

Supplementary information, Figure S4. Src is activated in L929 cells by TNF stimulation in a $G\gamma 10$ dependent but necrosome independent manner, related to Figure 5. (A) Cells were pretreated with or without PI3K inhibitor LY294002 (20 µM) for 12 hours and then challenged with TNF (10 ng/ml) for 12 hours. Cell viability was measured using PI exclusion. (B) Control and Gy10 knockdown cells were challenged with TNF (10 ng/ml) for 10 hours. Then the cellular potassium concentrations were measured as described in Experimental Procedures. (C) Control and $G\gamma 10$ knockdown cells were challenged with TNF (10 ng/ml) for 6 hours. Then the cellular calcium concentrations were measured as described in Experimental Procedures. (D) TNF induced Src activation depends on TNFR1. L929 cells were infected with lentivirus expressing TNFR1 shRNA or control shRNA. After 48 hours, knockdown efficiency of TNFR1 was determined using qRT-PCR. Cell viability was measured after stimulation with TNF (10 ng/ml) for 12 hours. Data in (A) - (D) depict mean \pm SEM of one representative experiment of three or more.