## Supplemental Information

## Extended Experimental Procedures

### Mouse Strains

*Eμ-Myc/+* mice have been previously characterized (Adams, 1985). *Rpl24*$^{BST/+}$ (L24+/-) mice have been previously characterized (Oliver, 2004). To generate *Eμ-Myc/+;Rpl24*$^{BST/+}$ mice, we intercrossed Eμ-Myc mice to *Rpl24*$^{BST/+}$ mice (Barna et al., 2008). CD19-Cre mice have been previously characterized (Rickert et al., 1997). *TetO-DN-4EBP1* mice have been previously characterized (Hsieh et al., 2010). ROSA26-rtTA*M2 mice have been previously characterized and were from Jackson Laboratories (Stock #006965) (Hochedlinger et al., 2005). ROSA26-Lox-STOP-Lox-rtTA-IRES-GFP mice have been previously characterized (Jackson Laboratories Stock #005670) (Belteki, 2005). The University of California San Francisco Institutional Animal Care and Use Committee approved all studies involving live mice.

### B Cell Isolation

Splenic B lymphocytes were isolated using Miltenyi Mouse B cell isolation kit (#130-090-862) in conjunction with Miltenyi magnetic separation columns (#130-042-401) per manufacturer instructions.

### Metabolite Extraction from B Cells

B lymphocytes were isolated using MACS separation, pelleted and then resuspended in 2mL methanol. To isolate the intracellular aqueous metabolites, chloroform and water were then added to the methanol in equal volumes (final solution 1:1:1 methanol:chloroform:water). The solution was vortexed and centrifuged to separate the aqueous and lipid phases.

For Magnetic Resonance experiments, the dried aqueous phase polar extracts (approximately 8 replicates per genotype) were then redissolved in 200 µl of 100 mM phosphate buffer (pH 7.0) prepared in 90% $H_2O$ - 10% $D_2O$ and containing 0.5 mM sodium 3-(trimethylsilyl)propionate-2,2,3,3-d4 (TMSP, Cambridge Isotope Laboratories) as internal reference (Ronen et al., 2001).

For HPLC and dNTP measurement, the aqueous phase was dried using a spin vac and resuspended in 250µL HPLC grade water. Subsequently, aqueous extracted metabolites were passed through a Costar Spin-X 0.22µm cellulose acetate column to remove any particulates prior to analysis by HPLC or PCR-based dNTP quantitation. Cellular dNTP concentrations were measured as described (Ferraro et al., 2009). HPLC was performed essentially as described using a Varian Microsorb 100-5 $C_{18}$ 250 x 4.6mm (particle size 5µm) column was utilized in conjunction with a Varian ProStar HPLC system (Smolenski et al., 1990).

### Metabolic Flux experiments using [$^{14}$C] formate, [8-$^{14}$C] hypoxanthine and [U-$^{14}$C] glucose

Purine metabolic labeling experiments were performed essentially as described (Boss and Erbe, 1982). [$^{14}$C] formate and [8-$^{14}$C] hypoxanthine were from American Radiolabeled Chemicals, (#ARC 0163A and #ARC 0364, respectively). Briefly, freshly isolated B cells were cultured in growth factor and serum free RPMI 1640 media at a density of 5x10$^6$/mL. Immediately after isolation, radiolabeled formate or hypoxanthine was added and cells were cultured for 2 hours. Cells were then harvested, pelleted and lysed in 1 ml of 0.4 N perchloric acid, heated at 100°C for 70 min, cooled on ice for 5 min, centrifuged at 1000 X g for 5 min, and the supernatant was applied to Bio-Spin Disposable Chromatography Columns (Bio-Rad #732-6008) packed with pre-equilibrated AG 50W-X4 Resin, 100-200 mesh (Bio-Rad #142-1341). The columns were washed 8 times with 1.0 ml of 0.1 N HCl, and the purines were eluted with 3 washes of 1.0 ml of 6N HCl. A 1.0mL aliquot of this eluate was added to 10 mL of scintillation fluid and radioactivity was measured by liquid scintillation counting.

