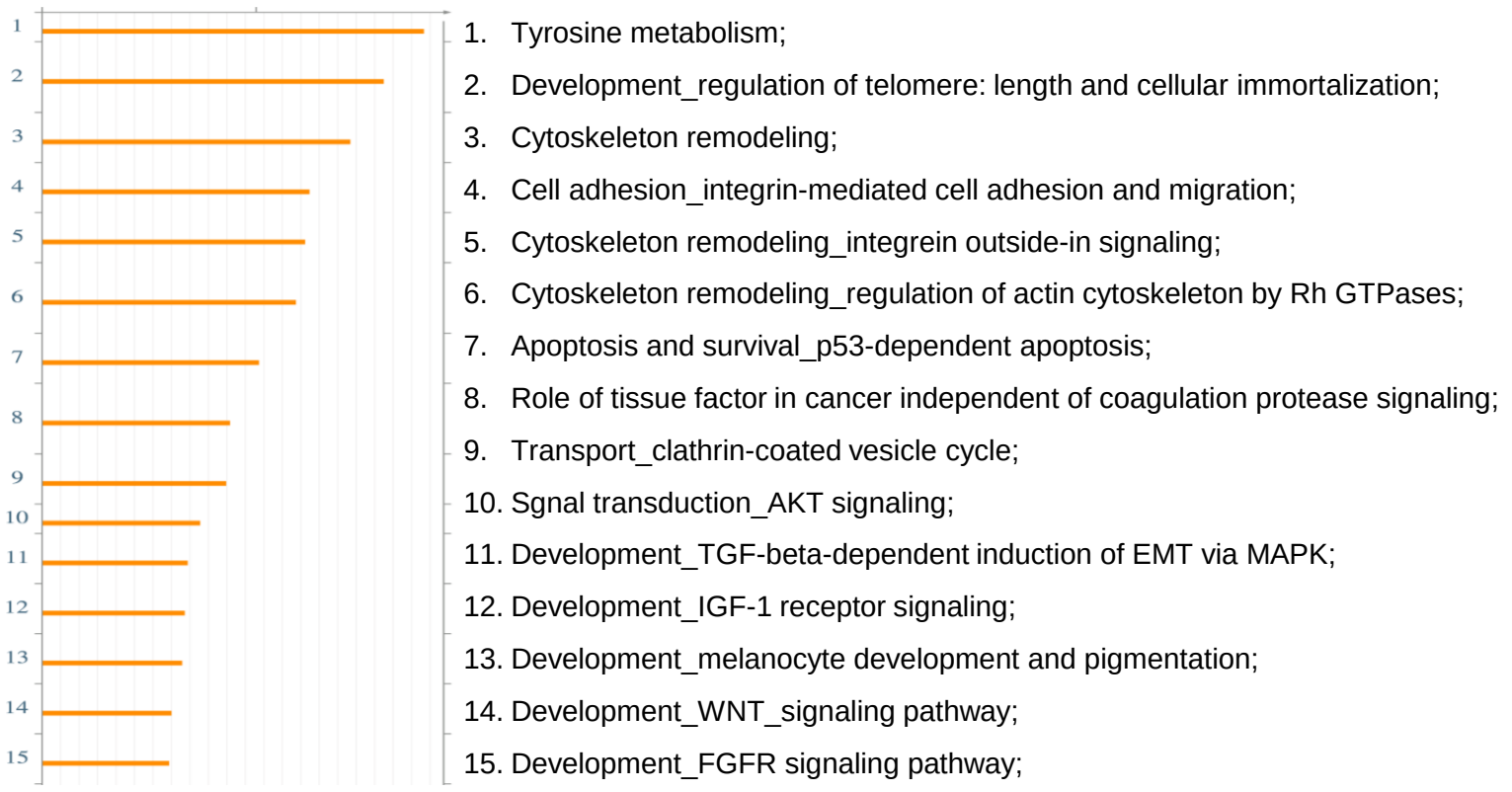
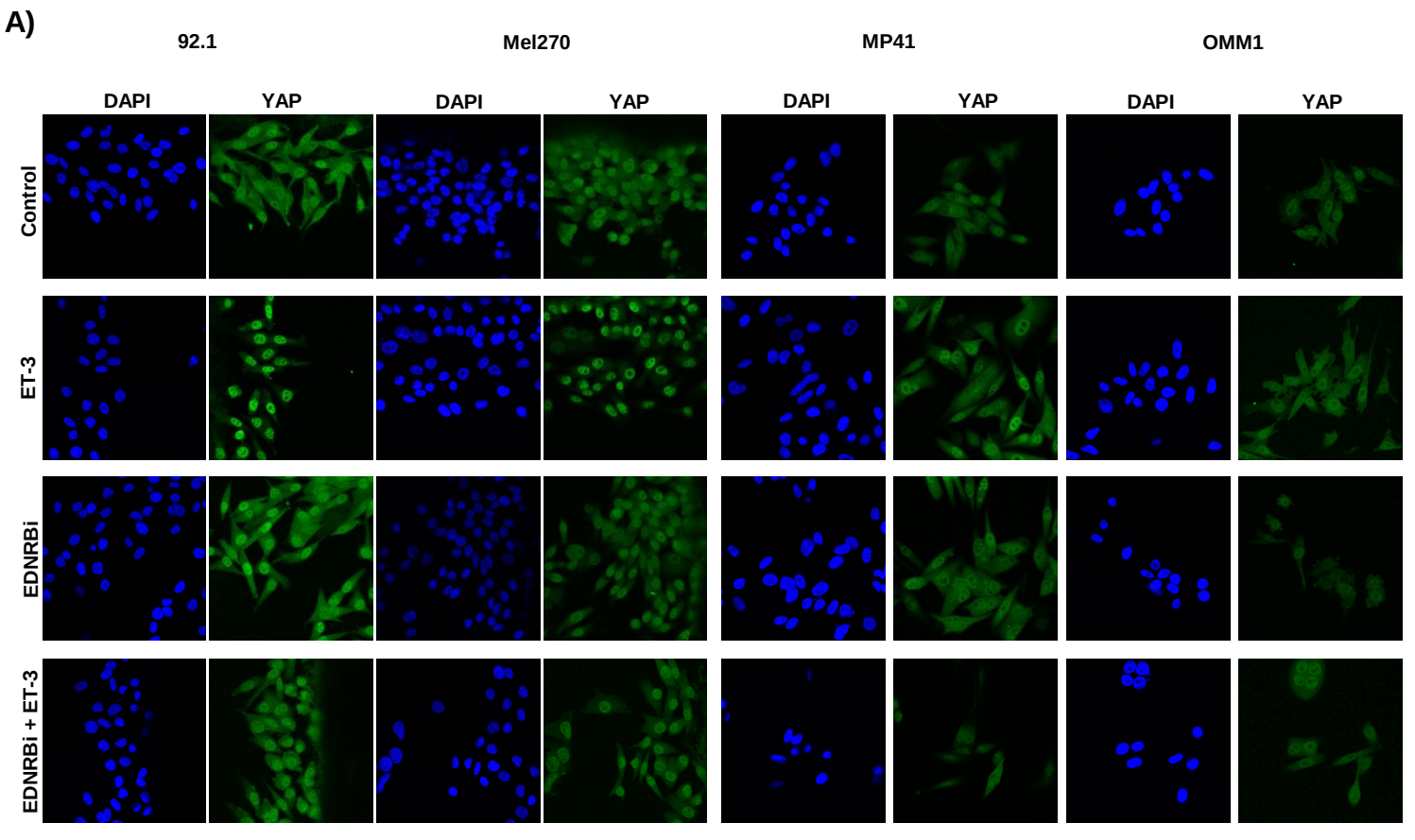
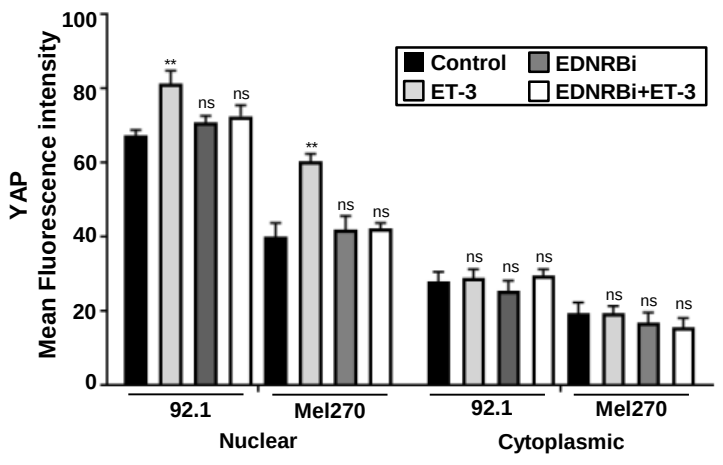
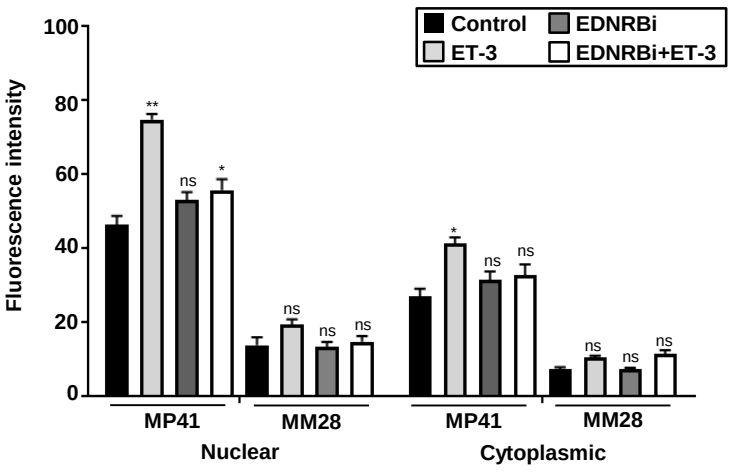


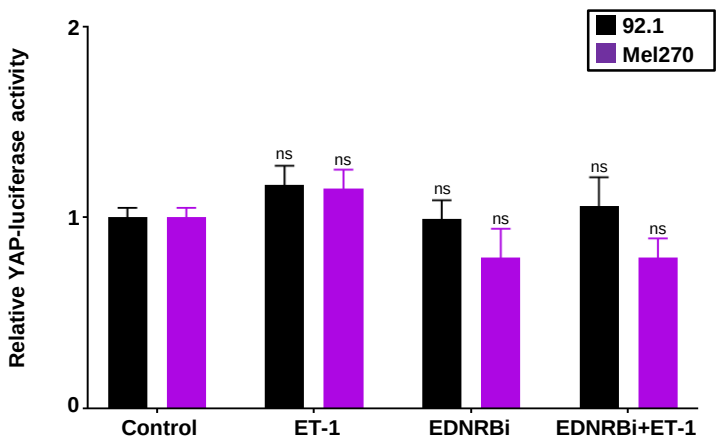
Supplemental Figure 1: Gene ontology network analysis of uveal melanoma cells following MEKi treatment (24h) and ABPP. Figure shows top 15 most significantly altered pathways.



