

Mitochondria





Supplementary information, Figure S6. Gy10 plays no role in necrosome formation but is required for accumulation of RIP1 and RIP3 in the heterotypic membrane fraction during the process of necroptosis, related to Figure 7. (A) Gy10 plays no role in TNF induced NF-kB and MAP kinase activation. Control and $G\gamma 10$ knockdown L929 cells were stimulated with TNF (10 ng/ml) for the indicated time periods. Cells were harvested and whole cell extracts were examined by Western blot to determine phosphorylation and protein levels of indicated proteins. (B) Control and $G\gamma 10$ knockdown L929 cells were challenged with TNF (10 ng/ml) plus zVAD (20 µM) for 3 hours. Cell viability was measured using PI exclusion. (C) L929 cells were pretreated with PP2 (2 µM) for 2 hours and were challenged with TNF (10 ng/ml) plus zVAD (20 µM) for 3 hours. Cell viability was measured using PI exclusion. (D) Src kinase inhibitor does not affect the formation of necrosomes. Flag RIP1 knock-in L929 cells were pretreated with PP2 (2 µM) for 2 hours and were challenged with TNF (10 ng/ml) and $zVAD (20 \mu M)$ for 3 hours. Immunoprecipitation and immunoblotting were performed as in Figure 7A. (E) Images of mitochondria fraction and heterotypic membrane (HeteroM) fraction by transmission electron microscopy. Data in (B), and (C) depict mean \pm SEM of one representative experiment of three or more.