Supporting Information

Radzyukevich *et al.* 10.1073/pnas.0804150106

SI Text

Western Blot Analysis. Immuno detection was performed as described previously (1) using α1- and α2-specific antibodies (α1 monoclonal α6f; University of Iowa Developmental Hybridoma Bank and IgG F(ab)2 secondary antibody; Jackson ImmunoResearch Laboratories; α2 polyclonal, affinity purified from α2-antisera generated using the synthetic HERED peptide (2, 3) with an anti-rabbit goat secondary antibody (Cortex Biochem). Blots were visualized by chemiluminescence (Amersham–Amersham Pharmacia) and quantified by densitometry (ImageQuant; Molecular Dynamics). Three replicate intensity measurements were obtained for each isoform from 4 mice of each genotype (12 observations per isoform per genotype). The intensities were adjusted for analysis to display obtain approximately equal average intensities for α1 and α1. Equality in the amount of protein loaded (20 μg of protein per lane for both isoforms) was verified using Ponceau S staining. The group mean intensities of Ponceau S bands bracketing the α-isoforms were compared using an unpaired t test for pooled data with equal variances and did not differ significantly between genotypes (P = 0.35 for α1; P = 0.71 for α2). Intensities of α1 and α2 bands were compared using a repeated mixed linear model (ANOVA, SAS procedure PROC MIXED), with genotype modeled as a fixed effect and mouse ID as a random effect.

Treadmill Exercise. Untrained male and female mice of 6–12 wk age were evaluated for their ability to perform physical exercise using a computerized treadmill (Omnipacer Treadmill LC/M; Accuscan Instruments). Mean body weight was 25.2 ± 0.7 g (n = 42) in WT and 26.7 ± 0.6 g (n = 47) in the $\alpha2^{R/R}$ mice starting pools ($P > 0.11$). The mice were run in the afternoon ≈10 h into the light cycle when the mice normally exhibited periodic motor activity. The treadmill logged interruptions in running time in 1-sec intervals. Performance was assessed using the number of interruptions (failure to run) accumulated in each 2-min interval. The mice were familiarized with the treadmill for 3–5 days before the test run by running 10 min per day at 10–14 m/min (e.g., ref. 4). Each test run was preceded by an acclimatization run at 10 m/min for 5 min.

Two exercise paradigms were used: (*i*) graded exercise, and (*ii*) high-intensity exercise. The graded exercise test subjected the mice to stepwise increases in intensity each 2-min interval, starting from 14 m/min up to a maximum speed of 30 m/min, at an incline of 6°. Running speed was increased by 2 m/min every 2 min, over 18 min. The high-intensity exercise test subjected the mice to constant high-intensity exercise at a fixed running speed of 24 m/min for 20 min at an incline of 6°. This speed was chosen to produce at least 90% of maximal oxygen consumption (5) and is the speed at which this measure is least sensitive to differences in body weight.

The mice showed a wide variability in their interest in and/or ability to perform treadmill running, consistent with a wide body of exercise studies on mice (6). To control for this innate variability, we introduced a post hoc criteria for inclusion in the analysis. All mice were subjected to both tests without preselection. The starting pool showed no selection for these criteria based on genotype (Table S1). After the graded test, the criterion for inclusion in the analysis was the ability to run at the 16 m/min speed for 2 min with fewer than 10 failures. Twenty-one WT and 20 $\alpha2^{R/R}$ mice met this criterion. After the high-intensity test, the criterion for inclusion in the analysis pool was the ability to have completed the first 2 min of running at 24 m/min with ≤60 failures. Eighteen WT and 20 $\alpha2^{R/R}$ mice met this criterion. The outcomes using these criteria distributed similarly among genotypes, confirming that the criteria did not select a priori for differences based on genotype. Stringent selection criteria are essential for assessing exercise performance in mice, particularly during high-intensity exercise, because mice have a limited operating range of exercise capacity. Mice have a high metabolic rate with a baseline cardiac output at 70% of maximal, compared with only 25% in humans and other mammals (7).

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Table S1. Performance of WT and $\alpha 2^{R/R}$ mice on the inclusion criteria for exercise tests

	All Mice			Analysis Pool		
	WT	$\alpha 2^{R/R}$	P	WT	$\alpha 2^{R/R}$	P
Graded	8.0 ± 1.9 (30)	11.2 ± 3.6 (30)	0.51	4.2 ± 1.1 (21)	3.3 ± 1.2 (20)	0.56
High-intensity	58.4 ± 5.5 (46)	60 ± 5.5 (45)	0.80	26.4 ± 4.7 (18)	26.9 ± 3.8 (20)	0.95