

## SUPPLEMENTS

**Supplemental Figure 1. IGF-1 reduced SMC senescence via IGF-1 receptor-dependent mechanism.** Aortic SMC were isolated from control mice (Con SMC) and from mice with IGF-1 receptor deficiency (IGF-1R-KO SMC) and cultured until passage 5 (P5) or passage 20 (P20). Cells were exposed to IGF-1 for 12h and  $\beta$ -Galactosidase ( $\beta$ -gal) staining was performed using the Senescence Detection Kit. A, representative images of  $\beta$ -Gal-positive cells. B, quantitative data. \* $P < 0.05$  for untreated P20 vs. untreated P5 SMC. # $P < 0.05$  for P20 Con SMC+IGF-1 vs, untreated P20 Con SMC. Empty bars, P5 SMC, solid bars, P20 SMC.

**Supplemental Figure 2. Long-term treatment with IGF-1 decreased activity of senescence-associated  $\beta$ -galactosidase (A) and reversed reduction in DNA telomere length (B).** Murine aortic SMC were cultured until passage 5 (P5) or passage 20 (P20). Cells were exposed to IGF-1 for 24 and 72hrs and activity of senescence-associated  $\beta$ -galactosidase (SA  $\beta$ -gal) (A), and DNA telomere length (B) were quantified using commercial assays in according with manufacturer's instructions. \* $P < 0.05$  for P20 vs. P5 SMC. # $P < 0.05$  for IGF-1-treated P20 SMC vs. untreated P20 SMC. Empty bars, P5 SMC, solid bars, P20 SMC.

**Supplemental Figure 3. SMC treatment with IGF-1 (72hrs) upregulated autophagy in P20 SMC.** P20 SMC were treated with 10 ng/ml IGF-1, or with 10  $\mu$ M Bafilomycin (Baf), or with combination of IGF-1 and Baf. LC3 II/I ratio was quantified by immunoblotting/densitometry and results were normalized per GAPDH levels. \* $P < 0.05$  vs. untreated cells. # $P < 0.05$  IGF-1+Baf, vs IGF-1.

**Supplemental Figure 4. IGF-1 increased mitochondrial mass.** A, P20 SMC were stained with MitoTracker Deep Red FM dye and the signal was quantified by confocal microscopy. B, mitochondrial copy number was assessed in P20 SMC by using Mouse mitochondrial DNA copy number kit. \* $P < 0.05$  for SMC+IGF-1 vs. untreated SMC.

**Supplemental Figure 5. SMC treatment with IGF-1 (72hrs) upregulated mitophagy.** P5 and P20 SMC were exposed to 10 ng/ml IGF-1 for 72hrs and stained with Mtpagy and Lyso dyes using Mitophagy detection kit (A). The number of vacuoles double positive for Mtpagy and Lyso was quantified per cell and shown on the graph (B). \* $P < 0.05$  for

untreated P20 vs. untreated P5 SMC. #P<0.05 for IGF-1 vs. no IGF-1. Empty bars, P5 SMC, solid bars, P20 SMC.

**Supplemental Figure 6. SiRNA-induced downregulation of Nrf2, Sirt3 and PINK1.** P20 SMC were transfected with 5nM Nrf2 siRNA (A) or Sirt3 siRNA (B) or PINK1 siRNA (C) or scrambled siRNA (control) and after 48 hours cells protein expression was assessed by immunoblotting.

