

Supplemental Figure 1

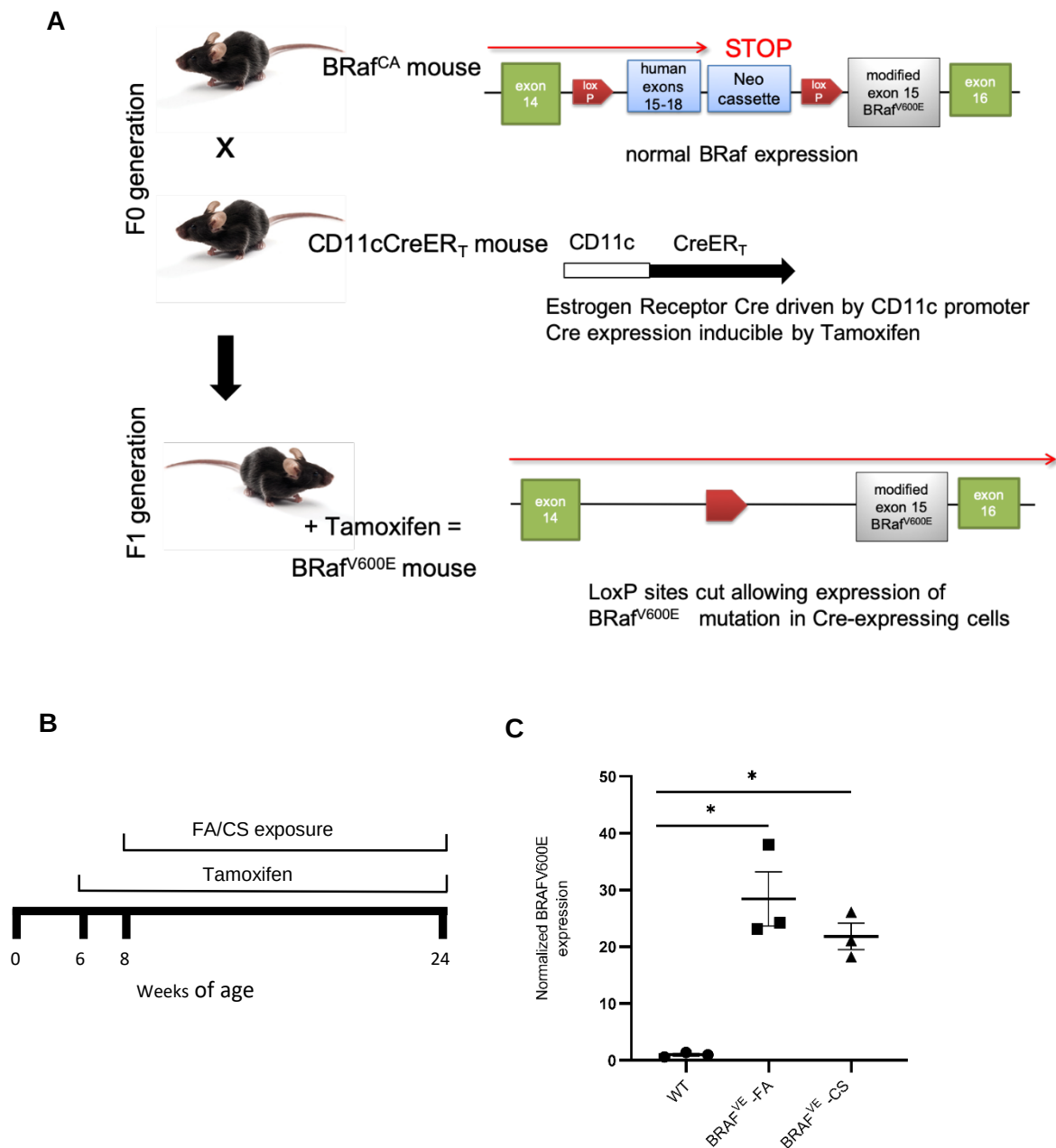


Fig. S1. Mouse model of PLCH. (A) Breeding scheme to generate BRAFV600E mutation in CD11c⁺ cells. **(B)** Scheme of PLCH mouse model exposures. CD11c-ERT- BRAF^{VE} mice were treated with Tamoxifen at 6 through 24 weeks of age and exposed to filtered air (FA) or cigarette smoke (CS) 8 through 24 weeks of age. **(C)** The expression of BRAF-V600E mRNA in lungs of WT mice and BRAF^{VE} mice that were exposed to FA or CS was measured by RT-PCR. Representative data are shown as the mean $\pm$ SEM. ANOVA with Tukey multiple comparisons test was used $*P < 0.05$.

