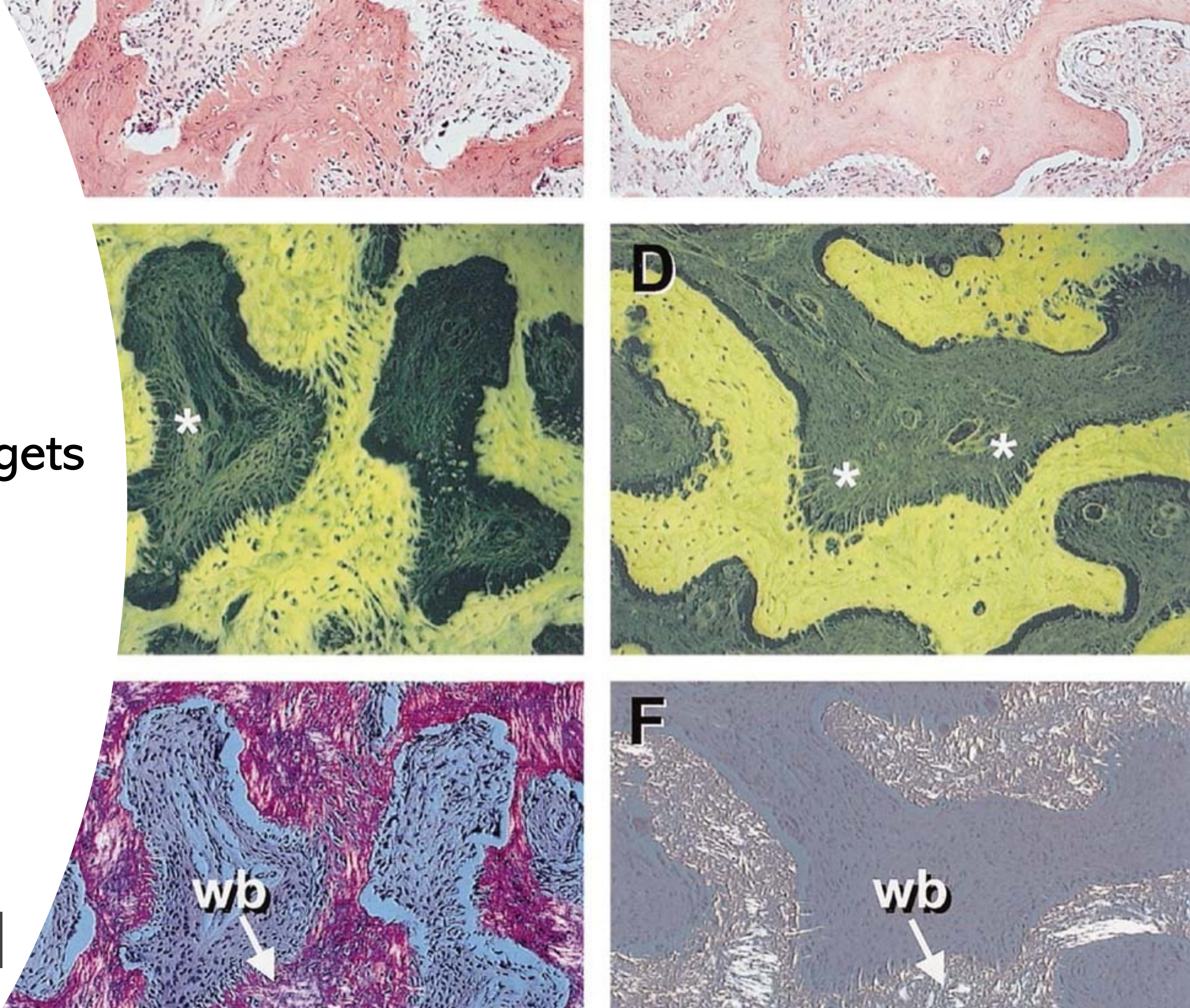
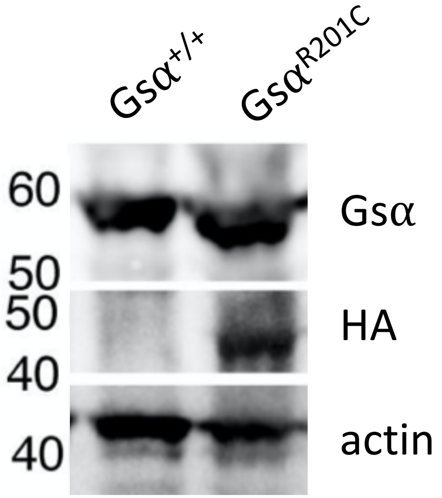
GNAS and HDAC8: potential therapeutic targets to restore differentiation of BMSCs in Fibrous Dysplasia

