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# Co-activation of invasion and survival contributes to transformation induced by exon 14 skipping of MET receptor in lung cancer

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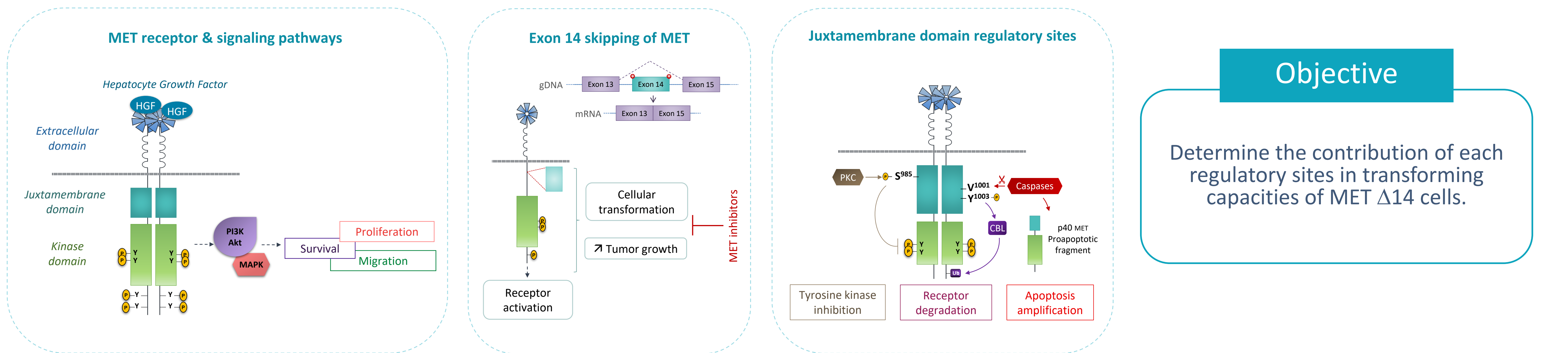
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## Introduction

MET is a tyrosine kinase receptor (RTK) expressed predominantly in epithelial cells and activated by HGF (Hepatocyte Growth Factor). Like other RTKs, its deregulation is associated to tumorigenesis in several cancers. Although the majority of RTK mutations affect the kinase domain, those affecting the MET receptor in lung cancer cause exon 14 skipping (MET Δ14). This alteration can lead to the aberrant activation of MET and which make these patients eligible for MET targeted therapies. The in frame skipping of exon 14 induces the loss of the juxtamembrane domain. This domain contains at least three negative regulation sites. Among them, phosphorylation of residues S985 and Y1003 respectively leads to inhibition of kinase activity and degradation of MET in response to its ligand HGF. Moreover, the ESVD1002 motif is a caspase cleavage site involved in pro-apoptotic activity in the absence of ligand.

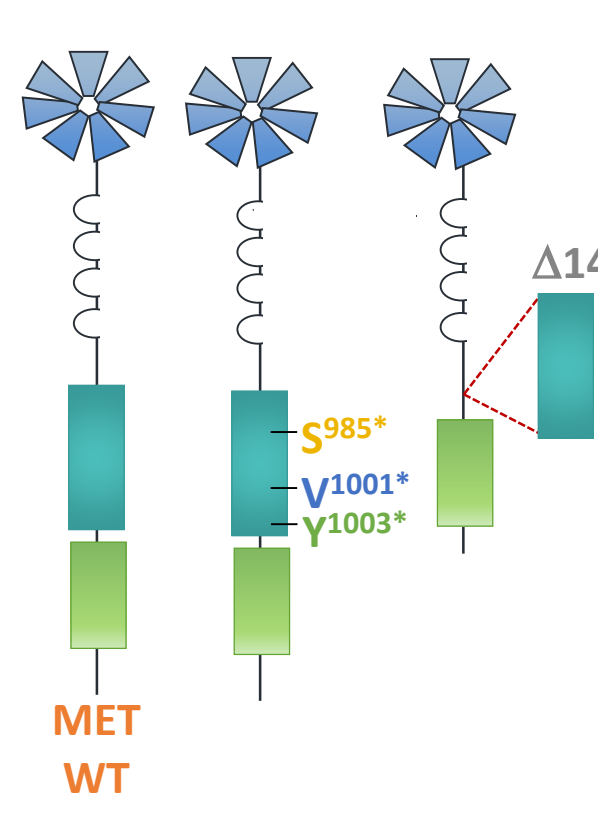


## Objective

Determine the contribution of each regulatory sites in transforming capacities of MET Δ14 cells.

