Marshall Supplemental Information

Supplemental Methods.

Reagents.

Unless otherwise stated chemicals were purchased from Sigma Aldrich (Steinheim, Germany). Antibodies were purchased from Abcam (Cambridge, UK) unless otherwise indicated. PCR primers were synthesized by Eurofins MWG Operon (Ebersberg, Germany) and Universal probes were from Roche (West Sussex, UK). The non-steroidal anti-androgen flutamide (1) was obtained from Tocris (Bristol, UK)

Western blotting

In order to analyse total concentrations of AR protein in hESC, cells were seeded at $2x10^5$/well in 6 well culture plates treatments with vehicle (ethanol) or DHT (10^{-8} M) for 8, 24 or 48h and performed in triplicate as detailed for mRNA quantification. The experiment was repeated 5 times. Cells were lysed with SBJ buffer [0.05M HEPES, 0.15M NaCl, 0.1M NaF, 0.01M $Na_2H_2P_2O_7$, 0.005M EDTA dehydrate, 0.25M sucrose, 1mM DTT, 1% Triton-X, 1mM Na_3VO_4]; a mini protease inhibitor tablet (Roche Diagnostics, Mannheim, Germany) and 1nM DHT were added immediately prior to addition to the cell monolayer. Samples from triplicate wells were pooled. Gel analysis was carried out using gels and reagents from Invitrogen (Paisley, UK). Total protein (20µg), was mixed with NuPAGE LDS sample buffer and NuPAGE reducing agent, heated for 10 minutes at 70°C and placed on ice before loading onto a 4-12% Bis-Tris gel. Molecular weight markers (Full Range Rainbow, Amersham Biosciences, Buckinghamshire UK) were added to one lane. Electrophoresis was carried out using MOPS SDS NuPAGE running buffer with added antioxidant at 200V for 50 minutes. Separated proteins were transferred onto Immobilon-FL membrane (Millipore, Ballerica, MA) overnight at 20V using transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol) thereafter membranes were incubated for 1h in 5% non-fat milk dissolved in TBS/0.05% Tween (BDH, Poole, UK). Membranes were incubated overnight at 4°C with rabbit anti-AR (1:200) and mouse anti-β-tubulin (1:1000) antibodies. After washing bound primary antibodies were detected by incubating the membrane for 1h in the dark at room temperature with labeled secondary

antibodies, (goat anti-rabbit IRDye 800 and goat anti-mouse IRDye 680) (both Licor Biosciences, Cambridge, UK). Antibodies were visualised using an Odyssey Infrared Imager (Licor Biosciences, Cambridge, UK) with Odyssey 3.0 software.

Legends for supplemental Figures

Supplemental Figure 1.

Full thickness sections from human endometrium recovered during the menstrual cycle stained with DAPI (blue) or antibody directed against AR (green); functional layer (top), myometrium (bottom).

Note intense immunostaining for AR in stromal cells in tissue adjacent to the uterine lumen in proliferative phase [green bar]. During the secretory phase the intensity of immunostaining in stromal cells is reduced in the upper [functional] layer and immunopositive cell nuclei are detectable in epithelial cells (arrows). In contrast, stromal cells in the basal compartment (asterisks) maintain intense immunopositive staining for AR throughout the cycle.

Supplemental Figure 2: Flow chart of bioinformatic data analysis.

Summary of steps taken to integrate and interrogate datasets from endometrium and prostate.

Supplemental Figure 3. Transfac analysis of promoter elements in putative endometrial androgen target gene set.

Genomic sequence (10Kb upstream and 4Kb downstream of the transcription start site) from each candidate was analyzed for predicted motifs for transcription factor binding sites (TFBS). Note the identification of multiple putative AR binding sites in each of the target gene set, consistent with data from the ChIP dataset (2) that was utilized during the initial bioinformatics analysis but also putative binding sites for other steroid receptors (ER, GR, PR) as well as transcription factors belonging to the AP1 and SP1 families that are know to participate in steroid-dependent regulation of gene expression (3).

Supplemental Figure 4. Immunoexpression of putative androgen-target genes in functional layer of human endometrium from the proliferative or secretory phases of the cycle.

