Supporting Information

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Fig. S1. CD11b⁺MMP9⁺Ly6G⁺ granulocytes are recruited to platelet–tumor cell aggregates within 2 h of tumor cell entry into the circulation. (A) Immunostaining for CD11b, Ly6G, and MMP9 in mouse lungs 2 h after the injection of 10⁶ MC38GFP tumor cells. Note that the vast majority of recruited cells coexpress CD11b, Ly6G, and MMP9. (Scale bar: 100 μ m.) (B) Immunostaining for CD11b (red), and platelets (GP1b β ; blue) in mouse lungs 2 h after the injection of 10⁴ MC38GFP cells (green). Note the enrichment of CD11b⁺ cells in the vicinity of the platelet–tumor cell aggregates. (Scale bar: 100 μ m.) (C) Number of CD11b⁺ cells localized within a 40- μ m radius from the center of MC38GFP cells. The average number of CD11b⁺ found in areas of equivalent size (5,026 μ m²; corresponding to the area of a circle of 40- μ m radius) in the same image was determined as control. Bars represent the mean \pm SEM (n = 52 cells from n = 4 mice). ****P* < 0.001 as determined by unpaired two-sided *t* test. (*D*) Immunostaining for CD11b (red), and platelets (GP1b β ; blue) in mouse lungs 1 min, 2 h, or 4 h after the injection of vehicle alone (HBSS) or in lungs of control noninjected mice. (Scale bar: 100 μ m.) No recruitment of platelets or granulocytes is observed when vehicle alone is injected. (*E*) Numbers of CD11b⁺ cells in the lungs of mice treated as in *D*. Bars represent the mean \pm SEM ($n \ge 12$ images from $n \ge 3$ mice). (*F*) Flow cytometry diagrams of viable CD45⁺ cells isolated from lungs collected at time 0 (no injection) or 2 h after the injection of 10⁶ MC38GFP cells. Cells were stained for CD45, CD11b, Ly6G, and Gr1, and with propidium iodide. Note that Ly6G⁺ cells also express high levels of CD11b and Gr1.



depletion inhibits the recruitment of MMP9⁺ cells to the lungs. Mice were treated with a platelet-depleting antibody or an IgG control 24 h before the i.v. injection of MC38GFP cells. Immunostaining for MMP9 (red), and platelets (GP1b β ; blue) in mouse lungs at the times indicated following the injection of MC38GFP cells (green). (Scale bar: 100 µm.) (*B*) Immunostaining for MMP9 (red) and platelets (GP1b β ; blue) in lungs from WT or *Itgb3^{-/-}* mice 2 h after the injection of MC38GFP cells (green). (Scale bar: 100 µm.) (*C*) Immunostaining for MMP9 (red) and platelets (GP1b β ; blue) in lungs from NOD SCID mice 2 h after the injection of MC38GFP or CellTracker Green-labeled CHO cells (green). Lungs of control (0; no injection) NOD SCID mice are also shown. (Scale bar: 100 µm.) (*D*) Immunostaining for CD11b (red) and platelets (GP1b β ; blue) in lungs from mice 2 h after i.v. injection of CellTracker Green-labeled Lewis lung carcinoma (LLC) or B16F10 tumor cells (green) into WT C57BL/6 mice. (Scale bar: 100 µm.)

