

Hypoxic & Reperfusion Responses are Selectively Altered During Non-Small Cell Lung Cancer Progression

INTRODUCTION

- Solid tumors undergo repeated cycles of hypoxia & reperfusion (h/r)
- These cycles occur hundreds to thousands of times & may function as a selective pressure mechanism influencing genomic & epigenetic alterations
- Studies have indicated that such selective pressure is exerted on specific oncogenic signaling pathways (i.e. p53 & PTEN)
- No systematic studies have examined acute hypoxia in specific pathway activation, alteration or termination
- This study is designed as a pilot to determine if the phosphoproteome, ubiquitylproteome & acetylproteome of normal bronchial epithelia (NBE) differs from that of non-small cell lung cancer (NSCLC) under hypoxic & normoxic conditions
- Our hypothesis is that acute hypoxia results in the specific selection of certain kinase-regulated pathways allowing tumor growth in a harsh microenvironment
- Future studies will verify that such alterations convey a selective growth &/or survival benefit by testing if cycles of acute hypoxia in NBE lead to genetic patterns of post-translational modification (PTM) seen in NSCLC cells

MATERIALS & METHODS

- Cell Lines from ATCC Life Science Research (Manassas, VA):
 - Human normal bronchial epithelial cells (NBE)
 - A549 (CCL-185)
 - SW1573 (CRL-2170)
 - H1650 (CRL5883)
- Culture Medium from ATCC Life Science Research:
 - Airway Epithelial Cell Basal Medium
 - ATCC-formulated F-12K Medium
 - ATCC-formulated Leibovitz's L-15 Medium
 - ATCC-formulated RPMI-1640 Medium
- Culture Conditions:
 - Normoxic: 37°C, 5% CO₂, 15% water vapor under atmospheric O₂
 - Hypoxic: 37°C, 1% O₂, 5% CO₂, 84% N₂ in Hypoxygen glove box (Frederick, MD)
 - Reperfusion: 37°C, 5% CO₂, 15% water vapor under atmospheric O₂
- Cells were grown to 70-80% confluency & were subsequently grown under normoxic, hypoxic or hypoxic with reperfusion conditions for 60 minutes under each condition
- Following growth, cells were lysed with NP-40 lysis buffer & 1X protease inhibitor cocktail from Thermo Scientific (Rockford, IL)
- Supernatant was isolated by centrifugation & proteins were extracted
- Western blots were run following standard procedure; blots were developed using a LI-COR Odyssey NIR imaging system (Lincoln, NE)

