Figure Legends

Figure S1:

Chromogenic staining (DAB, brown) of paraffin-embedded DLBCL cell lines DHL4, and Toledo, untreated and BCR crosslinked, with anti-pLYN (Y396), anti-pSYK (Y323), and anti-pBTK (Y551). Increased phosphorylation upon crosslinking is observed for DHL4. No significant phosphorylation is observed for Toledo in either untreated or crosslinked cells.

Figure S2:

(A) Schematic illustration of Tissuequest analysis workflow. Individual cells are first identified by finding the nuclei (DAPI). In case of cytoplasmic antigens as depicted here for pLYN, Tissuequest provides an algorithm that is growing in stained areas around the nucleus and creates a "cytoplasmic mask" that measures staining intensity within the cytoplasm only. The mean staining intensity within the cytoplasmic mask as exemplified here for pLYNis then plotted against the mean DAPI intensity and displayed as a dotplot in a flow cytometry-like fashion. The cutoffs are set based on negative controls using the forward and backward gating tools of the program that allow to connect individual dots to corresponding cell and *vice versa*. This leads to identification of negative and positive cells.
(B) Identical illustration with actual representative image from cytoplasmic pLYN analysis in DLBCL cell line LY7.

Figure S3:

Qualitative Western blot of whole cell lysates for untreated (BCR-) and crosslinked (BCR+) cell lines illustrated in Figure 1A, probed with anti-pLYN, anti-pSYK, and anti-pBTK. Blotting with anti-LYN, anti-SYK and anti-BTK confirmed protein expression in all cell lines and conditions.

Figure S4:

Chromogenic staining (DAB, brown) of two paraffin-embedded patient DLBCL specimens with anti-pLYN (Y396), anti-pSYK (Y323), and anti-pBTK (Y551). Cases are representative of an unambiguous BCR- (left) and BCR+ (right).

Figure S5:

(A) Schematic illustration of Tissuequest workflow extended to subcellular localization of FOXO1. In this example there are 83% of all cells that are positive for FOXO1 and 67% of the cells show cytoplasmic FOXO1 staining (67%). The ratio of these percentages is used to calculate the F_{cyt} score. (B) Identical illustration with actual representative image from FOXO1 analysis in DLBCL cell line LY7. In this analysis, 93% of cells expressed FOXO1 and 64% exhibited cytoplasmic staining. Note that some cells show exclusively cytoplasmic or nuclear staining while some cells show concomitant cytoplasmic and nuclear expression. Negative cells show only DAPI staining.

Figure S6:

(A) Chromogenic staining (DAB, brown) of paraffin-embedded DLBCL cell lines DHL4, and Toledo, untreated and BCR crosslinked, with anti- FOXO1. (B) Chromogenic staining (DAB, brown) of a representative $BCR^{+}F_{cyt}^{+}$ paraffin-embedded patient DLBCL specimen with anti-pLYN (Y396), anti-pSYK (Y323), anti-pBTK (Y551), and anti-FOXO1.

Figure S7:

(A) IHC staining of paraffin-embedded DLBCL cell lines U2932, DHL6 and Toledo, untreated and crosslinked, with anti-$pAKT_{sub}$ (red) and DAPI (blue). U2932 and DHL6 show significantly increased

AKT activation upon crosslinking, while Toledo shows constitutively high levels of $pAKT_{sub}$. Scatterplots of total intensity per cell for $pAKT_{sub}$, FOXO1 (Texas Red) vs. nuclear intensity (DAPI) from Tissuequest™ analysis of the same cells are shown below. The percent positive cells displayed in the upper right corner of each scatterplot if the average of three independent experiments.

(B) Qualitative Western blot with antibodies used for FOXO1 and AKT shows that steady-state cellular levels are unchanged upon crosslinking.

(C) Histogram of percent positive cells, untreated and crosslinked, determined by qIF for $pAKT_{sub}$, and percent ratio cells positive for FOXO1 using cytoplasmic vs. whole cell mask (F_{cyt}), in the entire DLBCL cell line panel. Cell lines are arranged in the same order as for Figure 2A (average of three independent experiments).

Figure S8: Comparison of *F*-scores for TMA with visual assessments by expert pathologists.

(A) Comparison of immunofluorescent images evaluated by an expert using a 3-tier system (0 – negative, 1 – weak positive, 2 – strong positive).

(B) Assessment by a second expert of a separately stained slide using light microscopy (DAB stain) and a 4-tier scoring system for cytoplasmic FOXO1. Box plot has following parameters: (■) mean, (—) median, box 25-75%, bar 10-90%, (●) min/max.

Figure S9: Comparison of FOXO1 cytoplasmic localization and BCR signaling in primary DLBCL tumors.

Scatterplot of ⟨pSYK,pBTK⟩ vs. pLYN for primary DLBCL patient specimens in a TMA analyzed by qIF for pLYN (Y396), pSYK (Y323) and pBTK (Y551) and CD20. Dashed lines represent criteria for active BCR signaling (⟨pSYK,pBTK⟩>13.5, pLYN>15). Specimens are categorized according to the F_{cyt}^{+}

score: F_{cyt}^{+}>60 (dark green), 60>F_{cyt}^{+}>40 (light green), F_{cyt}^{+}<40 $^{-}$(white). Representative fields of view for six cases stained for FOXO1 (green) and CD20 (red) are shown for selected specimens, Clockwise from top right (i) ⟨pSYK,pBTK⟩$^{+}$pLYN$^{+}F_{cyt}^{+}$ (BCR$^{+}F_{cyt}^{+}$), (ii) ⟨pSYK,pBTK⟩$^{+}$pLYN$^{+}F_{cyt}^{-}$ (BCR$^{+}F_{cyt}^{-}$), (iii) ⟨pSYK,pBTK⟩$^{-}$pLYN$^{+}F_{cyt}^{+}$ (low CD20^{+}), (iv) ⟨pSYK,pBTK⟩$^{-}$pLYN$^{+}F_{cyt}^{+}$ (heterogeneous), (v) ⟨pSYK,pBTK⟩$^{-}$pLYN$^{-}F_{cyt}^{-}$ (BCR$^{-}F_{cyt}^{-}$), (vi) ⟨pSYK,pBTK⟩$^{+}$pLYN$^{-}F_{cyt}^{-}$ (nuclear pSYK).