mass. Indeed, if mIL-6 and osteocalcin were in the same genetic pathway controlling exercise capacity, compound double heterozygous *Ocn*$^{+/-}$ *Il-6Hsa*$^{+/-}$ mice should exhibit the same deficit in exercise capacity as the one observed in *Il6*$_{Hsa}^{-/-}$ or *Ocn*$^{-/-}$ mice, whereas the single heterozygous mice would run normally. As shown in Figure 3G, this is exactly what was observed. However, and as importantly, muscle mass was not decreased in any type of muscle in *Ocn*$^{+/-}$ *Il-6Hsa*$^{+/-}$ mice (Figure 4B). These data indicate on the one hand that the mIL-6 regulation of muscle mass and muscle function occurs through different mechanisms. On the other hand, they show that mIL-6's regulation of muscle mass does not account for the mIL-6 regulation of exercise capacity.

To ascertain whether there are any abnormalities in type I or type II muscle fiber frequency in the absence of mIL-6, we used 2 methods. First, we performed immunohistochemistry for either the slow myosin heavy chain (type I) or the fast myosin heavy chain (type II) and, second, we analyzed gene expression in soleus muscle of myosin heavy chain isoform 2a (*MyHC-IIa*) and 2b (*MyHC-IIb*). In both cases there were no detectable differences in the abundance of type I or type II fibers in *Il6*$_{Hsa}^{-/-}$ and control soleus muscles (Figure 4, C–E and H). Likewise, a histological analysis of the soleus muscle in 3-month-old *Il6*$_{Hsa}^{-/-}$ mice and control littermates did not reveal any significant difference in myofiber cross-sectional area (CSA) between *Il6*$_{Hsa}^{-/-}$ and control soleus muscles (Figure 4G).

IL-6 favors osteoclastogenesis by signaling in osteoblasts. Any effort to unravel the cellular pathway whereby mIL-6 enhances exercise capacity during endurance exercise must start with the identification of the cell type in which mIL-6 signals in bone. For that purpose, we relied on a classical coculture assay between osteoblasts and bone marrow–derived osteoclast progenitor cells that were obtained from mice of different genotypes (19). IL-6 exerts its biological activities through 2 molecules, IL-6R and the glycoprotein gp130, the signal transducing partner of IL-6R (20). Because gp130 contributes to the signaling of other cytokines, defining the importance of IL-6 signaling in a given cell type in vivo requires the deletion of *Il6r* in that cell type.

All coculture experiments described below were performed in the presence of the soluble IL-6R (sIL-6R) (21). When *Il6r*$^{-/-}$ osteoblasts were cocultured in the presence of IL-6 with *Il6r*$^{fl/fl}$ osteoclast progenitor cells, the number of tartrate-resistant acid phosphatase–positive (TRAP-positive) osteoclasts and osteoclasts containing 4 or more nuclei were both significantly reduced compared with what was observed when both osteoblasts and osteoclast progenitor cells were derived from *Il6r*$^{fl/fl}$ mice or when *Il6r*$^{fl/fl}$ osteoblasts were cocultured with *Il6r*$^{-/-}$ osteoclast progenitor cells (Figure 5A). Furthermore, expression of markers of osteoclast differentiation or function, such as *Dc-stamp*, *Atp6vd02* (ATPase, H^+ transporting, V0 subunit D2), *Clcn7* (chloride voltage-gated channel 7), and *Acp5* (TRAP) was also significantly reduced in osteoclasts following coculture of *Il6r*$^{-/-}$ osteoblasts with *Il6r*$^{fl/fl}$ osteoclast progenitor cells compared with what was observed when *Il6r*$^{fl/fl}$ osteoblasts were cocultured with *Il6r*$^{fl/fl}$ or *Il6r*$^{-/-}$ osteoclasts progenitor cells (Figure 5B). We also performed coculture experiments using *Il6r*$^{-/-}$ osteoblasts and *Il6r*$^{-/-}$ osteoclast progenitor cells in the presence of sIL-6R. As shown in Figure 5C, there was limited generation of $TRAP^+$ multinucleated osteoclasts in this experiment. This does not exclude the formal possibility that *trans* IL-6 signaling contributes to osteoclastogenesis in other experimental contexts.

Lastly, we cocultured *Il6r*$^{fl/fl}$ osteoblasts or osteoclast progenitor cells that had been infected beforehand with an empty adenovirus or one expressing *Cre* recombinase. We verified in each case that infection with *Cre* achieved a deletion of *Il6r* above 65% (Supplemental Figure 2, A and B). Regardless of the presence or lack of IL-6 in the culture medium, we found that when *Il6r* was deleted in osteoblasts the number of TRAP-positive multinucleated osteoclasts obtained was significantly decreased compared with what was observed in cocultures of *Il6r*$^{fl/fl}$ osteoclast progenitor cells and *Il6r*$^{fl/fl}$ osteoblasts infected with an empty adenovirus or one expressing *Cre* recombinase (Figure 5, D and E, and Supplemental Figure 2C). Of note, the *RankL/Opg* ratio was significantly higher in control coculture experiments than when IL-6R had been deleted from osteoblasts (Supplemental Figure 2D). Taken together, these experiments support the notion that in the conditions of these assays, IL-6 acts primarily in cells of the osteoblast lineage to favor osteoclast differentiation.

