

Table S1. Sequence of primer pairs used for qPCR

| Gene Name | Forward (5'-3') | Reverse (5'-3') | Size (pb) |
|---------------------|-------------------------|--------------------------|------------------|
| CD24 | CTGCTCCTACCCACGCA | AGAGAGTGAGACCACGAAGAGA | 171 |
| CD34 | GCTTTGCTTGCTGAGTTTGCT | TCGTTTCTGTGATGTTTGTGTG | 212 |
| CD49 (ITGA2) | TGCCAGAGGAAGGAAAAGGA | TAACTTTGGACCGCTGGAGA | 229 |
| CD73 | TCCTCTCAATGATGCCGCTT | TTGCGTTGATGAATGGGCGA | 161 |
| ESR1 | GAAAGGTGGGATACGAAAAGACC | GCTGTTCTTCTTAGAGCGTTTGA | 163 |
| HMGA2 | AGAGTCCCTCTAAAGCAGCTCA | CAACTGCTGCTGAGGTAGAAATCG | 196 |
| IGF1 | GCTCTTCAGTTCGTGTGTGGA | GCCTCCTTAGATCACAGCTCC | 133 |
| IGF1R | CGTGAAGATCCGCCATTCTC | TCACTTCTCCATGCGGTAA | 236 |
| IGF2 | GGGCAGTGACAAAAGCAAG | CTCAACCCAAGCAACAGGGA | 120 |
| IGF2R | ACCAGCAAGGCACAAATCAC | GAGTCATCCACCAAGTAGGCA | 250 |
| IGFBP1 | TGGGACGCCATCAGTACCTA | CTCCTGATGTCTCCTGTGCC | 133 |
| IGFBP2 | GACAAGCATGGCCTGTACAAC | AGAAGAGATGACACTCGGGG | 145 |
| IGFBP3 | CCCTGCCGTAGAGAAATGGA | TTGGTGGTGTAGCCTGGGA | 200 |
| IGFBP4 | CATCATCCCCATCCCCAACTG | GAAGCTTCAACCCCGTCTT | 122 |
| IGFBP5 | CCAATTGTGACCGCAAAGGA | GTCGAAGGTGTGGCACTGAA | 152 |
| IGFBP6 | GGTCTACACCCCTAACTGCG | CTGCGGTTACATCCTGTGG | 189 |
| KIT | GACAGGCTCGTGAATGGCAT | GCACATCCACTGGCAGTACA | 124 |
| KLF11 | CCAGGTTGCCGGAAGACCTA | GCGTGACAGCTCATCCGAAC | 135 |
| MED12 | GGCCTCCCGATGTTTACC | GTTGGAAGTATCTTGGCAGG | 158 |
| PGR | AAGGAGTTGTGTCGAGCTCA | GTTTCACCATCCCTGCCAAT | 198 |
| PGR-B | TCCCCGAGTTAGGAGACGAGA | CTGTGGCTGTCGTTTGTCCC | 90 |
| PLGA1 | AGTCTGAGGCTTTTTCCATCAGT | GGGTCGTGTGTATGGAGGTG | 493 |
| PUM1 | GACGCTATGGTGGACTACTTCT | TGGAACGCACCTGATGTTCTG | 142 |
| XIAP | TGGCAGATTATGAAGCACGGAT | AGCCCTCCTCCACAGTGAAAG | 136 |

Table S2. List of Antibodies used for immunofluorescence analysis.

