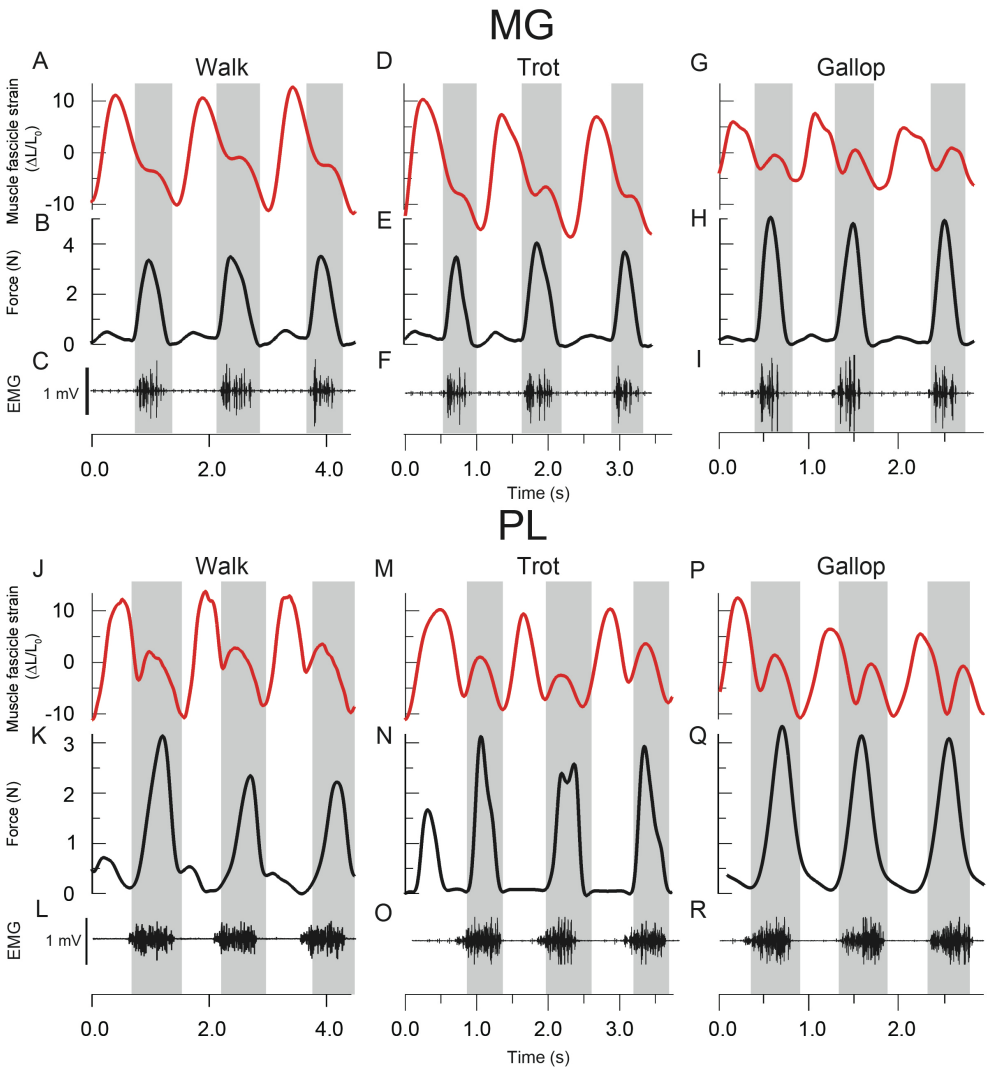

Figure S1. MG muscle fascicle strain (A, D, G), muscle force (B, E, H), and unamplified EMG (C, F, I) for level walk, trot, and gallop. PL muscle fascicle strain (J, M, P), muscle force (K, N, O), and unamplified EMG (L, O, R) for level walk, trot, and gallop. Gray regions represent the stance phase of each stride.

