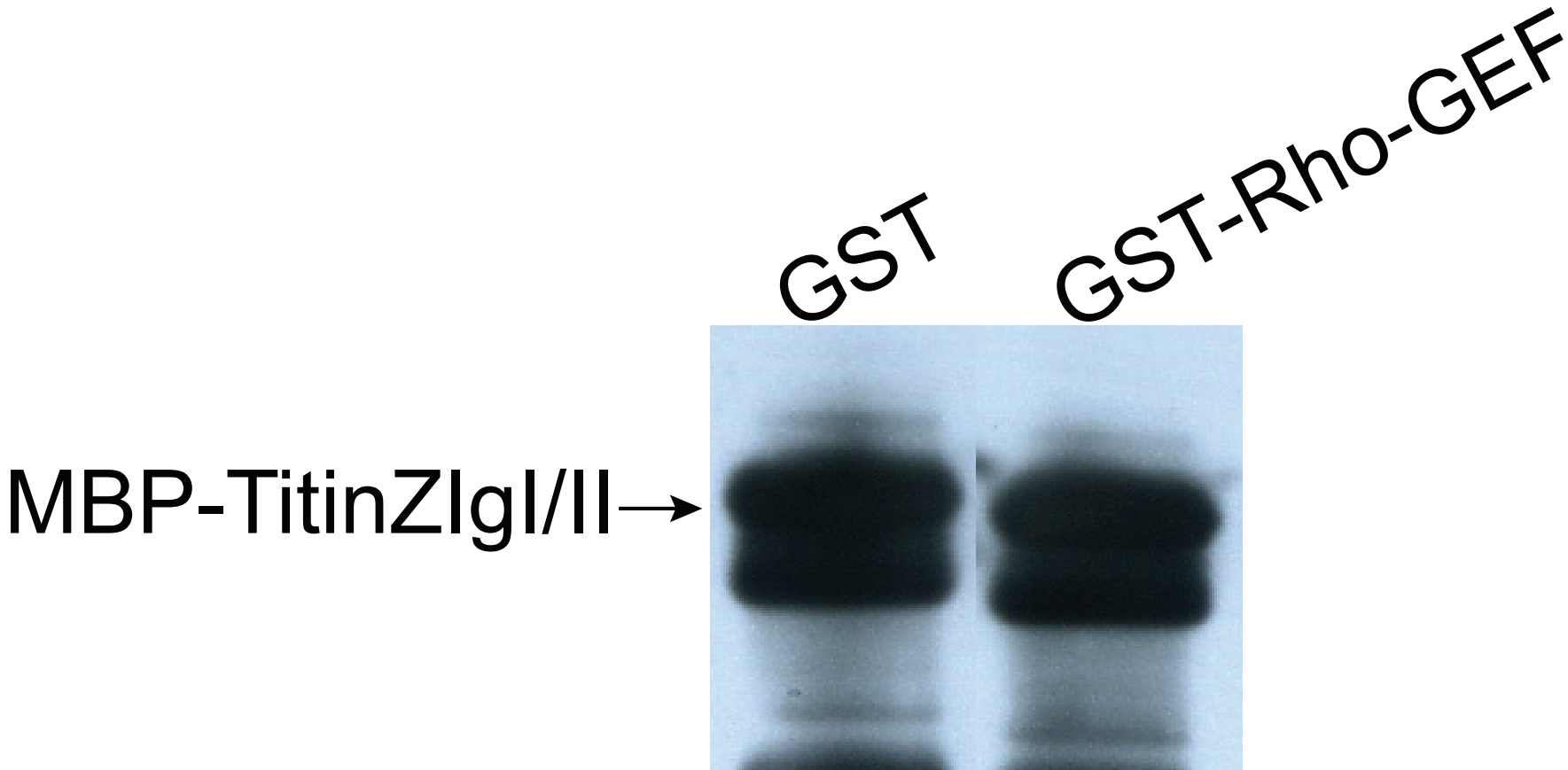
E08-03-0237 Bloch

Supplementary Figure 1: Effect of the Rho-GEF domain of obscurin on immunoblotting of titin epitopes. To test for epitope masking of the N-terminal Ig domains of titin by the Rho-GEF domain of obscurin, we blotted 500ng of a fusion construct containing the Ig I and II domains of titin (the same fusion construct used to generate our antibodies to titin at the Z-disk) and overlayed with either GST or GST fused to the Rho-GEF domain (1μg/μl) of obscurin. Binding of MBP-TitinZIgI/II to GST-Rho-GEF occurs under these conditions (see Figure 9K). We then probed the blot with the antibody used for immunofluorescence. The results show that the Rho-GEF domain of obscurin does not block binding of the antibody to the N-terminal epitopes of titin.

Supplementary Figure 1



Supplementary Table 1

A: Rho-GEF/PH RT	GCCACAGATCTGCTTCACCCA
B: Rho-GEF/PH Rev pGBKT7	GACGGATCCCTAGCCACGATCTGCTTCAC
C: Rho-GEF/PH For pGBKT7	AGTGAATTCGTCATCCAGGAGTTGCTGAGTTC
D: PH For pGBKT7	AGTGAATTCCCTATCCGCCAGGGTCAC
E: Rho-GEF Rev pGBKT7	ATCGGATCCCTAGCGCTGTGGCAGGGCAGA
F: RanBP9 108 For pGADT7	ACTTCCCGGGTCTTGCAGCGGGCCCCGGC
G: RanBP9 317 For pGADT7	TCCACCCGGGTGGGCTTCAAACACCAGGA
H: RanBP9 523 For pGADT7	TCCACCCGGGTCATCAATCATATTGCCAT
I: RanBP9 211 For pGADT7	TCCACCCGGGTGCCTGTGGGATTTATTAT
J: RanBP9 421 For pGADT7	TCCACCCGGGTGAAGCCATTGAAACAACA
K: RanBP9 729 Rev pGADT7	GCAGCTCGAGCTAATGTAGGTAGTCTTC
L: RanBP9 316 Rev pGADT7	GCAGCTCGAGCACAGTAGGATACAAATT
M: RanBP9 522 Rev pGADT7	GCAGCTCGAGTGCTTTATTTGATATTAC
N: RanBP9 420 Rev pGADT7	GCAGCTCGAGTCCCATTCTTCCTGGTAA
O: RanBP9 623 Rev pGADT7	GCAGCTCGAGAAAGTGGATCATTCTTTC
P: RanBP10 For pGADT7	ATGAATTCATGGCGGCAGCGACG
Q: RanBP10 Rev pGADT7	TATCTCGAGCTAGTGCAAGTAGTC
R: RanBP9 108 For pRSETC	CGACCTCGAGCTCTTGCAGCGGGCCCC
S: RanBP9 729 Rev pRSETC	GATCAAGCTTCTACTAATGTAGGTAGTC
T: RanBP9 108 For pMAL-c2x	ATCGCTAGCCTTGCAGCGGGCCCC
U: RanBP9 729 Rev pMAL-c2x	GATAAGCTTCTAATGTAGGTAGTC
V: RanBP9 108 For pGEX4T-1	AGCCCGGGTCTTGCAGCGGGCCCC
W: RanBP9 729 Rev pGEX4T-1	GTGCGGCCGCCTAATGTAGGTAGTC
X: RanBP9 108 For HcRed	ATCTCGAGCTCTTGCAGCGGGCCCC
Y: RanBP9 729 Rev HcRed	CGCGAAGCTTCTAATGTAGGTAGTC

All primers are listed 5' and 3'. For each indicated plasmid, the forward (For) and reverse (Rev) primers were used under standard conditions for the creation of plasmids.