



Fig. S1. Results of 13 generations of selection for the maximum swim-induced rate of oxygen consumption in the bank vole: Replicate line means (raw values, not adjusted for body mass). The mean number of individuals tested per replicate line and generation was 211 in the selected lines 36 in the control lines. In generation 12 the selection was relaxed (broken line). In generation 13th, from which individuals for this research were sampled, the maximum mass-adjusted swim-induced rate of oxygen consumption was 46% higher in the A-selected (both sexes) than Control lines (Adjusted Least Square Means from an ANCOVA model; A-selected: 5.28 ± 0.044 ; Control: 3.6 ± 0.046 mlO₂/min; $p < 0.0001$). The Sex \times line Type effect was nearly significant ($p = 0.06$), because in control lines females tended to achieve slightly higher mass-adjusted rates than males did (females: 3.68 ± 0.053 , males: 3.55 ± 0.051 mlO₂/min), whereas in the selected lines there was no difference between sexes (females: 5.28 ± 0.048 , males: 5.28 ± 0.049).

Table S1. Tissue processing protocol. After dissection and formalin buffering the muscles were stored in 70% Ethanol (Line EtOH-B, Linegal Chemicals, Poland) at room temperature (RT: 20 – 25°C) until the tissue processing procedure performed according to the below described protocol.

Process	Reagent	Time	Temperature
dehydration	80% Ethanol	1 h	RT
dehydration	96% Ethanol	1 h	RT
dehydration	96% Ethanol	45 min	RT
clearing	ST Ultra (Leica, Wetzlar, Germany)	45 min	RT
clearing	ST Ultra	45 min	RT
paraffin infiltration	ST Ultra + histoplast paraffin (Shandon, Thermo Scientific, Waltham, MA, USA) (1:1)	Overnight	56°C
paraffin infiltration	histoplast paraffin	6 h	56°C

Table S2. Preliminary analyses (Pearson correlations) of hind leg muscles (gastrocnemius with plantaris) in 16 individuals: repeatability and homogeneity of the mean fibre area and the mean saturation of PAS staining, calculated from four bundles within each of the three compartments (external, central and internal; see Methods) (N=16 in all the analyses).

(A) Analysis of repeatability of the measurements: correlations between the results obtained independently by two researchers for cross-sections made at 1/2 of the muscle length. Each researcher analysed a separate slide with the cross-section, and independently defined the compartments, chose four bundles, and performed all the measurements. Thus, the high correlations not only confirm high repeatability of the measurements, but also validate the protocol of choosing a representative set of fibres for the analyses.

Dependent Variable	Pearson correlation coefficients (p values) calculated separately for three compartments		
	External	Central	Internal
Mean fibre area (μm^2)	0.94 (0.000)	0.82 (0.000)	0.91 (0.000)
Mean saturation of PAS staining (dimensionless)	0.93 (0.000)	0.96 (0.000)	0.94 (0.000)

(B) Analysis of the muscle structure homogeneity along the muscle's length: correlations between the results of measurements made on the cross-sections at 1/3 and 1/2 of the muscle length. As the data for two independent cross-sections at 1/2 length were available, two correlation coefficients were calculated for each of the two variables and three compartments.

Dependent Variable	Compartment	Pearson correlation coefficients (p values)	
		1/3 vs 1/2 (1 st section)	1/3 vs 1/2 (2 nd section)
Mean fibre area (μm^2)	External	0.81 (0.000)	0.80 (0.000)
	Central	0.74 (0.001)	0.85 (0.000)
	Internal	0.80 (0.000)	0.75 (0.001)
Mean saturation of PAS staining (dimensionless)	External	0.93 (0.000)	0.88 (0.000)
	Central	0.74 (0.001)	0.85 (0.000)
	Internal	0.80 (0.000)	0.75 (0.001)

(C) Analysis of the muscle structure homogeneity across the muscle: correlations between the means calculated for external, central and internal compartments from the same cross-section.

Dependent Variable	Muscle length	Cross - section	Pearson correlation coefficients (p values)		
			External vs Central	Central vs Internal	Internal vs External
Mean fibre area (μm^2)	1/3		0.52 (0.040)	0.69 (0.003)	0.48 (0.061)
	1/2	1 st	0.73 (0.001)	0.79 (0.000)	0.56 (0.023)
		2 nd	0.68 (0.005)	0.78 (0.001)	0.61 (0.016)
Mean saturation of PAS staining (dimensionless)	1/3		0.75 (0.000)	0.93 (0.000)	0.88 (0.000)
	1/2	1 st	0.67 (0.005)	0.95 (0.000)	0.79 (0.000)
		2 nd	0.91 (0.000)	0.95 (0.000)	0.90 (0.000)

Table S3 Adjusted mean muscle fibre area (MA; back-transformed least square means and 95% confidence limits - LSM[95%CL]) in bank voles from the selected and control lines, and the results of nested ANCOVA models with body mass as covariate, performed separately for females (N=48) and males (N=47). In the analysis results of 12 bundles for each individual were used as observations (total number of bundles for females: n=576, males n=564). The mean muscle fibre area increased with the body mass in females (common slope [95%CL]: 0.006 [0.003 - 0.009] $\log(\mu\text{m}^2)/\text{g}$, $t_{549}=3.88$, $p=0.000$) but not in males (0.001 [-0.001 - 0.004] $\log(\mu\text{m}^2)/\text{g}$, $t_{536}=1.00$, $p=0.315$). Ndf - nominator degrees of freedom, Ddf – denominator degrees of freedom. The interaction term body mass \times line type for males was excluded from the model at $p=0.03$.

	Fixed factor	Factor Level	MA (μm^2) LSM (95%CL)	F statistic (Ndf, Ddf)	P value
Females	line type	selected	406 (367 - 450)	0.00 (1,6)	0.98
		control	406 (366 - 450)		
	Compartment	external	418 (384 - 456)	1.48 (2,12)	0.27
		central	399 (366 - 435)		
internal		401 (367 - 437)			
	line Type \times Compartment			1.12 (2,12)	0.36
Males	line type	selected	424 (356 - 505)	3.94 (1,6)	0.09
		control	389 (326 - 464)		
	Compartment	external	417 (358 - 486)	1.6 (2,12)	0.24
		central	403 (346 - 469)		
		internal	398 (342 - 464)		
	line Type \times Compartment			1.14 (2,12)	0.35