

Supplementary Figure 1. PLA positivity depends on HLA-DR expression in LN229 IDH1(D252G R132H). (A) IF detection of endogenous HLA-DR in LN229 IDH1(D252G R132H) (LN229 DG RH) by mouse anti-human HLA-DR (TAL1B5) antibody; green, HLA-DR; blue, DAPI; 40x objective. (B) IDH1(R132H)-HLA-DR-PLA on siCONTROL and siHLA-DRA treated LN229 IDH1(D252G R132H) co-

stained with TAL1B5; red, PLA; green, HLA-DR; blue, DAPI; left panels, 63x; right panels, 40x objectives, respectively. Representatives of 3 experiments each.

Supplementary Figure 2. Localization of PLA signal in LN229 IDH1(R132H) IVE2. (A) IF detection of retrovirally transfected IDH1(R132H) and IDH1(WT) in LN229 IVE2 and LN229 IDH1(WT); green, IDH1(R132H); blue, DAPI; 20x objective. Representative of 3 experiments. (B) IDH1(R132H)-HLA-DR-PLA on LN229 IDH1(R132H) IVE2 and IDH1(WT) co-stained with Annexin V (annexin); red, PLA signal; green, annexin; blue, DAPI. Representative of 3 experiments. (C) IDH1(R132H)-HLA-DR-PLA on LN229 IDH1(R132H) IVE2 co-stained with Rab7; red, PLA signal; green, Rab7; blue, DAPI. Experiment was performed once. White arrows, PLA signal in annexin (B) or Rab7 (C) positive areas, respectively; 63 objective.

Supplementary Figure 3. Correlation of IDH1(R132H) expression level and PLA signal in LN229 IDH1(R132H) IVE2. (A) Exemplary IDH1(R132H)-HLA-DR PLA on LN229 IDH1(R132H) IVE2; red, PLA signal; green, IDH1(R132H); blue, DAPI. (B) ImageJ algorithm analyzing IDH1R132H-expression (MFI, mean fluorescence intensity of IDH1R132H co-staining) and PLA signal on single cell level; red numbers, IDH1R132H+ cells; right panel, blue numbers, PLA signals. (C) Quantification of (B). (D) Correlation plot of (C); $n = 27$, Spearman's rho, $r = 0.6020$, $p = 0.0009$. (A, B) 63x objective.

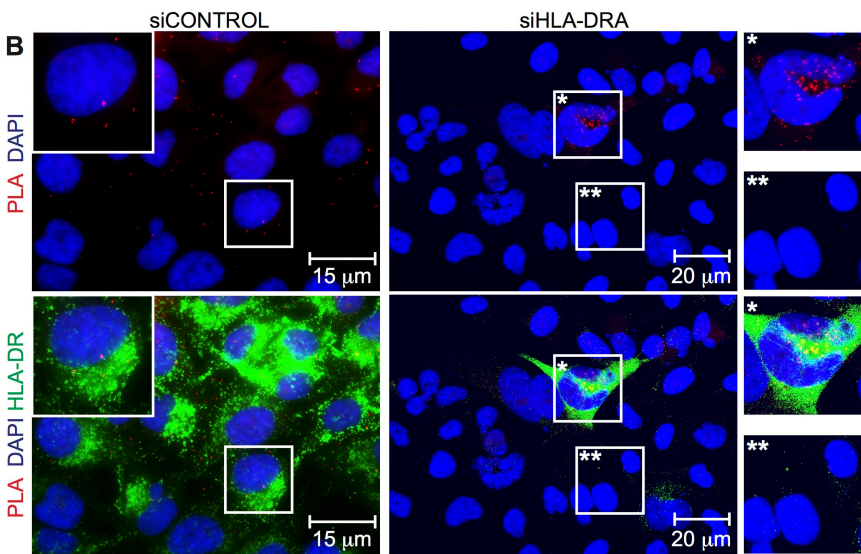
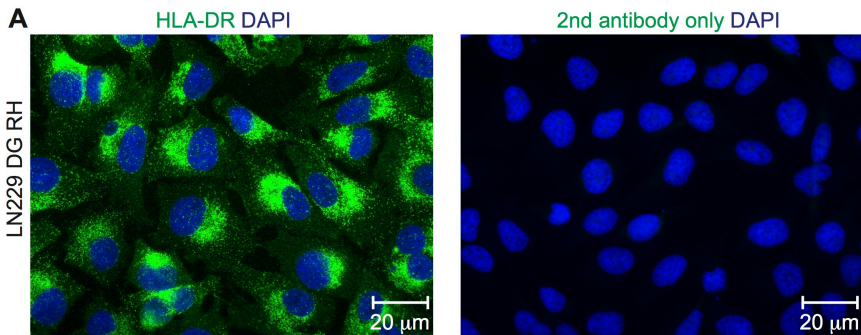
Supplementary Figure 4. IDH1(R132H) and HLA-DR expression and histological analysis of primary glioma cell lines NPH001 (A°III), NCH645 (secondary GBM), and NCH620 (secondary GBM). (A) HLA-DR IHC and IDH1(R132H) IHC on NPH001 and NCH645; 40x objective. (B) H&E staining of NPH001, NCH645, and NCH620; 20x objective. Representatives of 3 experiments each.

