SUPPLEMENTARY METHODS

Open field test

Locomotor activity in mice was tested in a novel open field as described previously (Beurel et al., 2013). Mice were placed in a Plexiglas open field arena (Med Associates, St Albans, VT) outfitted with photobeam detectors under soft overhead lighting, and activity was monitored using activity monitoring software (Med Associates). Distance travelled during each 5 min block was calculated.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Cytokines and chemokines in the hippocampus after one or two stresses.

Mice were subjected to one or two daily sessions of inescapable foot shocks separated by 24 hr. Hippocampi were collected 1, 6, 12, and 24 hr after a single session of inescapable shocks, or 0.25, 1, 3, and 6 hr after two daily sessions of inescapable foot shocks, followed by measurements of cytokines and chemokines by multiplex analysis. Hippocampi from 3-4 mice were pooled in each group for multiplex analysis.

Supplementary Figure 2. Cytokines and chemokines in the prefrontal cortex after one or two stresses.

Mice were subjected to one or two daily sessions of inescapable foot shocks separated by 24 hr. Prefrontal cortex was collected 1, 6, 12, and 24 hr after a single session of inescapable shocks, or 0.25, 1, 3, and 6 hr after two daily sessions of inescapable foot shocks, followed by measurements of cytokines and chemokines by multiplex analysis. The prefrontal cortex from 3-4 mice were pooled in each group for multiplex analysis.

Supplementary Figure 3. Effects of TLR4 deficiency on stress-induced increases in hippocampal cytokines and chemokines.

Wild-type and TLR4$^{-/-}$ mice were subjected to one or two daily sessions of inescapable foot shocks followed by measurements of cytokines and chemokines in the hippocampus by multiplex analysis. Hippocampi were collected 6, 12, and 24 hr after a single stress, or 1 and 3 hr after two stresses. Hippocampi from 3-4 mice in each group were pooled for multiplex analysis. (A) Hippocampal cytokines and chemokines that are diminished in TLR4$^{-/-}$ mice after one or two daily sessions of inescapable foot shocks compared to WT mice. (B) Hippocampal cytokines and chemokines that have similar or

larger increases in TLR4$^{-/-}$ mice compared to wild-type mice after a single or two daily sessions of inescapable foot shocks.

Supplementary Figure 4. Pretreatment with fluoxetine or TDZD-8 attenuate stress-induced cytokines and chemokines in mouse hippocampus.

Following two weeks of daily treatment with fluoxetine (Flx), TDZD-8 or saline (Sal), mice were subjected to two daily sessions of inescapable foot shocks and after 3 hr cytokines and chemokines in the hippocampus were measured by multiplex. Hippocampi from 3-4 mice were pooled in each group for multiplex analysis.

Supplementary Figure 5. Intranasal administration of HMGB1 siRNA blocked the accelerated rate of cytokine and chemokine increases in mouse hippocampus induced by a prior stress.

HMGB1 siRNA or scrambled siRNA was administered intranasally to mice 1 hr after the first of two daily sessions of inescapable foot shocks. Hippocampi were collected 3 and 12 hr after two daily sessions of inescapable foot shocks, followed by measurements of cytokines and chemokines by multiplex analysis. Hippocampi from 4 mice were pooled for multiplex analysis.

Supplementary Figure 6. Effects of stress on prefrontal cortex HMGB1 levels in the prefrontal cortex.

Prefrontal cortex HMGB1 levels 24 hr after a single session of inescapable foot shocks. n = 3-4 in each group.

Supplementary Figure 7. Behavior of TLR4$^{-/-}$ mice.

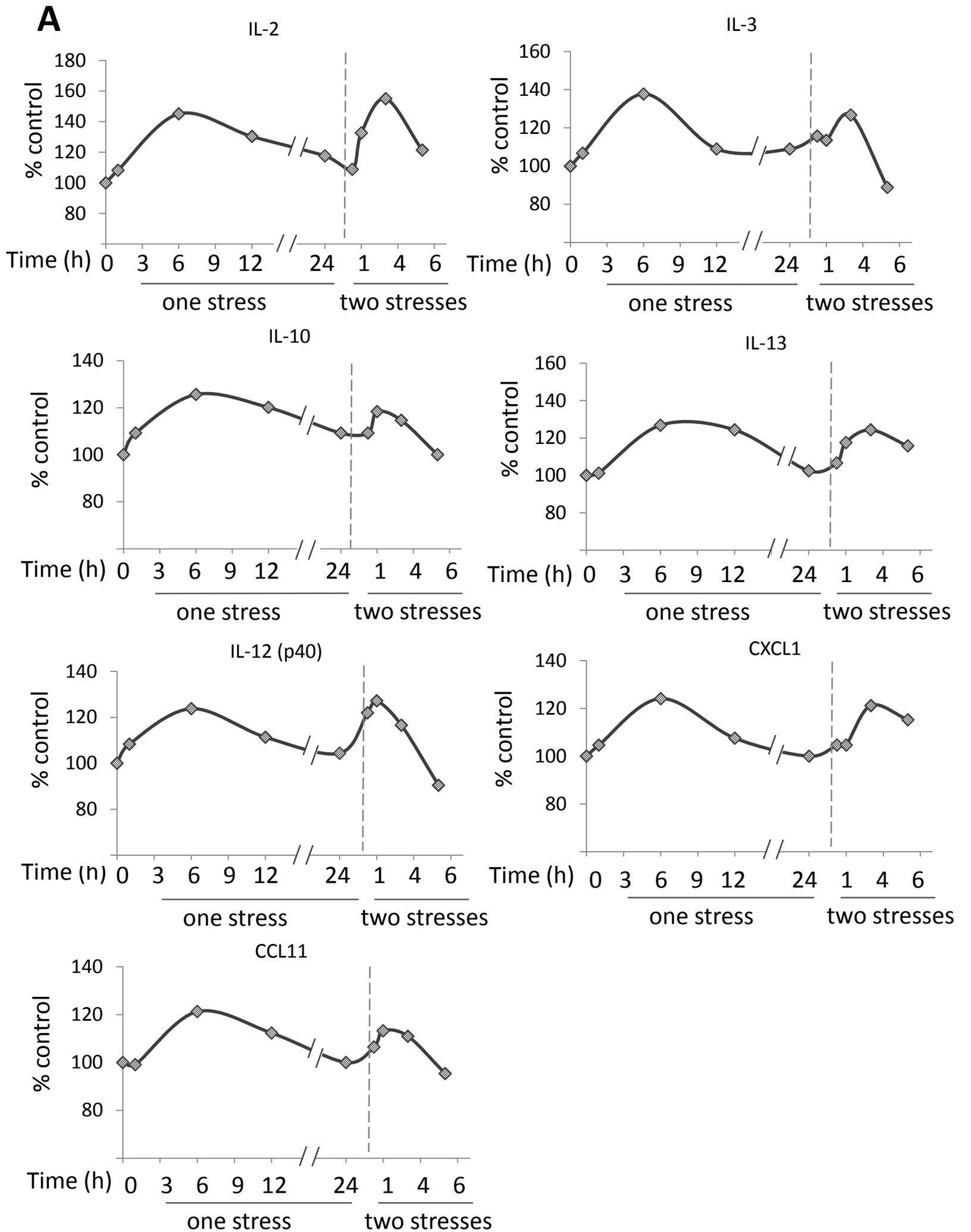
TLR4 deficiency did not alter (A) locomotor activity in a novel open filed (n = 4-5 mice per group) and (B) the sensitivity to foot shocks measured by latency to escape from escapable foot shocks (n = 8-14 mice per group).

Supplementary Table 1. Times of peak levels after one or two stresses for hippocampal cytokines and chemokines.

