Supplementary Material

The trajectory of gait development in mice

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1. Supplementary Methods – Detailed Methods Description

Gait Analysis

Apparatus. Gait data was collected using the DigiGait Imaging System (Mouse Specifics, Inc), an advanced gait analysis system with Ventral Plane Imaging Technology that generates digital paw prints from the animal as it runs on a motorized treadmill (Hampton et al., 2004). This system has been described in detail previouslyhttps://www.zotero.org/google-docs/?unSJpg (Hampton et al., 2004). Due to smaller body size of juvenile mice, 0.6 cm thick bumpers made from expanded PVC were placed on the sides of the apparatus using magnets to narrow the width of the run alley and thus increase the potential for straight, usable runs. The floor of the apparatus was made of tempered glass around which looped a transparent belt (PCV and HDPE blend) measuring 6 cm wide. The animal was sequestered to the 40.5 cm run alley by a transparent polycarbonate enclosure and illuminated to 1381 lux from above and below with LED panel lighting. A digital video camera (Basler Ace/Scout Camera with 12.5/16mm lens) was housed beneath the run alley to capture the ventral plane image of the animal, which was sent to the DigiGait software on a connected computer.

Procedure. Both male and female experimenters collected C57 gait data and the female experimenter collected FVB gait data. Pilot testing revealed FVB mice can adequately run on the DigiGait by P17. However, C57 mice could not run adequately until P21. Therefore, we chose to examine gait across the post-weaning, juvenile period (Figure 1A). All testing occurred during the light phase of the circadian cycle. For all habituation and testing sessions, the mice were placed in a holding room adjacent to the testing environment for a 30 min acclimation period prior to testing, and weighed. Prior to testing the paws of the FVB mice were dyed with red food coloring (McCormick) diluted into water to increase contrast with belly fur. The testing room overhead lights were off during testing for optimal video capture, however the holding room lights remained on to decrease disruptions of circadian rhythms. On P20, each mouse was habituated to the apparatus. This consisted of placing the animal on the stationary belt and starting the belt moving at 5 cm/s and slowly increasing the speed until 20 cm/s is reached allowing for at least 30 sec of run time. Because speed is the greatest influencer of gait, the speed of the treadmill during data collection was kept constant across all ages at 20 cm/s to allow for appropriate comparisons of forced gait across age. Testing occurred P21, P24, P27 and P30. For these test days, each mouse was placed individually on the apparatus. The belt was started at 10 cm/s until the animal started walking forward. Once the animal reached the front of the alley, the speed was increased to 20 cm/s. Because speed is the greatest influencer of gait, the speed of the treadmill during data collection was kept constant across all ages at 20 cm/s to allow for appropriate comparisons of forced gait across age. Once a usable run was acquired, the belt was stopped and the animal removed to the homecage. Criteria for a usable run included a consistent forward movement with no sliding, jumping, or side drift. The belt was cleaned with 70% EtOH between litters or as needed between mice and daily upon completion of testing.

Video Processing. To obtain optimal contrast between the mouse paws and coat color for recognition of paws for digital footprint generation and processing of videos through the DigiGait software pipeline, we post-processed the videos using ImageJ (Wayne Rasband, NIH, USA, RRID:SCR_003070). To determine optimal levels of brightness and contrast we used two videos from each time point and processed each multiple times using differing levels of brightness and contrast. The settings that resulted in the smoothest paw area contact plots were used (brightness adjustment of 19 and contrast adjustment of 135). These adjustments were then applied to all videos. The adjusted videos were exported as uncompressed .avi files to ensure compatibility with the DigiGait software.

Each video was then processed through the DigiGait Analysis software, as described previously (Hampton et al., 2004). This entailed applying filters to exclude the snout and adjust contrast for optimal digital footprint generation. Paw contact area plots were generated and validated against the video of the mouse. Any tracking errors were manually corrected. The digital footprints and paw contact area plots were then used to extract the

gait parameters used for analysis (Figure 1B). Gait was analyzed by quantifying components of the step cycle, or stride, broken into when a paw has contact with the ground, known as the stance phase, and when it is moving through the air, known as the swing phase (Figure 1B). The stance phase is further broken down into the paw braking phase (heel strike to full stance) and propulsion phase (full stance to toe push off). All our trials comprised at least 12 strides based on previous work suggesting 9 strides or more are required for DigiGait data processing (Hampton et al., 2004) (C57, *M*=19.0, range: 12.0-24.5; FVB, *M*=18.9, range: 13.0-25.5).

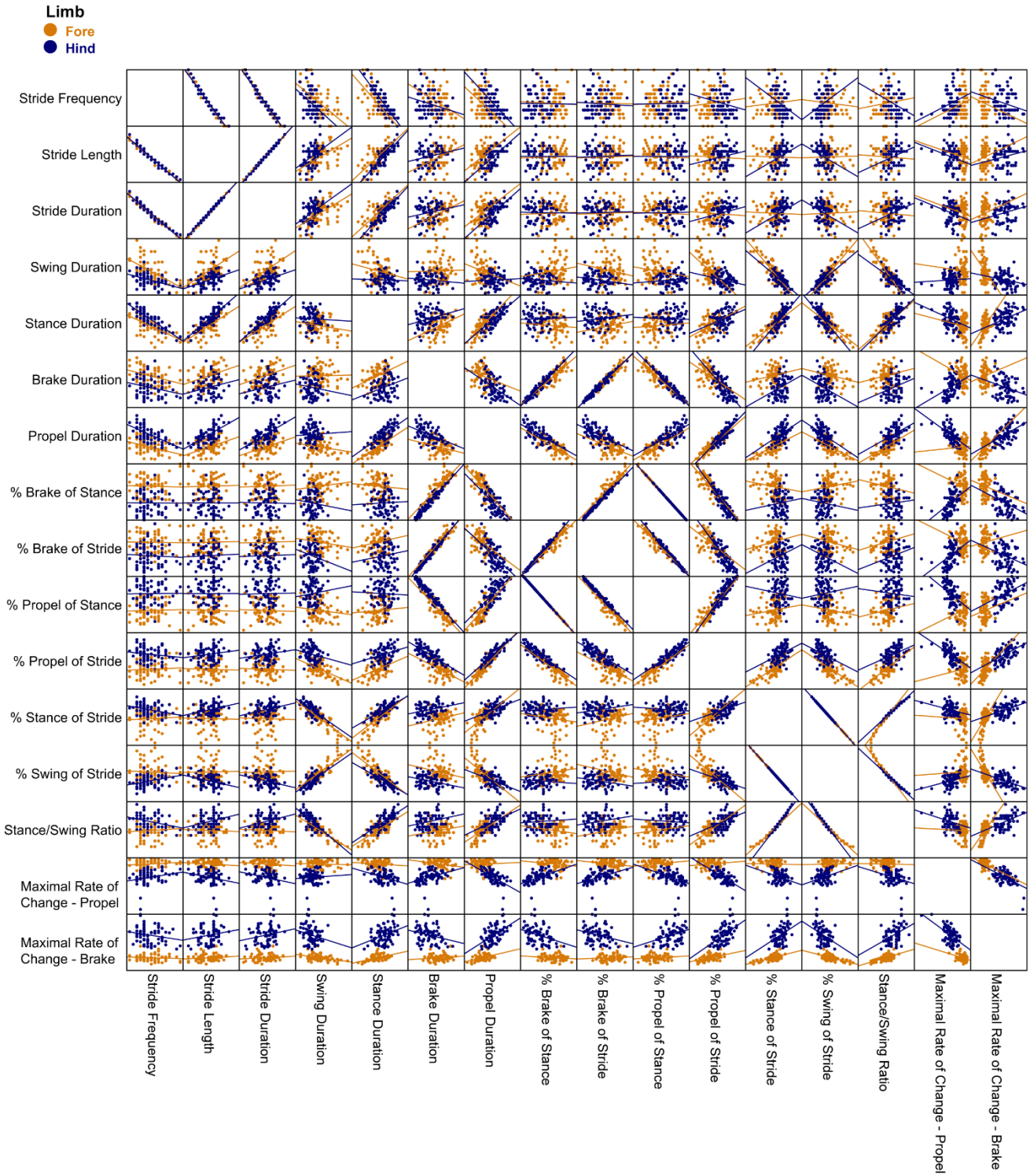
Selection of gait metrics for analysis. The DigiGait software analysis system outputs a comprehensive list of gait metrics. A concern with such a large list of variables is inflation of familywise error rate. To identify possible redundant variables, we generated scatterplot matrices. Pairs of variables that were visibly perfectly aligned were considered redundant and one variable within the pair was excluded from further analysis. The variables we excluded were stride duration, % brake of stride, % brake of stance, % propulsion of stride, % propulsion of stance, swing/stance ratio (Figures S1, S2), hindlimb shared stance time (Figures S3, S4), and ataxia coefficient (Figures S5, S6)

In addition to excluding variables based on redundancy, we also determined if variables required exclusion based on poor reliability. The post-video acquisition processing within the DigiGait software requires adjustment of filters to remove the snout and decrease noise from the digital paw prints, as well as manual corrections to errors within the paw contact area plots, introducing the possibility of inconsistencies across experimenters. We examined the inter-rater reliability of this processing between the measurements produced by two independent experimenters by calculating intraclass correlation coefficients (ICC) with their 95% confidence intervals using IBM SPSS Statistic software (v.25) based on absolute-agreement and two-way mixed-effects model. The data used for this were derived from an independent cohort of 10 FVB mice tested on P24, P27 and P30. For fore and hind limbs, respectively, 20/25 and 27/32 metrics showed excellent reliability (ICC ≥ .75) and another 2/25 and 2/32 showed good reliability (ICC = .60 - .74). The remaining metrics showed poor reliability (ICC ≤ .39; 3/25 and 3/32). ICCs are reported in Table 1. We excluded from further analysis any metrics with poor reliability: midline distance, axis distance, paw drag, and maximal rate of change of paw area contact during the propulsion phase. We also excluded tau propulsion despite good reliability because we felt this measure has not yet been defined or validated adequately in the literature and thus its usefulness is not clear.

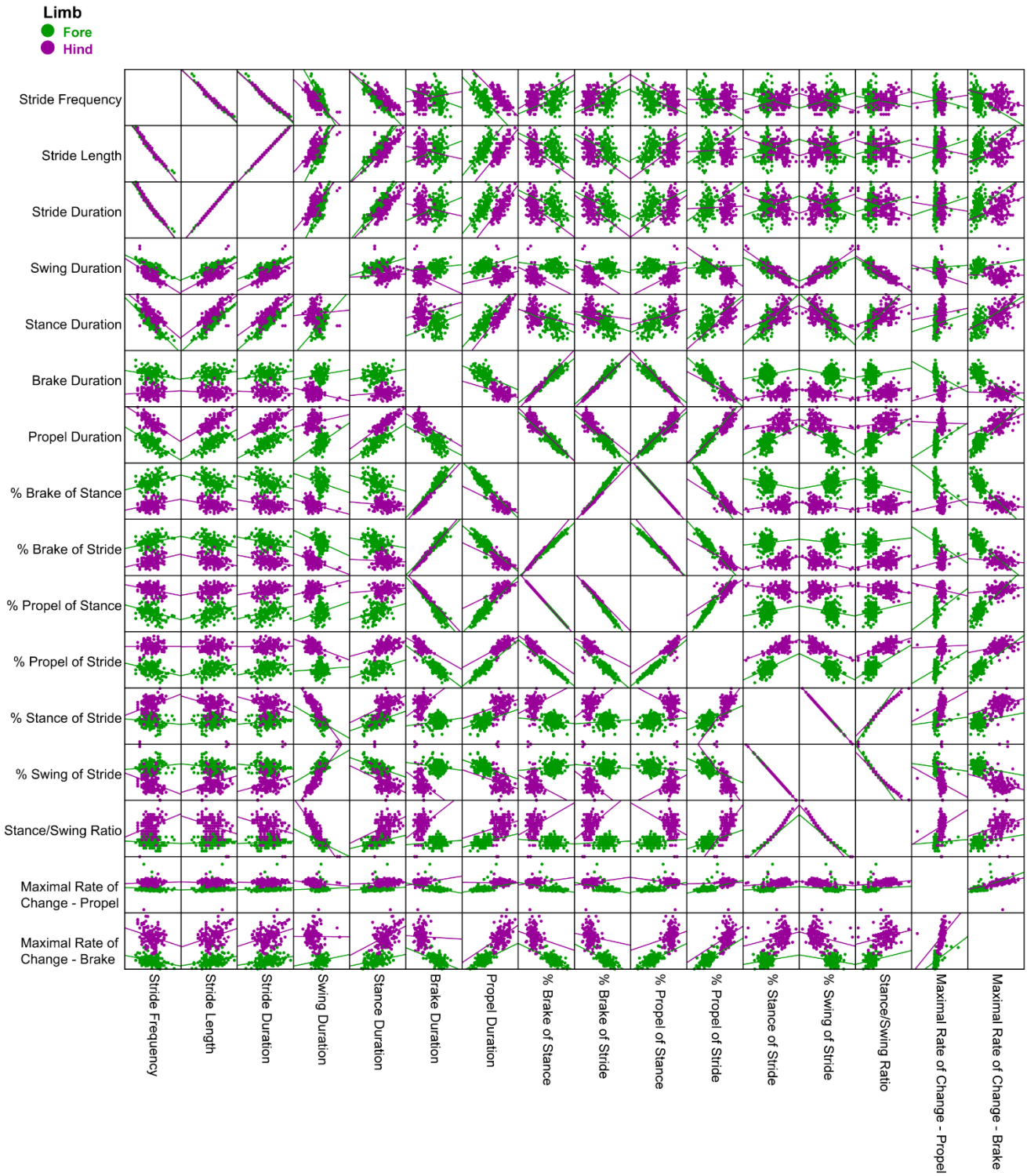
Body Length Quantification

Animal body length was measured by importing videos from the Digigait software into Ethovision (Noldus Information Technology, The Netherlands, RRID:SCR_000441). The animal's body was automatically detected using the contrast of the darker body against a lighter background. A custom script provided by Noldus was used to calculate its length based on the coordinates of the nose, center point and tail base. A length measurement was calculated from those three coordinates for every frame of the video and then averaged into one score per animal for analysis. Thus, this accounted for any differences that occurred due to extensions or contractions of the body during a stride. To validate this method of body length measurement, the body lengths of a subset of FVB and C57 mice were also measured manually from the same videos at three different time points and then averaged. Intraclass correlation coefficients (ICC) with their 95% confidence intervals were calculated between the manual and automated measurements using IBM SPSS Statistics software (v.25, RRID:SCR_002865) based on absolute-agreement and two-way mixed-effects model. The ICCs indicated excellent reliability between the manual and automated measurements (FVB, ICC = .972, 95% CI [.904, .992]; C57, ICC = .977, 95% CI [.955, .988]), providing confidence in and validation of our automated process for body length measurement.

