SUPPLEMENTAL INFORMATION FOR

LKB1 loss promotes endometrial cancer progression via CCL2-dependent recruitment of macrophages

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Figures S1-6, Tables S2-S3, and Legends

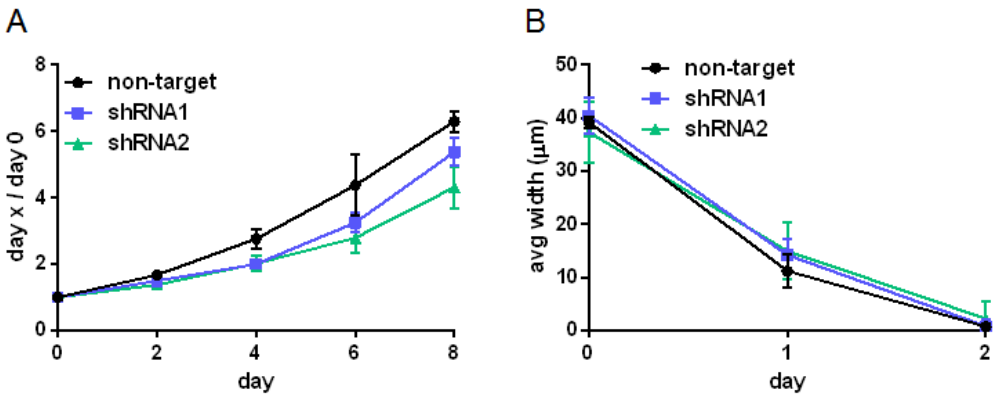

Figure S1. LKB1 knockdown does not affect growth rate or migration of EM cells. A) Growth curve of isogenic EM cells over an 8 day period. Values were obtained by calculating mean intensity of crystal violet staining per day normalized to day 0. B) Wound healing assay showing width of wound (µm) over time. Error bars=S.E.M.

