

SUPPLEMENTARY DATA

FIGURE S1

Absence of *mEgr3* and *mEgr4* expression in embryonic mouse limb tendons.

Adjacent transverse sections of E12.5 (A-D) or E18.5 (E,F) forelimbs were hybridized with mScx (A,F) mEgr1 (B), mEgr3 (C,E) and mEgr4 (D) probes (blue) and then incubated with the MF20 antibody (light brown) in order to visualize muscles (A,B,E,F). (G,H) Sagital sections of E12.5 embryos were hybridized with mEgr4 probe. mEgr3 and mEgr4 transcripts were not detected in E12.5 limbs, while mEgr1 (B, arrow) was detected in mScx expression domains (A, arrow) in forming tendons. In E18.5 limbs, mEgr3 was expressed in muscle spindles (E) but not in tendons visualized by mScx expression on adjacent sections (F), while mEgr4 was not expressed nor in muscle and tendons at this stage (data not shown). (G,H) mEgr4 was nevertheless expressed in the epithelium of the digestive track of E12.5 embryos, providing a positive control for mEgr4 probe. u, ulna, r, radius



FIGURE S2

Multiple expression sites for *Egr1* in chick embryos.

Wings of E11 (A,B) and E8 (C-F) chick embryos were sectioned longitudinally. (G,H) E8 chick embryos were sectioned laterally along the antero-posterior axis. Sections were hybridized with the DIG-labeled antisense probes (blue) for Egr1 (A,C-G), or Scx (B,H) and then incubated with the MF20 antibody (C,G,H) or with the HNK1 antibody (D) in order to visualize muscles and nerves, respectively. Arrows in A indicate Egr1 expression along a tendon visualized by Scx expression on adjacent sections (B). Arrows in C indicate Egr1 expression in tendons near the muscles, while the arrowhead point to the Egr1 expression at the neuromuscular junction. Arrowheads in D indicate Egr1expression along the nerves. Egr1 is also expressed in cartilage cells (E). Arrows in F indicate Egr1expression in forming feather buds. In axial regions, Egr1 transcripts are observed in a sub-region of Scx-positive domain, near axial muscles (G,H, arrows). Ca, cartilage, DRG, Dorsal Root Ganglia, NT, neural tube.



FIGURE S3

Egr2 expression is transient in embryonic chick limb tendons

Chick wings of E14 embryos were sectioned longitudinally. Adjacent sections were hybridized with the DIG-labeled antisense probes (blue) for Egr1 (A,D), Egr2 (B,E) or Scx (C,F) and then incubated with the MF20 antibody (light brown) in order to visualize muscles. Arrows show the Egr1 (A,D) expression and the absence of Egr2 expression (B,E) in tendons labeled with Scx (C,F). Arrowheads in A,B indicate feather buds. Insets in A,B,C show enlargements of feather buds at E14 expressing Egr1 and Egr2 but not Scx. m, muscle.



FIGURE S4

Forced-expression of control GPF vector does not activate the expression of tendon genes in neural tubes.

The neural tubes of chick embryos were electroporated with the pCA β -GFP construct as control. Adjacent transverse sections of different embryos were hybridized with the GFP probe to visualize the ectopic GFP expression, one day after electroporation (A,G, arrows). The corresponding adjacent sections were hybridized with probes for the following tendon-associated markers: *Collal* (B), *Col3a1* (C), *Col5a1* (D), *Col12a1* (E), *Col14a1* (F), *Scx* (H). No expression of all these tendon markers was induced after ectopic GFP expression in the right parts of the neural tubes (B-F,H, arrows). The revelation time of *in situ* hybridization was over left. The adjacent sections corresponding to different embryos are grouped accordingly. NT, neural tube, No, Notochord



FIGURE S5

Scx and Collal transcripts are observed in limb tendons from double Egr1^{-/-} Egr2^{-/-} mutant mice by *in situ* hybridization.

Longitudinal sections of E14.5 (A,B) and E18.5 (C-H) forelimbs from wild-type mice (A,C,E,G) and $Egr1^{-/-}Egr2^{-/-}$ double mutant mice (B,D,F,H) were hybridized with *mScx* (A-D), *mCol1a1* (E-H) probes (blue) and then incubated with the MF20 antibody (A,B, E-H). (C,D) were colored with Hemaluneosin. The expression of *mScx* (B,D) and *mCol1a1* (F,H) is observed in tendons of $Egr1^{-/-}Egr2^{-/-}$ double mutant mice compared to stage-matched control limbs (A,C,E,G). m, muscle. *Col1a1* expressing tendons are arrowed in G and H.