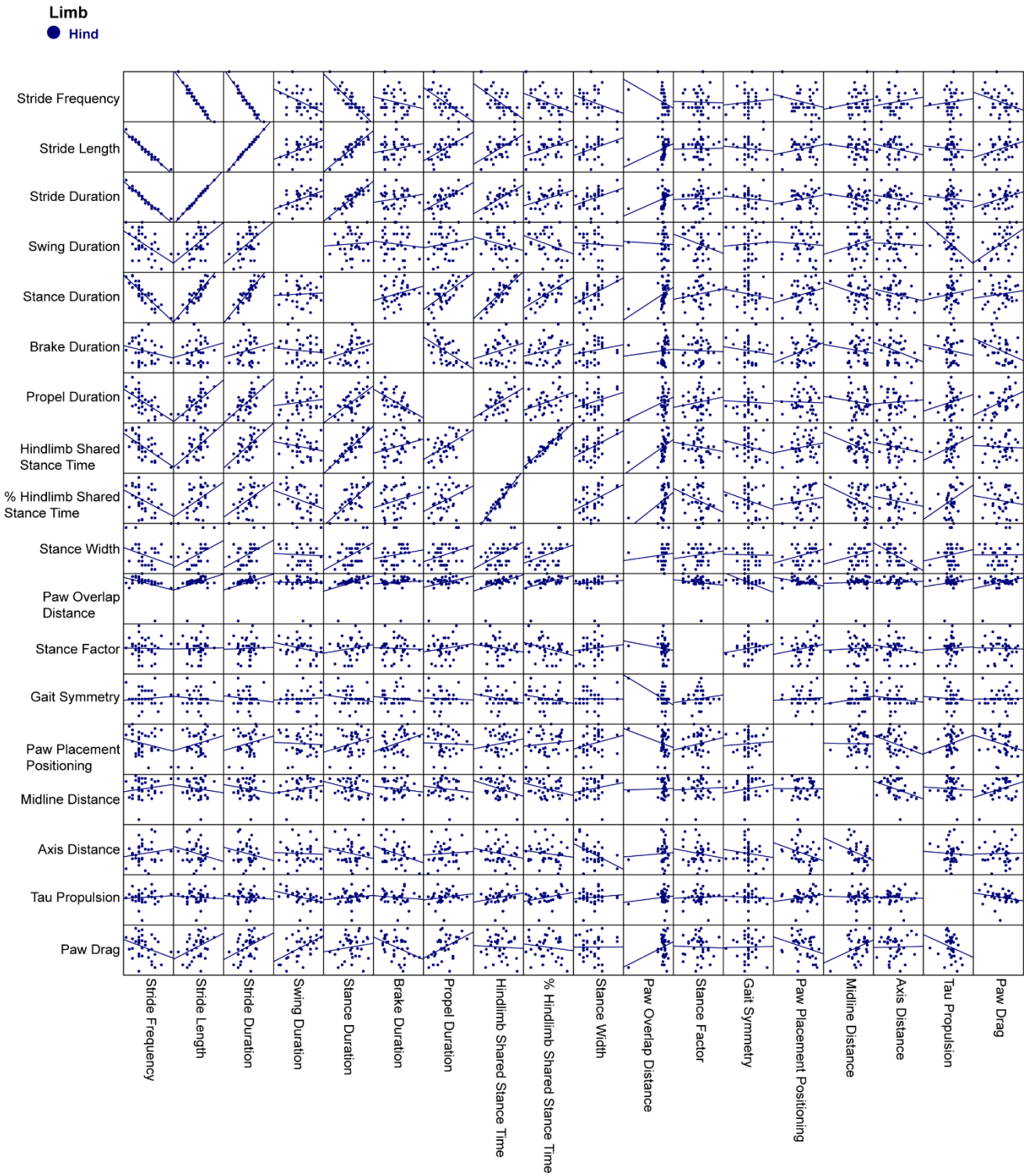
2. Supplementary Figures



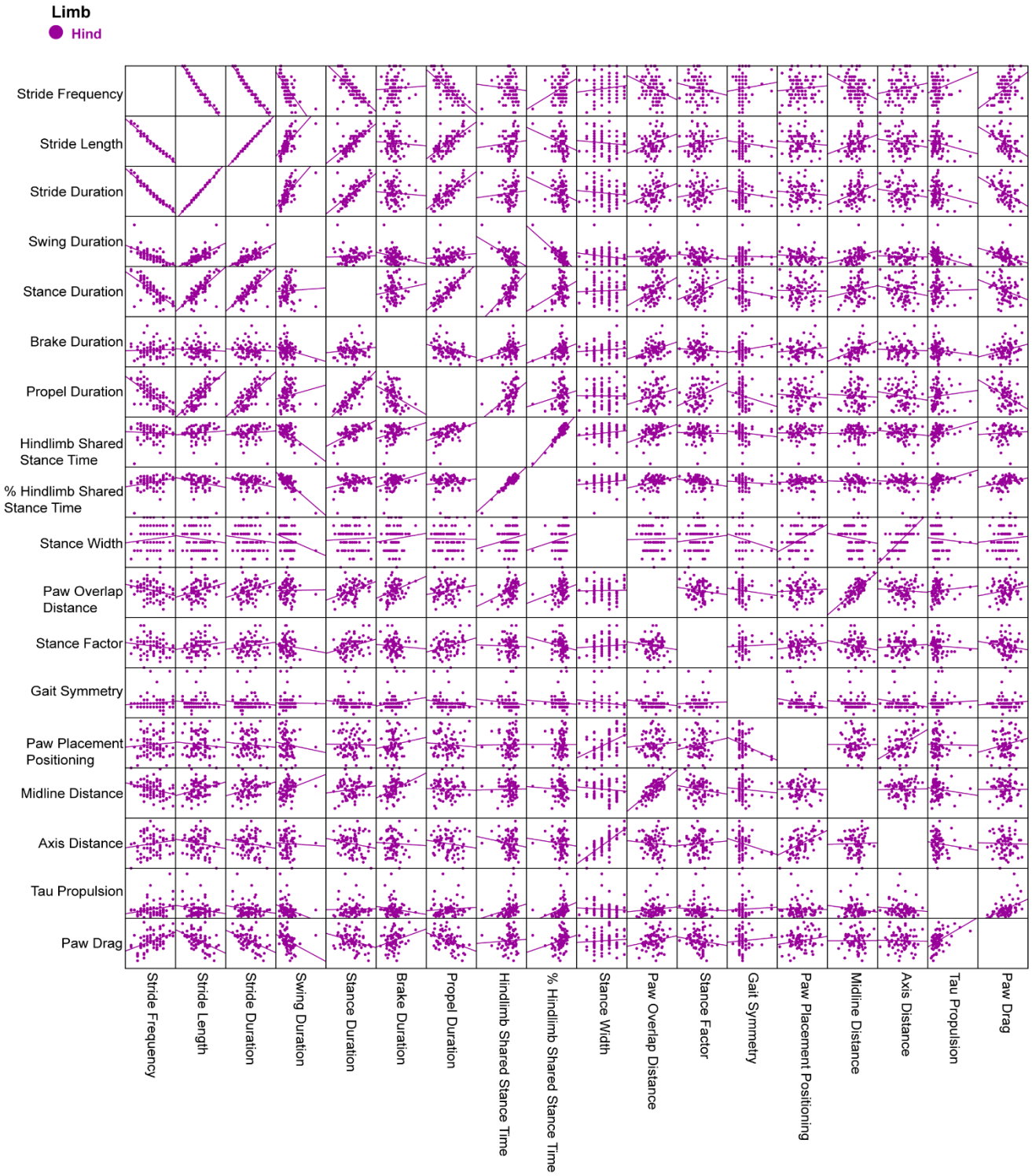
Supplementary Figure 1. Scatter matrices of spatiotemporal metrics measured in both fore and hindlimbs of C57 mice at P21, P24, P27 and P30. One variable from a pair that was considered close to or at perfect alignment was excluded from further analysis.



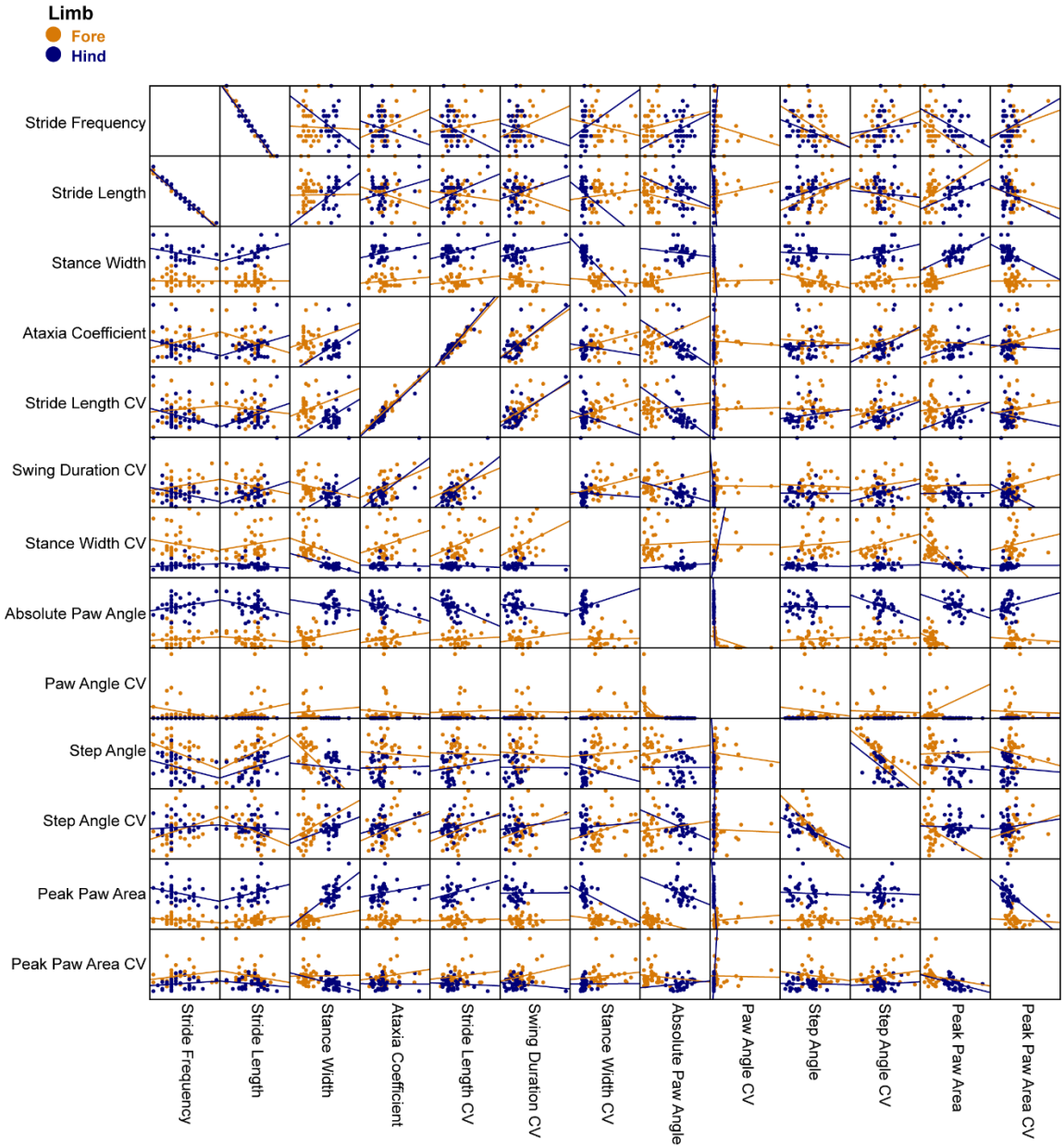
Supplementary Figure 2. Scatter matrices of spatiotemporal metrics measured in both fore and hindlimbs of FVB mice at P21, P24, P27 and P30. One variable from a pair that was considered close to or at perfect alignment was excluded from further analysis.