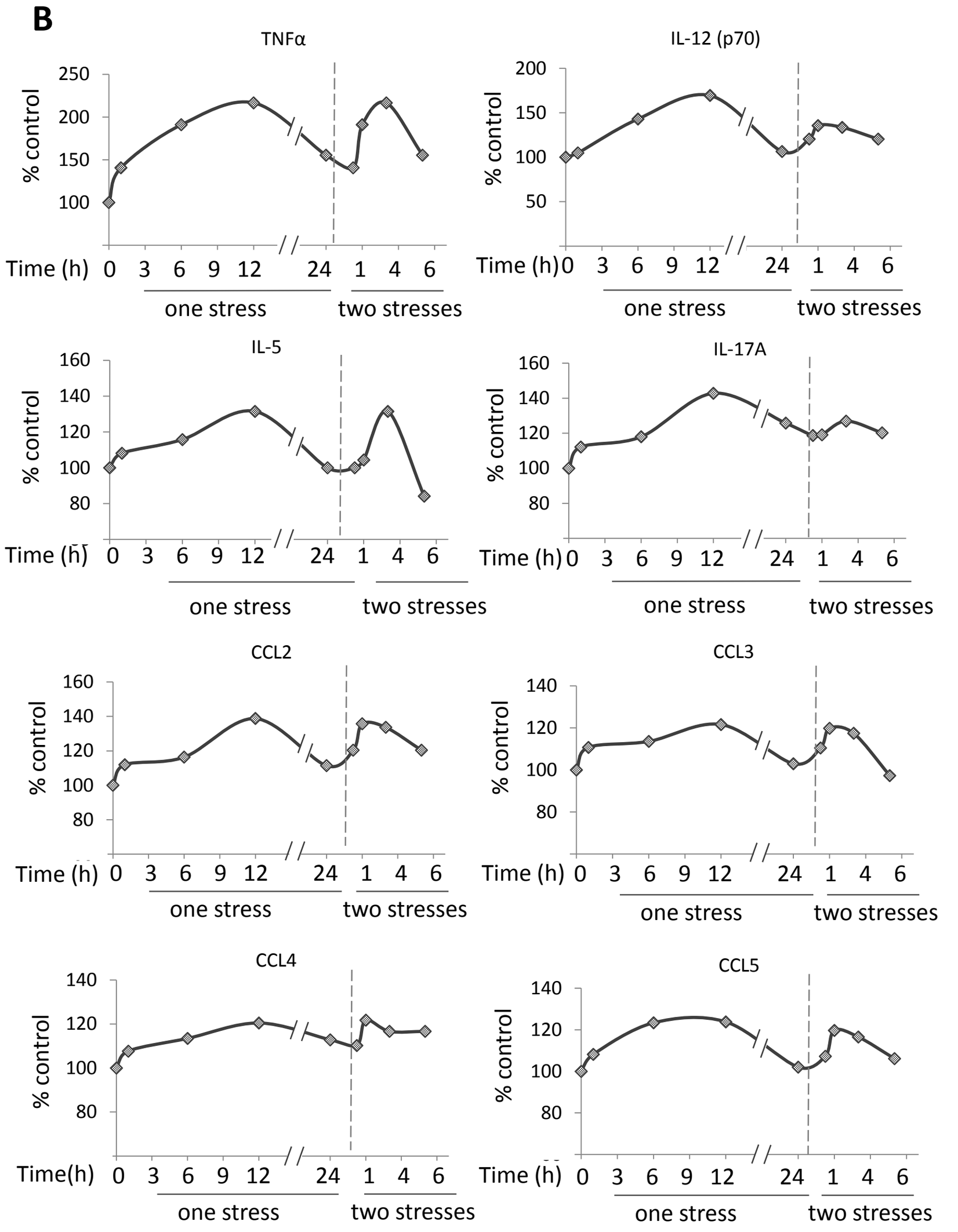
Supplementary Table 2. Reported effects of stress on rodent brain cytokine levels.

Supplementary Table 3. Reported effects of stress on cytokine increases induced by LPS.

Supplementary Figure 1



Supplementary Figure 1



Supplementary Figure 1

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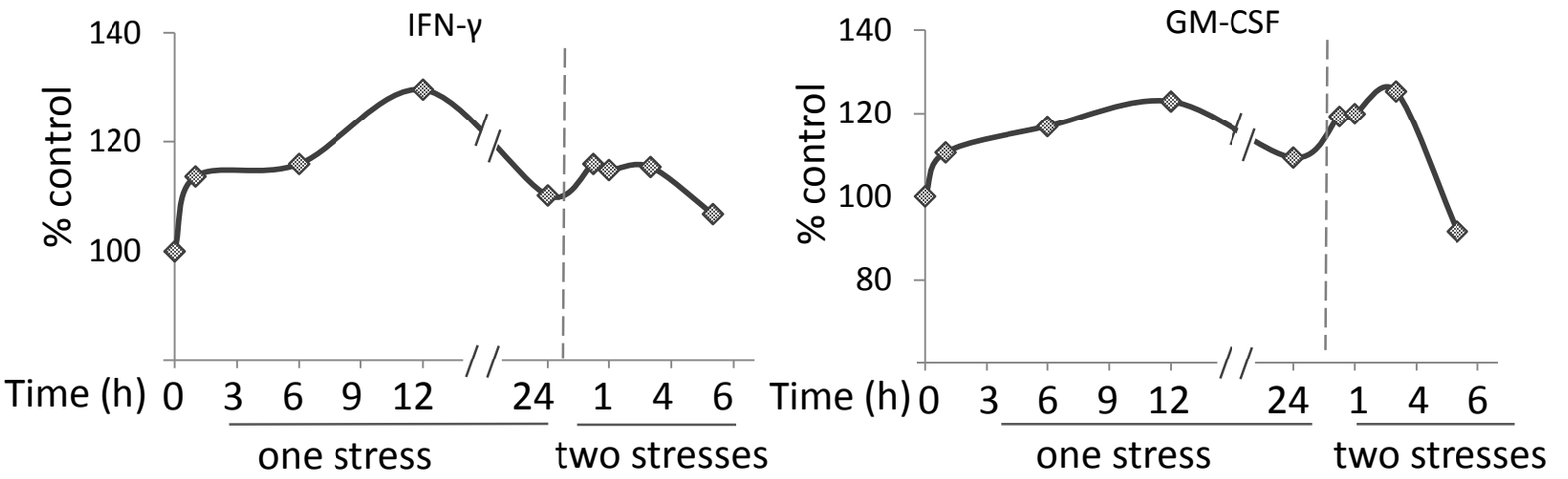

Supplementary Figure 1

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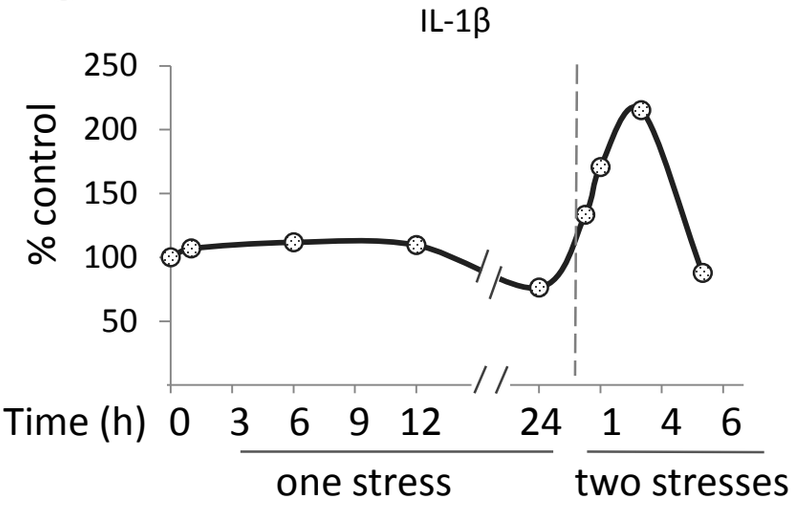

