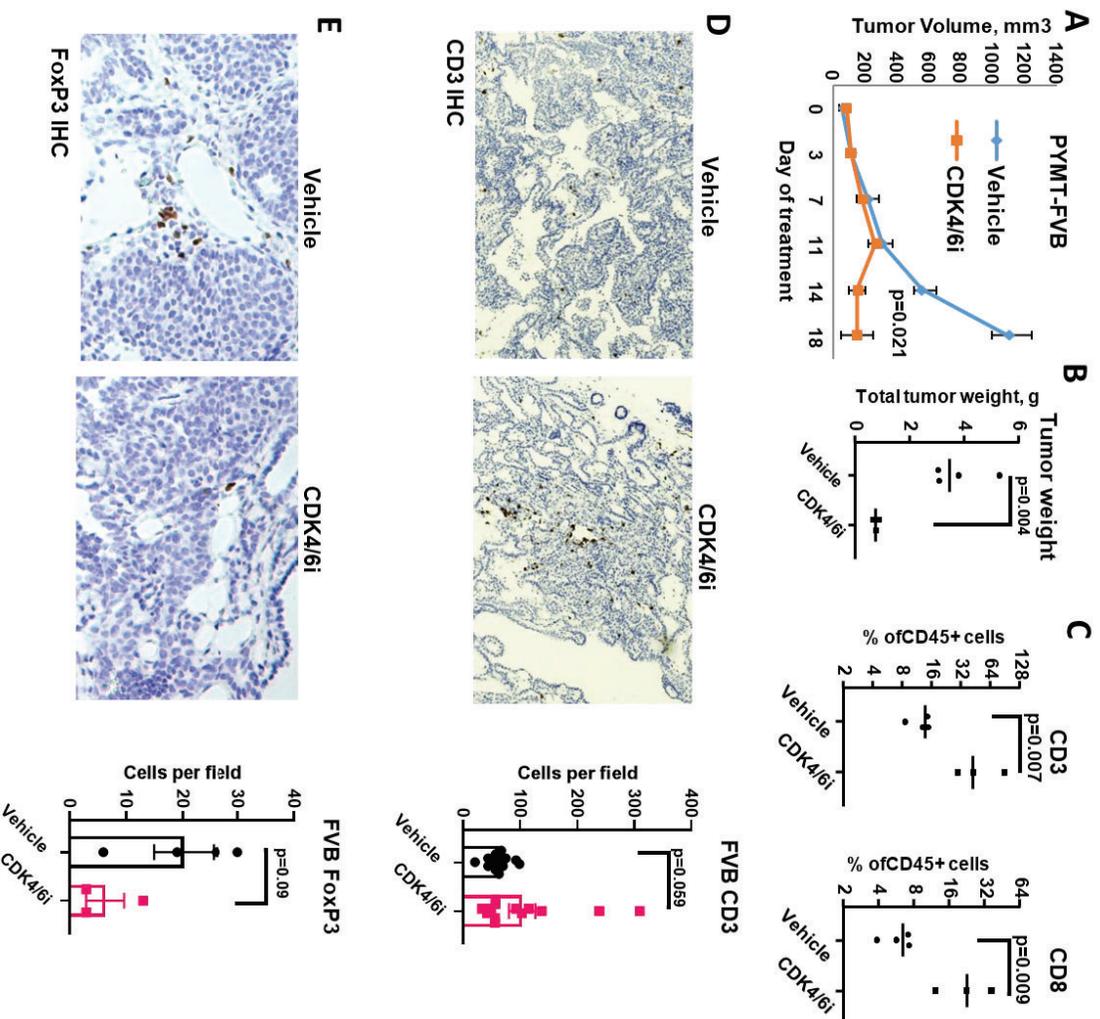


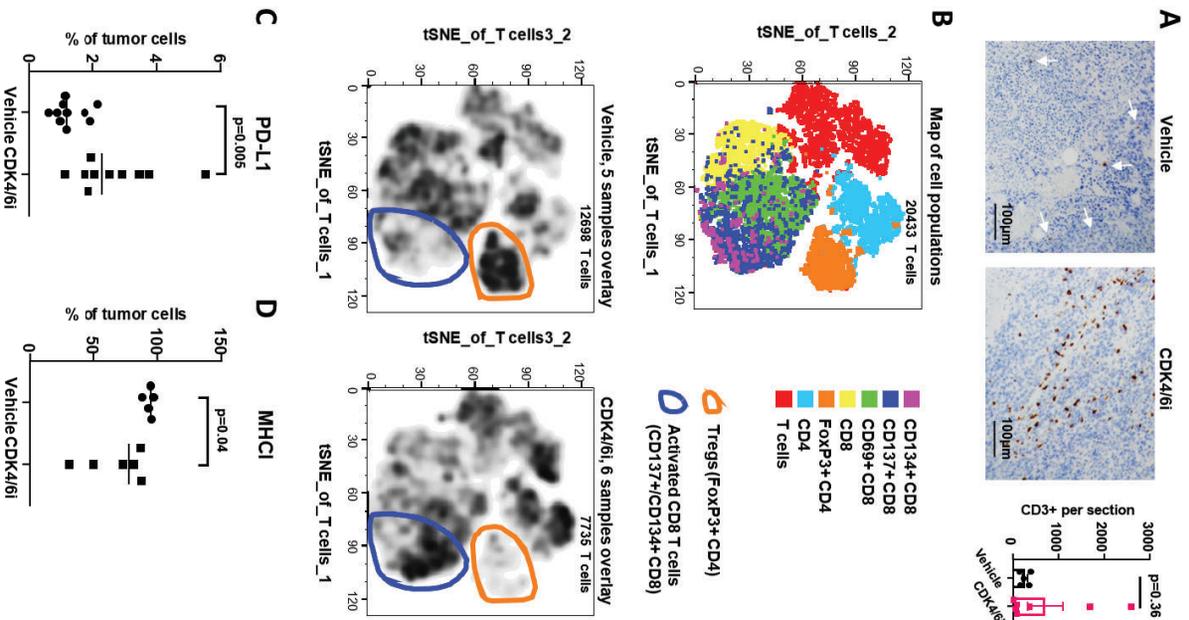
Table S1. Related to Figure 1. Comparison of the immune cell infiltrate in PYMT tumors from mice treated with vehicle or with CDK4/6i palbociclib (Palbo). ns – not significant, n/t – not tested, TAM – tumor associated macrophage, MDSC – myeloid-derived suppressor cell

Cell population	Markers (gated on live CD45+ cells)	Day 8: Average frequency \pm SD (% of CD45+)		Day 25: Average frequency \pm SD (% of CD45+)		Palbo effect	Palbo effect p value	Day effect p value	Interaction p value
		Vehicle n=9	Palbo n=9	Vehicle n=6	Palbo n=6				
B cells	CD19 ⁺	1.78 \pm 0.77	3.72 \pm 1.49	3.70 \pm 1.95	7.17 \pm 2.69	increase	0.0003	0.0003	ns
NKT	NK1.1 ⁺ /CD3 ⁺	5.05 \pm 1.05	6.06 \pm 1.56	3.22 \pm 1.39	2.71 \pm 1.22	ns	0.615	<0.0001	ns
NK	NK1.1 ⁺ /CD3 ⁻	5.34 \pm 2.99	5.58 \pm 1.57	3.01 \pm	2.41 \pm	ns	0.802	0.0008	ns