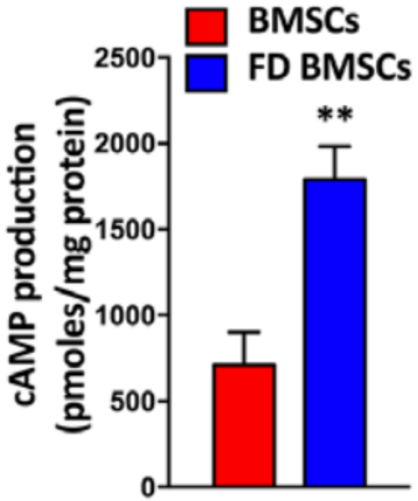


A. Brunone, M. L. Crudeli, E. Di Graci, F. Paci

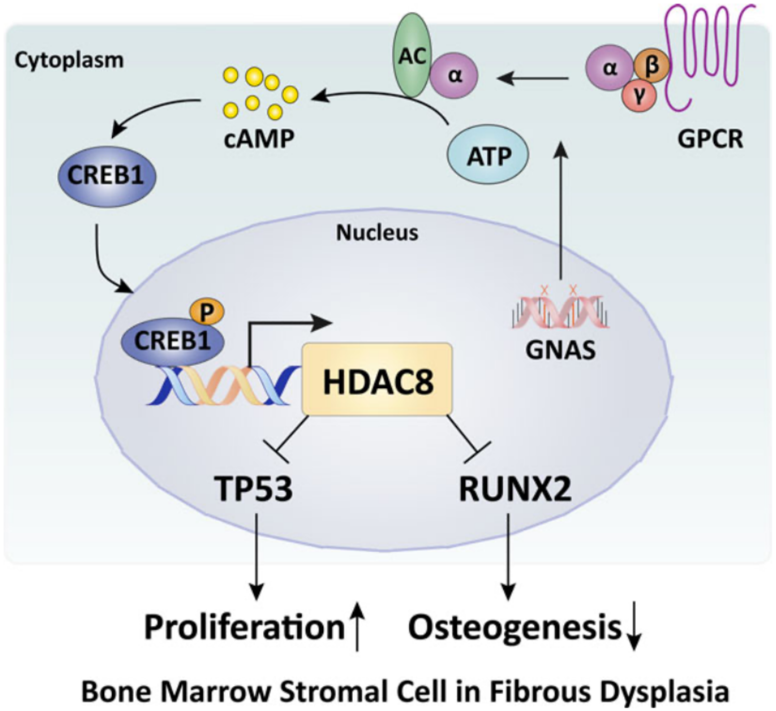
Role of GNAS and HDAC8 in Fibrous Dysplasia



Adapted by: Raimondo et al., 2020



Xiao, Fu, Zhu et al., 2018



Xiao, Fu, Zhu et al., 2018

FIRST AIM

Use of CRISPR/Cas13 on HDAC8 to interfere with HDAC8 activity in FD BMSCs

↓

Reduce HDAC8 activity to restore physiological osteogenic differentiation

SECOND AIM

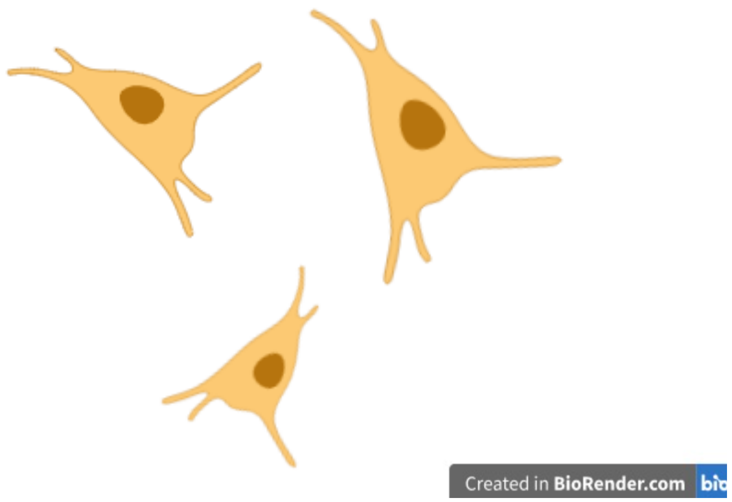
Use of CRISPR/Cas9 on GNAS mutated gene in human FD BMSCs

↓

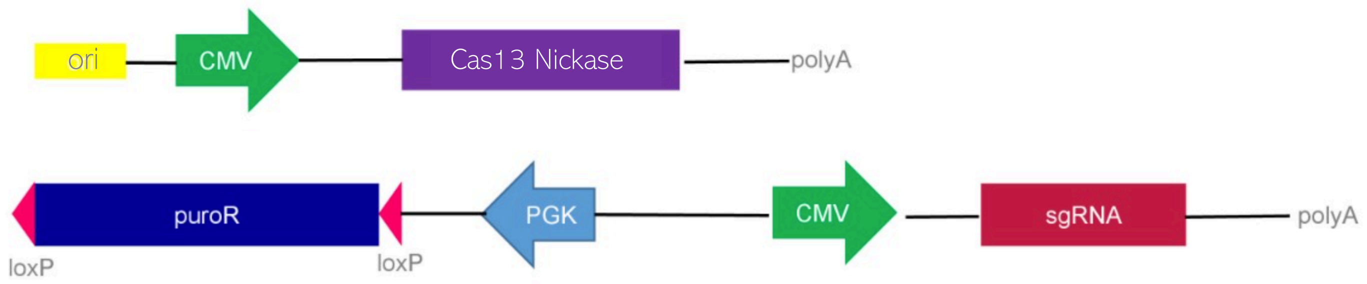
Restore WT Gsα phenotype

CRISPR/Cas13 on HDAC8 – Material

Human FD CD146/MCAM cells



Donor vector
pDEST-IRES-puro-polyA



HDAC8 sgRNA

1: 5-GACGTGTCTGATGTTGGCCTNGG-3
2: 5-GCGGAAGATGGAGGAGCCGGNGG-3
3: 5-GTAGCAATTAACTGGTCTGGNGG-3

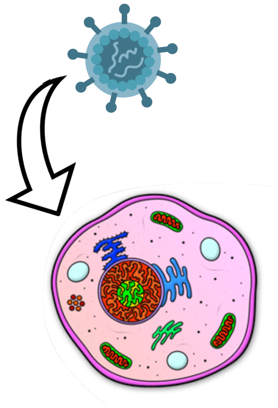
http://www.e-crisp.org

CRISPR/Cas13 on HDAC8 – Methods

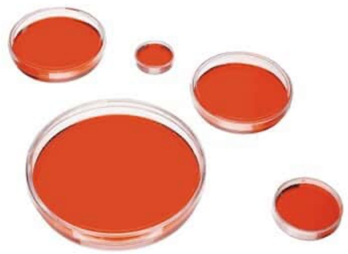
0 h | 24 h | 48 h | 72 h

Incubation at 37°C:
- 25 cm^2 plastic flasks
- DMEM medium
- 100 U/ml penicillin
- 100 µg/ml streptomycin
- 10% fetal bovine serum

