Supplementary Material: Interleukin-34 Enhances the Tumor Promoting Function of Colorectal Cancer-Associated Fibroblasts

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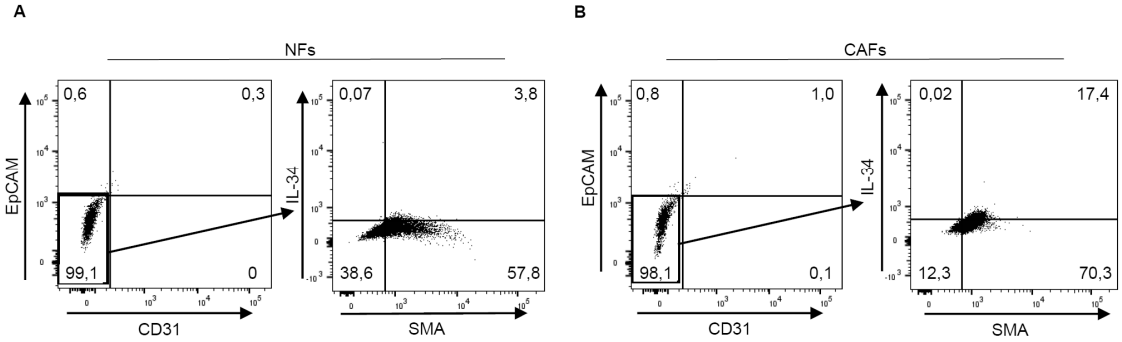

Figure S1. CAFs express interleukin-34 (IL-34). (**A**),(**B**). Left panels: representative dot-plots showing expression of CD31 and EpCAM in normal fibroblasts (NFs) (**A**) and cancer-associated fibroblasts (CAFs) (**B**) taken from tumoral and non-tumoral areas of one patient with colon cancer; right panels show expression of α-SMA and IL-34 in cells negative for CD31 and EpCAM. One of two experiments in which similar results were obtained is shown.

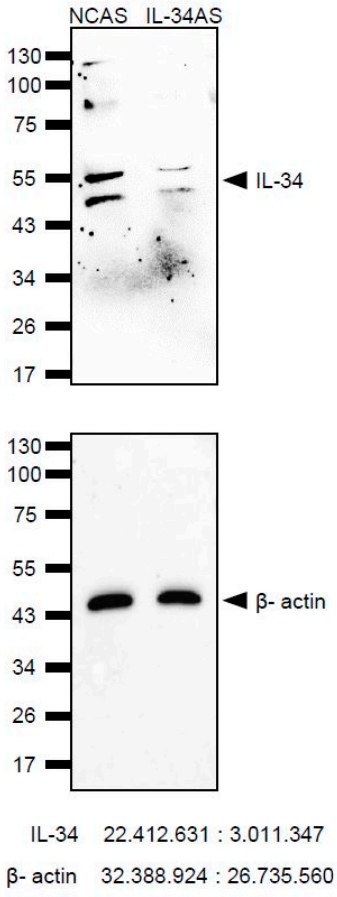

Figure S2. Uncropped Western Blot of Figure 3D.

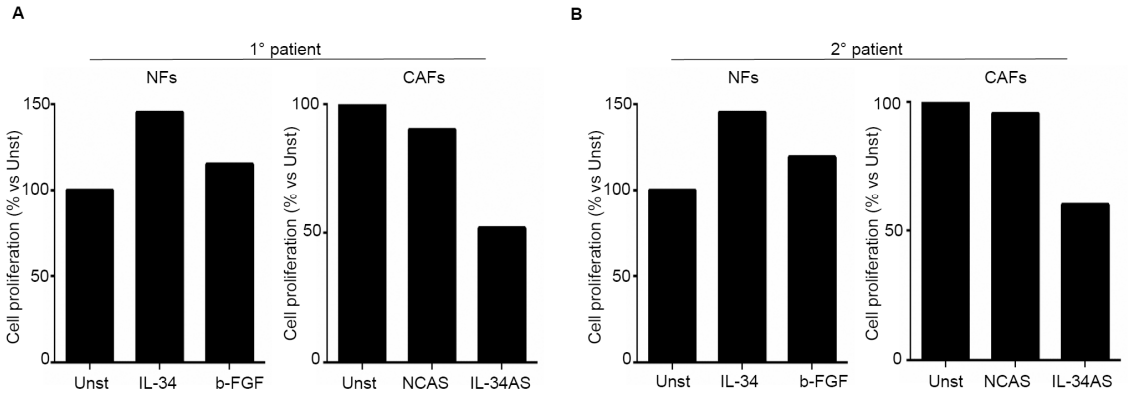

Figure S3. IL-34 enhances fibroblast proliferation. (**A**) 1°patient, (**B**), 2° patient. Left panels: serum-starved normal fibroblasts (NFs) were stimulated with IL-34 (50 ng/mL) or basic-fibroblast growth factor (b-FGF) (20 ng/mL) for 48 h. Right panels: cancer-associated fibroblasts (CAFs) were transfected with negative control antisense oligonucleotide (NCAS) or IL-34 antisense oligonucleotide (IL-34 AS) for 48 h. Cell proliferation was evaluated by 5-bromodeoxyuridine (BrdU) proliferation assay kit. Results of two experiments that analyzed cells of two patients are shown. Unst = unstimulated.

