Supplementary Material

Supplementary Figure and Table Legends

Supplement Figure 1. **Optimization of CRISPR KO in MKs. A-B**: Surface expression of αIIb (% CD41+) on day 13 MKs as measured by flow cytometry **A.** after transfection with negative control crRNA (CR) or three different crRNA (A, B, or C) predicted to target gene ITGA2B or **B.** after transfection of different numbers of CD34+ cells with negative control or ITGA2B crRNA B. **C.** Representative Sanger sequencing traces from day 13 MKs targeted with negative control or ITGA2B crRNA. Note the clearly defined single nucleotide peaks in control, whereas multiple overlapping nucleotide peaks surround the cut site, a characteristic of the accumulation of insertions and deletions. Insertions/deletions (indels) were estimated by Synthago software and in **D.** mean +/- SEM summary score of knockout efficiency is shown. **E.** Representative flow cytometry analysis of day 13 MK surface expression of CD41, GPVI, or B2M MK after transfection with negative control, ITGA2B, GP6, or B2M crRNA (see figure 2B for MK gating strategy). **F.** mean +/- SEM summaries of the percent of positive cells for each protein represented in E. Surface proteins analyzed are indicated on the y-axis, and crRNA targeted gene on the x-axis. N=3-6 individual cords per group. Paired t-tests.

Supplement Figure 2. **Expansion of CRISPR KO MKs.** Fold expansion of viable cells between day 6 and 13 of culture after CRISPR transfection of $0.5x10^6$ CD34+ cells on day 5 of culture.

Supplement Figure 3. **Microscopy analysis of CRISPR KO MKs.** Representative fluorescence confocal microscopy images of CRISPR KO MKs on day 13 of culture. MKs were fixed in suspension with 2% paraformaldehyde and centrifuged onto glass coverslips. Cells were permeabilized with 0.1% Triton X 100 and stained with phalloidin 488, wheat germ agglutinin (WGA) 555, and DAPI, and imaged by confocal microscopy. Cells ranged in size from 10-37 microns, which is consistent with previously published reports[1,2]. No apparent differences in morphology between groups were determined by an observer blinded to groups.

Supplement Figure 4. PKC activity in GP6 CRISPR KO MKs. Day 13 negative CRISPR and *GP6* CRISPR KO MKs at baseline or after 5 minute stimulation with thrombin (1 U/mL) or convulxin (1 μg/mL) were assessed by western blot for PKC activity using an antibody mixture that recognizes the Phospho-PKC Substrate Motif [(R/K)XpSX(R/K)]. GAPDH is used as loading control. Shown are MKs from 2 independent cords with densitometry analysis below each.

Supplement Figure 5. Mean Fluorescence intensity (MFI) of integrin activation and surface P-selectin of CRISPR KO MKs in response to platelet agonists. Day 13 CRISPR KO and control MKs were treated for 10 minutes with thrombin (1 U/mL), CRP (2 μg/mL), or 2-meSADP (200 nM); stained for activated alpha2b/beta3 (PAC-1), and P-selectin; and analyzed by flow cytometry as described in figure legend 2B. Shown are normalized mean +/- SEM summaries of the MFI for **A.** PAC-1 or **B.** P-selectin staining. n=3-9 independent cords per group. Mixed effects analysis with Dunnett's adjustment

for multiple comparisons. The unfilled circle represents an outlier (Grubb's) removed for statistical analysis.

Supplement Figure 6. MFI of integrin activation and surface P-selectin of RASGRP2 CRISPR KO MKs in response to short treatment with platelet agonists. day 13 CRISPR KO and control MKs were treated for 2 minutes with thrombin (THR, 1 U/mL), or 3 minutes with convulxin (CVX, 2 μg/mL) or CRP (2 μg/mL); stained for activated alpha2b/beta3 (PAC-1), and P-selectin; and analyzed by flow cytometry according to the gating strategy described in figure legend 2B. **A-B**: normalized mean +/- SEM summaries of **A.** PAC-1 MFI or **B.** P-selectin MFI. n=3 independent cords per group. paired t-tests, n=3 per group.