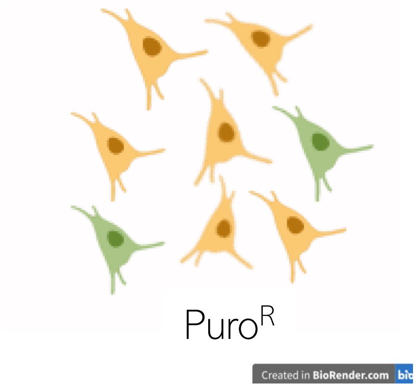
0 h – pLenti transfection:
- polybrene 8 mg/ml
- human FD BMSCs



24 h – Plating and proliferation

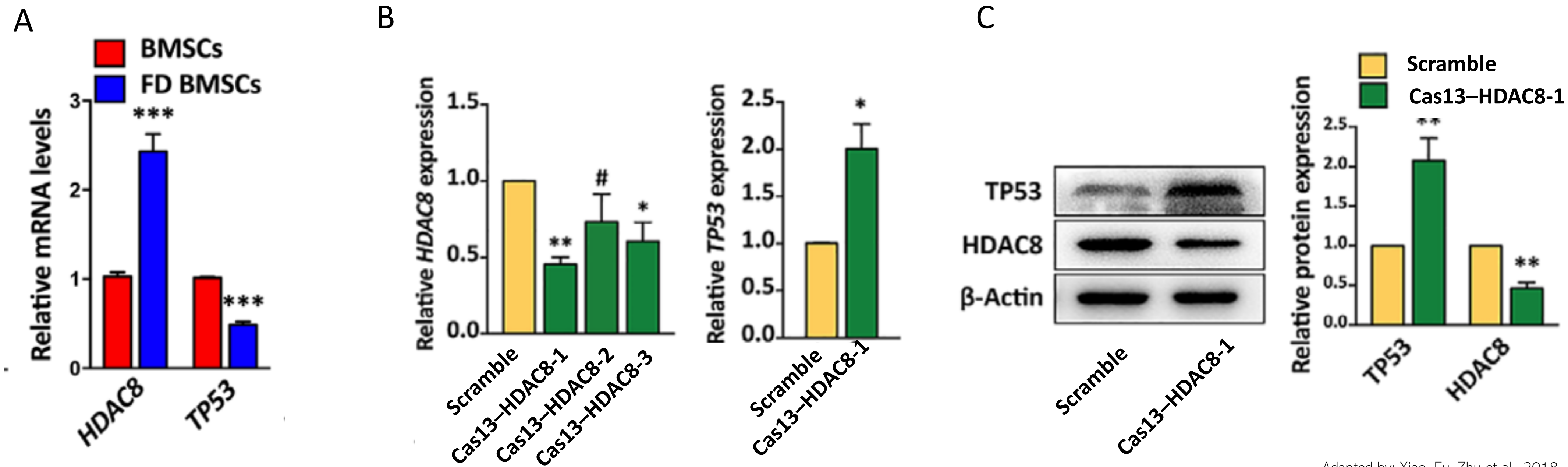


48 h – PuroR selection



72 h – Harvest

CRISPR/Cas13 on HDAC8 – *In vitro* results I

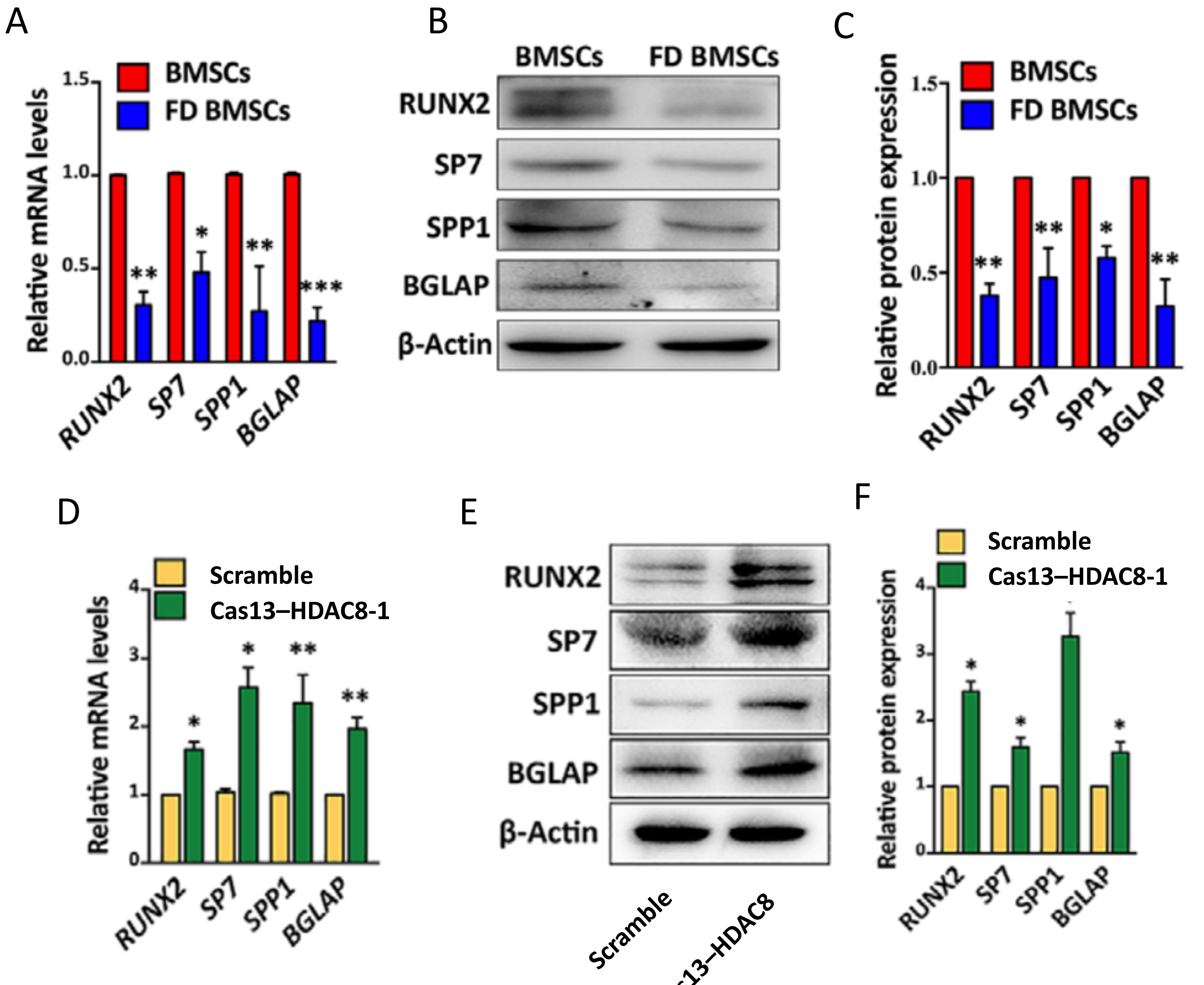


Adapted by: Xiao, Fu, Zhu et al., 2018

Graphic A. RT-PCR showing HDAC8 and TP53 mRNA level expression after FD BMSCs were cultured in osteogenesis induction medium for 7 days.

Graphic B-C. RT-PCR and western blot of HDAC8 and TP53 expression in FD BMSCs with CRISPR/Cas13 compared with the control.

