

Supplementary Information

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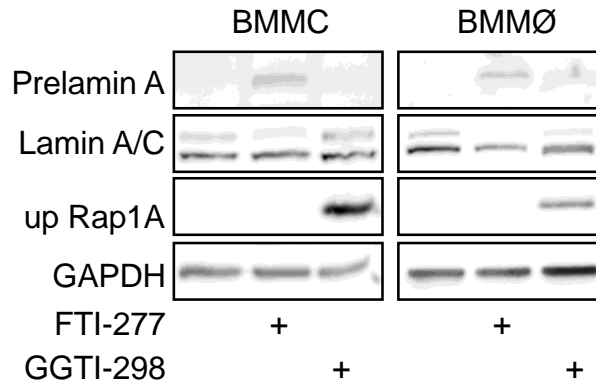


Figure S1. FTI-277 specifically inhibits protein farnesylation and GGTI-298 specifically inhibits protein geranyl-geranylation. BMMCs and BMMØ were treated with 5 μ M FTI-277 (72 h) or 5 μ M GGTI-298 (24 h). Levels of non-prenylated lamin (Prelamin A) and un-prenylated Rap1A (up Rap1A) were determined by Western blotting. Accumulation of Pre-lamin in FTI-277 incubated cells demonstrate inhibition of protein farnesylation. GGTI-298 had no effect on pre-lamin but abrogated geranyl-geranylation of Rap1A. Experiment was performed 3 times for each cell type and one representative Western blot image is shown.

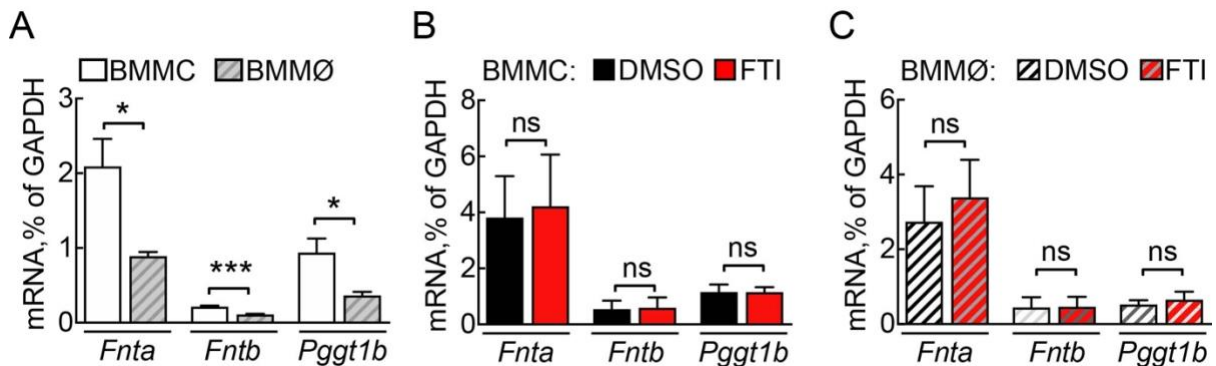


Figure S2. Expression of farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase) in mast cells and macrophages. (A) mRNA abundance of *Fnta* (α subunit of FTase and GGTase), *Fntb* (β subunit of FTase) and *Pgg1b* (β subunit of GGTase) in BMMCs and BMMØ was assessed by qPCR and normalized to the corresponding level of GAPDH expression. Results from n=4 independent experiments are shown. (B and C) Effect of FTI-277 on FTase and GGTase expression. BMMCs (B) and BMMØ (C) were treated with DMSO or 5 μ M FTI-277 for 72 h. Expression of *Fnta*, *Fntb* and *Pgg1b* genes was assessed by qPCR and normalized to GAPDH. Experimental number is n=3 for BMMC and n=2 for BMMØ from independent experiments. Statistical significance was evaluated with Student T-test. ns: p>0.05; *: p \le 0.01, ***: p \le 0.001.