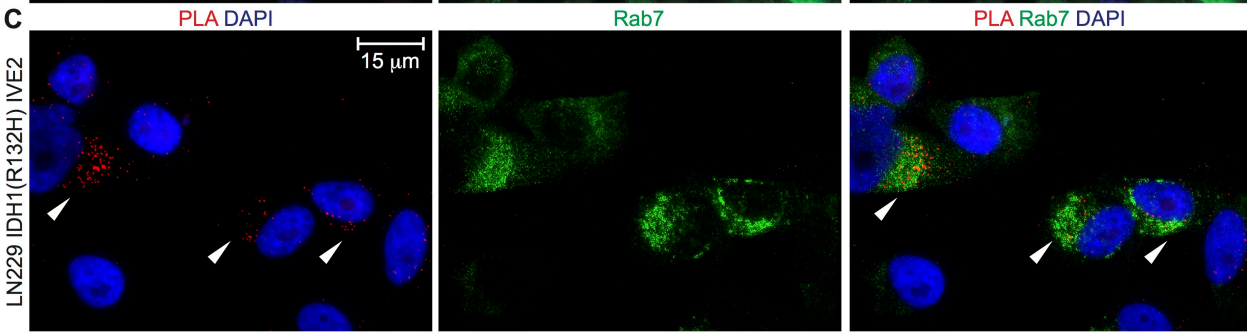
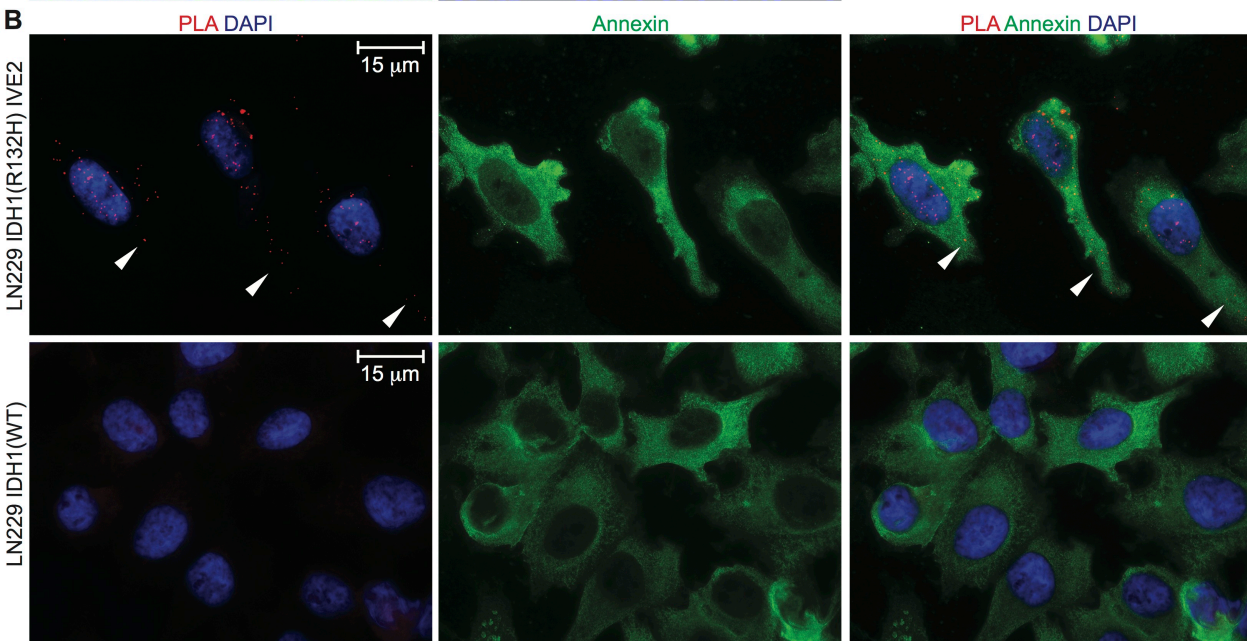
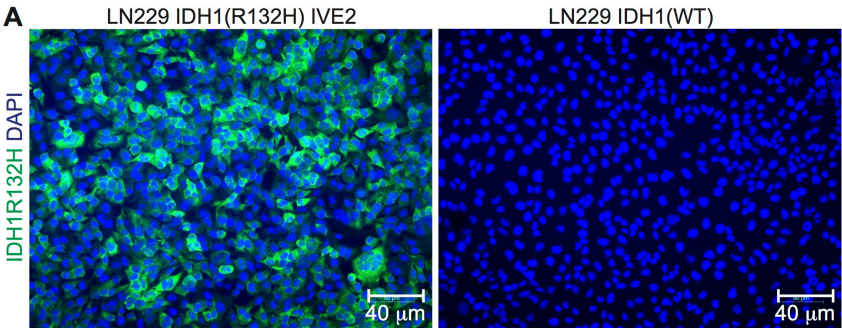
Supplementary Figure 5. NY-ESO-1 overexpression in glioma cell line LN229. IF of LN229 EV (empty vector) and LN229 NY (NY-ESO-1); green, NY-ESO-1; blue, DAPI; 40x objective. Representative of 3 experiments.

