**Supplemental Figure Legends**

**Supplemental Figure 1**

**S1A. The number of actively dividing cells decreases with time post-injury.** In Fig. 1, we utlilized EdU incorporation at 7 and 14 days to measure cell proliferation. However, EdU labeling is cumulative, allowing one to identify all cells that divided during the labeling period. To obtain snapshots of proliferation in real time, we also carried out PH3 labeling at these same time points post-PTBI. For this experiment, we collected newly eclosed male flies, subjected them to PTBI, and aged them for the specified time periods. We find similar numbers of proliferating cells at 24 hours and 7 days post-PTBI, and observe a strong reduction in proliferation at 14 days post-PTBI. At 24 hours, control brains have an average of 3 PH3+ cells (n=11 brains, 28 cells), while PTBI brains have an average of 11 PH3+ cells (n=17 brains, 181 cells). At 7 days, control brains have an average of 2 PH3+ cells (n=6 brains, 11 cells), while PTBI brains have an average of 12 PH3+ cells (n=7 brains, 82 cells). At 14 days, control brains have an average of 2 PH3+ cells (n=6 brains, 9 cells), while PTBI brains have an average of 4 PH3+ cells (n=6 brains, 24 cells). Unpaired t tests for PTBI to control comparisons at these 3 times points are p<0.0001, p<0.0005, and p<0.06, respectively.

**S1B. Robust proliferative responses decrease with age.** To explore whether age impacts the amount of cell proliferation that occurs post-injury, we compared newly eclosed adult males to animals aged to 7 days, 14 days, and 28 days prior to PTBI. We then used anti-PH3 to assay cell proliferation 24 hours after injury. Flies injured at within 6 hours of eclosion had an average of 11 PH3+ cells/brain (n=17 brains, 182 cells) compared to an average of 3 PH3+ cells/brain in age-matched controls (n=11 brains, 28 cells). Flies that were aged to 7 days, then subjected to PTBI had an average of 6 PH3+ cells/brain (n=11 brains, 65 cells) compared to age-matched controls which had an average of 2 PH3+ cells/brain (n= 5 brains, 12 cells). When flies were aged to 14 days prior to PTBI, and assayed 24 hours later, there was an average of 1 PH3+ cell/brain (n=8 brains, 11 cells) similar to age-matched controls which also averaged 1 PH3+ cell/brain (n=4 brains, 2 cells). 28-day uninjured control (n=4, 1 cell) and PTBI (n=3, 1 cell) flies both averaged 0 PH3+cells/brain. Unpaired t tests for PTBI to control comparisons at these 4 time points are p<0.0001, p<0.04, p<0.07, and p<0.84, respectively.

**S1C. Pulse-chase and continuously fed EdU animals have similar numbers of EdU+ cells at 7 days post-PTBI.** To assess whether mitotically active cells survive post-PTBI, we used two methods of feeding EdU, pulse-chase (flies are fed EdU for 4 days, then placed on standard sugar food for 3 days) and continuously fed (flies are fed EdU every day before being assayed). At 7 days post-PTBI, we find that pulse-chase PTBI brains have an average of 14 EdU+ cells (n=8, 108 cells) while continuously fed PTBI brains have an average of 9 EdU+ cells (n=7, 64 cells). Although there is a trend of fewer EdU+ cells in the continuously fed animals, an unpaired t test reveals that this is not significantly different (p<0.22). Error bars reflect the standard deviation (SD).

**S1D. Cell proliferation is not limited to the damaged right hemisphere.** In order to determine where cell proliferation occurs post-PTBI, we counted EdU+ cells in the left and right hemispheres of the central brain. At 7 days, the left hemispheres of control brains have an average of 1 EdU+ cells (n=9 brains, 5 cells) while PTBI brains have an average of 4 EdU+ cells (n=15 brains, 63 cells; p<0.04). In the right hemisphere, control brains have an average of 1 EdU+ cell (n=9 brains, 10 cells), while PTBI brains have an average of 7 EdU+ cells (n=15 brains, 109 cells; p<0.002). Error bars reflect the standard deviation (SD).

