

**Supplementary information**

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**A machine-vision-based frailty index for mice**

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In the format provided by the authors and unedited

Table S1: Testing batches

Batch	Males	Females	Total Mice	Repeated Mice
Batch 1	141	81	222	NA
Batch 2	173	146	319	105
Batch 3	57	45	102	33
Total	371	272	643	

Table S2: Video Features

Video Metrics			
Category	Name	Description	Units
Open Field	distance_cm	Sum of locomotor activity.	cm
Open Field	center_time_secs	Sum of time spent in center.	sec
Open Field	periphery_time_secs	Sum of time spent along any wall.	sec
Open Field	corner_time_secs	Sum of time spent in any corner.	sec
Open Field	center_distance_cm	Average distance from center across the video.	cm
Open Field	periphery_distance_cm	Average distance from nearest periphery across the video.	cm
Open Field	corner_distance_cm	Average distance from nearest corners across the video.	cm
Open Field	grooming_number_bouts	Sum of all grooming bouts in video.	~
Open Field	grooming_duration_secs	Average length of grooming bouts.	sec
Gait	angular_velocity	The first derivative of angle of a mouse, determined by the vector connecting the mouse's base of tail to its base of neck	deg/second
Gait	lateral_displacement	The difference between the minimum and maximum values of a reference point's perpendicular distance from the mouse's displacement vector for a stride for each frame of a stride, normalized by the mouse's body length. The reference points used are nose, base of tail, and tail tip.	~
Gait	limb_duty_factor	The amount of time that the paw is in contact with the ground divided by the full stride time, calculated and averaged for each hind paw.	~
Gait	speed_cm_per_sec	Speed is determined by the base of tail point.	cm/second
Gait	step_length	The distance that the right hind paw travels past the previous opposite paw strike. Step_length 1 uses left hind paw strike, while step_length2 uses right hind paw strike.	cm

Video Features			
Category	Name	Description	Units
Gait	step_width	The length of the shortest line segment that connects the right hind paw strike to the line that connects the left hind paw's toe-off location to its subsequent foot strike position.	cm
Gait	stride_length	The full distance that the left hind paw travels for a stride, from toe-off to foot-strike.	cm
Gait	temporal_symmetry	The difference in time between the left and right hindpaw strike, divided by the total strike time.	~
Gait	stride_count	Sum of all recorded strides in video.	strides
Gait	distance_cm_sc	Sum of locomotor activity, normalized by time spent in open field.	cm/second
Engineered	dAC	Distance between base of head and base of tail, normalized by the max dAC recorded.	cm
Engineered	dB	The distance between the mid-back point and the midpoint of the line AC.	cm
Engineered	aABC	The angle between the base of head point, mid-back point, and base of tail point.	degrees
Engineered	width	Width of the ellipse fit for the mouse calculated for all frames.	cm
Engineered	length	Length of the ellipse fit for the mouse calculated for all frames.	cm
Engineered	rearpaw	The distance between rearpaws calculated for all frames.	cm
Engineered	rear_count	Sum of rearing bouts in video.	~
Engineered	avg_rear_len	Average length of rearing bouts in video.	sec
End of Table			

Table S3: vFI Feature Correlation (Pearson) with FI score. P-value calculated using Wald Test with t-distribution of the test statistic.

