**Online supplementary information**

**Supplementary Figure S1. TRAIL-induced necroptosis at acidic pHe is dependent on RIPK1 and RIPK3.** (**a**) HT29 cells were transiently transfected with si RIPK1, si RIPK3, si RIPK1/si RIPK3 (si RIPK1/3) or si NT1 (non targeting siRNA used as negative control) for 72 h. Densitometry analysis of RIPK1 and RIPK3 expression carried out from three independent western blot experiments. Relative RIPK1 or RIPK3 expression was expressed in arbitrary units (AU) as percentage of absorbance measured in si NT1 transfected cells. (**b**) Densitometry analysis of RIPK1 and RIPK3 expression carried out in MEFs RIPK1, MEFs RIPK1 KO, MEFs RIPK3 and MEFs RIPK3 KO cells (three independent western blot experiments). Relative RIPK1 or RIPK3 expression was expressed in arbitrary units (AU) as percentage of absorbance measured in MEFs RIPK1. Mean ± SD. $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$ and $^{***}$ $P < 0.001$

**Supplementary Figure S2. TRAIL-induced necroptosis at acidic pHe is dependent on TRAIL death receptors.** (**a**) HT29 cells were treated with indicated concentrations of TRAIL-Flag, FasL-Flag (cross-linked with 2 µg/mL anti-Flag M2) or TNF, for 24 h, at physiological pHe 7.4 or acidic pHe 6.5. Percentage of cell death was estimated with a methylene blue viability assay as described in M&M. (**b**) HT29 cells were treated or not (NT) with 100 ng/mL of TRAIL-Flag (cross-linked with 2 µg/mL anti-Flag M2) for 24 h, after a 1 h pre-treatment with 10 µg/mL Etanercept (α TNF) or antagonistic antibodies directed against Fas (α Fas), DR4 (α DR4) or DR5 (α DR5), at pHe 7.4 or 6.5. Percentages of apoptosis and necrosis were estimated as described in M&M. (**c**) Jurkat cells were treated or not (NT) with 100 ng/mL FasL-Flag (cross-linked with 2 µg/mL anti-Flag M2) for 24 h, after a 1 h pre-treatment with 10 µg/mL antagonistic antibody directed against Fas (α Fas). Percentage of cell death was estimated as described in M&M. (**d**) L929 cells were treated or not (NT) with

100 ng/mL TNF and 20 µM z-VAD for 24 h, after a 1 h pre-treatment with 10 µg/mL Etanercept (α TNF). Percentage of cell death was estimated with a methylene blue viability assay. Mean ± SD. $^{\#}$ $P < 0.05$ and $^{\$\$\$, \#\#\#}$ $P < 0.001$

**Supplementary Figure S3. TRAIL-induced necroptosis at acidic pHe is dependent on TRAIL death receptors.** (**a**) HT29 cells were transiently transfected with siRNAs directed against TNFR1, Fas, DR4 and DR5 or with si NT1 (non targeting siRNA used as negative control). 72 h after transfection, cells were treated or not (NT) with 100 ng/mL TRAIL-Flag and 2 µg/mL anti-Flag M2 for 24 h, at physiological pHe 7.4 or acidic pHe 6.5. Percentages of apoptosis and necrosis were estimated as described in M&M. (**b**) Western blot analysis of TNFR1, Fas, DR4 or DR5 expression in HT29 cells was carried out 72 h after transfection (one representative of three independent experiments). Anti-human HSC70 was used as a control of protein loading. (**c**) Densitometry analysis of TNFR1, Fas, DR4 or DR5 expression was carried out in HT29 cells 72 h after transfection (three experiments). Relative TNFR1, Fas, DR4 or DR5 expression was expressed in arbitrary units (AU) as percentage of absorbance measured in si NT1 transfected cells. $^{*, \#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ and $^{***, \#\#\#}$ $P < 0.001$

**Supplementary Figure S4. TRAIL-induced necroptosis at acidic pHe is dependent on TRAIL death receptors.** (**a**) Jurkat cells were transiently transfected with a siRNA directed against Fas or with si NT1 (non targeting siRNA used as negative control). 72 h after transfection, cells were treated or not (NT) with 100 ng/mL FasL-Flag and 2 µg/mL anti-Flag M2 for 24 h. Percentages of apoptosis and necrosis were estimated as described in M&M. (**b**) L929 cells were transiently transfected with a siRNA directed against TNFR1 or with si NT1. 72 h after transfection, cells were treated or not (NT) with 100 ng/mL TNF and 20 µM z-VAD for 24 h. Percentage of cell death was estimated with a methylene blue viability assay as

