Supplemental Data for

Autophagy and Oxidative Stress in Gliomas with IDH1 Mutations

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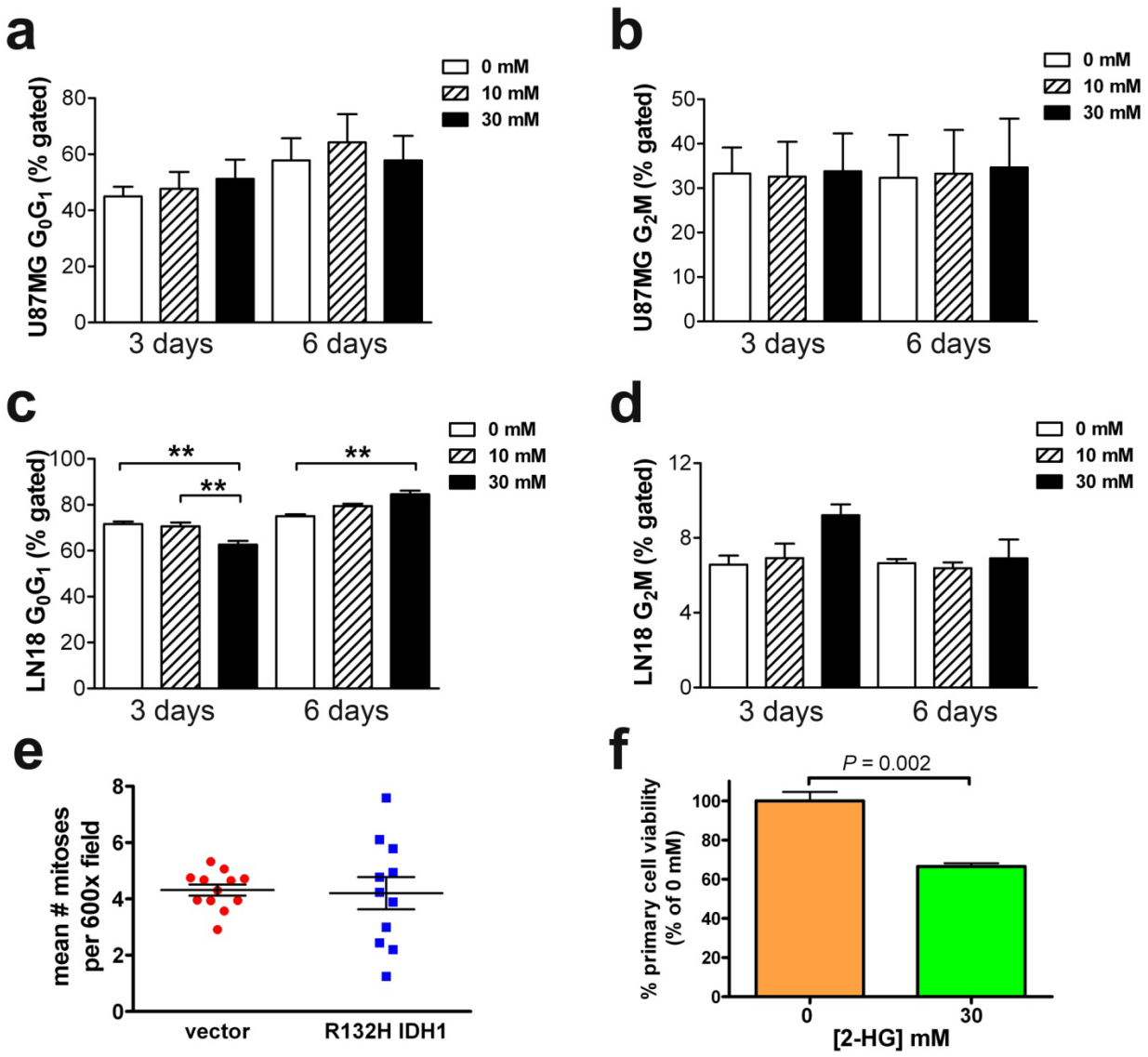
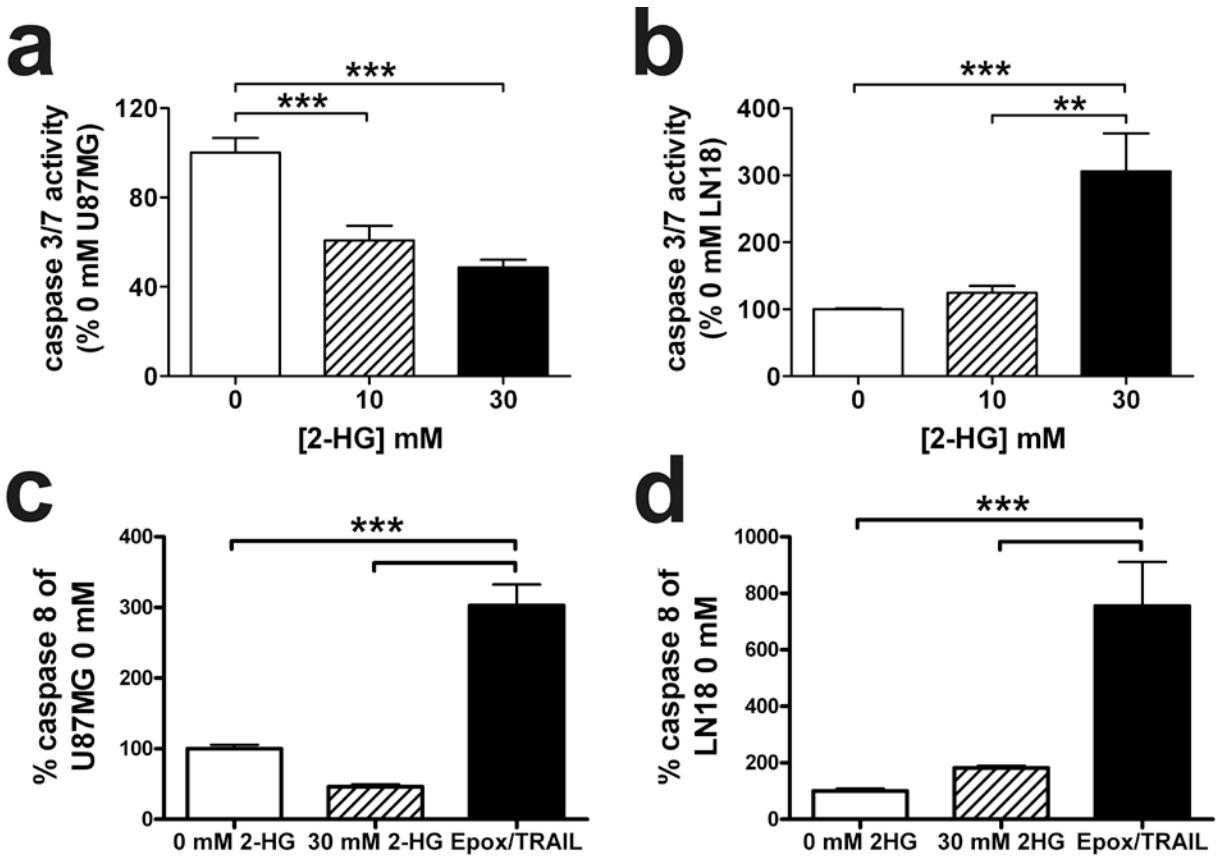
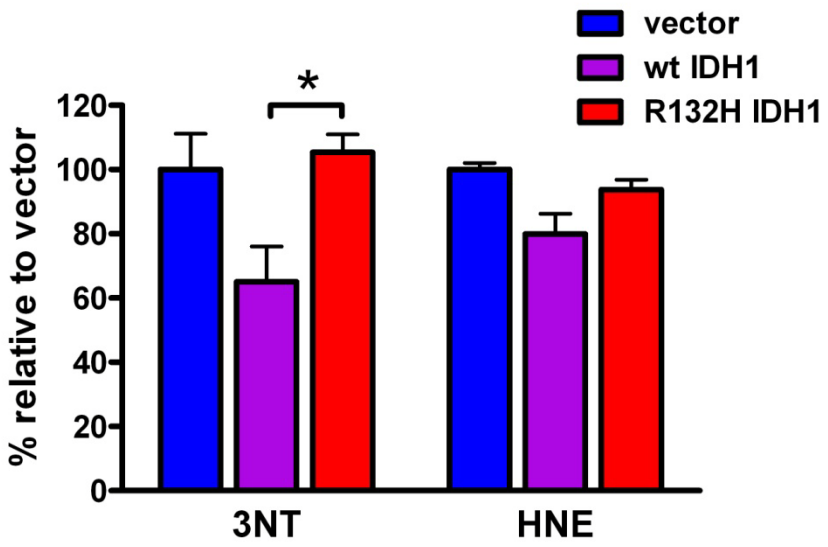


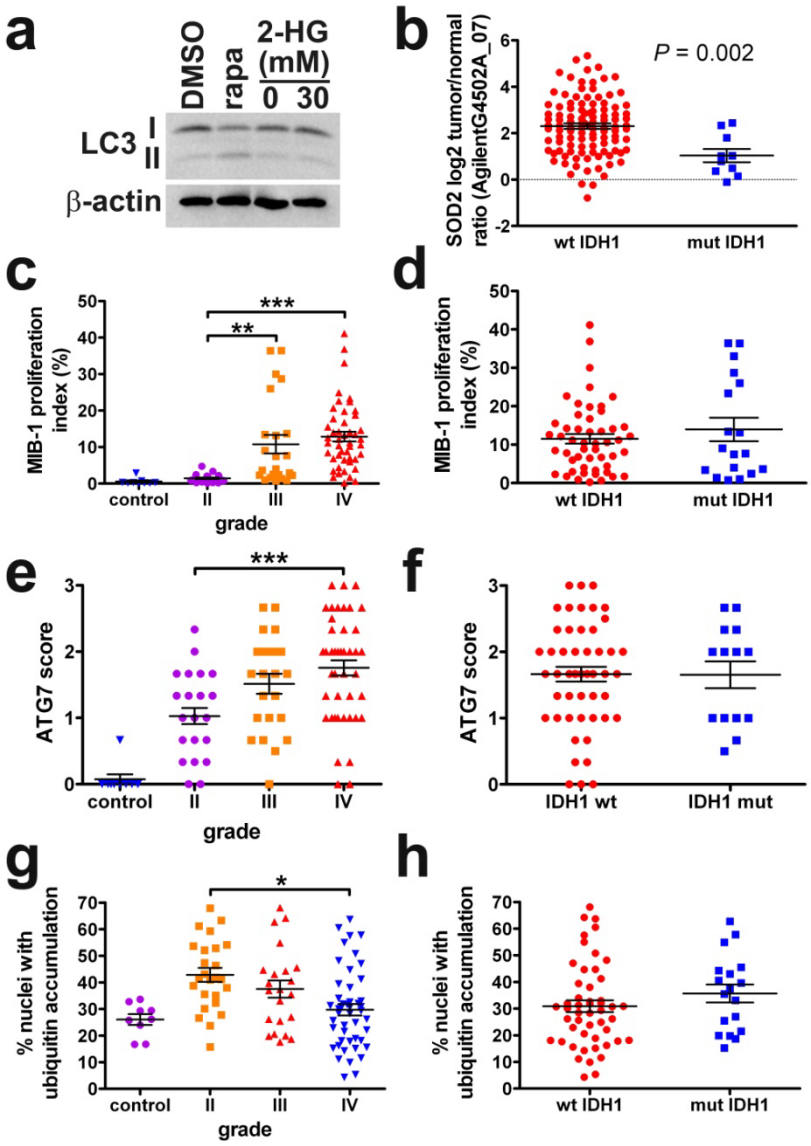
Supplemental Figure 1: Effect of 2-HG and mutant IDH1 on other cell cycle parameters. Both U87MG and LN18 cells were pulsed with 30 mM exogenous unmodified 2-HG *in vitro*. In U87MG cells, 2-HG had no effect on either G_0G_1 (a nor G_2M (b) phases, either at 3 or 6 days post treatment. In contrast, 2-HG temporarily and weakly reduced the % of cells in G_0G_1 after 3 days of treatment, but by 6 days the effect was the opposite (c). No significant G_2M changes were measured in LN18 cells at either 3 or 6 days of 2-HG treatment. Data for (a-d) represent mean ± SEM, n = 3. (e) There was no significant