

Supplementary Materials:

Detailed Characterization of Immune Cell Infiltrate and Expression of Immune Checkpoint Molecules PD-L1/CTLA-4 and MMR Proteins in Testicular Germ Cell Tumors Disclose Novel Disease Biomarkers

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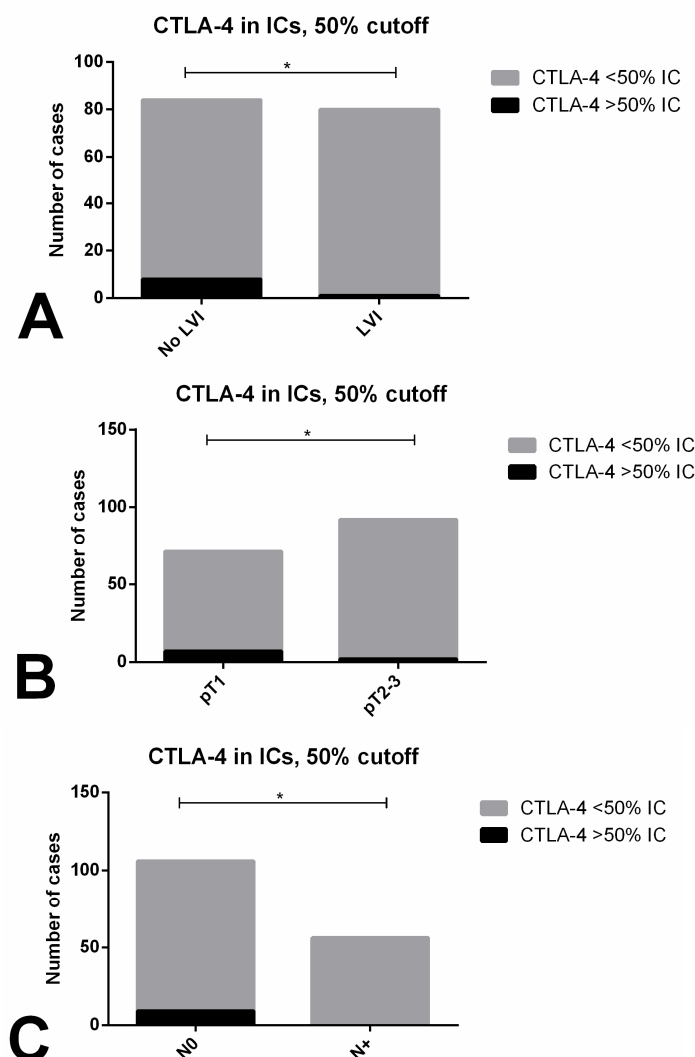


Figure S1. Immunoexpression of CTLA-4 in immune cells of testicular germ cell tumors and association with prognostic features. Association between CTLA-4 immunoexpression above the 50% cutoff in immune cells and lymphovascular invasion (**A**), pT stage (**B**) and N stage (**C**). Abbreviations: LVI—lymphovascular invasion; IC—immune cells. * refers to $p < 0.05$.

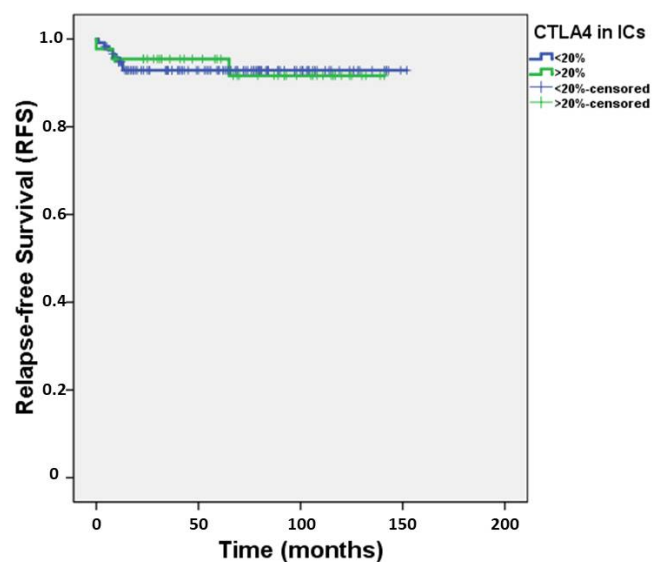


Figure S2. Relapse-free survival according to CTLA-4 immunoexpression in immune cells of testicular germ cell tumors. Abbreviations: IC—immune cells; RFS—relapse-free survival.