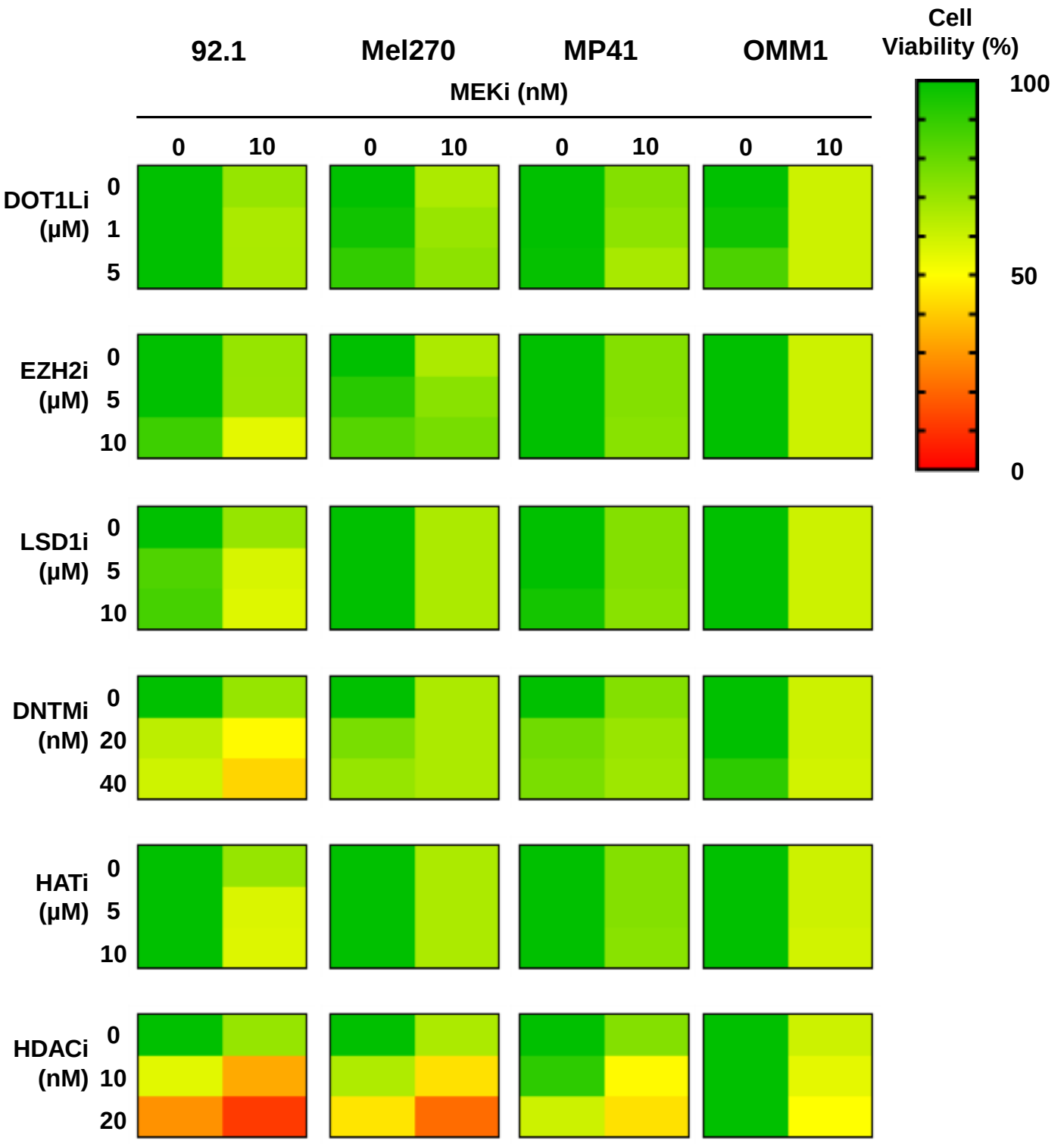




Supplemental Figure 2: ET-3 increases nuclear accumulation of YAP in 92.1, Mel270, MP41 and OMM1 cells. A) Cells were treated with either ET-3 (100nM, 1 hr), EDNRB antagonist (bosentan, 80μM) or ET-3 + bosentan (100nM and 80μM, respectively). Cells were fixed and stained for YAP. **B)** and **C)** Quantification of nuclear/cytoplasmic YAP staining from A).

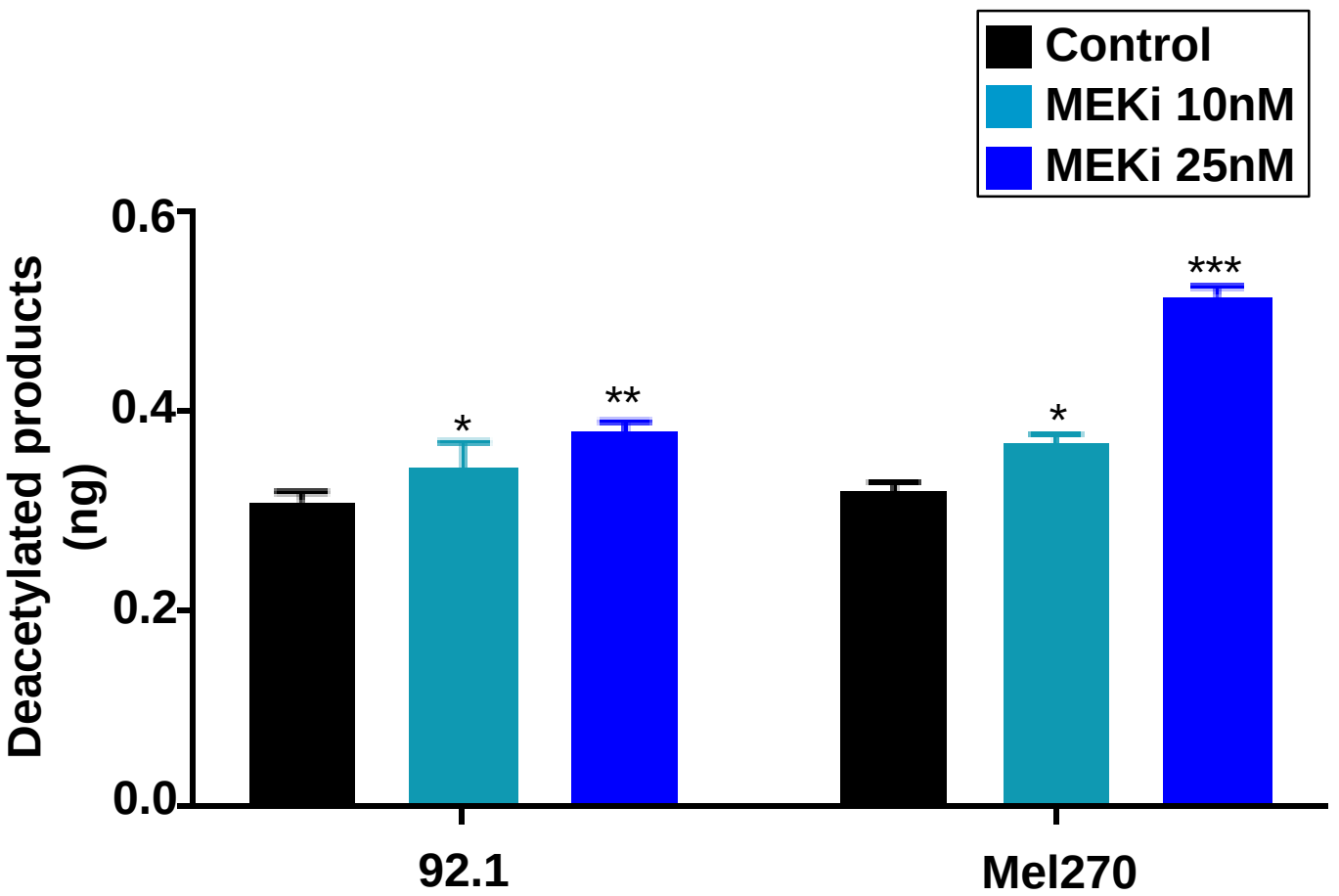


Supplemental Figure 3: ET-1 does not induce YAP reporter activity in 92.1 or Mel270 cells. Cells were treated with either ET-1 (100nM, 1 hr), EDNRB antagonist (bosentan, 80µM) or ET-1 + bosentan (100nM and 80µM, respectively).

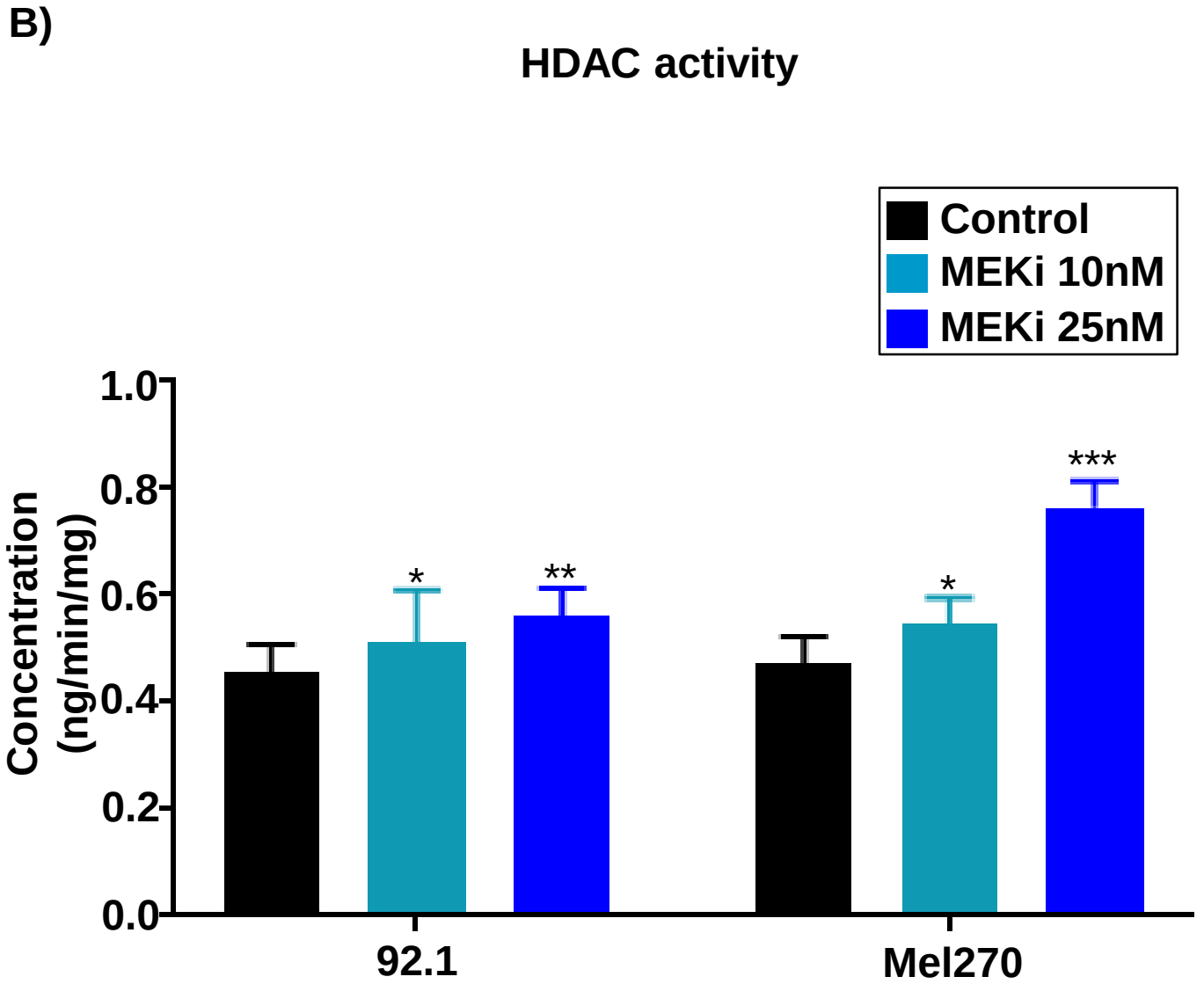


Supplemental Figure 4: HDAC inhibitors increase the cytotoxic effects of MEK inhibition. Data show heatmaps showing the inhibition of the growth of uveal melanoma cell lines (92.1, MP41, Mel270 and OMM1) treated with of a MEK inhibitor (trametinib, 10nM) in combination with inhibitors of DOT1 (EPZ5676), EZH2 (Tazemetostat), LSD1 (GSK 2879552), DNMT (decitabine), HAT (anacardic acid) and HDAC (panobinostat). Cells were treated with vehicle, epigenetic inhibitors (0-10 μM), MEK inhibitor (trametinib, 10nM) or the combination for 72 h before being subjected to the MTT assay.

