Supplemental data

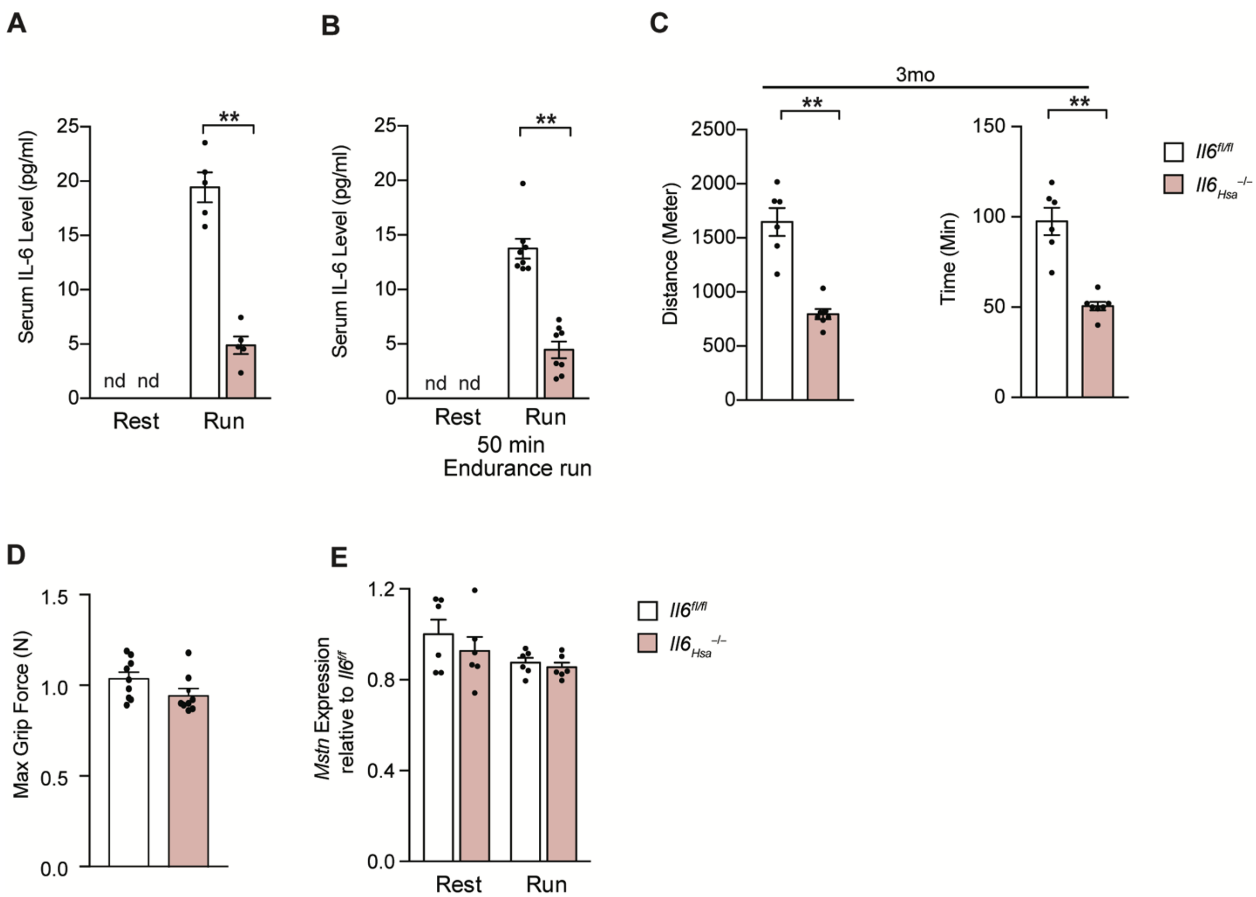
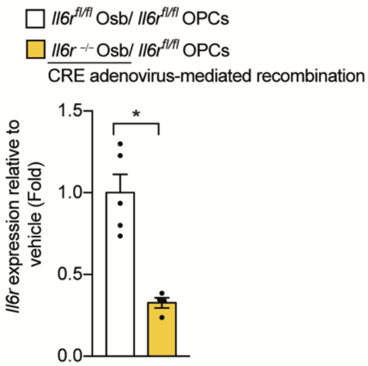


Figure S1. Related to Figure 2. Muscle derived IL-6 is needed for maximal exercise capacity.

