Supplementary Information

Results

Isolation of an IQGAP1 mutant with restored interaction with MA mutants

To further probe the interaction of IQGAP1 with MA, we sought mutants of IQGAP1 that would show restored interaction with the normally noninteracting MA mutants. Plasmid pGal4AD-IQGAP1 was subjected to mutagenesis by passage in the *E. coli* mutator strain XL1 Red, the mutagenized DNA was introduced into yeast along with various LexA-MA mutants, and rare blue colonies were recovered. A total of 250,000 colonies were screened; plasmids were recovered and sequenced, and their activity was confirmed by retesting. Two IQGAP1 mutants that showed strong interaction with MA mutant T4 were recovered repeatedly: mutant Y1627H (11 isolates) and double mutant Y1627H, E1550K (2 isolates). Tests showed that mutation E1550K was neither necessary nor sufficient for restoring the interaction, but that mutation Y1627H was fully able to restore T4 binding to wild-type levels (Figure S2A). Further tests showed that mutant Y1627H was also able to interact with wild-type MA and with MA mutants T1, T3, and T6, but no longer interacted with mutant T7 (Figure S2B). These results suggest that the Y1627H mutation broadly enhanced the interaction of IQGAP1 with many MA mutants in yeast.

If the restoration of MA binding by IQGAP1 were sufficient unto itself to restore a critical MA function, it was possible that the expression of the Y1627H mutant of IQGAP might support replication of the MA mutant viruses. To test this notion, 293T cells were transiently transformed with a full-length Flag-IQGAP1 Y1627H expression construct, and either wild-type or mutant versions of MuLV DNA. Virus was harvested and purified, and the virion proteins were analyzed by SDS-PAGE and Western blot (Figure S2C). Expression of the mutant IQGAP1 had no effect on the yield or processing of the wild-type, T1, T4, or T6 mutant viruses, even though the mutant IQGAP was

well-expressed in these cells. Thus, the Y1627H mutation did not functionally rescue the MA mutants in mammalian cells.

Materials and Methods

MA mutant plasmid constructs

The alanine scanning method was used to make mutations throughout the MA coding region of pNCA. The High Fidelity enzyme (Roche, Indianapolis, IN) was used to limit possible random mutagenesis. Mutations were created by overlap extension PCR (Ho et al., 1989) with pNCA as template DNA by using outside primers (forward primer pNCA/EcoRI 5′-TCGTCTTCAAGAATTCTCATG-3′; reverse primer XhoI/R2 5′-ATCCCAGTCTGGGCGCTCGAGGGGAAAAGCG-3′). The sense strand primers utilized for creating mutations were as follows, and the anti-sense primers were the complement:

T1, 5′-GCTGCAGCTGGCACCTTTAACCGAGACCTC-3′

T2, 5′-GCTGCAGCTAACCGAGACCTCATCACC-3′;

T3, 5′-GCTGCAGCTCTCATCACCCAGGTTAAG-3′;

T4, 5′- GCTGCAGCTCAGGTTAAGATCAAGGTC-3′;

T5, 5′- GCTGCAGCTATCAAGGTCTTTTCACCT-3′;

T6, 5′- GCTGCAGCTTTTTCACCTGGCCCGCAT-3′;

T7, 5′-GCTGCAGCTGGCCCGCATGGACACCCA-3′;

T8, 5′-GCTGCAGCTGGACACCCAGACCAGGTC-3′;

T9, 5′- GCTGCAGCTGACCAGGTCCCCTACATC-3′;

T10, 5′-GCTGCAGCTCCCTACATCGTGACCTGG-3′;

T11, 5′- GCTGCAGCTGTGACCTGGGAAGCCTTG-3′;

SMA6, 5′-GTGGCAGCAGCAGCCTTGGCTTTTGACCCCCCTCCCTGG-3′;

SMA8, 5′- GCCGCTGCTGCTGCTCCCCCTCCCTGGGTCAAG-3′;

SMA2, 5′-GTACACCCTAAGGCAGCAGCAGCAGCACCTCCATCCGCC-3′;

