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Supplementary Materials for

Tumor-Induced STAT3 Signaling in Myeloid Cells Impairs Dendritic Cell Generation by Decreasing PKCβII Abundance

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The PDF file includes:

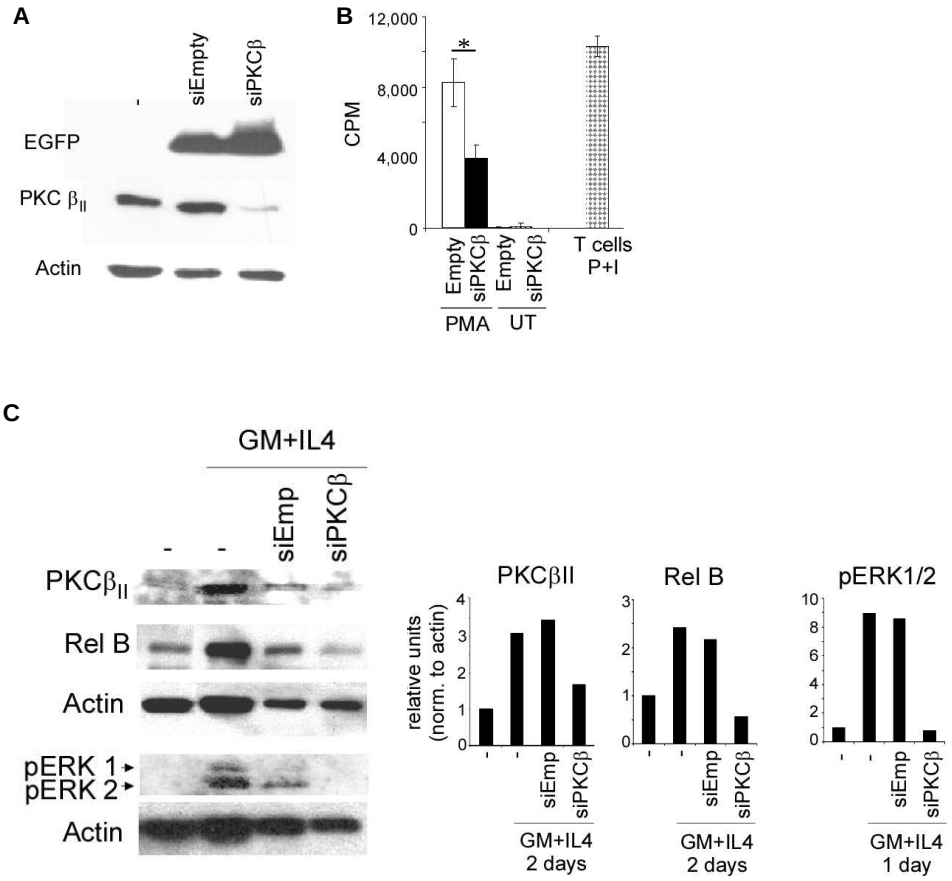

Fig. S1. Activation of the NF-κB and ERK pathways during the differentiation of myeloid progenitor cells into DCs is dependent on PKCβII. (**A** and **B**) K562 cells were left untransfected (-) or were transfected with either pEGFP-siEmpty or pEGFP-siPKCβ. (A) Three days after transfection, half of the cells were removed, whole-cell lysates were prepared, and samples were analyzed by Western blotting with antibodies against the indicated proteins. Blots are representative of three independent experiments. (**B**) The remaining cells were either left undifferentiated (UT) or were induced to differentiate with PMA for an additional 5 days. Cells were then analyzed for their ability to stimulate the proliferation of co-cultured allogeneic T cells. Data are means ± SD from three independent experiments. **P* < 0.05 by student's *t* test. (**C**) Monocytes were isolated from PBMCs and were left untransfected (-) or were transfected with either pEGFPsiEmpty or pEGFPsiPKCβ. After 1 day in culture, cells were left untreated or were treated with GM-CSF and IL-4 (GM+IL4) for an additional 1 to 2 days. Protein was isolated from day 1 untransfected cells, from undifferentiated monocytes, and from cells after 1 to 2 days in the presence of GM-CSF and IL-4. Western blotting analysis was then performed to detect pERK1/2 and actin in samples from cells cultured for 1 day in GM-CSF and IL-4, whereas PKCβII and RelB were analyzed in samples from cells after 2 days of culture with GM-CSF and IL-4. Left: Representative Western Blots. Right: Densitometric analysis of Western blots. Data are means from three independent experiments.