| Target | Reference | Dilution used |
|--|--------------------------------------|----------------------|
| CD24 | Abcam (ab199140) | 1:100 |
| CD34 | Dako | Ready to use |
| CD49b | Santa Cruz Biotechnology (sc-74466) | 1:50 |
| CD73 | Abcam (ab175396) | 1:50 |
| Desmin | Dako (IR606-Clone D33) | Ready to use |
| Era | Santa Cruz Biotechnology (sc-8002) | 1:100 |
| HMGA2 | Sigma (SAB2701959) | 1:100 |
| Ki67 | ABclonal (A2094) | 1:100 |
| c-KIT (CD117) | Ventana Medical Systems (790-2951) | Ready to use |
| PGR | Santa Cruz Biotechnology (sc-166169) | 1:100 |
| Vimentin | ThermoFisher (MA514564) | 1:1000 |
| Alexa Fluor 488 AffiniPure F(ab') ₂ Fragment Goat Anti-Rabbit IgG (H+L) | Jackson ImmunoResearch (111-546-003) | 1:1000 |
| Alexa Fluor Plus 594. Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody | ThermoFisher (A-32742) | 1:1000 |

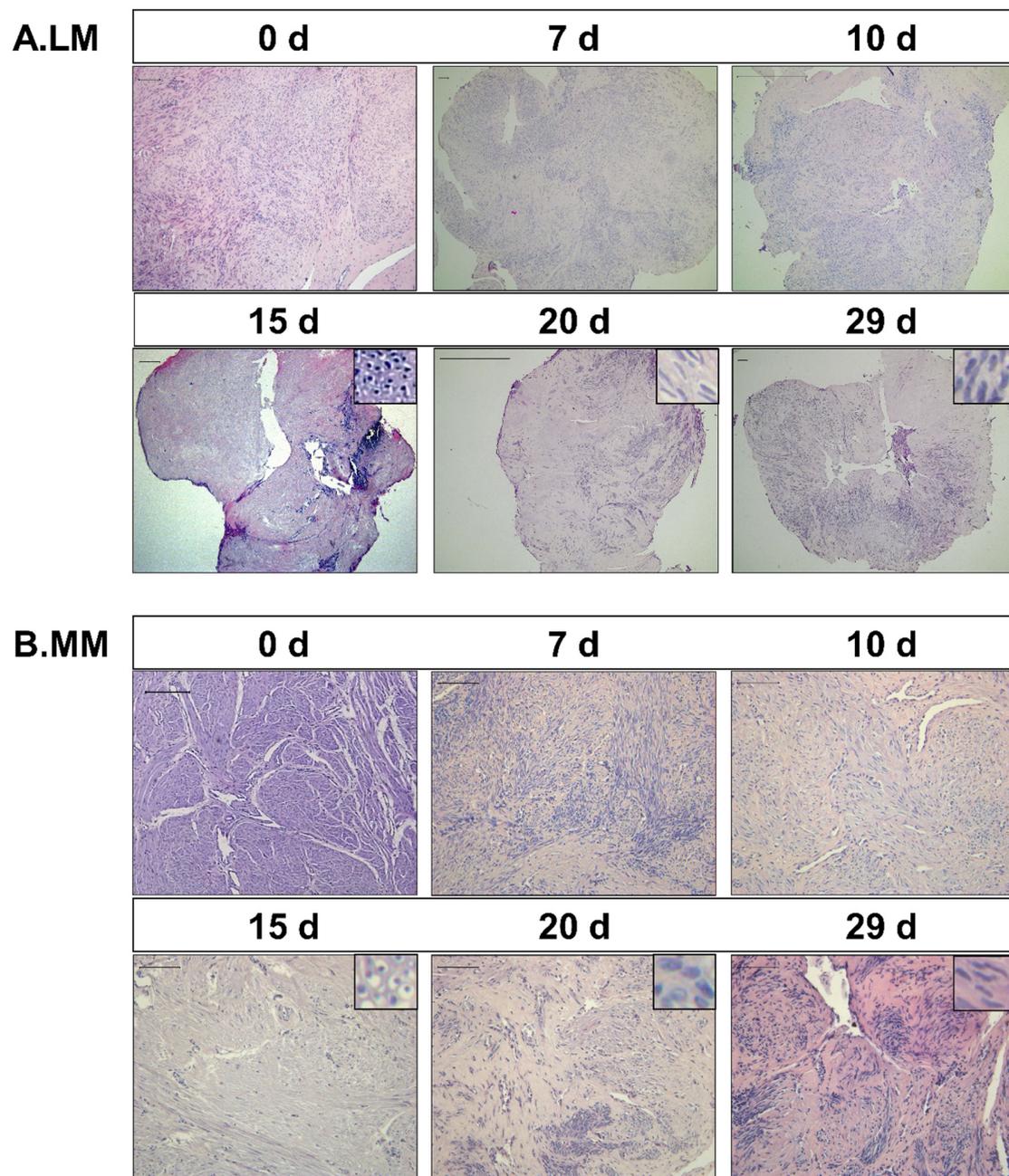


Figure S1. Cell repopulation of normal and tumor tissue as assessed by hematoxylin and eosin staining. Representative images of (A) myometrium (MM) and (B) leiomyoma (LM) organ sections H&E stained at baseline (0 d) and after 7, 10, 15, 20, and 29 days of culture. The inserts (top right) at long incubation times show a higher magnification detail of the cells. Scale bar, 100 μ m.

