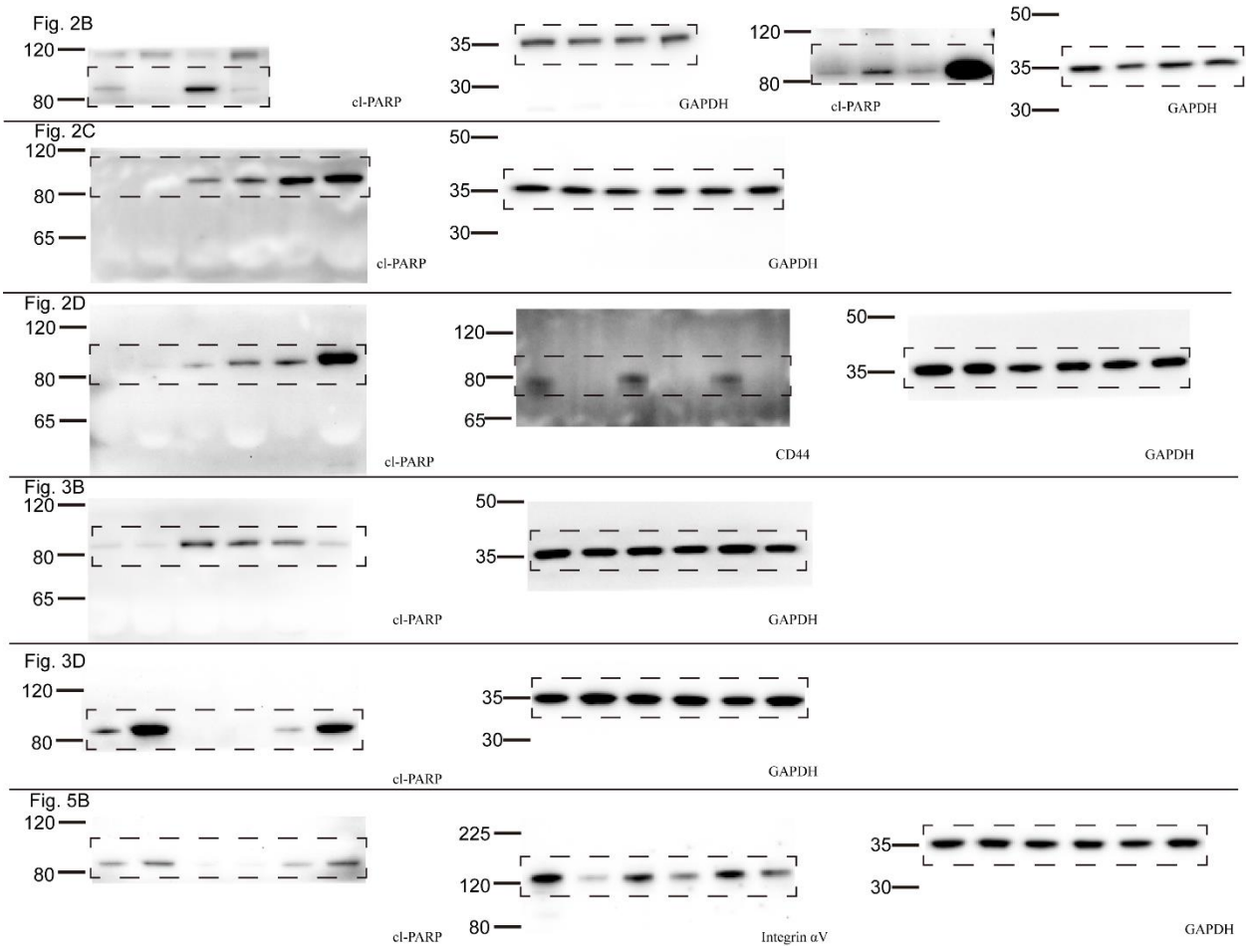


Figure S1. Uncropped chemiluminescent signals of all Western blot images shown in the manuscript. Ladders labeled in unit of kDa were indicated next to each blot. Band selections were indicated with dashed rectangles.

Figure S1 Cont.

