

Supplementary Figures and text

Title : Robust expression of LINE-1 retrotransposon encoded proteins in oral squamous cell carcinoma

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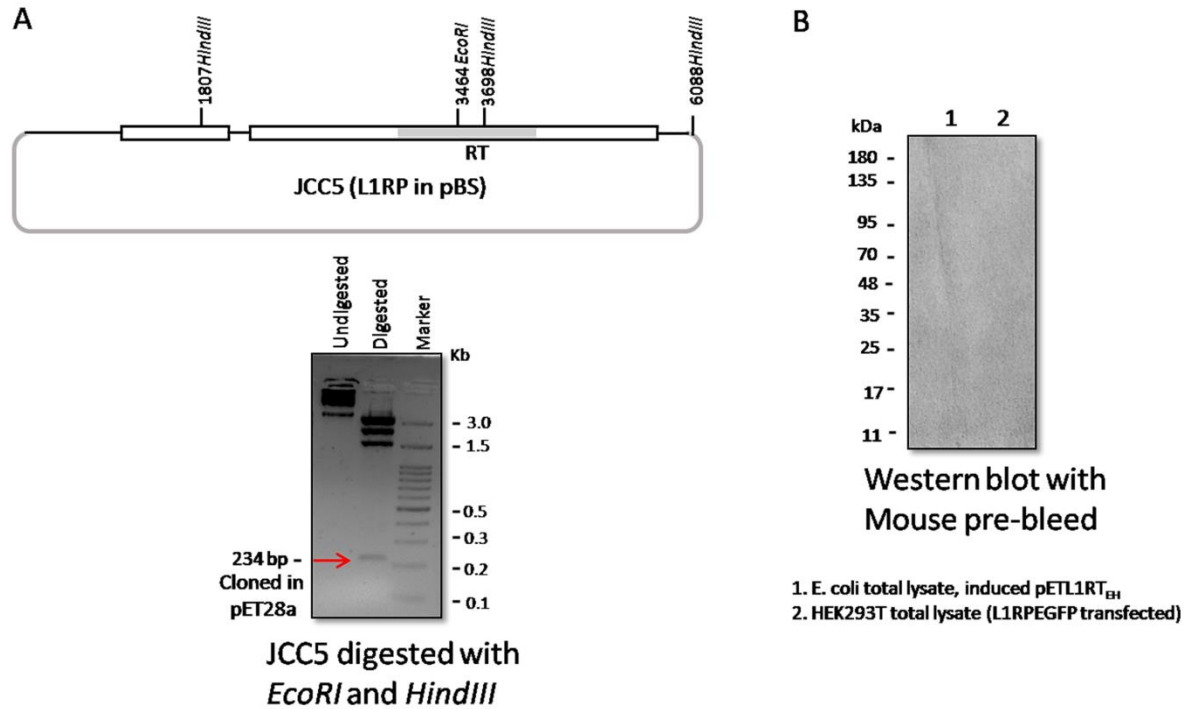
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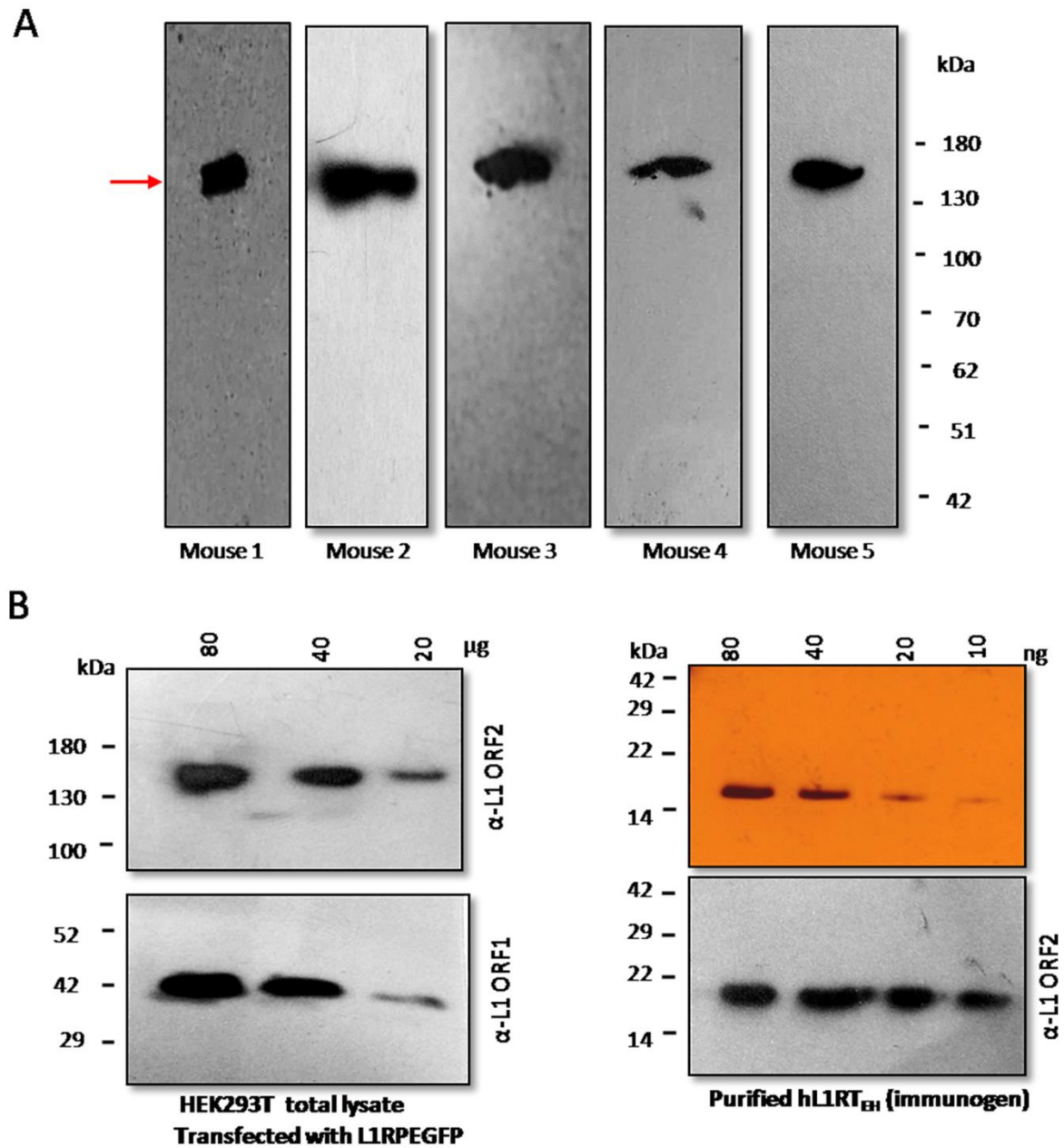
± These authors contributed equally

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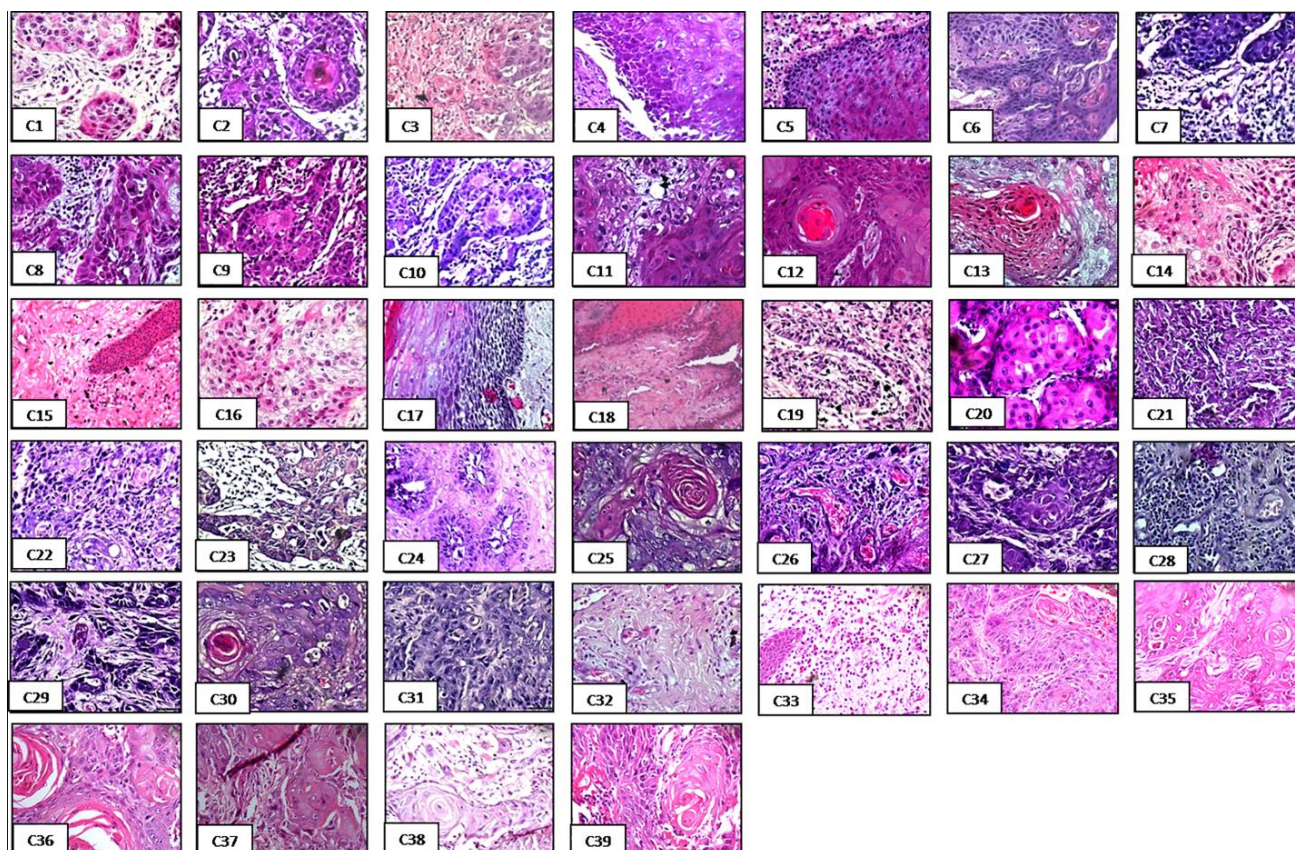
Supplementary Figures :



