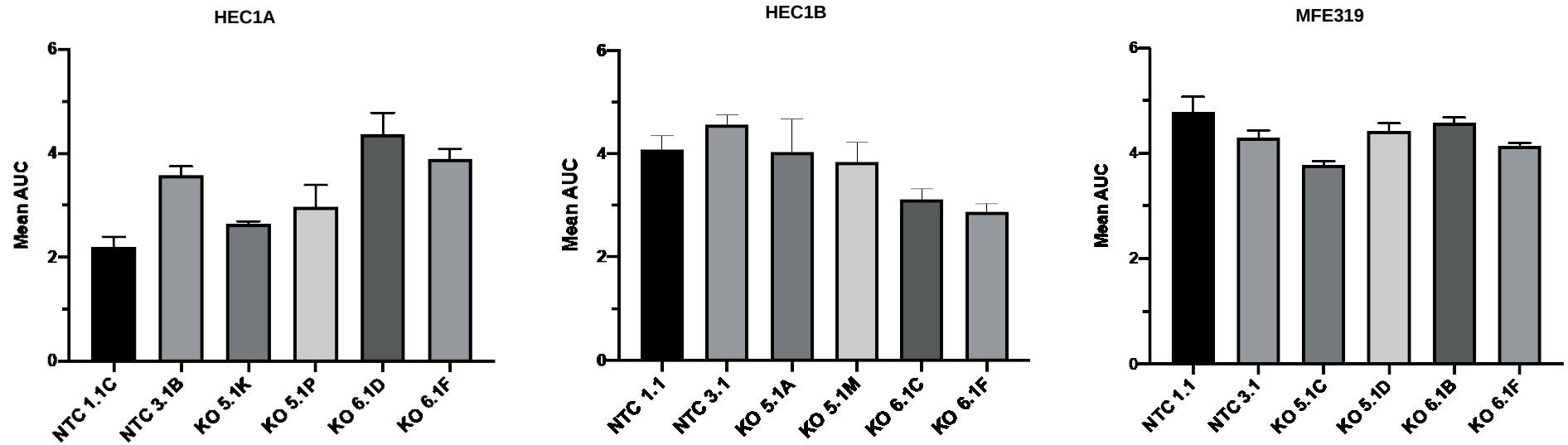


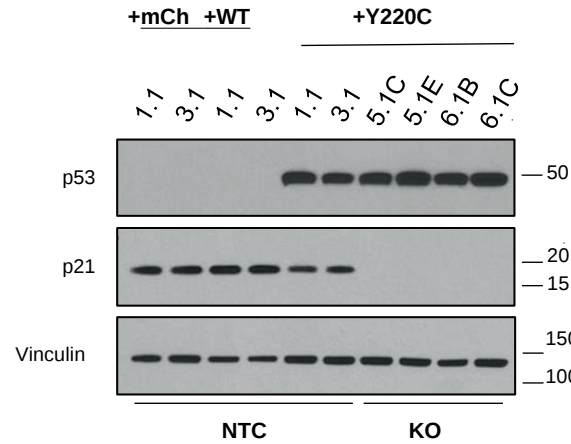
Supplemental Figure 1.



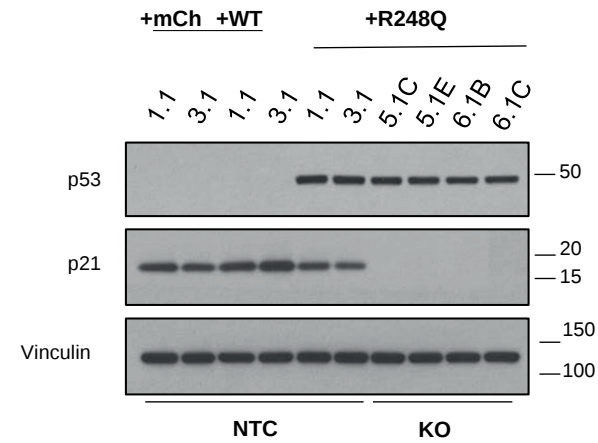
TP53 knockout via CRISPR/Cas9 in three EC cell lines with GOF/DN alleles and high VAF (presumed loss-of-heterozygosity) does not impact radiation response.

Supplemental Figure 2.

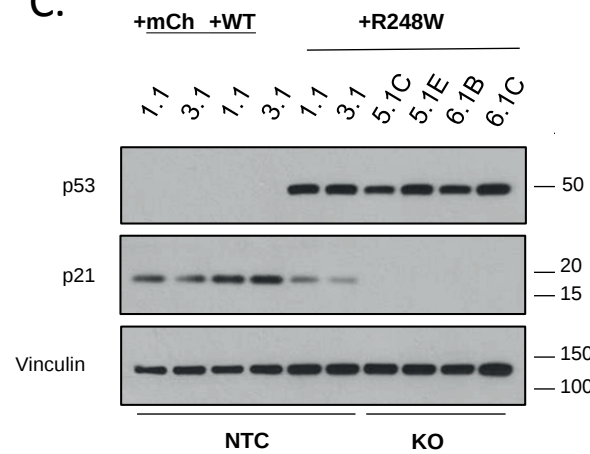
A.



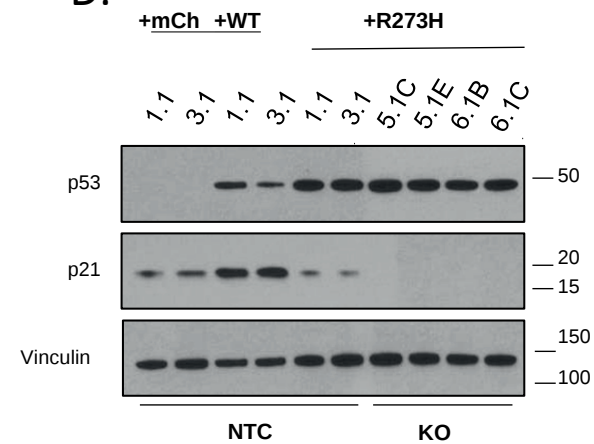
B.



C.

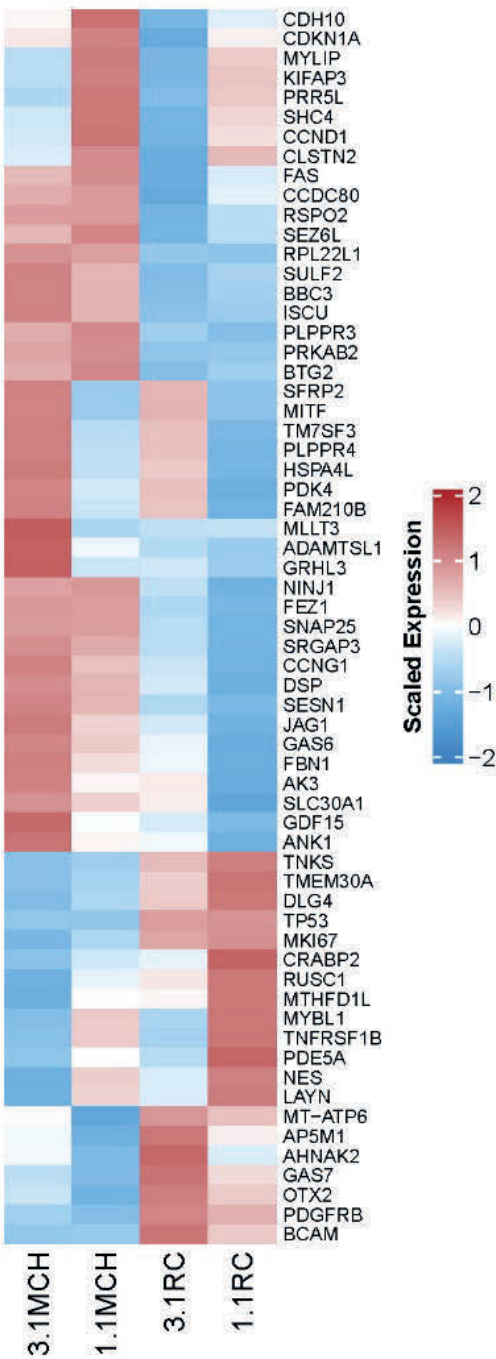


D.