T	CD3 ⁺	22.42 \pm 4.52	29.35 \pm 4.58	28.39 \pm 9.68	41.46 \pm 13.08	increase	0.002	0.005	ns
CD4	CD4 ⁺ /CD3 ⁺	7.55 \pm 1.32	8.58 \pm 2.00	11.48 \pm 3.08	14.31 \pm 8.41	ns	0.221	0.004	ns
LAG3 CD4	LAG3 ⁺ /CD4 ⁺ /CD3 ⁺	0.72 \pm 0.24	0.88 \pm 0.42	1.87 \pm 0.87	3.72 \pm 1.31	increase	0.001	<0.0001	0.005
TIM3 CD4	TIM3 ⁺ /CD4 ⁺ /CD3 ⁺	1.08 \pm 0.44	0.71 \pm 0.2	2.89 \pm 1.29	1.47 \pm 0.90	decrease	0.003	<0.0001	ns
CD8	CD8 ⁺ /CD3 ⁺	5.95 \pm 1.53	9.17 \pm 2.14	4.05 \pm 1.53	7.78 \pm 6.65	increase	0.01	ns	ns
LAG3 CD8	LAG3 ⁺ /CD8 ⁺ /CD3 ⁺	0.78 \pm 0.52	0.59 \pm 0.29	1.04 \pm 0.88	1.37 \pm 0.57	ns	0.743	0.02	ns
TIM3 CD8	TIM3 ⁺ /CD8 ⁺ /CD3 ⁺	0.86 \pm 0.63	0.84 \pm 0.44	0.36 \pm 0.42	0.51 \pm 0.32	ns	0.63	0.029	ns
CD11b	CD11b ⁺	61.22 \pm 9.93	55.47 \pm 3.83	60.48 \pm 9.35	50.48 \pm 16.56	decrease	0.049	ns	ns
Macrophages/ TAM	F4/80 ⁺	13.78 \pm 3.70	12.53 \pm 1.46	14.45 \pm 3.63	7.57 \pm 2.93	decrease	0.001	ns	0.019