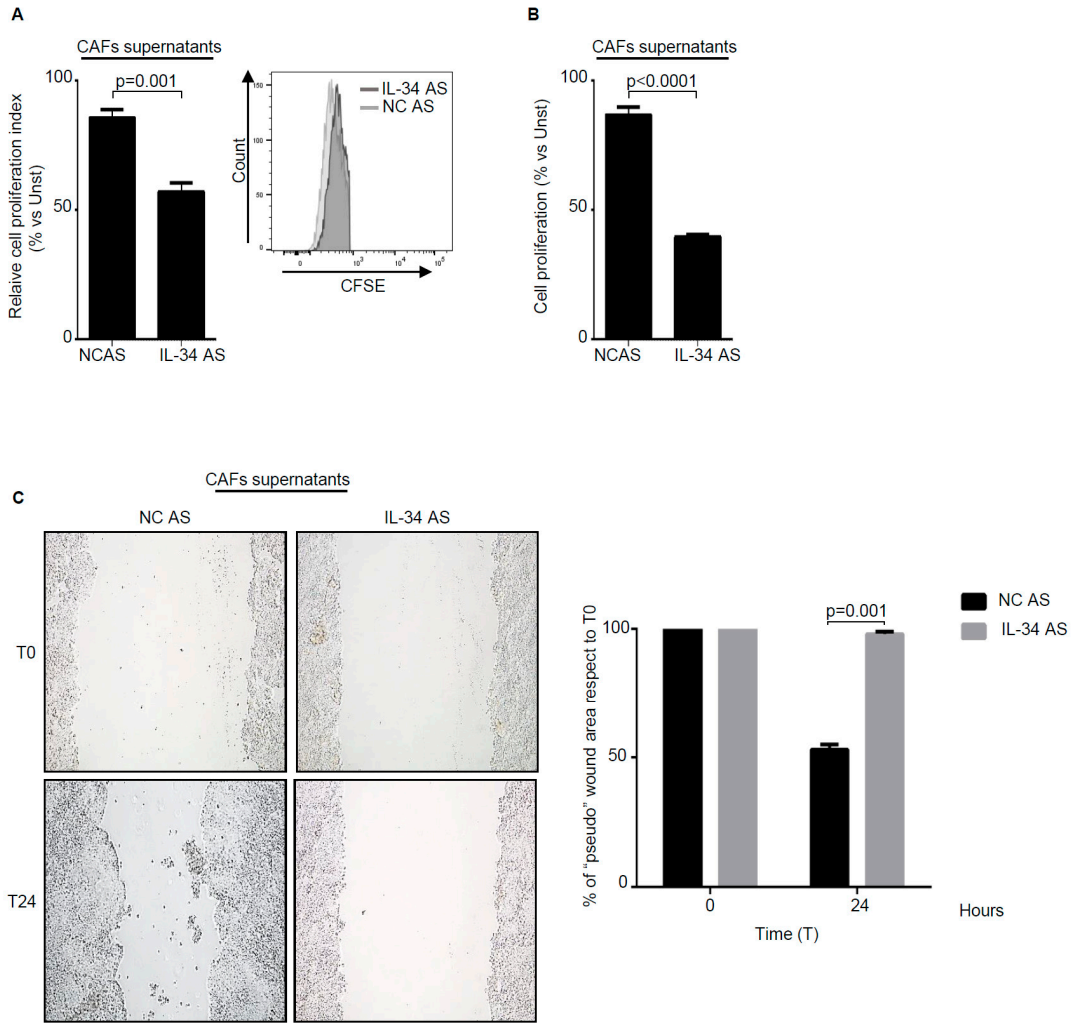

Figure S4. IL-34 produced by fibroblasts decreases HT-29 cell proliferation and migration. (**A**),(**B**) HT-29 cells were incubated with supernatants of CAFs previously transfected with either negative control

