOS expression is driven by estrogen signaling [43–45], and estrogen-induced FOS acts to repress progesterone receptor activity [46, 47] and upregulate MMP9 [48], processes that are also regulated by ARID1A [12, 30]. During the menstrual cycle, JUN is upregulated in the luminal and glandular epithelium during the proliferative phase [49]. JUN and FOS have been predicted to regulate cytokine secretion in endometriosis-associated macrophages [50]. Recently, the AP-1 inhibition was shown to reduce the size of endometriosis lesions and relieve hyperalgesia in a rat endometriosis model [51]. In our own studies, we identified AP-1 subunit expression in endometriotic lesions of ARID1A-PIK3CA mutant mice [13], suggesting a role for AP-1 in the invasion driven by ARID1A loss. Therefore, we sought to determine if AP-1 subunits play a role in the invasive processes occurring in endometriosis.

Here, we identify a role for AP-1 subunit JUNB in the regulation of EMT and invasion processes in endometriosis. JUNB is upregulated following ARID1A loss in endometriosis cells. We utilized siRNAs targeting JUNB to profile the effects of JUNB loss in endometriosis, with and without ARID1A co-knockdown. Via quantitative PCR (qPCR), we screened a panel of 66 genes related to invasion and migration processes and found that JUNB regulates the expression of invasion-promoting genes. While ARID1A knockdown promotes invasion, co-knockdown of JUNB rescues the invasive phenotype in endometriosis cells. JUNB may represent an important co-regulator in ARID1A-deficient invasive endometriosis.

Materials and Methods

Cell lines and cell culture

12Z cells are an immortalized endometriosis cell line derived from the epithelial component of a human endometriosis sample [52], which has been used in many studies of endometriosis [53–57]. 12Z cells were maintained in DMEM/F12 media (HyClone) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 1% penicillin/streptomycin (P/S). 12Z cells were seeded at a density of 40,000 cells/mL in DMEM/F12 media supplemented with 10% FBS and 1% L-glutamine. The following day, cells were transfected with 50 pmol/mL of siRNA (Dharmacon, ON-TARGETplus Non-targeting Pool, human ARID1A #8289 SMARTpool, and human JUNB #3726 SMARTpool) using the RNAiMax (ThermoFisher) lipofectamine reagent according to the manufacturer's instructions at a ratio of 1:1 volume:volume in OptiMEM (Gibco). After 24 hours, the

media was replaced. The following day, media was replaced with DMEM/F-12 media supplemented with 0.5% FBS, 1% P/S and 1% L-glutamine.

RNA isolation and qPCR

Cells were collected 72 hours-post siRNA transfection using the Quick-RNA Miniprep Kit (Zymo Research) for RNA or RIPA buffer (Cell Signaling) for protein. cDNA was synthesized from RNA, and real-time quantitative PCR was performed using PowerUp SYBR Green Master Mix (ThermoFisher) and the Applied Biosystems ViiA7 real-time PCR system. Primer pairs for human genes are described in Table 1. Three biological replicates were used for each condition and results were normalized to control gene RPL17.

Western blotting

Cells were collected 72 hours-post siRNA in RIPA buffer (Cell Signaling) to generate protein lysates. Protein lysates were quantified using the Micro BCA Protein Assay Kit (ThermoFisher) and a FlexSystem3 plate reader. Protein lysates were run on a 4–15% gradient SDS-PAGE gel (BioRad) and transferred to PVDF membrane using the TransBlot Turbo system (BioRad). Primary antibodies were used at the following dilutions: 1:1000 ARID1A (12354, Cell Signaling); 1:1000 β-Actin (8457, Cell Signaling); 1:1000 JunB (3753, Cell Signaling); 1:1000 Slug (9585, Cell Signaling); 1:1000 Snail (3879, Cell Signaling). Horseradish peroxidase (HRP) conjugated secondary antibodies (Cell Signaling) were used at a dilution of 1:2000. Clarity Western ECL Substrate (BioRad) was used for protein band visualization, and western blot exposures were captured using the ChemiDoc XRS+ imaging system (BioRad). Two experimental replicates were used for each condition.

Transwell invasion assay

12Z cells were seeded in 6-well dishes at a density of 50,000 cells per well. After 24 hours, cells were transfected with siRNA as described above. At 48 hours post-transfection, cells were trypsinized, and 100 μL of cell mixture containing 30,000 cells and 0.3 mg/mL Matrigel was seeded into transwell plates (8 μm pore polycarbonate membrane, Corning) pre-coated with 100 μL of 0.3 mg/mL Matrigel. After 1 hour, serum-free DMEM/F12 1% P/S, 1% L-glutamine media was added to the top chamber and DMEM/F12, 5% FBS, 1% P/S, 1% L-glutamine was added to the bottom chamber. After 16 hours, transwell units were transferred to plates containing 2 μg/mL calcein-AM in DMEM/F12. After 1 hour, media was aspirated from the top chamber and unmigrated cells were removed with a cotton swab. Images were collected using a Nikon Eclipse T*i* microscope in five non-overlapping fields per well. ImageJ software (National Institutes of Health) was used to quantify cells based on size and intensity. Five biological replicates were used for each condition.

