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Department of Animal Sciences

Mesenchymal progenitor cells in intramuscular connective tissue development

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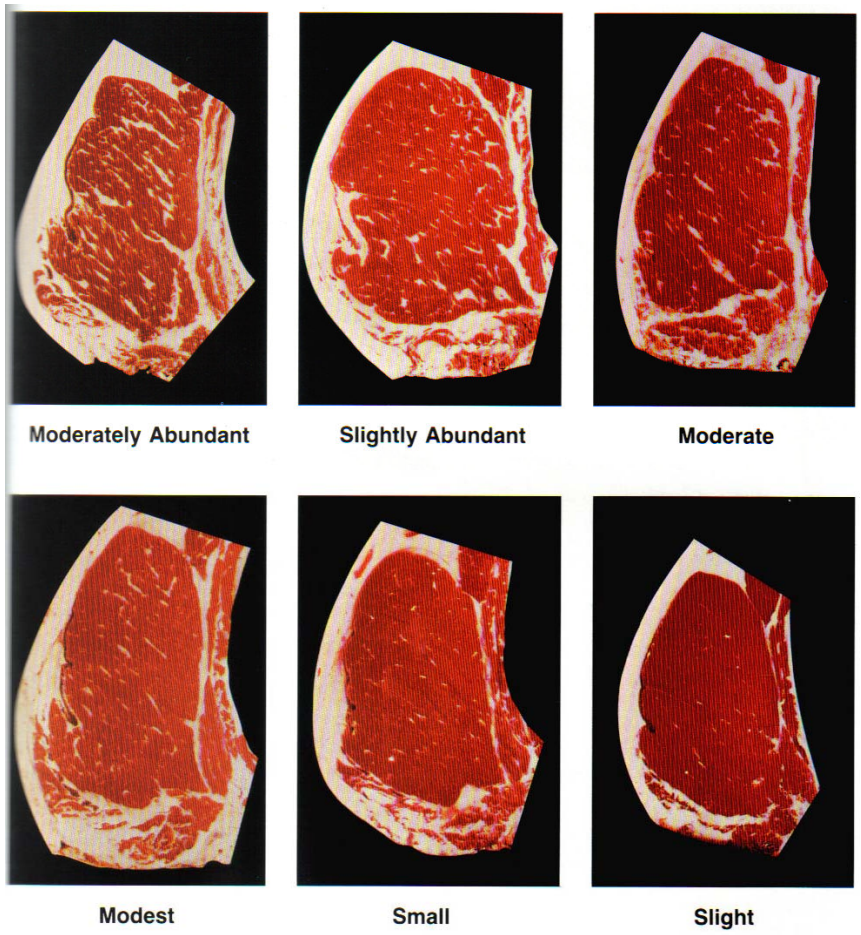
Beef Quality: marbling and tenderness

- Beef quality is mainly determined by marbling and tenderness.
- Marbling is the primary criterion for grading beef carcasses.
- Only carcasses with moderate to abundant marbling qualify for high quality grades.



Beef Quality: marbling and tenderness

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Beef tenderness is determined by:

- Myofibrillar effect: aging, stretching carcasses improves tenderness.
- Background toughness: connective tissue, mainly collagen and its cross-linking.
  - Both collagen content and cross-linking increase as animals become older.
  - Question: How to reduce collagen content and cross-linking in muscle?

Tenderness remains a top problem for beef

Collagen content and cross-linking, two critical control points:

- Fibroblast … er fibrotic proteins in connec…
- Fibroblasts secrete enzymes catalyzing collagen cross-linking.

How to reduce fibroblasts?

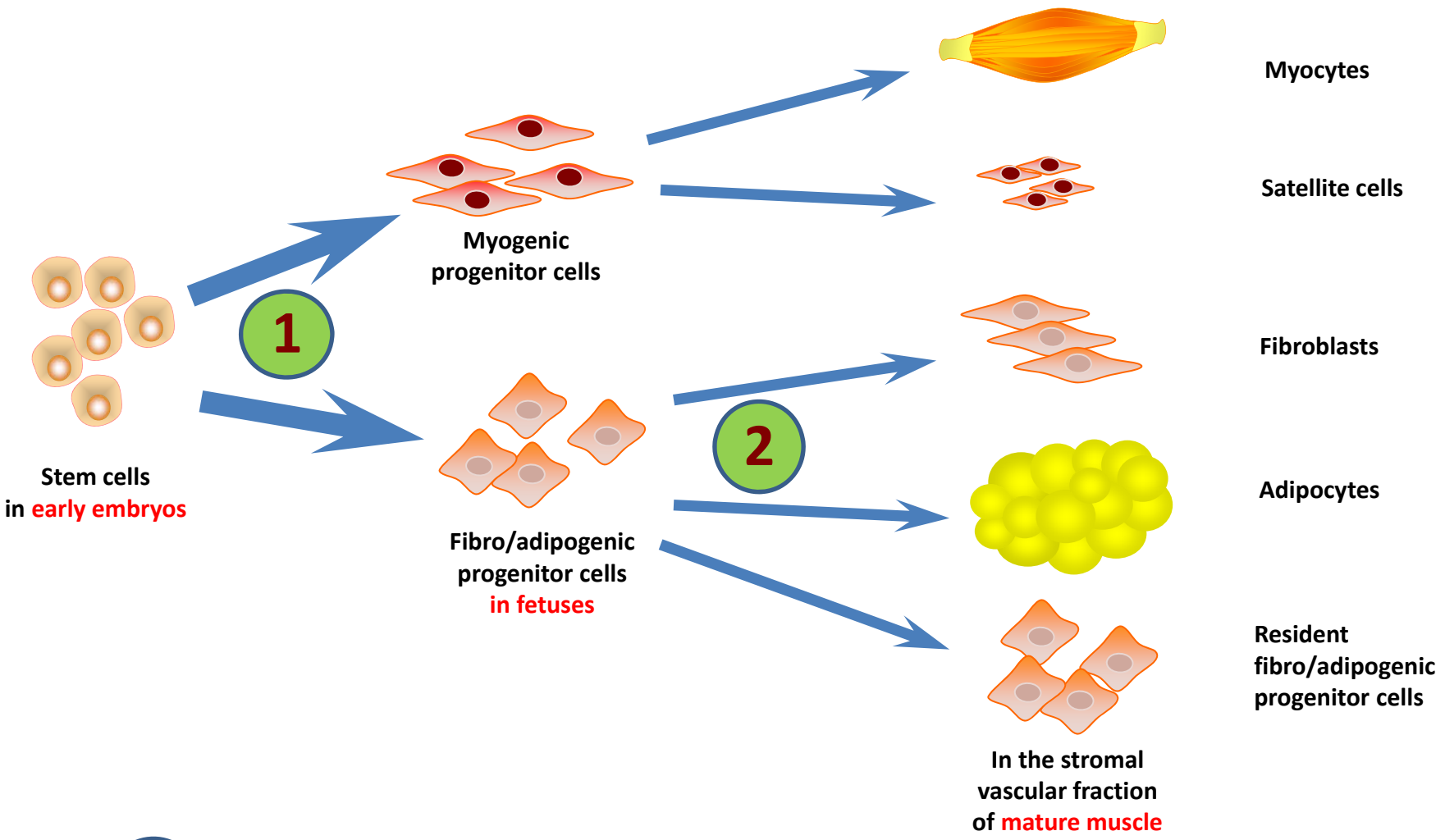
Collagen content

Collagen cross-linking

Tenderness remains a top problem for beef

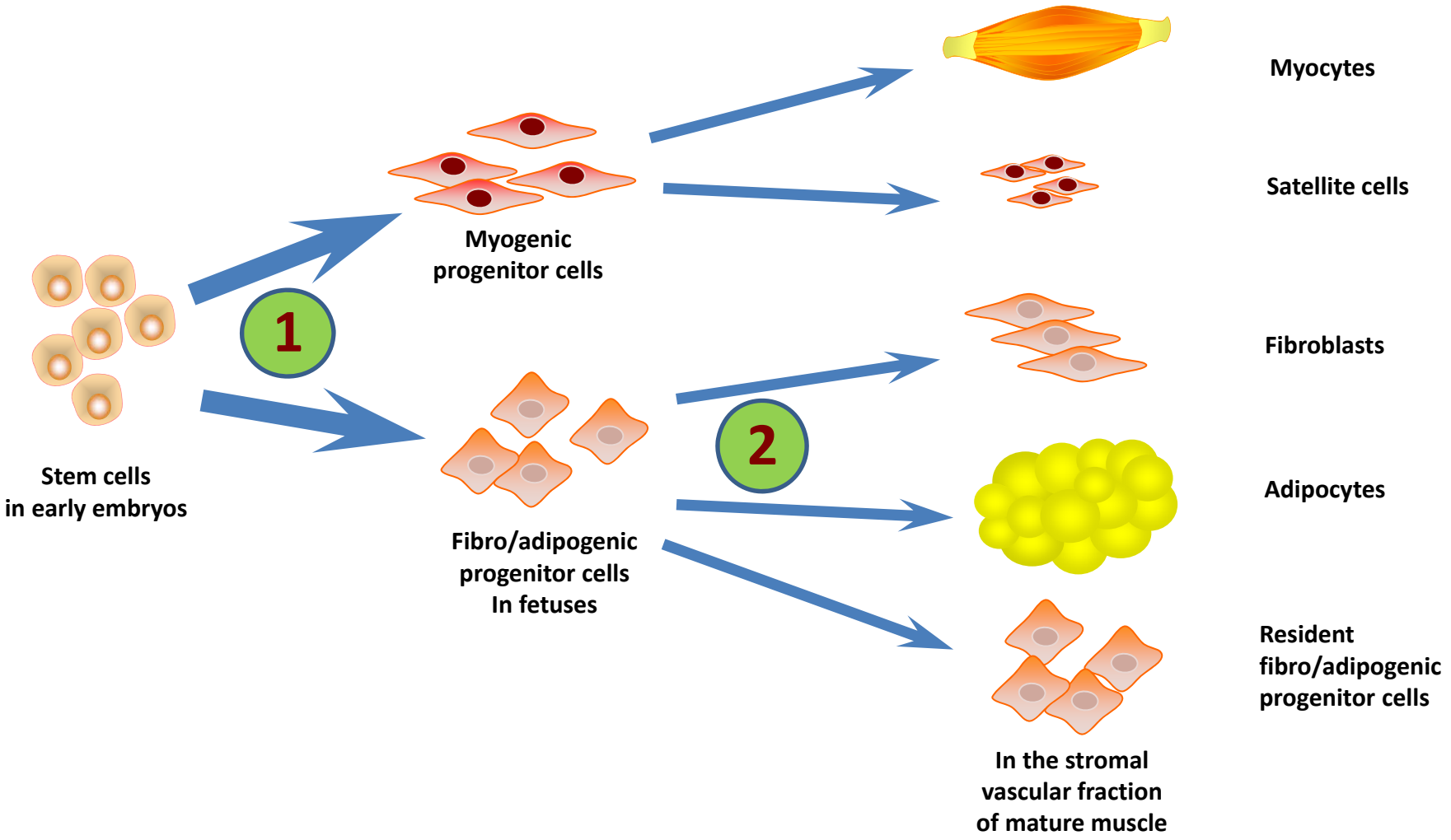
How to reduce fibroblasts?

- Fibroblasts are derived from mesenchymal multipotent cells, primarily during the early developmental stage.
- More importantly, fibroblasts and adipocytes share a common immediate progenitor cells, so called fibro/adipogenic cells (FAPs).

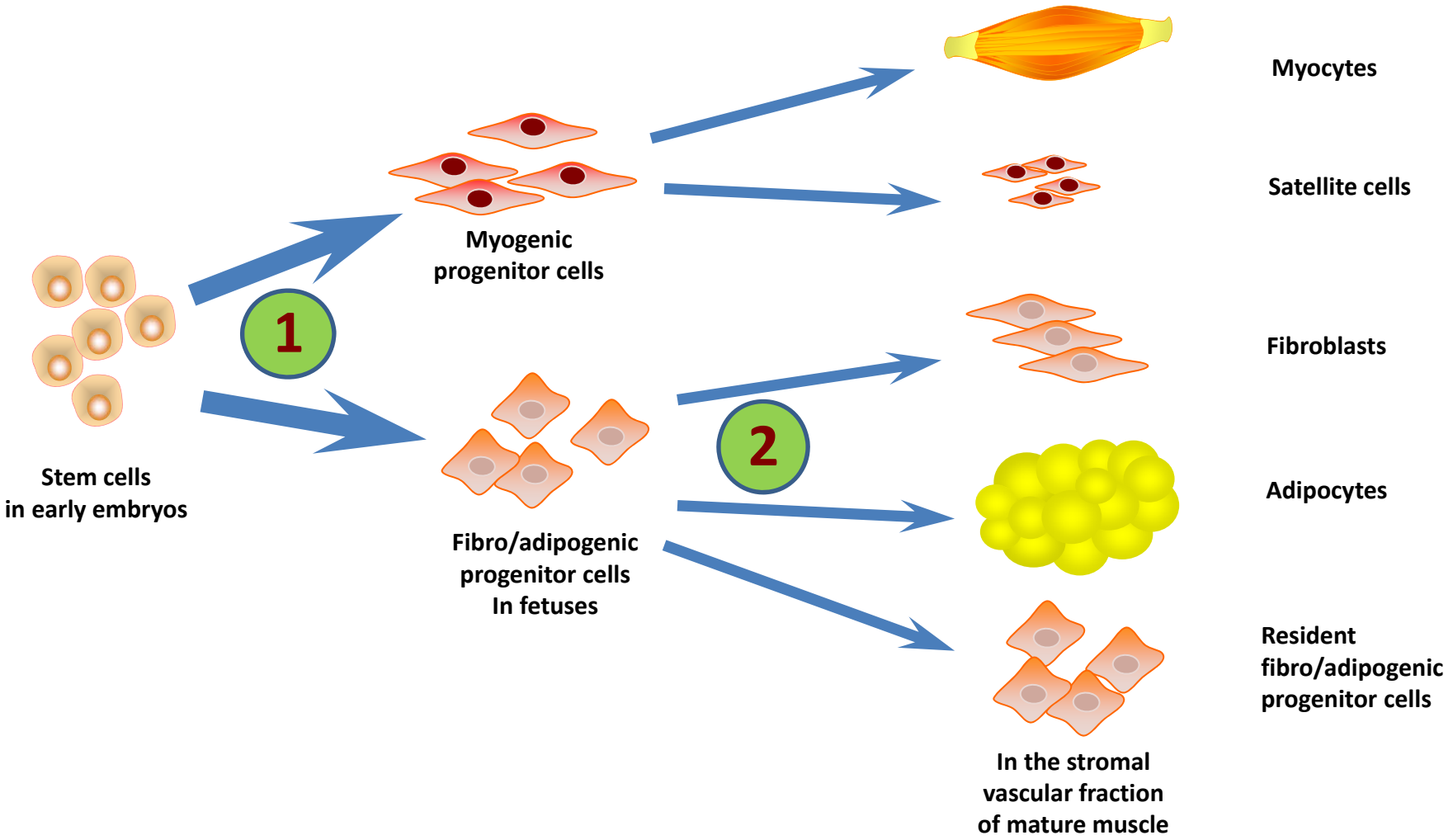


1 : Why do some stem cells become myogenic cells, while others become fibro/adipogenic cells?

2 : Why do some progenitor cells become adipocytes, while others become fibrogenic cells?



- The mechanisms regulating mesenchymal progenitor cell commitment to myogenesis, adipogenesis and fibrogenesis are poorly defined, especially in livestock.



- Tumor necrosis factor (TGF) β signaling pathway and Zfp423 transcription factor appear to have critical roles in determining progenitor commitments to either adipogenic or fibrogenic lineages.