Supplemental Figure 2

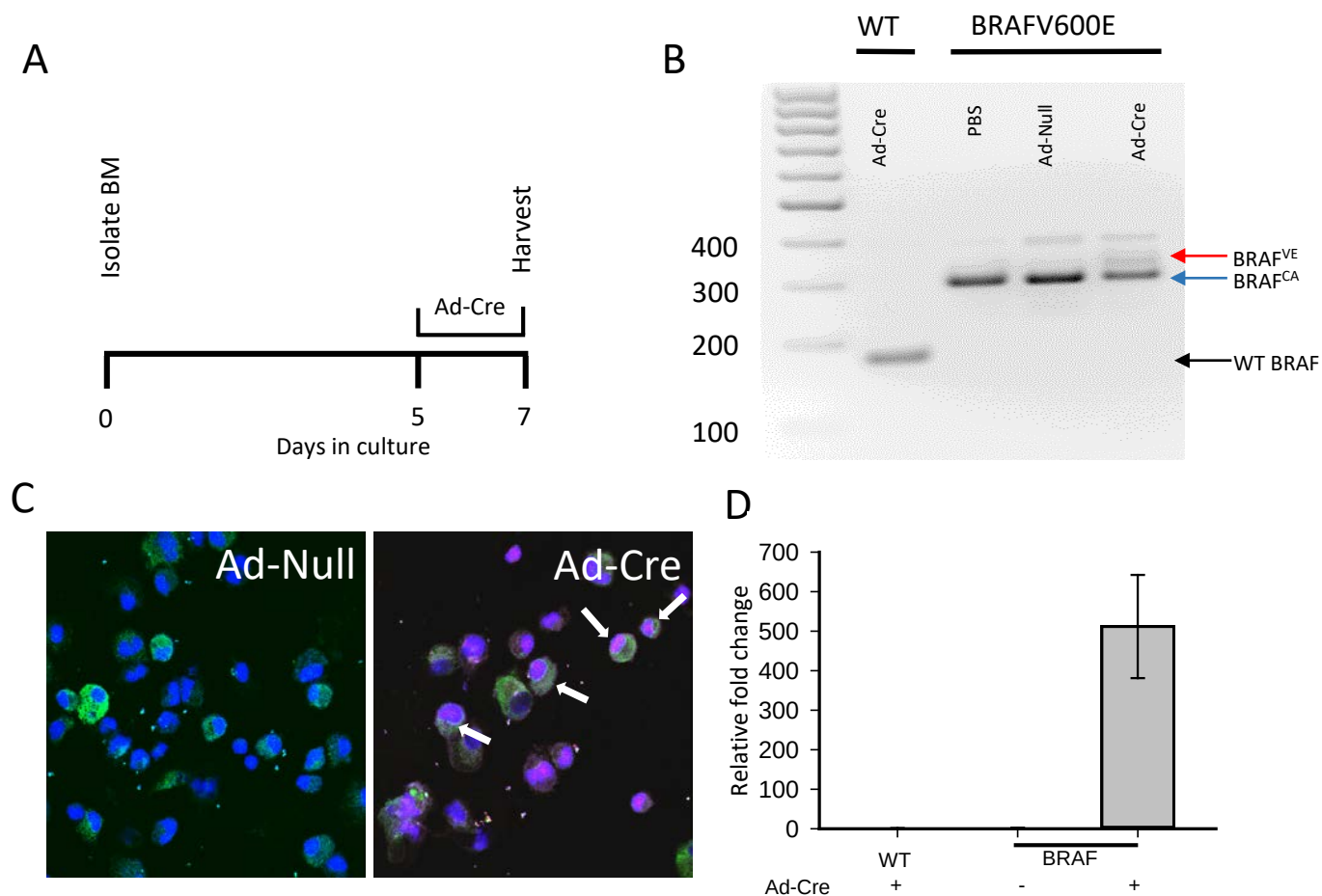


Fig. S2. Generation of BRAF^{VE} BMDC with Adenovirus-Cre. (A) Scheme of BRAF^{VE} BMDC generation. Ad-Cre with an mCherry reporter was added to the culture of BMDC at the indicate time and the cells were harvested at day 7 and used for assays. (B) WT and BRAF^{VE} BMDCs were lysed and DNA was amplified with BRAF primer. The product was than size fractionated on a 2% agarose gel. BRAF^{CA} represents the mutant BRAF before cre excision, BRAF^{ve} represents the mutant BRAF after cre excision and recombination. (C) Representative IF staining of the BMDCs generated from CD11c-ERT- BRAF^{VE} mice that were transfected with Ad-null and Ad-Cre for 2 days. Arrows point to cells that are positive for CD11c and mCherry staining (Blue – DAPI, Green – CD11c, Red – mCherry). Representation of experiments from 4 mice. (D) The expression of BRAF-V600E mRNA in WT BMDCs and BRAF^{VE} BMDCs with or without Ad-Cre transfection was measured by RT-PCR. Data are shown as the mean $\pm$ SEM of at least 5 independent experiments. ANOVA with Tukey multiple comparisons test was used * P < 0.05.

