Supplementary Materials

Materials and Methods

Determination of the methylation state of receptors *in vivo.* VB13 cells containing plasmid pGW100 expressing the different V5-tagged Tar variants were grown at 30ºC overnight in tryptone broth containing 50 μg/mL ampicillin. Overnight cultures were back-diluted 1:100 into the same medium and shaken at 30ºC until an OD_{600nm} of ~0.6. Cells were harvested by centrifugation, washed three times with 10 ml of 10 mM potassium phosphate buffer (pH 7.0) containing 0.1mM EDTA, and resuspended in 5 ml of 10 mM potassium phosphate (pH 7.0) containing 0.1 mM EDTA, 10 mM sodium DL-lactate, and 200 μg/ml of chloramphenicol. One ml aliquots were placed into scintillation vials and incubated for 10 min at 30ºC with shaking. The cells were then incubated for another 30 min at 30ºC after adding L-methionine to a final concentration of 100 μM. 100 μl of 100 mM L-aspartate or 10 mM $NiSO_4$ solutions, or 100 μl of buffer, were added to the cells, and they were incubated for another 20 min at 30^{o}C. Reactions were terminated by addition of 100 μl 100% trichloroacetic acid (TCA), and the samples were incubated on ice for 15 min. Denatured proteins were pelleted and subsequently washed with 1% TCA and acetone. The dried pellet was resuspended into 100 μl of 2X SDS loading buffer.

The resuspended pellets were subjected to 3 freeze/boil cycles of 10 min each to denature proteins. A 20 μl aliquot of each sample was loaded into a 7.5% acrylamide SDS-PAGE gel. Once electrophoresis was complete, proteins were transferred to nitrocellulose paper. An immunoblot was performed by adding anti-V5 epitope antibody (Invitrogen), and visualization

of proteins was achieved using goat-anti-mouse antibody conjugated to alkaline phosphatase (Bio-Rad). Standards were run as a mixture of Tar proteins containing equal proportions of V5-tagged versions of the EEEE, QEQE, and QQQQ forms of the otherwise wild-type Tar receptor produced within RP3098 containing pRD300. The Gln residues affect protein migration like methylated Glu residues, so that the standards should migrate like the unmethylated, doubly methylated, and quadrupally methylated forms of the receptor, respectively (Figure S3). Mutant and wild-type receptors were subjected to SDS-PAGE and immunoblotting to ensure that their steady-state *in vivo* level (Figure S2 and Table 1) were similar to the wild-type receptor. Briefly, HCB436 (ΔT *cheR*$^-$*B*$^-$) cells harboring various Tar receptors expressed from pGW100 were grown in TB and diluted to a standard O.D of 0.6 for all samples. The cells were then pelleted and resuspended in 2X SDS loading dye. The samples were then subjected to three cycles of boiling and freezing to lyse the cells. The samples were run on an SDS-PAGE gel and immunoblotted using an anti-V5 antibody (Invitrogen) as a primary antibody, and an HRP-conjugated goat anti-mouse secondary antibody (Invitrogen) as the secondary antibody. The supersignal HRP substrate (Thermo scientific) and Kodak X-ray film was used to detect the chemiluminescence. The blots were exposed for 45 seconds and quantitated using Image J (NIH). To ensure the mobility of the mutant receptors during SDS-PAGE was similar to wild-type receptor SDS-PAGE gels and immunoblots were performed (Figure S1). Briefly, all samples were grown in TB and diluted to a standard O.D. of 0.6. The cells were treated similar to that in Table S1 except that the methylation standards were TCA preciptitated as mentioned earlier. The samples were then loaded onto a large 7.5% SDS-PAGE gel and immunoblotted

using anti-V5 primary antibody (Invitrogen) and alkaline phosphatase-conjugated goat anti-mouse secondary antibody (Bio-Rad).

