Supplemental Information

Molecular mechanisms of immunocytokine IL-33-mediated stromal interactions in cancer metastasis

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Supplemental Figures and Tables

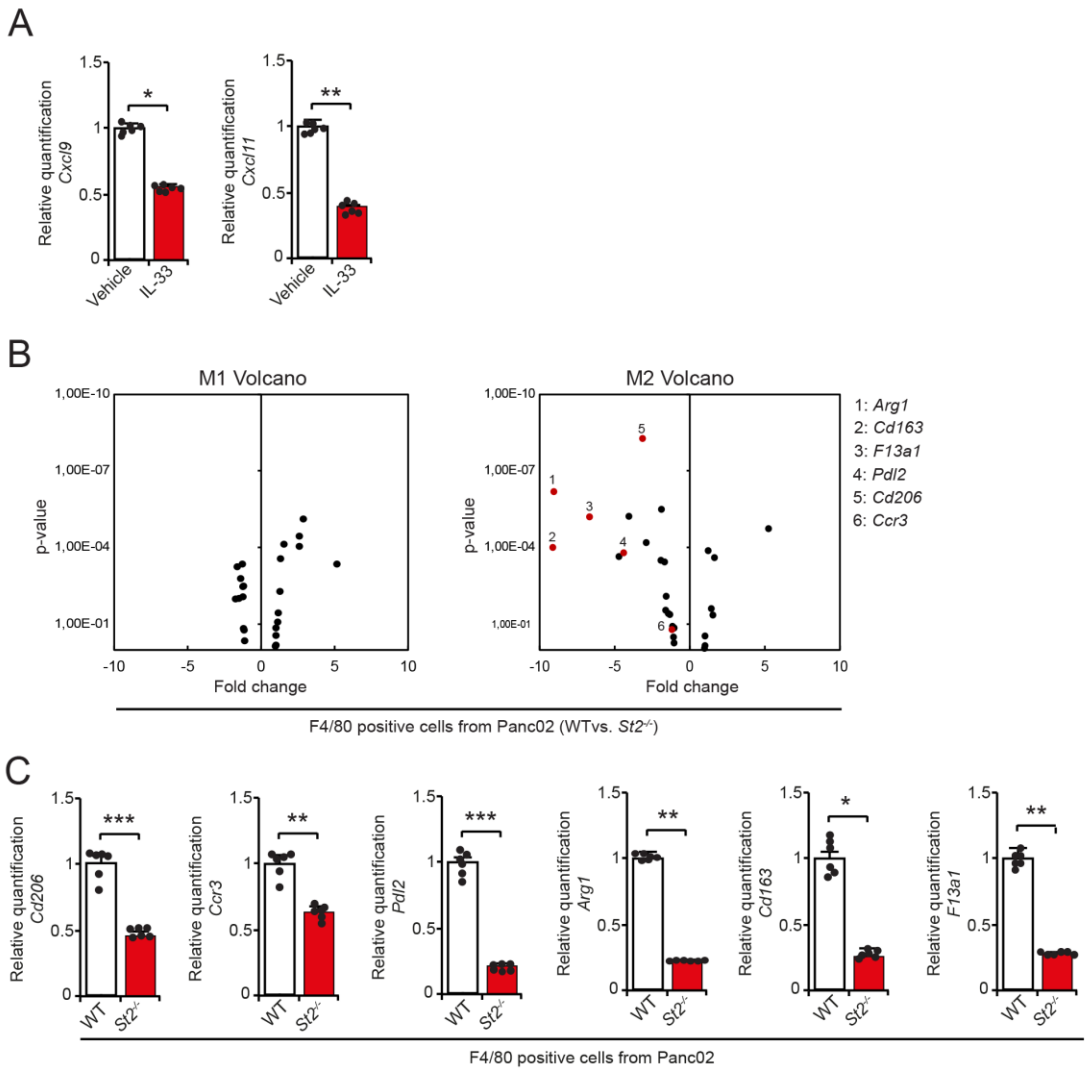

Figure S1. Polarization of M2-TAMs by IL-33.

(A) qPCR quantification of *Cxcl9* and *Cxcl11* mRNA expression levels in IL-33-stimulated macrophages (n = 6 samples per group).

(B) Volcano plots of a subset of M1- and M2-related genes by genome-wide expression profiling of F4/80^{+} cells sorted from Panc02 tumors grown in WT or *St2*$^{-/-}$ mice. Numbered dots (red) indicates (1) *Arg1*, (2) *Cd163*, (3) *F13a1*, (4) *Pdl2*, (5) *Cd206*, and (6) *Ccr3* genes identified by genome-wide expression profiling (n = 3 samples per group).

