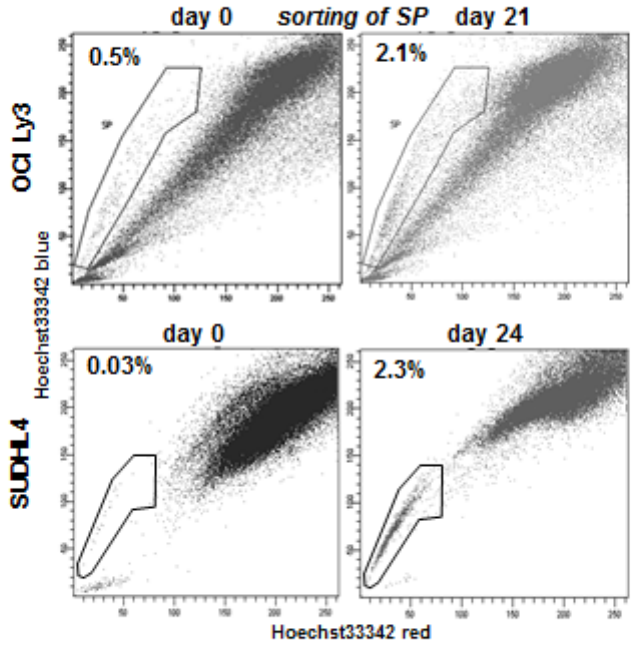
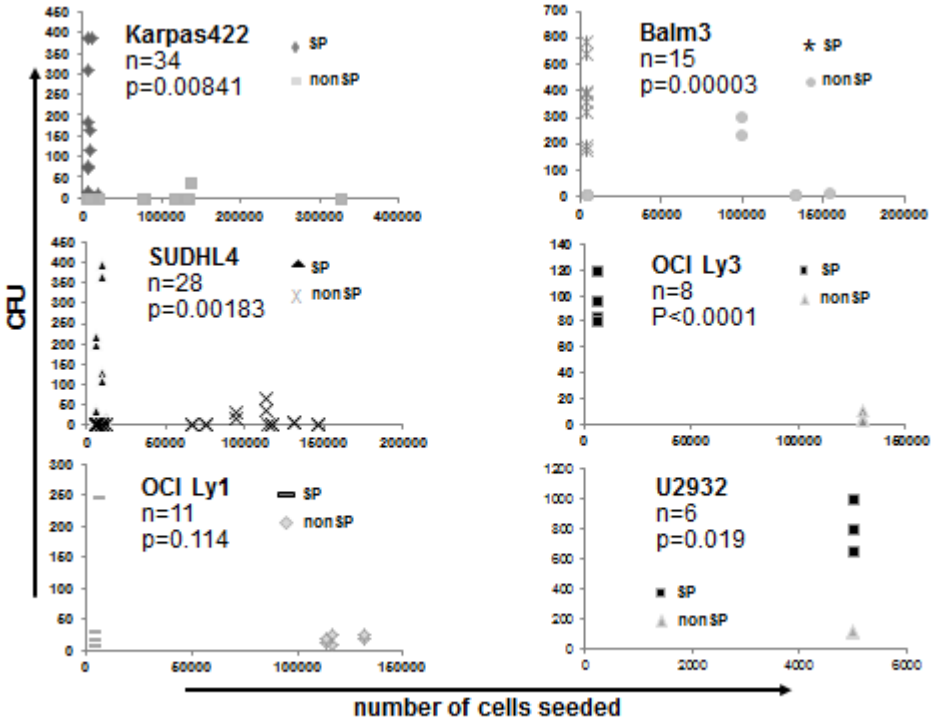
Supplemental Figures



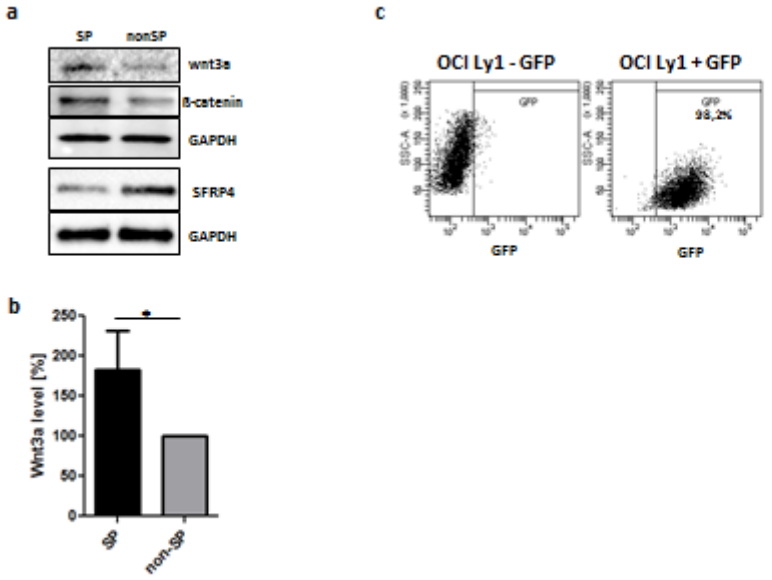
Supplemental Figure S1 Generation of SP and nonSP cells from sorted SP cells.

Following Hoechst33342 staining lymphoma SP cells were sorted by flow cytometry as boxed. NonSP cells died off, while SP cells expanded in cell culture for 21 days and 24 days, respectively, and were stained with Hoechst33342 again. The re-analysis showed resurgence of the characteristic patterns and proportions of SP and nonSP cells in the cell line cultures.