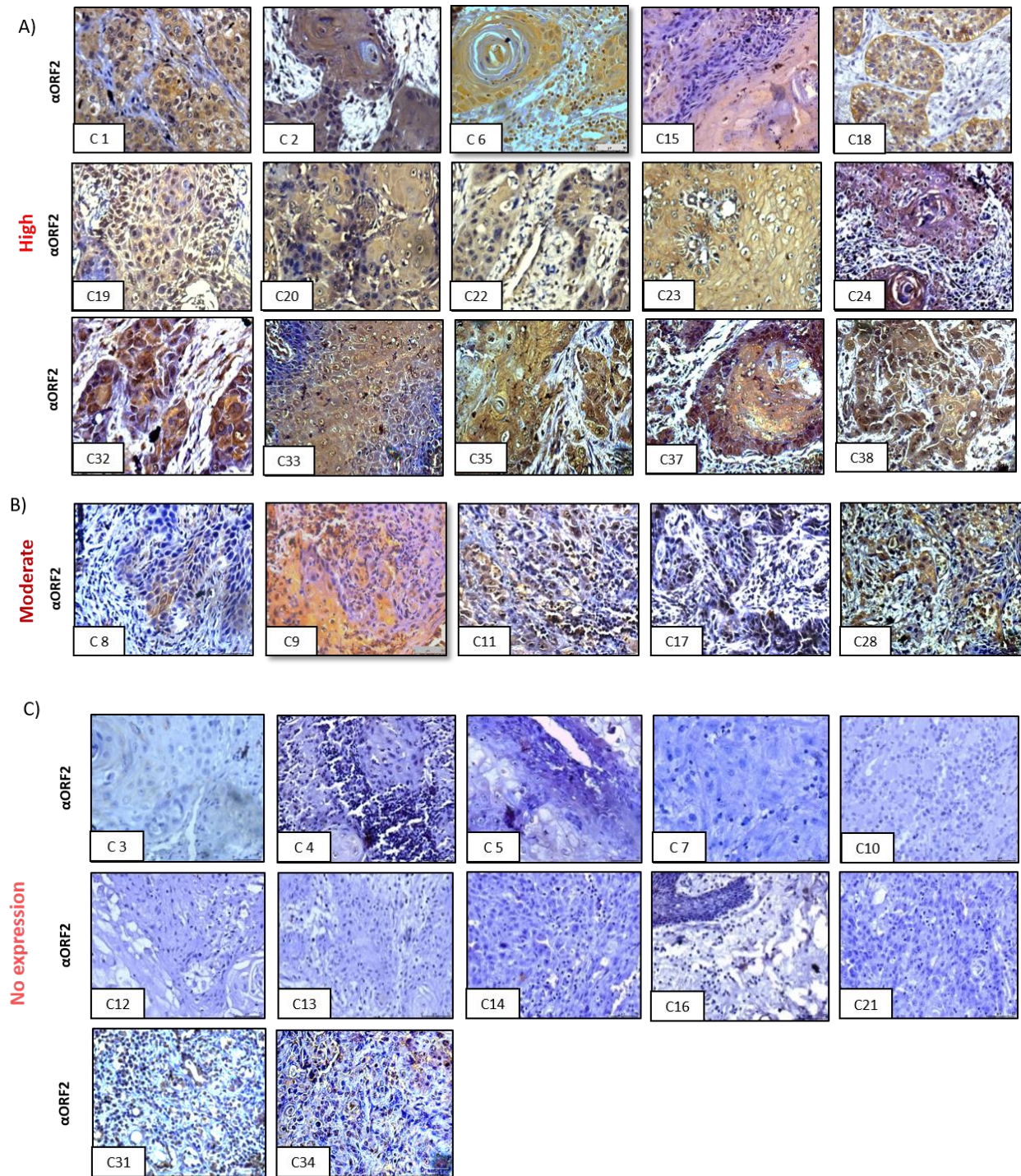
Supplementary figure 1: (A) Schematic map of JCC5 (L1RP in pBSKS plasmid) [37] and its restriction digestion with *EcoRI* and *HindIII* restriction enzymes. The digested product was resolved in 1.5% agarose gel. The 234 bp RT fragment (marked by arrow) was subcloned in Pet28a bacterial expression vector. (B) Western blot of L1RT_{EH} (immunogen) and full length exogenous L1ORF2p with non-immune mice sera.



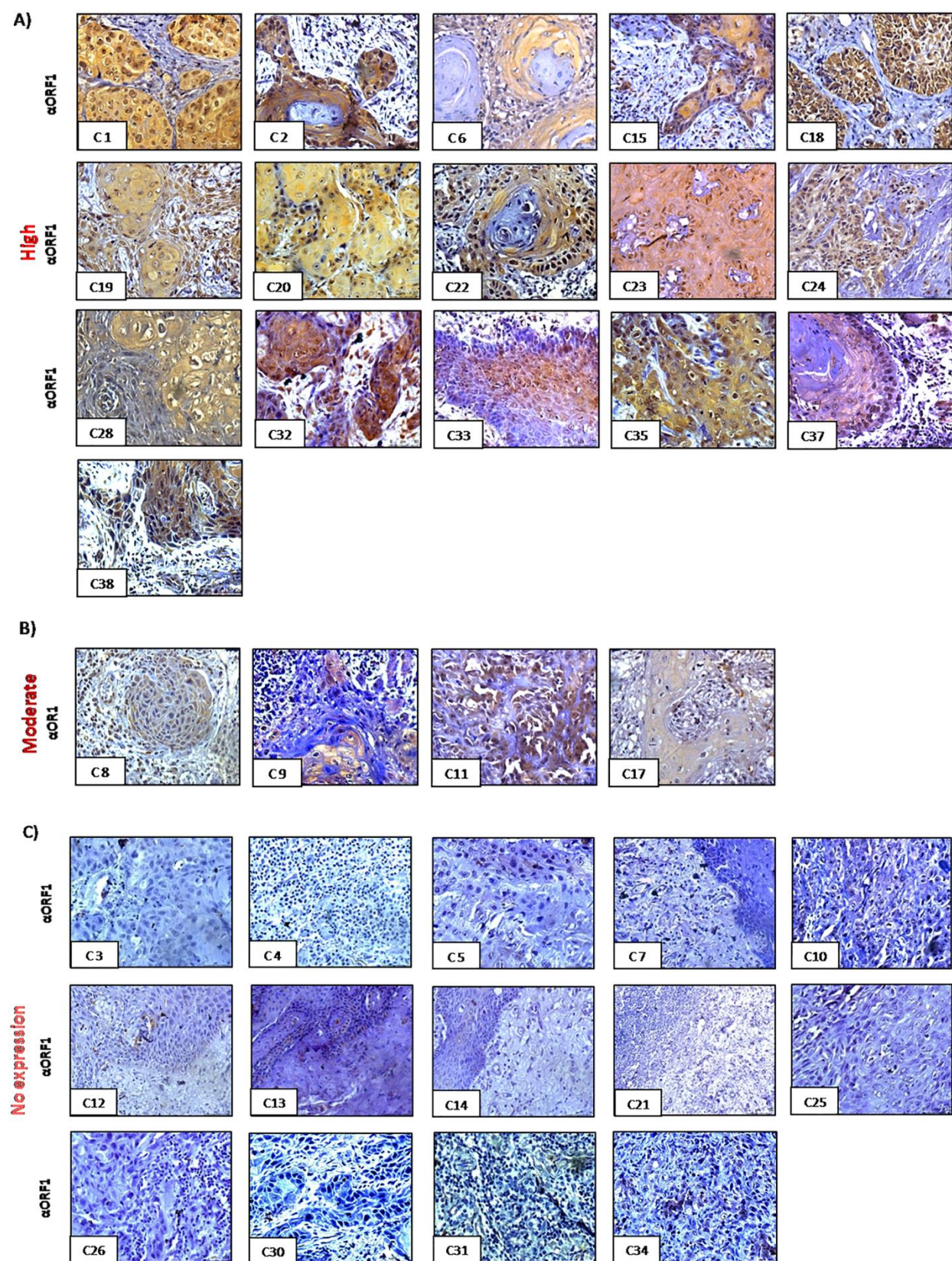
Supplementary figure 2: (A) Immune sera from five different mice were checked by Western blot showing a distinct 150 kDa band of exogenous L1ORF2p in HEK293T total lysate transfected with L1RPEGFP [38]. (B) Sensitivity analysis of L1 ORF2p antibody by Western blot and silver staining.



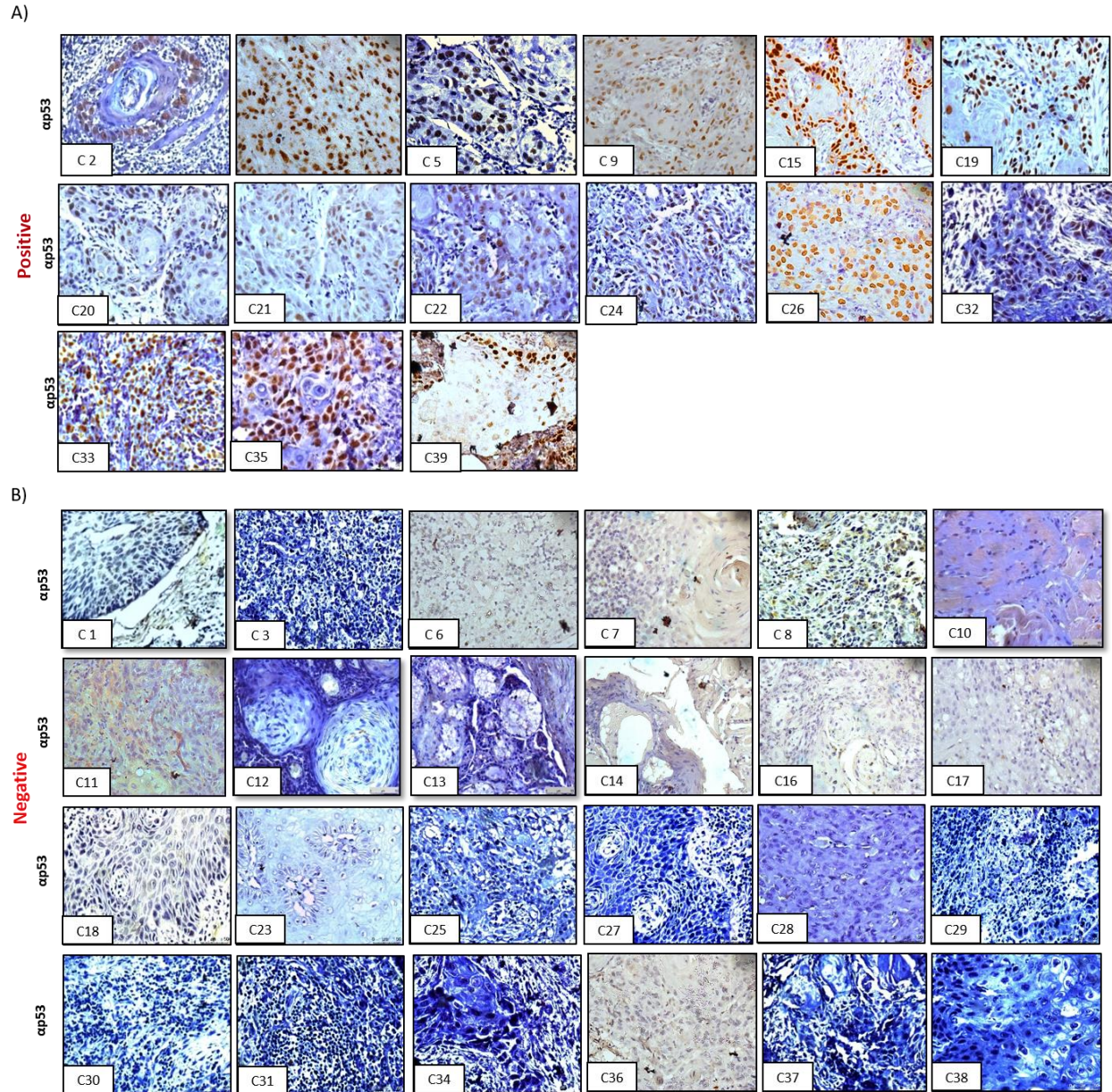
Supplementary figure 3: Hematoxylin-eosin stained section of post-operative OSCC samples.



