

Supplemental Table 1: Effect of diet and genotype on the incidence of prostate cancer in 5 month old TRAMP mice.

Genotype	Diet	n	TUMOR STAGE	
			Non-Cancer	Cancer
ERWT	Casein	175	53 (30%)	122 (70%)
ERWT	Genistein 300mg/kg	81	43 (53%)*	38 (47%)*
ERWT	Genistein 750mg/kg	25	17 (68%)*	8 (32%)*
ER α KO	Casein	80	7 (9%)*	73 (91%)*
ER α KO	Genistein 300mg/kg	25	1 (4%)*	24 (96%)*
ER β KO	Casein	51	13 (25%)	38 (75%)
ER β KO	Genistein 300mg/kg	23	5 (22%)	18 (78%)

Non-cancer was defined as normal, hyperplasia, and prostatic intraepithelial neoplasia.

Cancer was defined as well-, moderately-, and poorly differentiated carcinoma.

Each group was compared with ERWT casein.

** p<0.005 – very statistically significant; *** p<0.001 – extremely statistically significant.

Supplemental Table 2: Tumor incidence for TRAMP mice split by separate studies.

1. study 1033-01								
Genotype	Diet	n	TUMOR STAGE					
			Non-Cancer			Cancer		
			Normal	HYP	PIN	WDC	MDC	PDC
ER α WT	Casein	25	2 (8%)	4 (16%)	1 (4%)	13 (52%)	0	5 (20%)
ER α WT	Genistein 300mg/kg	28	1 (4%)	10 (36%)	7 (25%)	6 (21%)	0	4 (14%)
ER α KO	Casein	29	0	1 (3%)	0	25 (86%)	1 (3%)	2 (7%)
ER α KO	Genistein 300mg/kg	25	0	0	1 (4%)	23 (92%)	1 (4%)	0
2. study 1009-02								
Genotype	Diet	n	TUMOR STAGE					
			Non-Cancer			Cancer		
			Normal	HYP	PIN	WDC	MDC	PDC
ER α WT	Casein	27	0	5 (18.5%)	8 (30%)	9 (33%)	0	5 (18.5%)
ER α KO		31	0	1 (3%)	0	28 (90%)	0	2 (6%)
ER α WT	Daidzein 250 mg/kg	26	0	5 (19%)	8 (31%)	7 (27%)	0	6 (23%)
ER α KO		25	0	1 (4%)	2 (8%)	18 (72%)	2 (8%)	2 (8%)
3. study 1023-03								
Genotype	Diet	n	TUMOR STAGE					
			Non-Cancer			Cancer		
			Normal	HYP	PIN	WDC	MDC	PDC
ER α WT	Casein	22	0	1 (4%)	2 (9%)	14 (64%)	0	5 (23%)
ER α KO	Casein	20	0	1 (5%)	4 (20%)	15 (75%)	0	0

4. study 1001-04								
Genotype	Diet	n	TUMOR STAGE					
			Non-Cancer			Cancer		
			Normal	HYP	PIN	WDC	MDC	PDC
ER α WT	Casein	16	0	0	6 (37.5%)	8 (50%)	0	2 (12.5%)
5. study 1042-04								
Genotype	Diet	n	TUMOR STAGE					
			Non-Cancer			Cancer		
			Normal	HYP	PIN	WDC	MDC	PDC
ERWT	Casein	29	0	0	8 (28%)	14 (48%)	0	7 (24%)
ERWT	Genistein 300mg/kg	25	0	3 (12%)	10 (40%)	1 (4%)	1 (4%)	10 (40%)
ER β KO	Casein	25	0	0	4 (16%)	8 (32%)	0	13 (52%)
ER β KO	Genistein 300mg/kg	23	0	0	5 (22%)	8 (35%)	0	10 (43%)
6. study 1084-07								
Genotype	Diet	n	TUMOR STAGE					
			Non-Cancer			Cancer		
			Normal	HYP	PIN	WDC	MDC	PDC
ERWT	Casein	28	0	0	7 (25%)	16 (57%)	0	5 (18%)
ERWT	Genistein 300mg/kg	28	0	0	12 (43%)	8 (29%)	0	8 (29%)
ERWT	Genistein 750mg/kg	25	0	0	17 (68%)	2 (8%)	0	6 (24%)

