Supplemental Information:

Figures S1-S5 and Table S1.

Buechler...Turley, Figure S1

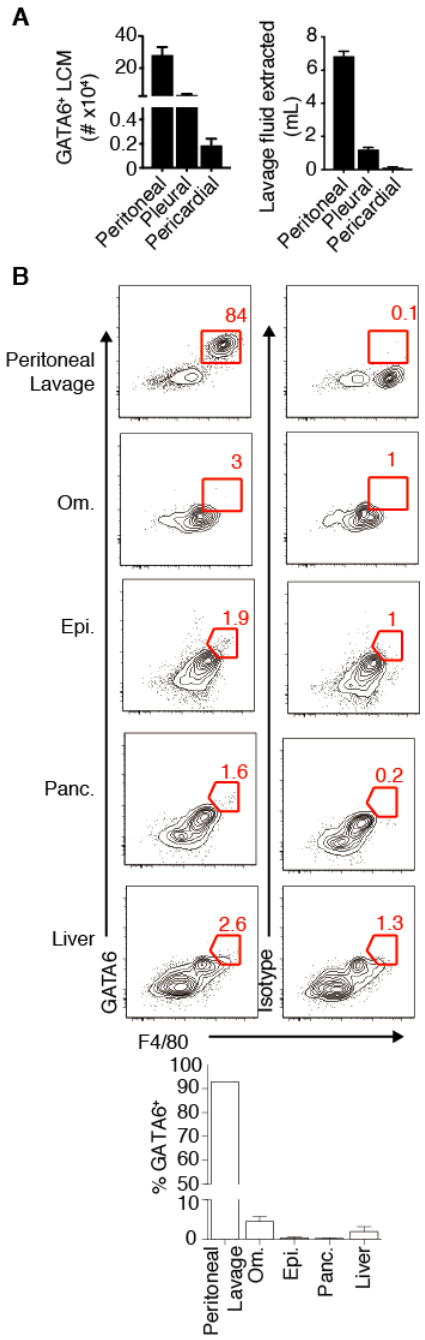

Figure S1. LCM numbers across cavities and mesothelial cells and fibroblasts at cavity surfaces exhibit *Wt1-Raldh1/2* axis.

Related to Figure 1

(A) GATA6^{+} LCM number across cavities (left) and lavage fluid extracted (right). (B) Frequency of GATA6^{+} F4/80^{+} cells in peritoneal lavage and select cavity-associated tissues (top) and quantification (bottom). (A) Data are from 12-13 mice across >3 experiments. (B) Data are from 3 experiments. Peritoneal lavage quantification is from one experiment, representative of ≥3. (A-B) Mean values + SEM are shown.