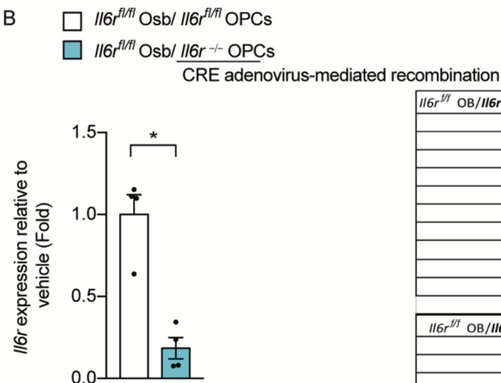
(A) Circulating IL-6 levels in 3-month-old male *$Il6^{f/f}$* and *$Il6_{Hsa}$-/-* mice at rest and after exercise. **(B)** Circulating IL-6 levels during 50 min endurance run of 3-month-old female *$Il6^{f/f}$* and *$Il6_{Hsa}$-/-* mice. (**C**) Performance during an endurance run of 3, and 6-month-old *$Il6^{f/f}$* and *$Il\text{-}6_{Hsa}$-/-* male mice. **(D)** Muscle strength determined as the maximal grip force in 3-month-old *$Il6^{f/f}$* and *$Il6_{Hsa}$-/-* female mice. (**E**) *Mstn* expression 3-month-old male *$Il6^{f/f}$* and *$Il6_{Hsa}$-/-* mice at rest and after exercise. All these experiments are representative of three independent experiments. Data shown in C, D were analyzed by 2-tailed unpaired t test and data in A and E Tukey's post hoc test. Results represent the mean+ SEM.

A



IL6r		Hprt		Δ Cp			
Il6r $^{f/f}$ OB/*Il6r* $^{f/f}$ Opc with Adeno Empty	34.57		27.34	0.006673			0.93655
	34.39		26.93	0.005708			0.8011
	34.27		27.44	0.008752			1.22826
	34.60		27.84	0.009254			1.29874
	34.88		27.30	0.005240	0.007126	1	0.73535
						Average	1
						Devest	0.25248
						SEM	0.11291
		Hprt		Δ Cp			
Il6r-/- OB/*Il6r* $^{f/f}$ Opc with adeno Cre	36.12		26.92	0.001696			0.237998
	35.87		27.37	0.002749			0.385771
	36.27		27.56	0.002399			0.336677
	36.10		27.44	0.002471			0.346844
						Average	0.32682
						Devest	0.06288
						SEM	0.03144
						t test	0.00292

B



Il6r $^{f/f}$ OB/*Il6r* $^{f/f}$ Opc with Adeno Empty	33.88		27.16	0.009482			1.11125
	33.80		26.28	0.005446			0.63821
	34.07		27.34	0.009373			1.09846
	34.16		27.49	0.009831			1.15207
					0.008533	*Average*	1
						Devest	0.24227
						SEM	0.12114
						t test	
Il6r $^{f/f}$ OB/*Il6r* $^{f/f}$ Opc with Adeno Cre	36.78		26.29	0.000692			0.08107
	37.65		27.05	0.000644			0.07544
	36.61		28.20	0.002932			0.34366
	36.62		27.68	0.002032			0.23816
					0.001575	*Average*	0.18459
						Devest	0.13013
						SEM	0.06507
						t test	0.01672

