



Venuto and Erickson, Supplementary Materials: *Figure S1, Tables S1–S4.*

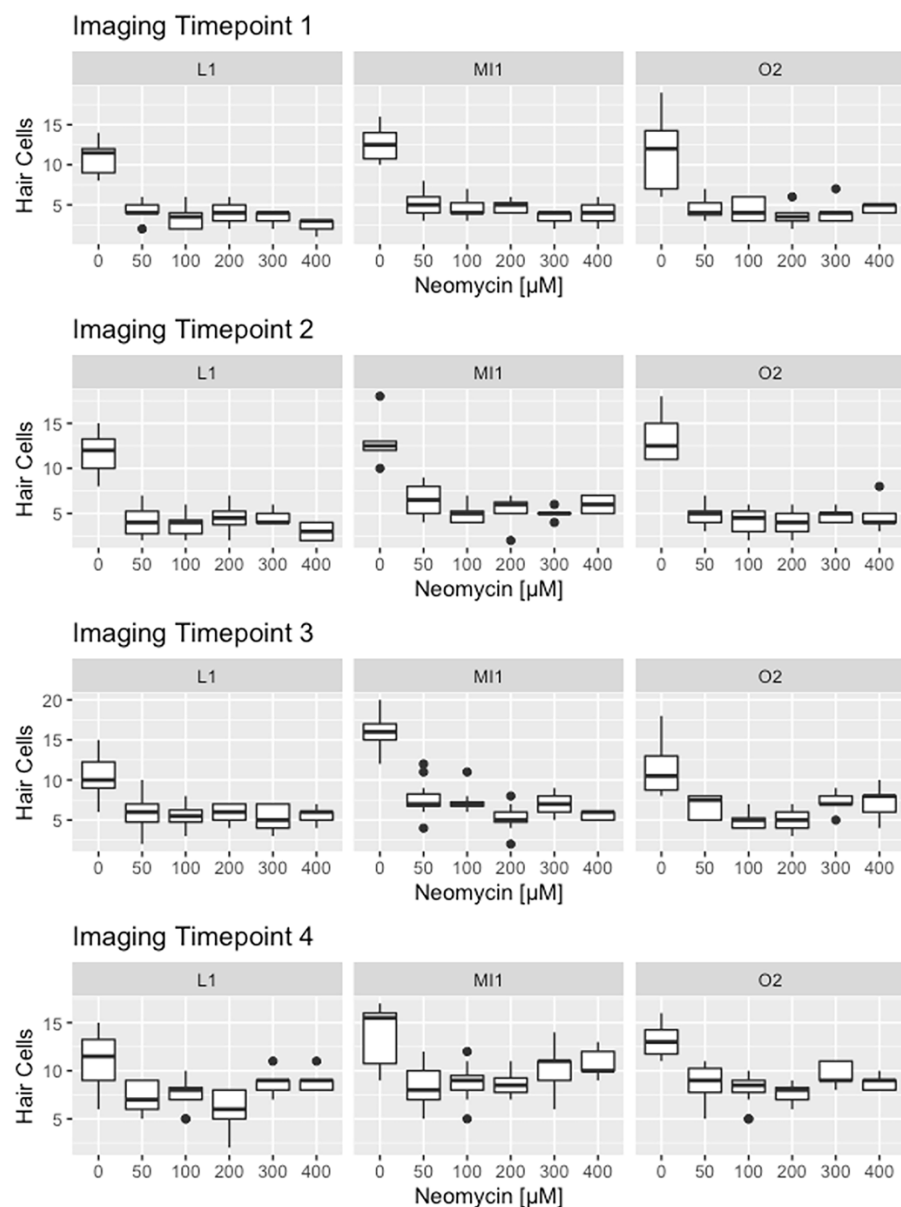


Figure S1. Box plot of total hair cell counts (GFP-positive) from individual neuromasts L1, MI1, and O2 at each imaging timepoint in the 12-hour treatment timeline.

Table S1. Average final percent larval survival (\pm standard deviation) at each neomycin concentration. The starting number of larvae are provided for each condition.

Neomycin Concentration	6 Hours		12 Hours	
0 μ M	96% \pm 6.9%	(n = 46)	94% \pm 11.0%	(n = 49)
50 μ M	62% \pm 45.2%	(n = 52)	86% \pm 18.7%	(n = 49)
100 μ M	24% \pm 33.3%	(n = 58)	67% \pm 34.4%	(n = 53)
200 μ M	13% \pm 13.7%	(n = 64)	43% \pm 41.8%	(n = 56)
300 μ M	0% \pm 0.0%	(n = 68)	42% \pm 20.2%	(n = 62)
400 μ M	0% \pm 0.0%	(n = 68)	18% \pm 6.7%	(n = 62)

Table S2. Average number of hair cells (\pm standard deviation) at imaging timepoints I1 – I4 (See Figure 1A) for neomycin concentrations 0, 50, 100, and 200 μ M.

