iScience, Volume *26*

Supplemental information

TRAIL-dependent apoptosis of peritoneal mesothelial cells by NK cells promotes ovarian cancer invasion

Anna Mary Steitz, Clarissa Schröder, Isabel Knuth, Corinna U. Keber, Leah Sommerfeld, Florian Finkernagel, Julia M. Jansen, Uwe Wagner, Sabine Müller-Brüsselbach, Thomas Worzfeld, Magdalena Huber, Vanessa M. Beutgen, Johannes Graumann, Elke Pogge von Strandmann, Rolf Müller, and Silke Reinartz

Supplemental Information

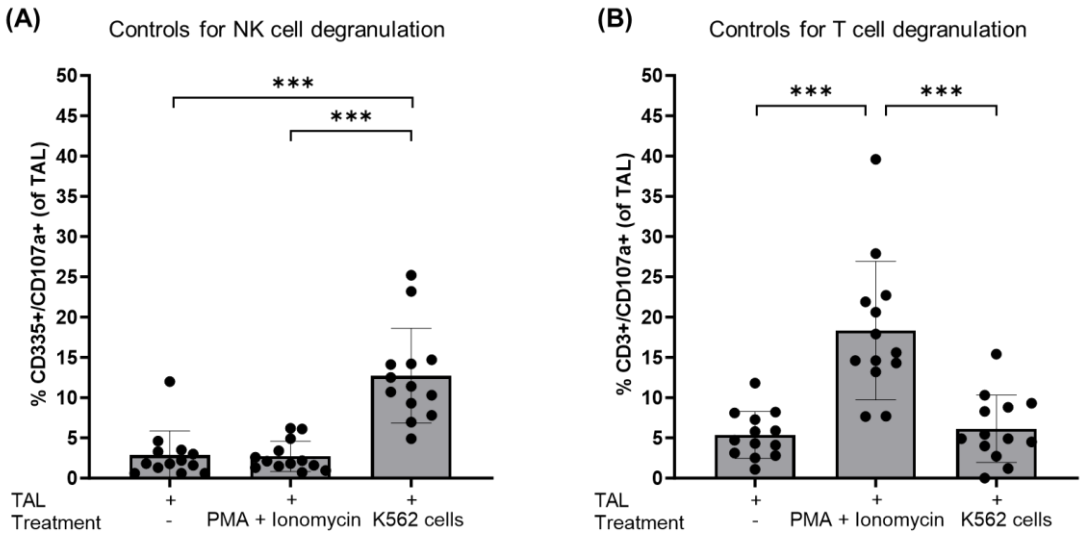

Figure S1. Controls for flow cytometric determination of NK- and T cell degranulation, related to Fig. 1 and 2.

(A, B) As a positive control for NK cell degranulation, TAL were co-cultured with the MHCI-deficient cell line K562 (ratio TAL:K562 10:1). As a positive control for T cell degranulation, TAL were treated with PMA and Ionomycin.

(A) The percentage of degranulating NK cells is shown gated on the CD335+/CD107a+ cells.

(B) The amount of degranulating T cells is shown as percentage of CD3+/CD107a+ cells. In both cases, untreated TAL were included as background controls for T and NK cell degranulation.

The mean of n=13 biological replicates of different patients is shown by horizontal bars and vertical error bars represent the standard deviation. *** FDR < 0.001; determined by paired t test and Benjamini-Hochberg adjustment.

