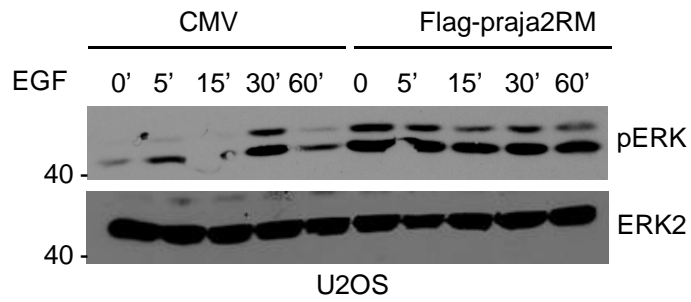
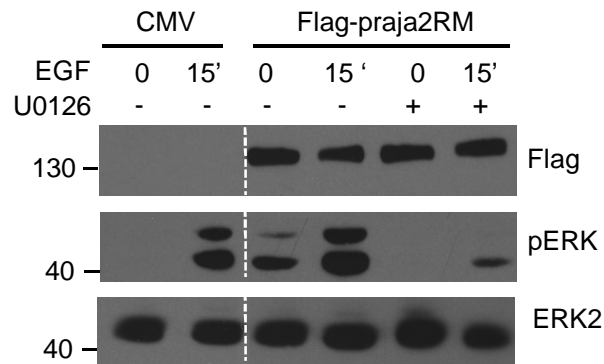


Supplementary figure S1. praja2 degrades KSR1 through the proteasome. Immunoblot of lysates from cells transiently transfected with Flag-praja2 vector (either wild type or ring mutant). Where indicated, the cells were treated with MG132 (10 μ M) and harvested at the indicated time points.

