Hodgkin/Reed-Sternberg cells induce GPNMB expression and release from macrophages to suppress T-cell responses to the Epstein-Barr virus-encoded LMP2A protein

Authors

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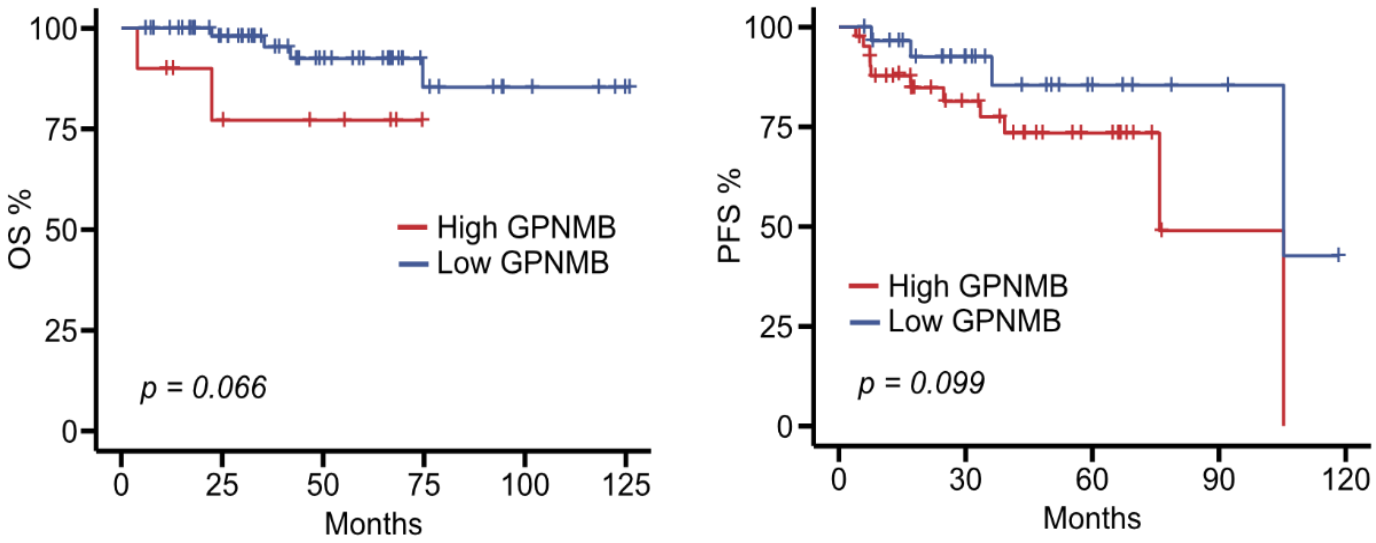
Supplementary Figure 1

