Supplemental Information

Supplemental Methods

HOMA-IR Homeostatic model assessment of insulin resistance (HOMA-IR) was employed to measure the fasting insulin levels in serum samples using a mouse Insulin ELISA kit (Cat. No. N04572, Jining Industrial, China) or a human Insulin ELISA kit (Cat. No. N191211, Jining Industrial, China). Fasting blood glucose levels were measured using the Aviva glucometer before killing animals after 6 h starvation. The values were calculated using the following formula: Fasting glucose (mg dL−1) × fasting insulin (mU L−1)/405.

Hyperglycemia models Glucose (Cat. No. MB2510, Meilunbio, China), fructose (Cat. No. MB2500, Meilunbio, China), and sucrose (Cat. No. MB2466, Meilunbio, China) were dissolved in drinking water at the dose of 2.5% w/v, 5% w/v, and 5% w/v. Drinking solutions were given ad libitum to the mice. The diabetes model was induced in mice by intraperitoneally injecting streptozotocin (Cat. No. MB1227, Meilunbio, China) at a single dose of 60 mg/kg. Food intake and water intake of mice were measured on a daily basis. Peripheral platelets and red blood cells were analysed by a haematology analyser (Cat. No. BC-2600, Mindray, China). The total protein and glucose in the urine were detected by a urine diagnosis kit (Cat. No. 8V, Urit, China) according to the manufacturer's instructions.

Enzyme-linked immunosorbent assay Serum samples were collected and were stored at –80°C till further analysis. Platelets were lysed in a RIPA buffer with the proteinase and phosphatase inhibitor cocktail (Cat. No. MA0151, Meilunbio, China; Cat. No. MB2678, Meilunbio, China; 1:100), followed by a 10-minute centrifugation. Supernatants were stored at –80°C till further analysis. Mouse Insulin ELISA (Cat. No.

N04572, Jining Industrial, China), human Insulin ELISA (Cat. No. N191211, Jining Industrial, China), and mouse TxA_2 ELISA (Cat. No. E-EL-0057c, Elabscience, China) were performed according to the manufacturer's protocol using the appropriate standard curve. Absorbance values were detected at 450 nm using a Synergy 2 Multi-Mode Microplate Reader (BioTek).

Transmission electron microscopy and scanning electron microscopy For Transmission electron microscopy, the platelets were fixed by 2.5% glutaraldehyde in Tyrode's buffer for 1.5 h at room temperature and then pelleted by centrifugation at 1500 g for 5 min. The pellet was washed and post-fixed with 1% osmium tetroxide in Tyrode's buffer supplemented with sucrose (25 mg/ml) for 2 h. The samples were dehydrated in ascending ethanol concentrations, acetone, propylene oxide, and embedded. Polymerization was performed under increasing temperatures from 37 °C to 60 °C for 3 d. Ultrathin sections were cut using an Ultracut E system (Leica, Germany) and stained with saturated aqueous uranyl acetate and lead citrate. The specimens were examined using a Tecnai G2 Spirit electron microscope (FEI, Netherlands) at an operating voltage of 120 kV. For scanning electron microscopy, cell samples were fixed in 2.5% glutaraldehyde in PBS buffer (pH 7.4) and post-fixed in 1% OsO_4. Dehydration was carried out in an ethanol dilution series (30%, 50%, 70%, and 90%, followed by 3× 100%). After critical drying with an automated critical point dryer (Cat. No. EM CPD300, Leica,), the samples were mounted on double-sided carbon tape on holders and plasma-coated with 8 nm gold. Pictures were taken using a conventional scanning electron microscope (Cat. No. Helios, Thermo Fisher).

Platelet aggregometry Turbidometric platelet aggregation was performed in platelet-rich plasma in response to 10 μM ADP (Cat. No. MB1706, Meilunbio, China). Aggregation was assessed as maximal aggregation at 6 min after agonist challenge. Results were recorded by an aggregometer (Cat. No. Aggram, Helena, UK).