**S1E. There is no significant difference in the number of PH3+ and EdU+ cells observed 24 hours post-PTBI.** We use two methods, anti-PH3 immunochemistry to label mitotic cells and EdU labeling of newly synthesized DNA to assay cell division. To assess the extent to which anti-PH3 and EdU labeling are comparable, we evaluated both control and PTBI brains with both methods at 24 hours. We expected to detect more EdU-labeled cells than anti-PH3 labeled cells post-PTBI because anti-PH3 transiently labels cells during M phase of the cell cycle while EdU labeling is cumulative. Instead, we observed similar numbers of labeled cells with the two assays. Specifically, in control brains, there were an average of 3 PH3+ cells (n=11 brains, 28 cells) and 2 EdU+ cells (n=6 brains, 13 cells). In PTBI brains, there were an average of 11 PH3+ cells (n=17 brains, 181 cells) and 11 EdU+ cells (n=6 brains, 65 cells). Thus, while control and injured brains displayed significant differences in cell proliferation with both assays (PH3: p<0.0001, EdU: p<0.0005), the number of proliferating cells detected with the two methods was not significantly different. Error bars reflect the standard deviation (SD). There are several potential explanations for this result. For instance, it could be that EdU does not efficiently penetrate the blood brain barrier and/or diffuse through the brain tissue. In this case, only a subset of proliferating cells would be labeled, and EdU labeling would underreport cell proliferation. Alternatively, some of the dividing cells may die. Indeed, DNA synthesis does precede cell death in some contexts (e.g. (Rimkus *et al.* 2008)). However, the fact that there are similar numbers of EdU positive cells in post-PTBI brains when animals are continuously fed EdU and when they are pulse-chased with EdU (**Fig. S1C**), supports the idea that most dividing cells are viable. It therefore seems likely that EdU does not efficiently penetrate the blood brain barrier and/or diffuse through the brain tissue, particularly in older animals.

**Supplemental Figure 2**

**S2A,B. PTBI decreases lifespan. A.** To assess the impact of PTBI on viability, we performed a lifespan assay with control and PTBI adult male flies. For each condition, >300 flies were assayed. Aging was carried out in vials containing 20-40 flies. Dead flies were counted and surviving flies placed on clean food every 2-3 days. Within the first 12 days, there was no significant difference in survival between control and injured flies. However, beyond 12 days, we saw a significant drop in survival of injured animals. Control males reached 50% survival at 70 days of age, while PTBI males reached 50% survival at 48 days. The maximum lifespan was 84 days for uninjured flies and 74 days for injured flies. This indicates that PTBI does impact lifespan. However, in the window of 0-12 days, there is no significant difference in survival, suggesting that death is more likely due to delayed secondary injury. Error bars reflect the standard deviation (SD). **B.** To test whether age at injury affects outcome, we compared flies injured at 0-6 hours, 14 days, and 28 days post-eclosion. Survival was assayed 24 hours later. Control flies at all ages and flies injured 0-6 hours post-eclosion, exhibited survival of 98-99% with no significant difference between age-matched control and PTBI flies (p<0.69). However, flies injured 14 days post-eclosion showed a significantly reduced survival of 88% (p<0.0001), while flies injured 28 days post-eclosion showed a further reduction to 75% survival (p<0.0001) compared to age-matched controls. These data indicate that age at the time of PTBI does affect survival. Error bars reflect the standard deviation (SD).

**S2C-D’. Cell death following PTBI is increased transiently after injury.** To directly test whether PTBI causes cell death, we used terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). TUNEL marks the terminal stages of death, both apoptotic and necrotic, when nuclear DNA has been cleaved and degraded by DNases (Grasl-Kraupp et al. 1995). We assayed cell death using TUNEL staining (in green) in control and PTBI brains at 4 hours, 24 hours and 10 days post-PTBI. DAPI is in blue. We observe a transient increase in cell death. At 4 hours post-PTBI, there are an average of 37 TUNEL+ cells/brain (n=5 brains, 183 cells) compared to only 13 TUNEL+ cells/brain (n=5 brains, 67 cells) in uninjured controls. At 24 hours post-PTBI, there are an average of 21 TUNEL+ cells/brain (n=5 brains, 107 cells) compared to only 11 TUNEL+ cells/brain (n=5 brains, 53 cells) in uninjured controls (compare **D** and **D’** to **C** and **C’**). And at 10 days post-PTBI, there are an average of 26 TUNEL+ cells/brain (n=5 brains, 132 cells) compared to 24 TUNEL+ cells/brain (n=5 brains, 121 cells) in uninjured controls. Unpaired t tests yield p values of p<0.003, p<0.02, and p<0.7 for the 3 time points, respectively. Error bars reflect the standard deviation (SD). Thus, although the total number of dying cells increases with age in the control brains, cell death due to PTBI appears to peak shortly after the injury such that by 10 days post-injury, there was no significant difference between control and injured brains (**F**).

**S2E,G,H. PTBI increases neurodegeneration.** To ask whether the observed cell death following PTBI corresponded to neurodegeneration, we used a standardized index (Cao et al. 2013) to analyze histological preparations from brains 25 days after PTBI. Controls exhibited little neurodegeneration at 25 days (**E**). In PTBI flies, we observed an increase in the number of lesions (**G**). Using the neurodegeneration index described in Cao et al., 2013, controls had an average neurodegeneration index score of 1.7+/-0.2, while PTBI flies had an average neurodegeneration index score of 3.0+/-0.4 (**H**). This represented a statistically significant difference (p<0.01). Error bars reflect the standard deviation (SD).