Title: MyD88 signaling is critical in the development of pancreatic cancer cachexia

Authors and affiliations:

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Supplementary Data

Fig. S1 Orthotopic (OT) model of pancreatic cancer cachexia. 3 x 10^6 KPC cells or PBS were directly implanted into mouse pancreas, and animals were euthanized 12 days post-implantation. (A) Male sham control vs. KPC tumor mice. (B) Female sham control vs. KPC tumor mice.

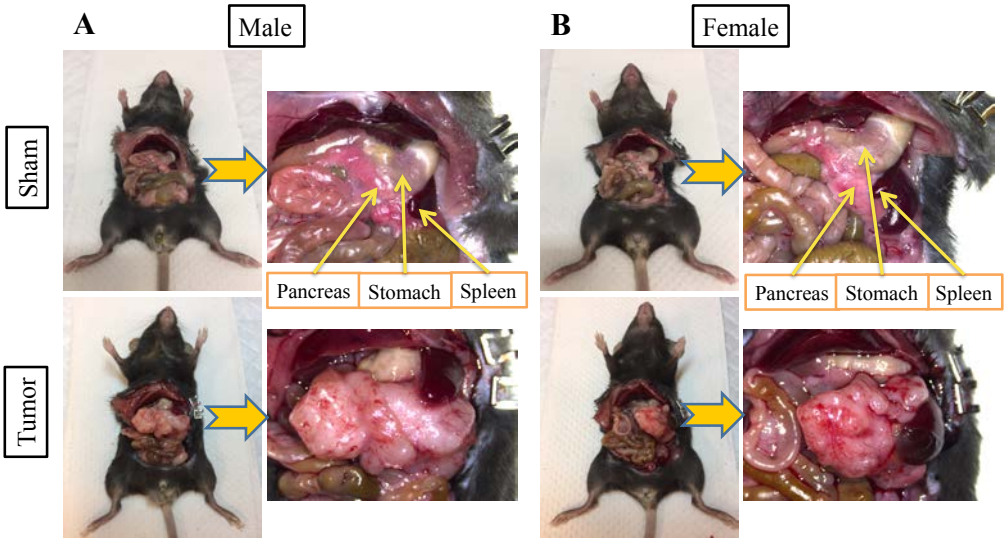
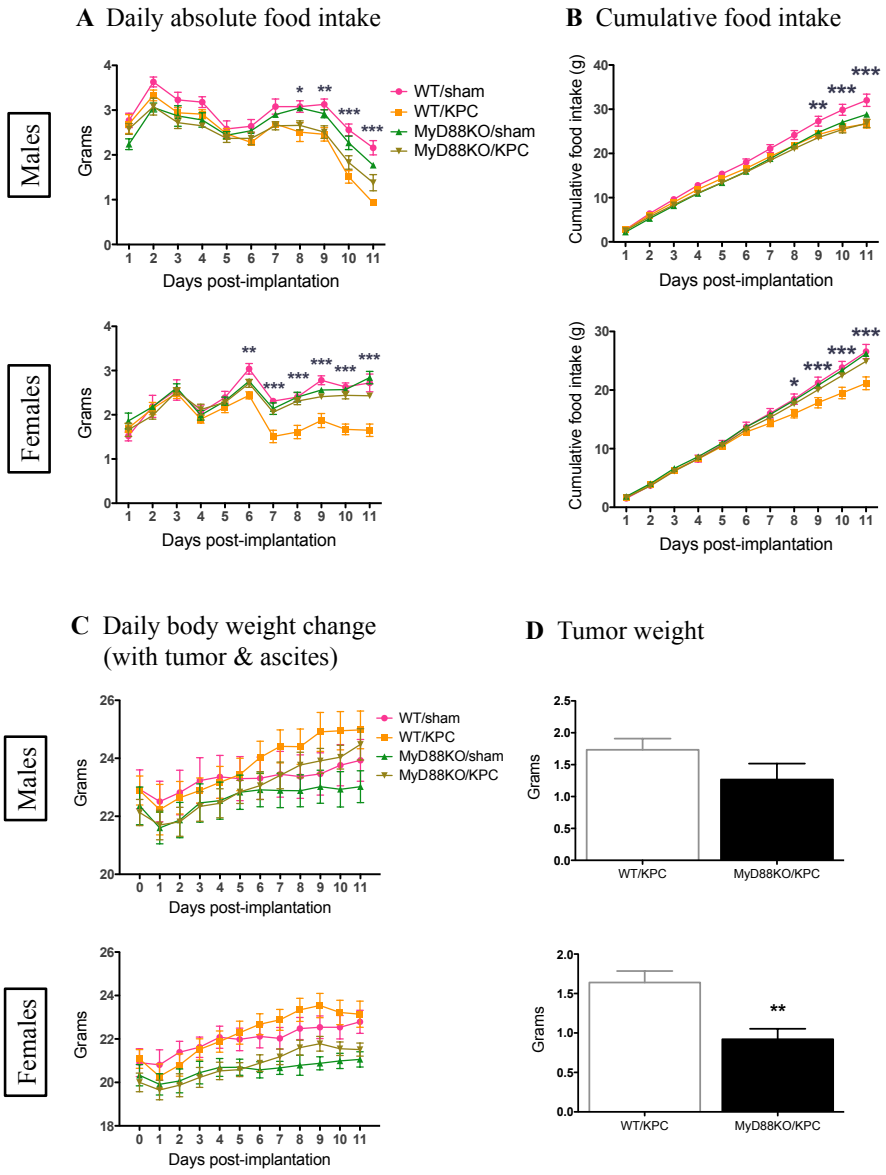