***In vivo* model of arterial thrombosis** Platelets were isolated from mice and treated *ex vivo* with vehicle or 20 μM MKT-077 (Cat. No. A12388, AdooQ Bioscience) solution for 30 min. After the treatment, platelets were labelled with 1 mM calcein (Cat. No. 148504-34-1, Sigma-Aldrich) solution for 20 min. At the same time, male C57BL/6 mice were anesthetized and mesenteric arterioles were exposed. $FeCl_3$ injury was performed by applying a filter paper saturated with 10% $FeCl_3$ onto the mesenteric arteriole for 2 min. After $FeCl_3$ injury, calcein-labelled mouse platelets were immediately injected intravenously. Thrombi started forming after approximately 5 minutes and the complete thrombosis process was recorded using fluorescent microscopy (Cat. No. TE2000-U, Nikon, Japan) for 10 minutes.

***In vitro* clot retraction** Clot formation was initiated by adding 300 μl platelet-rich plasma to siliconized vials containing 1 U/ml thrombin (Cat. No. MB1368, Meilunbio, China) and 20 mM $CaCl_2$ (Cat. No. 10005861, Sinopharm, China). Reaction tubes were gently mixed for 5 s, and were incubated at room temperature for 45 min. The retraction was quantified by measuring the volume of the remaining serum after clot formation.

Tail bleeding assay Animals were placed in a prone position. A distal 10 mm segment of the tail was amputated with a scalpel. The tail was immediately immersed in a 50-

ml tube containing pre-warmed isotonic saline. The tail tip was positioned about 2 cm below the body and was monitored for 10 min. Bleeding time was recorded.

Supplemental Tables

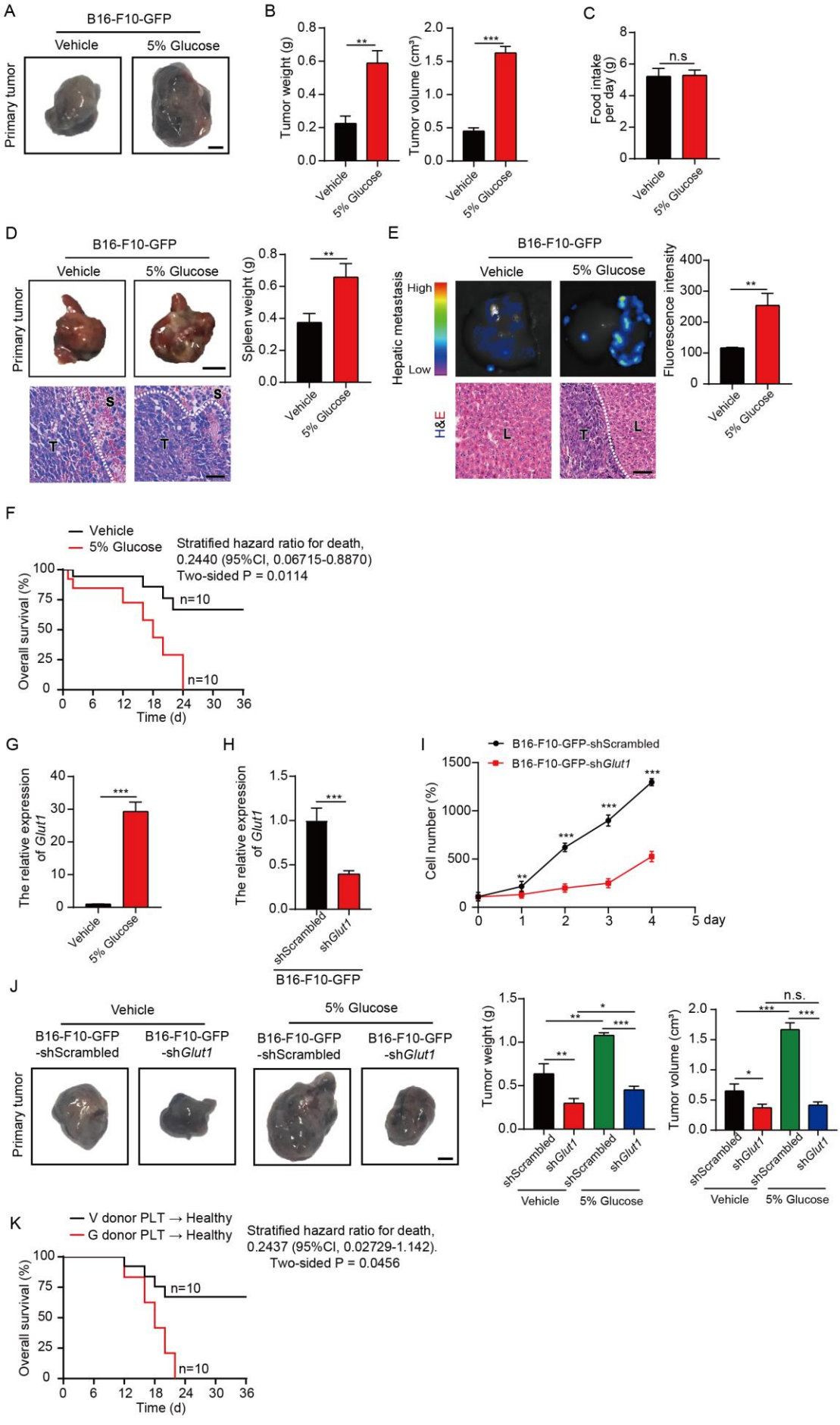
Table 1. Primer pairs used for glucose metabolism-related gene array in mouse MKs

