Supplementary Information

Necrotic cells influence migration and invasion of glioblastoma via NF-κB/AP-1-mediated IL-8 regulation

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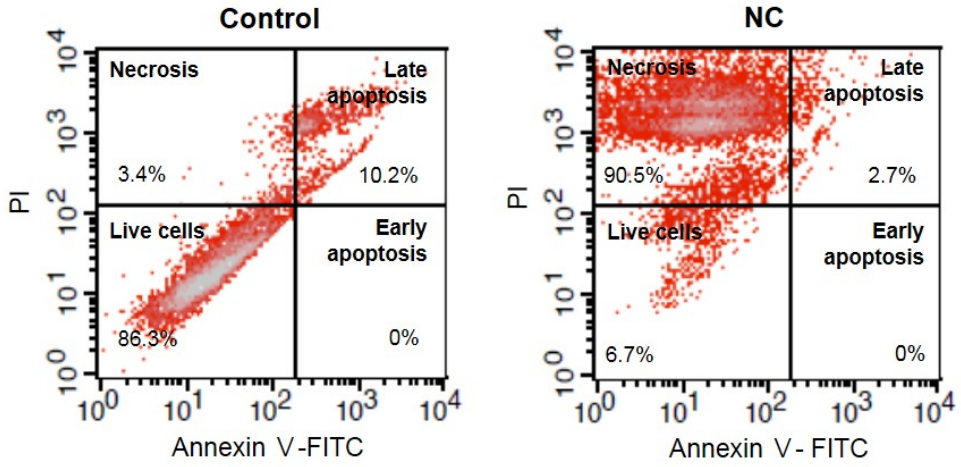
Supplementary Figure S1. Preparation of necrotic cells. CRT-MG cells were frozen and thawed through five cycles of liquid nitrogen-water bath. Cells were stained using Annexin Ⅴ-FITC and propidium iodide (PI), and then the quantitation of apoptosis/necrosis was determined by flow cytometry. The proportion of necrotic cells stained positive for PI was seen to consist of over 90% of prepared cells. NC, necrotic cells.

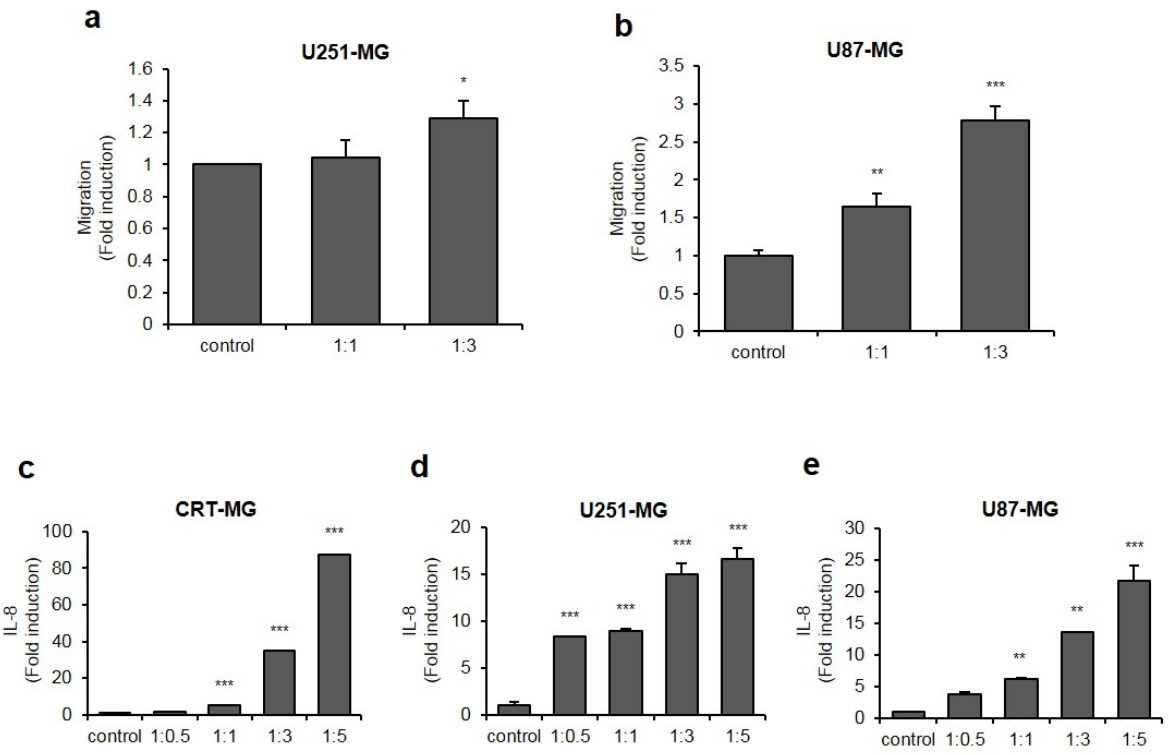
Supplementary Figure S2. Necrotic cells increase migration and IL-8 expression in U251-MG and U87-MG cells. (a,b) U251-MG and U87-MG cells were treated with necrotic cells for 0, 24 and 48 h. Migration activity was measured by calculating the area that advanced from boundary lines of scratch to cell-free space for 24 or 48 h. Data are presented as the fold induction compared with each untreated control cells. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. control. (c-e) CRT-MG, U251-MG and U87-MG cells were either untreated or treated with different ratios of necrotic cells (NC) for 24 h as indicated. After incubation, supernatants from each condition were collected and IL-8 protein levels were measured by ELISA. Data were presented as fold induction compared with control cells. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. control.

Supplementary Figure S3. Necrotic cells affect proliferation of CRT-MG cells. (a) CRT-MG cells (1×10^4) were seeded and treated with necrotic CRT-MG cells for 24 h. CCK-8 solution was further incubated for 4 h and absorbance was measured at 450 nm using a microplate reader to cell proliferation. $***P < 0.001$ *vs.* control. (b) CRT-MG cells treated with necrotic cells in the presence or absence of either neutralizing IL-8 antibody (2.5 μg/ml) or control IgG (2.5 μg/ml) for 24h. Proliferation was measured by CCK-8. $***P < 0.001$ *vs.* control. n.s., not significant. Data shown are representative of at least three experiments.

Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Fig. S3