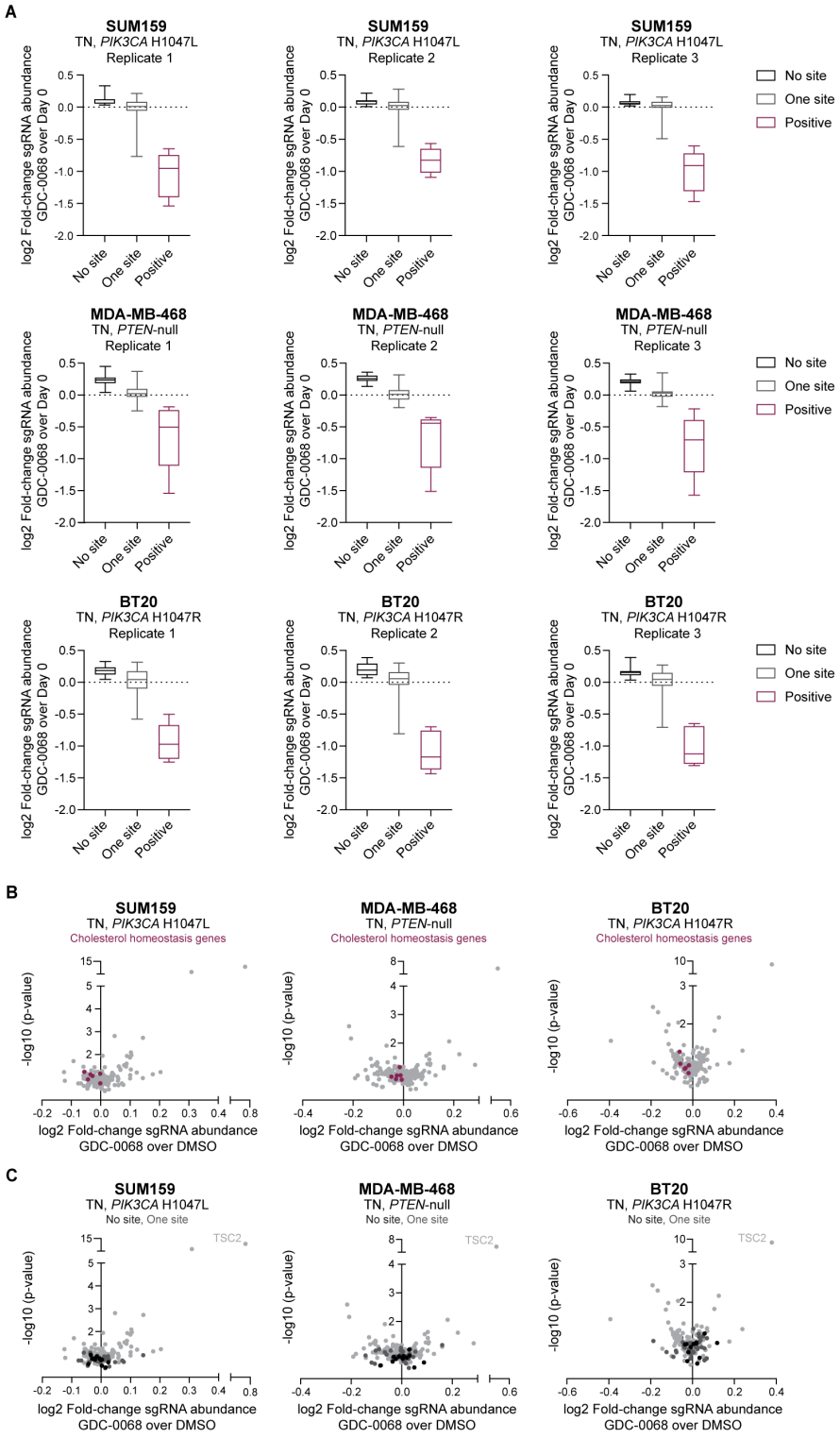
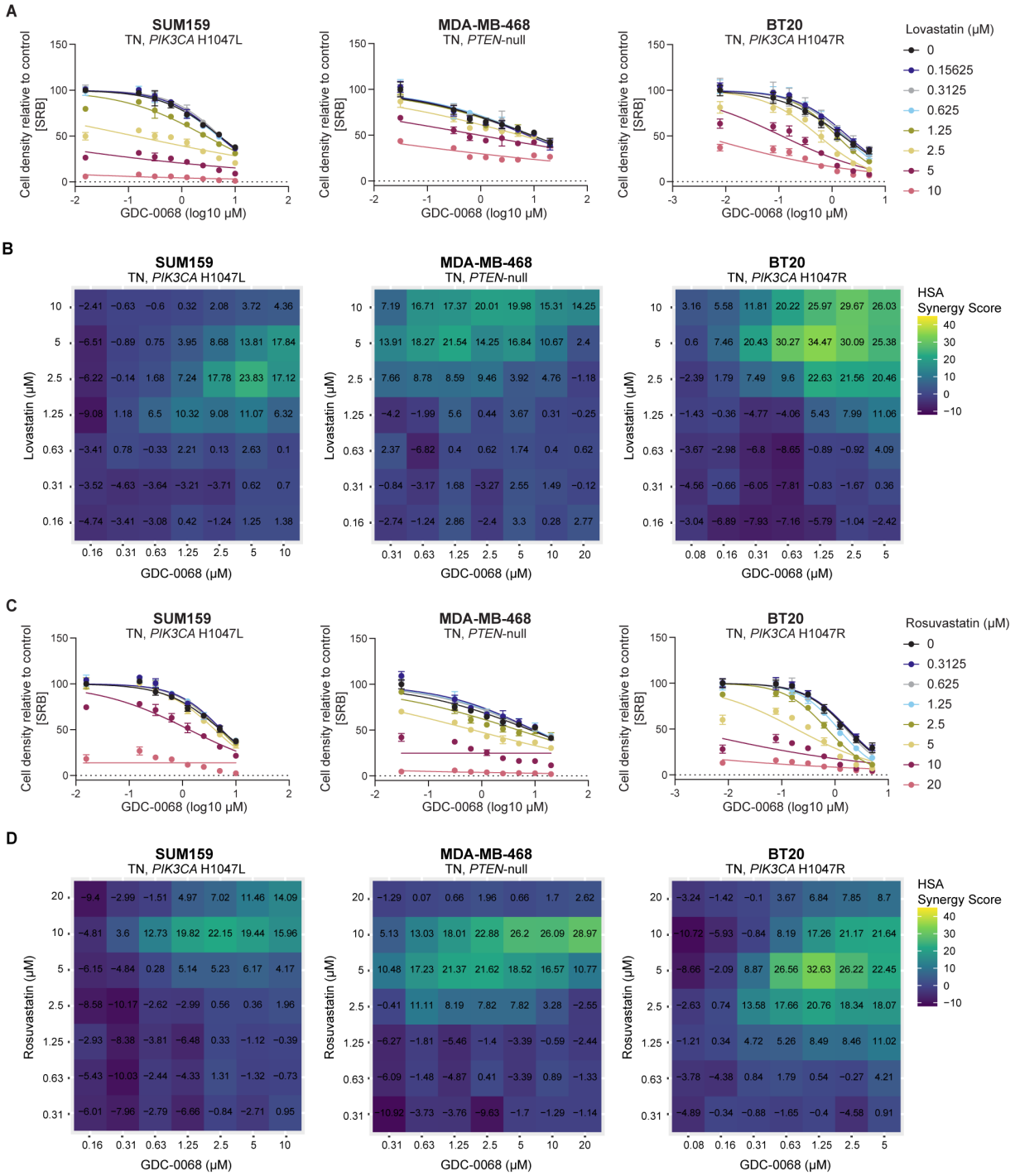


1 **Supplementary Figure S1. Custom CRISPR/Cas9 minipool library screen validates cholesterol**

2 **homeostasis hits in a panel of TNBC cells. A,** Comparison of the log2 fold-change in sgRNA

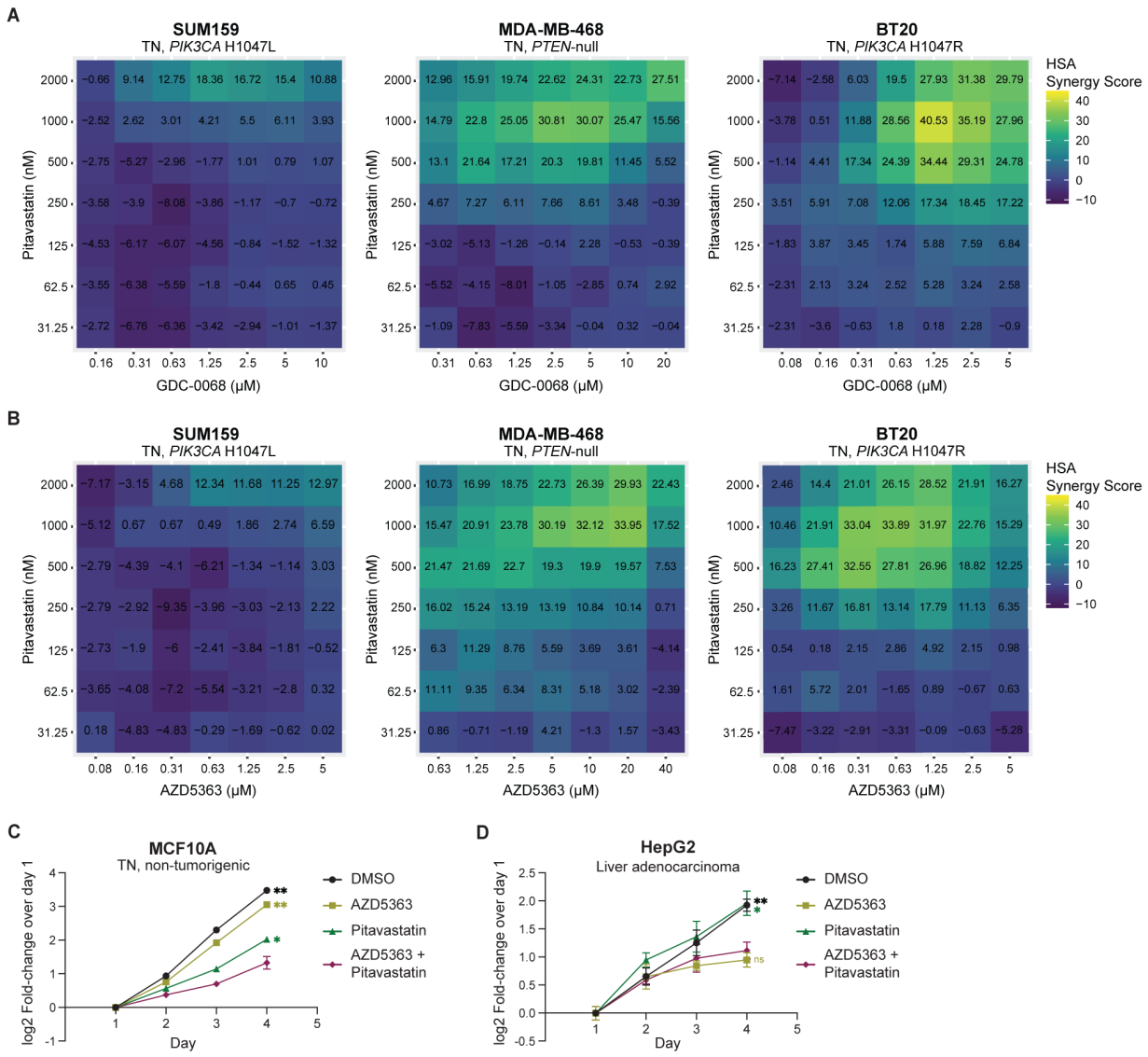
3 abundance for no site, one site and positive control sgRNAs in the GDC-0068 arm compared to the day 0

arm of the custom CRISPR/Cas9 minipool library screen. No site sgRNAs do not cut anywhere in the genome. One site sgRNAs introduce single cuts in the genome at intergenic sites that are predicted to have little to no deleterious effects. Positive control sgRNAs target genes that are essential for cell viability. Data are plotted as box and whisker plots by replicate for SUM159, MDA-MB-468 and BT20 cells. The box extends from the 25^{th} to 75^{th} percentiles, and whiskers extend from minimum to maximum values. **B,** Volcano plots showing log2 fold-change in sgRNA abundance in the GDC-0068 arm (SUM159: 4.34 μM, MDA-MB-468: 8.49 μM, BT20: 0.9 μM) of the custom CRISPR/Cas9 minipool screen compared to the DMSO arm versus -log10 (p-value). Cholesterol homeostasis genes with negative log2 fold-changes in all three TNBC cell lines are highlighted (*ABCA12*, *ABCB4*, *CYP39A1*, *SREBF2*, *TMEM97*, *VPS4B*). Data are represented as the mean of 3 technical replicates for each cell line. **C,** Volcano plots showing log2 fold-change in sgRNA abundance in the GDC-0068 arm of the custom CRISPR/Cas9 minipool screen compared to the DMSO arm versus -log10 (p-value). Controls are highlighted, including no site and one intergenic site controls and the positive control *TSC2*. Data are represented as the mean of 3 technical replicates for each cell line.



