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Idiopathic Pulmonary Fibrosis: Pulmonary functional reprise by reinfusion of CRISPR-Cas9 edited alveolar stem cells AT2

Alice Amico, Edoardo Brandi, Marco D'Angelo

Master U-STEM
Anno Accademico 2020-2021

Tutor: Dr. Mattia La Torre

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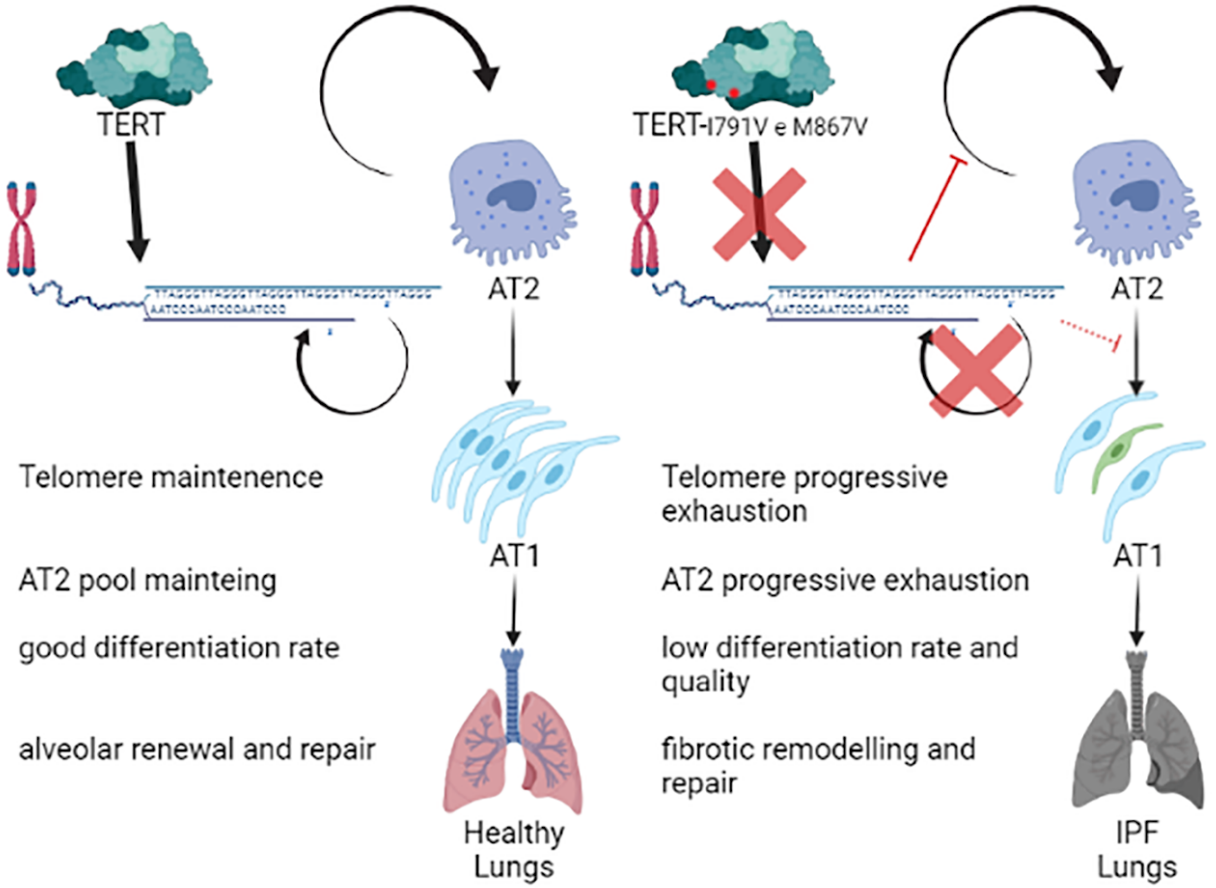

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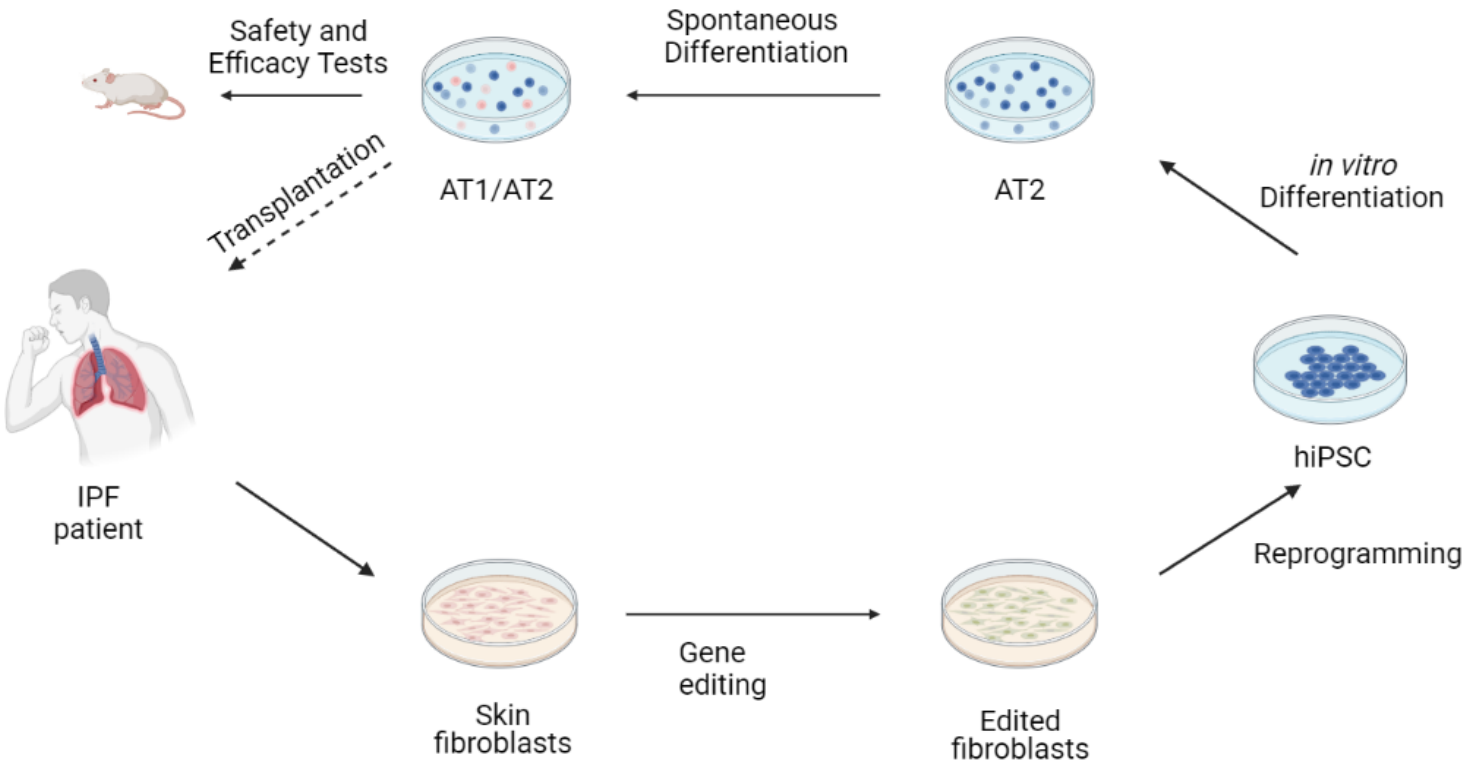
AIM: is it possible to recover IPF-lung functionality by patient derived edited AT2 cells infusion?