Supplement Figure 7. Microscopy analysis of COMMD7 KO MKs. A. Fluorescence confocal microscopy images of negative control versus *COMMD7* KO MKs (representatives shown from 3 independent cords) on day 13 of culture. MKs were prepared for immunofluorescence imaging as described in Supplement Figure Legend 3 with wheat germ agglutinin (WGA), phalloidin, and DAPI. The mean and median diameters of MKs as measured from 3 cords (104 Negative control and 95 *COMMD7* KO MKs) did not differ between groups (means: 16.1 ± 3.4 and 15.7 ± 3.0; medians: both 15.0). The diameters were consistent with previously published[2] diameters of human MKs found in neonates (mean 15.3 microns) and in adult bone marrow following transplant of cord blood CD34+ cells (median 16.7 microns)**. B.** Transmission Electron Microscopy (TEM) images (representatives shown from 3 independent cords) of

negative control versus CRISPR KO MKs on day 13 of culture. MKs were fixed in suspension in 2.5% glutaraldehyde in phosphate-buffered saline, followed by processing for TEM as previously described[3]. The yellow box in the top panels place the zoom images below where representative features are labeled in red as GR=Granules (note that granules were the size and shape of platelet granules, but generally less dense than those typically observed by TEM in platelets), DMS=Demarcation Membrane System, MT=Mitochondria, N=Nucleus. No obvious differences in morphology between groups were determined by an observer blinded to groups.

Supplement Figure 8. *COMMD7* in MKs mediates responses to platelet agonists, activation MFI and total P-selectin expression. A-E,G: negative control or COMMD7 KO day 13 MKs were kept at baseline or stimulated with convulxin (2 μg/mL) or thrombin (1 U/mL) for 3 or 10 minutes as indicated, and analyzed by flow cytometry. Viable MKs were gated as in Figure 2B. Shown is the normalized mean +/- SEM of MFI for **A-B)** Pac1, **C)** labeled fibrinogen, **D-E)** surface p-selection, or **G)** surface CD63. Paired t tests, n=5 independent cords per group. **F**: western blot analysis of total P-selectin protein in day 13 negative control and COMMD7 KO MKs. Densitometry analysis is shown on the right with GAPDH used as loading control. Paired t test, n=3 independent cords per group.

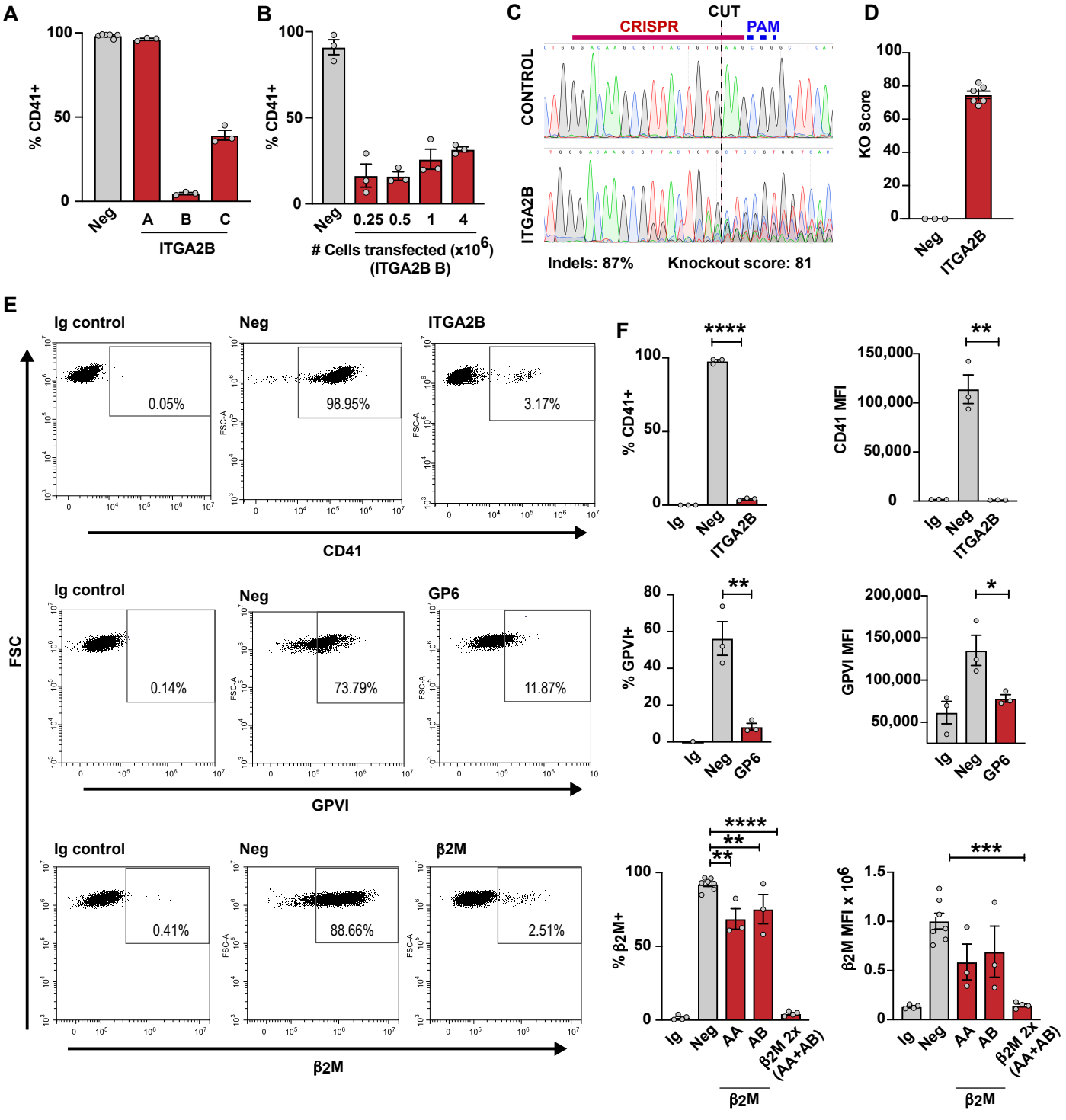
Supplement Figure 9. PKC activity in COMMD7 CRISPR KO MKs. Day 13 negative CRISPR and *COMMD7* CRISPR KO MKs at baseline or after 5 minute stimulation with thrombin (1 U/mL) or convulxin (1 μg/mL) were assessed by western blot for PKC