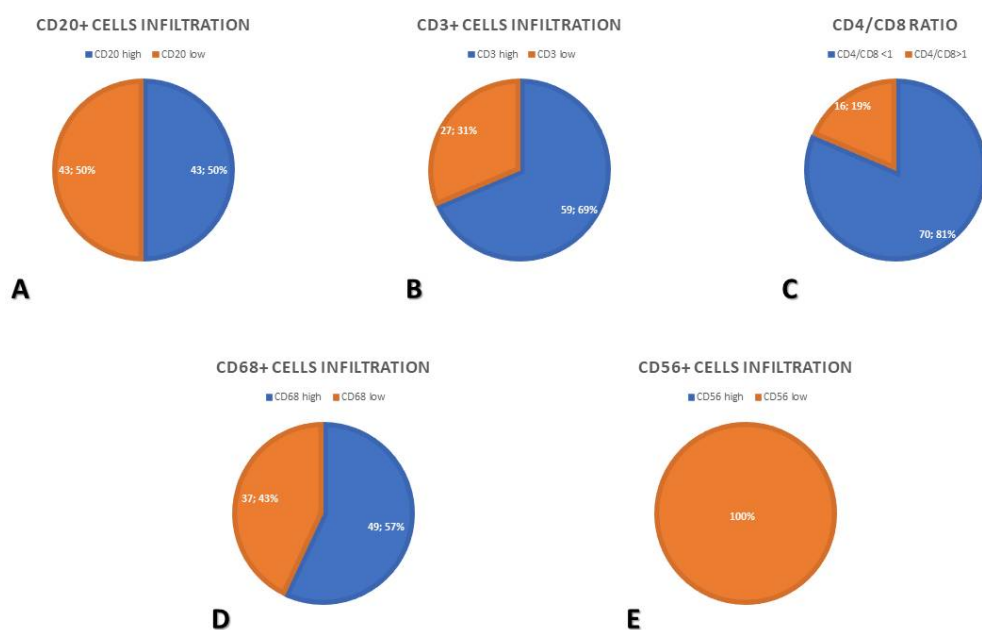


Figure S3. Characterization of the immune infiltrate populating pure seminomas. Categorization of tumors according to the amount of CD20-positive B-lymphocytes (A), CD3-positive T-lymphocytes (B), the CD4/CD8 ratio (C), CD68-positive macrophages (D) and CD56-positive natural killer cells (E).