(C) qPCR quantification of *Cd206*, *Ccr3*, *Pdl2*, Arg1, *Cd163* and *F13a1* mRNA levels in F4/80^{+} cells sorted from Panc02 tumors grown in WT or *St2*$^{-/-}$ mice (n = 6 samples per group).

Mean ± s.e.m., * p<0.05; **p<0.01; ***p<0.001. Student's *t*-test.

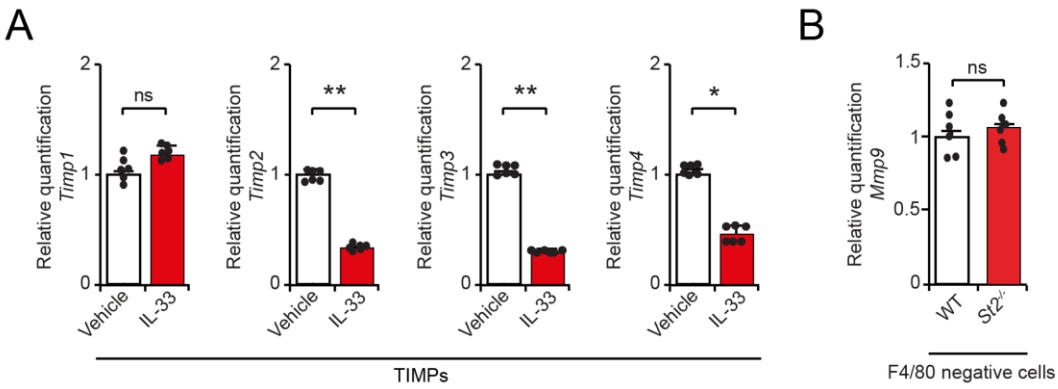

Figure S2. Downregulation of TIMPs in IL-33-stimulated macrophages.

(A) qPCR quantification of *Timp1*, *Timp2*, *Timp3*, and *Timp4* mRNA expression levels in IL-33-stimulated macrophages (n = 6 samples per group).

(B) qPCR quantification of *Mmp9* mRNA expression levels in F4/80^{-} cells sorted from Panc02 tumors grown in WT or *St2*$^{-/-}$ mice (n = 6 samples per group).

Mean ± s.e.m., * $p<0.05$; **$p<0.01$. ns, not significant. Student's *t*-test.

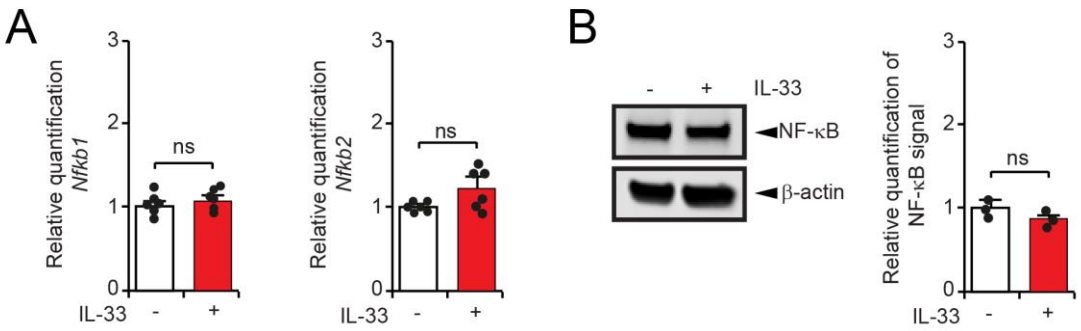

Figure S3. The effect of IL-33 on NF-κB expression levels.

(A) qPCR quantification of *Nfkb1 and Nfkb2* mRNA expression levels in IL-33-stimulated macrophages (n = 6 samples per group).

(B) Western immunoblot analysis of NF-κB expression level in IL-33-stimulated macrophages. Beta actin served as a loading control (n = 3 samples per group). Mean ± s.e.m., ns, not significant. Student's *t*-test.

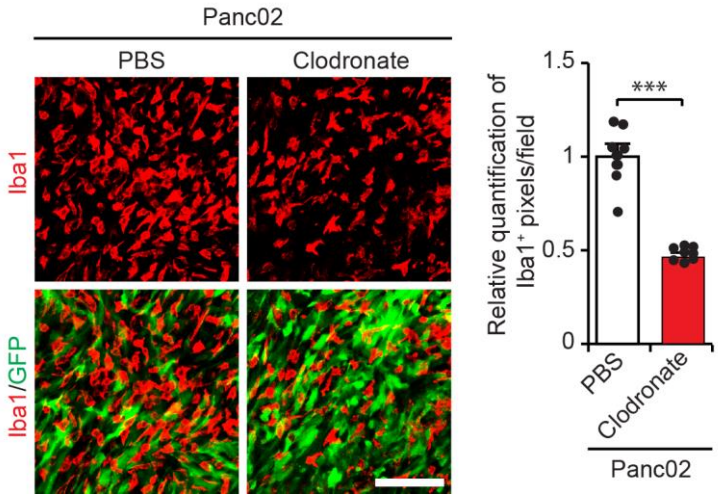

Figure S4. Ablation of tumor macrophages by clodronate.