activity using an antibody mixture that recognizes the Phospho-PKC Substrate Motif [(R/K)XpSX(R/K)]. GAPDH is used as loading control. Shown are MKs from 2 independent cords with densitometry analysis below each.

Supplement Table 1. RNA-seq analysis of predicted off-target transcripts and genes flanking COMMD7. RNA was isolated from day 13 negative control CRISPR and COMMD7 CRISPR treated MKs and sequenced. Shown are results from a differential expression analysis of the two genes flanking COMMD7 (DNMT3B, C20orf203) and all genes predicted as off targets (idtDNA). None of the targets were significantly decreased (adjusted p value <0.05) in COMMD7 KO MKs compared to controls. n=3 independent donors per group.

References

1. Levine RF. Isolation and Characterization of Normal Human Megakaryocytes. *Br. J. Haematol.* 1980;45(3):487–497.
2. Ignatz M, Sola-Visner M, Rimsza LM, et al. Umbilical Cord Blood Produces Small Megakaryocytes After Transplantation. *Biol. Blood Marrow Transplant.* 2007;13(2):145–150.
3. Schwertz H, Köster S, Kahr WHA, et al. Anucleate platelets generate progeny. *Blood*. 2010;115(18):3801–3809.



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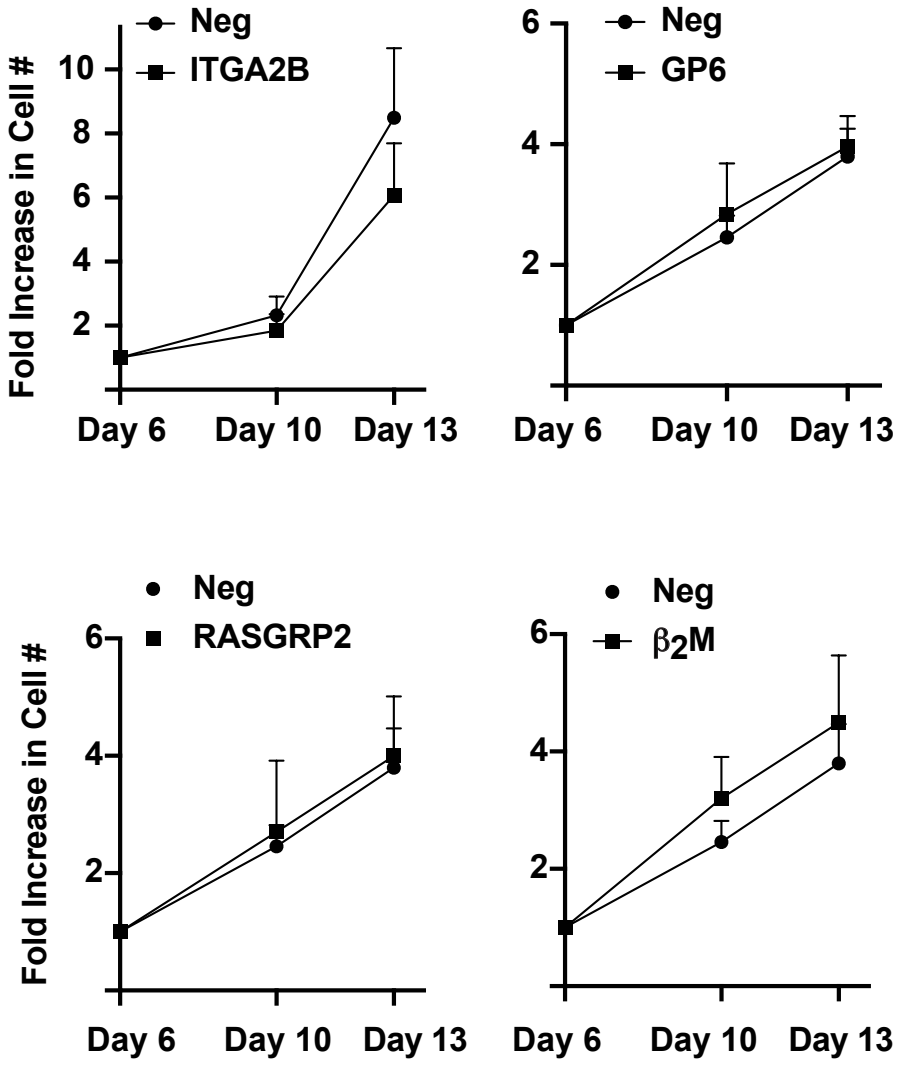
Supplement Figure 1



