Supplemental Figure 1. Hypoxia-dependent E1 protein expression by HYPR-Ad-mIL4 infected cells correlates with cellular HIF-1α levels. (A) Cellular HIF-1α protein levels were measured under normoxia (N) or hypoxia (H) in uninfected LN229 human glioblastoma cells on Days 1, 3, and 6 by Western blot analysis of total cell lysates. Actin was used as a loading control. Cellular HIF-1α protein expression was induced in response to hypoxia as early as Day 1 and continued through Day 6. In contrast, HIF-1α protein was not detectable under normoxia. **(B)** LN229 were infected at MOI 1 with HYPR-Ad-mIL4, *dl*309-Ad, HYPR-Ad#1 viruses or mock infected and then maintained under normoxia (N) or hypoxia (H). Ad E1B 55K (arrow) and E1B 21K were measured by Western blot analysis of total cell lysates at Days 1 and 3 postinfection. Cellular HIF-1β and actin were used as loading controls. Expression of E1B 21K and 55K were not detectable on Day 1. By Day 3, E1B 21K and 55K were both expressed in infected HYPR-Ad-mIL4 and HYPR-Ad#1 cells with increased levels under hypoxia while their expression by *dl*309-Ad was similar under normoxia and hypoxia. The asterisk indicates a non-specific band detected by the E1B 55K antibody.

Supplemental Figure 2. Hypoxia-dependent mIL-4 expression by HYPR-Ad-mIL4 infected cells. NHA **(A)** and D247MG **(B)** cells were infected at MOI 1 or 5, respectively, with AdLacZ, *dl*309-Ad, HYPR-Ad#1, HYPR-Ad-mIL4 or mock infected and then maintained under normoxia (N, white) or hypoxia (Hyp, black). mIL-4 in the conditioned media was measured by ELISA at the indicated days. The data represent the mean mIL-4 concentration (pg/ml) ± SD. Asterisks indicate a statistically significant (Students *t* test) increase in mIL-4 levels under hypoxia. In the NHA study **(A)**, the level

of mIL-4 under hypoxia vs. normoxia following HYPR-Ad-mIL4 infection was significantly increased by 26-fold on Day 3 ($p= 7.2 \times 10^{-10}$, left panel) and 14-fold on Day 6 ($p= 3 \times 10^{-8m}$, right panel). In the D247MG study **(B)**, a significant increase in mIL-4 levels under hypoxia was evident as early as Day 1 (10-fold increase, p=0.01, **left panel**) and continued through Day 3 (12-fold increase, p=0.029, **middle panel**) and Day 5 (13-fold increase, p=0.002, **right panel**).