RESULTS

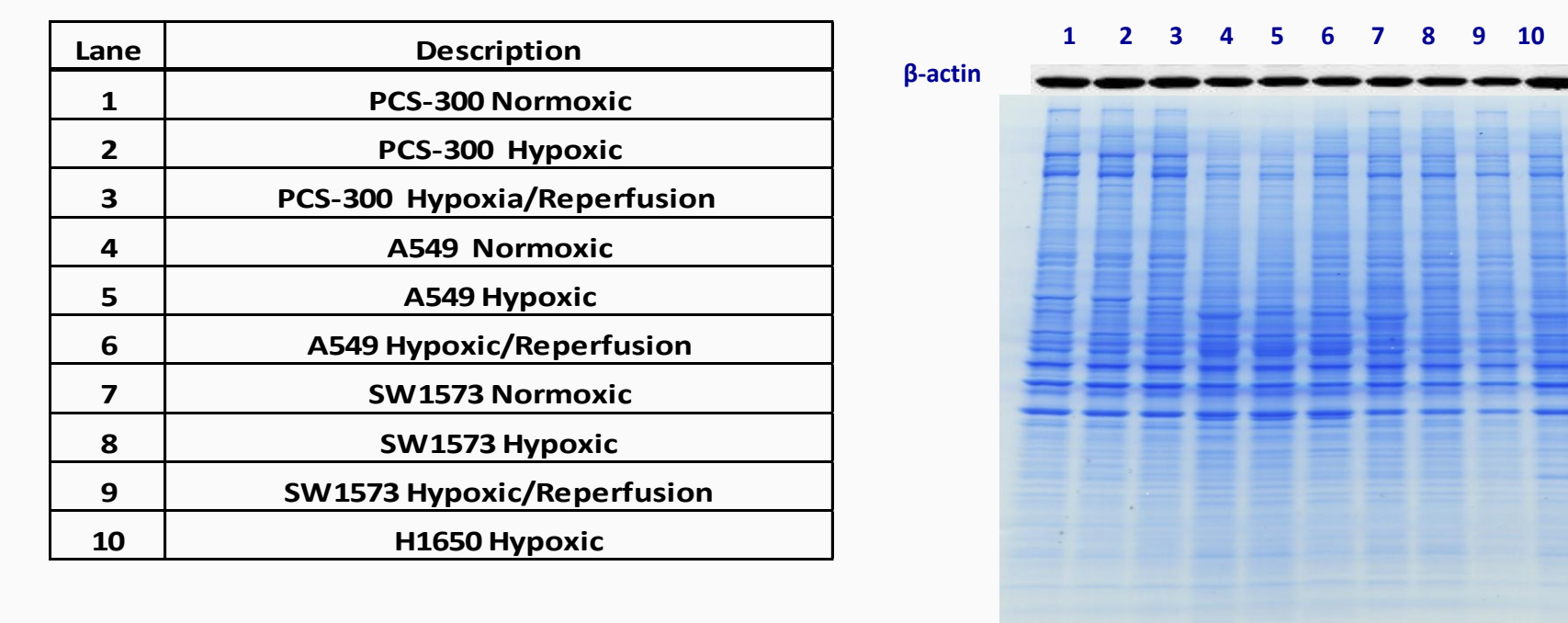


Figure 1: β -Actin & Coomassie blue Gel Stain showing protein quantification for subsequent Western blots

