Knockout mouse lines presenting with interesting abnormalities (mammalian phenotype [MP:0000001]) may receive additional testing outside of the normal pipeline in order to maximise information collected on that mouse line. Matched wild-type controls are also processed to identify phenotypic abnormalities arising from the targeted allele.

Top3b$^{gt(DC0348)WTSI}$

Topoisomerase (DNA) III beta

Genetic Background: 129P2/OlaHsd;C57BL/6NTac

Mutant Cell Line: DC0348 (SIGTR)

ES Cell Clone: E14TG2a

Genotypes Tested for Secondary Project

Homozygous (*Top3b*$^{gt(DC0348)WTSI/gt(DC0348)WTSI}$).

Relevant Pipeline Observations

Mice exhibit:

- No relevant pipeline observations.

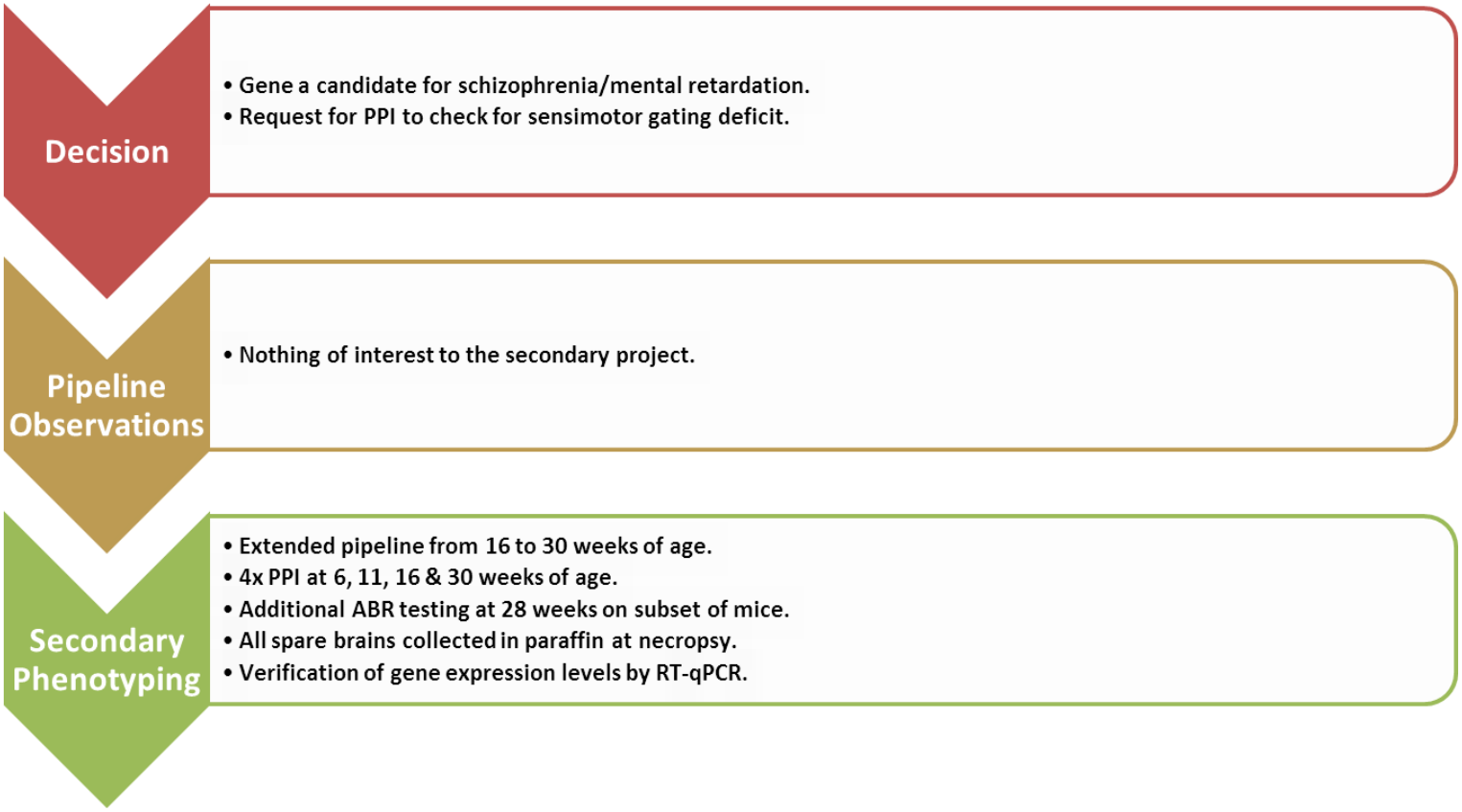
Secondary Project Procedure

Top3b allele gene trap mice were characterized on request as part of the Sanger Mouse Genetics Project's (Sanger MGP) high-throughput phenotyping pipeline. This was because the gene is one of five within a schizophrenia-mental retardation candidate interval. Sanger MGP was given the line of mice specifically in order to perform prepulse inhibition (PPI) testing.

Ten homozygous male and 10 homozygous female mutants, with age matched wild-type (WT) controls entered the pipeline between 3-4 weeks of age in 3 separate cohorts. They were placed onto a high-fat diet (21.4% crude fat content, Western RD, 829100, Special Diets Services) at 4 weeks of age and commenced the standardized phenotyping screens at that same time.

In addition to the normal testing schedule, these mice underwent PPI of the acoustic startle response screening 4 times at 6, 11, 16 and 30 weeks of age. A subset of the mice also received a second assessment of auditory brainstem response (ABR) at 28 weeks of age to ensure that both mutants and WTs were still able to hear for the final PPI screen. Due to the extended length of the pipeline, the plasma chemistry, haematology and peripheral blood lymphocytes tests were performed at 31 weeks of age instead of 16.

