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Supplemental Material to:

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Vemurafenib enhances MHC induction in *BRAF*^{V600E} homozygous melanoma cells

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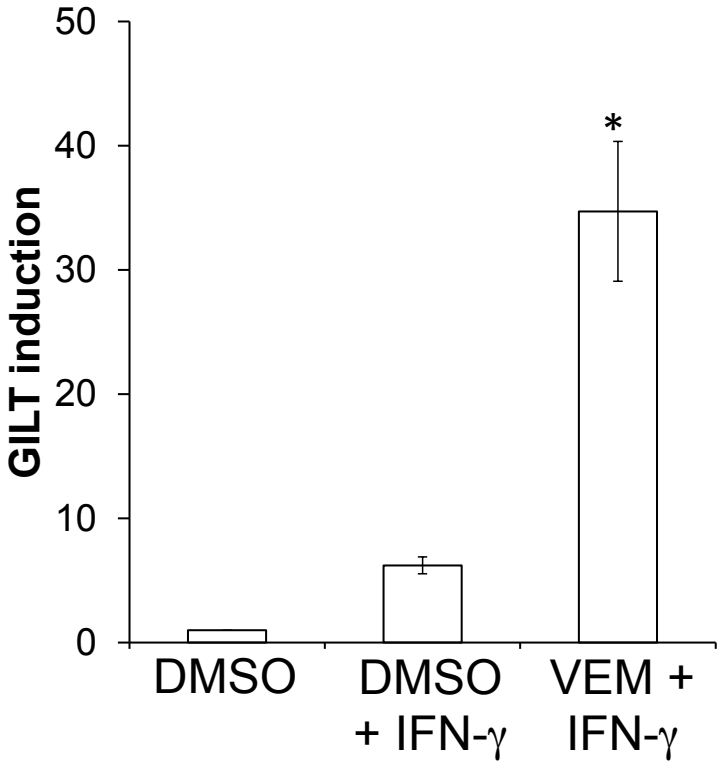
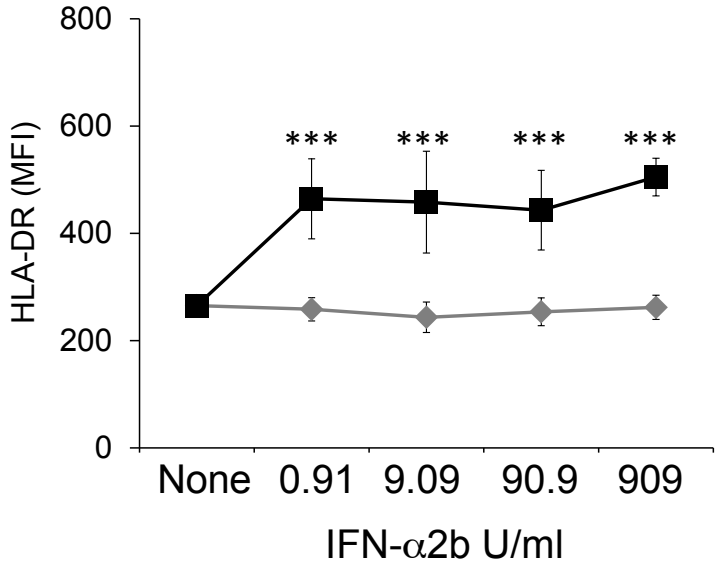


