

**A**

sequence	position	modification
IAKPNVSA <b>S</b> TQASR	Ser85	Phospho
SLEISQS <b>Y</b> TTTQR	Tyr370	Phospho
ST <b>T</b> PANLD <b>S</b> ESEHFFR	Thr1885, Ser1891	Phospho
SLAFEEG <b>S</b> QSTTISSLSEK	Ser1981	Phospho
Q <b>S</b> SQLEDRTTEAANR	Ser2592	Phospho

**B**

	Ser85	Tyr370
human ( <i>Homo sapiens</i> )	-----S <b>A</b> STQA-SRQKKM	SLEISQ---S <b>Y</b> TTTQRES
chimpanzee ( <i>Pan troglodytes</i> )	-----S <b>A</b> STQA-SRQKKM	SLEISQ---S <b>Y</b> TTTQRES
horse ( <i>Equus caballus</i> )	-----S <b>A</b> STQA-GRQKKM	SLEISQ---S <b>Y</b> ATTQREC
dog ( <i>Canis familiaris</i> )	-----S <b>A</b> STQA-TRQKKM	SLEISQ---S <b>Y</b> TTTQREF
rabbit ( <i>Oryctolagus cuniculus</i> )	-----S <b>A</b> STQA-SRQKKM	SLEISQ---S <b>Y</b> TTTQRES
mouse ( <i>Mus musculus</i> )	-----S <b>A</b> TTQS-SRQKKM	SVEISQ---S <b>Y</b> V-TQRES
frog ( <i>Xenopus tropicalis</i> )	-----S-----	-----S-----
fruitfly ( <i>Drosophila melanogaster</i> )	TQFNLEAGSQT-SGNGHF	-----KADEN
yeast ( <i>Saccharomyces cerevisiae</i> )	-----S <b>T</b> TNKL <b>S</b> LS <b>E</b> NRL	-LGITK <b>S</b> LL <b>T</b> <b>Y</b> FALNRKN

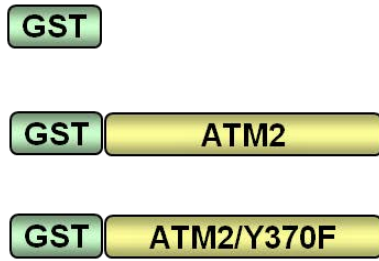
  

	Thr1885	Ser1891	Ser1981
human ( <i>Homo sapiens</i> )	TSRST <b>T</b> PANLD <b>S</b> ESEHFF	TSRST <b>T</b> PANLD <b>S</b> ESEHFF	SLAFEEG <b>S</b> QNTTISSLSE
chimpanzee ( <i>Pan troglodytes</i> )	TSRST <b>T</b> PANLD <b>S</b> ESEHFF	TSRST <b>T</b> PANLD <b>S</b> ESEHFF	SLAFEEG <b>S</b> QSTTISSLSE
horse ( <i>Equus caballus</i> )	TSRST <b>T</b> PANLD <b>S</b> ESEHIF	TSRST <b>T</b> PANLD <b>S</b> ESEHIF	SLTFEEG <b>S</b> QSTTISSLSE
dog ( <i>Canis familiaris</i> )	TSRST <b>T</b> PANLD <b>S</b> ESEHFF	TSRST <b>T</b> PANLD <b>S</b> ESEHFF	SLTFEEA <b>S</b> QNTAISSLSE
rabbit ( <i>Oryctolagus cuniculus</i> )	TSRST <b>T</b> PANLD <b>S</b> DSEHFF	TSRST <b>T</b> PANLD <b>S</b> DSEHFF	SLTFEEG <b>S</b> QNTTISSLSE
mouse ( <i>Mus musculus</i> )	ASRS <b>A</b> T <b>P</b> ANS <b>D</b> S <b>E</b> SENF <b>L</b>	ASRS <b>A</b> T <b>P</b> ANS <b>D</b> S <b>E</b> SENF <b>L</b>	SPTFEEG <b>S</b> QGTTISSLSE
frog ( <i>Xenopus tropicalis</i> )	-----S-----	-----S-----	-----S-----
fruitfly ( <i>Drosophila melanogaster</i> )	----T <b>A</b> P-----NSQ <b>E</b> IF	----T <b>A</b> P-----NSQ <b>E</b> IF	-----S-----
yeast ( <i>Saccharomyces cerevisiae</i> )	-----S-----	-----S-----	YLLFEEM <b>N</b> MPN-I-----