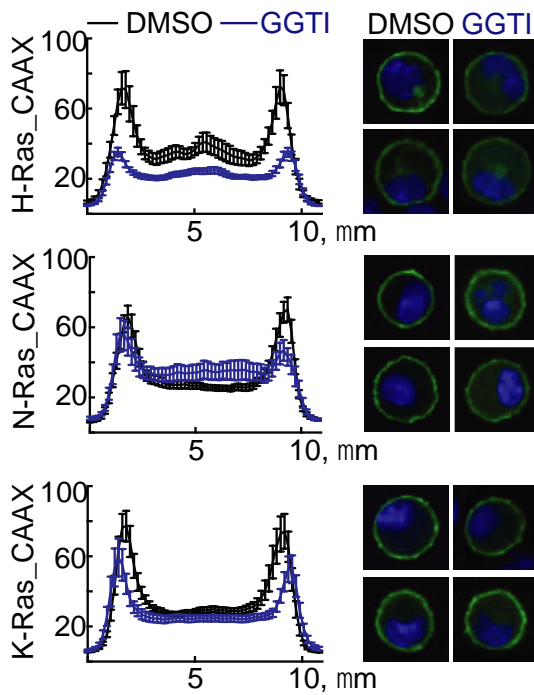


Figure S3. GGTI-298 causes re-localization of H-Ras from the plasma membrane to the cytosol. BMMCs were transfected with GFP-tagged C-terminal 25 amino acids (CAAX domain) of H-Ras, N-Ras or K-Ras. Afterwards we treated them with 5 μ M GGTI-298 for 24 h. Images of PFA-fixed cells were acquired by confocal microscopy. Plotted are GFP-intensity mean values \pm standard error of mean (SEM) of N>18 cells.

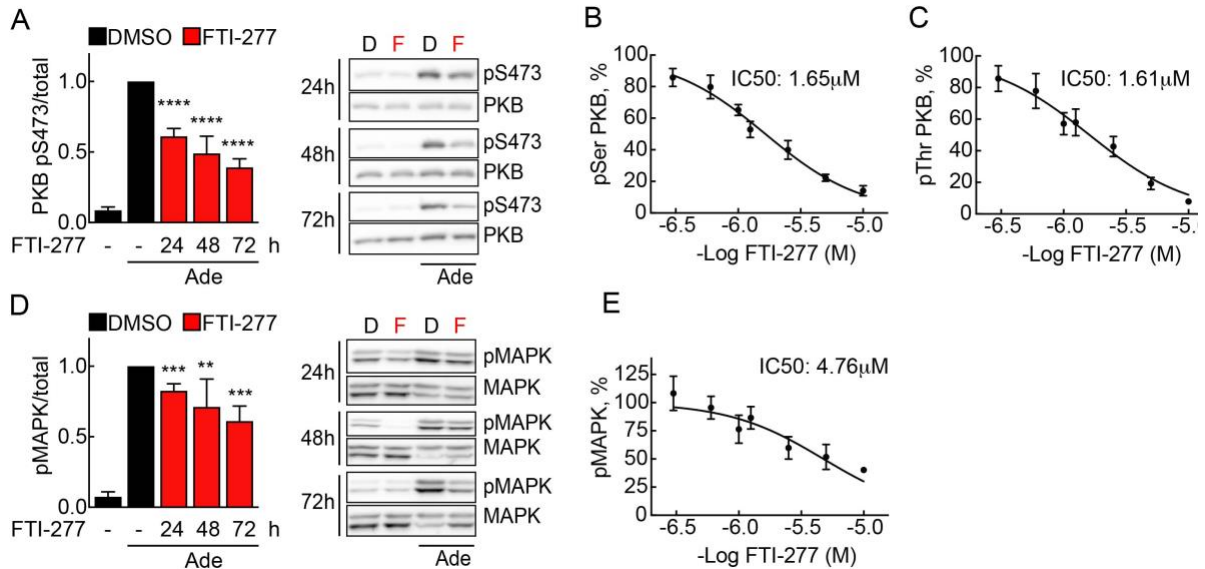


Figure S4 Time- and concentration-dependence of FTI-277 action on mast cells. BMMCs were treated with DMSO or 5 μ M FTI-277 for 24, 48 or 72 h. Cells were then starved for 4 h in IL3-free medium containing 2% FCS and either DMSO or 5 μ M FTI-277, followed by stimulation with 2 μ M adenosine. Phosphorylation of PKB at Ser473 (A) and pMAPK (D) were determined by Western blotting and normalized to the total PKB or MAPK levels, correspondingly. Each time point contains results from 3-7 experiments. (B, C, E) BMMCs were treated with increasing concentration of FTI-277 (0.3 μ M – 10 μ M) for 72 h, starved and stimulated as in (A). Levels of phosphorylation of PKB at Ser473 (B), pThr308 (C) and pMAPK (E) were quantified by western blotting, normalized to the total PKB or MAPK levels, and used to determine IC₅₀. Each dilution series was tested in n=5 experiments

and one-way ANOVA with Bonferroni's post hoc test was used to determine statistical significance. **: $p \leq 0.01$, ***: $p \leq 0.001$; ****: $p \leq 0.0001$. D=DMSO; F=FTI-277.