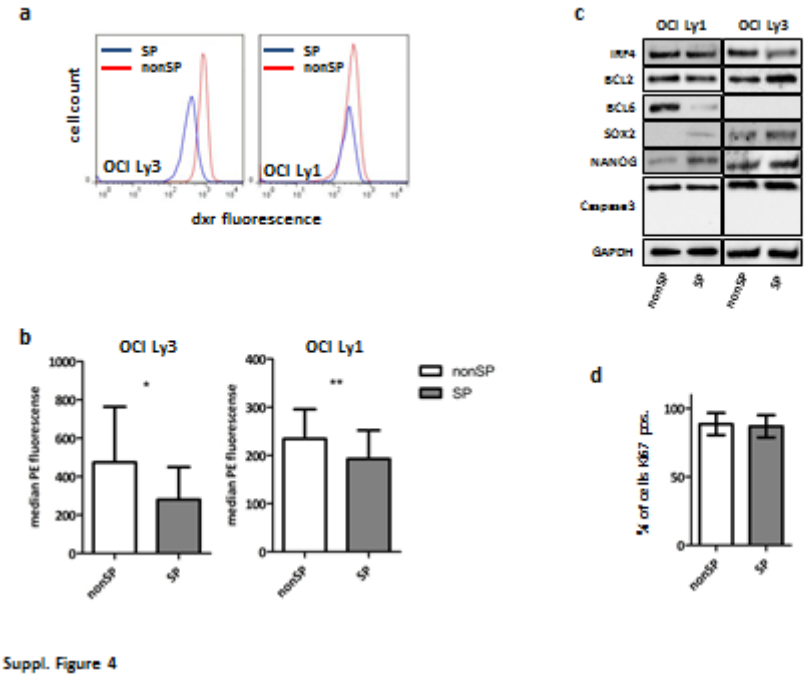
Supplemental Figure 2 Lymphoma SP cells are enriched in colony formation capacity.

After 15 days of culture in semisolide media, purified SP cells generated lymphoma colonies at high frequency, compared to no or only few colonies from corresponding nonSP cell preparations. The differences of colony numbers obtained from SP cells (dark symbols) versus those obtained from nonSP cells (light symbols) were significant for the cell lines Balm3, Karpas422, SUDHL4, OCI-Ly3, and U2932 each as indicated, but not for OCI-Ly1 (unpaired two sided t-test).



Suppl. Figure 3

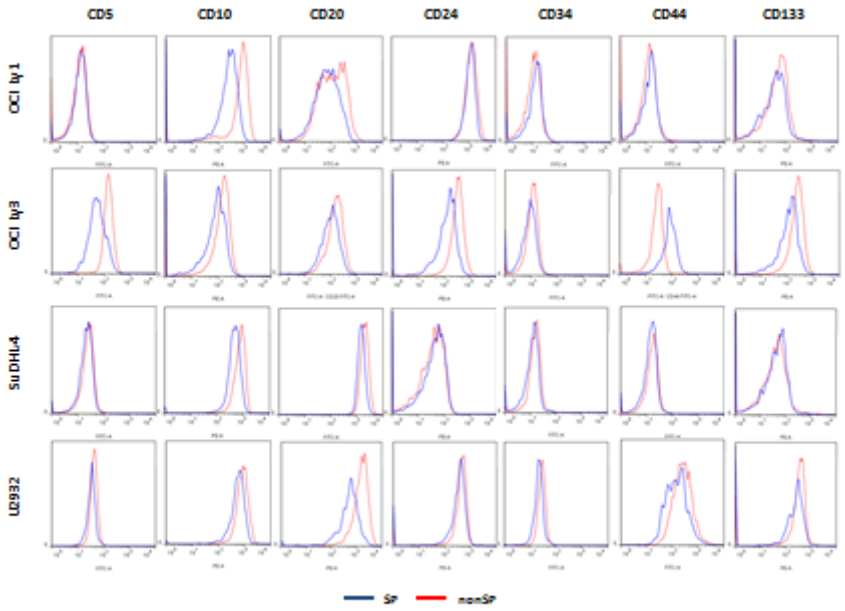
Supplemental Figure 3 Reciprocal expression of wnt3a and SFRP4 in cell line OCI-Ly1 and stability of transgenic GFP expression. (a) Increased levels of wnt3a and ß-catenin versus lower levels of SFRP4 in whole cell lysats of SP cells compared to nonSP cells of OCI Ly1, comparable to the situation in OCI Ly3 (Figure 3 c); (b) comparison of densitometric quantification wnt3a western blots from both SP and nonSP cells (n=4; p=0.0209 paired ons-sided t-test); (c) For cell tracking in mixed cultures, cell lines were transfected with the pLKO.3G lentiviral vector. Stability of GFP expression was routinely analysed by flow cytometry, here shown as uniform expression of GFP in OCI Ly1 cells after 2 weeks of culture.



Suppl. Figure 4

Supplemental Figure 4 Drug export from SP cells and cell cycle status of SP cells.

