

A

Gucy2c 56 DAVNEGLEIVRGRLQNAGLNVTV 78
+ ++E E V R+Q++G NVT+
NHERF3 430 NVLDEPYEKVVDRIQSSGKNVTI 452

Gucy2c 322 ALAYLNGILLFGHMLKIFLENGENI 346
+A G+L H++++ NGEN+
NHERF3 167 GVAMRAGVLADDHLIEV---NGENV 188

Gucy2c 374 DVDSTMVLLYTSVDTKKYKVLITYDTHVNKTYPV-DMSPTFTWKNSKLPNDITGRGP 429
D DS + ++ D LL D + Y + SP +++ +LPN P
NHERF3 300 DHDSVEMIRKGGDQTS---LLVVDKETDNMYRLAHFSPFLYYQSQELPNGSVKEAP 353

Gucy2c 708 LFLETAEEKELEVYLLVKNCWEEEDPEKRPDFKKIETTTLAKIFGLFHDQKNESYMDTLIRR 767
FL ++ E + +V+ C P ++ + + L +I G+F D++ + L+R+
NHERF3 22 FFLRIEKDTEGHLVRVVEKC---SPA EKAGLQDGRVL-RINGVFVDKEEHMQVVDLVRK 77

Gucy2c	Identities	Positives	Gaps
Region 1	9/23(39%)	16/23(69%)	0/23(0%)
Region 2	8/25(32%)	15/25(60%)	3/25(12%)
Region 3	14/57(25%)	23/57(40%)	4/57(7%)
Region 4	13/60(22%)	29/60(48%)	4/60(6%)

NHERF3

B

Gucy2c 114 RMGCVLIGP 122
R+ CV GP
NHERF4 481 RLSCVASGP 489

Gucy2c 888 RHAIIDIAKMAL 898
RHA D+A+ L
NHERF4 195 RHAHDVARAQL 205

Gucy2c 935 IKMPRYCLF 943
++ PR+CL
NHERF4 110 LERPRFCLL 118

Gucy2c 977 CQFLYEVVRGETYLKGRGNETTYWL---TGMKDQKFNL P 1011
C + + G + GN+ +WL TG ++ +P
NHERF4 224 CHIVKDEGGFGFSVTHGNQGPFWLVSTGGAAERAGVP 261

Gucy2c 1000 LTGMKDQK-----FNLPTPPTVENQQR 1021
L G +D++ LPT PT ++ R
NHERF4 39 LVGSRDRRGRVFSVPVPLPTNPPTTQHPT R 68

Gucy2c	Identities	Positives	Gaps
Region 1	5/9(56%)	6/9(66%)	0/9(0%)
Region 2*	6/11(55%)	8/11(72%)	0/11(0%)
Region 3*	4/9(44%)	7/9(77%)	0/9(0%)
Region 4	9/38(24%)	17/38(44%)	3/38(7%)
Region 5	9/30(30%)	14/30(46%)	8/30(26%)