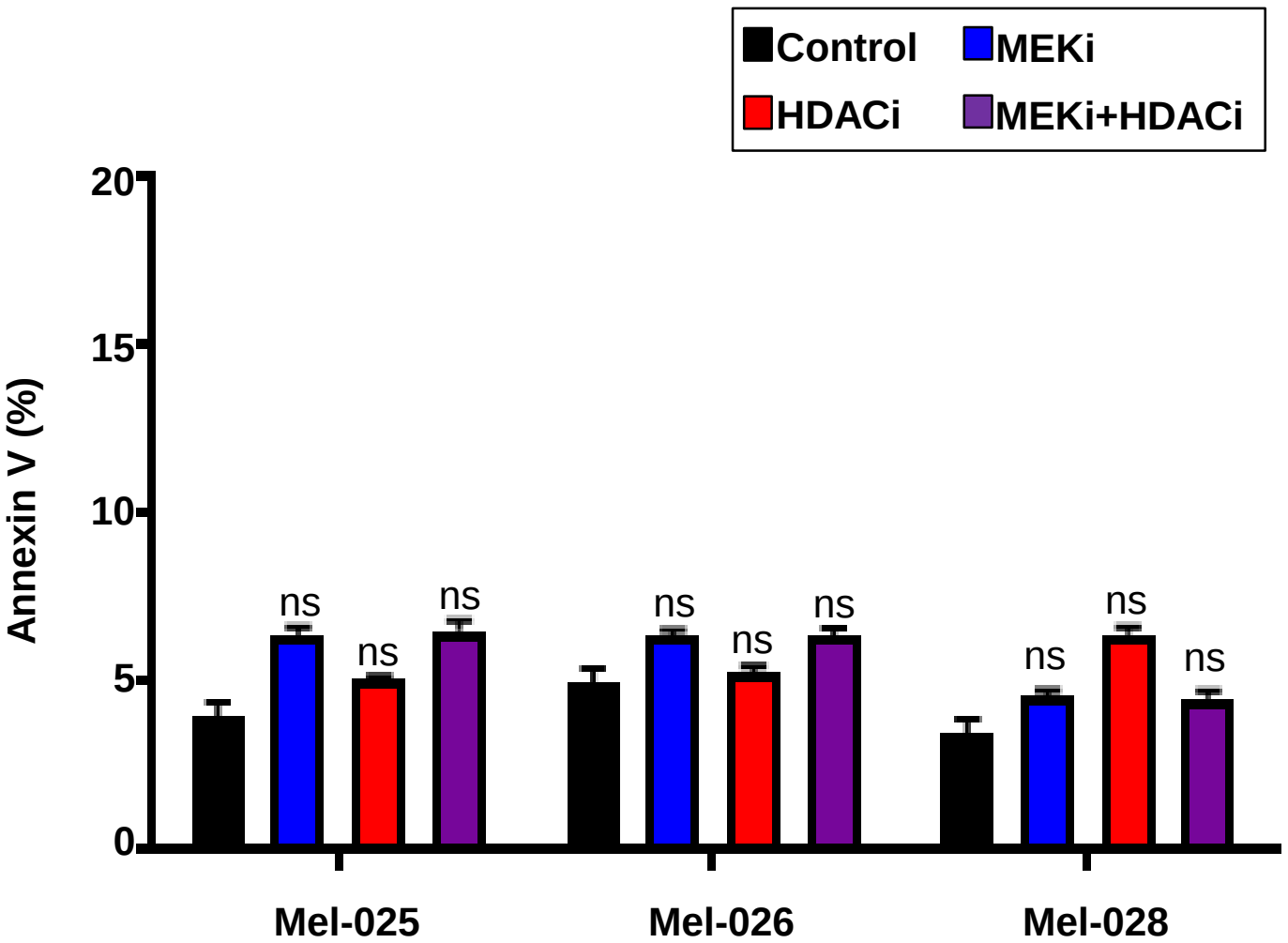
A)



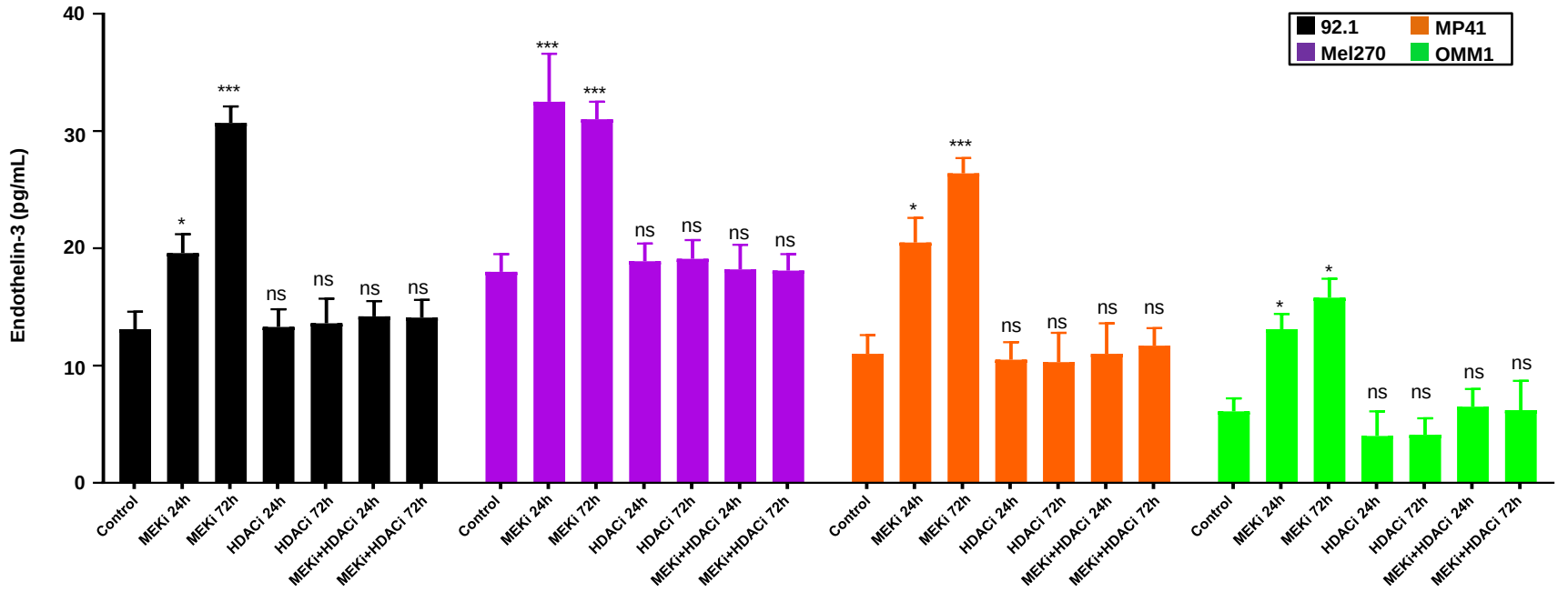
B)



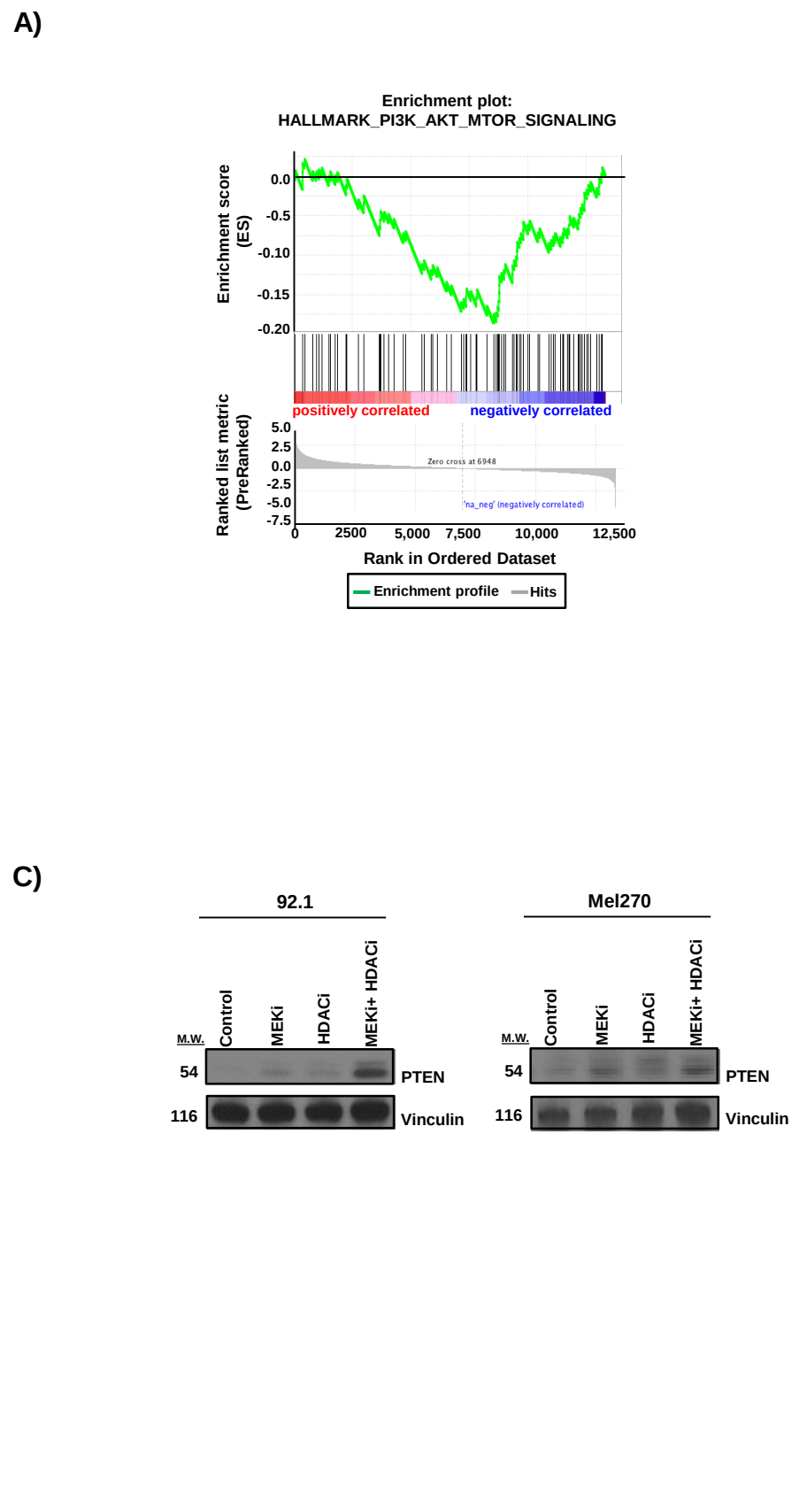
Supplemental Figure 5: MEK inhibition leads to an increase in global protein acetylation in uveal melanoma cells. 92.1 and Mel270 cells were treated for 72 h with trametinib (10 and 25 nM) before being assayed for total protein acetylation and HDAC activity.