7. study 1049-09-1

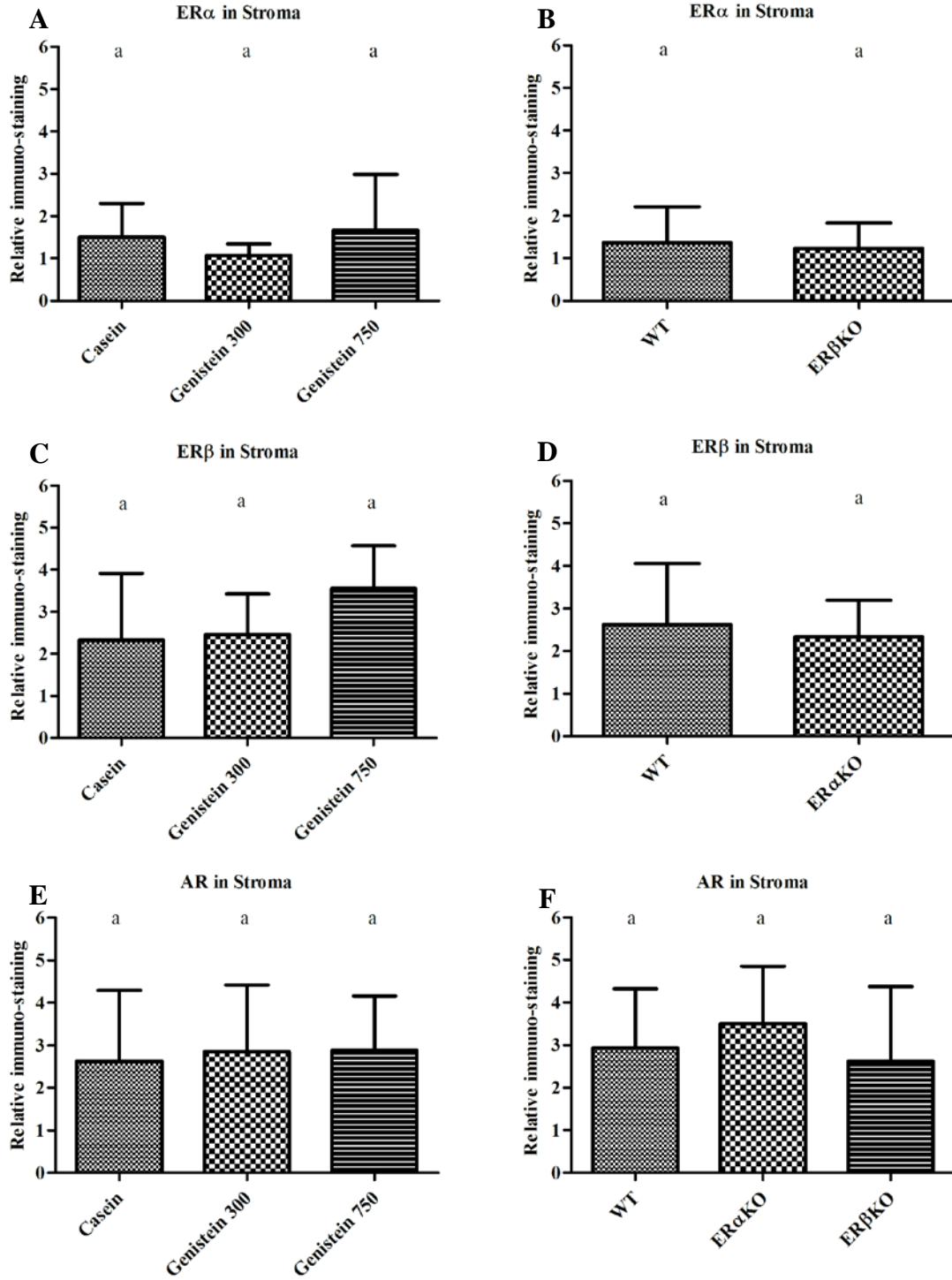
Genotype	Diet	n	TUMOR STAGE					
			Non-Cancer			Cancer		
			Normal	HYP	PIN	WDC	MDC	PDC
ERWT	Casein	28	0	0	9 (32%)	14 (50%)	0	5 (18%)
ER β KO	Casein	26	0	0	9 (35%)	10 (38%)	0	7 (27%)