antisense oligonucleotide (NCAS) or IL-34 antisense oligonucleotide (IL-34 AS) for 24 h. HT-29 cell proliferation was evaluated after 48 h by flow cytometry, and proliferation index was calculated with Modfit LT (Verity Software House, Inc., Topsham, ME, USA) (**A**) or by a 5-bromodeoxyuridine (BrdU) proliferation assay kit (Roche Diagnostics, Monza, Italy) (**B**). Data indicate mean ± SEM of four independent experiments in which cells of four patients were analyzed. Right insets in (**A**): representative histograms showing the expression of CFSE in HT-29 analyzed by flow cytometry. (**C**). Representative images of "pseudo" wound in monolayer of HT-29 cells treated with supernatants of CAFs previously transfected with either negative control antisense oligonucleotide (NCAS) or IL-34 antisense oligonucleotide (IL-34 AS) for 24 h. Cells were photographed at the time of scratch (T0) and examined for cell migration after 24 h from the specific stimulation. Right panel shows the % of "pseudo" wound area in a monolayer of HT-29 cells at the specific time point (T) with respect to area in T0 (defined as 100%), and the data are expressed as mean ± SEM of three independent experiments in which cells of three patients were analyzed.

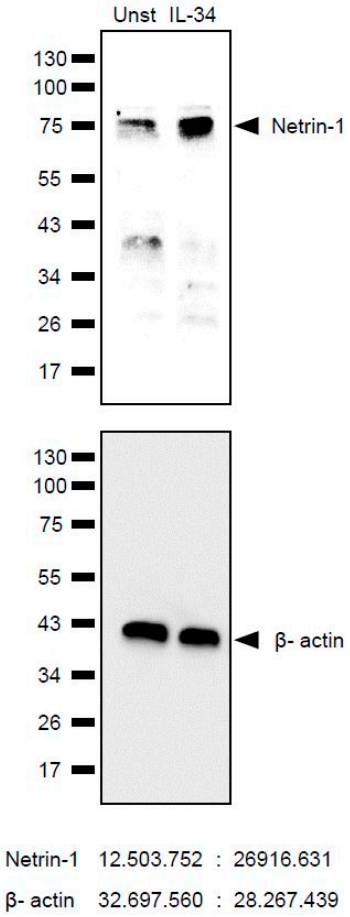

Figure S5. Uncropped Western Blot of Figure 7A.

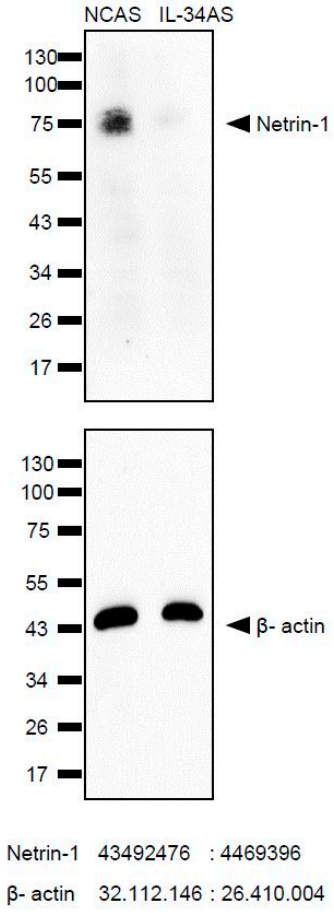

Figure S6 Uncropped Western Blot of Figure 7C.

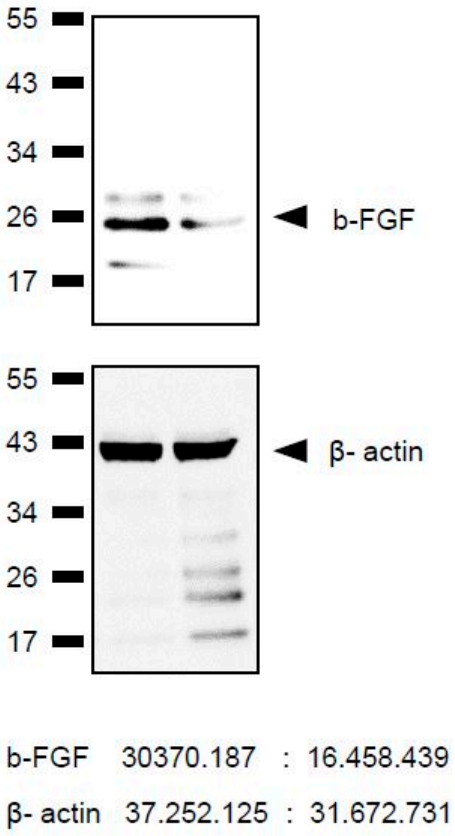

Figure S7. Uncropped Western Blot of Figure 7D.