Measurement of $^{14}$C-labelled formate incorporation into RNA, DNA, and protein was performed as described (White et al., 1975). Measurement of [U-$^{14}$C] glucose into purines, RNA, and DNA was performed using the protocols adapted from [$^{14}$C] formate incorporation with 0.2µCi/mL [U-$^{14}$C] glucose (specific activity 10mCi/mmol). Glucose oxidation was performed essentially as described (Gerhart-Hines et al., 2007) except cells were cultured in RPMI 1640 media. [U-$^{14}$C] glucose was from Perkin-Elmer (#NEC042V250UC).

**qRT-PCR analysis**

RNA was isolated from cells using Trizol reagent (Invitrogen) per manufacturer's instructions. cDNA was generated using High-Capacity cDNA reverse transcription kit (Applied Biosciences). Quantitative real-time PCR was performed using iQ SYBR green mix (BioRad) on a MyIQ2 instrument. Data was analyzed using the Δ-ΔCt method and data were normalized to expression of *β-Actin* unless otherwise specified. The following primers were used to measure the indicated mouse genes.

*β Actin* – Forward: 5'-GACATGGAGAAGATCTGGCA-3'
Reverse: 5'-GGTCTCAAACATGATCTGGGT-3'

*Prps2* – Forward: 5'-ATGAAGTGGACCGGATGGTT-3'
Reverse: 5'-GGTGGCACCAGCTGAGAGTA-3'

*Ppat* – Forward: 5'-AGGAATGTGGTGTGTTTGGGT-3'
Reverse: 5'-CAATACCAGCGCTCTCCTGA-3'

*Umps* – Forward: 5'-CCAATCACATTCCCATGCTC-3'
Reverse: 5'-AACACTGGCTCCGCTGGT-3'

*Ctps* – Forward: 5'-GTGTGCAGGTGCTCAAATCC-3'
Reverse: 5'-CAAGGGTACCCGGTAGATGG-3'

*Impdh2* – Forward: 5'-CGCAAGCCAAGAACCTCATA-3'
Reverse: 5'-AAGCGACGGGCATACTCAG-3'

*Atic* – Forward: 5'-TATGTGACCGGCACTATCGG-3'
Reverse: 5'-GCTTGTCCACCCATTCCTTC-3'

*Prps1* – Forward: 5'-CCTGCCATTTCTCGAATCAA-3'
Reverse: 5'-GTGGGTTCTCCTGATGGCTT-3'

*Rplp0* – Forward: 5'-GCAGACAACGTGGGCTCCAAGCAGAT-3'
Reverse: 5'-GGTCCTCCTTGGTGAACACGAAGCCC-3'

*c-Myc* – Forward: 5'-CCAACAGGAACTATGACCTCG-3'
Reverse: 5'-AGCAGCTCGAATTTCTTCCAG-3'

*Eif4e* – Forward: 5'-CAGGAGGTTGCTAACCCAGA-3'
Reverse: 5'-CTTAGAGATCAATCGAAGGTTTGC-3'

**Antibodies and Reagents**

Antibodies used were: PRPS2 (Abnova #H00005634-A01), β-Actin (Sigma #A5316), PPAT (Sigma #HPA036092), IMPDH2 (Sigma #HPA001400), CTPS (Epitomics #6603-1), UMPS (Abcam #ab80857), GAPDH (Cell Signaling #2118), RPS3 (Abcam #ab77772), ATIC (Abcam #ab33520), 4EBP1 (Cell Signaling #9644), Tubulin (Sigma #T8203). P-4EBP T37/46 (Cell Signaling #2855), P-eIF4E S209 (Cell Signaling #9741), eIF4E (BD Biosciences #610270), PRPS1/2 (Epitomics #S2623/abcam #ab38209), PAN PRPS (Santa Cruz #sc-376440), C-MYC (Epitomics #1472), cleaved PARP (Cell Signaling #9544). APC-conjugated rat-anti mouse B220 was from the UCSF hybridoma core. Control and mouse *Prps1* and *Prps2* siRNA were purchased from Dharmacon. FLAG-*Prps2* mRNA was generated from linearized plasmid DNA using the mMessage mMachine T7 Ultra Kit (Invitrogen). FLAG-*Prps2* mRNA was transfected into cells using TransIT transfection reagent (Mirus). MLN0128 was kindly provided by the Shokat Laboratory at UCSF. PD0325901 (referred to as PD901 in the text and figures) was from Sigma (catalog #PZ0162). Mitotracker Deep Red, Mitotracker Green and 2-NBDG dyes were from Life Technologies and stains were carried out according to manufacturer's protocols. 4-hydroxytamoxifen was from Sigma (catalog #H7904).