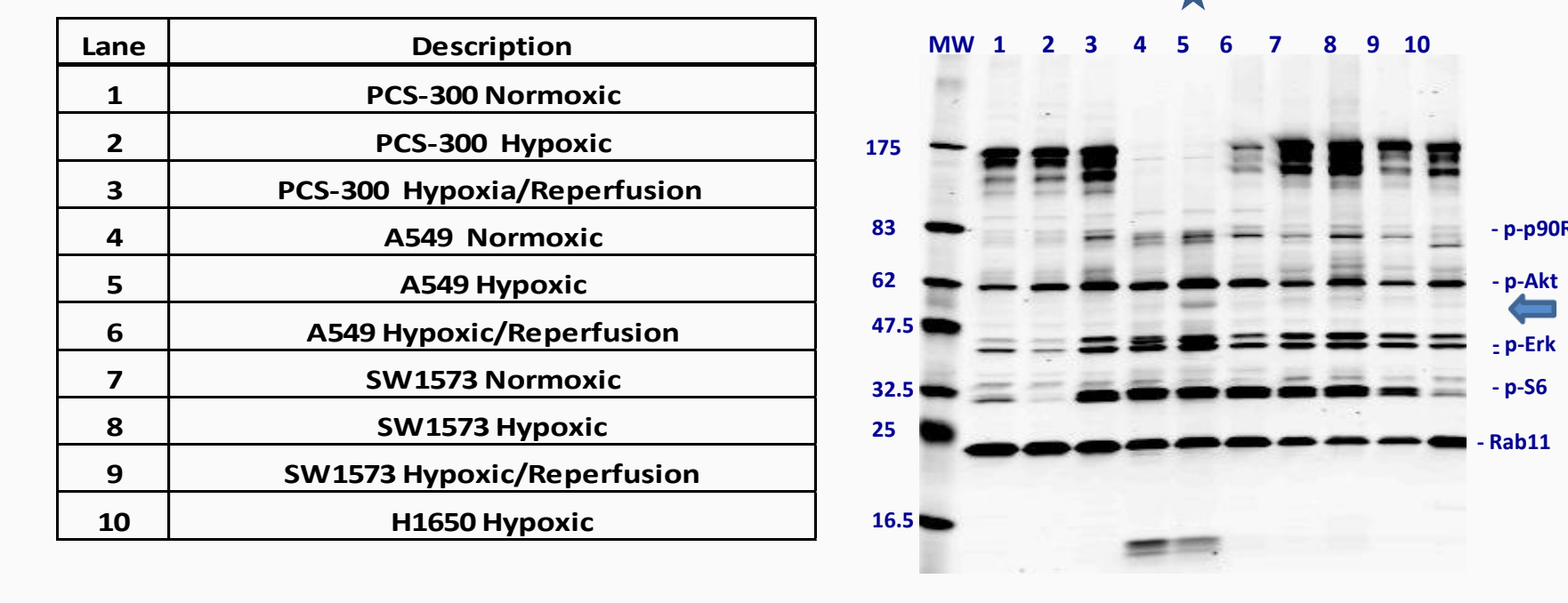


Figure 2: Western blot of PathScan[®] Multiplex

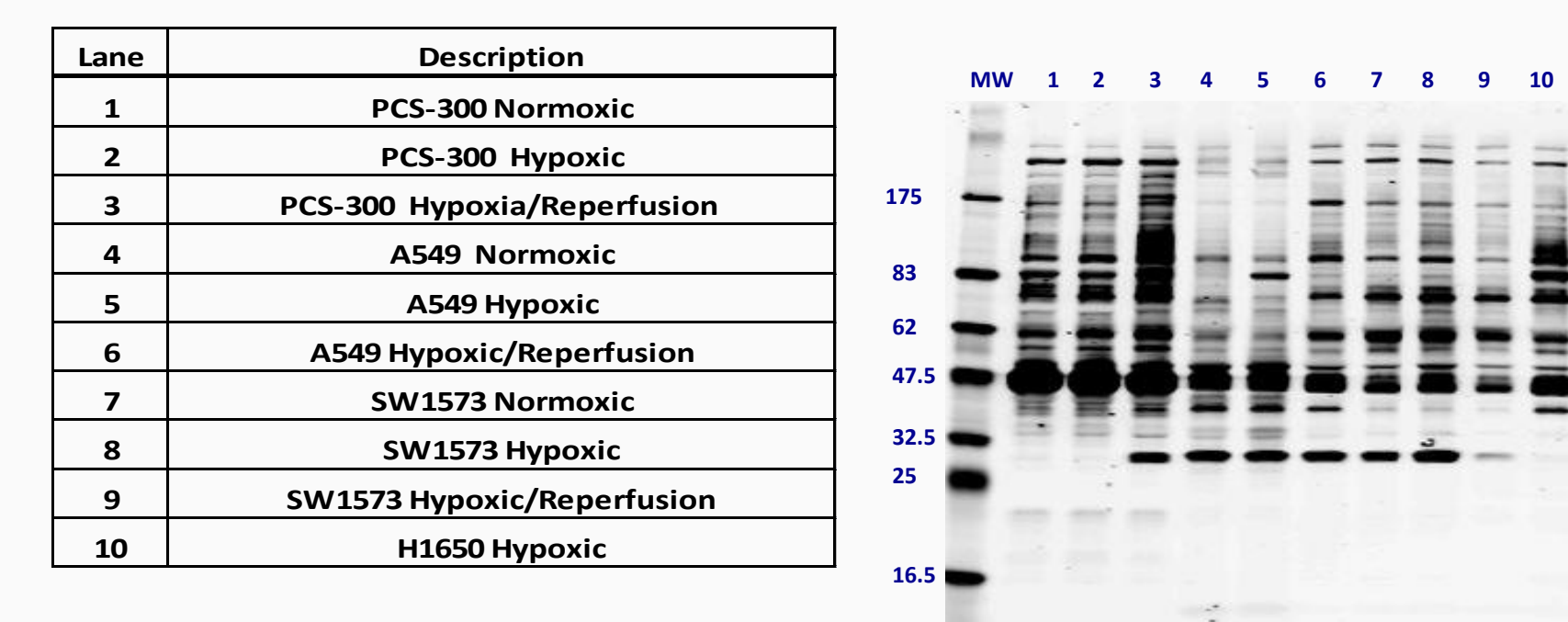


Figure 3: Western Blot of Akt Substrate: RXX(s/t)

