Supplementary Fig. S1. A schematic diagram of the methods, datasets and patient samples used to identify and characterize the 10 genes associated with poor survival in ovarian cancer. (A) Identification of the 10-gene signature. (B) Validation of the 10-gene signature in predicting poor overall survival.
Supplementary Fig. S2. Correlogram of the 10 poor outcome signature genes. The main diagonal shows the gene name. At the horizontal and vertical intersection of each gene, the Pearson correlation coefficient (center, in black) and the associated P value (top right corner, in red) are shown.
Supplementary Fig. S3. Validation of the 10-gene signature in a dataset composed of 10 individual datasets. The Kaplan-Meier Plotter (http://kmplot.com/) was used to validate the predictive power of the 10-gene signature in a combined microarray of 10 datasets shown on the right. Affymetrix probe IDs of the 10 genes, including 201792_at (AEBP1), 204320_at (COL11A), 203325_s_at (COL5A1), 213290_at (COL6A2), 215446_s_at (LOX), 210809_s_at (POSTN), 213139_at (SNAI2), 203083_at (THBS2), 201147_s_at (TIMP3), 204619_s_at (VCAN), were uploaded to the website program. The mean expression of all 10 genes was analyzed for overall survival of patients with serous histology (n=1,058). Patient samples were randomly split by upper tertile. The Kaplan-Meier plot shows the overall survival in two patient groups with ‘high’ (red) and ‘low’ (black) expression of the 10-gene signature.
Supplementary Fig. S4. ABI Open Array Real-time PCR of 9 poor outcome signature genes (*COL6A2 was not available on the array) in normal (N, n=8), primary (P, n=30), and metastatic (M, n=29) ovarian cancer samples. Each dot represents an individual patient sample. Bars indicate the mean +/- SEM. * indicates statistical significance (P<0.05).
**Supplementary Fig. S5.** Increase in *COL11A1* expression in recurrent tumors in comparison to primary ovarian tumors. Quantification of the *COL11A1 in situ* hybridization signal in matched pairs of primary ovarian cancer and recurrent/persistent metastasis from eight patients. H score = % positive stromal cells x intensity (0, 1+, 2+, 3+) under 10X objective. Each point represents the H score in a single field. Nine intratumoral fields were scored in each sample except for two samples in which fewer fields were scored due to minimal amount of tumor tissue. Data are presented as the mean +/- SEM.
Supplementary Fig. S6. Confinement of COL11A1 expression to intra- and peri-tumoral stroma. Representative low magnification image of COL11A1 in situ hybridization in a metastatic tumor nodule. Four different fields of the tumor nodule are shown at a higher magnification. Dotted lines demarcate tumor epithelium (E) and peritumoral stroma (S). N denotes a necrotic area. The counter stain is hematoxylin. Size bars, 1 mm (center image) and 100 µm (peripheral images).
Supplementary Fig. S7. Knockdown of COL11A1 in the A2780 ovarian cancer cell line. (A) Real-time PCR of COL11A1 in A2780 cells transduced with scrambled control shRNA (sh-scr) and A2780 cells transduced with five different shRNAs targeting COL11A1 (sh1-sh5). (B) Western blot analysis of COL11A1 protein expression. The membrane was exposed for different lengths of time to visualize the molecular marker and the COL11A1 band. GAPDH was used as a loading control. Thirty micrograms of cell lysates were loaded onto a mini-PROTEAN TGX gradient gel (4-20%). Protein was transferred to a 0.2 µm PVDF nitrocellulose membrane, which was incubated in blocking buffer for 1 hour and then incubated with primary antibodies against COL11A1 (Abcam ab64883; 1:500 dilution) and GAPDH (Fitzgerald 10R-G109A; 1:5000 dilution) for 1 hour at room temperature. Membranes were incubated with secondary antibodies (goat anti-rabbit IRDye 800 for COL11A1 and goat anti-mouse IRDye 680 for GAPDH; 1:5000 dilution) for 1 hour at room temperature. The signal was analyzed by the Li-Cor Odyssey system.