SMA3,5′-CCTCCGCCTCCTCTTGCAGCATCCGCCGCTTCTCTCCCCCTTGAA-3′;
SMA4,5′-GCCCCGTCTCTCGCAGCAGCAGCACCTCGTTCGACC-3′

These pNCA mutants were amplified by PCR and cloned into pSH2-1 by using outside primers (forward primer MA/BamHI 5′-GACGCGGATCCGTGGCCAGACTGTTACCACTCCC-3′; reverse primer MA/SalI 5′-CAACGCGTCGACCTAATAAAGGGAGGATCGAGGCGG-3′).
The presence of all mutations was confirmed by DNA sequencing.

Potential suppressor mutations identified in revertant virus DNAs were recreated along with the parental mutation in a clean plasmid background to confirm their suppressor activity. The mutations were recreated by overlap extension PCR (Ho et al., 1989) with either the wild-type pNCA or the respective mutant proviral vector, pNCA T1, T2,T4, or T6, as template DNA by using outside primers (forward primer pNCA/EcoRI 5′-TCGTCTTCAAGAATTCTCATG-3′ ; reverse primer XhoI/R2 5′-ATCCCAGTCTGGGCGCTCGAGGGGAAAAGCG-3′). The sense strand primers utilized for creating mutations were as follows, and the anti-sense primers were the complement:
MA T6 Rev 1: 5′-GCTGCAGTTTTTTCACCTGGCCCGCAT-3′
MA T4 Rev 5′-ACCTTTAACCGAGACGCTGTAGCTCAGGTTAAGATCAAG-3′
MA T1 F38L: (5′T1REV MA.T-C): 5′GTTACCCTCTGCTCTGCAGAATGGC-3′
MA T6 Rev2 F38L: (5′T1REV MA.T-C): same as MA T1 F38L.
MA F38L: 5′T1REV MA.T-C: same as MA T1 F38L.
MA T2 F38L: 5′T1REV MA.T-C: same as MA T1 F38L.
MA T4 F38L: 5′T1REV MA.T-C: same as MA T1 F38L.
The pNCA-derived revertants were subsequently used as template to amplify a 390-bp MA fragment which was cloned into pSH2-1 to test for interaction activity in yeast.

IQGAP plasmid constructs

The IQGAP1 DNA fragment used in the yeast two hybrid studies was the shortest cDNA clone recovered from the yeast two hybrid screen, aa 1440-1657. N-terminal and C-terminal deletion constructs as well as alanine substitution mutations of IQGAP1 (aa1440-1657) were created by PCR and cloned into pGADNOT.

N-terminal deletion constructions:

Reverse primer,

IQGAP1 3′Sal: 5′-ATCTCTACCAGTCGACTTACTTCCCATAGAACTTTTTGTTGAG-3′

The forward primers used are as follows:

5′NotI.IQGAP1.s18.KPM (1440) 5′-
AAGGAAAAAAGCGGCCGCAAGCCCATGAAGGAGGATAAC-3′

5′NotI.IQGAP1.s18.GLK (1460) 5′-
AAGGAAAAAAGCGGCCGCGGCCTAAAGAAGCTAACGGAG-3′

5′NotI.IQGAP1.s18.IND (1480) 5′-
AAGGAAAAAAGCGGCCGCATCAACGACATTGCCAAGGAT-3′

5′NotI.IQGAP1.s18.ELV (1500) 5′-
AAGGAAAAAAGCGGCCGCGAATTGGTAAAACTGCAGCAG-3′

5′NotI.IQGAP1.s18.EQV (1520) 5′-
AAGGAAAAAAGCGGCCGCGAGCAGGTGGACTACTACAAG-3′

5′NotI.IQGAP1.s18.GKV (1540) 5′-
AAGGAAAAAAGCGGCCGCGGCAAGGTCTCCAAAAAGCCT-3′

5′NotI.IQGAP1.s18.KYT (1560) 5′-
AAGGAAAAAAGCGGCCGCAAGTACACAGCAGCGAGGCTG-3′

5′NotI.IQGAP1.s18.ANQ (1580) 5′-
AAGGAAAAAAGCGGCCGCGCAAACCAATTTAAAAATGTT-3′

5′NotI.IQGAP1.s18.EVK (1600) 5′-
AAGGAAAAAAGCGGCCGCGAAGTAAAAGCCAAGTTCATG-3′
5′NotI.IQGAP1.s18.LLQ (1620) 5′-
AAGGAAAAAAGCGGCCGCTTGCTGCAGCTACAGTATGAA-3′

