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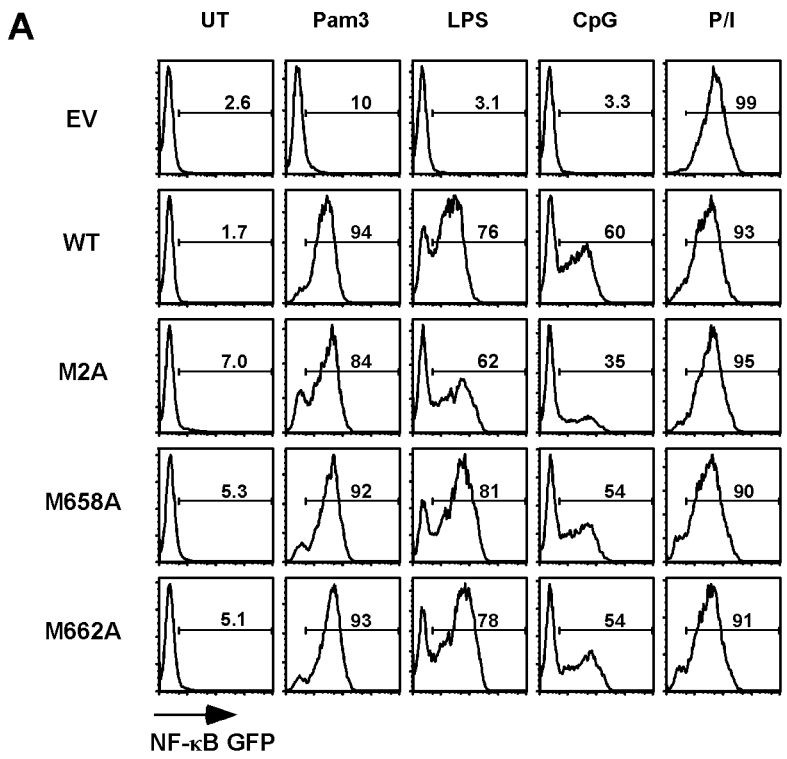
SUPLEMENTAL INFORMATION (1 table and 2 figures)

Definition of the Client-Binding Domain and the Substrate Specificity of gp96 (HSP90b1, GRP94) in Chaperoning Toll-Like Receptors And Integrins

Shuang Wu *et al*.



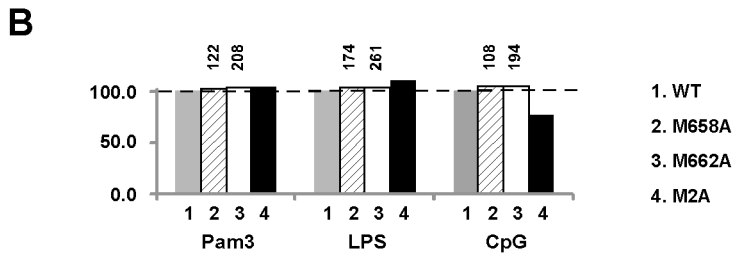


Figure S1. Cells with Met mutants of gp96 are fully responsive to TLR stimulation. *A*, NFκB-GFP activation after overnight (16-18hr) stimulation with nothing (UT), PMA/ionomycin (P/I) or various TLR ligands. Pam3CSK4, LPS, CpG, PMA/ionomycin concentration were described in methods section. Numbers refer to percentage of GFP-positive cells. *B*, Quantification of the mean fluorescence intensity (MFI) of NFκB-GFP reporter activation in *A*.

