## **Supplementary materials**

## Cell-type specific analysis of physiological action of estrogen in mouse oviducts

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**Figure S1.** IHC Staining of ESR1, Ki67, and PGR in uterine and oviduct samples collected from ovariectomized females treated with Veh or  $E_2$ . **A.** Staining of Ki67 and PGR in uterine samples after Veh or  $E_2$  treatment. **B-D.** Lower magnification of ESR1, Ki67, and PGR staining images from Fig. 1 of oviduct samples treated with Veh or  $E_2$ . All scale bars = 50  $\mu$ m.



Figure S2. Dot plots of top 10 differentially expressed genes in cell clusters comparing between Veh vs. E<sub>2</sub> treatment or InfAmp vs. IsthUTJ. A. Marker genes in all cell clusters including 0/ciliated (InfAmp), 1/secretory (InfAmp), 2/secretory (IsthUTJ), 3/fibroblast *Pdgfra*<sup>-</sup>, 4/muscle, 5/epithelial (subset), and 6/fibroblast *Pdgfra*<sup>+</sup> cell clusters. Epithelial cells are indicated by *Epcam*<sup>+</sup> and *Krt8*<sup>+</sup>, mesenchymal cells (fibroblasts and muscles) by *Vim*<sup>+</sup>, *Pdgfra*<sup>+</sup>, and *Twist2*<sup>+</sup>, muscle cells by *Act2a*<sup>+</sup> and *Myh11*<sup>+</sup>, ciliated cells by *Foxj1*<sup>+</sup> and *Ccdc153*<sup>+</sup>, secretory cells by *Ovgp1*<sup>+</sup>, and endothelial cells by *Pecam1*<sup>+</sup>. *Krt19* was expressed in both epithelial and fibroblasts. *Sprr2f, Serpina1e*, and *S100g* were expressed specifically in 5/epithelial cell subset.
B-K. The top 10 region- and E<sub>2</sub>-specific genes for each cell clusters are shown.



Figure S3. UCSC Genome Browser screenshots of ESR1 binding sites using ESR1 chromatin immunoprecipitation-sequencing (ChIP-seq) analysis from mouse uterine dataset (GSE36455)  $^{50,51}$  that were treated with E<sub>2</sub> (blue tracks) or Veh (pink tracks) for 1 hr. Significant increase of ESR1 binding sites (red arrows) on the promotors, TSSs, and intragenic regions were observed in a subset of genes identified in scRNA-seq analysis in the mouse oviduct to be enriched after E<sub>2</sub>

compared to Veh treatment in A. all cell clusters, B. ciliated cells, C. secretory cells, D. epithelial cell subset, E. fibroblasts, and F. muscle cells. Note that scales on y-axes for Veh and  $E_2$  tracks are actual, not fixed scales.



**Figure S4:** Dot plots of genes in (**A-B**) protease and (**C-D**) protease inhibitor family in cells isolated from Veh and E<sub>2</sub>-treated samples; including 0/ciliated, 1/secretory (InfAmp), 2/secretory (IsthUTJ), and 3/fibroblast *Pdgfra*<sup>-</sup>, 4/muscle, 5/epithelial (subset), and 6/fibroblast *Pdgfra*<sup>+</sup> cell clusters.



**Figure S5.** Dot plots of marker genes for each cell cluster from estrus and Veh- vs. E<sub>2</sub>-treated datasets combined, including 0/secretory (InfAmp), 1/ciliated (InfAmp), 2/secretory (IsthUTJ), 3/epithelial (subset), 4/fibroblast *Pdgfra*<sup>+</sup>, 5/fibroblast *Pdgfra*<sup>-</sup>, and 6/muscle cell clusters.