Supplemental Table 3: Body and organ weights of mice from different dietary groups.

Genotype	Diet	Weights (gm)			
		Body	Repro. Tract	Prostate	Testes
ERWT	Casein	28.90 ± 0.22 ^a	1.43 ± 0.10 ^a	0.41 ± 0.11 ^a	0.21 ± 0.004 ^a
ERWT	Genistein ³⁰⁰	28.98 ± 0.32 ^a	1.80 ± 0.27 ^{ad}	0.83 ± 0.28 ^a	0.20 ± 0.004 ^a
ERWT	Genistein ⁷⁵⁰	30.18 ± 0.68 ^b	1.35 ± 0.18 ^a	0.28 ± 0.17 ^a	0.21 ± 0.005 ^a
ER α KO	Casein	29.74 ± 0.52 ^a	3.02 ± 0.18 ^b	0.33 ± 0.03 ^a	0.17 ± 0.011 ^b
ER α KO	Genistein ³⁰⁰	32.38 ± 0.81 ^c	3.98 ± 0.24 ^c	0.43 ± 0.03 ^a	0.18 ± 0.018 ^{ab}
ER β KO	Casein	30.68 ± 0.50 ^a	2.21 ± 0.32 ^d	0.89 ± 0.28 ^a	0.21 ± 0.005 ^a
ER β KO	Genistein ³⁰⁰	28.28 ± 0.44 ^a	1.85 ± 0.40 ^{ad}	0.94 ± 0.44 ^a	0.21 ± 0.006 ^a

