Supplementary data

Supplementary Figure 1. Renné et al.

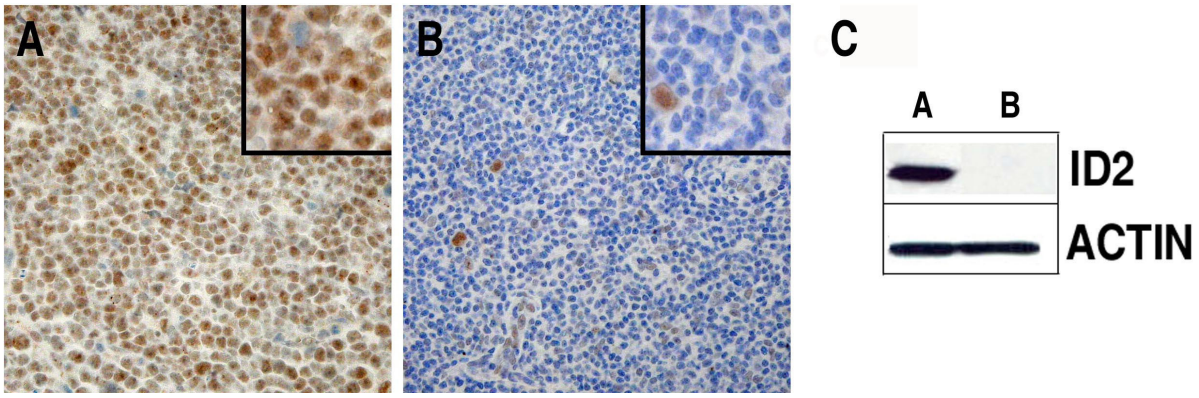


Figure 1. Specificity of the anti-ID2 antibody in immunohistochemistry with formalin-fixed paraffin-embedded tissues.

To demonstrate the specificity of the anti-ID2 antibody used for immunohistochemistry (IHC) and immunofluorescence (IF) in this study, we used a diffuse large B cell lymphoma (DLBCL) showing strong ID2 positivity and a DLBCL with only very few ID2 positive cells in IHC with sections from formalin-fixed paraffin-embedded samples of the lymphomas. From frozen samples of both cases several 10 μm sections were lysed in Laemmli buffer and used for Western blot analysis with the same anti-ID2 antibody. **A** and **B**: Staining of DLBCL with strong (A) and no (B) ID2 expression are shown. Presence of few ID2 positive cells in B confirmed the successful staining of also this case. The inserts show higher maginifications for both cases. **C**: Western blot of the cases shown in A and B with the anti-ID2 antibody. A 15 kD ID2-specific band was only observed for case A showing strong positivity in the IHC. Loading of equal amounts of lysats was controlled with an anti-actin antibody.

Supplementary Figure 2. Renné et al.