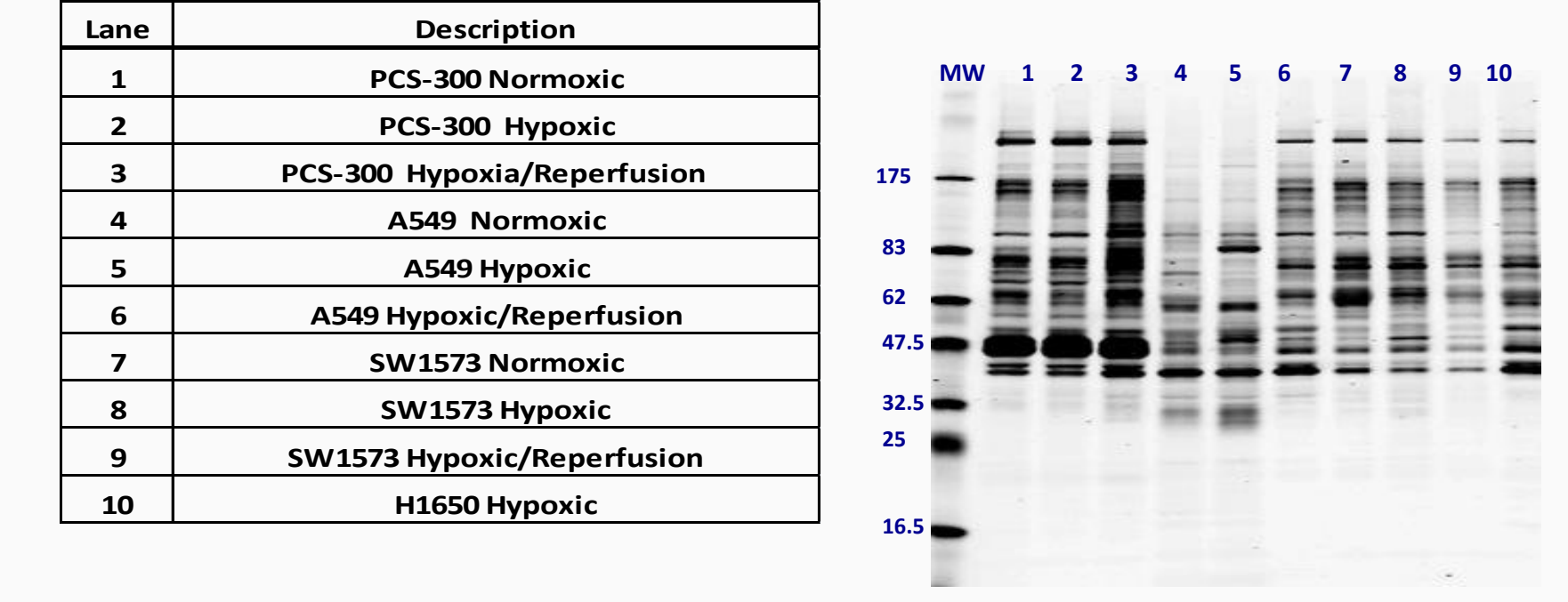


Figure 4: Western Blot of Akt Substrate: RXXXX(s/t)

