

Figures 1-4 Validation of CD24 and CD44 antibodies and double immunostaining CD44/CD24, canine tonsils

Figure 1. CD24 staining of lymph follicles and epithelium (x10) **Figure 2.** CD24 staining of a lymph follicle (x20) **Figure 3.** CD44 staining on basal layer of the epithelium (x20) **Figure 4.** Double immunostaining of CD24/CD44 on the epithelium (x20)

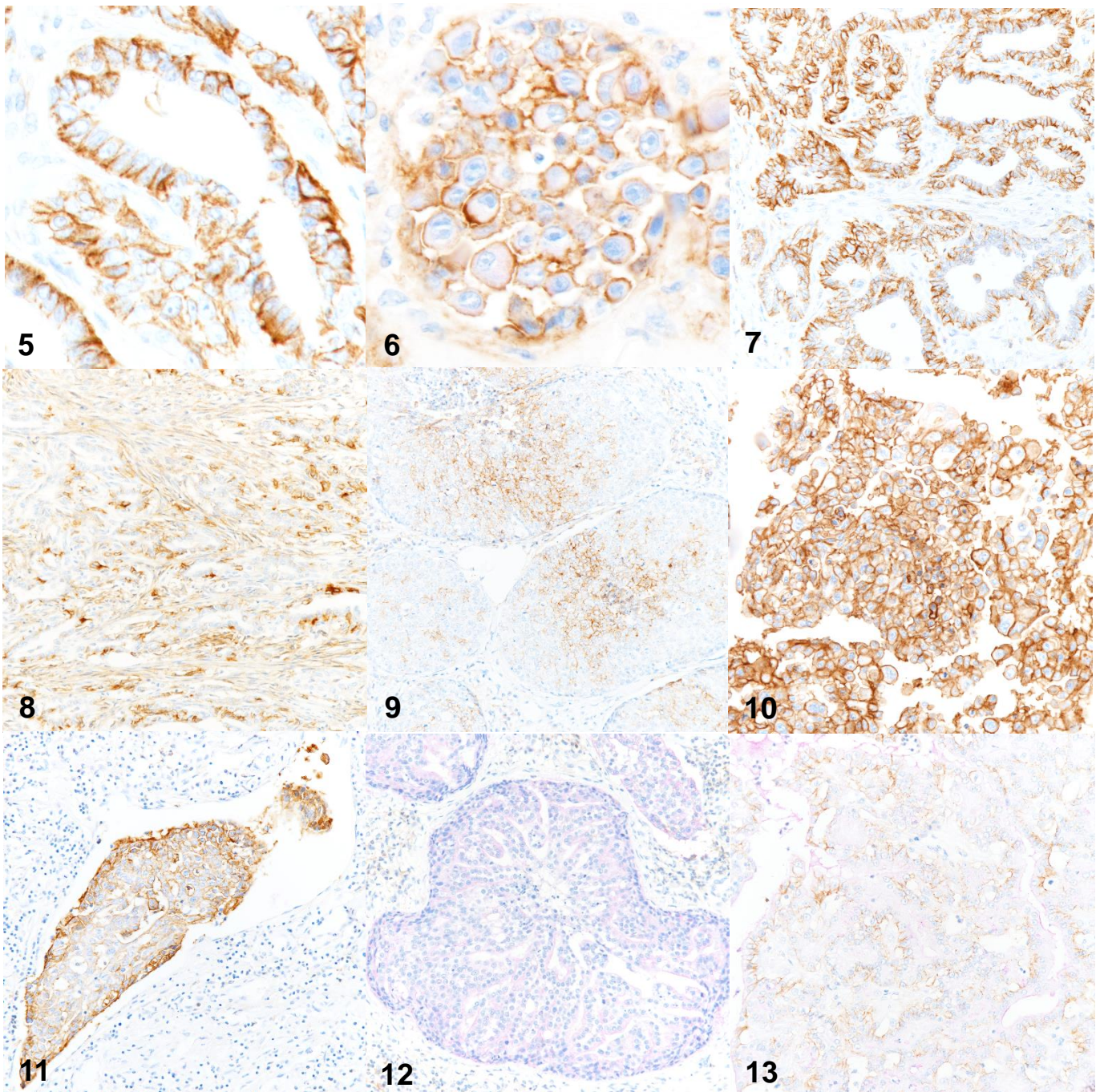
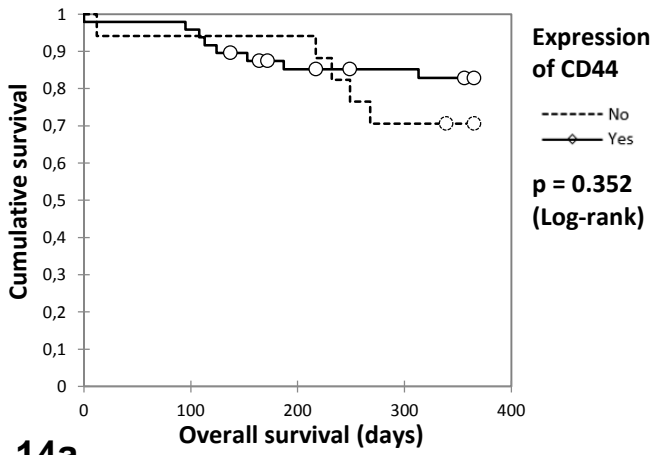
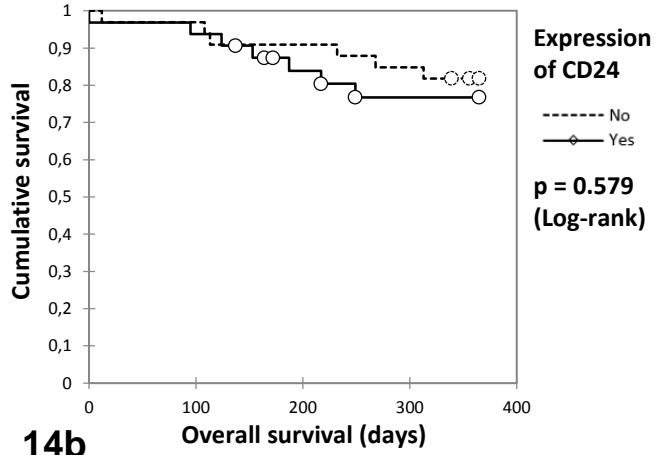