Supplemental Figure 3

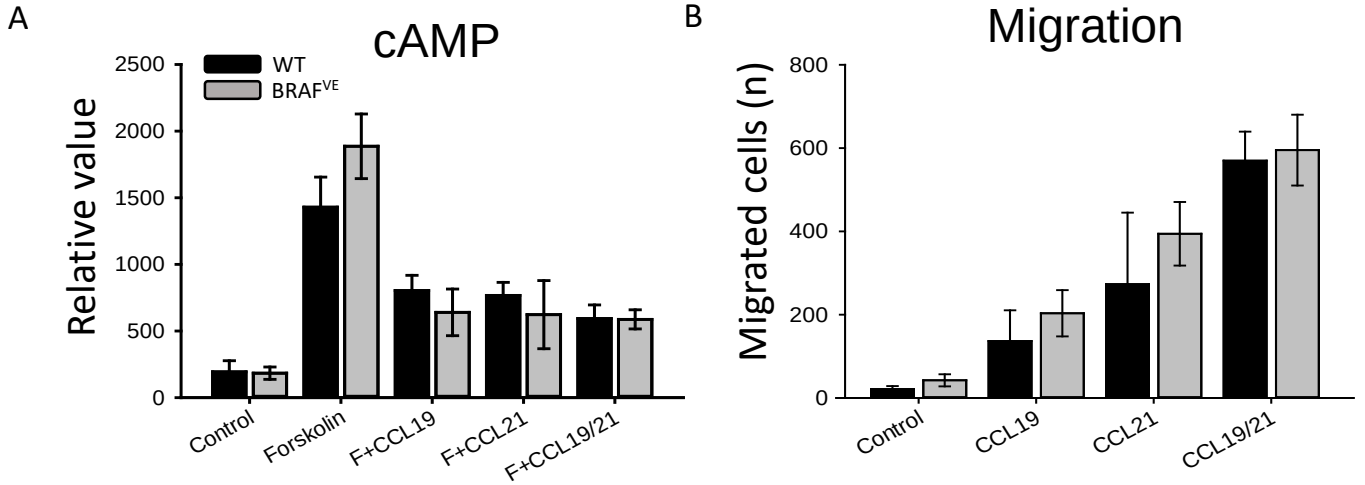


Fig. S3. BRAFV600E mutations do not alter CCR7 axis function. (A) Intracellular cAMP levels in BMDCs from WT and BRAFV600E mice following the administration of ad-Cre to induce BRAFV600E were measured in response to stimulation by CCL19, CCL21 or both (300ng/ml each). Data are shown as the mean $\pm$ SEM of 3 independent experiments. (B) The number of cells that migrated into the lower chamber of the transwell plate in response to stimulation by CCL19, CCL21 or both was determined by flow cytometry after 3 hours. Data are shown as the mean $\pm$ SEM of 3 independent experiments. ANOVA with Tukey multiple comparisons test was used $*P < 0.05$.

