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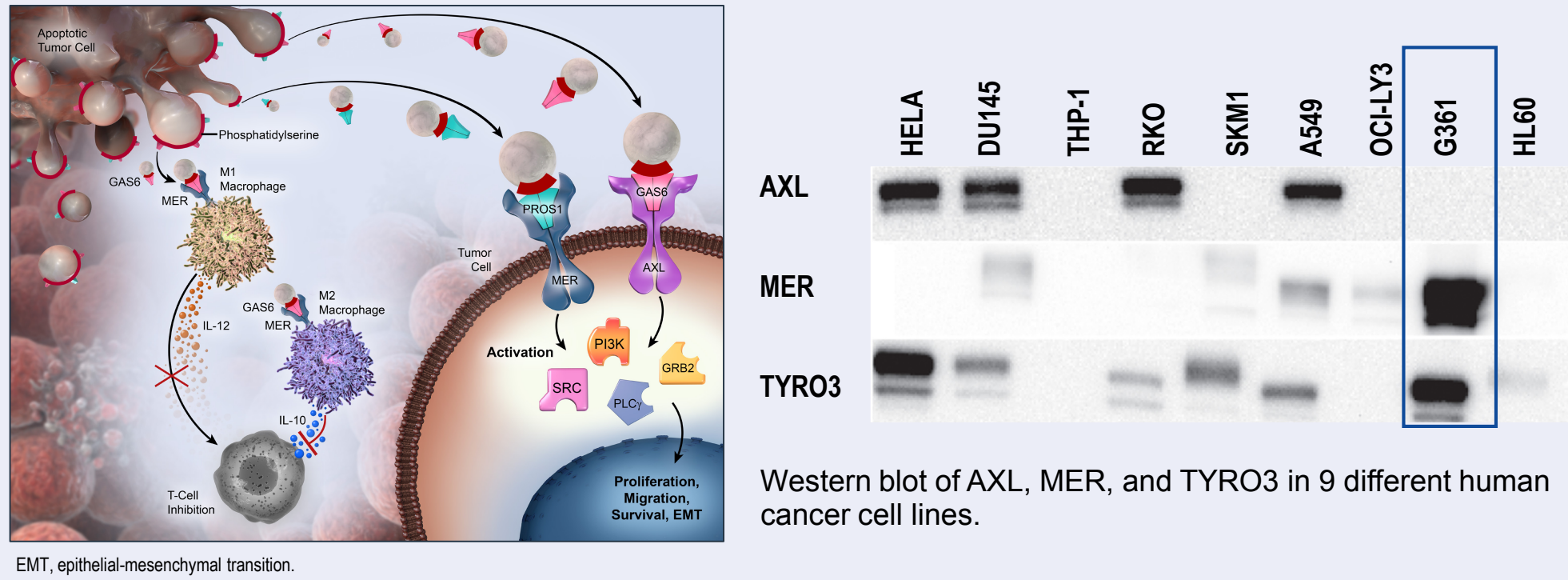
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Abstract

