SUPPLEMENTAL DATA AND METHODS

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Materials

DMSO (D2650), and thrombin (T9326-150UN) were from Sigma-Aldrich (St. Louis, MO USA). Calibration beads 3.5–4.0 μm were from Spherotech Inc (Lake Forest, IL USA). Anti-platelet serum was purchased from Cedarlane (Burlington, Ontario Canada). P-selectin ELISA kit and Proteome profiler array, mouse cytokine array panel A (ARY006) were purchased from R&D Systems (Minneapolis, MA USA). Clopidogrel was purchased from Clifford Hallam Healthcare (Keysborough, Australia). Soluble aspirin tablets (AsproClear) were derived from Bayer (Pymble NSW, Australia) and Clexane (enoxaparin sodium) was from Sanofi (Macquarie Park NSW, Australia).

Generation and maintenance of cell lines

Immortalised Eμ-*myc* mouse lymphoma cells 5849, A118C, AF40A and 1194 were all Immature B ($B220^+$, $cKit^-$, IgD^{low}, IgM^+) derived from spleens and lymph nodes of sick Eμ-*myc* mice and were generated and cultured as previously described [1]. A118C and AF40A were a kindly provided by Dr. Stanley Lee. They were maintained in high-glucose Dulbecco's modified Eagle's media (DMEM, Gibco) supplemented with 1mM HEPES, 13.5μM folic acid, 0.34mM L-Asparagine, 0.55mM L-Arginine, 22.2mM D-Glucose, 100U/mL penicillin-streptomycin, 10% (v/v) heat-inactivated foetal calf serum and 50μM β-mercaptoethanol. Cultures were maintained in a 10% CO_2 humidified 37°C incubator. GRANTA cells (kindly provided by the Huang laboratory, WEHI) and were grown in RPMI 1640 + 10% (v/v) heat-inactivated foetal calf serum in a 5% CO_2 humidified 37°C incubator

Lymphoma cell survival

To assess the effect of platelets on lymphoma cells; $1x10^7$ washed murine platelets/well or 1-fold PRMs were co-incubated with $5x10^4$ Eμ-*myc* cells in a flat-bottom 96-well plate at 37°C and 5% serum or under reducing serum concentrations (0-10% FBS) in media described above. At 24 or 48 hours post-stimulation, viability and cell number was assessed by Flow cytometry. Cell viability was determined by the exclusion of the nuclear dye, Fluoro-Gold; (Fluorochrome) and cell number using known amounts of APC-coated beads (Miltenyi

Biotech). Surface markers to detect Eμ-*myc* cells (CD19) and platelets (CD41) were included. Serum starvation for 24 h was performed prior platelet incubation when indicated.

Flow cytometry

Fluorescently conjugated anti–mouse antibodies used for flow-cytometric analyses were either purchased or produced by the WEHI monoclonal antibody production facility. WEHI antibodies: B220 (RA3-6B2), CD19 (1D3), cKit (CD117 clone:ACK-4) and IgM (5.1). BD Biosciences (Franklin Lakes NY, USA) antibodies: IgD (11-26c.2a), P-selectin (RB40.34) and CD41 (MWReg30). Data was acquired using the Fortessa flow cytometer or FACS Calibur flow cytometer and sorting performed on the FACSAria II flow cytometer (BD Biosciences). Analysis was performed using Flowjo software (TreeSTAR).

Purification of murine platelets

Murine washed platelets were purified as previously described [2]. Blood from C57BL/6 mice was obtained by cardiac puncture into 0.1 volume of Aster-Jandl anticoagulant (85 mM sodium citrate, 69 mM citric acid, and 20 mg/ml glucose, pH 4.6) [3]. Platelet-rich plasma (PRP) was obtained by centrifugation at 125 g for 8 min, followed by centrifugation of the supernatant buffy coat at 125 g for 8 min. Platelets were washed by two sequential centrifugations at 860 g for 5 min in 140 mM NaCl, 5 mM KCl, 12 mM trisodium citrate, 10 mM glucose, and 12.5 mM sucrose, pH 6.0 (buffer A). The platelet pellet was resuspended in 10 mM Hepes, 140 mM NaCl, 3 mM KCl, 0.5 mM $MgCl_2$, 10 mM glucose, and 0.5 mM $NaHCO_3$, pH 7.4 (buffer B) at a concentration of 1–5 x 10^8 cells/ml. The platelet count was determined by flow cytometric analysis by adding a known concentration of FACS calibration beads (Spherotech) on the FACS Calibur flow cytometer.