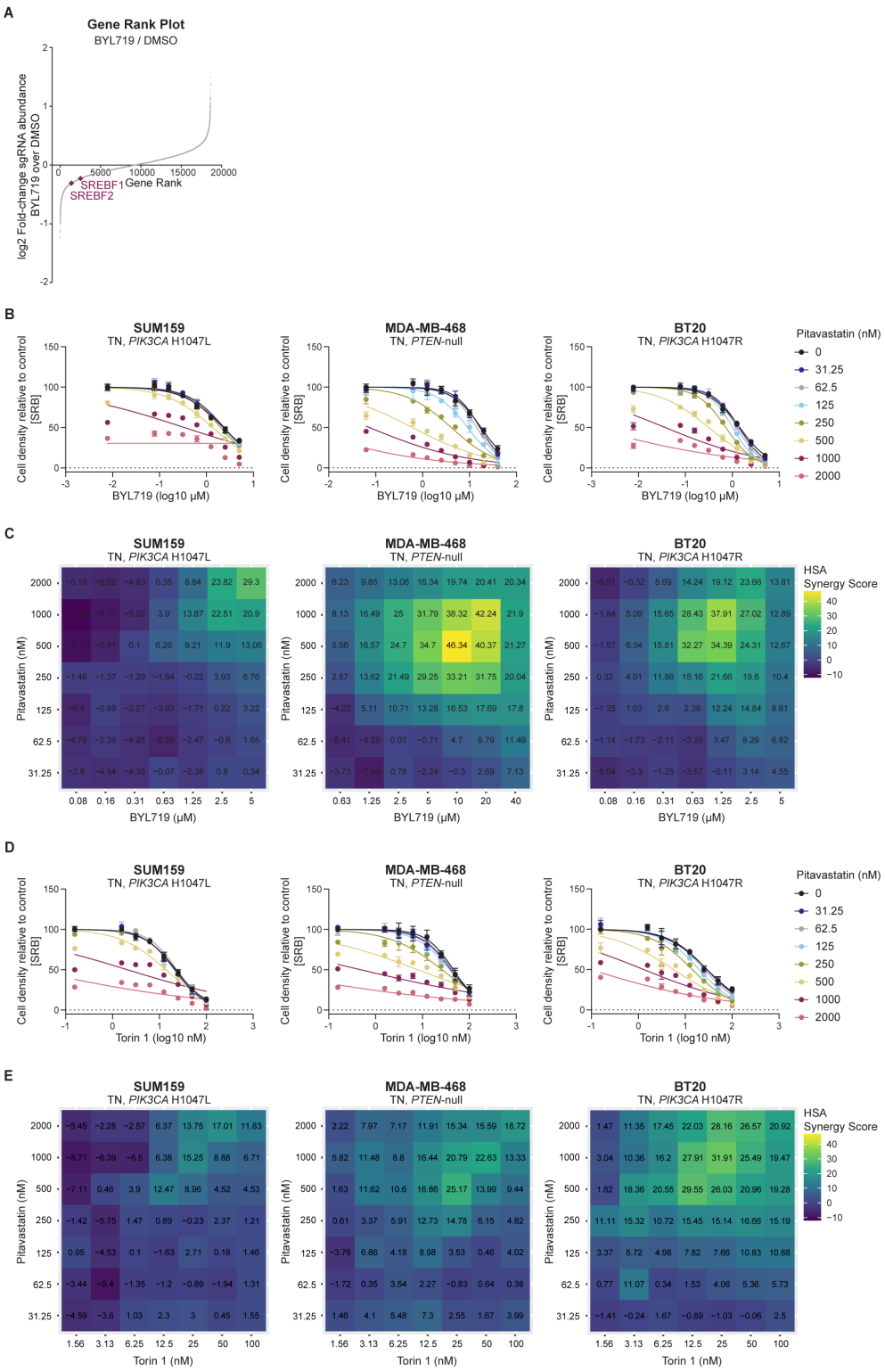
Supplementary Figure S2. Lovastatin and rosuvastatin synergize with AKT inhibitors in TNBC cells. A, TNBC cell lines (SUM159, MDA-MB-468, BT20) were treated with increasing doses of GDC-0068 (SUM159: 0-10 μM, MDA-MB-468: 0-20 μM, BT20: 0-5 μM) and lovastatin (0-10 μM) or **C,** rosuvastatin (0-20 μM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). **B,** TNBC cell lines (SUM159, MDA-MB-468, BT20) were

treated with increasing doses of GDC-0068 (SUM159: 0-10 μM, MDA-MB-468: 0-20 μM, BT20: 0-5 μM) and lovastatin (0-10 μM) or **D,** rosuvastatin (0-20 μM) for 72 hours, and cell density was measured by SRB assay. HSA synergy scores were calculated using SynergyFinder and are reported in the heatmaps (N=3 technical replicates).



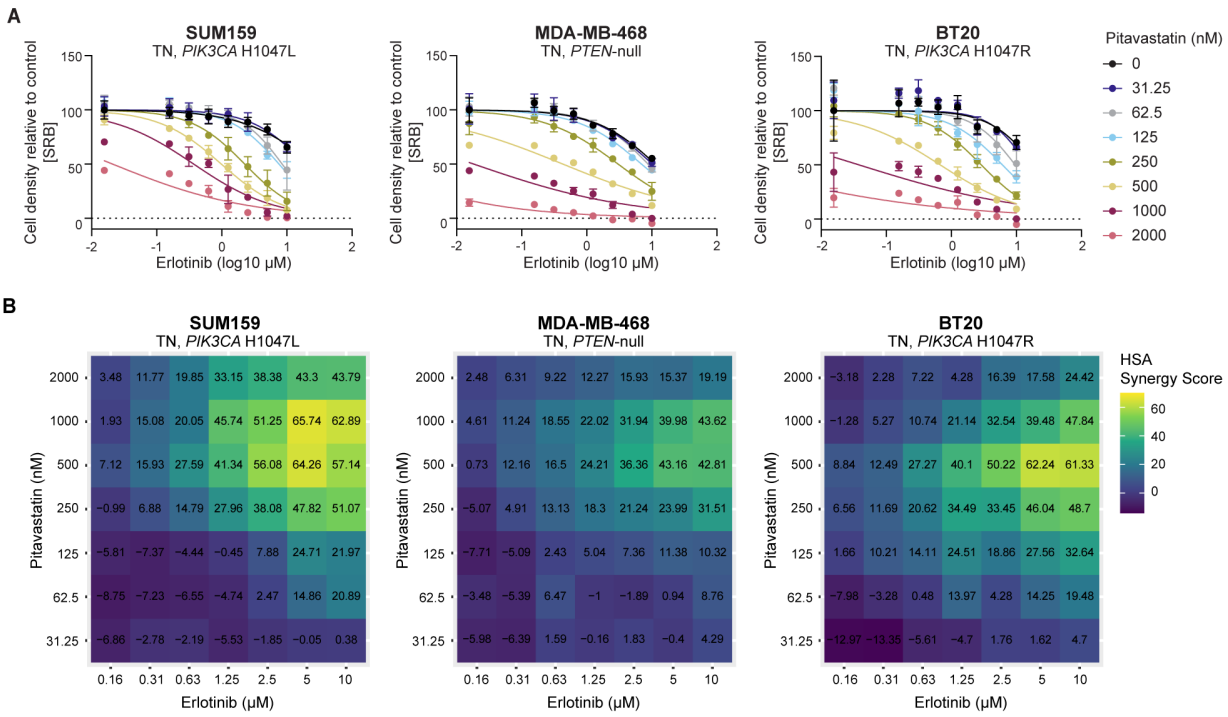
Supplementary Figure S3. Pitavastatin synergizes with AKT inhibitors in TNBC cells. A-B, TNBC cell lines (SUM159, MDA-MB-468, BT20) were treated with increasing doses of GDC-0068 (SUM159: 0-10 μM, MDA-MB-468: 0-20 μM, BT20: 0-5 μM) (**A**) or AZD5363 (SUM159: 0-5 μM, MDA-MB-468: 0-40 μM, BT20: 0-5 μM) (**B**) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. HSA synergy scores were calculated using SynergyFinder and are reported in the heatmaps (N=3 technical replicates). **C,** The triple-negative, non-tumorigenic mammary epithelial cell line, MCF10A, was treated with DMSO, 3 μM AZD5363, 2 μM pitavastatin or a combination of AZD5363 and pitavastatin for 72 hours, and cell density was measured daily by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance (ANOVA) with

Dunnett's multiple comparison test; asterisks (*) indicate significant differences compared to the AZD5363 and pitavastatin combination treatment on day 4 (*, p = 0.0332, **, p = 0.0021). **D,** The liver adenocarcinoma cell line, HepG2, was treated with DMSO, 20 μM AZD5363, 0.5 μM pitavastatin or a combination of AZD5363 and pitavastatin for 72 hours, and cell density was measured daily by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test; asterisks (*) indicate significant differences compared to the AZD5363 and pitavastatin combination treatment on day 4 (*, p = 0.0332, **, p = 0.0021).

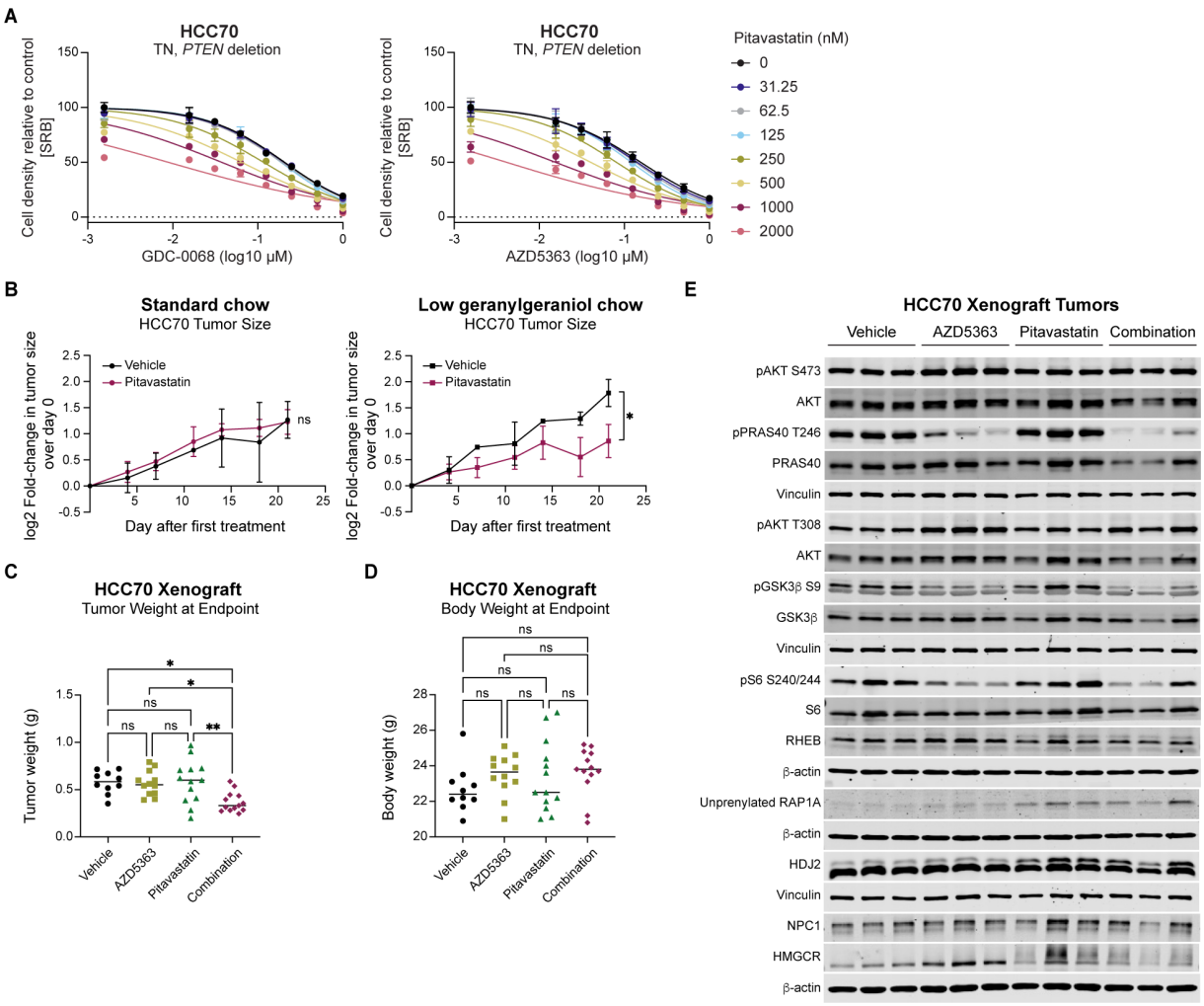


Supplementary Figure S4. Pitavastatin synergizes with the PI3Kα inhibitor BYL719 and the mTORC1/2 inhibitor Torin 1 in TNBC cells. A, Rank plots showing the log2 fold-change of each gene plotted against the rank dropout for the BYL719 treatment arm of the CRISPR/Cas9 screen compared to

the DMSO arm. The transcription factors *SREBF1* and *SREBF2* are highlighted. The plot was generated using MAGeCK with a read count cutoff of 50 (N=3 technical replicates). **B,** TNBC cell lines (SUM159, MDA-MB-468, BT20) were treated with increasing doses of BYL719 (SUM159: 0-5 μM, MDA-MB-468: 0-40 μM, BT20: 0-5 μM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). **C,** HSA synergy scores were calculated for the dose curves shown in **B** using SynergyFinder and are reported in the heatmaps (N=3 technical replicates). **D,** TNBC cell lines (SUM159, MDA-MB-468, BT20) were treated with increasing doses of Torin 1 (0-100 nM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=2 technical replicates). **E,** HSA synergy scores were calculated for the dose curves shown in **D** using SynergyFinder and are reported in the heatmaps (N=2 technical replicates).

