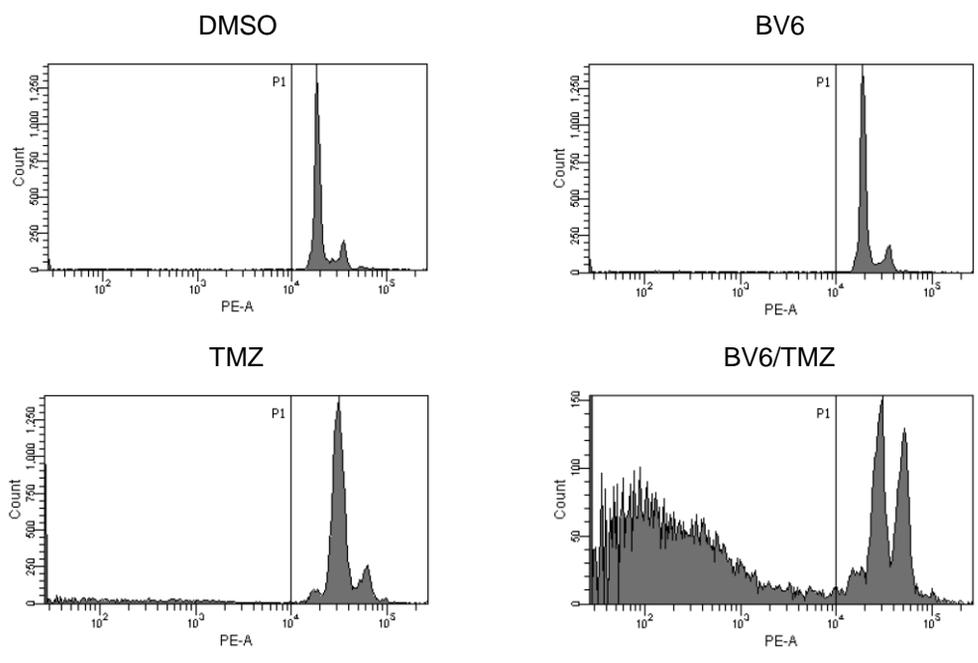
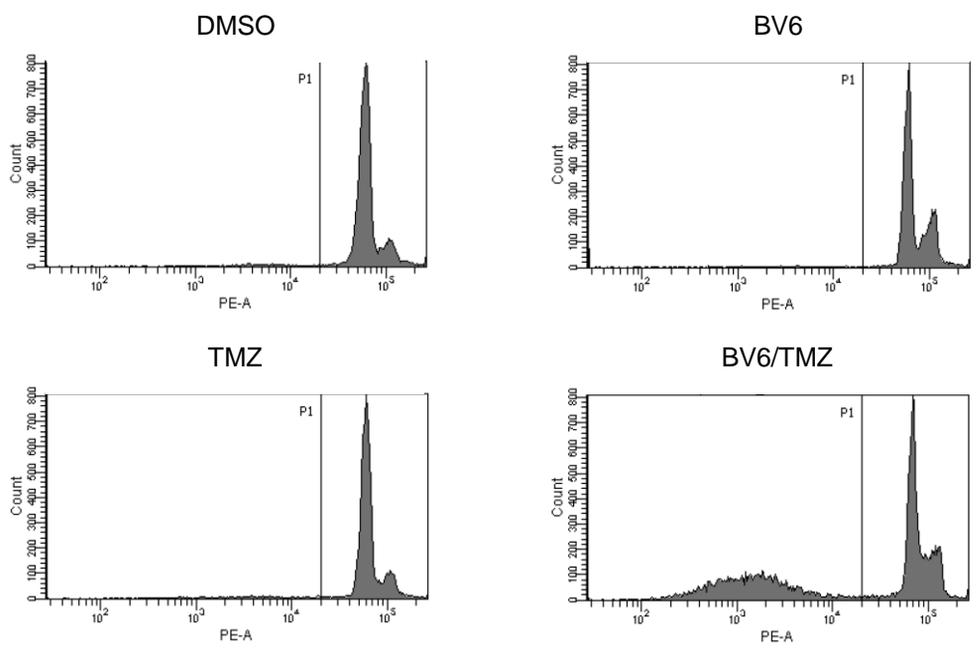