Bioinformatics and statistics

ClusterProfiler [58] was used to compute and visualize pathway enrichment from a list of gene symbols with respective gene universes. GO Biological Process gene sets were retrieved from MSigDB [59]. *ComplexHeatmap* [60] was used for hierarchical clustering by Euclidean distance and general heatmap visualization. *Eulerr* [61] was used to produce proportional Euler diagrams. *ggplot2* [62] was used for certain plotting applications. The

statistical computing language R [63] and GraphPad Prism 9 software were used for many applications throughout this manuscript.

Results

JUNB is upregulated in 12Z human endometriosis cells following ARID1A loss.

To explore the role of AP-1 in ARID1A-deficient endometriosis, we utilized our previously published RNA-seq dataset in which ARID1A knockdown was induced in 12Z human endometriosis cells by transfection with siRNA [12]. Knockdown of ARID1A resulted in significant differential expression of four of the seven major AP-1 subunits, upregulation of JUNB, FOSL1 and FOSL2, and downregulation of JUND (Fig. 1). Among these, the change in gene expression was greatest and most significant for JUNB (Fig. 1B), suggesting a more prominent role for JUNB as a transcription factor following ARID1A loss.

Previous studies have shown roles for JUNB in EMT [64, 65], therefore, we wanted to examine the function of JUNB in ARID1A-dependent EMT gene expression and invasion. To do so, we utilized siRNAs targeting JUNB (siJUNB) to knockdown JUNB in 12Z cells (Fig. 2). JUNB siRNA was used both in the presence and absence of ARID1A siRNAs (siARID1A), and knockdown was confirmed by western blot (Fig. 2A) and qPCR (Fig. 2B,C).

JUNB co-regulates genes affected by ARID1A loss.

Using our model of ARID1A and JUNB knockdowns in 12Z cells, we next wanted to determine the role of JUNB in promoting the invasive phenotype driven by ARID1A loss in ARID1A-deficient endometriosis. To do so, we utilized a panel of 66 gene primer pairs (Table 1) to determine differential expression of genes following knockdown of ARID1A, JUNB or both. These 66 genes were enriched for pathways related to extracellular matrix, cell adhesion and migration (Fig. 3A). Utilizing expression data from all 66 genes, principal component analysis was performed, and samples treated with the same conditions clustered together based on their principal components (Fig. 3B). The 66 genes, on average, were upregulated by ARID1A knockdown and downregulated by JUNB knockdown, such that the average gene expression was reduced in the ARID1A + JUNB co-knockdown relative to ARID1A knockdown alone (Fig. 3C). We then statistically compared expression across conditions and observed 20 of the 66 genes were significantly differentially expressed following ARID1A loss (Fig. 3D), 14 of the 66 genes following JUNB loss (Fig. 3E), and 27 of the 66 genes following loss of both ARID1A and JUNB (Fig. 3F). Of the 14 genes affected by JUNB loss alone, 5 were unaffected by ARID1A loss alone (Fig. 3G). These 5 genes, *FBLN2*, *THBS2*, *COL5A1*, *ITGAV*, and *CMTM3*, were all downregulated following JUNB knockdown (Fig. 3H). Relative to ARID1A knockdown alone, the ARID1A and JUNB co-knockdown resulted in differential expression of 13 of the 66 genes (Fig. 3I). Of these, 10 genes were affected also affected by ARID1A knockdown relative to control cells (Fig. 3J). Most of these genes were grouped together by hierarchical clustering, representing genes which were upregulated by ARID1A loss alone, and downregulated by an additional JUNB knockdown (Fig. 4). These results suggest a role for JUNB in regulating the processes of EMT and invasion, which define ARID1A-deficient endometriosis.

JUNB regulates EMT, extracellular matrix and cell adhesion in ARID1A-deficient endometriosis.

Among genes upregulated by ARID1A knockdown and rescued by JUNB co-knockdown were two master regulators of EMT [66], transcription factors *SNAI1* (Snail) and *SNAI2* (Slug), (Fig. 5A–C). *LOX* (lysyl oxidase), a protein which regulates extracellular matrix protein structure and has been previously shown to regulate invasiveness in 12Z cells [57], and LOX family member *LOXL2*, also followed this pattern, suggesting a role for JUNB in regulating extracellular membrane composition (Fig. 5D,E). We previously identified ICAM-1 (Intercellular Adhesion Molecule 1) as a marker of mutated endometrium in our ARID1A and PIK3CA mutant mouse model [12] which was observed in the peritoneal endometriotic lesions of those mice [13]. Here, we identify a role for JUNB in upregulating the *ICAM1* gene. Similarly, we previously identified upregulation VCAM1 (Vascular Cell Adhesion Molecule 1) following ARID1A loss [12], and here we find a role for JUNB in promoting that upregulation. These results suggest that JUNB regulates the same gene expression programs that promote cell invasion following ARID1A loss.

To test whether JUNB does regulate invasive phenotypes in endometriosis, we performed a transwell invasion assay. As previously described [12], loss of ARID1A promotes cellular invasion (Fig. 6). However, knockdown of JUNB reduces invasion, such that co-knockdown of ARID1A and JUNB rescues the invasive phenotype driven by knockdown of ARID1A alone, restoring cellular invasion to the level of the control cells (Fig. 6). These data suggest that JUNB represents an important transcription factor for the invasion of ectopic endometrium in endometriosis, and that JUNB co-regulates similar EMT programs which are affected by ARID1A loss.