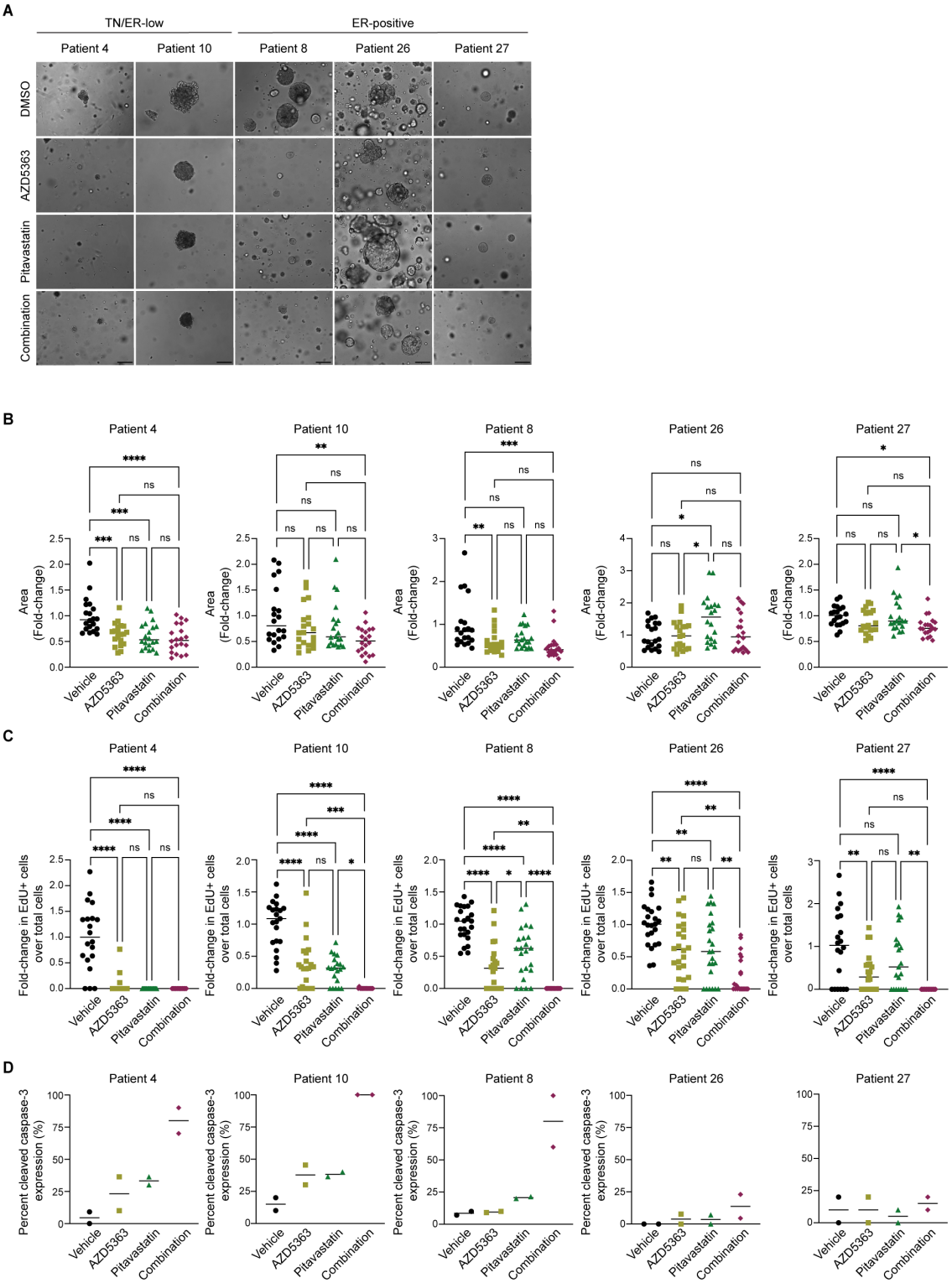


Supplementary Figure S5. Pitavastatin synergizes with the EGFR inhibitor erlotinib in TNBC cells. **A,** TNBC cell lines (SUM159, MDA-MB-468, BT20) were treated with increasing doses of erlotinib (0-10 μM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=2 technical replicates). **B,** HSA synergy scores were calculated for the dose curves shown in **A** using SynergyFinder and are reported in the heatmaps (N=2 technical replicates).



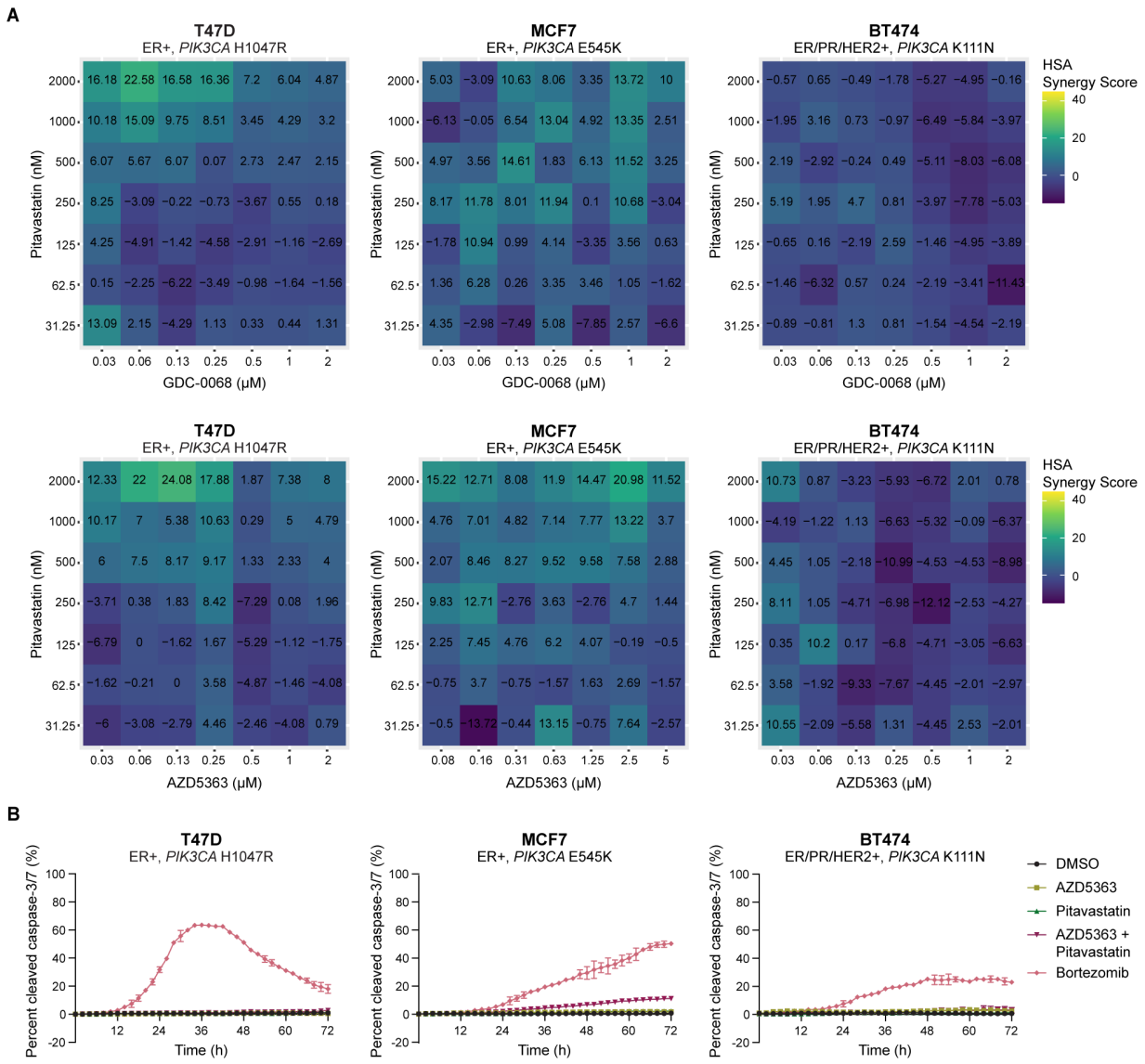
Supplementary Figure S6. AZD5363 and pitavastatin synergize in HCC70 cells *in vitro* and in mouse xenografts with on-target efficacy. A, HCC70 cells were treated with increasing doses of GDC-0068 (0-1 μM) or AZD5363 (0-1 μM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). **B,** In a pilot experiment, HCC70 cells were injected subcutaneously into NSG mice and tumors were allowed to grow for 21 days before starting treatments. Mice were maintained on a standard chow diet or switched to a low geranylgeraniol chow diet 3 days before starting treatments and were treated once daily with vehicle (0.5% carboxymethylcellulose, N=2 per diet) or 100 mg/kg pitavastatin (daily, N=3 per diet) for 20 days. Tumor size (mm^3) was measured every 3-4 days. Statistical analysis was performed using an unpaired, two-tailed parametric t-test (*, p = 0.0332). **C-E** represent data from the mouse experiment in **Fig. 3B-D**. **C,** Tumor weight was measured at the endpoint. **D,** Mouse body weight was measured at the endpoint.

For **C-D**, statistical analysis was performed using two-way analysis of variance (ANOVA) with Tukey's multiple comparison test; asterisks (*) indicate significant differences compared to the AZD5363 and pitavastatin combination treatment at the endpoint (*, $p = 0.0332$, **, $p = 0.0021$). **E,** Select tumors (3 per treatment group) were harvested 2 hours after the last AZD5363 treatment and 6 hours after the last pitavastatin treatment and immunoblotted for $pAKT^{Ser473}$, $pPRAS40^{Thr246}$, $pAKT^{Thr308}$, $pGSK3\beta^{Ser9}$, $pS6^{Ser240/244}$, RHEB, unprenylated RAP1A, HDJ2, NPC1, HMGCR, vinculin and β-actin.



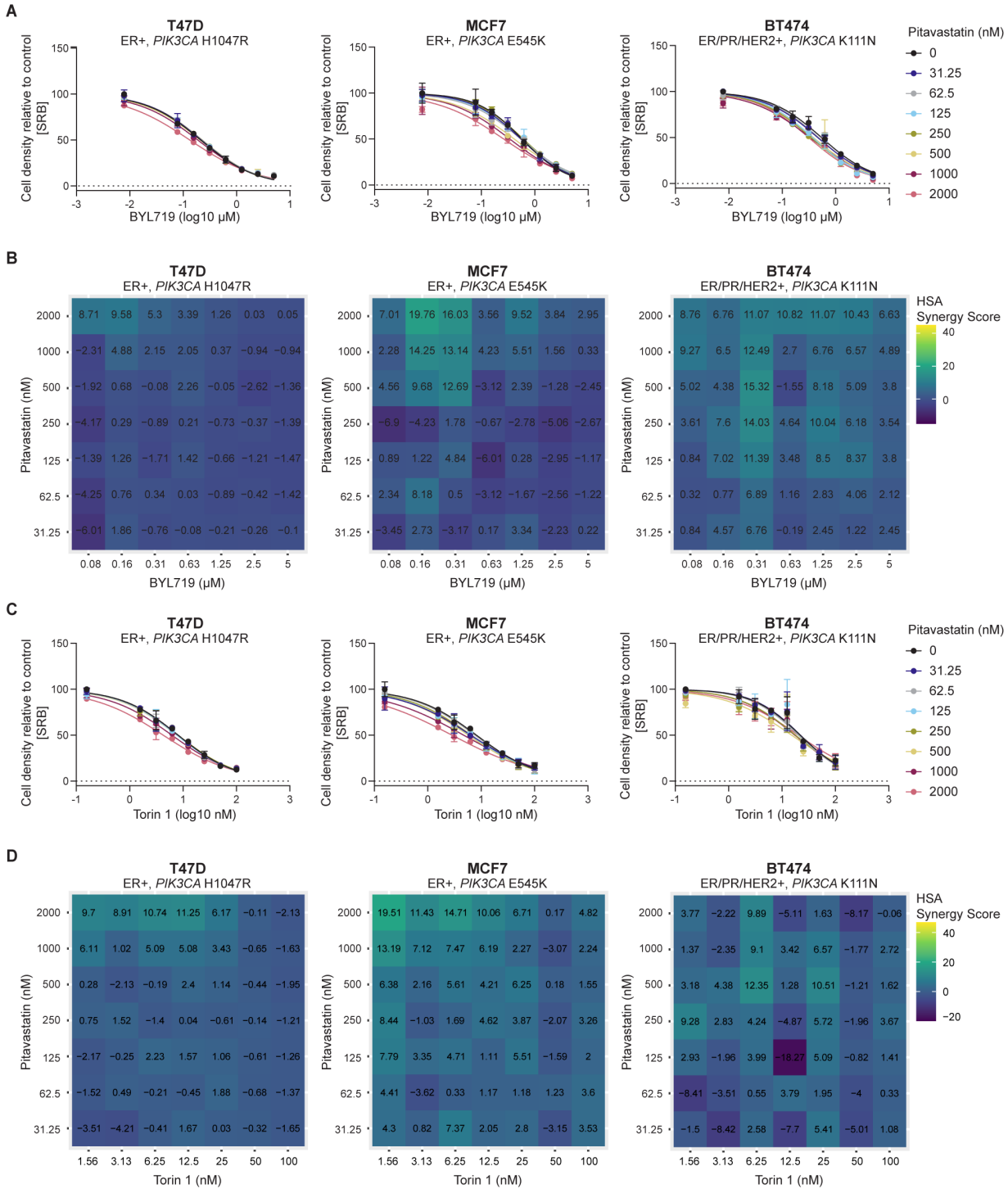
Supplementary Figure S7. TN/ER-low PDOs are sensitive to combination AZD5363 and pitavastatin. A, A panel of breast cancer PDOs were treated with DMSO, 1 μM AZD5363, 5 μM pitavastatin or the combination of AZD5363 and pitavastatin for 96 hours and organoid size and

morphology were assessed. A representative image for each PDO in each treatment condition is shown. Scale bars are 200 μm. **B,** The area of 20 PDOs per treatment condition was quantified and normalized to the vehicle-treated condition. **C,** EdU+ cells were quantified for 20 PDOs per treatment condition and normalized to total cell number and to the vehicle-treated condition. For **B-C**, statistical analysis was performed using two-way analysis of variance (ANOVA) with Tukey's multiple comparison test; asterisks (*) indicate significant differences (*, $p = 0.0332$, **, $p = 0.0021$, ***, $p = 0.0002$, ****, $p < 0.0001$). **D,** The percentage of cleaved caspase-3-expressing PDOs was quantified for 2 images per treatment condition.