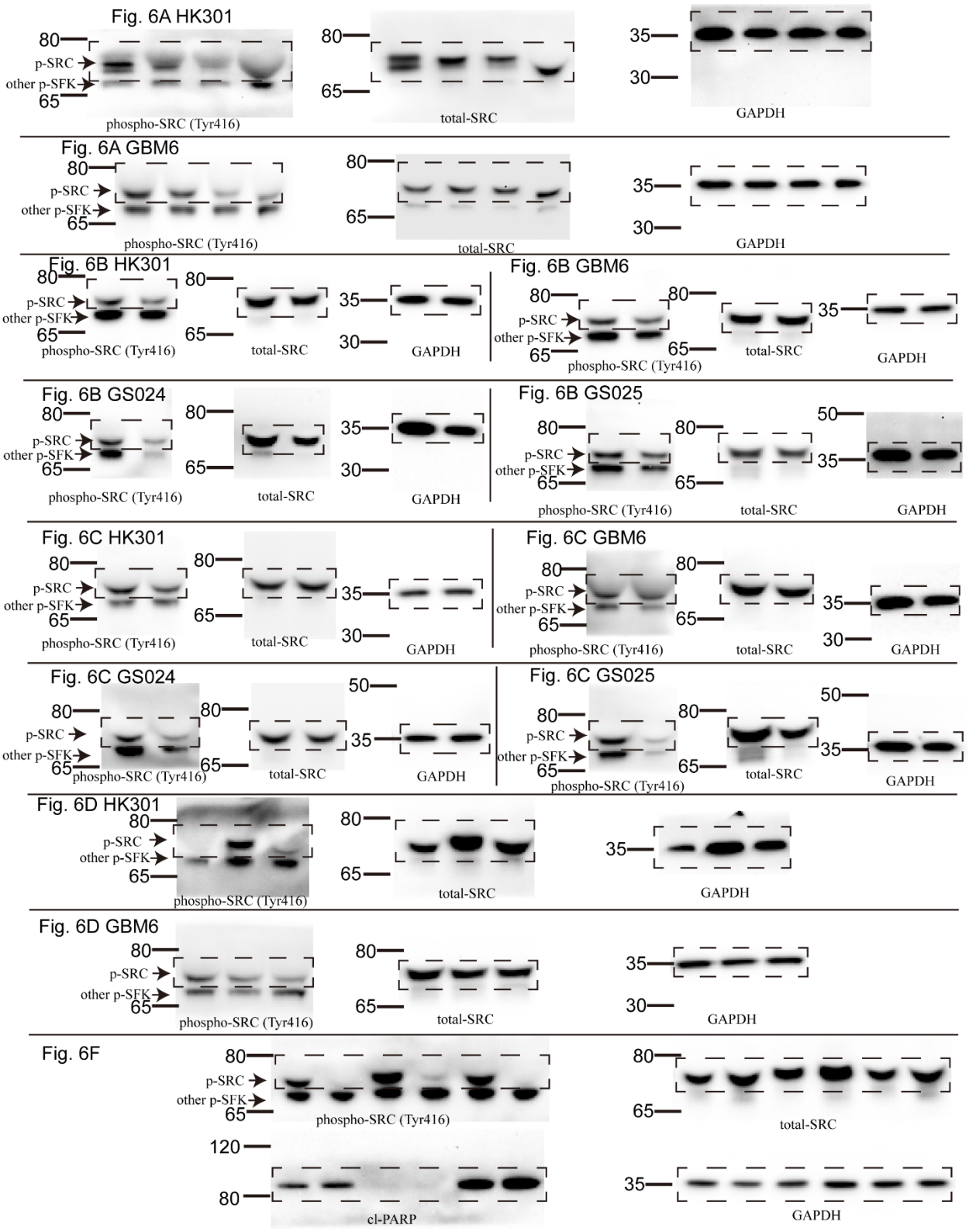
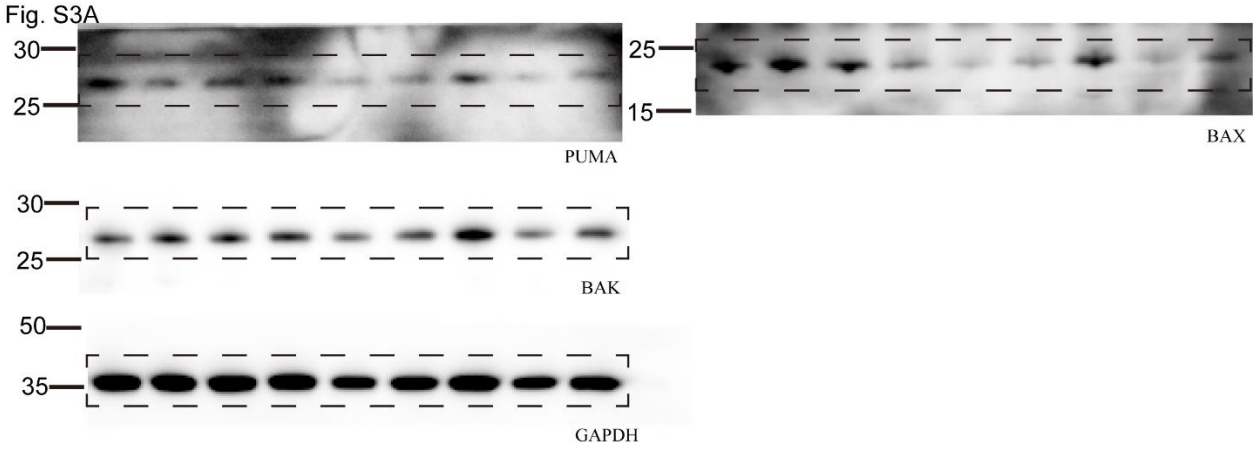
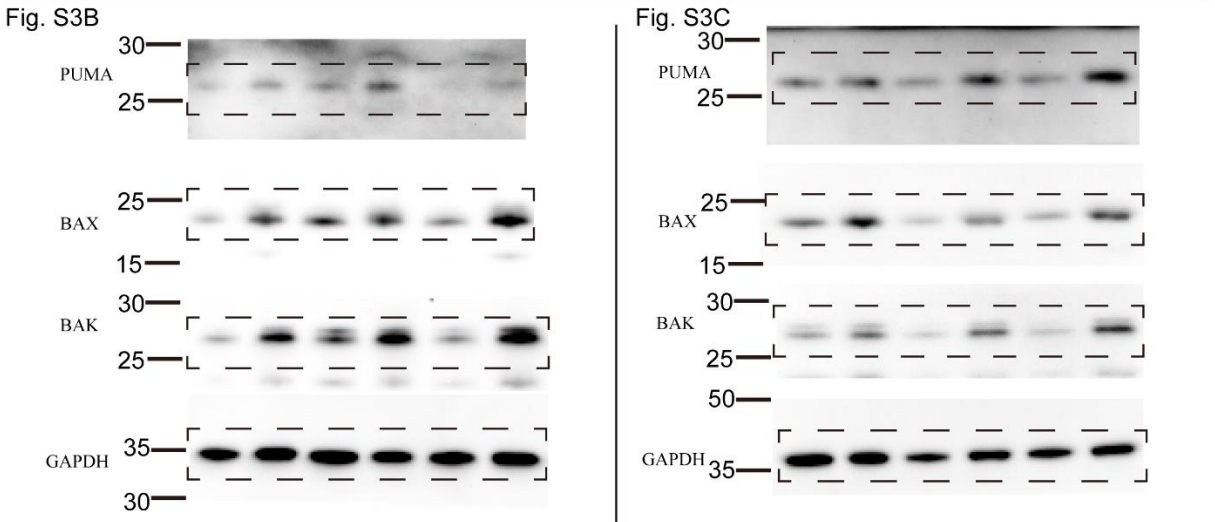


Figure S1 Cont.





