Supplementary Materials and Methods

Study design of animal experiments

All animal experiments were performed with approval from the local animal experimental ethics committee and according to the government of Baden-Wuerttemberg/Tuebingen.

NOD.CB17-Prkdc^{scid}/NcrCrl mice (Jackson laboratories) and C57BL6/J mice (Charles River) were maintained and bred at the animal facility of the University of Ulm in a pathogen-free environment (SPF IVC barrier) with a 14/10 day and night cycle as well as water and food ad libitum. All animal procedures were performed according to protocols approved by the state government of Baden-Wuerttemberg, Germany, following the animal welfare guidelines (Registration 1124 and 1128).

Cell line models:

Murine A20 cells (TIP-208, ATCC) were cultured in RPMI 1640 supplemented 10% FBS, 1% Penicillin/Streptomycin and 50 µM β-mercaptoethanol and maintained at a proliferation density of 1 million cells/ml. Ba/F3 were cultured in RPMI 1640 containing 10% FBS, 1% Penicillin/ Streptomycin and 10 ng/ml IL-3. The murine pre-B-cell line 300-19 was cultured in RPMI 1640 with 10% FBS, 1% L-glutamine, 1% Penicillin/Streptomycin, 1% non-essential amino acids, 1% sodium pyruvate and 50 µM β-mercaptoethanol. HEK-293 T cells (CRL-3216, ATCC) used for lentiviral production and the M210B4 stromal cells used in co-culture experiments were cultured in DMEM with 10% FBS and 1% Penicillin/Streptomycin. JVM2 cells were cultured in RPMI medium with 10% FBS, 1% L-glutamine, 1% Penicillin/Streptomycin.

Primary CLL patient samples:

Gene expression profiling was performed on RNA from 337 previously untreated CLL patient samples using Affymetrix Human Exon 1.0 ST Arrays. Data for core probe sets summarized on gene levels was normalized with the RMA algorithm from the aroma affymetrix package. Genomic aberrations were analyzed using FISH[1]. *IGHV* gene mutations status and gene mutations in *TP53, NOTCH1, SF3B1* were analyzed as previously described[2]. The analyses were conducted in accordance with the Declaration of Helsinki, approved by our institutional review board, and informed consent was obtained from all patients.

In vivo drug treatments:

Linsitinib (OSI-906; Selleckchem) was administered at 25 mg/kg by oral gavage once daily buffered in 30% PEG 400 + 0.5% Tween 80 + Propylene glycol.GS-649443 (Gilead) was formulated in 10% (v/v) Ethanol + 20% (v/v) Cremophor EL + 70% (v/v) saline Solution (0.9% w/v) to achieve 1 mg/ml and treated BID with 5 mg/kg by oral gavage.

Murine primary cells used in *ex vivo* and *in vitro* experiments

Ex vivo experiments were performed using viably frozen tumor cells stored in freezing medium with 45% FBS, 45% RPMI and 10% DMSO. The cells were thawed in RPMI containing 10% FBS. For functional studies viably frozen tumor cells from the 3rd or 4thtransfers were used.

In vitro drug treatments of murine primary cells

The GSK3 inhibitor (iGSK3) CHIR-99021 (Selleckchem) and the FOXO1 inhibitor (iFOXO1) AS1842856 (Calbiochem) were dissolved in DMSO to a stock concentration of 10mM. Viably frozen murine primary tumor cells from the 4th transfer were thawed and pre-treated with either iGSK3, iFOXO1 or with DMSO for 3 hours. After the pre-treatment time point, either iPI3K-δ or DMSO was added to each of the pre-treatment condition. The cells were further incubated for 12 hours after which RNA was isolated and expression changes in *Igf1r* was analyzed using RT-qPCR analysis.

Cell proliferation assay (MTS assay):

Impact of inhibitors on cells *in vitro* was analyzed using the MTS cell proliferation assay. Briefly, 20,000 cells per well were seeded in triplicates on flat bottom 96 well plates (Nunc-Thermo Scientific). Serial dilutions of the inhibitors were added to make up for a total volume of 100µl per well. Individual plates were set up for the day 0, day 2 and day 4 timepoints and incubated at 37°C. At the specified timepoints, 20µl of CellTiter 96® AQueous one solution cell proliferation assay reagent (Promega) was added to each well and the plates were further incubated at 37°C for 3 hours. The OD was measured at 492nm using the Multiskan™ GO Microplate spectrophotometer (Thermo Scientific). The whole experiments were repeated three times.

5-bormodeoxyuridine (BrdU) incorporation assay

BrdU incorporation assay was performed according to the manufacturer´s protocol (BD Pharmingen™ BrdU Flow Kit). In brief, mice were treated with 10mg/kg BrdU by intraperitoneal injection 1 hour before sacrifice. Approximately 5 million cells derived from a single cell suspension of the different organs were fixed in 70% ethanol. After washing, the cells were permeabilized and intracellular labeled with anti-BrdU and measured on BD LSRII analyzer.

Pappenheim's panoptic staining

To perform the blood smear, 5µl of peripheral blood where taken and spread on microscopy slides. After drying, the smears were fixed in methanol for 10 min. Pappenheim method of panoptic staining was performed for 3 minutes and remaining solution washed off. Representative pictures were taken using a Zeiss microscope at 40X magnification.

Immunocytochemistry

Cytospins were performed using 50,000 cells on Superfrost Menzel-Gläser slides (ThermoScientific) with Cytospin 4 (Thermo Scientific) at 1000rpm for 3 minutes. After spinning, cells were fixed in 2% ice-cold PFA for 10 minutes and rinsed twice with PBS. Permeabilization was made using 200µl of permeabillization solution (Triton X-100 0.5% in 1X PBS) for 30 minutes. After rinsing with PBS, the specimens were blocked in blocking buffer (5% goat serum albumin in PBS) for 1 hour. Each slide was incubated overnight at 4°C with 200µl of FOXO1 (C29H4) antibody (Cell Signaling), diluted 1:100 in antibody dilution buffer (1% BSA, 0.3% Triton X-100 in PBS). The slides were then incubated with 200µl of a 1:500 dilution of the secondary antibody (Cy3 goat anti-rabbit IgG (H+L) (life technologies) and incubated for 1h at room temperature in the dark. The slides were mounted using fluorescence mounting medium with DAPI (Vector Laboratories). The images were acquired using a Leica fluorescent microscope connected to Zeiss Axiocam camera.

Analysis of whole exome sequencing

After alignment of paired-end reads (100 bp) to mm10 with bwa-mem (0.7.10), the alignment was sorted and duplicates were removed using Picard 1.64. After local realignment (GATK 3.2.2) and coverage calculation (bedtools 2.17.0., Suppl.Table 4) we performed variant calling (samtools0.1.19) and identified somatic and germline variants (SNVs and InDels)

using VarScan 2 (2.3.7). The variants were annotated using Annovar (release 22Mar2015) and filtered based on annotation. We focused on non-synonymous somatic mutations affecting exons or splicing sites, subsequently we removed low confidence calls (supported by less as 4 reads and variants not supported by reads mapping to both strands). In addition we filtered out calls, which have entry in Genomic SuperDups and dbSNP 138 annotation databases. Filtered high confidence calls were subjected to visual inspection in Integrative Genomics Viewer (IGV). Similar filtering pipeline was applied to germline calls and after pre-filtering we assessed the presence of cancer-relevant gene mutations (n=83) in germline.

Analysis of pathways associated with mutated genes

The mutations identified exclusively in the GS-649443 resistant tumors were included to identify pathways associated with these genes. The Ingenuity pathway analysis (IPA) was used to identify signaling networks based on biological function of the enriched gene sets and relation to networks involved in diseases. Gene ontology analysis was performed using DAVID and biological processes implicated with the gene sets were analyzed. Protein-protein interactions were analyzed using STITCH analysis and related pathways were identified using the linked KEGG database search.

shRNA and overexpression constructs

For knockdown of *GSK3B*, a set of 3 different shRNAs were obtained from the TRC lentiviral human shRNA library (GE Healthcare Dharmacon, RHS4533-EG2932; Suppl.Table 5) For overexpression of murine IGF1R, pcDNA3.1 with *Igf1r* ORF was obtained from Genscript (OMu20664C; Accession.No NM_010513). The *Igf1r* ORF was shuttled into the SFLV–cDNA EGFP vector system.

Lentiviral transduction

Production of virus has been performed as described using the lentiX lentiviral expression system (Clontech) following the manufacturer´s instructions. For packaging the virus, a viral packaging vector (pAX2/GAG-Pol) and an envelope vector for the virus (pMD2/VSV-G) were used. Viruses harvested 24 and 48 hours after transfection were used for transduction of A20, Ba/F3 and 300-19 cells with 8mg/ml Polybrene. The cells were infected for 12 hours after which the virus was removed and the cells were allowed to recover for 2 days. Selection of transduced cells was performed by using 4µg/ml puromycin for A20 cells and 2µg/ml puromycin for Ba/F3 and 300-19 cells. Overexpression of *Igf1r* cloned in SFLV GFP vector

was performed on murine A20 cells as mentioned above however the transduced cells were selected by sorting for GFP expressing cells using BD FACS Aria II cell sorter.

B-cell receptor (BCR) stimulation using anti-IgM

Prior to BCR stimulation, the cells were cultured in RPMI 1640 medium with 0.5% cell culture grade BSA. The cells were then exposed to 10µg/ml anti-IgM polyclonal goat F(ab′)2 fragments to mouse IgM (for murine primary tumor cells, 300-19 or Ba/F3 cells) or 10µg/ml anti-IgG polyclonal goat F(ab′)2 fragments to mouse IgG for A20 cells (Jackson Immunoresearch). The cells were incubated for 10 minutes, washed with PBS and flash frozen with liquid nitrogen.