described in M&M. (**c**) Jurkat cells were transiently transfected with si Fas or with si NT1 for 72 h. Western blot analysis of Fas was carried in transfected cells. Anti-human HSC70 was used as a control of protein loading (one representative of three independent experiments). Densitometry analysis of Fas expression was carried out in Jurkat cells 72 h after transfection (three experiments). Relative Fas expression was expressed in arbitrary units (AU) as percentage of absorbance measured in siNT1 transfected cells. (**d**) L929 cells were transiently transfected with si TNFR1 or with si NT1 for 72 h. Western blot analysis of TNFR1 was carried in transfected cells. Anti-human HSC70 was used as a control of protein loading (one representative of three independent experiments). Densitometry analysis of TNFR1 expression was carried out in L929 cells 72 h after transfection (three experiments). Relative TNFR1 expression was expressed in arbitrary units (AU) as percentage of absorbance measured in siNT1 transfected cells. (**e**) HT29 cells were treated or not (NT) with 100 ng/mL TRAIL-Flag and 2 µg/mL anti-Flag M2 for indicated times, at physiological pHe 7.4 or acidic pHe 6.5. TNF extracellular concentration was determined by ELISA as described in M&M. Mean ± SD. $^{**}$ $P < 0.01$ and $^{###}$ $P < 0.001$

**Supplementary Figure S5. TRAIL-induced necroptosis at acidic pHe is dependent on PARP-1.** (**a**) HT29 cells were treated with 100 ng/mL TRAIL-Flag and 2 µg/mL anti-Flag M2, for the indicated times, at physiological pHe 7.4 or acidic pHe 6.5. Densitometry analysis of PAR expression was carried out from three independent western blot experiments. Relative PAR expression was expressed in arbitrary units (AU) as percentage of absorbance measured in cells at time 0. (**b**) HT29 cells were transiently transfected with a siRNA directed against PARP-1 or with si NT1 for 72 h. Densitometry analysis of PARP-1 expression was carried from three independent western blot experiments. Relative PARP-1 expression was expressed in arbitrary units (AU) as percentage of absorbance measured in si NT1 transfected cells. (**c**)

MEF PARP-1 or MEFS PARP-1 KO cells were transiently transfected with si PARP-1 or si NT1 for 72 h. Densitometry analysis of PARP-1 expression was carried out from three independent western blot experiments. Relative PARP-1 expression was expressed in arbitrary units (AU) as percentage of absorbance measured in siNT1 transfected cells. Mean ± SD. $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$ and $^{***}$ $P < 0.001$

