

SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

● Match to: EGFR_HUMAN Score: 3356
Epidermal growth factor receptor precursor (EC 2.7.10.1) (Receptor tyrosine-protein kinase ErbB-1)
Found in search of C:\Program Files\Matrix Science\Mascot Daemon\mgf\39
Untitled\mascot_daemon_merge.mgf

Nominal mass (M): 134190; Calculated pI value: 6.26

Taxonomy: [Homo sapiens](#)

Sequence Coverage: 56%

Matched peptides shown in **Red**

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1 MRPSGTAGAA LLALLAALCP ASRALEEKKV CQGTSNKLTQ LGTFEDHFLS
51 LQRMFNCEV VLGNLEITYV QRNYDLSFLK TIQEVAGYVL IALNTVERIP
101 LENLQIIRGN MYYENSYALA VLSNYDANKT GLKELPMRNL QEILHGAVRF
151 SNNPALCNVE SIQWRDIVSS DFLSNMSMDF QNHLGSCQKC DPSCPNGSCW
201 GAGEENCQKL TKIICAQQCS GRCRGKSPSD CCHNQCAAGC TGPRESDCLV
251 CRKFRDEATC KDTCPPLMLY NPTTYQMDVN PEGKYSFGAT CVKKCPRNYV
301 VDHGSCVRA CGADSYEMEE DGVRKCKKCE GPCRKVNGI GIGEFKDSLS
351 INATNIKHKF NCTISGDLH ILPVAFRGDS FHTPPLDPQ ELDILKTVKE
401 ITGFLLIQAW PENRDLHAF ENLEIRGRT KQHGQFSLAV VSLNITSLGL
451 RSLKEISDGD VISGNKNLC YANTINWKKL FGTSGQKTKI ISNRGENSCK
501 ATGQVCHALC SPEGCWGPEP RDCVSCRNVS RGRECVDKCN LLEGEPREFV
551 ENSECIQCHP ECLPQAMNIT CTGRGPDNCI QCAHYIDGPH CVKTCPAGVM
601 GENNTLVWKY ADAGHVCHLC HPNCTYGCTG PGLEGCPTNG PKIPSIATGM
651 VGALLLLLVV ALGIGLFMRR RHIVRKRTLR RLLQERELVE PLTPSGEAPN
701 QALLRILKET EFKIKVLGS GAFGTVYKGL WIPEGEKVKI PVAIKELREA
751 TSPKANKEIL DEAYVMASVD NPHVCRLLGI CLTSTVQLIT QLMPFGCLLD
801 YVREHKDNIG SQYLLNWCVQ IAKGMNYLED RRLVHRDLAA RNVLVKTPQH
851 VKITDFGLAK LLGAEEKEYH AEGGKVPIKW MALESILHRI YTHQSDVWSY
901 GVTWELMTF GSKPYDGIPA SEISSILEKG ERLPQPPICT IDVYMIMVKC
951 WMIDADSRPK FRELIIEFSK MARDPQRYLV IQGDERMHLP SPTDSNFYRA
1001 LMDEEDMDDV VDAEYLIPQ QGFFSSPSTS RTPLLSSLSA TSNNSTVACI
1051 DRNGLQSCPI KEDSFLQRYS SDPTGALTED SIDDTFLPVP EYINQSVPKR
1101 PAGSVQNPVY HNQPLNPAS RDPHYQDPHS TAVGNPEYLN TVQPTCVNST
1151 FDSPAHWAQK GSHQISLDNP DYQQDFFPKE AKPNGIFKGS TAENAEYLRV
1201 APQSSEFIGA
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Figure S1. MS analysis of EGFR. EGFR in the nuclear lysate was isolated by IP from MDA-MB 468 cells. The sequence coverage of EGFR was about 56% by tandem mass (MS/MS) spectrometric analysis.

