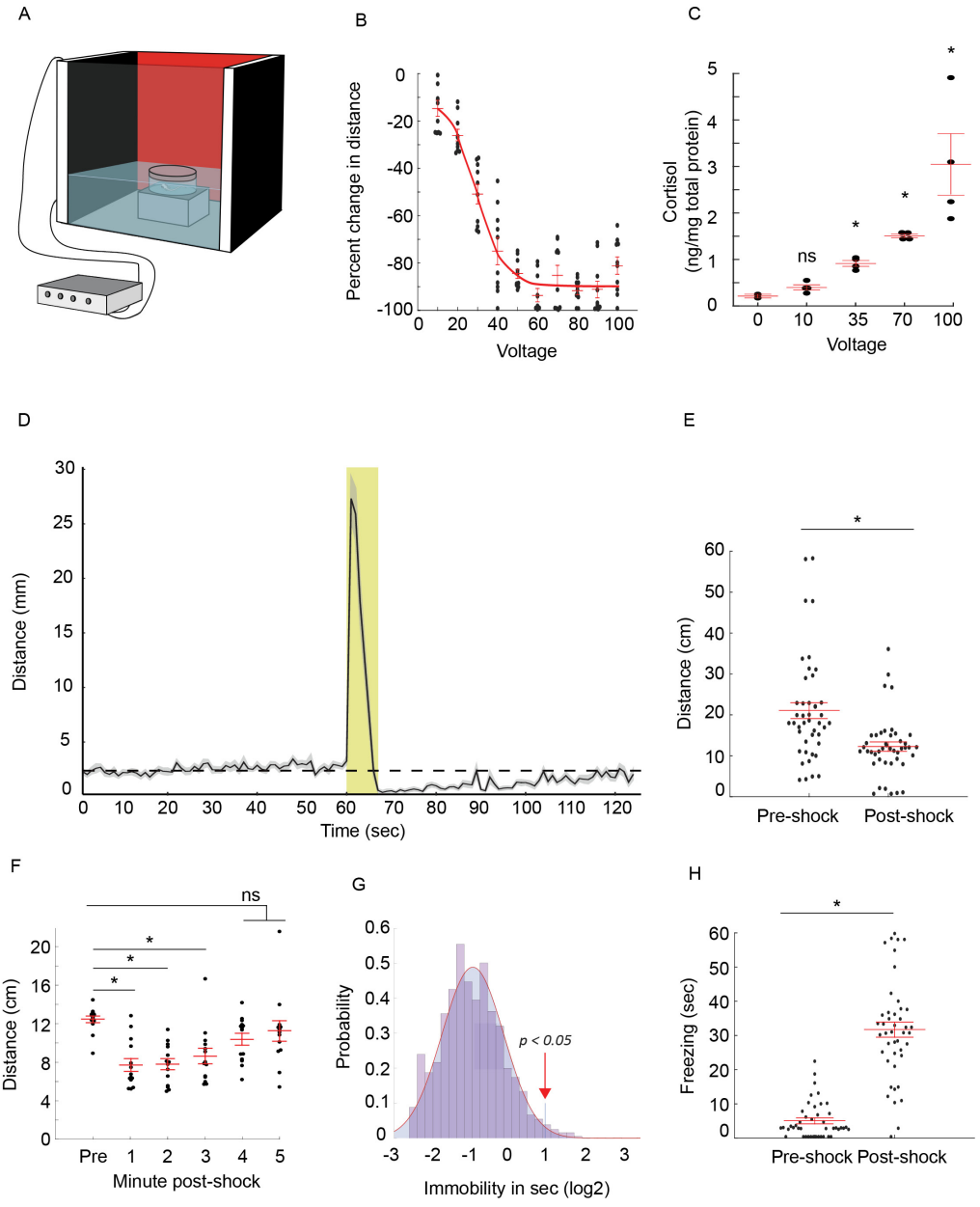

Figure S1. Electric field stimulation elicits fear responses in larval zebrafish; related to figures 1-3