Schematic Outline of Secondary Project



Pipeline Phenotyping Heat Map

Colony Prefix	Allele Name	Viability at weaning	Fertility	Weight Curves	Open Field	Modified SHIRPA	Grip Strength	Hot Plate	Dysmorphology	Indirect Calorimetry	Glucose Tolerance (ip)	Auditory Brainstem Response	Body Composition (DEXA)	X-ray Imaging	Stress Induced Hyperthermia	Eye Morphology	Plasma Chemistry	Haematology (CBC)	Peripheral Blood Lymphocytes	Tissue Biobank	Heart Weight
MHHK	Top3b<gt(DC0348)SIGTR>																				

Significant / Interesting
Not significant
Resources available

Material & Methods for Non-Pipeline Tests

Prepulse Inhibition

Three startle chambers were used to measure PPI (MED-ASR-PRO1; Med Associates Inc., St. Albans, VT). Testing at all but 30 weeks of age was performed in a Plexiglas cylinder 3.2 cm in diameter, while week 30 testing was performed in a 4.4 cm diameter cylinder due to the large size of some mice. Mice were placed into the cylinder which was then secured onto the startle platform within the sound attenuated chamber. Two speakers located beside the platform produced the white noise background (60 dB), 10 ms prepulse (63, 66 or 69 dB) and 40 ms startle (110 dB) stimuli (2.4-24.3 kHz). Mouse movements were detected by the load cell in the platform and transmitted to the computer where the amplitude of the first peak post startle or prepulse (in prepulse only trials) was calculated.

Each PPI testing session consisted of a 5 min acclimatization period in the chamber with background noise. This was followed by block 1, consisting of 15 trials of startle pulse only. Immediately thereafter block 2 commenced consisting of 48 randomised trials of either prepulse only or prepulse-startle with 50 ms between the prepulse and startle stimuli. Three different prepulse levels were tested for a total of 8 occurrences of each trial type. The inter-trial interval varied randomly from 20-30 sec.

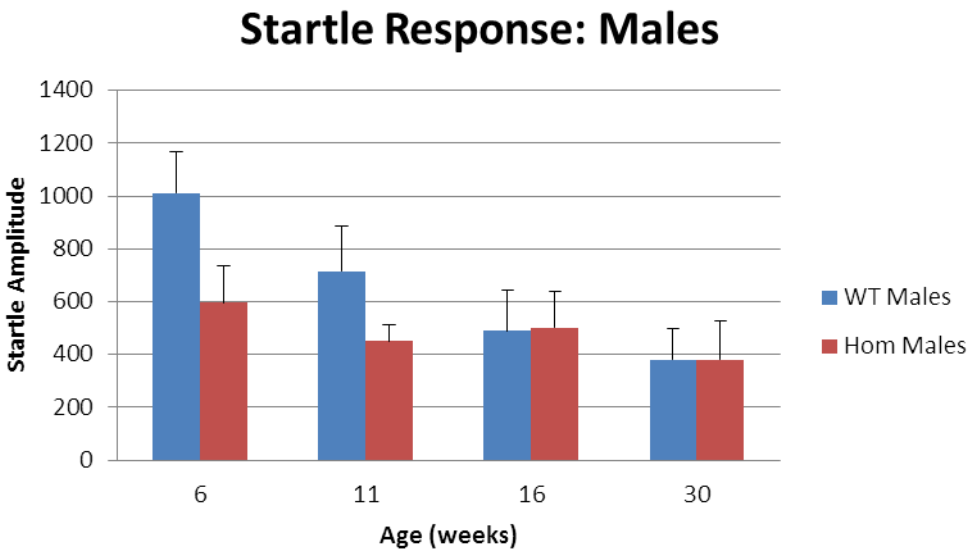
Statistical Analysis

To investigate differences in the startle response alone, a 2-tailed Student's or Welch's t-test was used, depending on the outcome of the Levene's test for equality of variance. Two-way repeat measure ANOVAs were used to examine the effect of genotype and prepulse level on prepulse only responses and prepulse inhibition. Significant ANOVAs were followed by post hoc Bonferroni tests.

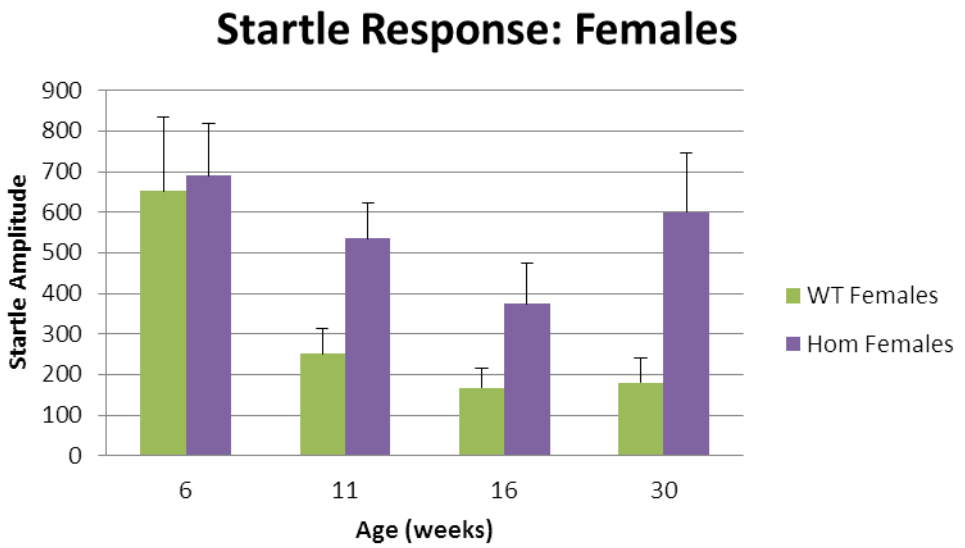
Differences were considered significant when $p<0.05$. For significant results, Cohen's d was calculated as a measure of effect size. Statistical analyses were performed using SPSS v16.0 and GraphPad Prizm v4.