Transforming growth factor (TGF)β and fibrogenesis

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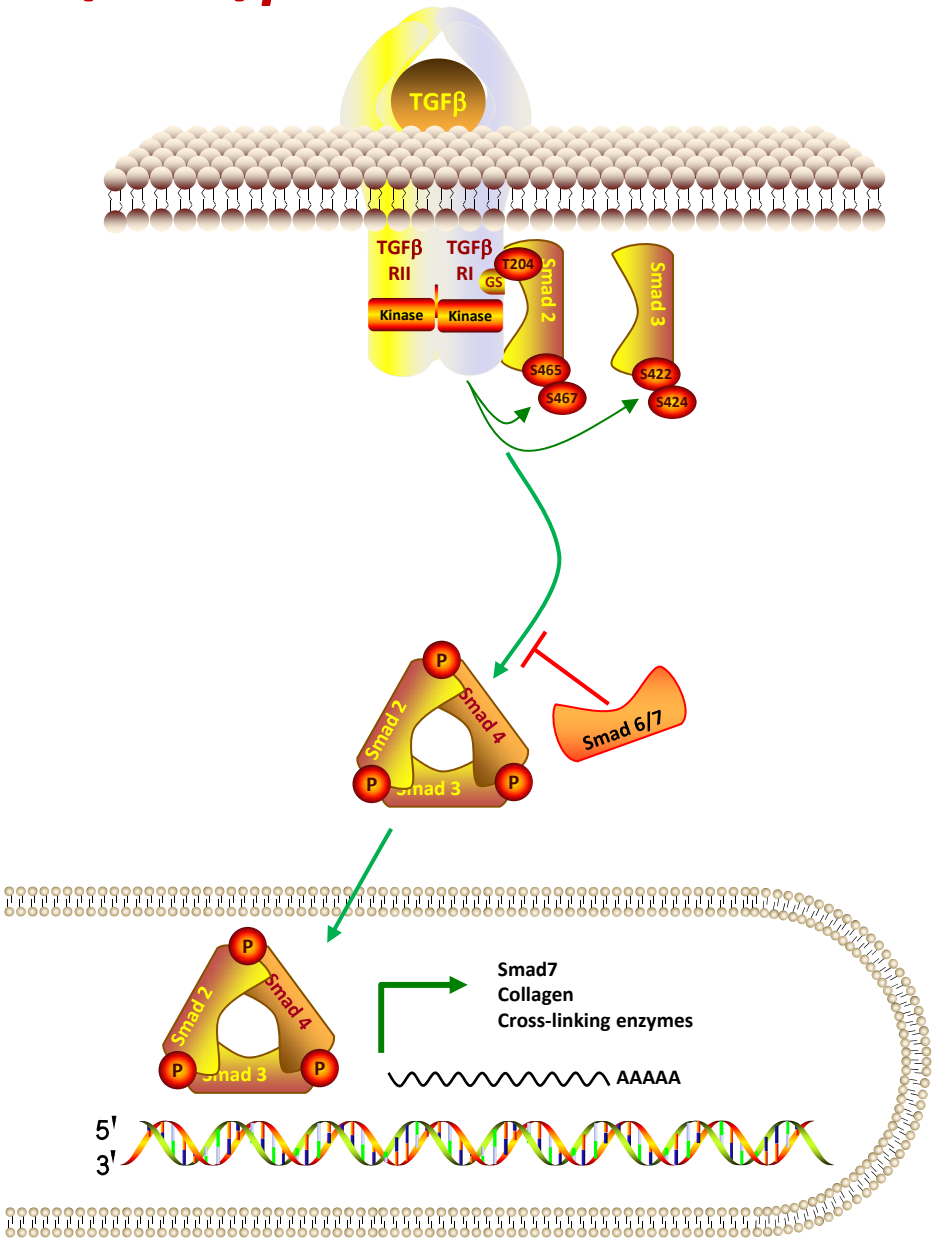
- TGFβ is a major signaling pathway promoting fibrogenesis.
- TGFβ signaling is enhanced by inflammation and other factors.
- Obesity is known to induce inflammation and TGFβ signaling.
- Fetal stage is critical for fibrogenesis.



Experimental design

- **Objective:** Thus, a maternal obesity sheep model was used to assess the role of TGF-β signaling on fibrogenesis during early development.
- **Animals:** Non-pregnant ewes were assigned to a control diet (Con, fed 100% of NRC nutrient recommendations, *n* = 6) or obesogenic diet (OB) fed 150% of NRC recommendations, *n* = 6) from 60 days before conception. Fetal *semitendinosus* (St) muscle was sampled at 135 days of gestation (term 148 days).
- **Methods:** Histochemical analyses, Hydroxyproline assay, Real-time PCR, Western blot analyses, Electrophoretic mobility shift assay (EMSA).

Huang et al., AJP- Endocrinology and Metabolism 298:1254-1260, 2010

Enhanced tumor necrosis factor (TNF)α expression in OB fetal muscle

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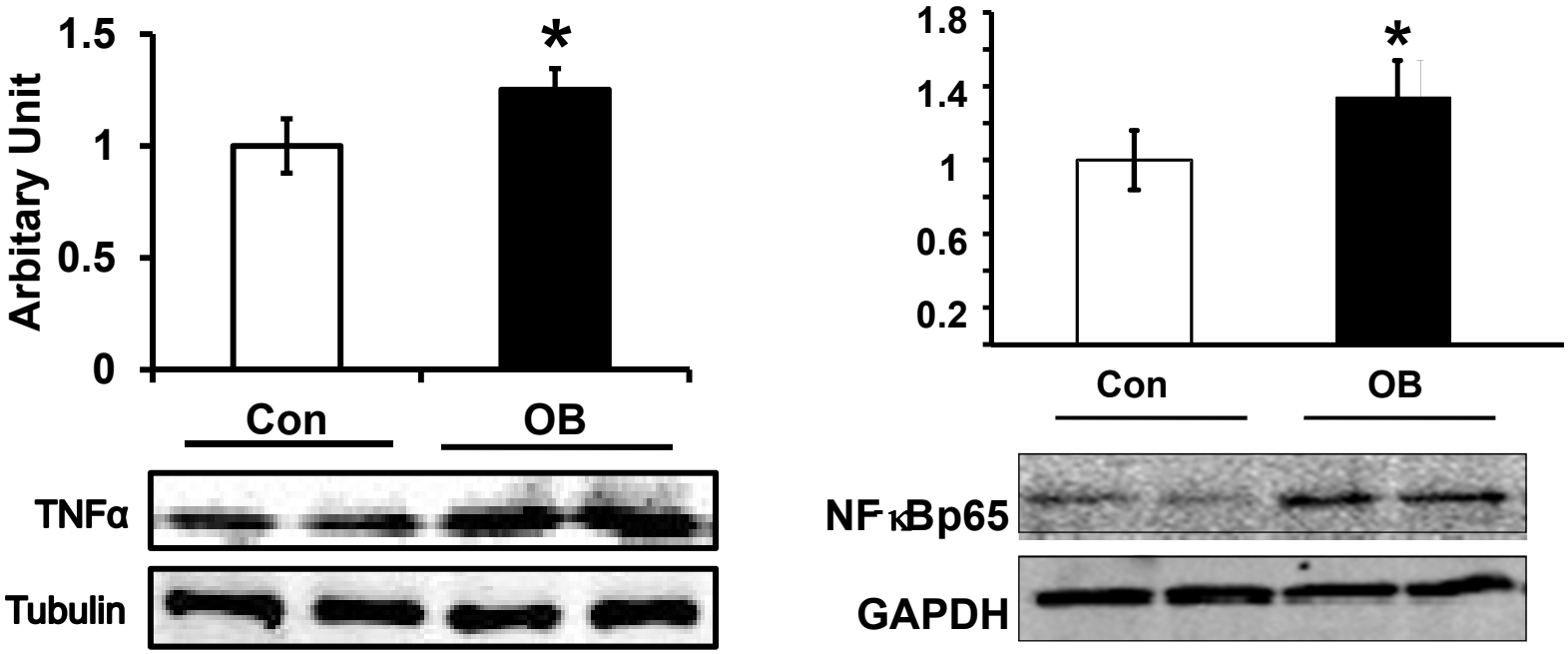

TNFα: A marker of systemic inflammation.
NF-κB: A major inflammatory signaling.

Xu et al., Endocrinology, 2010, 151: 380.

Transforming growth factor (TGF)β content increased in OB fetal muscle

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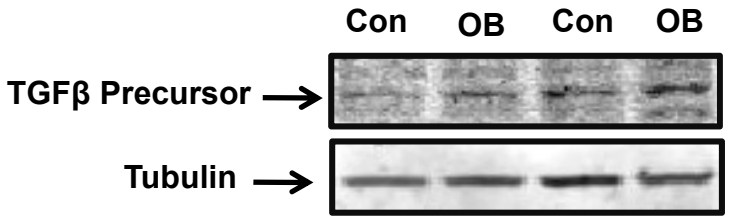

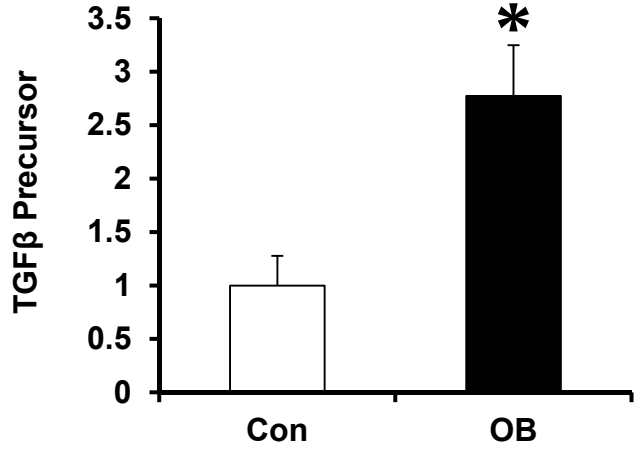

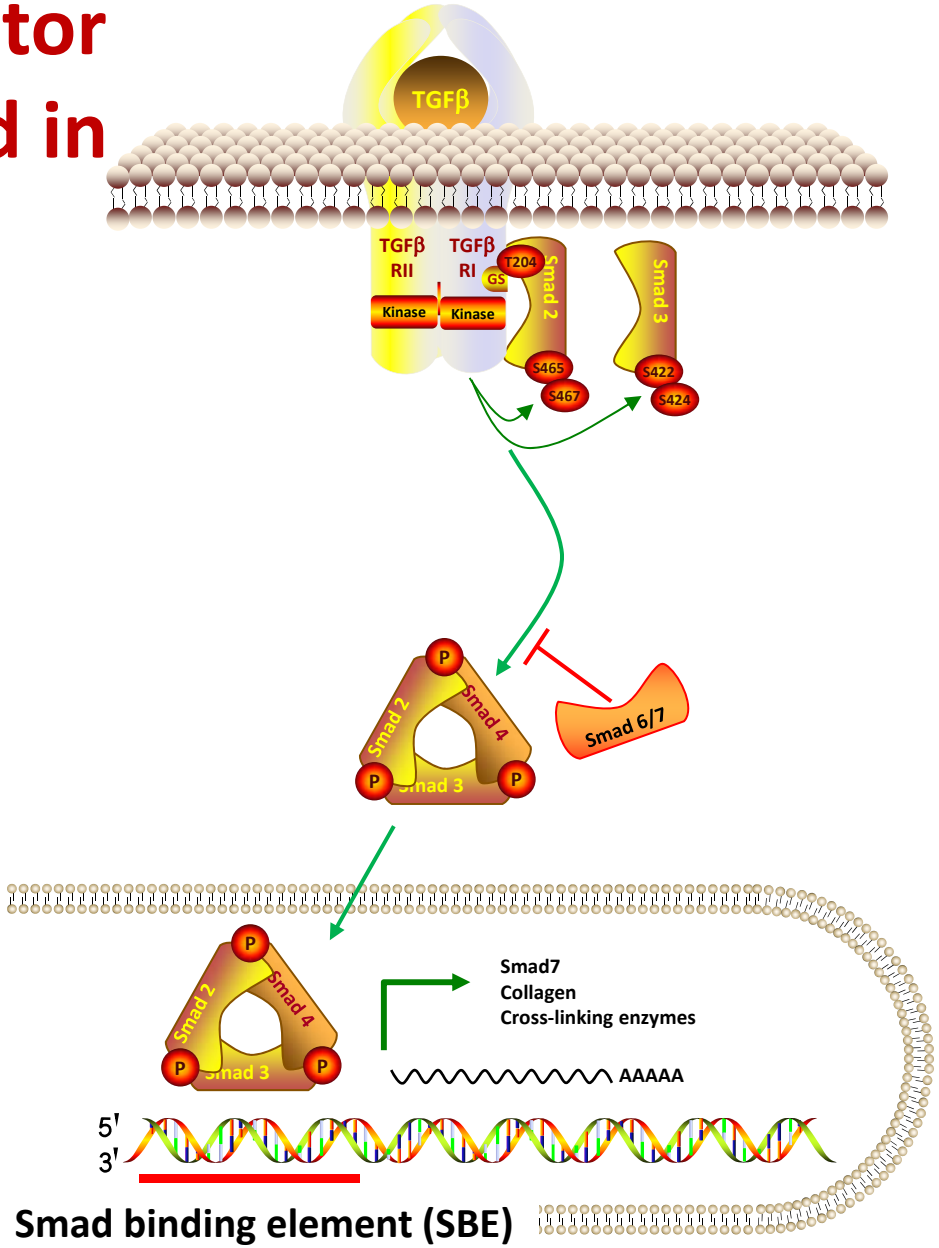

Huang et al., AJP- Endocrinology and Metabolism 298:1254-1260, 2010

Binding to Smad responsive element was increased in OB fetal muscle

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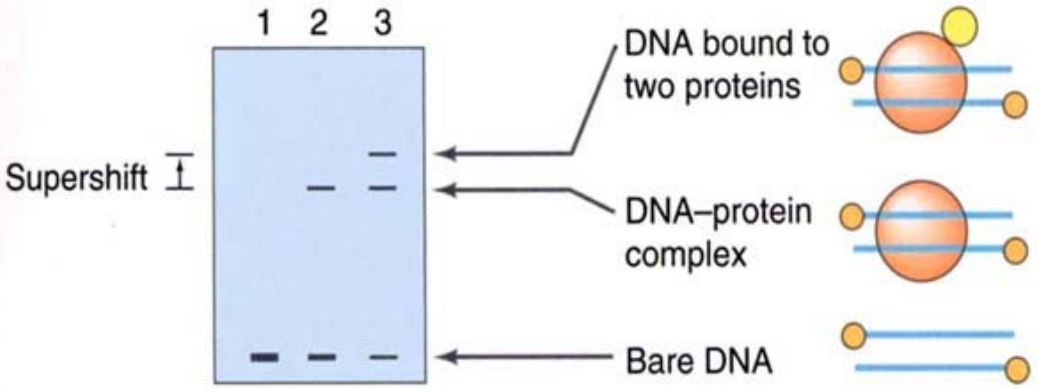
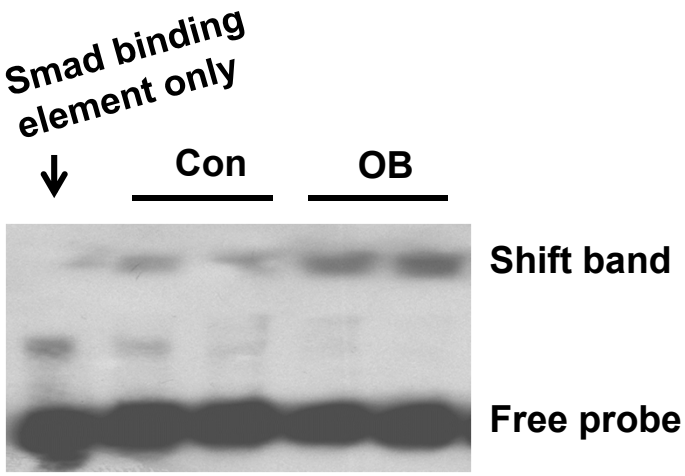
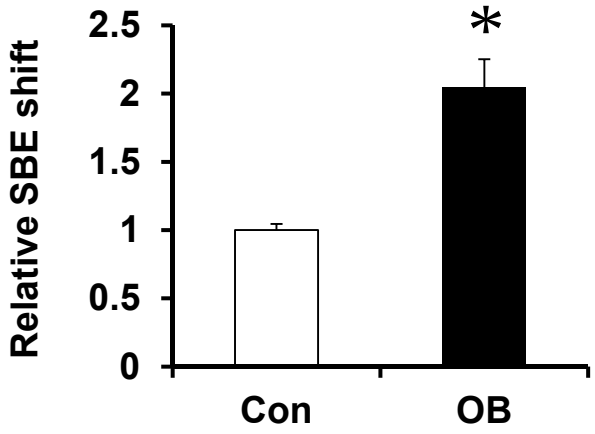


