Figure S3. DAF2-DA assay for detection of NO production. (A) NOS enzymatic activity visualized by NADPH-diaphorase staining in targeted overexpression of NOS to the PG cells (outlined) (phm>NOS) and control (phm>LacZ). Larvae were raised at 21°C and brain complexes were dissected from wandering larvae. Scale bars = 200μm. (B) NOS overexpression in the PG (phm>NOS) increases NO production in the PG cells (outlined). Measurement of nitric oxide (NO) production by the fluorescent reporter DAF2-DA. Larvae were raised at 21°C and brain complexes with the PG were isolated and stained at 117hr AED. Scale bar = 100μm. n: phm>LacZ = 29, phm>NOS = 30. Statistical analysis: mean +/- SEM. ** p<0.01 calculated by two-tailed Student’s t-test.