Online Resource 1

Hormone Receptor Expression Varies by Molecular Subtype, Age, and Sex in Pheochromocytoma and Paraganglioma: A Transcriptomic Analysis

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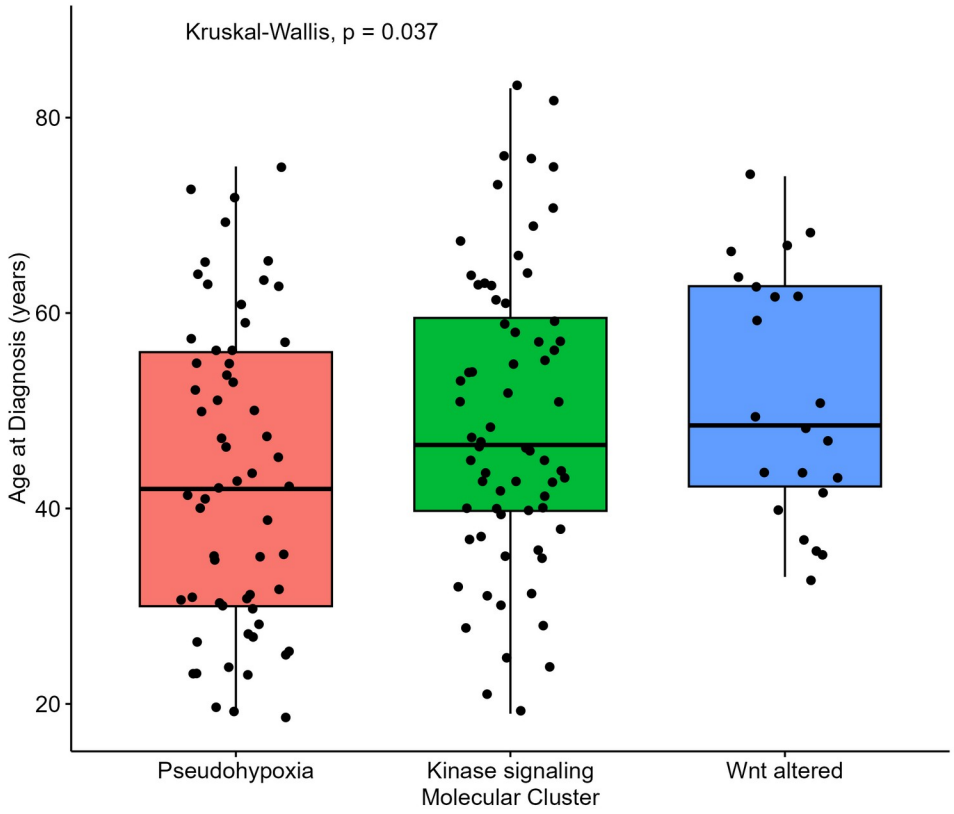


Figure S1. Age at diagnosis differs significantly by molecular cluster in PCPG. Boxplots show age at diagnosis (years) for the 151 patients in the pseudohypoxia (cluster 1), kinase signaling (cluster 2), and Wnt-altered (cluster 3) molecular clusters. The Kruskal-Wallis test identified a significant difference in age at diagnosis between molecular clusters ($p = 0.037$), with pseudohypoxia tumors presenting at a younger age than kinase signaling and Wnt-altered tumors. Abbreviation: PCPG, pheochromocytoma and paraganglioma.