P32 labeled Smad binding elements





Huang et al., AJP- Endocrinology and Metabolism 298:1254-1260, 2010

Expression of Smad7 was enhanced in OB fetal muscle

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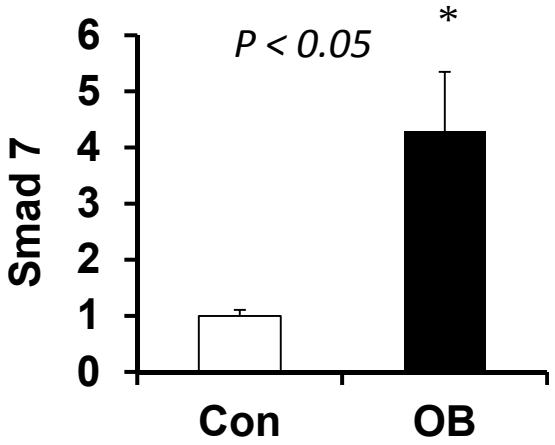

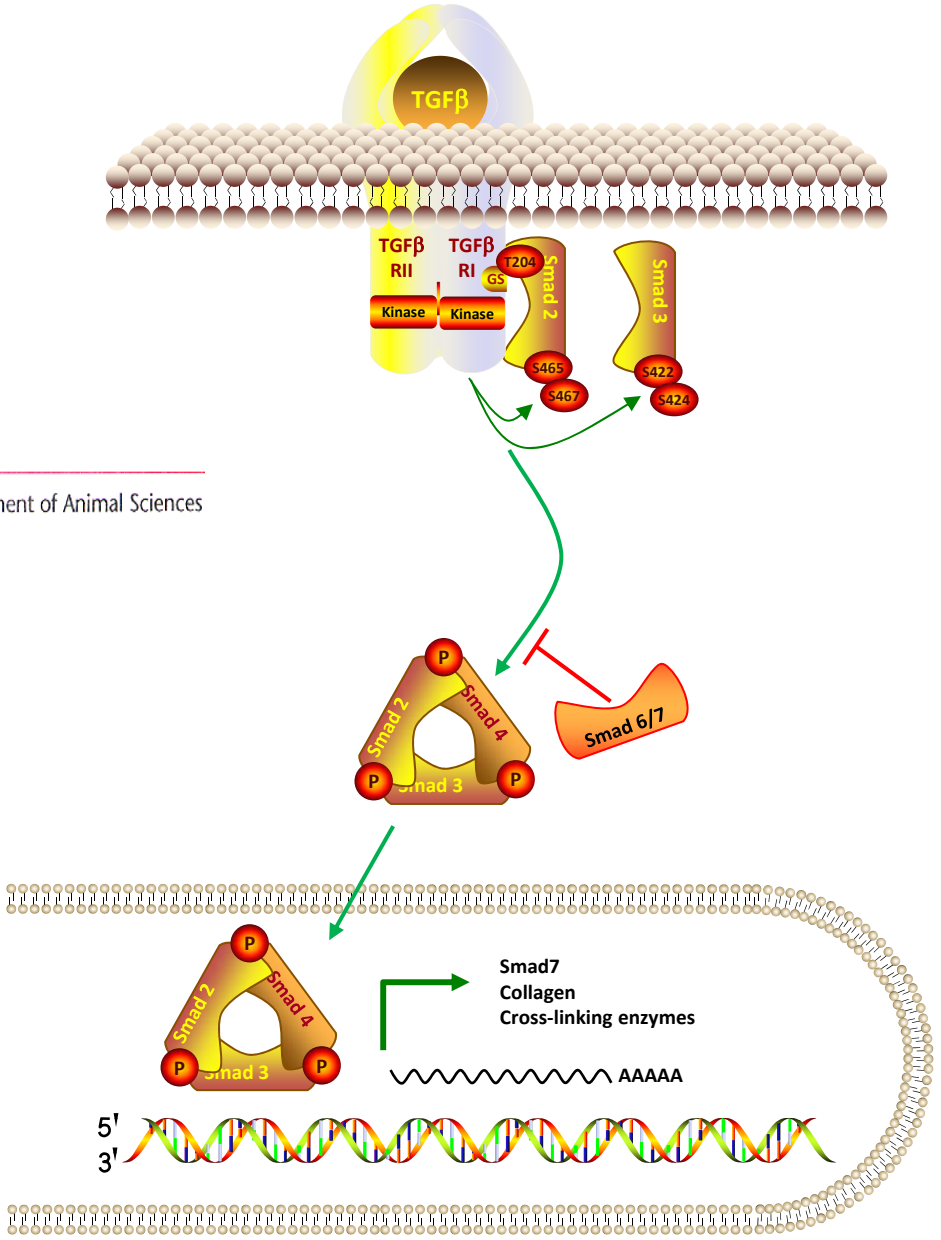

Huang et al., AJP- Endocrinology and Metabolism 298:1254-1260, 2010

Expression of TGFβ target genes was enhanced (Connective tissue content)

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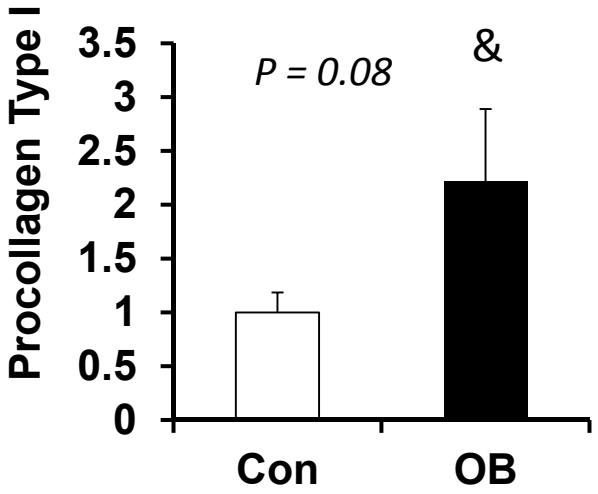

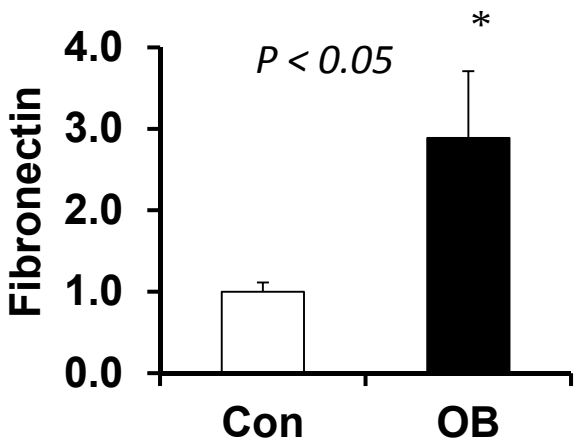

Procollagen: Precursor of collagen.
Fibronectin: Extracellular matrix glycoprotein.

Huang et al., AJP- Endocrinology and Metabolism 298:1254-1260, 2010

Expression of TGFβ target genes was enhanced (Collagen cross-linking)

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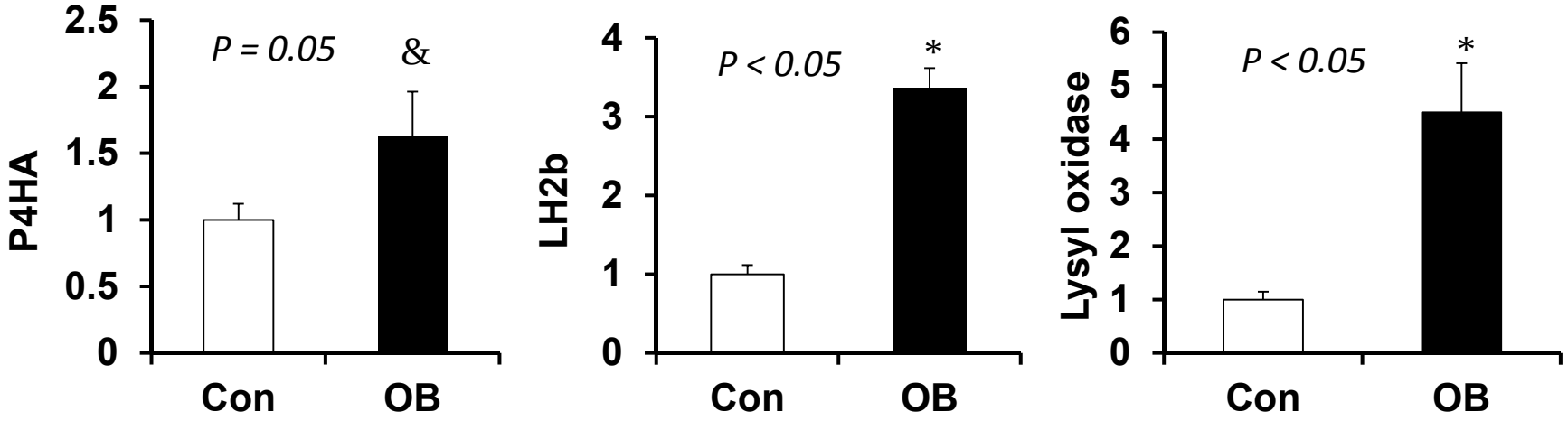

P4HA: prolyl 4-hydroxylase, formation of hydroxyproline.
LH2b: lysyl hydroxylase-2b, collagen cross-linking.
Lysyl oxidase: amine oxidase, collagen cross-linking.

Huang et al., AJP- Endocrinology and Metabolism 298:1254-1260, 2010

Transforming growth factor (TGF)β and fibrogenesis

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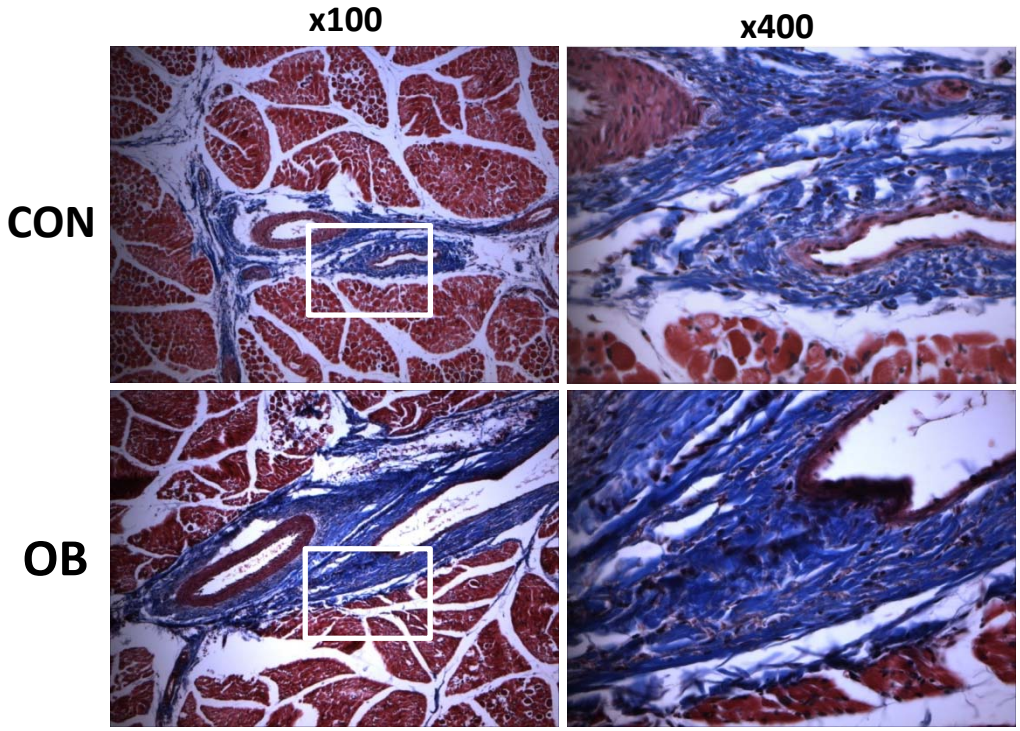
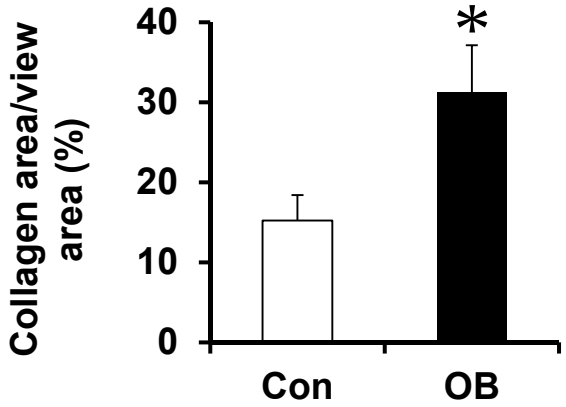
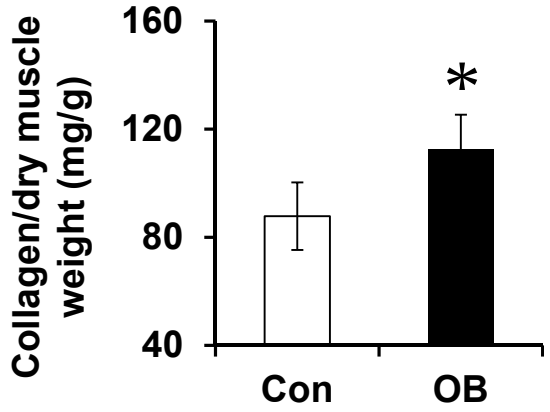
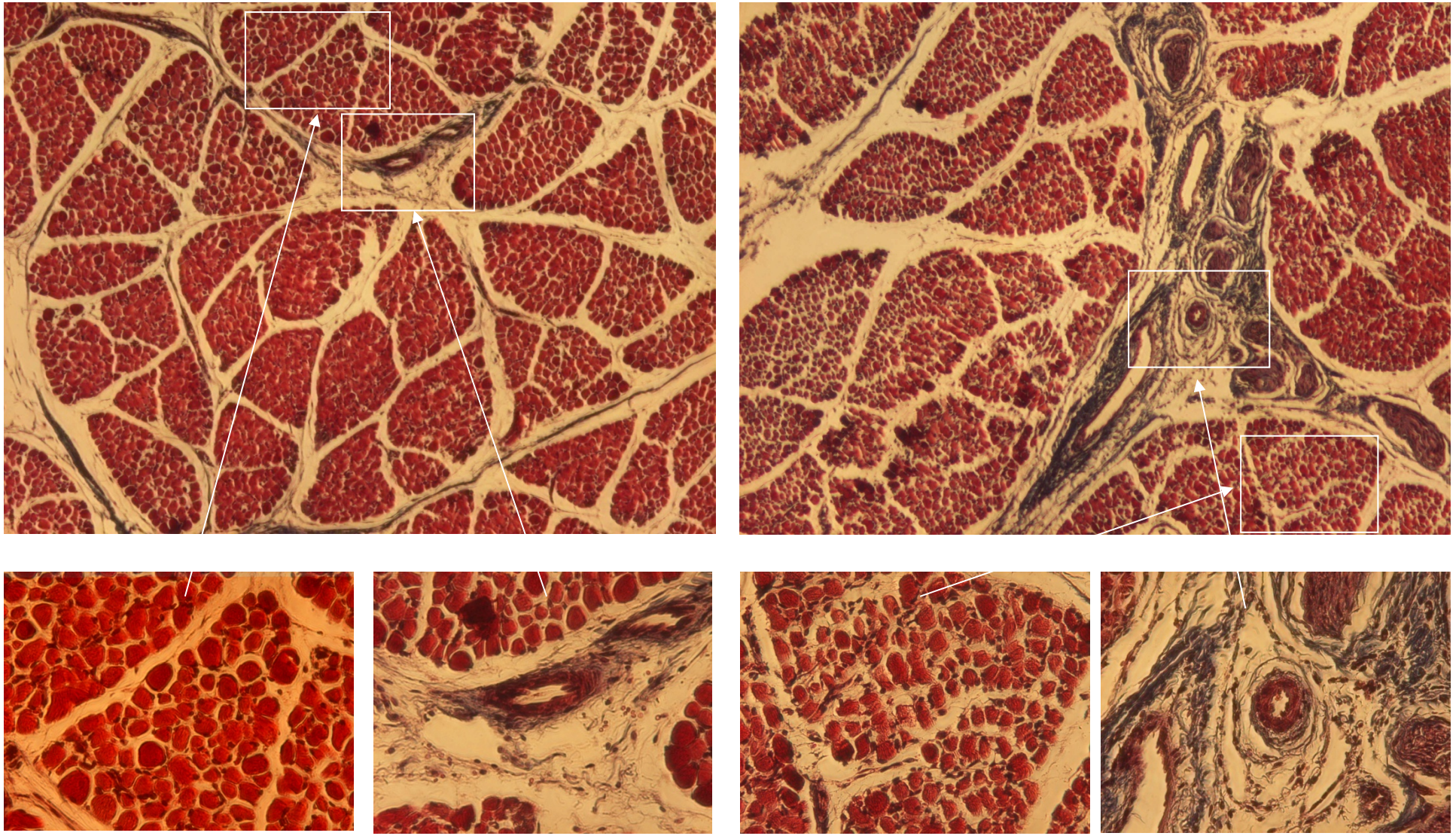






