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Bim contributes to the progression of Huntington's disease-associated phenotypes

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Supplementary Materials

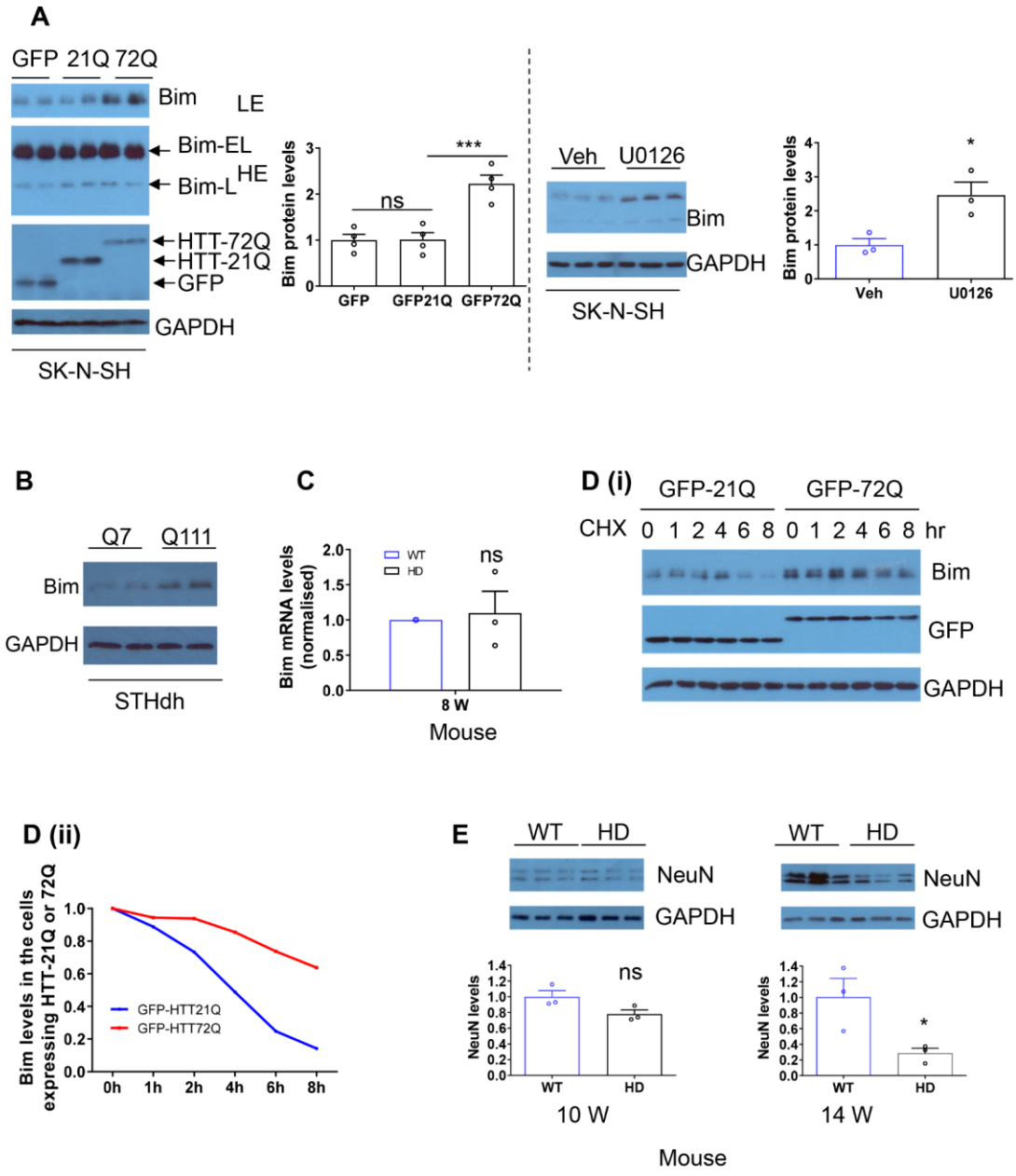

Figure S1. Bim reduction in HD models. (A) Left panels: SK-N-SH cells were transfected with GFP vector, GFP-HTT exon 1-21Q (HTT-21Q) or GFP-HTT exon 1-72Q (HTT-72Q) for 48 hours. Over 80% transfection efficiency was confirmed. The cell lysates were subjected to immunoblot with indicated antibodies. The higher exposure shows that Bim EL and Bim L can be detected in the cells. LE: lower exposure; HE: higher exposure. n=4. **Right panels**: The cell lysates of SK-N-SH cells treated with vehicle (DMSO) or MAPK inibitor U0126 (7 hours)

