The immune landscape of residual triple-negative breast cancers after neoadjuvant chemotherapy
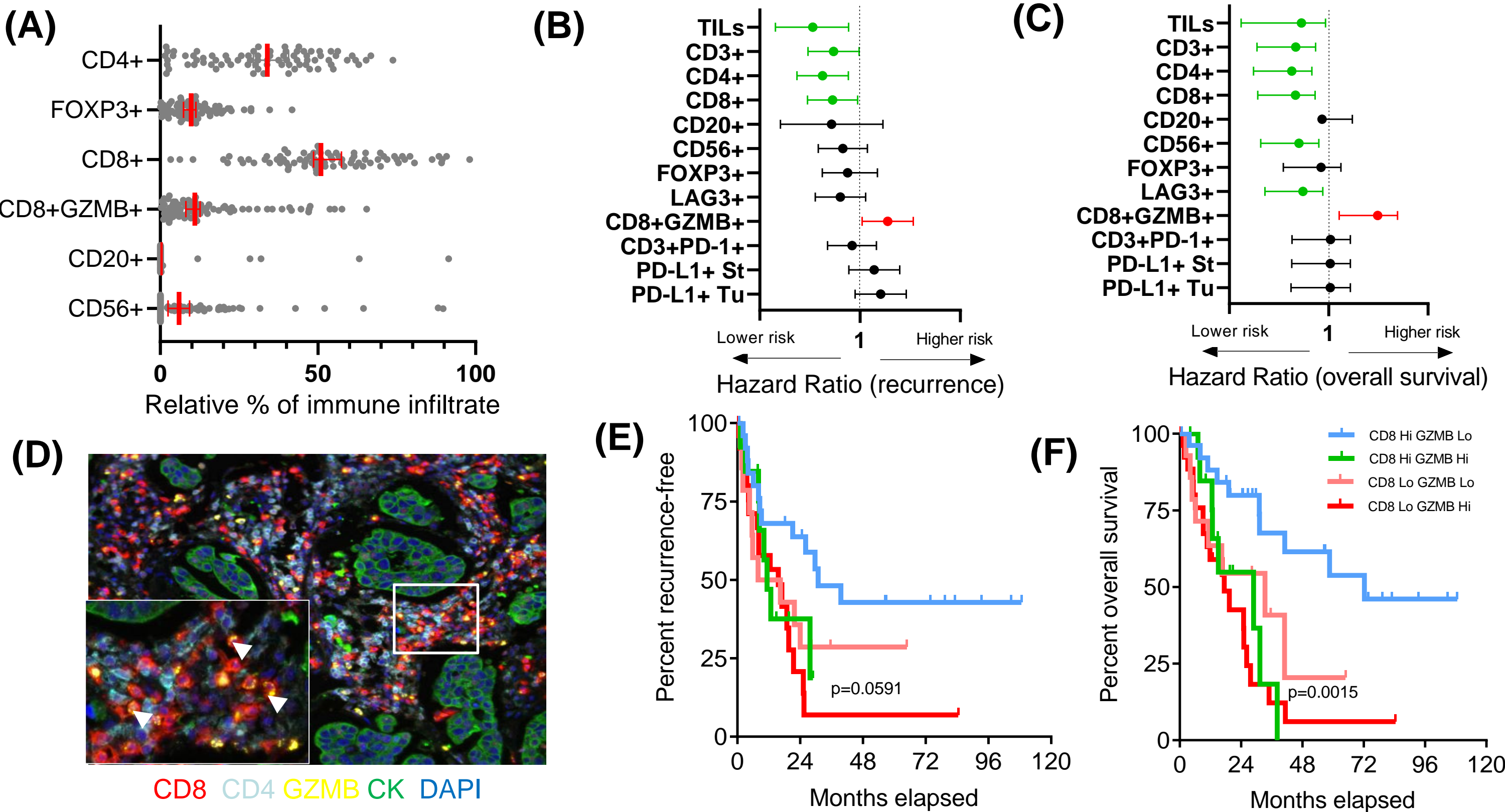
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High CD8+GZMB+/CD8 ratio conveys a worse prognosis in residual TNBC