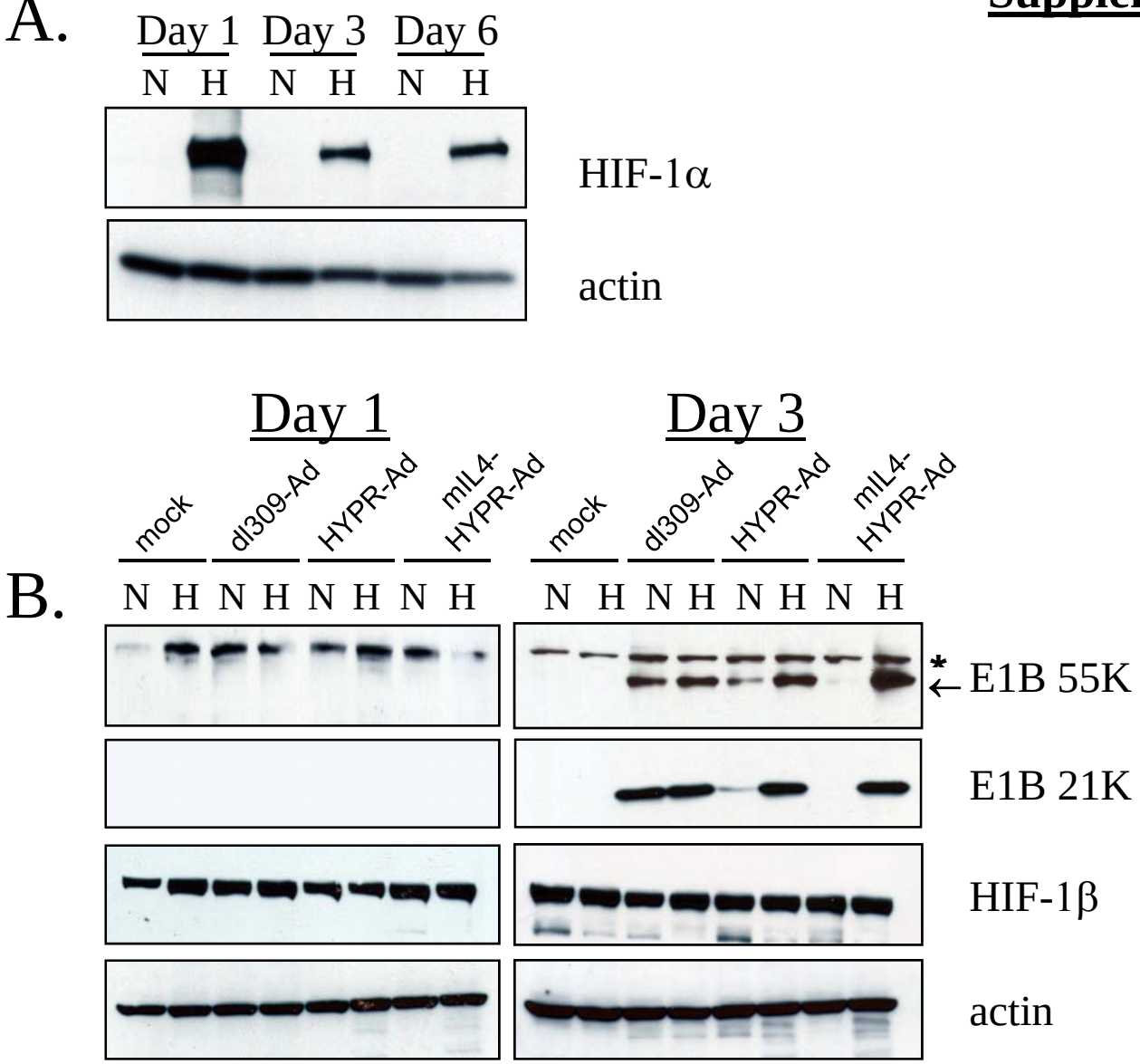
Supplemental Figure 3. HYPR-Ad-mIL4 selectively lyses hypoxic cells. U87ΔEGFR (**A**), D247MG (**B**), and normal human fibroblast (Hs68, **C**) cells were infected at MOI 1, 5, or 100, respectively, with AdLacZ, HYPR-Ad#1, HYPR-Ad-mIL4, *dl*309-Ad or mock infected and then maintained under normoxia (N) and hypoxia (Hyp). Cells were visually monitored for CPE. Shown are photographs (100x magnification) of crystal violet stained cells taken 8-10 days postinfection. HYPR-Ad-mIL4 and HYPR-Ad#1 elicited a hypoxia-dependent CPE response, with >95% of infected cells displaying CPE under hypoxia but not normoxia. The CPE inducing ability of these viruses was similar, with both viruses inducing comparable CPE under hypoxia at the same MOI and point in time. As expected, cells infected with *dl*309-Ad underwent >95% CPE under both normoxia and hypoxia. AdLacz infected cells showed no visual evidence of CPE, demonstrating that the hypoxic and viral infection conditions are not cytotoxic and that CPE resulted from viral replicative oncolysis.

Supplemental Figure 4. HYPR-Ad-mIL4 induces hypoxia-dependent cell death. LN229 (**A**), U251MG-T2 (**B**), and D247MG (**C**) cells were infected at MOI 25, 0.1, or 5,

respectively, with AdLacZ, dl309-Ad, HYPR-Ad#1, HYPR-Ad-mIL4 or mock infected and then maintained under normoxia (N) vs. hypoxia (Hyp). Cell viability was analyzed by MTT assay after 8, 9, and 8 days, respectively, of infection. The data represent the mean percent cytotoxicity ± SD.