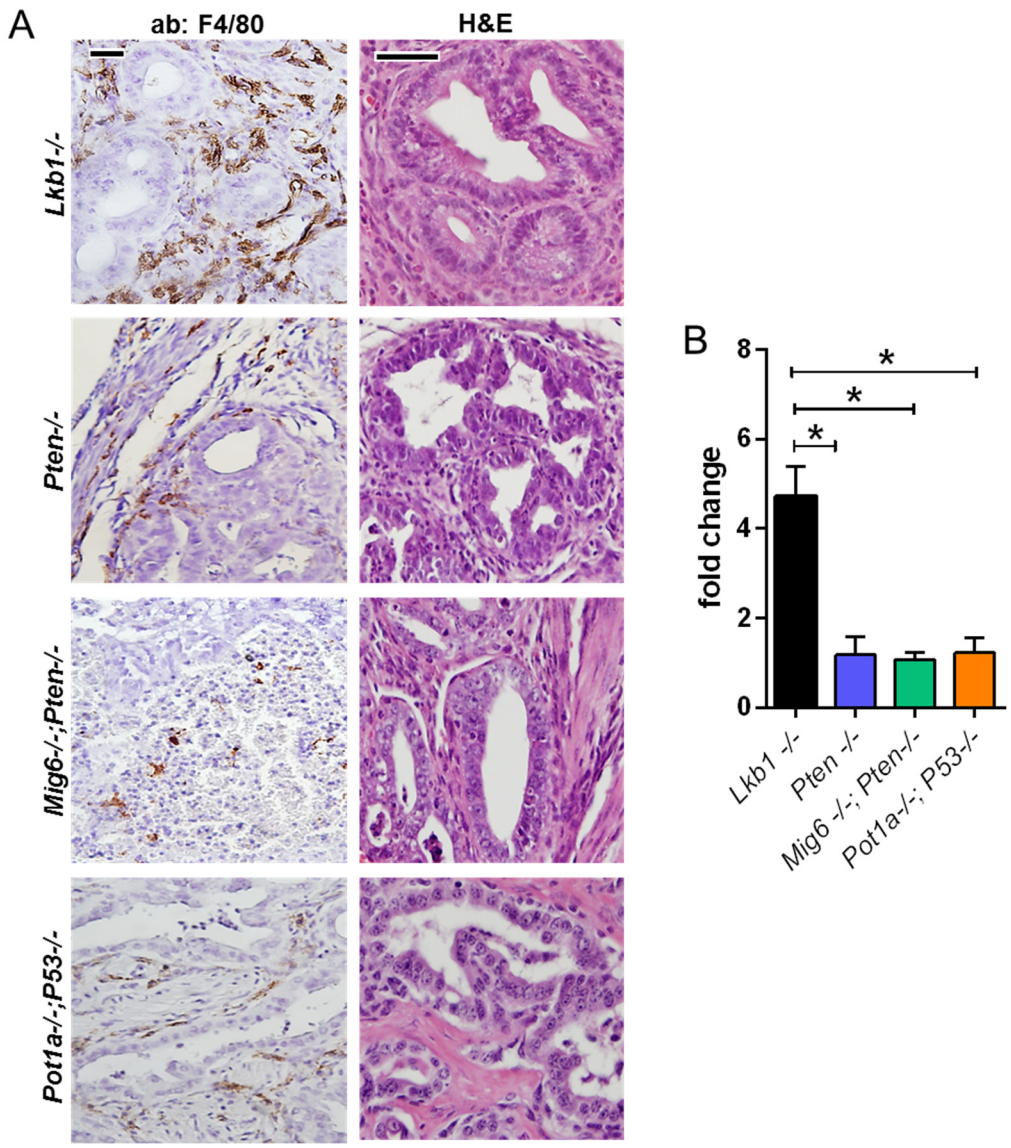

Figure S2. Macrophage infiltration distinguishes *Lkb1*-based from other murine endometrial cancer models. A) Representative F4/80 immunostaining of four murine endometrial cancer models showing greatest macrophage density in 12 week *Lkb1*$^{-/-}$ tumors, with H&E stains showing invasive tumor for each model. B) Fold change in F4/80+ cells between each cancer model and respective sibling controls (n=4 per experimental group and sibling controls for every model analyzed). *Lkb1*$^{-/-}$ mice displayed the greatest fold change in macrophage density (*P<0.01) per student's t test. Bars=50 µm; panels in the same column are all at the same magnification. Error bars=S.E.M.