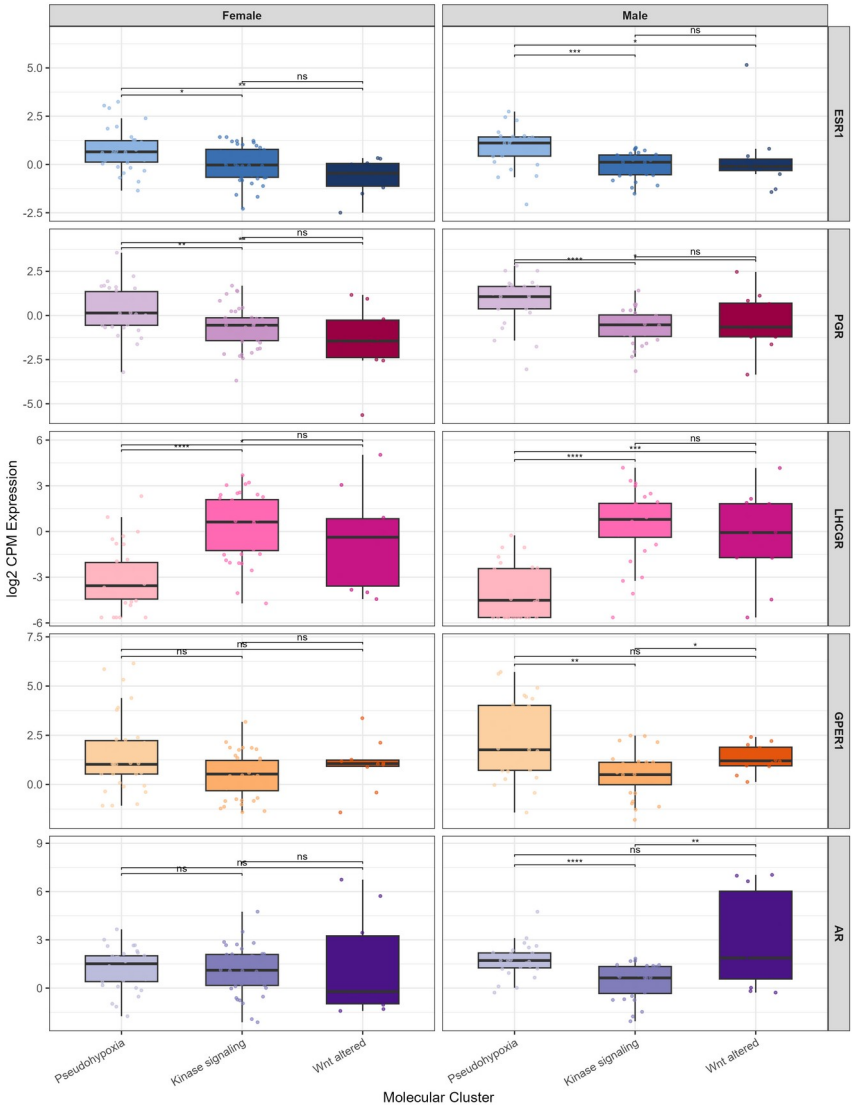


Figure S2. Hormone receptor expression by molecular cluster stratified by patient sex. Boxplots show normalized expression (log2 CPM) of ESR1, PGR, LHCGR, GPER1, and AR across molecular clusters (pseudohypoxia, kinase signaling, and Wnt-altered) stratified by patient sex (female and male). Cluster-differential expression of ESR1, PGR, and LHCGR was observed in both female and male patients. AR and GPER1 showed cluster differences in male patients only; in female patients, no pairwise AR or GPER1 comparison was significant after correction. P-values were calculated using pairwise Wilcoxon rank-sum tests with Benjamini–Hochberg correction and are displayed as: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; ns, not significant. Box plots show median ± interquartile range; individual points represent primary tumors (one per patient). Abbreviations: AR, androgen receptor; ESR1, estrogen receptor alpha; GPER1, G protein-coupled estrogen receptor 1; LHCGR, luteinizing hormone/choriogonadotropin receptor; log2 CPM, log2 counts per million; ns, not significant; PCPG, pheochromocytoma and paraganglioma; PGR, progesterone receptor.