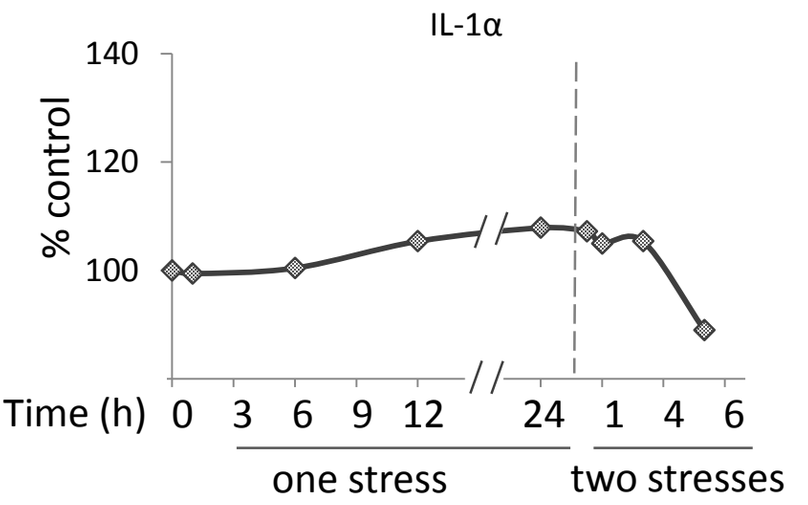

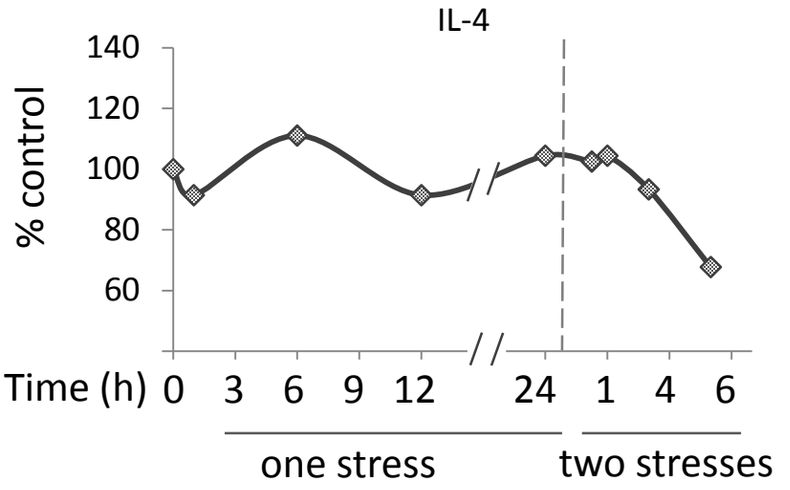

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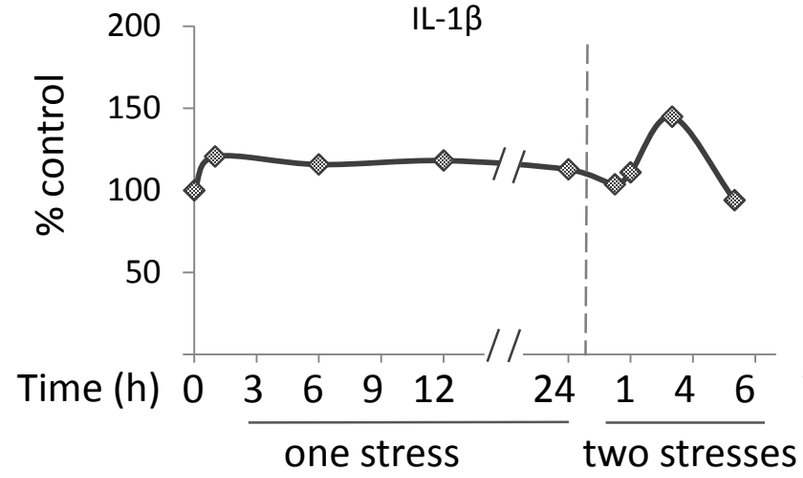