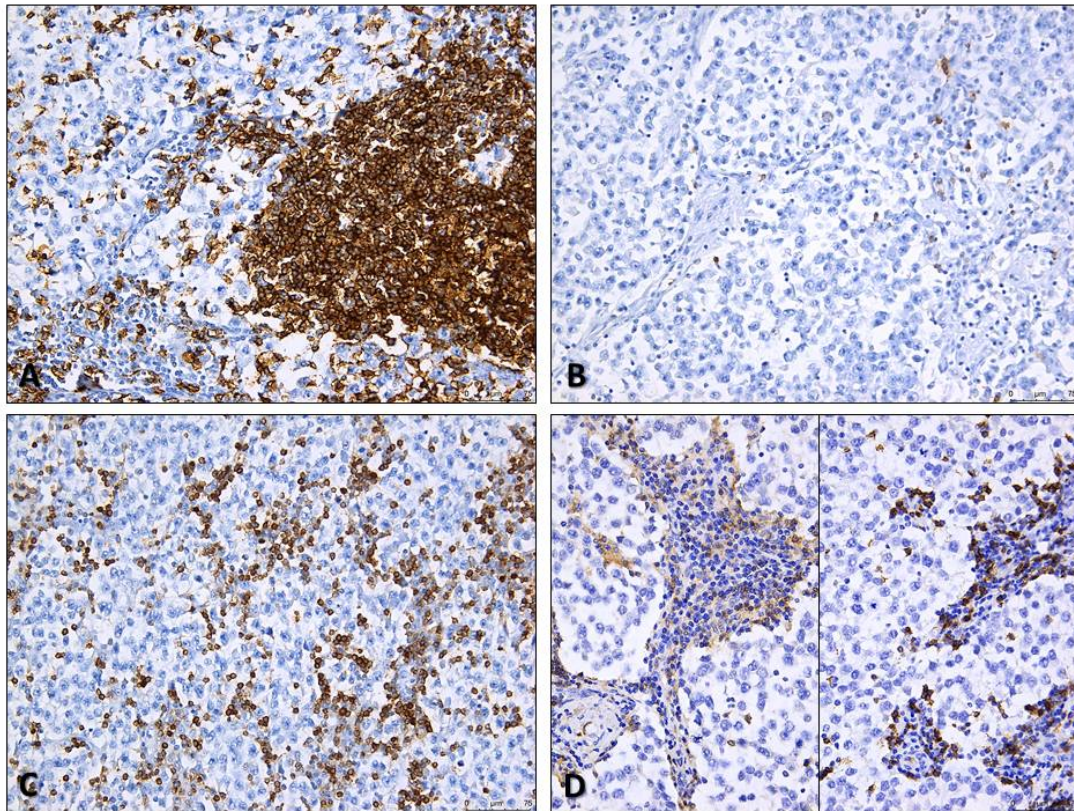


Figure S4. Illustrative examples of characteristics of the immune infiltrate in pure seminomas. **A**—Pure seminoma with abundant CD20-positive B-lymphocyte infiltration, both peripheral and intratumoral, forming follicles across the whole representative slide (200×) and **B**—Pure seminoma with very few CD20-positive B-cells within the fibrous septa across the whole slide (200×); **C**—Pure seminoma showing a high infiltration with CD3-positive T-lymphocytes (200×); **D**—Pure seminoma showing presence of both CD4-positive (left) and CD8-positive (right) T-lymphocytes (200×).

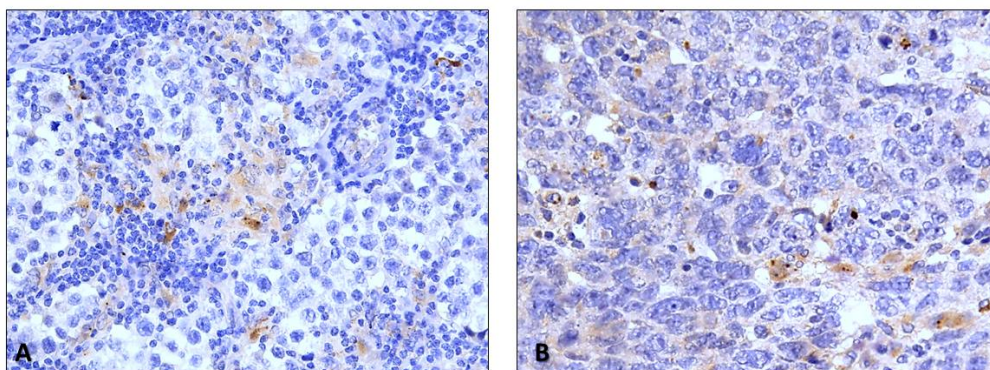


Figure S5. Absent and weak immunoexpression of CTLA-4 in tumor cells. **A**—Pure seminoma showing absence of specific staining with CTLA-4 in the tumor cells; **B**—Pure embryonal carcinoma showing very faint cytoplasmic staining for CTLA-4 in tumor cells, that was only depicted in high power (400×).

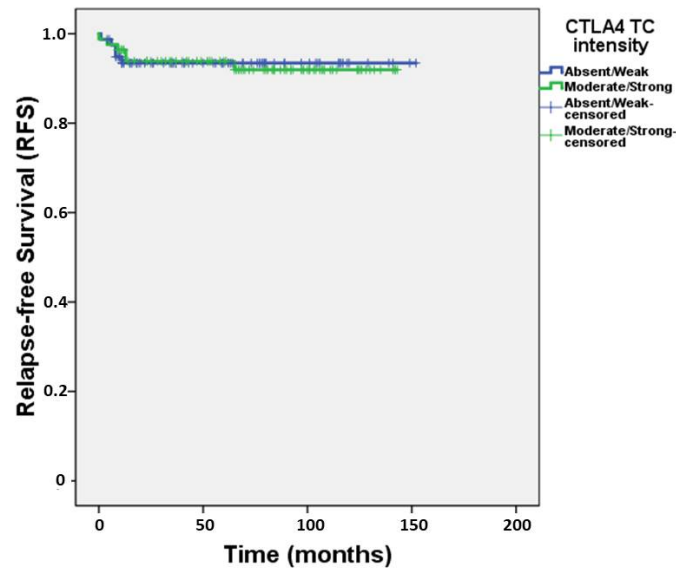


Figure S6. Relapse-free survival according to CTLA-4 immunoreactivity intensity in tumor cells of testicular germ cell tumors.