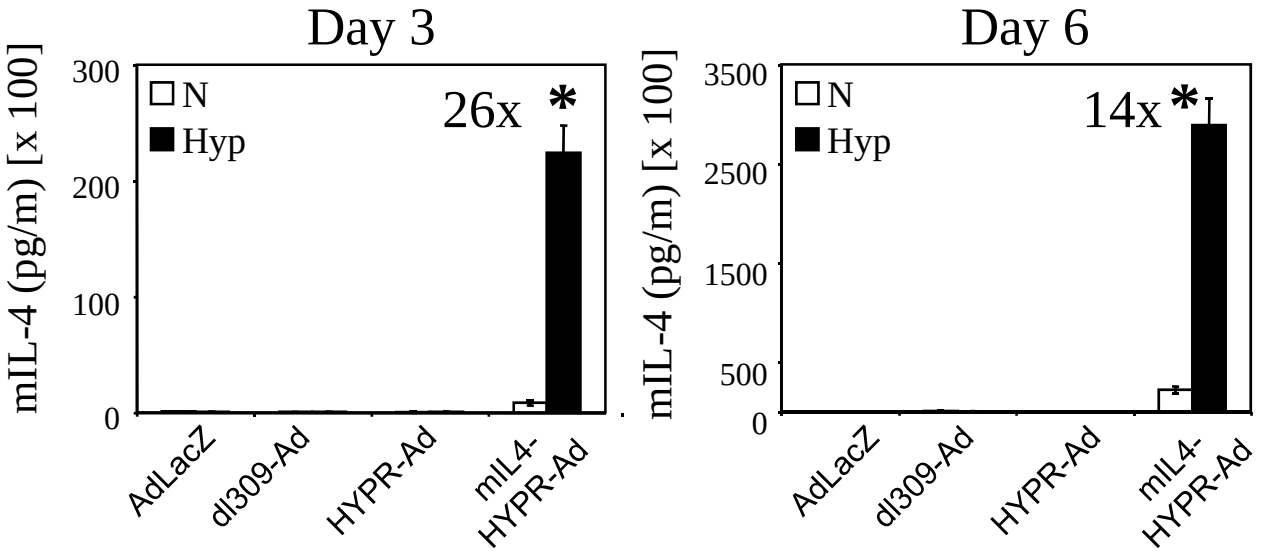
Supplemental Figure 5. HYPR-Ad-mIL4 therapy does not cause observable toxicity (change in animal weight) following injection intratumorally into s.c. established xenografts. Average animal weight (in grams) in the virus- (dl309-Ad, HYPR-Ad#1, HYPR-Ad-mIL4) and PBS- treatment groups in the (**A**) LN229, (**B**) U251MG-T2 (avg. size of 100 mm^3), and (**C**) U251MG-T2 (avg. size of 350 mm^3) tumor studies. See Fig. 3 above. The data represent the mean ± SD.