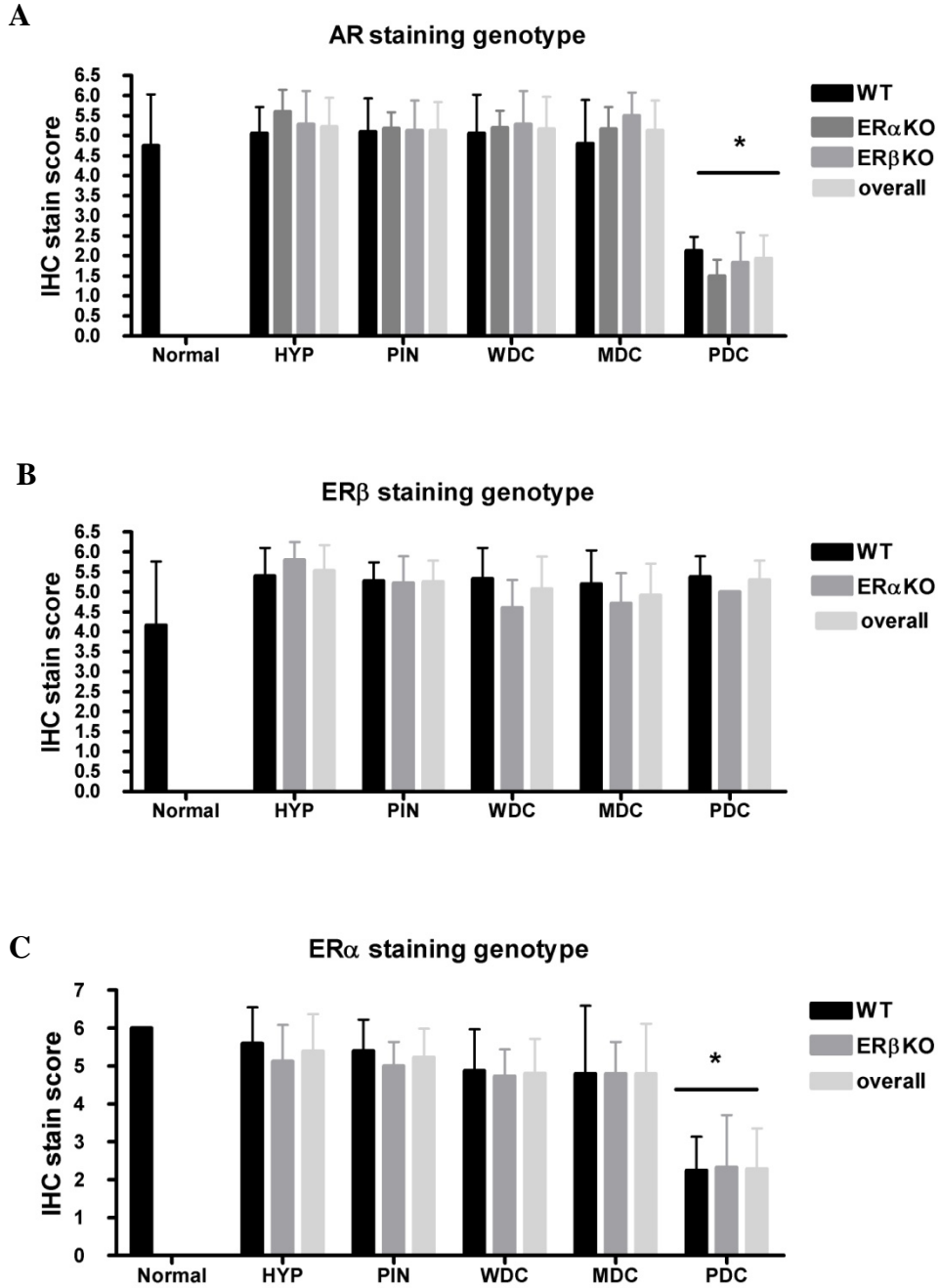
Means ± SEM. Values within columns with different letter superscripts are significantly different, p<0.05.