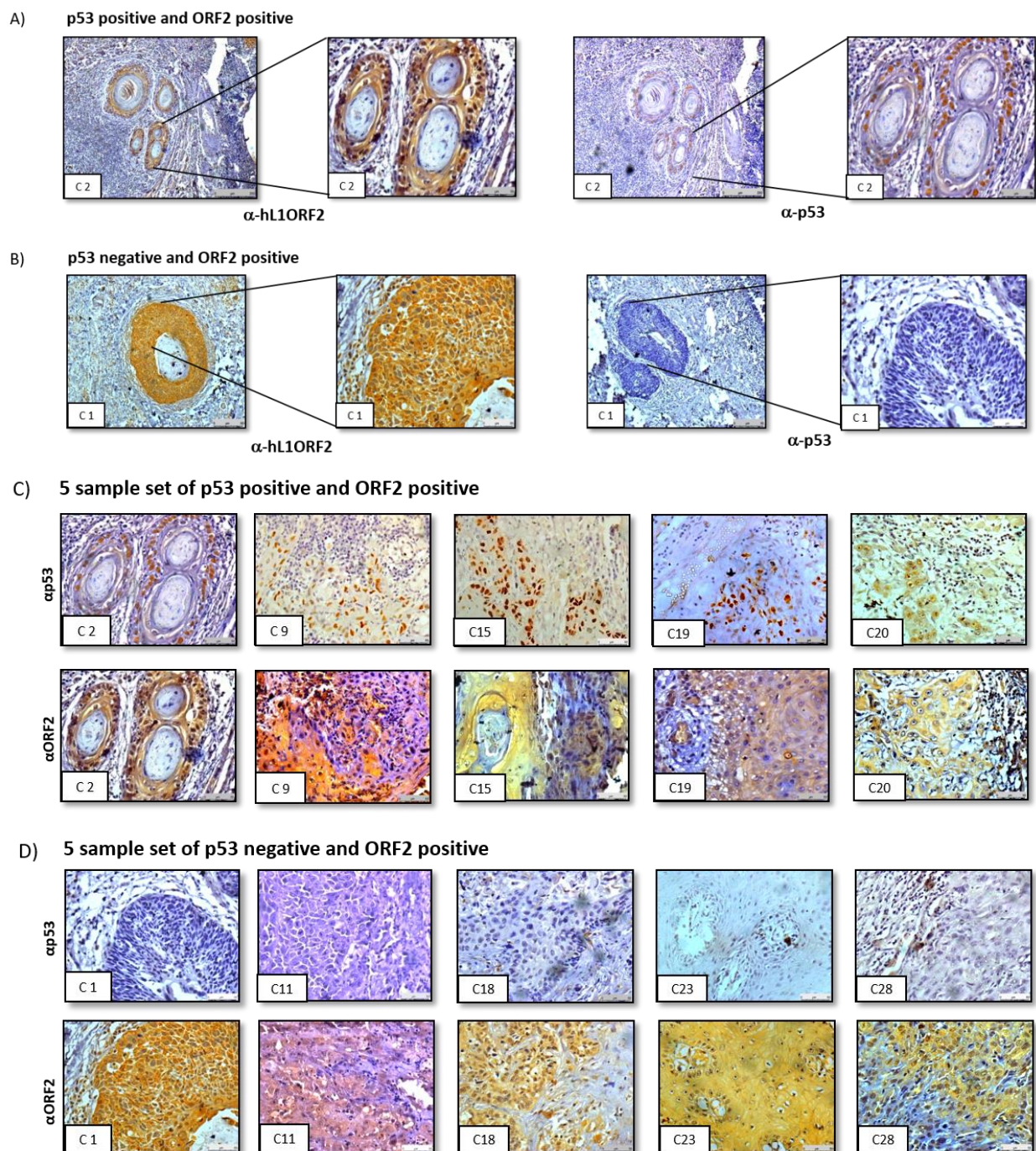
Supplementary figure 4: IHC analysis of L1ORF2p expression in post-operative OSCC samples. (A) High L1ORF2p expression. (B) Moderate expression of L1ORF2p. (C) Less or no expression of L1ORF2p.



Supplementary figure 5: IHC analysis of L1ORF1p expression in post-operative OSCC samples. (A) High L1ORF1p expression. (B) Moderate expression of L1ORF1p. (C) Less or no expression of L1ORF1p.



Supplementary figure 6: IHC analysis of p53 expression in post-operative OSCC samples. (A) Samples showing positive p53 expression. (B) Samples showing negative p53 expression.



Supplementary figure 7: IHC analysis of L1-ORF2p expression in p53 positive and p53 negative samples of OSCC. (A) IHC analysis of L1-ORF2p expression in p53 positive sample (C2). (B) IHC analysis of L1-ORF2p expression in p53 negative sample (C1). (C) Representative of five samples (C2, C9, C15, C19 and C20) showing L1-ORF2p expression in p53 positive

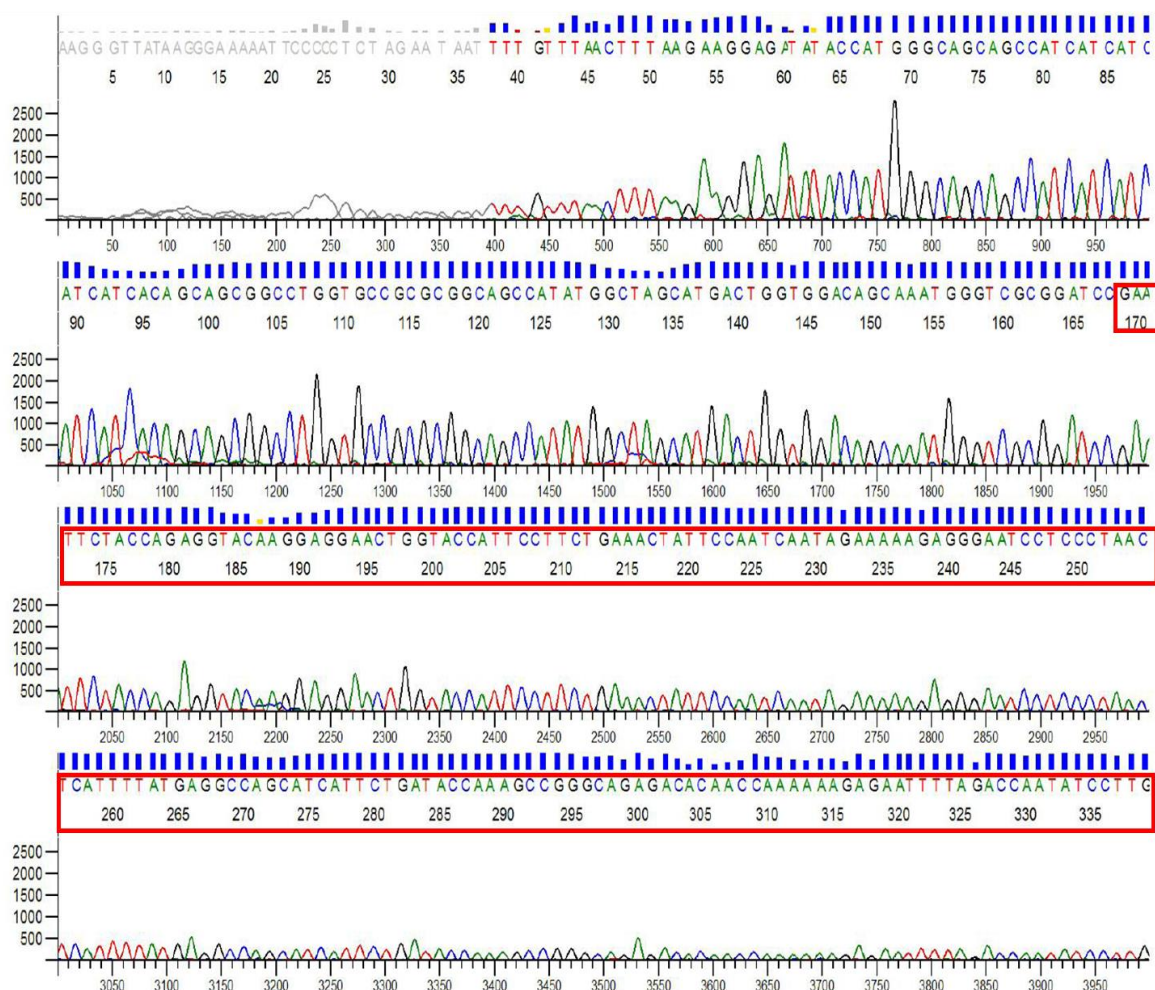
samples. (D) Representative of five samples (C1, C11, C18, C23 and C28) showing L1-ORF2p expression in p53 negative samples.

Supplementary text:

Cloned in pET 28a between ECOR1 and Hind III:

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAG
 CCATATGGCTAGCATGACTGGTGGACAGCAATGGGTGCGGGATCC GAATTCTACC
 AGAGGTACAAGGAGGAAGTGGTACCATTCTTCTGAACTATTCCAATCAATAGAA
 AAAGAGGGAATCCTCCCTAACTCATTATGAGGCCAGCATATTCTGATACCAAAG
 CCGGGCAGAGACACAACCAAAAAAGAGAATTTAGACCAATATCCTTGATGAACAT
 TGATGCAAAAATCCTCAATAAAATACTGGCAAACCGAATCCAGCAGCACATCAAA A
AGCTTGCGGCCGCACTGGAGCACCACCACCACCACTGA

Chromatogram from sanger sequencing of recombinant ORF2 protein:



Red box indicates the cloned sequence

Rat L1 (Acc No. DQ100473.1)(Kirilyuk et al. NAR 2008)

MGKKQNRKTGNSKTQSASPPPKERSSSPATEQSWMENDFDELREEGFRRSNYSELREDIQTGKGEVENFEKNLEECITR
ITNTEKCLKELMELKTKARELREECRLRSRCDQLEERVSAMEDEMNMKREGKFREKRIKRNEQSLQEIWDYVKRPNL
RLIGVPESDVENGTKLENTLQDIIQENFPNLARQANVQIQEIQRTPQRYSSRRATPRHIIVRFTKVEMKEKMLRAAREKG
RVTLKGKPIRLTADLSAETLQARREWGPIFNILKEKNFQPRISYPAKLSFISEGEIKYFIDKQMLRDFVTTTRPALKELLKEALN
MERNNRYQPLQNHAKM

MAKGKRKNPTNRSQDHSPSSEPRTPSPNPGHPNTPEKVDVDLKayLMMVEDIKKEFNNSLKEIQENTAKELQVLKE
KQENTAKELQVLKEKQENTTKQVEVLIEKQENTSKQVMEMNKITLCLKREVDTIKKTQSEATLEIETLGKKS GTIDASISN
RIQEMEERISGAEDSIENIGTTIKENGKCKILTQNIQEIQDTMRRPNLRIIGVDENEDFQLKGPANIFNKIIEENFPNLKKE
MPMNIQEAYRTPNRLDQKRNSSRHIIIRTPNALNKDRILKAVREKGQV TYKGKPIRITPDFSPETMKARRAWTDVIQTLR
EHKQPRLLYPAKLSITIDGETKVFHDKTKFTHYLSN PALQRIITEKKQYKDG NHALEKTRR

MARGKRRNPSNRNQDCMPSSEPNSPAKTNMEYPNTPEKQDLVSKSYLIMMLEDFKKDMNTLRETQEIIINKQVEAYRE
EWQKSLKEFQENTIKQMKELKMEIEAIKKEHMETTLDIENQKKRQGAVDTSFTNRIQEMEERISGAEDSIEIDSTVKDN
VKRRKLLVQNIQEIQDSMRRSNLRIIGIEESEDSQLKGPVNIFNKIIENFPNLKKEIPIGIQEAYRTPNRLDQKRNTSRHIIV
KTPNAQNKERILKAVREKQVQTYKGRPIRITPDFSPETMKARRSWTDVIQTLREHKCQPRLLYPAKLSINIDGETKIFHDK
TKFTQYLSTNPALQRIINGKAQHKEASYTLEARN