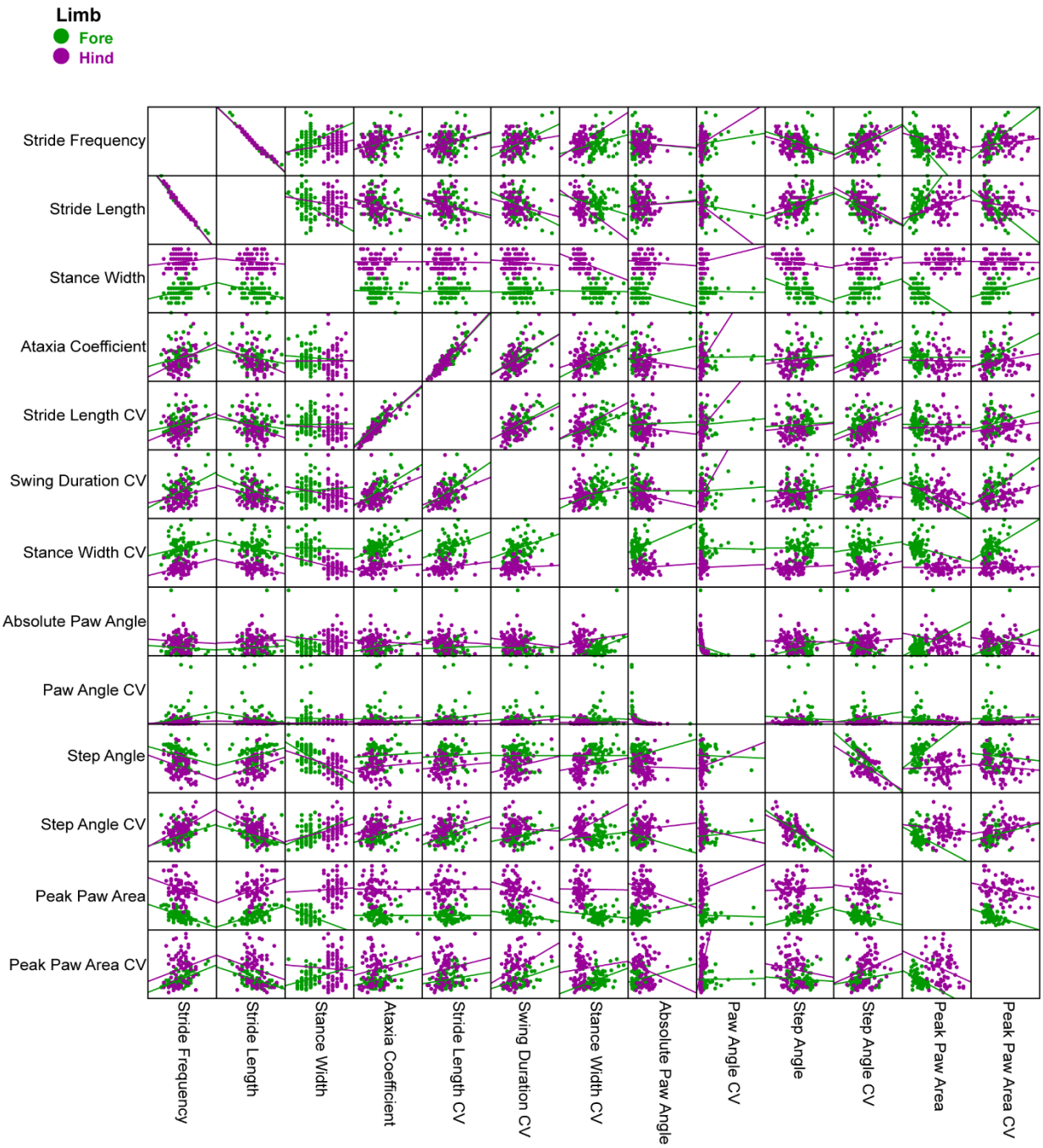
Supplementary Figure 3. Scatter matrices of spatiotemporal metrics measured in only the hindlimbs of C57 mice at P21, P24, P27 and P30. One variable from a pair that was considered close to or at perfect alignment was excluded from further analysis.



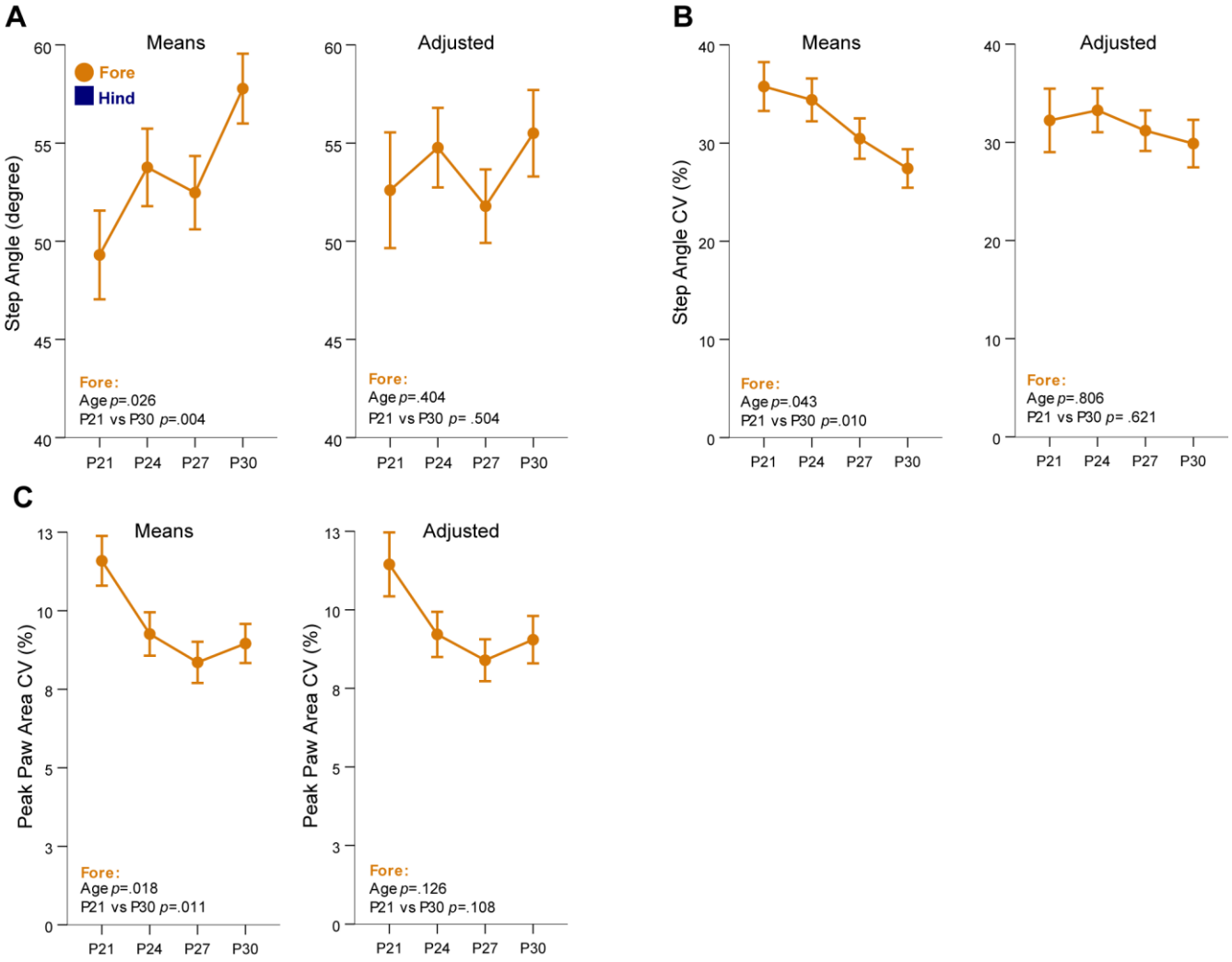
Supplementary Figure 4. Scatter matrices of spatiotemporal metrics measured in only the hindlimbs of FVB mice at P21, P24, P27 and P30. One variable from a pair that was considered close to or at perfect alignment was excluded from further analysis.



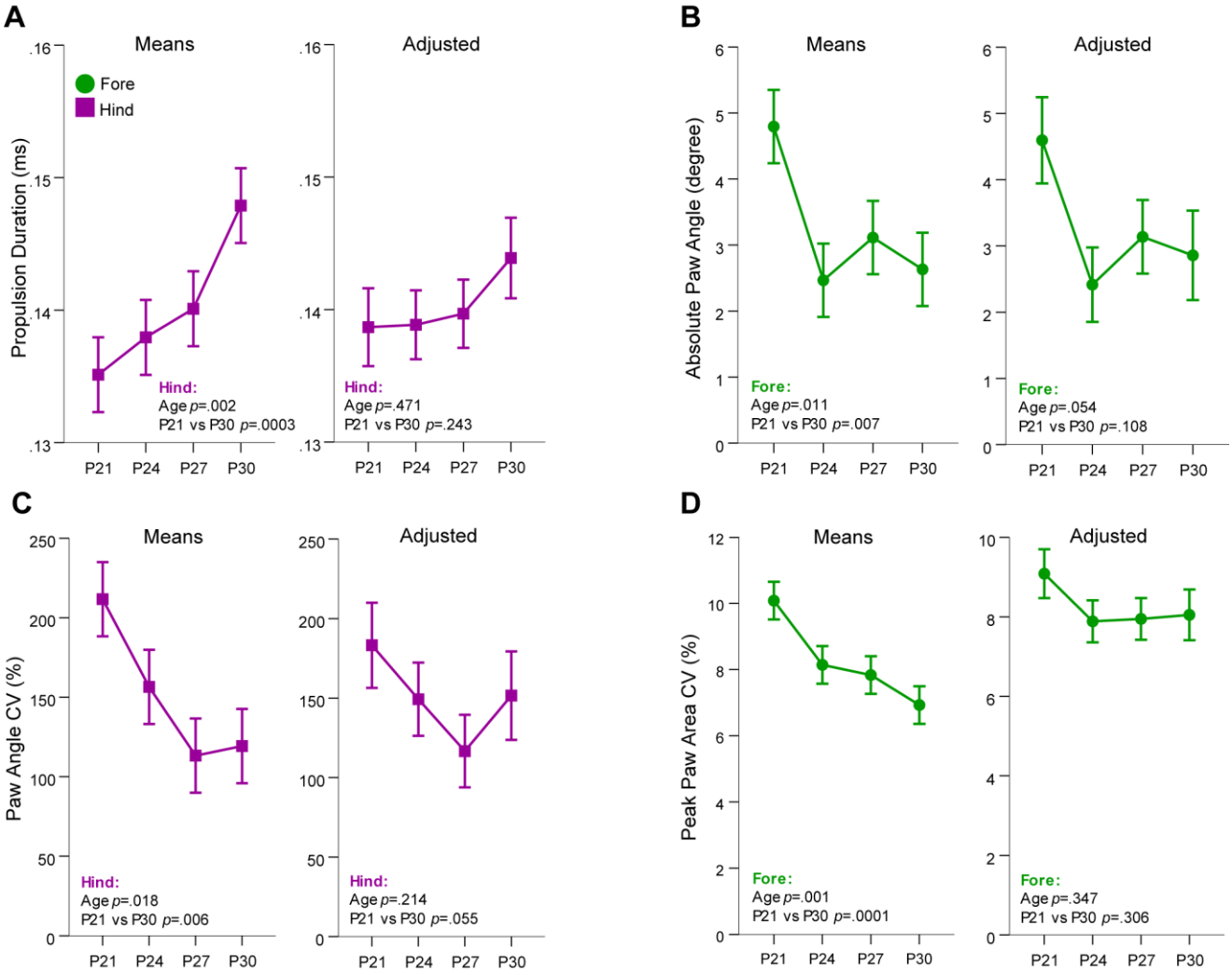
Supplementary Figure 5. Scatter matrices of postural and variability metrics measured in both fore and hindlimbs of C57 mice at P21, P24, P27 and P30. One variable from a pair that was considered close to or at perfect alignment was excluded from further analysis.



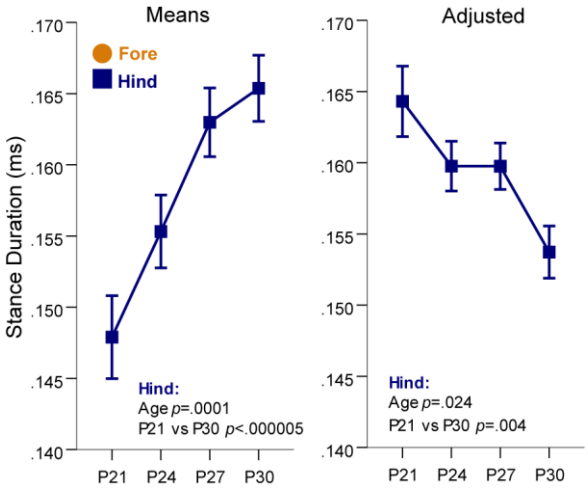
Supplementary Figure 6. Scatter matrices of Scatter matrices of postural and variability metrics measured in both fore and hindlimbs of FVB mice at P21, P24, P27 and P30. One variable from a pair that was considered close to or at perfect alignment was excluded from further analysis.