Tumor-infiltrating lymphocytes (TILs) in the residual disease are a positive prognostic factor, but how specific immune composition of the tumor guides outcome is unclear, resulting in a lack of understanding of how to employ immunotherapies in the adjuvant setting.
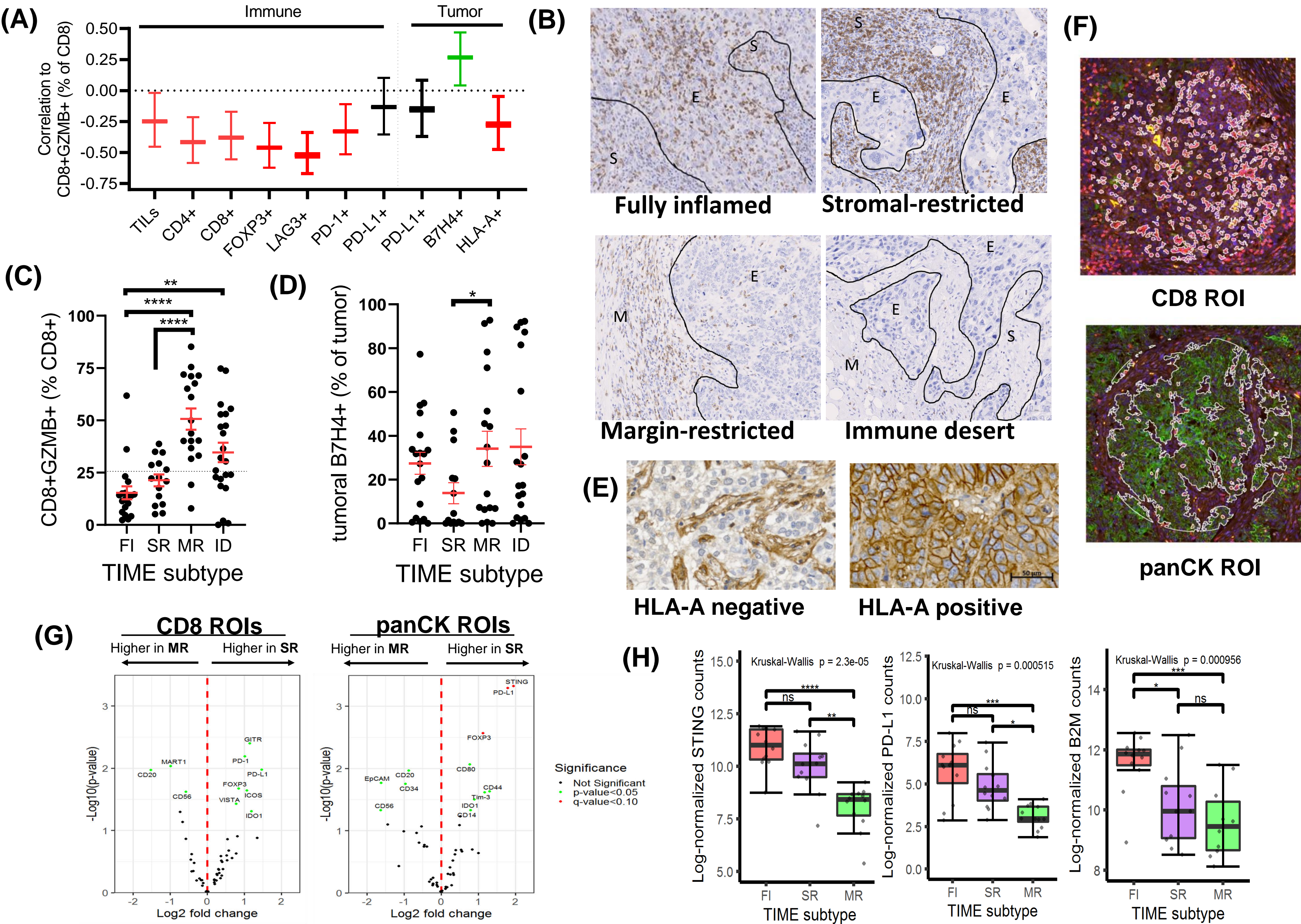

We assessed multiple immunologic biomarkers in a series of 99 residual TNBCs after NAC. H&E-scored TILs were highly correlated to CD3, CD4 and CD8 (p<0.001). While CD20 and CD56 positive cells only make up a small and non-significant proportion of the TILs composition. As plotted in the relative percentage graph where each dot represents a sample, error bars denote the median and 95%CI (A). Univariant Cox proportional hazard ratio analysis revealed TILs in residual tumors after NAC are predictive of both RFS and OS (p=0.0093, and p=0.0376, respectively), as previously demonstrated. Likewise, infiltration by T cell populations were associated with significantly improved outcome. Interestingly, the ratio cytotoxic CD8 cells (CD8+GZMB+) to CD8 cells were paradoxically associated with worse RFS and OS (B-C). Representative section showing dual CD8+GZMB+ on multiplex IF (D). Kalan-Meier curves show CD8+GZMB+ are a marker of negative prognosis even when adding total CD8 count into the analysis, suggesting independence (E-F).

Discussion

We have found a paradoxical association of CD8+GZMB+ T cells with a negative prognosis in NAC-treated TNBC. We hypothesize that while these T cells are poised for cytotoxic activity, they remain restricted through sub-localization outside the tumor core, downregulation of HLA-A on tumor cells preventing interaction with the T cell receptor, downregulation of STING thwarting type I interferon response, and upregulation of B7H4 expression, which has been shown to inhibit cytotoxic T cell activity. Patients with low CD8 in the tumor core and high CD8+GZMB+ in the post-NAC setting may benefit from adjuvant immunotherapy, particularly in combination with therapies that enhance MHC-I antigen presentation.

Loss of tumor HLA-A expression associates with marginal T cell localization and GZMB+ CD8 T cells

Next, we set out to explain this paradoxical finding. We found that CD8+GZMB+ T cell infiltration inversely correlated with TILs (p=0.0293, A) and showed a positive correlation with B7H4 tumor expression (p=0.0183, A). Then, we quantified CD8/mm² as described by Gruosso et al *J Clin Invest.* 2019, examples of TIME classification are shown on CD8 IHC images (B). CD8+GZMB+ high tumors mainly correspond to margin-restricted and immune-desert tumor immune microenvironment landscapes (C). Since CD8 T cells recognize antigens presented by MHC-I, we investigated HLA-A tumor expression. CD8+GZMB+ high cases showed decreased expression or loss of HLA-A in tumor cells (p=0.0189). In parallel, margin-restricted and immune-desert tumors presented lower HLA-A expression (D). Representative images of expression and loss of HLA-A are shown in (E). *In situ* spatial analysis of 58 proteins on tumor (panCK) and CD8+ T cell populations (F), showed lower STING and PD-L1 expression on tumor cells on a subset of MR tumors compared to stroma-restricted and fully-inflamed tumors (G, H).

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