Fig. S2 Daily food intake and gross body weight, and terminal tumor mass, ascites and fecal triglyceride. (A) Daily food intake post-implantation. **(B) Daily cumulative food intake post-implantation. (A and B) absolute amount of daily food intake related to Figure 1.** **(C)** Daily body weight post-implantation progressively increased in tumor mice of both genotypes due to tumor growth and ascites development. (D) Tumor weight on the day of sacrifice. (E) Ascites weight on the day of sacrifice. (F) Fecal triglyceride concentration in the late stage of PDAC cachexia. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, WT/sham vs. WT/KPC (A and B), WT/KPC vs. MyD88 KO/KPC (D), Sham vs. KPC (E) or WT/sham vs. WT/orlistat (F). Two-way ANOVA (A, B and E) or One-way ANOVA (F) with post-hoc Bonferroni-corrected t-test. Unpaired t-test (D), N = 5-6/group.



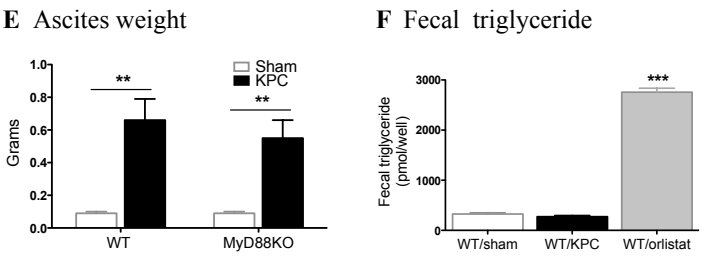


Fig. S3 IP KPC mice exhibit amelioration in behavioral phenotype when My88 signaling is blocked. In the absence of My88 signaling, IP KPC mice exhibit: (**A-C**) attenuated anorexia, (D) no difference in gross body weight change, (E) slightly smaller tumors. *, P < 0.05, **, P < 0.01, ***, P < 0.001, WT/sham vs. WT/KPC. Two-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 4-5/group.

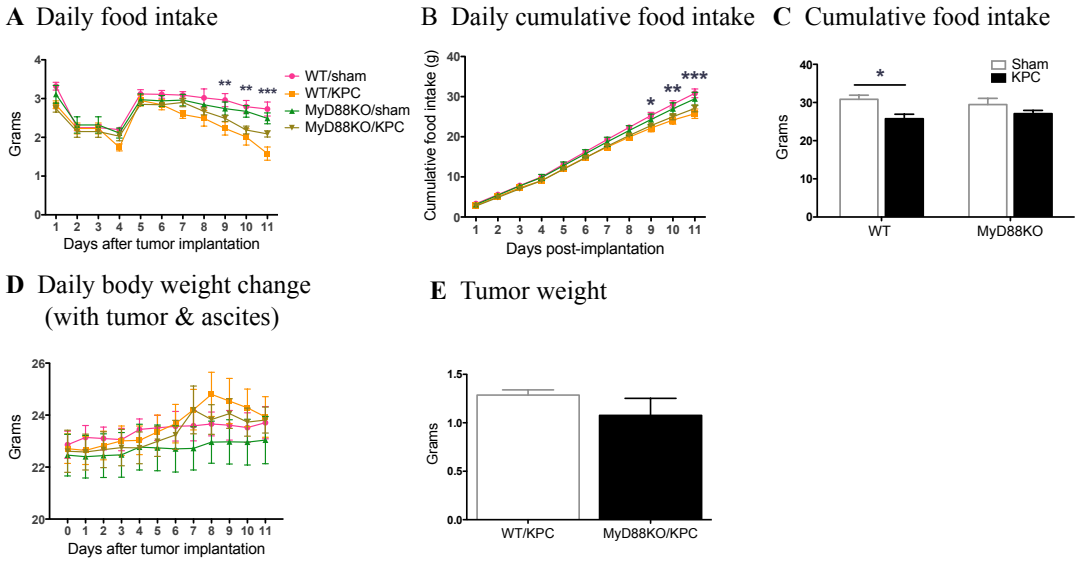


Fig. S4 Blockade of MyD88 signaling reduces lean mass loss in PDAC cachexia. Body composition was measured twice on the day of implantation and the day of sacrifice. (A) Absolute fat mass. (B) Absolute lean mass. **(C) Net gain in lean mass. (A-C) are related to Figure 2.** *, P < 0.05, **, P < 0.01, ***, P < 0.001, Sham vs. KPC. Two-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 5/group.