Discussion

In this study, we characterized a role for JUNB in the regulation of EMT, cell adhesion and extracellular matrix proteins in the context of endometriosis with and without ARID1A loss. Several publications have reported roles for AP-1 at accessible chromatin following ARID1A loss. In fibroblasts [39] and colorectal cancer cell lines [40], ARID1A loss results in decreased chromatin accessibility and enrichment of differentially accessible AP-1 sites. Interactions between SWI/SNF and AP-1 are reported to promote increased chromatin accessibility at enhancers [39, 40]. We previously identified ARID1A binding at AP-1 sites and differential chromatin accessibility at AP-1 sites following ARID1A loss [12] and that ARID1A and PIK3CA mutant endometriotic lesions in mice express AP-1 subunits [13]. Here, we characterized the upregulation of JUNB following ARID1A loss and regulation of common EMT programs by JUNB.

The differential accessibility at AP-1 binding sites [12] and an upregulation of JUNB protein expression following ARID1A loss suggest both an increase in activity of JUNB as well as an increase in available chromatin for JUNB to bind. A limitation of our study is that we did not distinguish between these two features, and it may be the case that JUNB expression alone is a driving feature of cellular invasion. Following JUNB knockdown alone, invasion of 12Z cells was reduced compared to control cells, suggesting a mechanism which does not entirely depend on ARID1A. Still, expression of several marker genes, including *SNAI2*,

were unchanged following knockdown of JUNB alone. Differences in the transcriptional response to JUNB knockdown may reflect differences in AP-1 dimerization, as JUNB may form protein dimers with any JUN or FOS family proteins.

Recently, we described a role for the histone acetyltransferase P300 in promoting invasive phenotypes in ARID1A-deficient endometriosis through the hyperacetylation of super-enhancers, such that knockdown of P300 or chemical inhibition of the histone acetyltransferase domain rescued the invasive phenotype [26]. AP-1 is known to interact with P300. AP-1 driven transcription of the HPV chromatin in cervical cancer cells depends on P300 recruitment, and P300 acetylation of AP-1 dimers enhances AP-1 binding to DNA [67]. AP-1 recruits P300 to mediate DNA looping at the MMP9 gene in response to TNF-alpha signaling in human leukemia cells, and P300 acetyltransferase activity is necessary for DNA looping [68]. In our model, P300 co-knockdown with ARID1A also rescues the gene expression of *SNAI1*, *SNAI2*, *LOX*, *LOXL2* and *VCAM1* [26]; genes we have shown here are co-regulated by JUNB. However, the regulation of invasive endometriosis by P300 appears be relevant only in the context of ARID1A loss, while JUNB appears to regulate invasion regardless of ARID1A expression. This may indicate a role for regulation of invasion by JUNB in all endometriosis subtypes, rather than just the deep infiltrating and ovarian endometriosis subtypes in which ARID1A is commonly mutated [14, 21–25]. In the case of certain genes including *SNAI2*, in which knockdown of JUNB alone has no effect on expression, there may be a shared mechanism by which JUNB and P300 co-regulate the invasive phenotype in the context of ARID1A loss. However, JUNB may regulate invasion and EMT by a mechanism independent of ARID1A and P300 in the context of other genes, such as *SNAI1*. Additional studies will be needed to further characterize the relationship between ARID1A and individual AP-1 subunits.

The medical treatment options for endometriosis are unsatisfactory, as they are mostly limited to oral contraceptives and progestins, which are only successful in two thirds of women with symptomatic endometriosis [69]. Inhibition of AP-1 was recently shown to reduce the size of endometriotic lesions while also relieving hyperalgesia in a rat model [51]. One study found that AP-1-mediated TNF-alpha signaling induces cytokine IL-6 in endometriotic stroma [70], while another study found that AP-1 binding to the MMP7 promoter was increased following treatment with EGF, suggesting its role in EMT processes as we have described here [71]. Therefore, we conclude that treatment of endometriosis using broad AP-1 inhibitors has the potential to reduce inflammation and lesion growth, while also inhibiting the formation of invasive deep infiltrating endometriosis. Further studies will be required to characterize the mutational contexts in which each AP-1 inhibition may alleviate each of these phenotypes.

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Availability of data and material

The previously published RNA-seq dataset analyzed herein is available at GEO: GSE121198. All other data generated or analyzed during this study are included in this published article.

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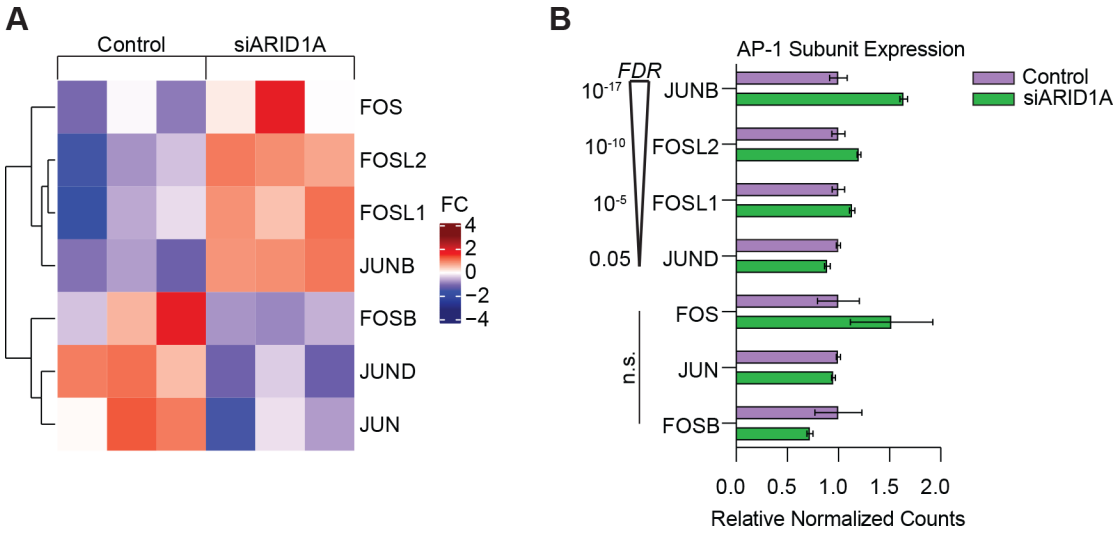