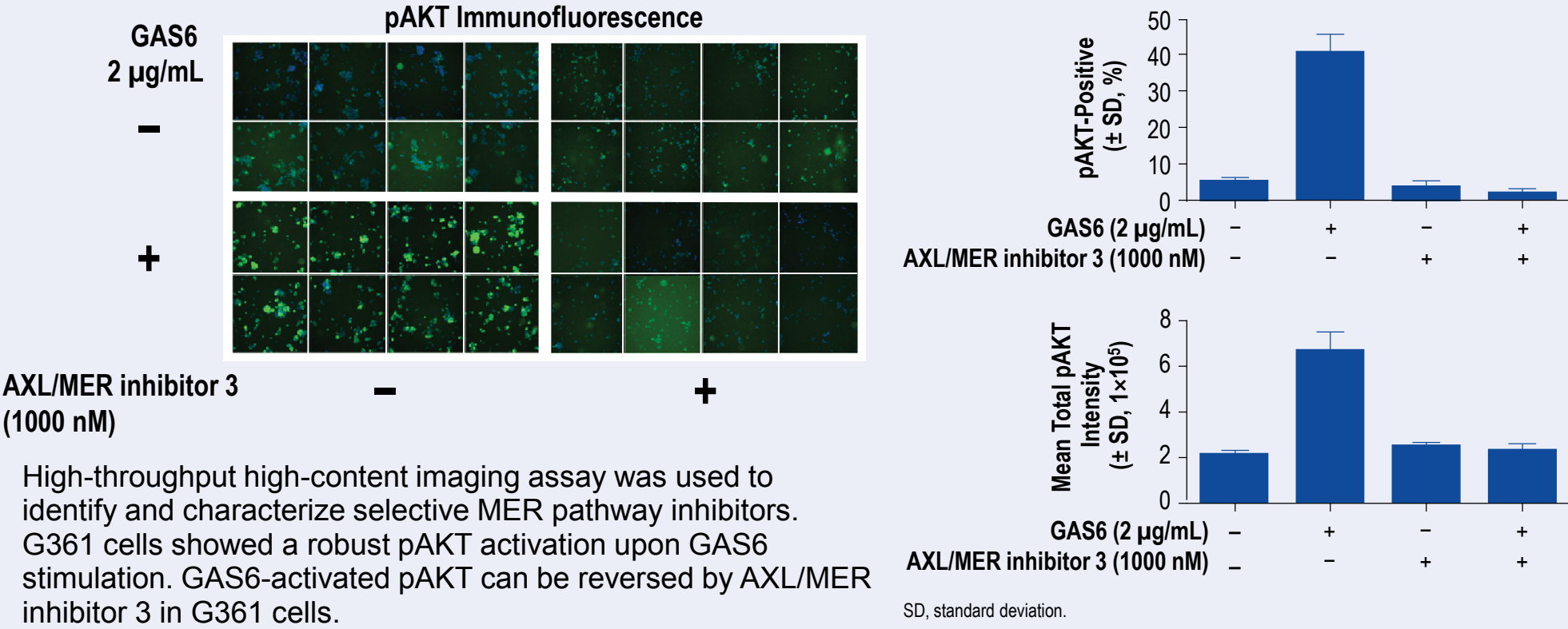
Abnormalities in receptor tyrosine kinases (RTKs) have been shown to be involved in a wide range of important biological activities in cancer, including cell proliferation, differentiation, and drug resistance. The TAM family of RTKs includes 3 family members: TYRO3, AXL, and MER. Binding of ligands to these receptors was reported to lead to activation of the TAM kinases, as well as PI3K/Akt, RAS/RAF/MAPK, and JAK/STAT signaling pathways in human cancer cells. Among these 3 TAM kinases, the MER signaling pathway represents an attractive target for the treatment of human cancers. MER phosphorylation is dependent on the ligand GAS6; however, ligand-activated phospho-MER is often not stable and is very difficult to detect without pervanadate pretreatment in human cancer cells and this has been a hurdle for developing a selective MER kinase inhibitor. Therefore, it is important to identify a specific pharmacodynamic marker to monitor MER kinase activity in human cancer cells. Here, we report the development of a phospho-AKT assay for characterization of MER kinase inhibitors in human G361 cells. We profiled the expression of MER among multiple human cancer cells and demonstrated that MER and TYRO3, but not AXL, show high levels of protein expression in human G361 melanoma cells. In G361 cells, phospho-AKT is induced by GAS6 (2 µg/mL) treatment and the induction of phospho-AKT can be reversed by AXL/MER tool compounds. To determine the role MER and TYRO3 play in the activation of phospho-AKT, either MER or TYRO3 was selectively depleted by siRNA knockdown. We demonstrate that GAS6-induced phospho-AKT is only dependent on MER kinase, but not TYRO3 in human G361 melanoma cells. In addition, using phospho-AKT as readout, a high-throughput cell-based assay was established in the G361 human melanoma cells for evaluation of compound potency in inhibiting MER pathway activation. Further, we observed a correlation in compound potency between inhibition of phospho-AKT in G361 cells and phospho-MER in MER-overexpressing BaF3 cells. In summary, we have demonstrated that GAS6-induced phospho-AKT can be a potential pharmacodynamic marker for inhibition of MER kinase and have successfully developed a cell-based functional assay for screening small molecule inhibitors of MER kinase for potential therapeutic utility in treating GAS6/MER-deregulated human cancers.

