In classical Hodgkin lymphoma the combination of the CCR5 antagonist maraviroc with trabectedin synergizes, enhances DNA damage and decreases three-dimensional tumor-stroma heterospheroid viability

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Supplementary Appendix

In classical Hodgkin lymphoma the combination of the CCR5 antagonist maraviroc with trabectedin synergizes, enhances DNA damage and decreases 3D tumor-stroma heterospheroid viability.

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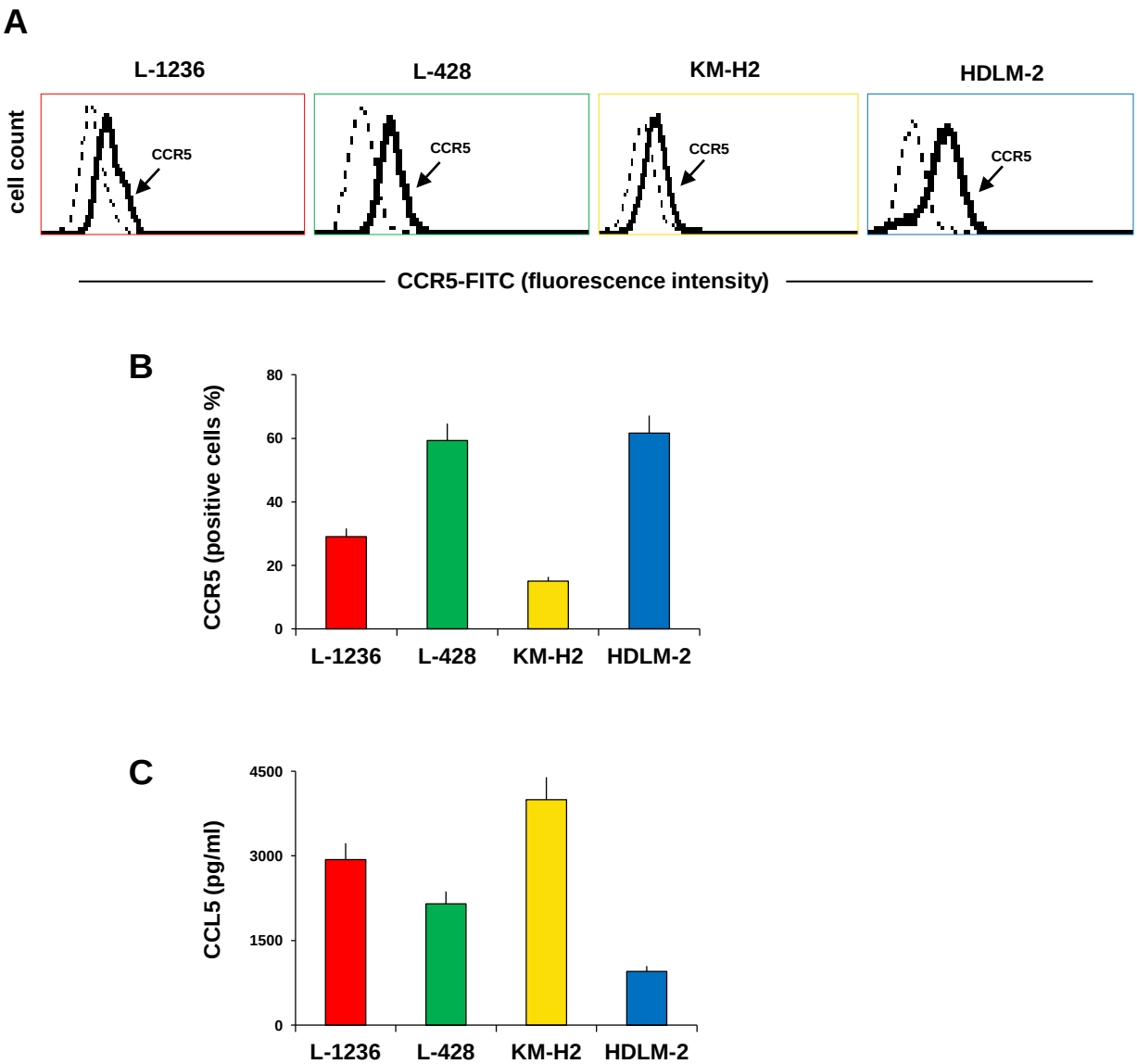
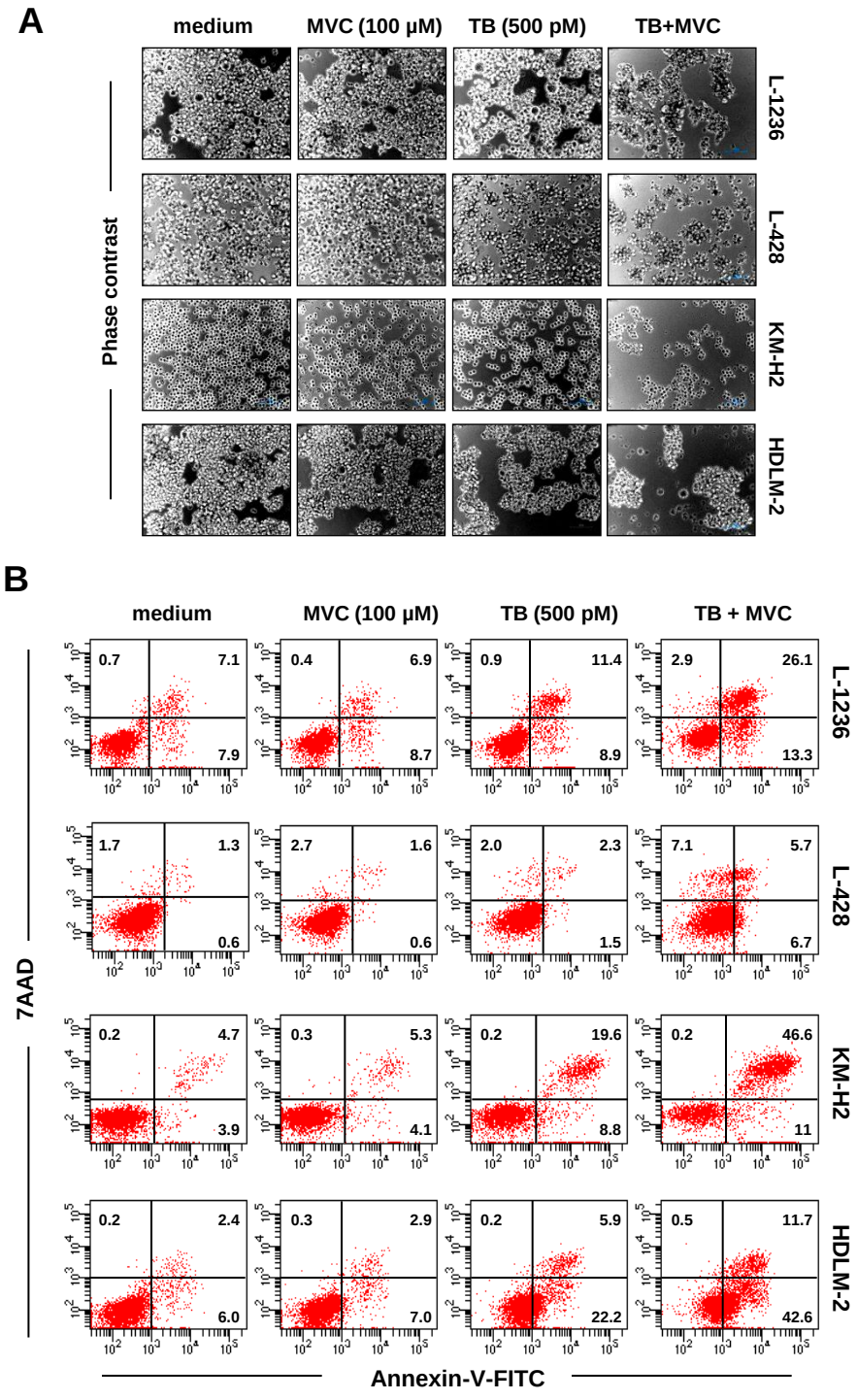
Supplemental Figure S1