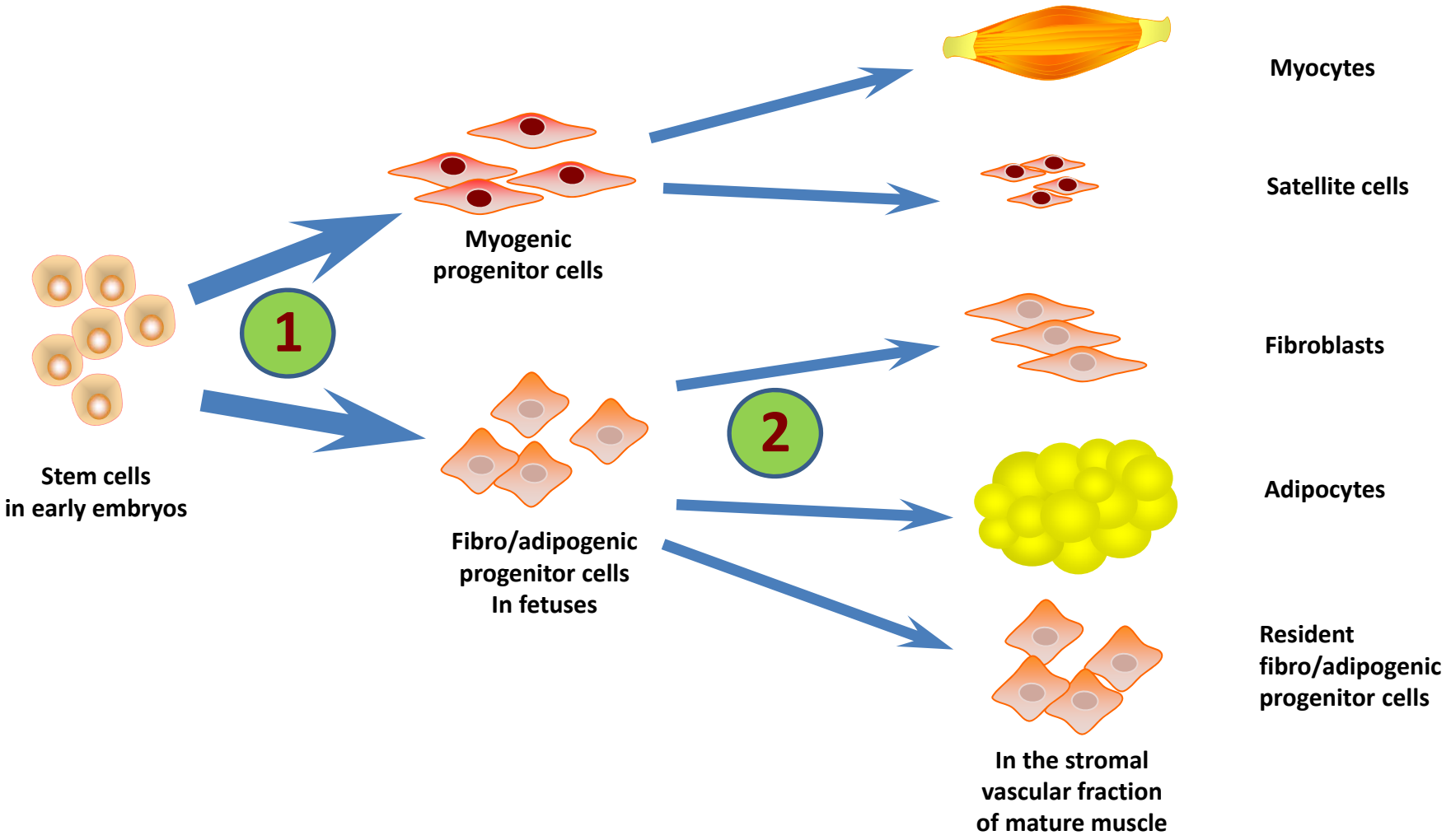
Huang et al., AJP- Endocrinology and Metabolism 298:1254-1260, 2010



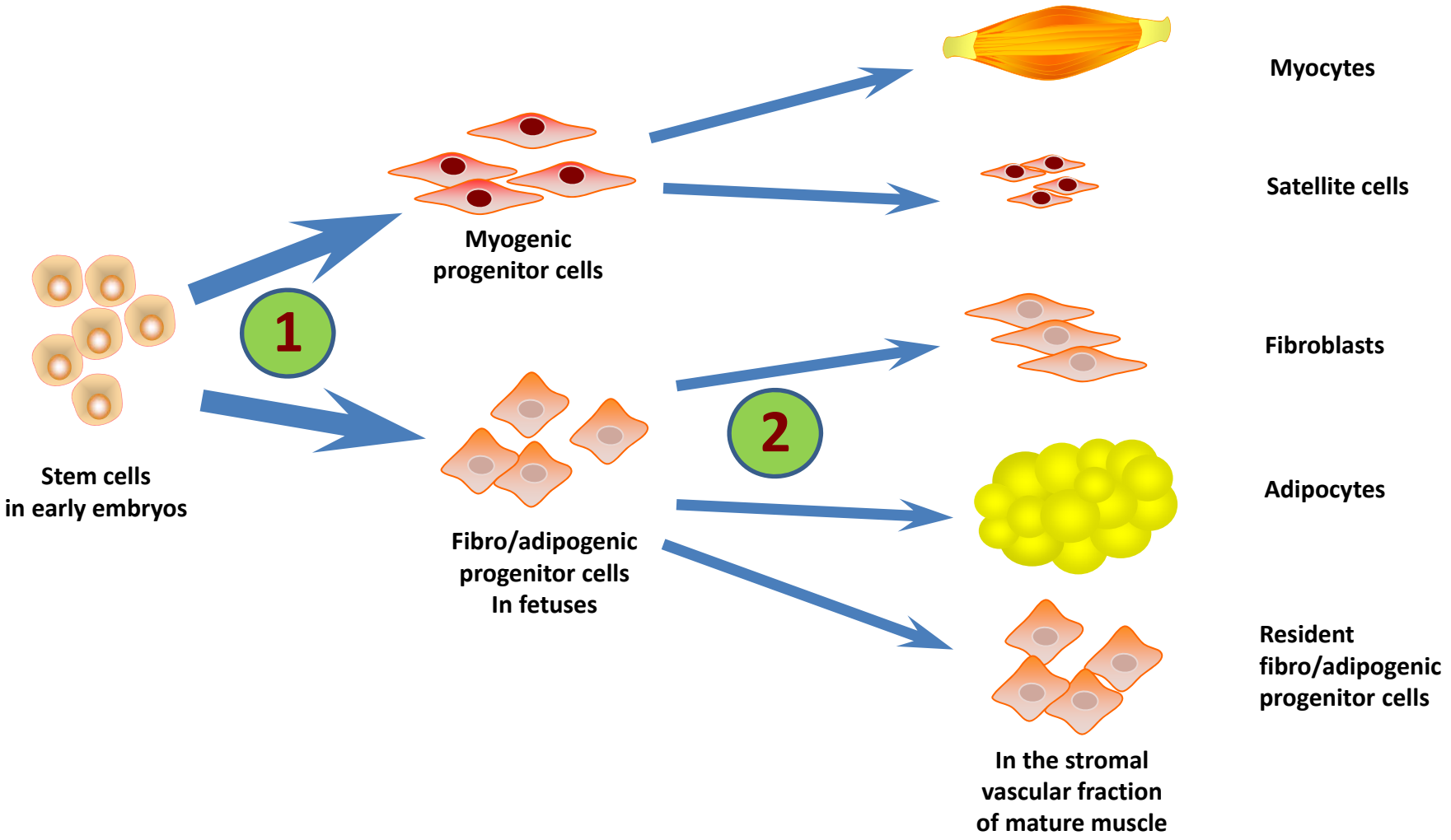
Con **OB**

Sheep fetal muscle at 135 dG when skeletal muscle matures (term day 148 gestation).

Xu et al., Endocrinology, 2010, 151: 380.



- It appears that the fibro/adipogenic pathway was enhanced in fetal muscle due to obesity and over-nutrition in early development – Control point 1.



- To further study mechanisms regulating lineage commitments of mesenchymal stem cells and progenitor cells, we used Wagyu and Angus cattle.

Wagyu cattle are known for its extremely high marbling

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- Wagyu cattle are known for their extremely high marbling.
- Due to their similarities in growth characteristics, but sufficient difference in marbling, Wagyu and Angus cattle are frequently compared.
- We sampled the *Sternomandibularis* muscle for analyses.

Wagyu muscle have enhanced adipogenesis

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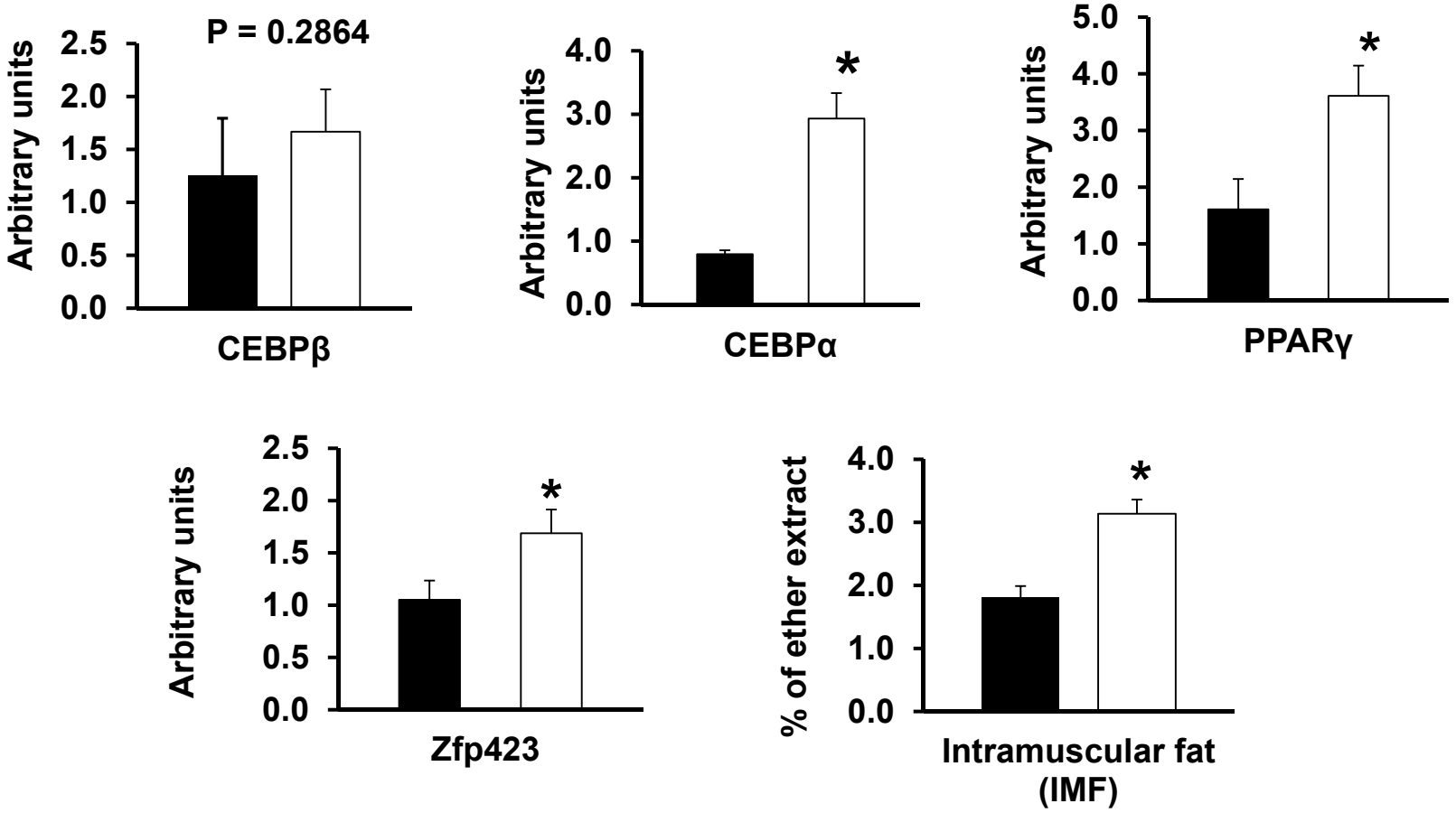

Duarte et al., J. Anim. Sci., 2013, 91: 2938

Wagyu cattle have higher fibrogenesis

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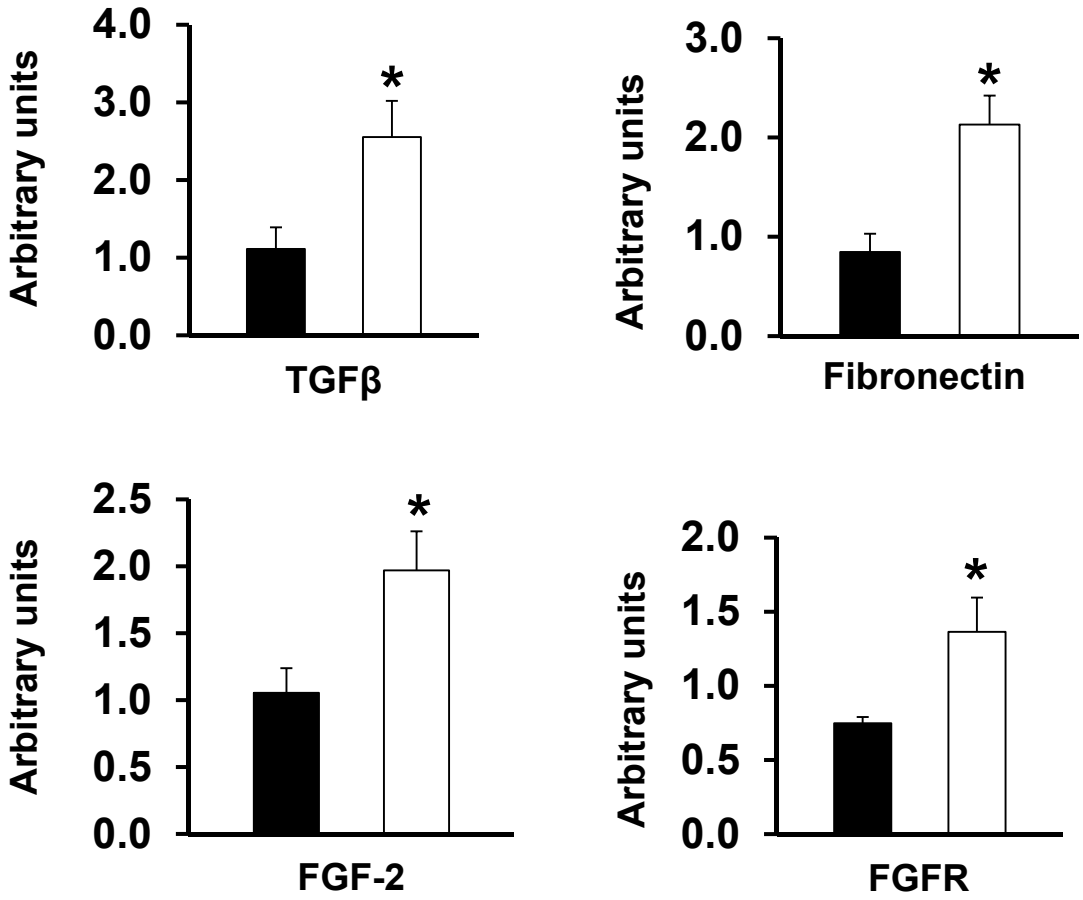

