Supplementary Figure Legends

Figure S1. Quantification of steroid receptor levels in breast cancer PDX UCD4 and UCD65. Tumors were grown in mice supplemented with continuous E2, E2+P4, or E2+MPA as described. **A**, Tumor sections were immunostained for ER, PR, and AR as described. Analysis was performed using Leica Imagescope software and an algorithm tuned for nuclear staining. Numbers indicate percent positive cells (sum of 1+, 2+, and 3+ intensities) plus/minus SEM. An n of 3-5 tumors were analyzed per condition except for [a], n=2 tumors. **B**, Immunoblot of AR, PR (A and B isoforms indicated), and ER(α) in UCD4 and UCD65 tumors supplemented with E2. Molecular weights are indicated. α-tubulin was used as a loading control. T47D cells were used as a control for PRA and PRB expression. PRA:PRB ratios were determined using LI-COR Image Studio software and are indicated for each tumor and cell line.

Figure S2. Final tumor masses for growth experiments described in Figure 1B and C. **A** and **B**, Tumors were grown in mice either in the absence of exogenously added hormones (placebo), or in the presence of continuous E2, E2+P4, or E2+MPA. **C**, UCD4 tumors were grown in mice supplemented with E2, then treated with either vehicle (E2+Veh), tamoxifen (E2+TAM) or MPA (E2+MPA). Final tumor masses at necropsy are plotted versus the number of days of incubation ± SEM. Groups were compared using ANOVA followed by a Tukey post-hoc multiple comparison test. Significance (P value) is indicated. n=6-8 tumors per condition. Experiments were performed a minimum of two times with the same statistically significant results. n=6 tumors per condition.

Figure S3. Progestin influence on estrogen-dependent growth of two additional ER+ breast cancer PDX. **A**, Tumors UCD12 and UCD15 were grown in mice either in the absence of exogenously added hormones (placebo), or in the presence of continuous E2, E2+P4, or

E2+MPA. Tumor volumes were measured weekly and plotted versus the number of days of incubation ± SEM. Tumor volumes and masses at the final time point were compared using ANOVA followed by a Tukey post-hoc multiple comparison test. Significance (P value) is indicated. n=6-8 tumors per condition. Experiments were performed a minimum of two times with the same statistically significant results. **B**, UCD12 and UCD15 tumor sections from E2 supplemented mice stained by IHC for ER, PR, and AR. Scale bars, 200 um.

Figure S4. PR is associated with the RNA Pol III complex in ER+PR+ breast cancer PDX. Co-IP of PR with POLR3A and POLR3B in UCD4 and UCD65 E2+P4 treated tumors was performed as described in methods with a different PR antibody (DAKO 1294).

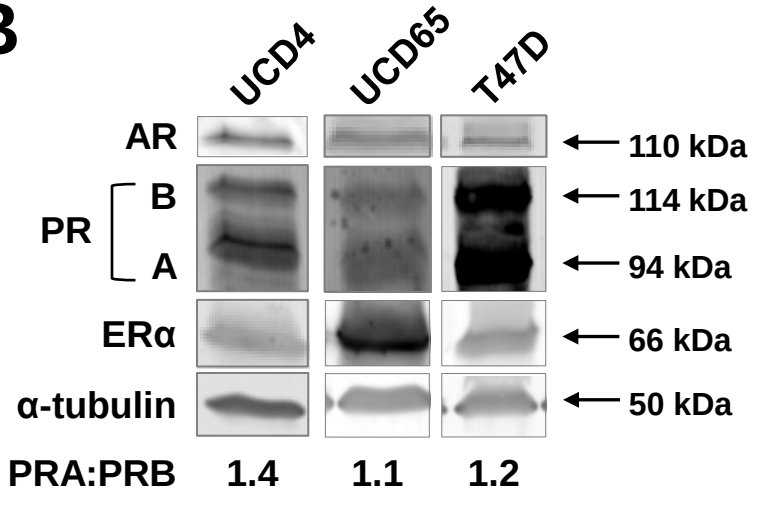
Figure S5. PR is associated with ER(α) in T47D cells supplemented with E2+P4. Co-immunoprecipitation (co-IP) was performed as described in methods. Here, T47D cells were treated with 10 nM E2+ ethanol vehicle (OH) or E2+ 100 nM P4 for 24 h. Co-IP on cell lysates was performed for PR (Ab sc-7208), ER (Ab sc-543), or IgG, followed by immunoblots for PR (A and B isoforms indicated) or ER. Molecular weights are indicated.

Supplementary Figure S1.