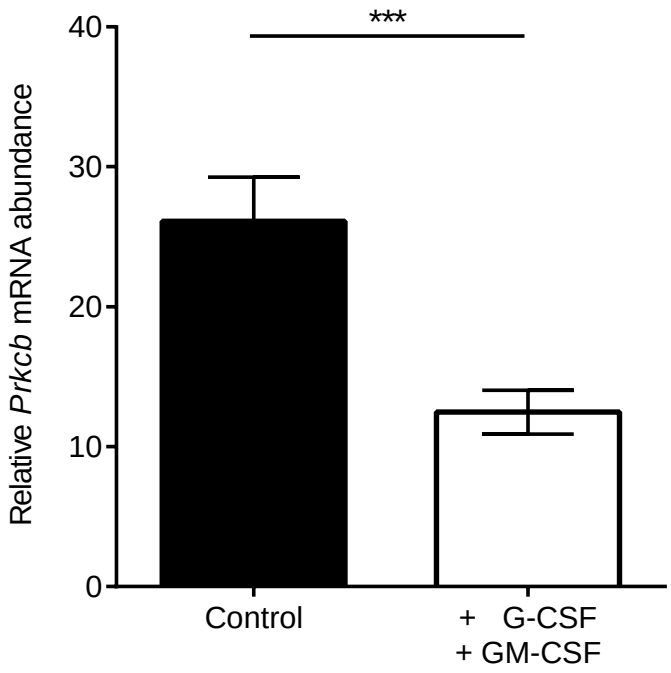

Fig. S2. G-CSF decreases the extent of *Prckb2* expression in freshly isolated murine bone marrow cells. *Prckb2* mRNA abundance was analyzed from gene expression profiles generated from freshly isolated BALB/c mouse bone marrow or bone marrow cells cultured in GM-CSF and G-CSF for 4 days, as described previously (*23*)(GSE21927). Data are means ± SD from three mice for each condition. ***$P < 0.005$ by student's *t* test.

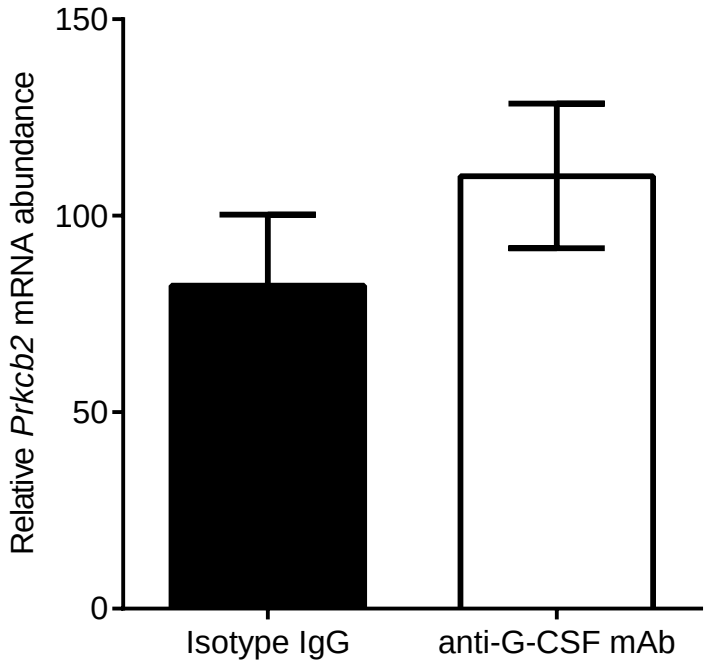

Fig. S3. Depletion of G-CSF increases the expression of *Prkcb2* in myeloid cells from tumor-bearing mice. Splenic CD11b^{+} myeloid cells were isolated from tumor-free mice and from mice bearing AT3 tumors after injection of an anti–G-CSF neutralizing antibody or an isotype IgG control, as described previously (*13*). The abundance of *Prkcb2* mRNA was determined by qPCR analysis. Data are means ± SEM from three mice for each condition. P = 0.1344 by student's t test.