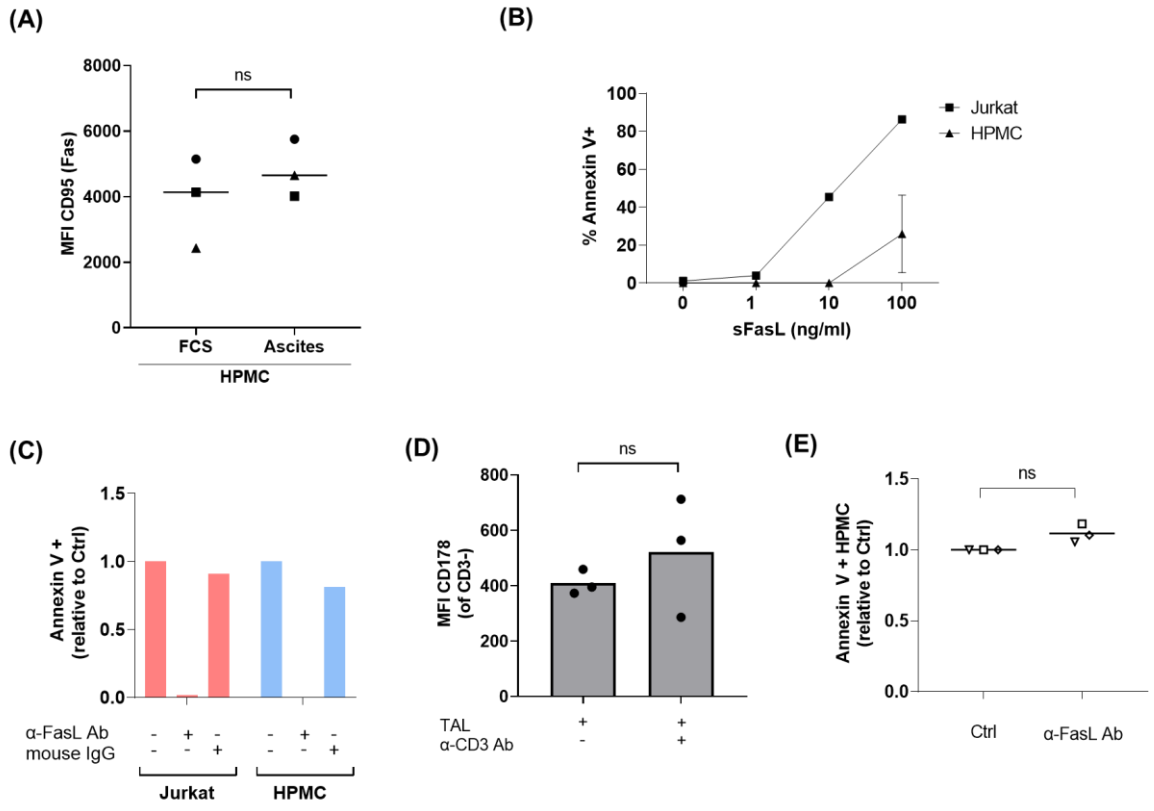

Figure S2. Fas / FasL- independent cell death in HPMC after co-culture with TAL, related to Fig. 2.

(A) Flow cytometric evaluation of Fas (CD95) expression in HPMC from HGSC patients cultured for 3 days jn OCMI/5% FCS (FCS) or OCMI/50% ascites pool (Ascites). The geometric MFI is shown and was calculated after subtracting the isotype control (n=3 patients).

(B) Sensitivity of HPMC against crosslinked sFasL compared to a Fas-sensitive cell line (Jurkat). After 24h incubation, apoptosis was measured by flow cytometry after Annexin V/ PI staining. Background apoptosis of untreated cells was subtracted (n=3 HPMC).

(C) The specificity of killing induced by crosslinked sFasL in HPMC and Jurkat cells was evaluated by adding blocking antibody α-FasL Ab. An irrelevant mouse IgG was included as control.

(D) The expression of FasL (CD178) on TAL (+/- stimulation with α-CD3 Ab) was assessed by flow cytometry. The geometric MFI is shown and was calculated after subtracting the isotype control (n=3 patients).

(E) TAL activated with α-CD3 Ab for 2 days were pretreated with blocking α-FasL Ab or a mouse IgG control (Ctrl) prior to the co-culture with HPMC (Ratio TAL:HPMC 10:1; n=3 patients). HPMC apoptosis was evaluated by Annexin V /PI staining and expressed relative to Ctrl.

Horizontal bars show the mean. p values were determined by two-sided, paired t-test (ns = non-significant, $p > 0.05$).