# Suppl. Fig. 1

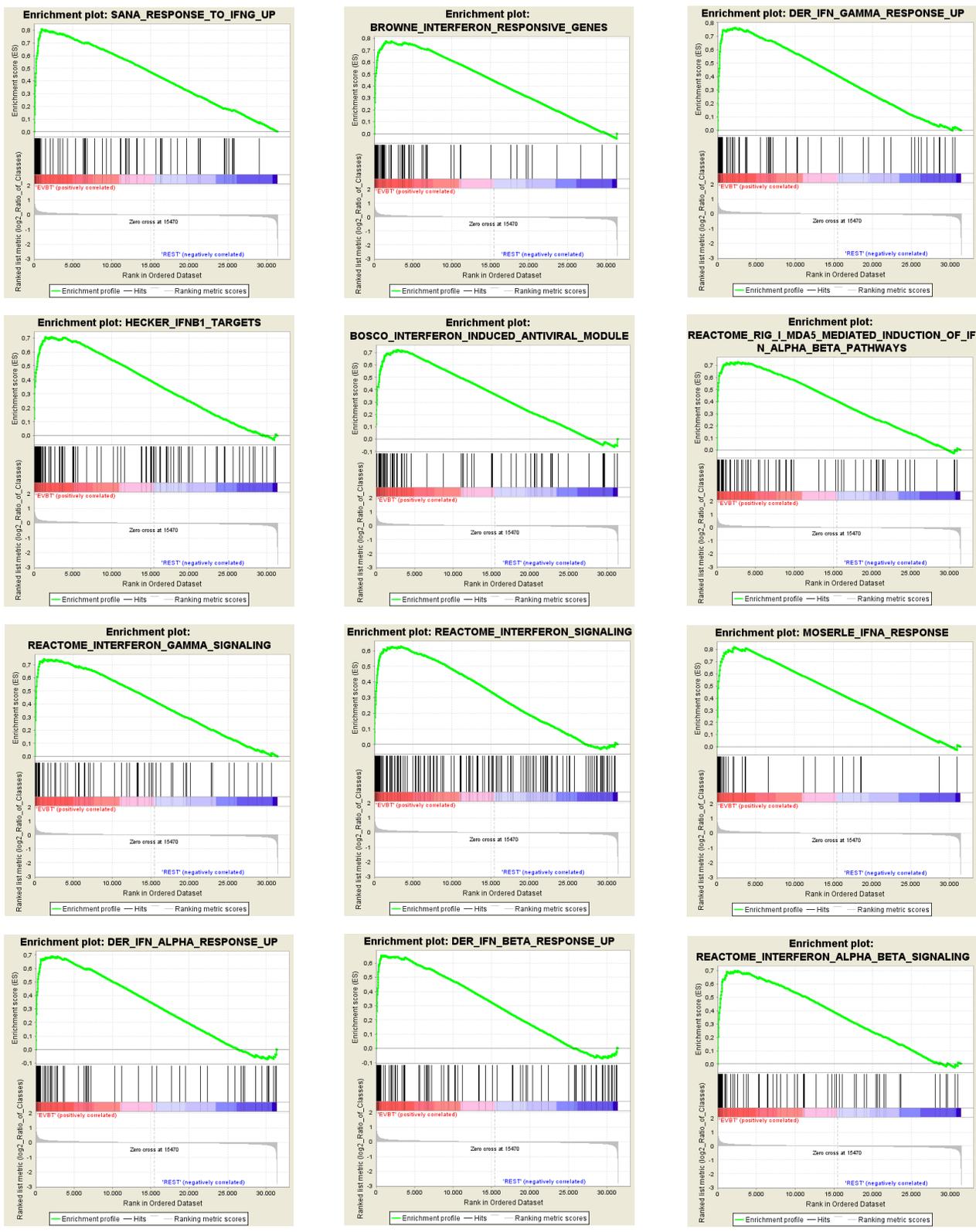
## A



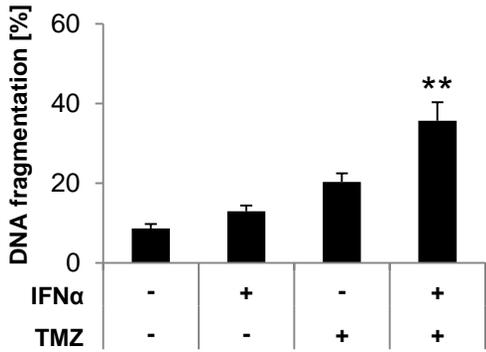
## B



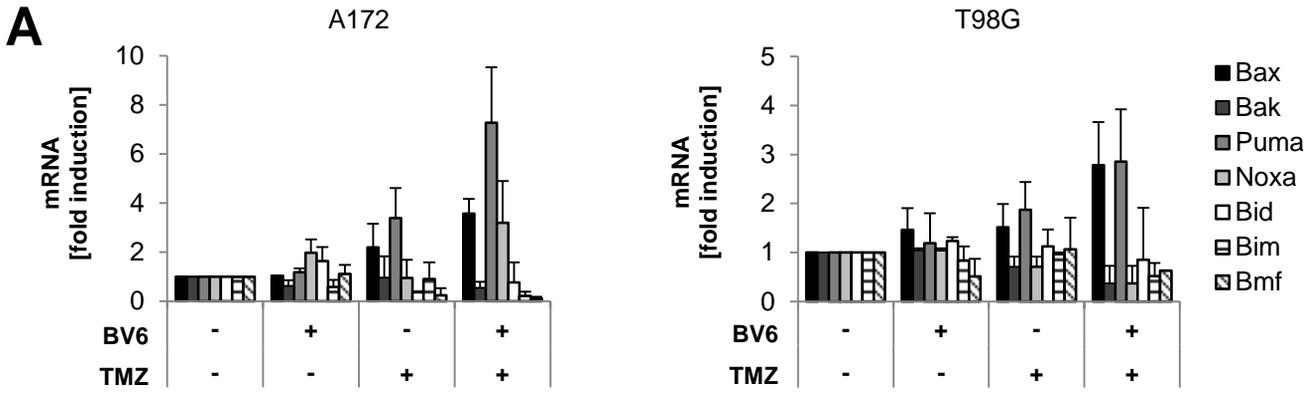
# Suppl. Fig. 2



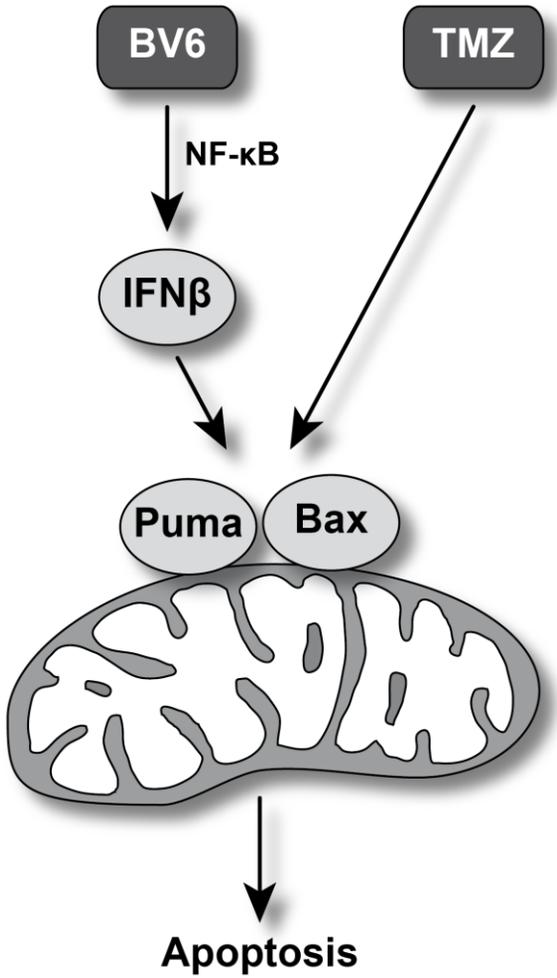
**Suppl. Fig. 3**



Suppl. Fig. 4



Suppl. Fig. 5



## 1 **Supplementary figure legends**

### 2 **Suppl. Figure 1: BV6 enhances TMZ-induced apoptosis**

3 A172 cells (a) or T98G cells (b) were treated for 144 hours with 100  $\mu$ M TMZ and/or  
4 or 2  $\mu$ M BV6 (A172) or 4  $\mu$ M BV6 (T98G) or DMSO. Apoptosis was determined by  
5 FACS analysis of DNA fragmentation of PI-stained nuclei. Representative graphs of  
6 three independent experiments performed in triplicate are shown.

7

### 8 **Suppl. Figure 2: BV6/TMZ co-treatment upregulates IFN-responsive genes**

9 A172 cells stably expressing I $\kappa$ B $\alpha$ -SR or EV were treated for 9 hours with 100  $\mu$ M  
10 TMZ and/or 2  $\mu$ M BV6 or DMSO. Whole-Genome expression profiling of three  
11 independent experiments was performed. Genes with similar regulation in A172 cells  