Protein extraction and Western Blotting

For total protein extraction, cells were lysed in RIPA buffer (150 mM sodium chloride, 1% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50mM Tris pH 8.0) supplemented with 1mM DTT, 0.5mM PMSF and phosphatase inhibitor cocktail, for 60 min at 4°C. The amount of protein in each sample was quantified using the Protein Assay (Bio-rad). Equal concentrations of proteins were analyzed on 12% polyacrylamide gels or 4-12% Nu-Page pre-cast gels and subsequently transferred onto PVDF membranes. The primary antibodies used are mentioned in Suppl.Table 6. Images were acquired using the western gel documentation system.

Protein expression analysis using Simple Western™

For total protein extraction, cells were harvested cells in Cell Signaling Technology lysis buffer containing: Protease Inhibitor Cocktail (Roche Diagnostics Corp), and phosphatase inhibitor sets 1 and 2 (EMD Millipore). Following 30 minutes on ice, cell lysates were cleared by centrifugation at 12,500 rpm for 10 minutes at 4°C. The amount of protein in each sample was quantified using Pierce™ BCA assay kit (ThermoFisher). Lysates with equal concentrations of protein were analyzed by Simple Western technology using Peggy Sue™ or Sally Sue™ instruments (Suppl.Table 7, ProteinSimple, San Jose, CA). Data were processed using Compass software (ProteinSimple).

Flow cytometric analysis

All antibodies which were used for flow cytometry are listed in Suppl.Table 8. Surface staining was performed in PBS containing 2% of FBS for 30 minutes at 4°C. After washing, samples were analyzed on a four-laser BD LSRII analyzer. FCS files were analyzed by using the FlowJo software version 7.

DiOC6/PI staining for cell viability

Loss of mitochondrial transmembrane potential following drug treatment was assessed using DiOC6/PI staining and FACS. After treatment for 4 days with inhibitors, cells were stained were stained with 45nM DiOC6 (Sigma Aldrich) in RPMI with 0.5% BSA for 20 minutes at 37°C. The cells were then stained with CD19-APC (eBioscience) for 20 minutes at room temperature. The cells were then centrifuged and resuspended in FACS staining buffer containing 2µg/ml PI and measured with FACS immediately. The CD19+ fraction within the DiOC6+ PI- population was evaluated.

RNA extraction and expression analysis using RT-qPCR

Total RNA was isolated using RNAeasy or DNA/RNA Allprep Kit (Qiagen) according to the manufacturer's instructions. RNA concentration was estimated using Nanodrop (Thermo scientific) and 400ng of RNA was reverse transcribed using the Reverse Transcription Kit (Promega). The cDNA was diluted 1:10 (for all genes) or 1:2 (for *Wt1*) prior to addition into the Q-PCR reaction mix. SybrGreen Supermix (Bio-rad) was used for the Q-PCR analysis as per the manufacturer´s protocol. Fold differences in gene expression was analyzed using the ΔΔ Ct method by normalizing to control sets as mentioned in the figure legends. Table of primers is presented in Suppl.Table 9.

Quantification and statistical analyses:

Statistical considerations

Statistical analyses were performed using Prism software version 6.0 (GraphPad). For non-normally distributed data sets the non-parametric Mann-Whitney U test was used. Categorical variables were compared using the two tailed Fisher's exact test. Dose response curves were compared using extra-sum-of-squares F test by including the best-fit parameters

such as using 95% confidence interval, LogIC50 and hillslope of the curves. Survival curves were analyzed using the log-rank-test (Mantel-Cox). Statistical significance was defined as $P<0.05$. All exact P values are provided in the figure legend.

Image analysis of western blots:

Intensities of individual bands in western blots were analyzed using Fiji ImageJ densitometry software. Phosphorylation levels of proteins were expressed as a relative measure compared to that of the total protein and to their respective loading controls (ACTIN or LAMIN B).

Immunocytochemistry Image analysis:

Ten different fields were obtained per slide at 60X magnification with equal camera exposure and the overlay images were created with the DAPI nuclear stain to define nuclear and cytoplasmic localization of FOXO1. A blinded scoring of FOXO1 localization: as present in the nucleus, present in nucleus and cytoplasm, expressed only in nucleus or as not expressed was performed using Fiji ImageJ. FOXO1 localization in each sample was calculated from the average of the scores from the 10 fields and expressed as percentage of total number of cells per field.

References:

1. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N. Engl. J. Med.* 2000;343(26):1910–6.
2. Stilgenbauer S, Schnaiter A, Paschka P, et al. Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial. *Blood*. 2014;123(21):3247–54.

Supplementary table legend

Suppl. Table 1.1: Mutations with ≥ 10% TVF identified using whole exome sequencing of different tumor clones derived from mice treated with vehicle (iPI3K-δ sensitive tumors) across the different transfers (1st transfer N=1; 2nd transfer N=1; 3rd transfer N=2; 4th transfer N=3). The table represents acquired mutations in every single tumor.

Suppl. Table 1.2: Mutations with ≥10% TVF identified using whole exome sequencing of different tumors clones derived from mice treated with GS-649443 (iPI3K-δ resistant tumors) across the different transfers (3rd transfer N=2; 4th transfer N=4). The table represents acquired mutations in every single tumor.

Suppl. Table 2.1: Ingenuity Pathway analysis (IPA) predicts signalling networks which are associated with the mutated genes. Top significant pathway is the cellular movement, immune cell trafficking and inflammatory response.

Suppl. Table 2.2: Analysis of protein interactions using STITCH to identify pathways associated with the mutated genes. Pathway enrichments were identified from the linked KEGG pathway database.

Suppl. Table 2.3: Gene ontology enrichment analysis was performed using (DAVID) on mutations identified in resistant tumor cells. Biological processes associated with the mutated gene sets are listed in the table.

Suppl. Table 3: Characteristics of CLL patient cohort used for *in vitro* analysis. Sample #7 showed high mRNA expression but lower protein expression (Fig. 6E). All samples except sample #11 were derived from either untreated patients or those with chemo/chemo-immunotherapy. Sample #11 was derived from a patient treated with rituximab+idelalisib for 10 months.

Suppl. Table 4: Coverage statistics for whole exome sequencing (WES) of different tumor clones derived from mice treated with vehicle (iPI3K-δ sensitive tumors) across the different transfers (1st transfer N=1; 2nd transfer N=1; 3rd transfer N=2; 4th transfer N=3) and those treated with GS-649443 (iPI3K-δ resistant tumors) in all transfer rounds (3rd transfer N=2; 4th transfer N=4).

Suppl. Table 5: Human shRNA sequences for the knockdown of *GSK3B.*

Suppl. Table 6: List of antibodies used in Western Blot analysis.

Suppl. Table 7: Table of antibodies which have been used for Simple Western™ analysis.

Suppl. Table 8: Flow cytometry antibodies and the respective clones.

Suppl. Table 9: Sequences of forward and reverse primers used for RT-qPCR analysis.

Suppl. Table 1.1

Sensitive: 1st round Vh-1		
Gene	**Mutation**	**TVF**
2810417H13Rik	NM_026515: c.C214G	15.79

Sensitive: 2nd round Vh-1		
Gene	**Mutation**	**TVF**
Wars	NM_001164488: c.737+2T>C	21.69
Csnk1g1	NM_173185: c.A398G	19.75
Xylt2	NM_145828: c.2017_2017delinsC-	17.5
Rtn3	NM_001003933: c.A707C	17
Prkag3	NM_153744: c.T527G	13.25
Pik3r5	NM_177320: c.G1459C	10.71
Nedd1	NM_008682: c.G1099A	20.78
Abca3	NM_001039581: c.G3319A	15.69
Col6a1	NM_009933: c.G2516A	10.13

Sensitive: 3rd round Vh-1		
Gene	**Mutation**	**TVF**
Abca3	NM_001039581: c.G3319A	60
Prkag3	NM_153744: c.T527G	50.56
Nedd1	NM_008682: c.G1099A	48.03
Csnk1g1	NM_173185: c.A398G	47.66
Rtn3	NM_001003933: c.A707C	41.9
Parp11	NM_181402: c.T560C	37.93
Vcan	NM_019389: c.T2171G	30.88
Xylt2	NM_145828: c.2017_2017delinsC-	19.05
Vmn2r65	NM_001105180: c.T1089G	16.13
Smc5	NM_001252685: c.G3005A	8.97
Wars	NM_001164488: c.737+2T>C	42.5
Klrb1b	NM_030599: c.G289C	33.33
Klrb1b	NM_030599: c.T275A	27.27
Slfn8	NM_181545: c.G1669A	12.24
Itih2	NM_010582: c.A1571C	11.76
Pabpc4	NM_130881: c.C74A	11.76
Fam46d	NM_001163104: c.A433G	8.75

Sensitive: 3rd round Vh-2		
Gene	**Mutation**	**TVF**
Abca3	NM_001039581: c.G3319A	59.62
Wars	NM_001164488: c.737+2T>C	52.94
Prkag3	NM_153744: c.T527G	48.65
Vcan	NM_019389: c.T2171G	47.44
Rtn3	NM_001003933: c.A707C	39.68
Ttn	NM_028004: c.A43832G	12.1
Tnk2	NM_001289443: c.G1078A	50
Nedd1	NM_008682: c.G1099A	46.85
Csnk1g1	NM_173185: c.A398G	44.74
Parp11	NM_181402: c.T560C	42.25
AY761185	NM_001012640: c.G102C	37.75
Slfn8	NM_181545: c.1975_1975delinsAGA-	19.23
Xylt2	NM_145828: c.2017_2017delinsC-	12.5