CD206 Macroph. (M2 like)	CD206 ⁺ /F4/80 ⁺	5.25 \pm 2.71	5.38 \pm 1.24	4.16 \pm 1.27	2.64 \pm 1.11	ns	0.317	0.009	0.231
MHCII Macroph. (M1 like)	I-A/I-E ⁺ /F4/80 ⁺	4.15 \pm 1.91	3.27 \pm 0.92	5.46 \pm 1.74	1.95 \pm 1.16	decrease	0.0005	ns	0.026
Monocytes/ mMDSC	Ly6C ^{high} /CD11b ⁺	25.83 \pm 5.02	20.66 \pm 2.49	22.8 \pm 2.27	16.87 \pm 6.15	decrease	0.002	0.04	ns
Neutrophils/ gMDSC	Ly6G ^{high} /CD11b ⁺	1.99 \pm 1.08	3.46 \pm 1.36	5.89 \pm 3.10	14.7 \pm 4.95	increase	<0.0001	<0.0001	0.001
PD-L1+ leukocytes	PD-L1 ⁺	33.86 \pm 6.81	39.94 \pm 8.63	n/t	n/t	ns	0.104	n/t	n/t

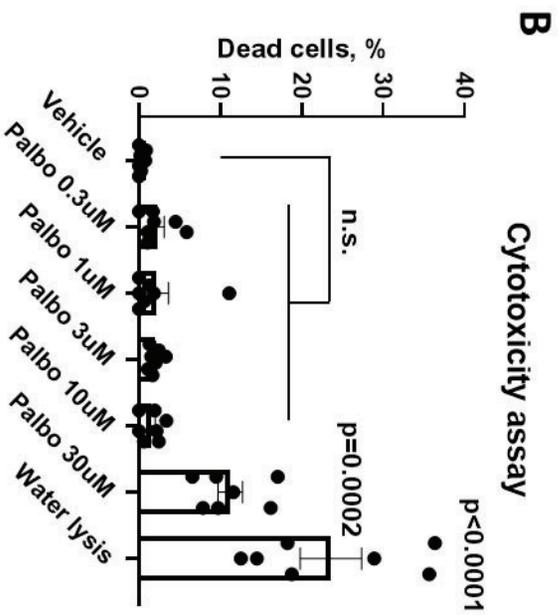
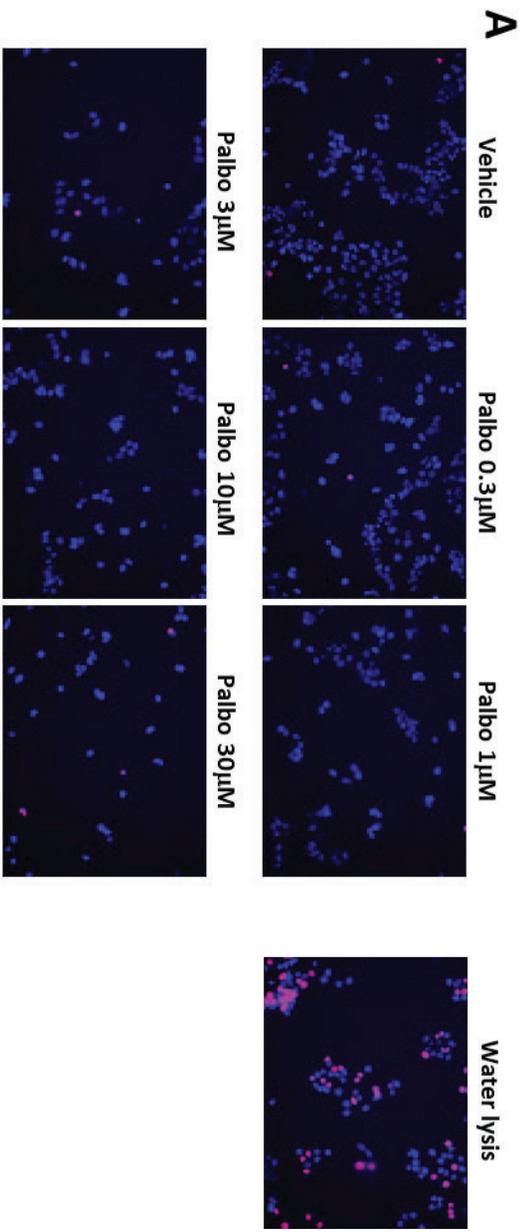
Supplementary figures



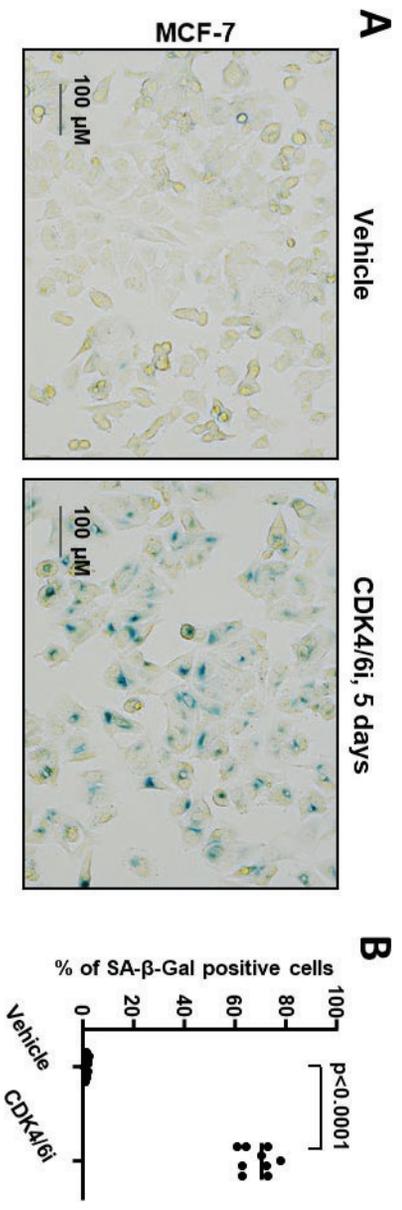
Supplementary Figure 1. Related to Figure 1. Palbociclib inhibits tumor growth and increases T cell infiltrate in transgenic PYMT model of breast cancer. A. Growth of spontaneous mammary tumors in PYMT FBV transgenic mice that were treated twice daily with vehicle or 100mg/kg palbociclib. Growth curves were built based on average volume of all fat pad tumor lesions per mouse (n=3-4 per group, mixed model), data are presented as mean total tumor volume \pm SEM). B. Comparison of the total weight of all tumor lesions per mouse in vehicle and palbociclib-treated mice using t-test (n=3-4). C. Percentages of CD3+ and CD3+/CD8+ cells in tumors of mice shown in A (n=3-4 mice, t-test). D. Representative microphotographs and quantification of CD3 IHC staining in tumors of transgenic PYMT FVB mice shown in A. Several random microscopic fields were acquired. Each dot represents a field (n=13-16 fields, t-test). E. Representative microphotographs and quantification of FoxP3 IHC staining in tumors of transgenic PYMT FVB mice shown in A (n=3-4 tumors, t-test).