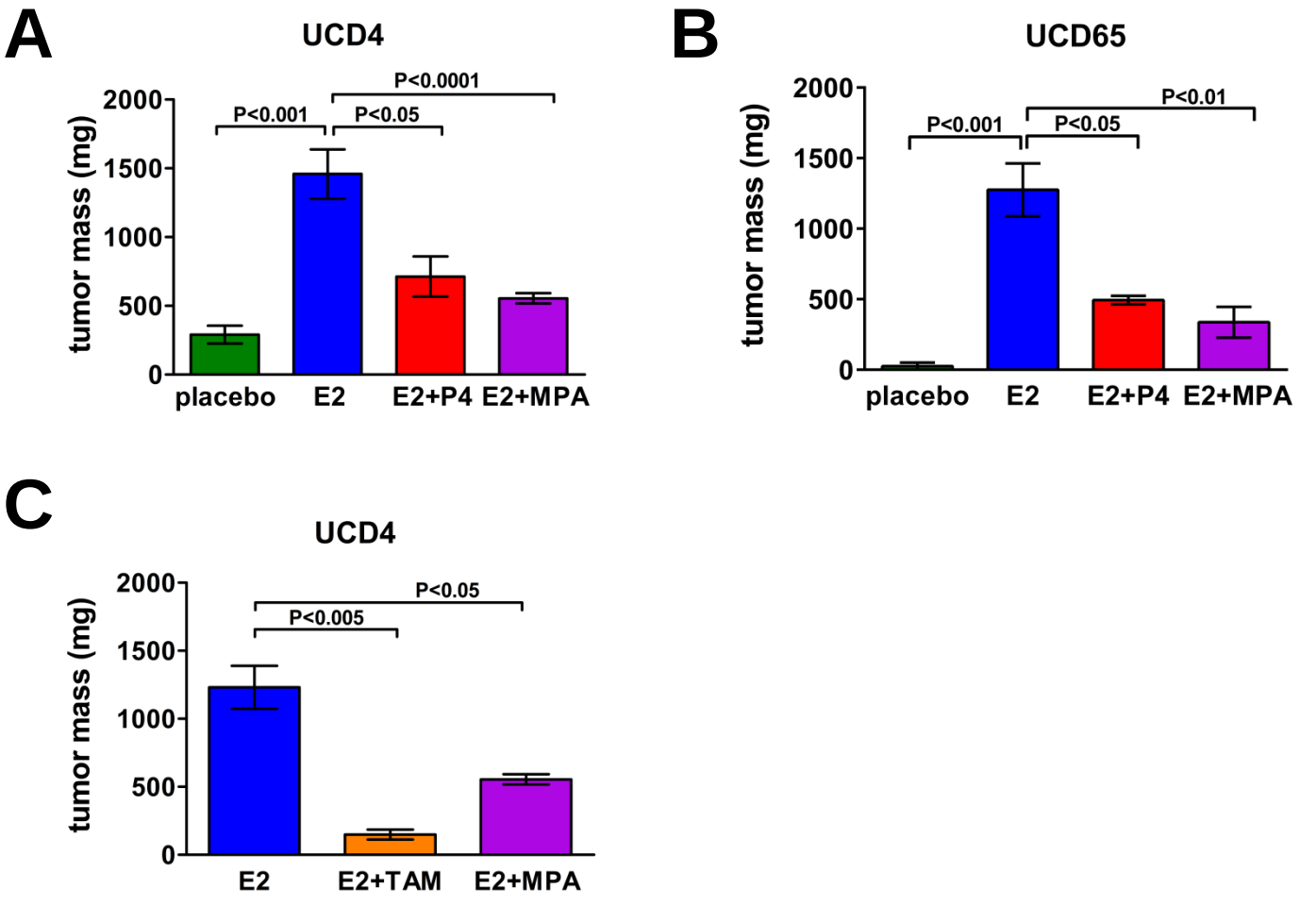
A

Tumor/stain	Hormone background		
UCD4	E2	E2+P4	E2+MPA
ER	82.1 ± 7.1	73.6 ± 1.4	81.9 ± 7.0
PR	83.5 ± 7.2	45.2 ± 14.9	50.4 ± 15.2
AR	51.6 ± 8.8	65.3 ± 7.3	93.2 ± 0.6[a]
UCD65			
ER	97.5 ± 1.4	94.6 ± 1.2	96.0 ± 2.7
PR	42.2 ± 12.7	52.7 ± 7.9	71.8 ± 16.3
AR	3.9 ± 2.9	7.7 ± 4.3	13.7 ± 8.2