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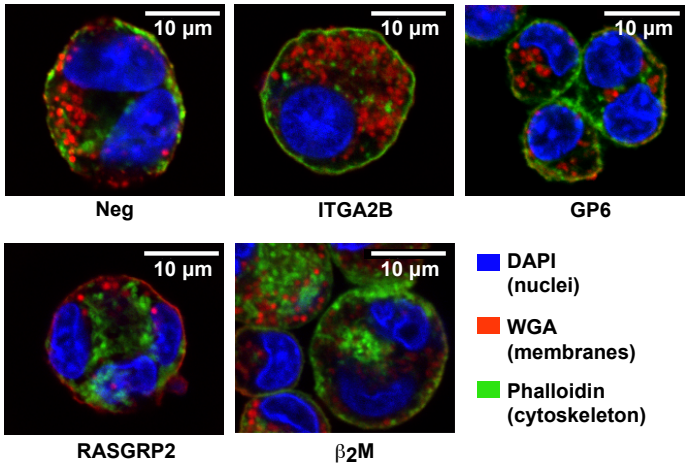

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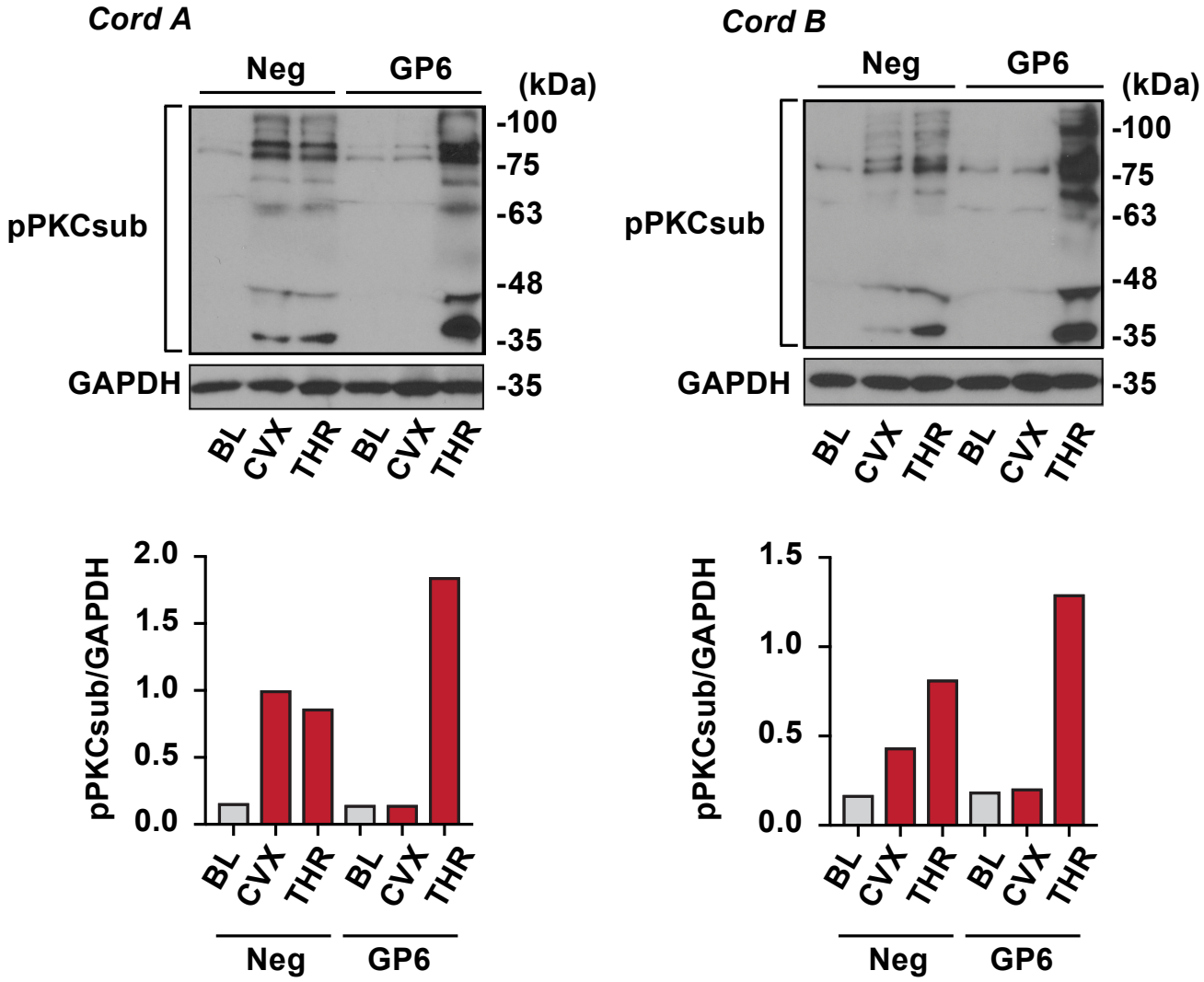

