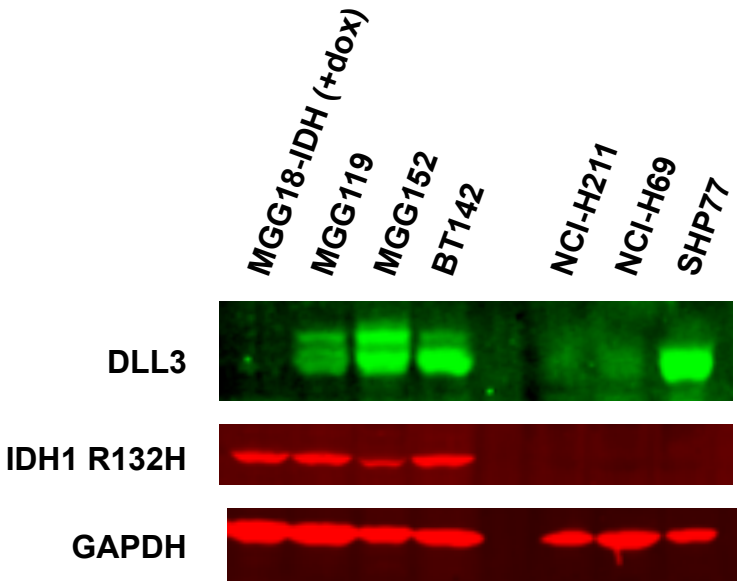
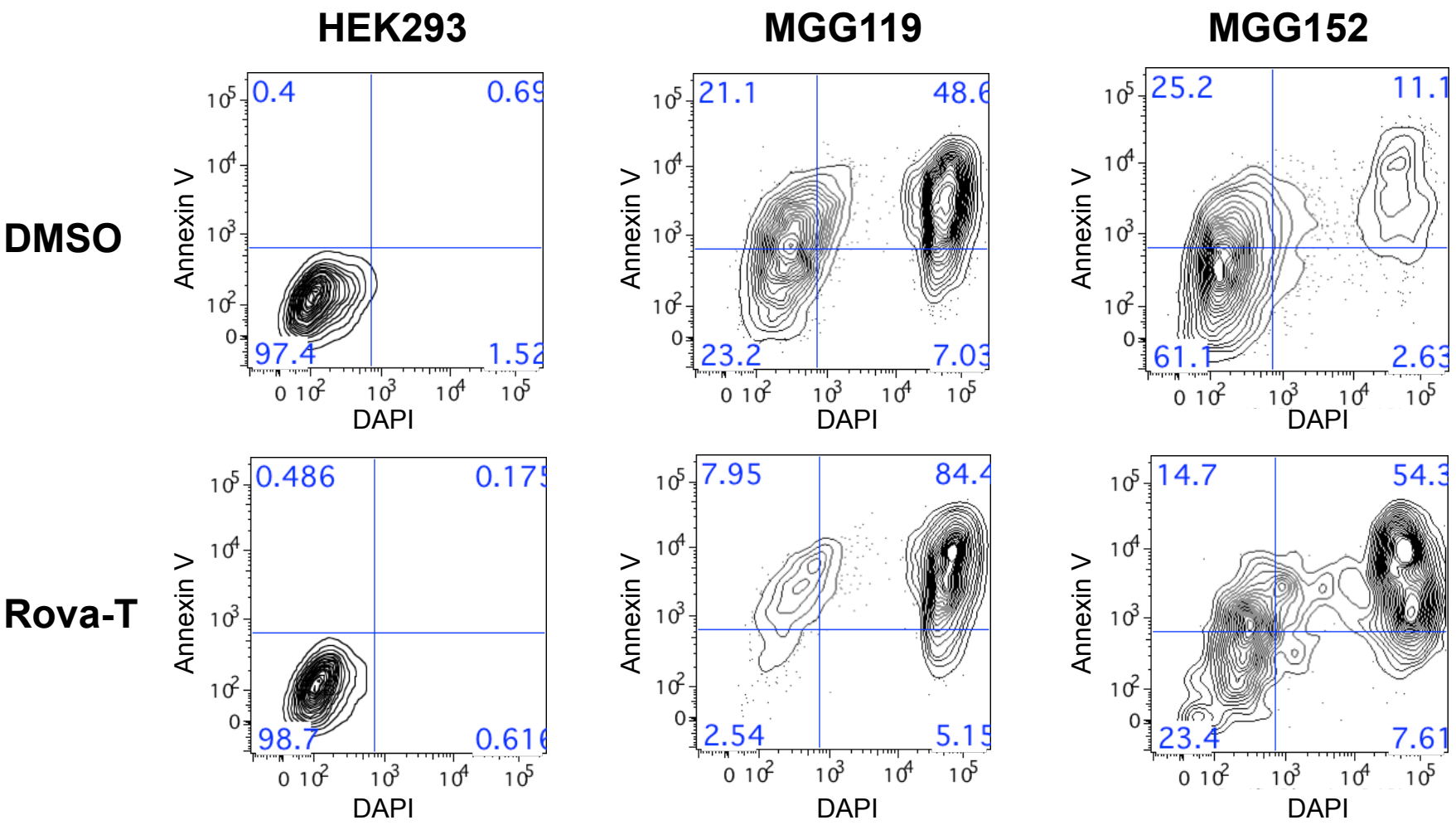
Supplementary Figure S1



Supplementary Figure S1. Western blot using antibodies against DLL3, the IDH1 R132H mutant enzyme, and GAPDH. Lane labels: MGG18-IDH (+dox): An *IDH* wildtype patient-derived glioblastoma tumorsphere line (MGG18) engineered with a tetracycline-inducible IDH1 R132H gene (MGG18-IDH), cultured with doxycycline for 72 hours; MGG119: An patient-derived glioblastoma tumorsphere line with endogenous IDH1 R132H. MGG152: An patient-derived glioblastoma tumorsphere line with endogenous IDH1 R132H. BT142: a publically available patient-derived glioblastoma tumorsphere line with endogenous IDH1 R132H (ref. 48); NCI-H211: cell line known to lack DLL3 expression (negative control). NCI-H69: Cell line with low to medium DLL3 expression (control). SHP77: Cell line with high DLL3 expression (positive control).