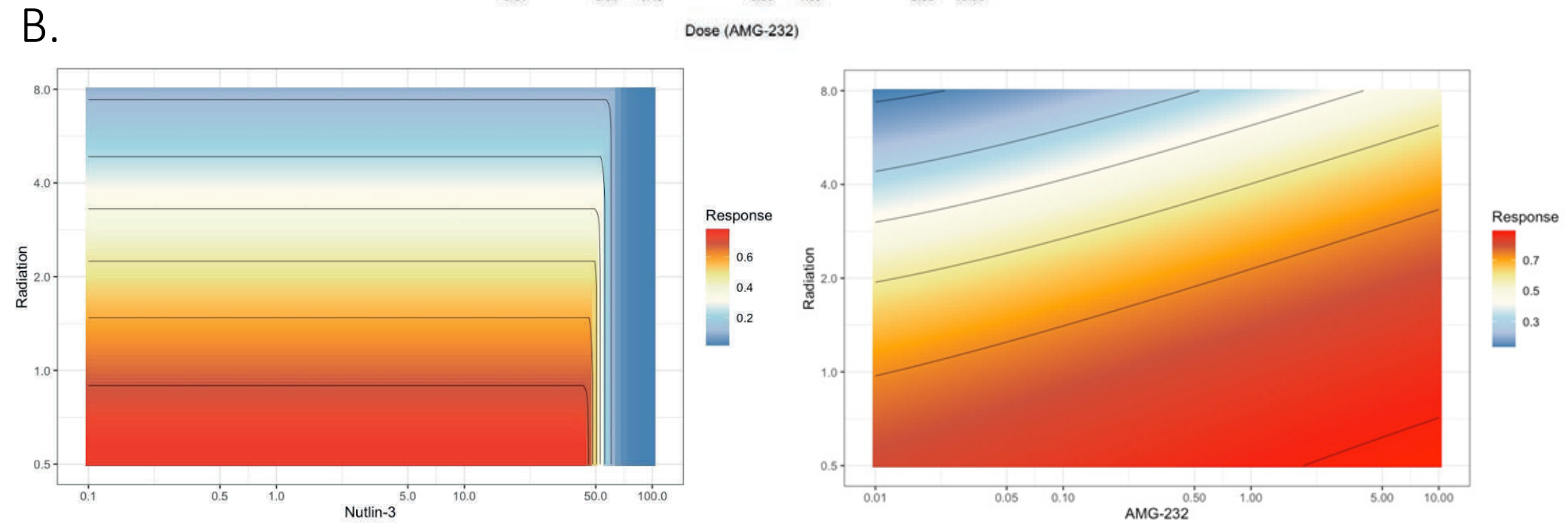
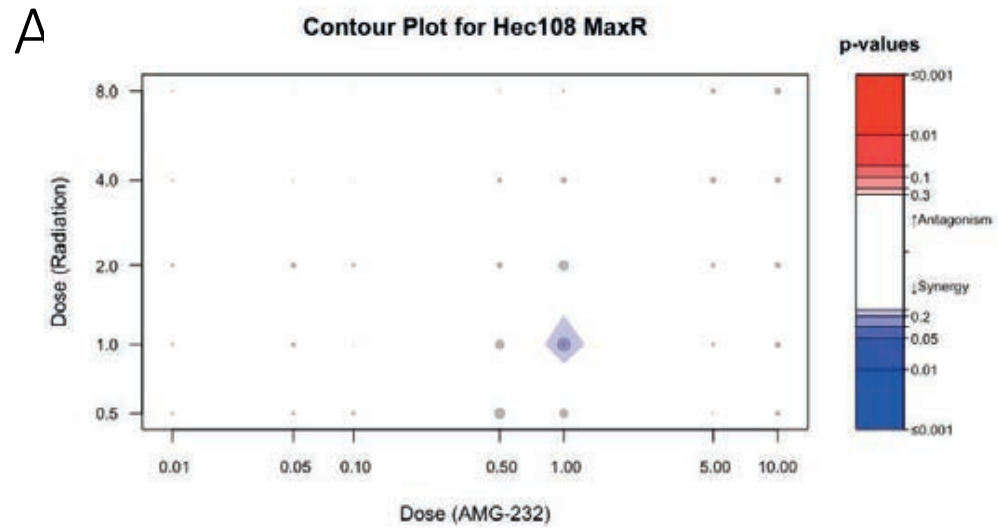
Western-blot analysis of 4 alternative TP53 alleles reveal abrogation of p21 signaling by the mutant allele in the presence of two wild-type alleles. As seen in the knock-out monoclonal lines (5.1C/E and 6.1B/C) expression of the mutant allele alone does not impact p21 signaling but confers significant accumulation of p53. Wild-type p53 is rapidly degraded by MDM2, thus there is variability in the western blot ability to pick up the wild-type p53 band (in the 1.1/3.1 mcherry and +WT), as seen in panel D versus A-C.

Supplemental Figure 3.



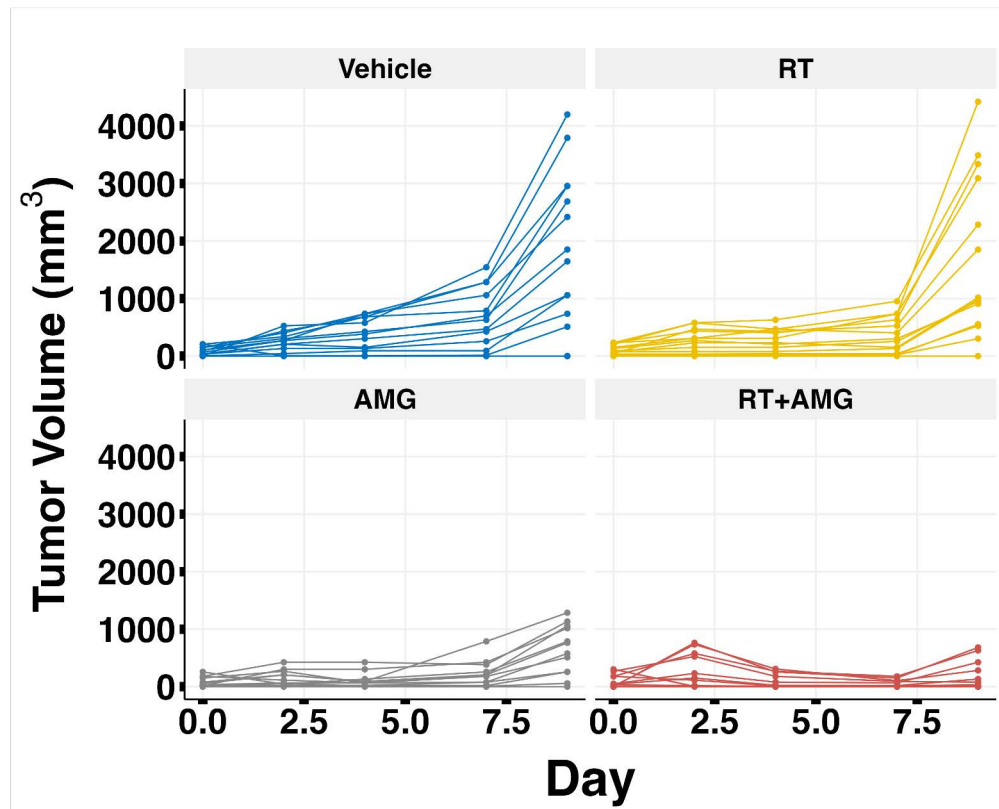
Heat-map of differential gene expression. In this experiment, two NTC (1.1 and 3.1) monoclonal isolates were transfected with mcherry and an R273C allele. Radiation was administered (2Gy) and RNA extracted 24 hours later. Differential gene expression between the lines with only wild-type alleles and those with wild-type + R273C can be appreciated. Notably TP53 expression is confirmed in our 1.1RC and 3.1RC constructs, confirming increased expression via our PGK promoter.

Supplemental Figure 4.



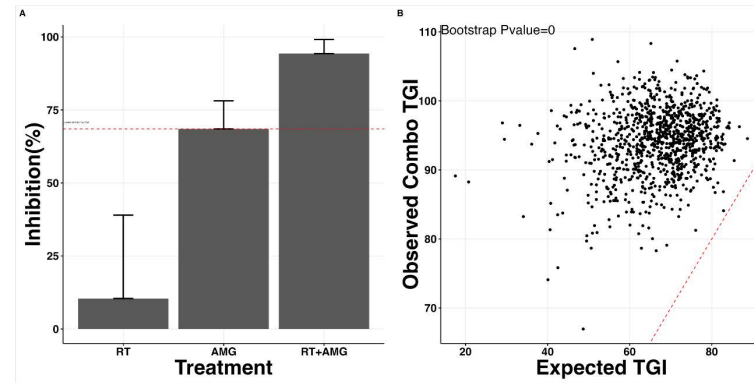
A) Contour plot of HEC108 and AMG-232 demonstrating weak synergy at 1nM and 1Gy dosing. **B)** Isobologram of Hec1B using radiation (y-axis) and drug concentration (x-axis). As noted there is no effect with the addition of Nutlin-3 to radiotherapy and a suggestion of an antagonistic effect at higher doses with AMG-232.

Supplemental Figure 5.

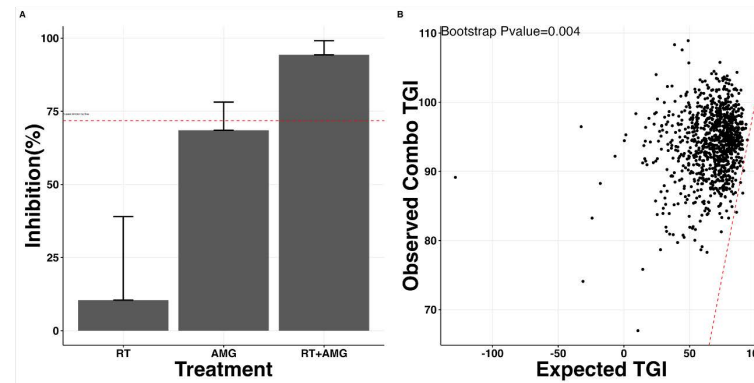


Individual xenograft tumor growth curves based on treatment group. Each line represents an individual tumor through the entire experiment. Each mouse had 2 tumors, one on each flank. We can observe that AMG-232 (bottom left) had a substantial impact on tumor size, while co-treatment with 1Gy of radiotherapy almost eliminated the tumors by day 7 post-treatment.

Supplemental Figure 6.