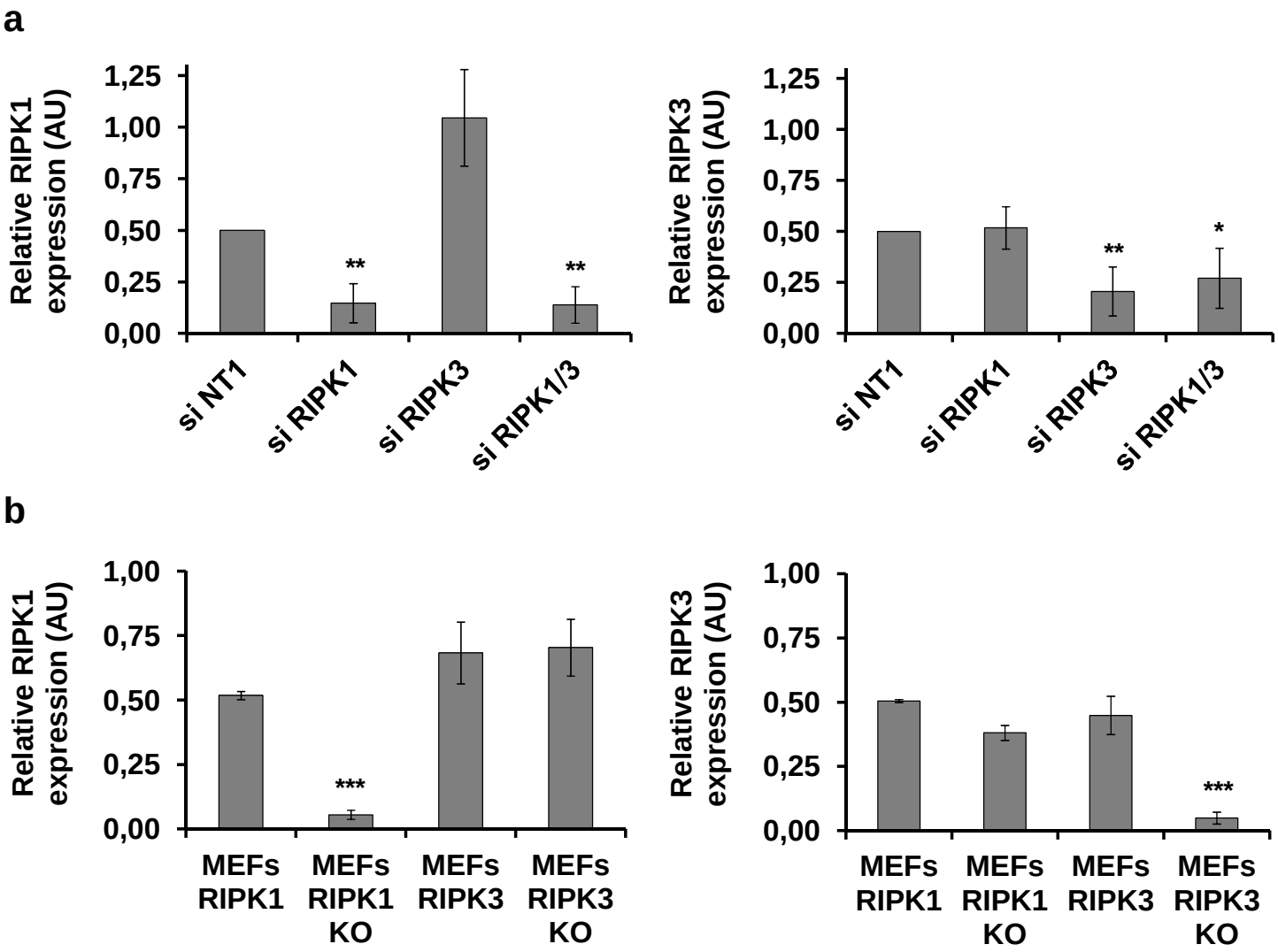
**Supplementary Figure S6. ROS production in TRAIL-induced necroptosis at acidic pHe**. (**a**) HT29 cells were treated or not (NT) with 100 ng/mL TRAIL-Flag and 2 µg/mL anti-Flag M2 for the indicated times, at acidic pHe 6.5. ROS production was measured by flow cytometry as described in M&M. Menadione was used as positive control. Left panel: flow cytometry kinetic analysis of superoxide anion ($O_2^{\cdot -}$) generation with DHE staining (one representative of three independent experiments is shown); right panel: quantitative analyses of superoxide anion generation measured in three independent experiments. Values are represented as percentage of cells with accumulated $O_2^{\cdot -}$. (**b**) HT29 cells were treated or not (NT) with 100 ng/mL TRAIL-Flag and 2 µg/mL anti-Flag M2 for 24 h, at pHe 6.5, after a 4 h pretreatment with 20 mM N-acetyl-cysteine (NAC), 100 µM Thiourea or 5 mM TEMPOL. ROS production was measured by flow cytometry as described in M&M. Quantitative analyses of superoxide anion generation measured in three independent experiments. Values are represented as percentage of cells with accumulated $O_2^{\cdot -}$. (**c**) HT29 cells were treated or not (NT) with 100 µM Menadione for 1 h, after a 4 h pretreatment with 20 mM NAC, 100 µM Thiourea or 5 mM TEMPOL. ROS production was measured by flow cytometry as described in M&M. Quantitative analyses of superoxide anion generation measured in three independent experiments. Values are represented as percentage of cells with accumulated $O_2$-. Mean ± SD. $^{*,\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$

**Supplementary Figure S7. ROS production is not involved in TRAIL-induced necroptosis at acidic pHe**. (**a**) HT29 cells were treated or not (NT) with 100 ng/mL TRAIL-Flag and 2 µg/mL anti-Flag M2 for 24 h, at physiological pHe 7.4 or acidic pHe 6.5, after a 4 h pretreatment with 20 mM NAC, 100 µM Thiourea or 5 mM TEMPOL. Percentages of apoptosis and necrosis were estimated as described in M&M. (**b**) PARP-1 activity was determined as described in M&M. (**c**) HT29 cells were treated or not (NT) with 100 ng /mL TRAIL-Flag and 2 µg/mL anti-Flag M2 for 8 h, at pHe 7.4 or 6.5, after a 4 h pretreatment with 20 mM NAC, 100 µM Thiourea or 5 mM TEMPOL. Intracellular ATP concentration was measured as described in M&M. (**d**) HT29 cells were treated or not (NT) with 150 mM propionate and 300 mM acetate for 24 h, after a 4 h pretreatment with 20 mM NAC or 5 mM TEMPOL. Percentage of cell death was estimated with a methylene blue viability assay as described in M&M. (**e**) ROS production was measured by flow cytometry as described in M&M. Quantitative analyses of superoxide anion generation measured in three independent experiments. Values are represented as percentage of cells with accumulated $O_2^{\cdot -}$. Mean ± SD. $^{\#}$ $P < 0.05$, $^{\#\#\#}$ $P < 0.001$