Gene name	Forward primer (5'- 3')	Reverse primer (5'- 3')
Pik3ca	CCACGACCATCTTCGGGTG	ACGGAGGCATTCTAAAGTCACTA
Akt	ATGAACGACGTAGCCATTGTG	TTGTAGCCAATAAAGGTGCCAT
Prkcb	GTGTCAAGTCTGCTGCTTTGT	GTAGGACTGGAGTACGTGTGG
Tas1r2	TGCTCTGCCTAAGCCAGTC	CTCTTCACGTTGGCATGGAGG
Aldh3a1	AATATCAGTAGCATCGTGAACCG	GGAGAGCCCCTTAATCGTGAAA
Pklr	TCAAGGCAGGGATGAACATTG	CACGGGTCTGTAGCTGAGTG
Dld	GAGCTGGAGTCGTGTGTACC	CCTATCACTGTCACGTCAGCC
Grp75	ATGGCTGGAATGGCCTTAGC	GCACCCTTGATTGCTTCTGATG
Bpgm	GGACCAGAAACTTAACAACGACG	CAGGCTGTGTGAATGGACCT
Pgam1	TCTGTGCAGAAGAGAGCAATCC	CTGTCAGACCGCCATAGTGT
Dlat	CTTTAGCCTCCAAAGCGAGAG	AGATTGTAAATGTTCCACCCTGG
Aldc	AGAAGGAGTTGTCGGATATTGCT	TTCTCCACCCCAATTTGGCTC
Ldha	TGTCTCCAGCAAAGACTACTGT	GACTGTACTTGACAATGTTGGGA
Cck	AAGAGCGGCGTATGTCTGTG	CATCCAGCCCATGTAGTCCC
Slc2a4	GTGACTGGAACACTGGTCCTA	CCAGCCACGTTGCATTGTAG
Gpr	TTTTCAGTGACATTCCTCGCC	GCACATAGACACAGAAGGGAGA
Plcb	GCCCCTGGAGATTCTGGAGT	GGGAGACTTGAGGTTCACCTTT
Akr1a1	AGCCTGGTCAGGTGAAAGC	GGCCTCCCCAATCTCAGTT
Slc2a5	CCAATATGGGTACAACGTAGCTG	GCGTCAAGGTGAAGGACTCAATA
Lct	AGTGAAAGTTTTCGTCTACACCC	CTGGACGTACAGCTCAGGAAG
Pgm3	TACCTGTGTATTGCACCAAAACT	AGAGCCAGGATAGCCTCAATC
Pkm	GCCGCCTGGACATTGACTC	CCATGAGAGAAATTCAGCCGAG
Mgam	ATGGAGAGTGACGTTGTAAACAC	GGAAGCGATTTGATGTCTGGTA
Pgk1	ATGTCGCTTTCCAACAAGCTG	GCTCCATTGTCCAAGCAGAAT
Gnat3	TAGGAGCCGAGAGGACCAAG	GCTGGTATTCAGATGCCCTTTC
Prkca	GTTTACCCGGCCAACGACT	GGGCGATGAATTTGTGGTCTT
Hk2	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
Ldhc	TTCACGGTGTAAGATTACTGTGG	TCGTATCAGCGTCAACAAGGG
Pfkm	TGTGGTCCGAGTTGGTATCTT	GCACTTCCAATCACTGTGCC
Slc2a1	CAGTTCGGCTATAACACTGGTG	GCCCCCGACAGAGAAGATG
Fbp1	CACCGCGATCAAAGCCATCT	AGGTAGCGTAGGACGACTTCA
Aldh5a	CGGTCAAGGAGAGGAGCTTAC	GGACTAGCCCTCGCTTATCTTT
Pgam2	TGGAACCAAGAGAACCGTTTC	TGGCATCTTTGATAGCGGTGG
Ald	AAATCTACCCTCTAGTACGGCAG	TTTCCCGGCACAAGACTCG
Eno3	CACAGCCAAGGGTCGATTCC	CCCAGGTATCGTGCTTTGTCT
Ldhbr	CATTGCGTCCGTTGCAGATG	GGAGGAACAAGCTCCCGTG
Eno1	TGCGTCCACTGGCATCTAC	CAGAGCAGGCGCAATAGTTTTA