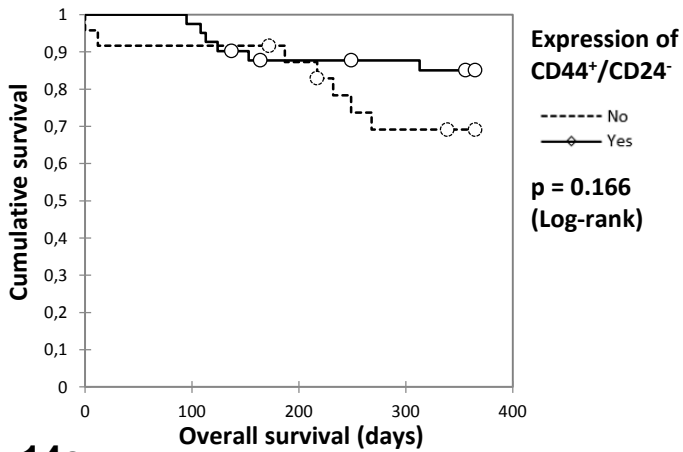
Figure 5 Luminal cells with a CD44⁺/CD24⁻ phenotype. CD44 is localized only on the basolateral domain of the cells (x40) **Figure 6** Anaplastic cells with a CD44⁺/CD24^{low} phenotype (x40). **Figures 7-10** Examples of staining with a CD44⁺/CD24⁻ phenotype in the four major histological types. **Figure 7** Tubular simple carcinoma, luminal component (x20). **Figure 8.** Tubular complex carcinoma, myoepithelial component (x20). **Figure 9.** Solid carcinoma, diffuse component (x20) **Figure 10.** Anaplastic carcinoma, diffuse component (x20). **Figure 11** Embolus in an anaplastic tumor stained with a CD44⁺/CD24⁻ phenotype (x20). **Figure 12** Grade 3 solid carcinoma stained with a CD24⁺/CD44⁻ phenotype (x20). **Figure 13** Grade 2 solid carcinoma stained with a CD44⁺/CD24⁺ phenotype (x20).