**Supplementary Figure S8. Concanavalin A-induced hepatitis is associated with TRAIL-induced necrotic cell death.** (**a**) C57Bl/6 mice were treated or not (NT=PBS, 4 mice) with 20 mg/Kg Con A for 6 h (five mice) or 10 h (four mice). Western blot analysis of HMGB1 or Cyclophilin A was carried out in lysates of liver tissues. Anti-human HSC70 was used as a control of protein loading. Densitometry analysis of HMGB1 or Cyclophilin A (CypA) expression was carried out. Mean relative HMGB1 or CypA expression was expressed in arbitrary units (AU) as percentage of absorbance measured in NT. (**b**) C57Bl/6 mice were treated or not (PBS, five mice) with 12 mg/Kg Con A for 10 h (eight mice). Western blot analysis of protein poly ADP-ribosylation (PAR), PARP-1 and caspase-3 was carried out in

lysates of liver tissues. Anti-human HSC70 was used as a control of protein loading. Densitometry analysis of PAR, PARP-1 or caspase-3 expression was carried out. Mean relative PAR, PARP-1 or caspase-3 expression was expressed in arbitrary units (AU) as percentage of absorbance measured in PBS. Mean ± SD. $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$ and $^{***}$ $P < 0.001$

**Supplementary Figure S1**



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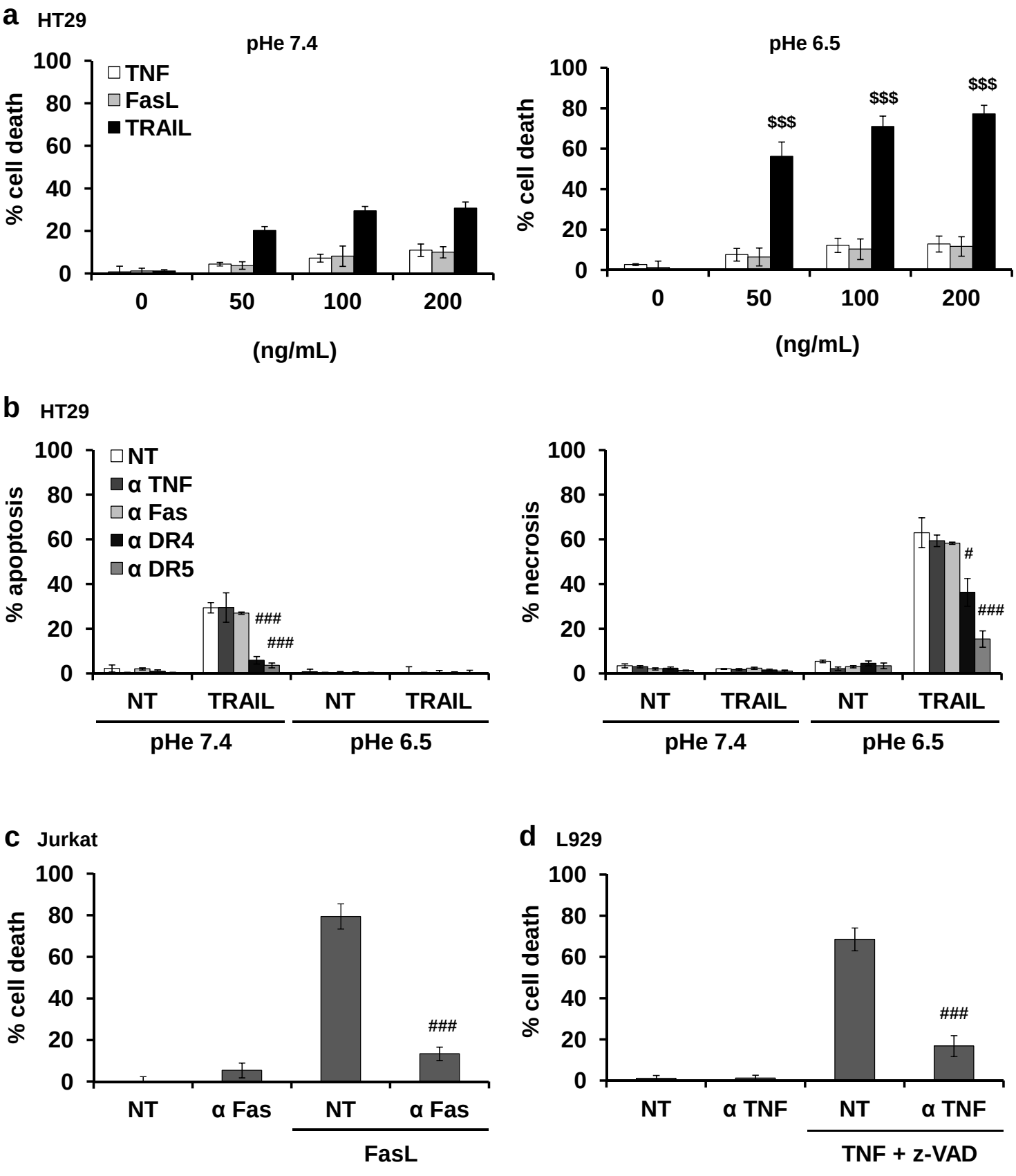