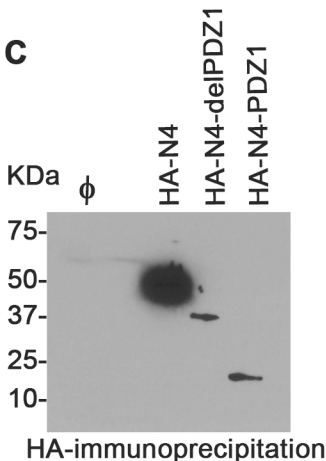
NHERF4

}Region 2

}Region 3

Catalytic regions

C



Supplementary figure 1

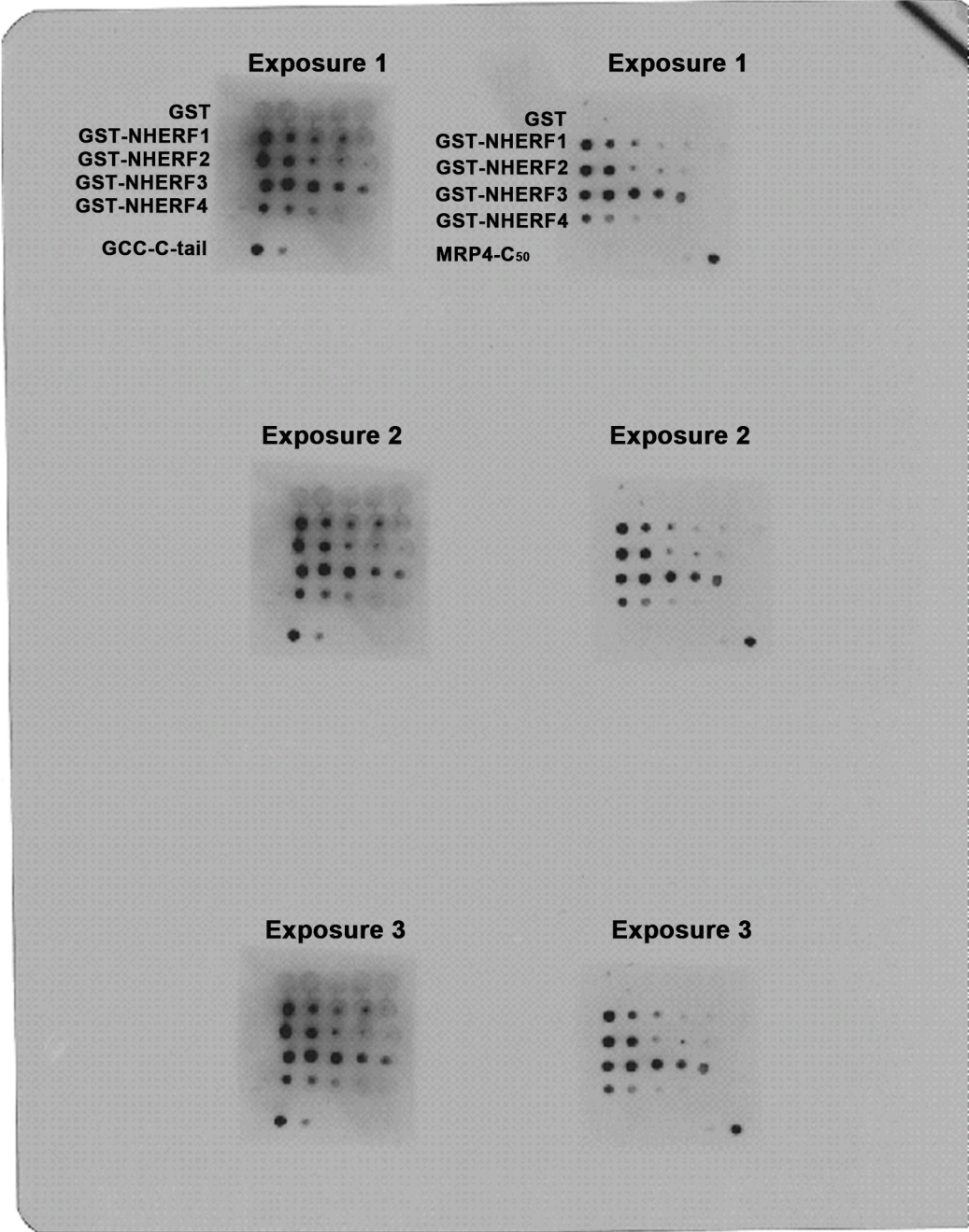
A.

Raw data :Figure 1C

Dot-blot analysis of GST-NHERF1-4 binding with the C-terminal 50 amino acids of GCC

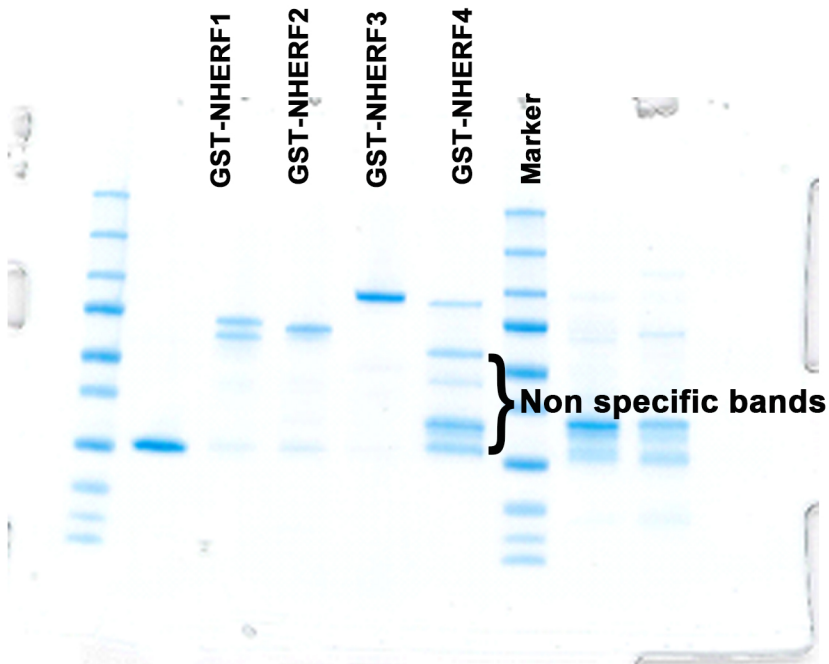
Raw data :Figure 4A

Dot-blot analysis of GST-NHERF1-4 binding with the C-terminal 50 amino acids of MRP4



