Supplementary materials

Figure S1

Hierarchical clustering of regulated genes from microarray data and GO analysis of differentially expressed genes. (A) Hierarchical clustering of regulated genes between each time was performed using Partek Genomics Suite. Data were clustered using the Euclidean algorithm. Red to blue in the heatmap indicated high to low expression levels. **(B)** Examples of GO terms with over 50 genes. The number of regulated genes included in the GO term was indicated in brackets. The x-axis showed the percentage of the total number of differentially expressed genes, for a particular GO term, in tendon progenitor cells at E11.5 (green lines) or in differentiated tendon cells at E14.5 (red lines).

Figure S2

Regulated genes between E14.5 tendon differentiated cells versus E11.5 tendon progenitors in the TGF-beta KEGG pathway N°4350. Up-regulated and down-regulated genes in limb tendon cells (E14.5 vs E11.5) were coloured in red and green, respectively, in the map of the KEGG TGF-beta pathway N°4350. Two genes, known to be transcriptionally activated by TGF-β ligand, the secreted factor, *Tgfbi* and the transcription factor, *Zeb2* and the TGF-β Receptor 3 (*Tgfbr3* or *Betaglycan*) were added to the pathway in brackets. These 3 genes were up-regulated in E14.5 tendon differentiated cells versus E11.5 tendon progenitors. All the extracellular components of the classical TGF-beta pathway were up-regulated in E14.5 cells versus E11.5 tendon progenitors.

Figure S3

Regulated genes between E14.5 tendon differentiated cells versus E11.5 tendon progenitors in MAPK KEGG pathway N°4010. Up-regulated and down-regulated genes in limb tendon cells (E14.5 vs E11.5) were coloured in red and green, respectively, in the map of the KEGG MAPK pathway N°4010. It is has to be noted that the number of genes classified in the MAPK pathway (KEGG N°04010) is high (259) and the MAPK pathway includes TGF-β pathway members.

Figure S4

RT-q-PCR analyses for tendon markers in E9.5, E10.5 and E12.5 mouse limbs.

(**A**) The relative mRNA levels of *Scx, Col1a1* and *Col1a2* were determined in E9.5, E10.5 and E12.5 mouse limbs. The *Scx* mRNA levels, at E9.5 were arbitrary established at 1. All the relative mRNA levels were calculated and compared to the *Scx* mRNA levels at E9.5. Based on the average number of RT-q-PCR cycles for *Scx*, we observed that *Scx* was already expressed in E9.5 limbs (ct: 26,77) and E10.5 (ct: 26,07). Both *Col1a* genes were highly expressed at E9.5, E10.5 and E12.5 (green bars) compared to *Scx* expression (purple bars). In addition, the *Col1a1* and *Col1a2* mRNA expression levels significantly increased between E10.5 and E12.5. (**B**) The relative mRNA levels of the differentiation tendon marker *Tnmd*, and the tendon genes identified in the transcriptome, *Aqp1*, *Thbs2* and *Thbs4* were determined at E9.5, E10.5 and E12.5 and compared to that of *Scx* at E9.5 mouse limbs. The *Scx* mRNA levels, at E9.5 were arbitrary established at 1. Based on the average number of RT-q-PCR cycles, we observed that *Tnmd* and *Aqp1* mRNA levels were not detectable in E9.5 and E10.5 limbs (above 31 cycle numbers). The mRNA levels of *Thbs2* (29 cycle numbers) and *Thbs4*

(30 cycle numbers) mRNA levels were very low in E9.5 and E10.5 limbs. However, the *Tnmd, Aqp1*, *Thbs2* and *Thbs4* mRNA expression levels were significantly increased at E12.5 compared to those at E9.5/E10.5. The mRNA expression levels of *Tnmd* (0,44), *Aqp1* (0,40), *Thbs2* (0,75) and *Thbs4* (1,15) genes at E12.5 were comparable to those of *Scx* at E9.5 (1), E10.5 (1,73) and E12.5 (1,15). The significant increase of late tendon gene expression highlighted the initiation of the tendon differentiation process at E12.5. (**C)** The relative mRNA levels of *Tgfb1, Tgfb2, Tgfb3* were determined and compared to that of *Scx* in E9.5 mouse limb explants. The *Scx* mRNA levels were arbitrary established at 1. Since all *Tgfb* mRNA levels were established to the same reference (*Scx* at E9.5), mRNA levels were comparable between *Tgfb* genes and stages. *Tgfb2* displayed the highest expression levels compared to those of other *Tgfb* genes at all stages, E9.5, E10.5 and E12.5. It has to be noted that *Tgfb3* expression levels significantly increased at E12.5 versus E9.5 or E10.5. The error bars represent standard deviation (SD). The asterisks in histograms indicate p-values, *<0.05, **<0.01 in unpaired student's t-test.

Figure S5

Schematic and simplified representation of the TGFβ/SMAD2/3 and FGF/ERK signalling pathways and their intracellular cross-talks. TGF-β ligands signal via the serine/threonine kinase type I Receptor (TGFβ−RI or other named ALK5) and its activator type II Receptor (TGFβ-RII). The TGF-β canonical intracellular pathway is the SMAD2/3, which regulates transcription in a cell context dependent manner. *Smad7* is considered as a common target gene of the TGFβ/SMAD2/3 pathway. FGF ligands signal via tyrosine kinase receptors. One main intracellular pathway downstream of FGF is the ERK MAPK pathway. *Pea3, Spry2* and *Dusp6* are considered as transcriptional readouts of active FGF/ERK pathway.

Positive and negative cross talks between the TGFβ/SMAD2/3 and FGF/ERK pathways are indicated with green and red dashed lines, respectively. TGF-β receptors can activate the ERK MAPK pathway (green dashed line). Conversely, the ERK MAPK pathway blocks the SMAD2/3 pathway by modulating SMAD3 function (red dashed line).

In our gain and loss-of-function experiments, we targeted both pathways at different levels. Protein recombinants were used to activate the TGF-β and FGF pathways. Specific inhibitors were used to block the TGF-β pathway at the level of the receptors (SB43) or at the level of the SMAD2/3 pathway (SIS3). PD18 blocks the ERK pathway.

Figure S6

Quantitative real-time PCR analyses of mRNA levels of tendon genes in E10.5 mouse limb explants (**A**) or in in E12.5 mouse limb explants (**B**), cultured for 24 hours with FGF or the PD18 ERK inhibitor. For each gene, the mRNA levels of control limbs were normalized to 1. (A) In E10.5 mouse limb explants, FGF did not modify the relative mRNA expression levels of tendon genes, while PD18 increased *Col1a1* and *Thbs2* expression, but not that of *Scx*. PD18 inhibitor dramatically decreased the expression levels of *Pea3* and *Spry2* genes. (B) In E12.5 mouse limb explants, FGF did not modify the relative mRNA expression levels of *Scx*, *Col1a1*, *Thbs2*, *Tnmd* and *Aqp1*, while increasing *Col1a2* and *Thbs4* expression. PD18 inhibitor significantly decreased the expression of *Col1a2*, *Pea3* and *Spry2* genes, in E12.5 mouse limbs. The error bars represent standard error (SEM). The asterisks in histograms indicate p values, *<0.05, **<0.01, ***<0.001 in unpaired student's t-test.