IL-6 signaling in osteoblasts is needed to enhance exercise capacity during endurance exercise. To determine whether the ability of IL-6 signaling in osteoblasts to enhance osteoclast differentiation explains, at least in part, why IL-6 increases exercise capacity we crossed *Il6r*$^{fl/fl}$ mice with *Ocn-Cre* mice that delete genes in differentiated osteoblasts after birth (Figure 6A and refs. 22, 23). We chose this *Cre* driver mouse because we had shown earlier that mIL-6 does not influence exercise capacity in any noticeable manner before 3 months of age, and therefore it seems unlikely that IL-6 functions primarily in osteoblast progenitor cells. This manipulation resulted in an efficient deletion of *Il6r* in differentiated osteoblasts but not in osteoclasts or other cell types or other tissues tested in *Il6r*$_{Osb}^{-/-}$ mice (Figure 6B). *Il6r*$_{Osb}^{-/-}$ mice were obtained at the expected Mendelian ratio, had a normal life span, and appeared overtly normal.

Despite a marked increase in circulating IL-6 levels during exercise, 3- and 6-month-old *Il6r*$_{Osb}^{-/-}$ mice displayed a severe decrease in their ability to perform endurance exercise compared with control mice (Figure 6, C and D, and Supplemental Figure 3A). In contrast, mutant mice lacking IL-6R in myofibers only ran as well as control mice (Figure 6E). The deficit in exercise capacity observed in mice lacking IL-6 signaling in osteoblasts was not caused by a decrease in muscle mass or a low bone mass because muscle mass, bone mass, and markers of bone resorption at rest were all similar between *Il6r*$_{Osb}^{-/-}$ and control mice, despite differences in markers of bone resorption during exercise (Supplemental Figure 3, B–D). These results indicate that IL-6 needs to signal in osteoblasts to favor exercise capacity.

Several lines of evidence indicate that the lack of secretion of bioactive osteocalcin is a major contributor to the deficit in exercise capacity of the *Il6r*$_{Osb}^{-/-}$ mice (Figure 6F). First, circulating osteocalcin levels did not increase in these mutant mice during endurance exercise (Figure 6, C–E). Second, compound heterozygous *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice exhibited a deficit in exercise capacity of similar severity to the one seen in *Ocn*$^{-/-}$ or *Il6r*$_{Osb}^{-/-}$ mice, but single heterozygous mice did not (Figure 6G and ref. 13). Likewise, circulating osteocalcin levels did not increase in *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice, as they did in control single heterozygous littermates during exercise