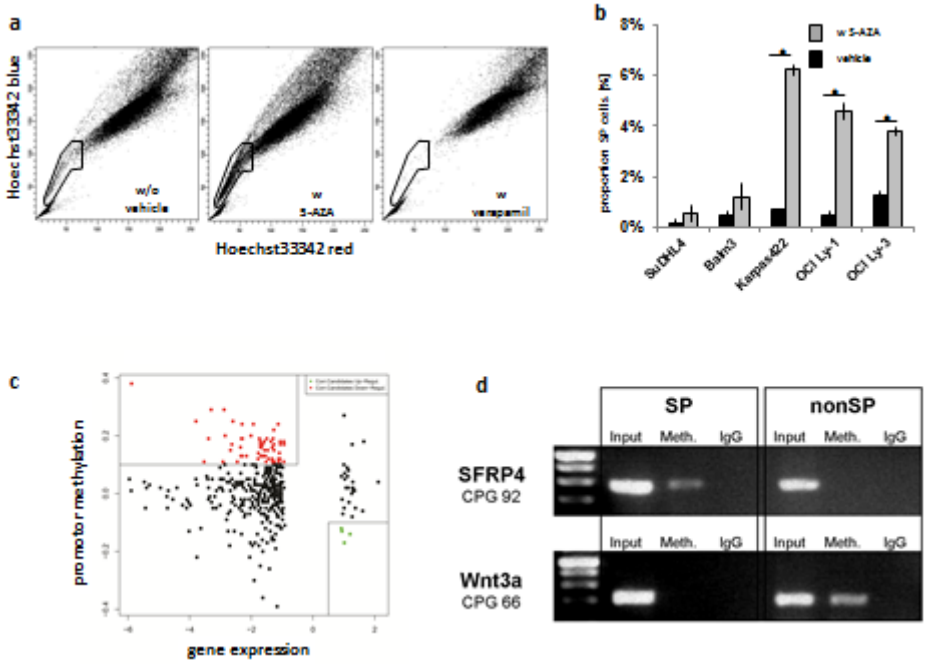
(a,b) Lymphoma cells were co-incubated at 37°C with Hoechst33342 and 10µM doxorubicin for 60 min, washed and doxorubicin efflux allowed for further 30 min at 37°C in medium containing Hoechst33342. Flow cytometry of cellular doxorubicin fluorescence with significantly higher retention of doxorubicin in nonSP compared to SP cells (representative examples in a, statistical analysis of median fluorescence intensity in b; OCI Ly1: paired t-test p=0.0015, n=6; OCI Ly3: paired t-test p=0.013, n=6). (c) Similar protein levels of IRF4, BCL2, BCL6, SOX2, NANOG, as well as lag of caspase3 cleavage in SP and nonSP cells of the cell lines OCI Ly1 and OCI Ly3. (d) Ki67 staining of OCI Ly3 nonSP and SP showed no cell difference in the number of positive cells.



Suppl. Figure 5

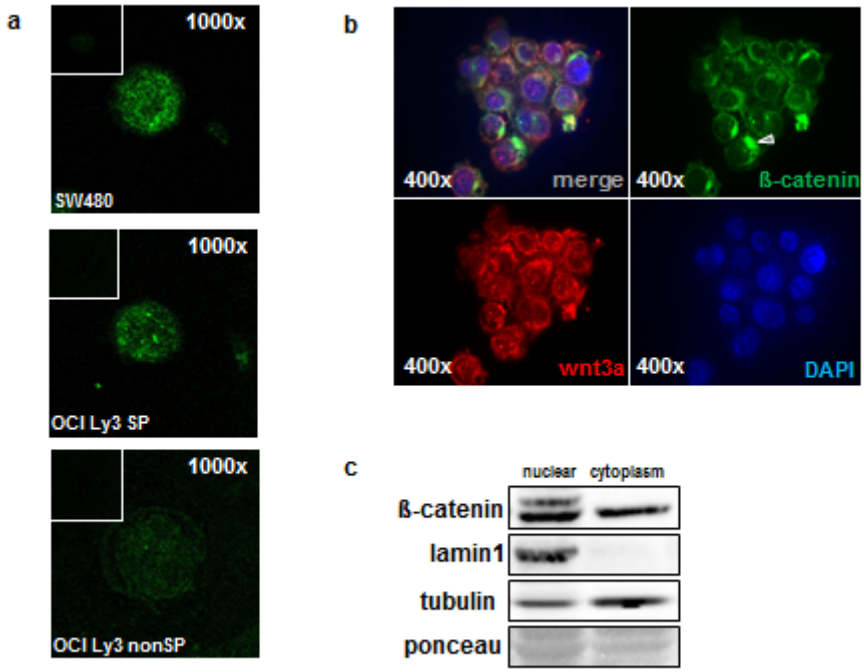
Supplemental Figure 5 Phenotype of SP vs. nonSP cells in DLBCL cell lines.

(a) The cell lines OCI Ly1, OCI Ly3 SuDHL-4 and U2932 were stained with Hoechst33342 for SP versus nonSP detection, stained with antibodies against the antigens indicated, and measured on a flow cytometer.



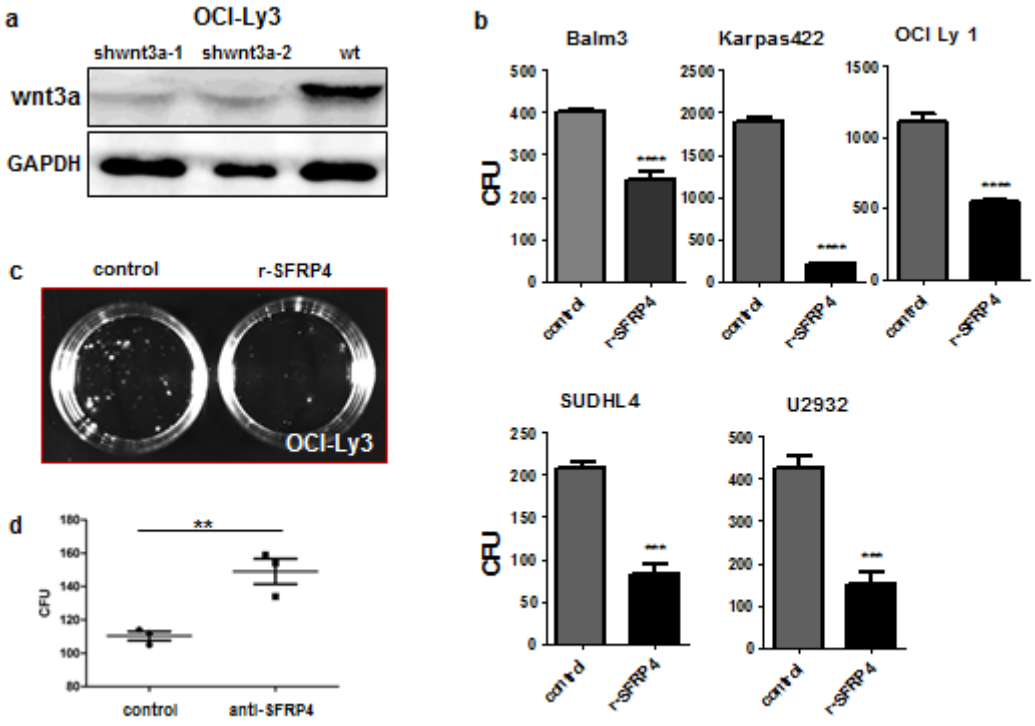
Supplemental Figure 6 Epigenetic regulation of SP phenotype.

