

## Supplementary Figure Legends

**Figure S1** Aligned sequences of VHS domain-containing proteins in the region corresponding to the C-terminal half of the domain. The secondary structure of the human STAM1 VHS domain is shown with solid bars to indicate  $\alpha$ -helices. Identically conserved residues that are not directly involved in binding are shaded gray.

**Figure S2** Additional SPR binding curves. (A) Full-length ESCRT-0. (B) STAM1 VHS domain. (C) STAM1 VHS-UIM. (D) Hrs DUIM. (E) Hrs FYVE-VHS-DUIM. (F) Vps27 tandem UIMs.

		$\alpha 5$	$\alpha 6$	$\alpha 7$	$\alpha 8$	
STAM1_Hs	79	IFHLEVCSRDFASEVS	.....NVLN.KGHPKVCEK	LKALMVEWTDEFKNDPQLS	.....LISAMIKNLKEQGVTFP.	143
STAM2_Hs	79	IFHLEVCSRDFATEVR	.....AVIKNKAHPKVCEK	LKSLMVEWSEEFQKDPQFS	.....LISATIKSMKEEGITFP.	144
Hse1_Sc	79	RLRQEISSKNFTSLLY	.....ALIESHSVHITLKKAVT	DVVKQLSDSFKDDPSLR	.....AMGDLYDKIKRKAPYLVQ	146
Hrs_Hs	78	TVHDEVANKQTMEEK	.....DLLKRQVEVNVRNKILYLI	QAWAHAFRNEPKYK	.....VVQDTYQIMKVEGHVFP.	143
Hrs_Dm	76	PVHEEVFTKENCEMFS	.....SFLESTPHENVRQKMLEL	VQTWAYAFRSDKYQ	.....AIKDTMTILKAKGHTFP.	141
Vps27_Sc	83	PFIKEICSREFMDTMEH	.....VILREDSNEELSELVKTILY	EYVAFKNDSQLN	.....YVAKVYDKLISRGIKFPE	150
GGA1_Hs	80	RFHDEVGKFRFLNELIKVV	SPKYLGSRTSEKVKNKILELLYS	WTVGLPEEVK	.....IAEAYQMLKKQGIVKSD	148
GGA2_Hs	96	KFHSEVAKFRFLNELIKVL	SPKYLGSWATGKVKGRVIEILFS	WTVWFPEDIK	.....IRDAYQMLKKQGIKQD	164
GGA3_Hs	79	RFHNEVGKFRFLNELIKVV	SPKYLGDRVSEKVKTKVIELLYS	WTMALPEEAK	.....IKDAYHMLKRQGIVQSD	147
GGA1_Sc	93	SIHLQISRKEFLNDLVKR	..FPEQPPLRYSKVQQMILEAIE	EWYQTICKHAS	YKDDLQYINDMHKLLKYKGYTFP.	165
GGA2_Sc	97	PFHLQISRKEFLNELVKR	..FPGHPPLRYSKIQRILITAI	EWYQTICKHSSYKNDMGY	IRDMHRLLLKYKGYAFP.	169
TOM1_Hs	84	RFHVLVASQDFVESVLVR	..TILPKNNPPTIVHDKVLNLI	QSWADAFRSPDLT	.....GVVTIYEDLRRKGLEFP.	152
TOM1L1_Hs	86	SFQSLIVKKEFVKENLVK	..LLNPRYNLPLDIQNRILNF	IKTWSQGFPGVDVS	.....EVKEVYLDLVKKGVPFP.	154