C-terminal deletions
The forward primer is: 5′NotI.IQGAP1.s18.KPM (1440) 5′-
AAGGAAAAAAGCGGCCGCAAGCCCATGAAGGAGGATAAC-3′
The reverse primers used are as follows:
S18.11.RAK, (1640) 5′-ACGCGTCGACTTATTTAGCTCTATCAAATAATTT-3′
S18.12.HYQ, (1620) 5′-ACGCGTCGACTTACTGATAATGCAACATGAAAGT-3′
S18.13.VGD, (1600) 5′-ACGCGTCGACTTAGTCTCCAACTTCTTCTGTTGG-3′
S18.14.EDL, (1580) 5′-ACGCGTCGACTTAAAGGTCTTCAATCTCCAGAAG-3′

Alanine substitution mutations (mts1-8) in IQGAP1 (aa1440-1657) were created by overlap extension PCR using pGadnot-IQGAP1 as a template (forward primer, 5′NotI.IQGAP1.s18.KPM (1440) and reverse primer, IQGAP1 3′Sal. The sense strand primers utilized for creating mutations were as follows, and the anti-sense primers were the complement:
IQ18mt1, 5′- GCAGCTGCAGCCGAATTGGTAAAACTGCAGCAG-3′;
IQ18mt2, 5′-GCAGCTGCAGCCCTGCAGCAGACGTACTCG-3′;
IQ18mt3, 5′-GCAGCTGCAGCCTACTCGGCGCTGAACTCT-3′;
IQ18mt4, 5′-GCAGCTGCAGCCAACTCTAAGGCCACCTTT-3′;
IQ18mt5, 5′-GCAGCTGCAGCCACCTTTTACGGCGAGCAG-3′;
IQ18mt6, 5′-GCAGCTGCAGCCGAGCAGGTGGACTACTAC-3′;
IQ18mt7, 5′-GCAGCTGCAGCCTACTACAAGAGCTACATC-3′;

IQ18mt8, 5′-GCAGCTGCAGCCTACATCAAAACCTGCTTG-3′;

Alanine substitution mutations (mts9-12) in IQGAP1 (aa1440-1657) were created by overlap extension PCR using pBluescript-IQGAP1 as a template (forward primer, 5′-AACAGCTATGACCATGATT-3′; reverse primer, 5′- CAACTGTTGGGAAGGGCG-3′) The sense strand primers utilized for creating mutations were as follows, and the anti-sense primers were the complement:
IQ18mt9, 5′- GCAGCTGCCGCATAAGTCGACCTCGAGGGGGGGCCC-3′
IQ18mt10, 5′-GCAGCTGCCGCATTCTATGGGAAGTAAGTC-3′
IQ18mt11, 5′-GCAGCTGCCGCACTCAACAAAAAGTTCTAT-3′
IQ18mt12, 5′-GCAGCTGCCGCACTGATCTTCCTTCTCAAC-3′

Varying lengths of full length murine IQGAP1 were generated by PCR and were cloned into pCMV2Flag (Sigma, St. Louis, MO) at the NotI and HindIII restriction sites. The template used was full length murine IQGAP1 cDNA which was obtained from Andre Bernards′ group at Harvard. The reverse primer used was:
IQGAP1.3′NotIw/stop: 5′
ACTCATACAGCGGCCGCTTACTTCCCATAGAACTTTTTGTTGAG-3′
The forward primers used are as follows:
Full-lengthIQGAP1 (pCDNA4B5′4nuc): 5′-
CCCAAGCTTGCCATGGACTCCGCCGCGGAGGAGGTTGATGGC-3′
IQDN3 (pCDNA4B 5′1921): 5′-
CCCAAGCTTGCCATGGACAGAACCCTGAGTGCCCTACGTTCT-3′
IQDN4 (pCDNA4B 5′2221): 5′-
CCCAAGCTTGCCATGGACCTTTGGCTGGCCAACGAAGGCTTG-3′