(a,b) Increased proportions of SP cells by exposure to 5-azacitidine (5-AZA, 72 h, 5 µM) in suspension cultures of lymphoma cell lines (shown for OCI-Ly3 in a, with control for Hoechst33342 staining by verapamil; further cell lines in b; n=3 each, *p<0.01, paired t-test). (c) Combined microarray-based comparative analysis of SP versus nonSP cells in gene expression (c, abscissa) and gene promotor methylation (c, ordinate) identifying genes with high expression levels / and promotor hypomethylation (green dots), as well as genes with low transcript levels / promotor hypermethylation (red dots) (c, here OCI Ly3, genes with transcript levels between +1.0 and -1.0 suppressed). (d) CpG-specific PCR after methylated DNA immunoprecipitation (MeDIP) showing, that CpG 92 of the SFRP4 promotor was hypermethylated while CpG 66 of wnt3a was hypomethylated, compared to nonSP cells each.



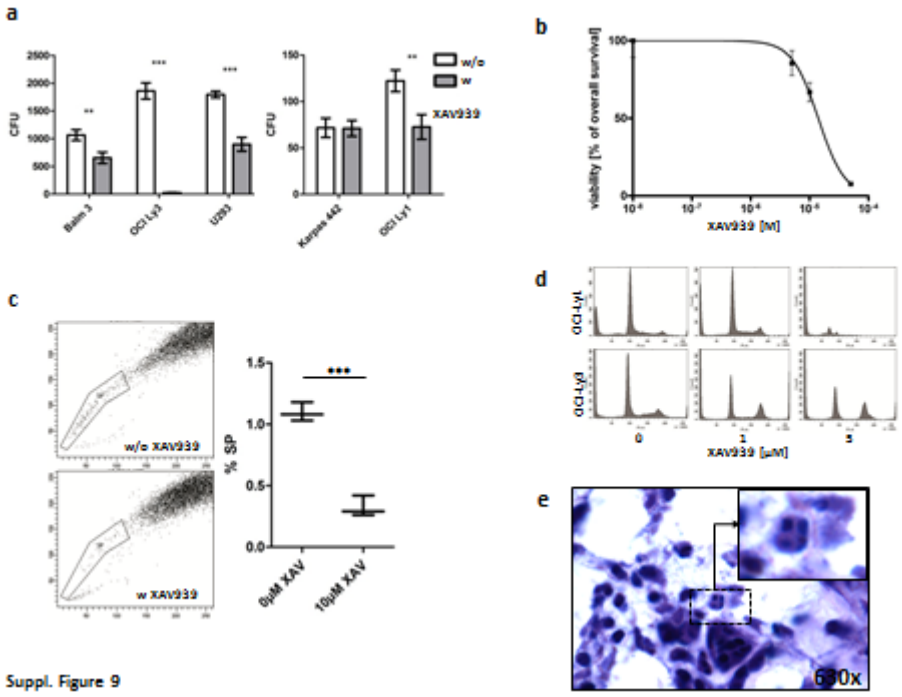
Supplemental Figure 7 Subcellular localization of ß-catenin in OCI-Ly3 subpopulations.

(a) Sorted cell populations showed nuclear ß-catenin at enhanced levels in SP cells, compared to the nuclei of nonSP cells (unsorted cells of colon cancer cell line SW480 as positive control; cytospin preparations, 1000x). (b) In unsorted whole cell preparations, ß-catenin was also detected in the cytoplasma of the lymphoma cells, accumulated at a distinct perinuclear region (arrowhead). (c) Subcellular fragmentation and western blot of analysis of unsorted OCI Ly3 lymphoma cells revealed ß-catenin both in the nuclear and in the cytoplasmic fractions of lymphoma cell lysates, with lamin1 as nuclear and tubulin as cytoplasmic marker, respectively.



Supplemental Figure 8 Silencing of wnt3a in OCI-Ly3 and role of SFRP4 for growth of lymphoma cell lines.