Duarte et al., J. Anim. Sci., 2013, 91: 2938

Wagyu cattle are higher collagen content

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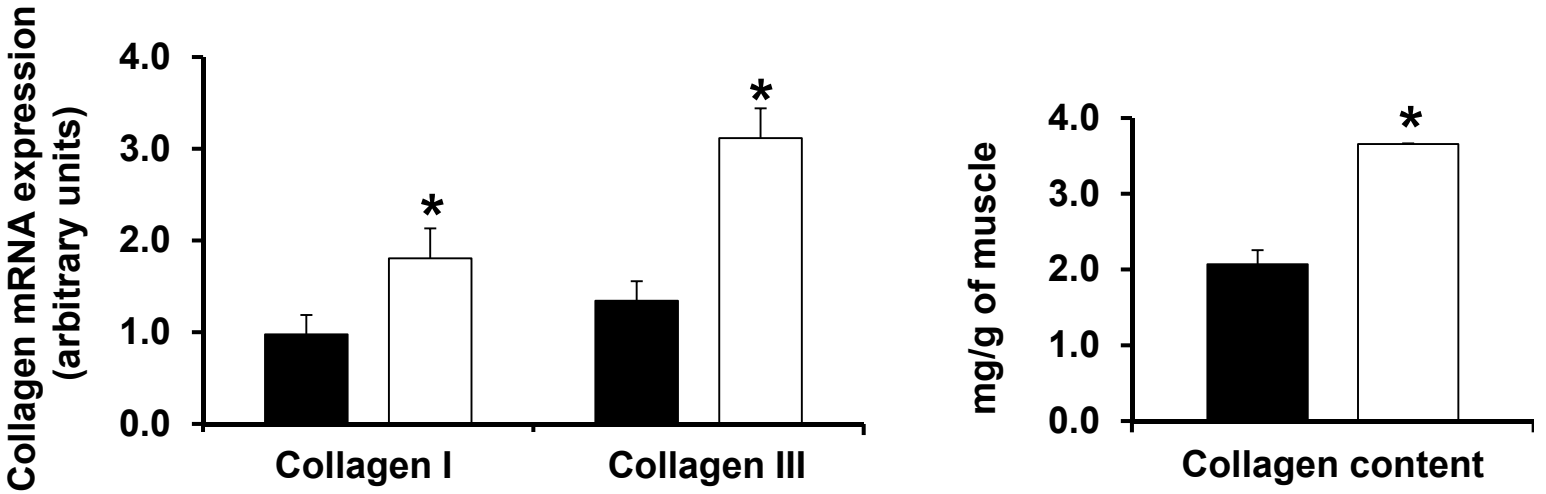

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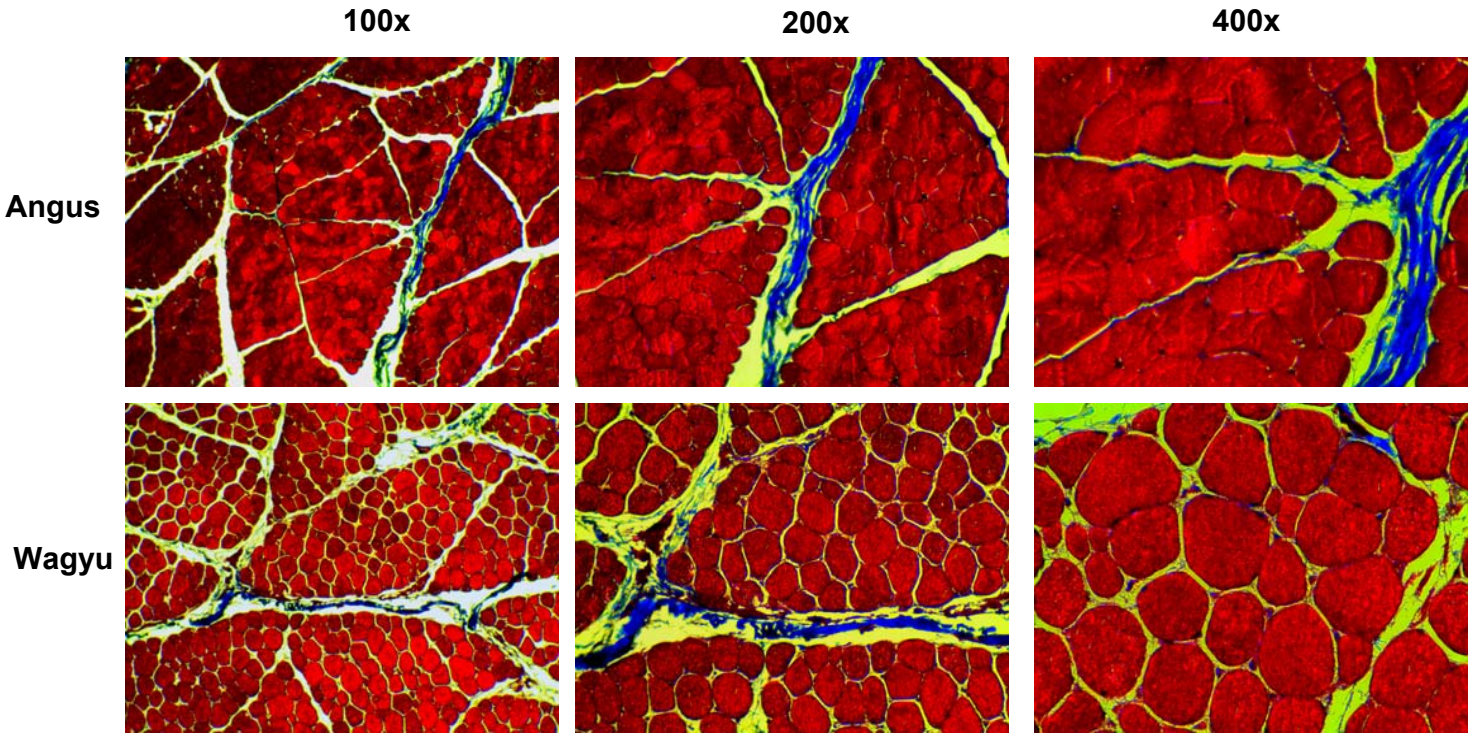

Duarte et al., J. Anim. Sci., 2013, 91: 2938

Wagyu have decreased myogenic pathway

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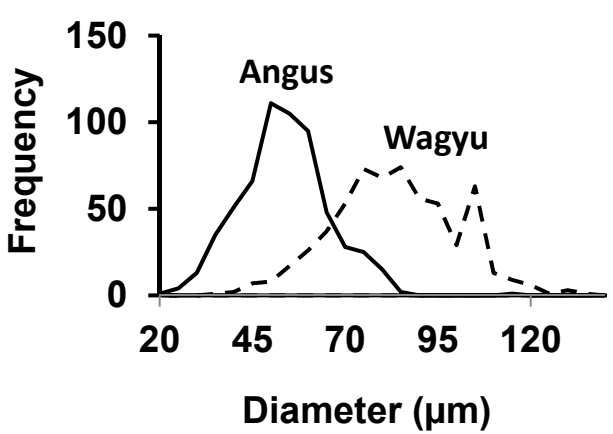
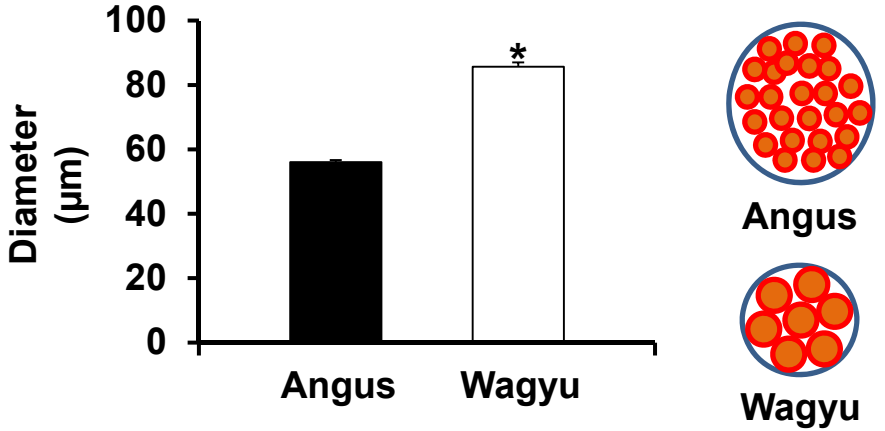
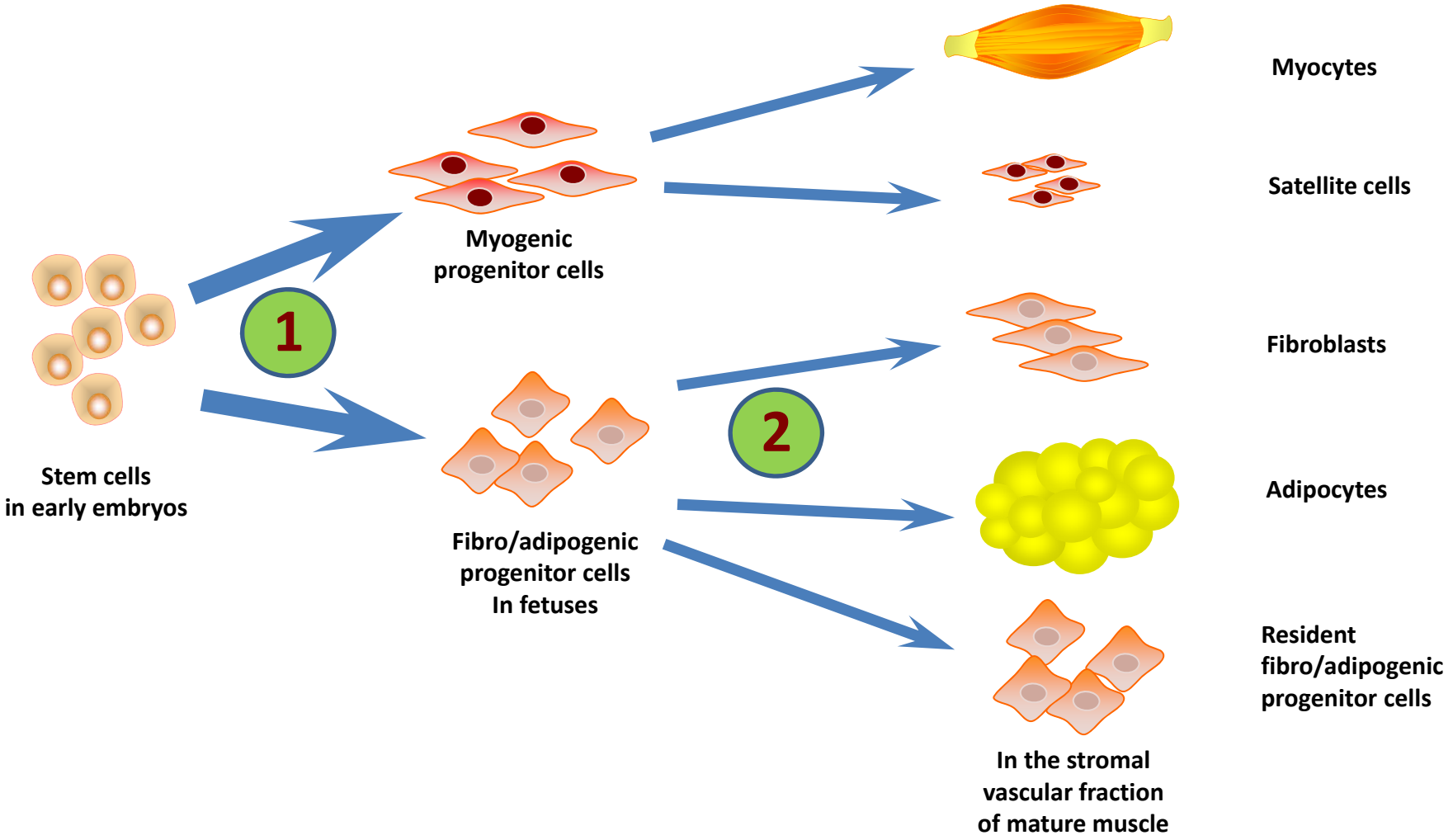




- Attenuated fetal myogenesis forms less muscle fibers.
- The larger muscle fiber diameter in Wagyu despite less muscle mass shows less muscle fiber numbers, indicating attenuated myogenesis during early development.

Duarte et al., J. Anim. Sci., 2013, 91: 2938



- It appears that both fibro/adipogenic pathway (1st question) and adipogenic differentiation (2nd question) are enhanced in Wagyu, which we are exploring.
- Our current studies focus on Zfp423.

Zfp423 in adipogenesis of fibro/adipogenic progenitor cells

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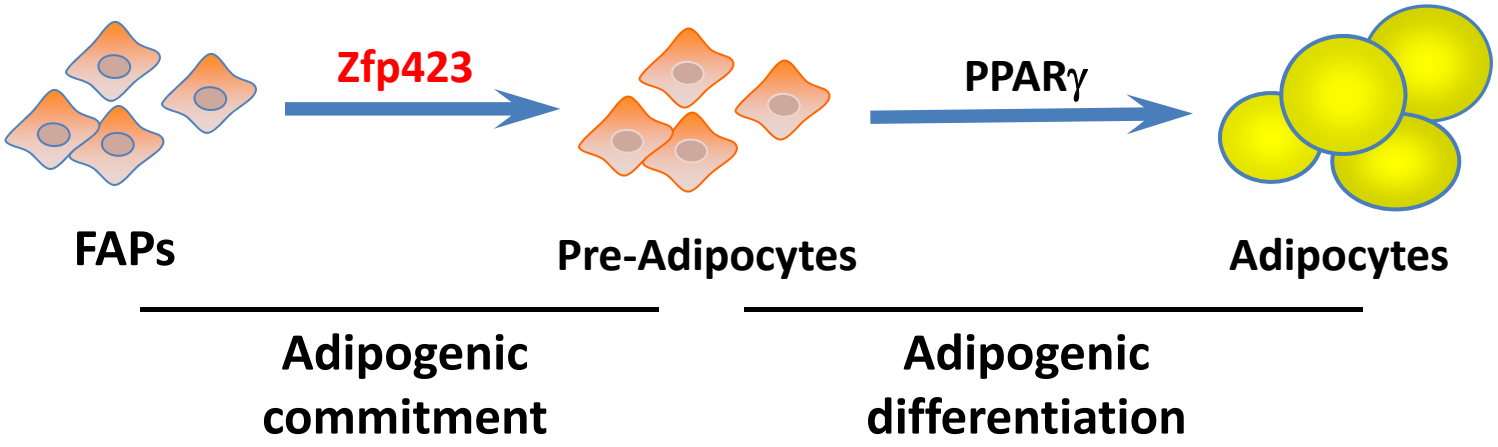
Key question 2: what determines the lineage commitment of fibro/adipogenic cells (FAPs)?

- Zinc finger protein (Zfp) 423 is a newly identified transcription factor regulating adipogenic commitment of FAPs, which induces adipogenic differentiation and reduces fibrogenesis.



What is Zfp423?

- We hypothesized that Zfp423 is critical for adipogenic differentiation of intramuscular fibro/adipogenic progenitor cells.

- Zfp423 is correlated with enhanced adipogenesis and reduced fibrogenesis in beef cattle.