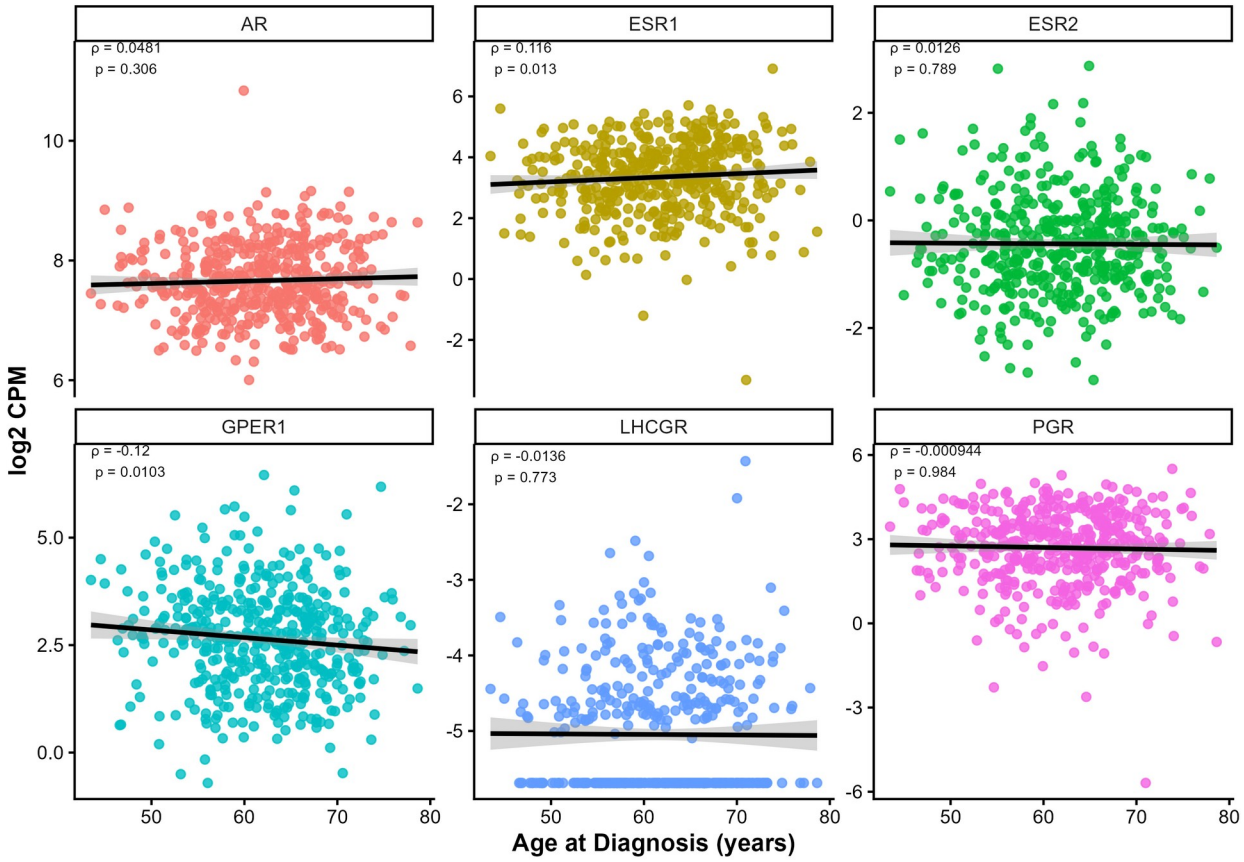


Figure S3. Hormone receptor (HR) expression in PRAD tumors varies with age at diagnosis. Expression levels of AR, ESR1, ESR2, GPER1, LHCGR, and PGR were analyzed in TCGA-PRAD primary tumors (one per patient; n = 455 with age available) using Spearman correlation with age at diagnosis. ESR1 expression showed a positive correlation with age (ρ = 0.116, p = 0.013), while GPER1 expression was negatively correlated with age (ρ = −0.120, p = 0.010). No significant correlations were observed for AR, ESR2, LHCGR, or PGR. Regression lines with 95% confidence intervals are shown. Abbreviations: AR, androgen receptor; ESR1/2, estrogen receptors 1 and 2; GPER1, G protein-coupled estrogen receptor 1; HR, hormone receptor; LHCGR, luteinizing hormone/choriogonadotropin receptor; PRAD, prostate adenocarcinoma; PGR, progesterone receptor.