Purification of platelet released molecules

Platelets were prepared and counted as described above and resuspended to 3x10^8 cells/ml in buffer B. PRMs were prepared as previously described [4], where washed platelets were activated with 1U/ml thrombin for 15 minutes at room temperature on a tube roller (an aliquot of platelets was taken at this time point for FACS confirmation of platelet activation by P-selectin surface expression). The vehicle control for PRM was buffer B with 1 U/ml thrombin. Activated platelets were centrifuged at 1500xg for 5 minutes and the supernatant (and vehicle control) was transferred to a 3-kDa molecular weight cut-off ultra-centrifugal filter device Amicon® (Merck Millipore). Supernatant was centrifuged at 3250xg until the volume was reduced to

500uL (approximately 50 minutes), achieving a 20-fold volumetric concentration. Aliquots of platelet released molecules were stored at -80°C until required.

Production of passage 1 Eμ-*myc* cells

Primary immature B ($B220^+$, $cKit^-$, IgD^{low}, IgM^+) Eμ-*myc* lymphoma cells (166, 5849 and 5903) were harvested from lymph nodes (axillary, brachial, inguinal and mesenteric) and spleen of terminally ill Eμ-*myc* mice. The harvested cells were then transplanted IV via the tail vein into recipient C57BL/6 wild-type mice to generate passage 1 (P1) Eμ-*myc* lymphoma cells (harvested from spleen and lymph nodes) subsequently used in transplant experiments.

Production of passage 1 Eμ-*Tcl-1* cells

In a similar manner to our investigations with the Eμ-*myc* mice we collected and characterised spleen tumours cells from Eμ-*Tcl-1* transgenic mice [5] by flow cytometry. The harvested cells were then transplanted IV via the tail vein into recipient C57BL/6 wild-type mice to generate passage 1 (P1) Eμ-*Tcl-1* cells (harvested from spleen) subsequently used in transplant experiments.

***In vivo* transplantation of Eμ-*Tcl-1* cells**

Wild-type C57BL/6, $Mpl^{-/-}$, Tpo^{Tg} were injected IV with 10^4 (P1) Eμ-*Tcl-1* CLL cells and analysed 100 days post tumour transplantation. Disease severity was measured by peripheral blood counts, spleen and lymph node (axillary, brachial, inguinal and mesenteric) weights.

Peripheral blood counts

Automated cell counts were performed on blood collected from the retro-orbital plexus or by cardiac puncture into Microtainer tubes containing EDTA (Sarstedt, Ingle Farm, SA Australia), using an ADVIA 2120 haematological analyser (Siemens, Munich Germany) equipped with a mouse analysis software module (Bayer).

B-cell subset analysis

Spleen cell suspensions were prepared by physical dissociation in 3 ml of BSS 2% FCS. Subsequently, 7 ml of Red Cell Removal Buffer (156 mM NH4Cl, 0.1 mM EDTA, and 12 mM NaHCO3) was added for 1 min at room temperature. After centrifugation the supernatants were aspirated and the pellet was resuspended in 5 ml of BSS 2% FCS. Cell suspensions were filtered

using a 100 μm cell strainer. B-cell populations were defined as ProB-PreB1 (B220$^+$ cKit$^+$), PreB2 (B220$^+$ cKit$^-$ IgD$^-$ IgM$^-$), Immature (B220$^+$ cKit$^-$ IgD$^-$ IgM$^+$), Mature (B220$^+$ cKit$^-$ IgD$^+$ IgM$^+$). Data was acquired using a LSRFortessa flow cytometer (BD, Franklin Lakes, NY, USA), and data analysis was performed using FlowJo software 10.3.0 (Treestar Inc, Ashland, USA).

Tumour liver infiltration

Whole livers were removed and fixed in 4% paraformaldehyde overnight. Livers were then dehydrated overnight and paraffin embedded using an automatic tissue processor. Sections were cut, mounted on slides and stained with haematoxylin and eosin (H&E). Entire slides were scanned using the Aperio ScanScope AT Slide scanner (Leica Biosciences) and processed on ImageScope. Whole liver sections were analysed in a blinded fashion using a custom macro in FIJI using the colour deconvolution plug-in [6] to quantify the total area of lymphocyte infiltration as percent whereby infiltrated tumour cells stain darker than surrounding healthy tissue.

Anti-platelet serum mediated thrombocytopenia

Acute thrombocytopenia was induced by IV injection of 100 μl of anti-platelet serum (APS) (Cedarlane) per 20 g body weight at a 1:25 pre-dilution. This reduces platelet counts to near undetectable levels within 24 h, followed by rebound thrombocytosis [2]. Male wild-type C57BL/6 mice were transplanted IV on day 1 with Eμ-*myc* 5849 tumour cells (2 x10^4) and acute thrombocytopenia was induced by APS IV injection on day 0 or alternatively on day 0, day 3 and day 6. Mice were analysed on day 16. Disease severity was measured by peripheral blood counts and spleen weight.