Table S1

Global list of genes regulated in mouse limb tendon cells during development. List of all significantly regulated genes in the microarray with no filter of probe expression. Column A correspond to the probe references in the Affymetrix microarray. Columns B, C and D correspond to gene symbol, gene title and transcript ID, respectively. Columns E, F, G correspond to the signal in Arbitrary Unit (AU) in the microarray for one probe at E11.5, E12.5 and E14.5, respectively. The mean of the signal has been established with the three biological replicates at each time point. Numbers below 500 (AU) are considered as reflecting low level of gene expression. Numbers above 8000 (AU) are considered as reflecting very high level of gene expression. Columns H, I and J correspond to fold-changes between E12.5 vs E11.5 (muscle-independent phase), E14.5 vs E12.5 (muscle-dependent phase) and E14.5 vs E11.5 (tendon differentiated cells vs tendon progenitors), respectively. Column K, L and M correspond to Gene Ontology Biological Process, Gene Ontology Cellular Component, Gene Ontology Molecular Function, respectively. In table S1, genes have been ordered according to the fold-enrichment of gene expression (from high to low levels) in E14.5 versus E11.5.

Table S2

List of up-regulated and down-regulated genes of the KEGG pathway N°4350 (TGF-beta) in limb tendon cells at different stages of development. Up-regulated and down-regulated genes are indicated in red and green, respectively. The TGF-beta pathways has been divided into sub-pathways as indicated in the KEGG pathway N°4350. Significant Fold-changes (superior to 1.5) are indicated in bold and in brackets. Underlined genes are not

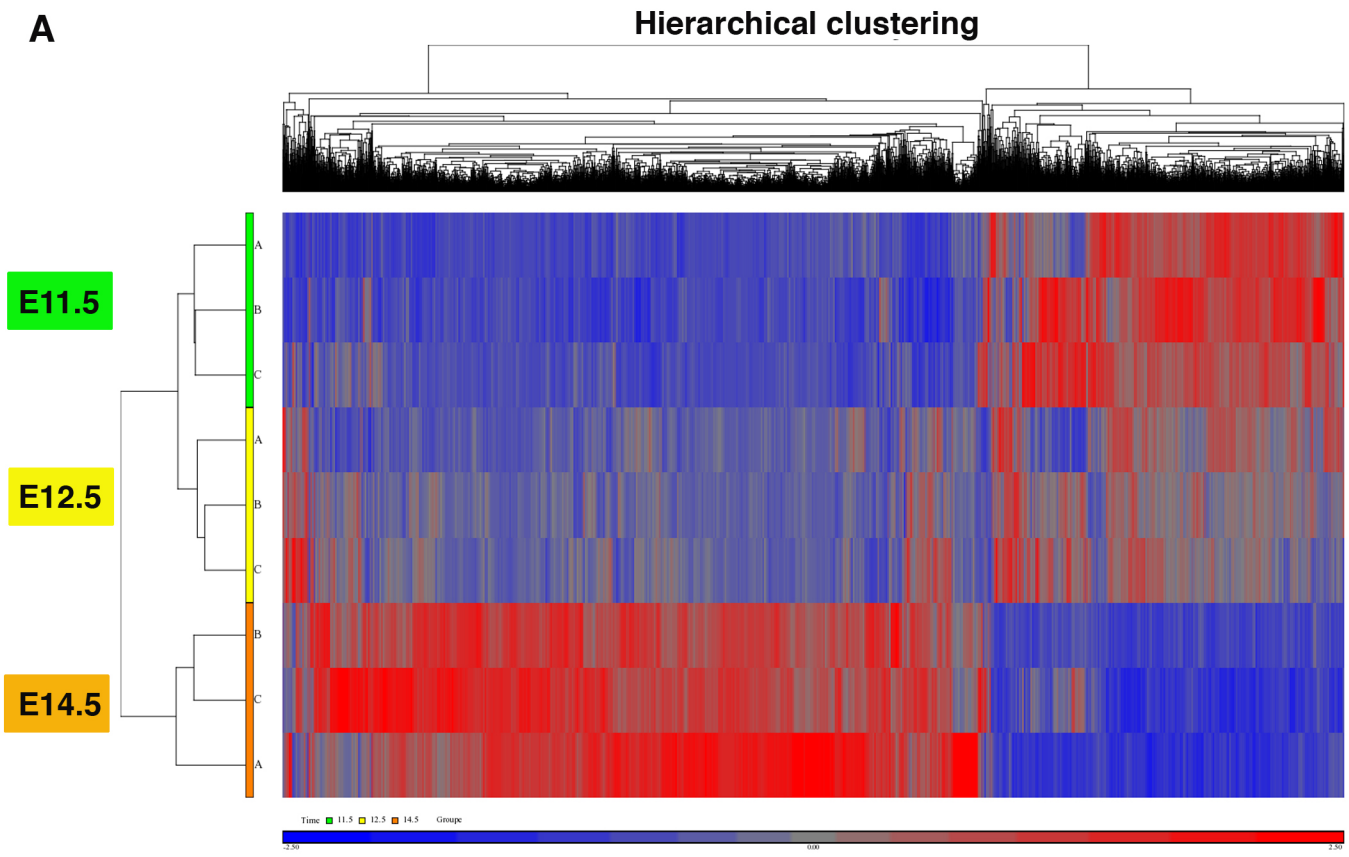
identified as being part of the KEGG pathway N°4350 but have been manually added as being part of the TGF-beta signalling pathway.