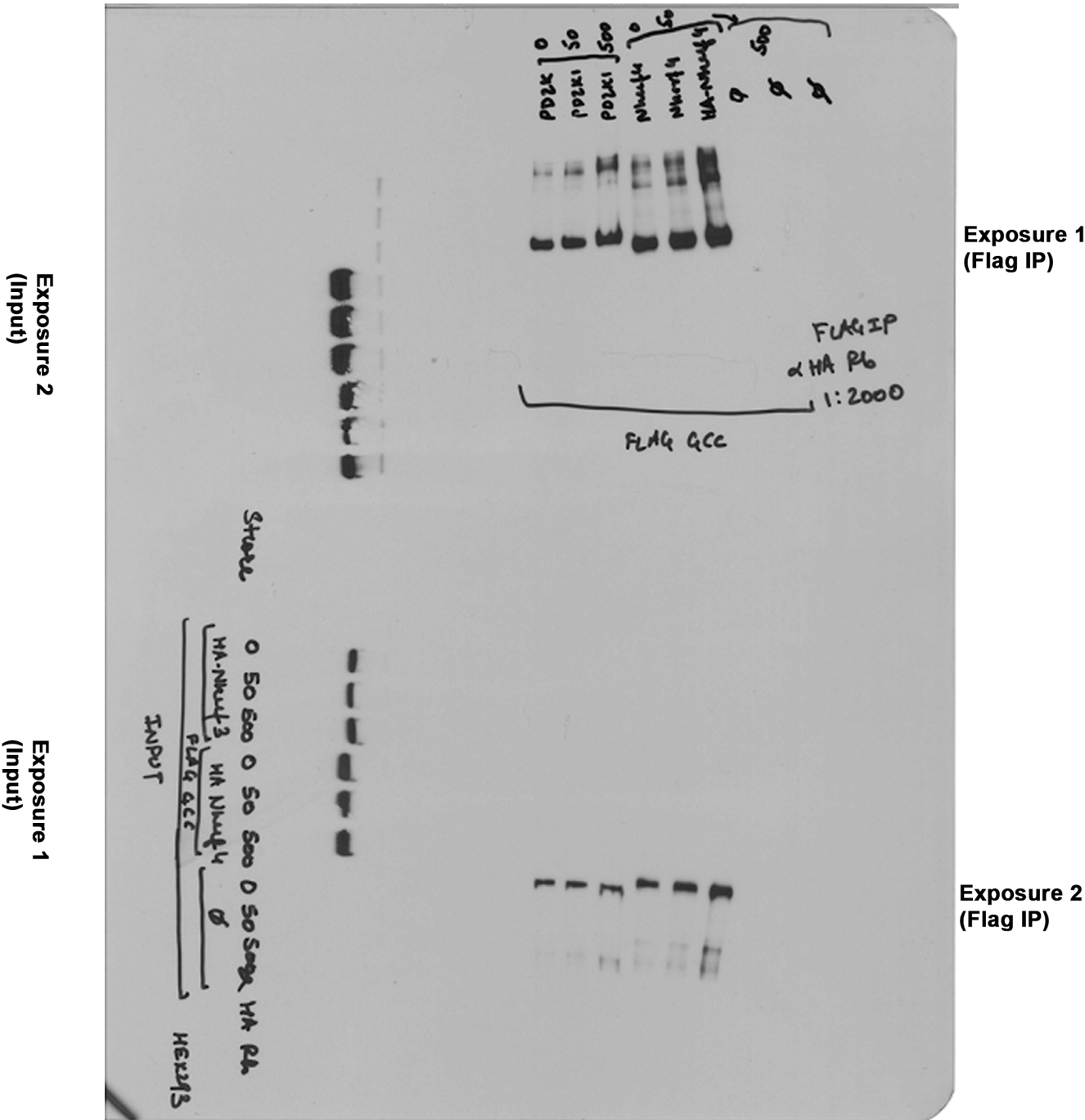
Supplementary figure 2

B. Raw data: Figure 1B



Additionally, we observe that the NHERF4 protein can efficiently immunoprecipitate GCC on par with NHERF3.