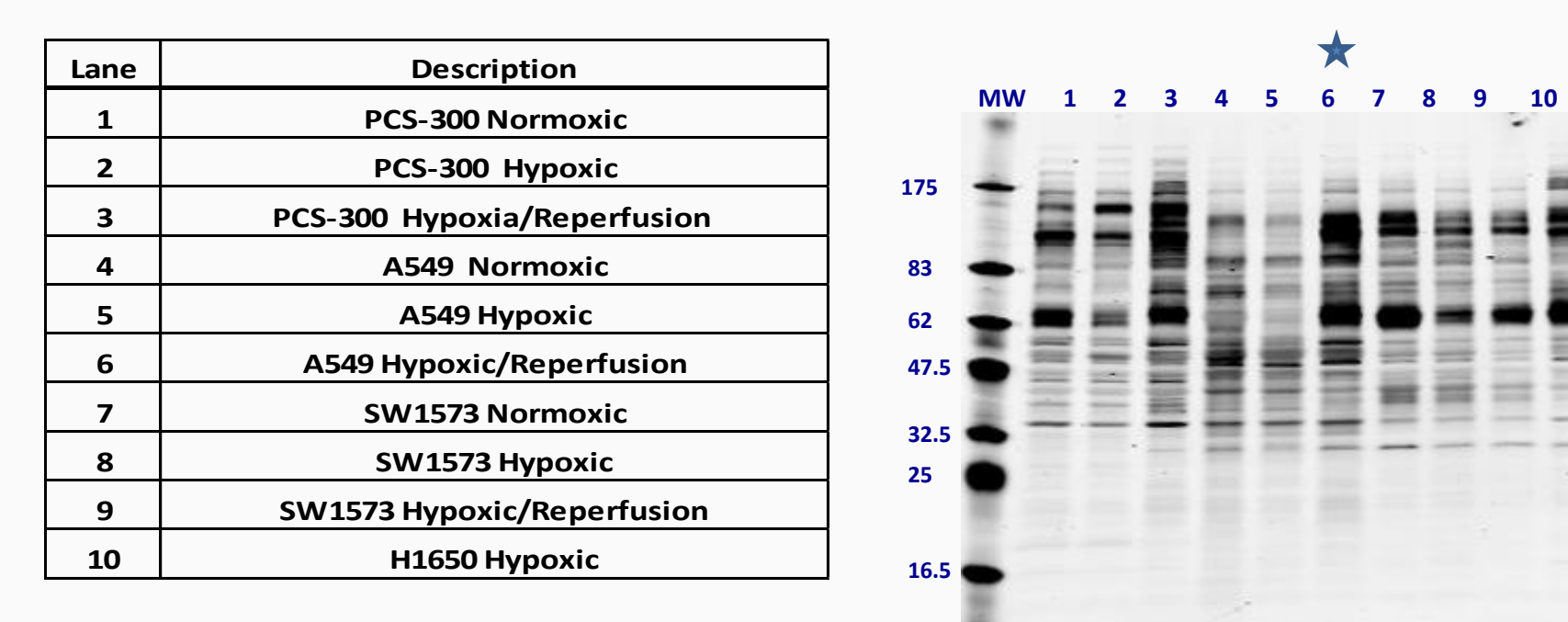


Figure 5: Western Blot of Phosphotyrosine: Y

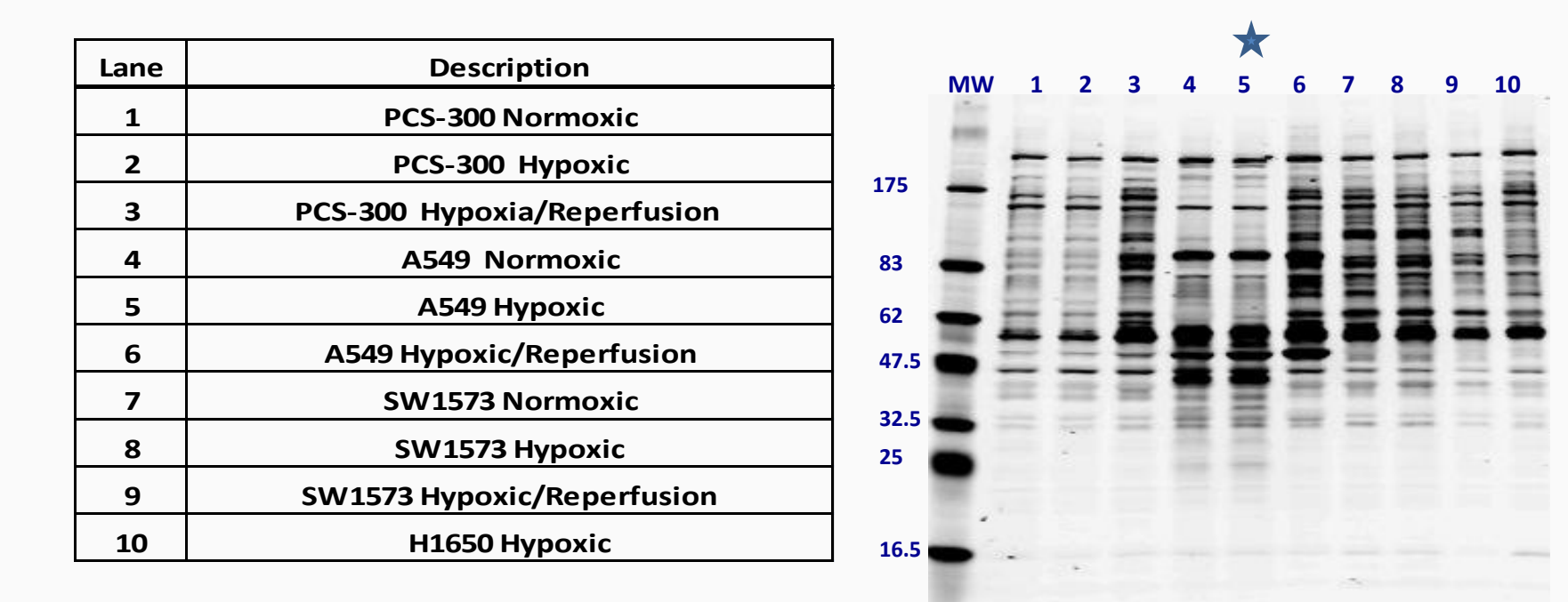