Table 2. Primer pairs used for *Grp75* promoter binding transcription factor gene array in mouse MKs

Gene name	Forward primer (5'- 3')	Reverse primer (5'- 3')
Esr1	CCTCCCGCCTTCTACAGGT	CACACGGCACAGTAGCGAG
Pax5	CCATCAGGACAGGACATGGAG	GGCAAGTTCCACTATCCTTTGG
Nr3c1	AGCTCCCCCTGGTAGAGAC	GGTGAAGACGCAGAAACCTTG
Gtf2ird1	TTCGTCCTCTAACCCAGAGTC	ACAGAATTAGGGTGAAGTTCGGA
Tob	AGAACAATCCAGACTAGCAGCA	GGGAACTTCACATCACAGCTC
Stat4	TGGCAACAATTCTGCTTCAAAAC	GAGGTCCCTGGATAGGCATGT
Hnf1a	GACCTGACCGAGTTGCCTAAT	CCGGCTCTTTCAGAATGGGT
Hoxd10	ACCTATGGAATGCAAACCTGTG	TCTGTCCAACTGTCTACTTGAGG
Hoxd9	GCACCCTCAGCAACTACTACG	AAAACTACACGAGGCGAACTC
Myc	ATGCCCCTCAACGTGAACTTC	CGCAACATAGGATGGAGAGCA
Sry	GCTGGGATGCAGGTGGAAAA	CCCTCCGATGAGGCTGATATT
Tfap2a	TTTTTCAGCTATGGACCGTCAC	GAAGTCGGCATTAGGGGTG
Lrf2	AATTCCAATACGATACCAGGGCT	GAGCGGAGCATCCTTTTCCA

