

Exogenous creatine supplementation promotes tumor metastasis via megakaryocyte creatine kinase B-STAT5B signaling

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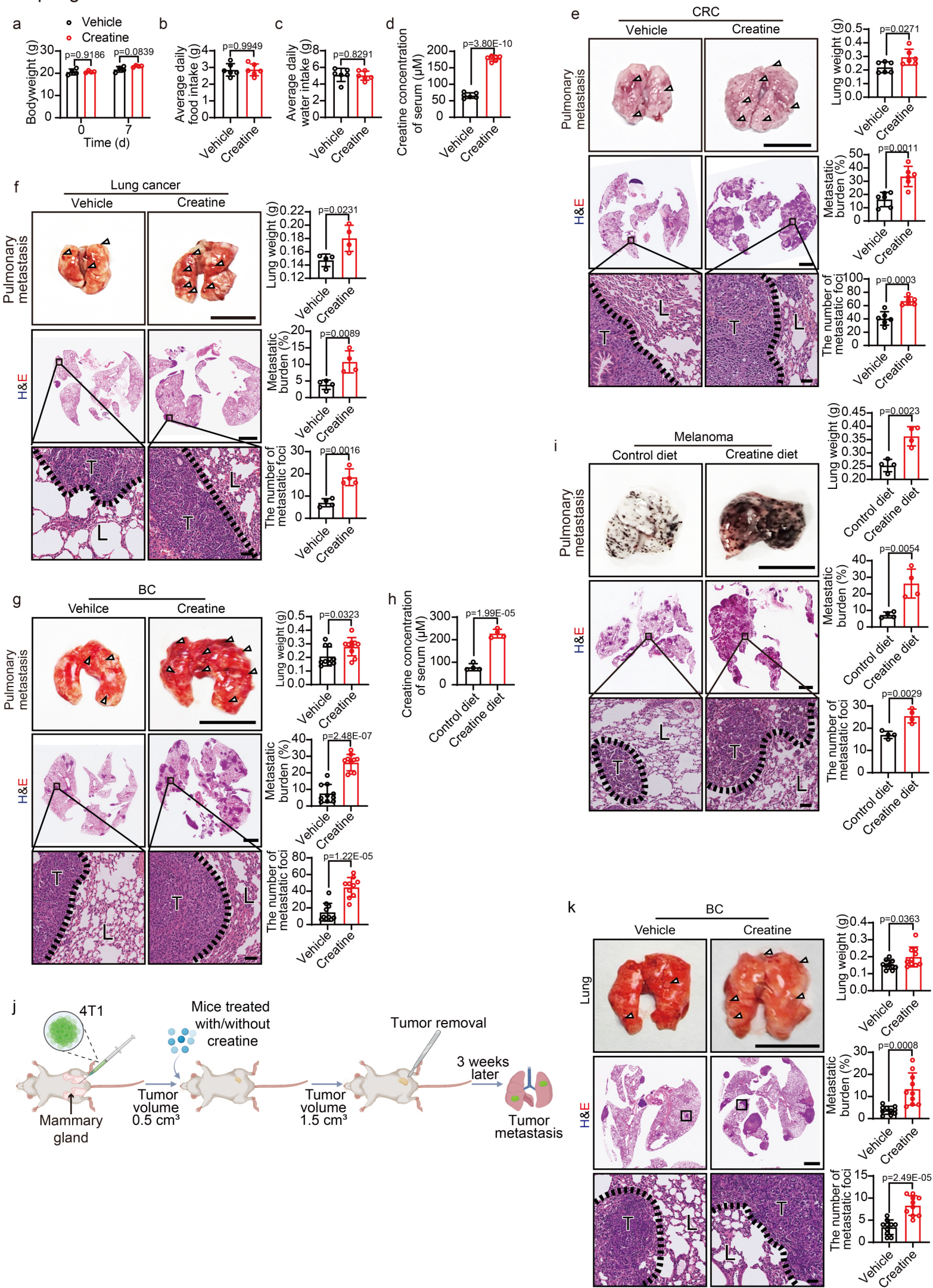
Running title: megakaryocytes confer creatine-induced metastasis

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Supplementary Figures and Figure Legends

2

Sup Fig.1



Supplementary Figure 1. Exogenous creatine promotes tumor metastasis in various tumor models