***In vivo* imaging (IVIS imaging)**

P1 5849 lymphoma cells were transduced with pMSCV-Luc2-IRES-mCherry retroviral vector [7] in the presence of retronectin. Successfully transduced cells were washed in PBS and 10^4 cells injected IV into wild-type C57BL/6 albino and *Tpo*^{Tg} C57BL/6 albino recipient mice. To visualize tumour burden, recipient mice were injected IP with 50mg/kg D-luciferin potassium salt (Caliper Life Sciences) made in PBS. Mice were anaesthetized with isoflurane inhalant and imaged using the Xenogen IVIS spectrum live-imaging system (Perkin Elmer) to detect luciferase bioluminescence 10 minutes after administration of the D-luciferin substrate.

Tumour burden was quantified 7 days post tumour injection by measuring the total photon flux per second emitted from a region of interest (ROI) drawn around the whole mouse.

Quantification of soluble P-selectin in serum

For sP-selectin ELISA, blood (≥ 200μL) was collected from healthy and tumour bearing mice either by retro-orbital bleed into a non-heparinised capillary, or cardiac puncture and left to clot for 2 hours at room temperature. The clotted blood was centrifuged at 2000 *g* for 20 minutes and serum was stored at -80°C until required. Serum was diluted 1:50 in buffer supplied by the manufacturer and the ELISA was performed according to manufacturer's instructions.

Cytokine analysis of BM fluid

Proteome profiler array, mouse cytokine array panel A (R&D #ARY006) includes CXCL13/BCA-1, C5/C5a, G-CSF, GM-CSF, I-309, Eotaxin, sICAM-1, INF-γ, IL-1a, IL-1β, IL-1rα, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-12 p70, IL-16, IL-17, IL-23, IL-27, IP-10, I-TAC, KC, M-CSF, JE, MCP-5, MIG, MIP-1α, MIP-1β, MIP-2, RANTES, SDF-1, TARC, TIMP-1, TNF-α, and TREM-1.

RNA sequencing and gene expression analysis of blood residing Eμ-*myc* cells

mCherry^{+}Eμ-*myc* 5849 2x10^4 cells were transplanted IP into age and gender matched Tpo^{Tg} and wild-type mice (WT). CD19 mCherry double positive and fluorogold negative cells were sorted on a FACSAria II flow cytometer (BD Biosciences) from blood of tumour bearing mice day 15 (1 WT, 1 Tpo^{Tg}) and day 19 (3 WT, 2 Tpo^{Tg}) after tumor transplant. Blood was drawn into EDTA and RBCs were depleted before sorting. After sorting, cell pellets were frozen down and all RNA samples were prepared at the same time using RNeasy mini kit according to the RNeasy mini handbook from Qiagen (Germantown, MD, USA). mRNA libraries were prepared and samples were sequenced at the Australian Genome Research Facility (AGRF) on the Illumina HiSeq platform to obtain 100bp single end reads per sample.

Reads were aligned to the mm10 build of the mouse genome using the STAR software package v2.5.3a [8]. Over 96% of reads mapped to the reference genome for each sample. Following alignment, successfully mapped reads were summarized into gene-level counts using the featureCounts function [9] from the Rsubread software package v1.27.4 [10] where an average of 71% of reads were assigned to genes for all samples. Genes were identified using NCBI RefSeq

annotation. Differential expression analyses were carried out using limma v3. 3.50.0 [11]and edgeR v3.36.0 [12].

Expression-based filtering for lowly expressed genes was performed using edgeR's filterByExpr function with default parameters. A total of 10,802 genes remained for downstream analysis. Compositional differences between the libraries were normalized using the trimmed mean of M-values (TMM) method [13]. Read counts were transformed to log-counts-per-million before applying the RUVIII method from the ruv software package v0.9.7.1 [14] with k=2 to remove unwanted variation. Differential expression between Tpo^{Tg} and WT samples was assessed using linear models and robust empirical Bayes moderated t-statistics with a trended prior variance (the limma-trend pipeline) [15]. The false discovery rate (FDR) was controlled below 5% using the Benjamini and Hochberg method [16]. The mean-difference plot was generated using the plotMD function in limma. Pathway analyses were performed on differentially expressed genes to test for overrepresentation of biological pathways as defined by Molecular Signatures Database (MSigDB) hallmark gene sets [17,18] using the fry gene set test. The GEO accession number is GSE199782.

Pharmacological inhibition of platelet function and coagulation

Wild-type C57BL/6 male mice were transplanted IV with immortalised Eμ-*myc* 5849 cells ($2x10^4$). Mice were euthanised and analysed at the same time once the first mouse in the experimental cohort reached an ethical end point. Disease severity was measured by peripheral blood counts and spleen weight.