Figure S1

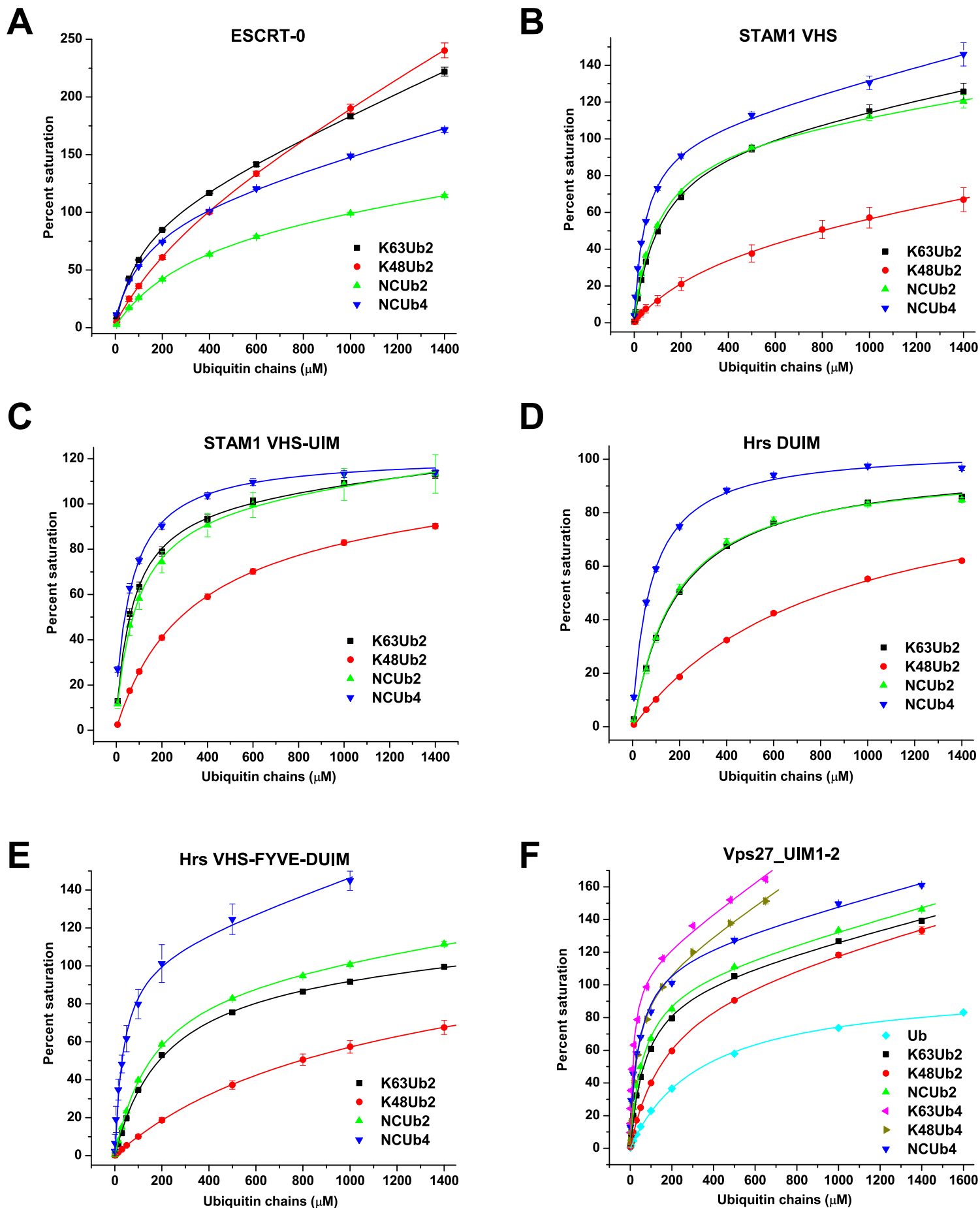


Figure S2

Table S1. List of bacterial expression constructs.

Plasmid Name	DNA insert codes for:
pGST2-STAM1-VHS	Human STAM1 (2-143)
pGST2-STAM-VHS-UIM	Human STAM1 (2-200)
pGST1-STAM-UIM	Human STAM1 (158-200)
pGST1-Hrs-VHS	Human Hrs(2-143)
pGST1-Hrs-DUIM	Human Hrs (250-289)
pGST1-GGA1-VHS	Human GGA1 (1-148)
pGST1-GGA2-VHS	Human GGA2 (1-164)
pGST1-GGA3-VHS	Human GGA3 (1-147)
pGST1-TOM1-VHS	Human TOM1 (1-152)
pGST1-TOM1L1-VHS	Human TOM1L1 (1-154)
pGST2-Hse1-VHS	Sc Hse1 (1-146)
pGST1-Vps27-VHS	Sc Vps27 (2-150)
pGST1-Gga1-VHS	Sc Gga1 (1-165)
pGST1-Vps27-UIM1-2	Sc Vps27 (245-330)

Table S2. List of plasmids for yeast experiments.

plasmid	Description
<i>VPS27</i> /YCplac111	<i>VPS27</i> wild-type cassette/YCplac111
<i>HSE1</i> /YCplac22	<i>HSE1</i> wild-type cassette/YCplac22
<i>HSE1</i> <sup>ΔUIM</sup> /YCplac22	<i>HSE1</i> cassette A170Q/S174D/YCplac22
<i>HSE1</i> <sup>ΔVHS</sup> /YCplac22	<i>HSE1</i> cassette W25A/L29D/YCplac22
<i>HSE1</i> <sup>ΔVHSAUIM</sup> /YCplac22	<i>HSE1</i> W25A/L29D/ A170Q/S174D/YCplac22
<i>VPS27</i> <sup>ΔUIM1-2</sup> /YCplac111	<i>VPS27</i> cassette A266Q/S270D/A309Q/S313D/YCplac111
<i>VPS27</i> <sup>ΔVHSAUIM1-2</sup> /YCplac111	<i>VPS27</i> cassette L28A/L32D/ A266Q/S270D/A309Q/S313D/YCplac111
pGO45	GFP-tagged Carboxypeptidase S/pRS426 (Odorizzi et al. 1998)