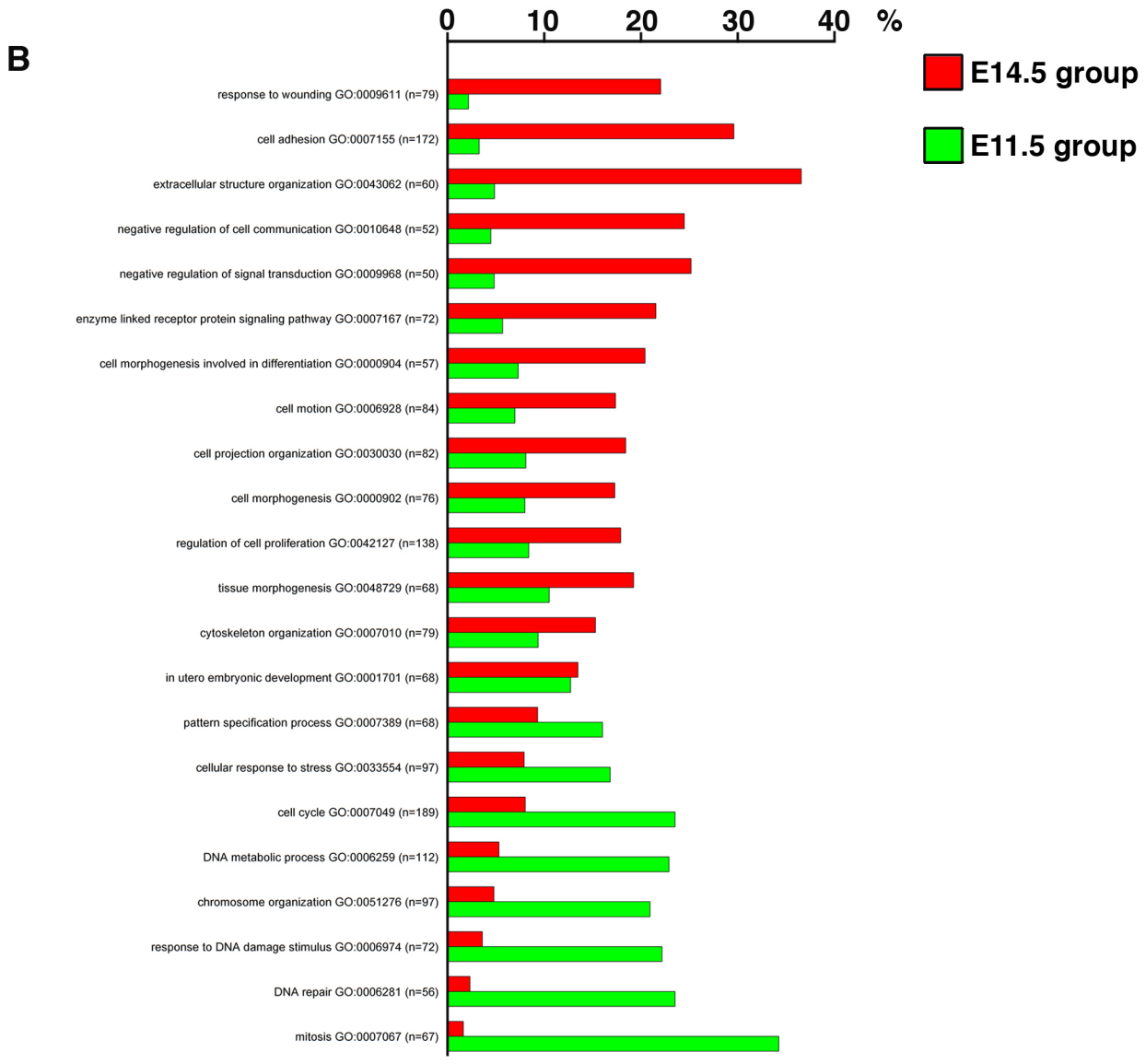
Table S3

List of up-regulated and down-regulated genes of the KEGG pathway N°4010 (MAPK) in limb tendon cells at different stages of development. Up-regulated and down-regulated genes are indicated in red and green, respectively.

Table S4

List of primers used for RT-q-PCR analyses


A
Hierarchical clustering
E11.5
E12.5
E14.5
A
B
C
A
B
C
B
C
A
Time 11.5 12.5 14.5 Group
-2.50
0.00
2.50


B
0
10
20
30
40
%
E14.5 group
E11.5 group
response to wounding GO:0009611 (n=79)
cell adhesion GO:0007155 (n=172)
extracellular structure organization GO:0043062 (n=60)
negative regulation of cell communication GO:0010648 (n=52)
negative regulation of signal transduction GO:0009968 (n=50)
enzyme linked receptor protein signaling pathway GO:0007167 (n=72)
cell morphogenesis involved in differentiation GO:0000904 (n=57)
cell motion GO:0006928 (n=84)
cell projection organization GO:0030030 (n=82)
cell morphogenesis GO:0000902 (n=76)
regulation of cell proliferation GO:0042127 (n=138)
tissue morphogenesis GO:0048729 (n=68)
cytoskeleton organization GO:0007010 (n=79)
in utero embryonic development GO:0001701 (n=68)
pattern specification process GO:0007389 (n=68)
cellular response to stress GO:0033554 (n=97)
cell cycle GO:0007049 (n=189)
DNA metabolic process GO:0006259 (n=112)
chromosome organization GO:0051276 (n=97)
response to DNA damage stimulus GO:0006974 (n=72)
DNA repair GO:0006281 (n=56)
mitosis GO:0007067 (n=67)