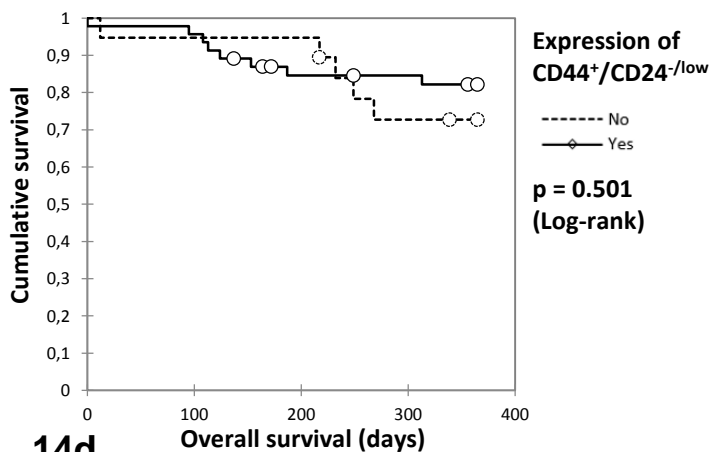
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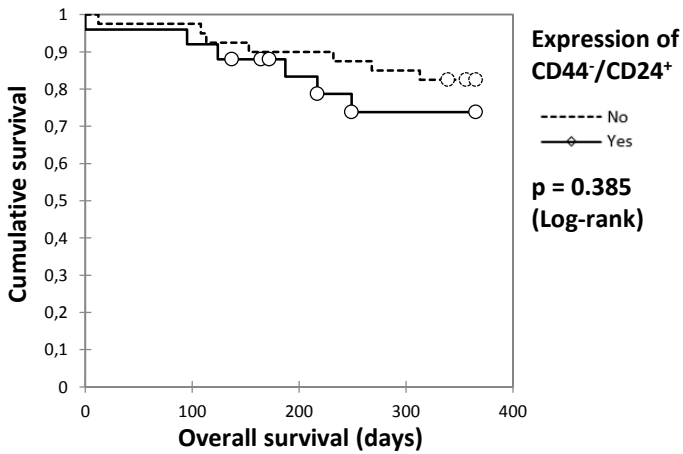
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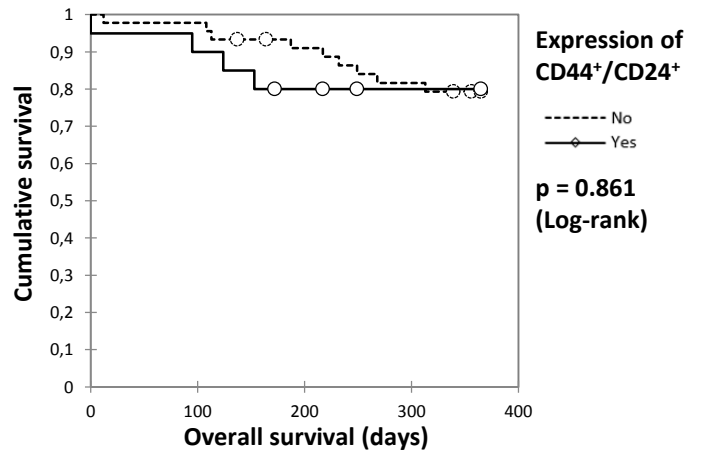
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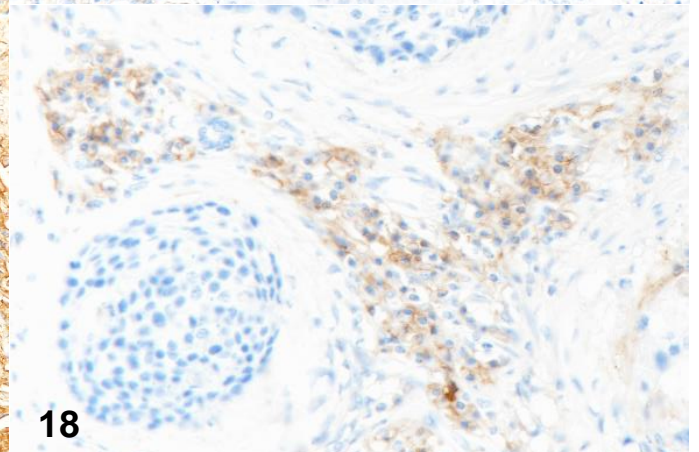