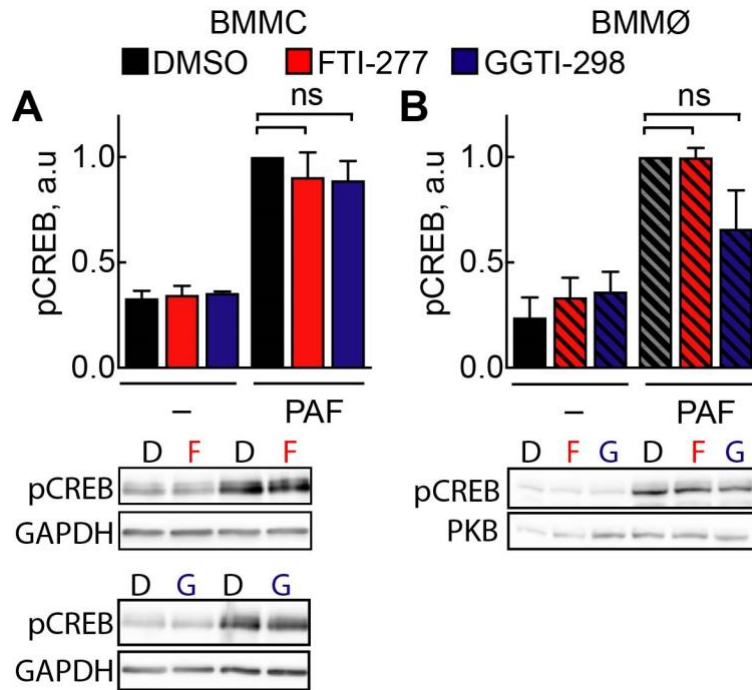


Figure S5. Neither FTI-277 nor GGTI-298 affect PAF-signaling in BMMC or BMMØ. BMMCs and BMMØ were treated with DMSO, 5 μ M FTI-277 (72 h) or 5 μ M GGTI (24 h), starved for 4 h and activated with 1 μ M PAF. Phosphorylation of cyclic AMP-responsive element-binding protein (CREB) at Ser133 was determined by Western blot analysis of cell lysates and normalized GAPDH (BMMC) or PKB (BMMØ). N=3-4 for each data point. Significant relationship was tested with one-way ANOVA with Bonferroni correction. ns: $p > 0.05$. D=DMSO; F=FTI-277; G=GGTI-298.

Table S1. Primer Sequences used for qPCR

Gene	Accession-No	Primer sequence 5' to 3'
Mm_GAPDH	NM_008084.3	F: CTGCACCACCAACTGCTTAG R: CCATCCACAGTCTTCTGGGTG
Mm_p84	AY753194.1	F: ATAGAGCAGGTGGCTAGCGA R: CAGAAGGTCACCGACACAGTG
Mm_p101	NM_177320.2	F: GACATCCTACAGGAAGTCCTTCTC R: TCAGCGCAATGCCTGTCCAT
Mm_p110g	NM_020272.2	F: CCCTGGTGATCGAGAAATGC R: GTCTTGGCGCAGATCATCAC
Mm_NRas	NM_010937.2	F: ATGAGGACAGGCGAAGGGTTC R: TCACACTTGTTGCCTACCAGCAC
Mm_KRas4A	XM_006506919.3	F: GATGTGCCTATGGTCCTGGTAGG R: GCATCCTCCACTCTCTGTCTTGTC
Mm_KRas4B	NM_021284.6	F: GATGTGCCTATGGTCCTGGTAGG R: GCATCGTCAACACCCTGTCTTGTC
Mm_HRas	NM_008284.2	F: GGCAGGGCGTGGAGGATG R: GCAGCCAGGACCACTCTCATCG
Mm_RRas	NM_009101.2	F: TGCCATTAACGACAGGCAG R: TGTTCCCAACCAACACAATG
Mm_RRas2	NM_025846.2	F: GGCAATAAAGCTGACCTGGA R: ATCCTGATCTTTGCCGATG
Mm_MRas	NM_008624.3	F: CCACCAGCTCATTCTGCGTGCAAGG R: CCTTGGTCCCTGGTGACTTTCCTTAGG
Mm_TNFalpha	NM_013693.2	F: ATCCGCGACGTGGAAGCTG

		R: CGAAGTTCAGTAGACAGAAGA
Mm_IL6	NM_031168.2	F: ACAACCACGGCCTTCCCTACTT R: CACGATTTCCCAGAGAACATGTG
Mm_Fnta	NM_008033.3	F: AGCATCGACAGTGGGTCATTC R: GACGAAGTGTCTTTGGTTCCAC
Mm_Fntb	NM_145927.2	F: GAGAAGATCCAGGAGGTCTTCAG R: CTCATAGGCATCTGTCAGTTGTC
Mm_Pggt1b	NM_172627.3	F: CCATCAAAGAATCCAGGAGCAG R: ATCCACACGGCCTAAGTCATCTC