C

D

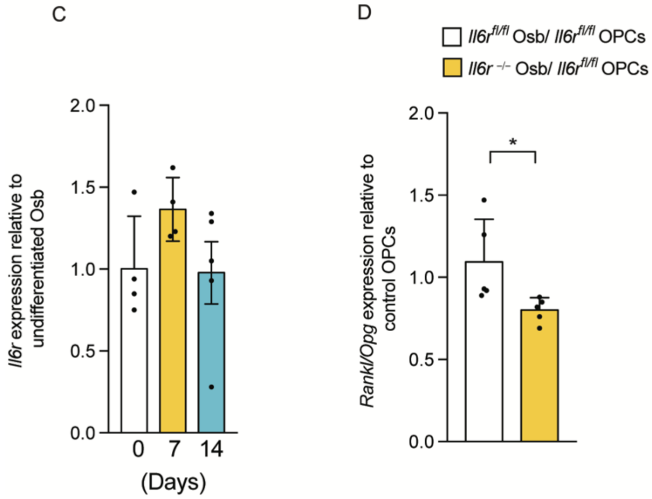


Figure S2: Related to Figure 5: IL-6 favors osteoclastogenesis by signaling in osteoblasts.

(A) Deletion efficiency of *Il6r* in osteoblasts. Co-culture of *Il6r* $^{f/f}$ osteoblasts infected with either adenovirus expressing the empty vector or Cre recombinase with *Il6r* $^{f/f}$ osteoclast precursor cells. (**B**) Deletion efficiency of *Il6r* in osteoclast precursor cells. Co-culture of *Il6r*$^{f/f}$ osteoblasts with *Il6r*$^{f/f}$ osteoclast precursor cells infected with either adenovirus expressing the empty vector or Cre. (**C**) Expression level of *Il6r* mRNA in the cultured osteoblasts isolated from 4days calvarias with at different time points (0,7,14days) in osteoblast mineralization medium, n=4. (**D**) Ratio of *Rankl* and *Opg* expression in co-culture of (1) *Il6r*$^{f/f}$ osteoblasts with *Il6r* $^{f/f}$ OPCs, (2) *Il6r*$_{Osb}$*-/-* osteoblasts with *Il6* $^{f/f}$ OPCs in presence of IL-6 and sIL-6r. These experiments are representative of 3 independent experiments. Data in A, B and D by 2-tailed unpaired t test whereas C were analyzed by one-way ANOVA followed by Tukey's post hoc test. Error bars represent SEM. Results represent the mean+ SEM. *P < 0.05, **P < 0.01.