In the dominant negative studies, 293T cells were cotransfected with 3 μg of the wild-type proviral vector (either pNCA or pNCS) and either 6 μg, 9 μg, or 12 μg of the IQGAP1 expression vector in 100x15 mm plates using the calcium phosphate method. Cells and supernatants were harvested 48 hrs post-transfection.

Constructs for expression of IQGAP fragments in mammalian cells were derived by PCR. The sequences of the primers for generation of N-terminal Flag-tagged IQDN4 were as follows: forward primer, IQGAP1.5'NotI.Flag, 5'-AAGGAAACTCATACAGCGGCCGCGCCATGGACTACAAAGACGATGACGACAAGCTT-3'; reverse primer, IQGAP1.3'NotIw/stop, 5'-AAGGAAACTCATACAGCGGCCGCTTACTTCCCATAGAACTTTTTGTTGAG-3' The template used for this PCR reaction was pCMV2Flag-IQDN4. The PCR product, wild-type Flag-IQDN4, was then cloned into pCDNA4/TO/myc-HisB vector (Invitrogen, Carlsbad, CA), and the full length inserts were confirmed by sequencing.

Construction of plasmids for *in vitro* binding assays

A C-terminal fragment of IQGAP1 was amplified by PCR and cloned into the NotI and SalI restriction sites of pGex-5x-2 (Amersham Biosciences, Piscataway, NJ), thus generating GST-IQGAP1 (s18). The template for the PCR was pCMV2Flag-IQDN4.
A C-terminal fragment of IQGAP1 Y1627H was amplified by PCR and cloned into the NotI and SalI restriction sites of pGex-5x-2 (Amersham Biosciences), thus generating GST-IQGAP1 Y1627H (s18 Y1627H). The template for the PCR was full length pCMV2Flag-IQGAP1 Y1627H. The primers used were forward primer, S18/5'SalI.fwd: 5'-ATCTATCCGTCGACAAAGCCCATGAAGGAGGATAAC-3', and reverse primer, IQGAP1.3'NotI w/stop.
MA and MA mutants were amplified by PCR and cloned into pMALC2 (NEB, Ispwich, MA), thus generating MBP-MA wild-type or MBP-MA mutants (T4, T5, SMA6, T11,

T4Rev, T1Rev, T6Rev1, and T6Rev2). The template for PCR was either the wild-type proviral vector, pNCA, or the respective mutant proviral vector, pNCA T4, T5, SMA6, T11, T4Rev, T1Rev, T6Rev1, or T6Rev2. The primers used were as follows: MA/5′ EcoRI: 5′-ATCCGAATTCATGGGCCAGACTGTTACCACT-3′ and 3′pSH2-1 Sal(MA)Rev: 5′-CAACGCGTCGACCTAATAAAGGGAGGATCGAGGCGG-3′.

XL1 Red Mutagenesis

Gal4AD-IQGAP1 (aa 1440-1657) was transformed into the XL1-Red mutator strain (Stratagene, LaJolla, CA) to generate random mutations. The XL1-Red strain is deficient in three of the primary DNA repair pathways in *E.coli*, the *mutS*, *mutD* and *mutT*, making its mutation rate approximately 5,000-fold higher than that of its wild-type parent. This mutant library was transformed into yeast and screened for second site suppressors that would store interaction to MA-non-interacting mutants such as MA T4 by qualitative filter lift assay. The yeast DNA was then purified and electroporated into KC8 to isolate plasmids from yeast. The DNAs were sequenced after purification. There were two mutants identified: Y1627H and double mutant, Y1627H and E1550K. Since there were no other mutations in the coding region, the region was amplified and cloned into an un-mutagenized backbone of pGadnot. The primers used were IQGAP1 3′Sal and 5′NotI.IQGAP1.s18.KPM (1440).

