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Supplemental Information

PAI-1-Dependent Inactivation of SMAD4-Modulated Junction and Adhesion Complex in Obese Endometrial Cancer

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Table S1: Patients information for tissue microarrays from endometrial tumors. Related to Figure 1.

Age	27-50 (mean=41.5, total, n=35)
Histology	
	Endometrioid (94%)
	Clear cell (3%)
	Carcinosarcoma (3%)
FIGO Stage	
	I (86%)
	II (6%)
	III & IV (8%)
BMI	
	20-29 (20%)
	30-39 (37%)
	≥ 40 (43%)

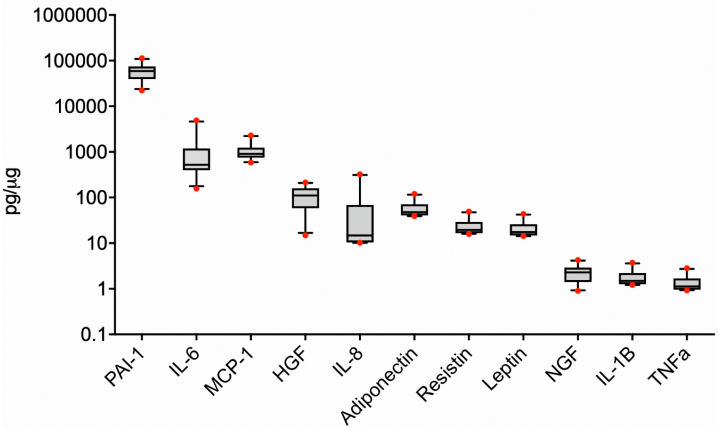

Figure S1: Abundant PAI-1 in conditioned media of adipose stromal cells. Related to Figure 1.

Conditioned media of 10 adipose stromal cell (ASC) lines were examined by FlexMap multiplexing assays for a panel of adipokines and cytokines. Secreted protein levels (pg), normalized to total proteins (µg) of cells in culture at the time of harvest, show about two orders of magnitude more abundance of PAI-1 compared to the other adipokines/cytokines.