Sensitive: 4th round Vh-1		
Gene	**Mutation**	**TVF**
Rtn3	NM_001003933: c.A707C	42.11
Vcan	NM_019389: c.T2171G	39.86
Vmn2r65	NM_001105180: c.T1089G	13.82
Syne1	NM_153399: c.T2795C	10.92
Clec14a	NM_025809: c.A1074T	10.28
Tnk2	NM_001289443: c.G1078A	58.82
Cntn3	NM_008779: c.G1303A	44.44
Abca3	NM_001039581: c.G3319A	44
Prkag3	NM_153744: c.T527G	43.1
Parp11	NM_181402: c.T560C	38.1
Nedd1	NM_008682: c.G1099A	35.79
Csnk1g1	NM_173185: c.A398G	34.78
Wars	NM_001164488: c.737+2T>C	31.82
Kmt2c	NM_001081383: c.7198_7198delinsGAG-	11.69
Traf3ip3	NM_153137: c.491-2A>TCTC-	10

Sensitive: 4th round Vh-2		
Gene	**Mutation**	**TVF**
Prkag3	NM_153744: c.T527G	30.43
Xylt2	NM_145828: c.2017_2017delinsC-	15.87
Vmn2r65	NM_001105180: c.T1089G	13.71
Tnk2	NM_001289443: c.G1078A	62.26
Vcan	NM_019389: c.T2171G	47.92
Csnk1g1	NM_173185: c.A398G	47.52
Abca3	NM_001039581: c.G3319A	46.97
Nedd1	NM_008682: c.G1099A	46.43
Rtn3	NM_001003933: c.A707C	45.81
Wars	NM_001164488: c.737+2T>C	44.03
Kcnc4	NM_145922: c.A247G	28

Sensitive: 4th round Vh-3		
Gene	**Mutation**	**TVF**
Tnk2	NM_001289443: c.G1078A	51.02
Csnk1g1	NM_173185: c.A398G	47.37
Nedd1	NM_008682: c.G1099A	39
Rtn3	NM_001003933: c.A707C	38.71
Parp11	NM_181402: c.T560C	38.46
Tanc2	NM_181071: c.A2638G	18.69
Abca3	NM_001039581: c.G3319A	57.14
AY761185	NM_001012640: c.G102C	45.51
Prkag3	NM_153744: c.T527G	42.55
Wars	NM_001164488: c.737+2T>C	40.7
Vcan	NM_019389: c.T2171G	40.65
Slfn8	NM_181545: c.1975_1975delinsAGA-	24
Katna1	NM_011835: c.A1286G	16.92
Nhlrc2	NM_025811: c.T211C	12.04
Krt81	NM_001166157: c.A347C	11.74
2410002F23Rik	NM_025880: c.763_764insAACA	10

Suppl. Table 1.2

Resistant: 3rd round I-5		
Gene	**Mutation**	**TVF**
Grb2	NM_008163 c.67dupG	82.35
Rtn3	NM_001003933: c.A707C	44.12
Baz2b	NM_001001182: c.T998G	36.36
Irak1bp1	NM_001168240: c.A528C	30.86
Usp17lb	NM_201409: c.A259C	30.68
Mpdz	NM_001305284: c.T2965C	26.58
Chrna7	NM_007390: c.1294_1294delinsC-	23.28
Ttn	NM_028004: c.A43832G	12.81
2410002F23Rik	NM_025880: c.760_761insAGATA	10.59
Csnk1g1	NM_173185: c.A398G	50.62
Nedd1	NM_008682: c.G1099A	45.04
Vcan	NM_019389: c.T2171G	42.33
Parp11	NM_181402: c.T560C	31.46
Xirp2	NM_001024618: c.A8851G	13.54

Resistant: 3rd round I-6		
Gene	**Mutation**	**TVF**
Abca3	NM_001039581: c.G3319A	63.22
Csnk1g1	NM_173185: c.A398G	49.16
Prkag3	NM_153744: c.T527G	48.39
Parp11	NM_181402: c.T560C	47.17
Nedd1	NM_008682: c.G1099A	46.75
Vcan	NM_019389: c.T2171G	46.55
Rtn3	NM_001003933: c.A707C	45.5
Cd44	NM_001039151: c.G943A	44.66
AY761185	NM_001012640: c.G102C	43.16
Slc39a12	NM_001012305: c.T466C	43.01
Fat1	NM_001081286: c.A4220G	41.18
Morc2b	NM_177719: c.C1976T	39.86
Myct1	NM_026793: c.T46G	37.89
Prkd1	NM_008858: c.T605C	37.57
Ccp110	NM_182995: c.A3007C	37.31
Klhl29	NM_001164493: c.A35G	37.25
Osbpl1a	NM_207530: c.A1133G	29.61
Ptk2	NM_007982: c.G1603A	27.49
Slfn8	NM_181545: c.1975_1975delinsAGA-	26.67
Amy2b	NM_001190404: c.C604T	24.05
2410002F23Rik	NM_025880: c.763_764insAACA	15.74
2410002F23Rik	NM_025880: c.760_761insAGATA	15.24
Clpx	NM_001044389: c.9_10insTGCGGCGCTTGTACC	10.67

Resistant: 4th round I-1		
Gene	**Mutation**	**TVF**
Abca3	NM_001039581: c.G3319A	53.61
Vcan	NM_019389: c.T2171G	50.31
Wars	NM_001164488: c.737+2T>C	49.59
Nedd1	NM_008682: c.G1099A	44.77
Rtn3	NM_001003933: c.A707C	41.62
Csnk1g1	NM_173185: c.A398G	41.61
Lypd8	NM_027339: c.A405C	37.59
Hrh1	NM_001252642: c.T353C	35.61
Mylk	NM_139300: c.C53T	32.28
Ddhd1	NM_001039106: c.T755C	30.61
Prkag3	NM_153744: c.T527G	28.26
Elp3	NM_001253812: c.C857T	25
Rps12	NM_011295: c.G358A	23.98
Gabra4	NM_010251: c.G191A	23.32
Gc	NM_008096: c.T11G	23.23
Cyp19a1	NM_007810: c.A20C	21.36
Epb4.1l3	NM_013813: c.A44G	21.25
Plce1	NM_019588: c.A5912C	20.69
Klhl29	NM_001164493: c.A35G	20
Fat3	NM_001080814: c.C3513G	19.61
Mboat1	NM_153546: c.T811G	19.31
Crim1	NM_015800: c.A1241G	18.08
Tubal3	NM_001033879: c.A890C	17.83
Anks4b	NM_028085: c.C416T	17.43
B4galnt3	NM_198884: c.G2942A	16.19
Siglec1	NM_011426: c.444dupC	15.38
Itga1	NM_001033228: c.A3175C	14.58
Pcdh18	NM_130448: c.C1735T	13.88
Tshb	NM_001165940: c.T315G	12.36
Clca4b	NM_001033199: c.G1417A	11.99
Foxp2	NM_053242: c.1766+2T>G	10.71

Resistant: 4th round I-2		
Gene	**Mutation**	**TVF**
Vcan	NM_019389: c.T2171G	49.28
Csnk1g1	NM_173185: c.A398G	48.18
Parp11	NM_181402: c.T560C	46.38
Rtn3	NM_001003933: c.A707C	45.63
Abca3	NM_001039581: c.G3319A	45.28
Pramel1	NM_031377: c.T1145C	39.53
Wars	NM_001164488: c.737+2T>C	37.25
B230219D22Rik	NM_181278: c.A8C	33.33
Gpc5	NM_175500: c.A1580C	32.41
Srfbp1	NM_026040: c.A1000C	30
Clpx	NM_001044389: c.9_10insTGCGGCGCTTGTACC	13.33

Resistant: 4th round I-3		
Gene	**Mutation**	**TVF**
Abca3	NM_001039581: c.G3319A	59.04
Prkag3	NM_153744: c.T527G	56.9
Tnk2	NM_001289443: c.G1078A	55.77
Csnk1g1	NM_173185: c.A398G	54.17
Nedd1	NM_008682: c.G1099A	45.67
Wars	NM_001164488: c.737+2T>C	45.54
Vcan	NM_019389: c.T2171G	44.83
Parp11	NM_181402: c.T560C	39.73
Rtn3	NM_001003933: c.A707C	38.59
Sned1	NM_172463: c.T2111G	36.47
Ddhd1	NM_001039106: c.T755C	33.33
Fat3	NM_001080814: c.C3513G	32.73
Pcdh18	NM_130448: c.C1735T	30.17
Fat3	NM_001080814: c.T3568G	29.24
Gdf10	NM_145741: c.C1421T	26.21
Rps12	NM_011295: c.G358A	24.34
Clca4b	NM_001033199: c.G1417A	23.71
Mboat1	NM_153546: c.T811G	23.56
Elp3	NM_001253812: c.C857T	23.08
Siglec1	NM_011426: c.444dupC	22.89
Eml5	NM_001081191: c.G2357A	22.69
Anks4b	NM_028085: c.C416T	22.68
Slfn8	NM_181545: c.1975_1975delinsAGA-	22.58
B4galnt3	NM_198884: c.G2942A	22.09
Ccdc106	NM_146178: c.T422G	20.48
Itga1	NM_001033228: c.A3175C	20.25
Tmem132e	NM_001304439: c.T1007G	16.85
Tubal3	NM_001033879: c.A890C	16.28
Gc	NM_008096: c.T11G	15.71
Hrh1	NM_001252642: c.T353C	14.29
Plce1	NM_019588: c.A5912C	13.99
Mylk	NM_139300: c.C53T	13.46
Lypd8	NM_027339: c.A405C	11.76
Calcoco2	NM_001271018: c.T938A	10.85
4930407I10Rik	NM_001166475: c.A3406C	10.62