**Figure S6.** UMAP plot of clusters of cells from human fallopian tubes with different cell-type markers; epithelial cells ( $EPCAM^+$ ), fibroblast ( $PDGFRA^+$ ), muscle cells (MYH11), endothelial cells ( $PECAM1^+$ ), immune cells ( $CD52^+$ ), secretory epithelial cells ( $OVGP1^+$ ), ciliated epithelial cells ( $FOXJ1^+$ ), epithelial cells in the infundibulum and the ampulla identified in mice ( $PDXK^+$ ), fibroblast and muscle cells identified in mice ( $DCN^+$ ), secretory epithelial cells in the isthmus identified in mice ( $SERPINA1^+$ ), epithelial cells in the UTJ identified in mice ( $CRABP2^+$ ).

## Supplementary Tables:

**Table S1:** Top 25 genes and *p*-values (*p*) enriched in each cell cluster identified in Veh- and E<sub>2</sub>treated samples; 0/ciliated (InfAmp), 1/secretory (InfAmp), 2/secretory (IsthUTJ), 3/ fibroblast *Pdgfra*<sup>-</sup>, 4/muscle, 5/epithelial (subset), and 6/fibroblast *Pdgfra*<sup>+</sup> clusters.

	0/	1/						4/					
0/ gene	n 0/	1/ gene	1/n	2/ gene	2/ n	3/ gene	3/ n	3/ <i>p</i> gene		5/ gene	5/ n	6/ gene	6/ n
o/ gene	P	gene	- <i>P</i>	-/ gene	<i>-, p</i>	o, gene		Sene	6 34E	o, gene	8.94E	o, gene	4 96E
Ccdc153	0	Ovgp1	0	Fxvd3	0	Igfbp6	0	Acta2		Sprr2f -95		Dcn -67	
		o or or o	-			-8/*F*	, , , , , , , , , , , , , , , , , , ,		6.74E	~	3.92E		2.29E
Tppp3	0	Aldoc	0	Fxyd4	0	Upk3b	0	Tagln	-113	Car2	-91	Gsn	-64
								Ŭ	7.06E		2.96E		5.03E
Elof1	0	Ier3	0	Wfdc2	0	Dcn	0	Myl9	-113	Tmem213	-78	Lgals1	-47
									1.72E		1.13E		2.34E
Chchd10	0	Gstm2	0	Id2	0	Rarres2	0	Tpm2	-110	Gm28940	-75	Serpinf1	-45
4933434E2				Slc25a4					3.92E	4933408J1	1.46E		3.62E
ORik	0	Plet1	0	8	0	Gas6	0	Tpm1	-110	7Rik	-75	Cd63	-45
									1.25E	A530040E	1.60E		4.50E
Mtl	0	Rpl32	0	Calb1	0	Aebp1	0	Myl6	-109	14Rik	-75	Lum	-44
~				~		~			9.06E		2.43E		5.74E
Gm19935	0	Plat	0	Grn	0	Serping1	0	Mustn1	-104	Zfp366	-75	Mirg	-41
T	0	D 1	0	Pdzklip	0	<i>C</i> 2	0	C 1	1.23E	C= 17(()	2.6/E	Mand	7.38E
1mem212	0	KCN1	0	1	0	Csrp2	0	Csrp1	-101 5.70E	Gm4/002	-/5	Myrji	-41 7.52E
Eam 182h	0	Dn114	0	10000	0	Cfh	0	Elma	5./0E	Magad	2.86E	016.945	/.52E
Fum1050	0	Крі14	0	Апрер	0	Cjn	0	гти	-94 2.94E	npus4	-73 2.04E	0117845	-41 7.52E
Unrah 3	0	Krt18	0	Galm	0	Spare	0	Sparell	2.04L 03	Gm11112	5.04E	Fornl	7.52E 41