Fig. 1 |. Differential expression of AP-1 subunits following ARID1A knockdown.

a, Clustering of 7 canonical AP-1 subunits based on expression in control and siARID1A 12Z cells. **b,** Relative normalized counts expression data of AP-1 subunits from control cells and siARID1A cells, ranked by *DEseq2* FDR.

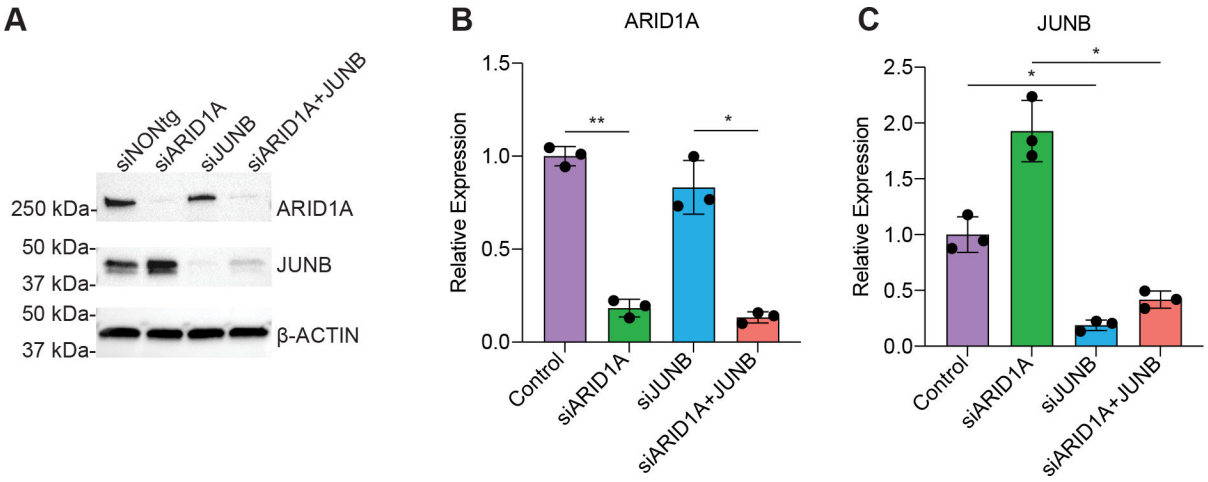

Fig. 2 |. Knockdown of ARID1A and JUNB by siRNA transfection.

a, Western blot showing ARID1A, JUNB, and β-Actin protein expression following siRNA treatment (n = 2 independent experiments). **b-c,** Gene expression of ARID1A and JUNB by qPCR, normalized to RPL17 (mean ± s.d). Statistic is *t*-test, *FDR < 0.05, **FDR < 0.01.