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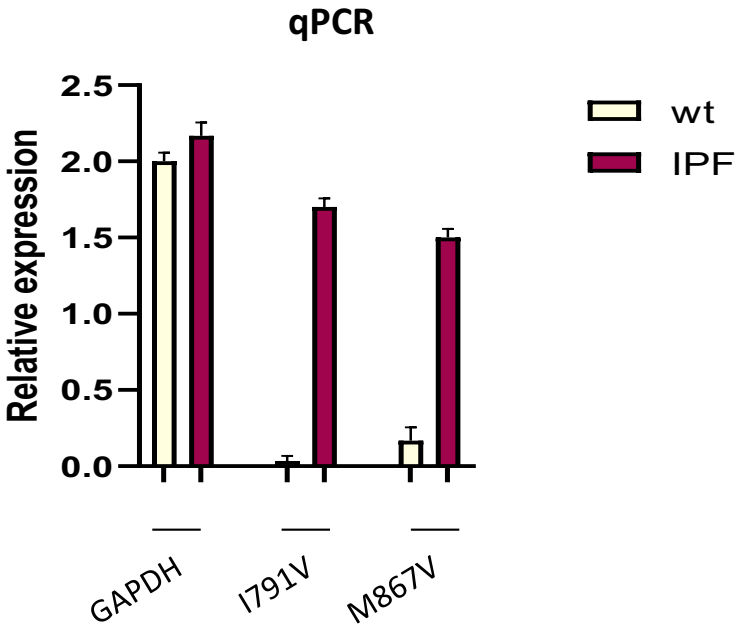
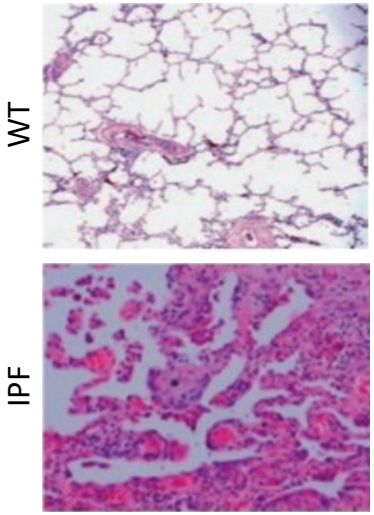
IPF-fibroblast

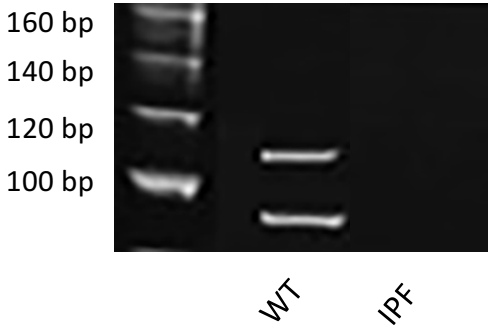
IPF- Mutation Primer design

Adapted from Zapparoli, G. V. *et al. BMC Cancer* (2013)





Adapted from Manolescu *et al* D. *Clin Interv Aging* (2018)



Adapted from Yoshizaki K et al. *BMC Vet Res*. 2021

The recruited IPF-patient shows both mutations of interest (I791V and M867V)

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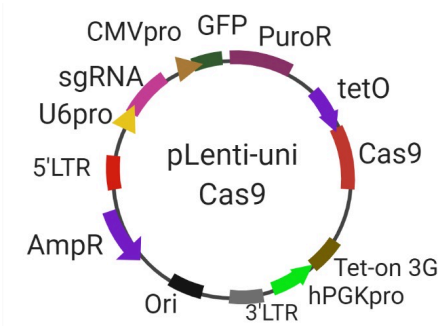


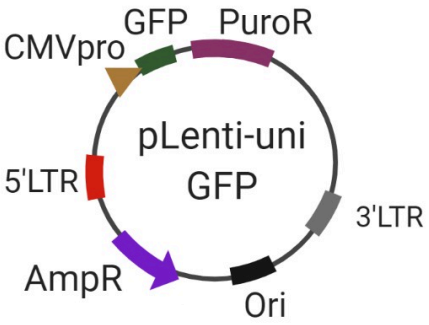
Patient derived fibroblast

Target sequence:

AGAAATCATCCACCAAACGCAGG

LEFT ARM
ACAAGAAATCATCCACCAAA

RIGHT ARM
CGCAGGAGCAGCCTAAA



Patient derived fibroblast

Patient derived-fibroblasts was transfected using two lentiviral vectors to obtain two cell lines: **Cas 9** (edited and labeled) and **GFP** (labeled)

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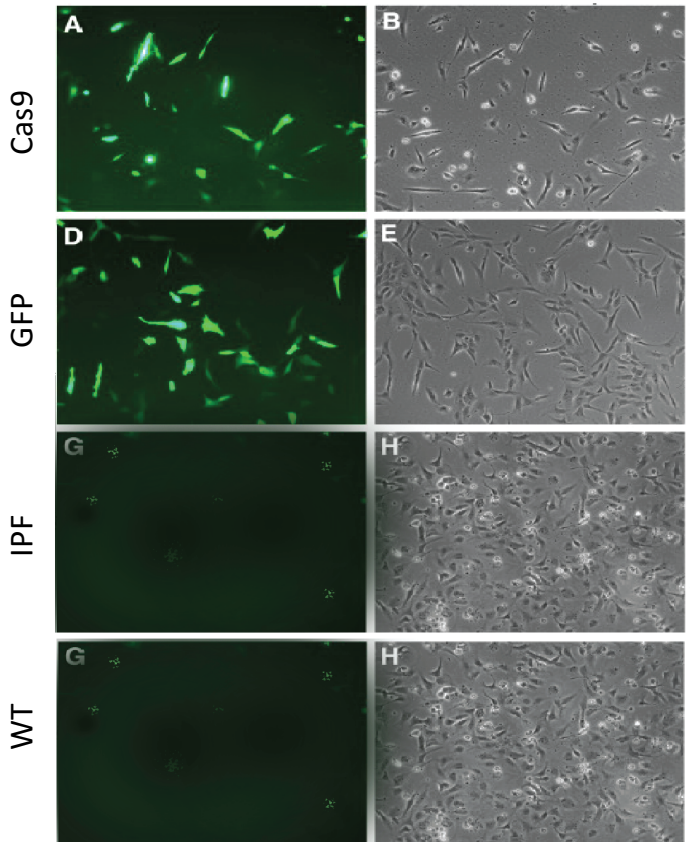
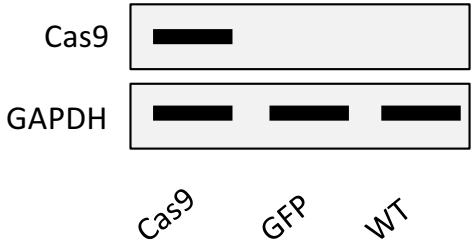
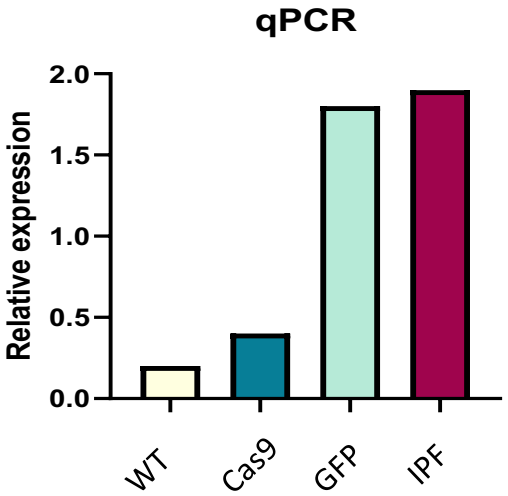
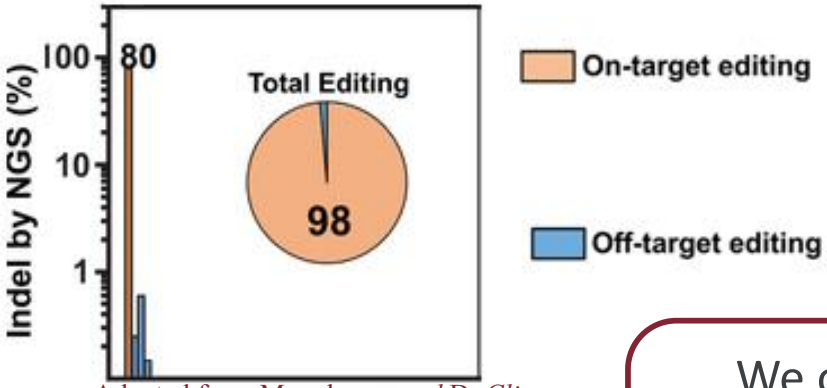