difference in Mib-1 proliferation index between vector and R132H IDH1 U87MG xenografts, n = 11. (f) Primary GBM culture 10932 showed 2-HG toxicity similar to that seen in the 2169 primary GBM cells (Figure 1j).



Supplemental Figure 2: Effect of 2-HG on caspase 3/7 and caspase 8 activities. Even after changing the media 3 days after initial 2-HG pulse (with readdition of 2-HG), by 6 days the same suppressive or inductive effects of 2-HG on caspase 3/7 activity were seen in U87MG and LN18 cells, respectively (a, b), as was seen when maintaining cells in unchanged media for 6 days (Figure 2a & b). Likewise, after 6 days of 2-HG treatment, there were trends toward reduced and increased caspase 8 in U87MG and LN18 cells, respectively, though these differences were not significant (c & d). Data for (a-d) represent mean ± SEM, n = 3. $^{**}P < 0.01$, $^{***}P < 0.001$



Supplemental Figure 3: Effect of mutant IDH1 on oxidative stress markers. U87MG glioma cells overexpressing wild-type IDH1 (purple bars) or R132H IDH1 (red bars) showed no significant differences in levels of either 3-nitrotyrosine (3NT) or 4-hydroxy-2-nonenal (HNE) compared to GFP-vector controls (blue bars). In particular, while cells overexpressing wt IDH1 had reduced 3NT levels compared to mutant IDH1 cells (*$P < 0.05$), there was no difference between mutant IDH1 and GFP-vector controls. Data represent mean ± SEM, n = 3.



Supplemental Figure 4: Autophagy in LN18 cells and other markers of proliferation, autophagy, and MnSOD, in patient-derived IDH1-mutant gliomas. In contrast to U87MG cells (Figure 4c), LN18 cells pulsed with 2-HG showed no change in LC3-I to LC3-II conversion in response to 24 hours of 2-HG (a), although the rapamycin positive control did induce some conversion. Similar results were observed after 48 hours (not shown). (b) Analysis of TCGA GBMs with known IDH1 mutation status (Veerhaak et al, Cancer Cell. 2010 Jan 19;17(1):98-110) showed that SOD2 mRNA, which encodes MnSOD, was about 50% lower in IDH1-mutant GBMs compared to wild-type tumors, consistent with data shown in Figure 5F and in the text. TMA analysis of grades II-IV gliomas showed increasing Mib-1 proliferation index (c) and ATG7 (e), and decreasing nuclear ubiquitin accumulation (g), with increasing WHO grade. However, there were no significant differences among pooled grades III and IV tumors according to IDH1 status in any of those parameters (d, f, h). In all scatterplots, each data point represents the average score from 3 separate TMA cores in a given tumor, data are expressed as mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$. Control brain tissues are surgical resections of nonneoplastic epileptic foci.