Supplementary Information 2

Demographic Patterns and Immunophenotypic Characterization of Tumor–Immune Interactions in Canine Cutaneous Mast Cell umors

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Supplementary Figures

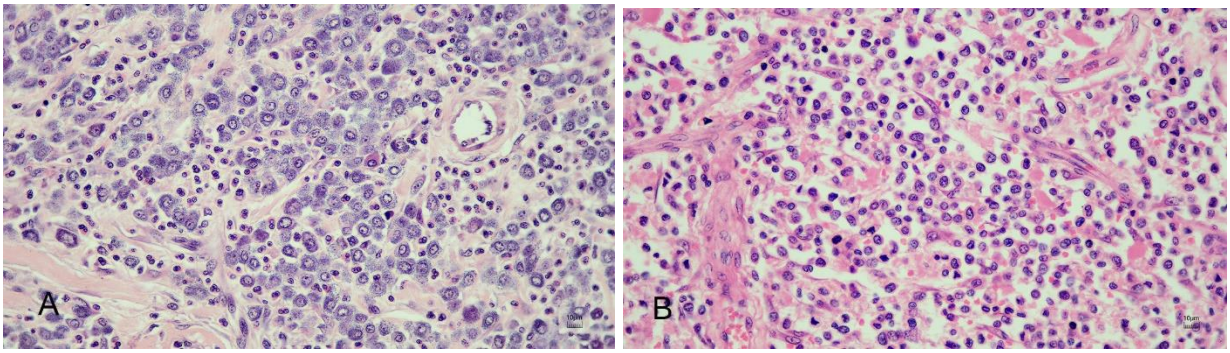

Figure S1. Representative histological features of canine cutaneous mast cell tumors (MCTs) stained with hematoxylin and eosin (H&E) at 40× magnification. (A) Low-grade MCT showing well-differentiated mast cells with round nuclei, mild anisokaryosis, and sparse mitotic figures within a moderately fibrotic stroma.(B) High-grade MCT demonstrating pleomorphic, poorly differentiated mast cells with marked anisokaryosis, increased mitotic activity, and multifocal necrosis. Scale bar = 10 μm.

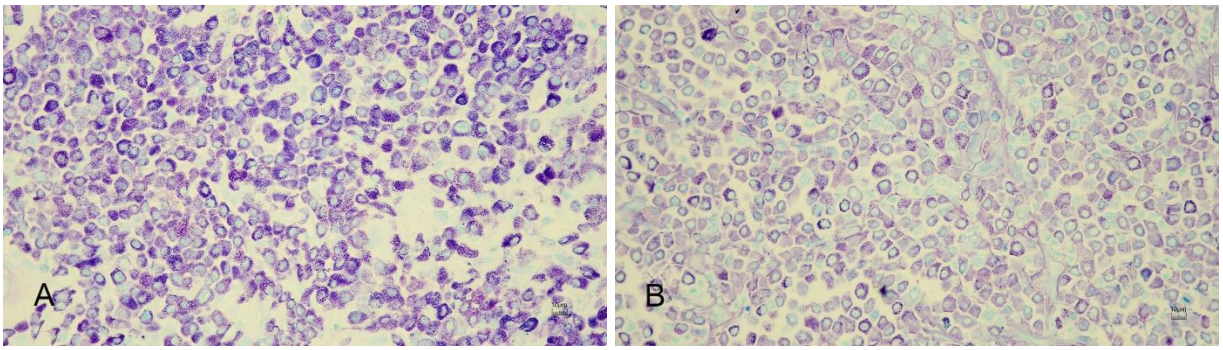

Figure S2. Toluidine blue staining of canine cutaneous mast cell tumors (MCTs) at 40× magnification. (A) Low-grade MCT displaying abundant, well-granulated mast cells with metachromatic cytoplasmic granules and mild nuclear atypia. (B) High-grade MCT characterized by fewer granulated mast cells, increased nuclear pleomorphism, and diminished metachromasia. Scale bar = 10 μm.