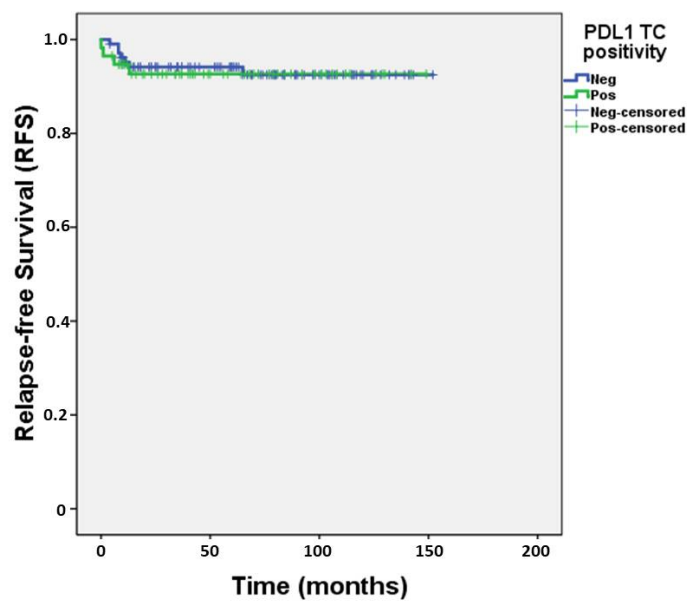
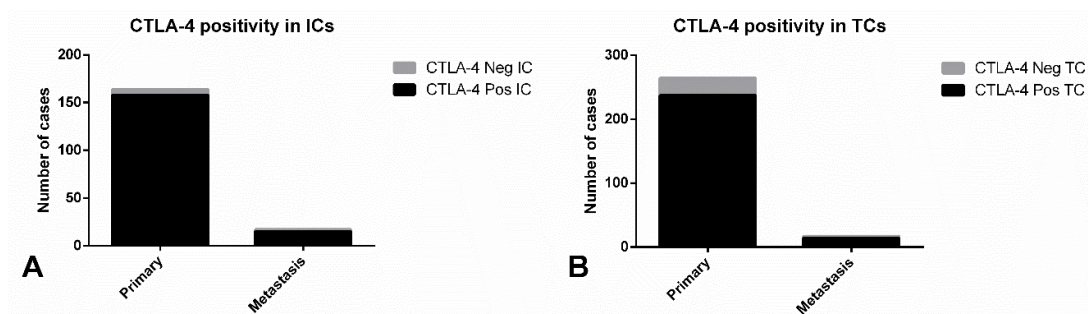


Figure S7. Relapse-free survival according to PD-L1 immunoreactivity in tumor cells of testicular germ cell tumors.



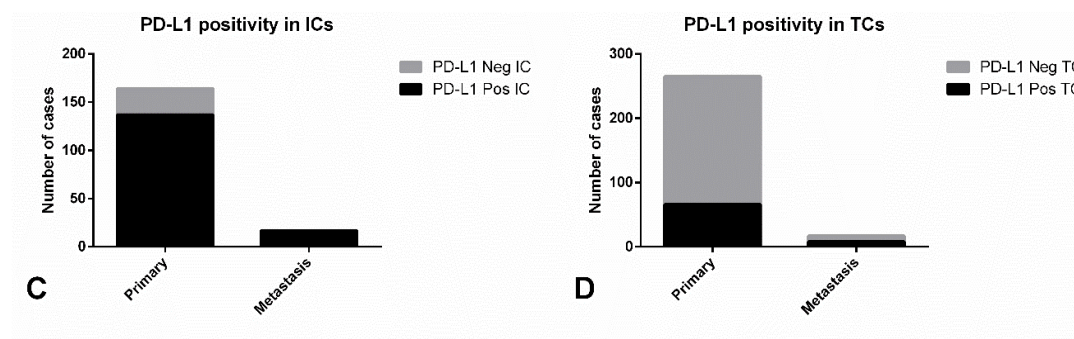


Figure S8. Immunoexpression of PD-L1 and CTLA-4 among primary and metastatic testicular germ cell tumor samples, **A** and **C**—Positivity in immune cells among primary and metastatic tumors; **B** and **D**—Positivity in tumor cells among primary and metastatic tumors.