v preos	0	K/110	0	Guim	1 16F	Spure	0	Spurcii	1.88F	0117712	3 10F	F030018	7 53F
Foril	0	Kctd14	0	Cndn?	-302	C3	0	Mvh11	-89	Gm29325	-75	B13Rik	-41
1700016K1	Ŭ	iiciui i	0	Chup2	6.99E	05	0	myn11	6.44E	0112/020	3.12E	Gm2901	7.58E
9Rik	0	Rps5	0	Rnf128	-296	Ogn	0	Mvlk	-89	Gm13963	-75	0	-41
1110017D1		I ···			1.08E	- 8 -			8.24E		3.13E	-	7.79E
5Rik	0	Emb	0	Ybx1	-288	Upk1b	0	Des	-88	Col28a1	-75	Tbr1	-41
		Seleno			8.76E				1.01E		3.24E		7.86E
Dynlrb2	0	т	0	Ifitm 1	-281	Nbl1	0	Lgals1	-87	Gm26945	-75	Tdrd5	-41
					1.79E				6.19E		3.24E		7.96E
Gm867	0	Rps9	0	Cldn10	-274	Nkain4	0	Vim	-87	Gm40190	-75	Ecel1	-41
1700007K1					3.98E				5.18E		3.38E	AI18237	8.03E
3Rik	0	Rps27a	0	Crabp2	-272	Cldn15	0	Cald1	-83	Gm16630	-75	1	-41
<i>a</i> . 10.			0	<i>a i</i>	1.01E			<i>a</i>	9.12E	G. 2000.6	3.77E	A930009	8.10E
Cfap126	0	Eeflal	0	Gstol	-271	Rspol	0	Crip1	-83	Gm38096	-75	AISRik	-41
<i>C L</i> 1	0	D 111	0	9530014 D07D:1	1.08E	<i>C</i> 1	0	a · 2	3.52E	0.55	3.77E	Gm3904	8.24E
Calm1	0	RplII	0	BU/Rik	-2/0	Gasi	0	Cavins	-/0	Gpr55	-/3	3	-41
Sector	0	En 1	0	4833423 E24D:L	3.04E	D 2	0	Dalld	3.30E	Daal10h	3.80E	76-107	8.48E
Snin	0	гпі	0	E24Kik	-200 1.60E	D2m	0.00E	гини	-/3 6.41E	Kasilob	-73 4 14E	ZJP407	-41 0.07E
Dnah5	0	Rns15a	0	$Cd\theta$	-259	Iaf1	+00	Acth	-73	Gm5160	4.14E	Gnall	9.97E
Dhuno	Ŭ	npsisu	0	Cu>	1.46E	15/1	4.65E	new	1.43E	4430106G	4 16F	Iaky13-	1.01E
Tm4sf1	0	Rn l 23	0	Tspan8	-217	Col3a1	-307	Mfoe8	-69	13Rik	-75	1gkv15-	-40
111115/1	v	10125	3.59E	Ispano	1.30E	corsur	2.51E	11/800	3.11E	15100	4.29E	55 1	1.04E
Dvnll1	0	Slc1a3	-308	Klf5	-214	Efemp1	-303	Igfbp7	-67	Serinc4	-75	Mcmdc2	-40
, <u>-</u>			5.41E	<i>y</i> -	8.55E	J. 17-	1.55E	GV T	7.06E		4.40E	Gm4062	1.09E
Rsph1	0	Rps4x	-306	Pla2g4a	-205	Cavin1	-295	Malat1	-64	Slc47a1	-75	1	-40
		1	6.17E		1.03E		9.40E		4.00E		4.60E	Gm4091	1.10E
Nudt4	0	Cd81	-305	Sox17	-202	Tmsb10	-288	Calm2	-60	Gm33280	-75	0	-40
	1		4.42E		7.82E		1.26E		2.39E		4.80E		1.10E
Dnajc7	0	Rpl10	-303	Car2	-195	Crip1	-285	Actn1	-59	Rgs6	-75	Slc13a1	-40

**Table S2:** Top 500 genes and *p*-values differentially expressed in Veh- vs. E<sub>2</sub>-treated samples or InfAmp vs. IsthUTJ in all 7 cell clusters combined, 0/ciliated, 1 and 2/secretory, 3 and 6/fibroblast, 4/muscle, and 5/epithelial (subset) cell clusters. Please refer to file Table S2.xlsx.

