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Supplemental Information

Potentiating Oncolytic Virus-Induced

Immune-Mediated Tumor Cell Killing

Using Histone Deacetylase Inhibition

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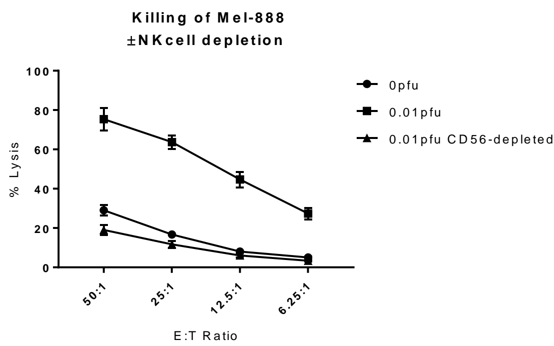
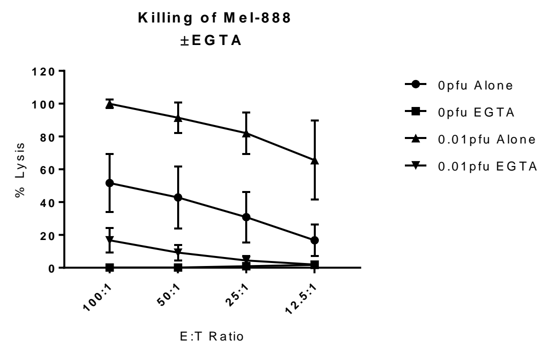
Supplementary Figure 1