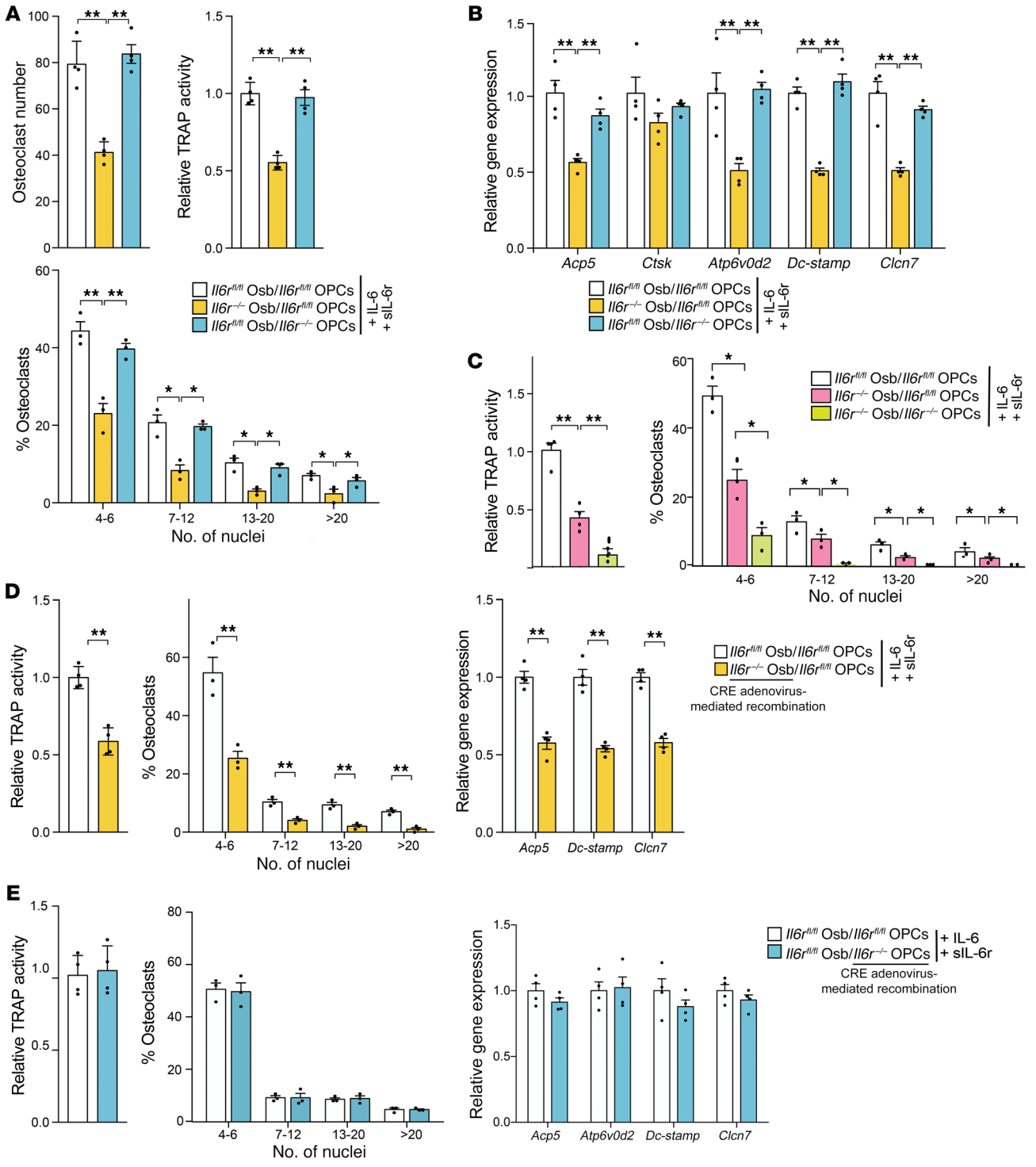

Figure 5. IL-6 favors osteoclastogenesis by signaling in osteoblasts. (**A** and **B**) Coculture of *Il6r*$^{fl/fl}$ osteoblasts with *Il6r*$^{fl/fl}$ osteoclast precursor cells (OPCs), *Il6r*$_{osb}$$^{-/-}$ osteoblasts with *Il6*$^{fl/fl}$ OPCs, and *Il6r*$^{fl/fl}$ osteoblasts with *Il6r*$_{osb}$$^{-/-}$ OPCs in the presence of IL-6 and sIL-6r. (**A**) Quantification of the number of osteoclasts (OCs), TRAP activity, and the number of nuclei per TRAP$^+$ osteoclast. (**B**) Expression of resorbing-activity markers *Acp5*, cathepsin K (*Ctsk*), *Atp6v0d2*, *Dc-stamp*, and *Clcn7* in mouse osteoclasts (WT OCs). (**C**) Coculture in the presence of IL-6 and sIL-6r of (i) *Il6r*$^{fl/fl}$ osteoblasts with *Il6r*$^{fl/fl}$ OPCs, (ii) *Il6r*$_{osb}$$^{-/-}$ osteoblasts with *Il6*$^{fl/fl}$ OPCs, or (iii) *Il6r*$^{-/-}$ osteoblasts with *Il6r*$_{osb}$$^{-/-}$ OPCs. TRAP activity and quantification of the number of osteoclasts. (**D** and **E**) *IL6r*$^{-/-}$ osteoblasts were generated by infecting *Il6r*$^{fl/fl}$ osteoblasts with adenovirus expressing *Cre* recombinase and coculturing with *Il6r*$^{fl/fl}$ osteoblasts in the absence or presence of IL-6 in the culture medium. Similarly, *IL6r*$^{-/-}$ OPCs were generated by infecting *Il6r*$^{fl/fl}$ OPCs with adenovirus expressing *Cre* recombinase and coculturing with *Il6r*$^{fl/fl}$ osteoblasts in the absence or presence of IL-6. (**D**) Quantification of TRAP activity, of the number of nuclei per TRAP$^+$ osteoclast, and gene expression of resorption markers (*Acp5*, *Dc-stamp*, and *Clcn7*). (**E**) Cocultures in the presence of IL-6 in the culture medium, quantification of TRAP activity, the number of nuclei per TRAP$^+$ osteoclast, and expression of resorption markers (*Acp5*, *Atp6v0d2*, *Dc-stamp*, and *Clcn7*). These results are representative of 3 independent experiments with triplicate samples. Data in **A**–**E** were analyzed by 2-way ANOVA followed by Tukey's post hoc test; the relative TRAP activity data in **A** and **C**–**E** were analyzed by 2-tailed unpaired *t* test. Error bars represent SEM. $^*P < 0.05$; $^{**}P < 0.01$.

