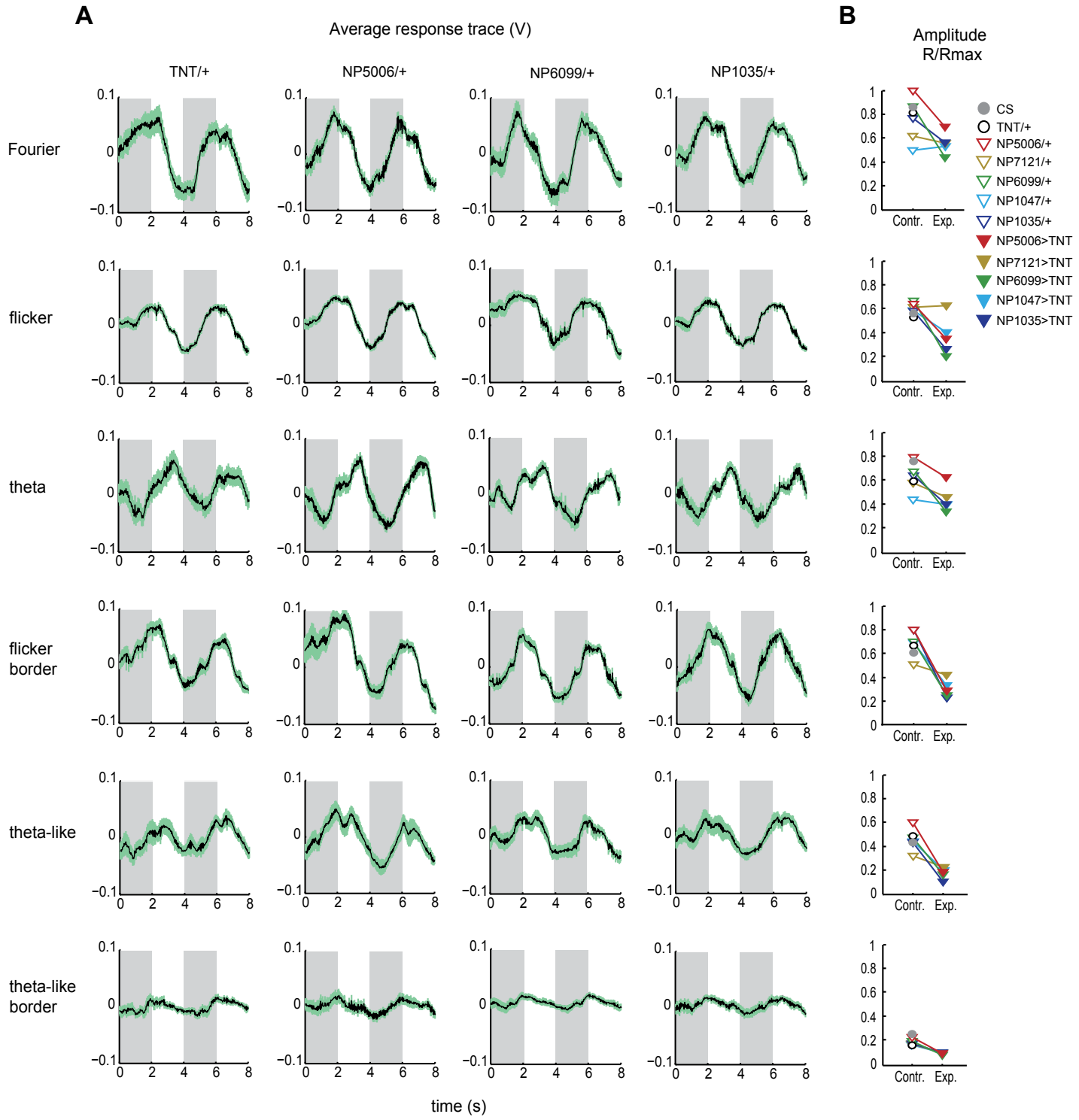


Fig. S1. Optomotor response traces in four control lines and relative amplitude R/R_{max} in all lines to each type of motion stimulus. (A) The average traces of three heterozygous Gal4 controls and UAS-TNT control to six types of stimuli. For the theta stimulus, the ratio of the internal dot speed to theta bar speed is –1.0. Each panel displays the average response trace in black flanked by the standard error in green, in which the fly strain is marked on the top of each column. Only the first two cycles of each average trace was plotted for clarity. (B) The relative amplitude R/R_{max} of the average traces in various fly strains to six types of motion stimuli. A general decrease in R/R_{max} was induced in almost all experimental groups irrespective of stimulus type. Exp., experimental groups including five LT10 and/or LT11 blocked lines; Contr., control groups including heterozygous Gal4 controls, UAS-TNT control and the CS strain. Ten to 25 flies were used for each data point.