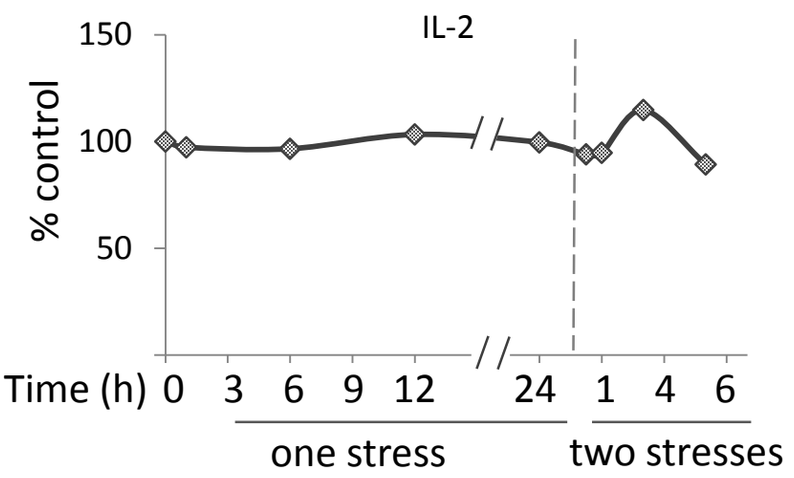

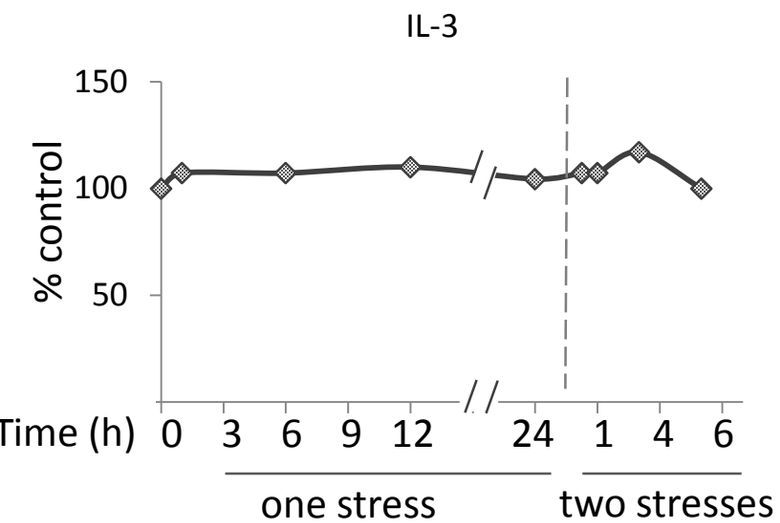

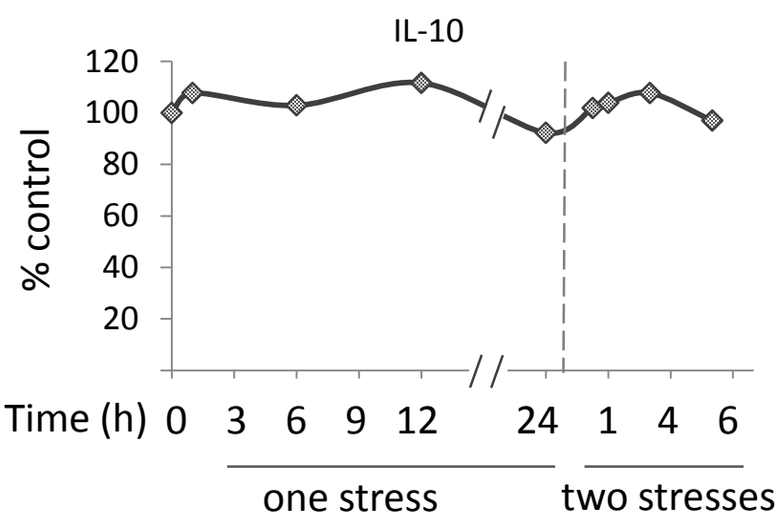

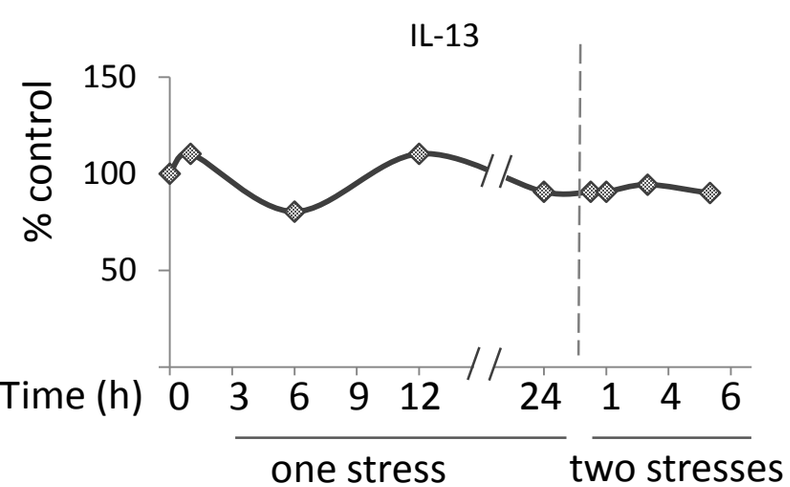

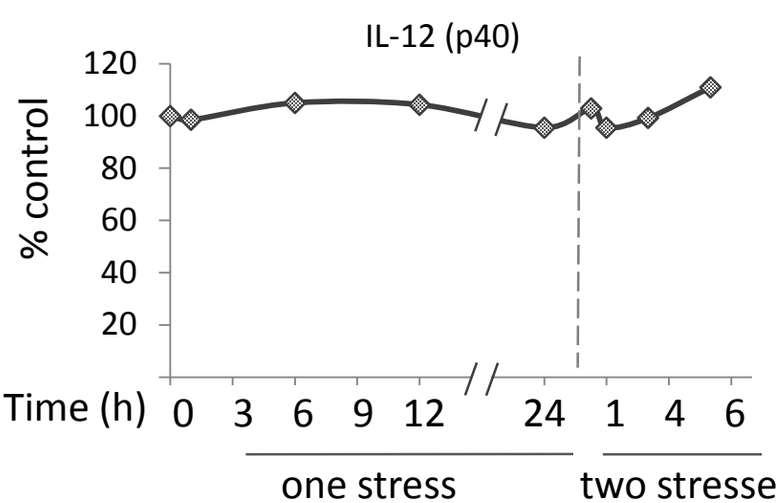

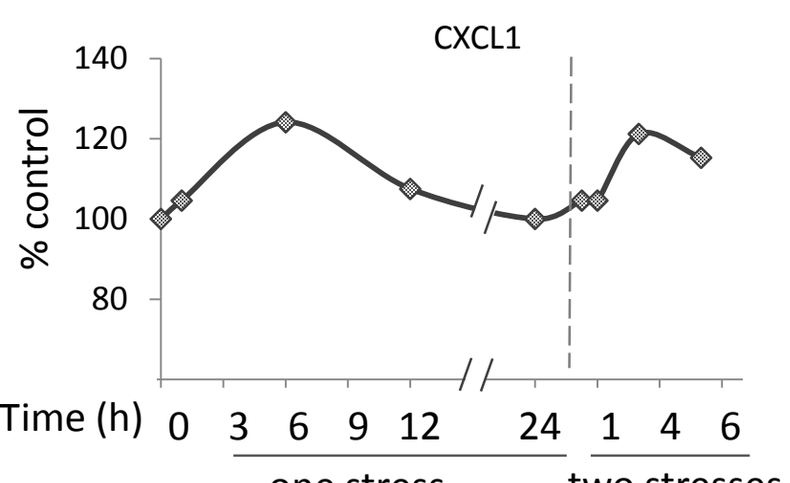

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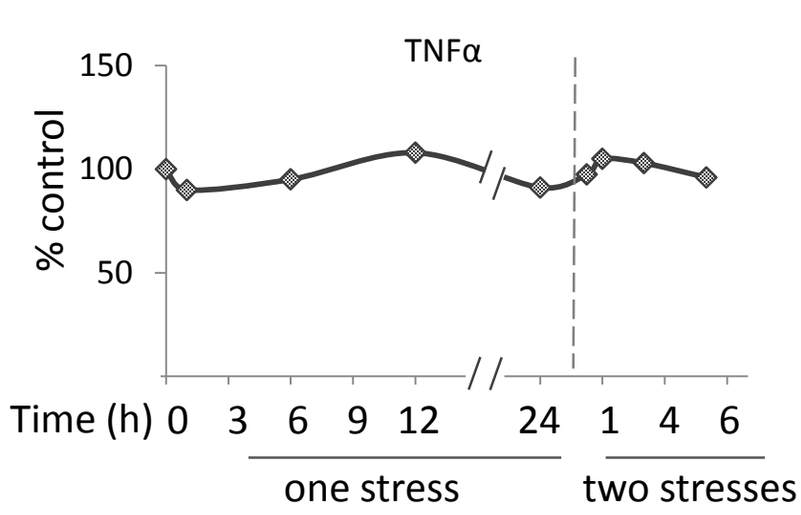