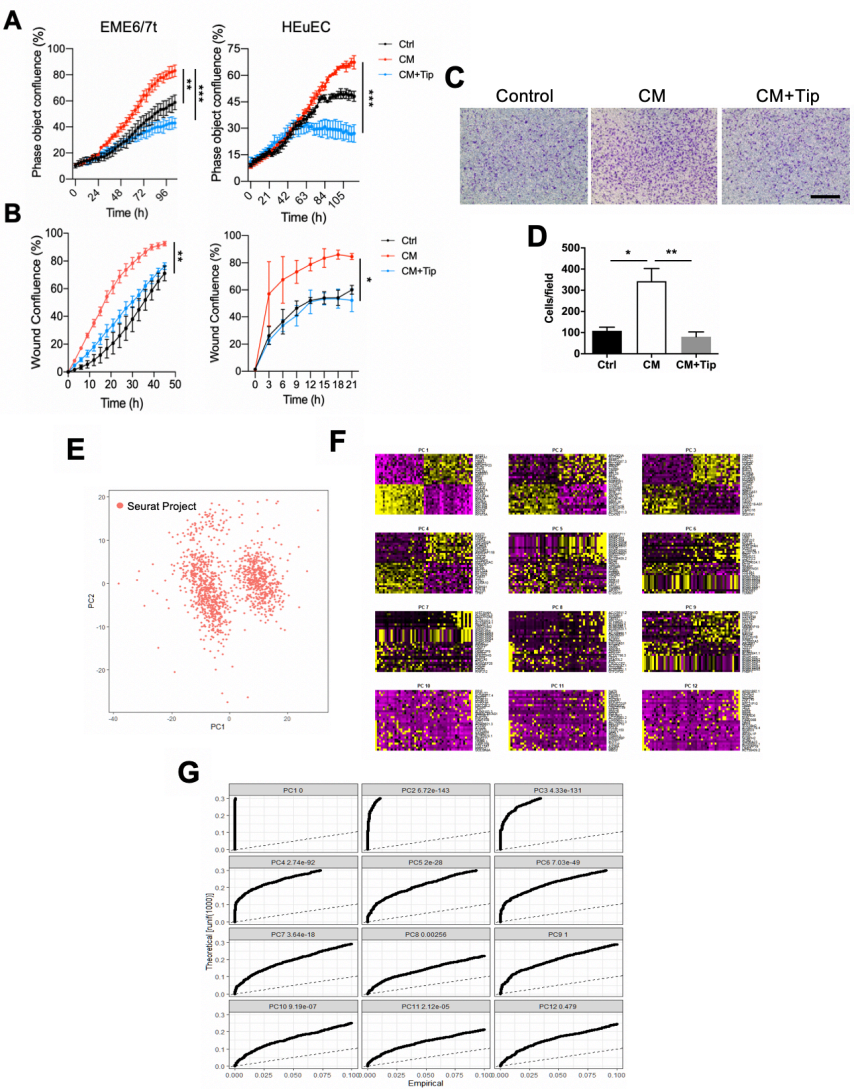

Figure S2: PAI-1-initiated pro-tumorigenic phenotype in endometrial epithelial cells. Related to Figure 2. Immortalized EME6/7t endometrial epithelial cells (EECs) and an additional source of un-immortalized HEuEC primary cells were exposed to ASC-CM in the presence or absence of PAI-1 antagonist, Tip. Proliferation (**A**) and migration (**B**) rates in quadruplicate were determined using a live-cell imaging system IncuCyte. (**C-D**) Invasion assay of EME6/7t cells exposed to ASC-CM and CM+Tip, showing significantly enhanced invasive potential of EME6/7t cells after CM exposure, which was reversed by Tip. Scale: 500 µm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (**E**) Principal component analysis (PCA) utilizing the most variable genes. (**F**) Heatmaps showing the genes and cells ordered by PCA scores and used to select principal components. Yellow indicating high gene expression, and purple indicating low expression. (**G**) The JackStraw plots displaying p-values for each principal component. Dashed line, uniform distribution; Solid curve, the significant principal component.

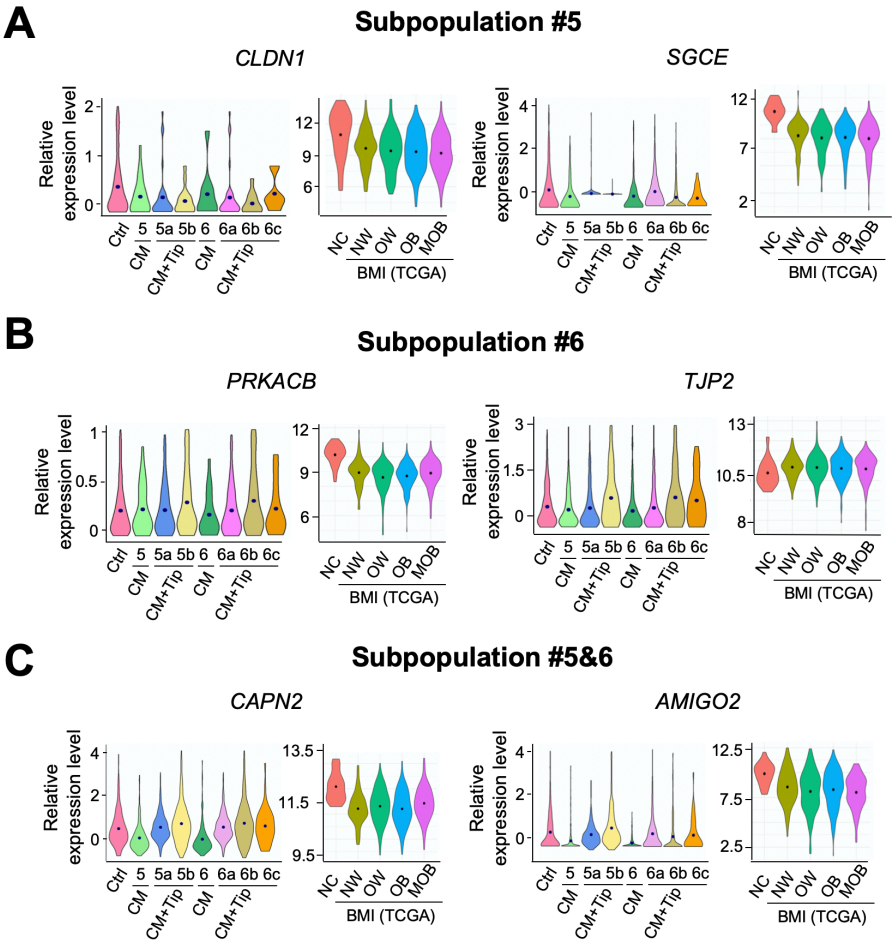

Figure S3: Representative BMI-associated JAC genes were regulated both in EEC subpopulations #5 and 6 after exposure to ASC-CM. Related to Figure 3.

