

Supplementary Data

ZNF238 antibody production. To generate polyclonal antibodies in rabbits against ZNF238, the C-terminal part of the protein, comprising the last 15 amino acids (CVRDWTLEDSSQELWK) was synthesized and conjugated to KLH before injection (OpenBiosystems). The specificity of the antibody was tested on a protein lysate obtained from HEK293T cells stably expressing ZNF238 with a Flag tag at its C-terminal. The protein lysate was analysed by western blot either with Flag Ab (1/5000), ZNF238 Ab (1/5000) or ZNF238 Ab (1/5000) plus peptide used for rabbit immunization (10 μ L of 5mg/mL).

Phosphorylation assay. 293T cells were transfected with ZNF238-Flag for 24 hours. ZNF238-Flag was immunoprecipitated from whole lysate with either the ZNF238 antibody followed by incubation with protein A sepharose beads or with FlagM2 beads. The immunocomplexes were divided into three equal samples and incubated 20 minutes at 37°C with calf intestinal phosphatase (CIP) buffer (promega) (10ul) or CIP buffer (10uL) plus CIP (10uL of 1U/uL) or CIP buffer plus CIP and PBS. The samples were then analyzed by Western blot.

SupplementaryFigure 1: The sequence of ZNF238 is highly conserved during evolution.

The amino-acid sequence of mouse ZNF238 protein (NP_038943.3) is shown aligned with its human ortholog (NP_006343.2) using the Clustal multiple sequence alignment program. 519 amino acids /522 are identical (*) between the two sequences.

Supplementary Figure 2: ZNF238 antibody characterization

Western blot of total protein lysate from control HEK293T cells (lanes CTR) or HEK293T stably transfected with ZNF238-Flag (lane SL).

A) The ZNF238 antibody recognizes 2 main protein isoforms at the same size to those recognized by the anti-Flag tag antibody. The ZNF238 antibody also detects a non-specific band at about 60kDa (asterisk). The specificity of this antibody is also demonstrated in the competition experiment (right panel) with the peptide used to generate the ZNF238 antibody in rabbits. In this experiment the ZNF238 antibody was pre-incubated for 1 h with 50 μ g of the ZNF238 C-terminal peptide used for the rabbit immunizations.

B) The upper band of the doublet recognized by the ZNF238 antibody is highly reduced following phosphatase treatment.

Supplementary Figure 3: ZNF238 expression suppresses U87MG cell colony formation.

Re-expression of ZNF238 following doxycycline treatment (+DOX) in a clone of U87MG cells is able to highly decrease colony formation when compared with untreated cells (-DOX).

NP_038943.3 (Mouse) MEFPDHSRHLQCLSEQRHQGFLCDCTVLVGDAQFRAHRAVLASCSMYFHLFYKDQLDKR 60
 NP_006343.2 (Human) MEFPDHSRHLQCLSEQRHQGFLCDCTVLVGDAQFRAHRAVLASCSMYFHLFYKDQLDKR 60

NP_038943.3 DIVHLNSDIVTAPAFALLEFMYEGKLFKDLPIEDVLAASYLHMYDIVKVCCKKLKEK 120
 NP_006343.2 DIVHLNSDIVTAPAFALLEFMYEGKLFKDLPIEDVLAASYLHMYDIVKVCCKKLKEK 120

NP_038943.3 ATTEADSTKKEEDASSCSKVESLSDGSSHMAGDLPSDEDEGEDDKLNILPSKRDLAAEP 180
 NP_006343.2 ATTEADSTKKEEDASSCSKVESLSDGSSHMAGDLPSDEDEGEDDKLNILPSKRDLAAEP 180
 *****:*****:*****

NP_038943.3 GNMWMRLPSDSAGIPQAGGEAEPHATAAGKTVASPCSSTESLSQRSVTSVRDSADVDCVL 240
 NP_006343.2 GNMWMRLPSDSAGIPQAGGEAEPHATAAGKTVASPCSSTESLSQRSVTSVRDSADVDCVL 240

NP_038943.3 DLSVKSSLSGVENLNSSYFSSQDVLRSNLVQVKVEKEASCDESVDVGTNDYDMEHSTVKES 300
 NP_006343.2 DLSVKSSLSGVENLNSSYFSSQDVLRSNLVQVKVEKEASCDESVDVGTNDYDMEHSTVKES 300