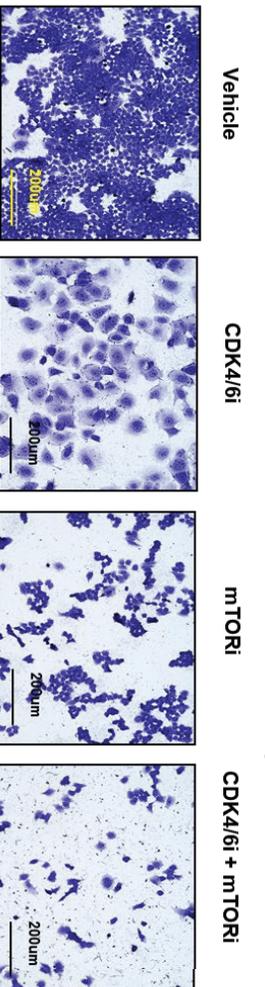
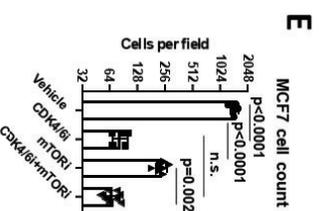
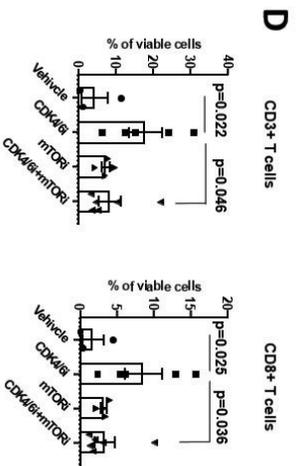
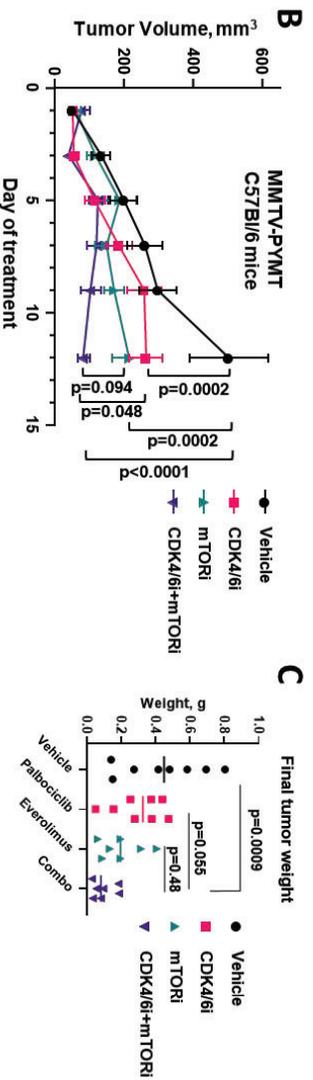
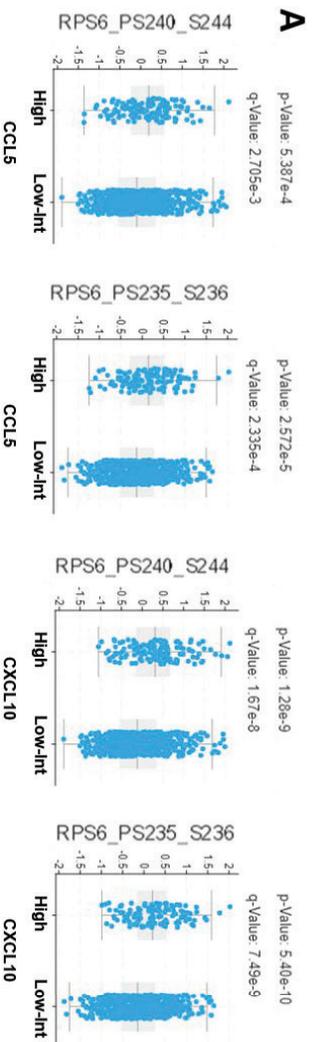
Supplementary Figure 2. Related to Figure 1. Palbociclib affects the levels and phenotype of tumor-infiltrating T cells and surface expression of receptors involved in tumor-immune interaction on malignant cells *in vivo*. **A.** Representative microphotographs and quantification of CD3 IHC staining in PYMT tumors from female C57Bl/6 mice treated with vehicle or 100 mg/kg palbociclib. Arrows point out infrequent CD3+ cells in vehicle treated group (n=5-7 individual tumors, t-test). **B.** Results of the dimension reduction analysis to visualize overall state of T cell phenotypes in tumors of vehicle and palbociclib-treated mice. These results complement individual parameter data shown in Fig. 1F-K. Populations of Tregs and activated CD8+ T cells are circled. **C.** Results of the flow cytometry analysis of PD-L1 expression of tumor cells from mice shown in Fig. 1F. Tumors were collected at 8-day time points (n=9, t-test). **D.** Results of the flow cytometry analysis of MHC class I expression on tumor cells from experiment described in Fig. 1F (n=5-6 individual tumors, t-test).



Supplementary Figure 3. Related to Figure 3. Palbociclib is not cytotoxic at 0.3–10 μ M concentrations. MCF7 cells were treated for 5 days with vehicle or CDK4/6i palbociclib at indicated concentrations. Control cells were killed by incubation in water for 10 min. Dead cells were visualized with PI and all cells were identified by hoechst 33342 staining. A. Representative microphotographs of treated cells. B. Percentages of dead cells in indicated treatment groups counted in random fields (n=7 random fields, one-way ANOVA).