Violin plots showing expression changes of (**A**) BMI-associated JAC genes corresponding to subpopulation #5, (**B**) BMI-associated JAC genes corresponding to subpopulation #6, (**C**) BMI-associated JAC genes that correspond to both subpopulations #5 and 6. The expression of genes in immortalized endometrial epithelial cells EME6/7t with or without exposure to ASC-CM (-/+ tiplaxtinin, Tip; plots on left) and in TCGA endometrial cancer cohort stratified by obesity status (plots on right). NC, non-cancer control; and cancer patients: NW, normal weight; OW, overweight; Ob, obese; MOB, morbidly obese.

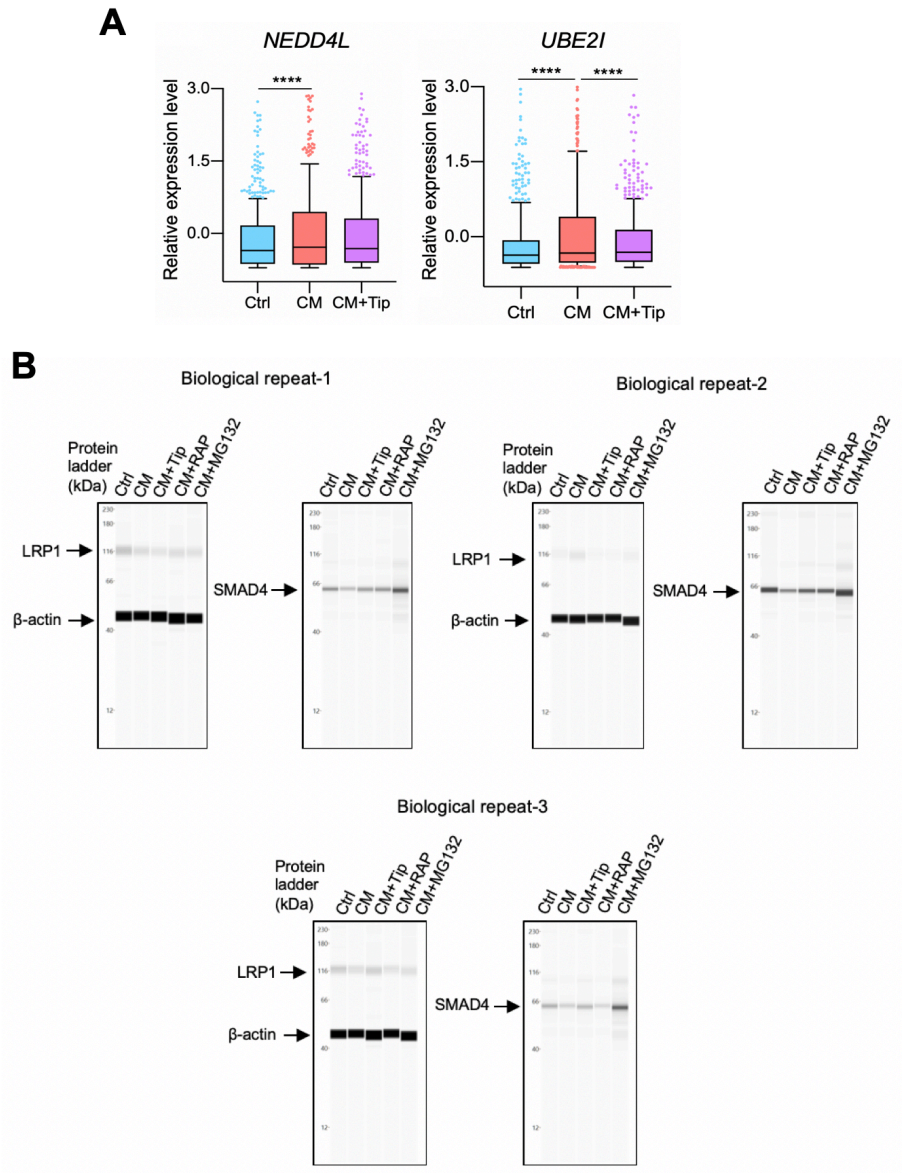

Figure S4: PAI-1 upregulates *NEDD4L* and *UBE2I* genes and suppresses SMAD4 protein expression but not LRP1 in endometrial epithelial cells. Related to Figure 4.