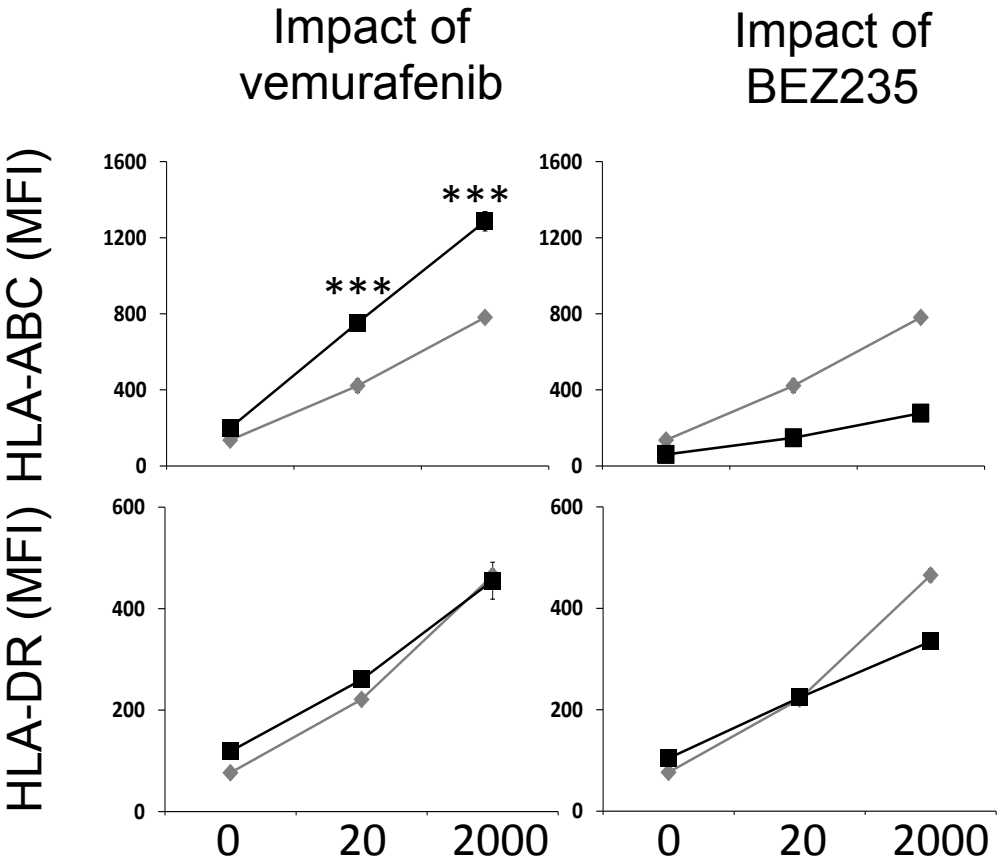
Supplementary Figure S1. The induction of gamma-interferon-inducible lysosomal thiol reductase (GILT) mRNA is enhanced by vemurafenib. A375 cells were pre-treated with vehicle (DMSO) or vemurafenib (VEM, 10 μM) for 60 minutes prior to the addition of IFN-γ (2000 U/ml). Steady state mRNA levels of GILT were analyzed 72 hours after the addition of IFN-γ using quantitative real-time RT-PCR and are expressed relative to cells treated with DMSO. The *y*-axis represents the average GIFT fold induction of four independent experiments. (*, $p<0.05$ paired Student's t test, as compared to cells pre-treated with DMSO and treated with IFN-γ)



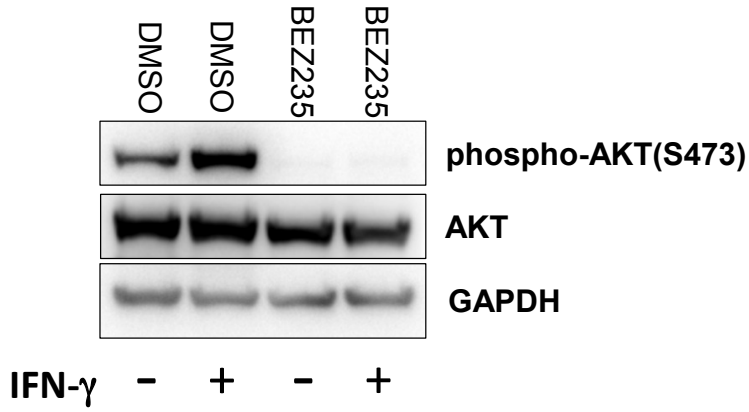
Supplementary Figure S2. Vemurafenib enhances MHC class II induction by IFN-α2b in A375 cells. A375 cells were left untreated (none) or pre-treated with vehicle (DMSO, gray diamonds) or vemurafenib (0.5 μM, black squares) for 60 minutes prior to the addition of IFN-α2b at the concentrations indicated long the *x*-axis. Cell surface MHC class II (HLA-DR) expression was measured using flow cytometry 72 hours following the addition of IFN-α2b. The *y*-axis represents the average mean fluorescence intensity (MFI) from 5 experiments. The error bars represent the standard deviation. (***, $p<0.001$, repeated measures ANOVA, as compared to cells treated with the same concentration of IFN-α2b and DMSO)

Supplementary Table T1. Summary of cell lines used.