were subjected to immunoblot with indicated antibodies. n=3. The relative Bim protein levels (versus GAPDH) were quantified. The mean of Bim/GAPDH in control group was set as 1. Statistical analyses were performed with One-way ANOVA (left). ***: P<0.001; ns: not significant, or T-test (Right). *: P=0.0265. **(B)** The cell lysates of STHdhQ7 (WT HTT) or STHdhQ111 (mHTT) striatal cells were subjected to immunoblot with indicated antibodies. **(C)** qPCR for Bim mRNA levels was performed with mRNAs from the striata of WT or HD mice (8 weeks). The data show the relative values, as the mRNA levels in each WT group were set as 1. n=3 (3 male (M) for both WT and HD mice), triplicate for each mouse sample. Data are mean±SEM. Statistical analysis was performed by T-test. ns: not significant. **(D)** SK-N-SH cells (6 wells) were transfected with GFP-HTT-21Q or GFP-HTT-72Q. After 24 hours, over 80% transfection efficiency was confirmed, and the cells were treated with the protein synthesis inhibitor cycloheximide (CHX, 50 μg/ml) for 0, 1h, 2h, 4h, 6h, 8h, respectively. (i) The cells were lysed and subjected to immunoblot with anti-Bim, GFP and GAPDH. (ii) The relative Bim levels (versus GAPDH) were quantified. The mean of Bim/GAPDH in each control group was set as 1. **(E)** The lysates of striata from WT or HD mice (10 or 14 weeks) were subjected to immunoblot with NeuN (a neuron marker) or GAPDH. The relative NeuN levels were quantified. The mean of NeuN/GAPDH in each control group was set as 1. n=3 (3M for all cases). Data are mean±SEM. Statistical analyses were performed with T-test. ns: not significant; *: P=0.0420.

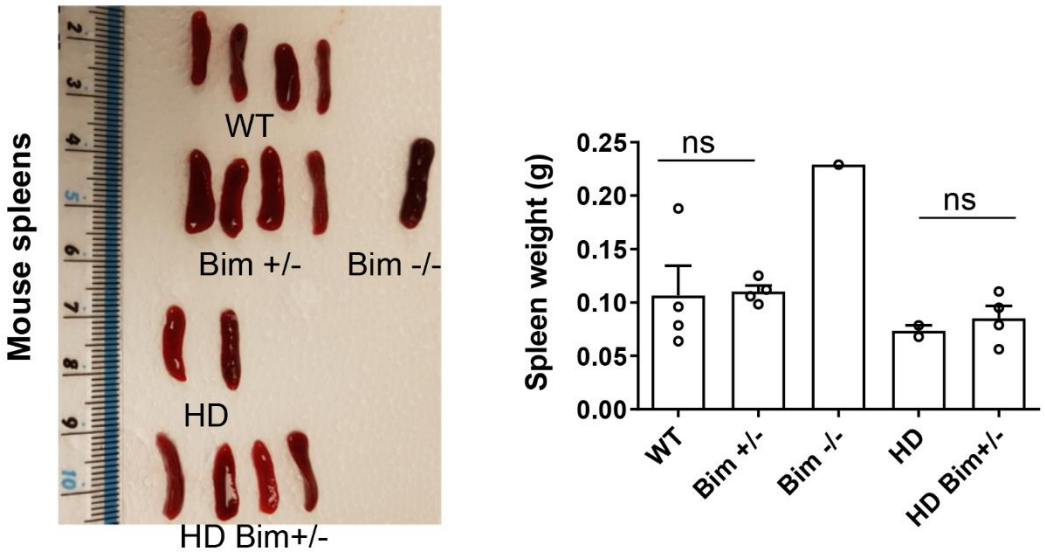

Figure S2. Bim reduction does not cause adverse effects on the size and weight of spleen in mice. The spleens from 12-week WT (4M), Bim+/- (3M/1F), Bim-/- (1M), HD (2M) and HD Bim+/- (4M) mice were harvested and weighed. Data are mean±SEM. Statistical analysis was performed with T-Test. ns: not significant.