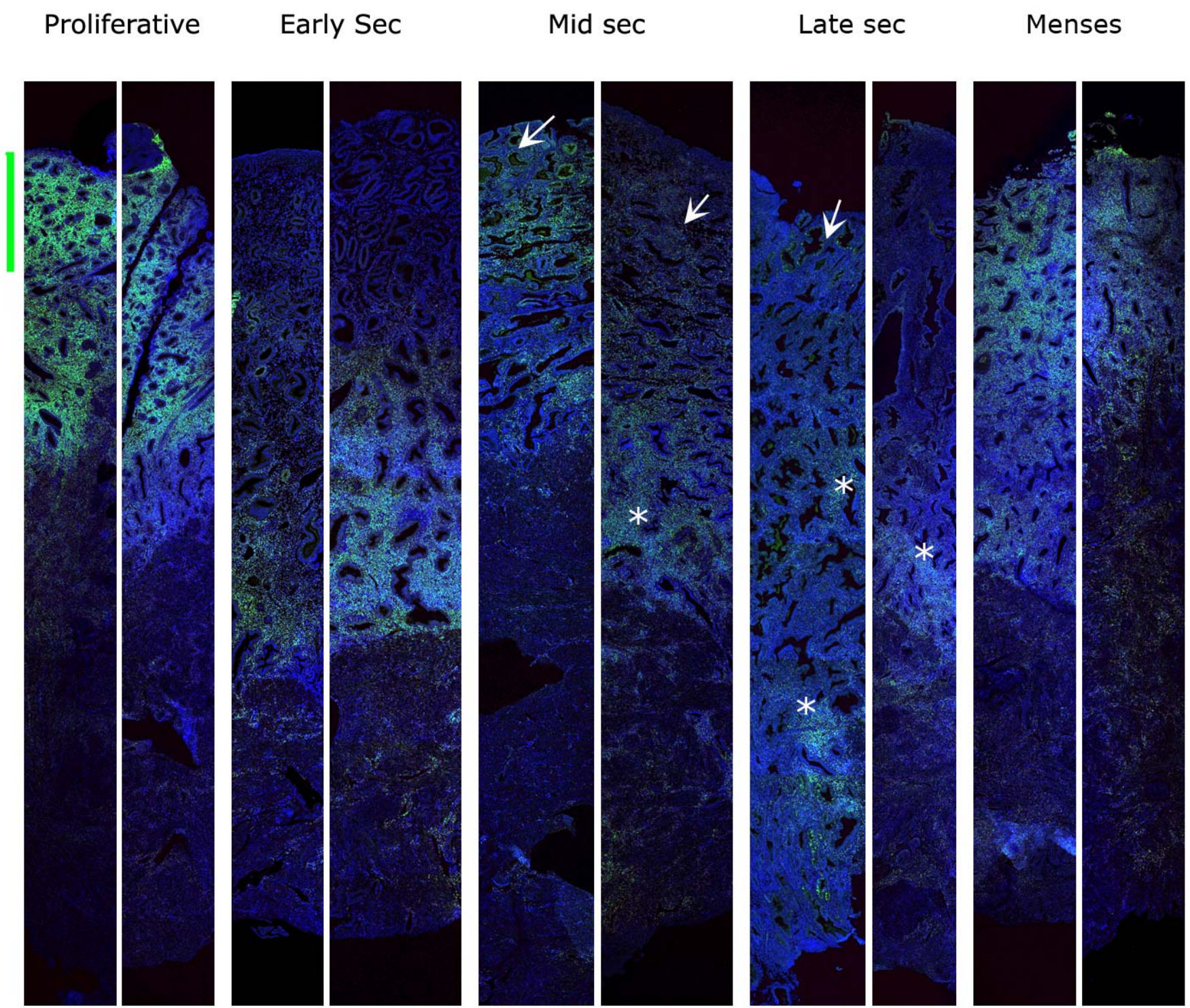
Middle panels show high power views of the stromal compartment during the proliferative phase. Insets in left hand panels are negative controls. Expression of PMAIP1, CD44, PRUNE 2, CITED2 and MAOA (not shown) was detected in both the stromal (s) and glandular (g) compartments.

Supplemental Figure 5. Characterisation of primary hESC and validation of expression of proteins encoded by androgen target gene set.