Resistant: 4th round I-4		
Gene	**Mutation**	**TVF**
Csnk1g1	NM_173185: c.A398G	47.71
Abca3	NM_001039581: c.G3319A	44.55
Vcan	NM_019389: c.T2171G	44.22
Nedd1	NM_008682: c.G1099A	44.2
Rtn3	NM_001003933: c.A707C	42.77
AY761185	NM_001012640: c.G102C	40.15
Wars	NM_001164488: c.737+2T>C	39.42
Parp11	NM_181402: c.T560C	37.17
Klhl9	NM_172871: c.A1370C	34.13
Chpt1	NM_001146690: c.T572C	31.5
Dnah10	NM_019536: c.A7880G	30.28
Enc1	NM_007930: c.A1055G	25.84
Il2	NM_008366: c.141_141delinsGCA-	21.43
Grb2	NM_008163: c.T421C	20.99
Arhgef10l	NM_001112722: c.G347A	13.79
Kdm6a	NM_009483: c.A4271C	12.56
Xirp2	NM_001024618: c.A8851G	11.43
Ttn	NM_028004: c.A43832G	10.48
Dock5	NM_177780: c.1815_1815delinsA-	10.08
Ednra	NM_010332: c.C964T	10.05
Adcy9	NM_009624: c.G1148A	10

Suppl. Table 2.1: Signaling networks associated with the mutated genes (Ingenuity pathway analysis)

ID	Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
1	CD44, CHRNA7, CYP19A1, DOCK5, ENC1, GC, ITGA1, MYLK, PLCE1, PTK2, SIGLEC1	21	11	Cellular Movement, Immune Cell Trafficking, Inflammatory Response
2	ANKS4B, CCDC106, CCP110, DNAH10, FAT3, GABRA4, GPC5, PCDH18, RPS12, SNED1, SRFBP1	21	11	Lipid Metabolism, Small Molecule Biochemistry, Organismal Development
3	ARHGEF10L, B4GALNT3 , BAZ2B, CHPT1, CLCA4, CRIM1, DDHD1, Foxp2, MYCT1, OSBPL1A	21	11	Molecular Transport, Gene Expression, Behavior
4	ADCY9, EDNRA, PTK2, GDF10, IL2, MPDZ, PRKD1, SLC39A12,TSHB	17	9	Cell-To-Cell Signaling and Interaction, Cell-mediated Immune Response, Cellular Development
5	CLPX, ELP3, EML5, GRB2, HRH1, IRAK1BP1, KDM6A	12	7	Carbohydrate Metabolism, Small Molecule Biochemistry, Dermatological Diseases and Conditions
6	FAT1, KLHL29, MBOAT1, TUBAL3, XIRP2	10	6	Post-Translational Modification, Cellular Function and Maintenance, Developmental Disorder

Suppl. Table 2.2

Pathways associated with the mutated genes (STITCH)

# ID	Pathway description	Observed gene count	FDR	Matching genes in the network (labels)
5205	Proteoglycans in cancer	10	4.52E-07	*Cbl,Cd44,Egfr,Frs2,Gab1,Grb2,Plce1,Ptk2,Pxn,Sos1*
4012	ErbB signaling pathway	7	1.16E-06	*Cbl,Egfr,Gab1,Grb2,Ptk2,Shc1,Sos1*
4510	Focal adhesion	9	1.47E-06	*Bcar1,Egfr,Grb2,Itga1,Mylk,Ptk2,Pxn,Shc1,Sos1*
5100	Bacterial invasion of epithelial cells	6	9.18E-06	*Bcar1,Cbl,Gab1,Ptk2,Pxn,Shc1*
4020	Calcium signaling pathway	7	6.49E-05	*Adcy9,Chrna7,Ednra,Egfr,Hrh1,Mylk,Plce1*
4062	Chemokine signaling pathway	7	6.49E-05	*Adcy9,Bcar1,Grb2,Ptk2,Pxn,Shc1,Sos1*
5220	Chronic myeloid leukemia	5	0.00011	*Cbl,Gab2,Grb2,Shc1,Sos1*
4810	Regulation of actin cytoskeleton	7	0.000155	*Bcar1,Egfr,Itga1,Mylk,Ptk2,Pxn,Sos1*
5206	MicroRNAs in cancer	6	0.000155	*Cd44,Egfr,Grb2,Shc1,Sos1,Spry2*
4014	Ras signaling pathway	7	0.000169	*Egfr,Gab1,Gab2,Grb2,Plce1,Shc1,Sos1*
4540	Gap junction	5	0.000193	*Adcy9,Egfr,Grb2,Sos1,Tubal3*
4915	Estrogen signaling pathway	5	0.000273	*Adcy9,Egfr,Grb2,Shc1,Sos1*
4722	Neurotrophin signaling pathway	5	0.000716	*Frs2,Gab1,Grb2,Shc1,Sos1*
5214	Glioma	4	0.000716	*Egfr,Grb2,Shc1,Sos1*
4320	Dorso-ventral axis formation	3	0.000823	*Egfr,Grb2,Sos1*
4912	GnRH signaling pathway	4	0.00262	*Adcy9,Egfr,Grb2,Sos1*
4015	Rap1 signaling pathway	5	0.00843	*Adcy9,Bcar1,Egfr,Plce1,Prkd1*
5213	Endometrial cancer	3	0.00869	*Egfr,Grb2,Sos1*
5223	Non-small cell lung cancer	3	0.00974	*Egfr,Grb2,Sos1*
4910	Insulin signaling pathway	4	0.0125	*Cbl,Grb2,Shc1,Sos1*
5211	Renal cell carcinoma	3	0.0145	*Gab1,Grb2,Sos1*
4630	Jak-STAT signaling pathway	4	0.0164	*Cbl,Grb2,Sos1,Spry2*
4664	Fc epsilon RI signaling pathway	3	0.0164	*Gab2,Grb2,Sos1*
4917	Prolactin signaling pathway	3	0.0193	*Grb2,Shc1,Sos1*
4080	Neuroactive ligand-receptor interaction	5	0.0211	*Chrna7,Ednra,Gabra4,Hrh1,Tshb*
5215	Prostate cancer	3	0.0292	*Egfr,Grb2,Sos1*
5200	Pathways in cancer	5	0.0343	*Cbl,Egfr,Grb2,Ptk2,Sos1*
4151	PI3K-Akt signaling pathway	5	0.0429	*Egfr,Grb2,Itga1,Ptk2,Sos1*
4660	T cell receptor signaling pathway	3	0.0429	*Cbl,Grb2,Sos1*

Suppl. Table 2.3

Biological processes associated with the gene mutations (DAVID)

No.	GO Term	Biological process	Count	%	P-Value	Fold Enrichment	FDR
1.	GO:0007155	cell adhesion	7	11.290	0.0021	5.117	2.918
2.	GO:0007265	Ras protein signal transduction	3	4.838	0.0074	22.630	9.992
3.	GO:0000187	activation of MAPK activity	3	4.838	0.0187	13.995	23.304
4.	GO:0050728	negative regulation of inflammatory response	3	4.838	0.0241	12.225	29.006
5.	GO:0001764	neuron migration	3	4.838	0.0461	8.577	48.436
6.	GO:0048010	vascular endothelial growth factor receptor signaling pathway	2	3.225	0.0669	28.363	62.130
7.	GO:0007205	protein kinase C-activating G-protein coupled receptor signaling pathway	2	3.225	0.0746	25.324	66.299
8.	GO:0007156	homophilic cell adhesion via plasma membrane adhesion molecules	3	4.838	0.0747	6.525	66.324
9.	GO:0051928	positive regulation of calcium ion transport	2	3.225	0.0797	23.636	68.820
10.	GO:0007399	nervous system development	4	6.451	0.0860	3.761	71.680
11.	GO:0001934	positive regulation of protein phosphorylation	3	4.838	0.0918	5.780	74.108

Suppl. Table 3

Characteristics of CLL patient cohort used for *in vitro* analysis

Sample	Sex	Age	IGHV	TP53	FISH	Previous/current Therapies	*IGF1R* mRNA expression
#1	female	69	Mut	n.a.	normal	Untreated	Low
#2	female	54	Mut	WT	13q-	Untreated	Low
#3	male	65	Mut	n.a.	13q-	Untreated	Low
#4	male	61	Unmut	WT	trisomy 12, 14q-, t(14;18)	Chemo-immunotherapy	Low
#5	male	64	Unmut	n.a.	13q14.3	Untreated	Low
#6	male	49	Unmut	n.a.	13q-	Chemo-immunotherapy	Low
#7	female	70	Mut	WT	13q-	Untreated	High
#8	male	55	Unmut	n.a.	13q-	Untreated	High
#9	male	71	Mut	Mut	normal	Chemo-immunotherapy	High
#10	male	76	Mut	n.a.	13q-	Chemotherapy	High
#11	female	69	n.a.	n.a.	13q-, 17p-	Prior: Chemo-immunotherapy Current: Rituximab+Idelalisib	High
#12	male	48	Mut	n.a.	normal	Chemo-immunotherapy	High

Suppl. Table 4

Coverage statistics for exome sequencing

File name	bp on target [%]	Average Coverage	No Coverage	1x Coverage	10x Coverage	15x Coverage	50x Coverage	120x Coverage	200x Coverage
TCL1-192 tumor 1	47.56	126.929	0.13	99.87	99.05	98.17	82.10	49.03	38.52
TCL1-192 tumor 2	49.28	174.777	0.14	99.86	99.14	98.52	89.00	65.89	56.59
Sens: 1st round Vh-1	38.91	85.9038	0.14	99.86	98.60	97.08	69.83	29.13	19.88
Sens: 2nd round Vh-1	42.90	80.9129	0.15	99.85	98.20	96.21	65.02	25.85	17.69
Sens: 3rd round Vh-1	49.64	119.011	0.22	99.78	98.26	96.92	77.97	45.32	35.53
Sens: 3rd round Vh-2	40.02	111.973	0.12	99.88	99.33	98.76	87.17	36.63	6.92
Sens: 4th round Vh-1	47.51	124.91	0.11	99.89	99.37	98.83	88.05	56.44	43.36
Sens: 4th round Vh-2	44.75	128.207	0.12	99.88	99.35	98.85	89.29	59.28	46.07
Sens: 4th round Vh-3	39.01	100.156	0.12	99.88	99.17	98.45	83.49	42.25	27.88
Res: 3rd round I-5	505.10	118.079	0.18	99.82	97.45	94.64	71.44	47.93	39.93
Res: 3rd round I-6	505.10	118.079	0.18	99.82	97.45	94.64	71.44	47.93	39.93
Res: 4th round I-1	48.06	153.051	0.12	99.88	99.47	99.09	91.88	68.82	57.83
Res: 4th round I-2	40.11	107.214	0.14	99.86	99.26	98.62	84.93	46.20	32.14
Res: 4th round I-3	43.09	131.326	0.13	99.87	99.40	98.93	89.61	47.37	13.67
Res: 4th round I-4	47.22	156.366	0.12	99.88	99.46	99.06	92.00	58.45	23.34