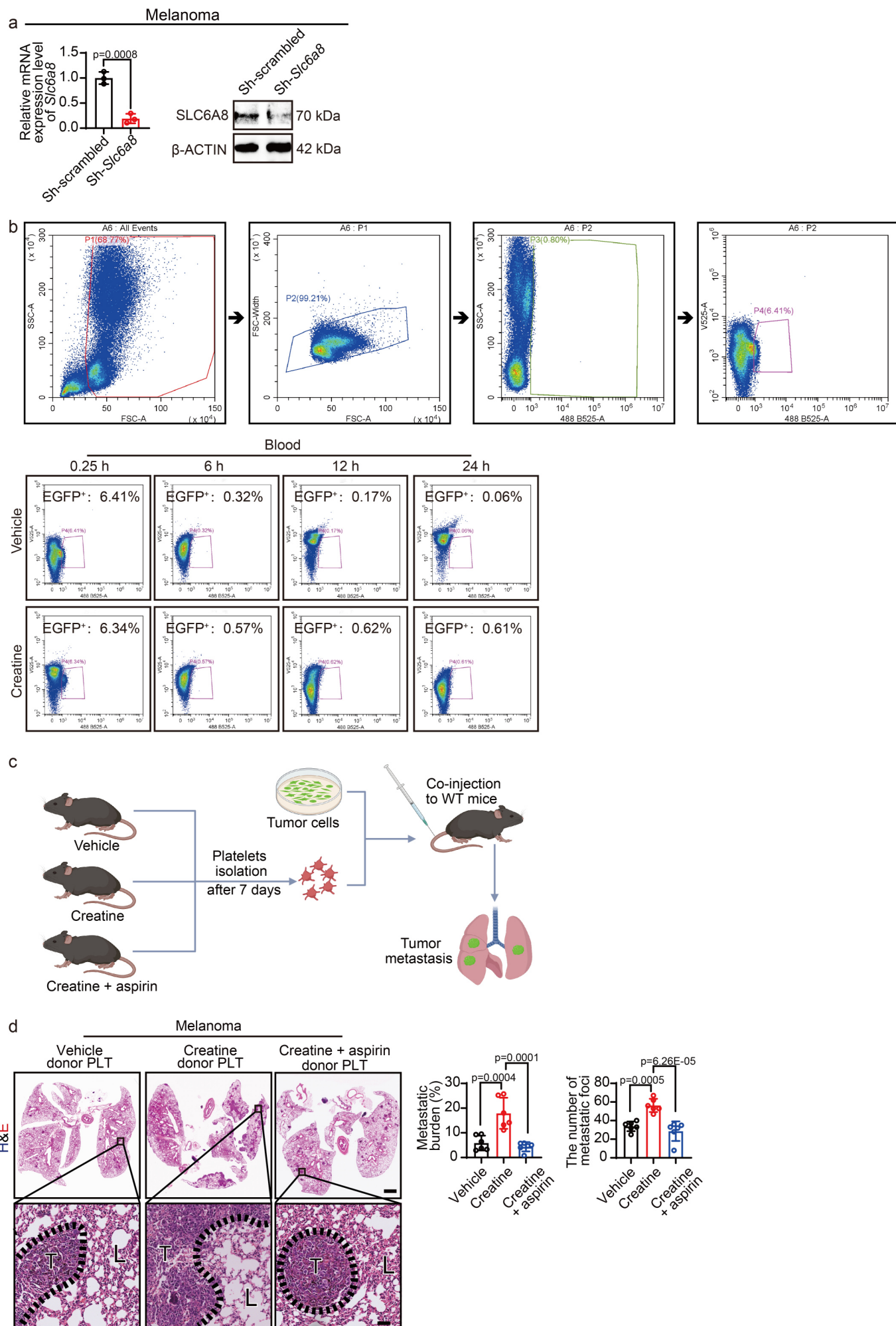
a Quantification of bodyweight in vehicle- or creatine-treated mice ($n = 4$ mice per group). **b, c** Healthy mice were treated with vehicle or creatine and the average food intake and water intake were quantified ($n = 6$ tests per group). **d** Circulating creatine concentrations in vehicle- or creatine-treated mice after 7 days of treatment ($n = 6$ mice per group). **e-g** Male C57BL/6 mice were treated with vehicle or creatine for 7 days and were then injected i.v. with MC38 murine CRC cells (**e**) or LLC murine lung cancer cells (**f**). Female BALB/c mice were treated with vehicle or creatine for 7 days and were then injected i.v. with 4T1 murine breast cancer cells (**g**). Representative lung pictures and H&E histological analysis of lung metastasis. Dashed lines mark the borders between tumor (T) and lung (L) tissues. Arrowhead, visible metastatic nodules. Scale bar in upper panels, 1 cm. Scale bar in middle panels, 2 mm. Scale bar in lower panels, 50 μm . Quantifications of lung weight ($n = 6, 4$, and 10 mice per group in **e, f, g**, respectively). Quantifications of metastatic burden and the number of metastatic foci ($n = 6, 4$, and 10 mice per group in **e, f, g**, respectively). **h** Circulating creatine concentrations in mice fed with control diet or creatine diet ad libitum for 7 days ($n = 4$ mice per group). **i** Mice were fed with control diet or 5% w/w creatine diet ad libitum and were then injected i.v. with melanoma cells. Representative lung pictures and H&E histological analysis of lung metastasis. Dashed lines mark the borders between tumor (T) and lung (L) tissues. Scale bar in upper panels, 1 cm. Scale bar in middle panels, 2 mm. Scale bar in lower panels, 50 μm . Quantifications of lung weight ($n = 4$ mice per group). Quantifications of metastatic burden and the number of metastatic foci ($n = 4$ mice per group). **j** Schematic diagram of the spontaneous metastasis model of 4T1 breast cancer in BALB/c mice (created in BioRender. Ruibo, C. (2026)

1 <https://BioRender.com/ey8rzg9>). **k** Representative lung pictures and H&E histological
2 analysis of lung metastasis. Dashed lines mark the borders between tumor (T) and lung
3 (L) tissues. Arrowhead, visible metastatic nodules. Scale bar in upper panels, 1 cm.
4 Scale bar in middle panels, 2 mm. Scale bar in lower panels, 50 μ m. Quantifications of
5 lung weight ($n = 10$ mice per group). Quantifications of metastatic burden and the
6 number of metastatic foci ($n = 10$ mice per group). Statistical significance in **a, b, c, d,**
7 **e, f, g, h, i,** and **k** was determined by unpaired two-tailed Student's *t*-test. Source data
8 are provided as a Source Data file. Data presented as mean $\pm$ s.d..