CRISPR/Cas13 on HDAC8 – *In vitro* results II

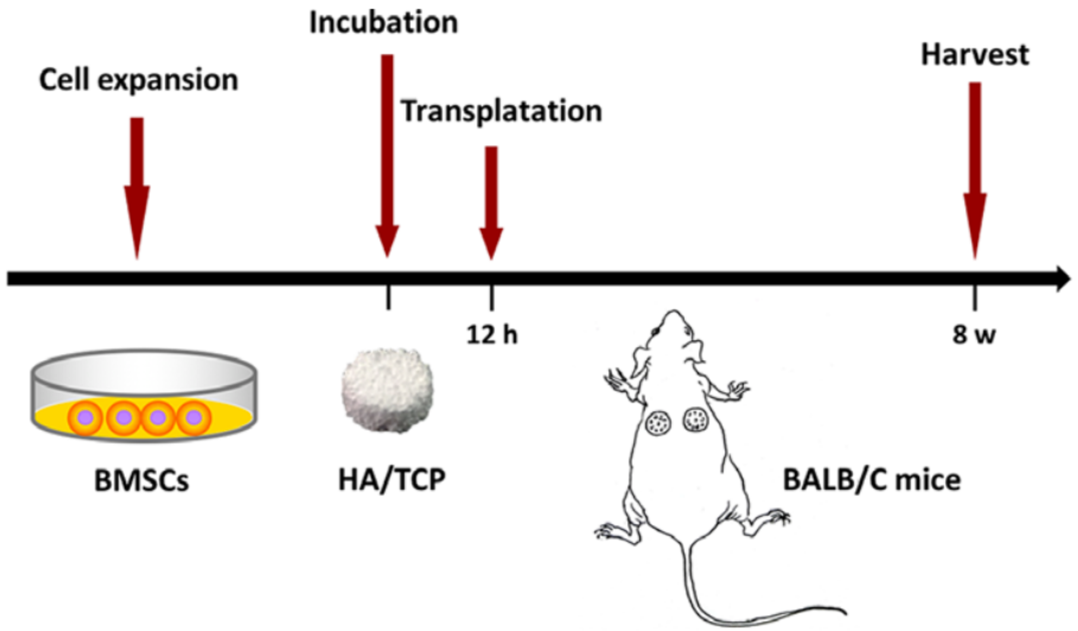


Adapted by: Xiao, Fu, Zhu et al., 2018

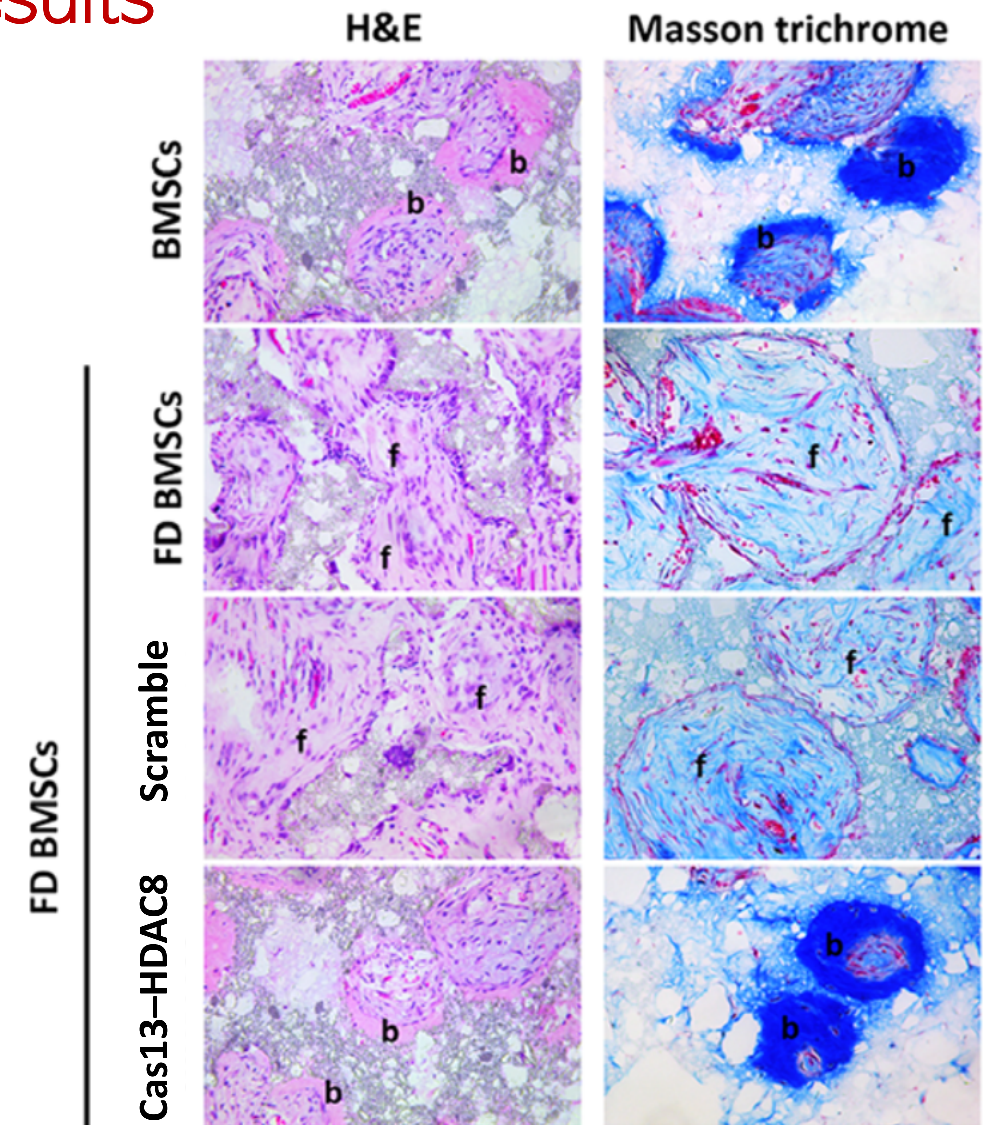
Graphics A-C. 1: RT-PCR showing osteogenisis marker mRNA expression. 2-3: western blot analysis of osteogenesis markers. Data collected from FD BMSCs in osteogenesis induction medium for 7 days.

Graphics D-F. RT-PCR and western blot of osteogenesis marker expression in FD BMSCs with CRISPR/Cas13 compared with the control.