A

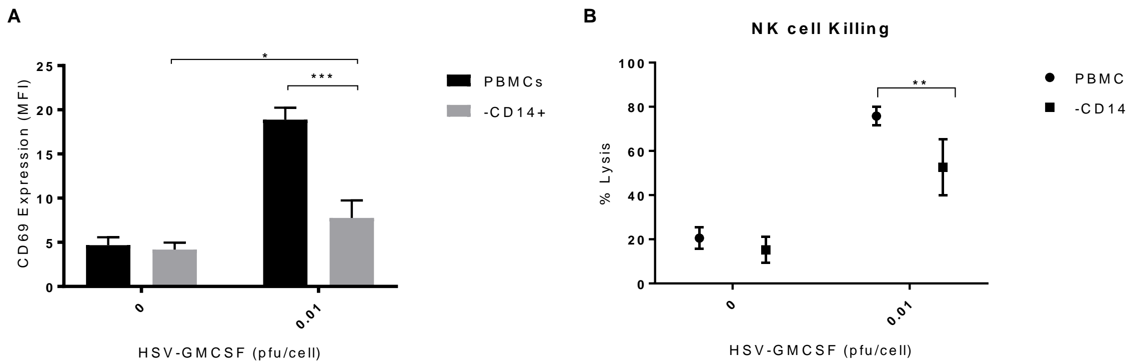


B



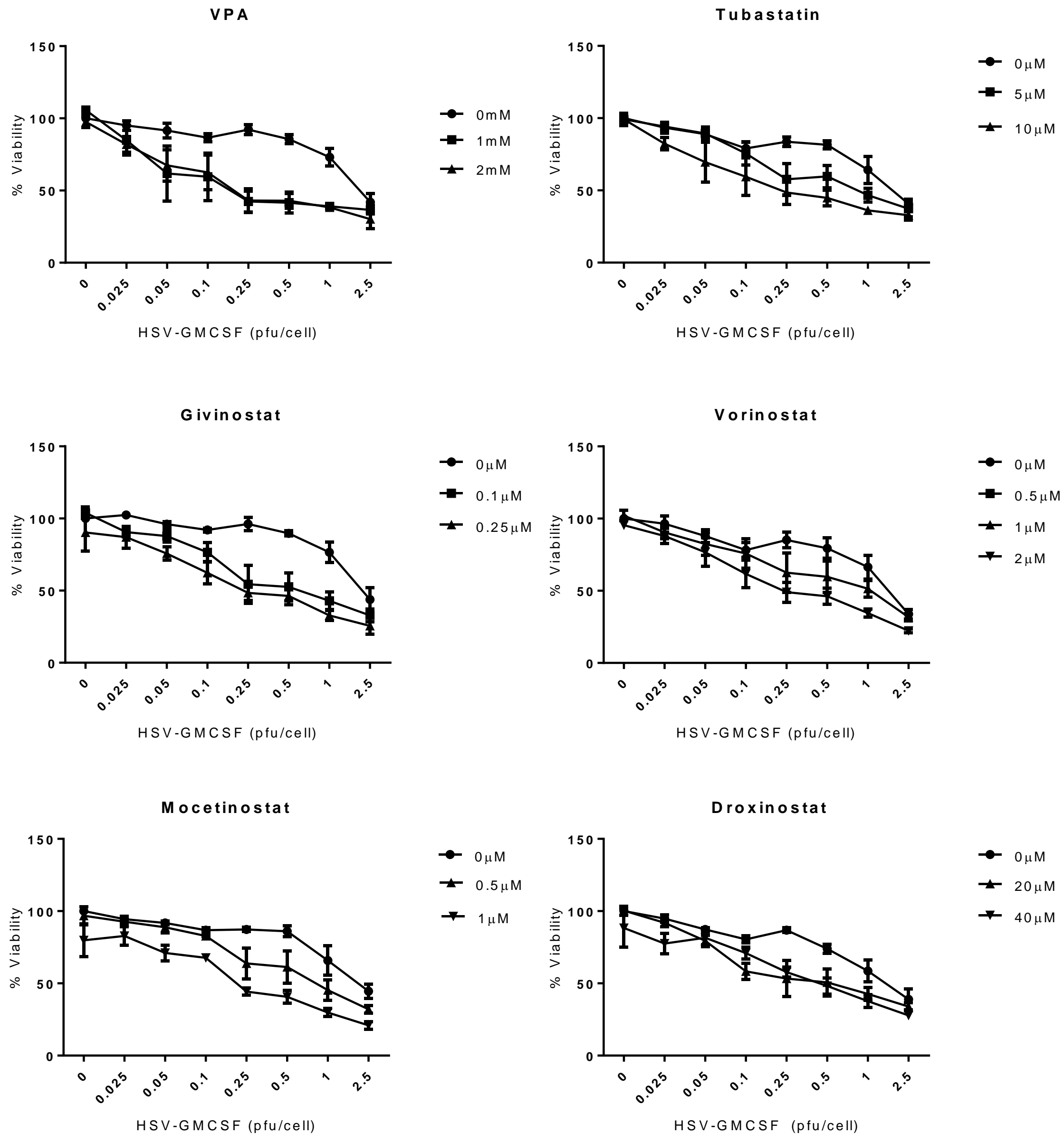
Supplementary Figure 1. NK cells are responsible for lysis of MEL888 target cells. A. PBMC were either left untreated (0 pfu/cell) or treated with 0.01pfu/cell HSV^{GM-CSF} overnight, before PBMC were then either left intact (0.01pfu) or NK cells were depleted using $CD56^{+}$ MACS selection (0.01pfu CD56-depleted) prior to ^{51}Cr release assays. The mean percentage lysis of ^{51}Cr-labelled MEL888 tumor cell targets is shown (n=3; ±SEM). **B**. PBMC were either left untreated (0 pfu/cell) or treated with 0.01pfu/cell HSV^{GM-CSF} overnight. Percentage lysis of ^{51}Cr-labelled MEL888 targets was determined in the presence or absence of 2mM EGTA (n=2, ±SEM).