Supplemental Figure 1

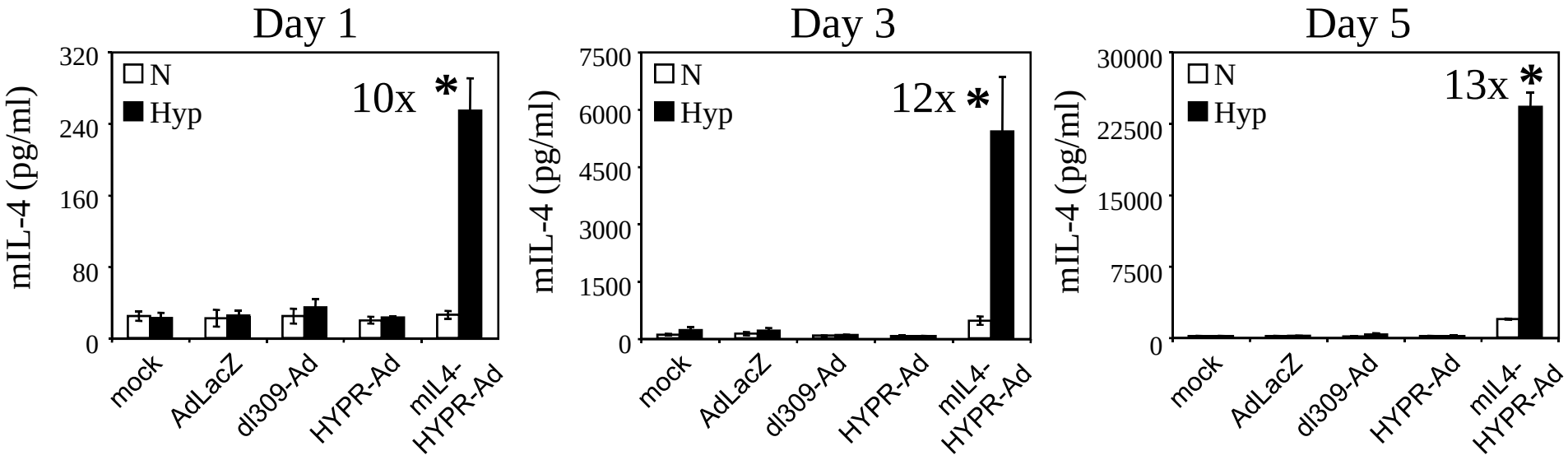


Supplemental Figure 2

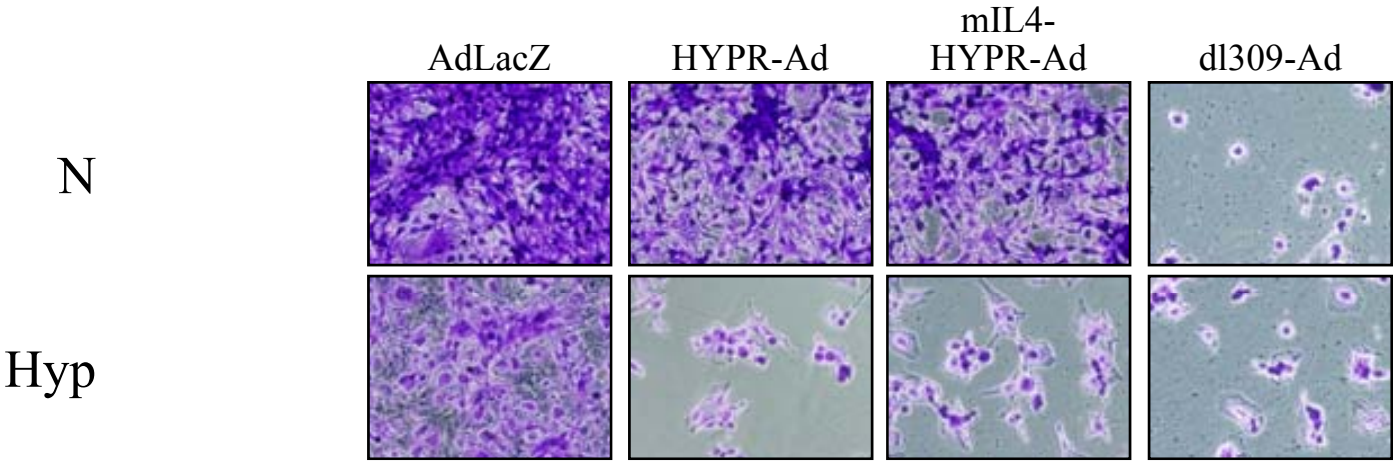
A. Normal Human Astrocytes