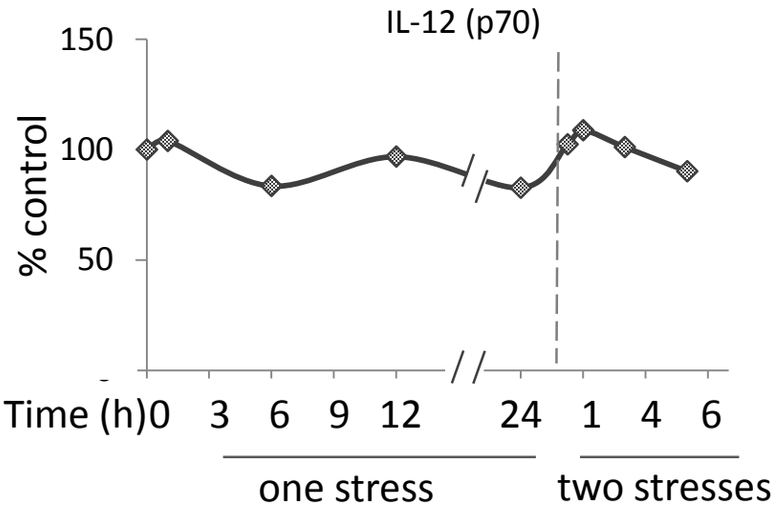

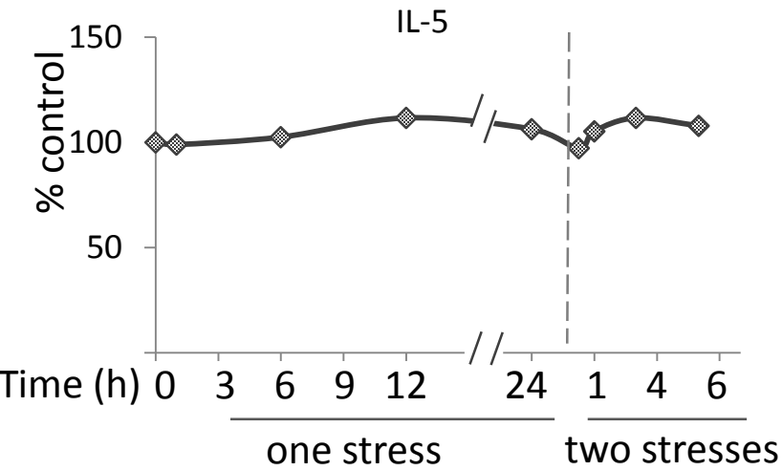

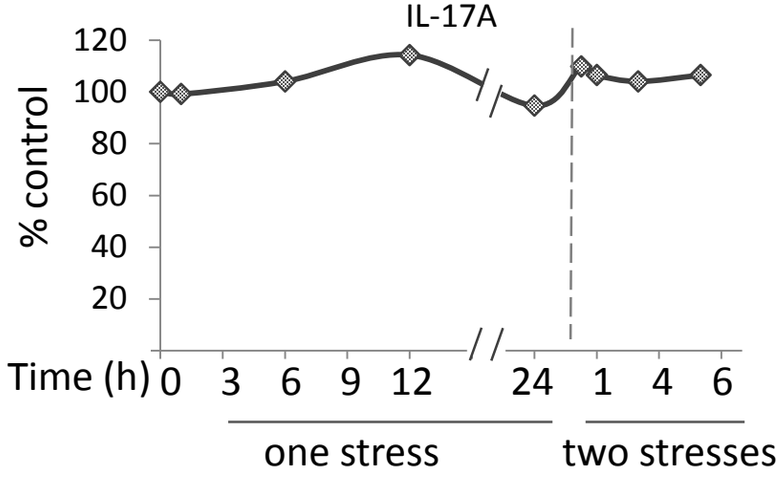

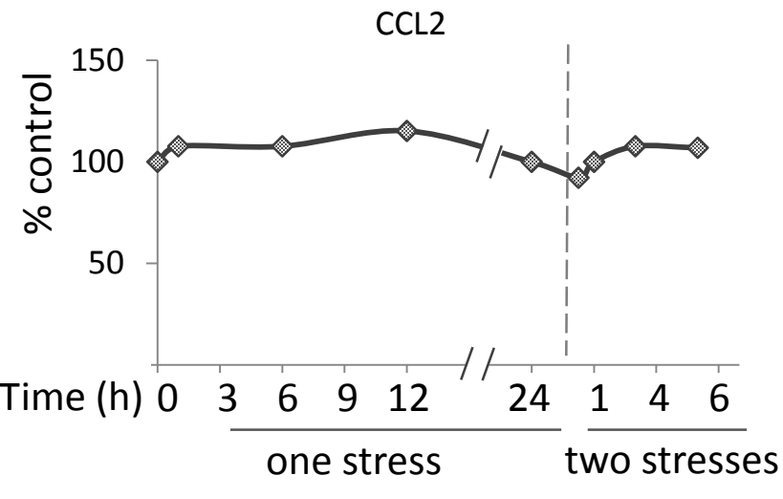

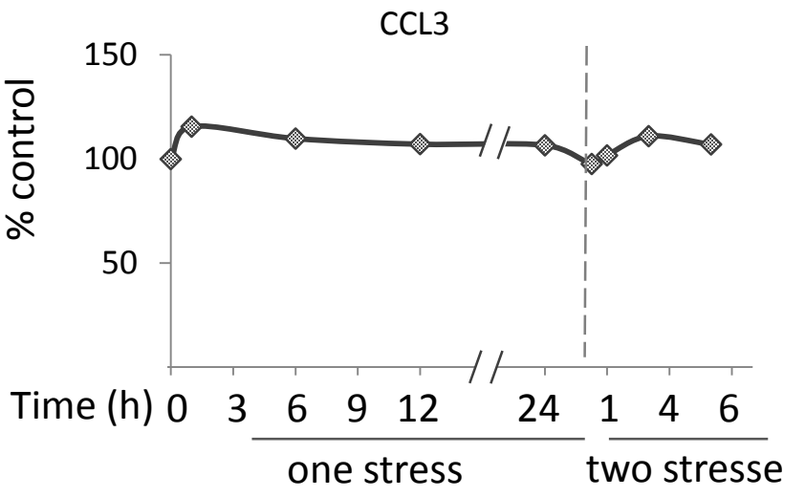

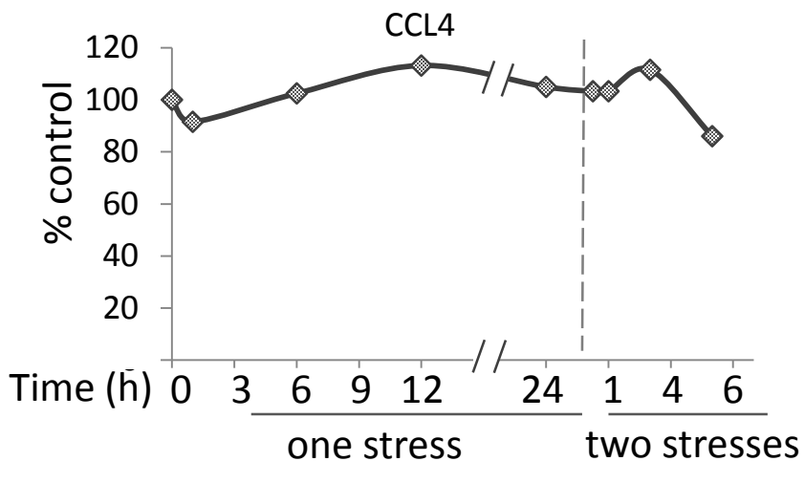

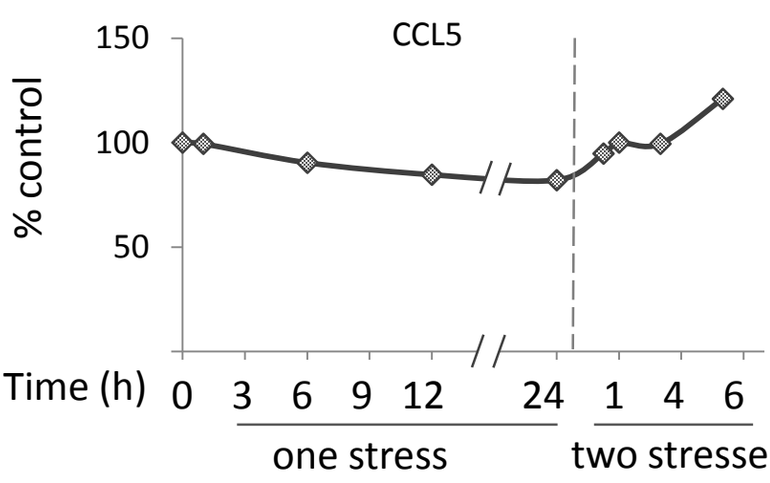

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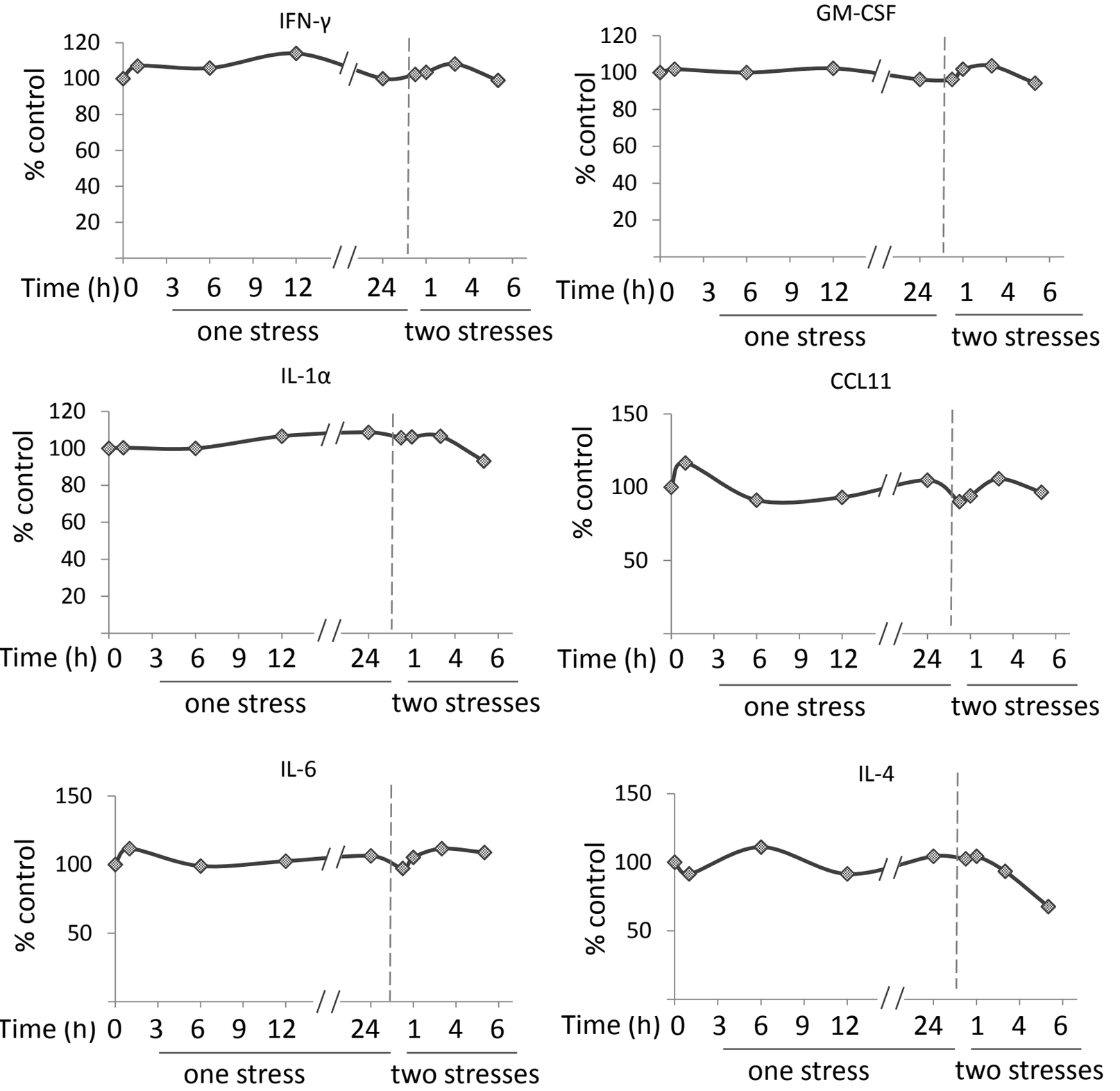

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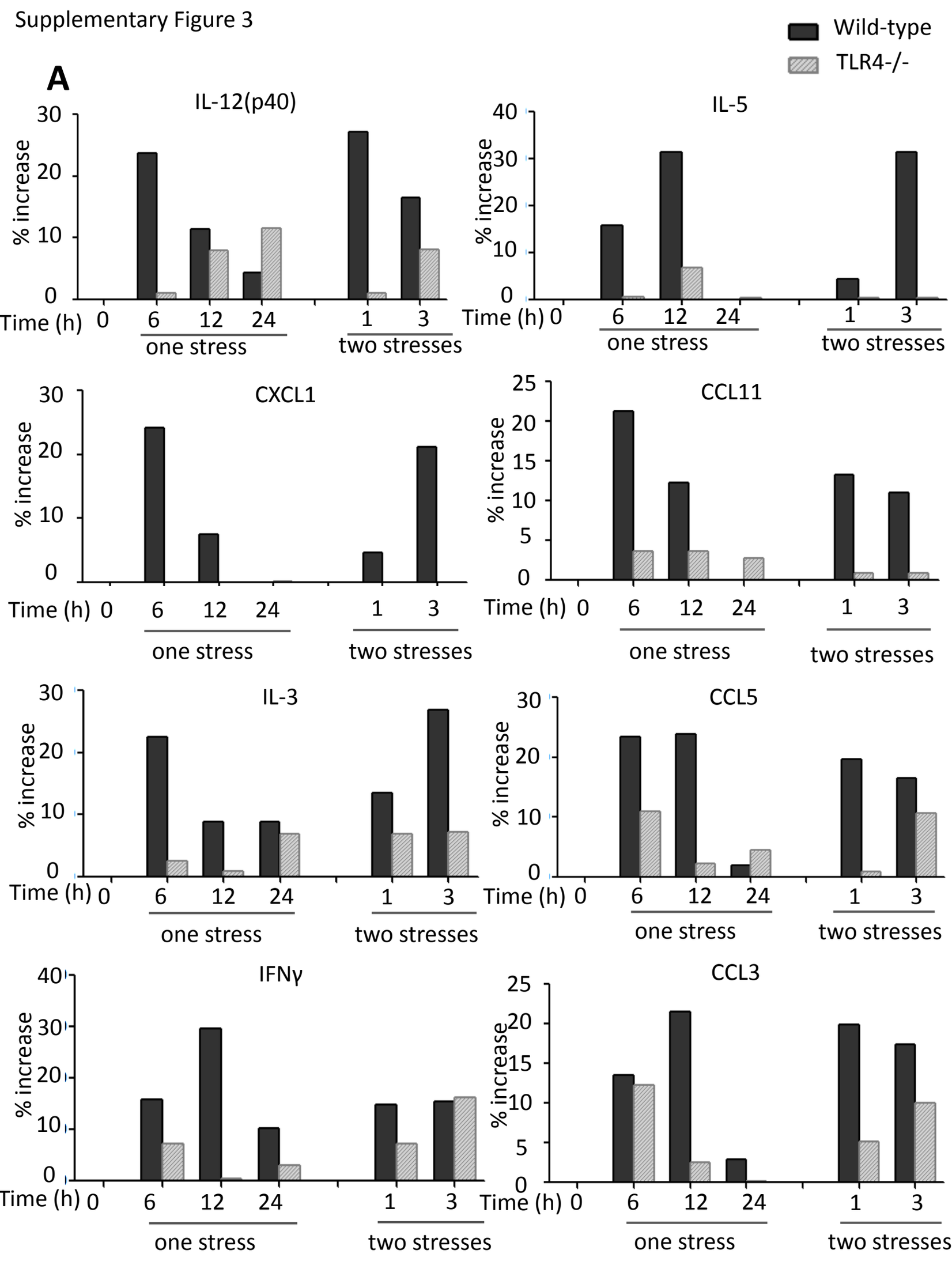