Supplementary Figure 6. NY-ESO-1-HLA-DR PLA on melanoma cell lines SK-Mel-37 and SK-Mel-23. (A) Negative control to NY-ESO-1 IF in SK-Mel-37; green, secondary antibody only; blue, DAPI; 40x objective. Representative of 3 experiments. (B) Western blot detecting endogenous NY-

ESO-1 in SK-Mel-23 (negative control) and SK-Mel-37 and myc-tagged (*) NY-ESO-1 overexpressed in LN229; tubulin, loading control; LN229 EV, empty vector, negative control. Representative of 3 experiments. (C) NY-ESO-1-HLA-DR PLA on SK-Mel-23 (negative control); red, PLA signal; blue, DAPI; 63x objective. Experiment was performed once. (D and E) NY-ESO-1-HLA-DR PLA co-stained for HLA-DR (D) and NY-ESO-1 (E) on SK-Mel-37 treated with siHLA-DRA, siNY-ESO-1, and siCONTROL; red, PLA signal; green, HLA-DR, NY-ESO-1, respectively; blue, DAPI; 40x objective (D); (E) confocal acquisition (100x); red arrow, PLA signal; white arrows, siNY-ESO-1 transfected cells. Representative of 2 experiments.

Supplementary Figure 7. In-depth analysis of PLA positivity in tumor tissue in situ. (A) Correlation of PLA positivity and HLA-DR expression (semiquantitative; -, negative; +, low; ++, moderate; +++, strong) in IDH1(R132H)+ tumors; n = 20, n(PLA positive) = 10, n(PLA negative) = 10, p = 0.08471, wilcoxon rank sum test; if HLA-DR- excluded, n = 15, n(PLA positive) = 10, n(PLA negative) = 5, p = 0.7274, wilcoxon rank sum test. (B) IDH1(R132H)-HLA-DR PLA co-stained for endothelial marker CD34 on tumor tissues p016 (63x objective) and p017 (40x objective); red, PLA signal; green, CD34; blue, DAPI. White arrows indicate CD34 positivity. Each patient was analyzed once.



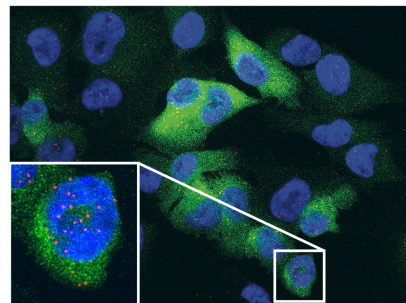
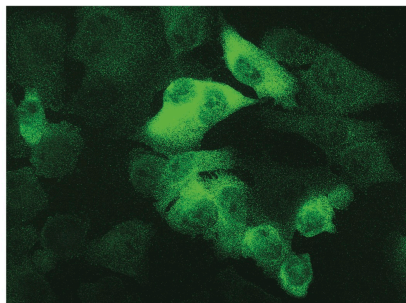
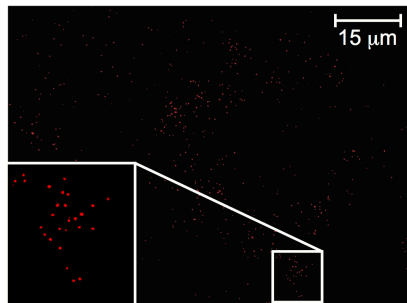