Aspirin treatment. Aspirin was administered at a dose of approximately 25 mg/kg/day for the duration of the experiment. Mice were pre-treated with aspirin for 1 week prior to Eμ-*myc* IV injection. Soluble aspirin tablets (AsproClear) were dissolved in water to a concentration of 25 mg/ml. Aspirin solution was injected into animal hydropacs (~400mL volume) at a 1:80 dilution. Assuming each animal drinks on average between 3 and 4 mL of water each day [19] 25mg/mL dilution equates to 4.7 to 5mg aspirin each day for a 25g mouse (20mg/kg dosage). At a dose of approximately 25 mg/kg/day for the duration of the experiment.

Clopidogrel treatment. Mice were dosed daily via oral gavage with 200μl Clopidogrel at 50mg/kg in 0.5% methylcellulose. Drug treatment was initiated 1 day prior to tumour cell transplant and continued throughout the experiment. Vehicle-treated mice received 200μl 0.5% methylcellulose by oral gavage at the same timing and frequency as the Clopidogrel-treated mice.

Low molecular weight heparin treatment. Clexane (enoxaparin sodium) and vehicle (saline) was administered *s.c.* at 5 and 10 mg/kg starting 1 day prior tumour transplant.

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SUPPLEMENTAL TABLES

Supplemental Table 1. Upregulated inflammatory genes.

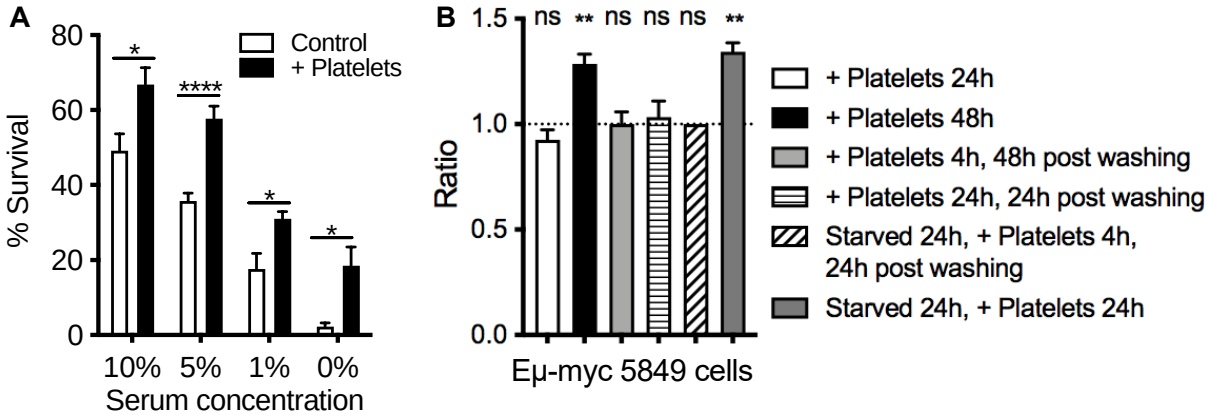
39 upregulated protein-coding inflammatory genes in blood-residing Eμ-*myc* lymphoma cells in Tpo^{Tg} mice compared to blood-residing Eμ-*myc* lymphoma cells in wild-type mice. Genes are sorted after P-values starting with highest significance.