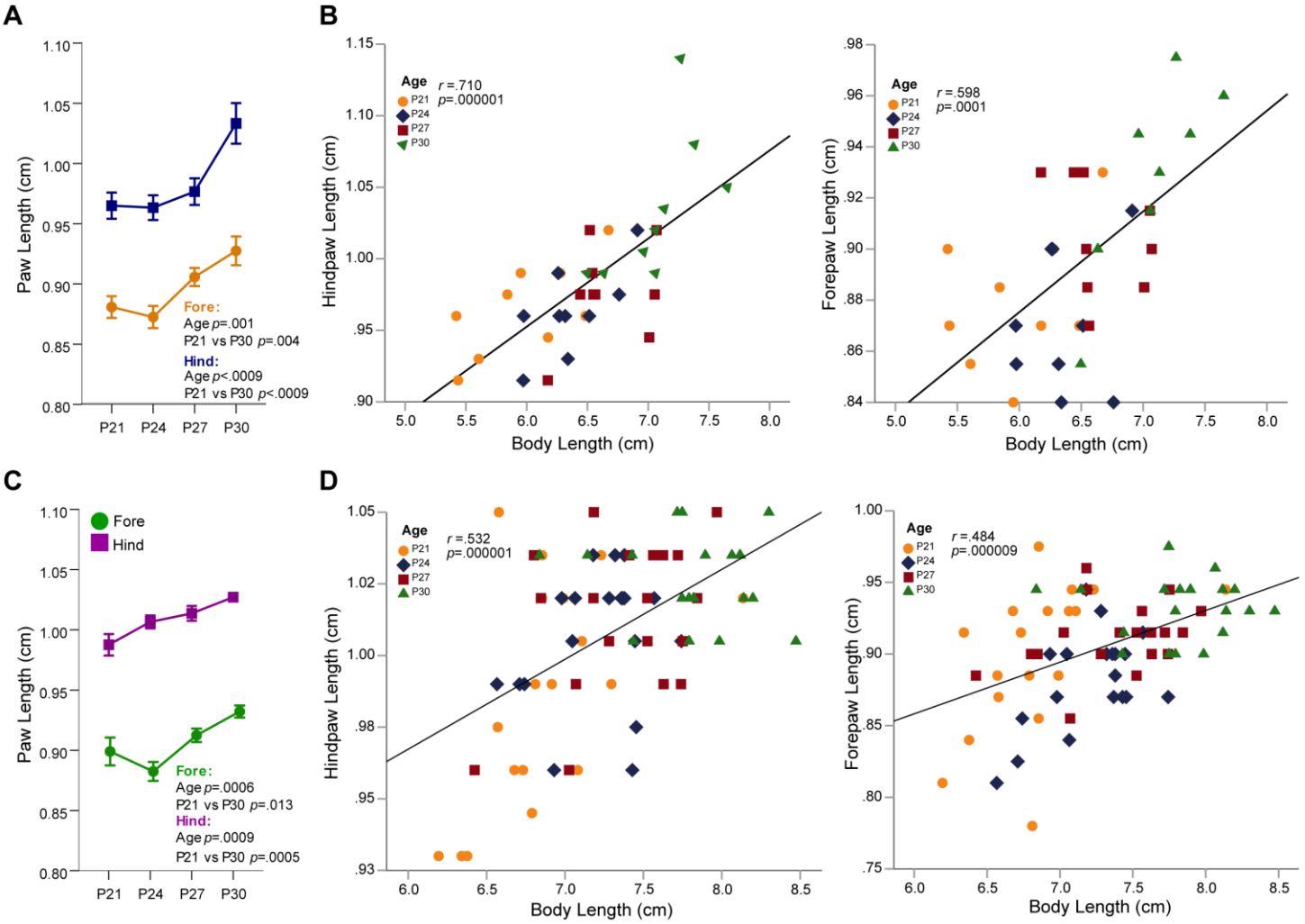
Supplementary Figure 7. Gait metrics that only reflected change in body length in C57 mice. Raw data means and covariate adjusted means are presented for all three variables. (**A**) Step Angle, (**B**) step angle coefficient of variance (CV), and (**C**) peak paw area CV appeared to significantly change with age in C57 mice. However, after adjusting for differences in body length from P21 to P30, these variables no longer significantly changed with age. Data are means ± SEM and covariate adjusted means ± SEM.



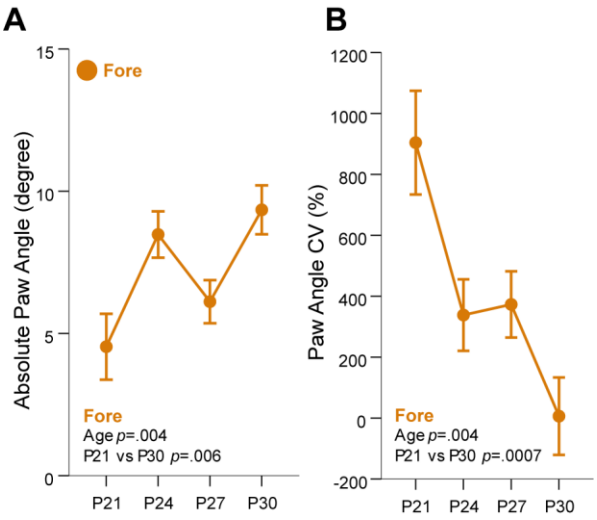
Supplementary Figure 8. Gait metrics that only reflected change in body length in FVB mice. Raw data means and covariate adjusted means are presented for all variables. (**A**) Propulsion duration, (**B**) absolute paw angle, (**C**) paw angle coefficient of variance (CV), and (**D**) peak paw area CV appeared to significantly change with age in FVB mice. However, after adjusting for differences in body length from P21 to P30, these variables no longer significantly changed with age. Data are means ± SEM and covariate adjusted means ± SEM.



Supplementary Figure 9. The directional trajectory of hindlimb stance duration in C57 from P21 to P30 is flipped when adjusted for body length changes. Hindlimb stance duration raw means and covariate adjusted means are presented for C57 mice. Before adjusting for body length, stance duration appeared to significantly increase. However, after adjusting for differences in body length from P21 to P30, stance duration significantly decreased from P21 to P30. Data are means ± SEM and covariate adjusted means ± SEM.



Supplementary Figure 10. Scatter matrices of Scatter matrices of postural and variability metrics measured in both fore and hindlimbs of FVB mice at P21, P24, P27 and P30. (A) C57 fore- and hindpaw lengths increase with age, as measured at full stance. Data are means ± SEM. (**B**) Scatterplots of the strong positive relationship between body length and fore- and hindpaw lengths from P21 to P30 in C57 mice. All data represented. **(C)** FVB fore- and hind paw lengths increase with age, as measured at full stance. Data are means ± SEM. (**D**) Scatterplots of the strong positive relationship between body length and fore- and hindpaw lengths from P21 to P30 in FVB mice. All data represented.



Supplementary Figure 11. Absolute paw angle and paw able coefficient of variation for C57 forelimbs. (**A**) The postural metric absolute paw angle significantly changed across P21 to P30 for the forelimbs in C57 mice, but meaning of the change across time is unclear. (**B**) The variability of this metric, paw angle CV, significantly decreased from P21 to P30.

3. Supplementary Tables

Supplementary Table 1. Statistical output for C57 gait data.

OUTCOME	NORMAL	LMM	parameters	*t*	sig.	LMM (adjusted for body length)	parameters	*t*	sig.
%Hindlimb Shared Stance	normal	Age F(3,44.19)=1.92, p=0.140	P21 vs. P30	-2.140	0.036	Age F(3,38.60)=1.27, p=0.300	P21 vs. P30	1.118	0.268
			P24 vs. P30	-0.406	0.686	Body Length F(1,49.65)=12.30, p=0.0010	P24 vs. P30	1.904	0.061
			P27 vs. P30	-0.405	0.687		P27 vs. P30	1.252	0.216
% Stance Fore	P30 nonnormal	Age F(3,44.79)=8.15, p=0.0002	P21 vs. P30	-3.099	0.003	Age F(3,43.93)=8.48, p=0.0001	P21 vs. P30	-2.989	0.004
			P24 vs. P30	0.906	0.368	Body Length F(1,68.81)=1.89, p=0.174	P24 vs. P30	-0.254	0.800
			P27 vs. P30	0.853	0.398		P27 vs. P30	0.035	0.972
% Stance Hind	normal	Age F(3,44.28)=2.43, p=0.077	P21 vs. P30	-2.438	0.017	Age F(3,37.90)=1.07, p=0.373	P21 vs. P30	1.068	0.290
			P24 vs. P30	-1.048	0.299	Body Length F(1,51.43)=12.52, p=0.0009	P24 vs. P30	1.470	0.146
			P27 vs. P30	0.001	0.999		P27 vs. P30	1.707	0.093
% Swing Fore	P30 nonnormal	Age F(3,44.79)=8.15, p=0.0002	P21 vs. P30	3.099	0.003	Age F(3,43.93)=8.48, p=0.0001	P21 vs. P30	2.989	0.004
			P24 vs. P30	-0.906	0.368	Body Length F(1,68.81)=1.89, p=0.174	P24 vs. P30	0.254	0.800
			P27 vs. P30	-0.853	0.398		P27 vs. P30	-0.035	0.972
% Swing Hind	normal	Age F(3,44.28)=2.43, p=0.077	P21 vs. P30	2.438	0.017	Age F(3,37.90)=1.072, p=0.37	P21 vs. P30	-1.068	0.290
			P24 vs. P30	1.048	0.299	Body Length F(1,51.43)=12.52, p=0.0009	P24 vs. P30	-1.470	0.146
			P27 vs. P30	-0.001	0.999		P27 vs. P30	-1.707	0.093
Absolute Paw Angle Fore	normal	Age F(3,51.45)=5.02, p=0.004	P21 vs. P30	-2.404	0.019	Age F(3,43.11)=5.21, p=0.004	P21 vs. P30	-2.876	0.006
			P24 vs. P30	0.467	0.642	Body Length F(1,42.18)=2.83, p=0.10	P24 vs. P30	-0.680	0.499
			P27 vs. P30	-2.551	0.014		P27 vs. P30	-2.950	0.005
Absolute Paw Angle Hind	normal	Age F(3,50.47)=2.45, p=0.07	P21 vs. P30	1.957	0.054	Age F(3,46)=1.12, p=0.35	P21 vs. P30	-0.365	0.716
			P24 vs. P30	0.540	0.591	Body Length F(1,49.4)=5.59, p=0.022	P24 vs. P30	-0.974	0.333
			P27 vs. P30	-0.899	0.373		P27 vs. P30	-1.689	0.097
Brake Duration Fore	normal	Age F(3,48.73)=2.96, p=0.04	P21 vs. P30	-0.003	0.998	Age F(3,47.34)=4.77, p=0.005	P21 vs. P30	2.293	0.025
			P24 vs. P30	-1.624	0.109	Body Length F(1,65.08)=8.55, p=0.005	P24 vs. P30	0.606	0.546
			P27 vs. P30	0.963	0.340		P27 vs. P30	2.277	0.026
Brake Duration Hind	normal	Age F(3,50.84)=15.62, p=2.5E-07	P21 vs. P30	-6.631	0.000	Age F(3,42.82)=7.13, p=0.0005	P21 vs. P30	-3.817	0.0003
			P24 vs. P30	-1.527	0.131	Body Length F(1,49.21)=0.56, p=0.460	P24 vs. P30	-0.795	0.429
			P27 vs. P30	-1.989	0.053		P27 vs. P30	-1.622	0.110
Gait Symmetry	normal	Age F(3,60.82)=0.78, p=0.510	P21 vs. P30	1.093	0.278	Age F(3,50.57)=0.40, p=0.76	P21 vs. P30	0.244	0.808
			P24 vs. P30	-0.167	0.868	Body Length F(1,45.56)=0.52, p=0.476	P24 vs. P30	-0.541	0.590
			P27 vs. P30	-0.421	0.676		P27 vs. P30	-0.606	0.547
Max. Rate Contact Change Fore	normal	Age F(3,49.46)=2.45, p=0.075	P21 vs. P30	-1.417	0.161	Age F(3,46.24)=2.03, p=0.12	P21 vs. P30	1.068	0.290
			P24 vs. P30	-0.310	0.757	Body Length F(1,56.51)=7.02, p=0.010	P24 vs. P30	1.355	0.180
			P27 vs. P30	1.491	0.142		P27 vs. P30	2.451	0.017
Max. Rate Contact Change Hind	normal	Age F(3,49.07)=1.82, p=0.156	P21 vs. P30	0.826	0.412	Age F(3,49.14)=4.94, p=0.004	P21 vs. P30	3.549	0.001
			P24 vs. P30	-0.498	0.620	Body Length F(1,43.91)=16.22, p=0.0002	P24 vs. P30	1.716	0.091
			P27 vs. P30	1.623	0.111		P27 vs. P30	2.843	0.006
Paw Overlap Distance	P30 nonnormal	Age F(3,41.29)=1.69, p=0.183	P21 vs. P30	-0.233	0.816	Age F(3,36.41)=4.92, p=0.006	P21 vs. P30	2.850	0.006
			P24 vs. P30	1.546	0.127	Body Length F(1,42.38)=16.53, p=0.0002	P24 vs. P30	3.771	0.0004
			P27 vs. P30	1.509	0.139		P27 vs. P30	2.716	0.009
Paw Angle CV Fore	P21, P24, P27, P30 nonnormal	Age F(3,45.88)=3.87, p=0.015	P21 vs. P30	2.952	0.004	Age F(3,41.67)=5.14, p=0.004	P21 vs. P30	3.595	0.001
			P24 vs. P30	0.519	0.606	Body Length F(1,49.66)=4.66, p=0.036	P24 vs. P30	1.778	0.080
			P27 vs. P30	1.784	0.081		P27 vs. P30	2.476	0.016
Paw Angle CV Hind	normal	Age F(3,60.39)=1.93, p=0.134	P21 vs. P30	1.775	0.080	Age F(3,53.27)=1.25, p=0.302	P21 vs. P30	1.487	0.142
			P24 vs. P30	1.931	0.058	Body Length F(1,45.36)=0.11, p=0.739	P24 vs. P30	1.806	0.076
			P27 vs. P30	0.604	0.549		P27 vs. P30	0.671	0.505
Paw Placement Positioning	normal	Age F(3,47.39)=0.74, p=0.54	P21 vs. P30	-1.418	0.160	Age F(3,46.53)=0.363, p=0.780	P21 vs. P30	0.865	0.390
			P24 vs. P30	-0.797	0.429	Body Length F(1,63.03)=5.20, p=0.026	P24 vs. P30	0.857	0.394
			P27 vs. P30	-0.924	0.360		P27 vs. P30	0.237	0.813
Peak Paw Area CV Fore	normal	Age F(3,58.55)=3.63, p=0.018	P21 vs. P30	2.617	0.011	Age F(3,43.12)=2.01, p=0.126	P21 vs. P30	1.635	0.108
			P24 vs. P30	0.327	0.745	Body Length F(1,40.57)=0.05, p=0.832	P24 vs. P30	0.152	0.880
			P27 vs. P30	-0.646	0.522		P27 vs. P30	-0.670	0.506
Peak Paw Area CV Hind	normal	Age F(3,48.55)=9.13, p=6.7E-05	P21 vs. P30	4.959	0.000005	Age F(3,40.34)=4.26, p=0.011	P21 vs. P30	3.362	0.001
			P24 vs. P30	2.454	0.017	Body Length F(1,46.70)=0.02, p=0.901	P24 vs. P30	2.052	0.044
			P27 vs. P30	0.698	0.489		P27 vs. P30	0.699	0.487
Peak Paw Area Fore	normal	Age F(3,47.67)=1.27, p=0.30	P21 vs. P30	-0.710	0.480	Age F(3,43.62)=2.26, p=0.095	P21 vs. P30	2.045	0.045
			P24 vs. P30	-0.452	0.653	Body Length F(1,50.62)=11.38, p=0.001	P24 vs. P30	1.573	0.121
			P27 vs. P30	1.186	0.241		P27 vs. P30	2.398	0.020
Peak Paw Area Hind	normal	Age F(3,45.74)=1.51, p=0.22	P21 vs. P30	-0.066	0.947	Age F(3,45.79)=4.68, p=0.006	P21 vs. P30	3.457	0.001
			P24 vs. P30	-0.769	0.445	Body Length F(1,41.81)=23.05, p=2.04E-05	P24 vs. P30	1.901	0.062
			P27 vs. P30	1.284	0.206		P27 vs. P30	2.868	0.006
Propulsion Duration Fore	normal	Age F(3,49.42)=17.50, p=7.4E-08	P21 vs. P30	-6.312	2.2E-08	Age F(3,47.26)=7.94, p=0.0002	P21 vs. P30	-2.757	0.008
			P24 vs. P30	-0.910	0.366	Body Length F(1,63.77)=2.56, p=0.114	P24 vs. P30	0.328	0.744
			P27 vs. P30	-1.005	0.319		P27 vs. P30	-0.174	0.863
Propulsion Duration Hind	normal	Age F(3,47.93)=2.26, p=0.09	P21 vs. P30	0.809	0.421	Age F(3,47.30)=8.14, p=0.0002	P21 vs. P30	4.740	0.00001
			P24 vs. P30	-1.244	0.218	Body Length F(1,45.13)=30.25, p=1.7E-06	P24 vs. P30	1.970	0.053
			P27 vs. P30	0.859	0.395		P27 vs. P30	2.622	0.011