Yellow shaded part from human L1ORF1 was used to make L1ORF1 specific antibody

10

>Rat L1ORF2

MNIKGNNNHYSLSLNINGLNSPIKRHRLTNWIRNEDPAFCCLQETHLRDKDRHYLRVKGWKTTFQANGQKKQAGVAI
 LISNKNFQLKVIKKDKEGHFIFIKGKIHQDELSILNIYAPNTRAPTYVKETLLKLKTHIAPHTIIVGDFNTPLSSMDRSWKQK
 LNSDVDRLREVMSQMDLTDIYRTFYPAKAGYTFFSAPHGTFSKIDHIIQKQTGLNRYRKIEIIPCVLSDHHGLKLVFNNNK
 GRMPTYTWKLNNALLNDNLVKEEIKKEIKNFLEFNENENTTYSNLWDTMKAVLRGKLIASACRKKQERAYVSSMTAHL
 KALEQKEANTPRRSRRQEIIKLRAEINQVETKRTIERINRTKSWFFEKINKIDKPLARLTRGHRECVQINKIRNEKGDITDS
 EEIQKIIRSYYKNLYSTKFENLQEMDYFLDRYQVSKLNQEQLNLPITPKEIEAVIKGLPTTKSPGPDGFSAEFYQTFIED
 LIPILSKLFHKIETDGALENSFYESTITLIPKPHKDTTKKGNFRPISLMNIDAKILNKILANRIQEHKTIHHHDQVGFIPGMQG
 WFNIRKTINVIHYINKLKEQNHMIISLDAEKAFDKIQHPFMIKVLERIGIQGPYLNIVKAIYSKPVANIKLNGEKLEAIPLKSG
 TRQGCPSPYLFNIVLEVLARAIQQKEIKGMQIGKEEVKISLFADDMIVYLSDPKSSTRELLKLNINFSKVAGYKINSNKS
 AFLYTKKEQAEKEIRETTPFIIDPNNIKYLGVALTKQVKDLYNKNFKTLKKEIEEDLRRWKDLPCSWIGRINIVKMAILPKAI
 YRFNAIPIKIPIQFFKELDRITICKFIWNNKKPRIAKAILNNKRTSGGITPELKQYRAIVIKTAWYWRDRQIDQWNRIEDP
 EMNPHTYGHILFDKGAKTIQWKKDSIFSKWCWFNWRATCRRMQIDPCLSPCTKLKSKWIKDLHIKPDTLKLEEKLKGH
 LEHMGTKGNFLNKTTPMAYALRSRIDKWDLIKLSQFCKAKDTPVTRTKRQPTDWEKIFTNPTTDRGLISKIYKELKKLDRRE
 TNNPIKKWGSELNKEFTAEECRMAEKHLKKCSTSLVIREMQIKTTLRFHLLTPVRLAKIKNSGDSRCWRGCGERGTLHC
 WWDCLRVKPFWKSVMRFLRKLDIELPEDPAIPLGIYPKDASTYKRDTCSTMFIAALFIARKWKEPRCPSTEEWIQKM
 WYIYTMEYSAIKNNKFMKFVGKWLELENIILSELTQSQKDIHGMHSLISGY

CLUSTAL multiple sequence alignment of Human, Mice and Rat L1ORF2 protein sequences
 Yellow shaded part from human L1ORF2 was used to make L1ORF2 specific antibody

Human_ORF2	-----MTGSTSHITILTNLINGLNSAIKRHRLASWIKSQDPSVCCIQETHLTCRDTHR 53
ORF2_GF21	MPTLTTKITGSNNYLSLISLNINGLNSPIKRHRLTDWLHKQDPTFCCLQETHLREKDRHY 60
RAT_ORF2	-----MNIKGNNNHYSLSLNINGLNSPIKRHRLTNWIRNEDPAFCCLQETHLRDKDRHY 55
	:*::*:***** :*****:*.::*:**:*:***** :* *
Human_ORF2	LKIKGWRKIYQANGKQKKAGVAILVSDKTDKPTKIKRDKEGHYIMVKGSIQEEELTILN 113
ORF2_GF21	LRVKGWKTIFQANGLKKQAGVAILISDKIDFQPKVIKKDKEGHFILIKGKILQEELSILN 120
RAT_ORF2	LRVKGWKTTFQANGQKKQAGVAILISNKNFQLKVIKKDKEGHFIFIKGKIHQDELSILN 115
	*::**:. :**** :*:*****:*. :* :. **:*:*****:*.**.* :*:**:
Human_ORF2	IYAPNTGAPRFIKQVLSDLQRDLSDHTLIMGDFNTPLSTLDRSTRQKVNKDTQELNSALH 173
ORF2_GF21	IYAPNARAATFIKDTLVKLKAHIAPHTIIVGDLNTPLSMDRSWKQKLNDRDTPVKLTEVMK 180
RAT_ORF2	IYAPNTRAPTYVKETLLKLKTHIAPHTIIVGDFNTPLSSMDRSWKQKLNVDRLREVMS 175
	*****: * :*:*. :* :. : **:*:*****:***** :*:*. * . * .. :
Human_ORF2	QADLIDIYRTLHPKSTEYTFSSAPHHTYSKIDHIVGSKALLSKCRTEIITNYLSDHSAI 233
ORF2_GF21	QMDLTDIYRIFNPKTGYTFSSAPHGTFSKIDHIIHGKTGLNRYKNIEIVPCILSDHHGL 240
RAT_ORF2	QMDLTDIYRTFYPAKAGYTFFSAPHGTFSKIDHIIQKQTGLNRYRKIEIIPCVLSDHHGL 235
	* ** ***** : **:. ***** *:*****:* *: *. :. : ** : **** .. :
Human_ORF2	KLELRIKNTQSRSTTWKLNNLLNDYVWVHNEMKAEIKMFFETNENKDTTYQNLWDFAKA 293
ORF2_GF21	RLIFNNNIKNGKPTFTWKLNNLLNDTLVKEGIKKEIKDFLEFNENEATTYPNLWDTMKA 300
RAT_ORF2	KLVFNNN-KGRMPTYTWKLNNALLNDNLVKEEIKKEIKNFLEFNENENTTYSNLWDTMKA 294
	:* :. : : ***** ***** :*: :* *** *: ***** :*****:*****
Human_ORF2	VCRGKFIALNAYKRRQERSKIDTLTSQLEKEKQEQTSHKASRRQEITKIRAELEKEIETQ 353
ORF2_GF21	FLRGKLIASLSTSKKKRERAHTSSLTTHLKALEKKEANSPPKRSRRQEIIKLGRGEINQVETR 360
RAT_ORF2	VLRGKLIASACRKKQERAYVSSMTAHLKALEQKEANTPRRSRRQEIIKLRAEINQVETK 354
	. ***:***. :*:*** :*:*** **:* . : ***** *:*.***:***:
Human_ORF2	KTLQKINESRSWFFERINKIDRPLARLIKKKREKNQIDTIKNDKGDITTDPTIEIQTIRE 413
ORF2_GF21	RTIQRINQTRSWFFFEKINKIDKHLARLTRGQRDKILINKIRNEKGDITTDPEEIQTIRS 420
RAT_ORF2	RTIERINRTKSWFFFEKINKIDKPLARLTRGHRECVQINKIRNEKGDITTDSEEIQKIIRS 414
	:*::**:*:*****:*****: ***** :*: :* :* :*:*:***** ***** **.