Figure 6: Western Blot of MAPK Substrate: PXS

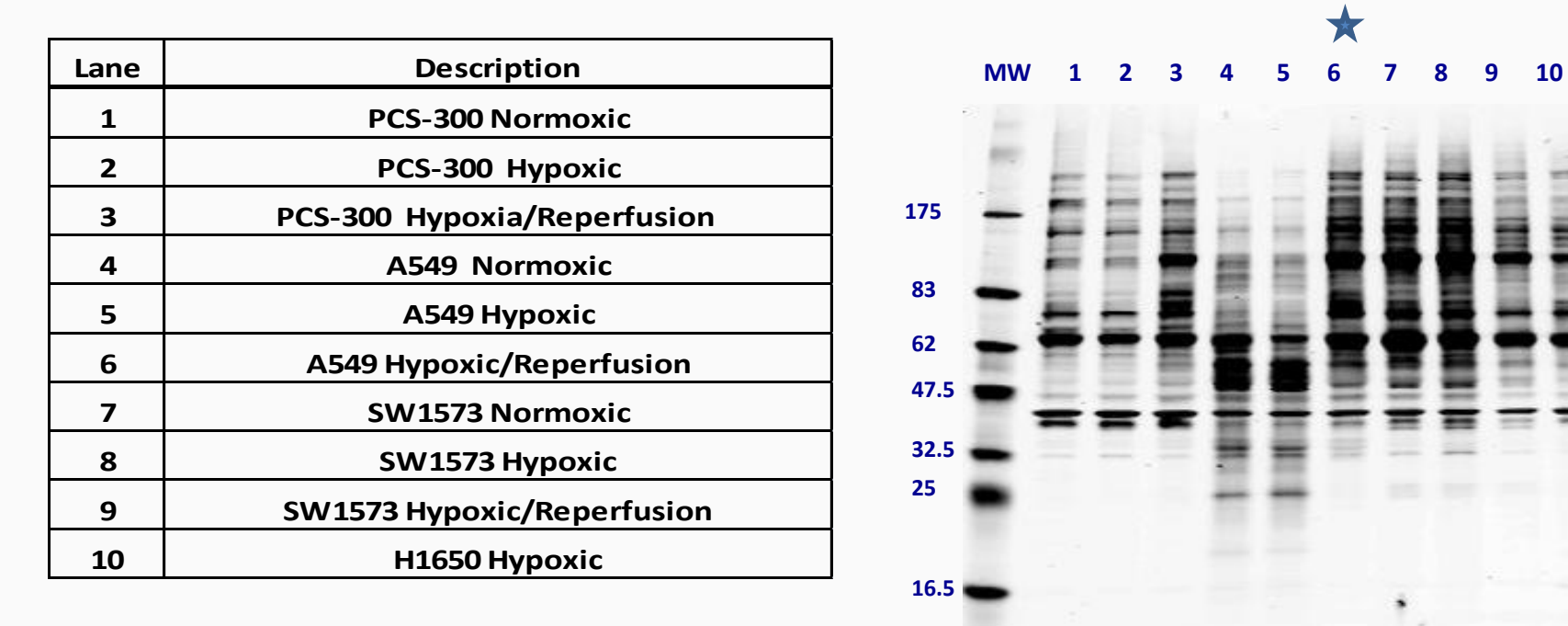


Figure 7: Western Blot of CDK Substrate: (K/R)sPX(K/R)