Supplementary Figure S2



Supplementary Figure S2. **Rova-T induces apoptosis in DLL3-expressing *IDH* mutant glioma tumorspheres.** HEK293 (DLL3 negative, left column), MGG119 (DLL3 expressing *IDH* mutant patient-derived glioma tumorsphere, middle column) and MGG119 (DLL3 expressing *IDH* mutant patient-derived glioma tumorsphere, right column) were treated with DMSO (top row) or 100 pM of SC16LD6.5 (Rova-T) (bottom row) for 7 days. Apoptosis was then measured by staining with Annexin V according to the manufacturer's instructions (BD Pharmingen) in combination with DAPI. Apoptotic cells (top right quadrant) were measured using a FACScan flow cytometer (BD Biosciences), and the relative cell distribution (shown as percentage in the quadrants, in blue) was analyzed using Flo Jo software (FloJo, LLC).

Supplementary Table S5. DLL3 Immunohistochemistry Summary (Discovery Set)

	n	H-score median (95% CI)	*P*	DLL3+ cells median (95% CI)	*P*	DLL3+ tumor cells				
						0%	1-5%	6-49%	50-79%	≥80%
***IDH* wildtype GBM**	17	15 (0-50)	**0.0007** (3 major groups)	15% (5-40%)	**0.002** (3 major groups)	2	6	5	4	0
MGMT status										
Unmethylated	4	4.5 (0-15)		5% (0-15%)		1	2	1	0	0
Methylated	12	27.5 (0-75)	0.11	25% (5-60%)	0.12	1	3	4	4	0
Classifier Subclass										
Mesenchymal	6	0 (0-20)		5 (0-20)		2	3	1	0	0
Non-mesenchymal	10	42.5 (9-100)	**0.019**	35% (5-65)	**0.004**	0	2	4	4	0
***IDH* mutant glioma**	19	250 (50-270)	**0.0014** (*vs IDH*wt GBM)	80% (40-90%)	**0.003** (*vs IDH*wt GBM)	3	1	2	2	11
1p/19q codeleted	9	270 (170-300)		90% (65-90%)		0	0	1	1	7
Non-codeleted	10	85 (0-260)	**0.003**	55% (0-80%)	**0.003**	3	1	1	1	4
Glioma variants	10	0 (0-50)		0.5% (0-70%)		5	2	1	1	1

Immunostains were scored by two independent teams and prior to determination of the molecular status of the tumors. The Abbvie Stemcentrx team generated H-scores and the NYU team reported the percent of DLL positive (DLL3+) tumor cells.
Abbreviations: *IDH*wt, *IDH* wildtype

Supplementary Table S6. DLL3 Immunohistochemistry Summary (Validation Set)

	n	H-score median (95% CI)	*P*	DLL3+ cells median (95% CI)	*P*	DLL3+ tumor cells				
						0%	1-5%	6-49%	50-79%	≥80%
***IDH* wildtype GBM**	26	10 (1-20)		5% (1-10%)		6	8	10	2	0
MGMT status										
Unmethylated	14	15 (1-30)		7.5% (1-10%)		2	5	7	0	0
Methylated	10	6 (0-30)	0.29	3% (0-10%)	0.31	4	3	2	1	0
Classifier Subclass										
Mesenchymal	6	0.5 (0-30)		0.5% (0-10)		3	1	2	0	0
Non-mesenchymal	17	10 (2-20)	0.08	5% (1-10)	0.14	2	7	6	2	0
IDH* mutant glioma	22	130 (100-180)	**0.0000043** (*vs IDH*wt GBM)	60% (50-70%)	**0.00000017** (*vs IDH*wt GBM)	2	0	2	15	3
1p/19q codeleted	6	180 (100-170)		60% (50-90%)		0	0	0	5	1
Non-codeleted	10	150 (80-210)	0.59	65% (40-80%)	0.33	0	0	2	6	2
Recurrent IDH mutant glioma*	14	210 (80-240)	0.085 (*vs* non-rec *IDH*mut)	70% (50-80%)	0.16 (*vs* non-rec *IDH*mut)	0	0	2	8	4
Paired tumors										
Initial	11	180 (100-210)		60% (50-70%)		0	0	0	10	1
Recurrent	11	210 (50-240)	0.61 (*vs* initial)	70% (50-80%)	0.77 (*vs* initial)	0	0	1	7	3

Immunostains were scored by two NYU neuropathologists who reported the percent of DLL positive (DLL3+) tumor cells.
Abbreviations: *IDH*wt, *IDH* wildtype; *IDH*mut, *IDH* mutant; non-rec, non-recurrent;
*9 *IDH* mutant patients, including 3 paired recurrences (total 12 tumors) had unknown 1p/19q chromosome status.