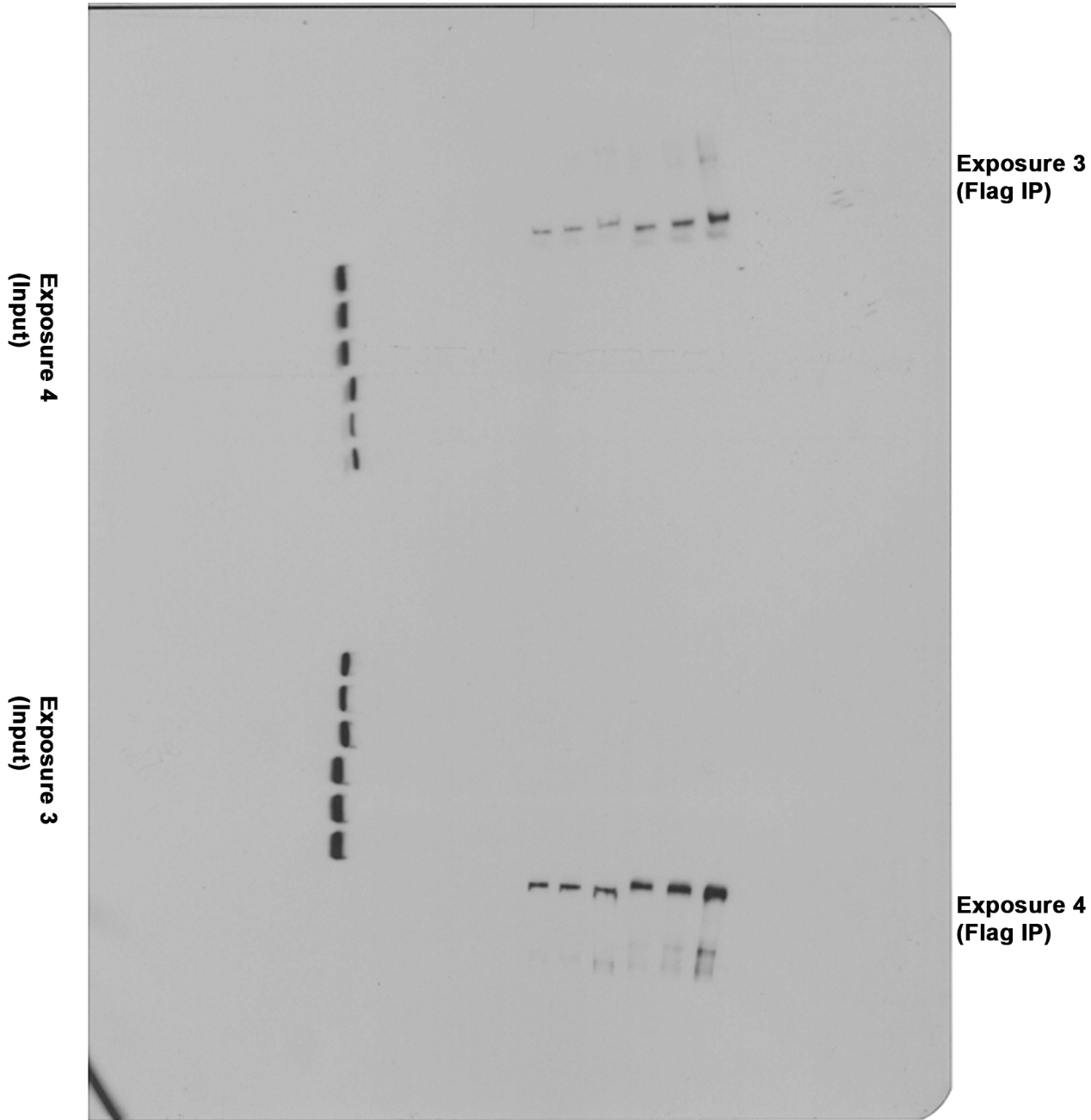
A. Raw data: Figure 3E
(HA-tagged protein IP & Input)