Cells were incubated with antibodies directed against (A) stromal cell marker (CD10) and (B) androgen receptor (AR). Cells at passage 4, note positive immunostaining in the cytoplasm and perinuclear regions respectively. Scale bars 20 μm. (C) Western blot confirming expression AR in primary hESC, AR upper band green, tubulin lower band red. Lanes 1-3 hESCs treated with DHT 10^{-8} M, lanes 4-6 hESC treated with ethanol, 3 separate experiments, M= molecular markers. Note increased concentrations of AR in cells treated with DHT in agreement with previous reports. Expression of proteins encoded by *PMAIP1* (D), *PRUNE2* (E) and *CITED2* (F) was confirmed. Note that whilst CITED2 was exclusively nuclear, PRUNE2 was cytoplasmic and PMAIP1 was in both the peri-nuclear and cytoplasmic compartments. Scale bars all 20 μm.

Supplemental references.

1. **Labrie F** 1993 Mechanism of action and pure antiandrogenic properties of flutamide. Cancer 72 Supplement 12:3817-3827
2. **Wang Q, Li W, Liu XS, Carroll JS, Janne OA, Keeton EK, Chinnaiyan AM, Pienta KJ, Brown M** 2007 A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. Mol Cell 27:380-392
3. **Safe S, Kim K** 2008 Non-classical genomic estrogen receptor (ER)/specificity protein and ER/activating protein-1 signaling pathways. J Mol Endocrinol 41:263-275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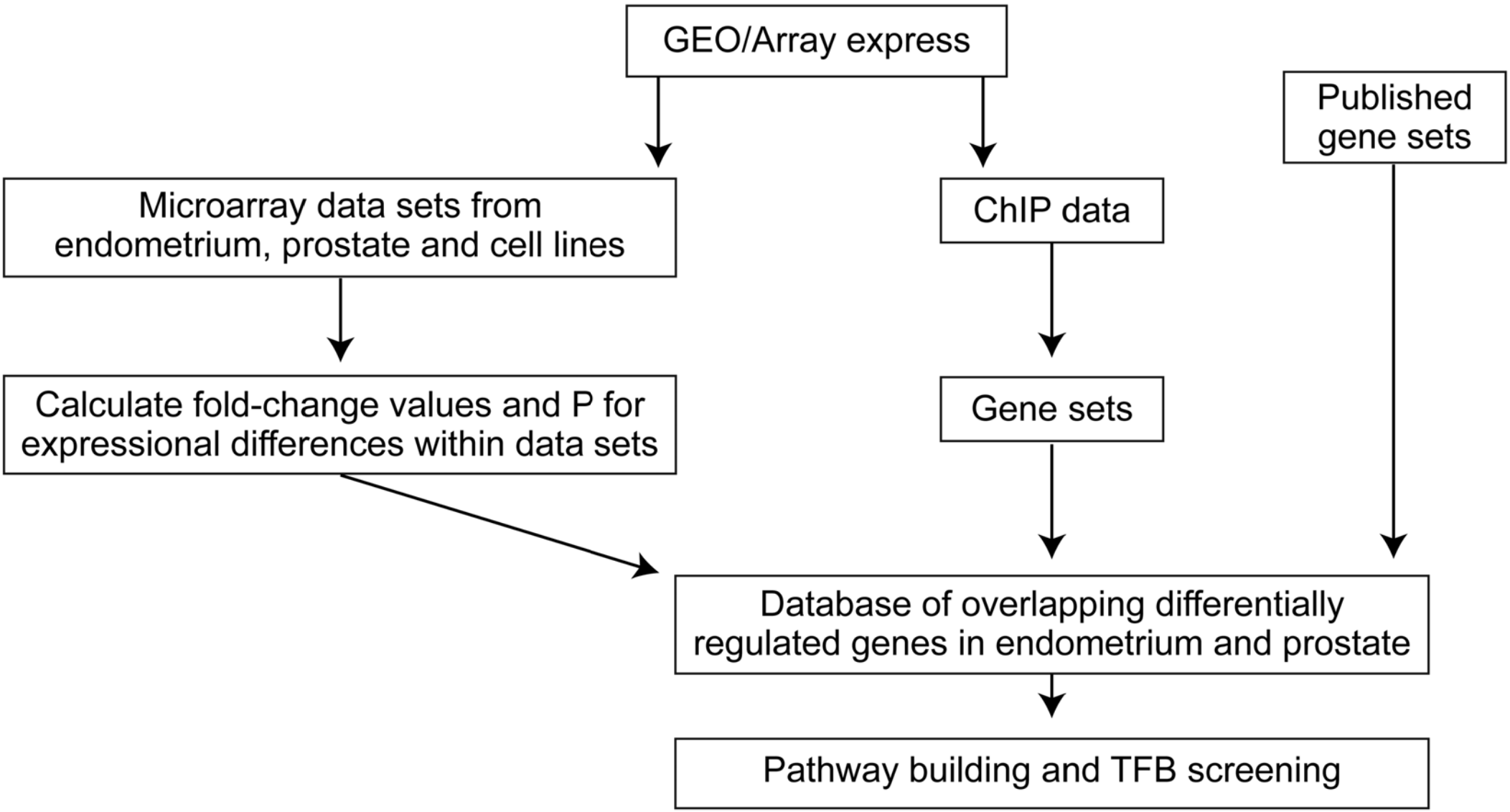
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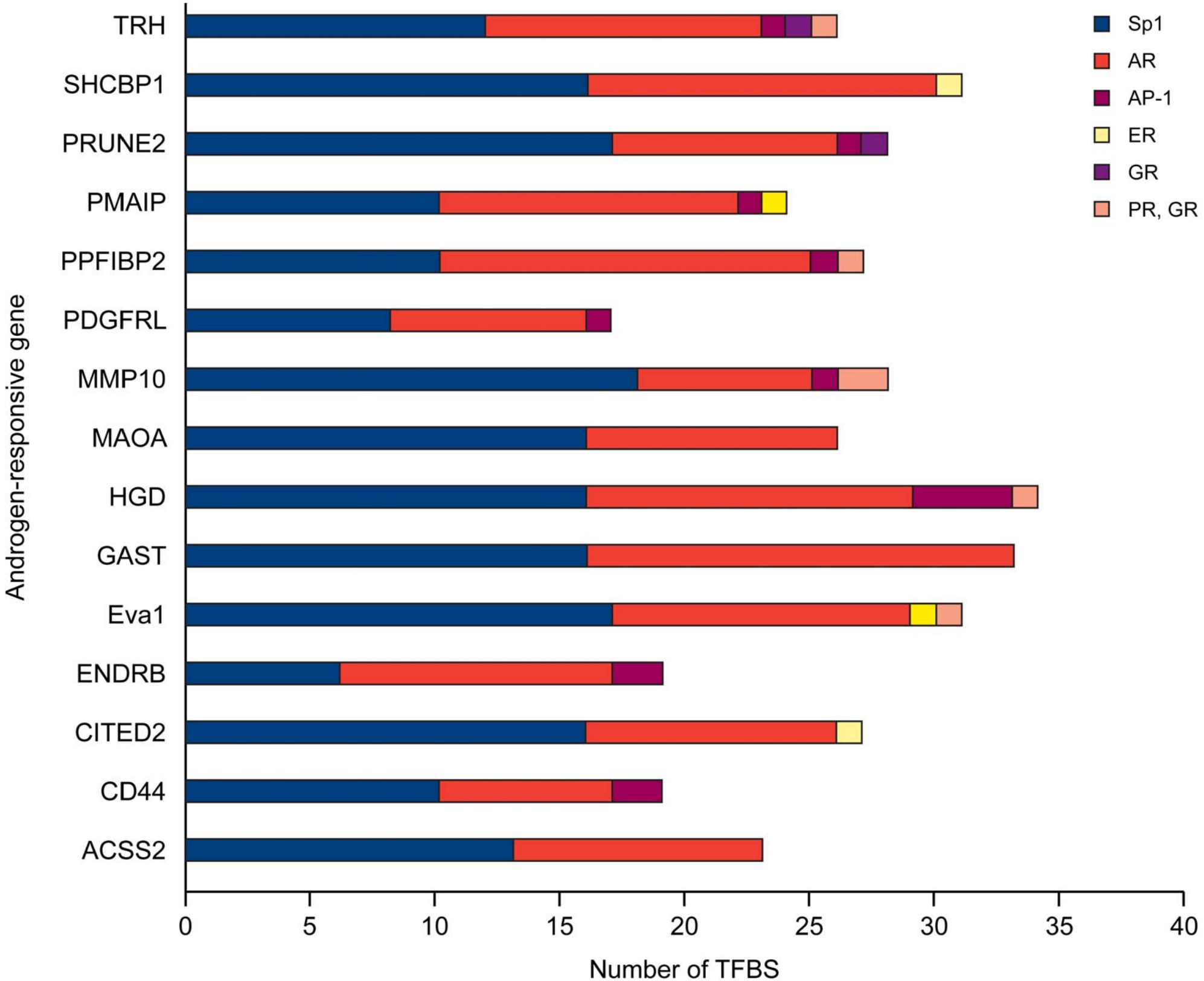