Expression of AXL/MER in Multiple Human Cancer Cell Lines

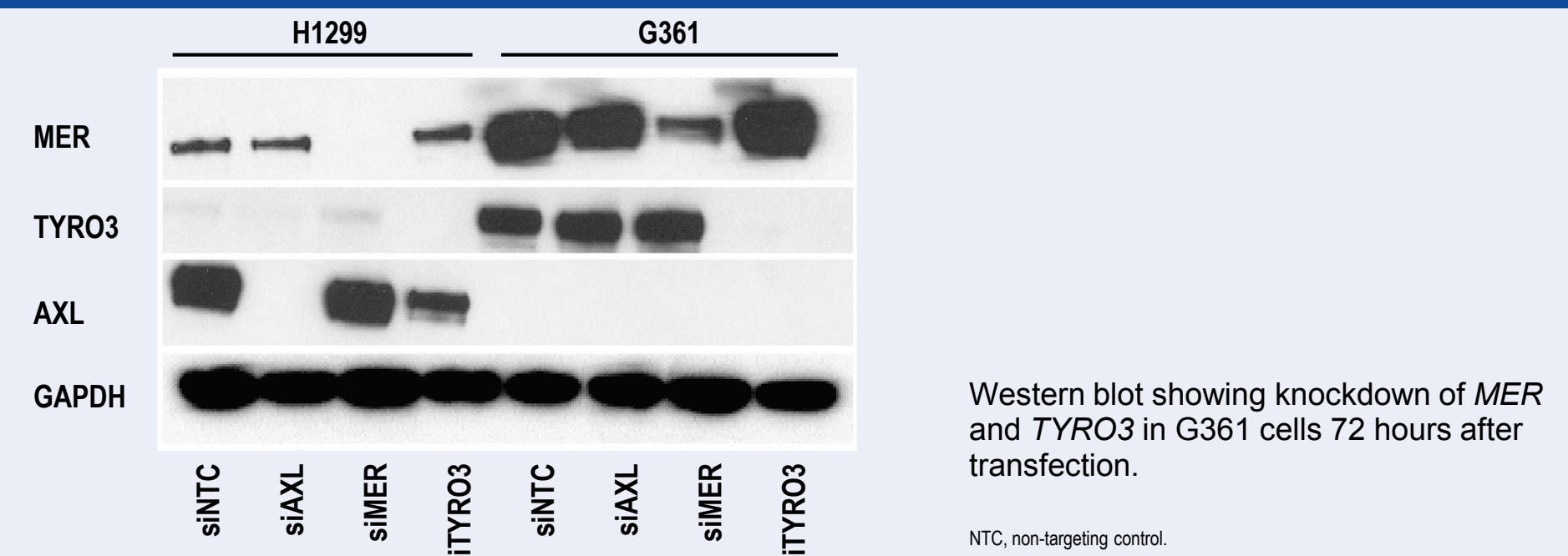


Western blot of AXL, MER, and TYRO3 in 9 different human cancer cell lines.