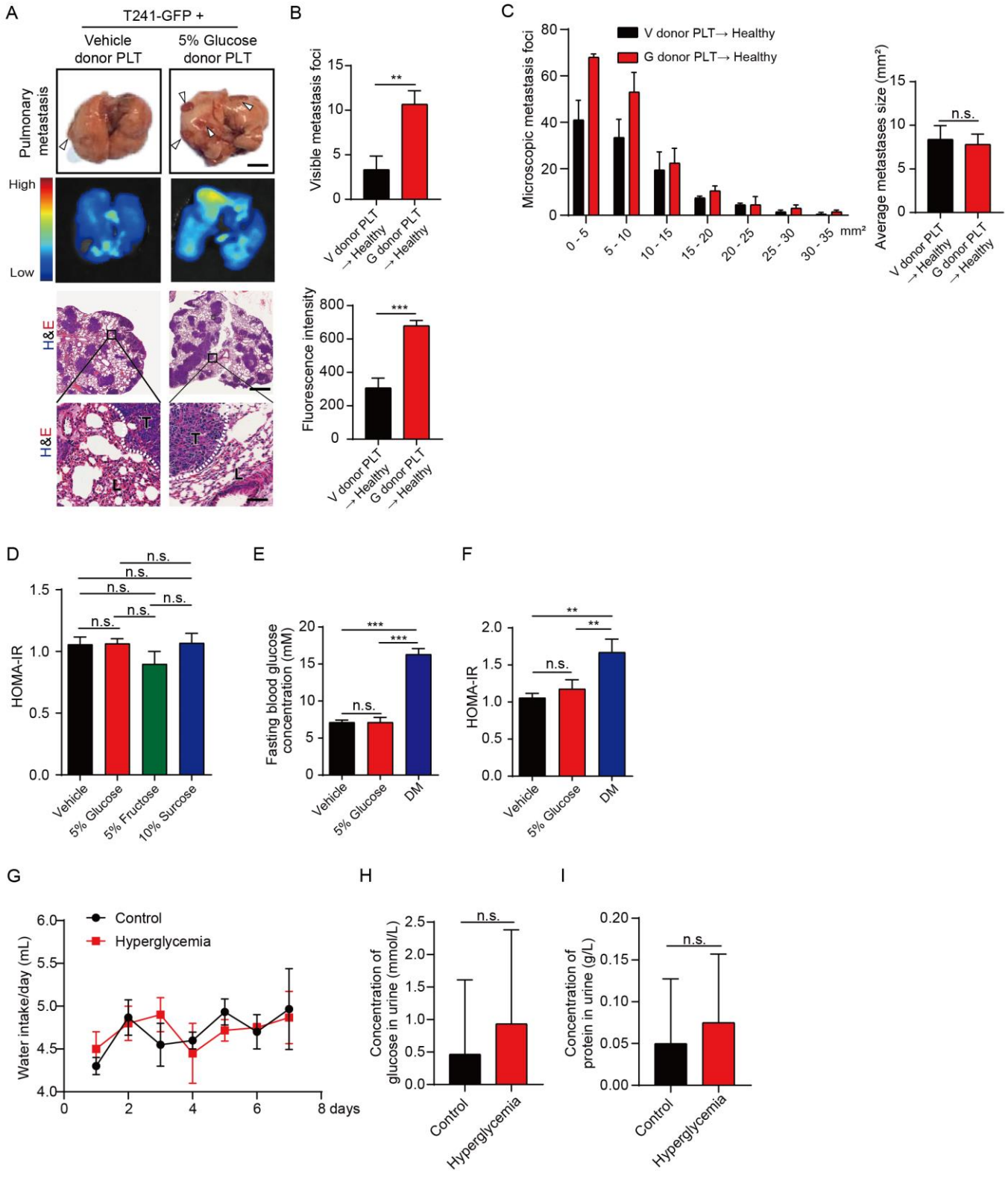
Supplemental Figures and Figure Legends


A
B16-F10-GFP
Vehicle
5% Glucose
Primary tumor
B
Tumor weight (g)
Tumor volume (cm³)
Vehicle
5% Glucose
C
Food intake per day (g)
n.s
D
Spleen weight (g)
S
T
E
Hepatic metastasis
High
Low
H&E
L
Fluorescence intensity
F
Vehicle
5% Glucose
Stratified hazard ratio for death, 0.2440 (95%CI, 0.06715-0.8870)
Two-sided P = 0.0114
Overall survival (%)
n=10
Time (d)
G
The relative expression of Glut1
H
shScrambled
shGlut1
I
B16-F10-GFP-shScrambled
B16-F10-GFP-shGlut1
Cell number (%)
day
J
B16-F10-GFP -shScrambled
B16-F10-GFP -shGlut1
K
V donor PLT → Healthy
G donor PLT → Healthy
Stratified hazard ratio for death, 0.2437 (95%CI, 0.02729-1.142).
Two-sided P = 0.0456