vFI feature correlation with FI score.						
Features	All Mice		Males		Females	
	Cor	Pvalue	Cor	Pvalue	Cor	Pvalue
tip_tail_lateral_displacement_iqr	0.629	0.000	0.582	0.000	0.659	0.000
median_step_width	0.561	0.000	0.513	0.000	0.559	0.000
median_width	0.556	0.000	0.561	0.000	0.497	0.000
step_length2_iqr	0.551	0.000	0.595	0.000	0.421	0.000
base_tail_lateral_displacement_iqr	0.547	0.000	0.498	0.000	0.614	0.000
median_rearpaw	0.545	0.000	0.493	0.000	0.552	0.000
step_length1_iqr	0.544	0.000	0.584	0.000	0.418	0.000
step_width_iqr	0.504	0.000	0.474	0.000	0.507	0.000
median_length	0.494	0.000	0.386	0.000	0.552	0.000
stride_length_iqr	0.481	0.000	0.483	0.000	0.425	0.000
avg_bout_len	-0.467	0.000	-0.456	0.000	-0.448	0.000
temporal_symmetry_iqr	0.458	0.000	0.485	0.000	0.350	0.000
dB_median	0.455	0.000	0.382	0.000	0.496	0.000
median_speed_cm_per_sec	-0.442	0.000	-0.493	0.000	-0.335	0.000
dB_nongait_median	0.429	0.000	0.328	0.000	0.561	0.000
dAC_nongait_median	0.402	0.000	0.320	0.000	0.440	0.000
median_stride_length	-0.395	0.000	-0.480	0.000	-0.189	0.002
rears_0_5	-0.390	0.000	-0.362	0.000	-0.391	0.000
dAC_stdev	-0.317	0.000	-0.341	0.000	-0.225	0.000
limb_duty_factor_iqr	0.314	0.000	0.346	0.000	0.184	0.002
dAC_min	0.290	0.000	0.323	0.000	0.232	0.000
median_tip_tail_lateral_displacement	0.275	0.000	0.126	0.012	0.558	0.000
rear_count	-0.274	0.000	-0.348	0.000	-0.155	0.010
speed_cm_per_sec_iqr	-0.247	0.000	-0.325	0.000	-0.165	0.006
rears_5_10	-0.237	0.000	-0.259	0.000	-0.169	0.005
aABC_nongait_stdev	-0.227	0.000	-0.361	0.000	-0.063	0.297
corner_distance_cm	-0.224	0.000	-0.263	0.000	-0.118	0.049
aABC_stdev	-0.222	0.000	-0.340	0.000	-0.097	0.108
aABC_min	0.221	0.000	0.257	0.000	0.176	0.003
median_step_length1	-0.216	0.000	-0.293	0.000	-0.014	0.823
angular_velocity_iqr	0.209	0.000	0.175	0.000	0.348	0.000
grooming_number_bouts	-0.190	0.000	-0.217	0.000	-0.094	0.119
dB_nongait_min	0.186	0.000	0.191	0.000	0.214	0.000
periphery_distance_cm	-0.178	0.000	-0.246	0.000	-0.039	0.521
median_step_length2	-0.178	0.000	-0.256	0.000	-0.016	0.794
distance_cm	-0.161	0.000	-0.229	0.000	-0.027	0.655
median_temporal_symmetry	0.160	0.000	0.174	0.001	0.138	0.024
grooming_duration_secs	-0.151	0.000	-0.111	0.029	-0.185	0.002
median_base_tail_lateral_displacement	0.149	0.000	-0.003	0.950	0.511	0.000
aABC_median	-0.148	0.000	-0.093	0.065	-0.149	0.013
periphery_time_secs	-0.115	0.003	-0.093	0.067	-0.140	0.020
Distance cm/sc	-0.105	0.006	-0.182	0.000	0.068	0.259
dB_nongait_max	-0.102	0.009	-0.095	0.061	-0.121	0.046
dAC_nongait_stdev	-0.096	0.014	-0.144	0.005	-0.042	0.490

vFI Feature correlation with FI score (Table continued)						
Features	All Mice		Males		Females	
	Cor	Pvalue	Cor	Pvalue	Cor	Pvalue
nose_lateral_displacement_iqr	-0.095	0.013	-0.164	0.001	0.121	0.045
corner_time_secs	-0.087	0.024	-0.013	0.803	-0.164	0.006
center_distance_cm	-0.074	0.057	-0.119	0.018	0.006	0.921
dAC_median	0.071	0.066	0.047	0.352	0.131	0.030
dB_max	0.064	0.100	0.030	0.557	0.063	0.297
aABC_nongait_median	-0.059	0.128	-0.007	0.888	-0.120	0.046
center_time_secs	0.043	0.266	0.046	0.364	0.011	0.855
dB_stdev	0.041	0.285	0.006	0.910	0.057	0.343
stride_count	-0.034	0.378	-0.023	0.649	0.052	0.392
dB_nongait_stdev	-0.031	0.432	-0.080	0.117	0.030	0.615
median_angular_velocity	0.025	0.514	-0.012	0.807	0.108	0.073
median_limb_duty_factor	0.007	0.853	-0.033	0.517	0.092	0.127
median_nose_lateral_displacement	-0.006	0.885	-0.034	0.501	0.160	0.008
End of Table						

Table S4: vFI feature correlation (Pearson) with age. P-value calculated using Wald Test with t-distribution of the test statistic.

