**Supplemental Figures**

**Supplemental Figure 1. Representative FACS plots.** Representative gating strategies for all immune cell analysis from D4M3.A tumors after four days after beginning CDK4/6i + MEKi treatment. **A.** Identification of live (Zombie UV-), CD45.2+ cells was accomplished as shown.  **B.** Gating strategy for CD8+ cells (CD4-, CD8+ of CD45.2+) and their relative phenotypic markers (CD44, PD-1, Ki67, or GrzB).  **C.** Representative gating of T regulatory cells (FoxP3+, CD4+ cells) of CD4+ cells shown in (B) and NK cells (NK1.1+, CD4-) of CD45.2 shown in (A).  **D.** Representative gating strategy for CD4+ NK cells of the CD45.2+ population. **E.** Representative plots of MHC-I or MHC-II by CD45.2. **F.** Representative plots of IDO-1, LGalS9, or PD-L1 by CD45.2. **G.** Representative plots of OX40L or CD137L by CD11c (of CD45.2 from A) all of live cells.

**Supplemental Figure 2. MEK plus CDK4/6 inhibition does not alter infiltration of regulatory T cells or NK cells.** D4M3.A mouse melanoma tumors were treated for 4 days and tumors were analyzed for: **A.** Tregs (FOXP3+, CD4+) and **B.** NK cells (NK1.1+). Graphed are mean and SD.

**Supplemental Figure 3. MEK plus CDK4/6 inhibition does not regulate PD-L1 expression on tumor or immune cells.** Mice bearingD4M3.A mouse melanoma tumors were treated for 4 days with vehicle, MEKi alone, CDK4/6i alone and MEKi plus CDK4/6i and tumors were analyzed for the expression of immune-related markers in CD45-negative and CD45-positive populations. **A.** IDO expression. **B.** LGalS9 expression. **C.** PD-L1 expression. **D.** Mean fluorescence intensity (MFI) of PD-L1 on CD45+ immune cells. Graphed are mean and SD. **E.** Human melanoma cell lines, 1205Lu (*BRAF* V600E) and WM1366 (*NRAS* Q61L) were treated with CDK4/6i (palbociclib) and IFN-γ for 24 hours before western blotting for PD-L1 expression and reduction of phospho-RB1. HSP90 serves as the loading control.

**Supplemental Figure 4. Animal survival for T cell co-stimulatory molecules following MEK and CDK4/6 targeting. A.** Survival for C57BL/6 mice bearing D4M tumors following OX40 agonist treatment as in Figure 4. The experiment was ended when tumors were 1000 mm3. **B.** Survival for C57BL/6 mice bearing D4M tumors following CD137L blocking antibody treatment. Dots indicate when animals were censored due to euthanizing when tumors were less than 1000 mm3. Significance was assessed as described in Materials and Methods.