CRISPR/Cas13 on HDAC8 – *In vivo* results



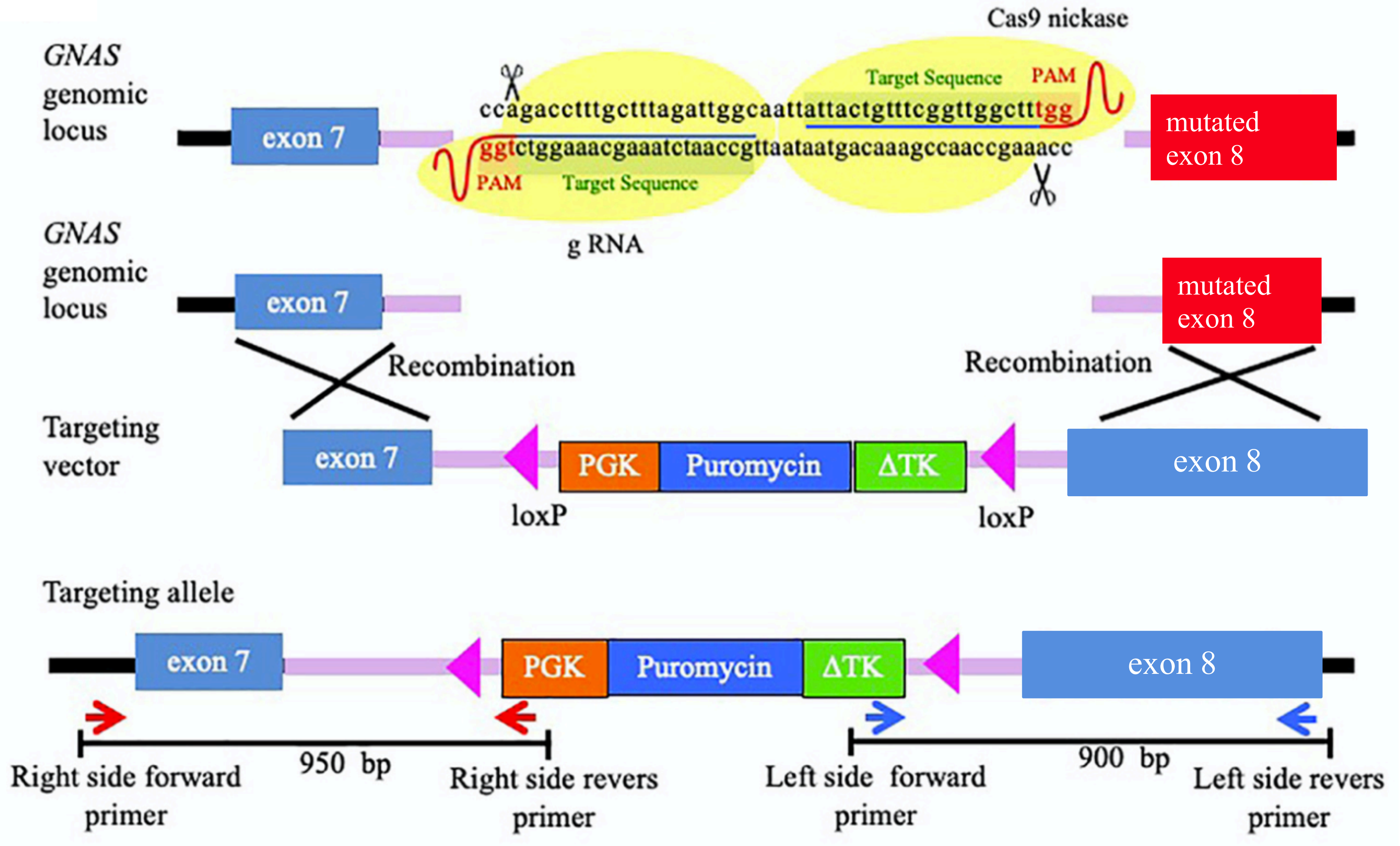
Adapted by: Xiao, Fu, Zhu et al., 2018



Adapted by: Xiao, Fu, Zhu et al., 2018

b = bone tissue
ft = fibrous tissue

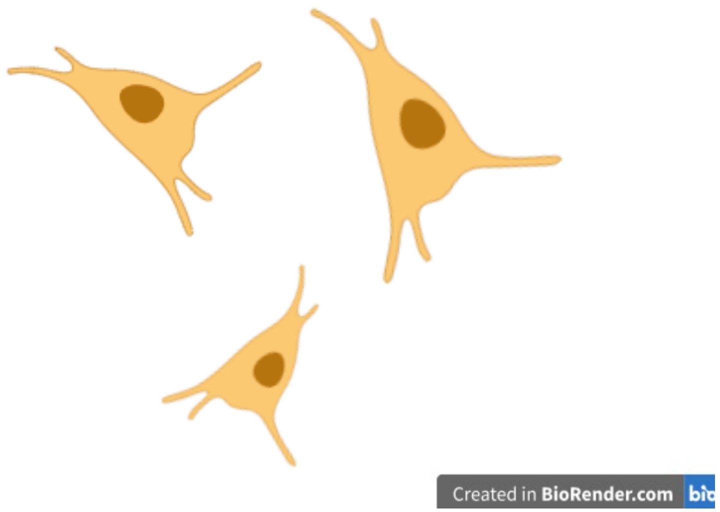
CRISPR/Cas9 strategy – Material and Methods