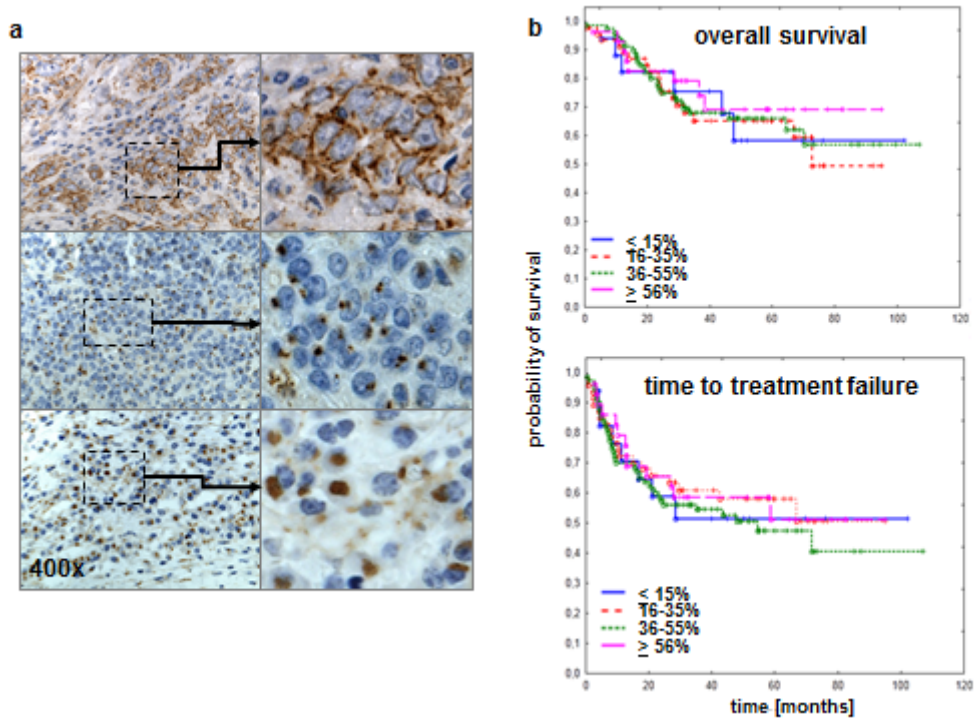
(a) Wnt3a was silenced in OCI-Ly3 lymphoma cells by transduction with two independend lentiviral shRNA constructs, shwnt3a-1 and -2. (b) In colony assays, r-SFRP, added once at start of the cultures at a concentration of 100 ng/ml, reduced the number of colonies in all cell lines (example of CFU for OCI-Ly3; c, two-sided t-test, *** $p<0.001$, **** $p<0.001$). (d) Addition of neutralizing antibody against SFRP4 increased CFU activity (d, OCI-Ly3, two-sided t-test, $p=0.0089$).



Suppl. Figure 9

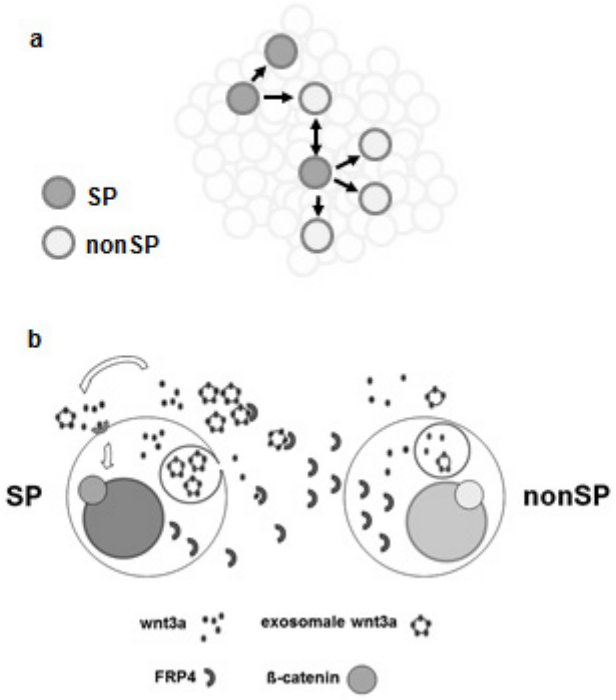
Supplemental figure 9 Tankyrase inhibition impedes tumor growth in lymphoma cell lines.

(a) $5x10^3$ lymphoma cells of each cell line were exposed to XAV939 or DMSO (5 µM, 4h), seeded in semisolid media, and colony formation counted on day 14. XAV939 reduced CFU numbers in all cell lines except Karpas422 (two-sided t-test, **= $p<0.01$, ***= $p<0.001$). (b) XAV939 exerted direct short-term cytotoxicity at concentrations >50 µM (OCI-Ly3; MTT-test for 48 h). (c) XAV939 reduced the proportion of SP cells in lymphoma cell culture (OCI-Ly3; 10 µM XAV939, 48 h; two-sided t-test, $p<0.001$). (d) XAV939 induced cell cycle arrest and apoptosis in OCI-Ly1 and OCI-Ly3 (d; XAV939 as indicated, 72 h). (e) Following direct application to the tumor *in ovo*, XAV930 altered histological morphology (compare Figure 6). OCI-Ly3 lymphomas treated with *in vivo* with direct application of XAV939 (10 µM, day 8 *in ovo*) exhibited sparse infiltrates of tumor cells with mostly condensed, partially fractionated nuclei (hematoxylin stain; w insert)



Supplemental figure 10 Staining patterns for ß-catenin in DLBCL and Kaplan-Meier estimates of survival according to ß-catenin levels in DLBCL patients.

(a) Paraffin fixed samples of aggressive lymphoma tumors were stained with a specific antibody against ß-catenin, and three representative staining patterns (cytoplasmic upper row, perinuclear middle panel and nuclear lower row) are depicted (magnification in right panel, compare figure 6 f). (b) For 258 patients clinical outcome parameters within the DSHNHL trials NHL B1 and B2, evaluating CHOP versus CHOEP as primary therapy for aggressive lymphoma patients, were available. Cumulative survival analysis revealed no impact from the amount of positive cells per sample on overall survival (upper panel) or time to treatment failure (lower panel).



