

Appendix

The association of the JNK scaffold protein, WDR62, with the mixed lineage kinase 3, MLK3

Miriam Hadad, Sharon Aviram, Ilona Darlyuk-Saadon, Ksenya Cohen-Katsenelson, Alan J.
Whitmarsh, Ami Aronheim

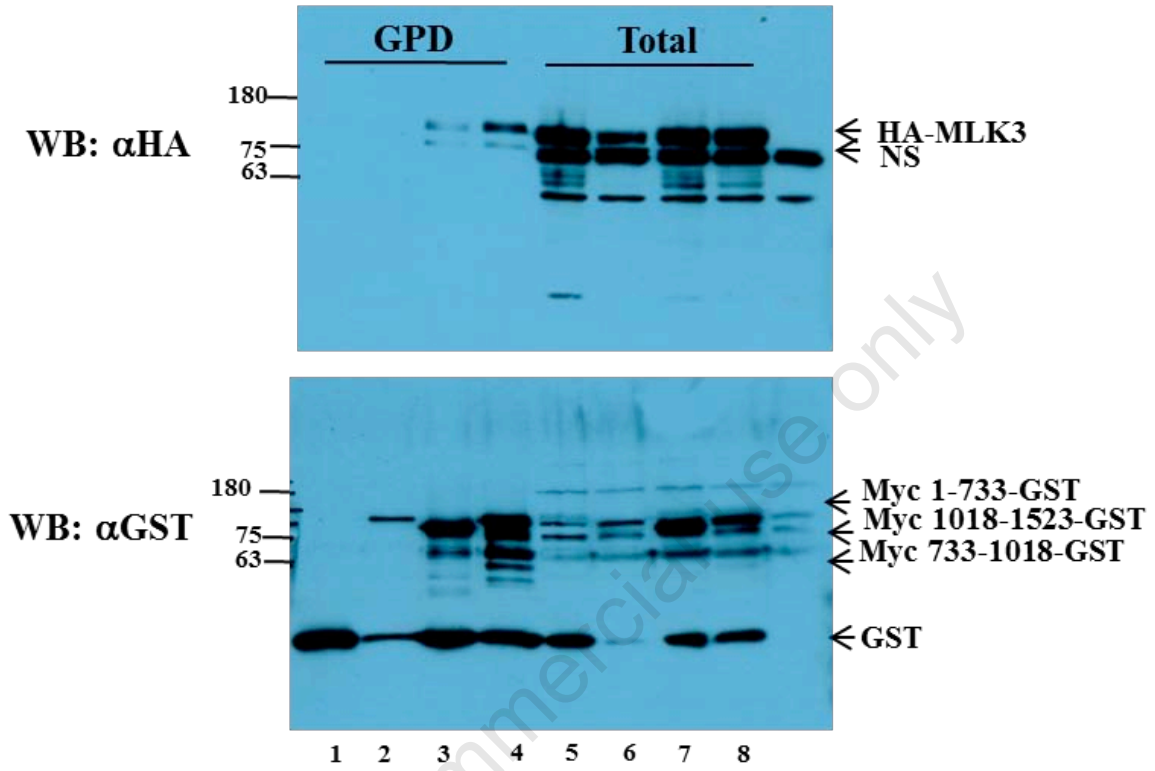
Supplemental Table and Figures

Supplemental Table 1. Expected size (kDa) for all construct used.

Figure	Construct	Expected size (kDa)
2, 3, 4	Myc-1-1523-GST	198.5
2	Myc-1-733-GST	111.6
2, 3, 4	Myc-733-1018-GST	62.3
3, 4	Myc-1018-1284-GST	60.2
2	Myc-1018-1523-GST	86.5
4	Myc-1212-1284-GST	38.9
4	Myc-1212-1523-GST	65.2
1, 5	Myc-1-1523-GST	168.5
2	Myc-733-971-GST	27.2
2	Myc-733-1018-GST	32.3
2	Myc-1018-1212-GST	22.3
2	Myc-1031-1212-GST	20.9
2	Myc-1212-1523-GST	35.2
1, 2, 3, 4	HA-MLK3	94
3	HA-MLK3 1-114	13.5
3	HA-MLK3 115-399	32.2
3	HA-MLK3 400-591	22
3	HA-MLK 592-487	29
5	Flag-MLK3	94
1	HA-MLK2	107
1	HA-ZAKa	89.8
1	HA-DLK	96.5
5	HA-JNKK2-JNK1	94.5
4	CDC42QL	21

