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Supplementary Materials for

Lin28a induces SOX9 and chondrocyte reprogramming via HMGA2 and blunts cartilage loss in mice

Yohan Jouan *et al.*

Corresponding author: Martine Cohen-Solal, martine.cohen-solal@inserm.fr

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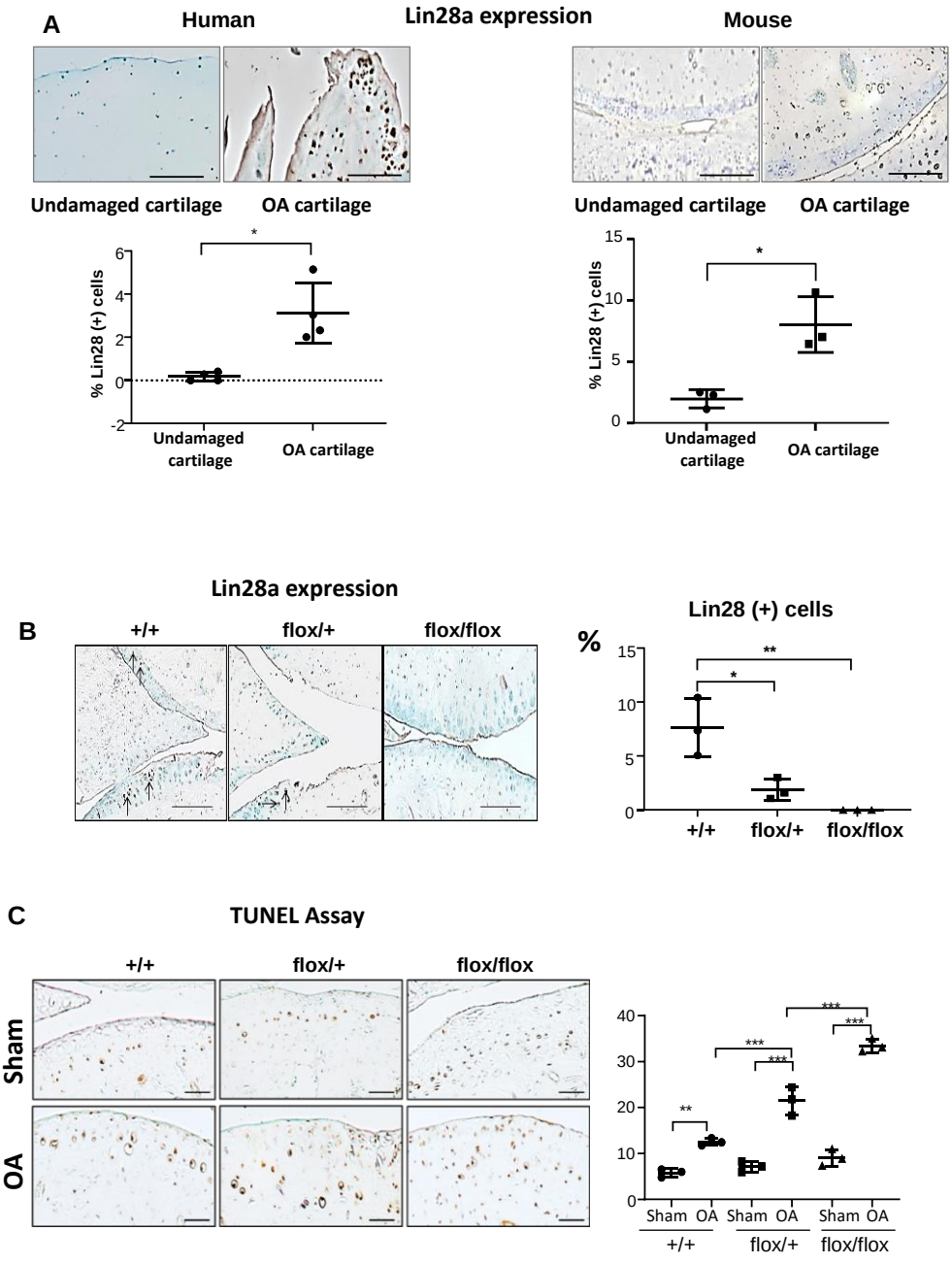
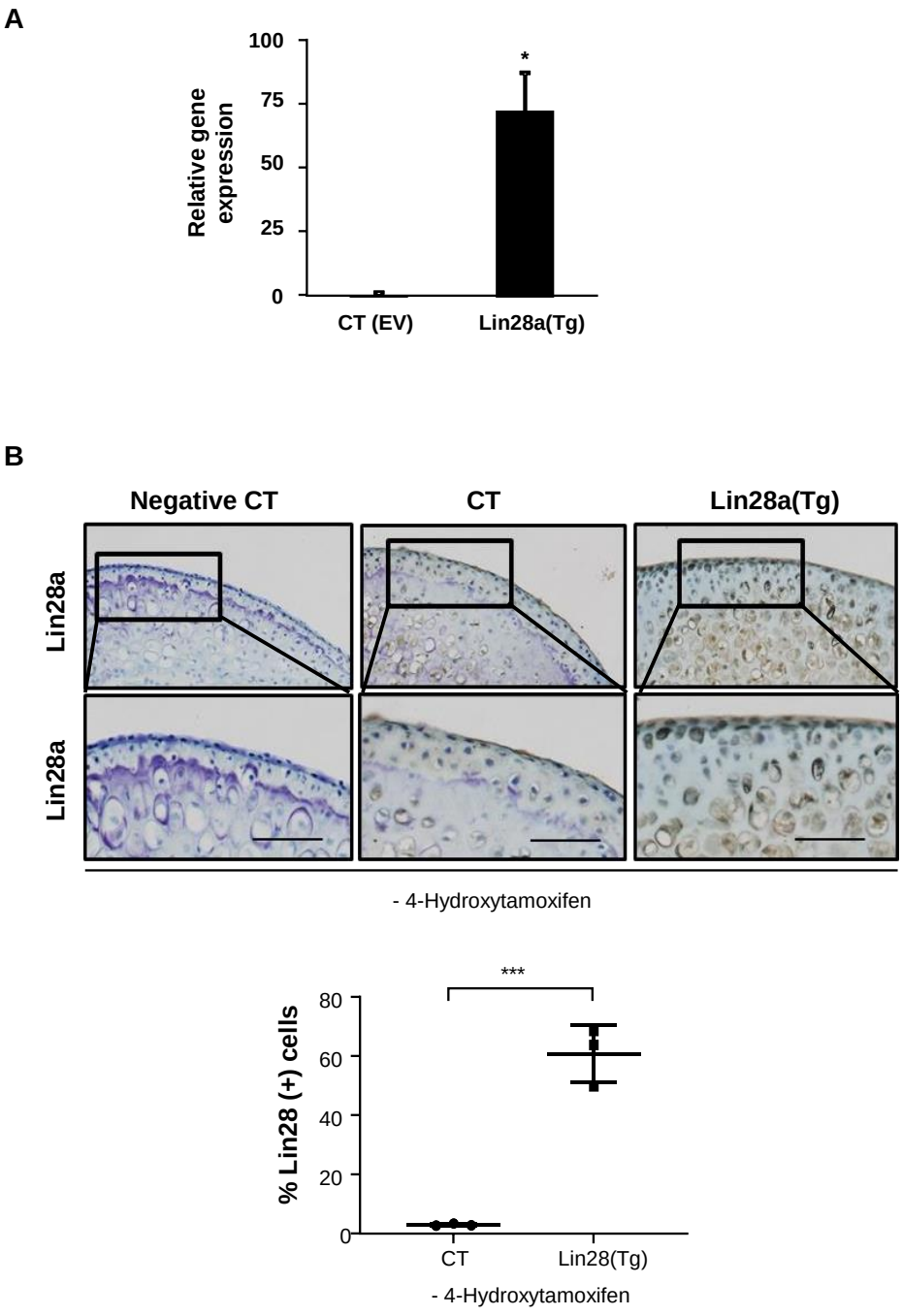
This PDF file includes:

Figs. S1 to S6

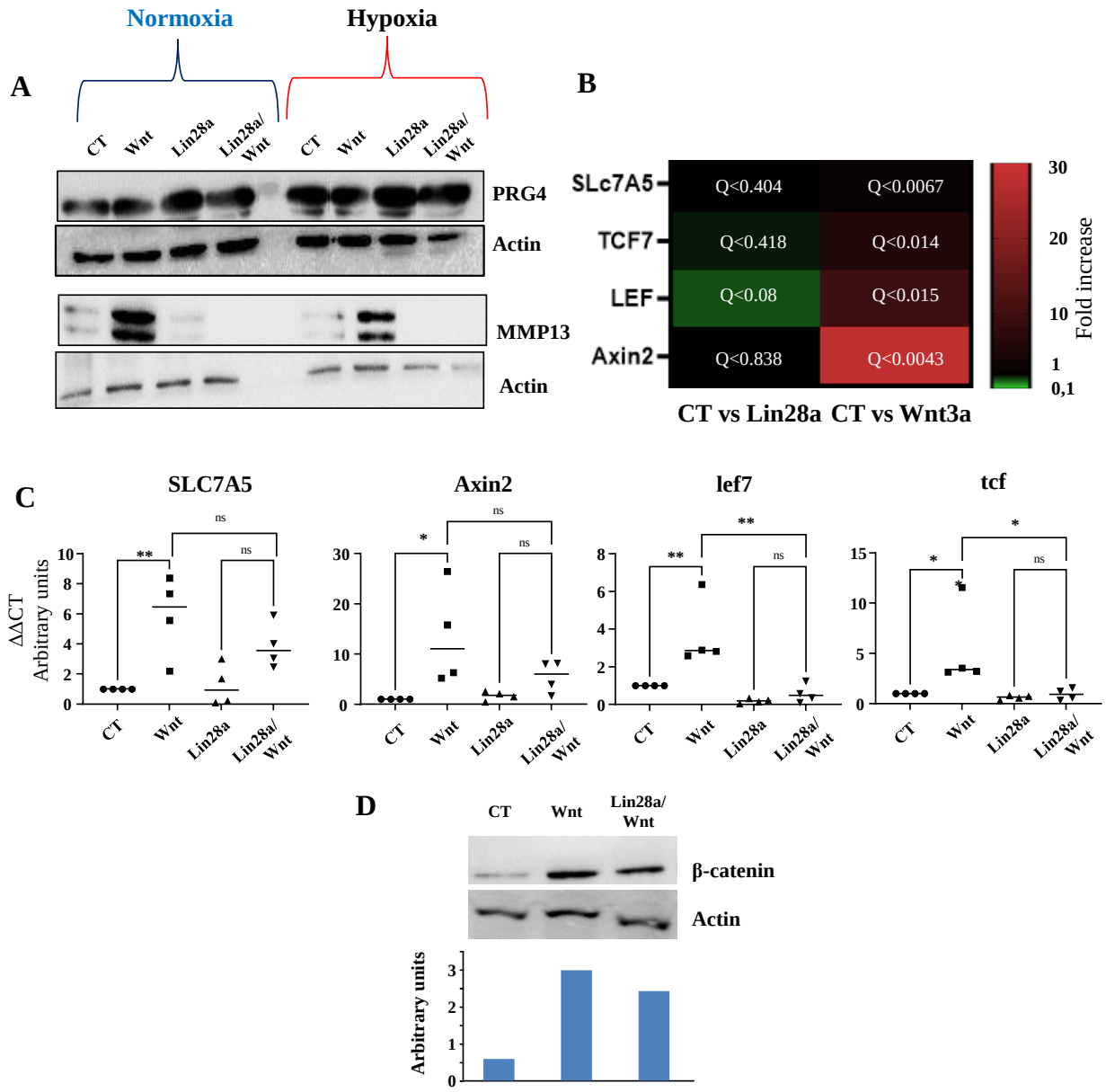


Supplementary figure S1: Expression of Lin28a in tissues of humans and mice with osteoarthritis (OA)