	Total Hair Cells (GFP+)	Functional Hair Cells (FM+)	Total Hair Cells (GFP+)	Functional Hair Cells (FM+)
Timepoint I1	6 Hours	6 Hours	12 Hours	12 Hours
0 μ M	12.7 \pm 2.7	10.7 \pm 2.8	10.6 \pm 2.9	9.0 \pm 2.8
50 μ M	4.3 \pm 1.3	0.4 \pm 0.4	5.0 \pm 1.3	1.5 \pm 1.0
100 μ M	4.1 \pm 1.3	0.4 \pm 0.4	4.3 \pm 1.4	1.5 \pm 1.1
200 μ M	3.8 \pm 1.3	0.4 \pm 0.4	4.3 \pm 1.1	0.3 \pm 0.3
Timepoint I2				
0 μ M	12.4 \pm 2.7	11.1 \pm 2.7	11.4 \pm 1.5	10.2 \pm 1.9
50 μ M	4.7 \pm 1.5	1.2 \pm 1.2	5.6 \pm 1.9	2.5 \pm 1.3
100 μ M	4.3 \pm 1.5	0.8 \pm 0.8	4.6 \pm 1.2	2.0 \pm 1.3
200 μ M	4.3 \pm 1.6	1.0 \pm 1.0	5.1 \pm 1.3	2.1 \pm 1.2
Timepoint I3				
0 μ M	12.9 \pm 3.1	11.7 \pm 3.5	12.2 \pm 3.7	10.2 \pm 3.3
50 μ M	6.8 \pm 2.5	1.5 \pm 1.2	6.7 \pm 1.3	1.7 \pm 1.2
100 μ M	5.9 \pm 1.9	0.8 \pm 0.6	5.9 \pm 1.4	0.8 \pm 0.7
200 μ M	5.6 \pm 1.5	0.7 \pm 0.7	5.2 \pm 1.1	0.2 \pm 0.2
Timepoint I4				
0 μ M	13.6 \pm 2.9	11.6 \pm 3.4	13 \pm 2.6	12.1 \pm 2.8
50 μ M	8.1 \pm 2.0	3.7 \pm 1.5	8.3 \pm 1.9	5.6 \pm 2.1
100 μ M	8.0 \pm 1.8	2.3 \pm 1.5	8.4 \pm 1.7	4.1 \pm 2.1
200 μ M	7.2 \pm 2.1	1.6 \pm 1.1	7.7 \pm 1.7	2.8 \pm 2.2

Table S3. Related to Figures 4A, B – Results of a Kruskal-Wallis ANOVA with Dunn post-test comparing hair cell death between the 50, 100, and 200 μM neomycin concentrations at imaging timepoints I1 and I3 in the 6-hour and 12-hour treatment timelines.

Total Hair Cells (GFP+) at I1				Total Hair Cells (GFP+) at I3			
Conc. 1	Conc. 2	Interval	P-value	Conc. 1	Conc. 2	Interval	P-value
50 μM	100 μM	6 Hours	1	50 μM	100 μM	6 Hours	1
50 μM	200 μM	6 Hours	1	50 μM	200 μM	6 Hours	1
100 μM	200 μM	6 Hours	1	100 μM	200 μM	6 Hours	1
50 μM	100 μM	12 Hours	1	50 μM	100 μM	12 Hours	1
50 μM	200 μM	12 Hours	1	50 μM	200 μM	12 Hours	0.107
100 μM	200 μM	12 Hours	1	100 μM	200 μM	12 Hours	1

Functional Hair Cells (FM+) at I1				Functional Hair Cells (FM+) at I3			
Conc. 1	Conc. 2	Interval	P-value	Conc. 1	Conc. 2	Interval	P-value
50 μM	100 μM	6 Hours	1	50 μM	100 μM	6 Hours	1
50 μM	200 μM	6 Hours	1	50 μM	200 μM	6 Hours	0.462
100 μM	200 μM	6 Hours	1	100 μM	200 μM	6 Hours	1
50 μM	100 μM	12 Hours	1	50 μM	100 μM	12 Hours	1
50 μM	200 μM	12 Hours	0.042	50 μM	200 μM	12 Hours	0.197
100 μM	200 μM	12 Hours	0.077	100 μM	200 μM	12 Hours	1

Table S4. Related to Figure 4C – Results of a Kruskal-Wallis ANOVA with Dunn post-test comparing the 6-hour treatment timeline to the 12-hour treatment timeline at imaging timepoint 3 for both the total (GFP+) and functional (FM+) hair cells at the 50, 100, and 200 μM neomycin concentrations.

Neomycin Concentration	Hair Cell Status	P-value
50 μM	GFP +	0.898
100 μM	GFP +	0.884
200 μM	GFP +	0.321
50 μM	FM+	0.719
100 μM	FM+	0.485
200 μM	FM+	0.778