(**A**) Single-cell RNA-seq analysis reveals increased expression levels of *NEDD4L* and *UBE2I* in immortalized endometrial epithelial cells EME6/7t exposed to ASC-CM relative to cells additionally treated with a PAI-1 antagonist (Tip) or control EECs. ****$p < 0.0001$. (**B**) Capillary Western immunoassays showing protein expression levels of LRP1 and SMAD4 of EME6/7t cells in three independent experiments (three biological replicates).

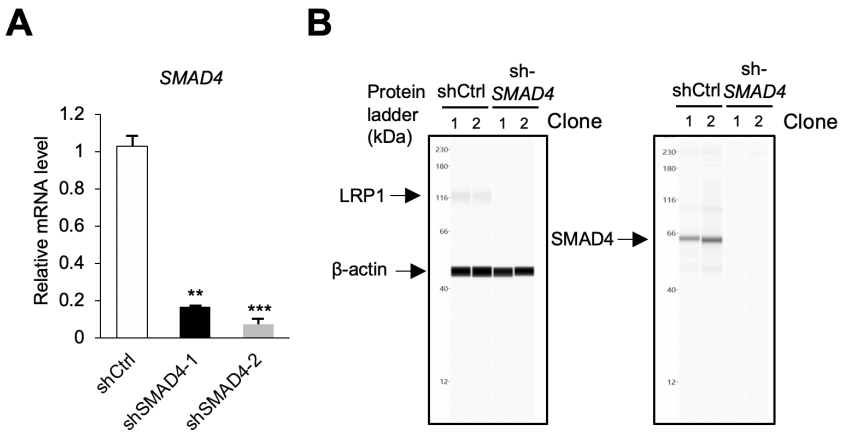

Figure S5: Expression levels of SMAD4 and LRP1 in two *SMAD4* table knockdown clones.

Related to Figure 5.

(**A**) Bar plots showing efficient *SMAD4* knockdown in EME6/7t cells in the two stable sh*SMAD4* clones relative to control shCtrl clone. **p < 0.01, ***p < 0.001. (**B**) Capillary Western immunoassays showing protein expression levels of LRP1 and SMAD4 in the two stable shCtrl clones or two stable sh*SMAD4* clones of EME6/7t cells.

Table S2: Patients information for CyTOF analysis. Related to Figure 6.

Case#	Age	BMI	Ethnicity	FIGO Stage	FIGO Grade	Histology	Type
P07	61	20.5	African American	IB	3	Papillary serous	2
P08	56	21.7	non-Hispanic, white	IIIC2	3	Papillary serous	2
P09	72	28.0	Hispanic	IA	1	Endometrioid	1
P14	35	30.0	Hispanic	II	3	Endometrioid	1
P06	43	34.1	Hispanic	IA	1	Endometrioid	1
P15	56	34.7	non-Hispanic, white	IA	2	Endometrioid	1
P03	31	41.6	Hispanic	IA	1	Endometrioid	1
P10	53	48.1	Hispanic	IA	1	Endometrioid	1
P11	29	55.0	Hispanic	IB	1	Endometrioid	1