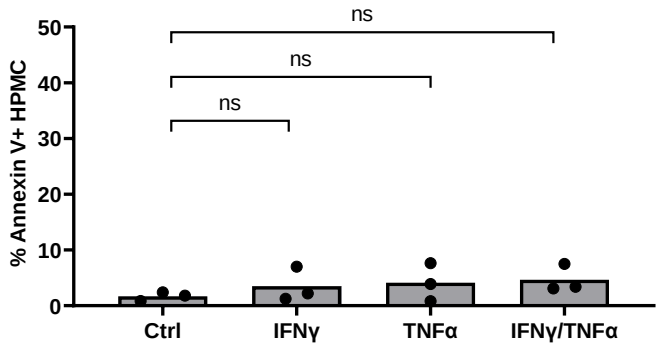

Figure S3. TNFα und IFNγ failed to induce cytotoxicity in HPMC, related to Fig. 2.

HPMC from HGSC patients were treated for 6 hours with IFNγ (20ng/ml), TNFα (100 ng/ml) alone or combined. Apoptosis induction in HPMC was determined by flow cytometric analysis after Annexin V / PI staining. Untreated cells were included as controls (Ctrl). The percentage of Annexin V + HPMC is given for n=3 patients. Horizontal bars show the mean. p values were determined by two-sided, paired t-test (ns= not significant).

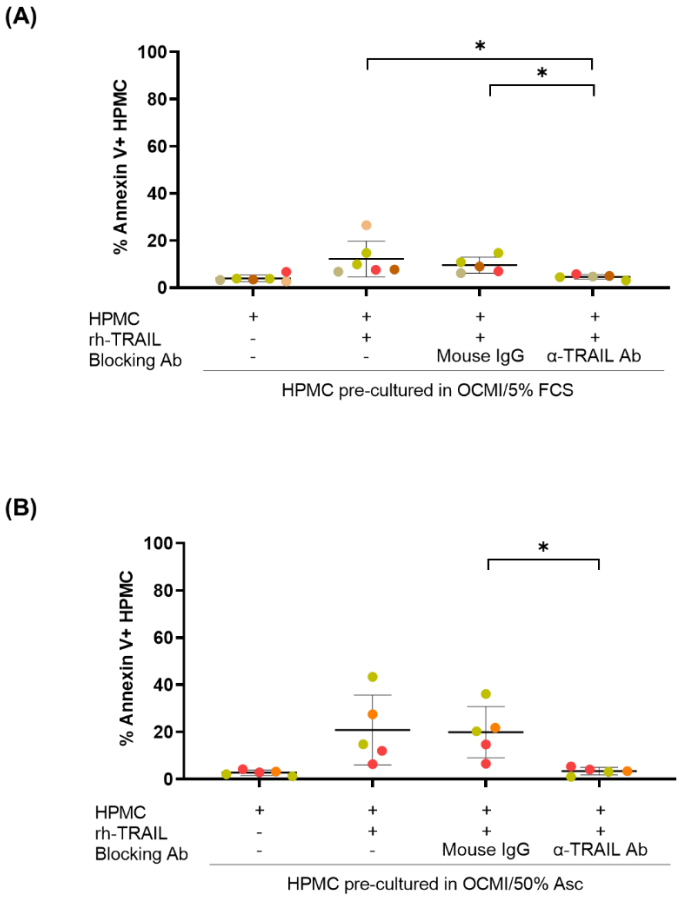

Figure S4. Impact of ascites on the sensitivity of HPMC to apoptosis induction by rh-TRAIL, related to Fig. 2.

(A, B) HPMC from HGSC patients were treated with rh-TRAIL for apoptosis induction as measured by flow cytometric Annexin V / PI staining. Where indicated, a blocking α-TRAIL blocking Ab or an irrelevant mouse IgG as blocking control were added prior to incubation with rh-TRAIL. Experiments were conducted with HPMC pre-cultured in OCMI/5% FCS media (n=6 replicates as indicated by different colors) (A) and in OCMI/50% ascites media (n=5 as indicated by different colors) (B). The mean is shown by horizontal bars and vertical error bars represent the standard deviation. * FDR < 0.05; determined by paired t test and Benjamini-Hochberg adjustment.