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Chemokine/cytokine	MC38GFP	Platelets	Platelets + MC38GFP
Pf4	32.4 ± 0.6	1,169.9 ± 39.8	500.0 ± 4.2
CXCL2	31.5 ± 1.9	778.1 ± 36.5	852.5 ± 16.3
sP-selectin	68.0 ± 11.0	699.2 ± 50.3	813.5 ± 29.0
CXCL5	10.0 ± 2.6	222.4 ± 3.9	510.0 ± 1.4
CXCL7	7.7 ± 0.4	220.9 ± 10.1	849.6 ± 26.8
IL1α	9.6 ± 1.9	74.3 ± 13.7	47.0 ± 14.1
IGFBP6	1.4 ± 0.6	64.1 ± 16.3	35.0 ± 2.8
LEPR	3.7 ± 1.3	61.4 ± 5.9	21.0 ± 0.0
CCL20	18.3 ± 3.9	58.6 ± 2.0	67.5 ± 33.2
Leptin	3.7 ± 0.0	58.1 ± 11.7	28.0 ± 2.8
IL13	5.9 ± 0.6	57.2 ± 14.4	28.0 ± 0.0
sIL3Rβ	11.9 ± 1.3	56.3 ± 7.8	75.0 ± 60.8
IL1β	7.8 ± 0.6	48.9 ± 1.3	6.0 ± 1.4
CCL2	39.7 ± 0.6	48.9 ± 2.6	59.5 ± 2.1
IL2	15.5 ± 2.6	46.2 ± 2.6	26.0 ± 0.0
VEGF	3.7 ± 1.3	43.8 ± 22.8	16.5 ± 2.1
MMP3	38.2 ± 0.8	41.5 ± 1.7	42.7 ± 1.4
IGF1	17.2 ± 1.1	40.6 ± 3.8	50.7 ± 0.9
sL-selectin	25.1 ± 0.6	39.7 ± 5.2	36.5 ± 0.7
IL9	40.6 ± 0.6	39.7 ± 6.5	42.5 ± 3.5
CCL5	5.5 ± 0.0	37.4 ± 12.4	12.0 ± 2.8
XCL1	24.7 ± 6.5	35.5 ± 2.0	27.5 ± 2.1
IL3	3.2 ± 0.6	34.6 ± 22.8	13.5 ± 3.5
VCAM1	9.1 ± 1.3	34.2 ± 9.1	32.0 ± 1.4
CXCL12	28.3 ± 0.0	34.2 ± 0.0	31.0 ± 1.4
CXCL1	6.4 ± 0.0	33.7 ± 5.9	6.5 ± 2.1
IGFBP3	9.6 ± 1.9	31.4 ± 11.7	30.5 ± 9.2
CCL19	4.1 ± 0.6	30.5 ± 1.3	13.5 ± 0.7
CCL2/	17.4 ± 0.0	29.1 ± 0.7	30.5 ± 4.9
	26.3 ± 3.4	28.1 ± 0.4	30.1 ± 2.7
	3.2 ± 0.0	27.7 ± 0.5	22.0 ± 1.4
	23.3 ± 0.0	27.7 ± 0.0	27.5 ± 0.7
IFINγ sE solostin	20.5 ± 2.0	27.7 ± 0.0	32.5 ± 2.1
CYCL16	12.7 ± 0.0 10.0 ± 1.3	27.5 ± 0.0	20.0 ± 3.9
CXCL10	10.0 ± 1.5	20.5 ± 0.7	40.5 ± 4.5
II 12 n70	15.5 ± 0.0	22.0 ± 0.5 21.2 + 2.6	15.0 ± 2.0 25.5 ± 3.5
II 10	13.3 ± 1.5 14.2 ± 0.6	21.2 ± 2.0 20 3 + 9 1	180 ± 28
CCI 24	14.2 ± 0.0 16.0 + 1.9	20.3 ± 0.0	25.5 + 3.5
SCE	5.9 ± 0.6	20.3 ± 3.9	31.0 ± 5.7
ΤΝΕα	19.6 ± 4.5	19.8 + 2.0	27.5 ± 0.7
CCL3	3.2 + 0.6	19.4 + 2.6	17.5 ± 0.7
1L12p40/p70	15.1 + 0.6	19.4 + 10.4	20.0 + 1.4
Dtk	21.5 ± 2.6	19.3 ± 8.0	16.0 ± 4.5
CCL9	4.1 ± 0.6	18.5 ± 3.9	19.5 ± 0.7
VEGFR2	10.9 ± 6.4	18.1 ± 1.3	16.7 ± 3.6
IL15	20.2 ± 1.5	17.5 ± 0.4	19.9 ± 0.9
TPO	4.6 ± 1.3	17.1 ± 2.0	8.0 ± 0.0
sTNFRII	15.1 ± 0.6	16.6 ± 5.2	34.0 ± 1.4
M-CSF	7.8 ± 0.6	16.2 ± 0.7	19.5 ± 0.7
CCL11	2.3 ± 0.6	16.2 ± 2.0	25.5 ± 40.3
CXCL13	7.8 ± 0.6	15.7 ± 1.3	11.0 ± 2.8
GITR	10.3 ± 1.1	14.5 ± 2.1	15.1 ± 1.4
AXL	4.6 ± 1.3	13.8 ± 1.3	8.5 ± 0.7
FasL	10.0 ± 2.6	13.4 ± 2.0	12.0 ± 1.4
CCL25	8.2 ± 1.3	12.5 ± 0.7	17.5 ± 2.1
CCL1	3.7 ± 0.0	12.0 ± 0.0	13.5 ± 2.1
IL4	13.2 ± 0.6	12.0 ± 1.3	18.0 ± 1.4
CD30T	5.5 ± 0.0	12.0 ± 2.6	6.5 ± 0.7
ITAC	12.7 ± 2.3	11.8 ± 1.7	13.2 ± 3.2
CD40	3.2 ± 0.6	11.5 ± 0.7	8.5 ± 3.5
IL17BR	14.1 ± 1.1	11.0 ± 0.4	16.4 ± 0.5
TIMP1	10.5 ± 0.6	10.6 ± 0.7	21.5 ± 0.7