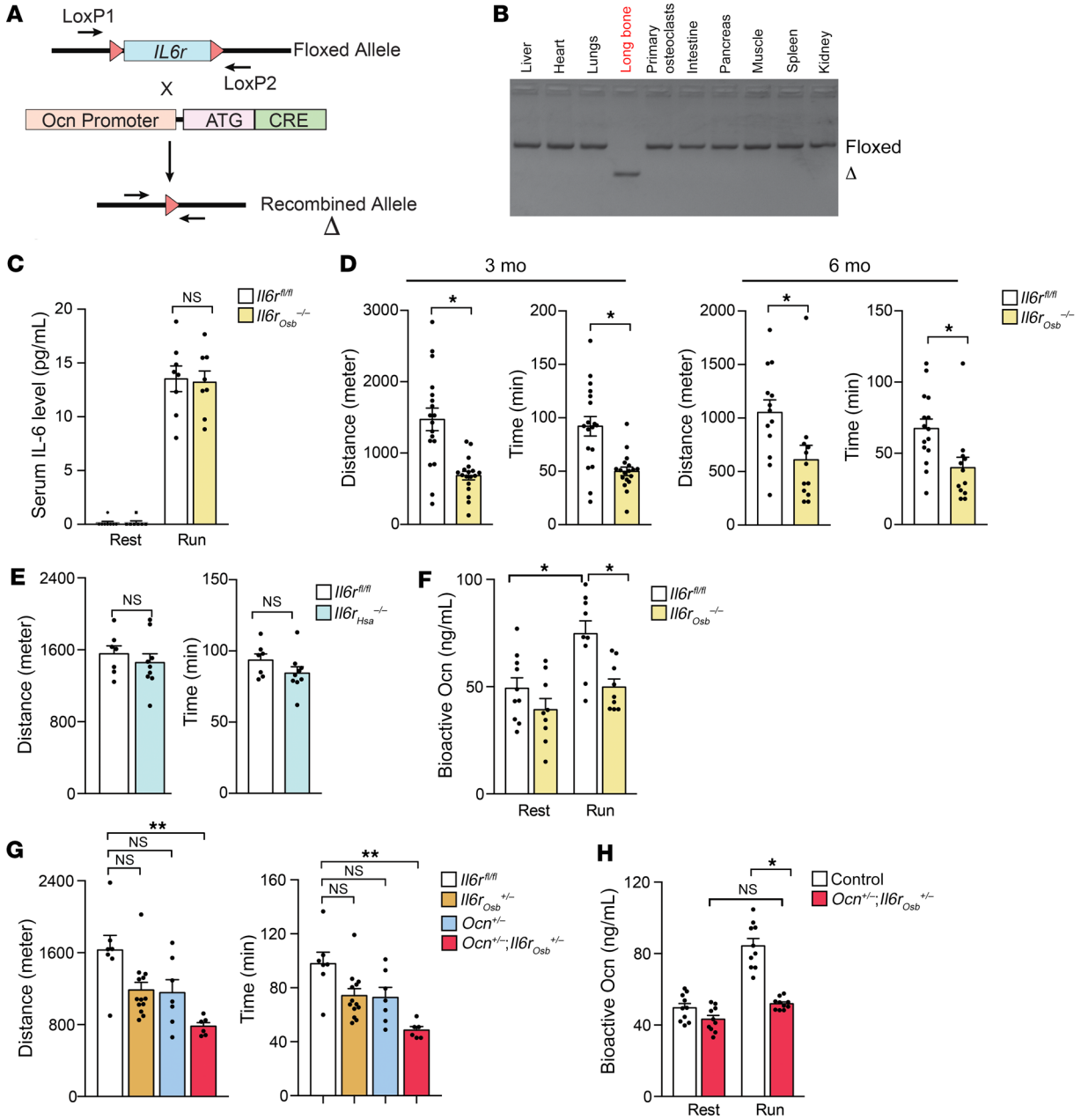

Figure 6. IL-6 signaling in osteoblasts is needed to enhance exercise capacity during endurance exercise. (**A**) Crossing of *Il6r*$^{fl/fl}$ mice with *Ocn-Cre* mice to delete *Il6r* in differentiated osteoblasts after birth and generate *Il6r*$_{Osb}^{-/-}$ mice. (**B**) Detection of *Il6r* deletion by PCR on genomic DNA isolated from various tissues of *Il6r*$_{Osb}^{-/-}$ mice. (**C**) Circulating IL-6 levels in 3-month-old *Il6r*$^{fl/fl}$ and *Il6r*$_{Osb}^{-/-}$ mice before and after exercise, $n = 8$. (**D**) Performance during an endurance run of 3- and 6-month-old *Il6r*$^{fl/fl}$ and *Il6r*$_{Osb}^{-/-}$ mice, $n = 12$–18. (**E**) Performance during an endurance run of 3-month-old *Il6r*$^{fl/fl}$ and *Il6r*$_{Hsa}^{-/-}$ mice, $n = 7$–9. (**F**) Circulating Ocn levels at rest and after exercise in bones of 3-month-old *Il6r*$^{fl/fl}$ and *Il6r*$_{Osb}^{-/-}$ mice, $n = 9$–10. (**G**) Performance during an endurance run of 3-month-old control (*Il6r*$_{Osb}^{+/-}$, *Ocn*$^{+/-}$, and WT) and *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice, $n = 7$–13. (**H**) Circulating Ocn levels in 3-month-old controls (WT, *Il6r*$_{Osb}^{+/-}$, and *Ocn*$^{+/-}$) and *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice at rest and after exercise, $n = 10$ each. These results are representative of 4 independent experiments. Data were analyzed by 1-way ANOVA followed by Tukey's post hoc test. Data presented as the mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$.

(Figure 6H). Third, an injection of IL-6 did not correct the deficit in exercise capacity in *Il6r*$_{Osb}^{-/-}$ or in *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice even though it increased their circulating IL-6 levels (Figure 7, A–C, and Supplemental Figure 3E). Fourth and conversely, an injection of osteocalcin increased exercise capacity in both *Il6r*$_{Osb}^{-/-}$ and *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{-/-}$ mice (Figure 7, D–G).

mIL-6 favors glucose uptake and catabolism in myofibers during exercise through osteocalcin. In the last set of experiments we explored whether the decrease in exercise capacity caused by the absence of either muscle-derived IL-6 or IL-6 signaling in osteoblasts was secondary to a lack of substrate availability (24) or rather, as it is the case when osteocalcin signaling in myofibers is disrupted, to a decrease in substrate uptake and catabolism in muscle (13).

Glucose homeostasis assayed by glucose tolerance and insulin sensitivity tests and liver gluconeogenesis assayed by a pyruvate tolerance test were unaffected by the postnatal deletion of *Il6*

