

Supplementary Table 1. Frailty assessment scoring according to Rizzo et al.⁸⁾

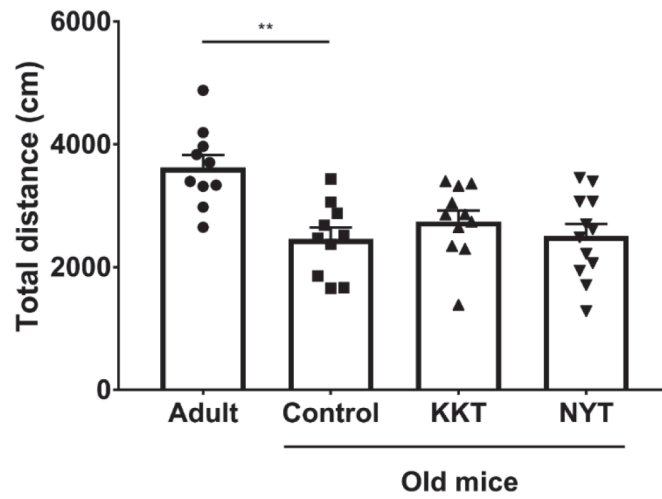
Parameter	Scoring
Alopecia	0 = normal fur density 0.5 = < 25% fur loss 1 = > 25% fur loss
Loss of fur color	0 = normal color 0.5 = focal grey/brown changes 1 = fur color changes throughout body
Coat condition	0 = smooth, sleek, shiny coat 0.5 = coat is slightly ruffled 1 = unkempt and un-groomed, matted appearance
Dermatitis	0 = absent 0.5 = focal lesions 1 = widespread or multifocal lesions
Loss of whiskers	0 = no loss 0.5 = reduced number of whiskers 1 = absence of whiskers
Piloerection	0 = no piloerection 0.5 = involves fur at base of neck only 1 = piloerection
Cataracts	0 = no cataract 0.5 = small opaque spot on one eye 1 = clear evidence of spots on both eyes
Eye discharge/swelling	0 = normal 0.5 = slight swelling and/or secretions 1 = obvious bulging and/or secretions
Microphthalmia	0 = normal size 0.5 = one or both eyes slightly small or sunken 1 = one or both eyes very small or sunken
Corneal opacity	0 = normal 0.5 = minimal clouding on one eye 1 = marked clouding on both eyes
Nasal discharge	0 = no discharge 0.5 = small amount of discharge 1 = obvious discharge, both nares
Distended abdomen	0 = absent 0.5 = slight bulge 1 = abdomen clearly distended
Malocclusions	0 = mandibular longer than maxillary incisors 0.5 = teeth slightly uneven 1 = teeth very uneven and overgrown
Menace reflex	0 = always responds 0.5 = no response to 1 or 2 approaches 1 = no response to 3 approaches
Rectal prolapse	0 = no prolapse 0.5 = small amount of rectum tissue visible below tail 1 = rectum clearly visible below tail
Penile Prolapse	0 = no prolapse 0.5 = small amount of prolapsed tissue visible 1 = prolapsed tissue clearly visible
Diarrhoea	0 = none 0.5 = softened/smeared stool or bedding near rectum 1 = feces plus blood and bedding near rectum
Body condition score	0 = bones palpable, not prominent 0.5 = bones prominent 1 = bones very prominent
Tumours	0 = absent 0.5 = < 1.0 cm 1 = > 1.0 cm or multiple smaller tumours
Kyphosis	0 = absent 0.5 = mild curvature 1 = clear evidence of hunched posture
Gait disorder	0 = no abnormality 0.5 = abnormal gait but animal can still walk 1 = marked abnormality, impairs ability to move
Tremor	0 = no tremor 0.5 = slight tremor 1 = marked tremor; animal cannot rear
Breathing rate/depth	0 = normal 0.5 = modest change in breathing rate and/or depth 1 = marked changes in rate/depth, gasping
Vestibular disturbance	0 = absent 0.5 = mild head tilt and/or slight spin when lowered 1 = severe disequilibrium
Vision loss	0 = before vibrascope contact 0.5 = upon vibrascope contact 1 = does not reach (nose in contact with surface)
Tail stiffening	0 = no stiffening, tail curls around finger 0.5 = tail responsive but does not curl 1 = tail completely unresponsive
Righting Reflex	0 = normal 0.5 = lands on side or back but rights itself immediately 1 = lands on back, fails to right itself