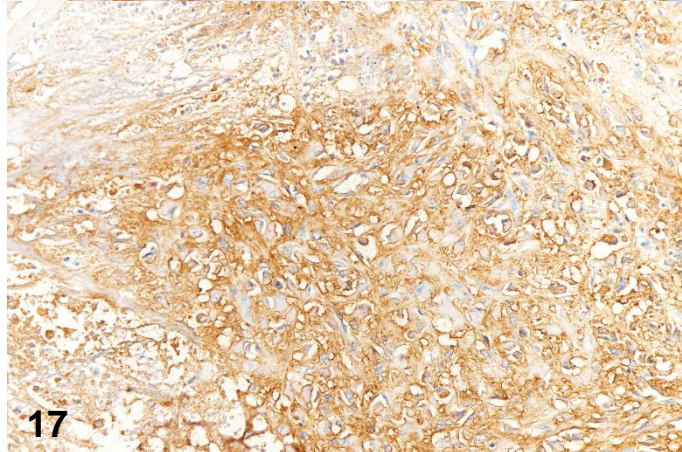
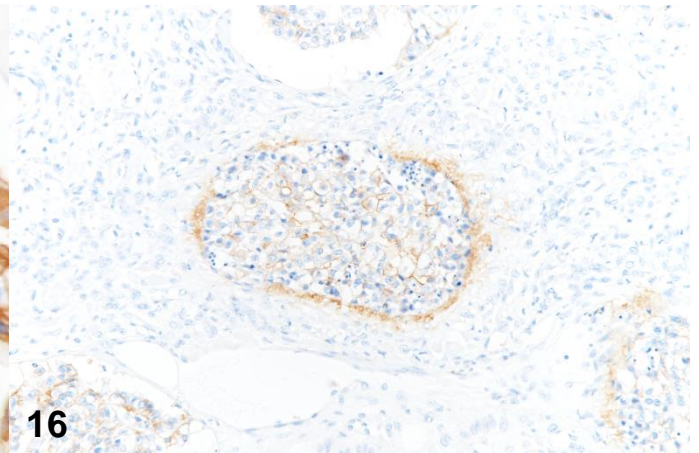
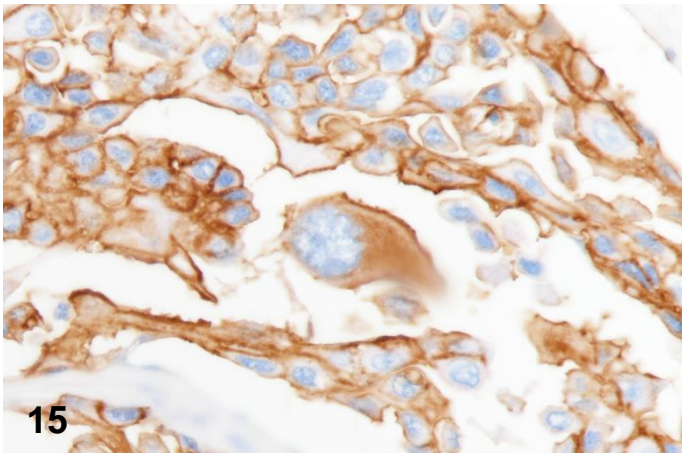
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Figure 14 Kaplan-Meier overall survival curves of group with expression or absence of (a) CD44 (b) CD24, (c) CD44⁺/CD24⁻, (d) CD44⁺/CD24^{-/low}, (e) CD44⁻/CD24⁺, (f) CD44⁺/CD24⁺