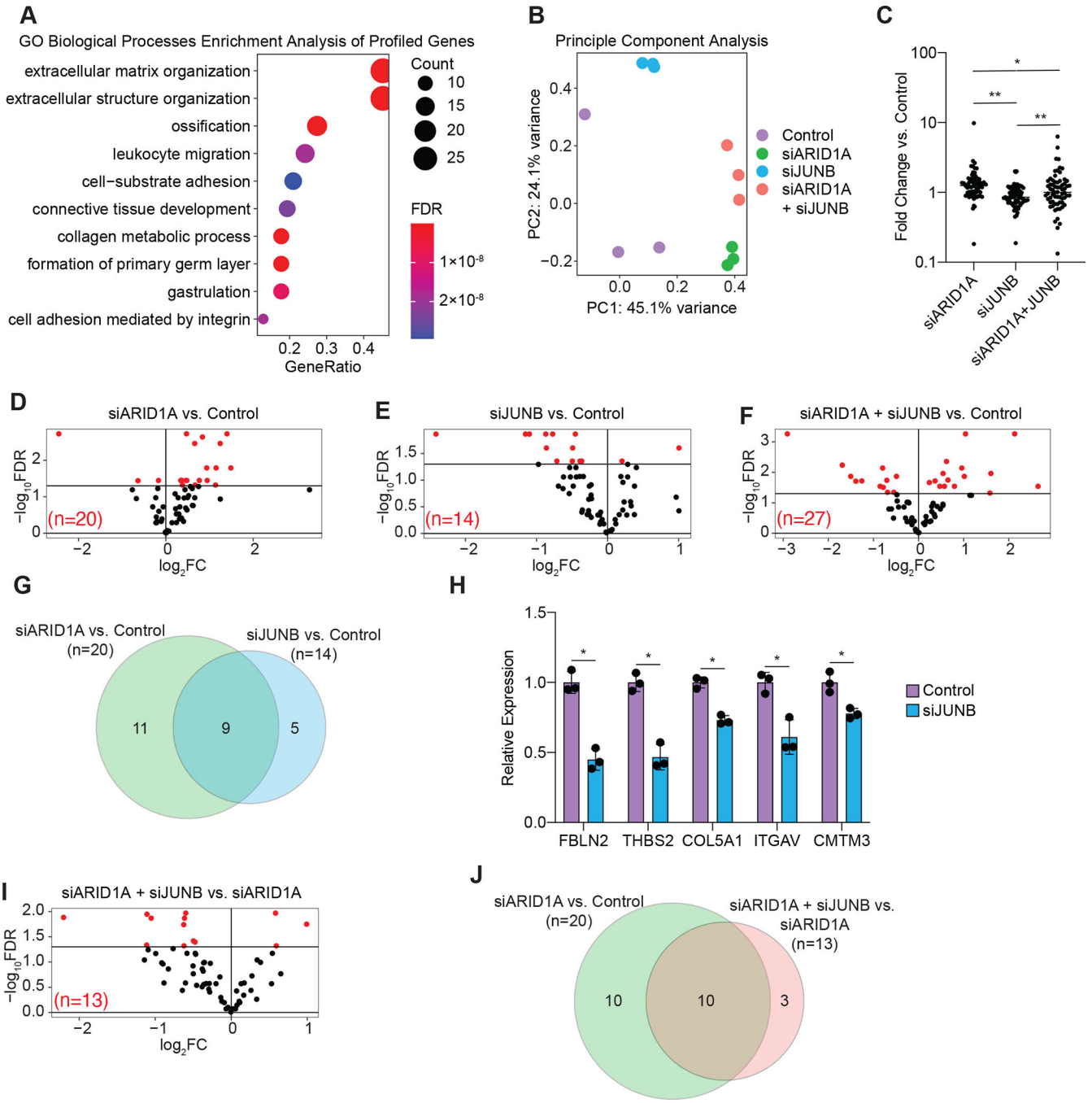

Fig. 3 |. Differential gene expression following knockdown of ARID1A, JUNB or both.

a, Pathway enrichment analysis of 66 genes tested for mSigDb Gene Ontology (GO) Biological Processes. **b,** Principal component analysis of gene expression data for each sample. **c,** Mean fold change value for each condition relative to control. **d-f,** Volcano plot of 66 genes following ARID1A loss (d), JUNB loss (e) or both (f) relative to control. Red dots represent genes with significance FDR < 0.05. **g,** Proportional Euler diagram displaying differentially-expressed genes from siARID1A and siJUNB. **h,** Relative gene expression of 5 genes uniquely dysregulated following JUNB knockdown (mean ± s.d). **i,**

Volcano plot of 66 genes following ARID1A and JUNB knockdown compared to ARID1A knockdown alone. **j**, Proportional Euler diagram displaying differentially-expressed genes from siARID1A compared to control and siARID1A + siJUNB compared to siARID1A alone. Statistic is *t*-test, *FDR < 0.05, **FDR < 0.01.