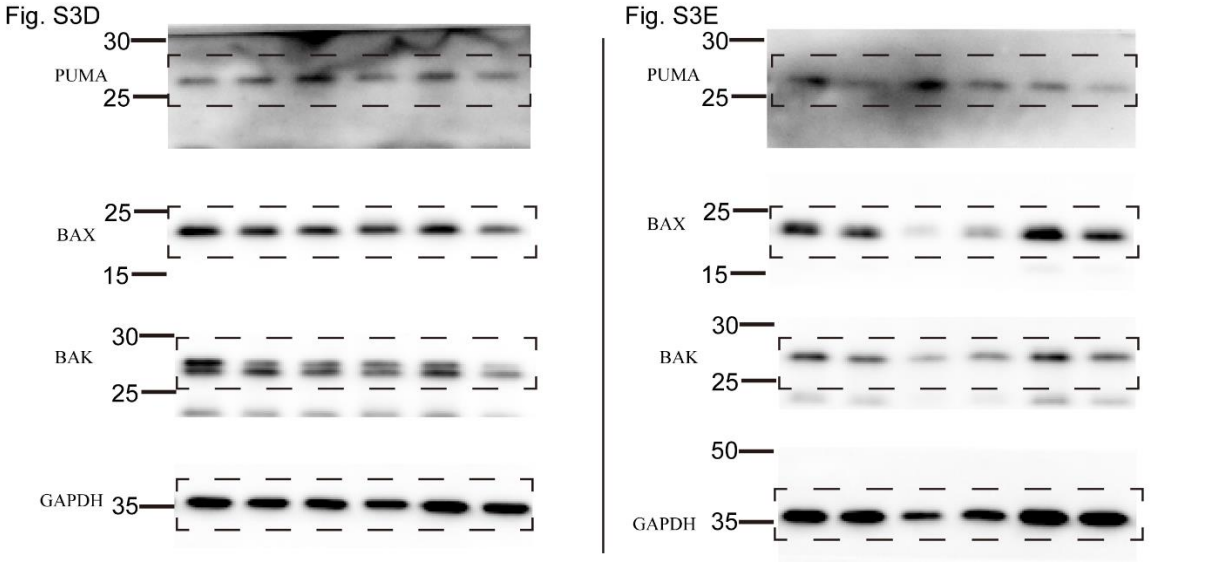


Figure S1 Cont.

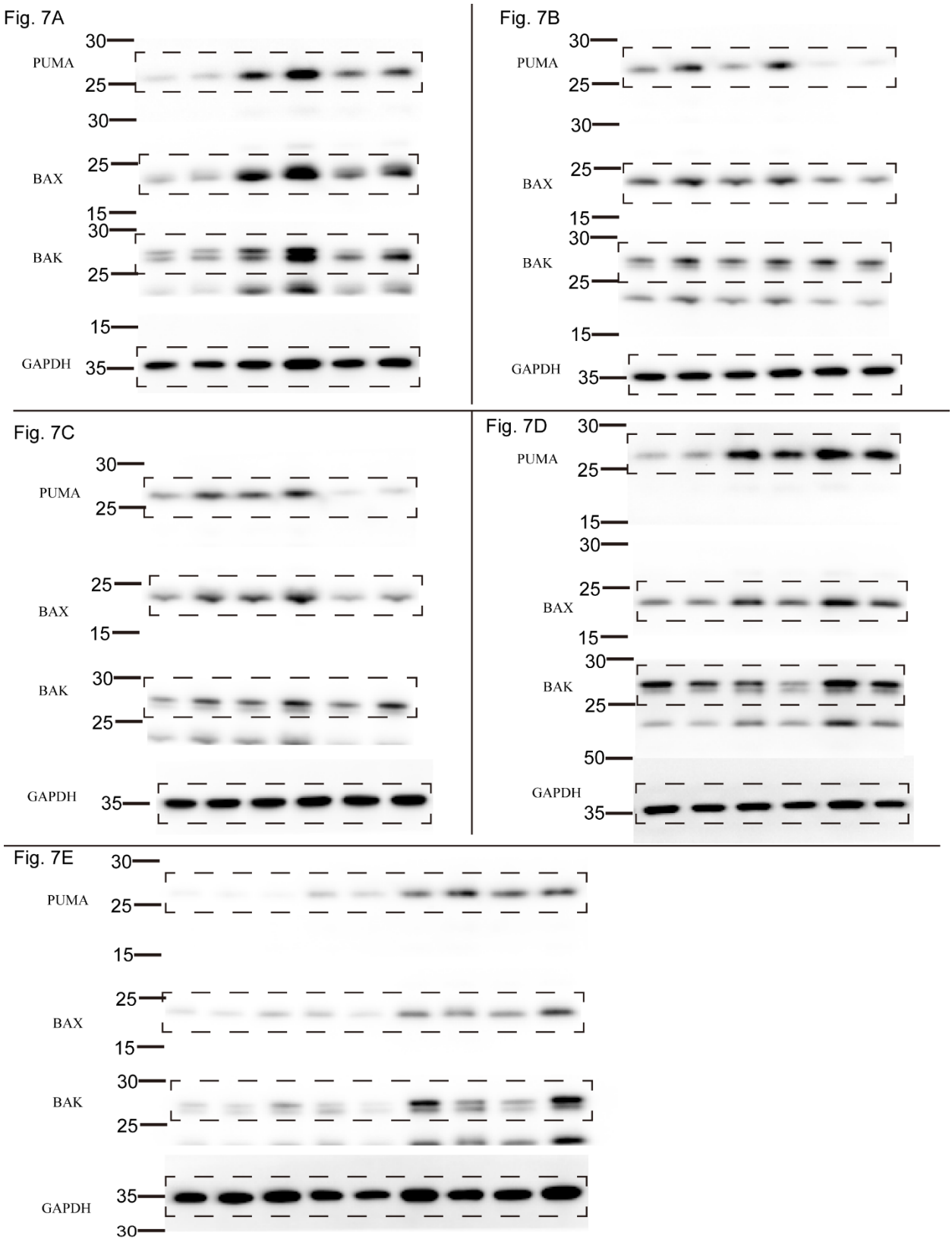


Figure S1 Cont.