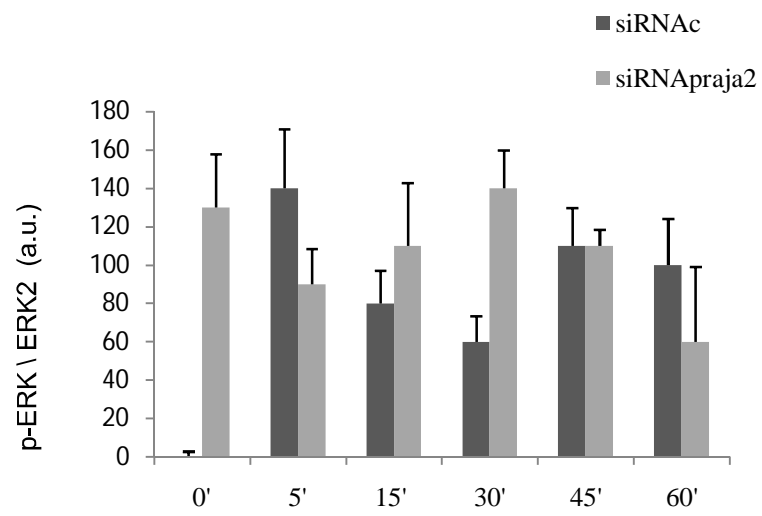
A



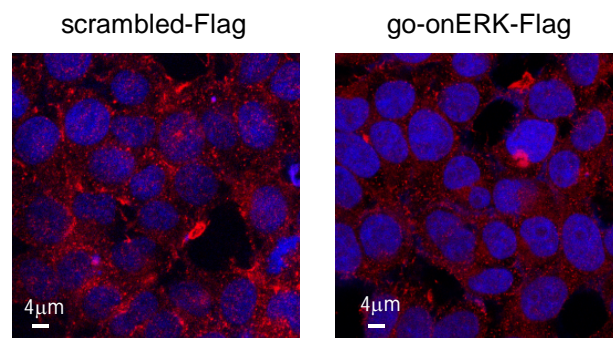
B



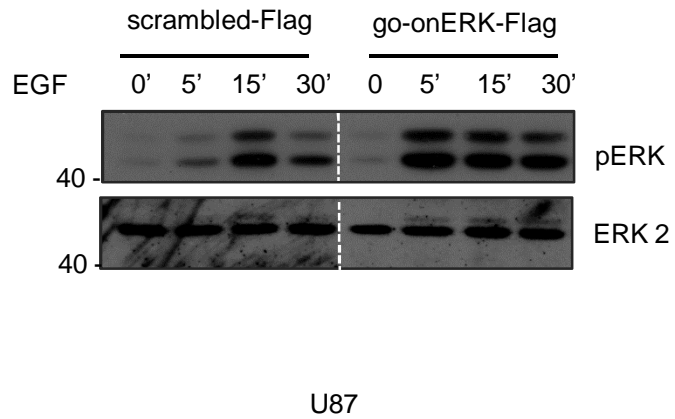
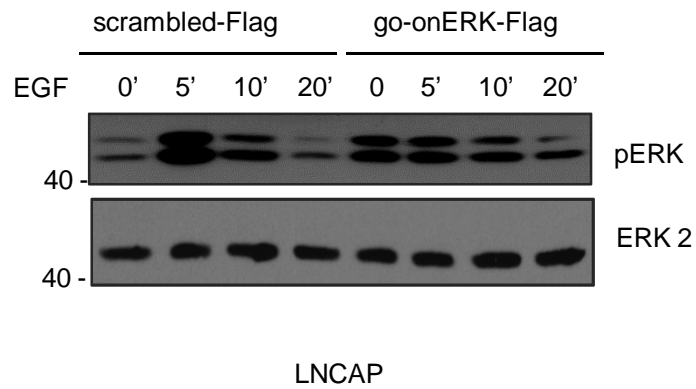
Supplementary Figure S2. praja2 regulates ERK phosphorylation by EGF. Osteosarcoma cells (U2OS) (**A**) or HEK 293 cells (**B**) were transiently transfected with empty vector (CMV) or flag-praja2rm (RM) vector and treated with EGF (100ng/ml). HEK293 are pretreated for 15 minutes with U0126 (5 μ m) (**B**). Lysates were immunoblotted with the indicated antibodies.



Supplementary figure S3. Quantitative analysis of experiment showed in 4C .



Supplementary figure S4. Entry of go-onERK-Flag into the cells. HEK293 cells, treated for eight hours with scrambled-Flag or go-onERK-Flag peptides, were immunostained with monoclonal anti-Flag antibody. Nuclei were stained with DRAQ5. Images were collected and analyzed by confocal microscopy.



Supplementary figure S5. LNCAP and U87 cells were pretreated (8 hours) with the synthetic peptides(1 μ M) (scrambled-Flag and go-onERK-Flag-Flag) and stimulated with EGF. Lysates were immunoblotted for P-ERK and ERK

