

Fig. S1. (A) A western blot where increasing amounts of zebrafish brain homogenate (10 - 50 µg) were loaded on 10% SDS-PAGE gel and transferred to a nitrocellulose membrane. ECL detection of Hsd11b2 (bands outlined by red rectangle) using the chicken-anti-Hsd11b2 antibody. (B) Fluorescent detection of total protein on the same blot using SYPRO Ruby Red Protein Blot Stain. (C) A linear regression analysis of Hsd11b2/total protein band intensity in proportion to increased protein loading showing the linear range of detection, represented by the black trendline (10 - 40  $\mu$ g, R<sup>2</sup> = 0.9913), and that above 40 µg, the signal begins to plateau and sample loading does not exhibit the expected increase in band intensity, represented by the grey dashed trendline (10 - 50  $\mu$ g, R<sup>2</sup> = 0.5673). (D) A western blot where 20  $\mu$ g aliquots of zebrafish brain homogenate were loaded into each lane and then divided into 3 pieces following transfer (red line). From left to right, the split blots were incubated in 0.5 µg ml<sup>-1</sup> chickenanti-Hsd11b2 (38-KDa), pre-immune serum, and 0.5 µg mL<sup>-1</sup> chicken-anti-Hsd11b2 containing 5 µg mL<sup>-1</sup> immunizing peptide. There was no immunoreactivity observed in the pre-immune or immunizing peptide sections proving the specificity of the chickenanti-Hsd11b2 antibody.



**Fig. S2.** Representative western blots showing **(A)** Hsd11b2 protein expression determined using chicken-anti-Hsd11b2 antibody, and **(B)** total protein determined using SYPRO Ruby Red Protein Blot Stain following exposure to a single (solid blue) and repeat (blue outline) 1-min air exposure stressor and recovery in the brains of adult zebrafish. Representative western blots of **(C)** Hsd11b2 protein expression determined using chicken-anti-Hsd11b2 antibody, and **(D)** total protein determined using SYPRO Ruby Red Protein Blot Stain following 24 h of social stress in the brains of dominant (Dom) and subordinate (Sub) zebrafish; group-housed (GH) fish are included as a control. IC = internal control from pooled brain homogenates.



**Fig. S3.** Effects of an acute (closed blue circles) and repeat (open blue circles) 1-min air exposure stressor on *hsd11b2* transcript abundance in brain zone 1 (telencephalon, olfactory bulbs; n=2 fish pooled per sample). **(A)** Pooled brain zone 1 *hsd11b2* transcript abundance after exposure to an acute, and **(B)** repeat acute stressor. Gene transcript abundance is normalized to the mean expression of two housekeeping genes (*ef1a* and *rpl8*). Data is presented as individual data points, with solid lines and whiskers representing the mean ± SEM for each timepoint (*hsd11b2* transcript abundance acute stressor n = 3-4, repeat acute stressor n = 3). Differences in *hsd11b2* mRNA levels following acute and repeat acute stress exposure were determined using a one-way ANOVA with a Tukey's *post-hoc* test (*P* > 0.05).



**Fig. S4.** Effects of an acute (closed blue circles) and repeat (open blue circles) 1-min air exposure stressor on *pcna* transcript abundance in the pooled brain zone 1 (telencephalon, olfactory bulbs; n=2 fish pooled per sample). **(A)** Pooled brain zone 1 *pcna* transcript abundance after exposure to an acute, and **(B)** repeat acute stressor. Gene transcript abundance is normalized to the mean expression of two housekeeping genes (*ef1a* and *rpl8*). Data is presented as individual data points, with solid lines and whiskers representing the mean ± SEM for each timepoint (*pcna* transcript abundance acute stressor n = 3-4, repeat acute stressor n = 3). Outliers removed from treatment groups represented by black triangles. Differences in *pcna* mRNA levels following acute (*P* < 0.05) and repeat acute stress (*P* > 0.05) exposure were determined using a Kruskal Wallis test followed by Dunn's *post-hoc* test. Letters indicate significant differences among groups within a panel; groups which have the same letter are not significantly different from one another.



Fig. S5. Effects of an acute (closed blue circles) and repeat (open blue circles) 1-min air exposure stressor on gr and mr transcript abundance in the pooled brain zone 1 (telencephalon, olfactory bulbs; n=2 fish pooled per sample). (A) Pooled brain zone 1 gr transcript abundance after exposure to an acute, and (B) repeat acute stressor. (C) Pooled brain zone 1 mr transcript abundance after exposure to an acute, and (D) repeat acute stressor. Gene transcript abundance is normalized to the mean expression of two housekeeping genes (*ef1a* and *rpl8*). Data is presented as individual data points, with solid lines and whiskers representing the mean  $\pm$  SEM for each timepoint (*gr* transcript abundance acute stressor n = 2-4, repeat acute stressor n= 3; mr transcript abundance acute stressor n = 2-4, repeat acute stressor n = 3). The 6 h post-stress recovery timepoint is omitted for gr and mr mRNA abundance due to an insufficient sample size. Differences in gr and mr mRNA levels following acute and repeat acute stress exposure were determined using a Kruskal Wallis test followed by Dunn's post-hoc test except for acute stress mr expression where a one-way ANOVA followed by Tukey's *post-hoc* test was used. Letters indicate significant differences among groups within a panel; groups which have the same letter are not significantly different from one another.