**Plasmids and Retrovirus Constructs**
Constructs encoding *MycER* retroviruses have been previously described (Barna et al., 2008). FLAG-tagged mouse *Prps2* plasmid was generated by TOPO cloning the FLAG-*Prps2* PCR fragment into the pCRII TOPO vector downstream of the T7 promoter or into pGL3-promoter containting wild-type or ΔPRTE 5'UTR of *Prps2* with SV40 promoter sequences replaced by the T7 promoter sequence. Luciferase reporter constructs were generated by cloning the mouse *Prps2* or *Prps1* 5'UTR region into the pGL3 promoter vector (Promega) using the HindIII and NcoI sites. Site-directed mutagenesis of the *Prps2* 5'UTR PRTE sequence was performed using the following oligos.

Forward ΔPRTE – 5'-cggtagcagtagtctgcatcgcagagcgcgctcctcagtc-3'
Reverse ΔPRTE – 5'-gactgaggagcgcgctctgcgatgcagactactgctaccg-3'
Forward PRTE transversion – 5'-cggtagcagtagtctgcatcgcagAGGGAAAGAGGAAGAGGagcgcgctcctcagtc-3'
Reverse PRTE transversion – 5'-gactgaggagcgcgctCCTCTTCCTCTTTCCCTctgcgatgcagactactgctaccg-3'

The dominant negative mutant of 4EBP1 (DN-4EBP1) cDNA has been previously described (Hsieh et al., 2010). The DN-4EBP1 cDNA was subcloned into pMSCV-hygro to generate a constitutive mammalian expression vector for luciferase reporter experiments. Retroviruses containing shRNA hairpins targeting *Prps2* were cloned into a pMSCV vector harboring palmitoylated GFP cDNA subcloned downstream of the PGK promoter. The U6 promoter was cloned into the multiple cloning site and the following shRNA target sequences were used.

Control non-targeting shRNA: 5'-CAACAAGATGAAGAGCACCAA-3'
Mouse *Prps2* shRNA: 5'- GTGGTTATTTGGTCGTTAATT-3'
Human *PRPS2* shRNA: 5'- TGCAGTGCTTGTATTGGTTTAA-3'

The retrovirus expressing inducible shRNA targeting mouse *Prps2* (Tet-PRPS2 shRNA MSCV-pGFP) was constructed in the following manner. First, the mouse *Prps2* shRNA sequence above was cloned into the Tet-pLKO puro vector (Addgene #21915) and the puromycin resistance gene was replaced with the palmitoylated GFP gene. Then, the region containing the Tet-inducible H1 promoter of this vector through the pGFP gene was subcloned into the MCS of the retroviral pMSCV 2.2 vector.

Amino acid substitution mutants of Myc were generated by site-directed mutagenesis of Myc-Blast pWZL vector (Addgene #10674) using the primers:

P57S Forward: 5'-TGGAAGAAATTCGAGCTGCTGAGCACCCCGCCCCTG-3'
P57S Reverse: 5'-CAGGGGCGGGGTGCTCAGCAGCTCGAATTTCTTCCA-3'
T58A Forward: 5'-AGAAATTCGAGCTGCTGCCCGCCCCGCCCCTGTC-3'
T58A Reverse: 5'-GACAGGGGCGGGGCGGGCAGCAGCTCGAATTTCT-3'
F138C Forward: 5'-ACTGTATGTGGAGCGGCTGTTCGGCCGCCGC-3'
F138C Reverse: 5'-GCGGCGGCCGAACAGCCGCTCCACATACAGT-3'

**Cell Culture**
NIH3T3 cells and primary wild-type and transformed mouse embryo fibroblasts (MEFs) were cultured in DMEM containing 10% fetal bovine serum (FBS) and penicillin/streptomycin. To generate doxycycline-inducible DN-4EBP1 MEFs, we intercrossed TetO-4EBP mice (Hsieh et al., 2010), with ROSA26-rtTA*M2 mice (Jackson Laboratories Stock #006965) (Hochedlinger et al., 2005). For doxycycline-induced expression of DN-4EBP1, cells were treated with 1μg/mL doxycycline dissolved in water. Embryos were harvested at 13.5 d.p.c., fetal liver, head and limbs were removed, embryos were minced with a razor and single cells were obtained by trypsin digestion. To transform MEFs, we transduced them with retroviruses generated from Myc-pWZL and HRas-pBABE plasmids. Daudi, Raji, U266 and JJN-3 cells were purchased from American Type Culture Collection and maintained in RPMI1640 media supplemented with 10% FBS.