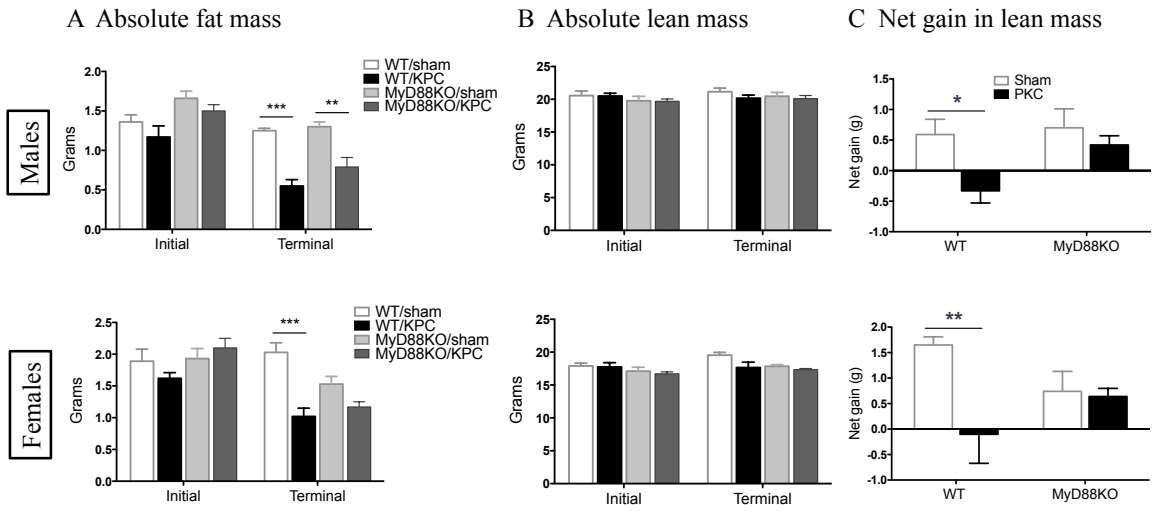


Fig. S5 IP KPC mice exhibit reduced lean mass loss and fatigue when MyD88 signaling is absent. Body composition was measured twice on the day of implantation and the day of sacrifice. (A) Fat mass gain. (B) Lean mass gain. (C) Sum of fat mass and lean mass gain. Gain% is the net gain normalized to baseline. (D) Absolute fat mass. (E) Absolute lean mass. (F) Locomotor activity (LMA) within 12-h dark phase (active phase). Movement counts were monitored post-implantation throughout entire course of experiment. *, P < 0.05, **, P < 0.01, ***, P < 0.001, Sham vs. KPC (A-E) or WT/sham vs. WT/KPC (F). Two-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 5/group.

