Supplementary figures

Comprehensive analysis of the human SH3 domain family reveals a wide variety of non-canonical specificities

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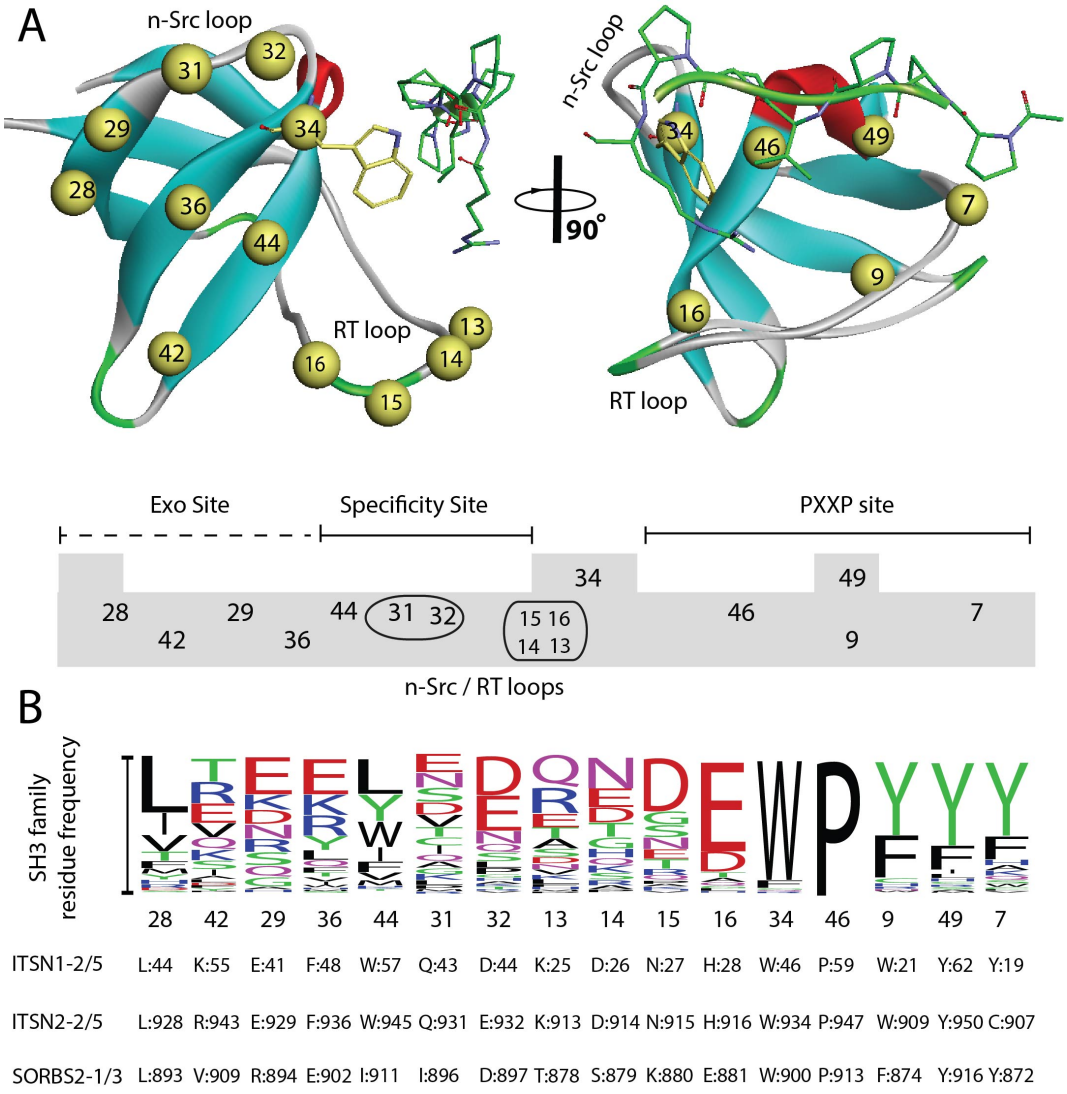
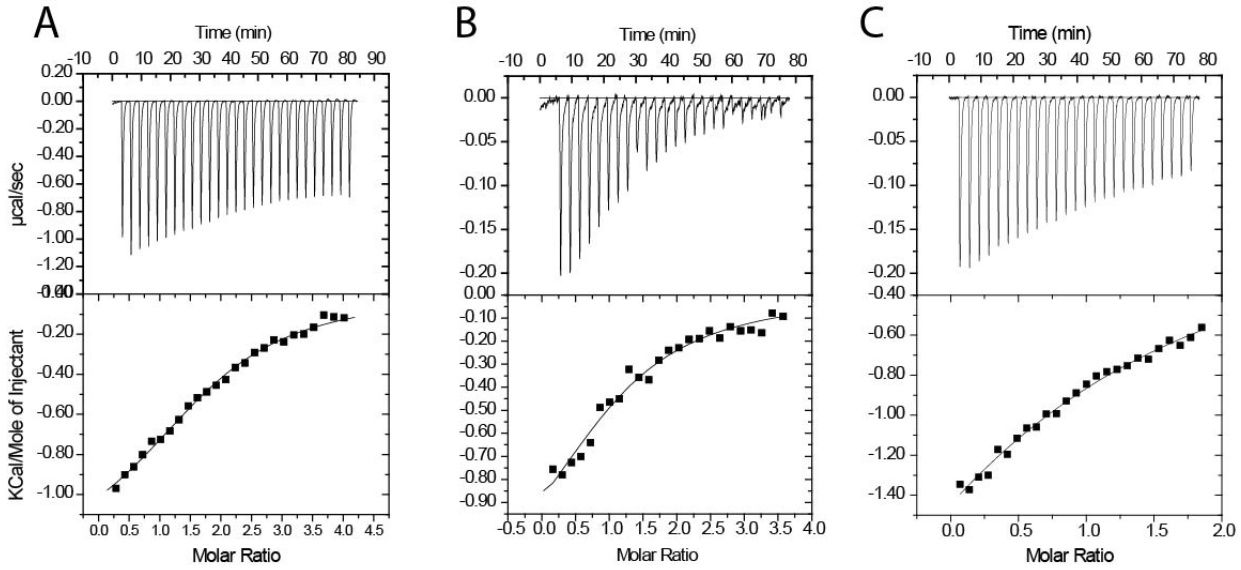