Buechler...Turley, Figure S2

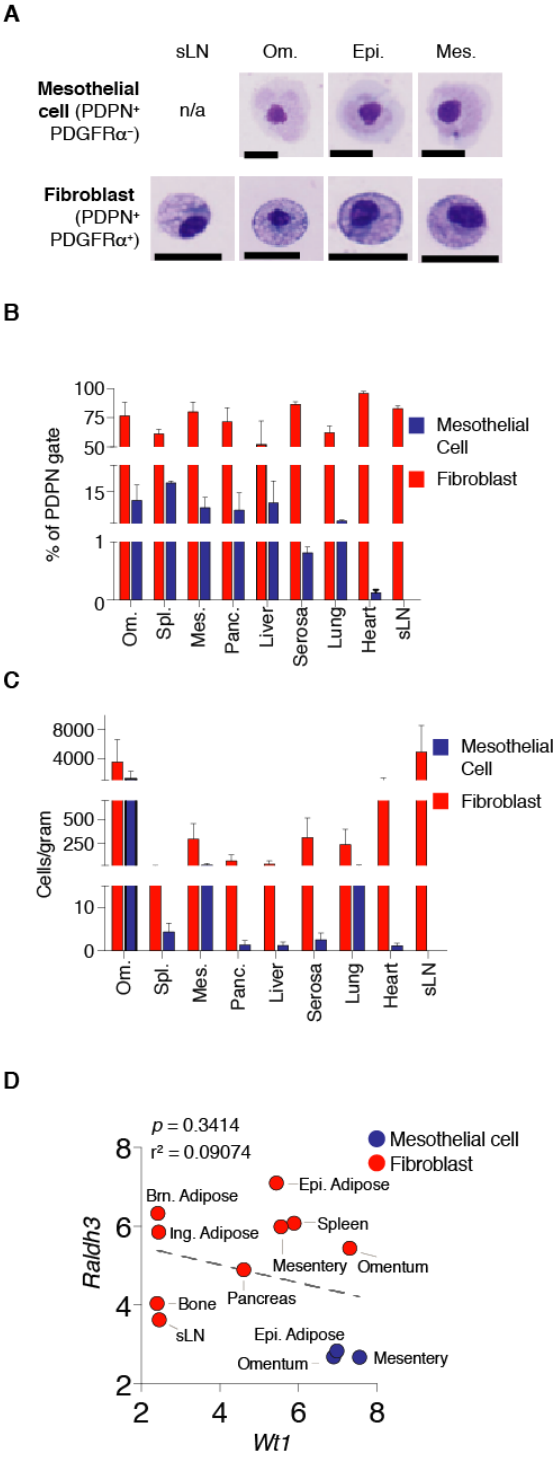

Figure S2. *Wt1*+ mesothelial and fibroblastic stromal cell identification and expression of *Wt1* and *Raldh3*.

Related to Figure 3

(A) FACS sorted samples after cytospin and stained with Wright-Giemsa stain. Scale bar indicates 25uM. (B) Frequency of mesothelial cells and fibroblasts in PDPN+ gate in cavity and non-cavity tissues. (C) Mesothelial cells and fibroblasts per gram of tissue. (D) Correlation between *Raldh3* and *Wt1* in FACS-sorted mesothelial cells (blue dots) and fibroblasts (red dots). Gene expression across biological replicates (TPM + 5 (Log_2)) were averaged to generate data points. (A) Data are representative of 2 samples. (B, C) Data are from 3 experiments with 1 mouse per experiment. (D) 3 independent biological replicates were sequenced for each cell type, except spleen and pancreas fibroblasts (n = 2). (B-C) Mean values + SEM are shown. (D) p value and r-squared values determined by linear regression.

