SUPPLEMENTAL FIGURES

Supplemental Figure 1. Induction of Hsp70 by Ad-Hsp70 or Hsp70-ATPase mutant constructs. ***A)*** Hsp70 protein in MLEC was detected by western blot after a time-course of Ad-Hsp70 or Ad-Ctrl treatment. β-actin as loading control. ***B)*** Hsp70 protein in cell culture supernatant was measured by ELISA a time course of Ad-Hsp70 or Ad-Ctrl treatment. The values are expressed as mean ± SD. **p*<0.01 *vs* corresponding Ad-Ctrl (experiments were performed in triplicates). WT, TLR4$^{-/-}$ or Hsp70$^{-/-}$ MLEC were transfected with human WT Hsp70, Hsp70 K71E mutant or SHAM control. Hsp70 ***(C)*** or GFP ***(D)*** mRNA expression was measured by real time RT-PCR. ***(E)*** Hsp70 protein in MLEC was detected by western blot. β-actin was used as loading control. Arrows show the endogenous Hsp70 and GFP-Hsp70 fusion bands. ***(F)*** Hsp70 ELISA assays in MLEC supernatants. The values are expressed as mean ± SD. **p*<0.05, *vs* Ctrl; $^{\#}p$<0.05, *vs* corresponding WT hyperoxia (experiments were performed in triplicates).

Supplemental Figure 2. Hsp70 deletion correlates with decreased secreted Hsp70 in MLEC and mouse lungs during hyperoxia. WT or Hsp70$^{-/-}$ MLEC were treated with Ad-Ctrl or Ad-Hsp70 and exposed to 72h of hyperoxia. RA, room air control. ***A)*** Hsp70 ELISA assay in MLEC supernatant. ***B)*** Hsp70 mRNA expression in MLEC was measured by real time RT-PCR. The values are expressed as mean ± SD. **p*<0.05 *vs* RA Ad-Ctrl WT MLEC; ***p*<0.05 *vs* hyperoxia Ad-Ctrl WT MLEC; $^{\#}p$<0.05 *vs* corresponding Ad-Ctrl MLEC (experiments were performed in triplicates). WT or Hsp70$^{-/-}$ mice were treated with Ad-Ctrl or Ad-Hsp70 and exposed to 72h of hyperoxia, then sacrificed. RA, room air control. ***C)*** Hsp70 ELISA assay in mice BAL fluid. ***D)***