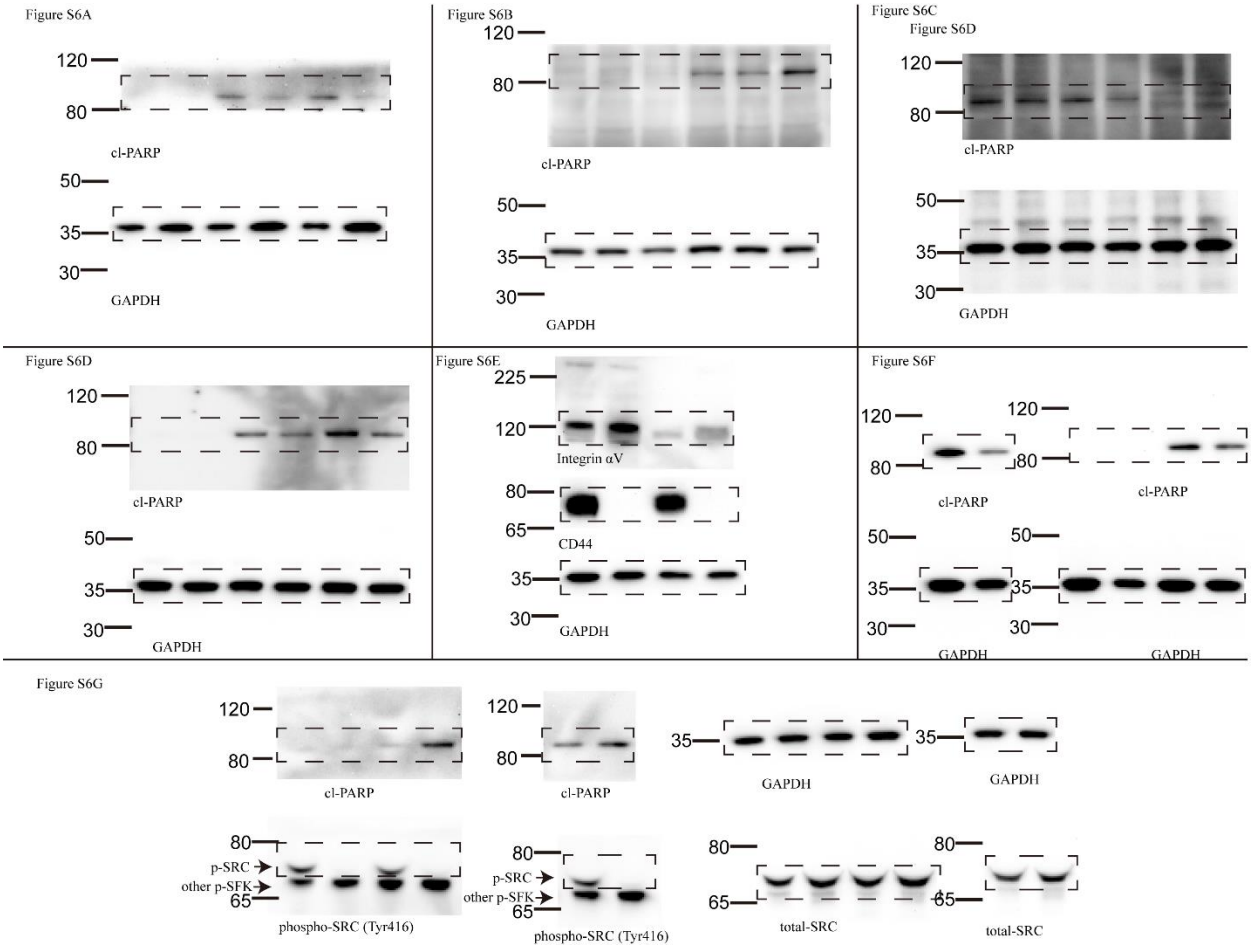


Figure S2

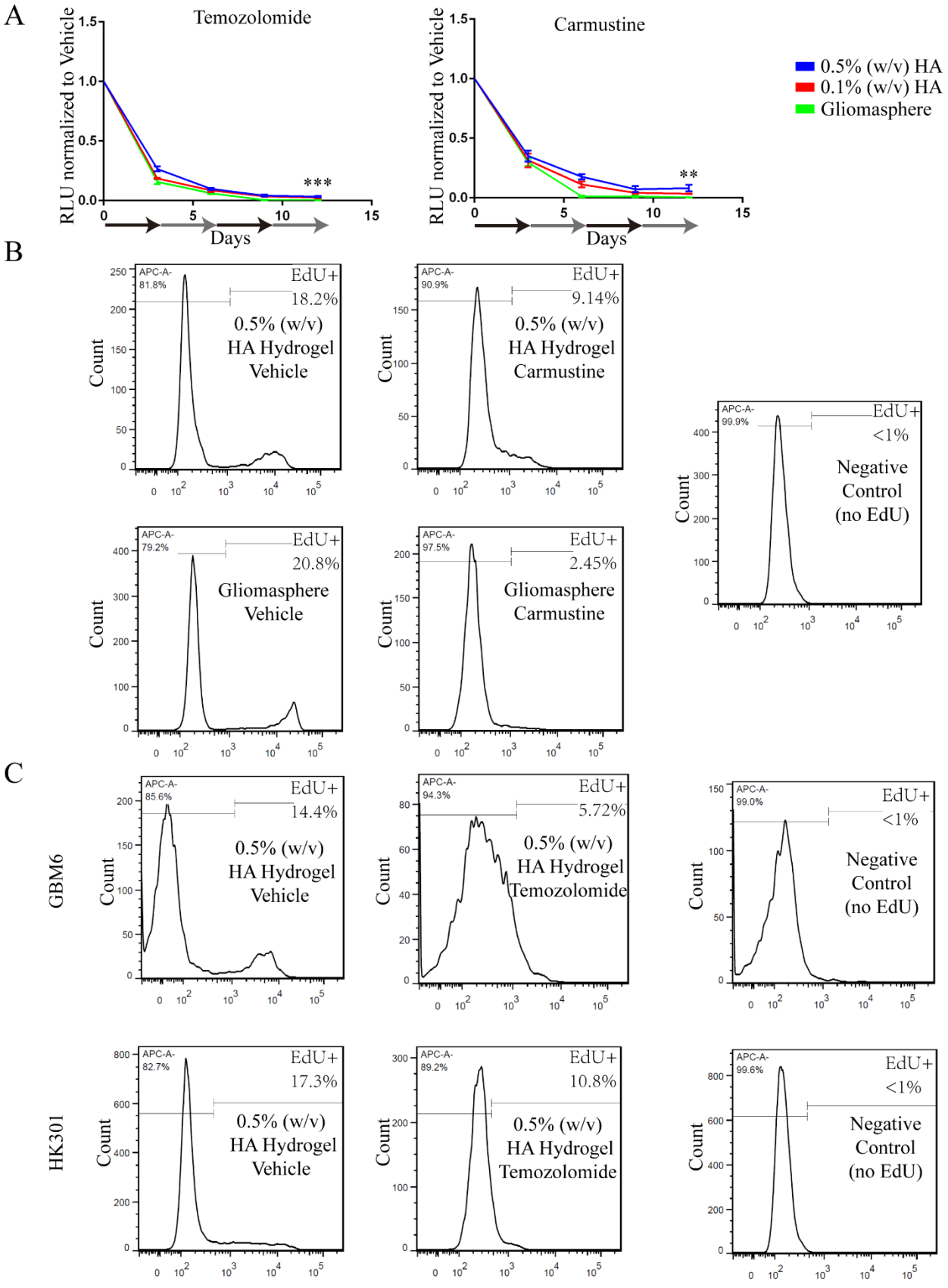

A
Temozolomide
Carmustine
RLU normalized to Vehicle
Days
***
**
0.5% (w/v) HA
0.1% (w/v) HA
Gliomasphere
B
EdU+ 18.2%
0.5% (w/v) HA Hydrogel Vehicle
EdU+ 9.14%
0.5% (w/v) HA Hydrogel Carmustine
EdU+ <1%
Negative Control (no EdU)
EdU+ 20.8%
Gliomasphere Vehicle
EdU+ 2.45%
Gliomasphere Carmustine
C
GBM6
EdU+ 14.4%
0.5% (w/v) HA Hydrogel Vehicle
EdU+ 5.72%
0.5% (w/v) HA Hydrogel Temozolomide
EdU+ <1%
Negative Control (no EdU)
HK301
EdU+ 17.3%
0.5% (w/v) HA Hydrogel Vehicle
EdU+ 10.8%
0.5% (w/v) HA Hydrogel Temozolomide
EdU+ <1%
Negative Control (no EdU)
Count
APC-A-

Figure S2. A) Relative luminescence units (RLU) were normalized with background and noise. Normalized RLU of treated GBM6 cells were normalized to non-treated samples and then the signal before treatment (day 0) for each condition. Two-way ANOVA (culture condition, time) was performed. Error bars show standard deviations (n=3). ** $p<0.01$, *** $p<0.001$. B) Representative flow cytometry graph showing proliferation rate of HK301 cells (EdU incorporation over 2.5 hrs) 3 days after 50μM carmustine or vehicle treatment. C) Representative flow cytometry graphs showing proliferation rate of HK301 and GBM6 cells (EdU incorporation over 2.5 hrs) 3 days after 500μM temozolomide or vehicle treatment. The EdU positive (EdU+) cell population represents the percentage of cells that went through the S phase of cell division while EdU was present in the culture medium.