Adapted from Badakov, R. & Jaźwińska, *Cytotechnology* (2006)







Adapted from Manolescu *et al* D. *Clin Interv Aging* (2018)

We observed proper integration for the lentiviral vectors and expression of carried genes

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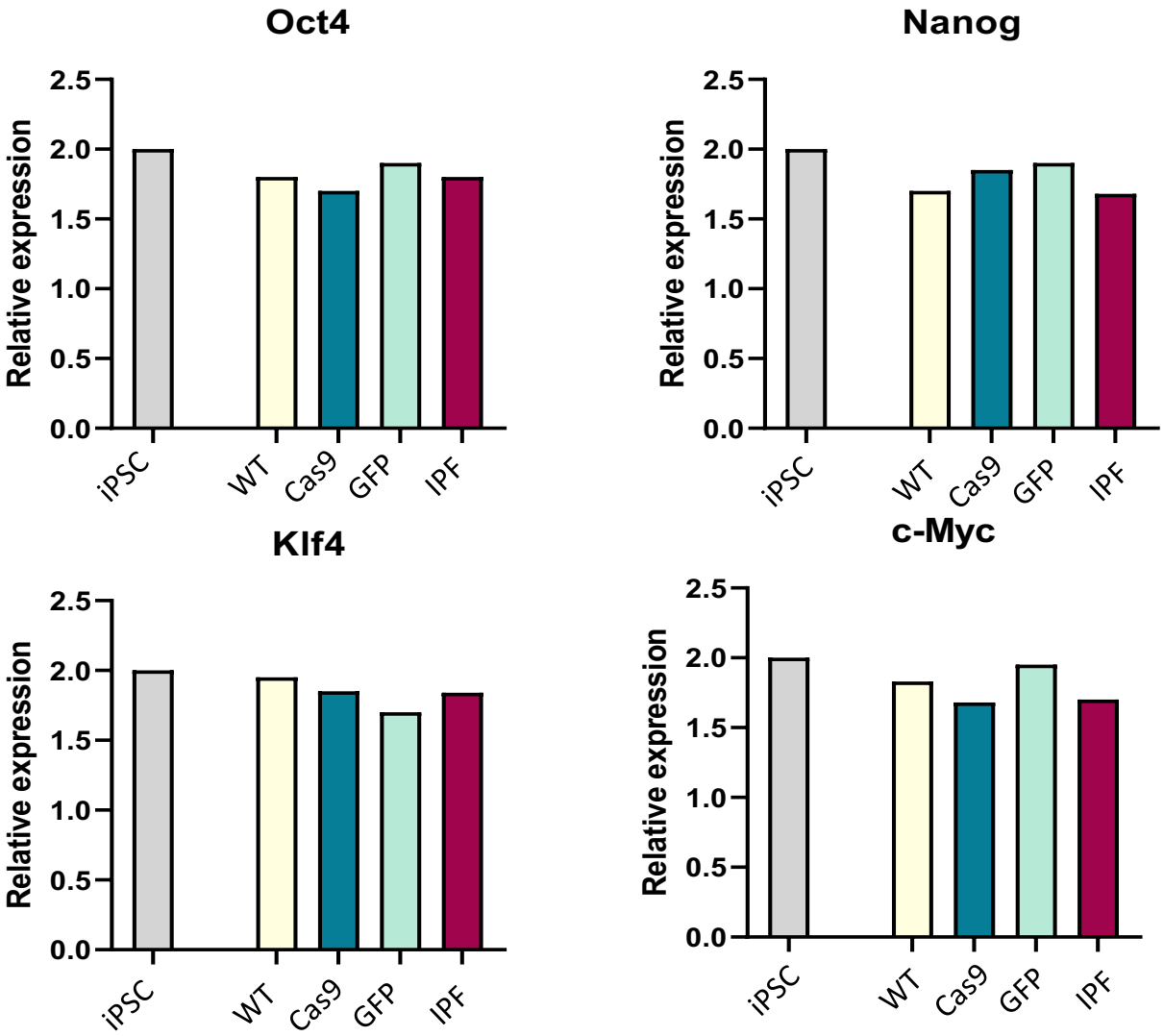

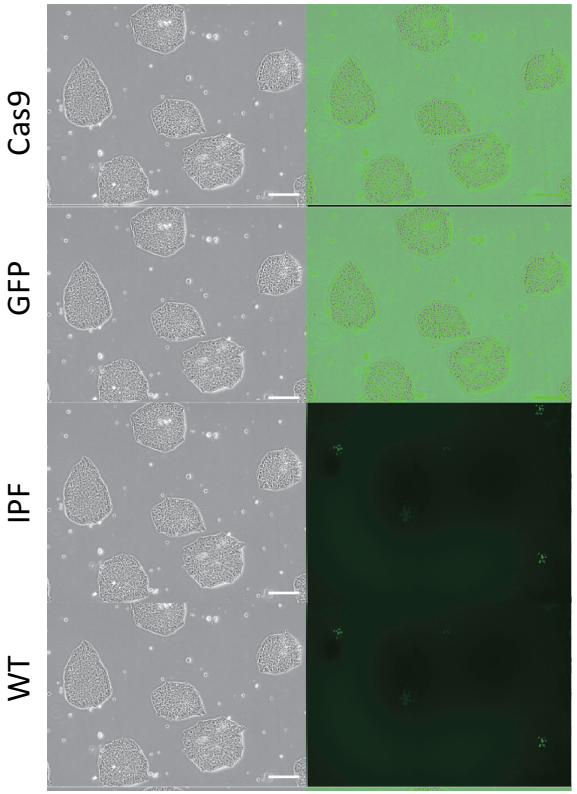

Adapted from Ohnuki M, Takahashi K. *Philos Trans R Soc Lond B Biol Sci*. 2015

mRNA transfection of IPF patient edited and labeled cells was used to obtain iPSC cell lines