Mm, *mus musculus*; F, forward primer; R, reverse primer

Table S2. Sequences of codon-optimized 3xHA-Ras, used for expression in HEK293 cells

3xHA_H-Ras	<p>GGTACCACCATGTATCCTTACGATGTGCCTGACTATGCCTATCCTTACGATGTGC CAGATTACGCTTATCCCTACGATGTGCCAGATTACGCCAAGCTTGACACAGAGTA CAAACCTGGTGGTGGTGGGAGCTGGCGGAGTCGGGAAGAGCGCACTGACCATCCAG CTGATTTCAGAACCCTTCGTGGACGAGTACGATCCCACAATCGAAGACTCCTATC GGAAACAGGTTCGTGATCGATGGCGAGACATGTCTGCTGGACATCTGGATACCGC CGGACAGGAGGAATACAGTGTATGCGGGATCAGTATATGCGCACAGGGGAAGGC TTCCTGTGCGTGTTCGCCATTAACAATACTAAGAGTTTTGAGGACATCCATCAGT ACCGAGAACAGATTAAGAGGGTCAAAGATTCAGACGATGTGCCATGGTCCCTGGT GGGAAACAAGTGCACCTGGCCGCTAGAACTGTGGAGAGCCGGCAGGCACAGGAT CTGGCACGCTCCTACGGGATCCCTTATATTGAAACCTCTGCAAAGACACGACAGG GCGTCGAGGACGCTTTCTATACCCTGGTGAGGGAAATCAGACAGCACAAGCTGAG GAAACTGAACCCCTCCTGACGAATCTGGCCCTGGCTGTATGTCTGTAAGTGCGTG CTGTCTTGACTCGAG</p>
3xHA_N-Ras	<p>GGTACCACCATGTATCCTTACGATGTGCCTGACTATGCCTATCCTTACGATGTGC CAGATTACGCTTATCCCTACGATGTGCCAGATTACGCCAAGCTTGACACAGAGTA CAAACCTGGTGGTGGTGGGAGCTGGCGGAGTCGGGAAGAGCGCACTGACCATCCAG CTGATTTCAGAACCCTTCGTGGACGAGTACGATCCCACAATCGAAGACTCCTATC GGAAACAGGTTCGTGATCGATGGCGAGACATGTCTGCTGGACATCTGGACACCGC CGGCCAGGAGGAGTACAGCGCCATGCGCGACCAGTACATGCGCACCGGCGAGGGC TTCCTGTGCGTGTTCGCCATCAACAACAGCAAGAGCTTCGCCGACATCAACCTGT ACCGCGAGCAGATCAAGCGCGTGAAGGACAGCGACGACGCTGCCATGGTGTCTGGT GGGCAACAAGTGCACCTGCCACCCGACCGTGGACACCAAGCAGGCCACAGG CTGGCCAAGAGCTACGGCATCCCCTTCATCGAGACCAGCGCCAAGACCCGCCAGG GCGTGGAGGACGCTTCTACACCCTGGTGCGGAGATCCGCCAGTACCGCATGAA GAAGCTGAACAGCAGCGACGACGGCACCCAGGGCTGCATGGGCCTGCCCTGCGTG GTGATGTGACTCGAG</p>
3xHA_K-Ras	<p>GGTACCACCATGTATCCTTACGATGTGCCTGACTATGCCTATCCTTACGATGTGC CAGATTACGCTTATCCCTACGATGTGCCAGATTACGCCAAGCTTGACACAGAGTA CAAACCTGGTGGTGGTGGGAGCTGGCGGAGTCGGGAAGAGCGCACTGACCATCCAG CTGATTTCAGAACCCTTCGTGGACGAGTACGATCCCACAATCGAAGACTCCTATC GGAAACAGGTTCGTGATCGATGGCGAGACATGTCTGCTGGACATCTGGACACCGC CGGCCAGGAGGAGTACAGCGCCATGCGCGACCAGTACATGCGCACCGGCGAGGGC TTCCTGTGCGTGTTCGCCATCAACAACACCAAGAGCTTCGAGGACATCCACCACT ACCGCGAGCAGATCAAGCGCGTGAAGGACAGCGAGGACGCTGCCATGGTGTCTGGT GGGCAACAAGTGCACCTGCCAGCCGACCGTGGACACCAAGCAGGCCACAGGAC CTGGCCCCGAGCTACGGCATCCCCTTCATCGAGACCAGCGCCAAGACCCGCCAGG GCGTGGACGACGCTTCTACACCCTGGTGCGGAGATCCGCAAGCACAAGGAGAA GATGAGCAAGGACGGCAAGAAGAAGAAGAAGAAGAGCAAGACCAAGTGCGTGATC ATGTGACTCGAG</p>