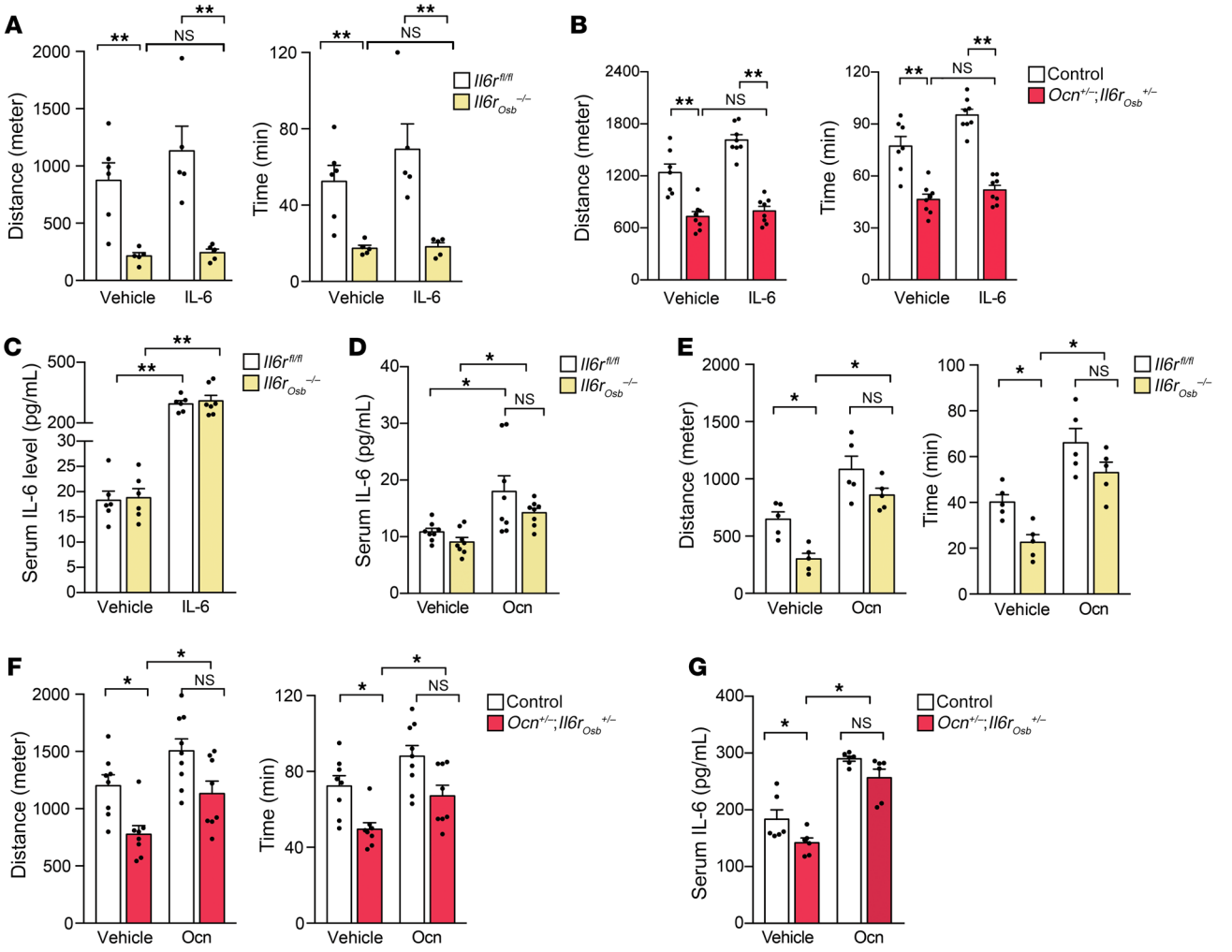

Figure 7. IL-6 signaling in osteoblasts is needed to enhance exercise capacity during endurance exercise. (**A**) Performance during an endurance run of 3-month-old *Il6r*$^{fl/fl}$ and *Il6r*$_{Osb}^{-/-}$ mice after an i.p. injection of IL-6 (3 ng/g), $n = 6$. (**B**) Performance during an endurance run of 3-month-old *Il6r*$_{Osb}^{+/-}$ *Ocn*$^{+/-}$ and control (*Il6r*$_{Osb}^{+/-}$, *Ocn*$^{+/-}$, and WT) mice after an i.p. injection of IL-6 (3 ng/g), $n = 5$. (**C**) Circulating IL-6 levels during an endurance exercise in 3-month-old *Il6r*$^{fl/fl}$ and *Il6r*$_{Osb}^{-/-}$ mice after i.p. injection of IL-6 (3 ng/g), $n = 6$. (**D**) Circulating IL-6 levels in 3-month-old *Il6r*$^{fl/fl}$ and *Il6r*$_{Osb}^{-/-}$ mice after i.p. injection of Ocn (120 ng/g), $n = 8$. (**E**) Performance during an endurance exercise of 3-month-old *Il6r*$^{fl/fl}$ and *Il6r*$_{osb}^{-/-}$ mice treated with osteocalcin (Ocn, 120 ng/g), $n = 5$. (**F**) Performance during an endurance exercise of 3-month-old *Il6r*$_{Osb}^{+/-}$ *Ocn*$^{+/-}$, *Il6r*$_{Osb}^{+/-}$, *Ocn*$^{+/-}$, and WT mice after an i.p. injection of Ocn (120 ng/g), $n = 8$. (**G**) Circulating IL-6 levels in 3-month-old controls (WT, *Il6r*$_{Osb}^{+/-}$, and *Ocn*$^{+/-}$) and *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice treated with Ocn (120 ng/g), $n = 6$. These results are representative of 4 independent experiments. Data were analyzed by 1-way ANOVA followed by Tukey's post hoc test. Results presented as the mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$.

from myofibers (Supplemental Figure 4, A–E). Likewise, expression in the liver of *Pepck* and *G6pase* that encode 2 key enzymes of liver gluconeogenesis was similar in *Il6*$_{Hsa}^{-/-}$, *Il6r*$_{Osb}^{-/-}$, and control mice before and after exercise, thus indicating that gluconeogenesis is not overtly decreased in the absence of mIL-6 or of IL-6 signaling in osteoblasts (Supplemental Figure 4, F–I). The absence of any detectable decrease in substrate availability in mice lacking either mIL-6 or IL-6R in osteoblasts prompted us to test whether substrate uptake and catabolism in myofibers were decreased in the absence of mIL-6 or IL-6 signaling in osteoblasts.