GAS6 Induces pAKT in G361 Cells

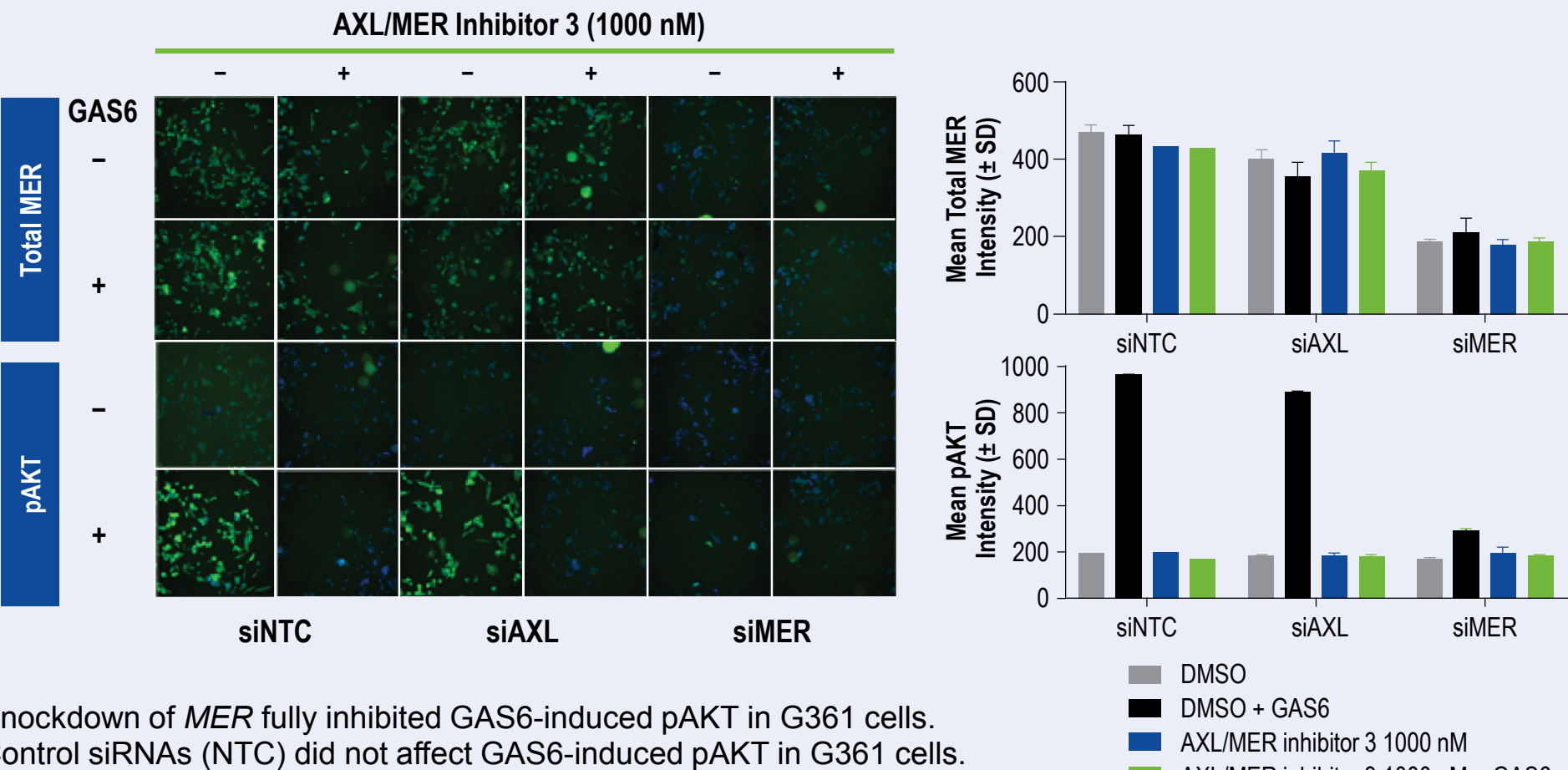


Knockdown of MER and TYRO3 by siRNA in G361 Cells