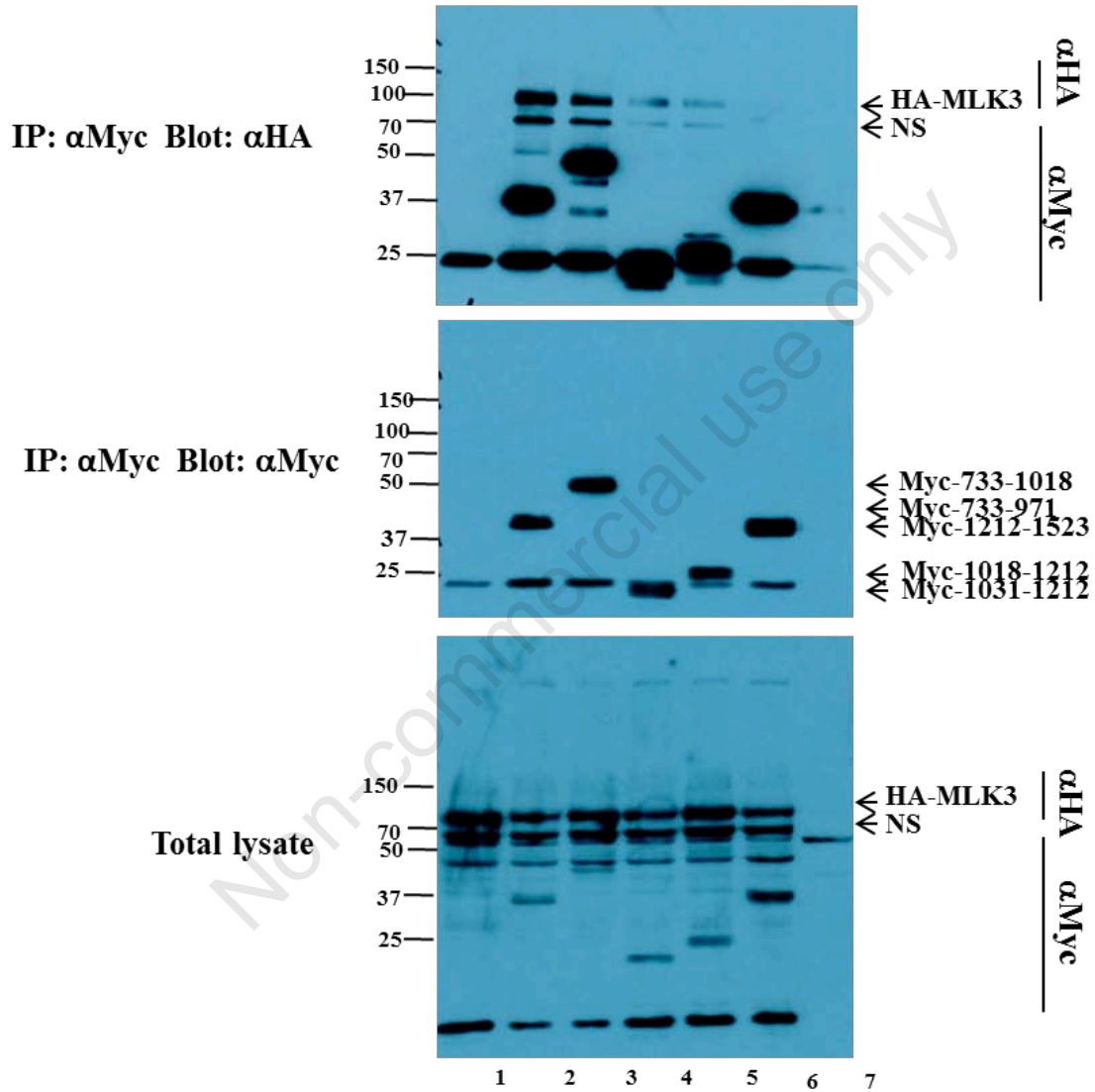
Supplemental Figure 1. The entire gel from Figure 2B is shown.