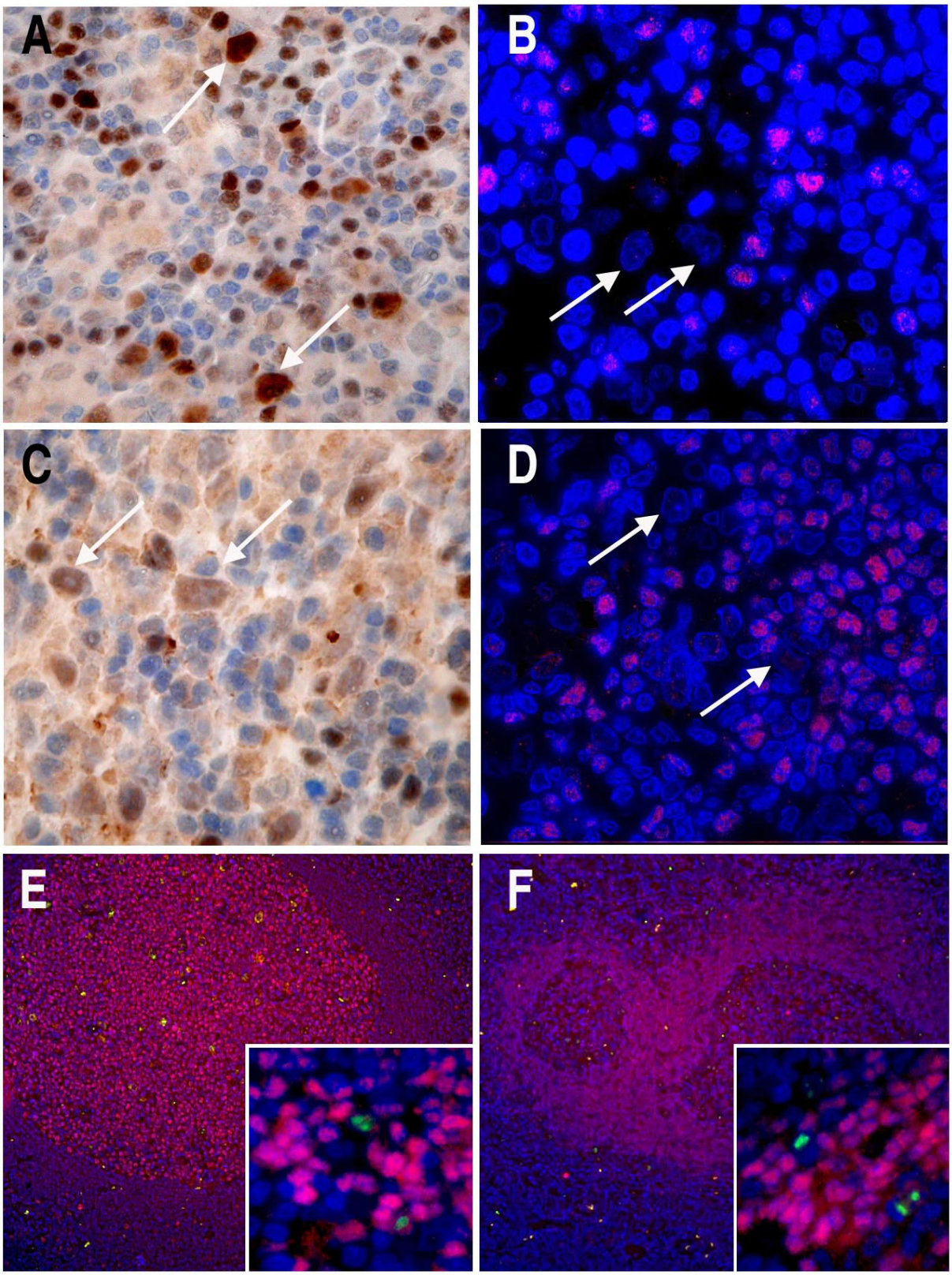
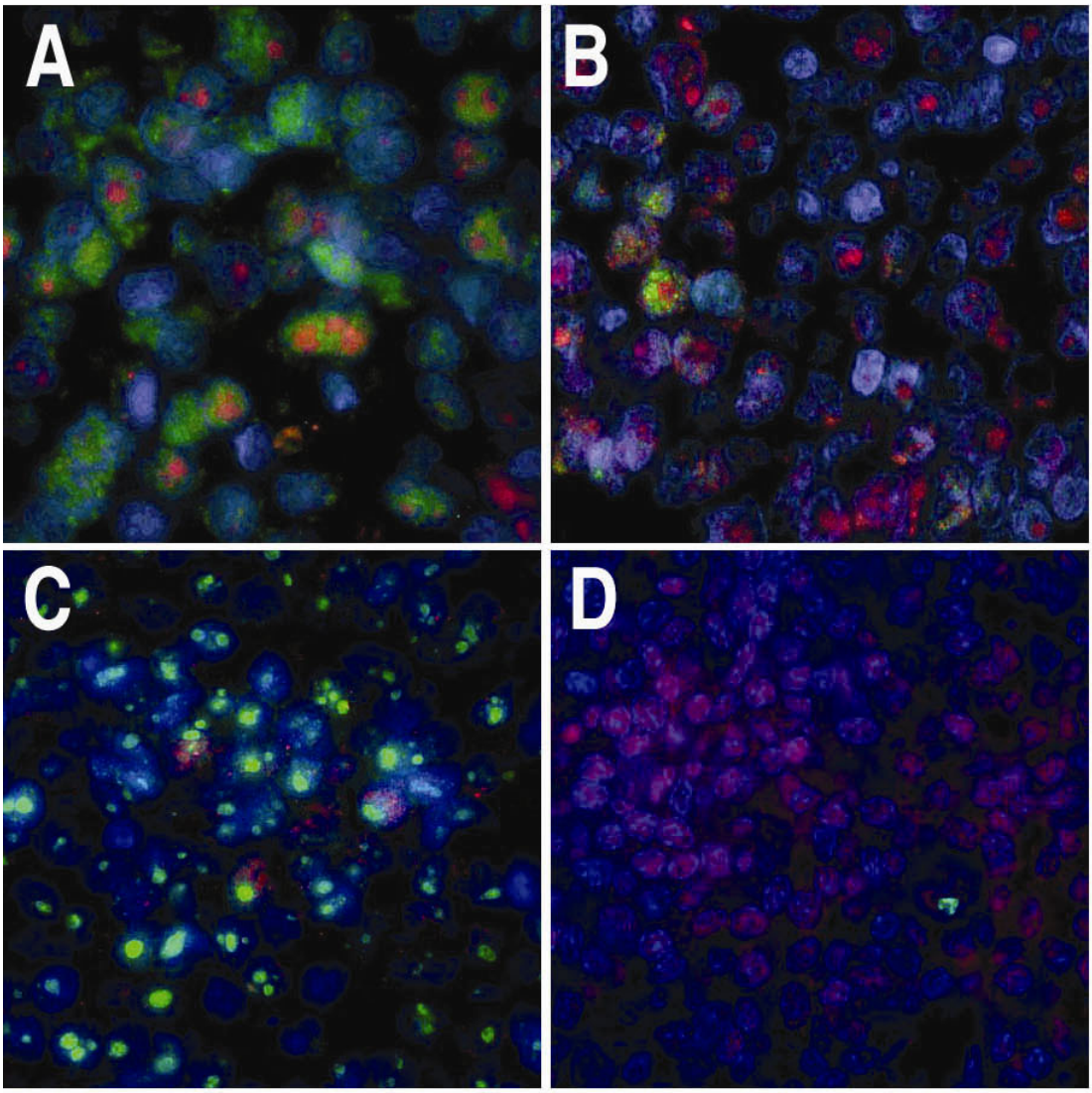