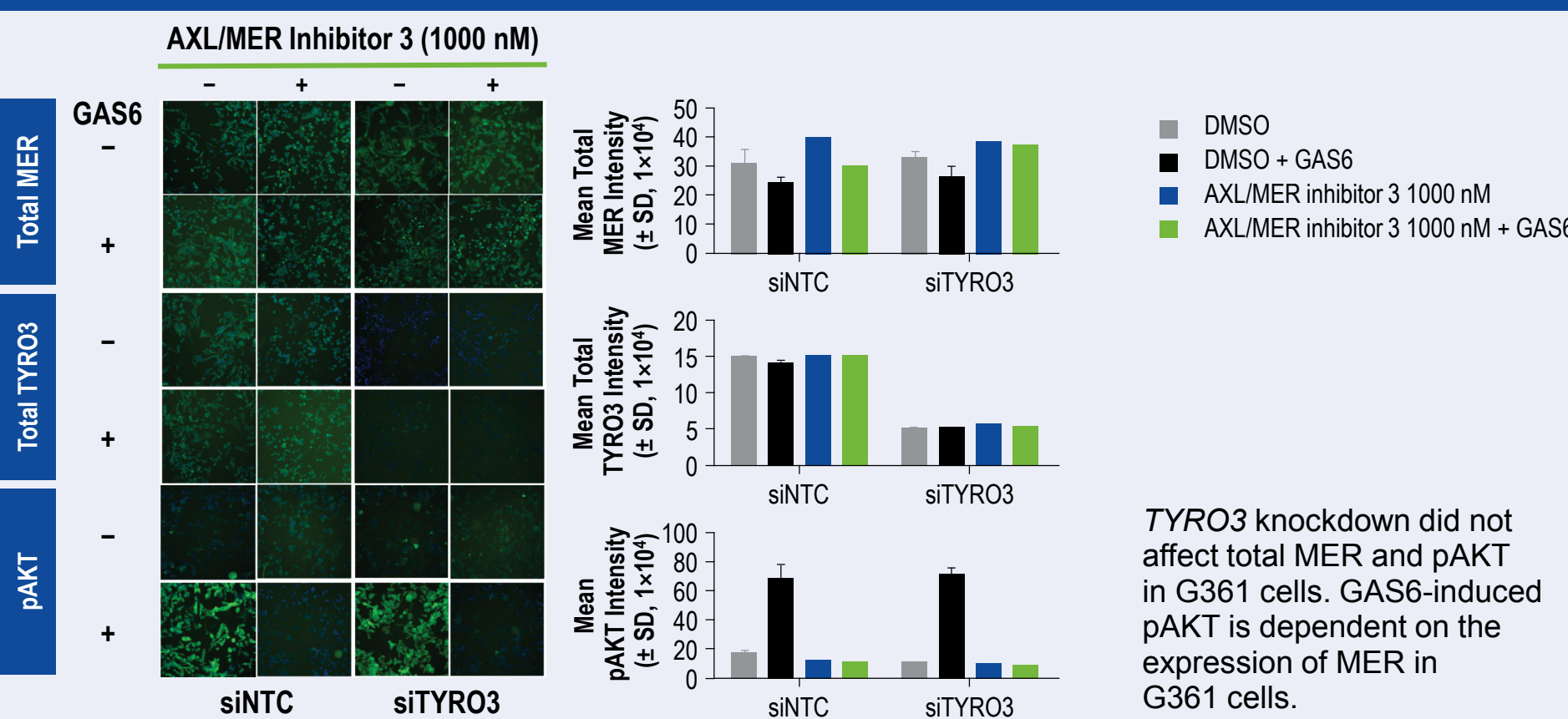


Western blot showing knockdown of MER and TYRO3 in G361 cells 72 hours after transfection.