A



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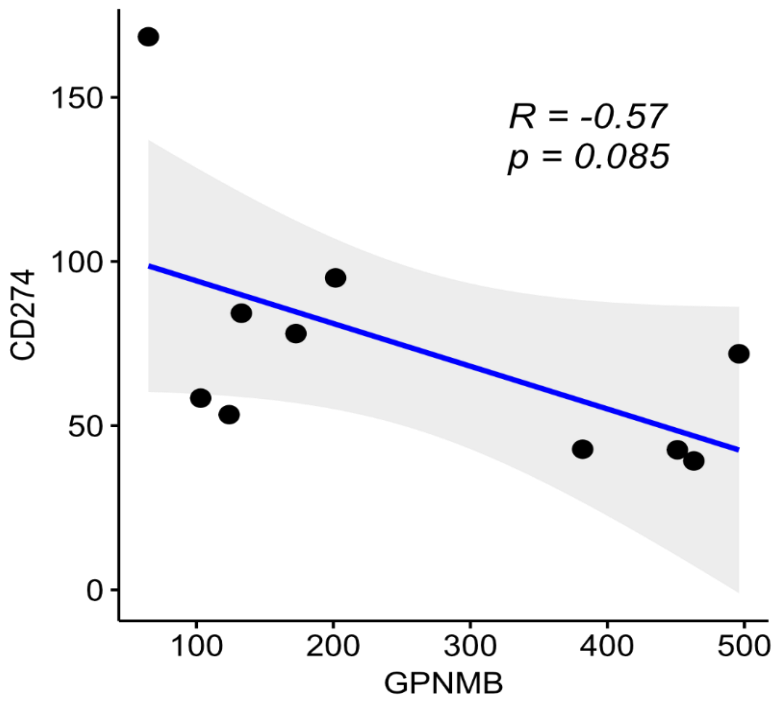
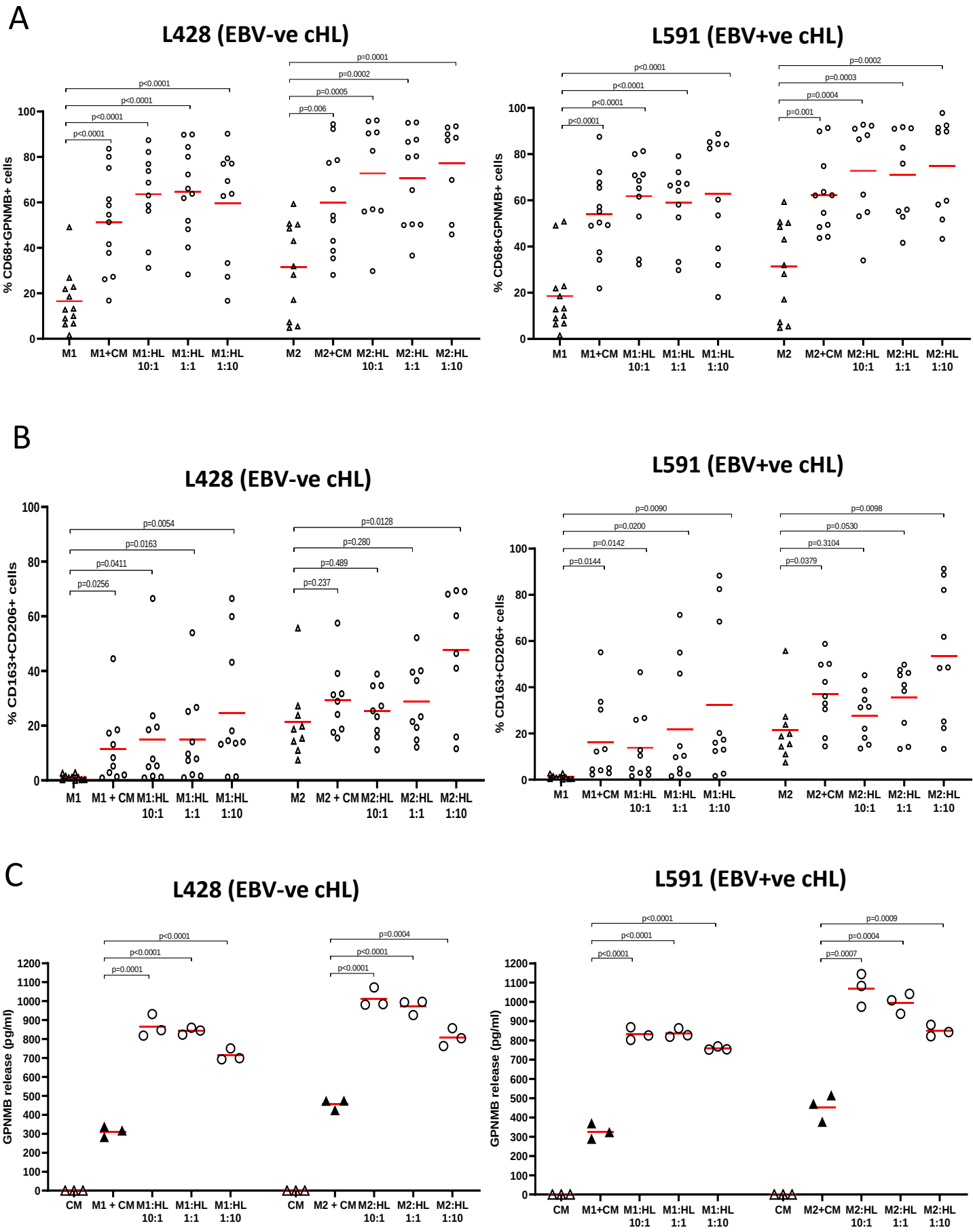


Supplementary Figure 1: GPNMB expression in primary cHL A) Survival analysis comparing GPNMB high versus GPNMB low tumors by Kaplan-Meier curves. Overall survival (OS) and progression free survival (PFS) were reduced in GPNMB high tumors compared to low tumors, but these differences were only of borderline significance. **B)** Correlation between PD-L1 (CD274) expression and GPNMB expression in cHL determined by Nanostring GeoMx in the macrophage enriched compartment (defined by CD68 expression).