Phenotyping Data of Interest (Significant Changes)

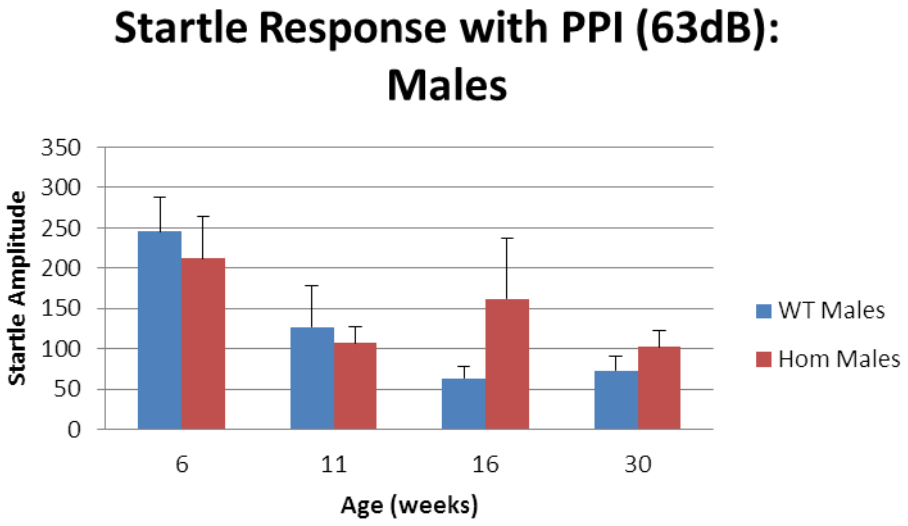
In-Life Phenotyping

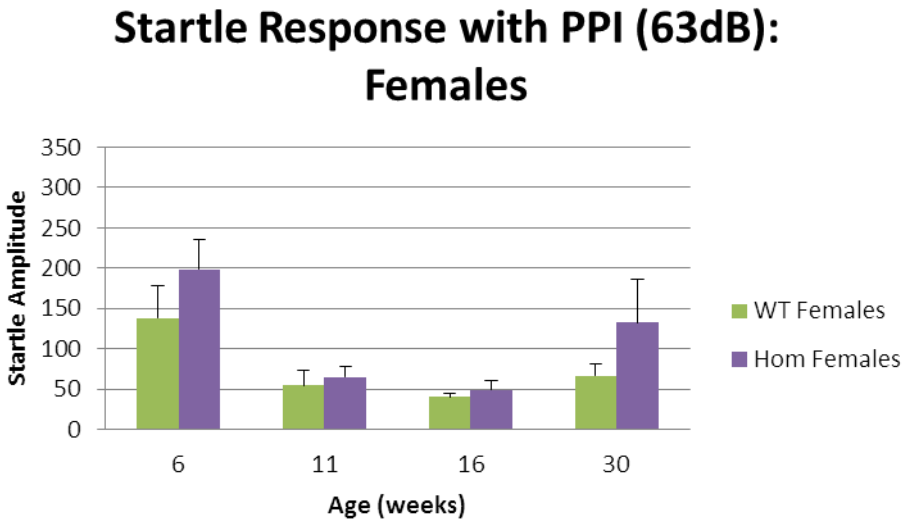


There were no statistically significant differences in startle response between WT and mutant males at any of the tested ages. Startle response in WT males showed a statistically significant decrease between weeks 6 and 16 ($p<0.01$), and weeks 6 and 30 ($p<0.001$). The same trend was observed in the mutant males but this was not statistically significant.

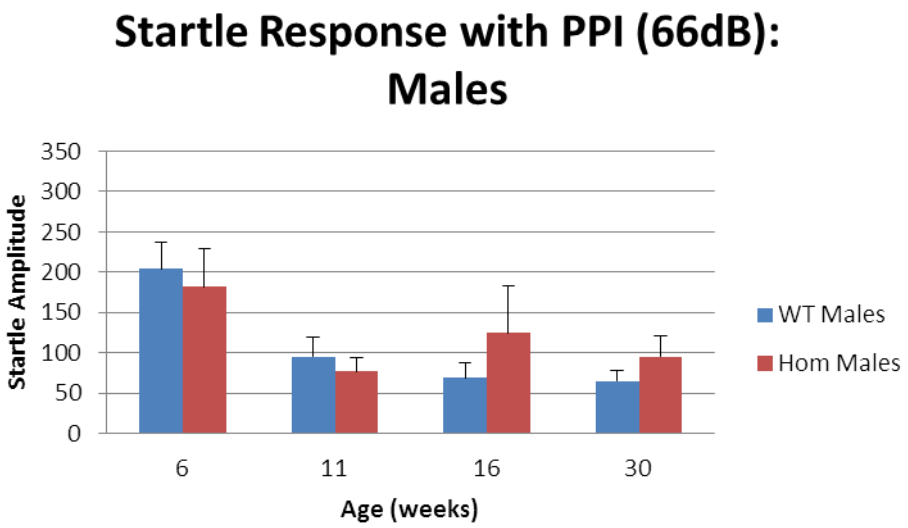


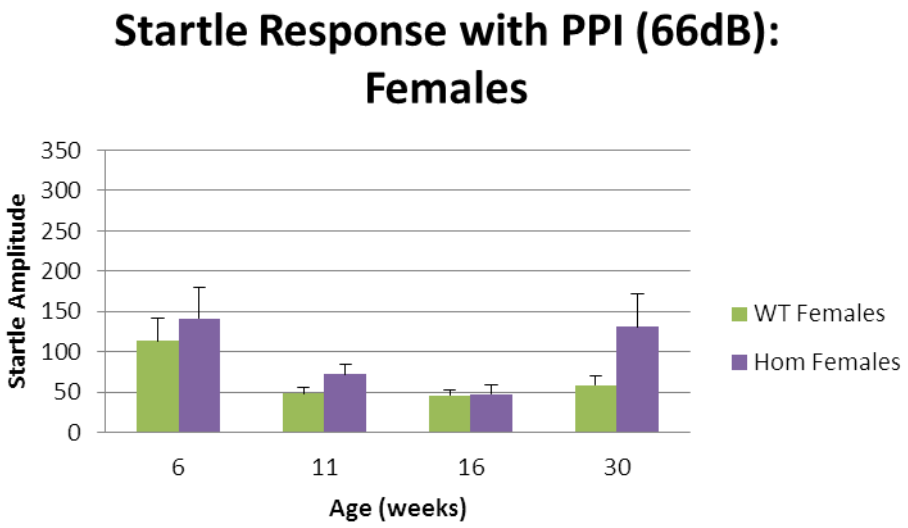
There was a statistically significant difference between the startle only response of WT and mutant female mice at 11 weeks ($\bar{x}_{WT}$ = 250, $\bar{x}_{KO}$ = 535, p=0.016, *d*=-1.20) and 30 weeks ($\bar{x}_{WT}$ = 180, $\bar{x}_{KO}$ = 600, p=0.016, *d*=-1.20). In both cases, the mutants showed an increased average response to the startle pulse compared to WTs. The same trend was observed at 16 weeks of age although this did not reach statistical significance (p=0.085). Similar to the males, WT female startle response was significantly lower between weeks 6 and 11 (p<0.01), weeks 6 and 16 (p<0.001) and between weeks 6 and 30 (p<0.01). Mutant females only showed a significant difference in their startle response between weeks 6 and 16 (p<0.05).