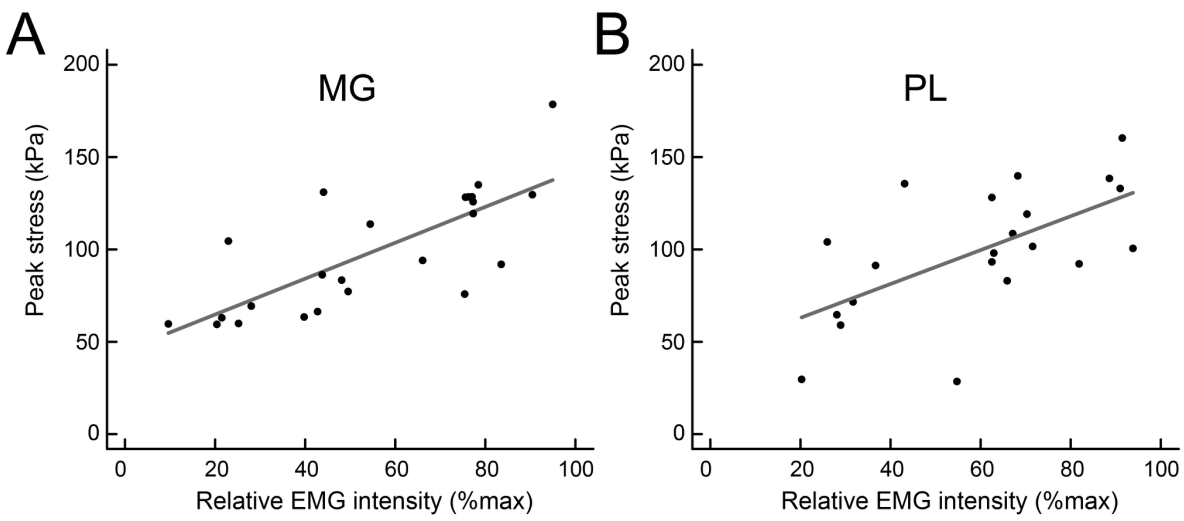

Figure S2. Peak stress (kPa) as a function of relative EMG intensity for the MG (A) and PL (B). The relationship between peak stress and relative EMG intensity evaluated with linear regression is significant for both MG ($p<0.01$ and $R^2=0.58$) and PL ($p<0.01$ and $R^2=0.38$).