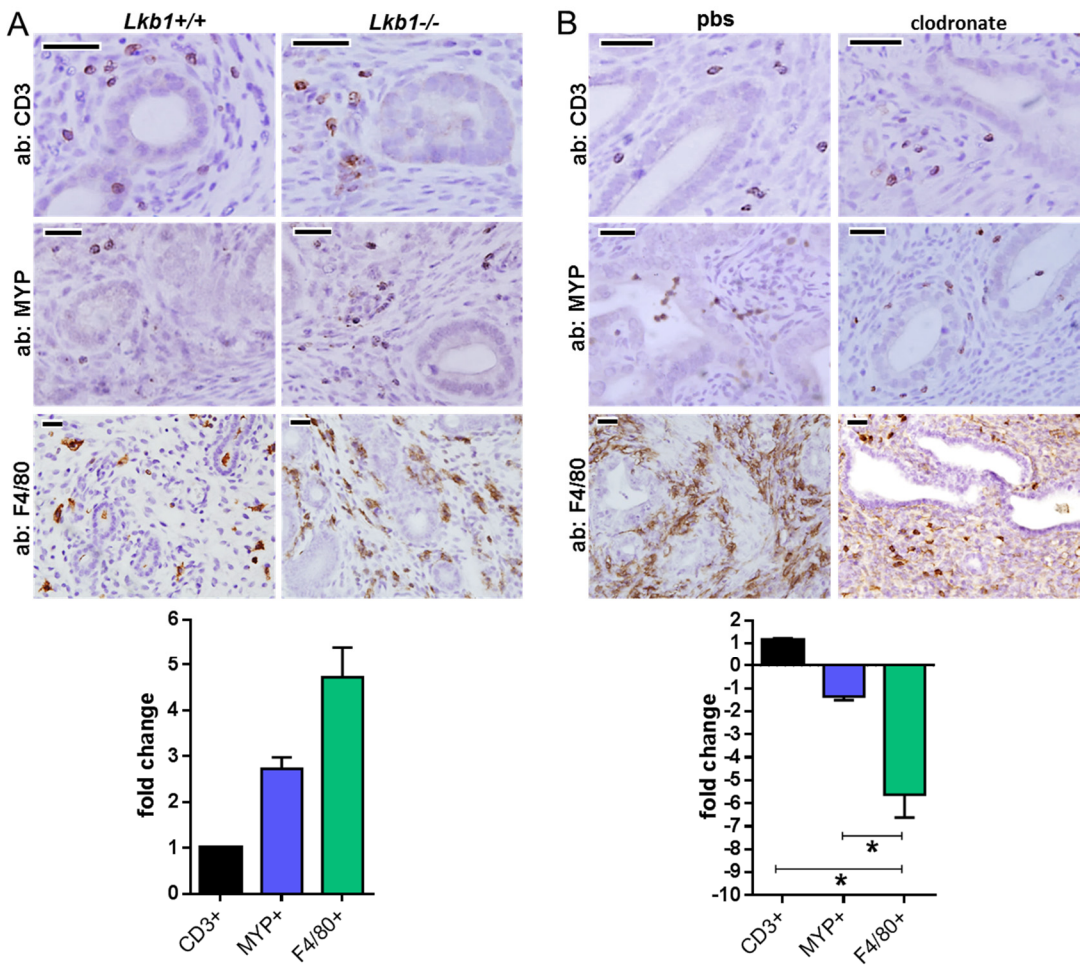

Figure S3. Quantitation of leukocyte infiltrates in *Lkb1*$^{-/-}$ tumors. A) Immunostaining of tumors with mature lymphocyte marker CD3, neutrophil marker myeloperoxidase (MYP), and macrophage marker F4/80 in 12 week-old animals. Graph shows quantitation of lymphocytes, neutrophils, and macrophages in uterine tissue sections normalized to sibling controls (n=4 for *Lkb1*$^{+/+}$ and n=4 *Lkb1*$^{-/-}$ mice). Positive cells were counted in 5 separate fields and normalized by total area for every mouse analyzed followed by fold change calculation. B) Immunostaining of CD3, MYP, and F4/80 in *Lkb1*$^{-/-}$ animals treated with liposomal PBS or clodronate. Graph shows quantitation of lymphocytes, neutrophils, and macrophages in clodronate treated animals normalized to controls (n=4 per treatment group). F4/80+ cells showed significantly greater depletion following clodronate treatment than neutrophils and lymphocytes (*P<0.05 per student's t test). Bars=50 μm in all panels. Error bars=S.E.M.

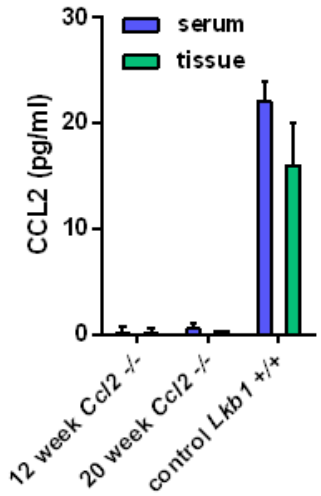

Figure S4. Validation of *Ccl2*$^{-/-}$ transgenic mouse line. A) ELISA on serum and uterine tissue showing undetectable amounts of CCL2 in younger (12 week) and older (20 week) *Ccl2*$^{-/-}$ mice. *Lkb1*$^{+/+}$ mice, which are *Ccl2*$^{+/+}$, served as a positive control for CCL2 detection.