HSA



BLISS

In Vivo Highest Single Agent (top) and BLISS (bottom) synergy analyses for AMG-232 and Radiotherapy. As seen on the bar graphs (left) tumor growth inhibition (TGI) exceeded an additive effect under both models. Bootstrap analyses using both models (right) confirmed statistically significant synergy compared to expected TGI, as denoted by the dashed line.

Supplemental Table 1.

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
p53 Pathway	9/200	3.59E-06	1.26E-04	8.283096	103.8415782	CDKN1A;BTG2;SESN1;NINJ1;CCNG1;FAS;TM7SF3;TP53;BLCAP
Apoptosis	6/161	4.44E-04	0.007778	6.639215	51.24539677	CDKN1A;BTG2;CCND1;GADD45B;PLPPR4;FAS
TNF-alpha Signaling via NF-kB	6/200	0.001375	0.016047	5.294039	34.88214092	CDKN1A;BTG2;CCND1;GADD45B;NINJ1;IER2
Mitotic Spindle	3/199	0.119967	0.422972	2.553139	5.414022257	RICTOR;KIFAP3;KIF15
Hypoxia	3/200	0.121286	0.422972	2.54005	5.358499671	CDKN1A;HOXB9;CCNG2
G2-M Checkpoint	3/200	0.121286	0.422972	2.54005	5.358499671	CCND1;MKI67;KIF15
Estrogen Response Early	3/200	0.121286	0.422972	2.54005	5.358499671	SOX3;CCND1;MYBL1
Myogenesis	3/200	0.121286	0.422972	2.54005	5.358499671	CDKN1A;GADD45B;SCHIP1
mTORC1 Signaling	3/200	0.121286	0.422972	2.54005	5.358499671	CDKN1A;BTG2;CCNG1
E2F Targets	3/200	0.121286	0.422972	2.54005	5.358499671	CDKN1A;MKI67;TP53

Hallmark pathway analyses using differential gene expression data 24 hours after radiotherapy exposure.

Supplemental Table 2.

Target	Target Sequence	F oligo (BsmBI site in red)	R oligo (BsmBI site in red)
NTC 1.1	GTATTACTGATATTGGTGGG	CACCGGTATTACTGATATTGGTGG G	AAACCCACCAATATCAGTAATAC C
NTC 3.1	TCAACCCAGCGCACCGTTG	CACCGTCAACCCAGCGCACCGTT G	AAACCAACGGTGCGCTGGGGTTG AC
TP53 5.1	TGAGGGCAGGGGAGTACTGT	CACCGTGAGGGCAGGGGAGTACT GT	AAACACAGTACTCCCCTGCCCTCA C
TP53 6.1	GTTGCAAACCAGACCTCAGG	CACCGGTTGCAAACCAGACCTCA GG	AAACCTGAGGTCTGGTTTGCAAC C

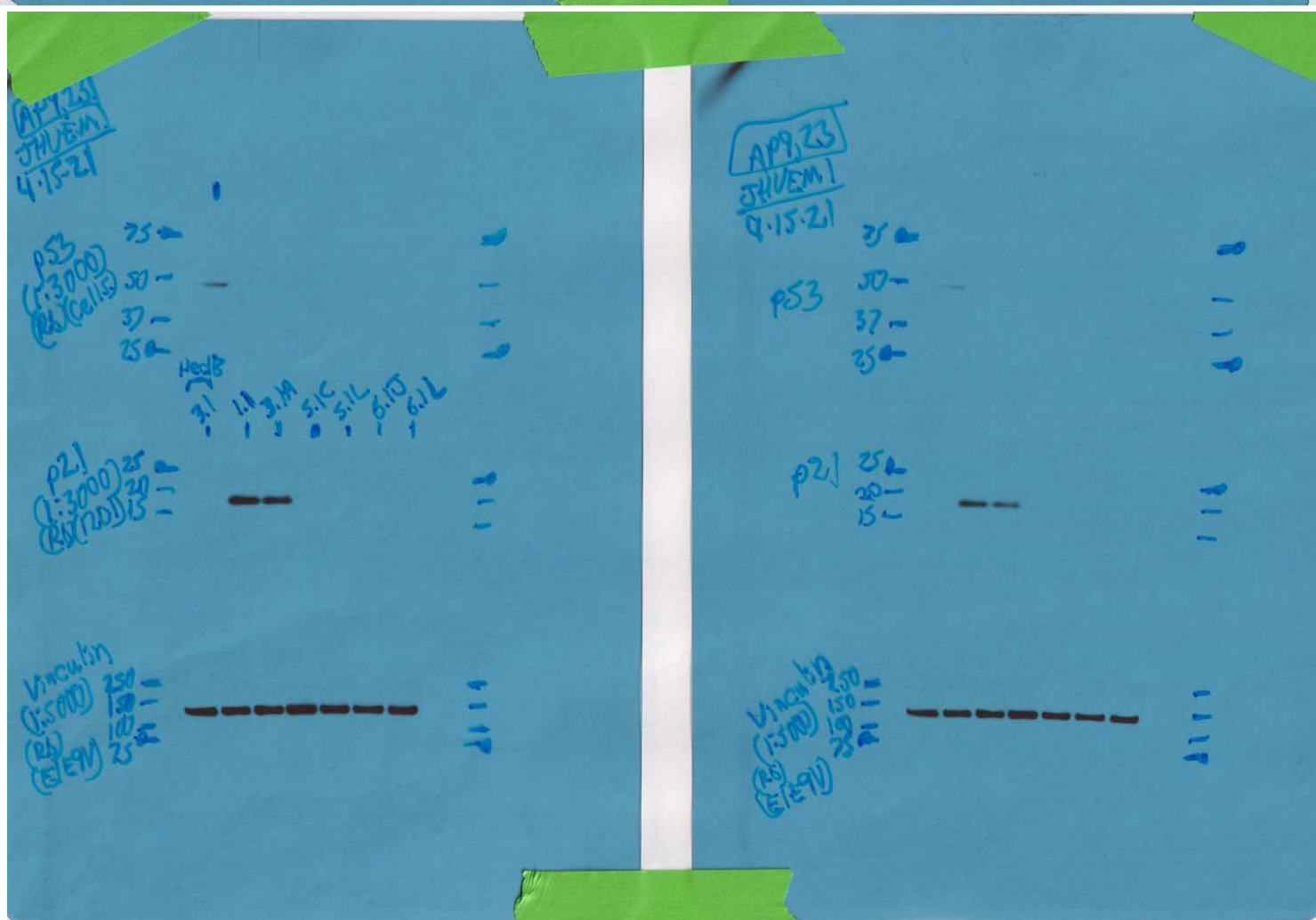
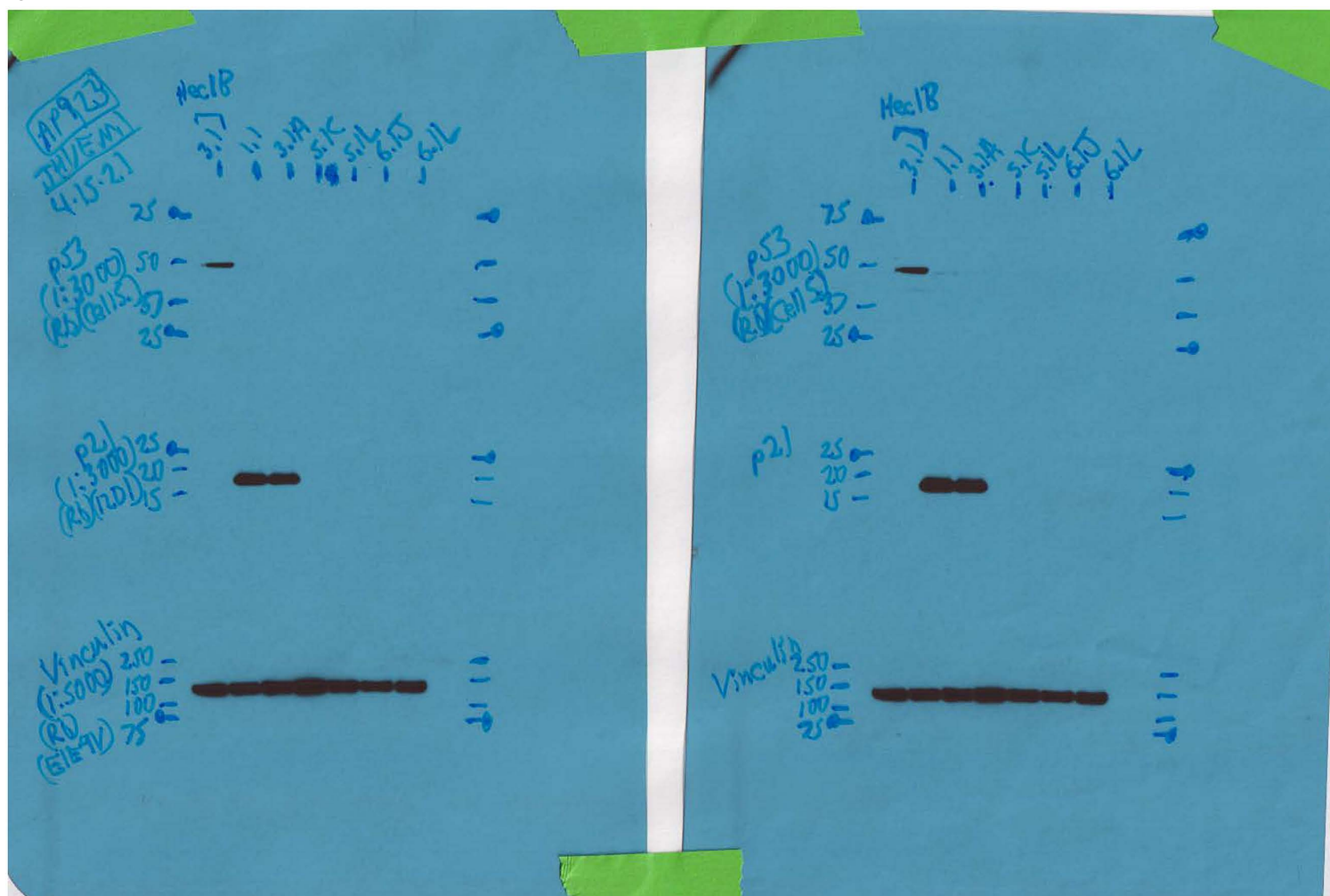
CRISPR/Cas9 guide-RNA sequences. A designed 5.1 targets exon 5 and 6.1 targets exon 6.