## Strategies

### Juxtamembrane domain mutations

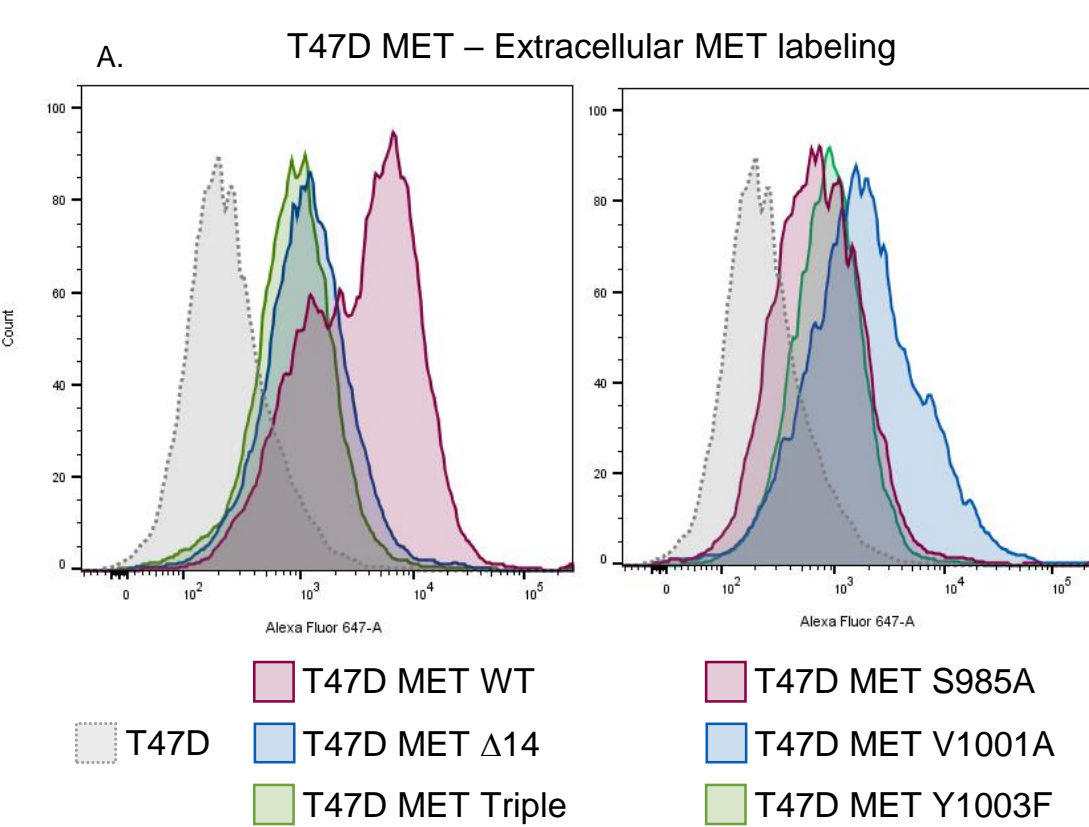


**T47D**  
Epithelial breast cell line  
No MET expression

### MET cell lines obtained by stable transfection

Validation of T47D MET lines

→ T47D MET WT, Δ14, S985A, V1001A, Y1003F and Triple



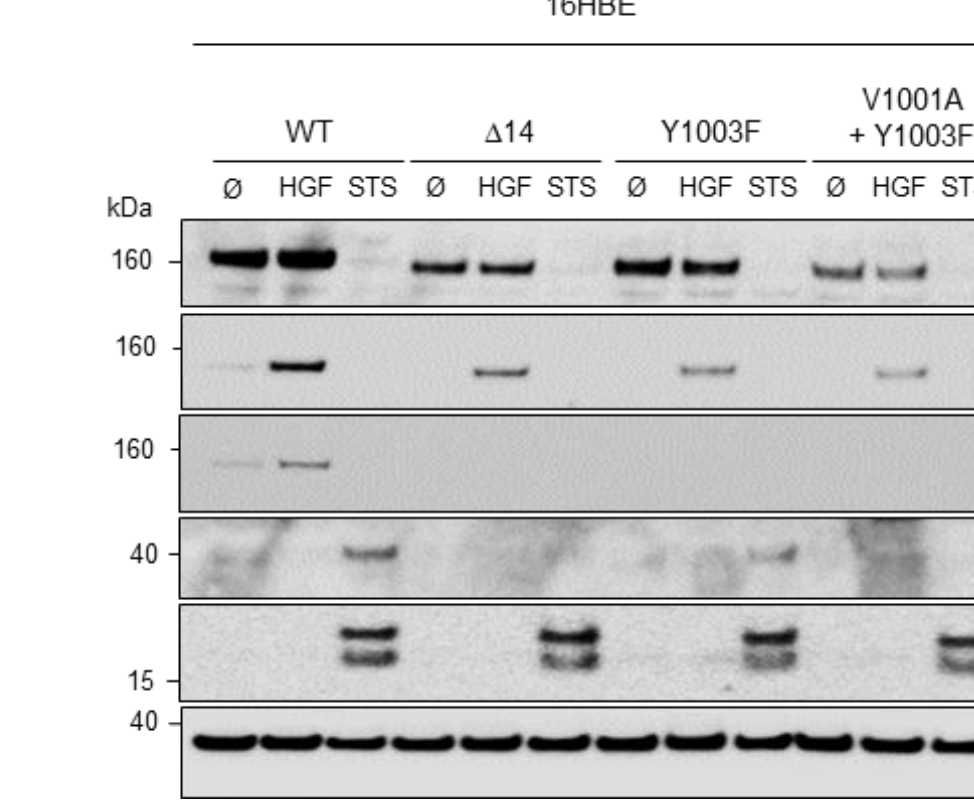
**Figure 1 : Validation of MET expression in stable T47D MET lines.** MET expression was determined by flow cytometry (A) and immunofluorescence (B) using an antibody against extracellular domain of MET on T47D, T47D MET WT or mutants.

**16HBE**  
Epithelial pulmonary cell line  