Kinase domain of KSR2 is in PDB (2Y4I)
Sequence alignment with KSR1 shows very high conservation
Identified Peptide 862-**DLQERPFSS**-872 in KSR1 is analogous to 913-**EQEERPTFTT**-923

```

SP|Q8IVT5|KSR1_HUMAN MDRRAALFAAAMGKFKKCCGCCDAAAAECACAAASRALQCCGQLQFLIDISIGSLRCLRT 60
SP|Q6VAB6|KSR2_HUMAN -----MDE-----ENMTKSEEQQLSLQKALQCCQLVQNMIDLSISNLECLRT 43
          * *
          : : *
          : : *

SP|Q8IVT5|KSR1_HUMAN KCAVSNDLTQQEIRTLKLVRYICTQRQCKLSVAPGERTPELNSYPRFSDMLYTFNVRP 120
SP|Q6VAB6|KSR2_HUMAN KCATSNDLTQKQIRTLKLVRYIKYFSSRLSCKKVALQERNAELDGFQLRHMFRIVDVRK 103
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN EVVQEI-PRDLTLDALLEMNEAKVKETLRRCCAGCDECCRLQYALTCLRKVTGLGGEHKE 179
SP|Q6VAB6|KSR2_HUMAN EVLLEISPGQLSLEDLLEMTDEQVCETVRYKCANREECARLNASLSCLRNVMSGCGNLSK 163
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN DSSWSSLDARRESCGCPSTDTLSAASLPWPCSSQLG-RA-----GNSAQCF-- 225
SP|Q6VAB6|KSR2_HUMAN -QDWIQWPTTETCKENN-PVCPPEPTPWIRTELSQSPRVPSKCVQHYCHTSPTPGAPV 221
          * *
          * *

SP|Q8IVT5|KSR1_HUMAN ---RSISVSAALPASDSPTPSFSEGLSD-TCIFLEA-SCRLT--PRALHSFITPTTQQLR 278
SP|Q6VAB6|KSR2_HUMAN TEVDRLTVDAYPCLCFP--PPLSCHRSLFPSPFRQRAVARTPPRTFNIVITVTPCPTPMR 280
          * * * *
          * * * *

SP|Q8IVT5|KSR1_HUMAN RETELKFPRTPPPPSRKVFQLLPSPFTLRSKFSSESLQCNRIDVSSMR----- 327
SP|Q6VAB6|KSR2_HUMAN KKNLKPCTPPPSRKLHLIPCFALERSKFSSEFQLCHRVDKHAHTPKAKKSKPLMLK 340
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN -----FDSLSCSP 335
SP|Q6VAB6|KSR2_HUMAN IHSVCSCEMIPSQQRSPILLSERSLSRFVCGHAPFLPSTPPVETIANFSANTLSVPRMS 400
          * *
          * *

SP|Q8IVT5|KSR1_HUMAN QMVERDIGLSVTHRFSTKSWLSQVCHVCQKSMIFCVKCKHCRLECKNKCTKEAPACRISF 395
SP|Q6VAB6|KSR2_HUMAN QIPRDLGNSIKHRFSTKYWMSQTCVCGKGLFGLKCNCKFLCKNKCTKEAPCHLLI 460
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN LP---LTRLRTEVSPSDINNVDRAAEPHFC---TLPK-ALTKKHPFAMHLDSSSN 447
SP|Q6VAB6|KSR2_HUMAN IERGDPARLVRTESVPCDINNPLRKPFRYSDLISQLPKTKNKIKDHFVPTQPDSSSN 520
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN P8STTSSTPSSPAPFPTSSNFPSSATTPMPSPGQDRSRFNFAAYFIHERQQEIFFVPSA 507
SP|Q6VAB6|KSR2_HUMAN P8STTSSTPSSPAPFLPSPATPPSPLEPSPQCTRQWFMNLPASHYTKYKQEIFFVVP 580
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN GRCWKLLIAESLKENAFNISAFANAAPLPEAADCTRLDDQPKADVLEAEAEAEPEAC 567
SP|Q6VAB6|KSR2_HUMAN VFET-----PTRAPQVILHPVTSNPILEGNPLQIEVEPTS-----EN---EEV 621
          * *
          * *

SP|Q8IVT5|KSR1_HUMAN KSEAEDDEDEVDDLPSRRRPHRCPISEKASQTSVYLQEWDPFQVELCEPIGOCGRMCRV 627
SP|Q6VAB6|KSR2_HUMAN HDEAEYSEDDFEEMLSL-LSARSFPRKASQTSIFLQEWDPFQELICELIGCRFGQV 680
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN HRCRWGCEVAIRLLEMDGCHQOHLKLFKIVMNYRQTRRENVVLFMGACMNPPLAITTS 687
SP|Q6VAB6|KSR2_HUMAN YHCRWGEVAIRLIDIERDNEQLKAFKREVMATRQTRRENVVLFMGACMSPPLAITTS 740
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN LCKCRTLSFVDRDPTSLDINKTRQIAQEIINGMCYLEAKCIVRKLKSRNVFYDNCKVV 747
SP|Q6VAB6|KSR2_HUMAN LCKCRTLYSVVRDARIVLDVNRQIAQEIIVFGMCYLEAKCILEFDLRSRNVFYDNCKVV 800
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN ITDFLFCISCVVRECRRENQLKLSHDWLCYLAPEIVREMPCKDEEDQLPFSKAADVYAF 807
SP|Q6VAB6|KSR2_HUMAN ITDFLFSISCVLQACRRDFLRIQNCWLCHLAPEIIRQLSPDTEEDKLPFSKESDVEAL 860
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN CTVWYELQARDWPLENQAFAASIWQIGSGECKRVLTSVSLCKEVSEILSACNAFDLQER 867
SP|Q6VAB6|KSR2_HUMAN CTIWYELAREWPFKTQPAEATIWQMT--CHEFNLSQIGMKKEISDILLFCNAFEQEER 918
          * * * * *
          * * * * *

SP|Q8IVT5|KSR1_HUMAN DFEGLMDMLEKLPENRRLSHPCHFWRBADINGSKVPRFERFGLQVLESNPFM 923
SP|Q6VAB6|KSR2_HUMAN DFEGLMDMLEKLP----- 950
          * *
          * *

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Supplementary figure S6. alignment of human KSR1 (KSR1_HUMAN, Q8IVT5) with KSR2 sequence (KSR2_HUMAN, Q6VAB6)