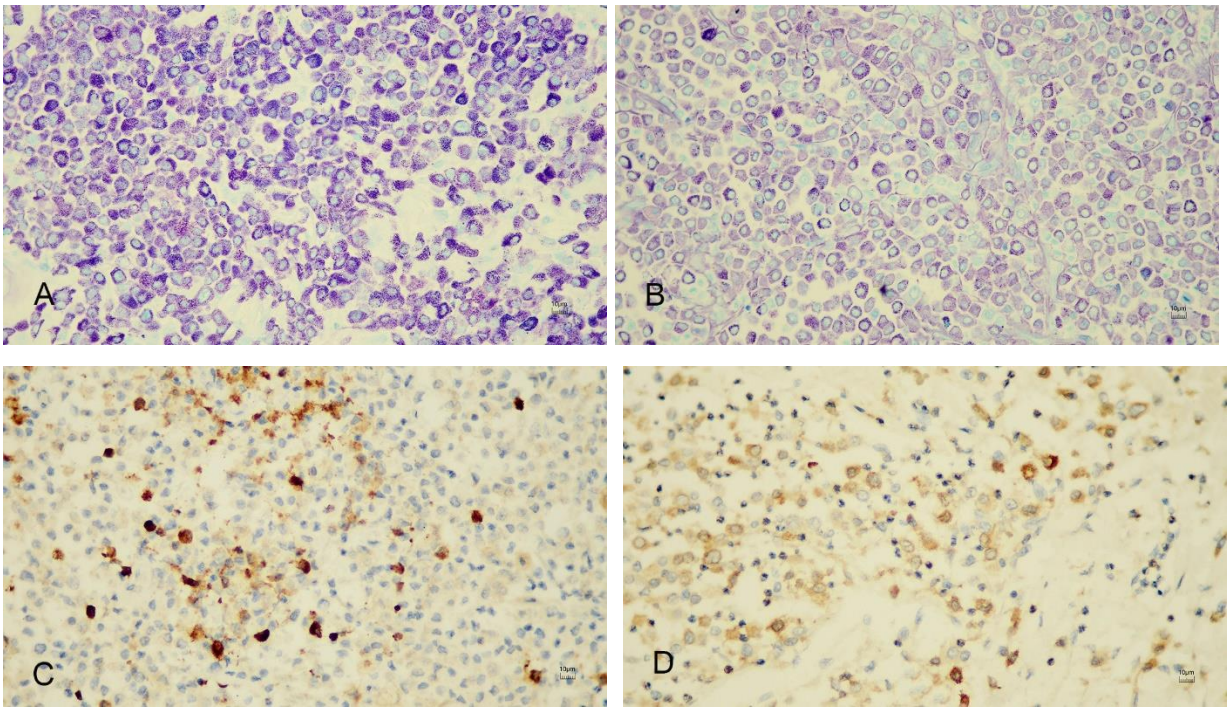

Figure S3. Histochemical and immunohistochemical identification of mast cells in canine cutaneous mast cell tumors (MCTs) at 40× magnification. (A) Low-grade MCT stained with toluidine blue, showing numerous well-differentiated mast cells containing dense metachromatic granules. (B) High-grade MCT with reduced numbers of granulated mast cells and diminished metachromasia. (C) Low-grade MCT demonstrating strong cytoplasmic immunoreactivity for mast cell tryptase, highlighting abundant mature mast cells. (D) High-grade MCT exhibiting weaker and more heterogeneous tryptase staining, consistent with reduced mast cell differentiation. Scale bar = 10 µm.