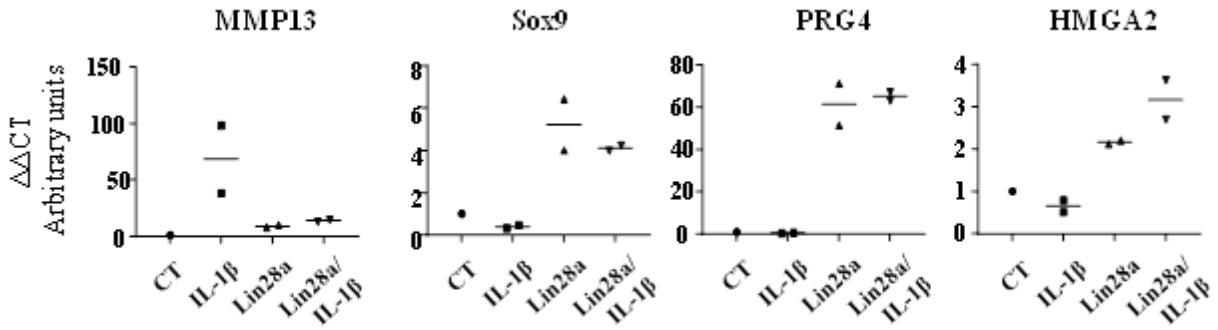
(A) Immunohistochemistry and quantification of Lin28a expression in humans and mice in undamaged cartilage and OA cartilage (Scale bar, 200 µm for humans and 100 µm for mouse). (B) Immunohistochemistry and quantification of Lin28a expression in cartilage of Lin28a control (CT; +/+), heterozygous deleted (flox/-) and homozygous deleted (flox/flox) mice 8 weeks after OA induction (scale bar, 100 µm). (C) Immunohistochemistry and quantification of apoptotic cells in +/+, flox/- and flox/flox mice under sham and OA treatment (scale bar, 100 µm).