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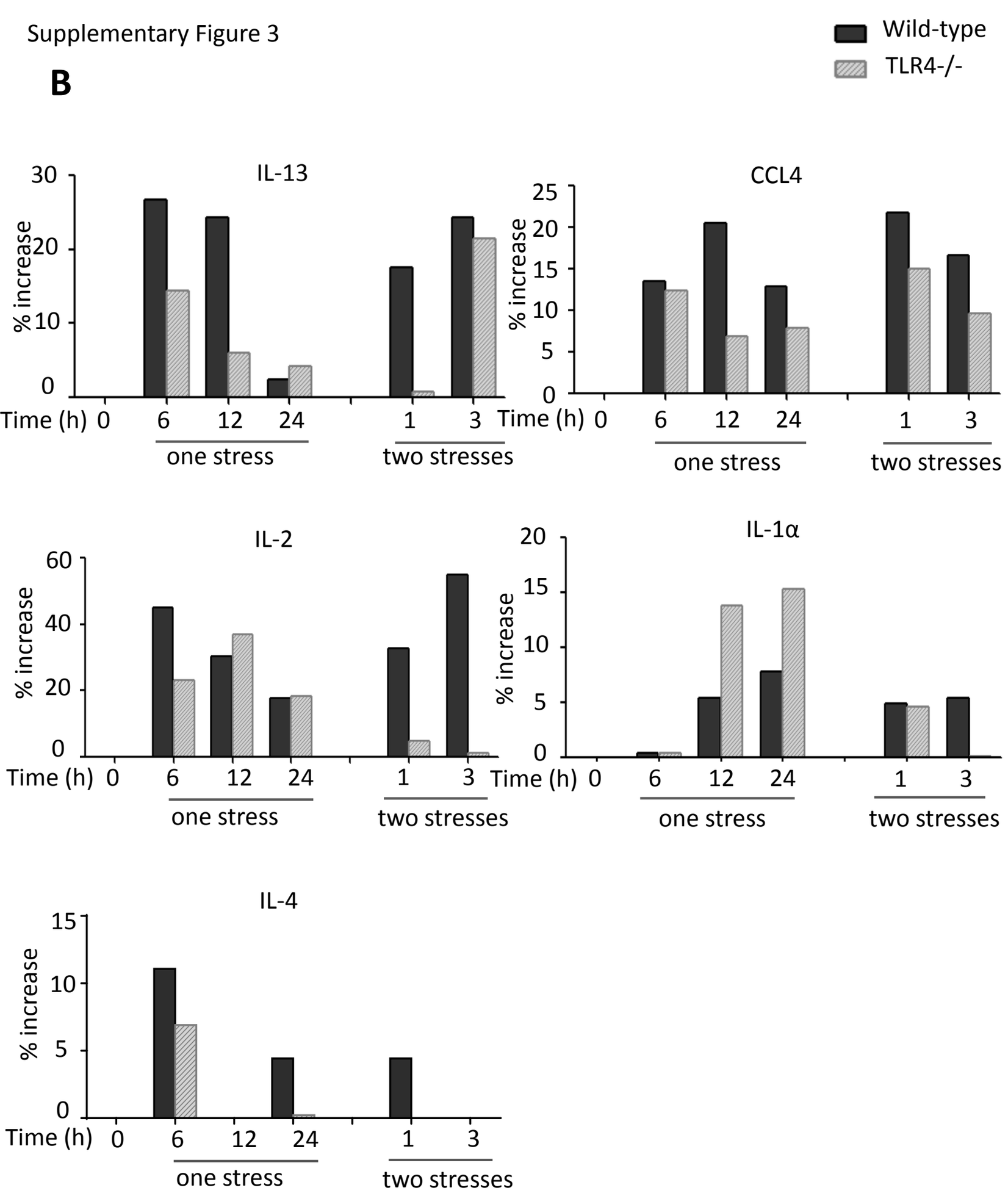

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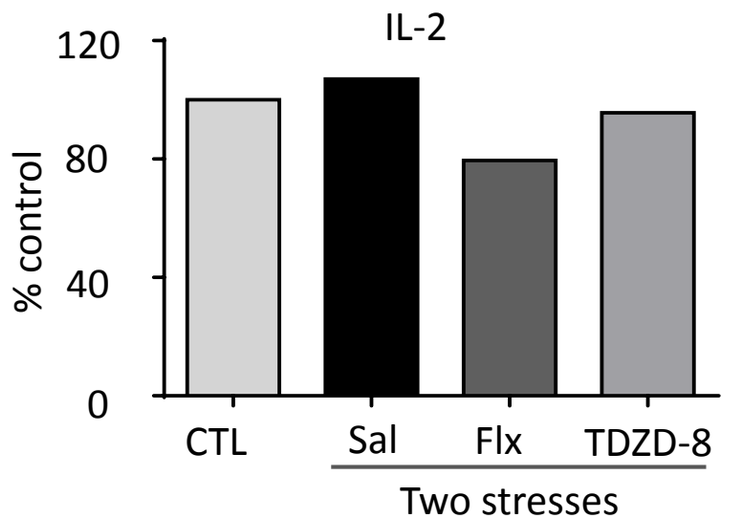