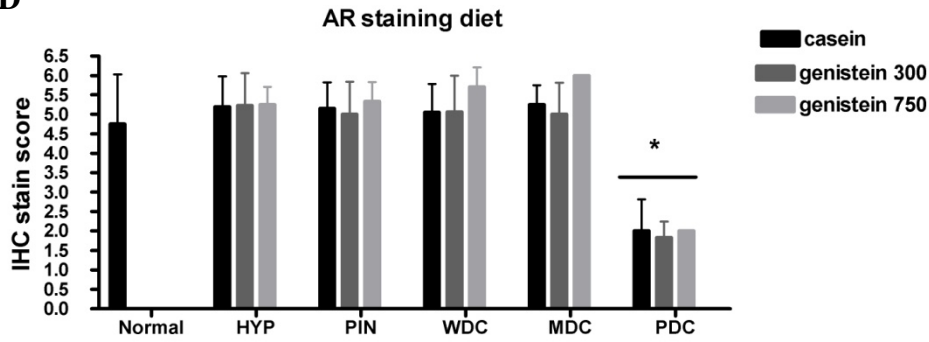
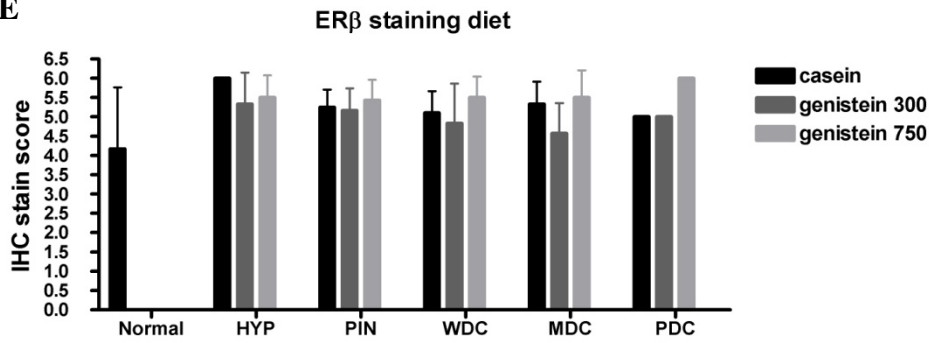
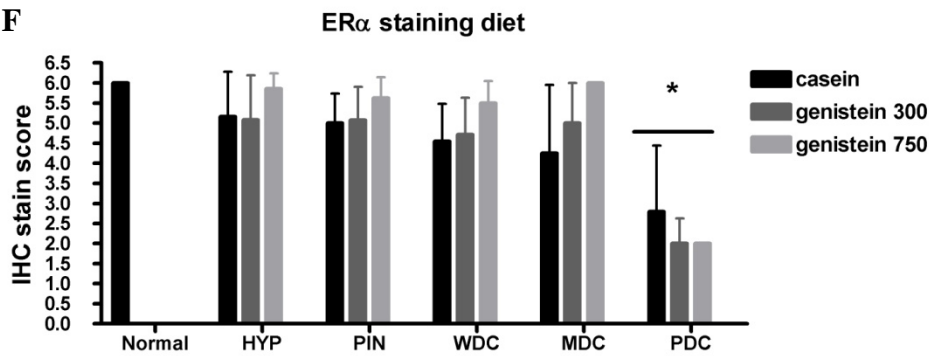
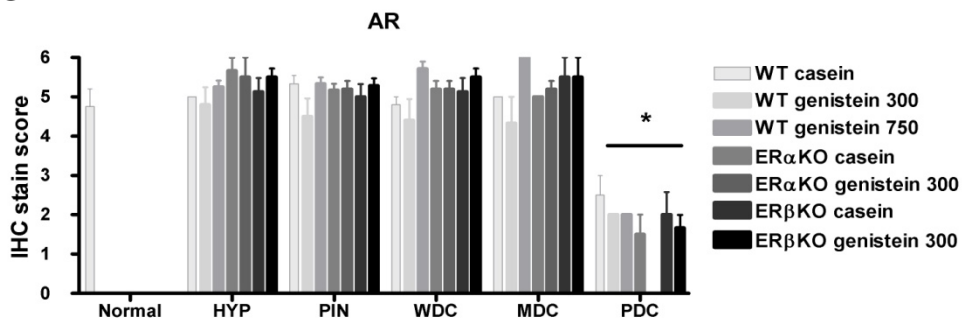
Supplemental Figure 1: Additional analysis of stromal IHC scores