Supplemental Figure 4

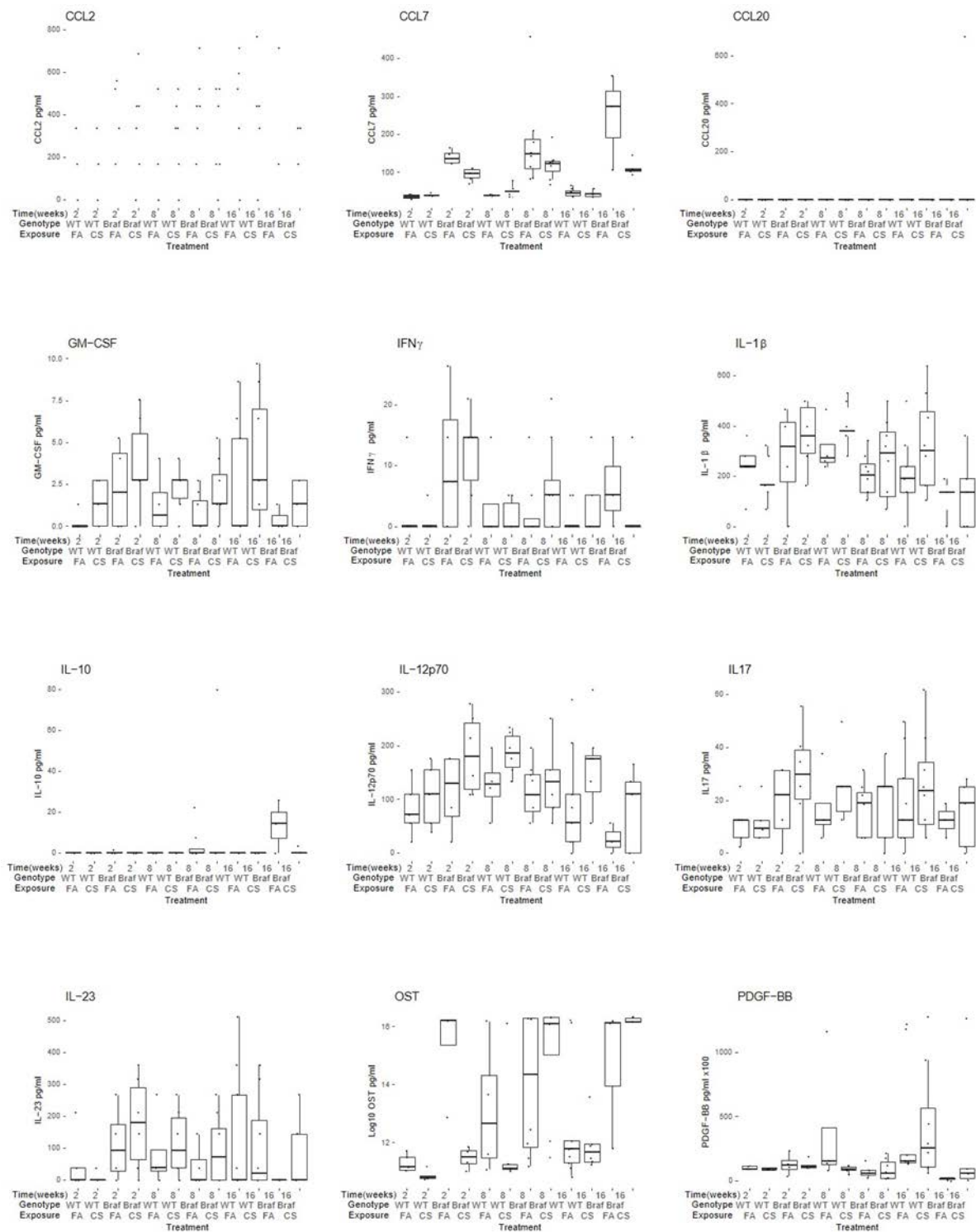


Fig. S4. Serum chemokine/cytokine levels in mice. Peripheral blood was collected from WT and BRAFVE mice exposed to FA/CS for the indicated period and the chemokine/cytokine levels in the serum were measured by multiplex ELISA (Luminex). Each data point represents an individual animal.

Supplemental Figure 5

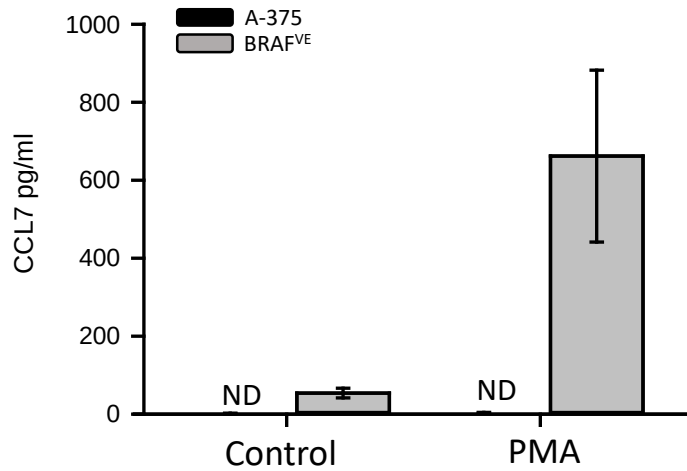


Fig. S5. A-375 melanoma cell line does not secrete CCL7. A-375 cells and BRAF^{VE} BMDCs were cultured with/without PMA overnight. The supernatant was collected and CCL7 secretion