Expression pattern of XBP1(S) in human B-cell lymphomas

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Methods

Immunofluorescent staining was performed on human tonsil sections after heat mediated antigen retrieval. Primary antibodies were mouse anti-XBP1(S) clone 143F, rabbit anti-BLIMP1 (rabbit polyclonal), goat anti-PAX5 polyclonal (Santa Cruz Biotechnology). Secondary antibodies for immunofluorescence were Alexa Fluor 488 donkey anti-mouse, Alexa Fluor 594 donkey anti-rabbit, and biotinylated donkey anti-goat coupled with AMCA-Avidin (Vector Labs, UK) for blue fluorescence. Images were taken with a 63x/1.25 oil (total magnification: 630x) objective using a Zeiss Axio Imager Z1 imaging fluorescence microscope (Carl Zeiss Jena GmbH, Jena, Germany) with a Digital Microscope Camera ProgRes MF (Jenoptik, Jena, Germany). Images were captured and processed using the ISIS3 image capture system (MetaSystems, Altlussheim, Germany). Images were subject to thresholding, with lower thresholds set at the median fluorescence. Composite and single channel images are shown.
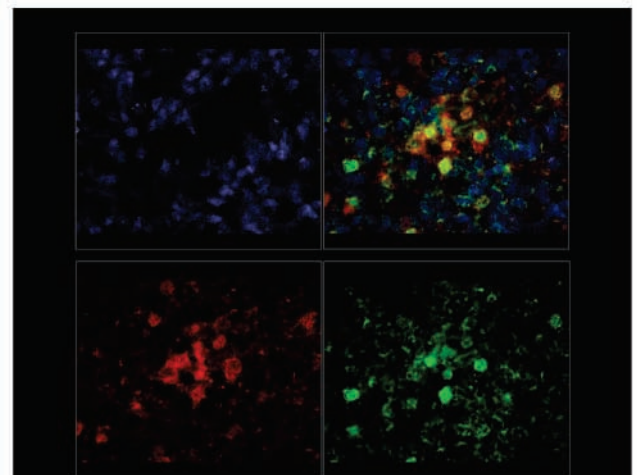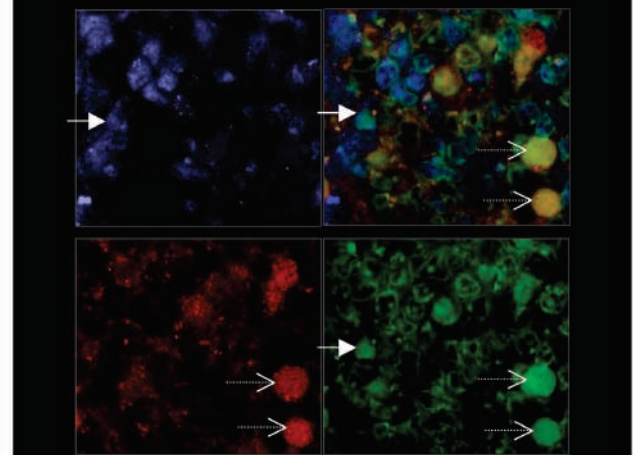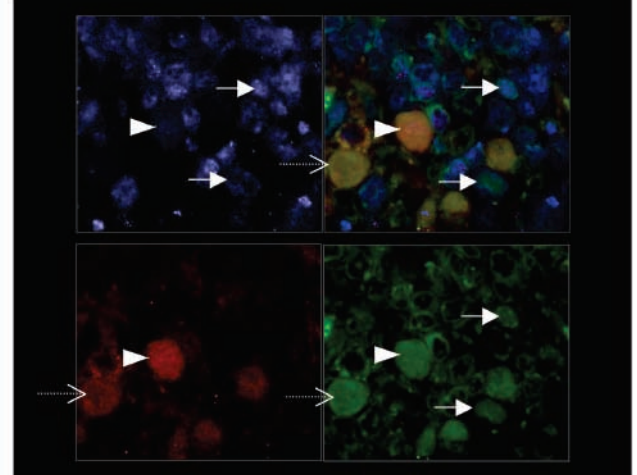

Online Supplementary Figure S1. XBP1(S) expression is observed in BLIMP1 and PAX5 expressing cells. Three representative fields are shown from reactive tonsil tissue. In each case composite and single channel images are shown. PAX5 in blue, XBP1(S) in green, BLIMP1 in red, and composite image top right. (A) Shows image as in Figure 1 E with single channel images. (B) and (C) additional representative fields at higher magnification. In (B) and (C) open arrows identify nuclei co-expressing BLIMP1 and XBP1(S) in the absence PAX5. Arrow heads identify nuclei co-expressing BLIMP1, XBP1(S) and PAX5. Solid arrows identify nuclei co-expressing BLIMP1 and XBP1(S) in the absence of PAX5.