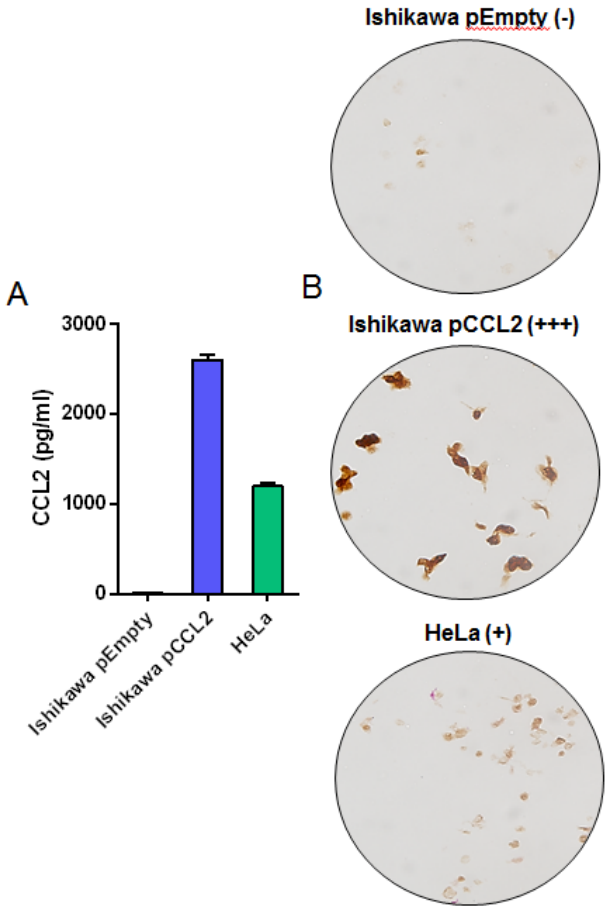

Figure S5. Validation of human CCL2 antibody for TMA studies. A) ELISA on media conditioned for 48h from Ishikawa endometrial cell line transfected with either empty vector or *CCL2* cDNA. HeLa cells, which were non-transfected, are also shown. As expected, cDNA transfection resulted in significantly higher levels of CCL2 in the media compared to empty vector. HeLa cells endogenously produced an intermediate level of CCL2. B) CCL2 immunostaining of cell lines following fixation in 10% buffered formalin and paraffin-embedding to simulate clinical pathology laboratory conditions.