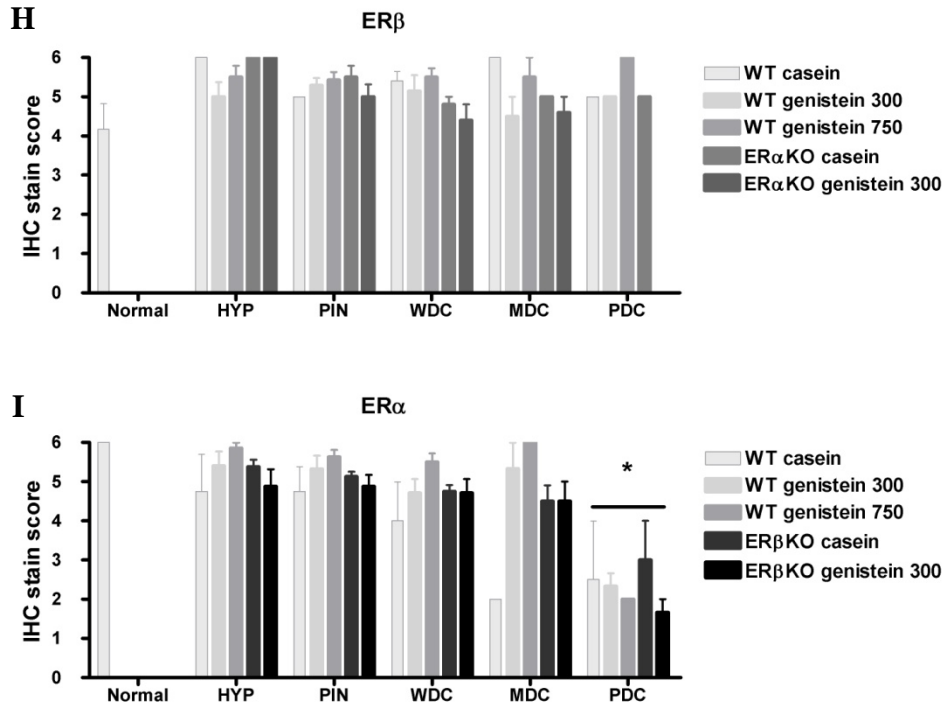


No significant changes are seen in ER α , ER β , or AR staining when comparing diet or genotype. **A, C, E.** analysis of IHC scores by comparing diet; **B, D, F.** analysis of IHC scores by comparing genotype. Error bars show standard deviation.

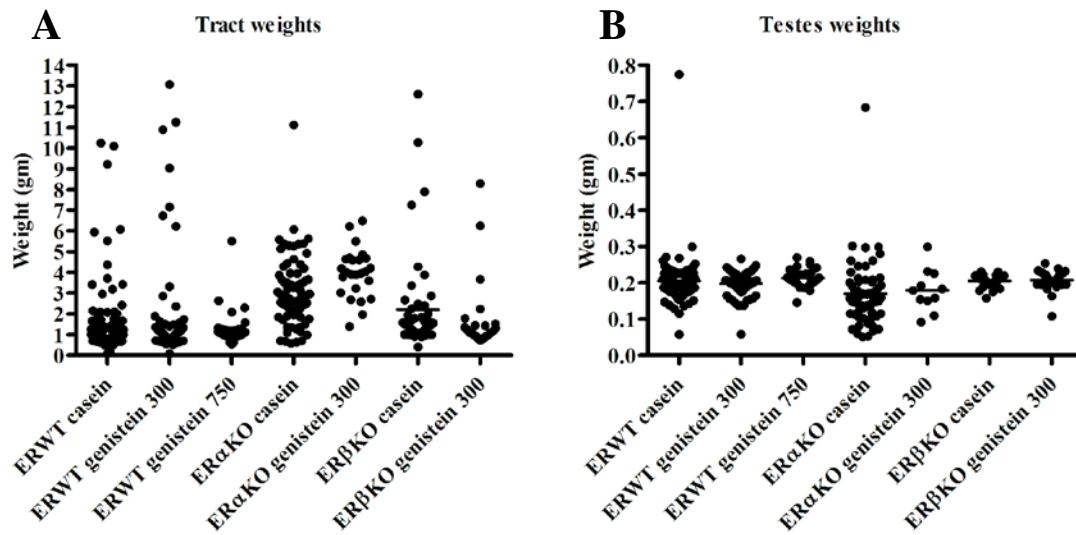
Supplemental Figure 2: Additional analysis of epithelial IHC scores