Supplemental Table 3.

Name/mutation	F primer (mutated site underlined)	R primer (mutated site underlined)
attL flanking sites	(L1) CGTTGTAAAACGACGGCCAGTC	(L2) GCCAGGAAACAGCTATGACCATG
TP53 R273H	AGCTTTGAGGTGCATGTTTGTGCCTGT	ACAGGCACAAACATGCACCTCAAAGCT
TP53 R273C	AGCTTTGAGGTGTGTGTTTGTGCCTGT	ACAGGCACAAACACACACCTCAAAGCT
TP53 R248Q	GGCGGCATGAACCAGAGGCCCATCCTC	GAGGATGGGCCTCTGGTTCATGCCGCC
TP53 R248W	GGCGGCATGAACTGGAGGCCCATCCTC	GAGGATGGGCCTCCAGTTCATGCCGCC
TP53 Y220C	GTGGTGGTGCCCTGTGAGCCGCCTGAG	CTCAGGCGGCTCACAGGGCACCACCAC

TP53 variant site-directed mutagenesis primer sequences with nucleotide substitutions.

Figure 1 - Panel C (JHUEM1)



AP1
JHUEM
1-21-21 25
p53 50
(1:3000) 37
(R5)(CS) 25
p21 25
(1:3000) 25
(R5)(CS) 10
250
150
100
75
+10min Nutlin3, 18hrs

Hec18
JHUEM
-21-21
-1-1
-3-1
-5-1C
-5-1E
-6-1B
-6-1C
-3-1E
-1-1
-3-1
-5-1C
-5-1E
-6-1B
-6-1C
111111
111111
111111

AP1
JHUEM
1-21-21 25
p53 50
(1:3000) 37
(R5)(CS) 25
p21 25
(1:3000) 25
(R5)(CS) 10
250
150
100
75
+10min Nutlin3, 18hrs

Hec18
JHUEM
-21-21
-1-1
-3-1
-5-1C
-5-1E
-6-1B
-6-1C
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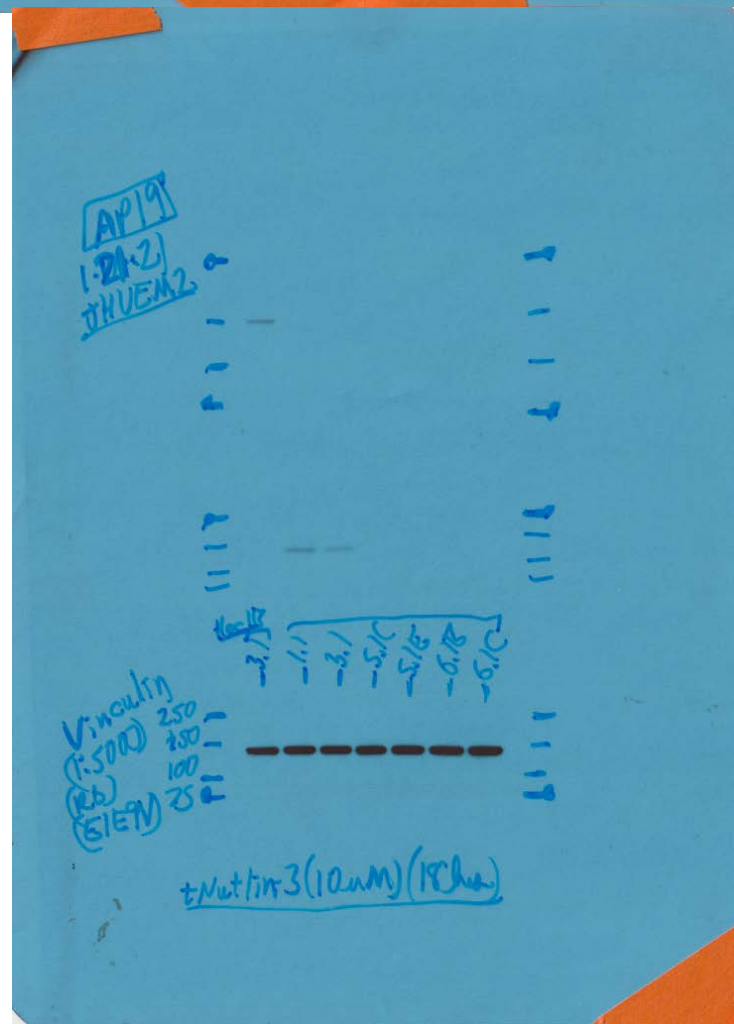


Figure 1 - Panel D (JHUEM2 - 5.1 TP53 KO Addback)

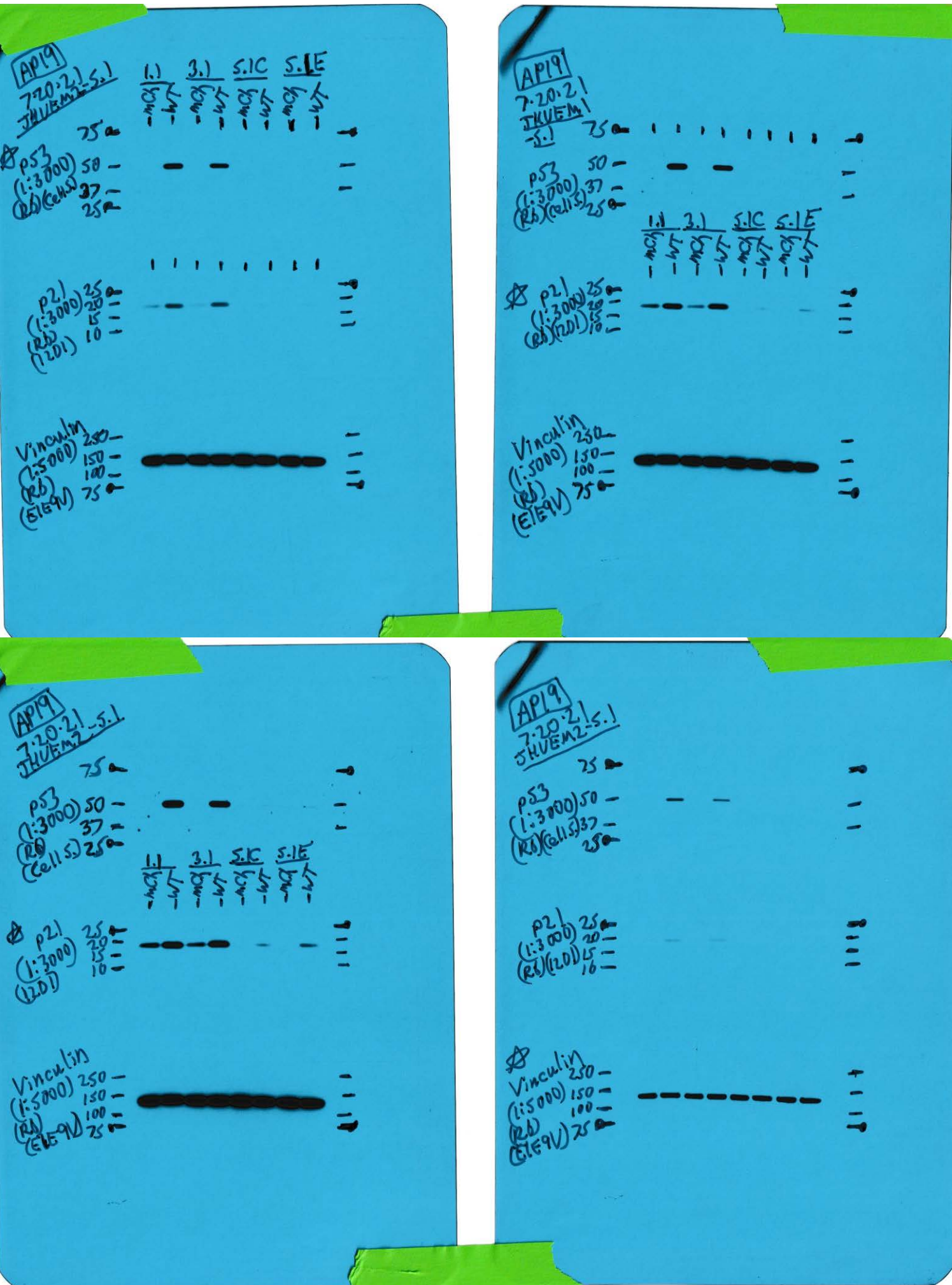


Figure 1 - Panel D (JHUEM2 - 6.1 TP53 KO Addback)