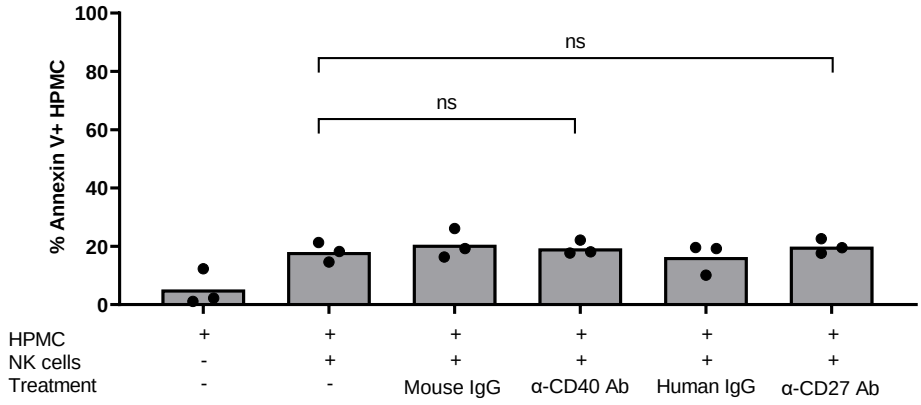

Figure S5. Impact of CD40 and CD27 signaling on activation of ascites-derived NK cells, related to Fig. 3.

NK cells were pre-treated with agonistic mouse α-CD40 and human α-CD27 Ab stimulation prior to HPMC co-culture. Irrelevant mouse and human IgG was included as controls. The amount of apoptotic HPMC (% Annexin V+ cells) after co-culture with NK-cells is presented (n=3 patients). The mean is shown by horizontal bars. p values were determined by two-sided, paired t-test and Benjamini-Hochberg adjustment (ns = non-significant).