Supplementary Figure 4. Related to Figure 3. Palbociclib induces senescence marker expression in breast cancer cells. A. Images of MCF7 cells cultured in the presence of 1 μ M palbociclib for either 8 days or for 5 days followed by drug washout and additional 3 days of culture with drug vehicle only. Control cells were treated with vehicle for 8 days. **B.** Representative images of MCF7 cells treated with vehicle or 1 μ M of CDK4/6i palbociclib for 5 days and analyzed for the presence of senescence-associated β -galactosidase. **C.** Statistical analysis of the percentages of senescence-associated β -galactosidase-positive cells (n=9, t- test).



Supplementary Figure 5. Related to Figure 4. Effect of combined mTOR and CDK4/6 blockade on tumor growth and T cell recruitment. **A.** cBLO portal analysis of the phosphorylation of mTOR target pS6 on residues S240/244 and S235/236 in human breast cancer tumors expressing high vs low-intermediate levels of CCL5 and CXCL10 (n=960, TCGA dataset). **B.** Growth of injected PYMT tumors in female c57Bl/6 mice treated once a day with 100 mg/kg CDK4/6i palbociclib, 5 mg/kg mTORi everolimus, combination of both or vehicle for 12 days (n=6-7, mixed model, error bars represent SEM). **C.** Final tumor weight from experiment described in B. **D.** Results of the flow cytometry analysis of CD3⁺ (pan-T), and CD3⁺CD8⁺ (cytotoxic T cells) cells in tumor cell suspensions from PYMT tumor-bearing C57Bl/6 mice treated with 100 mg/kg CDK4/6i palbociclib, 5 mg/kg mTORi everolimus, combination of both, or vehicle for 8 days. Cells were gated on live cells based on viability dye staining (n=3-6, one-way ANOVA). **E.** Numbers of MCF7 cells treated with 2µM mTOR inhibitor rapamycin (mTORi), 5µM CDK4/6i palbociclib, or combination of both for 5 days. Cells were counted in random microscopic fields after crystal violet staining (n=6, t-test). **F.** Representative microphotographs of cells from experiment in E.