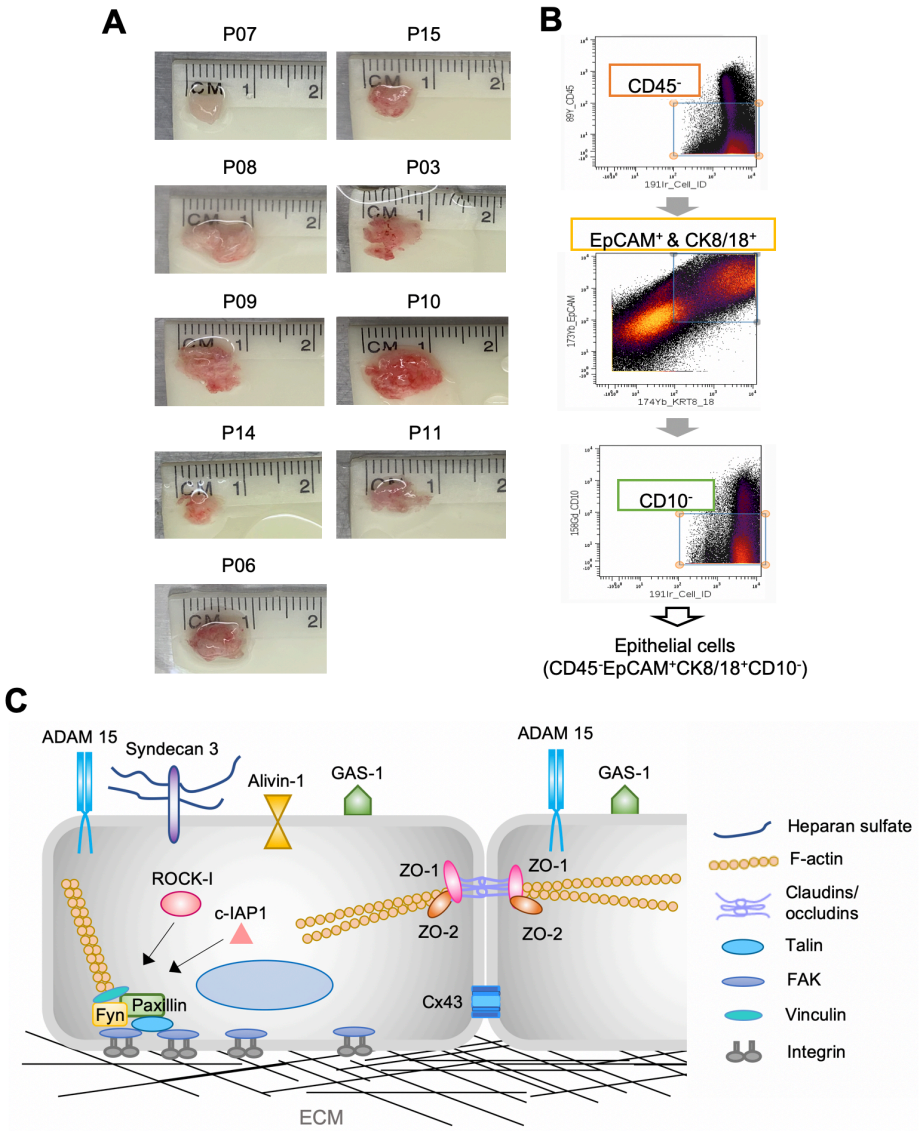

Figure S6: Isolating epithelial tumor cells from tissue of endometrial cancer patients and a schematic overview of JAC functions in epithelial cells. Related to Figure 6.

(**A**) Tumor appearance from endometrial cancer patients. (**B**) CyTOF gating strategies to analyze epithelial cells from endometrial tumors. (**C**) Apical surface protein, growth arrest-specific 1 (Gas1), apical junction (ADAM15, syndecan 3 (SDC3), and AMIGO2), and tight junction (zonula occludens (ZO)1 and ZO2) maintain epithelial polarity. Gap junction (connexin 43, Cx43) connects cell to cell and mediates intercellular communication. Rho-associated protein kinase 1 (ROCK1) and cellular inhibitor of apoptosis protein 1 (cIAP1) are focal adhesion regulators. Paxillin and Fyn associated with integrins adhere to the extracellular matrix (ECM).

Table S3: CyTOF antibody panel. Related to Figure 6.