0

5

10

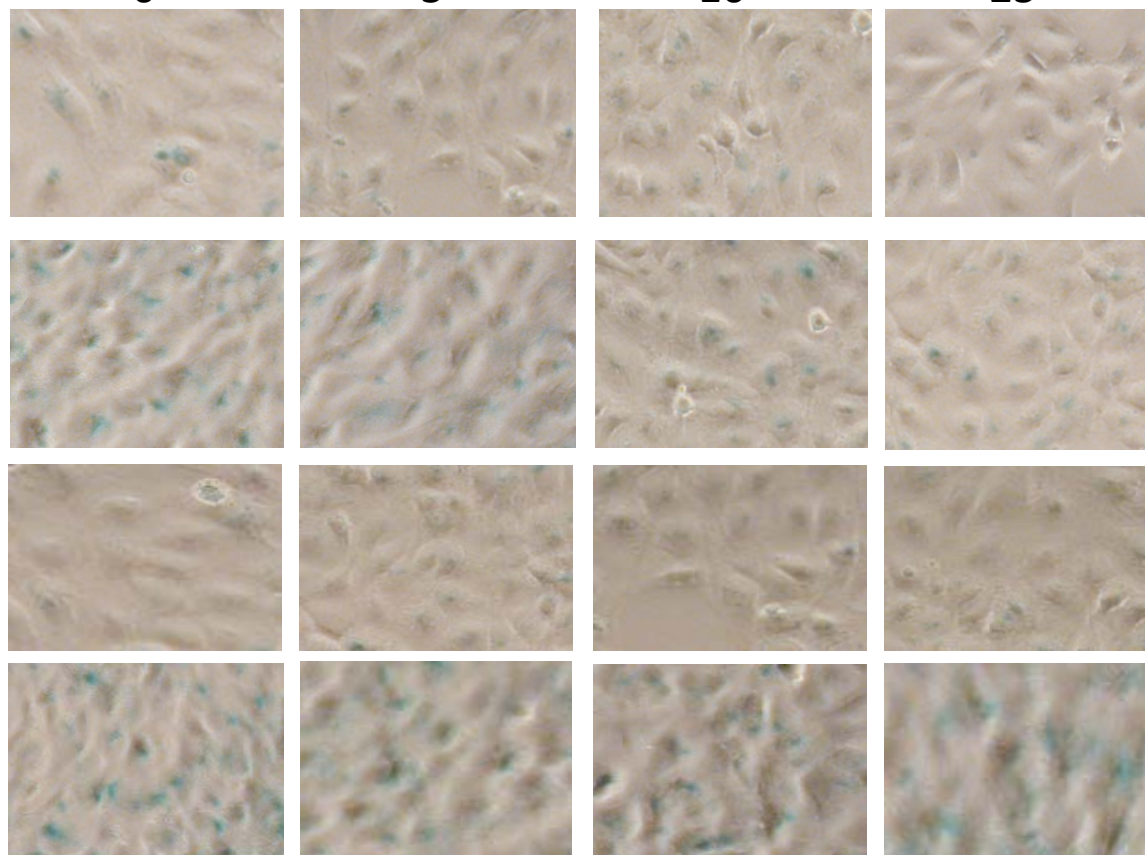
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A