adapted by: Watanabe et al., 2020

CRISPR/Cas9 on GNAS – Material and methods

Human FD CD146/MCAM cells

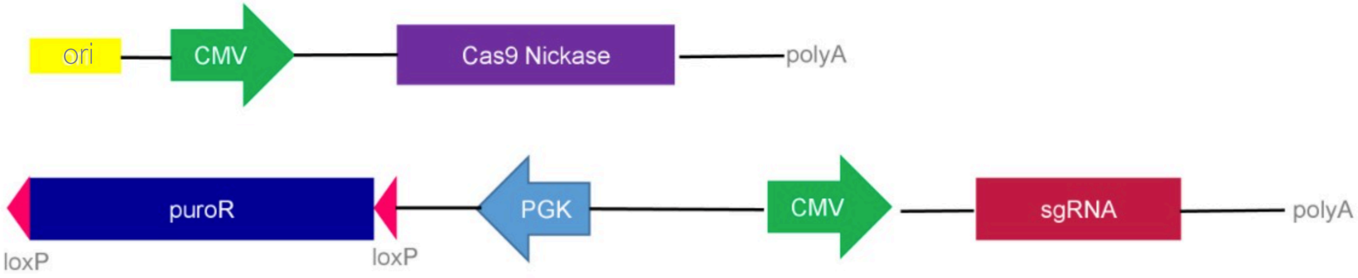


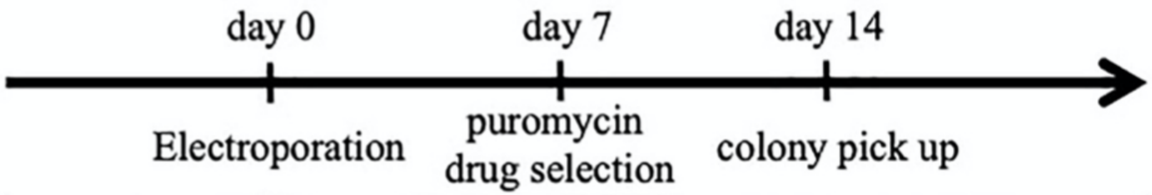
GNAS gsRNA

1: 5-GAGGCGATTGAAGTACGTGCNGG-3
2: 5-GCTGCTTCTAGGTAATGCGGNGG-3

http://www.e-crisp.org

Donor vector pDEST-IRES-puro-polyA

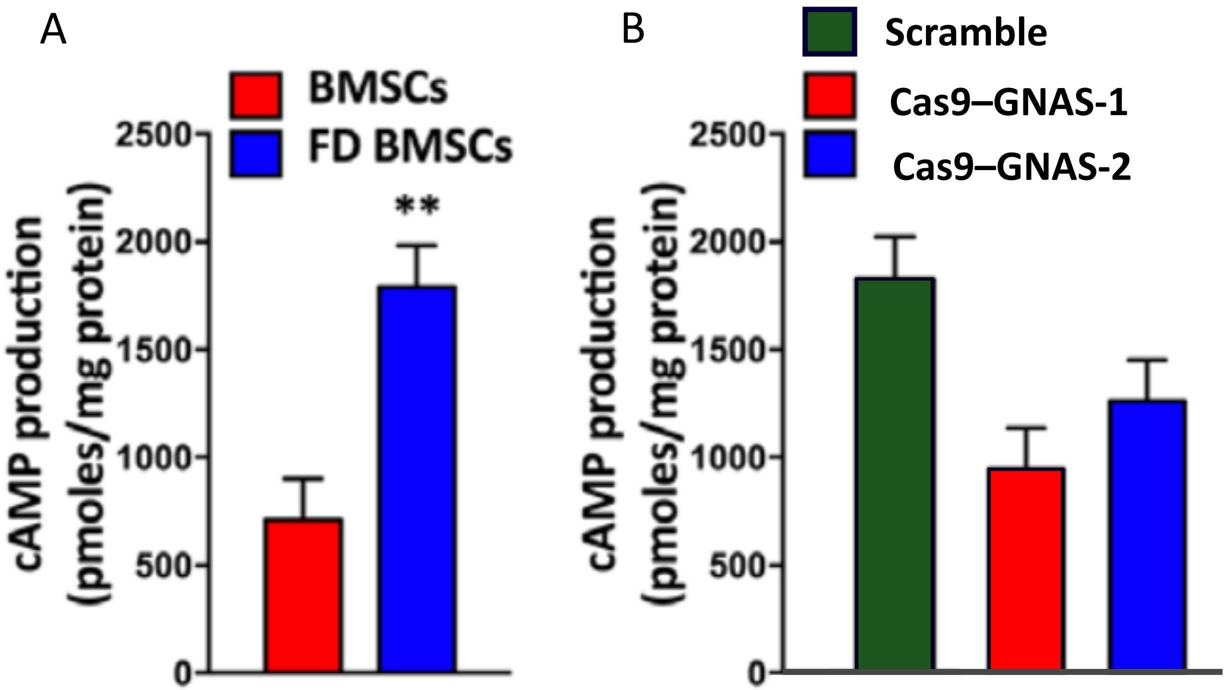




adapted by: *Watanabe et al.*, 2020