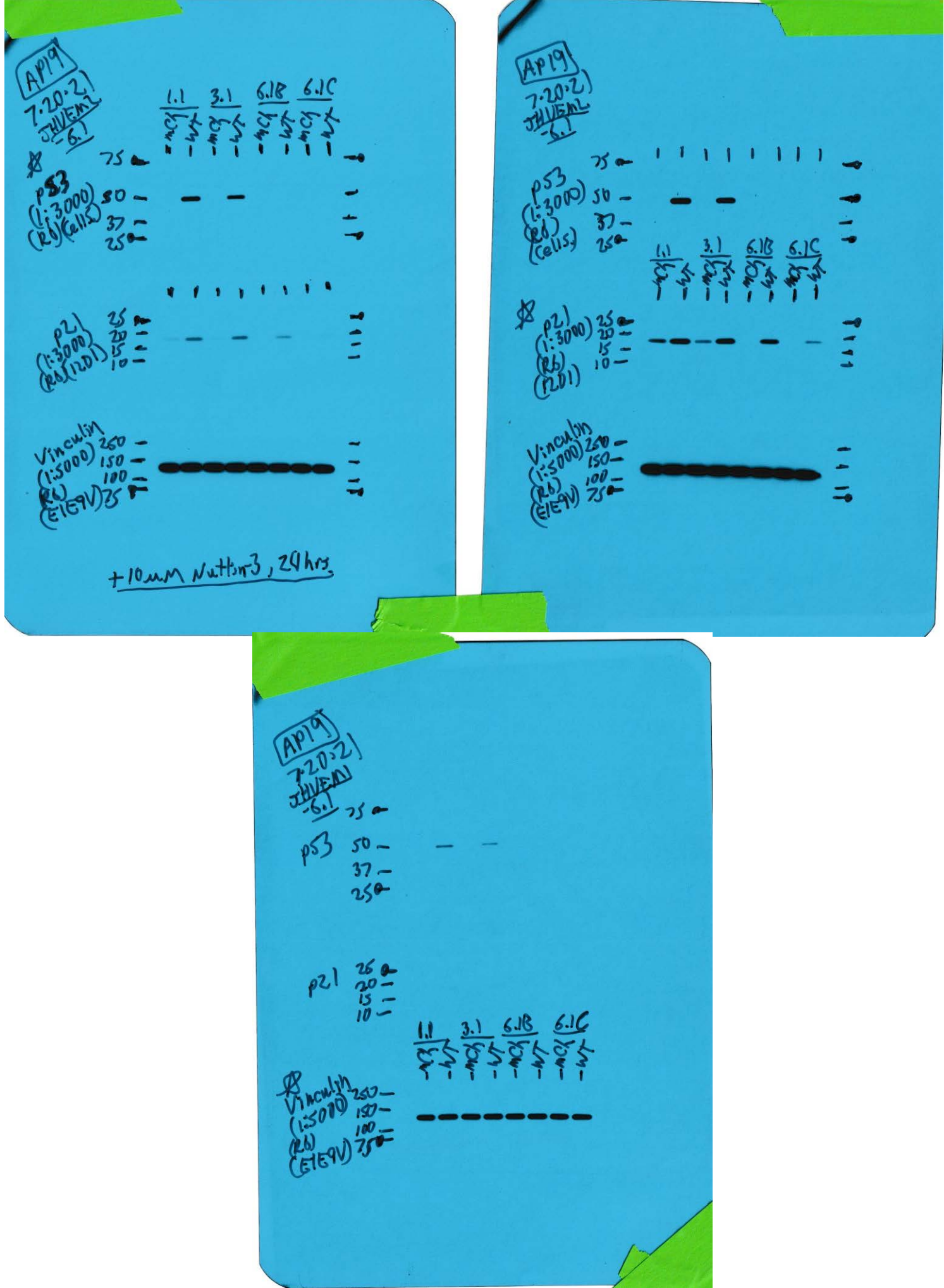
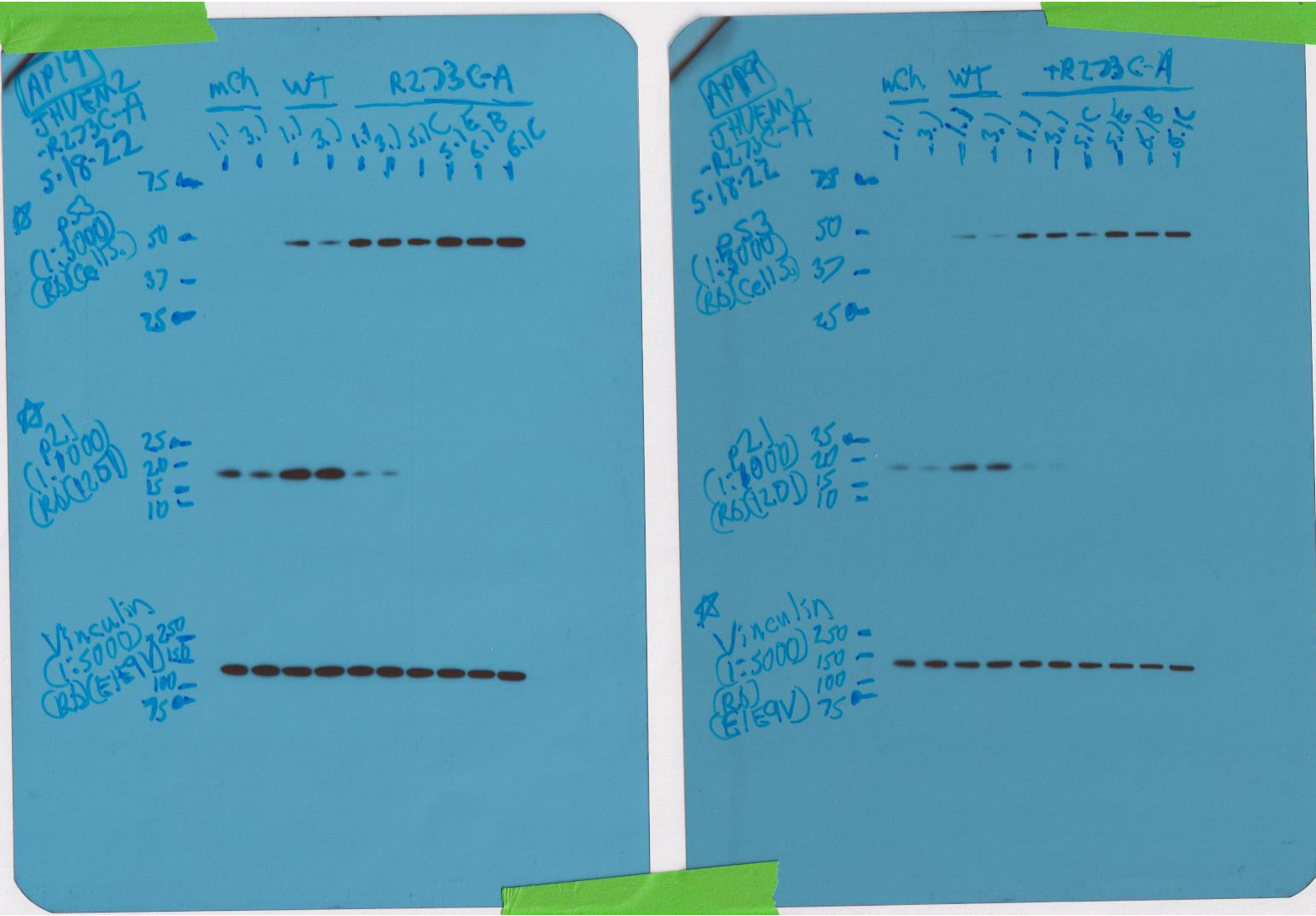


Figure 2 - Panel A (JHUEM2 + R273C Variant Dominant-Negative)



API9
JHVEN2
12-11-20

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10

NTC3.1
JHVEN2
API9
R222
R224
R226
R228
R230
R232
R234
R236
R238
R240
R242
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Figure 3 Panel B - (Nutlin-3 Response JHUEM2/Hec108/Hec1B)

