

# L-Proline Prevents Endoplasmic Reticulum Stress in Microglial Cells Exposed to L-Azetidine-2-carboxylic Acid

Jordan Allan Piper <sup>1</sup>, Nour Al Hammouri <sup>1</sup>, Margo Iris Jansen <sup>1</sup>, Kenneth J. Rodgers <sup>2</sup>,  
Giuseppe Musumeci <sup>3</sup>, Amolika Dhungana <sup>4</sup>, Sahar Masoumeh Ghorbanpour <sup>4</sup>, Laura A Bradfield <sup>4</sup>  
and Alessandro Castorina <sup>1,\*</sup>

## Supplementary Figures

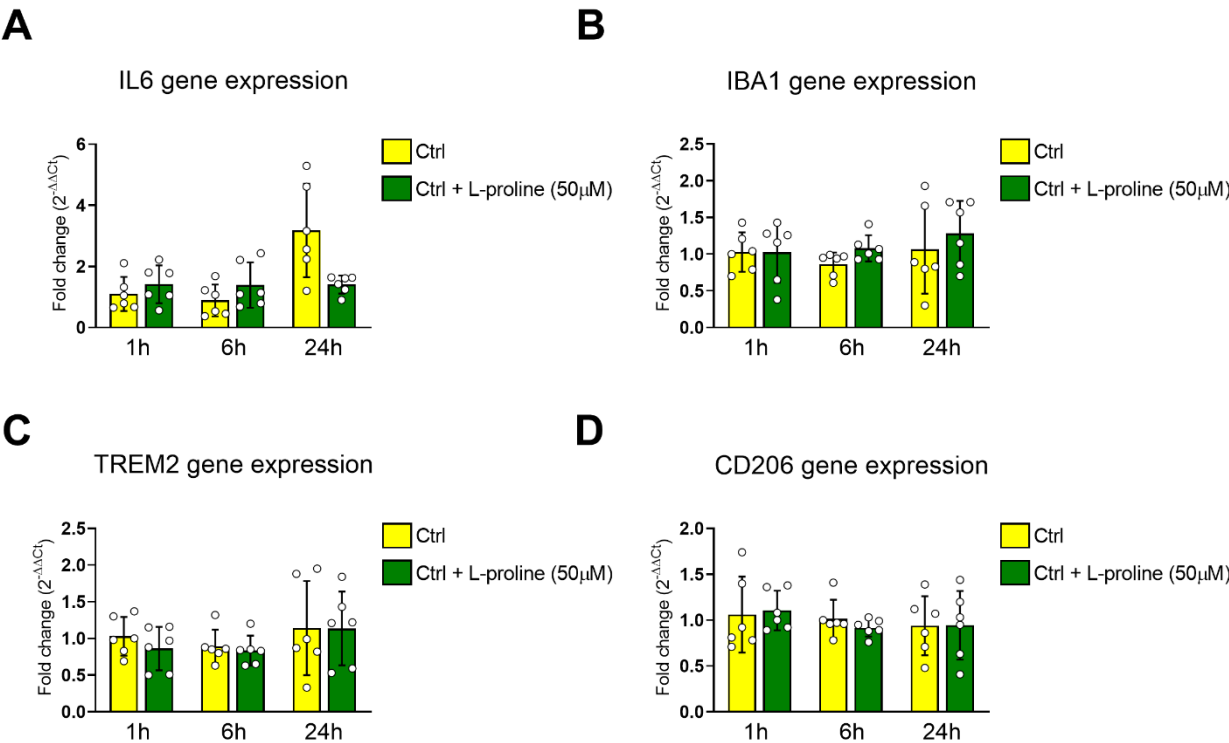


Figure S1. Effects of L-proline treatment alone on the expression pro- and anti-inflammatory genes. Gene expression in untreated cells or treated with L-proline (50 μM) at 1, 6 and 24 h was measured using real-time qPCR and quantified using the  $\Delta\Delta C_t$  method after normalization to the S18 housekeeping gene. Bar graphs display comparative gene expression changes of the following inflammation-related genes: (A) IL6, (B) IBA1, (C) TREM2 and (D) CD206. Results are presented as mean fold changes vs Ctrl  $\pm$  SEM, obtained from (n=6) biological replicates.