Consistent with this notion, we found that glucose uptake, measured by the uptake of ^{3}H-2-deoxyglucose (^{3}H-2-DG), was decreased in oxidative muscles of both *Il6*$_{Hsa}^{-/-}$ and *Il6r*$_{Osb}^{-/-}$ mice compared with their respective control littermates (Figure 8, A and B). Moreover, expression of *Pgma1* that is necessary for glycolysis was decreased in oxidative muscles of *Il6*$_{Hsa}^{-/-}$ mice after exercise (Figure 8C). A similar decrease in glucose uptake was observed in *Ocn*$^{+/-}$ *Il6*$_{Hsa}^{+/-}$ and *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice, further supporting the notion that osteocalcin mediates the positive effect of mIL-6 on glucose uptake in myofibers during exercise (Figure 8, D and E). To further demonstrate that this is the case we injected osteocalcin or IL-6 into *Il6*$_{Hsa}^{-/-}$, *Il6r*$_{Osb}^{-/-}$, *Ocn*$^{+/-}$ *Il6*$_{Hsa}^{+/-}$, or *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice. Osteocalcin injections normalized glucose uptake in muscle in all mutant mouse strains regardless of whether they increased circulating IL-6 levels or not (Figure 8, F–I, Supplemental Figure 4, J–M, and Table 1). IL-6 injections increased circulating IL-6 levels in all mutant mouse strains and it corrected the deficit in glucose uptake only in *Il6*$_{Hsa}^{-/-}$ and *Ocn*$^{+/-}$ *Il6*$_{Hsa}^{+/-}$ mice in which circulating osteocalcin levels increased after exercise, but not in *Il6r*$_{Osb}^{-/-}$ or *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice in which circulating osteo-