Figure S3

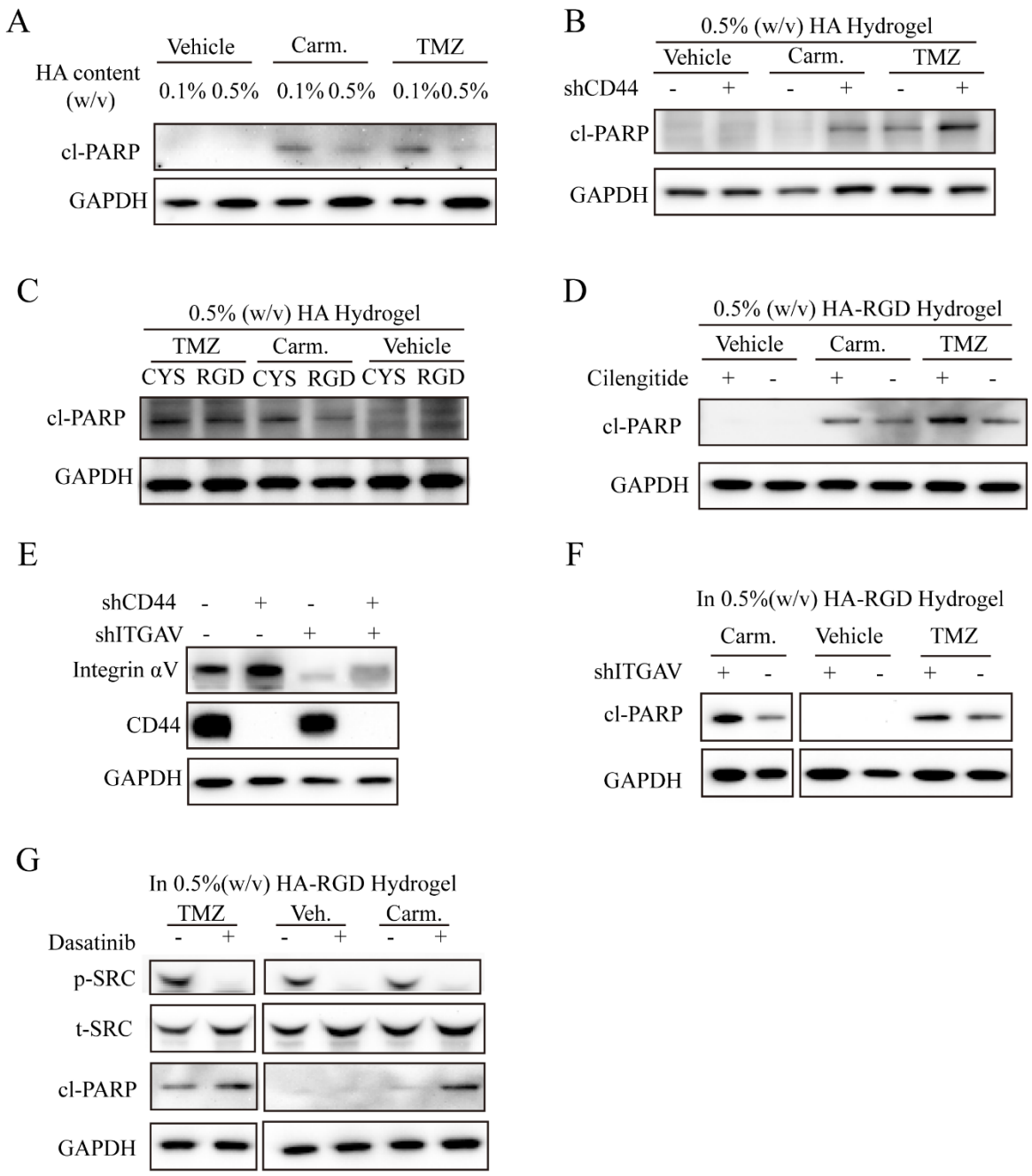


Figure S3. Representative phase contrast images of GS024 cells cultured in different hydrogel conditions. 50μM cilengitide, 100nM dasatinib, or vehicle were added to the cell cultures 8 days after encapsulation and incubated for 24hr. Scale bars=200μm. Arrows indicate cells displaying invasive morphology.

Figure S4

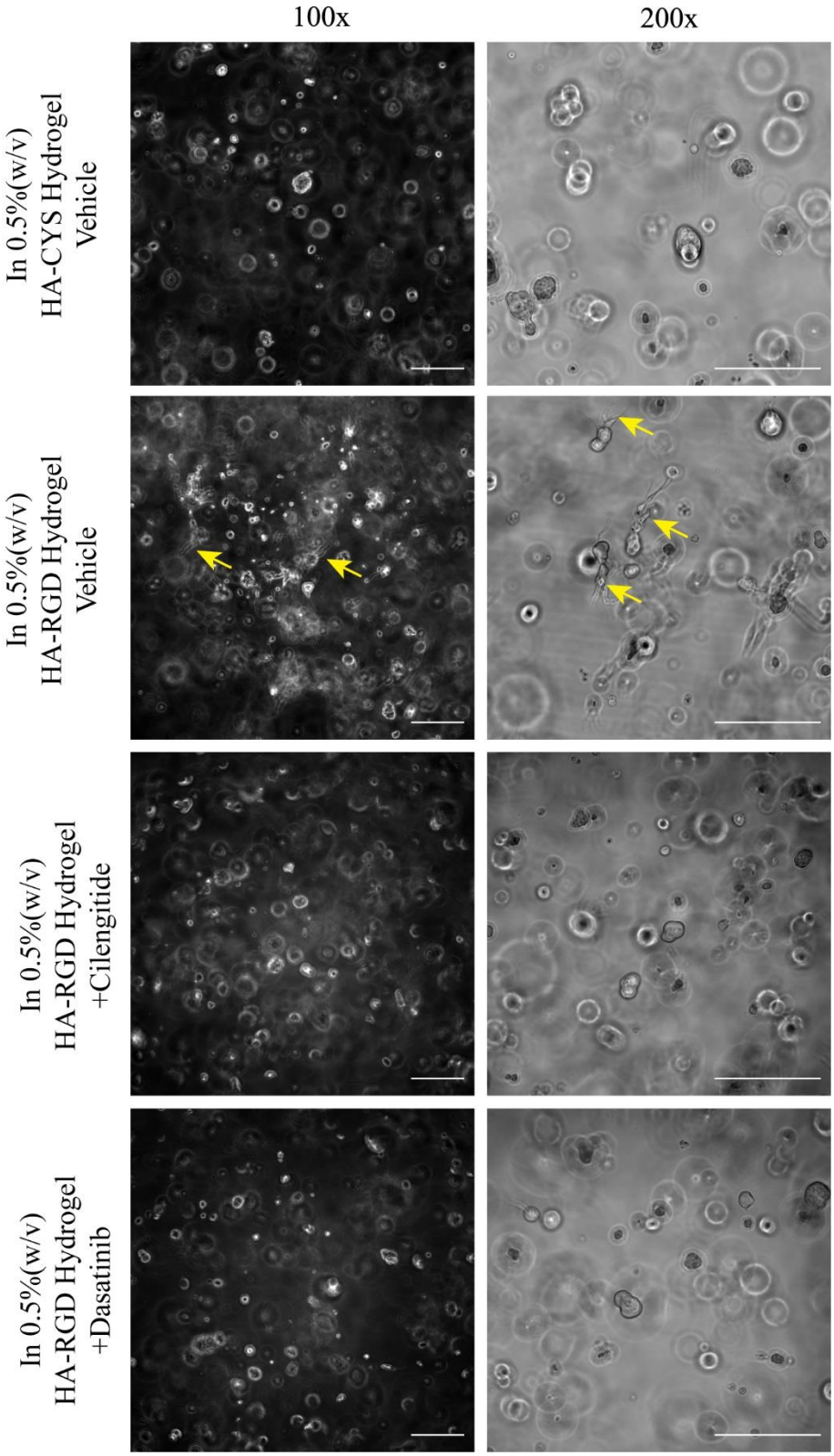


Figure S4. Representative phase contrast images of GS024 cells cultured in different hydrogel conditions. 50μM cilengitide, 100nM dasatinib, or vehicle were added to the cell cultures 8 days after encapsulation and incubated for 24hr. Scale bars=200μm. Arrows indicate cells displaying invasive morphology.