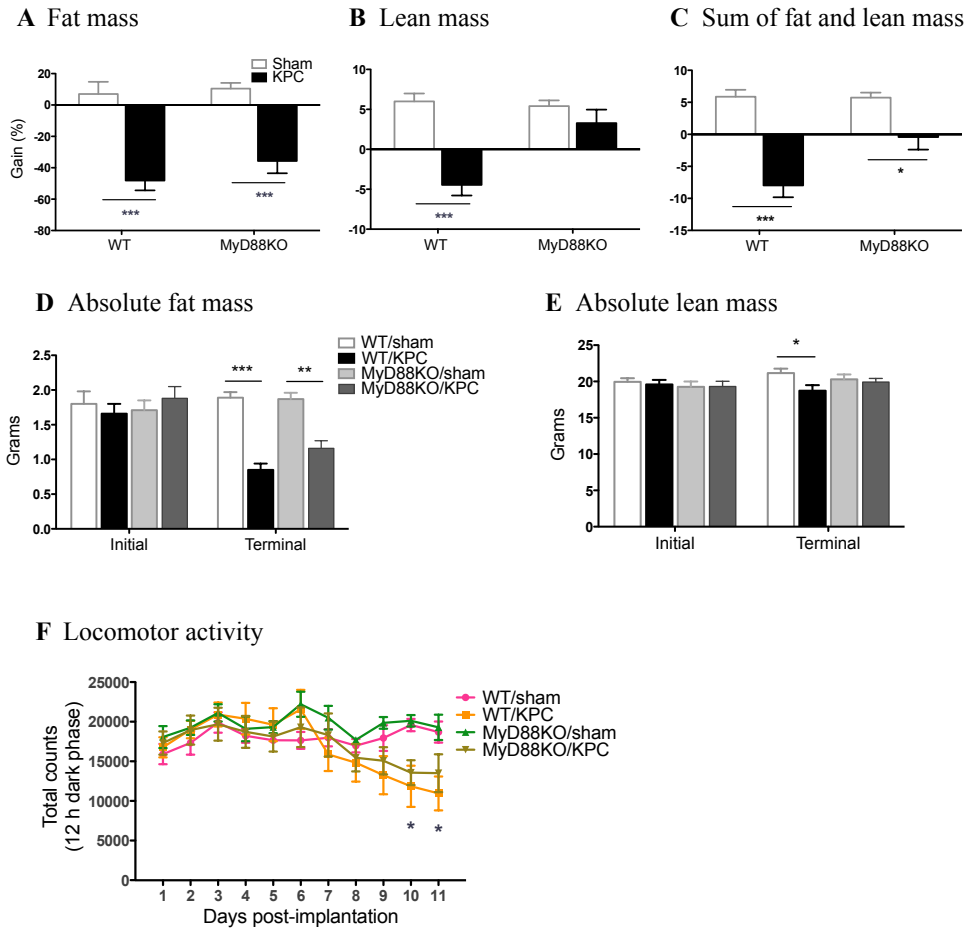


Fig. S6 MyD88 signaling does not affect body temperature during development of PDAC cachexia. (A) Body temperature at nighttime in male mice in the last 6 days before sacrifice. (B) Body temperature at nighttime in female mice in last 7 days before sacrifice. Both WT and MyD88 KO tumor mice developed a similar degree of hypothermia, particularly during the middle of dark phase (active phase). (C) Body temperature in male mice with IP implantation. N = 5/group.

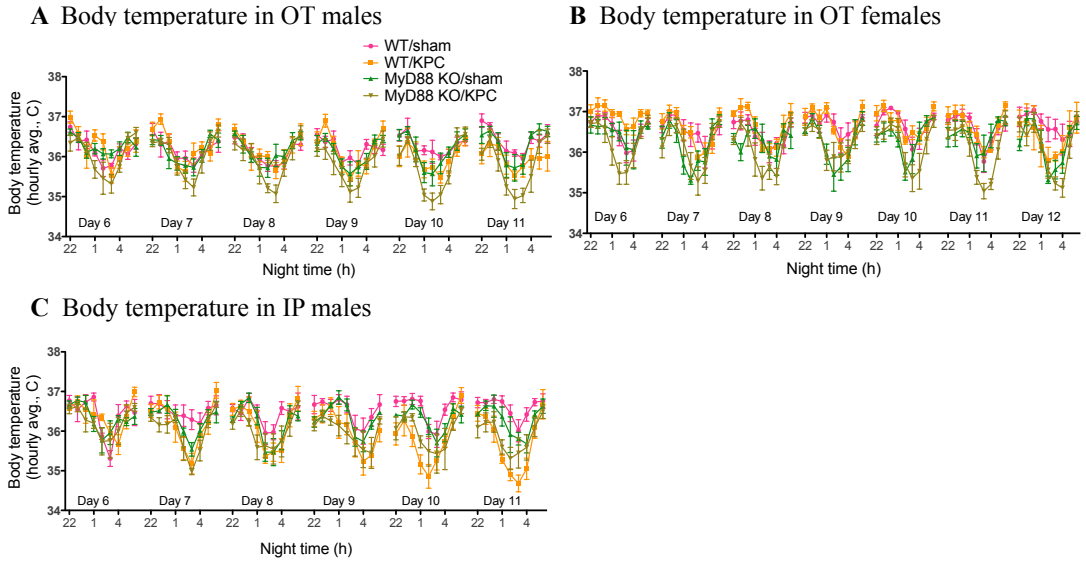


Fig. S7 MyD88 signaling is essential for muscle catabolism in PDAC cachexia. Mice were euthanized on day 11-12 post-implantation. (A) Absolute gastrocnemius weight (average of both left and right gastrocnemii). (B) Relative gastrocnemius weight. (C) Absolute heart weight. (D) Relative heart weight. Relative muscle weight was normalized to initial body weight. (A-D) are related to Figure 4 (A) and (B). *, P < 0.05, **, P < 0.01, ***, P < 0.001, Sham vs. KPC. Two-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 5/group.

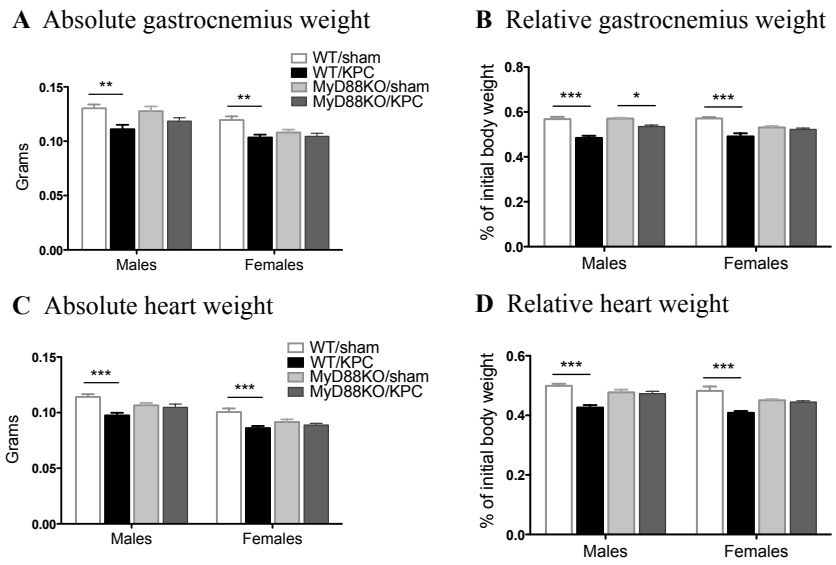


Fig. S8 MyD88 KO IP KPC mice exhibit reduced muscle catabolism. Male mice with IP implantation were sacrificed on day 11 post-implantation. (A) Gastrocnemius and heart weight loss. Muscle weight was normalized to initial body weight, and presented as loss (%) to sham mice of the same genotype. (B) Absolute weight of gastrocnemius and heart. (C) Relative weight of gastrocnemius and heart (relative to initial body weight). *, $P < 0.05$, **, $P < 0.01$, **, $P < 0.01$, WT/KPC vs. MyD88/KPC (A), Sham vs. KPC (B and C). Two-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 4-5/group.