(A) Diagram of testing chamber. (B) Larvae display an intensity dependent decrease in total distance traveled during 1 min post-shock. The red line represents a statistical fit to the data points (n=10 larvae per data point, 4-point sigmoid nonlinear regression, R^2=0.99). (C) The rise in cortisol levels post-shock also depended on voltage intensity (n=4 pools of 15 larvae, Kruskal-Wallis ANOVA H=18.33; p<0.01, Dunn's post-test, p<0.05 for 35, 70 and 100 V). (D) Mean locomotor activity (black) relative to pre-shock levels (dashed line), and standard error (light gray) for wild-type larvae (n=44) exposed to electric shock (gold box, 5 sec). There was no correlation between level of hyperactivity during shock and the change in distance traveled post-shock (Pearson's correlation R = 0.24, p=0.11), suggesting that variability between individuals was not due to differences in sensing shock. (E) Total distance traveled during 1 min pre- and post-shock intervals (n=44; Wilcoxon W=-868.0, p<0.0001). (F) By 4 min post-shock, larvae resume pre-shock levels of locomotor activity (n=14, Kruskal-Wallis ANOVA H=28.49; p<0.0001, Dunn's post-test p<0.05 for 1,2 and 3 min post-shock). (G) Histogram depicting duration of immobility bouts (log2) in freely swimming larvae, with the corresponding probability distribution function overlaid (red). The red arrow indicates duration of immobility exceeding p=0.05. (H) Freezing durations are significantly increased following shock (n=44, Mann-Whitney U=88.5; p<0.0001). Black circles in B, C, E, F and H correspond to individual larvae, and red bars represent the mean ±SEM. In E and H, pre- and post-shock measurements correspond to 1-min intervals. No significance (ns) and significance below p=0.05 (*) are denoted.