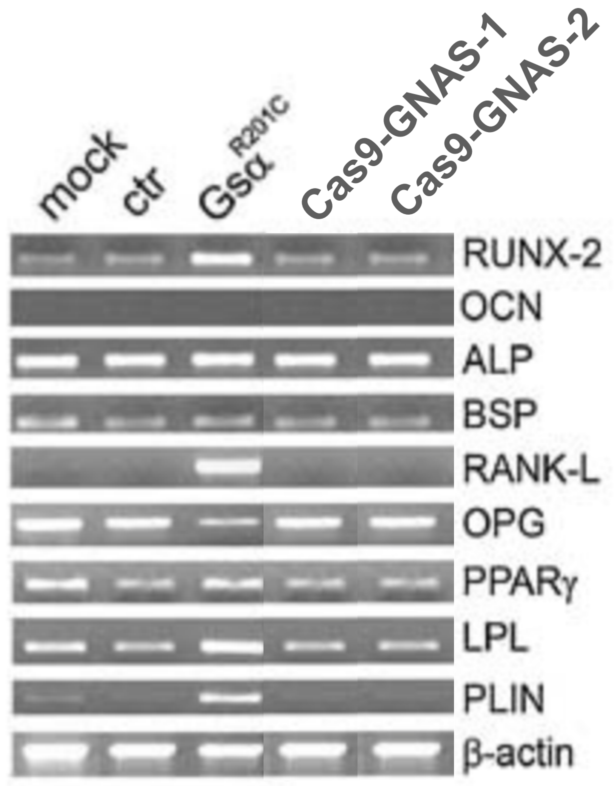
CRISPR/Cas9 strategy - *In vitro* results

A



adapted by: *Xiao, Fu, Zhu et al.*, 2018

C

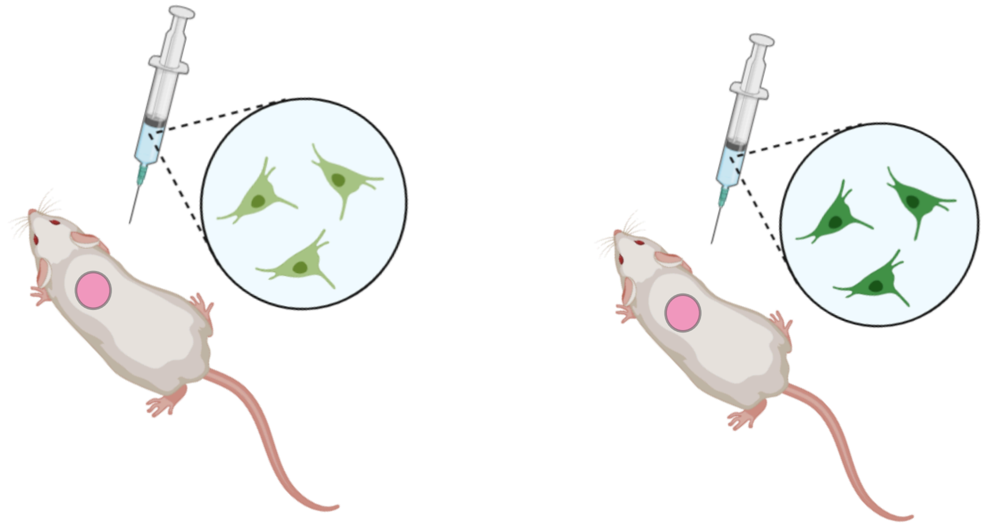


adapted by: *Piersanti et al.*, 2010

Graphic A-B. cAMP levels in FD BMSCs and control cells analyzed by ELISA .

Graphic C. RT-PCR showing osteogenic marker quantification in Gsα^{WT}-transduced BMSCs.