Human_ORF2	YYKHL YANKLENLEEMDTFLD TYTLPRLNQEEVESLNR PITGSEIVAIINSLPTKKSPGP 473
ORF2_GF21	FYKSL YSTKLENLDEM DKFLDKYQV PKLNQDQVDLLNSPISPK EIEAVINSLPAKKSPGP 480
RAT_ORF2	YYKNLYSTKFENLQEMDYFLDRYQVSKLNQE QINQLNNPITPK EIEAVIKGLPTKKSPGP 474
	: ** **: .: **: **: ** * : : **: **: : * ** : . ** *: : **: **: *****
Human_ORF2	DGFTA E F Y Q R Y K E E L V P F L L K L F Q S I E K E G I L P N S F Y E A S I I L I P K P G R D T T K K E N F R P I 533
ORF2_GF21	DGFS A E F Y Q T F K E D L I P V L H K L F H R I E V E G T L P N S F Y E A T I T L I P K P Q K D P T K I E N F R P I 540
RAT_ORF2	DGFS A E F Y Q T F I E D L I P I L S K L F H K I E T D G A L P N S F Y E S T I T L I P K P H K D T T K K G N F R P I 534
	: ** *: ** *: : *: *: * ** *: * : * * ** *: : * ** *: : * * * ** *
Human_ORF2	SLMNIDAKILNKILANRIQQHIKKL IHHDQVGFIPGMQGWFNIRKSINVIQHINRAKDN 593
ORF2_GF21	SLMNIDAKILNKILANRIQEHIKEI IHPDQVGFIPGMQGWFNIRKSINVIHYINKLKDN 600
RAT_ORF2	SLMNIDAKILNKILANRIQEHIKTI IHHDQVGFIPGMQGWFNIRKTNVIHYINKLKEQN 594
	: *****: ** *: ** * *****: *****: ** *: *: *
Human_ORF2	HMIISIDAEKAFDKIQPFMLKTLNKLIGDGYFKIIRAIYDKPTANIILNGQKLEAFPL 653
ORF2_GF21	HMIISLDAEKAFDKIQHPFMKVLERSGIQGPYLNIIKAIYKPVANIKVNGEKLEAIP 660
RAT_ORF2	HMIISLDAEKAFDKIQHPFMKVLERIGIQGPYLNIVKAIYKPVANIKLNGEKLEAIP 654
	: *****: *****: ** *: *: : ** *: *: : ** *: ** *: ** *: ** *: ** *
Human_ORF2	KTGTRQGCPLSPLLFNIVLEVLARAI RQEKEIKGIQLGKEEVKLSLFADDMIVYLENP 713
ORF2_GF21	KSGTRQGCPLSPYLFNIVLEVLARAI RQKEIKGIQIGKEEVKISLFADDMIVYISDP 720
RAT_ORF2	KSGTRQGCPLSPYLFNIVLEVLARAI RQKEIKGMQIGKEEVKISLFADDMIVYLSDP 714
	: *: ***** *****: *****: *: *****: *****: .: *
Human_ORF2	SAQNLLKLISNFSKVS GYKIN VQKSQAFLYTNNRQTESQIMGELPFTIASKRIKYLGIQL 773
ORF2_GF21	STRELNLINSFGEVAGYKINSNKSM AFLYTKNKQAEKEIRETTPFSIVTNNIKYLGV 780
RAT_ORF2	STRELLKLNNFSKVAGYKINSNKSV AFLYTKEKQAEKEIRETTPFIIDPNNIKYLGVA 774
	: *: **: **. *: : *: ***** : ** *****: *: *: * ** * :. *****: *
Human_ORF2	TRDVKDLFKENYKPLLKEIKEETNKWKNIPCSWVGRINIVKMAILPKVIYRFNAIPIK 833
ORF2_GF21	TKEVKDLYDKNFKSLKKEIKEDLRRWKDLPCSWIGRINIVKMAILPKAIYRFNAIPIK 840
RAT_ORF2	TKQVKDLYNKNFKTLKKEIEEDLRRWKDLPCSWIGRINIVKMAILPKAIYRFNAIPIK 834
	: *: *****: .: *: * ** *: *: .: *: *****: *****: *****: *
Human_ORF2	MTFFTELEKTTLKFIWNQKRARI AKSILSQKNKAGGITLPDFKLYYKATVTKTAWYWYQN 893
ORF2_GF21	TQFFNELEGAICKFIWNKKPRIAKTLLKDKRTSGGITMPDLKLYYRAIVIKTAWYWYRD 900
RAT_ORF2	IQFFKELDRTICKFIWNKKPRIAKAILNNKRTSGGITIPELKQYYRAIVIKTAWYWYRD 894
	: ** *: : *****: *****: *: *: .: *****: *: ** *: * *****: :
Human_ORF2	RDIDQWNRTPESEIMPHIYNYLIFDKPEKNKQWKGKDSL FNKWCWENWLAICRKLKLP 953
ORF2_GF21	RQVDQWNRIEDPEMNPHTYGHLIFDKGAKTIQWKKDSIFNNWCWHNLLSCRRMRIYP 960
RAT_ORF2	RQIDQWNRIEDPEMNPHTYGHLIFDKGAKTIQWKKDSIFSKWCWENWRATCRMQIDPCL 954
	: *: ***** * *: ** *: ***** * . ** ** *: .: ** ** ** *: : : * *
Human_ORF2	TPYTKINSRWIKDLNVKPKTIKLEENLGITIQDIGVGKDFMSKTPKAMATKDKIDKWDL 1013
ORF2_GF21	SPCTKVKSKWIKELHIKPETLKLIEEKVGKSL EDMGTGEKFLNRTALACSVRSRIDKWDL 1020
RAT_ORF2	SPCTKLKSKWIKDLHIKPDTLKLIEEKLGHLEHMG TGKNFLNKTMPAYALRSRIDKWDL 1014
	: * ** *: : *****: *: ** *: * ** *: : .: *: .: *: .: * * : : *****
Human_ORF2	IKLSFCTAKETTIRVNRQPTTWEKIFATYSSDKGLISRIYNELKQIYKKKTNNPIKKWA 1073
ORF2_GF21	MKLQSFCAKADTVNKT KRPTDWERIFTYPSDRGLISNIYKELKKVD FRKSNNPIKKWG 1080
RAT_ORF2	IKLQSFCAKADTVVRTKRQPTDWEKIFTNPTDRGLISKIYKELKKLDRRETNNPIKKWG 1074
	: *: ** *: **. *: .: * ** ** *: : .: *: *****: *****: : : *****.
Human_ORF2	KDMNRHFSKEDIYAAKHKMKCSSSLAIREMQIKT TMR YHLTPVRMAI IKKSGNNRCWRG 1133
ORF2_GF21	SELNKEFSPEEYRMAEKHLKKCSTSLIIREMQIKT TLR FHLTPVRMAIKNSGDSRCWRG 1140
RAT_ORF2	SELNKEFTAEECRMAEKHLKKCSTSLVIREMQIKT TLR FHLTPVRLAKIKNSGDSRCWRG 1134
	: .: *: .: *: * : *: *****: ** *****: *: *****: * ** *: .: *****
Human_ORF2	CGEIGTLLHCWWDCKLVQPLWKS VWRFLRDLLELIPDPAIPLLGIYPNEYKSCCYKDT 1193
ORF2_GF21	CGERGTLHCWWDCLVQPLWKS VWRFLRKL DIVLPEDPAIPLLGIYPEDAP-TGKKDTC 1199
RAT_ORF2	CGERGTLHCWWDCLVKPFWKS VWRFLRKL DIELPEDPAIPLLGIYPK DAS-TYKR DTC 1193
	: ** * *****: *: *: *****: *: : * *****: *****: : *****: : ** *
Human_ORF2	TRMFIAALFTIAKTWNQPKCPT MIDWIKKMWHIYTM EY YAAIKNDEFISFVGTWMMKLE 1253
ORF2_GF21	STMFI AALFIIARSWKEPRCPSTE EWIQKMWYIYTM EY YSAIKKN EFMKFLAKWMDLEGI 1259
RAT_ORF2	STMFI AALFIIARKWKEPRCPSTE EWIQKMWYIYTM EY YSAIKNNKFMK FV GKWLELENI 1253
	: : ***** ** *: .: *: **: : *: *****: *****: *****: *: .: .: .: .: ** *

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Human_ORF2      ILSKLSQEQTCKHRIFSLIGGN1275
ORF2_GF21      ILSEVTHSQRNSHNMYSLISGY1281
RAT_ORF2       ILSELTQSQKDIHGMHSLISGY1275
                ***:::..*: * :.***.*
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>hL1ORF2RT

EFYQRYKEELVPFLCLKFQSIKEGILPNSFYEASIIILPKPGRDTTKKENFRPISLMNIDAKILNKILANRIQQHIKKL

>mL1ORF2RT

EFYQTFKEDLIPVLHKLFRHIEVEGTLPNSFYEATITLIPKPQKDPTKIENFRPISLMNIDAKILNKILANRIQEHIKEI

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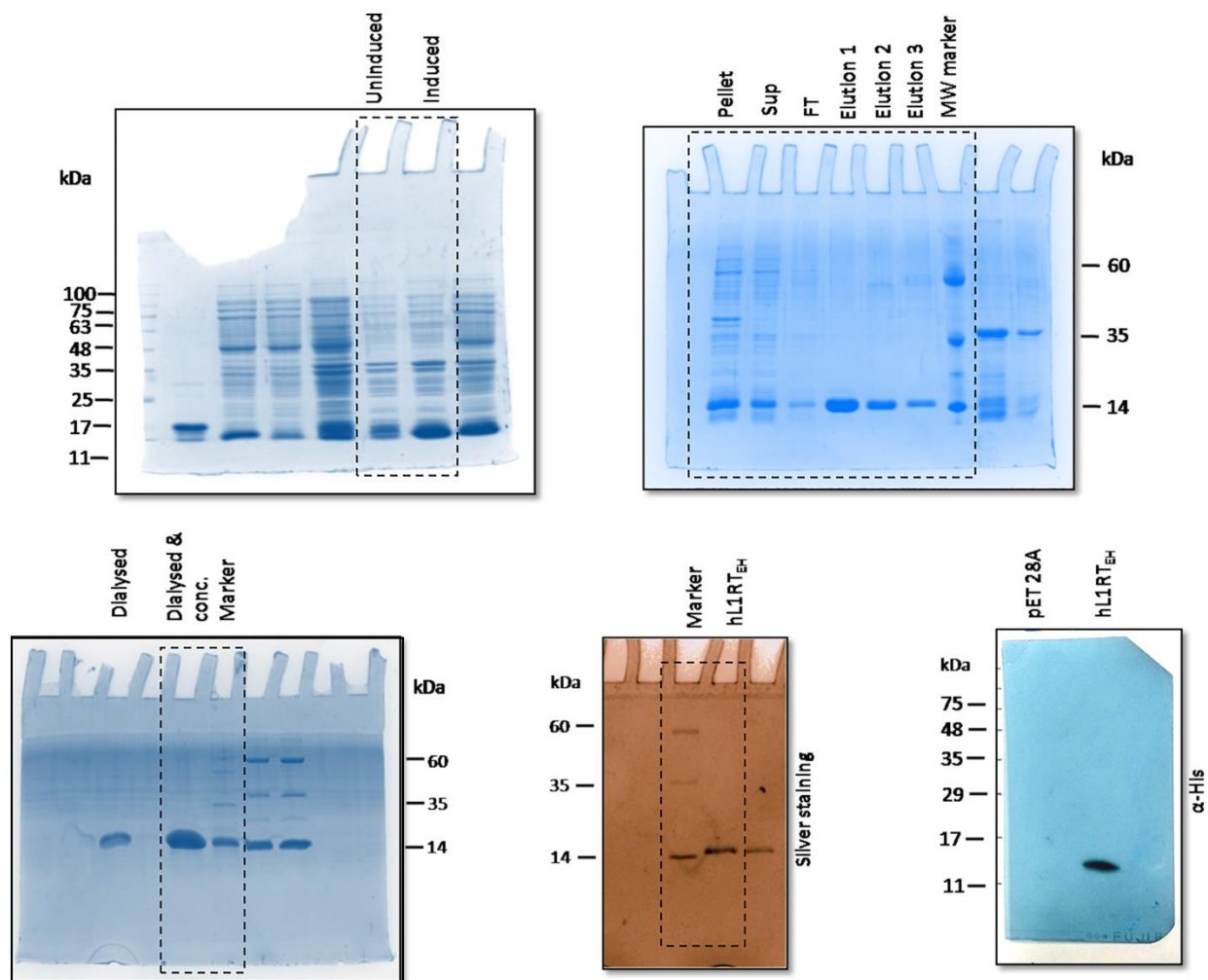
CLUSTAL(1.2.4) multiple sequence alignment

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hL1ORF2RT      EFYQRYKEELVPFLCLKFQSIKEGILPNSFYEASIIILPKPGRDTTKKENFRPISLMNI  60
mL1ORF2RT      EFYQTFKEDLIPVLHKLFRHIEVEGTLPNSFYEATITLIPKPQKDPTKIENFRPISLMNI  60
rL1ORF2RT      EFYQTFIEDLIPILSKLFHKIETDGALPNSFYESTITLIPKPHKDTTCKGNFRPISLMNI  60
                **** : *:*:*.* ***: ** :* *****:.* ***** :* ** *****
hL1ORF2RT      DAKILNKILANRIQQHIKKL          80
mL1ORF2RT      DAKILNKILANRIQEHIKEI        80
rL1ORF2RT      DAKILNKILANRIQEHIKTI       80
                *****:*** :
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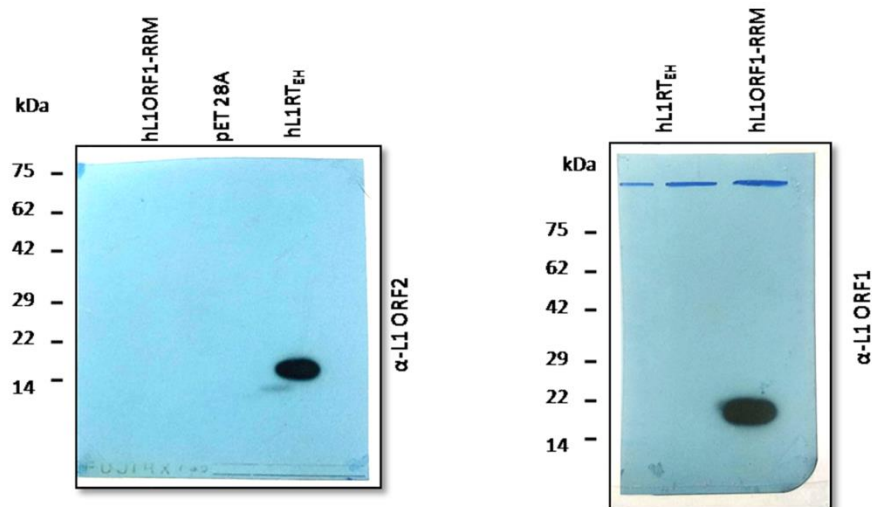
Percent Identity Matrix - created by Clustal2.1