Supplemental Figure 1. Glucose promotes tumor cell growth via GlUT1

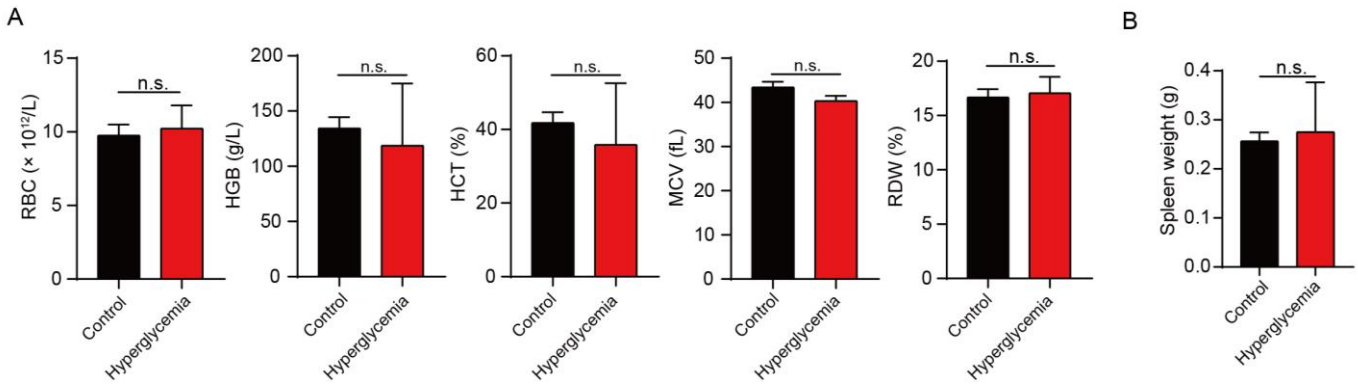
(A) Mice were treated with a 5% glucose solution and were subcutaneously implanted with B16-F10-GFP melanoma cells. Representative tumor pictures are shown. Scale bar, 2 mm. (B) Quantifications of tumor weight and tumor volume (n = 6 animals per group). (C) Quantifications of food intake per day in control or 5% glucose treated tumor-bearing mice (n = 6 animals per group). (D) Mice were treated with a 5% glucose solution and were surgically implanted with tumor cells in the spleen. Representative tumor pictures are shown. H&E histological analysis of the tumor. Dashed lines mark the borders between tumor and spleen tissues. S: Spleen; T: tumor. Scale bar in upper panels: 2 mm. Scale bar in lower panels: 100 μm. Quantifications of spleen weight (n = 6 animals per group). (E) Representative fluorescent micrographs of the liver. H&E histological analysis of liver metastases. Dashed lines mark the borders between tumor and liver tissues. L: liver; T: tumor. Scale bar in lower panels: 100 μm. Quantifications of liver fluorescence intensity (n = 6 animals per group). (F) Mice were treated with vehicle or 5% glucose for 7 days and were then injected i.v. with B16-F10-GFP tumor cells. Survivals of mice were monitored for 36 days. (G) qPCR quantification of *Glut1* mRNA expression levels in tumor implanted in control or hyperglycaemic mice (n = 6 samples per group). (H) qPCR quantification of *Glut1* mRNA expression levels in B16-F10-GFP tumor cells transfected with or without *Glut1*-shRNA (n = 3 samples per group). (I) Cell proliferation assay of B16-F10-GFP tumor cells transfected with or without *Glut1*-shRNA (n = 5 samples per group). (J) Mice were treated with a 5% glucose solution and were i.v. injected with tumor cells transfected with or without sh*GLUT1*. Representative tumor pictures are shown. Scale bar, 2 mm. Quantifications of tumor weight and tumor volume (n = 6 animals per group). (K) Platelets were isolated from control or hyperglycemic donor mice and were mixed with B16-F10-GFP

melanoma cells and co-injected into healthy recipient mice. Survivals of mice were monitored for 36 days. *$p<0.05$; **$p<0.01$, ***$p<0.001$. NS = not significant. Data presented as mean ± s.e.m..