Figure S5

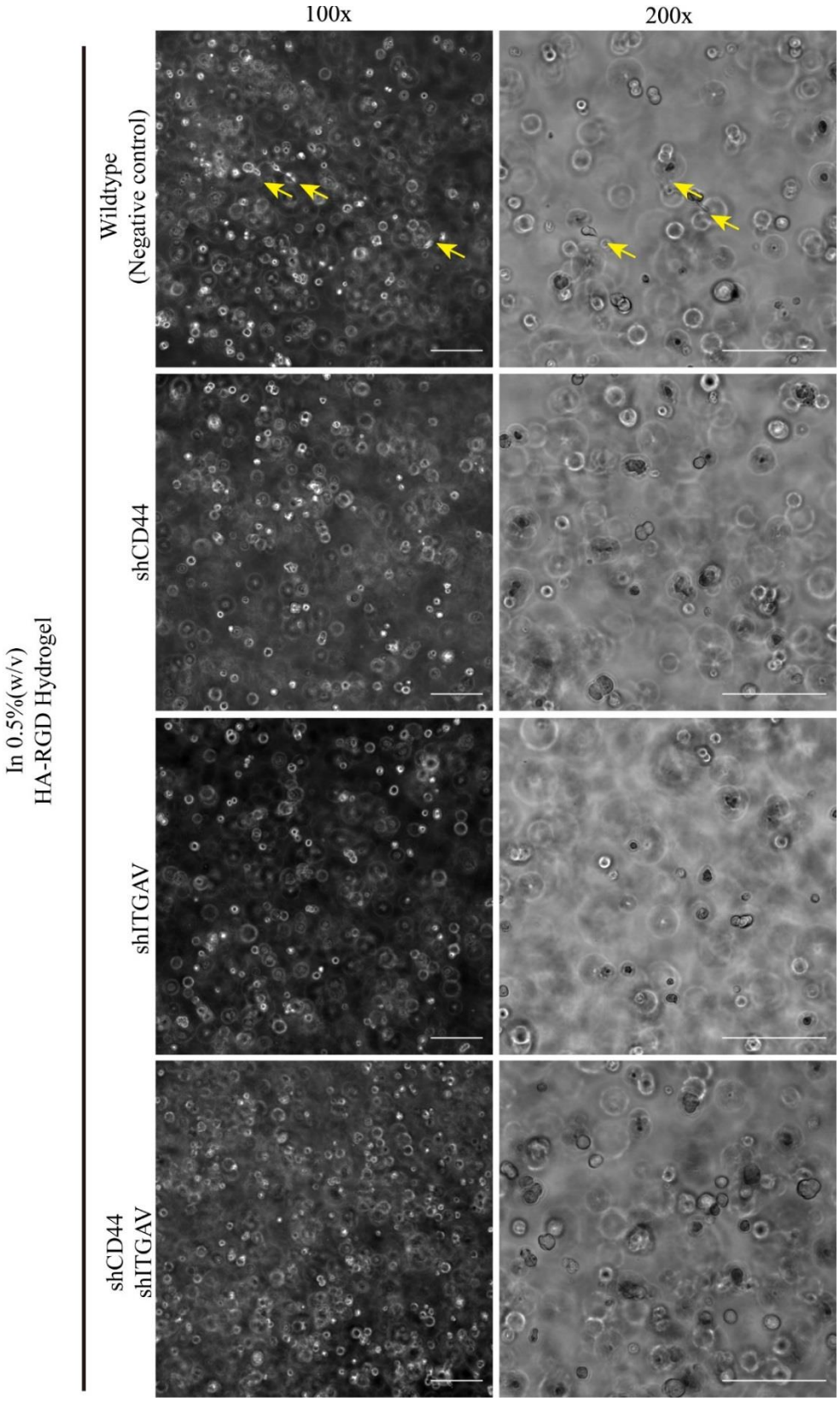


Figure S5. Representative phase contrast images of GBM6 cells cultured in different viral transduction conditions 8 days after encapsulation in 0.5%(w/v)HA-RGD hydrogels. Scale bars=200µm. Arrows indicate cells displaying invasive morphology.