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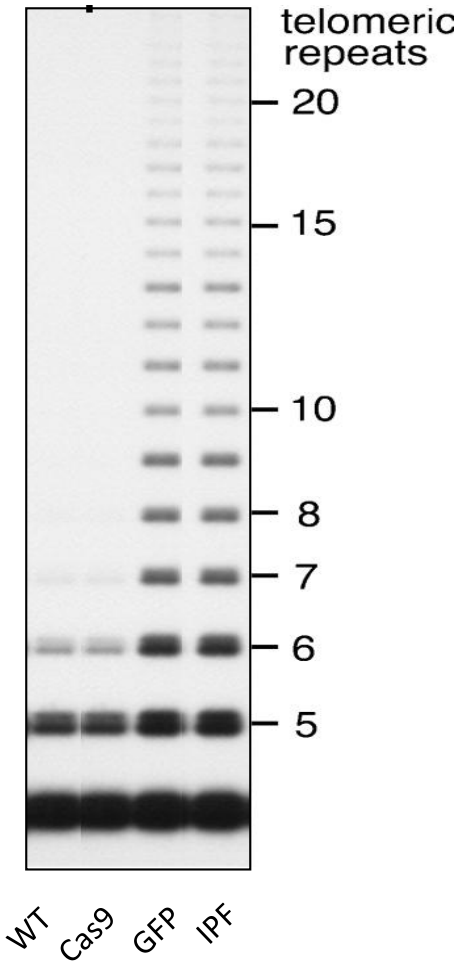
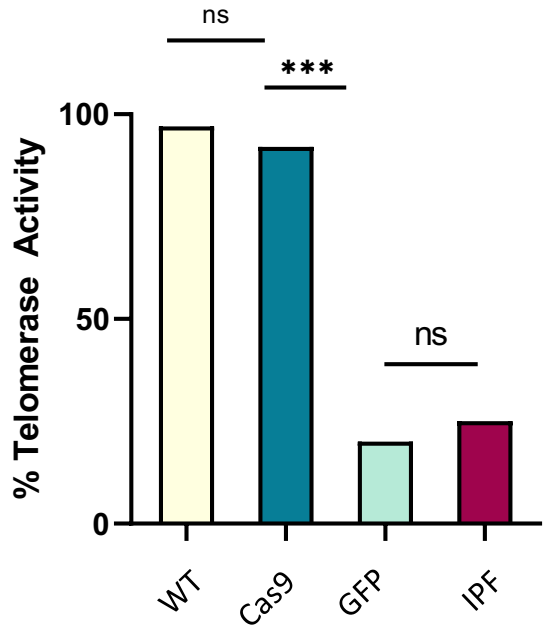
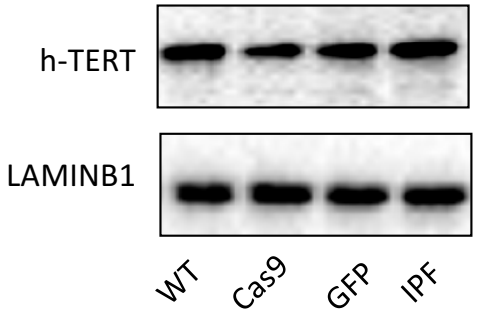
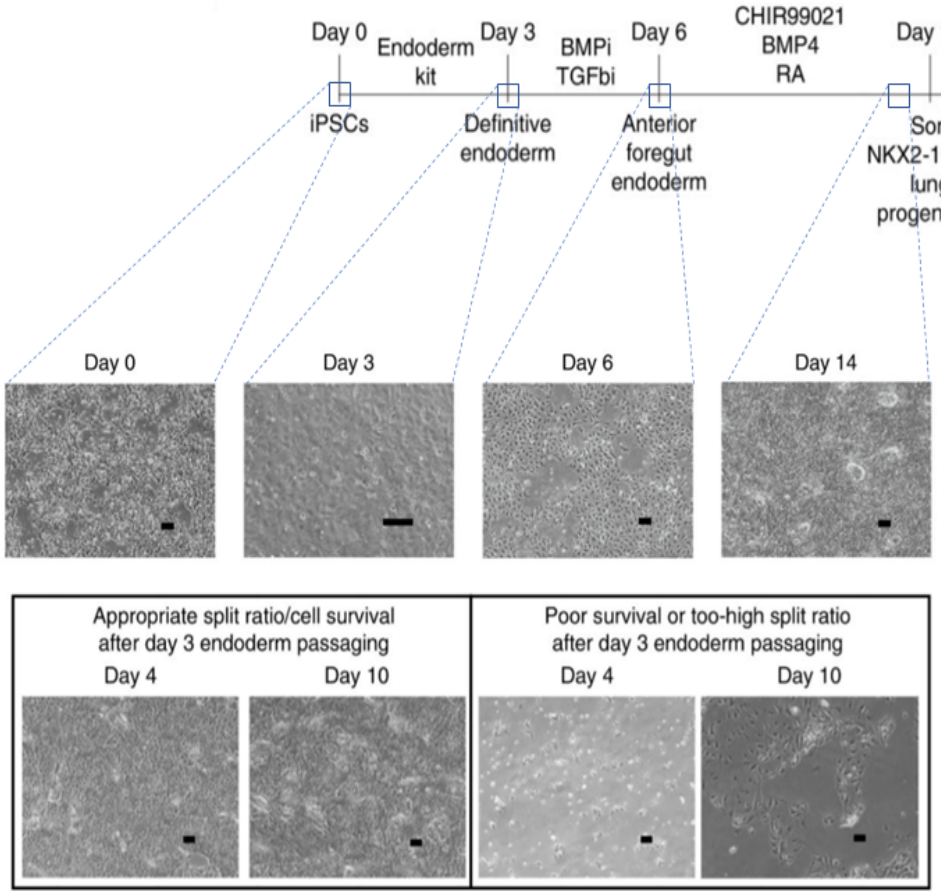


Adapted from Garforth SJ, Wu YY, Prasad VR. *Biochem J*. 2006

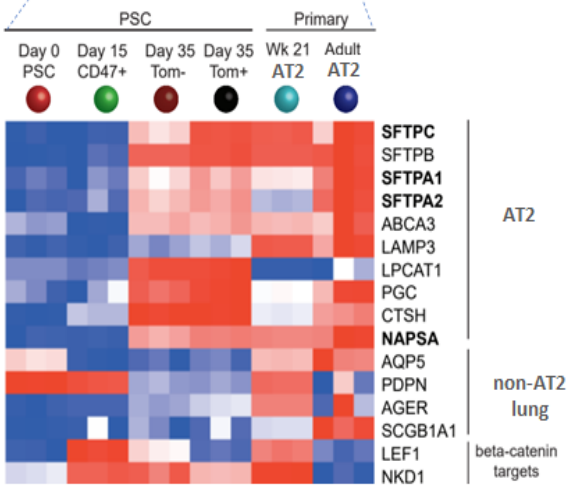




A non-protein level dependent TERT functional recovery is showed by the Cas9 line



Adapted from Jacob A et al. *Cell Stem Cell*.



Adapted from Jacob A et al. *Cell Stem Cell*.