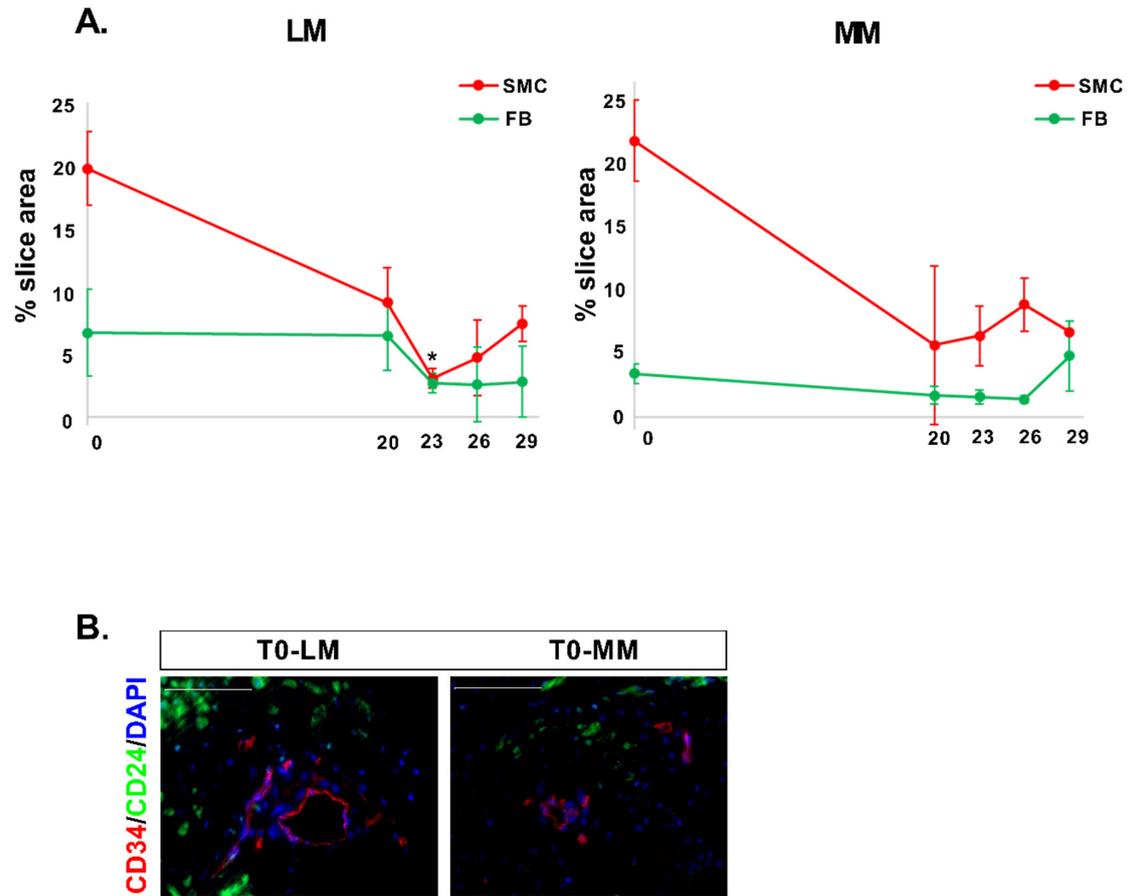


Figure S2. Proportion of smooth muscle cells and fibroblast after long-term culture and immunostaining of blood vessels. (A) Graph represents the percentage of slice area occupied by smooth muscle cells (SMC) and fibroblasts (FB) in leiomyoma (LM) and myometrium (MM) slices at T0 and after 20, 23, 26, and 29 days of culture. For each time point, 3 MM and 3 LM were analyzed. A significant decrease in SMCs was observed in LM after 23 days of culture. Data represent the mean \pm S.E.M. * $p < 0.05$ compared to control at time zero. (B) IF staining of CD34 (red) and CD24 (green) in leiomyoma (LM) and myometrium (MM) at baseline (T0). Endothelial cells from blood vessels were CD34⁺/CD24⁻. DAPI stained cell nucleus (blue). Scale bar, 100 μ m.

