

Cell Reports, Volume 39

Supplemental information

Stromal AR inhibits prostate tumor progression by restraining secretory luminal epithelial cells

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Supplemental Figures

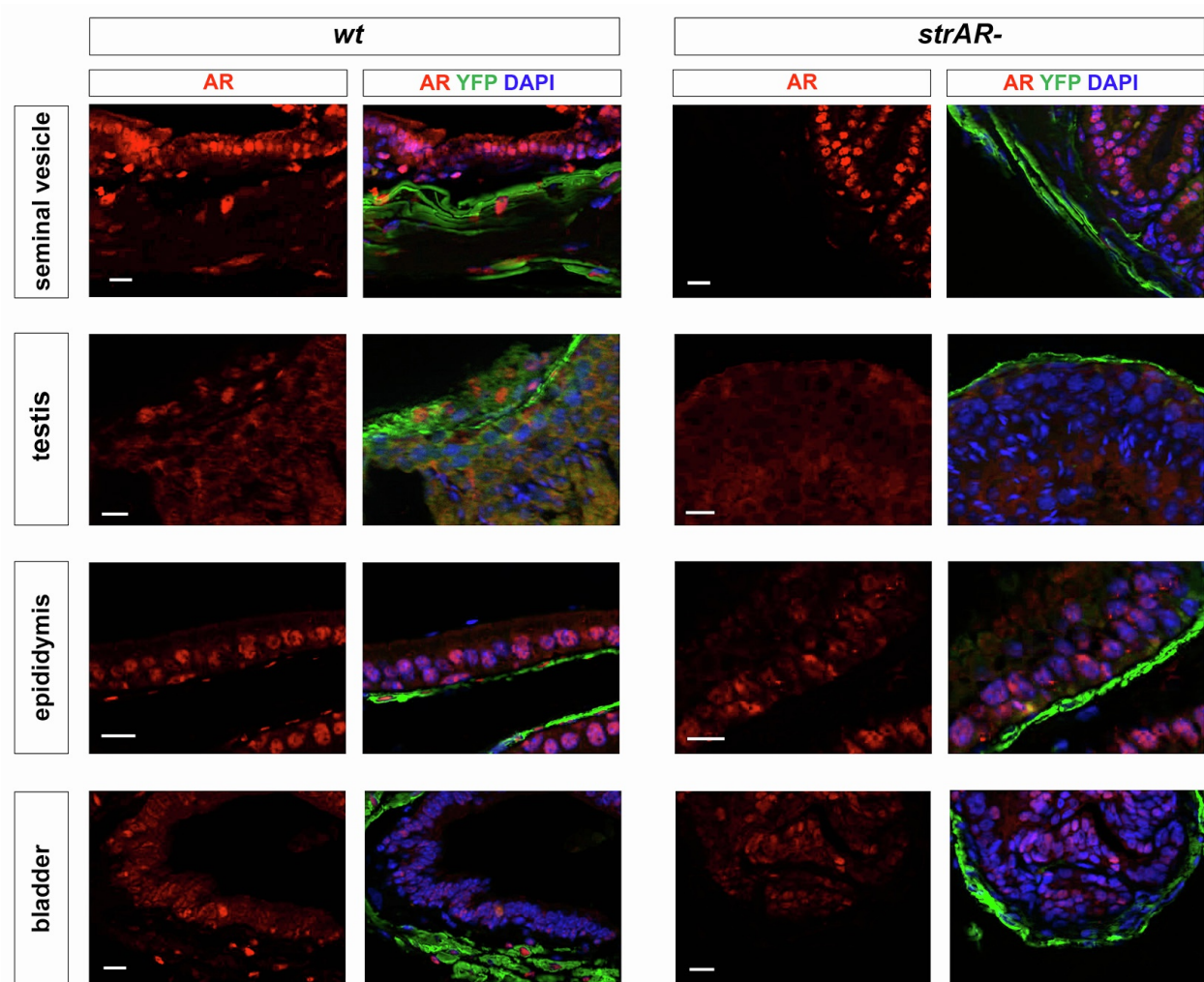


Figure S1. *Myh11-CreER^{T2}* deletes stromal AR in other tissues. Related to Figure 1. IF staining showing that AR (red) was deleted in SMCs (green) of the seminal vesicle, testis, epididymis, and bladder in *str^{AR-}* mice. Scale bar, 20 μm .