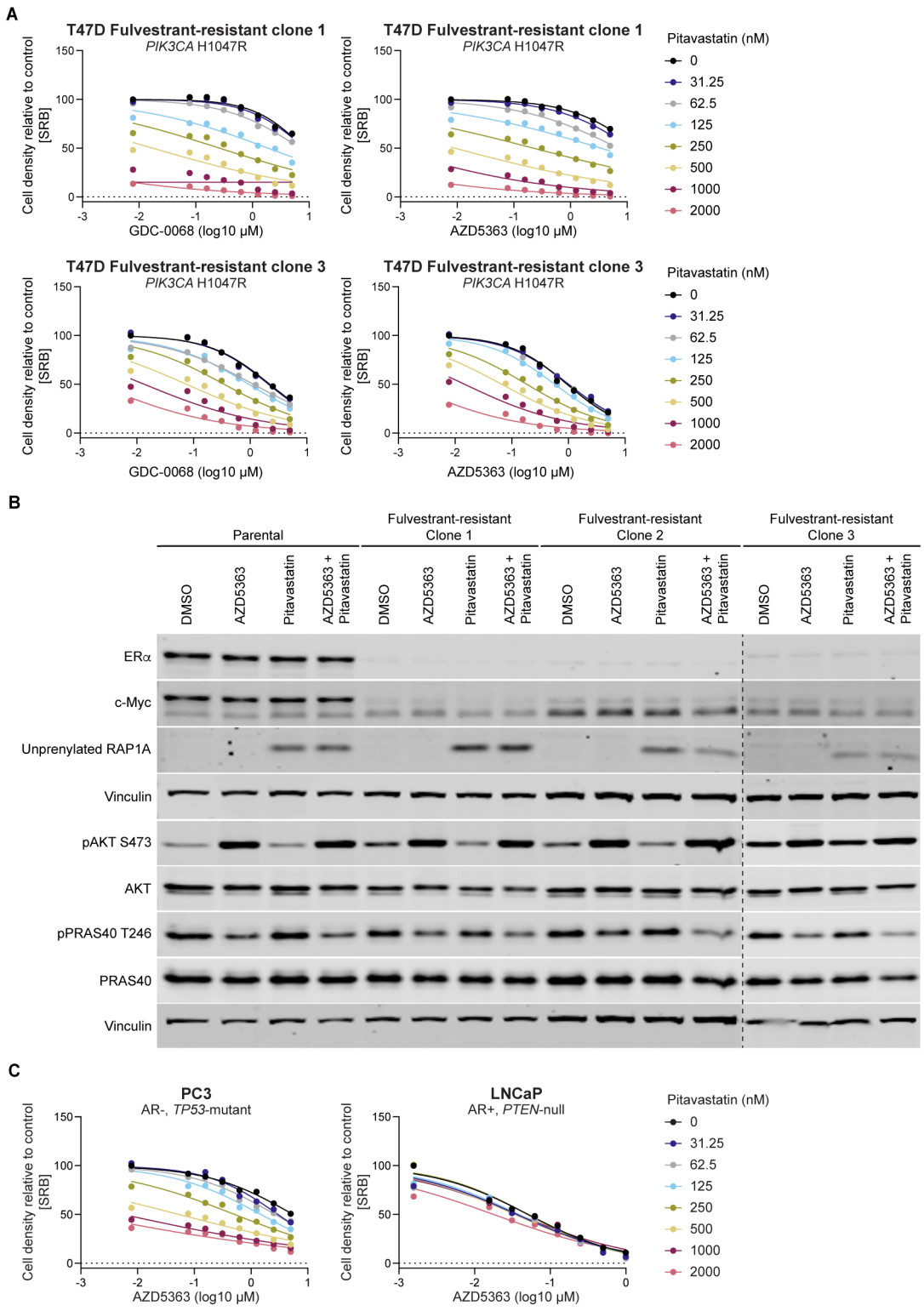
Supplementary Figure S8. Pitavastatin does not synergize with AKT inhibition to induce cell death in ER-positive breast cancer cells. A, ER-positive breast cancer cell lines (T47D, MCF7, BT474) were treated with increasing doses of GDC-0068 (0-2 μM) or AZD5363 (T47D: 0-2 μM, MCF7: 0-5 μM, BT474: 0-2 μM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. HSA synergy scores were calculated using SynergyFinder and are reported in the heatmaps (N=3 technical replicates). **B,** ER-positive breast cancer cell lines were treated with DMSO, AZD5363 (T47D: 0.25 μM, MCF7: 1.25 μM, BT474: 0.25 μM), pitavastatin (2 μM), a combination of AZD5363 and pitavastatin or bortezomib (10 μM) for 72 hours, and total cell number (rapid red nuclear dye) and number of dead cells

(cleaved caspase-3/7 dye) were measured every 2 hours for 72 hours by Incucyte live-cell analysis. Data are represented as mean ± SD of percent cleaved caspase-3/7 signal (N=4 technical replicates).



101 **Supplementary Figure S9. Pitavastatin does not synergize with the PI3Kα inhibitor BYL719 or the**
102 **mTORC1/2 inhibitor Torin 1 in ER-positive breast cancer cells. A,** ER-positive breast cancer cell lines
103 (T47D, MCF7, BT474) were treated with increasing doses of BYL719 (0-5 μM) and pitavastatin (0-2000
104 nM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD

(N=3 technical replicates). **B,** HSA synergy scores were calculated for the dose curves shown in **a** using SynergyFinder and are reported in the heatmaps (N=3 technical replicates). **C,** ER-positive breast cancer cell lines (T47D, MCF7, BT474) were treated with increasing doses of Torin 1 (0-100 nM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=2 technical replicates). **D,** HSA synergy scores were calculated for the dose curves shown in **C** using SynergyFinder and are reported in the heatmaps (N=2 technical replicates).

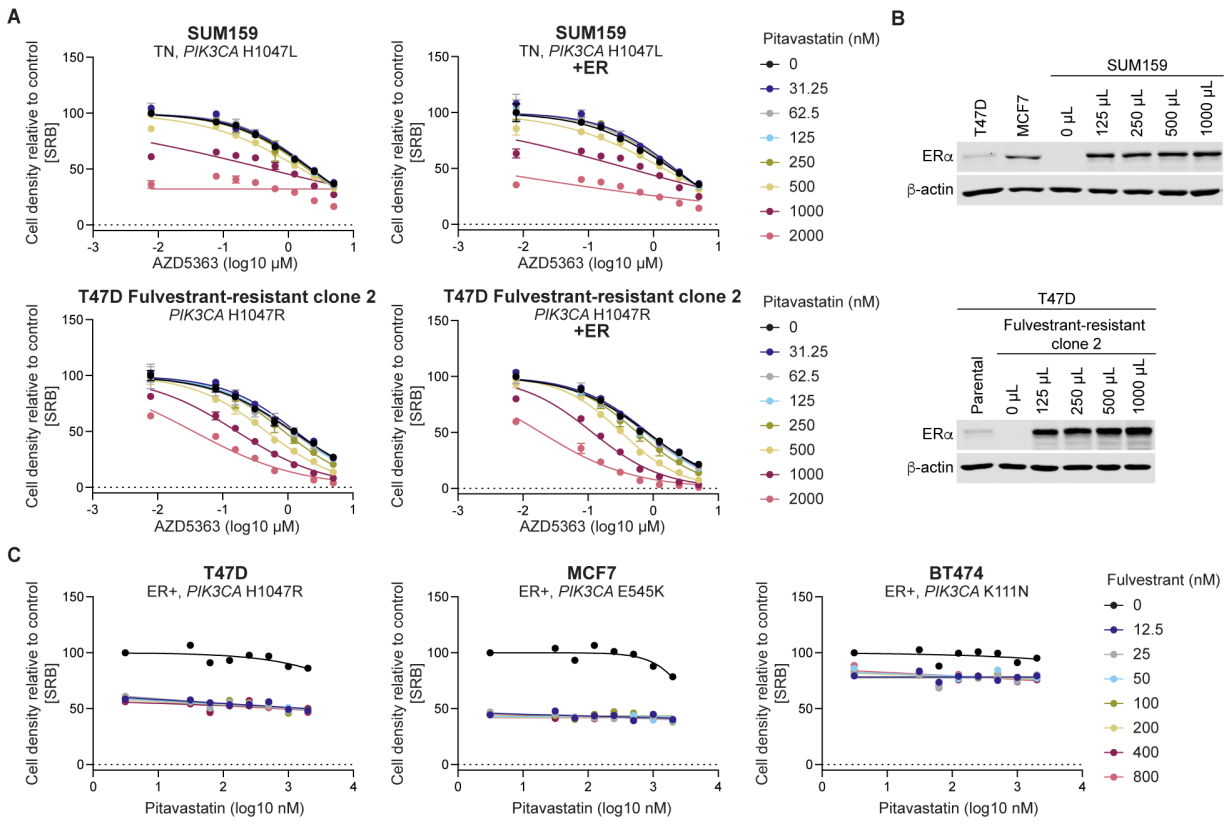


111 **Supplementary Figure S10. Pitavastatin synergizes with AKT inhibition in fulvestrant-resistant**

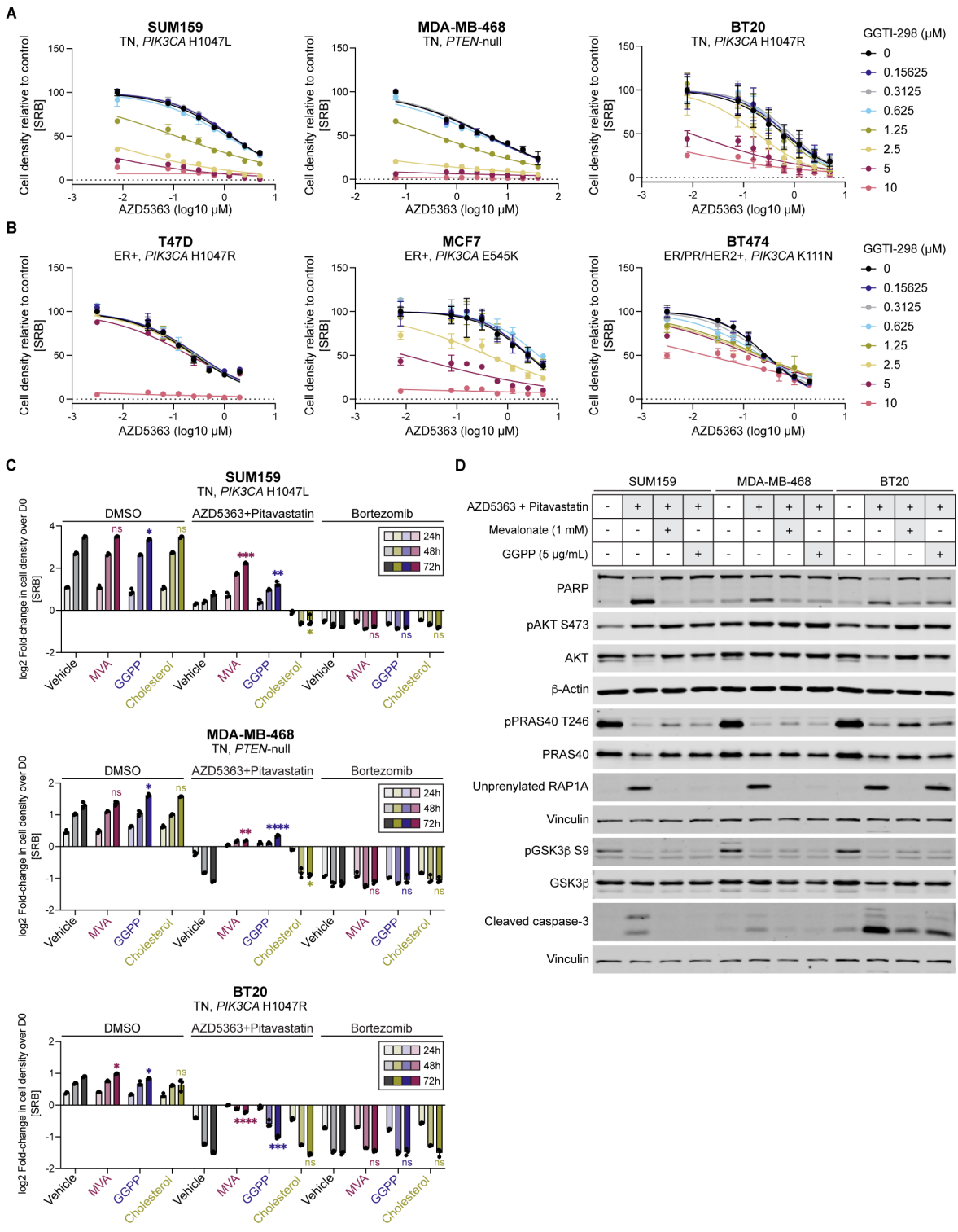
112 **T47D cells. A,** Parental and fulvestrant-resistant T47D cells were treated with increasing doses of GDC-

113 0068 (0-5 μM) or AZD5363 (0-5 μM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was

measured by SRB assay (N=1 technical replicate). **B,** Immunoblots of ERα, c-Myc, unprenylated RAP1A, vinculin, $pAKT^{Ser473}$ and $pPRAS40^{Thr246}$ in parental and fulvestrant-resistant T47D cells treated with DMSO, AZD5363 (0.25 μM), pitavastatin (0.5 μM) or the combination of AZD5363 and pitavastatin for 72 hours. Fulvestrant-resistant clone 3 samples were run on a separate gel. **C,** AR-negative (PC3) and AR-positive (LNCaP) prostate cancer cells were treated with increasing doses of AZD5363 (PC3: 0-5 μM, LNCaP: 0-1 μM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay (N=1 technical replicate).



Supplementary Figure S11. Modulation of ER expression does not affect pitavastatin sensitivity. A, ER-negative breast cancer cell lines (SUM159, fulvestrant-resistant T47D clone 2) were transduced with lentiviral vectors to overexpress estrogen receptor (ER) and were maintained in culture for approximately 1 week. Parental and ER-overexpressing cells were then treated with increasing doses of AZD5363 (0-5 μM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=2 technical replicates). **B,** Immunoblots of ERα and β-actin in SUM159 and fulvestrant-resistant T47D cells overexpressing ER with pHAGE-ESR1 compared to ER-positive breast cancer cell lines (T47D, MCF7) and parental T47D cells. **C,** ER-positive breast cancer cell lines (T47D, MCF7, BT474) were treated with increasing doses of fulvestrant (0-800 nM) and pitavastatin (0-2000 nM) for 72 hours, and cell density was measured by SRB assay. Data represent a single technical replicate (N=1 technical replicate).