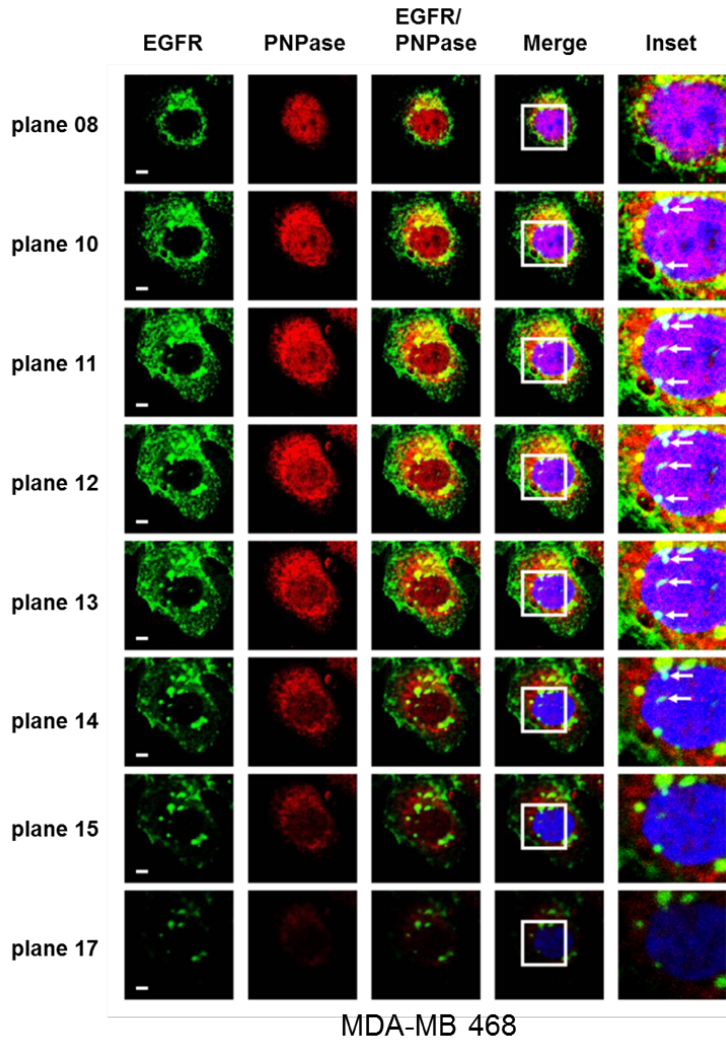


Figure S2. Co-localization of EGFR and PNPase in the nucleus at 10 min post-IR. MDA-MB 468 cells were treated with 4 Gy IR. After 10 min, cells were fixed with paraformaldehyde, examined by immunofluorescence staining with anti-EGFR antibody and anti-PNPase antibody, and observed under a confocal microscopy captured using a Zeiss LSM 710 laser microscope. For sequential photosections, a single cell was dissected into 27 focal sections with a thickness of 0.5 μm each. The nucleus (blue signal) spanned between focal planes 8-17. Co-localization of EGFR and PNPase in the nucleus, as the white spots indicated by arrows in the merged images, is shown from planes 10-14 in the insets. Boxed areas are shown in detail in the insets. Bar, 5 μm .

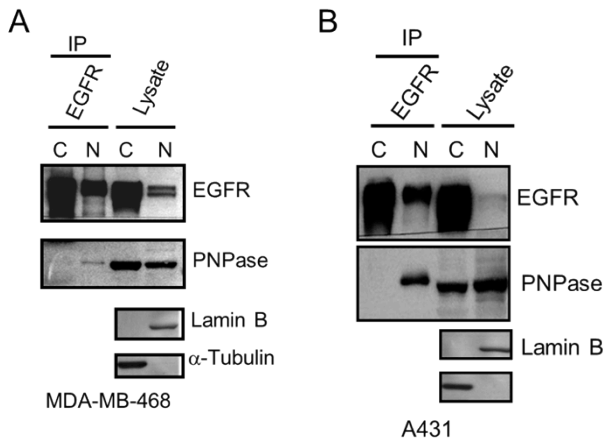


Figure S3. Nuclear EGFR interacts with PNPase. The interaction between EGFR and PNPase in cytosolic (C) or nuclear (N) lysates from MDA-MB 468 cells (A) and A431 cells (B) were verified by IP (immunoprecipitation)/IB (immunoblotting) with anti-EGFR/anti-PNPase antibody, respectively.