Supplementary figure 3

B. Raw data: Figure 3E
(HA-tagged protein IP & Input _ Different exposure)

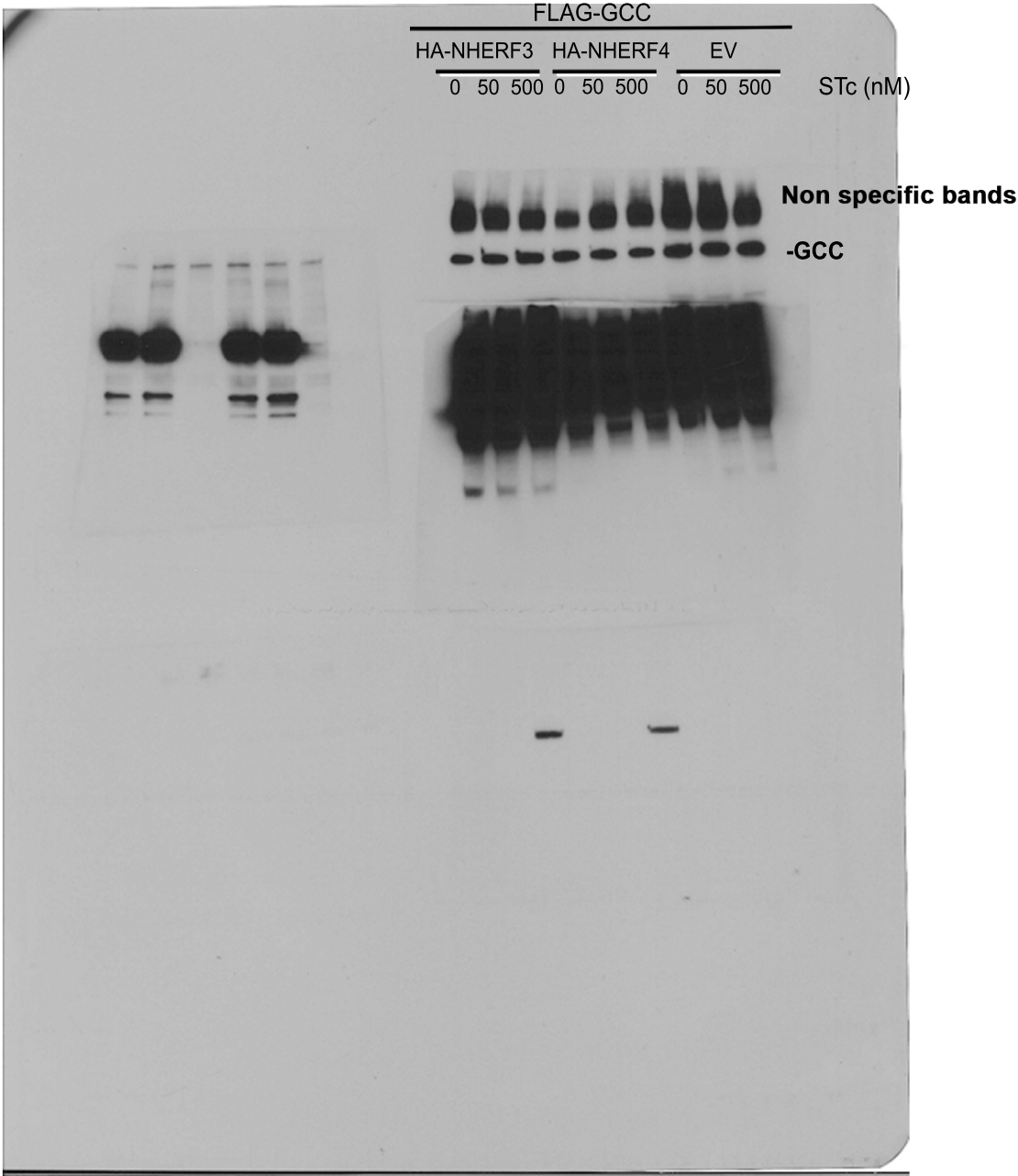
FLAG-GCC									STc (nM)
HA-NHERF3			HA-NHERF4			EV			
0	50	500	0	50	500	0	50	500	