Suppl. Table 5

shRNA sequences for the knockdown of GSK3B

Target gene	Clone ID	Mature Antisense 5´ to ´3
GSK3B shRNA 1	TRCN0000010551	TCTTTCCAAACGTGACCAGTG
GSK3B shRNA 2	TRCN0000010552	TTTCTACCAACTGATCCACAC
GSK3B shRNA 3	TRCN0000000822	TTTGGTAGTTTGACATTTGGG

Suppl. Table 6

List of antibodies which have been used in Western blot analysis

Antibody	Catalog Number	Molecular weight [kDa]	2nd antibody	Dilution of stock	Incubation time
p-IGF1R	#3021 CST	95	rabbit	1:500	Over night 4°C
IGF1R	#9750 CST	95	rabbit	1:500	Over night 4°C
p-AKT^{Thr308}	#4056 CST	60	rabbit	1:500	Over night 4°C
AKT	#9272 CST	60	rabbit	1:1000	Over night 4°C
p-ERK (E-4)	# sc-7383 Santa Cruz	44 → p-ERK1 42 → p-ERK2	mouse	1:1000	Over night 4°C
ERK1/2 (K-23)	#sc-94 Santa Cruz	44	rabbit	1:1000	Over night 4°C
LAMIN B (C-20)	#sc-6216 Santa Cruz	67	goat	1:500	Over night 4°C
β-ACTIN	#sc-1615 Santa Cruz	42	goat	1:500	Over night 4°C
P-FOXO	#9461 CST	82	rabbit	1:500	Over night 4°C
FOXO	#2880 CST	80	rabbit	1:500	Over night 4°C

In 5% BSA + TBS-T 0.1%

Suppl. Table 7

List of antibodies which have been used in Simple Western™ analysis

Antibody	Catalog number	Molecular Weight [kDa]	2nd antibody	Dilution of stock	Incubation time
IGF1R	#3027 CS	95	rabbit	1:50 In ProteinSimple antibody diluent II	30 minutes
p-AKT^{Ser473}	#4058 CS	60	rabbit	1:25 In ProteinSimple antibody diluent II	30 minutes
AKT	#2920 CS	60	rabbit	1:100 In ProteinSimple antibody diluent II	30 minutes
p-ERK$^{Tyr202/204}$	#9101 CS	44 → p-ERK1 42 → p-ERK2	rabbit	1:50 In ProteinSimple antibody diluent II	30 minutes
ERK1/2	#9102 CS	44 → ERK1 42 → ERK2	rabbit	1:100 In ProteinSimple antibody diluent II	30 minutes
PI3K-α	#4255 CS	110	rabbit	1:50 In ProteinSimple antibody diluent II	30 minutes
PI3K-β	#3011 CS	110	rabbit	1:100 In ProteinSimple antibody diluent II	30 minutes
PI3K-δ	#sc-7176 Santa Cruz	110	rabbit	1:100 In ProteinSimple antibody diluent II	30 minutes
PI3K-γ	#ABD-026L Jena Bioscience	110	mouse	1:50 In ProteinSimple antibody diluent II	30 minutes
β-ACTIN	#4968 CS	45	rabbit	1:250 In ProteinSimple antibody diluent II	30 minutes

Suppl. Table 8

Flow cytometry antibodies and respective clones

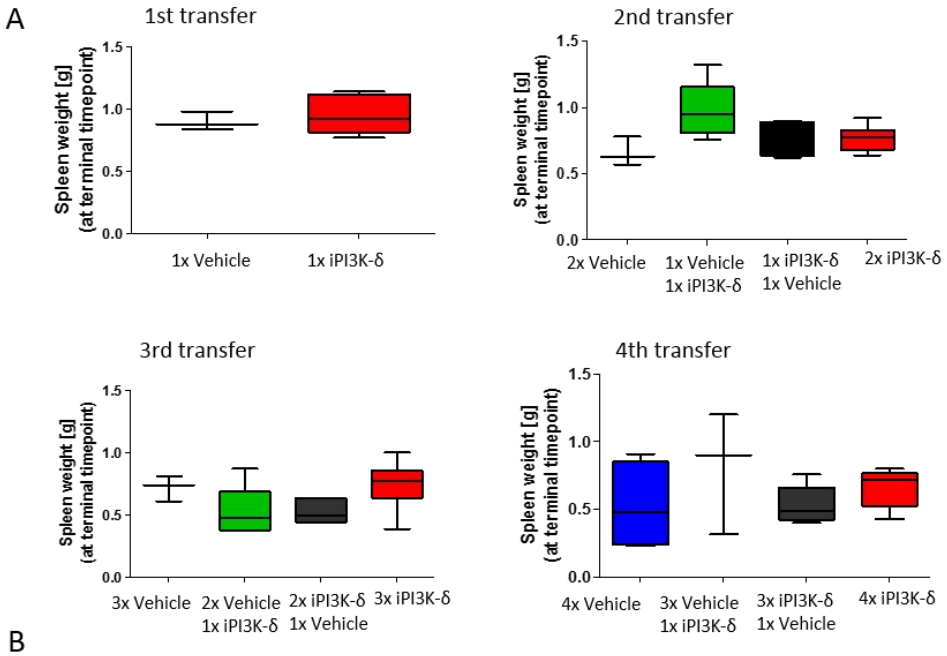
Antibody	Clone	Cat. Nr.	Vendor
Anti-Mouse CD5 PE	53-7-3	12-0051-82	eBioscience
Anti-Mouse CD19 APC	eBio1D3	17-0193-82	eBioscience
Anti-Mouse CD45.2 Alexa Fluor 700	104	56-0454-82	eBioscience
Anti-Mouse CD3 Pacific Blue	17A2	100214	BioLegend
Anti-Mouse CD11b PE-Cyanine7	M1/70	25-0112-82	eBioscience
Anti-Hu/Mo CD45R (B220) PerCP-Cyanine5.5	RA3-6B2	45-0452-82	eBioscience
Anti-Mouse CD45.1 APC/Cy7	A20	110716	BioLegend

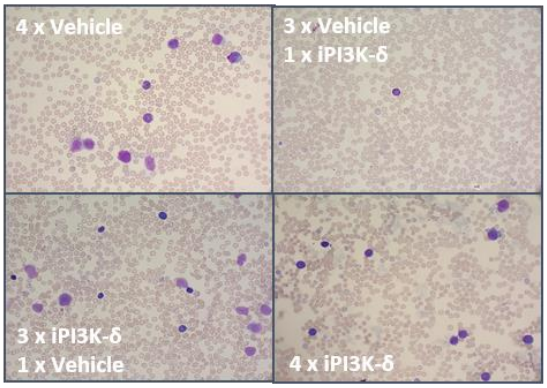
Suppl. Table 9

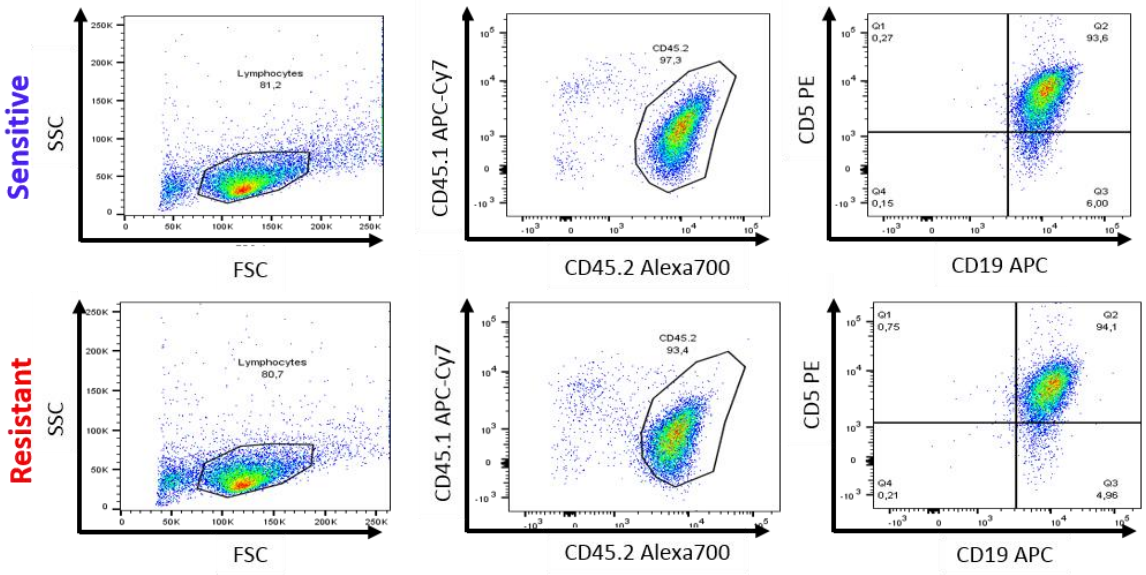
Primers for gene expression analysis using RT-qPCR analysis