PLA

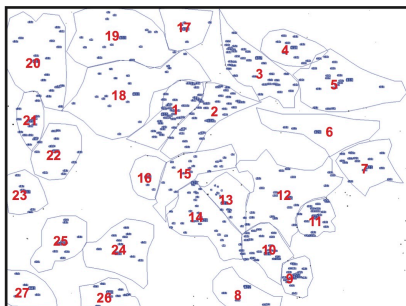
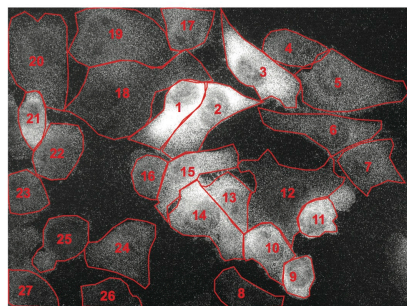
IDH1R132H

PLA IDH1R132H DAPI

A



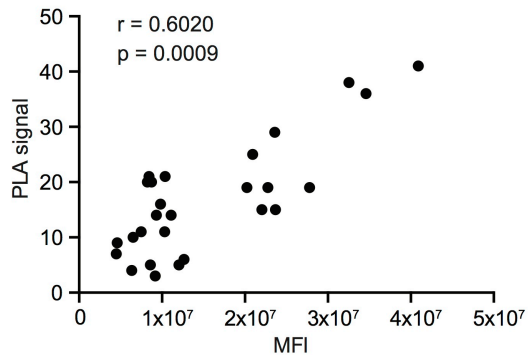
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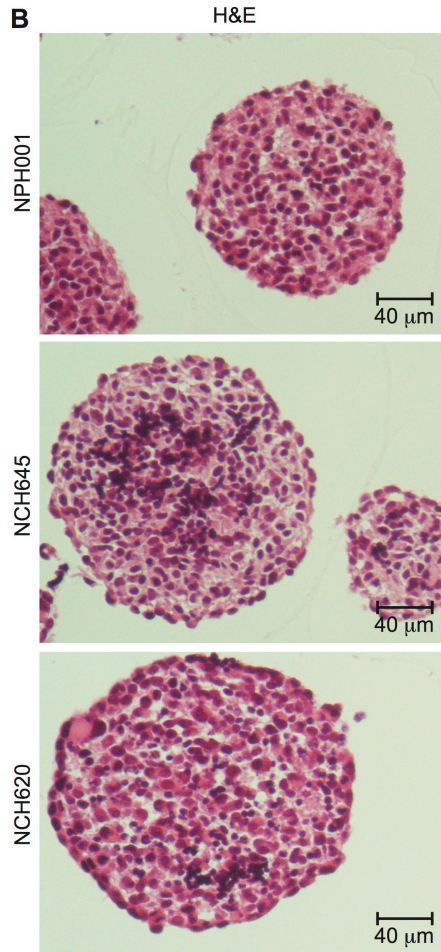
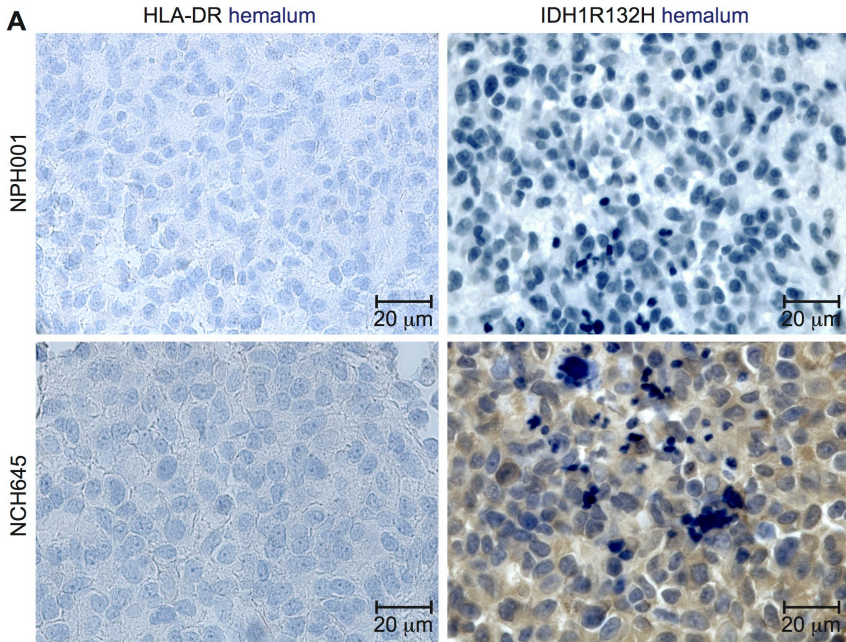


