Expanded View Figures

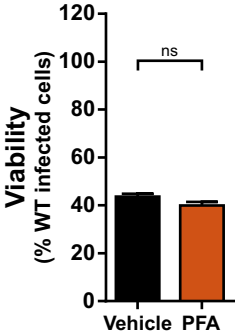

Figure EV1. Murine cytomegalovirus DNA replication is not required for necroptosis in sensitive fibroblasts.
Relative viability of 3T3-SA cells infected with M45*mut*RHIM compared to WT MCMV (MOI = 5) in the presence or absence of 200 μg/ml phosphonoformic acid (PFA). n.s., not significant ($P > 0.05$) by two-tailed unpaired Student's *t*-test. Error bars indicate SD ($n = 3$ biological replicates).

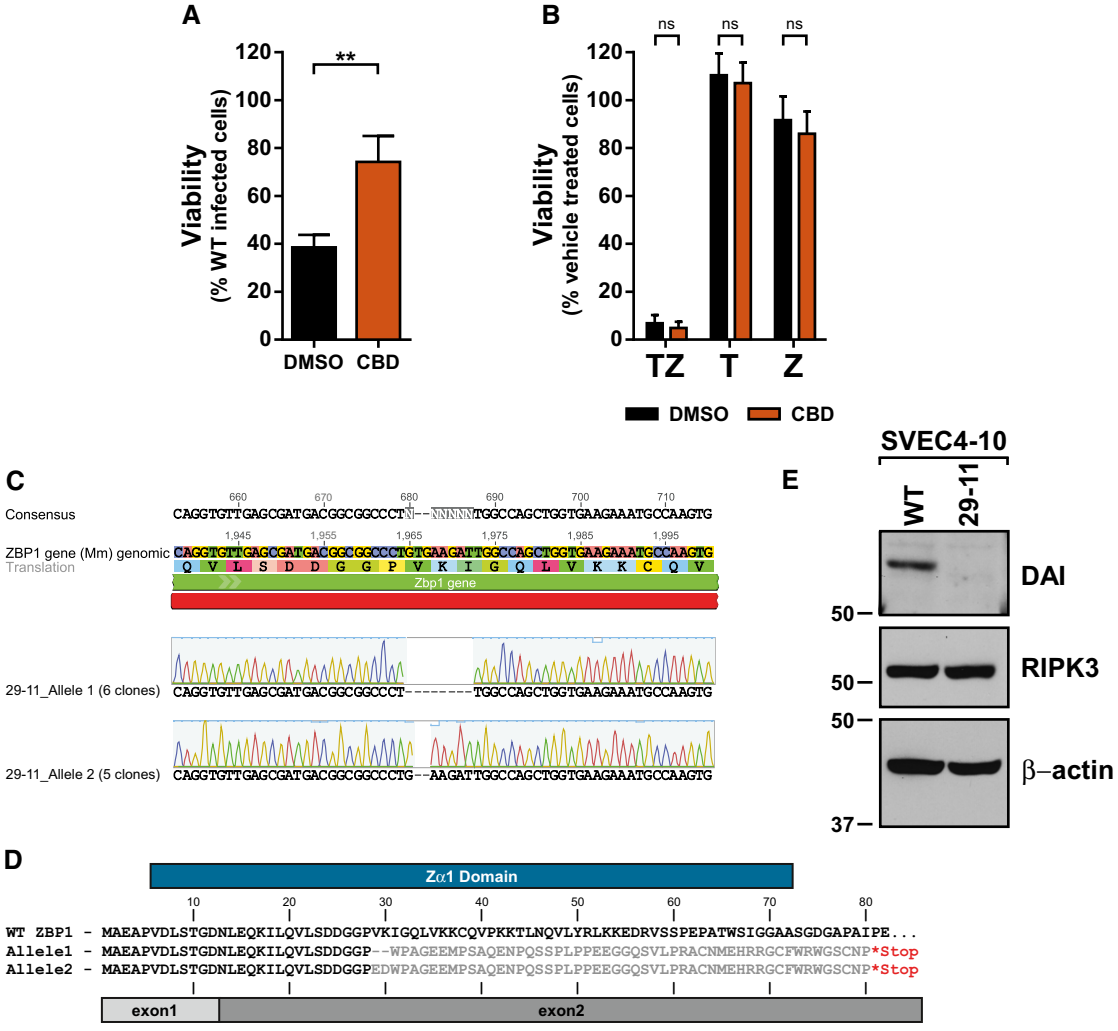

Figure EV2.