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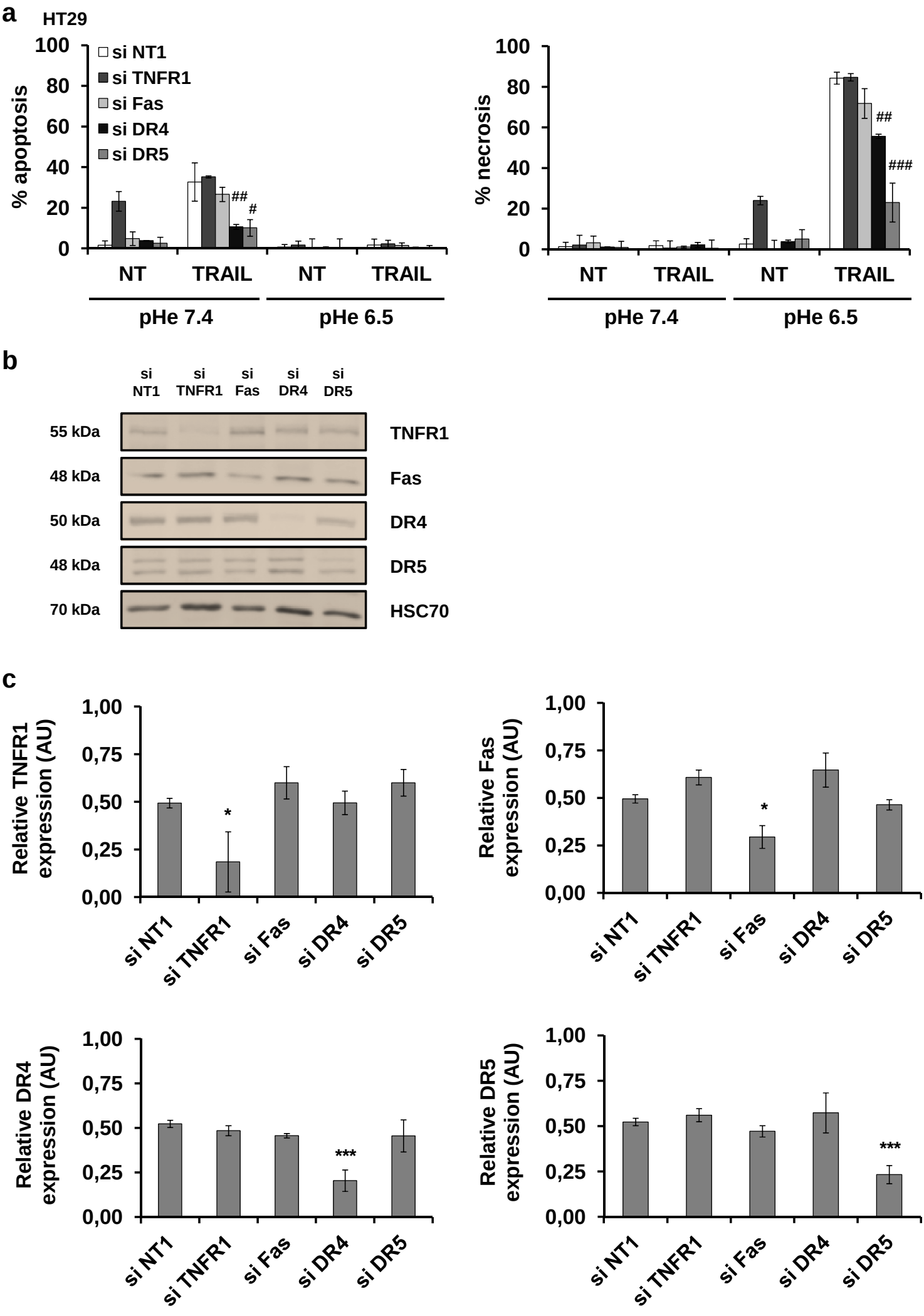