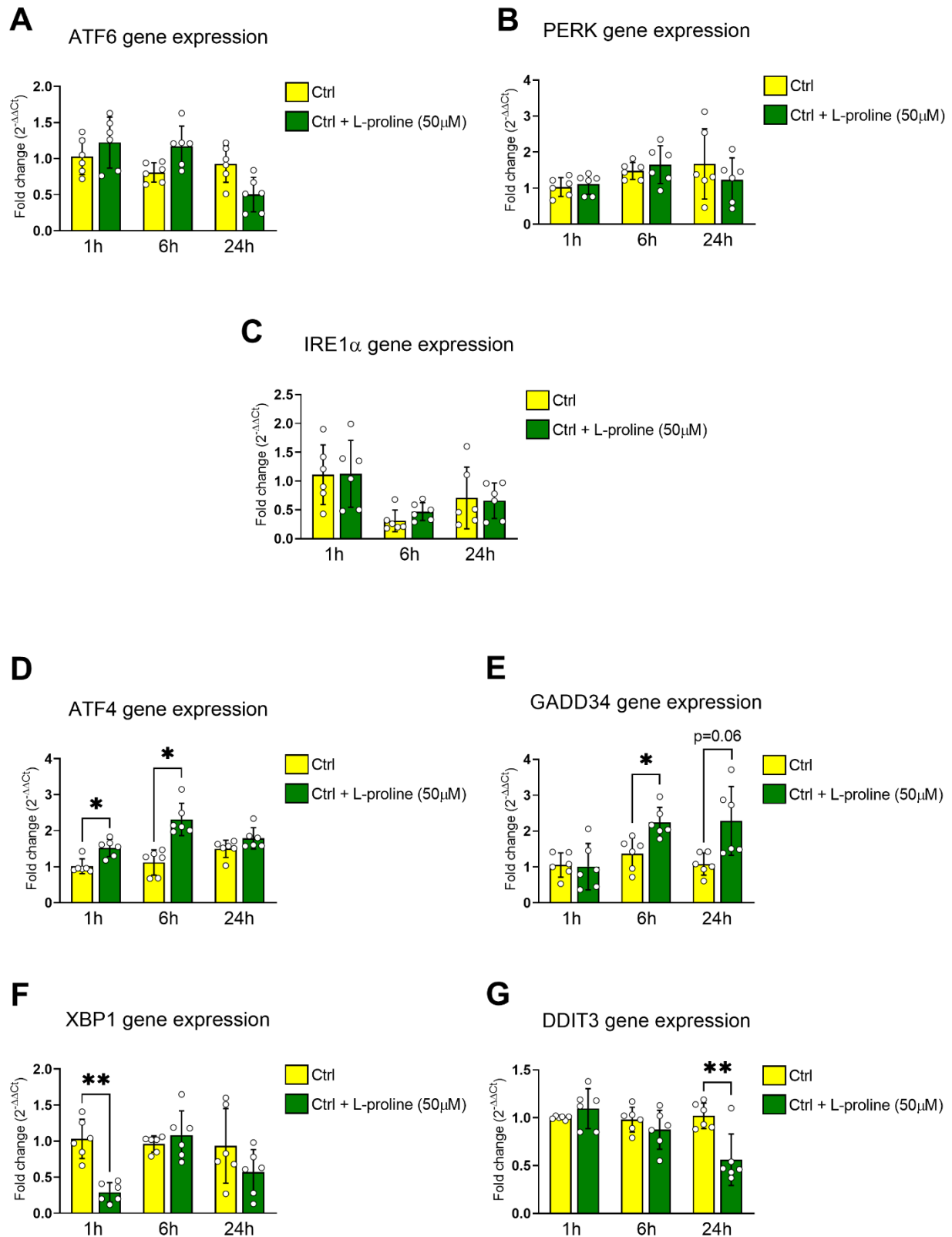


Figure S2. Effects of AZE treatment on the expression of ER stress / UPR genes. Gene expression was measured using real-time qPCR and quantified using the  $\Delta\Delta C_t$  method after normalization to the S18 housekeeping gene. Bar graphs show the differential expression of the following upstream and downstream regulators of the ER stress / UPR response; ATF6, PERK (aka EIF2AK3), IRE1 $\alpha$  (aka ERN1), ATF4, GADD34, XBP1 and DDIT3. Relative expression levels of (A) ATF6, (B) PERK, (C) IRE1 $\alpha$ , (D) ATF4, (E),

GADD34, (F) XBP1 and (G) DDIT3 transcripts were measured in untreated (Control) and L-proline-treated BV2 microglial cells at various times (1, 6 and 24 h, respectively). Results are presented as mean fold changes of Controls  $\pm$  SEM of three independent experiments, each run using two biological replicates per experiment (n=6). \*p<0.05 or \*\*p<0.01 vs Ctrl, as determined by repeated measures ANOVA followed by Tukey post-hoc tests.