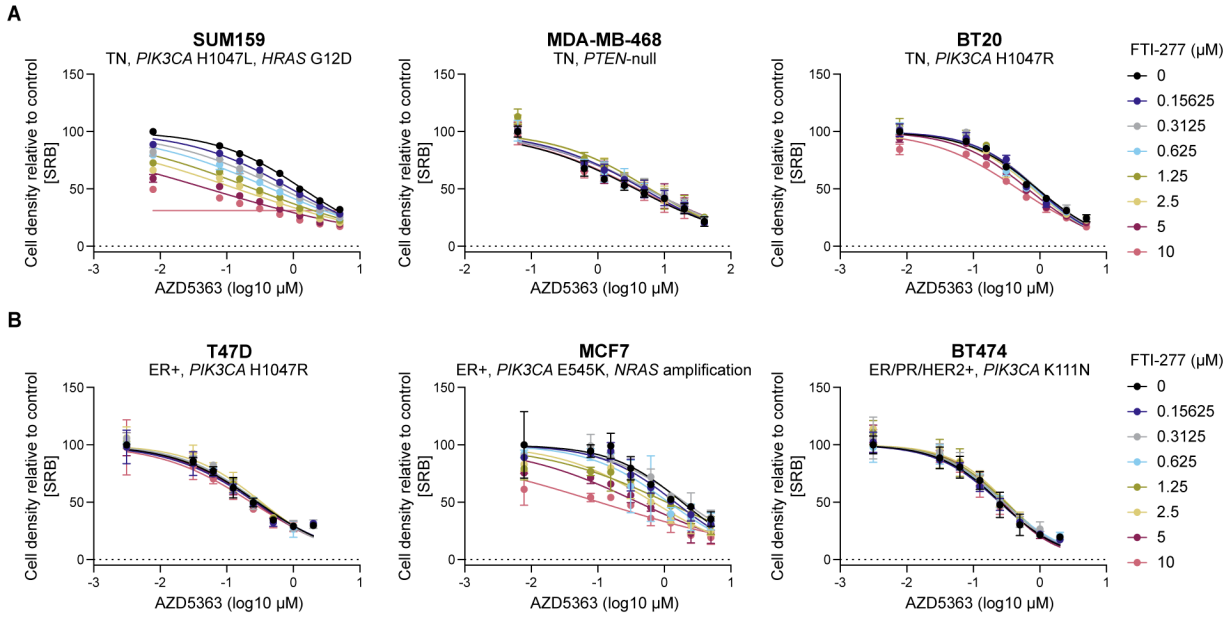


132 **Supplementary Figure S12. AKT inhibitors synergize with cholesterol biosynthesis inhibition to**

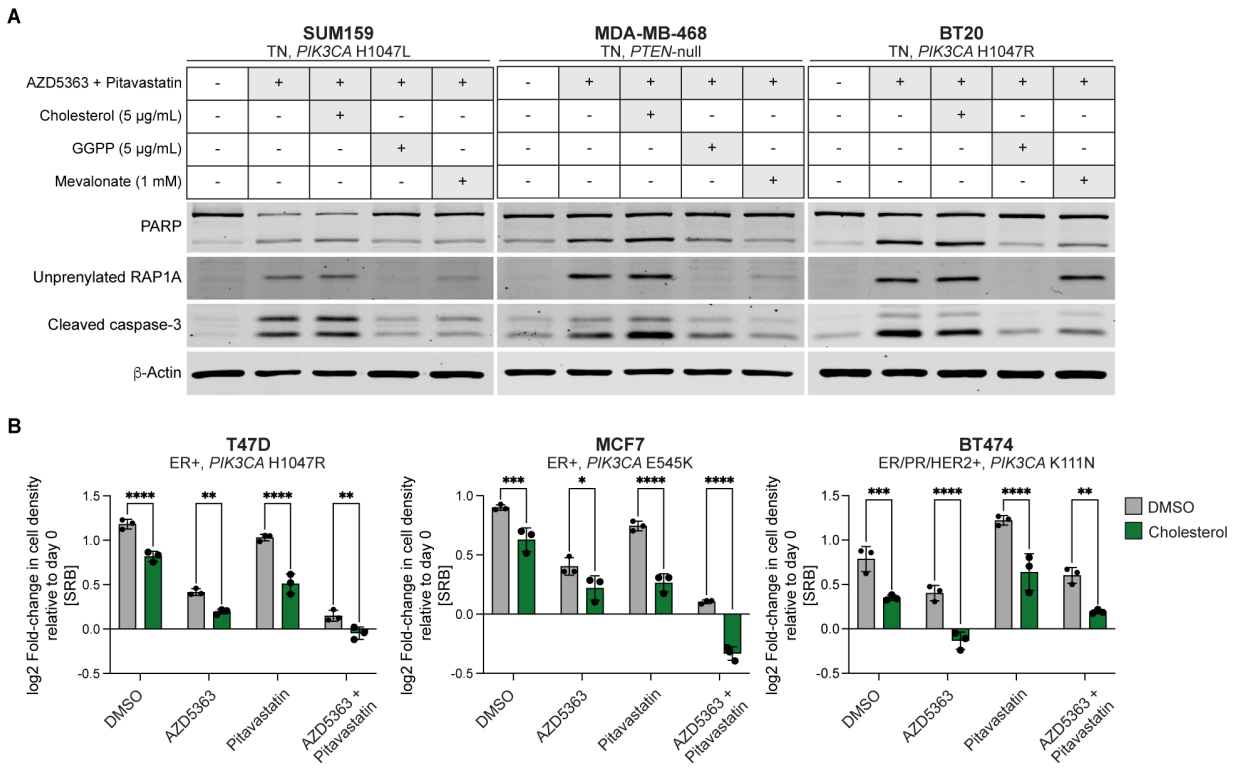
133 **deplete GGPP. A,** TNBC cell lines (SUM159, MDA-MB-468, BT20) were treated with increasing doses of

134 AZD5363 (SUM159: 0-5 μM, MDA-MB-468: 0-40 μM, BT20: 0-5 μM) and GGTI-298 (0-10 μM) for 72

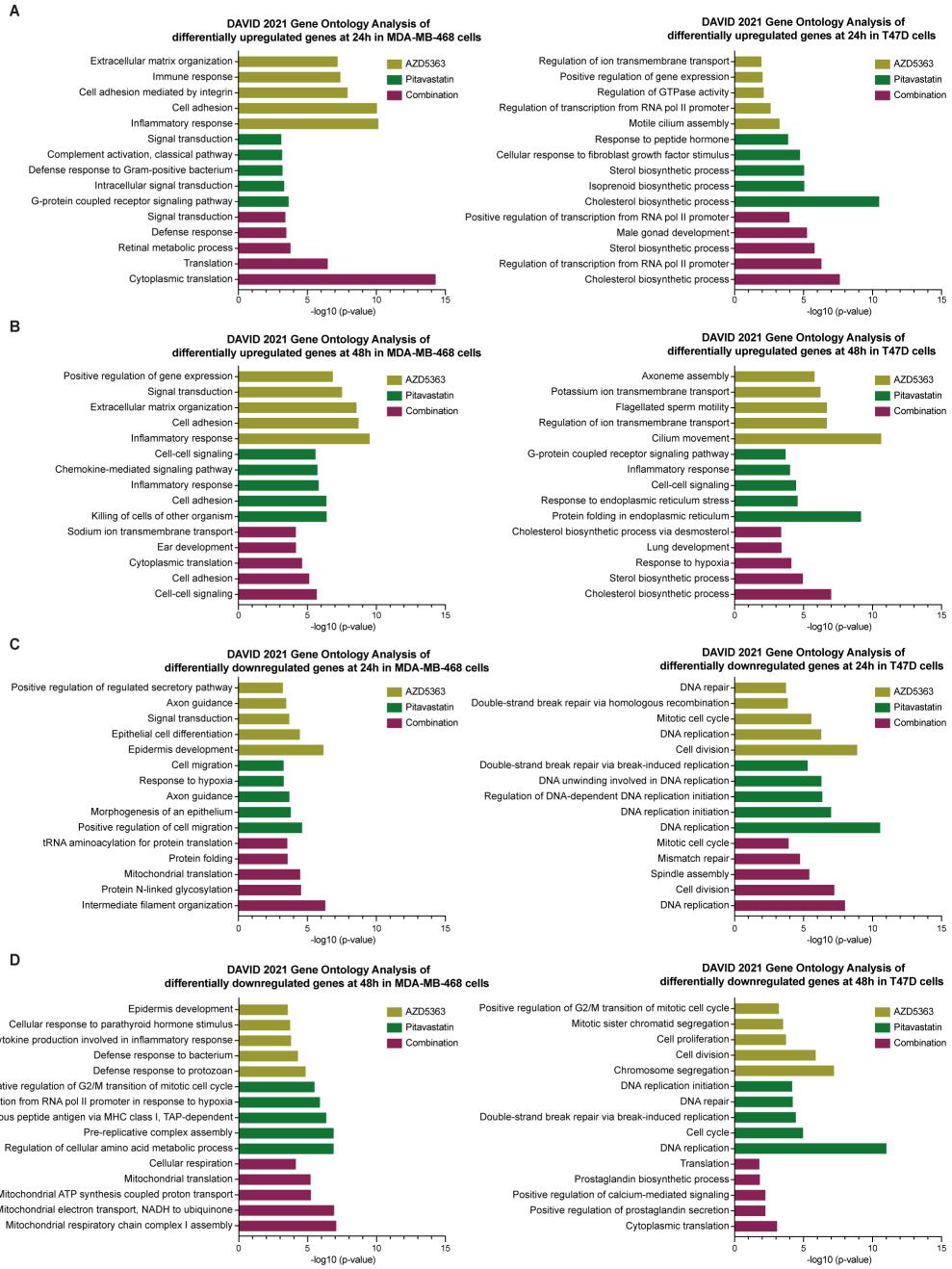
hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=2 technical replicates). **B,** ER-positive breast cancer cell lines (T47D, MCF7, BT474) were treated with increasing doses of AZD5363 (T47D: 0-2 μM, MCF7: 0-5 μM, BT474: 0-2 μM) and GGTI-298 (0-10 μM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=2 technical replicates). **C,** TNBC cell lines (SUM159, MDA-MB-468, BT20) were treated with DMSO, a combination of AZD5363 (SUM159: 5 μM, MDA-MB-468: 15 μM, BT20: 1.25 μM) and pitavastatin (SUM159: 4 μM, MDA-MB-468: 2 μM, BT20: 2 μM) or bortezomib (10 μM) and supplemented with vehicle (7:3 MeOH:NH_4OH), mevalonate (1 mM), GGPP (5 μg/mL) or cholesterol (5 μg/mL) for 24, 48 or 72 hours. Cell density was measured by SRB assay at each time point. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed for the 72-hour time point using two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test; asterisks (*) indicate significant differences compared to the 72-hour vehicle supplemented condition within the DMSO, AZD5363 and pitavastatin or bortezomib treatment group (*, $p = 0.0332$, **, $p = 0.0021$, ***, $p = 0.0002$, ****, $p < 0.0001$). **D,** Immunoblots of PARP, $pAKT^{Ser473}$, $pPRAS40^{Thr246}$, unprenylated RAP1A, $pGSK3\beta^{Ser9}$, cleaved caspase-3, β-actin and vinculin in TNBC cell lines (SUM159, MDA-MB-468, BT20) treated with DMSO or a combination of AZD5363 (SUM159: 5 μM, MDA-MB-468: 15 μM, BT20: 1.25 μM) and pitavastatin (SUM159: 4 μM, MDA-MB-468: 2 μM, BT20: 2 μM) and supplemented with vehicle (7:3 MeOH:NH_4OH), mevalonate (1 mM), GGPP (5 μg/mL) or cholesterol (5 μg/mL) for 22 hours.



Supplementary Figure S13. Farnesyltransferase inhibition with FTI-277 synergizes with AZD5363 in *RAS*-altered breast cancer cells. A, TNBC cell lines (SUM159, MDA-MB-468, BT20) were treated with increasing doses of AZD5363 (SUM159: 0-5 μM, MDA-MB-468: 0-40 μM, BT20: 0-5 μM) and FTI-277 (0-10 μM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=2 technical replicates). **B,** ER-positive breast cancer cell lines (T47D, MCF7, BT474) were treated with increasing doses of AZD5363 (T47D: 0-2 μM, MCF7: 0-5 μM, BT474: 0-2 μM) and FTI-277 (0-10 μM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=2 technical replicates).