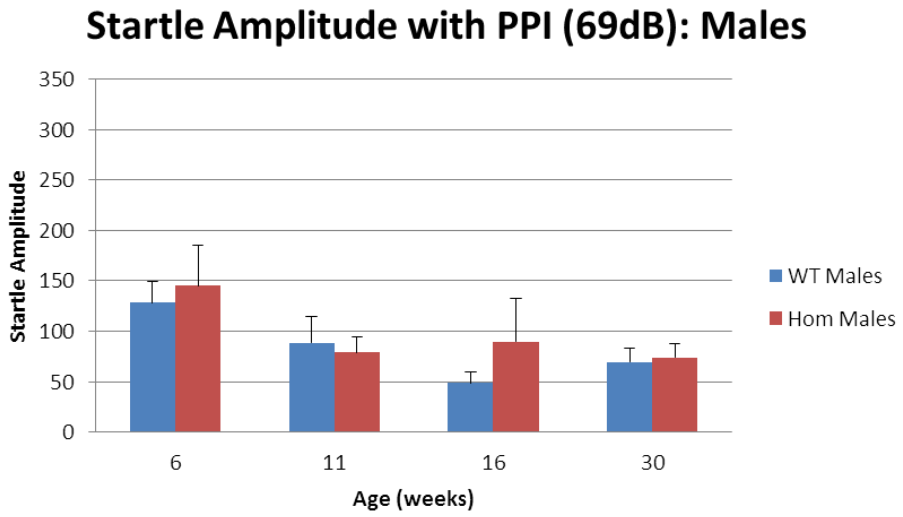


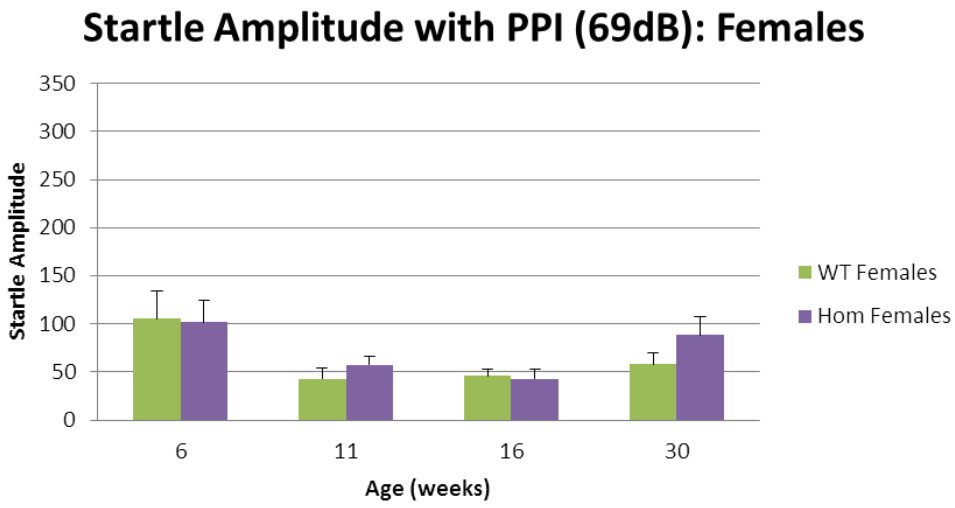
There were no statistically significant differences in absolute response to prepulse (at 63 dB) plus startle between WT and mutant males or females at any age tested.



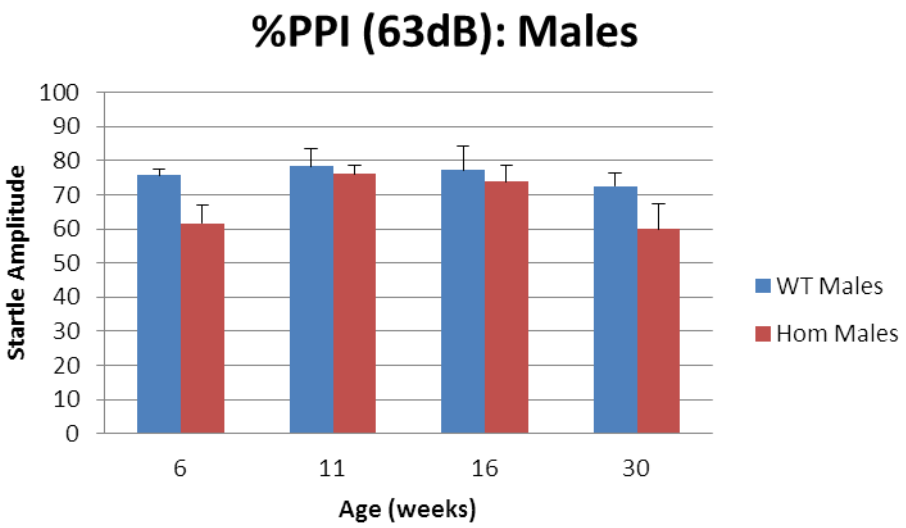


There were no statistically significant differences in absolute response to prepulse (at 66 dB) plus startle between WT and mutant males or females at any age tested.