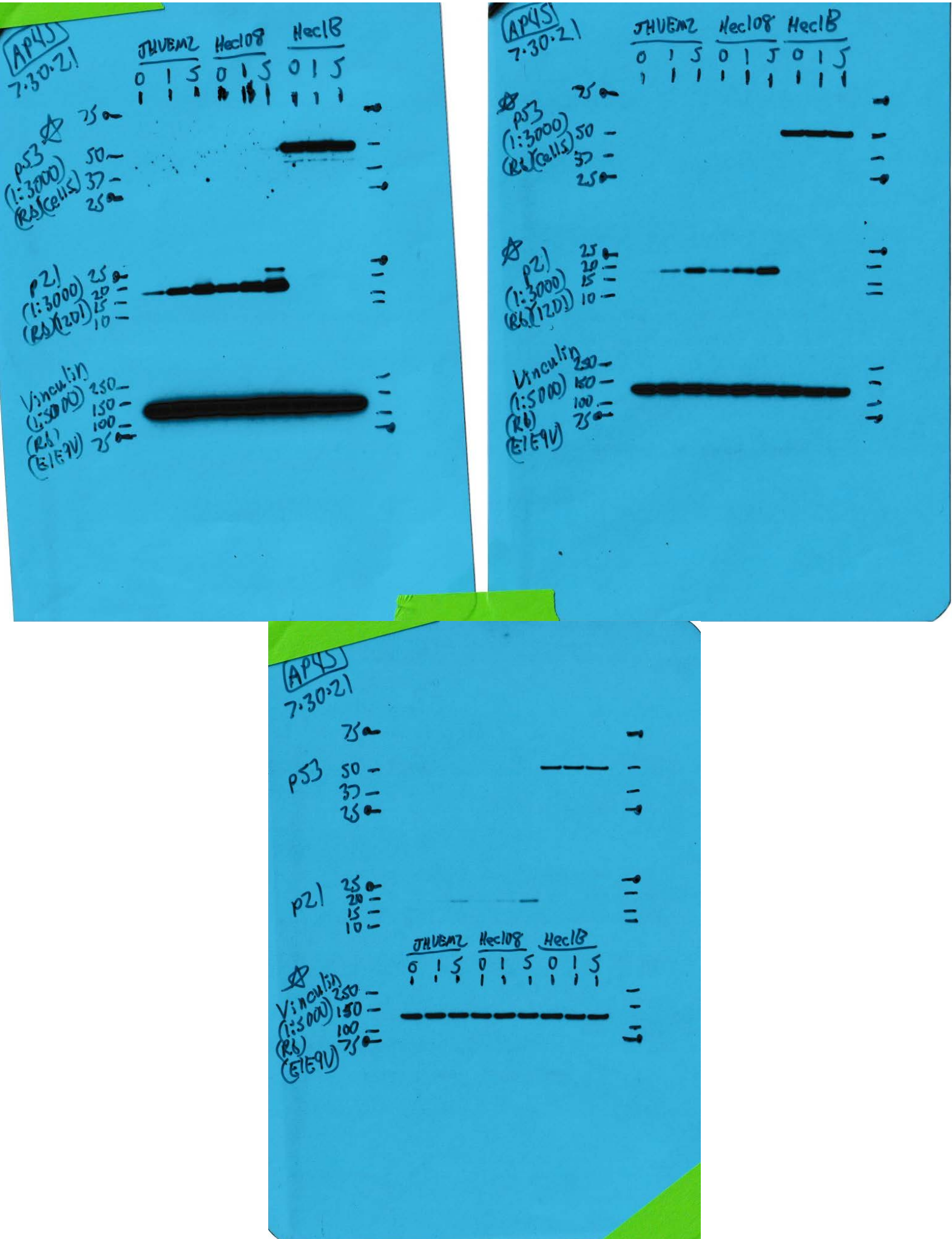


Figure 3 Panel B- (Nutlin-3 Response JHUEM2/Hec108 SubFigure)