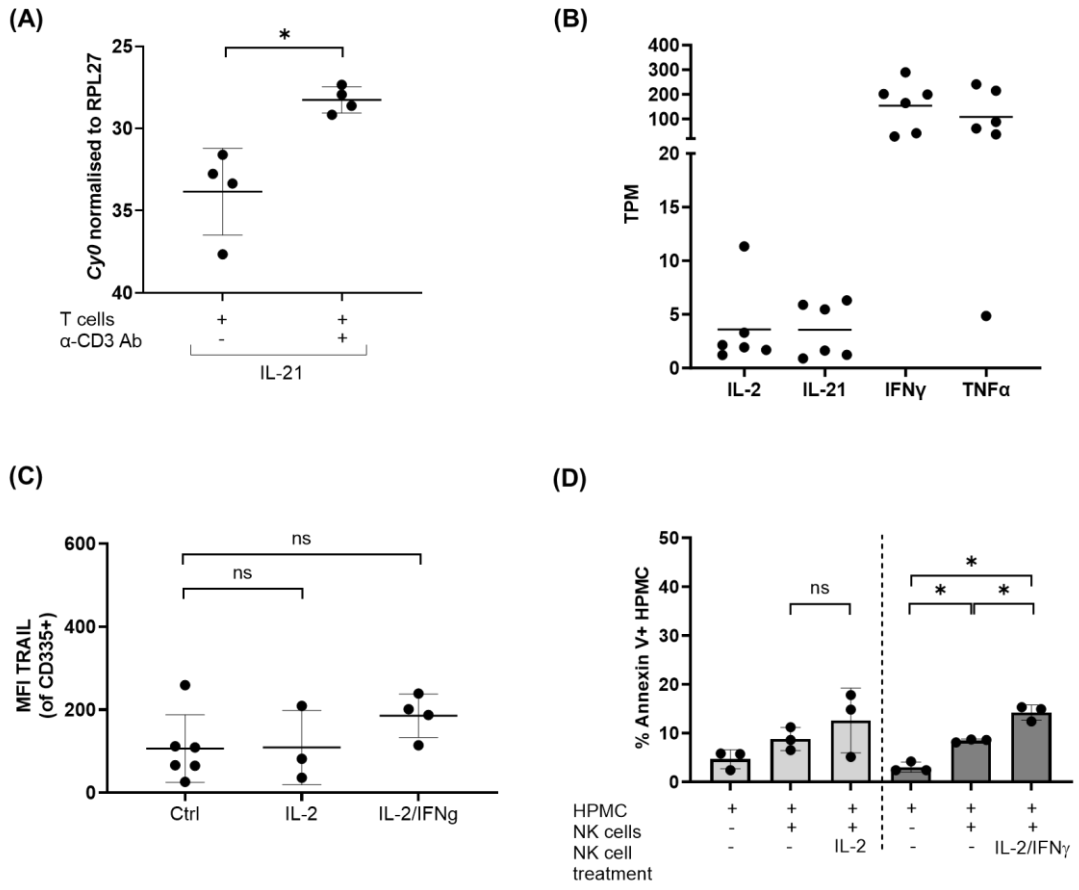

Figure S6. TRAIL regulation in NK cells by cytokines secreted by activated T cells, related to Fig. 3.

(A) Validation of IL-21 mRNA expression in ascites-derived T cells (+/- α-CD3 Ab stimulation) by RT-qPCR (n=4 patients).

(B) RNASeq data showing the TPM values of IL-2, IL21, IFNγ and TNFα gene expression in *ex vivo* TAT (n=6 patients). [S1]

(C) Flow cytometric evaluation of TRAIL expression on ascites-derived NK cells after stimulation with rh-IL-2 alone or combined with IFNγ. NK cells were left unstimulated as background control. The geometric MFI is shown and was calculated after subtracting the isotype control (n=3-4 patients).

(D) The cytotoxicity of NK cells after stimulation with rh-IL-2 +/- rh-IFNγ was determined after co-culture with HPMC. The amount of apoptotic HPMC (% Annexin V+ cells) is presented (n=3 patients).

The mean is shown by horizontal bars and vertical error bars represent the standard deviation. p values were determined by two-sided, paired t-test (ns = non-significant, *p<0.05). For multiple testing in panel D, * FDR < 0.05; determined by paired t test and Benjamini-Hochberg adjustment.