Con SMC

P5 SMC

P20 SMC

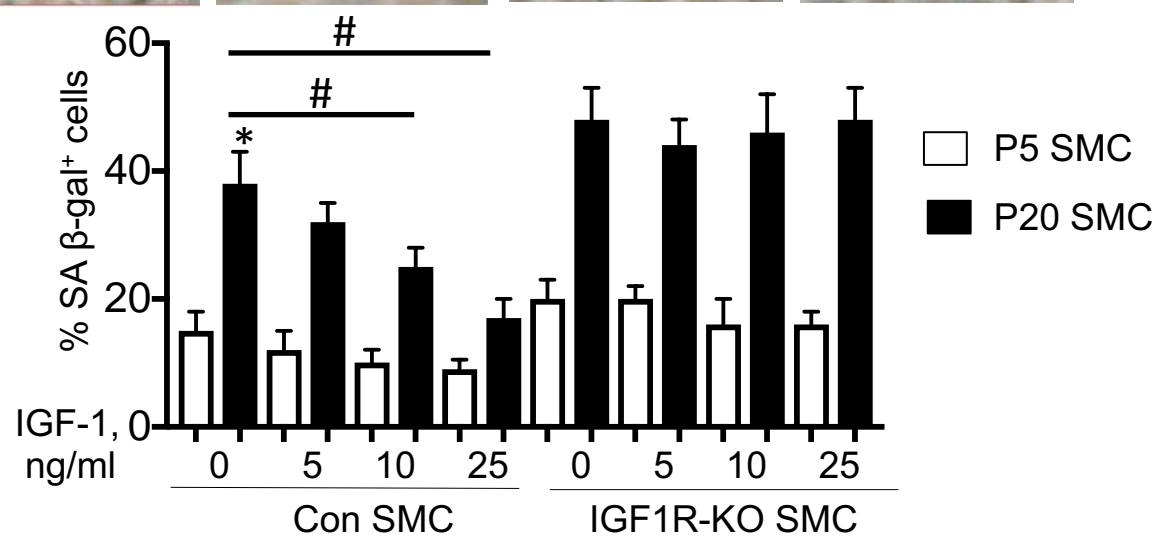


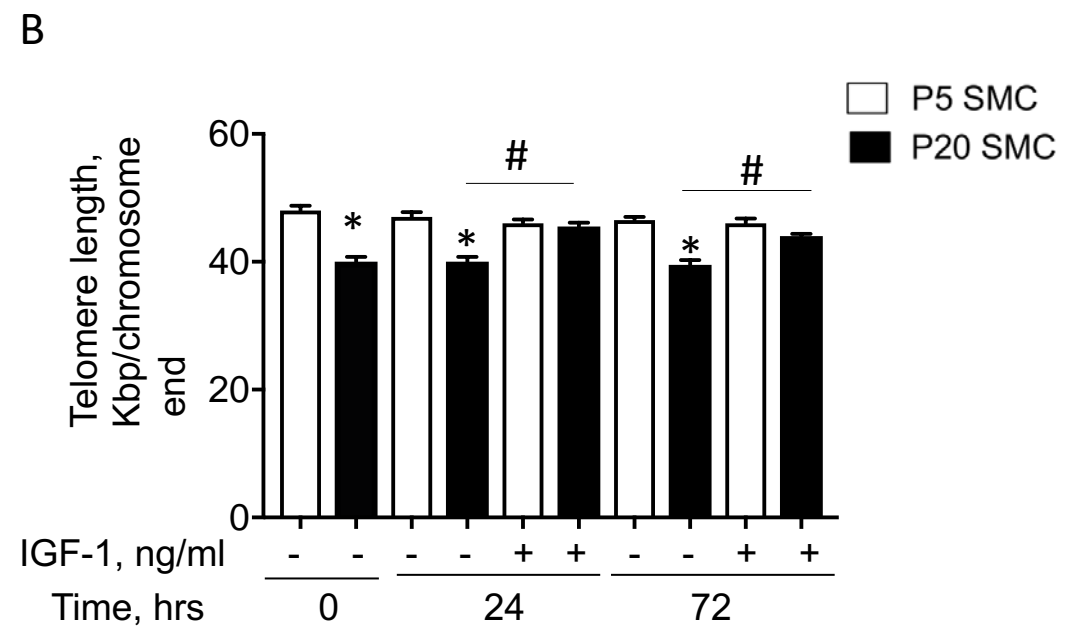
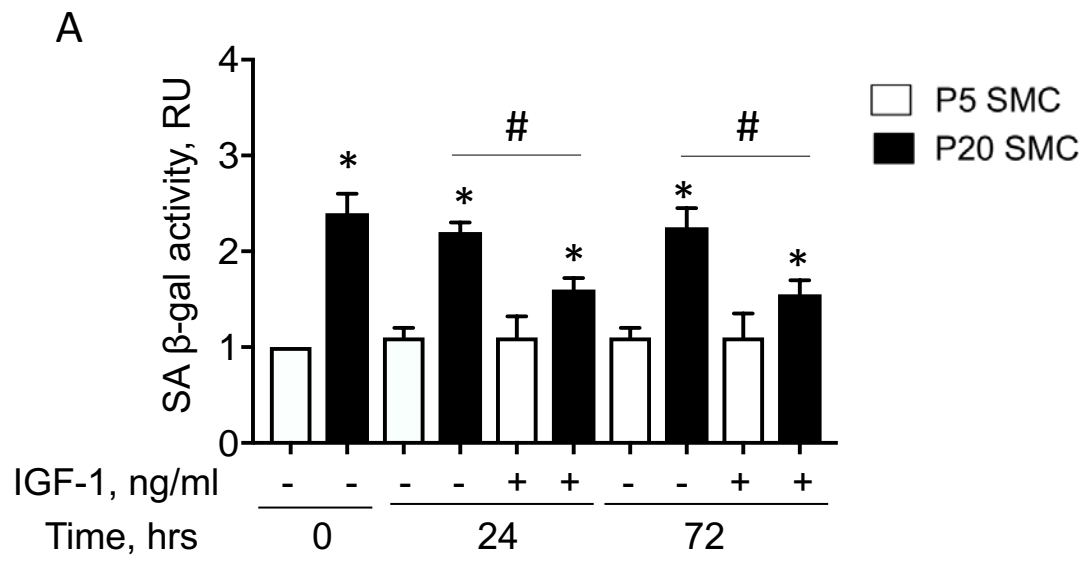
IGF-1R-KO SMC

