Supplemental Material

PDGFRβ regulates craniofacial development through homodimers and functional heterodimers with PDGFRα

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Supplemental materials provided:

Supplemental Tables (1):

- Supplemental Table S1. Primers used in qRT-PCR analyses.

Supplemental Figure Legends

Supplemental Figures (3)

- Supplemental Figure S1, related to Figure 4. Extracellular matrix proteins with unaltered localization in *Pdgfra* mutant facial blebs.
- Supplemental Figure S2, related to Figure 4. Expression of extracellular matrix transcripts and proteins is unaltered in *Pdgfra* mutant cranial skin.
- Supplemental Figure S3, related to Figure 5. Antibodies used in the proximity ligation assay are highly specific.

Supplemental Tables

Supplemental Table S1. Primers used in qRT-PCR analyses.

Transcript	Forward primer (5' to 3')	Reverse primer (5' to 3')
mB2m	ACTGACCGGCCTGTATGCTA	TGAAGGACATATCTGACATCTCTA
mPdgfra	GATAGTGGAGAACCTGTTGC	TCAGTCTCTGTTCGTCCAGG
mPdgfrb	CTACAATGCCATCAAGAGGG	AACTCCTCATCTACCTGCTG
mItga6	GATGGGAGTCACTGTTCAGA	ATCGCAGAAACTCCAGTCTC
mItgb4	CTTTGATGCCATCCTGCAGA	CCTGTGTCTTGTATTGGGTG
mLama3	GTCAACCTCACCTTGGATCT	GCTTCTTGCCCAAACTGTTC
mLamb3	GTGGATTGACCAAACCTGAG	CTCTTGTCTAGGTCCAGCTG
mLamc2	CCTGCAACTGCCACTCAAAG	CTGGGTCACAGTCACACTTG
mCol4a1	GTCTGGCTTCTGCTGCTCTT	TTTTGTCCTGGTGGTCCATG
mFras1	GCTGGCGGAATTTGCAGTAT	GCAATCCACAGCACTTCTCC
mFrem1	GTTGGGAAACTCTCTCCACA	CGGATGATGTTACAGTCTGG
mFrem2	CATGGCTACCTTCTCAACCT	CAGTCGAAAATGCTGGTTGG
mHspg2	CTGAGGACACAGAGACTGTC	GCTGTACTCAATGGACCGAG