Symbol	GeneID	logFC	AveExpr	t	P.Value	adj.P.Val	B
Plec	18810	0.20832027	9.28647219	9.66209364	4.32E-06	0.00686737	4.72037042
Plaur	18793	0.50058286	4.66867192	8.272431	1.56E-05	0.00701648	3.55984432
Rabgap1l	29809	0.25589858	7.51760154	7.45059094	3.62E-05	0.00948352	2.77195991
Ahnak	66395	0.16316701	11.8655589	7.34221112	4.06E-05	0.00975671	2.66178158
B4galt5	56336	0.15603342	6.81586378	6.65536905	8.75E-05	0.01281922	1.92699093
Ager	11596	0.62510012	2.21075326	6.63978018	8.91E-05	0.01281922	1.90955556
Myo1g	2E+05	0.18996745	7.85676044	6.42793093	0.00011424	0.01310044	1.66915291
Mxd1	17119	0.31454711	4.79682482	6.41810829	0.00011558	0.01310044	1.65784862
P2rx4	18438	0.34869478	3.82744145	6.26649274	0.00013855	0.013606	1.48156609
Cd86	12524	0.29503012	4.48107059	6.23670439	0.00014362	0.01360617	1.44653194
Klf4	16600	0.29705386	4.78542639	6.08042248	0.00017376	0.01454476	1.26055489
Tnfrsf1b	21938	0.2995342	3.86717847	5.76151639	0.00025888	0.01635346	0.86955275
Ecm1	13601	0.43089392	3.07696944	5.73477065	0.00026785	0.01646938	0.83604927
Macf1	11426	0.13778582	9.44028669	5.68625559	0.00028499	0.01646938	0.77499254
P2rx7	18439	0.55712145	2.72900936	5.62880506	0.00030683	0.01656086	0.7022163
Abca1	11303	0.52566875	3.36973696	5.48337242	0.00037067	0.01819158	0.51567881
Il10ra	16154	0.28144598	5.70584442	5.23186683	0.00051758	0.02039172	0.18522502
Irf7	54123	0.29902421	6.30995843	5.0714181	0.00064344	0.02173515	-0.0308192
Klf6	23849	0.18707191	7.23887692	4.90906286	0.00080501	0.02408794	-0.2535774
Muc1	17829	0.54460728	2.21063191	4.77443369	0.00097216	0.02667527	-0.4414433
Pdlim7	67399	0.3402256	3.46196137	4.6459513	0.0011668	0.02876052	-0.6233712
Ddx58	2E+05	0.29696547	4.38335397	4.59987416	0.00124645	0.02937868	-0.6892375
Btg2	12227	0.22455234	6.04727962	4.42517138	0.00160554	0.03222182	-0.9419128
B4galt1	14595	0.14339188	6.4516156	4.41235269	0.00163593	0.03238503	-0.9606338
Capn1	12333	0.16348229	5.65085173	4.26396367	0.00203597	0.03647193	-1.1791122
Lrrc8b	4E+05	0.25834087	3.76973188	4.25094534	0.00207574	0.03667661	-1.1984325
Emp3	13732	0.09474549	8.032672	4.24631418	0.00209008	0.03667661	-1.2053114
Naip5	17951	0.23610593	3.67975818	4.19312993	0.00226258	0.0376523	-1.2845282
Adgre1	13733	0.7444456	0.1917997	4.16990923	0.00234259	0.03844243	-1.3192406
Slc11a1	18173	0.64285154	0.83689155	4.14298068	0.00243915	0.03904169	-1.3595903
Tlr7	2E+05	0.56148307	1.07197141	4.12370479	0.00251087	0.03975032	-1.3885353
Flt3l	14256	0.20056438	3.87439594	4.08548712	0.00265975	0.04073208	-1.4460748
Gnai2	14678	0.07663752	8.86703885	4.05913481	0.00276786	0.04132965	-1.4858662
Capg	12332	0.1797919	7.41123107	3.98728753	0.00308693	0.04358826	-1.5948268
Gpr183	3E+05	0.28829992	4.40997407	3.93613607	0.0033377	0.04535078	-1.6728139
Cdc42se2	72729	0.09789407	7.1480812	3.92023654	0.00341997	0.04567321	-1.6971234
Casp7	12369	0.15390735	4.53483097	3.90180267	0.00351805	0.04634385	-1.7253478
Nrp1	18186	0.7176459	0.09086008	3.86001791	0.00375153	0.04784416	-1.7894827
Itga2b	16399	1.30427651	1.77051074	3.82382162	0.00396701	0.0490855	-1.8452134

Description
plectin
plasminogen activator, urokinase receptor
RAB GTPase activating protein 1-like
AHNAK nucleoprotein (desmoyokin)
UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5
advanced glycosylation end product-specific receptor
myosin IG
MAX dimerization protein 1
purinergic receptor P2X, ligand-gated ion channel 4
CD86 antigen
Kruppel-like factor 4 (gut)
tumor necrosis factor receptor superfamily, member 1b
extracellular matrix protein 1
microtubule-actin crosslinking factor 1
purinergic receptor P2X, ligand-gated ion channel, 7
ATP-binding cassette, sub-family A (ABC1), member 1
interleukin 10 receptor, alpha
interferon regulatory factor 7
Kruppel-like factor 6
mucin 1, transmembrane
PDZ and LIM domain 7
DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
B cell translocation gene 2, anti-proliferative
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1
calpain 1
leucine rich repeat containing 8 family, member B
epithelial membrane protein 3
NLR family, apoptosis inhibitory protein 5
adhesion G protein-coupled receptor E1
solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
toll-like receptor 7
FMS-like tyrosine kinase 3 ligand
guanine nucleotide binding protein (G protein), alpha inhibiting 2
capping protein (actin filament), gelsolin-like
G protein-coupled receptor 183
CDC42 small effector 2
caspase 7
neuropilin 1
integrin alpha 2b