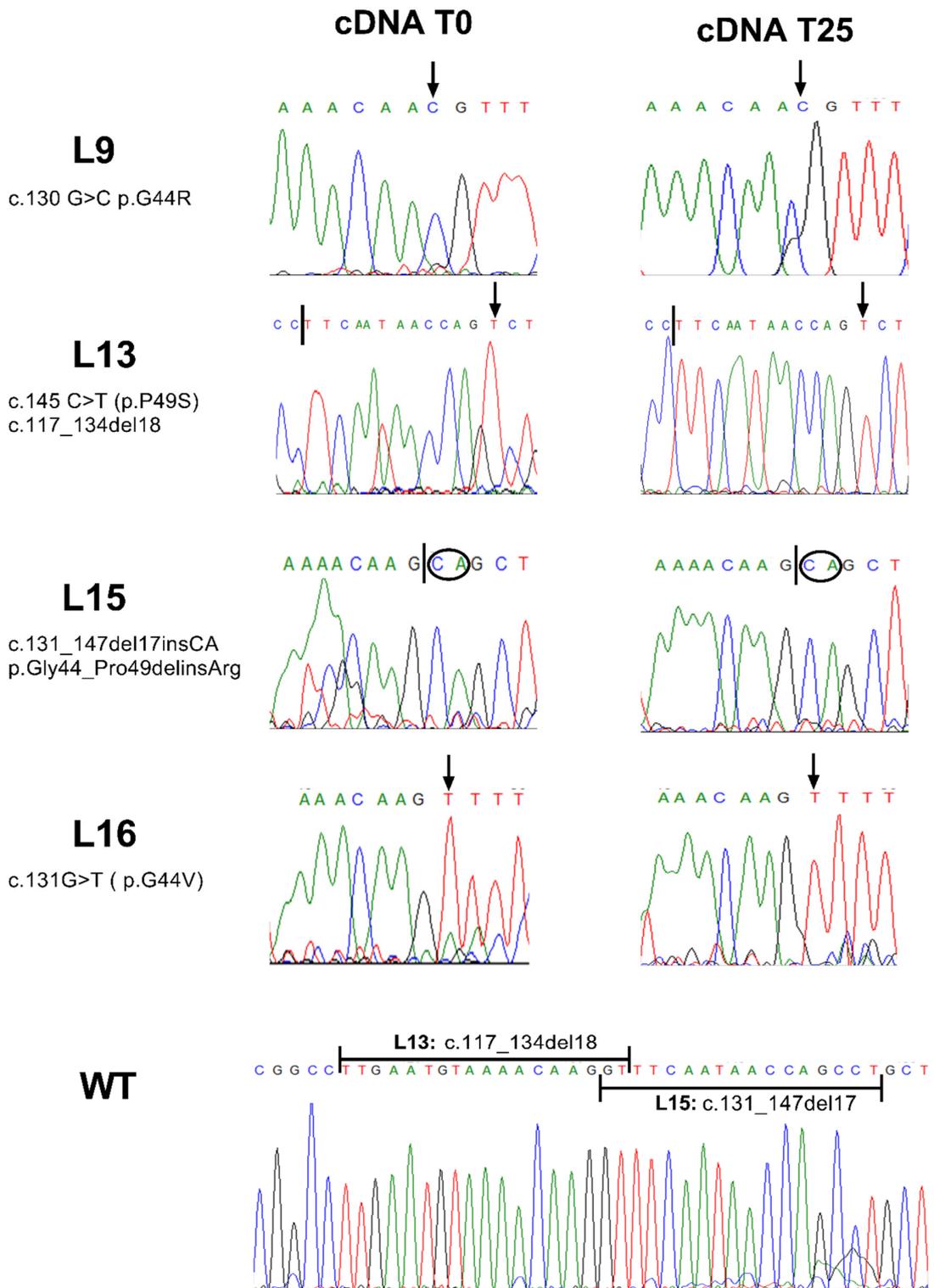


Figure S3. Driver mutations are maintained in organ cultures at long-term culture.

Sequence electropherograms illustrating different types of somatic mutations in *MED12* in the four leiomyomas (LM). cDNA sequencing demonstrated point mutation in the hot spot codon 44 in two tumors (L9 and L16), one tumor acquired an indel mutation (L15) and the remaining tumor presented a deletion together with a point mutation (L13). Mutant alleles were preferentially expressed at baseline (T0) and after 25 days of culture (T25). The bottom electropherogram represents the wild type sequence (WT) of *MED12* exon 2. Arrows point to single base substitutions. A short vertical black line indicates the position of the nucleotide where deletion starts.