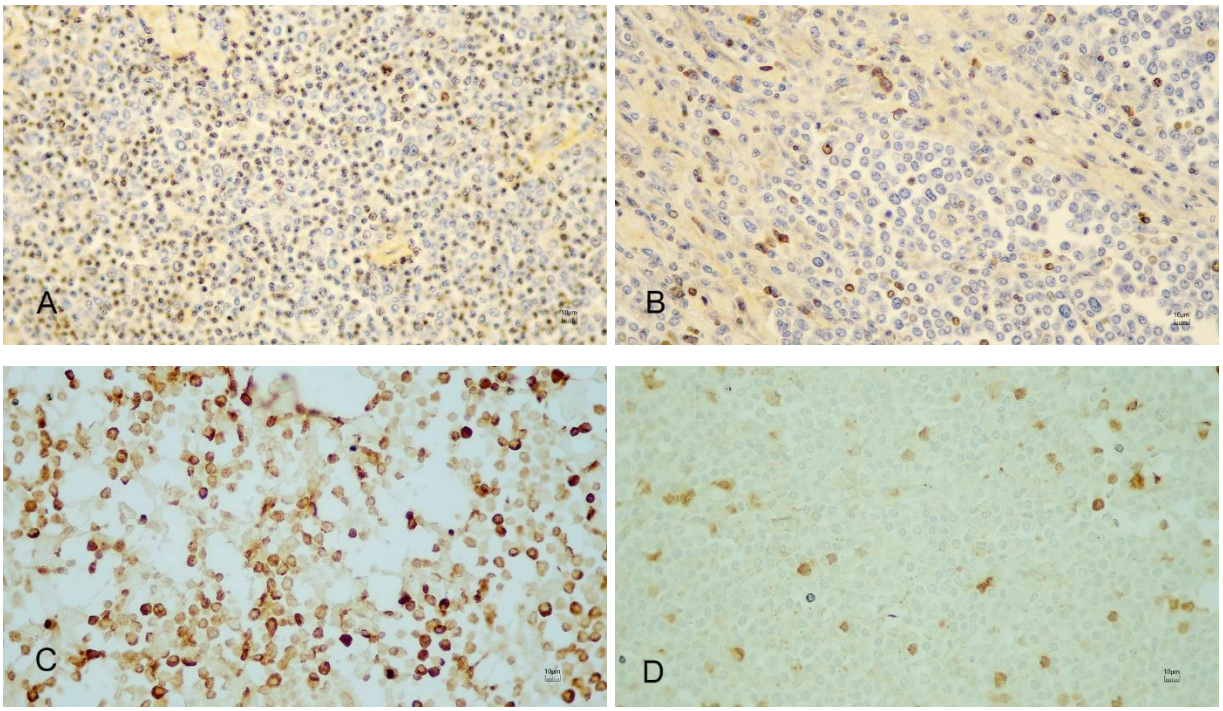

Figure S4. Immunohistochemical expression of MHC class I and class II molecules in canine cutaneous mast cell tumors (MCTs), 40× magnification. (A) Low-grade MCT exhibiting strong MHC I immunoreactivity in numerous neoplastic cells. (B) High-grade MCT showing reduced and patchy MHC I staining. (C) Low-grade MCT with diffuse and intense MHC II expression, consistent with enhanced antigen-presenting activity. (D) High-grade MCT demonstrating weak or heterogeneous MHC II staining in the tumor microenvironment. Scale bar = 10 μm.