Figure EV2. Ciliobrevin-D treatment protects from MCMV-induced necroptosis and characterization of DAI knockout 29-11 cell lines.

A Relative viability of SVEC4-10 cells infected with M45*mut*RHIM compared to WT MCMV (MOI = 5) in the presence or absence of 100 μM Ciliobrevin-D (CBD). $^{**}P < 0.01$; two-tailed unpaired Student's *t*-test. Error bars indicate SD ($n = 3$ biological replicates).

B Relative viability of SVEC4-10 cells treated with TNF (T), zVAD (Z), or TNF + zVAD-fmk (TZ) for 6 h in the presence or absence of 100 μM CBD. n.s., not significant ($P > 0.05$) by two-tailed unpaired Student's *t*-test. Error bars indicate SD ($n = 3$ biological replicates).

C Representative results of sequencing and alignment of the DAI/ZBP1 exon 2 locus in 29-11 cells. Amplicons generated from 29-11 genomic DNA using ZBPSurveyor primers were cloned, and 11 independent clones sequenced.

D Amino acid alignment of protein products predicted from the sequencing results in (C). Gray bars denote the boundaries of exon 1 (light) and exon 2 (dark) of DAI/ZBP1, and blue bar represents the defined Zα1 domain.

E IB analysis to detect DAI/ZBP1, RIPK3, and β-actin from SVEC-10 parental and 29-11 cell lines.