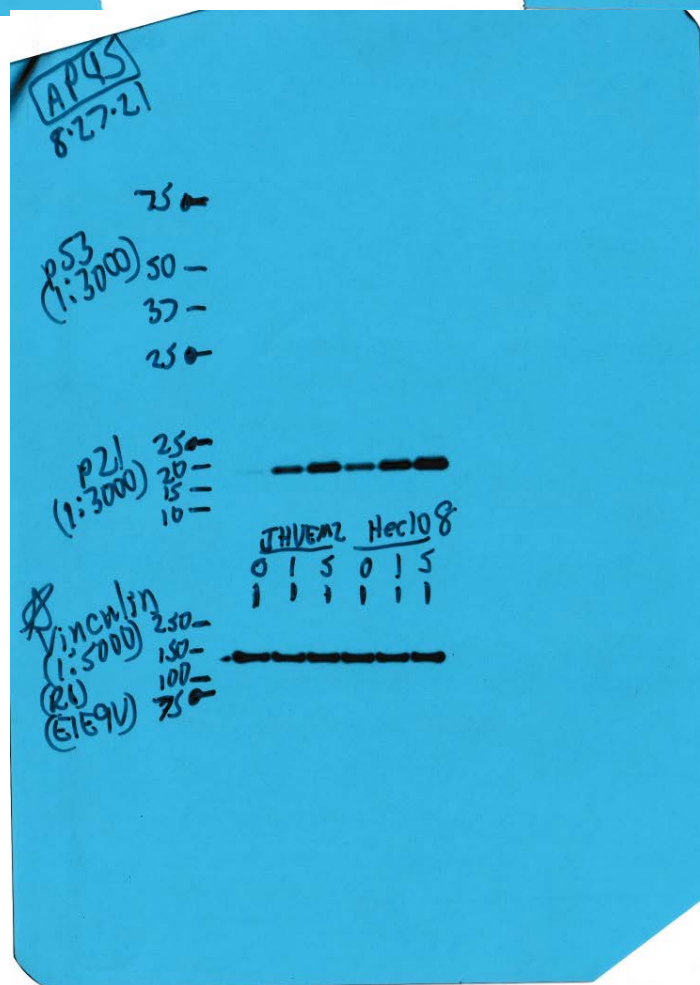
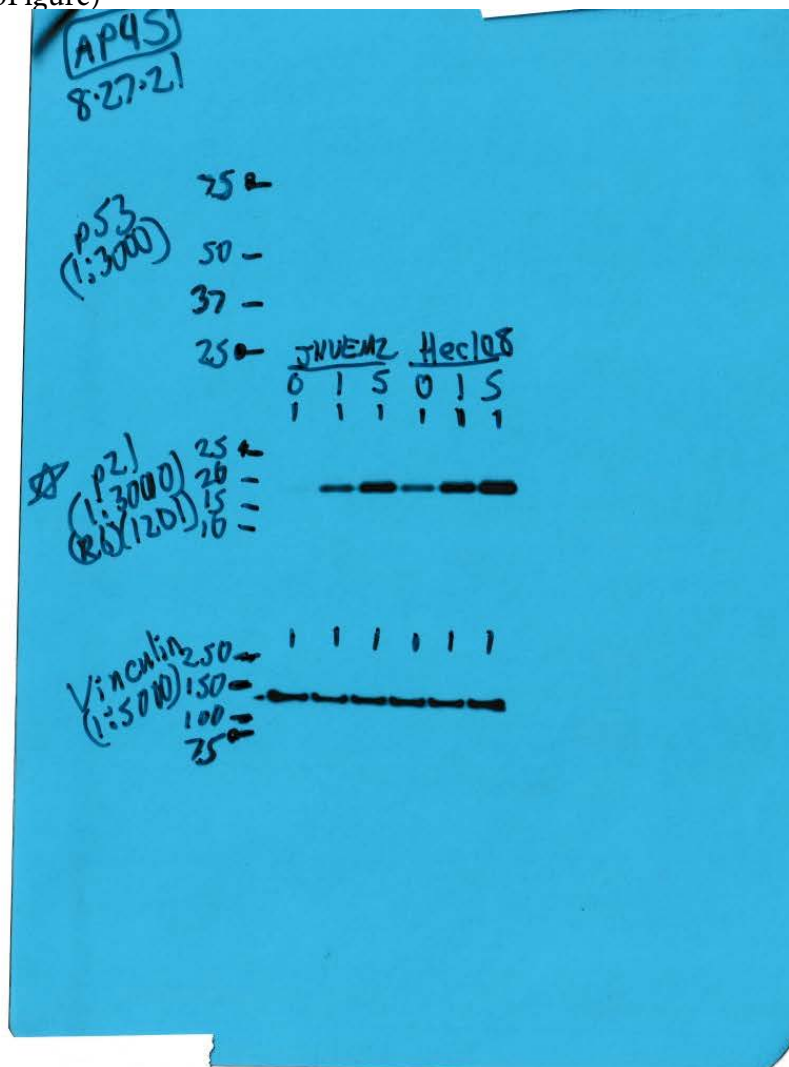
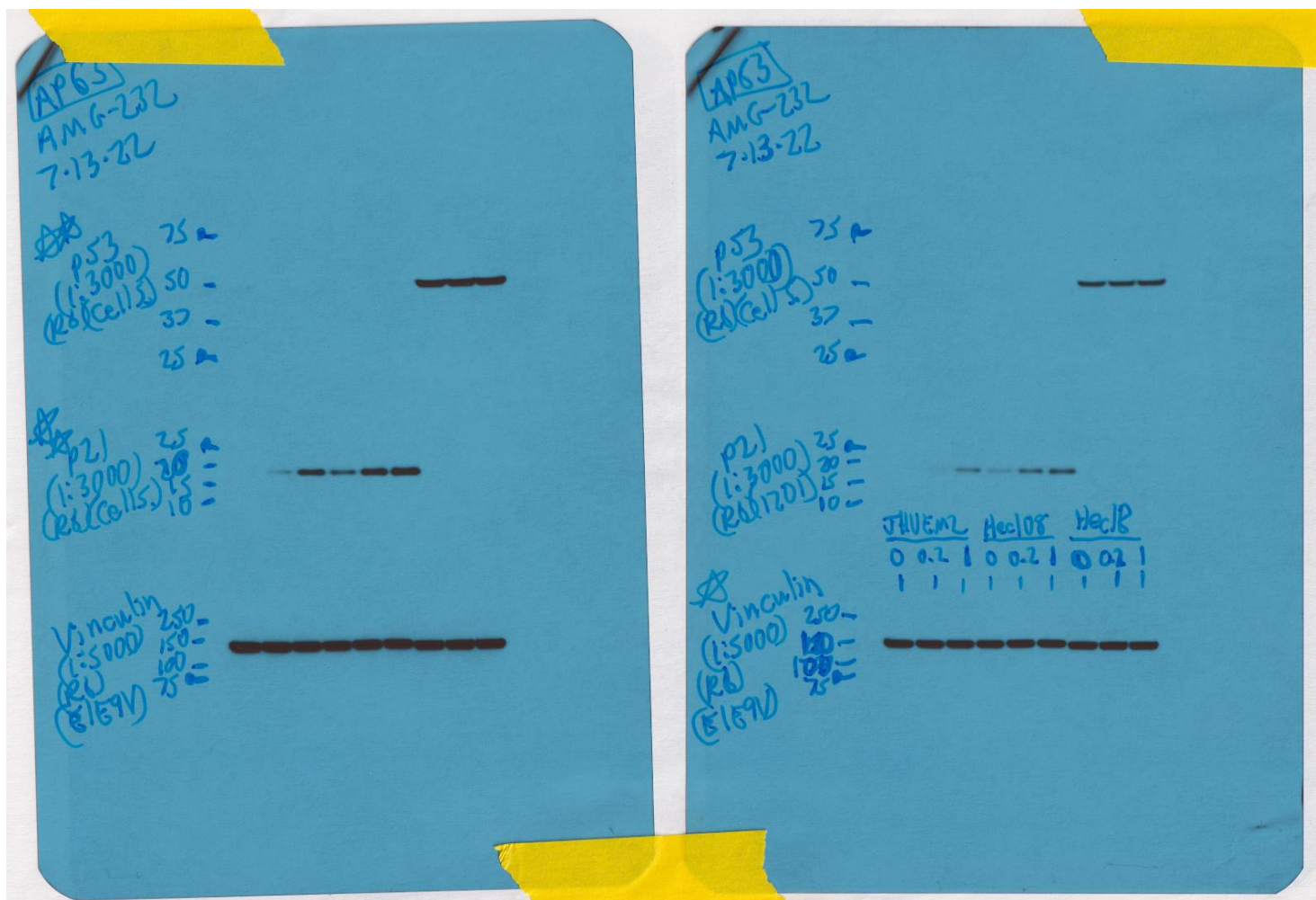
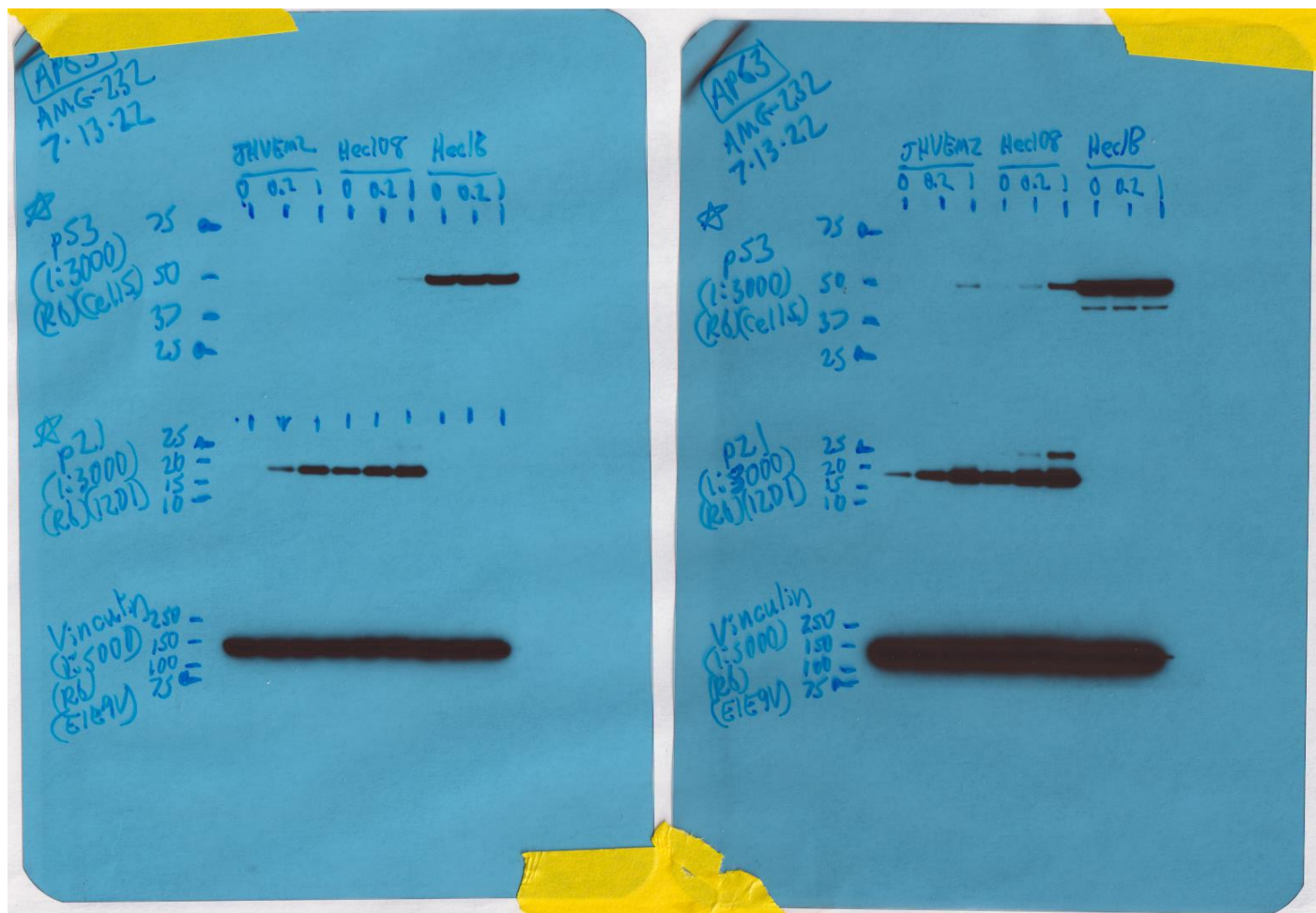
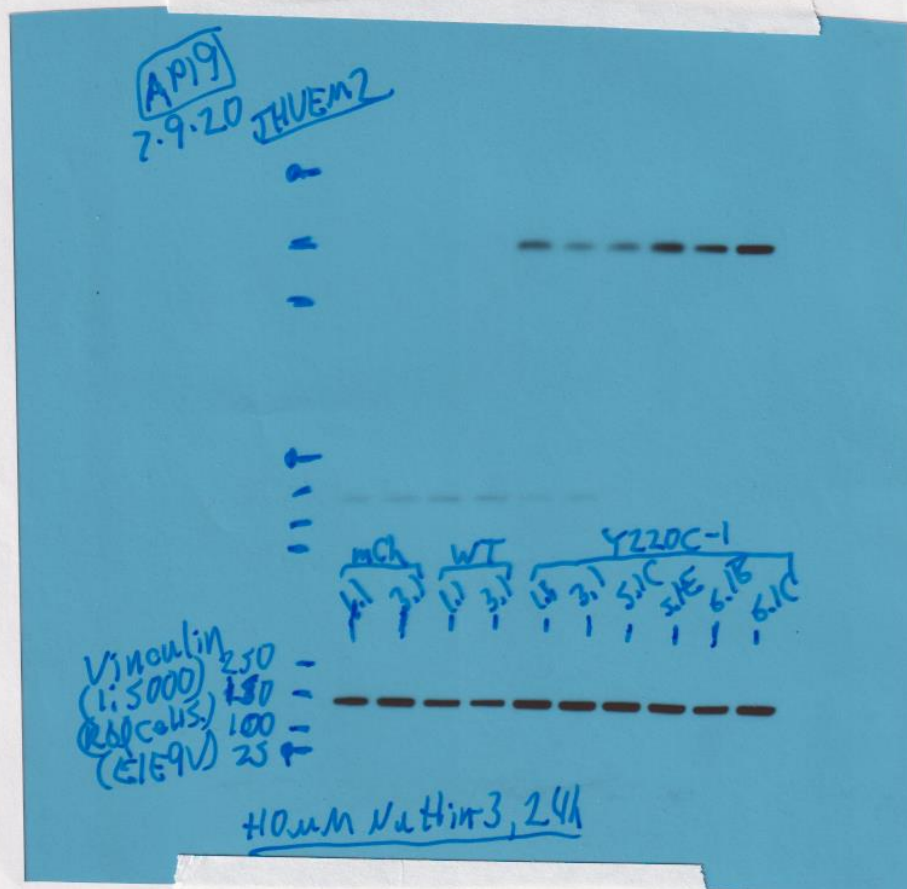
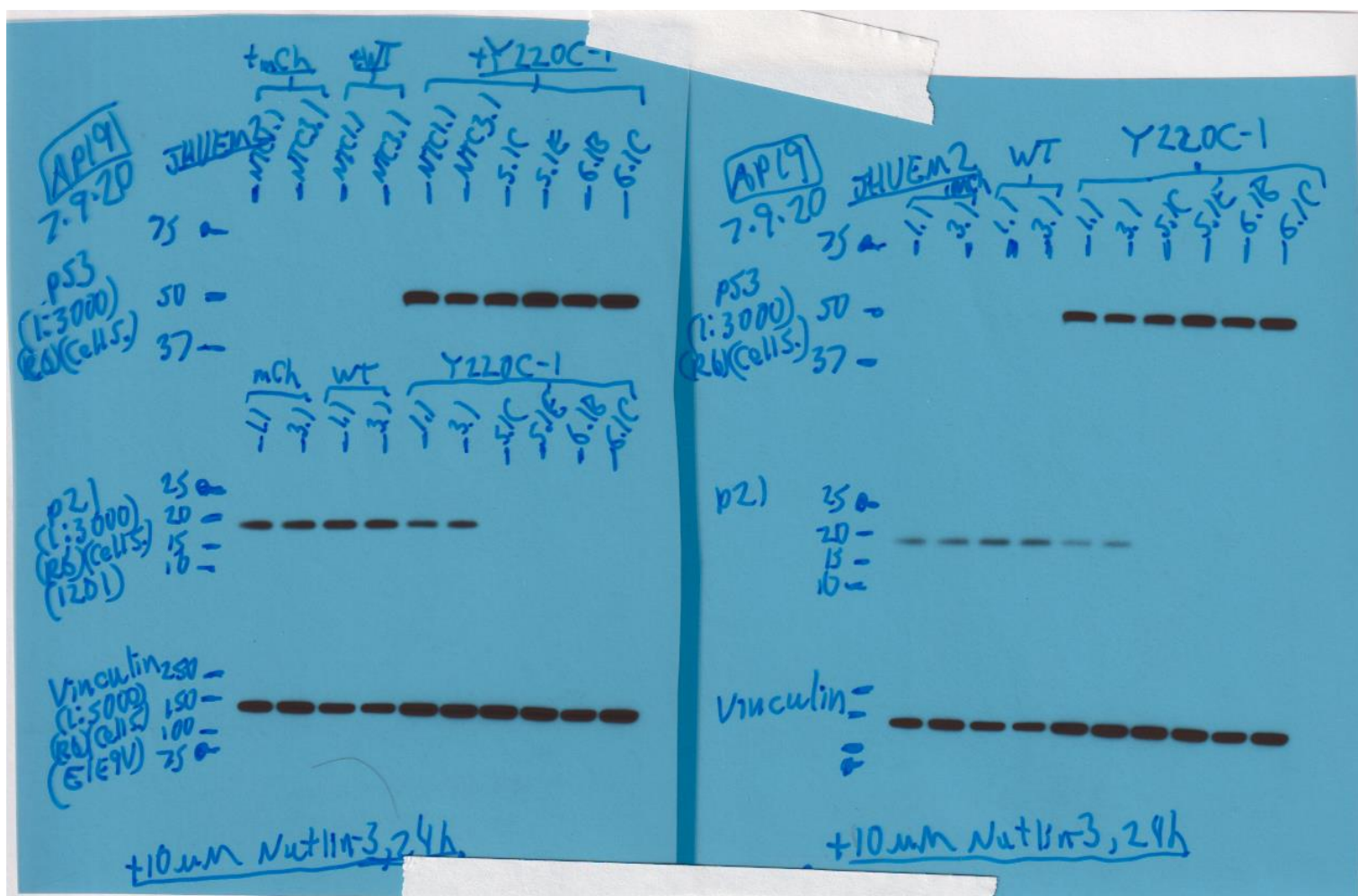
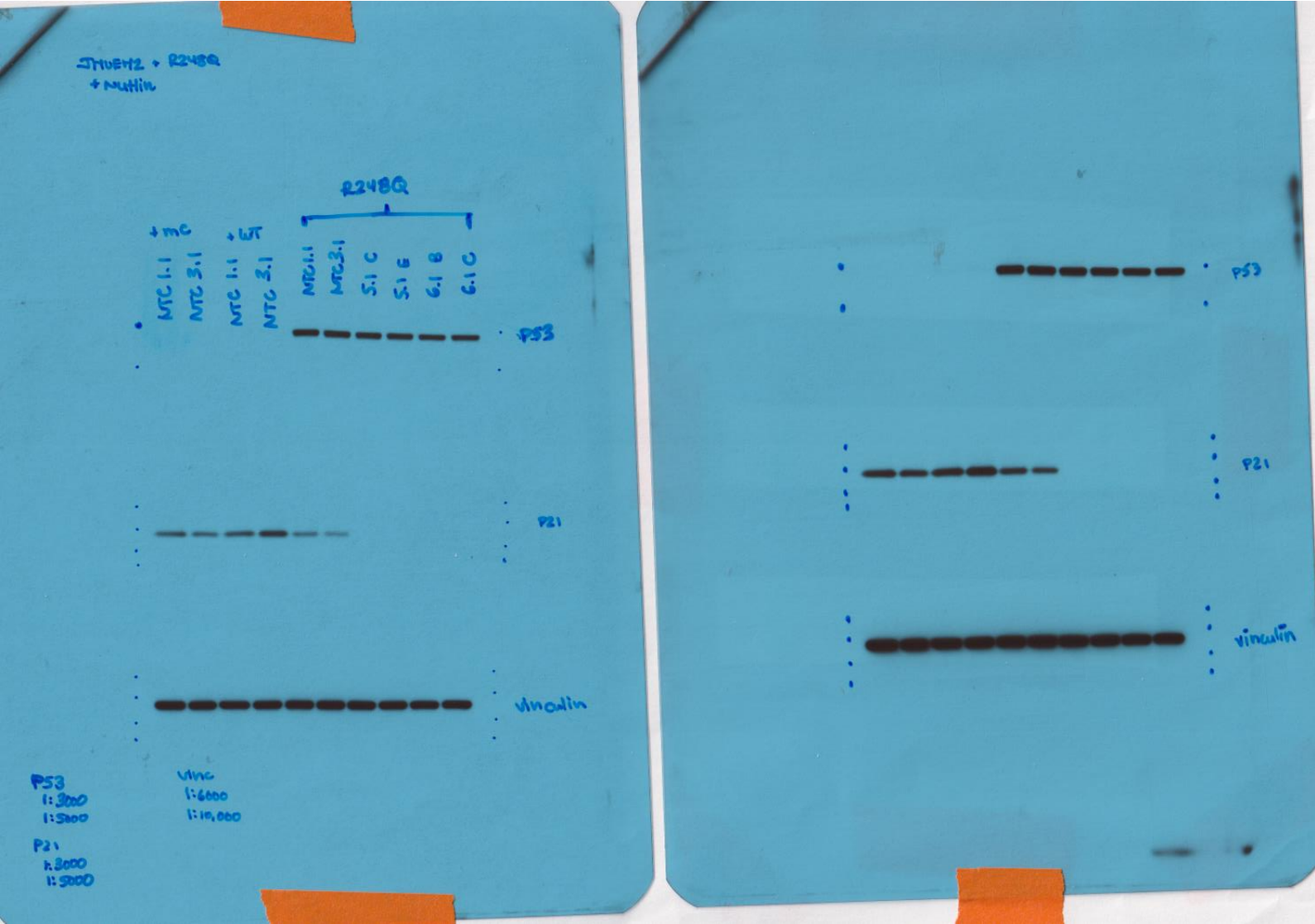