GST:	+	-	-	-	+	-	-	-	-
1-733-GST:	-	+	-	-	-	+	-	-	-
733-1018-GST:	-	-	+	-	-	-	+	-	-
1018-1523-GST:	-	-	-	+	-	-	-	+	-
HA-MLK3:	+	+	+	+	+	+	+	+	-

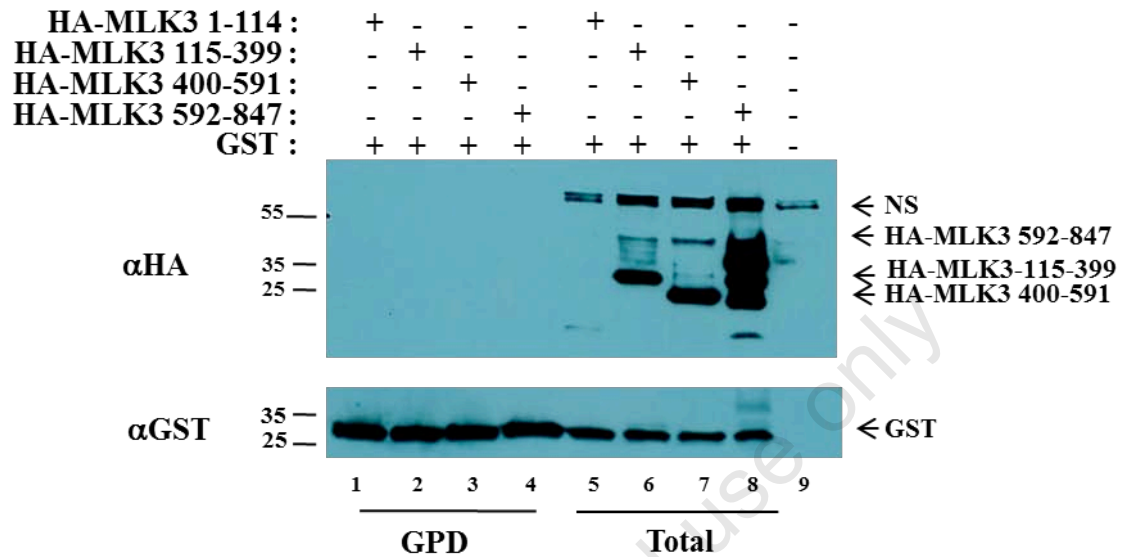


Supplemental Figure 2. The entire gel from Figure 2D is shown.

Myc-733-971:	-	+	-	-	-	-	-
Myc-733-1018:	-	-	+	-	-	-	-
Myc-1031-1212:	-	-	-	+	-	-	-
Myc-1018-1212:	-	-	-	-	+	-	-
Myc-1212-1523:	-	-	-	-	-	+	-
HA-MLK3:	+	+	+	+	+	+	-



Supplemental Figure 3. GST pull down for GST protein and various MLK3 deletion mutants used in Figure 3. HEK-293T cells were transfected with the indicated plasmids. GST-containing complexes were isolated from cell lysates by GST pull down (GPD). Eluted protein complexes were subjected to Western blotting with either anti-HA (upper panel) or anti-GST (lower panel) antibodies. The migration of the relevant proteins is indicated by arrows.



Supplemental Figure 4. Validation of CDC42QL GTPase activity. HEK-293T cells were transfected with the indicated plasmids. Cell lysate was subjected to Western blot analysis with anti-Phospho-MLK3 (Top panel) and anti-HA antibody (bottom Panel).