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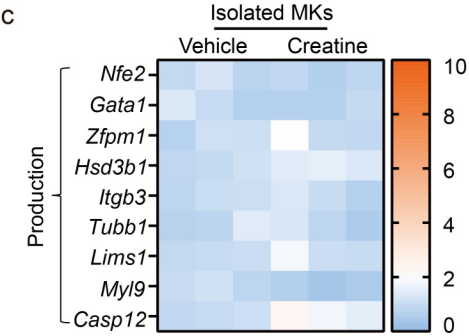
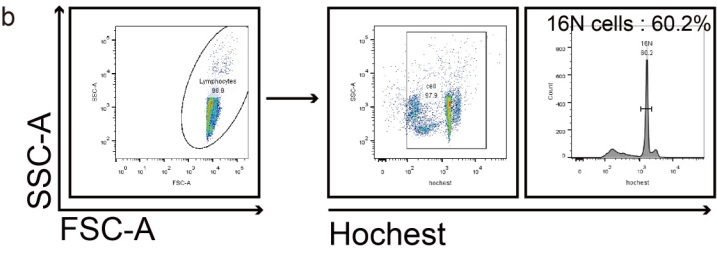
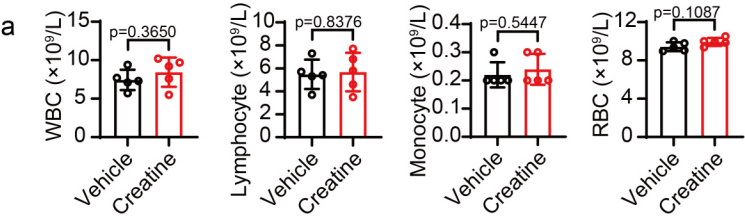
Sup Fig.2



Supplementary Figure 2. Creatine-promoted metastasis is platelet activation-dependent

a QPCR quantification of *Slc6a8* mRNA expression in melanoma cells transfected with ShRNA of scrambled or *Slc6a8* ($n = 3$ samples per group). Western blot of SLC6A8 in melanoma cells transfected with ShRNA of scrambled or *Slc6a8*. β -ACTIN marks the loading amount in each lane. Blots have been repeated twice. Samples derive from the same experiment but different gels, with different antibodies processed in parallel. **b** Gating strategies and representative images of flow cytometry of GFP⁺ tumor cells in the peripheral blood from vehicle- or creatine-treated mice at various timepoints. **c** Schematic diagram of the adoptive platelet transfer model. Platelets were isolated from vehicle- or creatine-treated donor mice receiving various drug treatments and were co-injected with melanoma cells into healthy recipient mice (created in BioRender. Ruibo, C. (2026) <https://BioRender.com/9d97ycz>). **d** H&E histological analysis of lung metastasis. Dashed lines mark the borders between tumor (T) and lung (L) tissues. Scale bar in upper panels, 2 mm. Scale bar in lower panels, 50 μ m. Quantifications of metastatic burden and the number of metastatic foci ($n = 6$ mice per group). Statistical significance in **a** was determined by unpaired two-tailed Student's *t*-test. Statistical significance in **d** was determined by one-way ANOVA. Source data are provided as a Source Data file. Data presented as mean $\pm$ s.d..

Sup Fig.3

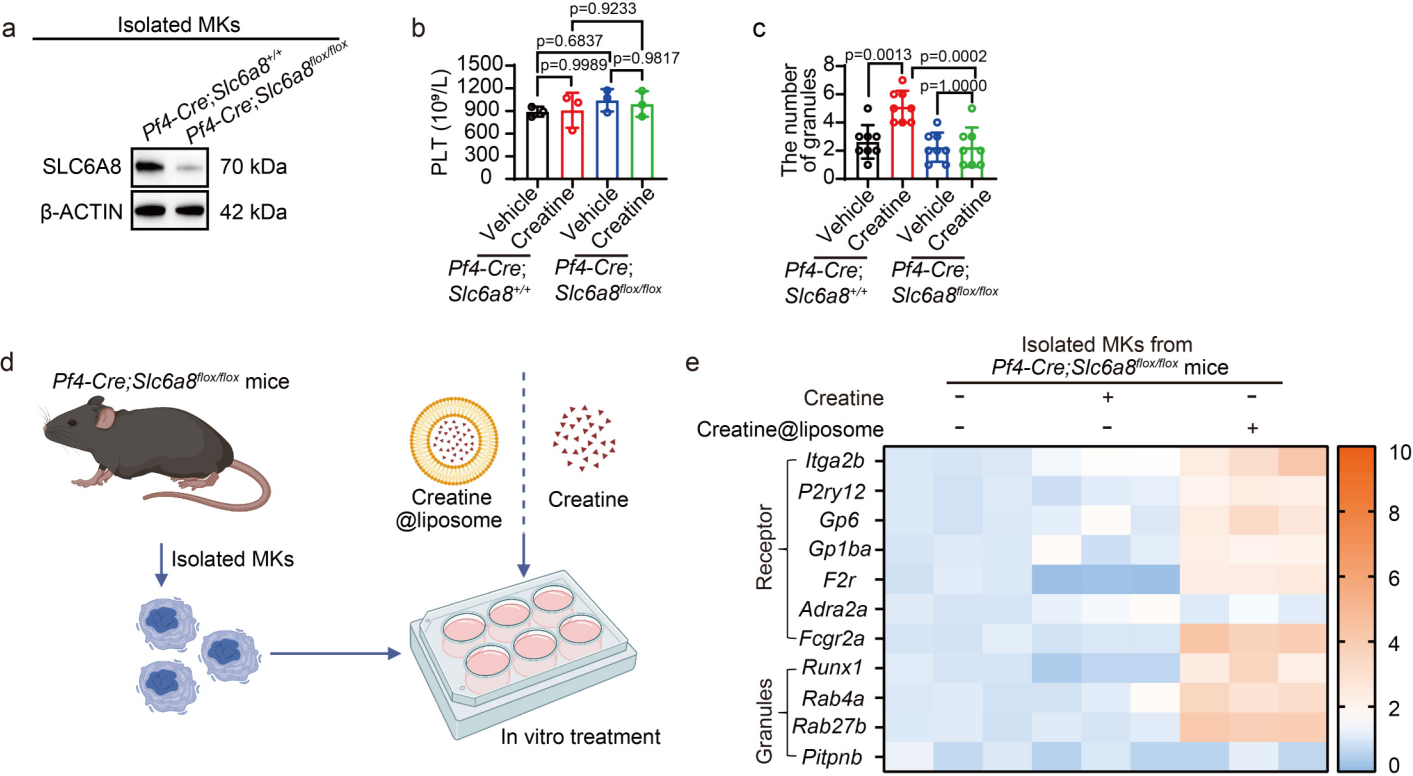


1 **Supplementary Figure 3. Creatine insignificantly affects thrombocytopoiesis-**
2 **associated genes**