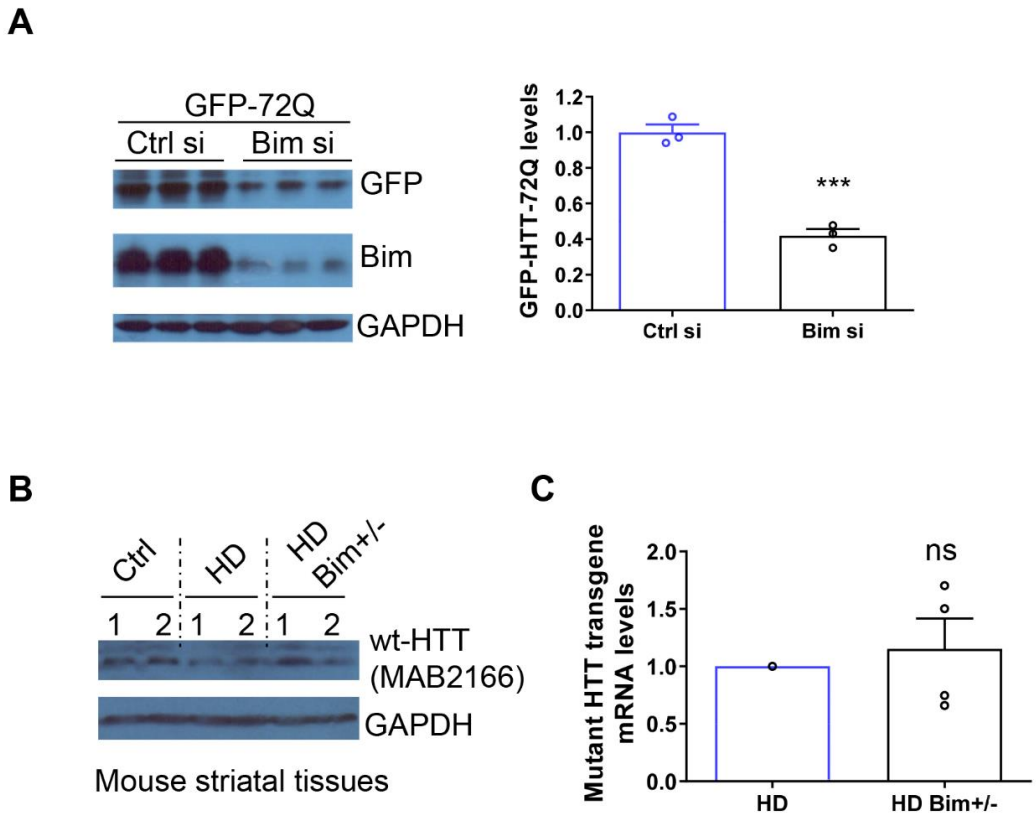

Figure S3. Bim reduction reduces mHTT levels. (A) SK-N-SH cells were transfected with HTT-21Q/vector, HTT-72Q/vector or HTT-72Q/Bim-siRNA. After 48 hours, the cells were harvested. The cellular lysates were subjected to immunoblot with anti-GFP, Bim or GAPDH. The relative GFP-HTT-72Q levels were quantified. The mean of Bim/GAPDH in control group was set as 1. n=3. Data are mean±SEM. Statistical analyses were performed with T-test. ***: P=0.0006. **(B)** The lysates of striata from 12-week WT, HD or HD Bim+/- mice (all male) were subjected to immunoblot with the antibodies indicated. Note that HTT monoclonal antibody (MAB2166) recognises HTT aa181-810, but does not react with the human transgene protein product HTT N171-82Q in HD mice. **(C)** qPCR measures human mutant HTT transgene mRNA levels in HD or HD Bim+/- mice (12 weeks). The data show the relative values, as the mRNA levels in each HD group were set as 1. n=4 mice (4M), triplicate for each mouse sample. Data are mean±SEM. Statistical analyses were performed by T-tests. ns: not significant.