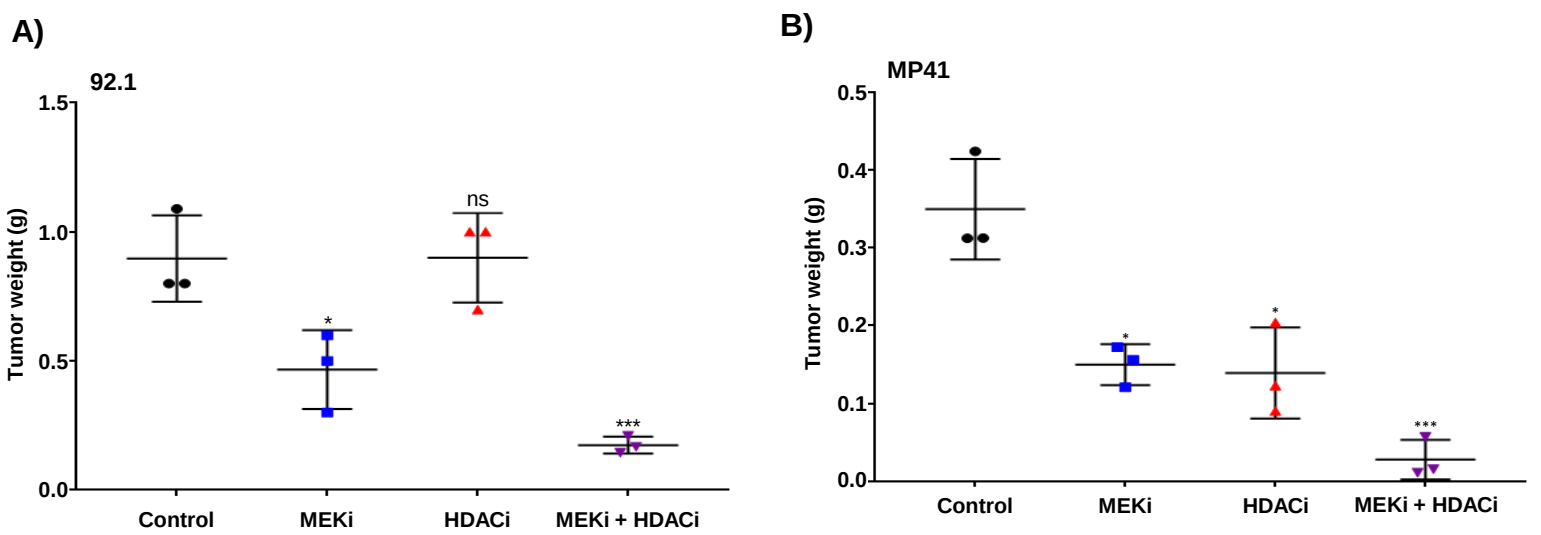
Supplemental Figure 6: MEKi, HDACi and the MEKi-HDACi combination do not affect the survival of primary uveal melanocytes. 3 uveal melanocyte lines (MEL-025, MEL-206, MEL-028) were treated with vehicle, MEKi (trametinib,10nM), HDACi (panobinostat, 10nM) or the combination for 72 h. Apoptosis was measured by FITC-Annexin-V binding and flow cytometry.



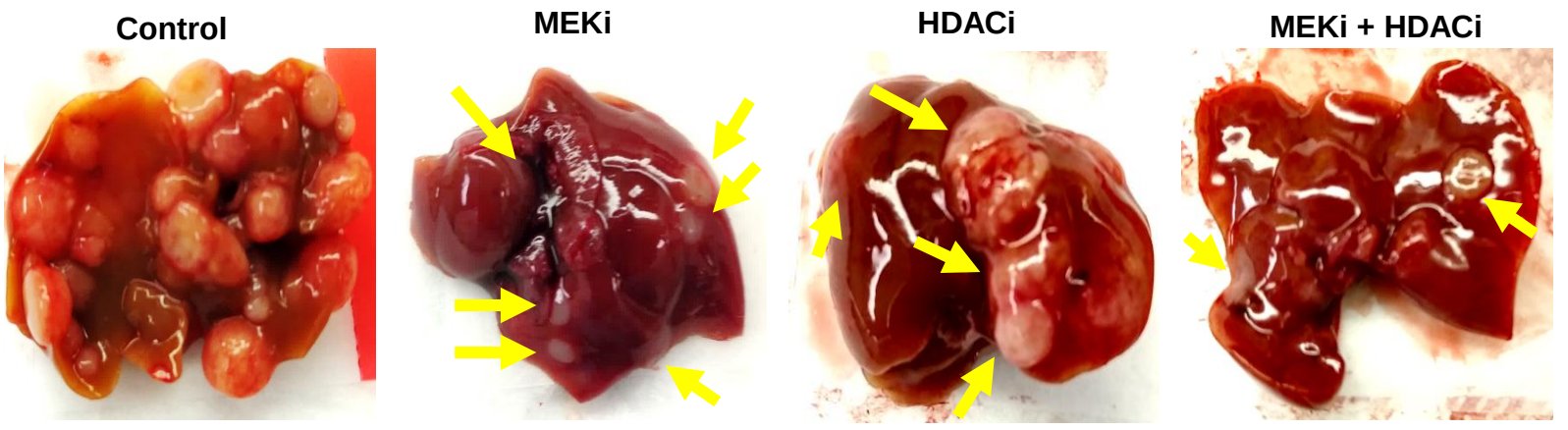
Supplemental Figure 7: HDACi suppresses MEKi-induced release of ET-3 from uveal melanoma cells. Uveal melanoma cell lines were treated with vehicle, MEKi (trametinib,10nM), HDACi (panobinostat, 10nM) or a combination of the two drugs for 24 or 72 h. ET-3 concentrations were measured by ELISA.



Supplemental Figure 8: Negative modulation of PI3K-AKT pathway by combinatory treatment with MEKi+HDACi. A) GSEA analysis of RNA-Seq experiments of the comparison between MEKi-treated only and MEKi+HDACi-treated uveal melanoma cells (24h) shows a decrease in PI3K-AKT-mTOR pathway activity. **B)** Heatmap shows the comparison between MEKi vs. Control, HDACi vs. Control and MEKi+HDACi vs. MEKi only. **C)** MEKi+HDACi combination increases expression of PTEN in uveal melanoma cells by Western Blot.



Supplemental Figure 9: The MEK-HDACi combination delivers durable responses in the 92.1 uveal melanoma xenograft model. After subcutaneous inoculation and formation of palpable tumors of 92.1 or MP41 cells, mice were treated with vehicle (Control group), MEKi (Trametinib, 1mg/kg po daily), HDACi (Panobinostat, 20mg/kg, IP, 3X week) or the combination for 31 days. Data shows final mean tumor weights from each group **A)** 92.1 cell line and **B)** MP41 cell line.



Supplemental Figure 10: Macroscopic findings of liver tumor formation after inoculation of MP41 by tail vein injection. Pictures were taken after 7 weeks (4 weeks until tumors were visible on MRI and then 3 weeks of treatment with vehicle (Control group), MEKi (Trametinib, 1mg/kg po daily), HDACi (Panobinostat, 20mg/kg, IP, 3X week) or the combination. Multiple foci of liver metastases are indicated (yellow arrows).

Figure 2C

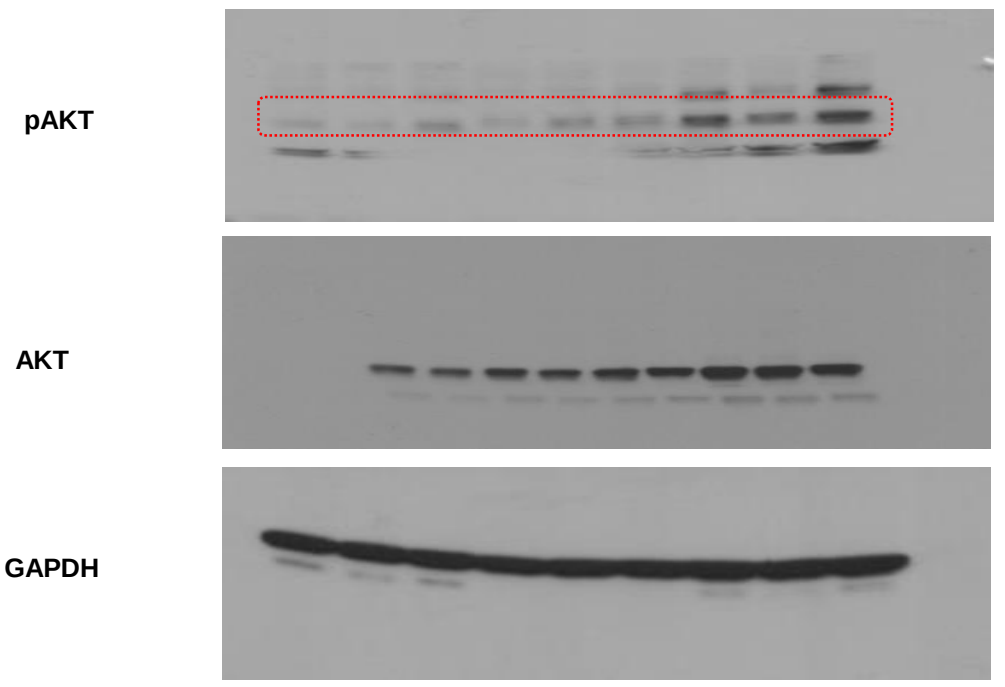


Figure 3D

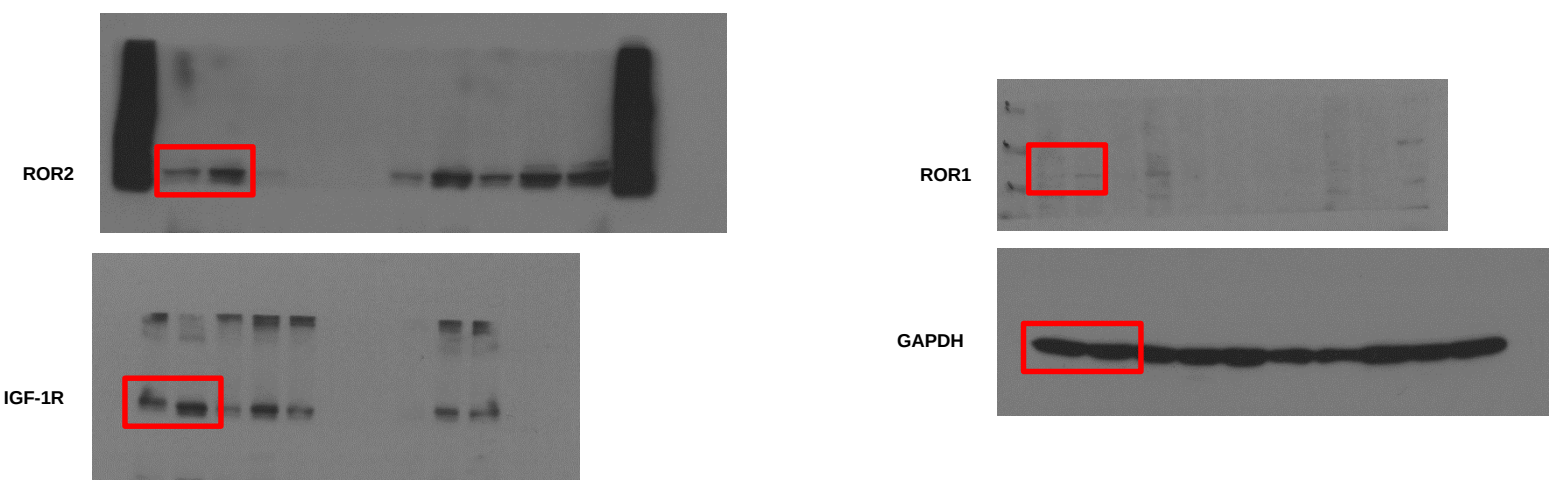
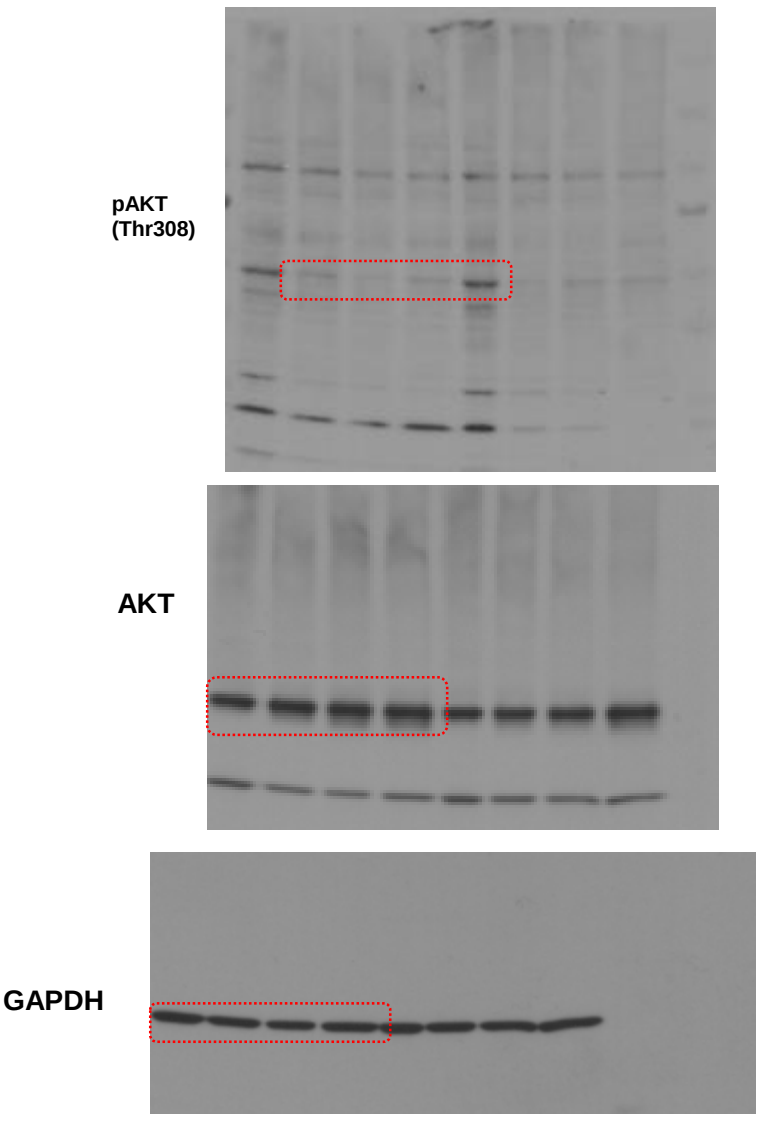