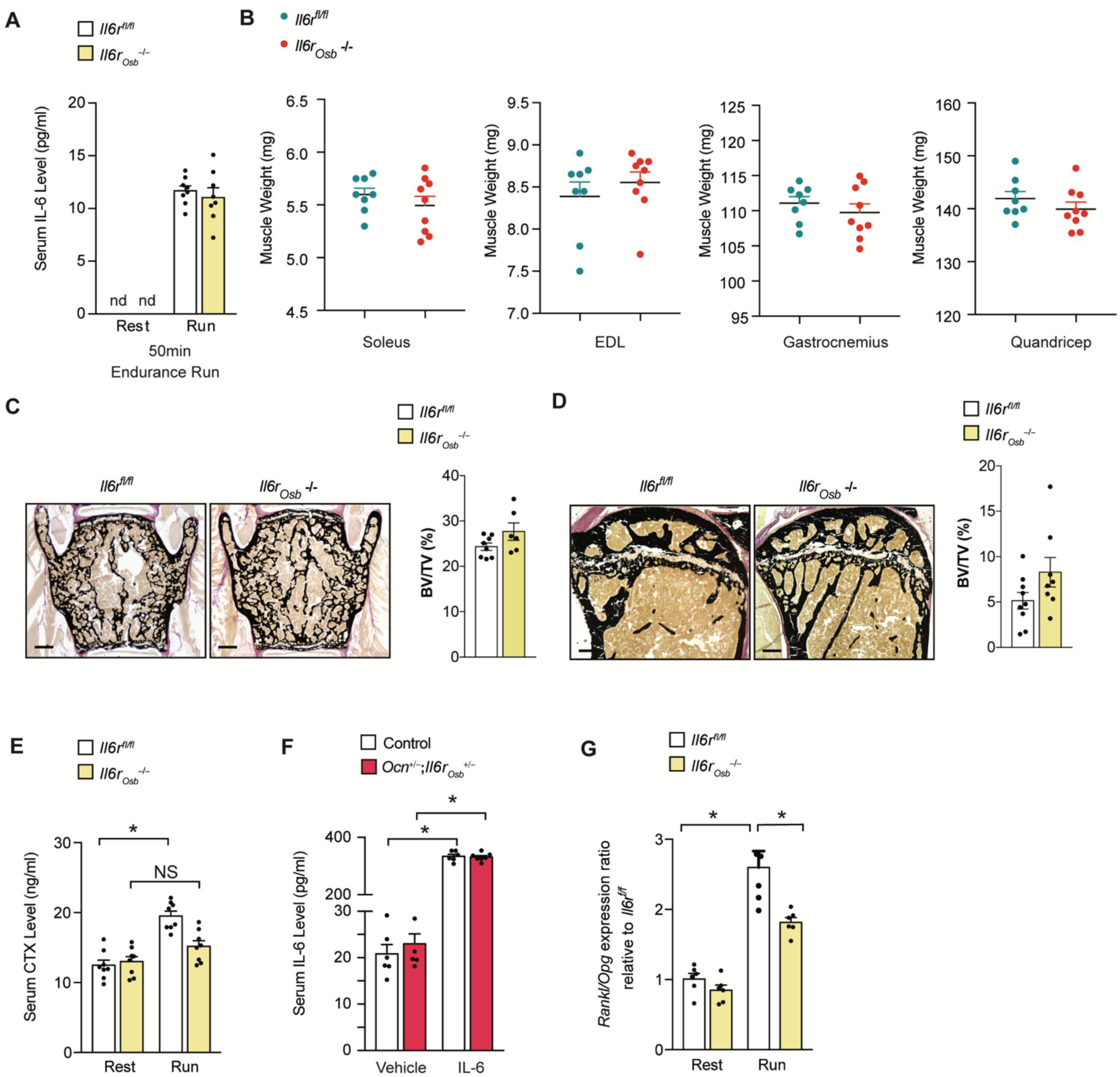
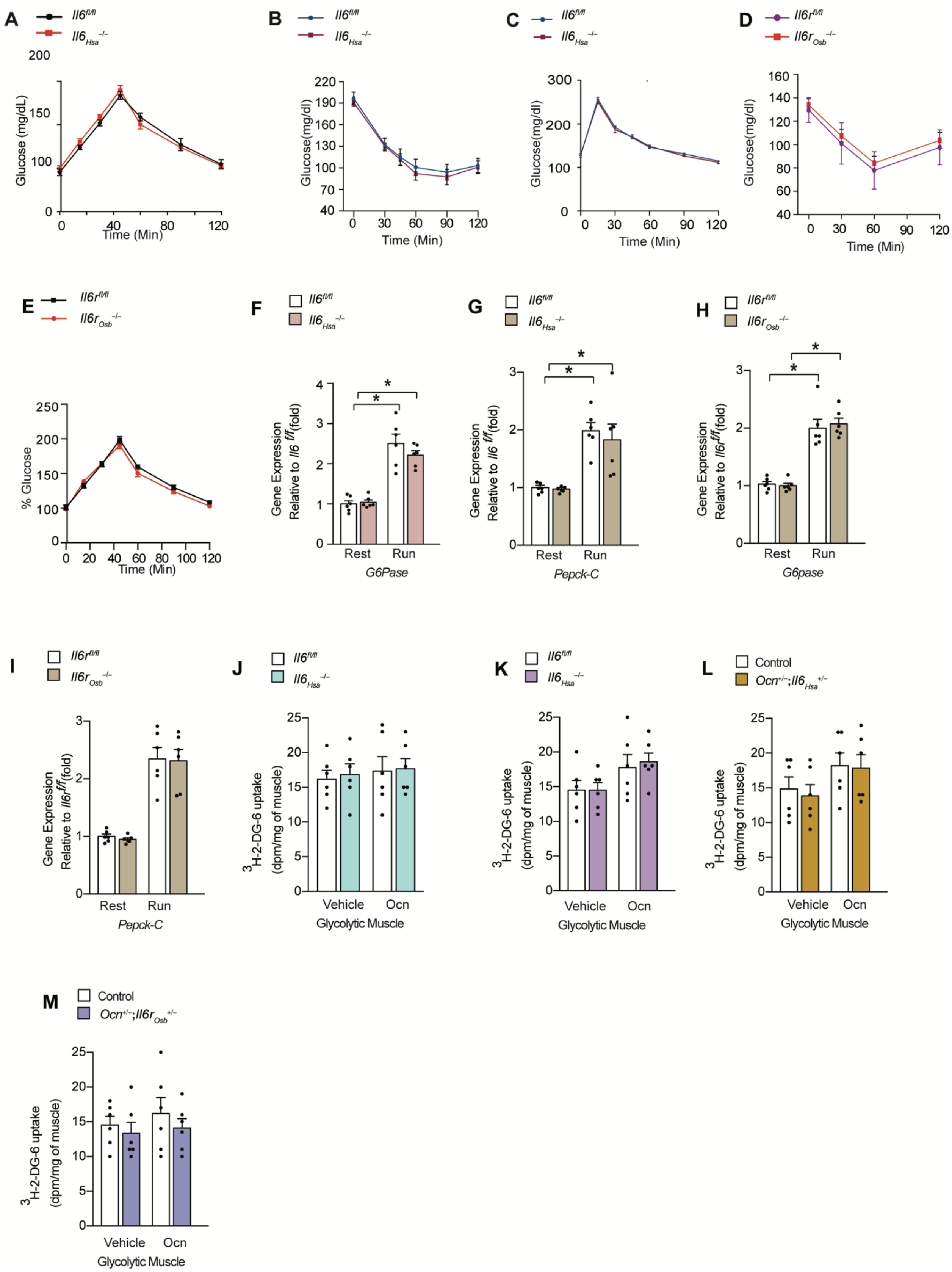