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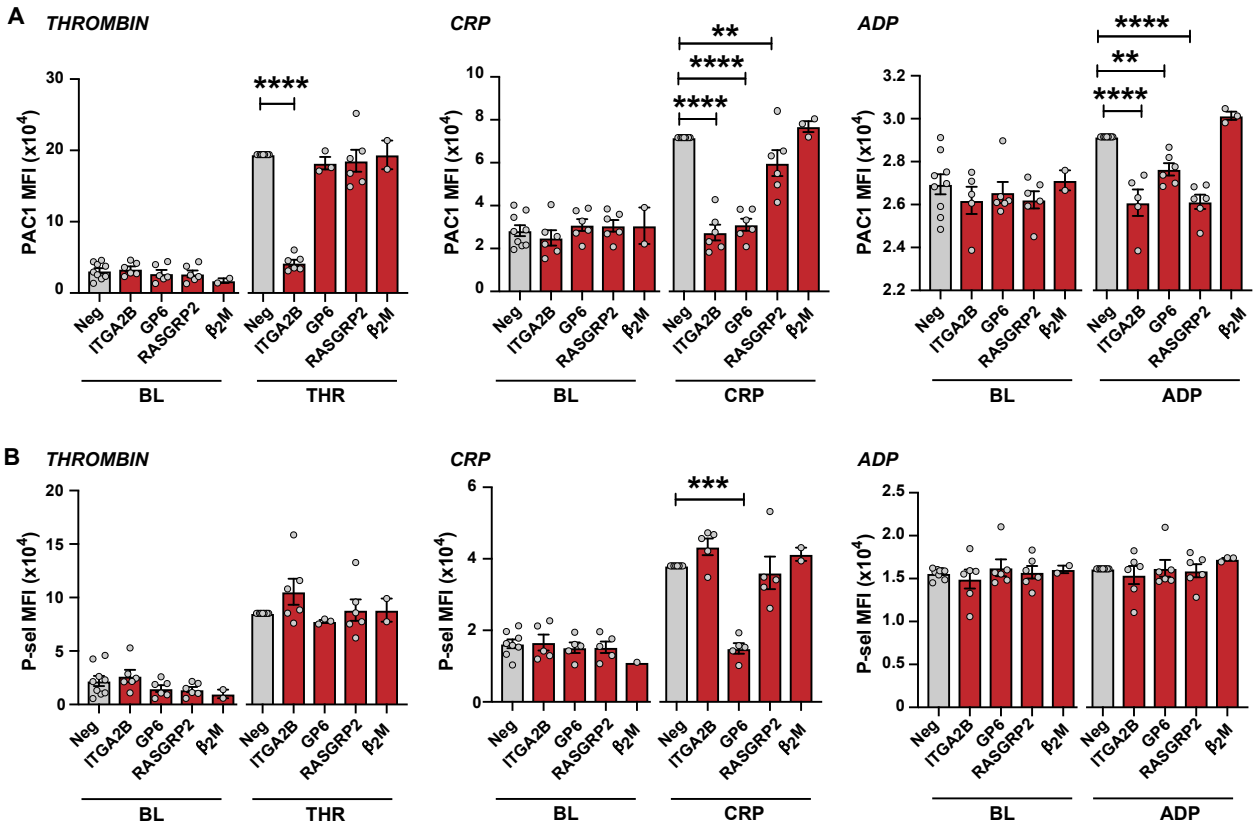