Supplementary Table 2: Total 27 items of Frailty assessment score

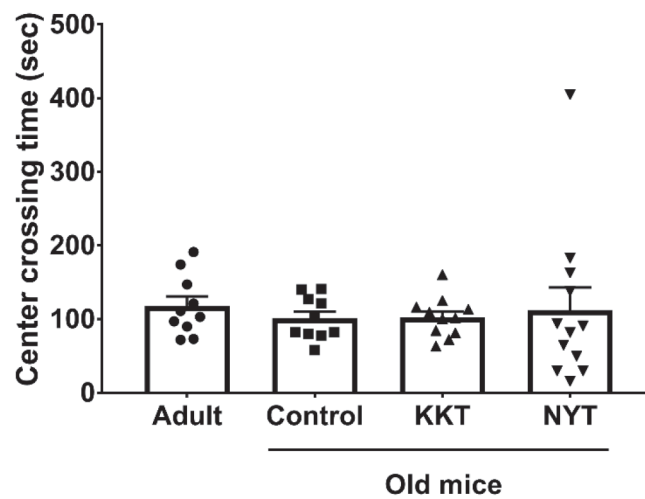
	Frailty assessment score (Average \pm SEM)			
	Adult (N=10)	Old-control (N=10)	Old-KKT (N=11)	Old-NYT (N=12)
Alopecia	0	0.05 \pm 0.05	0	0
Loss of fur color	0	0.6 \pm 0.16	0.23 \pm 0.08	0.17 \pm 0.07
Coat condition	0	0.6 \pm 0.16	0	0
Dermatitis	0.25 \pm 0.13	0	0	0
Loss of whiskers	0.05 \pm 0.05	0.9 \pm 0.07	0.45 \pm 0.11	0.58 \pm 0.14
Piloerection	0	0.2 \pm 0.13	0	0
Cataracts	0	0	0	0
Eye discharge/swelling	0	0.10 \pm 0.07	0.09 \pm 0.06	0.04 \pm 0.04
Microphthalmia	0	0.1 \pm 0.10	0.05 \pm 0.05	0
Corneal opacity	0	0	0	0
Nasal discharge	0	0	0	0
Distended abdomen	0	0	0	0
Malocclusions	0	0	0.14 \pm 0.10	0.13 \pm 0.09
Menace reflex	0	0.10 \pm 0.07	0.09 \pm 0.06	0.17 \pm 0.09
Rectal prolapse	0	0	0	0
Prolapse*	0	0	0	0
Diarrhoea	0	0	0	0
Body condition score	0	0.25 \pm 0.08	0.18 \pm 0.08	0.08 \pm 0.06
Tumours	0	0.10 \pm 0.10	0.05 \pm 0.05	0
Kyphosis	0	0.05 \pm 0.05	0	0
Gait disorder	0	0.05 \pm 0.05	0	0
Tremor	0	0.10 \pm 0.07	0	0.04 \pm 0.04
Breathing rate/depth	0	0	0	0
Vestibular disturbance	0.10 \pm 0.07	0.25 \pm 0.08	0.23 \pm 0.08	0.29 \pm 0.07
Vission loss	0.10 \pm 0.07	0.10 \pm 0.07	0.36 \pm 0.12	0.08 \pm 0.06
Tail stiffening	0.35 \pm 0.13	0.85 \pm 0.08	0.86 \pm 0.07	0.96 \pm 0.04
Righting Reflex	0	0.05 \pm 0.05	0.05 \pm 0.05	0

* Penile Prolapse

(A)



(B)



Supplementary Figure 1. Effect of ninjin'yoeito (NYT) and kamikihito (KKT) on locomotor activity in old mice

An open field test for evaluation of locomotor activity was performed at week 4. The apparatus (Neuroscience Inc., Tokyo, Japan) consisted of a square floor (50 cm × 50 cm) enclosed by walls (25 cm high). Each mouse was placed in the center of the apparatus and monitored with the video tracking system for 7 min. (A) The total distance traveled on the open field and (B) center crossing time were determined by using the analysis software (Act-2, Neuroscience Inc.). N=10-12/group. One-way analysis of variance + Bonferroni's multiple comparison test was used to calculate significance. *: P < 0.05 vs. Old-control.