Stance Duration Fore	normal	Age F(3,46.76)=16.38, p=2.1E-07	P21 vs. P30	-6.450	1.2E-08	Age F(3,41.75)=3.73, p=0.018	P21 vs. P30	-1.211	0.231
			P24 vs. P30	-2.263	0.027	Body Length F(1,53.19)=21.31, p=2.5E-05	P24 vs. P30	0.787	0.434
			P27 vs. P30	-0.177	0.860		P27 vs. P30	1.647	0.105
Stance Duration Hind	normal	Age F(3,46.45)=8.60, p=0.0001	P21 vs. P30	-4.818	8.1E-06	Age F(3,47.55)=3.44, p=0.024	P21 vs. P30	2.969	0.004
			P24 vs. P30	-3.139	0.003	Body Length F(1,45.45)=107.42, p=1.5E-13	P24 vs. P30	2.220	0.030
			P27 vs. P30	-0.879	0.384		P27 vs. P30	2.554	0.014
Stance Factor Fore	normal	Age F(3,50.18)=0.33, p=0.801	P21 vs. P30	-0.010	0.992	Age F(3,45.10)=0.43, p=0.734	P21 vs. P30	-0.700	0.487
			P24 vs. P30	0.158	0.875	Body Length F(1,52.82)=0.89, p=0.350	P24 vs. P30	-0.413	0.681
			P27 vs. P30	-0.763	0.449		P27 vs. P30	-1.030	0.307
Stance Factor Hind	normal	Age F(3,55.90)=2.53, p=0.07	P21 vs. P30	1.379	0.172	Age F(3,48.01)=2.58, p=0.065	P21 vs. P30	1.159	0.251
			P24 vs. P30	-0.773	0.442	Body Length F(1,52.26)=0.120, p=0.742	P24 vs. P30	-0.438	0.663
			P27 vs. P30	1.607	0.114		P27 vs. P30	1.631	0.108
Stance Width CV Fore	normal	Age F(3,51.98)=0.83, p=0.48	P21 vs. P30	0.801	0.426	Age F(3,47.64)=0.87, p=0.462	P21 vs. P30	0.961	0.340
			P24 vs. P30	1.167	0.247	Body Length F(1,55.14)=0.330, p=0.567	P24 vs. P30	1.281	0.205
			P27 vs. P30	-0.231	0.819		P27 vs. P30	-0.003	0.997
Stance Width CV Hind	P27 nonnormal	Age F(3,59.17)=3.69, p=0.017	P21 vs. P30	0.572	0.569	Age F(3,52.89)=3.35, p=0.026	P21 vs. P30	-0.728	0.469
			P24 vs. P30	3.192	0.002	Body Length F(1,54.95)=2.19, p=0.145	P24 vs. P30	1.795	0.077
			P27 vs. P30	1.490	0.142		P27 vs. P30	0.931	0.355
Stance Width Fore	normal	Age F(3,44.84)=3.06, p=0.038	P21 vs. P30	1.519	0.133	Age F(3,41.53)=3.57, p=0.022	P21 vs. P30	1.841	0.070
			P24 vs. P30	-1.209	0.231	Body Length F(1,61.57)=1.27, p=0.263	P24 vs. P30	-0.201	0.842
			P27 vs. P30	-0.784	0.437		P27 vs. P30	-0.180	0.858
Stance Width Hind	normal	Age F(3,46.90)=6.04, p=0.001	P21 vs. P30	-0.310	0.758	Age F(3,44.49)=8.58, p=0.0001	P21 vs. P30	2.598	0.012
			P24 vs. P30	-3.635	0.001	Body Length F(1,61.09)=13.01, p=0.0006	P24 vs. P30	-0.732	0.467
			P27 vs. P30	-1.992	0.052		P27 vs. P30	-0.328	0.744
Step Angle CV Fore	normal	Age F(3,52.06)=2.91, p=0.043	P21 vs. P30	2.641	0.010	Age F(3,48.07)=0.327, p=0.806	P21 vs. P30	0.497	0.621
			P24 vs. P30	2.424	0.018	Body Length F(1,55.37)=2.74, p=0.104	P24 vs. P30	0.954	0.343
			P27 vs. P30	1.168	0.248		P27 vs. P30	0.470	0.640
Step Angle CV Hind	normal	Age F(3,54.33)=0.15, p=0.93	P21 vs. P30	0.401	0.690	Age F(3,41.97)=0.105, p=0.956	P21 vs. P30	0.319	0.751
			P24 vs. P30	0.452	0.653	Body Length F(1,44.06)=0.004, p=0.949	P24 vs. P30	0.407	0.686
			P27 vs. P30	-0.100	0.920		P27 vs. P30	-0.076	0.939
Step Angle Fore	normal	Age F(3,52.34)=3.34, p=0.026	P21 vs. P30	-2.952	0.004	Age F(3,48.04)=0.993, p=0.404	P21 vs. P30	-0.672	0.504
			P24 vs. P30	-1.526	0.132	Body Length F(1,55.61)=2.89, p=0.095	P24 vs. P30	-0.228	0.820
			P27 vs. P30	-2.197	0.033		P27 vs. P30	-1.464	0.148
Step Angle Hind	normal	Age F(3,56.09)=1.14, p=0.34	P21 vs. P30	-1.602	0.114	Age F(3,47.17)=0.87, p=0.464	P21 vs. P30	-1.148	0.255
			P24 vs. P30	0.110	0.912	Body Length F(1,50.23)=0.011, p=0.916	P24 vs. P30	0.030	0.976
			P27 vs. P30	-0.590	0.558		P27 vs. P30	-0.593	0.556
Stride Frequency Fore	normal	Age F(3,49.42)=7.26, p=0.0004	P21 vs. P30	4.360	4.4E-05	Age F(3,45.34)=1.004, p=0.400	P21 vs. P30	-1.476	0.145
			P24 vs. P30	2.847	0.006	Body Length F(1,50.29)=46.51, p=1.1E-08	P24 vs. P30	-1.075	0.286
			P27 vs. P30	0.771	0.444		P27 vs. P30	-1.517	0.135
Stride Frequency Hind	normal	Age F(3,50.22)=6.32, p=0.001	P21 vs. P30	4.033	0.0001	Age F(3,45.78)=0.997, p=0.403	P21 vs. P30	-1.661	0.102
			P24 vs. P30	2.785	0.007	Body Length F(1,45.90)=47.43, p=1.4E-08	P24 vs. P30	-0.956	0.343
			P27 vs. P30	0.862	0.393		P27 vs. P30	-1.179	0.244
Stride Length CV Fore	normal	Age F(3,57.24)=1.74, p=0.169	P21 vs. P30	1.650	0.103	Age F(3,48.07)=1.40, p=0.254	P21 vs. P30	1.534	0.130
			P24 vs. P30	2.013	0.048	Body Length F(1,49.80)=0.33, p=0.570	P24 vs. P30	1.981	0.052
			P27 vs. P30	0.637	0.527		P27 vs. P30	0.789	0.434
Stride Length CV Hind	normal	Age F(3,45.95)=1.64, p=0.194	P21 vs. P30	-0.774	0.441	Age F(3,42.26)=3.13, p=0.035	P21 vs. P30	1.830	0.072
			P24 vs. P30	1.337	0.186	Body Length F(1,54.39)=9.34, p=0.003	P24 vs. P30	3.004	0.004
			P27 vs. P30	0.816	0.419		P27 vs. P30	1.985	0.052
Stride Length Fore	normal	Age F(3,49.58)=8.02, p=0.0002	P21 vs. P30	-4.458	0.00003	Age F(3,44.89)=1.16, p=0.334	P21 vs. P30	1.408	0.164
			P24 vs. P30	-2.963	0.004	Body Length F(1,48.47)=48.38, p=8.2E-09	P24 vs. P30	0.950	0.346
			P27 vs. P30	-0.558	0.580		P27 vs. P30	1.717	0.092
Stride Length Hind	normal	Age F(3,50.57)=6.66, p=0.0007	P21 vs. P30	-4.090	0.0001	Age F(3,46.64)=0.994, p=0.404	P21 vs. P30	1.604	0.114
			P24 vs. P30	-3.040	0.003	Body Length F(1,46.46)=47.17, p=1.46E-08	P24 vs. P30	0.690	0.493
			P27 vs. P30	-0.948	0.348		P27 vs. P30	1.085	0.283
Swing Duration CV Fore	normal	Age F(3,61.32)=1.28, p=0.29	P21 vs. P30	0.527	0.600	Age F(3,49.61)=1.09, p=0.361	P21 vs. P30	0.027	0.978
			P24 vs. P30	1.837	0.070	Body Length F(1,46.37)=0.212, p=0.648	P24 vs. P30	1.271	0.208
			P27 vs. P30	1.316	0.195		P27 vs. P30	1.133	0.262
Swing Duration CV Hind	P30 nonnormal	Age F(3,45.40)=0.17, p=0.91	P21 vs. P30	-0.497	0.621	Age F(3,43.70)=0.48, p=0.700	P21 vs. P30	1.024	0.310
			P24 vs. P30	-0.469	0.640	Body Length F(1,56.28)=3.05, p=0.086	P24 vs. P30	0.704	0.484
			P27 vs. P30	-0.680	0.500		P27 vs. P30	0.062	0.951
Swing Duration Fore	normal	Age F(3,44.57)=2.24, p=0.10	P21 vs. P30	-0.377	0.707	Age F(3,42.33)=5.46, p=0.003	P21 vs. P30	2.967	0.004
			P24 vs. P30	-2.271	0.027	Body Length F(1,66.75)=17.00, p=0.0001	P24 vs. P30	0.832	0.408
			P27 vs. P30	-1.001	0.322		P27 vs. P30	1.018	0.313
Swing Duration Hind	normal	Age F(3,47.86)=0.80, p=0.50	P21 vs. P30	-1.095	0.277	Age F(3,38.42)=0.265, p=0.850	P21 vs. P30	0.003	0.998
			P24 vs. P30	-1.469	0.146	Body Length F(1,48.81)=0.92, p=0.342	P24 vs. P30	-0.581	0.563
			P27 vs. P30	-0.952	0.347		P27 vs. P30	-0.506	0.615

Supplementary Table 2. Statistical output for FVB gait data.