Supplementary Figure 2



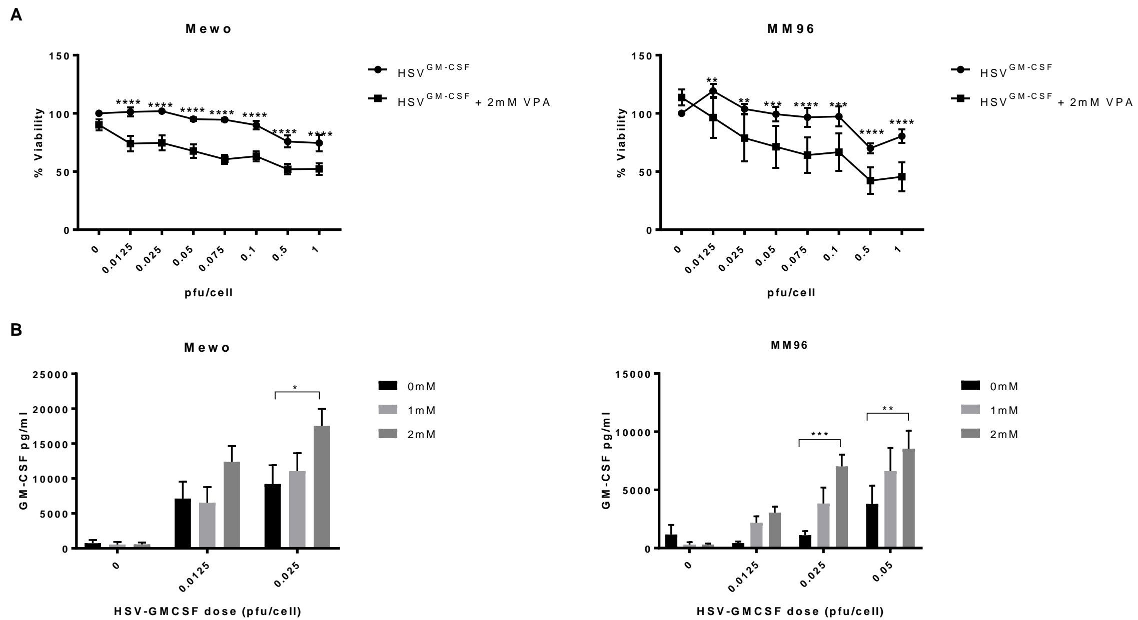
Supplementary Figure 2. Depletion of CD14+monocytes from PBMC decreases NK cell activation. A. Heathy donor PBMC (±CD14^{+} monocyte depletion) were treated with HSV^{GM-CSF} overnight and the expression of CD69 on NK cells was determined by flow cytometry. Data shows the average mean fluorescence intensity of CD69 expression on NK cells (n=4, +SEM). **B.** Heathy donor PBMC (±CD14^{+} monocyte depletion) were treated with HSV^{GM-CSF} overnight, co-cultured with MEL888 cells for 4hrs, and target cell lysis was determined by ^{51}Cr release. Data shows the average percentage tumor cell lysis (n=4, ±SEM).

Supplementary Figure 3



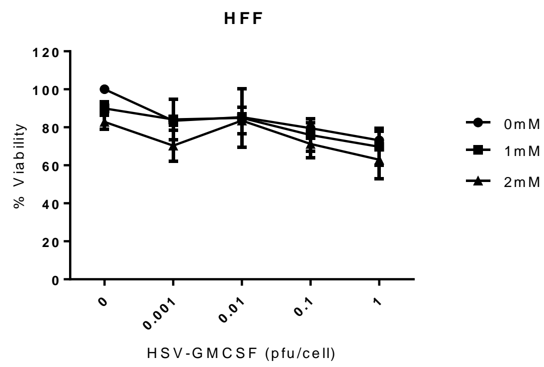
Supplementary Figure 3. Multiple HDACi enhance HSV$^{GM\text{-}CSF}$-induced cytotoxicity. Melanoma cell lines were seeded and treated with a range of HDACi at sub toxic doses for 24hrs, prior to the addition of HSV$^{GM\text{-}CSF}$ at concentrations ranging from 0 to 2.5pfu/cell. Cells were left for a further 48hrs and cell viability was determined by MTT assay. Data shown is the average of at least three independent experiments, ±SEM.