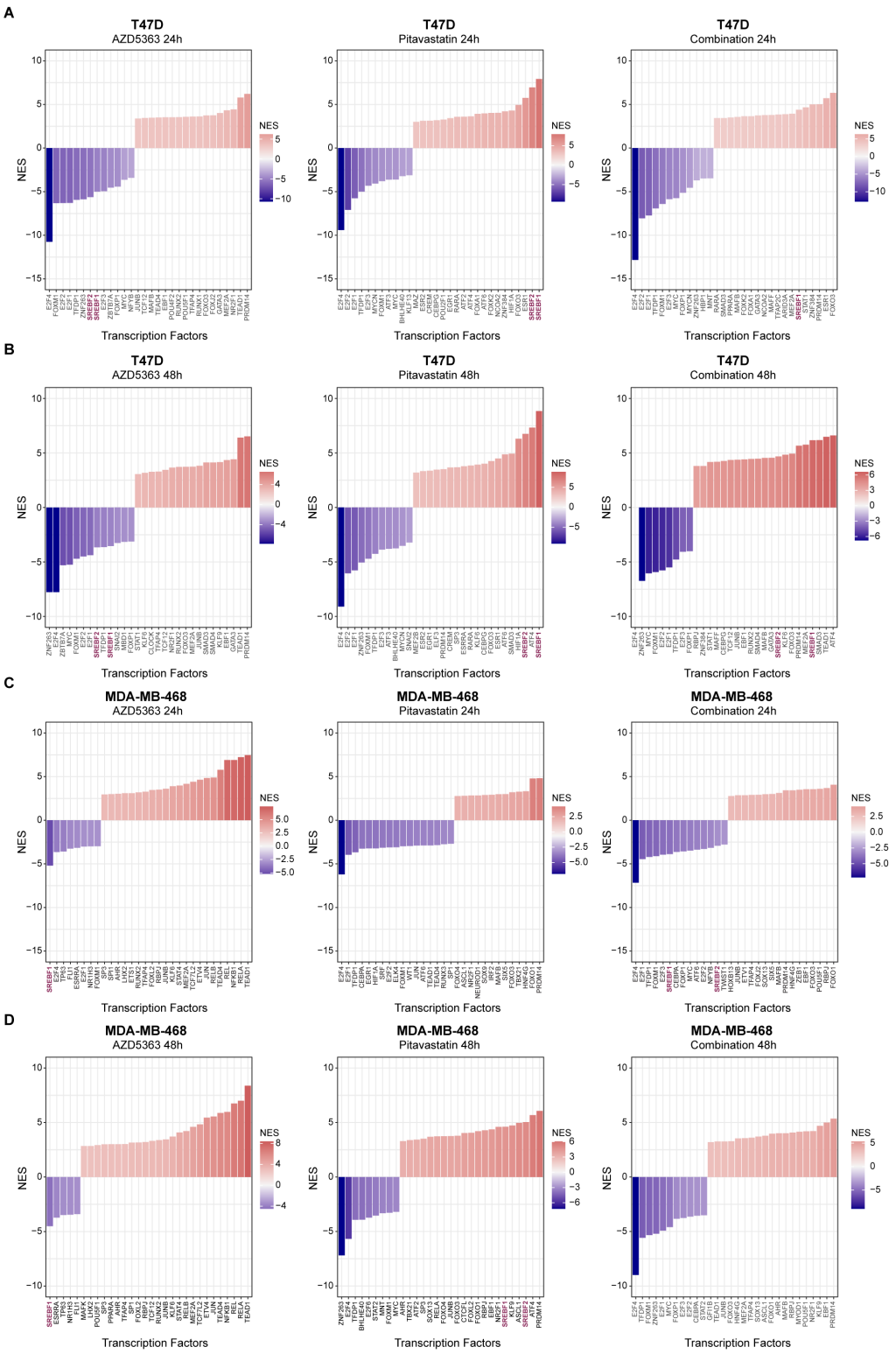


Supplementary Figure S14. Cholesterol supplementation potentiates the cytotoxicity of AZD5363 and pitavastatin in TN and ER+ breast cancer cells. A, Immunoblots of PARP, unprenylated RAP1A, cleaved caspase-3 and β-actin in TNBC cell lines (SUM159, MDA-MB-468, BT20) treated with DMSO or a combination of AZD5363 (SUM159: 5 µM, MDA-MB-468: 15 µM, BT20: 1.25 µM) and pitavastatin (SUM159: 4 µM, MDA-MB-468: 2 µM, BT20: 2 µM) and supplemented with vehicle (7:3 MeOH:NH_4OH), cholesterol (5 µg/mL), GGPP (5 µg/mL) or mevalonate (1 mM) for 22 hours. **B,** ER-positive breast cancer cell lines (T47D, MCF7, BT474) were treated with DMSO, AZD5363 (T47D: 0.25 µM, MCF7: 1.25 µM, BT474: 0.25 µM), pitavastatin (2 µM) or a combination of AZD5363 and pitavastatin for 72 hours and supplemented with DMSO or 5 µg/mL cholesterol. Cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance (ANOVA) with Šidák's multiple comparison test; asterisks (*) indicate significant differences between the mean of DMSO-supplemented cells and the mean of cholesterol-supplemented cells for each treatment (*, p = 0.0332, **, p = 0.0021, ***, p = 0.0002, ****, p < 0.0001).



Supplementary Figure S15. Cholesterol biosynthesis is uniquely upregulated after pitavastatin treatment in ER+ breast cancer cells. A-D, DAVID 2021 gene ontology analysis of differentially regulated genes after AZD5363, pitavastatin or combination treatment for 24 or 48 hours in MDA-MB-468 and T47D cells from RNA-sequencing data. Pathways are plotted against the -log10 (p-value). **A,** Pathways enriched in uniquely upregulated genes in MDA-MB-468 and T47D cells at 24 hours. **B,** Pathways enriched in uniquely upregulated genes in MDA-MB-468 and T47D cells at 48 hours. **C,**

Pathways enriched in uniquely downregulated genes in MDA-MB-468 and T47D cells at 24 hours. **D,** Pathways enriched in uniquely downregulated genes in MDA-MB-468 and T47D cells at 48 hours.

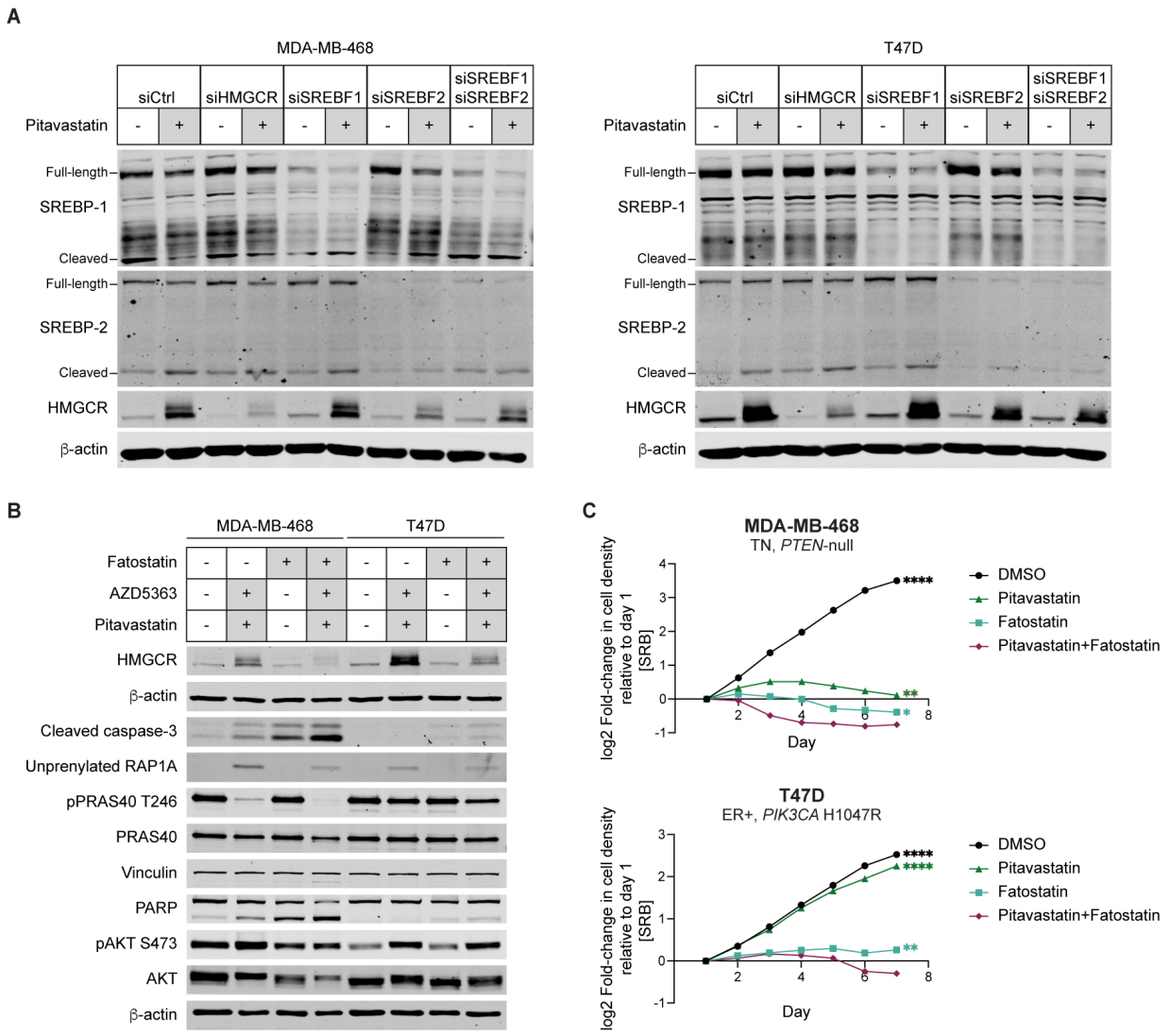


182 **Supplementary Figure S16. The predicted transcription factor activities of *SREBF1* and *SREBF2***

183 **are uniquely upregulated after AZD5363 and pitavastatin treatment in ER+ breast cancer cells. A-**

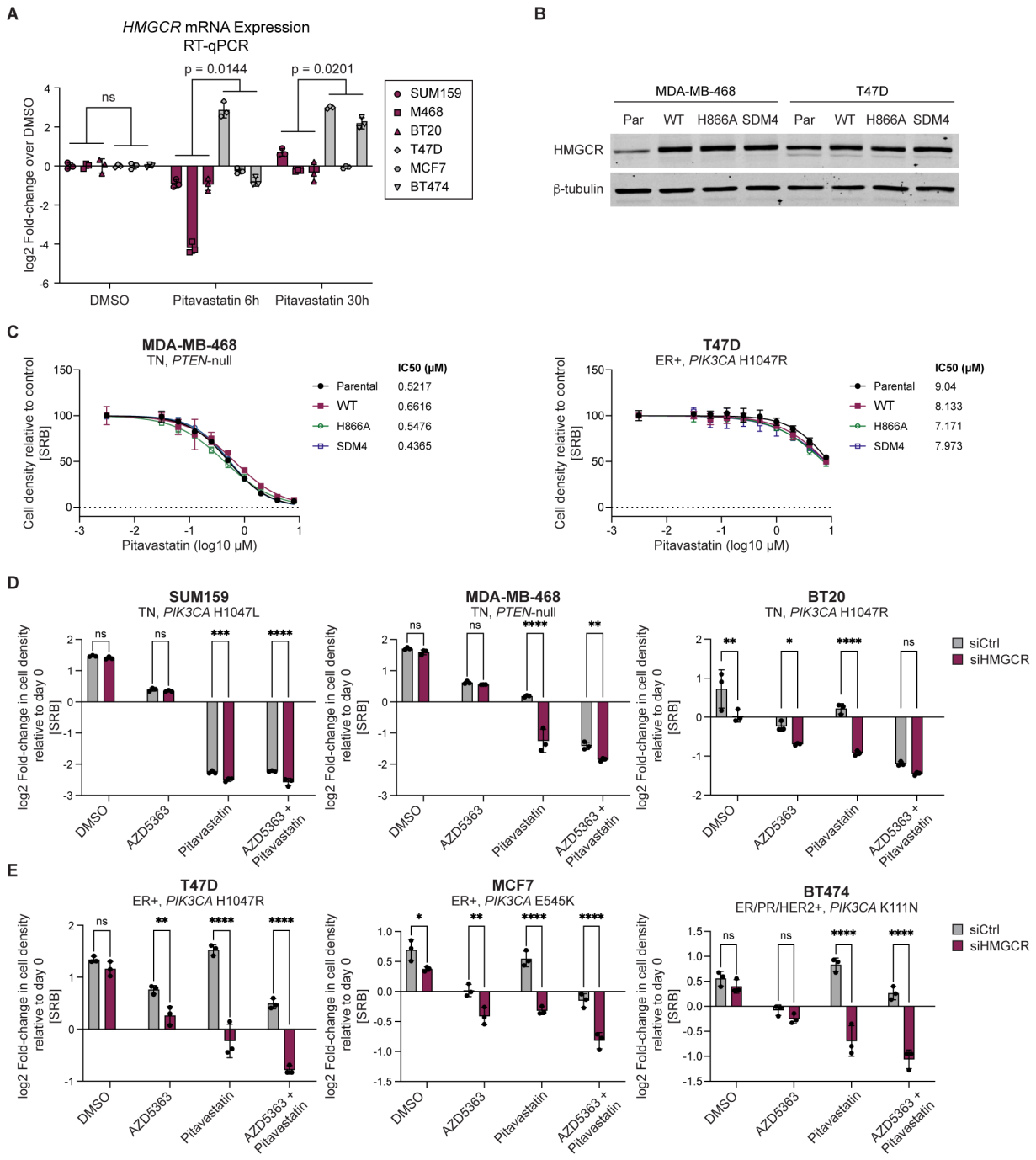
184 **D,** DoRothEA transcription factor activity prediction of RNA sequencing data in T47D cells after 24 (**A**) or

48 hours (**B**) of treatment and in MDA-MB-468 cells after 24 (**C**) or 48 hours (**D**) of treatment. Normalized enrichment scores (NES) are plotted for transcription factors with activity that is predicted to be significantly altered by drug treatment. *SREBF1* and *SREBF2* are highlighted.