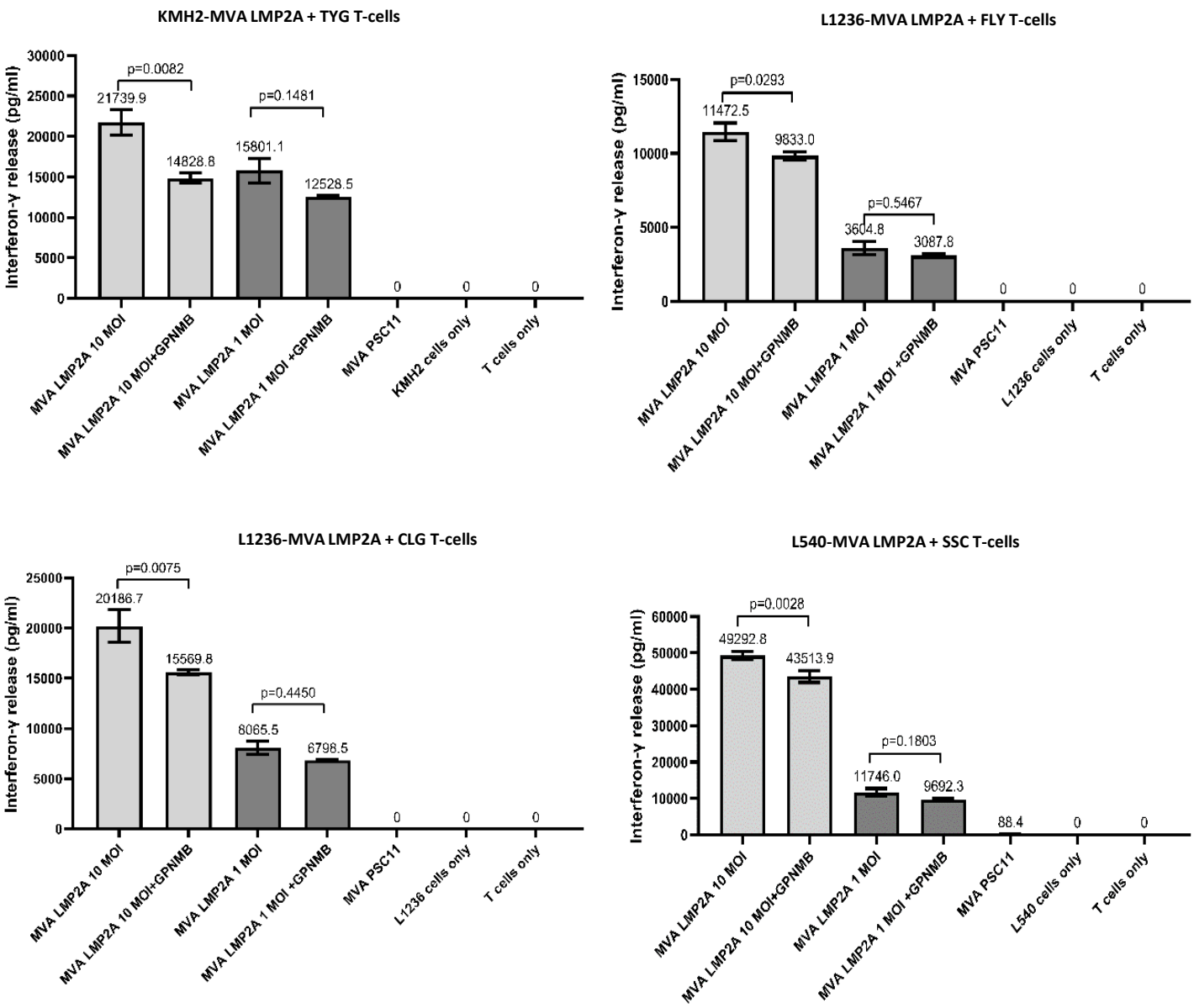
Supplementary Figure 2



Supplementary Figure 2: cHL cells induce GPNMB expression and release from macrophages and their polarization to M2 phenotype. A) Flow cytometry for GPNMB expression on M1 or M2 macrophages following their culture in L428 or L591 cHL-derived conditioned media (+CM) or their direct co-culture with L428 and L591 cells at different macrophage:HL cell ratios for 24h. Macrophages generated from at least nine different individuals were tested per condition. Anti-GPNMB-PE antibody (HOST5DS) and CD68/PE-Texas Red (Thermo Fisher, eBioscience, Waltham, MA, USA) were used. **B)** Flow cytometry for CD163+CD206+ M2 marker expression on M1 or M2 macrophages following their culture in L428 and L591 CM or by their co-culture with L428 or L591 cells as in A). CD163/APC and CD206/PE-Cy7 (Thermo Fisher) antibodies were used. Macrophages generated from at least nine different individuals were tested per condition. **C)** ELISA measurement of GPNMB release by M1 and M2 macrophages exposed to L428 or L591 CM or directly co-cultured with L428 or L591 cells for 24h. GPNMB Duoset ELISA kit (R&D Systems, Minneapolis, MN, USA) was used. Shown are the results of three separate donors. Means (solid bars) for all experiments were compared by Student's t-test.

Supplementary Figure 3

A