3 **a** WBC, lymphocyte, monocyte, and RBC analysis in whole blood from vehicle- or
4 creatine-treated mice ($n = 5$ mice per group). **b** Gating strategies and Hoechst 33342
5 staining of isolated MKs. MK polyploidy was validated. **c** Heatmap of
6 thrombocytopoiesis-associated genes of isolated primary MKs from vehicle- or
7 creatine-treated mice ($n = 3$ mice per group). Statistical significance in **a** was
8 determined by unpaired two-tailed Student's *t*-test. Source data are provided as a
9 Source Data file. Data presented as mean $\pm$ s.d..

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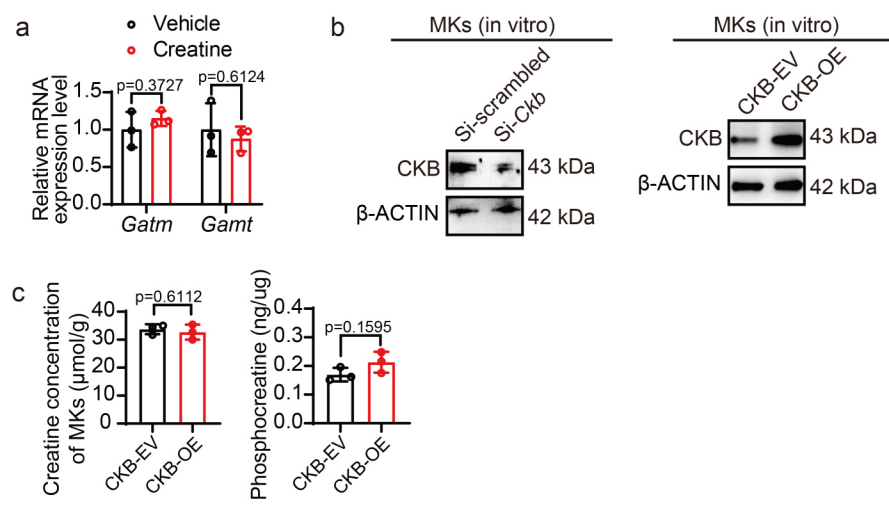
Sup Fig.4



Supplementary Figure 4. Genetic depletion of *Slc6a8* in MKs does not affect platelet counts.

a Western blot of SLC6A8 in isolated primary MKs from wt or *Pf4 Cre Slc6a8^{flox/flox}* mice. β -ACTIN marks the loading amount in each lane. Blots have been repeated twice. Samples derive from the same experiment but different gels, with different antibodies processed in parallel. **b** Platelet counts analysis in whole blood from vehicle- or creatine-treated wt or *Pf4 Cre Slc6a8^{flox/flox}* mice ($n = 3$ mice per group). **c** Quantifications of granules in transmission electron microscopy micrographs of platelets isolated from vehicle- or creatine-treated wt or *Pf4 Cre Slc6a8^{flox/flox}* mice ($n = 8$ random fields per group). **d** Schematic diagram of the in vitro study in which freshly isolated MKs from *Slc6a8^{flox/flox}* mice were treated with creatine or creatine@liposome (created in BioRender. Ruibo, C. (2026) <https://BioRender.com/urkzg4m>). **e** Heatmap of platelet receptor- and granule-associated genes of isolated primary MKs treated with creatine or creatine@liposome. ($n = 3$ samples per group). Statistical significance in **b** and **c** was determined by one-way ANOVA. Source data are provided as a Source Data file. Data presented as mean $\pm$ s.d..