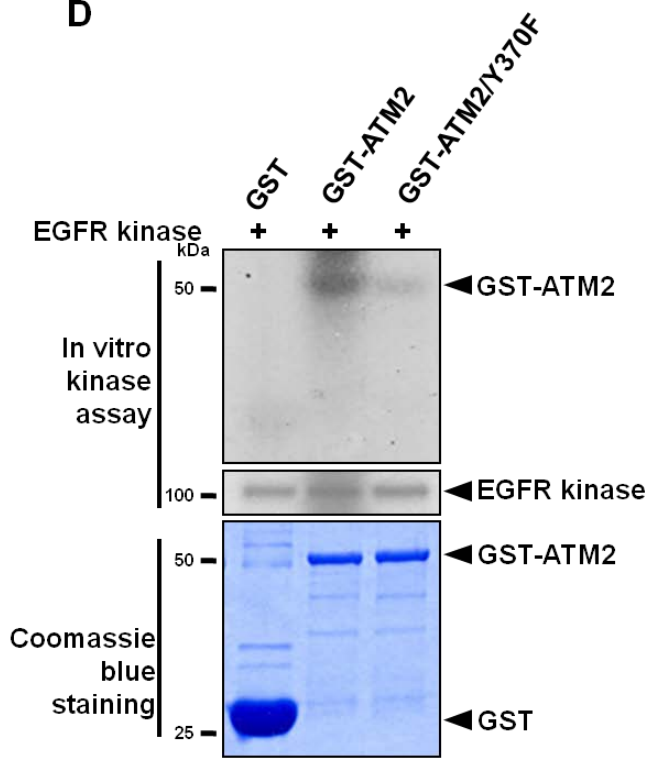
  

	Ser2592
human ( <i>Homo sapiens</i> )	ITKNVPK <b>Q</b> S <b>S</b> QLDED <b>R</b> TE
chimpanzee ( <i>Pan troglodytes</i> )	ITKNVPK <b>Q</b> S <b>S</b> QLDED <b>R</b> TE
horse ( <i>Equus caballus</i> )	ITKS <b>A</b> PK <b>Q</b> S <b>S</b> QLDED <b>R</b> TE
dog ( <i>Canis familiaris</i> )	ITKN <b>A</b> PK <b>Q</b> S <b>S</b> QLDED <b>R</b> TE
rabbit ( <i>Oryctolagus cuniculus</i> )	ITKN <b>A</b> PK <b>Q</b> S <b>S</b> QLDED <b>R</b> ME
mouse ( <i>Mus musculus</i> )	ITK <b>S</b> T <b>S</b> KEN <b>S</b> H <b>L</b> DED <b>R</b> TE
frog ( <i>Xenopus tropicalis</i> )	LTK <b>N</b> AP <b>K</b> Q <b>I</b> <b>S</b> QLDED <b>R</b> ME
fruitfly ( <i>Drosophila melanogaster</i> )	-----N <b>T</b> ER <b>S</b> G
yeast ( <i>Saccharomyces cerevisiae</i> )	-----K <b>I</b> Q