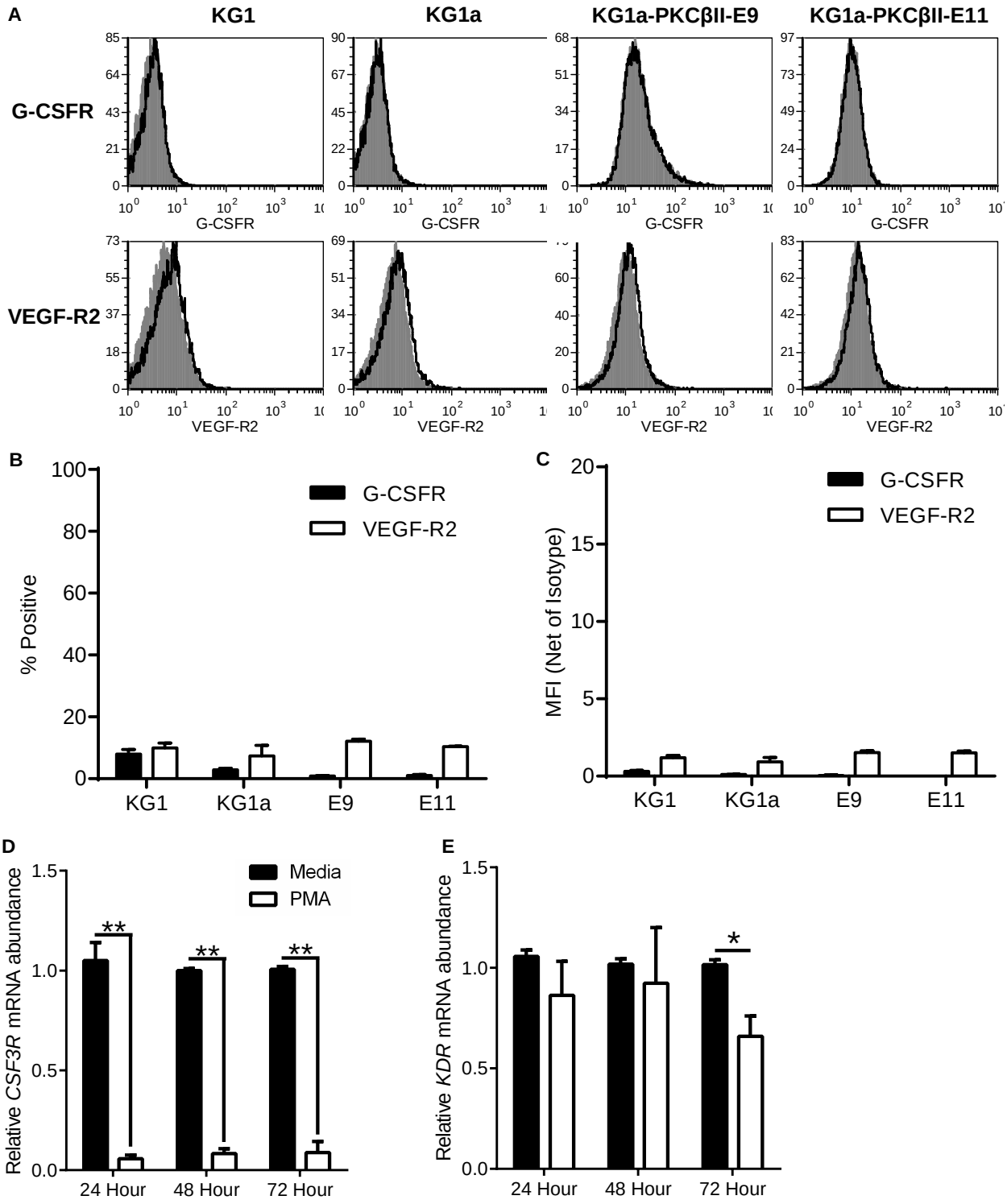

Fig. S4. Effect of PKCβII activation on the expression of G-CSFR and VEGFR2. (**A** to **C**) The presence of G-CSFR and VEGFR2 on the surface of KG1 cells, KG1a cells, and KG1a-PKCβII-GFP clones E9 and E11 was analyzed by flow cytometry. (A) Representative flow cytometry plots. Gray histograms show isotype controls, whereas black lines indicate specific staining. (B) Percentages of viable cells that have cell-surface G-CSFR and VEGFR2,

determined relative to staining with an isotype control antibody. Data are means ± SEM from three independent experiments. (C) MFIs, less those for isotype antibody staining, for the indicated receptors. Data are means ± SEM from three independent experiments. (**D** and **E**) PKC activation decreases the abundance of mRNAs for cytokine and growth factor receptors that are activated by TCM. KG1 cells were treated in CM in the absence or presence of PMA for up to 72 hours. Total RNA was collected, and gene expression was analyzed by qPCR. Data are means ± SD from three independent experiments, except for *VEGFR2* at 24 and 48 hours, where n = 5. **P* < 0.05, *****P* < 0.001 by student's *t* test.

Table S1. Characteristics of breast cancer patients and healthy controls. All donors were female, and all patient samples were collected before any treatment. ER, estrogen receptor status (positive or negative) on tumor cells; PR, progesterone receptor status (positive or negative) on tumor cells. TMN Tumor staging: T status: Primary tumor grade; N status: Regional lymph node grade; M status: metastatic grade.