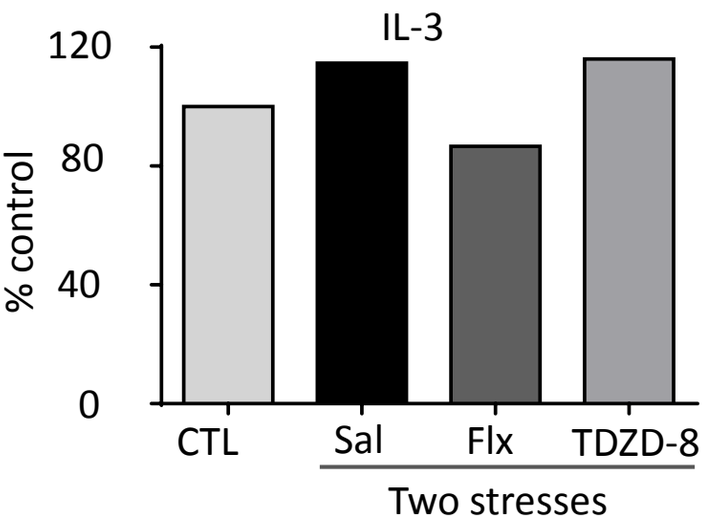

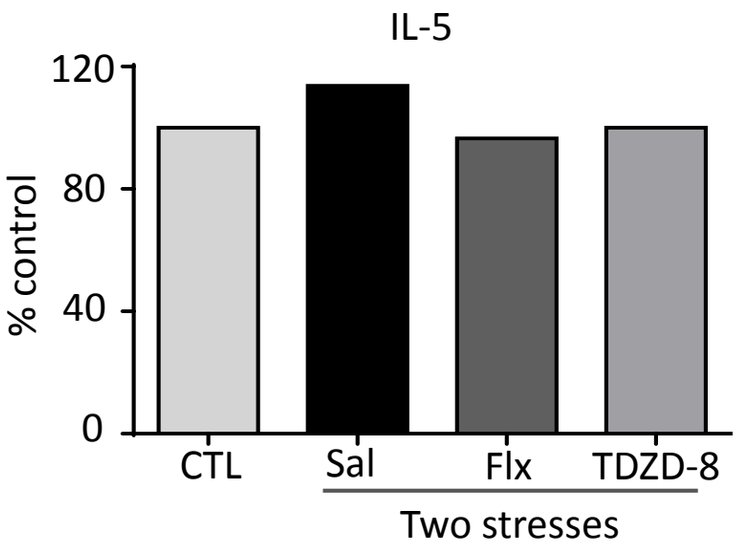

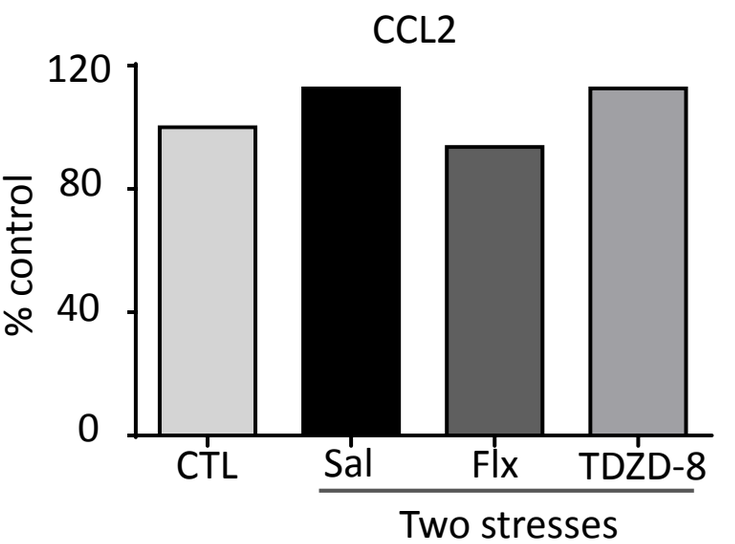

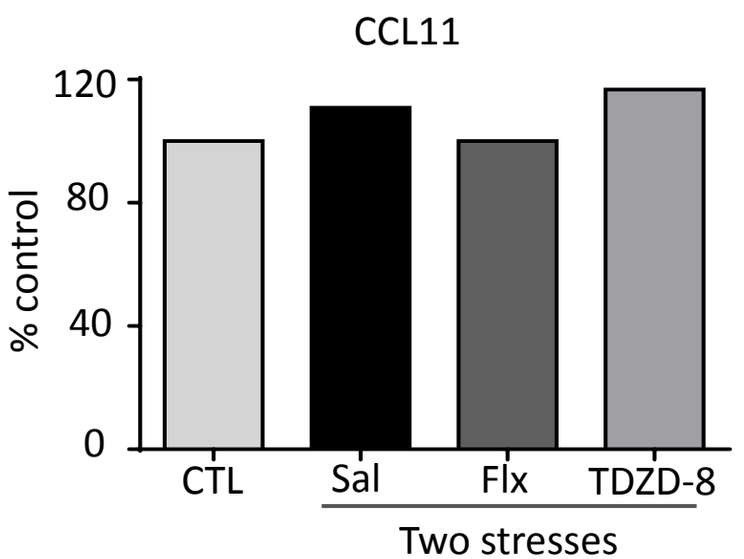

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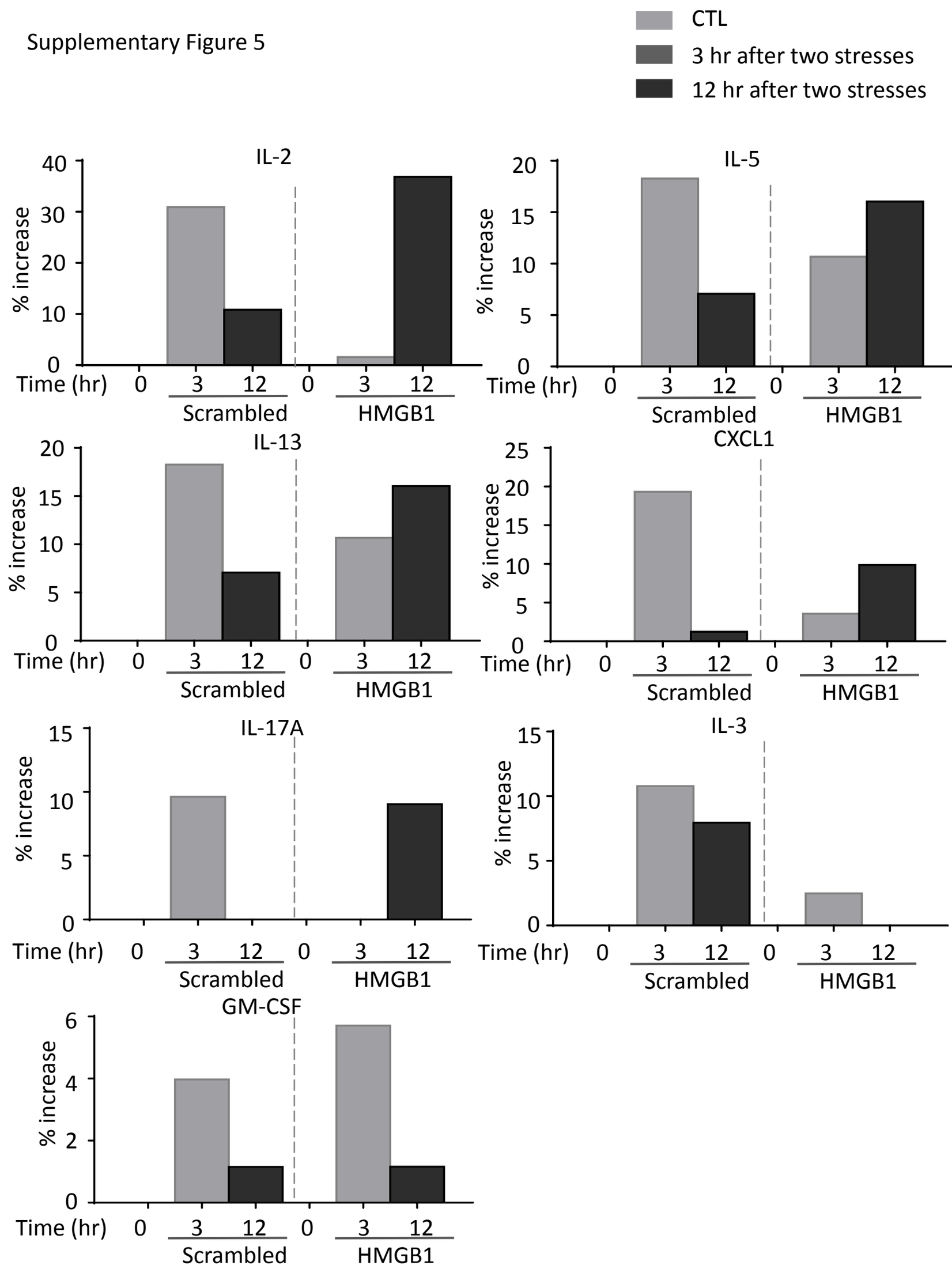