Supplemental Figure 11 Schematic overview. (a) Lymphoma SP cells give rise to SP cell and nonSP cells, and nonSP cells revert to SP cells, resulting in tumor growth. (b) In the tumor, wnt3a secreted from SPs and chaperoned by exosomes fosters tumor growth, while sFRP4 from nonSP cells has a suppressive effect. While certain wnt3a levels appear critical for autonomous growth of SP cells as well as persistence of nonSP cells in the tumor (quorum sensing), sFRP4 limits such stimulatory effects of wnt3a (quorum quenching).

Supplementary experimental procedures

Cells, antibodies, small molecules, plasmids and vectors

The previously described diffuse large B-cell lymphoma (DLBCL) cell lines SUDHL4, U2932 and Karpas422 were obtained from a public depository (DSMZ, Braunschweig, Germany), the cell line Balm-3 (42) was kindly provided by B. Glass and the cell OCI-Ly1 as well as OCI-Ly3 (43-45) from the Ontario Cancer Instituts; the cell lines were propagated in RPMI 1640 supplemented with 25 mM HEPES, GlutaMAX I (Gibco-BRL), 1x penicillin/streptomycin (Sigma, Biochrom) and 10% heat-inactivated fetal calf serum (Gibco-BRL). The cell lines (L-Wnt3a (ATCC #CRL-2647) and L-cells (ATCC #CRL-2648) were kindly provided by T.Pukrop. Approval for the collection and analysis of patient samples after

specific informed consent was obtained from the Institutional Review Board of the University Medicine Goettingen, and was conducted according to the Declaration of Helsinki. Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described (45). For CFU-assays, cell numbers as indicated were seeded in triplicates into 1 mL methylcellulose medium (reach bio, colonygel, tm1101). Cells were allowed to form colonies by incubating at 37°C, counted on day 14 of culture. Specific clonogenicity was expressed as number of CFUs per dish, or fraction of control in %.

For propagation of human lymphoma cells *in ovo*, fertilized chick eggs were incubated at 37.8°C and 80% relative humidity. A window was made into the egg shell at day three and sealed with tape. Eggs were placed back in the incubator and at day 10 the lymphoma cells (5×10^5 or as indicated in Matrigel/serum; B&D) were applied on the chorionalantoic membrane (CAM). Tumors were studied on day 17 of chick development, when the embryo was sacrificed and the CAM excised for analysis. For propagation of lymphoma cells in the mouse, 6- to 8-week-old C.B-17 scid/scid (SCID) bred in our own breeding facility maintained under defined flora conditions, were injected with lymphoma cells according to a protocol approved by the local and regional Animal Protocol Review Committees. Directly after flow cytometry purification, SP or nonSP lymphoma cells at the cell numbers indicated in 100 µl Matrigel/serum (B&D) were injected subcutaneously. The mice were sacrificed after development of visible local tumor growth. The animals were dissected, macroscopically visible tumor lesions documented and the primary tumor excised for further analysis.

For SP cell analysis or sorting, the mononuclear cells were stained with the fluorescent dye Hoechst 33342 (Sigma) in a concentration of 5 mg/ml (37°C for 2 hours) as previously described (3). The SP population, characterized by the most rapid efflux Hoechst 33342, was identified and sorted by its fluorescence profile in dual wavelength analysis (405/30 and 670/40 nm) after excitation at 350 nm on a triple-laser instrument (ARIA II, Becton-Dickinson); data were analyzed with CellQuest (Becton-Dickinson) software.

The primary antibodies used in this study for the applications were as indicated: anti-flotillin-2 (clone 29, BD-Pharmingen), anti-ß-catenin (polyclonal rabbit antibody kindly provided by F. Brembeck), anti-wnt3a (Clone # 217804, R&D Systems), anti-SFRP4 (AF1827, R&D Systems), anti-kappa-light-chain (polyclonal anti-kappa-FITC/lambda-PE, Beckmann-Coulter), anti-CD19 (J4.119, Beckman-Coulter). Secondary antibodies against mouse or rabbit immunoglobulin were obtained from SantaCruz.

Exosome preparation and quantification

Exosomes were prepared by differential centrifugation according to standard protocols (6). Following incubation of $5x10^7$ lymphoma cells for 48h in complete exosome-free medium, cells and larger debris were removed by centrifugation for 10 min (10 min., 500 g, 4°C). The supernatant was centrifuged again (20 min., 10000 g; 4°C; Beckman L8-55 ultracentrifuge, rotor Ti32) to remove intermediate size particles. Subsequently the supernatant containing exosomes was filtered (0.22 µM Millex GP), and again centrifuged (240 min., 120 000g, 4°C; Beckman L8-55 ultracentrifuge, rotor Ti32) to obtain the exosome pellet, which was washed once in PBS, and finally resuspended in 50 µl PBS for further applications. Exosomes were quantified by measuring whole protein according to standard protocols (BioRad-DC-Protein-Assay), Western blot detecting flotillin-2 in comparison with whole cells or control exosome preparations, as previously described (28). For sucrose gradient separation, purified exosomes were loaded onto the top of a step gradient consisting layers of 2.25 M, 2.0 M, 1.75 M, 1.5 M, 1.25 M, 0.75 M, 0.5 M and 0.25 M sucrose. The samples were centrifuged for 2.5 h at 110,000 x g in a Beckmann SW32 Ti. Eight 3.75 ml fractions were collected and diluted in 10 µM HEPES to a total volume of 30 ml. Fractions were pelleted for 2.5 h at 110,000 x g and dissolved in RLT buffer with 1% protease inhibitor cocktail (Sigma). Samples were analyzed by SDS PAGE and western blotting as described below.