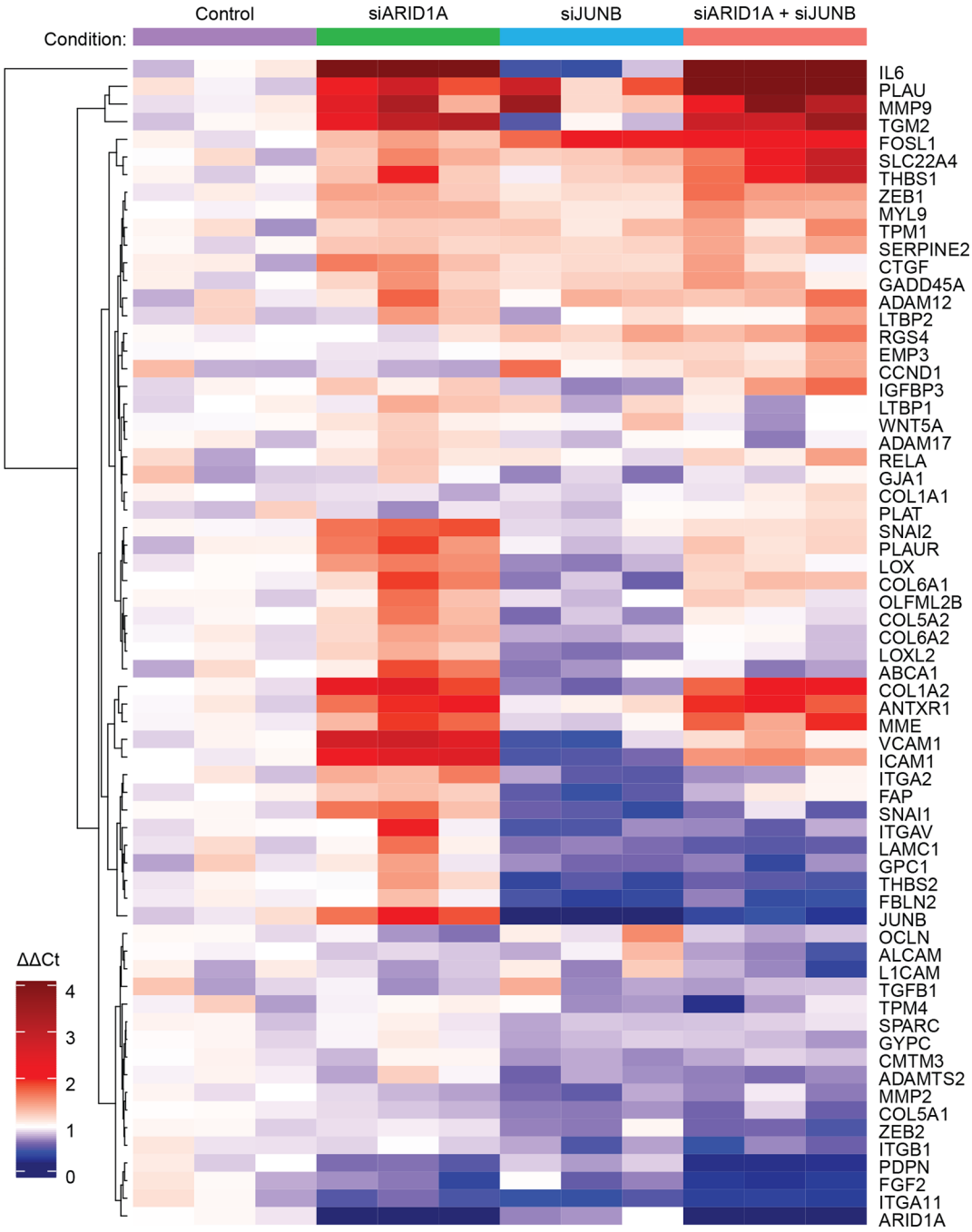

Fig. 4 |. Relative gene expression of all genes in all samples.

Hierarchical clustering of fold change values (ΔΔCt) for control, siARID1A, siJUNB and siARID1A + siJUNB samples.

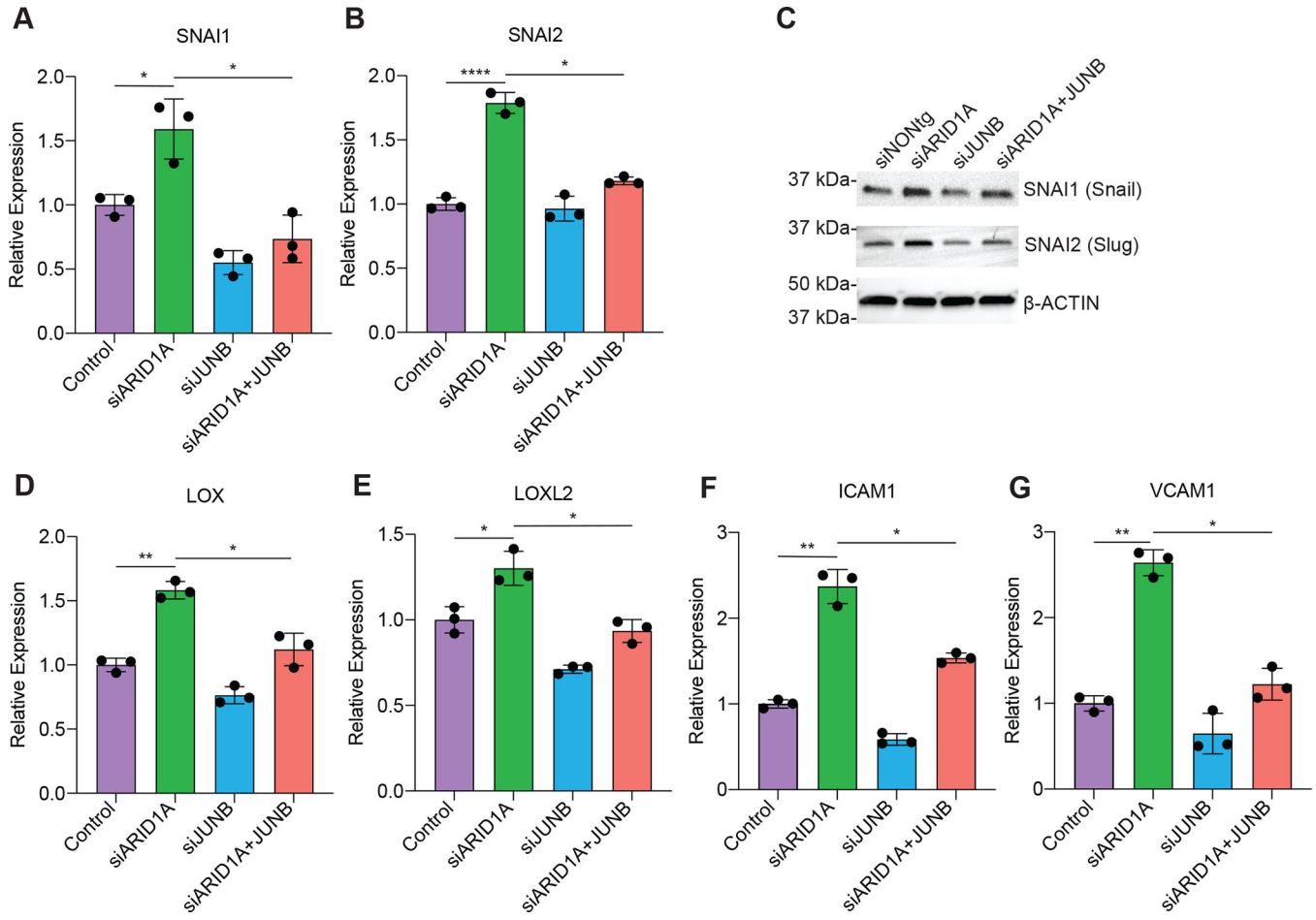

Fig. 5 |. Expression of genes rescued by JUNB co-knockdown.