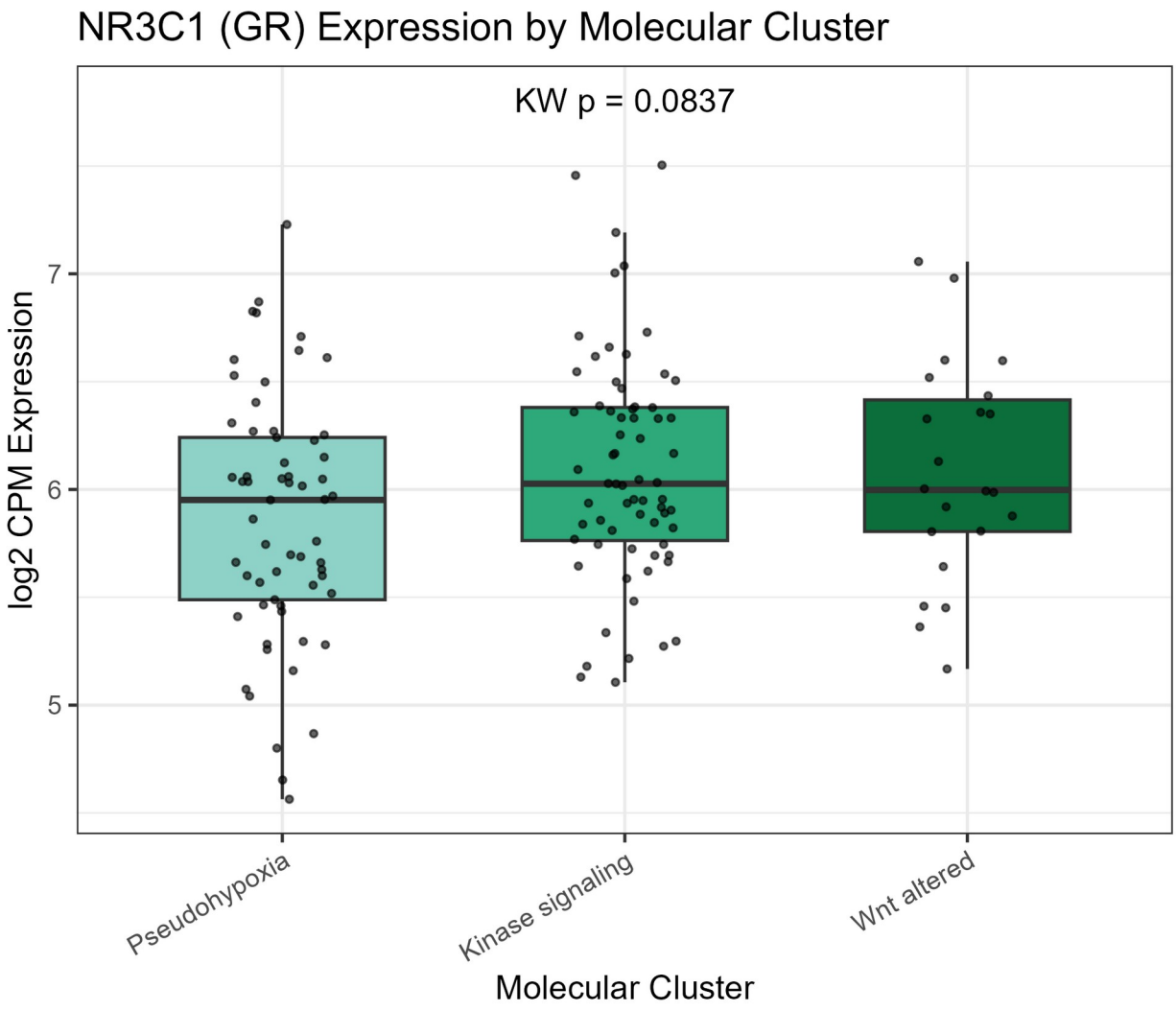


Figure S4. NR3C1 (glucocorticoid receptor) expression across PCPG molecular clusters. Boxplots show normalized expression (log2 CPM) of NR3C1 across molecular clusters (pseudohypoxia, kinase signaling, and Wnt-altered). No significant difference in NR3C1 expression was observed across clusters (Kruskal-Wallis $\chi^2 = 4.96$, df = 2, p = 0.084). Box plots show median ± interquartile range; individual points represent primary tumors (one per patient). Abbreviations: GR, glucocorticoid receptor; KW, Kruskal-Wallis; log2 CPM, log2 counts per million; NR3C1, nuclear receptor subfamily 3 group C member 1; PCPG, pheochromocytoma and paraganglioma.