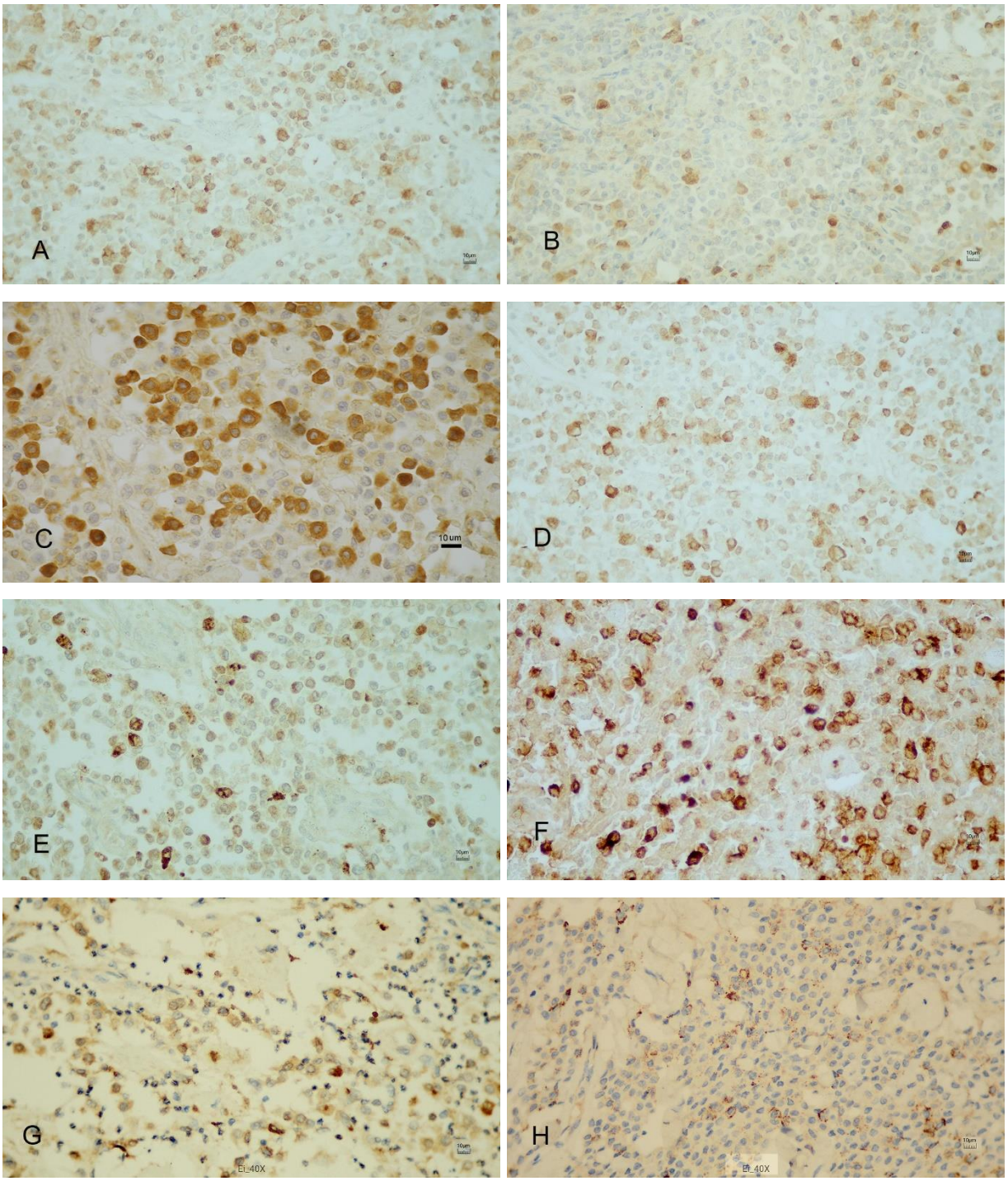

Figure S5. Immunohistochemical detection of tumor-infiltrating lymphocyte (TIL) subsets in canine cutaneous mast cell tumors (MCTs), 40× magnification. (A) Low-grade MCT showing dense infiltration of $CD4^+$ T-helper cells with strong membranous staining. (B) High-grade MCT

with markedly reduced $CD4^+$ cell density and weaker staining intensity. (C) Low-grade MCT exhibiting moderate infiltration of $CD8^+$ cytotoxic T lymphocytes (CTLs). (D) High-grade MCT with sparse $CD8^+$ T-cell presence and reduced immunoreactivity. (E) Low-grade MCT with abundant $CD25^+$ regulatory T cells displaying intense membrane and cytoplasmic staining. (F) High-grade MCT showing diminished CD25 expression with scattered weakly positive cells. (G) Low-grade MCT with rare $CD57^+$ lymphocytes. (H) High-grade MCT demonstrating increased but heterogeneously distributed $CD57^+$ cells, suggestive of activated or senescent T/NK subsets.Scale bar = 10 μm.