Figure 3 Panel D- (AMG-232 Response JHUEM2/Hec108/Hec1B)





Supplementary Figure 2 - R248Q



JHVEN2
AP19

7-17-20

p53
(1:3000) 50 -
37 -

+mCh		+WT		+R248Q-B					
1:1	3:1	1:1	3:1	1:1	3:1	5:1C	5:1E	6:1B	6:1C
1	1	1	1	1	1	1	1	1	1

p21
(1:3000) 25 -
20 -
15 -
10 -Vinculin
(1:5000) 50 -
150 -
100 -
75 -+10 μ M Nutlin3, 24h.JHVEN2
AP19

7-17-20

p53
(1:3000) 50 -
(Rb) (cells) 37 -

+mCh		+WT		+R248Q-B					
1:1	3:1	1:1	3:1	1:1	3:1	5:1C	5:1E	6:1B	6:1C
1	1	1	1	1	1	1	1	1	1

p21
(1:3000) 25 -
(Rb) (cells) 20 -
(1201) 15 -
10 -Vinculin
(1:5000) 250 -
(Rb) (cells) 150 -
(169V) 100 -
75 -+10 μ M Nutlin3, 24h.AP19
JHVEN2

7-17-20

p53
(1:3000) 50 -
(Rb) (cells) 37 -

+mCh		WT		+R248W-2					
1:1	3:1	1:1	3:1	1:1	3:1	5:1C	5:1E	6:1B	6:1C
1	1	1	1	1	1	1	1	1	1

p21
(1:3000) 25 -
(Rb) (cells) 20 -
(1201) 15 -
10 -Vinculin
(1:5000) 250 -
(Rb) (cells) 150 -
(169V) 100 -
75 -+10 μ M Nutlin3, 24h.AP19
JHVEN2

7-17-20

p53
(1:3000) 50 -
37 -

+mCh		+WT		+R248W-2					
1:1	3:1	1:1	3:1	1:1	3:1	5:1C	5:1E	6:1B	6:1C
1	1	1	1	1	1	1	1	1	1

p21
(1:3000) 25 -
20 -
15 -
10 -Vinculin
(1:5000) 250 -
150 -
100 -
75 -+10 μ M Nutlin3, 24h.

Supplementary Figure 2 - R273H