1: hL1ORF2RT	100.00	76.25	73.75
2: mL1ORF2RT	76.25	100.00	83.75
3: rL1ORF2RT	73.75	83.75	100.00

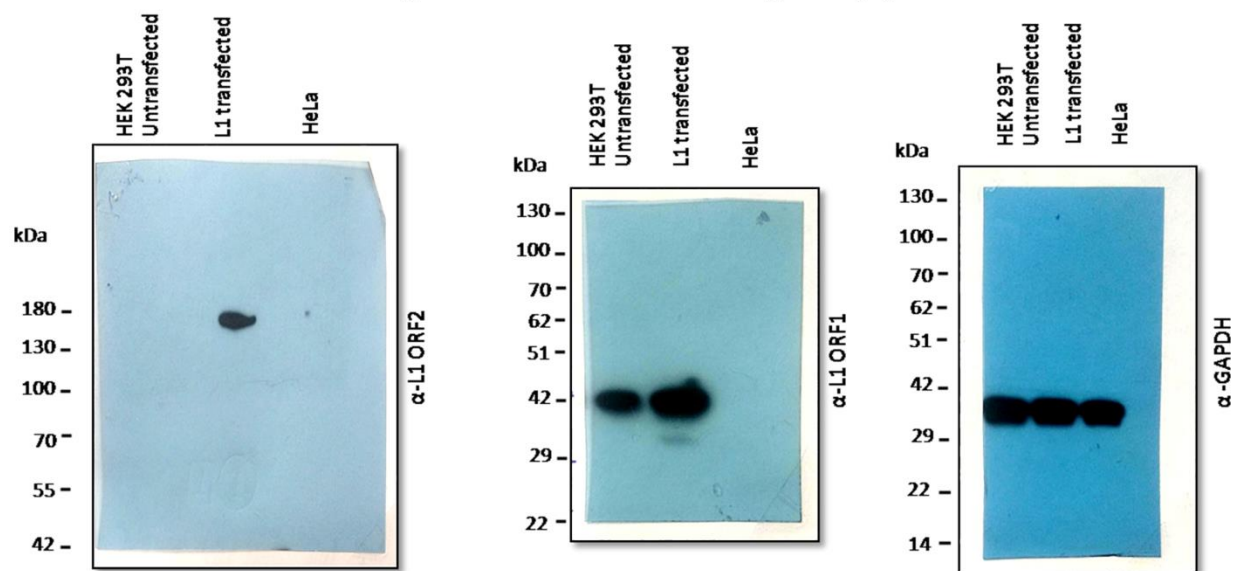
Original Gels and Immunoblots shown in main figures attached :



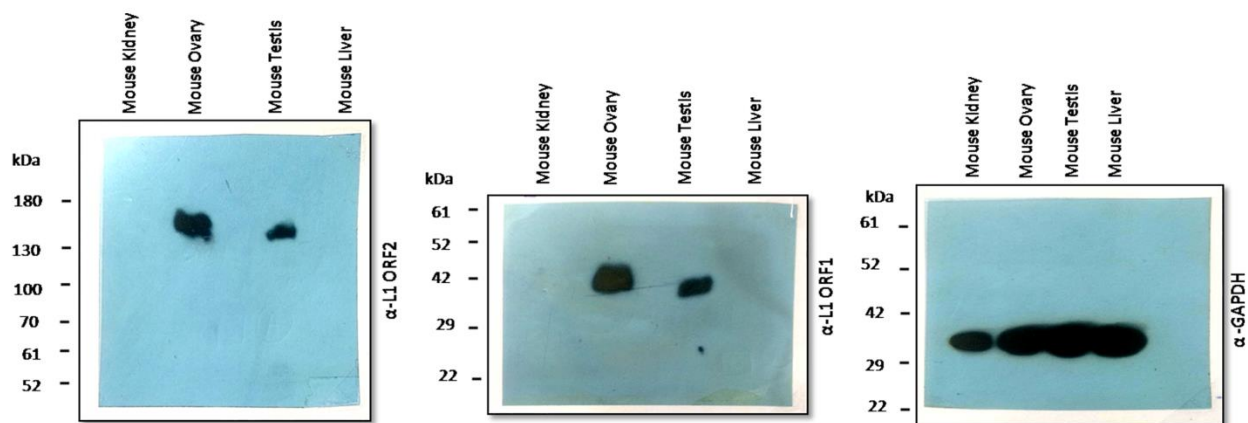
Original SDS Page and western blots of Main Figure 1



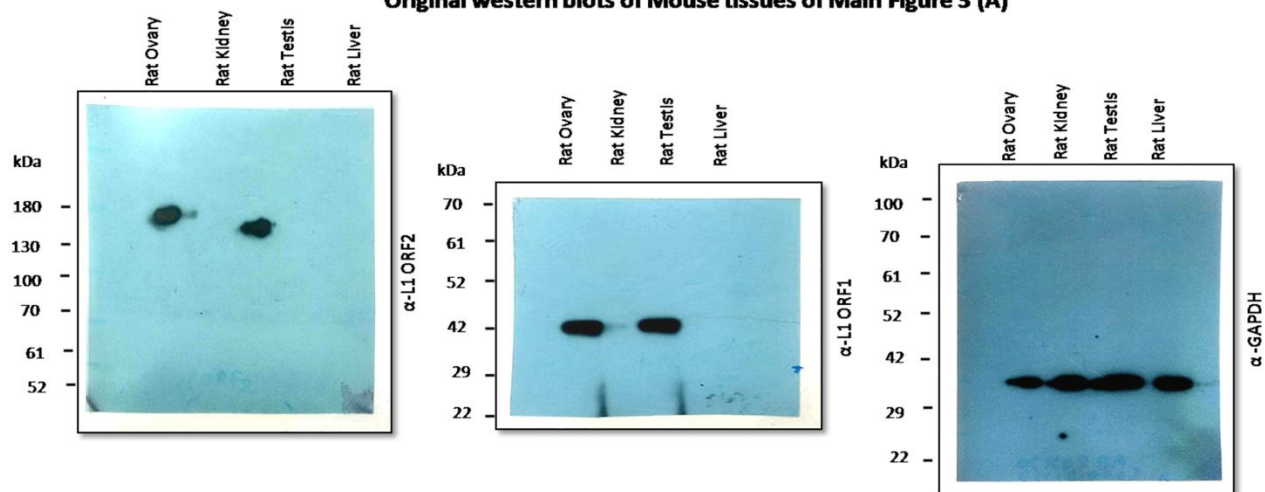
Original western blots of Main Figure 2 (A)



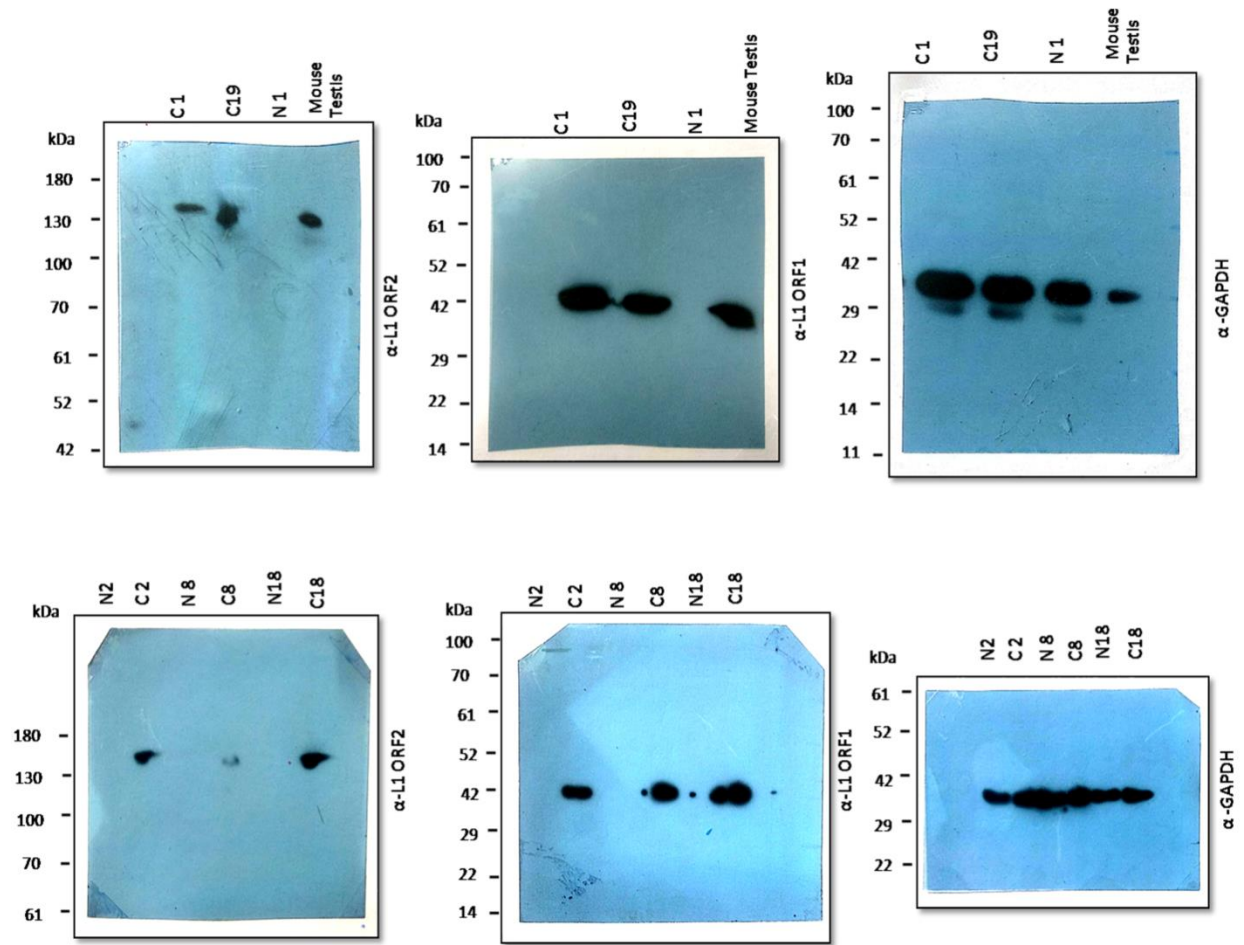
Original western blots of Main Figure 2 (B)



Original western blots of Mouse tissues of Main Figure 3 (A)

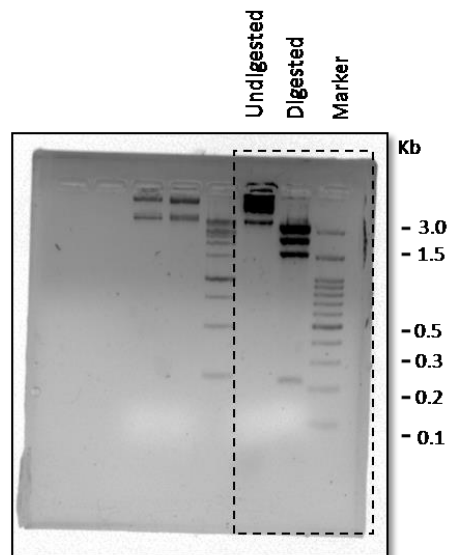


Original western blots of Rat tissues of Main Figure 3 (B)

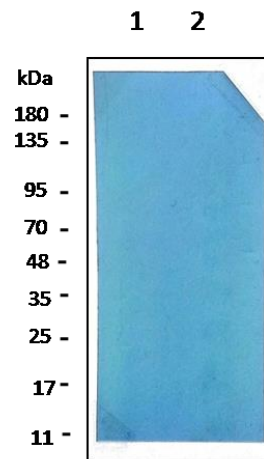


Original western blots of Main Figure 6 (B)

Original Gels and Immunoblots shown in supplementary figures attached :