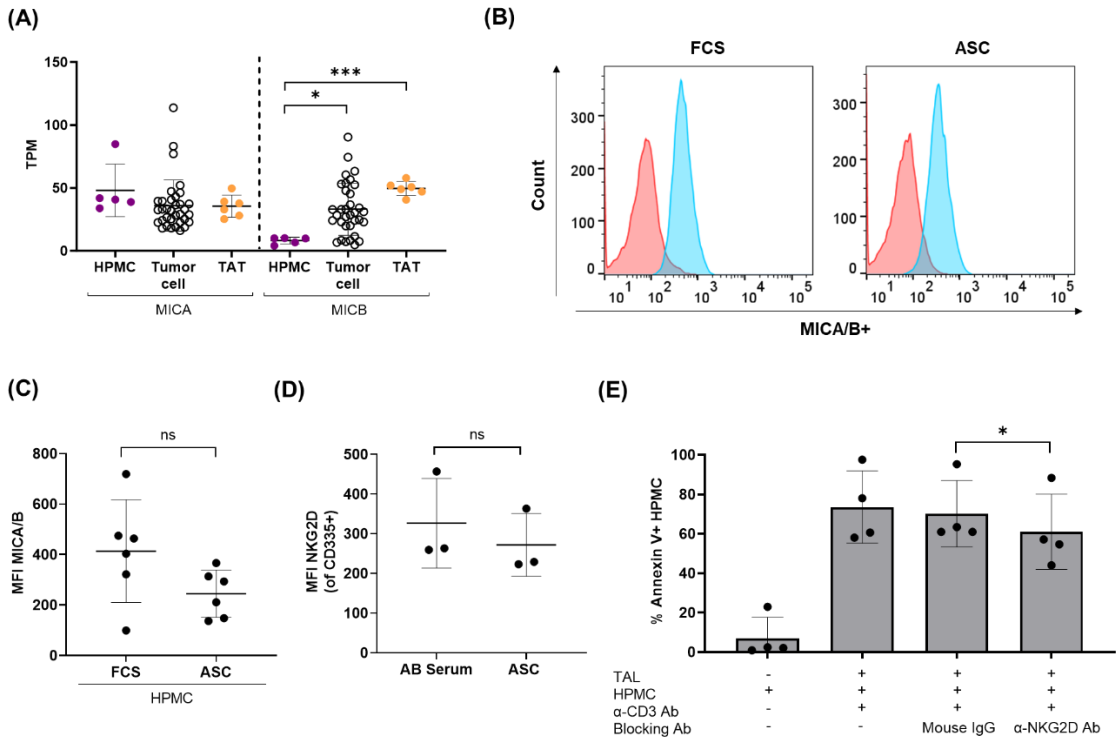

Figure S7. Impact of NKG2D-MICA/B crosstalk on NK cell-mediated HPMC apoptosis, related to Fig. 4.

(A) Expression of MICA and MICB genes in HPMC (from omentum), tumor cells and TAT (from ascites) from HGSC patients according to RNASeq data sets as described previously. [S1] TPM values are depicted from n= 5 HPMC, n=34 tumor cells and n=6 TAT of different patients.

(B, C) MICA/B expression in HPMC cultured either in OCMI/5% FCS (FCS) or OCMI/50% ascites (ASC) was validated on protein level by flow cytometry. **(B)** Representative histograms of MICA/B expression in HPMC. MICA/B expression is depicted in blue and the isotype control in red. **(C)** The geometric mean MFI of MICA/B expression (minus the isotype control) is shown for HPMC of n=6 patients.

(D) NKG2D expression on NK cells was determined by flow cytometry dependent on the culture in AB-serum or in ascites (n=3 patients).

(E) Apoptosis induction in HPMC by TAL (+/- α-CD3 Ab treatment) was measured by Annexin V staining of CD45- cells (n=4 patients). To evaluate the impact of NKG2D-based interaction on HPMC apoptosis, TAL were treated with α-NKG2D blocking Ab prior to HPMC co-culture. An irrelevant mouse IgG was included as control.

The mean is shown by horizontal bars bars and vertical error bars represent the standard deviation. p values were determined by two-sided, paired t-test (ns= non-significant, *p<0.05). For multiple testing in panel A, * FDR < 0.05; *** FDR < 0.001; determined by unpaired t test and Benjamini-Hochberg adjustment.