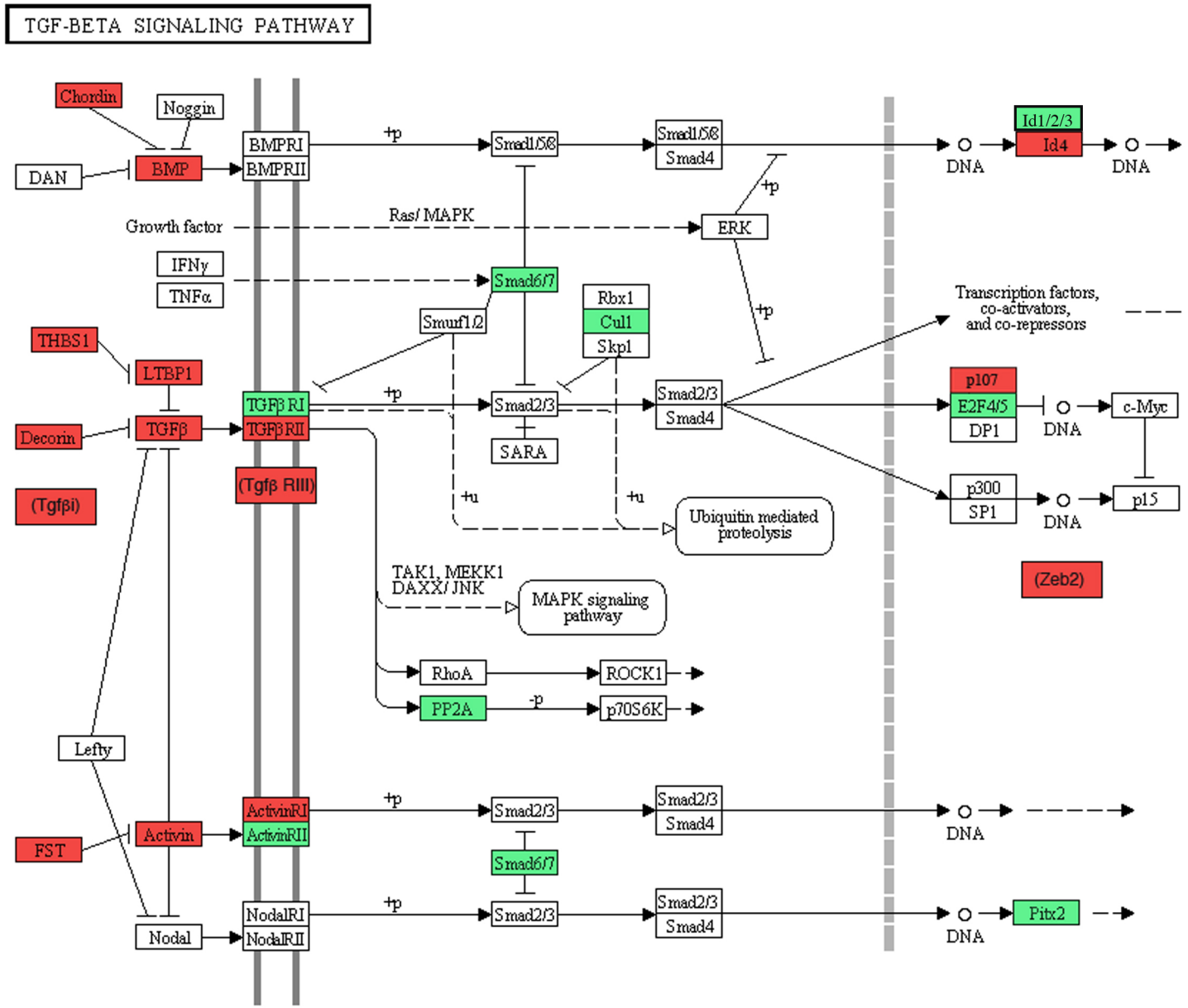
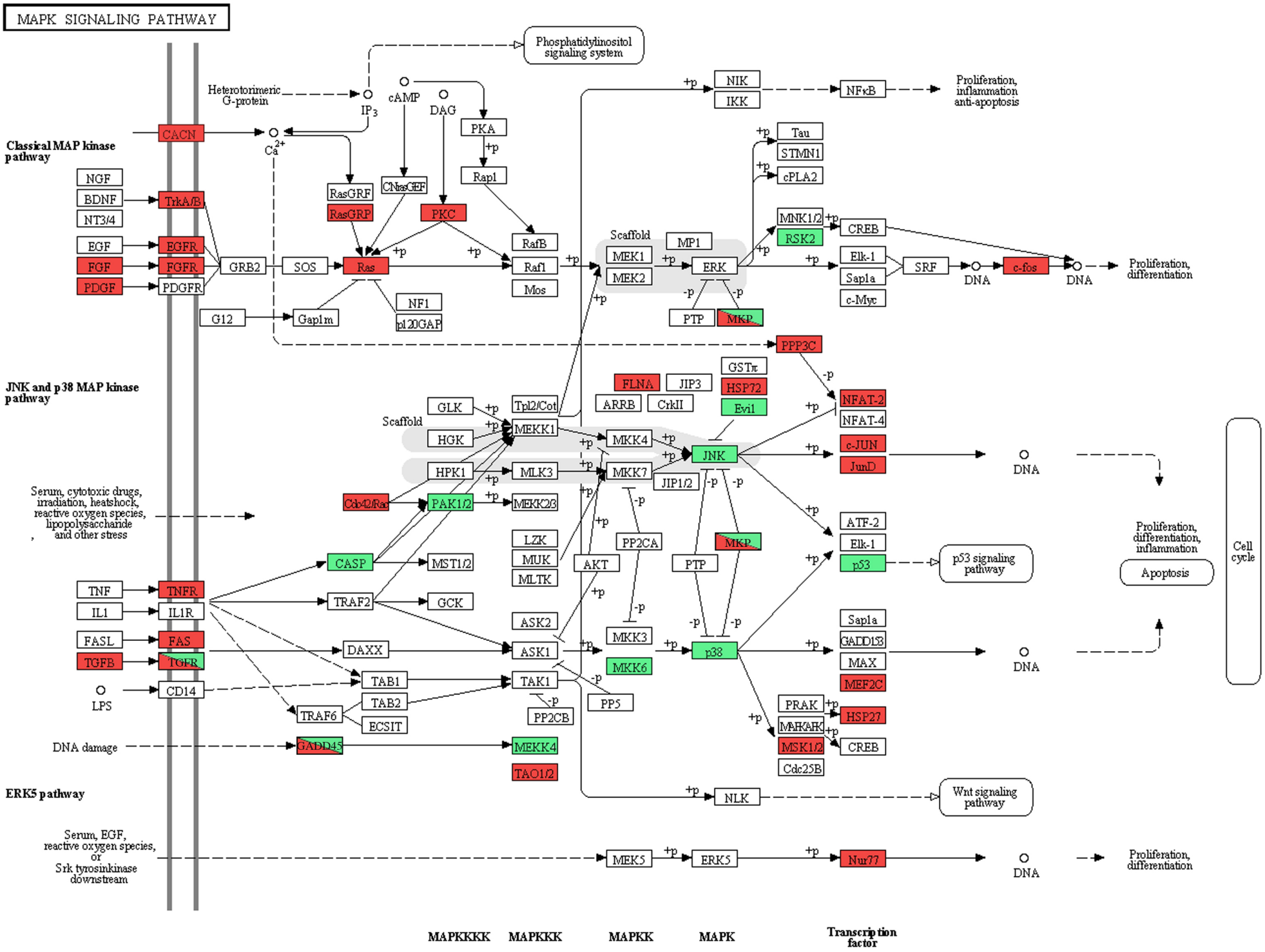
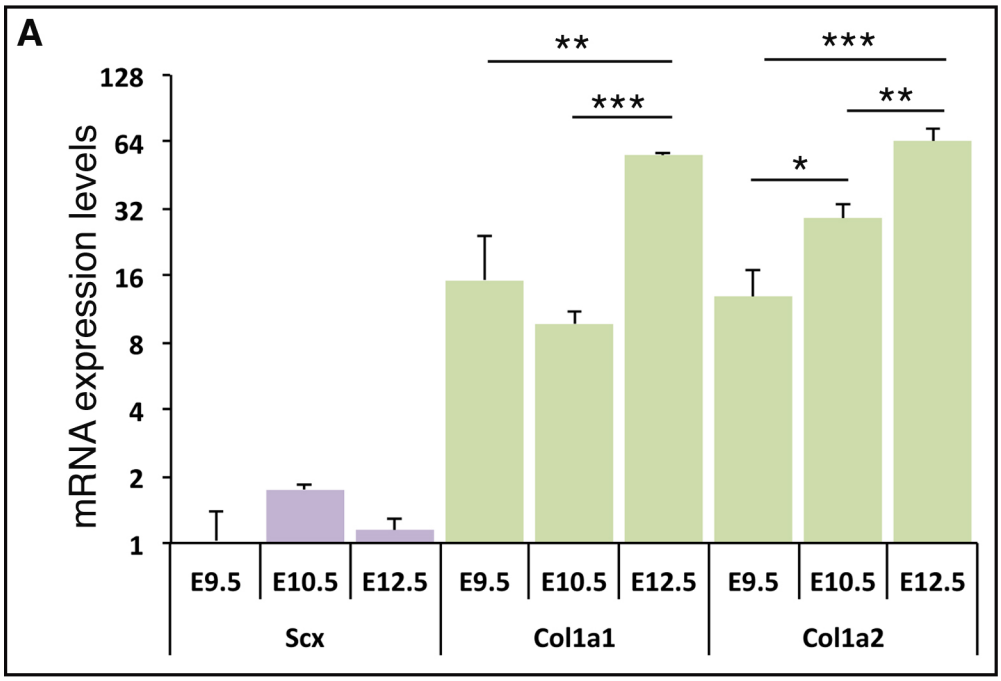
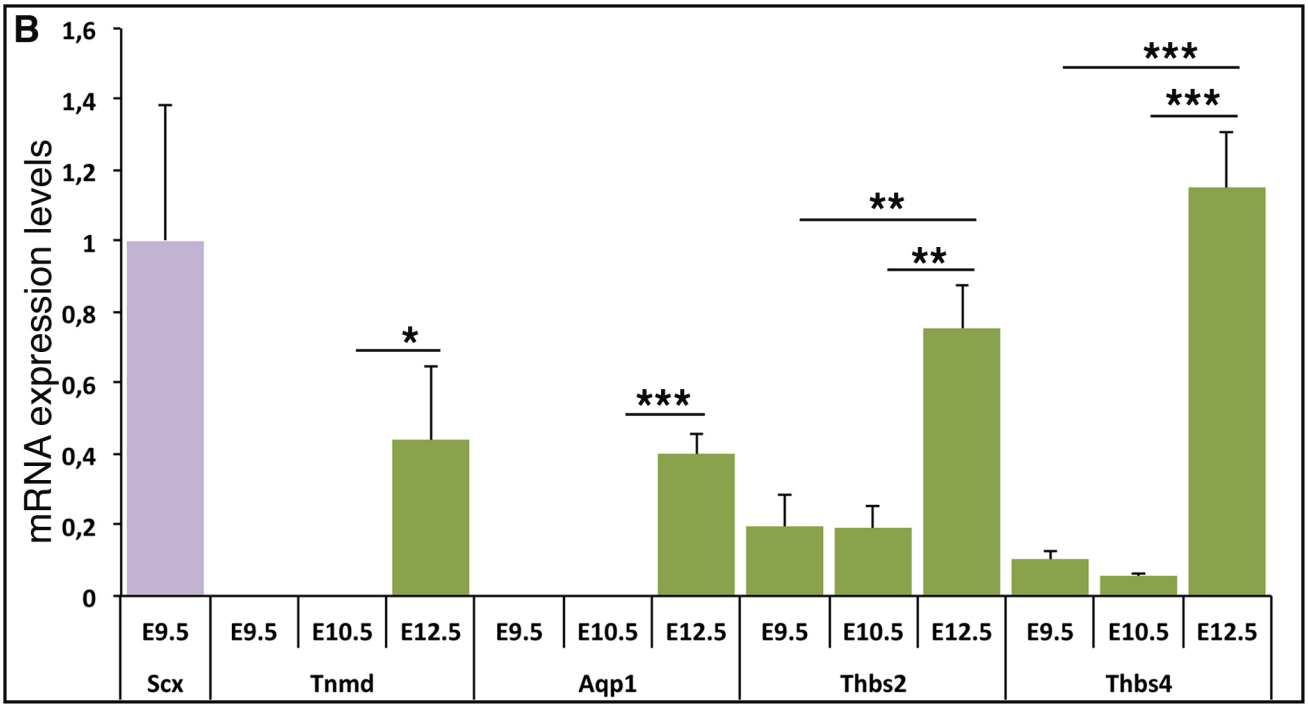