D**E****F****G**



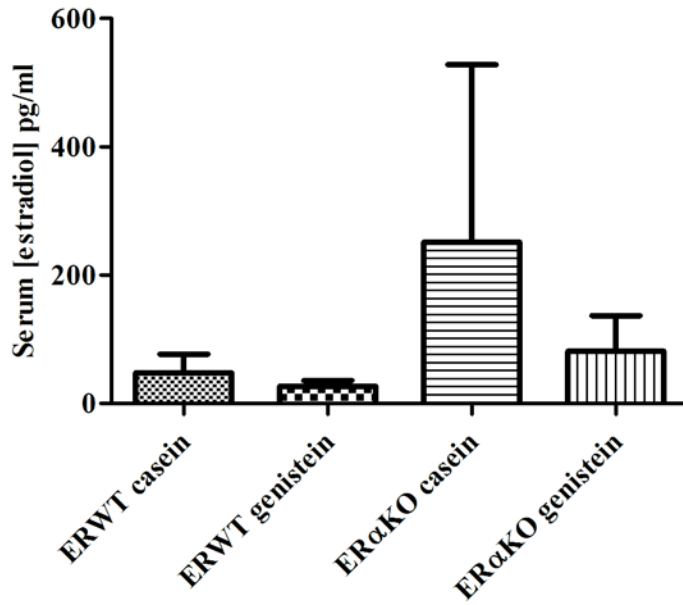
No significant changes are seen in ER α , ER β , or AR staining when comparing genotype and/or diet. **A-C.** analysis of IHC scores by comparing genotype; A) n = 66, from studies 1,5, 6, and 7, B) n = 45, from studies 1, 2, 6, and 7, C) n=49 from studies 1, 5, 6, and 7 (See Supplemental Table 2). **D-F.** analysis of IHC scores by comparing diet; D) n = 54, from studies 1,5, and 6, E) n = 36, from studies 1, and 6, F) n=40 from studies 1, 5, and 6 (See Supplemental Table 2). **G-I.** analysis of IHC scores by comparing diet and genotype; G) n = 54, from studies 1,5, and 6, H) n = 36, from studies 1, and 6, I) n=40 from studies 1, 5, and 6 (See Supplemental Table 2). Error bars show standard deviation, * indicates p<0.05



Supplemental Figure 3: TRAMP mouse urogenital tract (A) and testes (B) weights.

ERαKO mice had significantly lower testes weights which corresponds to previous reports (34) and the observed lower fertility in both ERαKO mice (S1) and one human male (S2, S3). There is still some controversy regarding the main ER in the testis - Gustafsson found ERβ to be the main receptor in testis (S4) while Katzenellenbogen's data support an ERα leading role (S5). We did not observe any significant differences in testis weight between ERWT and ERβKO mice.

Supplemental Figure 4. Estradiol in ER α KO and ERWT mice fed genistein or control diets.