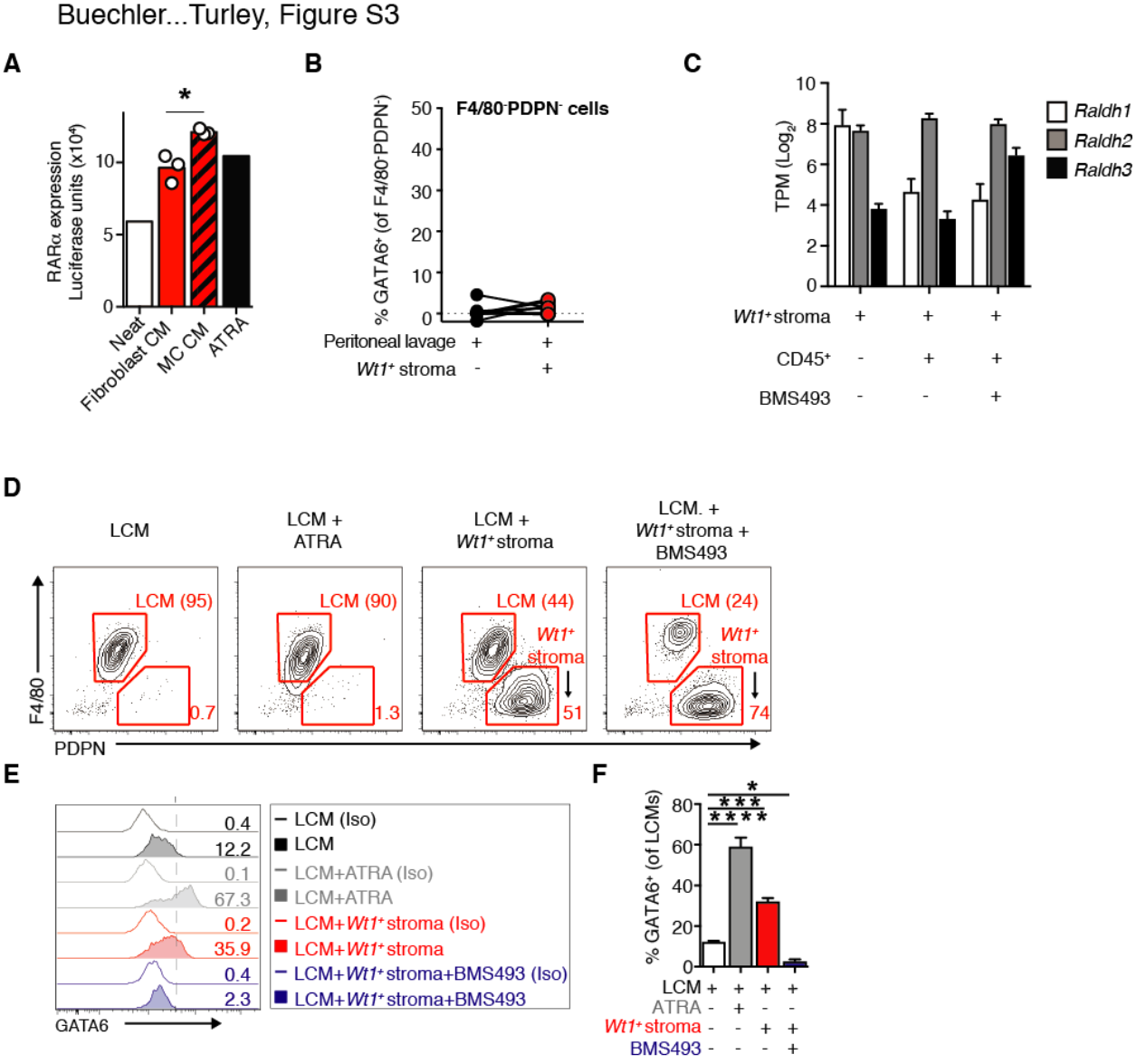

Figure S3. *Wt1*+ mesothelial and fibroblastic stromal cells produce active retinoic acid and maintain GATA6 in LCM.

Related to Figures 4 and 5

(A) Luciferase expression in RARα-luciferase cells after 24 hours of stimulation with media or conditioned media from mesothelial cells, fibroblasts or ATRA. (B) Quantification of the frequency of live GATA6$^+$ F4/80$^-$PDPN$^-$ cells after culture with autologous omental *Wt1*$^+$ stromal cells. (C) Expression (TPM; transcripts per million) of *Raldh1, Raldh2, Raldh3* in FACS-sorted omental *Wt1*$^+$ stromal cells after 1 week in culture as noted. (D) Representative gating FACS-sorted co-culture assays. Numbers in parentheses represent frequency. (E) Representative GATA6 expression on peritoneal LCMs. Numbers represent frequency of GATA6$^+$ LCMs. Shaded histograms represent isotype staining. (F) Frequency of GATA6$^+$ peritoneal LCM after treatment with ATRA, co-cultured with omental *Wt1*$^+$ stromal cells with cell-cell contact or co-cultured with *Wt1*$^+$ stromal cells and BMS493. (A) Each dot represents 1 conditioned media lot. (B, E-F) GATA6 expression was determined by GATA6 staining minus isotype. Data are from ≥3 (B-C, F) or representative of 3 (D-E) experiments. 5 mice were pooled for FACS-sorted to generate cells in D-F. *$p<0.05$, ***$p<0.0005$, ****$p<0.0001$, as determined by unpaired student's t-test (A) or Dunnett's multiple comparison post-test (F). Data are represented as mean (A-B), + SEM (C, F).