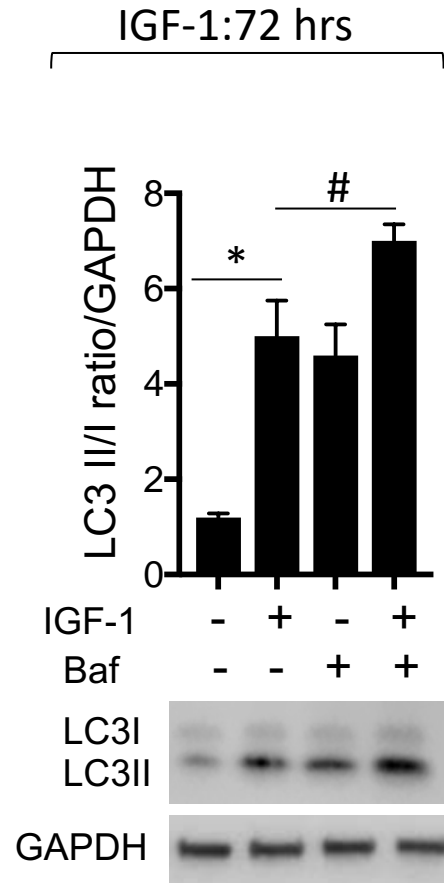
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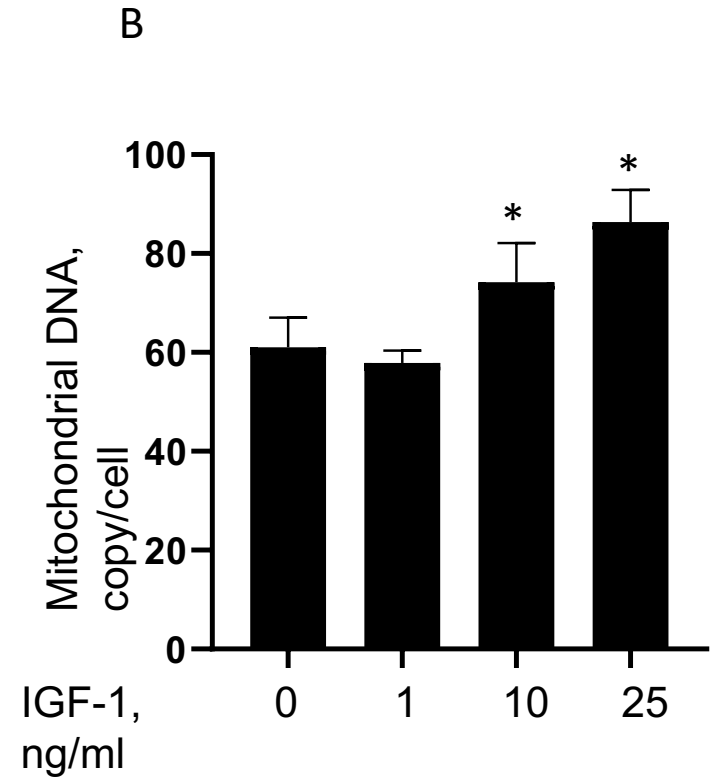
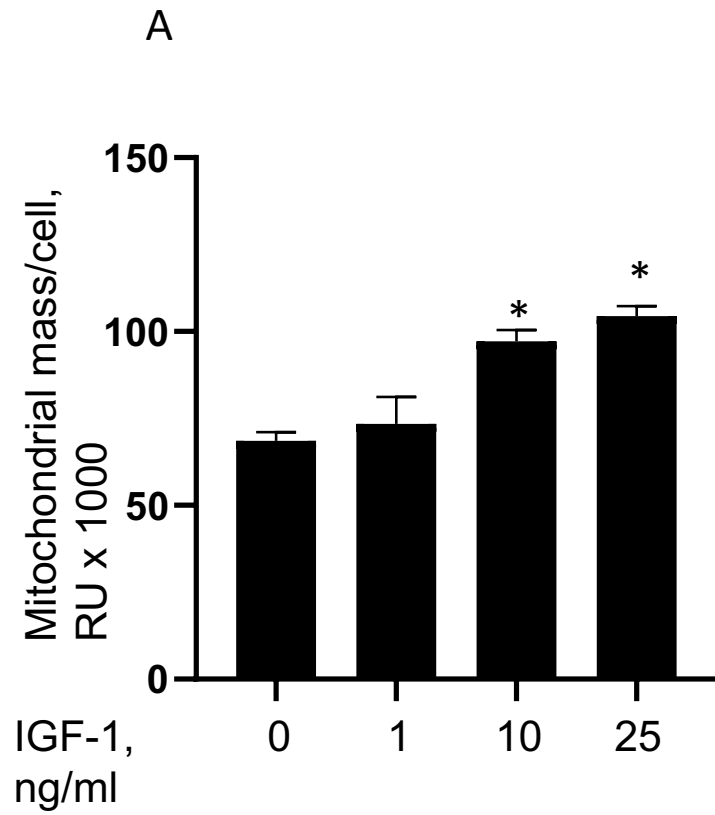
P20 SMC