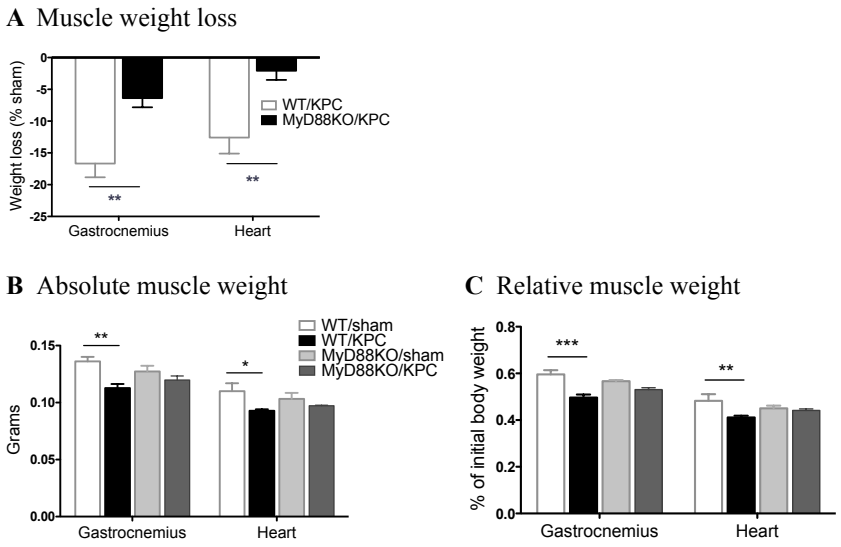


Fig. S9 MyD88 signaling is essential for excessive muscle catabolism in PDAC cachexia. Mice were euthanized on day 11-12 post-implantation. (A) Gene expression in gastrocnemius. (B) Gene expression in heart. Data was presented as relative fold change to WT/sham group. (A) and (B) are related to Figure 4 (C) and (D). *, $P < 0.05$, ***, $P < 0.001$, WT/Sham vs. WT/KPC. One-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 5/group.

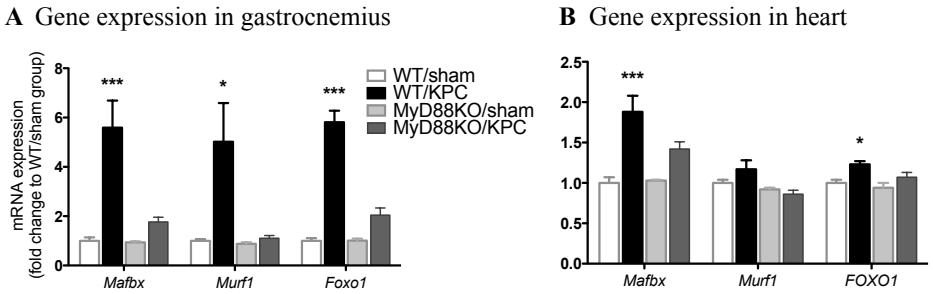


Fig. S10 Pancreatic cancer-induced systemic inflammation is diminished in MyD88 KO IP mice. Male mice with IP implantation were sacrificed on day 11 post-implantation. (A) White blood cell (WBC), neutrophil and lymphocyte counts. (B) Serum IL-6 concentration. ***, P < 0.001, WT/sham vs. WT/KPC. Two-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 4-5/group.