C

cell	MFI / cell	PLA / cell
1	4,089872e+007	41
2	3,460013e+007	36
3	3,2555176e+007	38
4	1,0320998e+007	11
5	1,0384579e+007	21
6	9,175482e+006	3
7	9,306426e+006	14
8	4,496312e+006	7
9	2,0238572e+007	19
10	2,093166e+007	25
11	2,7804776e+007	19
12	8,236265e+006	20
13	2,3682438e+007	15
14	2,360654e+007	29
15	2,2756188e+007	19
16	1,2028082e+007	5
17	1,265542e+007	6
18	8,417153e+006	21
19	9,820301e+006	16
20	8,739797e+006	20
21	2,204244e+007	15
22	1,109273e+007	14
23	8,594265e+006	5
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26	4,593632e+006	9
27	6,336205e+006	4

D

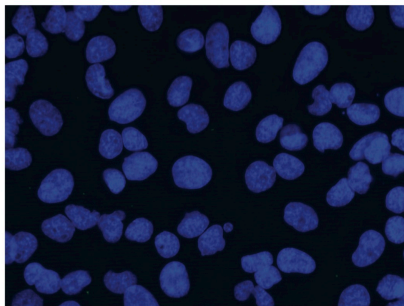
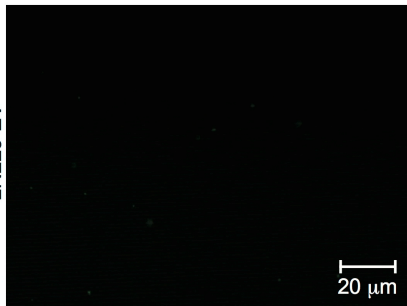




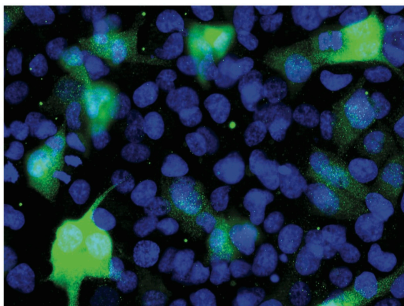
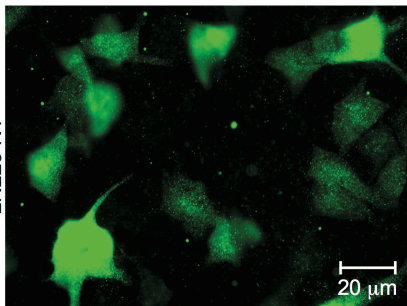
NY-ESO-1