OUTCOME	NORMAL	LMM	estimates	*t*	sig.	LMM (adjusted for body length)	estimates	*t*	sig.
%Hindlimb Shared Stance	normal	Age F(3,48.51)=7.58, p=0.0003	P21 vs. P30	-4.421	0.00003	Age F(3,48.981)=7.219 p=.0004	P21 vs. P30	-4.152	0.00009
			P24 vs. P30	-2.772	0.007	Body Length F(1,75.261)=1.732, p=.192	P24 vs. P30	-3.070	0.003
			P27 vs. P30	-0.573	0.569		P27 vs. P30	-1.191	0.238
% Stance Fore	normal	Age F(3,52.07)=2.03, p=0.121	P21 vs. P30	-1.728	0.088	Age F(3,51.440)=1.352 p=.267	P21 vs. P30	0.508	0.613
			P24 vs. P30	0.335	0.739	Body Length F(1,64.023)=7.224, p=.009	P24 vs. P30	1.708	0.092
			P27 vs. P30	-0.473	0.638		P27 vs. P30	0.751	0.456
% Stance Hind	normal	Age F(3,50.01)=9.93, p=3.04E-05	P21 vs. P30	-4.571	0.00002	Age F(3,50.437)=10.498 p=.00002	P21 vs. P30	-4.520	0.00002
			P24 vs. P30	-2.698	0.009	Body Length F(1,74.647)=2.737, p=.102	P24 vs. P30	-3.212	0.002
			P27 vs. P30	0.357	0.722		P27 vs. P30	-0.565	0.574
% Swing Fore	normal	Age F(3,52.07)=2.03, p=0.121	P21 vs. P30	1.728	0.088	Age F(3,51.440)=1.352 p=.267	P21 vs. P30	-0.508	0.613
			P24 vs. P30	-0.335	0.739	Body Length F(1,64.023)=7.224, p=.009	P24 vs. P30	-1.708	0.092
			P27 vs. P30	0.473	0.638		P27 vs. P30	-0.751	0.456
% Swing Hind	normal	Age F(3,50.0)=9.93, p=3.0E-05	P21 vs. P30	4.571	0.00002	Age F(3,50.437)=10.498 p=.00002	P21 vs. P30	4.520	0.00002
			P24 vs. P30	2.698	0.009	Body Length F(1,74.647)=2.737, p=.102	P24 vs. P30	3.212	0.002
			P27 vs. P30	-0.357	0.722		P27 vs. P30	0.565	0.574
Absolute Paw Angle Fore	P21 nonnormal	Age F(3,42.23)=4.18, p=0.011	P21 vs. P30	2.760	0.007	Age F(3,42.074)=2.751 p=.054	P21 vs. P30	1.627	0.108
		Friedman's Test:	P24 vs. P30	-0.214	0.831	Body Length F(1,56.325)=0.343, p=.560	P24 vs. P30	-0.489	0.626
		$\chi2(3,N=19)=5.286$, p=.152	P27 vs. P30	0.672	0.506		P27 vs. P30	0.351	0.727
Absolute Paw Angle Hind	normal	Age F(3,52.72)=0.67, p=0.58	P21 vs. P30	1.288	0.202	Age F(3,53.529)=1.557 p=.211	P21 vs. P30	2.070	0.042
			P24 vs. P30	0.941	0.350	Body Length F(167.102)=2.662, p=.107	P24 vs. P30	1.677	0.098
			P27 vs. P30	1.116	0.270		P27 vs. P30	1.757	0.084
Brake Duration Fore	normal	Age F(3,52.29)=0.89, p=0.45	P21 vs. P30	-1.266	0.209	Age F(3,52.673)=0.496 p=.687	P21 vs. P30	-0.016	0.987
		Friedman's Test:	P24 vs. P30	-0.472	0.638	Body Length F(1,74.065)=1.745, p=.191	P24 vs. P30	0.321	0.749
		$\chi2(3,N=19)=6.206$, p=.102	P27 vs. P30	0.321	0.749		P27 vs. P30	0.934	0.354
Brake Duration Hind	normal	Age F(3,52.26)=4.56, p=0.007	P21 vs. P30	-3.386	0.001	Age F(3,52.177)=2.801 p=.049	P21 vs. P30	-2.404	0.019
			P24 vs. P30	-0.831	0.408	Body Length F(1,65.993)=0.009, p=.924	P24 vs. P30	-0.656	0.514
			P27 vs. P30	-1.177	0.245		P27 vs. P30	-1.010	0.316
Gait Symmetry	P21, P24, P27, nonnormal	Age F(3,58.11)=0.577, p=0.632	P21 vs. P30	0.862	0.391	Age F(3,58.383)=0.381 p=.767	P21 vs. P30	-0.275	0.784
		Friedman's Test:	P24 vs. P30	0.862	0.391	Body Length F(1,59.600)=1.976, p=.165	P24 vs. P30	0.058	0.954
		$\chi2(3,N=19)=2.692$, p=.442	P27 vs. P30	1.277	0.208		P27 vs. P30	0.657	0.513
Max. Rate Contact Change Fore	normal	Age F(3,48.74)=8.45, p=0.0001	P21 vs. P30	-2.513	0.014	Age F(3,48.024)=8.273 p=.0002	P21 vs. P30	-2.462	0.016
			P24 vs. P30	-3.877	0.0002	Body Length F(1,73.296)=0.823, p=.367	P24 vs. P30	-3.760	0.0003
			P27 vs. P30	-4.896	0.00001		P27 vs. P30	-4.723	0.00002
Max. Rate Contact Change Hind	normal	Age F(3,47.67)=10.09, p=3.0E-05	P21 vs. P30	-5.121	2.25E-06	Age F(3,48.107)=10.984 p=.00001	P21 vs. P30	-5.344	9.47E-07
			P24 vs. P30	-5.002	4.03E-06	Body Length F(1,72.083)=4.890, p=.030	P24 vs. P30	-5.591	4.21E-07
			P27 vs. P30	-3.606	0.0007		P27 vs. P30	-4.489	0.00004
Paw Angle CV Fore	P21, P24, P30 nonnormal	Age F(3,41.66)=2.56, p=0.07	P21 vs. P30	-2.317	0.023	Age F(3,42.031)=2.046 p=.122	P21 vs. P30	-1.953	0.055
		Friedman's Test:	P24 vs. P30	-0.976	0.332	Body Length F(1,57.606)=0.142, p=.707	P24 vs. P30	-1.030	0.306
		$\chi2(3,N=19)=7.926$, p=.048	P27 vs. P30	-2.116	0.041		P27 vs. P30	-2.068	0.044
Paw Angle CV Hind	P24, P30 nonnormal	Age F(3,51.84)=3.67, p=0.018	P21 vs. P30	2.833	0.006	Age F(3,52.626)=1.543 p=.214	P21 vs. P30	0.724	0.472
		Friedman's Test:	P24 vs. P30	1.179	0.242	Body Length F(1,69.880)=4.084, p=.047	P24 vs. P30	-0.063	0.950
		$\chi2(3,N=19)=5.716$, p=.126	P27 vs. P30	-0.217	0.829		P27 vs. P30	-1.142	0.258
Paw Overlap Distance	normal	Age F(3,55.98)=33.05, p=2.0E-12	P21 vs. P30	-9.557	1.16E-14	Age F(3,54.359)=12.790 p=1.9E-06	P21 vs. P30	-5.079	2.68E-06
			P24 vs. P30	-5.105	2.69E-06	Body Length F(1,73.109)=13.448, p=.0005	P24 vs. P30	-2.534	0.013
			P27 vs. P30	-3.383	0.001		P27 vs. P30	-1.141	0.258
Paw Placement Positioning	normal	Age F(3,52.59)=0.87, p=0.463	P21 vs. P30	-1.062	0.291	Age F(3,52.296)=0.416 p=.742	P21 vs. P30	0.018	0.986
			P24 vs. P30	-1.219	0.227	Body Length F(1,59.145)=1.449, p=.233	P24 vs. P30	-0.440	0.661
			P27 vs. P30	0.036	0.971		P27 vs. P30	0.529	0.599
Peak Paw Area CV Fore	normal	Age F(3,52.12)=6.06, p=0.001	P21 vs. P30	4.040	0.0001	Age F(3,49.542)=1.128 p=.347	P21 vs. P30	1.030	0.306
			P24 vs. P30	1.635	0.106	Body Length F(1,68.060)=9.369, p=.003	P24 vs. P30	-0.194	0.847
			P27 vs. P30	1.436	0.157		P27 vs. P30	-0.143	0.887
Peak Paw Area CV Hind	normal	Age F(3,55.29)=31.80, p=4.4E-12	P21 vs. P30	9.044	1.33E-13	Age F(3,54.239)=15.958 p=1.5E-07	P21 vs. P30	5.344	1.01E-06
			P24 vs. P30	4.547	0.00002	Body Length F(1,69.664)=4.445, p=.039	P24 vs. P30	2.806	0.006
			P27 vs. P30	0.909	0.367		P27 vs. P30	-0.168	0.867
Peak Paw Area Fore	normal	Age F(3,48.81)=8.80, p=0.00009	P21 vs. P30	-1.251	0.215	Age F(3,47.398)=9.342 p=.00006	P21 vs. P30	-1.540	0.128
			P24 vs. P30	-3.034	0.003	Body Length F(1,69.947)=0.824, p=.367	P24 vs. P30	-3.056	0.003
			P27 vs. P30	-4.880	0.00001		P27 vs. P30	-4.698	0.00002
Peak Paw Area Hind	normal	Age F(3,48.69)=5.51, p=0.002	P21 vs. P30	-3.558	0.001	Age F(3,48.118)=6.291 p=.001	P21 vs. P30	-3.862	0.0002
			P24 vs. P30	-3.668	0.0005	Body Length F(1,73.124)=3.208, p=.077	P24 vs. P30	-4.149	0.00009
			P27 vs. P30	-3.220	0.002		P27 vs. P30	-3.846	0.0003
Propulsion Duration Fore	normal	Age F(3,51.83)=5.31, p=0.003	P21 vs. P30	-1.631	0.107	Age F(3,46.904)=3.710 p=.017	P21 vs. P30	1.299	0.198
			P24 vs. P30	-1.146	0.256	Body Length F(1,73.391)=14.104, p=.0003	P24 vs. P30	1.042	0.301
			P27 vs. P30	-3.622	0.001		P27 vs. P30	-1.315	0.194
Propulsion Duration Hind	normal	Age F(3,54.22)=5.86, p=0.002	P21 vs. P30	-3.821	0.0003	Age F(3,50.093)=0.853 p=.471	P21 vs. P30	-1.176	0.243
			P24 vs. P30	-3.356	0.001	Body Length F(1,74.708)=6.166, p=.015	P24 vs. P30	-1.428	0.158
			P27 vs. P30	-3.398	0.001		P27 vs. P30	-1.538	0.129
Stance Duration Fore	normal	Age F(3,54.86)=5.25, p=0.003	P21 vs. P30	-2.925	0.005	Age F(3,50.573)=4.218 p=.010	P21 vs. P30	1.233	0.221
			P24 vs. P30	-1.807	0.075	Body Length F(1,74.488)=29.772, p=6.1E-07	P24 vs. P30	1.330	0.188
			P27 vs. P30	-4.243	0.00009		P27 vs. P30	-1.173	0.246