Supplementary Figure 4



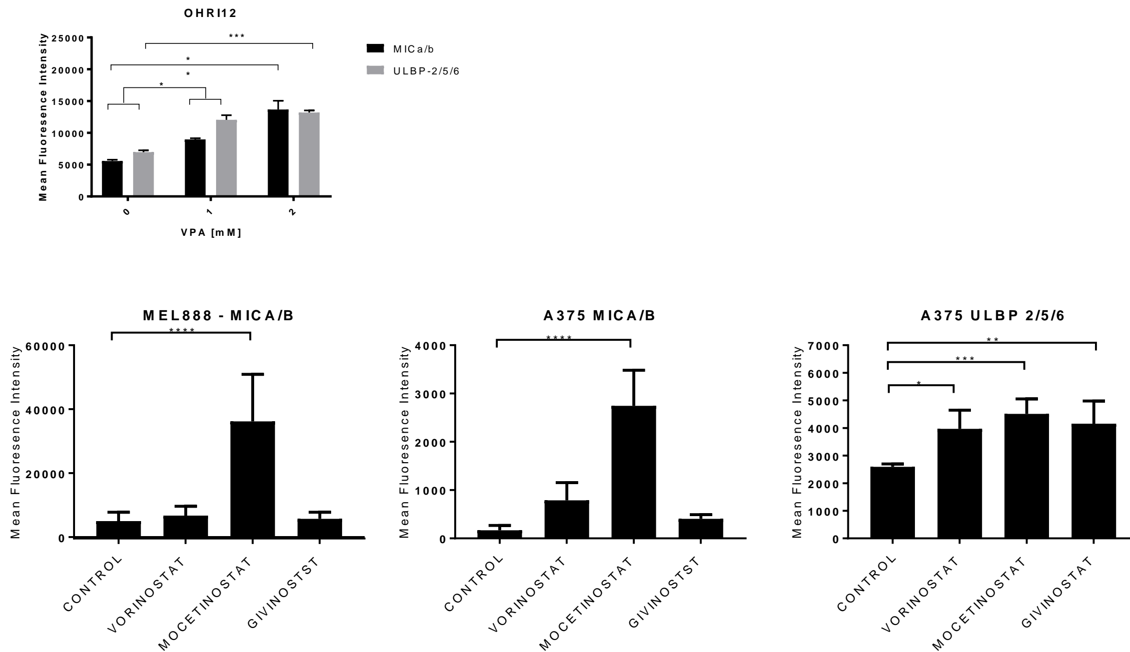
Supplementary Figure 4: VPA enhances HSV^{GMCSF} cytotoxicity and transgene expression. A. Melanoma cell lines (MeWo and MM96) were seeded and treated with VPA for 24hrs prior to the addition of HSV$^{GM\text{-}CSF}$ at concentrations ranging from 0 to 1pfu/cell. Cells were left for a further 48hrs and cell viability was determined by MTT assay. Data shown is the average of at least four independent experiments ±SEM. **B.** VPA-treated cells were treated with HSV$^{GM\text{-}CSF}$ for 24hrs and GM-CSF production was determined by ELISA. Data shows the mean of at least five independent experiments +SEM. Statistical significance is denoted by *p<0.05, **p<0.01, p***< 0.005, ****p<0.0001.