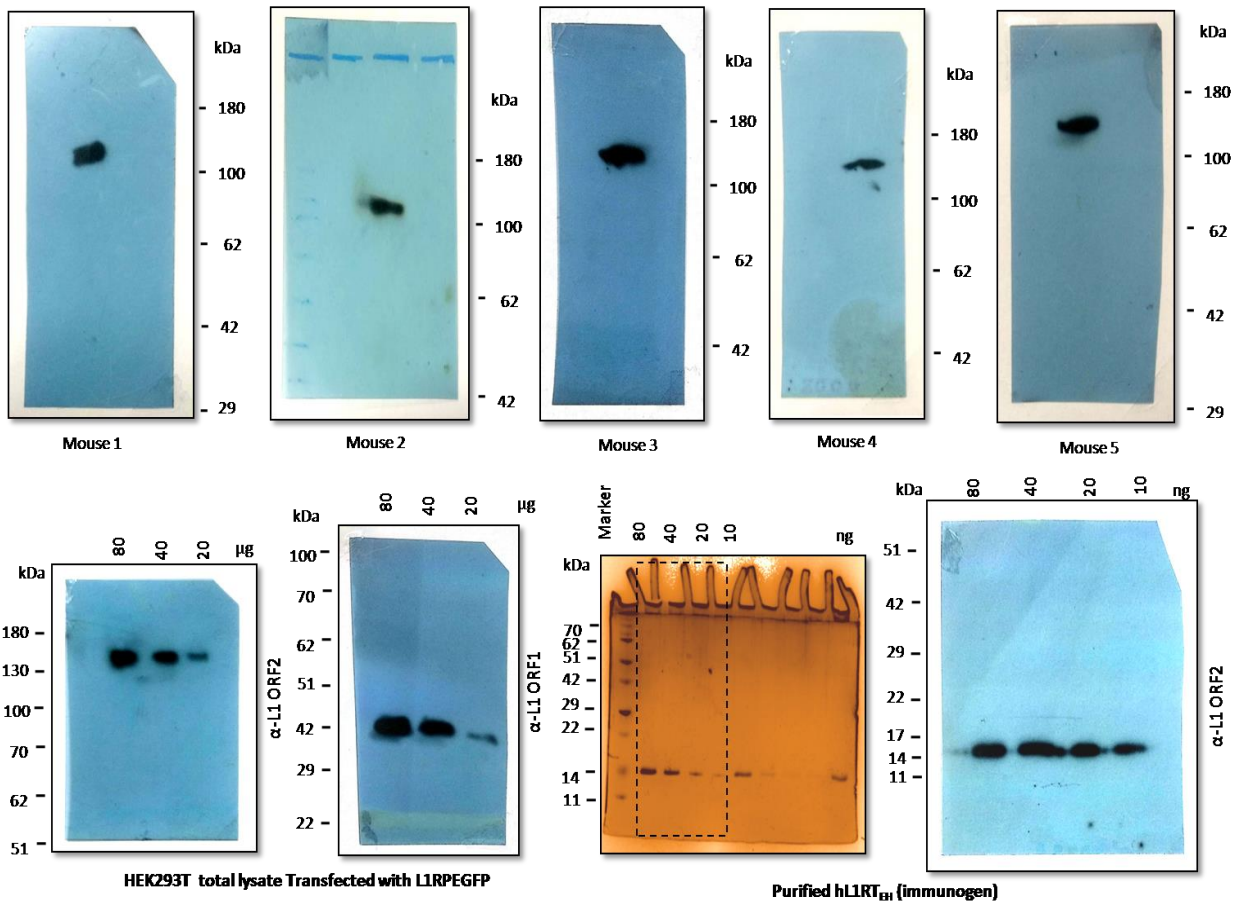
JCC5 digested with
EcoRI and *HindIII*



Western blot with
Mouse pre-bleed

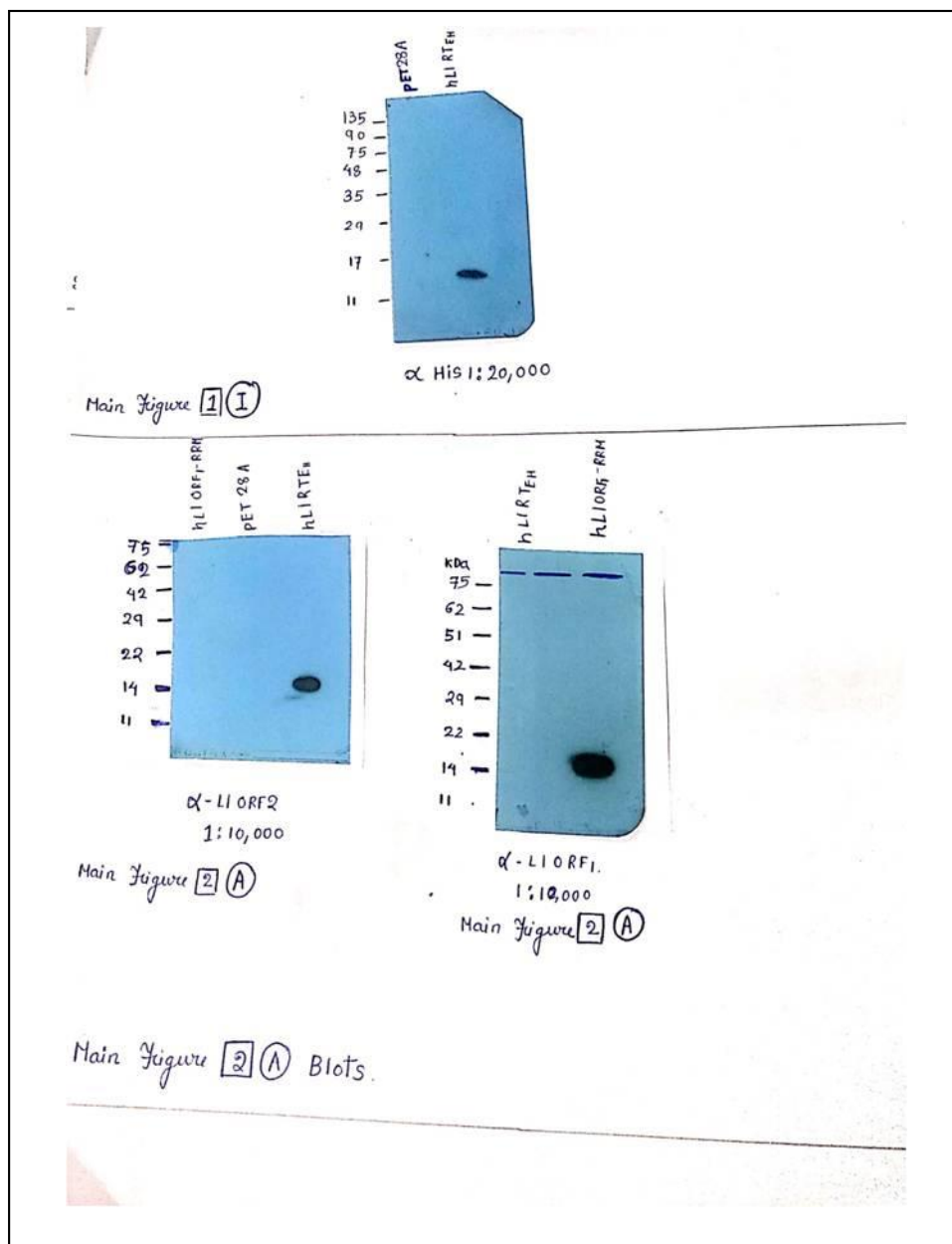
1. *E. coli* total lysate, induced pETL1RT_{EH}
2. HEK293T total lysate (L1RPEGFP transfected)

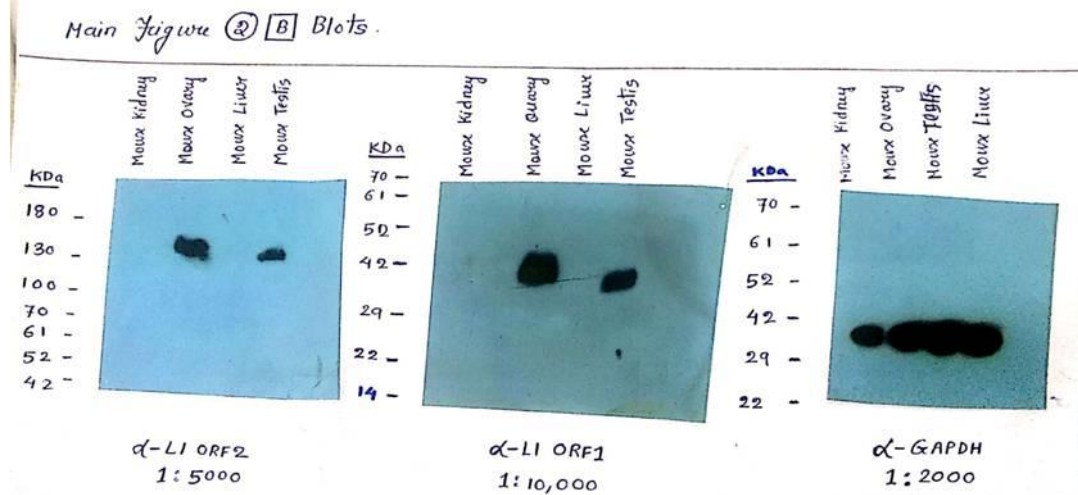
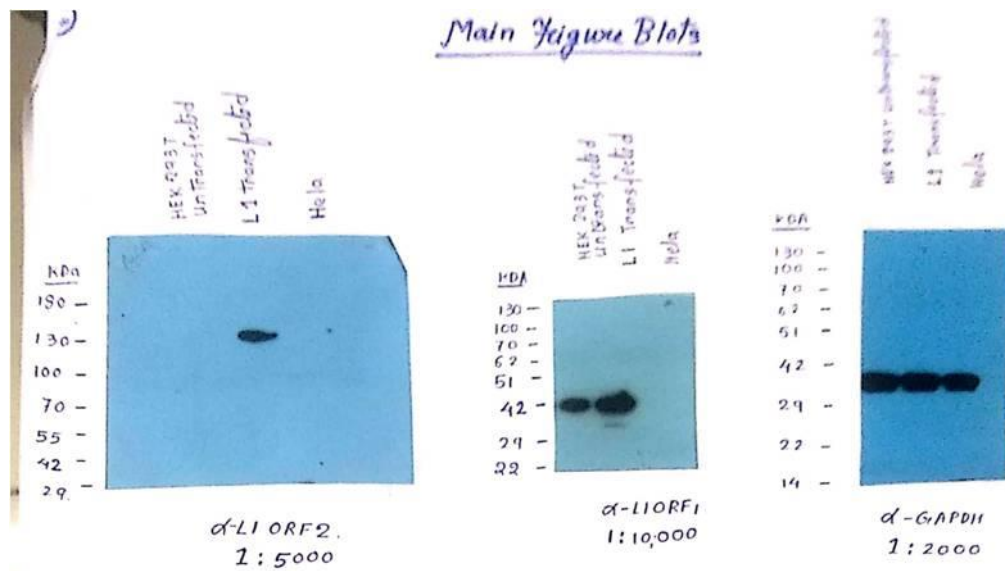
Original Agarose gel and western blot of Supplementary Figure 1