MET WT expression

### CRISPR-Barcoding • Endogenous MET mutation genome editing

Validation of 16HBE MET lines

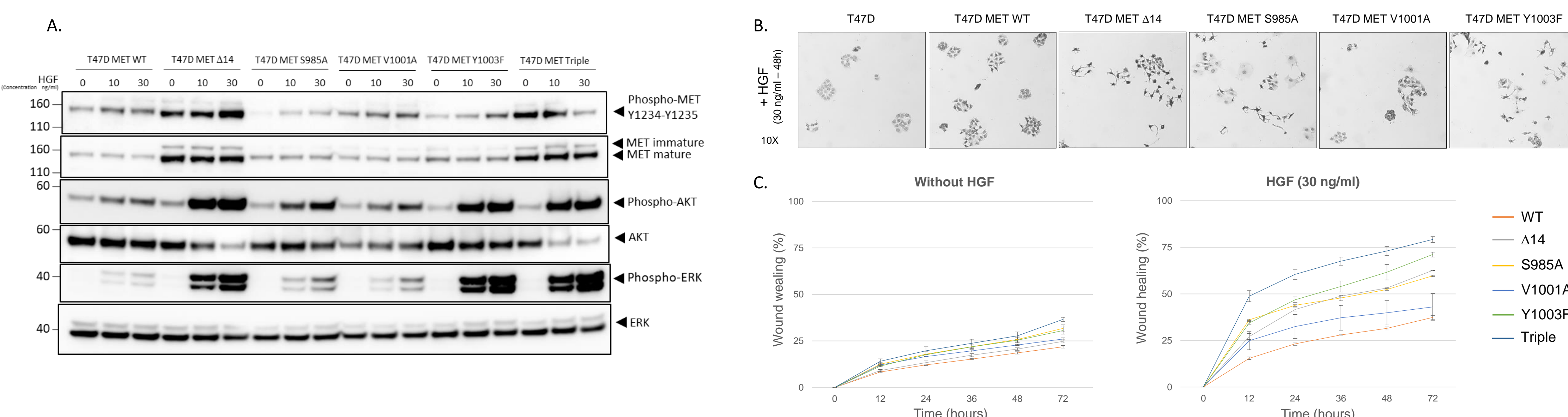
→ 16HBE MET WT, Δ14, Y1003F and V1001A + Y1003F



**Figure 2 : Validation of MET mutations in stable CRISPR 16HBE MET lines.** Expression or phosphorylation of MET, MET-Y1003, p40MET, ERK and caspase 3 cleaved was determined by Western Blot with protein extracts from CRISPR 16HBE MET WT or mutant treated or not by HGF (20 ng/ml – 30 min) or staurosporine (1 μM – 7h).