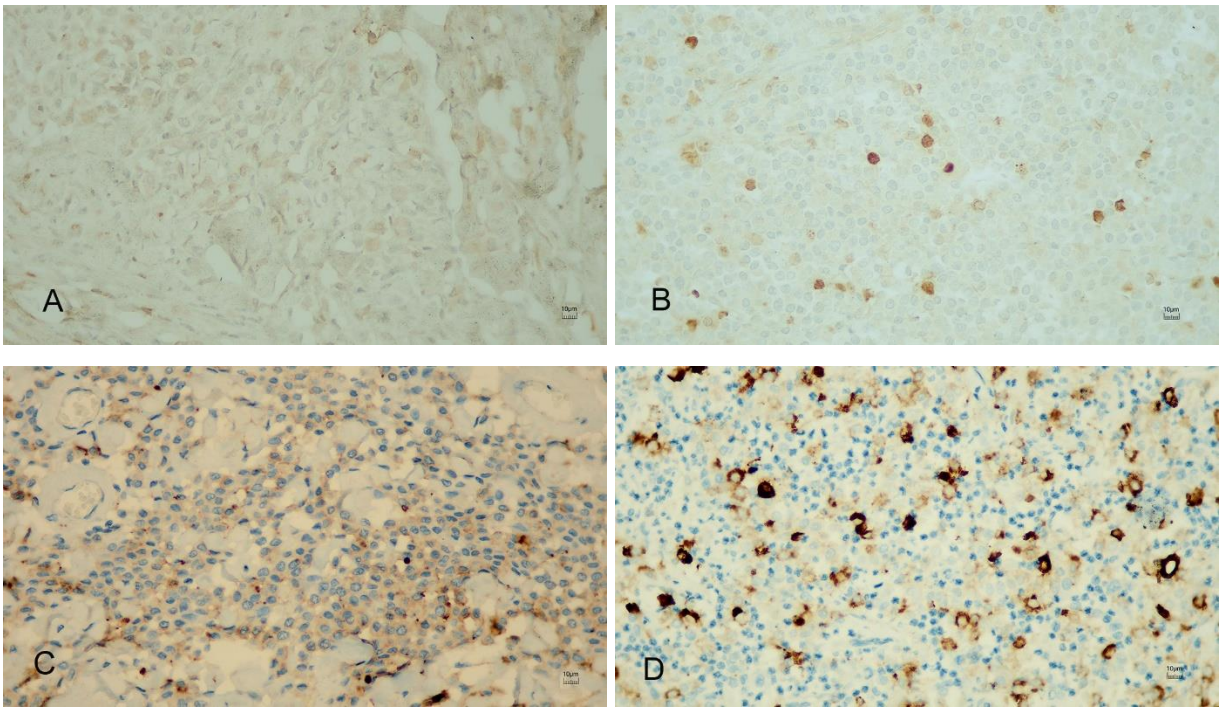

Figure S6. Immunohistochemical expression of immune checkpoint molecules PD-1 and PD-L1 in canine cutaneous mast cell tumors (MCTs), 40× magnification. (A) Low-grade MCT with focal PD-1 expression on scattered cells within the tumor microenvironment. (B) High-grade MCT exhibiting increased and stronger PD-1 immunoreactivity. (C) Low-grade MCT demonstrating mild PD-L1 expression in neoplastic cells. (D) High-grade MCT showing increased and stronger PD-L1 staining in tumor cells. Scale bar = 10 μm.