**Sucrose Gradient Fractionation and Polysome Profiling**
Sucrose gradient fractionation and polysome profiling were performed as described (Hsieh et al., 2012). Upon polysome fractionation, RNA was extracted using Trizol reagent in conjunction with PureLink RNA isolation kits (Invitrogen). RNA isolated from each fraction was reverse transcribed as described above. For qRT-PCR

analysis of polysomal fractions, data are expressed relative to 5S rRNA expression and subsequently represented as a fraction of total mRNA for either *Prps1* or *Prps2*. 5S rRNA primers used were: Forward – 5'-GCCCGATCTCGTCTGATCT-3' and Reverse – 5'-AGCCTACAGCACCCGGTATT-3'.

**Fetal Liver Hematopoietic Stem Cell Culture, Retroviral Preparation, Infection, and Transplantation**

Fetal liver hematopoietic stem cell culture, infection and transplantation were performed essentially as described with slight variation (Zuber et al., 2010). Day 14.5 pregnant mice from a *Eμ-Myc/+* transgenic × Black 6 wild-type cross were sacrificed to obtain fetal livers, which were minced and grown at approximately $3\times10^6$ cells/mL in media supporting hematopoietic stem cell (HSC) growth (50% DMEM, 50% Iscove's modified Dulbecco's Medium (Gibco), supplemented with 10% fetal calf serum, 2% L-glutamine (200 mM), 100 U/mL penicillin/streptomycin, 50μM β-mercaptoethanol, 2 ng/mL recombinant murine interleukin-3, 2 ng/mL recombinant murine interleukin-6, and 10 ng/mL recombinant murine stem cell factor (all cytokines from Peprotech) at 37°C in a humidified 5% $CO_2$ incubator.

Retroviruses were prepared by transfecting HEK293 cells with Tet-PRPS2 shRNA MSCV-pGFP and pCL-Eco (Addgene #12371) plasmids using polyfect (Qiagen). 12 hours later, media was replaced with media containing 50% DMEM, 50% Iscove's modified Dulbecco's Medium (Gibco), supplemented with 10% fetal calf serum, 2% L-glutamine (200 mM), 100 U/mL penicillin/streptomycin, and 50μM β-mercaptoethanol. 24 hours later, virus-containing supernatant was collected, media was replaced and supernatant was filtered through a 0.45μm syringe filter. Viral supernatant was collected up to 72 hours post-transfection.

Spinoculation of HSCs was performed 3 days after harvest and culture of HSCs. In a six well dish, approximately $3\times10^6$ cells were infected by spinoculation three times with five hours between infections. Briefly, viral supernatant was supplemented with the cytokines at concentrations listed above as well as 4μg/mL polybrene (Sigma). For each round of infection, 1.5mL of supplemented filtered viral supernatant was added and cells were spun for 15 minutes at 1500rpm.

Two days after the last spinoculation, efficacy of infection was assessed by FACS analysis to determine the percentage of GFP positive cells. For all transplanted recipient mice, greater than 60% of donor HSCs were infected. Three days after spinoculation, HSCs were spun down, resuspended in PBS and $5\times10^6$ living HSCs were injected via tail vein into recipient lethally irradiated recipient mice. After one month of recovery post-transplantation wherein mice were placed on an antibiotic regimen to prevent infection, mice were segregated into two cohorts and treated with either vehicle (water) or 2mg/mL doxycycline via their drinking water. Mice were subsequently palpated twice weekly to monitor for development of lymph tumors. For apoptosis measurement upon acute administration of doxycycline, 500μL of a 1mg/mL solution of doxycycline was administered to mice via ip injection. 24 hours later, B cells were isolated for apoptosis measurement or western blot analysis.