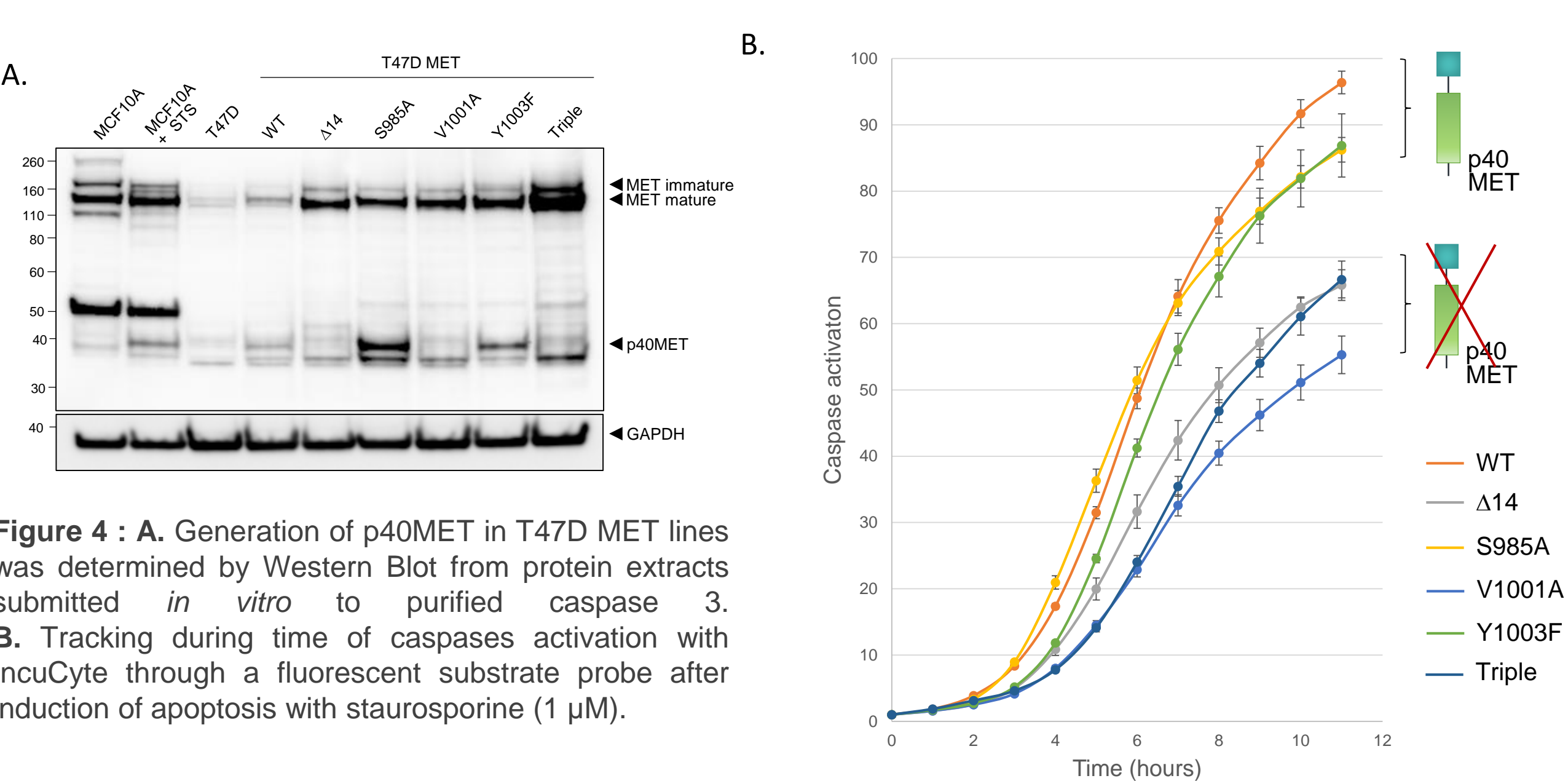
## Results

### HGF stimulation overactivates signalling pathways and migratory capacities in MET Δ14 and Y1003F cells



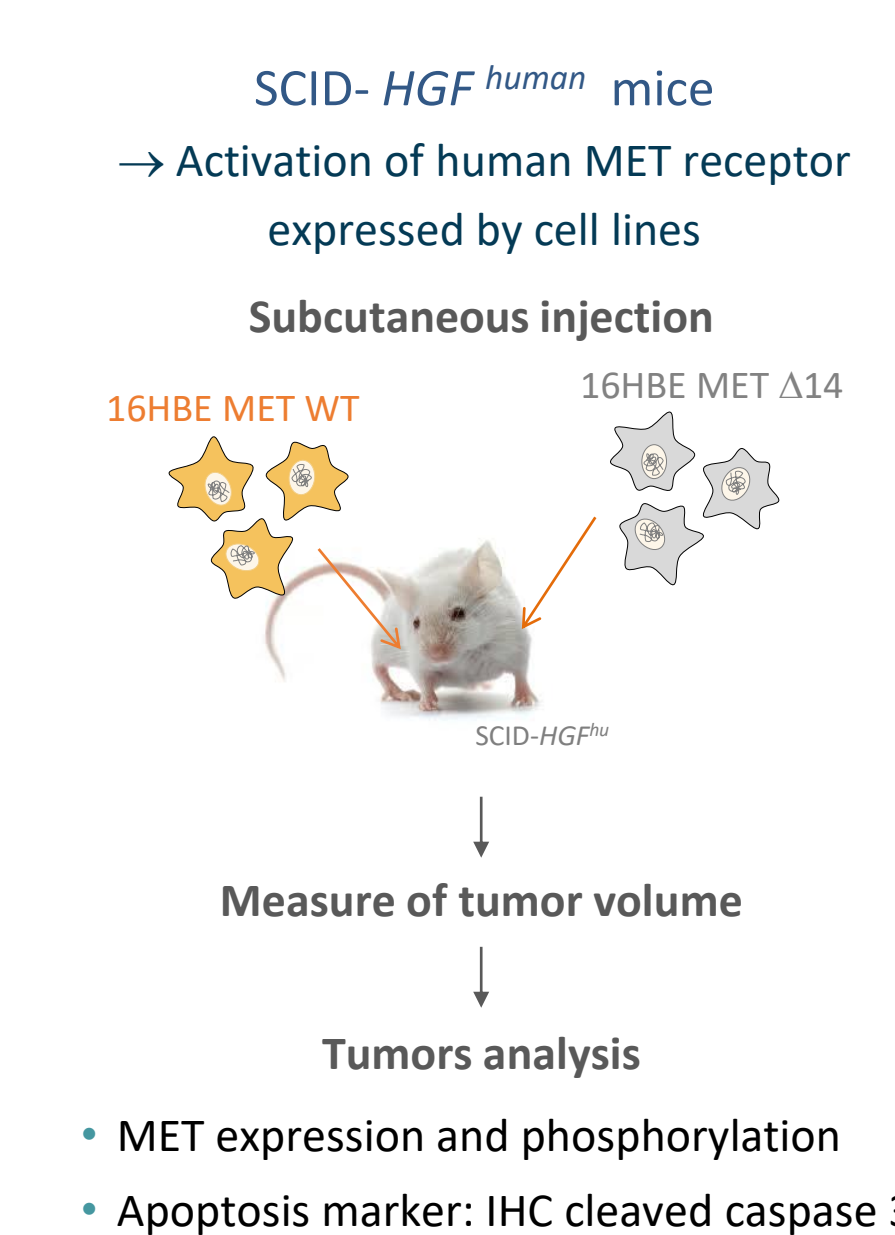
**Figure 3 : A.** Expression and phosphorylation of MET, AKT and ERK were determined by Western Blot with protein extracts from T47D MET WT or mutants treated or not by HGF at 10 or 30 ng/ml for 30 min. **B.** Scattering assay by microscopy (40X magnification) of T47D, T47D MET WT or mutants fixed and stained Eosine-Carazzi after 48h of HGF stimulation (30 ng/ml). **C.** Wound healing assay of T47D MET WT or mutant. After cells seeding, injury was performed and cells were stimulated or not by HGF at 30 ng/ml during several days. Wound healing was tracked by videomicroscopy and quantified by IncuCyte software. **D.** Unsupervised analysis of transcriptional responses on Affymetrix microarray from total RNAs extracted from T47D MET WT or mutants treated by HGF at 30 ng/ml during 24h (biological triplicates).