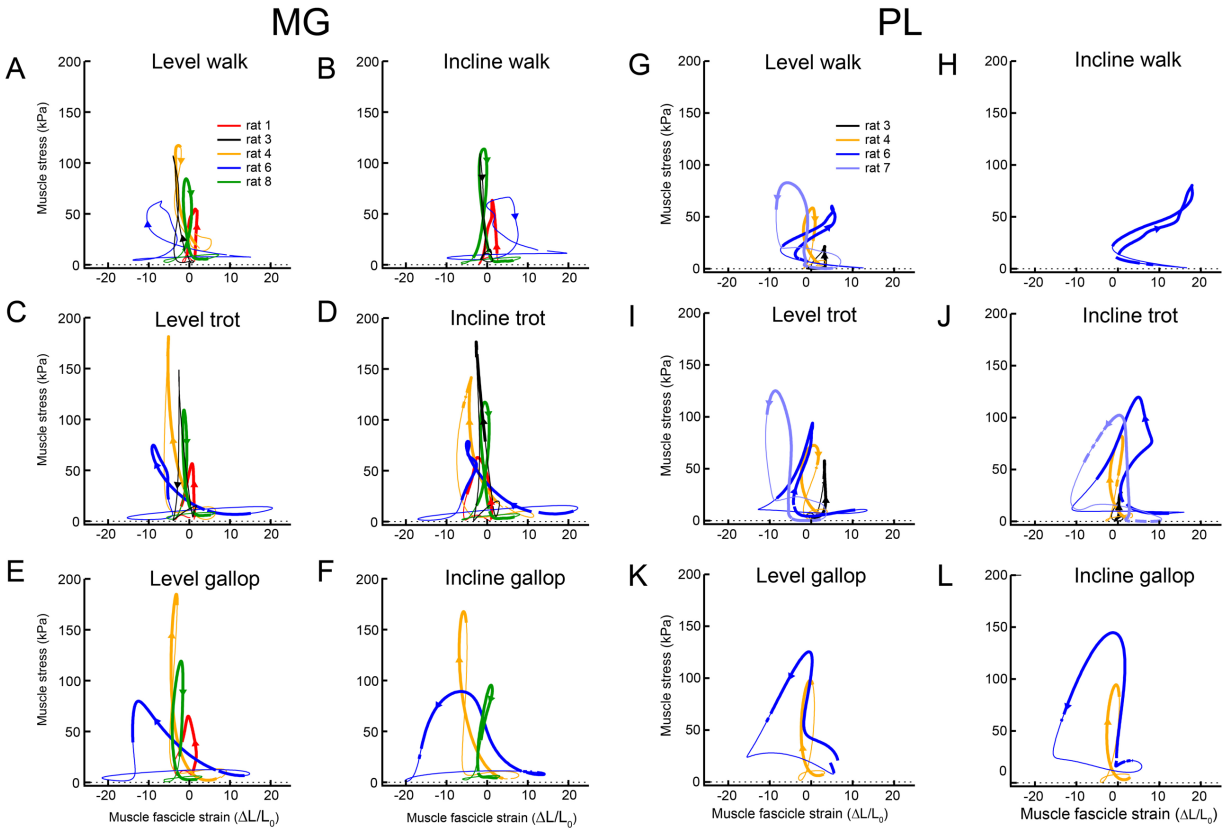

Figure S3. Mass-specific work loops of the MG (A-F) and PL (G-L) plotted as muscle stress versus fascicle strain, for comparison among multiple individuals. The direction of muscle stress relative to strain over the course of a complete stride cycle is shown by the arrows, together with the net mass-specific work (J kg^{-1}) performed by the muscle for each condition. Muscle activation (EMG) timing is indicated by the bold portion of each loop.

Table S1. Muscle architecture of medial gastrocnemius (MG) and plantaris (PL)

Muscle	Muscle mass (g)	Muscle length (mm)	Fascicle length (mm)	Pennation angle (degrees)	PCSA (mm^2)
MG	0.87 ± 0.08	31.13 ± 1.06	11.00 ± 0.93	26.4 ± 3.3	63.35 ± 14.39
PL	0.36 ± 0.03	35.15 ± 2.24	10.12 ± 0.21	18.4 ± 5.1	30.14 ± 3.66

Data are presented as mean ± s.e.m.

Table S2. Coefficients of variation across strides within an individual (individual) and across individuals (group) for level walk, trot, and gallop

	Walk		Trot		Gallop	
	Individual	Group	Individual	Group	Individual	Group
MG EMG intensity	17.0%	59.1%	20.0%	49.3%	19.2%	23.2%
PL EMG intensity	23.8%	63.1%	11.2%	52.9%	8.0%	13.4%
MG peak stress	6.9%	28.3%	6.7%	32.2%	12.1%	34.8%
PL peak stress	8.0%	38.3%	5.4%	28.1%	6.6%	2.8%
MG net strain	90.2%	83.1%	48.3%	71.5%	121.7%	145.0%
PL net strain	795.3%	531.1%	116.7%	100.0%	78.5%	76.6%
MG net work	56.0%	889.9%	4352.1%	69.7%	73.4%	281.0%
PL net work	187.3%	868.6%	31.9%	2320.1%	27.5%	355.3%

Table S3. P-values for non-parametric tests for the effect of gait and grade across rats

	Effect of gait: Level			Effect of gait: Incline			Effect of grade		
	Walk-Trot	Walk-Gallop	Trot-Gallop	Walk-Trot	Walk-Gallop	Trot-Gallop	Walk	Trot	Gallop
MG EMG intensity	0.16	**0.03**	0.06	0.06	0.13	0.13	0.31	0.16	0.06
PL EMG intensity	0.28	0.13	0.13	0.06	0.06	0.06	0.78	0.50	0.88
MG peak stress	**0.02**	0.06	0.13	0.06	0.13	0.56	0.44	0.38	0.22
PL peak stress	0.06	0.25	0.25	0.25	0.25	0.19	0.75	0.50	0.81
MG net strain	0.96	0.69	0.41	0.78	0.63	0.69	0.78	0.97	1.00
MG shortening strain	0.15	0.78	0.91	0.69	0.63	0.69	0.69	0.84	1.00
MG lengthening strain	0.96	0.78	0.31	0.59	1.00	0.69	0.50	0.28	0.88
PL net strain	0.89	0.88	0.38	1.00	0.50	0.13	1.00	0.94	0.25
PL shortening strain	0.11	0.38	0.75	0.50	0.75	0.63	0.50	0.91	0.75
PL lengthening strain	0.89	0.88	0.63	0.75	0.50	0.25	0.75	0.78	0.25
MG net work	**0.02**	0.31	0.69	0.13	0.25	0.38	0.06	0.06	0.25
MG positive work	**0.02**	0.19	0.31	0.13	0.50	0.63	0.56	0.06	0.13
MG negative work	**0.02**	0.56	0.69	0.69	0.25	0.63	0.19	0.78	0.25
PL net work	0.78	0.50	0.50	NA	NA	0.50	NA	0.13	0.25
PL positive work	0.78	0.25	0.50	NA	NA	0.50	NA	0.31	0.25
PL negative work	0.69	0.75	0.75	NA	NA	1.00	NA	0.13	0.50

Bolded values indicate p-value < 0.05