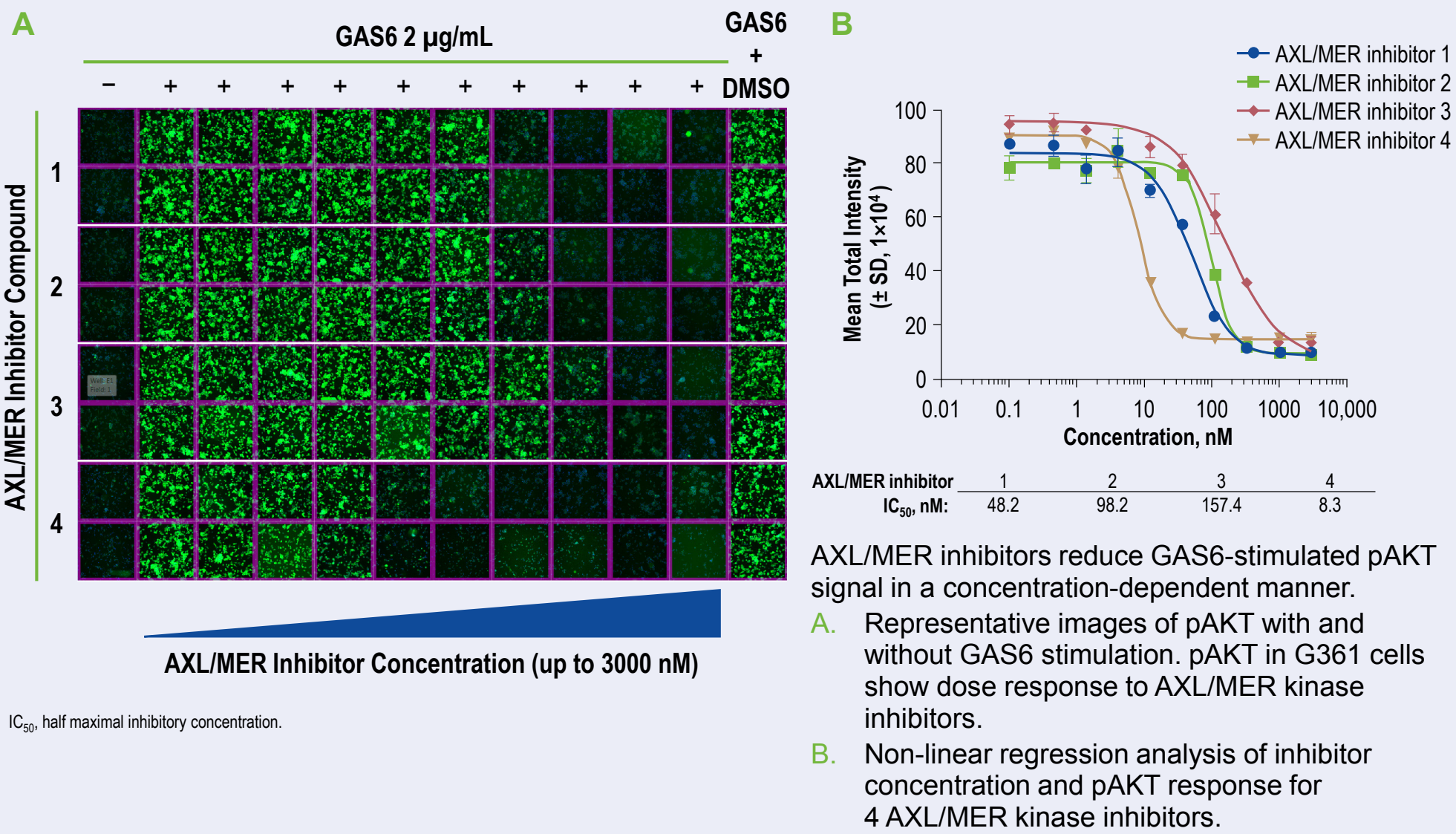
Knockdown of MER Inhibits pAKT in G361 Cells



