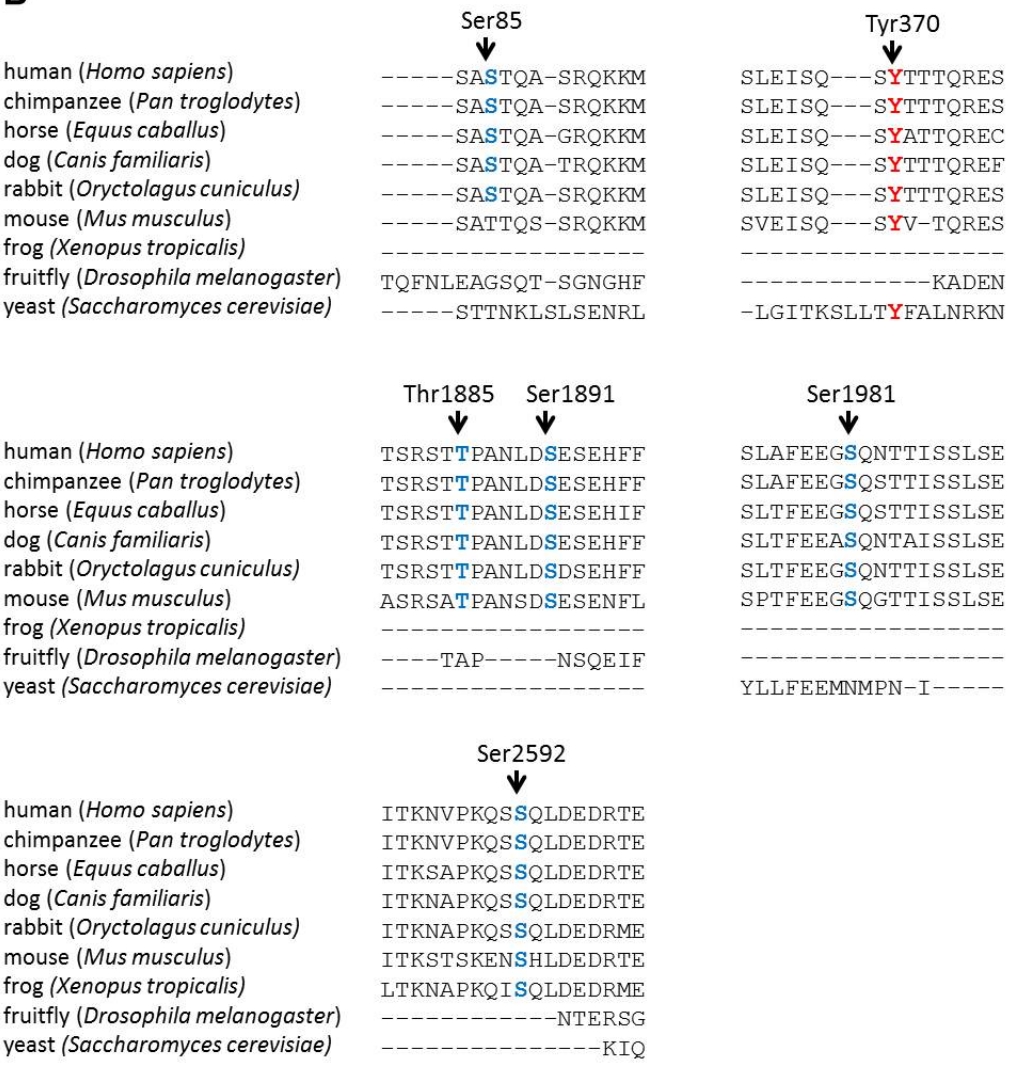
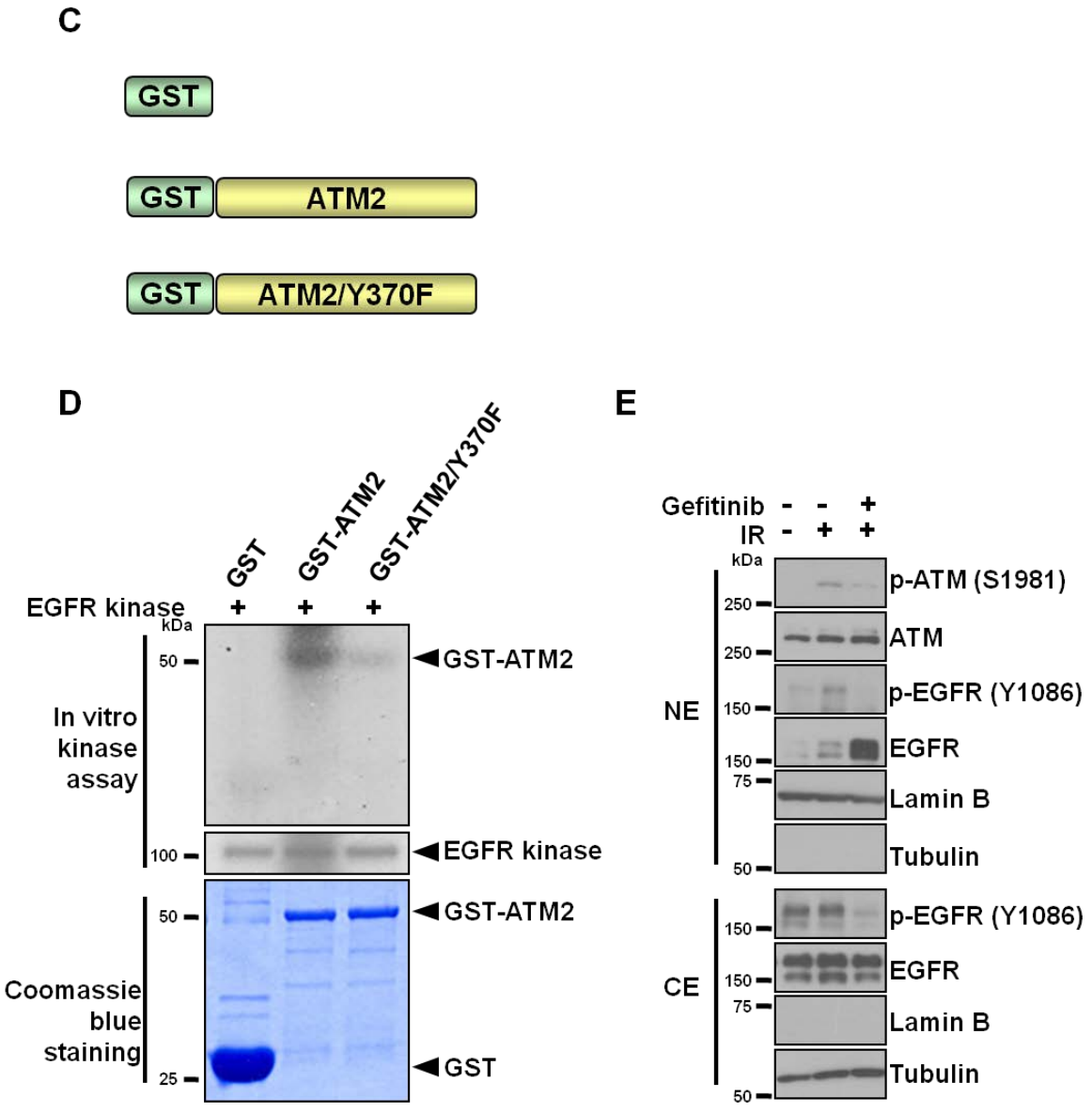