### Loss of p40MET generation leads to better survival of MET Δ14 and V1001A cells

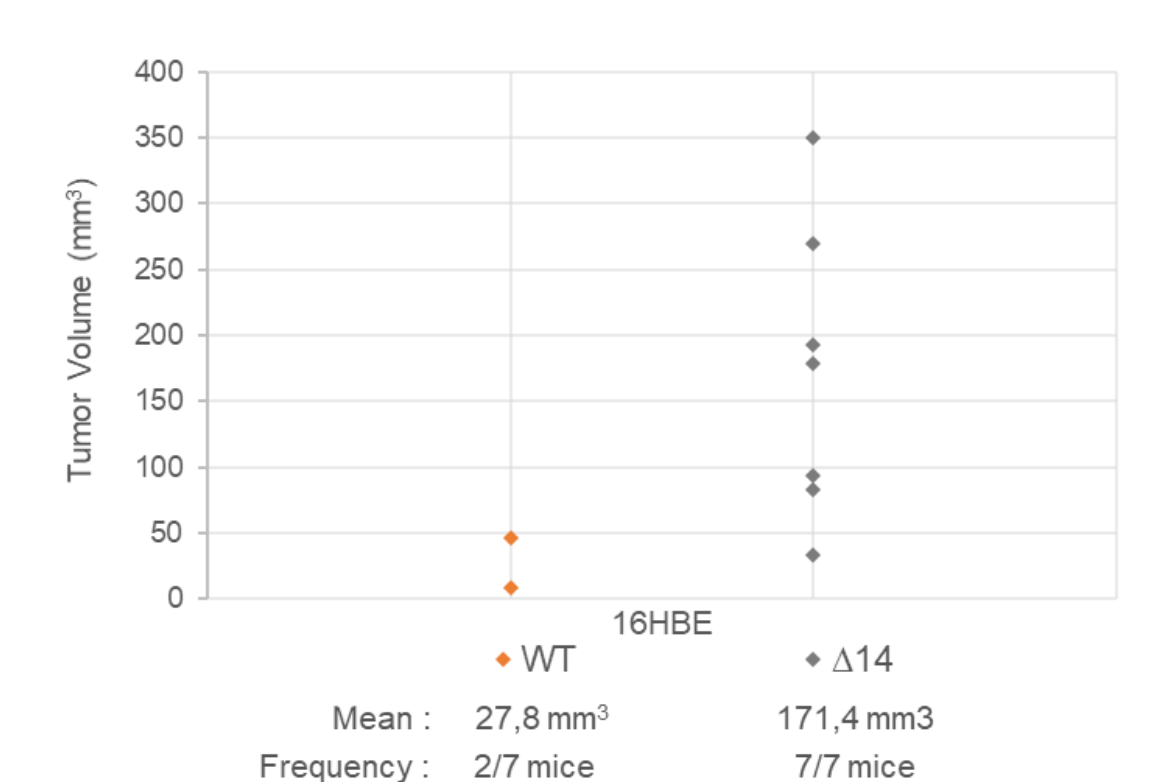


**Figure 4 : A.** Generation of p40MET in T47D MET lines was determined by Western Blot from protein extracts submitted *in vitro* to purified caspase 3. **B.** Tracking during time of caspases activation with IncuCyte through a fluorescent substrate probe after induction of apoptosis with staurosporine (1 μM).

### Consequences on tumorigenesis



### End-point measure of tumor volume from 16HBE lines



**Figure 5 : 16HBE MET WT and Δ14 cells (2.10<sup>6</sup> cells) were xenografted in the flank of SCID-HGF<sup>hu</sup> mice and they were monitored until the tumor was excised (9 weeks after injection).**

→ Next step : injection of 16HBE MET WT, Δ14, Y1003F and V1001A + Y1003F

## Conclusion

We demonstrate that HGF stimulation of MET Δ14 receptor induces sustained and stronger activation of the main signaling pathways associated with an increase of migration. Moreover, in absence of HGF, MET Δ14 cells are more resistant to apoptosis. Therefore transforming capacities of MET exon 14 skipping can be the result of the loss of at least two regulatory sites. We are currently confirming these results in a normal pulmonary epithelial cell line (16HBE) and we will evaluate the consequence of MET exon 14 skipping in animal model of tumorigenesis. This original mechanism of loss of several regulatory processes calls into question the efficacy of therapies offered to MET Δ14 patients. Indeed, these patients can be treated by tyrosine kinase inhibitors targeting MET or with pro-apoptotic chemotherapies. Thus, the use of TKIs seems relevant since we demonstrated stronger HGF-induced signaling in MET Δ14 cells. Nevertheless, the resistance to apoptosis observed could lead to of therapeutic failure in the context of pro-apoptotic chemotherapies.