Figure 3E

92.1



Supplemental Figure 11: Uncropped blots

Figure 3F

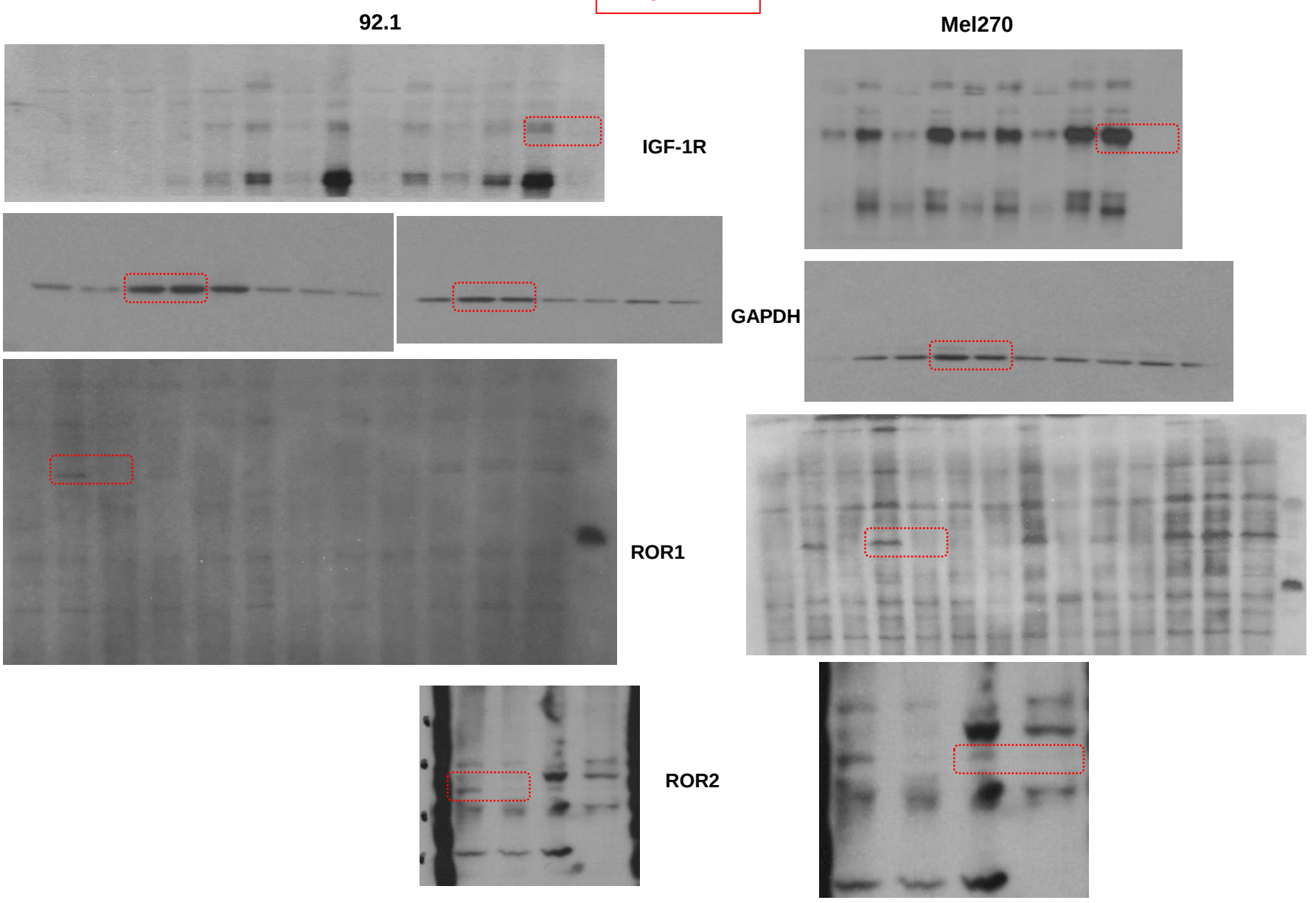
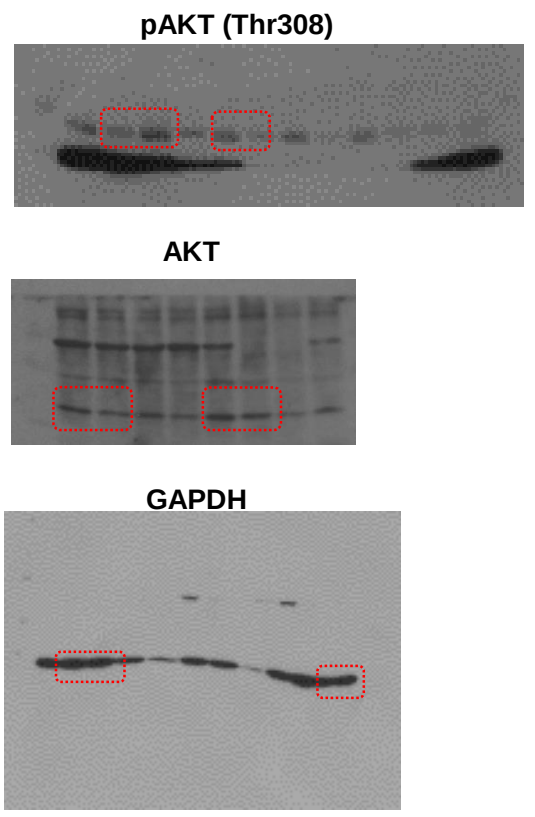


Figure 3G



Supplemental Figure 11: Uncropped blots

Figure 4F

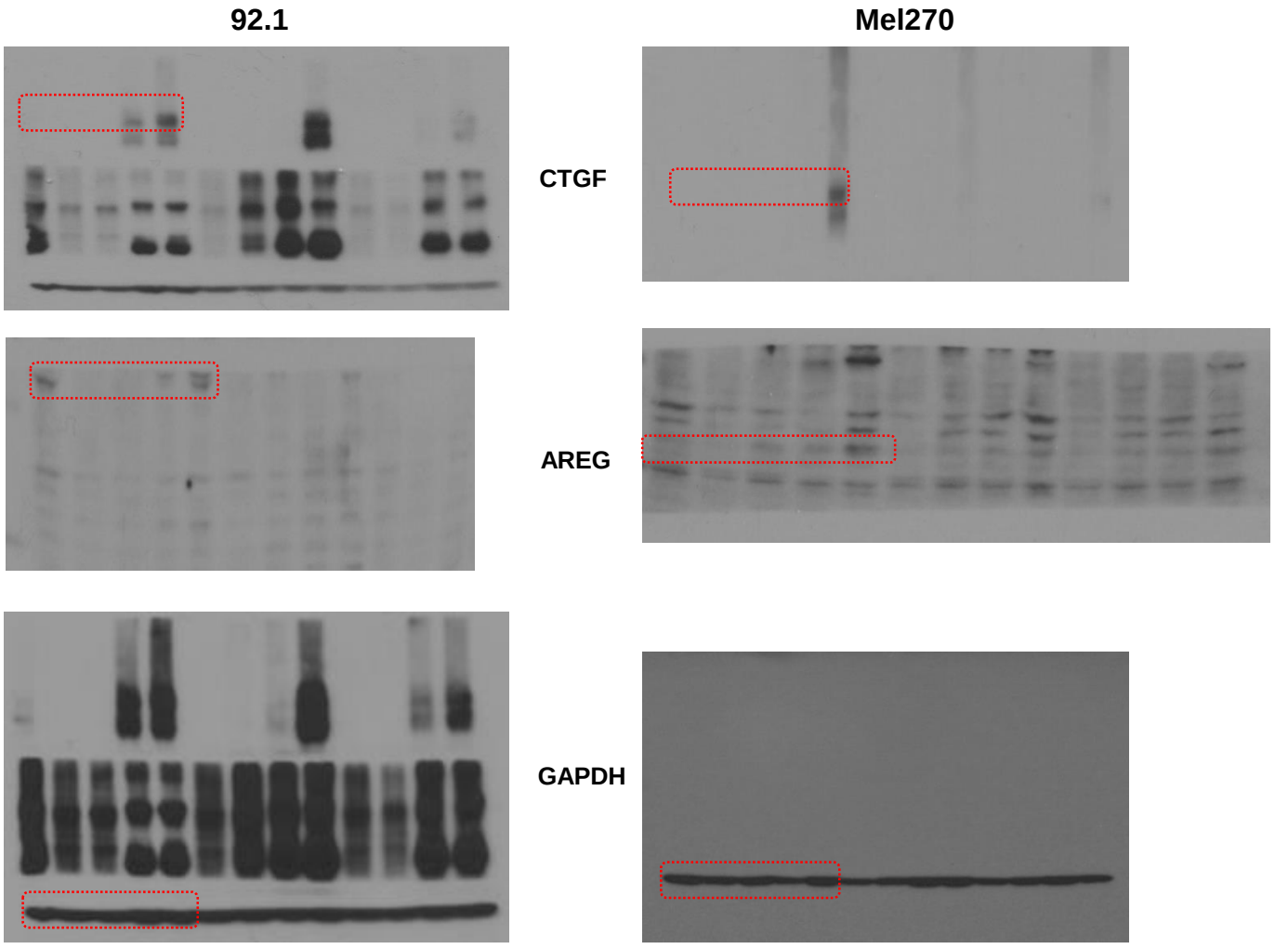
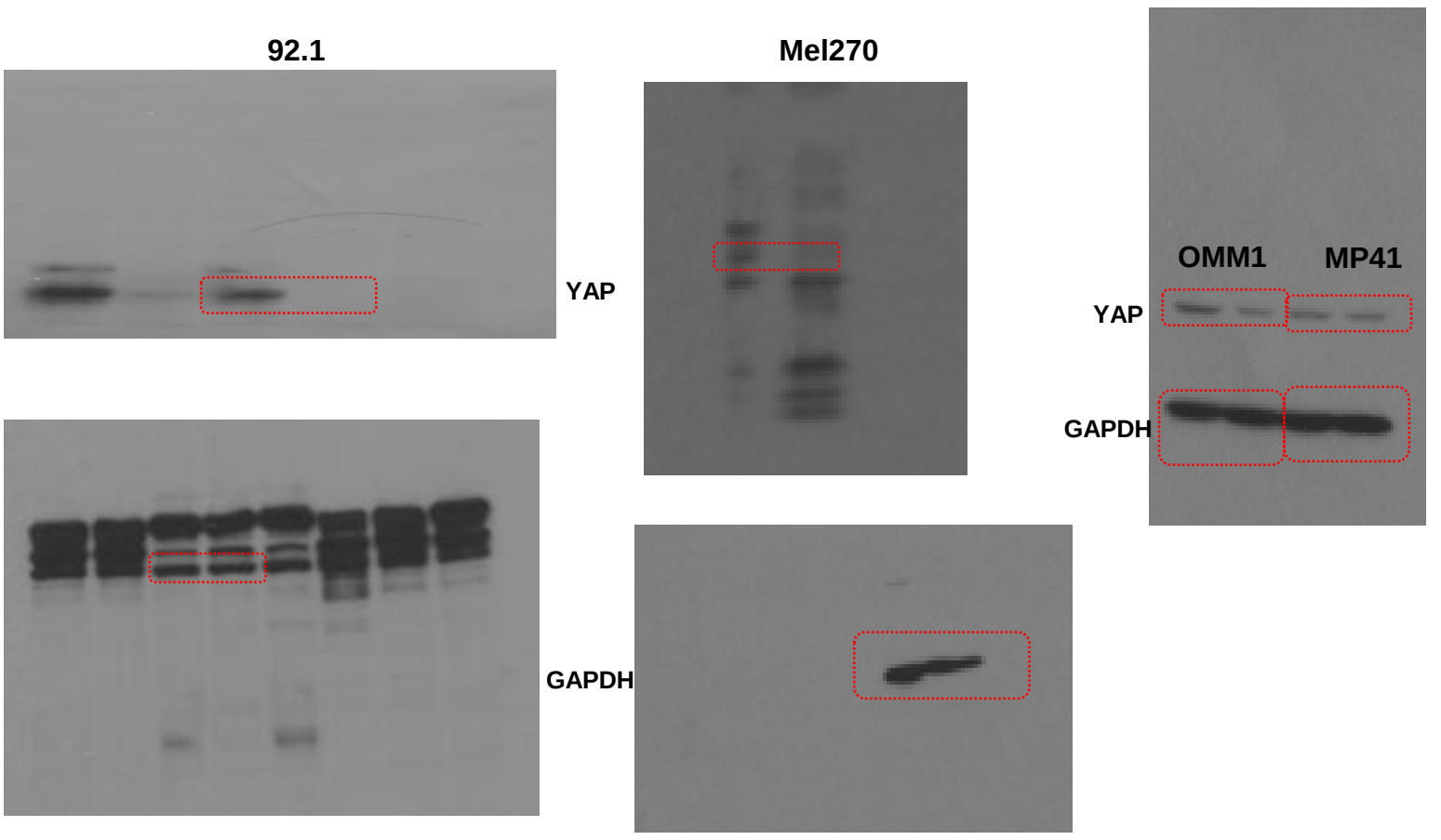