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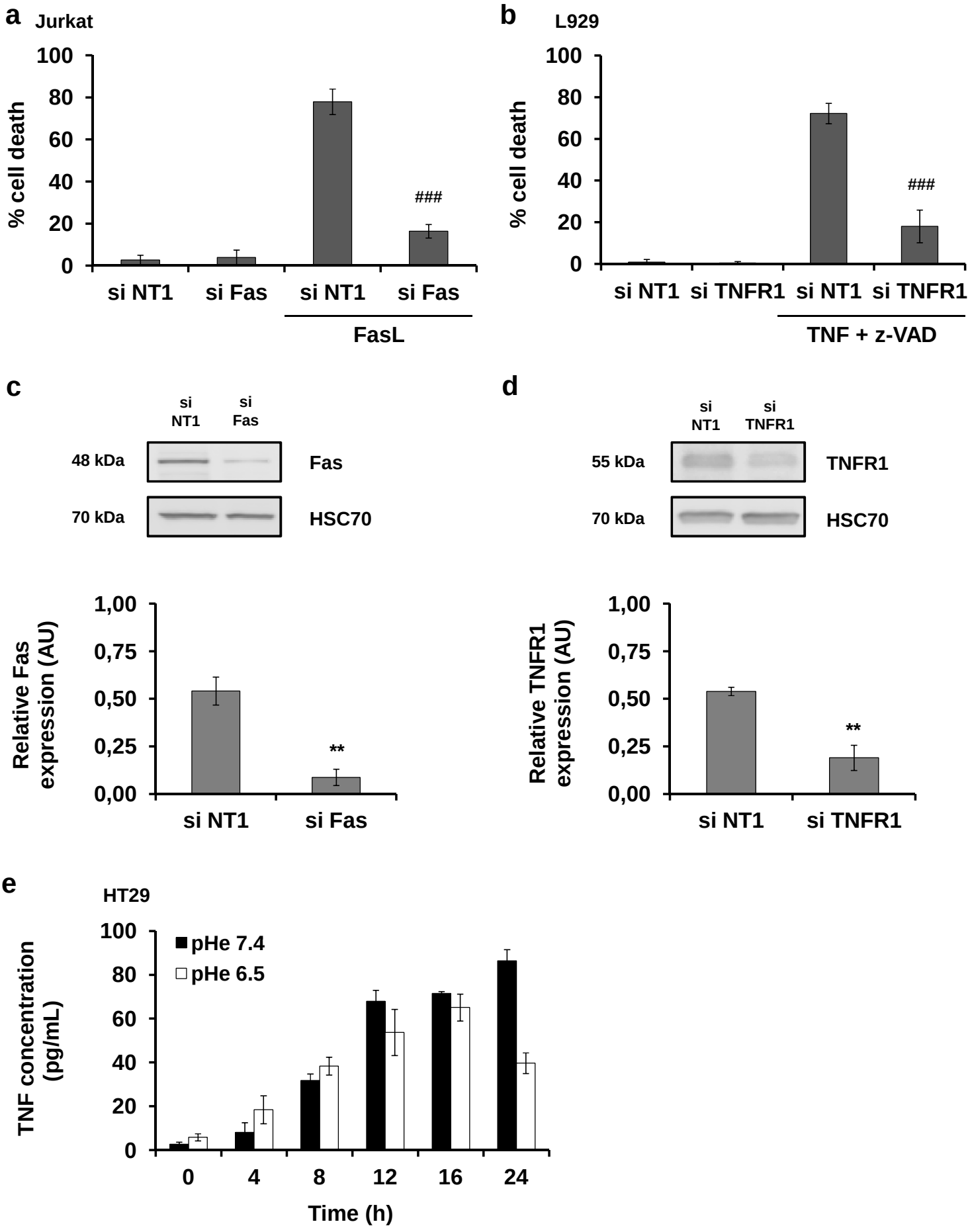

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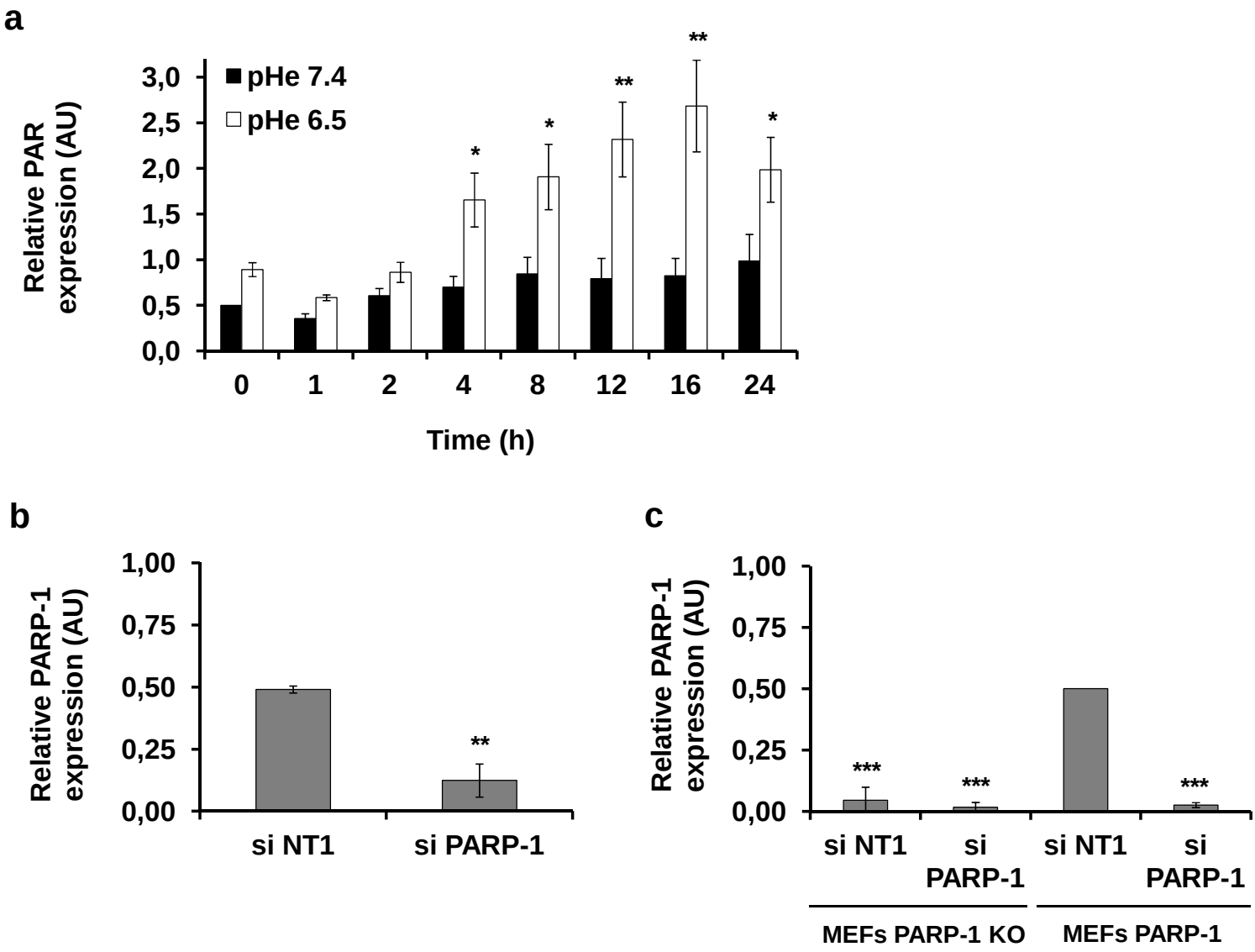