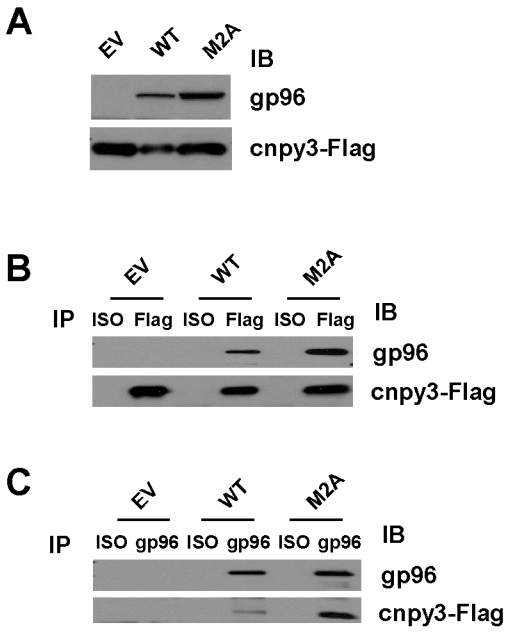


Figure S2. M2A mutant can still interact with co-chaperone cnpy3. *A*, Expression of gp96 mutants and cnpy3-Flag in transduced gp96-null E4.126 cells. *B*, IP of cnpy3-Flag followed by IB for cnpy3-Flag or gp96. *C*, IP of gp96 followed by IB for cnpy3-Flag or gp96.

Table S1. Primers used for generating gp96 mutants.

Mutants	Primer sequences
ΔCBD	N-piece Forward: 5'-ggaagatctgccaccatgagggtcctgtgg-3' Reverse: 5'-tttcgaacgttttcttttgactggcctgactggccacaagagcacaggga-3' C-piece Forward: 5'-tccctgtgctcttgtggccagtcaggccagtcaaaagaaaacgttcgaaa-3' Reverse: 5'-cacacgggattcatagcgaga-3'
AA1	1st set Forward: 5'-ctcttgtggccagtcagatcatgaaggcacaagc-3' Reverse: 5'-gcttgtgccttcatgatctgactggccacaagag-3' 2nd set Forward: 5'-ctcttgtggccagtcagtatgcagcgtctgccgccgcggcggcgatcatgaaggcacaagc-3' Reverse: 5'-gcttgtgccttcatgatcgccgccgcggcggcagacgctgcatactgactggccacaagag-3'
AA2	Forward: 5'-gcacaagcatacgcgacgggcaaggccatctctacaaatgccgctgccagtcaaaag-3' Reverse: 5'-cttttgactggcagcggcatttgtagagatggccttgcccgtcgcgtatgcttgtgc-3'
M2A	Forward: 5'-atggatggtctggcaacgcggagaggatcgcgaaggcacaagcatacc-3' Reverse: 5'-ggtatgcttgtgccttcgcgatcctctccgcgttgccagaccatccat-3'
M658A	Forward: 5'-tatggatggtctggcaacgcggagaggatcatgaaggc-3' Reverse: 5'-gccttcatgatcctctccgcgttgccagaccatccata-3'
M662A	Forward: 5'-ggcaacatggagaggatcgcgaaggcacaagcatacca-3' Reverse: 5'-tggtatgcttgtgccttcgcgatcctctccatgttgcc-3'
NTD+CBD	Forward: 5'-atagcggccgcgccaccatgagggtcctgtg-3' Reverse: 5'-tatgcggccgcttacttatcgtcgtcatccttgtaatcatagtaatttgtagagatgt-3'
NTDΔCBD	Forward: same as NTD+CBD Reverse: 5'-tatgcggccgcttacttatcgtcgtcatccttgtaatcctgactggccacaagagcaca-3'
CTD+CBD	1st rd Forward: 5'-cctgctgaccttcgggttcgtcagagctgattacaaggatgacgacgataagtatggatggtctggcaacatg-3' 2nd set Forward: 5'-atagcggccgcgccaccatgagggtcctgtgggtgttgggcctctgctgtgtcctgctgaccttcgggttcg-3' Reverse: 5'-atagcggccgcacacgggattcatagcgaga-3'
CTDΔCBD	1st rd Forward: 5'-cctgctgaccttcgggttcgtcagagctgattacaaggatgacgacgataaggccagtcaaaagaaaacgttc-3' 2nd set Forward: same as CTD+CBD Reverse: same as CTD+CBD