TGF-BETA SIGNALING PATHWAY
Chordin
Noggin
DAN
BMP
BMPRI
BMPRII
+p
Smad1/5/8
Smad1/5/8
Smad4
DNA
Id1/2/3
Id4
DNA
Growth factor
Ras/ MAPK
ERK
+p
IFNγ
TNFα
Smad6/7
Rbx1
Cul1
Skp1
Smurf1/2
+p
Transcription factors,
co-activators,
and co-repressors
THBS1
LTBP1
TGFβ RI
TGFβ RII
+p
Smad2/3
Smad2/3
Smad4
p107
E2F4/5
DP1
DNA
c-Myc
Decorin
TGFβ
(Tgfβ RIII)
SARA
(Tgfβi)
+u
+u
Ubiquitin mediated
proteolysis
p300
SP1
DNA
p15
(Zeb2)
TAK1, MEKK1
DAXX/ JNK
MAPK signaling
pathway
RhoA
ROCK1
PP2A
-p
p70S6K
Lefty
ActivinRI
ActivinRII
+p
Smad2/3
Smad2/3
Smad4
DNA
FST
Activin
Smad6/7
NodalRI
NodalRII
+p
Smad2/3
Smad2/3
Smad4
Pitx2
DNA
Nodal

Figure S2


MAPK SIGNALING PATHWAY
Classical MAP kinase pathway
JNK and p38 MAP kinase pathway
ERK5 pathway
Phosphatidylinositol signaling system
Heterotorimeric G-protein
cAMP
DAG
IP3
Ca2+
CACN
PKA
Rap1
RasGRF
RasGRP
CNasGEF
PKC
NGF
BDNF
NT3/4
TrkA/B
EGF
FGF
PDGF
EGFR
FGFR
PDGFR
GRB2
SOS
Ras
RafB
Raf1
Mos
G12
Gap1m
NF1
p120GAP
Scaffold
MP1
MEK1
MEK2
ERK
PTP
MKP
NIK
IKK
NFκB
Proliferation, inflammation anti-apoptosis
Tau
STMN1
cPLA2
MNK1/2
RSK2
CREB
Elk-1
Sap1a
c-Myc
SRF
DNA
c-fos
Proliferation, differentiation
PPP3C
GSTπ
HSP72
Evi1
FLNA
JIP3
ARRB
CrkII
GLK
HGK
HPK1
Tpl2/Cot
MEKK1
MLK3
MEKK2/3
Scaffold
MKK4
MKK7
JIP1/2
JNK
NFAT-2
NFAT-4
c-JUN
JunD
ATF-2
Elk-1
p53
p53 signaling pathway
Proliferation, differentiation, inflammation
Apoptosis
Cell cycle
Serum, cytotoxic drugs, irradiation, heatshock, reactive oxygen species, lipopolysaccharide and other stress
Cdc42/Rac
PAK1/2
CASP
MST1/2
LZK
MUK
MLTK
AKT
PP2CA
PTP
MKP
TNF
IL1
FASL
TGFB
TNFR
IL1R
FAS
TGFR
TRAF2
GCK
ASK2
ASK1
DAXX
MKK3
MKK6
p38
Sap1a
GADD153
MAX
MEF2C
HSP27
CREB
PRAK
MAPKAPK
MSK1/2
Cdc25B
LPS
CD14
TAB1
TAB2
ECSIT
TAK1
PP2CB
PP5
TRAF6
DNA damage
GADD45
MEKK4
TAO1/2
NLK
Wnt signaling pathway
Serum, EGF, reactive oxygen species, or Src tyrosinkinase downstream
MEK5
ERK5
Nur77
MAPKKKK
MAPKKK
MAPKK
MAPK
Transcription factor


A
mRNA expression levels
128
64
32
16
8
4
2
1
**
***
***
**
*
E9.5
E10.5
E12.5
E9.5
E10.5
E12.5
E9.5
E10.5
E12.5
Scx
Col1a1
Col1a2


B
mRNA expression levels
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
***
***
**
**
*
***
E9.5
E9.5
E10.5
E12.5
E9.5
E10.5
E12.5
E9.5
E10.5
E12.5
E9.5
E10.5
E12.5
Scx
Tnmd
Aqp1
Thbs2
Thbs4

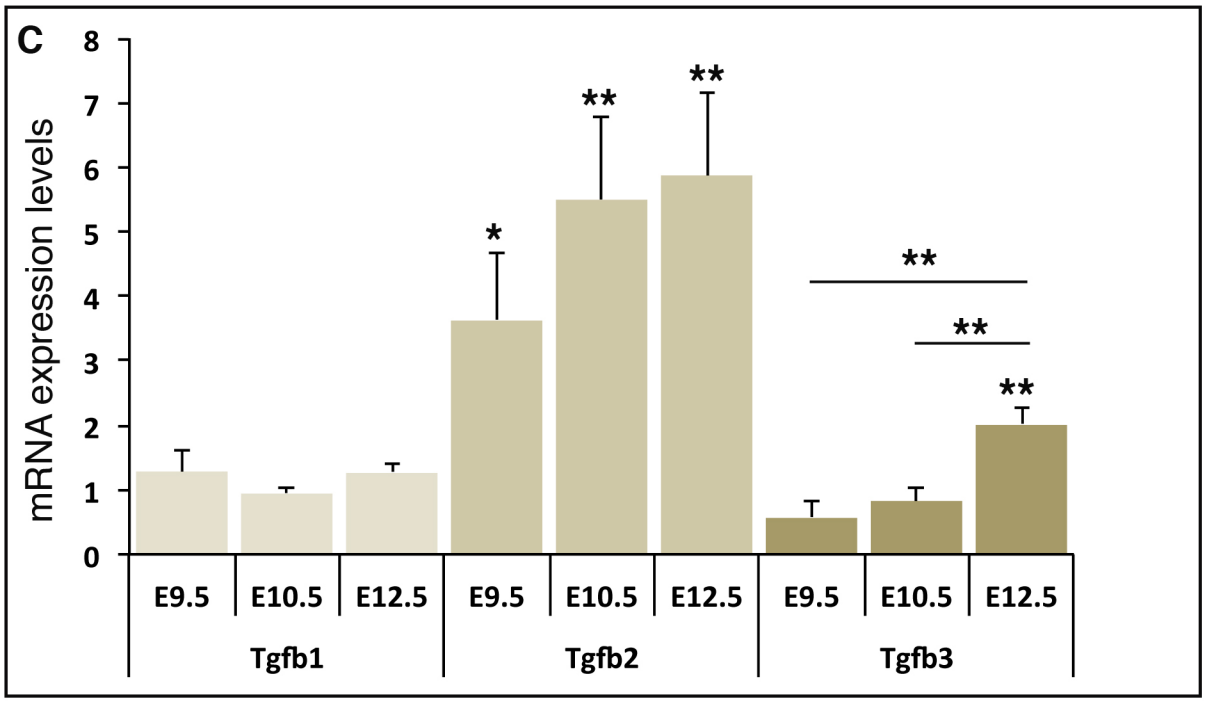

C
mRNA expression levels
8
7
6
5
4
3
2
1
0
*
**
**
**
**
**
E9.5
E10.5
E12.5
E9.5
E10.5
E12.5
E9.5
E10.5
E12.5
Tgfb1
Tgfb2
Tgfb3