**Table S3:** Gene ontology (GO) biological processes (BPs) enriched in Veh- vs. E<sub>2</sub>-treated samples or InfAmp vs. IsthUTJ in all 7 cell clusters combined, 0/ciliated, 1 and 2/secretory, and 3 and 6/fibroblast. Data were analyzed using the top 500 genes identified in Table S2. PANTHER overrepresentation test was used as analysis type with Fisher's test, FDR correction was included when applicable. There were no significant BPs enriched in 4/muscle and 5/epithelial (subset) cell clusters. Please refer to file Table S3.xlsx.

**Table S4:** Top 1,000 genes and GOBPs enriched in endogenous (estrus) vs. exogenous E<sub>2</sub>treated samples in all 7 cell clusters combined. Data were analyzed using the top 1,000 genes identified. PANTHER overrepresentation test was used as analysis type with Fisher's test and FDR correction. Please refer to file Table S4.xlsx.

0/								4/						7/		8/		9/		10/	10/
gene	0/ p	1/ gene	1/ p	2/ gene	2/ p	3/ gene	3/ p	gene	4/ p	5 gene	5/ p	6/ gene	6/ p	gene	7/ p	gene	8/ p	gene	9/ p	gene	р
0	2.27E-		2.29E-	0	1.82E	KRTAP5-	1.76E	HLA-	7.25		1.19E-		1.37		4.46E	PTCR	2.68	CCL2	2.42E	0	2.40
IL7R	179	SPARCL1	182	WFDC2	-165	ASI	-130	DPB1	E-81	LYZ	63	GSN	E-21	FTH1	-15	Α	E-13	1	-11	IFI27	E-07
	1.52E-		3.18E-		7.30E		3.25E	HLA-	2.68	AC02065	4.38E-		2.07		5.89E		2.75	CLD	2.64E	EGFL	2.85
CD69	151	SFRP4	176	CLU	-165	HMMR	-130	DPA1	E-80	6.1	63	CFD	E-21	SRGN	-15	GZMB	E-13	N5	-11	7	E-07
	1.01E-		4.79E-		2.83E		6.17E	HLA-	6.77		1.62E-		7.21		1.50E	PPP1R	2.84	CAVI	2.92E		3.15
KLRB1	140	DCN	170	KRT18	-161	BNIPL	-130	DQA1	E-76	CSTA	57	DCN	E-21	EMP3	-14	14B	E-13	N2	-11	LIFR	E-07
	3.61E-		6.22E-		9.24E	RNF217-	8.52E	HLA-	1.43		2.03E-		7.73	SCNN1	1.32E	JCHAI	2.85		2.94E		3.39
BTG1	124	Cl1orf96	163	ELF3	-153	ASI	-130	DRB1	E-72	S100A9	57	IGFBP6	E-21	G	-13	N	E-13	TFF3	-11	CAVI	E-07
	2.12E-		2.72E-		3.19E	TMEM25	9.70E		6.17		2.19E-	SERPIN	9.13	DLGAP	1.35E		2.94		4.17E	MT1	3.76
CD52	120	IGFBP4	162	CLDN4	-147	5A	-130	CD74	E-71	TYROBP	53	GI	E-21	2	-13	IGKC	E-13	TFPI	-11	M	E-07