Donor	Age	Grade	ER	PR	T Status	N Status	M Status
Patient Donor 1	60-64	III-NOS	+	+	X	2a	0
Patient Donor 2	45-49	III-C	+	+	2	3a	0
Patient Donor 3	65-69	III-A	+	-	1	2	0
Patient Donor 4	40-44	IV	+	+	3	0	1
Patient Donor 5	80-84	III-C	-	-	Any	3	0
Patient Donor 6	65-69	IV	+	-	4b	1	1
Patient Donor 7	65-69	III-A	+	+	1	2	0
Patient Donor 8	60-64	IV			2	2b	1
Patient Donor 9	50-54	IV	+	+	3	3a	1
Patient Donor 10	70-74	III-A	+	+	1	2	0
Patient Donor 11	80-84	III-B	+	+	4	Any	0
Patient Donor 12	45-49	III-C	+	+	Any	3	0
Patient Donor 13	50-54	III-A	+	+	3	2	0
Patient Donor 14	75-79	III-A	+	+	2	2	0
Patient Donor 15	55-59	III-C	+	-	1c	3a	0
Patient Donor 16	70-74	III-A	+	+	2	2	0
Patient Donor 17	65-69	IV	+	+	4d	0	1
Patient Donor 18	50-54	III-C	+	+	1c	3a	0

Non-Patient Donor 1	60-64
Non-Patient Donor 2	45-49
Non-Patient Donor 3	65-68
Non-Patient Donor 4	40-44
Non-Patient Donor 5	65-69
Non-Patient Donor 6	65-69
Non-Patient Donor 7	50-54
Non-Patient Donor 8	40-44
Non-Patient Donor 9	50-54
Non-Patient Donor 10	50-54
Non-Patient Donor 11	45-49
Non-Patient Donor 12	75-79
Non-Patient Donor 13	60-64
Non-Patient Donor 14	65-69
Non-Patient Donor 15	55-59

Table S2: Oligonucleotides used in this study and their application.

Quantitative real-time PCR and reverse-transcription PCR		
PRKCB2	Forward:	5`- AAG GGC TGA TGA CCA AAC AC -3`
	Reverse:	5`- CGG TCG AAG TTT TCA GCA TT -3`
ACTB	Forward:	5`- CCC AGC ACA ATG AAG ATC AAG ATC AT -3`
	Reverse:	5`- ATC TGC TGG AAG GTG GAC AGC GA -3`
Prkcb2	Forward:	5`- CGC CCT CCG GCA GAA GAA CG -3`
	Reverse:	5`- GTC AGT GCC GCA GAG GCT GG -3`
Actb	Forward:	5`- CCT AAG GCC AAC CGT GAA AAG -3`
	Reverse:	5`- GAG GCA TAC AGG GAC AGC ACA-3`
CSF3R	Forward:	5`- CCC CCA ATC CAT GGC CTG AGG GCT -3’
	Reverse:	5`- TGG GGC ATG GGA GGG AGC CAT T -3’
IL6RA	Forward:	5`- CCT GGC AAG ACC CCC ACT CCT -3`
	Reverse:	5`- TAA GTG CCT GCA TGG GGG TGG A -3`
KDR	Forward:	5`- TGT ACC AAG CCA GGA GGG CCA -3`
	Reverse:	5`- TCC ACA AAT CCA GAG CTG GCT GA -3`
Site-directed mutagenesis (bolded-underlined sequence indicates the mutated nucleotides)		
Putative STAT3 binding site #1	Sense:	5’- GAC AGA TGA CGG CAC CTG GAG ATA TTT TAA **CCC** TGT AGA TAC CTC TTG C -3’
	Antisense:	5’- GCA AGAGGT ATC TAC A**GG G**TT AAA ATA TCT CCA GGT GCC GTC ATC TGT C -3’
Putative STAT3 binding site # 2 & 3	Sense:	5’- GAA TTT GTG AAG ACG AGT CAG AAA TGA ATG AAA CCC GGA ACC CTA TTG ATC TAC TGA AAT CCT TCC TCC CCA CAC T -3’
	Antisense:	5’- AGT GTG GGG AGG AAG GAT TTC AGT AGA TCA ATA GGG TTC CGG GTT TCA TTC ATT TCT GAC TCG TCT TCA CAA ATT C -3’
Putative STAT3 binding site #4	Sense:	5`- CCC TAT GTT ACA GTT GGG G**CC C**CG GAG TCG TTT TGC AGA GG -3`
	Antisense:	5`- CCT CTG CAA AAC GAC TCC G**GG G**CC CCA ACT GTA ACA TAG GG -3`
ChIP assay		
PRKCB promoter	Forward:	5`- AGG AGA GAG TCC TGG TTG GA -3`
	Reverse:	5`- GTA GGG GAG GAG CGA TCT TT -3`