was measured by ELISA. Data shown are the mean $\pm$ SEM of 3 independent experiments. ND, Not detected.

Supplemental Figure 6

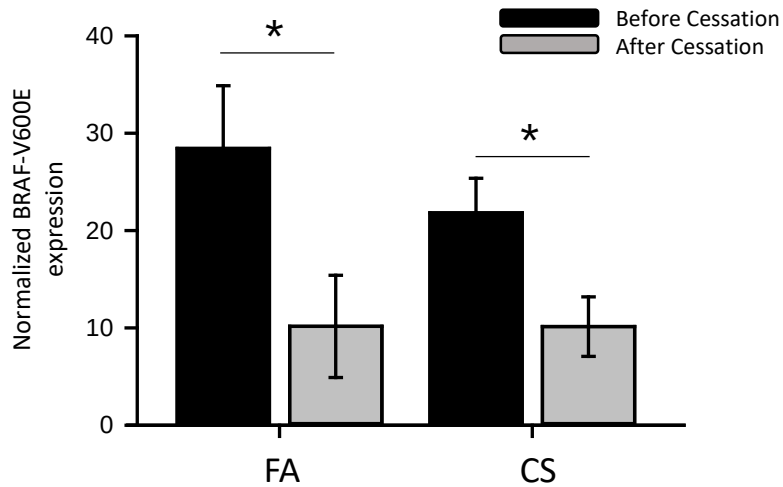


Fig. S6. Decreased BRAF-V600E expression in lung CD11c cells after cessation of CS exposure and tamoxifen treatment. CD11c cells were isolated and lysed from mice were exposed to CS and Tamoxifen for 4 months and 1 month after rotation to FA and regular chow. The mRNA of BRAF-V600E was measured by RT-PCR (n=4 mice per group). ANOVA with Tukey multiple comparisons analysis was used. *P < 0.05. Data represent mean +/- SEM.