Stance Duration Hind	normal	Age F(3,53.20)=0.96, p=0.417	P21 vs. P30	-6.315	1.69E-08	Age F(3,49.505)=2.862 p=.046	P21 vs. P30	-2.644	0.010
			P24 vs. P30	-4.397	0.00004	Body Length F(1,74.508)=10.501,	P24 vs. P30	-1.947	0.055
			P27 vs. P30	-4.857	0.00001	p=.002	P27 vs. P30	-2.420	0.019
Stance Factor Fore	normal	Age F(3,53.72)=8.14, p=0.0001	P21 vs. P30	1.796	0.077	Age F(3,54.845)=4.700 p=.005	P21 vs. P30	0.868	0.389
			P24 vs. P30	-1.315	0.192	Body Length F(1,65.043)=0.438, p=.511	P24 vs. P30	-1.471	0.146
			P27 vs. P30	-1.792	0.079		P27 vs. P30	-1.908	0.061
Stance Factor Hind	normal	Age F(3,54.13)=15.43, p=2.3E-07	P21 vs. P30	1.472	0.145	Age F(3,53.181)=1.486 p=.229	P21 vs. P30	1.925	0.058
			P24 vs. P30	0.304	0.762	Body Length F(1,70.251)=1.516, p=.222	P24 vs. P30	0.920	0.360
			P27 vs. P30	0.148	0.883		P27 vs. P30	0.710	0.481
Stance Width CV Fore	normal	Age F(3,54.47)=0.21, p=0.888	P21 vs. P30	-0.608	0.545	Age F(3,56.373)=0.875 p=.460	P21 vs. P30	-1.463	0.148
			P24 vs. P30	-0.708	0.481	Body Length F(1,51.300)=2.402, p=.127	P24 vs. P30	-1.376	0.173
			P27 vs. P30	-0.314	0.755		P27 vs. P30	-0.829	0.411
Stance Width CV Hind	normal	Age F(3,52.76)=1.34, p=0.271	P21 vs. P30	1.632	0.107	Age F(3,52.867)=0.354 p=.786	P21 vs. P30	0.012	0.990
			P24 vs. P30	0.879	0.382	Body Length F(1,67.868)=3.085, p=.084	P24 vs. P30	-0.177	0.860
			P27 vs. P30	1.624	0.111		P27 vs. P30	0.641	0.524
Stance Width Fore	normal	Age F(3,51.90)=0.231, p=0.874	P21 vs. P30	0.730	0.468	Age F(3,47.735)=.186 p=.906	P21 vs. P30	-0.453	0.652
			P24 vs. P30	0.401	0.689	Body Length F(1,75.985)=2.070, p=.154	P24 vs. P30	-0.465	0.643
			P27 vs. P30	0.000	1.000		P27 vs. P30	-0.739	0.463
Stance Width Hind	normal	Age F(3,54.81)=0.990, p=0.405	P21 vs. P30	-0.637	0.526	Age F(3,55.454)=1.147 p=.338	P21 vs. P30	-0.940	0.350
			P24 vs. P30	-1.498	0.138	Body Length F(1,75.475)=.480, p=.491	P24 vs. P30	-1.635	0.106
			P27 vs. P30	-0.329	0.744		P27 vs. P30	-0.632	0.529
Step Angle CV Fore	normal	Age F(3,51.71)=0.21, p=0.890	P21 vs. P30	-0.717	0.476	Age F(3,52.059)=1.150 p=.338	P21 vs. P30	-1.826	0.072
			P24 vs. P30	-0.448	0.656	Body Length F(1,63.293)=3.590, p=.063	P24 vs. P30	-1.380	0.172
			P27 vs. P30	-0.082	0.935		P27 vs. P30	-0.901	0.371
Step Angle CV Hind	normal	Age F(3,52.19)=2.60, p=0.06	P21 vs. P30	1.221	0.226	Age F(3,51.063)=2.550 p=.066	P21 vs. P30	-0.360	0.720
			P24 vs. P30	1.442	0.154	Body Length F(1,75.767)=3.385, p=.070	P24 vs. P30	0.202	0.841
			P27 vs. P30	-0.954	0.345		P27 vs. P30	-1.757	0.084
Step Angle Fore	normal	Age F(3,51.25)=0.18, p=0.912	P21 vs. P30	-0.667	0.507	Age F(3,52.446)=0.159 p=.923	P21 vs. P30	0.511	0.611
			P24 vs. P30	-0.114	0.910	Body Length F(1,56.512)=2.310, p=.134	P24 vs. P30	0.666	0.507
			P27 vs. P30	-0.323	0.748		P27 vs. P30	0.305	0.761
Step Angle Hind	normal	Age F(3,53.08)=0.15, p=0.929	P21 vs. P30	-0.263	0.793	Age F(3,58.741)=0.316 p=.814	P21 vs. P30	0.767	0.446
			P24 vs. P30	-0.556	0.580	Body Length F(1,51.207)=2.250, p=.140	P24 vs. P30	0.234	0.815
			P27 vs. P30	-0.578	0.566		P27 vs. P30	0.005	0.996
Stride Frequency Fore	normal	Age F(3,54.42)=7.07, p=0.0004	P21 vs. P30	2.439	0.017	Age F(3,52.105)=4.336 p=.008	P21 vs. P30	-1.392	0.168
			P24 vs. P30	1.707	0.092	Body Length F(1,75.998)=24.818,	P24 vs. P30	-1.174	0.244
			P27 vs. P30	4.168	0.0001	p=3.6E-06	P27 vs. P30	1.324	0.190
Stride Frequency Hind	normal	Age F(3,54.09)=7.02, p=0.0005	P21 vs. P30	2.330	0.022	Age F(3,50.760)=4.136 p=.010	P21 vs. P30	-1.379	0.172
			P24 vs. P30	1.541	0.128	Body Length F(1,75.608)=23.107,	P24 vs. P30	-1.268	0.209
			P27 vs. P30	4.061	0.0002	p=.000008	P27 vs. P30	1.196	0.236
Stride Length CV Fore	normal	Age F(3,57.11)=1.73, p=0.170	P21 vs. P30	-1.677	0.098	Age F(3,56.683)=1.671 p=.183	P21 vs. P30	-1.681	0.097
			P24 vs. P30	-1.605	0.113	Body Length F(1,61.257)=0.419, p=.520	P24 vs. P30	-1.716	0.090
			P27 vs. P30	-0.030	0.976		P27 vs. P30	-0.289	0.773
Stride Length CV Hind	normal	Age F(3,54.92)=0.478, p=0.700	P21 vs. P30	-0.434	0.665	Age F(3,54.683)=0.788 p=.506	P21 vs. P30	-1.097	0.276
			P24 vs. P30	0.640	0.524	Body Length F(1,61.420)=1.317, p=.256	P24 vs. P30	-0.033	0.973
			P27 vs. P30	-0.218	0.828		P27 vs. P30	-0.677	0.501
Stride Length Fore	normal	Age F(3,53.60)=7.32, p=0.0003	P21 vs. P30	-2.521	0.014	Age F(3,50.170)=4.024 p=.012	P21 vs. P30	1.176	0.243
			P24 vs. P30	-2.098	0.040	Body Length F(1,75.959)=22.304,	P24 vs. P30	0.708	0.481
			P27 vs. P30	-4.411	0.00005	p=.00001	P27 vs. P30	-1.570	0.122
Stride Length Hind	normal	Age F(3,53.58)=8.19, p=0.0001	P21 vs. P30	-2.696	0.009	Age F(3,49.715)=4.110 p=.011	P21 vs. P30	0.908	0.367
			P24 vs. P30	-2.102	0.039	Body Length F(1,75.693)=20.333,	P24 vs. P30	0.618	0.538
			P27 vs. P30	-4.582	0.00003	p=.00002	P27 vs. P30	-1.752	0.085
Swing Duration CV Fore	normal	Age F(3,51.78)=1.36, p=0.266	P21 vs. P30	-1.708	0.092	Age F(3,51.110)=1.902 p=.141	P21 vs. P30	-2.117	0.038
			P24 vs. P30	-0.654	0.515	Body Length F(1,58.880)=1.552, p=.218	P24 vs. P30	-1.205	0.232
			P27 vs. P30	0.137	0.891		P27 vs. P30	-0.395	0.695
Swing Duration CV Hind	normal	Age F(3,50.61)=2.77, p=0.051	P21 vs. P30	2.590	0.012	Age F(3,50.603)=1.306 p=.283	P21 vs. P30	1.496	0.139
			P24 vs. P30	1.166	0.247	Body Length F(1,58.395)=0.389, p=.535	P24 vs. P30	0.683	0.497
			P27 vs. P30	0.140	0.889		P27 vs. P30	-0.132	0.895
Swing Duration Fore	normal	Age F(3,52.81)=3.36, p=0.026	P21 vs. P30	-1.332	0.187	Age F(3,51.581)=2.728 p=.053	P21 vs. P30	1.247	0.216
			P24 vs. P30	-1.779	0.079	Body Length F(1,69.908)=11.086,	P24 vs. P30	0.213	0.832
			P27 vs. P30	-3.169	0.003	p=.001	P27 vs. P30	-1.248	0.217
Swing Duration Hind	normal	Age F(3,50.31)=5.15, p=0.003	P21 vs. P30	1.740	0.086	Age F(3,48.861)=8.474 p=.0001	P21 vs. P30	3.616	0.001
			P24 vs. P30	0.786	0.435	Body Length F(1,75.690)=10.861,	P24 vs. P30	2.532	0.014
			P27 vs. P30	-2.118	0.039	p=.002	P27 vs. P30	-0.183	0.856

Supplementary Table 3. Descriptive statistics for C57 and FVB gait data.

Outcome	Age	C57 Unadjusted		C57 Adjusted		FVB Unadjusted		FVB Adjusted	
		Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
% Hindlimb Shared Stance	P21	39.599	7.930	43.680	9.643	43.434	8.596	42.158	9.890
	P24	43.111	6.970	44.521	6.620	47.618	8.596	47.295	8.707
	P27	43.222	6.564	42.559	6.113	52.308	8.596	52.460	8.639
	P30	43.814	6.335	40.508	7.224	53.242	8.596	54.690	10.226
% Stance Fore	P21	57.831	4.257	56.801	5.695	59.492	1.686	60.022	1.880
	P24	61.785	3.766	61.461	3.937	60.466	1.686	60.600	1.619
	P27	61.600	3.548	61.787	3.599	60.113	1.686	60.050	1.604
	P30	60.992	3.452	61.759	4.433	60.311	1.686	59.709	1.952
% Stance Hind	P21	62.893	3.078	64.582	3.734	64.842	3.320	64.237	3.782
	P24	64.041	2.714	64.601	2.556	66.632	3.320	66.478	3.343
	P27	64.724	2.556	64.467	2.356	68.921	3.320	68.993	3.318
	P30	64.723	2.478	63.406	2.807	68.703	3.320	69.389	3.907
% Swing Fore	P21	42.169	4.257	43.199	5.695	40.508	1.686	39.978	1.880
	P24	38.215	3.766	38.539	3.937	39.534	1.686	39.400	1.619
	P27	38.400	3.548	38.213	3.599	39.887	1.686	39.950	1.604
	P30	39.008	3.452	38.241	4.433	39.689	1.686	40.291	1.952
% Swing Hind	P21	37.107	3.078	35.418	3.734	35.158	3.320	35.763	3.782
	P24	35.959	2.714	35.399	2.556	33.368	3.320	33.522	3.343
	P27	35.276	2.556	35.533	2.356	31.079	3.320	31.007	3.318
	P30	35.277	2.478	36.594	2.807	31.297	3.320	30.611	3.907
Absolute Paw Angle Fore	P21	5.685	4.549	4.536	5.791	4.795	2.778	4.595	3.253
	P24	8.966	3.978	8.481	4.078	2.466	2.778	2.415	2.804
	P27	5.897	3.762	6.119	3.790	3.113	2.778	3.137	2.778
	P30	8.468	3.580	9.347	4.292	2.632	2.778	2.858	3.378
Absolute Paw Angle Hind	P21	24.467	4.069	22.994	5.135	4.526	1.851	4.895	2.146
	P24	22.955	3.560	22.439	3.573	4.337	1.851	4.430	1.847
	P27	21.659	3.364	21.969	3.315	4.374	1.851	4.330	1.830
	P30	22.442	3.207	23.540	3.816	3.855	1.851	3.437	2.229
Brake Duration Fore	P21	0.049	0.011	0.055	0.014	0.055	0.010	0.056	0.011
	P24	0.046	0.010	0.047	0.010	0.057	0.010	0.057	0.010
	P27	0.051	0.009	0.050	0.009	0.059	0.010	0.058	0.010
	P30	0.049	0.009	0.046	0.011	0.058	0.010	0.056	0.012
Brake Duration Hind	P21	0.023	0.013	0.025	0.017	0.022	0.006	0.022	0.007
	P24	0.040	0.011	0.040	0.012	0.026	0.006	0.026	0.006
	P27	0.039	0.010	0.038	0.011	0.026	0.006	0.026	0.006
	P30	0.044	0.010	0.043	0.012	0.028	0.006	0.027	0.007
Gait Symmetry	P21	1.009	0.029	1.006	0.036	1.002	0.022	0.999	0.025
	P24	1.000	0.025	0.999	0.026	1.002	0.022	1.001	0.022
	P27	0.998	0.024	0.999	0.024	1.005	0.022	1.005	0.021
	P30	1.001	0.022	1.003	0.027	0.997	0.022	1.001	0.026