***Eμ-Myc/+* Tumor Cell Culture, Infection, Transplantation and Monitoring**

*Eμ-Myc/+* tumor cells were harvested, cultured and infected with Tet-PRPS2 shRNA MSCV-pGFP essentially as described (Schmitt et al., 2000). After transplantation via tail vein of approximately $5\times10^6$ live tumor cells into syngeneic recipients, tumors were allowed to engraft for several days. Between days 5 and 7 post transplantation, blood samples from tail bleeds were subjected to FACS analysis to assay for tumor take by measurement of GFP positive B220 positive tumor cells. Upon detection of GFP positive population of circulating B220 positive cells, mice were placed in either vehicle (water) or doxycycline (2mg/mL in water) treatment regimens representing day 0 of the survival curve.

**Genotyping Protocol for *Prps2$^{null}$* mice**

*Prps2$^{null}$* mice were genotyped using: Forward 5'-ACATTGCCATAAGGAATTATCAGAG-3' and Reverse 5'-GGCGCCAGCCTGCTTT-3' to detect the mutant allele and Forward 5'-TGCCAGTTATCACCGCTCA-3' and Reverse 5'-GCTGCCCACACTTCACTCTT-3' to detect the wild-type allele. Promega GoTaq was used to amplify genomic DNA and cycling conditions were as follows:

1. 94° for 1.5min
2. 94° for 30sec
3. 55° for 1min

4. 72° for 1min
5. Repeat cycles 2-4 ×35
6. 72° for 2min
7. 4° for ∞

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**Figure S1. Measurement of Ribo- and Deoxyribonucleotides in Myc overexpressing cells, Related to Figure 1.** (A) Measurement of various purine, pyrimidine, and pyridine ribonucleotides via HPLC. (B) Measurement of deoxynucleotide triphosphates via [3H] nucleotide PCR incorporation assay. (C) Prps1 mRNA levels in primary B lymphocytes isolated from 4-6 week old mice from the indicated genotypes. For all experiments, error bars represent standard deviation, N=3-6 mice per condition, **P<0.01 ,*P<0.05 by student's t-test.

**Figure S2. Specificity of *Prps2*-targeting siRNA, Related to Figure 2.** (A) *Prps1* and *Prps2* mRNA levels in primary B lymphocytes isolated from 4-6 week old mice from wild-type mice after transduction with either control (ctrl) or *Prps2*-specific siRNA. Error bars represent standard deviation, N=3, **P<0.01 by student's t-test.

**Figure S3. *Myc* is a serum responsive gene and *Myc* overexpression can increase PRPS2 protein levels in NIH3T3 cells, Related to Figure 3.** (A) MYC protein was assessed by western blot in serum-starved NIH3T3 cells treated with 20% Fetal Bovine Serum for the indicated times. (B) *Myc* mRNA was measured by qRT-PCR in cells treated as in (A). (C) *Myc* mRNA association with polysomal fractions was measured and expressed as a fraction of total *Myc* mRNA in the conditions indicated in NIH3T3 cells. (D) Empty vector control or Myc-Blast pWZL retroviruses were used to generate stable NIH3T3 cell lines. Western blotting (D) and qRT-PCR (E) was performed to assess the expression of the indicated protein and mRNA targets, respectively. For (B) and (E), mRNA expression data are normalized to *β-Actin*. For (B), (C) and (E), error bars represent standard deviation, N=3, **P<0.01, *P<0.05 by student's t-test.

**Figure S4. *Prps2* is regulated at the translational level by a specific cis-acting sequence element within its 5'UTR, Related to Figure 4.** (A) qRT-PCR measurement of indicated mRNA levels from B lymphocytes isolated from compound transgenic mice treated with vehicle (-dox) or doxycycline for 6 hours to drive inducible expression of dominant-negative-4EBP1 (DN-4EBP1). (B) qRT-PCR measurement of indicated mRNA levels from B lymphocytes isolated from specified genotypes treated as denoted. (C) Sequences of mouse *Prps2* and *Prps1* 5' untranslated regions (UTRs). Red underlined region denotes PRTE sequence, bold AUG represents start codon, numbering is relative to translational start codon. (D) Luciferase activity driven from a PRPS2 5'UTR reporter with the pyrimidine-rich translational element harboring transversion mutations was measured from cells transfected with empty vector or a vector overexpressing DN-4EBP1. For (A) and (B), mRNA levels are normalized to expression of *β-Actin*. For (A), (B), and (D), error bars represent standard deviation. N=3 for (A) and (B), N=4 for (D). **P<0.01, *P<0.05 by student's t-test.