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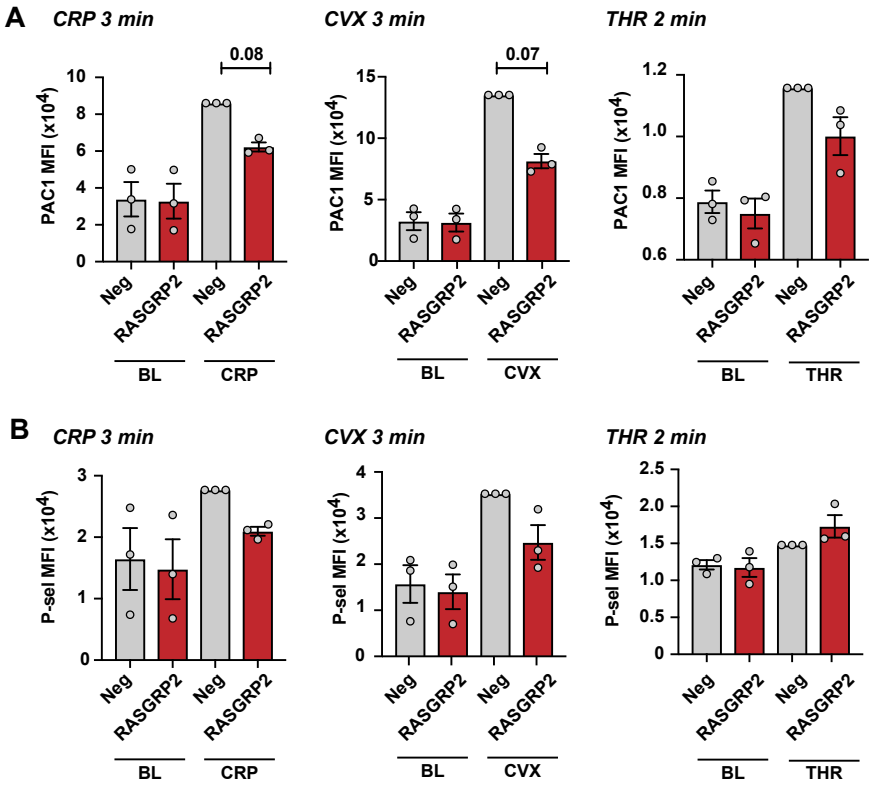

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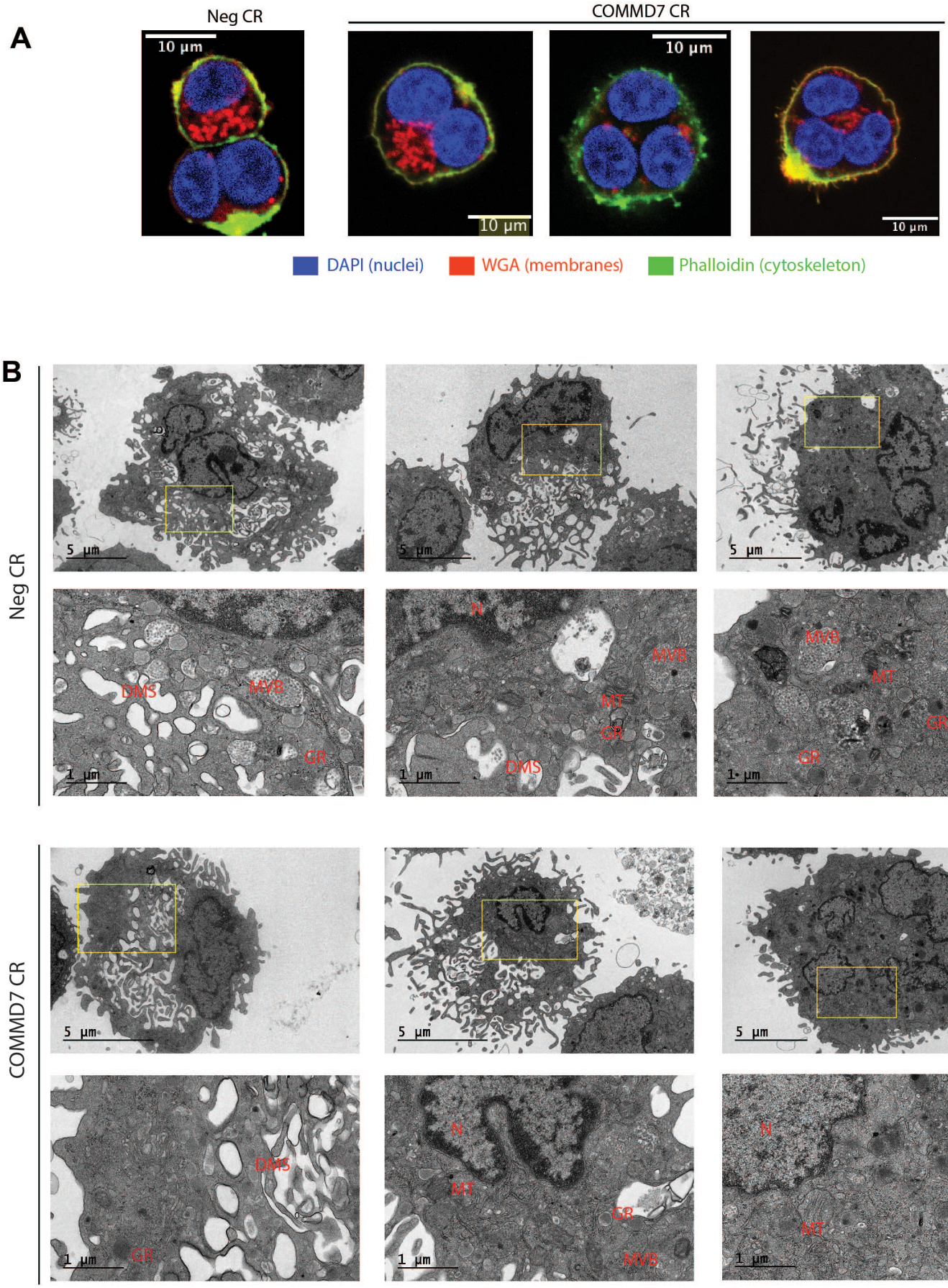