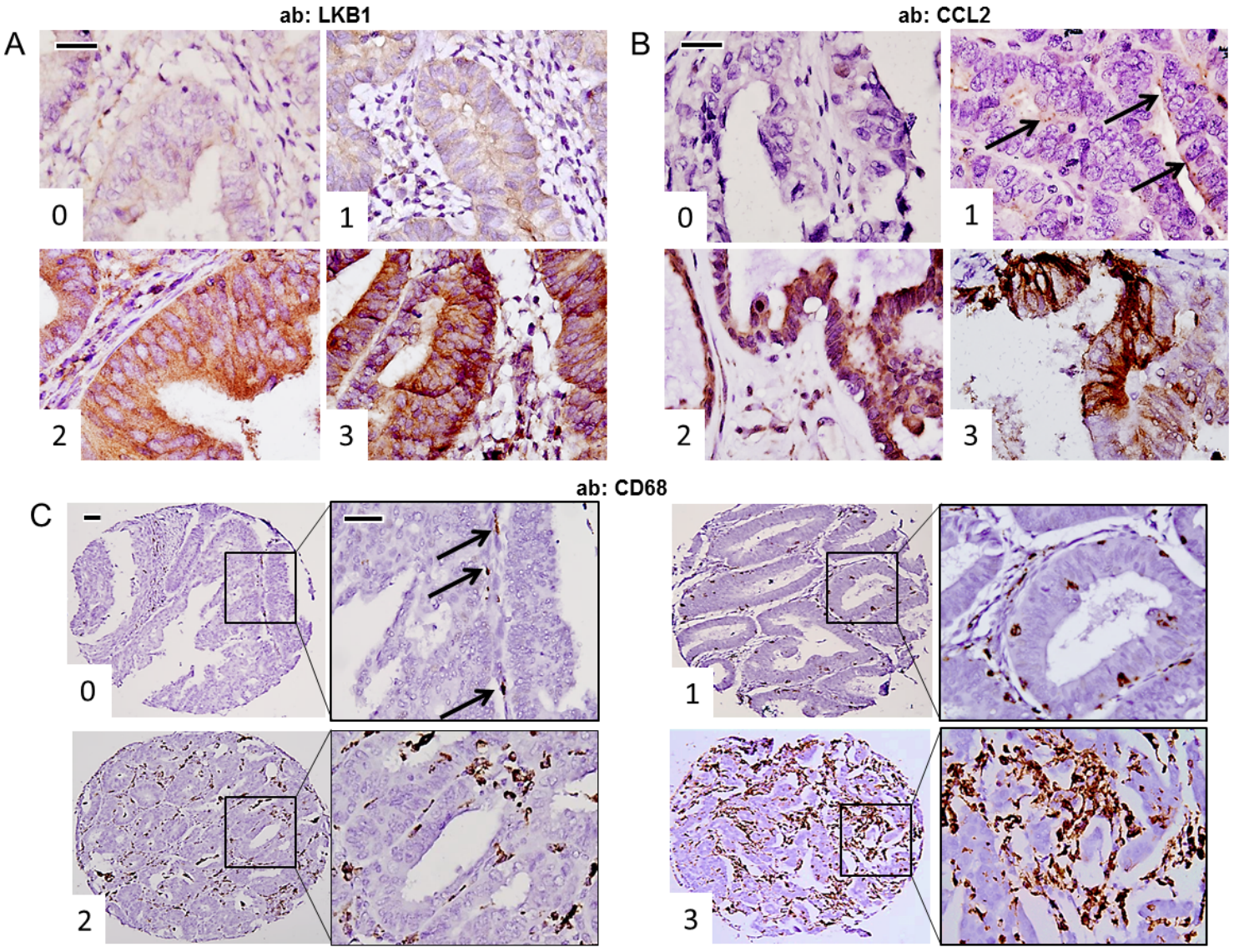

Figure S6. Histological scoring schema for LKB1, CCL2, and CD68 expression by immunohistochemical analysis of human endometrial adenocarcinomas. Immunohistochemical staining of A) LKB1 B) CCL2 or C) CD68 in representative cases illustrating 0-3 scoring system employed to analyze the TMA. For LKB1 and CCL2, only staining in epithelium was scored. For CD68, which was used for macrophage quantitation, staining was evaluated in the epithelial and stromal compartments combined (insets show higher magnification). Arrows in panels B and C highlight positive staining. See methods section for detailed explanation of scoring schema for each marker. Bars=50 μm in all panels.

Receptor binding (n=29 genes) P=7x10^{-4}		
Probe ID	***Gene notation***	***Gene name***
ILMN_1720048	CCL2	chemokine (C-C motif) ligand 2
ILMN_2161577	CXCL6	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
ILMN_2212999	KIF5C	kinesin family member 5C
ILMN_1809364	NTF3	neurotrophin 3
ILMN_1802653	EBI3	Epstein-Barr virus induced 3
ILMN_1699651	IL6	interleukin 6 (interferon, beta 2)
ILMN_1782098	SMO	smoothened, frizzled family receptor
ILMN_1740938	APOE	apolipoprotein E
ILMN_2149164	SFRP1	secreted frizzled-related protein 1
ILMN_1682636	CXCL2	chemokine (C-X-C motif) ligand 2
ILMN_1681721	OASL	2'-5'-oligoadenylate synthetase-like
ILMN_1773352	CCL5	chemokine (C-C motif) ligand 5
ILMN_1791679	DNER	delta/notch-like EGF repeat containing
ILMN_1752562	CXCL5	chemokine (C-X-C motif) ligand 5
ILMN_2350634	EFEMP1	EGF containing fibulin-like extracellular matrix protein 1
ILMN_1728478	CXCL16	chemokine (C-X-C motif) ligand 16
ILMN_1791759	CXCL10	chemokine (C-X-C motif) ligand 10
ILMN_1766914	MFAP4	microfibrillar-associated protein 4
ILMN_1691364	STAT1	signal transducer and activator of transcription 1, 91kDa
ILMN_2105573	CCL3L3	chemokine (C-C motif) ligand 3-like 3
ILMN_1669617	GRB10	growth factor receptor-bound protein 10
ILMN_2160428	IL1RAPL1	interleukin 1 receptor accessory protein-like 1
ILMN_2372124	HNF4A	hepatocyte nuclear factor 4, alpha
ILMN_1745242	PLSCR1	phospholipid scramblase 1
ILMN_1785699	PTHLH	parathyroid hormone-like hormone
ILMN_1758418	TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b
ILMN_1672022	EPHA4	EPH receptor A4
ILMN_2160428	IL1RAPL1	interleukin 1 receptor accessory protein-like 1
ILMN_1676449	SLIT2	slit homolog 2 (Drosophila)