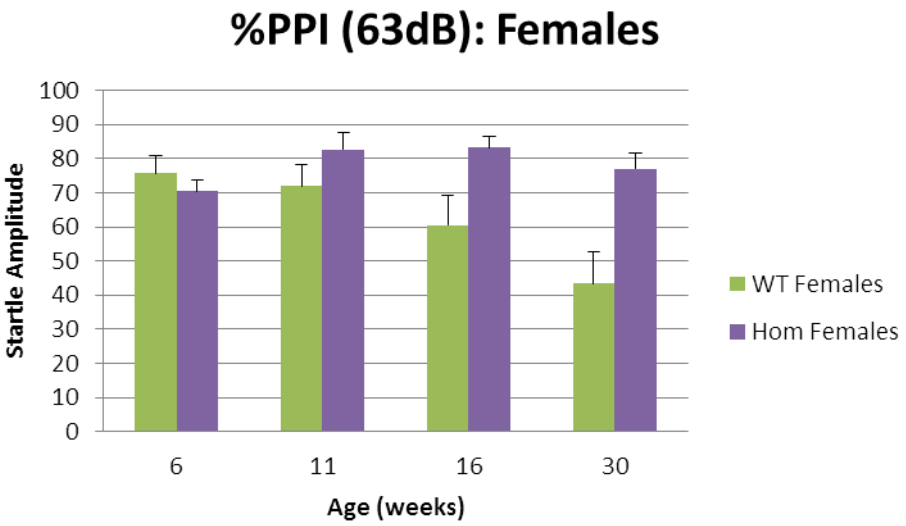




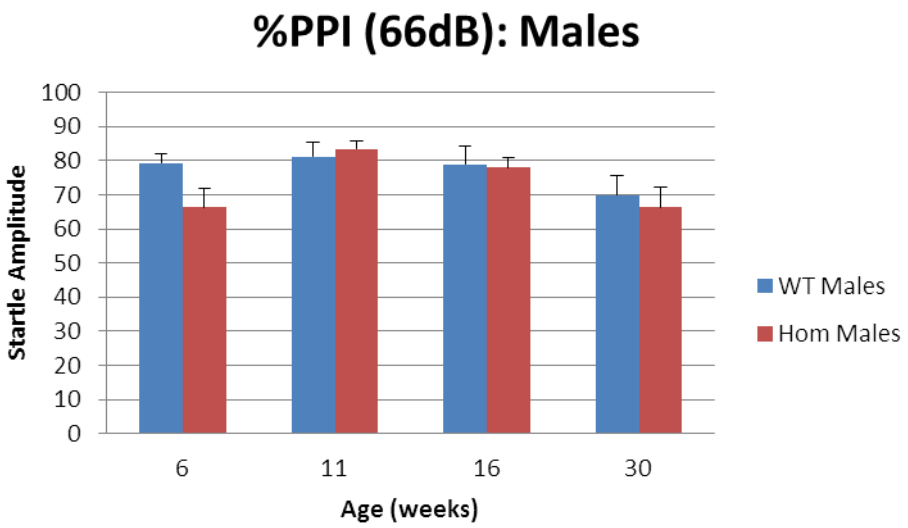
There were no statistically significant differences in absolute response to prepulse (at 69 dB) plus startle between WT and mutant males or females at any age tested.



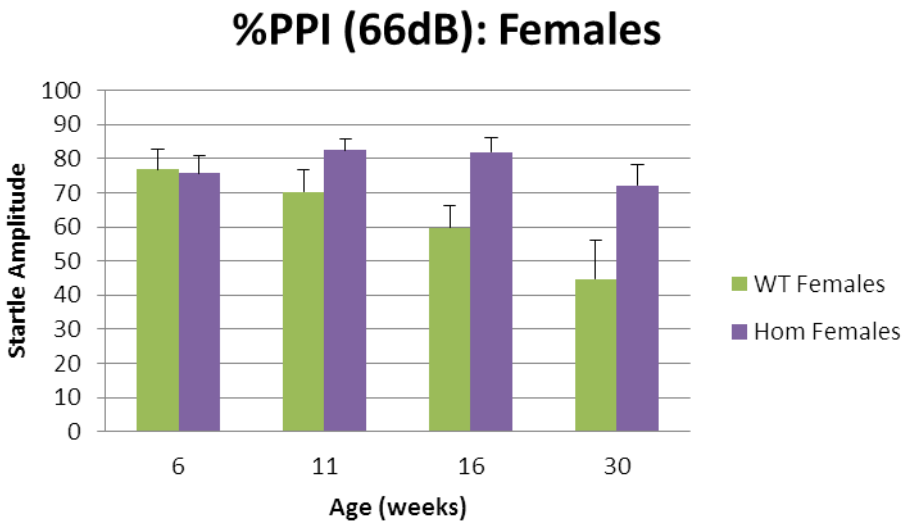
A statistically significant difference in average %PPI between WT and mutant males was seen at 6 weeks of age only (p=0.027) with a 18.7% decrease in %PPI compared to WTs ($\bar{x}_{WT}$ = 75.68, $\bar{x}_{KO}$ = 61.56, *d*=1.22).



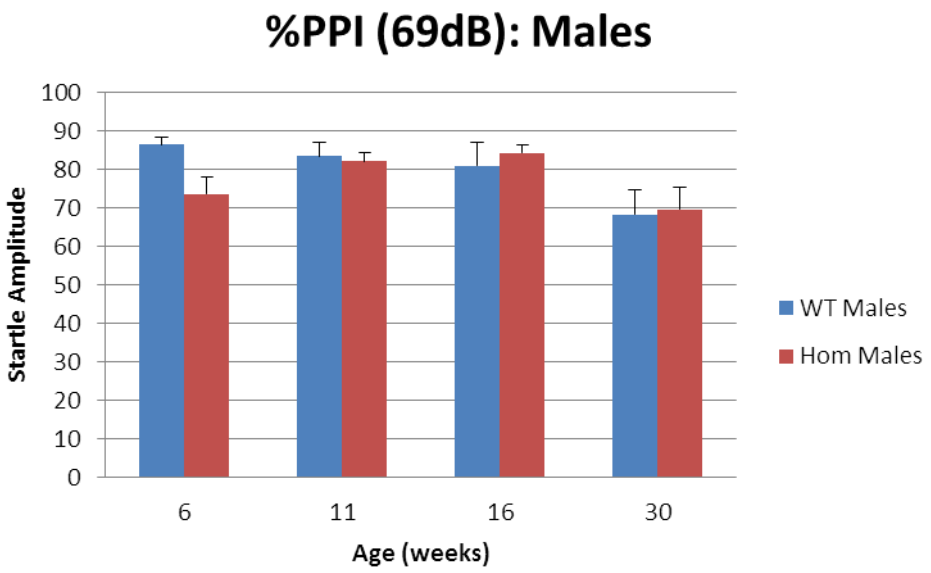
Statistically significant differences in average %PPI between WTs and mutant females were seen at 16 and 30 weeks of age (p=0.011 and p=0.014 respectively). At 16 weeks, a 37.5% increase in %PPI was seen ($\bar{x}_{WT}$ = 60.54, $\bar{x}_{KO}$ = 83.25, *d*=-0.32) and at 30 weeks, a 77.4% increase in %PPI was seen ($\bar{x}_{WT}$ = 43.47, $\bar{x}_{KO}$ = 77.12, *d*=-1.54).