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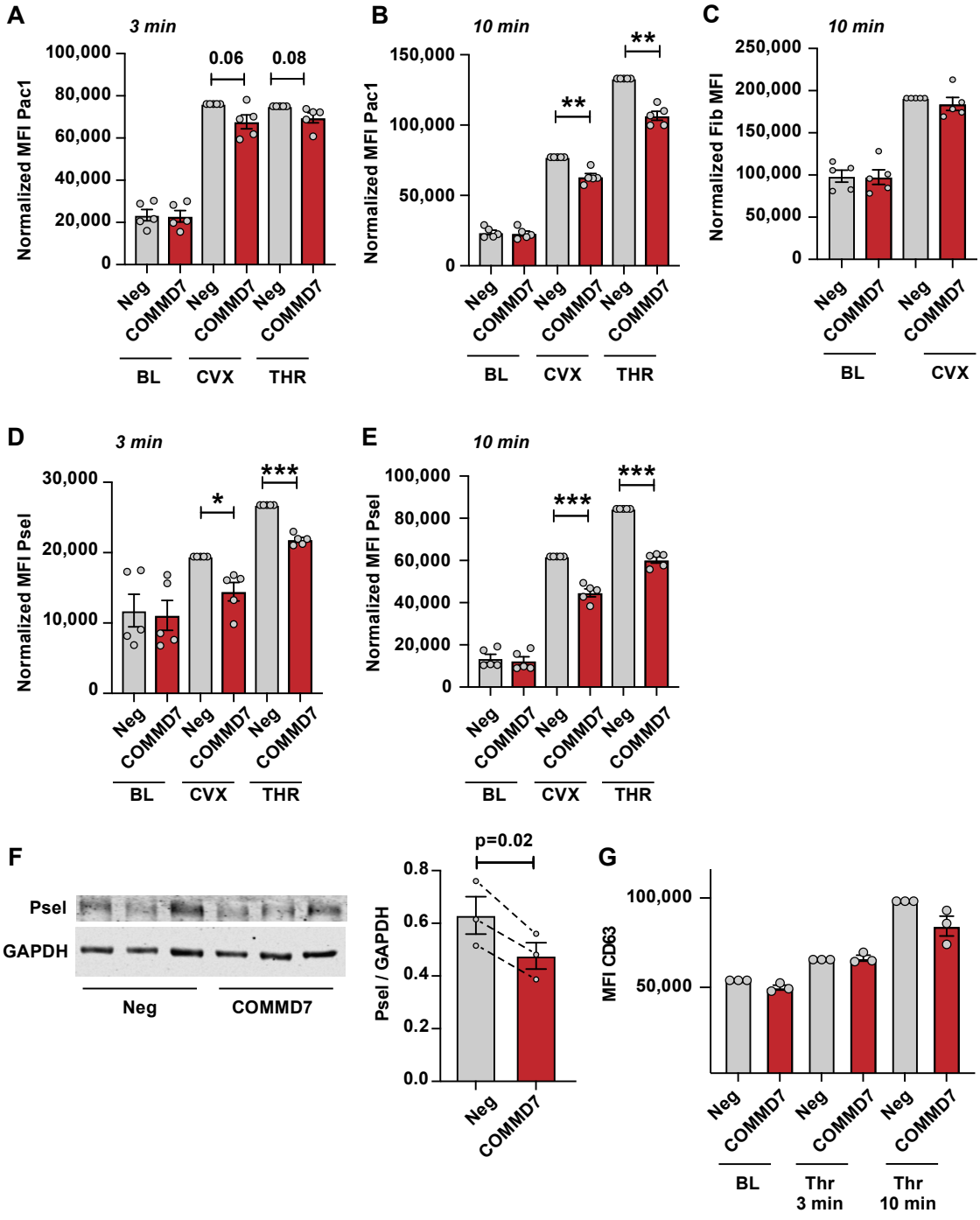