Endogenous Wnt-signaling was performed by TOP/FOP reporter assay. $5x\ 10^4$ HEK293 cells in triplets per condition were plated out the day before. Cells were transfected by lipofectamine2000 with 6.25ng TOP or FOP plasmid, respectively, and 5ng of renilla TK-plasmid for internal normalization. 8 hours after transfection, cells were stimulated with exosome suspension (100µg) in 200µL exosome-depleted DMEM. As negative control, cells were incubated in 200µL exosome-depleted DMEM. As positive controls, supernatant of L-cells with ectopic Wnt3a-expression and 200ng of recombinant wnt3A were applied to the target cells. Cells were harvested after 36 hours and the reporter activity measured by luminometer. As small molecules were used at the concentrations indicated: XAV939, SB-216763, IWR1, IWP2 (all from Sigma-Aldrich). For silencing wnt3a and SFRP4 validated specific shRNA sequences were used (The RNAi Consortium, www.broadinstitute.org/rnai/trc: shwnt3a: shWnt3a I - TRCN0000089120; shWnt3a II-TRCN0000089122; shSFRP4: Clone Id TRCN0000014490) were cloned into pLKO.1-eGF

(Addgene), and lentiviral particles produced in HEK293T producer cell line with the plasmids pCMV-ΔR8.91 (containing gag, pol and rev genes) und pMD.G (VSV-G expressing plasmid) following standard protocols.

Gene expression, DNA methylation

For gene expression analysis 300 ng of purified RNA were labelled with biotin, according to the Affymetrix Gene Human Arrays 1.0 ST protocol. Biotinilated RNA was hybridized 16hrs at 45° C with rotation (60rpm) into individual Affymetrix Gene Human Arrays 1.0 ST. GeneChips were washed and stained in the GeneChip® Fluidics Station 450 (Affymetrix) according to GeneChip® Whole Transcript (WT) Sense Target Labeling Assay manual (Affymetrix) instructions. Microarrays were scanned in a GeneChip® Scanner 3000 7G (Affymetrix) and quality control was evaluated by Affymetrix software (Expression ConsoleTM). Intensity data were extracted using Affymetrix's AGCC Software (version 2.0) and analyzed using the R packages Affy (1) and Limma (7) of Bioconductor (2). The gene expression data analysis consisted of the following steps: 1. between-array normalization, 2. probe summary with the rma algorithm, 3. fitting the data to a linear model and 4. detection of differential gene expression. Quantile-normalization was applied to the log2-transformed intensity values as a method for between-array normalization, to ensure that the intensities had similar distributions across arrays (5). To find genes with significant expression changes between groups, empirical Bayes statistics were applied to the data by moderating the standard errors of the estimated values (7). *P*-values were obtained from the moderated t-statistic and corrected for multiple testing with the Benjamini–Hochberg method (4). The *P*-value adjustment guaranteed a smaller number of false positive findings by controlling the false discovery rate (fdr). For each gene, the null hypothesis, that there is no change between SP and nonSP fractions, was rejected when its fdr was lower than 0.05. The results are available at GEO (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53079).

The HumanMethylation27 BeadChip (Illumina, San Diego, CA, USA) uses Infinium technology to perform genome-wide screening of DNA methylation patterns. In detail, the HumanMethylation27 panel targets 27 578 unique CpG sites located within the proximal promoter regions of transcription start sites of 14 475 consensus coding sequencing (CCDS) in the NCBI Database (Genome Build 36). In addition, 254 assays cover 110 miRNA promoters. On average, two assays were selected per CCDS gene and from 3 to 20 CpG sites for >200 cancer-related and imprinted genes. Further specifications of the platform can be found at http://www.illumina.com/products/infinium_humanmethylation27_beadchip_kits.ilmn. 200

ng DNA was applied per BeadChip according to the manufacturer's instructions. Initial data analysis was performed with the BeadStudio Methylation Module. DNA methylation values, described as beta values, were recorded for each locus in each sample. Only few CpG sites did not reveal reliable signals, as determined by their detection P-value, and were therefore excluded from further analysis. Raw data were quantile normalized. Statistical analysis and clustering by Euclidian distance was performed using the MultiExperiment Viewer (MeV, TM4). To functionally characterize the differentially methylated genes between SP and NSP, we searched for over-represented Gene Ontology terms using DAVID (8,9). The analysis to detect enriched Gene Ontology (GO) terms was carried out separately for methylated and demethylated promotors to identify groups of genes with similar functions. The results of significant GO terms (p-value < 0.01 oder 0.05) are listed in supplemental table 1. MeDIP assay was performed using the Methylamp Methylated DNA Capture (MeDIP) Kit (P1015, Epigentek) protocol according to the manufacturer's instructions. PCR was performed to detect gDNA after meDIP. The Wnt3a primer (us 5´-CAACCCGACTTCCGCGCCAT-3´; ds 5-TGCCGAAGGAGCCCGTCTCA-3´) yielded an amplicon of 214 bp. The SFRP4 primer (us 5´-CGAGATGCACACGCCACGGT-3´; ds 5´- ACA GCA CGC AGG AGA ACG CC-3´) yielded an amplicon of 272 bp.

