**Modeling Neuroimmune Interactions in Human Subjects and Animal Models to Predict Subtype Specific Multidrug Treatments for Gulf War Illness**

**Detailed Data Collection Methods**

## Gulf War Illness Exposure Paradigm and Treatment

All animal experiments were performed using protocols approved by the Centers for Disease Control-National Institute for Occupational Safety and Health Institutional Animal Care and Use Committee (CDC-NIOSH IACUC). Adult male C57BL/6J mice (n=5-7 mice per group), 8-12 weeks of age were purchased from Jackson Labs (Bar Harbor, ME). Mice were exposed to CORT (200 mg/L in 0.6% EtOH; Steraloids, Newport, RI, USA) in the drinking water for 4 days. On day 5, mice received a single intraperitoneal injection of DFP (4 mg/kg, MilliporeSigma, St. Louis, MO, USA) or physiological saline. After the initial exposure, mice received intermittent 4-day bouts of CORT water every other week (4 days on/10 days off) for a total of 11 weeks. Following the final 4 day CORT exposure, mice received a single subcutaneous injection of LPS (0.5 mg/kg, MilliporeSigma) or physiological saline. Mice were sacrificed by decapitation at 6 hours following this exposure.

## Tissue Preparation

Immediately after decapitation, whole brains were removed from the skull with the aid of blunt curved forceps. Cortices were dissected free hand on a thermoelectric cold plate (Model TCP-2, Aldrich Chemical Co., Milwaukee, WI, USA) using a pair of fine curved forceps (Roboz, Washington, DC, USA). Brain regions were frozen at -85 °C and used for subsequent isolation of total RNA.

## qRT-PCR

The total RNA from the cortex were isolated using Trizol® reagent (Thermo Fisher Scientific, Waltham, MA, USA) and Phase-lock heavy gel (Eppendorf, AG Hamburg, Germany), and purified using RNeasy mini-spin columns (Qiagen, Valencia, CA, USA). Total RNA (1 ug) was reverse transcribed to cDNA using Superscript III and oligo (dT)12-18 primers (Thermo Fisher Scientific, Waltham, MA, USA) in a 20 µL reaction. Real-time

PCR analysis of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and of the proinflammatory mediators, TNFα, IL-6, CCL2, IL-1β, leukemia inhibitor factor (LIF), and oncostatin M (OSM) was performed in an ABI7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in combination with TaqMan® chemistry as previously described. All PCR amplifications (40 cycles) were performed in a total volume of 50 μL, containing 1 μL cDNA, 2.5 uL of the specific Assay of Demand primer/probe mix (Thermo Fisher Scientific, Waltham, MA, USA), and 25 uL of Taqman® Universal master mix (Thermo Fisher Scientific, Waltham, MA, USA). Sequence detection software (version 1.7; Applied Biosystems/Thermo Fisher Scientific, Waltham, MA, USA) was used to determine threshold cycle (CT) values.