**Supplementary Figure Legends**

**Figure S1** Representative CT scans showing A) the popliteal fossa nodular melanoma (PFNM) and B) groin lymphadenopathy (LN). C) Volumes of the lesions calculated from CT scans (% relative to the initial volume), showing a mixed response to therapies and eventual tumor progression.

**Figure S2** Representative whole exome sequencing reads at the PIK3CAE545 locus for all 5 biopsy timepoints, showing the G->A change resulting in PIK3CAE545K.

**Figure S3** Variant allele frequency of PIK3CAE545K detected by ddPCR in plasma-derived cfDNA. Numbers indicate days post-MEK+CDK4i treatment completion.

**Figure S4** pS6/S6 and pRB/Rb western blot quantitation versus GI50 for the indicated drug treatments in A) SB2 and B) WM1366 cells. Bars represent the quantification of the phosphorylated protein normalized for protein total levels. Red dots represent GI50s calculated from the growth curves showed in Fig 2A-F.

**Figure S5.** A-B) Effect of Rapamycin or PF-4708671 as single agents in GFP-, PIK3CAWT orPIK3CAE545K cell lines. C-F) Effect of Rapamycin or PF-4708671 on MEKi and/or CDK4i in GFP- or PIK3CAWT-expressing SB2 and WM1366 cell lines. Cell titer is normalized to control group (DMSO 0.01%) cell confluence after 72h of drug treatments. Error bars represent SEM.

**Figure S6.** Effect of MEKi+CDK4i ± S6Ki in hTERT-immortalized melanocytes. Cell titer is normalized to control group (DMSO 0.01%) cell confluence after 72h of drug treatments. Error bars represent SEM.

**Figure S7.** pS6/S6 and pRB/Rb western blot quantitation versus GI50 for the indicated drug treatments in A) SB2E545K and B) WM1366E545K cells.. Bars represent the quantification of the phosphorylated protein normalized for protein total levels. Red dots represent GI50s calculated from the growth curves showed in Fig 3A-B.

**Figure S8** CFSE proliferation assays for A) SB2E545K and B) WM1366E545K cells. CFSE measures dye retention in non-dividing cells. The Y-axis denotes 1/%CFSE retained, meaning the lower the value the more dye was retained. Error bars represent SEM.

**Fig S9**. A-B) Quantitation of cell cycle phases in PIK3CAWT-expressing SB2 and WM1366 cells with the indicated drug treatments, by flow cytometric measurement of propidium iodide 24h after drug treatments (300nM). Representative experiment out of 3 replicates. C-D) Quantitation of apoptosis by measurement of Caspase3/7 activity. FUs are the percentage of apoptotic over total cell confluence after 72h of drug treatments (300 nM). Error bars represent SEM.

**Figure S10.** Annexin V quantification in PIK3CAE545K- or PIK3CAWT-expressing A) SB2 and B) WM1366 cells after 72h of treatment with the indicated drugs (300 nM). FUs are calculated as percentage of apoptotic (red) cells over total cell confluence. Error bars represent SEM.

**Figure S11.** pS6/S6 and pRB/Rb western blot quantitation versus percentage of proliferating cells for the indicated drug treatments. Bars represent the quantification of the phosphorylated protein normalized for protein total levels. Red dots represent the percentage of proliferating cells calculated from the Propidium Iodide assays (% of G2+S cells).

**Figure S12.** Representative pictures of pH3 IHC assay on SB2 xenograft sections. pH3 fluorescent signal is in green, nuclei are stained with DAPI (blue). Bars represent 100 µm.

**Figure S13.** Representative pictures of TUNEL assay on SB2 xenograft sections. TUNEL fluorescent signal is in red, nuclei are stained with DAPI (blue). Bars represent 100 µm.