	4.62E-		1.93E-		1.06E		1.45E	HLA-	1.01		1.94E-		3.92	SLC30A	1.37E		3.28	SPTB	8.16E		4.09
CD3E	112	IGFBP7	161	KRT8	-146	TEPP	-129	DRA	E-70	CTSS	52	MGP	E-20	2	-13	MZB1	E-13	NI	-11	EMP1	E-07
	3.31E-		1.11E-		7.82E		1.46E	HLA-	3.01	-	1.01E-		6.66		1.40E		3.48	S100	1.97E	RNAS	6.45
RPS12	110	LGALSI	160	SLPI	-145	PRCI	-129	DMA	E-69	FTL	50	FBLN5	E-20	NCAPH	-13	PLD4	E-13	A10	-10	EI	E-07
CDAD	5.79E-	GEDDI	2.89E-	CDICDA	3.56E	ND 1777 (	1.57E	HLA-	1.09	<i></i>	3.00E-	DI (CO	1.04	G100.45	1.56E	10 55	3.54	WI FL	5.98E	VED	6.53
CD3D	110	SFRP1	158	CRISP3	-123	WNT7A	-129	DQBI	E-67	TKT	49	PLAC9	E-19	S100A5	-13	IRF/	E-13	KLF4	-10	NFIB	E-07
TSC22	1.14E-	CDAL	2.95E-	WDTIO	3.66E	** • •	1.82E	MS4A6	6.83	1.0.11.00	3.47E-		1.80	(CD) (	1.57E		4.44	HLA-	7.85E	SOCS	7.44
D3	109	CD81	155	KRT19	-119	ILII	-129	A	E-60	LGALS2	49	LTBP4	E-19	ASPM	-13	TTM2C	E-13	E	-10	3	E-07
11 ( D.G.2	4.66E-	TD1 (2	3.92E-	ELV (CI	5.18E	AL137077	2.25E	GPRI8	4.66	G100.40	5.06E-	CDE	2.98	71121	1.58E	CL ICA	5.29	VI DA	1.15E	GNG	7.46
HARS2	106	IPM2	154	ELN-ASI	-116	.2	-129	3	E-59	S100A8	49	CPE	E-19	ZHXI	-13	CLIC3	E-13	KLF2	-09		E-07
LINC02	5.22E-		4./3E-	AC02090	/.50E	AC09/359	2.86E	SPCN	7.18	DCAD	6.4/E-	SEMA3	5.79	71157()	1.58E	IDEO	7.06	AKAP	1.41E	1M4S	9.85
315	106	RARRES2	155	9.3	-110	.2	-129	SKGN	E-59	PSAP	48	C	E-19	ZNF/03	-13	IKF8	E-13		-09	F I	E-07
	5.32E-	SELENO	8.09E-	AC11939	1.31E	100 120	3.03E		2.08	TUDCI	9.85E-	GEDDO	1.50	AF12/5	1.59E	CNUL	1.20 E.12	GNG	1.86E	VDV2	1.34
KPLP1	106	P	151	0.2	-115	ADRA2B	-129	AIF1	E-38	THBSI	48	SFRP2	E-18	//.4	-13	GNAIS	E-12		-09	IBAS	E-06
AC0229	8./4E-	TCLO	1.41E-	AC01163	2.10E	KONIA	3.38E		5.39		2.12E-		1.80	270	1.64E	GRAS	1.39	LMO	1.8/E	CCD	2.40
10.1 C10.ruf	100	IGM2	14/ 1.26E	2.1	-115	ACNJ4	-129	TVDOD	E-38	AIFI	4/ 1.20E	TIMPS	E-18	3/9	-13	P SEC(1	E-12	2	-09 2.22E	COSB	E-00
C100rj	1.00E- 105	DTCDS	1.20E- 145	EDCAM	3.13E	SUOV	3.42E		5.59 E 55	10182	1.20E-	MEAD5	2.89 E 19	CDVID	1.00E	DEC01	1.00 E 12	VDV2	2.22E	1211	2.44 E 06
91	2.120	FIGDS	145 6.47E	EFCAM	-115	SUUA	-129	Γ	E-33	AF 152	40 1.74E	MFAF J	2.01	4 <i>C</i> 0020	-13		E-12	I DA J V AN	-09 2.12E	A2M TSC2	E-00