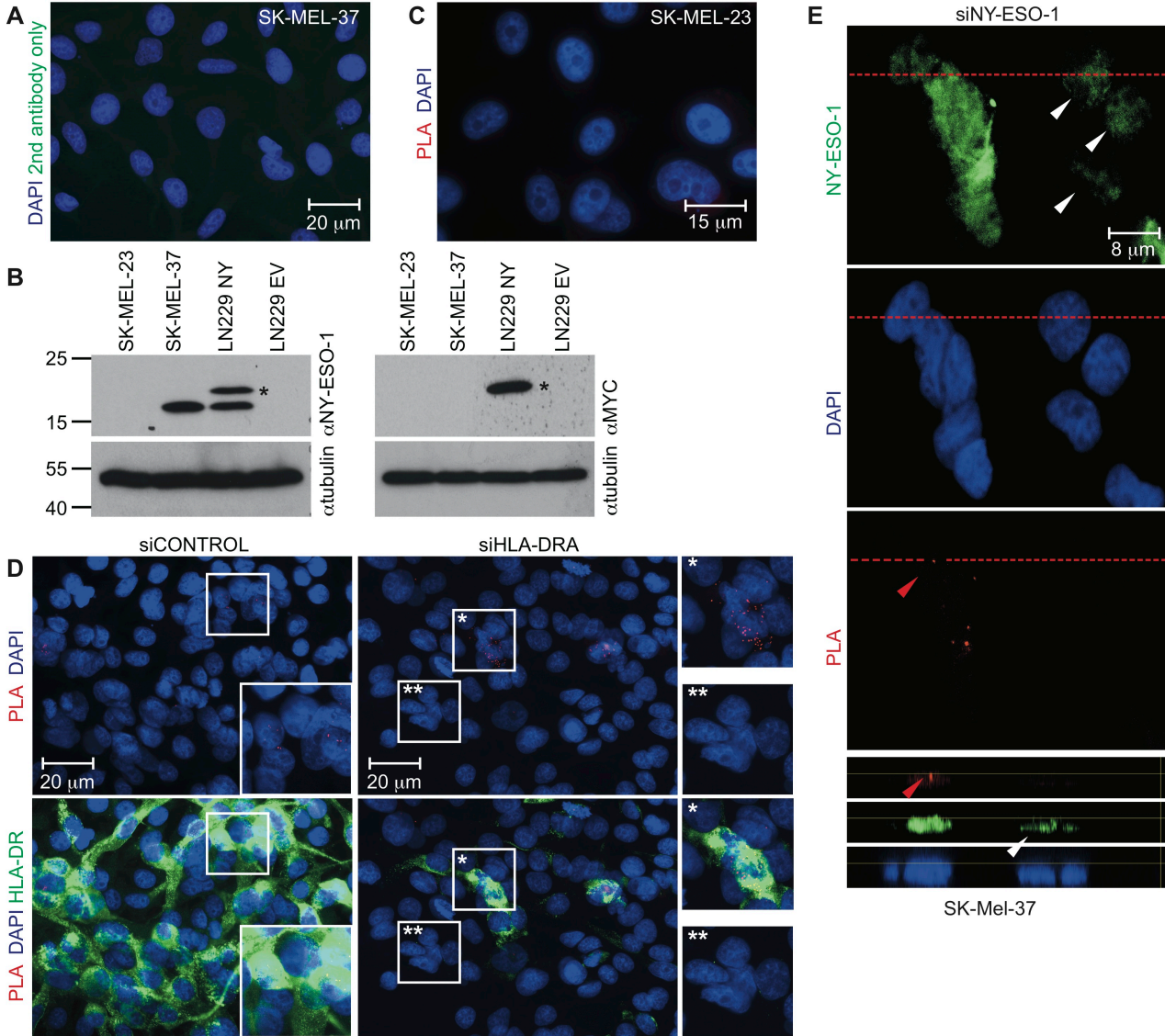
NY-ESO-1 DAPI

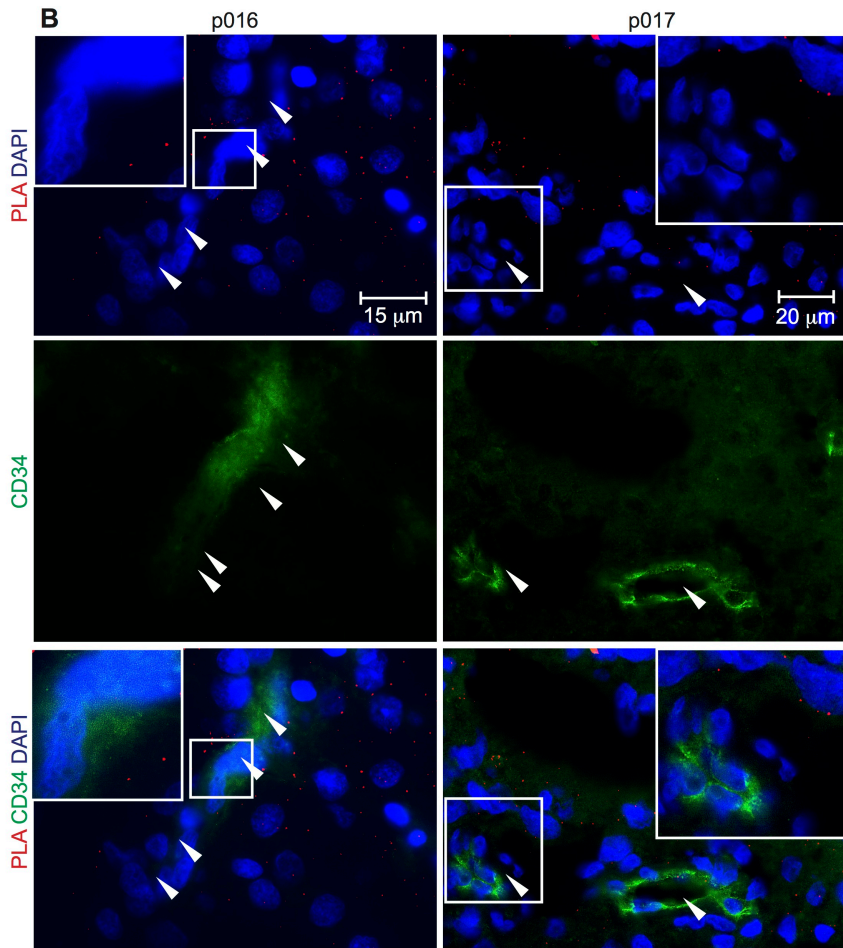
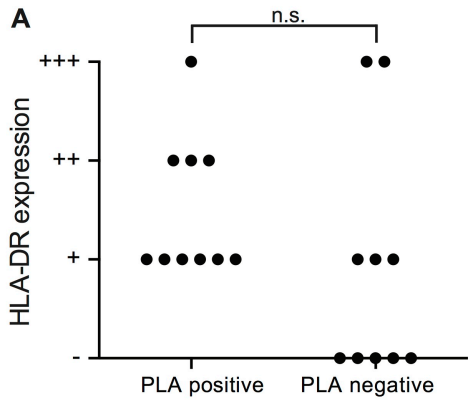
LN229 EV