Table S2, Part 1. Gene ontology analysis of common LKB1 regulated transcripts. Illumina probe ID, gene notation, and gene names are shown for two highly significant gene ontology categories (Receptor binding, n=29 genes, P=7x10^{-4}; Extracellular region part, n=31 genes, P=7x10^{-6}) per hypergeometric test.

Extracellular region part (n=31 genes) P=7x10^{-6}		
Probe ID	***Gene notation***	***Gene name***
ILMN_2073758	MMP12	matrix metallopeptidase 12 (macrophage elastase)
ILMN_2068104	TFPI2	tissue factor pathway inhibitor 2
ILMN_1720048	CCL2	chemokine (C-C motif) ligand 2
ILMN_1802653	EBI3	Epstein-Barr virus induced 3
ILMN_2196328	POSTN	periostin, osteoblast specific factor
ILMN_1805665	FLRT3	fibronectin leucine rich transmembrane protein 3
ILMN_1699651	IL6	interleukin 6 (interferon, beta 2)
ILMN_2132982	IGFBP5	insulin-like growth factor binding protein 5
ILMN_1740938	APOE	apolipoprotein E
ILMN_2149164	SFRP1	secreted frizzled-related protein 1
ILMN_2341229	CD34	CD34 molecule
ILMN_1676449	SLIT2	slit homolog 2 (Drosophila)
ILMN_1751375	ENAM	enamelin
ILMN_1711514	COCH	coagulation factor C homolog, cochlin (Limulus polyphemus)
ILMN_2153495	WNT7B	wingless-type MMTV integration site family, member 7B
ILMN_2054019	ISG15	ISG15 ubiquitin-like modifier
ILMN_1752562	CXCL5	chemokine (C-X-C motif) ligand 5
ILMN_1791759	CXCL10	chemokine (C-X-C motif) ligand 10
ILMN_1784459	MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)
ILMN_1766914	MFAP4	microfibrillar-associated protein 4
ILMN_1750373	KAL1	Kallmann syndrome 1 sequence
ILMN_2105573	CCL3L3	chemokine (C-C motif) ligand 3-like 3
ILMN_1785402	LTBP1	latent transforming growth factor beta binding protein 1
ILMN_1785699	PTHLH	parathyroid hormone-like hormone
ILMN_1758418	TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b
ILMN_1682636	CXCL2	chemokine (C-X-C motif) ligand 2
ILMN_1773352	CCL5	chemokine (C-C motif) ligand 5
ILMN_2350634	EFEMP1	EGF containing fibulin-like extracellular matrix protein 1
ILMN_1728478	CXCL16	chemokine (C-X-C motif) ligand 16
ILMN_1670305	SERPING1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
ILMN_1805665	FLRT3	fibronectin leucine rich transmembrane protein 3

Table S2, Part 2. Gene ontology analysis of common LKB1 regulated transcripts. Illumina probe ID, gene notation, and gene names are shown for two highly significant gene ontology categories (Receptor binding, n=29 genes, P=7x10^{-4}; Extracellular region part, n=31 genes, P=7x10^{-6}) per hypergeometric test.

Age	**(yr)**
Median	59
Mean	58
Range	24-89
Stage	**(%)**
I (n=114)	65%
II (n=17)	10%
III (n=16)	9%
IV (n=20)	11%
unstaged (n=8)	5%
Grade	**(%)**
I (n=71)	41%
II (n=62)	35%
III (n=42)	24%
Histology	**(%)**
Endometrioid (n=161)	92%
other (n=14)	8%

Table S3. Characteristics of 175 patients in the study population. Clinical data for patients and endometrial tumors represented in TMA. “Other” histotypes include serous and clear cell carcinoma.