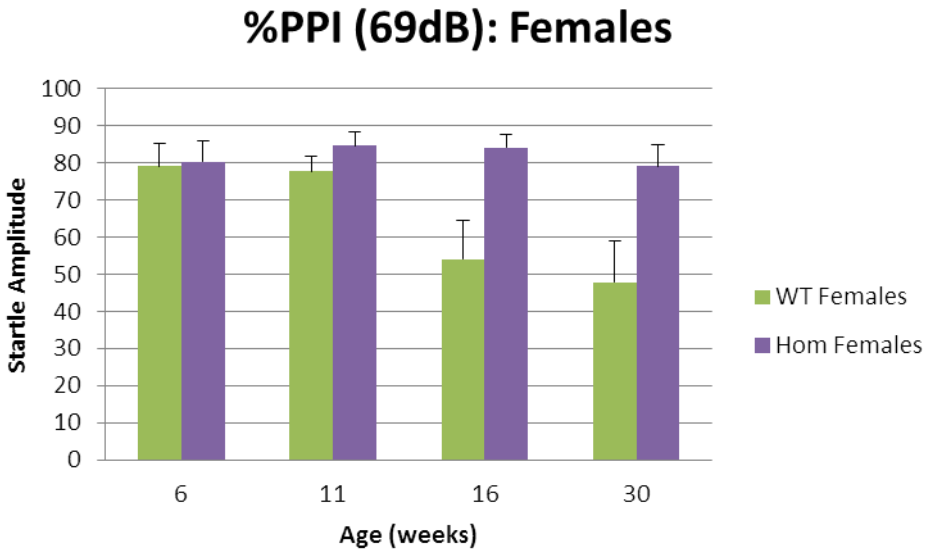
A statistically significant difference in average %PPI between WT and mutant males was seen at 6 weeks of age only (p=0.027) with a 16.5% decrease in %PPI compared to WTs ($\bar{x}_{WT}$ = 79.37, $\bar{x}_{KO}$ = 66.28, *d*=1.04).



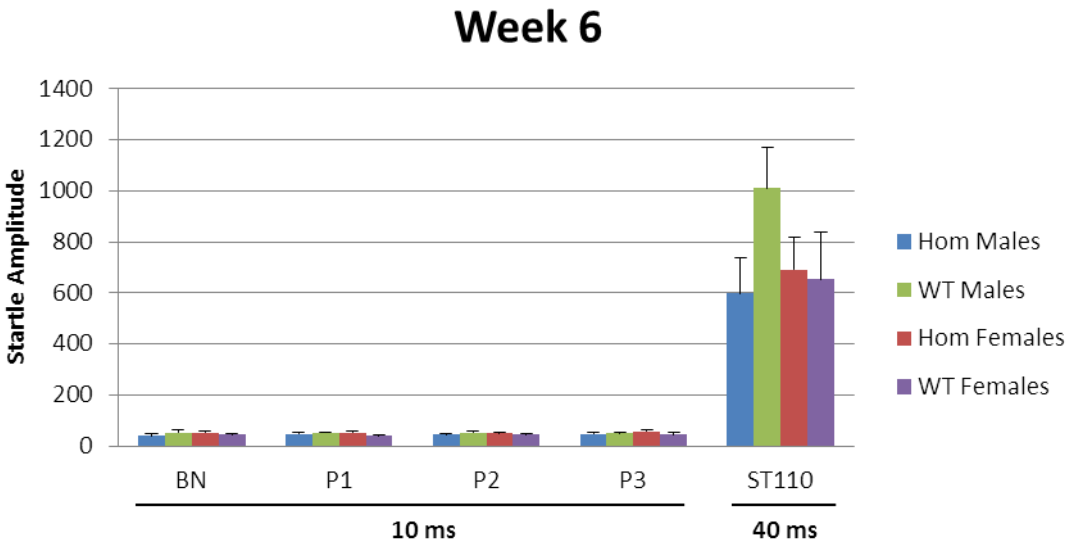
Statistically significant differences in average %PPI between WTs and mutant females were seen at 16 and 30 weeks of age (p=0.011 and p=0.014 respectively). At 16 weeks, a 37.2% increase in %PPI was seen ($\bar{x}_{WT}$ = 59.73, $\bar{x}_{KO}$ = 81.93, *d*=-0.22) and at 30 weeks of age, a 61.5% increase in %PPI was seen ($\bar{x}_{WT}$ = 44.70, $\bar{x}_{KO}$ = 72.20, *d*=-0.99).



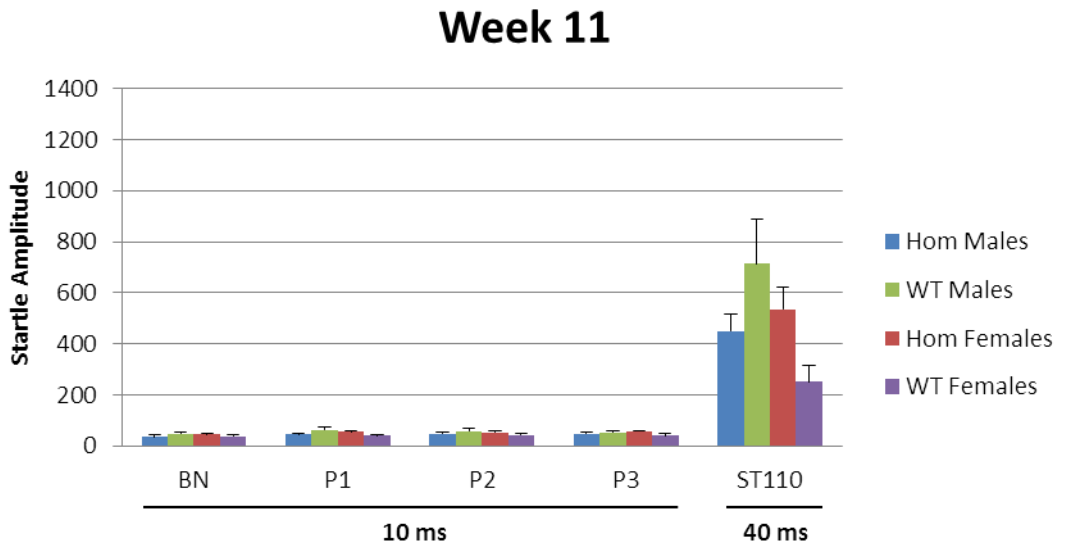
A statistically significant difference in average %PPI between WT and mutant males was seen at 6 weeks of age only (p=0.027), with a 14.7% decrease in %PPI compared to controls ($\bar{x}_{WT}$ = 86.41, $\bar{x}_{KO}$ = 73.72, *d*=1.27).