CRISPR/Cas9 strategy - *In vivo* transplant expectations



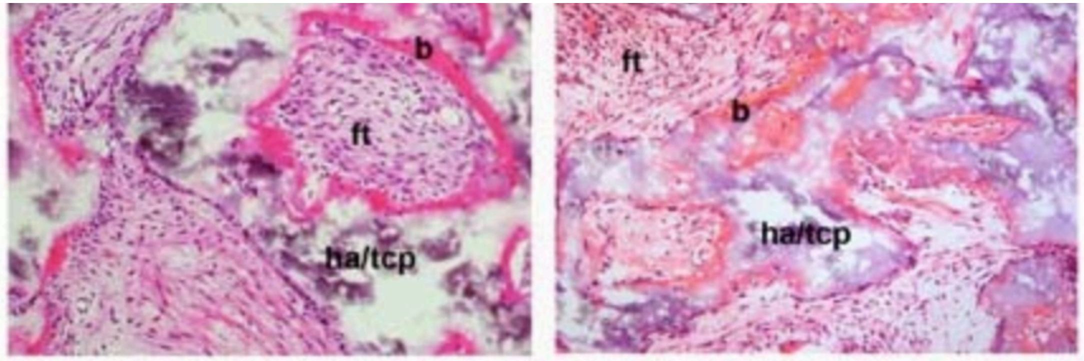
 Scramble BMSCs

 Cas9-GNAS-1 BMSCs

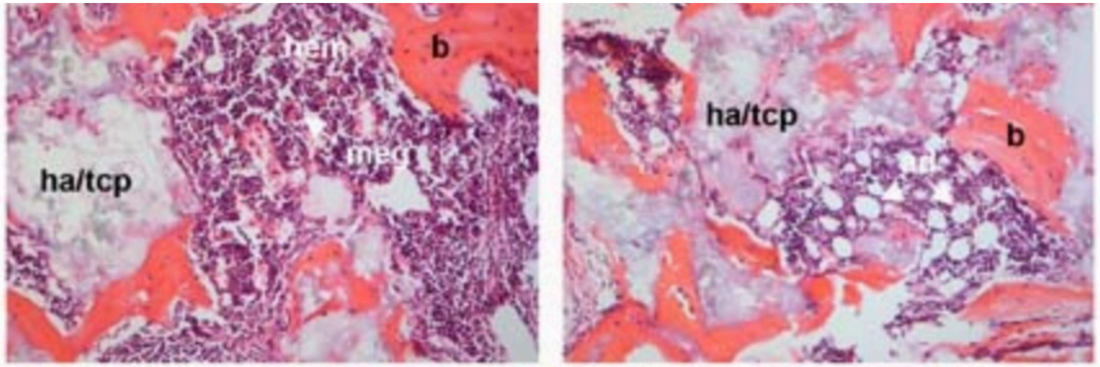
 HA intraossicle

Created in BioRender.com

Scramble BMSCs



Cas9-GNAS-1 BMSCs



adapted by: Piersanti et al., 2010

b = formation of abundant bone
ft = fibrous tissu
ha/tcp = hydroxyapatite/tricalcium phosphate
hem, meg = megakaryocytes
ad = adypocites

Conclusions

CRISPR/Cas13 strategy

- *In vitro* collected data show restoration of normal mRNA and protein expression levels in FD BMSCs transfected with CRISPR/Cas13 donor vector;
- *In vivo* treatment in mice shows normal bone tissue developement, resulting in a WT-like phenotype;
- These results suggest that HDAC8 could be a potential hopeful therapeutic target to fight Fibrous Dysplasia in BMSCs.

CRISPR/Cas9 strategy

- Innovative CRISPR technology allows to correct specifically the GNAS mutation to effectively heal Fibrous Dysplasia;
- *In vitro* experiment demonstrates restoration of cAMP normal level, resulting in a WT-like expression of osteogenic markers;
- *In vivo* experiments showed ossicles formation in treated mice.

Costs

MATERIALS

•12X lentiCRISPR v2 Addgene	€ 952.30
•16X 3-weeks-old male BALB/C mice Jax	€ 271.00
•1X QIAGEN OneStep RT-PCR Kit (100) Qiagen	€ 519.00
•2X QIAquick PCR Purification Kit (50) Qiagen	€ 208.00
•3X RUNX2 Wstern blot kit (AWBK41328) Avasysbio	€ 1,394.00
•2L DMEM – Dulbecco's modified Eagle Medium Thermofischer	€ 76.00
•10 mg Puromycin Sigmaaldrich	€ 63.50
•1X Trichrome Stain Kit (ab150686) Abcam	€ 312.26
•1X H&E Staining Kit (ab245880) Abcam	€ 131.20
TOTAL	**€ 3,927.26**

STAFF

•1X Class V Researcher	€ 60,084.02
•2X Class III Researcher	€ 109,736.68
•1X Post PhD student	€ 19,012.76
•1X PhD student	€ 16,067.88
•1X Animal technician	€ 28,500.00
TOTAL per Year	**€ 233,401.34**

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References

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- *Piersanti et al.* Transfer, analysis, and reversion of the fibrous dysplasia cellular phenotype in human skeletal progenitors. J Bone Miner Res. 2010; 25(5):1103-16.
- *Raimondo et al.* Changes in gene expression in human skeletal stem cells transduced with constitutively active Gsα correlates with hallmark histopathological changes seen in fibrous dysplastic bone. PLoS ONE 2020; 15(1): e0227279.
- *Saggio.* Perils and Promises of Therapeutic Approaches for the Stem Cell Disease Fibrous Dysplasia. Stem Cells Translational Medicine 2019; 8:110–111
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- *Yu Fu et al.* Histone deacetylase 8 suppresses osteogenic differentiation of bone marrow stromal cells by inhibiting histone H3K9 acetylation and RUNX2 activity. The International Journal of Biochemistry & Cell Biology (2014); 54:68–77.