Figure 4I



Supplemental Figure 11: Uncropped blots

Figure 6G

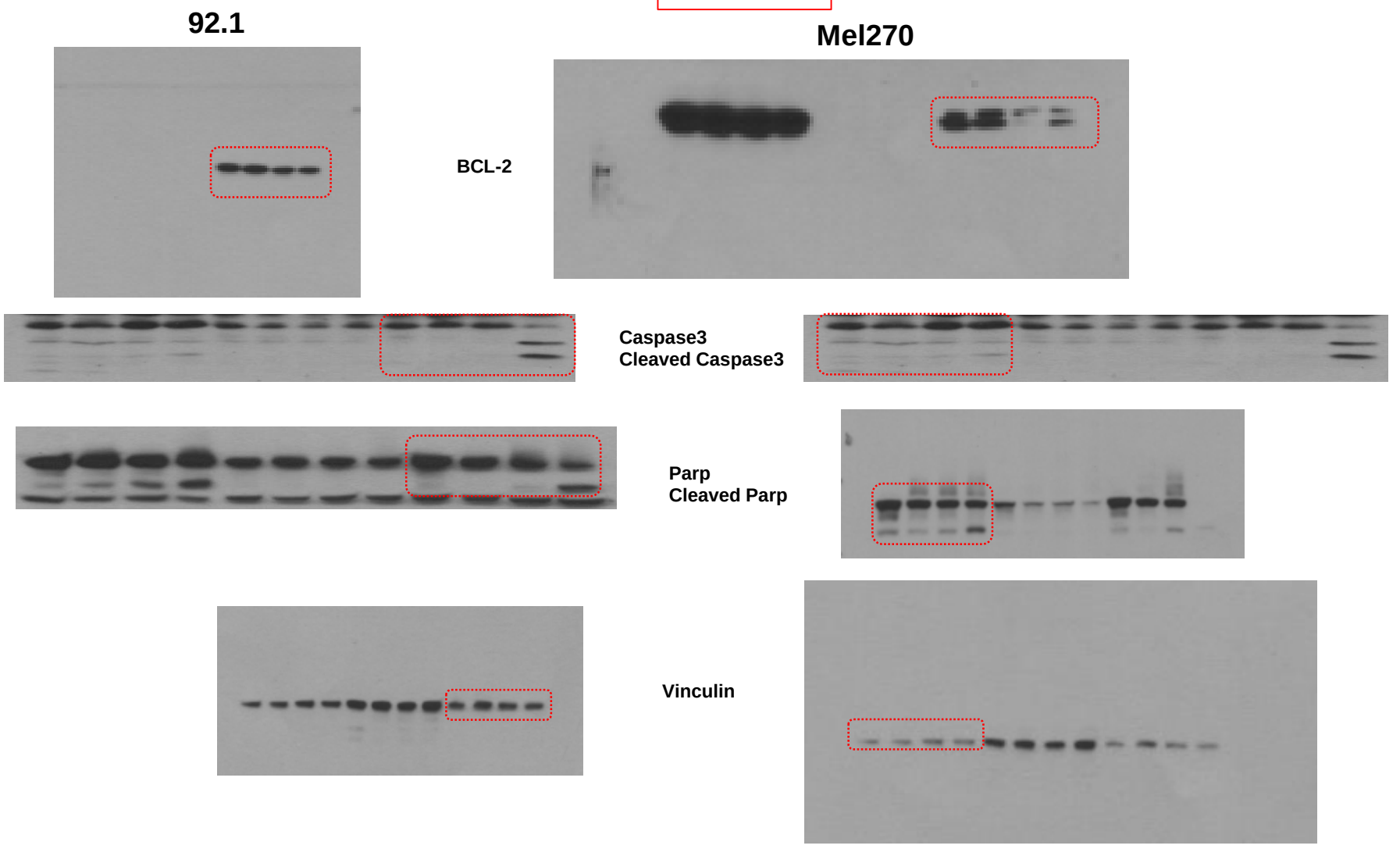
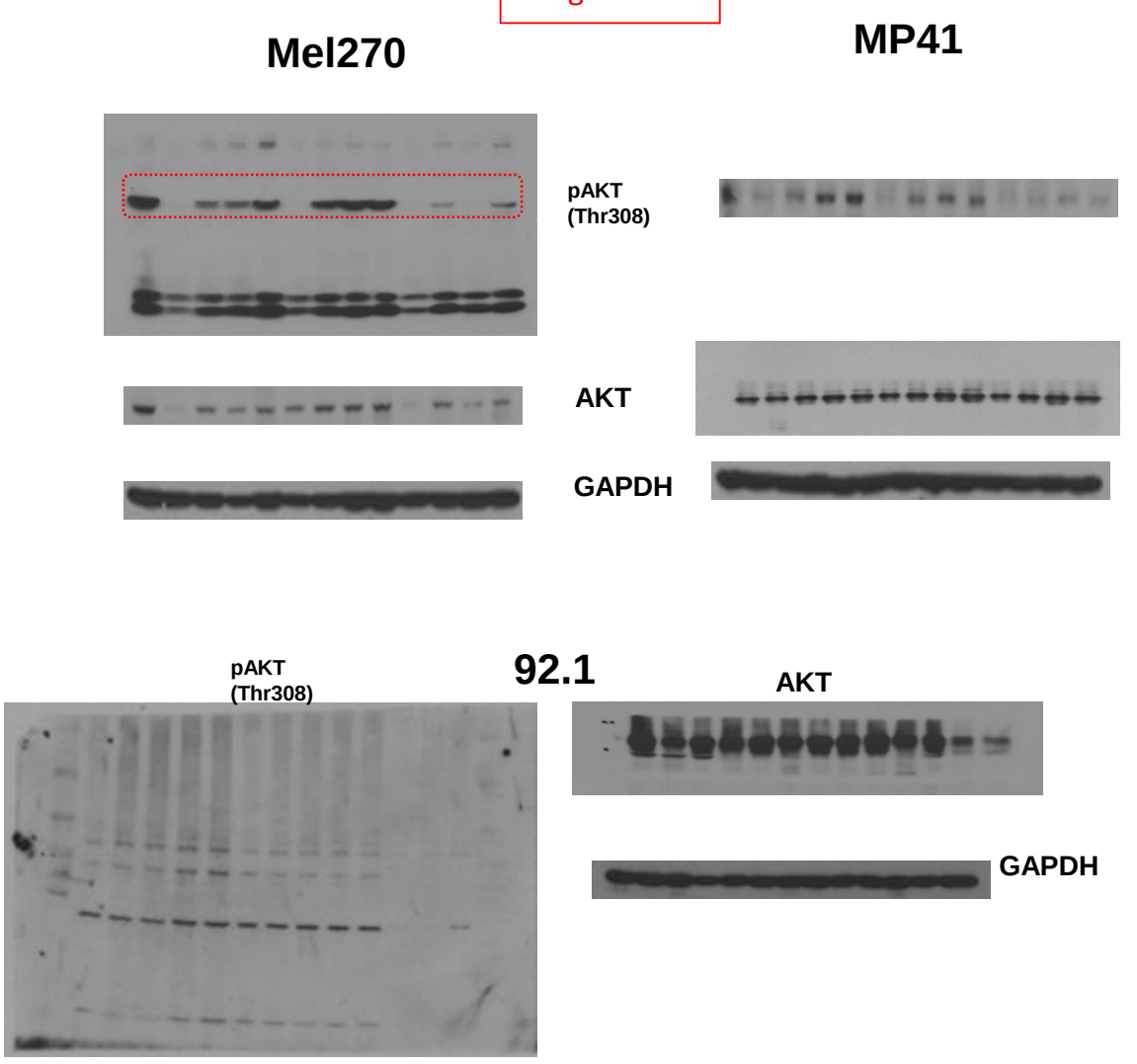