a-b, Relative expression of *SNAI1* (a) and *SNAI2* (b) in all samples (mean ± s.d). **c,** Protein expression of SNAI1, SNAI2 and β-Actin protein expression following siRNA treatment (n = 2 independent experiments). **d-g,** Relative expression of *LOX* (d), *LOXL2* (e), *ICAM1* (f) and *VCAM1* (g) in all samples (mean ± s.d). Statistic is *t*-test, *FDR < 0.05, **FDR < 0.01.

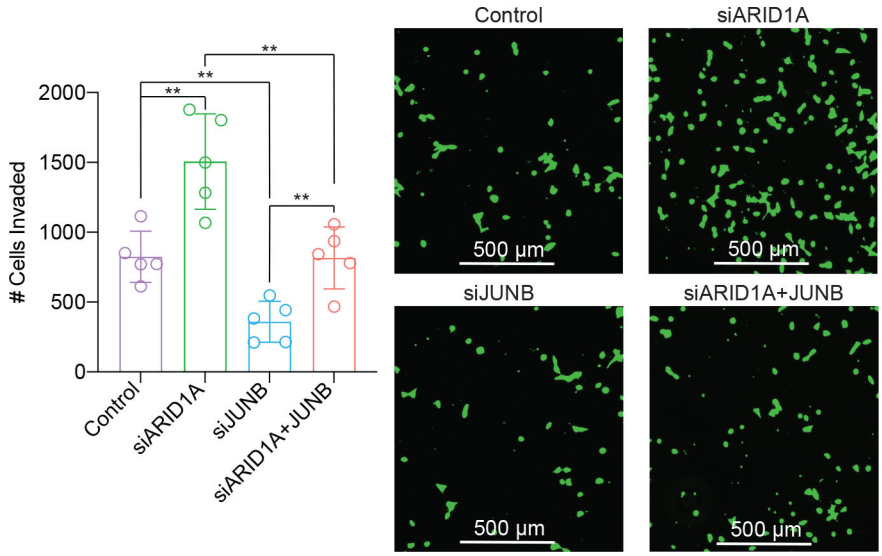

Fig. 6 |. Transwell invasion assay following knockdown of ARID1A, JUNB or both.

Invasion of 12Z cells following 48h siRNA treatment. Data represents five biological replicates (mean ± s.d; **P < 0.01; two-sided unpaired *t*-test).

Table 1 |

List of primers used for qPCR analysis of human genes

Gene	Forward primer	Reverse primer
ABCA1	AACGAGACTAACCAGGCAATC	ACACAATACCAGCCCAGAAC
ADAM12	TCTGCCTGAATCGTCAATGTC	AAACTTGTCACAGAAGGGAGG
ADAM17	TTGGCAAGTGTAAGGATGGG	TTGTTCAGCATCGACATAGGG
ADAMTS2	TCCAAAACCTTCATTGCCATG	GTATTTGTACGTCAGTGAGACCC
ALCAM	TGGCAATATCACATGGTACAGG	AGCCTTGGTTGTCTTGTACTC
ANTXR1	GCCTCCACAAAATTGCATCAG	GGAAAGTGTAAACCGAGCATG
ARID1A	AGATGGGACACCCAAGACAG	CTTCCTCTCAGGCTCACCAC
CCND1	CATCTACACCGACAACTCCATC	TCTGGCATTTTGGAGAGGAAG
CMTM3	CTACTTTGCTATCTCCATCACGG	CCTTGTTTGAGGAATTTGGCC
COL1A1	GGCCCAGAAGAACTGGTACA	GGCCCAGAAGAACTGGTACA
COL1A2	GATTGAGACCCTTCTTACTCCTGAA	GGGTGGCTGAGTCTCAAGTCA
COL5A1	CACAACTTGCCTGATGGAATAACA	GCAGGGTACAGCTGCTTGGT
COL5A2	AGCAAACCCATCCAGTGTAC	TGGCTGTATTAGGTGATTGGTG
COL6A1	TCAAGTCCTTCACCAAGCG	ATCTCCACCTCGTCACTGTA
COL6A2	GACCAGGACACCATCAACC	TCACCCATGTTGCCCTTG
CTGF	ACCAATGACAACGCCTCC	TTGGAGATTTTGGGAGTACGG
EMP3	CTGAATCTCTGGTACGACTGC	AACATGAACAGGATGAAGGAGAG
FAP	AGCTTCCTCGTCCAATTCAG	TGGATCTCCTGGTCTTTGTTTC
FBLN2	ATATGCTCCTGTTTTCCCGG	GGTTGACACAGTAGAAGGATCC
FGF2	TATGTCGTGGAAGCACTGGA	CCATCTTGAGGTGGAAGGGT
FOSL1	GGGCATGTTCCGAGACTTC	CTCATGGTGTTGATGCTTGG
GADD45A	GGGAAAGTCGCTACATGGATC	GTGTAGGGAGTAACTGCTTGAG
GJA1	ACATGAATTACAGCCACTAGCC	ACAATTGAGTGGAATCTTGATGC
GPC1	GACTATTGCCGAAATGTGCTC	GCTGCCGATGACACTCTC
GYPC	ACGGAGTTTGCTGAGAGTG	GGAGGCATTCAGGGAAGTG
ICAM1	CAGCACGTACCTCTATAACCG	CCAATATGGGAAGGCCGAG
IGFBP3	CAGAGCACAGATACCCAGAAC	AGCACATTGAGGAACTTCAGG
IL6	CCACTCACCTCTTCAGAACG	CATCTTTGGAAGGTTCAGGTTG
ITGA11	GTGCCTATGACTGGAATGGAG	CGACCGATGTGACTGTGTAC
ITGA2	TCCGCACAAAGTATTCCCAG	AGGCACCAATAGACACATCG
ITGAV	AGAATCAAGGAGAAGGTGCC	GGCGAGTTTGGTTTTCTGTC
ITGB1	CAGCGCATATCTGGAAATTTGG	TCTCCAGCAAAGTGAAACCC
JUNB	GGACACGCCTTCTGAACG	CGGAGTCCAGTGTGGTTTG
L1CAM	TATGATTCCAACCAGGGTCAC	TCAATGCCTTCCAGCTCAG
LAMC1	GACCTCTATCAAGATACGTGGG	AAAACTGCCCTCCATATCCC
LOX	ACATTCGCTACACAGGACATC	TTCCCACTTCAGAACACCAG
LOXL2	AGGTTGCAGAATCCGATTACTC	TGTTTAAGAGCCCGCTGAAG