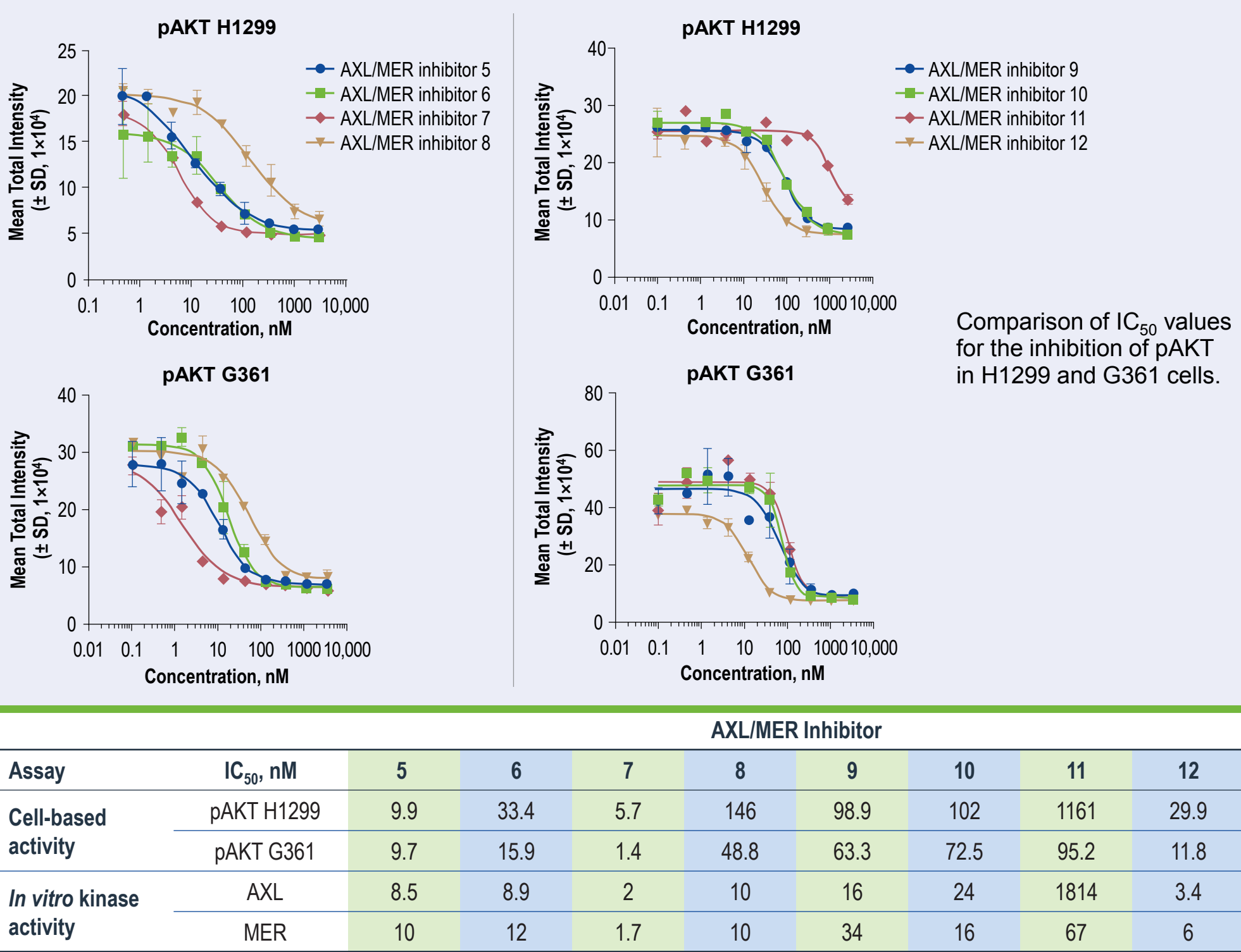
Knockdown of TYRO3 Did NOT Inhibit pAKT in G361 Cells