Protein name	Gene name	Clone	Metal	Cellular functions
Gas1	*GAS1*		166Er	Cooperate with hedgehog signals
AMIGO2	*AMIGO2*	30G9.1G2	143Nd	Decrease cell adhesion/migration
SDC3	*SDC3*		141Pr	Cooperate with hedgehog signals
ADAM15	*ADAM15*	23G9	154Sm	Bind to αvβ3 and α5β1 integrin to mediate cell motility
Cx43	*GJA1*		162Dy	Assemble to gap-junction intercellular communication channels
ZO1	*TJP1*	1A12	169Tm	Interaction with F-actin and occludin in tight junction complexes
ZO2	*TJP2*	3E8D9	146Nd	Interaction with F-actin and occludin in tight junction complexes
ROCK1	*ROCK1*	EP786Y	161Dy	Cooperate with zonula adherens organization, RhoA signaling, and cortical tension
Paxillin	*PXN*	Y113	160Gd	As a docking protein to recruit signaling proteins to focal adhesions and coordinate downstream signaling
Fyn	*FYN*		153Eu	Associate with Src and focal adhesion kinase (FAK) to regulate cell migration
cIAP1	*BIRC2*		167Er	Control Rho GTPases signaling for focal adhesion formation
TGFR-2	*TGFBR2*		165HO	The ligand-binding receptor for TGFβ signaling
SMAD2	*SMAD2*	31H15L4	152Sm	Form complex with SMAD3 or/and SMAD4 to induce TGFβ signaling
SMAD4	*SMAD4*	253343	164Dy	A central mediator of TGFβ signaling and induces a tumor suppressive effect
CK 8/18	*KRT 8/18*	C51	174Yb	Cytoskeletal proteins and be induced by endometrial cancer invasion
EpCAM	*EPCAM*		173Yb	As an epithelial tumor cell marker and associate with epithelial proliferation