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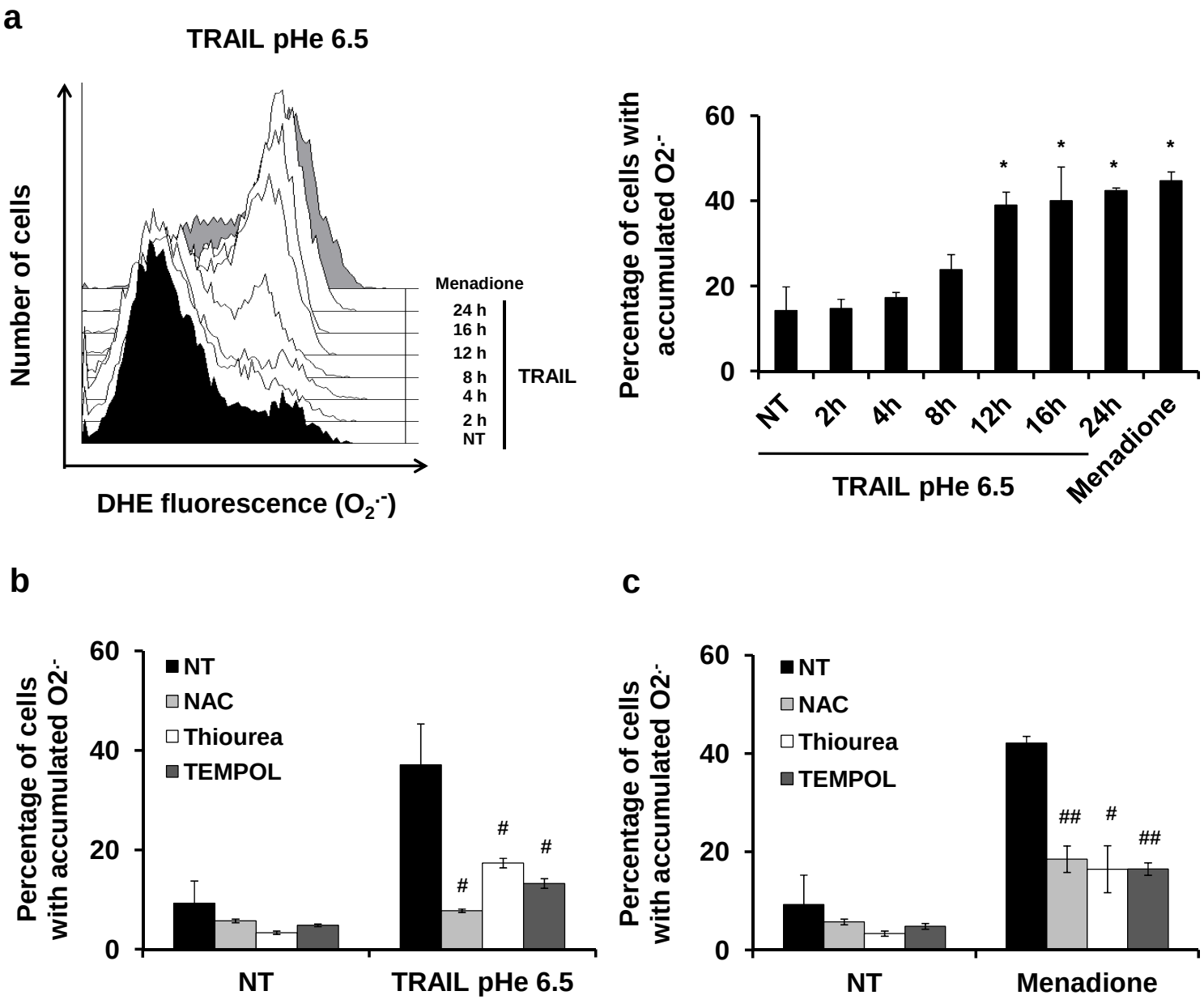