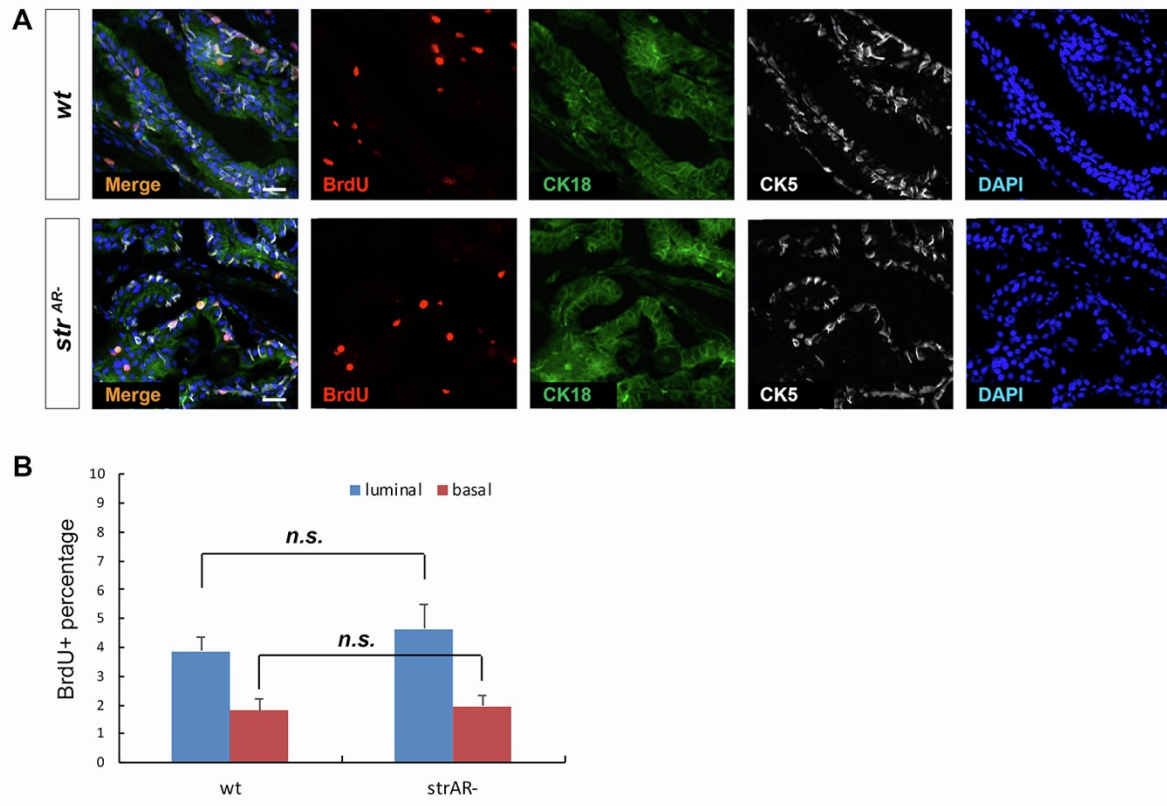


Figure S2. BrdU assay comparing epithelial cell proliferation between str^{AR-} and wt prostate at 11 months of age. Related to Figure 1. (A) Representative IF images with each individual channel. CK18 and CK5 mark luminal and basal cells, respectively. **(B)** Quantitation of the proportions of BrdU+ cells in luminal or basal cells of each sample. n.s., not significant by student t -test.