12 expressing I $\kappa$ B $\alpha$ -SR served as control for background expression of non-NF- $\kappa$ B-  
13 stimulated genes. GSEA was performed comparing TMZ/BV6-treated cells to all  
14 other settings. Enrichment plots of IFN signaling-mediated gene sets out of the top  
15 100 regulated gene sets upon BV6/TMZ-treatment are shown.

16

### 17 **Suppl. Figure 3: IFN $\alpha$ sensitizes glioblastoma cells to TMZ-induced apoptosis**

18 A172 cells were treated for 120 hours with 100  $\mu$ M TMZ and/or 1 ng/ml IFN $\alpha$  or  
19 DMSO. Apoptosis was determined by FACS analysis of DNA fragmentation of PI-  
20 stained nuclei. Mean values + SD of three independent experiments performed in  
21 triplicate are shown; \*p<0.05; \*\*p<0.01.

22

### 23 **Suppl. Figure 4: BV6/TMZ-mediated upregulation of Puma and Bax**

24 (a) A172 cells (left) or T98G cells (right) were treated for 48 hours with 100  $\mu$ M TMZ  
25 and/or 2  $\mu$ M BV6 (A172) or 4  $\mu$ M BV6 (T98G) or DMSO. Bax, Bak, Puma, Noxa, Bid,  
26 Bim and Bmf mRNA levels were analyzed by qRT-PCR, normalized to 28S rRNA

27 expression and fold increase in mRNA levels are shown. Mean values + SD of two  
28 independent experiments performed in duplicate are shown.

29 (b) A172 cells (left) or T98G cells (right) were treated for 48 hours with 100  $\mu$ M TMZ  
30 and/or 2  $\mu$ M BV6 (A172) or 4  $\mu$ M BV6 (T98G) or DMSO. Expression levels of Bax  
31 and Puma were analyzed by western blotting, asterisks indicate unspecific bands.  
32 Expression of  $\beta$ -actin served as loading control. A representative experiment of two  
33 independent experiments is shown.

34

35 **Suppl. Figure 5: BV6-mediated upregulation of IFN $\beta$  sensitizes glioblastoma**  
36 **cells to TMZ-induced mitochondrial apoptosis**

37 Smac mimetic BV6-induced NF- $\kappa$ B-activation promoted transcriptional upregulation  
38 of IFN $\beta$ . IFN $\beta$  and TMZ cooperated to induce apoptosis in glioblastoma cells.  
39 Cooperative upregulation of the proapoptotic Bcl-2 family proteins Puma and Bax via  
40 BV6/TMZ treatment contributed to BV6/TMZ-induced apoptosis.

41

42