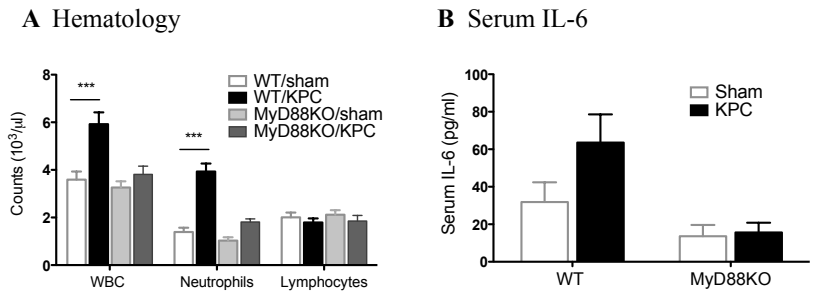
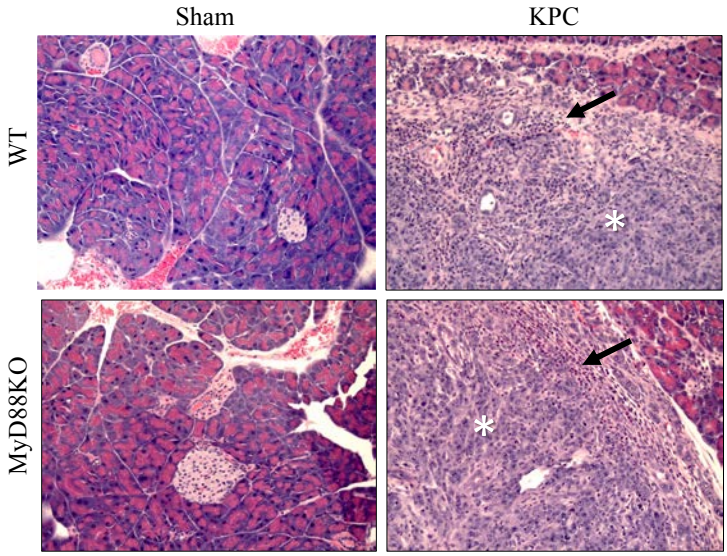
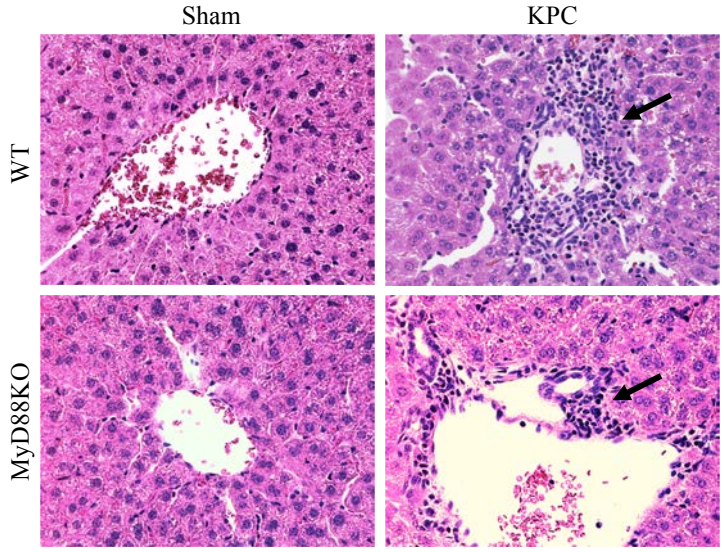


Fig. S11 Histopathology of orthotopical KPC compared with sham in WT vs. MyD88 KO mice. (A) Sections of the pancreas from sham mice revealed no acute or chronic inflammation, whereas, sections of tumor (*) in WT/KPC and MyD88 KO/KPC mice both showed marked acute inflammation in and around tumor (arrows). (B) Both WT and MyD88 KO KPC mice also had portal-based mixed inflammation (arrow). (C) Liver weights were modestly increased in MyD88 KO/KPC mice. (D) Compared with sham mice, both WT and MyD88 KO KPC mice had increased lymphoid infiltration of splenic red pulp and conspicuous megakaryocytes (arrows), consistent with extramedullary hematopoiesis in these animals. (E) Increased spleen weights were identical between WT and MyD88 KO KPC mice. Tissue sections were stained with haematoxylin and eosin (H&E) and photographed with 20× (A, D) and 40× (B) objective lens. *, P < 0.05, ***, P < 0.001, Sham vs. KPC. Two-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 5/group.

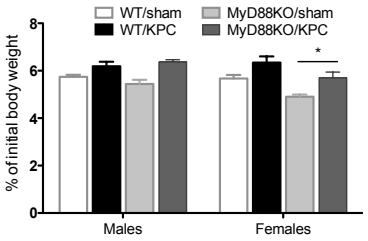
A Pancreas and tumor (H&E staining, 20X)



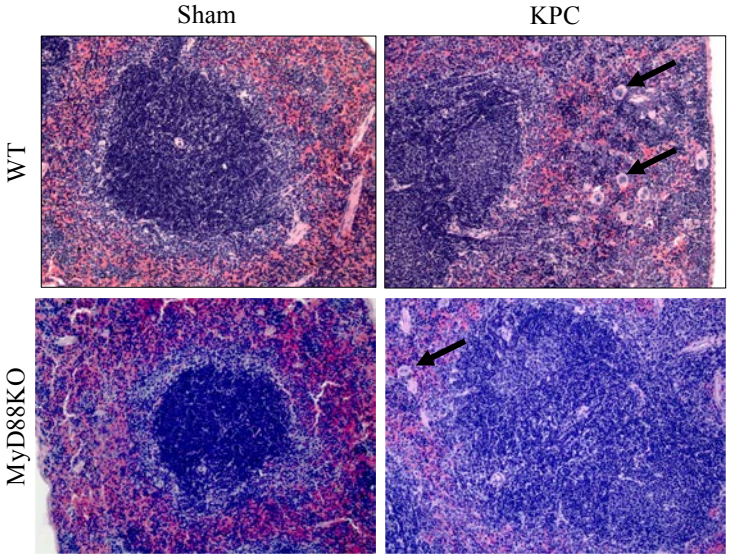
B Liver (H&E staining, 40X)



C Liver weights



D Spleen (H&E staining, 20X)



E Spleen weights

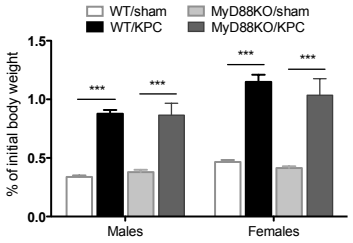


Fig. S12 Pancreatic cancer-induced systemic and CNS inflammatory gene expression. Mice were euthanized on day 11-12 post-OT implantation. (A and B) Liver inflammatory gene expression. (C) Hypothalamic inflammatory gene expression. (D) Cortex inflammatory gene expression. Data was presented as relative fold change to WT/sham group. (A-D) are related to Figure 6 (C-F). *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, Sham vs. KPC. One-way ANOVA with post-hoc Bonferroni-corrected t-test. N = 5/group.

