

Table 1. Yeast two-hybrid analysis of class E VPS factor interactions

| VP16-activation domain fusion | | GAL4 DNA-binding domain fusion | | | | | | | | | | | | | | | |
|-------------------------------|------------|--------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------|------------|------------|-------------|-------------|-------------|-------|
| | | ESCRT-I | ESCRT-II | | | ESCRT-III | | | | | | | | | Other | | |
| | | Tsg101 | EAP45 | EAP20 | EAP30 | CHMP6 | CHMP4A | CHMP4B | CHMP4C | CHMP2A | CHMP2B | CHMP3 | CHMP1A | CHMP1B | AIP-1/ALIX | VPS4 | None |
| ESCRT-I | Tsg101 | 2.12 ± 0.31 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| | VPS 28 | 3.28 ± 0.23 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| ESCRT-II | EAP45 | <0.05 | <0.05 | 2.1 ± 0.09 | 3.57 ± 0.15 | 0.05 ± 0.01 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| | EAP20 | <0.05 | 3.33 ± 0.06 | <0.05 | 3.66 ± 0.14 | 3.49 ± 0.10 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| | EAP30 | <0.05 | 3.51 ± 0.03 | 3.53 ± 0.11 | 0.97 ± 0.08 | 0.98 ± 0.14 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| ESCRT-III | CHMP 6 | <0.05 | 0.65 ± 0.10 | 3.47 ± 0.17 | 3.27 ± 0.33 | <0.05 | 1.66 ± 0.21 | 3.54 ± 0.13 | 2.82 ± 0.20 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| | CHMP4A | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 2.84 ± 0.27 | 3.22 ± 0.14 | 2.08 ± 0.15 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 3.3 ± 0.04 | <0.05 | <0.05 |
| | CHMP4B | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.85 ± 0.15 | 1.60 ± 0.19 | 0.79 ± 0.16 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 2.27 ± 0.66 | <0.05 | <0.05 |
| | CHMP4C | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.64 ± 0.15 | 1.73 ± 0.09 | 2.93 ± 0.66 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 1.85 ± 0.19 | <0.05 | <0.05 |
| | CHMP 5 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| | CHMP2A | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.83 ± 0.18 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 3.32 ± 0.02 | <0.05 |
| | CHMP2B | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| | CHMP3 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.20 | <0.14 | 3.76 ± 0.26 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| | CHMP1A | <0.08 | <0.08 | <0.08 | <0.08 | <0.08 | <0.08 | <0.20 | <0.14 | <0.08 | <0.08 | 0.5 ± 0.12 | 0.13 ± .03 | 0.16 ± 0.02 | 3.18 ± 0.41 | 0.08 ± 0.02 | <0.05 |
| | CHMP1B | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | 0.8 ± 0.06 | <0.05 | <0.05 | 2.94 ± 0.17 | <0.05 |
| Other | AIP-1/ALIX | 0.33 ± 0.01 | <0.05 | <0.05 | <0.05 | <0.05 | 3.34 ± 0.08 | 2.73 ± 0.94 | 3.03 ± 0.20 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.54 ± 0.83 | <0.05 | <0.05 |
| | VPS4 | <0.05 | <0.05 | <0.05 | <0.05 | 0.08 ± 0.01 | <0.05 | <0.20 | <0.14 | 3.4 ± 0.05 | <0.05 | <0.05 | 3.4 ± 0.14 | 2.71 ± .32 | <0.05 | <0.05 | <0.05 |
| | Hrs | 1.88 ± 0.38 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.20 | <0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| | None | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.20 | 0.14 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

Each of the mammalian class E VPS factors was screened in the yeast two-hybrid assay for interaction with each of the others. The table shows the mean ± SD of optical density values obtained in a liquid β-galactosidase assay performed on three independent pools of yeast transformants. Positive signals are indicated by shading. Very weak signals obtained with GAL4-CHMP1B/VP16-CHMP1A, GAL4-ALIX/VP16-CHMP1A, and GAL4-CHMP-6/VP16-VPS4 were less than 2-fold above background and were not considered positive. Of these transformants, only the CHMP1A:CHMP1B interaction was found to be detectable by coprecipitation analysis. The GAL4 DNA-binding domain fusions of VPS28, HMP5, and Hrs were strong transcriptional activators in the absence of a coexpressed VP16-activation domain fusion protein and were therefore excluded from the analysis.