Gene sets
Database of overlapping differentially regulated genes in endometrium and prostate
Pathway building and TFB screening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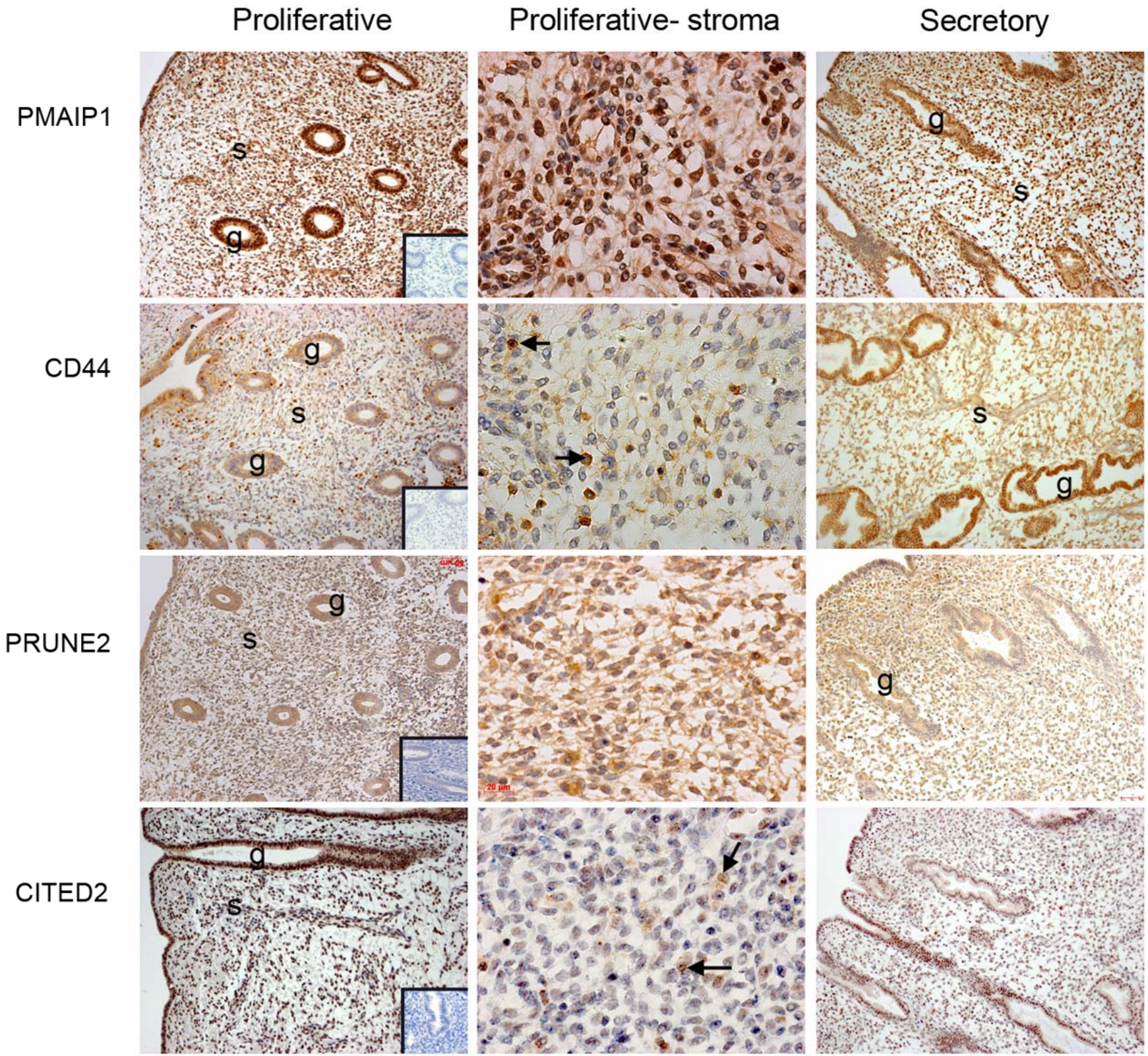
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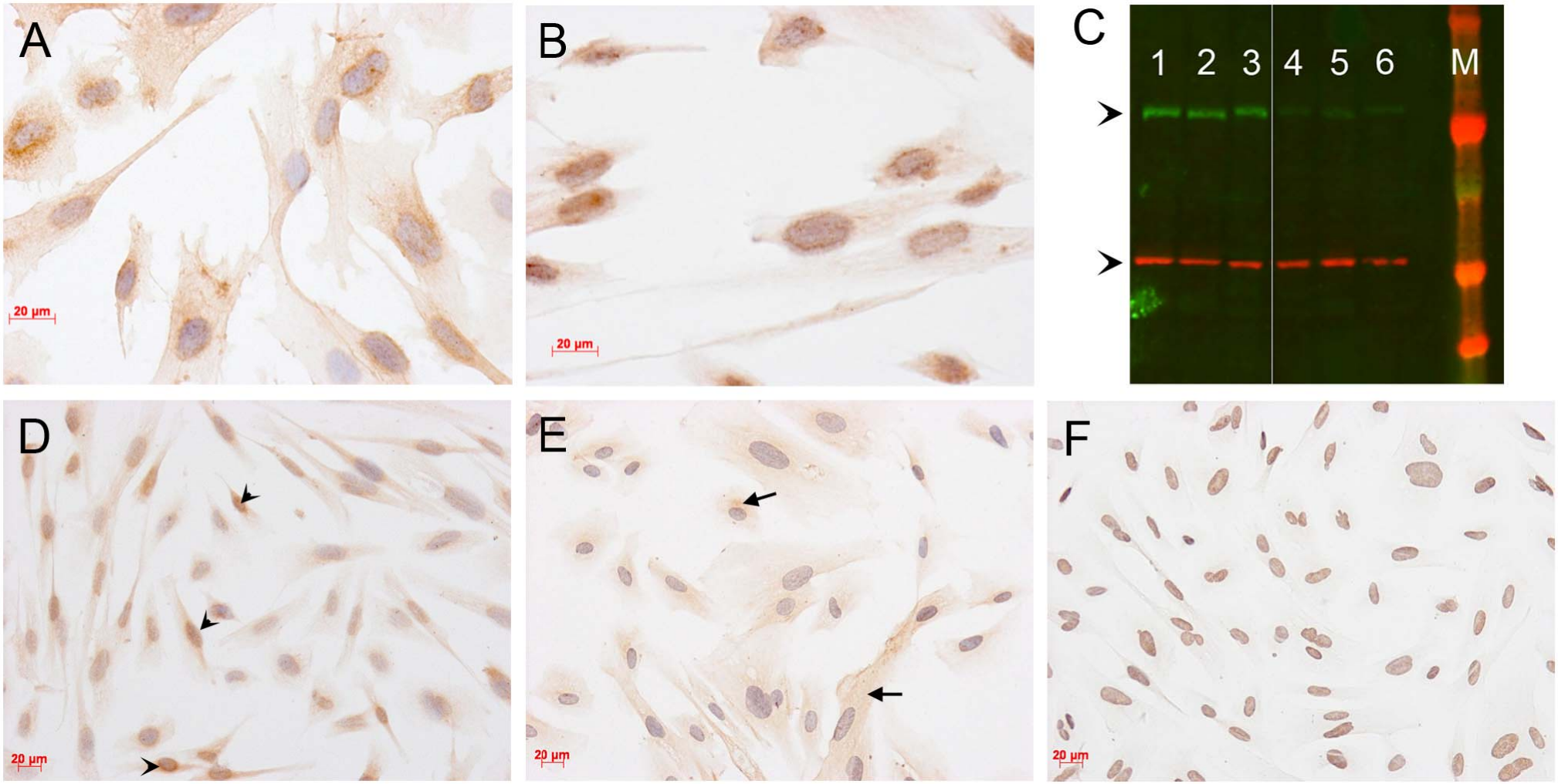
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Table 1. Gene sets interrogated to provide an endometrial androgen target gene set