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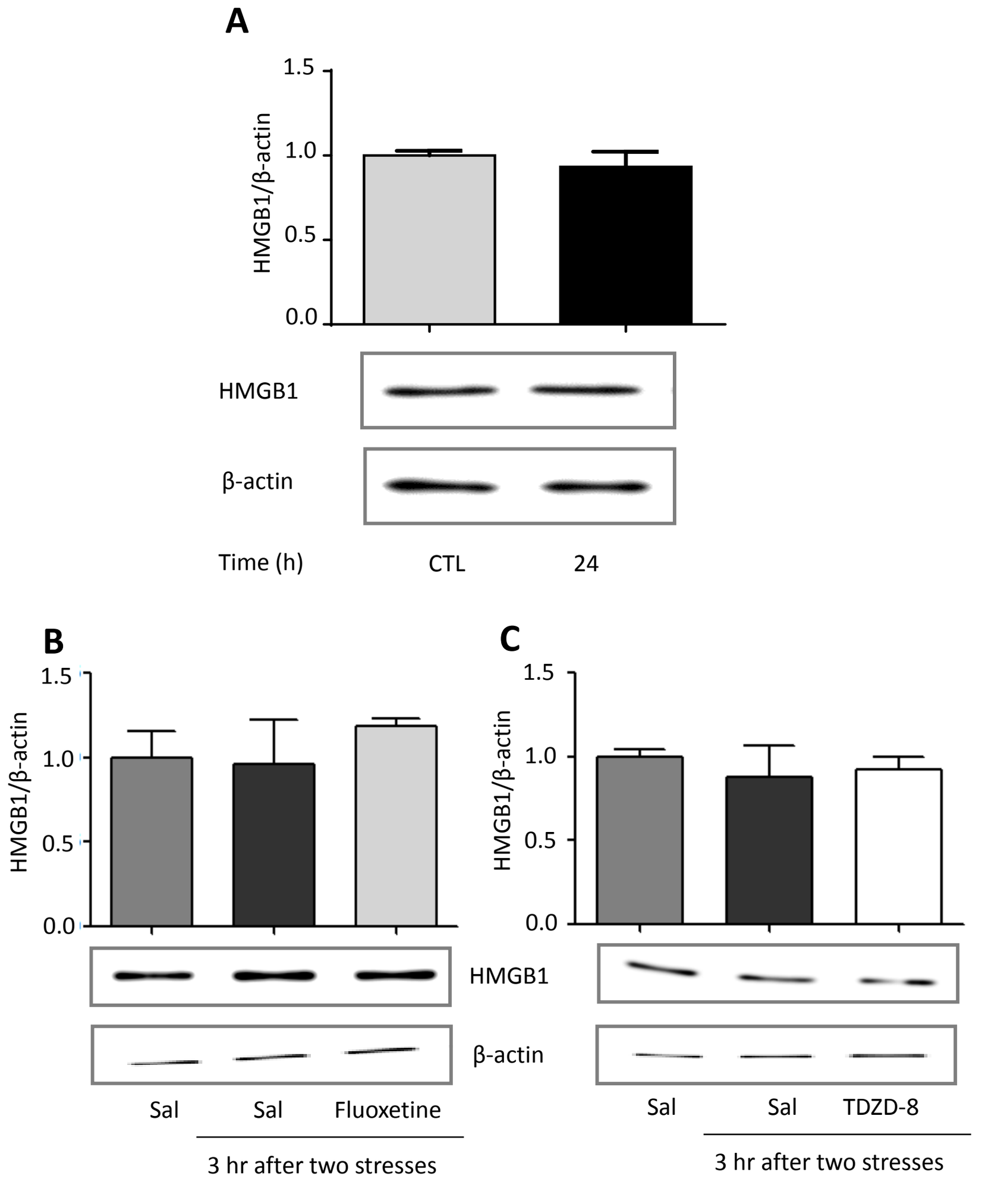
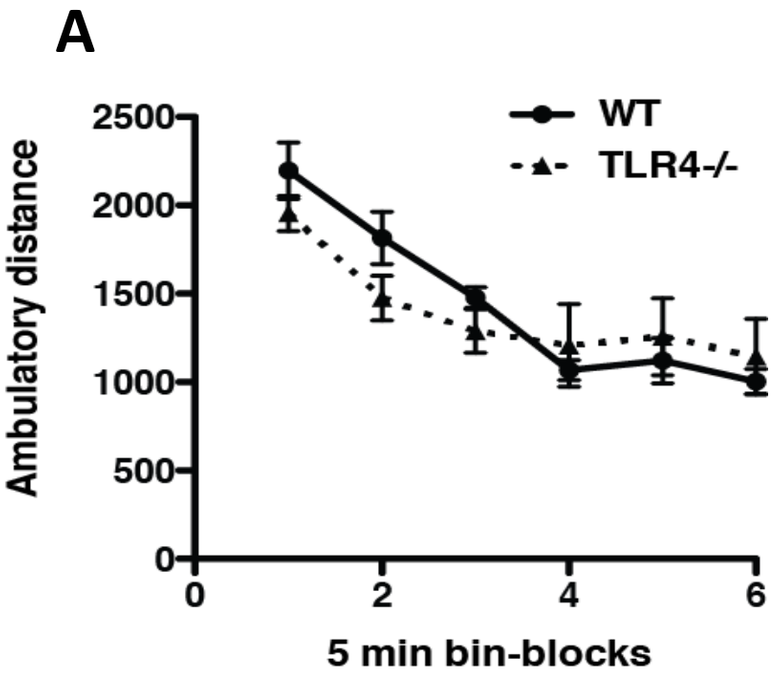
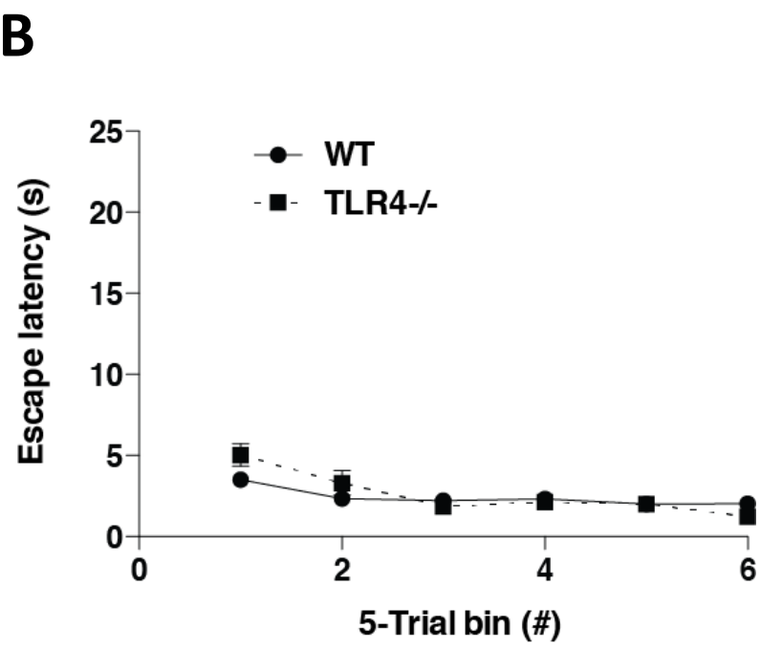


Supplementary Figure 7





Supplementary Table 1: Times of peak levels after one or two stresses for hippocampal cytokines.

Time of peak levels		**One stress**	
		6 hr	**12 hr**
Two stresses	**1 hr**	IL-10, IL-12(p40), CCL11	IL-12(p70), CCL2, CCL3, CCL4, CCL5, IFNγ
	3 hr	IL-2, IL-3, IL-13, CXCL1	TNFα, IL-6, IL-5, IL-17A, GM-CSF

Supplementary Table 2: Reported effects of stress on rodent brain cytokine levels

Stress	Cytokines	Region	Stress effects	Time points after stress	Reference
Immobilization stress	TNFα	Cortex	Increase	Immediately	Madrigal et al., 2002
Acute social defeat stress;	IL-6	Prefrontal cortex	Increase	1.25 hr	Audet et al., 2011
Inescapable foot shocks; inescapable tail shocks	IL-1β, IL-10	Hypothalamus	Increase	Various times	Nguyen et al., 2000; O'Connor et al., 2003; Deak et al, 2005; Blandino et al., 2006; Blandino et al., 2009
Chronic restraint stress	IL-1β, IL-6	Prefrontal cortex	Increase	Immediately	Gárate et al., 2013
Repeated social defeat stress	IL-1β	Brain microglia	Increase	14 hr	Wohleb et al., 2011
Chronic mild stress	TNFα, IL-18, IL-4, IL-6	Hippocampus, prefrontal cortex, hypothalamus	Increase	Immediately	Gárate et al., 2011; You et al., 2011
	IL-10 TGF-β	Hippocampus, cortex	Decrease		
Inescapable foot shocks; inescapable tail shocks; forced swim test	IL-1β	Hippocampus, cortex	No effect	Various times	Nguyen et al., 2000; Deak et al., 2003;
Inescapable foot shocks;	IL-6, IL-10	Hippocampus, cortex	No effect	Immediately	Blandino et al., 2009

Supplementary Table 3: Reported effects of stress on cytokine increases induced by LPS

Stress	Cytokines	Region	Stress effects on LPS response	Time points after LPS	Reference
Repeated social defeat stress	TNFα, IL-1β	Brain	Increase	3 hr	Quan et al., 2001
Inescapable tail shocks	IL-1β	Hippocampus, hypothalamus cortex	Increase	1, 2hr	Johnson et al., 2002; Johnson et al., 2003; Johnson et al., 2004
Chronic unpredictable mild stress	TNFα, IL-1β	Prefrontal cortex	Increase	2 hr	Munhoz et al., 2006; De Pablos et al., 2006
Inescapable tail shocks	IL-1β	Hippocampal microglia	Increase	4 hr	Frank et al., 2007
Chronic unpredictable mild stress	TNFα, IL-1β	Hippocampus	Increase	6 hr	Espinosa-Oliva et al., 2011
Repeated social defeat stress	TNFα, IL-1β	Brain macrophages	Increase	4, 24 hr	Wohleb et al., 2012