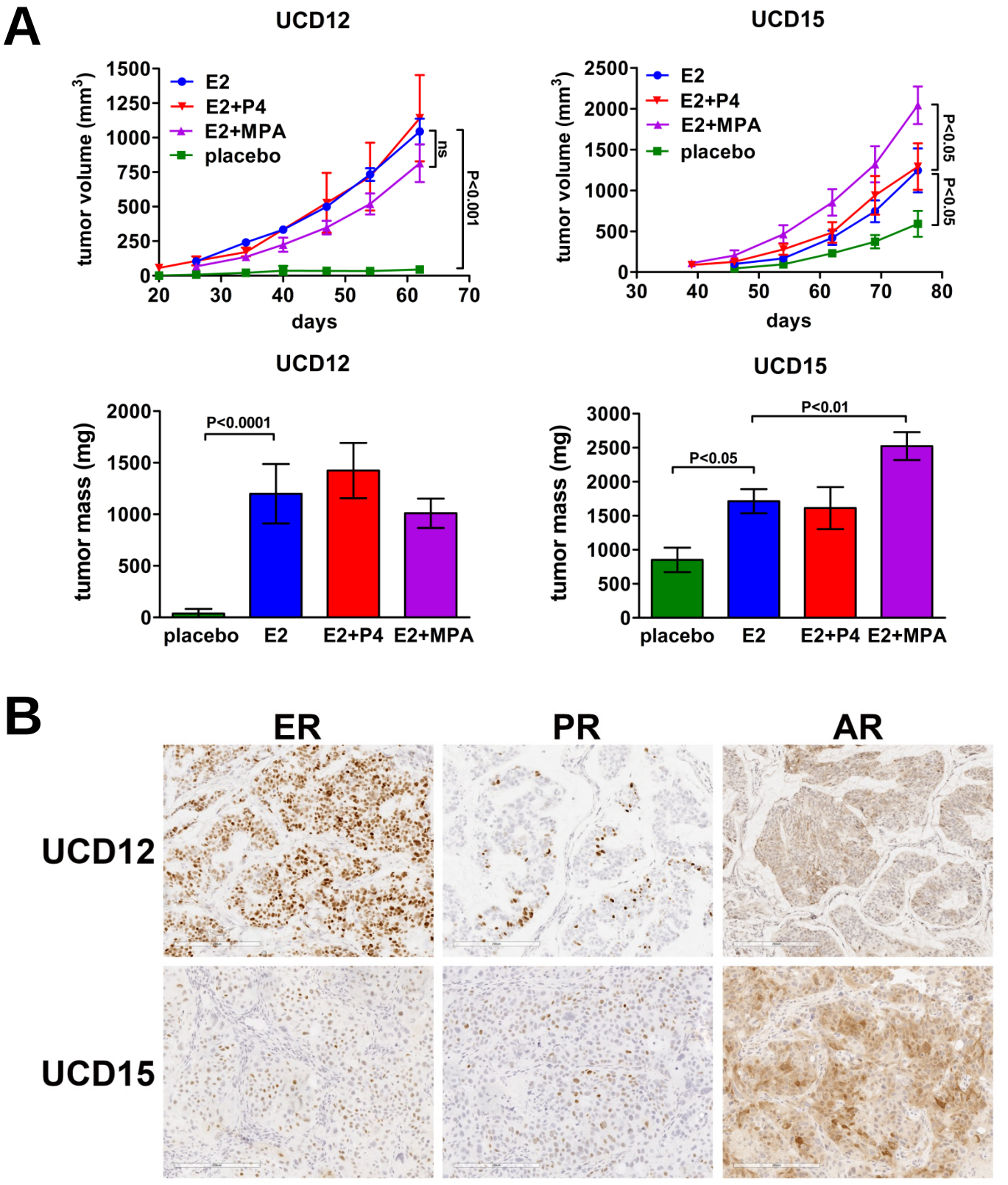
B



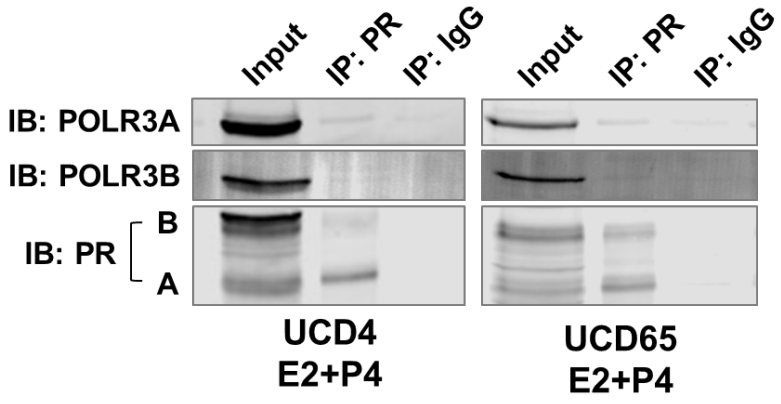
Supplementary Figure S2.



Supplementary Figure S3.



Supplementary Figure S4.



Supplementary Figure S5.