Buechler...Turley, Figure S4

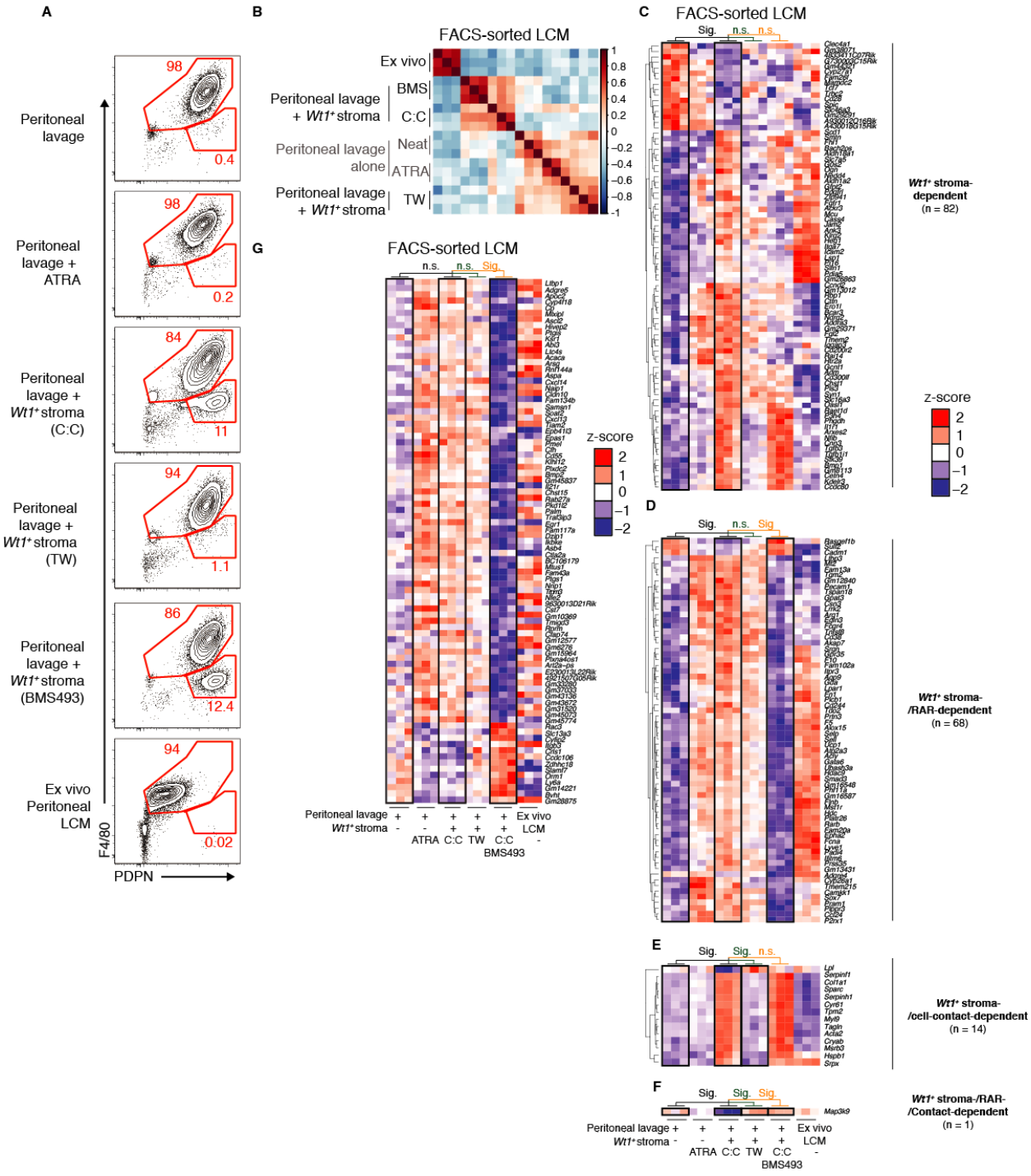

Figure S4. *Wt1*⁺ stromal cells program LCM identity.

Related to Figure 5.