Supplemental Figure Legends

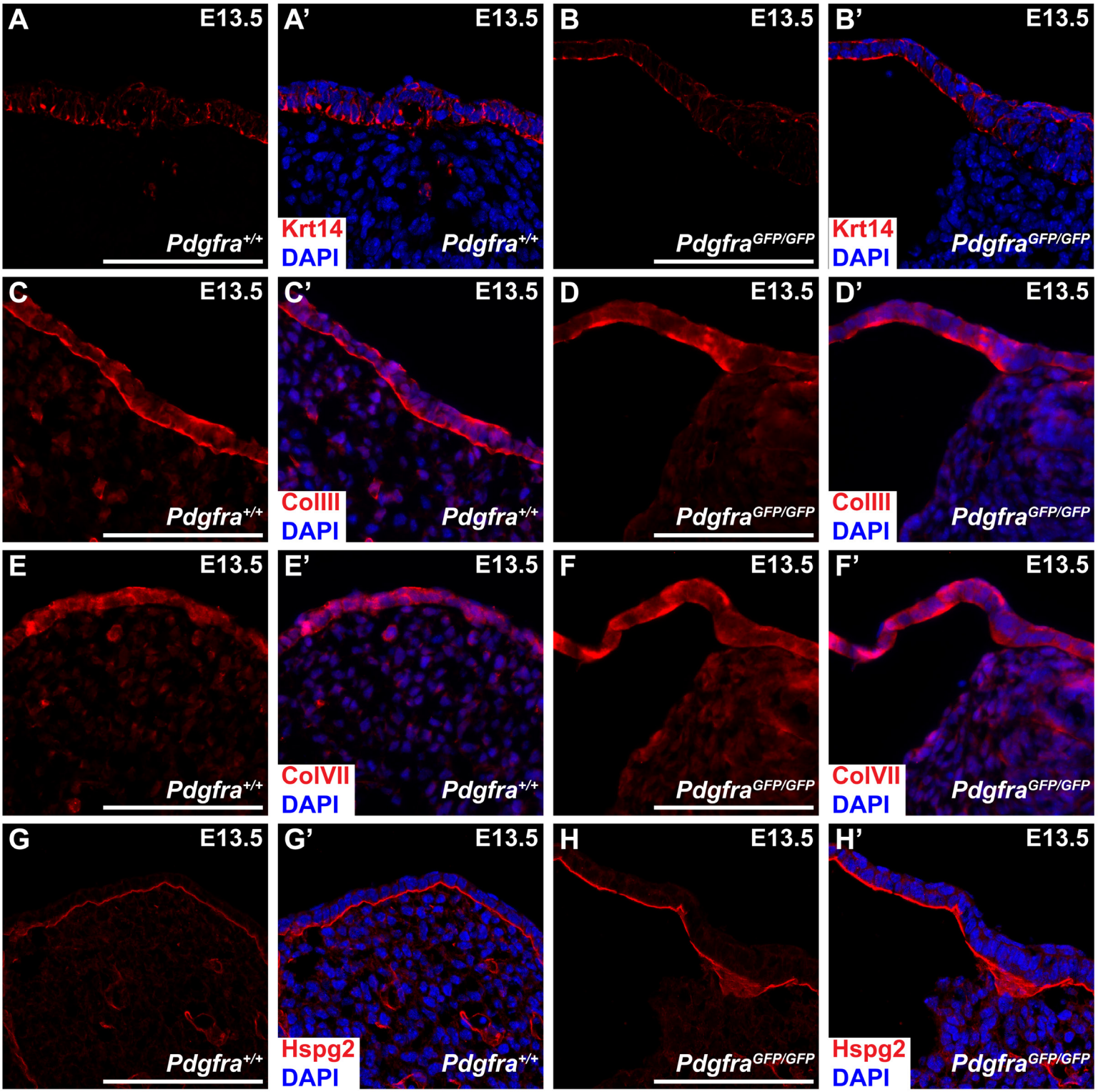
Supplemental Figure S1, related to Figure 4. Extracellular matrix proteins with unaltered localization in *Pdgfra* mutant facial blebs. (*A-H'*) Expression of Keratin 14 (red; *A-B'*), Collagen type III (red; *C-D'*), Collagen type VII (red; *E-F'*) and Hspg2 (red; *G-H'*) as assessed by immunofluorescence analyses of E13.5 wild-type (*A,A',C,C',E,E',G,G'*) versus $Pdgfra^{GFP/GFP}$ (*B,B',D,D',F,F',H,H'*) craniofacial surface tissue. Localization of Keratin 14, Collagen type III, Collagen type VII and Hspg2 were unchanged between wild-type and mutant tissues. Nuclei were stained with DAPI (blue; *A',B',C',D',E',F',G',H'*). Scale bars, 100 μm.

Supplemental Figure S2, related to Figure 4. Expression of extracellular matrix transcripts and proteins is unaltered in *Pdgfra* mutant cranial skin. (*A*) Bar graph depicting qRT-PCR values revealing reduced expression of *Lamb3* in E13.5 $Pdgfra^{GFP/GFP}$ whole cranial skin as compared to that of wild-type. No significant changes in transcript expression in the mutant samples were detected for *Itga6, Itgb4, Lama3, Lamc2, Col4a1, Fras1, Frem1, Frem2* and *Hspg2*. Data are presented as mean ± SEM. n.s., not significant. *, $p<0.05$. (*B-D*) Western blot analysis of E13.5 whole cranial skin lysates revealed similar levels of Integrin α6 (*B*), Collagen type IV (*C*) and Frem1 (*D*) protein between wild-type and $Pdgfra^{GFP/GFP}$ samples. β-tubulin served as a loading control. WB, Western blot.