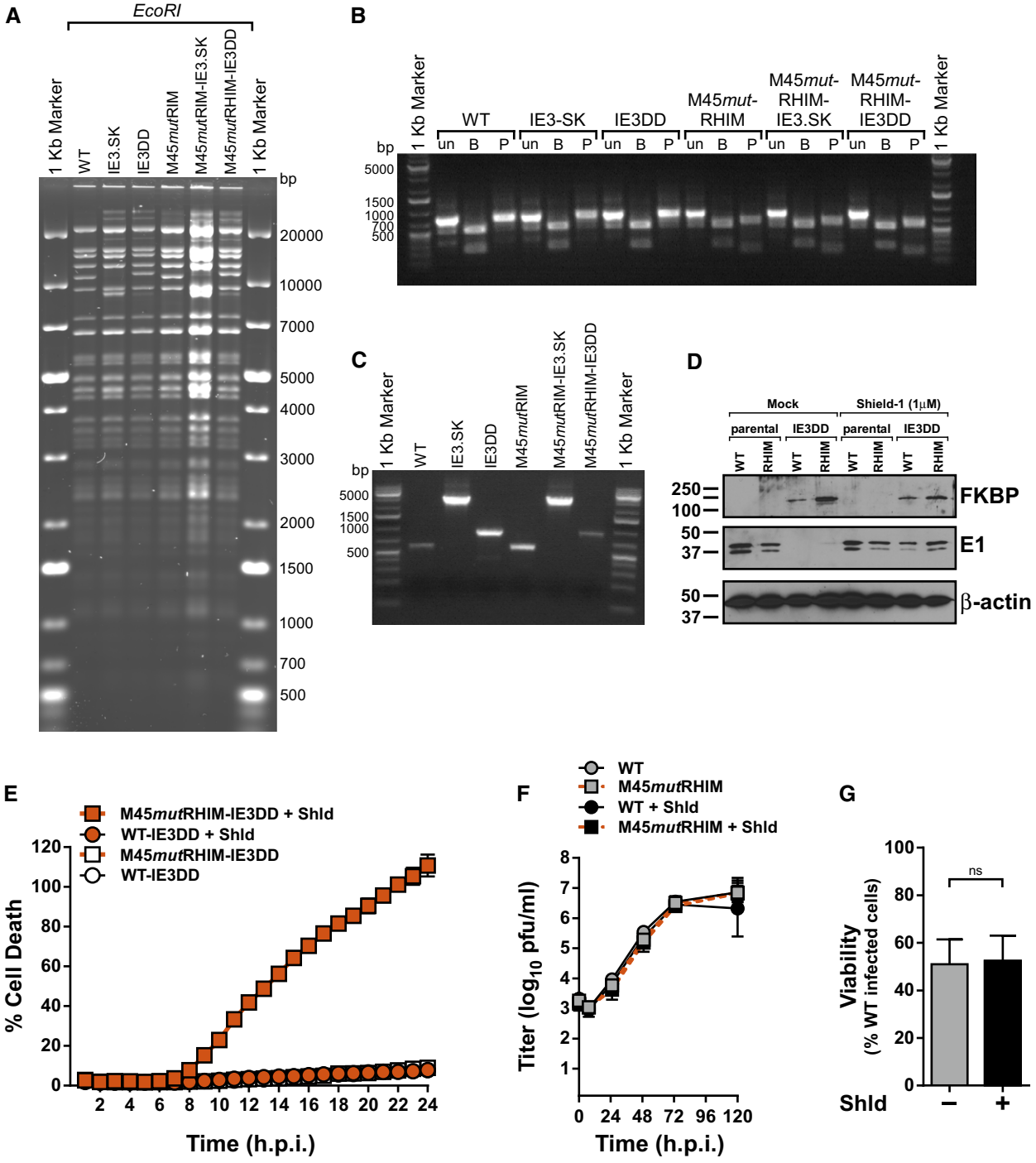

Figure EV3.

Figure EV3. Generation and characterization of IE3-DD viruses.

A RFLP analysis of parental WT, IE3.SK, IE3DD, parental M45*mut*RHIM, M45*mut*RHIM –IE3.SK, and M45*mut*RHIM-IE3DD bacmids digested with *Eco*RI.

B Agarose gel analysis of PCR amplicons generated from bacmids described in (A) with primers HS36 and HS37. Amplicons were digested with the indicated restriction endonuclease to diagnose the M45*mut*RHIM locus. un, undigested; B, *Bam*HI; P, *Puv*II.

C Agarose gel analysis of PCR amplicons generated from bacmids described in (A) with primers HS09 and HS10 to diagnose insertions at the end of IE3 exon 5.

D IB analysis to detect the DD-domain (FKBP), E1, and β-actin from NIH3T3 cells infected 13 h with parental or IE3DD WT or M45*mut*RHIM (RHIM) MCMV (MOI = 1) in the presence or absence of 1 μM Shield-1.

E Kinetics of cell death of 3T3-SA cells infected with WT or M45*mut*RHIM (MOI = 5) in the presence or absence of 1 μM Shield-1 (Shld), measured in real time by Sytox green incorporation. Error bars indicate SD (n = 4 biological replicates).

F Multistep replication levels of parental WT and M45*mut*RHIM viruses (MOI = 0.05) in NIH3T3 cells in the presence or absence of 1 μM Shld. Error bars indicate SD (n = 3 biological replicates).

G Relative viability of SVEC4-10 cells infected with M45*mut*RHIM compared to WT MCMV in the absence or presence of 1 μM Shld. n.s., not significant ($P > 0.05$) by two-tailed unpaired Student's t-test. Error bars indicate SD (n = 3 biological replicates).