Figure S4

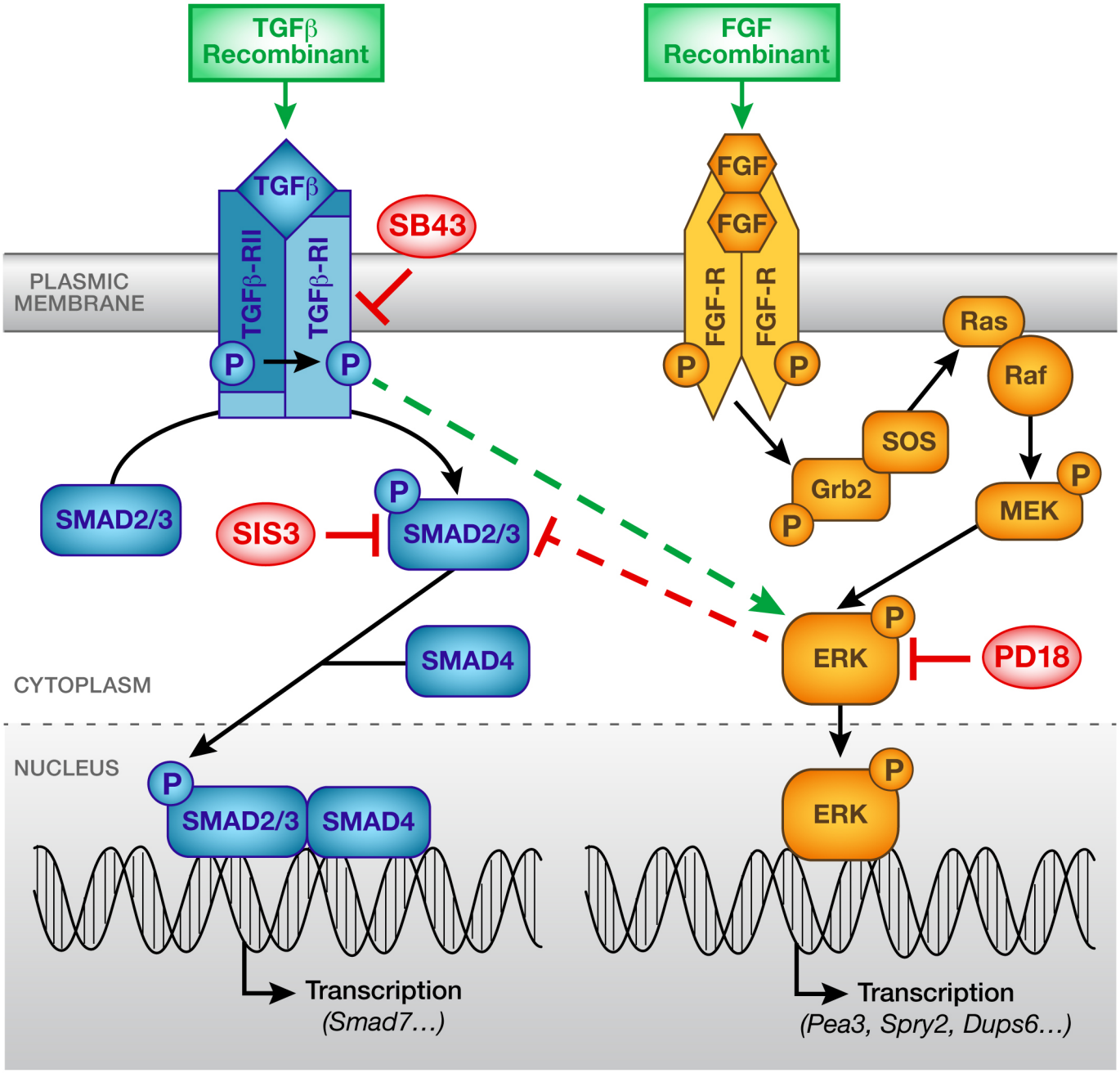
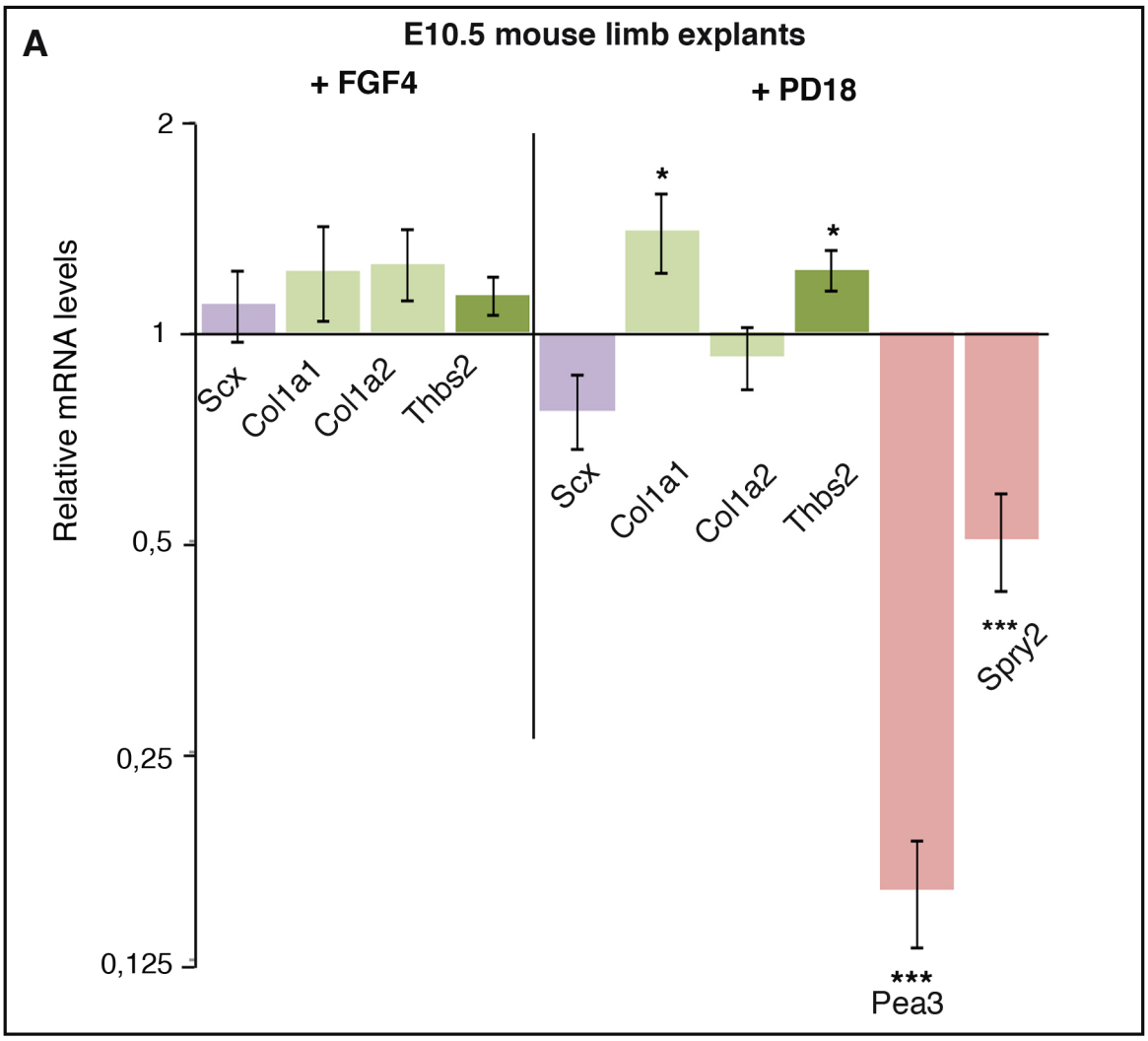

