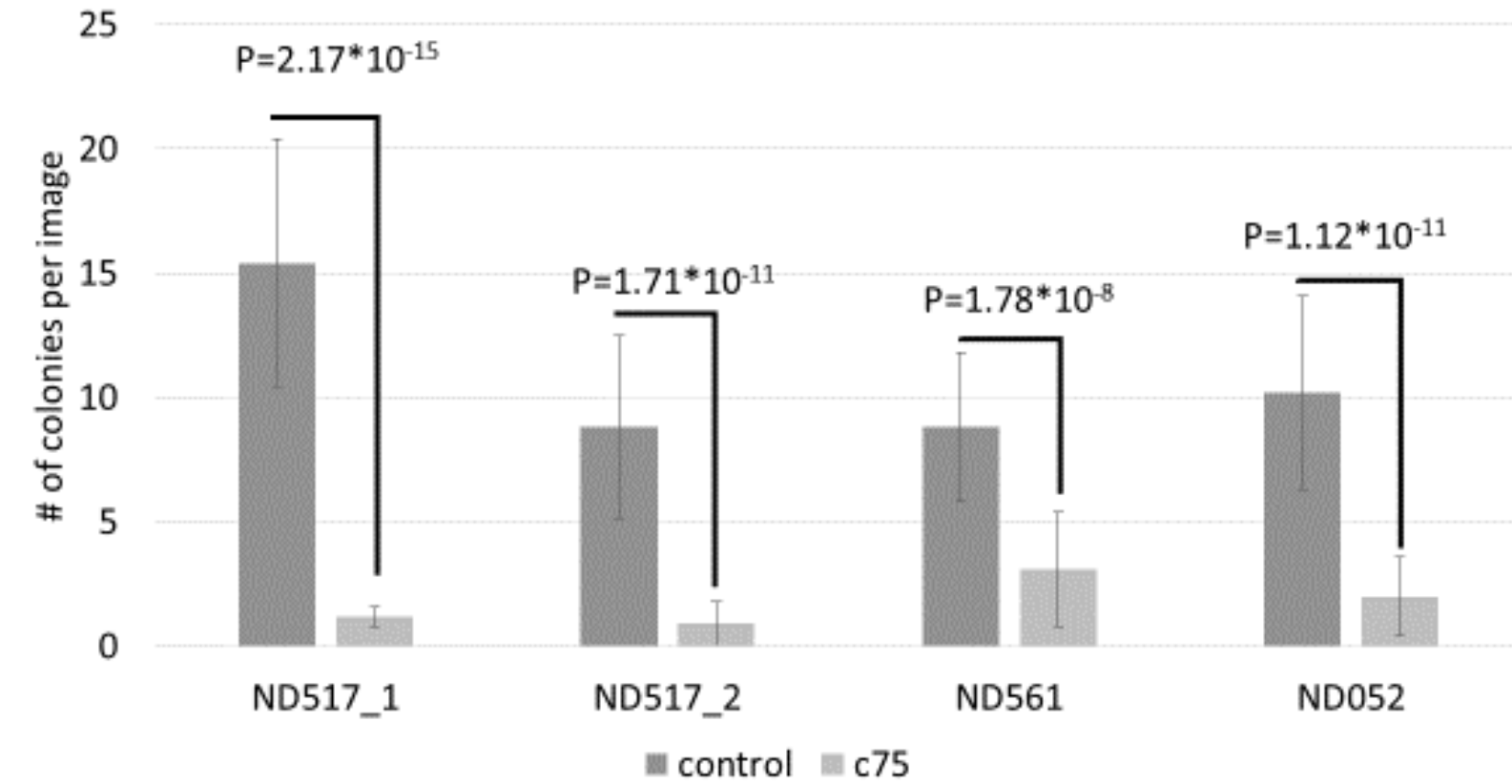
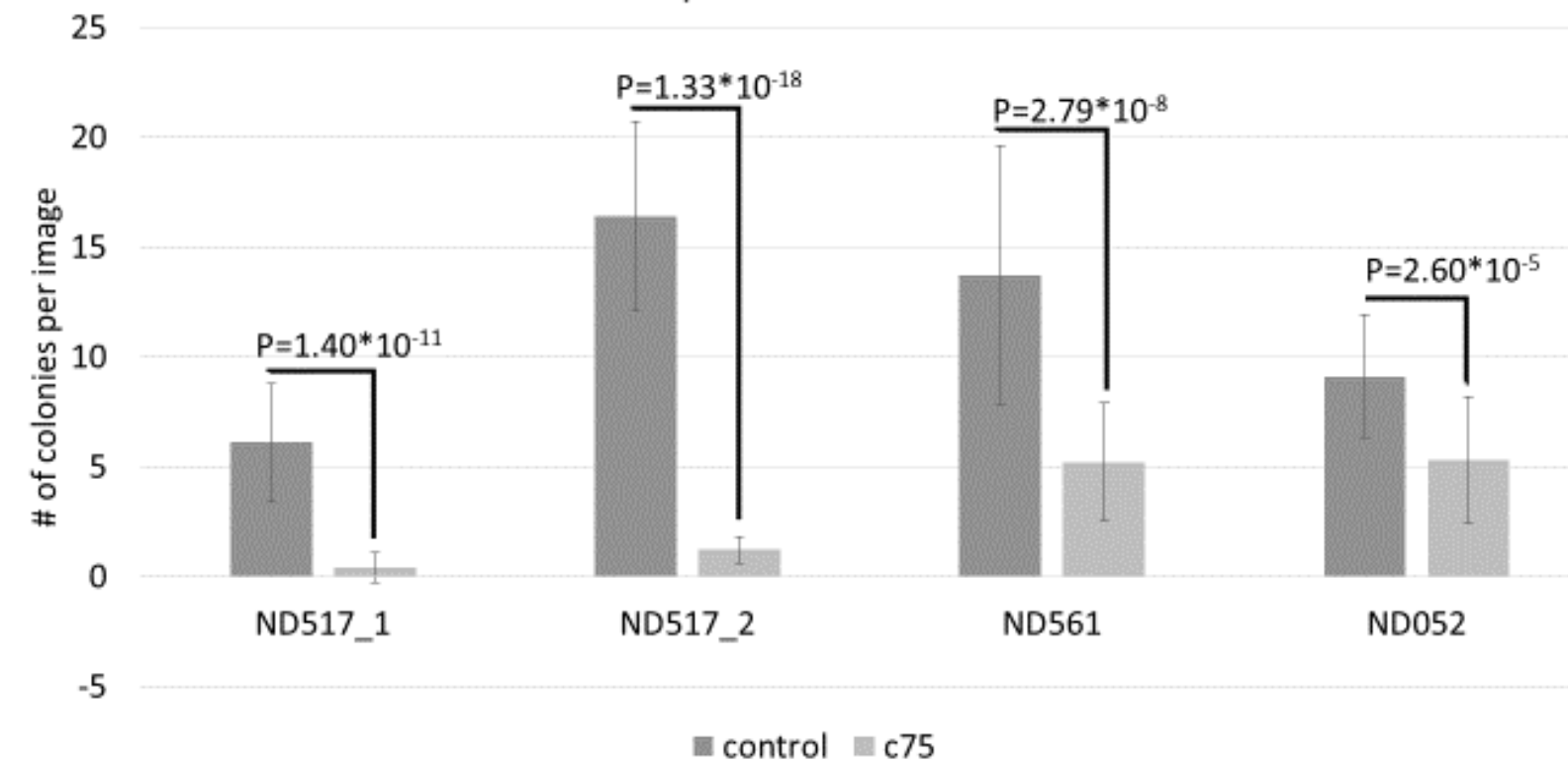