Figure S3: Related to Figure 6. mIL-6 favors exercise capacity in part through osteocalcin

Circulating IL-6 levels during 50 min endurance run of 3-month-old female *Il6*$^{f/f}$ and *Il6*$_{Hsa}$-/- mice, n=8. (**B**) Weight of hindlimb muscles such as Soleus, EDL, Gastrocnemius and quadriceps in 3month-old *Il6r*$^{f/f}$ and *Il6r*$_{osb}$-/- mice, n=8-10. (**C-D**) Bone histology and histomorphometry of (**C**) lumbar vertebrae, n=6-8 (**D**) tibiae from 6-month-old *Il6r*$^{f/f}$ and

$Il6r_{osb}^{-/-}$ mice, n=6-8. (**E)** Circulating IL-6 levels in 3-month-old controls: WT, *$Il6r_{Osb}$+/-, Osteocalcin +/-* and *$Il6r_{Osb}$+/-; Osteocalcin +/-* mice treated with IL-6 (3ng/g), n=5-7. (**F**) Circulating CTX levels in 5-month-old *$Il6r^{f/f}$* and *$Il6r_{osb}^{-/-}$* mice n=6. (**G**) *Rankl/Opg* expression ratio in the Femur of 3-month-old *$Il6r^{f/f}$* and *$Il6r_{osb}^{-/-}$* mice n=6. These experiments are representative of 3 independent experiments. Data in B, C and D by 2-tailed unpaired t test whereas A, E, F and G were analyzed by one-way ANOVA followed by Tukey's post hoc test. Error bars represent SEM. Results represent the mean+ SEM. $^*P < 0.05$, $^{**}P < 0.01$.