(A) Immunohistochemical staining and quantification of $Iba1^+$ (red) macrophages in PBS- or clodronate-treated Panc02 tumors. Tumor cells are GFP^+ (n = 8 random fields per group, scale bar = 100 μm).
Mean ± s.e.m., ***p<0.001. Student's *t*-test.

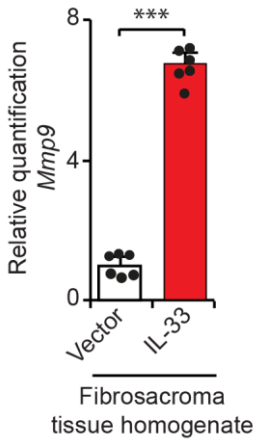

Figure S5. Production of MMP9 in fibrosarcoma.

(A) qPCR quantification of *Mmp9* mRNA expression levels in vector or IL-33-overexpressing fibrosarcoma tumors (n = 6 samples per group). Mean ± s.e.m., ***p<0.001. Student's *t*-test.

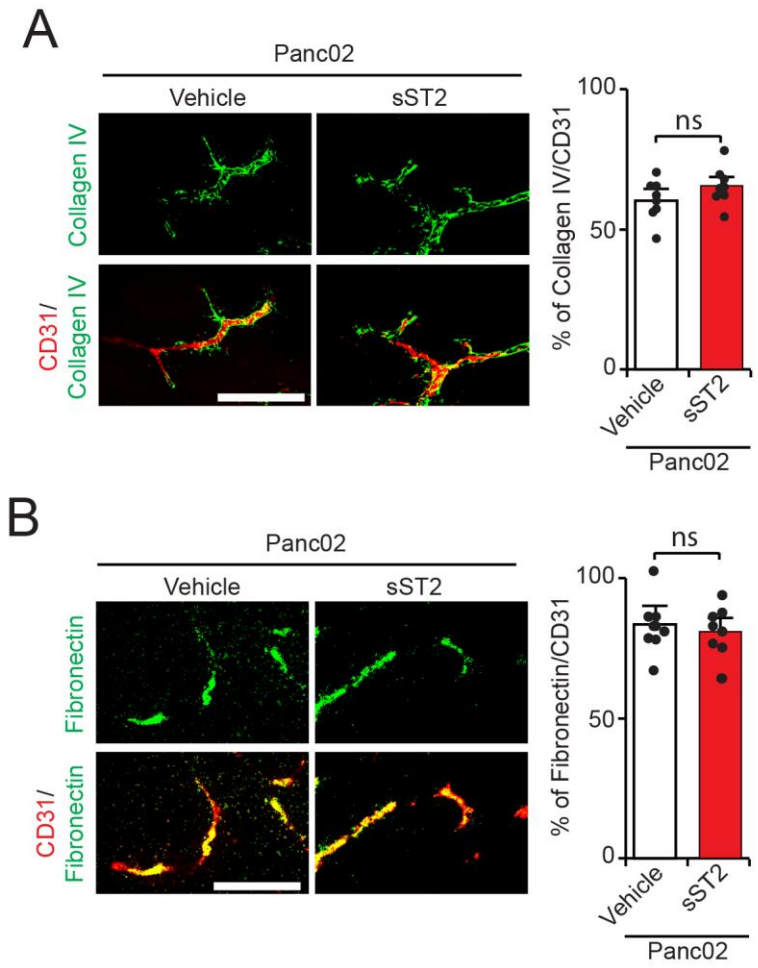

Figure S6. Impact of IL-33-ST2 signaling on collagen IV and fibronectin contents in tumors.

(A) Immunohistochemical staining and quantification of collagen IV$^+$ (green) and CD31$^+$ (red) structures in vehicle- or sST2-treated Panc02 tumors. Quantification of the percentage of collagen IV$^+$/CD31$^+$ signals per field (n = 8 random fields per group, scale bar = 100 μm).

(B) Immunohistochemical staining and quantification of fibronectin$^+$ (green) and CD31$^+$ (red) structures in vehicle- or sST2-treated Panc02 tumors. Quantification of the percentage of fibronectin$^+$/CD31$^+$ signals per field (n = 8 random fields per group, scale bar = 100 μm). Mean ± s.e.m., ns, not significant. Student's *t*-test.