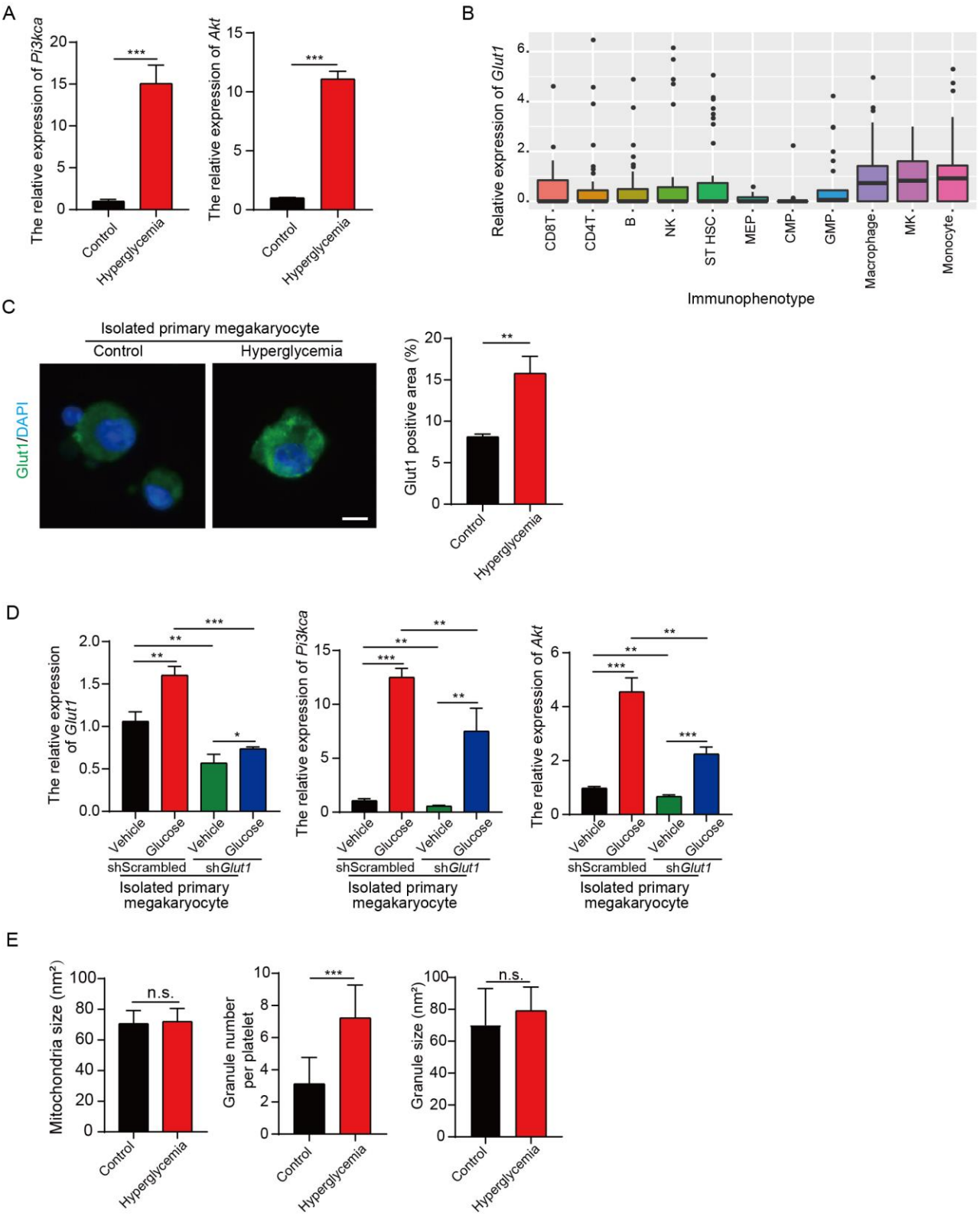
Supplemental Figure 2. Glucose promotes metastasis without altering insulin sensitivity

(A) Platelets were isolated from control or hyperglycemic donor mice and were mixed with T241-GFP tumor cells and co-injected into healthy recipient mice. Representative lung pictures are shown. White arrowheads point to visible metastatic nodules. Scale bar in lung pictures: 3 mm. Representative fluorescent lung micrographs are shown. H&E histological analysis of lung metastasis. Dashed lines mark the borders between tumor and lung tissues. L: lung; T: tumor. Scale bar in upper panels: 2 mm. Scale bar in lower panels: 100 μm. (B) Quantifications of visible surface lung metastatic nodules. Quantifications of lung fluorescence intensity (n = 8 animals per group). (C) Quantifications of metastasis rate analysis. Whole lung microscopic metastasis distribution was analysed by size and number. Quantifications of average metastasis size (n = 8 lungs per group). (D) HOMA-IR levels of the vehicle-, 5% glucose-, 5% fructose-, and 10% sucrose-treated mice (n = 6 samples per group). (E-F) Fasting blood glucose levels and HOMA-IR levels in vehicle-treated, 5% glucose-treated, and STZ-induced diabetic mice (n = 6 samples per group). (G-I) Healthy mice were treated with vehicle or 5% glucose for 7 days and the water intake, urine glucose, and urine protein levels were quantified (n = 6 animals per group). *p<0.05; **p<0.01, ***p<0.001. NS = not significant. Data presented as mean ± s.e.m..