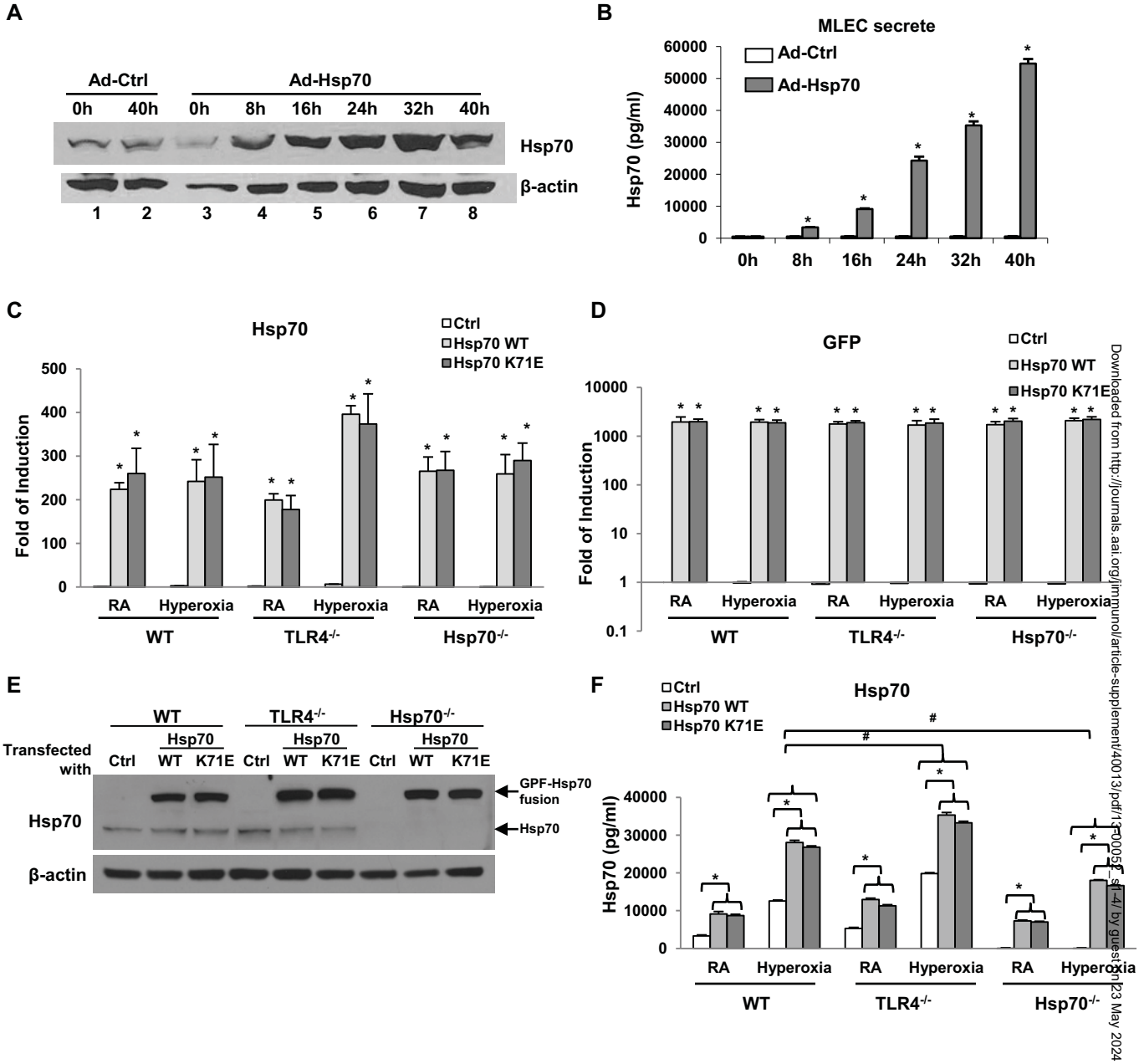
Hsp70 mRNA expression in lung lysates was measured by real time RT-PCR. The values are expressed as mean ± SD. *p<0.05 *vs* RA Ad-Ctrl WT mice; **p<0.05 *vs* hyperoxia Ad-Ctrl WT mice; $^{\#}p$<0.05 *vs* corresponding Ad-Ctrl mice (n=5 for each group).

Supplemental Figure 3. Methyl β–cyclodextrin orsoluble Hsp70 antibody blocked the ability of Ad-Hsp70 to decrease hyperoxia-induced LDH release and apoptosis in MLEC. WT or TLR4$^{-/-}$ MLEC were pretreated with methyl β–cyclodextrin (MBC, 2.5mM) for 6hr, then treated with Ad-Ctrl or Ad-Hsp70 and exposed to 72h of hyperoxia. ***A)*** LDH activity assay from MLEC supernatant. ***B)*** Graphical quantitation of flow cytometry analysis of apoptosis. The values are expressed as mean ± SD. *p<0.05, *vs* corresponding –MBC RA Ad-C group, **p<0.05, *vs* corresponding Ad-C group, $^{\#}p$<0.05, *vs* corresponding –MBC group. ***C)*** WT MLEC were pretreated with soluble Hsp70 antibody (Hsp70 Ab, 1μg/ml) for 1hr, then treated with Ad-Ctrl or Ad-Hsp70 and exposed to 72h of hyperoxia. Graphical quantitation of flow cytometry analysis of apoptosis. The values are expressed as mean ± SD. *p<0.05, *vs* corresponding –Hsp70 Ab RA Ad-C group, **p<0.05, *vs* corresponding Ad-C group, $^{\#}p$<0.05, *vs* corresponding –Ab group (experiments were performed in triplicates).