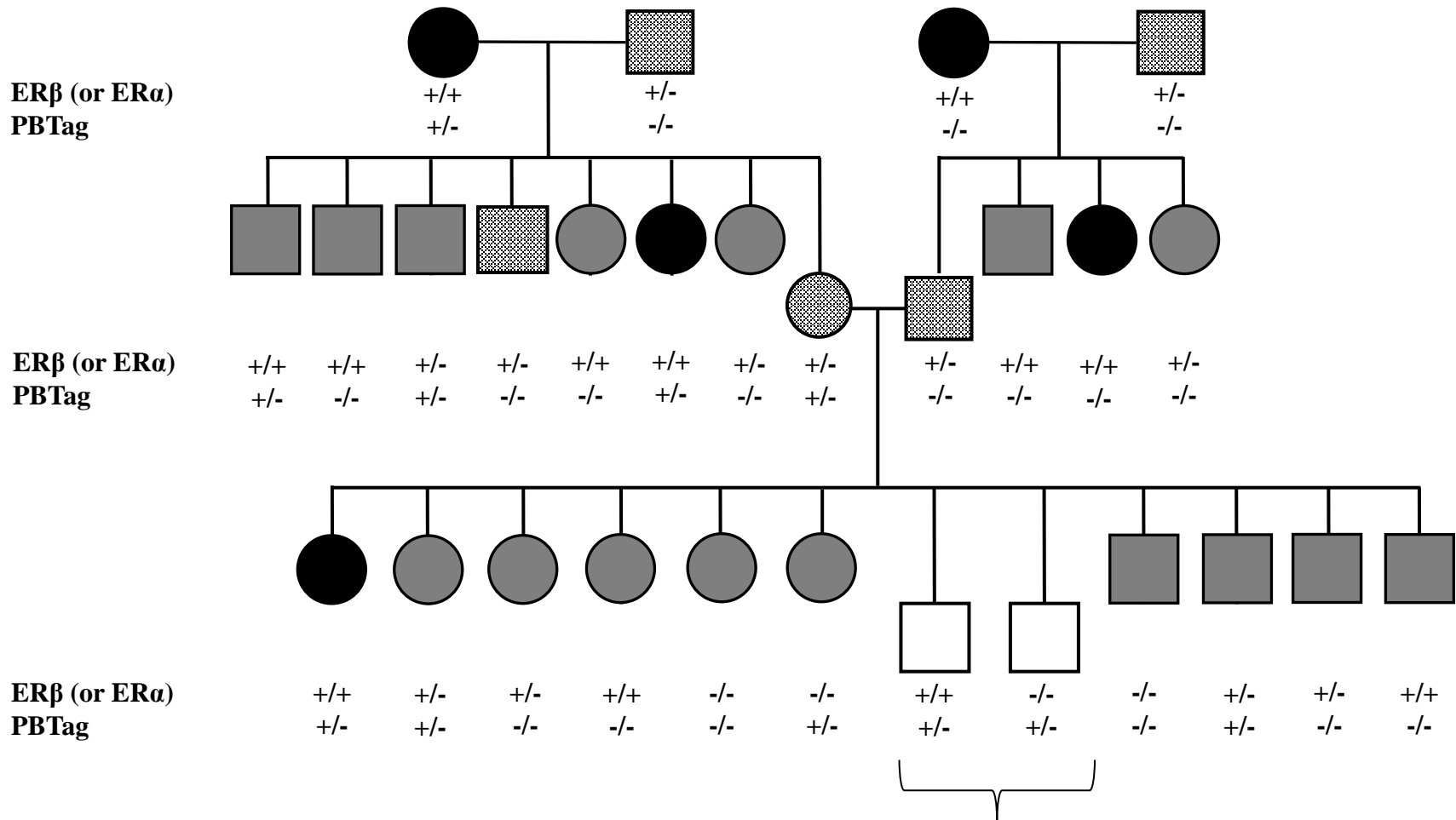
No significant effect on estradiol levels when comparing diet or genotype to controls.

Error bars show standard deviation

Supplemental References

- S1. Ebling FJ, Brooks AN, Cronin AS, Ford H, Kerr JB. Estrogenic induction of spermatogenesis in the hypogonadal mouse. *Endocrinology* 2000;141(8):2861-9.
- S2. Simpson ER. Genetic mutations resulting in estrogen insufficiency in the male. *Mol Cell Endocrinol* 1998;145(1-2):55-9.
- S3. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med* 1994;331(16):1056-61.
- S4. Makinen S, Makela S, Weihua Z, Warner M, Rosenlund B, Salmi S, Hovatta O, Gustafsson JA. Localization of oestrogen receptors alpha and beta in human testis. *Mol Hum Reprod* 2001;7(6):497-503.
- S5. Zhou Q, Clarke L, Nie R, Carnes K, Lai LW, Lien YH, Verkman A, Lubahn D, Fisher JS, Katzenellenbogen BS, Hess RA. Estrogen action and male fertility: roles of the sodium/hydrogen exchanger-3 and fluid reabsorption in reproductive tract function. *Proc Natl Acad Sci U S A* 2001;98(24):14132-7.

Breeding Strategy for (ER α KO, ER β KO, ERWT)/TRAMP Mice



Animals represented with white were used in the study. Animals represented in gray were to be culled. Animals represented with hatched backgrounds could be used as breeders.