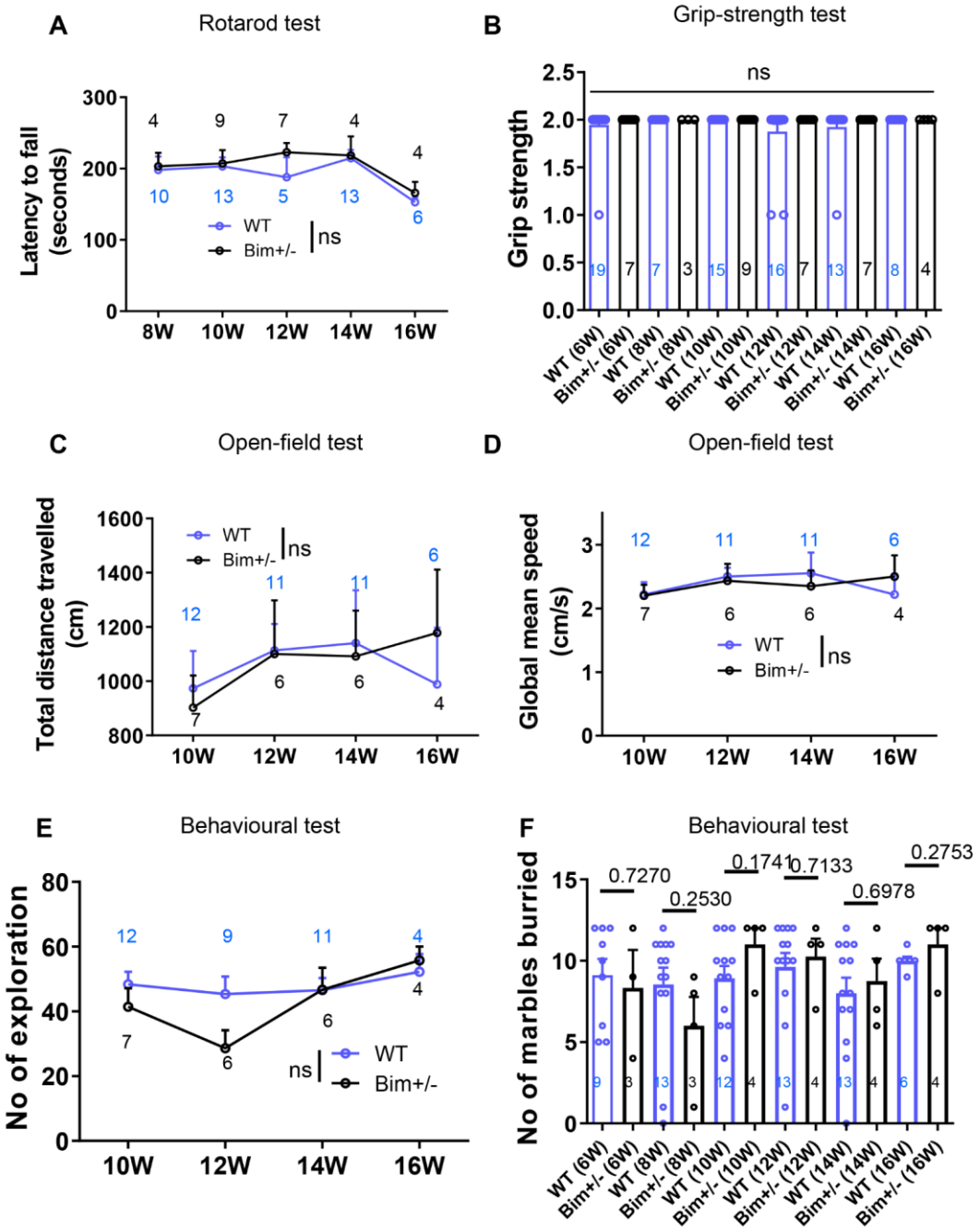

Figure S4. The HD-associated locomotor activity and phenotypes do not change significantly in Bim+/- mice. (A) Rotarod test was performed in WT or Bim+/- mice at indicated ages. Data are mean±SEM. n numbers are indicated in the figure. The statistical analysis was performed by two-way ANOVA test. ns: not significant. Table S2 lists the details of mouse genders for this experiment. **(B)** Grip strength test was performed in WT or Bim+/- mice at the indicated ages. Data are mean±SEM. n numbers are indicated in the figure. The

statistical analysis was performed by two-way ANOVA test. ns: not significant. Table S2 lists the details of mouse genders for this experiment. **(C-D)** Open field test was performed in WT or Bim+/- mice at the indicated ages for total distance travelled (cm) (C) and global mean speed (cm/s) (D). Data are mean±SEM. The statistical analysis was performed by two-way ANOVA test. n numbers are indicated in the figure. ns: not significant. Table S2 lists the details of mouse genders for this experiment. **(E)** The total number of explorations (the total number of visits to familiar and novel objects) in WT or Bim+/- mice at indicated ages, was digitally recorded in the Novel object recognition test. Data are mean±SEM. n numbers are indicated in the figure. The statistical analysis was performed by two-way ANOVA test. ns: not significant. Table S2 lists the details of mouse genders for this experiment. **(F)** Marble burying test was used for the mice at indicated ages. Data are mean±SEM. n numbers are indicated in the figure. The statistical analysis was performed by T-tests. ns: not significant. Table S2 lists the details of mouse genders for this experiment.