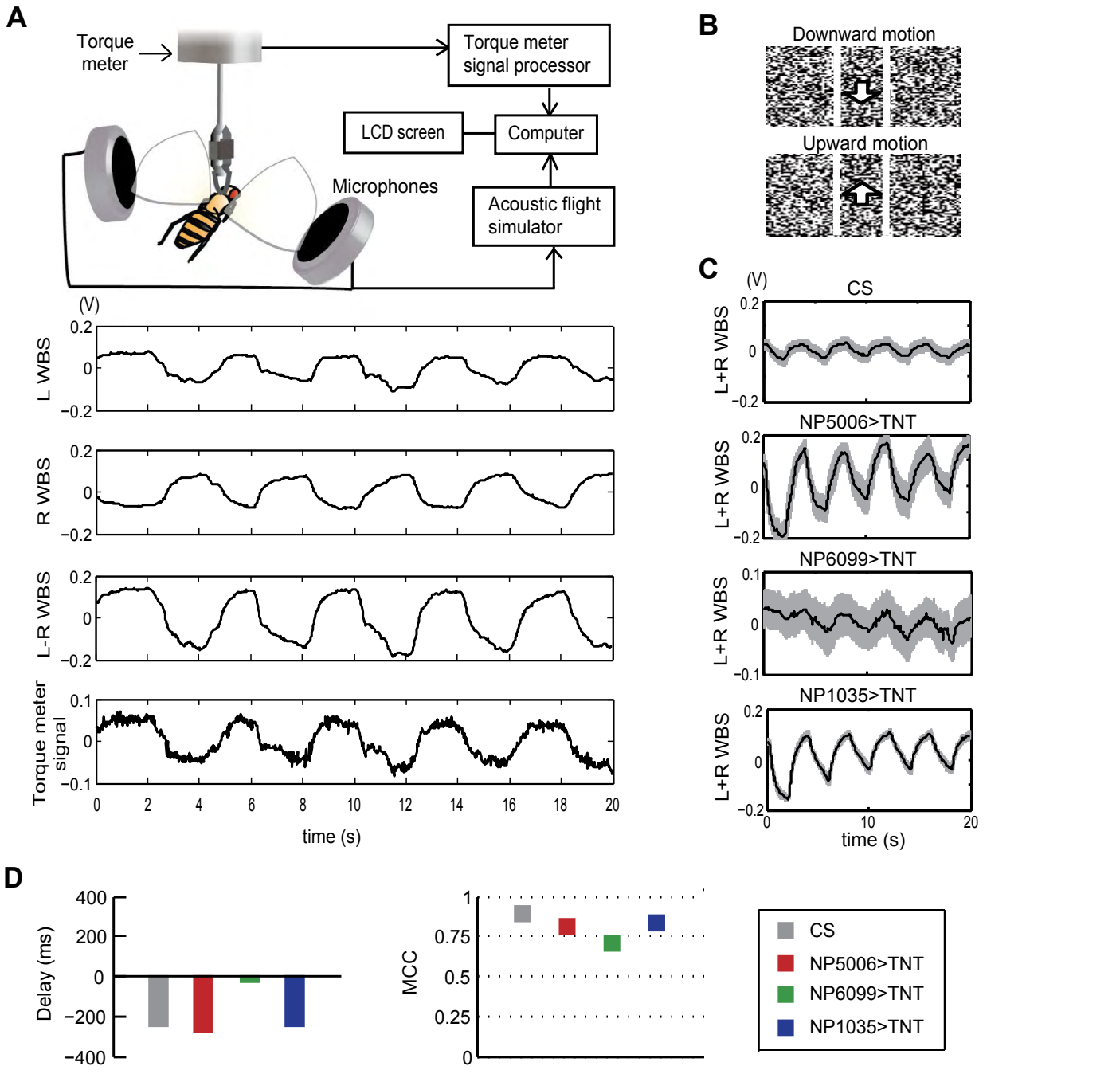
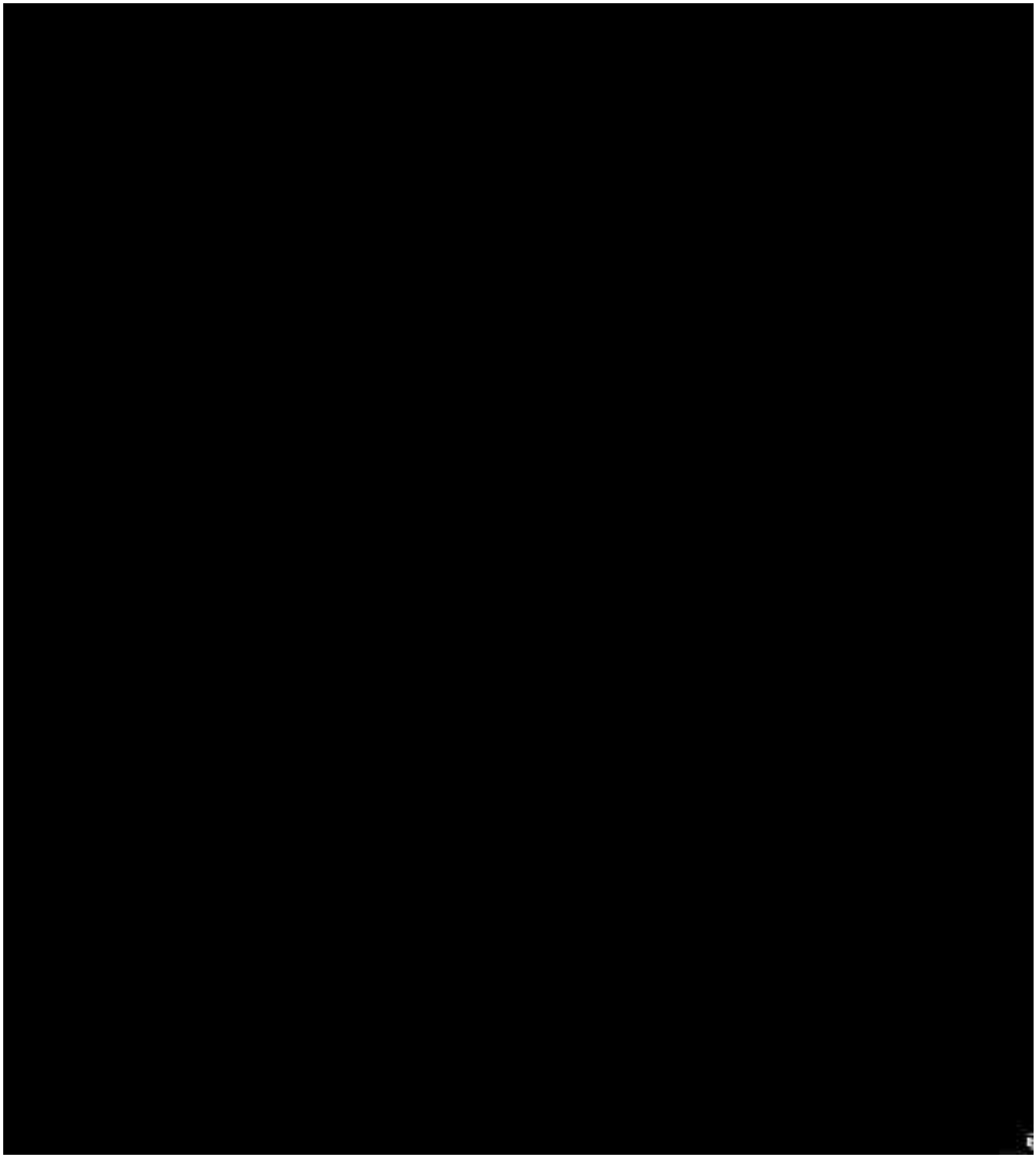