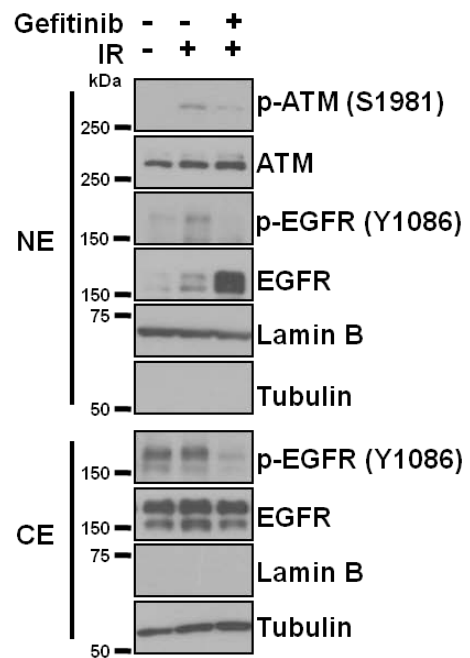
C

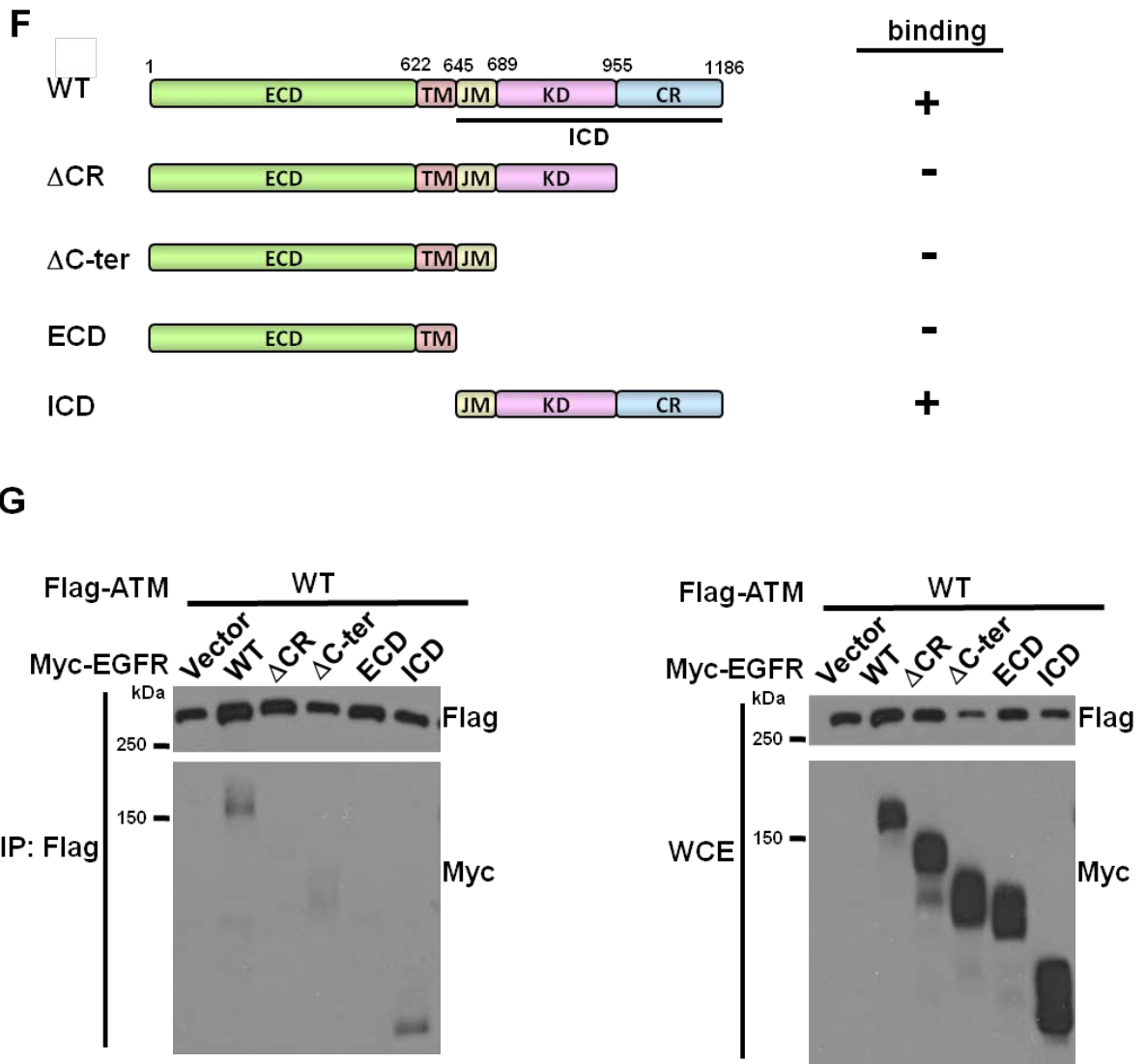


D



E





**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  $\gamma$ -<sup>32</sup>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 ( $\Delta$ CR); C-terminal deletion ( $\Delta$ C-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.