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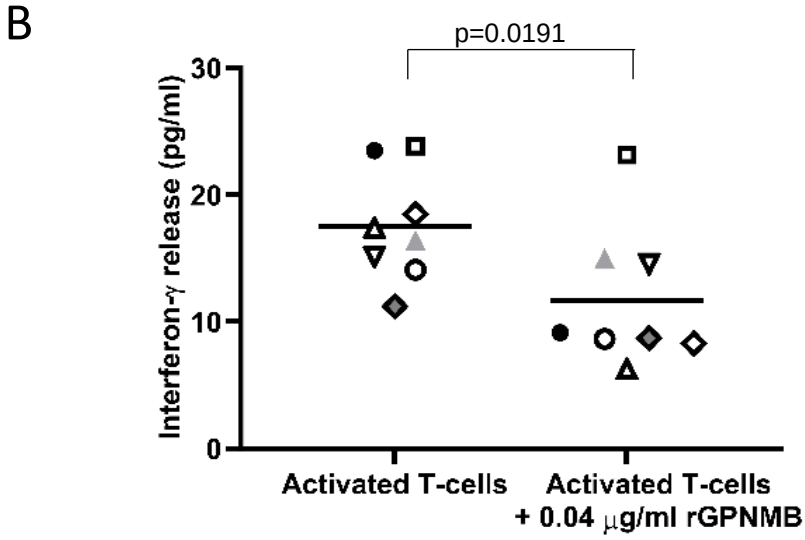

Supplementary Figure 3: Low dose soluble recombinant rGPNMB inhibits T-cell recognition of cHL lines *in vitro*. A) ELISA measurement of interferon-γ release by T-cells co-cultured with cHL cell lines for 18h. cHL cells were infected with MVA LMP2A (MOI of 1 or 10) or negative control virus, MVA-pSC11 (MOI 10). An optimal dose of soluble recombinant GPNMB (rGPNMB, 0.04μg/ml) was tested using the same TYG and CLG-specific CD8+ T-cell clones (as in Figure 3) and additional CD8+ T-cell clones specific for the HLA-A2-restricted epitope FLY (LMP2 amino acids 356-364) and HLA-A11-restricted epitope SSC (LMP2 amino acids 340-350). For the latter, HLA-A11-positive L540 HL line was also used as the target cells. Means (solid bars) were compared by Student's t-test. **B)** ELISA measurement of interferon-γ release by primary PBMCs from eight independent donors in the presence/absence of 0.04μg/ml rGPNMB. PBMCs were activated using a range of six different concentration of soluble CD3/CD28 activator (Stemcell technologies, Vancouver, Canada, 10971; 0.125, 0.25, 0.5, 1, 2, 5μl/ml). All CD3/CD28 concentrations tested had a similar effect on T-cell activation (data not shown). The data shown are means (solid bars) of means of all CD3/CD28 activators contractions tested for all eight donors and compared by Student's t-test.