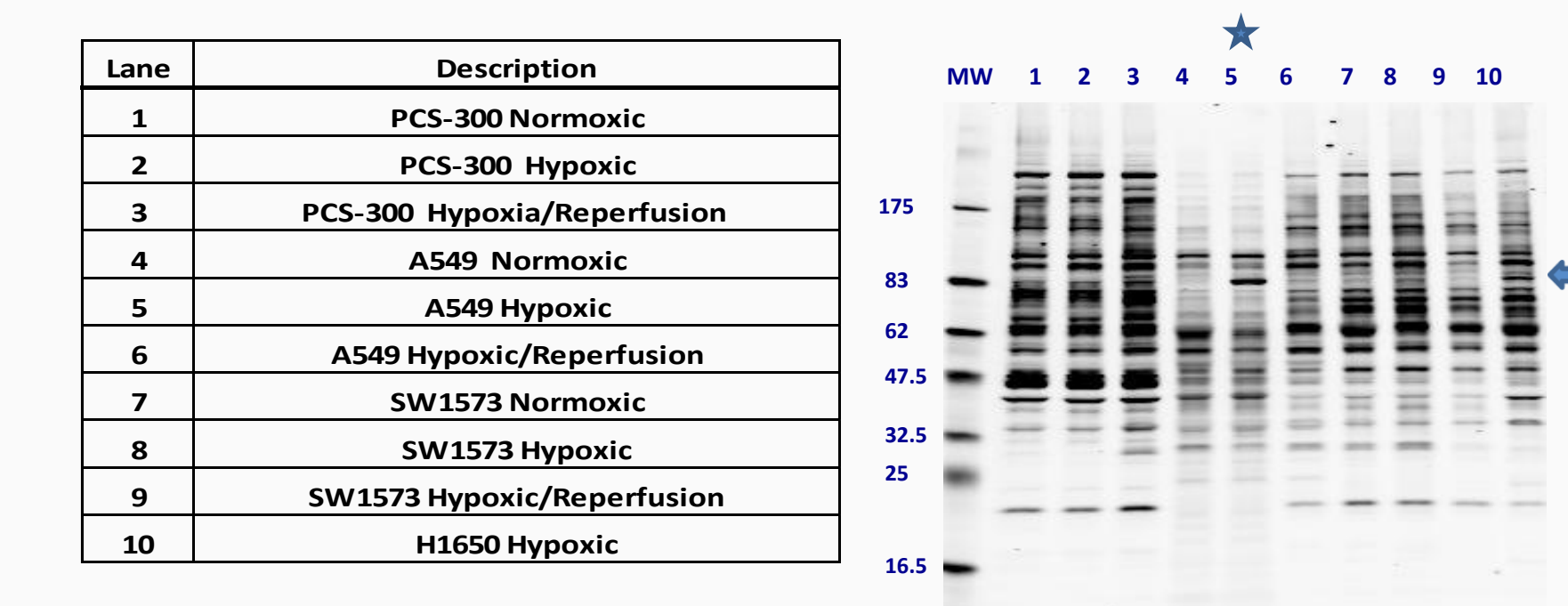


Figure 8: Western Blot of PKA Substrate: (K/R)(K/R)X(s/t)

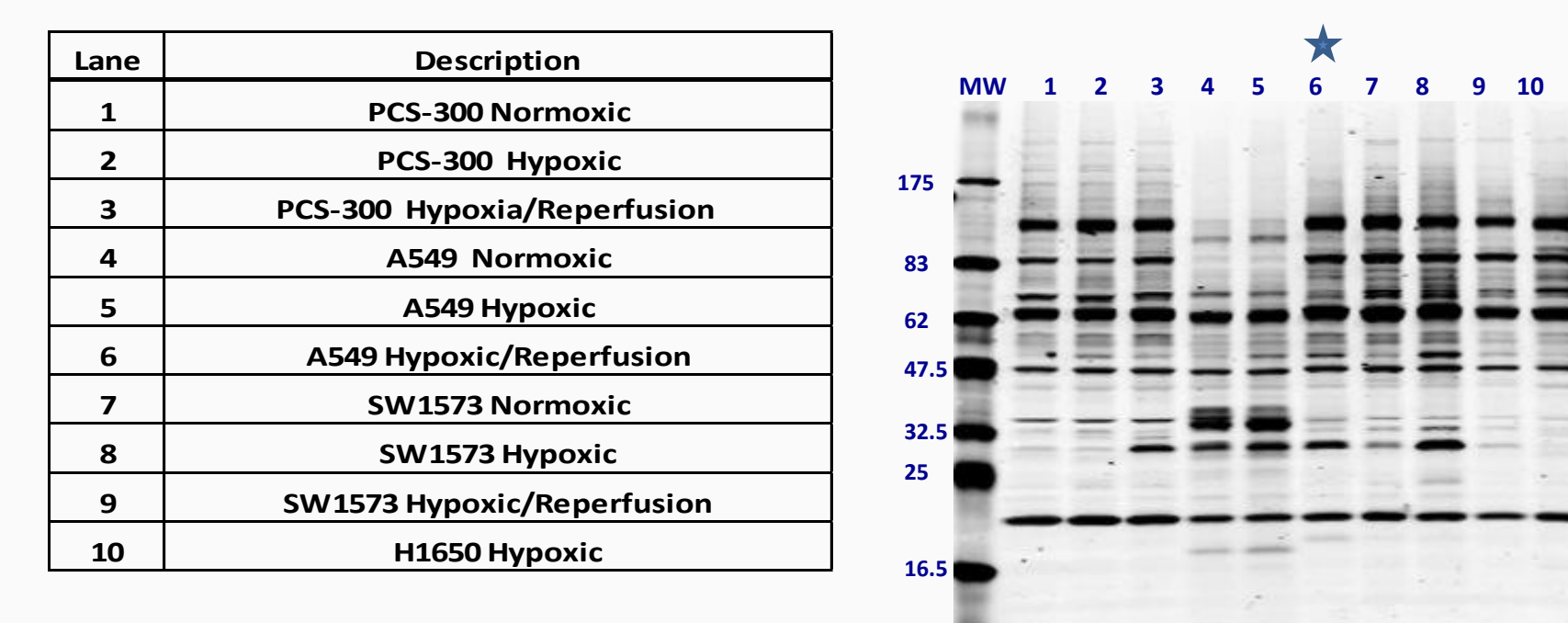


Figure 9: Western Blot of PKC Substrate: (K/R)XsX(K/R)