AT2 cell lines were obtained from iPSC lines through a 41 days-differentiation protocol

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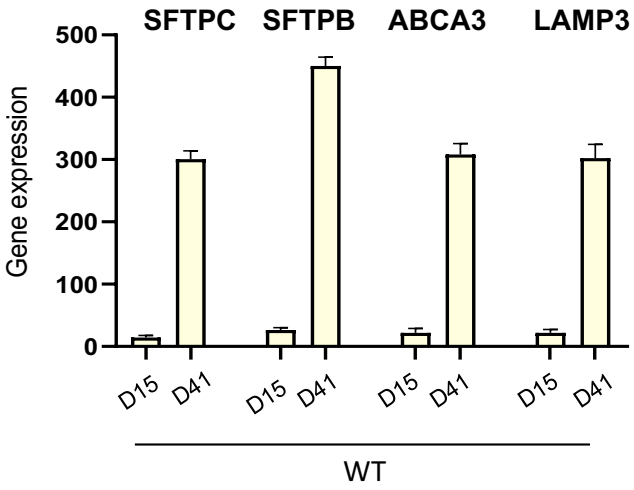

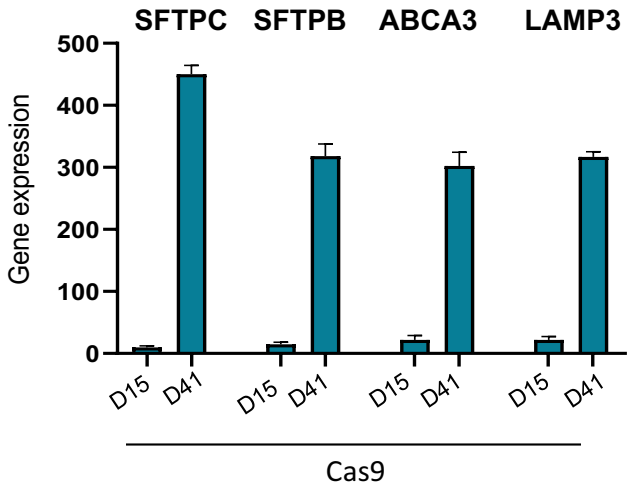

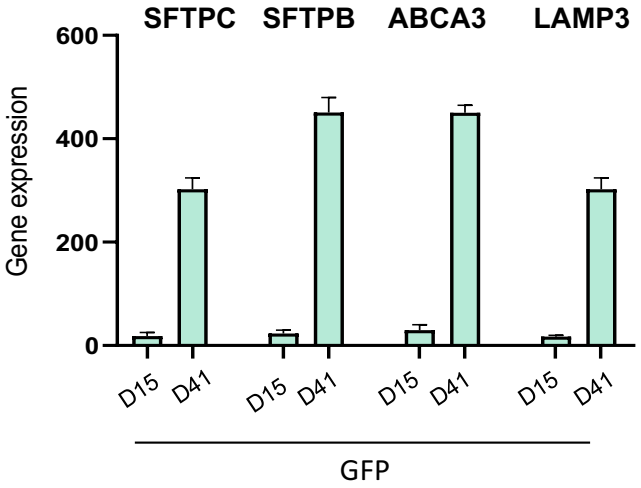

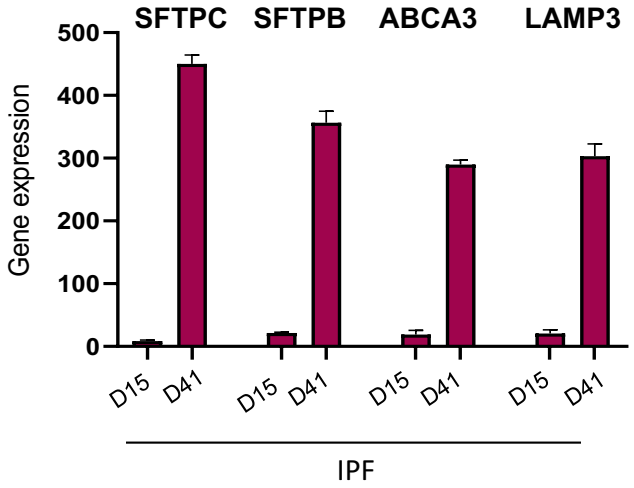

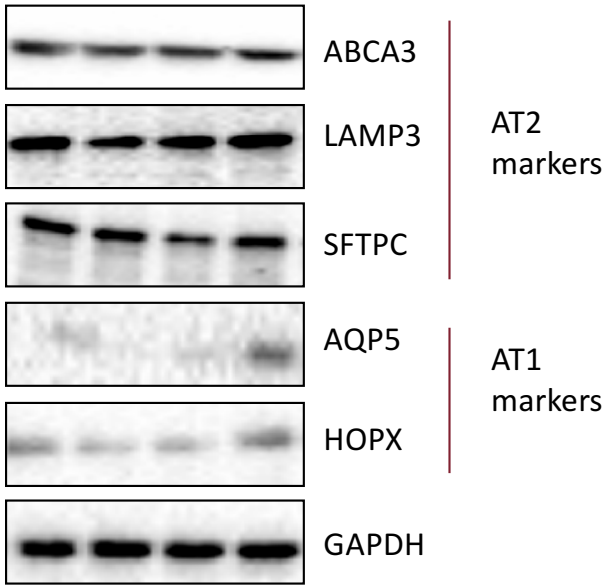

Each of the 4 differentiated lines showed expression of AT2 cells genes comparable to WT

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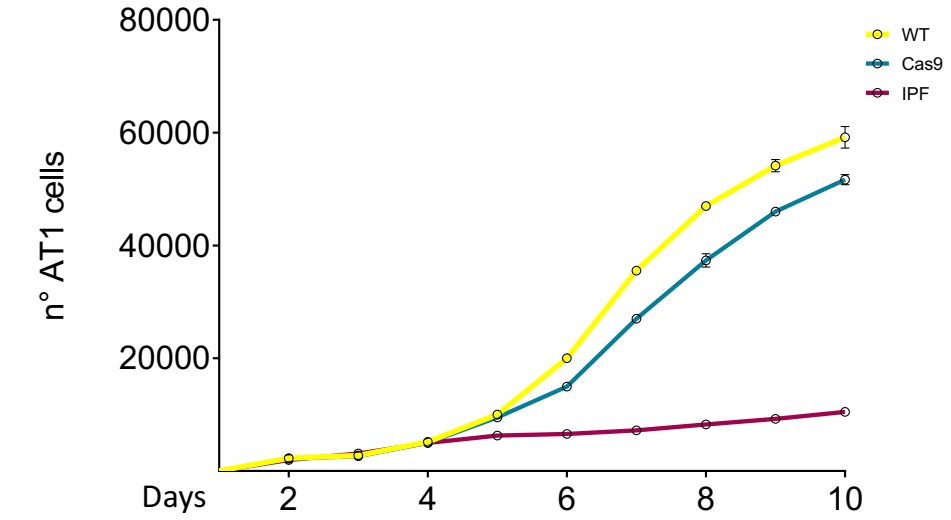