Sup Fig.5

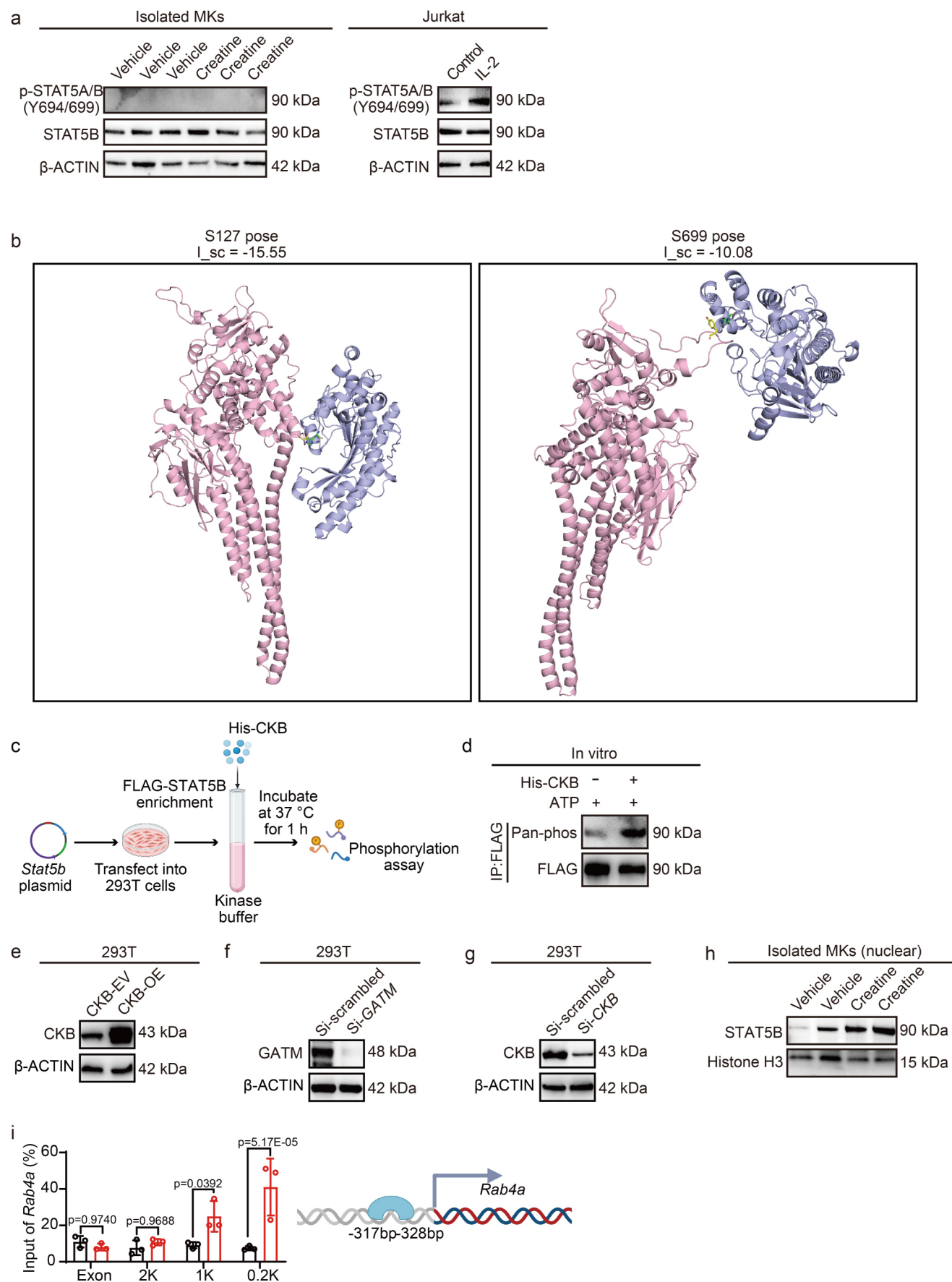


1 **Supplementary Figure 5. Exogenous creatine insignificantly affects creatine**
2 **synthesis enzymes**

3 **a** QPCR quantification of *Gatm* and *Gamt* mRNA expression in freshly isolated MKs
4 from vehicle- or creatine-treated mice ($n = 3$ mice per group). **b** Western blot of CKB
5 in si-scrambled- or si-*Ckb* (mouse)-transfected MKs in vitro. Western blot of CKB in
6 empty vector- or *Ckb* overexpression vector-transfected MKs in vitro. β -ACTIN marks
7 the loading amount in each lane. Blots have been repeated twice. Samples derive from
8 the same experiment but different gels, with different antibodies processed in parallel.
9 **c** Intracellular creatine concentrations in empty vector- or *Ckb* overexpression vector-
10 transfected MKs in vitro ($n = 3$ mice per group). Intracellular phosphocreatine
11 concentrations in empty vector- or *Ckb* overexpression vector-transfected MKs in vitro
12 ($n = 3$ mice per group). Statistical significance in **a** and **c** was determined by unpaired
13 two-tailed Student's *t*-test. Source data are provided as a Source Data file. Data
14 presented as mean $\pm$ s.d..