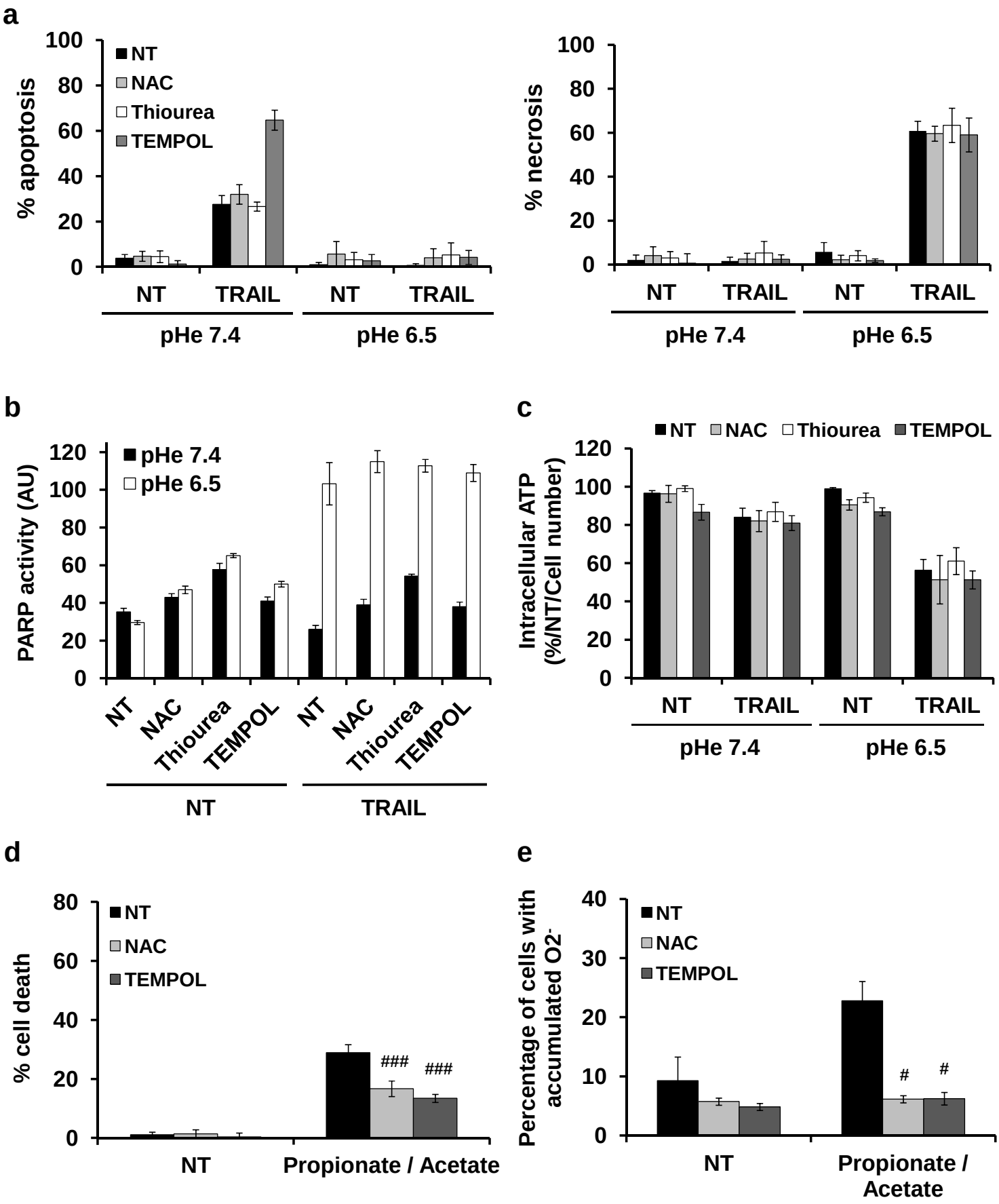


**Supplementary Figure S7**



**Supplementary Figure S8**