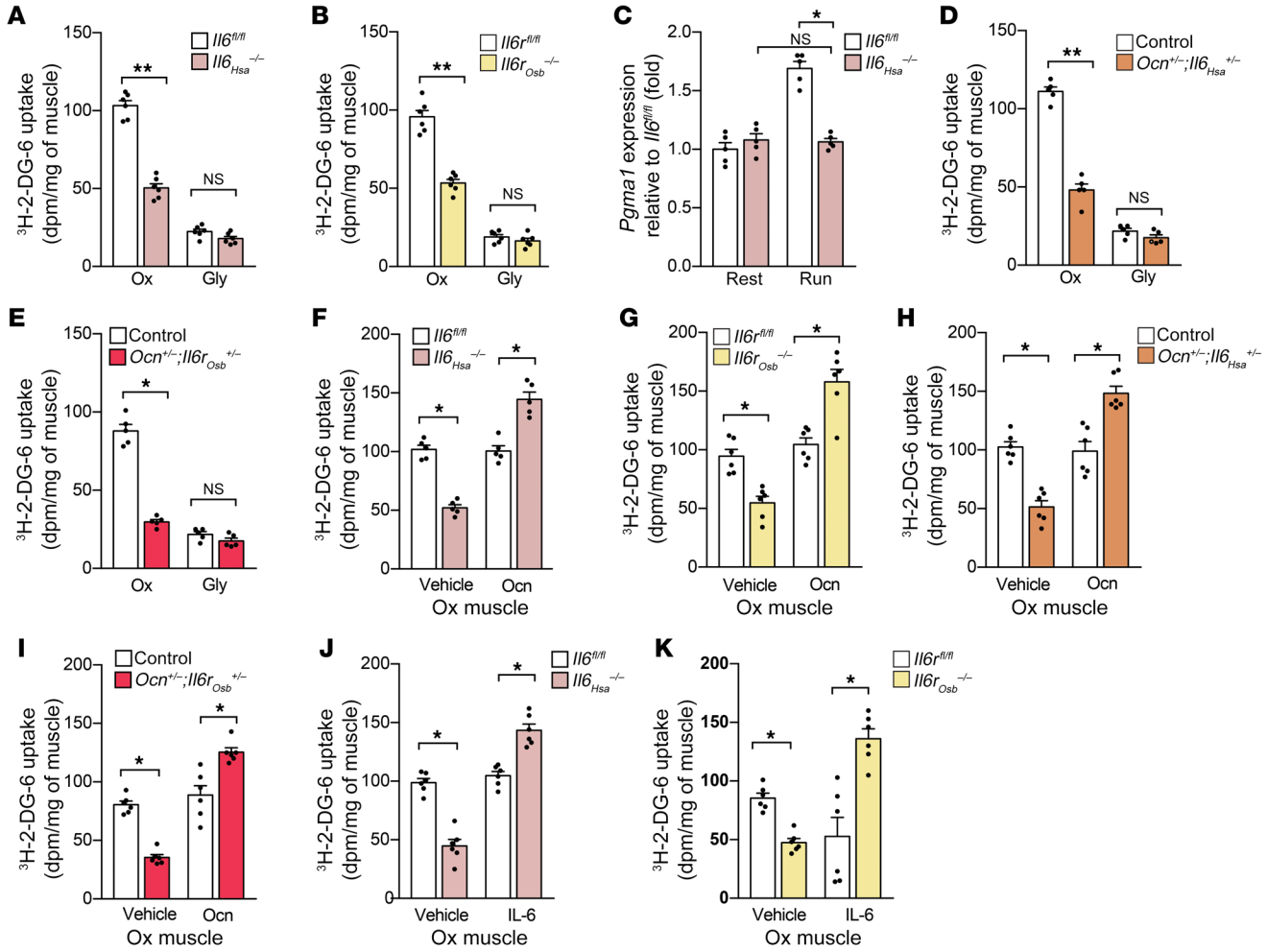

Figure 8. mIL-6 favors glucose uptake and catabolism in myofibers during exercise through osteocalcin. (**A** and **B**) Uptake of ^{3}H-2-DG in glycolytic (Gly, white quadriceps) and oxidative (Ox, red quadriceps) muscle in 3-month-old mice. (**A**) *Il6*$^{fl/fl}$ versus *Il6*$_{Hsa}^{-/-}$ and (**B**) *Il6r*$^{fl/fl}$ versus *Il6r*$_{Osb}^{-/-}$ mice after exercise, $n = 6$. (**C**) *Pgma1* expression at rest and after exercise in gastrocnemius muscle of 3-month-old *Il6*$^{fl/fl}$ versus *Il6*$_{Hsa}^{-/-}$ mice, $n = 5$ (3 replicates for each mouse). (**D** and **E**) Uptake of ^{3}H-2-DG in oxidative (Ox, red quadriceps) muscle in 3-month-old (**D**) controls (*Il6*$_{Hsa}^{+/-}$ and *Ocn*$^{+/-}$) versus *Ocn*$^{+/-}$ *Il6*$_{Hsa}^{+/-}$, and (**E**) controls (*Il6r*$_{Osb}^{+/-}$ and *Ocn*$^{+/-}$) versus *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice, $n = 6$. (**F**–**I**) Uptake of ^{3}H-2-DG in oxidative (Ox, red quadriceps) muscle after exercise in 3-month-old (**F**) *Il6*$^{fl/fl}$ versus *Il6*$_{Hsa}^{-/-}$, (**G**) *Il6r*$^{fl/fl}$ versus *Il6r*$_{Osb}^{-/-}$, (**H**) controls (WT, *Il6*$_{Hsa}^{+/-}$, and *Ocn*$^{+/-}$) versus *Ocn*$^{+/-}$ *Il6*$_{Hsa}^{+/-}$, and (**I**) controls (WT, *Il6r*$_{Osb}^{+/-}$, and *Ocn*$^{+/-}$) versus *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice treated with osteocalcin (Ocn, 120 ng/g) i.p., $n = 6$. (**J** and **K**) Uptake of ^{3}H-2-DG in oxidative (Ox, red quadriceps) muscle after exercise in 3-month-old (**J**) *Il6*$^{fl/fl}$ versus *Il6*$_{Hsa}^{-/-}$ and (**K**) *Il6r*$^{fl/fl}$ versus *Il6r*$_{osb}^{-/-}$ mice treated with IL-6 (3 ng/g) i.p., $n = 6$. These results are representative of 3 independent experiments. Data were analyzed by 1-way ANOVA followed by Tukey's post hoc test. Results presented as the mean ± SEM. *$P < 0.05$; **$P < 0.01$.

calcin levels did not increase following an IL-6 injection (Figure 8, J and K, and Table 1).

mIL-6 favors fatty acid uptake and catabolism in myofibers during exercise through osteocalcin. Expression in white adipose tissue of hormone-sensitive lipase (*Hsl*) and adipose triglyceride lipase (*Atgl*) that are necessary for lipolysis was similar in *Il6*$_{Hsa}^{-/-}$ and control mice before and after exercise (Supplemental Figure 5A). Circulating levels of nonesterified fatty acids (NEFAs) and triglycerides were either normal or increased in *Il6*$_{Hsa}^{-/-}$ and *Il6r*$_{Osb}^{-/-}$ mice compared with their control littermates before and after exercise (Figure 9, A and B, and Supplemental Figure 5, B and C). Circulating glucagon and glycerol levels were also unchanged, as was the expression of mitochondrial biogenesis markers in *Il6*$_{Hsa}^{-/-}$ and control mice before and after exercise (Supplemental Figure 5, D–F).

We also studied fatty acid (FA) uptake and catabolism. As seen in mice lacking osteocalcin signaling in myofibers (13), expression of *Fatp1*, which facilitates the uptake of long-chain FAs into cells and of *Cpt1b*, which promotes their transports across the mitochondrial membrane (25), was markedly decreased in muscle of *Il6*$_{Hsa}^{-/-}$, *Ocn*$^{+/-}$ *Il6*$_{Hsa}^{+/-}$, *Il6r*$_{Osb}^{-/-}$, and *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice compared with their respective controls after exercise (Figure 9, C–F). Furthermore, phosphorylation at Ser563 of HSL, the enzyme that hydrolyzes intracellular triglycerides into free FAs, was decreased in muscle of *Il6*$_{Hsa}^{-/-}$, *Ocn*$^{+/-}$ *Il6*$_{Hsa}^{+/-}$, *Il6r*$_{Osb}^{-/-}$, and *Ocn*$^{+/-}$ *Il6r*$_{Osb}^{+/-}$ mice (Figure 9, G–J). Collectively, these results support the notion that mIL-6 favors adaptation to exercise by promoting the production by bone of bioactive osteocalcin that in turn increases the uptake and catabolism of nutrients, i.e., glucose and FAs, in myofibers.

Discussion

This study was designed to address 2 questions related to IL-6 regulation of exercise capacity. The first one, prompted by the pleio-

Table 1. Summary of effects of intraperitoneal injection of osteocalcin or IL-6 during endurance run in different mouse strains

Genotype	Treatment	Glucose Uptake	Ocn Levels	IL-6 Levels	Run
Il6$_{Hsa}^{-/-}$	Vehicle	Low	↓	↓	↓
	Ocn (120 ng/g)	Rescued	↑	↑	↑
	IL-6 (3 ng/g)	Rescued	↑	↑	↑
Il6r$_{Osb}^{-/-}$	Vehicle	Low	↓	↓	↓
	Ocn (120 ng/g)	Rescued	↑	↑	↑
	IL-6 (3 ng/g)	Low	→	↑	↑
Ocn$^{+/-}$ *Il6*$_{Hsa}^{+/-}$	Vehicle	Low	↓	↓	↓
	Ocn (120 ng/g)	Rescued	↓	↑	↑
	IL-6 (3 ng/g)	Rescued	↑	↑	↓
Ocn$^{+/-}$ *Il6r*$_{Osb}^{+/-}$	Vehicle	Low	↓	↓	↓
	Ocn (120 ng/g)	Rescued	↑	↑	↑
	IL-6 (3 ng/g)	Low	→	↑	↓

tropic expression of *Il6*, was to determine to what extent does muscle contribute to the surge of circulating IL-6 levels during exercise. The second one was to define the cellular and molecular mechanisms used by IL-6 and/or mIL-6 to increase exercise capacity.