B

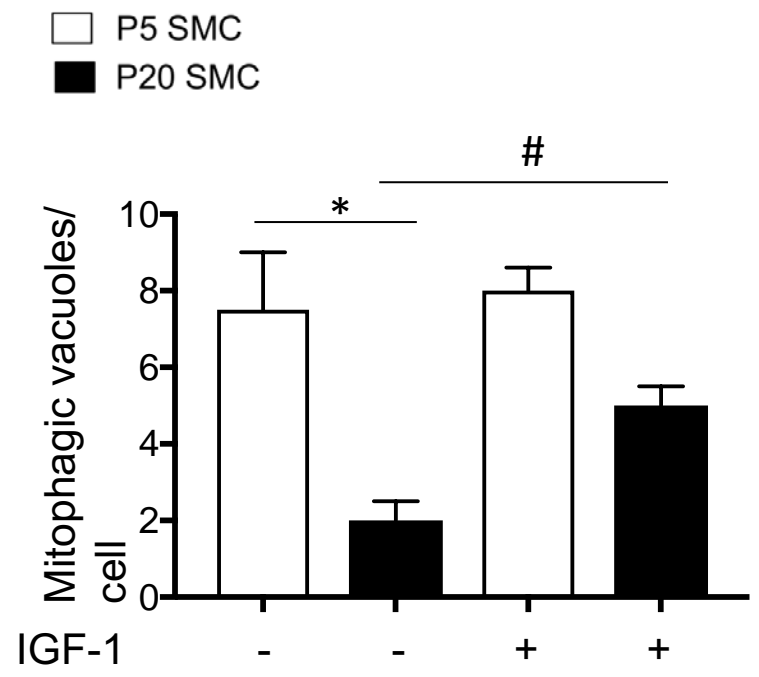
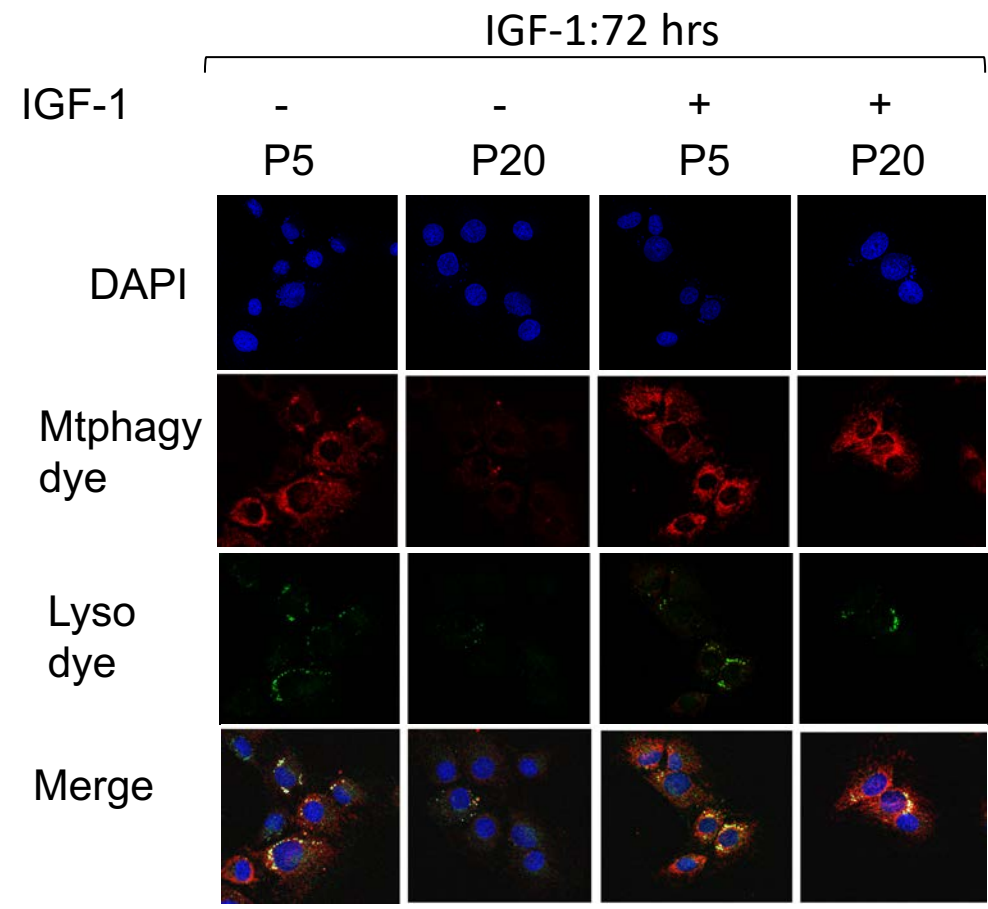








Suppl.Fig.5



Suppl.Fig.6