Gene	Forward primer	Reverse primer
LTBP1	CACCTGCGATTGCTTTGATG	AACACTTGTAGGAACCATCGG
LTBP2	GGGCTCCTTCAACTGTCTATG	GGCTGTTTTCACACTTCCAG
MME	CAACCTCAGAAACAGCAACTTG	ACTTCTCGGATCTGTGCAATC
MMP2	ACCCATTTACACCTACACCAAG	TGTTTGCAGATCTCAGGAGTG
MMP9	CGAACTTTGACAGCGACAAG	CACTGAGGAATGATCTAAGCCC
MYL9	TGATTGACCAGAACCGTGATG	TGGTGAGGAACATGGTGAAG
OCLN	CAAAGGGAAGAGCAGGAAGG	TGTTGATCTGAAGTGATAGGTGG
OLFML2B	GCAACACCCTGGTAGAGTTC	TACGGGAGCTTGTAGGAATTG
PDPN	TCAGAAAGCACAGTCCACG	TATGATTCCAACCAGGGTCAC
PLAT	AAACCCAGATCGAGACTCAAAG	ACCCATTCCCAAAGTAGCAG
PLAU	GGGAGATGAAGTTTGAGGTGG	AGATGGTCTGTATAGTCCGGG
PLAUR	ACAACGACACCTTCCACTTC	GGCAGATTTTCAAGCTCCAG
RELA	AATGGCTCGTCTGTAGTGC	TGCTCAATGATCTCCACATAGG
RGS4	TTCATCTCAGTCCAGGCAAC	GGAATCCTTCTCCATCAGGTTG
RPL17	GACCTTGTGTCCAGCCCCAT	ACGAAAAGCCACGAAGTATCTG
SERPINE2	CATCGCCTCCCTGGTTTATAG	GTAGTCGTTGCTTTGCATGG
SLC22A4	TTCAGGACTCGGAATATTGCC	GAGAGGAAACAGTTCAGGTAGG
SNAI1	ACAAGCACCAAGAGTCCG	ATGGCAGTGAGAAGGATGTG
SNAI2	AGCATTTCAACGCCTCCA	GGATCTCTGGTTGTGGTATGAC
SPARC	CGACTCTTCCTGCCACTTC	GGAATTCGGTCAGCTCAGAG
TGFB1	GGAAATTGAGGGCTTTCGC	ATGAGAAGCAGGAAAGGCC
TGM2	CCACACCTACAAATACCCAGAG	CAAAGTCACTGCCCATGTTC
THBS1	CTCCCCTATGCTATCACAACG	AGGAACTGTGGCATTGGAG
THBS2	GACCTCATAGACAGCTTCGC	CACTAGGTGGACGTTCTGAAG
TPM1	AGTACAAAGCCATCAGCGAG	GGAGTCTTGGGAGAAGTGAAG
TPM4	CTCGTAAGCTGGTCATCCTG	ATGCAGCCTCCAGAGATTTC
VCAM1	TCTACGCTGACAATGAATCCTG	AGGGCCACTCAAATGAATCTC
WNT5A	TCGCCCAGGTTGTAATTGAAG	TGAGAAAGTCCTGCCAGTTG
ZEB1	AAGTGGCGGTAGATGGTAATGT	AAGGAAGACTGATGGCTGAAAT
ZEB2	AAGAGAACTTTTCCTGCCCTC	ATTTGAACTTGCGATTACCTGC