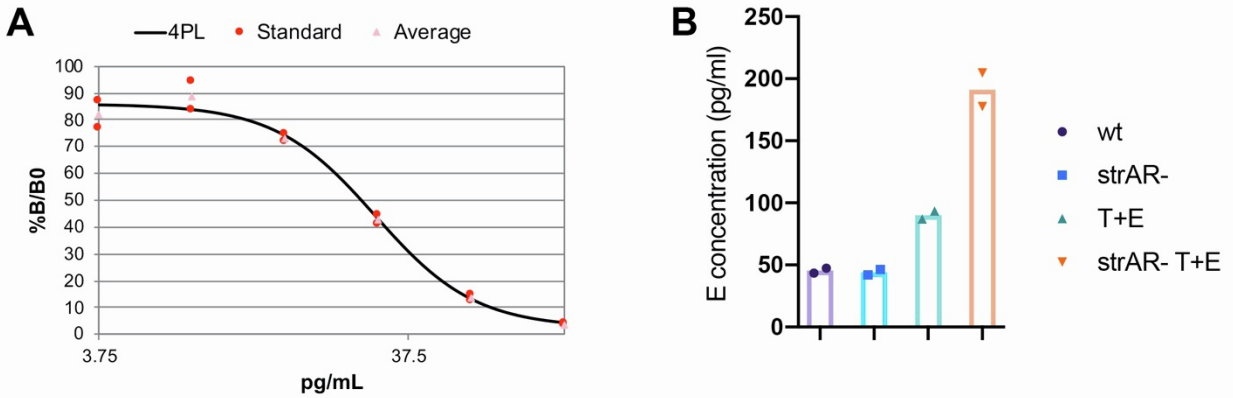


Figure S3. ELISA measurement of serum estrogen levels after T+E treatment. Related to Figure 2. (A) Standard curve created by the Arbor Assays Estradiol Serum EIA kit. (B) Quantitation of serum estradiol levels in age-matched *wt*, *str^{AR-}*, and *T+E* and *str^{AR-} T+E* mice 8 months after tube implantation. N=2 biological replicates in each group.

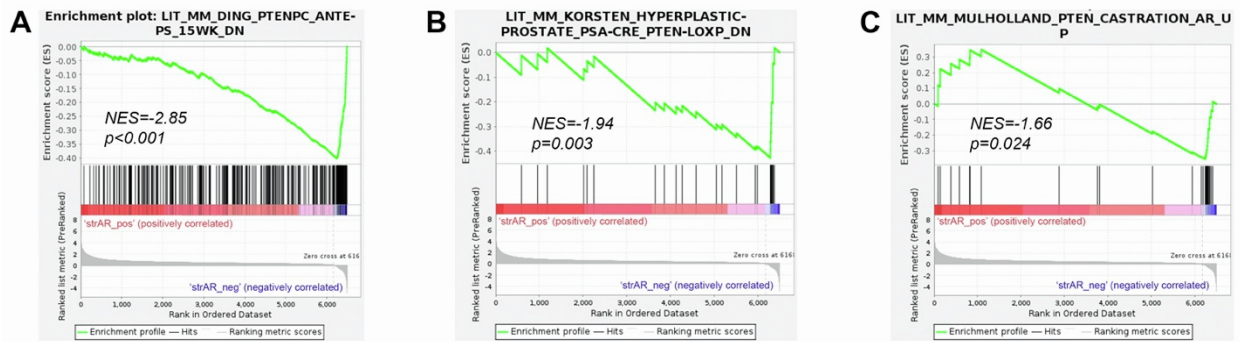


Figure S4. GSEA comparing differentially expressed genes in secretory luminal cells of *str^{AR-} T+E* vs *T+E* prostate. Related to Figure 3. (A-C) Genes downregulated in *str^{AR-} T+E* vs *T+E* are enriched in two independent published gene signatures of downregulated genes of *Pten*-null tumors vs. wt (A, B) and a published gene signature of AR-responsive genes (C).