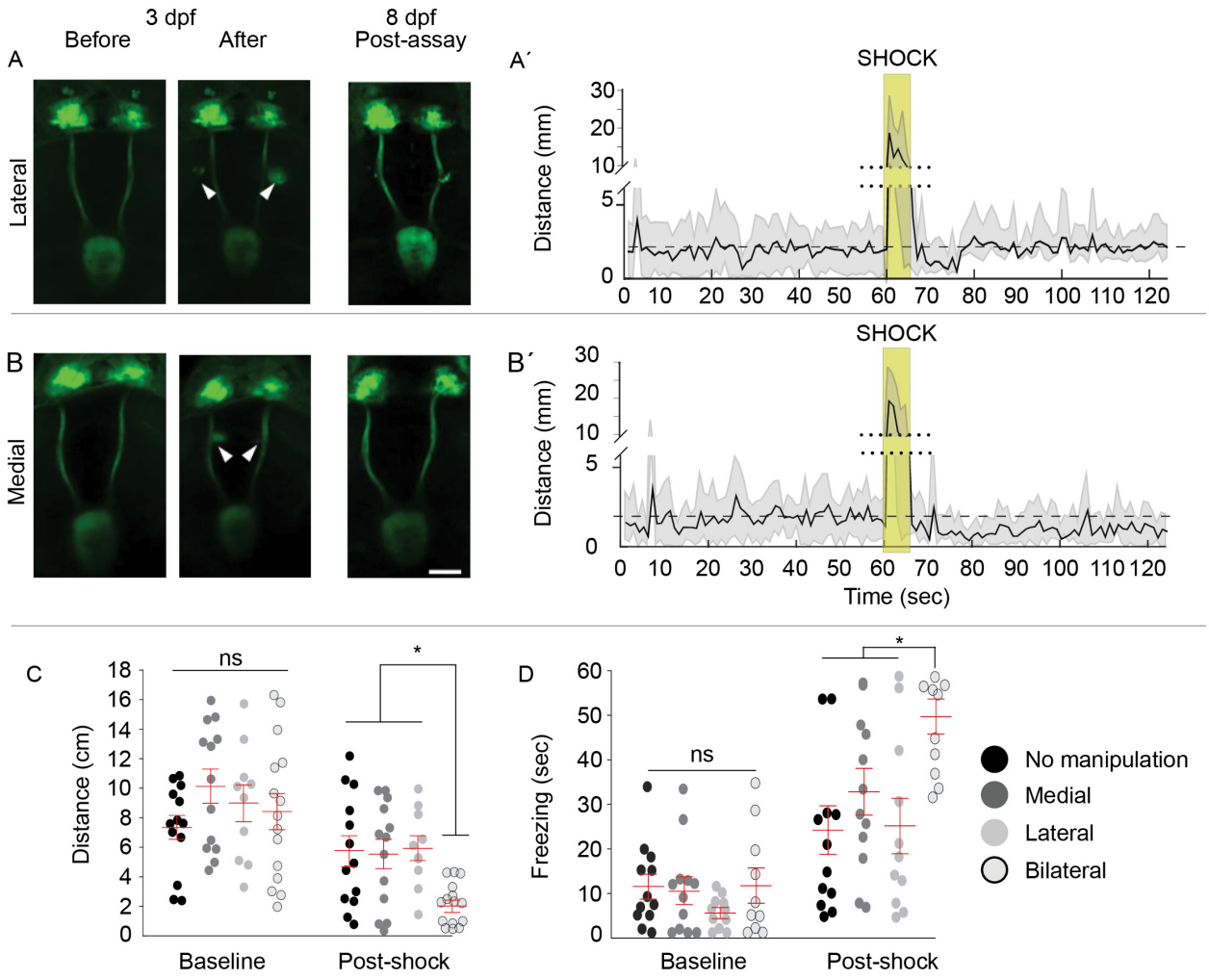

Figure S2. Increased freezing after shock in larvae with bilaterally severed FR; related to Figure 1

(A-B) Live confocal images of the Hb-IPN pathway in *Tg(gng8:nfsB-GFP-CAAX)* controls with lesions (white arrow heads) (A) lateral (n=12) or (B) medial (n=11) to the FR captured at 3 d before and after laser exposure, and 5 day later following behavioral testing. Scale bar is 50 µm. (C) Total distance traveled significantly decreased post-shock in larvae with bilaterally severed FR (n=15; Kruskal-Wallis ANOVA H=27.3; p<0.0001; Dunn's post test, p<0.05) compared to uncut controls (n=13) or those with lesions medial (n=13) or lateral (n=10) to the FR. (D) Freezing was also significantly increased (Kruskal-Wallis ANOVA H=28.22, p<0.0001; Dunn's post-test, p<0.05). Horizontal bars superimposed over scatter plots of individual larvae (circles) represent mean ±SEM for 1-min pre- and post-shock intervals. No significance (ns) and significance below p=0.05 (*) are denoted.