vFI Feature Correlation with Age						
Features	All Mice		Males		Females	
	Cor	Pvalue	Cor	Pvalue	Cor	Pvalue
tip_tail_lateral_displacement_iqr	0.760	0.000	0.728	0.000	0.795	0.000
median_step_width	0.662	0.000	0.609	0.000	0.695	0.000
median_width	0.648	0.000	0.646	0.000	0.628	0.000
step_length2_iqr	0.638	0.000	0.686	0.000	0.503	0.000
step_length1_iqr	0.637	0.000	0.691	0.000	0.494	0.000
base_tail_lateral_displacement_iqr	0.637	0.000	0.595	0.000	0.707	0.000
median_rearpaw	0.636	0.000	0.579	0.000	0.668	0.000
step_width_iqr	0.609	0.000	0.583	0.000	0.611	0.000
stride_length_iqr	0.564	0.000	0.575	0.000	0.502	0.000
avg_bout_len	-0.559	0.000	-0.559	0.000	-0.530	0.000
median_length	0.540	0.000	0.447	0.000	0.605	0.000
temporal_symmetry_iqr	0.537	0.000	0.550	0.000	0.450	0.000
dB_nongait_median	0.500	0.000	0.418	0.000	0.620	0.000
dB_median	0.491	0.000	0.402	0.000	0.570	0.000
median_speed_cm_per_sec	-0.482	0.000	-0.508	0.000	-0.401	0.000
dAC_nongait_median	0.421	0.000	0.339	0.000	0.470	0.000
rears_0_5	-0.420	0.000	-0.380	0.000	-0.442	0.000
median_stride_length	-0.411	0.000	-0.499	0.000	-0.170	0.005
dAC_stdev	-0.399	0.000	-0.420	0.000	-0.302	0.000
dAC_min	0.381	0.000	0.390	0.000	0.359	0.000
median_tip_tail_lateral_displacement	0.351	0.000	0.191	0.000	0.698	0.000
limb_duty_factor_iqr	0.334	0.000	0.379	0.000	0.171	0.004
aABC_min	0.303	0.000	0.328	0.000	0.271	0.000
aABC_nongait_stdev	-0.295	0.000	-0.369	0.000	-0.204	0.001
aABC_stdev	-0.291	0.000	-0.357	0.000	-0.231	0.000
rear_count	-0.274	0.000	-0.313	0.000	-0.192	0.001
speed_cm_per_sec_iqr	-0.272	0.000	-0.319	0.000	-0.218	0.000
rears_5_10	-0.260	0.000	-0.248	0.000	-0.240	0.000
grooming_number_bouts	-0.244	0.000	-0.241	0.000	-0.184	0.002
median_base_tail_lateral_displacement	0.226	0.000	0.078	0.120	0.647	0.000
median_step_length1	-0.215	0.000	-0.288	0.000	0.010	0.868
grooming_duration_secs	-0.209	0.000	-0.172	0.001	-0.247	0.000
median_temporal_symmetry	0.207	0.000	0.228	0.000	0.174	0.004
angular_velocity_iqr	0.207	0.000	0.175	0.000	0.352	0.000
corner_distance_cm	-0.199	0.000	-0.170	0.001	-0.175	0.004
dB_nongait_min	0.179	0.000	0.231	0.000	0.149	0.013
nose_lateral_displacement_iqr	-0.161	0.000	-0.215	0.000	0.052	0.393
distance_cm	-0.155	0.000	-0.184	0.000	-0.062	0.305
dAC_nongait_stdev	-0.152	0.000	-0.200	0.000	-0.093	0.125
periphery_distance_cm	-0.152	0.000	-0.168	0.001	-0.071	0.239
median_step_length2	-0.135	0.000	-0.212	0.000	0.055	0.359
aABC_median	-0.130	0.001	-0.090	0.074	-0.108	0.074
center_distance_cm	-0.114	0.003	-0.159	0.002	-0.025	0.683
Distance cm/sc	-0.092	0.017	-0.147	0.003	0.071	0.237

vFI feature correlation with age (Table continued)						
Features	All Mice		Males		Females	
	Cor	Pvalue	Cor	Pvalue	Cor	Pvalue
dB_stdev	0.077	0.048	0.069	0.171	0.031	0.611
dB_nongait_max	-0.076	0.050	-0.083	0.103	-0.077	0.205
corner_time_secs	-0.075	0.052	0.021	0.685	-0.196	0.001
periphery_time_secs	-0.074	0.056	-0.018	0.716	-0.148	0.014
dB_nongait_stdev	-0.071	0.067	-0.123	0.015	0.002	0.979
dB_max	0.070	0.070	0.058	0.247	0.015	0.805
dAC_median	0.062	0.108	0.017	0.736	0.170	0.005
median_nose_lateral_displacement	-0.046	0.233	-0.077	0.126	0.139	0.021
median_angular_velocity	0.037	0.341	0.008	0.872	0.111	0.065
aABC_nongait_median	-0.034	0.378	-0.003	0.949	-0.074	0.223
stride_count	-0.024	0.537	-0.013	0.800	0.072	0.231
median_limb_duty_factor	-0.022	0.571	-0.042	0.406	0.041	0.500
center_time_secs	-0.002	0.958	-0.033	0.512	0.012	0.846
End of Table						

Table S5: Manual FI item correlation (Pearson) with age. P-value calculated using Wald Test with t-distribution of the test statistic.