Figure 2. Differences in the sensitivity of IHC and IF stainings for E2A and PAX5.

For each of two cases of classical Hodgkin lymphoma (HL) two sections were stained with the same concentrations of E2A (1:200 dilution) or PAX5 (1:50) and bound primary antibody was either visualised by IHC with horseradish-peroxidase and 3.3-diaminobenzidine as substrate or with a 1:100 dilution of Alexa 594 Fluor chicken-anti-rabbit (E2A) or Alexa Fluor 594 chicken-anti-goat (PAX5) antibody. **A** and **B**: E2A visualised with IHC (A) or IF (B) in the same classical HL case. **C** and **D**: PAX5 visualised with IHC (C) or IF (D) in the same case of classical HL. With both antibodies HRS cells (marked by arrows) were stained in IHC but were negative in IF while small bystander cells were still positive for E2A and PAX5 in the IF stainings. **E** and **F**: IF double stainings for ID2/E2A (E) and ID2/PAX5 (F) with a tonsil showed the expected staining patterns. ID2 is shown in green, E2A and PAX5 are shown in red. Inserts show higher magnifications.

Supplementary Figure 3. Renné et al.



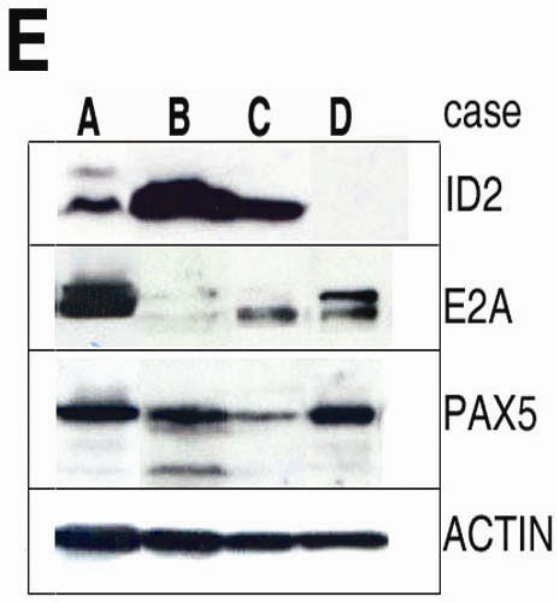


Figure 3. Differences in protein expression recognised by double IF are reflected by differences in protein expression as detected by Western blotting.

To demonstrate that comparisons of protein expression levels between different cases in IF double stainings are reliable and can also be recognised in Western blot analysis, we used four DLBCL cases with different expression of ID2, E2A and PAX5 in IF double stainings with formalin-fixed paraffin-embedded material. From all four cases also frozen material was available for lysate preparation for Western blot analysis. **A** and **B**: ID2/E2A double IF for cases 1 and 2. ID2 is shown in red and E2A in green. **C** and **D**: ID2/PAX5 double IF for cases 3 and 4 with ID2 in green and PAX5 in red. **E**: Western blot analysis of cases 1-4 for ID2, E2A, PAX5 and actin to control equal loading of the lanes. For both IF double stainings the Western blot analysis reflected the observed expression differences.