Supplementary figure 3

C. Raw data:Figure 3E

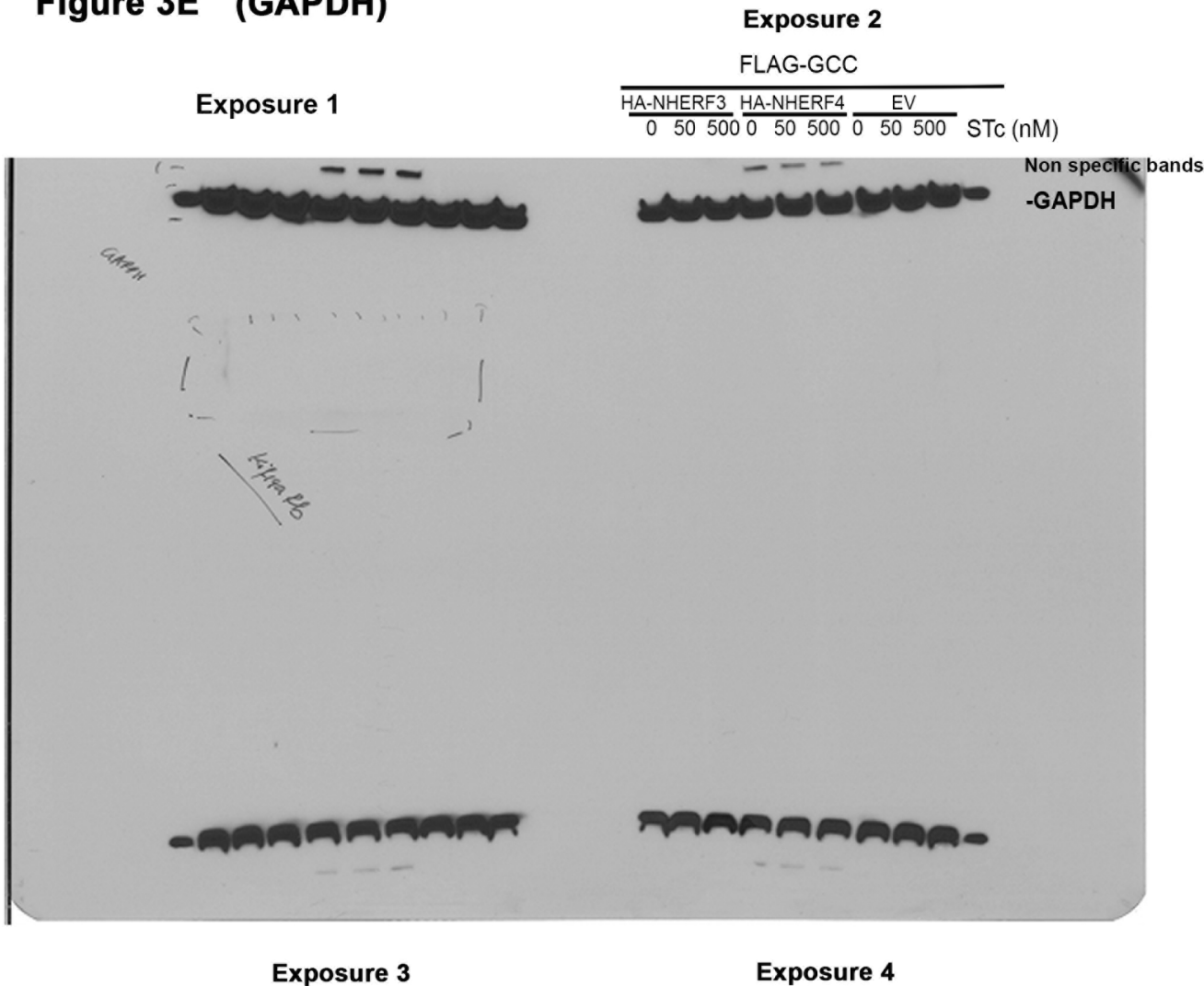
(GCC)



Supplementary figure 3

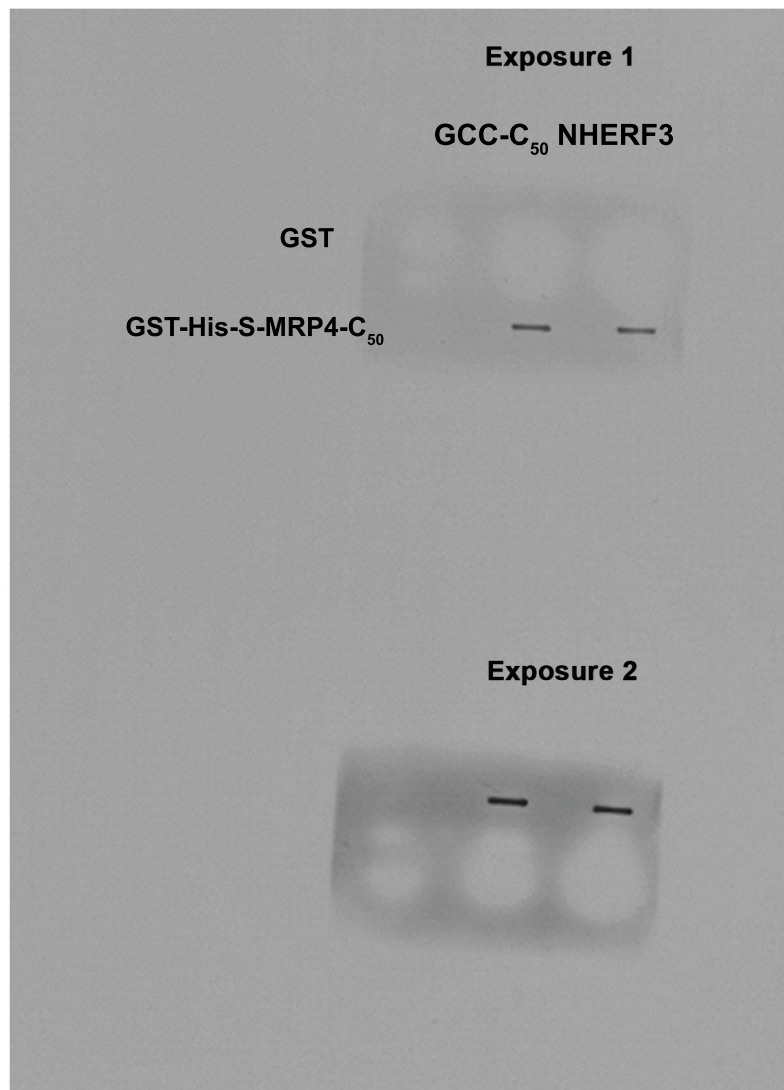
D. Raw data:

Figure 3E (GAPDH)



Raw data: Figure 4B

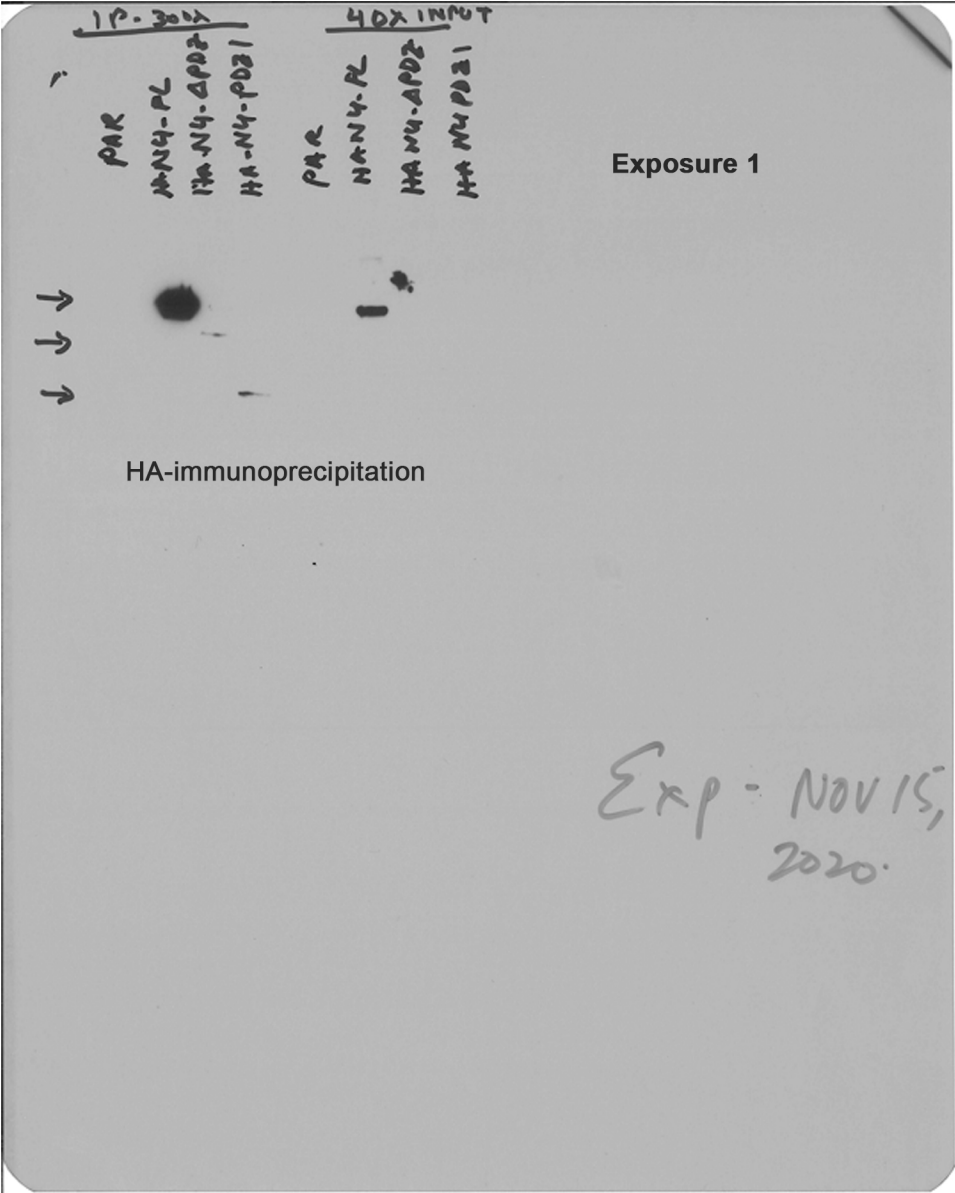
The macromolecular complex formation assay between
MRP4C50 and GCCC50 via NHERF3