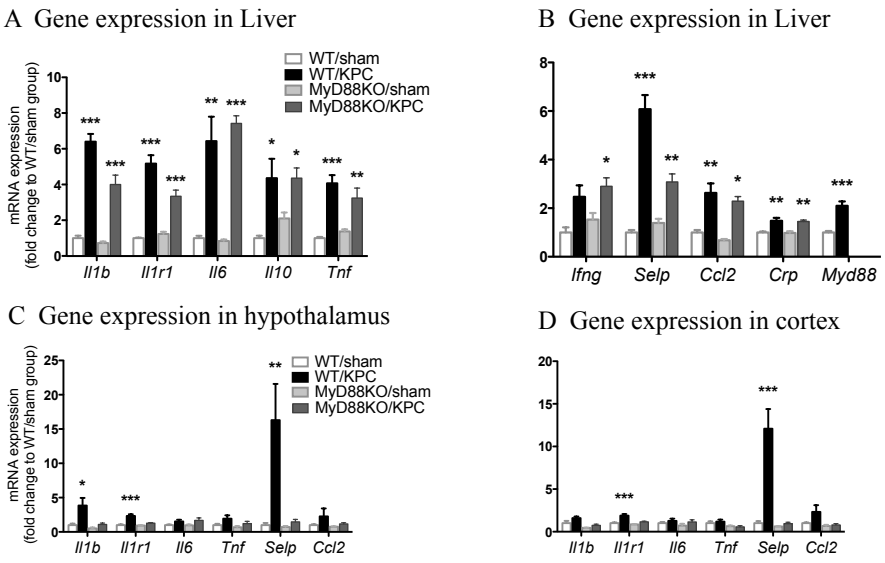


Fig. S13 MyD88 signaling contributes to high mortality in mice with IP PDAC. Sex-, age- and body weight-matched WT and MyD88 KO mice were implanted with 3 x 10^6 KPC cells through IP route. (A) Survival curves in males and females. (B) Initial body weight pre-tumor implantation. (C) Terminal tumor weight. **, P < 0.01, ***, P < 0.001, WT/KPC vs. MyD88 KO/KPC. Comparison of survival curves with Log-rank (Mantel-Cox) test (A) or unpaired t-test (C). N = 7-8/group.

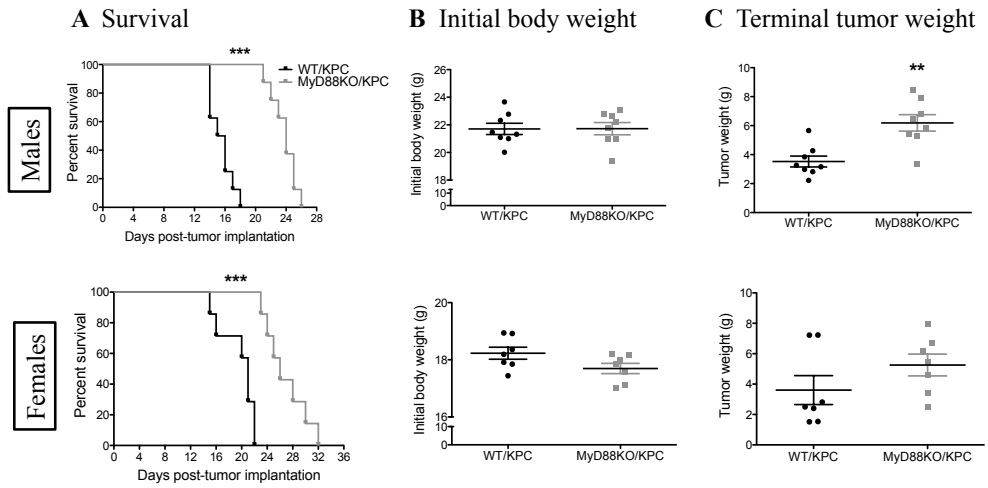


Table S1 Pancreatic cancer induces neutrophil-dominant leukocytosis and anemia in OT males. Leukocytosis but not anemia is associated with MyD88 signaling. Mice were sacrificed on day 11 post-implantation. Whole blood was analyzed for hematological parameters. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, Sham vs. KPC. Two-way ANOVA with post-hoc Bonferroni-corrected t-test.