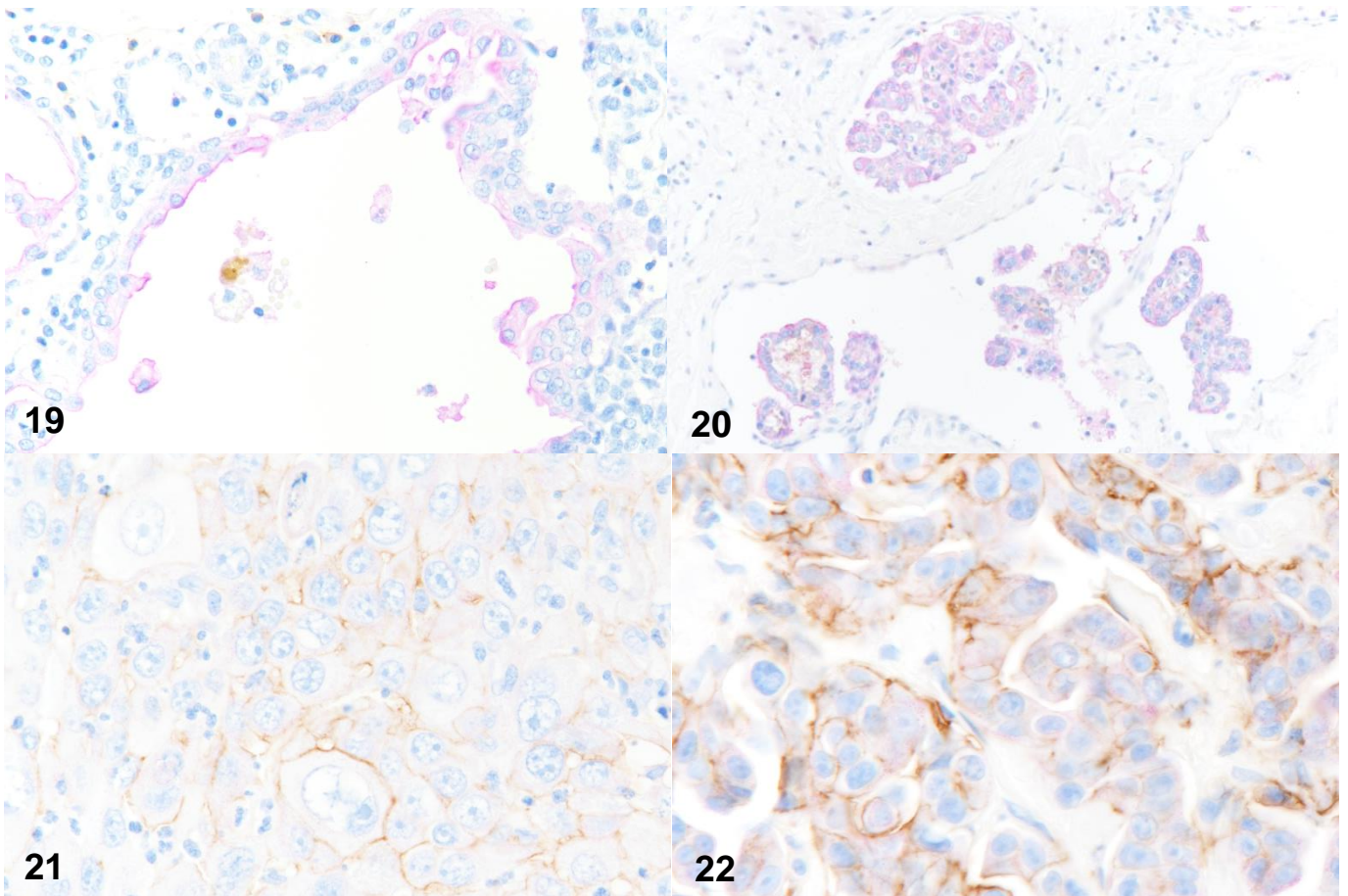
Supplementary data



Figures 15-18. Aspecific stainings of CD44

Figure 15. Aspecific CD44 cytoplasmic staining of a neoplastic cell affected with major karyomegaly. (x40) **Figure 16.** Aspecific staining of CD44 forming a ring around the infiltrated tumor cells in an anaplastic carcinoma. (x20) The phenomenon is most likely linked to necrosis. **Figure 17.** Massive aspecific CD44 staining affecting the stroma of an anaplastic carcinoma highly infiltrated with neoplastic cells and most likely associated to necrosis (x20). **Figure 18.** Aspecific staining of plasmacytes on a mammary carcinoma (x20).

Supplementary data



Figures 19 – 22 Aspecific stainings and observations on CD24

Figure 19. Aspecific staining of CD24 on the apical membrane of luminal tumor cells (x20)

Figure 20 Emboli showing an intense CD24 staining. On some of them, a CD24 apical layer is visible (x20). **Figure 21** CD44⁺CD24⁺ staining in cells with massive karyomegaly and increase in cell size (x40). **Figure 22** CD44⁺CD24⁺ staining in cells presenting no major changes in nuclei (x40)