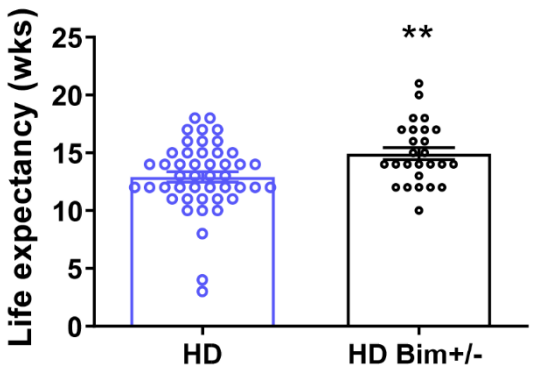

Figure S5. Bim reduction increases the lifespan in HD mice. The average life expectancy of HD or HD Bim+/- mice is plotted. Data are mean±SEM. n=47 (HD mice); n=26 (HD Bim+/- mice). wks: weeks. The statistical analysis was performed by T-test *: P=0.0064.

Table S1. The details of animal genders for behavioural assays in Fig. 4 and 5

Test	Age	WT (n number)	HD (n number)	HD Bim+/- (n number)
Rota rod	6 weeks	12 (F=4/M=8)	15 (F=4/M=11)	10 (F=4/M=11)
	8 weeks	13 (F=1/M=12)	13 (F=2/M=11)	15 (F=6/M=9)
	10 weeks	21 (F=4/M=17)	15 (F=1/M=14)	20 (F=8/M=12)
	12 weeks	12 (F=1/M=11)	12 (F=3/M=9)	13 (F=8/M=5)
	14 weeks	17 (F=2/M=15)	15 (F=5/M=10)	13 (F=6/M=7)
Global mean speed	6 weeks	16 (F=4/M=12)	11 (F=3/M= 8)	15 (F=5/M=10)
	8 weeks	15 (F=0/M=15)	15 (F= 2/M=13)	16 (F=6/M=10)
	10 weeks	17 (F=1/ M=16)	17 (F=0/M=17)	20 (F=8/M=12)
	12 weeks	15 (F=5/M=10)	18 (F=6/M=12)	16 (F=8/M=8)
	14 weeks	10 (F=1/M=10)	10 (F=3/M=7)	12 (F=8/M=4)
Total distance travelled	6 weeks	17 (F=4/M=13)	13 (F=3/M=10)	16 (F=5/M=11)
	8 weeks	14 (F=0/M=14)	15 (F=2/M=13)	14 (F=6/ M=8)
	10 weeks	15 (F=1/M=14)	15 (F=1/M=14)	20 (F=7/M=13)
	12 weeks	14 (F=4/M=10)	14 (F=3/M=11)	15 (F= 8/M=17)
	14 weeks	11 (F=1/M=10)	10 (F=2/M=8)	11 (F=8/M=3)
Discrimination ratio (Duration)	6 weeks	15 (F=4/M=11)	12 (F=3/M=9)	17 (F=5/M=12)
	8 weeks	15 (F=0/M=15)	15 (F=2/M=13)	14 (F=5/M=9)
	10 weeks	18 (F=1/M=17)	17 (F=2/M=15)	22 (F=8/M=14)
	12 weeks	14 (F=3/M=11)	15 (F=4/M=11)	12 (F=7/M=5)
	14 weeks	12 (F=1/M=11)	10 (F=3/M=7)	12 (F=8/M=4)
Discrimination ratio (Visits)	6 weeks	17 (F=4/M=13)	11 (F=3/M=8)	17 (F=5/M=12)
	8 weeks	12 (F=0/M=12)	11 (F=1/M=10)	13 (F=3/M=10)
	10 weeks	15 (F=1/M=14)	16 (F= 2/M=14)	22 (F=8/M=14)
	12 weeks	15 (F=4/M=11)	19 (F= 6/M=13)	12 (F=7/M= 5)
	14 weeks	17 (F=2/M=15)	13 (F=3/M=10)	10 (F=6/M=4)
No. of explorations	6 weeks	17 (F=4/M=13)	12 (F=3/M=9)	17 (F=5/M=12)
	8 weeks	15 (F=0/M=15)	15 (F=2/M=13)	16 (F=6/M=10)
	10 weeks	18 (F=1/M=17)	17 (F=2/M=15)	20 (F=7/M=13)
	12 weeks	9 (F=2/M=7)	11 (F=2/M=9)	13 (F=5/M=8)
	14 weeks	14 (F=2/M=12)	12 (F=2/M=10)	12 (F=8/M=4)
No of marbles buried	6 weeks	13 (F=1/M=12)	11 (F=1/M=10)	13 (F=4/M=9)
	8 weeks	14 (F=4/M=10)	10 (F=4/M= 6)	12 (F=2/M=10)
	10 weeks	16 (F=1/M=15)	17 (F=3/M=14)	10 (F=3/M= 7)
	12 weeks	17 (F=4/M=13)	13 (F=2/M=11)	11 (F=4/M=7)
	14 weeks	18 (F=4/M=14)	11 (F=5/M=6)	9 (F=3/M=6)
Grip strength	6 weeks	23 (F=5/M=18)	21 (F=5/M=16)	20 (F=11/M=9)
	8 weeks	11 (F=4/M=7)	10 (F=3/M=7)	13 (F=5/M=8)
	10 weeks	20 (F=2/M=18)	17 (F=4/M=13)	17 (F=6/M=11)
	12 weeks	18 (F=7/M=11)	19 (F=8/M=11)	21 (F=9/M=12)
	14 weeks	18 (F=5/M=18)	15 (F=5/M=10)	13 (F=5/M=8)
Lifespan			47 (F=22/M=25)	26 (F=16/M=10)