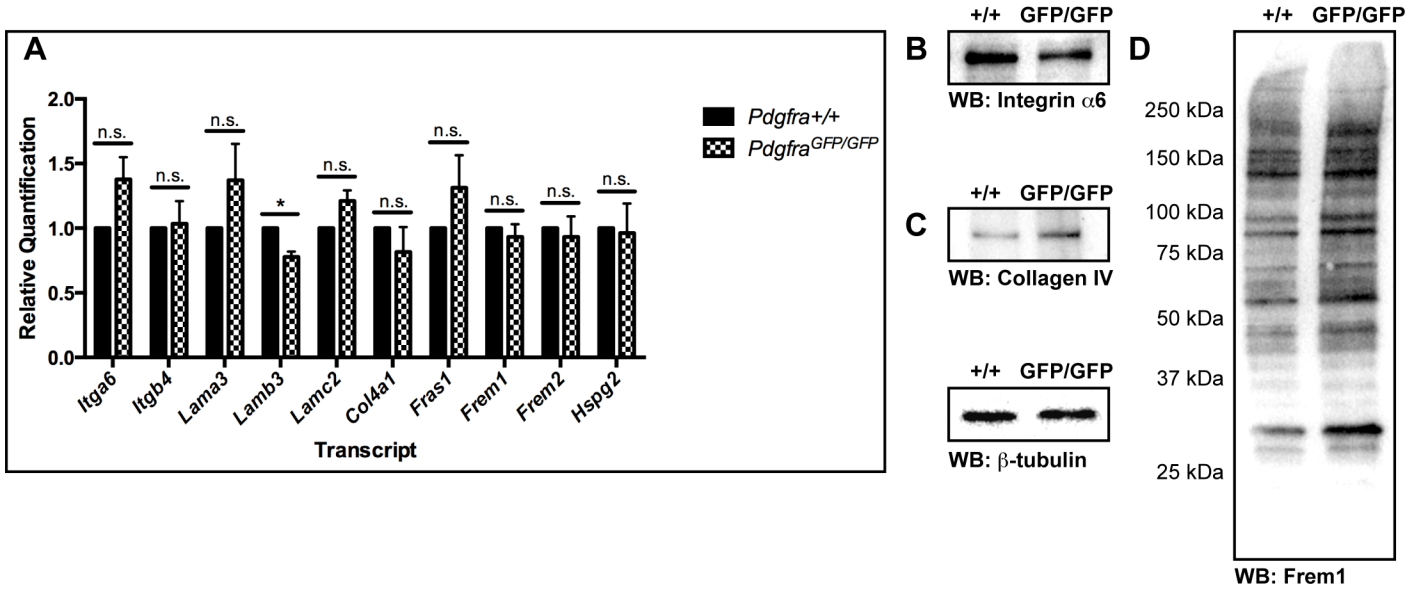
Supplemental Figure S3, related to Figure 5. Antibodies used in the proximity ligation assay are highly specific. (*A-B'*) Expression of PDGFRα (red) as assessed by

immunofluorescence analyses in E12.5 *Pdgfra*$^{fl/fl}$*;Wnt1-Cre*$^{+/+}$ (*A,A'*) and *Pdgfra*$^{fl/fl}$*;Wnt1-Cre*$^{+/Tg}$ (*B,B'*) craniofacial sections. Note the significant reduction in fluorescence intensity detected in the mandibular mesenchyme of the conditional knock-out embryo. Expression of PDGFRα in the epithelium of both genotypes is non-specific. (*C-D'*) Expression of PDGFRβ (red) as assessed by immunofluorescence analyses in E13.5 *Pdgfrb*$^{fl/fl}$*;Wnt1-Cre*$^{+/+}$ (*C,C'*) and *Pdgfrb*$^{fl/fl}$*;Wnt1-Cre*$^{+/Tg}$ (*D,D'*) craniofacial sections. Note the significant reduction in fluorescence intensity detected in the maxillary and mandibular mesenchyme of the conditional knock-out embryo. Nuclei were stained with DAPI (blue; *A',B',C',D'*). Md, mandible; Mx, maxilla. Scale bars, 100 μm.

Fantauzzo_FigS1



Fantauzzo_FigS2



Fantauzzo_FigS3