



Supplementary Figure. S5. Long-term effects of P^{124S} MEK1 expression on population doubling and p-ERK levels. (A) Impact of regulated expression of P^{124S} MEK1 on long-term growth of M238. Stable lines expressing empty vector, FLAG-MEK1 WT or FLAG-MEK1 P124S were cultured with different levels of doxycycline, enabling low (0.1 ng/ml) or high (0 ng/ml) expression levels of exogenous MEK1. Equal number of cells were serially plated every 4 days and fold growth calculated by dividing the number of viable cells (trypan-blue negative) at the end of each four day period over the starting seeding number. **(B)** Long-term, regulated expression of WT MEK or P^{124S} MEK and corresponding levels of ERK activation in a clonogenic assay (Fig. 2G). Doxycycline-controlled expression of vector (control), FLAG-MEK1 WT, vs. FLAG-MEK1 P124S in the V^{600E} B-RAF melanoma cell line, M238. Protein lysates (192 h post seeding at 0, 0.1, and 100 ng/ml doxycycline) were probed by Western blotting for the indicated phospho- and total protein levels. Tubulin, loading control.