EDC4	3.12E- 105	J	0.4/E-	AVADE	4.00E	WAIT7P	3./IE 120	SCVI	1.45 E 54	TVMD	1./4E-	CCDC8	2.91 E 19	AC0020	1.09E	2	3.00 E 12	NAN V2	3.12E	15C2 2D1	5.44 E 06
EDC4	105 5.54E	I SELENO	145 1.40E	AKAF0 4107964	-115 1.90E	WINI/D	-129 2 79E	SUKI	E-34	TIME	40 1.74E	0	E-10	91.2	-13 1.72E	5	E-12	MMD	-09 4 21E	2D1	2.50
EARDA	5.54E- 105	M	1.49E- 120	AL0/804	1.60E	I AMD3	3.78E	CTSZ	1.// E 54	VCAN	1./4E- 46		7.77 E 18	CYCLO	1./3E 12	ADEC	7.02 E 12	MMA N1	4.21E	וחו	5.39 E 06
TADI 4	5 71E-	11/1	1.70E-	4.2	3 /1F	BAGAINT	-12) 4.48E	CISL	7.50	VCAN	1.61E-	ADIIID	1.71	4C0076	-13 173E	AKEU	0.05	NNM	-0) 1 12F	ID1	3.63
KCNE5	105	TAGIN	135	11	-114	3	-120	RGS10	F-54	CVBB	1.0112-	IIAPI	F-17	34.3	-13	NR3C1	F-12	T	-00	GSN	5.05 E-06
KCWEJ	1.03E-	INGLN	135 185E-	1.1	-114 3.63E	5	5 16E	HI 4-	1 32	CIDD	3 58E-	UAI I	2.15	DMERP	-13 1.77E	WASCI	0.71	ARIA	6.76E	SPRV	3.67
KIFC1	104	NBL1	131	03	-114	TSFM	-129	DMB	F-53	\$100412	44	ACKR3	E-17	1	-13	CD74	E-12	A A	-09	1	E-06
iiii ci	1.68E-	NBEI	2.67E-	0.5	5.65E	151 111	5 28E	OGERI	2.92	TNERSE	1.11F-	nemits	2.85	F4M86	1 78E	CDTT	1.04	4DIR	7 24F	IIAC	3.82
MATN4	104	RAMP1	129	DNAH17	-114	DCAF4	-129	1	E-53	$\frac{1}{R}$	43	\$100410	E-17	R2	-13	AIRG	F-11	F	-09	A	E-06
MPDU	5 28E-	TO INT I	2 44E-	AC02723	6.17E	AC010618	5 30E	- 1	9 54	10	8.66E-	5100110	4 52	TMEM2	1.82E	PMEP	1 72	ANXA	8.43E	SOXI	4 04
1	104	<i>IGFBP6</i>	125	7.2	-114	.3	-129	RGS1	E-53	UPP1	43	COL6A2	E-17	55B	-13	Al	E-11	2	-09	7	E-06
-	5.77E-		9.18E-	SLC35F	9.72E	AC051619	5.46E		1.12		1.94E-	2010112	4.60		1.83E		1.74	IGFR	1.07E		6.49
KCNB2	104	IFITM3	125	1	-114	.5	-129	MS4A7	E-50	MAFB	41	COL1A2	E-17	ZBTB12	-13	PLP2	E-11	P7	-08	ID3	E-06
AP0007	8.87E-	LAPTM4	3.76E-	-	1.34E		5.58E	FCERI	2.49	HLA-	5.73E-		4.66		1.88E	LDLR	2.00		1.13E	THB	7.44
87.1	104	A	124	IL13	-113	SPATA12	-129	G	E-50	DRA	41	IGFBP5	E-17	RSPO3	-13	AD4	E-11	LAYN	-08	D	E-06
AC0076	9.43E-		3.63E-	TACSTD	1.48E	TMEM25	5.69E		6.93		7.01E-		5.47	AC1185	1.88E		3.06	RAB1	1.13E	ADIR	1.01
20.2	104	PGRMC1	123	2	-113	4-AS1	-129	CD83	E-48	LAPTM5	41	PMP22	E-17	53.1	-13	RPS3A	E-11	1A	-08	F	E-05
	-				-		-		-						-		· · · · ·				