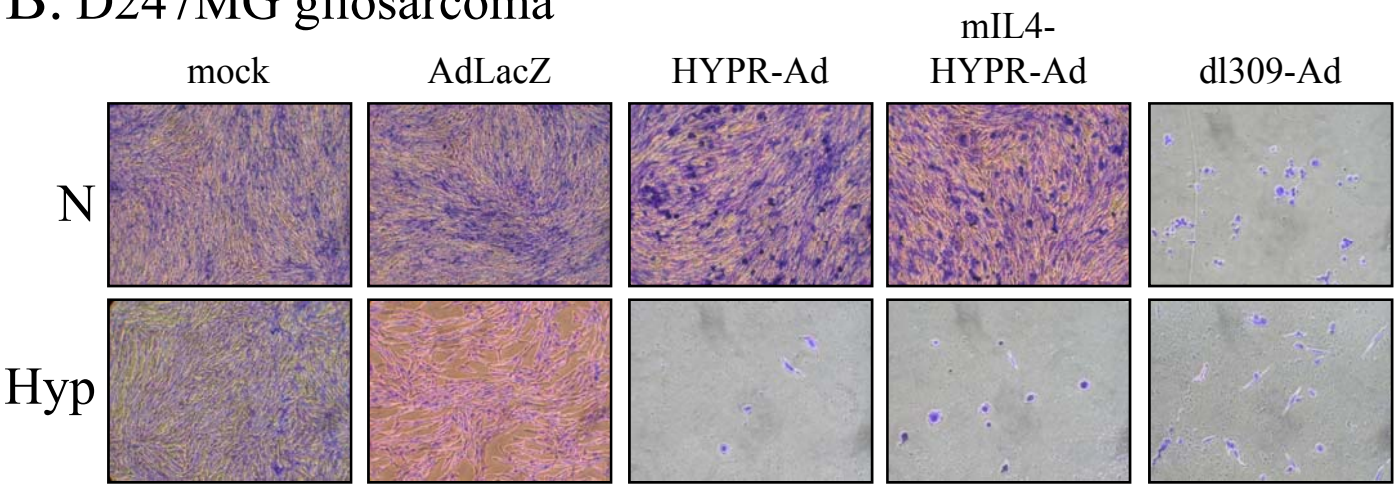
B. D247MG glioma



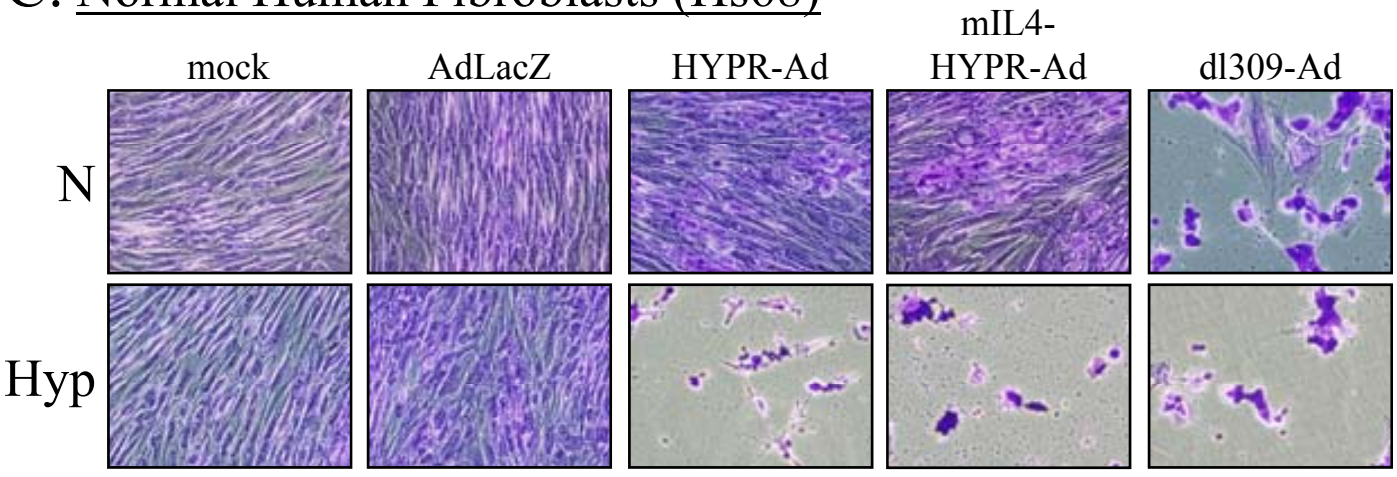
A. U87ΔEGFR glioma



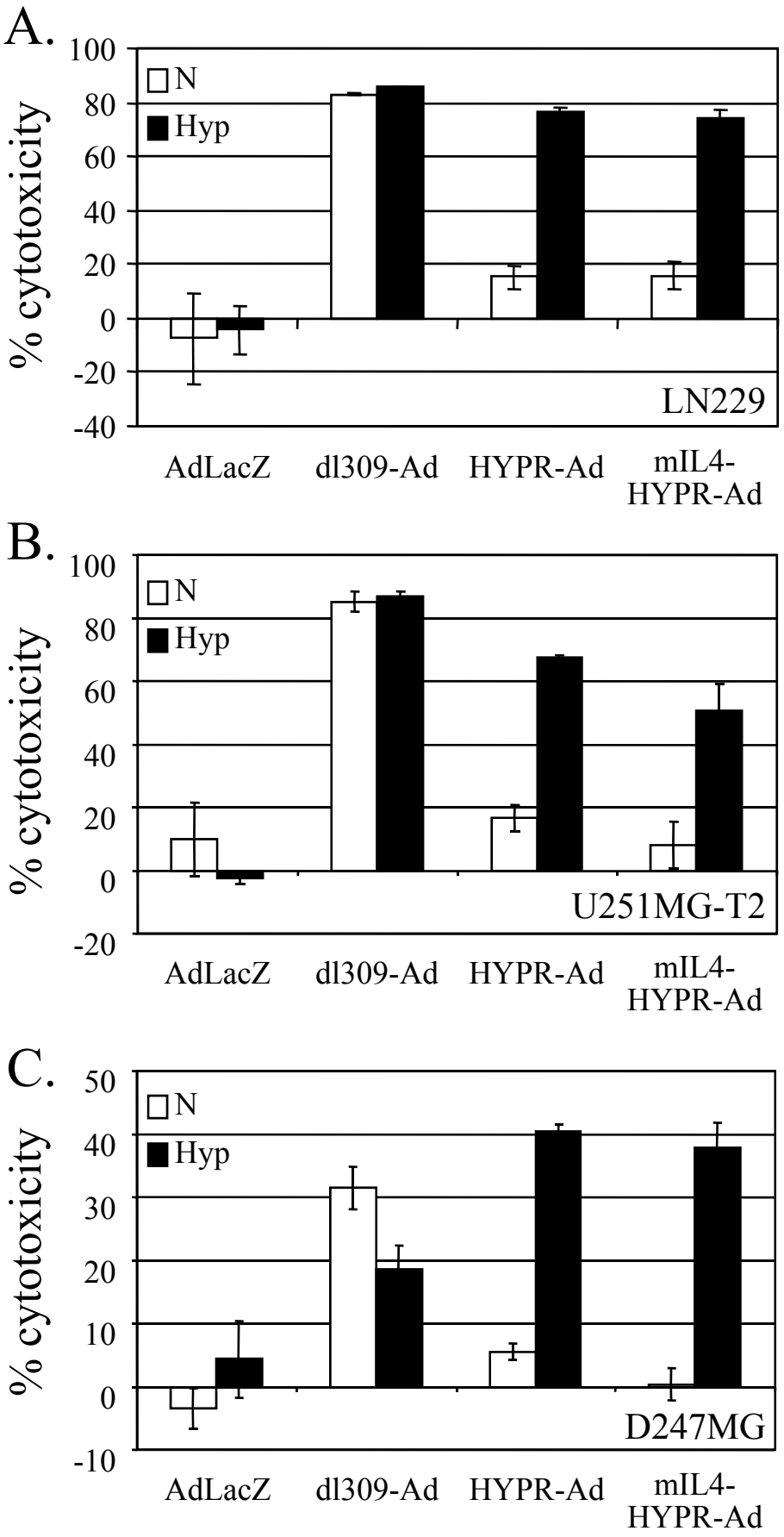