(A) Representative gating for FACS sorting of LCM or *Wt1*+ stromal cells from co-cultures or ex vivo. Numbers represent cell frequency. (B) Sample similarity heatmap showing spearman correlation between samples calculated from normalized, log transformed, mean-centered expression of top 500 most variable transcripts. (C) *Wt1*+ stromal cell-dependent genes were significantly different in LCM sorted from peritoneal lavage alone compared to LCMs sorted from peritoneal lavage:*Wt1*+ stromal cell co-culture (66 genes went up in co-culture with *Wt1*+ stromal cells; 16 down). (D) *Wt1*+ stromal cell-/RAR-dependent genes were those significantly different in (C) and between LCMs sorted from peritoneal lavage:*Wt1*+ stromal cells and LCMs sorted from peritoneal lavage:*Wt1*+ stromal cells with BMS493 treatment (65 genes from went up in co-culture with *Wt1*+ stromal cells and significantly changed when cultured with *Wt1*+ stromal cells with BMS493; 3 went down in these comparisons). *Wt1*+ stromal cell-/cell-contact-dependent genes were those significantly different in (C) and significantly different between LCMs sorted from peritoneal lavage:*Wt1*+ stromal cell co-cultures in a transwell format (13 genes from went up in in co-culture with *Wt1*+ stromal cells and significantly changed when cultured with *Wt1*+ stromal cells in transwell; 1 went down in these comparisons). *Wt1*+ stromal cell-/RAR-/cell-contact-dependent genes were those significantly different in comparisons made in (C), (D) and (E) (n = 1). (F) Differentially expressed genes in FACS-sorted LCM (same threshold as (C)) between peritoneal lavage:*Wt1*+ stromal cells (C:C) compared to peritoneal lavage:*Wt1*+ stromal cells (C:C) with BMS493 (n = 85). (A-G) Co-culture with cell contact (C:C), Co-culture with 0.4uM transwell membrane separation (TW), ATRA, BMS493. Cells were cultured at a ratio of 4-11:1(Peritoneal

lavage:*Wt1*+ stromal cells) for 6 days. Three independent biological replicates were sorted for each treatment.