Supplementary figure 4

A. Raw data:Supplementary Figure 1C

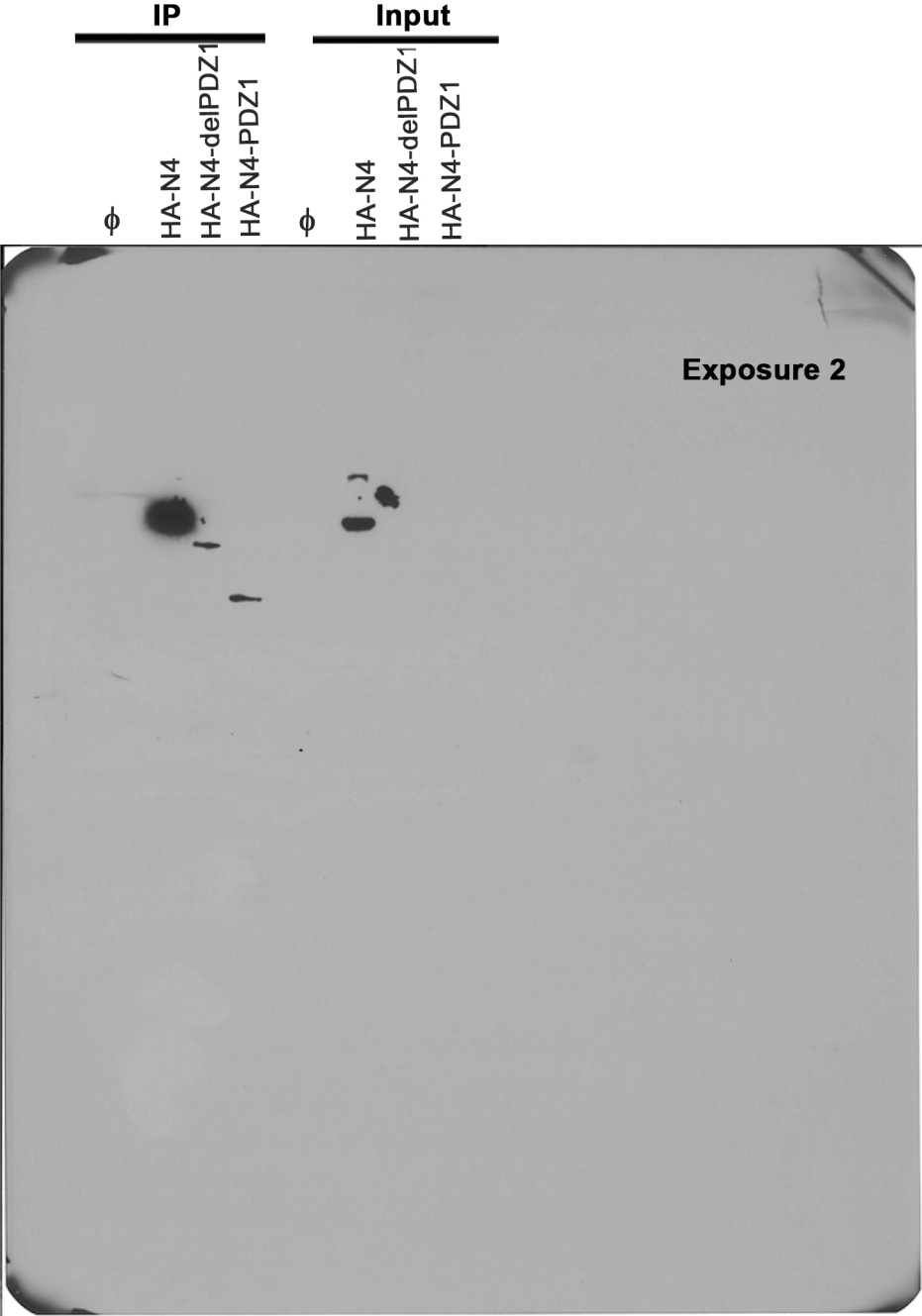
HA-immunoprecipitation of HA-tagged NHERF4 full-length, NHERF4 PDZ1, NHERF4 ΔPDZ1



Supplementary figure 5

B. Raw data:Supplementary Figure 1C

HA-immunoprecipitation of HA-tagged NHERF4 full-length, NHERF4 PDZ1, NHERF4 ΔPDZ1



Supplementary figure 5