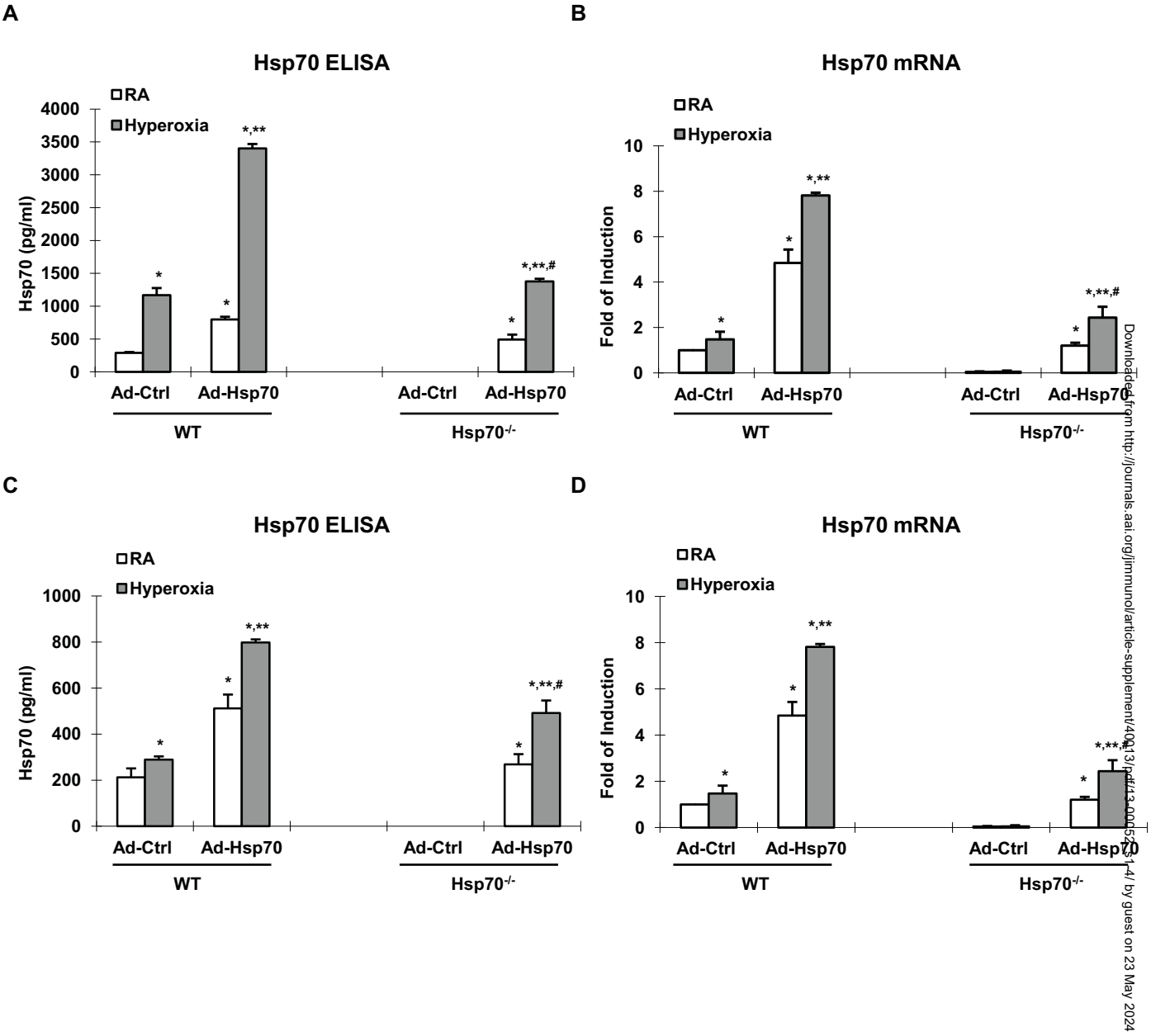
Supplemental Figure 4. Hsp70-mediated protection is independent of MyD88. WT or MyD88$^{-/-}$ MLEC were treated with Ad-Ctrl or Ad-Hsp70 and were exposed to 72h of hyperoxia. RA, room air control. ***A)*** LDH activity of MLEC was determined. ***B)*** Graphical quantitation of flow cytometry analysis of apoptosis in MLEC. The values are

expressed as mean ± SD. *p<0.05, *vs* Ad-Ctrl/RA WT; $^{\#}p$<0.05 *vs* Ad-Ctrl/hyperoxia WT (experiments were performed in triplicates).