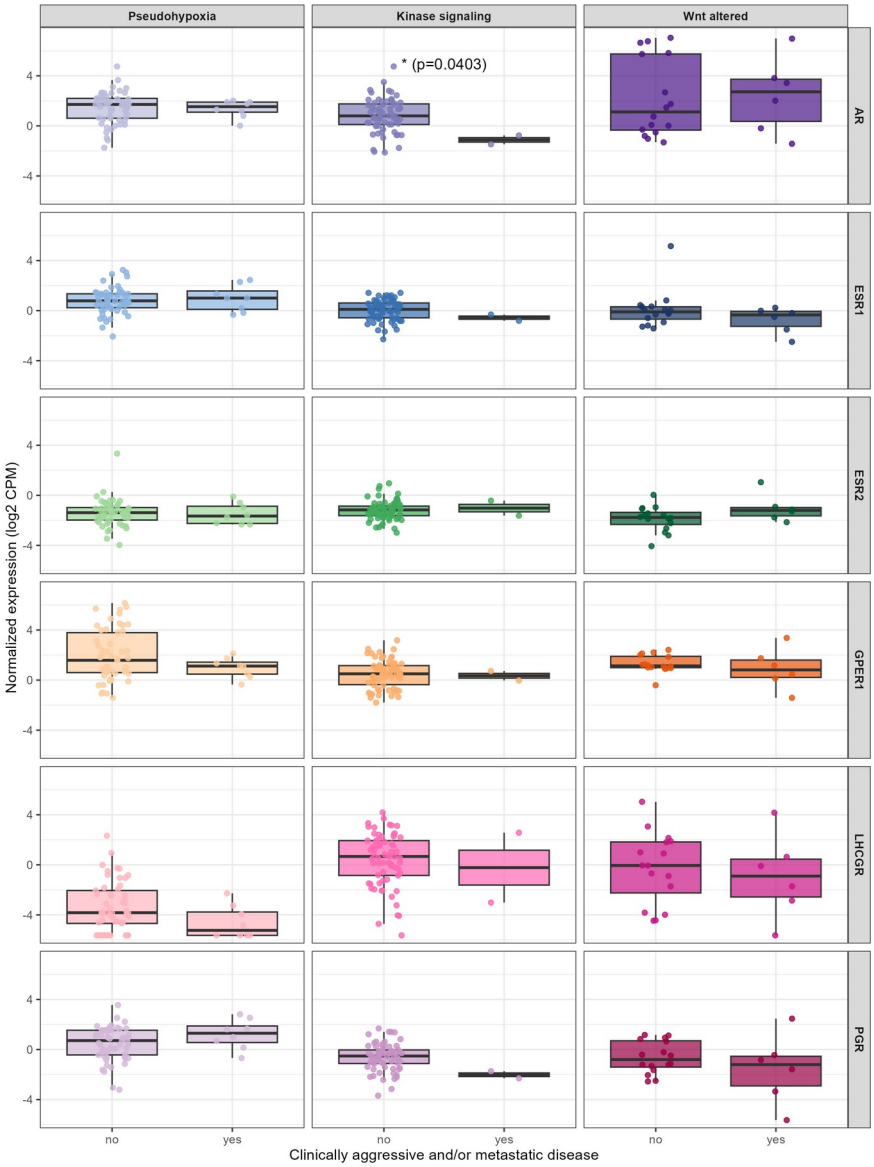


Figure S5. Hormone receptor expression in PCPG tumors by molecular cluster and presence of clinically aggressive and/or metastatic disease. Boxplots show normalized expression (log2 CPM) of AR, ESR1, ESR2, GPER1, LHCGR, and PGR stratified by molecular cluster (pseudohypoxia, kinase signaling, Wnt-altered) and clinical aggressiveness status (no vs. yes). In the kinase signaling cluster, low AR expression was associated with clinically aggressive and/or metastatic disease ($p = 0.040$; two affected patients). Within the pseudohypoxia cluster, LHCGR expression was lower in patients with clinically aggressive and/or metastatic disease, but the difference was not statistically significant ($p = 0.086$). P-values are from Wilcoxon rank-sum tests; only $p < 0.05$ are labeled. Abbreviations: AR, androgen receptor; ESR1/2, estrogen receptors 1 and 2; GPER1, G protein-coupled estrogen receptor 1; HR, hormone receptor; LHCGR, luteinizing hormone/choriogonadotropin receptor; PCPG, pheochromocytoma and paraganglioma; PGR, progesterone receptor.