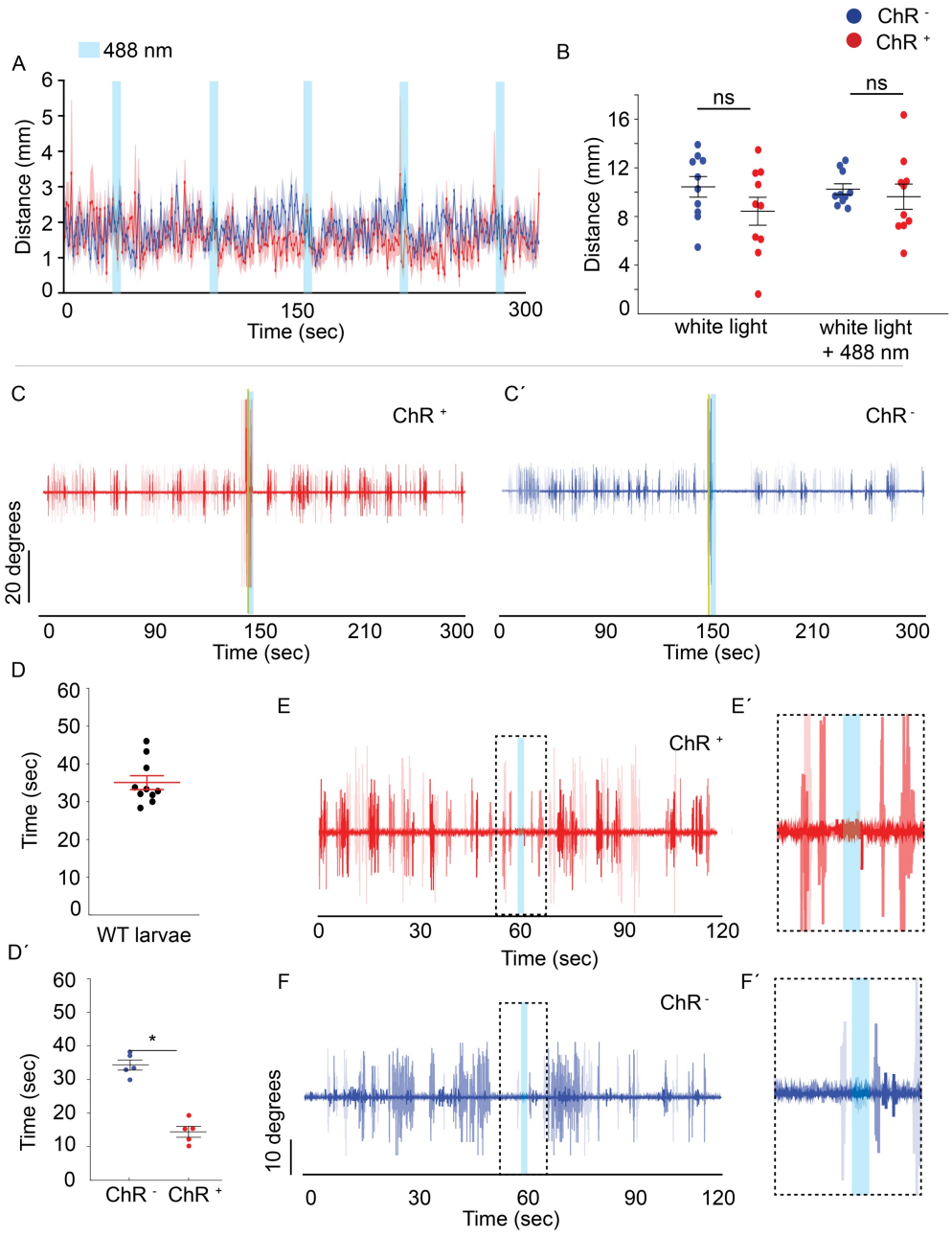

Figure S3. Optogenetic activation of dHb expedites recovery of tail movements following shock in head affixed larvae; related to Figures 2 and 4

(A) Mean activity (blue and red lines) and the standard error (shaded) for larvae expressing ChR2-mCherry in the dHb (red) or control siblings lacking the transgene (blue). Timing of blue light exposure (blue box) indicated. (B) Mean distance traveled during blue light exposure (white light + 488 nm) and 5-sec period immediately preceding it (white light). Neither blue light exposure (Mann-Whitney U=53.0; p>0.05) nor optogenetic activation of the dHb (Mann-Whitney U=60.0; p > 0.05) changes locomotor activity. Circles in B correspond to individual larvae and black bars represent the mean ±SEM. No significance (ns) is denoted. (C-C´) Overlay of three representative traces of tail movements of head affixed larvae expressing (C) ChR2 in the dHb (shades of red) or (C´) controls lacking ChR2 (shades of blue). Shock (gold line) and 488 nm light (blue box) exposure indicated. (D) Initiation of tail movements post-shock in wild-type larvae (n=10) (D´) ChR2 mediated activation of the dHb reduced the time lag (n=5 per group; Mann Whitney U=0, p<0.01). (E-F) Overlay of three representative traces of tail deflections by head affixed larvae (E) expressing (red) or (F) not expressing (blue) ChR2 in the dHb. Exposure to 488 nm light is indicated (blue box). (E´, F´) Enlargement of dashed box in panel E and F. Circles in B, D and D´ correspond to individual larvae, and black bars represent mean ±SEM. Significance below p=0.05 (*) is denoted.