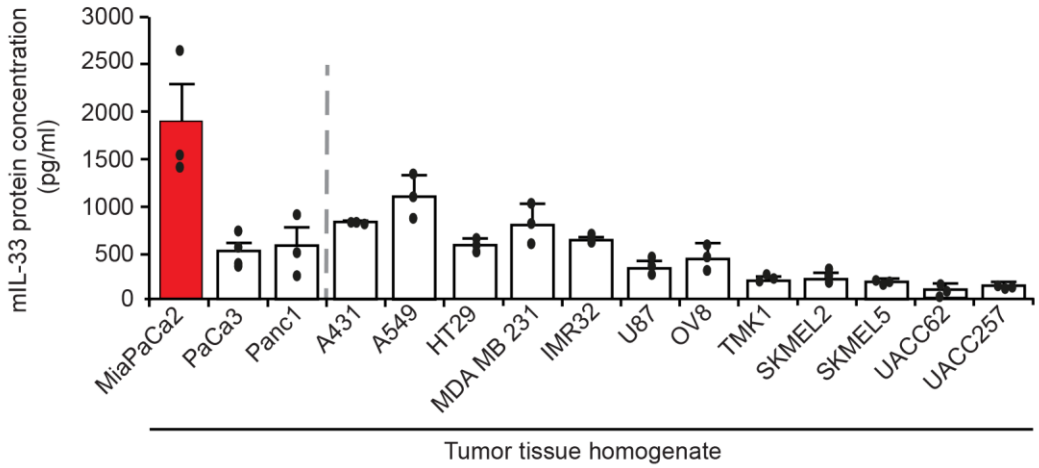

Figure S7. ELISA analysis of IL-33 protein in various human tumors.
IL-33 protein levels in various tumor tissues (n = 3 samples per group). Mean ± s.e.m.

Table S1. List of antibodies for immunoblotting

Antibody	**Catalog no.**	**Company**
Beta actin	3700	Cell Signaling
ERK	4695	Cell Signaling
Phospho-ERK	9101	Cell Signaling
IκBα	4812	Cell Signaling
Phospho-IκBα	2859	Cell Signaling
p38	9212	Cell Signaling
Phospho-p38	4631	Cell Signaling
NF-κB	6956	Cell Signaling
Donkey anti-Mouse (800CW)	926-32212	LI-COR
Donkey anti-Rabbit (680RD)	926-68073	LI-COR

Table 2. **List of primers**

Target	**Forward (5’ - 3’)**	**Reverse (5’ - 3’)**
m*Gaphd*	CCAGCAAGGACACTGAGCAA	GGGATGGAAATTGTGAGGGA
m*Mmp1a*	ACTACAACTGACAACCCAAGAAAG	AAGTGTCTCTTAGCTGGGCAC
m*Mmp2*	TTTCTATGGCTGCCCCAAGG	GTCAAGGTCACCTGTCTGGG
m*Mmp8*	GTCCCAAGTGGACACACACT	GGTTGAAAGGCATGGGCAAG
m*Mmp9*	GTCCAGACCAAGGGTACAGC	ATACAGCGGGTACATGAGCG
m*Mmp12*	CTGTGACTGTACCAAGCCAT	CTCCTGTGCTTAAGGAGGCT
m*Mmp13*	AGAAGTGTGACCCAGCCCTA	GGTCACGGGATGGATGTTCA
m*Cd206*	TGGGCAACATCGAGCAGAAT	TGCAGGGTTGACATGAGACC
m*Pdl2*	TTGTCTCCTTCTGTCTCCCAAC	TCAAAATCGCACTCCAGGCT
m*Ccr3*	TCTACCGGCCCTCACATACC	TTCAATCCAGAGAGCACCTCC
m*Arg1*	GAACTCTGATCGTAGCTGCCT	GAATCCCGAGGCAGAAGTCC
m*Cd163*	CACGGCACTCTTGGTTTGTG	CTCTGAATGACCCCCGAGGA
m*F13a1*	GTCCCGCCCAATAACTCCAA	CCCTCTGCGGACAATCAACT
m*Timp1*	GATCGGGGCTCCTAGAGACA	GCTGGTATAAGGTGGTCTCGT
m*Timp2*	CATGCTGGGGTTTCTAGCCA	GCATGACGGGAGTAAGGGAG
m*Timp3*	CCCTTGCATCTTTCCCCTGT	GGCCTCACCTCAAGTCTGTC
m*Timp4*	CTCTTGTCCTGCAAGTCCCC	CCTGGAGGGAAAATGCTTGT
m*Nfkb1*	ATGTAGTTGCCACGCACAGA	TGTAAAATGCATAAAACGGG GAAA
m*Nfkb2*	GCAGCACTAACTTTCTGCCC	GATAGGGGCCATCAGCTGTC