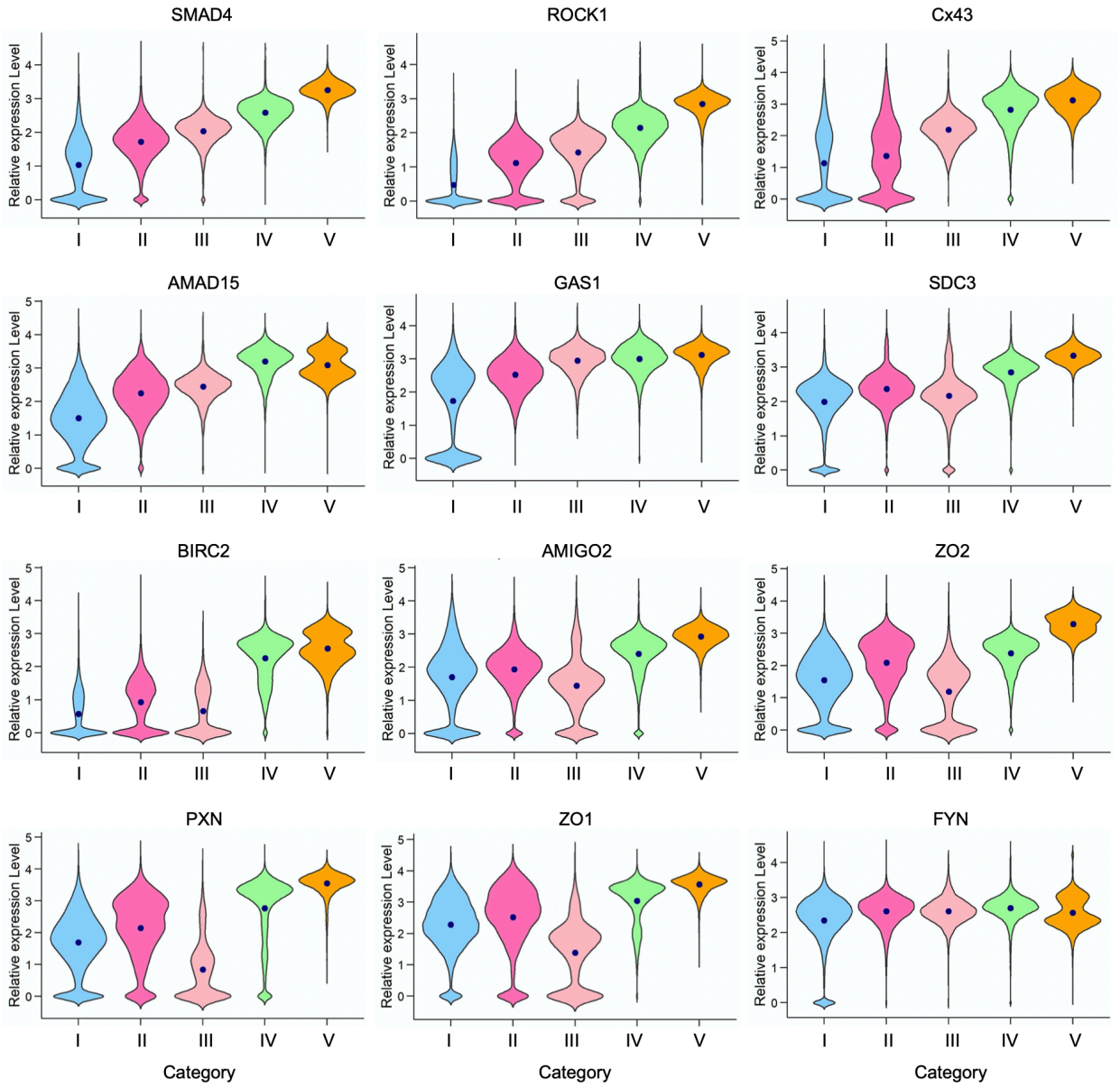

Figure S7: Violin plots showing expression patterns of SMAD4 and 11 proteins associated with junction and adhesion complex (JAC) within the five categories. Related to Figure 7.

Table S4: Primer sequences for ChIP-qPCR. Related to STAR Methods.

Gene	Sequence 5'	Sequence 3'
SMAD4 #1	GTGGCGCCTGTGATTTGCTTCT	CCTTGCCATGTGGTGCTCTCC
SMAD4 #2	CTTTGTTCCAGCCTCACTT	TAGTTACCTATTGCTGTATAACAATCTC
AMIGO2 #1	CCAACTCTTCCTCCCCT	TAACAAATCTGCCTGACCACA
AMIGO2 #2	TAATAATAATCCCAGAGCAGACCT	GGAAATGAGAGAGAGAGAACG
SDC3	TGCCAAAACAAGGGAGTC	GTGTGTCATTTCAAGGGTCTA
PXN	CACAGCCACCCTCCCTA	TGTTATTATTACCCAATTTCCGAGT
ADAM15	ATGACACACAATAGGGGC	TTCACATTCAGTTTGCTTCACGA
BIRC2	TGGAGAGGGCACAAACC	CAACTGGACGCTCATCG
FYN #1	GGTTCTGATTTAGGGGGTTT	CTCAAGGTGCTGTCTCCA
FYN #2	AGCAAAAGTCTGGAGGAAG	CGAGGTTTTTTGGGGGT
GAS1 #1	AGCTTCCCACGGCAGACCAA	GGAGGCCCAGCACTAGGAGATTC
GAS1 #2	CGTGCCCATTTATTCCGTCTTCT	GCGCCAGGCATCGTTTTCA
GJA1	CCAAGTAGAGGCGTTCA	GGCTGGTAAATGTGGAAGG
ROCK1	GAGAGGTCCAAAGTCCAG	GTTCGTTCATTCATTCATTGTGT
TJP2	TTCGACCCCCGCCTGATTC	CCGCAACCCGCCCTGAG
TJP1	GGTCTCGAACTCCTGGGCTCAT	CAGTCCTCGTTGCACGGTAAT

Table S5: Primer sequences for real-time qPCR. Related to STAR Methods.

Gene	Sequence 5'	Sequence 3'
AMIGO2	TGCTGGTCTTTTGGTATCGTA	GCACGAAAGGAACCATTGA
BIRC2	GGTTTTTATTATGTGGGTCGC	GCTGTTCAAGAAGATGAGGA
SDC3	GGTCACACTGCTCATCTATC	GAACTCCTCCTGCTTGTC
PXN	CACCCAACAGCAGACAC	TTGAAATCCGACAGCGAAG
FYN	CCCAACTACAACAACTTCCAC	GCAAGGTCCCCGTATGAGA
TJP1	ACGGACCAGTTTTCTCG	TGGGTAGGGCTGTTTGT
GAS1	CCGCTACCTGACCTACT	TGTTCTCCTTGACCGACT
GJA1	GGTGACTGGAGCGCCTTAG	GCGCACATGAGAGATTGGGA
ROCK1	ATGAAGATGAATAAGGAAGGCA	AAATACCCCAACCGACC
TJP2	GGGAAGGTCGCTGCTATTGT	CTCTCGCTGTAGCCACTCC
ADAM15	GAGGGACACACTTTGGAGAA	GACCACCAAGCCTCTGA
SMAD4	CTCATGTGATCTATGCCCGTC	AGGTGATACAACTCGTTCGTAGT