Maximal Rate of Paw Contact Change Fore	P21	31.023	9.421	35.140	11.930	43.887	9.579	42.929	11.112
	P24	33.726	8.260	34.911	8.176	41.475	9.579	41.232	9.870
	P27	37.128	7.786	36.300	7.542	41.463	9.579	41.577	9.799
	P30	34.384	7.476	31.385	8.953	49.956	9.579	51.043	11.466
Maximal Rate of Paw Contact Change Hind	P21	90.209	22.890	103.906	26.867	88.823	16.074	84.895	19.036
	P24	82.738	20.023	86.646	19.128	91.074	16.074	90.078	16.988
	P27	93.411	18.926	91.488	17.792	99.080	16.074	99.548	16.871
	P30	85.400	18.033	76.536	19.898	110.125	16.074	114.581	19.622
Paw Overlap Distance Hind	P21	2.115	1.146	2.816	1.351	1.071	0.384	1.209	0.405
	P24	2.587	1.004	2.811	0.937	1.536	0.384	1.571	0.361
	P27	2.529	0.947	2.344	0.868	1.742	0.384	1.726	0.359
	P30	2.183	0.907	1.691	1.006	1.963	0.384	1.807	0.417
Paw Angle CV Fore	P21	673.906	665.245	904.115	851.238	136.512	295.803	122.773	347.138
	P24	255.219	582.385	338.322	586.882	249.486	295.803	246.002	299.119
	P27	420.303	549.753	373.145	542.982	169.963	295.803	171.600	296.327
	P30	175.676	525.441	6.461	635.558	329.741	295.803	345.327	360.590
Paw Angle CV Hind	P21	26.287	10.288	26.816	13.028	211.679	116.804	183.218	133.856
	P24	26.247	9.002	26.417	9.339	156.490	116.804	149.272	115.227
	P27	23.143	8.504	23.022	8.689	113.253	116.804	116.643	114.143
	P30	21.627	8.112	21.274	9.650	119.275	116.804	151.564	139.072
Paw Placement Positioning Hind	P21	0.468	0.144	0.525	0.186	0.300	0.082	0.311	0.094
	P24	0.494	0.126	0.512	0.127	0.296	0.082	0.299	0.081
	P27	0.495	0.119	0.485	0.116	0.325	0.082	0.324	0.081
	P30	0.519	0.115	0.478	0.142	0.324	0.082	0.311	0.098
Peak Paw Area CV Fore	P21	11.587	3.953	11.446	5.089	10.077	2.839	9.086	3.075
	P24	9.257	3.457	9.219	3.598	8.138	2.839	7.887	2.646
	P27	8.355	3.269	8.396	3.345	7.831	2.839	7.949	2.621
	P30	8.954	3.111	9.049	3.770	6.926	2.839	8.050	3.194
Peak Paw Area CV Hind	P21	8.639	2.331	8.691	3.079	19.677	4.264	18.607	4.822
	P24	7.038	2.039	7.052	2.131	14.145	4.264	13.873	4.150
	P27	6.054	1.928	6.045	1.974	9.860	4.264	9.988	4.111
	P30	5.697	1.835	5.664	2.294	8.960	4.264	10.174	5.010
Peak Paw Area Fore	P21	0.458	0.135	0.530	0.165	0.688	0.104	0.678	0.120
	P24	0.468	0.118	0.488	0.114	0.651	0.104	0.649	0.108
	P27	0.514	0.111	0.500	0.106	0.634	0.104	0.635	0.107
	P30	0.482	0.107	0.431	0.123	0.720	0.104	0.731	0.124
Peak Paw Area Hind	P21	1.053	0.230	1.213	0.261	1.071	0.185	1.034	0.218
	P24	1.016	0.201	1.062	0.186	1.081	0.185	1.071	0.194
	P27	1.118	0.190	1.098	0.173	1.128	0.185	1.133	0.193
	P30	1.057	0.182	0.953	0.194	1.241	0.185	1.283	0.225

Propulsion Duration Fore	P21	0.085	0.014	0.089	0.018	0.089	0.015	0.095	0.015
	P24	0.104	0.012	0.105	0.012	0.092	0.015	0.093	0.013
	P27	0.104	0.011	0.103	0.011	0.085	0.015	0.085	0.013
	P30	0.106	0.011	0.103	0.014	0.096	0.015	0.089	0.016
Propulsion Duration Hind	P21	0.125	0.020	0.140	0.022	0.135	0.014	0.139	0.015
	P24	0.115	0.017	0.120	0.015	0.138	0.014	0.139	0.013
	P27	0.124	0.016	0.121	0.014	0.140	0.014	0.140	0.013
	P30	0.121	0.016	0.110	0.016	0.148	0.014	0.144	0.015
Stance Factor Fore	P21	1.000	0.086	0.987	0.113	1.018	0.060	1.014	0.071
	P24	1.004	0.075	1.000	0.078	0.966	0.060	0.965	0.061
	P27	0.987	0.071	0.990	0.072	0.960	0.060	0.961	0.060
	P30	1.001	0.068	1.010	0.085	0.988	0.060	0.993	0.073
Stance Factor Hind	P21	1.033	0.062	1.037	0.082	1.021	0.045	1.027	0.052
	P24	1.000	0.055	1.001	0.057	1.006	0.045	1.008	0.045
	P27	1.034	0.052	1.033	0.053	1.004	0.045	1.003	0.045
	P30	1.011	0.049	1.009	0.061	1.002	0.045	0.994	0.054
Stance Duration Fore	P21	0.134	0.013	0.144	0.015	0.144	0.014	0.151	0.013
	P24	0.149	0.011	0.152	0.010	0.148	0.014	0.150	0.011
	P27	0.155	0.011	0.153	0.010	0.144	0.014	0.143	0.011
	P30	0.156	0.010	0.149	0.011	0.154	0.014	0.146	0.013
Stance Duration Hind	P21	0.148	0.015	0.164	0.012	0.157	0.013	0.161	0.013
	P24	0.155	0.013	0.160	0.009	0.164	0.013	0.165	0.011
	P27	0.163	0.012	0.160	0.008	0.166	0.013	0.165	0.011
	P30	0.165	0.012	0.154	0.009	0.175	0.013	0.171	0.013
Stance Width CV Fore	P21	19.171	9.484	20.129	12.580	14.014	3.791	13.371	4.287
	P24	19.819	8.298	20.111	8.661	13.911	3.791	13.748	3.789
	P27	16.772	7.841	16.573	8.008	14.313	3.791	14.389	3.761
	P30	17.239	7.475	16.580	9.402	14.666	3.791	15.395	4.428
Stance Width CV Hind	P21	6.767	2.686	6.092	3.460	7.534	2.186	7.072	2.498
	P24	8.392	2.349	8.192	2.413	7.058	2.186	6.941	2.150
	P27	7.274	2.221	7.413	2.239	7.382	2.186	7.437	2.130
	P30	6.377	2.113	6.825	2.570	6.538	2.186	7.062	2.596
Stance Width Fore	P21	2.120	0.263	2.174	0.353	1.458	0.170	1.431	0.187
	P24	1.950	0.231	1.966	0.241	1.442	0.170	1.435	0.163
	P27	1.981	0.217	1.972	0.221	1.426	0.170	1.430	0.162
	P30	2.020	0.209	1.981	0.269	1.426	0.170	1.457	0.194
Stance Width Hind	P21	3.073	0.263	3.230	0.325	2.037	0.154	2.024	0.178
	P24	2.881	0.231	2.929	0.222	2.005	0.154	2.002	0.155
	P27	2.994	0.217	2.964	0.203	2.053	0.154	2.054	0.153
	P30	3.093	0.209	2.981	0.247	2.063	0.154	2.078	0.185
Step Angle CV Fore	P21	35.756	12.442	32.232	16.187	17.879	6.945	16.293	7.992
	P24	34.398	10.889	33.257	11.147	18.415	6.945	18.012	6.890
	P27	30.452	10.283	31.186	10.308	19.137	6.945	19.326	6.826
	P30	27.408	9.817	29.872	12.096	19.285	6.945	21.085	8.301

Step Angle CV Hind	P21	34.271	11.444	34.406	14.936	25.094	7.546	23.511	8.428
	P24	34.320	10.007	34.358	10.437	25.308	7.546	24.907	7.329
	P27	32.841	9.466	32.811	9.690	21.260	7.546	21.449	7.265
	P30	33.102	9.004	33.021	11.088	22.675	7.546	24.471	8.738
Step Angle Fore	P21	49.302	11.285	52.597	14.717	61.505	9.316	63.163	10.672
	P24	53.759	9.873	54.765	10.128	62.963	9.316	63.384	9.277
	P27	52.474	9.329	51.787	9.363	62.432	9.316	62.234	9.196
	P30	57.773	8.894	55.500	11.002	63.263	9.316	61.382	11.065
Step Angle Hind	P21	42.038	11.684	41.826	15.312	53.026	10.122	54.715	11.500
	P24	47.103	10.218	47.043	10.663	52.189	10.122	52.618	10.129
	P27	45.249	9.664	45.295	9.894	52.079	10.122	51.878	10.050
	P30	46.800	9.194	46.943	11.379	53.779	10.122	51.863	11.889
Stride Frequency Fore	P21	4.394	0.355	4.069	0.362	4.242	0.364	4.068	0.348
	P24	4.235	0.311	4.140	0.251	4.161	0.364	4.116	0.304
	P27	4.058	0.293	4.128	0.233	4.289	0.364	4.310	0.301
	P30	4.001	0.280	4.226	0.269	4.021	0.364	4.219	0.360
Stride Frequency Hind	P21	4.363	0.346	4.050	0.349	4.234	0.345	4.080	0.326
	P24	4.233	0.303	4.144	0.245	4.155	0.345	4.116	0.286
	P27	4.072	0.286	4.141	0.228	4.274	0.345	4.292	0.284
	P30	4.008	0.273	4.218	0.259	4.042	0.345	4.218	0.337
Stride Length CV Fore	P21	18.025	5.681	18.566	7.396	13.991	3.606	13.711	4.195
	P24	18.330	4.968	18.497	5.169	14.065	3.606	13.994	3.640
	P27	16.462	4.699	16.346	4.799	15.671	3.606	15.705	3.608
	P30	15.639	4.470	15.273	5.490	15.701	3.606	16.018	4.351
Stride Length CV Hind	P21	12.543	4.280	14.773	5.400	12.593	3.523	12.107	4.085
	P24	14.708	3.747	15.392	3.697	13.661	3.523	13.538	3.535
	P27	14.110	3.538	13.647	3.408	12.817	3.523	12.875	3.504
	P30	13.385	3.379	11.858	4.057	13.025	3.523	13.576	4.240
Stride Length Fore	P21	4.658	0.387	5.012	0.390	4.839	0.420	5.029	0.400
	P24	4.830	0.339	4.933	0.272	4.905	0.420	4.953	0.350
	P27	5.050	0.320	4.973	0.252	4.782	0.420	4.759	0.347
	P30	5.096	0.305	4.852	0.290	5.097	0.420	4.882	0.414
Stride Length Hind	P21	4.706	0.381	5.050	0.385	4.853	0.401	5.024	0.382
	P24	4.833	0.334	4.929	0.271	4.932	0.401	4.975	0.336
	P27	5.026	0.315	4.949	0.252	4.808	0.401	4.788	0.333
	P30	5.103	0.301	4.871	0.286	5.111	0.401	4.917	0.396
Swing Duration CV Fore	P21	24.963	8.376	24.328	10.744	18.839	4.734	18.133	5.463
	P24	27.449	7.326	27.244	7.614	20.257	4.734	20.078	4.726
	P27	26.440	6.927	26.593	7.080	21.294	4.734	21.378	4.684
	P30	23.839	6.593	24.245	7.958	21.123	4.734	21.924	5.670

Swing Duration CV Hind	P21	19.867	6.699	21.937	8.809	21.423	5.306	21.023	6.184
	P24	19.988	5.866	20.623	6.025	19.278	5.306	19.177	5.351
	P27	19.781	5.536	19.365	5.550	17.737	5.306	17.785	5.302
	P30	20.710	5.295	19.275	6.628	17.538	5.306	17.992	6.418
Swing Duration Fore	P21	0.098	0.015	0.109	0.018	0.098	0.008	0.101	0.009
	P24	0.093	0.013	0.096	0.013	0.097	0.008	0.098	0.008
	P27	0.097	0.012	0.095	0.012	0.095	0.008	0.095	0.008
	P30	0.100	0.012	0.093	0.014	0.101	0.008	0.097	0.009
Swing Duration Hind	P21	0.088	0.010	0.089	0.013	0.086	0.013	0.090	0.014
	P24	0.087	0.009	0.087	0.009	0.082	0.013	0.084	0.012
	P27	0.088	0.008	0.088	0.008	0.075	0.013	0.074	0.012
	P30	0.090	0.008	0.089	0.010	0.080	0.013	0.075	0.014

4. Supplementary Discussion

Strain Comparisons: While different strains of mice often show differential performance in in many behavioral assays (Eisener-Dorman et al., 2011; Keum et al., 2016; Liu et al., 2011; Martin et al., 2014; S. S. Moy et al., 2004; Sheryl S. Moy et al., 2008), our study was not designed to directly compare the two strains tested so our ability interpret any differences is limited. The differences observed may arise from size discrepancies, as FVB mice are consistently larger, or experimental parameters, such as different sample sizes. It is also possible that motor development in the FVB mouse occurs earlier than in the C57 mouse, as suggested by the fact that FVB mice were able to run successfully on the DigiGait at a younger age (P17) than C57 mice (P21, pilot data not shown). Future direct comparison studies are needed to validate strain differences suggested by the present study. In any case, our results provide a reference dataset of gait maturation for two commonly used mouse strains, often employed as the genetic backgrounds for models of disease.

Body Length: We also recognized that the rapid growth in body size across the age range examined could confound our gait data, and indeed we found that more than a third of all metrics were significantly influenced by body length. Decreased stride frequency, or cadence, and increased stride length have been suggested as markers of gait maturity in humans, however they are mainly driven by limb lengthening and thus likely do not reflect underlying motor circuit maturation - a more relevant phenotype for brain disorders like NDD. In both strains examined, we initially observed a comparable pattern of decreased stride frequency and increased stride length. However, body length significantly impacted both of these variables, and controlling for this influence revealed that neither stride frequency nor length changed with age from P21 to P30. Thus, we presented the data both before and after controlling for the impact of body size on each gait parameter to highlight the possibility of erroneous interpretations when body size is not considered, and to help define those features that could reflect true differences in CNS circuits rather than simply changes in limb length.

5. Supplementary References

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