Methods

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Animal:

- The *Sternocleidomastoid* muscle was sampled from the carcass of an Angus heifer (20 months of age) immediately after slaughter.
- Stromal vascular cells were separated, immortalized by over-expression of telomerase, and cloned.
- Three clones with high adipogenic and low adipogenic potential respectively were selected.

Zfp423 in adipogenesis of fibro/adipogenic progenitor cells

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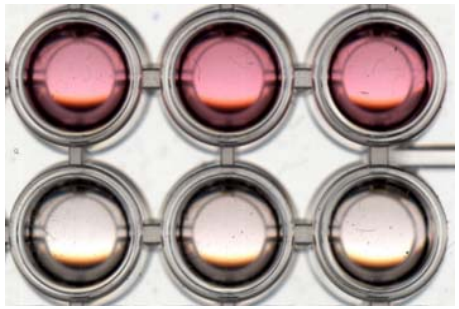
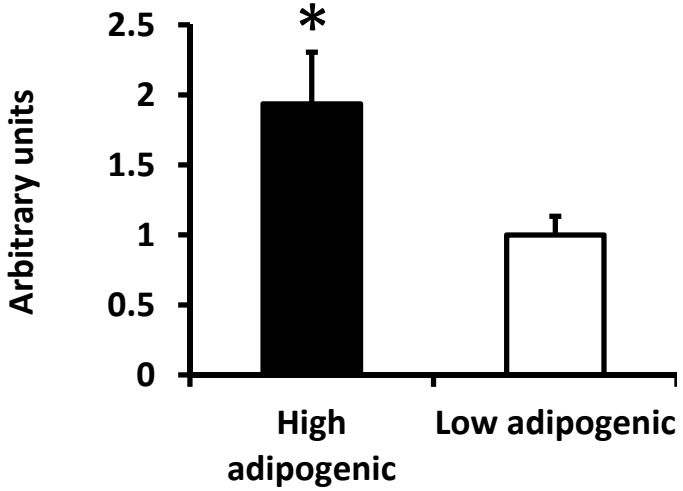
A



High adipogenic

Low adipogenic

B



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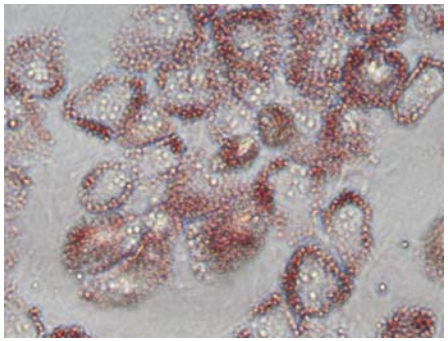

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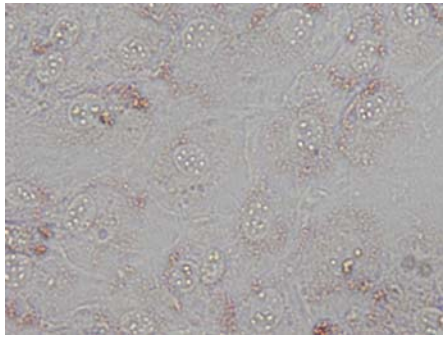

Huang et al., PLOS one, 2012, 7: e47496

mRNA expression of Zfp423 and other genes

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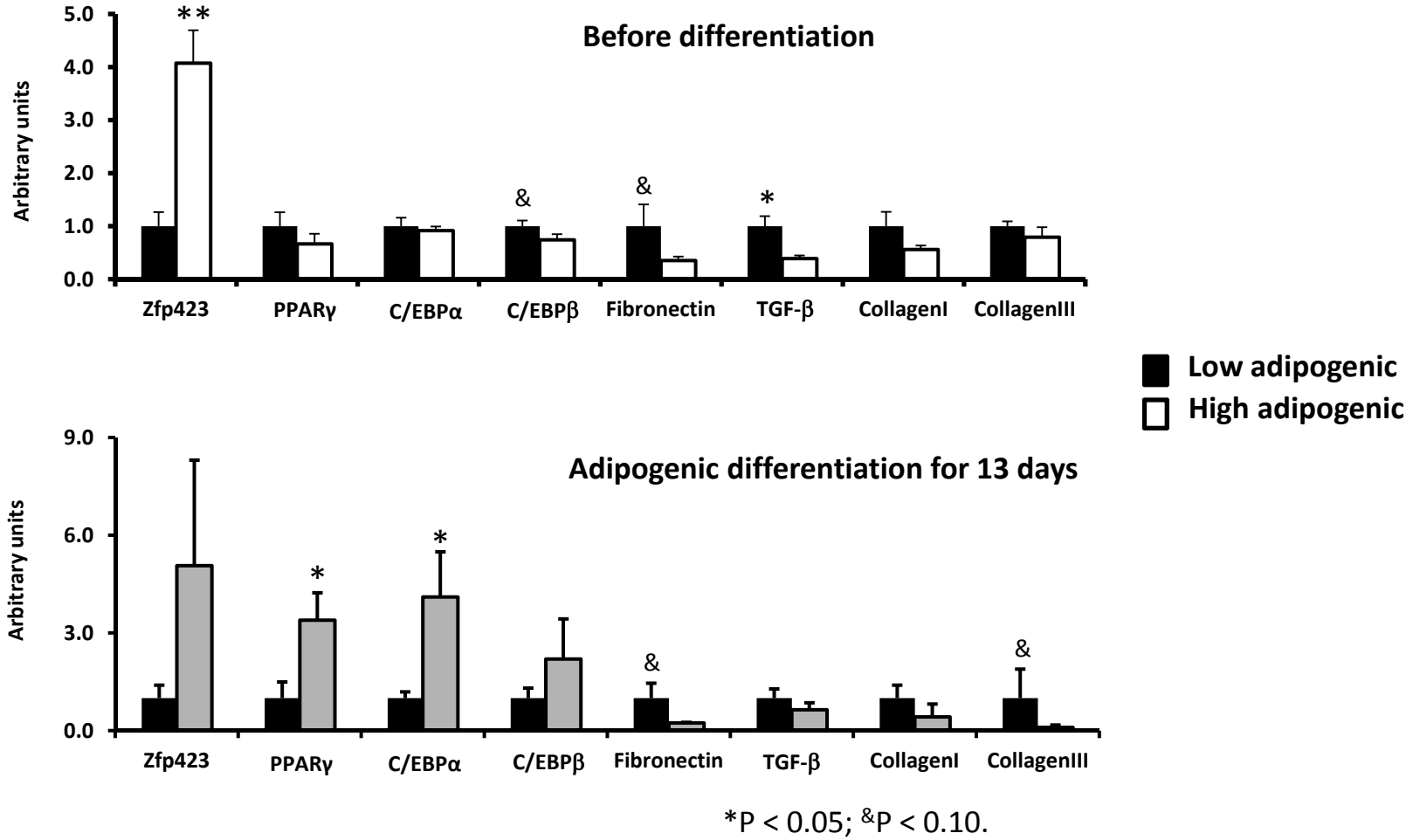

*P < 0.05; &P < 0.10.

Huang et al., PLOS one, 2012, 7: e47496

TGFβ signaling was higher in Low Adipogenic Cells

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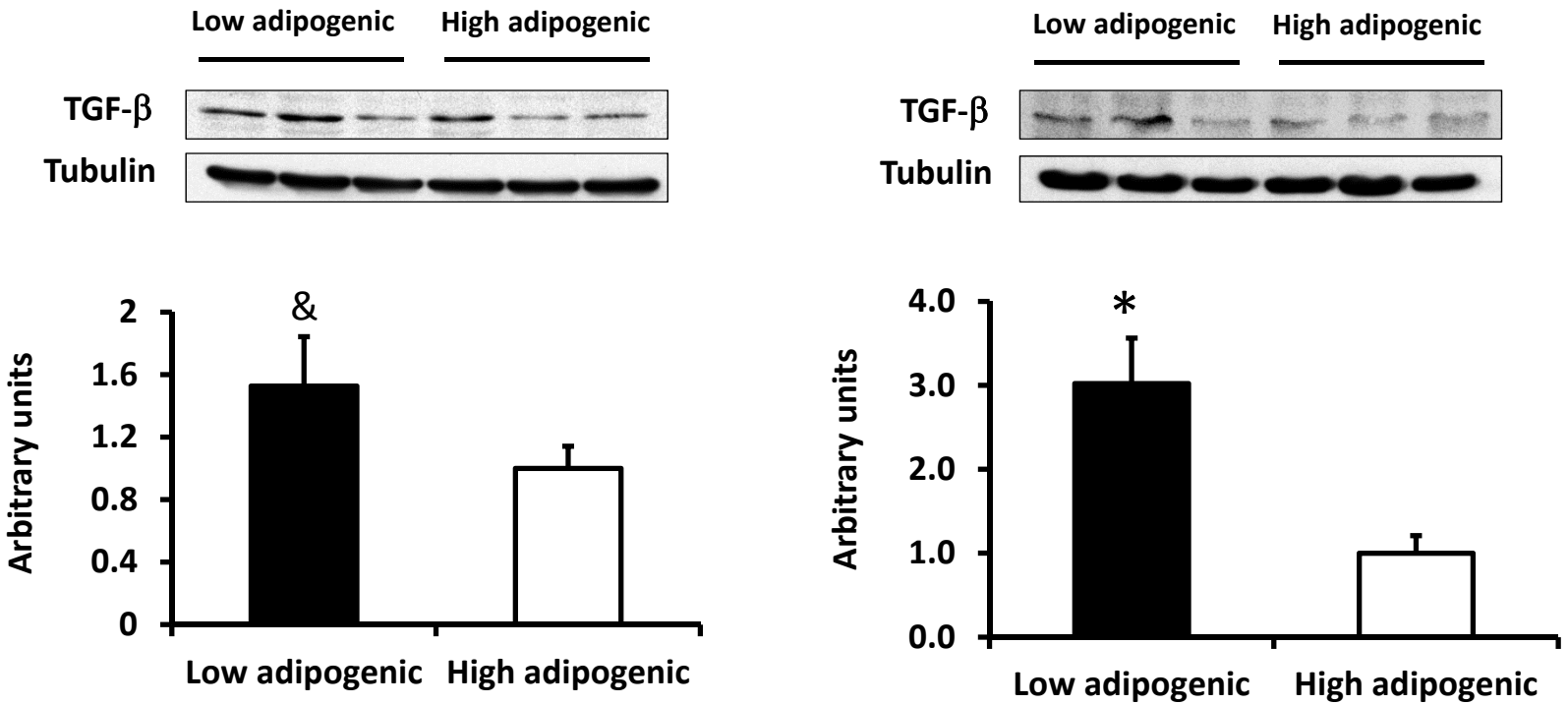

Before adipogenic differentiation

After adipogenic differentiation

Huang et al., PLOS one, 2012, 7: e47496

Zfp423 over-expression in low adipogenic cells enhances their adipogenic differentiation

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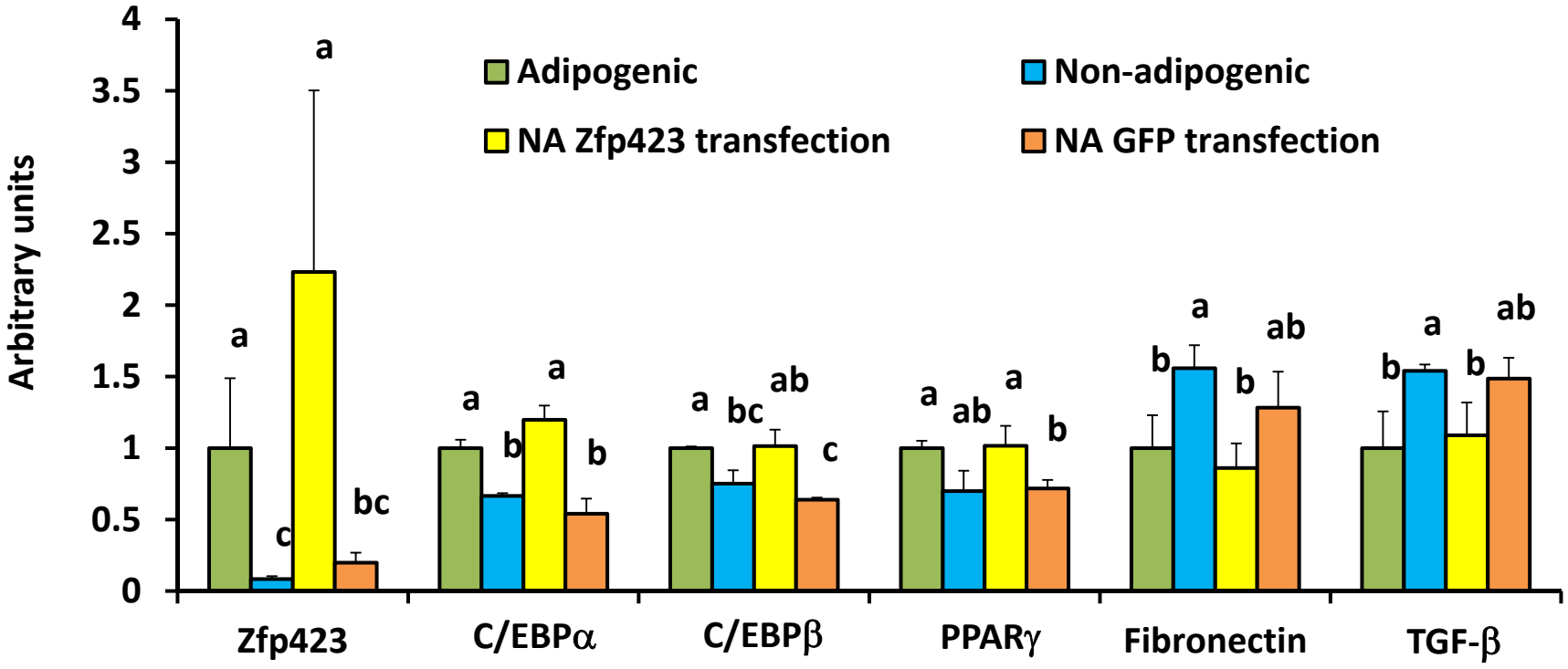

(Bars marked with different letter differ, n =3.)