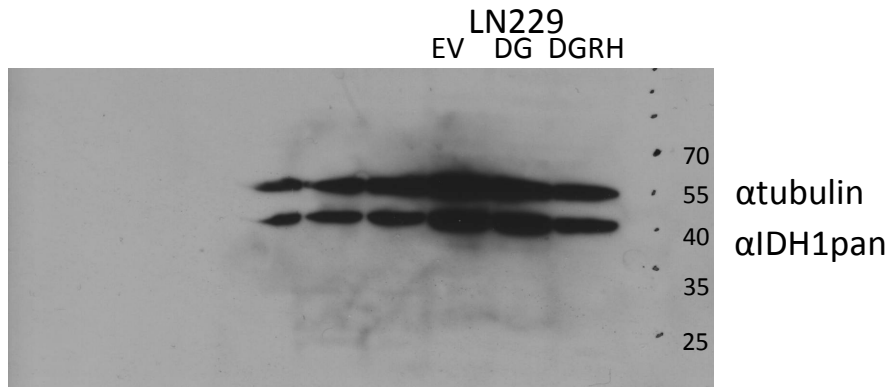
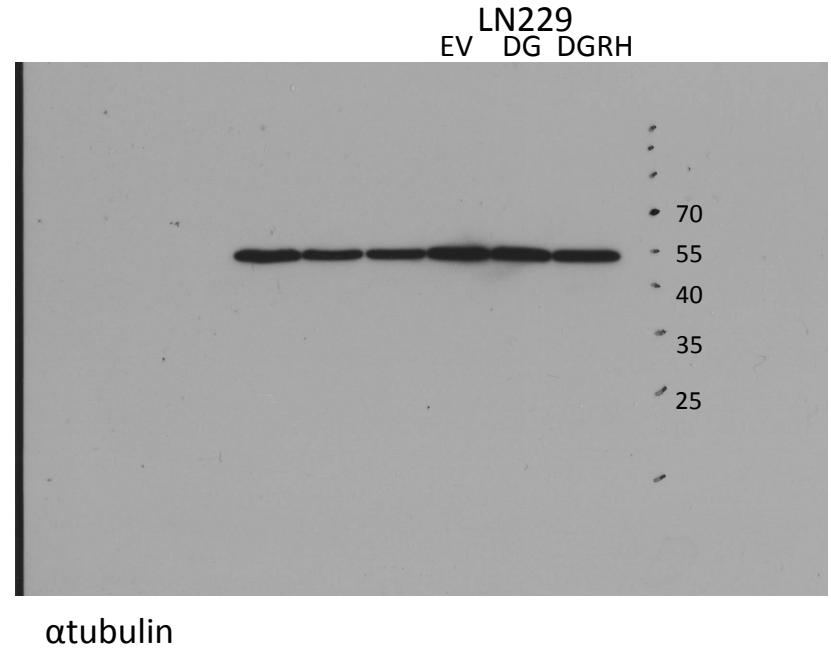
LN229 NY