Table S1. Cont	t
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Chemokine/cytokine	MC38GFP	Platelets	Platelets + MC38GFP
IL17	2.3 ± 0.6	10.6 ± 4.6	0.0 ± 2.8
VEGFR1	2.9 ± 0.4	10.4 ± 5.4	13.2 ± 0.5
XCP1	1.9 ± 0.4	10.1 ± 1.7	23.4 ± 0.5
Shh-N	7.4 ± 0.0	9.8 ± 0.4	9.3 ± 1.4
bFGF	11.1 ± 0.0	9.8 ± 1.3	9.9 ± 1.4
MDC	8.0 ± 0.8	9.5 ± 0.0	11.9 ± 1.4
CD30L	9.1 ± 1.3	9.2 ± 2.6	11.0 ± 0.0
OPG	142.8 ± 0.0	8.9 ± 0.0	272.3 ± 7.7
MMP2	11.9 ± 1.9	8.6 ± 0.4	10.3 ± 0.9
CCL12	3.7 ± 0.0	8.3 ± 1.3	9.5 ± 0.7
CX3CL1	7.3 ± 0.0	7.4 ± 0.0	12.5 ± 0.7
IL5	11.4 ± 0.6	7.4 ± 0.0	12.0 ± 0.0
sTNFRI	3.2 ± 0.6	6.5 ± 2.6	9.5 ± 0.7
TROY	3.2 ± 0.0	6.2 ± 0.4	9.3 ± 0.5
IL7	8.5 ± 0.0	6.2 ± 0.4	7.4 ± 1.4
ICAM1	6.1 ± 2.6	6.2 ± 0.4	7.4 ± 0.5
IGFII	4.8 ± 0.8	6.2 ± 0.4	7.1 ± 0.0
TRANCE	5.0 ± 0.4	5.3 ± 0.0	7.7 ± 0.9
FLT3 ligand	5.0 ± 0.4	5.3 ± 0.0	7.1 ± 0.0
OPN	5.3 ± 0.8	5.0 ± 0.4	5.5 ± 0.5
Pro-MMP9	4.0 ± 0.4	4.7 ± 0.8	6.4 ± 0.0
CXCL9	3.7 ± 0.0	4.6 ± 1.3	9.0 ± 1.4
CXCL15	3.4 ± 0.4	4.4 ± 0.4	6.4 ± 0.9
HGFR	3.4 ± 0.4	3.9 ± 0.4	5.1 ± 0.9
VEGFD	1.9 ± 0.4	3.6 ± 0.8	4.8 ± 0.5
VEGFR3	2.4 ± 0.4	3.6 ± 0.0	6.4 ± 3.6
TSLP	2.4 ± 0.4	3.3 ± 0.4	6.4 ± 0.0
GM-CSF	5.5 ± 0.0	2.8 ± 0.0	7.0 ± 0.0
Fcγ RIIB	1.6 ± 0.0	2.7 ± 0.4	3.2 ± 0.0
DPPIV/CD26	1.1 ± 0.0	1.8 ± 0.0	2.9 ± 0.5
IGFBP2	1.6 ± 0.8	1.8 ± 0.8	2.9 ± 0.5
IL6	4.6 ± 0.0	0.0 ± 2.0	5.0 ± 1.4
GCSF	2.7 ± 0.0	0.0 ± 1.3	3.0 ± 1.4

Protein levels determined with antibody arrays incubated with supernatants from MC38GFP cells, platelets, or platelets plus MC38GFP cells (arbitrary units; mean \pm SEM).