Addressing the first question through genetic means in the mouse established that the majority of IL-6 molecules detected in general circulation during an endurance exercise originate from myofibers. The analysis of *Il6*$_{Hsa}^{-/-}$ mice revealed several other aspects of IL-6 biology of great importance for our understanding of the regulation of exercise capacity during endurance exercise. The first one is that mIL-6 is necessary to achieve optimal exercise capacity. Second, mIL-6 is also responsible for the majority of the increase in circulating osteocalcin levels that occurs during endurance exercise. Third, the fact that an injection of osteocalcin largely rescued the deficit in exercise capacity in both *Il6*$_{Hsa}^{-/-}$ and *Il6*$_{Hsa}^{+/-}$ *Ocn*$^{+/-}$ mice suggested that osteocalcin mediates, at least in part, the ability of IL-6 to increase exercise capacity. This assumption does not exclude the possibility that IL-6 might increase exercise capacity through additional means.

A less anticipated observation, related to muscle biology, that the analysis of *Il6*$_{Hsa}^{-/-}$ mice revealed is that mIL-6 is necessary to maintain muscle mass in adult mice. A question this finding raised is whether this decrease in muscle explains, at least in part, the decrease in endurance exercise that the *Il6*$_{Hsa}^{-/-}$ mice experience. To address this question we relied on genetic epistasis, reasoning that if indeed the decrease in muscle function was secondary to the decrease in muscle mass then compound heterozygous mice lacking 1 allele of *Ocn* and 1 allele of *Il6* in muscle should experience either both phenotypes, i.e., a decrease in muscle mass and muscle function, or none of them. That *Il6*$_{Hsa}^{+/-}$ *Ocn*$^{+/-}$ mice exhibit the same deficit in exercise capacity as *Il6*$_{Hsa}^{-/-}$ mice but have a normal muscle mass indicates that the decrease in muscle function is not a consequence of the decrease in muscle mass. Of note, the decrease in muscle function during aerobic exercise also develops in *Ocn*$^{-/-}$ mice in the face of a normal muscle mass, further indicating that muscle function is not dependent on muscle mass (13, 26). This has important implications for the treatment of the decrease in muscle function in various sarcopenic syndromes. Of note, we did not detect any overt histological changes in muscle of mice lacking mIL-6 or its receptor in osteoblasts.

Underscoring its biological importance, we note that the crosstalk between IL-6 regulation of exercise capacity and osteocalcin extends beyond the mouse because an injection of osteocalcin increases circulating IL-6 levels in nonhuman primates, as it does in rodents (13). Furthermore, circulating osteocalcin also increased following a 12-week-long training intervention in humans, as it does in mice after a single bout of endurance exercise. Importantly for our purpose, this increase in circulating osteocalcin levels in humans was dependent on IL-6 signaling, as is the case in the mouse. This conservation of the interplay between exercise, osteocalcin, and IL-6 was a further incentive to decipher the mechanisms whereby IL-6 regulates adaptation to exercise and the release of osteocalcin from bone.

We first established through cell-based assays that IL-6 signals primarily in osteoblasts to favor osteoclast differentiation and the release of bioactive osteocalcin. These results, obtained using genetic approaches in cell culture, are in full agreement with previous reports (19) but do not exclude the possibility that IL-6 may also act on osteoclast progenitor cells at a level we could not detect in our experimental conditions. They also do not exclude the possibility that the source of IL-6 signaling in osteoblasts might be the resorbed extracellular matrix rather than the general circulation. Regardless, these observations were an obvious incentive to analyze the consequences for exercise capacity of deleting IL-6R from osteoblasts. We used for that purpose a *Cre* driver mouse that deletes genes in differentiated osteoblasts because the deficit in exercise capacity observed in the absence of mIL-6 does not develop before 3 months of age, thus suggesting it is not the result of a developmental process (22). The inactivation of IL-6 signaling in differentiated osteoblasts resulted in a severe deficit in exercise capacity that develops in the face of a normal muscle mass. These results contrast with those obtained after the deletion of the IL-6R in myofibers because this latter deletion does not hamper in any measurable way the ability of mice to perform an endurance exercise. They also indicate that muscle mass does not necessarily predict muscle function during exercise.

Importantly, *Il6r*$_{Osb}^{-/-}$ mice did not experience an increase in circulating osteocalcin during exercise and their deficit in exercise capacity was corrected by osteocalcin but not by IL-6. Taken together, these results identify osteocalcin, a hormone known to improve muscle function during exercise (13), as a major mediator of this function of IL-6 signaling in osteoblasts. Although we did not detect in our experimental setting any measurable influence of mIL-6 in the generation of glucose and FAs, the 2 substrates of myofibers during exercise, we do not exclude the possibility that such a mechanism of action may also contribute to mIL-6's ability to enhance exercise capacity at a level that was not detected by the assays we used (9).

The cross-regulation between IL-6 and osteocalcin presented in an earlier study (13) and this study raises the question of which