**Figure S5. PRPS2, but not PRPS1, expression is required for cancer cell survival, Myc-dependent increases in purine nucleotide biosynthesis and Myc-driven tumor progression, Related to Figure 5.** (A) Specificity of *Prps1*- and *Prps2*-targeting siRNA SmartPool hairpins were tested via qRT-PCR for *Prps1* or *Prps2* mRNA levels in wild-type (WT) as well as Myc+Ras-transformed mouse embryo fibroblasts transduced for 24 hours. (B) $^{14}$C-labeled formate incorporation into purine nucleotides was assessed in wild-type (WT) and Myc+Ras transformed MEFs 24 hours post transfection with control (ctrl), *Prps1*-, or *Prps2*-targeting siRNAs. $^{14}$C-labeled formate incorporation into (C) RNA, and (D) DNA was assessed by scintillation counting upon siRNA-mediated knockdown of PRPS1 or PRPS2 in primary splenic B lymphocytes. (E) *Prps2* and *Prps1* mRNA levels were measured in mouse embryo fibroblasts expressing control or *Prps2*-targeting shRNA. (F) Survival curves of *Eμ-Myc*/+ tumor transplanted mice. Cells were infected with retroviruses encoding constitutive GFP and doxycycline-inducible *Prps2* shRNA hairpin and subsequently transplanted via tail vein to syngeneic animals. Upon engraftment, mice were dosed with vehicle (-dox) or 2mg/mL doxycycline (+dox) in their drinking water. For all graphs, error bars represent standard deviation, ***P<0.001, **P<0.01, and *P<0.05 by student's t-test. N=3 for (A) and (E), N=6 for (B), (C), and (D).

**Figure S6. The generation and characterization of *Prps2$^{null}$* mice and B lymphocytes, Related to Figure 6.** (A) Schematic depicting *Prps2$^{null}$* targeting vector in genomic context of the *Prps2* gene. (B) Western blot using indicated antibodies to probe embryonic stem cell lysates from wild-type (WT) or *Prps2$^{null}$* cells. (C) qRT-

PCR analysis of mRNA levels of *Prps1* or *Prps2* in indicated tissues from WT or *Prps2*$^{null}$ mice. Data are expressed as relative to *Rplp0* expression and then normalized to WT tissue. (D) *Prps1* (white bars) and *Prps2* (black bars) mRNA abundance was quantified by qRT-PCR performed on tissues indicated from WT mice. mRNA levels are normalized to *Rplp0*. (E) Haematoxylin and Eosin stained tissue sections from wild-type (WT) or *Prps2*$^{null}$ spleens. (F) Cell size of WT (red histogram) or *Prps2*$^{null}$ (blue histogram) B220+ B cells was assessed by FACS analysis. (G) Cell cycle distribution of splenic B cells purified from WT or *Prps2*$^{null}$ mice. (H) Annexin V staining was performed to assess the degree of apoptosis of primary B lymphocytes from WT and *Prps2*$^{null}$ mice. For all graphs, error bars represent standard deviation, and N=3.

**Figure S7. Myc-overexpressing Cells Require PRPS2 Function to Maintain Glucose Homeostasis, Related to Figure 7.** (A) Primary splenic B lymphocytes from the indicated genotypes were isolated and stained with 2-NBDG to measure glucose uptake. (B) The rate of lactate production by primary B lymphocytes from the indicated genotypes was assessed upon harvesting culture medium after a 6hr incubation. Primary splenic B lymphocytes freshly isolated from mice from the indicated genotypes were labeled with [U-$^{14}$C]-glucose and the fate of glucose was assessed by scintillation counting of $^{14}$C radioactive signal from captured $CO_2$ (C), purine nucleotides (D), RNA (E), or DNA (F). FACS analysis was performed to assess mitochondrial mass (G), and mitochondrial membrane potential (H) using the indicated dyes. (I) Western blotting was performed on human cells infected with retroviruses expressing control (ctrl) or PRPS2-targeting shRNAs and probed with the indicated antibodies. For all graphs, error bars represent standard deviation, N=3 for (A), (G), (H), N=6 for (B), (C), (D), (E), (F), ***P<0.001, **P<0.01, *P<0.05 by student's t-test.