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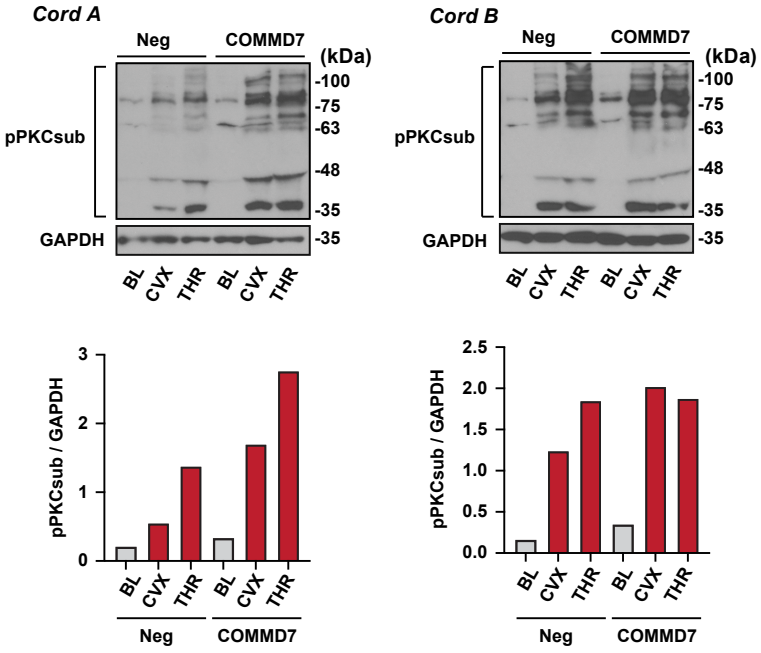

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Supplement Figure 9

gene_name	description	Direction	log2FoldChange	padj	Predicted Target Sequence	LOCUS
CHMP1B	charged multivesicular body protein 1B	Down	-0.25828825	0.19068993	ACCTTCAATCTGATCATTCT	chr18:11852421
C20orf203	chromosome 20 open reading frame 203	Down	-0.07551727	0.89169981	NA - Flanks COMMD7	chr20:32629625-32653981
TTLL4	tubulin tyrosine ligase like 4	Down	-0.04569523	0.9334525	ACCTCCAGCCAGATCAGGCT	chr2:218740068
ENO1	enolase 1	Up	0.618978665	1.03E-05	ACCTCCAGTCAGATCGTTCT	chr1:8870742
MAP2K3	mitogen-activated protein kinase kinase 3	Up	0.465150697	0.0021866	ACCCATGGCCCAGCGCCCAA	chr17:21288008
DNMT3B	DNA methyltransferase 3 beta	Up	0.322030301	0.04679013	NA - Flanks COMMD7	chr20:32760385-32811356
ZC3H12A	zinc finger CCCH-type containing 12A	Up	0.45124044	0.09849093	ACCTATGCCCCATCAGGCAG	chr1:37482999
PDK1	pyruvate dehydrogenase kinase 1	Up	0.199944223	0.3240794	ACAAGCAATCAGTTCAGTTT	chr2:172596721
PASK	PAS domain containing serine/threonine kinase	Up	0.230957466	0.37178375	TCCAGCAATCAGCTCAGTCT	chr2:241132914
FOXO1	forkhead box O1	Up	0.029586738	0.94500825	ACATATGGCCAATCCAGCAT	chr13:40560198

Supplement Table 1