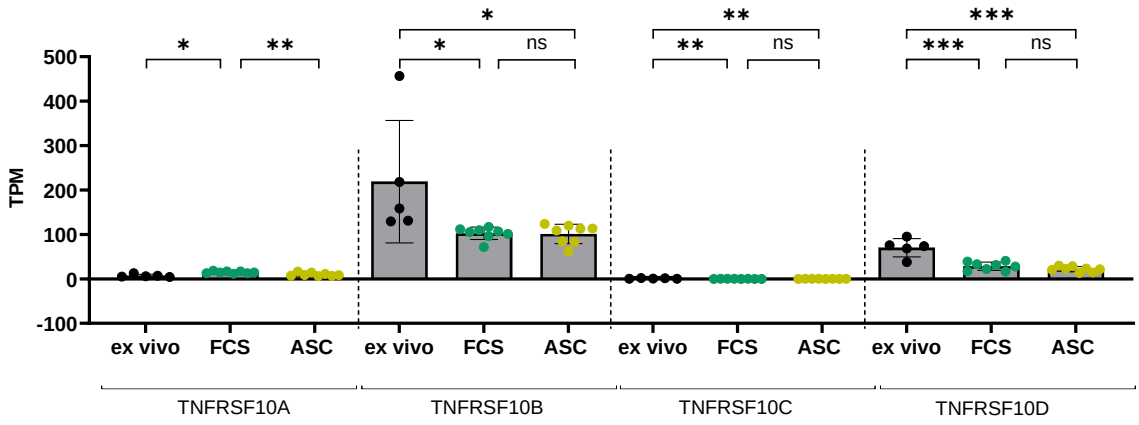

Figure S8. Expression of TRAIL receptor genes in *ex vivo* compared to cultured HPMC from HGSC patients, related to Fig. 4.

RNASeq data sets showing the TPM values of TNFRSF10A, TNFRSF10B, TNFRSF10C and TNFRSF10D gene expression are depicted for *ex vivo* HPMC (n=5 patients) and HPMC cultured in OCMI/5% FCS (FCS, n=8 patients) and in OCMI/50% ascites pool (ASC, n=8 patients). [S1] The mean is shown by horizontal bars and vertical error bars represent the standard deviation. * FDR < 0.05; ** FDR < 0.01; *** FDR < 0.001; determined by unpaired t test and Benjamini-Hochberg adjustment (ns = non-significant).

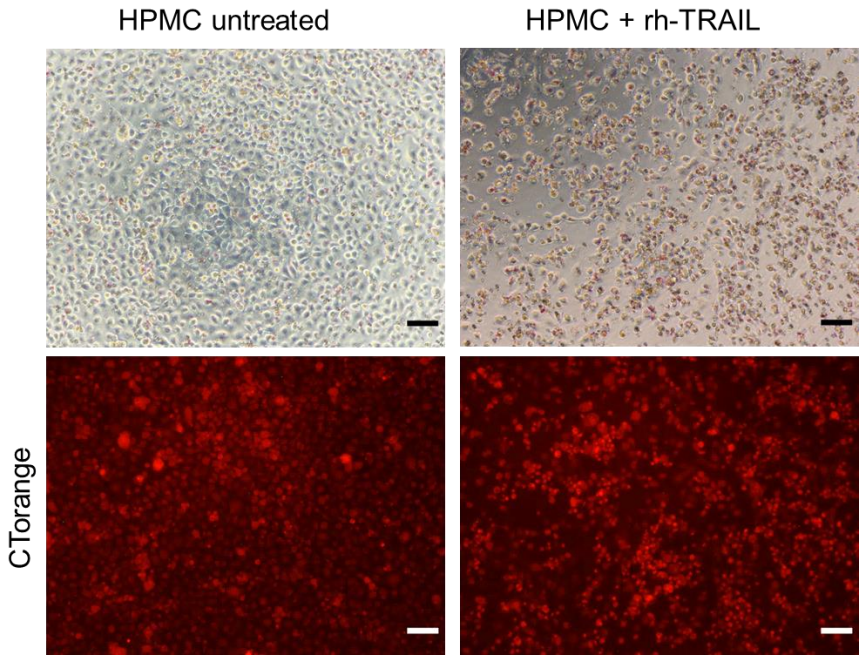

Figure S9. Microscopic pictures depicting the TRAIL-mediated clearance of the HPMC monolayer, related to Fig. 6.

HPMC stained with CTorange were grown to confluence on a collagen I gel overnight prior to treatment +/- rh-TRAIL. TRAIL-induced loss of HPMC is visible as holes in the monolayer and additionally detected under the fluorescence microscope as reduction of CTorange-labeled cells. Scale bars = 100µm