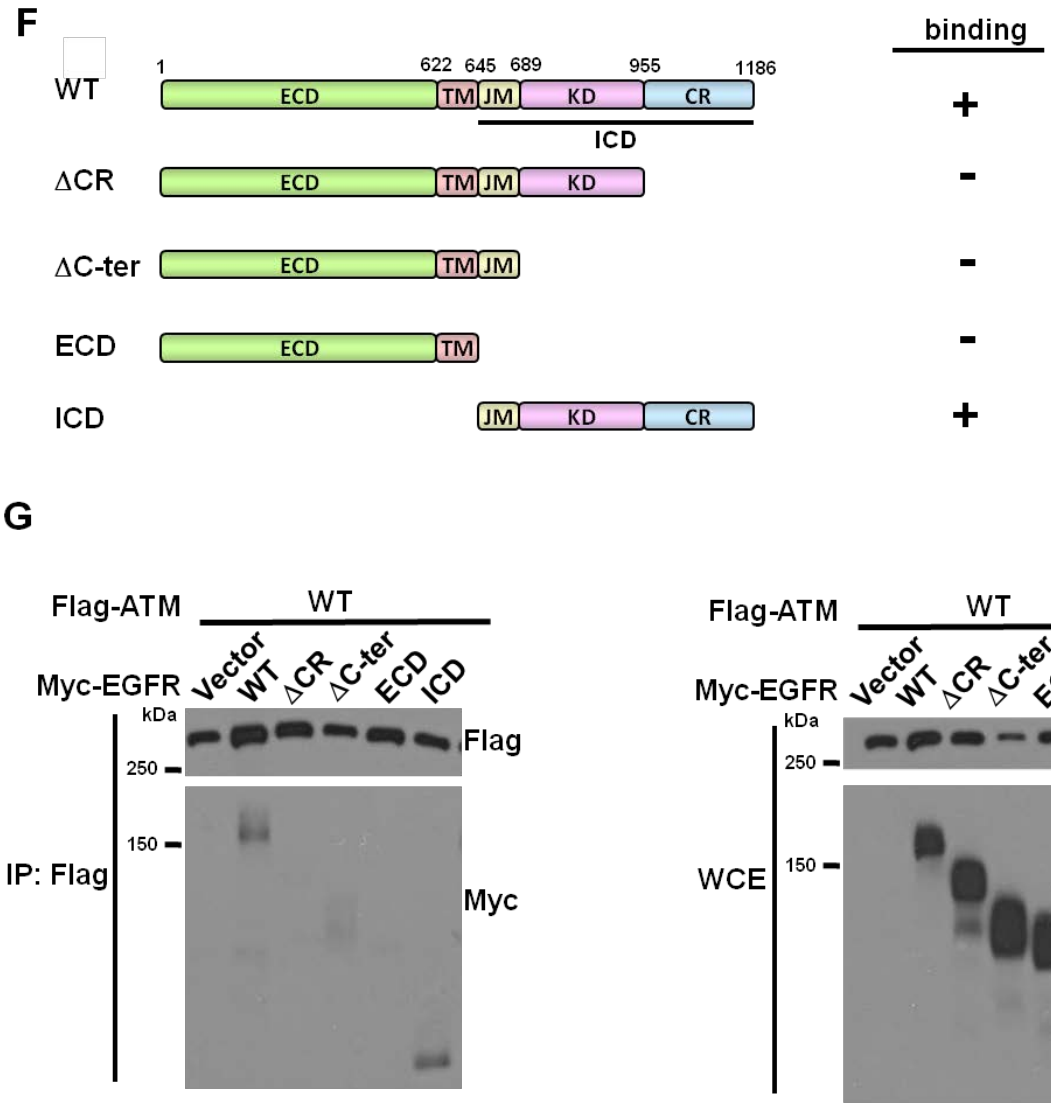
A