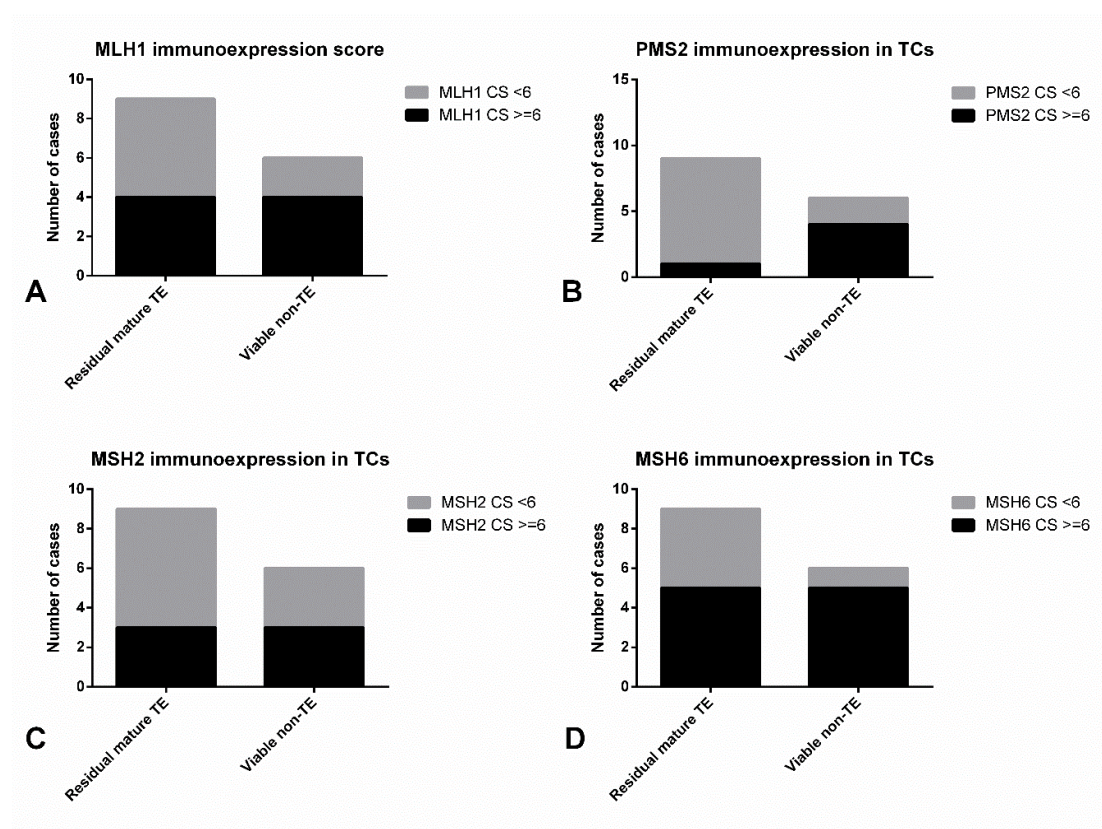


Figure 9. Immunoexpression of mismatch repair proteins in metastatic testicular germ cell tumor samples. Combined score for MLH1 (**A**), PMS2 (**B**), MSH2 (**C**) and MSH6 (**D**) among residual mature teratoma and non-teratoma viable disease.

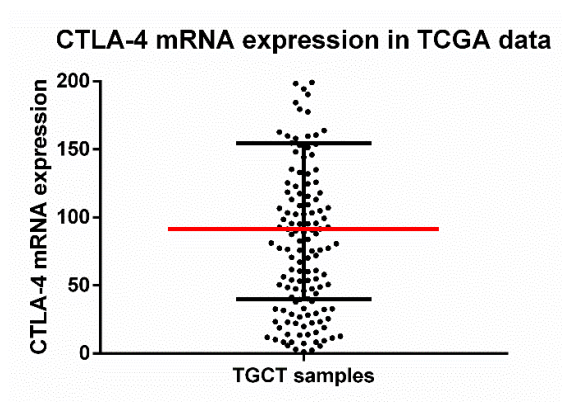


Figure S10. mRNA expression of CTLA-4 in the TCGA database for Testicular Germ Cell Tumors. TGCT—testicular germ cell tumors.

Tables S1–S4 are in a separately file



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