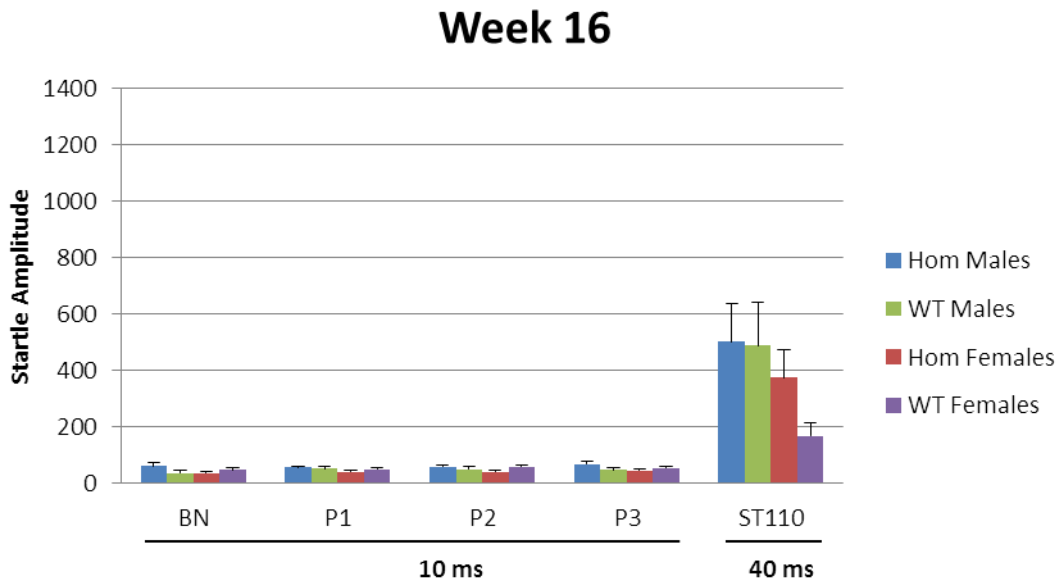
Statistically significant differences in average %PPI between WTs and mutant females were seen at 16 and 30 weeks of age (p=0.011 and p=0.014 respectively). At 16 weeks, at 69dB, a 55.7% increase in %PPI was seen ($\bar{x}_{WT}$ = 54.01, $\bar{x}_{KO}$ = 84.11, *d*=-0.64). At 30 weeks of age, at 69dB, a 65.7% increase in %PPI was seen ($\bar{x}_{WT}$ = 47.74, $\bar{x}_{KO}$ = 79.13, *d*=-1.18).



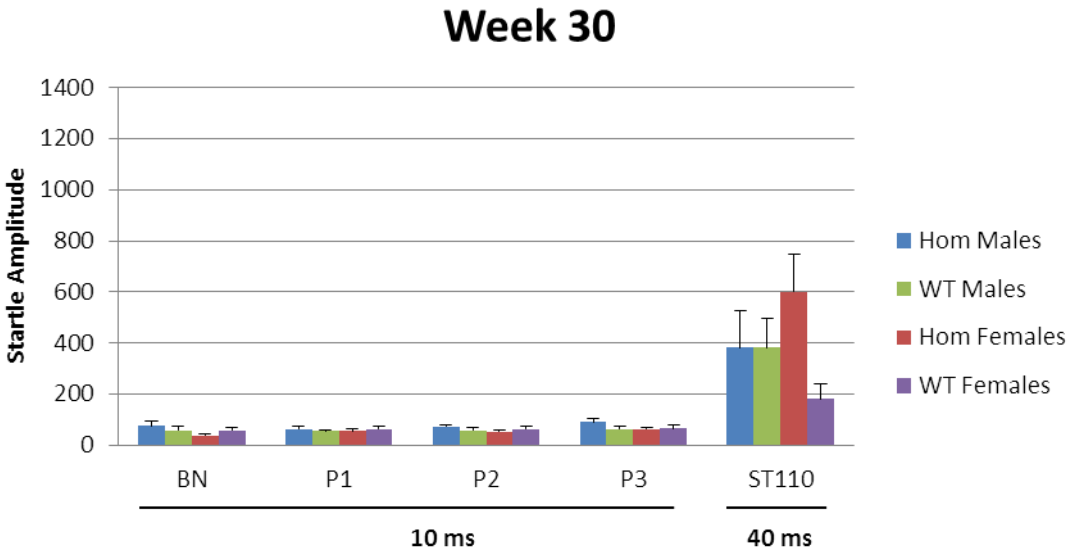
There were no statistically significant differences in response between WT and mutant males or females at 6 weeks of age for BN, P1, P2 or P3.