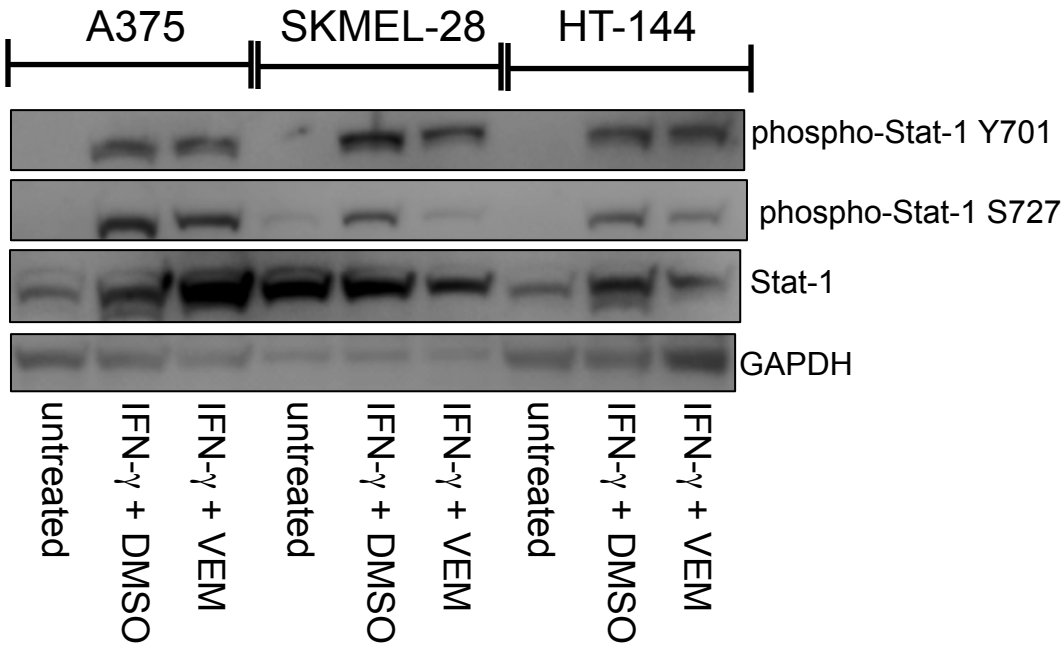
Cell line	Source	Catalogue Number	BRAF Status
A375	ATCC	CRL-1619	V600E Homozygous
HT-144	ATCC	HTB-63	V600E Homozygous
MALME-3M	ATCC	HTB-65	V600E Heterozygous
MeWo	ATCC	HTB-64	Wild type
SK-MEL2	ATCC	HTB-68	Wild type
SK-MEL3	ATCC	HTB-69	V600E Heterozygous
SK-MEL5	ATCC	HTB-70	V600E Heterozygous
SK-MEL28	ATCC	HTB-72	V600E Homozygous
UACC-62	NCI		V600E Homozygous



Supplementary Figure S3. Impact of vemurafenib and BEZ235 on MHC induction in BRAF^{V600E} homozygous UACC-62 cells. *Left panels*, UACC-62 cells were pre-treated with vehicle (DMSO, gray diamonds) or vemurafenib (VEM, 0.5 μM, black squares) for 60 minutes prior to the addition of IFN-γ at the doses indicated along the *x*-axis. Averaged mean fluorescence intensity (MFI) values from three experiments are shown for MHC class I (top panel) and class II (bottom panels) as measured using flow cytometry 72 hours after IFN-γ treatment. (***, $p<0.001$, ANOVA, as compared to cells pre-treated with DMSO) *Right panels*, a comparison between DMSO pre-treated cells (gray diamonds) and BEZ235-pre-treated cells (black squares) is shown.



Supplementary Figure S4. The dual PI3K/mTOR inhibitor BEZ235 decreases levels of phosho-AKT. A375 cells were treated with vehicle (DMSO) or BEZ235 (0.5 μM) alone of 60 minutes prior to the addition of IFN-γ (2000 U/ml). Cell lysates were prepared 72 hours after the addition of IFN-γ and levels of phospho-AKT (serine 473), AKT, and GAPDH visualized via western blot.



Supplementary Figure S5. The impact of vemurafenib on Stat1 phosphorylation. A375, SKMEL-28, and HT-144 cells were left untreated or received vehicle (DMSO) or vemurafenib (VEM, 0.5 μM) 60 minutes prior to the addition of IFN-γ (2000 U/ml). Whole cell lysates were prepared 24 hours after the addition of IFN-γ and levels phospho-Stat1(at tyrosine 701 and serine 727) assessed by western blot. Levels of Stat1 and GAPDH are also shown.