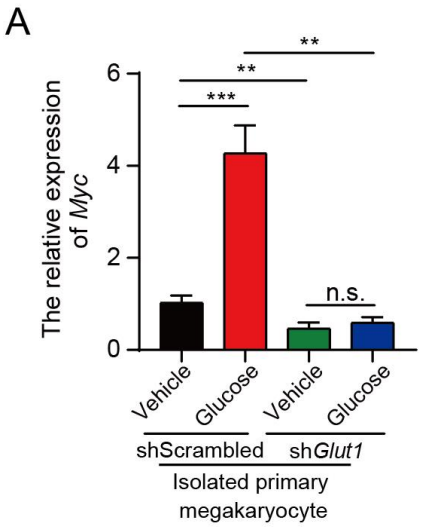
Supplemental Figure 3. RBC parameters and spleen weight are not affected by hyperglycemia

(A) RBC, HGB, HCT, MCV, and RDW analysis in whole blood from control or hyperglycaemic groups. (n = 6 samples per group). (B) Quantifications of spleen weight in control and hyperglycemia groups (n = 6 samples per group). NS = not significant. Data presented as mean ± s.e.m..



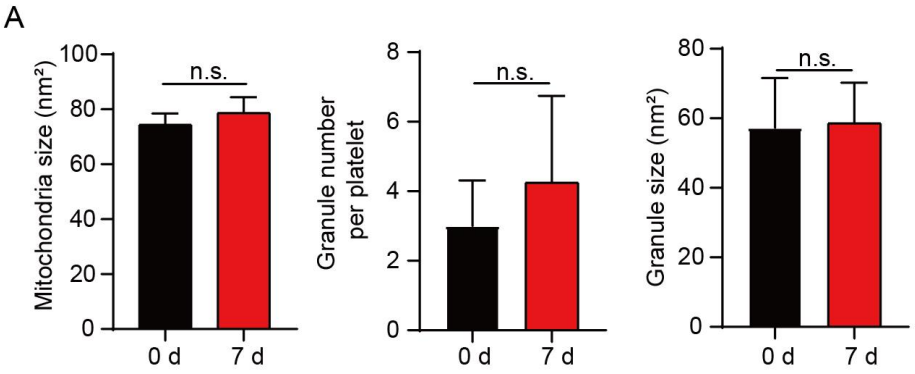
Supplemental Figure 4. Glucose activates MKs and increases platelet granule number

(A) qPCR quantification of *Pik3ca* and *Akt* mRNA expression levels in MKs isolated from control or hyperglycemia group (n = 3 samples per group). (B) *Glut1* expression levels in various cell types in bone marrow. Data were collected from the Atlas of Mouse Blood Cells. (C) GLUT1 (green) and DAPI (blue) immunofluorescent staining micrographs of isolated MKs from control or hyperglycaemic mice. Quantifications of $GLUT1^+$ signals per cell (n = 6 random fields per group). Scale bar, 15 μm. (D) Isolated healthy MKs were transfected with or without *Glut1*-shRNA and then treated with normal (5.5 mM) or high (12.5 mM) dose of glucose *in vitro*. qPCR quantification of *Glut1*, *Pik3ca* and *Akt* mRNA expression levels in various groups (n = 6 samples per group). (E) Quantifications of mitochondria size, granule number, and granule size per platelet (n = 6 random fields per group). *p<0.05; **p<0.01, ***p<0.001. NS = not significant. Data presented as mean ± s.e.m..



Supplemental Figure 5. Glucose-driven MYC expression in MKs is GLUT1 dependent

(A) Isolated healthy MKs were transfected with or without *Glut1*-shRNA and then treated with normal (5.5 mM) or high (12.5 mM) dose of glucose *in vitro*. qPCR quantification of *Myc* mRNA expression levels in various groups (n = 6 samples per group). **p<0.01; ***p<0.001. NS = not significant. Data presented as mean ± s.e.m..



Supplemental Figure 6. Glucose administration does not change mitochondria or granule sizes in human platelets

(A) Quantifications of mitochondria size, granule number, and granule size per platelet (n = 6 random fields per group). NS = not significant. Data presented as mean ± s.e.m..