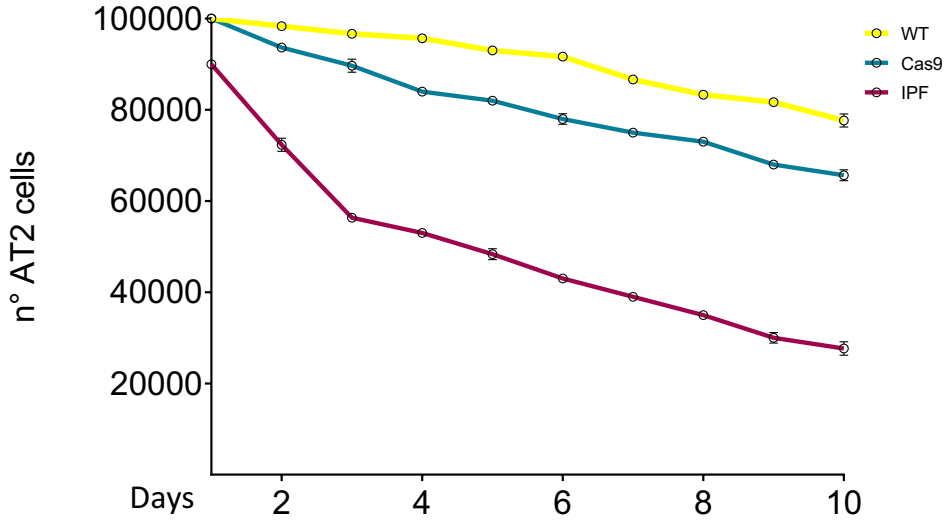

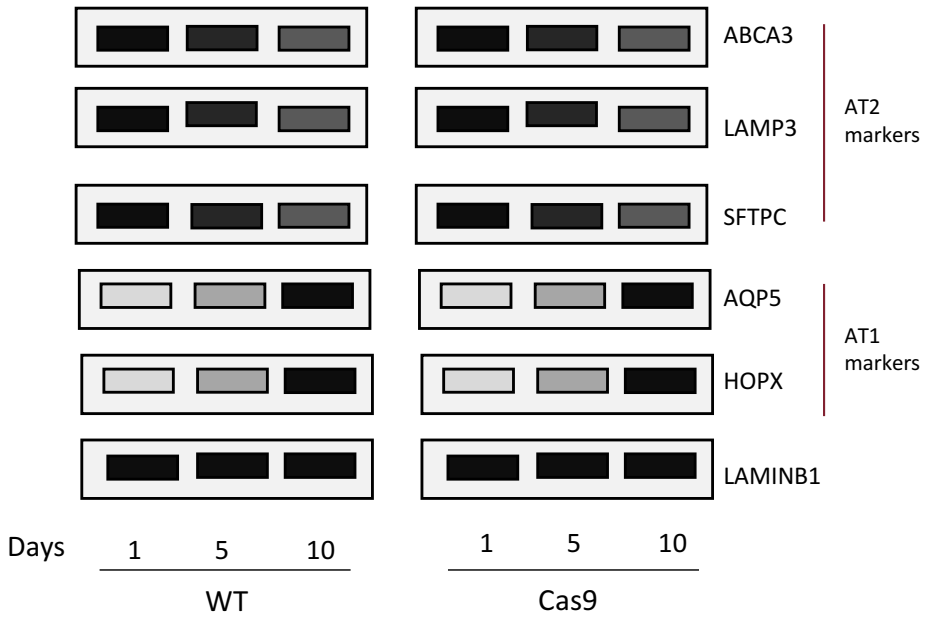

Cas9 showed functional recovery: AT2 cells staminal pool is conserved, while differentiation and proliferation to AT1 cells is recovered

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TERT -/-

Bleomycin
low dose

IPF
model

Povedano, Juan M et al. - 2015

TERT -/-

WT

Povedano, Juan M et al. - 2015

We used TERT -/- mice treated with bleomycin as animal model for *in vivo* treatment

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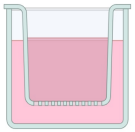
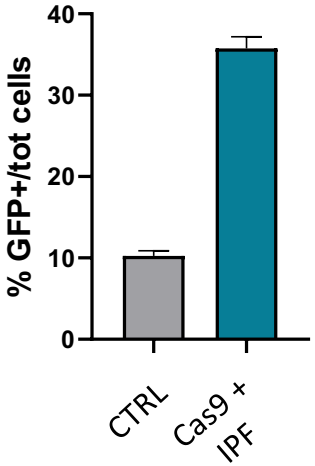
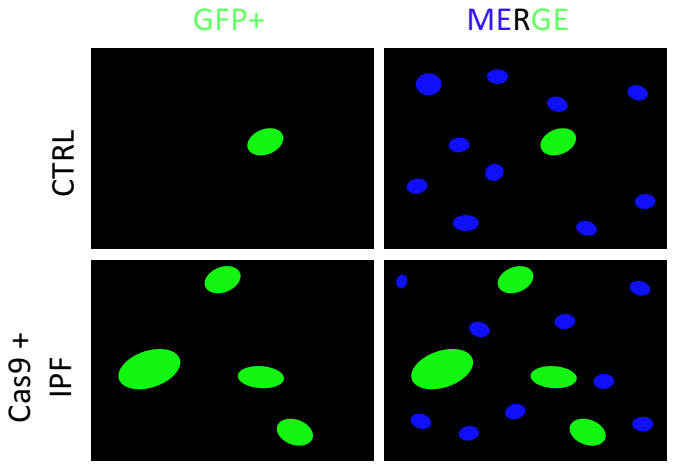
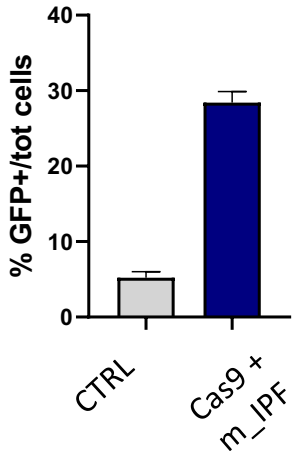


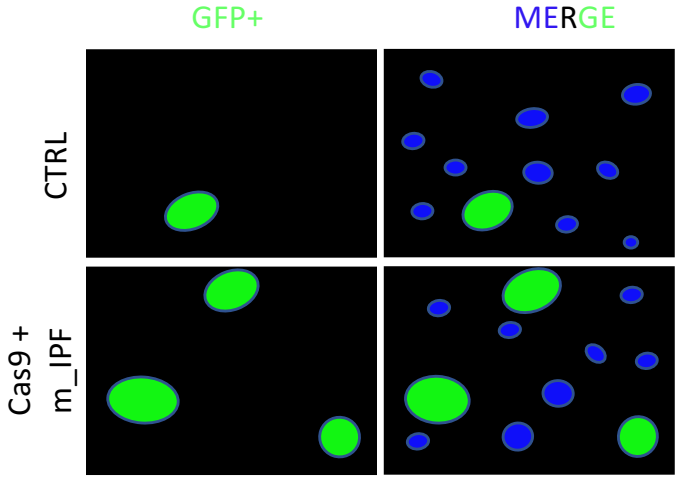
AT2_Cas9 + AT2_IPF

AT2_Cas9 + mouse-AT2 (isolated from IPF model)









AT2 edited cells in both of the human and mice cell cultures showed an advantage compared to GFP, WT and IPF lines. Therefore, we believe AT2 cells to be effective in restoring AT2 cells pool *in vivo*.