Synonyms
AA591047|AU042537|EBS1|PCN|PLTN|Plec1
Cd87|u-PAR|uPAR
5830411O09Rik|8430421H08Rik|9630005B12Rik|AW049894|HHL|Hh1|mKIAA0471
DY6
9430078I07Rik|AW049941|AW539721
RAGE
E430002D17Rik
AW122478|Mad|Mad1
AI504491|AW555605|D5Ertd444e|P2X4
B7|B7-2|B7.2|B70|CLS1|Cd28l2|ETC-1|Ly-58|Ly58|MB7|MB7-2|TS/A-2
EZF|Gklf|Zie
CD120b|TNF-R-II|TNF-R2|TNF-R75|TNF-alphaR2|TNFBR|TNFR80|TNFRII|TNFalpha-R2|Tnfr-1|Tnfr2|p75
AI663821|p85
ABP620|Acf7|Aclp7|MACF|R74989|mKIAA0465
AI467586|P2X(7)|P2X7R
ABC-1|Abc1
AW553859|CDw210|CDw210a|IL-10R1|IL-10RA|Il10r|mIL-10R
-
AI448727|BCD1|C86813|CPBP|Copeb|FM2|FM6|Ierepo1|Ierepo3|R75280|Zf9
CD227|EMA|Muc-1
LMP
6430573D20Rik|C330021E21|RIG-I|RLR-1
AA959598|APRO1|Pc3|TIS21
B-1,4-GalT|B-1,4-GalT1|GalT|Ggtb|Ggtb2|b4Gal-T1|beta-1,4-GalT|beta-1,4-GalT1|beta4Gal-T1
Capa-1|Capa1|mu-calpin
R75581|Ta-lrrp|mKIAA0231
H-4|H4|HNMP-1|MI-35|Ymp
Birc1e|Lgn1|Naip-rs3
DD7A5-7|EGF-TM7|Emr1|F4/80|Gpf480|Ly71|TM7LN3
Bcg|Ity|Ity1|Lsh|Nramp|Nramp1|ity
-
Flt3lg|Ly72L
C76432|Galphai2|Gia|Gnai-2
gCap39|mbh1
Ebi2
2810404F18Rik|AA408783|AA536669|SPEC2
AI314680|CMH-1|ICE-IAP3|Mch3|caspase-7|mCASP-7
C530029I03|NP-1|NPN-1|Npn1|Nrp
AI172977|CD41|CD41B|GpIIb|alphaIIb

SUPPLEMENTAL FIGURES

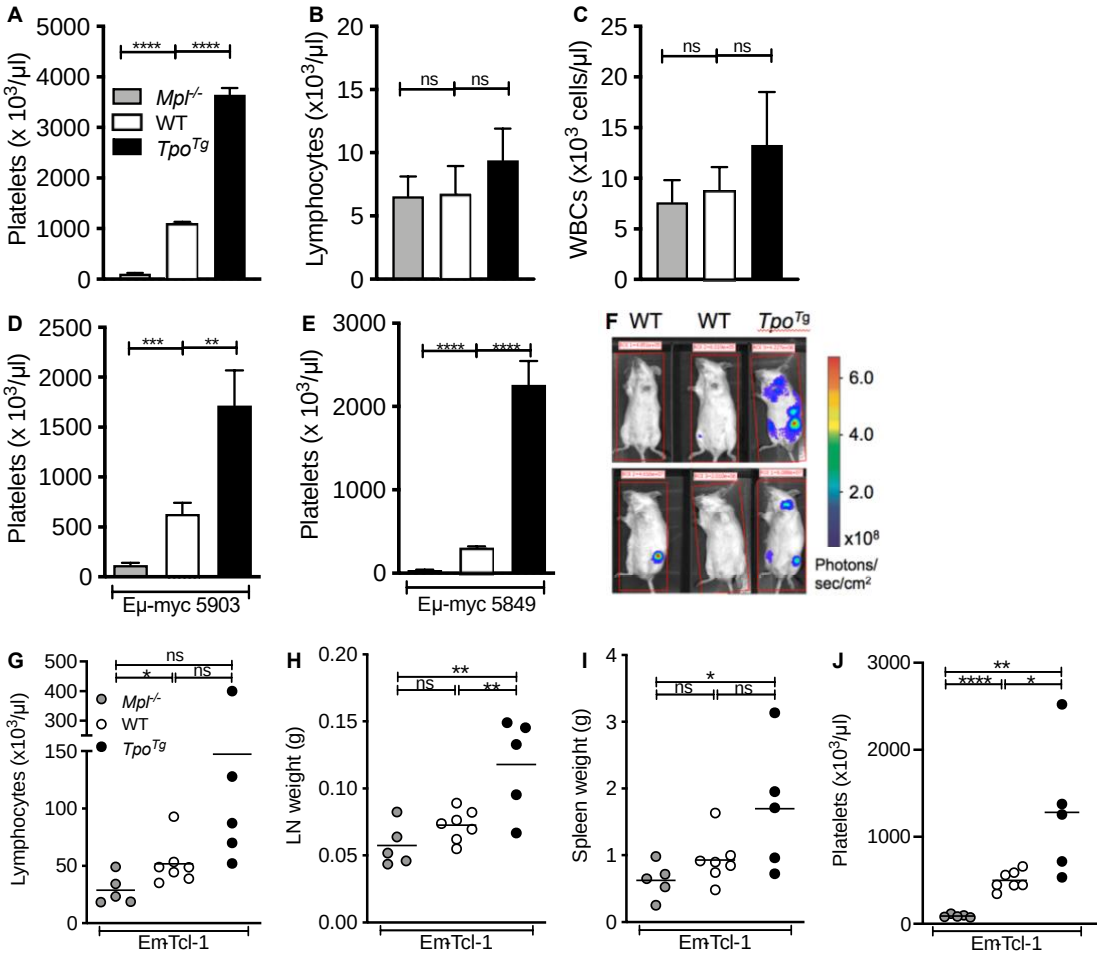


Supplemental Figure 1:

Platelets promote survival of Eμ-*myc* cell lines.