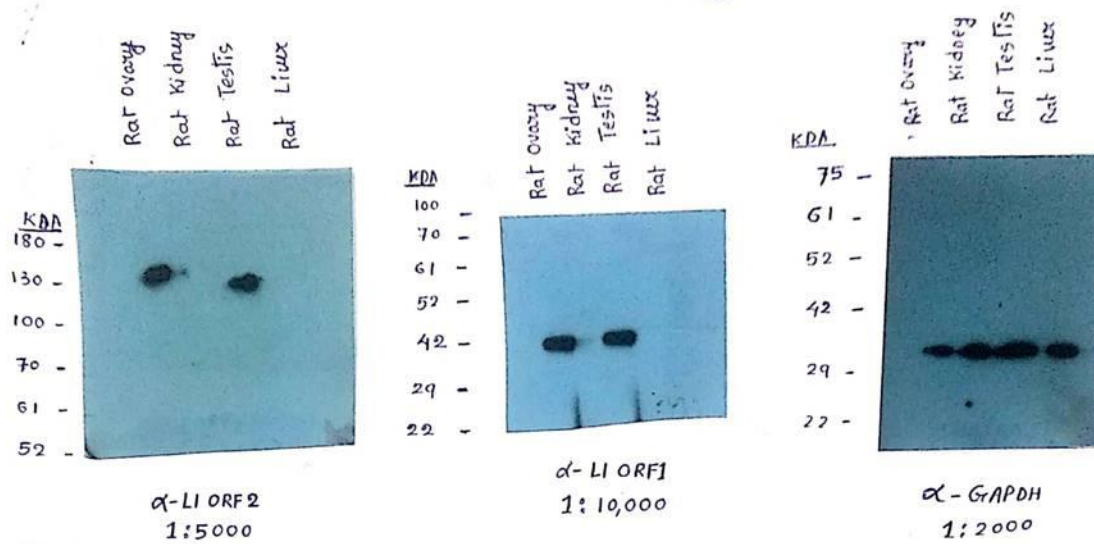
Original gel and western blots of Supplementary Figure 2

Original Hand written hard copies of Immunoblots shown in main figures attached :

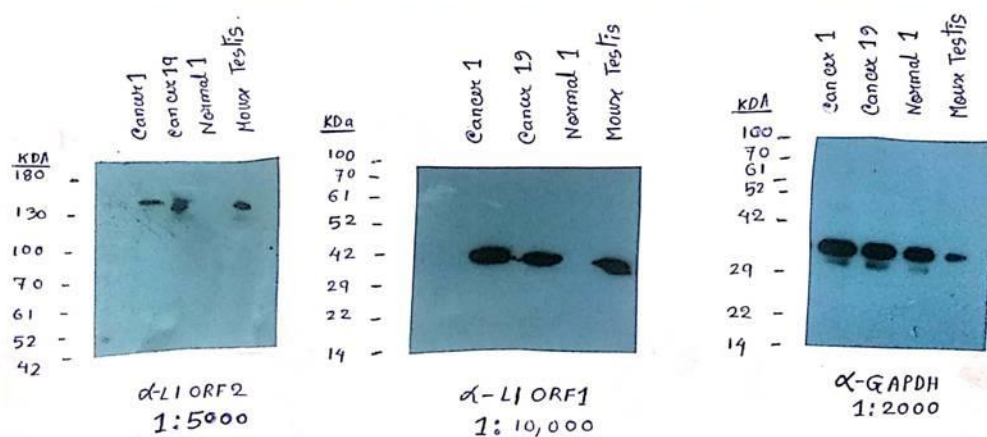




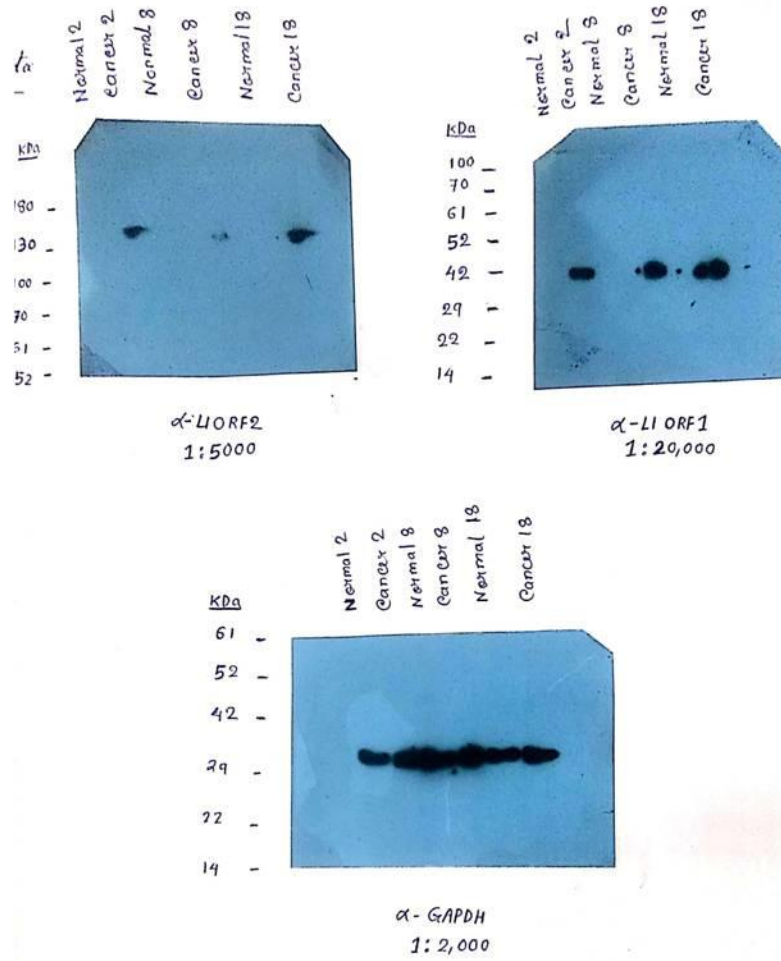
Main Figure (3) A Moux Tissue Blots



Main Figure (3) B Rat tissue Blots

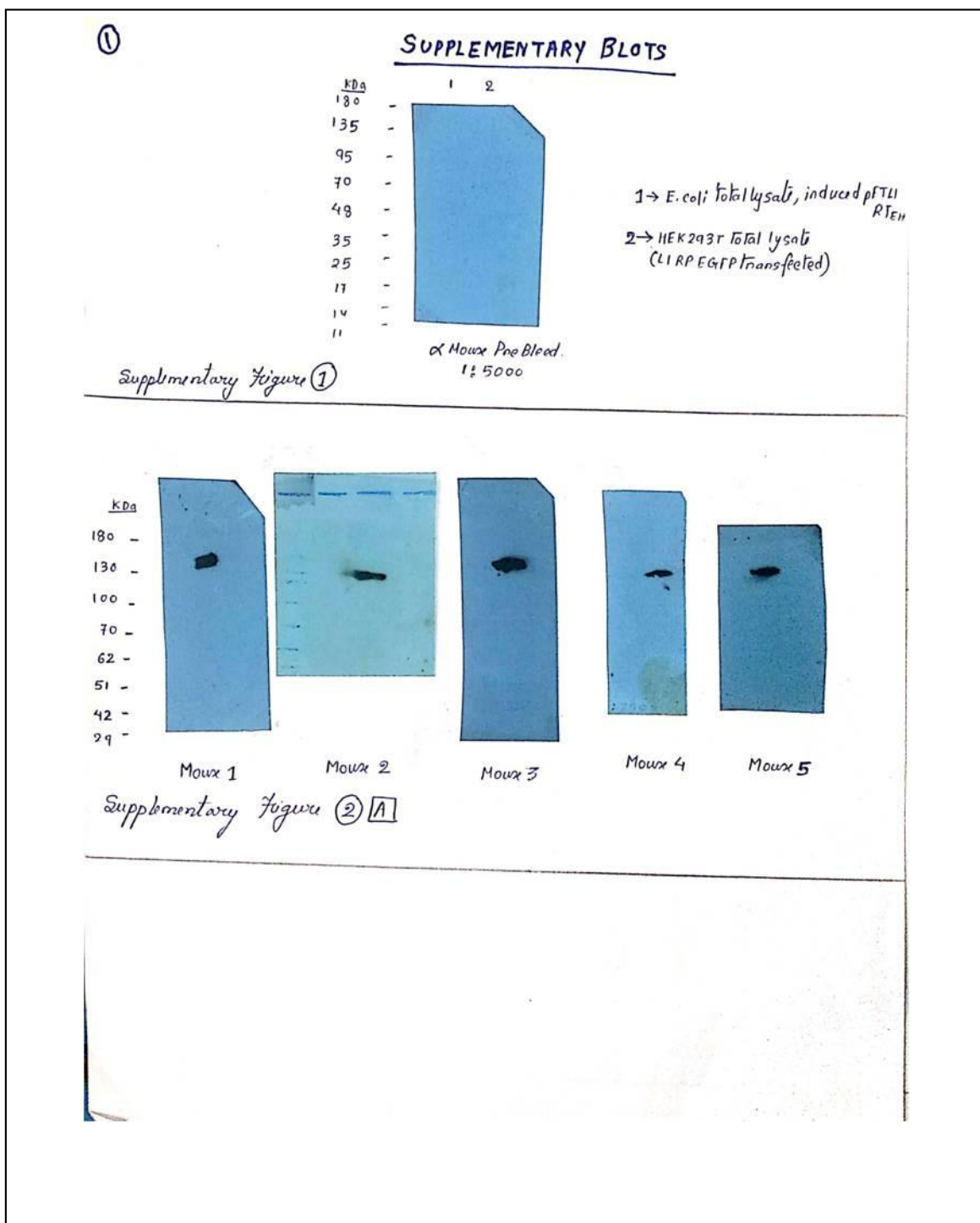


Main Figure (6) B Cancer Tissue Blots



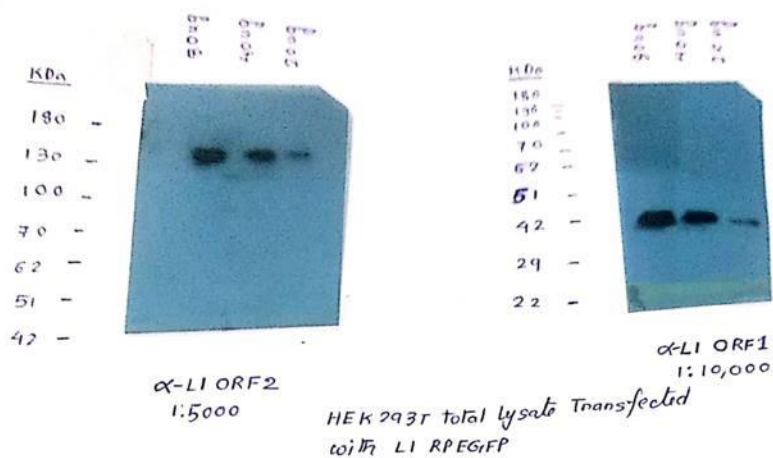
Main Figure (C) (B) Cancer Tissue Blots.

Original Hand written hard copies of Immunoblots shown in supplementary figures attached :

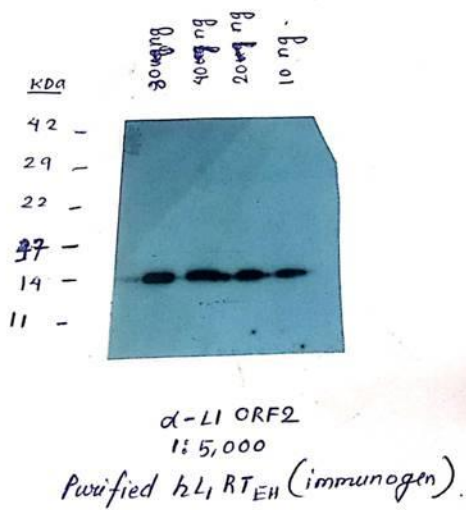




SUPPLEMENTARY BLOTS



Supplementary Figure 2 **B**



Supplementary Figure 2 **B**