A)

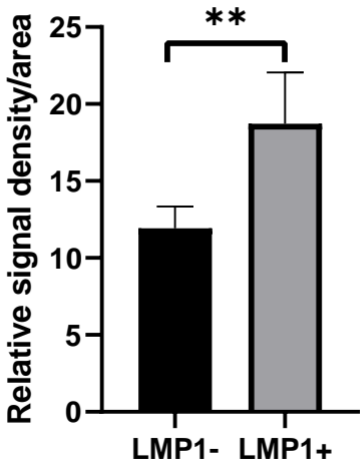
Average colony number
24hr post C75 treatment

**B)**

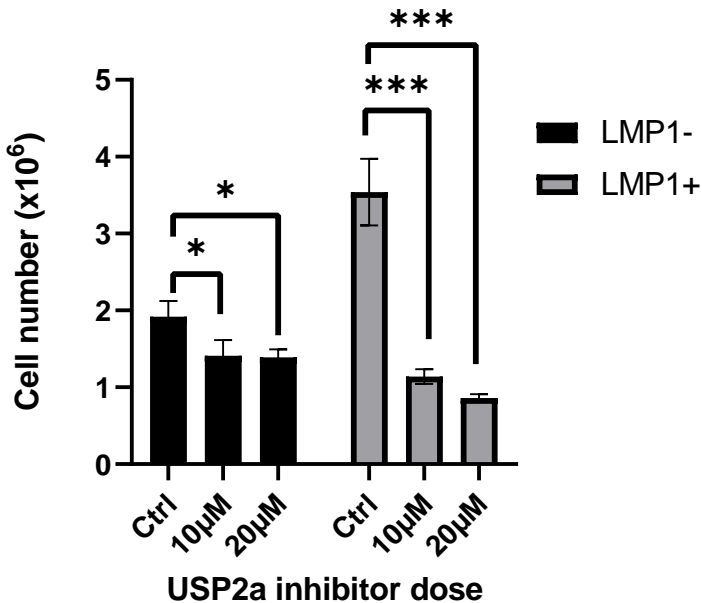
Average colony number
48hr post C75 treatment