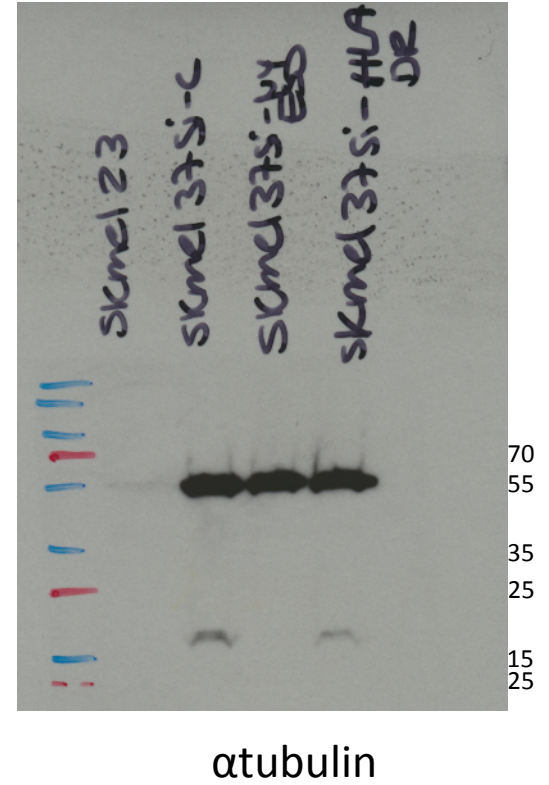
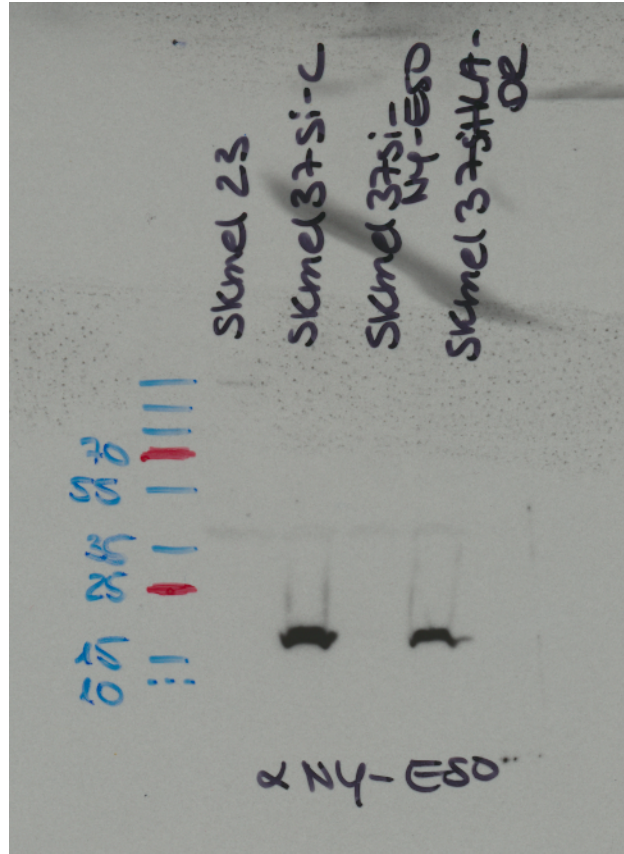
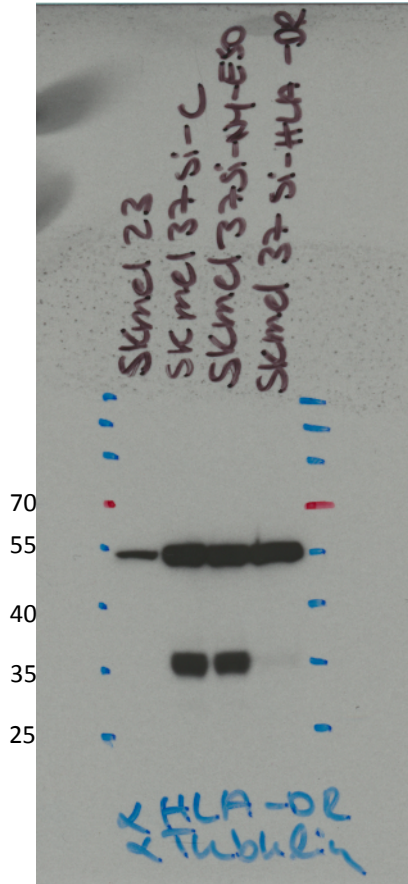


Full unedited gels for Figure 2E



all same membrane

Full unedited gels for Figure 6F



same membrane

Full unedited gel for Supplementary Figure 6B