Huang et al., PLOS one, 2012, 7: e47496

Zfp423 over-expression in low adipogenic cells enhances their adipogenic differentiation

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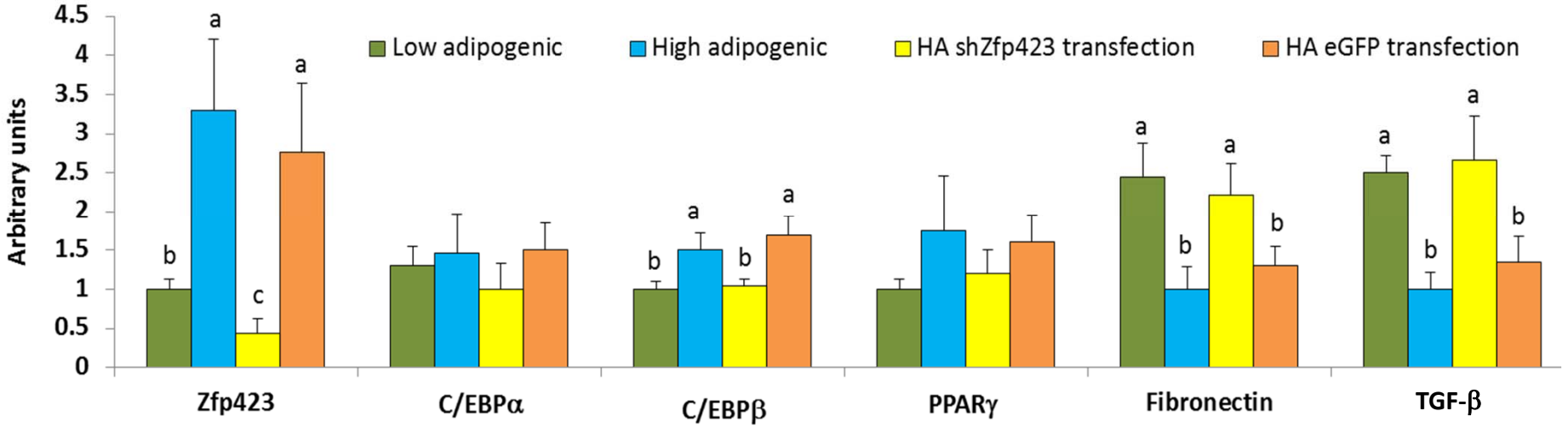

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Zfp423 in adipogenesis of fibro/adipogenic progenitor cells

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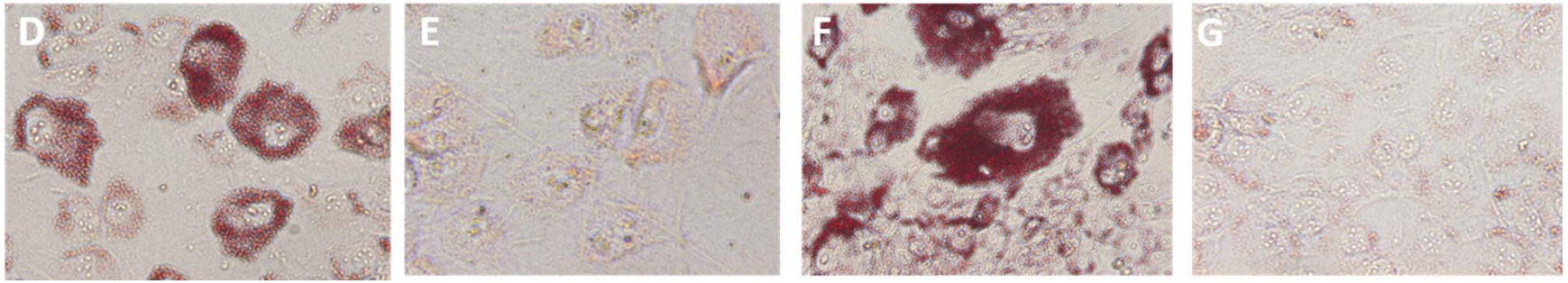
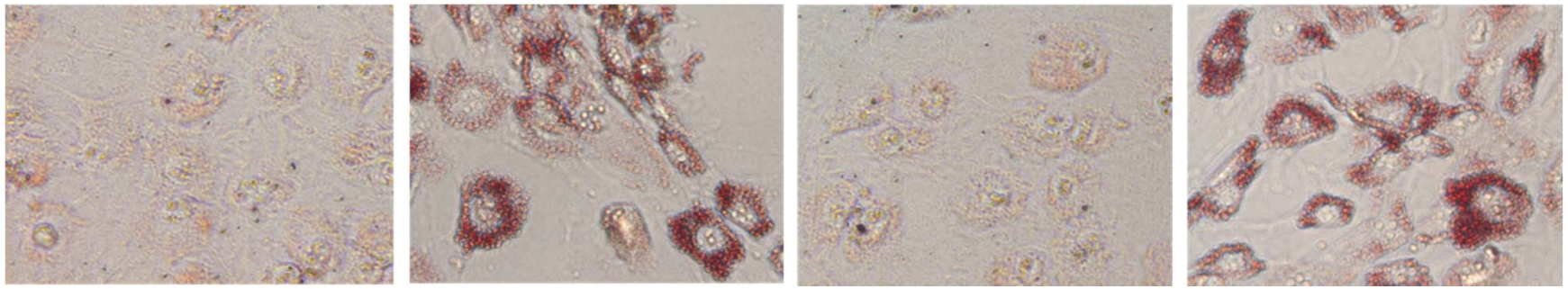


High adipogenic | Low adipogenic | Low adipogenic + Zfp423 | Low adipogenic + eGFP



Low adipogenic | High adipogenic | High adipogenic + shZfp423 | High adipogenic + GFP

Huang et al., PLOS one, 2012, 7: e47496

Why does Zfp423 express at a higher level in high adipogenic cells?

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- Both high and low adipogenic cells are from the same bovine animal.
- They have identical genetic composition.
- The difference in their adipogenic and fibrogenic differentiation should be due to epigenetic modifications.
- Because these cells have been immortalized and cloned, only stable epigenetic modifications, or DNA methylation, is expected to maintain.

Zfp423 promotor contains rich GC sites, and higher methylation in low compared to high adipogenic cells

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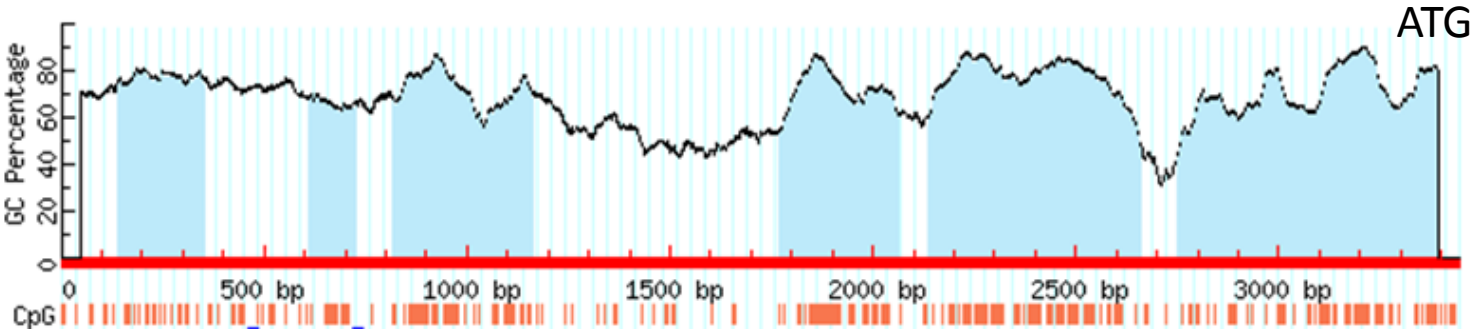

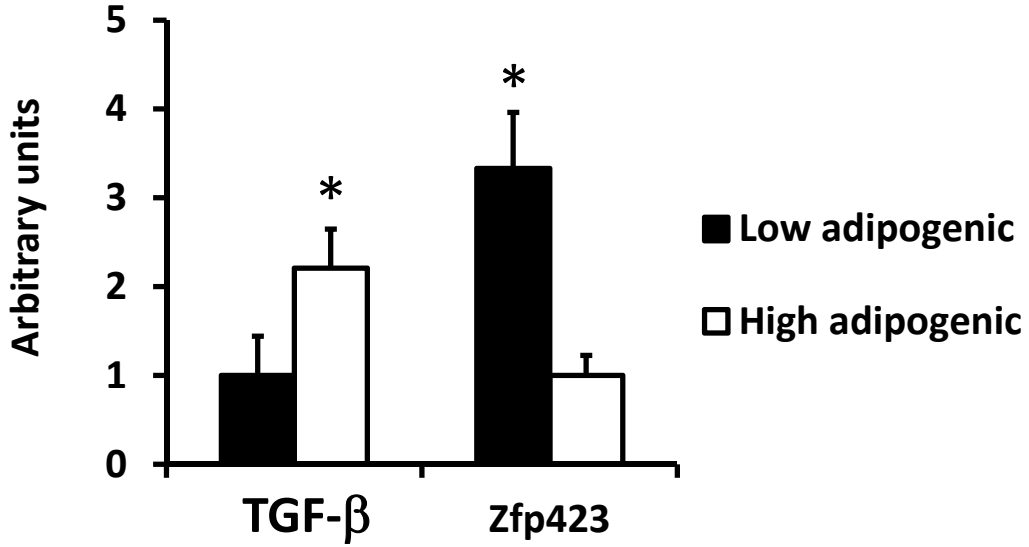

Methylated DNA immunoprecipitation (MeDIP) was used to measure the DNA methylation of Zfp423 promoter.

Huang et al., PLOS one, 2012, 7: e47496

Zfp423 in adipogenesis of fibro/adipogenic progenitor cells

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Department of Animal Sciences

- Why do TGFβ and Zfp423 expression differ between low and high adipogenic cells?
- DNA methylation is a stable epigenetic modification which inhibits gene expression, determining cell phenotypes.
- TGFβ DNA methylation was higher, while Zfp423 DNA methylation was lower in low adipogenic cells, consistent with higher adipogenic and low fibrogenic differentiation of FAPs.

Huang et al., PLOS one, 2012, 7: e47496

What regulates Zfp423 expression?

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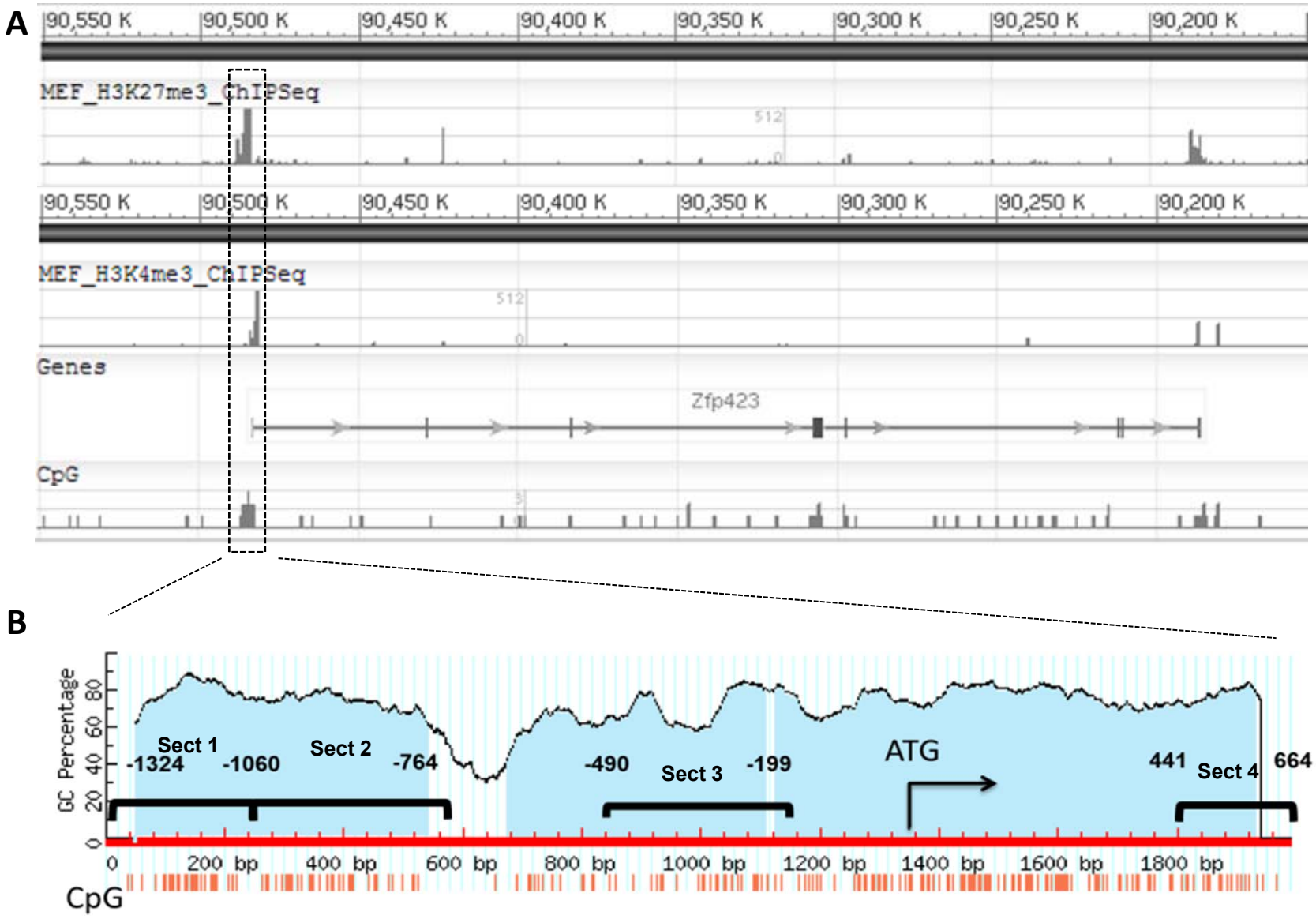
- We hypothesized that epigenetic modifications have key roles in regulating Zfp423 expression.
- Epigenetic modifications are mainly referring to DNA methylation and histone modifications.
- Histone modifications include histone methylation and acetylation, and others.

What regulates Zfp423 expression?

- Polycomb repressive complex 2 (PGC2) catalyzes repressive histone modifications, H3K27me3.
- Trithorax group proteins (TrxG) catalyze permissive histone modifications, H3K4me3.
- Both H3K27me3 and H3K4me3 co-exist in key developmental genes, forming “bivalent” status.
- Lack of stimulation, repressive histone modifications convert to DNA methylation for permanent silencing.

Zfp423 promoter has a “bivalent” status



Yang et al., Diabetes, 2013, 62:3727

Maternal obesity enhances Zfp423 expression in fetal tissue via inducing epigenetic changes

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- To test the role of epigenetic changes in the regulation of Zfp423 expression, we used a diet-induced obesity pregnant mouse model.
- Female mice were fed either a control diet (Con) or an obesogenic diet (OB) for two months to induce obesity.
- Fetal mice at E14.5 were collected for analyses, when early adipose development has initiated.