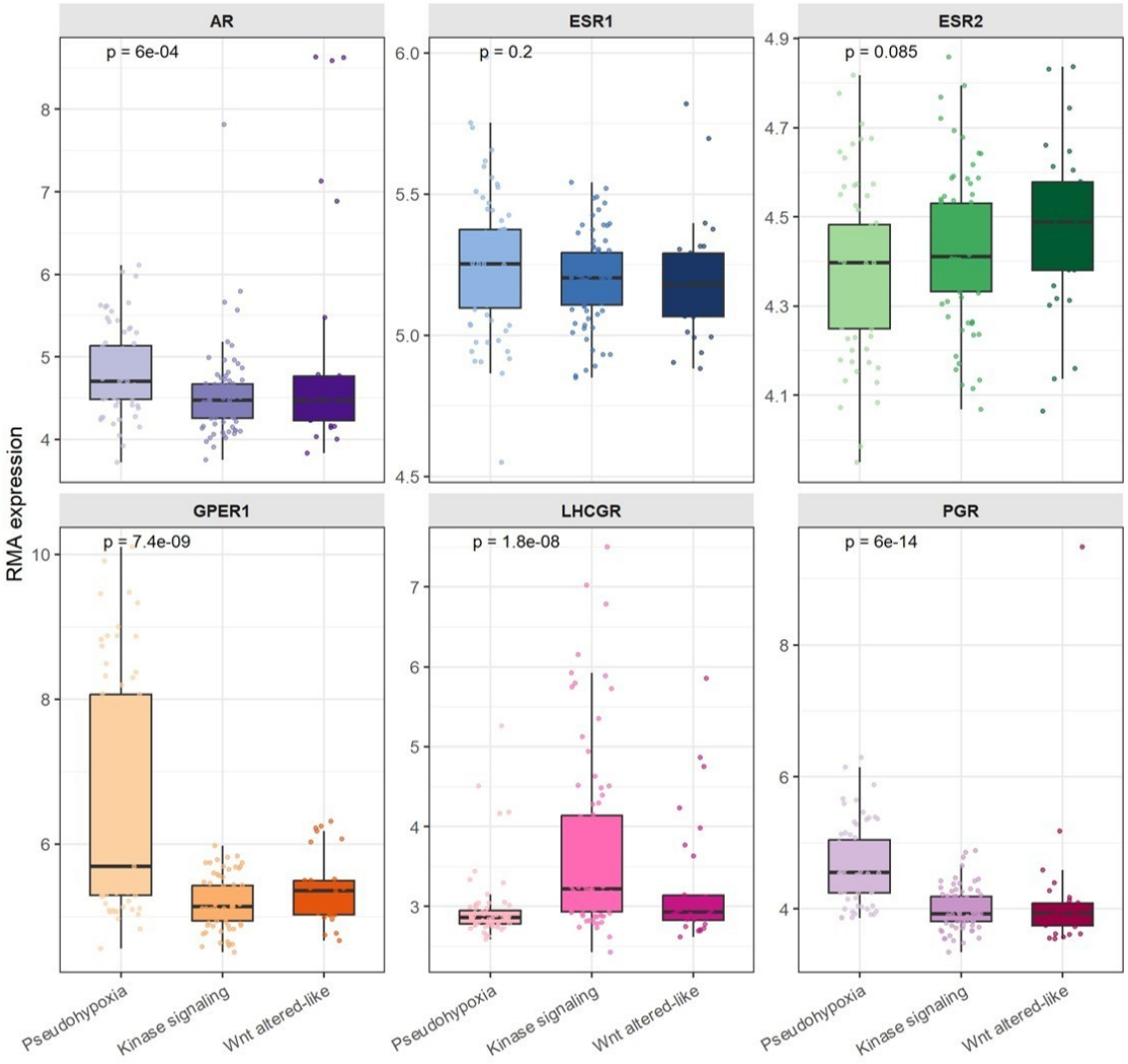


Figure S6. External validation of hormone receptor expression across molecular subtypes in the E-MTAB-733 PCPG cohort. Published E-MTAB-733 transcriptome annotations were harmonized into broader biologic classes corresponding to pseudohypoxia (C1A/C1B), kinase signaling (C2A), and Wnt-altered-like tumors (C2B); C2C samples were excluded from the primary validation analysis. Boxplots show normalized RMA expression of AR, ESR1, ESR2, GPER1, LHCGR, and PGR across the harmonized external classes. P-values were calculated using the Kruskal-Wallis test. Abbreviations: AR, androgen receptor; ESR1, estrogen receptor alpha; ESR2, estrogen receptor beta; GPER1, G protein-coupled estrogen receptor 1; LHCGR, luteinizing hormone/choriogonadotropin receptor; PGR, progesterone receptor; RMA, robust multi-array average; C1A/C1B, pseudohypoxia-related transcriptome classes; C2A, kinase-signaling-related transcriptome class; C2B, Wnt-altered-like transcriptome class.