Supplementary Figure 5



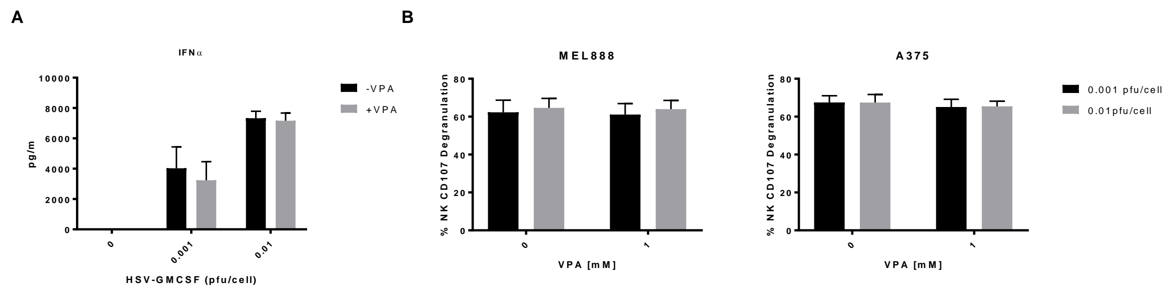
Supplementary Figure 5. VPA does not enhance HSV$^{GM\text{-}CSF}$ cytotoxicity in normal HFF. HFF were seeded and treated with 0, 1 or 2mM VPA for 24hrs, prior to the addition of HSV$^{GM\text{-}CSF}$ at concentrations ranging from 0 to 1pfu/cell. Cells were left for a further 48hrs and cell viability was determined by MTT assay (n=3, ±SEM).

Supplementary Figure 6



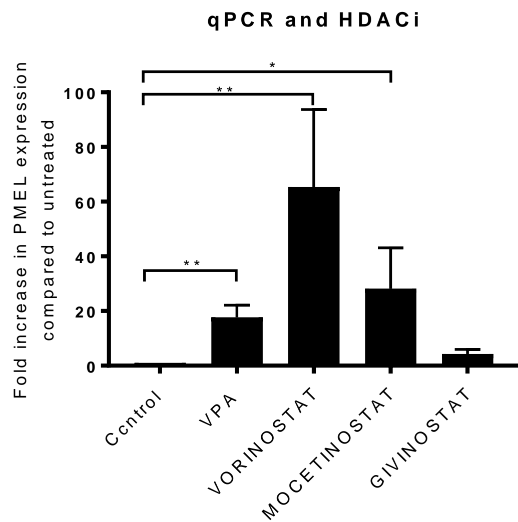
Supplementary Figure 6: VPA up-regulates NK cell activatory ligands on primary melanoma cells and alternative HDACi modulate MICA/B and ULBP2/5/6 expression. A. Expression of NK ligands (MICA/B and ULBP2/5/6) on the surface of primary melanoma cells (OHRI12) was determined by flow cytometry. Cells were treated with VPA at indicated doses for 48hr (n=3, +SEM). **B**. MEL888 and A375 cells were treated with 2mM VPA, 2μM vorinostat, 1μM mocetinostat and 0.2μM givinostat for 48hrs and the expression of MICA/B and ULBP2/5/6 was determined by flow cytometry (n=3, +SEM).

Supplementary Figure 7



Supplementary Figure 7. Pre-treatment of PBMC with VPA does not prevent IFNα production or NK cell activation. **A**. IFNα production from PBMC pre-treated ±VPA for 4 hrs followed by $HSV^{GM\text{-}CSF}$ treatment (0, 0.01 and 0.001pfu/cell) overnight was determined by ELISA (n=3, +SEM); un-bound VPA was removed prior to addition of $HSV^{GM\text{-}CSF}$. **B**. Healthy donor PBMC were treated ±VPA for 4 hours followed by $HSV^{GM\text{-}CSF}$ treatment (0.01 and 0.001pfu/cell) overnight. PBMC were co-cultured with melanoma targets and NK cell ($CD56^{+}$/$CD3^{-}$) CD107 degranulation was determined by flow cytometry. Data shows the mean percentage of NK cells expressing CD107 after co-culture with MEL888 and A375 cell targets (n=3, +SEM).

Supplementary Figure 8



Supplementary Figure 8. Alternative HDACi up-regulate PMEL TAA expression. PMEL mRNA expression levels in A375 cells were quantified by qRT-PCR relative to EF1α housekeeping control following treatment with 2mM VPA (n=6, +SEM), 2µM vorinostat, 1µM mocetinostat and 0.1µM givinostat for 48hrs (n=3, +SEM).