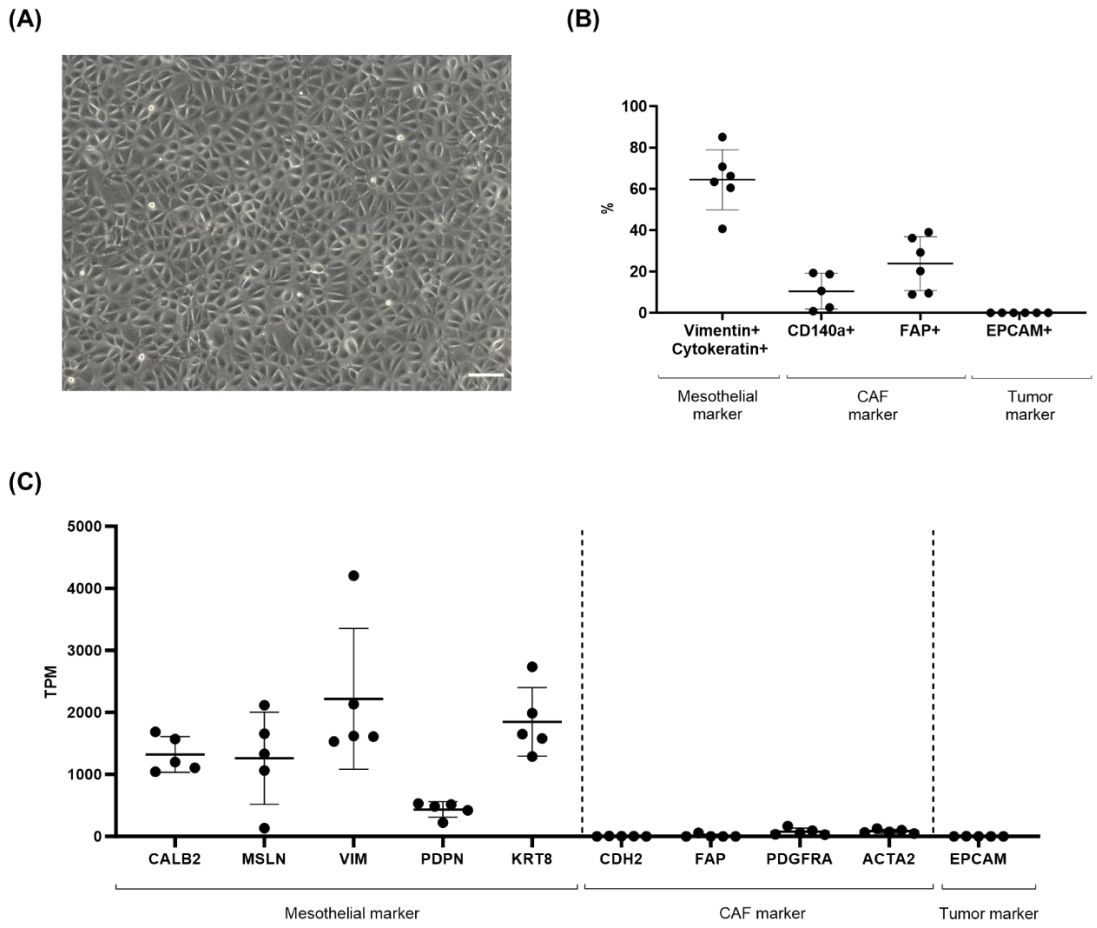

Figure S10.

Validation of mesothelial phenotype of HPMC, related to Fig. 1-6.

(A) Representative microscopic picture showing the cobblestone-like morphology of cultured HPMC. Scale bar = 100µm.

(B) Flow cytometric analysis of mesothelial marker (cytokeratin, vimentin), CAF-specific marker (FAP, CD140a) and tumor marker (EpCAM) in HPMC.

(C) RNASeq data sets showing the TPM values of mesothelial, CAF- and tumor-specific marker gene expression in HPMC after isolation from omentum (n=5 patients) [S1]. The mean is shown by horizontal bars and vertical error bars represent the standard deviation.

Supplemental References

S1. Sommerfeld L, Finkernagel F, Jansen JM, Wagner U, Nist A, Stiewe T, et al. The multicellular signalling network of ovarian cancer metastases. Clinical and Translational Medicine 2021. doi:10.1002/ctm2.633.