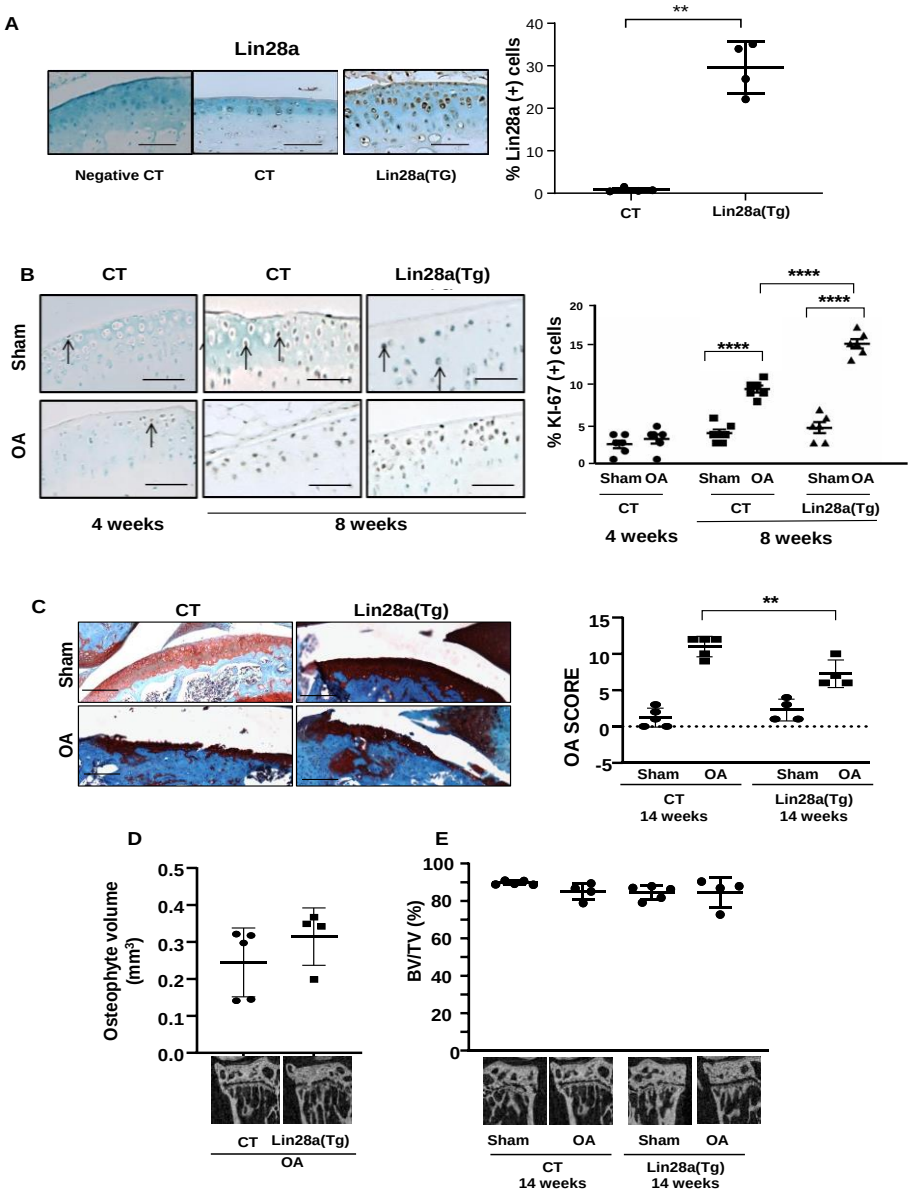
Supplementary figure S2: Lin28a increased anabolism and decreased catabolism in chondrocytes
Primary chondrocytes were transduced with Lin28a lentivirus [Lin28(TG)] or empty vector [Ct(EV)]. (A) Lin28a gene expression was assessed by RT-qPCR in response to [Lin28(TG)] or [Ct(EV)] transduction on primary chondrocytes. Data are mean ± SEM. *P<0.05 compared with controls. (B) Immunohistochemistry and quantification of Lin28a expression in explants from wild-type and Lin28a(Tg) mice treated or not with Wnt3a. 4-Hydroxitamoxifen was used to induce in vitro recombination. Negative condition = IgG control (scale bar, 100 µm). Data are mean ± SEM. ***P<0.001



Supplementary figure S3: Interactions of Lin28a and Wnt canonical pathway
(A) Wild-type (WT) mouse primary chondrocytes were transduced with Lin28a lentivirus [Lin28a(TG)] or empty vector [CT (EV)] and cultured for 48h 1% O_2 or 21% in the presence of Wnt3a conditioned medium (Wnt3a-CM) to trigger chondrocyte catabolism. Western blot analysis was performed for PRG4 and MMP13. **(B)** Heatmap representing the fold-increase in the expression on Wnt canonical gene targets based on RNAseq analysis between CT and Lin28a overexpressing chondrocytes or CT and Wnt3a-treated chondrocytes. **(C)** RTqPCR analysis of Wnt canonical target gene expression extracted in WT mouse primary chondrocytes transduced with Lin28a lentivirus [Lin28a(Tg)] or empty vector [CT (EV)] and cultured for 48h 1% O_2 in the presence of Wnt3a conditioned medium (Wnt3a-CM) to trigger chondrocyte catabolism. (D) Western Blot analysis of β-catenin expression,*p<0.05 and ** p<0.01 compared with control.