Buechler...Turley, Figure S5

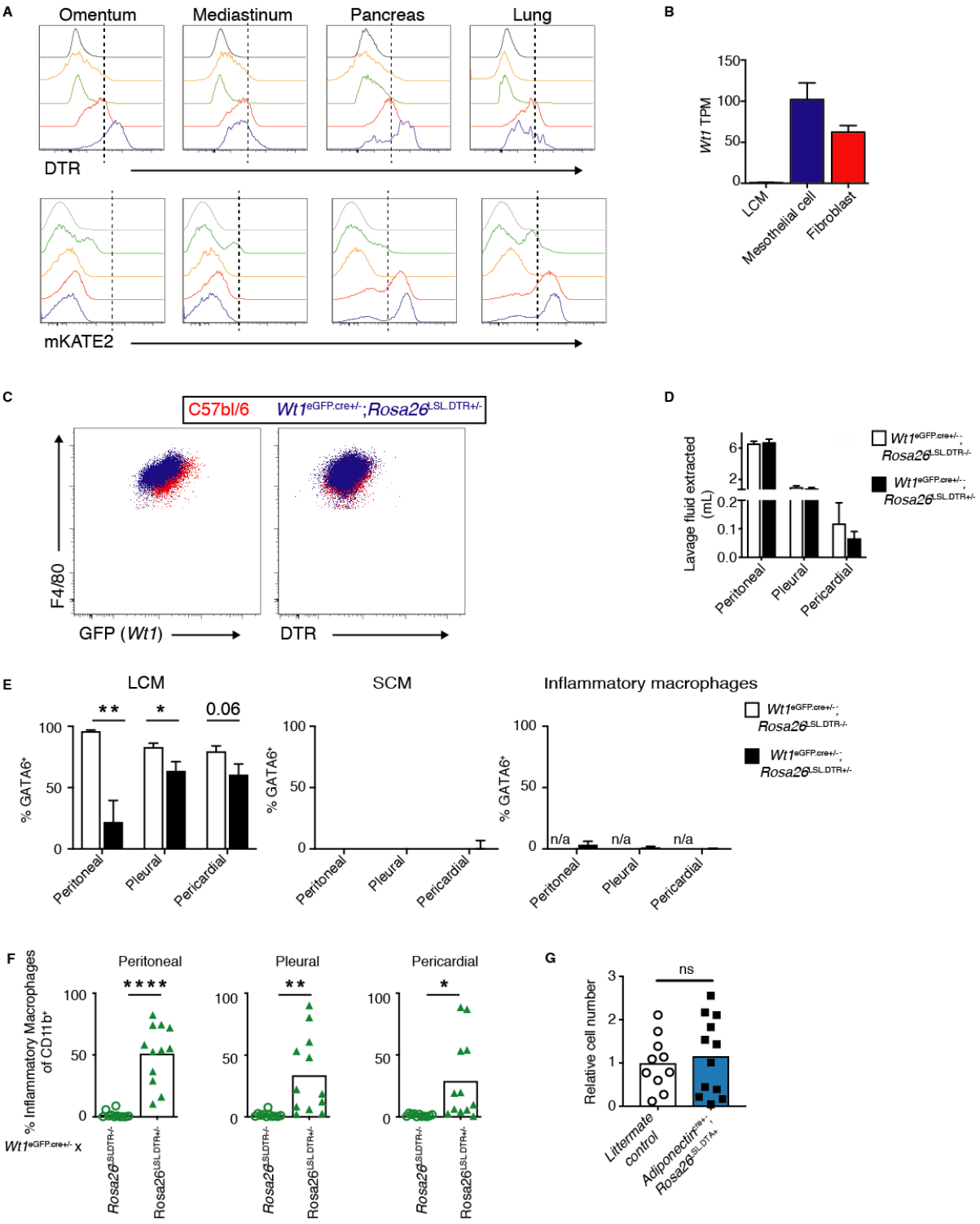

Figure S5. *Wt1*+ stromal cells are critical for LCM homeostasis.

Related to Figure 6.

(A) GFP, DTR and mKATE2 gating in *Wt1*$^{eGFP.cre}$;*Rosa26*$^{LSL.DTR}$ (top) and *Wt1*$^{eGFP.cre+/-}$;*Rosa26*$^{LSL.mKATE2+/-}$ (bottom). CD45$^-$EpCAM$^-$CD31$^-$PDPN$^+$PDGFRα^- mesothelial cells (blue), CD45$^-$EpCAM$^-$CD31$^-$PDPN$^+$PDGFRα^+ fibroblasts (red), CD45$^-$EpCAM$^-$PDPN$^{-/+}$CD31$^+$ endothelial cells (orange) and CD45$^-$EpCAM$^-$PDPN$^-$CD31$^-$ double negative cells (green) and SSC^{low}CD45$^+$ hematopoietic cells (black). (B) *Wt1* expression in LCMs, omental mesothelial cells and omental fibroblasts. TPM = transcripts per million reads. (C) DTR expression in LCM. Cells gated on peritoneal LCM. C57bl/6 control used to account for autofluorescence in GFP channel. (D) Lavage fluid extracted. (E) Frequency of GATA6$^+$ LCM, SCM and inflammatory macrophages in *Wt1*$^{eGFP.cre+/-}$;*Rosa26*$^{LSL.DTR-/-}$ and *Wt1*$^{eGFP.cre+/-}$;*Rosa26*$^{LSL.DTR+/-}$ mice 24 hours after DT-administration. Inflammatory macrophages were excluded from analysis of *Wt1*$^{eGFP.cre+/-}$;*Rosa26*$^{LSL.DTR-/-}$ mice due to low cell number (n/a) (F) Frequency of inflammatory macrophages in cavity spaces 24 hours after administration of DT in *Wt1*$^{eGFP.cre+/-}$;*Rosa26*$^{LSL.DTR-/-}$ and *Wt1*$^{eGFP.cre+/-}$;*Rosa26*$^{LSL.DTR+/-}$ mice 24 hours. LCM were gated as in Figure 6, SCM were gated as Live CD45$^+$Gr1$^-$SiglecF$^-$CD11b$^+$MHCII$^+$F4/80$^-$, inflammatory macrophages were gated as in CD45$^+$Gr1$^-$SiglecF$^-$CD11b$^+$MHCII$^-$F4/80$^+$. (G) Relative number of LCMs in littermate and *Adiponectin*$^{cre+}$;Rosa26$^{LSL.DTA+}$ mice. LCMs were gated as CD11b$^+$ICAM2$^+$. (B) LCMs and omental *Wt1*$^+$ stromal cells were FACS sorted and RNAseq was performed. Data are from three biological replicates. Data are from 4 (D, E (pleural and pericardial cavity) or 2 experiments peritoneal cavity (E) or 3 experiments (G) or representative of 2-4 experiments (C). Each dot represents 1 mouse (F, G). Relative number was determined by multiplying the total cell number in the lavage by the percentage of CD11b$^+$ICAM2$^+$

cells. *p<0.05, **p<0.005, ****p<0.0001, as determined by unpaired student's t-test (E-G). Mean (F, G) or mean +SEM (B, D, E) are shown.