Species	Target gene	Forward primer 5′- 3′	Reverse primer 5′- 3′
mouse	*Ak4*	AAAGGATCGCCCAGAACTTT	GCCACGTCACCAACTTCC
mouse	*Angt2*	ACATGCACCGTGAACGAG	TGATGAGTCAATTGTCTGGTAGG
mouse	*bFGF*	CGGCTCTACTGCAAGAACG	TGCTTGGAGTTGTAGTTTGACG
mouse	*Brca1*	TTGAGACACGCGCTTAACCT	ATCAAGTTCACTGTCTTCCATTTCT
mouse	*Brca2*	GAAGGAAGCACTGAGATTTGC	ACACCGCTGTGTTGTGTCTT
mouse	*Cdkn3*	GATGAAGAACAGACTCCAATTCAA	AACCTGGAAGAGCACATAAACC
mouse	*Erbb2ip*	GTTCTCACTATGGCAGCTCTAGG	GACTGGATGTCTGCGGATG
mouse	*Igf1r*	GAGAATTTCCTTCACAATTCCAT	CACTTGCATGACGTCTCTCC
mouse	*Pdgfα*	CACCAGCAGCGTCAAGTG	TTCCTGACATACTCCACTTTGG
mouse	*Pdgfβ*	CTGTGATCGAGAATGGCTACG	CAGCAATTTCTACATCTCCCAGT
mouse	*Rabl5*	ATTCGAGCTCTGGGACTGTG	CCATGAGCATCCTTCATCAG
mouse	*Rasgrp3*	TGCACCGATGGTATTTATCCT	CTCCACTGGCATTCCGATAC
mouse	*Rna Pol*	CATCAACCAGGTGGTACAGC	GATTCTGGAACTCAACACTCTCC
mouse	*Tec*	ATTGAATATCACAAGCACAATGC	CCCTTTGTACTGACCGGGTA
mouse	*Tnfawp8/1*	AACTGAAGGCTTGACACATCC	GGCTCTTCGTGCTGAAGGT
mouse	*Ube2e3*	GGAGCTAGCAGAGATAACCCTTG	ATCTCCTTTAGGCCCAGCAC
mouse	*Wt1*	CAGATGAACCTAGGAGCTACCTTAAA	TCTGCCCTTCTGTCCATTTC
human	*Igf1R*	TTCAGCGCTGCTGATGTG	AAGTTCCCGGCTCATGGT
human	*RPL19*	ATCGATCGCCAGATGTATCA	GCGTGCTTCCTTGGTCTTAG
human	*Gsk3ß*	GACATTTCACCTCAGGAGTGC	GTTAGTCGGGCAGTTGGTGT
human	*Foxo1*	GTGGGGCAACCTGTCGTA	TTCTCGGCTGAGCTCTCG
human	*Foxo3*	GCTAAGCAGGCCTCATCTCA	TTCCGTCAGTTTGAGGGTCT
human	*Foxo4*	ACGAGTGGATGGTCCGTACT	GTGGCGGATCGAGTTCTTC

Suppl. Figure 1







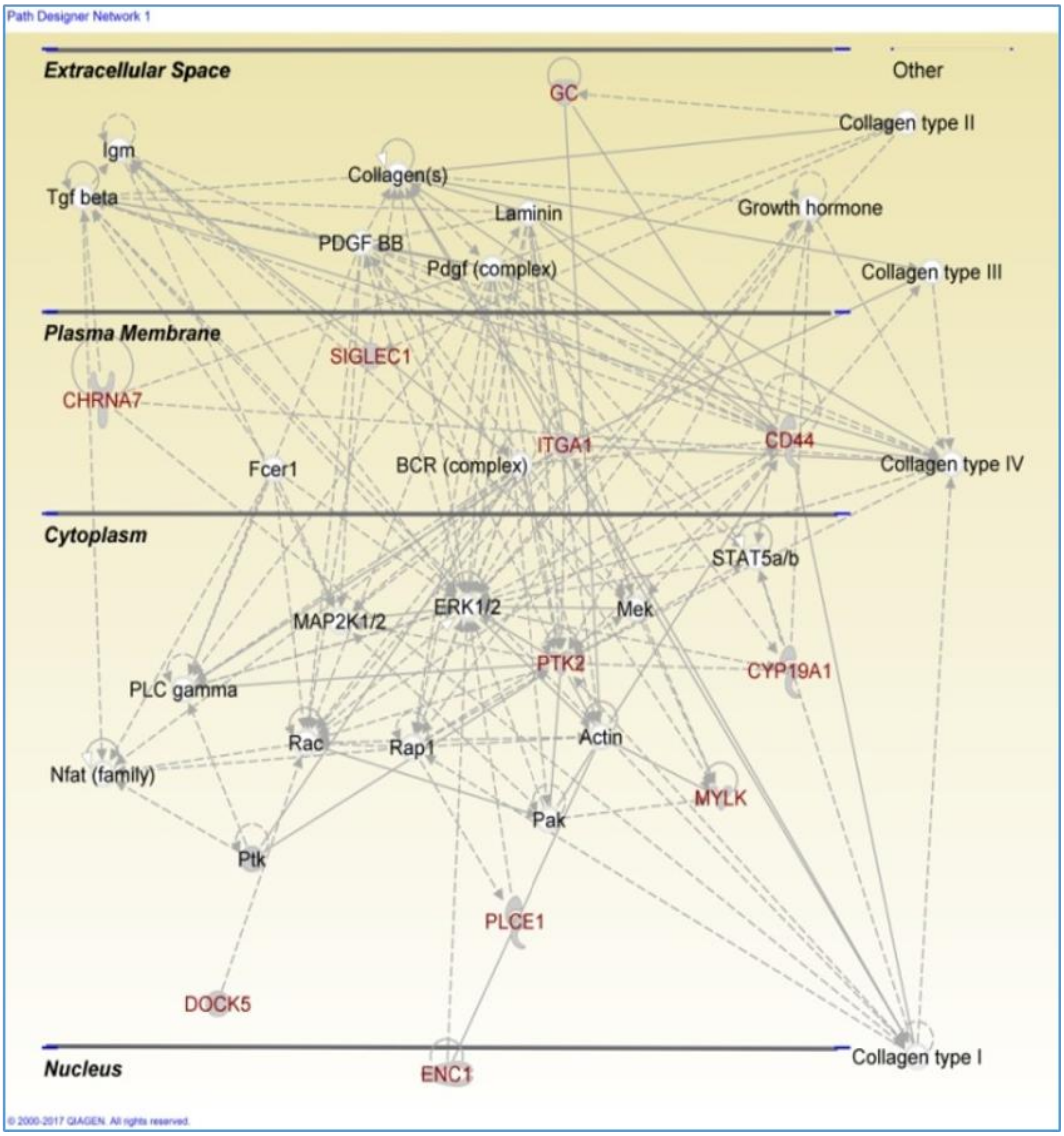
Suppl. Fig. 1

(A) Spleen weights at the terminal time point (Fig 2A, 2B) of mice in the different treatment groups. Similar spleen weights between the different treatment groups, indicate that all mice became critically sick because of an increase in tumor burden at the terminal time point.

(B) Representative blood smears of mice from the 4th transfer that were sacrificed after 5 days of treatment start. The groups included mice which received tumors that were previously treated with the vehicle (3X vehicle) or inhibitor (3X inhibitor) in the previous transfers. Subsequently, the mice were treated with either vehicle or GS-649443 in the 4th round for 5 days.

(C) Representative FACS blots showing CLL tumor cell marker expression of CD19 and CD5 in sensitive and resistant cells. Cells of sensitive and resistant mice display similar morphologies.

Suppl. Figure 2

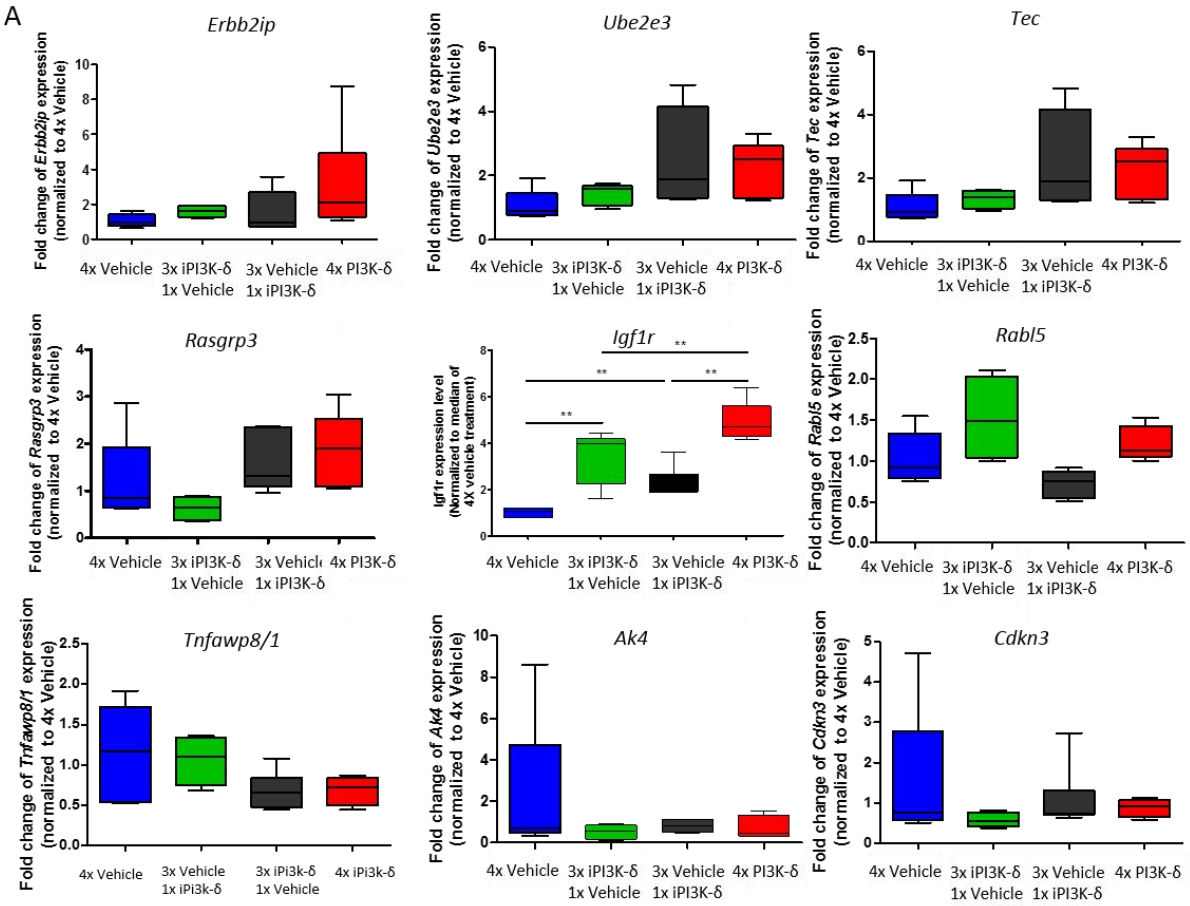


Suppl. Fig. 2

Pathway generated using Ingenuity Pathway Analysis (IPA) from mutated genes identified in the resistant tumor cell clones. The pathway corresponds to the top enriched functional network namely, cellular movement, immune cell trafficking and inflammatory response (Suppl.Table 2.1).