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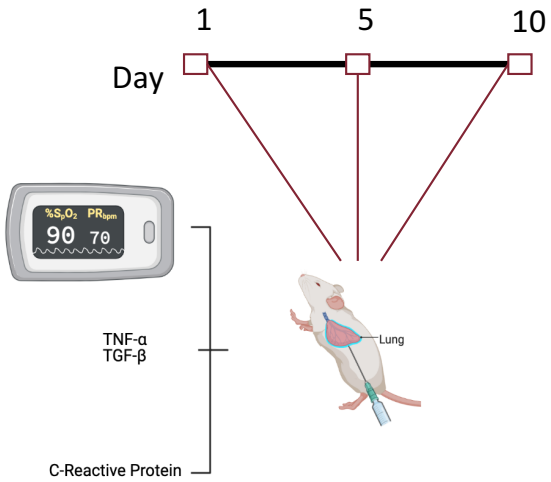
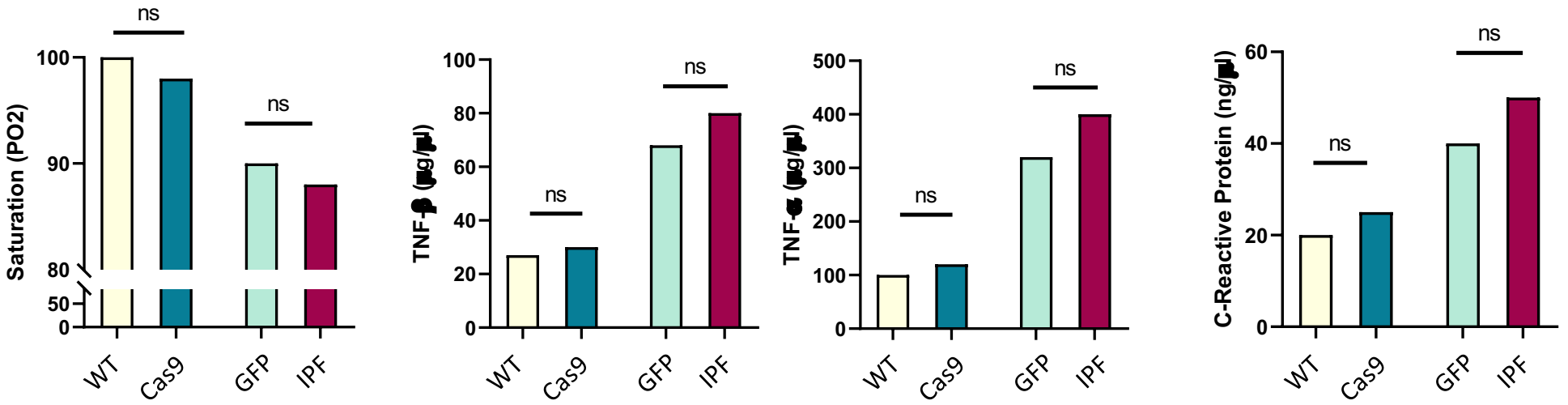


After 10 days by the injection of Cas9 cells in the IPF mouse model a reprisal of the AT2 cells functionality is observed, as showed by the comparable to WT levels of the selected markers



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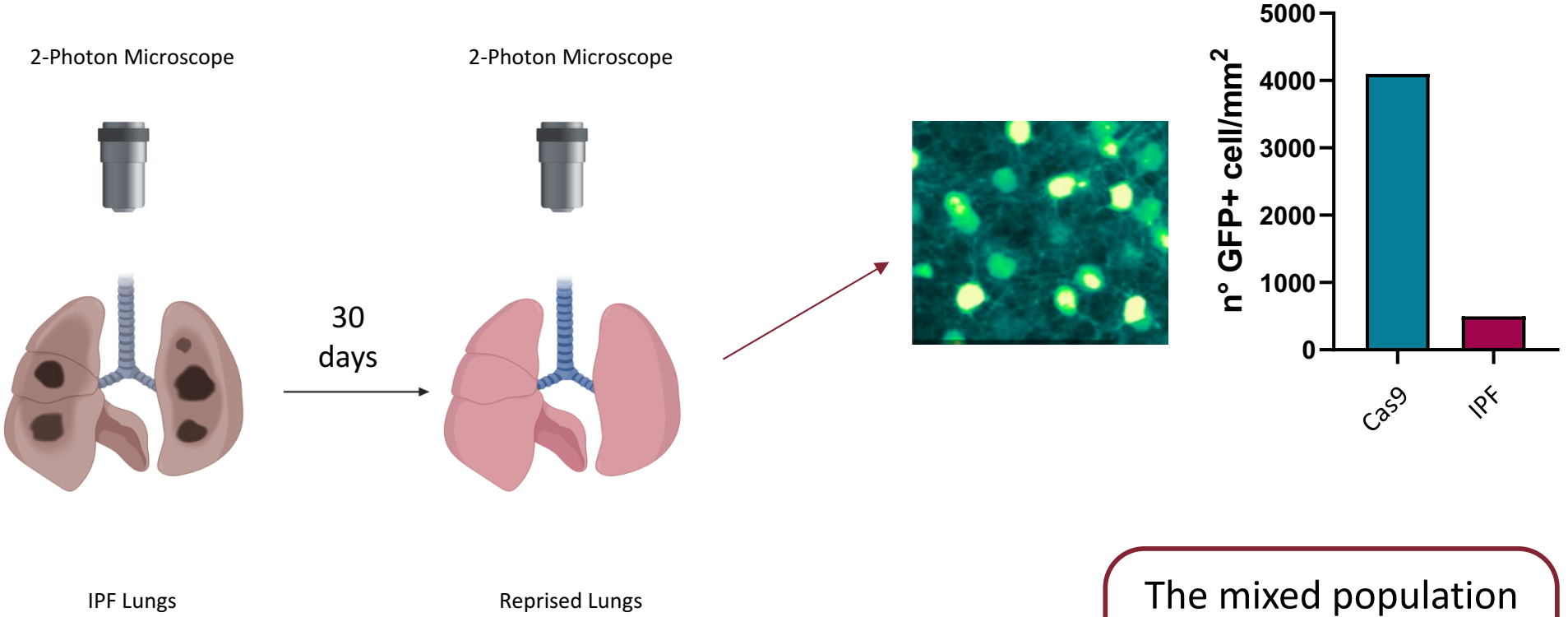

The mixed population (AT1/AT2) is mantained *in vivo* 30 days after injection

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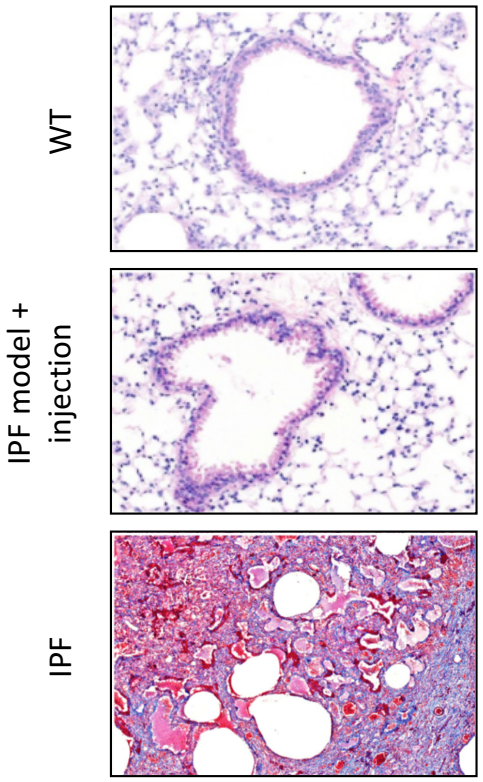
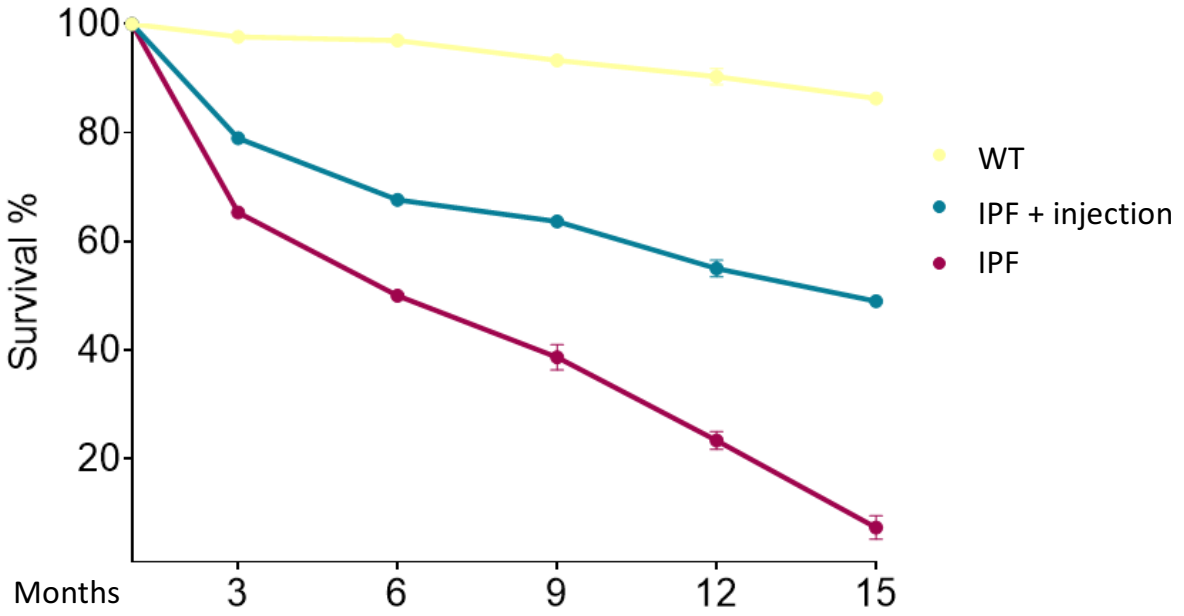