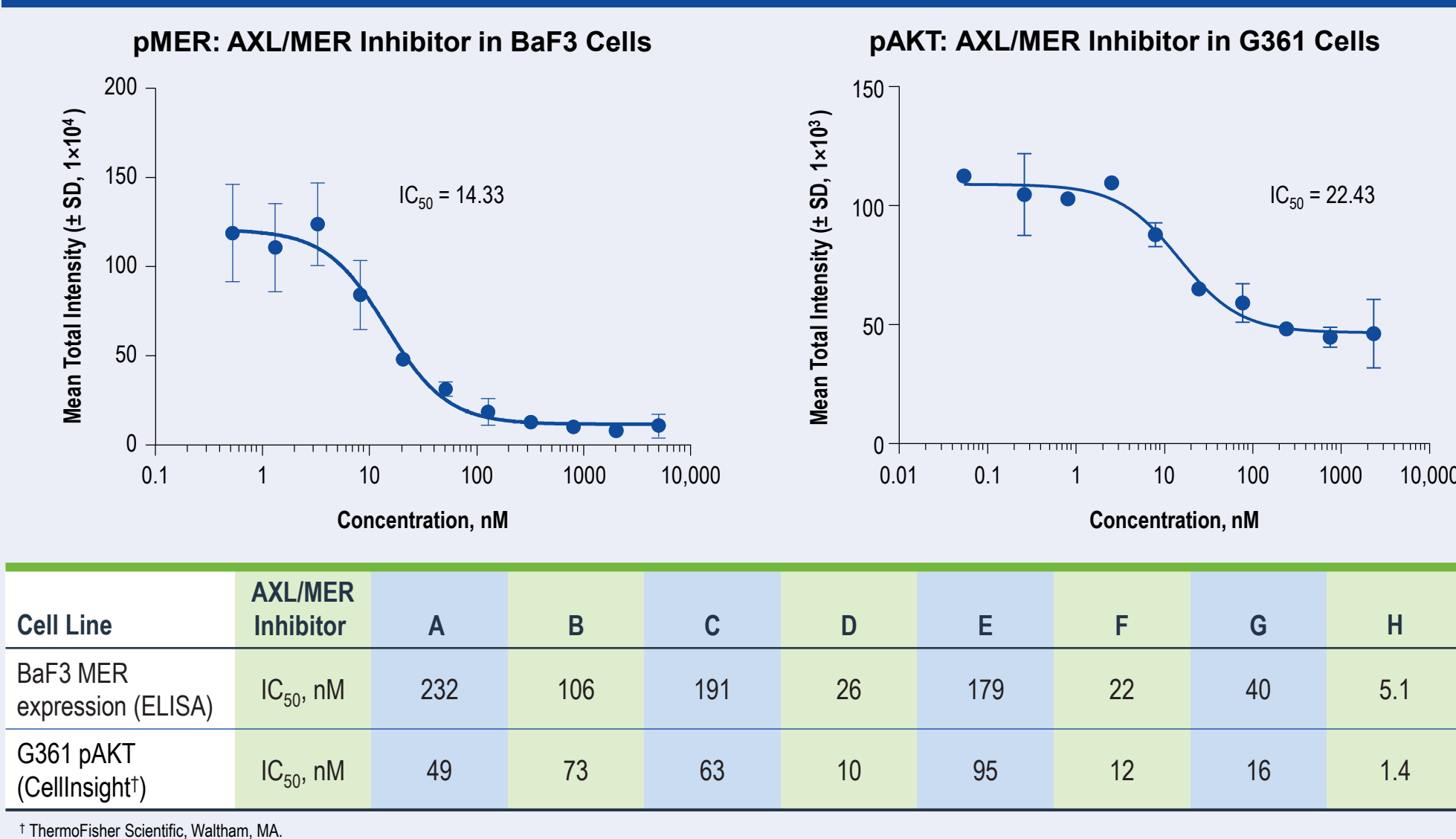
Evaluating Cellular Potency of AXL/MER Inhibitors



Comparison of IC50 for pAKT Inhibition Between H1299 and G361 Cell Lines



Relationship Between pMER and pAKT in 2 Different Cell Assays



Conclusions