B. D247MG gliosarcoma



C. Normal Human Fibroblasts (Hs68)

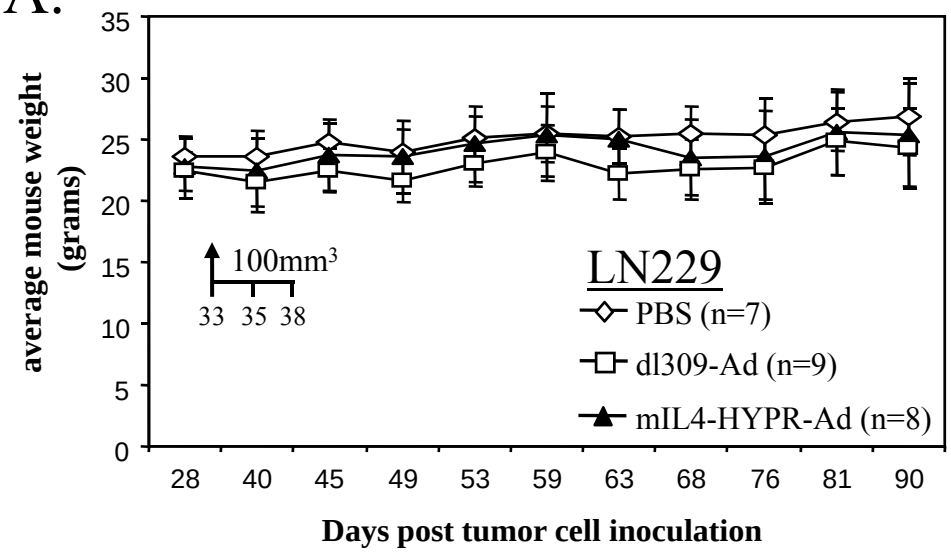


Supplemental Figure 3



Supplemental Figure 4

A.