Suppl. Figure 3

A



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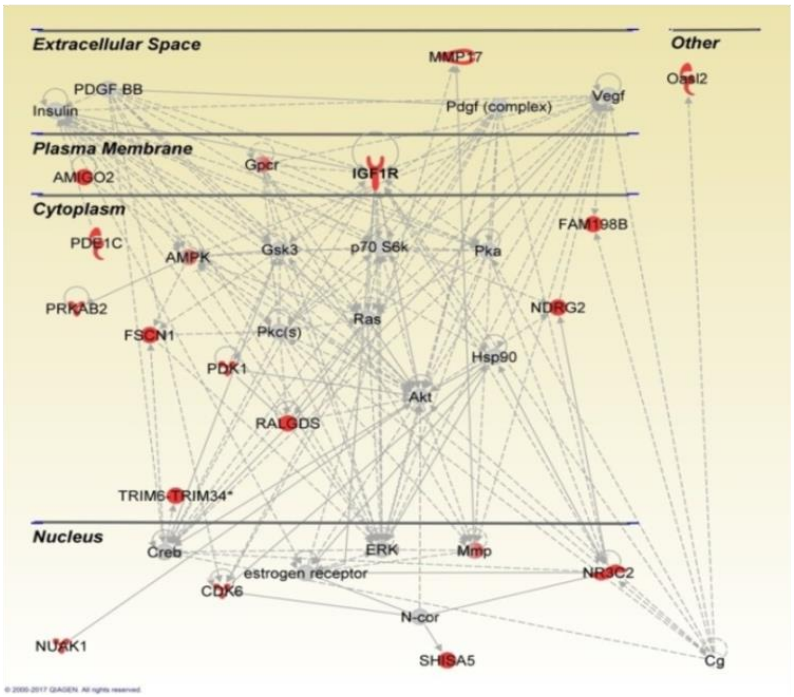
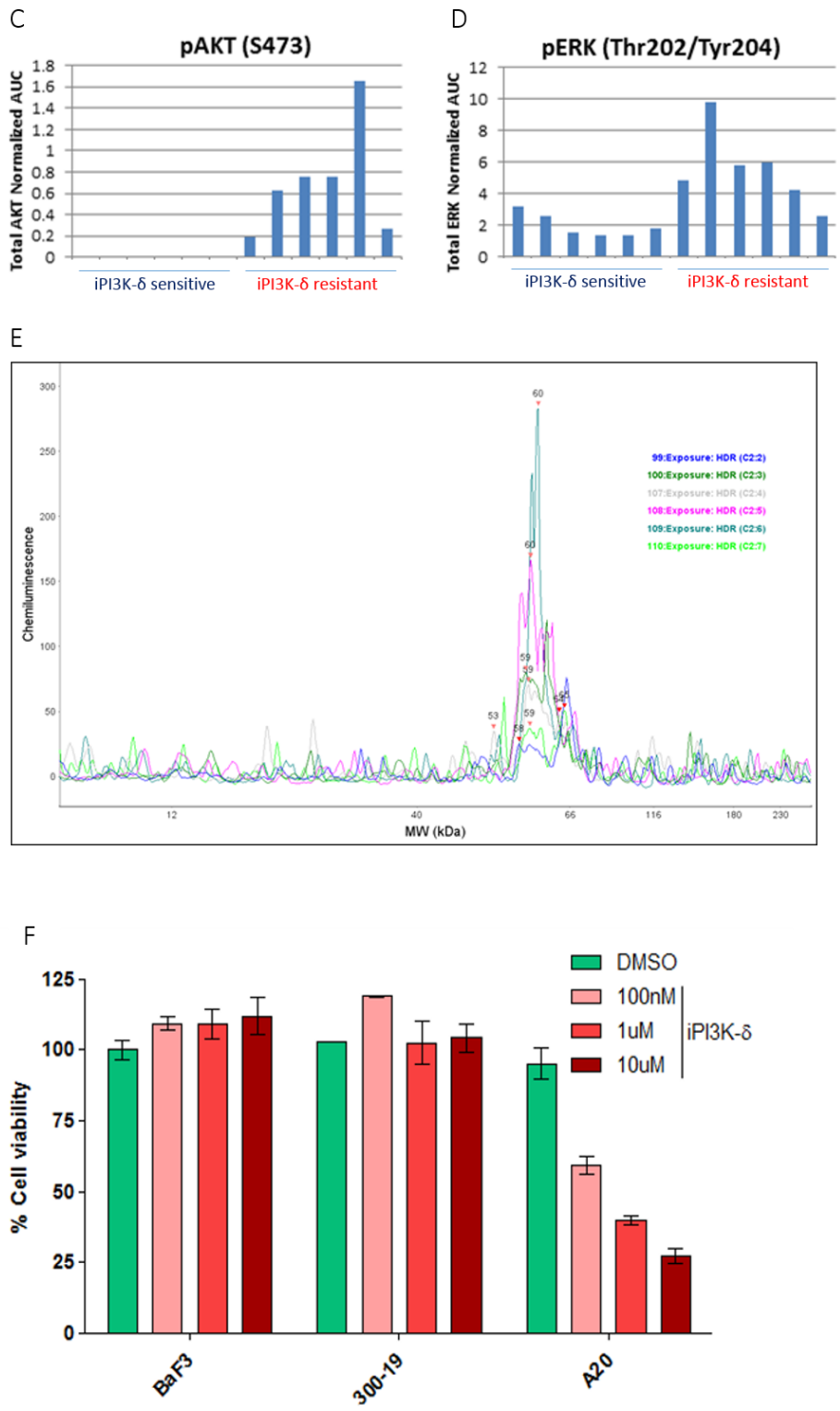




Suppl. Figure 3

(A) RT-qPCR validation of the genes identified with RNAseq to be differentially expressed in the PI3K-δ inhibitor resistant tumor cells in comparison to sensitive tumor cells. Tumors obtained from the 4th transfer were used for the analysis.

(B) Pathway generated using Ingenuity Pathway analysis (IPA) based on the differentially expressed genes from RNAseq analysis. The pathway corresponds to the top deregulated signaling network, namely, growth factor receptor signaling.

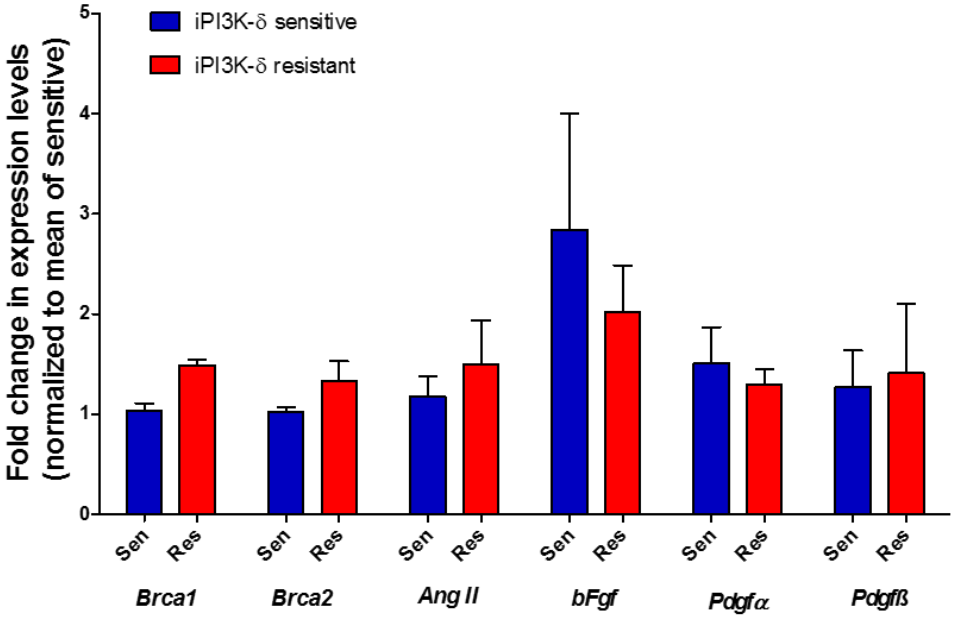
(C) Quantification of phospho AKT and (D) phospho ERK bands from Simple Western in Fig 3B.

(E) Chemiluminescence corresponding to specific detection of phospho AKT at ~60kDa in Fig 3B

(F) Cell viability measured by MTS assay after treatment of murine BaF3, 300-19 and A20 with DMSO, 100nM, 1µM and 10µM of GS-649443 for 2 days.