Features	All Mice		Males		Females	
	Correlation	p-value	Correlation	p-value	Correlation	p-value
Piloerection	0.721	0.000	0.641	0.000	0.797	0.000
Kyphosis	0.703	0.000	0.660	0.000	0.725	0.000
Gait.disorders	0.674	0.000	0.631	0.000	0.694	0.000
Coat.condition	0.661	0.000	0.675	0.000	0.597	0.000
Body.condition	0.551	0.000	0.519	0.000	0.569	0.000
Distended.abdomen	0.548	0.000	0.523	0.000	0.527	0.000
Loss.of.fur.colour	0.444	0.000	0.442	0.000	0.326	0.000
Tumours	0.319	0.000	0.327	0.000	0.239	0.000
Vestibular.disturbance	0.317	0.000	0.297	0.000	0.310	0.000
Breathing.rate.depth	0.307	0.000	0.290	0.000	0.319	0.000
Righting.Reflex	0.287	0.000	0.254	0.000	0.347	0.000
Tremor	0.280	0.000	0.212	0.000	0.381	0.000
Tail.stiffening	0.261	0.000	0.262	0.000	0.230	0.000
Loss.of.whiskers	0.244	0.000	0.276	0.000	0.370	0.000
Vision.loss..Visual.Placing.	0.213	0.000	0.189	0.000	0.334	0.000
Alopecia	0.195	0.000	0.149	0.003	0.438	0.000
Eye.discharge.swelling	0.069	0.072	0.049	0.332	0.103	0.081
Menace.reflex	-0.055	0.156	-0.027	0.600	-0.078	0.188
Corneal.opacity	0.032	0.412	0.020	0.696	0.071	0.231
Cataracts	0.007	0.860	-0.020	0.692	0.055	0.351
Dermatitis	0.007	0.862	0.042	0.410	-0.009	0.875
Malocclusions	-0.007	0.863	0.009	0.860	-0.038	0.521
Microphthalmia	0.002	0.966	0.015	0.764	-0.019	0.745
Nasal.discharge						
Rectal.prolapse						
Vaginal.uterine.						
Diarrhea						



Table S6: Manual Frailty Scoring Rubric (modified from [2])

System/ Parameter	Clinical assessment of deficit	Scoring
Weight	Weigh the mouse at habituation.	Actual body weight (g)
Alopecia	Loss of fur color	0 = normal fur density 0.5 = < 25% fur loss 1 = > 25% fur loss
Alopecia	Inspect for signs of fur loss	0 = normal fur density 0.5 = < 25% fur loss 1 = > 25% fur loss
Loss of fur colour	Note any change in fur colour from black to grey or brown	0 = normal colour 0.5 = focal grey/brown changes 1 = grey/brown fur throughout body
Dermatitis	Document skin lesions	0 = absent 0.5 = focal lesions (e.g. neck, flanks, under chin) 1 = widespread or multifocal lesions
Loss of whiskers	Inspect the animal for signs of a reduction in the number of whiskers	0 = no loss 0.5 = reduced number of whiskers 1 = absence of whiskers
Coat condition	Inspect the animal for signs of poor grooming	0 = smooth, sleek, shiny coat 0.5 = coat is slightly ruffled 1 = unkempt and un-groomed, matted appearance
Piloerection	Observe the animal and look for signs of piloerection, in particular on the back of the neck	0 = no piloerection 0.5 = involves fur at base of neck only 1 = widespread piloerection
Cataracts	Visual inspection of the mouse to detect opacity in the centre of the eye	0 = no cataract 0.5 = small opaque spot 1 = clear evidence of opaque lens
Cataracts	Visual inspection of the mouse to detect opacity in the centre of the eye	0 = no cataract 0.5 = small opaque spot 1 = clear evidence of opaque lens
Eye discharge/swelling	Visual inspection of the mouse to detect ocular discharge and swelling of the eyes.	0 = normal 0.5 = slight swelling and/or secretions 1 = obvious bulging and/or secretions
Microphthalmia	Inspect eyes	0 = normal size 0.5 = one or both eyes slightly small or sunken 1 = one or both eyes very small or sunken
Corneal opacity	Visual inspection of the mouse to superficial white spots and/or clouding of the cornea	0 = normal 0.5 = minimal changes in corneal 1 = marked clouding and/or spotting of cornea
Nasal discharge	Visual inspection of the mouse to detect nasal discharge	0 = no discharge 0.5 = small amount of discharge 1 = obvious discharge, both nares