Figure legends

Figure S1. Baseline receptor methylation levels for wild-type and mutant Tar receptors in strains with and without CheR and CheB. A.) Wild-type and MLLT(G_{1-4}) baseline methylation patterns in buffer in the presence (+CH_3) and absence (-CH_3) of methylation machinery. The methylation standard (Std.) consists of methylation states EEEE (uppermost band), QEQE (middle band), and QQQQ (lowest band). B.) Baseline methylation patterns for G_0 through G_8 in the absence and presence of methylation machinery. C.) The MLLT (-2 through +4) baseline methylation patterns with and without CheR and CheB. All receptors were tagged with a C-terminal V5-epitope tag and detected using an anti-V5 antibody.

Figure S2. Expression levels of mutant Tar receptors relative to wild-type Tar. A.) Expression levels of wild-type Tar, G_1 through G_9, and MLLT(G_{1-4}). B.) Expression levels of wild-type Tar and MLLT mutants (-4 through +5). All receptors were tagged with a C-terminal V5-epitope tag and detected using an anti-V5 antibody. All samples were normalized to a OD_{600nm} of 0.6.

Figure S3. Methylation patterns for wild-type Tar and the -1 Tar mutant. *In vivo* methylation patterns of Tar receptors are shown for VB13 cells expressing V5-tagged wild-type or the -1 mutant receptors after 20 min exposure to 1 mM aspartate (Asp), 10 mM $NiSO_4$ (Ni^{2+}), or buffer

(-). Migration standards (Std.) of the EEEE, QEQE, and QQQQ forms of wild-type Tar are also shown.

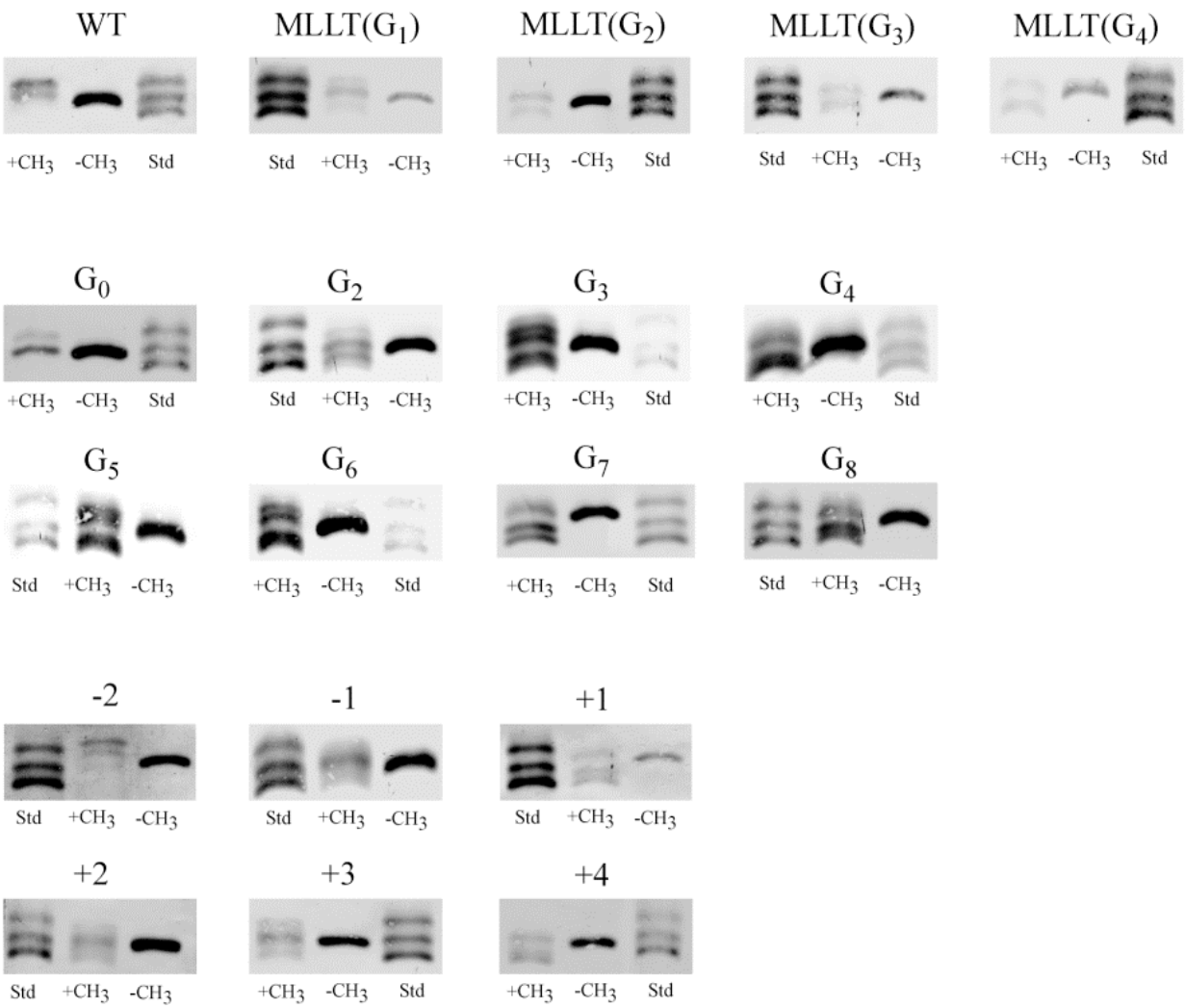
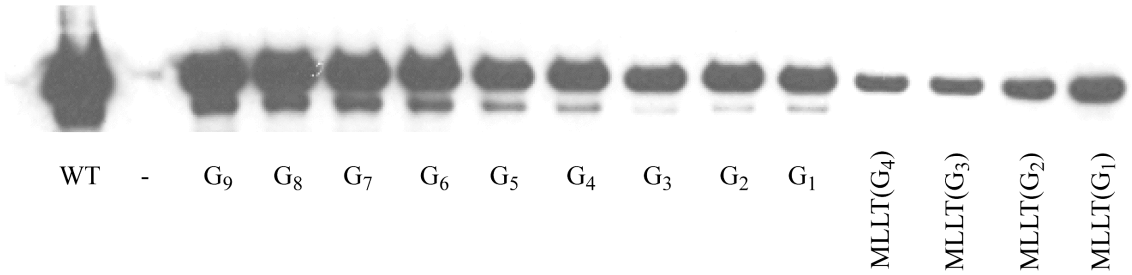