Fig. S1: SH3 domain residues involved in peptide recognition

(A) A representative SH3 domain fold is shown to highlight the PXXP site, specificity site and the exo site (PDB ID: 1SEM). Important residues for binding are labeled in the structure according to their position in the sequence alignment of the SH3 family with phage display results (**Table S1**), and are linked to the schematic representation of the binding pockets, shown below

the structure. **(B)** The amino acid frequency is shown for each position important in peptide recognition. The logo representation is obtained from a sequence alignment of the successfully phaged SH3 domains (**Table S1**). The residue correspondence to the ITSN1-2/5, ITSN2-2/5 and SORBS2-1/3 structures are shown below the frequencies.



Model: OneSites
Chi^2/DoF = 355.2
N = 1.80 ± 0.0516 Sites
K = 1.87E4 ± 1.88E3 M-1
DH = -1329 ± 58.12 cal/mol
DS = -80.1 cal/mol/deg
Kd = 53.5 ± 5.3 uM

Model: OneSites
Chi^2/DoF = 1325
N = 1.13 ± 0.144 Sites
K = 2.62E4 ± 6.47E3 M-1
DH = -1428 ± 245.2 cal/mol
DS = 15.4 cal/mol/deg
Kd = 38.2± 9.4 uM

Model: OneSites
Chi^2/DoF = 617.6
N = 1.14 ± 0.308 Sites
K = 8.26E3 ± 2.72E3 M-1
DH = -4443 ± 1816 cal/mol
DS = 3.02 cal/mol/deg
Kd = 121.1 ± 39.9 uM

Fig. S2: Analysis of several SH3-peptide binding affinities using ITC

(A) 90 µM ITSN1(II/V)-SH3 with 1 mM of peptide WRDSSGYVMGPW; **(B)** 40 µM SORBS2(I/III)-SH3 with 0.5 mM of peptide LRTGEAYLRYVD; **(C)** 50 µM SORBS2(I/III)-SH3 with 1 mM of peptide RLPLRPPLPHTS.

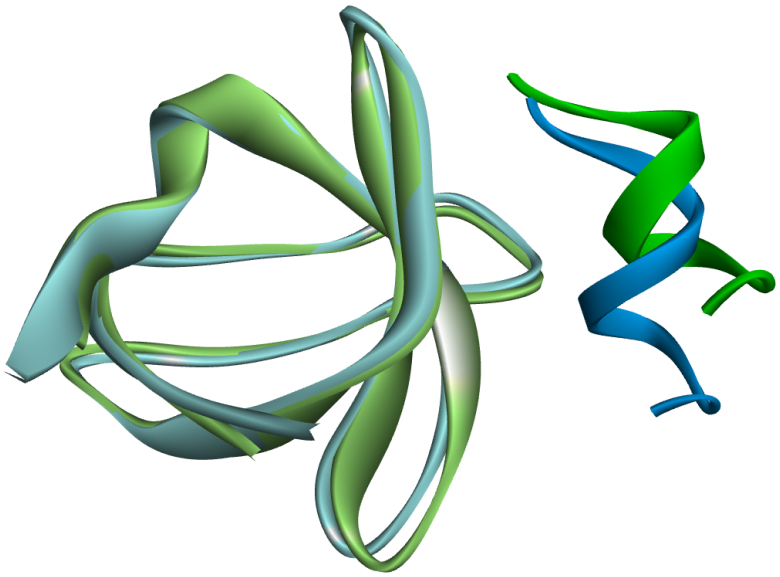


Fig. S3: Structural superposition of ITSN1-2/5 and ITSN2-2/5 peptide complexes (green and blue ribbons, respectively).