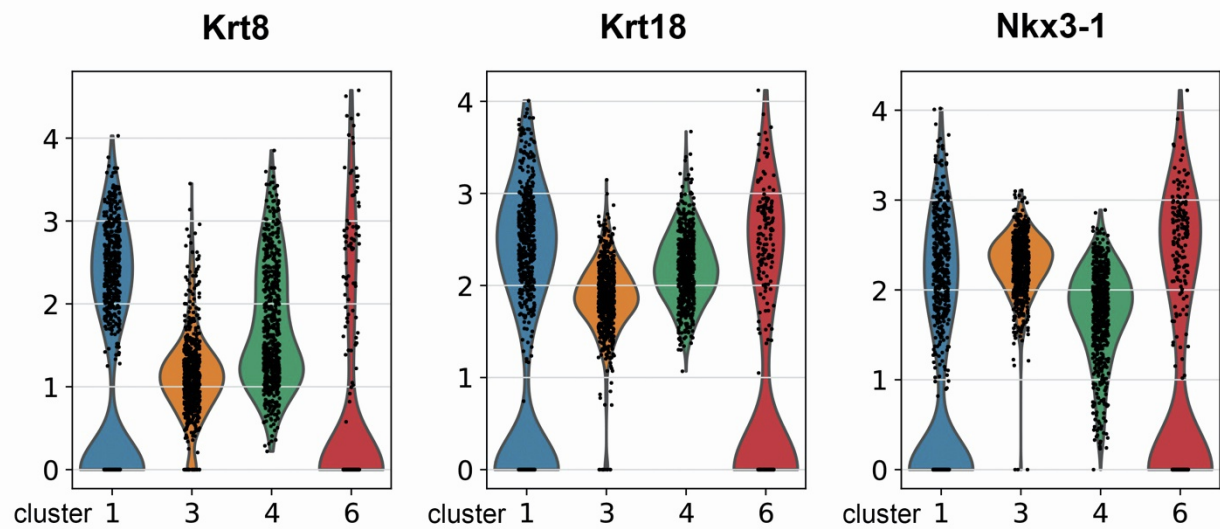


Figure S5. Violin plots showing expression of luminal marker genes *Krt8*, *Krt18*, and *Nkx3.1* in clusters 1, 3, 4, and 6. Related to Figure 4. Clusters 1 and 6 include cells that failed to pass the minimal detection threshold at the base, but generally have cells with higher luminal marker expression levels than clusters 3 and 4.

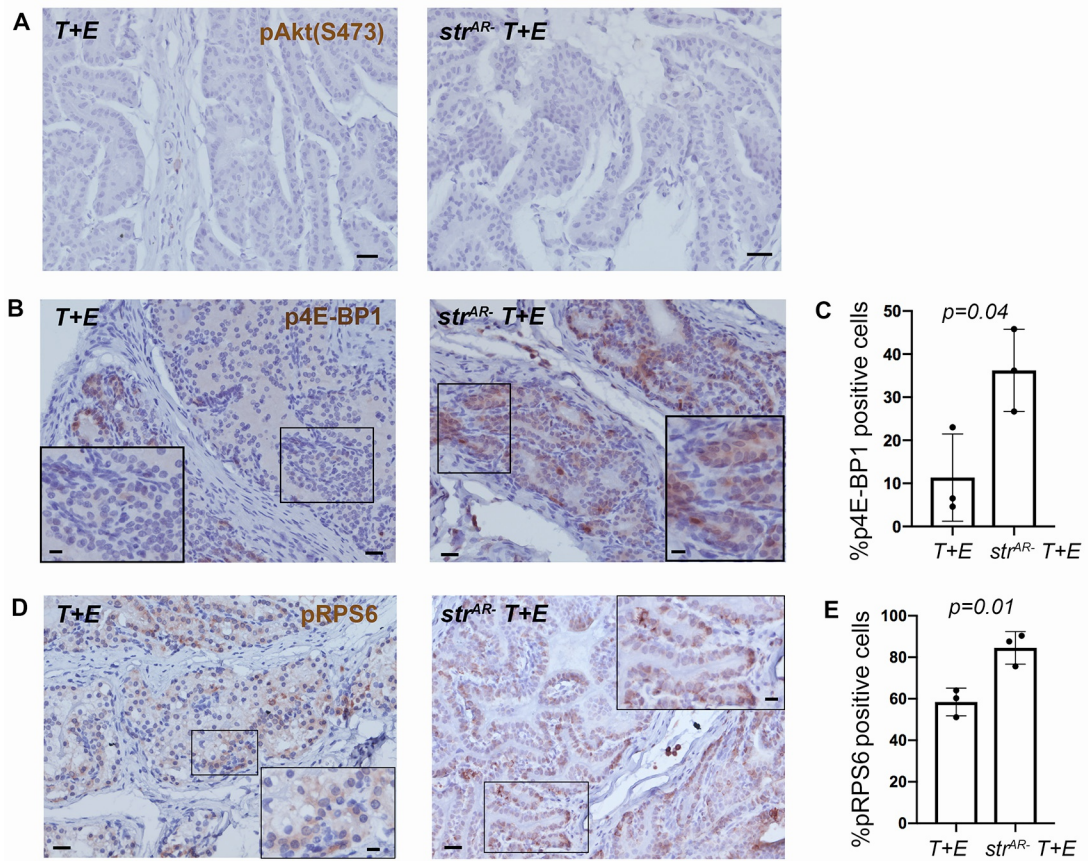


Figure S6. Higher PI3K-mTORC1 activity in *str^{AR-} T+E* tumors. Related to Figure 4.

(A,B,D) Representative IHC images showing pAkt(S473) (A), p4E-BP1 (B), and pRPS6(S235/236) (D) staining in *T+E* (left) and *str^{AR-} T+E* (right) tissues. Scale bars, 50 μ m. Scale bars in inset, 10 μ m. (C,E) Quantitation of percentages of p4E-BP1 positive cells (C) and pRPS6(S235/236) positive cells (E) in the tumor epithelium. N=3 animals per group. P values calculated by student's *t*-test.