A. Eμ-*myc* cell line A118C was co-incubated with murine platelets for 48 h at 37°C and 0-10% serum and % survival was assessed by flow cytometry. n=3-10 per serum concentration.

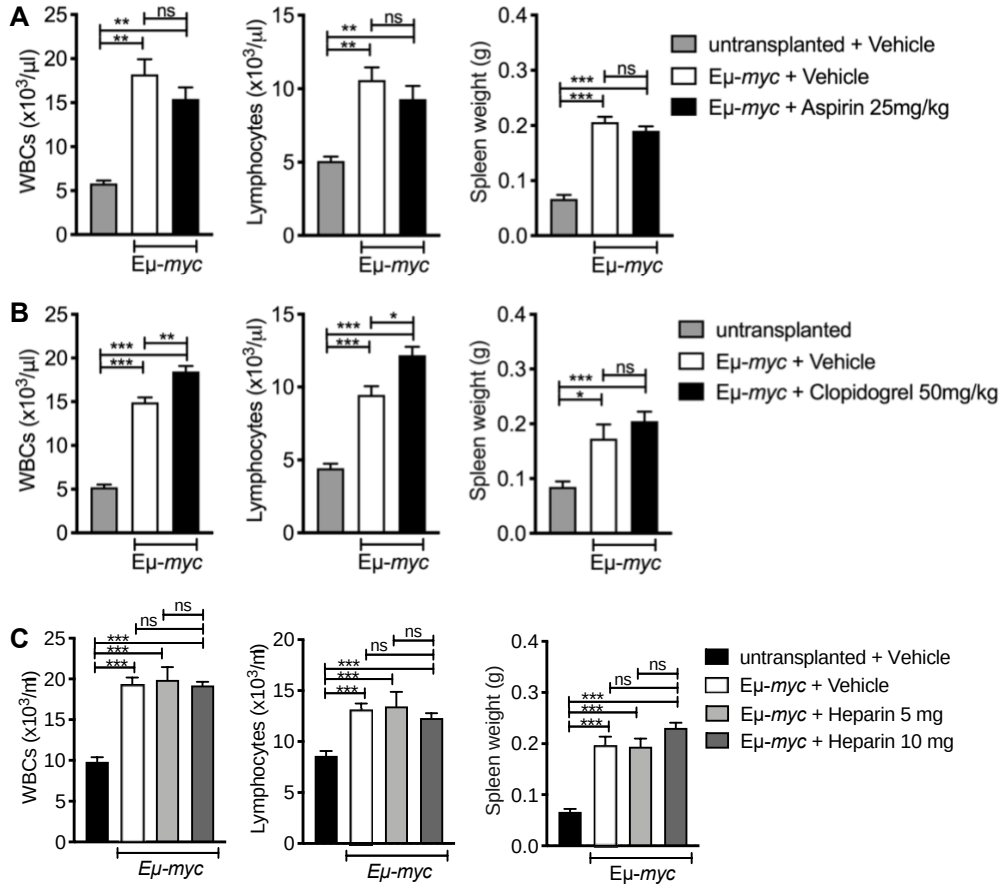
B. Eμ-*myc* cell line 5849 was co-incubated with murine platelets for indicated times at 37°C and 5% serum and % survival was assessed by flow cytometry. Data is presented as ratio compared to control. It is indicated if serum starvation for 24 h was performed prior platelet incubation. n=3-7 per condition. Results are presented as mean ± SEM. Students unpaired t-test. * $p<0.05$; ** $p<0.005$; ****$p<0.0001$; ns=not significant.