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Sup Fig.6

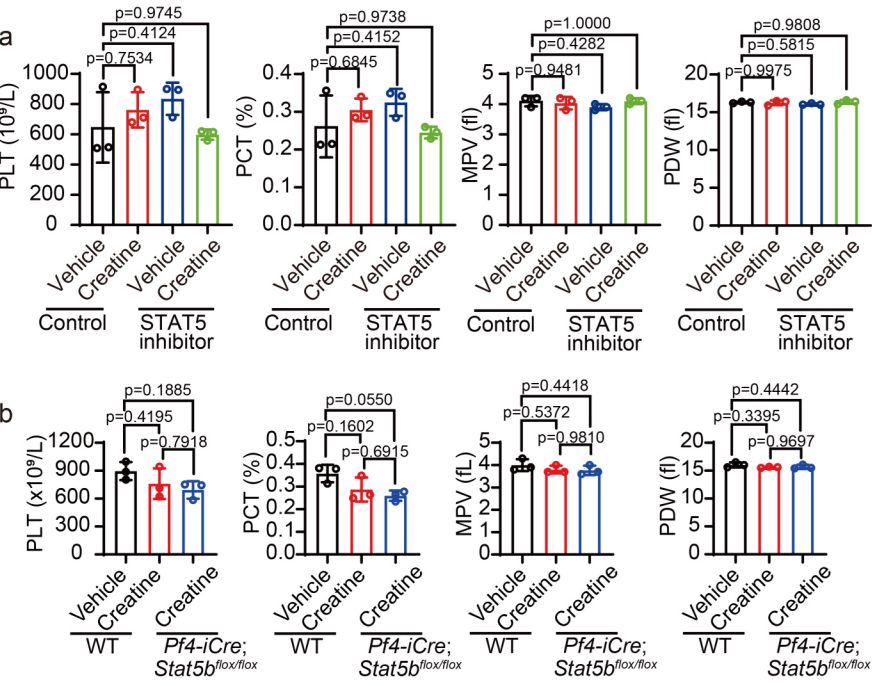


1 **Supplementary Figure 6. Molecular docking of CKB and STAT5B**

2 **a** Western blot of phosphorylation of Y699 in STAT5B and total STAT5B in freshly
3 isolated MKs from vehicle- or creatine-treated mice. IL-2-stimulated Jurkat human T
4 cell line served as positive control. β -ACTIN marks the loading amount in each lane. **b**
5 Predicted binding poses of CKB and STAT5B showing the highest binding affinity
6 pose for CKB with STAT5B-S127 site and STAT5B-Y699 site, respectively. CKB and
7 STAT5B are shown in light blue and light pink ribbon, respectively. The S127 and
8 Y699 residues in STAT5B are shown in yellow sticks. The H66 residues in CKB are
9 shown in green sticks. **c** Schematic diagram of the in vitro kinase assay. FLAG-
10 STAT5B and His-CKB were co-incubated in a kinase buffer containing ATP. FLAG-
11 STAT5B was then purified for the phosphorylation test. **d** Western blot of
12 phosphorylation of the STAT5B-FLAG co-incubated with or without His-CKB. FLAG
13 marks the loading amount in each lane. **e** Western blot of CKB in empty vector- or *Ckb*
14 overexpression vector-transfected 293T cells in vitro. β -ACTIN marks the loading
15 amount in each lane. **f** Western blot of GATM in si-scrambled- or si-*Gatm*-transfected
16 293T cells in vitro. β -ACTIN marks the loading amount in each lane. **g** Western blot of
17 CKB in si-scrambled- or si-*Ckb* (human)-transfected 293T cells in vitro. β -ACTIN
18 marks the loading amount in each lane. **h** Nuclear fractions were separated from freshly
19 isolated MKs from vehicle- or creatine-treated mice. Western blot of STAT5B in total
20 nuclear fraction. Histone H3 marks the loading amount in each lane. **i** ChIP assay of
21 STAT5B binds to different promoter regions of *Rab4a*. Nonimmune IgG was used for
22 input. Coding region (exon) served as controls ($n = 3$ samples per group). Schematic
23 diagram of the DNA binding model. Blots in **a**, **d**, **e**, **f**, **g**, and **h** have been repeated
24 twice. Samples derive from the same experiment but different gels, with different
25 antibodies processed in parallel. Schematics in **c** and **i** were created in BioRender.

1 Ruibo, C. (2026) <https://BioRender.com/5hsv4us>. Statistical significance in **i** was
2 determined by two-way ANOVA. Source data are provided as a Source Data file. Data
3 presented as mean $\pm$ s.d..

Sup Fig.7

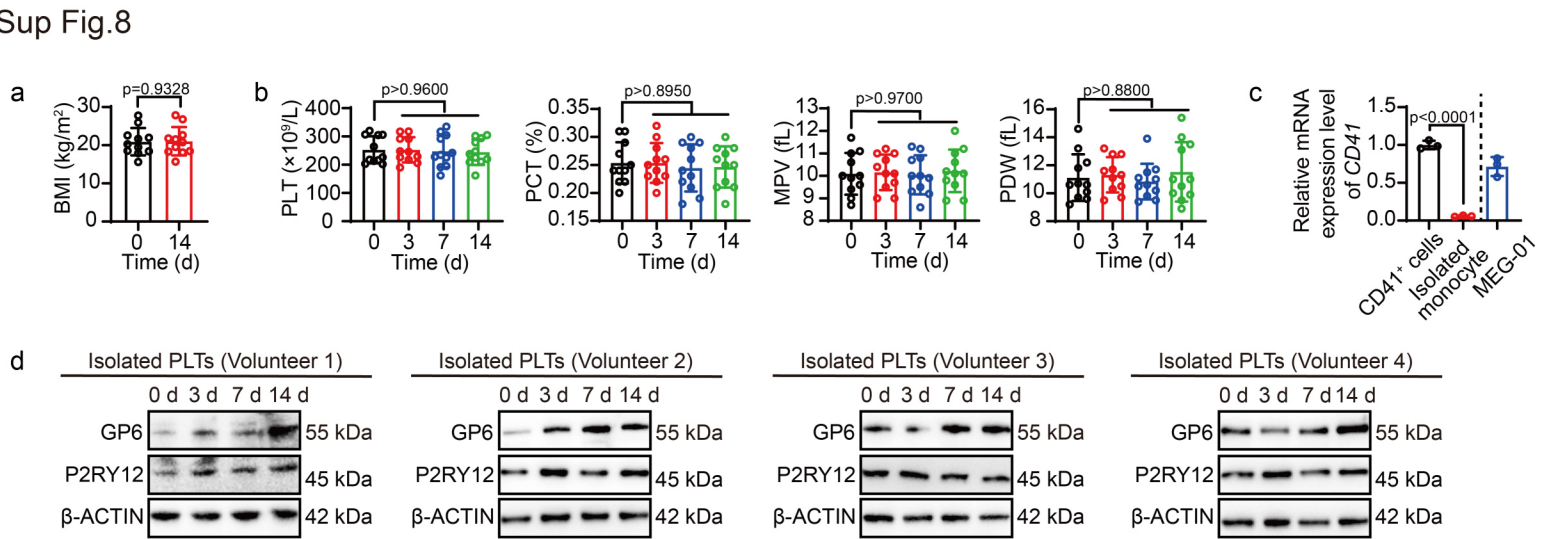


1 **Supplementary Figure 7. Pharmacological inhibition of STAT5 or MK-specific**
2 ***Stat5b* depletion insignificantly affects platelet count**

3 **a** Platelet counts (PLT), plateletcrit (PCT), platelet distribution width (PDW), mean
4 platelet volume (MPV) analysis in whole blood from vehicle- or creatine-treated mice
5 with or without STAT5-specific inhibitor administration ($n = 3$ samples per group). **b**
6 Platelet counts (PLT), plateletcrit (PCT), platelet distribution width (PDW), mean
7 platelet volume (MPV) analysis in whole blood from vehicle- or creatine-treated wt or
8 Pf4 iCre *Stat5b*^{flox/flox} mice ($n = 3$ samples per group). Statistical significance in **a** and
9 **b** was determined by one-way ANOVA. Source data are provided as a Source Data file.