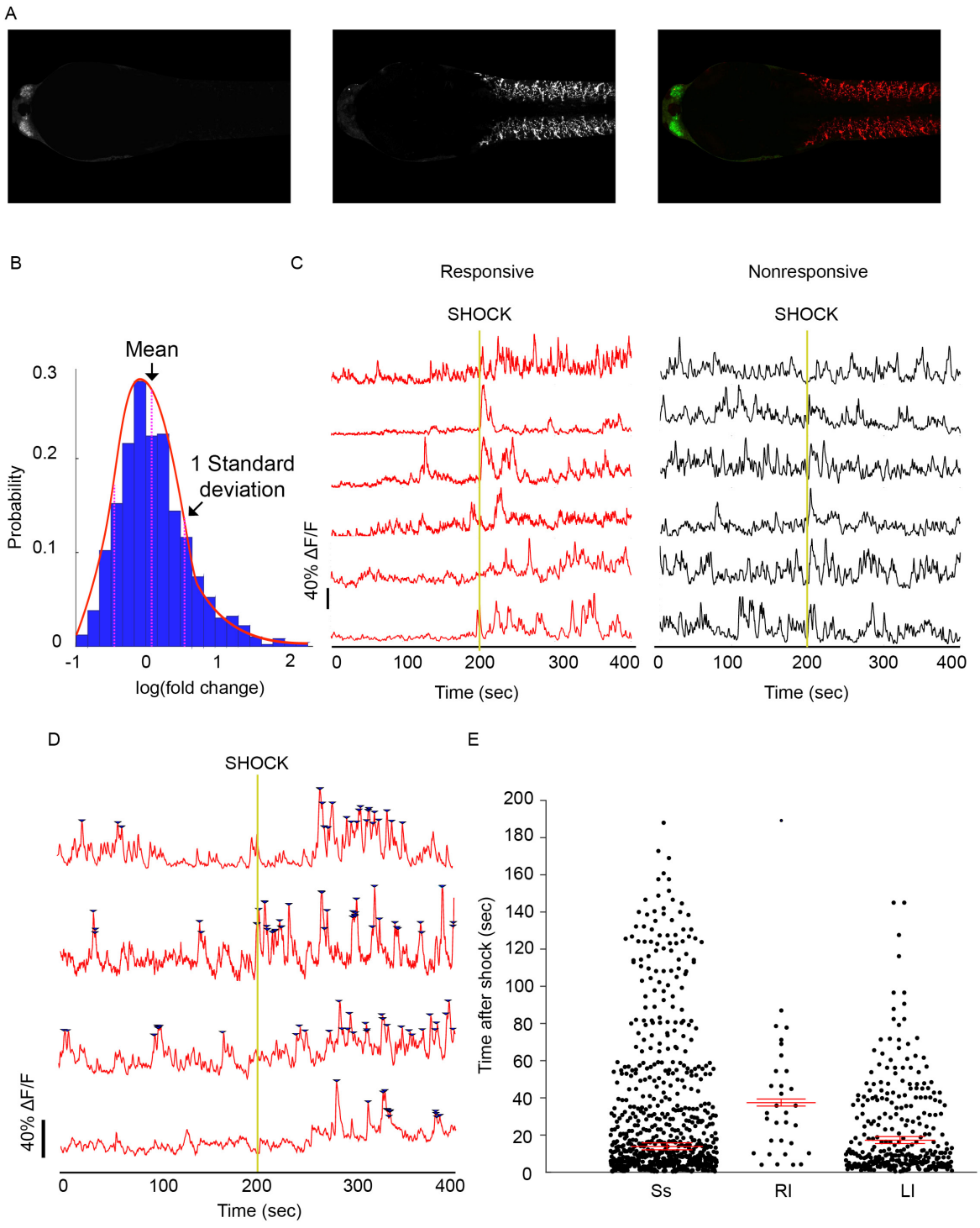

Fig S4. Increase in dHb neuronal activation post-shock; related to Figure 4

(A) Live imaging of 8 day *Tg(gng8:gal4);Tg(UAS:GCaMP7a)* larva paralyzed with rhodamine conjugated α-bungarotoxin. Dorsal view, anterior to the left. Left panel shows GCaMP7a labeling, middle panel is rhodamine labeling and right panel is merged image. Background fluorescence was masked using Fiji and Matlab. (B) Histogram of log fold change in GCaMP7a activation (ln) of 4195 neurons post-shock, with corresponding probability density function (solid red line). Mean and standard deviation indicated by vertical dashed lines. Neurons were defined as responsive when their fold change in activity exceeded 1 standard deviation. (C) Representative traces of fluorescence (ΔF/F) for responsive (red) and non-responsive neurons (black). (D) Representative traces of responsive neurons with firing events (blue arrowheads) identified using the *findpeaks* function in MATLAB. (E) Distribution of timing to first firing event post-shock for responsive neurons in the dHb of situs solitus larvae, and those with left or right isomerized dHb.