Supplemental Figure 1

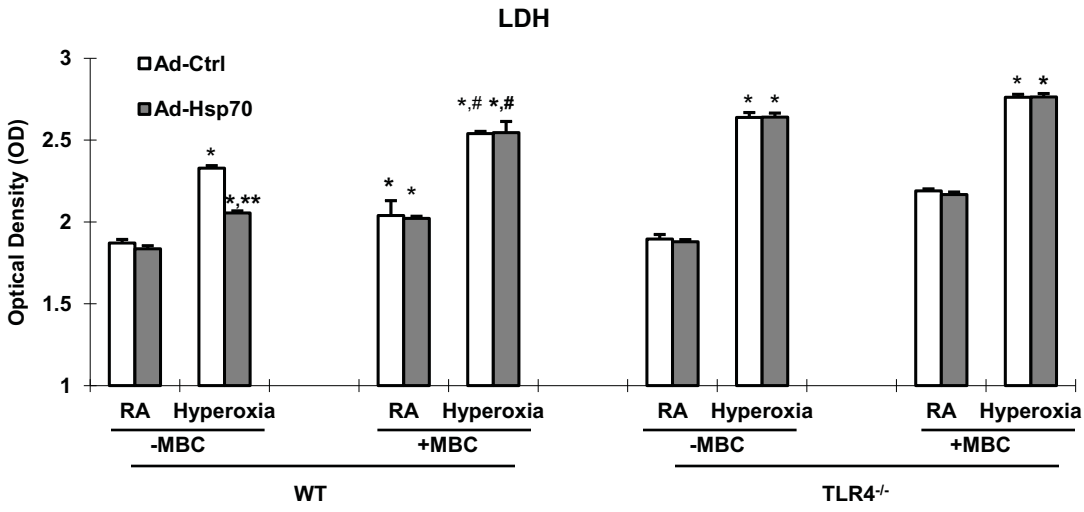


Supplemental Figure 2



Supplemental Figure 3

A



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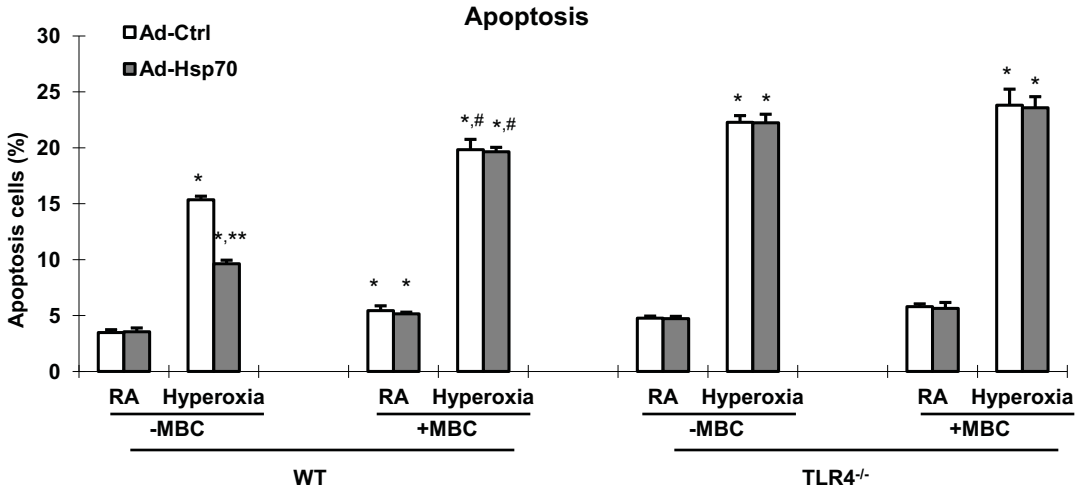

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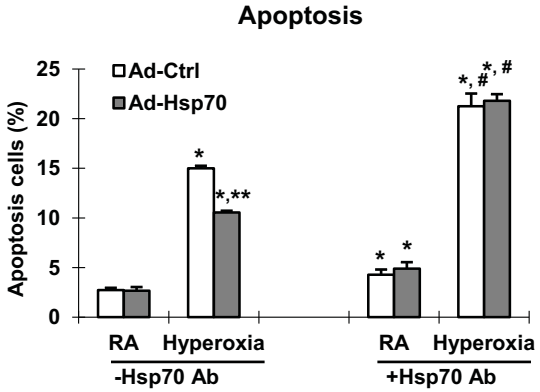

Supplemental Figure 4