DNA methylation was lower in Zfp423 of fetal tissue of obese mothers

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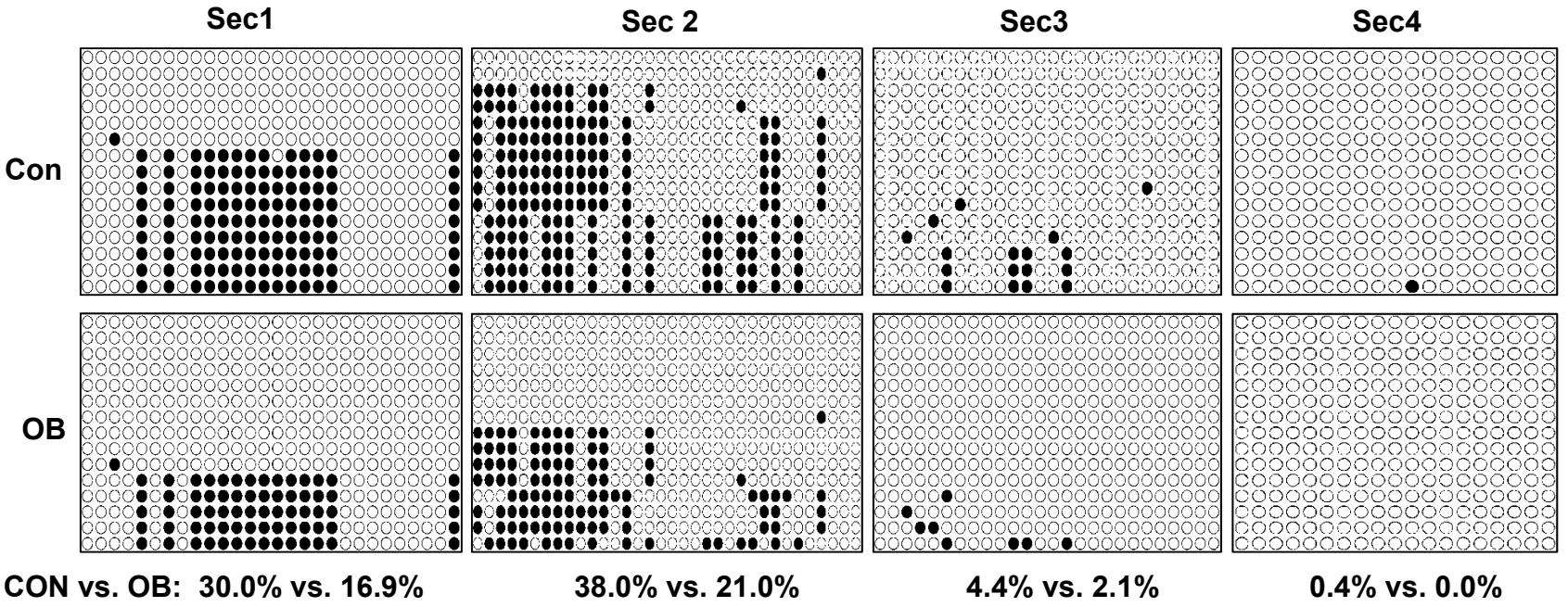
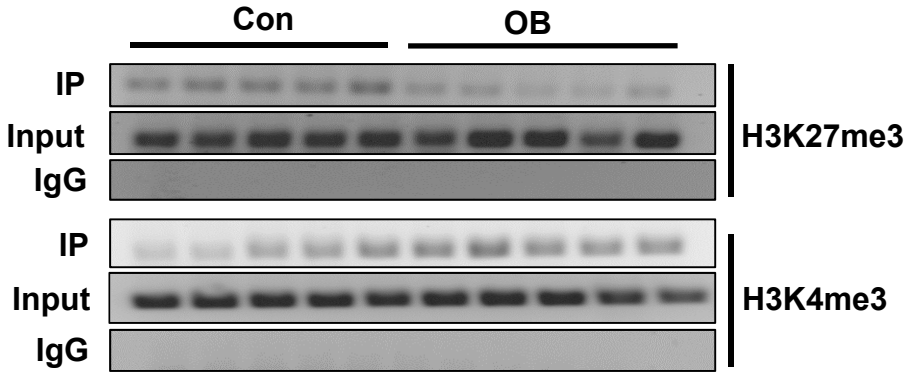
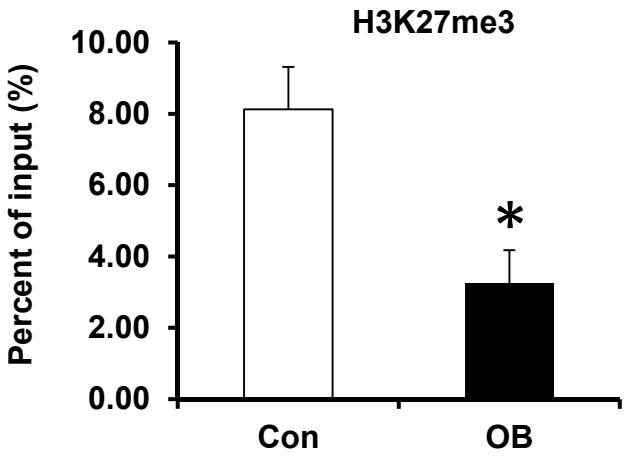
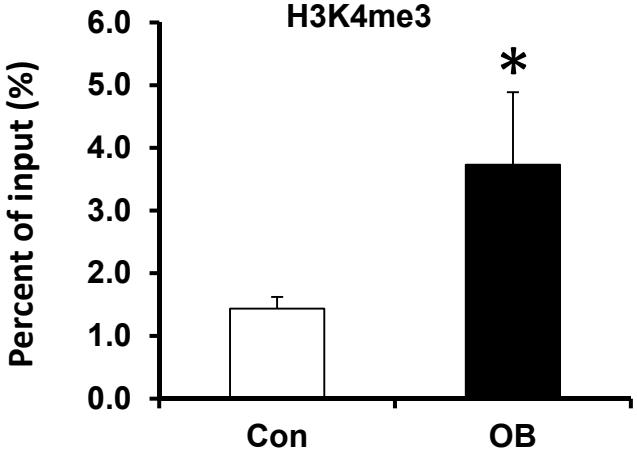


Yang et al., Diabetes, 2013, 62:3727

Inhibitory histone modification, H3K27me3 is lower, and permissive modification, H3K4me3, is higher in OB







Yang et al., Diabetes, 2013, 62:3727

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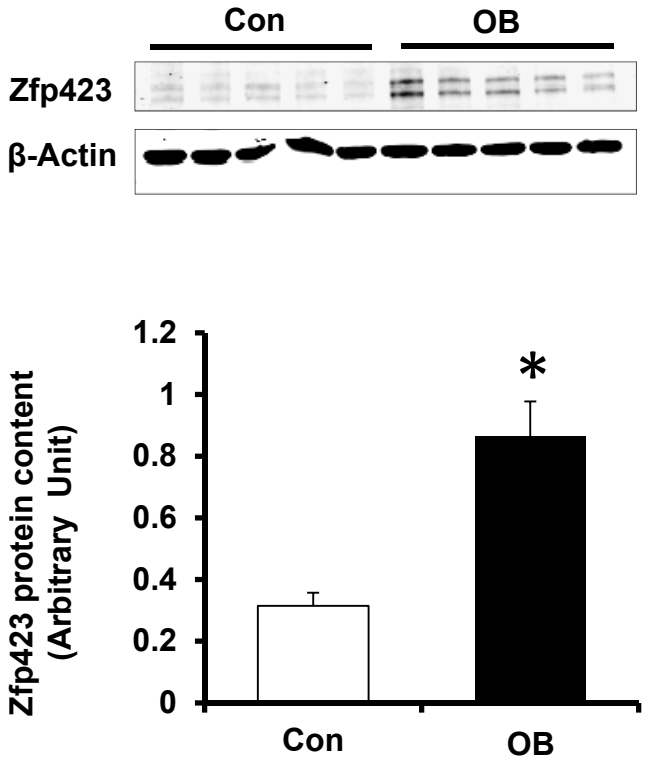

Yang et al., Diabetes, 2013, 62:3727

Proposed mechanism linking maternal obesity to epigenetic modifications in the Zfp423 promoter

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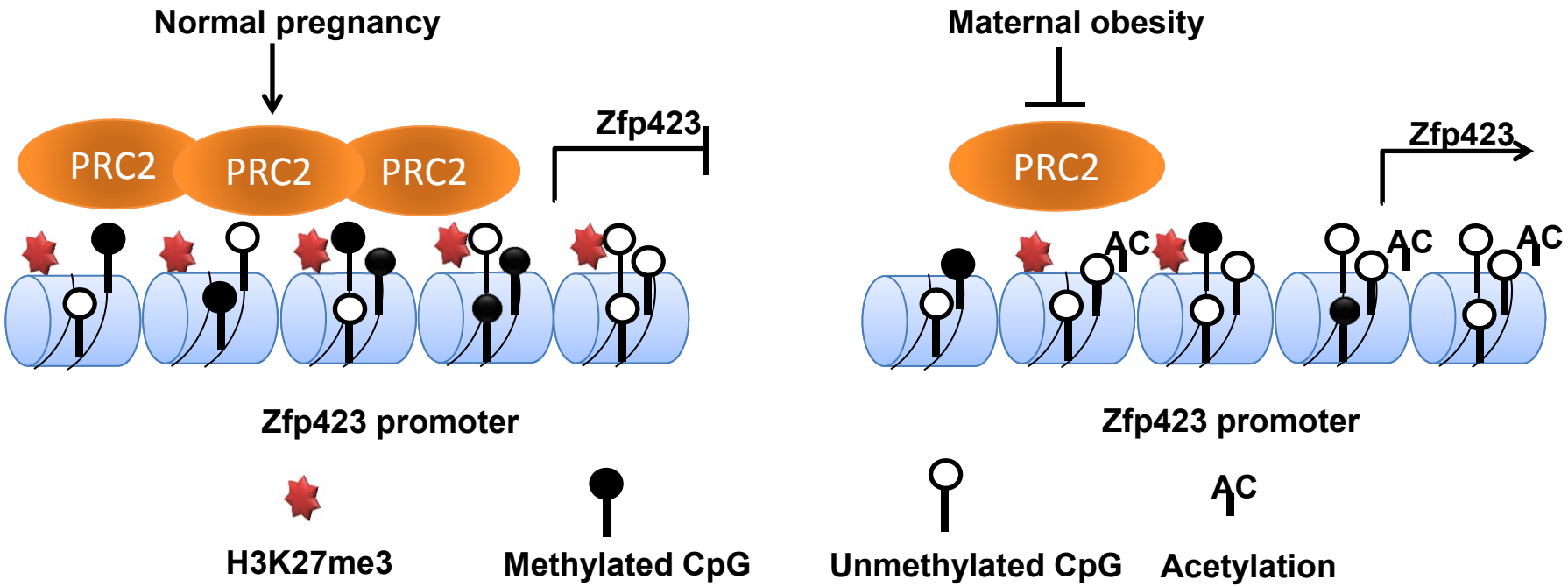
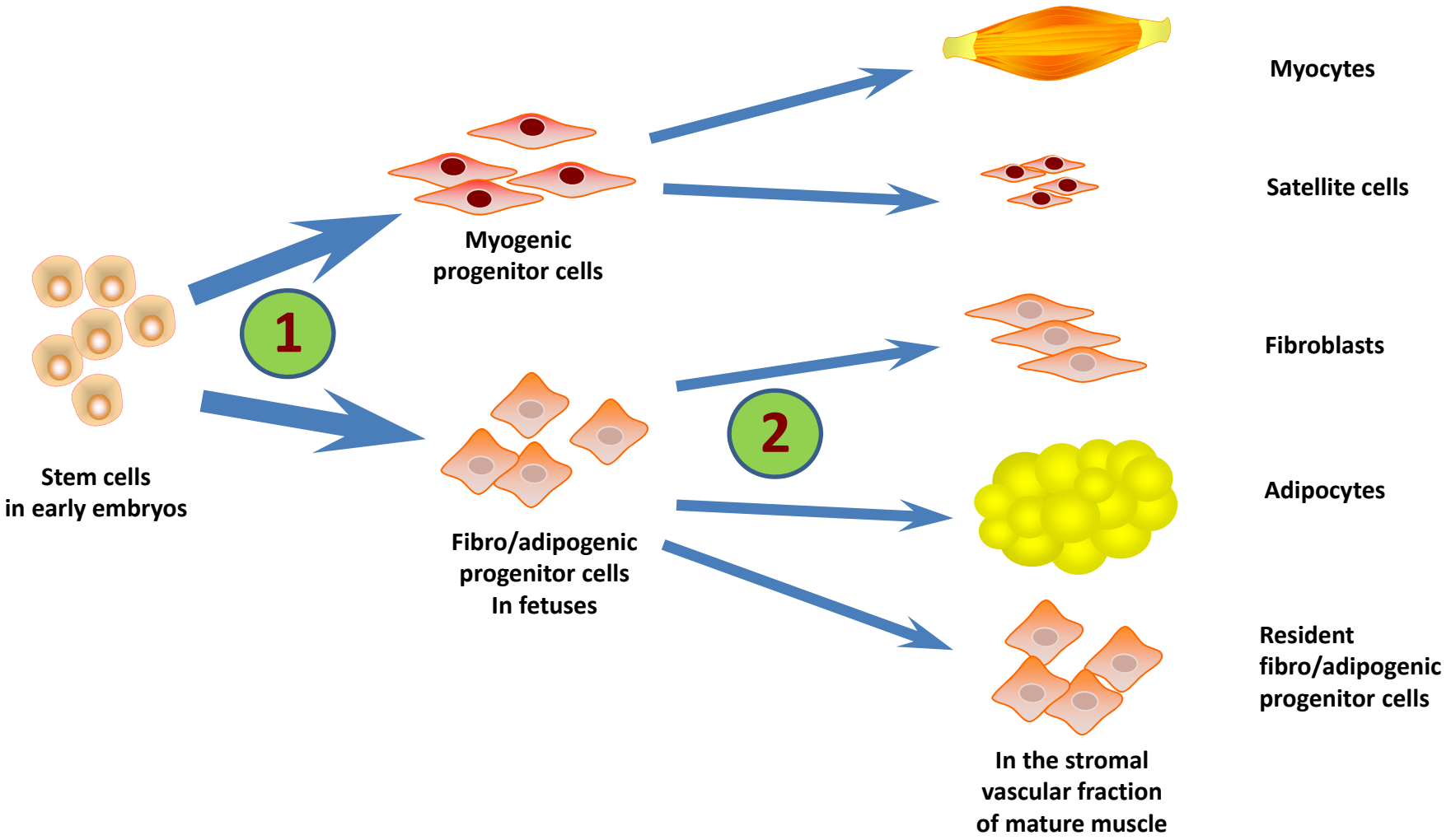
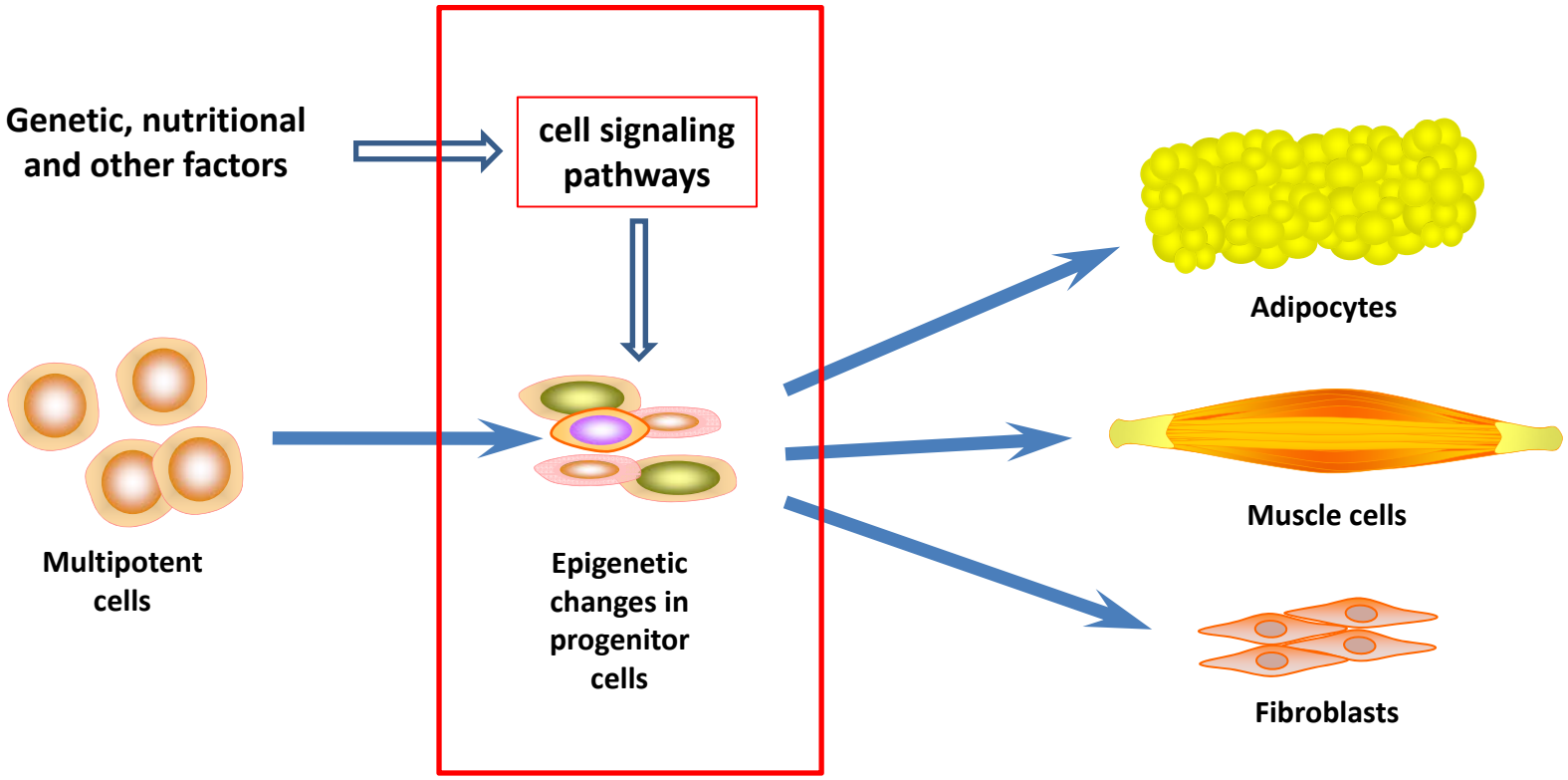




- In summary, there are two major control points for reducing intramuscular fibrogenesis:
  - **Stem cells to either myogenic or FAP lineage.**
  - **FAPs to either adipogenic or fibrogenic lineage.**

Mechanisms regulating mesenchymal progenitor cell differentiation



What controls mesenchymal progenitor cell differentiation?

- *Critical questions.*
- If we know these answers,
  - We can:
    - Improve lean/fat ratio and production efficiency.
    - Reduces connective deposition and increase tenderness of beef.
    - Enhance intramuscular adipogenesis and marbling.

Funding support:


Thanks!
Comments and suggestions?