Fig. S2. First-order vertical motion perception is not impaired by blocking LT10 and/or LT11. (A) Schematic diagram of experimental setup for simultaneously recording the wing beat strength (WBS) and the yaw torque. A fly was suspended from a torque meter and between two microphones in the meantime. The left (L) and right (R) WBS were recorded by the acoustic flight simulator (AFS) in real-time (see Materials and methods), and the yaw torque was simultaneously measured by the torque meter. Fourier motion (the same as shown in Fig. 1C) was presented to CS flies. The bottom panels show the traces of a single sample fly: L, R and L–R WBS (recorded by the AFS) and the yaw response (recorded by the torque meter). The results indicated that the L–R WBS was proportional to the yaw response. Experiments shown in B–D were performed using the AFS alone. (B) The vertical motion stimulus was similar to theta-like motion except that the theta-like object was always static, and the direction of coherent dot motion within the theta-like object during the 20 s stimulus was not constant but altered between up and down with a period of 4 s. White thick arrows show the direction of dot motion, 2 s for downward and 2 s for upward in each stimulus cycle. White vertical bars did not appear in the actual stimuli and were used only to mark the borders of moving individual dots. (C) Steering responses of TNT driven by three Gal4 lines and the CS strain to the stimulus in B. Each average pitch optomotor response trace (black curve) was flanked by the standard error (grey). The optomotor response in pitch was calculated as the L+R WBS, the sum of the signals of the two wings. (D) The lag and MCC index from cross-correlation analysis to average pitch optomotor response traces in C. These blocking lines showed robust and quick steering responses to the vertical motion stimulus, as CS flies did. Ten to 25 flies were used for each data point in C and D.