Table S2. The details of animal genders for behavioural assays in Fig. S4

Test	Age	WT (n number)	Bim +/- (n number)
Rota rod			
	8 weeks	10 (F=1/M=9)	4 (F=0/M=4)
	10 weeks	13 (F=3/M=10)	9 (F=1/M=8)
	12 weeks	5 (F=0/M=5)	7 (F=1/M=6)
	14 weeks	13 (F=3/M=10)	4 (F=0/M=4)
	16 weeks	6 (F=1/M=5)	4 (F=1/M=3)
Global mean speed			
	10 weeks	12 (F=0/M=12)	7 (F=1/M=6)
	12 weeks	11 (F=4/M=7)	6 (F=1/M=5)
	14 weeks	11 (F=1/M=10)	6 (F=1/M=5)
	16 weeks	6 (F=1/M=5)	4 (F=1/M=3)
Total distance travelled			
	10 weeks	12 (F=0/M=12)	7 (F=1/M=6)
	12 weeks	11 (F=4/M=7)	6 (F=1/M=5)
	14 weeks	11 (F=1/M=10)	6 (F=1/M=5)
	16 weeks	6 (F=1/M=5)	4 (F=1/M=3)
No. of explorations			
	10 weeks	12 (F=0/M=12)	7 (F=1/M=6)
	12 weeks	9 (F=2/M=7)	6 (F=1/M=5)
	14 weeks	11 (F=1/M=10)	6 (F=1/M=5)
	16 weeks	4 (F=1/M=3)	4 (F=1/M=3)
No of marbles buried	6 weeks	9 (F=0/M=9)	3 (F=1/M=2)
	8 weeks	13 (F=4/M=9)	3 (F=0/M=3)
	10 weeks	12 (F=1/M=11)	4 (F=0/M=4)
	12 weeks	13 (F=3/M=10)	4 (F=0/M=4)
	14 weeks	13 (F=3/M=11)	4 (F=1/M=3)
	16 weeks	6 (F=2/M=4)	4 (F=1/M=3)
Grip strength	6 weeks	19 (F=7/M=12)	7 (F=1/M=6)
	8 weeks	7 (F=1/M=6)	3 (F=0/M=3)
	10 weeks	15 (F=3/M=12)	9 (F=1/M=8)
	12 weeks	16 (F=5/M=11)	7 (F=0/M=7)
	14 weeks	13 (F=3/M=10)	7 (F=1/M=6)
	16 weeks	8 (F=0/M=8)	4 (F=1/M=3)