10 Data presented as mean $\pm$ s.d..

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Supplementary Figure 8. Exogenous creatine supplementation increases hyperactive platelets in humans

a Quantifications of BMI of volunteers before and after 14 days of creatine treatment ($n = 11$ volunteers per group). **b** Platelet counts (PLT), plateletcrit (PCT), platelet distribution width (PDW), mean platelet volume (MPV) analysis in whole blood from volunteers at various timepoints ($n = 11$ volunteers per group). **c** QPCR quantification of *CD41* mRNA expression in freshly isolated $CD41^+$ cells, isolated monocytes, and MEG-01 cells ($n = 3$ samples per group). **d** For functional study, 4 out of 11 volunteers were randomly selected and platelets were isolated from peripheral blood. Western blot of GP6 and P2RY12 in isolated platelets from each volunteer at various timepoints. β -ACTIN marks the loading amount in each lane. Blots have been repeated twice. Samples derive from the same experiment but different gels, with different antibodies processed in parallel. Statistical significance in **a** and **c** was determined by unpaired two-tailed Student's *t*-test. Statistical significance in **b** was determined by one-way ANOVA. Source data are provided as a Source Data file. Data presented as mean $\pm$ s.d..

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Supplementary Tables

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1 **Supplementary Table 1. Baseline characteristics of 11 healthy volunteers**

2

Gender	Female	6
	Male	5
Age		20.91 ± 1.45
Height (cm)		165.09 ± 7.08
Weight (kg)		57.64 ± 14.16
BMI (kg/m²)		20.94 ± 3.59
NSAID	Yes	0
	No	11

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1 **Supplementary Table 2. Primers for qPCR and ChIP used in the experiment**
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Primer Name	Primers (5'-3')
Primers for qPCR	
m- <i>Rab4a</i> -F	CTCCAGGTTCGCACAAGAGAA
m- <i>Rab4a</i> -R	TCCTTGACACTGCATGAAAG
m- <i>Plcg1</i> -F	ATCCAGCAGTCCTAGAGCCTG
m- <i>Plcg1</i> -R	GGATGGCGATCTGACAAGC
m- <i>Gp1ba</i> -F	CATTCCCAGCACACTTGTAGT
m- <i>Gp1ba</i> -R	TGAGGTGAGTGAAATGCACCA
m- <i>Orai1</i> -F	GATCGGCCAGAGTTACTCCG
m- <i>Orai1</i> -R	TGGGTAGTCATGGTCTGTGTC
m- <i>Itga2b</i> -F	TTCTTGGGTCCTAGTGCTGTT
m- <i>Itga2b</i> -R	CGCTTCCATGTTTGTCTTATGA
m- <i>P2ry12</i> -F	ATGGATATGCCTGGTGTCAACA
m- <i>P2ry12</i> -R	AGCAATGGGAAGAGAACCTGG
m- <i>F2r</i> -F	TGAACCCCCGCTCATTCTTTC
m- <i>F2r</i> -R	CCAGCAGGACGCTTTCATTTT
m- <i>Adra2a</i> -F	GTGACACTGACGCTGGTTTG
m- <i>Adra2a</i> -R	CCAGTAACCCATAACCTCGTTG
m- <i>Gp6</i> -F	CTTTCTTCTGTATTGGGCTGTGT
m- <i>Gp6</i> -R	CGTCCAGCATTACTTCTTTCCA
m- <i>Fcgr2a</i> -F	AGGGCCTCCATCTGGACTG
m- <i>Fcgr2a</i> -R	GTGGTTCTGGTAATCATGCTCTG
m- <i>Actb</i> -F	GGCTGTATTCCCCTCCATCG
m- <i>Actb</i> -R	CCAGTTGGTAACAATGCCATGT
m- <i>Gatm</i> -F	GCTTCCTCCCGAAATTCCTGT
m- <i>Gatm</i> -R	CCTCTAAAGGGTCCCATTCTG
m- <i>Gamt</i> -F	CACGCACCTGCAAATCCTG
m- <i>Gamt</i> -R	TACCGAAGCCCCTTCCAAGA
m- <i>Ckb</i> -F	AGTTCCTGATCTGAGCAGC
m- <i>Ckb</i> -R	GAATGGCGTCGTCCAAAGTAA
m- <i>Runx1</i> -F	GATGGCACTCTGGTCACCG
m- <i>Runx1</i> -R	GCCGCTCGGAAAAGGACAA
m- <i>Rab27b</i> -F	CGTCAGGAAAAGCGTTTAAGGT
m- <i>Rab27b</i> -R	AGAAGCTCTGTTGACTGGTGA
m- <i>Pitpnb</i> -F	TCCATTCACTCAAGACCAAGAGA
m- <i>Pitpnb</i> -R	AACCCTTCTTACGCATTGTTTCT
m- <i>Nfe2</i> -F	TCCTCAGCAGAACAGGAACAG
m- <i>Nfe2</i> -R	GGCTCAAAAGATGTCTCACTTGG
m- <i>Gata1</i> -F	TGGGGACCTCAGAACCCTTG
m- <i>Gata1</i> -R	GGCTGCATTTGGGGAAGTG
m- <i>Zfp1</i> -F	AGGAAACAGAGCAATCCCCG