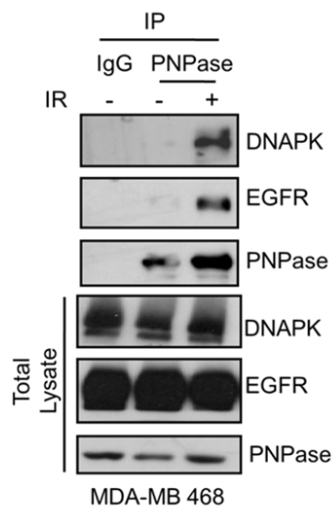


Figure S4. IR increases the association of PNPase/EGFR/DNAPK complex. MDA-MB 468 cells were irradiated with or without 4 Gy IR. After 10 min, total lysates were harvested and individually immunoprecipitated with antibodies against IgG or PNPase, followed by SDS-PAGE separation and IB (upper panel). The endogenous expression level of indicated protein was examined by IB (lower panel).

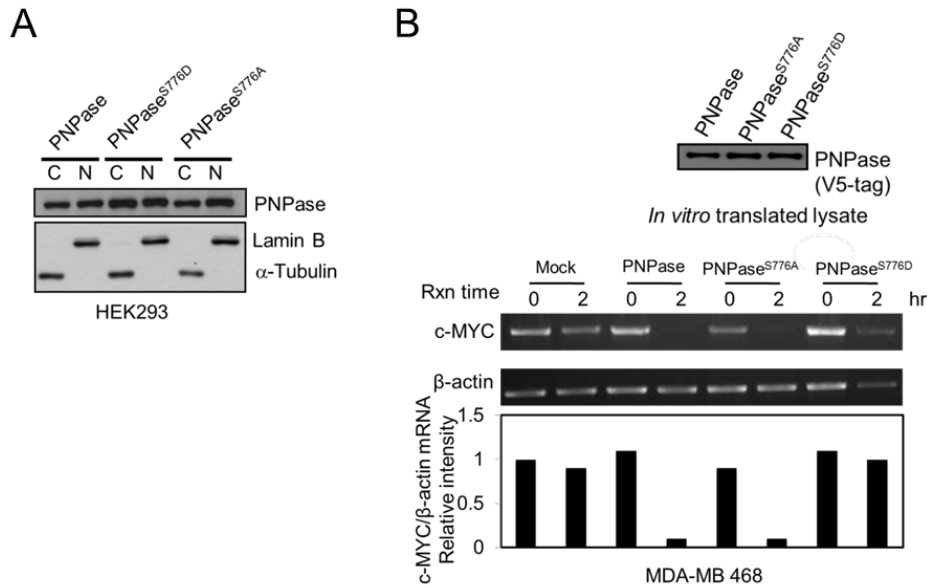


Figure S5. The enzymatic activity and nuclear translocation of PNPase. (A) HEK293 cells were transfected with the wild-type, S776A, or S776D mutant of PNPase plasmid. After 24h, the cell lysate was extracted as C: cytosol and N: nucleus fractions. The expression of exogenous PNPase variants in each fraction was determined by IB with anti-V5 antibody. (B) Each PNPase variant was *in vitro* translated by TNT coupled reticulocyte lysate system, and the expression of each recombinant PNPase protein was determined by IB (top). The ribonuclease activity of each *in vitro* translated PNPase was examined as aforementioned (bottom). The bar graph is the relative level of c-MYC mRNA normalized to β -actin.

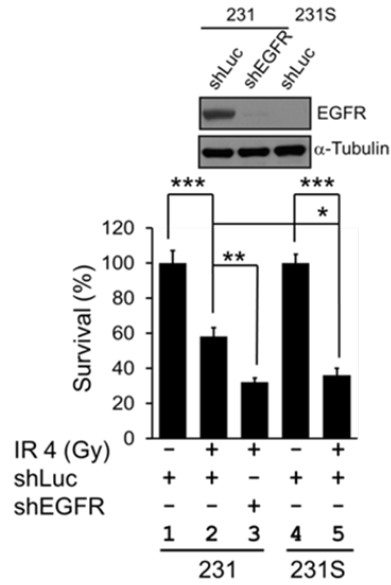


Figure S6. The enzymatic activity and nuclear translocation of PNPase. Each shRNA-infected cell lines of MDA-MB 231 radio-resistant (231) and radio-sensitive (231S) cells were irradiated with or without 4 Gy IR. Cell viability determined after 2 days. ***, $p < 0.005$, **, $p < 0.01$, *, $p < 0.05$ by *t*-test.