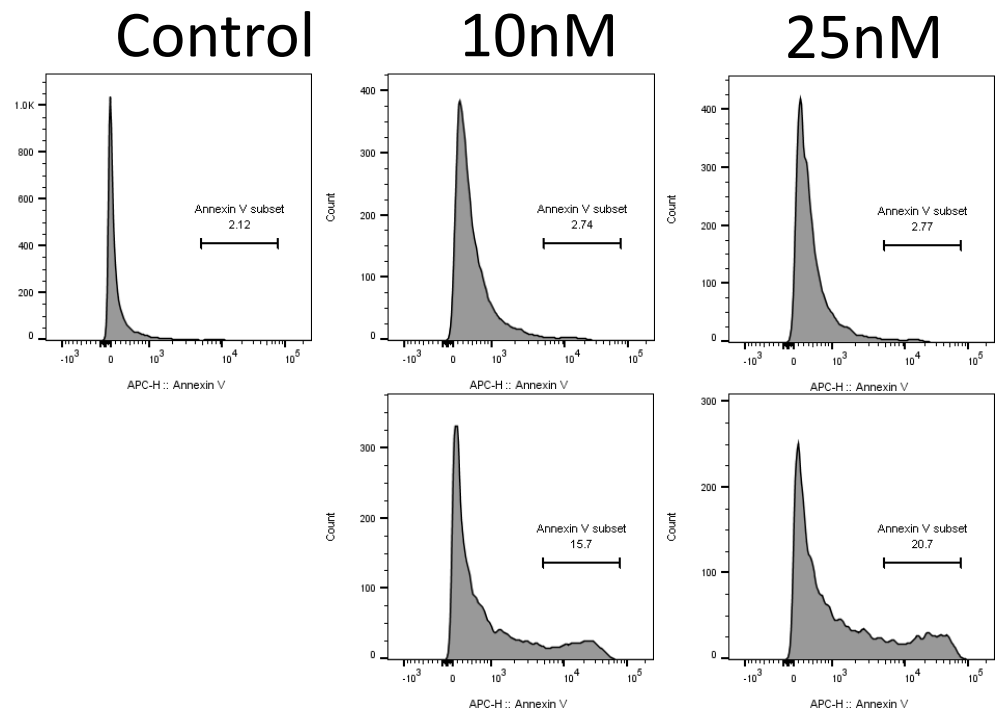
Figure 7A



Supplemental Figure 11: Uncropped blots

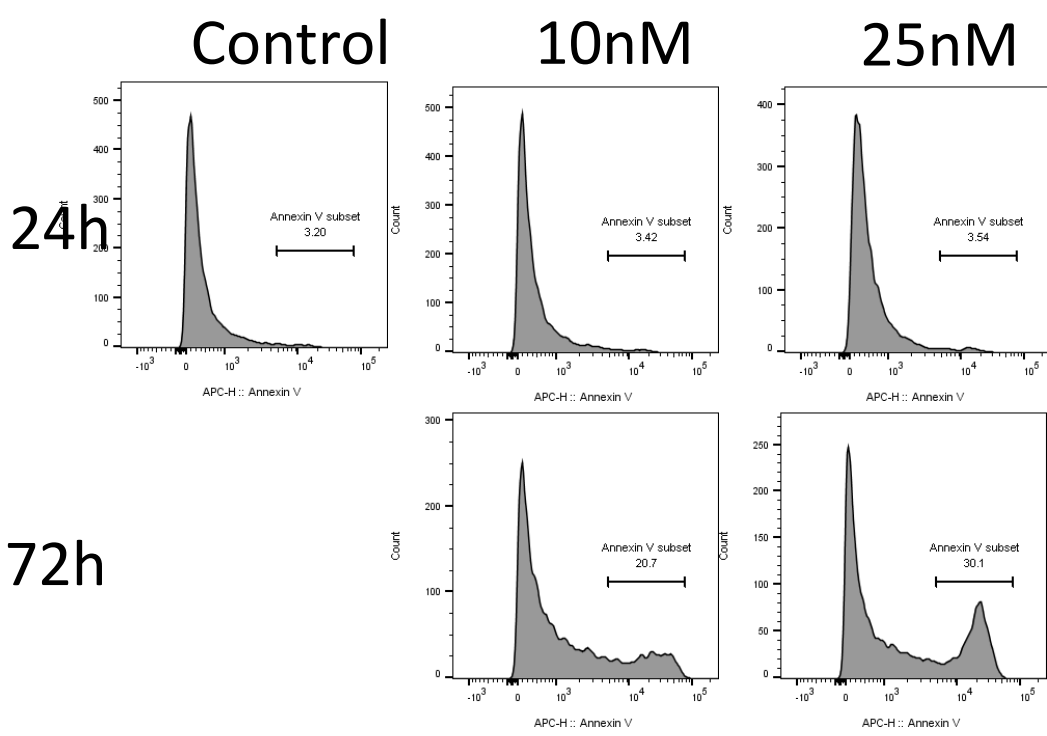
92.1

Figure 1D



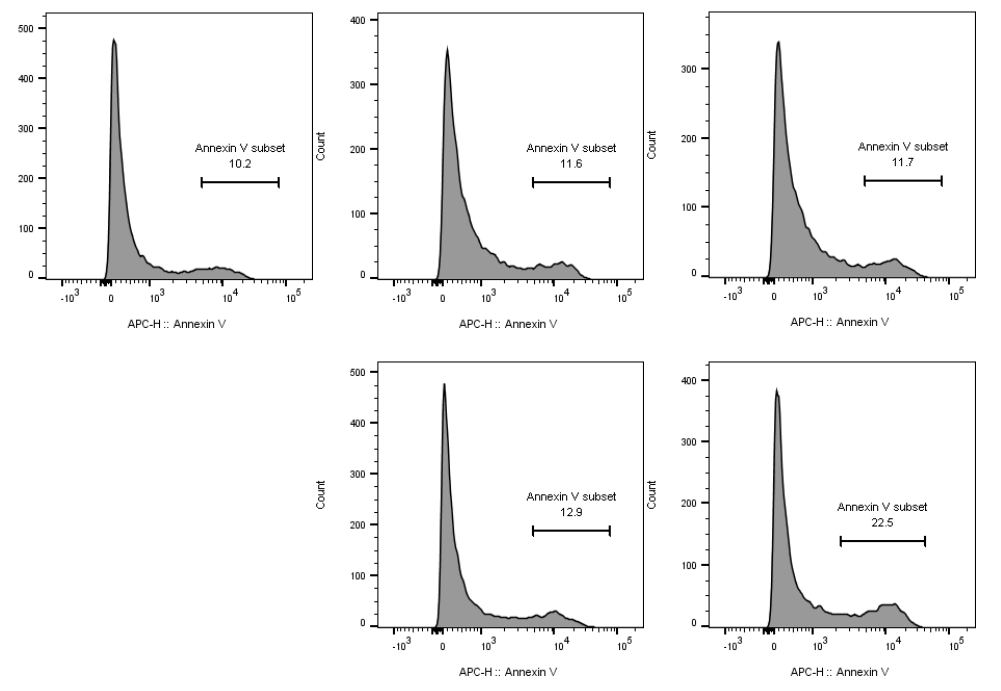
MP41

Figure 1D



Mel270

Figure 1D



OMM1

Figure 1D

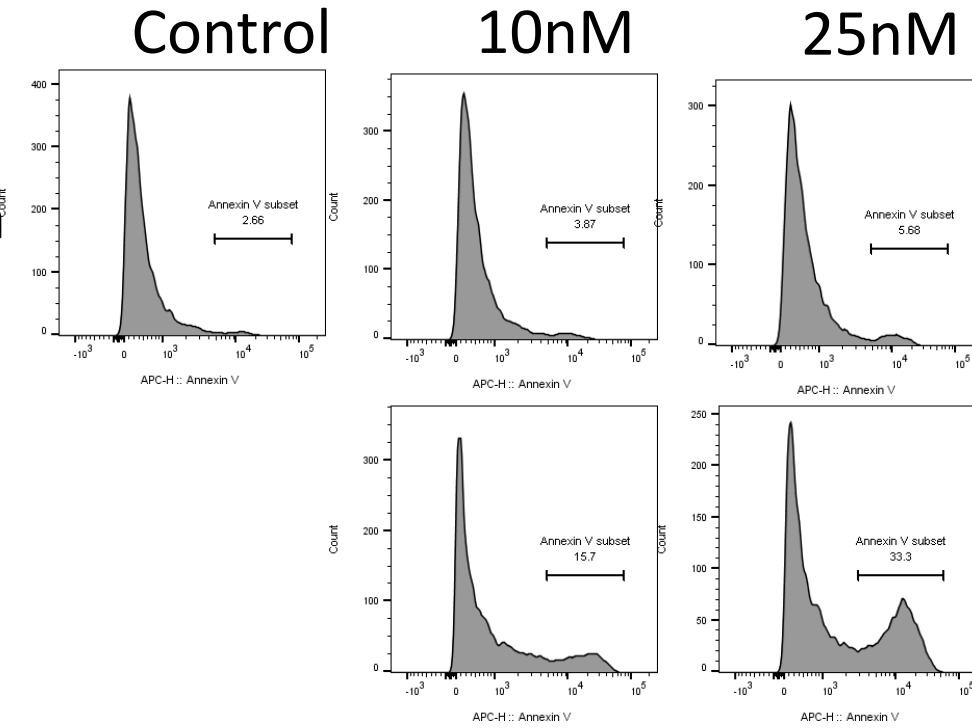
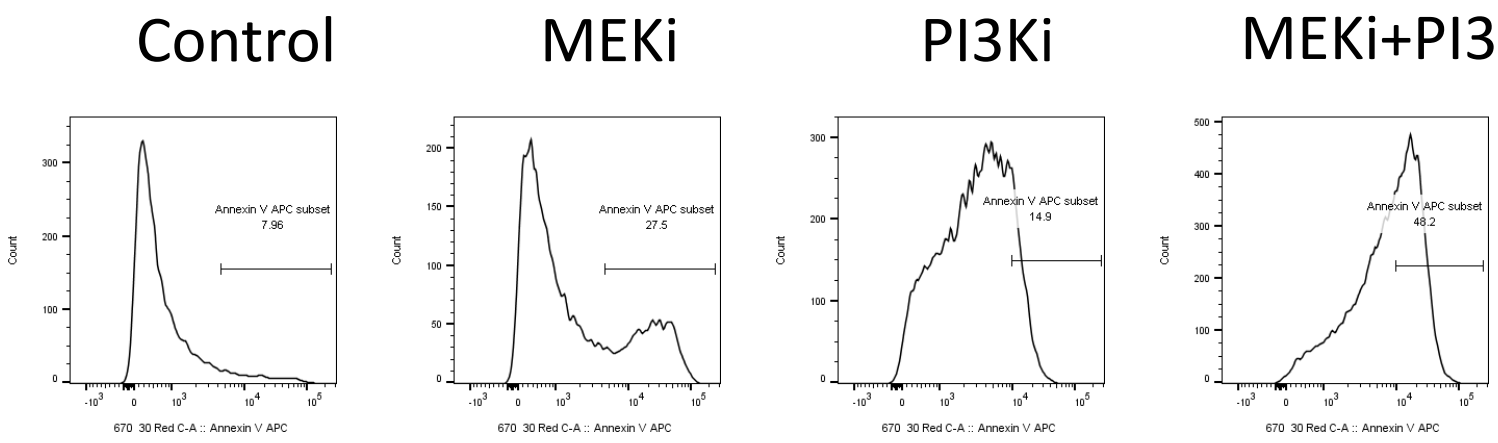
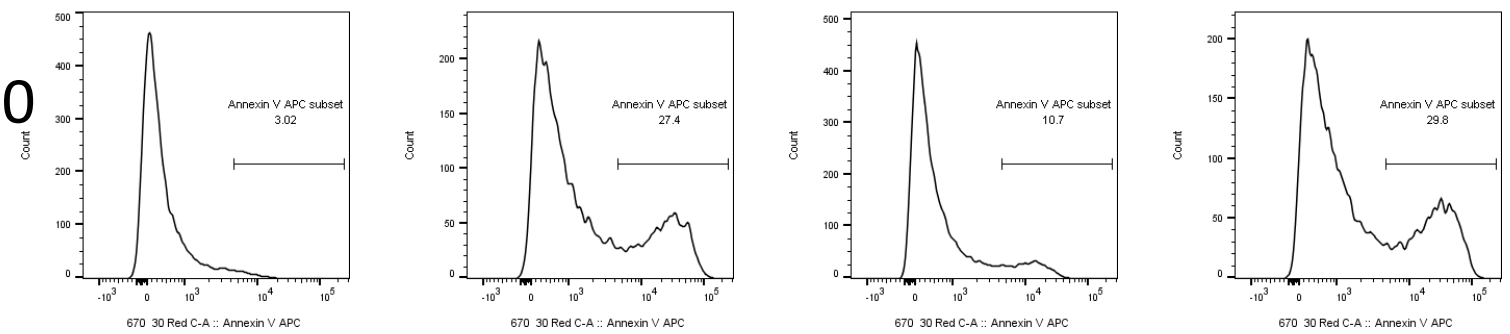