System/ Parameter	Clinical assessment of deficit	Scoring
Rectal prolapse	Grasp the mouse by the base of the tail to detect signs of rectal prolapse	0 = no prolapse 0.5 = small amount of rectum visible below tail 1 = rectum clearly visible below tail
Penile prolapse	Grasp the mouse by the base of the tail to detect signs of vaginal/uterine or penile prolapse	0 = no prolapsed 0.5 = small amount of prolapsed tissue visible 1 = prolapsed tissue clearly visible
Diarrhoea	Grasp the mouse and invert it to check for signs of diarrhoea. Also look for fecal smearing in home cage	0 = none 0.5 = some feces or bedding near rectum 1 = rectum clearly visible below tail
Body condition score	Place mouse on flat surface, hold tail base and manually assess the flesh/fat that covers the sacroiliac region (back and pubic bones)	0 = bones palpable, not prominent 0.5 = bones prominent or barely felt 1 = bones very prominent or not felt due to obesity
Gait disorders	Observe the freely moving animal to detect abnormalities such as hopping, wobbling, circling, wide stance and weakness.	0 = no abnormality 0.5 = abnormal gait but animal can still walk 1 = marked abnormality, impairs ability to move
Menace reflex	Move an object (cotton swab) towards the mouse's face (between the eyes) 3 times. Record whether the mouse blinks in response.	0 = always responds 0.5 = no response to 1 or 2 approaches 1 = no response to 3 approaches
Tremor	Observe the freely moving animal to detect tremor, both at rest and when the animal is trying to climb up an incline	0 = no tremor 0.5 = slight tremor 1 = marked tremor; animal cannot climb
Tumours	Observe the mice to look for symmetry. Hold the base of the tail and manually examine mice for visible or palpable tumours	0 = absent 0.5 = <1.0 cm 1 = >1.0 cm or multiple smaller tumours
Kyphosis	Inspect the mouse for curvature of the spine or hunched posture. Run your fingers down both sides of the spine to detect abnormalities.	0 = absent 0.5 = mild curvature 1 = clear evidence of hunched posture
Breathing rate/depth	Observe the animal. Note the rate and depth of breathing as well as any gasping behaviour	0 = normal 0.5 = modest change in breathing rate and/or depth 1 = marked changes in rate/depth, gasping
Tail stiffening	Grasp the base of the tail with one hand, and stroke the tail with a finger of the other hand. The tail should wrap freely around the finger when mouse is relaxed	0 = no stiffening 0.5 = tail responsive but does not curl 1 = tail completely unresponsive
Malocclusions	Grasp the mouse by the neck scruff, invert and expose teeth. Look for uneven, overgrown teeth	0 = mandibular longer than maxillary incisors 0.5 = teeth slightly uneven 1 = teeth very uneven and overgrown

System/ Parameter	Clinical assessment of deficit	Scoring
Distended abdomen	Hold the mouse vertically by the base of their tail and tip backwards over your hand. Excess fluid visible as a bulge below the rib cage	0 = absent 0.5 = slight budge 1 = abdomen clearly distended
Vestibular disturbance	Hold the base of the tail and lower mouse towards a flat surface. Inspect for head tilt, spinning, circling, head tuck or trunk curling	0 = absent 0.5 = mild head tilt and/or slight spin when lowered 1 = severe disequilibrium
Righting Reflex	The mouse is restrained in the supine position (belly up) above the bench ( 25cm) and the evaluator quickly and briskly lets go and moves hand away from the mouse. Normal response of the animal is to land on its feet	0 = normal 0.5 = lands on side or back but rights itself immediately after 1 = lands on back, fails to right itself
Vision loss (Visual Placing)	Lower mouse towards a grid cage lid surface. Evaluate the position the mouse reaches towards the surface	0 = before vibrassee contact 0.5 = upon vibrassee contact 1 = does not reach (nose in contact with surface)
End of Table		