Gal4AD-IQGAP1 E1550K was generated by overlap extension PCR mutagenesis with Gal4AD-IQGAP1 (aa 1440-1657) used as template DNA. The outside primers used were: IQGAP1 3′SalI and 5′NotI.IQGAP1.s18.KPM (1440).
The sense strand primers utilized for creating mutations were as follows, and the anti-sense primers were the complement:
IQGAP1.E2K.1550 5'-TCCAAAAAGCCTAGGAAAATGAAAGGCAAGAAA-3′

pCMV2Flag-IQGAP1 Y1627H was generated in an effort to determine if this mutant could restore virus replication of non-interacting, replication defective mutants to wild-type levels. This mutant was generated by overlap extension PCR mutagenesis with full length murine IQGAP1 used as template DNA. The outside primers were:
IQGAP1.3′NotIw/stop: 5′-
ACTCATACAGCGGCCGCTTACTTCCCATAGAACTTTTTGTTGAG-3′
IQGAP1.5′HindIII (pCDNA4B5′4nuc): 5′-
CCCAAGCTTGCCATGGACTCCGCCGCGGAGGAGGTTGATGGC-3′

The sense strand primers utilized for creating mutations were as follows, and the anti-sense primers were the complement:
FL-IQGAP1 Y1627H (IQ4.Y2H. 1627):
5'-TTGCTGCAGCTACAGCATGAAGGAGTTGCAGTT-3′

RNAi Interference (RNAi) Expression Constructs

Oligonucleotides encoding short hairpin RNAs targeting human IQGAP1 or IQGAP2 were ligated into BglII and HindIII restriction sites of pSuperRetro-Puro (Oligoengine, Seattle, WA). pSuperRetroZeo was generated by replacing the Puromycin cassette with a Zeocin cassette at the AgeI and NsiI restriction sites. The sequences of the oligonucletides used in vector construction were as follows; number in parentheses indicates targeting to either IQGAP1 or 2..
46(1) sense: 5′-GATC CCC GGAAAGCTCTGGCAATTTA TTCAAGAGA
TAAATTGCCAGAGCTTTCC TTTTT GGAA A-3′
46(1) anti-sense: 5′- AGCT T TTCC AAAAA GGAAAGCTCTGGCAATTTA
TCTCTTGAA TAAATTGCCAGAGCTTTCC GGG-3′
28(1) sense: 5′-GATC CCC GTCAGAACGTGGCTTATGA TTCAAGAGA