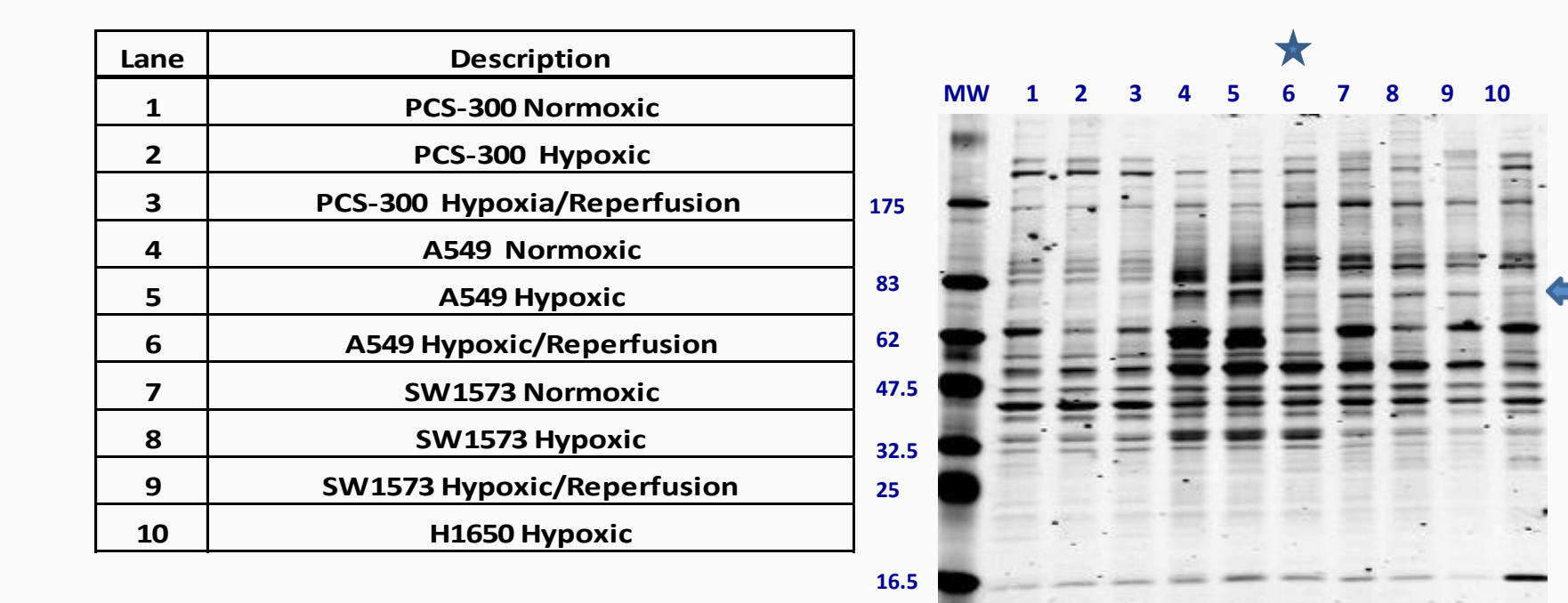


Figure 10: Western Blot of Acetylated-Lysine

RESULTS

- Antibodies (Abs) directed phospho-motifs, ubiquitin-motifs & acetyl-motifs indicate numerous PTMs responsive to changes in oxygen growth conditions
- Results indicate multiple PTMs occur in NSCLC that differ from those in NBE
- Such alterations could convey a selective advantage to the tumor cells by altering signal transduction pathways, activation status, differential complex formation (phosphorylation), protein degradation, localization or complexation (ubiquitylation) & protein confirmation/activity status (acetylation)
- Of note, the NSCLC lines selected for their notably different oncogenic properties displayed limited common PTMs in response to h/r
- PTM responses common to NBE & NSCLC are hypothesized to be critical regulatory steps necessary for cell survival, while those PTMs lost are hypothesized to be inhibitory growth regulatory steps selected against during rounds of acute hypoxia during tumorigenesis
- PTMs gained in NSCLC but absent in NBE may confer a growth advantage that has been acquired during acute hypoxia-selected oncogenesis

CONCLUSIONS

- Differential PTM responses exist between NBE & NSCLC under micro-environmental acute hypoxic challenge justifying a larger study comparing h/r induced PTM profiles
- PTM profiles are potentially instrumental in both diagnosis & prognosis - particularly in relation to radiation & genotoxic therapy resistance
- Current clinical methodologies offer poor prediction of the hypoxic state & acute hypoxia-induced aggressiveness & resistance of tumors
- This research will provide key insights into determining these factors & thus influence treatment options
- Verification that PTM alterations convey selective growth advantages suggest that there are novel, non-redundant nodes suitable for therapeutic targeting
- Future studies will evaluate if repeated cycles of h/r in NBE & NBE cells transformed with oncogenic Kras(G12D) or a dominant negative form of p53 [p53(R175H)] or combined Kras(G12D)+p53(R175H) lead to h/r-induced PTM profiles seen in mature cancers

ACKNOWLEDGEMENTS

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