Table S1. Results of hematology assay in OT male mice

Hematoclinical parameters	WT/sham N=5	WT/KPC N=5	MyD88KO/sham N=5	MyD88KO/KPC N=5
Leukocytes (×10³/μL):				
WBC	4.71±0.41	10.57±0.73***	5.94±0.80	7.24±0.57
Neutrophils	1.02±0.06	7.04±0.49***	0.90±0.15	3.54±0.49**
Lymphocytes	3.45±0.36	3.12±0.28	4.77±0.65	3.46±0.28
Monocytes	0.24±0.02	0.36±0.05	0.26±0.04	0.21±0.03
Eosinophils	0.00±0.00	0.06±0.01	0.00±0.00	0.02±0.01
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Erythrocytes:				
RBC (×10⁶/μL)	9.15±0.03	7.72±0.21*	8.61±0.17	7.69±0.20
Hb (g/dL)	12.42±0.13	10.22±0.29***	11.96±0.30	10.56±0.34
HCT (%)	40.88±0.26	33.40±0.89***	38.52±0.80	34.34±0.72***
MCV (fL)	44.80±0.32	43.30±0.24*	44.72±0.29	44.68±0.27
MCH (pg)	13.58±0.19	13.22±0.14	13.90±0.23	13.72±0.10
MCHC (g/dL)	30.32±0.22	30.58±0.24	31.06±0.47	30.72±0.37
Thrombocytes:				
PLT (×10³/μL)	825.20±31.76	849.80±40.23	741.20±17.55	629.20±35.41

Table S2 Pancreatic cancer induces neutrophil-dominant leukocytosis and anemia in OT females. Leukocytosis but not anemia is associated with MyD88 signaling. Mice were sacrificed on day 11 post-implantation. Whole blood was analyzed for hematological parameters. *, $P < 0.05$, ***, $P < 0.001$, Sham vs. KPC. Two-way ANOVA with post-hoc Bonferroni-corrected t-test.

Table S2. Results of hematology assay in OT female mice

Hematoclinical parameters	WT/sham N=6	WT/KPC N=6	MyD88KO/sham N=6	MyD88KO/KPC N=6
Leukocytes (×10³/μL):				
WBC	3.59±0.29	9.58±1.32***	2.70±0.29	4.74±0.44*
Neutrophils	0.95±0.14	5.25±0.59***	0.78±0.13	2.55±0.32
Lymphocytes	2.44±0.22	3.95±0.93	1.79±0.15	2.01±0.16
Monocytes	0.19±0.02	0.34±0.07	0.13±0.02	0.16±0.01
Eosinophils	0.01±0.00	0.03±0.01	0.01±0.00	0.02±0.00
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Erythrocytes:				
RBC (×10⁶/μL)	9.12±0.10	7.74±0.25	8.81±0.20	8.11±0.16
Hb (g/dL)	13.08±0.23	11.32±0.37*	12.07±0.21	11.10±0.22
HCT (%)	41.52±0.33	33.88±1.20***	38.27±0.77	34.63±0.72***
MCV (fL)	45.57±0.73	43.75±0.26*	43.43±0.31	42.67±0.26
MCH (pg)	14.37±0.36	14.60±0.09	13.70±0.14	13.67±0.13
MCHC (g/dL)	31.50±0.42	33.40±0.27*	31.57±0.33	32.05±0.22
Thrombocytes:				
PLT (×10³/μL)	710.33±44.46	865.83±44.62	822.67±55.16	810.33±46.99

Table S3 Pancreatic cancer induces neutrophil-dominant leukocytosis and anemia in IP males. Leukocytosis but not anemia is associated with MyD88 signaling. Mice were sacrificed on day 11 post-implantation. Whole blood was analyzed for hematological parameters. *, $P < 0.05$, ***, $P < 0.001$, Sham vs. KPC. Two-way ANOVA with post-hoc Bonferroni-corrected t-test.

Table S3. Results of hematology assay in IP male mice

Hematoclinical parameters	WT/sham N=4	WT/KPC N=5	MyD88KO/sham N=4	MyD88KO/KPC N=5
Leukocytes (×10³/μL):				
WBC	3.59±0.34	5.92±0.50***	3.26±0.26	3.81±0.35
Neutrophils	1.39±0.18	3.93±0.34***	1.03±0.14	1.81±0.13
Lymphocytes	2.01±0.20	1.79±0.17	2.12±0.19	1.85±0.24
Monocytes	0.18±0.02	0.18±0.02	0.11±0.01	0.12±0.01
Eosinophils	0.01±0.01	0.02±0.01	0.00±0.00	0.02±0.01
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Erythrocytes:				
RBC (×10⁶/μL)	8.74±0.15	8.04±0.23	8.87±0.15	7.79±0.19
Hb (g/dL)	12.08±0.14	11.30±0.37	12.25±0.23	10.48±0.16*
HCT (%)	39.25±0.53	35.48±0.92***	39.05±0.82	34.24±0.56***
MCV (fL)	44.93±0.50	44.14±0.25	44.05±0.33	43.94±0.42
MCH (pg)	13.83±0.15	14.06±0.14	13.83±0.06	13.48±0.25
MCHC (g/dL)	30.78±0.42	31.82±0.44	31.38±0.32	30.62±0.46
Thrombocytes:				
PLT (×10³/μL)	709.00±149.33	807.40±33.50	853.50±23.08	792.60±25.68