sequence	position	modification
IAKPNVSA**S**TQASR	Ser85	Phospho
SLEISQS**Y**TTTQR	Tyr370	Phospho
ST**T**PANLD**S**ESEHFFR	Thr1885, Ser1891	Phospho
SLAFEEG**S**QSTTISSLSEK	Ser1981	Phospho
QS**S**QLDEDRTEAANR	Ser2592	Phospho

B




C
GST
GST
ATM2
GST
ATM2/Y370F
D
GST
GST-ATM2
GST-ATM2/Y370F
EGFR kinase
+
+
+
kDa
50
100
In vitro kinase assay
GST-ATM2
EGFR kinase
50
25
Coomassie blue staining
GST-ATM2
GST
E
Gefitinib
IR
kDa
250
250
150
150
75
50
NE
p-ATM (S1981)
ATM
p-EGFR (Y1086)
EGFR
Lamin B
Tubulin
150
150
75
50
CE
p-EGFR (Y1086)
EGFR
Lamin B
Tubulin



Supplementary information, Figure S1. ATM is tyrosine phosphorylated at residue 370. **(A)** Mass spectrometry analysis results of immunoprecipitated (IP) exogenous Flag-tagged ATM from HeLa cell nuclear extracts after 10 Gy IR. Sequence: ATM peptide analyzed by mass spectrometry. Position: phosphorylation residue on ATM. **(B)** Alignment of ATM partial sequences from human to yeast. Red Y indicates human ATM tyrosine 370 (Y370) is conserved

among all mammalian species and yeast. Frog possesses shorter form of ATM, which lacks the N-terminal region. **(C)** Schematic of GST only (GST), GST-fused ATM2 and GST-ATM2-Y370F (GST-ATM2/Y370F) constructs. ATM2 indicates ATM residues 250-522. **(D)** Recombinant GST, GST-ATM2 and GST-ATM2/Y370F proteins were incubated with purified recombinant human EGFR kinase (His672-Ala1210) *in vitro*, analyzed by SDS-PAGE, and detected by γ-^{32}P exposure (kinase assay) or Coomassie brilliant blue staining. **(E)** HeLa cells were serum starved, treated with DMSO or gefitinib*, and stimulated with or without 10 Gy IR. The resulting cells were harvested for nuclear fractionation, followed by co-immunoprecipitation assays as shown in Figure 1D and 1E. NE, nuclear extract; CE, cytosolic extract. *Gefitinib actually significantly increases the levels of nuclear EGFR in HeLa cells, and this has been repeatedly observed. This is an interesting observation that will require further investigation. **(F)** Interactions between various EGFR domain structures and ATM. Myc-tagged EGFR wild type (WT); C-terminal regulatory region deletion (ΔCR); C-terminal deletion (ΔC-ter), intracellular domain deletion (ECD), and extracellular domain deletion (ICD). (+) or (−) on the right indicates positive or negative interaction, respectively. ECD: extracellular domain; TM: transmembrane; JM: juxtamembrane; KD: kinase domain; CR: C-terminal regulatory region; ICD: intracellular domain. **(G)** HEK 293T cells were transfected with plasmids as indicated and harvested for Co-IP assay followed by Western blot analysis after IR stimulation. WCE: whole cell extract.