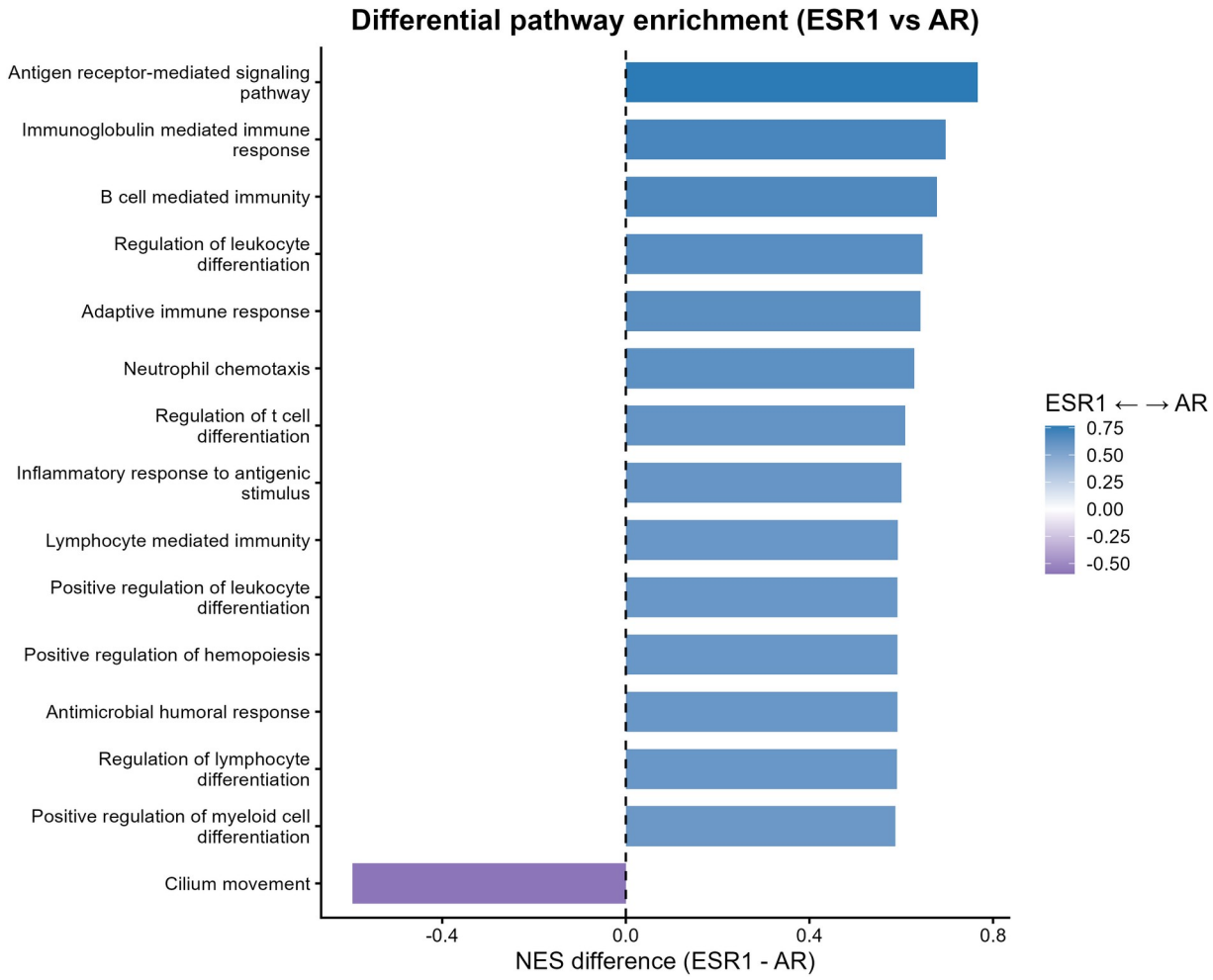


Figure S7. Differential pathway enrichment in ESR1-high versus AR-high pseudohypoxia PCPG. Differential pathway enrichment plot comparing normalized enrichment scores (NES) between ESR1-high and AR-high pseudohypoxia (cluster 1) tumors ($\Delta NES = NES_{ESR1} - NES_{AR}$). Positive values (blue) indicate pathways more strongly enriched in ESR1-high tumors; negative values (purple) indicate pathways more strongly enriched in AR-high tumors. The 15 pathways with the largest absolute ΔNES are shown. Immune-related pathways, including antigen receptor-mediated signaling, immunoglobulin-mediated immune response, and B cell-mediated immunity, were preferentially enriched in ESR1-high tumors, whereas cilium movement was relatively enriched in AR-high tumors. Abbreviations: AR, androgen receptor; ESR1, estrogen receptor alpha; NES, normalized enrichment score; ΔNES, difference in normalized enrichment score.