TCATAAGCCACGTTCTGAC TTTTT GGAA A-3′

28(1) anti-sense: 5′-AGCT T TTCC AAAAA GTCAGAACGTGGCTTATGA

TCTCTTGAA TCATAAGCCACGTTCTGAC GGG-3′

30(1) sense: 5′- GATC CCC GGAGAGACCTTGACTGAAA TTCAAGAGA

TTTCAGTCAAGGTCTCTCC TTTTT GGAA A-3′

30(1) antisense: 5′- AGCT T TTCC AAAAA GGAGAGACCTTGACTGAAA

TCTCTTGAA TTTCAGTCAAGGTCTCTCC GGG-3′

53(2) sense: 5′-GATC CCC GGACACGTGTCATCCGAAA TTCAAGAGA

TTTCGGATGACACGTGTCC TTTTT GGAA A-3′

53(2) antisense: 5′-AGCT T TTCC AAAAA GGACACGTGTCATCCGAAA

TCTCTTGAA TTTCGGATGACACGTGTCC GGG-3′

65(2) sense: 5′-GATC CCC GCCGAAGCCTCATGATAAA TTCAAGAGA

TTTATCATGAGGCTTCGGC TTTTT GGAA A-3′

65(2) antisense:5′-AGCT T TTCC AAAAA GCCGAAGCCTCATGATAAA

TCTCTTGAA TTTATCATGAGGCTTCGGC GGG-3′

52(2) sense: 5′- GATC CCC GTGTCCTGCTAGATATAGA TTCAAGAGA

TCTATATCTAGCAGGACAC TTTTT GGAA A-3′

52(2) antisense: 5′- AGCT T TTCC AAAAA GTGTCCTGCTAGATATAGA

TCTCTTGAA TCTATATCTAGCAGGACAC GGG-3′

Quantitation of viral replication assays

Levels of RT activity or CA protein detected by phosphoimager or autoradiography were scanned and quantified by using Image J software (Abramoff et al., 2004). Background was subtracted before normalizing to controls.

Supplementary Figure 1 legend

Figure S1: The residues contacting IQGAP lie on a side surface of the MA trimer. An image of the HIV-1 MA trimer is shown, with monomers colored in pink, teal, and blue (Hill et al., 1996) (Protein data bank, reference 1HIW structure coordinates). The sequence of the M-MuLV MA protein was aligned with that of the HIV-1 MA in accord with a structure-based sequence alignment prepared with pairwise superimpositions (Riffel et al., 2002). The HIV-1 residues corresponding to the altered residues in the M-MuLV replication and interaction defective mutants (T1, T2, T4, T5, T6, T11, SMA6, and SMA8) are highlighted in red on the blue monomer. The residue corresponding to the altered residue in MA revertant F38L is highlighted in yellow.

Figure S2: Characterization of IQGAP mutants with restored interaction with mutant MA baits in yeast two-hybrid system. (A) Yeast expressing the indicated Gal4AD-IQGAP1 mutants and LexA-MA proteins were scored for β-galactosidase expression as before. (B) Characterization of the interaction of the Y1627H mutant of IQGAP1 with various MA mutants. (C) Virion production of MA mutants in cells expressing IQGAP1 or IQGAP-Y1627H. 293T cells were transfected with various MA mutant DNAs as indicated, with or without tagged IQGAP Y1627H; culture medium and cell lysates were harvested, and the yields of virions were assessed by Western blot developed with anti-CA antiserum (upper panel). Expression of Gag in cell lysates was examined similarly (middle panel), and expression of tagged IQGAP was confirmed with anti-Flag antibody (lower panel).

Figure S3: Quantitation of viral replication assays for various MA mutants. (A) Levels of RT on day 4 postinfection , shown in Figure 1C, were quantitated and plotted relative to wild-type (100%). (B) Levels of CA released from transfected cells, shown in Figure 1D upper panel, were quantitated and plotted relative to wild-type (100%). (C) Levels of

RT on day 4 postinfection , shown in Figure 2C, were quantitated and plotted relative to wild-type (100%). (D) Levels of CA released from cells expressing virus and IQDN4, as shown in Figure 5B, were quantitated and plotted relative to control (100%). (E) Levels of CA released from cells expressing virus and IQDN3, as shown in Figure 5C, were quantitated and plotted. (F) Levels of RT in medium at various days postinfection of cells expressing various RNAi hairpins as indicated, shown in Figure 6B, were quantitated and plotted.

Figure S4: RNAi-mediated knock-down of IQGAP1 and 2 reduces the susceptibility of cells to transduction by an M-MuLV vector. 293T cell lines stably expressing the indicated pairs of hairpin RNAs were challenged by infection with an MuLV vector expressing hygro^r and plated in medium containing 200 μg/ml hygromycin, and the resulting colonies were counted after 10 days.

Supplementary
Table S1. MA interacting proteins recovered from two-hybrid screen.

Gene Name	Description (Hodges et al., 2002)	Number of times recovered
IQGAP1	IQ motif-containing GTPase activating protein 1	7
VAV	Vav 1 proto-oncogene, a guanyl-nucleotide exchange factor	4
DNAJ/HSP	Heat shock protein that may be involved in protein folding	1
Transketolase	Transfers a two carbon ketol from a ketose to an aldose	5
DOK3	PH and PTB domain-containing hematopoietic cell-specific adaptor protein	5
Others	N/A	21
Total	N/A	43