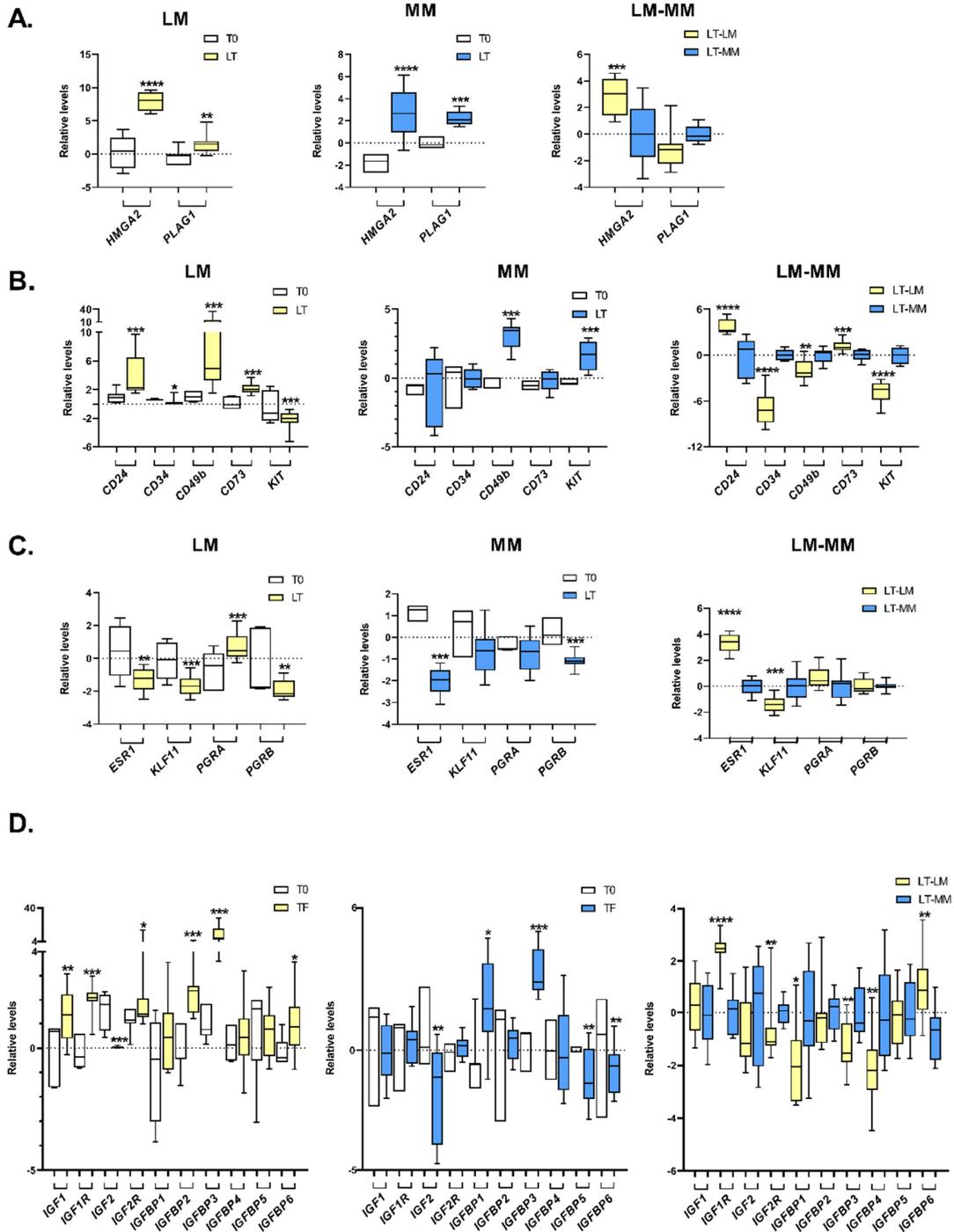


Figure S4. Quantitative PCR data showing differentially expressed genes in leiomyoma and myometrium organ culture.

(A) Box-plot showing relative expression levels of *HMGA2* and *PLGAI* in leiomyoma (LM) and myometrium (MM) organ slices. Long-term culture slices from 4 LMs (16 slices) and 3 MMs (12 slices) displayed significant *HMGA2*, *PLGAI* upregulation compared to their corresponding T0 slices. Increased expression of *HMGA2* was detected in LM compared to MM slices at long-term culture, in agreement with IF data.

(B) Box-plot of relative expression levels of stem cell surface marker (*CD24*, *CD34*, *CD73*, and *KIT*) in long-term culture of 16 slices corresponding to 4 leiomyomas (LM) and 12 slices corresponding to 3 myometria (MM) compared to their corresponding T0 (left and middle graphics). Normal and tumor slices were also compared at long-term culture (right graphic).

(C) Box-plot of relative expression levels of *KLF11* transcription factor and progesterone isoforms A (*PGRA*) and B (*PGRB*) and estrogen receptor alpha (*ESR1*) in long-term culture of 4 leiomyomas (LM, 14 slices) and 3 myometria (MM, 12 slices) compared to their corresponding T0 (left and middle graphics). Normal and tumor slices were also compared at long-term culture (right graphic).

(D) Box-plot of relative expression levels of *IGF1*, *IGF2*, *IGF1R*, *IGF2R*, and associated binding proteins (*IGFBP1-IGFBP6*) in long-term culture of 4 leiomyomas (LM, 16 slices) and 3 myometria (MM, 12 slices) compared to its corresponding T0 (left and right graphics). Normal and tumor slices were also compared at long-term culture (bottom graphic). * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.