Suppl. Figure 4

A



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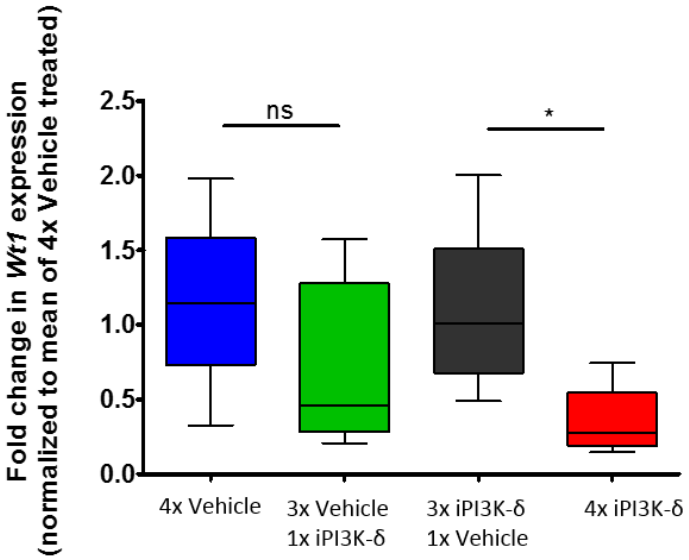

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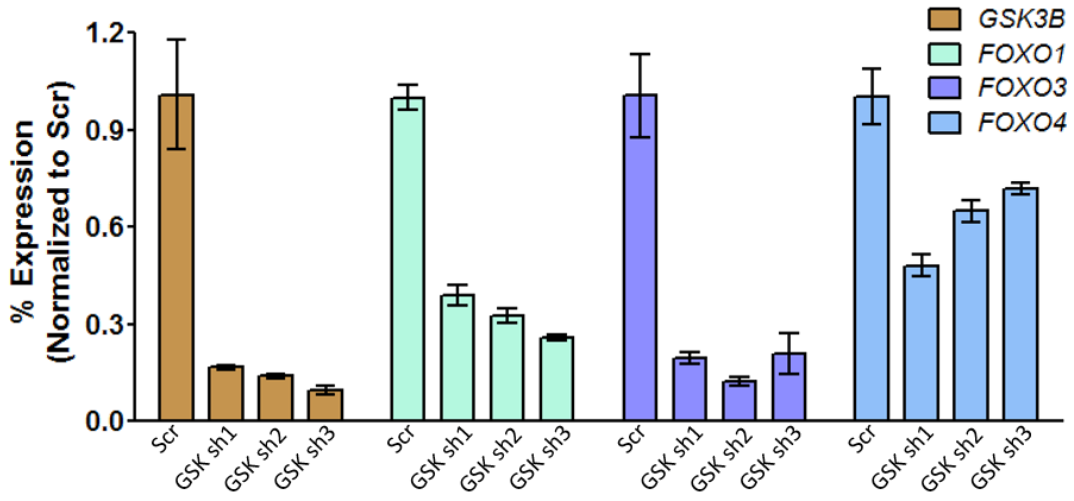
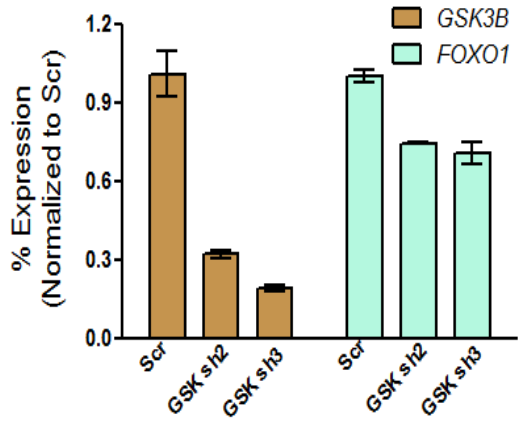


D



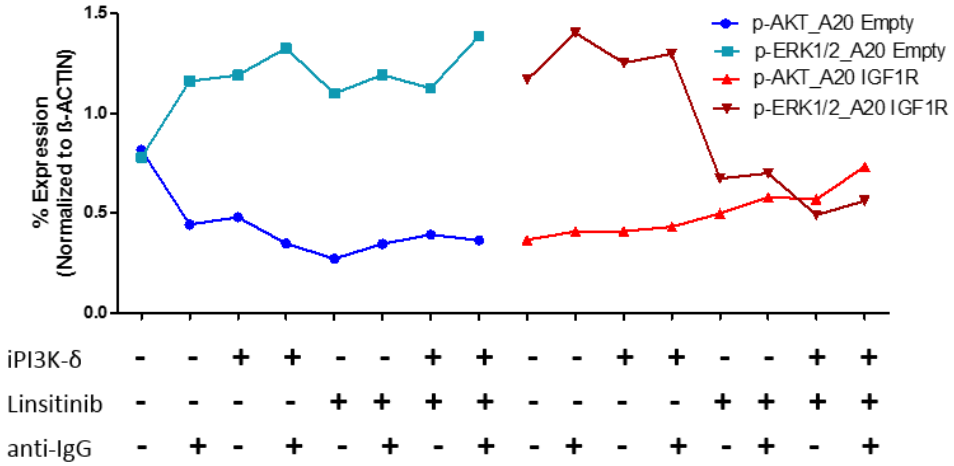
(A) RT-qPCR analysis for expression of genes described to be transcriptional regulators of *Igf1r* in tumors from the 4th transfer that were either treated with vehicle (iPI3K-δ sensitive) or GS-649443 (iPI3K-δ resistant)across the different treatment rounds.

(B) RT-qPCR analysis for expression levels of the *Wt1*, a transcriptional repressor of *Igf1r* in tumors from the 4th transfer. Significant downregulation (P=0.0173, Mann-Whitney test) was observed in the resistant tumors treated with GS-649443 compared to vehicle treatment.

(C) RT-qPCR analysis for expression levels of *GSK3B*, *FOXO1*, *FOXO3* and *FOXO4* upon knockdown of GSK3B in the human NHL cell line JVM2 using 3 different shRNAs (GSK sh1, GSK sh2 and GSK sh3) and (D) in MEC-1 cells using 2 different shRNAs (GSK3 sh2 and GSK3 sh3). No knockdown of GSK3B was observed in GSK3 sh1in Mec1 cells. Expression levels of the genes were normalized to that of scrambled vector (Scr) transduced cells.

Suppl. Figure 5

A



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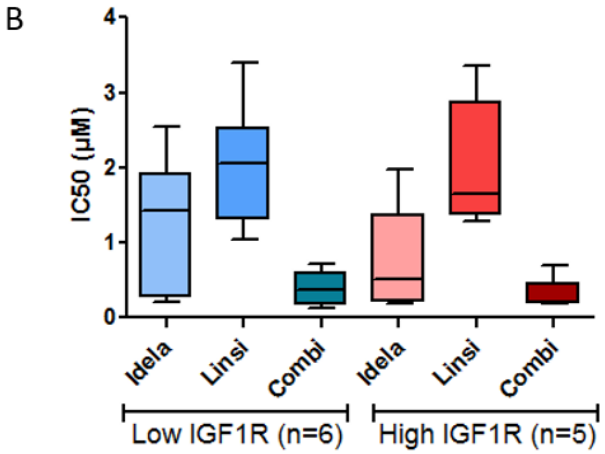

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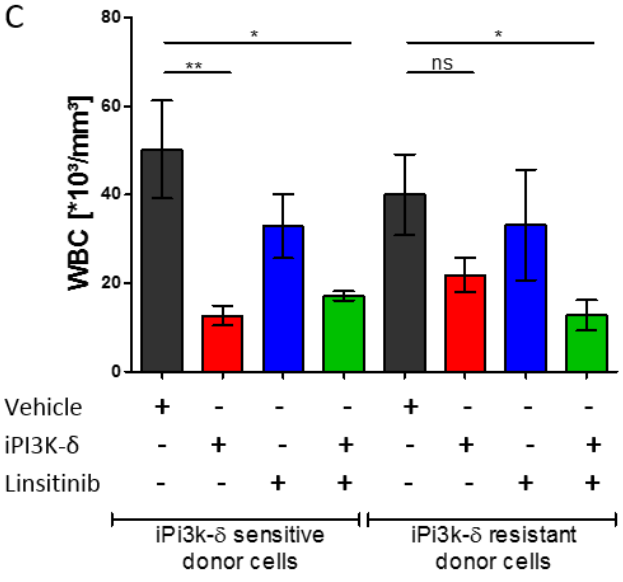

Suppl. Figure 5

(A) Quantification of Fig.6C, were A20 cells, transduced with empty vector or *Igf1r* cDNA, were treated with DMSO, GS-649443 or linsitinib in the presence or absence of anti-IgG stimulation.
p-AKT and p-ERK levels have been quantified and normalized to ß-Actin.

(B) Comparison of IC50 from high and low IGF1R expressing samples, (excluding idelalisib treated patient sample) after treatment with idelalisib, linsitinib or the combination for 4 days. Viability assessed using DiOC6/PI staining and FACS.

(C) WBC count after 5 days of treatment from mice transplanted with iPI3K-δ sensitive or resistant tumors from the 3rd round and subsequently treated with either vehicle, GS-649443, linsitinib or the combination of GS-649443 and linsitinib in the 4th round. Significant reduction of WBC was observed in the iPI3k-δ sensitive group treated with GS-649443 (vehicle vs. iPI3K-δ P=0.0087) or with the combination treatment (vehicle vs. linsitinib+ iPI3K-δ; P=0.0173). In the iPI3k-δ resistant group, combination treatment of GS-649443 and linsitinib led to significant reduction in WBC count (vehicle vs. linsitinib+iPI3K-δ; P=0.0173). All P values were obtained from Mann-Whitney test.

Suppl. Figure 6: Graphical abstract