Supplemental Figure 2:

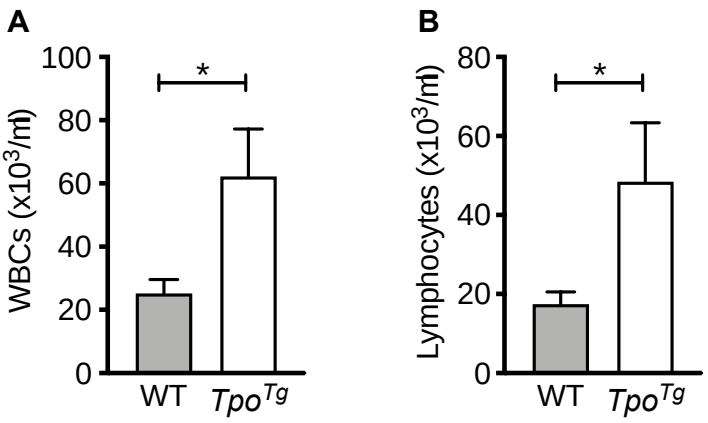
Characterisation of Eμ-*myc* and Eμ-*Tcl-1* transplanted and untransplanted mice

A. Platelet counts in adult wild-type (WT); (n=40), $Mpl^{-/-}$ (n=14) and Tpo^{Tg} (n=28) mice. Mean ± SEM. **B-C.** Lymphocyte and WBC counts in adult WT; (n=7), $Mpl^{-/-}$ (n=19) and Tpo^{Tg} (n=6) mice. Mean ± SD. Students unpaired t-test. **D.** Platelet counts 23 days post 10,000 Eμ-*myc* 5903 passage 1 cell *i.v.* transplant into WT, $Mpl^{-/-}$, and Tpo^{Tg} mice. Mean ± SEM. n=9-10 mice per genotype. Students unpaired t-test. **E.** Platelet counts 17 days post 10,000 Eμ-*myc* 5849 passage 1 cell *i.v.* transplant into WT (n=27), $Mpl^{-/-}$ (n=18), and Tpo^{Tg} (n=16) mice. Mean ± SEM. Students unpaired t-test. **F.** Representative image of IVIS imaging (4 WT albino and 2 Tpo^{Tg} albino mice) 7 days post tumour injection of 10,000 mCherry-luciferase tagged Eμ-*myc* 5849 tumour cells. **G-J.** Altered Eμ-*Tcl*-1 leukaemia progression in Tpo^{Tg} and $Mpl^{-/-}$ mice. 10,000 Eμ-*Tcl*-1 passage 1 tumour cells were injected *i.v.* 100 days prior to analysis. **G.** Lymphocyte counts, **H.** Lymph node weight, **I.** Spleen weight and **J.** Platelet count. Each symbol represents an individual mouse. Mean. Students unpaired t-test. * $p<0.05$; ** $p<0.005$; *** $p<0.001$; **** $p<0.0001$; ns=not significant.



Suppl. Figure 3: Pharmacological inhibition of platelet function and coagulation.

A. WBC and lymphocyte counts and spleen size from male wild-type C57BL/6 mice transplanted *i.v.* with immortalized Eμ-*myc* 5849 cells ($2x10^4$) and administered aspirin at 25mg/kg orally in drinking water with mice being pre-treated 1 week prior Eμ-*myc* tumour injection. n=10 aspirin, n=10 vehicle (water) and n=3 untransplanted + vehicle (water) mice. Data is combined from two separate experiments in which mice were analysed 17 and 20 days post tumour transplant. **B.** WBC and lymphocyte counts and spleen size from male mice transplanted *i.v.* with immortalised Eμ-*myc* 5849 cells ($2x10^4$) and administered clopidogrel by oral gavage daily (50 mg/kg, n=6) or vehicle (0.5% methylcellulose, n=6) starting 1 day prior to tumour transplant. Untransplanted control mice were included (n=4). Mice were analysed 19 days post tumour transplant. **C.** WBC and lymphocyte counts and spleen size from male mice transplanted *i.v.* with immortalised Eμ-*myc* 5849 cells ($2x10^4$) and administered low molecular weight heparin (LMWH) enoxaparin sodium *s.c.* daily at 5 and 10 mg/kg starting 1 day prior to tumour transplant. n=6 5mg/kg, n=6 10mg/kg, n=12 saline (vehicle), n=4 untransplanted + saline control mice. Data is combined from two separate experiments in which mice were analysed 14 days post tumour transplant Mean ± SEM. Students unpaired t-test. * $p<0.05$; ** $p<0.005$; *** $p<0.001$; **** $p<0.0001$; ns=not significant.



Supplemental Figure 4:

Characterisation of Eμ-*myc* bearing mice included in RNA sequencing

mCherry$^+$Eμ-*myc* 5849 transplanted mice included in RNA sequencing of sorted mCherry$^+$Eμ-*myc* cells. **A.** WBC counts and **B.** lymphocyte counts in wild-type (WT) and *Tpo*^{Tg} mice post mCherry$^+$Eμ-*myc* 5849 2x10^4 cell *i.p.* transplant. Cells were collected day 15 (1 WT, 1 *Tpo*^{Tg}) and day 19 (3 WT, 2 *Tpo*^{Tg}) after transplant. Mean ± SEM. Students unpaired t-test. * $p<0.05$;