Dataset	Tissue or cell type	Platform	Details	Reference
GSE4888+	Human endometrium	Array (Affymetrix)	Normal from menstrual cycle [extracted data based on genes expressed in P>MS]	(1)
n/a	Human endometrial stromal cells	Array (Illumina 6v1)	+/- siRNA against AR +decidualisation 72h	(2)
n/a	Rat uterus (ovx)	Array (Affymetrix)	+/- Mibolerone (AR agonist)	(3)
GSE7868	LnCAP cells (prostate)	ChIP	+/- DHT, time course	(4)
E-TABM-233*	LnCAP cells DuCAP cells (prostate)	AR ChIP-ChIP	+/- R1881, 1 hour	(5)

+ Accessed from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/)

* Accessed from Array Express (http://www.ebi.ac.uk/microarray-as/ae/)

1. Talbi S, *et al.* (2006) Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology* 147(3):1097-1121.
2. Cloke B, *et al.* (2008) The androgen and progesterone receptors regulate distinct gene networks and cellular functions in decidualizing endometrium. *Endocrinology* 149(9):4462-4474.
3. Nantermet PV, *et al.* (2005) Androgenic induction of growth and differentiation in the rodent uterus involves the modulation of estrogen-regulated genetic pathways. *Endocrinology* 146(2):564-578.

4. Wang Q, *et al.* (2007) A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. *Mol Cell* 27(3):380-392.
5. Massie CE, *et al.* (2007) New androgen receptor genomic targets show an interaction with the ETS1 transcription factor. *EMBO Rep* 8(9):871-878.

Table 2. Primers and probes for quantitative PCR

Target Gene	Accession no.	Primer	Primer	Roche probe
ACSS2	NM_018677	CTTTGTCACCTTGTGTGATGG	ATGGGGCCAATCTTTTCTCT	41
AR	Nm_000044	GCCTTGCTCTCTAGCCTCAA	GTCGTCCACGTGTAAGTTGC	
CD44	NM_000610	GACACCATGGACAAGTTTTGG	CGGCAGGTTATATTCAAATCG	13
CITED2	NM_006079	ACTACATGCCGGATTTGCAC	CAATCTCGGAAGTGCTGGTT	49
ENDRB	NM_000115	ATCGTCATTGACATCCCTATCA	GCTTACACATCTCAGCTCCAAA	53
HGD	NM_000187	CCAGGTGGTTACACGGTCAT	TTGAACGGGGAGACATCC	37
MAOA	NM_000240	GGCCACATGTTCGACGTAGT	TTTGGCAGCAGATAGTCCTG	10
MMP10	NM_002425	CAAAAGAGGAGGACTCCAACA	TTCACATCCTTTTCGAGGTTG	61
PDGFL	NM_006207	GTCAAGTACCAGCTGCTCTAC	GCCAAGATGGTTGTTGAG	48
PMAIP1/NOXA	NM_021127	GGAGATGCCTGGGAAGAAG	CCTGAGTTGAGTAGCACACTCG	67
PPFBP2	NM_003621	GAATGGAAGCTAAAGGCCACT	ATCTTTCAGGGCCACCTGTT	41
SHCBP1	NM_024745	CCCTTGCAATGGAGCATGT	TTCATCCTCCTCTTCTTCATCC	25

Target Gene	**Accession no.**	**Commercial Supplier**	**Catalogue number**
MPZL2/EVA1	NM_005797	ABI inventory	Hs00170684_m1
PRUNE 2/BMCC1	NM_138818	ABI inventory	Hs00322421_m1
GAST	NM_000805	ABI inventory	Hs0099489_m1
TRH	NM_007117	ABI inventory	Hs01099852_g1

Table 3. Antibodies, dilutions and suppliers

Abcam, San Francisco, CA; Imgenex, San Diego, CA; Thermoscientific, Fremont, CA;

Protein	Raised in	Dilution for tissue sections	Dilution for primary ESC	Supplier
AR	rabbit	1:100	1:20	Abcam
CD10	mouse	N/A	1:50	ThermoScientific
CD44	rabbit	1:200	1:20	Abcam
CITED2	mouse	1:1000	1:50	Abcam
MAOA	goat	1:300	1:100	Abcam
PMAIP1	mouse	1:200	1:50	Imgenex
PRUNE 2	goat	1:500	1:200	Abcam