WT
+CH3 -CH3 Std
MLLT(G1)
Std +CH3 -CH3
MLLT(G2)
+CH3 -CH3 Std
MLLT(G3)
Std +CH3 -CH3
MLLT(G4)
+CH3 -CH3 Std
G0
+CH3 -CH3 Std
G2
Std +CH3 -CH3
G3
+CH3 -CH3 Std
G4
+CH3 -CH3 Std
G5
Std +CH3 -CH3
G6
+CH3 -CH3 Std
G7
+CH3 -CH3 Std
G8
Std +CH3 -CH3
-2
Std +CH3 -CH3
-1
Std +CH3 -CH3
+1
Std +CH3 -CH3
+2
Std +CH3 -CH3
+3
+CH3 -CH3 Std
+4
+CH3 -CH3 Std

Figure S1

A.


WT
-
G9
G8
G7
G6
G5
G4
G3
G2
G1
MLLT(G4)
MLLT(G3)
MLLT(G2)
MLLT(G1)

B.

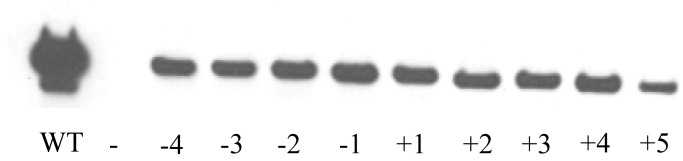

WT
-
-4
-3
-2
-1
+1
+2
+3
+4
+5

Figure S2

Figure S3