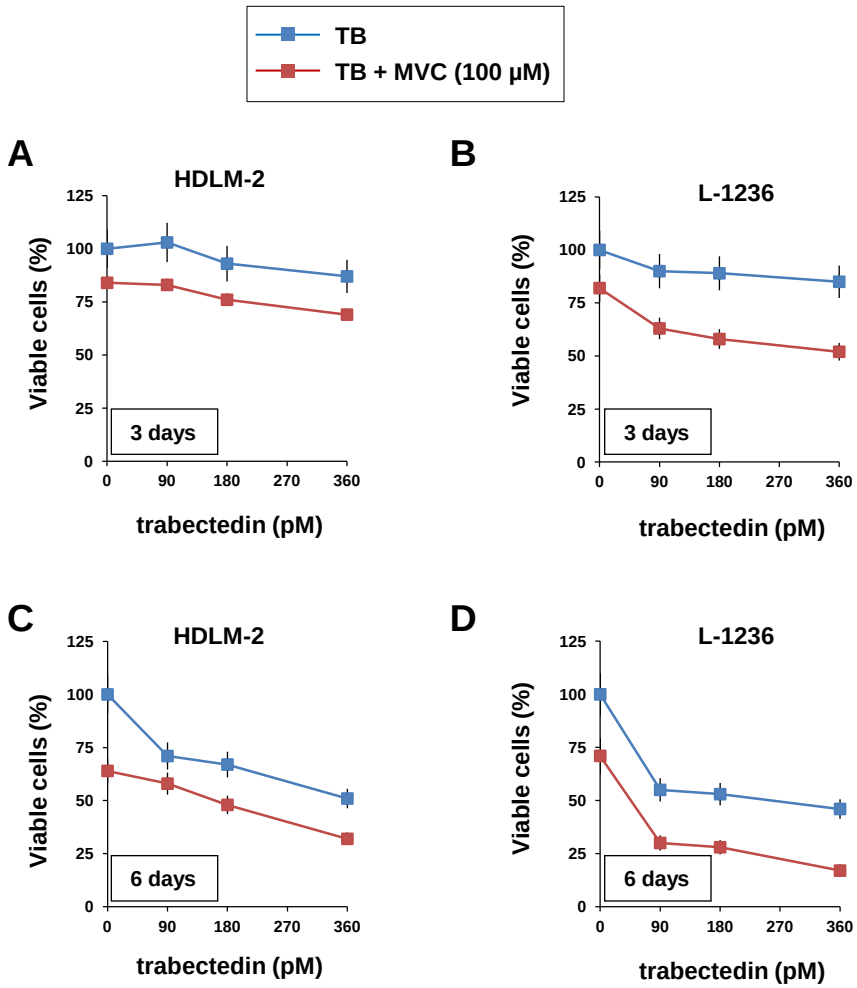
Supplemental Figure S1. CCR5 expression and CCL5 secretion by HRS cells. (A) CCR5 expression was analyzed using the monoclonal mouse anti-CCR5 mAb (clone 45531; R&D Systems) followed by the FITC-conjugated goat anti-mouse IgG (Jackson Immuno Research). Assay results were detected by flow cytometry on a BD FACSCanto II flow cytometer. Data were analyzed using BD FACSDiva v.8.0.1 software (BD Biosciences, Milano, Italy). **(B)** Percentage of CCR5 positive cells (flow cytometry) **(C)** cHL cells were seeded at 2.0 × 10^5/ml in RPMI-1640 plus 10% FCS, and medium collected after 72 h. CCL5 was quantified using commercially available ELISA kit (Immunological Sciences). Three biological replicates tested in duplicate (n=6) and results are expressed as mean and SD.

Supplemental Figure S2



Supplemental Figure S2. Maraviroc enhanced trabectedin cytotoxicity.
HRS cells were treated with maraviroc, trabectedin, or their combination. **(A)** Phase contrast microscopy showing the cytotoxic effects of the drugs after 24h treatment. **(B)** Representative cytofluorimetric dot blots of the cells double stained with Annexin-V-FITC (Thermo Fisher Scientific) and 7AAD (BD Pharmingen), and analyzed by flow cytometry after 48h treatment.
MVC, maraviroc; TB, trabectedin.

Supplemental Figure S3



Supplemental Figure S3. Maraviroc alone and in combination with trabectedin decreased cell viability of 3D heterospheroids formed by HRS cells and cHL-MSCs. (A, C) HDLM-2 and **(B, D)** L-1236 were cultured under non-adherent conditions with cHL-MSCs to form heterospheroids (24h). Then cells were cultured with trabectedin (0-360 pM), maraviroc (100 µM) alone or in combination. After 3 and 6 days, cell viability was evaluated with Presto Blue assay. Values are mean and SD of three experiments. MVC, maraviroc; TB, trabectedin.