A
Il6fl/fl
Il6Hsa−/−
Glucose (mg/dL)
Time (Min)
B
Il6fl/fl
Il6Hsa−/−
Glucose(mg/dl)
Time (Min)
C
Il6fl/fl
Il6Hsa−/−
Glucose(mg/dl)
Time (Min)
D
Il6rfl/fl
Il6rOsb−/−
Glucose(mg/dl)
Time (Min)
E
Il6rfl/fl
Il6rOsb−/−
% Glucose
Time (Min)
F
Il6fl/fl
Il6Hsa−/−
Gene Expression Relative to Il6 f/f(fold)
Rest
Run
G6Pase
G
Il6fl/fl
Il6Hsa−/−
Gene Expression Relative to Il6 f/f(fold)
Rest
Run
Pepck-C
H
Il6rfl/fl
Il6rOsb−/−
Gene Expression Relative to Il6r f/f(fold)
Rest
Run
G6pase
I
Il6rfl/fl
Il6rOsb−/−
Gene Expression Relative to Il6r f/f(fold)
Rest
Run
Pepck-C
J
Il6fl/fl
Il6Hsa−/−
3H-2-DG-6 uptake (dpm/mg of muscle)
Vehicle
Ocn
Glycolytic Muscle
K
Il6fl/fl
Il6Hsa−/−
3H-2-DG-6 uptake (dpm/mg of muscle)
Vehicle
Ocn
Glycolytic Muscle
L
Control
Ocn+/−;Il6Hsa+/−
3H-2-DG-6 uptake (dpm/mg of muscle)
Vehicle
Ocn
Glycolytic Muscle
M
Control
Ocn+/−;Il6rOsb+/−
3H-2-DG-6 uptake (dpm/mg of muscle)
Vehicle
Ocn
Glycolytic Muscle

Figure S4: Related to Figure 8. mIL-6 favors glucose uptake and catabolism in myofibers during exercise through osteocalcin.

(A) Blood glucose levels during a PTT in 3-month-old *Il6*$^{f/f}$ and *Il6*$_{Hsa}$*-/-* littermate mice, n=6. **(B)** Blood glucose levels during an ITT of 3-month-old *Il6*$^{f/f}$ and *Il6*$_{Hsa}$*-/-* mice, n=6. (**C-E**) Blood glucose levels during a (**C**) GTT (**D**) ITT (**E**) PTT in 3-month-old *Ilr6*$^{f/f}$ and *Il6r*$_{Osb}$*-/-* mice, n=6. (**F-G**) Expression of (F) *G6Pase,* (G) *Pepck-c* at rest and after exercise in livers of 3-month-old *Il6*$^{f/f}$ and *Il6*$_{Hsa}$*-/-* mice, n=6. (**H-I**) Expression of (H) *G6Pase,*(**I**) *Pepck-c* at rest and after exercise in livers of 3-month-old *Il6r*$^{f/f}$ and *Il6r*$_{Osb}$*-/-* mice, n=6. (**J-M**) Uptake of ^{3}H-2-DG in glycolytic (Gly, white quadricep) muscles treated with Ocn after exercise in 3-month-old mice, n=6 **(J)** *Il6*$^{f/f}$ vs *Il6*$_{Hsa}$-/- **(K)** *Il6r*$^{f/f}$ vs *Il6r*$_{osb}$*-/-.* (**L**) Controls (WT, *Il6*$_{Hsa}$+/- and *Osteocalcin*+/-) vs *Osteocalcin+/-*;*Il6*$_{Hsa}$+/- **(M)** controls (WT, *Il6r*$_{Osb}$+/- and *Osteocalcin*+/-) vs *Osteocalcin +/-*;*Il6r*$_{Osb}$+/- n=6. These experiments are representative of 3 independent experiments. Data were analyzed by 1-way ANOVA followed by Tukey's post hoc test. Error bars represent SEM. Results represent the mean+ SEM. *P < 0.05.