Adapted from Cony, F. Get al. *Pesquisa Veterinária Brasileira* (2019)



After 30 days by the injection of Cas9 cells in IPF mice, a tissue regeneration and replacement of the fibrotic areas is showed when compared to untreated IPF mice. Furthermore, an increase of survival up to 15 Mo. is observed

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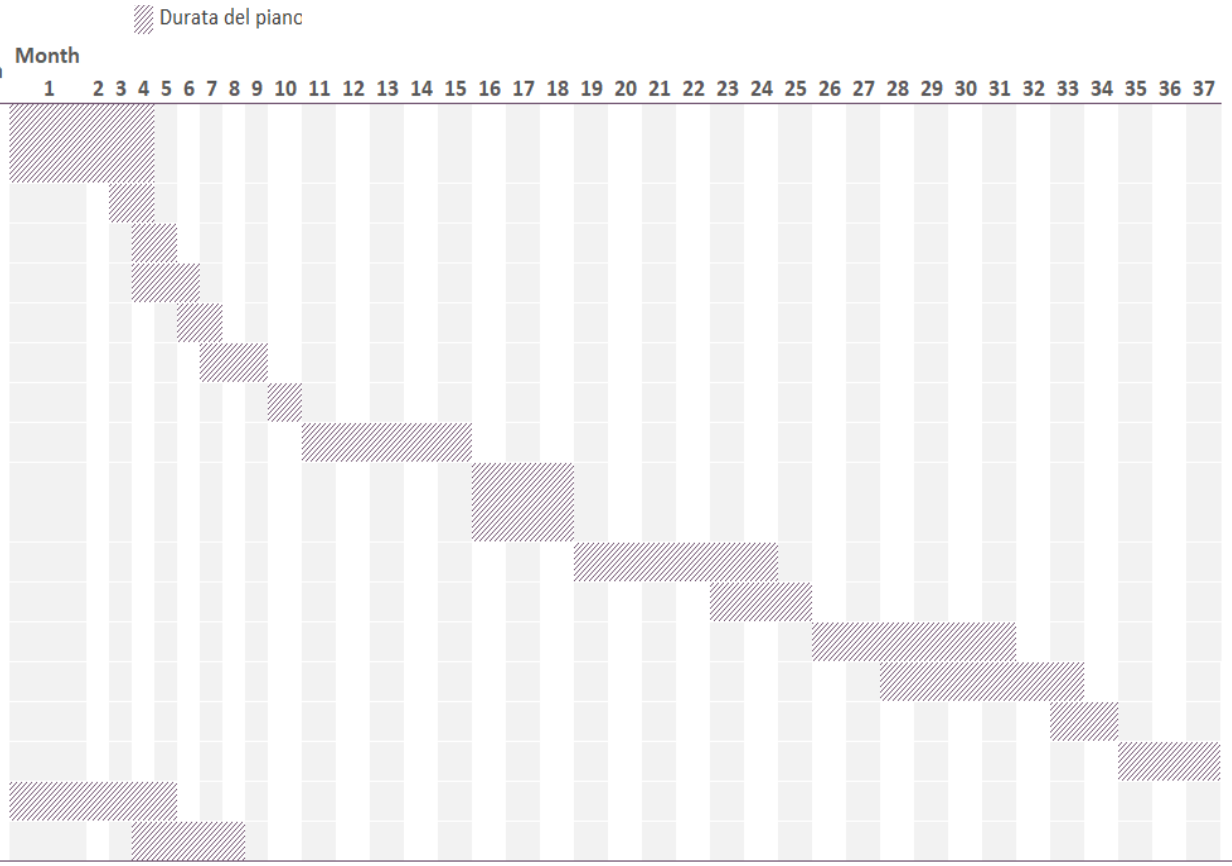
Project Planning

Project activities	planned start	expected duration
Patient and healty subject recruitment	1	4
Primary Fibroblast colture derivation	3	2
Mutation control check	4	2
Trasfection procol set-uo on HEK-293	4	3
Lentiviral Trasfection on Fb	6	2
Reprogramming	7	3
Tert functionality control	10	1
Differentiation iPSc into AT2	11	5
AT2 functional and differentiation control	16	3
IPF mouse model obtaining	19	6
Growth advantages assay and control	23	3
In vivo- AT2 intraoulmonary injection	26	6
monitoring models rescue	28	6
histological section assay	33	2
Results divulgation	35	4
ethical committee submission	1	5
animal ethical committee submission	4	5



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Budget

Costs	Total Budget	Co-Funding	List of costs proposed for funding to the backer	Percentage of total proposed to the backer
Staff salary	0,00	0,00	Not permitted	0
Researchers' contracts	80.000,00	0,00	80.000,00	23,73887
Equipment (leasing-rent)	0,00	0,00	0,00	0
Supplies	140.000,00	0,00	140.000,00	41,54303
Model costs	60.000,00	0,00	60.000,00	17,80415
Subcontracts	0,00	0,00	0,00	0
Patients' costs	3.500,00	0,00	3.500,00	1,038576
IIT services and databases	0,00	0,00	0,00	0
Publication costs	4.000,00	0,00	4.000,00	1,186944
Conferences	3.500,00	0,00	3.500,00	1,038576
Travels	6.000,00	0,00	6.000,00	1,780415
Overheads	40.000,00	0,00	40.000,00	11,86944
Coordination costs	0,00	0,00	0,00	0
Total	**337.000,00**	**0,00**	**337.000,00**	**100**

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Future perspectives

- Follow up after 30 days in terms of survival, safety and efficacy
- *In vivo* step in human
- Is it possible to cryoconserve patient derived edited cells in a AT2-state?
- Is there an alternative to intrapulmonary injection?
- Does this protocol properly work on other mutations or sporadic form of IPF?

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Bibliography

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- *Povedano, Juan M et al. – 2015*
- Cony, F. Get al. *Pesquisa Veterinária Brasileira* (2019)