Buechler…Turley. Table S1

Related to Figure 5

Peritoneal LCM hallmark genes	*Wt1*+ stromal regulation	References
Gata6	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014)
Rarb	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014)
Padi4	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014)
Cd93		Gautier (2012), Okabe (2014), Gautier (2014)
Serpinb2		Gautier (2012), Okabe (2014), Rosas (2014)
F5	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014), Rosas (2014)
Tgfb2		Gautier (2012), Okabe (2014), Rosas (2014)
Arg1	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014), Gundra (2014)
Lyz1		Gautier (2012), Okabe (2014), Gautier (2014)
Flnb		Gautier (2012), Okabe (2014), Rosas (2014)
Ank		Gautier (2012), Okabe (2014), Rosas (2014), Gundra (2014)
Rai14	*Wt1*+ stroma-dependent	Gautier (2012), Okabe (2014), Rosas (2014)
Hdc	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014), Gautier (2014)
Wnt2		Gautier (2012), Okabe (2014), Rosas (2014)
Vmn2r26		Gautier (2012), Okabe (2014), Rosas (2014)
Apoc2	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014), Gundra (2014)
Aqp9	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014), Rosas (2014)
F13a1		Gautier (2012), Okabe (2014), Gundra (2014)
Egln3	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014), Rosas (2014)
Stard13		Gautier (2012), Okabe (2014), Rosas (2014)
Marco		Gautier (2012), Okabe (2014), Gundra (2014)
Nfe2	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014)
Fn1	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014)
Prg4		Gautier (2012), Okabe (2014)
Icam2	*Wt1*+ stroma-dependent	Gautier (2012), Okabe (2014)

Lrg1		Gautier (2012), Okabe (2014)
Emilin2		Gautier (2012), Okabe (2014)
Thbs1		Gautier (2012), Okabe (2014)
Saa3		Gautier (2012), Okabe (2014)
Calml4		Gautier (2012), Okabe (2014)
Mstr1	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Okabe (2014)
Cgnl1		Gautier (2012), Okabe (2014)
Lama3		Gautier (2012), Gundra (2014)
Cxcl13	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Gundra (2014)
Clec4d		Gautier (2012), Gundra (2014)
Cmah		Gautier (2012), Rosas (2014)
Fgfr1	*Wt1*+ stroma-dependent	Gautier (2012), Rosas (2014)
Garnl3		Gautier (2012), Rosas (2014)
Selp	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Rosas (2014)
1110032E23Rik		Gautier (2012), Rosas (2014)
Naip1	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Rosas (2014)
Dnahc12		Gautier (2012), Gautier (2014)
Gbp1		Gautier (2012), Gautier (2014)
Car6		Gautier (2012), Gautier (2014)
Slfn4		Gautier (2012), Gautier (2014)
Sell	*Wt1*+ stroma-/RAR-dependent	Gautier (2012), Gautier (2014)
Hpse		Gautier (2012), Gautier (2014)
S100a4		Gautier (2012), Gautier (2014)
Slfn1	*Wt1*+ stroma-dependent	Gautier (2012), Gautier (2014)
Tspan32		Gautier (2012), Gautier (2014)
F7		Gautier (2012), Gautier (2014)
Cav1		Gautier (2012), Gautier (2014)
Hp		Gautier (2012), Okabe (2014), Gundra (2014)
Gm1673		Okabe (2014), Gundra (2014)
Ltbp1	*Wt1*+ stroma-/RAR-dependent	Okabe (2014), Rosas (2014)
Kcnn4		Gautier (2014), Gundra (2014)
Nuf2		Gautier (2014), Gundra (2014)
Apoc1		Gautier (2014), Gundra (2014)
Rgs2		Gautier (2014), Gundra (2014)
Ldhb		Gautier (2014), Gundra (2014)
Aspa	*Wt1*+ stroma-/RAR-dependent	Gautier (2014)

Table S1. List of peritoneal LCM hallmark genes. Gene name, whether expression of this gene in LCM requires *Wt1*+ stromal cells, RAR signaling or both and references are listed. Hallmark genes were derived from the references listed.