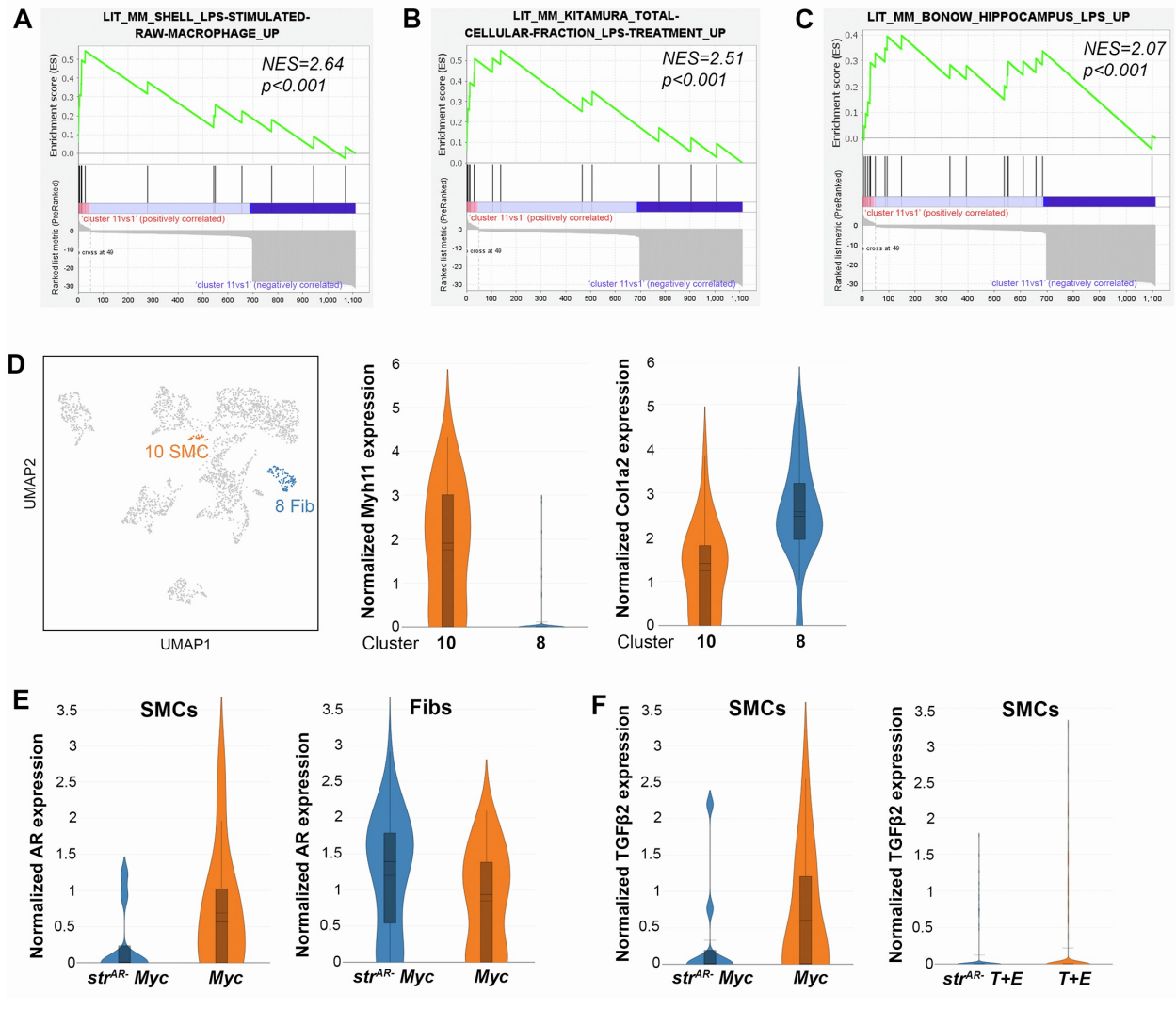


Figure S7. GSEA analyses of clusters 11 and 1 and marker expressions in SMCs and fibroblasts. Related to Figure 5. (A-C) GSEA showing enrichment of upregulated genes in cluster 11 vs cluster 1 in three published LPS-induced gene signatures. **(D)** Violin plots showing expression of *Myh11* (middle panel) and *Col1a2* (right panel) in clusters 10 and 8 of the UMAP (left panel). **(E)** Violin plots comparing *AR* expression in SMCs (left) and fibroblasts (right) between the *Myc* and *str^{AR-} Myc* samples. **(F)** Violin plots comparing *TGFβ2* expression in SMCs between the *Myc* and *str^{AR-} Myc* samples (left) and between the *T+E* and *str^{AR-} T+E* samples (right).