Figure S6

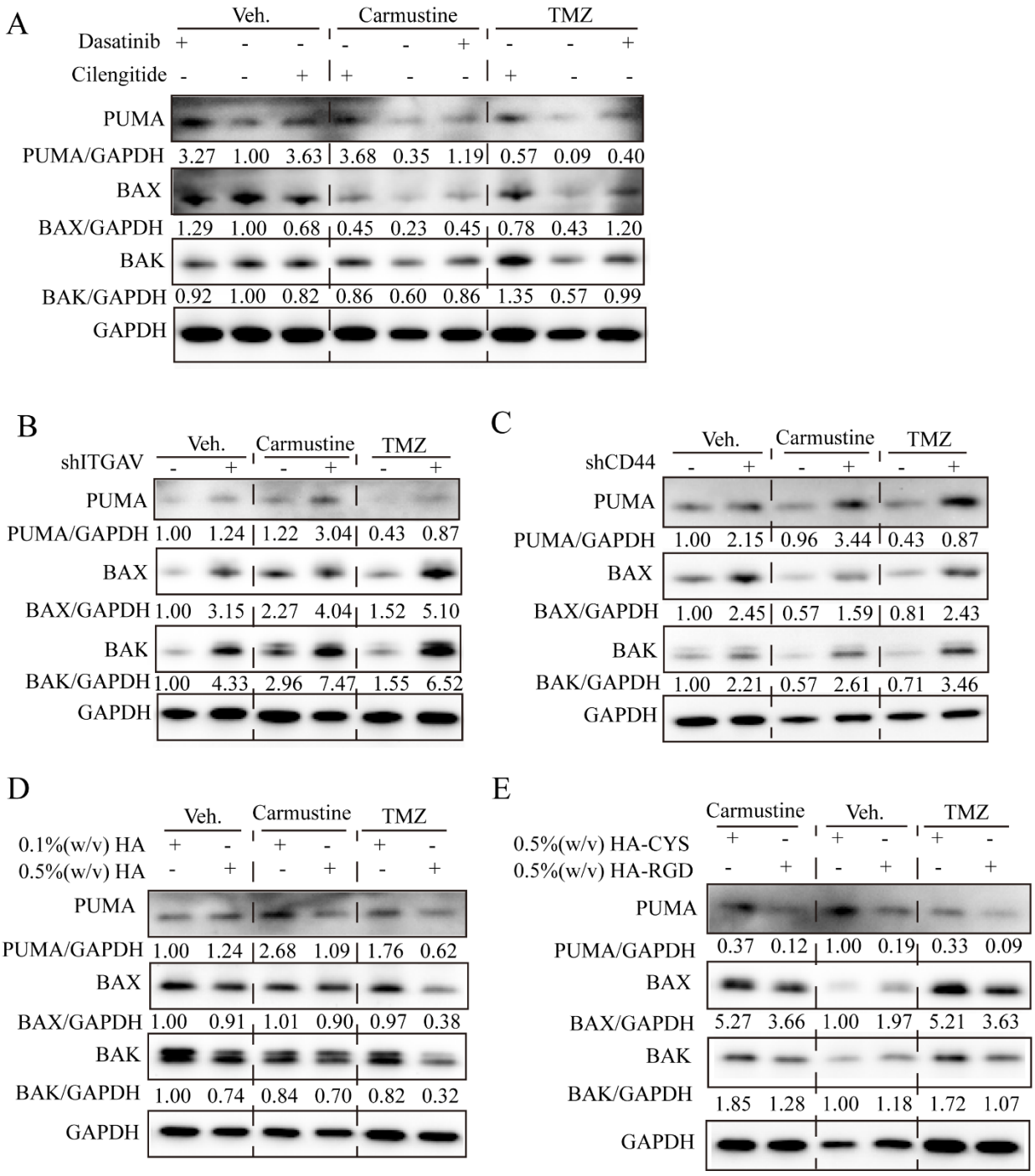


Figure S6. Representative Western blot images of GBM6 cultured in various hydrogel properties, genetic knockdown conditions and treatments. The cells were cultured in 0.5%HA(w/v)-RGD hydrogels throughout panel A and B, and no RGD mimetic peptide was incorporated into the hydrogels in panel C and D. All culture lysates were collected 72hr after chemotherapy or vehicle treatments. Densitometry analysis of integrated band intensities normalized to GAPDH was indicated in each panel.

Figure S7

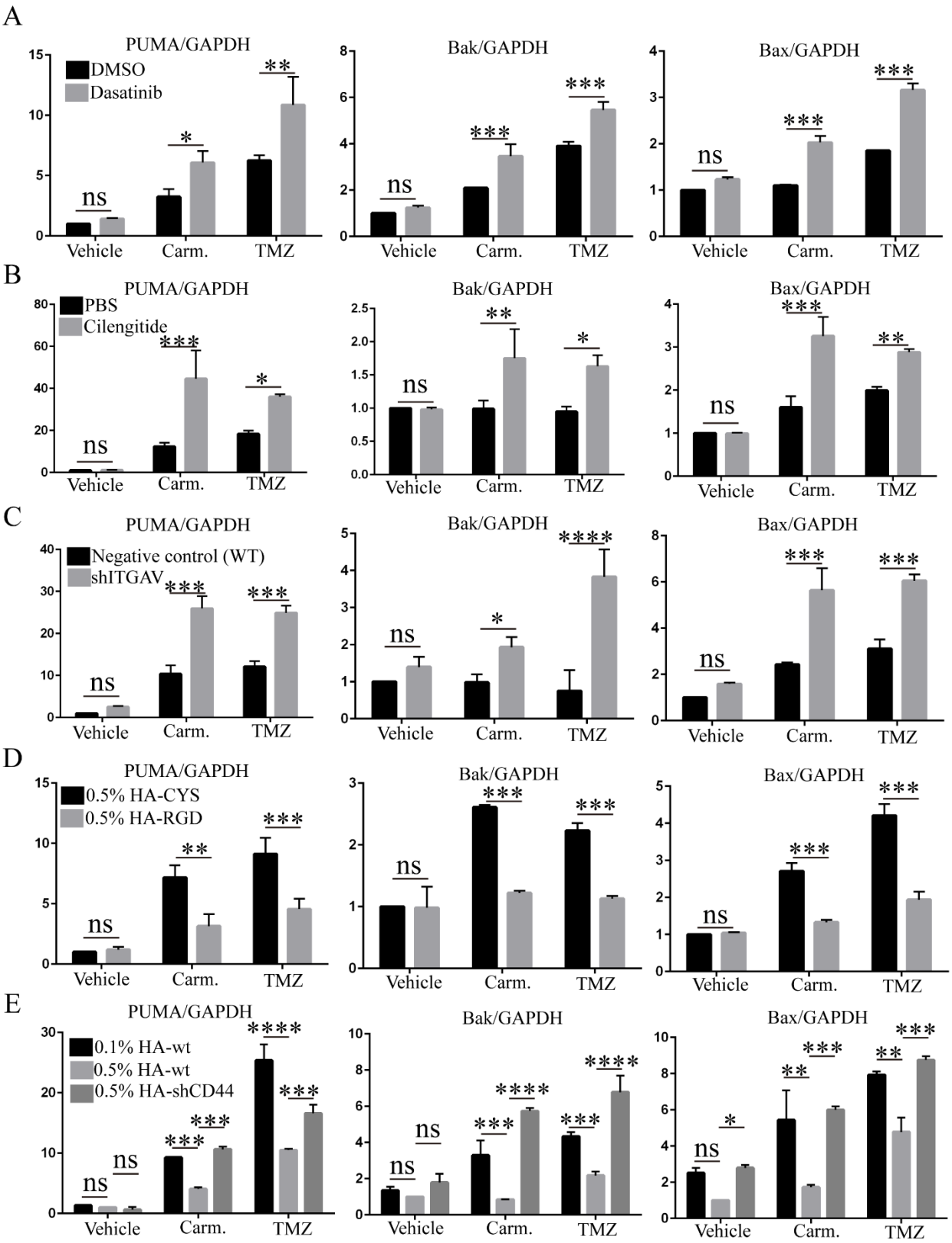


Figure S7. Densitometry quantification of Western blotting data represented in Figure 7. Student's *t* test was used to compare pro-apoptotic factor expression within each treatment arm. **p*<0.5, ***p*<0.01, ****p*<0.001, *****p*<0.0001, "ns" represents non-significance. n=3 to 5.