USP2a densitometry FASN-IP



USP2a inhibition impairs proliferation of LMP1+ cells



| Donor number | Age | Sex | HLA-A | HLA-B | HLA-C |
|---------------------|------------|------------|-----------------|-------------------|-------------------|
| ND500 (B-cells 1) | 25 | F | A 02:06, 11:01 | B 15:02, 40:01 | C 04:03, 08:01 |
| ND560 (B-cells 2) | 21 | F | A 02:06, 24:02 | B 15:25, 40:02 | C 03:04, 04:03 |
| ND492 (B-cells 4) | 37 | M | A 01:01, 03:01 | B 07:02, 37:01 | C 06:02, 07:02 |
| ND451 (B-cells 6) | 31 | F | A 29:01, 68:01 | B 15:17, 39:01 | C 07:01, 12:03 |
| ND052 | 56 | M | A2 | B62, B44 | N/A |
| ND517 | 28 | F | A 11: 01, 30:02 | B 18: 01, B 51:01 | C 05: 01, B 15:02 |
| ND561 | 25 | M | A 23:01, 30:01 | B 13:02, 49:01 | C 06:02, 07:01 |

1 **Supplemental table 1. Complete metabolite comparison of primary B-cells vs**
2 **matched LCLs.**

3

4 **Supplemental table 2. Donor information for primary B-cells.**

5 De-identified, purified human B-cells were obtained from the Human Immunology Core of
6 the University of Pennsylvania, where HLA typing was performed. Age, sex, and MHC I
7 types are provided above to show heterogeneity of donors.

8

9 **Supplemental Figure 1. Cell colony size is decreased by FASN inhibition during**
10 **EBV-immortalization.** 10 million cells per group were collected from three donors (one
11 donor was assayed at two independent times) and infected with B95.8 strain EBV
12 24hours prior to treatment. Cells were imaged on a Nikon TE2000 Inverted Microscope
13 at 4x magnification 24 (A) and 48 hours (B) post C75 treatment. 30 randomized, non-
14 overlapping images from each group were collected and analyzed on ImageJ.
15 Considering the average colony size of a healthy cell formation, a threshold of ≥ 1000
16 pixels² was set before using the “analyze colony” feature to determine number of cell
17 formations per image that met the “healthy threshold”. The values from the 30 images
18 were then averaged to determine the number of colonies formed on average per donor
19 group per image.

20

21 **Supplemental Figure 2. USP2a stabilizes FASN protein in DG75 cells.** 10 μ g of
22 polyclonal rabbit antibody to FASN or normal rabbit IgG was added to the lysate of 10
23 million LMP1- or LMP1+ DG75 cells, respectively. Magnetic protein-A conjugated beads

24 were utilized to immunoprecipitate FASN/IgG binding proteins. Beads were boiled in 2x
25 laemmli buffer and ran on a western blot beside 10% protein lysate input and blotted for
26 FASN and USP2a signal. Densitometry analysis was performed on Invitrogen iBright
27 Analysis Software, with signal density/area from IgG control lanes subtracted from IP
28 lanes. IgG normalized IP signal was then normalized to input signal density/area. Data
29 shown is representative of three independent co-IP assays, averaged. The above graph
30 is representative of three independent assays, averaged.

31

32 **Supplemental Figure 3. USP2a inhibition ablates proliferation in DG75 cells.** 1

33 million LMP1- or LMP1+ DG75 cells were treated with DMSO control, 10 μ M ML364, or
34 20 μ M ML364. At 24 hours, cells were counted with trypan blue to exclude dead cells.

35 Statistics are comparing proliferation of each treatment group within the same cell line.

36 Error bars represent standard deviation of three independent experiments. P values for

37 significant differences (Student's t-test) are summarized by three asterisks ($p < 0.001$), two

38 asterisks ($p < 0.01$), or one asterisk ($p < 0.05$).

39

40