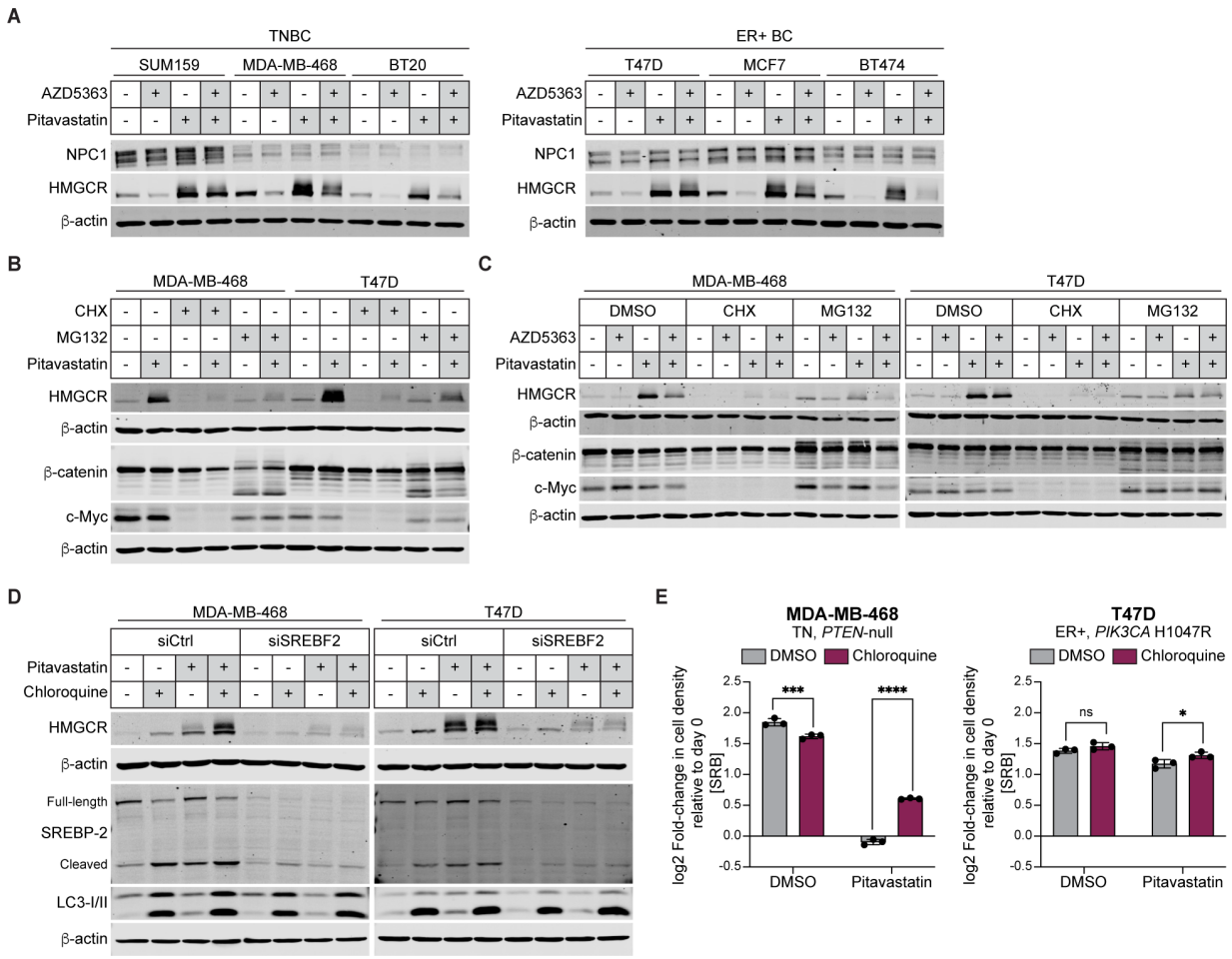
Supplementary Figure S17. *SREBF2* depletion or inhibition sensitizes breast cancer cells to pitavastatin by limiting HMGCR upregulation. A, Immunoblots of SREBP-1, SREBP-2, HMGCR and β-actin in MDA-MB-468 and T47D cells with siRNA knockdown of control (Ctrl), *HMGCR*, *SREBF1*, *SREBF2* or both *SREBF1* and *SREBF2* and treatment with DMSO or 2 μM pitavastatin for 24 hours. **B,** Immunoblots of HMGCR, cleaved caspase-3, unprenylated RAP1A, pPRAS40^{Thr246}, PARP, pAKT^{Ser473} and β-actin in MDA-MB-468 and T47D cells treated with DMSO or 10 μM fatostatin for 24 hours, followed by treatment with AZD5363 (MDA-MB-468: 15 μM, T47D: 0.25 μM) and 2 μM pitavastatin for 24 hours. **C,** MDA-MB-468 and T47D cells were pre-treated with DMSO or 10 μM fatostatin for 2 hours, followed by treatment with DMSO or 2 μM pitavastatin for 7 days, and cell density was measured daily by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using

two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test; asterisks (*) indicate significant differences compared to the pitavastatin and fatostatin combination treatment on day 7 (*, $p = 0.0332$, **, $p = 0.0021$, ***, $p = 0.0002$, ****, $p < 0.0001$).



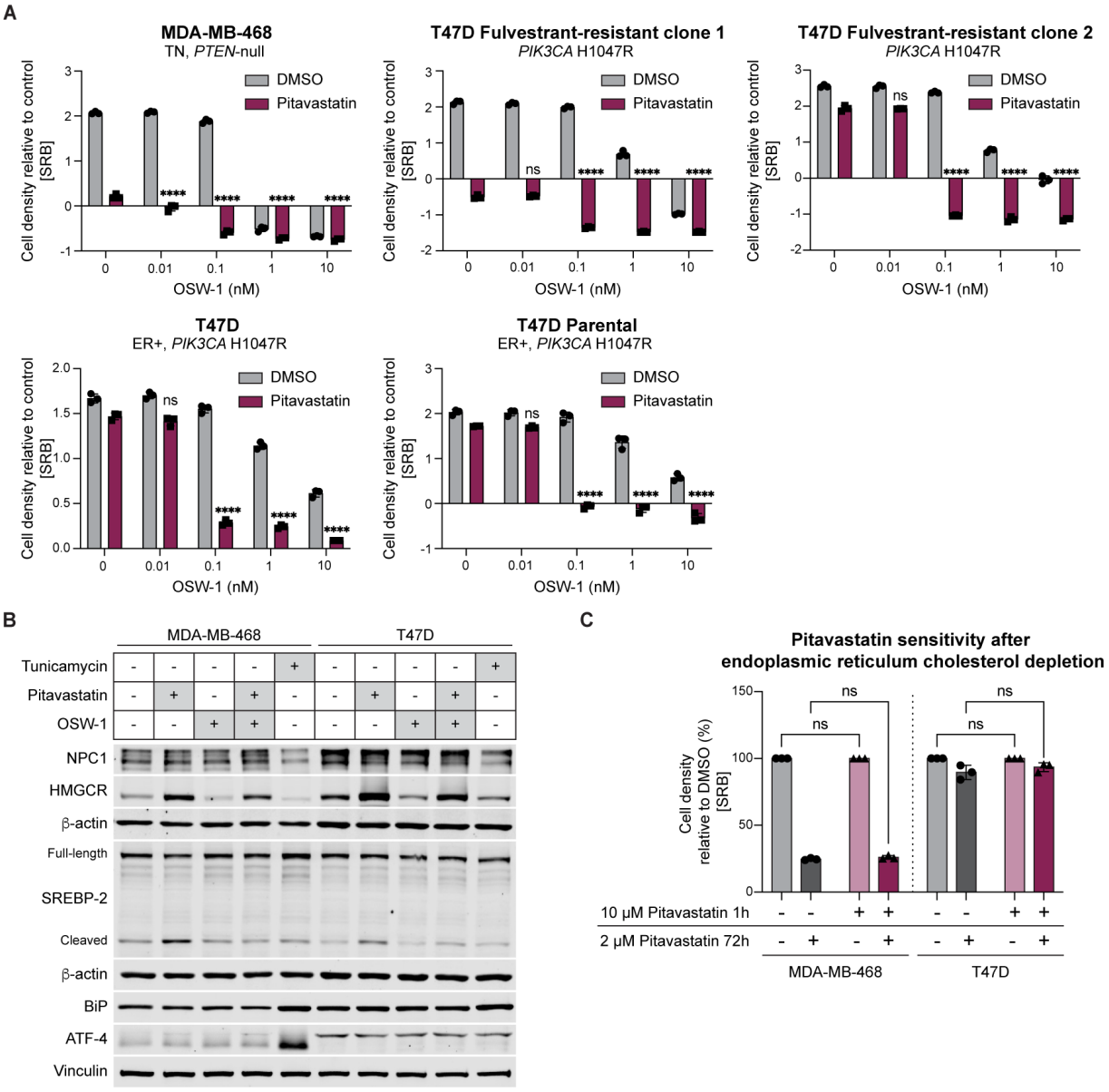
Supplementary Figure S18. Depletion of HMGCR sensitizes ER+ breast cancer cells to pitavastatin and combination AZD5363 and pitavastatin. A, TN (SUM159, MDA-MB-468, BT20) and ER-positive (T47D, MCF7, BT474) breast cancer cell lines were treated with DMSO or 2 μM pitavastatin for 6 or 30 hours, and *HMGCR* mRNA expression was measured by RT-qPCR. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance

(ANOVA) with Šidák's multiple comparison test. TNBC cell lines were compared to ER-positive breast cancer cell lines in each condition. **B,** Immunoblots of HMGCR and β-tubulin in parental and HMGCR-expressing MDA-MB-468 and T47D cells. Cells are expressing wild-type (WT), H866A or E559A, K691A, D767A and H866A pLenti6/V5-HMGCR. **C,** MDA-MB-468 and T47D cells expressing the pLenti6/V5-HMGCR constructs described in **B** were treated with a range of concentrations of pitavastatin (0-8 μM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). **D,** TNBC cell lines (SUM159, MDA-MB-468, BT20) were transfected with siControl (siCtrl) or siHMGCR for 24 hours and then treated with DMSO, AZD5363 (SUM159: 5 μM, MDA-MB-468: 15 μM, BT20: 1.25 μM), pitavastatin (SUM159: 4 μM, MDA-MB-468: 2 μM, BT20: 2 μM) or a combination of AZD5363 and pitavastatin for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). **E,** ER-positive breast cancer cell lines (T47D, MCF7, BT474) were transfected with siControl (siCtrl) or siHMGCR for 24 hours and then treated with DMSO, AZD5363 (T47D: 0.25 μM, MCF7: 1.25 μM, BT474: 0.25 μM), pitavastatin (2 μM) or a combination of AZD5363 and pitavastatin for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). For **D-E,** statistical analysis was performed using two-way analysis of variance (ANOVA) with Šidák's multiple comparison test to compare the mean of siCtrl cells to the mean of siHMGCR cells for each treatment (*, $p = 0.0332$, **, $p = 0.0021$, ***, $p = 0.0002$, ****, $p < 0.0001$).



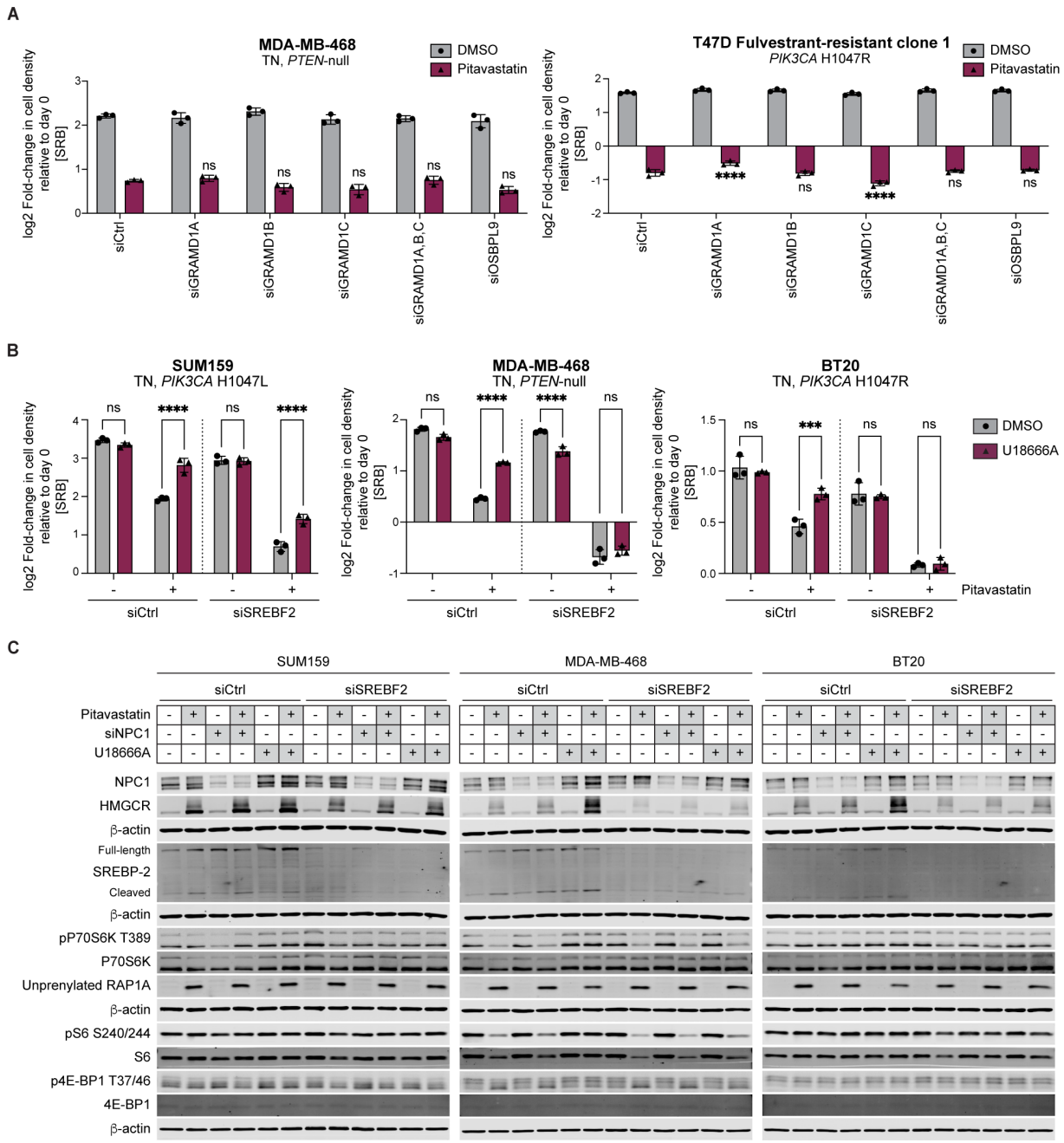
Supplementary Figure S19. Pitavastatin-induced HMGCR upregulation is mediated by SREBP-2-dependent new synthesis of HMGCR. A, Immunoblots of NPC1, HMGCR and β-actin in a panel of TN (SUM159, MDA-MB-468, BT20) and ER-positive (T47D, MCF7, BT474) breast cancer cells treated with DMSO or a combination of AZD5363 (SUM159: 2.5 μM, MDA-MB-468: 10 μM, BT20: 1.25 μM, T47D: 0.25 μM, MCF7: 1.25 μM, BT474: 0.25 μM) and 2 μM pitavastatin for 24h. **B,** Immunoblots of HMGCR, β-catenin, c-Myc and β-actin in MDA-MB-468 and T47D cells pre-treated with DMSO, 10 μg/mL cycloheximide (CHX) or 10 μM MG132 for 2 hours, followed by treatment with DMSO or 2 μM pitavastatin for 24 hours. **C,** Immunoblots of HMGCR, β-catenin, c-Myc and β-actin in MDA-MB-468 and T47D cells pre-treated with DMSO, 10 μg/mL cycloheximide (CHX) or 10 μM MG132 for 2 hours, followed by treatment with DMSO, AZD5363 (MDA-MB-468: 10 μM, T47D: 0.25 μM), 1 μM pitavastatin or the combination of AZD5363 and pitavastatin for 24 hours. **D,** Immunoblots of HMGCR, SREBP-2, LC3-I/II and β-actin in MDA-MB-468 and T47D cells transfected with siControl (siCtrl) or siSREBF2 for 24 hours

followed by treatment with DMSO, 50 μM chloroquine, 2 μM pitavastatin or chloroquine and pitavastatin for 24 hours. **E,** MDA-MB-468 and T47D cells were co-treated with DMSO or 2 μM pitavastatin and DMSO or 5 μM chloroquine for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance (ANOVA) with Šidák's multiple comparison test to compare the mean of DMSO-treated cells to the mean of chloroquine-treated cells for each treatment (*, $p = 0.0332$, ***, $p = 0.0002$, ****, $p < 0.0001$).