m- <i>Zfp1</i> -R	CAGGTGGGCTCACATCTTCT
m- <i>Hsd3b1</i> -F	TGGACAAAGTATTCCGACCAGA
m- <i>Hsd3b1</i> -R	GGCACACTTGCTTGAACACAG
m- <i>Itgb3</i> -F	CCACACGAGGCGTGAAGCTC
m- <i>Itgb3</i> -R	CTTCAGGTTACATCGGGGTGA
m- <i>Tubb1</i> -F	CTGGGAGGTGATCGGGGAA
m- <i>Tubb1</i> -R	GCACATACTTCTTACCGTAGGCT
m- <i>Lims1</i> -F	CAGCGTCCTTGAAGCTAATGG
m- <i>Lims1</i> -R	TTACTGTTACGATCTTCTCAGC
m- <i>My19</i> -F	ACAGCGCCGAGGACTTTTC
m- <i>My19</i> -R	AGACATTGGACGTAGCCCTCT
m- <i>Casp12</i> -F	AGACAGAGTTAATGCAGTTTGCT
m- <i>Casp12</i> -R	TTCACCCACAGATTCCTTCC
h- <i>RAB4A</i> -F	GTCCGTGACGAGAAGTTATTACC
h- <i>RAB4A</i> -R	TGAGCGCACTTGTTTCCAAA
h- <i>PLCG1</i> -F	GGAAGACCTCACGGGACTTTG
h- <i>PLCG1</i> -R	GCGTTTTTCAGGCGAAATTCCA
h- <i>Orai1</i> -F	GACTGGATCGGCCAGAGTTAC
h- <i>Orai1</i> -R	GTCCGGCTGGAGGCTTTAAG
h- <i>ITGA2B</i> -F	GATGAGACCCGAAATGTAGGC
h- <i>ITGA2B</i> -R	GTCTTTTCTAGGACGTTCCAGTG
h- <i>P2RY12</i> -F	CACTGCTCTACACTGTCCTGT
h- <i>P2RY12</i> -R	AGTGGTCCTGTTCCAGTTTG
h- <i>GP6</i> -F	TCCCGGCCATGAAGAGAAGT
h- <i>GP6</i> -R	TTACGTCCCCTCCTGACGAC
h- <i>GP1BA</i> -F	CTGTGAGGTCTCCAAAGTGGC
h- <i>GP1BA</i> -R	GTGAGGCGAGTGTAAGGCATC
h- <i>F2R</i> -F	CCACCTTAGATCCCCGGTCAT
h- <i>F2R</i> -R	GTGGGAGGCTGACTACAAACA
h- <i>ADRA2A</i> -F	TCGTCATCATCGCCGTGTTT
h- <i>ADRA2A</i> -R	AAGCCTTGCCGAAGTACCAG
h- <i>FCGR2A</i> -F	TTTGAGATGAGTAATCCCAGCCA
h- <i>FCGR2A</i> -R	TCAGGCCCAGTCTCCATTTTA
h- <i>ACTB</i> -F	CATGTACGTTGCTATCCAGGC
h- <i>ACTB</i> -R	CTCCTTAATGTCACGCACGAT
h- <i>RUNX1</i> -F	CTGCCCATCGCTTTCAAGGT
h- <i>RUNX1</i> -R	GCCGAGTAGTTTTTCATCATTGCC
h- <i>RAB27B</i> -F	TAGACTTTCGGGAAAAACGTGTG
h- <i>RAB27B</i> -R	AGAAGCTCTGTTGACTGGTGA
h- <i>PITPNB</i> -F	CTGTTCAAGAGTATCAGGTTGGG
h- <i>PITPNB</i> -R	GGCACTTTGCTCTTTAGGTGA

Primers for ChIP	
m- <i>Rab4a</i> -promoter-200-F	CTCAGGTGGGTCAAACCTCCC
m- <i>Rab4a</i> -promoter-200-R	GGTCACCAGAACTCTCCTGC
m- <i>Rab4a</i> -promoter-1000-F	ACCTCTGCACCTTGGCAGTTT
m- <i>Rab4a</i> -promoter-1000-R	ATCCAGTACACCGCAGGAAC
m- <i>Rab4a</i> -promoter-2000-F	ACCTCTGCACCTTGGCAGTTT
m- <i>Rab4a</i> -promoter-2000-R	TGGCCTCTGATCCTATCCAGT
m- <i>Rab4a</i> -exon-F	CATCACCAGCCGAGAAACCT
m- <i>Rab4a</i> -exon-R	GTCCTTCTTGTTCCCGCAGA
m- <i>P2ry12</i> -promoter-200-F	AGGAGGACAGGAGAGGCAAT
m- <i>P2ry12</i> -promoter-200-R	TTCCTGTCAGAGGACCTGGA
m- <i>P2ry12</i> -promoter-1000-F	AACCGCTTGACTCTGGGAAG
m- <i>P2ry12</i> -promoter-1000-R	CATGTGATCTGGGGTGGTGG
m- <i>P2ry12</i> -promoter-2000-F	TACACCAGCTGTCGAGCAAG
m- <i>P2ry12</i> -promoter-2000-R	GGTAGACATGGATCCGGCTG
m- <i>P2ry12</i> -exon-F	TTGCACGGATTCCCTACACC
m- <i>P2ry12</i> -exon-R	GAAGCAGCCCCTTGGGTAAT
m- <i>Gp6</i> -promoter-200-F	ATGTGCCAATGGAGAGGCAA
m- <i>Gp6</i> -promoter-200-R	CTGCCAGGTCCAAAGGTCAT
m- <i>Gp6</i> -promoter-1000-F	GTCAGGAATGAGGTATGTTTTTCAC
m- <i>Gp6</i> -promoter-1000-R	TGCTATTTCTCCTGCTCTAGCC
m- <i>Gp6</i> -promoter-2000-F	ACGTGGGTGTTAATCCAGCC
m- <i>Gp6</i> -promoter-2000-R	CACGGCTTTGTGGTCAAGTG
m- <i>Gp6</i> -exon-F	ATGCCTTGGTGCCACGATTA
m- <i>Gp6</i> -exon-R	TCCCTCTGCTGAAAGCCAAG

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