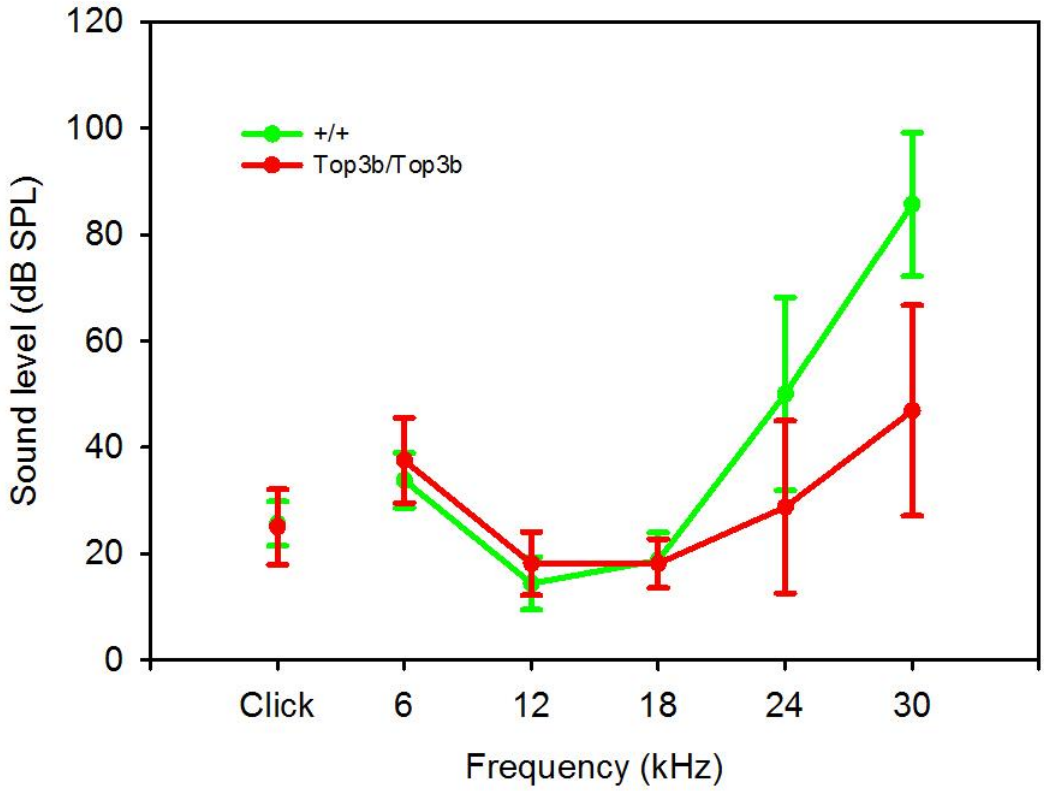
There was no statistically significant differences in response between WT and mutant males at 11 weeks of age for BN, P1, P2 or P3. A statistically significant difference was seen between responses to prepulse alone for female controls and mutants at 11 weeks of age (p=0.047). At prepulse level 1, mutant females showed a 35.1% increase in response compared to WTs ($\bar{x}_{WT}$ = 40.05, $\bar{x}_{KO}$ = 54.11, *d*=-1.03). At prepulse level 2, a 27.4% increase in response compared to WTs was seen ($\bar{x}_{WT}$ = 41.91, $\bar{x}_{KO}$ = 53.38, *d*=-0.76). At prepulse level 3, a 38.2% increase in response compared to WTs was seen ($\bar{x}_{WT}$ = 40.63, $\bar{x}_{KO}$ = 56.15, *d*=-1.04).



There were no statistically significant differences in response between WT and mutant males or females at 16 weeks of age for BN, P1, P2 or P3.

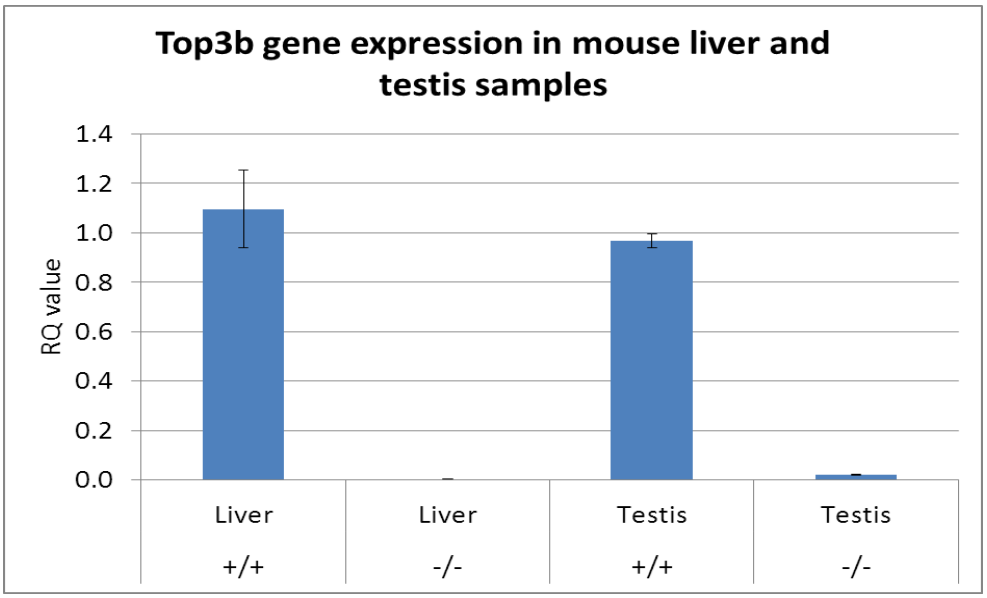


There were no statistically significant differences in response between WT and mutant males or females at 30 weeks of age for BN, P1, P2 or P3.



Despite genotype differences in high frequency (≥24 kHz) ABR thresholds detected at 28 weeks, the bulk of the startle stimulus energy is in the frequency range where the hearing sensitivity of the two genotypes are equivalent.

Ex-Vivo Quality Control Phenotyping/Check



Gene expression levels of Top3b in mutant mice were verified by RT-qPCR. Approximately 15-20 mg of liver or testis was homogenised and RNA extracted using a Qiagen RNeasy fibrous tissue extraction kit. 1 µl (~200 ng) of total RNA was used in a 10 µl reaction using a TaqMan RNA-to-CT One Step kit (Applied Biosystems). A TaqMan probe spanning exons 2-3 of Top3b, flanking the gene trap insertion point (Applied Biosystems probe Mm01344818_m1), was used in a multiplex reaction with either a primer-limited B2M or a GAPD endogenous control (Applied Biosystems). Reactions were performed in triplicate on an ABI Viia7 qPCR machine and analysed with Viia7 1.1 software, using the relative quantification module. Reactions performed using the B2M endogenous probe gave a higher variation within the technical replicates and across the WT controls than with the GAPD probe, and thus data is shown using the GAPD endogenous controls.

Some Top3b expression was observed in homozygotes, although the levels were much lower than those in the WT controls (0.3% and 2.3% of WT levels for liver and testis respectively).