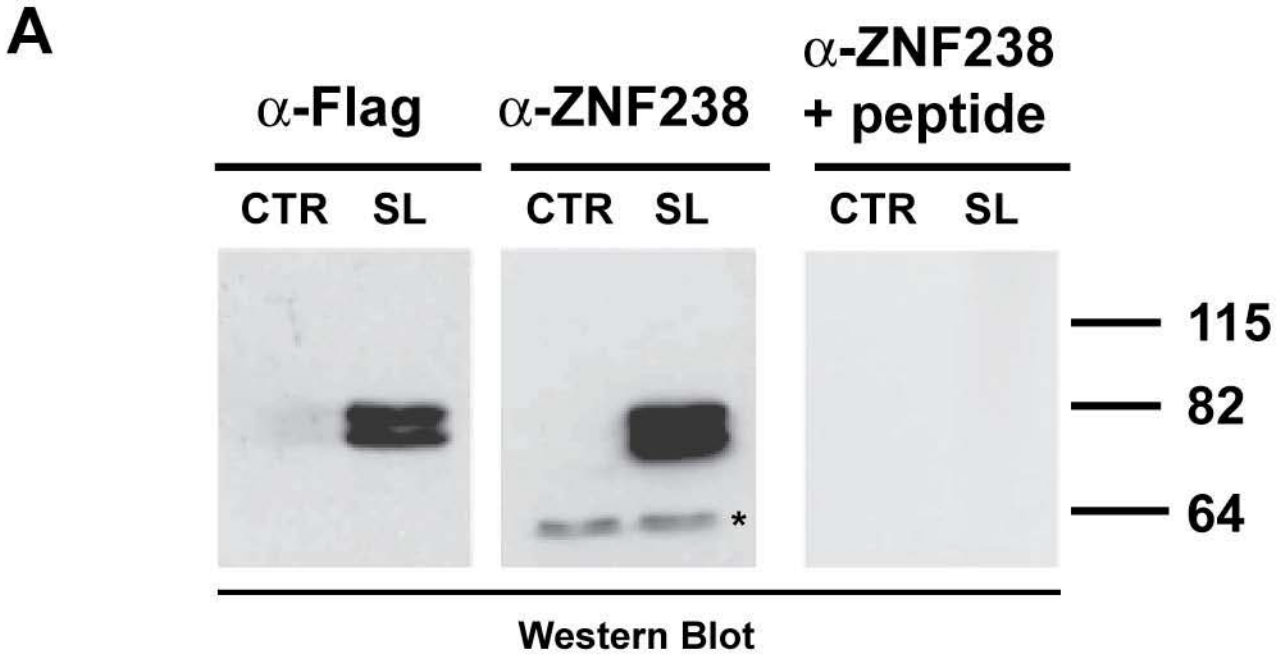
NP_038943.3 VSTNNRVQYEPAHLAPLREDSVLRDREDKASDDEMMTPESERVQVEGGMENSLLPYVS 360
 NP_006343.2 VSTNNRVQYEPAHLAPLREDSVLRDREDKASDDEMMTPESERVQVEGGMENSLLPYVS 360

NP_038943.3 NILSPAGQIFMCPLCNKVPFSPHILQIHLSTHFREQDGIRSKPAADVNVPTCSLCKGKTF 420
 NP_006343.2 NILSPAGQIFMCPLCNKVPFSPHILQIHLSTHFREQDGIRSKPAADVNVPTCSLCKGKTF 420

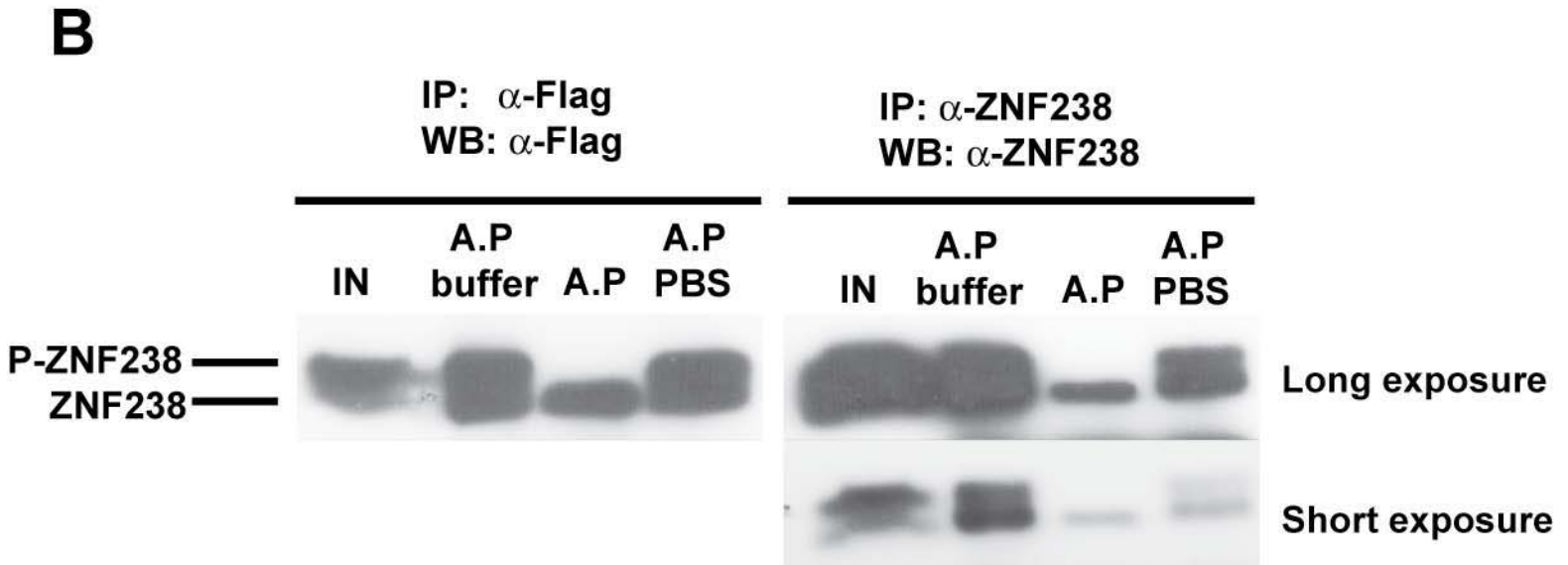
NP_038943.3 CMYTLKRHERTHSGEKPYTCTQCGKSFQYSHNLSRHAVVHTREKPHACKWCERRFTQSGD 480
 NP_006343.2 CMYTLKRHERTHSGEKPYTCTQCGKSFQYSHNLSRHAVVHTREKPHACKWCERRFTQSGD 480

NP_038943.3 LYRHIRKHFCELVNSLSVKSEALSLPTVRDWTLEDSSQELWK 522
 NP_006343.2 LYRHIRKHFCELVNSLSVKSEALSLPTVRDWTLEDSSQELWK 522

"*" : identical residues
 ":" : conserved substitutions
 "." : semi-conserved



CTR: HEK293T cells
 SL: HEK293T clone expressing ZNF238-Flag



U87MG