Table S3. ORF sequences of GFP-tagged Ras isoforms used for cellular localization assays (C-terminal 25 amino acids in blue)

Start	GFP	Ras (C-term 25 AA)	Restriction site
GFP-HRasCAAX Plasmid 1672	ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCCATCCTGGTCGAGC TGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGA TGCCACCTACGGCAAGCTGACCCTGAAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCC GCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGG CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGC GCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCA TCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAA CAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAAC TTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACC AGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCT GAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTC CTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACA AGTCCGGACTCAGATCT (BglII) CAGCACAAGCTGAGGAACTGAACCCCTCCTG ACGAATCTGGCCCTGGCTGTATGTCTGTAAAGTGGTGTCTTGA GAATTC (E coRI)		
GFP-NRasCAAX Plasmid 1673	ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCCATCCTGGTCGAGC TGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGA TGCCACCTACGGCAAGCTGACCCTGAAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCC GCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGG CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGC GCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCA TCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAA CAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAAC TTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACC AGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCT GAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTC CTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACA AGTCCGGACTCAGATCT (BglII) CAGTACCGCATGAAGAAGCTGAACAGCAGCG ACGACGGCACCCAGGGCTGCATGGGCCTGCCCTGCGTGGTGATGTGA GAATTC (E coRI)		
GFP-KRasCAAX Plasmid 1674	ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCCATCCTGGTCGAGC TGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGA TGCCACCTACGGCAAGCTGACCCTGAAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCC GCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGG CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGC GCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCA TCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAA CAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAAC TTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACC AGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCT GAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTC CTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACA AGTCCGGACTCAGATCT (BglII) CGCAAGCACAAGGAGAAGATGAGCAAGGACG GCAAGAAGAAGAAGAAGAAGAGCAAGACCAAGTGGTGTATCATGTGA GAATTC (E coRI)		

Table S4. Antibodies used for immunoblotting

Antibody		Company/Cat. Nr.
p110g (aa 97-335)	Clone H1 (<i>the russian</i>), mouse IgG2a	Alexis, 804-230-L001 (currently no commercial source)
p101 (aa 508)	clone D32A5, rabbit IgG	Cell Signaling, 5569S
p84 (aa 1-162)	Polyclonal, rabbit serum	Wymann Lab, rabbit 1, serum1
K-Ras (aa 54-189)	Clone F234, mouse IgG2a	Santa Cruz, Sc-30
N-Ras	Clone F155, mouse IgG1	Santa Cruz, sc31n
H-Ras	Clone Y132, rabbit IgG	Abcam, ab32417
R-Ras (aa 11-31)	Rabbit IgG	Abcam, ab47536
Lamin	Polyclonal, Goat IgG	Santa Cruz, sc-6215
Pre-Lamin	Polyclonal, goat IgG	Santa Cruz, sc-6214
Rap1A/B	Clone 26B4, rabbit IgG	Cell signaling, 2399
pS473 PKB	clone 193H12, Rabbit IgG	Cell Signaling, 4058
pT308 PKB	clone 244F9, Rabbit IgG	Cell Signaling, 4056
PKB	clone 40D4, Mouse IgG1	Cell Signaling, 2920
pMAPK (pERK-1&2)	mouse monoclonal anti-MAPK, activated, diphosphorylated ERK-1&2, clone MAPK-YT, ascites fluid IgG1	Sigma-Aldrich, M8159
MAPK (ErK-1, aa351-368)	polyclonal rabbit anti-ERK-1 (aa351-368)	Sigma-Aldrich, M7927
pS133 CREB	Clone 87G3, rabbit IgG	Sigma-Aldrich, 9198
GAPDH	clone GAPDH-71.1, mouse IgM	Sigma-Aldrich, G8795
Tubulin	clone DM1A, mouse IgG1	Sigma-Aldrich, T9026
Anti-Rabbit IgG-Peroxidase conjugate	Polyclonal, Goat	Sigma-Aldrich, A6154
Anti-Rabbit IgG Peroxidase conjugate	Polyclonal, Goat	Sigma-Aldrich, A4416

Anti-Goat IgG-HRP conjugate	Polyclonal, Donkey	Santa Cruz, sc-2020
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