Supplemental Tables

Table S1. *Hi-Myc* model H&E scores and histology. Related to Figure 2.

Mouse ID	Experiment	Score	Histology
425	ctl-3m	5.04	PIN1, dilated ducts, papillary and cribriform structures
426	ctl-3m	4.74	hyperplasia
427	ctl-3m	3.82	hyperplasia
2370	ctl-3m	2.25	overall normal, hyperplasia
2806	ctl-3m	3.22	overall normal, hyperplasia
2878	ctl-3m	3.81	hyperplasia
9018	ctl-3m	1.03	overall normal, some autolysis
9074	ctl-3m	2.14	overall normal, hyperplasia
9076	ctl-3m	2.86	overall normal, hyperplasia
401	AR-3m	3.28	hyperplasia, inflammation
402	AR-3m	5.42	PIN1
1494	AR-3m	4.15	hyperplasia
1496	AR-3m	4.73	hyperplasia, focal PIN1
2919	AR-3m	5.87	PIN2, atypia
2923	AR-3m	4.20	hyperplasia
2975	AR-3m	5.35	PIN3, dilated ducts, atypia in cribriform area
9136	AR-3m	3.06	overall normal, hyperplasia
9165	AR-3m	3.41	hyperplasia
9166	AR-3m	3.93	hyperplasia
9167	AR-3m	6.31	focal PIN3, atypia,cribriform, columnar cells,pleomorphism
9168	AR-3m	3.90	hyperplasia
1101	ctl-9m	4.01	PIN1
2369	ctl-9m	3.97	hyperplasia
2371	ctl-9m	3.77	hyperplasia
2372	ctl-9m	4.39	focal PIN1
2460	ctl-9m	3.22	hyperplasia
2519	ctl-9m	3.61	hyperplasia
1103	AR-9m	4.46	PIN1, focal proliferation to focal cribriform, mild atypia
2337	AR-9m	6.14	PIN3, dilated ducts, proliferation, mild atypia, periductal inflammation
2368	AR-9m	5.43	PIN1, focal thick bridges of epithelium across glands, hyperplasia, marked atypia, cribriform
9045	AR-9m	6.57	PIN2
9046	AR-9m	4.58	focal PIN1, proliferation to cribriform, columnar cells
9047	AR-9m	4.57	PIN1, focal proliferation, mild atypia

Table S2. T+E2 model H&E scores and histology. Related to Figure 2.

Mouse ID	Experiment	Score	Histology
293	ctl-4m	7.0	PIN1
330	ctl-4m	5.0	hyperplasia
1198	ctl-4m	5.5	hyperplasia
2736	ctl-4m	3.5	overall normal, hyperplasia
9384	ctl-4m	3.0	overall normal, hyperplasia
9437	ctl-4m	3.0	overall normal, hyperplasia
9625	ctl-4m	4.5	hyperplasia
1092	AR-4m	5.5	hyperplasia
1123	AR-4m	6.5	PIN1, inflammation
1125	AR-4m	7.0	PIN1, inflammation
1134	AR-4m	6.5	PIN1
1135	AR-4m	5.5	hyperplasia, necrosis
1136	AR-4m	7.5	PIN3, epithelial hyperplasia
1201	AR-4m	7.5	focal PIN3, cribriform, columnar and atypical cells
1203	AR-4m	6.0	hyperplasia, focal PIN1
9446	AR-4m	3.0	overall normal, hyperplasia
9612	AR-4m	5.0	hyperplasia
9615	AR-4m	7.0	hyperplasia, focal PIN1
251	ctl-8m	4.0	overall normal, hyperplasia
1283	ctl-8m	4.5	hyperplasia
1483	ctl-8m	5.5	hyperplasia
1485	ctl-8m	6.0	hyperplasia, focal PIN1
5436	ctl-8m	6.0	PIN1
451	AR-8m	6.5	PIN1, epithelial hyperplasia
579	AR-8m	9.0	PIN3, epithelial filling gland, gland enlargement
1087	AR-8m	6.0	focal PIN3, hyperplasia, gland filled with epithelial cells
1279	AR-8m	4.5	hyperplasia, focal PIN1, inflammation
5419	AR-8m	7.0	PIN1, epithelial hyperplasia, gland enlargement
5421	AR-8m	7.5	PIN2, epithelial hyperplasia
5423	AR-8m	8.0	PIN2