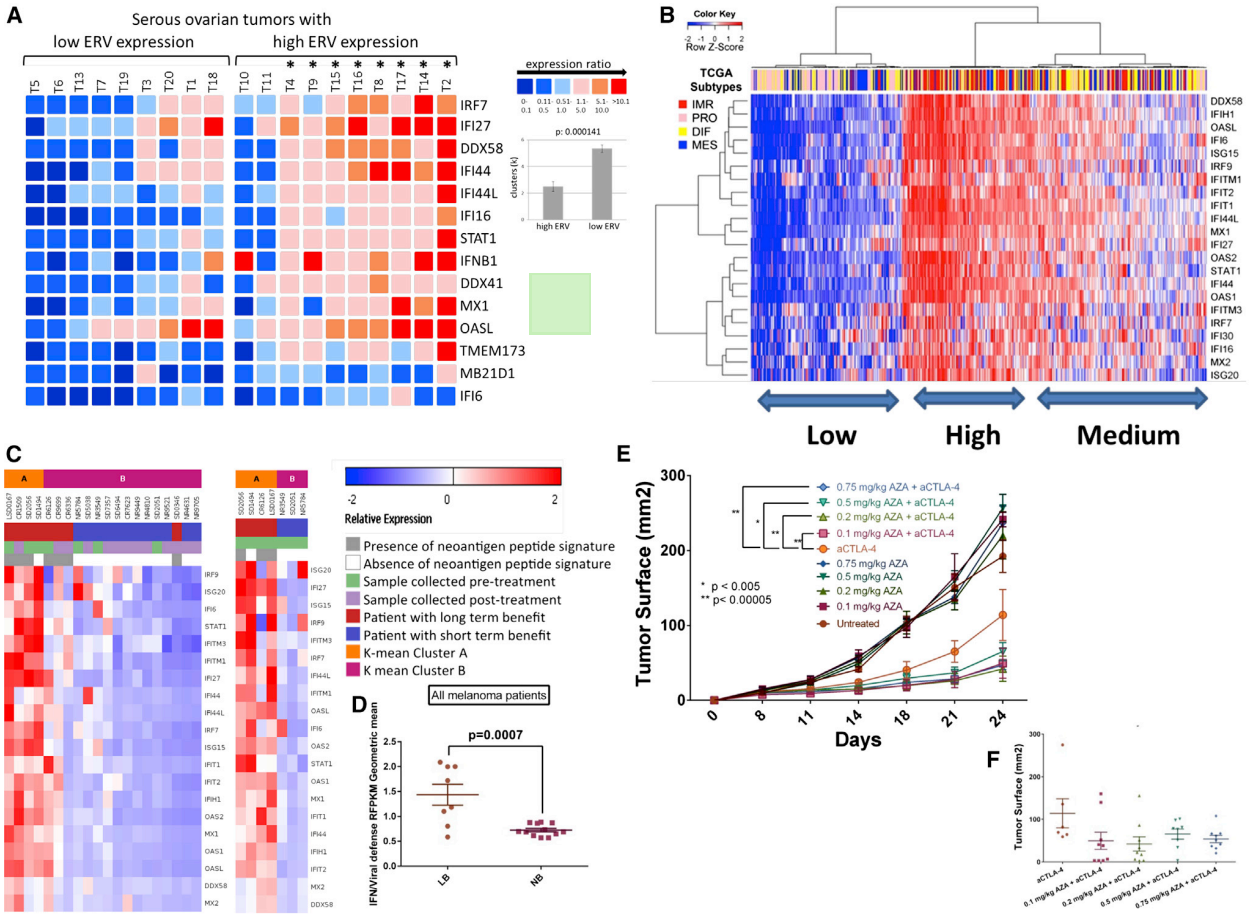

Figure 7. Aza-Upregulated Viral Defense Genes Are Significantly Correlated with ERVs in Primary Tumors and Correlate with Sensitivity to Immune Therapy

(A) Heatmap comparing basal levels of viral defense genes and ERVs in primary EOC. The cut-off for lower or higher ERVs was the mean control tissue value of 237.57 ± 83.05 molecules/ng RNA. Mean ISGs of the high ERV ovarian tumor (T) cohort (n = 10) is 12.65-fold higher than the mean of ISGs of the low ERV cohort (n = 9). The (*) denotes that eight of ten high ERV tumors had significantly higher ISG expression compared to the low ERV tumors. ISG expression is organized according to low and high ERV expression cohorts in arbitrary units; color code from blue to red shows increasing ISG expression. For clusters (k = 6), differences are significant between the high ERV expression (2.5 ± 0.37) and the low ERV expression cohort (5.33 ± 0.28).

(B) Interferon-stimulated viral defense genes upregulated at least 2-fold by Aza in EOC cell lines (right y axis) were used to cluster EOC tumors for RNA-seq data (blue, low; red, high) from The Cancer Genome Atlas (TCGA). EOC TCGA subtypes are shown: DIF (differentiated), IMR (immune reactive), MES (mesenchymal), and PRO (proliferative).

(C and D) Viral defense gene signature is upregulated in tumors from anti-CTLA-4-treated metastatic melanoma patients who derived durable clinical benefit (complete response, partial response, or progression free-survival >6 months as previously described) (Snyder et al., 2014) compared to those without benefit. Tumors collected pre-CTLA-4 treatment and shortly post-treatment are shown.

(D) y axis = RFPKM mean of viral defense genes in all melanoma patients.

(E and F) Tumor responses of mice injected with B16-F10 cells and treated with either PBS, anti-CTLA-4, Aza, or both anti-CTLA-4 and Aza. Data represent results from one of two independent experiments with identical results, each with n = 10 per arm. Y axis = mean tumor surface, error bars ± SEM.

See also Figure S7 and Tables S5 and S6.

DISCUSSION

Our present data now provide functional context for our earlier reports that DNMTis induce a complex set of immune pathway responses in tumor cells (Li et al., 2014; Wrangle et al., 2013). DNMTis trigger cytoplasmic dsRNA sensing, central to cellular viral defense responses, and activate interferon in EOC and colon cancer cells by disrupting DNMTs. This activation could induce tumor attraction of lymphocytes (Ivashkiv and Donlin, 2014). There are some important implications for one of the most exciting new developments in cancer treatment, immune checkpoint therapy (Brahmer et al., 2010, 2012; Berger et al., 2008; Leach et al., 1996; Topalian et al., 2015; Hodi et al., 2010; Weber et al., 2015) and for underlying mechanisms inherent to both tumor and host cells for reversal of immune tolerance in tumor infiltrating T-lymphocytes (Pardoll, 2012).