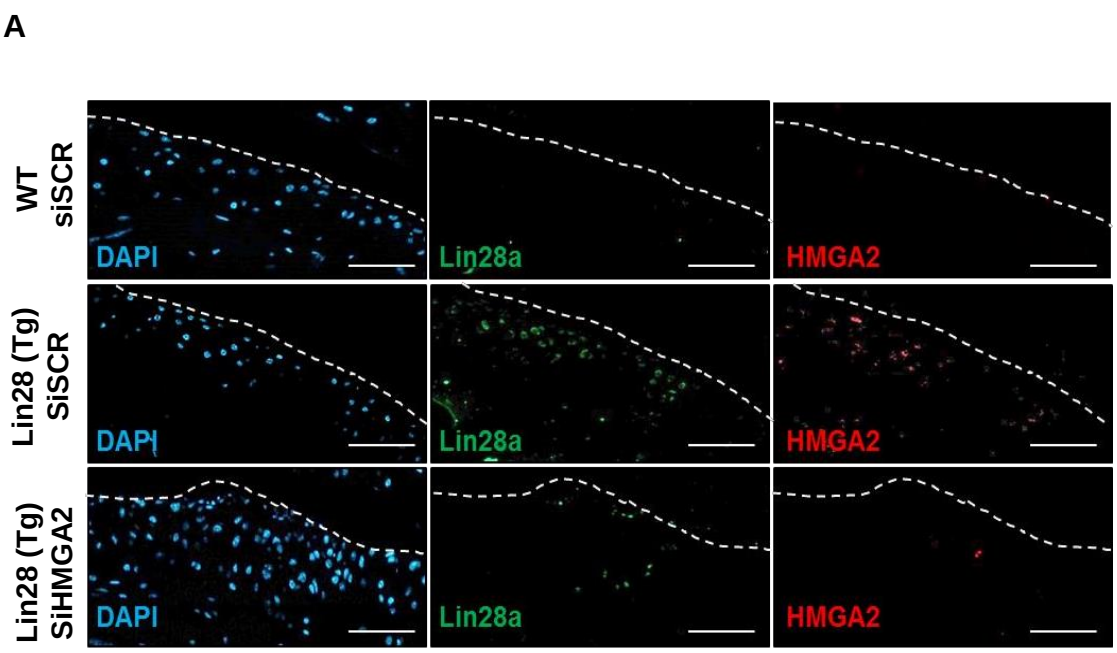


Supplemental Fig S4: Lin28a modulated IL1-induced catabolism
RTqPCR analysis of mRNA expression of chondrocyte catabolic gene (MMP13), chondrocyte anabolic genes (SOX9, PRG4) and Lin28a target (HMGA2) in chondrocytes from WT primary chondrocytes transduced with Lin28a lentivirus [Lin28a(Tg)] or empty vector [CT (EV)] and cultured for 48h 1% O_2 in the presence of IL-1 (1ng/ml).



Supplementary figure S5: Lin28a overexpression reduced OA phenotype in preventive or regenerative mouse models.

(A) Immunohistochemistry and quantification of Lin28a expression in Ct and Lin28a(Tg) mice 8 weeks after OA induction, negative condition = IgG control (Scale bar = 100 µm). (B) Immunohistochemistry and quantification of Ki-67 expression in sham or OA CT and Lin28a(Tg) mice at 4 and 8 weeks post-OA with regenerative treatment (Scale bar = 100 µm). (C) Safranin-O staining and quantification of OA score for articular cartilage in mice at 14 weeks post-OA induction (scale bar, 100 µm).(D) Osteophyte volume analyzed by microtomography in mice at 14 weeks post-OA induction. (E) Subchondral BV/TV analyzed by microtomography in mice at 14 weeks post-OA induction. Data are mean ± SEM. **P<0.01 *** P<0.005 ****P<0.001.



Supplementary figure S6: HMGA2 inhibition reversed Lin28a protective effects in vivo

Lin28a and HMGA2 staining by confocal immunofluorescence in CT siSRC, Lin28a(Tg) siSCR and Lin28a(Tg) siHMGA2 OA mice.

The datasets generated during and/or analyzed in the current study will be available from the corresponding author and to the following link: https://doi.org/10.5281/zenodo.6553754