TGFβ
Recombinant
FGF
Recombinant
TGFβ
TGFβ-RII
TGFβ-RI
SB43
PLASMIC
MEMBRANE
FGF
FGF
FGF-R
FGF-R
P
P
P
P
Ras
Raf
SOS
Grb2
MEK
P
P
SMAD2/3
SIS3
P
SMAD2/3
SMAD4
P
ERK
PD18
CYTOPLASM
NUCLEUS
P
SMAD2/3
SMAD4
P
ERK
Transcription
(Smad7…)
Transcription
(Pea3, Spry2, Dups6…)

Figure S5


A
E10.5 mouse limb explants
+ FGF4
+ PD18
Relative mRNA levels
2
1
0,5
0,25
0,125
Scx
Col1a1
Col1a2
Thbs2
Scx
Col1a1
Col1a2
Thbs2
Spry2
Pea3
*
*
***
***

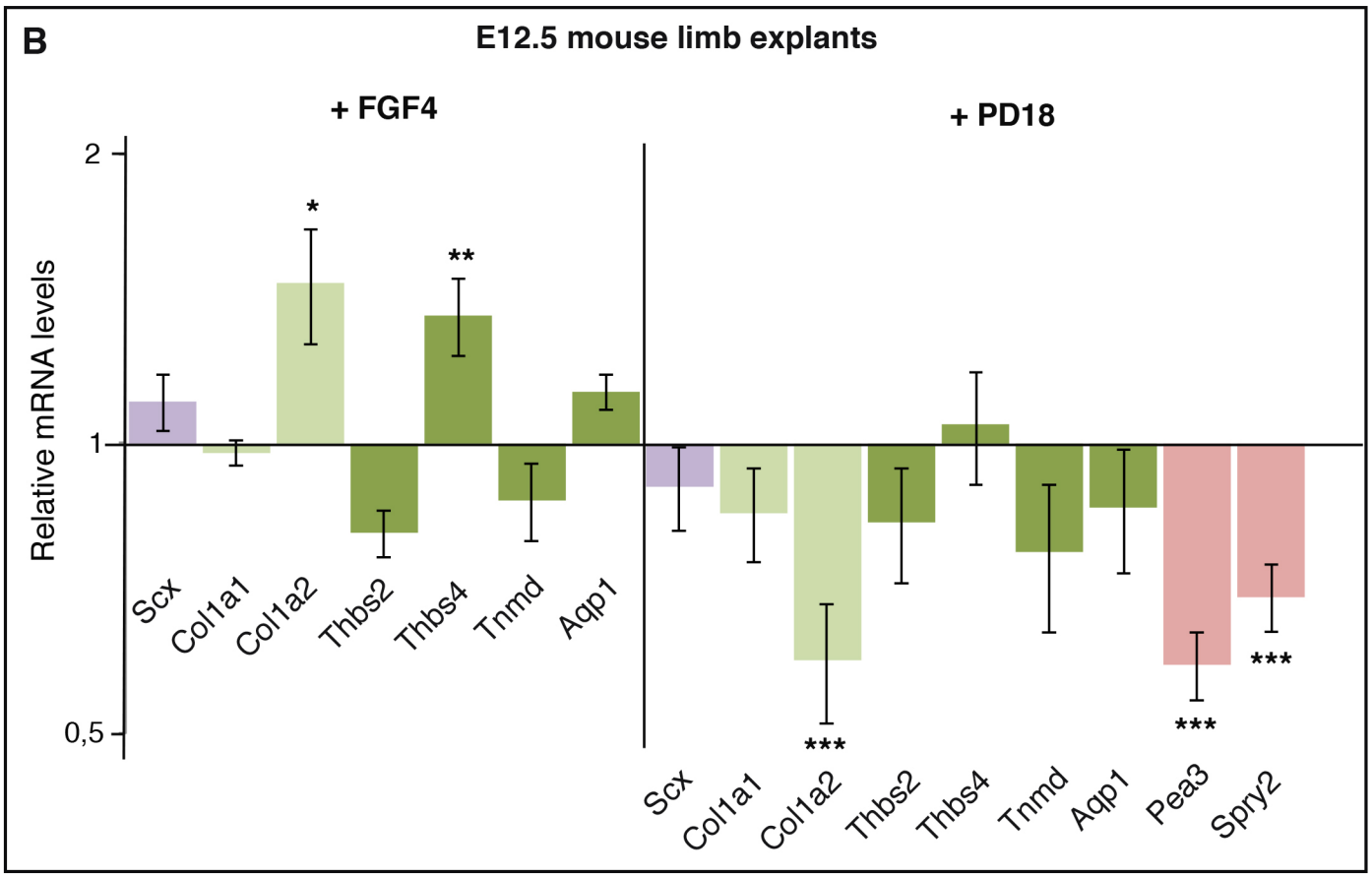

B
E12.5 mouse limb explants
+ FGF4
+ PD18
Relative mRNA levels
2
1
0,5
Scx
Col1a1
Col1a2
Thbs2
Thbs4
Tnmd
Aqp1
Scx
Col1a1
Col1a2
Thbs2
Thbs4
Tnmd
Aqp1
Pea3
Spry2
*
**
***
***
***

Figure S6

Download Table S1

Table S2. List of upregulated and downregulated genes of the KEGG pathway N°4350 (TGF-BETA) in limb tendon cells at different stages of development.

			List of upregulated and downregulated genes in tendon cells during development, identified as being part of the TGF-β signalling pathway KEGG N°04350 (**x**) Fold-changes		
Stages of development **(Number of regulated genes / total number of genes in the pathway)**	p-values	Sub-pathways	Extracellular components	Receptors	Intracellular components
E11.5 vs E12.5 **(11 +1 genes / 85)**	2.8 E-3	BMP	Bmp6 (**4,89**)		
		TGF-β	Dcn (**4,75**), Gdf5 (**3,29**), Tgfb2 (**1,75**), Tgfbi (**1,84**), Thbs1 (**1,76**), Thbs2 (**2,11**), Comp/Thbs5 (**2,62**),	TgfbR2 (**2,34**)	
		Activin/Nodal	Fst (**2,47**), Inhba (**2,33**),		Pitx2 (**-1,99**)
E12.5 vs E14.5 **(17 +2 genes / 85)**	2.2 E-2	BMP	Chrd (**2,22**)		Id1 (**-1,9**), Id2 (**-2,1**), Id4 (**2,12**)
		TGF-β	Dcn (**7,70**), Ltpb1 (**2,22**), Tgfb3 (**2,69**), Thbs1 (**2,09**), Thbs2 (**15,43**), Thbs3 (**2,65**), Thbs4 (**13,02**)	TgfbR2 (**2,93**), TgfbR3 (**2,20**)	Zeb2 (**4,18**)
		Activin/Nodal	Fst (**2,18**), Inhba (**2,11**), Inhbb (**4,26**)	Acvr2b (**-2,89**)	Pitx2 (**-2,78**)
E11.5 vs E14.5 **(27 +2 genes / 85)**	5.1 E-3	BMP	Chrd (**2,72**), Bmp5 (**2,47**), Bmp6 (**9,12**),		Id4 (**2,80**)
		TGF-β	Dcn (**36,57**), Ltpb1 (**2,78**), Tgfb2 (**2,14**), Tgfb3 (**3,52**), Tgfbi (**2,80**), Thbs1 (**3,69**), Thbs2 (**32,20**), Thbs3 (**3,42**), Thbs4 (**22,54**), Comp/Thbs5 (**29,05**)	TgfbR1/Alk5 (**-1,93**), TgfbR2 (**6,87**), TgfbR3 (**3,53**)	Cul1 (**-2,17**), E2f5 (**-2,17**), Ppp2rlb (**-1,81**), p107/rbl2 (**3,34**), Smad7 (**-2,06**), Zeb2 (**5,49**)
		Activin/Nodal	Fst (**5,49**), Inhba (**4,93**), Inhbb (**7,44**)	Acvrl1/Alk1 (**2,23**), Acvr1/Alk2 (**1,74**), Acvr2b (**-4,96**)	Pitx2 (**-4,7**)

Table S3. List of upregulated and downregulated genes of the KEGG pathway N°4010 (MAPK) in limb tendon cells at different stages of development.