Microscopy

For fluorescence microsocopy, cells were fixed at RT using 3.7% paraformaldehyde for 20 minutes, with the subsequent quenching of any unspecific binding using 50 mM NH_4Cl for 15 minutes and permeabilization with 0.05% Triton X-100 in PBS for 15 minutes. Primary antibodies were diluted 1:100 in PBS for 1 hour. After washing twice with PBS and incubating with 10% goat serum, the primary antibodies were visualized using goat secondary antibodies at a dilution of 1:500 in PBS coupled to Cy3 (Dianova). Samples were mounted in Fluoromount (DAKO) and analyzed with the following microscopes: Zeiss Axio Imager.Z1 (Carl Zeiss, Göttingen, Germany; Fig 2**g**), LSM 510 Meta (Carl Zeiss, Göttingen, Germany, Figure 3**e**, suppl. Figures 7**a,b**). CAM tumors were photographed with Leica MZ FLIII stereo microscope with DFC290 HD camera and Leica Application Suite Software LAS Version 3.8 (Leica, Wetzlar, Germany; Figure 2**c**). Tumor histology of CAM tumors after HE stain were documented with Olympus BX41 microscope (Olympus, Hamburg, Germany; Figures 5**b**, 6**f**, 9**e**,10**a**). CFU in semisolid media culture dishes were placed on a Stemi SV 11 (Carl Zeiss, Göttingen, Germany) inverted light platform, and photos taken with a hp Photosmart R742 (Palo Alto, USA; Figures 2**a** and 4**c,** suppl. Figure 8**c**) camera. The data were exported as

TIFF files and arranged using Adobe PhotoShop® without further modification of the primary image. Immunoelectron microscopy was performed according to the Tokuyasu method. Cell and exosome samples were fixed with 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M sodium phosphate [pH 7.4] at room temperature for 30 minutes, before the cells were postfixed with 4% paraformaldehyde and 0.1% glutaraldehyde on ice for 2 hours. After being washed twice with PBS – 0.02% glycine, the cells were embedded in 10% gelatin, cooled on ice, and cut into small blocks. The blocks were infused with 2.3 M sucrose overnight and stored in liquid nitrogen. Ultrathin cryosections were cut from the frozen samples and labeled with primary antibodies detected with protein A conjugated to gold (PAG). In the case of monoclonal antibodies, a polyclonal rabbit anti-mouse bridging antibody (Sigma) was used prior to detection with PAG. Sections were contrasted with uranyl acetate methyl cellulose on ice for 10 minutes, embedded in the same solution, and examined with a Phillips CM120 electron microscope. In preparation of whole cell imaging, cells were fixed in 2% glutaraldehyde in 0.1 M PBS, pH 7.4, for 2 hours, postfixed in 1% OsO_4 for 1 hour, dehydrated in ethanol, and then embedded in Epon.

SDS-PAGE, Western blot and PCR

For SDS-PAGE total cell lysates were prepared in CelLytic M (Sigma), supplemented with 1 mM Na_3VO_4, 10 mM Na_2MoO_4 and proteinase inhibitor cocktail (Sigma). 25 µg, or amounts as indicated, of protein were run on standard SDS-PAGE. Protein transfer was completed using 30V for 60 minutes on Hyperbond-C Extra (Amersham Biosciences), and blocked with 5% BSA (Sigma) in 0.1% Tris-buffered-saline. After washing, membranes were probed against the indicated antigens following the manufacturers´ recommendations for the antibodies. Secondary HRP-conjugated antibodies against anti-rabbit or anti-mouse were purchased from Santa Cruz. For chemo luminescence detection standard ECL (Pierce) was used. For quantification of mRNA, qRT/PCR of wnt3a, SFRP4, axin2, c-myc and GAPDH transcripts were performed in triplicates on a Taqman cycling machine (ABI Prism 7900HT Sequence Detection System, Applied Biosystems) following standard protocols. Briefly, the SYBR green kit (Quiagen) was used according to the manufacturer's protocols, with 40 cycles of denaturation (15 seconds at 95°C), annealing (45 seconds at 58°C) and elongation (60 seconds at 72°C) followed by a melting curve analysis. Subsequently, the threshold PCR cycle number (*CT*) was obtained when the increase in the fluorescence signal of the PCR product indicated exponential amplification. This value was normalized to the threshold PCR cycle number obtained for Beta-actin mRNA from a parallel sample. The hwnt3a primer (us 5´-GGAAGGTTCCATGAAGCGAGTCGG-3´; ds 5-CTCTGCGAAGTCCCTGTCCTCTCC-

3′) yielded an amplicon of 240bp, the sFRP4-Primer (us 5′-ACACCCTCTTAAGCAGCACCAG-3′; ds 5′-AGGGTGGATGTCCTGGGAAGTAAG-3′) an amplicon of 143bp, the Axin2-Primer (us 5′-GAGATATCCAGTGATGCGCTGAC-3′; ds 5′-AATTCCATCTACACTGCTGTCCGT-3′) an amplicon of 66bp, the c-myc-Primer (us 5′-AAGAGGGTCAAGTTGGACAGTTGC-3′; ds 5′-TTTCGGTTGTTGCTGATCTGTCT-3′) an amplicon of Beta-Actin primer (us 5′-CACACTGTGCCCATCTACGA-3′; ds 5′-TGAGGATCTTCATGAGCTAGTCAG-3′) an amplicon of 100 bp.

References

1. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics. 2004;20:307-315.

2. Gentleman RC, Carey VJ, Bates DM et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004;5:R80.

3. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J.Exp.Med. 1996;183:1797-1806.

4. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. Stat.Med. 1990;9:811-818.

5. Irizarry RA, Hobbs B, Collin F et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics. 2003;4:249-264.

6. Raposo G, Nijman HW, Stoorvogel W et al. B lymphocytes secrete antigen-presenting vesicles. J.Exp.Med. 1996;183:1161-1172.

7. Smyth GK, Michaud J, Scott HS. Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics. 2005;21:2067-2075.

8. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protoc. 2009;4(1):44-57.*

9. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res. 2009;37(1):1-13.*