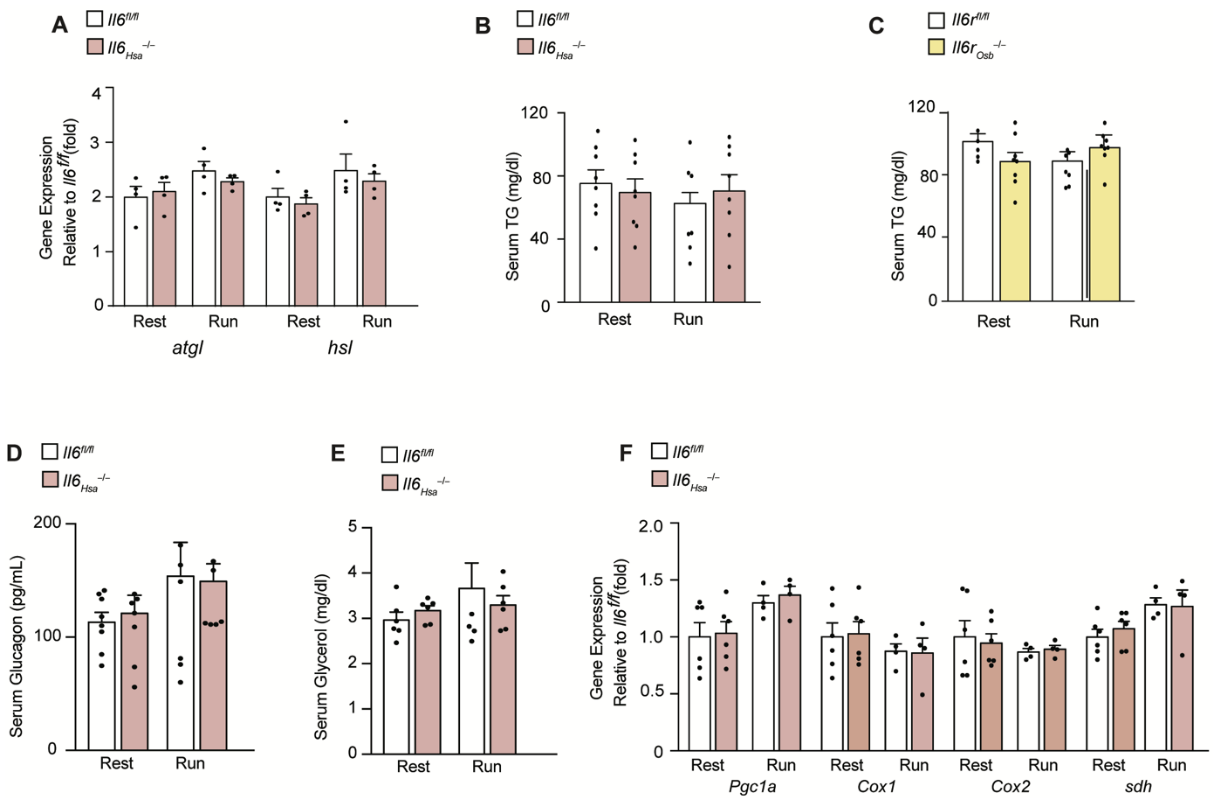


Figure S5: Related to Figure 9. mIL-6 favors fatty acid uptake and catabolism in myofibers during exercise through osteocalcin.

(**A**) *Hsl, atgl* expression at rest and after exercise in white adipose tissue of 3-month-old $Il6^{f/f}$ and $Il6_{Hsa}$-/- mice, n=4. (**B-C**) Serum triglycerides levels at rest and after exercise in 3-month-old (**B**) $Il6_{Hsa}$-/- and (**C**) $Il6r_{Osb}$-/- and their respective controls ($Il6^{f/f}$ and $Il6r^{f/f}$), n=8. (**D**) Circulating glucagon and (**E**) glycerol levels at rest and after exercise in 3-month-old $Il6_{Hsa}$-/- and $Il6r_{Osb}$-/- mice and their respective controls, n=8. (**F**) *Pgc1*a*, Cox1, Cox2, SDH* expression at rest and after exercise in gastrocnemius muscles of 3- month-old $Il6^{f/f}$ and $Il6_{Hsa}$-/- mice, n=6. These experiments are representative of three independent experiments. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Error bars represent SEM. Results represent the mean+ SEM. *P < 0.05.