		List of upregulated and downregulated genes in tendon cells during development, identified as being part of the MAPK signaling pathway KEGG N°4010		
Stages of development (**number of regulated genes / total number of genes in the pathway**)	p-values	Ligands	Receptors	Intra-cellular components
E11.5 vs E12.5 **(17 genes / 259)**	9.21 E-2	Fgf1, Fgf12, Tgfb2	Cacna1d, Cacna2d3, EgfR, TgfbR2	Rac3, Gadd45b, Mapk8ip2 Kinases: Pkca, Pkcb, Rps6ka3/RSK2, Rps6ka4/MSK2 MAPKK: Map2k6 MAPK: Mapk12/p38 DNA-binding proteins: Mef2c
E12.5 vs E14.5 **(34 genes / 259)**	1.77 E-1 (NS)	Fgf7, Fgf11, Fgf12, Pdgfa, Tgfb3	Fas, Cacna2d3, Cacnb4, Cacna1h Traf6, EgfR, Ntrk2, TgfbR2	Rasgrp2, Gadd45a, Gadd45b, Flnc Kinases: Taok3, Rps6ka2, Rps6ka4/MSK2, Rps6ka6 MAPKK: Map2k6 MAPK: Mapk10, Mapk8ip2, Mapk12/p38 MAP Kinase Phosphatases: Dusp3, Dusp6, Dusp8, Dusp9, Dusp10 DNA-binding proteins: Mecom/Evi1, Mef2c, Nfatc2, Nur77/Nr4a1
E11.5 vs E14.5 **(57 genes / 259)**	5.6 E-2	Fgf7, Fgf11, Fgf12, Fgf13, Fgf16, Pdgfa, Pdgfb Tgfb2, Tgfb3	Cacna1d, Cacna2d1 Cacna2d2, Cacna2d3, Cacnb1, Cacnb4, Cacng4, EgfR, FgfR4, Ntrk2, Fas, TgfbR1, TgfbR2, TnfR/Tnfrsf1a	Rac3, Rras, Rasgrp2, Casp3, Ppp3cb, Gadd45a, Gadd45b, Flnc Kinases: Pak1, Prkca, Rps6ka4/MSK2, Taok3 MAPKKK: Map3k4 MAPKK: Map2k6 MAPK: Mapk8/Jnk, Mapk12/p38 MAP Kinase Phosphatases: Dusp1, Dusp3, Dusp6, Dusp8, Dusp9, Dusp10, Dusp16 DNA-binding proteins: c-Fos, c-Jun, Jund, Hspa1b, Hspa2, Hspb1, Nfatc2, Mecom/Evi1, Mef2c, Nur77/Nr4a1, Trp53

Primers used for quantitative RT-PCR

Acvrl1……Fwd 5’ TCCTTCTGCAACCACAACGTGTCT
Rev 5’ ATCAGAGGCAGATGGGCATCAACT
Avcr2b……....Fwd 5’ GCTCATGAACGACTTTGTGGCTGT
Rev 5’ ACTGCTTGTCCTGAAGTGGGAAGA
Aqp1……...Fwd 5’ CAATTCACTTGGCCGCAATGACCT
Rev 5’ TACCAGCTGCAGAGTGCCAATGAT
BmpR1a…….Fwd 5’ TGGGAGTGGATCTGGATTGCCTTT
Rev 5’ TACCAACCTGCCGAACCATCTGAA
Col1a1……Fwd 5’ TGGAGAGAGCATGACCGATG
Rev 5’ GAGCCCTCGCTTCCGTACT
Col1a2……Fwd 5’ CCAGCGAAGAACTCATACAGC
Rev 5’ GGACACCCCTTCTACGTTGT
Col2a1……Fwd 5’ TTCCACTTCAGCTATGGCGA
Rev 5’ GACGTTAGCGGTGTTGGGAG
Gapdh……...…..Fwd 5’ TTGTGGAAGGGCTCATGACC
Rev 5’ TCTTCTGGGTGGCAGTGATG
Hprt ……Fwd 5’AGGGCATATCCAACAACAAACTT
Rev 5’GTTAAGCAGTACAGCCCCAAA
Id1……..Fwd 5’ TGAACGGCGAGATCAGTGCCTT
Rev 5’ AAGATGCGATCGTCGGCTGGAA
Id2…….....Fwd 5’ ATCACCAGAGACCTGGACAGAACC
Rev 5’ ATTCAGATGCCTGCAAGGACAGGA
Id3……..Fwd 5’ CATCTCCCGATCCAGACAGCTGAG
Rev 5’ AGCTCCTCTTGTCCTTGGAGATCA
Id4……..Fwd 5’ ACTCACCGCGCTCAACACT
Rev 5’ AATGCTGTCACCCTGCTTGTTCAC
Pea3…….. Fwd 5’ TCCCCTACCACCATGGAGAG
Rev 5’ GGGAGTCATAGGCACTGGAGTAAA
Scx…….Fwd 5’ CCTTCTGCCTCAGCAACCAG
Rev 5’ GGTCCAAAGTGGGGCTCTCCGTGACT
Smad7……Fwd 5’ CCCTCCTCCTTACTCCAGATACCCAAT

Rev 5’ ATCTGGACAGCCTGCAGTTGGTTT
Sox9……...Fwd 5’ AGTACCCGCATCTGCACAAC
Rev 5’ CCTCCACGAAGGGTCTCTTCT
Spry2……. Fwd 5’ TGCACATCGCTGGAAGAAGAGGAT
Rev 5’ CCAGCAGGCTTAGAACACATCTGA
Tgfb1……. Fwd 5’ TTTGGAGCCTGGACACACAGTACA
Rev5’ TGTGTTGGTTGTAGAGGGCAAGGA
Tgfb2 ……Fwd 5’ GAATAAAAGCGAAGAGCTCGAGG
Rev 5’ GAGGTGCCATCAATACCTGCA
Tgfb3…….Fwd 5’ CGGAGCACAATGAACTGGC
Rev 5’ AAACCTTAGAGGTAATTCCTTTGGG
Tgfbi……..Fwd 5’ GCCCTTGAAATCTTCAAACAGGCGTC
Rev 5’ TCCTCTCCAGTAACCGCTGATAGACA
TgfbR1……..Fwd 5’ ACAACATCAGGGTCTGGATCAGGT
Rev 5’ CCAAACCGACCTTTGCCAATGCTT
TgfbR2……..Fwd 5’ AAATTCCCAGCTTCTGGCTCAACC
Rev 5’ AGCACTCGGTCAAAGTCTCACACA
TgfbR3……..…Fwd 5’ CAGAAGCTGCCAAAGTGTGTGACT
Rev 5’ ATCATGGTCCAGATCATGGTGGCA
Thbs2…….Fwd 5’ AGGTGCATCTCGAGAGAGTCACT
Rev 5’ CTGCAAACACGAGATGGACATTC
Thbs4……..Fwd 5’ AGGGTGTCGGGATCAACTTTGCTA
Rev 5’ ACACACGCTCCATTCTGACACTCA
Tnmd……. Fwd 5’ AACACTTCTGGCCCGAGGTAT
Rev 5’ AAGTGTGCTCCATGTCATAGGTTTT
Zeb2……...Fwd 5’ AGCACCACCTGAAAGAACACCTGA
Rev 5’ AGGACCCAGAATGAGAGAAGCGTT
18S…….Fwd 5’ GGCGACGACCCATTCG
Rev 5’ ACCCGTGGTCACCATGGTA