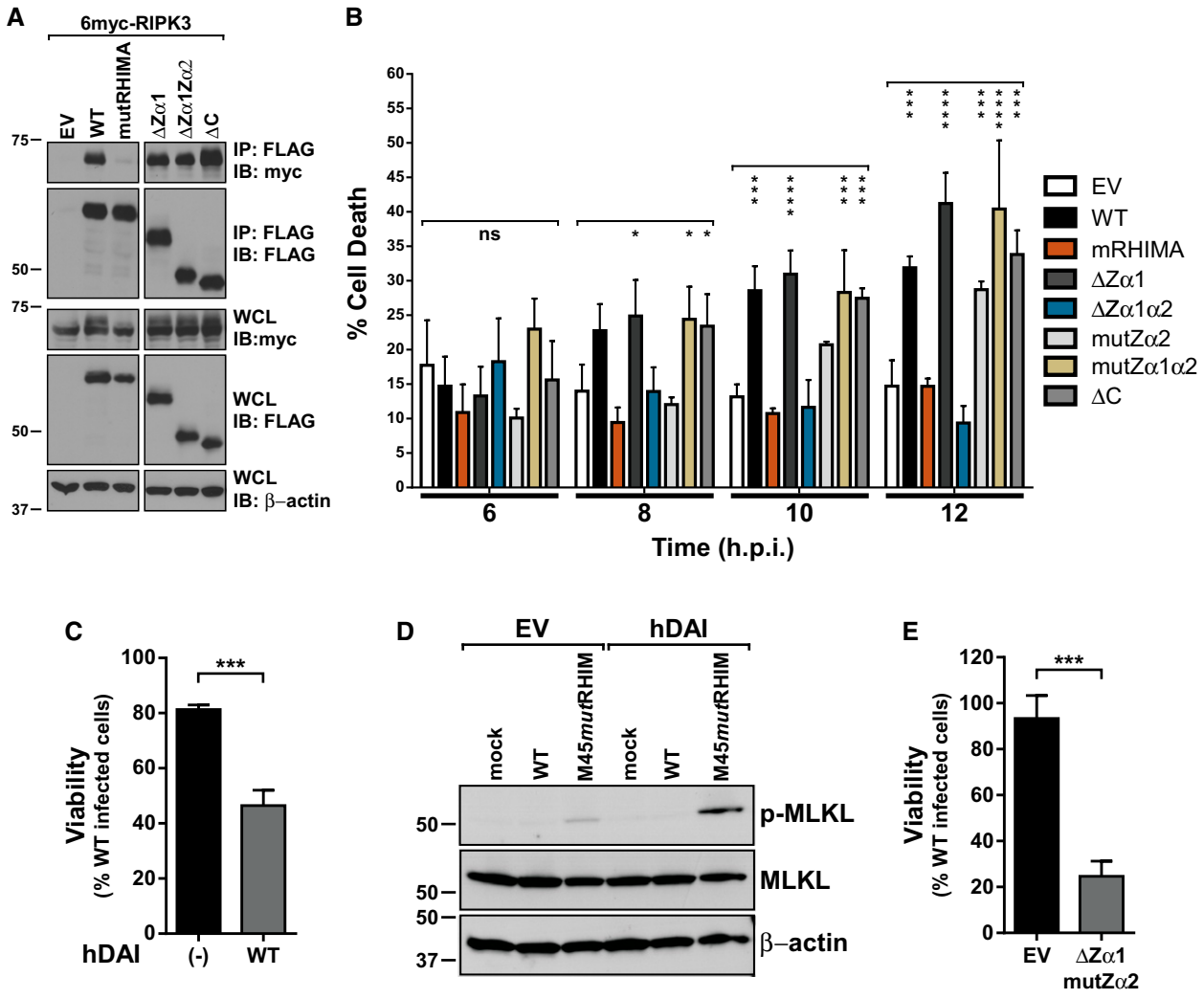

Figure EV4.

Figure EV4. Mutation of DAI's RNA binding domain delays necroptosis, and human DAI can mediate necroptosis.

A Immunoprecipitation (IP) and IB analysis to detect FLAG-DAI/ZBP1, myc-RIPK3, and β-actin from 293T cells transfected with vectors expressing 6myc-RIPK3 and different 3XFLAG-DAI/ZBP1 constructs, and subjected to IP with anti-FLAG M2 agarose beads.

B Cell death of DAI/ZBP1-reconstituted 29-11 cell lines infected with M45*mut*RHIM MCMV (MOI = 5); 6, 8, 10, and 12 h post-infection times were extracted from Fig 5D–F and replotted for clarity. ****$P < 0.0001$; ***$P < 0.001$; *$P < 0.05$; n.s., not significant ($P > 0.05$) by one-way ANOVA with Dunnett's multiple comparisons test, compared to EV. Error bars indicate standard deviation from the mean (SD; $n = 4$ biological replicates).

C Relative viability of HT-29 cells with or without hDAI expression infected with M45*mut*RHIM compared to WT MCMV. ***$P < 0.001$ by two-tailed unpaired Student's *t*-test. Error bars indicate SD ($n = 3$ biological replicates).

D IB analysis to detect p-MLKL, total MLKL, and β-actin from HT-29 cells with or without hDAI expression infected with WT or M45*mut*RHIM for 10 h.

E Relative viability of 29-11 cells reconstituted with empty vector (EV) or DAI-ΔZα1mutZα2 infected with M45*mut*RHIM compared to WT MCMV. ***$P < 0.001$ by two-tailed unpaired Student's *t*-test. Error bars indicate SD ($n = 3$ biological replicates).