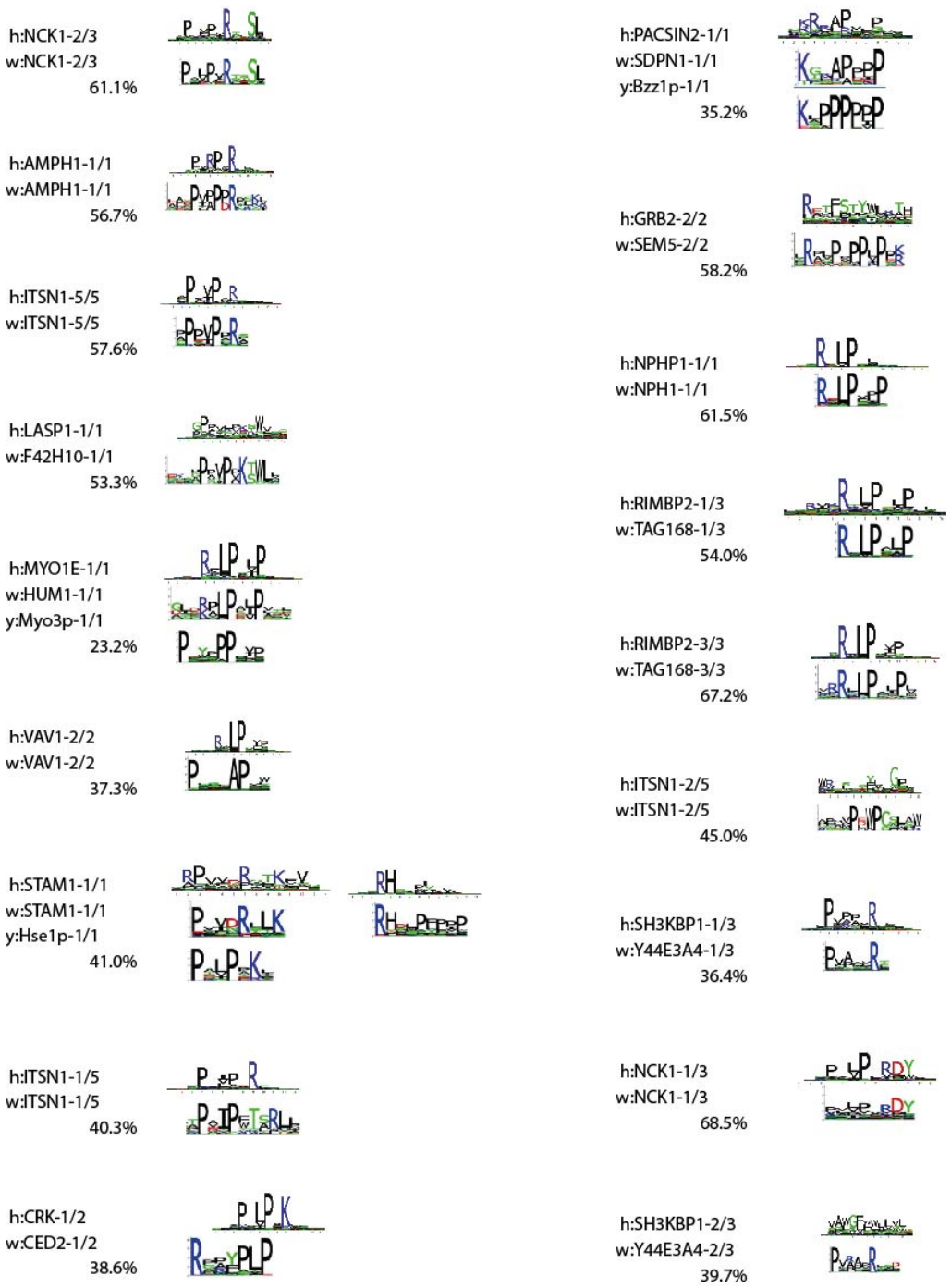


Fig. S4: SH3 domain binding specificities for the yeast (y), worm (w) and human (h) orthologs with available phage display data.

Orthologs are identified using inParanoid8 software and yeast/worm logos are obtained from previous publication (Gfeller et al., 2011). Sequence identities between SH3 domain pairs are shown.