Movie 1. Six types of motion stimuli. The movie displays six types of motion stimuli lasting one cycle as presented in our experiments. The dot speed within the theta object is –40 dots s^{-1}. The bottom panel below the Fourier, theta, flicker border and flicker bar motion stimuli is a space–time plot illustrating how one row (indicated by the hollow arrow above) chosen from the stimulus image evolves with time (vertical axis). The bottom panel below the theta-like motion stimulus is the continuous change in position of the stripe area (marked by the dashed frame) within which the dots are moving in the direction indicated by the white arrow. The bottom panel below the theta-like border motion stimulus is the continuous change in position of two individual columns whose dots are moving in the direction indicated by the arrows.

Table S1. Mean response amplitudes of individual flies (10^{-1} V)

Stimulus	CS	TNT/+	NP5006/+	NP5006>TNT	NP7121/+	NP7121>TNT	NP6099/+	NP6099>TNT	NP1047/+	NP1047>TNT	NP1035/+	NP1035>TNT
Fourier	1.81±0.06^{a}	1.87±0.13^{a}	1.95±0.06^{a}	1.47±0.13^{a,b}	1.22±0.08^{b}	1.06±0.05^{b,c}	1.80±0.21^{a}	0.90±0.05^{c}	1.10±0.06^{b}	1.25±0.07^{b}	1.73±0.09^{a}	1.12±0.09^{b}
Flicker	1.11±0.07^{b}	1.02±0.07^{b,c}	1.14±0.06^{b}	0.86±0.05^{b,c,d}	1.11±0.07^{b}	1.15±0.08^{a,b}	1.23±0.10^{a}	0.72±0.04^{d}	1.04±0.07^{b}	0.88±0.04^{b,c,d}	1.02±0.09^{b}	0.76±0.05^{c,d}
Theta	1.79±0.07^{a}	1.65±0.09^{a}	1.72±0.06^{a}	1.35±0.11^{a,b,c}	1.15±0.09^{b,c}	0.98±0.07^{c,d}	1.51±0.16^{a,b}	0.75±0.06^{d}	1.00±0.03^{c,d}	1.10±0.07^{b,c,d}	1.61±0.07^{a}	0.94±0.09^{c,d}
Flicker border	1.13±0.09^{b,c}	1.39±0.11^{a,b}	1.57±0.09^{a}	0.78±0.06^{c,d}	1.01±0.06^{c}	0.94±0.05^{c,d}	1.35±0.11^{a,b}	0.78±0.06^{c,d}	1.37±0.10^{a,b}	0.72±0.05^{d}	1.51±0.08^{a,b}	0.61±0.06^{d}
Theta-like	0.91±0.06^{b,c,d}	1.30±0.13^{a,b}	1.39±0.09^{a}	0.71±0.09^{d}	0.86±0.05^{c,d}	0.86±0.05^{c,d}	1.19±0.11^{a,b,c}	0.67±0.06^{d}	1.21±0.14^{a,b,c}	0.55±0.04^{d}	1.13±0.07^{a,b,c}	0.56±0.05^{d}
Theta-like border	0.74±0.04^{a}	0.59±0.06^{a}	0.65±0.04^{a}	0.60±0.06^{a}	0.56±0.04^{a}	0.63±0.04^{a}	0.62±0.06^{a}	0.67±0.05^{a}	0.57±0.04^{a}	0.56±0.05^{a}	0.59±0.03^{a}	0.63±0.05^{a}

The mean response amplitudes ($\bar{R}_{single}$) of individual flies in each strain (top row) to each stimulus are presented ±s.e.m. (N=10–25). Neuron blocking line data are shaded grey. A one-way ANOVA with Bonferroni correction for multiple comparisons was performed. Means of groups are not significantly different if they share a letter, whereas the means of any groups are significantly different in cases where they do not share a letter. $P \leq 0.05$ was considered significant.