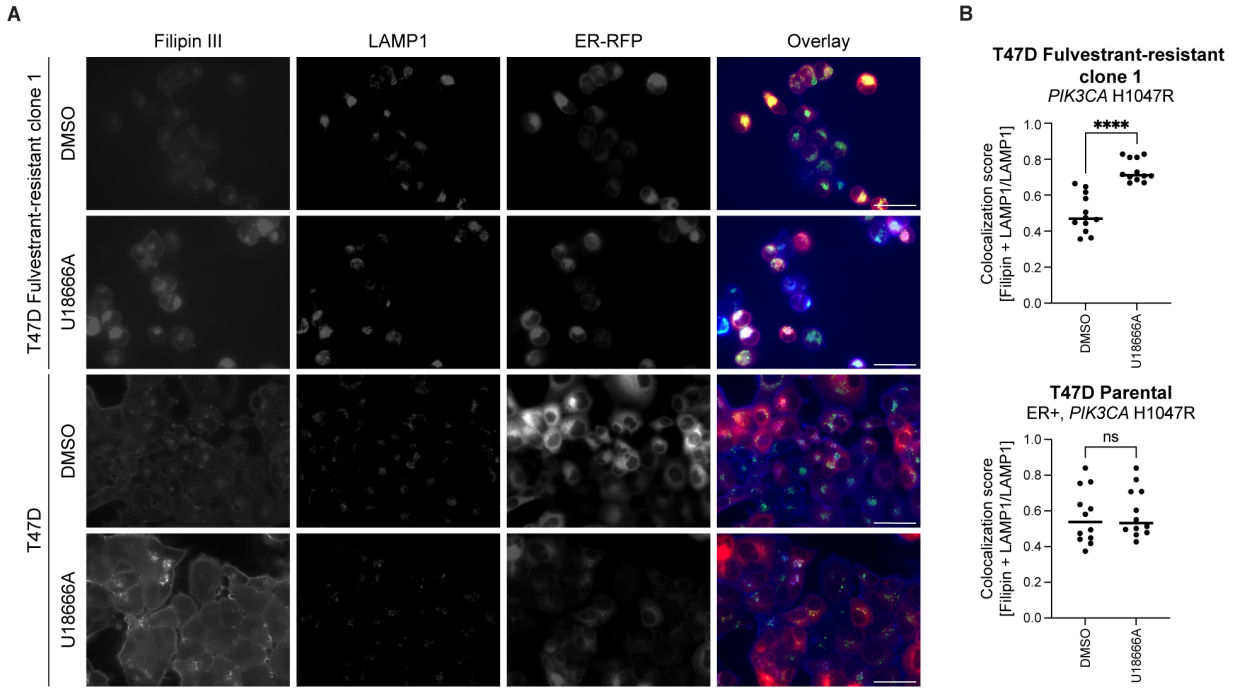
Supplementary Figure S20. Accumulation of endoplasmic reticulum cholesterol sensitizes breast cancer cells to pitavastatin. A, A panel of TN (MDA-MB-468, T47D fulvestrant-resistant clones 1 and 2) and ER-positive (T47D, parental T47D) breast cancer cells were co-treated with DMSO or 2 μM pitavastatin and increasing concentrations of OSW-1 (0-10 nM) for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test; asterisks (*) indicate significant differences compared to pitavastatin treatment without OSW-1 (****, $p < 0.0001$). **B,** Immunoblots of NPC1, HMGCR, SREBP-2, BiP, ATF-4, β-actin and vinculin in MDA-MB-

468 and T47D cells treated with 2 μM tunicamycin, 2 μM pitavastatin, 0.1 nM OSW-1 or the combination of pitavastatin and OSW-1 for 24 hours. **C,** MDA-MB-468 and T47D cells were seeded into RPMI supplemented with 10% lipid-depleted serum and treated with 10 μM pitavastatin for 1 hour. The media was then changed to fresh RPMI supplemented with 10% fetal bovine serum (complete serum), and cells were treated with DMSO or 2 μM pitavastatin for 72 hours. Cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance (ANOVA) with Tukey's multiple comparison test.



Supplementary Figure S21. Depletion or inhibition of the cholesterol trafficking protein NPC1 rescues SREBP-2 activation after pitavastatin treatment in TNBC. A, TNBC cell lines (MDA-MB-468, fulvestrant-resistant T47D clone 1) were transfected with siControl (siCtrl), siGRAMD1A, siGRAMD1B, siGRAMD1C, all 3 siGRAMD1s or siOSBPL9 for 24 hours and then treated with DMSO or 2 μM pitavastatin for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance

(ANOVA) with Dunnett's multiple comparison test; asterisks (*) indicate significant differences compared to the pitavastatin-treated siControl (siCtrl) condition for each cell line (****, $p < 0.0001$). **B,** TNBC cells (SUM159, MDA-MB-468, BT20) were transfected with siControl (siCtrl) or siSREBF2 and then treated with DMSO or 1 μM U18666A and DMSO or 2 μM pitavastatin for 72 hours, and cell density was measured by SRB assay. Data are represented as mean ± SD (N=3 technical replicates). Statistical analysis was performed using two-way analysis of variance (ANOVA) with Šidák's multiple comparison test (***, $p = 0.0002$, ****, $p < 0.0001$). **C,** Immunoblots of NPC1, HMGCR, SREBP-2, pP70S6K^{Thr389}, unprenylated RAP1A, pS6$^{Ser240/244}$, p4E-BP1$^{Thr37/46}$ and β-actin in a panel of TNBC cells (SUM159, MDA-MB-468, BT20) transfected with siControl (siCtrl) or siSREBF2 and siCtrl or siNPC1 for 24 hours and then treated with DMSO or 1 μM U18666A and DMSO or 2 μM pitavastatin for 24 hours.



Supplementary Figure S22. Fulvestrant-resistant T47D cells have dysregulated cholesterol trafficking compared to parental T47D cells. A, Fulvestrant-resistant clone 1 and parental T47D cells expressing endoplasmic reticulum RFP (ER-RFP; red) were treated with DMSO or 1 μM U18666A for 24 hours. Cells were fixed with 4% formaldehyde and stained with Filipin III (blue) and a LAMP1 antibody (green). Representative images are shown. Scale bars are 50 μm. **B,** Quantification of Filipin III and LAMP1 co-localization normalized to total LAMP1 from 12 non-overlapping fields. Statistical analysis was performed using an unpaired, two-tailed parametric t-test (****, $p < 0.0001$).

Supplementary Table S1. Patient-derived organoid characteristics.

Patient	Pathology	Mutations
4	Triple-negative breast cancer (primary)	-
8	ER-positive metastatic breast cancer (bone)	*PIK3CA* H1047R
10	ER-low metastatic breast cancer (ascites)	*AKT* E17K
26	ER-positive metastatic breast cancer (bone)	-
27	ER-positive invasive ductal carcinoma	-

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Supplementary Table S2. RT-qPCR primers.

Gene	Forward Primer	Reverse Primer
18S	5’-CTTAGAGGGACAAGTGGCG-3’	5’-ACGCTGAGCCAGTCAGTGTA-3’
HMGCR	5’-TCTAGTGAGATCTGGAGGATCCAA-3’	5’-AGGGATGGGAGGCCACAAAG-3’

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Supplementary Table S3. Plasmids.

Plasmid	Source	Selection Marker
pHAGE-ESR1	Addgene, 116737	Puromycin
pLenti6/V5-HMGCR	Cloned HMGCR from pCMV-SPORT6-hHMGCR1 (Addgene, 86085) into pLenti6/V5-p53_wt p53 (Gift from Muranen Lab; Addgene, 22945)	Blasticidin
pLenti6-ER-mRFP	Cloned ER-mRFP from ER-mRFP (Addgene, 62236) into pLenti6/V5-p53_wt p53 (Gift from Muranen lab; Addgene, 22945)	Blasticidin

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Supplementary Table S4. Antibodies for immunoblotting.

	Antibody	Manufacturer	Identifier	Host	Dilution
Primary antibody	PARP	Cell Signaling Technology	9542	Rabbit	1:1000
	pAKT S473	Cell Signaling Technology	4060	Rabbit	1:1000
	pAKT T308	Cell Signaling Technology	2965	Rabbit	1:1000
	AKT	Cell Signaling Technology	4691	Rabbit	1:1000
	pPRAS40 T246	Cell Signaling Technology	2997	Rabbit	1:1000
	PRAS40	Cell Signaling Technology	2691	Rabbit	1:1000
	pGSK3β S9	Cell Signaling Technology	9336	Rabbit	1:1000
	GSK3β	Cell Signaling Technology	9315	Rabbit	1:1000
	pS6	Cell Signaling Technology	5364	Rabbit	1:1000
	S6	Cell Signaling Technology	2217	Rabbit	1:1000
	pP70S6K	Cell Signaling Technology	9234	Rabbit	1:1000
	P70S6K	Cell Signaling Technology	2708	Rabbit	1:1000
	p4E-BP1	Cell Signaling Technology	2855	Rabbit	1:1000
	4E-BP1	Cell Signaling Technology	9452	Rabbit	1:1000
	Unprenylated RAP1A	Santa Cruz Biotechnology	sc-373968	Mouse	1:50
	RHEB	Abnova	H00006009-M01	Mouse	1:50
	HDJ2	ThermoFisher Scientific	MA5-12748	Mouse	1:1000
	PARP	Cell Signaling Technology	9542	Rabbit	1:1000
	Cleaved caspase-3	Cell Signaling Technology	9664	Rabbit	1:1000
	NPC1	Cell Signaling Technology	33422	Rabbit	1:1000
	HMGCR	Invitrogen	MA5-31336	Mouse	1:1000
	SREBP-1	Abcam	ab28481	Mouse	1:500

	SREBP-2	BD Biosciences	557037	Mouse	1:250
	Estrogen receptor α (ERα)	Cell Signaling Technology	8644	Rabbit	1:1000
	c-Myc	Cell Signaling Technology	5605	Rabbit	1:1000
	β-catenin	Cell Signaling Technology	8480	Rabbit	1:1000
	LC3-I/II	Cell Signaling Technology	4108	Rabbit	1:1000
	BiP	Cell Signaling Technology	3183	Rabbit	1:1000
	ATF-4	Cell Signaling Technology	11815	Rabbit	1:1000
	β-actin	Cell Signaling Technology	4970	Rabbit	1:3000
	Vinculin	Cell Signaling Technology	13901	Rabbit	1:1000
Secondary antibody	IRDye® 800CW Goat anti-Rabbit IgG (H + L)	LI-COR	926-32211	Goat	1:20,000
	IRDye® 800CW Goat anti-Mouse IgG (H + L)	LI-COR	926-32210	Goat	1:20,000
	IRDye® 680CW Goat anti-Rabbit IgG (H + L)	LI-COR	926-68021	Goat	1:20,000
	IRDye® 680CW Goat anti-Mouse IgG (H + L)	LI-COR	926-68070	Goat	1:20,000

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Supplementary Table S5. siRNA Product information.

Target	siRNA	Sequence	Horizon Discovery Catalog ID
SREBF1	Individual	GCGCACUGCUGUCCACAAA	J-006891-05-0005
SREBF2	Individual	GCACACUGGUUGAGAUCCA	J-009549-05-0005
HMGCR	Pool	GUGAGAAUGUUAUUGGAUA, GGUCGAAGAUCAAUUUACA, GAACAAGUUAUUACCCUAA, GAGCAGUGACAUUAUAAUU	L-009811-00-0005
NPC1	Pool	GGACAACUAUACCCGAAUA, GAAGAAAGCCCGACUUAUA, GCGAACGGCUUCUAAAUUU, GAUGAGACCAAUUGUGAUA	L-008047-00-0005
GRAMD1A	Individual	CCACUUAUAAGCAGCGUAA	J-014143-17-0005
GRAMD1B	Individual	GAGUGAAUGUUACGUGAUA	J-026529-13-0005
GRAMD1C	Individual	GUAUAAAGAAAGUCGGGAA	J-020545-17-0005
OSBPL9	Individual	CCAAAGCGCUUAAUAGAUU	J-009912-05-0005
ON-TARGETplus Non-targeting Pool	Pool	UGGUUUACAUGUCGACUAA, UGGUUUACAUGUUGUGUGA, UGGUUUACAUGUUUUCUGA, UGGUUUACAUGUUUUCCUA,	D-001810-10-20

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Supplementary Table S6. Key reagents.

Reagent	Supplier	Product number
BYL719	Active Biochem	A-1214
GDC-0068	MedChemExpress	RG7440
AZD5363 (capivasertib)	Cayman Chemicals (*in vitro* studies), AstraZeneca (*in vivo* studies)	15406
ARQ 092	Cayman Chemicals	1313881-70-7
MK-2206	Cayman Chemicals	11593
Torin 1	Tocris Bioscience	4247
Lovastatin	Selleckchem	S2061
Rosuvastatin	Selleckchem	S2169
Pitavastatin	Selleckchem	S1759
Bortezomib	Cayman Chemicals	10008822
Fulvestrant	Selleckchem	S1191
GGTI-298	Selleckchem	S7466
FTI-277	Selleckchem	S7465
Fatostatin	Selleckchem	S9785
Cycloheximide	Cayman Chemicals	14126
MG132	Cayman Chemicals	10012628
Chloroquine	Sigma-Aldrich	C6628
Tunicamycin	Sigma-Aldrich	T7765
U18666A	Cayman Chemicals	Cholesterol Cell-Based Detection Assay Kit, 10009779
OSW-1	MedChemExpress	HY-101213
Mevalonate	Sigma-Aldrich	90469
GGPP	Cayman Chemicals	63330
Cholesterol	Sigma-Aldrich	C4951

Filipin III	Cayman Chemicals	Cholesterol Cell-Based Detection Assay Kit, 10009779
Puromycin	Corning	61-385-RA
Blasticidin	InvivoGen	ant-bl-1

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