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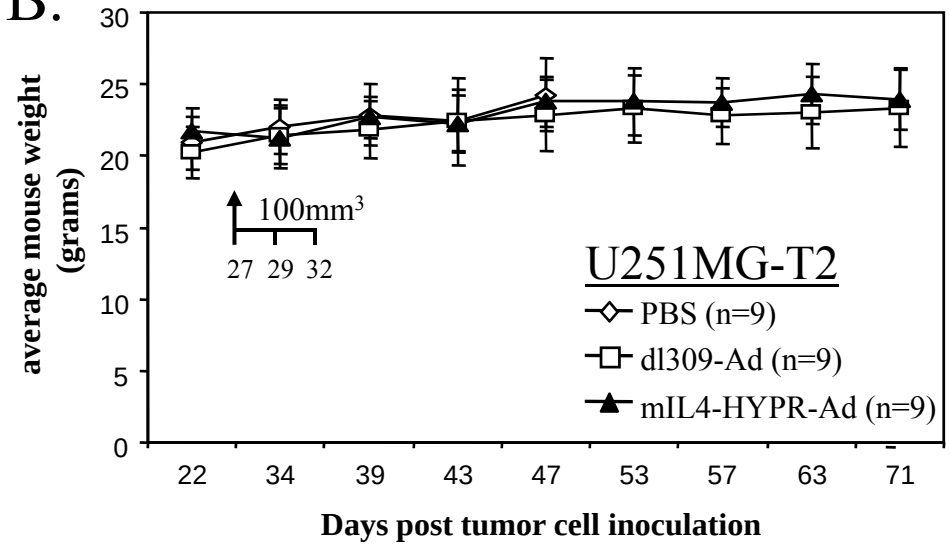
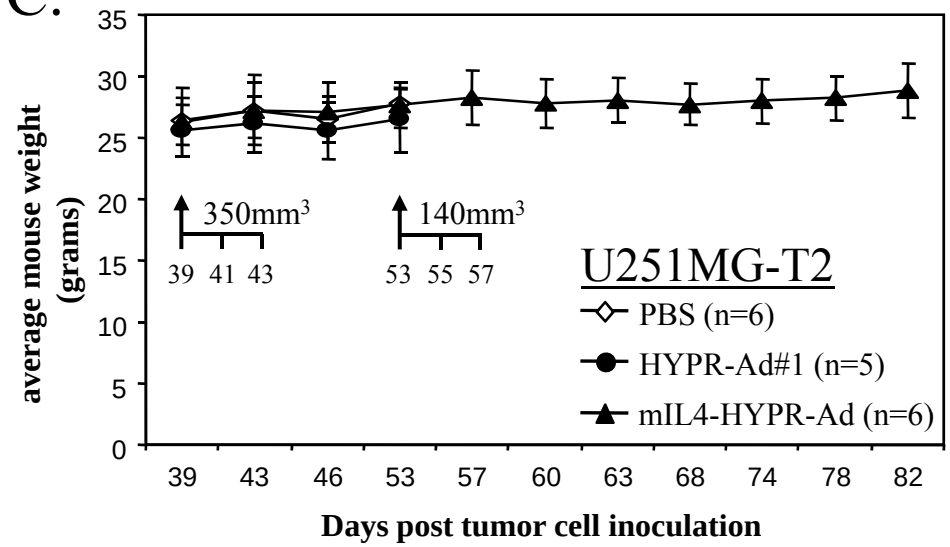


C.



Supplemental Figure 5