Figure 2D

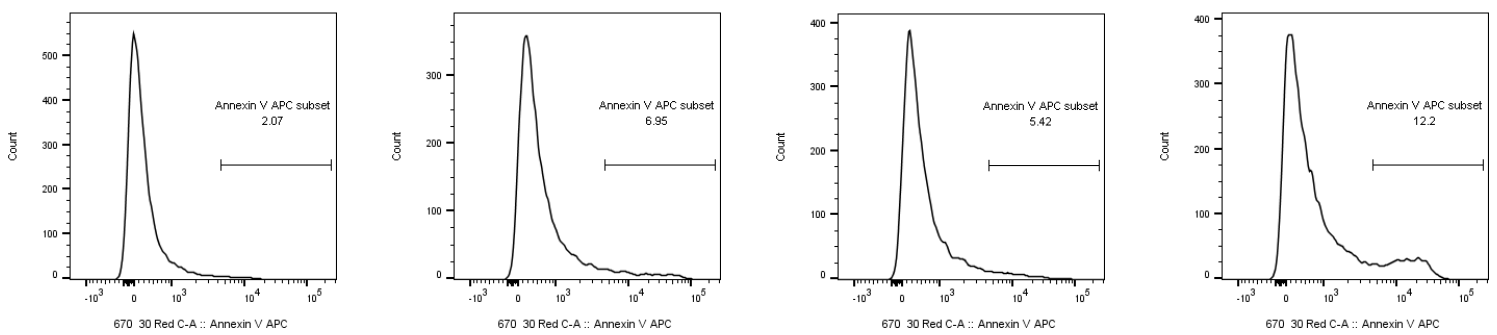
92.1



Mel270



MP41



Supplemental Figure 12: Flow cytometry plots

Figure 3H

siControl

siROR1/2

siIGF-1R

Control

MEKi

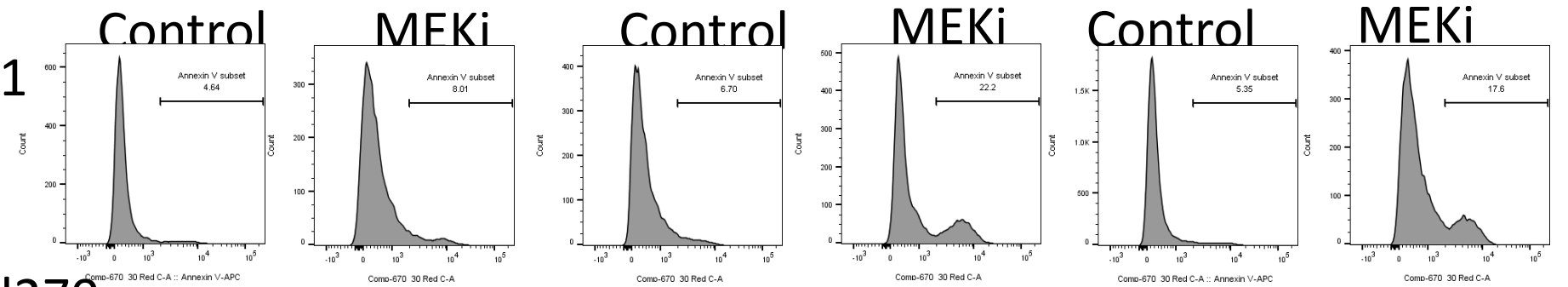
Control

MEKi

Control

MEKi

92.1



Mel270

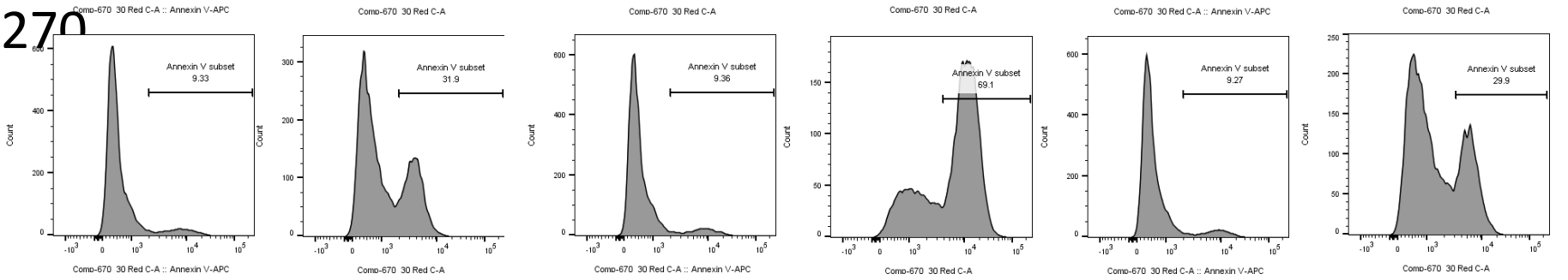


Figure 4J

siControl

siYAP

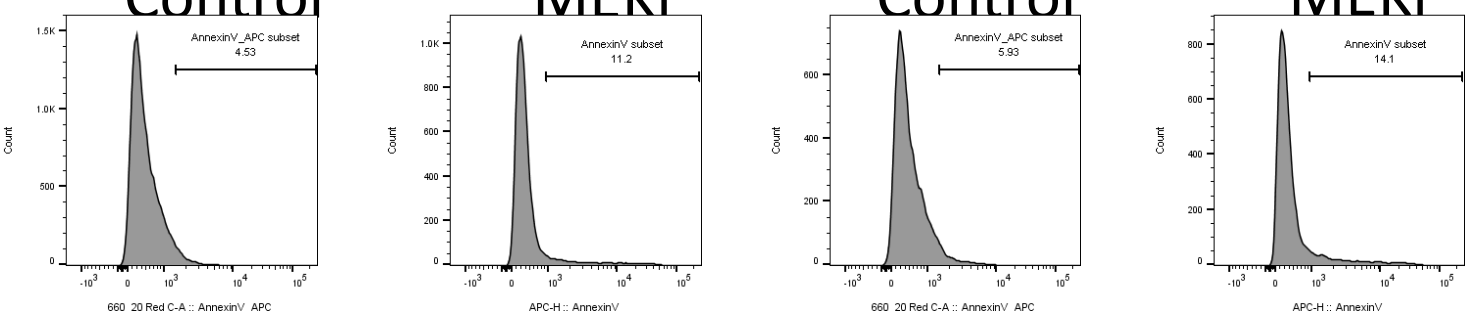
Control

MEKi

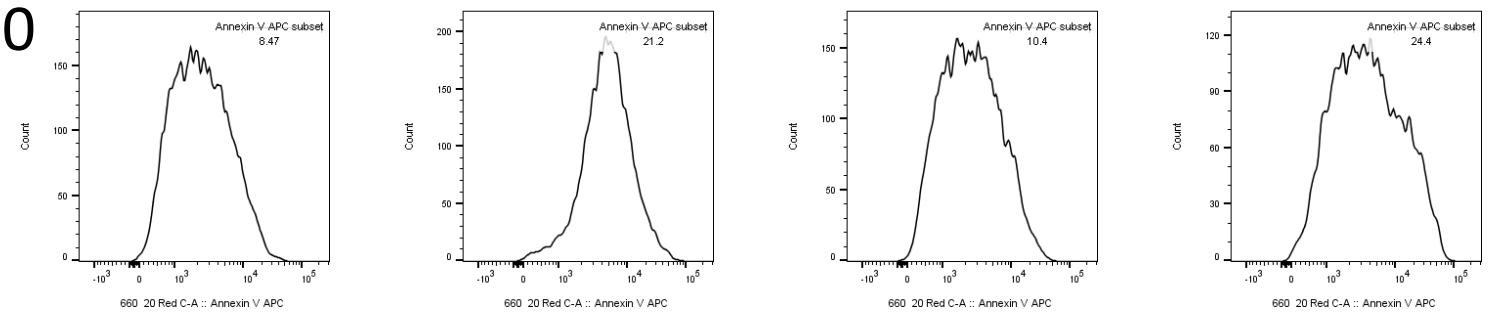
Control

MEKi

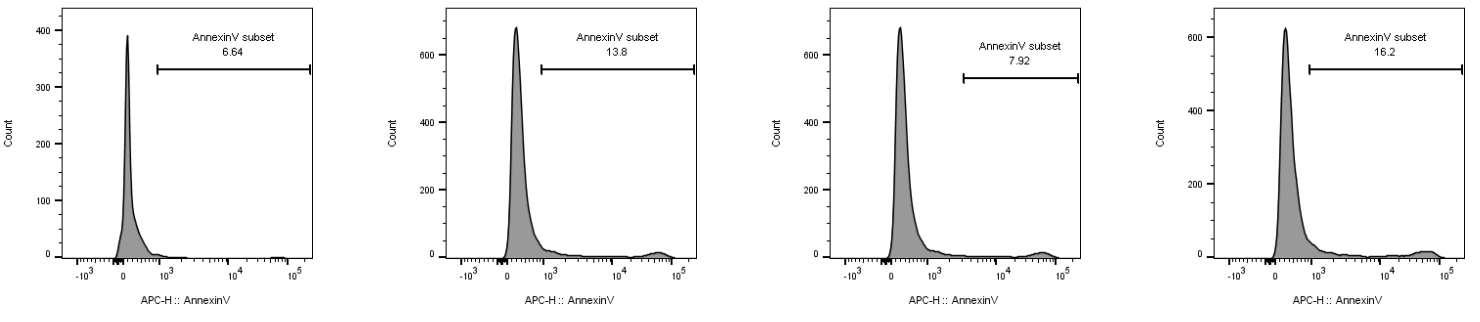
92.1



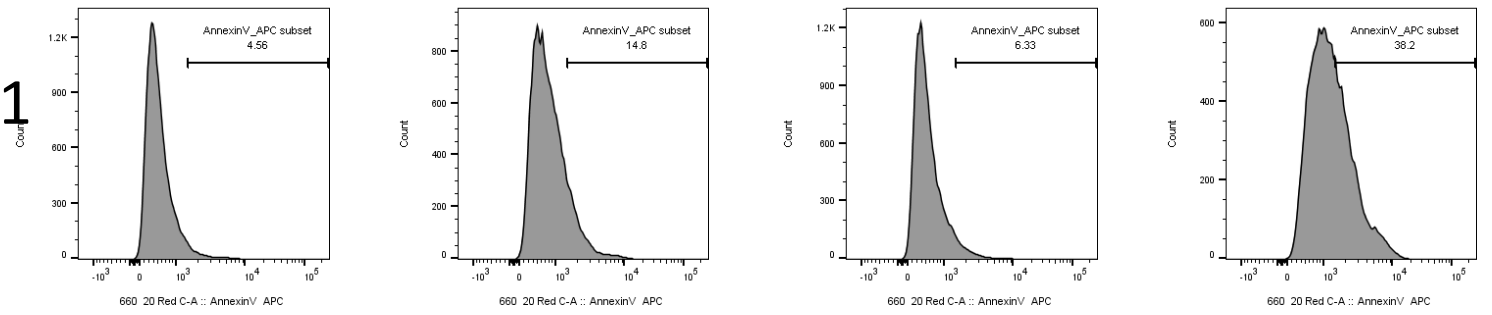
Mel270



MP41



OMM1



Supplemental Figure 12: Flow cytometry plots

Figure 6F

Supplemental Figure 12: Flow cytometry plots